Phenylenediamine-based bivalent glycocyclophanes: synthesis and analysis of the influence of scaffold rigidity and ligand spacing on lectin binding in cell systems with different glycomic profiles

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General experimental conditions Optical rotations were determined with a Perkin-Elmer 241 model polarimeter at the sodium D line at 20 °C. NMR spectra were recorded with Varian 300 MHz, 400 MHz, 500 MHz and 600 MHz spectrometers. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0), HOD for D_2O (δ 4.80) for ^{1}H-NMR and $CDCl_3$ (δ 77.0) for ^{13}C-NMR at 20 ºC. ^{13}C-NMR signals were assigned with the aid of DEPT-135, HSQC and HMBC. ^{1}H-NMR signals were assigned with the aid of COSY and TOCSY. Coupling constants (J) are reported in Hertz. IR spectra were recorded with a Mattson Galaxy Series IR 3000 using either thin film between NaCl plates or KBr discs, as specified. Melting points were measured on a Gallenkamp melting point apparatus. Elemental analysis was performed on an Exeter Analytical CE440 elemental analyser. Low and high resolution mass spectra were measured on either a Quattro *micro*™ LC-MS/MS or a Micromass LC time-of-flight (LCT) mass spectrometers and were measured in positive and/or negative mode as indicated in each case. TLC was performed on aluminium sheets precoated with Silica Gel 60 (HF_{254}, E. Merck) and spots visualized by UV and charring with H_2SO_4-EtOH (1:20) and/or PMA, $KMnO_4$, ninhydrine or mostaine solutions. Flash column chromatography was carried out using Silica Gel 60 (0.040-0.630 mm, E. Merck) and using a stepwise solvent polarity gradient (starting from the conditions indicated and increasing the polarity) correlated with TLC mobility. Reaction solvents were freshly dried and distilled where stated: acetonitrile, toluene and dichloromethane from calcium hydride; MeOH from magnesium turnings and tetrahydrofuran from sodium wire. Alternatively, dichloromethane, tetrahydrofuran and MeOH were used as obtained from Pure-Solv drying solvent system. Anhydrous DMF and pyridine were used as purchased from Sigma-Aldrich. Molecular

sieves are activated 4 Å molecular sieves. Semi-preparative HPLC was carried out using a Waters 600E HPLC system with a flow rate of 10 mL/min. The semi-preparative column used was reverse phase YMC-Pack ODS-AQ (S5μm, 250 x 20 mm). Wavelength for preparative HPLC was 259 nm.

Zemplén deacetylation Sodium methoxide in MeOH (1.0 M solution freshly prepared) was added to compound (2.5 mmol) in MeOH (25 mL) which had been pre-cooled on an ice bath. The reaction mixture was stirred at 0 ºC for 50 min. The solvent was evaporated under reduced pressure and the residue was dissolved in water and acidified to pH 6 by addition of Amberlite-H^+ resin. The resin was removed by filtration and the filtrate was freeze-dried to give the deacetylated product.

Allyl 2,4-di-*O*-acetyl-1-*O*-allyl-β-D-glucopyranosiduronate 13 Allyl alcohol (0.45 mL, 6.48 mmol) was added to trichloroacetimidate **12** (2.82 g, 5.89 mmol) in dry dichloromethane (68 mL) in the presence of molecular sieves, under N_2 and at room temp. The reaction mixture was stirred for 30 min and then cooled on an ice bath for 15 min. A solution of boron trifluoride etherate (0.37 mL, 2.94 mmol) in dry dichloromethane (4 mL) was then added slowly *via* cannula and the mixture stirred for 1.5 h on an ice-water bath that was allowed to attain room temp. The molecular sieves were removed by filtration and the filtrate washed with satd aq $NaHCO_3$, dried ($MgSO_4$), filtered and the solvent was removed under diminished pressure to give a white solid. Chromatography (cyclohexane-EtOAc, 2:1) gave the intermediate[18] as a white solid (1.42 g, 64%); R_f = 0.55 (cyclohexane-EtOAc 1:1); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 5.90-5.77

(m, 1H, alkene CH=), 5.30-5.17 (m, 4H, overlapping H-4, H-3, CH_2=), 5.07-5.01 (m, 1H, H-2), 4.61 (d, J 7.5, 1H, H-1), 4.39-4.33 (m, 1H, OCH_2), 4.13-3.99 (m, 2H, overlapping OCH_2, H-5), 3.76 (s, 3 H, CO_2CH_3), 2.04, (s, 3H, $OCOCH_3$), 2.02 (s, 6 H, $OCOCH_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 170.1, 169.4, 169.3 (CO), 167.3 (CO_2CH_3), 133.1 (alkene CH), 117.8 (alkene CH_2), 99.5, (C-1), 72.6, 72.1, 71.2, 70.1 (C-2 to C-5), 69.4 (OCH_2), 52.9, (CO_2CH_3), 20.6, 20.62, 20.05 (each $OCOCH_3$); LRMS (ES): 397.0 $[M+Na]^+$. A solution of lithium hydroxide monohydrate in 0.4 M water-THF-MeOH (9:4:1; 7 mL) was added to this glycoside (143 mg, 0.38 mmol) and the mixture cooled on an ice bath and stirred for 2 h. Water was added and the solution acidified to pH 3 by addition of Amberlite-H^+ resin. The resin was removed by filtration and lyophilisation gave the deprotected intermediate[19] as a white solid (77 mg, 86%); ^{1}H-NMR (D_2O, 300 MHz): δ = 6.05-5.92 (m, 1H, CH=), 5.42-5.28 (m, 2H, CH_2=), 4.56 (d, J 7.8, 1H, H-1), 4.43-4.19 (m, 2H, OCH_2), 3.91 (d, J 9.3, 1H, H-5), 3.61-3.50 (m, 2H, overlapping H-3, H-4), 3.38-3.32 (t, J 8.1, 1H, H-2); ^{13}C-NMR (D_2O, 75 MHz): δ = 173.5 (CO_2H), 133.2 (CH), 118.8 (CH_2), 101.1 (d, C-1), 75.4, 75.0, 72.8, 71.4 (C-2 to C-5), 70.7 (OCH_2). This intermediate (1.34 g, 4.08 mmol) was suspended in acetic anhydride (15 mL) in the presence of molecular sieves (4Å) and stirred at 85 ºC for 2 h. The solvent was co-evaporated with toluene and the residue (brown oil) was dried under high vacuum. The oil was dissolved in cyclohexane-EtOAc (1:1), filtered to remove the solid material and the filtrate eluted through a short column of silica gel (cyclohexane-EtOAc, 1:1) to give the 6,3-lactone intermediate as a colourless oil (888 mg, 72%);[20] R_f = 0.57 (cyclohexane-EtOAc, 1:1); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 5.91-5.77 (m, 1H, CH=), 5.33-5.20 (m, 2H, CH_2=), 5.13-5.07 (m, 2H, overlapping H-2, H-3), 4.93 (s, 1H, H-1), 4.87-4.84 (t, J

3.9, 1H, H-4), 4.29-4.22 (m, 1H, OCH_2), 4.26 (d, J 3.0, 2H, H-5), 4.06-3.99 (m, 1 H, OCH_2), 2.18, 2.09 (each s, 3H, $OCOCH_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 170.6, 169.7, 168.9 (each CO), 132.5 (CH), 118.6 (CH_2), 98.4 (C-1), 71.3, 69.1 (each CH), 68.7 (OCH_2), 68.1, 67.9 (each CH), 20.6, 20.5 (each CH_3); LRMS (ES): 323 $[M+Na]^+$, 623 $[2M+Na]^+$. Allyl alcohol (3 mL, 44 mmol) followed by NaOAc (100 mg, 1.22 mmol) were added to **12** (726 mg, 2.32 mmol) in dry THF (5 mL) in the presence of molecular sieves, at room temp and under N_2 and the mixture stirred at room temp for 30 h. The mixture was then filtered and the solvent was removed under diminished pressure. The residue (brown oil) was dissolved in dichloromethane (20 mL) and washed with brine. The aq phase was further extracted with dichloromethane (2 x 10 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and the solvent was removed under diminished pressure. Chromatography (cyclohexane-EtOAc, 2:1) of the residue gave **13** as a white solid (350 mg, 41%); R_f = 0.44 (cyclohexane-EtOAc, 1:1); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 5.96-5.77 (m, 2H, CH=), 5.37-5.10 (m, 5H, overlapping CH_2= and H-4), 4.93-4.88 (dd, J 7.5, 9.3, 1H, H-2), 4.68-4.53 (m overlapping with d, J 7.5, 3H, CO_2CH_2 and H-1), 4.37-4.30 (m, 1H, OCH_2), 4.11-4.04 (m, 1H, OCH_2), 3.99-3.96 (d, J 9.6, 1H, H-5), 3.80-3.72 (m, 1H, H-3), 2.89 (d, J 6.3, 1H, OH), 2.11, 2.06 (each s, 3H, $OCOCH_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 170.6, 170.3, 167.0 (each CO), 133.3, 131.2 (each CH), 119.3, 117.6 (each CH_2), 99.4 (C-1), 73.7, 73.0, 72.7, 71.9 (C-2 to C-5), 70.0 (OCH_2), 66.5 (CO_2CH_2), 20.8, 20.7 ($OCOCH_3$); LRMS (ES): 381.1 $[M+Na]^+$; IR (film from dichloromethane): 3510, 2958, 1753, 1373, 1226, 1132, 1085 cm^{-1}.

Allyl 2,4-di-*O*-acetyl-1-*O*-allyl-α-D-glucopyranosiduronate 17 Zemplén deacetylation of **16**[20] (940 mg, 2.61 mmol) gave a de-*O*-acetylated intermediate as a yellow foam (589 mg, 96%); $[\alpha]_D$ = +16.8 (*c* 0.15, H_2O); ^{1}H-NMR (D_2O, 300 MHz): δ = 6.06-5.93 (m, 1H, CH=), 5.42-5.28 (m, 2H, CH_2=), 5.03 (d, J 3.9, 1H, H-1), 4.29-4.09 (m, 2H, OCH_2), 4.16 (d, J 10.2, 1H, H-5), 3.78-3.72 (t, J 9.3, 1H, H-3), 3.65-3.57 (dd, J 3.6, 9.6, 1H, H-2) overlapping with 3.60-3.54 (t, J 9.6, 1H, H-4); ^{13}C-NMR (D_2O, 75 MHz): δ = 173.1 (CO_2H), 133.3 (CH), 118.4 (CH_2), 97.6 (C-1), 72.6, 71.4, 70.8 (each CH), 69.0 (OCH_2); HRMS (ES): calcd 233.0661 $[M-H]^-$, found 233.0654. This intermediate (1.56 g, 6.30 mmol) was added to acetic anhydride (23 mL) in the presence of molecular sieves and was then stirred at 85 ºC for 2 h. The solvent was co-evaporated with toluene and the residue (brown oil) was dried at high vacuum. The oil was dissolved in cyclohexane-EtOAc (1:1), filtered, and the filtrate was passed through a short column of silica eluting with cyclohexane-EtOAc (1:1). This gave a 6,3-lactone intermediate as a yellow oil (1.63 g, 86%); R_f = 0.55 (cyclohexane-EtOAc, 1:1); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 5.90-5.77 (m, 1H, CH=), 5.43-5.41 (dd, J 3.3, 3.9, 1H, H-2), 5.31-5.23 (m, 2H, CH_2=), 5.15-5.12 (td, J 4.2, 0.9, 1H, H-3), 5.00-4.99 (d, J 3.0, 1H, H-1), 4.82-4.79 (m, 1H, H-4), 4.44-4.37 (m and overlapping d, 3H, J 2.4, OCH_2, and H-5), 4.17-4.10 (m, 1H, OCH_2), 2.31, 2.11 (each s, 3H, $OCOCH_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 169.4, 168.2, 167.3 (each CO), 131.7 (CH), 117.3 (CH_2), 93.9 (C-1), 72.2 (CH), 70.0 (OCH_2), 69.5, 67.5, 65.2 (each CH), 19.5, 18.6 (each $OCOCH_3$); LRMS (ES): 301.0 $[M+H]^+$, 623 $[2M+Na]^+$. Allyl alcohol (2.5 mL, 36.7 mmol) was added to the 6,3 lactone (1.63 g, 5.33 mmol) in dry THF (8 mL) in the presence of NaOAc (217 mg, 2.65 mmol) and molecular sieves at room temp and under N_2 and the mixture stirred for 24 h.

Filtration and removal of the solvent under diminished pressure gave a brown oil. This oil was dissolved in dichloromethane (30 mL), washed with brine, the aq phase was further extracted with dichloromethane (2 x 15 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and the solvent was removed under diminished pressure. Chromatography of the residue (cyclohexane-EtOAc, 2:1) gave **17** as a white solid (1.03 g, 75 %); $[\alpha]_D$ = +20.8 (*c* 0.18, $CHCl_3$); R_f = 0.45 (cyclohexane-EtOAc, 1:1); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 5.98-5.78 (m, 2H, CH=), 5.39-5.20 (m, 4H, CH_2=), 5.17-5.15 (d, J 3.6, 1H, H-1), 5.11-5.04 (t, J 9.6, 1H, H-4), 4.83-4.79 (dd, J 3.9, 10.2, 1H, H-2), 4.69-4.55 (m, 2H, CO_2CH_2), 4.31-4.28 (d, J 10.2, 1H, H-5), 4.25-4.09 (m and overlapping t, 3H, J 9.6, OCH_2 and H-3), 4.06-3.99 (m, 1H, OCH_2), 2.45 (d, J 5.4, 1H, OH), 2.13, 2.08 (each s, 3H, $OCOCH_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 170.7, 170.4, 167.8 (each CO), 133.0, 131.2 (each CH), 118.5, 118.2 (each CH_2=), 95.4 (C-1), 72.9, 72.0, 69.4 (each CH), 69.1 (OCH_2), 68.7 (CH), 66.6 (CO_2CH_2), 20.9, 20.8 (each $OCOCH_3$); HRMS (ES): $[M+H]^+$ calcd 359.1342, found 359.1349; IR (film from dichloromethane): 3480, 2996, 1748, 1374, 1230, 1050 cm^{-1}.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4-di-*O*-acetyl-1-*O*-allyl-α-D-glucopyranosiduronic acid 18 The allyl ester **17** (363 mg, 1.01 mmol) and **15** (550 mg, 1.12 mmol) in dry dichloromethane (24 mL) and in presence of molecular sieves were stirred at room temp and under N_2 for 20 min. The reaction mixture was cooled on an ice bath and to it 0.04 M TMSOTf in dry dichloromethane (2.5 mL, 0.1 mmol) was added dropwise. The reaction mixture was stirred on an ice bath for 30 min and solid $NaHCO_3$ (250 mg) was added and stirring continued for 5 min. The mixture was filtered

and the solvent removed under diminished pressure. Chromatography (dichloromethane-EtOAc 5:1) gave a protected disaccharide as a white solid (465 mg, 65%); $[\alpha]_D$ = +9.68 (*c* 0.32, $CHCl_3$); R_f = 0.6 (dichloromethane-EtOAc 5:1); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 5.99-5.78 (m, 2H, CH=), 5.40-5.39 (dd, J 1.2, 2.7, 1H, CH_2=), 5.35-5.16 (m, 7H, overlapping CH_2=, H-4 GlcA, H-1 GlcA, H-3 Man, H-4 Man), 5.07-5.06 (m, 2H, overlapping H-1 Man, H-2 Man), 4.90-4.85 (dd, J 3.9, 10.2, 1H, H-2 GlcA), 4.70-4.52 (m, 2H, CO_2CH_2), 4.30-3.88 (m, 7H, overlapping H-5 Man, H-6_a Man, H6_b Man, H-3 GlcA, H-5 GlcA, OCH_2), 2.15, 2.12, 2.11, 2.04, 2.03, 1.98 (each s, 3H, $OCOCH_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 170.7, 170.0, 169.8, 169.7, 169.68, 169.6, 167.5 (each CO), 132.8, 131.1 (each CH), 119.6, 118.4 (each CH_2), 98.2 (C-1 Man), 95.0 (C-1 GlcA), 75.2, 71.5, 70.9, 69.7, 69.3 (each CH), 69.0 (OCH_2), 68.7, 68.5 (CH), 66.7 (CO_2CH_2), 65.7 (CH), 62.1 (C-6 Man), 20.8, 20.7 (2s), 20.6 (each $COCH_3$); HRMS (ES): calcd 711.2112 $[M+Na]^+$, found 711.2088; IR (film from dichloromethane): 3352, 2958, 1746, 1605, 1524, 1431, 1371, 1229 cm^{-1}. Removal of the allyl ester from this disaccharide intermediate (217 mg, 0.315 mmol) as described for **14** gave **18** as a pale yellow foam (98 mg, 48%); $[\alpha]_D$ = +15.0 (*c* 0.28, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 5.89-5.78 (m, 1H, CH=), 5.34-5.17 (m, 7H, overlapping of alkene CH_2=, H-4 GlcA, H-1 GlcA, H-3 Man, H-4 Man, CO_2H), 5.08-5.07 (m, 2H, overlapping of H-1Man, H-2 Man), 4.89-4.85 (dd, J 3.6, 9.6, 1H, H-2 GlcA), 4.31-4.28 (d, J 9.6, 1H, H-5 GlcA), 4.26-4.01 (m, 6H, overlapping H-5 Man, H-6a Man, H6b Man, H-3 GlcA, OCH_2O, 2.15, 2.13 (each s, 3H, $OCOCH_3$), 2.11(s, 6H, $OCOCH_3$), 2.03, 1.98 (each s, 3H, $OCOCH_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 170.3, 170.1, 169.9, 169.8, 169.7 (each CO), 132.8 (d, CH), 118.5 (t, CH_2), 98.2 (C-1 Man), 94.9 (C-1 GlcA), 75.1, 71.5, 70.7, 69.6, 69.2 (each d, CH), 69.1 (t,

OCH_2), 68.6, 65.8 (each d, CH), 62.2 (t, C-6 Man), 20.9, 20.8, 20.7 (3s), 20.6 (each q, $OCOCH_3$); HRMS (ES): $[M-H]^-$ calcd 647.1823, found 647.1819; IR (film from dichloromethane): 3541, 2962, 1748, 1431, 1372, 1226, 1141 cm^{-1}.

1,4-Di-[(*N*-(1-methoxycarbonyl)methylamino-2-oxoethyl)-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4-di-*O*-acetyl-1-*O*-allyl-α-D-glucopyranuronamido]-benzene 21a The Ugi reaction of **18** (98 mg, 0.151 mmol) as described for **14** gave, after chromatography (dichloromethane-MeOH, 98:2), **21a** as an off-white solid (81 mg, 72%); $[\alpha]_D$ = +14.3 (*c* 0.5, $CHCl_3$); R_f = 0.35 (dichloromethane-MeOH, 95:5); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 7.54 (s, 4H, aromatic H), 6.88-6.85 (br t, 2H, NH), 5.99-5.60 (m, 2H, CH=), 5.49-5.42 (t, J 9.3, 2H, H-4 GlcA), 5.28-5.22 (t, J 9.9, 2H, H-4 Man) overlapping with 5.22-5.15 (m, 6H, overlapping of alkene CH_2= and H-3 Man), 5.06-5.04 (t, J 4.8, 2H, H-2 Man), 4.96-4.91 (m, 4H, overlapping of H-1 GlcA, H-1 Man), 4.69-4.65 (dd, J 3.3, J 10.2, 2H, H-2 GlcA), 4.58-4.53 (d, J 15.3, 2H, NCH_2CONH), 4.36-4.33 (d, J 9.9, 2H, H-5 GlcA), 4.21-3.95 (m, 14H, overlapping $NHCH_2CO$, H-5 Man, H-6_a Man, H6_b Man, H-3 GlcA, NCH_2CONH), 3.86-3.66 (m, 10H, overlapping OCH_2, CO_2CH_3), 2.17, 2.12, 2.07, 2.04, 2.02, 1.87 (each s, 3H, $OCOCH_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 170.9, 170.3, 170.2, 170.1, 169.9, 169.8, 169.4, 168.0, 167.6 (each CO), 142.4 (aromatic C), 133.0 (alkene CH), 129.8 (aromatic CH), 118.4 (alkene CH_2), 98.5 (C-1 Man), 95.3 (C-1 GlcA), 75.7, 71.8, 71.6, 70.9, 69.9, 69.5 (each CH), 68.9 (OCH_2), 68.2, 66.1 (each CH), 62.4 (C-6 Man), 54.6 (NCH_2CONH), 52.4 (CO_2CH_3), 41.3 ($NHCH_2CO_2CH_3$), 21.1, 21.0, 20.9, 20.8 (2s), 20.7 (each $OCOCH_3$); HRMS (ES): calcd 1649.5029 $[M+Na]^+$, found 1649.5031; IR (film from dichloromethane): 3589, 3352,

2996, 1747, 1670, 1437, 1372, 1223, 1041 cm^{-1}.

1,4-Di-[(*N*-(1-methoxycarbonyl)methylamino-2-oxoethyl)-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4-di-*O*-acetyl-1-azido-1-deoxy-β-D-glucopyranuronamido]-benzene 22a The Ugi reaction of **19**[21] (62 mg, 0.098 mmol) as described for **14** gave, after chromatography (EtOAc-MeOH, 99:1), **22a** as a white amorphous solid (48 mg, 62%); R_f 0.52 (EtOAc: MeOH, 1:9); $[\alpha]_D$ + 37.2 (*c* 0.57, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.59 (s, 4H, aromatic H), 6.83 (t, *J* 5.5, 2H, N*H*CH_2), 5.45 (t, *J* 9.4 , 2H, H-4, GlcA), 5.28 (t, *J* 10.1, 2H, H-4 Man), 5.12 (dd, *J* 10.1, 3.3 Hz, 2H, H-3 Man), 5.07 (dd, *J* 3.0, 2.1, 2H, H-2 Man), 4.86 (d, *J* 1.4, 2H, H-1 Man), 4.81 (t, *J* 9.0, 2H, H-2 GlcA), 4.54 (d, *J* 15.6, 2H, NC*H*$_2$$CO_2$Me), 4.28-4.23 (m, 4H, overlapping NC*H*$_2$$CO_2$Me, H-1 GlcA), 4.16 (dd, *J* 12.6, 3.7, 2H, H-6a Man), 4.13-3.97 (m, 8H, overlapping H-6b Man, NHC*H*$_2$$CO_2$Me, H-5 GlcA), 3.95 (td, *J* 10.2, 2.8, 2H, H-5 Man), 3.80 (t, *J* 9.3, 2H, H-3 GlcA), 3.74 (s, 6H, OC*H*$_3$), 2.16, 2.12, 2.08, 2.06, 2.01, 1.95 (each s, each 6H, COC*H*$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.8, 170.2 (2s), 169.8, 169.7, 169.3, 168.0, 165.8 (each CO), 141.9 (aromatic C), 129.6 (aromatic *C*H), 99.7 (C-1 Man), 83.3, 80.5, 73.5, 71.4, 70.3, 70, 69.6, 69.0, 65.5 (C-1 GlcA, C2-5 GlcA and Man), 62 (C-6 Man), 54.6 (*C*H_2), 52.5 (O*C*H_3), 41.4 (*C*H_2), 21.0 (3s), , 20.9 (2s), 20.8 (each CO*C*H_3); LRMS (ES): 1597.4 $[M+H]^+$, 1619.5 $[M+Na]^+$; HRMS (ES): Calcd 1619.4533 $[M+Na]^+$, found 1619.4469. IR (film on NaCl): 2123, 1751, 1672, 1438, 1375, 1222, 1040 cm^{-1}.

Di-(α-D-mannopyranosyl)-tetraethylene ether 11 To imidate **15** (0.397 g, 0.81 mmol) in dry CH_2Cl_2 (6 mL) and in the presence of 4 Å molecular sieves was added tetraethylene glycol (69 μL, 0.4 mmol). The reaction was stirred for 30 min at room temp before cooling to 0 ˚C and for a further 20 min before the dropwise addition of BF_3OEt_2 (50 μL, 0.4 mmol). The reaction was then stirred for 16 h, diluted with CH_2Cl_2 (10 mL), $NaHCO_3$ (solid, 15 mg) added, and left to stir for a further 5 min before filtering through celite. Removal of solvent under diminished pressure gave a yellow oil. Chromatography (cyclohexane-EtOAc, 1:1, then dichloromethane-EtOAc, 1:9) gave the dimeric glycoside as a clear oil (0.078 g, 23%); R_f 0.38, (EtOAc: CH_2Cl_2, 4:1), $[\alpha]_D$ + 37.2 (*c* 0.85, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.34 (dd, *J* 10.0, 3.5, 2H, H-3), 5.27 (t, *J* 11.8, 2H, H-4), 5.25 (dd, *J* 3.4, 1.7, 2H, H-2), 4.86 (d, *J* 1.5, 2H, H-1), 4.28 (dd, *J* 12.2, 5.0, 2H, H-6a), 4.09 (dd, *J* 12.2, 2.4, 2H, H-6b), 4.05 (ddd, *J* = 7.2, 5.1, 2.6, 2H, H-5), 3.70-3.62 (m, 16H, 8 OCH_2), 2.14, 2.09, 2.03, 1.97 (each s, each 6H, $COCH_3$) ; ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.8, 170.2, 170.1, 169.9 (each $COCH_3$), 98.0 (C-1), 71.0, 70.9, 70.2 (each OCH_2), 69.8, 69.3, 69.7, 66.44, C-2—5), 67.6 (OCH_2) 62.7 (C-6), 21.1, 21.0, 20.9, 20.8 (each $COCH_3$); HRMS (ES): calcd 855.3134 $[M+H]^+$, found 855.3152 $[M+Na]^+$; calcd 877.2954, found 877.2956. IR (film on NaCl): 3451, 1745, 1370, 1225, 1137, 1085, 1049 cm^{-1}. This dimer (93.7 mg, 0.11 mmol) was dissolved in MeOH-water (1:1, 5 mL), to which was added dropwise a solution of 0.14 M LiOH in MeOH-water-THF (5:2:1, 1 mmol) at 0 ˚C. After 4 h NaH_2PO_4 (1 M) was added and the solvent was removed under diminished pressure. Chromatograpy of the residue (EtOAc to EtOAc-MeOH, 9:1) gave **11** as a clear syrup (41 mg, 71%); R_f 0.19 (EtOAc: MeOH, 1:1), $[\alpha]_D$ + 29 (c 1.5, MeOH) ; ^{1}H-NMR (300 MHz, D_2O): δ 4.91 (d, *J* 1.4, 2H, H-1), 3.98 (dd, *J* 3.2,

1.6, 2H, H-2), 3.94-3.64 (m, 26H, overlapping signals of H-3, H-4, H-5, H-6a, H-6b, 8 OCH_2) ; ^{13}C-NMR (75 MHz, D_2O): δ 100.1 (d, C-1), 72.9, 70.7, 70.1 (each *C*H) 69.8, 69.8, 69.7, (each O*C*H_2) 66.9 (*C*H), 66.6 (t, O*C*H_2) 61.1 (t, C-6) ; LRMS (ES): 541.0 $[M+Na]^+$. HRMS (ES): calcd 541.2108, found 541.2117 $[M+Na]^+$.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-1,2:5,6-di-isopropylidene-β -D-glucofuranoside 24 Trichloroacetimidate **15** (2.38 g, 4.854 mmol) and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (1.053 g, 4.045 mmol) were dried together under vacuum for 2 h in the presence of 4Å molecular sieves (3.5 g). To this mixture was added dry dichloromethane (10 mL) and the solution was stirred at room temp for 2 h. TMSOTf (75 μL, 0.4045 mmol) was added dropwise at –20 °C under N_2 and after 10 min $NaHCO_3$ (solid, 0.03 g) was added. The mixture was stirred for 10 min, filtered through celite and the solvent was removed. Chromatography (cyclohexane-EtOAc, 3:1 to 2.5:1 to 2:1) gave **24** as a white foam (1.84 g, 64%); R_f 0.56, (Cy: EtOAc, 1:1); $[\alpha]_D$ + 24.1 (*c* 0.78, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): *δ* 5.91 (d, *J* 3.6, 1H, H-1 Glc), 5.32-5.22 (m, 3H, H-2 to H-4 Man), 5.11 (s, 1H, H-1 Man), 4.58 (d, *J* 3.6, 1H, H-2 Glc), 4.30-4.26 (m, 2H, overlapping H-3 Glc and H-6a Man), 4.22-4.13 (m, 3H, overlapping signals of H-5 Glc, H-6b Man, H-6b Glc), 4.05 (dd, J 8.5, 2.9 Hz, 1H, H-4, Glc), 4.04-4.02 (m, 1H, H-5 Man), 3.95 (dd, J 8.4, 5.2, 1H, H-6 Glc), 2.14, 2.10, 2.05, 1.98 (each s, 3H, COCH_3), 1.49, 1.38, 1.32, 1.29 (each s, 3H, OCCH_3); ^{13}C-NMR (150 MHz, $CDCl_3$): *δ* 170.8, 170.0, 169.9, 169.7 (each *C*OCH_3), 112.3, 109.6 (each O*C*O), 105.4 (C-1 Glc), 98.9 (C-1 Man), 84.0 (C-2), 81.7, 81.6 (C-3 and C-4 Glc), 72.4 (C-5 Glc), 69.5 (C-5 Man), 69.2 (C-4 Man), 69.0 (C-2 Man), 68 (C-6 Glc), 66.6 (C-3 Man), 62.9 (C-6 Man), 27.1, 27.0, 26.5,

25.2 (each OC*C*H$_3$) 21.0, 20.9 (2s), 20.8 (each CO*C*H$_3$); LRMS (ES): HRMS (ES): calcd 613.2108 $[M+Na]^+$, found 613.2092 . Anal. calcd. for $C_{26}H_{38}O_{15}$: C, 52.88; H, 6.49; Found: C, 52.59; H, 6.28. IR (KBr): 2990, 2941, 1736, 1375, 1225, 1141, 1079, 1046, 850 cm^{-1}.

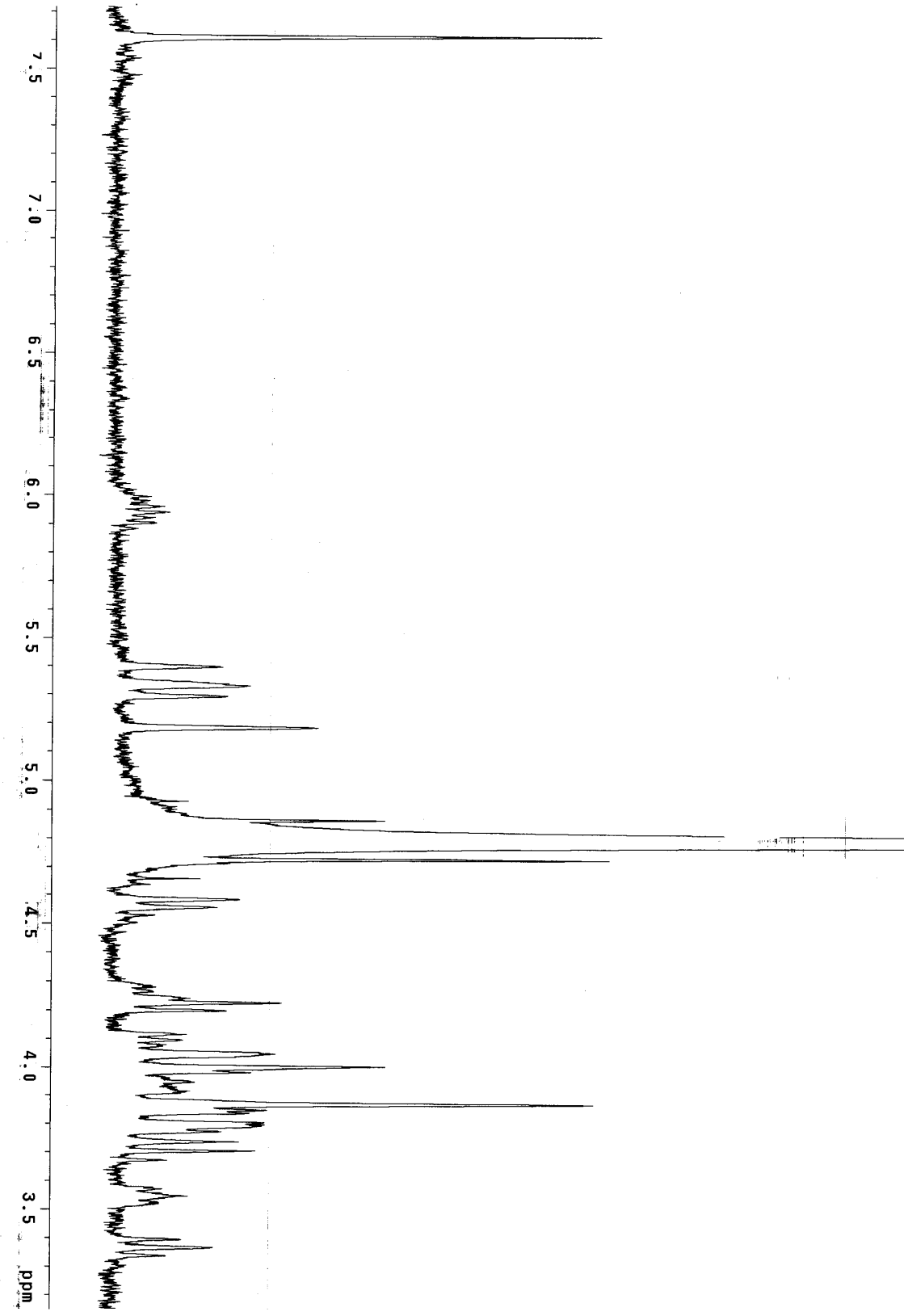
2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl amine 25 A solution of **24** (0.94 g, 1.59 mmol) in 80% acetic acid (50 mL) was heated at 100 ºC for 5 h and was then concentrated under reduced pressure. The excess acetic acid was azeotropically removed by coevaporation with MeOH (10 mL x 2), and then CH_2Cl_2 (10 mL x 2) to give the intermediate as a white foam (89%); LRMS (ES): 509.2 $(M-H)^-$, HRMS (ES): 533.1498 $(M+Na)^+$. This intermediate was dissolved in pyridine: acetic anhydride (1:1, 10 mL) and stirred overnight at room temp. The volatile components were removed under diminished pressure and any remaining excess volatile reagents were azeotropically removed with toluene (10 mL x 2) and then CH_2Cl_2 (10 mL x 2). The organic layer was extracted with EtOAc, washed with H_2O, dried ($MgSO_4$) and the solvent was removed. Chromatography (cyclohexane-EtOAc, 4:1 to 1:1) gave the per-*O*-acetylated intermediate as a white foam (0.274 g, 78% for two steps); R_f 0.59 (cyclohexane-EtOAc, 1:1), $[\alpha]_D$ + 48.1 (*c* 1.41, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, αα:αβ, 1.3:1) data for the α isomer: δ 6.31 (d, *J* 3.7, 1H, H-1 Glc), 5.30 (t, *J* 10.1, 1H, H-4 Man), 5.24-5.12 (m, 3H, overlapping signals of H-4 Glc, H-3 Man), 5.07 (dd, *J* 3.2, 2.0, 1H, H-2 Man), 5.05 (dd, *J* 9.9, 3.7, 1H, H-2 Glc), 5.00 (d, *J* 1.9, 1H, H-1 Man), 4.26-3.96 (m, 7H, overlapping signals of H-5,6 Glc and Man, H-3 Glc); selected peaks for the β isomer: δ 5.62 (d, *J* 8.1, 1H, H-1 Glc), 5.32 (t, *J* 10.1, 1H, H-4 Man), 5.09 (dd, *J* 3.2,

2.2, 1H, H-2 Man), 4.93 (d, *J* 2.0, 1H, H-1 Man) 3.89 (t, *J* 9.3, 1H, H-3 Glc), 3.73 (ddd, *J* 10.0, 4.7, 2.4, 1H, H-5 Glc), ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 170.9, 170.8 (2s), 170.2 (2s), 169.8 (2s), 169.7 (3s), 169.3 (2s), 168.8, 99.8, 98.9, 92.1, 89.5, 81.4, 77.4, 73.1, 71.5, 70.9, 70.3, 70.0, 69.9, 69.7, 69.0, 68.9, 68.7, 68.6, 65.8, 65.5, 62.3, 61.9, 61.8, 21.1, 21.0, 20.9, 20.8, 20.7; HRMS (ES): calcd 701.1905 $[M+Na]^+$, found 701.1915. Anal. Calcd. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64; Found: C, 49.27; H, 5.50. IR (KBr): 2966, 1753, 1437, 1374, 1227, 1141, 1041 cm^{-1}. This peracetate (0.615 g, 0.907 mmol) was dissolved in dry CH_2Cl_2 (7 mL) under an atmosphere of N_2. To this solution at room temp was added azidotrimethylsilane (0.3 mL, 2.27 mmol) followed $SnCl_4$ (0.05 mL, 0.45 mmol, dropwise). The reaction was stirred for 15 h at room temp and then diluted with dichloromethane (10 mL) and satd $NaHCO_3$ (15 mL) was added and the mixture stirred for 1 h. The mixture was extracted with CH_2Cl_2, washed with satd $NaHCO_3$ (2 x 20 mL), H_2O (2 x 20 mL), dried ($MgSO_4$) and the solvent removed. Chromatography of the residue (cyclohexane-EtOAc gradient elution, 3:1 to 1:1) gave the glycosyl azide as a white foam (0.384 g, 64%); R_f 0.28, (cyclohexane-EtOAc, 1:1); $[\alpha]_D$ -7.5 (1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 5.29 (t, *J* 10.1, 1H, H-4 Man) 5.14 (dd, *J* 9.9, 9.6, H-4 Glc) 5.09 (dd, *J* 10.1, 3.3, 1H, H-3 Man), 5.04 (dd, *J* = 3.3, 2.1, 1H, H-2 Man), 4.95 (t, *J* 9.1, 1H, H-2 Glc), 4.89 (d, *J* 2.0, 1H, H-1 Man), 4.44 (d, *J* 8.9, 1H, H-1 Glc), 4.21 (dd, *J* 12.5, 4.9, 1H, H-6b Glc), 4.16 (dd, *J* 12.6, 3.7, 1H, H-6a Glc), 4.11 (dd, *J* 12.6, 2.5, 1H, H-6a Man), 4.07 (dd, *J* 12.6, 2.4, 1H, H-6b Man), 3.95 (ddd, *J* = 10.1, 3.4, 2.5, 1H, H-5 Man), 3.82 (t, *J* = 9.4, 1H, H-3 Glc), 3.66 (ddd, *J* = 10.1, 4.8, 2.5, 1H, H-5 Glc), 2.13, 2.12, 2.11, 2.08, 2.07, 1.99, 1.93 (each s, each 3H, COC*H*$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.9, 170.8, 170.3, 169.8 (2s), 169.7, 169.5 (each *C*OCH_3), 99.8 (C-1 Man),

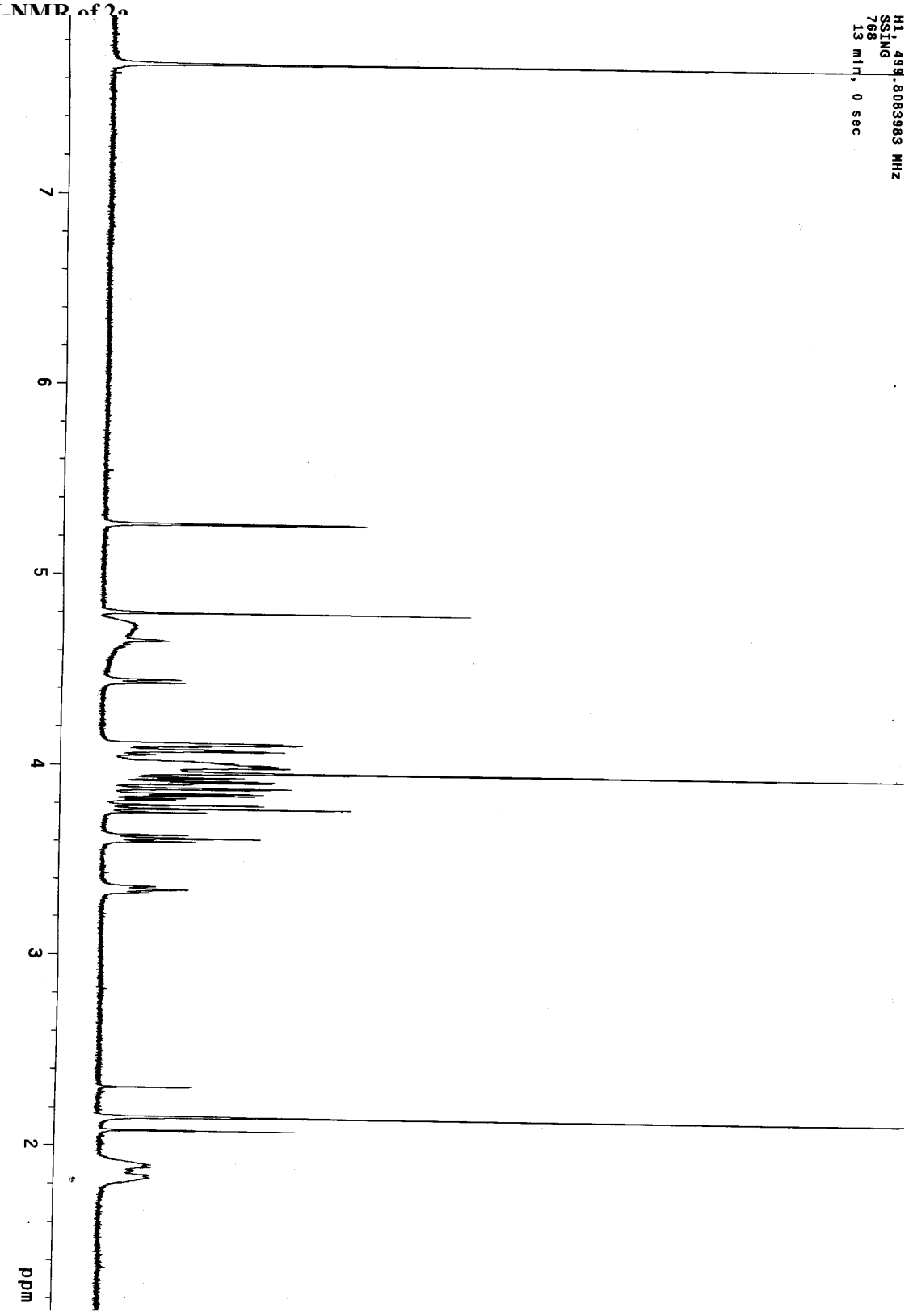
88.1 (C-1 Glc), 81.6 (C-3 Glc), 74.4 (C-5 Glc), 71.9 (C-2 Glc), 70.0, 69.7, 69.0, 68.6, (C-2,3,5 Man, C-4 Glc), 65.4, (C-4 Man), 62.0, 61.9 (C6 Glc and Man), 21.2, 21.1, 21.0, 20.9 (2s), 20.8 (each CO*C*H_3); HRMS (ES): calcd 684.1864 $[M+Na]^+$, found 684.1882; calcd 660.1888 $[M-H]^-$, found 660.1884. IR (KBr): 3232, 2927, 2852, 2122, 1751, 1627, 1375, 1226, 1041 cm^{-1}. This glycosyl azide (0.108 g, 0.163 mmol) was dissolved in EtOAc (10 mL) to which was added 10% Pd-C (15 mg) and the mixture stirred for 16 h under H_2. The solution was then filtered through celite and the solvent removed under diminished pressure. Chromatography (cyclohexane-EtOAc gradient elution, 1:1 to 3:2 then dichloromethane-EtOAc, 9:1) gave **25** as a white foam (81 mg, 78%); R_f 0.23 (EtOAc-CH_2Cl_2, 9:1); $[\alpha]_D$ +18.2 (*c* 1.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, β:α, 82:18): δ (β-anomer, **25**) 5.29 (t, *J* 10.1, 1H, H-4 Man), 5.13 (dd, *J* 10.1, 3.3, 1H, H-3 Man), 5.09 (t, *J* 9.7, 1H, H-4 Glc), 5.06 (dd, *J* 3.2, 2.2, 1H, H-2 Man), 4.94 (d, *J* 1.9, 1H, H-1 Glc), 4.80 (t, *J* 9.2, 1H, H-2 Glc), 4.22-4.15 (m, 2H, overlapping H-6b Man and Glc), 4.10-4.04 (m, 3H, overlapping H-6a Man and Glc, H-1 Glc), 4.00 (ddd, *J* 10.1, 3.6, 2.6, 1H, H-5 Man), 3.84 (t, *J* 9.3, 1H, H-3 Glc), 3.55 (ddd, *J* 10.1, 4.9, 2.4, 1H, H-5 Glc), 2.13 (2s), 2.10 (2s), 2.08, 2.01, 1.95 (each s, 3H, COC*H*$_3$); selected NMR data for the α anomer 5.17 (dd, *J* 10.1, 3.3, 1H), 4.97 (d, *J* 1.8, 1H), 4.27 (ddd, *J* 9.8, 4.6, 2.8, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.8, 170.6, 170.3, 170.1, 169.7, 169.6, 169.5, (each *C*OCH_3), 99.2 (C-1 Man) 85.2 (C-1 Glc), 81.5 (C-3 Glc) 73.4 (d, C-2 Glc), 72.9 (C-5 Glc), 69.6, 69.6, 69.5 (C-2,5 Man, C-4 Glc), 68.9 (C-3 Man), 65.4 (C-4 Glc), 62.4 (C-6 Glc), 61.9 (C-6 Man) 21.1, 20.9, 20.8, 20.7 (2s), 20.6 (each CO*C*H_3); HRMS (ES): calcd 658.1959 $[M+Na]^+$, found 658.1957 $[M+Na]^+$. Anal calcd for $C_{26}H_{37}NO_{17}$: C, 49.13; H,

5.87; N, 2.20 ; Found: C, 48.53; H, 5.90; N, 1.90. IR (KBr) 3429, 2920, 2361, 1759, 1375, 1234, 1136, 1071, 1040 cm^{-1}; LRMS: 636.2 $[M+H]^+$.

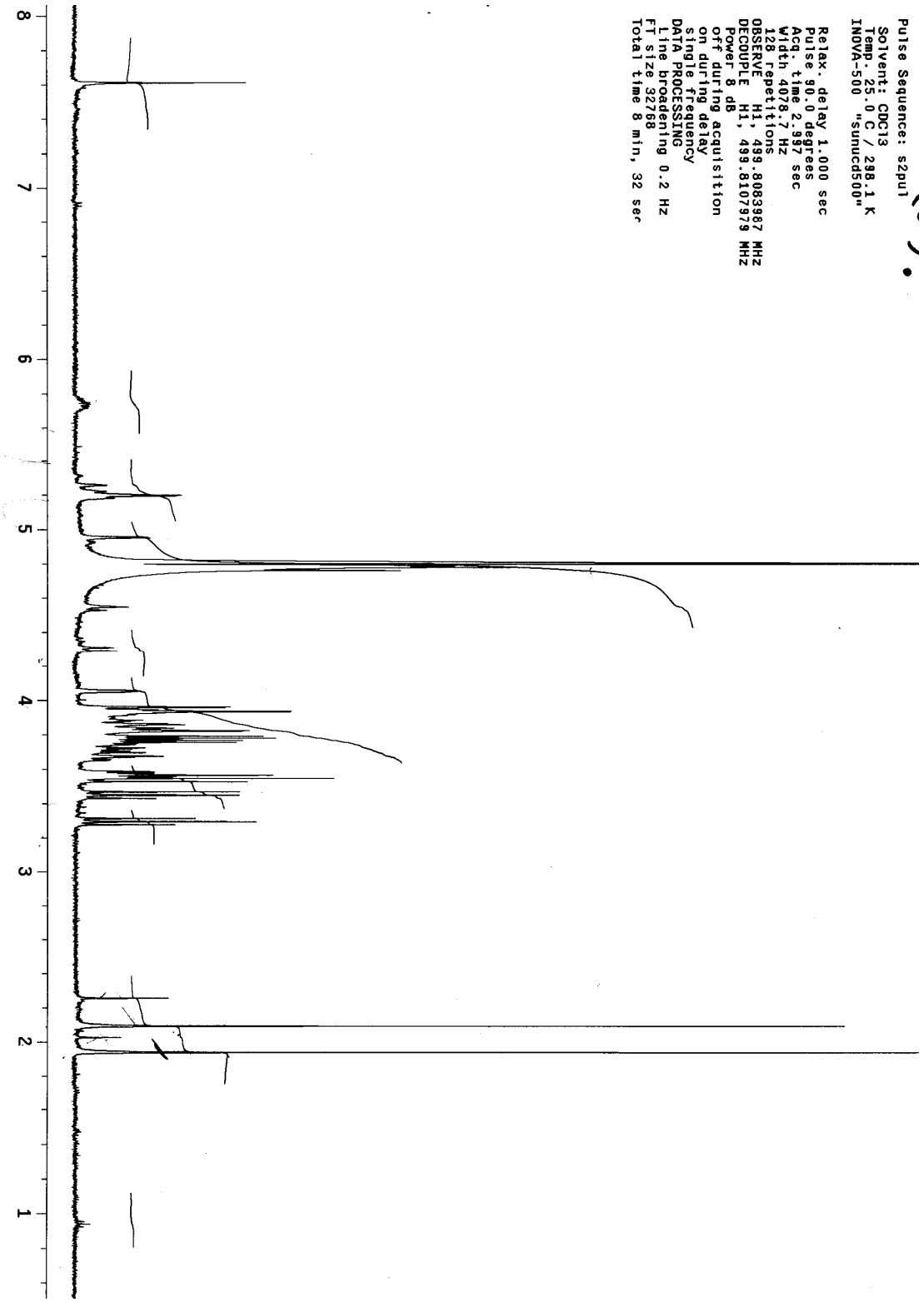
^{1}H-NMR of 1a



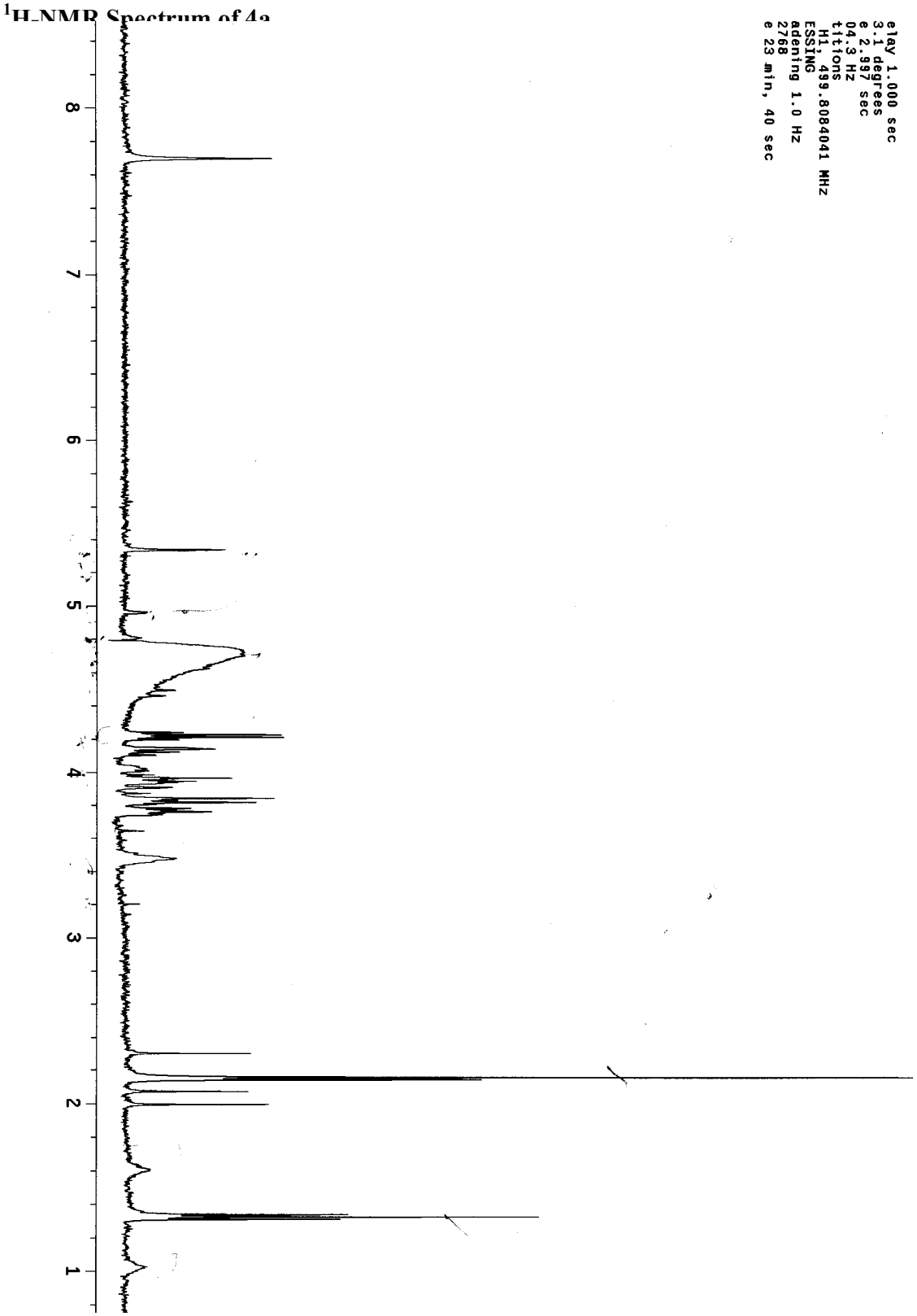
^{1}H-NMR of 2a



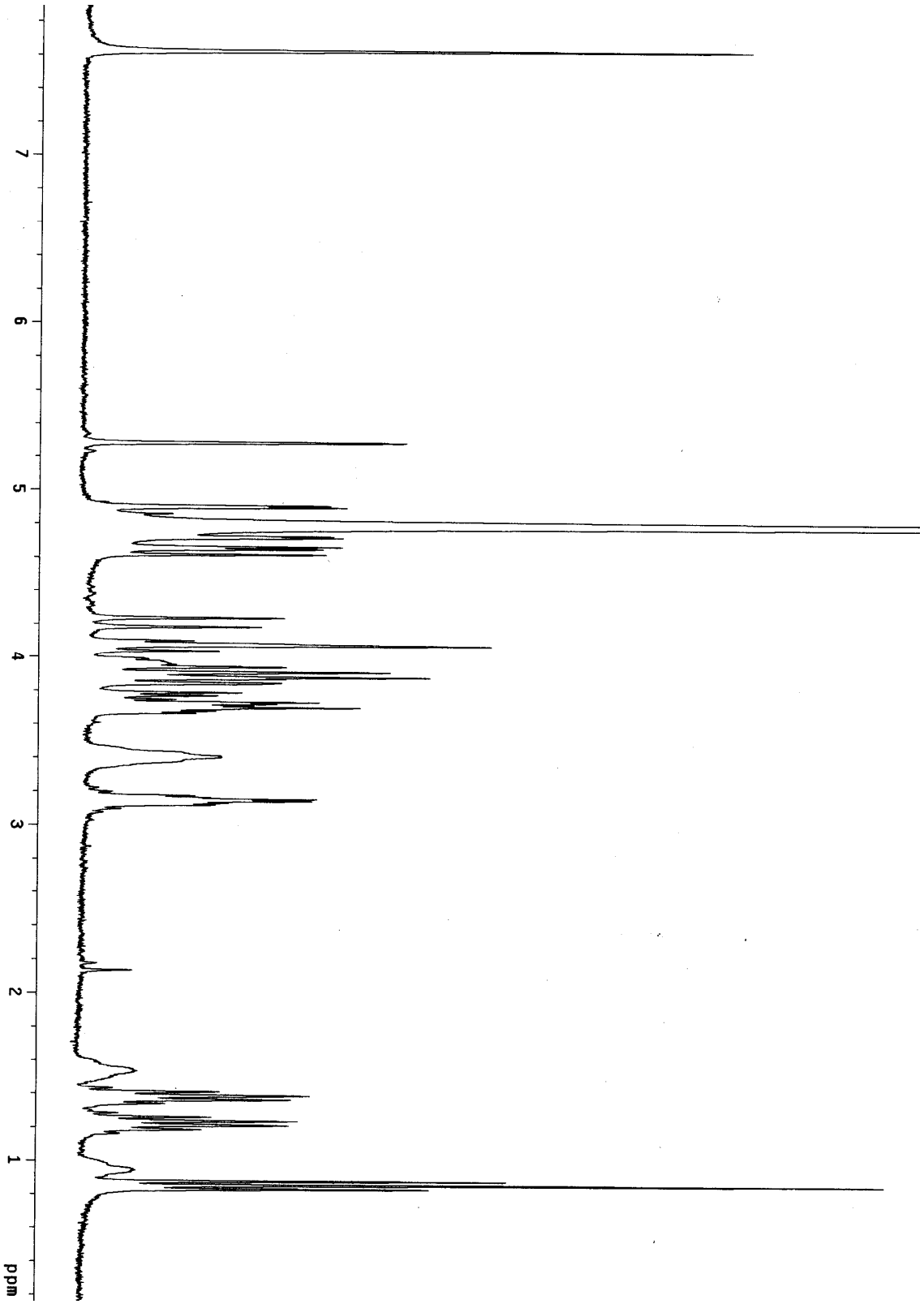
^{1}H-NMR Spectrum of 3a



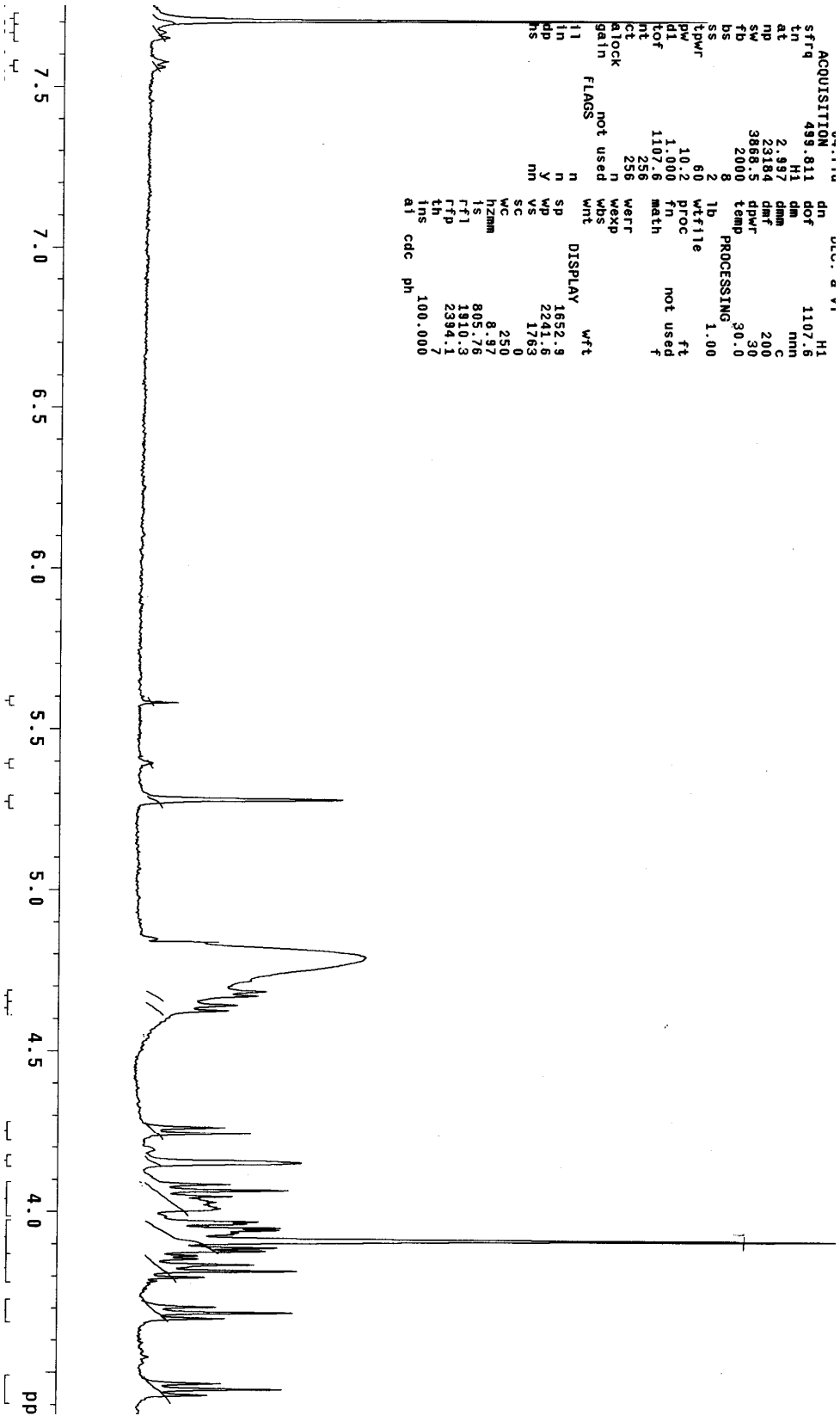
^{1}H-NMR Spectrum of 4a



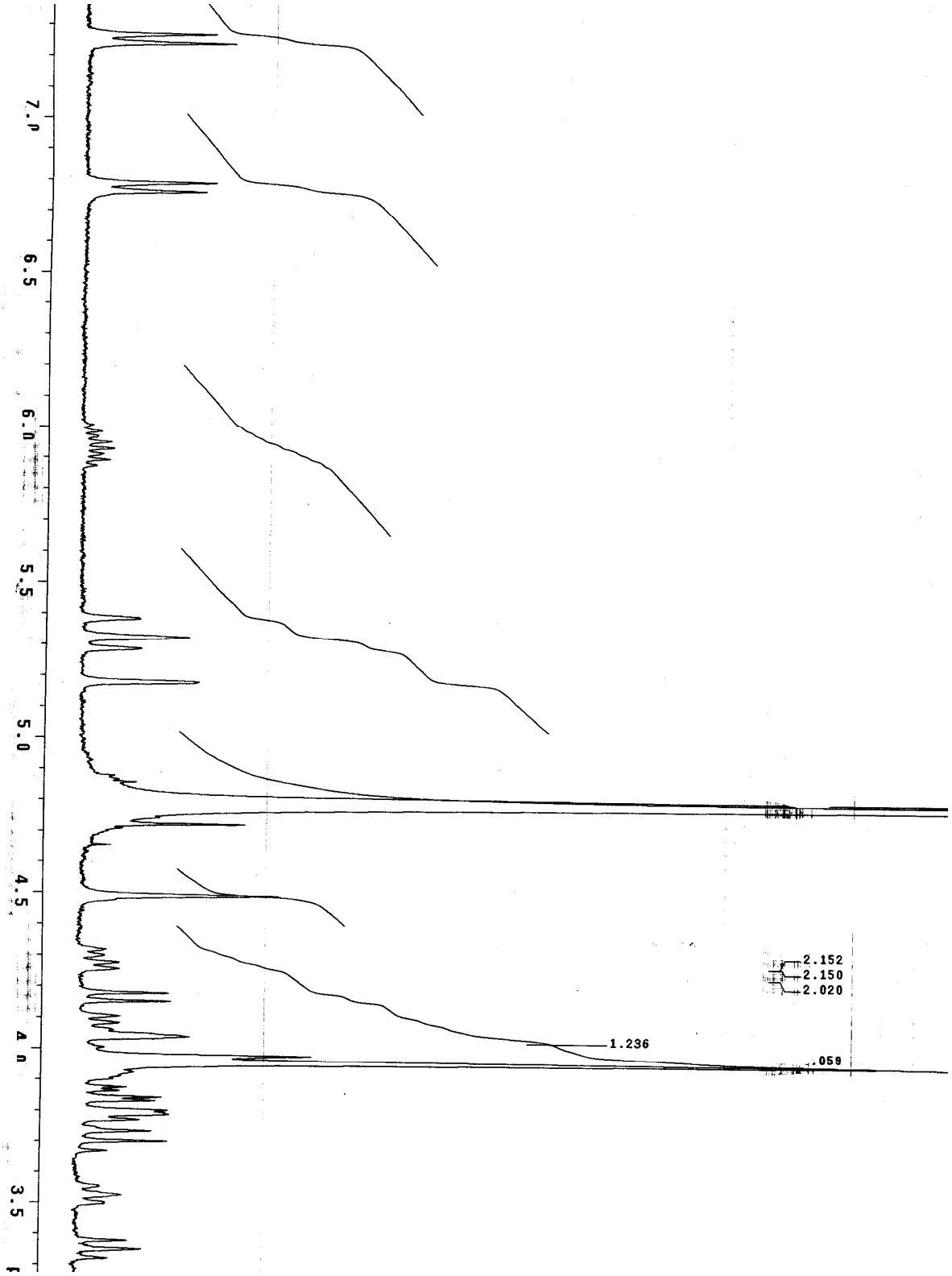
^{1}H-NMR Spectrum of 4b



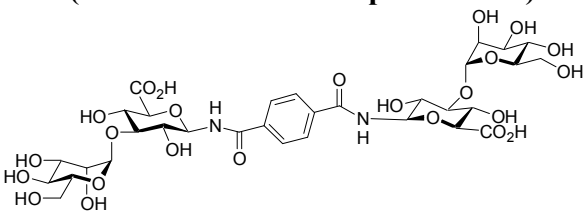
^{1}H-NMR of 5a

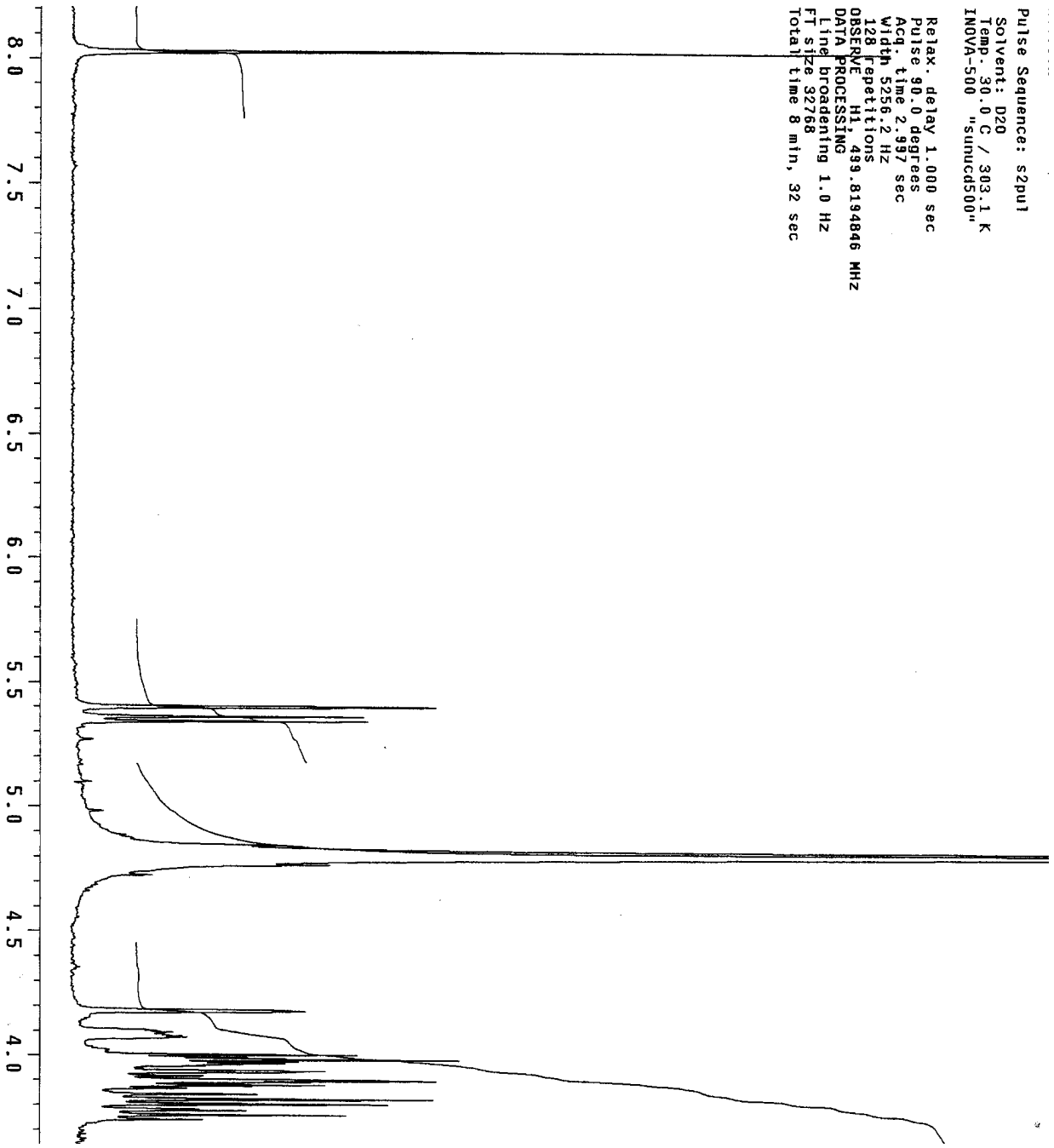


^{1}H-NMR Spectrum of 6a

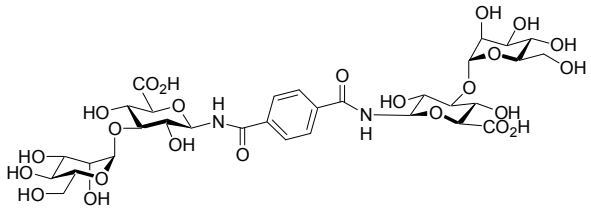


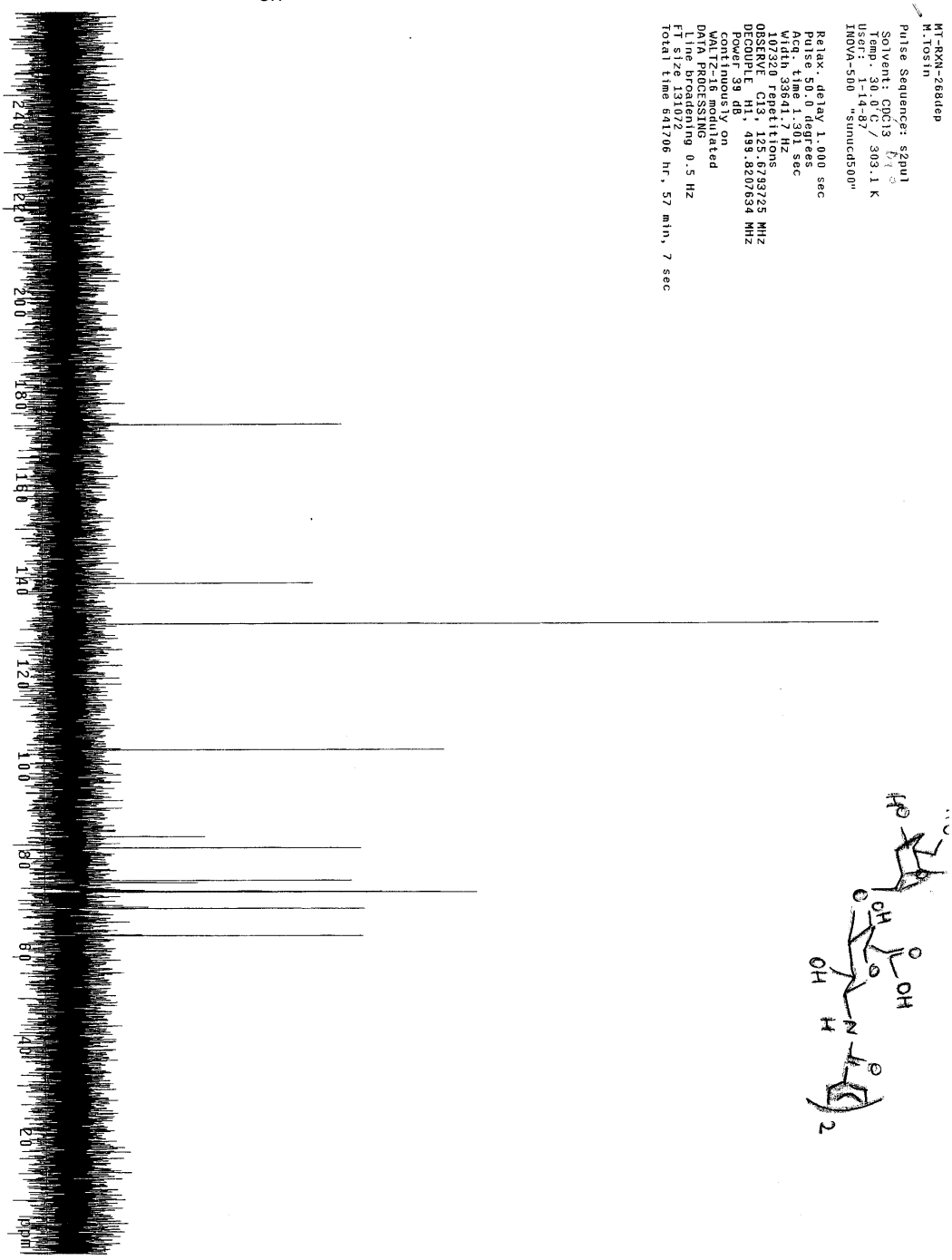
^{1}H-NMR Spectrum of 7c (obtained after HPLC purification)



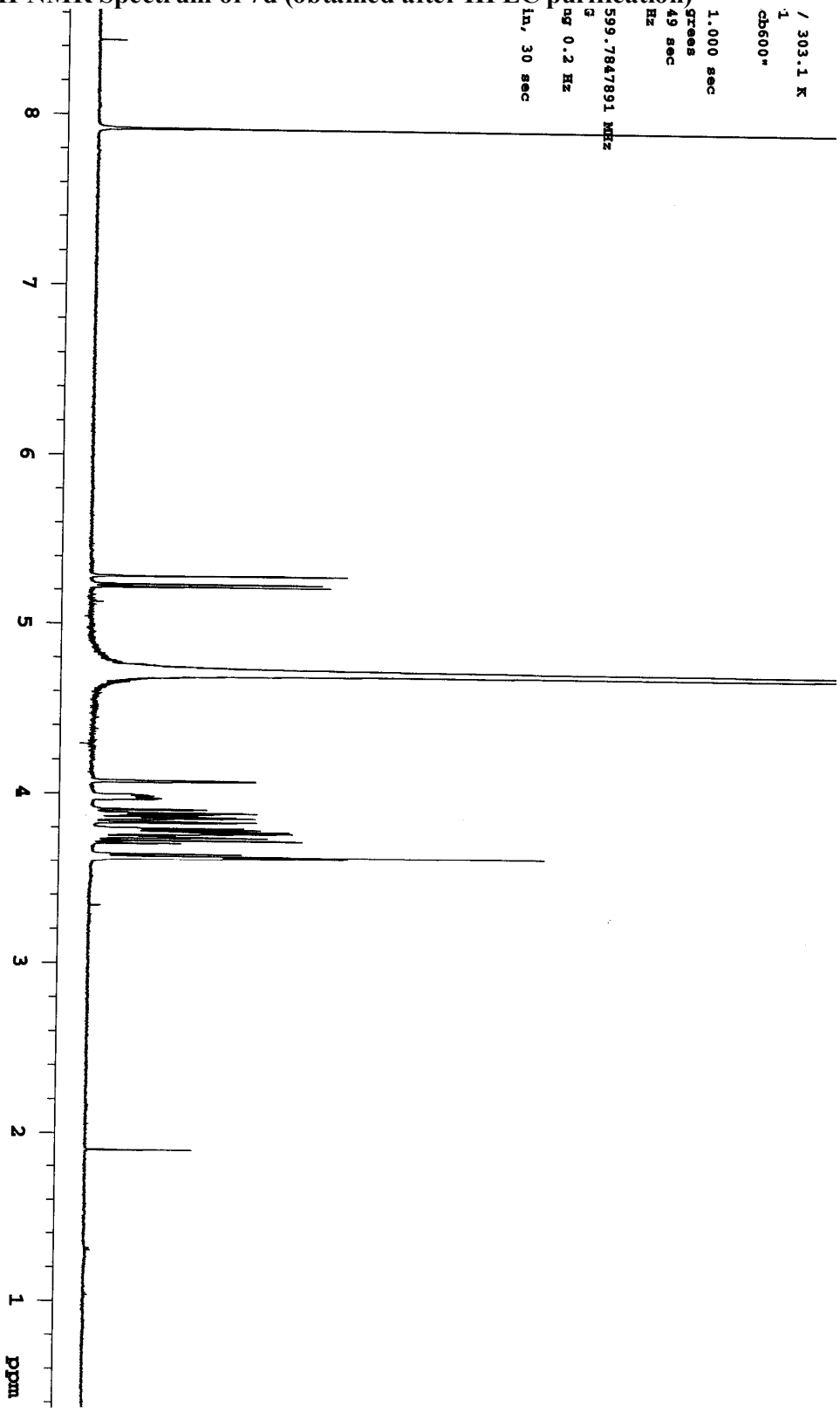


^{13}C-NMR of 7c

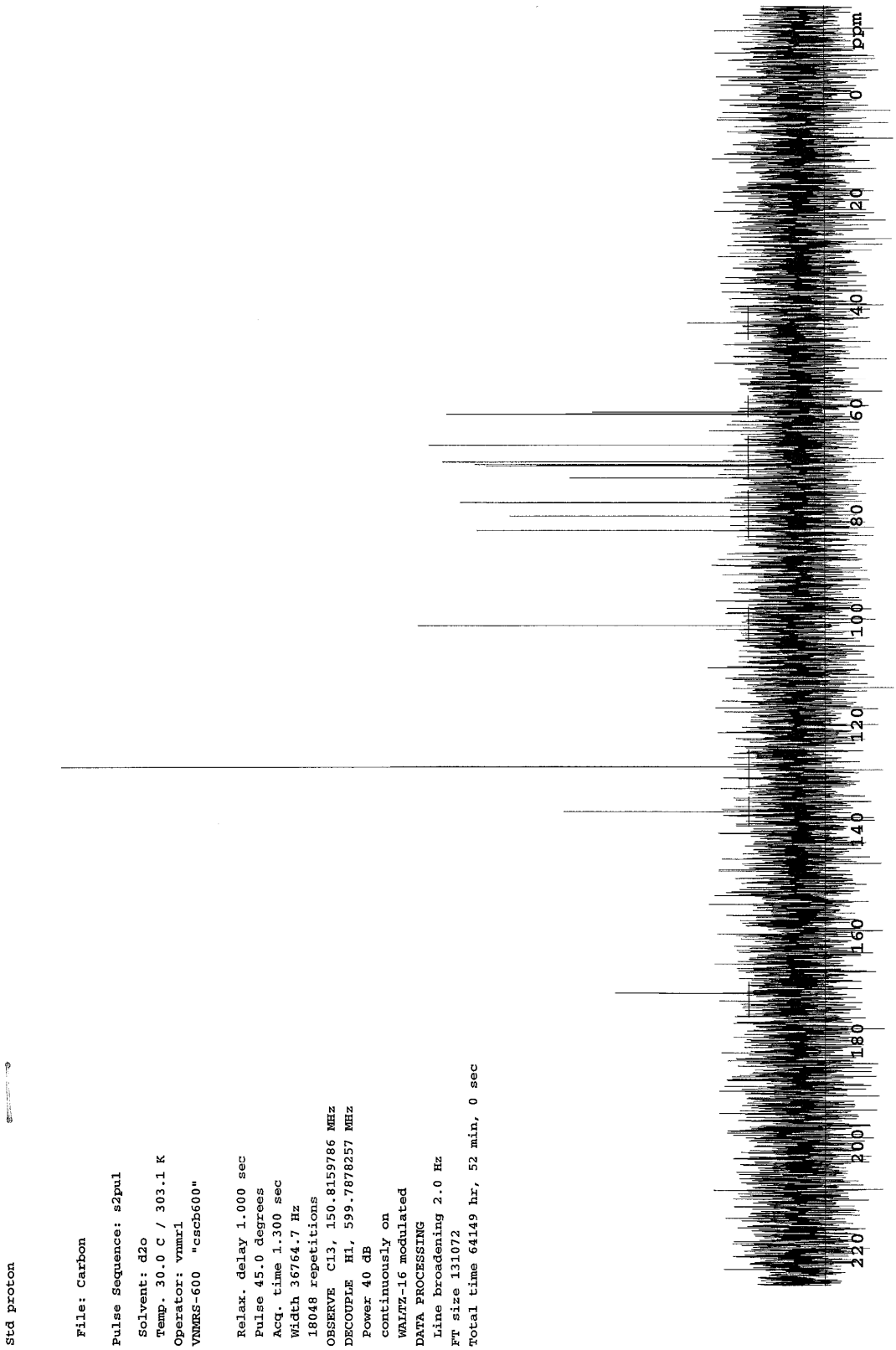




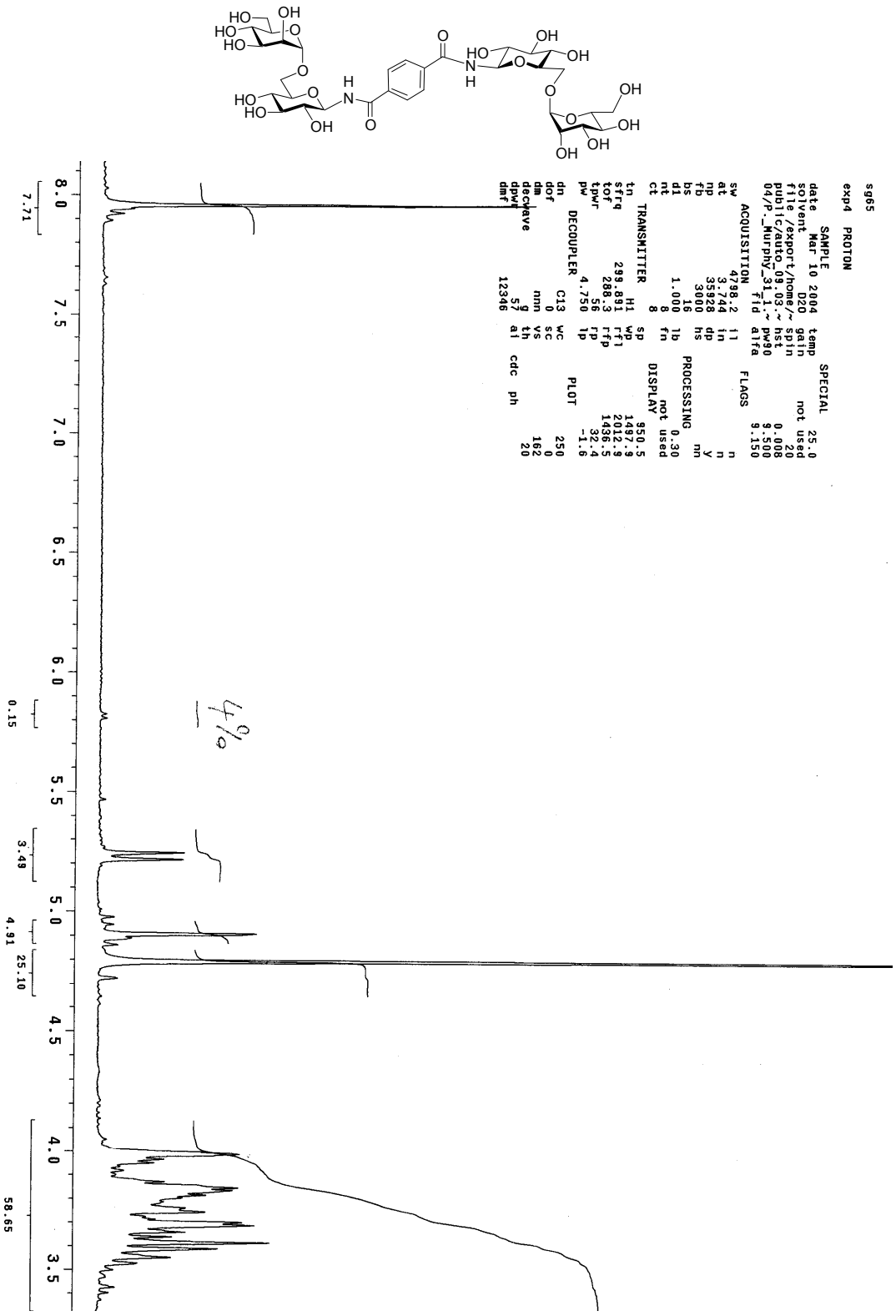
^{1}H-NMR Spectrum of 7d (obtained after HPLC purification)



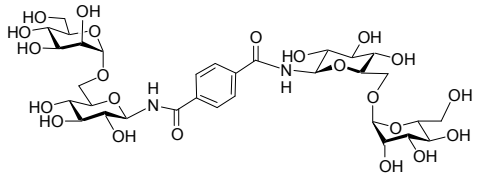
^{13}C-NMR Spectrum of 7d (obtained after HPLC purification)

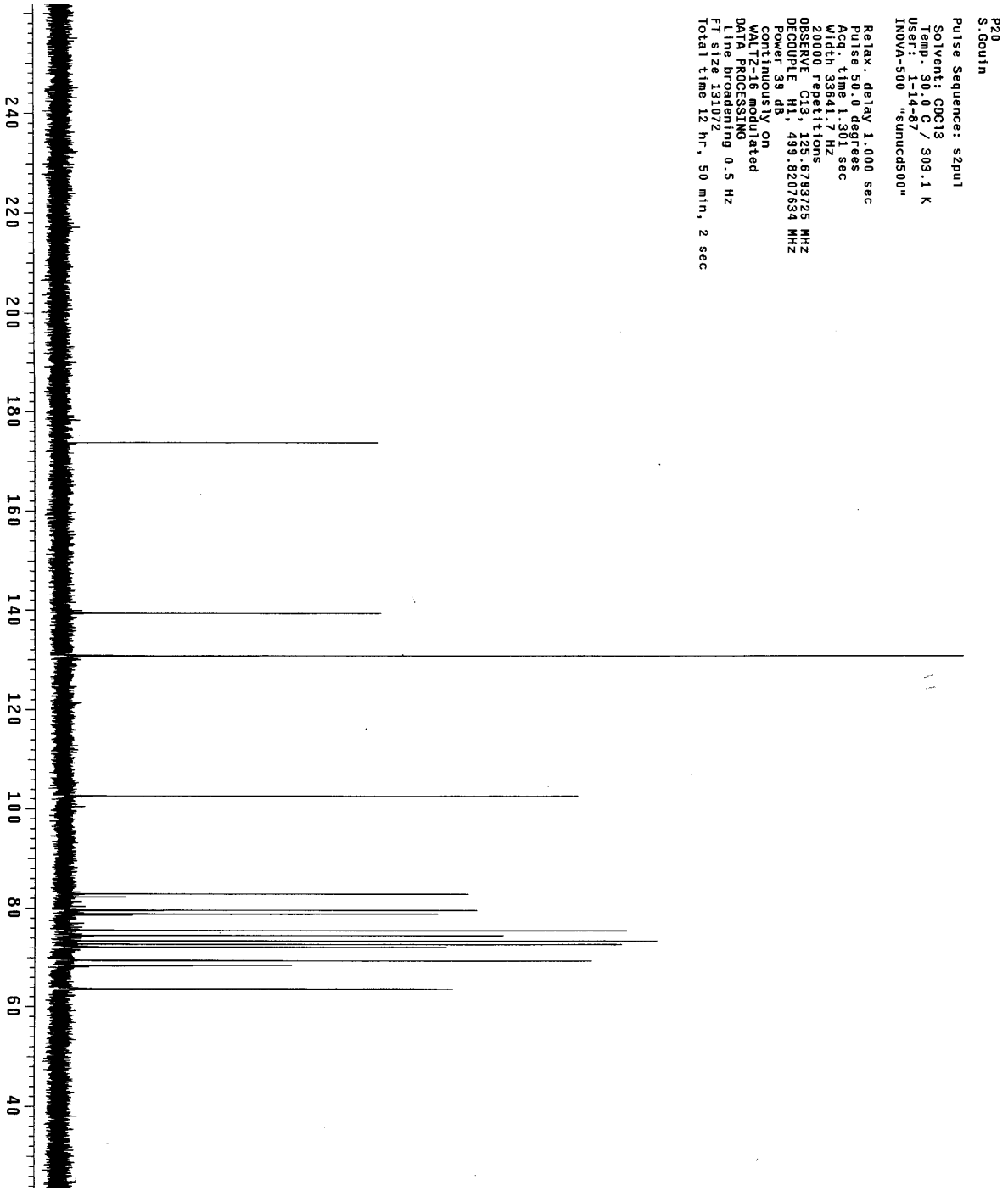


^{1}H-NMR Spectrum of 8 (obtained after HPLC purification)

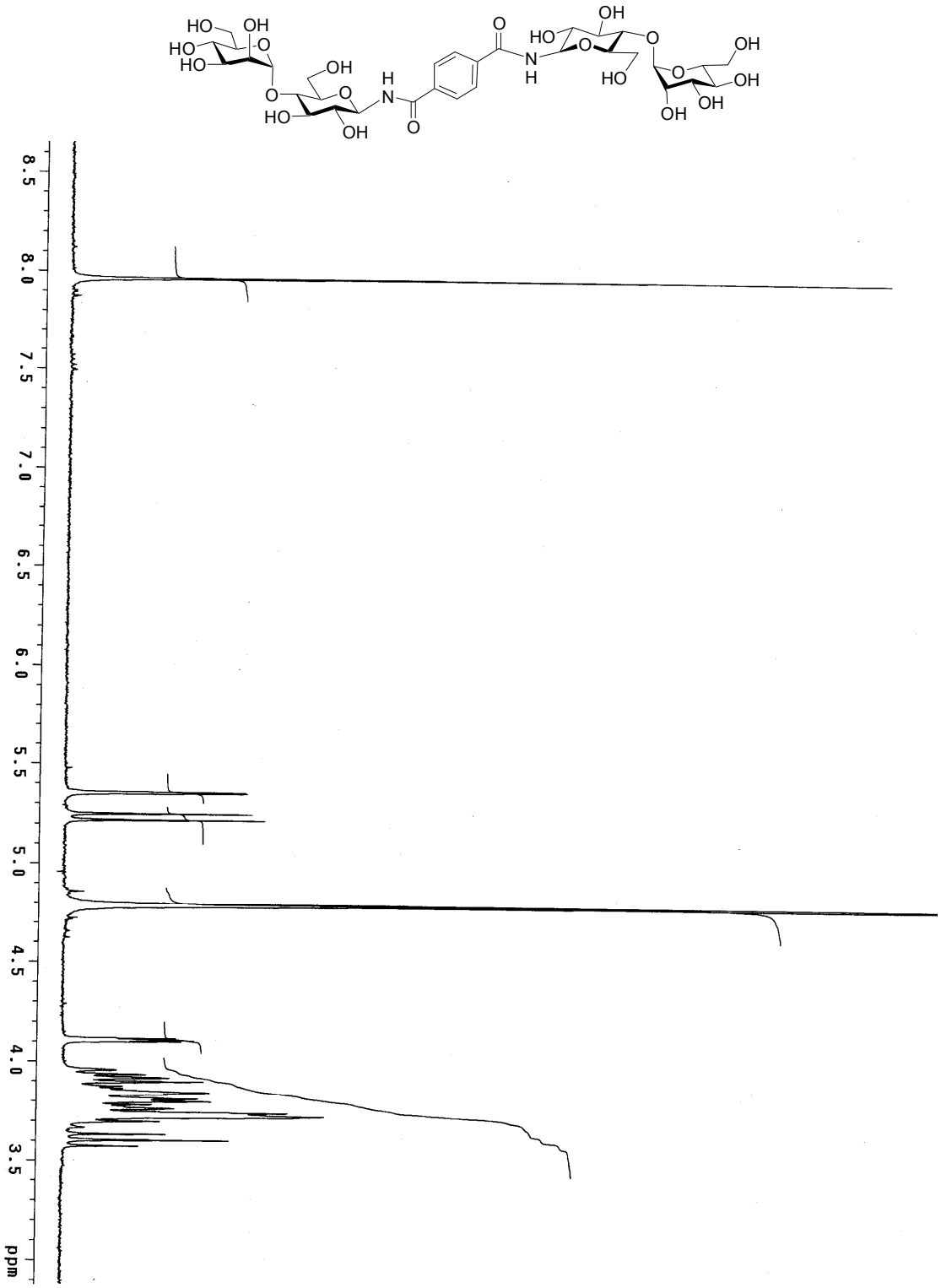


^{13}C-NMR Spectrum of 8 (obtained before HPLC)

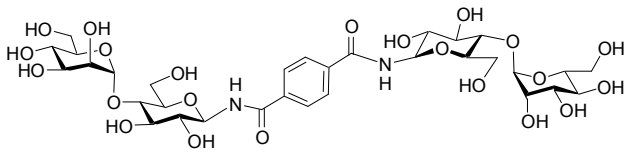


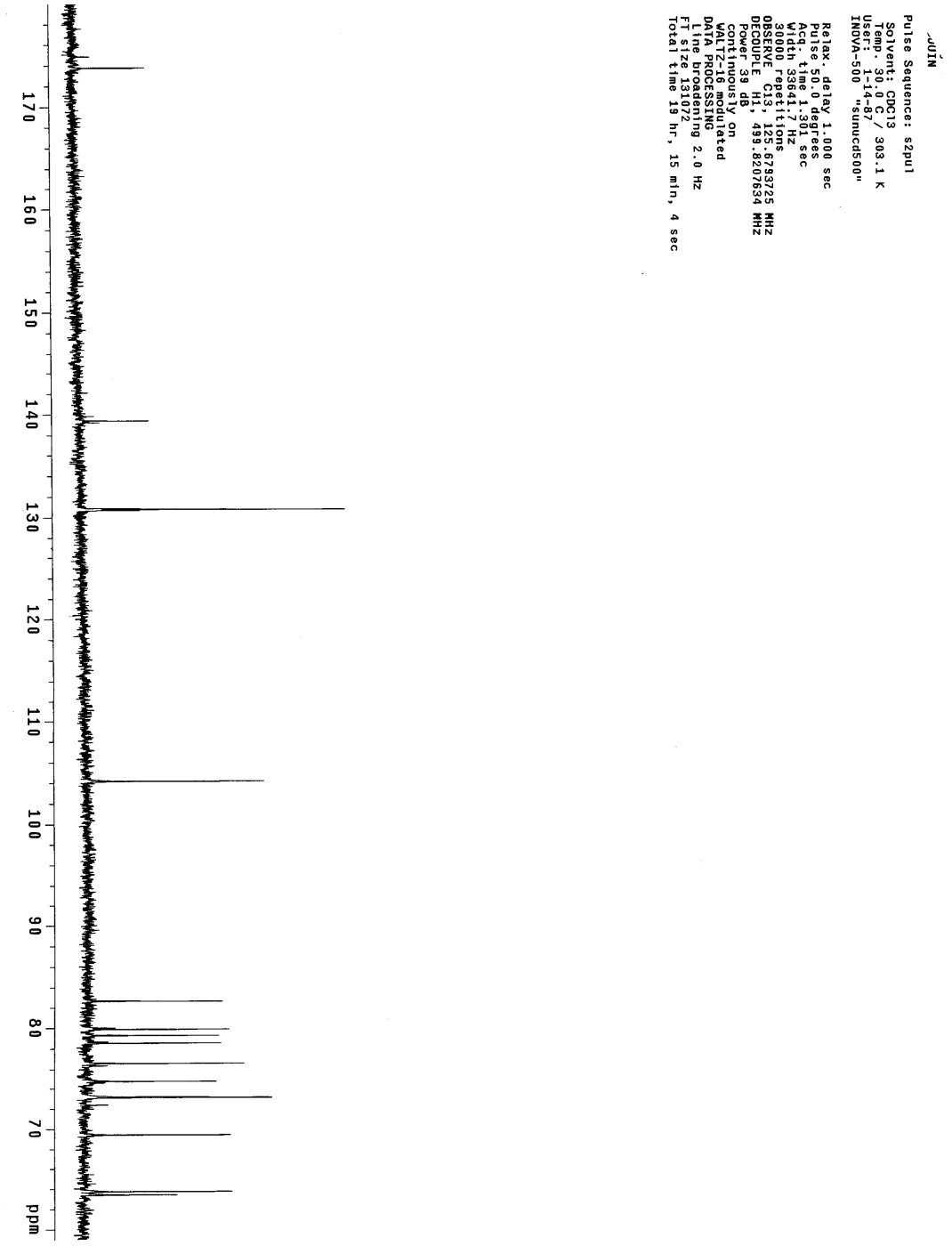


^{1}H-NMR Spectrum of 9 (obtained after HPLC)

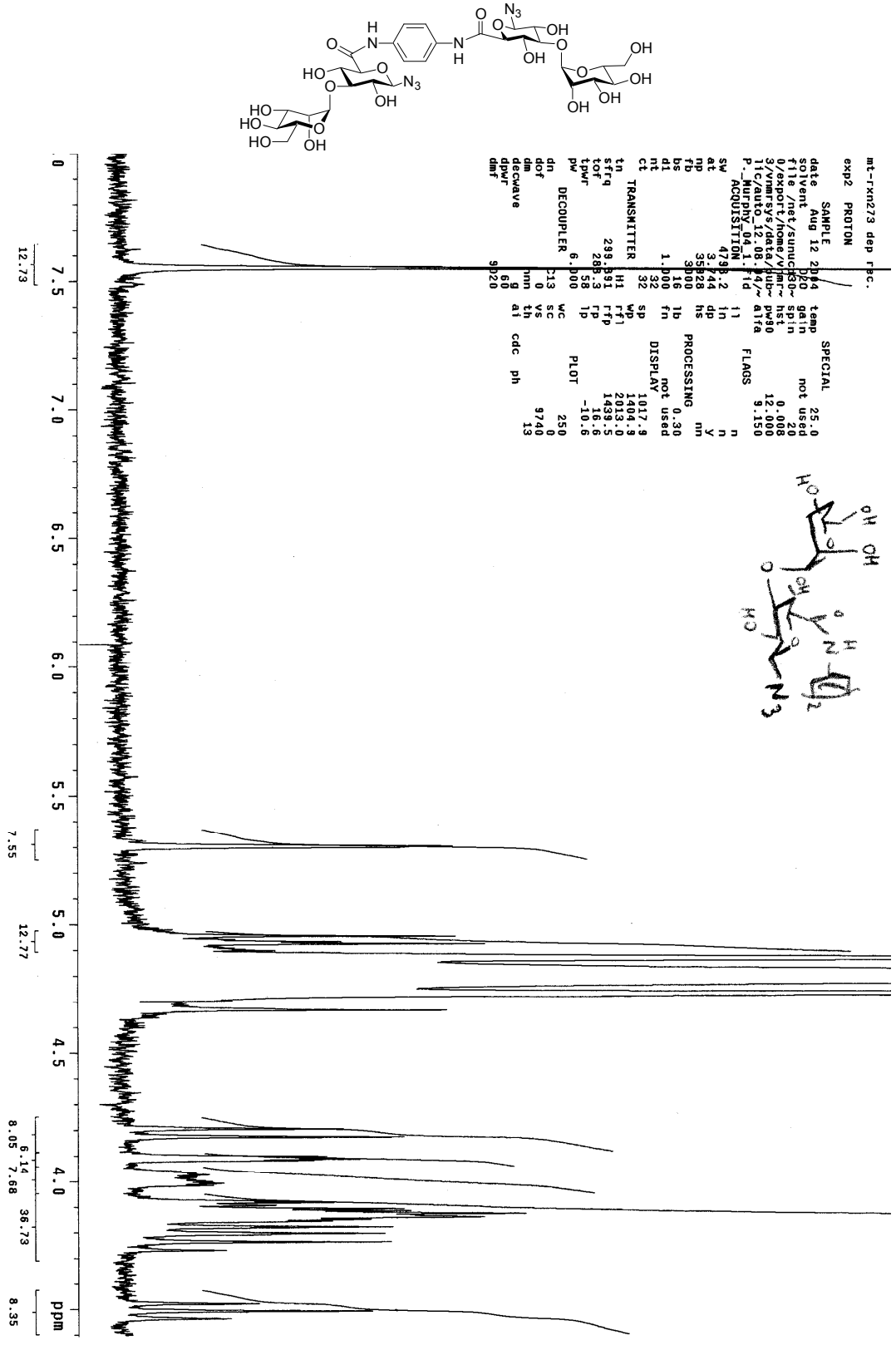


^{13}C-NMR Spectrum of 9 (obtained before HPLC purification)

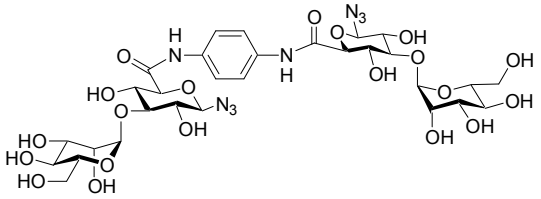


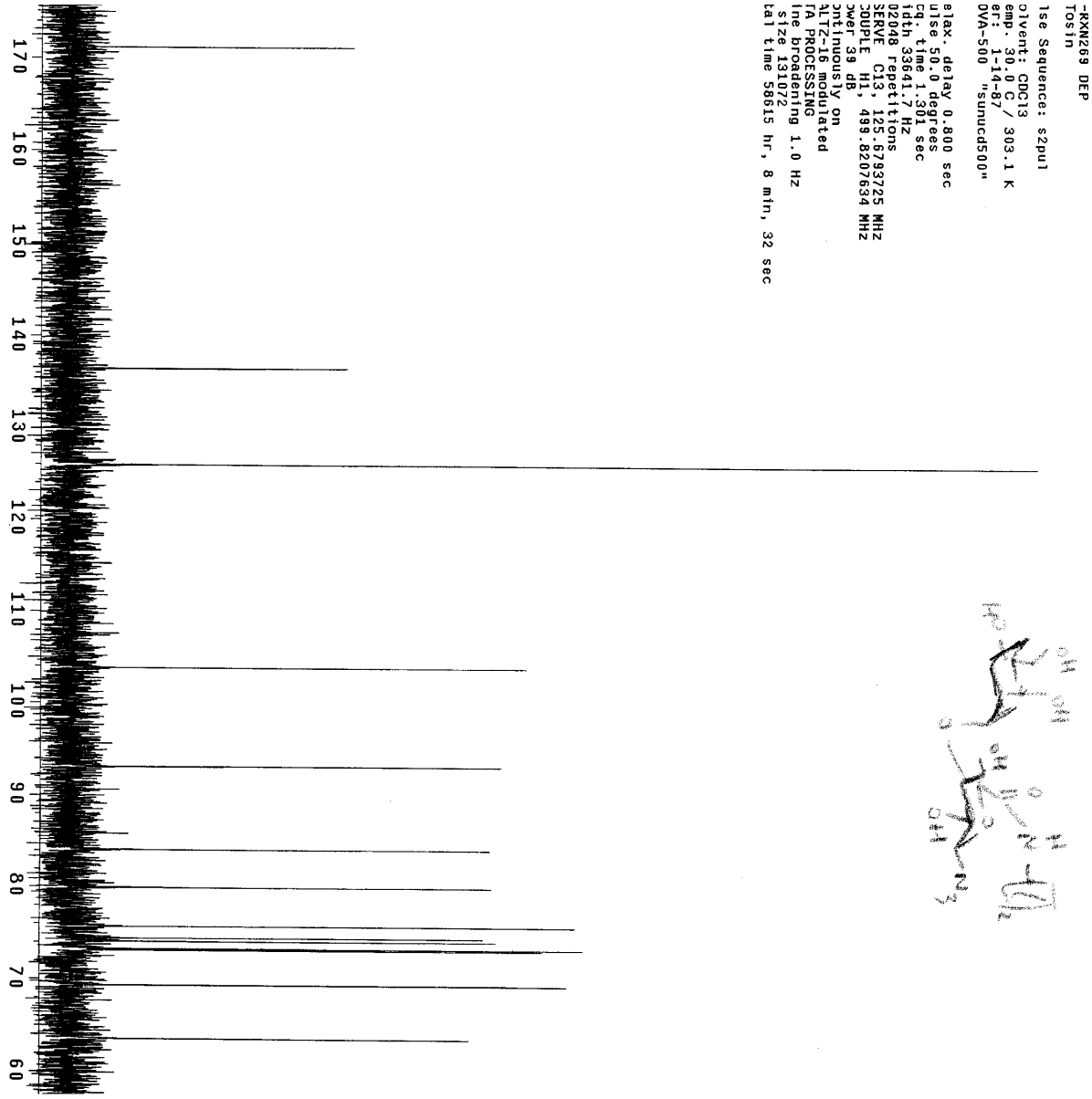


^{1}H-NMR of 10

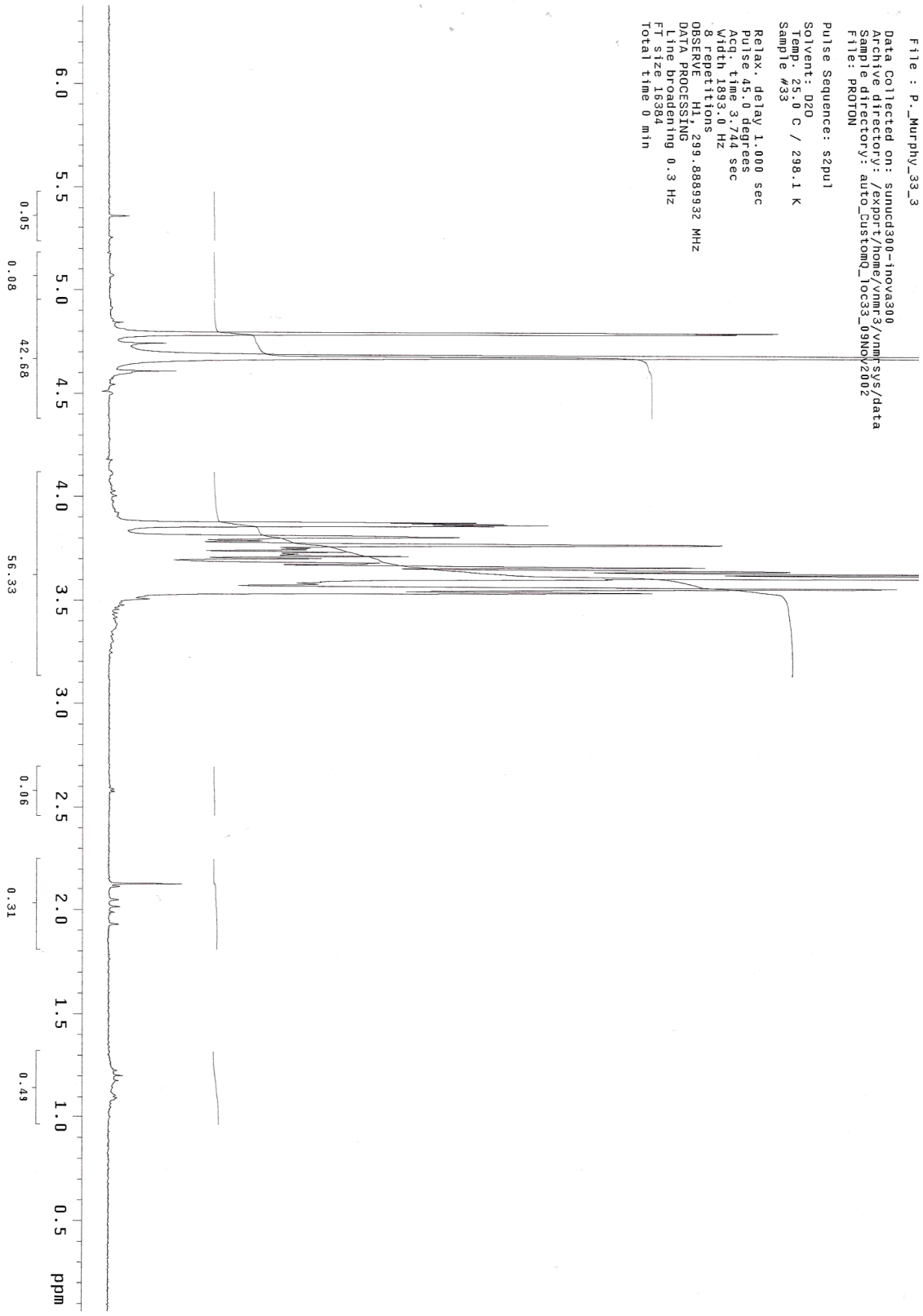


^{13}C-NMR of 10

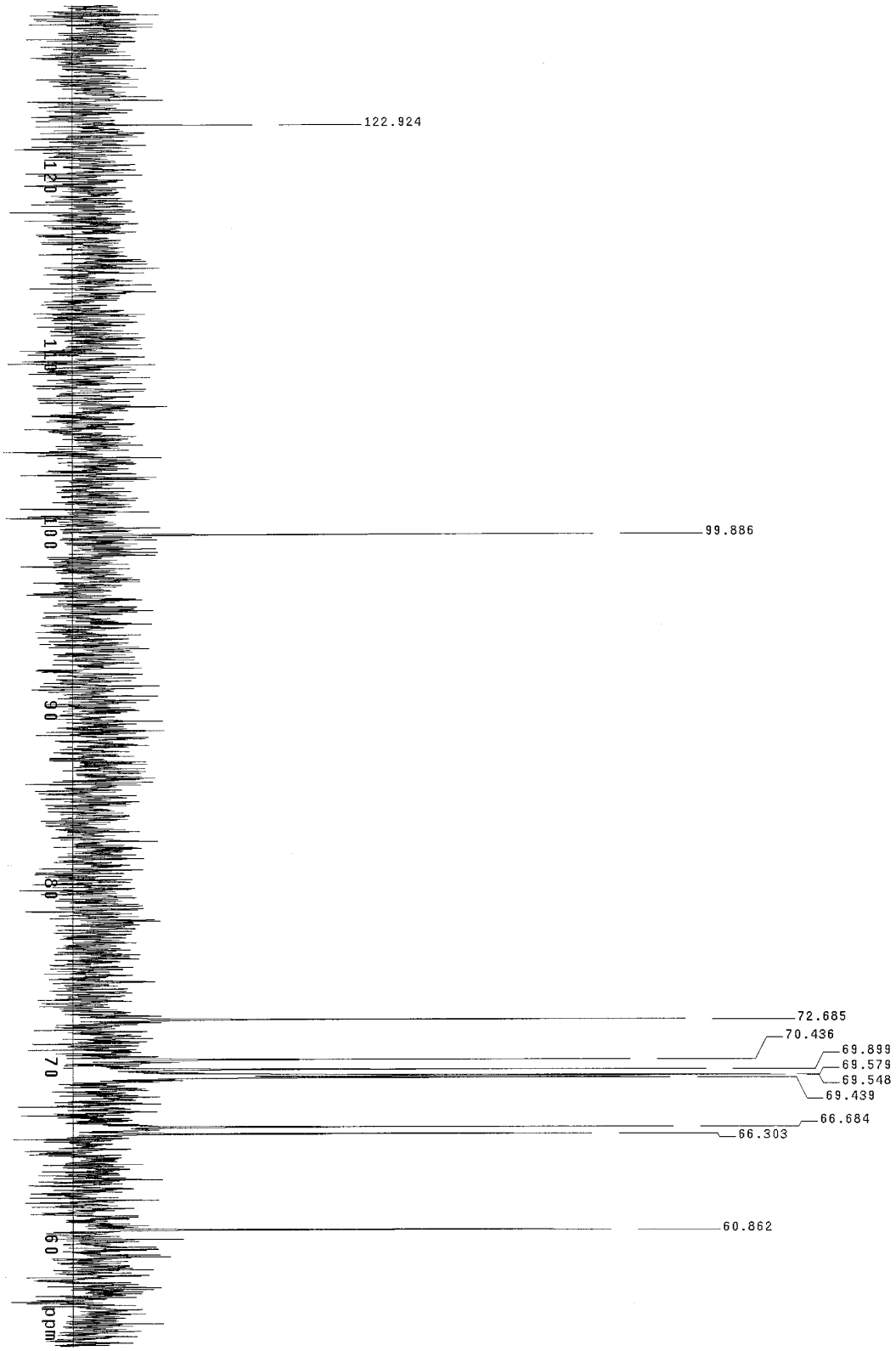




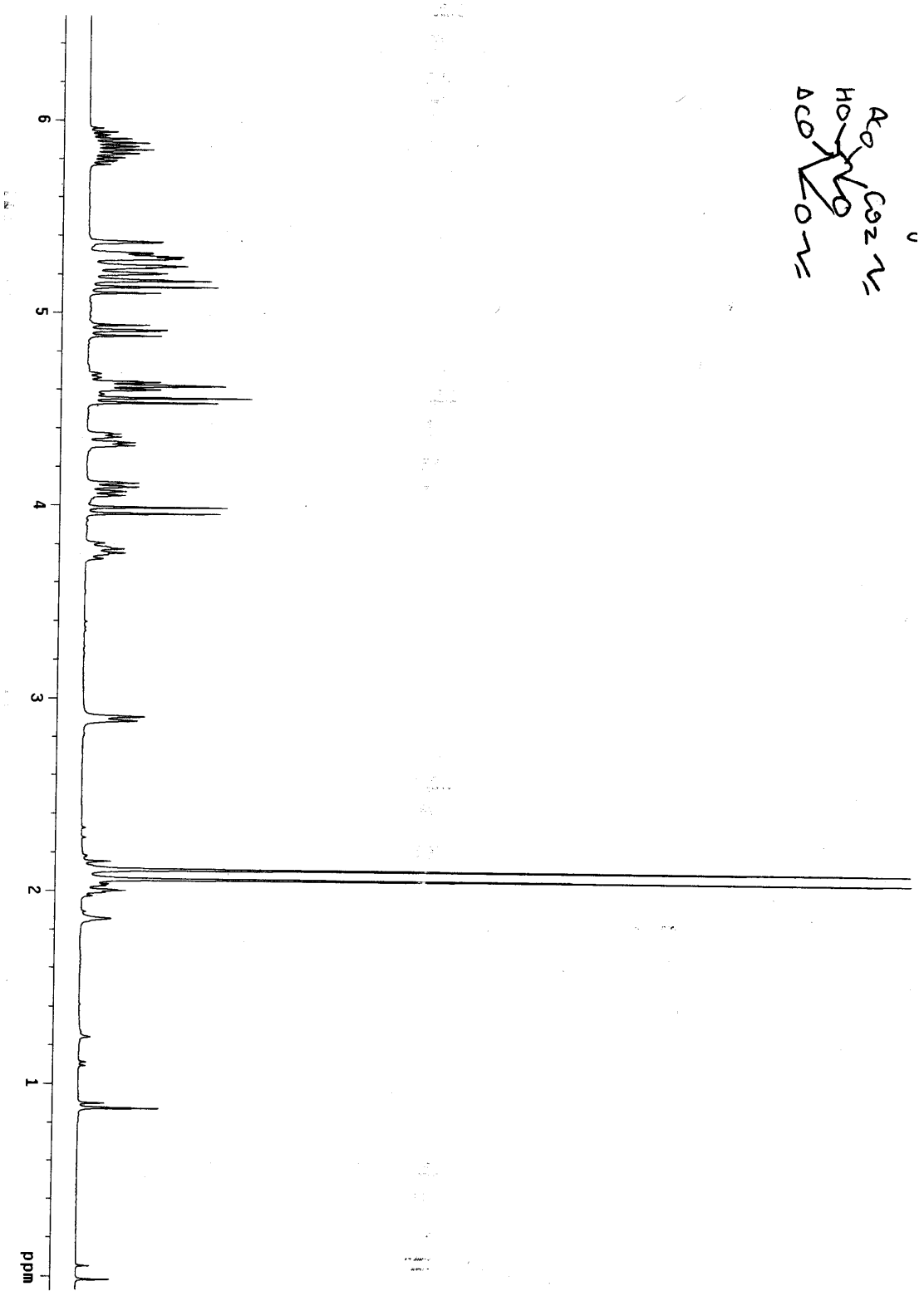
^{1}H-NMR of 11



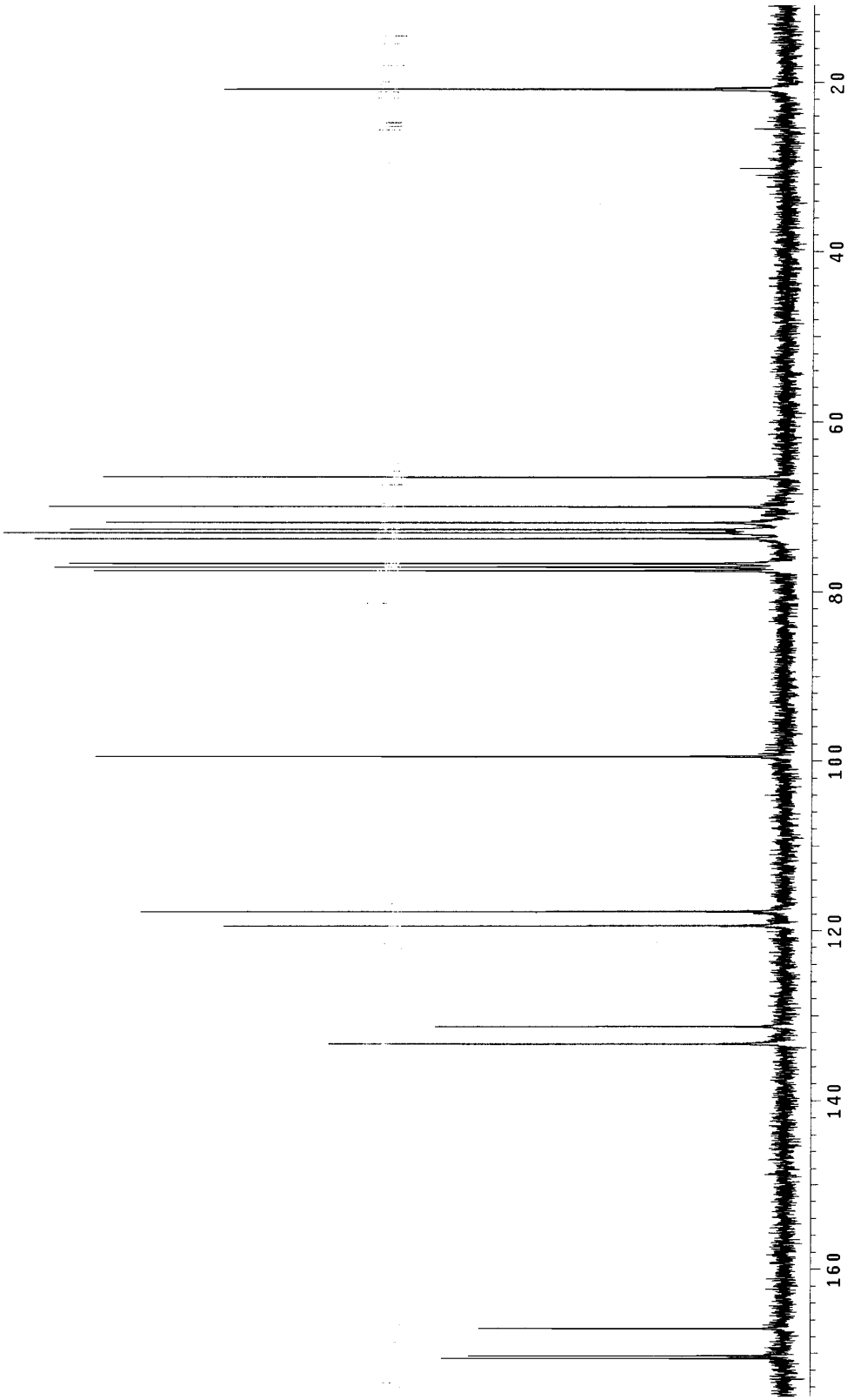
^{13}C-NMR of 11



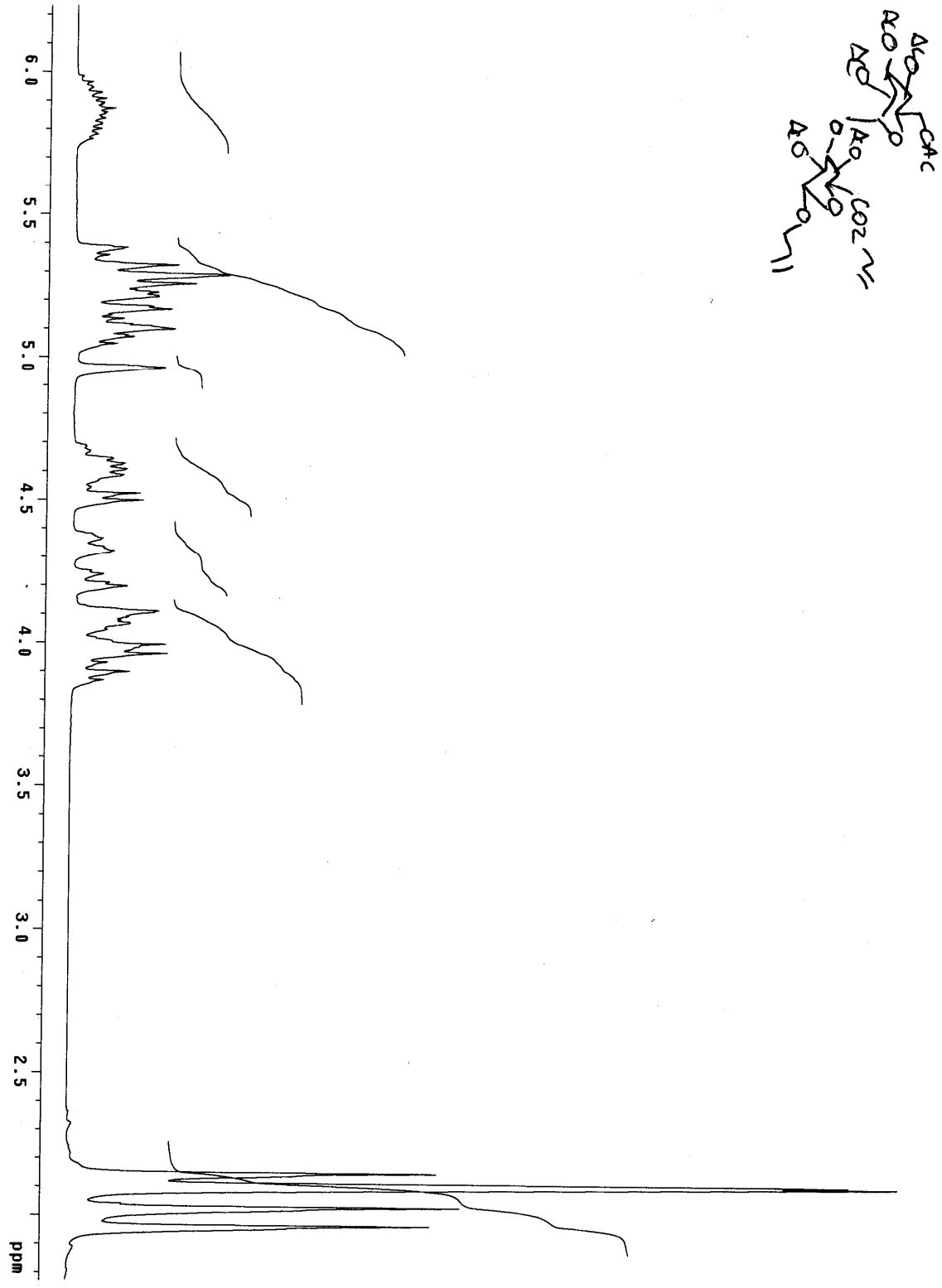
^{1}H-NMR of 13



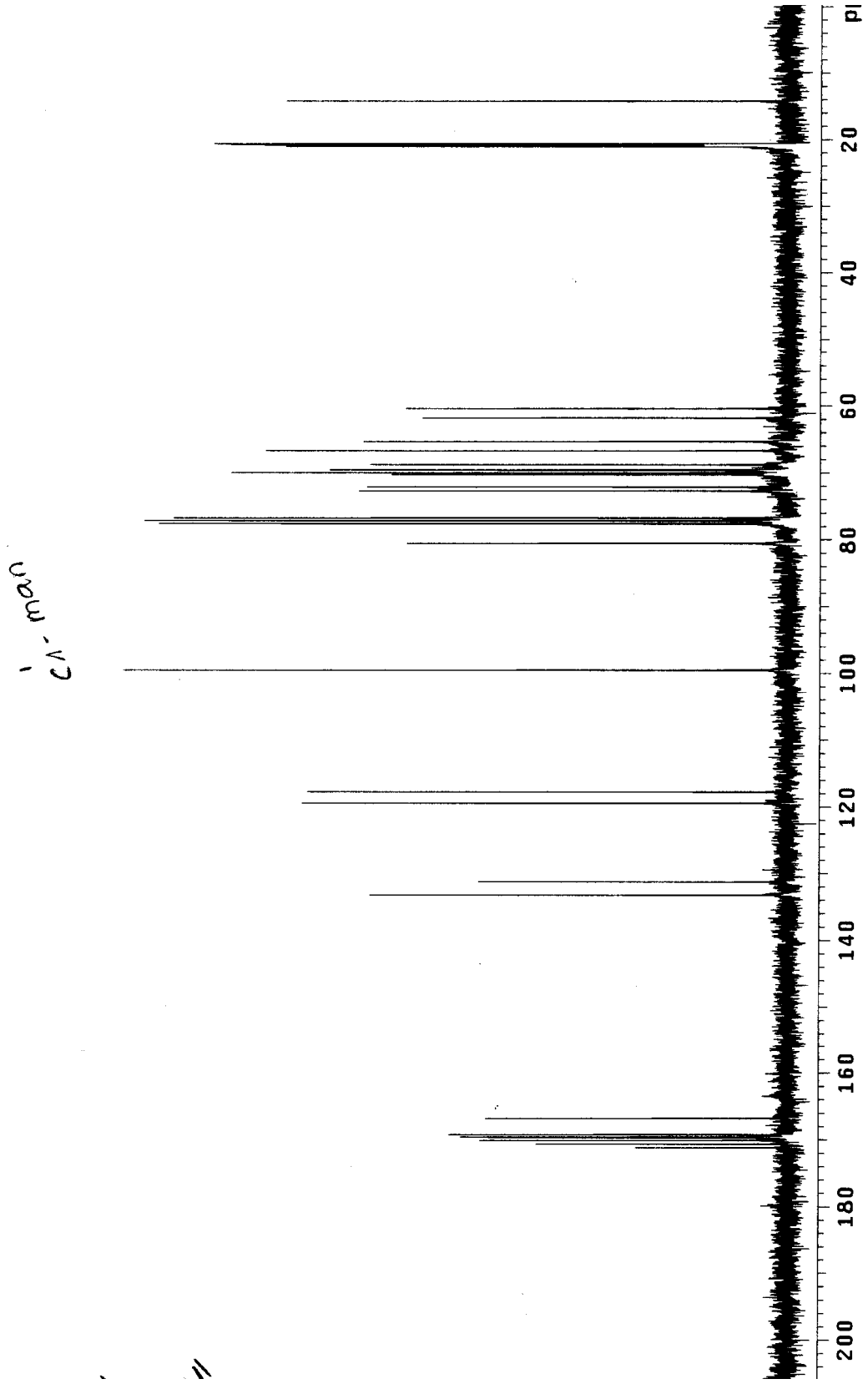
^{13}C-NMR of 13



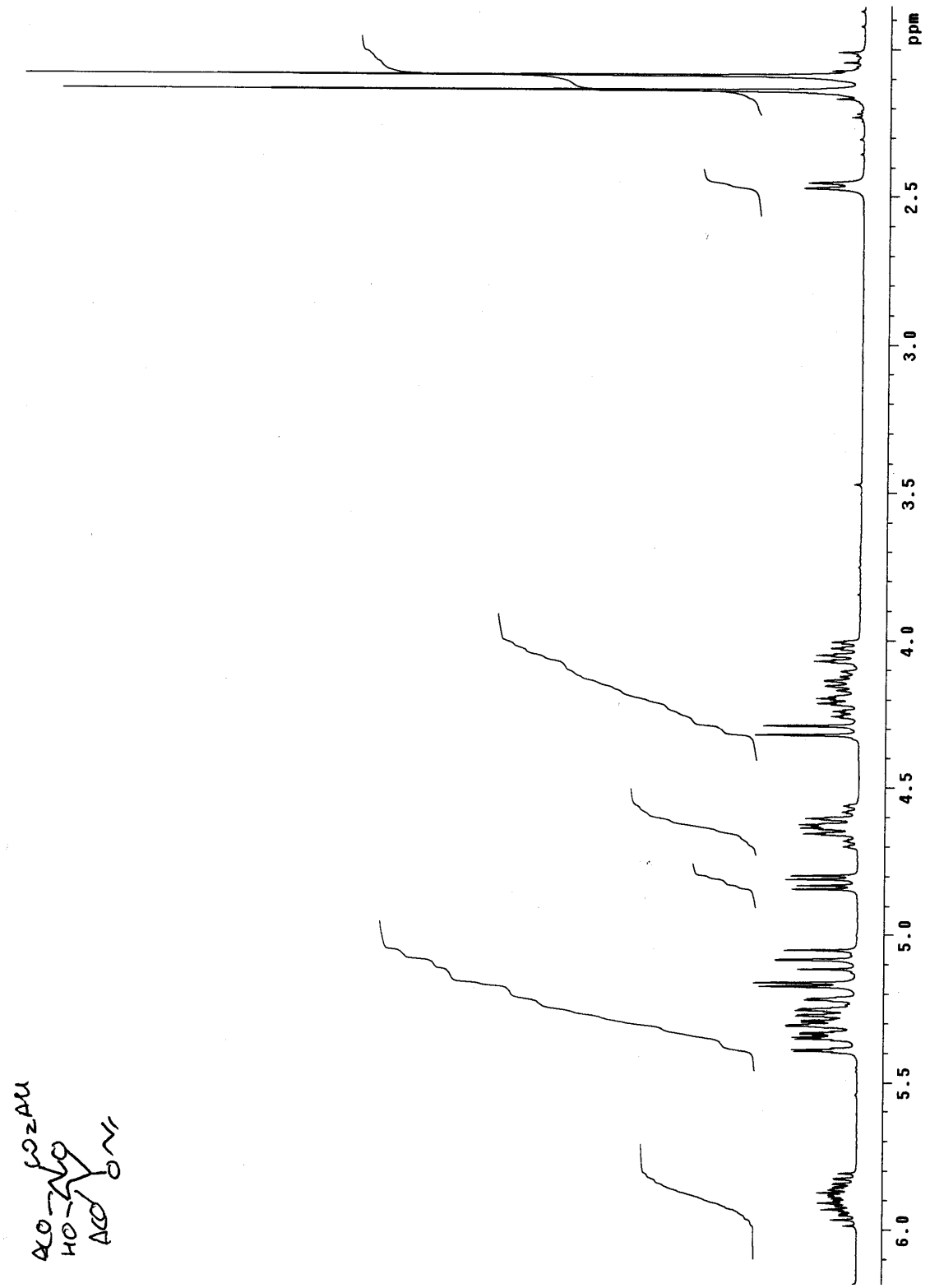
^{1}H-NMR of 14



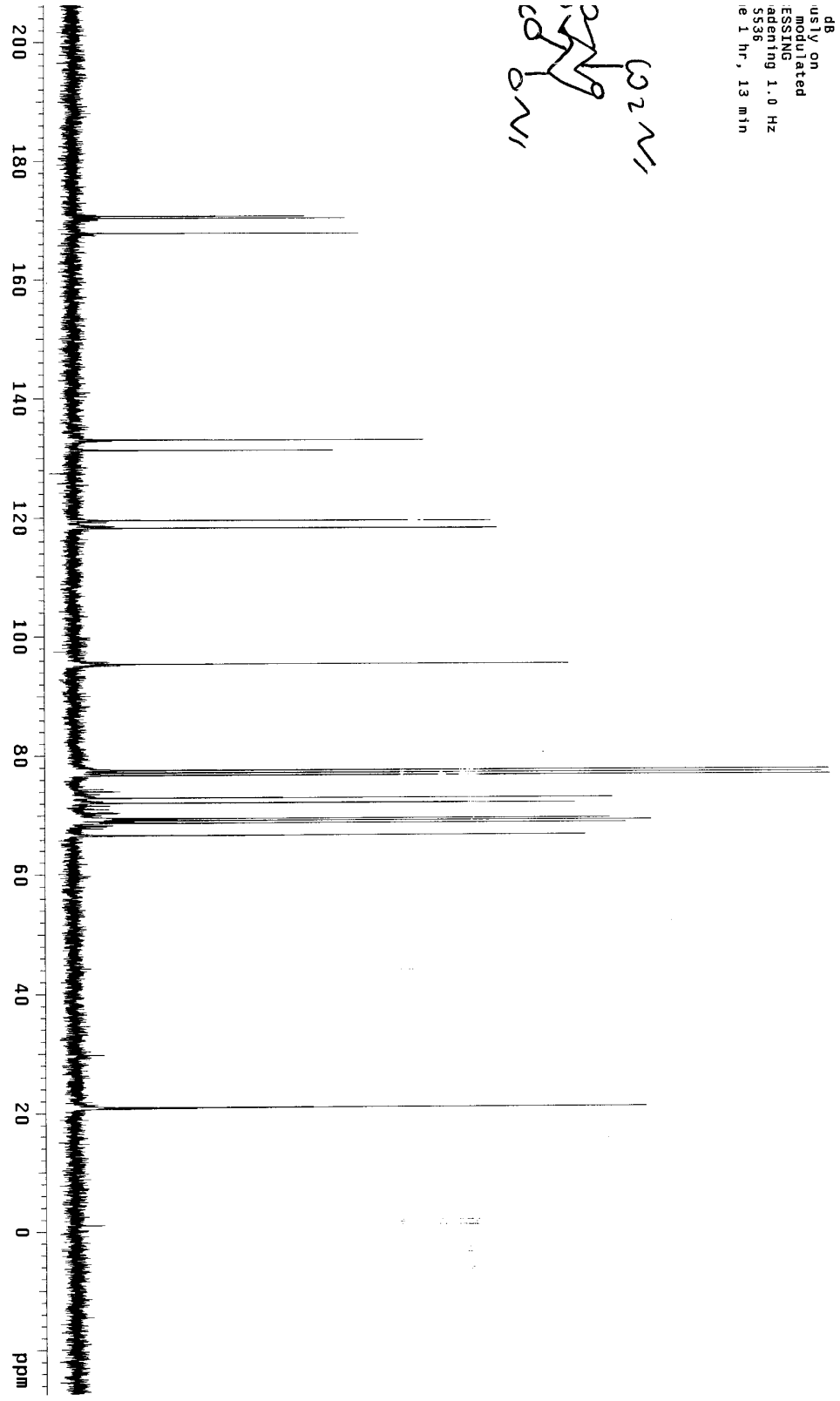
^{13}C-NMR of 14



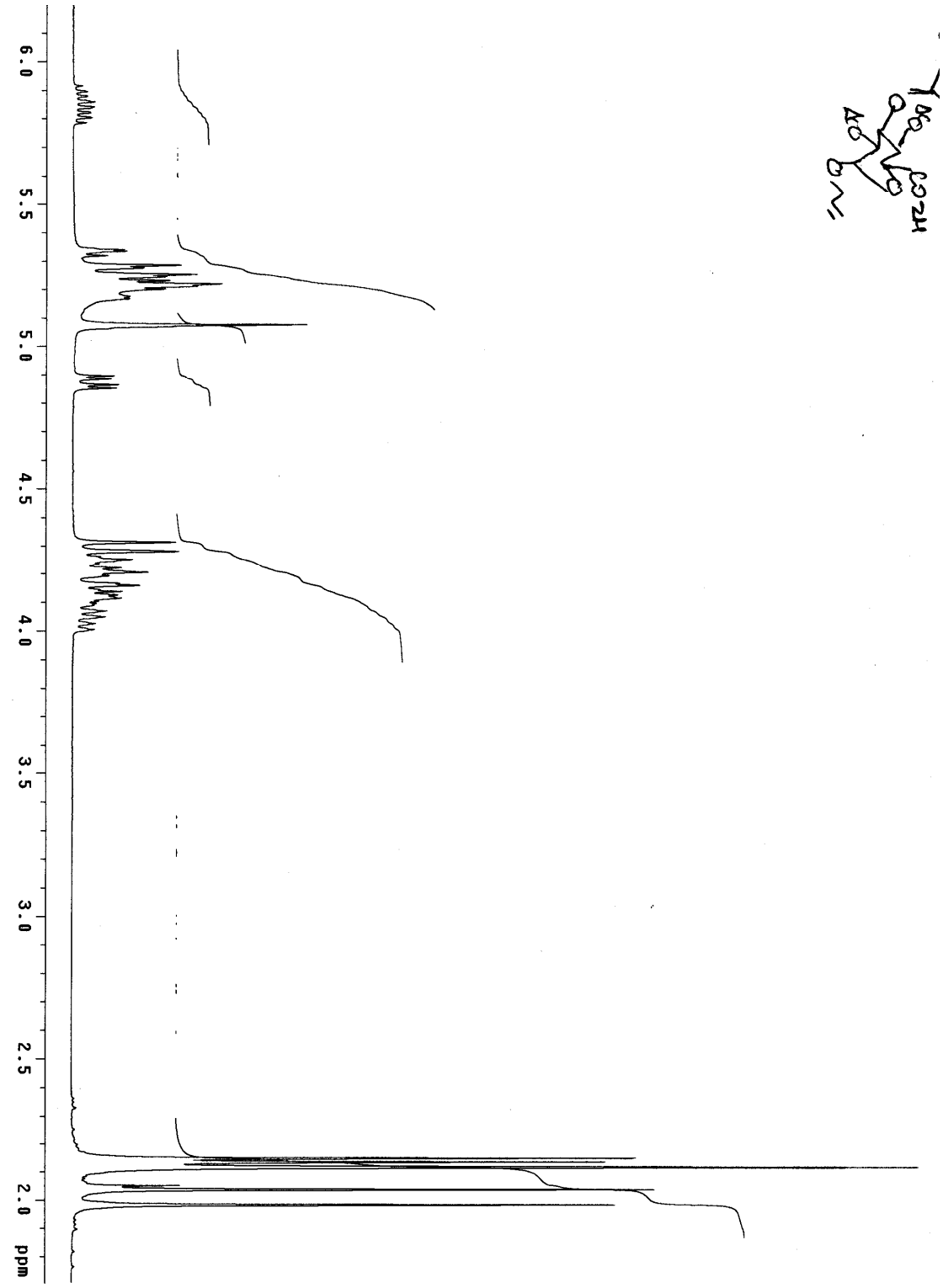
^{1}H-NMR of 17



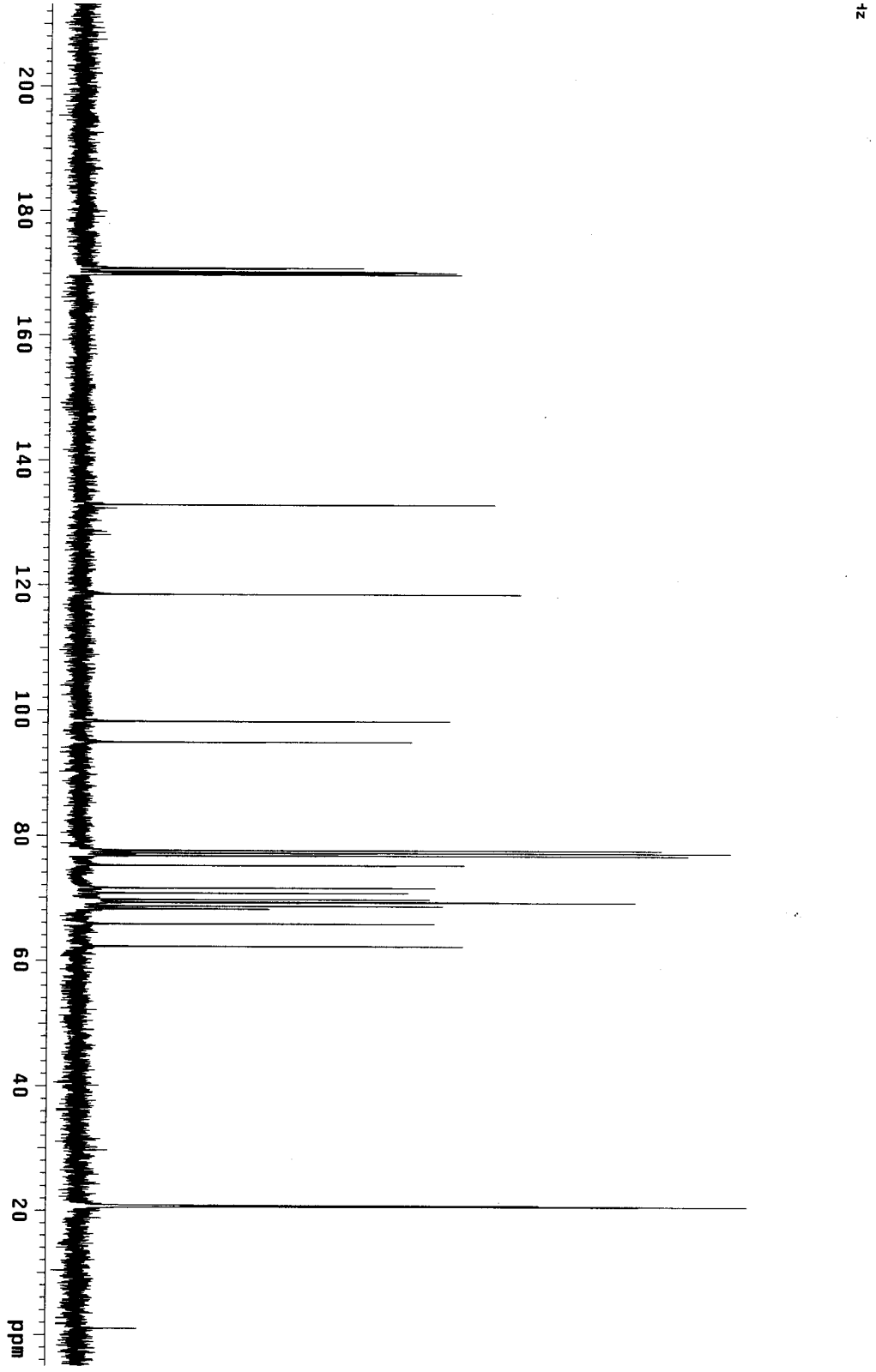
^{13}C-NMR of 17



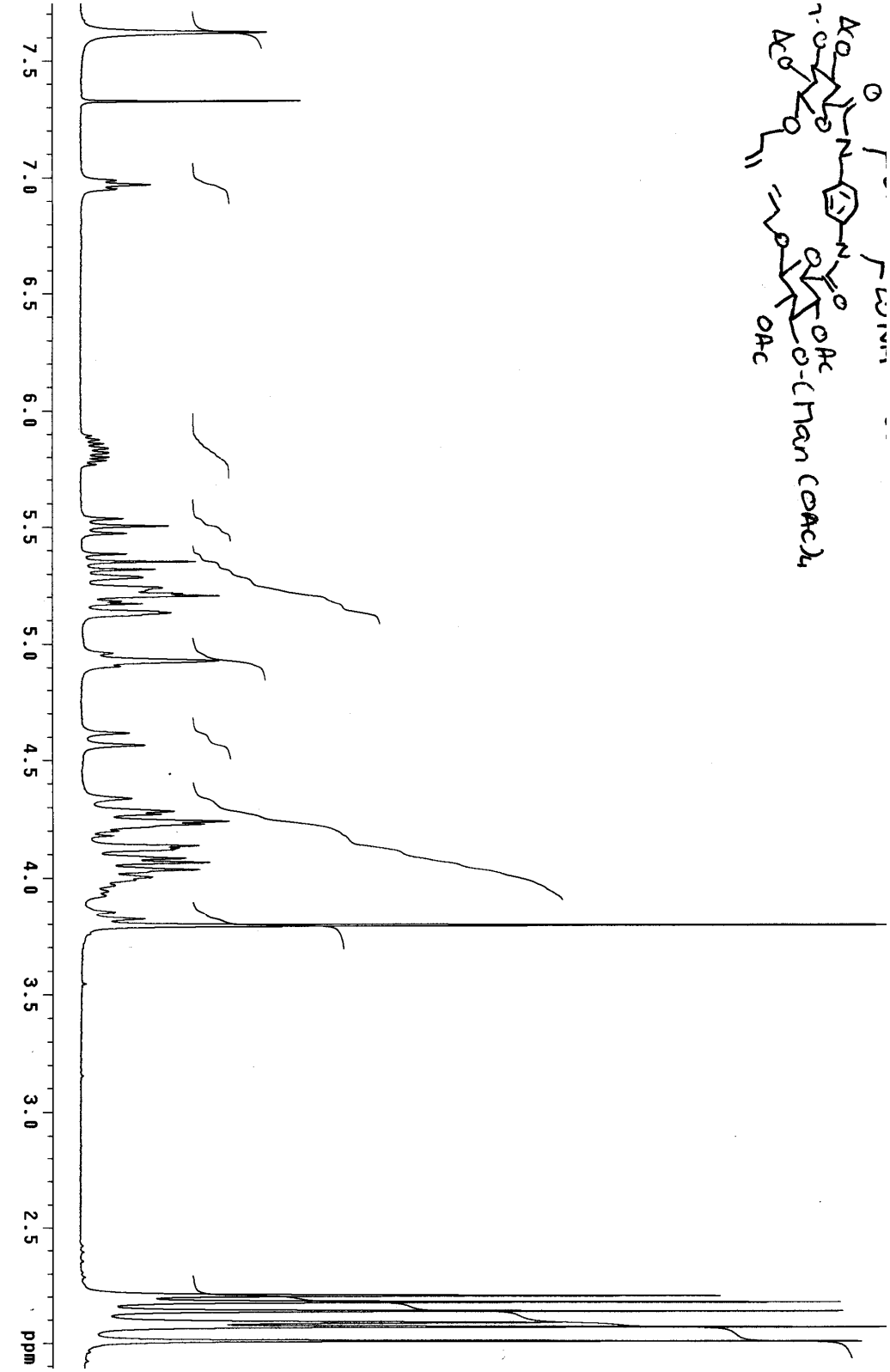
^{1}H-NMR of 18



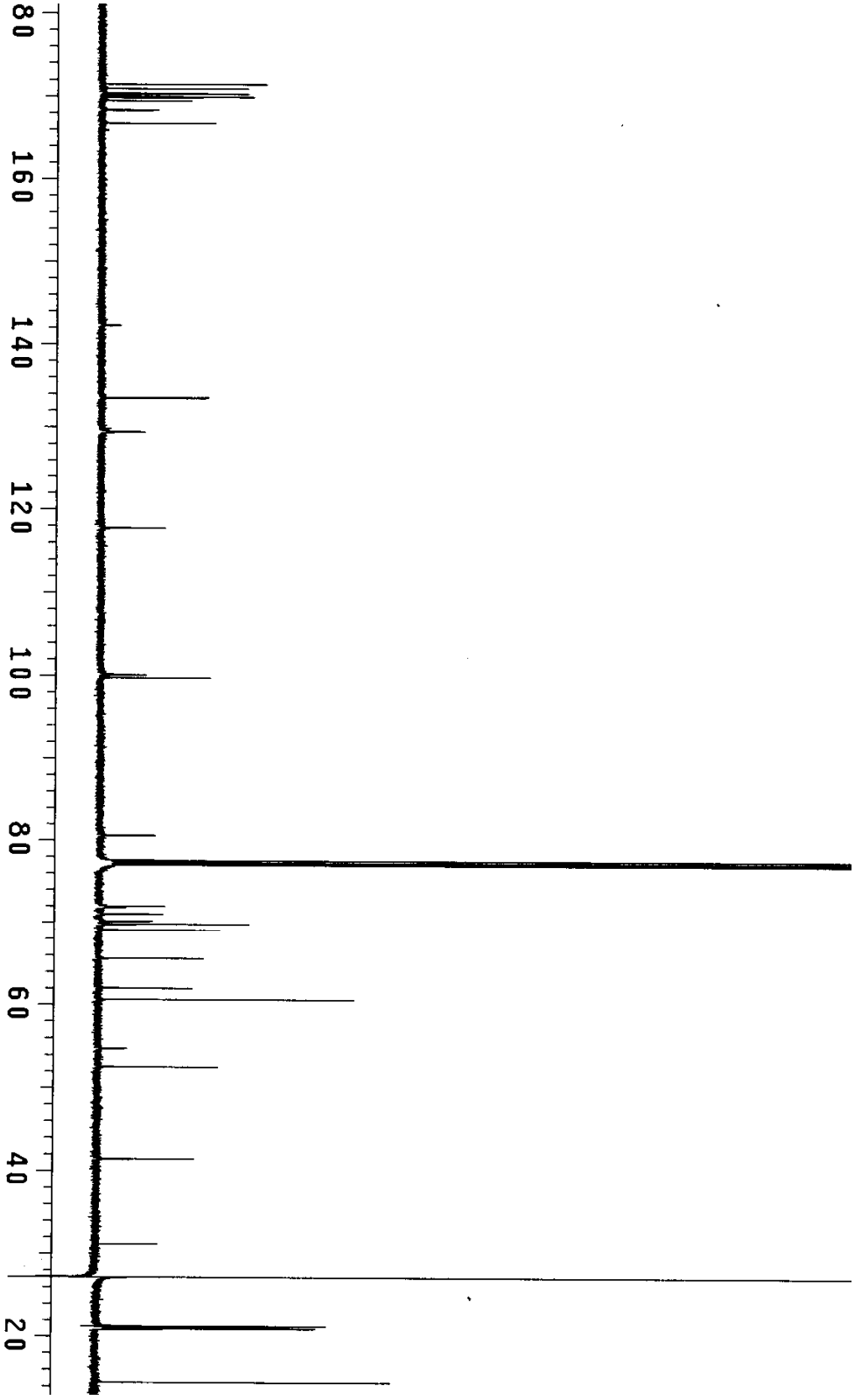
^{13}C-NMR of 18



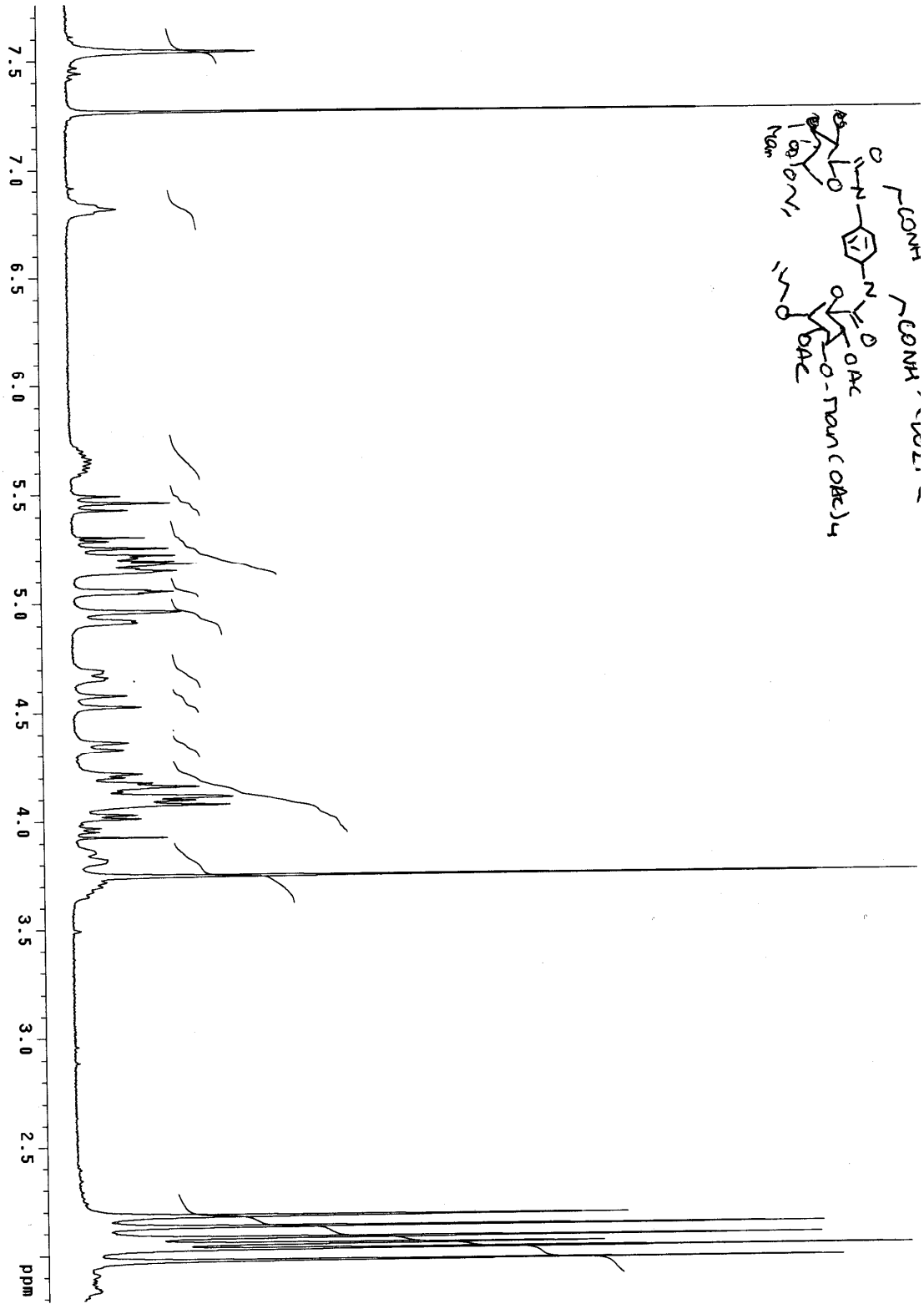
^{1}H-NMR of 20a



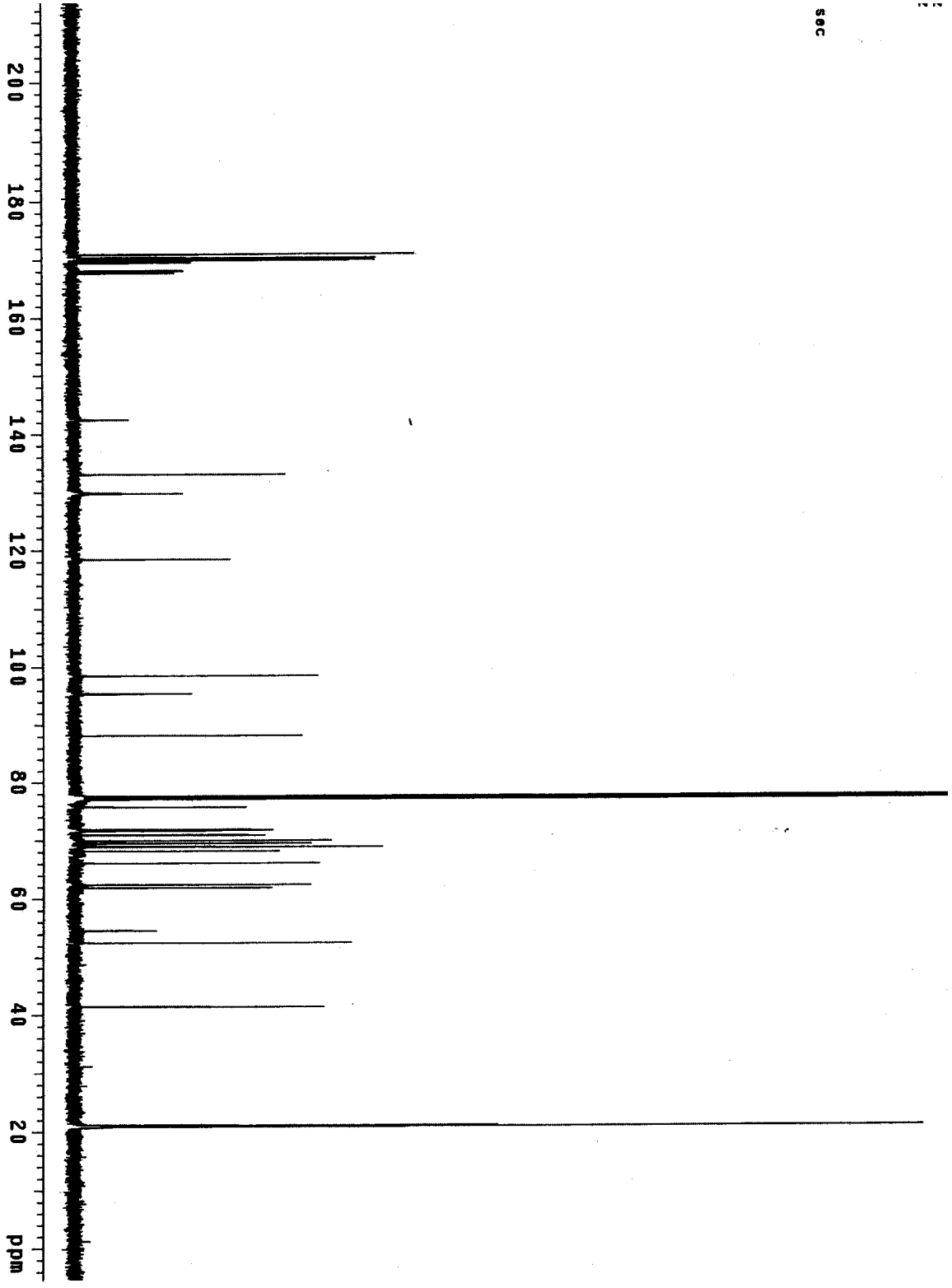
^{13}C-NMR of 20a



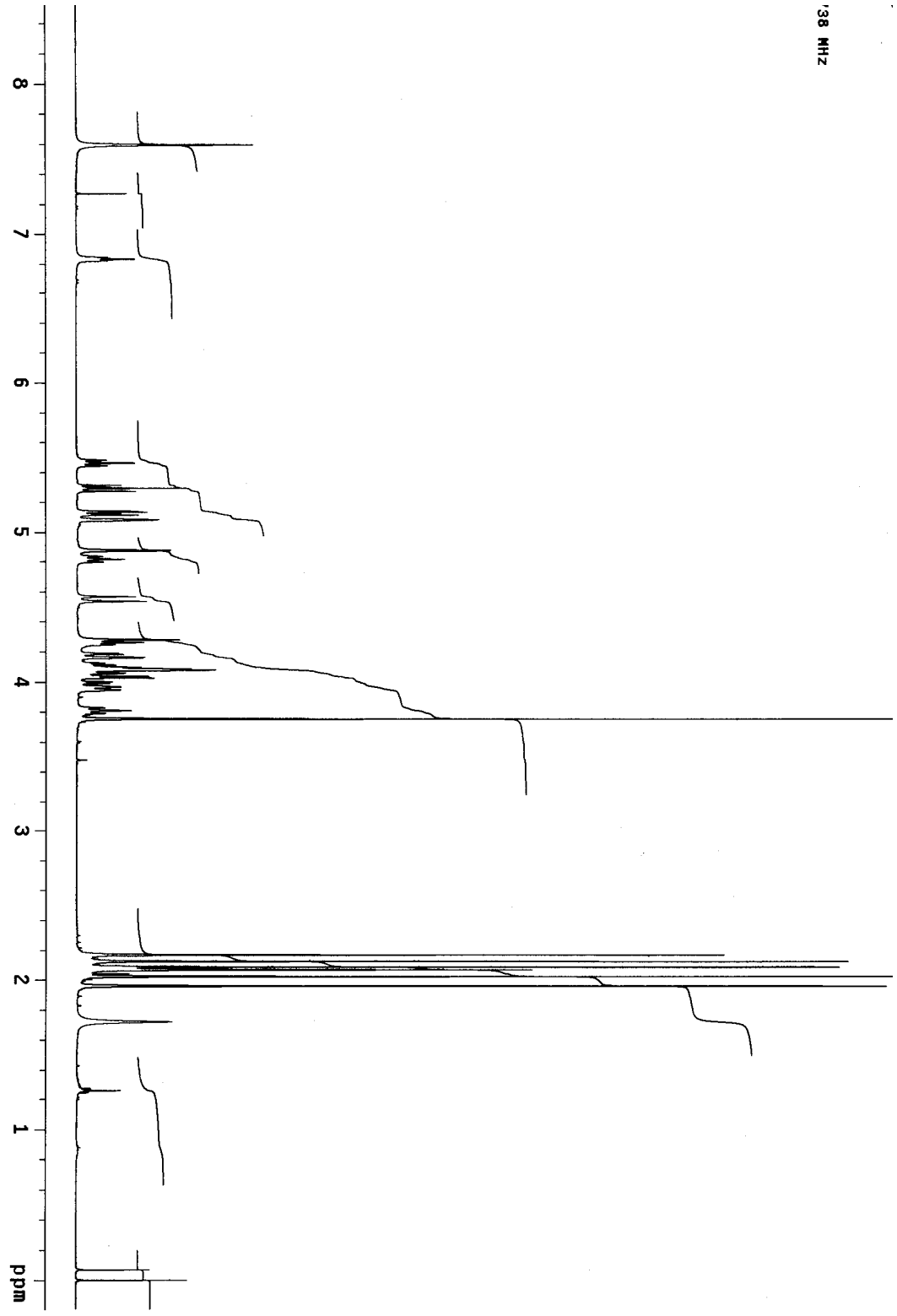
^{1}H-NMR of 21a



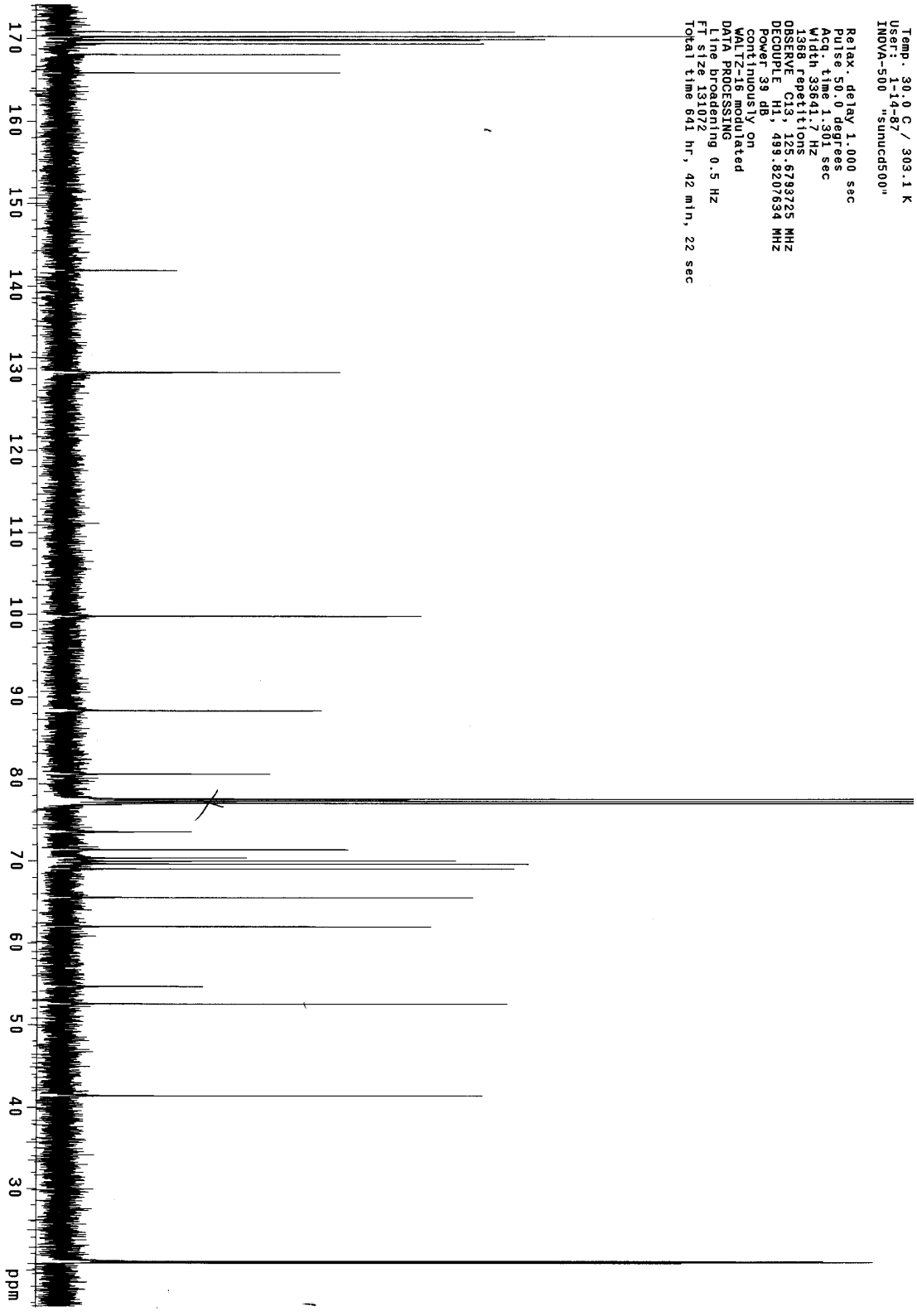
^{13}C-NMR of 21a



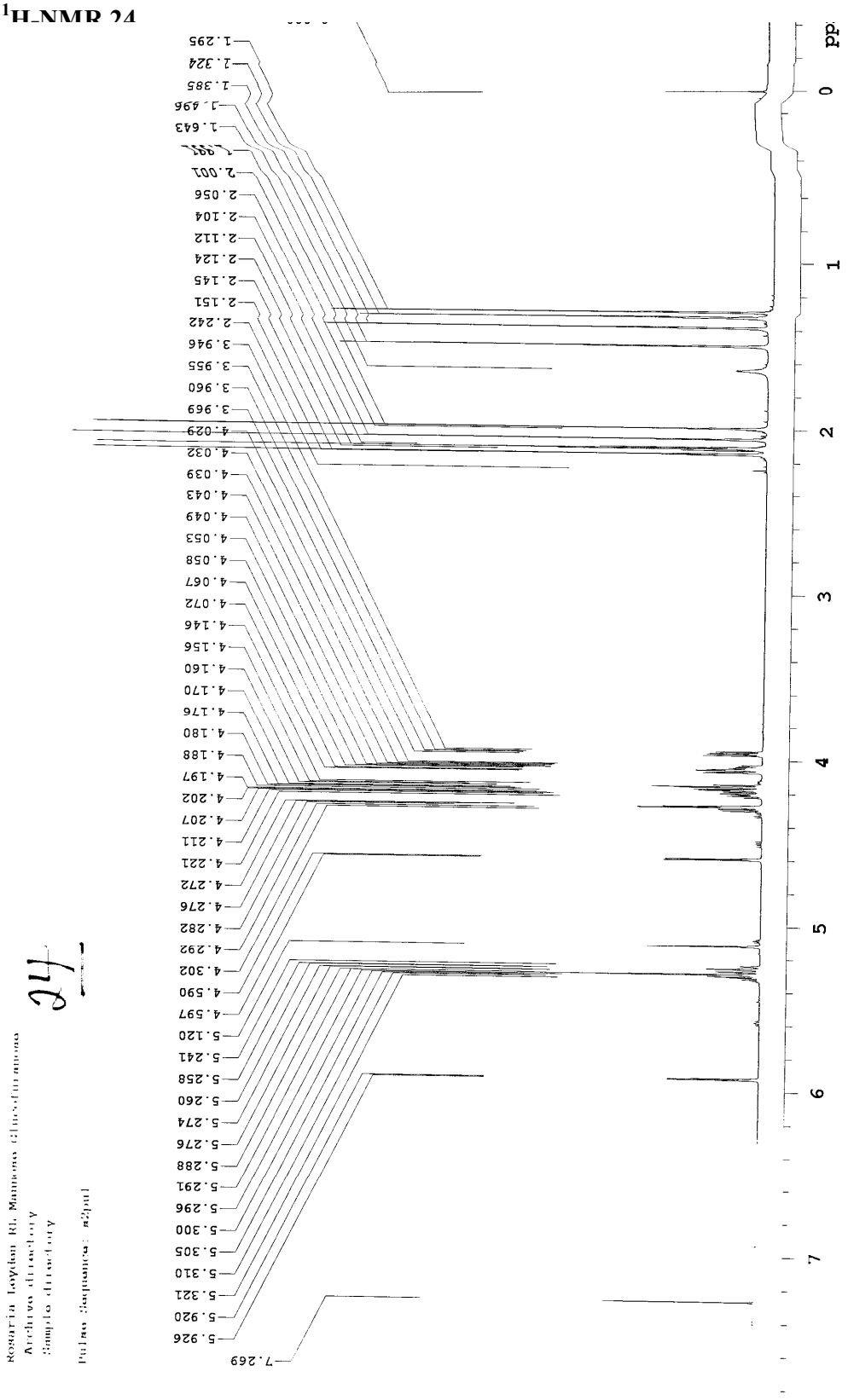
^{1}H-NMR of 22a



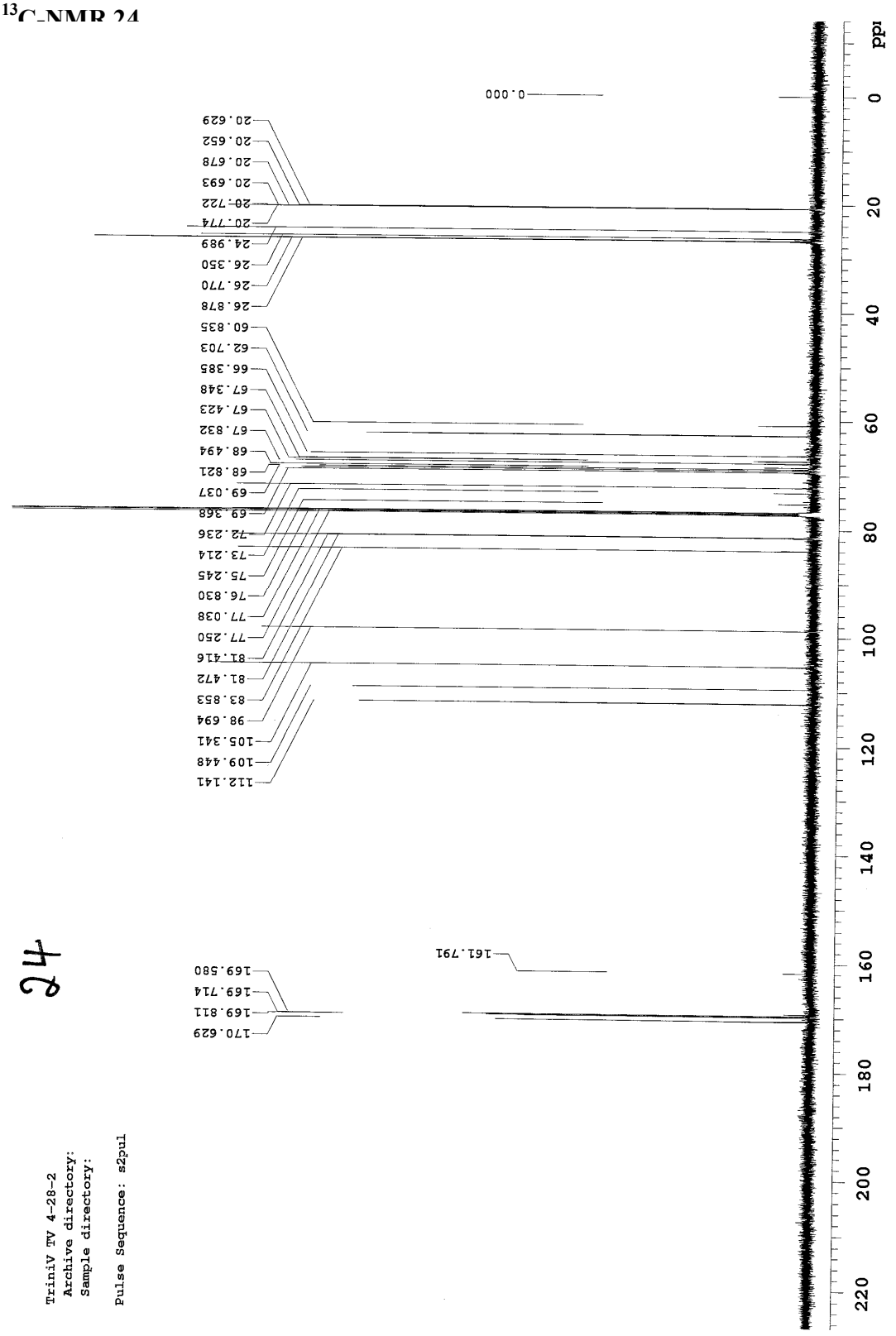
^{13}C-NMR 22a



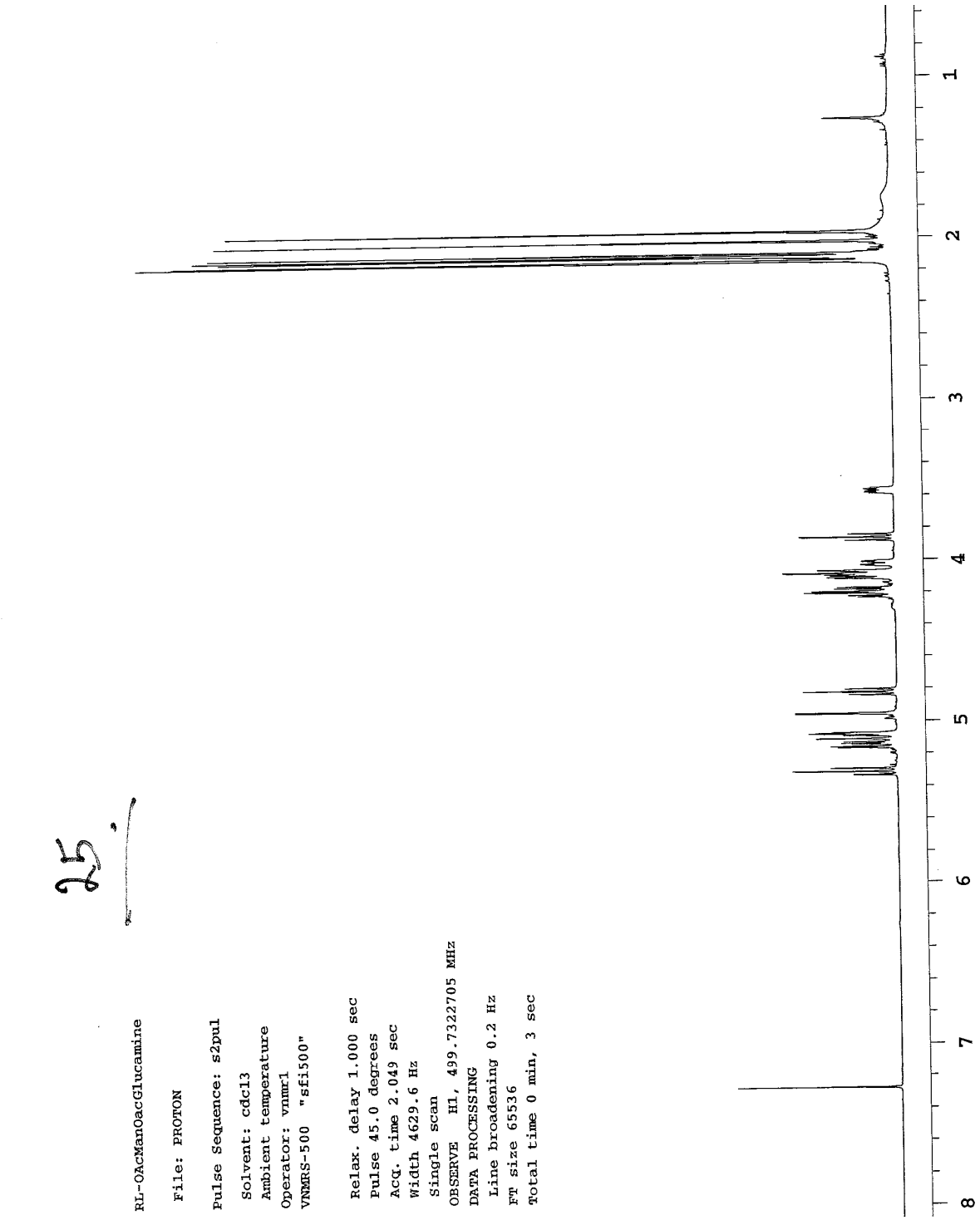
^{1}H-NMR 24



^{13}C-NMR 24



^{1}H-NMR 25



^{1}H-NMR of 26