Supporting Information

Design and Synthesis of Activity Probe for Glycosidases

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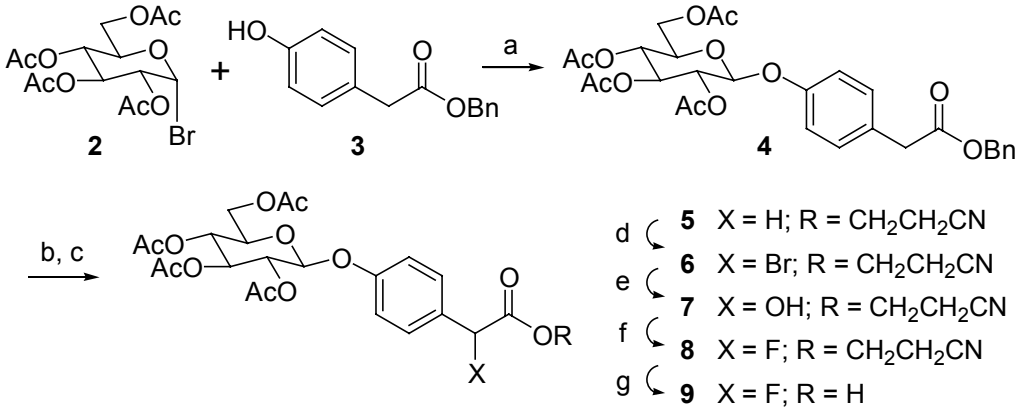
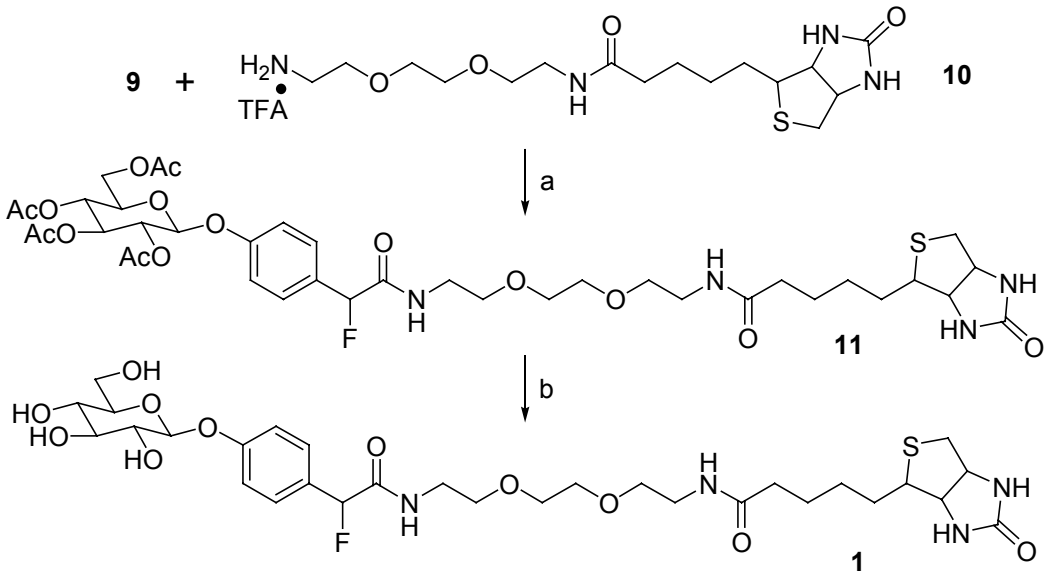
Experimental procedures and characterization, including copies of ^{1}H and ^{13}C NMR, for compounds **1**, **4**–**9**, and **11** as well as conditions for the labeling of β-glucosidase with probe **1** are included.

Scheme 1[a]



[a](a) AgOTf, CH_2Cl_2 (67%); (b) H_2, Pd/C (91%); (c) DCC, DMAP, $HOCH_2CH_2CN$, CH_2Cl_2 (95%); (d) NBS, CCl_4, hν (92%); (e) $AgNO_3$, acetone/H_2O (55%); (f) DAST, CH_2Cl_2 (85%); (g) DBU, CH_2Cl_2 (95%).

Scheme 2[a]



[a](a) DCC, HOBt, TEA (83%); (b) Na_2CO_3, MeOH (92%).

Experimental:

General methods. All reagents and starting materials were obtained from commercial suppliers (Acros, Aldrich and Merck) and were used without further purification. IR spectra were recorded on a Nicolet 550 series II spectrometer. ^1H and ^{13}C NMR were recorded using a Brucker AC-300 or Bruker Avance 400 spectrometer. The proton and carbon chemical shifts are given in ppm using $CDCl_3$ (δ_H 7.24 and 77.0) as internal standard. High resolution mass spectra were recorded with a JEOL-102A mass spectrometer. Analytical TLC (silica gel, 60F-54, Merck) and spots were visualized under UV light and/or phosphomolybdic acid-ethanol. Column chromatography was performed with Kiesegel 60 (70-230 mesh) silica gel (Merck). Melting points are reported without correction.

[4-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-acetic acid benzyl ester (4): Benzyl *p*-hydroxyphenylacetate **3** (265 mg, 1.1 mmol) and AgOTf (421 mg, 1.6 mmol) in the presence of 3A molecular sieves was dissolved/suspended in 10 mL of CH_2Cl_2 The mixture was cooled in an ice bath and a solution of tetra-*O*-acetyl-glucopyranosyl bromide **2** (675 mg, 1.6 mmol) in 5 mL of CH_2Cl_2 was slowly added. The reaction mixture was kept in the dark for 2 h. It was quenched by adding 1 mL of DIEA. The yellow precipitate was filtered off. The filtrate was concentrated to dryness and the residue was subjected to silica gel column chromatography eluted with *n*-hexane/EtOAc (7/3). The β-glucoside **4** (420 mg) was obtained in 67% yield. R_f = 0.45 (*n*-hexane/EtOAc = 1/1), mp 75–78°C. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.34-7.25 (m, 5 H), 7.17 (d, *J* = 6.6 Hz, 2 H), 6.92 (d, *J* = 6.6 Hz, 2H), 5.29-5.21 (m, 2

H, H-2 + H-4), 5.11 (dd, *J* = 11.7, 9.9 Hz, 1H, H-3), 5.08 (s, 2H), 5.03 (d, *J* = 7.6 Hz, 1 H, H-1), 4.26 (dd, *J* = 12.6, 5.3 Hz, 1 H, H-6), 4.13 (dd, *J* = 12.6, 2.4 Hz, 1 H, H-6'), 3.83 (m, 1 H, H-5), 3.59 (s, 2 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.97 (s, 3 H), 1.96 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.3 (C), 170.4 (C), 170.1 (C), 169.3 (C), 169.2 (C), 155.9 (C), 135.7 (C), 130.4 (CH), 128.8 (CH), 128.6 (CH), 128.5 (CH), 128.1 (C), 117.0 (CH), 99.1 (CH), 72.6 (CH), 71.9 (CH), 71.1 (CH), 68.2 (CH), 66.6 (CH_2), 61.8 (CH_2), 40.3 (CH_2), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 20.5 (CH_3). IR (KBr): 3038, 2964, 2945, 1760, 1512, 1450, 1369, 1239, 1140, 1047, 978, 922 cm^{-1}. HRMS calcd for $C_{29}H_{33}O_{12}$ $(M + 1)^+$ 573.1972, found 573.1969.

[4-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-acetic acid 2-cyanoethyl ester (5): To a solution of compound **4** (85 mg, 0.15 mmol) in 5 mL of EtOAc and a few drops of MeOH was added a spatula of 10% Pd/C. The system was evacuated and filled with H_2 three times. It was kept under H_2 atmosphere with a balloon and stirred at rt for 1 h. The Pd/C catalyst was filtered off through Celite 535, and the filtrate concentrated. The acid intermediate (65 mg, 91%) was obtained as a white foam after silica gel column chromatography eluted with MeOH/$CHCl_3$ (5/95). R_f = 0.51 (MeOH/$CHCl_3$ = 1/9). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.17 (d, *J* = 6.7 Hz, 2 H), 6.92 (d, *J* = 6.7 Hz, 2H), 5.29-5.20 (m, 2 H, H-2 + H-4), 5.13 (dd, *J* = 9.7, 9.3 Hz, 1 H, H-3), 5.03 (d, *J* = 7.5 Hz, 1 H, H-1), 4.25 (dd, *J* = 12.3, 5.3 Hz, 1 H, H-6), 4.13 (dd, *J* = 12.3, 2.4 Hz, 1 H, H-6'), 3.82 (m, 1 H), 3.56 (s, 2 H), 2.03 (s, 3 H), 2.01 (s, 3 H), 1.99 (s, 3 H), 1.98 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.1 (C), 170.6 (C), 170.2 (C), 169.4 (C), 169.3 (C), 156.0 (C), 130.5 (CH), 128.2 (C), 117.0 (CH), 99.0 (CH), 72.6 (CH), 71.9 (CH), 71.1 (CH), 68.2 (CH), 61.9 (CH_2), 40.0 (CH_2), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 20.5 (CH_3); IR (neat): 3389, 1760, 1523, 1372, 1227, 1037 cm^{-1}. HRMS calcd for $C_{22}H_{27}O_{12}$ $(M + 1)^+$ 483.1503; found 483.1499. To a solution of the acid intermediate (575 mg, 1.19 mmol), 3-hydroxypropionitrile (98 μL, 1.43 mmol) and DMAP (15 mg, 0.11 mmol) in 50 mL of CH_2Cl_2 cooled in an ice bath was slowly injected 4 mL of DCC solution (0.45 M in CH_2Cl_2). The reaction mixture was allowed to warm to rt and stirred for 10 h. The white precipitate was filtered off and the filtrate concentrated. The oil residue was dissolved in 60 mL of EtOAc and washed with 5% citric acid (×3), 10% $NaHCO_3$ (×3) and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The resulting oil was chromatographed on silica gel (EtOAc/ $CHCl_3$ = 2/8) to give compound **5** as an oil (606 mg, 95%). R_f = 0.33 (EtOAc/ $CHCl_3$ = 3/7). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.18 (dd, *J* = 6.6, 2.1 Hz, 2 H), 6.92 (dd, *J* = 6.6, 2.1 Hz, 2 H), 5.28-5.19 (m, 2 H, H-2 + H-4), 5.12 (dd, *J* = 9.9, 9.3 Hz, 1H, H-3), 5.03 (d, *J* = 7.6 Hz, 1 H, H-1), 4.26-4.22 (m, 3 H), 4.12 (dd, J = 12.3, 2.5 Hz,1 H, H-6), 3.80 (m, 1 H,

H-5), 3.59 (s, 2 H), 2.66 (t, J = 6.3 Hz, 2 H), 2.03 (s, 3 H), 2.01 (s, 3H), 2.00 (s, 3 H), 1.99 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.9 (C), 170.5 (C), 170.1 (C), 169.3 (C), 169.2 (C), 156.1 (C), 130.4 (CH), 128.1 (C), 117.1 (CH), 116.6 (C), 99.0 (CH), 72.6 (CH), 71.9 (CH), 71.1 (CH), 68.2 (CH), 61.8 (CH_2), 59.0 (CH_2), 39.9 (CH_2), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 17.9 (CH_2). IR (neat): 3336, 2936, 2850, 2259, 1747, 1517, 1424, 1228, 1155, 1037 cm^{-1}. HRMS calcd for $C_{25}H_{30}NO_{12}$ $(M + 1)^+$ 536.1768; found 536.1772.

[4-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-bromoacetic acid 2-cyanoethyl ester (6): To a solution of compound **5** (100 mg, 0.19 mmol) in 15 mL of CCl_4 was added NBS (36.6 mg, 0.21 mmol) and a catalytic amount of AIBN. The reaction flask was equipped with a condenser, and purged and filled with N_2 three times. It was irradiated with a 100W tungsten lamp for 1 h until white precipitate formed. The precipitate was filtered off and the filtrate concentrated. It was subjected to silica gel column chromatography eluted with EtOAc/$CHCl_3$ (2/8) to give the ester product **6** (105 mg, 92%) as a white foam. R_f = 0.33 (EtOAc/ $CHCl_3$ = 3/7). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.47 (d, J = 8.6 Hz, 2 H), 6.95 (d, J = 8.6 Hz, 2 H), 5.33 (s, 1 H), 5.27-5.23 (m, 2 H, H-2 + H-4), 5.18-5.07 (m, 2 H, H-1 + H-3), 4.35-4.31 (m, 2 H), 4.24 (dd, J = 11.8, 5.3 Hz, 1 H, H-6), 4.15 (dd, J = 11.8, 0.8 Hz, 1H, H-6'), 3.84 (m, 1 H, H-5), 2.70 (t, J = 6.3 Hz, 2 H), 2.04 (s, 3H), 2.01 (s, 3 H), 2.00 (s, 3 H), 2.00 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5 (C), 170.1 (C), 169.3 (C), 169.2 (C), 167.7 (C), 157.4 (C), 130.2 (CH), 129.7 (C), 117.1 (CH), 116.2 (C), 98.5 (CH), 72.5 (CH), 72.1 (CH), 71.0 (CH), 68.1 (CH), 61.8 (CH_2), 60.3 (CH_2), 45.0 (CH), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 17.7 (CH_2). IR (neat): 2982, 1753, 1510, 1385, 1221, 1037 cm^{-1}. HRMS calcd for $C_{25}H_{29}{}^{79}BrNO_{12}$ (M + 1) 614.0873; found 614.0872.

[4-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-hydroxyacetic acid 2-cyanoethyl ester (7): Compound **6** (150.0 mg, 0.24 mmol) was dissolved in 5 mL of acetone. A solution of $AgNO_3$ (83.0 mg, 0.48 mmol) and Ag_2CO_3 (33.1 mg, 0.12 mmol) in 5 mL of H_2O was added. A dark greenish precipitate was formed immediately. The reaction mixture was stirred at rt for 30 min. It was filtered and the filtrate was concentrated to dryness. The residual oil was dissolved in 50 mL of EtOAc and washed with 5% citric acid (×3), 10% $NaHCO_3$ (×3) and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The resulting oil was chromatographed on silica gel (EtOAc/ $CHCl_3$ = 4/6) to give compound **7** as a white foam (74.0 mg, 55%). R_f = 0.40 (EtOAc/ $CHCl_3$ = 6/4). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (d, J = 8.4 Hz, 2 H), 6.91 (d, J = 8.4 Hz, 2 H),

5.22-5.02 (m, 5 H), 4.28 (m, 1 H), 4.19-4.15 (m, 2 H), 4.07 (dd, *J* = 12.2, 1.7 Hz, 1 H, H-6), 3.83 (m, 1 H, H-5), 3.74 (d, *J* = 3.3 Hz, 1 H, OH), 2.61-2.54 (m, 2 H), 1.98 (s, 3 H), 1.96 (s, 3 H), 1.96 (s, 3 H), 1.94 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.6 (C), 170.4 (C), 170.0 (C), 169.3 (C), 169.2 (C), 156.8 (C), 132.5 (C), 127.8 (CH), 116.8 (CH), 116.3 (C), 98.6 (CH), 72.4 (CH), 72.1 (CH), 71.7 (CH), 70.8 (CH), 68.0 (CH), 61.7 (CH_2), 59.7 (CH_2), 20.5 (CH_3), 20.4 (CH_3), 20.4 (CH_3), 20.4 (CH_3), 17.6 (CH_2). IR (neat): 3481, 2969, 2259, 1746, 1517, 1372, 1227, 1182, 1044 cm^{-1}. HRMS calcd for $C_{25}H_{30}NO_{13}$ (M + 1) 552.1718; found 552.1729.

[4-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-fluoroacetic acid 2-cyanoethyl ester (8): To an ice-cooled solution of compound **7** (130.0 mg, 0.23 mmol) in 10 mL of CH_2Cl_2 was added 70 μL (0.46 mmol) of DAST. The reaction mixture was allowed to warm to rt and stirred for 15 h. It was quenched by adding small amount of silica gel and 1.5 mL of MeOH. It was concentrated and the fluorinated product **8** (110.0 mg, 85%) was obtained as a white foam after silica gel column chromatography eluted with EtOAc/$CHCl_3$ (2/8). R_f = 0.33 (EtOAc/ $CHCl_3$ = 3/7). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (d, *J* = 8.5 Hz, 2 H), 6.97 (d, *J* = 8.5 Hz, 2 H), 5.74 (d, *J* = 47.2 Hz, 1 H, C*H*F), 5.27-5.18 (m, 2 H), 5.12-5.06 (m, 2 H), 4.34 (m, 1 H), 4.31-4.17 (m, 2 H), 4.10 (d, J = 12.3 Hz, 1 H, H-6), 3.84 (m, 1 H, H-5), 2.71-2.61 (m, 2 H), 2.00 (s, 3 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.97 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3 (C), 170 (C), 169.2 (C), 169.1 (C), 167.8 (d, *J* = 28.8 Hz, C), 157.7 (C), 128.3 (d, J = 20.5 Hz, C), 128.2 (CH), 117.1 (CH), 116.1 (C), 98.5 (CH), 88.4 (d, *J* = 185.0 Hz, *C*HF), 72.4 (CH), 72.0 (CH), 70.9 (CH), 68.0 (CH), 61.7 (CH_2), 59.6 (CH_2), 20.5 (CH_3), 20.4 (CH_3), 20.4 (CH_3), 20.4 (CH_3), 17.7 (CH_2). ^{19}F NMR ($CDCl_3$) δ -179.0 (d, *J* = 49.6 Hz), -179.3 (d, *J* = 50.0 Hz). IR (neat): 2250, 1753, 1523, 1379, 1247, 1037 cm^{-1}. HRMS calcd for $C_{25}H_{29}O_{12}NF$ (M + 1) 554.1674; found 554.1678.

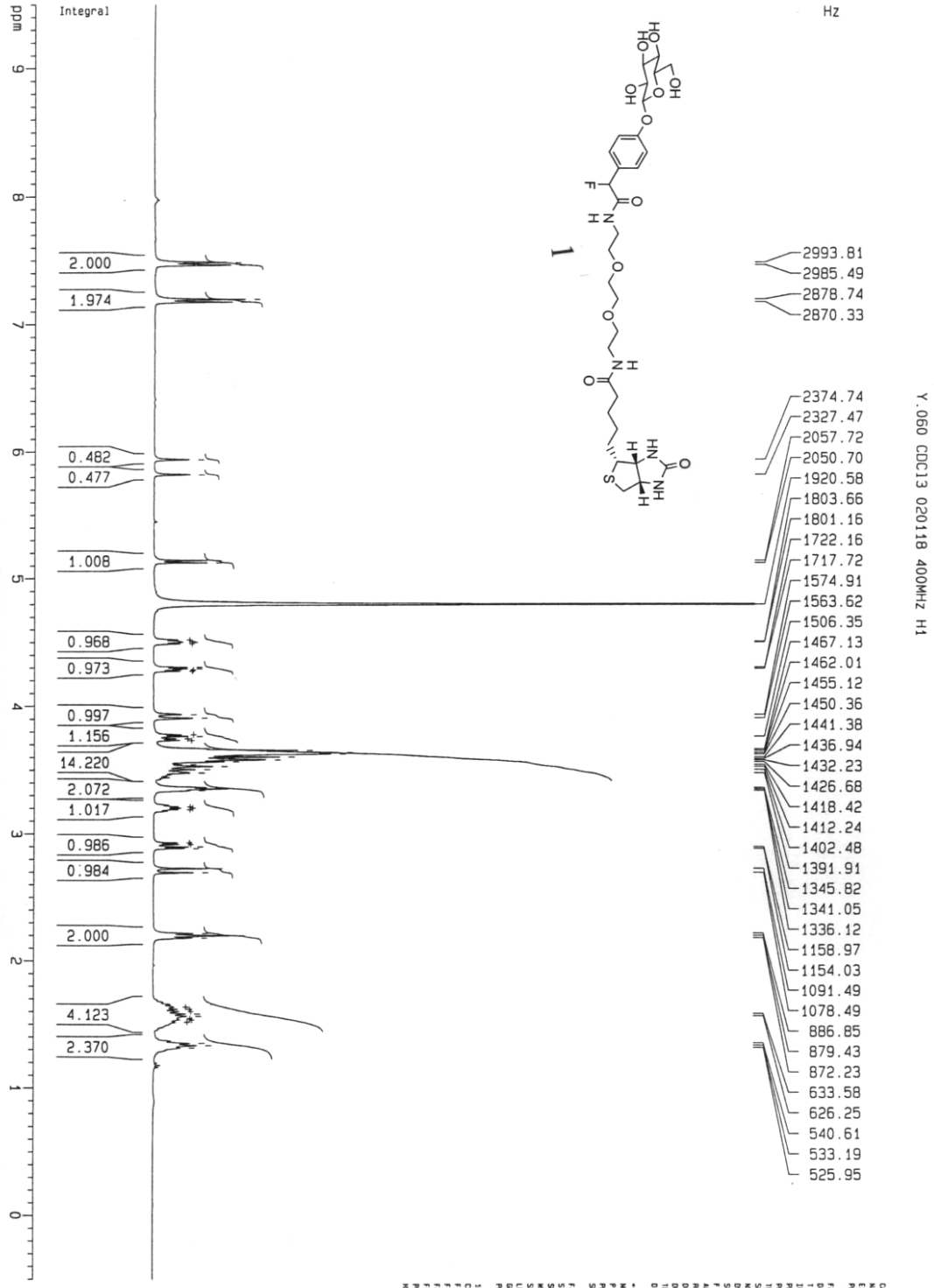
Triethylammonium [4-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-fluoro-acetate (9): To a solution of the fluoride **8** (130.0 mg, 0.23 mmol) in 5 mL of CH_2Cl_2 was added 50 μL (0.46 mmol) of DBU. The reaction mixture was stirred at rt for 30 min. EtOAc (50 mL) was then added, and it was washed with 5% citric acid (×3), H_2O (×1) and brine (×1). The organic phase was dried over anhydrous Na_2SO_4 and filtered. Since the α-fluoroacid was not stable, it was stored as the triethylammonium salt. This was achieved by adding 1 mL of TEA to the filtrate. The triethylammonium salt **9** was obtained as a solid (103.0 mg, 95%) after concentration. R_f = 0.25 (MeOH/$CHCl_3$ = 3/7), mp 111–113°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 (d, *J* = 8.5 Hz, 2 H), 6.84 (d, *J* = 8.5 Hz, 2 H), 5.53 (d, *J* = 50.4 Hz,

1 H, C*H*F), 5.23-5.14, (m, 2 H), 5.06 (dd, *J* = 11.2, 9.5 Hz, 1 H, H-3), 4.98 (d, *J* = 7.7 Hz, 1 H, H-1), 4.97 (d, *J* = 7.7 Hz, 1 H, H-1'), 4.19 (dd, *J* = 12.3, 5.5 Hz, 1 H, H-6), 4.06 (dd, *J* = 12.3, 2.4 Hz, 1 H, H-6'), 3.78 (m, 1 H, H-5), 2.97 (q, *J* = 7.5 Hz, 6 H), 1.98 (s, 3 H), 1.96 (s, 3 H), 1.96 (s, 3 H), 1.94 (s, 3 H), 1.16 (t, *J* = 7.5 Hz, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.2 (d, *J* = 22.0 Hz, C), 170.4 (C), 170.0 (C), 169.2 (C), 169.1 (C), 156.6 (C), 133.1 (d, *J* = 20.5 Hz, C), 128.0 (CH), 116.6 (CH), 99.0 (CH), 90.8 (d, *J* = 183.6 Hz, *C*HF), 72.5 (CH), 71.8 (CH), 71.0 (CH), 68.1 (CH), 61.8 (CH_2), 44.8 (CH_2), 20.5 (CH_3), 20.4 (CH_3), 20.4 (CH_3), 20.4 (CH_3), 8.2 (CH_3). ^{19}F NMR ($CDCl_3$) δ -170.2 (d, *J* = 53.6 Hz), -170.6 (d, *J* = 53.6 Hz). IR (neat): 3402, 1747, 1615, 1517, 1379, 1234, 1063, 1031 cm^{-1}. HRMS calcd for $C_{28}H_{41}FNO_{12}$ (M + TEA + 1) 602.2613; found 602.2624.

***N*-[4-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-fluoroacetyl-*N'*-biotinyl-3, 6-dioxaoctane-1,8-diamine (11):** To a solution of the triethylammonium salt of α-fluoroacid **9** (65.0 mg, 0.11 mmol) and compound **10** (61.0 mg, 0.13 mmol) in 3 mL of DMF was added HOBt (22.0 mg, 0.16 mmol), TEA (250 μL) and EDCI (31.1 mg, 0.16 mmol). The reaction mixture was stirred at rt for 24 h. DMF was then removed under high vacuum. The residual oil was dissolved in 40 mL of EtOAc. It was washed with 5% citric acid (×3), 10% $NaHCO_3$ (×3) and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The biotinylated product **11** (77.0 mg, 83%) was obtained as an oil after silica gel column chromatography eluted with MeOH/$CHCl_3$ (5/95). R_f = 0.33 (MeOH/$CHCl_3$ = 1/9). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, *J* = 8.3 Hz, 2 H), 7.26 (bs, 1H, NH), 6.95 (d, *J* = 8.3 Hz, 2 H), 6.72 (bs, 1 H, NH), 6.61 (bs, 1 H, NH), 5.87 (bs, 1 H, NH), 5.75 (d, *J* = 48.2 Hz, 1 H, C*H*F), 5.35-5.22 (m, 2 H), 5.20-5.12 (m, 2 H), 4.46 (bs, 1 H), 4.28-4.15 (m, 2 H), 4.09 (d, *J* = 11.9 Hz, 1 H, H-6), 3.92 (m, 1 H, H-5), 3.62-3.30 (m, 12 H), 3.05 (br, 1 H), 2.80 (dd, *J* = 12.6, 5.0 Hz, 1 H), 2.55 (d, *J* = 12.5 Hz, 1 H), 2.13 (dd, *J* = 7.4, 4.9 Hz, 2 H), 3.01 (s, 3 H), 1.98 (s, 3 H), 1.98 (s, 3 H), 1.97 (s, 3H), 1.68-1.48 (m, 4 H), 1.38-1.28 (m, 2 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3 (C), 170.5 (C), 170.1 (C), 169.3 (C), 169.2 (C), 168.6 (d, *J* = 22.1 Hz, C), 164.2 (C), 157.5 (C), 129.8 (d, *J* = 19.0 Hz, C), 128.4 (CH), 116.8 (CH), 98.6 (CH), 91.2 (d, *J* = 185.8 Hz, *C*HF), 72.5 (CH), 71.9 (CH), 70.9 (CH), 70.0 (CH_2), 69.9 (CH_2), 69.8 (CH_2), 69.3 (CH_2), 68.0 (CH), 61.7 (CH_2), 61.6 (CH), 60.1 (CH), 55.6 (CH), 40.3 (CH_2), 38.9 (CH_2), 38.8 (CH_2), 35.8 (CH_2), 28.2 (CH_2), 27.9 (CH_2), 25.5 (CH_2), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 20.5 (CH_3). ^{19}F NMR ($CDCl_3$) δ -175.4 (d, *J* = 51.2 Hz), -175.8 (d, *J* = 51.2 Hz). IR (neat): 3350, 2929, 1753, 1694, 1661, 1569, 1550, 1517, 1379, 1228, 1050. cm^{-1}. HRMS calcd for $C_{38}H_{54}FN_4O_{15}S$ (M + 1) 857.3291; found 857.3297.

***N*-Biotinyl-*N'*-[4-(β-D-glucopyranosyloxy)-phenyl]-fluoroacetyl-3,6-dioxaoctane-1,8-diamine (1):** To a solution of biotinylated compound **11** (72.0 mg, 0.084 mmol) in 5 mL of MeOH was added 35.6 mg of Na_2CO_3. The mixture was stirred at rt for 1 h. It was filtered through Celite and filtrate concentrated. The target product **1** was obtained as a white foam (53.0 mg, 92%) after silica gel column chromatography eluted with MeOH/$CHCl_3$ (3/7). R_f = 0.33 (MeOH/$CHCl_3$ = 3/7). ^{1}H NMR (D_2O, 400 MHz) δ 7.47 (d, *J* = 8.4 Hz, 2 H), 7.19 (d, *J* = 8.4 Hz, 2 H), 5.88 (d, *J* = 47.3 Hz, 1 H, C*H*F), 5.13 (d, *J* = 7.0 Hz, 1 H, H-1), 4.51 (dd, *J* = 7.4, 4.9 Hz, 1 H), 4.29 (dd, *J* = 4.4, 7.8 Hz, 1 H), 3.92 (d, *J* = 11.3 Hz, 1 H), 3.67 (dd, *J* = 12.4, 5.5 Hz, 1H), 3.70-3.43 (m, 14 H), 3.35 (t, *J* = 4.8 Hz, 2 H), 3.21 (m, 1 H), 2.91 (dd, *J* = 13.0, 4.9 Hz, 1 H), 2.71 (d, *J* = 13.0 Hz, 1 H), 2.20 (t, *J* = 7.3 Hz, 2 H), 1.70-1.45 (m, 4 H), 1.38-1.27 (m, 2 H). ^{13}C NMR (D_2O, 100 MHz) δ 176.7 (C), 171.3 (d, *J* = 24.0 Hz, C), 165.3 (C), 158.2 (C), 129.6 (CH), 129.3 (C), 117.1 (CH), 100.3 (CH), 91.2 (d, *J* = 183.0 Hz, *C*HF), 76.5 (CH), 75.9 (CH), 73.2 (CH), 69.8 (CH_2), 69.8 (CH_2), 69.8 (CH), 69.2 (CH_2), 69.0 (CH_2), 62.3 (CH), 60.9 (CH_2), 60.4 (CH), 55.7 (CH), 40.0 (CH_2), 39.2 (CH_2), 39.0 (CH_2), 35.7 (CH_2), 28.3 (CH_2), 28.0 (CH_2), 25.5 (CH_2). ^{19}F NMR (D_2O) δ -169.7 (d, *J* = 49.2 Hz), -169.8 (d, *J* = 50.4 Hz). IR (KBr): 3380, 2939, 2883, 1698, 1549, 1512, 1462, 1239, 1083, 1059 cm^{-1}. HRMS calcd for $C_{30}H_{46}FN_4O_{11}S$ (M + 1) 689.2868; found 689.2885.

Labeling of β-glucosidase with probe 1: Purified β-glucosidase (2.0 μg) was incubated with 1 mM of probe **1** in 50 mM of phosphate buffer (pH 7.0) at 37^{o}C for 30 min. The reaction products were separated by 8% SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. The membrane was blocked with 10% nonfat dry milk, washed with TTBS (0.05% Tween-20, 20 mM Tris pH 7.6, 137 mM NaCl), and treated with a streptavidin-horseradish peroxidase conjugate (Amersham-Phamacia, 1:2000 dilution) in TTBS containing 1% nonfat dry milk for 1 h at 25^{o}C. Visualization of bound streptavidin-horseradish peroxidase conjugate was achieved by treating the membrane with ECL chemiluminescence reagents (Amersham-Phamacia) and exposed to film for 1-30 min before development.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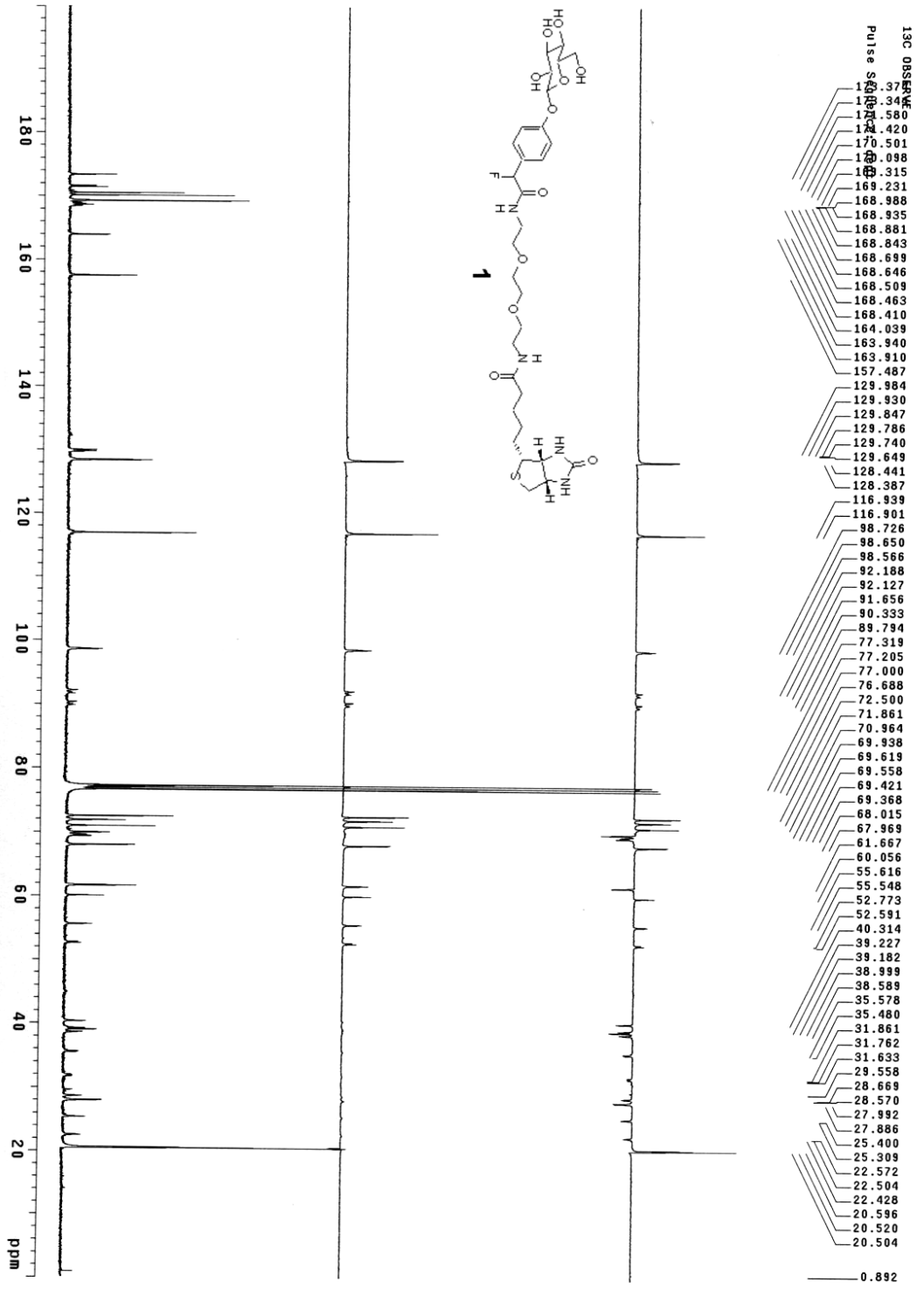
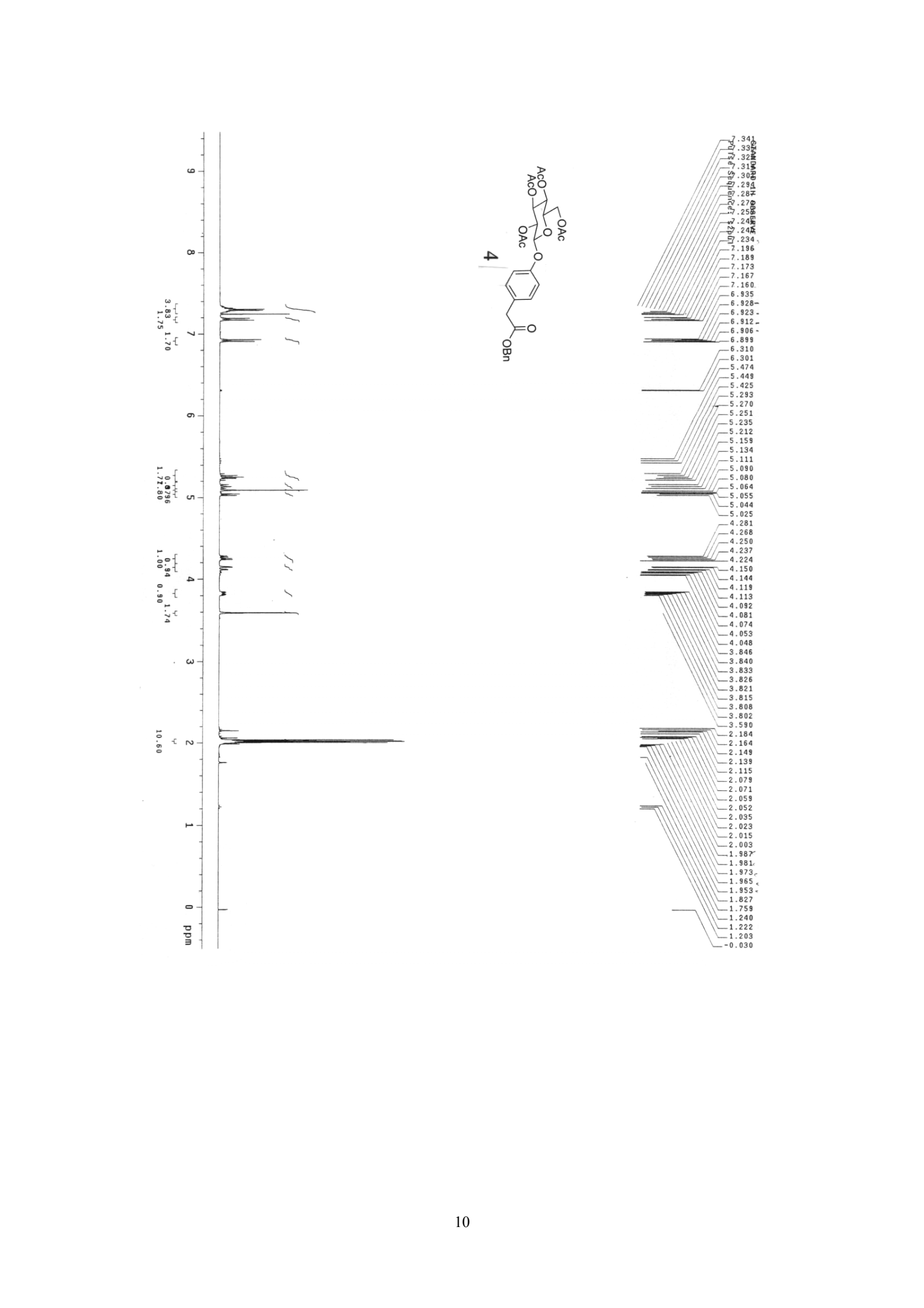
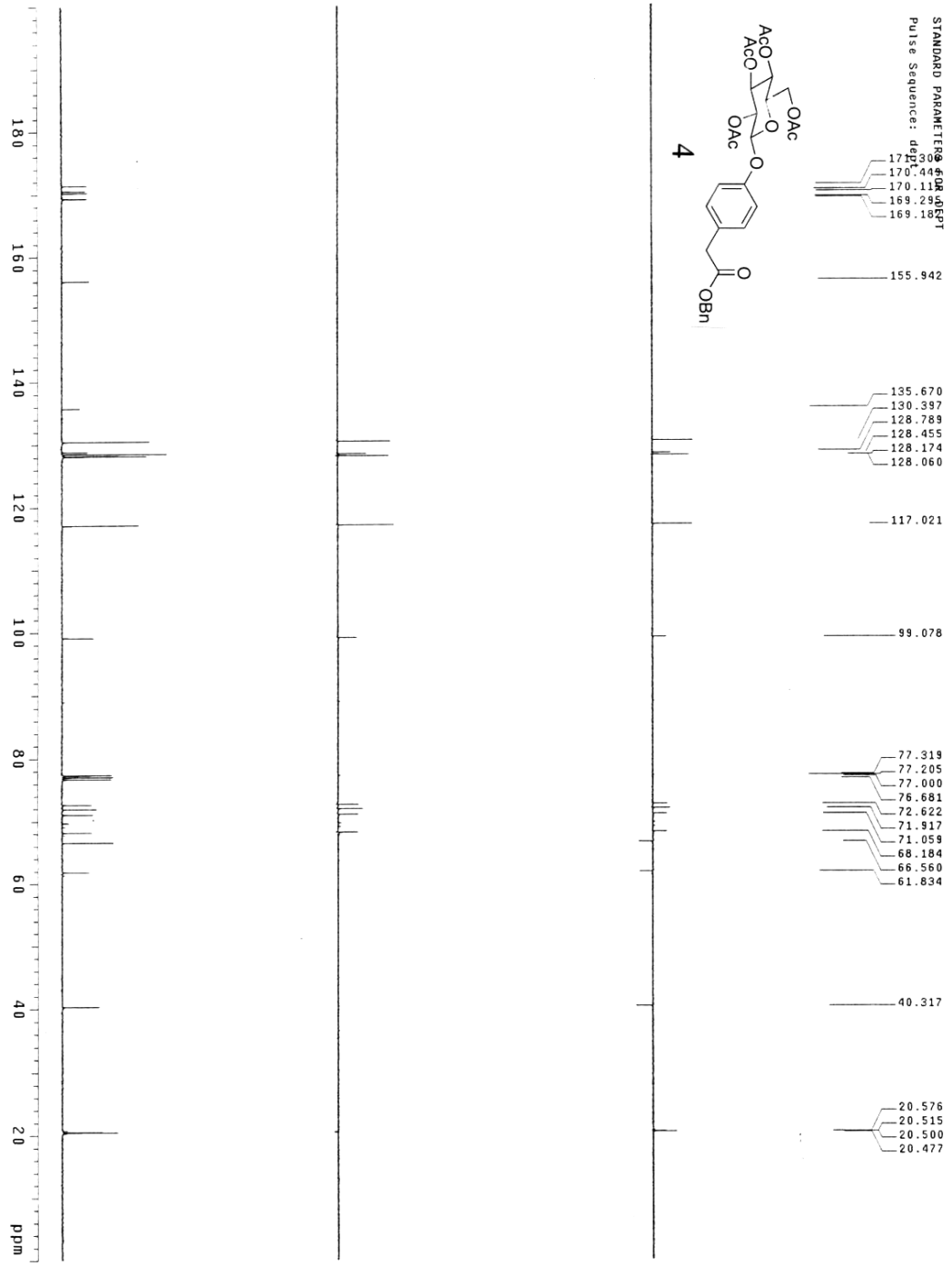
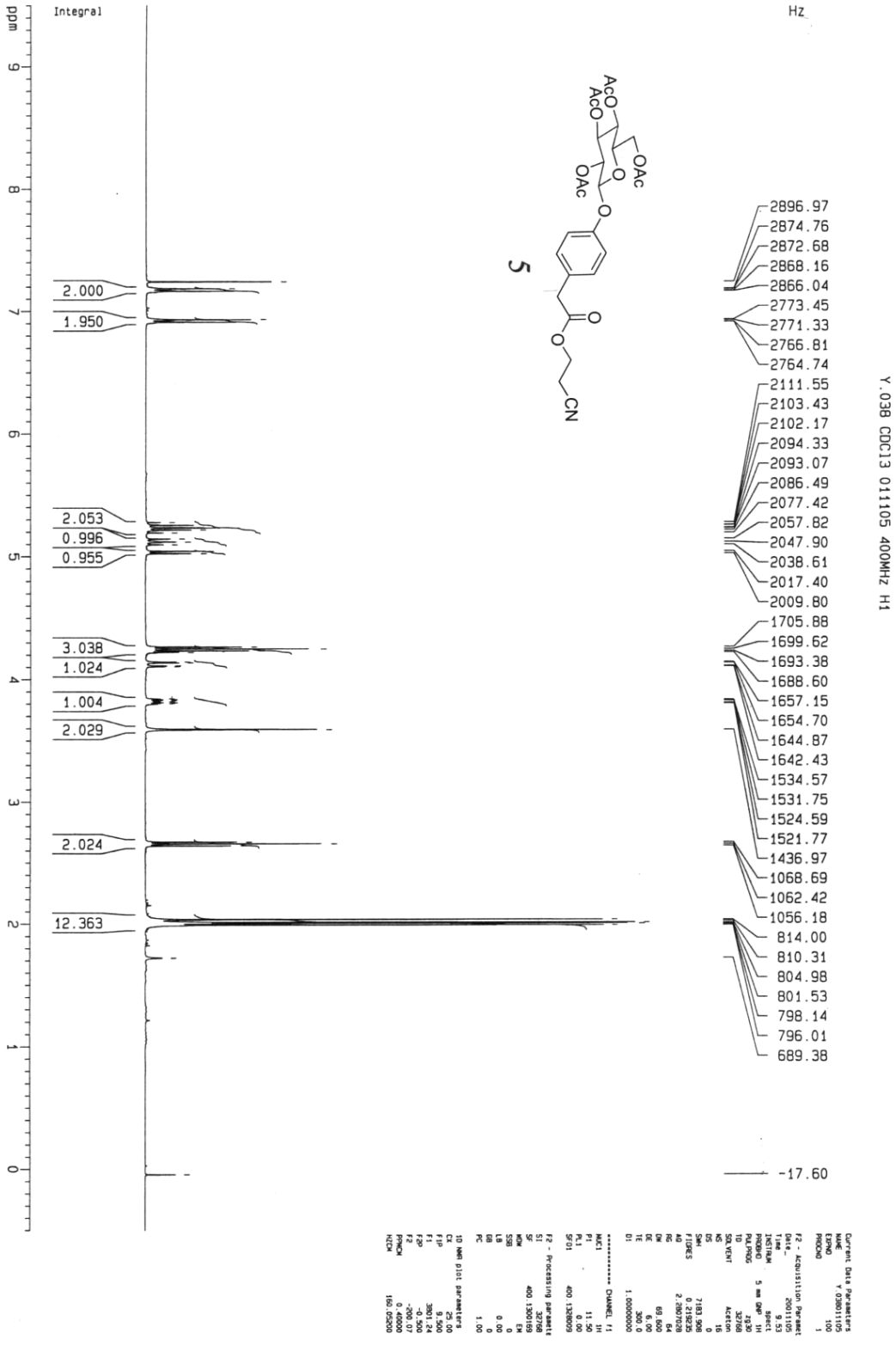
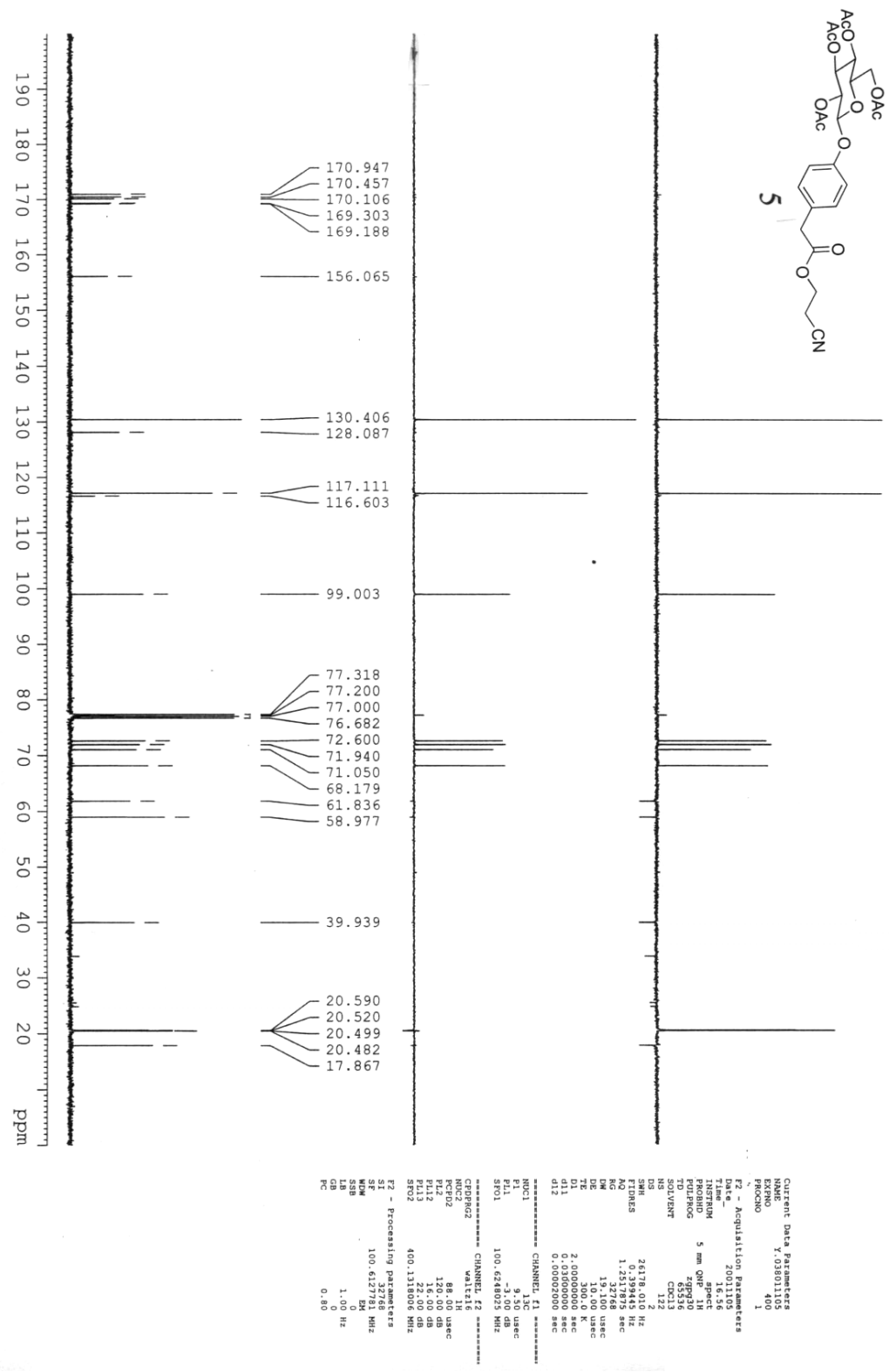
2374.74
2327.47
2057.72
2050.70
1920.58
1803.66
1801.16
1722.16
1717.72
1574.91
1563.62
1506.35
1467.13
1462.01
1455.12
1450.36
1441.38
1436.94
1432.23
1426.68
1418.42
1412.24
1402.48
1391.91
1345.82
1341.05
1336.12
1158.97
1154.03
1091.49
1078.49
886.85
879.43
872.23
633.58
626.25
540.61
533.19
525.95
Y.060 CDCl3 020118 400MHz H1

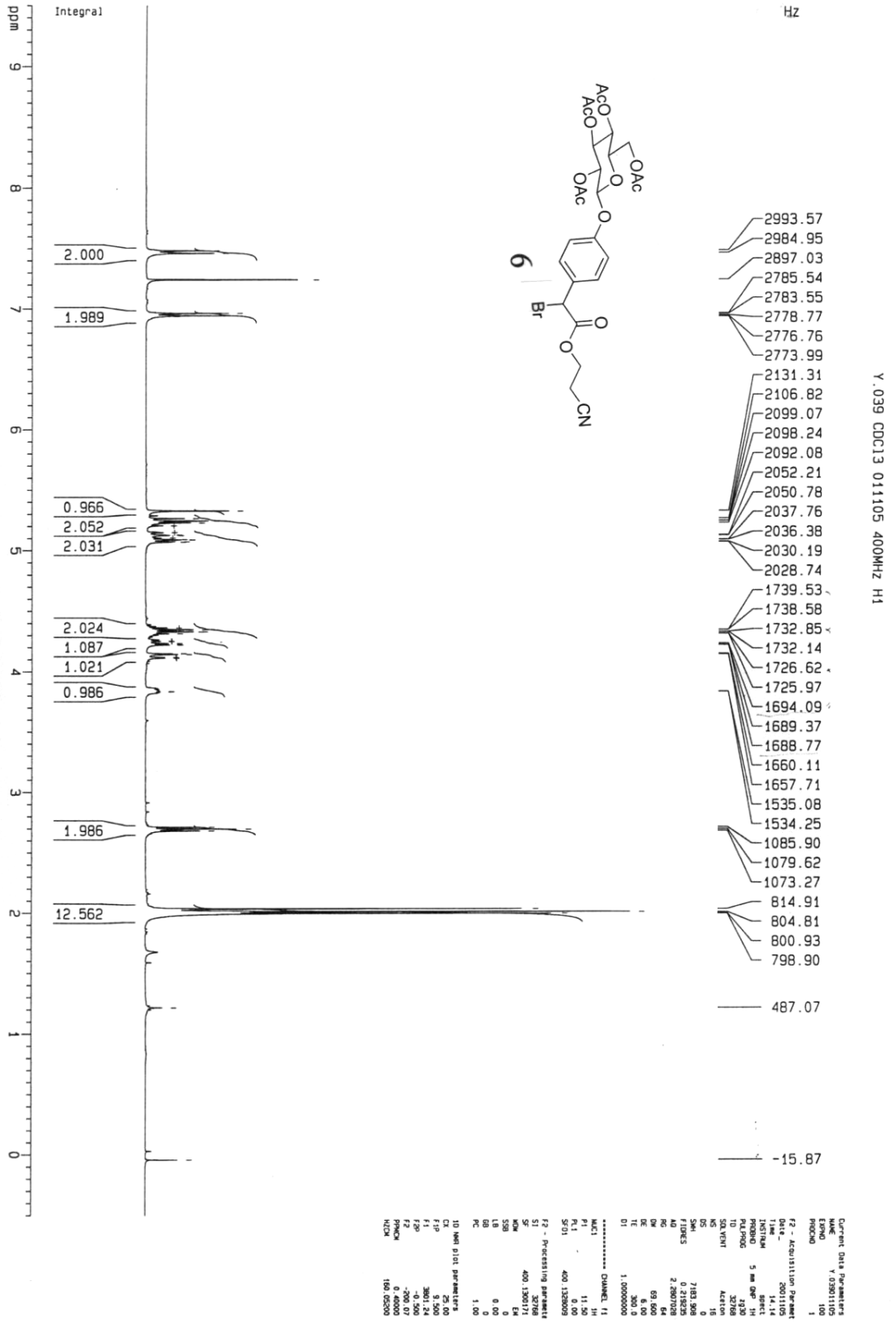

13C OBSERVE
Pulse Sequence
1
ppm
180
160
140
120
100
80
60
40
20
170.501
169.231
168.988
168.935
168.881
168.843
168.699
168.646
168.509
168.463
168.410
164.039
163.940
163.910
157.487
129.984
129.930
129.847
129.786
129.740
129.649
128.441
128.387
116.939
116.901
98.726
98.650
98.566
92.188
92.127
91.656
90.333
89.794
77.319
77.205
77.000
76.688
72.500
71.861
70.964
69.938
69.619
69.558
69.421
69.368
68.015
67.969
61.667
60.056
55.616
55.548
52.773
52.591
40.314
39.227
39.182
38.999
38.589
35.578
35.480
31.861
31.762
31.633
29.558
28.669
28.570
27.992
27.886
25.400
25.309
22.572
22.504
22.428
20.596
20.520
20.504
0.892


4
AcO
AcO
AcO
OAc
O
O
OBn
7.341
7.333
7.327
7.319
7.305
7.294
7.285
7.276
7.258
7.245
7.234
7.196
7.189
7.173
7.167
7.160
6.935
6.928
6.923
6.912
6.906
6.899
6.310
6.301
5.474
5.449
5.425
5.293
5.270
5.251
5.235
5.212
5.159
5.134
5.111
5.090
5.080
5.064
5.055
5.044
5.025
4.281
4.268
4.250
4.237
4.224
4.150
4.144
4.119
4.113
4.092
4.081
4.074
4.053
4.048
3.846
3.840
3.833
3.826
3.821
3.815
3.808
3.802
3.590
2.184
2.164
2.149
2.139
2.115
2.079
2.071
2.059
2.052
2.035
2.023
2.015
2.003
1.987
1.981
1.973
1.965
1.953
1.827
1.759
1.240
1.222
1.203
-0.030
3.83
1.75
1.70
1.78
1.80
0.96
1.00
0.94
0.90
1.74
10.60
9
8
7
6
5
4
3
2
1
0
ppm

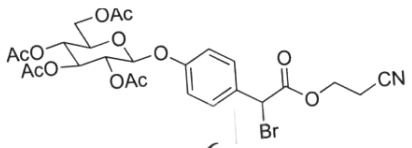

STANDARD PARAMETERS FOR DEPT
Pulse Sequence: dept
4
AcO
AcO
AcO
OAc
O
OAc
O
O
OBn
171.308
170.446
170.118
169.295
169.181
155.942
135.670
130.397
128.789
128.455
128.174
128.060
117.021
99.078
77.319
77.205
77.000
76.681
72.622
71.917
71.059
68.184
66.560
61.834
40.317
20.576
20.515
20.500
20.477
180
160
140
120
100
80
60
40
20
ppm


Integral
ppm
2.000
1.950
2.053
0.996
0.955
3.038
1.024
1.004
2.029
2.024
12.363
AcO
AcO
OAc
OAc
O
O
O
O
CN
5
Hz
2896.97
2874.76
2872.68
2868.16
2866.04
2773.45
2771.33
2766.81
2764.74
2111.55
2103.43
2102.17
2094.33
2093.07
2086.49
2077.42
2057.82
2047.90
2038.61
2017.40
2009.80
1705.88
1699.62
1693.38
1688.60
1657.15
1654.70
1644.87
1642.43
1534.57
1531.75
1524.59
1521.77
1436.97
1068.69
1062.42
1056.18
814.00
810.31
804.98
801.53
798.14
796.01
689.38
-17.60
Y.038 CDCl3 011105 400MHz H1

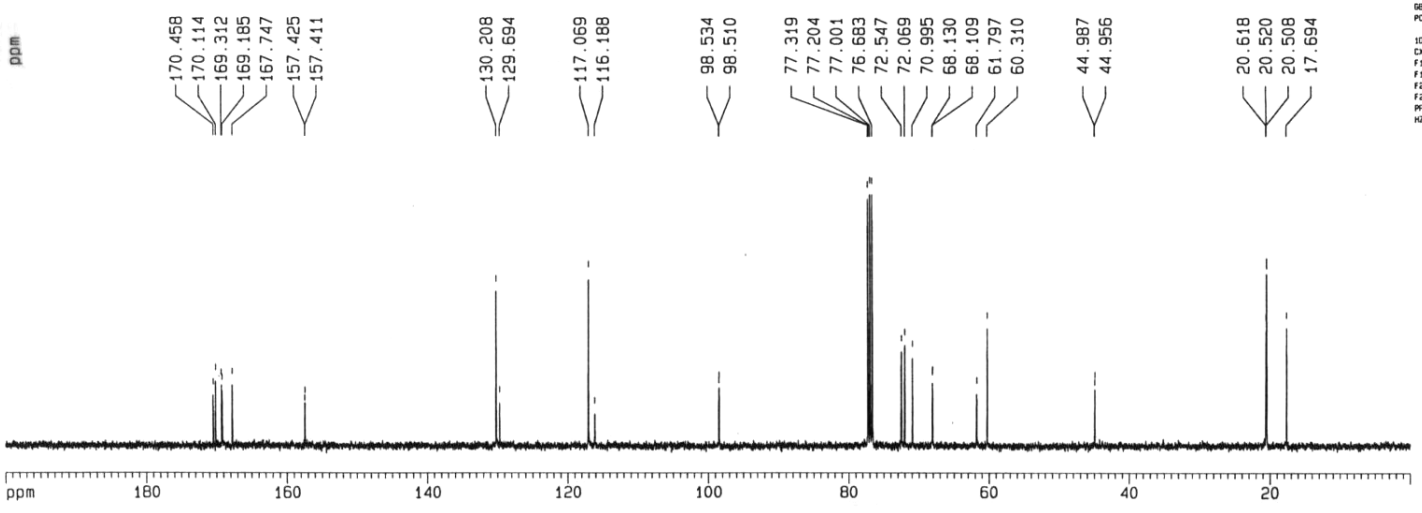

AcO
AcO
AcO
OAc
O
O
OAc
O
O
O
CN
5
170.947
170.457
170.106
169.303
169.188
156.065
130.406
128.087
117.111
116.603
99.003
77.318
77.200
77.000
76.682
72.600
71.940
71.050
68.179
61.836
58.977
39.939
20.590
20.520
20.499
20.482
17.867
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
Current Data Parameters
NAME 5
EXPNO 400
PROCNO 1
F2 - Acquisition Parameters
Date_ 20111115
Time_ 16.56
INSTRUM spect
PROBHD 5 mm QNP 1H
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 122
DS 2
SWH 26178.010 Hz
FIDRES 0.399431 Hz
AQ 1.2517875 sec
RG 32768
DW 19.100 usec
DE 10.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
d12 0.00002000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 9.50 usec
PL1 -3.00 dB
SFO1 100.6248025 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 88.00 usec
PL2 120.00 dB
PL12 16.00 dB
PL13 22.00 dB
SFO2 400.1318006 MHz
F2 - Processing parameters
SI 32768
SF 100.6127781 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 0.80


Y.039 CDCl3 011105 400MHz H1
Hz
Integral
ppm
2993.57
2984.95
2897.03
2785.54
2783.55
2778.77
2776.76
2773.99
2131.31
2106.82
2099.07
2098.24
2092.08
2052.21
2050.78
2037.76
2036.38
2030.19
2028.74
1739.53
1738.58
1732.85
1732.14
1726.62
1725.97
1694.09
1689.37
1688.77
1660.11
1657.71
1535.08
1534.25
1085.90
1079.62
1073.27
814.91
804.81
800.93
798.90
487.07
-15.87
2.000
1.989
0.966
2.052
2.031
2.024
1.087
1.021
0.986
1.986
12.562
6
Br
AcO
AcO
OAc
OAc
O
O
O
O
CN

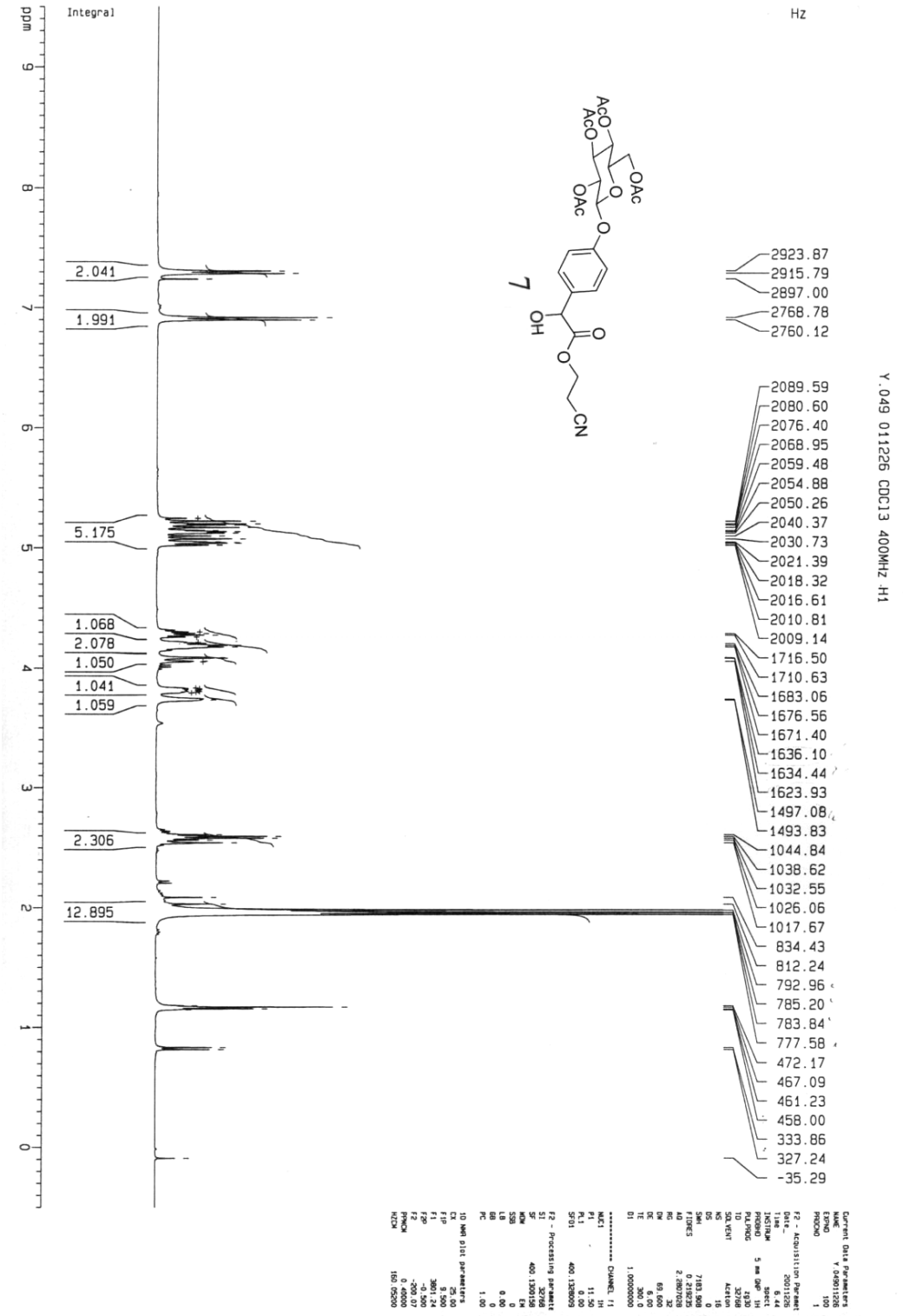
Y.039 CDCl3 011105 400MHz C13 (250p to -10p)

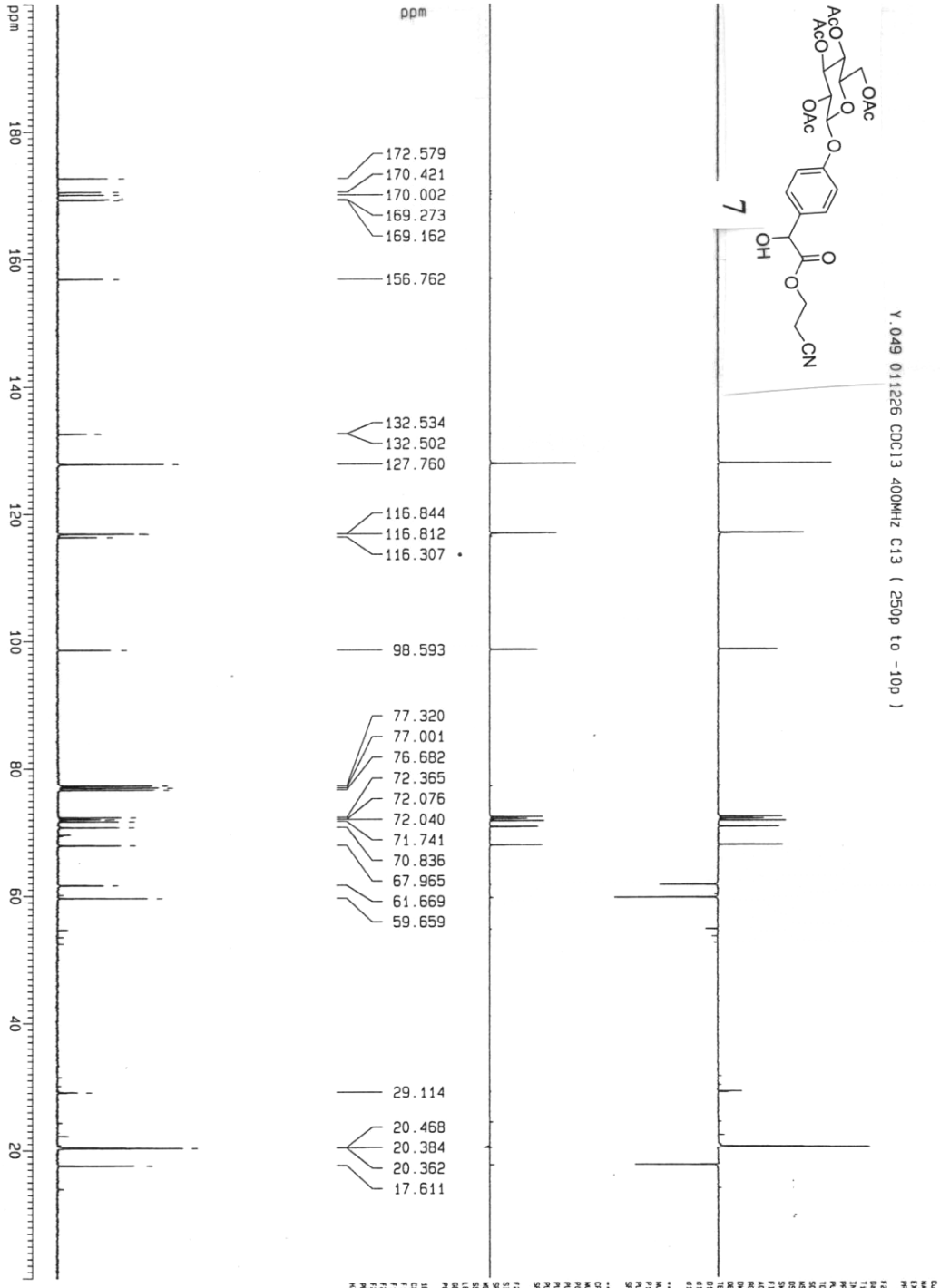


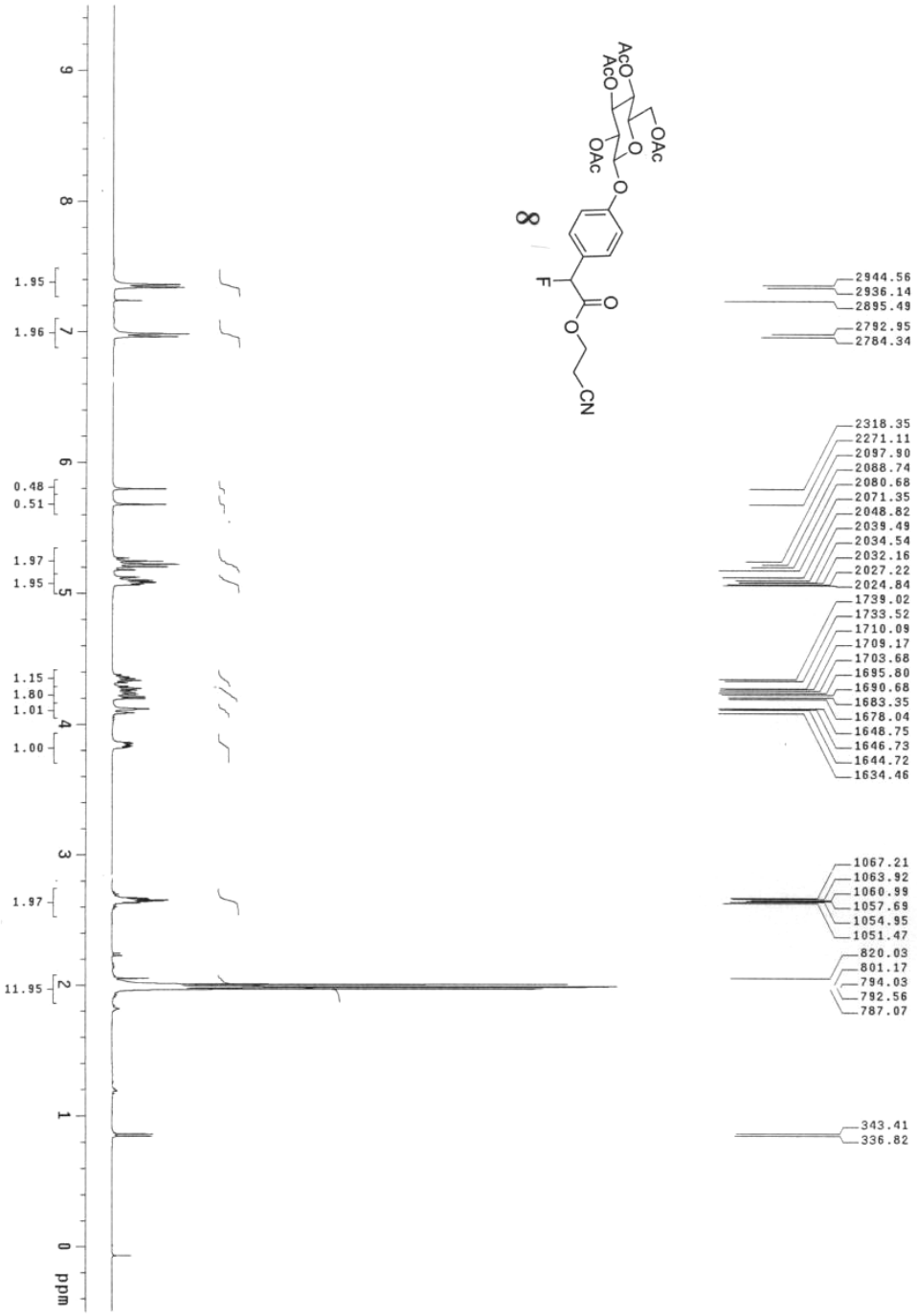
6



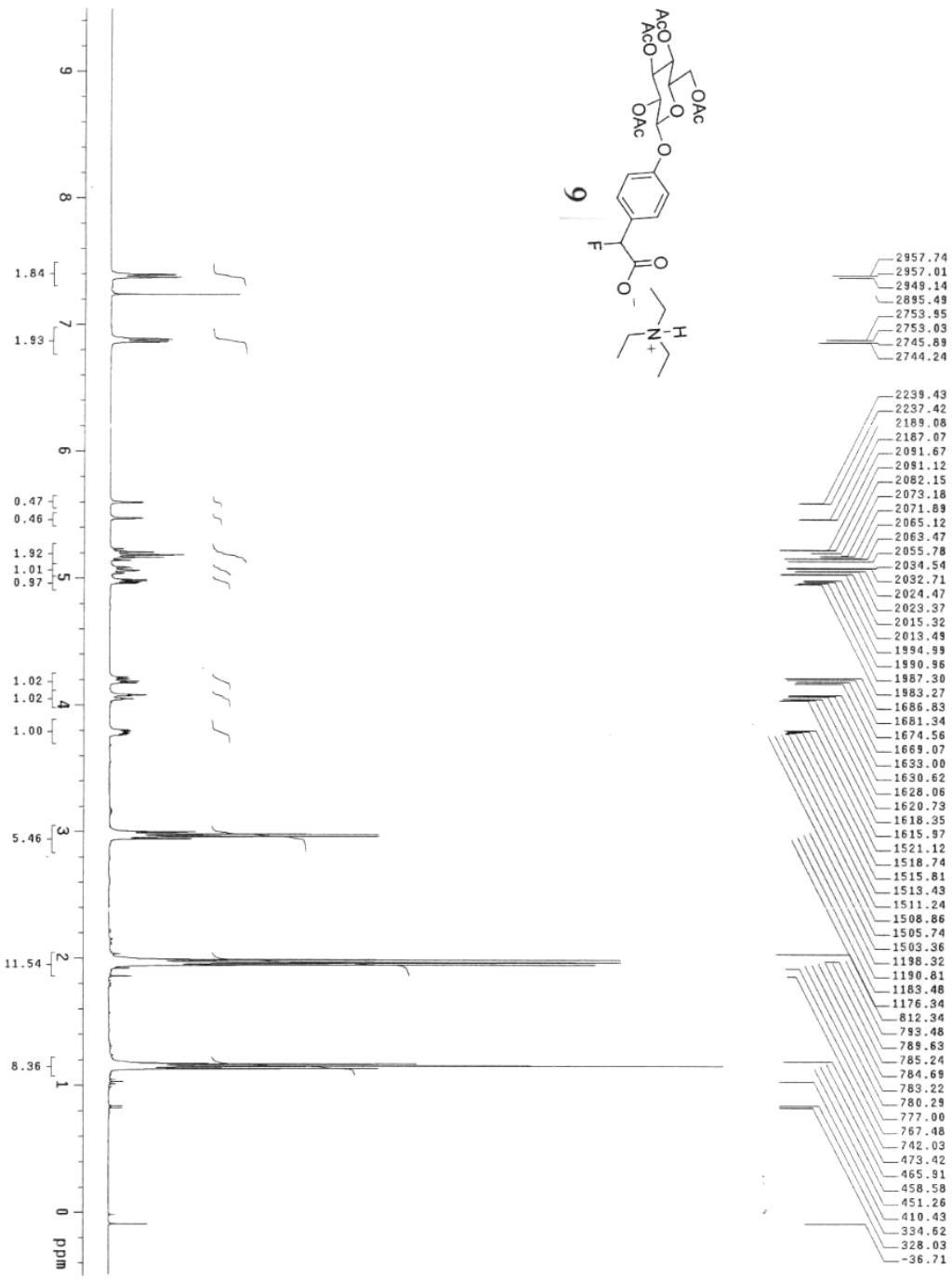

```
Current Data Parameters
NAME                Y.039011105
EXPNO                       400
PROCNO                        1

F2 - Acquisition Paramet
Date_              20011105
Time                   14.17
INSTRUM                spect
PROBHD     5 mm QNP  1H
PULPROG             zgpg30
TD                     65536
SOLVENT                CDCl3
NS                       278
DS                         2
SWH                26178.010
FIDRES             0.399445
AQ                 1.2517875
RG                     32768
DW                     19.100
DE                     10.00
TE                     300.0
D1                 2.00000000
d11                0.03000000
d12                0.00002000

============ CHANNEL f1
NUC1                      13C
P1                        9.50
PL1                      -3.00
SFO1             100.6248025

============ CHANNEL f2
CPDPRG2                waltz16
NUC2                       1H
PCPD2                     88.00
PL2                      120.00
PL12                      16.00
PL13                      22.00
SFO2             400.1318006

F2 - Processing paramete
SI                         32768
SF                100.6127757
WDW                           EM
SSB                            0
LB                          1.00
GB                             0
PC                          0.80

1D NMR plot parameters
CX                         25.00
F1P                       200.000
F1                      20122.55
F2P                       0.000
F2                         0.00
PPMCM                   8.00000
HZCM                  804.90222
```

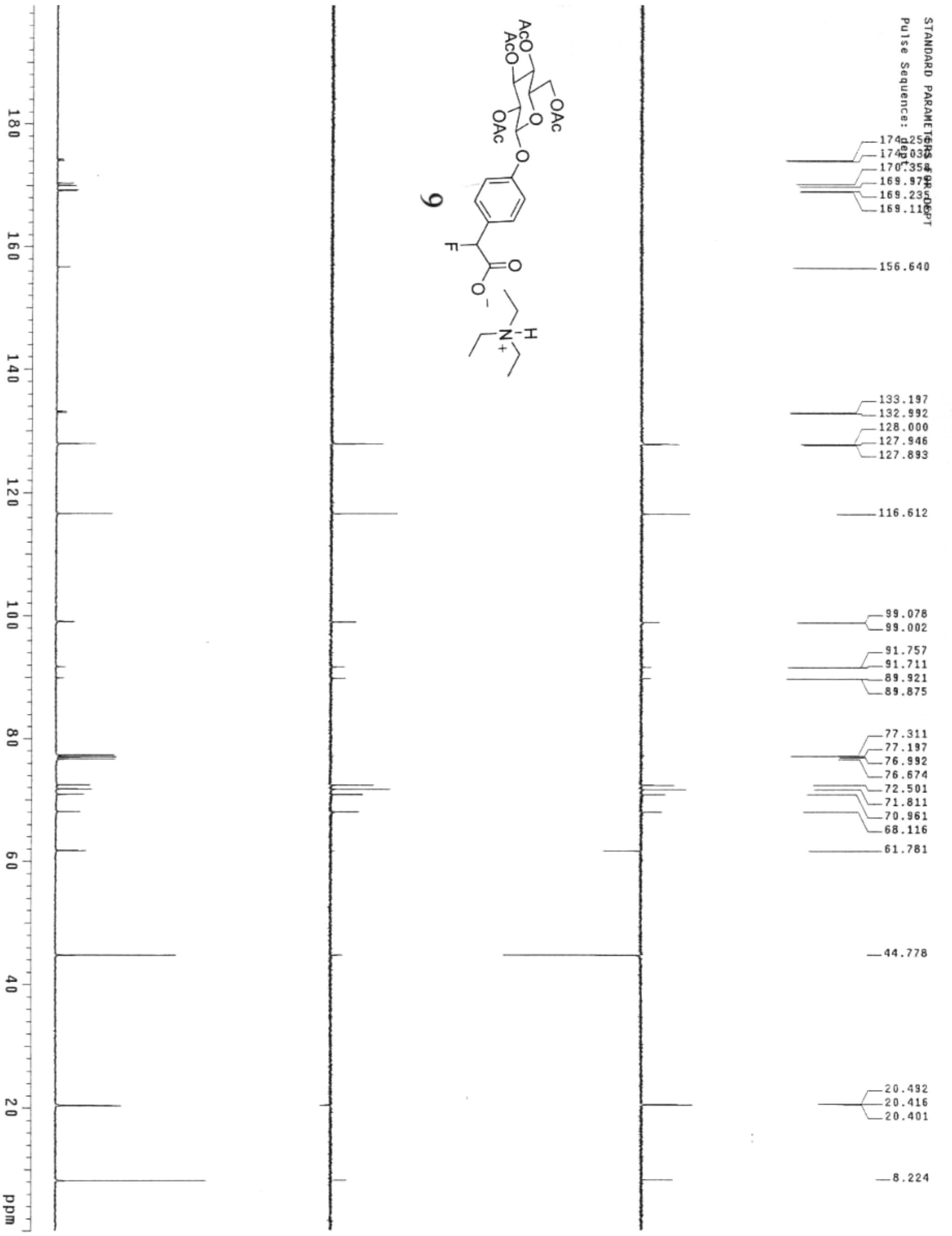

Integral
Hz
ppm
2.041
1.991
5.175
1.068
2.078
1.050
1.041
1.059
2.306
12.895
AcO
AcO
OAc
OAc
O
O
7
OH
O
O
CN
2923.87
2915.79
2897.00
2768.78
2760.12
2089.59
2080.60
2076.40
2068.95
2059.48
2054.88
2050.26
2040.37
2030.73
2021.39
2018.32
2016.61
2010.81
2009.14
1716.50
1710.63
1683.06
1676.56
1671.40
1636.10
1634.44
1623.93
1497.08
1493.83
1044.84
1038.62
1032.55
1026.06
1017.67
834.43
812.24
792.96
785.20
783.84
777.58
472.17
467.09
461.23
458.00
333.86
327.24
-35.29
Y.049 011226 CDCl3 400MHz H1

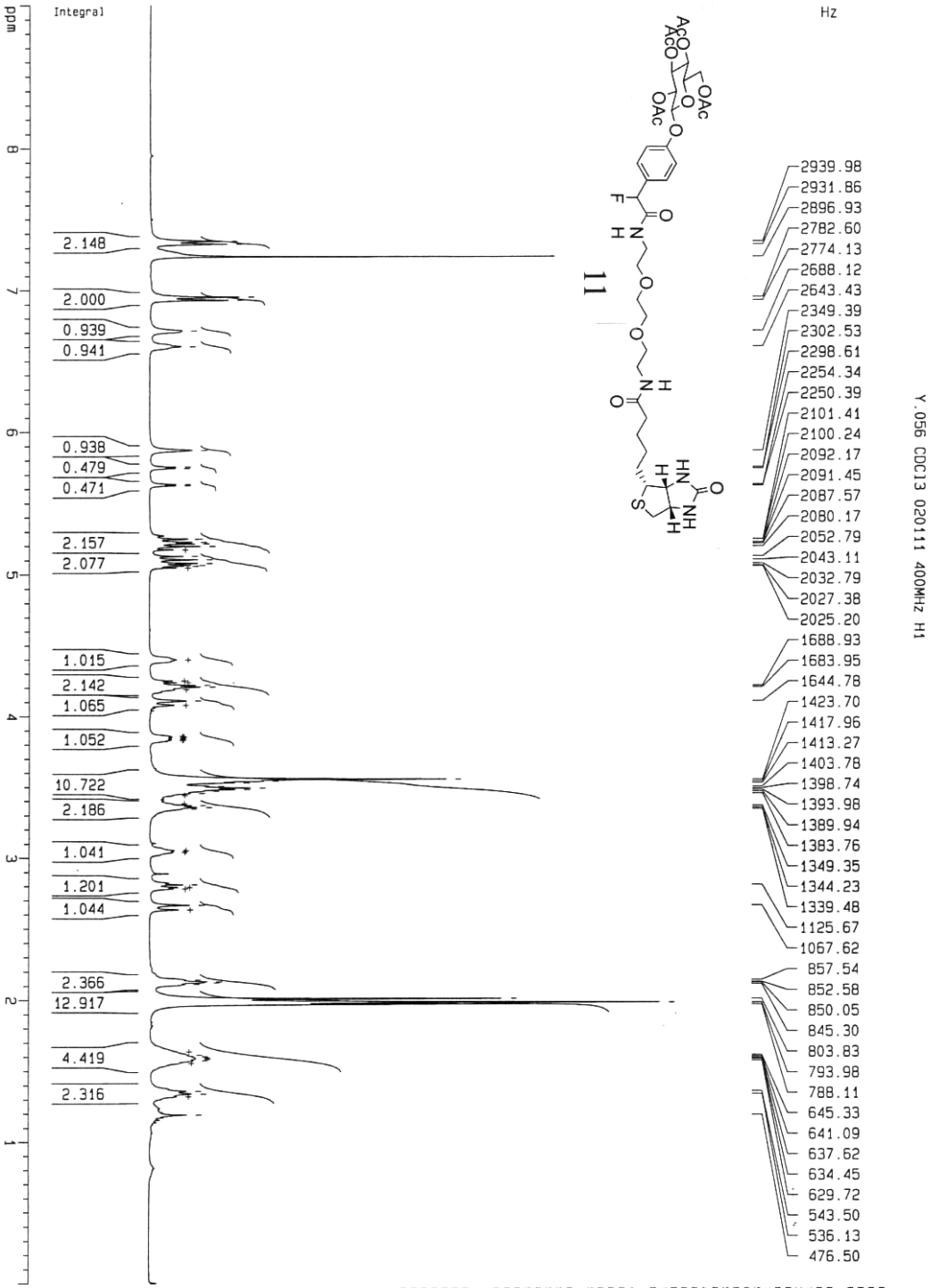

Y.049 011226 CDCl3 400MHz C13 (250p to -10p)
OAc
AcO
AcO
OAc
O
O
OH
O
O
CN
7
ppm
172.579
170.421
170.002
169.273
169.162
156.762
132.534
132.502
127.760
116.844
116.812
116.307
98.593
77.320
77.001
76.682
72.365
72.076
72.040
71.741
70.836
67.965
61.669
59.659
29.114
20.468
20.384
20.362
17.611
ppm
180
160
140
120
100
80
60
40
20

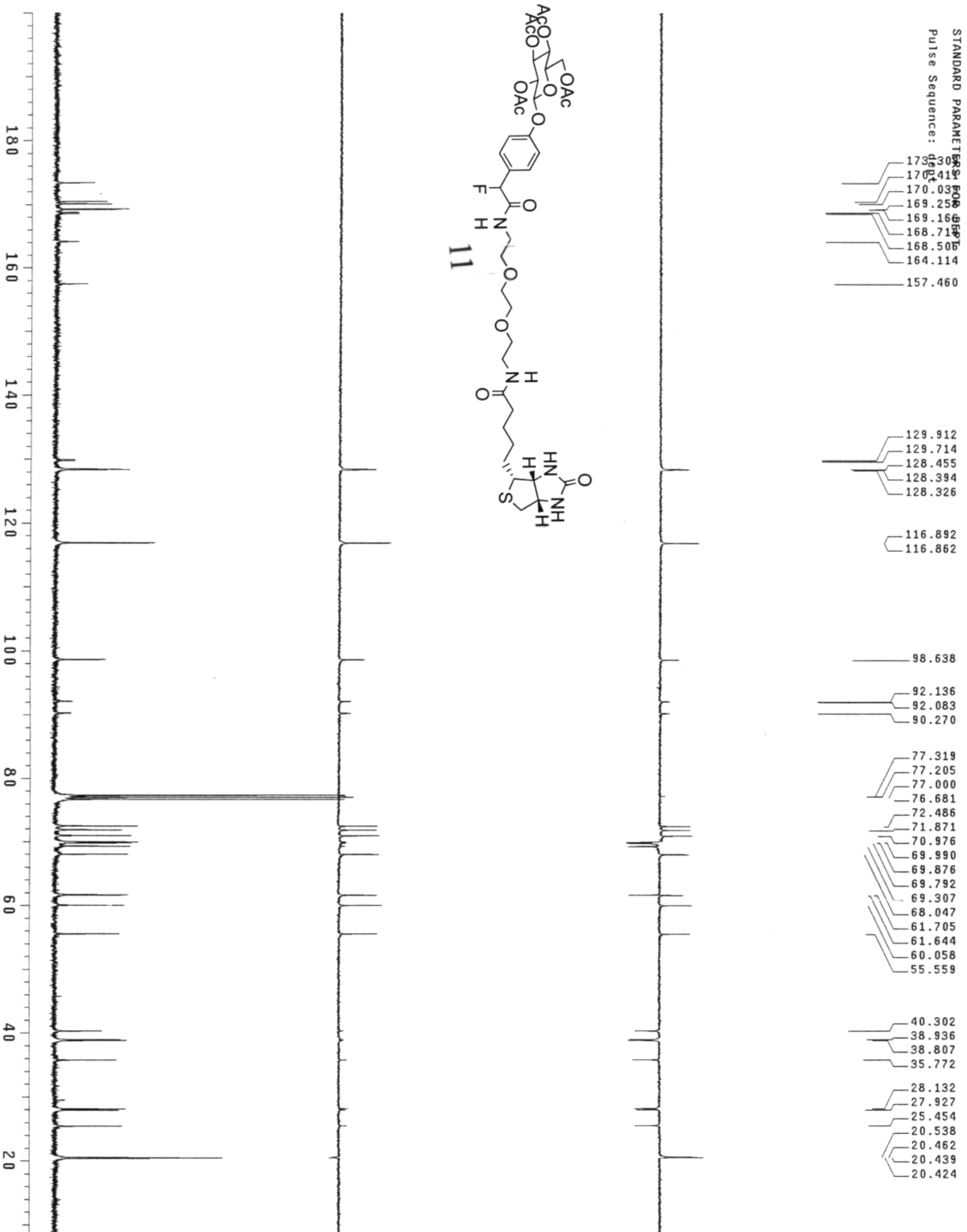

8
AcO
AcO
AcO
OAc
OAc
O
O
F
O
O
CN
1.95
1.96
0.48
0.51
1.97
1.95
1.15
1.80
1.01
1.00
1.97
11.95
ppm
2944.56
2936.14
2895.49
2792.95
2784.34
2318.35
2271.11
2097.90
2088.74
2080.68
2071.35
2048.82
2039.49
2034.54
2032.16
2027.22
2024.84
1739.02
1733.52
1710.09
1709.17
1703.68
1695.80
1690.68
1683.35
1678.04
1648.75
1646.73
1644.72
1634.46
1067.21
1063.92
1060.99
1057.69
1054.95
1051.47
820.03
801.17
794.03
792.56
787.07
343.41
336.82

STANDARD PARAMETERS FOR DEPT
Pulse Sequence: dept
170.322
169.976
169.225
169.094
167.961
167.672
157.756
157.733
128.364
128.303
128.273
128.242
128.212
128.159
117.067
116.141
98.494
98.464
89.336
89.276
87.485
87.424
77.319
77.000
76.681
72.433
71.962
70.915
68.047
61.727
59.649
22.351
20.492
20.416
20.401
17.677
AcO
AcO
OAc
OAc
O
O
F
O
O
CN
8
180
160
140
120
100
80
60
40
20
ppm


9
1.84
1.93
0.47
0.46
1.92
1.01
0.97
1.02
1.02
1.00
5.46
11.54
8.36
ppm
2957.74
2957.01
2949.14
2895.49
2753.95
2753.03
2745.89
2744.24
2239.43
2237.42
2189.08
2187.07
2091.67
2081.12
2082.15
2073.18
2071.89
2065.12
2063.47
2055.78
2034.54
2032.71
2024.47
2023.37
2015.32
2013.49
1994.99
1990.96
1987.30
1983.27
1686.83
1681.34
1674.56
1669.07
1633.00
1630.62
1628.06
1620.73
1618.35
1615.97
1521.12
1518.74
1515.81
1513.43
1511.24
1508.86
1505.74
1503.36
1198.32
1190.81
1183.48
1176.34
812.34
793.48
789.63
785.24
784.69
783.22
780.29
777.00
767.48
742.03
473.42
465.91
458.58
451.26
410.43
334.62
328.03
-36.71


STANDARD PARAMETERS FOR DEPT
Pulse Sequence: dept
AcO
AcO
OAc
OAc
O
F
O
O⁻
H
N+
9
170.358
169.975
169.235
169.110
156.640
133.197
132.992
128.000
127.946
127.893
116.612
99.078
99.002
91.757
91.711
89.921
89.875
77.311
77.197
76.992
76.674
72.501
71.811
70.961
68.116
61.781
44.778
20.492
20.416
20.401
8.224
180
160
140
120
100
80
60
40
20
ppm


Y.056 CDCl3 020111 400MHz H1
Integral
Hz
ppm
2.148
2.000
0.939
0.941
0.938
0.479
0.471
2.157
2.077
1.015
2.142
1.065
1.052
10.722
2.186
1.041
1.201
1.044
2.366
12.917
4.419
2.316
11
2939.98
2931.86
2896.93
2782.60
2774.13
2688.12
2643.43
2349.39
2302.53
2298.61
2254.34
2250.39
2101.41
2100.24
2092.17
2091.45
2087.57
2080.17
2052.79
2043.11
2032.79
2027.38
2025.20
1688.93
1683.95
1644.78
1423.70
1417.96
1413.27
1403.78
1398.74
1393.98
1389.94
1383.76
1349.35
1344.23
1339.48
1125.67
1067.62
857.54
852.58
850.05
845.30
803.83
793.98
788.11
645.33
641.09
637.62
634.45
629.72
543.50
536.13
476.50


STANDARD PARAMETERS FOR DEPT
Pulse Sequence: dept
169.166
168.506
164.114
157.460
129.912
129.714
128.455
128.394
128.326
116.892
116.862
98.638
92.136
92.083
90.270
77.319
77.205
77.000
76.681
72.486
71.871
70.976
69.990
69.876
69.792
69.307
68.047
61.705
61.644
60.058
55.559
40.302
38.936
38.807
35.772
28.132
27.927
25.454
20.538
20.462
20.439
20.424
11
180
160
140
120
100
80
60
40
20
ppm