Supporting information

Design, Synthesis and Properties of Boat-Shaped Glucopyranosyl Nucleic Acid

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Contents

General Procedures. Dichloromethane, DMF and pyridine were distilled from CaH_2 and the other reagents used as received from commercial suppliers. Melting point was measured with a Yanagimoto micro melting point apparatus and is uncorrected. ^{1}H-NMR (400 MHz), ^{13}C-NMR (100.5 MHz) and ^{31}P-NMR (161.8 MHz) were recorded on JEOL JNM-ECS-400 spectrometers. Chemical shift are reported in parts per million referenced to internal tetramethylsilane (0.00 ppm), residual $CHCl_3$ (7.26 ppm) or methanol (3.31 ppm) for ^{1}H-NMR, and chloroform-d_1 (77.16 ppm) or methanol-d_4 (49.00 ppm) for ^{13}C-NMR. Relative to 85% H_3PO_4 as external standard for ^{31}P-NMR. IR spectra were recorded on a JASCO FT/IR-4200 spectrometers. Optical rotations were recorded on a JASCO DIP-370 instrument. Mass spectra were measured on JEOL JMS-700 mass spectrometers. MALDI-TOF mass spectra were recorded on a Bruker Daltonics Autoflex II TOF/TOF mass spectrometer. For column chromatography, Fuji Silysia PSQ-100B or FL-100D silica gel was used. For high performance liquid chromatography (HPLC), SHIMADZU LC-6AD, SPD-10AV$_{VP}$ and CTO-10A$_{VP}$ were used. Thermal denaturation experiments were carried out on SHIMADZU UV-1650 and UV-1800 spectrometers equipped with a T_m analysis accessory.

1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-5-*C*-(hydroxymethyl)-β-D-glucopyranose (2). Compound **1** (2.56 g, 5.51 mmol)[1] was dissolved in ethanol (100 mL) and CH_2Cl_2 (40 mL). The solution was cooled to −78 ˚C. Ozone was bubbled through the solution until appearance of a pale blue color (3 h). After nitrogen bubbling, $NaBH_4$ (825 mg, 21.8 mmol) was added to the solution, and the resultant solution was allowed to warm to rt over 1 h. After addition of saturated aq. NH_4Cl, the reaction mixture was extracted with CH_2Cl_2, the organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1/1 to 1/2) to give compound **2** (1.83 g, 71%) as a white foam; $[\alpha]_D^{26}$ -25.5 (c 1.0, $CHCl_3$); IR ν_{max} (KBr): 1750, 2941, 3510 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.99 (3 H, s), 2.00 (3 H, s), 2.08 (3 H, s), 2.12 (3 H, s), 2.89 (1 H, brs), 3.63 (1 H, d, *J* = 13 Hz), 3.94 (1 H, t, *J* = 8 Hz), 4.07 (1 H, d, *J* = 13 Hz), 4.13 (1 H, d, *J* = 12 Hz), 4.18 (1 H, d, *J* = 12 Hz), 4.60 (1 H, d, *J* = 11 Hz), 4.64 (1 H, d, *J* = 11 Hz), 5.20 (1 H, t, *J* = 8 Hz), 5.46 (1 H, d, *J* = 8 Hz), 5.82 (1 H, d, *J* = 8 Hz), 7.23 – 7.36 (5H, m); ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ 20.7, 20.7(5), 20.7(8), 20.9, 59.6, 63.8, 70.0, 71.4, 74.4, 77.7, 77.9, 89.1, 127.7, 128.0, 128.5, 137.5, 169.0(8), 169.1(1), 170.6; MS (FAB) *m/z* 491 $[M+Na]^+$; HRMS (FAB): Calcd for $C_{22}H_{28}NaO_{11}$ $[M+Na]^+$: 491.1524. Found: 491.1523.

1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-5-*C*-(tosyloxymethyl)-β-D-glucopyranose (3). To a solution of compound **2** (396 mg, 0.85 mmol) in pyridine (4.2 mL) was added *p*-toluenesulfonyl chloride (322 mg, 1.69 mmol) and the resultant mixture was stirred at room temperature for 18 h under N_2 atmosphere. After addition of saturated aq. $NaHCO_3$, the reaction mixture was extracted with AcOEt, the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1/1) to give compound **3** (450 mg, 86%) as a white foam; $[\alpha]_D^{27}$ -32.2 (c 1.0, $CHCl_3$); IR ν_{max} (KBr): 1598, 1756, 2959, 3033 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.97 (3 H, s), 1.98 (3 H, s), 2.01 (3 H, s), 2.06 (3 H, s), 2.46 (3 H, s), 3.94 (1 H, dd, *J* = 6, 7 Hz), 4.00 (1 H, d, *J* = 12 Hz), 4.17 (1 H, d, *J* = 10 Hz), 4.23 (1 H, d, *J* = 10 Hz), 4.30 (1 H, d, *J* = 12 Hz), 4.63 (1 H, d, *J* = 12 Hz), 4.67 (1 H, d, *J* = 12 Hz), 5.04 (1 H, t, *J* = 6 Hz), 5.28 (1 H, d, *J* = 7 Hz), 6.01 (1 H, d, *J* = 6 Hz), 7.26 – 7.37

(7H, m), 7.80 (2 H, d, *J* = 8 Hz); ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ 20.6(8), 20.7(4), 20.9, 21.0, 21.8, 64.1, 67.4, 68.5, 71.0, 73.8, 76.1, 76.5, 90.3, 127.9, 128.1, 128.2, 128.6, 130.1, 132.4, 137.4, 145.4, 168.9, 169.2(7), 169.3(3), 170.1; MS (FAB) *m/z* 645 $[M+Na]^+$; HRMS (FAB): Calcd for $C_{29}H_{34}NaO_{13}S$ $[M+Na]^+$: 645.1612. Found: 645.1618.

1-{2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-5-*C*-(tosyloxymethyl)-β-D-glucopyranosyl}thymine (4). To a stirred solution of compound **3** (440 mg, 0.71 mmol) and thymine (134 mg, 1.06 mmol) in dry CH_3CN (6.3 mL) was added *N,O*-bis(trimethylsilyl)acetamide (BSA) (0.52 mL, 2.13 mmol) and the mixture was refluxed until clear solution was obtained. After cooling the reaction mixture to 0 ˚C, trimethylsilyltriflate (0.19 mL, 1.06 mmol) was added and the reaction mixture was refluxed for 5 h. The reaction mixture was diluted with AcOEt, washed with saturated aq. $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1/1 to 1/2) to give compound **4** (460 mg, 94%) as a white foam; $[\alpha]_D^{26}$ -49.6 (c 1.0, $CHCl_3$); IR ν_{max} (KBr): 1598, 1694, 1756, 2959, 3074, 3220 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.90 (3 H, s), 1.92 (3 H, s), 1.98 (3 H, s), 2.04 (3 H, s), 2.46 (3 H, s), 3.97 (1 H, d, *J* = 12 Hz), 4.11 – 4.20 (3H, m), 4.43 (1 H, d, *J* = 12 Hz), 4.63 (2 H, s), 5.15 (1 H, t, *J* = 9 Hz), 5.35 (1 H, d, *J* = 9 Hz), 6.13 (1 H, d, *J* = 9 Hz), 7.09 (1 H, s), 7.23 – 7.39 (7H, m), 7.85 (2 H, d, *J* = 8 Hz), 8.41 (1 H, s); ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ 12.7, 20.6, 20.7, 21.8, 64.3, 66.9, 70.2, 71.6, 74.9, 77.2, 78.2, 112.0, 127.9, 128.1(7), 128.2(1), 128.7, 130.2, 132.3, 134.9, 137.4, 145.6, 150.5, 163.3, 169.2, 169.5, 170.1; MS (FAB) *m/z* 689 $[M+H]^+$; HRMS (FAB): Calcd for $C_{32}H_{37}N_2O_{13}S$ $[M+H]^+$: 689.2011. Found: 689.1984.

1-{2,4,6-Tri-*O*-acetyl-3-*O*-(phenoxythiocarbonyl)-5-*C*-(tosyloxymethyl)-β-D-glucopyranosyl}thymine (5). To a solution of compound **4** (450 mg, 0.65 mmol) in AcOEt (13 mL) was added 20% $Pd(OH)_2$/C (230 mg). The reaction mixture was stirred under H_2 at room temperature for 14 h, filtered and concentrated. The obtained crude alcohol was dissolved in CH_2Cl_2 (2.9 mL), phenyl chlorothionoformate (0.16 mL, 1.16 mmol), triethylamine (0.24 mL, 1.74 mmol) and *N,N*-dimethyl-4-aminopyridine (ca. 7 mg, ca. 0.06 mmol) were added, and the reaction mixture was stirred at room temperature for 1 h under N_2 atmosphere. Furthermore phenyl chlorothionoformate (45 μL, 0.33 mmol) and triethylamine (68 μL, 0.49 mmol) were added to the mixture and the mixture was stirred at room temperature for 30 min. After concentration, resultant crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1/1 to 1/2) to give compound **5** (340 mg, 71%, over 2 steps) as a white foam; $[\alpha]_D^{27}$ -54.9 (c 1.0, $CHCl_3$); IR ν_{max} (KBr): 1598, 1695, 1755, 3076, 3190 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.95 (3 H, s), 2.02 (3 H, s), 2.03 (3 H, s), 2.08 (3 H, s), 2.43 (3 H, s), 4.06 (1 H, d, *J* = 12 Hz), 4.16 (1 H, d, *J* = 12 Hz), 4.39 (1 H, d, *J* = 11 Hz), 4.55 (1 H, d, *J* = 11 Hz), 5.35 (1 H, t, *J* = 9 Hz), 5.55 (1 H, d, *J* = 9 Hz), 6.23 (1 H, t, *J* = 9 Hz), 6.34 (1 H, d, *J* = 9 Hz), 6.99 (2H, d, *J* = 8 Hz), 7.14 (1 H, s), 7.27 – 7.44 (5H, m), 7.91 (2 H, d, *J* = 8 Hz), 8.97 (1 H, s); ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ 12.8, 20.6, 20.7, 20.8, 21.8, 64.2, 66.4, 68.9, 69.6, 80.2, 112.3, 121.6, 127.0, 128.4, 129.8, 130.2, 132.0, 134.5, 145.7, 150.3, 153.3, 163.3, 169.0, 169.5, 170.0, 194.4; MS (FAB) *m/z* 735 $[M+H]^+$; HRMS (FAB): Calcd for $C_{32}H_{35}N_2O_{14}S_2$ $[M+H]^+$: 735.1524. Found: 735.1536.

1-{2,4,6-Tri-*O*-acetyl-3-deoxy-5-*C*-(tosyloxymethyl)-β-D-glucopyranosyl}thymine (6). To a solution of compound **5** (578 mg, 0.79 mmol) in dry toluene (7.9 mL) was added tris(trimethylsilyl)silane (0.30 mL, 0.98 mmol) and azobisisobutyronitrile (ca. 3 mg, ca. 0.02 mmol) and the resultant mixture was stirred at 80 ˚C for 3 h under N_2 atmosphere. Further tris(trimethylsilyl)silane (0.07 mL, 0.24 mmol) was added to the mixture and the mixture was stirred at 80 ˚C for 1 h. After concentration, the obtained crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1/1 to 1/2) to give compound **6** (451 mg, 99%) as a white foam; $[\alpha]_D^{27}$ -35.3 (c 1.0, $CHCl_3$); IR ν_{max} (KBr): 1598, 1694, 1744, 3080, 3207 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.93 (3 H, s), 2.01 – 2.08 (10 H, m), 2.46 – 2.53 (4 H, m), 4.06 (1 H, d, *J* = 12 Hz), 4.16 (1 H, d, *J* = 12 Hz), 4.29 (1 H, d, *J* = 11 Hz), 4.53 (1 H, d, *J* = 11 Hz), 5.06 (1 H, ddd, *J* = 5, 10, 10 Hz), 5.18 (1 H, dd, *J* = 5, 11 Hz), 6.01 (1 H, d, *J* = 10 Hz), 7.03 (1 H, s), 7.38 (2H, d, *J* = 8 Hz), 7.84 (2 H, d, *J* = 8 Hz), 8.10 (1 H, s); ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ 12.7, 20.7(6), 20.7(8), 20.9, 21.8, 30.6, 64.0, 64.9, 66.6, 66.8, 77.9, 112.0, 128.2, 130.2, 132.3, 134.8, 145.6, 150.4, 163.4, 169.3, 169.7, 170.2; MS (FAB) *m/z* 583 $[M+H]^+$; HRMS (FAB): Calcd for $C_{25}H_{31}N_2O_{12}S$ $[M+H]^+$: 583.1592. Found: 583.1604.

1-{3-deoxy-5-*C*-(tosyloxymethyl)-β-D-glucopyranosyl}thymine (7). To a solution of compound **6** (400 mg, 0.69 mmol) in CH_3OH (9 mL) was added potassium carbonate (285 mg, 2.06 mmol) and the resultant mixture was stirred at room temperature for 15 min. After addition of H_2O, the reaction mixture was extracted with AcOEt, the organic layer was dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, $CHCl_3/CH_3OH$ = 5/1) to give compound **7** (300 mg, 96%) as a white foam; $[\alpha]_D^{28}$ -32.7 (c 1.0, MeOH); IR ν_{max} (KBr): 1598, 1694, 2949, 3065, 3350 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) δ 1.75 (1 H, ddd, *J* = 12, 12, 12 Hz), 1.88 (3 H, s), 2.27 (1 H, ddd, *J* = 5, 5, 12 Hz), 2.45 (3 H, s), 3.55 (1 H, d, *J* = 11 Hz), 3.63 (1 H, d, *J* = 11 Hz), 3.69 – 3.75 (1 H, m), 4.12 (1 H, d, *J* = 11 Hz), 4.12 (1 H, d, *J* = 5, 12 Hz), 4.51 (1 H, d, *J* = 11 Hz), 5.70 (1 H, d, *J* = 10 Hz), 7.45 (2H, d, *J* = 8 Hz), 7.52 (1 H, s), 7.85 (2 H, d, *J* = 8 Hz); ^{13}C-NMR (100.5 MHz, CD_3OD) δ 12.4, 21.6, 37.6, 63.9, 65.8, 67.2, 67.8, 80.7, 82.0, 111.6, 129.2, 131.2, 133.6, 138.3, 146.7, 152.8, 166.3; MS (FAB) *m/z* 457 $[M+H]^+$; HRMS (FAB): Calcd for $C_{19}H_{25}N_2O_9S$ $[M+H]^+$: 457.1275. Found: 457.1290.

1-{3-deoxy-4,6-*O*-isopropylidene-5-*C*-(tosyloxymethyl)-β-D-glucopyranosyl}thymine (8). To a solution of compound **7** (300 mg, 0.66 mmol) in dry acetone (6.6 mL) were added 2,2'-dimethoxypropane (0.10 mL, 0.81 mmol) and (+)-10-camphorsulfonic acid (16 mg, 0.07 mmol) and the resultant mixture was stirred at room temperature for 20 h under N_2 atmosphere. Further 2,2'-dimethoxypropane (0.08 mL, 0.66 mmol) was added to the mixture and the mixture was stirred at room temperature for 4 h. Again, 2,2'-dimethoxypropane (0.08 mL, 0.66 mmol) was added to the mixture and the mixture was stirred at room temperature for 2 h. After addition of saturated aq. $NaHCO_3$, the reaction mixture was extracted with AcOEt, the organic layer was dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, $CHCl_3/CH_3OH$ = 10/1) to give compound **8** (300 mg, 92%) as a white foam; $[\alpha]_D^{27}$ -26.8 (c 1.0, $CHCl_3$); IR ν_{max} (KBr): 1598, 1711, 2990, 3449 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) δ 1.22 (3 H, s), 1.48 (3 H, s), 1.69 (1 H, ddd, *J* = 12, 12, 12 Hz), 1.88 (3 H, d, *J* = 1 Hz), 2.12 (1 H, ddd, *J* = 4, 4, 12 Hz), 2.44 (3 H, s), 3.65 (1 H, d, *J* = 11 Hz), 3.75 (1 H, d, *J* = 11 Hz), 3.90 – 3.96 (1 H, m), 4.09 (1 H, dd, *J* = 4, 12 Hz), 4.48 (1 H, d, *J* = 11 Hz),

4.63 (1 H, d, *J* = 11 Hz), 5.77 (1 H, d, *J* = 9 Hz), 7.44 (2H, d, *J* = 8 Hz), 7.52 (1 H, d, *J* = 1 Hz), 7.84 (2 H, d, *J* = 8 Hz); ^{13}C-NMR (100.5 MHz, CD_3OD) δ 12.3, 19.2, 21.6, 29.4, 33.8, 64.5, 65.2, 68.5, 70.9, 73.3, 82.1, 101.8, 112.0, 129.2, 131.2, 133.7, 138.1, 146.7, 152.7, 166.2; MS (FAB) *m/z* 497 $[M+H]^+$; HRMS (FAB): Calcd for $C_{22}H_{29}N_2O_9S$ $[M+H]^+$: 497.1588. Found: 497.1611.

1-(3-deoxy-4,6-*O*-isopropylidene-2-*O*,5-*C*-methylene-β-D-glucopyranosyl)thymine (9). To a solution of compound **8** (293 mg, 0.59 mmol) in DMF (6 mL) was added sodium hydride (71 mg, 60% in oil, 1.77 mmol) and the resultant mixture was stirred at 60 ˚C for 10 h under N_2 atmosphere. After addition of saturated aq. NH_4Cl, the reaction mixture was extracted with AcOEt, the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The obtained crude product was purified by column chromatography (SiO_2, *n*-hexane/AcOEt = 1/1 to 1/2) to give compound **9** (167 mg, 87%) as a white foam; $[\alpha]_D^{26}$ +71.6 (c 1.0, $CHCl_3$); IR ν_{max} (KBr): 1694, 2885, 2993, 3185 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.42 (3 H, s), 1.53 (3 H, s), 1.86 – 1.92 (1 H, m), 1.97 (3 H, d, *J* = 1 Hz), 2.05 (1 H, ddd, *J* = 2, 10, 16 Hz), 3.61 (1 H, d, *J* = 11 Hz), 3.87 (1 H, dd, *J* = 2, 10 Hz), 3.92 (1 H, d, *J* = 11 Hz), 4.11 (1 H, ddd, *J* = 2, 5, 10 Hz), , 4.31 (1 H, ddd, *J* = 2, 2, 2 Hz), 4.57 (1 H, d, *J* = 10 Hz), 6.02 (1 H, dd, *J* = 1, 2 Hz), 7.37 (1H, d, *J* = 1 Hz); ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ 13.1, 18.8, 27.9, 29.0, 63.7, 65.2, 66.0, 66.2, 68.2, 84.5, 100.0, 110.6, 133.5, 150.2, 164.0; MS (FAB) *m/z* 325 $[M+H]^+$; HRMS (FAB): Calcd for $C_{15}H_{21}N_2O_6$ $[M+H]^+$: 325.1394. Found: 325.1379.

1-(3-deoxy-2-*O*,5-*C*-methylene-β-D-glucopyranosyl)thymine (10). Compound **9** was dissolved in AcOH/H_2O (3:2, 5 mL) and stirred at room temperature for 13 h. The solvent was removed under reduced pressure and the residue co-evaporated with toluene. The crude product was purified by column chromatography (SiO_2, $CHCl_3$/CH_3OH = 10/1) to give compound **10** (167 mg, 87%) as a colorless solid. A part of the solid was recrystallized from CH_3CN for x-ray crystallography; mp 119-121 ˚C (CH_3CN); $[\alpha]_D^{25}$ +101.1 (c 1.0, CH_3OH); IR ν_{max} (KBr): 1692, 2943, 3036, 3385 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) δ 1.82 – 1.88 (1 H, m), 1.91 (3 H, d, *J* = 1 Hz), 2.09 (1 H, ddd, *J* = 1, 10, 15 Hz), 3.63 (1 H, d, *J* = 12 Hz), 3.71 (1 H, d, *J* = 12 Hz), 3.90 (1 H, dd, *J* = 2, 10 Hz), 4.10 – 4.18 (3 H, m), 5.98 (1 H, m), 7.62 (1H, d, *J* = 1 Hz); ^{13}C-NMR (100.5 MHz, CD_3OD) δ 12.6, 31.8, 62.5, 63.6, 64.8, 67.4, 78.3, 84.7, 110.9, 136.3, 152.0, 166.4; MS (FAB) *m/z* 285 $[M+H]^+$; HRMS (FAB): Calcd for $C_{12}H_{17}N_2O_6$ $[M+H]^+$: 285.1081. Found: 285.1079.

1-{3-deoxy-6-*O*-(4,4'-dimethoxytrityl)-2-*O*,5-*C*-methylene-β-D-glucopyranosyl}thymine (11). To a solution of compound **10** (40 mg, 0.14 mmol) in pyridine (1 mL) was added 4,4′-dimethoxytrityl chloride (71 mg, 0.21 mmol) and the resultant mixture was stirred at room temperature for 2 h under a N_2 atmosphere. After addition of H_2O, the reaction mixture was extracted with AcOEt, the organic layer was dried over Na_2SO_4, and concentrated. The obtained crude product was purified by column chromatography (SiO_2, 0.5% triethylamine in *n*-hexane/AcOEt = 1/2 to AcOEt only) to give compound **11** (78 mg, 94%) as a white foam; $[\alpha]_D^{30}$ +48.3 (c 1.0, $CHCl_3$); IR ν_{max} (KBr): 1508, 1582, 1607, 1682, 2836, 2933, 3059, 3188, 3461 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.86 – 1.92 (1 H, m), 1.98 (3 H, d, *J* = 1 Hz), 2.07 (1 H, ddd, *J* = 2, 10, 16 Hz), 2.13 (1 H, d, *J* = 4 Hz), 3.12 (1 H, d, *J* = 10 Hz), 3.42 (1 H, d, *J* = 10 Hz), 3.70 (1 H, dd, *J* = 2, 10 Hz), 3.80 (6 H, s), 4.13 (1 H, d, *J* = 10 Hz), 4.29 – 4.35 (2 H, m), 6.01 (1 H, m), 6.84 – 6.88 (4 H, m), 7.23 – 7.36 (7

H, m), 7.43 – 7.45 (2 H, m), 7.61 (1H, d, *J* = 1 Hz), 9.03 (1 H, s); ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ 13.1, 30.1, 55.4, 62.6, 64.1, 64.2, 65.6, 76.2, 83.6, 86.8, 110.4, 113.5(6), 113.6(0), 127.3, 127.9, 128.3, 130.0(0), 130.0(1), 134.2, 135.0, 135.3, 144.4, 150.1, 158.8(7), 158.9(0), 164.0; MS (FAB) *m/z* 609 $[M+Na]^+$; HRMS (FAB): Calcd for $C_{33}H_{34}N_2NaO_8$ $[M+Na]^+$: 609.2207. Found: 609.2216.

1-[4-*O*-{2-cyanoethoxy(diisopropylamino)phosphino}-3-deoxy-6-*O*-(4,4'-dimethoxytrityl)-2-*O*,5-*C*-methylene-β-D-glucopyranosyl]thymine (12). To a solution of compound **11** (260 mg, 0.45 mmol) in dry CH_3CN (4.5 mL) were added *N*,*N*-diisopropylethylamine (0.23 mL, 1.34 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (0.15 mL, 0.67 mmol) and the resultant mixture was stirred at 0 ˚C for 3 h under a N_2 atmosphere. The reaction mixture was concentrated and the obtained crude product was purified by column chromatography (SiO_2, 0.5% triethylamine in *n*-hexane/AcOEt = 1/2 to AcOEt only) to give a 10:1 diastereomixture of compound **12** (270 mg, 77%) as a white foam; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.06 (6 H, d, *J* = 7 Hz), 1.12 (6 H, d, *J* = 7 Hz), 1.99 – 2.05 (1 H, m), 2.11 – 2.15 (1 H, m), 2.27 – 2.40 (2 H, m), 2.44 (3 H, s), 3.31 – 3.52 (6 H, m), 3.80 (6 H, s), 3.99 (1 H, d, *J* = 10 Hz), 4.07 (1 H, d, *J* = 10 Hz), 4.50 – 4.54 (1 H, m) , 4.59 (1 H, brs), 6.26 (1 H, brs), 6.85 (4 H, d, *J* = 9 Hz), 7.23 – 7.37 (7 H, m), 7.48 (2 H, d, *J* = 7 Hz), 8.13 (1H, s), 8.25 (1H, s), 9.29 (1H, s); the peaks at 0.91, 2.15, 2.57, 3.60, 4.07, 4.13, 4.21, 4.33, 6.15, and 6.80 ppm are derived from the other diastereomer; ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ 12.8, 20.3, 20.4, 24.4, 24.7, 24.8, 30.4, 30.4, 43.2, 43.3, 55.4, 58.0, 62.3, 64.1, 64.3, 64.6, 65.5, 76.3, 76.4, 83.6, 86.3, 110.7, 113.2, 113.2, 117.7, 127.1, 127.9, 128.5, 130.4, 130.5, 134.2, 135.6, 135.7, 144.8, 150.1, 158.7, 158.7, 163.9; ^{31}P-NMR (161.8 MHz, $CDCl_3$) δ 147.1, 151.2; MS (FAB) *m/z* 787 $[M+H]^+$; HRMS (FAB): Calcd for $C_{42}H_{52}N_4O_9P$ $[M+H]^+$: 787.3466. Found: 787.3466.

1-[4-*O*-{2-cyanoethoxy(diisopropylamino)phosphino}-3-deoxy-6-*O*-(4,4'-dimethoxytrityl)-2-*O*,5-*C*-methylene-β-D-glucopyranosyl]-5-methyl-4-(1,2,4-triazol-1-yl)-2-pyrimidinone (13). To a stirred suspension of 1,2,4-triazole (154 mg, 2.22 mmol) in acetonitrile (6.7 mL) was added phosphoryl chloride (48 μL, 0.62 mmol) at 0 ˚C, and the whole was stirred at 0 ˚C for 10 min. Triethylamine (0.36 mL, 2.55 mmol) was added and the reaction mixture was stirred at 0 ˚C for 40 min. A solution of compound **12** (53 mg, 0.07 mmol) in acetonitrile (1.3 mL) was added to the mixture and stirring was continued at room temperature for 5 h. The reaction mixture was poured into saturated aq. $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated. Purification by column chromatography (0.5% triethylamine in *n*-hexane/AcOEt = 1/2 to AcOEt only) afforded a white foam, which was further purified by precipitation to give a 10:1 diastereomixture of compound **13** (50 mg, 89%) as a white foam; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.06 (6 H, d, *J* = 7 Hz), 1.12 (6 H, d, *J* = 7 Hz), 1.99 – 2.05 (1 H, m), 2.11 – 2.15 (1 H, m), 2.27 – 2.40 (2 H, m), 2.44 (3 H, s), 3.31 – 3.52 (6 H, m), 3.80 (6 H, s), 3.98 – 4.01 (1 H, m), 4.07 (1 H, d, *J* = 10 Hz), 4.50 – 4.54 (1 H, m) , 4.59 (1 H, brs), 6.26 (1 H, brs), 6.85 (4 H, d, *J* = 9 Hz), 7.23 – 7.37 (7 H, m), 7.48 (2 H, d, *J* = 7 Hz), 8.13 (1H, s), 8.25 (1H, s), 9.29 (1H, s); the peaks at 0.93, 2.41, 2.57, 3.79, 4.10, and 6.81 ppm are derived from the other diastereomer; ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ 17.4, 20.4, 20.5, 24.4, 24.4, 24.7, 24.7, 30.0, 30.1, 43.2, 43.3, 55.4, 57.8, 58.0, 62.2, 64.1, 64.2, 64.5, 64.7, 76.6, 76.7, 85.2, 86.3, 106.3, 113.2, 113.2, 117.8, 127.1, 128.0, 128.5, 130.4, 130.5, 135.6, 135.7, 144.7, 145.3, 145.8, 153.6, 153.9, 158.5, 158.7; ^{31}P-NMR (161.8 MHz,

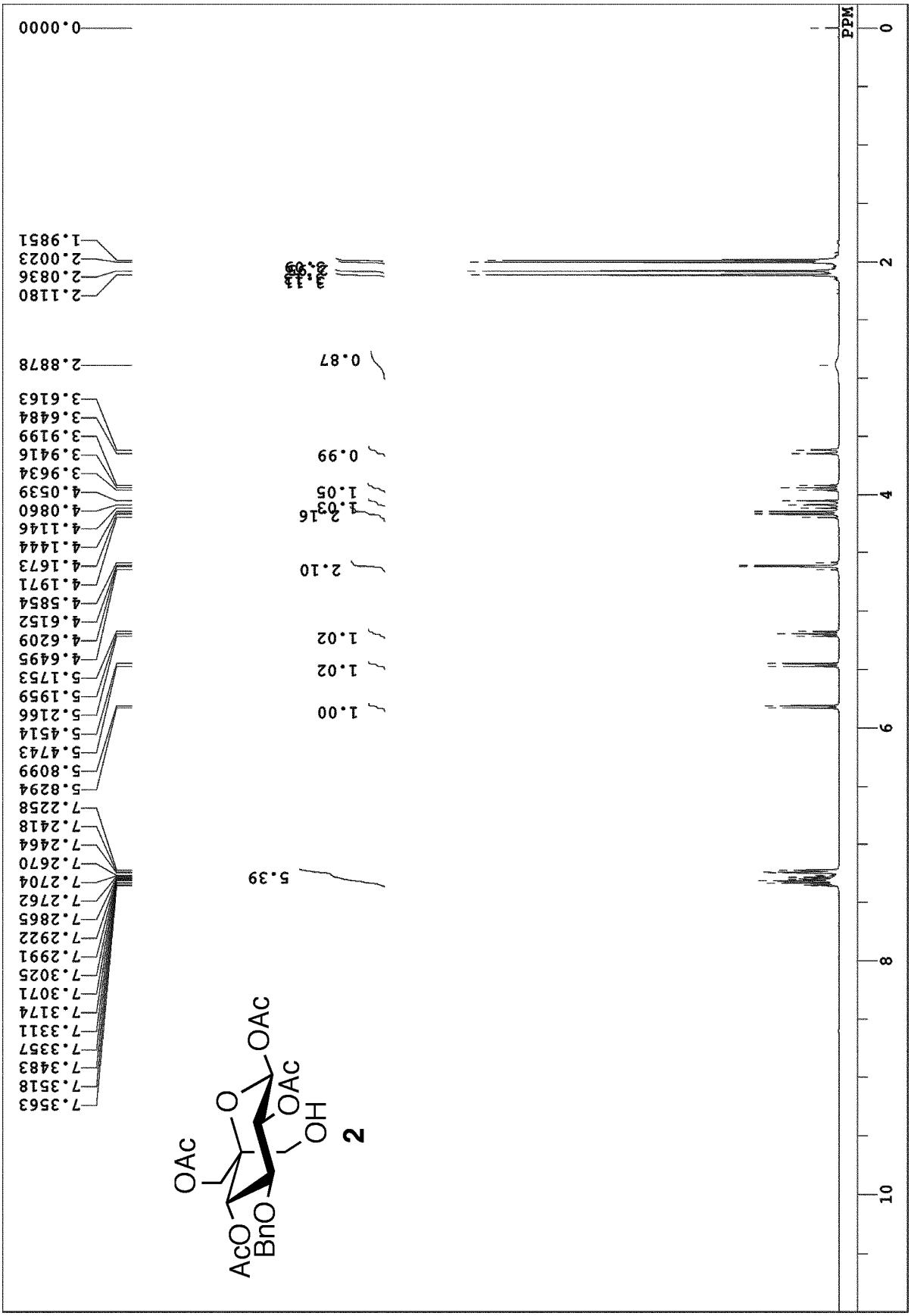
$CDCl_3$) δ 146.7, 151.5; MS (FAB) *m/z* 838 $[M+H]^+$; HRMS (FAB): Calcd for $C_{44}H_{53}N_7O_8P$ $[M+H]^+$:838.3688. Found: 838.3713.

Oligonucleotides synthesis

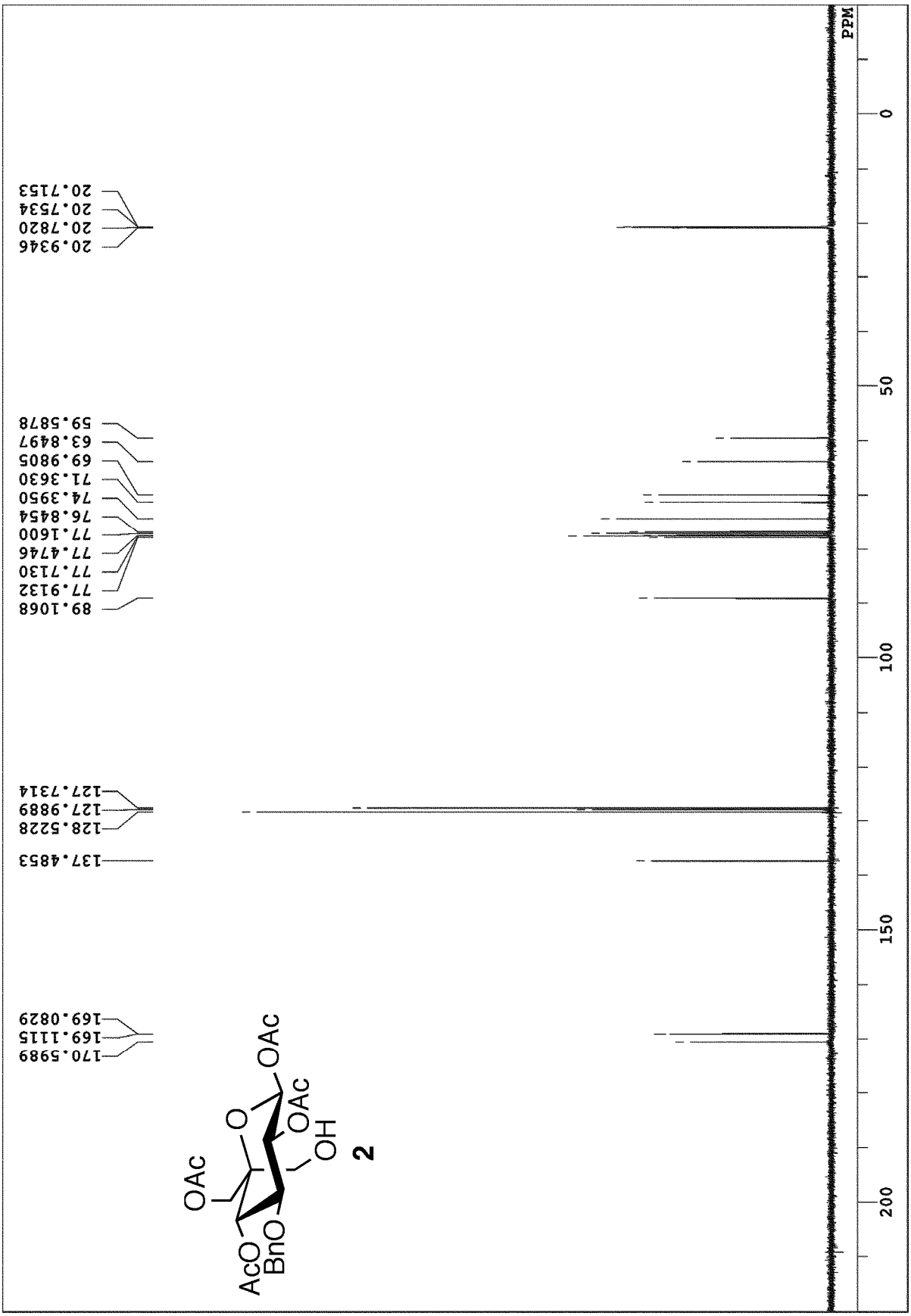
Synthesis of the XX-modified ONs was performed on an Applied Biosystems Expedite™ 8909 Nucleic Acid Synthesis System on a 0.2 μmol scale using a phosphoramidite coupling protocol and 5-[3,5-bis(trifluoromethyl)phenyl]-1*H*-tetrazole as the activator. The concentration of each phosporamidite was 0.067 M. The coupling times of phosphoramidite **12** and **13** were prolonged from 90 seconds to 6 minutes. Coupling yields were checked by trityl monitoring and were estimeted to be over 95%. The solid-supported ONs (DMTr-on) were treated with concentrated ammonium hydroxide solution at 55 ˚C for 12 h, and then concentrated. The crude ONs were roughly purified with a Sep-Pak Plus C_{18} Environmental Cartridge, and then carefully by RP-HPLC using Waters XBridge™ OST C18 2.5 μm (10 x 50 mm) with a linear gradient of MeCN (6-12% over 30 min for ON **14**-**17**, 6-9% over 30 min for ON **18**, **19**) in 0.1 M triethylammonium acetate buffer (pH = 7.0). The purity of the ONs was analyzed by RP-HPLC on a Waters XBridge™ Shield RP 18 2.5 μm (4.6 x 50 mm) and characterized by MALDI-TOF mass spectrometry.

^{1}H-, ^{13}C- and ^{31}P-NMR spectra of new compounds

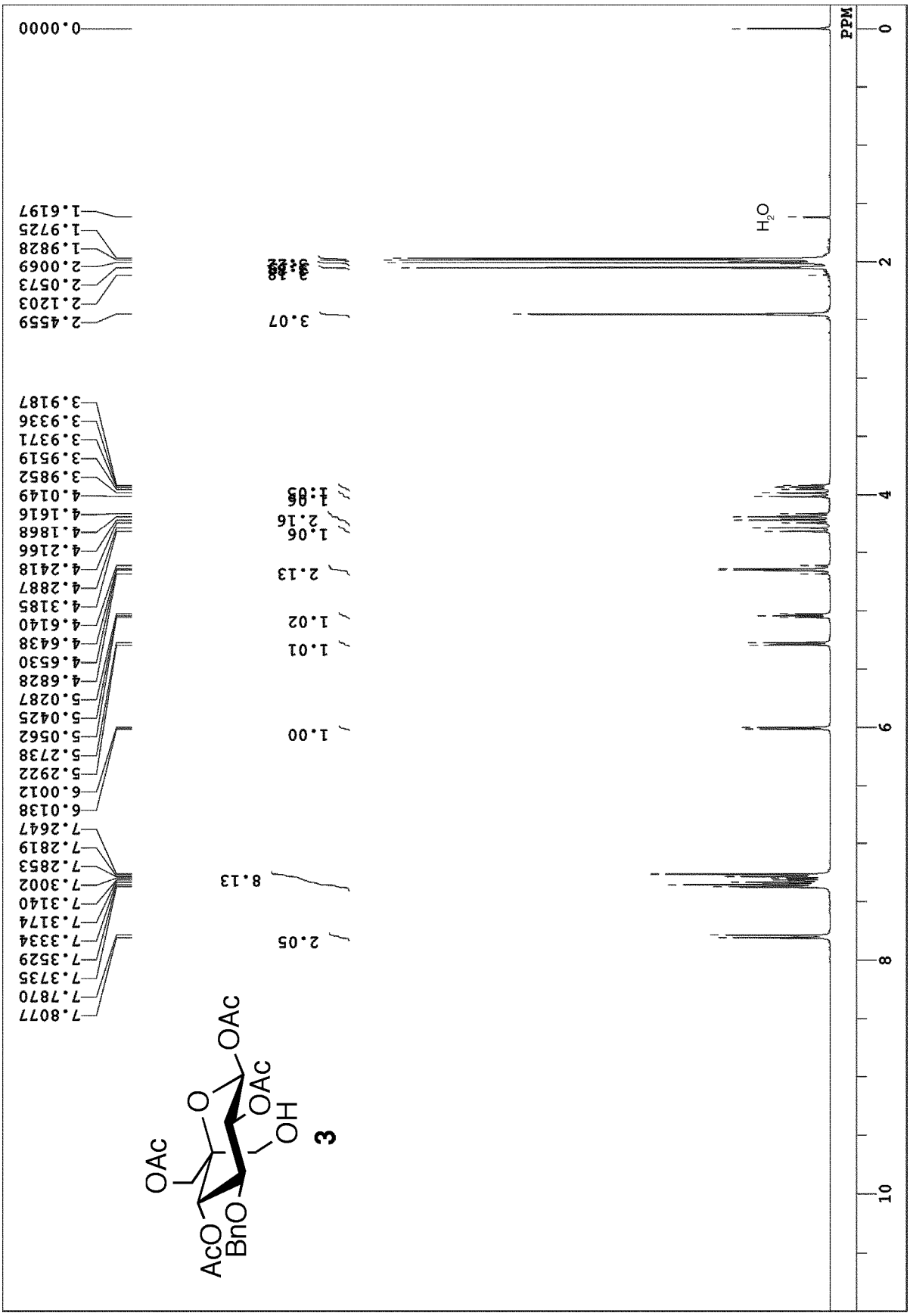
Compound **2** (^{1}H-NMR, 400 MHz, $CDCl_3$)



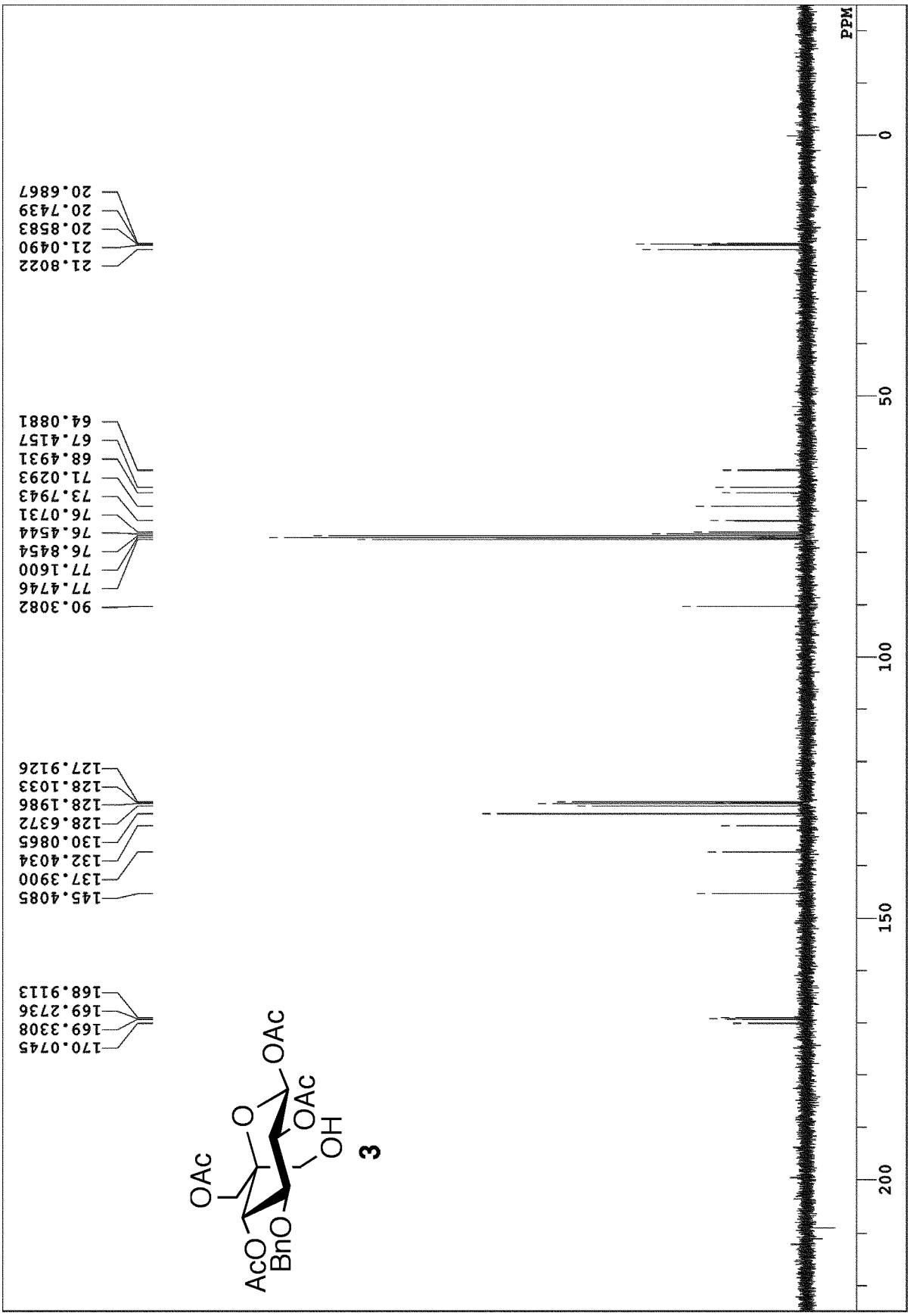
Compound **2** (^{13}C-NMR, 100.5 MHz, $CDCl_3$)



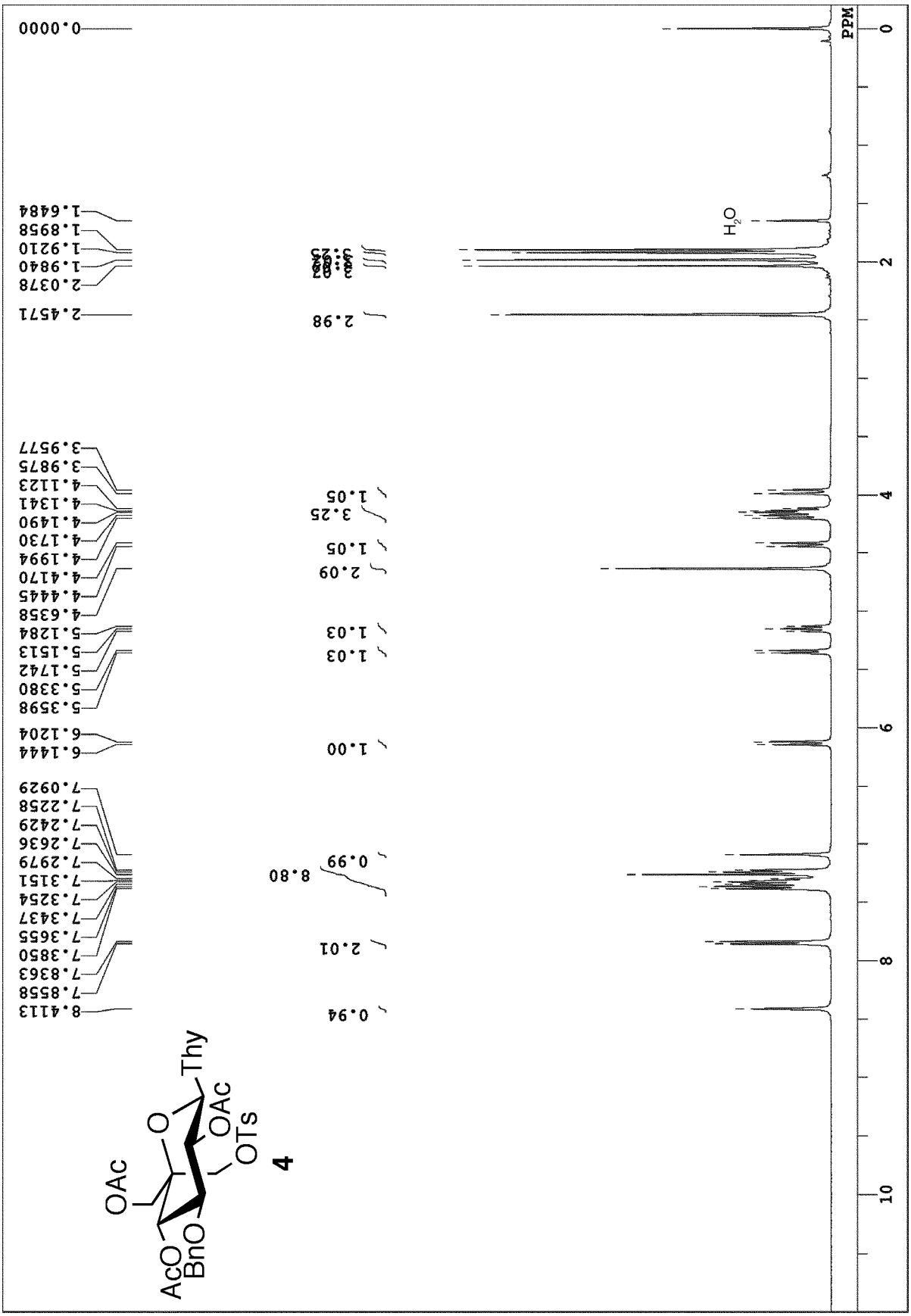
Compound **3** (^{1}H-NMR, 400 MHz, $CDCl_3$)



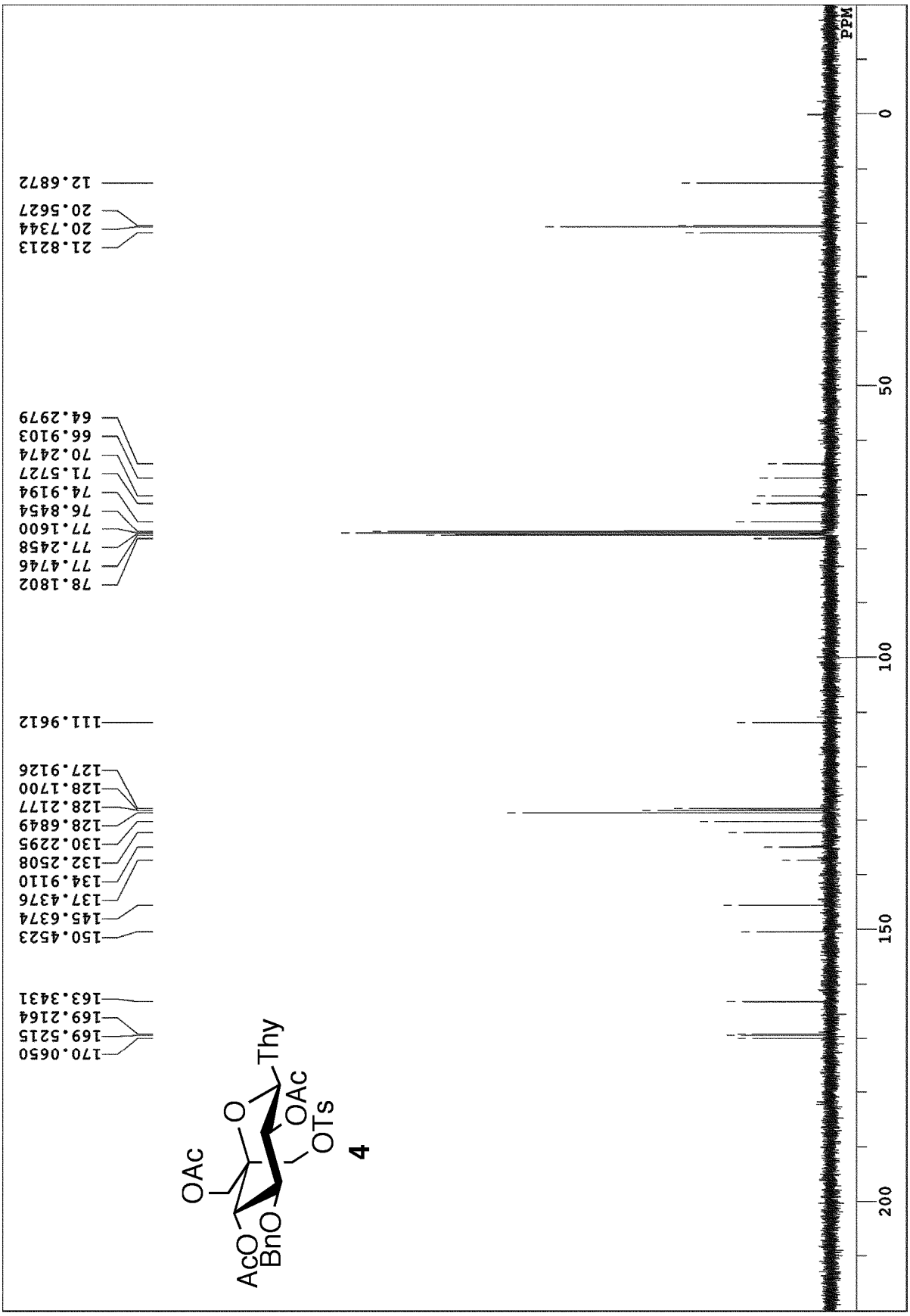
Compound **3** (^{13}C-NMR, 100.5 MHz, $CDCl_3$)



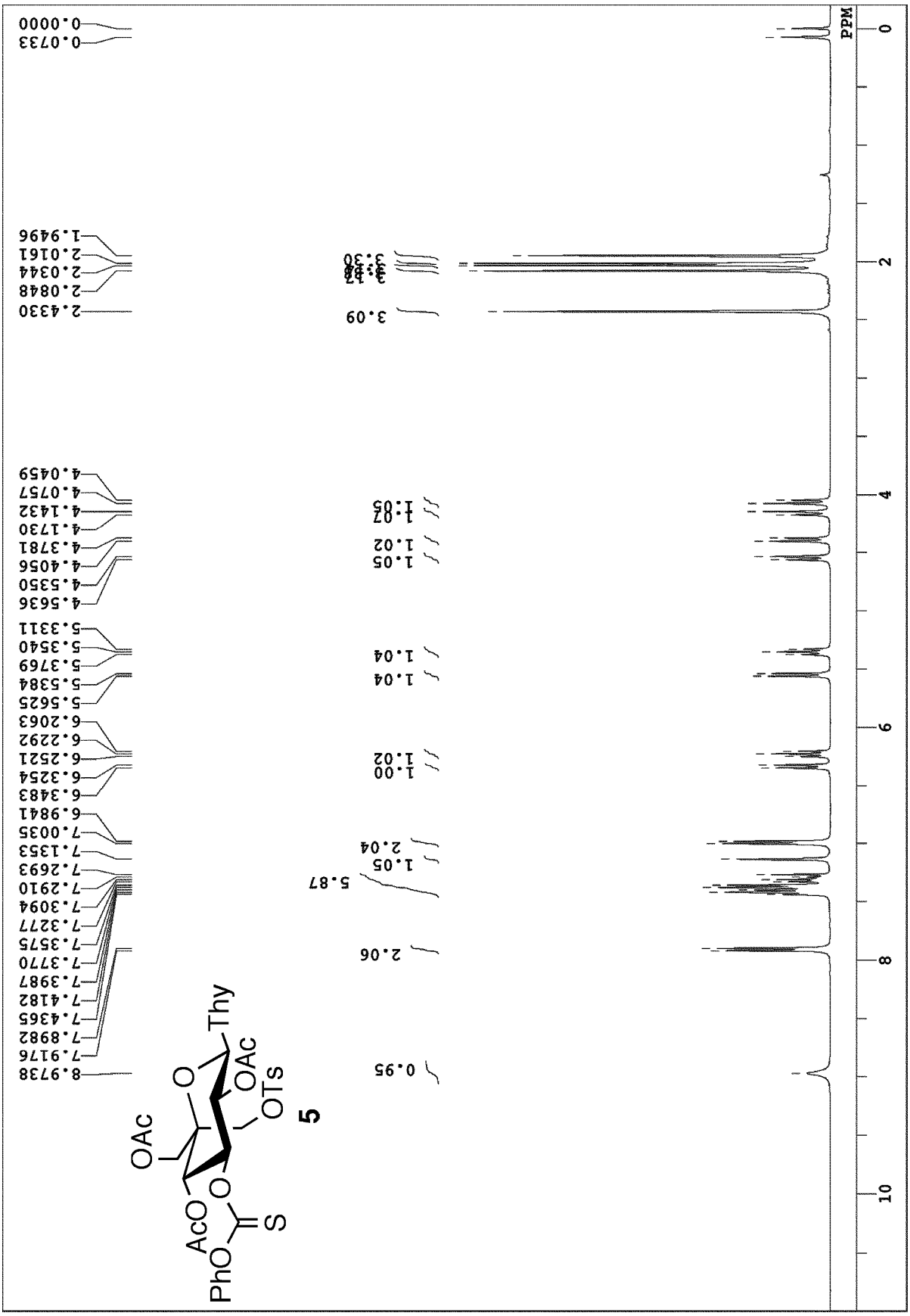
Compound **4** (^{1}H-NMR, 400 MHz, $CDCl_3$)



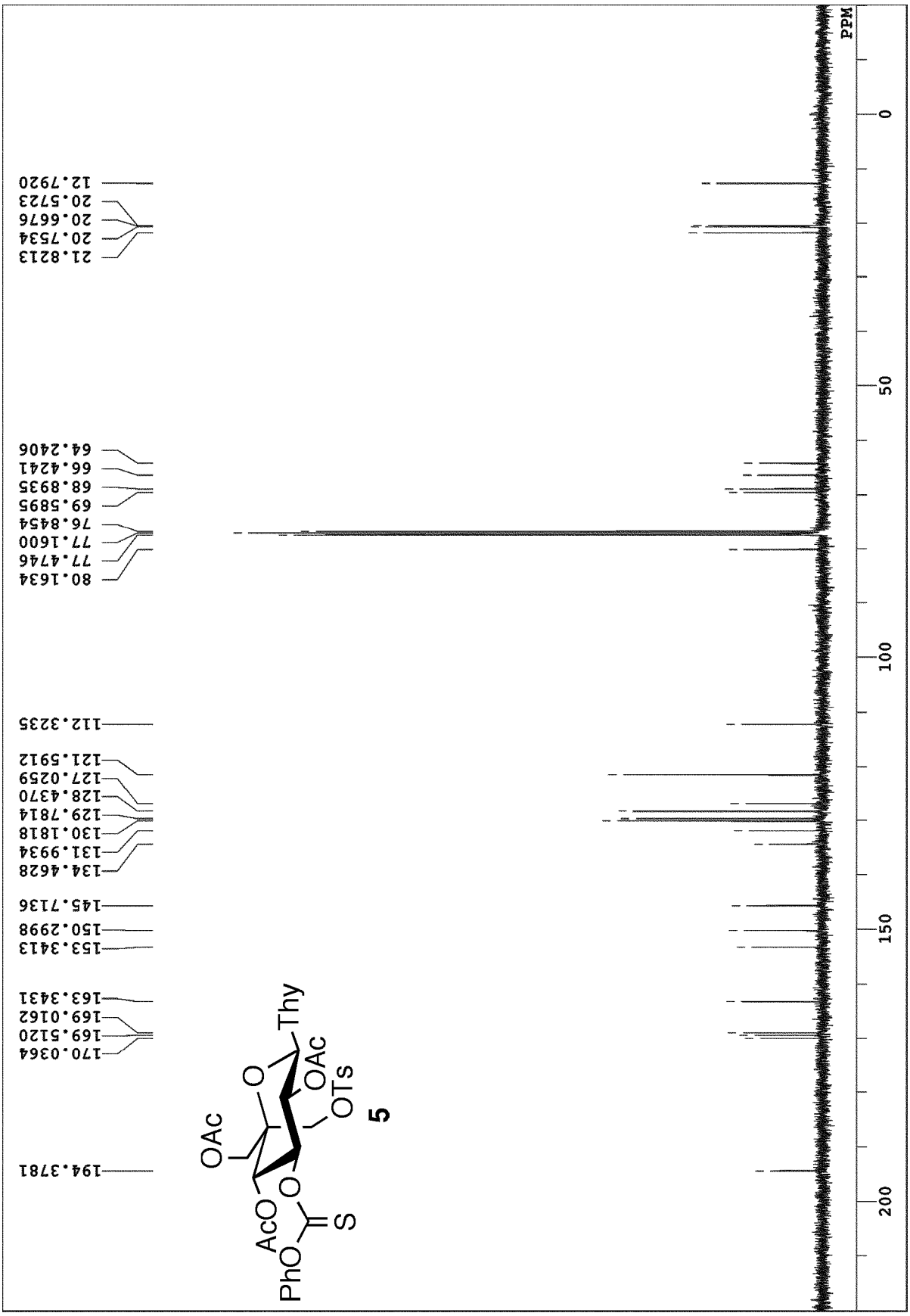
Compound **4** (^{13}C-NMR, 100.5 MHz, $CDCl_3$)



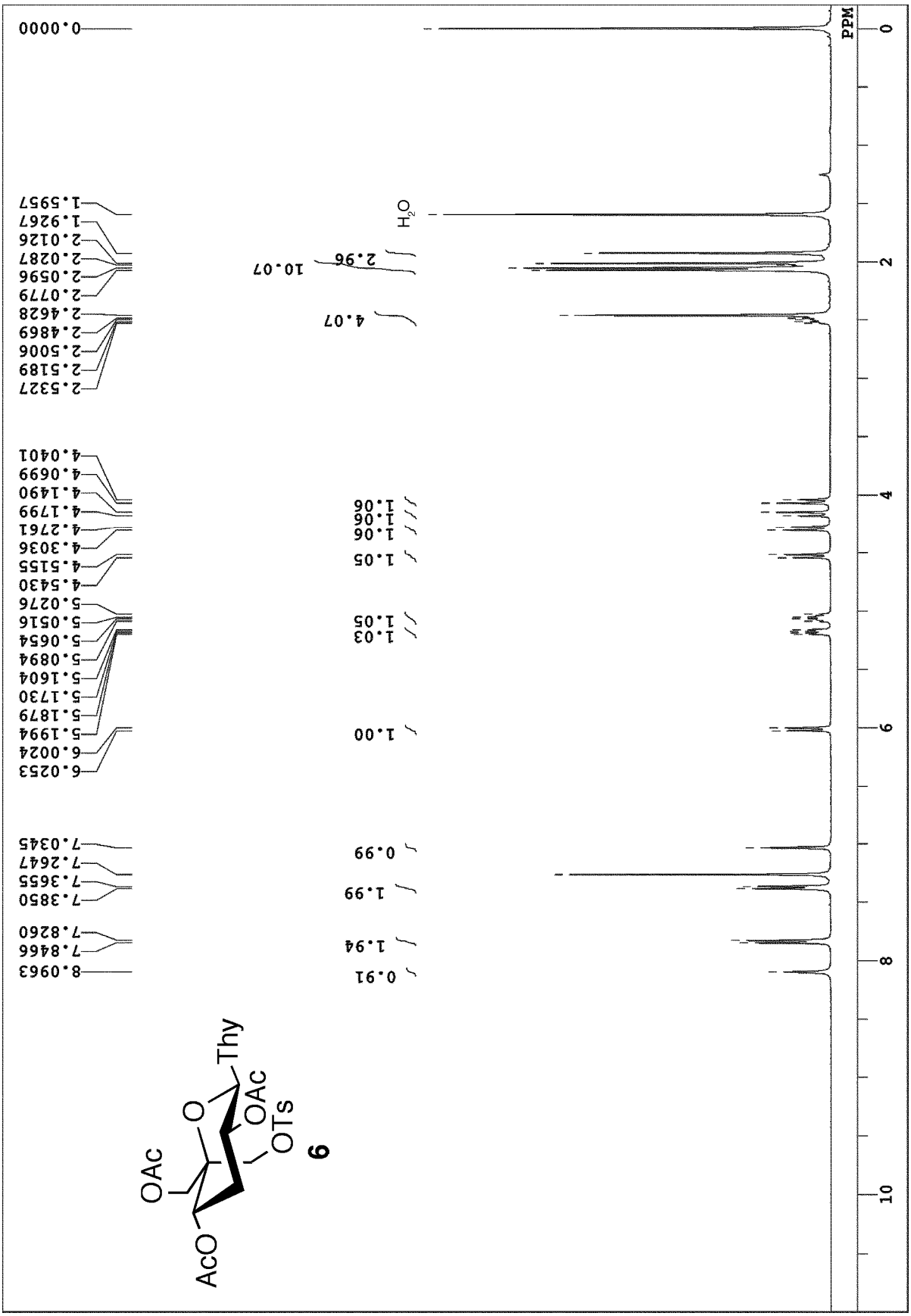
Compound **5** (^{1}H-NMR, 400 MHz, $CDCl_3$)



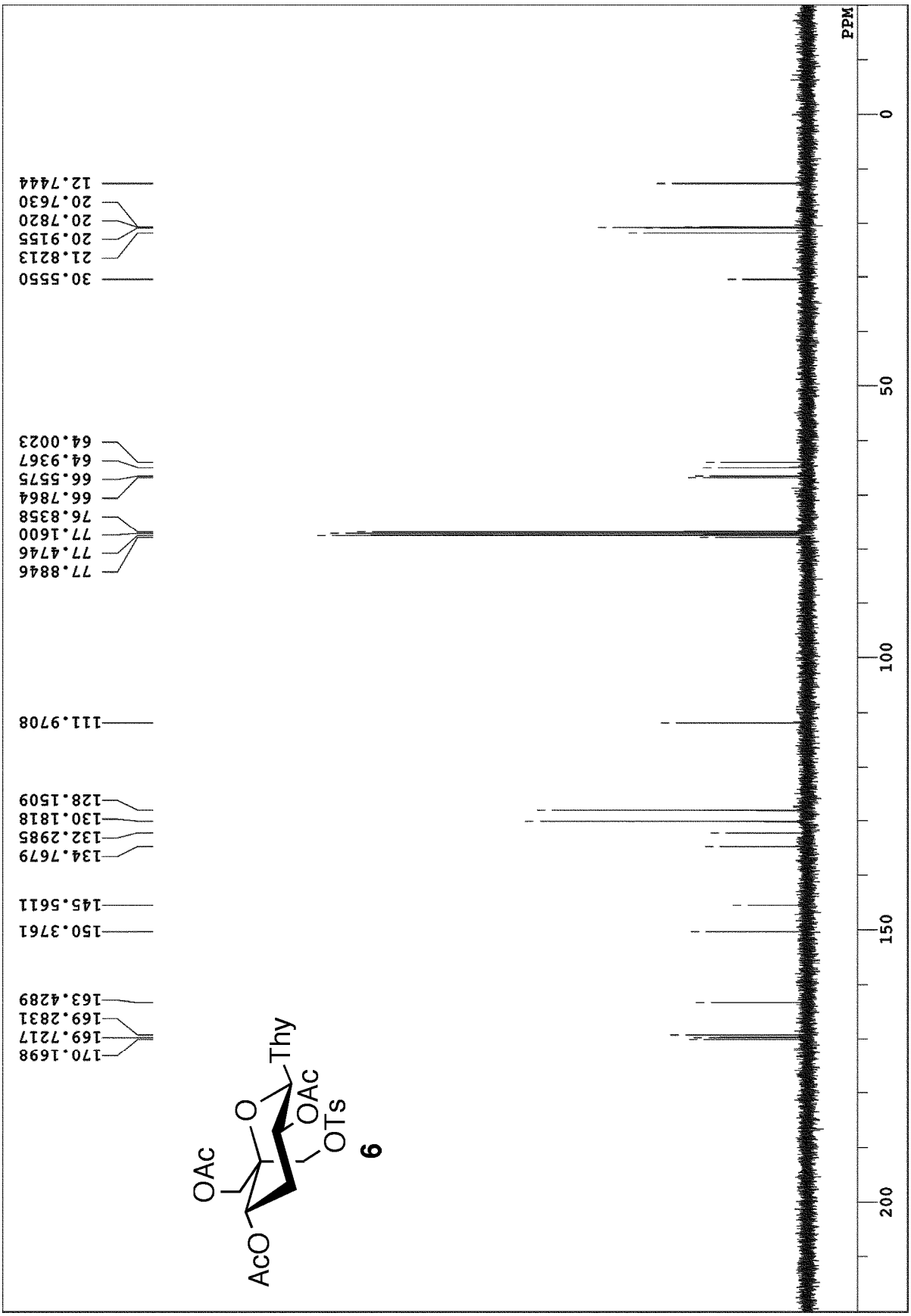
Compound **5** (^{13}C-NMR, 100.5 MHz, $CDCl_3$)



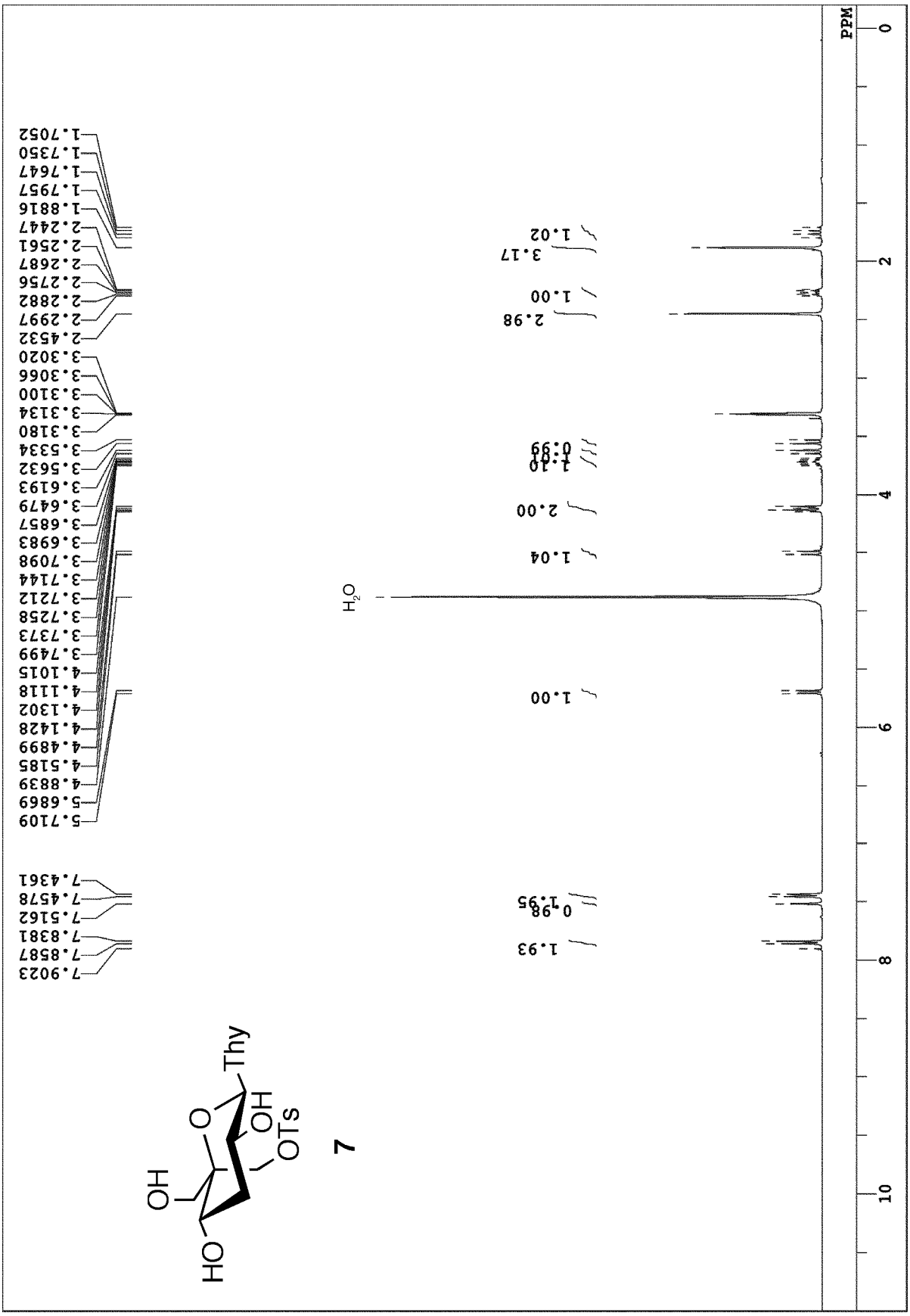
Compound **6** (^{1}H-NMR, 400 MHz, $CDCl_3$)



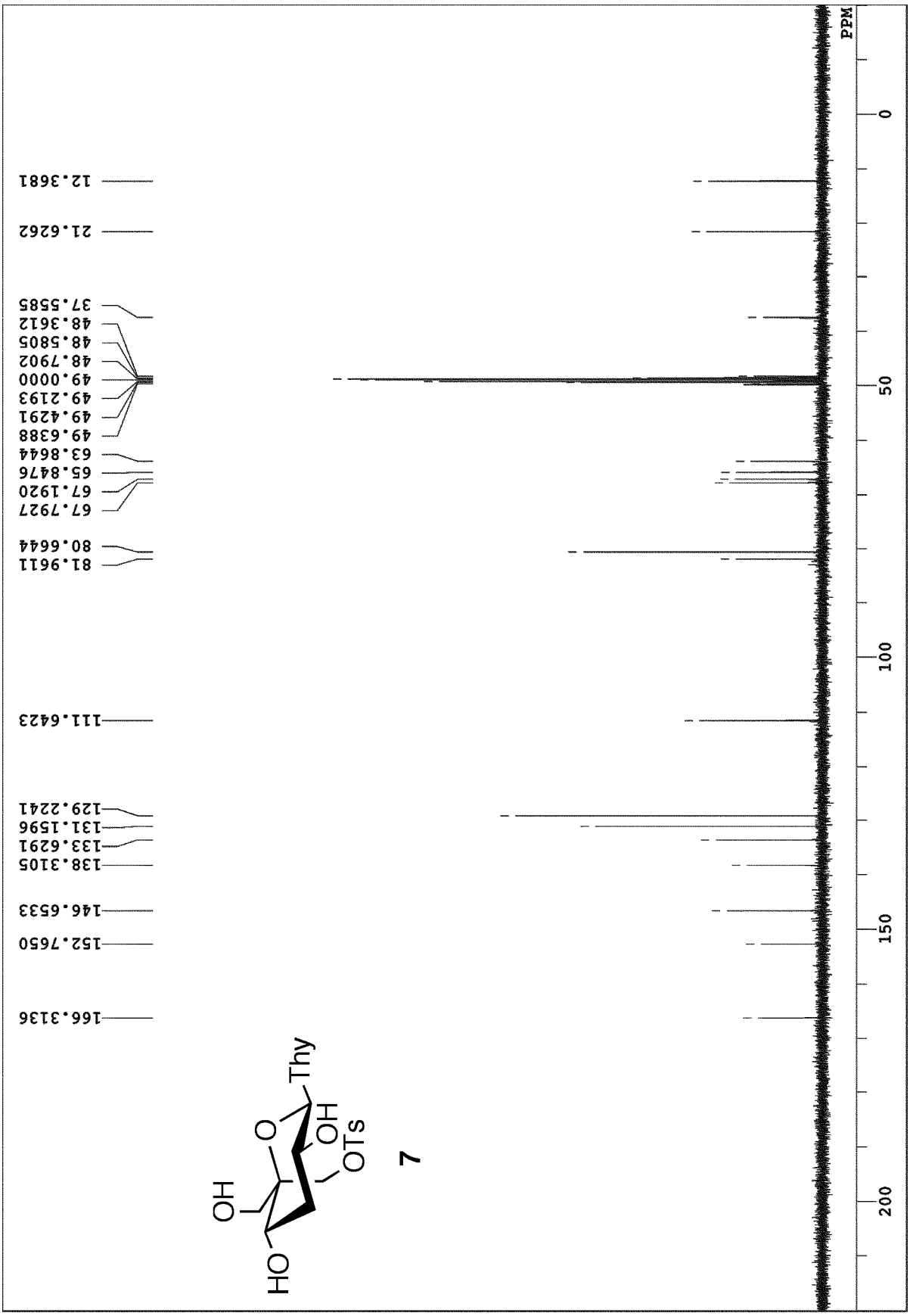
Compound **6** (^{13}C-NMR, 100.5 MHz, $CDCl_3$)



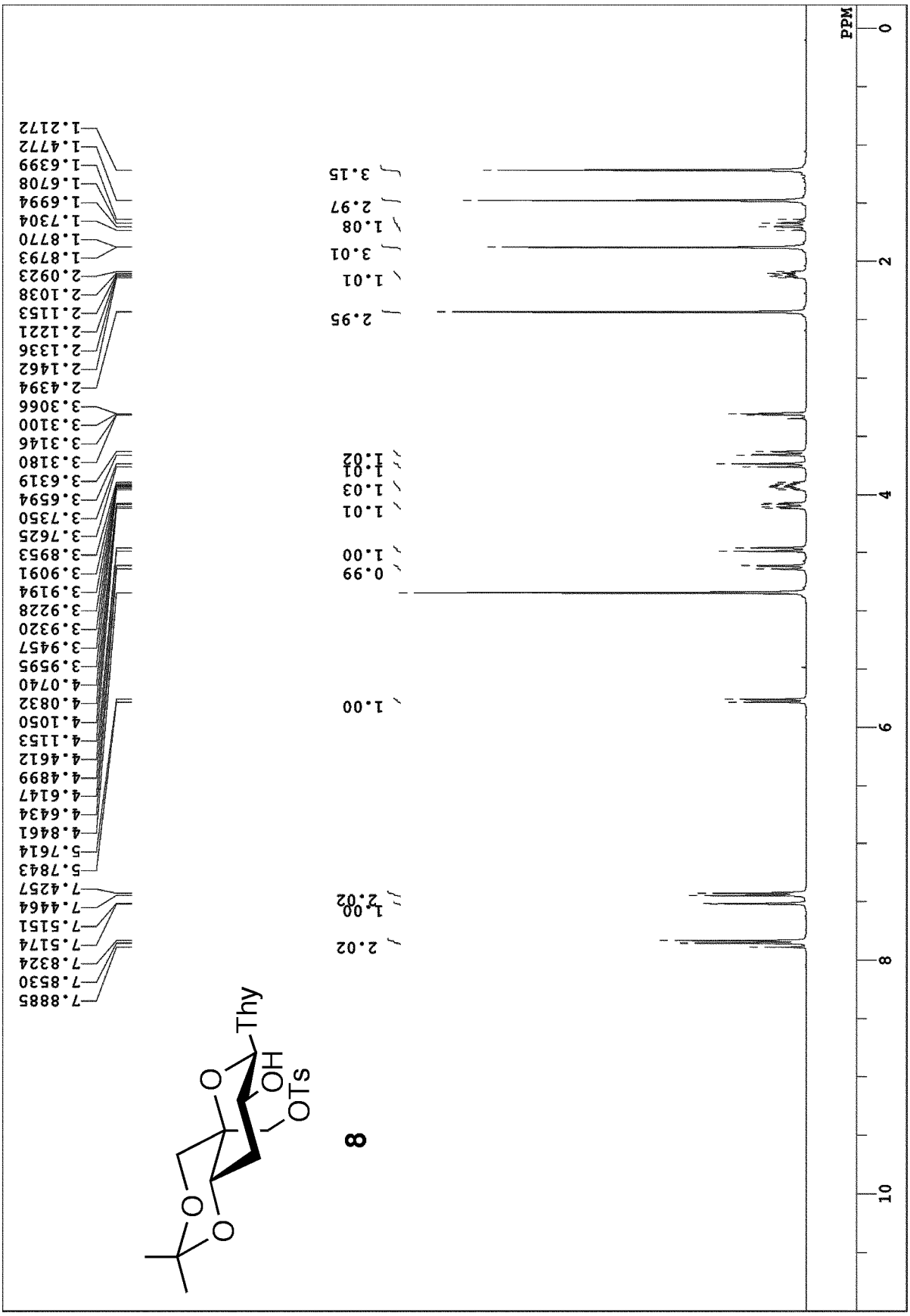
Compound **7** (^{1}H-NMR, 400 MHz, CD_3OD)



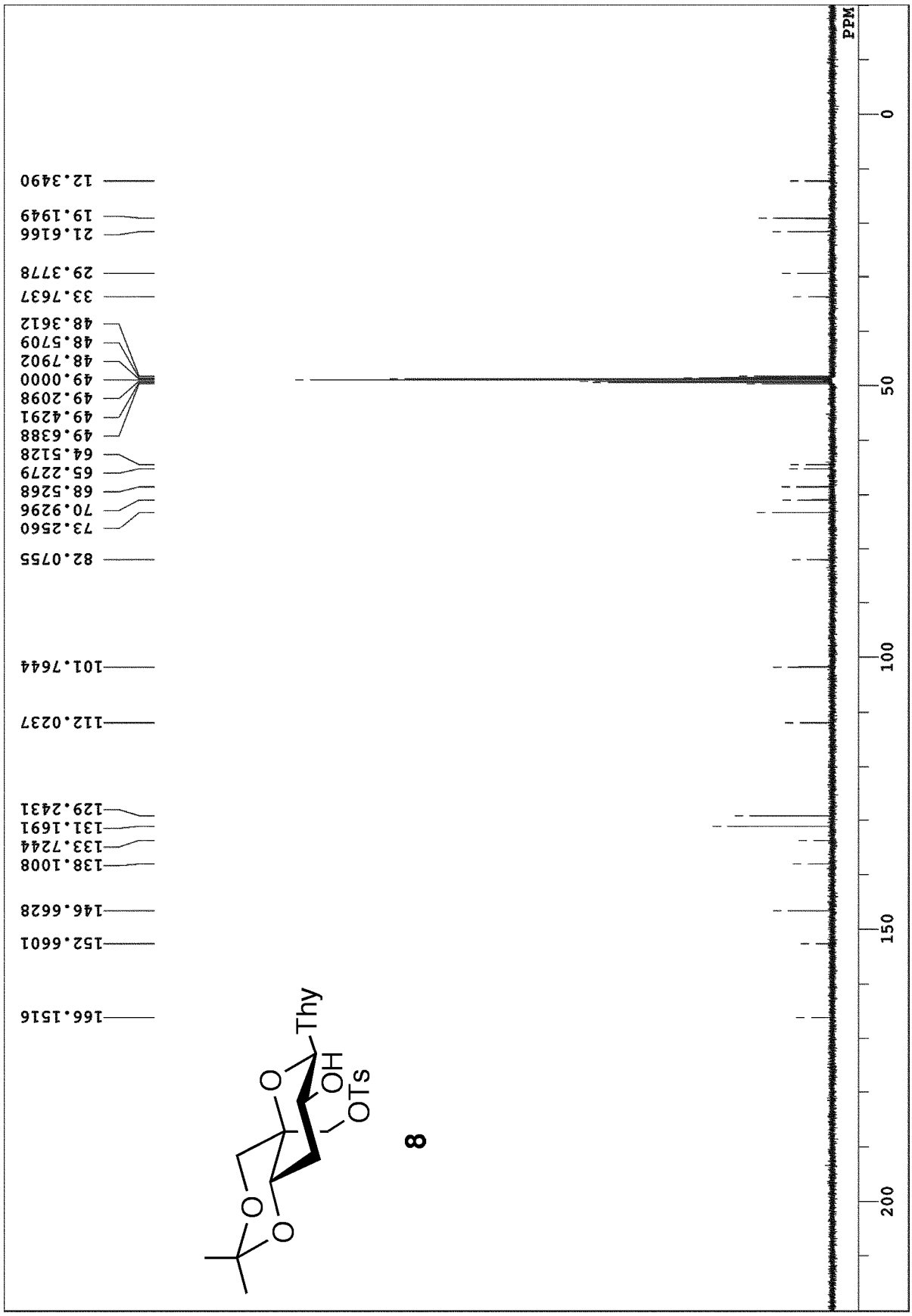
Compound **7** (^{13}C-NMR, 100.5 MHz, CD_3OD)



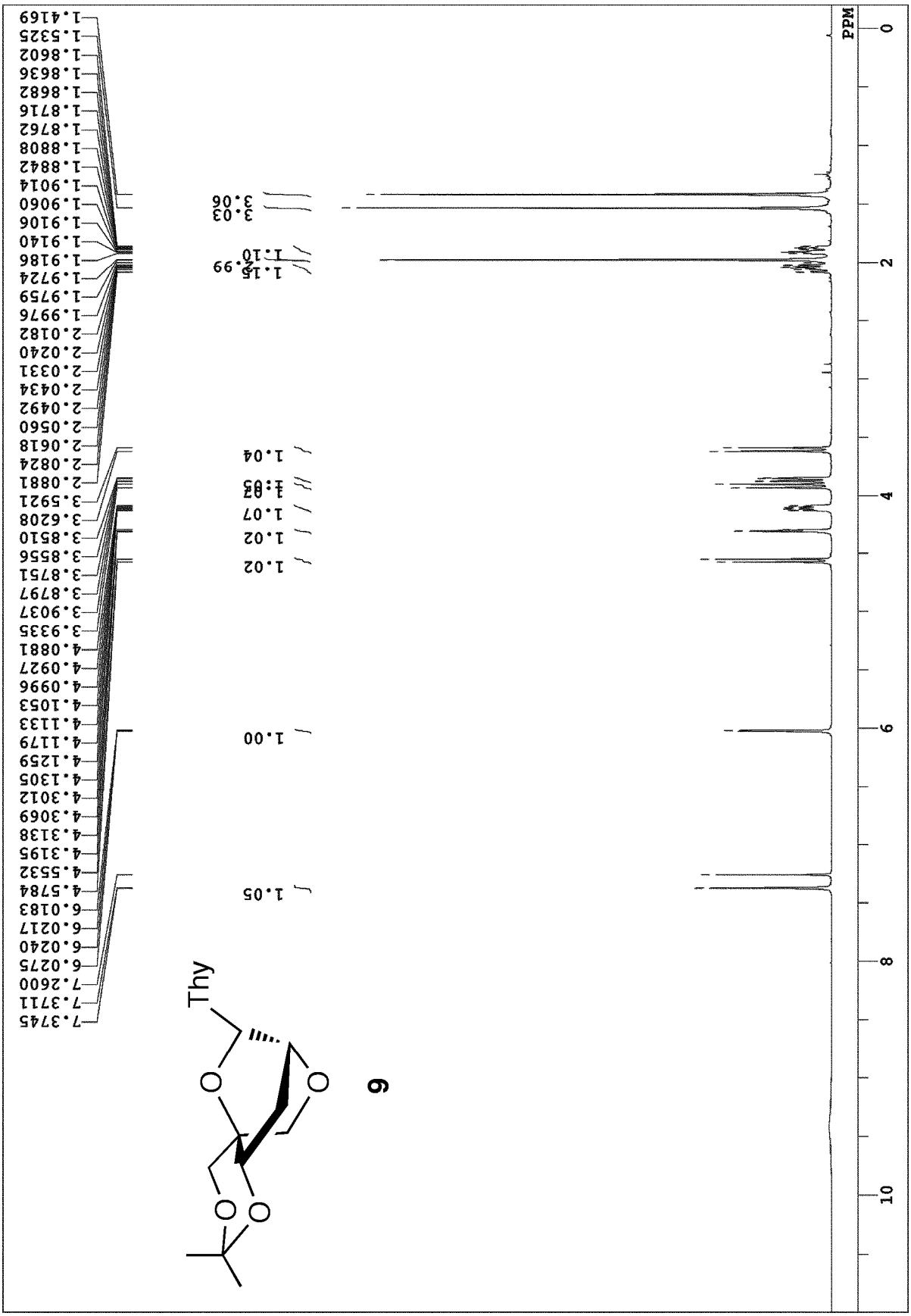
Compound **8** (^{1}H-NMR, 400 MHz, CD_3OD)



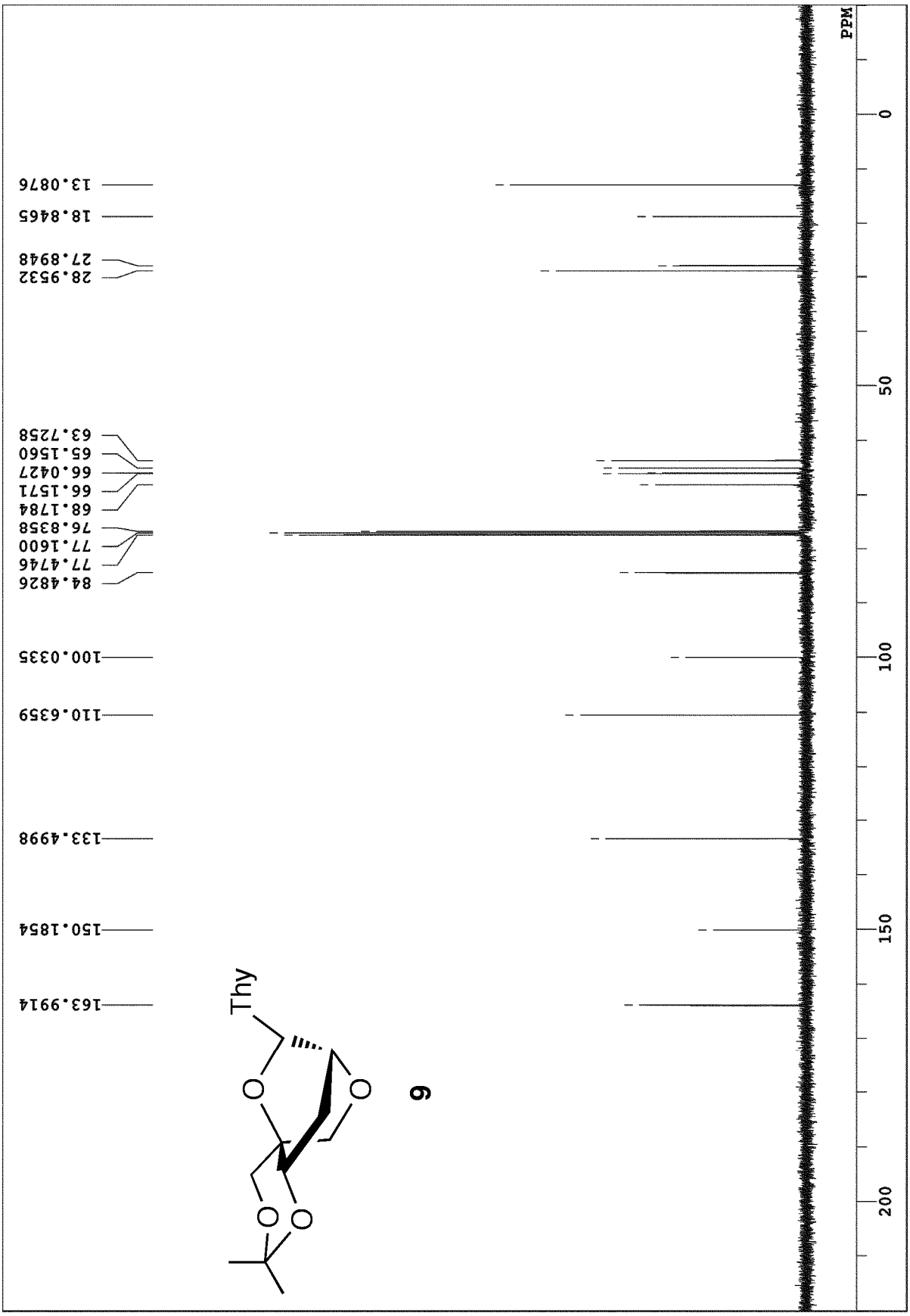
Compound **8** (^{13}C-NMR, 100.5 MHz, CD_3OD)



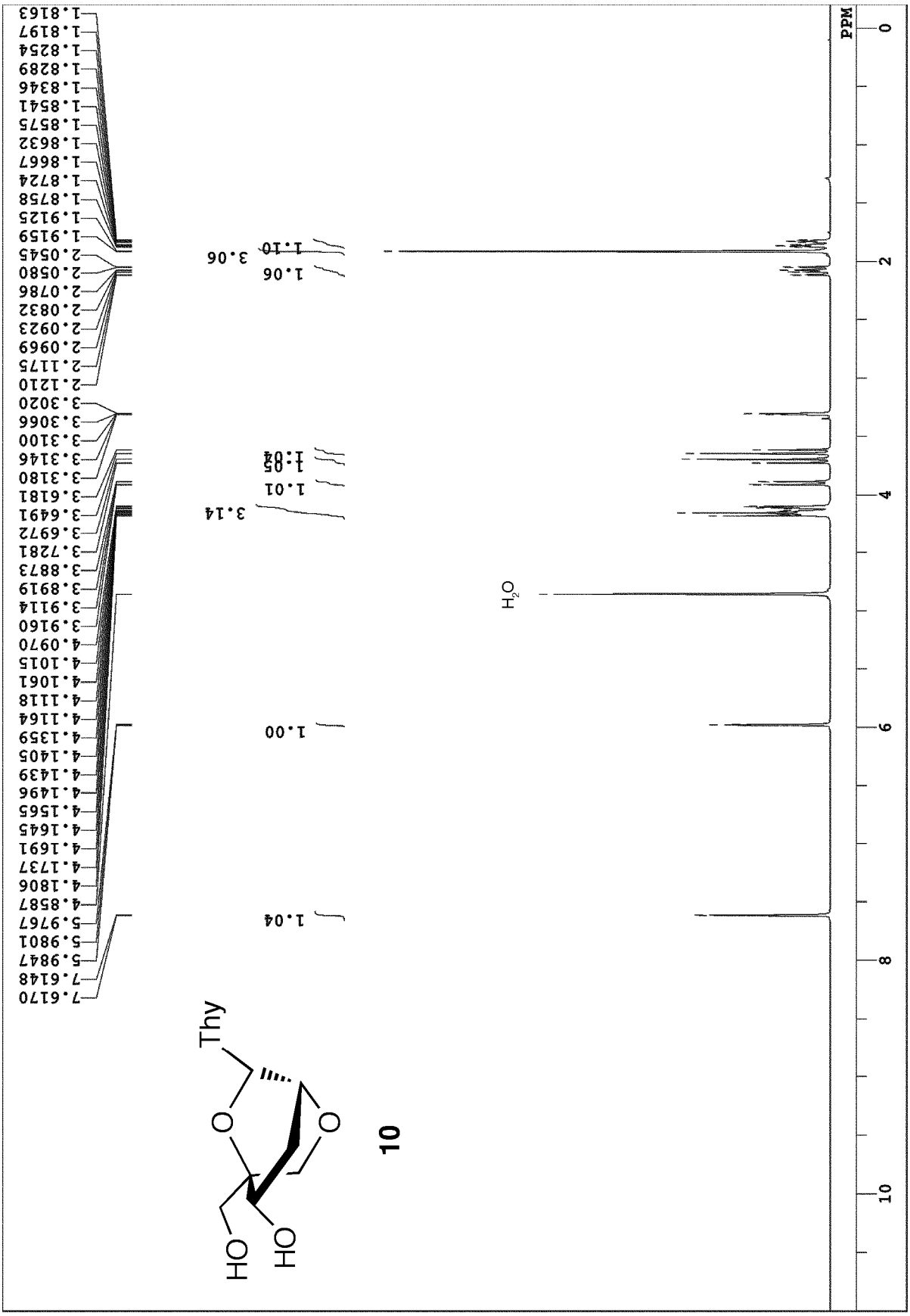
Compound **9** (^{1}H-NMR, 400 MHz, $CDCl_3$)



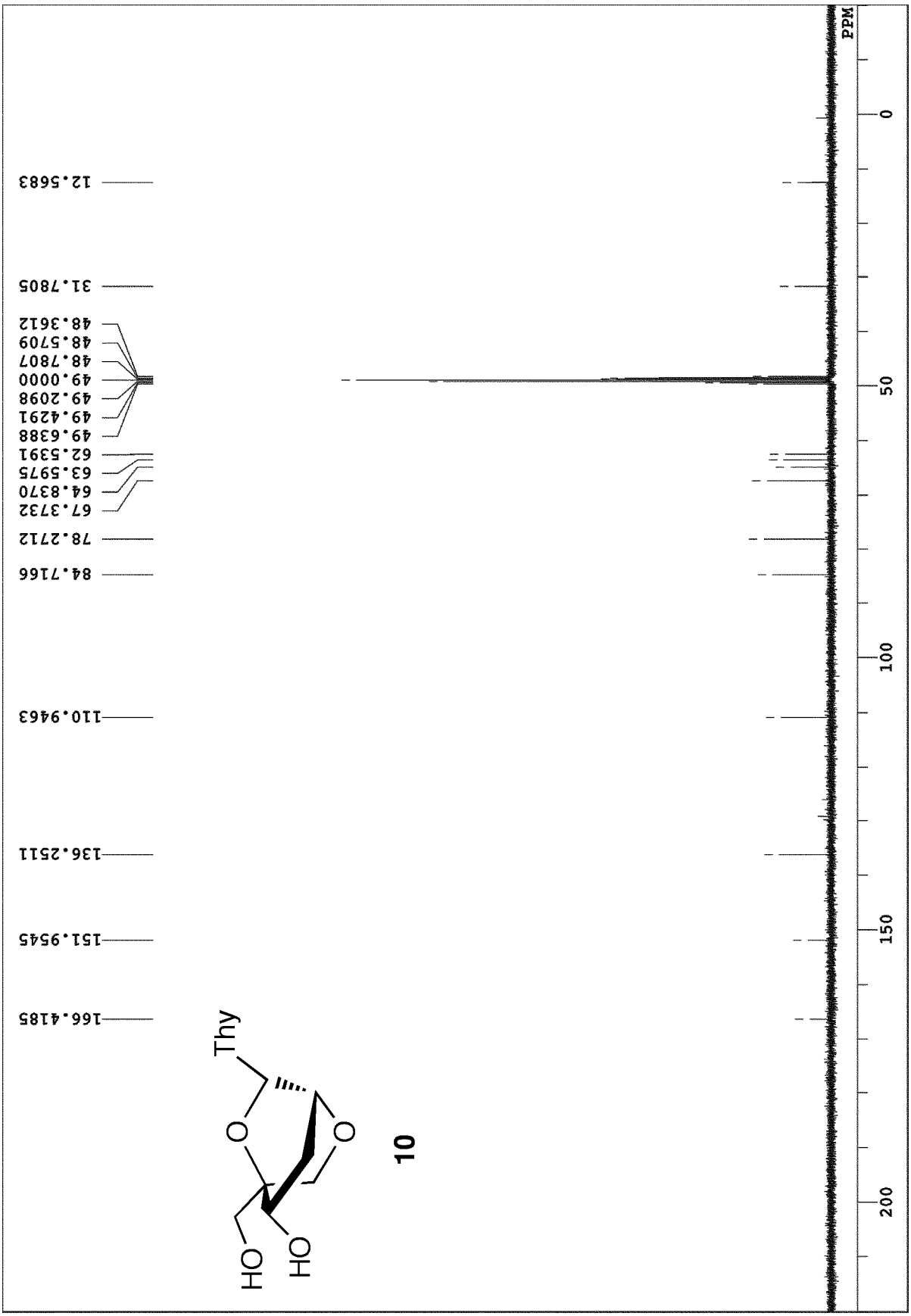
Compound **9** (^{13}C-NMR, 100.5 MHz, $CDCl_3$)



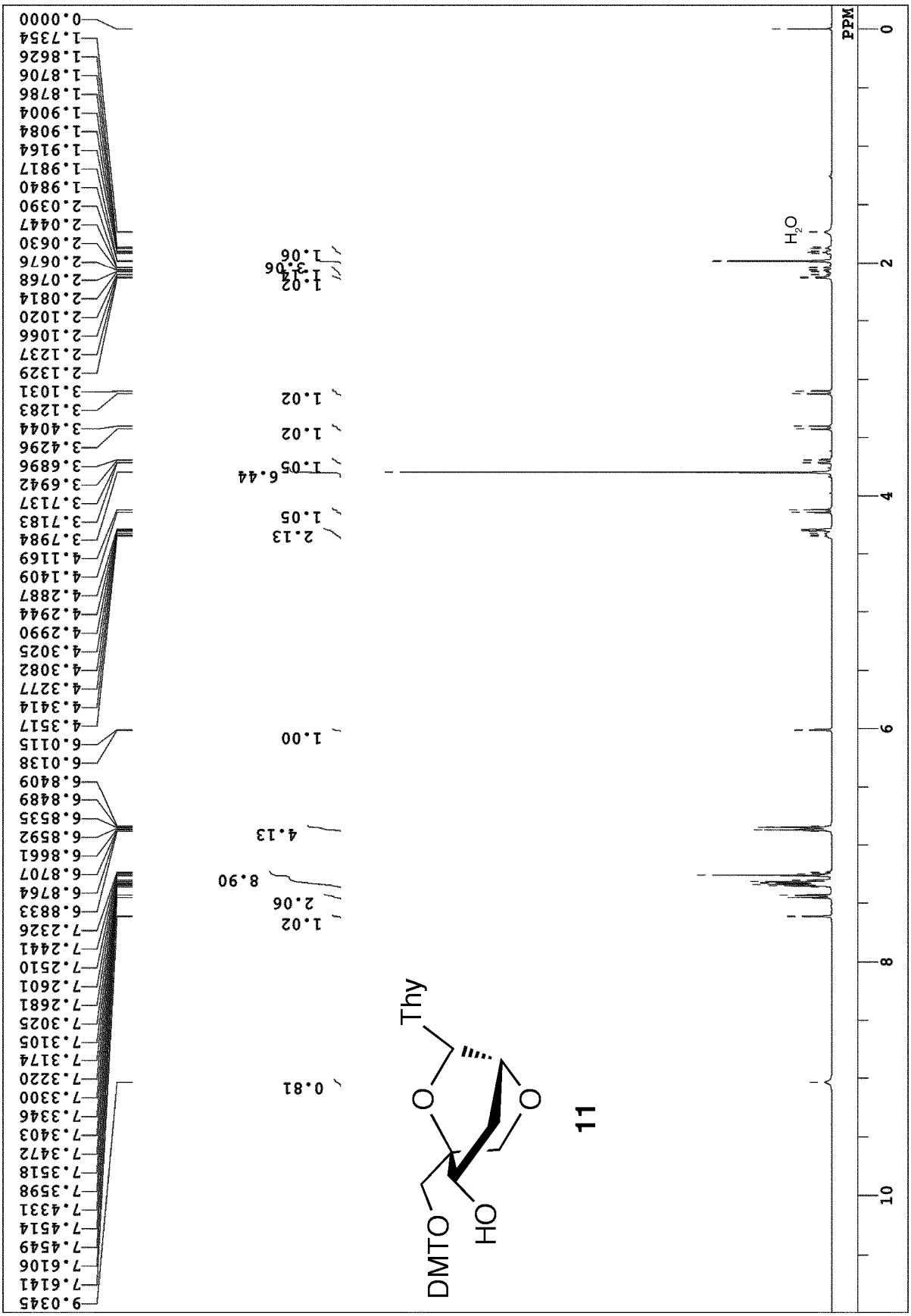
Compound **10** (^{1}H-NMR, 400 MHz, CD_3OD)



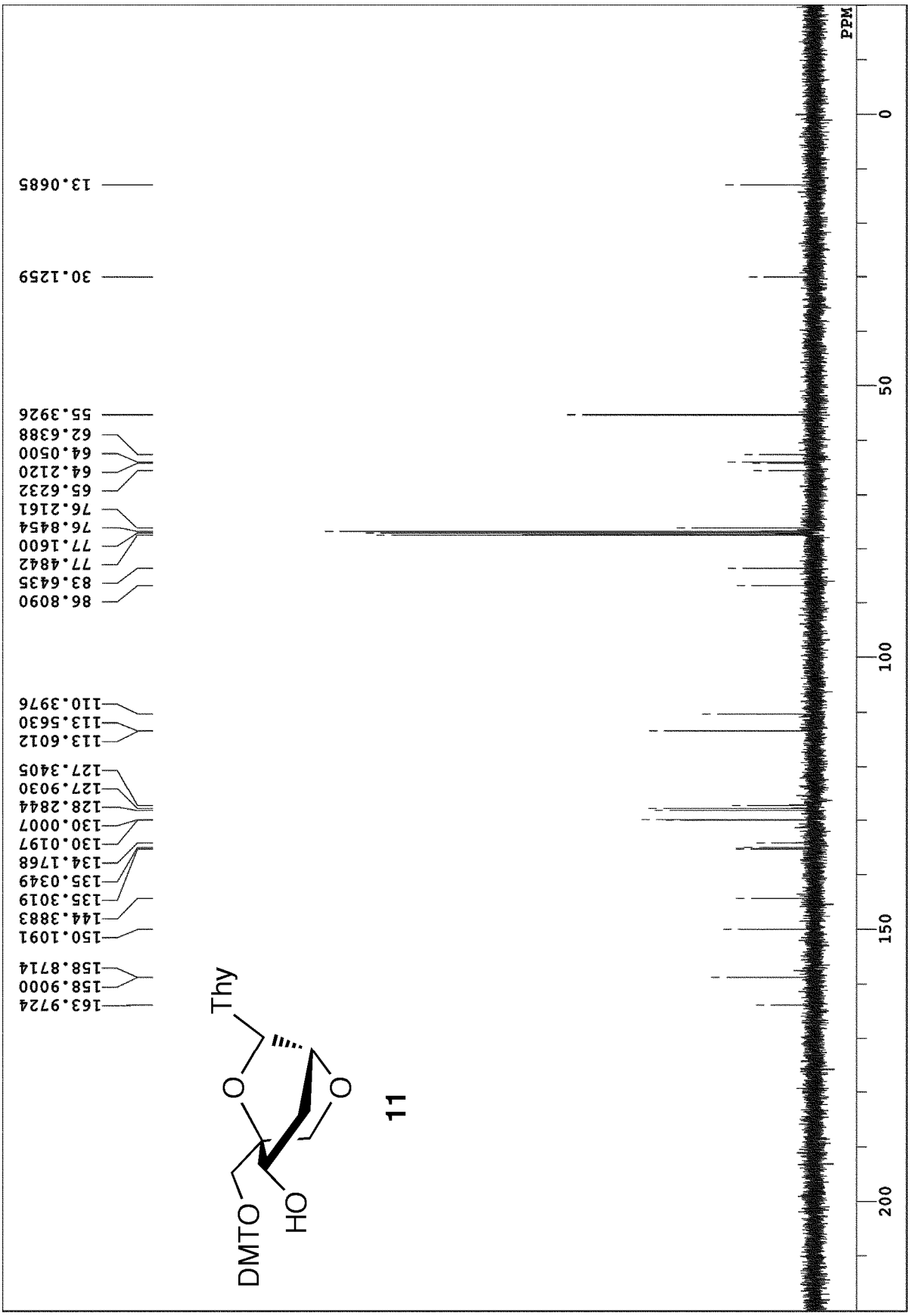
Compound **10** (^{13}C-NMR, 100.5 MHz, CD_3OD)



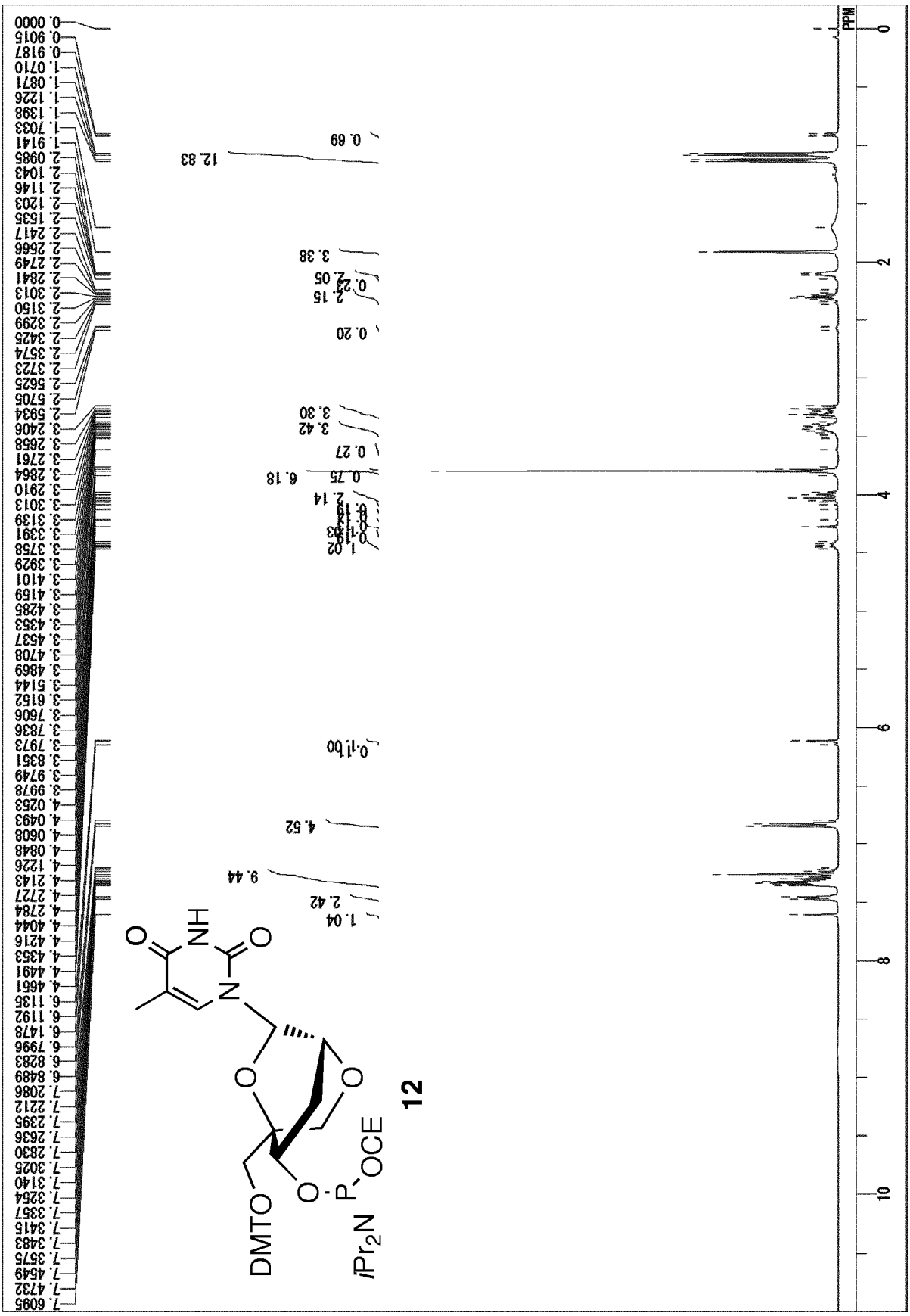
Compound **11** (^{1}H-NMR, 400 MHz, $CDCl_3$)



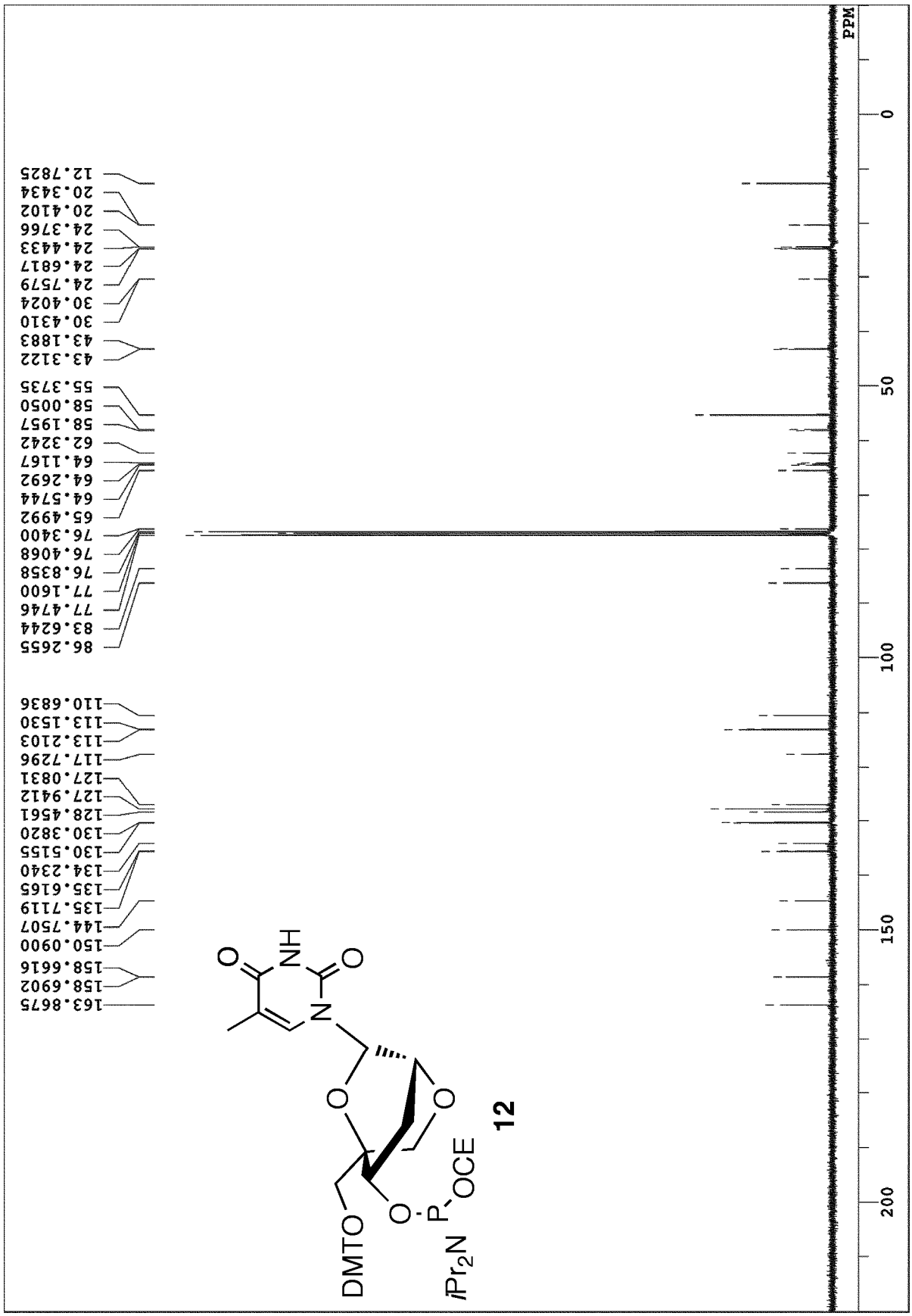
Compound **11** (^{13}C-NMR, 100.5 MHz, $CDCl_3$)



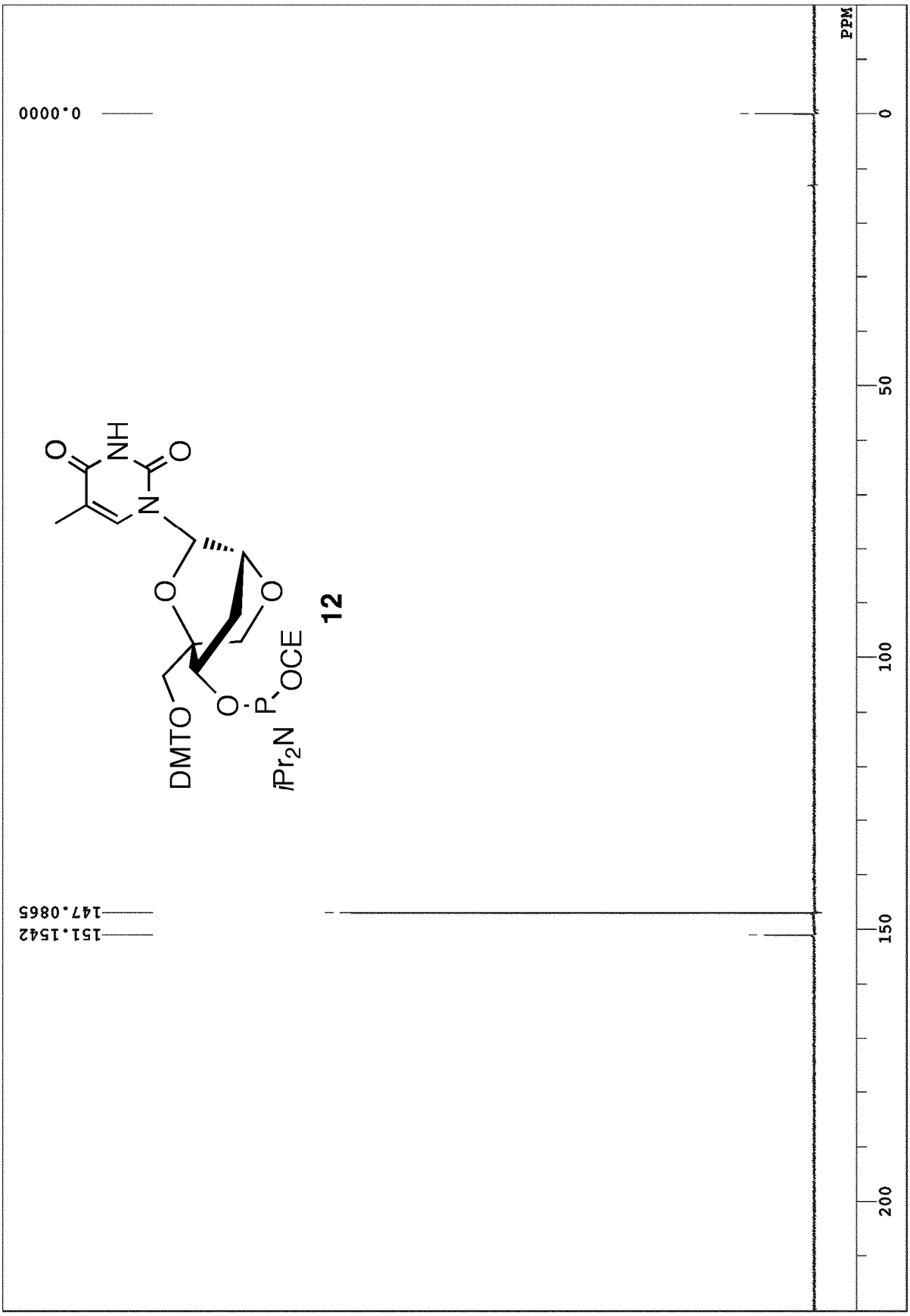
Compound **12** (^{1}H-NMR, 400 MHz, $CDCl_3$)



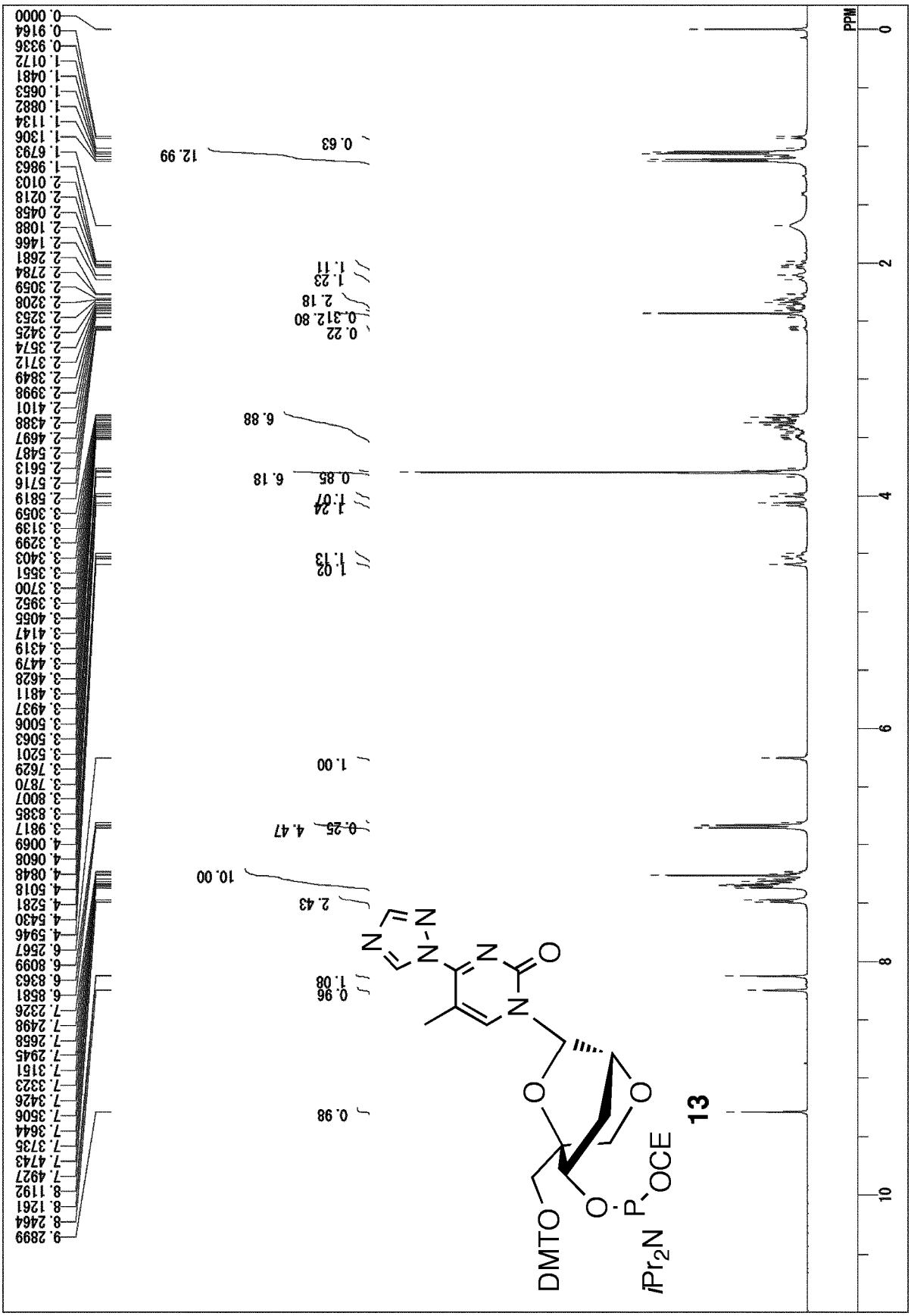
Compound **12** (^{13}C-NMR, 100.5 MHz, $CDCl_3$)



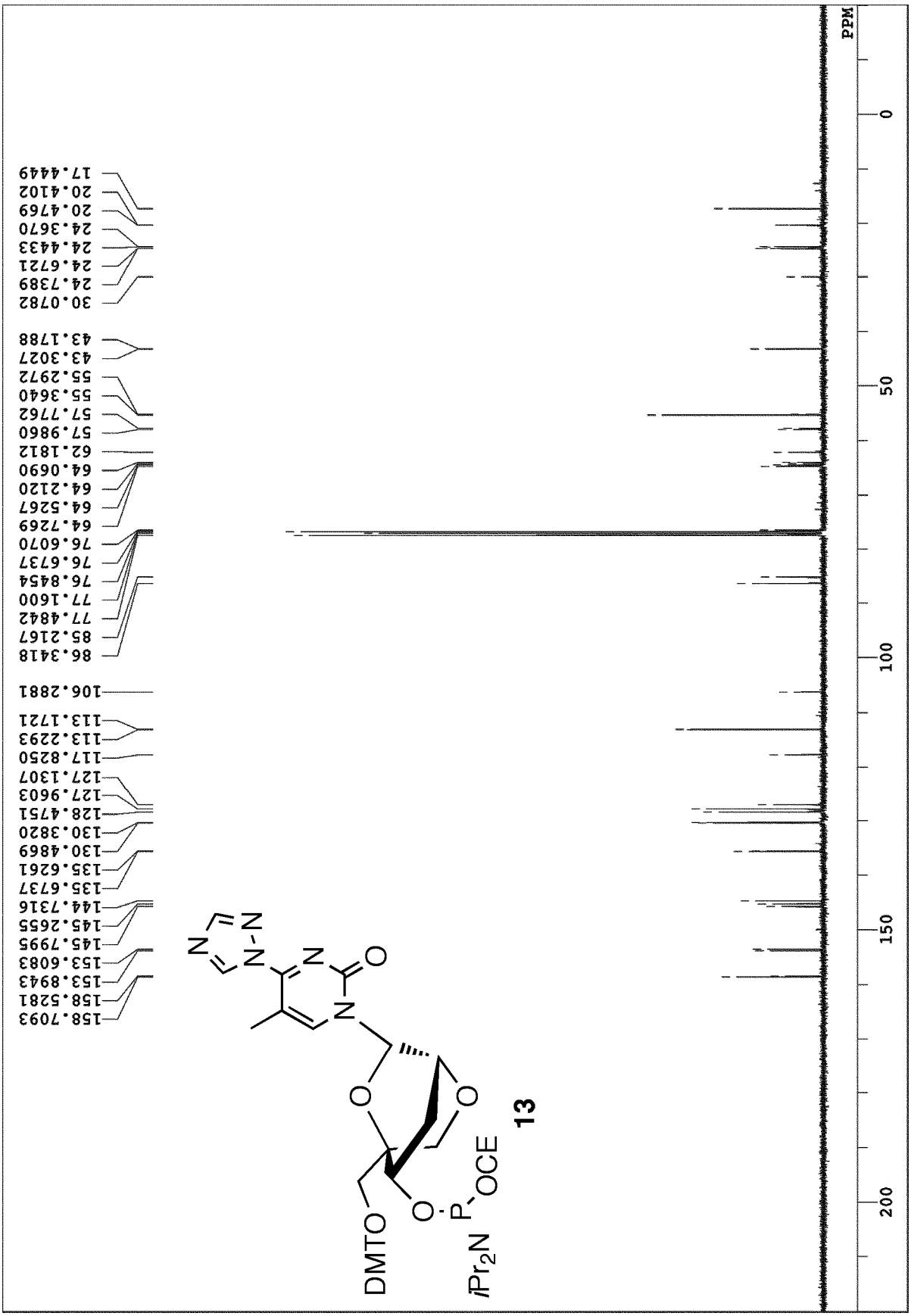
Compound **12** (^{31}P-NMR, 161.8 MHz, $CDCl_3$)



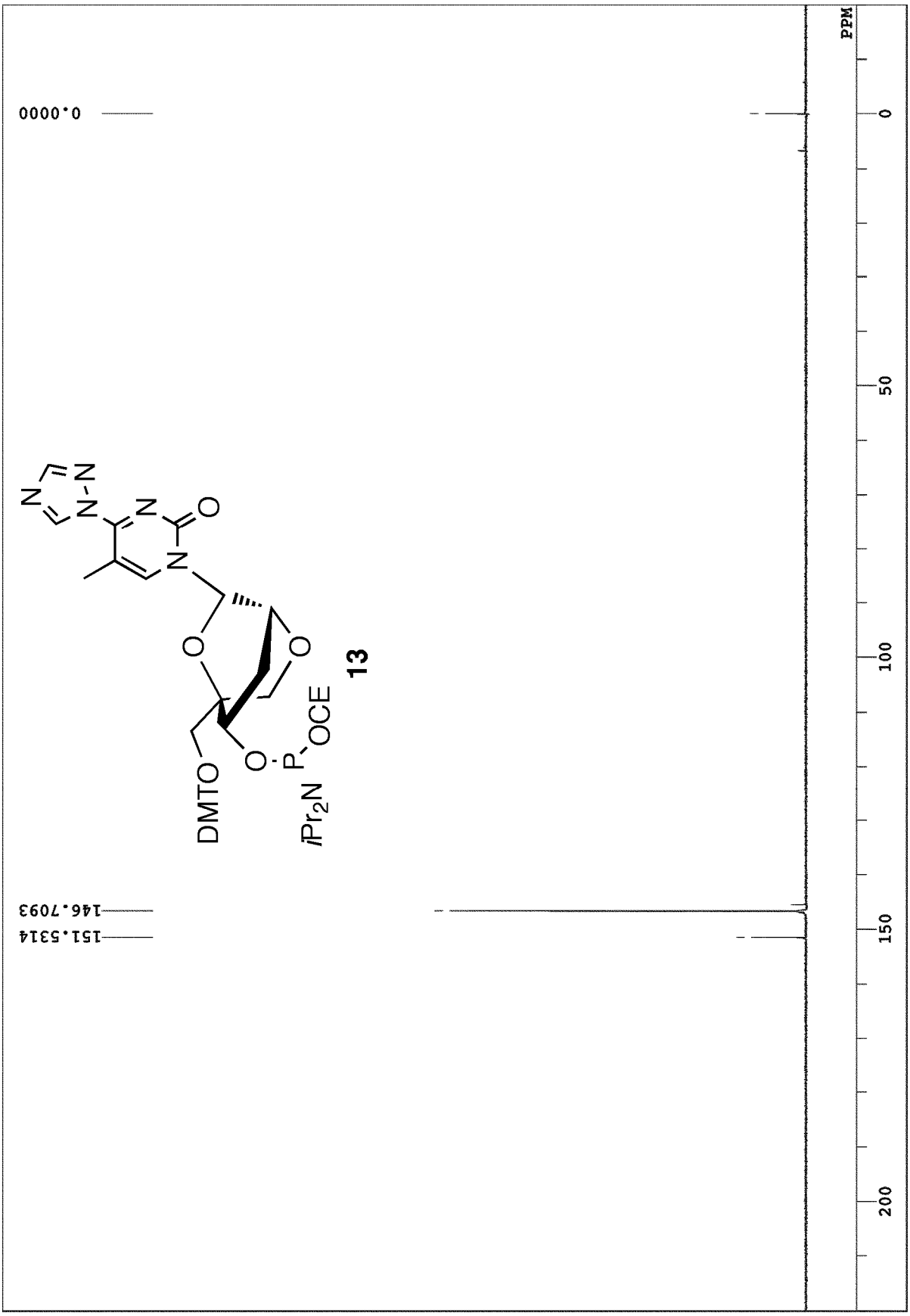
Compound **13** (^{1}H-NMR, 400 MHz, $CDCl_3$)



Compound **13** (^{13}C-NMR, 100.5 MHz, $CDCl_3$)



Compound **13** (^{31}P-NMR, 161.8 MHz, $CDCl_3$)



References

(S1) Blériot, Y.; Vadivel, S. K.; Herrera, A. J.; Greig, I. R.; Kirby, A. J.; Sinaÿ, P. *Tetrahedron* **2004**, *60*, 6813; which is the same as reference 14 in the manuscript.