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Electronic Supplementary Information for

Synthesis and Properties of 2'-*O*,4'-*C*-Spirocyclopropylene Bridged Nucleic Acid (scpBNA), an Analogue of 2',4'-BNA/LNA Bearing a Cyclopropane Ring

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Contents

1. General

Dry dichloromethane, *N,N*-dimethylformamide, tetrahydrofuran, acetonitrile and pyridine were used as purchased. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and ^{31}P NMR (162 MHz) spectra were recorded on a JEOL JNM-ECS-400 spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a JEOL JNM-AL-300 spectrometer. Chemical shift values are expressed in δ values (ppm) relative to internal tetramethylsilane (0.00 ppm), residual $CHCl_3$ (7.26 ppm) or CHD_2OD (3.31 ppm) for ^{1}H NMR, and internal tetramethylsilane (0.00 ppm), chloroform-d_1 (77.16 ppm) or methanol-d_4 (49.00 ppm) for ^{13}C NMR, and 85% H_3PO_4 (0.00 ppm) as external standard for ^{31}P NMR. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Optical rotations were recorded on a JASCO DIP-370 instrument. MALDI-TOF mass spectra of all new compounds were measured on SpiralTOF JMS-S3000. MALDI-TOF mass spectra of oligonucleotides were measured 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 flash column chromatography, Fuji Silysia PSQ-60B or FL-60D silica gel was used. For high performance liquid chromatography (HPLC), SHIMADZU LC-10AT$_{vp}$, SPD-10A$_{vp}$ and CTO-10$_{vp}$ were used.

2. Synthesis of scpBNA monomer and phosphoramidite.

3,5-Di-*O*-benzyl-4-*C*-formyl-l,2-*O*-isopropylidene-α-D-ribopentofuranose (2)

The compound **2** was prepared *via* a different procedure from the reported one (Morita, K.; Takagi, M.; Hasegawa, C.; Kaneko, M.; Tsutsumi, S.; Sone, J.; Ishikawa, T.; Imanishi, T.; Koizumi, M. *Bioorg. Med. Chem.* **2003**, *11*, 2211–2226.). To the solution of **1** (7.38 g, 18.5 mmol) in dry dichloromethane (100 mL) was added Dess-Martin periodinane (9.41 g, 22.2 mmol) at 0 ºC, and the reaction mixture was stirred at room temperature for 40 min under N_2 atmosphere. After completion of the reaction, saturated aq. $Na_2S_2O_3$ and saturated aq. $NaHCO_3$ were added, and the resulting mixture was further stirred for 10 min. The organic layer was then removed under reduced pressure, and the residual aqueous solution was extracted with Et_2O. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to afford **2** (7.61 g, quant.) as a colorless

oil.

Compound **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.60 (s, 3H), 3.61, 3.67 (AB, *J* = 11.0 Hz, 2H), 4.36 (d, *J* = 4.4 Hz, 1H), 4.46, 4.52 (AB, *J* = 12.2 Hz, 2H), 4.59, 4.71 (AB, *J* = 11.9 Hz, 2H), 4.60 (dd, *J* = 3.7, 4.4 Hz, 1H), 5.84 (d, *J* = 3.7 Hz, 1H), 7.21–7.37 (m, 10H), 9.91 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.1, 26.5, 69.1, 72.8, 73.8, 78.3, 79.6, 89.7, 104.8, 114.1, 127.7, 127.8, 128.0, 128.1, 128.4, 128.5, 137.0, 137.5, 200.0; IR (KBr): 2985, 2973, 2866, 1731, 1496, 1213, 1165, 1103, 1020, 739, 699 cm^{-1}; $[\alpha]_D^{29}$ +27.1 (c 1.03, MeOH); HRMS (MALDI) Calcd. for $C_{23}H_{26}O_6Na$ $[M+Na]^+$: 421.1622, Found 421.1620.

3,5-Di-*O*-benzyl-4-*C*-carboxy-l,2-*O*-isopropylidene-α-D-ribopentofuranose (3)

To the solution of **2** (7.61 g, 19.1 mmol) in acetonitrile (100 mL) was added aq. sodium dihydrogen orthophosphate (0.2 M, 20 mL, 3.82 mmol) and aq. hydrogen peroxide (30 wt.%, 2.3 mL, 21.0 mmol). After aq. sodium chlorite (0.75 M, 38 mL, 28.6 mmol) was added dropwise to the reaction mixture at 0 ºC, the reaction mixture was stirred at room temperature for 1 h. After addition of $Na_2S_2O_3$, the resulting mixture was stirred at room temperature for 10 min. The organic layer was then removed under reduced pressure, and the residual aqueous solution was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to afford **3** (7.61 g, 96%) as a white solid.

Compound **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.58 (s, 3H), 3.72, 3.77 (AB, *J* = 10.8 Hz, 2H), 4.30 (d, *J* = 4.5 Hz, 1H), 4.49, 4.55 (AB, *J* = 11.9 Hz, 2H), 4.65 (dd, *J* = 4.3, 4.5 Hz, 1H), 4.69, 4.80 (AB, *J* = 11.9 Hz, 2H), 5.83 (d, *J* = 4.3 Hz, 1H), 7.21–7.40 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3, 26.4, 71.8, 73.4, 74.0, 78.1, 78.5, 104.9, 114.6, 127.8, 128.0, 128.2, 128.4, 128.6, 128.7, 136.5, 137.4, 170.0; IR (KBr): 3171, 2985, 2937, 2870, 1768, 1497, 1163, 1098, 1020, 740, 698 cm^{-1}; $[\alpha]_D^{26}$ +42.3 (c 1.01, MeOH); HRMS (MALDI) Calcd. for $C_{23}H_{26}O_7Na$ $[M+Na]^+$: 437.1571, Found 437.1570.

3,5-Di-*O*-benzyl-l,2-*O*-isopropylidene-4-*C*-methoxycarbonyl-α-D-ribopentofuranose (4)

To the solution of **3** (7.61 g, 18.4 mmol) in dry *N,N*-dimethylformamide (30 mL) was added $NaHCO_3$ (15.4 g, 184 mmol) and iodomethane (2.86 mL, 45.9 mmol) at 0 ºC under N_2 atmosphere. After stirring at room temperature for 20 h, saturated aq. $Na_2S_2O_3$ was added and the resulting

mixture was extracted with Et_2O. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to afford **4** (7.35 g, 93%) as a white solid.

Compound **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 3H), 1.64 (s, 3H), 3.67, 3.82 (AB, *J* = 10.3 Hz, 2H), 3.75 (s, 3H), 4.25 (d, *J* = 5.0 Hz, 1H), 4.49, 4.54 (AB, *J* = 11.9 Hz, 2H), 4.59, 4.77 (AB, *J* = 12.2 Hz, 2H), 4.67 (dd, *J* = 4.2, 5.0 Hz, 1H), 5.89 (d, *J* = 4.2 Hz, 1H), 7.24–7.27 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.3, 27.3, 73.0, 73.7, 73.9, 79.4, 80.5, 89.7, 127.7, 127.7, 127.8, 127.9, 128.4, 128.6, 137.7, 169.4; IR (KBr): 2985, 2949, 2869, 1763, 1733, 1497, 1160, 1106, 1028, 738, 698 cm^{-1}; $[\alpha]_D^{27}$ +31.5 (c 1.00, MeOH); HRMS (MALDI) Calcd. for $C_{24}H_{28}O_7Na$ $[M+Na]^+$: 451.1727, Found 451.1732.

3,5-Di-*O*-benzyl-4-*C*-(1-hydroxycyclopropyl)-l,2-*O*-isopropylidene-α-D-ribopentofuranose (5)

To the solution of **4** (12.5 g, 29.0 mmol) in dry tetrahydrofuran (290 mL) was added tetraisopropyl orthotitanate (8.59 mL, 29.0 mmol) and 1M ethylmagnesium bromide in tetrahydrofuran (145 mL, 145 mmol) at 0 ºC under N_2 atmosphere. After stirring at room temperature for 6 h, saturated aq. NH_4Cl was added and the organic layer was then removed under reduced pressure. The residual aqueous solution was filtered through celite and extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane : AcOEt = 6 : 1) to afford **5** (6.80 g, 55%) as a yellow paste.

Compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56–0.68 (m, 3H), 1.16–1.21 (m, 1H), 1.39 (s, 3H), 1.61 (s, 3H), 3.37 (s, 1H), 3.48, 3.93 (AB, *J* = 9.8 Hz, 2H), 4.33 (d, *J* = 5.7 Hz, 1H), 4.43, 5.00 (AB, *J* = 11.6 Hz, 2H), 4.45, 4.54 (AB, *J* = 12.0 Hz, 2H), 4.84 (dd, *J* = 4.5, 5.7 Hz, 1H), 5.88 (d, *J* = 4.5 Hz, 1H), 7.26–7.39 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.64, 11.2, 27.1, 27.9, 56.3, 73.1, 73.8, 75.5, 80.3, 82.0, 89.1, 106.6, 114.6, 127.5, 127.6, 127.8, 128.0, 128.6, 128.8, 137.9, 138.2; IR (KBr): 2935, 2867, 1496, 1454, 1252, 1099, 1027, 741, 699 cm^{-1}; $[\alpha]_D^{29}$ +93.5 (c 1.02, MeOH); HRMS (MALDI) Calcd. for $C_{25}H_{30}O_6Na$ $[M+Na]^+$: 449.1935, Found 449.1939.

3,5-Di-*O*-benzyl-4-*C*-[1-(*tert*-butyldimethylsilyloxy)cyclopropyl]-l,2-*O*-isopropylidene-α-D-ribo pentofuranose (6)

To the solution of **5** (2.55 g, 5.99 mmol) in dry dichloromethane (50 mL) was added 2,6-lutidine (2.09 mL, 18.0 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (2.75 mL, 12.0 mmol) at 0 ºC under N_2 atmosphere. After stirring at room temperature for 2 h, saturated aq. $NaHCO_3$ was added and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane : AcOEt = 15 : 1 to 5 : 1) to afford **6** (2.92 g, 90%) as a yellow oil.

Compound **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ –0.06 (s, 3H), –0.02 (s, 3H), 0.57–0.77 (m, 3H), 0.75 (s, 9H), 1.20–1.25 (m, 1H), 1.34 (s, 3H), 1.43 (s, 3H), 3.46, 3.92 (AB, *J* = 9.5 Hz, 2H), 4.00 (d, *J* = 5.7 Hz, 1H), 4.42, 4.61 (AB, *J* = 12.0 Hz, 2H), 4.52, 4.86 (AB, *J* = 11.4 Hz, 2H), 4.95 (dd, *J* = 4.5, 5.7 Hz, 1H), 5.87 (d, *J* = 4.5 Hz, 1H), 7.19–7.43 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –3.4, –3.2, 7.7, 10.2, 17.8, 25.7, 27.1, 28.5, 57.5, 73.4, 73.8, 76.2, 80.0, 83.3, 90.3, 106.1, 114.5, 126.8, 126.9, 127.6, 127.8, 127.8, 128.5, 138.0, 139.2; IR (KBr): 2929, 2858, 1497, 1455, 1279, 1254, 1106, 1040, 733, 696 cm^{-1}; $[\alpha]_D^{29}$ +53.6 (c 1.01, MeOH); HRMS (MALDI) Calcd. for $C_{31}H_{44}O_6NaSi$ $[M+Na]^+$: 563.2799, Found 563.2809.

1-[3,5-Di-*O*-benzyl-4-*C*-[1-(*tert*-butyldimethylsilyloxy)cyclopropyl]-2-*O*-methanesulfonyl-β-D-ribopentofuranosyl]thymine (10)

To the solution of **6** (8.04 g, 14.9 mmol) in acetic acid (17.0 mL, 0.30 mol) was added acetic anhydride (28.2 mL, 0.30 mol) and trifluoroacetic acid (3.20 mL, 44.7 mmol) at 0 ºC. After stirring at room temperature for 5 h, saturated aq. $NaHCO_3$ was added and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product **7** (9.43 g) was used immediately for the next reaction without further purification.

To the solution of **7** (9.43 g) in dry acetonitrile (140 mL) was added thymine (5.63 g, 44.6 mmol), *N,O*-bis-trimethylsilylacetoamide (18.2 mL, 74.3 mmol) and trimethylsilyl trifluoromethanesulfonate (4.03 ml, 22.3 mmol) at room temperature under N_2 atmosphere. After refluxing for 2 h, saturated aq. $NaHCO_3$ was added and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to afford **8** (8.26 g). The crude product **8** was used for the next reaction without further

purification. For an analysis of compound **8**, a small amount of the crude product was purified by silica gel column chromatography (SiO_2, *n*-hexane : AcOEt = 3 : 1).

To the solution of **8** (8.26 g) in tetrahydrofuran (150 mL) was added aq. methylamine (40 wt.%, 30.4 mL, 0.73 mol) at 0 ºC, and the reaction mixture was stirred at room temperature for 4 h. After completion of reaction, the resulting mixture was concentrated and extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product **9** (7.50 g) was used for the next reaction without further purification. For an analysis of compound **9**, a small amount of the crude product was purified by silica gel column chromatography (SiO_2, *n*-hexane : AcOEt = 2 : 1).

To the solution of **9** (7.50 g) in dry pyridine (120 mL) was added methanesulfonyl chloride (1.43 mL, 18.5 mmol) at 0 ºC, and the reaction mixture was stirred at room temperature for 4 h under N_2 atmosphere. After addition of water, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane : AcOEt = 3 : 2) to afford **10** (7.39 g, 72%, 4 steps) as a white solid.

Compound **7**: ^{1}H NMR (300 HMz, $CDCl_3$) δ –0.07 (s, 3/2H), –0.02 (s, 3/2H), 0.00 (s, 3/2H), 0.04 (s, 3/2H), 0.59–0.83 (m, 4H), 0.75 (s, 9/2H), 0.78 (s, 9/2H), 1.92 (s, 3/2H), 1.98 (s, 3/2H), 2.01 (s, 3/2H), 2.09 (s, 3/2H), 3.44 (d, *J* = 9.9 Hz, 1/2H), 3.57 (d, *J* = 9.9 Hz, 1/2H), 3.91 (d, *J* = 9.9 Hz, 1/2H), 3.94 (d, *J* = 10.2 Hz, 1/2H), 4.30 (d, *J* = 5.1 Hz, 1/2H), 4.40–4.59 (m, 7/2H), 4.67 (d, *J* = 2.4 Hz, 1/2H), 4.71 (d, *J* = 2.4 Hz, 1/2H), 4.88 (d, *J* = 3.9 Hz, 1/2H), 4.92 (d, *J* = 3.6 Hz, 1/2H), 5.44 (t, *J* = 5.1 Hz, 1/2H), 5.57 (d, *J* = 5.7 Hz, 1/2H), 6.20 (d, *J* = 5.1 Hz, 1/2H), 6.39 (d, *J* = 5.1 Hz, 1/2H), 7.26–7.39 (m, 10H); HRMS (MALDI) Calcd. for $C_{32}H_{44}O_8NaSi$ $[M+Na]^+$: 607.2698, Found 607.2701.

Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$) δ –0.01 (s, 3H), 0.03 (s, 3H), 0.65–0.74 (m, 2H), 0.78 (s, 9H), 0.95–1.03 (m, 2H), 1.56 (d, *J* = 1.2 Hz, 3H), 1.97 (s, 3H), 3.63, 4.03 (AB, *J* = 9.8 Hz, 2H), 4.46 (d, *J* = 5.0 Hz, 1H), 4.50, 4.95 (AB, *J* = 11.3 Hz, 2H), 4.62, 4.72 (AB, *J* = 11.6 Hz, 2H), 5.50 (dd, *J* = 5.0, 8.9 Hz, 1H), 6.23 (d, *J* = 8.9 Hz, 1H), 7.26–7.44 (m, 10H), 7.66 (d, *J* = 1.2 Hz, 1H), 7.86 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ –3.3, –3.0, 7.3, 10.7, 12.3, 18.0, 20.8, 25.8, 58.1, 74.1, 74.4, 75.3, 75.3, 80.9, 84.9, 87.8, 111.6, 127.4, 127.6, 128.0, 128.3, 128.4, 129.0, 136.1, 137.1, 138.7, 150.8, 163.6, 170.8; IR (KBr): 3499, 2955, 2929, 1714, 1683, 1470, 1274, 1233, 1127, 1075,

1036, 733, 699 cm^{-1}; $[\alpha]_D^{24}$ –46.9 (c 0.99, MeOH); HRMS (MALDI) Calcd. for $C_{35}H_{46}N_2O_8NaSi$ $[M+Na]^+$: 673.2916, Found 673.2917.

Compound **9**: ^1H NMR (300 MHz, $CDCl_3$) δ 0.03 (s, 3H), 0.06 (s, 3H), 0.68–0.79 (m, 3H), 0.81 (s, 9H), 0.94–0.98 (m, 1H), 1.60 (d, *J* = 1.4 Hz, 3H), 2.86 (d, *J* = 12.0 Hz, 1H), 3.60, 4.02 (AB, *J* = 9.6 Hz, 2H), 4.21 (d, *J* = 5.4 Hz, 1H), 4.51–4.60 (m, 3H), 4.69 (B part of an AB system, *J* = 11.7 Hz, 1H), 5.20 (B part of an AB system, *J* = 10.8 Hz, 1H), 5.83 (d, *J* = 8.1 Hz, 1H), 7.32–7.42 (m, 10H), 7.59 (d, *J* = 1.4 Hz, 1H), 8.38 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –3.2, –2.9, 7.4, 10.8, 12.3, 18.1, 25.9, 58.2, 74.1, 74.5, 74.7, 75.8, 82.7, 86.8, 87.8, 111.4, 127.9, 128.0, 128.2, 128.5, 128.7, 129.0, 136.1, 137.1, 137.9, 151.1, 163.5; IR (KBr): 3422, 2955, 2929, 1699, 1470, 1277, 1254, 1129, 1087, 1036, 751, 698 cm^{-1}; $[\alpha]_D^{26}$ –45.1 (c 1.00, MeOH); HRMS (MALDI) Calcd. for $C_{33}H_{44}N_2O_7NaSi$ $[M+Na]^+$: 631.2810, Found 631.2814.

Compound **10**: ^1H NMR (300 MHz, $CDCl_3$) δ –0.01 (s, 3H), 0.04 (s, 3H), 0.58–1.02 (m, 4H), 0.78 (s, 9H), 1.56 (s, 3H), 2.89 (s, 3H), 3.63, 4.03 (AB, *J* = 9.8 Hz, 2H), 4.37 (d, *J* = 4.8 Hz, 1H), 4.63, 4.71 (AB, *J* = 11.6 Hz, 2H), 4.78, 4.94 (AB, *J* = 11.0 Hz, 2H), 5.58 (dd, *J* = 4.8, 8.7 Hz, 1H), 6.23 (d, *J* = 8.7 Hz, 1H), 7.26–7.41 (m, 10H), 7.60 (s, 1H), 8.13 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –3.4, –3.1, 7.1, 10.6, 12.2, 17.9, 25.7, 38.2, 57.9, 73.9, 74.0, 75.1, 77.2, 81.0, 84.5, 87.6, 111.9, 127.4, 127.5, 127.9, 128.2, 128.4, 128.9, 135.3, 136.7, 138.3, 150.6, 163.2; IR (KBr): 3414, 2926, 1696, 1454, 1363, 1127, 1072, 1038, 748, 698 cm^{-1}; $[\alpha]_D^{31}$ –48.2 (c 0.96, MeOH); HRMS (MALDI) Calcd. for $C_{34}H_{46}N_2O_9NaSiS$ $[M+Na]^+$: 709.2586, Found 709.2582.

1-[(1*R*,4*R*,6*R*,7*S*)-7-benzyloxy-4-benzyloxymethyl-2,5-dioxaspiro(bicyclo[2.2.1]heptane-3,1'-cyclopropan)-6-yl]thymine (13)

To the solution of **10** (3.19 g, 4.64 mmol) in tetrahydrofuran/ ethanol (150 mL, 3 : 2) was added 4M aq. sodium hydroxide (60 mL, 0.23 mol) at 0 ºC, and the reaction mixture was stirred at room temperature for 12 h. After addition of aq. HCl, the resulting mixture was concentrated and extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product **11** (2.71 g) was used for the next reaction without further purification. For an analysis of compound **11**, a small amount of the crude product was purified by column chromatography (SiO_2, *n*-hexane : AcOEt = 1.7 : 1).

To the solution of **11** (2.71 g) in dry pyridine (50 mL) was added trifluoromethanesulfonic

anhydride (3.65 mL, 22.3 mmol) at 0 ºC, and the reaction mixture was stirred at room temperature for 12 h under N_2 atmosphere. After addition of water, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product **12** (4.12 g) was used immediately for the next reaction without further purification.

To the solution of **12** (4.12 g) in tetrahydrofuran (250 mL) was added 1M tetrabutylammonium fluoride in tetrahydrofuran (13.9 mL, 13.9 mmol) at 0 ºC, and the reaction mixture was stirred at room temperature for 2 h. After completion of reaction, the reaction mixture was concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane : AcOEt = 3 : 2) to afford **13** (710 mg, 32%, 3 steps) as a white solid.

Compound **11**: ^{1}H NMR (400 MHz, $CDCl_3$) δ –0.06 (s, 3H), 0.00 (s, 3H), 0.57–0.91 (m, 4H), 0.74 (s, 9H), 1.80 (d, *J* = 0.9 Hz, 3H), 3.81, 4.15 (AB, *J* = 9.9 Hz, 2H), 4.15 (s, 1H), 4.22 (dd, *J* = 3.7, 11.9 Hz, 1H), 4.63, 4.76 (AB, *J* = 11.5 Hz, 2H), 4.67, 4.72 (AB, *J* = 11.9 Hz, 2H), 4.96 (d, *J* = 11.9 Hz, 1H), 6.03 (d, *J* = 3.7 Hz, 1H), 7.26–7.44 (m, 11H), 8.13 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –3.4, –3.2, 7.9, 10.9, 12.6, 17.8, 25.6, 58.1, 73.2, 74.3, 74.4, 74.5, 86.9, 87.2, 87.4, 108.8, 127.0, 127.5, 128.2, 128.4, 129.0, 129.0, 135.6, 137.4, 137.9, 149.9, 163.5; IR (KBr): 2954, 1703, 1669, 1472, 1286, 1254, 1097, 1042, 738, 696 cm^{-1}; $[\alpha]_D^{30}$ +36.3 (c 1.00, MeOH); HRMS (MALDI) Calcd. for $C_{33}H_{44}N_2O_7NaSi$ $[M+Na]^+$: 631.2810, Found 631.2813.

Compound **12**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.07 (d, *J* = 0.9 Hz, 3H), 0.66–0.88 (m, 13H), 1.25 (s, 3H), 3.50, 3.62 (AB, *J* = 7.7 Hz, 2H), 4.45, 4.56 (AB, *J* = 8.7 Hz, 2H), 4.60 (d, *J* = 2.7 Hz, 1H), 4.64, 4.75 (AB, *J* = 8.7 Hz, 2H), 5.58 (dd, *J* = 2.7, 4.2 Hz, 1H), 6.49 (d, *J* = 4.2 Hz, 1H), 7.29–7.41 (m, 10H), 7.46 (d, *J* = 0.6 Hz, 1H), 8.08 (s, 1H).

Compound **13**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.65–0.76 (m, 2H), 0.91–1.01 (m, 2H), 1.62 (d, *J* = 0.9 Hz, 3 H), 3.50, 3.63 (AB, *J* = 11.0 Hz, 2H), 4.04 (s, 1H), 4.51–4.59 (m, 4H), 4.70 (B part of an AB system, *J* = 12.0 Hz, 1H), 5.73 (s, 1H), 7.26–7.39 (m, 10H), 7.51 (d, *J* = 0.9 Hz, 1H), 8.33 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 5.3, 9.9, 12.4, 64.1, 68.4, 72.2, 74.0, 77.1, 87.1, 87.6, 110.3, 127.7, 127.8, 127.8, 128.2, 128.6, 128.7, 128.7, 135.1, 137.3, 137.5, 150.0, 164.1; IR (KBr): 3512, 3031, 1693, 1455, 1269, 1108, 1054, 761, 738, 699 cm^{-1}; $[\alpha]_D^{22}$ +55.3 (c 1.00, MeOH); HRMS (MALDI) Calcd. for $C_{27}H_{28}N_2O_6Na$ $[M+Na]^+$: 499.1840, Found 499.1829.

2,2'-Anhydro-1-[3,5-di-*O*-benzyl-4-*C*-(1-hydroxycyclopropyl)-β-D-arabinopentofuranosyl]thymine (14)

To the solution of **10** (459 mg, 0.67 mmol) in tetrahydrofuran (25 mL) was added 1M tetrabutylammoniumfluoride in tetrahydrofuran (0.67 mL, 0.67 mmol) at 0 ºC, and the reaction mixture was stirred at room temperature for 5 h. After addition of water, the resulting mixture was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, $CHCl_3$: CH_3OH = 50 : 1 to 20 : 1) to afford **14** (290 mg, 91%) as a white solid.

Compound **14**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.65–0.75 (m, 4H), 1.92 (d, *J* = 0.9 Hz, 3H), 3.24 (s, 1H), 3.31, 3.60 (AB, *J* = 10.5 Hz, 2H), 4.24, 4.33 (AB, *J* = 12.2 Hz, 2H), 4.59–4.63 (m, 2H), 4.85 (B part of an AB system, *J* = 11.7 Hz, 1H), 5.36 (dd, *J* = 2.1, 6.2 Hz, 1H), 6.15 (d, *J* = 6.2 Hz, 1H), 7.08–7.12 (m, 3H), 7.26–7.40 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.0, 10.8, 14.2, 56.5, 70.9, 73.3, 73.8, 85.9, 87.0, 89.7, 90.8, 118.9, 128.0, 128.1, 128.1, 128.5, 128.7, 129.0, 130.2, 136.1, 136.9, 159.6, 172.6; IR (KBr): 3330, 3069, 2923, 1665, 1633, 1556, 1487, 1128, 1087, 736, 700 cm^{-1}; $[\alpha]_D^{26}$ –2.24 (c 1.00, MeOH); HRMS (MALDI) Calcd. for $C_{27}H_{29}N_2O_6$ $[M+H]^+$: 477.2020, Found 477.2024.

1-[(1*R*,4*R*,6*R*,7*S*)-7-benzyloxy-4-benzyloxymethyl-2,5-dioxaspiro(bicyclo[2.2.1]heptane-3,1'-cyclopropan)-6-yl]thymine (13)

To the solution of **14** (1.62 g, 3.40 mmol) in *N,N*-dimethylformamide (35 mL) was added potassium carbonate (1.41 g, 10.2 mmol) at 0 ºC, and the reaction mixture was stirred at 90 ºC for 20 h. After addition of water, the resulting mixture was extracted with Et_2O. The combined organic layer was washed with water 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 afford **13** (1.23 g, 77%) as a white solid.

1-[(1*R*,4*R*,6*R*,7*S*)-2,5-Dioxaspiro(bicyclo[2.2.1]heptane-3,1'-cyclopropan)-7-hydroxy-4-hydroxymethyl-6-yl]thymine (15)

To the solution of **13** (2.58 g, 5.46 mmol) in AcOEt (50 mL) was added palladium hydroxide 20% on carbon (1.24 g). The reaction flask was degassed a few times with H_2 and the reaction mixture

was stirred at room temperature for 1 h under H_2 atmosphere. After completion of reaction, the reaction mixture was filtered, washed by AcOEt and concentrated. The crude product was purified by column chromatography (SiO_2, *n*-hexane : AcOEt = 1 : 5) to afford **15** (1.53 g, 95%) and **16** (70 mg, 5%) as white solids.

Compound **15**: ^{1}H NMR (300 MHz, CD_3OD) δ 0.70–0.92 (m, 4H), 1.89 (d, *J* = 1.1 Hz, 3H), 3.56, 3.74 (AB, *J* = 12.8 Hz, 2H), 4.19 (s, 1H), 4.32 (s, 1H), 5.63 (s, 1H), 7.79 (d, *J* = 1.1 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 5.1, 9.9, 12.6, 56.8, 68.7, 71.9, 81.0, 88.1, 89.9, 110.7, 137.0, 151.9, 166.5; IR (KBr): 3479, 3076, 1695, 1472, 1269, 1105, 1041 cm^{-1}; $[\alpha]_D^{20}$ +25.2 (c 1.01, MeOH); HRMS (MALDI) Calcd. for $C_{13}H_{16}N_2O_6Na$ $[M+Na]^+$: 319.0901, Found 319.0882.

Compound **16**: ^{1}H NMR (300 MHz, CD_3OD) δ 0.96–1.01 (m, 6H), 1.89 (d, *J* = 1.2 Hz, 3H), 2.29–2.41 (m, 1H), 3.65, 3.73 (AB, *J* = 11.6 Hz, 2H), 4.15 (d, *J* = 5.3 Hz, 1H), 4.53 (dd, *J* = 5.3, 8.3 Hz, 1H), 5.93 (d, *J* = 8.3 Hz, 1H), 7.95 (d, *J* = 1.2 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 12.5, 17.4, 18.9, 32.3, 63.6, 74.1, 75.6, 88.5, 92.0, 111.8, 138.9, 153.2, 166.4; IR (KBr): 3375, 2968, 1692, 1474, 1279, 1114, 1087 cm^{-1}; $[\alpha]_D^{30}$ –25.7 (c 1.04, MeOH); HRMS (MALDI) Calcd. for $C_{13}H_{20}N_2O_6Na$ $[M+Na]^+$: 323.1214, Found 323.1212.

1-[(1*R*,4*R*,6*R*,7*S*)-2,5-Dioxaspiro(bicyclo[2.2.1]heptane-3,1'-cyclopropan)-4-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-7-hydroxy-6-yl]thymine (17)

To the solution of **15** (873 mg, 2.95 mmol) in dry pyridine (60 mL) was added 4,4'-dimethoxytrityl chloride (1.50 g, 4.42 mmol) at 0 ºC, and the reaction mixture was stirred at room temperature for 9 h under N_2 atmosphere. After addition of water, the resulting mixture was extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, 0.5% triethylamine in *n*-hexane : AcOEt = 1 : 1 to 1 : 5) to afford **17** (1.72 g, 97%) as a white solid.

Compound **17**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.48–0.54 (m, 1H), 0.74–0.96 (m, 3H), 1.73 (s, 3H), 2.18 (d, *J* = 9.6 Hz, 1H), 3.16, 3.33 (AB, *J* = 11.0 Hz, 2H), 3.79 (s, 3H), 3.80 (s, 3H), 4.30 (d, *J* = 9.6 Hz, 1H), 4.43 (s, 1H), 5.76 (s, 1H), 6.85 (d, *J* = 8.4 Hz, 4H), 7.22–7.35 (m, 7H), 7.45 (d, *J* = 7.8 Hz, 2H), 7.65 (s, 1H), 8.39 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 5.3, 9.6, 12.7, 55.3, 58.0, 68.0, 72.6, 79.7, 86.8, 87.0, 88.0, 110.6, 113.4, 127.2, 128.1, 128.2, 130.1, 130.2, 134.8, 135.2, 135.4, 144.4, 150.1, 158.8, 164.3; IR (KBr): 3430, 2933, 1696, 1509, 1254, 1177, 1053, 829, 757 cm^{-1};

$[\alpha]_D^{21}$ –16.2 (c 1.00, MeOH); HRMS (MALDI) Calcd. for $C_{34}H_{34}N_2O_8Na$ $[M+Na]^+$: 621.2207, Found 621.2208.

1-[(1*R*,4*R*,6*R*,7*S*)-7-[(2-Cyanoethoxy)(diisopropylamino)phosphinoxy]-2,5-dioxaspiro(bicyclo[2.2.1]heptane-3,1'-cyclopropan)-4,4'-dimethoxytrityloxymethyl -6-yl]thymine (18)

To the solution of **17** (192 mg, 0.32 mmol) in dry acetonitrile (4 mL) was added *N,N*-diisopropylethylamine (0.17 mL, 0.96 mmol) and 2-cyanoethyl-*N,N*-diisopropyl-phosphoramidochloridite (0.11 mL, 0.48 mmol) at 0 ºC under N_2 atmosphere. After stirring at room temperature for 5 h, the reaction mixture was concentrated and the crude product was purified by column chromatography (SiO_2, 0.5% triethylamine in *n*-hexane : AcOEt = 2 : 1) to afford **18** (222 mg, 87%) as a white solid.

Compound **18**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.40–0.44 (m, 1H), 0.71–0.87 (m, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 3H), 1.15 (d, *J* = 6.6 Hz, 3H), 1.67 (s, 3/2H), 1.68 (s, 3/2H), 2.37–2.41 (m, 1H), 2.53–2.63 (m, 1H), 3.15–3.30 (m, 2H), 3.49–3.57 (m, 3H), 3.63–3.73 (m, 1H), 3.79 (s, 3H), 3.80 (s, 3H), 4.36 (d, *J* = 6.9 Hz, 1/2H), 4.41 (d, *J* = 8.7 Hz, 1/2H), 4.60 (s, 1/2H), 4.63 (s, 1/2H), 5.77 (s, 1H), 6.82–6.87 (m, 4H), 7.24–7.35 (m, 7H), 7.43–7.45 (m, 2H), 7.70 (s, 1/2H), 7.73 (s, 1/2H), 8.21 (s, 1H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.6; LRMS (FAB) m/z = 799 $(M+H)^+$; HRMS (FAB) Calcd. for $C_{43}H_{52}O_9N_4P$ 799.3472, Found 799.3475.

Figure S1. Compound **2** (^{1}H NMR, $CDCl_3$, 400MHz)

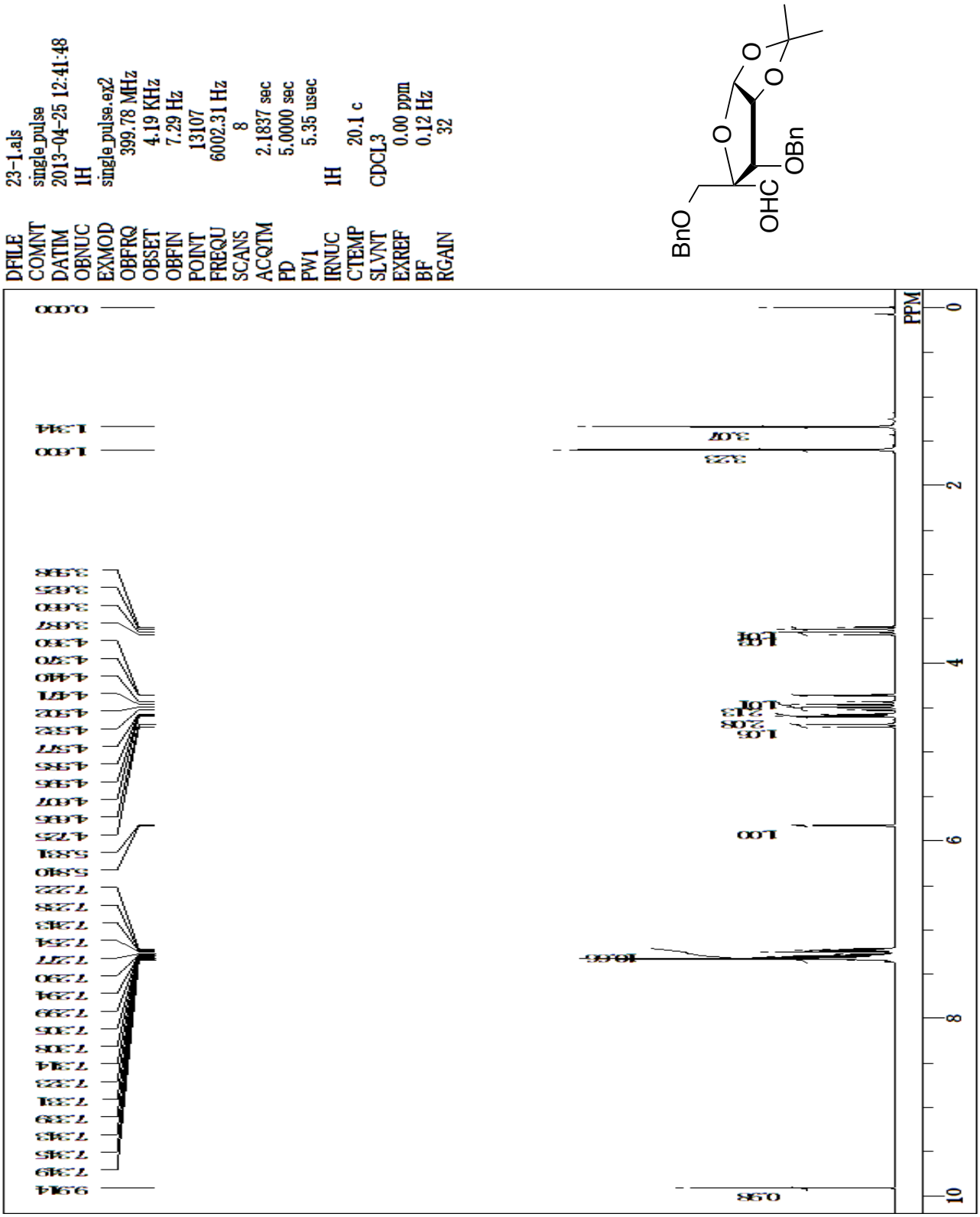


Figure S2. Compound **2** (^{13}C NMR, $CDCl_3$, 100 MHz)

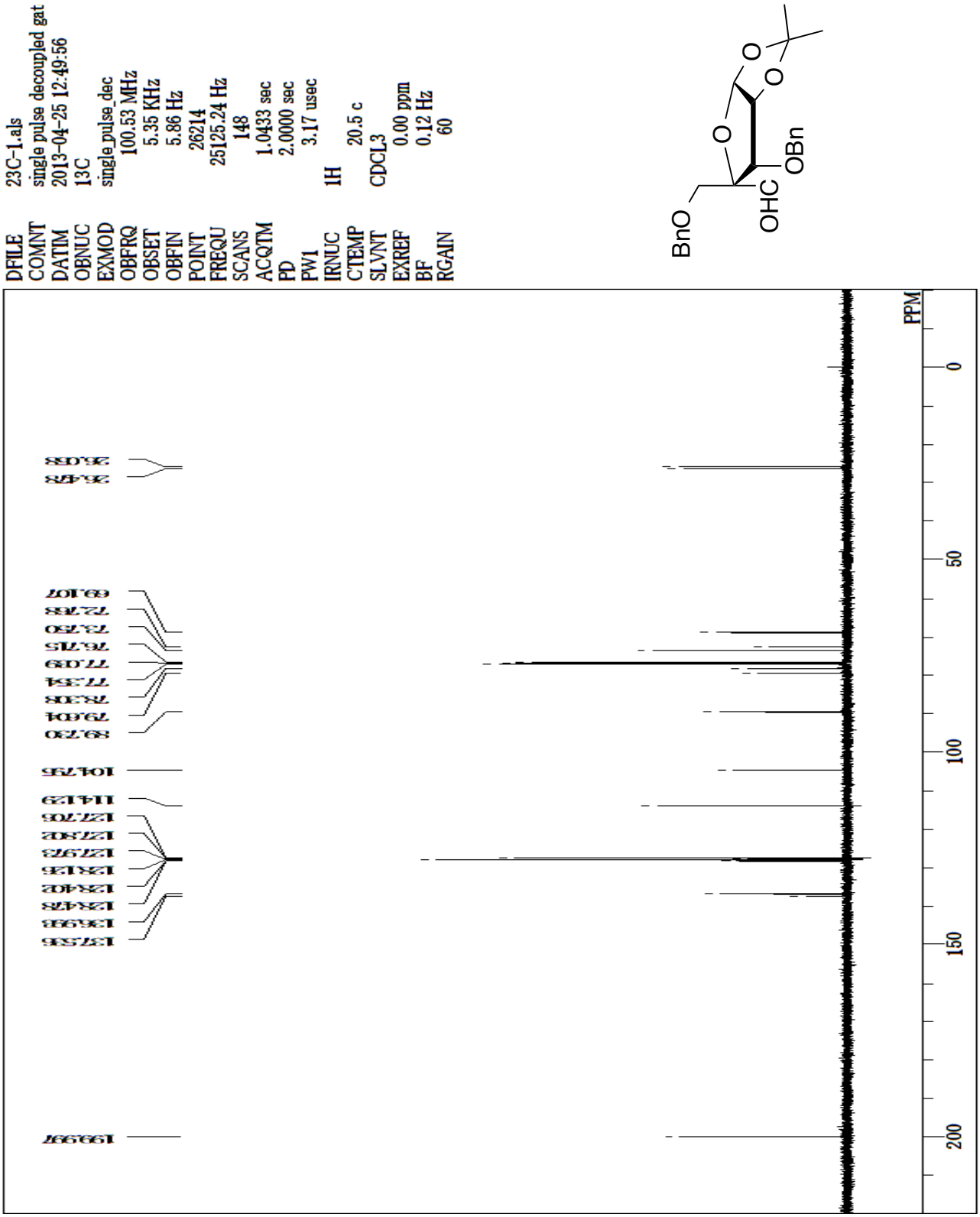


Figure S3. Compound **3** (^{1}H NMR, $CDCl_3$, 400 MHz)

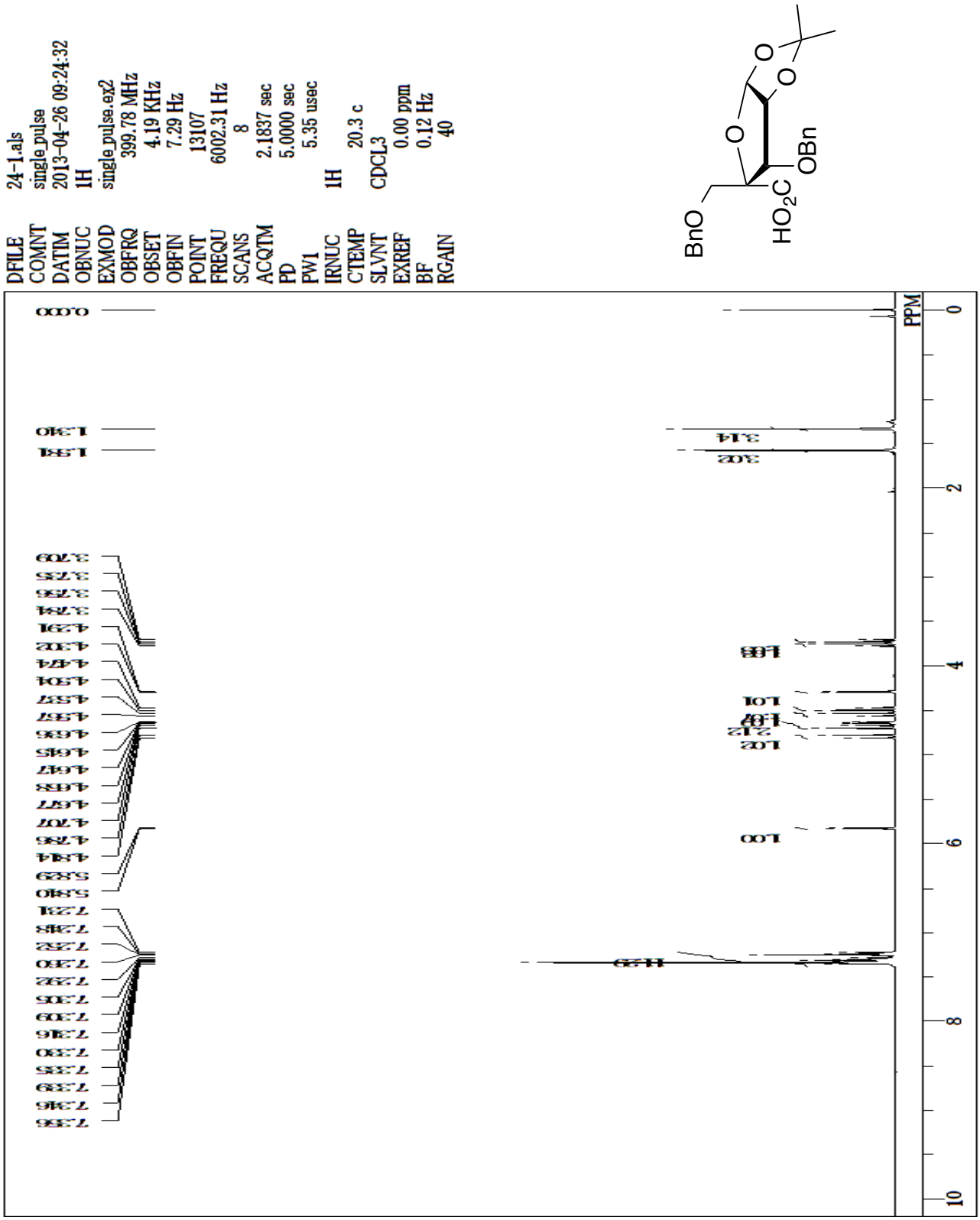


Figure S4. Compound **3** (^{13}C NMR, $CDCl_3$, 100 MHz)

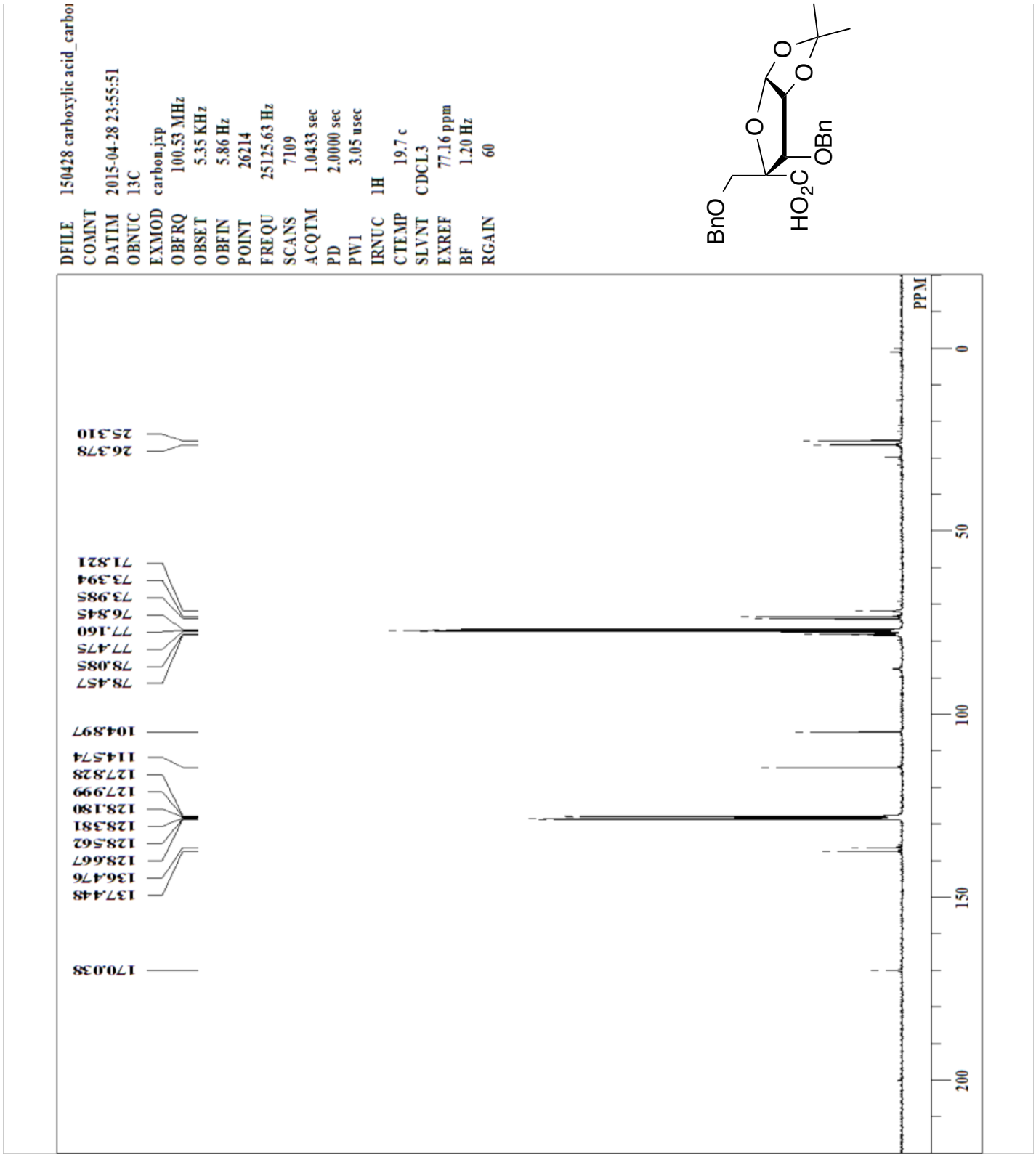


Figure S5. Compound **4** (^{1}H NMR, $CDCl_3$, 400 MHz)

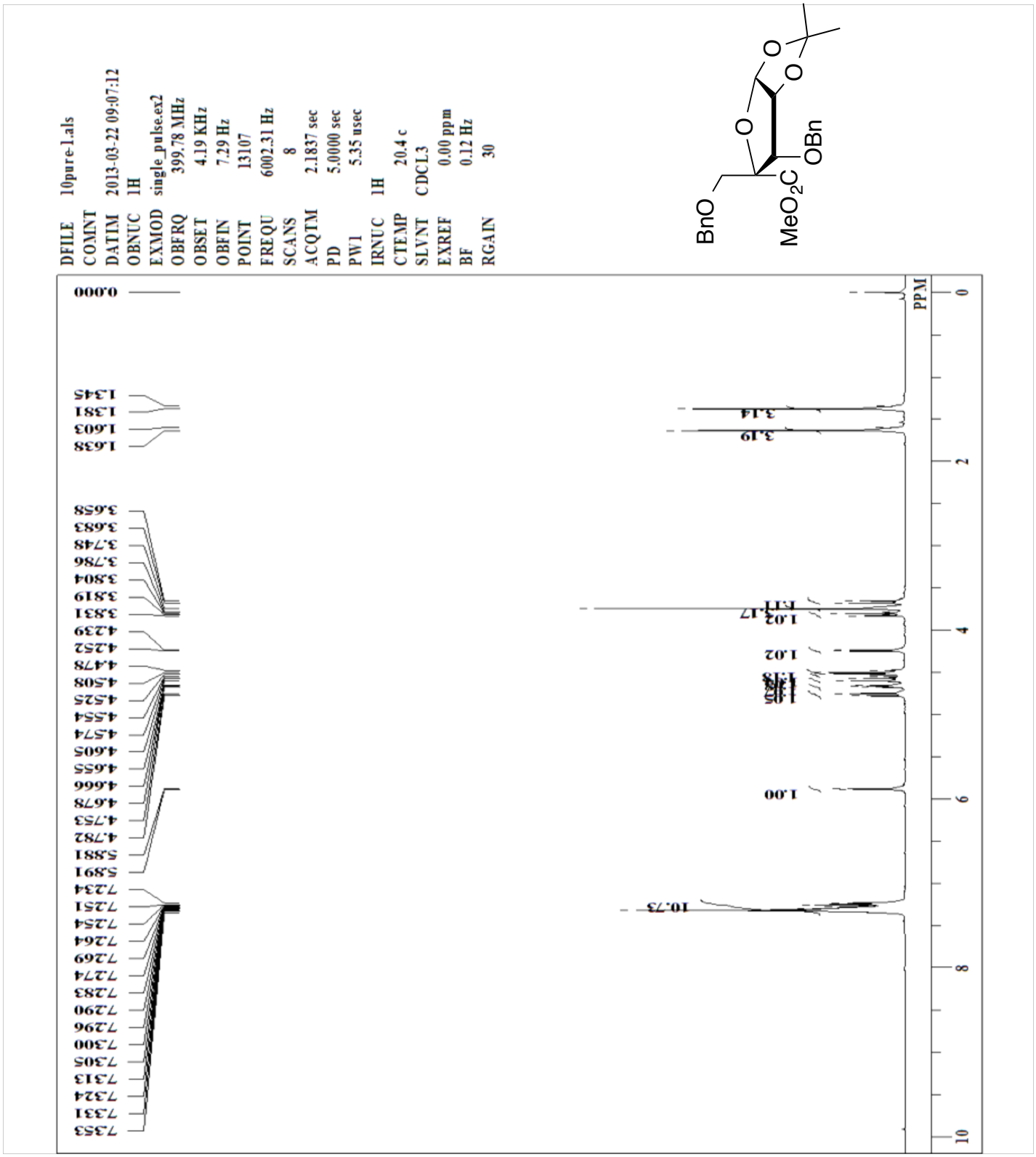


Figure S6. Compound **4** (^{13}C NMR, $CDCl_3$, 100 MHz)

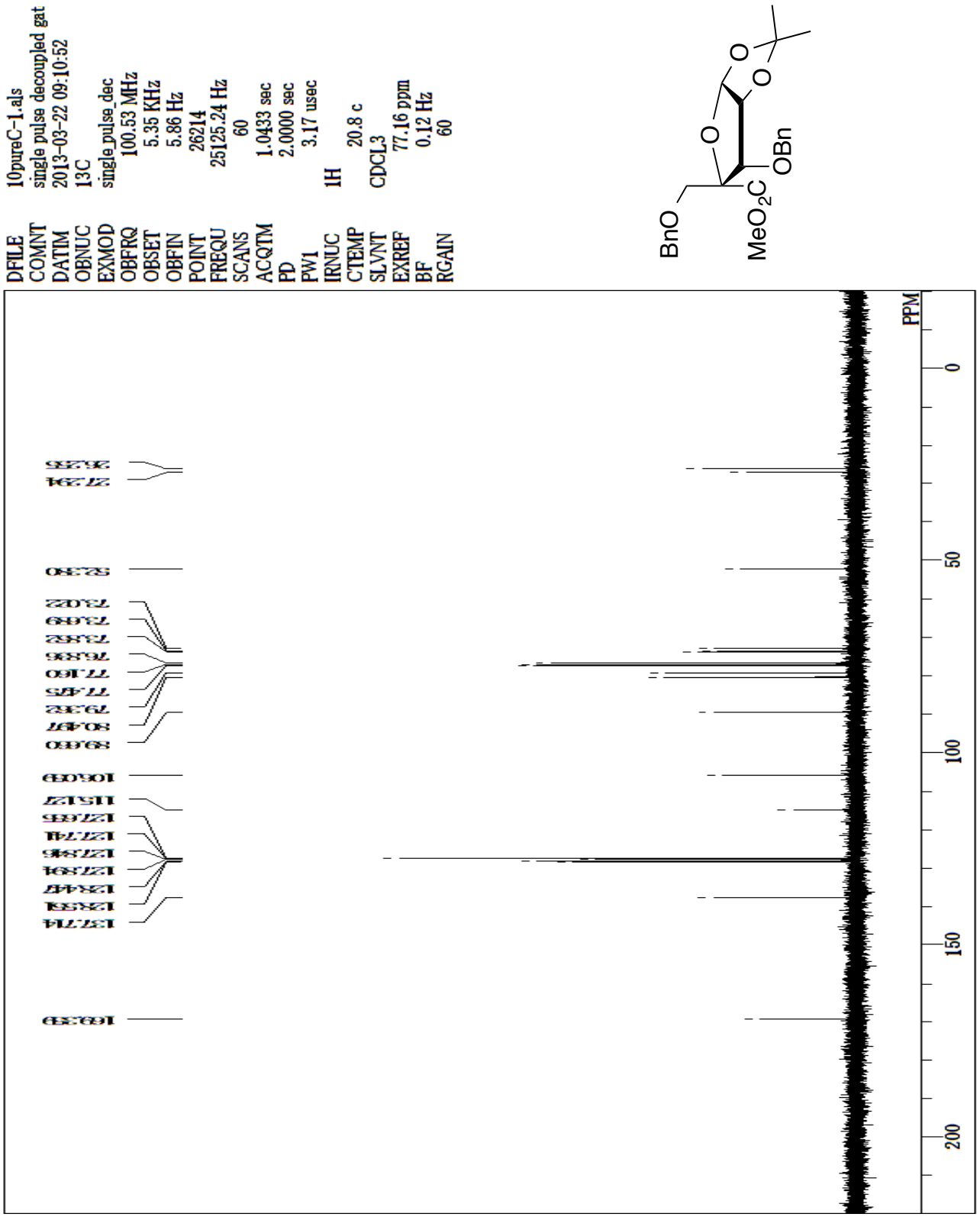


Figure S7. Compound **5** (^{1}H NMR, $CDCl_3$, 300 MHz)

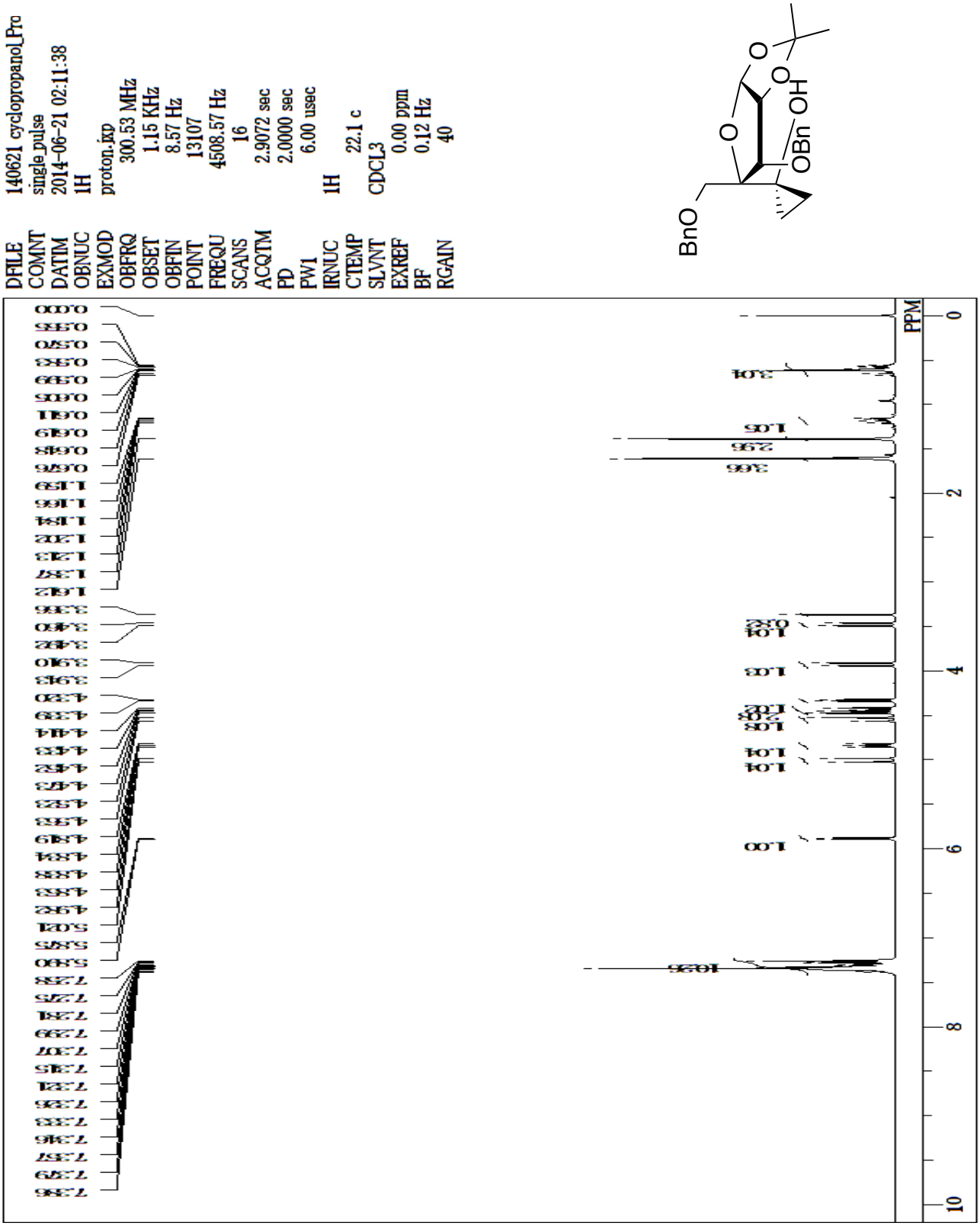


Figure S8. Compound **5** (^{13}C NMR, $CDCl_3$, 100 MHz)

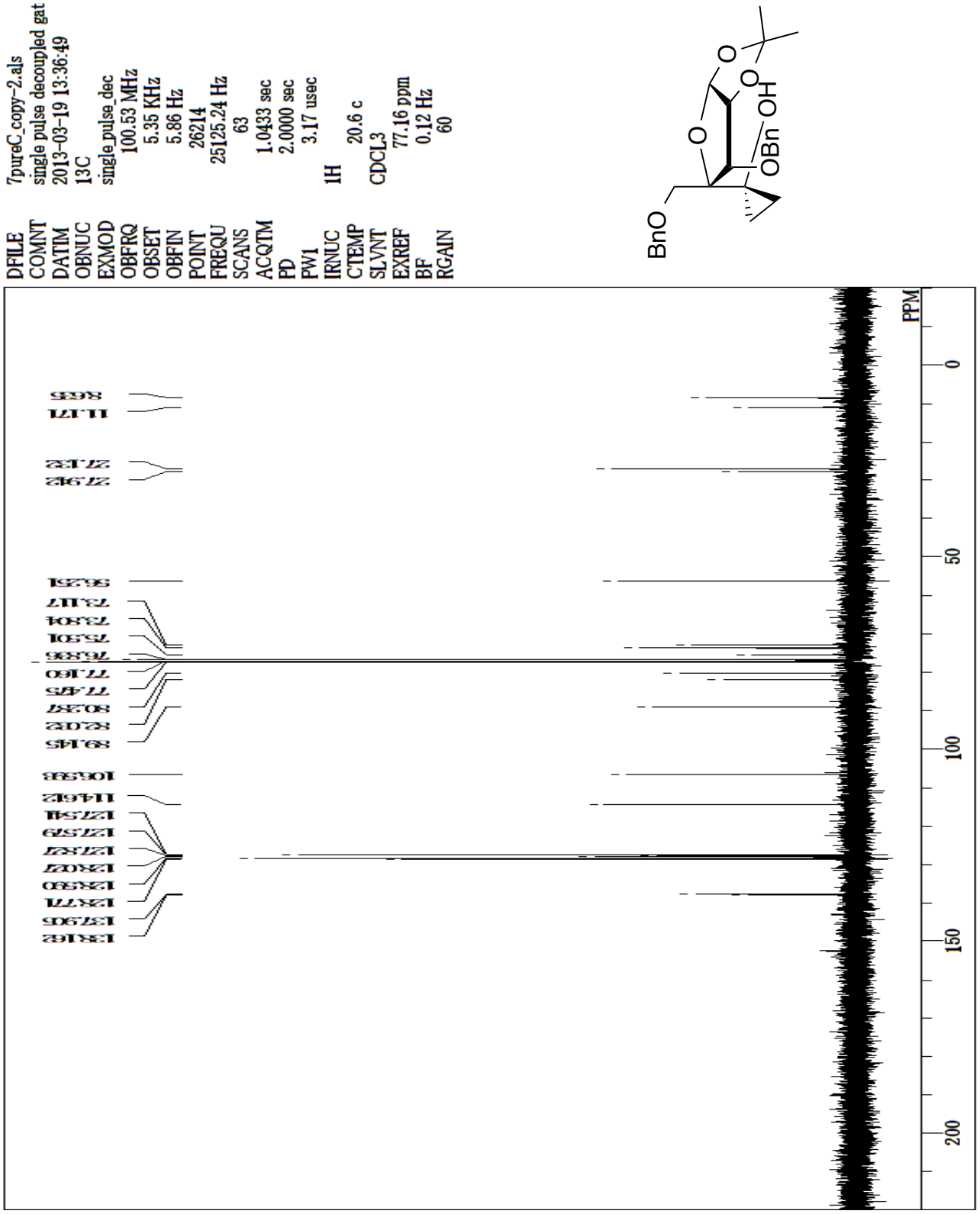


Figure S9. Compound **6** (^{1}H NMR, $CDCl_3$, 300 MHz)

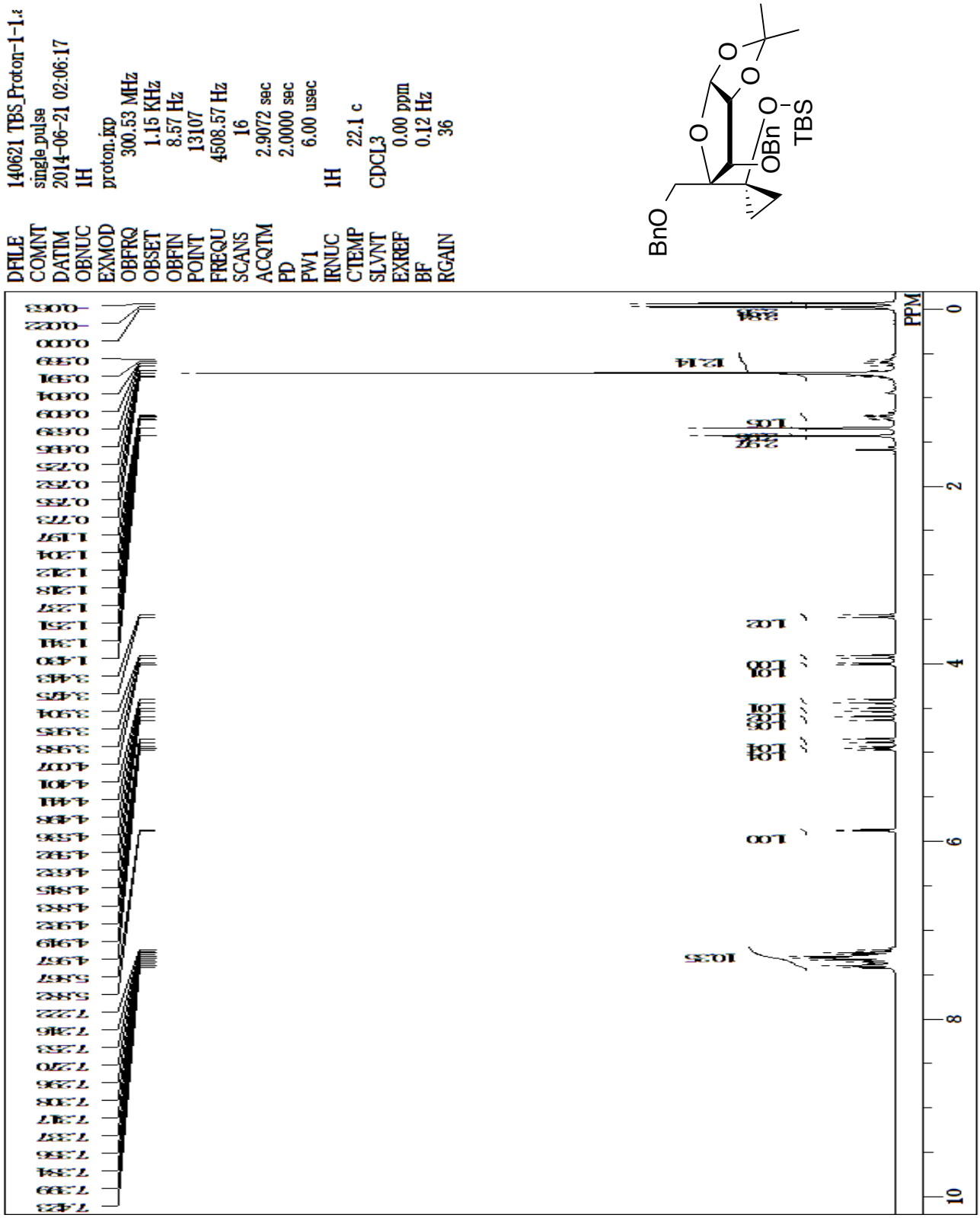


Figure S10. Compound **6** (^{13}C NMR, $CDCl_3$, 100 MHz)

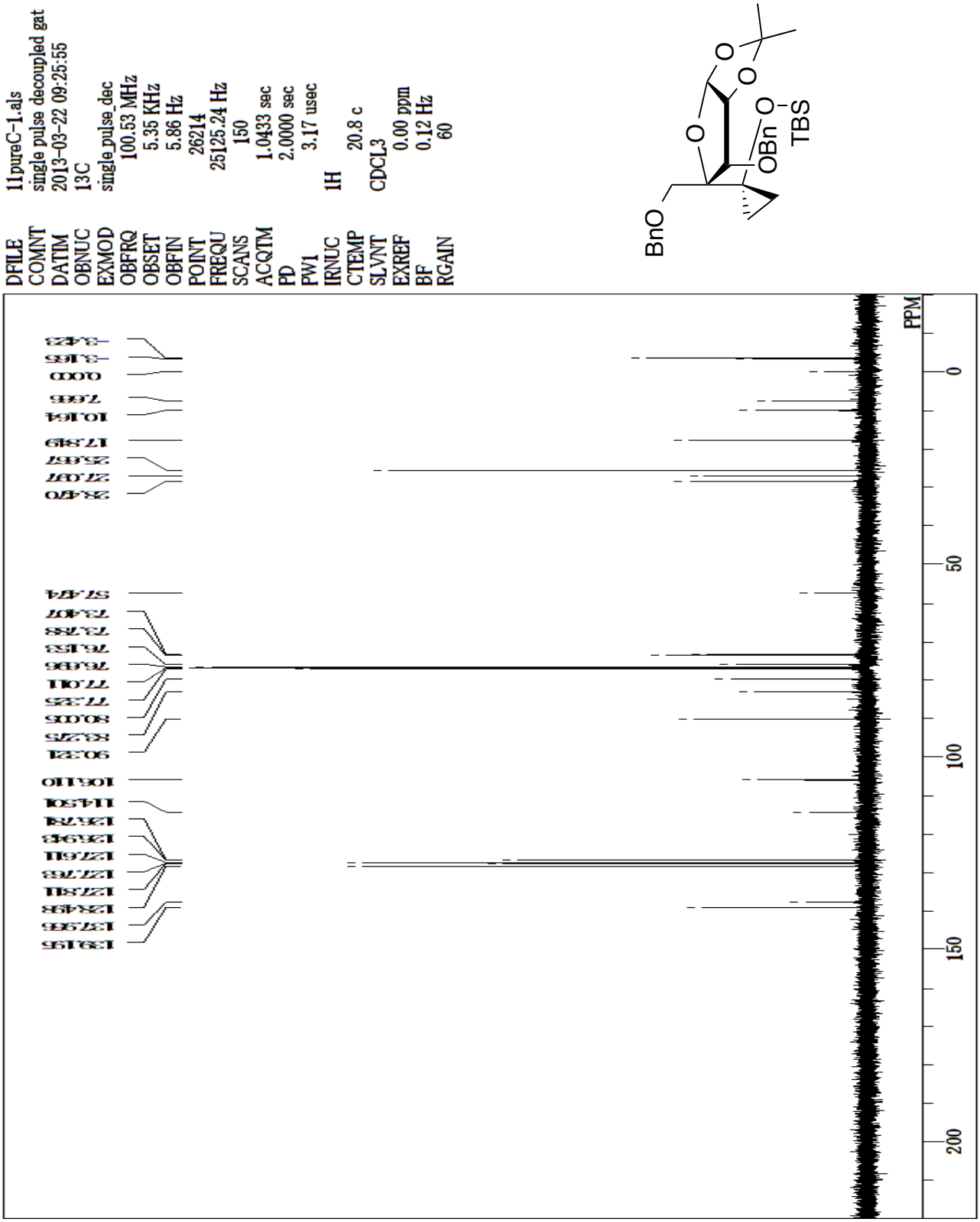


Figure S11. Compound **8** (^{1}H NMR, $CDCl_3$, 300 MHz)

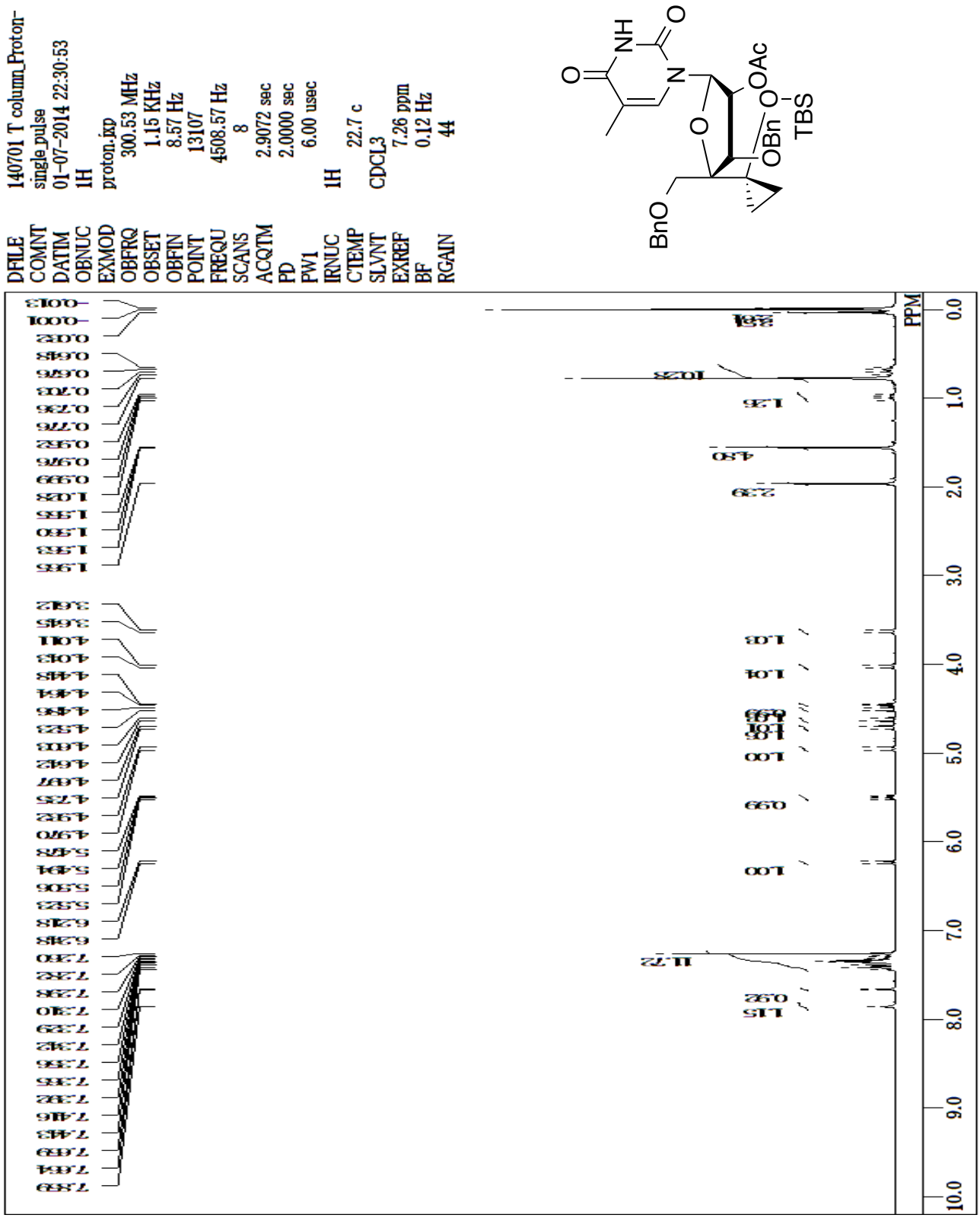


Figure S12. Compound **8** (^{13}C NMR, $CDCl_3$, 75 MHz)

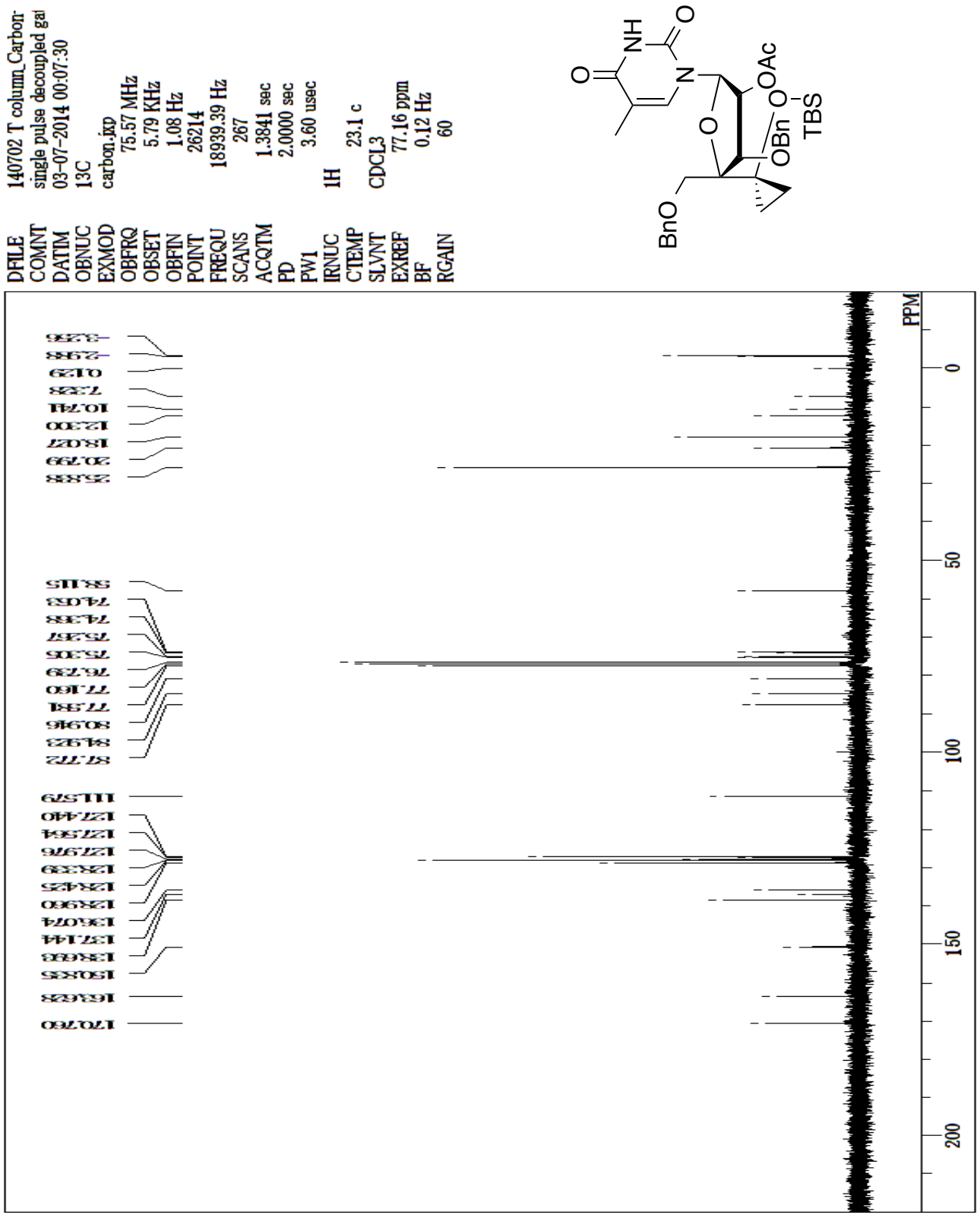


Figure S13. Compound **9** (^{1}H NMR, $CDCl_3$, 300 MHz)

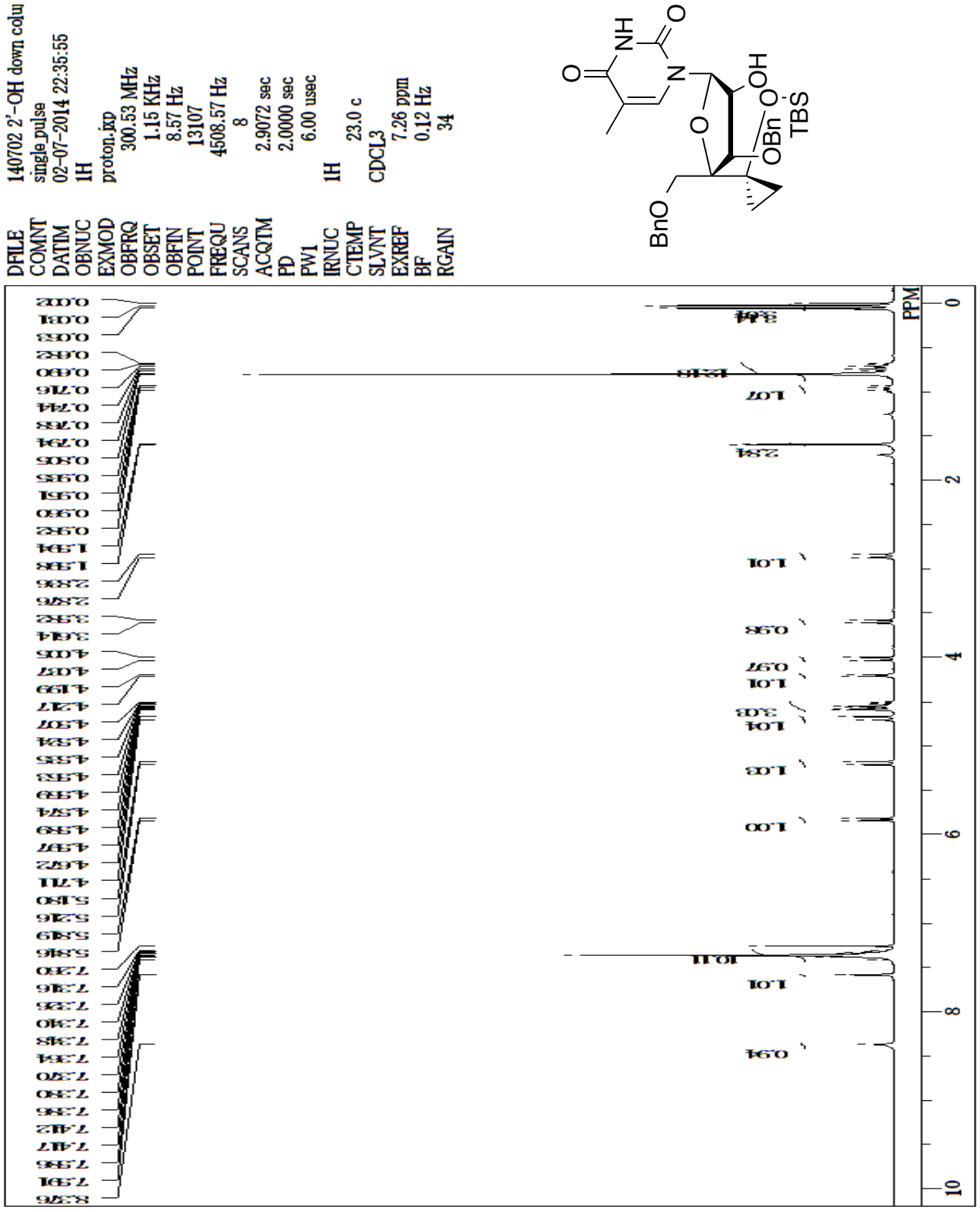


Figure S14. Compound **9** (^{13}C NMR, $CDCl_3$, 100 MHz)

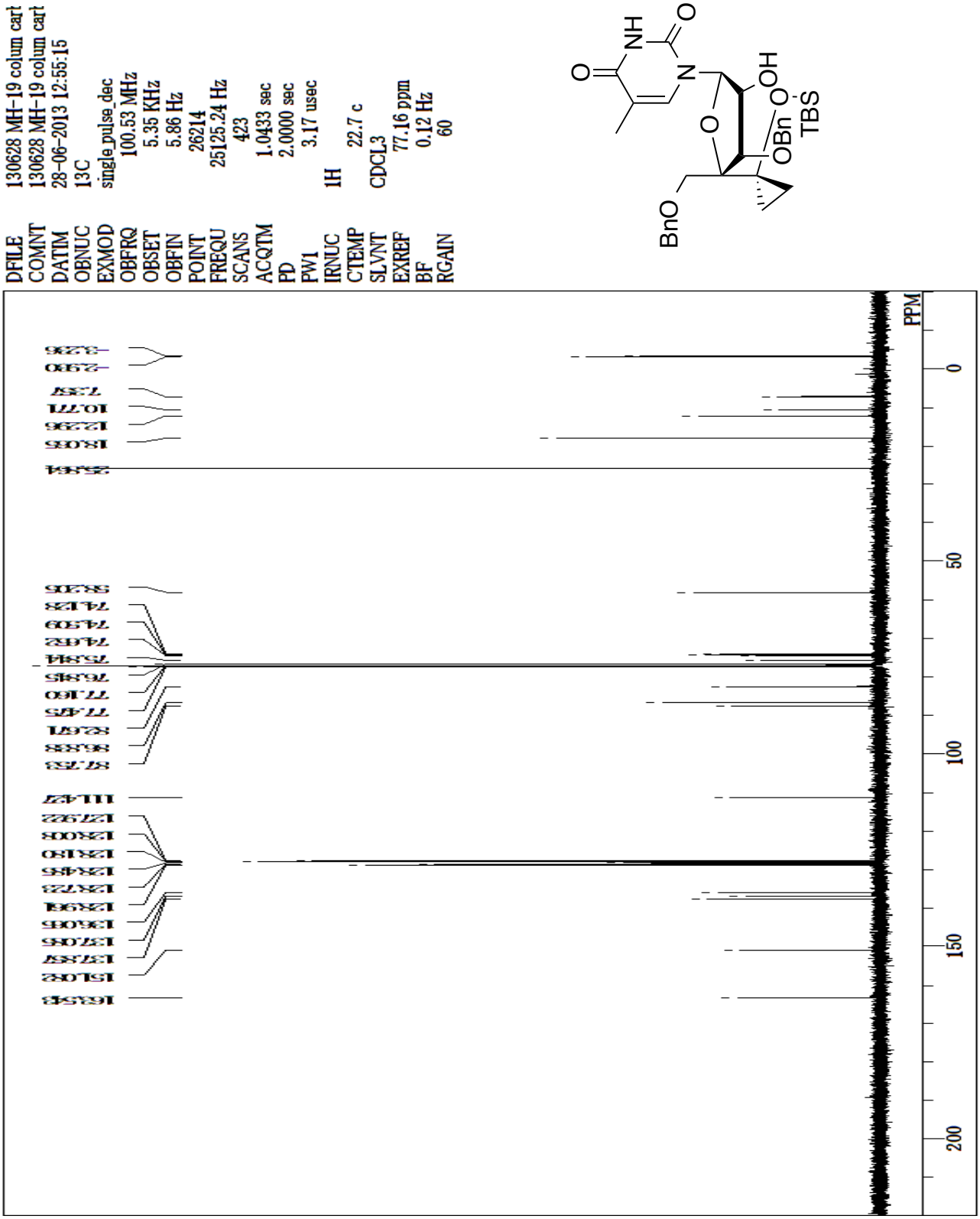


Figure S15. Compound **10** (^{1}H NMR, $CDCl_3$, 300 MHz)

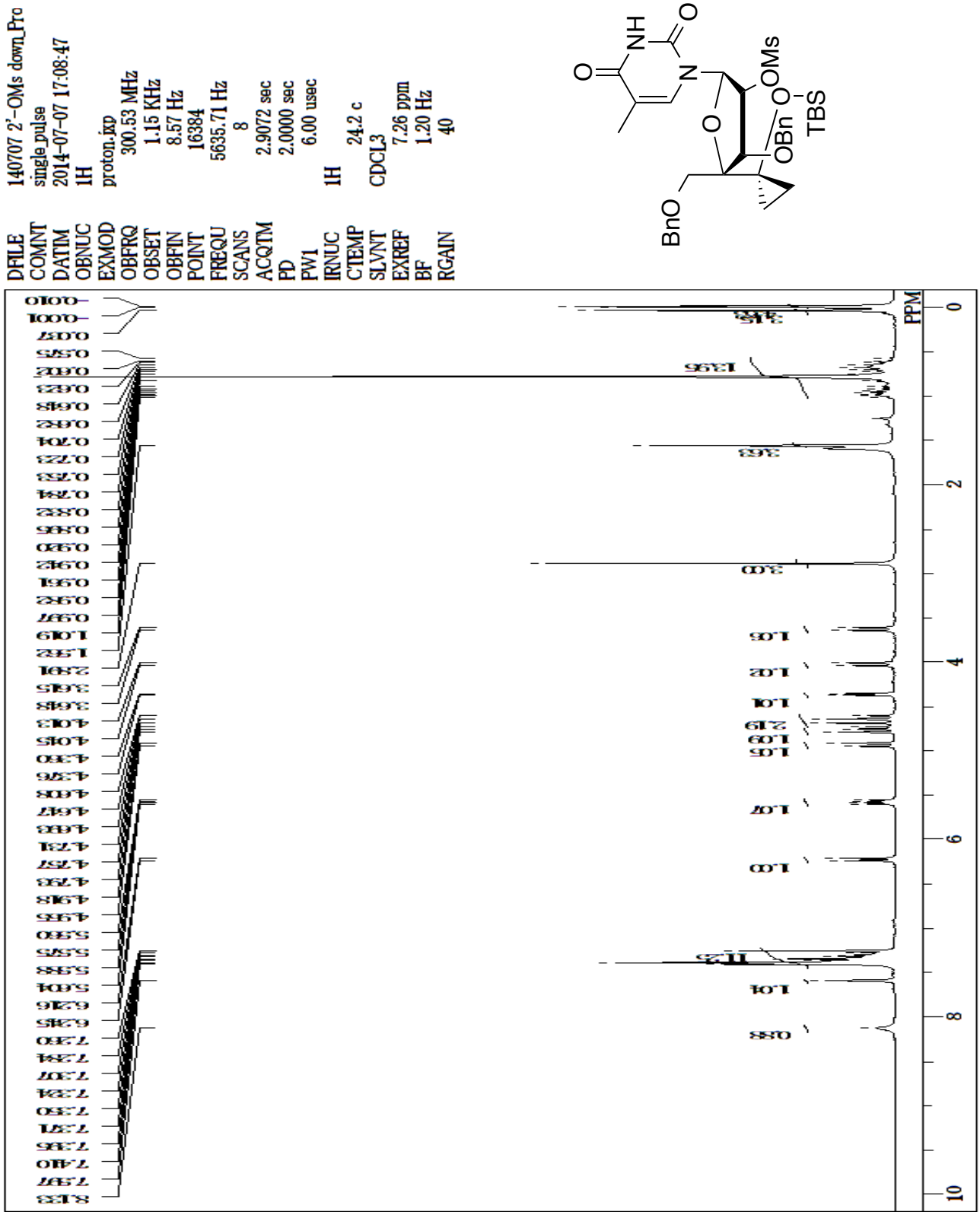


Figure S16. Compound **10** (^{13}C NMR, $CDCl_3$, 100 MHz)

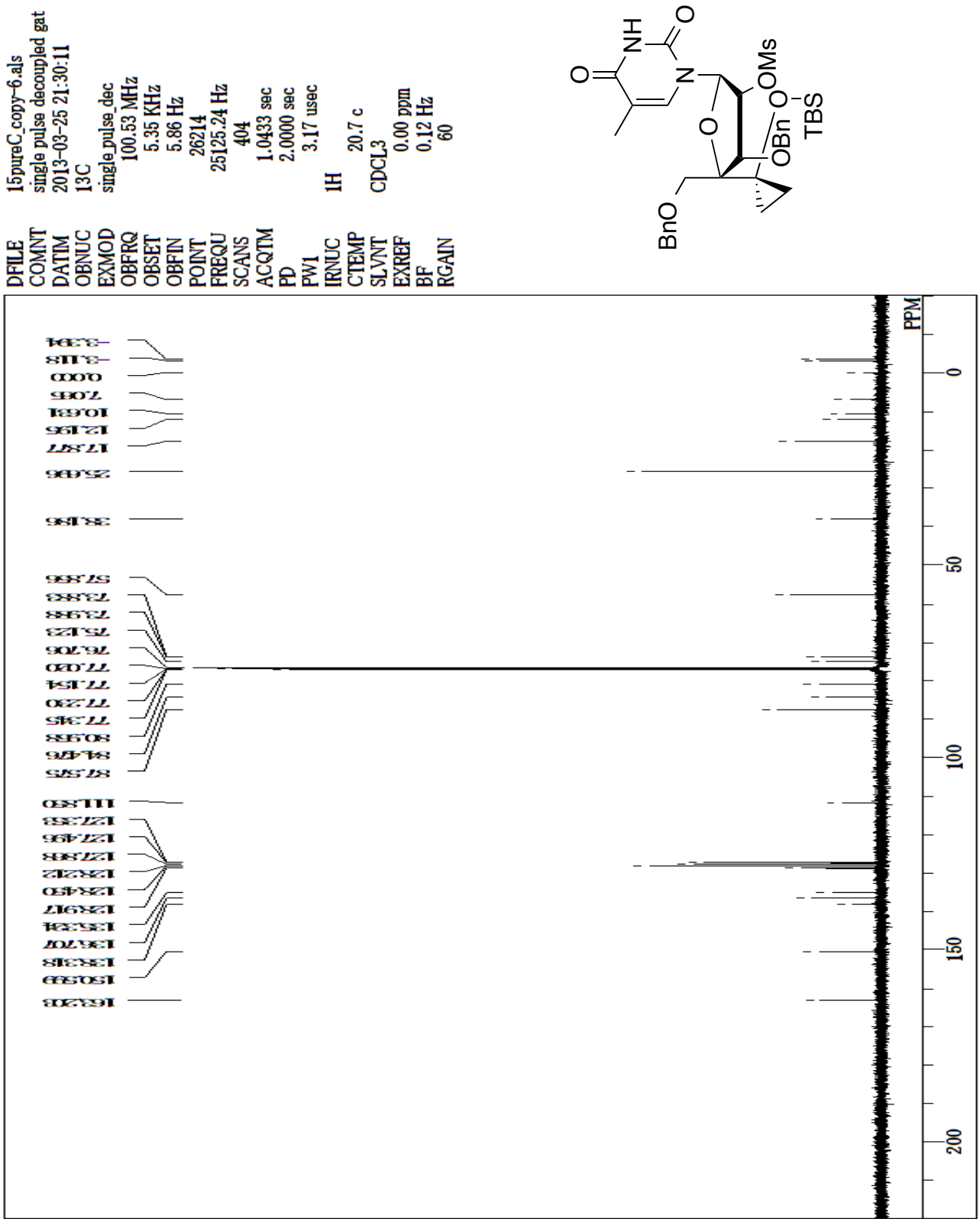


Figure S17. Compound **11** (^{1}H NMR, $CDCl_3$, 400 MHz)

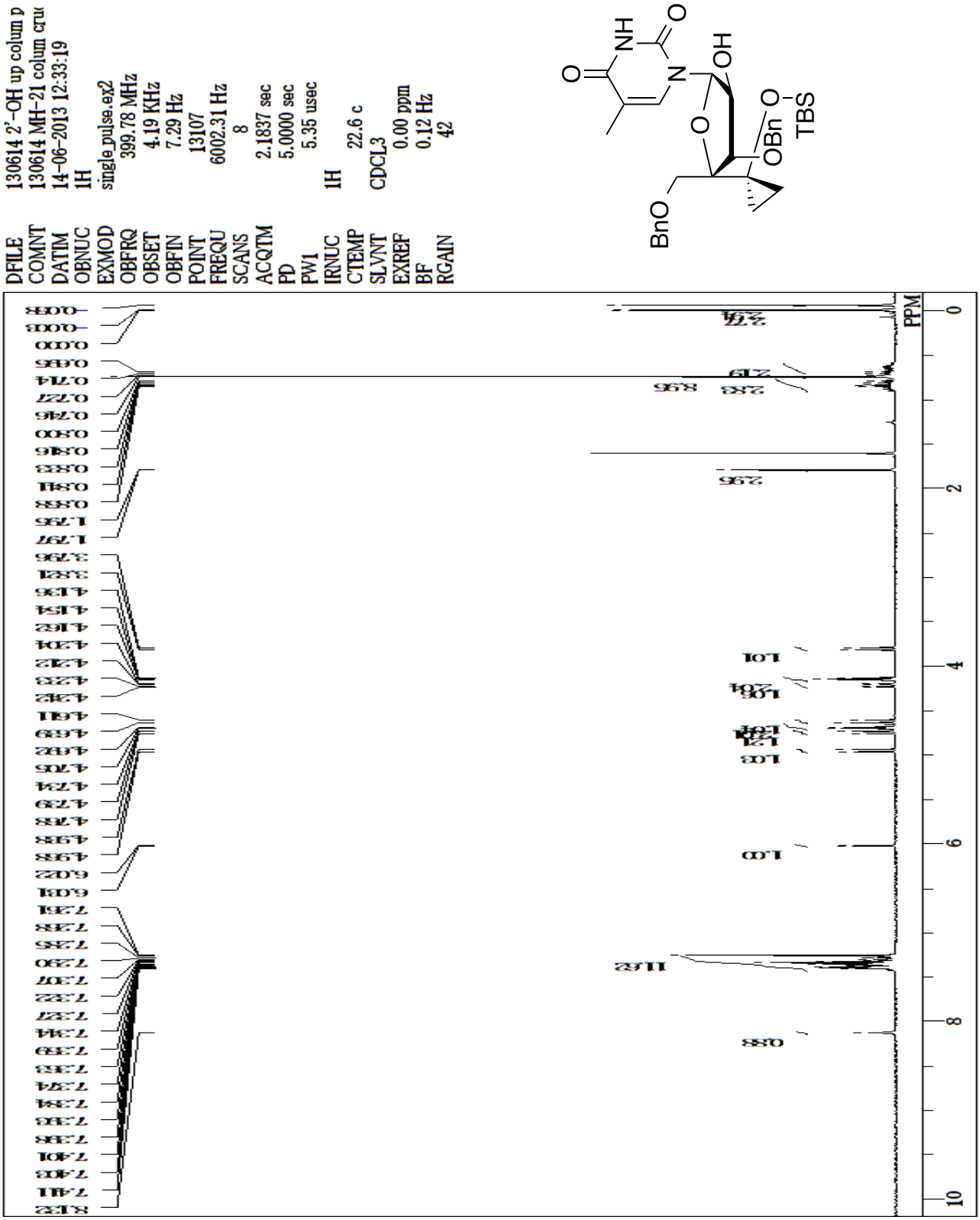


Figure S18. Compound **11** (^{13}C NMR, $CDCl_3$, 100 MHz)

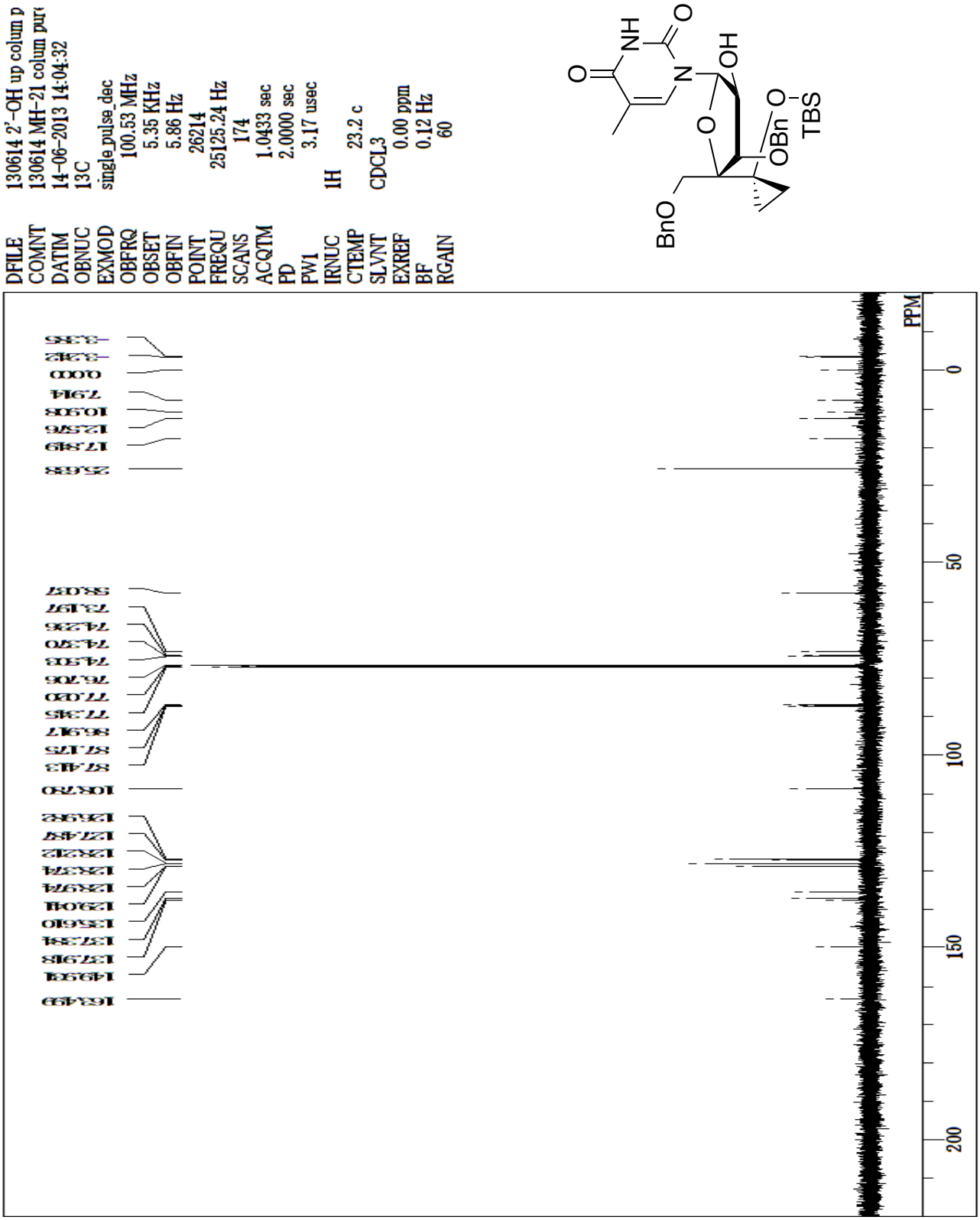


Figure S19. Compound **13** (^{1}H NMR, $CDCl_3$, 300MHz)

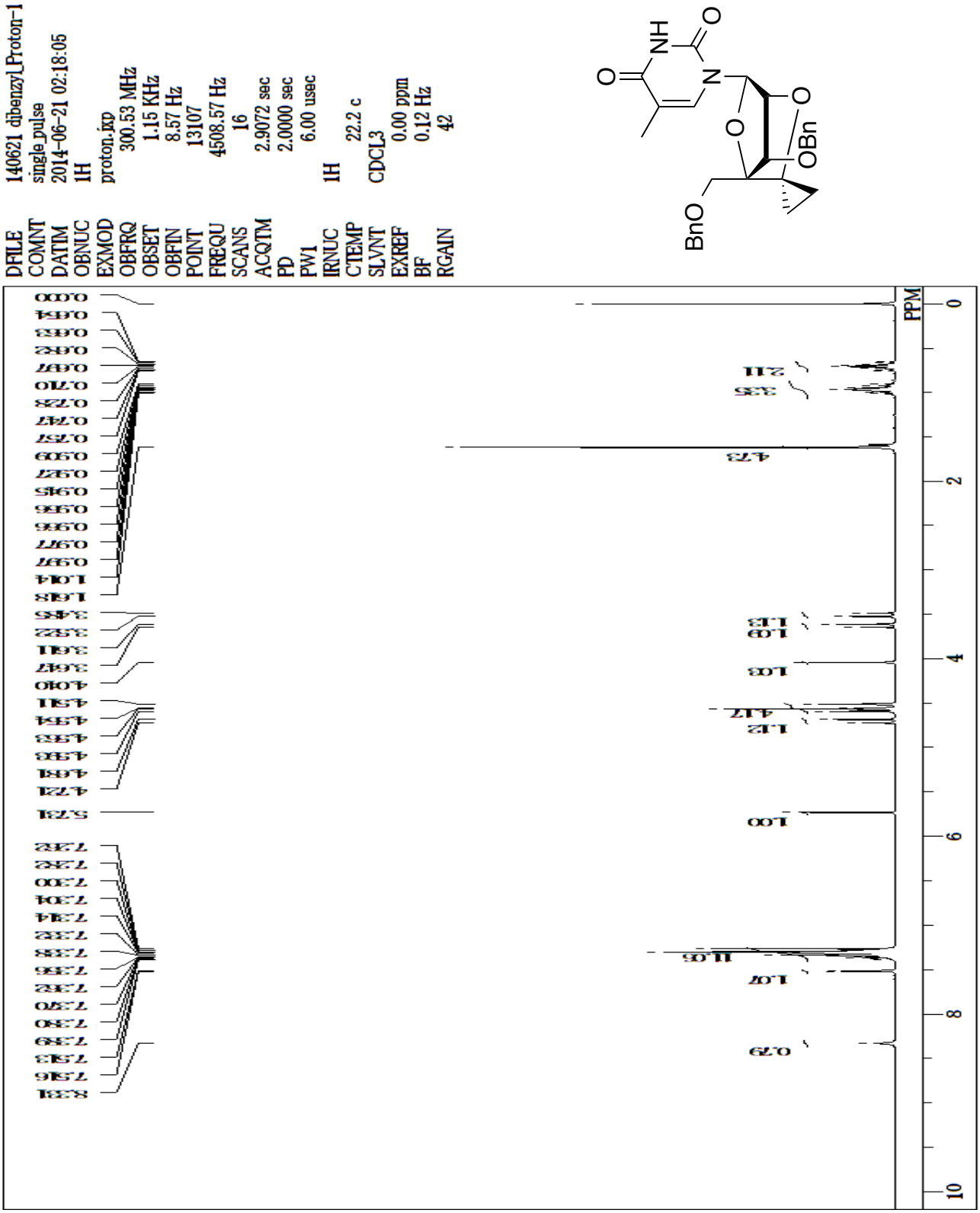


Figure S20. Compound **13** (^{13}C NMR, $CDCl_3$, 100MHz)

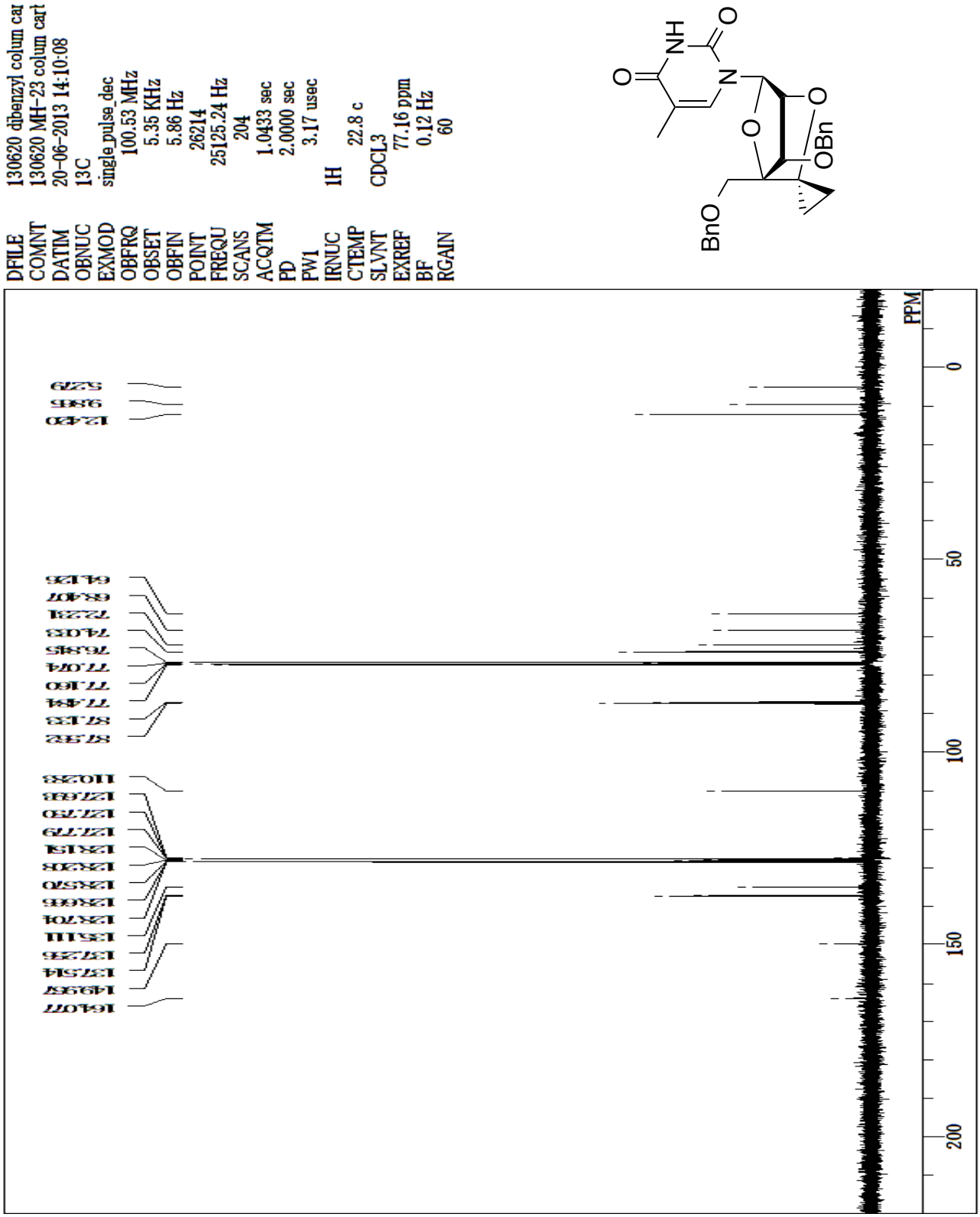


Figure S21. Compound **14** (^{1}H NMR, $CDCl_3$, 300 MHz)

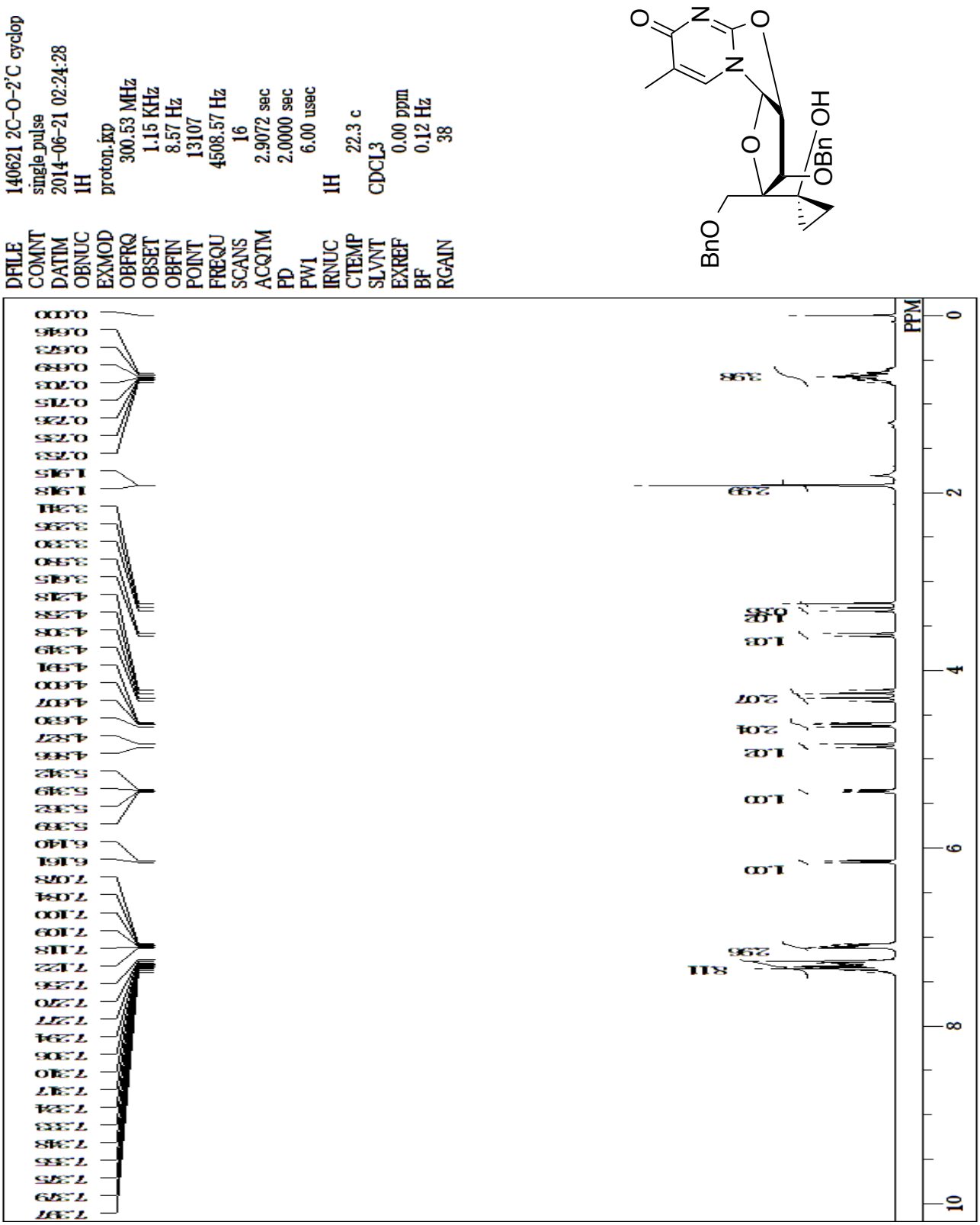


Figure S22. Compound **14** (^{13}C NMR, $CDCl_3$, 75 MHz)

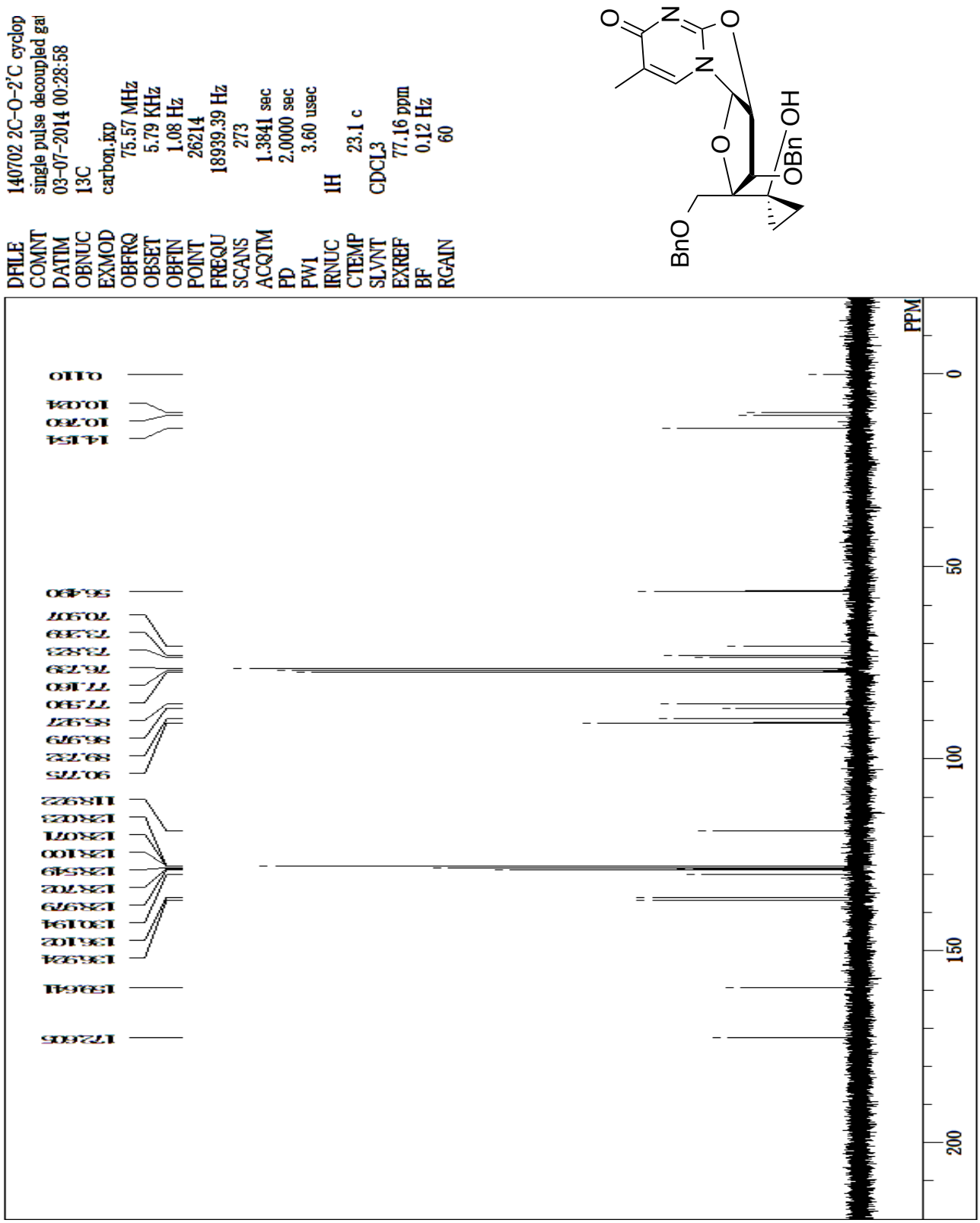


Figure S23. Compound **15** (^{1}H NMR, CD_3OD, 300 MHz)

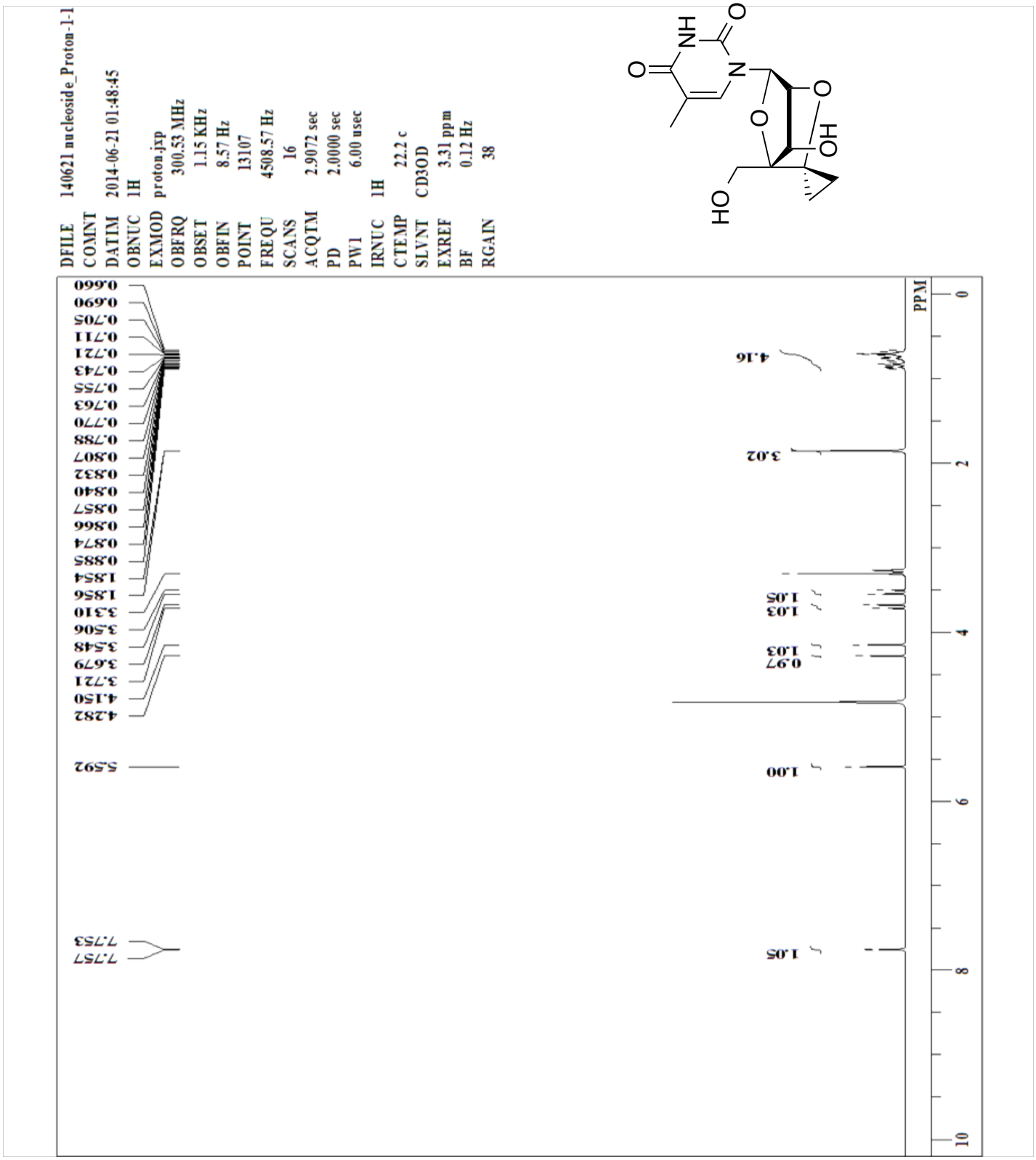


Figure S24. Compound **15** (^{13}C NMR, CD_3OD, 75 MHz)

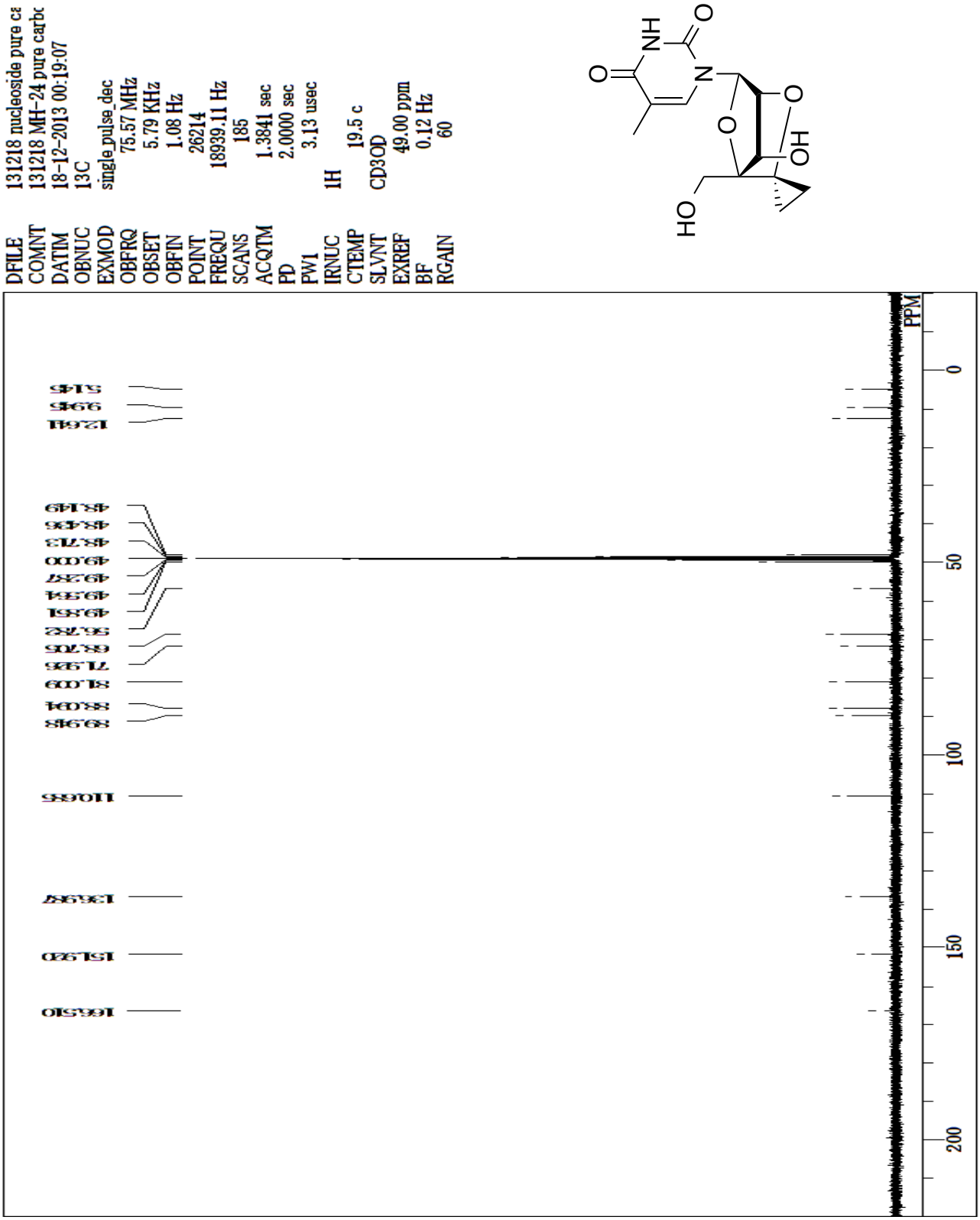


Figure S25. Compound **16** (^{1}H NMR, CD_3OD, 300 MHz)

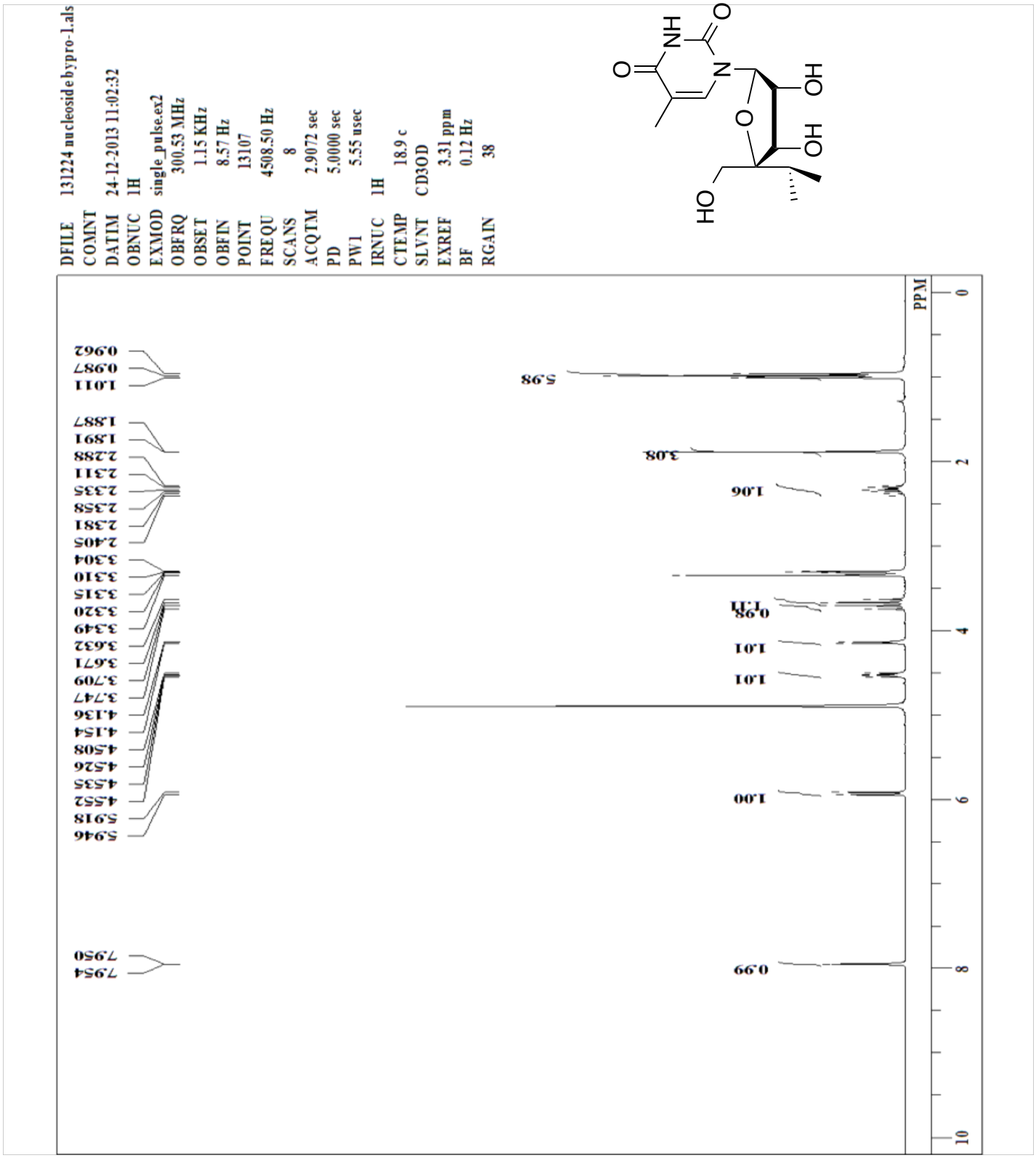


Figure S26. Compound **16** (^{13}C NMR, CD_3OD, 75 MHz)

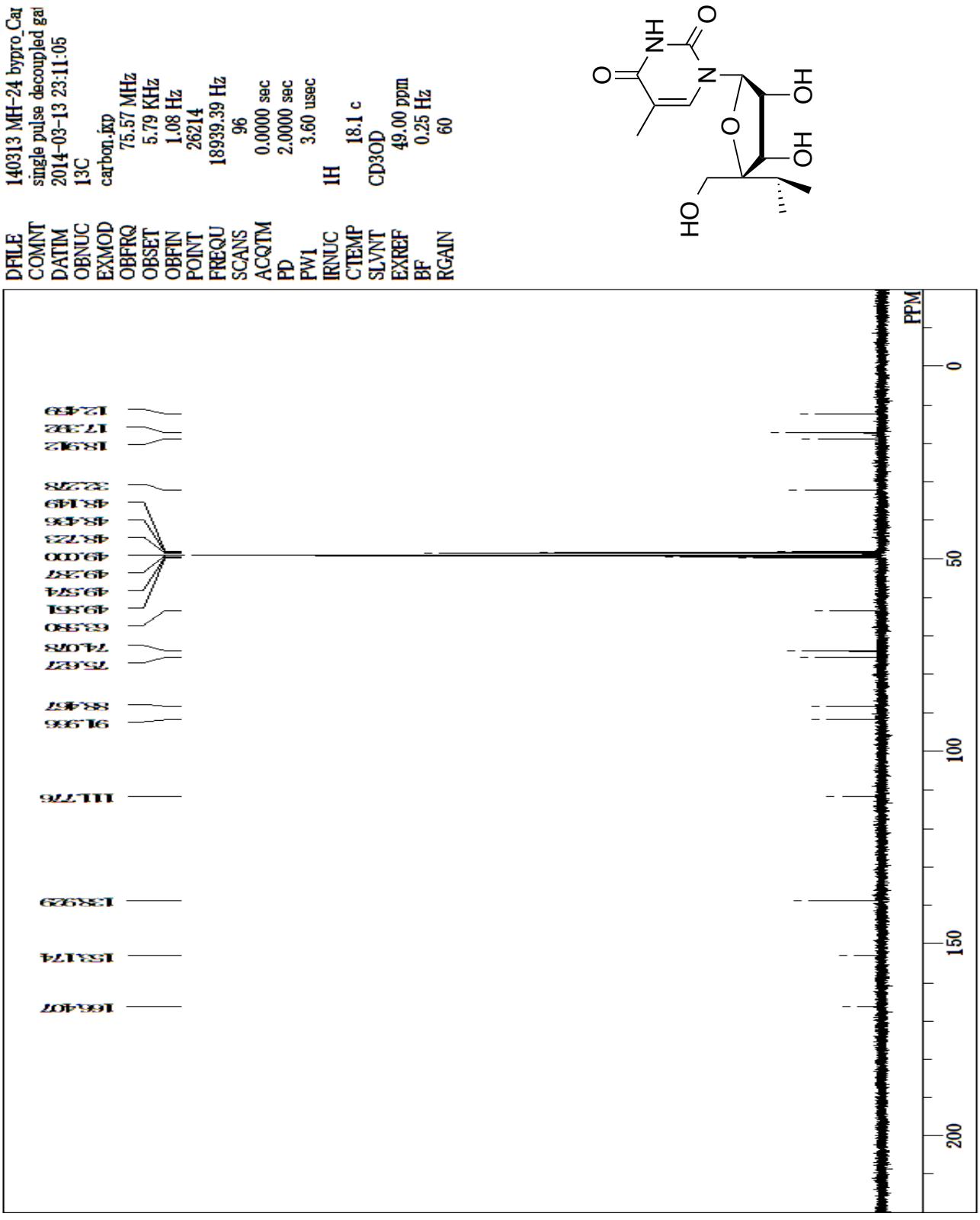


Figure S27. Compound **17** (^{1}H NMR, $CDCl_3$, 300 MHz)

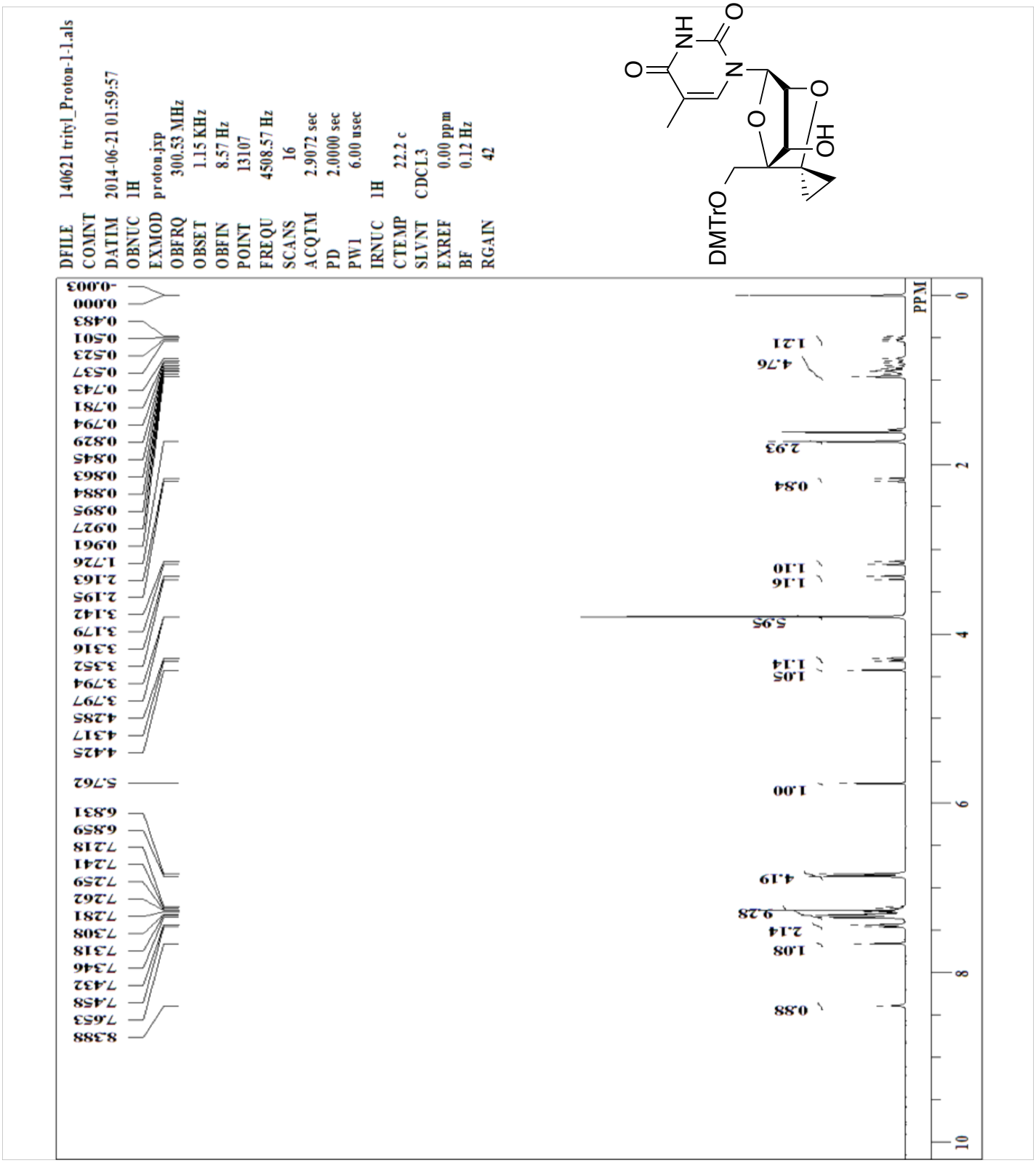


Figure S28. Compound **17** (^{13}C NMR, $CDCl_3$, 100 MHz)

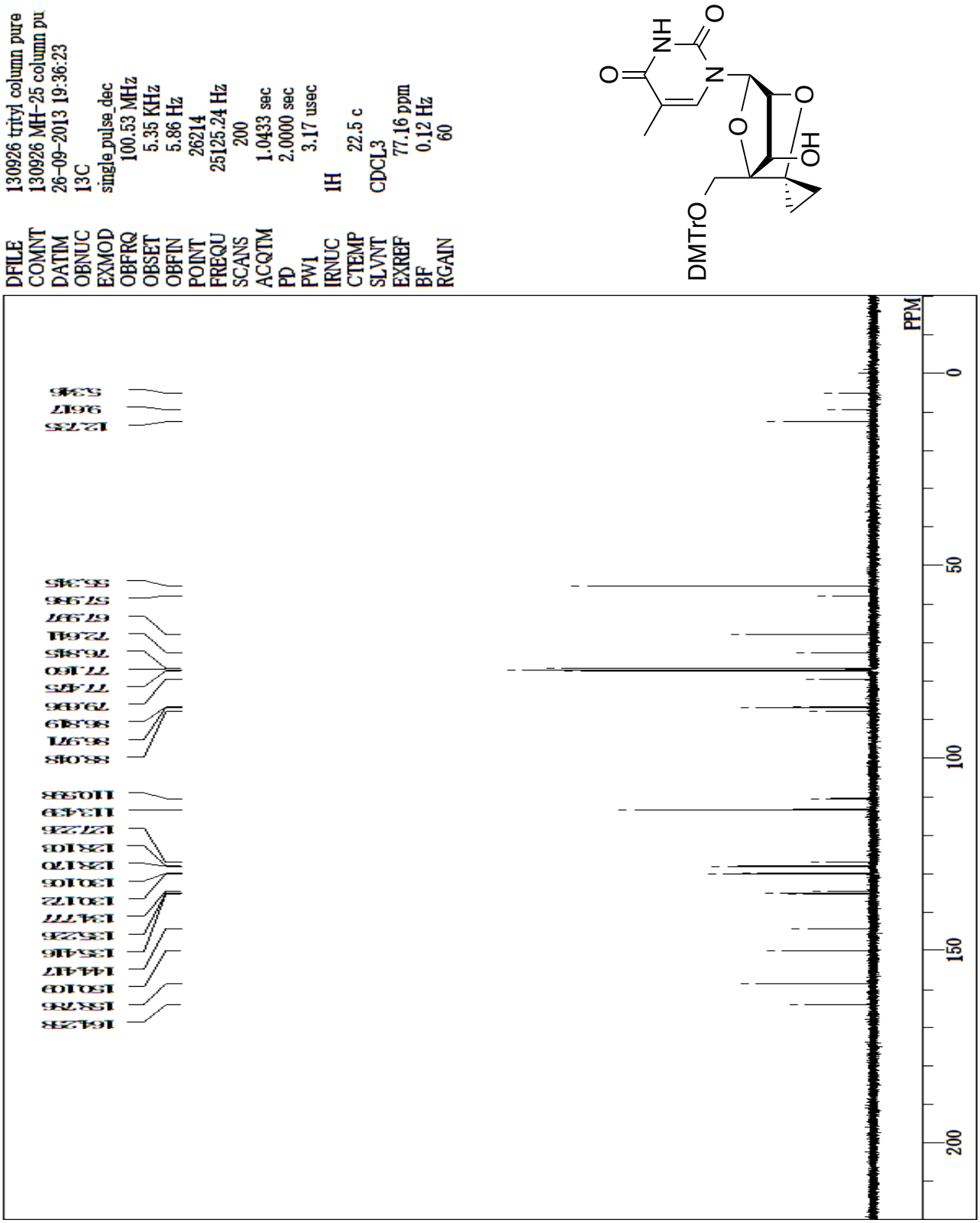


Figure S29. Compound **18** (^{1}H NMR, $CDCl_3$, 300 MHz)

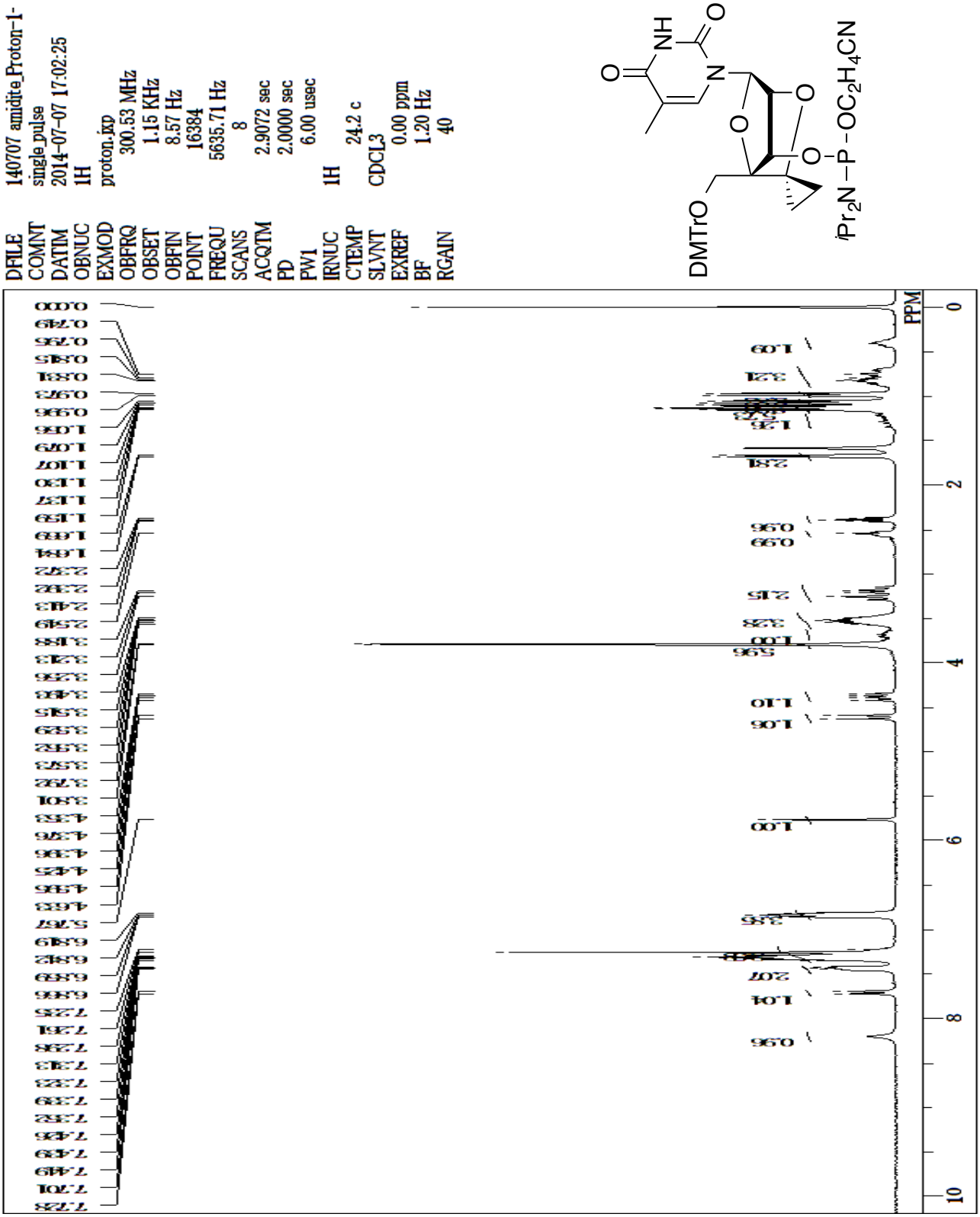
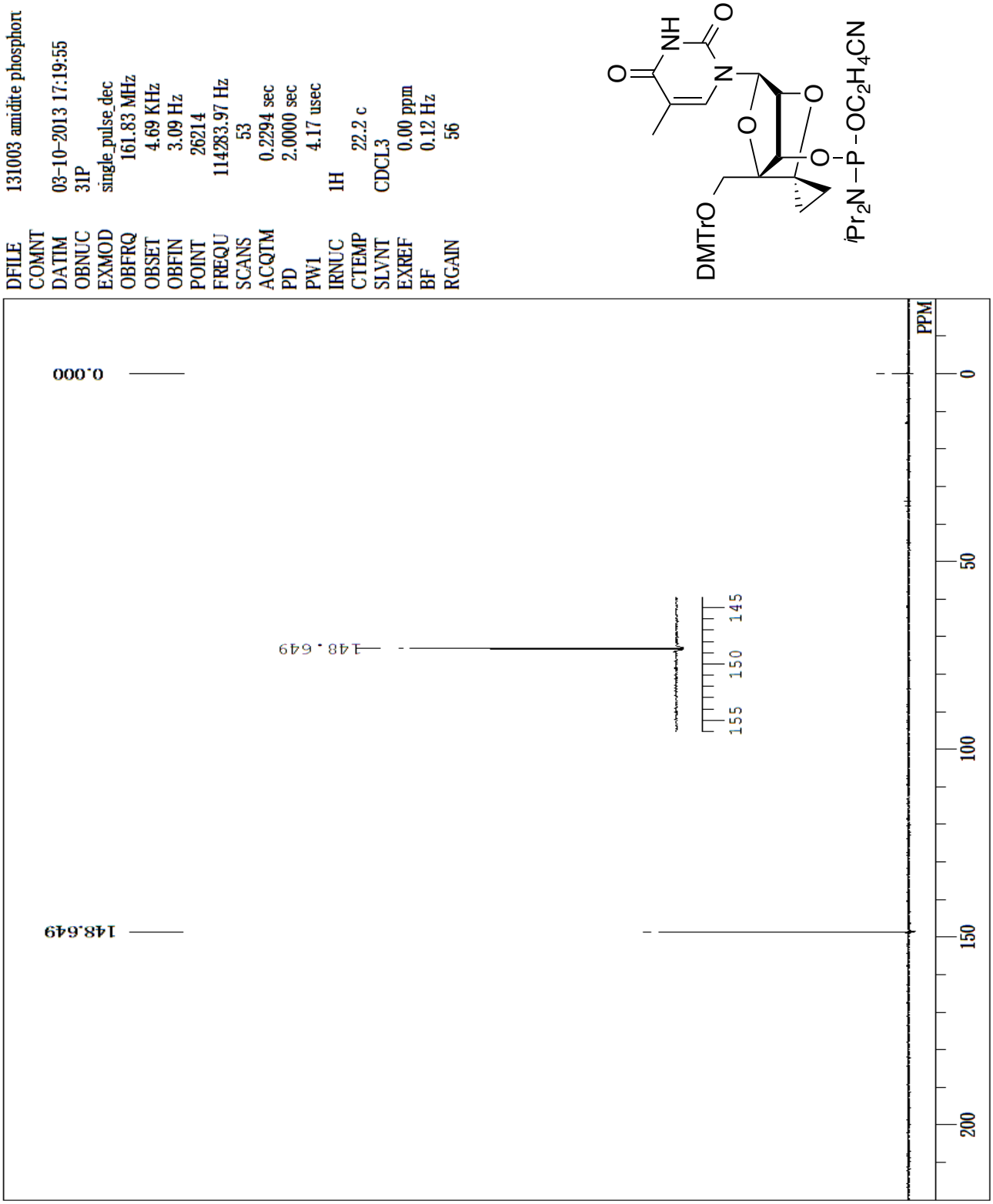


Figure S30. Compound **18** (^{31}P NMR, $CDCl_3$, 162 MHz)



3. Synthesis, purification and characterization of oligonucleotides

Synthesis of oligonucleotides modified with scpBNA was performed on an Applied Biosystems Expedite™ 8909 Nucleic Acid Synthesis System on 0.2 μmol scale of **ON2**–**ON6** and 1.0 μmol scale of **ON14** according to the standard phosphoroamidite protocol and 5-[3,5-bis(trifluoromethyl)-phenyl]-1*H*-tetrazole as the activator. In the case of 0.2 μmol scale, the coupling time of phosphoramidite **16** was prolonged from 32 seconds to 8 minutes. In the case of 1.0 μmol scale, that was prolonged from 40 seconds to 10 minutes. The synthesis was carried out in trityl on mode and the solid supported oligonucleotides were treated with concentrated ammonium hydroxide at 55 ºC for 12 h. The **ON2**–**ON6** and the **ON14** were briefly purified with Sep-Pak® Plus C_{18} Cartridge and Sep-Pak® Plus C_{18} Environmental Cartridge, respectively. The **ON2**–**ON6** and the **ON14** were further purified by reverse-phase HPLC with Waters XTerra MS C_{18} 2.5 μm (10 x 50 mm) columns with a linear gradient of MeCN (6 to 12% over 30 min) in 0.1 M triethylammonium acetate buffer (pH 7.0). The purity of the oligonucleotides were analyzed by reverse-phase HPLC with Waters XTerra MS C_{18} 2.5 μm (4.6 x 50 mm) columns and characterized by MALDI-TOF mass spectrometer.

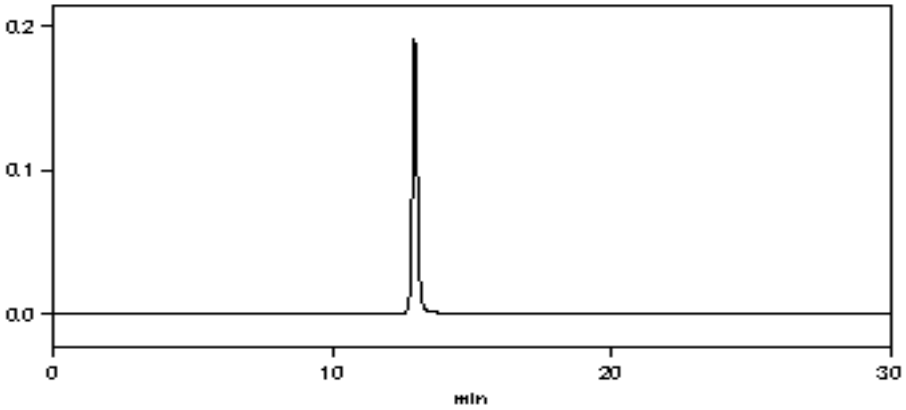
Table S1. Yields and MALDI-TOF MS data for the oligonucleotides.

oligonucleotides[a]		Yield (%)	MALDI-TOF MS calcd $[M-H]^-$	MALDI-TOF MS found $[M-H]^-$
5'-d(GCGTT**X**TTTGCT)-3'	**ON2**	26	3686.4	3686.8
5'-d(GCGTT**XX**TTGCT)-3'	**ON3**	12	3740.5	3741.0
5'-d(GCGTT**XXX**TGCT)-3'	**ON4**	8	3794.5	3794.6
5'-d(GCGTT**X**T**X**TGCT)-3'	**ON5**	36	3740.5	3740.3
5'-d(GCG**X**T**X**T**X**TGCT)-3'	**ON6**	41	3794.5	3794.6
5'-d(TTTTTTTT**X**)-3'	**ON14**	23	2728.8	2728.5

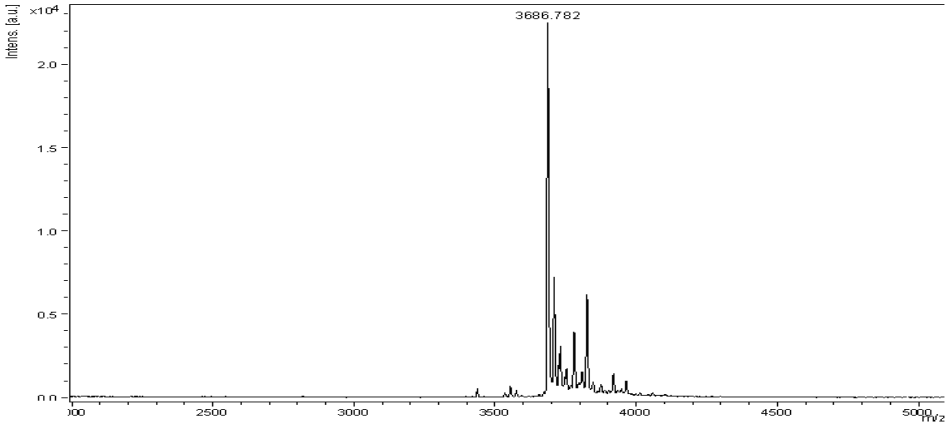
[a] **X** = scpBNA

Figure S31. MALDI-TOF MS spectra and HPLC charts of all new oligonucleotides.

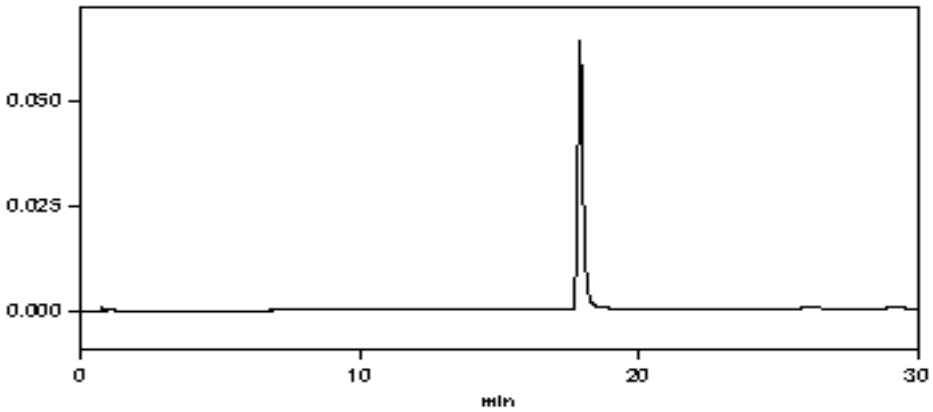
HPLC (ON2)



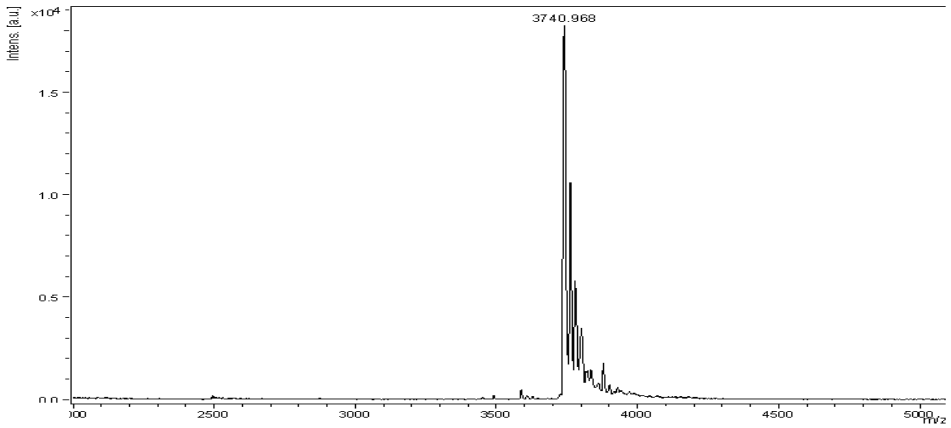
MALDI–TOF MS (ON2)



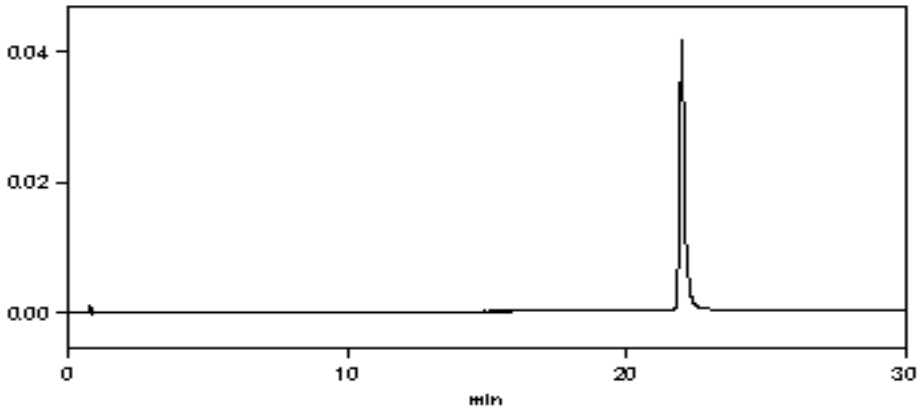
HPLC (ON3)



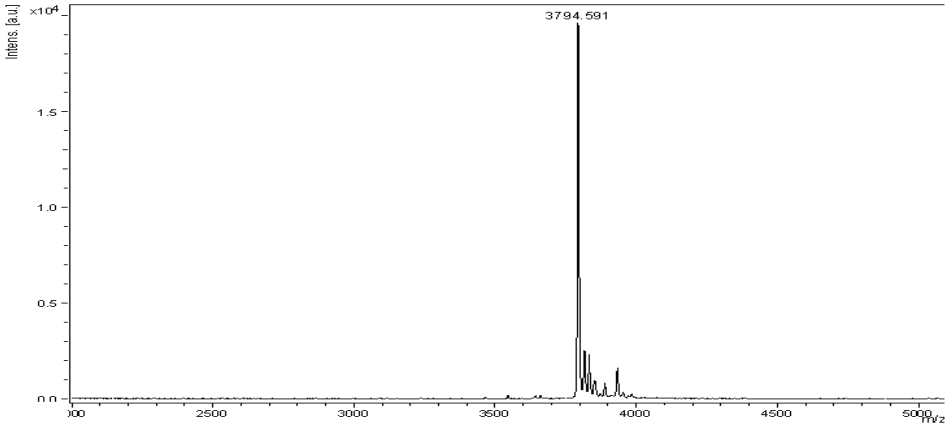
MALDI–TOF MS (ON3)



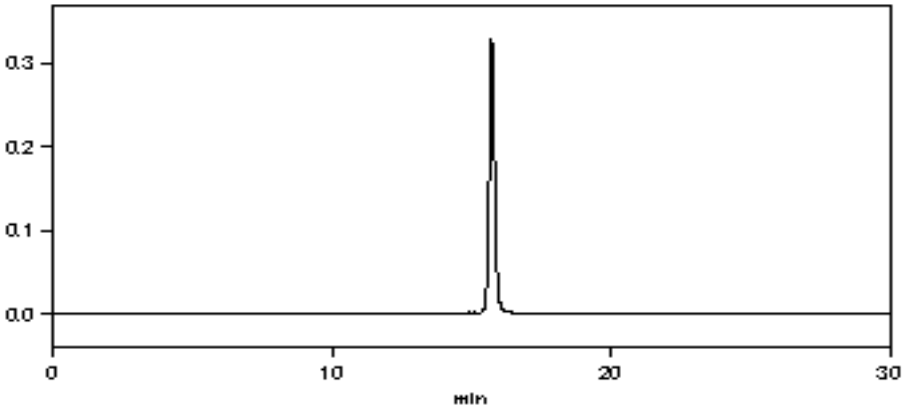
HPLC (ON4)



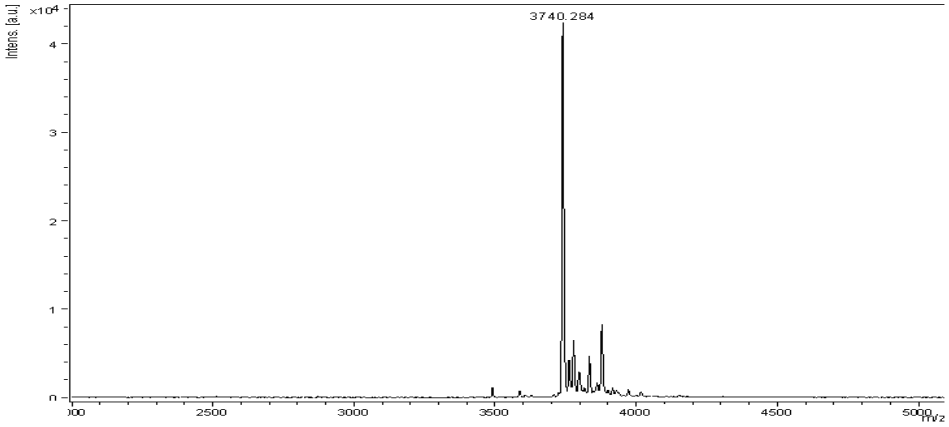
MALDI–TOF MS (ON4)



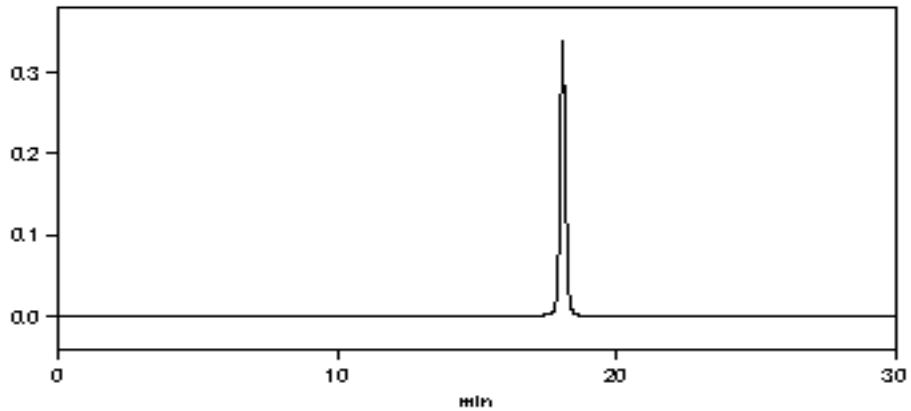
HPLC (ON5)



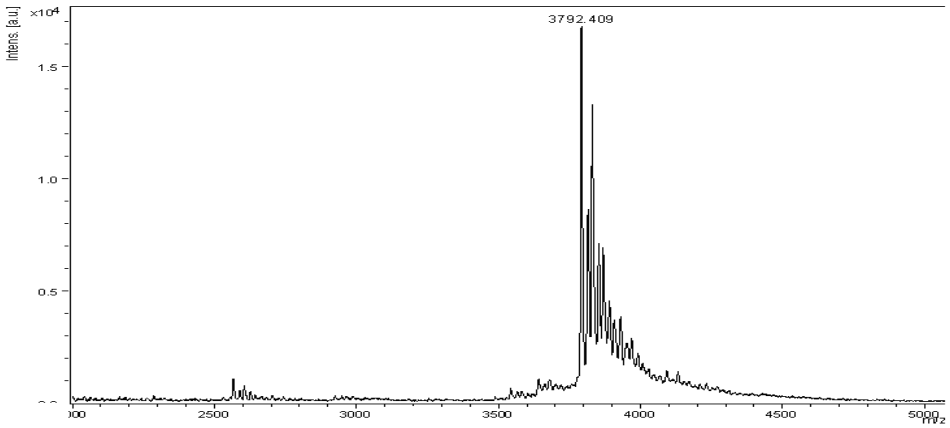
MALDI–TOF MS (ON5)



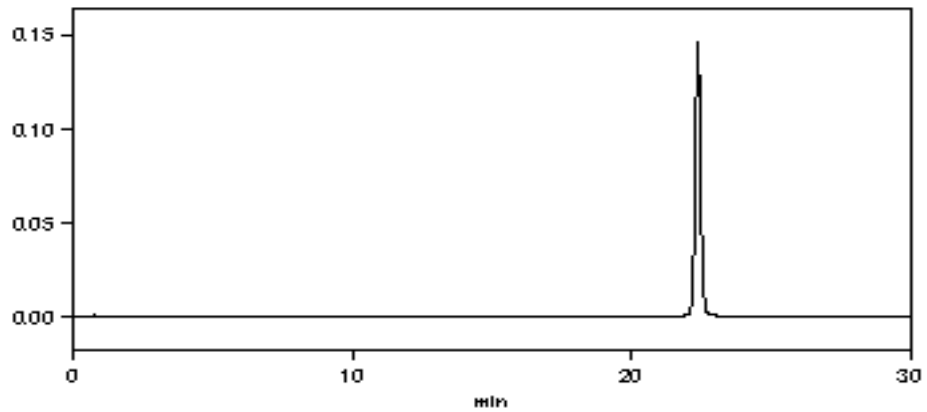
HPLC (ON6)



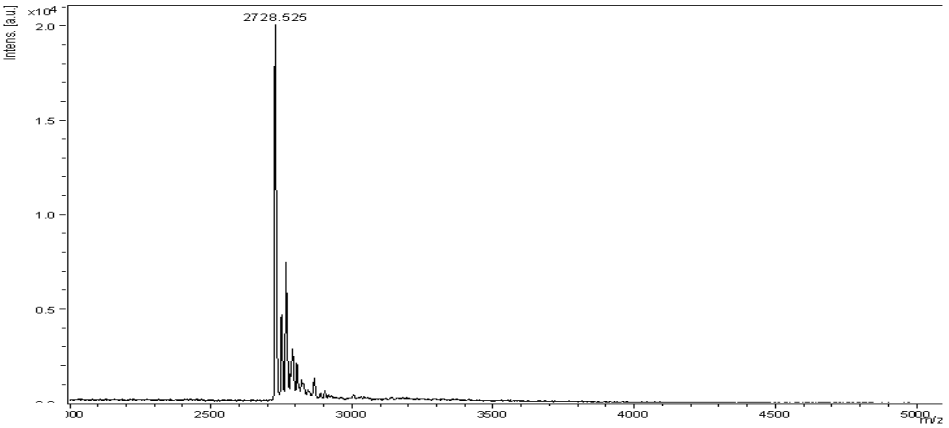
MALDI–TOF MS (ON6)



HPLC (ON14)



MALDI–TOF MS (ON14)



4. UV melting experiments

The UV melting experiments were carried out on SHIMADZU UV-1650PC and SHIMADZU UV-1800 spectrometers equipped with a T_m analysis accessory. Equimolecular amounts of the target RNA or DNA strand and oligonucleotide were dissolved in buffer (10 mM phosphate buffer at pH 7.2 containing 100 mM NaCl) to give final strand concentration of 4 μM. The samples were annealed by heating at 100 ºC followed by slow cooling to room temperature. The melting profile was recorded at 260 nm from 5 to 90 ºC at a scan rate of 0.5 ºC /min. The T_m value was calculated as the temperature of the half-dissociation of the formed duplexes based on the first derivative of the melting curve.

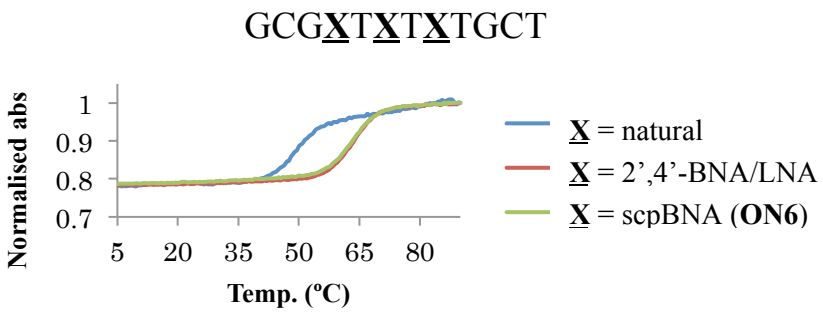


Figure S32. UV melting curves for the duplexes formed between representative oligonucleotides and ssRNA. The sequences of oligonucleotides and ssRNA are 5'-d(GCGXTXTXTGCT)-3' and 5'-d(AGCAAAAAACGC)-3', respectively.

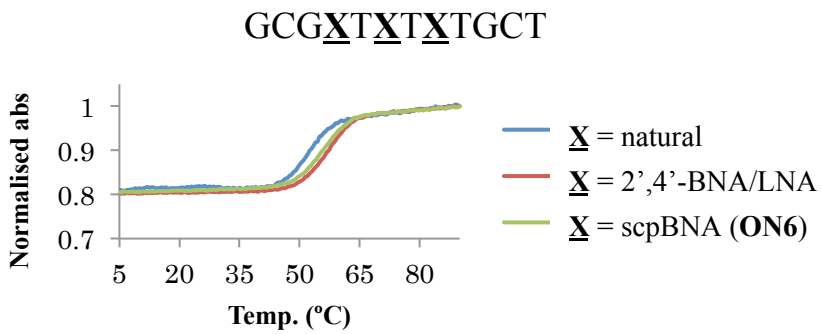
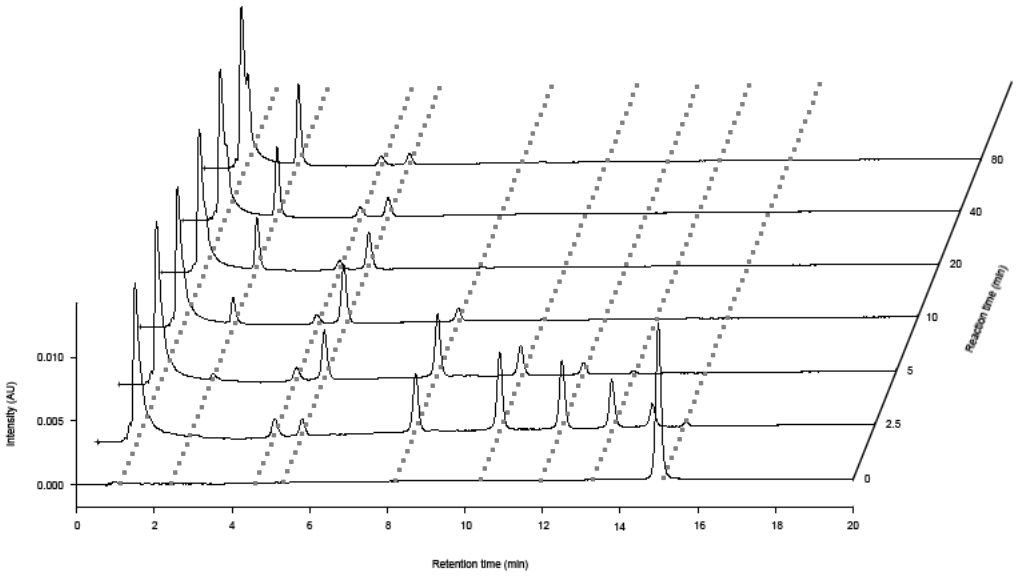


Figure S33. UV melting curves for the duplexes formed between representative oligonucleotides and ssDNA. The sequences of oligonucleotides and ssDNA are 5'-d(GCGXTXTXTGCT)-3' and

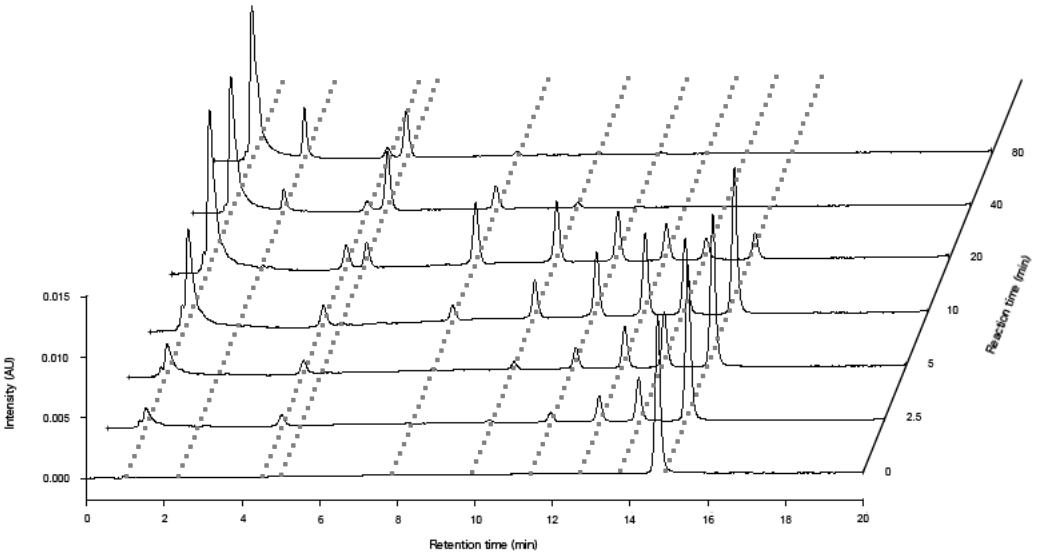
5’-r(AGCAAAAAACGC)-3’, respectively.

5. Nuclease resistance study

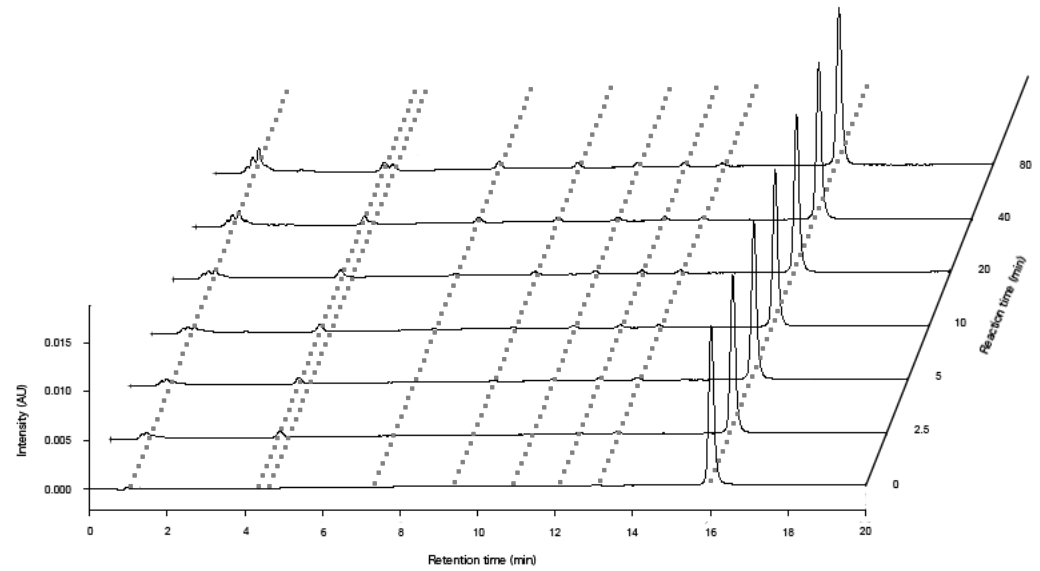
The sample solutions were prepared by dissolving 0.56 nmol of oligonucleotides in 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM $MgCl_2$. In each sample solutions, 0.14 µg CAVP was added and the cleavage reaction was carried out at 37 ºC. A portion of each reaction mixture was removed at time intervals and heated at 90 ºC for 2.5 min to deactivate the nuclease. Aliquots of the timed samples were analyzed by reverse-phase HPLC with Waters XBridge^{TM} OST C_{18} 2.5 µg (4.6 x 50 mm) columns to evaluate the amount of intact oligonucleotides remaining. The percentage of intact oligonucleotide in each sample was calculated and plotted against the digestion time to obtain a degradation curve.



Natural (**ON12**)

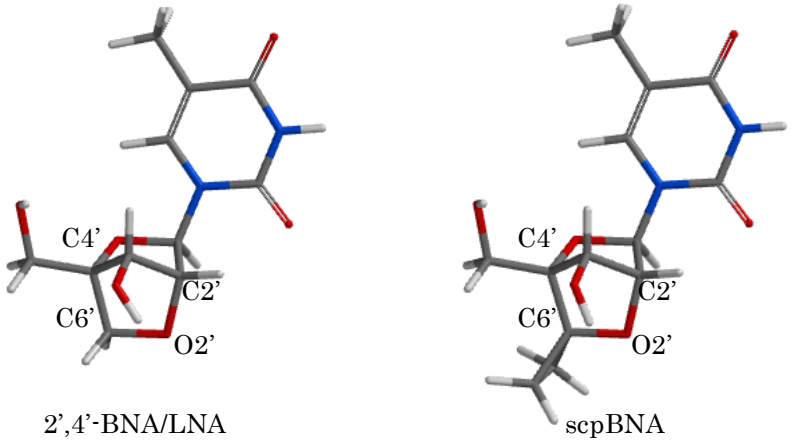


2',4'-BNA/LNA (**ON13**)



scpBNA (**ON14**)

Figure S34. Nuclease resistance of 5'-(TTTTTTTT**X**)-3' against *Crotalus admanteus* venom phosphodiesterase (CAVP). Column: Waters XBridge^{TM} OST C_{18} 2.5 µg (4.6 x 50 mm). Mobile Phase: Linear gradient of MeCN (6 to 12 % over 20 min) in 0.1 M triethylammonium acetate (pH 7.0). Flow rate: 1.0 mL/min. Detection: Absorbance at 260 nm.



2’,4’-BNA/LNA scpBNA

	2',4'-BNA/LNA	scpBNA
ν_0:	–0.71º	–1.74º
ν_1:	–35.74º	–34.78º
ν_2:	54.45º	53.92º
ν_3:	–56.87º	–57.17º
ν_4:	37.30º	37.72º
ν_{max}:	57.74º	57.54º
P:	19.44º	20.44º
O2'-C6'-C4':	102.96º	105.56º
C2'-O2'-C6':	104.29º	103.31º
δ:	62.13º	61.86º

Figure S35. Energy-minimized structures, endocyclic sugar torsion angles ν_0-ν_4, maximum torsion angle ν_{max}, pseudorotation phase angle *P*, bond angles of O2’-C6’-C4’ and C2’-O2’-C6’ and phosphate backbone torsion angle δ of 2’,4’-BNA/LNA and scpBNA. Theoretical calculation was carried out using HF/6-31G** basis set (Spartan ’10, Wavefunction, Inc). *P* and ν_{max} are calculated as follows: $\tan P = (\nu_4 + \nu_1 - \nu_3 - \nu_0)/(2 \cdot \nu_2 \cdot (\sin 36º + \sin 72º))$; $\nu_{max} = \nu_2 / \cos P$.

7. Mismatch discrimination and thermodynamic data of scpBNA

Table S2. T_m values (ºC) of duplexes formed between ONs and ssRNA with or without one base mismatch [a]

	T_m ($\Delta T_m = T_m$ [mismatch] – T_m [match]) (ºC)			
oligonucleotides	**Z** = A	G	C	U
ON1	48[b]	43 (–5)[b]	32 (–16)[b]	33 (–15)[b]
ON2	53	47 (–6)	35 (–18)	38 (–15)
ON7	52	47 (–5)	36 (–16)	39 (–13)

[a] Conditions: 10 mM phosphate buffer (pH 7.2), 100 mM NaCl, and 4 μM each oligonucleotide. The T_m values reflect the average of at least three measurements. The sequences of oligonucleotides are 5'-d(GCGTT**X**TTTGCT)-3' (**X** = natural thymidine (**ON1**), scpBNA-T (**ON2**), and 2',4'-BNA/LNA-T (**ON7**)). The sequence of ssRNA is 5'-r(AGCAAA**Z**AACGC)-3'. [b] Reference S1.

Table S3. T_m values (ºC) of duplexes formed between ONs and ssDNA with or without one base mismatch [a]

	T_m ($\Delta T_m = T_m$ [mismatch] – T_m [match]) (ºC)			
oligonucleotides	**Z** = A	G	C	T
ON1	52[b]	41 (–11)[b]	37 (–15)[b]	38 (–14)[b]
ON2	53	43 (–10)	38 (–15)	40 (–13)
ON7	53	42 (–11)	38 (–15)	41 (–12)

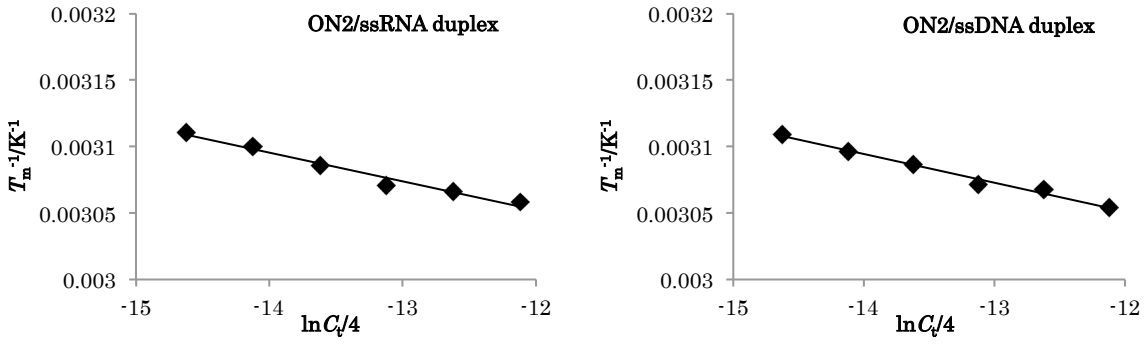
[a] Conditions: 10 mM phosphate buffer (pH 7.2), 100 mM NaCl, and 4 μM each oligonucleotide. The T_m values reflect the average of at least three measurements. The sequences of oligonucleotides are 5'-d(GCGTT**X**TTTGCT)-3' (**X** = natural thymidine (**ON1**), scpBNA-T (**ON2**), and 2',4'-BNA/LNA-T (**ON7**)). The sequence of ssDNA is 5'-d(AGCAAA**Z**AACGC)-3'. [b] Reference S1.

Table S4. Thermodynamic data of duplexes formed between ONs and ssRNA or ssDNA [a,b]

duplexes	ΔH^o (kcal mol^{-1})	ΔS^o (cal K^{-1} mol^{-1})	ΔG^o_{310K} (kcal mol^{-1})
ON1/ssRNA	–98.4[c]	–282[c]	–10.9[c]
ON2/ssRNA	–92.0	–257	–12.3
ON7/ssRNA	–87.6[c]	–244[c]	–12.0[c]
ON1/ssDNA	–84.6[c]	–235[c]	–11.6[c]
ON2/ssDNA	–92.0	–257	–12.4
ON7/ssDNA	–83.2[c]	–230[c]	–11.9[c]

[a] Conditions: 10 mM phosphate buffer (pH 7.2), 100 mM NaCl, and 0.89−10.9 μM each oligonucleotide (six data points). The T_m values reflect the average of at least three measurements. The sequences of oligonucleotides are 5'-d(GCGTT**X**TTTGCT)-3' (**X** = natural thymidine (**ON1**), scpBNA-T (**ON2**), and 2',4'-BNA/LNA-T (**ON7**)). The sequences of ssRNA and ssDNA are 5'-r(AGCAAAAAACGC)-3' and 5'-d(AGCAAAAAACGC)-3', respectively. [b] These values are calculated by Van't Hoff plots with six data points. See also Figure S36. [c] Reference S2.

Figure S36. Van't Hoff plots of T_m values of duplexes formed between **ON2** and ssRNA or ssDNA.



8. Supplementary references

S1 Y. Mitsuoka, T. Kodama, R. Ohnishi, Y. Hari, T. Imanishi, S. Obika, *Nucleic Acids Res.*, 2009, **37**, 1225–1238.

S2 K. Morihiro, T. Kodama, Kentefu, Y. Moai, R. N. Veedu, S. Obika, *Angew. Chem. Int. Ed.* 2013, **52**, 5074–5078.