Supporting Information

DNA duplexes and triplex-forming oligodeoxynucleotides incorporating modified nucleosides which can form stable and selective triplexes

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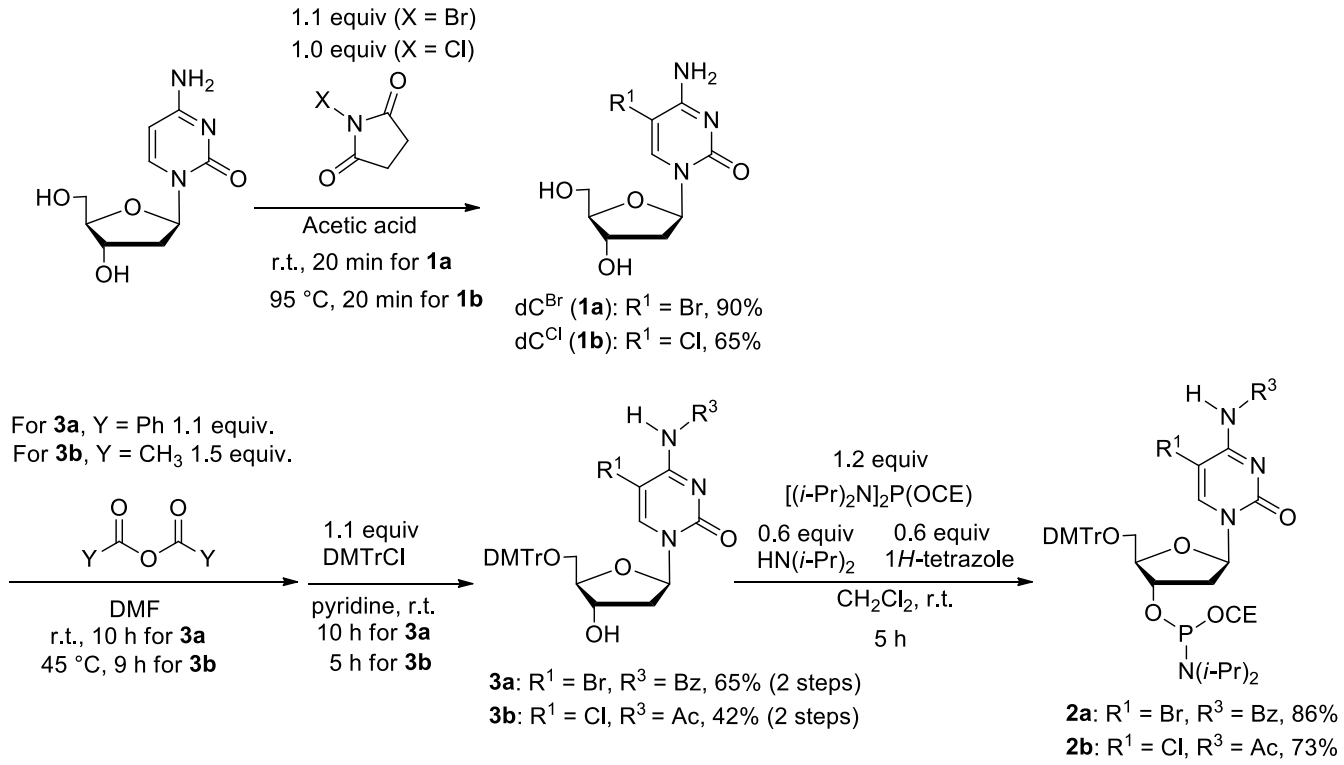
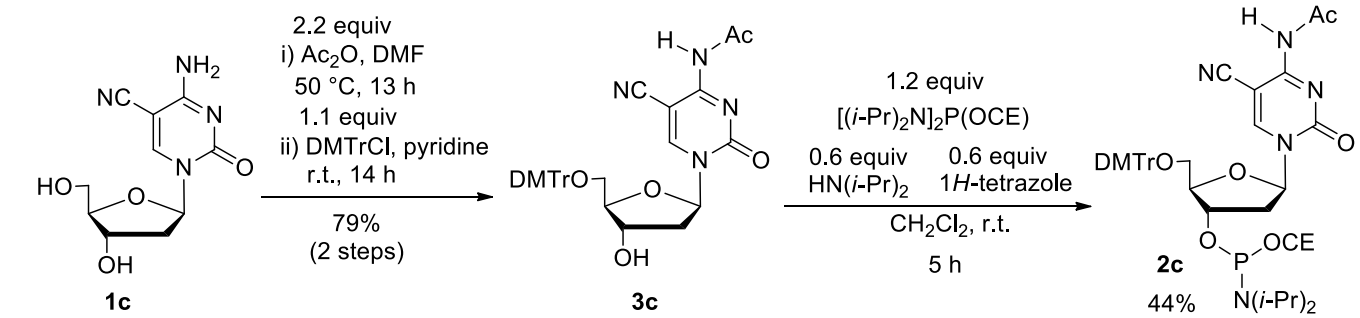
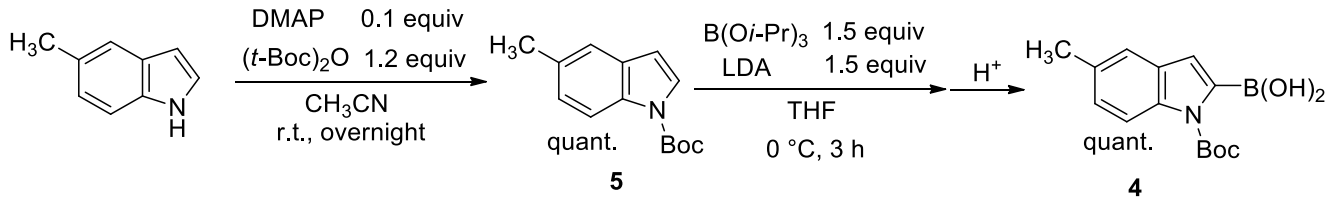
Contents



Scheme S1. Synthesis of nucleoside **1a**, **1b** and their phosphoramidites (**2a** and **2b**).



Scheme S2. Synthesis of nucleoside **2c**.



Scheme S3. Synthesis of 1-*N*-(*t*-Boc)-5-methyl-indole 2-borate (**4**).

General Methods

The dry solvents were purchased and stored over molecular sieves 4A. ^{1}H, ^{13}C, and ^{31}P NMR spectra were obtained at 500, 126, and 203 MHz, respectively. The chemical shifts were measured from DMSO-d_6 (2.49 ppm) for ^{1}H NMR, DMSO-d_6 (39.5 ppm) for ^{13}C NMR and 85% phosphoric acid (0.0 ppm) for ^{31}P NMR. Oligonucleotides were purified on anion-exchange high performance liquid chromatography (HPLC) at 50 °C with a linear gradient (10–67%) of solvent I (1 M NaCl in 25 mM phosphate buffer (pH 6.0), 10% acetnitrile) in solvent II (25 mM phosphate buffer (pH 6.0), 10% acetnitrile) was used at a flow rate of 1.0 mL/min for 40 min. MALDI-TOF mass was performed using 3-hydoroxypicolinic acid (100 mg/mL) in H_2O-diammonium hydrogen citrate (100 mg/mL) in H_2O (10 : 1, v/v) as a matrix.

Experimental procedure

5-Bromo-deoxycytidine (1a)[17, 18] Deoxycytidine (15.0 g, 66.0 mmol) and *N*-bromosuccinimide (12.9 g, 72.6 mmol) was dissolved in acetic acid (150 mL). The reaction mixture was stirred at room temperature for 20 min. Subsequently, the reaction mixture was cooled to room temperature in the water bath and then pyridine was added to neutralize. The solvents were removed under reduced pressure. The crude product was purified by chromatography on silica gel 60 N with $CHCl_3$-MeOH to give **1a** (18.2 g, 90%). R_f 0.25 ($CHCl_3$-CH_3OH, 4 : 1); IR (KBr) ν_{max} 3379, 3057, 1628, 1491, 1074 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.26 (1H, s), 7.86 (1H, br), 6.98 (1H, br), 6.07 (1H, t, J = 6.4 Hz), 5.22 (1H, br), 5.12 (1H, br), 4.21 (1H, d, J = 2.2 Hz), 3.78 (1H, d, J = 3.2 Hz), 3.61 (1H, m), 3.55 (1H, m), 2.17-2.12 (1H, m), 2.02-1.98 (1H, m); ^{13}C-NMR (DMSO-d_6) δ 162.0, 153.9, 142.1, 87.5, 86.4, 85.6,70.0, 60.9, 40.9. ESI-MS *m*/*z* calcd for $C_9H_{13}BrN_3O_4$+ [M + H]+ 306.0084; found 306.0076.

4-*N*-Benzoyl-5-bromo-5'-*O*-(4, 4'-dimethoxytrityl)-deoxycytidine (3a) Compound **1a** (3.0 g, 9.8 mmol) was treated with benzoic anhydride (2.4 g, 10.8 mmol) in DMF (40 mL) at room temperature for

10 h. After the completion of the reaction, solvent was evaporated. Ethyl acetate was added, and the precipitates were filtrated and dried under vacuum to give 3.4 g of the 4-*N*-bezoyl-5-bromo-deoxycytidine which was used without further purification. 3.0 g of this compound was treated with 4, 4'-dimethoxytrityl chloride (3.1 g, 8.0 mmol) in dry pyridine (73 mL) at room temperature for 10 h. The reaction was quenched with methanol and the solvent was evaporated. The crude product was purified by C-200 silica gel chromatography with hexane-ethyl acetate-0.5% pyridine to give **3a** (4.0 g, 65%, 2 steps). R_f 0.10 (*n*-hexane-EtOAc, 3 : 2); IR (KBr) ν_{max} 3433, 3065, 2932, 1709, 1578, 1508, 1248, 1175, 1094, 1034, 827, 712 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 12.79 (1H, br), 8.18-8.14 (3H, br), 7.61 (1H, m), 7.51 (2H, m), 7.40 (2H, m), 7.33-7.28 (6H, m), 7.22 (1H, m), 6.91-6.89 (4H, m), 6.12 (1H, t, *J* = 6.5Hz), 5.37 (1H, d, *J* = Hz), 4.28 (1H, br), 3.97 (1H, s), 3.72 (6H, s), 3.27-3.19 (2H, br), 2.32 (2H, br); ^{13}C-NMR (DMSO-d_6) δ 158.1, 144.7, 135.5, 135.3, 132.9, 129.7, 128.5, 128.0, 127.6, 126.8, 113.3, 86.2, 86.0, 70.2, 63.5, 55.1. ESI-MS *m*/*z* calcd for $C_{37}H_{35}BrN_3O_7$+ [M + H]+ 712.1653; found 712.1667.

5-Chloro-deoxycytidine (1b)[18-20] In a manner similar to that described for the synthesis of **1a**, deoxycytidine (11.0 g, 48.4 mmol) and *N*-chlorosuccinimide (6.5 g, 50.0 mmol) were treated in acetic acid (100 mL). The reaction mixture was stirred at 95 °C for 20 min. The purification by chromatography gave **1b** (8.2 g, 65%). R_f 0.19 ($CHCl_3$-CH_3OH, 4 : 1); IR (KBr) ν_{max} 3367, 3267, 3117, 1655, 1491, 1340, 1090 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.19 (1H, s), 7.83 (1H, br), 7.20 (1H, br), 6.08 (1H, t, *J* = 6.4 Hz), 5.21 (1H, d, *J* = 4.2 Hz), 5.18 (1H, m), 4.21 (1H, m), 3.78 (1H, m), 3.62 (1H, m), 3.56 (1H, m), 2.14 (1H, m), 2.00 (1H, m); ^{13}C-NMR (DMSO-d_6) δ 162.1, 154.3, 139.9, 99.5, 88.1, 86.1, 70.6, 61.6, 41.4. ESI-MS *m*/*z* calcd for $C_9H_{13}ClN_3O_4$+ [M + H]+ 262.0589; found 262.0573.

4-*N*-Acetyl-5-chloro-5'-*O*-(4, 4'-dimethoxytrityl)-deoxycytidine (3b) Compound **1b** (4.6 g, 17.4 mmol) was treated with acetic anhydride (2.5 ml, 26.1 mmol) in DMF (70 mL) at 45 °C for 9 h. The reaction was quenched with ethanol and the solvents were removed in vacuo. The crude product was

coevaporated three times with pyridine and dissolved in pyridine (174 mL). 4, 4'-Dimethoxytrityl chloride (7.4 g, 19.1 mmol) was added to the reaction mixture and stirred for 5 h at room temperature. After the completion of the reaction, isopropanol (20 mL) was added and the reaction mixture was evaporated. The crude product was extracted with water/ethyl acetate and ethyl acetate layer was collected and evaporated. Crude product was purified by 60 N silica gel chromatography with hexane-ethyl acetate-0.5% pyridine to give **3b** (4.4 g, 42%, 2 steps). R_f 0.24 ($CHCl_3$-CH_3OH, 9 : 1); IR (KBr) ν_{max} 3379, 2934, 1655, 1508, 1252, 1178, 1095, 1034, 829 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.89 (1H, br), 8.19 (1H, s), 7.38 (2H, d, J = 7.6 Hz), 7.32-7.18 (7H, m), 6.89-6.88(4H, m), 6.05 (1H, t, J = 6.2 Hz), 5.36 (1H, d, J = 4.6), 4.28 (1H, m), 3.98 (1H, m), 3.72 (6H, s), 3.27-3.18 (2H, m), 2.38-2.34 (1H, m), 2.26 (3H, s), 2.25-2.20 (1H, m); ^{13}C-NMR (DMSO-d_6) δ 158.1, 144.7, 135.4, 135.3, 129.7, 128.0, 127.6, 126.8, 113.3, 86.8, 86.2, 86.0, 70.0, 63.2, 55.1, 40.8, 25.0. ESI-MS *m/z* calcd for $C_{32}H_{33}ClN_3O_7$+ [M + H]+ 606.2002; found 606.2028.

4-*N*-Acetyl-5-cyano-5'-*O*-(4, 4'-dimethoxytrityl)-deoxycytidine (3c) 5-Cyanodeoxycytidine[21, 22] (200 mg, 0.79 mmol) was converted to **3c** in the manner similar to that described for the synthesis of **3b**. 5-Cyanodeoxycytidine and acetic anhydride (164 μL, 1.74 mmol) were treated in DMF (7.9 mL) at 50 °C for 13 h. After the quenching of the reaction and the removal of the solvents by evaporation, the crude product was treated with 4, 4'-dimethoxytrityl chloride (337 mg, 0.87 mmol) for 14 h. After being quenched with methanol and subsequent extraction, the ethyl acetate layer was evaporated. Collection of the white precipitates from diethylether gave **3c** (373 mg, 79%, 2 steps). R_f 0.25 (*n*-hexane-EtOAc, 1 : 4); IR (KBr) ν_{max} 3387, 3056, 2935, 2230, 1670, 1508, 1250, 1034, 829 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 10.97 (1H, br), 8.56 (1H, s), 7.37-7.36 (2H, m), 7.31-7.19 (7H, m), 6.89-6.87 (4H, m), 6.00 (1H, t, J = 6.1 Hz), 5.33 (1H, d, J = 4.6 Hz), 4.18 (1H, m), 4.00 (1H, m), 3.73 (6H, s), 3.27 (1H, m), 3.17 (1H, m), 2.37 (1H, m), 2.24 (1H, m), 2.13 (3H, s); ^{13}C-NMR (DMSO-d_6) δ 158.1, 144.7, 135.5, 135.3, 129.6,

127.9, 127.6, 126.7, 113.9, 113.3, 87.8, 86.5, 85.9, 85.8, 69.9, 63.4, 55.0, 40.5, 23.8. ESI-MS calcd for $C_{33}H_{32}N_4NaO_7$+ [M + Na]+ 619.2163; found 619.2106.

4-*N*-Benzoyl-5-bromo-5'-*O*-(4, 4'-dimethoxytrityl)-deoxycytidine 3'-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (2a) Compound **3a** (1.5 g, 2.1 mmol) was coevaporated with anhydrous acetnitrile. To a stirred solution of **3a,** diisopropylamine (179 μL, 1.3 mmol), and 1*H*-tetrazole (89 mg, 1.3 mmol) in dichloromethane (21 mL) was added 2-cyanoethyl *N, N, N', N'*-tetraisopropylphosphoramidite (763 mg, 2.5 mmol). The solution was stirred at room temperature under argon for 5 h. The reaction was diluted with water, and extracted with chloroform. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by C-200 silica gel chromatography with ethyl acetate-hexane-0.5% pyridine to give **2a** (1.7 g, 86%). R_f 0.39, 0.45 (n-hexane-EtOAc, 3 : 2); ^{1}H-NMR (DMSO-d_6) δ 12.74 (1H, br), 8.22 (1H, br), 8.14 (2H, br), 7.61 (1H, m), 7.51 (2H, m), 7.40 (2H, m), 7.33-7.26 (6H, m), 7.22 (1H, m), 6.88 (4H, m), 6.11 (1H, m), 4.48 (1H, m), 4.11-4.07 (1H, m), 3.72 (6H, m), 3.70-3.65 (1H, m), 3.62-3.45 (3H, m), 3.29-3.23 (2H, m), 2.75 (1H, t, J = 5.9 Hz), 2.64 (1H, t, J = 5.9 Hz), 2.49-2.31 (2H, m), 1.13-1.10 (8H, m), 0.97 (3H, m); ^{13}C-NMR (DMSO-d_6) δ 158.2, 144.7, 135.3, 135.2, 132.9, 129.7, 129.4, 128.9, 128.5, 128.0, 127.7, 127.6, 127.4, 126.8, 119.0, 118.8, 113.3, 112.8, 86.1, 86.0, 85.1, 84.7, 72.7, 72.6, 72.2, 72.1, 63.1, 62.8, 58.4, 58.3, 55.1, 42.6, 42.5, 24.4, 24.3, 24.2, 19.9, 19.8; ^{31}P-NMR (DMSO-d_6) δ 148.7, 148.4. ESI-MS calcd for $C_{46}H_{51}BrN_5NaO_8P$+ [M + Na]+ 934.2551; found 934.2515.

4-*N*-acetyl-5-chloro-5'-*O*-(4, 4'-dimethoxytrityl)-deoxycytidine 3'-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (2b) Compound **3b** (2.0 g, 3.2 mmol) was converted to **2b** in the same procedure for **2a**. To a stirred solution of **3b,** diisopropylamine (0.28 mL, 2.0 mmol), and 1*H*-tetrazole (139 mg, 2.0 mmol) in dichloromethane (33 mL) was added 2-cyanoethyl *N, N, N', N'*-

tetraisopropylphosphoramidite (1.2 g, 4.0 mmol). The purification gave **2b** (2.0 g, 73%). R_f 0.51, 0.63 (*n*-hexane-EtOAc, 4 : 1); ^{1}H-NMR (DMSO-d_6) δ 9.91 (1H, br), 8.22 (1H, s), 7.39-7.38 (2H, m), 7.31-7.22 (7H, m), 6.87 (4H, m), 6.05 (1H, m), 4.50 (1H, m), 4.12-4.08 (1H, m), 3.72 (6H, s), 3.69-3.46 (4H, m), 3.29 (2H, m), 2.75 (1H, t, J = 5.7 Hz), 2.64 (1H, t, J = 5.7 Hz), 2.47-2.36 (2H, m), 2.26 (3H, m), 1.13-1.09 (10H, m), 0.972 (2H, m); ^{13}C-NMR (DMSO-d_6) δ 170.0, 158.2 152.5, 149.6, 144.6, 141.8, 135.3, 135.2, 129.7, 128.0, 127.6, 126.8, 123.9, 119.0, 115.7, 113.3, 102.2, 86.8, 86.1, 86.0, 85.2, 84.8, 72.6, 72.4, 72.1, 71.9, 62.9, 62.6, 58.5, 58.4, 58.3, 58.2, 55.1, 43.8, 42.7, 42.6, 25.0, 24.4, 24.3, 23.4, 22.3, 19.8; ^{31}P-NMR (DMSO-d_6) δ 148.7, 148.5. ESI-MS calcd for $C_{41}H_{49}ClN_5NaO_8P$+ [M + Na]+ 828.2899; found 828.2853.

4-*N*-acetyl-5-cyano-5'-*O*-(4, 4'-dimethoxytrityl)-deoxycytidine 3'-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (2c) Compound **3c** (200 mg, 0.34 mmol) was converted to **2c** in the same procedure for **2a**. To a stirred solution of **3c,** diisopropylamine (28 μL, 0.20 mmol), and 1*H*-tetrazole (14 mg, 0.20 mmol) in dichloromethane (3.3 mL) was added 2-cyanoethyl *N, N, N', N'*tetraisopropylphosphoramidite (121 mg, 0.40 mmol). The purification gave **2c** (117 mg, 44%). R_f 0.63, 0.70 (*n*-hexane-EtOAc, 1 : 4); ^{1}H-NMR (DMSO-d_6) δ 10.99 (1H, br), 8.64 (1H, m), 7.31-7.19 (9H, m), 6.82-6.86 (4H, m), 6.04-5.99 (1H, m), 4.40 (1H, m), 4.12 (1H, m), 3.73 (6H, s), 3.72-3.65 (1H, m), 3.56-3.43 (3H, m), 3.35-3.19 (2H, m), 2.74 (1H, t, J = 5.9 Hz), 2.63 (1H, t, J = 5.9 Hz) 2.54-2.35 (2H, m), 2.14 (3H, s), 0.96 (3H, m); ^{13}C-NMR (DMSO-d_6) δ 203.3, 158.2, 144.7, 135.4, 135.3, 135.2, 129.7, 128.0, 127.6, 127.6, 126.8, 118.8, 114.0, 113.3, 87.8, 86.0, 85.9, 85.1, 72.5, 63.2, 62.8, 60.5, 58.4, 58.3, 55.0, 42.7, 42.6, 24.3, 24.2, 19.8; ^{31}P-NMR (DMSO-d_6) δ 148.8, 148.6. ESI-MS calcd for $C_{42}H_{49}N_6NaO_8P$+ [M + H]+ 819.3242; found 819.3260.

1-*N*-(*tert*-butoxycarbonyl)-5-methyindol (5) To a solution of 5-methylindole (3.0 g, 22.8 mmol) and *N*, *N*-dimetylaminopyridine (278 mg, 2.28 mmol) in acetonitrile (50 mL) was added Boc anhydride (6.0

g, 27.3 mmol). After being stirred at room temperature overnight, the mixture was quenched with methanol and the solvent was removed by evaporation. The resulting residue was purified by C-200 silica gel chromatography with ethyl acetate-hexane to give **5** (5.3 g, quant). R_f 0.55 (*n*-Hexane-EtOAc, 9 : 1); IR ($CHCl_3$) ν_{max} 1724 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.91 (1H, d, J = 8.5 Hz), 7.60 (1H, d, J = 3.7 Hz), 7.39 (1H, s), 7.12 (1H, d, J = 8.5 Hz), 6.61 (1H, d, J = 3.7 Hz), 2.37 (3H, s), 1.61 (9H, s); ^{13}C-NMR (DMSO-d_6) δ 149.1, 131.6, 130.4, 126.1, 125.5, 120.9, 114.3, 109.3, 107.2, 83.6, 27.7, 20.9. ESI-MS calcd for $C_{14}H_{18}NO_2$+ [M + H]+ 232.1332; found 232.1336.

1-*N*-(*tert*-butoxycarbonyl)-5-methyindol-2-borate (4) To the solution of **5** (8.8 g, 38 mmol) in THF (32 mL) was added triisopropyl borate (13 mL, 57 mmol). The solution was cooled to 0 °C in an ice bath, and LDA (2.0 M, 57 mmol) was added over 1 h. After 2 h, the reaction was poured into the phosphate buffer (pH 6.0) then extracted with chloroform. The organic layer was collected, dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was precipitated from chloroform to give **4** (10.5 g, quant). R_f 0.17 (*n*-hexane-EtOAc, 4 : 1); IR (KBr) ν_{max} 3373, 3153 ,1690, 1541, 1379, 1121 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.96 (1H, d, J = 8.4 Hz), 7.34 (1H, s), 7.09 (1H, d, J = 8.4 Hz), 6.55 (1H, s), 2.37 (3H, s), 1.59 (9H, s); ^{13}C-NMR (DMSO-d_6) δ 149.9, 134.5, 131.3, 130.9, 125.3, 120.5, 114.2, 112.0, 83.8, 27.6, 21.0. ESI-MS calcd for $C_{14}H_{18}BNNaO_4$+ [M+H]+ 298.1221; found 298.1265.

10-(β-D-2-deoxyribos-1-yl)-3-methyl-pyrimido[4,5-d]pyrimido[1,6-a]indol (1f) 5-iodo-deoxycytidine[18] (1.5 g, 4.3 mmol), palladium acetate (29 mg, 0.13 mmol), TPPTS (193 mg, 0.34 mmol), Na_2CO_3 (991 mg, 9.4 mmol) and **4** (2.3 g, 8.5 mmol) were placed in a round-bottomed flask under argon. Degassed H_2O-CH_3CN (1:1, v/v, 85 mL) was added, and the mixture was stirred at 45 °C for overnight. The mixture was evaporated under reduced pressure. The residue was diluted with MeOH, filtered, and concentrated under reduced pressure. The product was precipitated from diethylether to

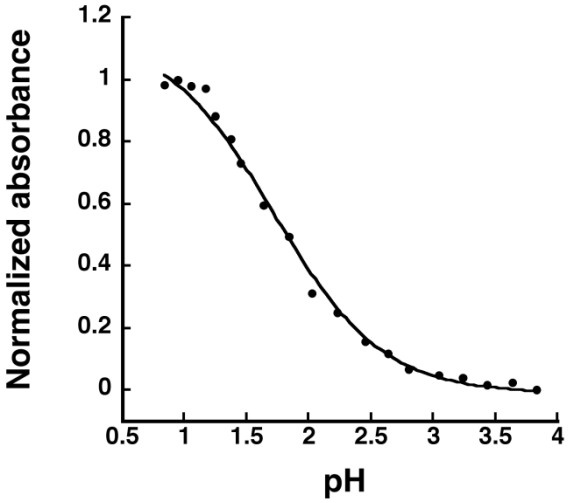
give **1f** (1.0 g, 63%). R_f 0.44 ($CHCl_3$-CH_3OH, 9 : 1); IR (KBr) ν_{max} 3366, 3067, 2924, 1718, 1655, 1352, 1263, 1097, 812 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.9 (1H, br), 9.31 (1H, s), 8.22 (1H, d, J = 8.3 Hz), 7.40 (1H, s), 7.12 (1H, d, J = 8.3 Hz), 6.85 (1H, s), 6.15 (1H, t, 5.5 Hz), 5.50 (1H, s), 5.32 (1H, d, J = 3.9 Hz), 4.30 (1H, m), 3.91 (1H, m), 3.83 (1H, m), 3.70 (1H, m), 2.40 (3H, s), 2.39-2.34 (1H, m), 2.21-2.18 (1H, m); ^{13}C-NMR (DMSO-d_6) δ 157.5, 153.3, 140.2, 133.0, 131.5, 130.2, 130.1, 124.6, 119.8, 114.7, 97.0, 95.9, 87.7, 86.7, 68.5, 60.1, 41.1, 21.2. ESI-MS calcd for $C_{19}H_{19}N_4O_7$+ [M + H]+ 383.1350; found 383.1312.

β-D-1-(3-methyl-pyrimido[4, 5-d]pyrimido[1, 6-a]indol-10-yl)-5-*O*-(4, 4'-dimethoxytrityl)-2-deoxyribose (3f) Compound **1f** (0.80 g, 2.1 mmol) was coevaporated three times with pyridine and dissolved in pyridine (20 mL). Dimethoxytrityl chloride (0.99 g, 2.9 mmol) was added to the reaction mixture and stirred for 6 h at room temperature. After the completion of reaction, methanol was added and reaction mixture was evaporated. The crude product was purified by C-200 silica gel chromatography with $CHCl_3$-MeOH-0.5% pyridine to give **3f** (1.3 g, 89%). R_f 0.13 (*n*-hexane-EtOAc, 1 : 4); IR (KBr) ν_{max} 3367, 2934, 1734, 1647,1551, 1508, 1350, 1254, 1177, 1095, 1034, 829 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 12.0 (1H, br), 8.68 (1H, s), 8.18 (1H, d, J = 8.3 Hz), 7.39 (2H, m), 7.29-7.26 (6H, m), 7.18 (2H, m), 7.09 (1H, m), 6.84 (4H, m), 6.24-6.20 (2H, m), 5.37 (1H, br), 4.24 (1H, br), 4.10 (1H, br), 3.64-3.62 (6H, m), 3.36-3.34 (2H, m), 3.27-3.24 (1H, m), 2.45-2.43 (1H, m), 2.39 (3H, s), 2.31-2.26 (1H, m); ^{13}C-NMR (DMSO-d_6) δ 158.1, 157.8, 153.3, 147.1, 144.6, 139.1, 135.6, 135.5, 132.8, 131.4, 129.9, 129.7, 129.6, 129.4, 127.9, 127.8, 126.8, 124.7, 119.8, 114.7, 113.3, 97.6, 96.2, 87.2, 86.5, 85.9, 70.3, 63.7, 54.9, 40.9, 21.1. ESI-MS calcd for $C_{40}H_{36}N_4NaO_7$+ [M + H]+ 707.2476; found 707.2474.

β-D-1-(3-methyl-pyrimido[4,5-d]pyrimido[1,6-a]indol-10-yl)-5-*O*-(4, 4'-dimethoxytrityl)-2-deoxyribose-3-(2-cyanoetyl *N,N*-diisopropylphosphoramidite) (2f) Compound **3f** (1.0 g, 1.5 mmol) was converted to **2f** in the procedure same as that for **2a**. To a stirred solution of **3f,** diisopropylamine

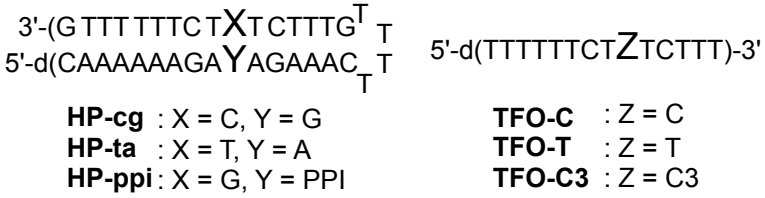
(124 μL, 0.88 mmol), and 1*H*-tetrazole (61 mg, 0.88 mmol) in dichloromethane (20 mL) was added 2-cyanoethyl *N, N, N', N'*-tetraisopropylphosphoramidite (0.53 mg, 1.8 mmol). The purification gave **2f** (0.94 g, 73%). R_f 0.38, 0.51 (*n*-hexane-EtOAc, 3 : 2); ^{1}H-NMR (DMSO-d_6) δ 12.0 (1H, br), 8.75 (1H, m), 8.19 (1H, d, J = 8.3 Hz), 7.39 (2H, m), 7.28-7.21 (6H, m), 7.18 (2H, m), 7.10 (1H, d, J = 8.3Hz), 6.88-6.82 (4H, m), 6.32 (1H, m), 6.23 (1H, m), 4.47 (1H, m), 4.22 (1H, m), 3.73-3.29 (12H, m), 2.76 (1H, t, J = 5.9 Hz), 2.64 (1H, t, J = 5.9 Hz), 2.61-2.54 (1H, m), 2.46-2.44 (1H, m), 2.39 (3H, s), 1.11 (9H, m), 0.98 (3H, m); ^{13}C-NMR (DMSO-d_6) δ 158.2, 157.8, 153.2, 147.1, 144.5, 139.5, 139.3, 135.4, 135.3, 132.9, 131.4, 123.0, 129.7, 129.4, 128.0, 127.8, 126.9, 124.8, 119.8, 119.0, 118.8, 114.7, 113.3, 97.8, 96.4, 87.3, 86.1, 86.0, 85.5, 85.2, 73.0, 72.9, 72.7, 72.5, 63.1, 63.0, 58.4, 58.3, 55.1, 55.0, 43.8, 42.7, 42.6, 24.3, 23.4, 22.3, 21.1, 19.8; ^{31}P-NMR (DMSO-d_6) δ 150.6, 150.4. ESI-MS calcd for $C_{49}H_{53}N_6NaO_8$+ [M + H]+ 907.3555; found 907.3593.

Measurement of the pK_a of 5-cyanodeoxycytidine



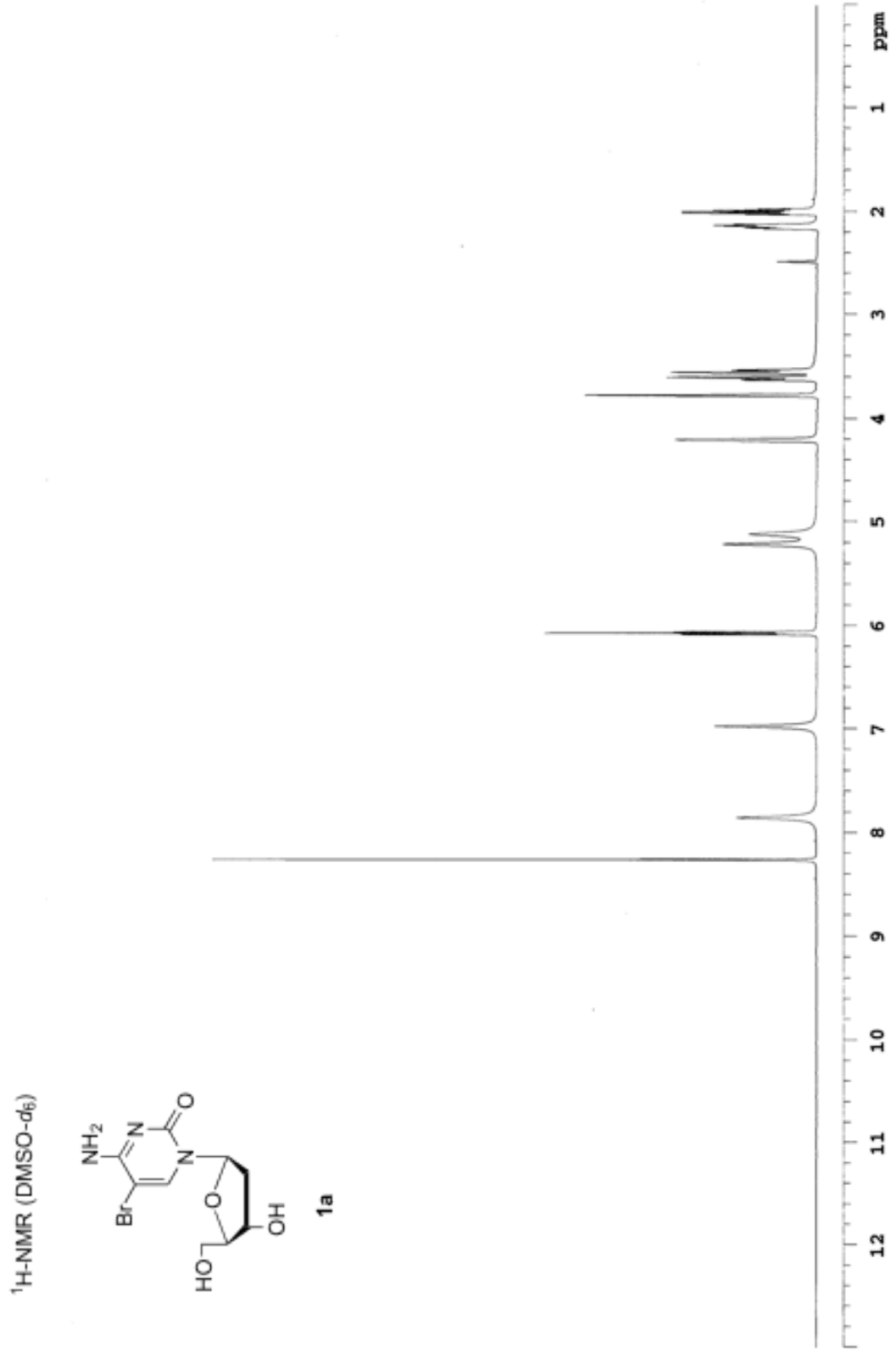
The pK_a value of the N3 of 5-cyanodeoxycytidine was determined by plotting normalized relative absorbances at 294 nm against pH values. The plots were fitted to the theoretical curve. The pK_a was determined to be 1.7. The concentration of the nucleoside was 70 μM, and the measurements were carried out in 10 mM citrate buffer, 10 mM NaCl, 10 mM $MgCl_2$.

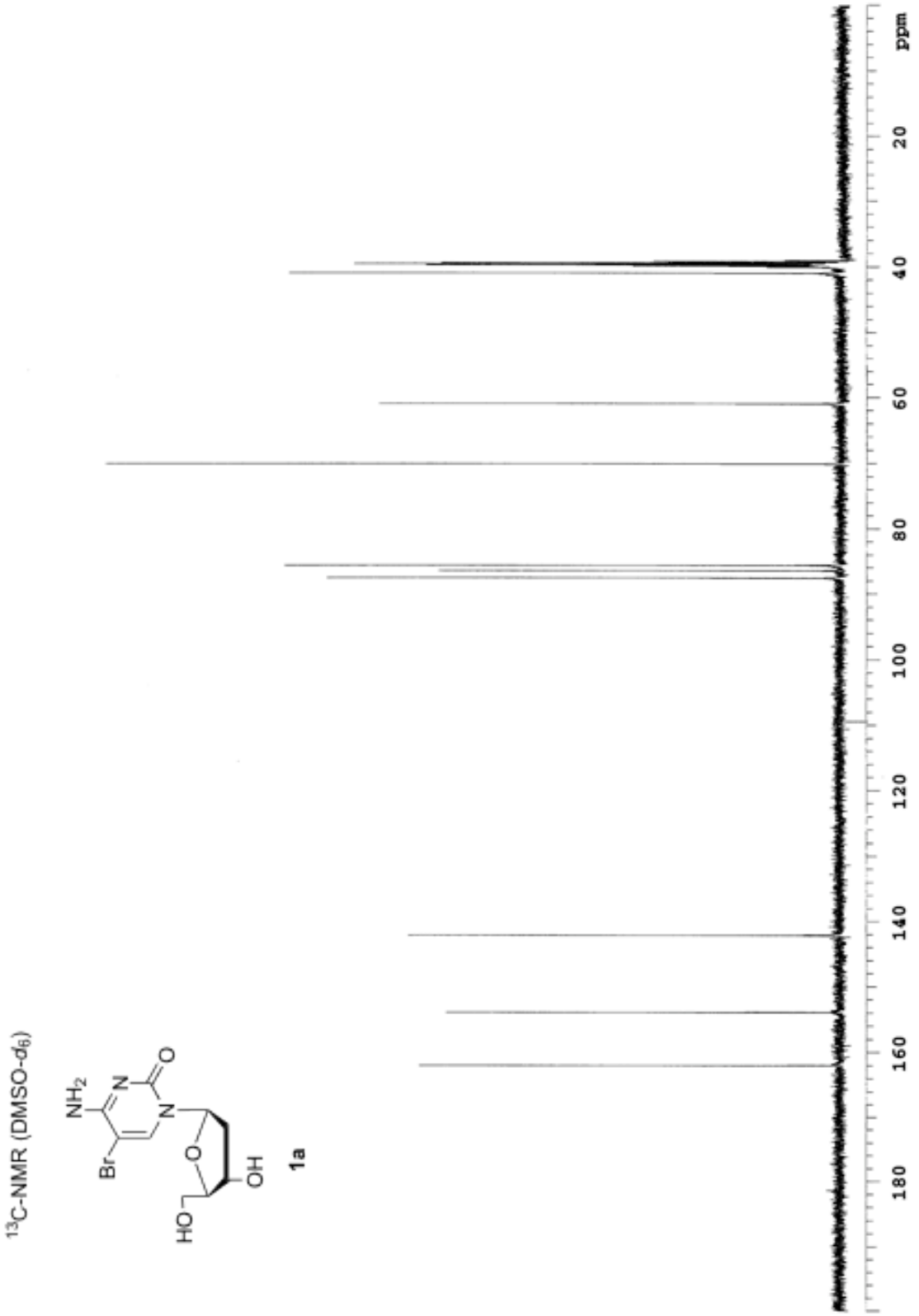
T_m values of several triplexes in pH 7.0 condition

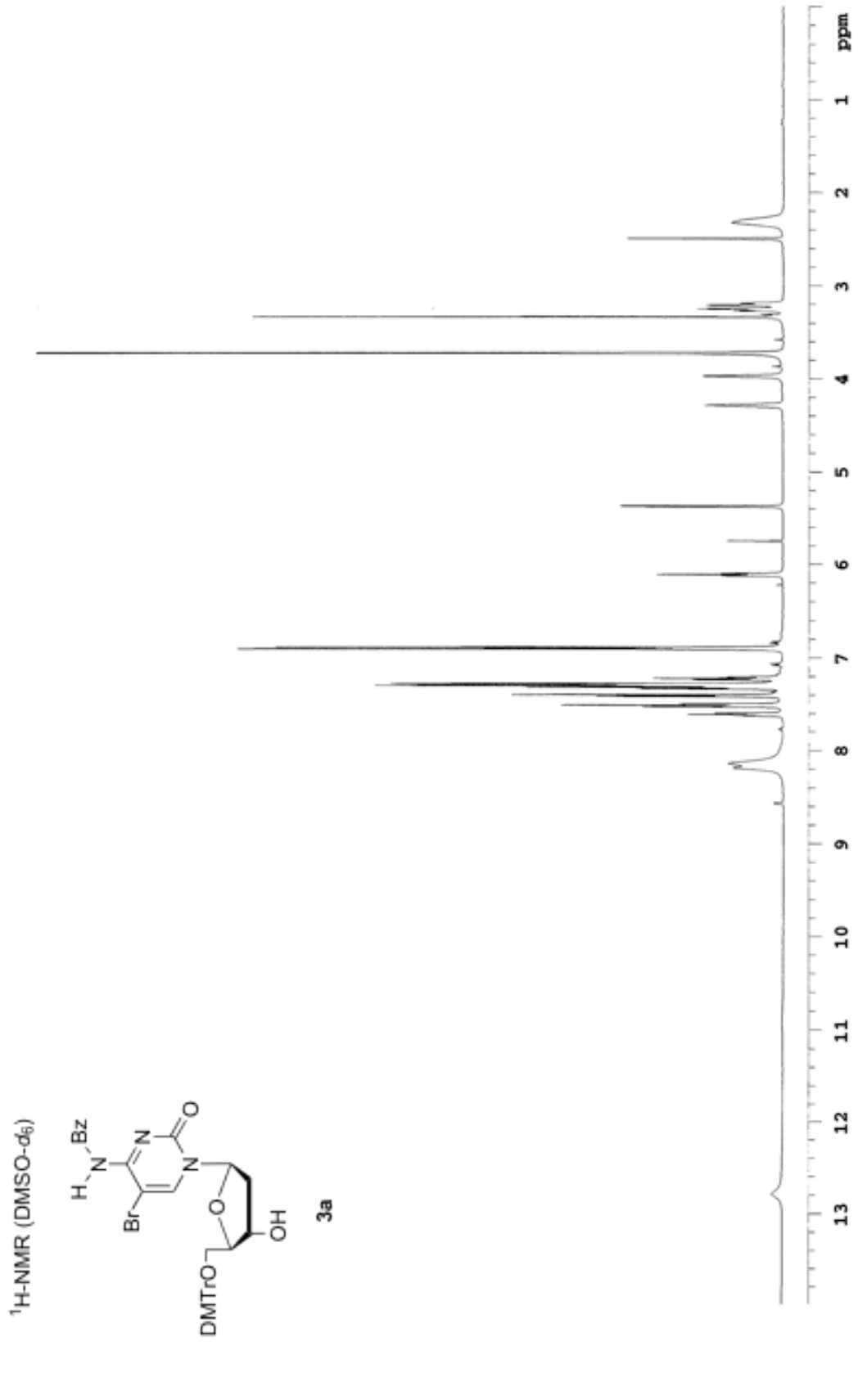


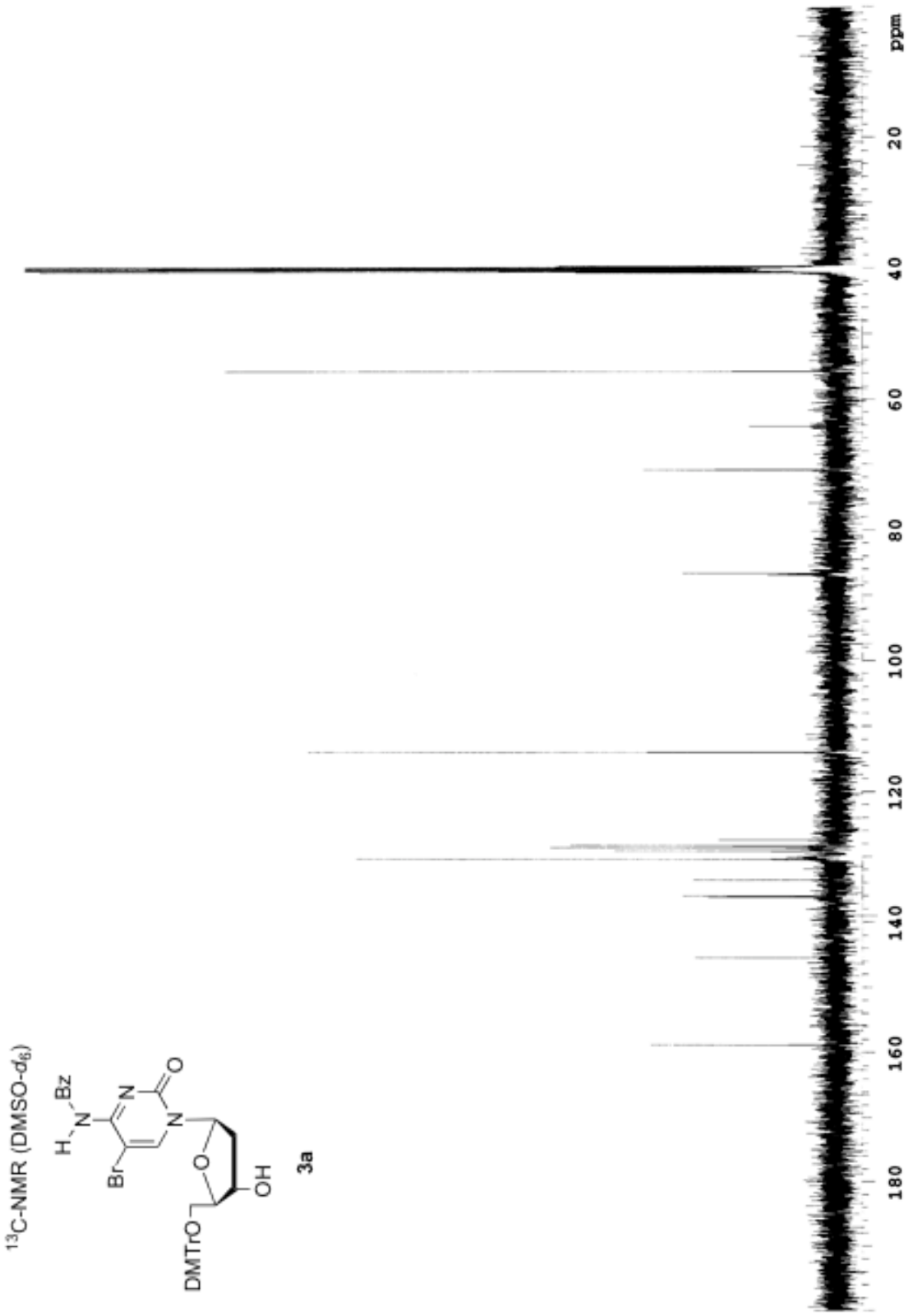
	TFO-C Z = C HP-cg (X-Y = C-G)	TFO-T Z = T HP-ta (X-Y = T-A)	TFO-C3 Z = C3 HP-gppi (X-Y = G-PPI)	
T_m	**29**	**30**	**35**	[°C]

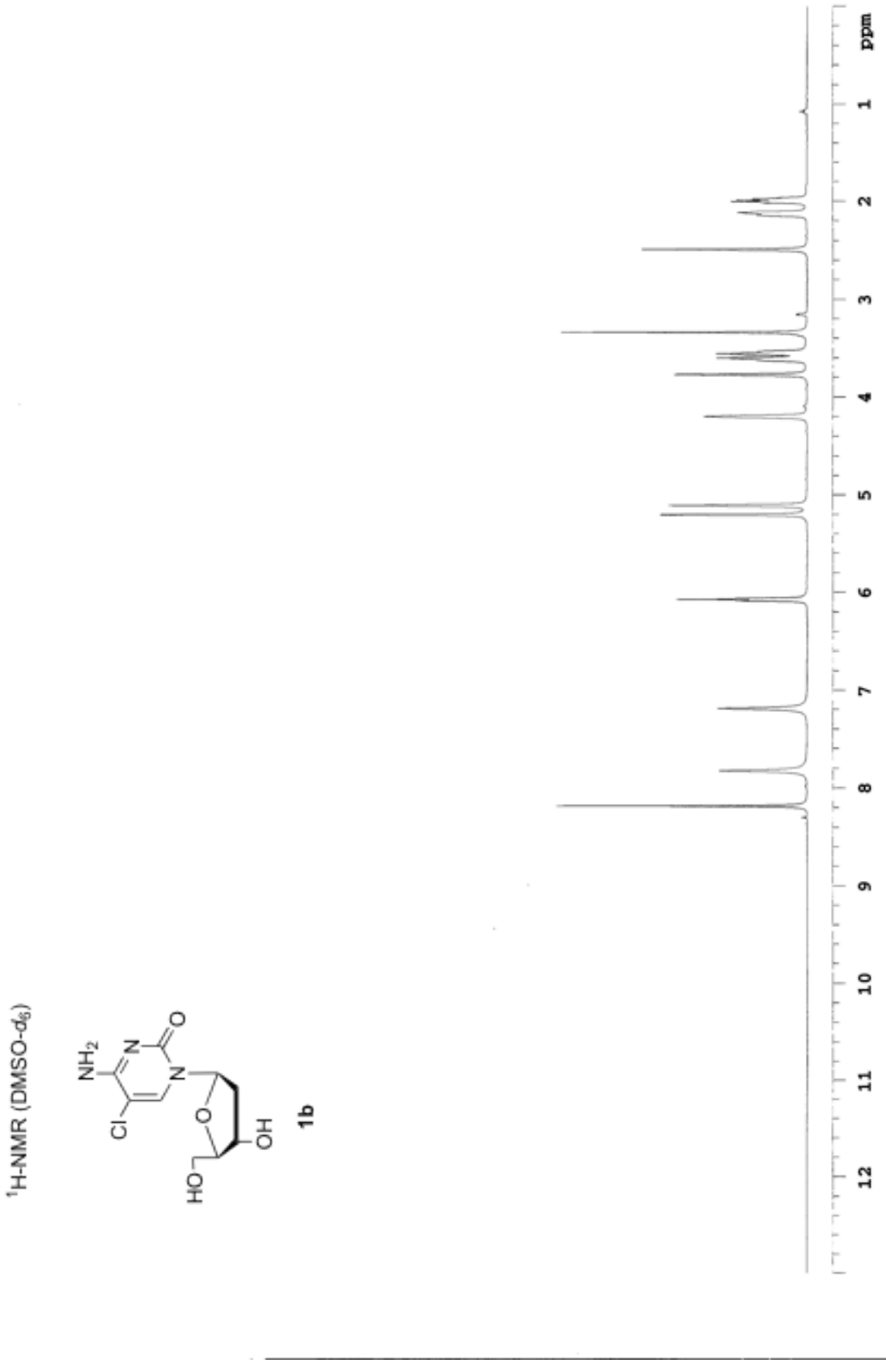
T_m values of C-G•C+, T-A•T, P-PPI•C3 triads in pH 7.0 condition. 10 mM $MgCl_2$, 500 mM NaCl, 10 mM sodium cacodylate. Oligonucleotides were mixed in a 1:1 ratio at a concentration of 2.0 mM.

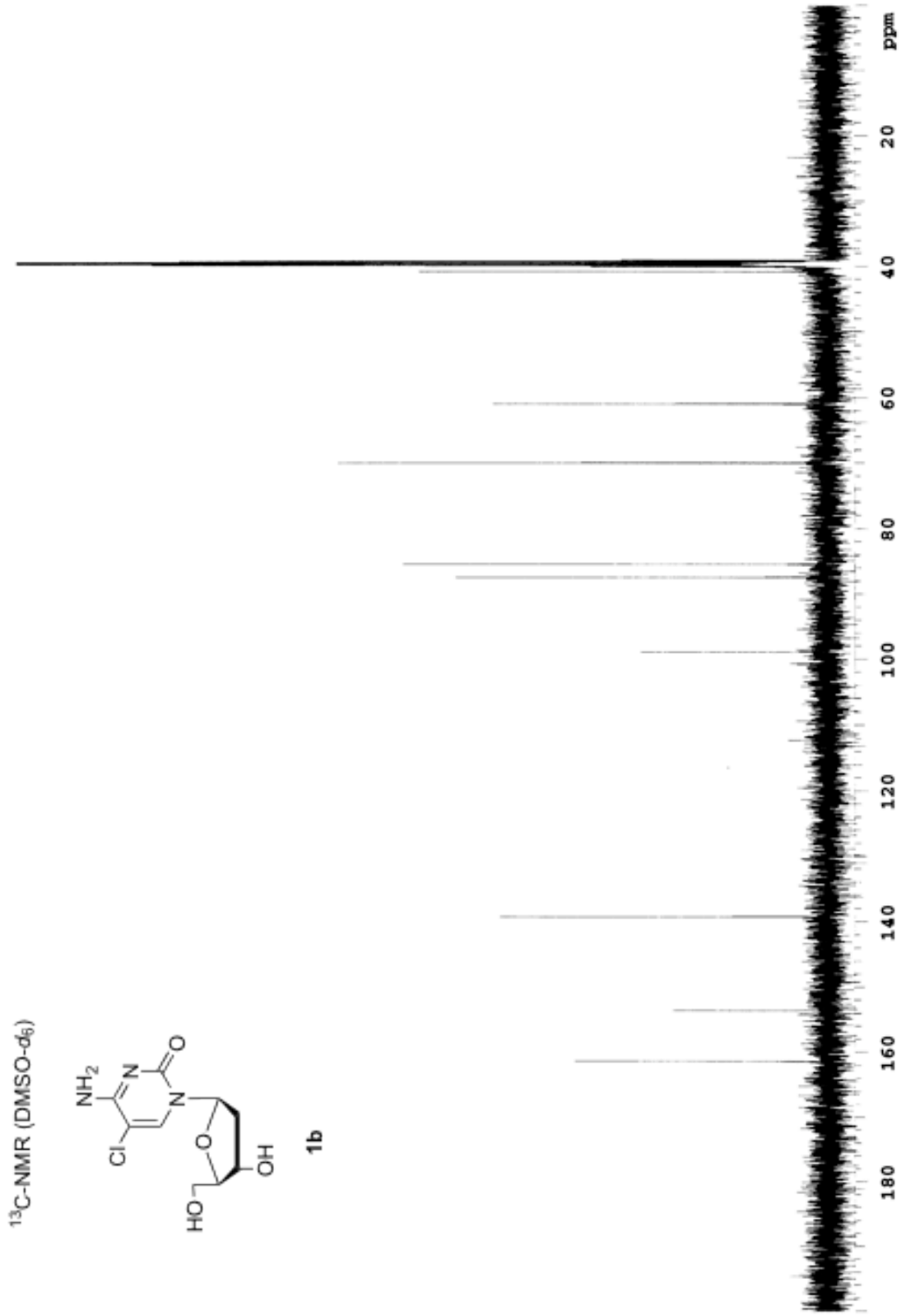

^{1}H-NMR (DMSO-d_6)
NH$_2$
Br
HO
OH
1a
ppm
1
2
3
4
5
6
7
8
9
10
11
12

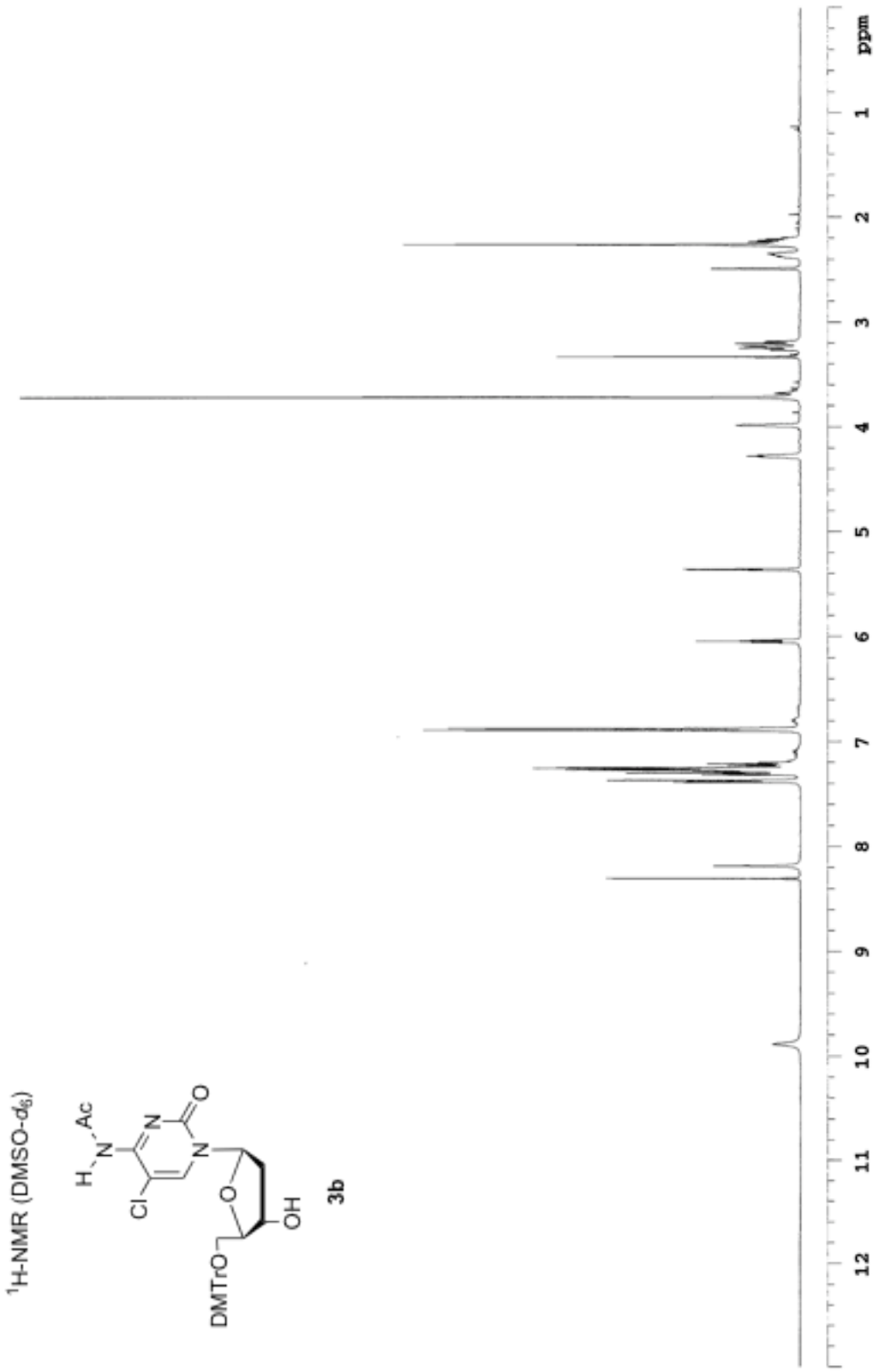


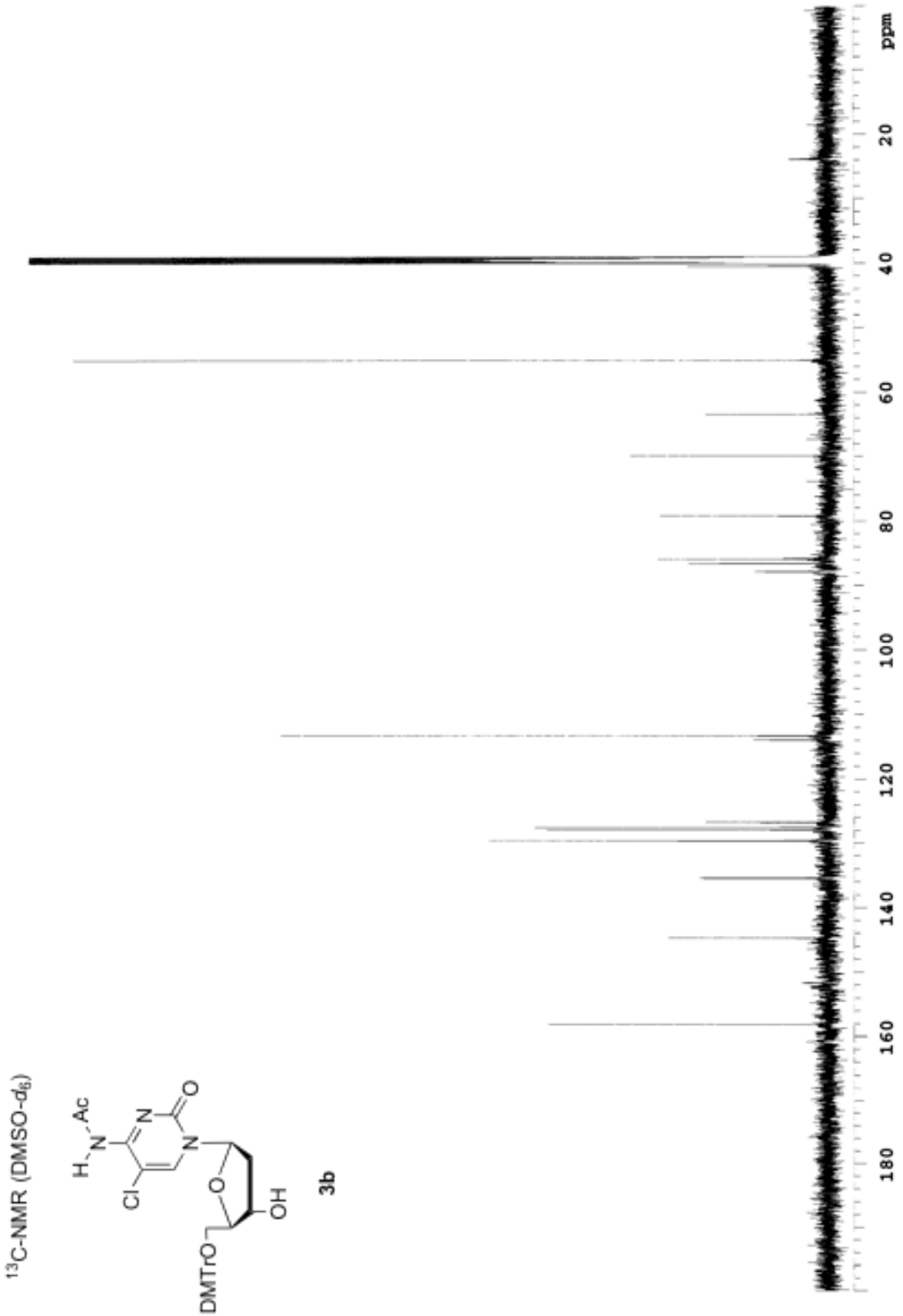

^{1}H-NMR (DMSO-d_6)
Bz
H
N
N
O
N
Br
O
OH
DMTrO
3a
ppm
1
2
3
4
5
6
7
8
9
10
11
12
13

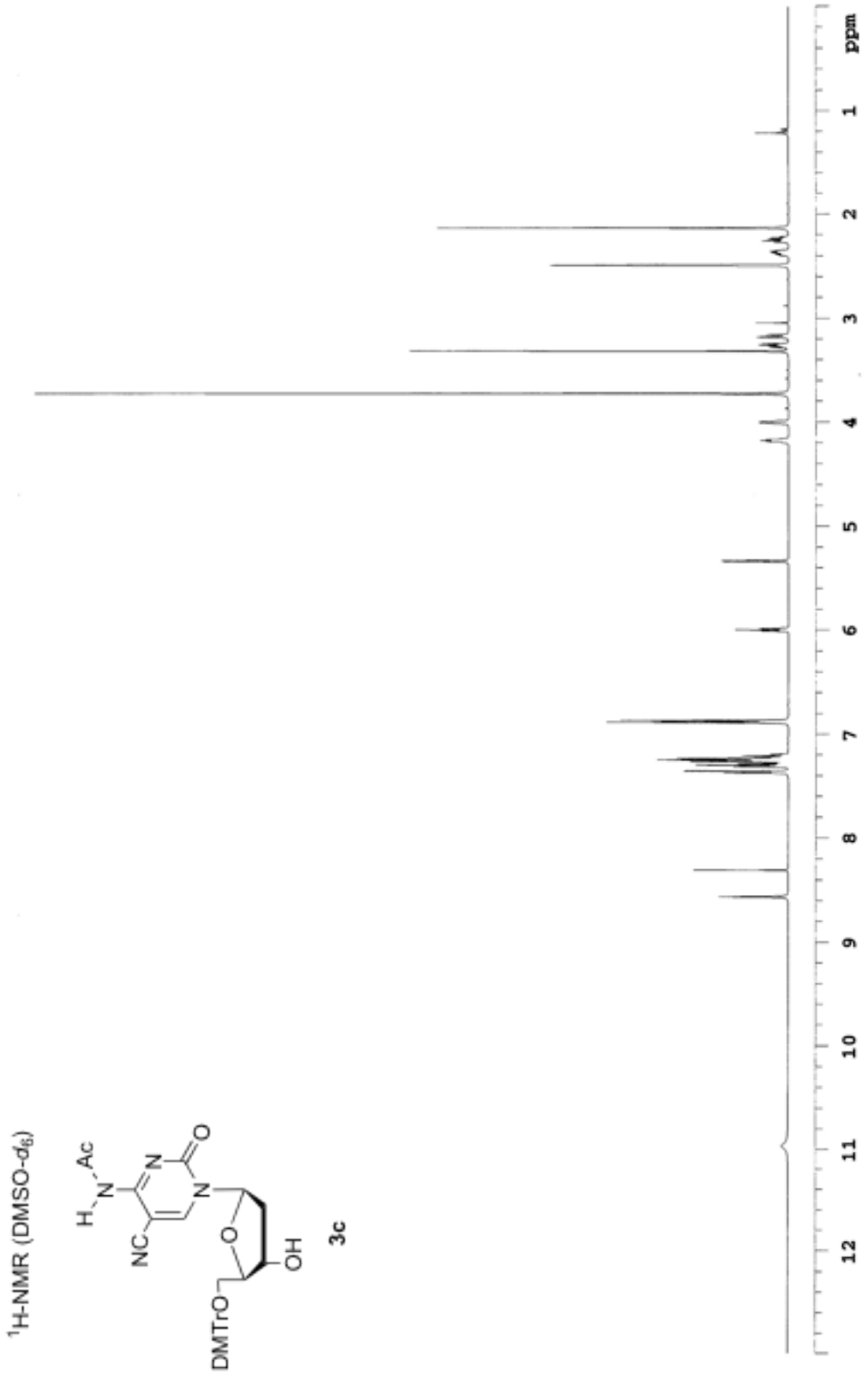



^{1}H-NMR (DMSO-d_6)
NH_2
N
O
N
Cl
O
OH
HO
1b
ppm
1
2
3
4
5
6
7
8
9
10
11
12

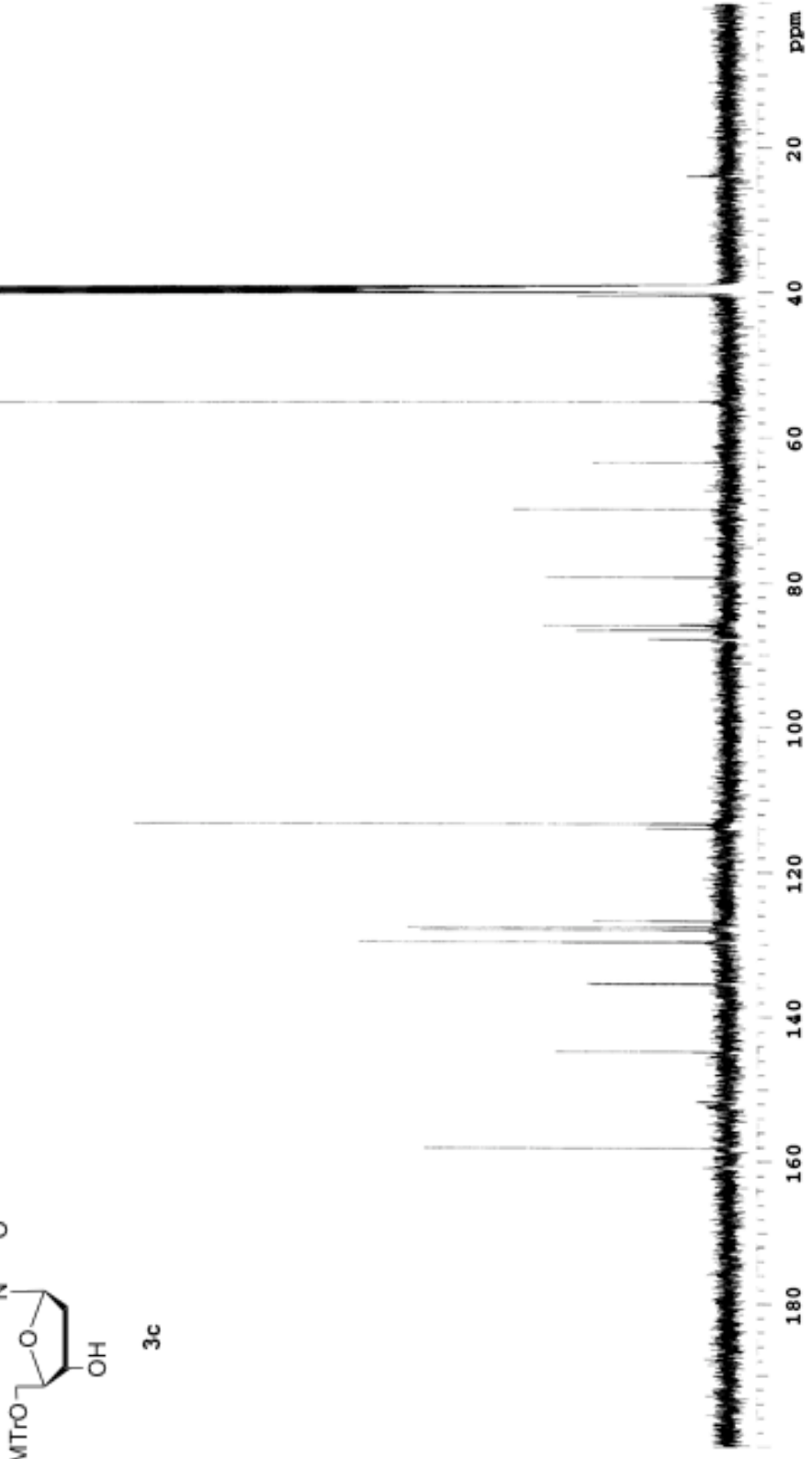


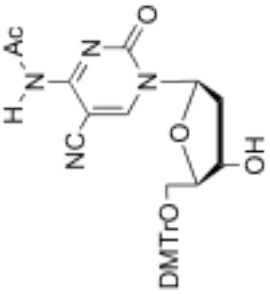




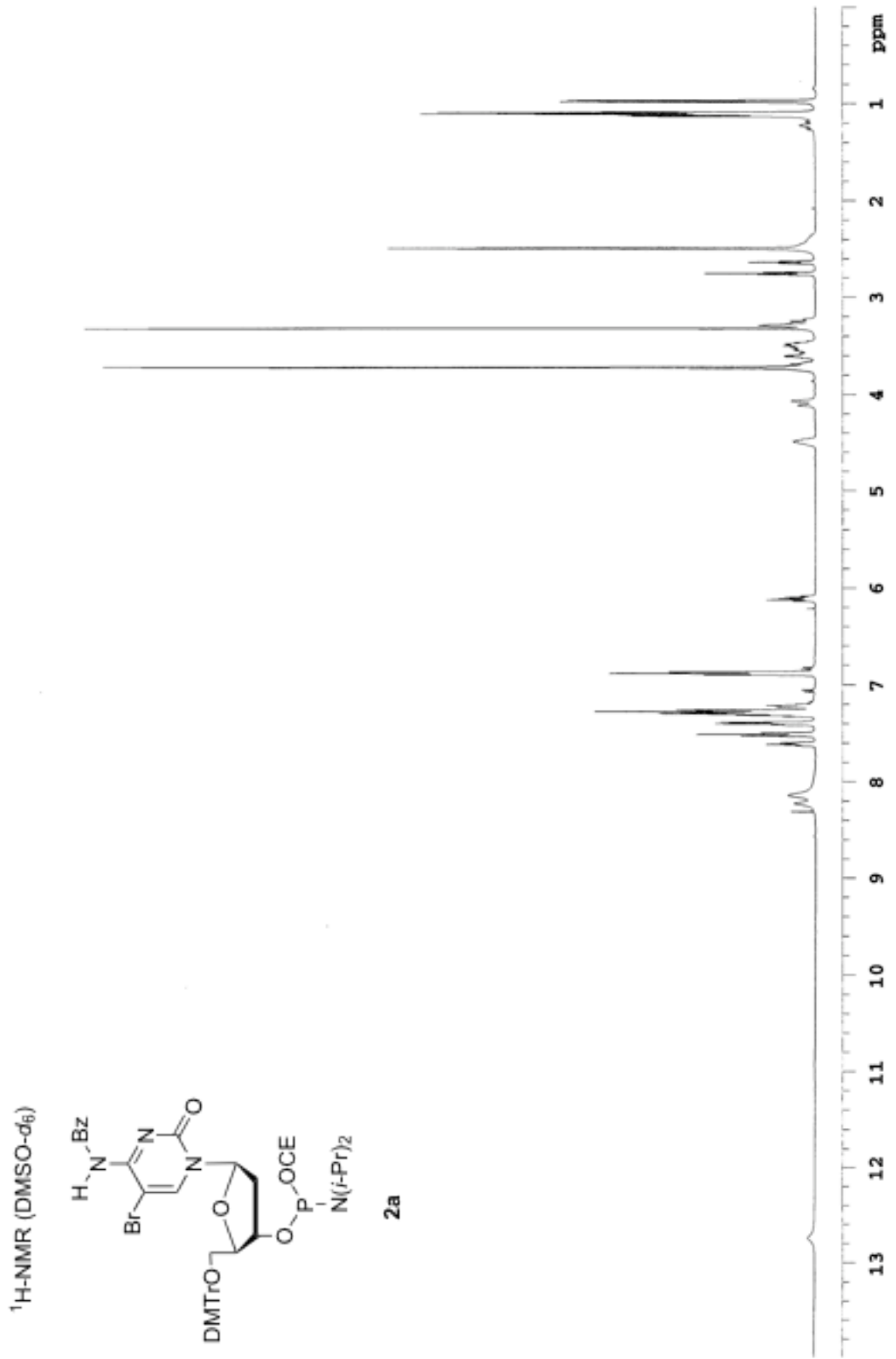


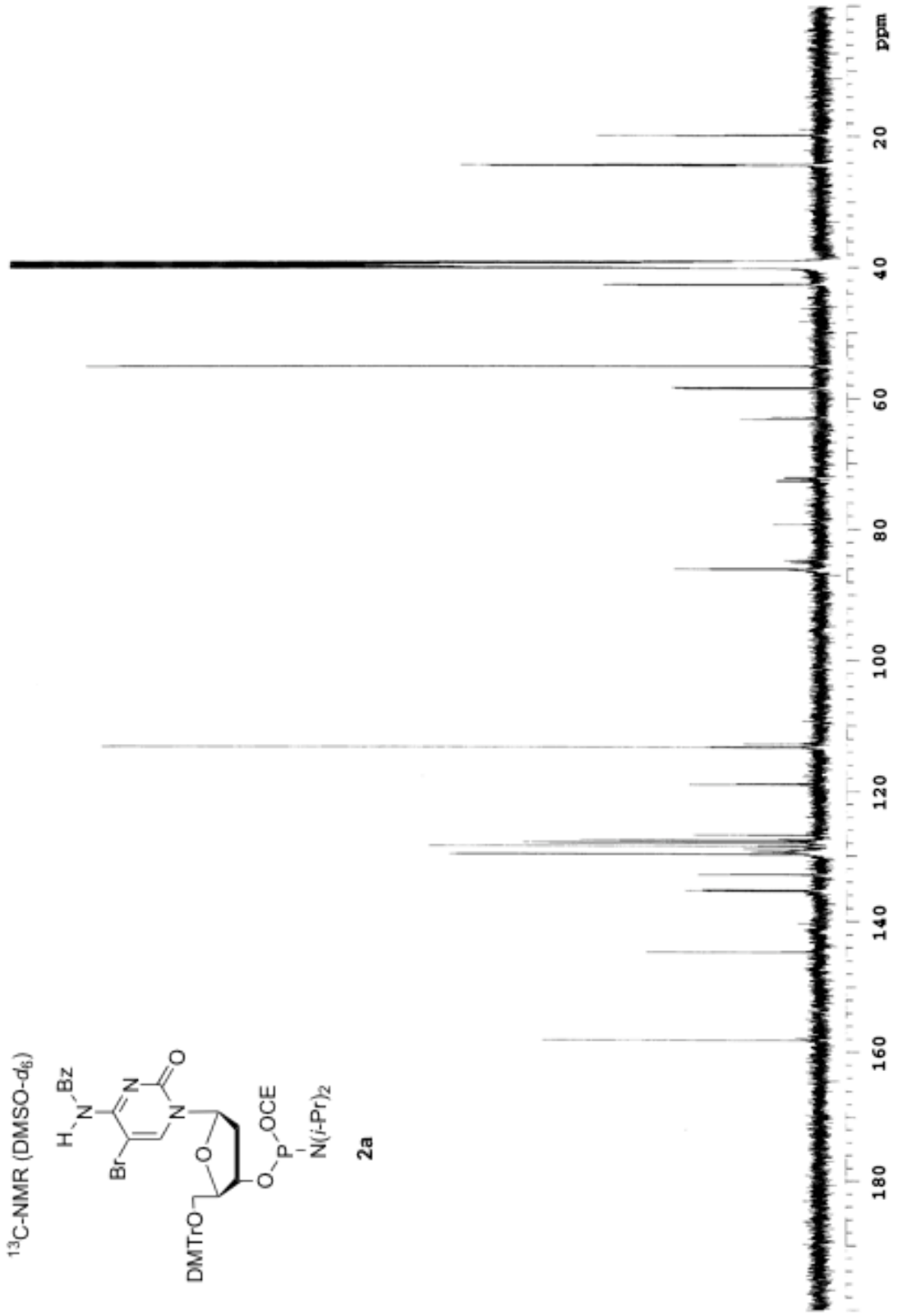
^{13}C-NMR (DMSO-d_6)

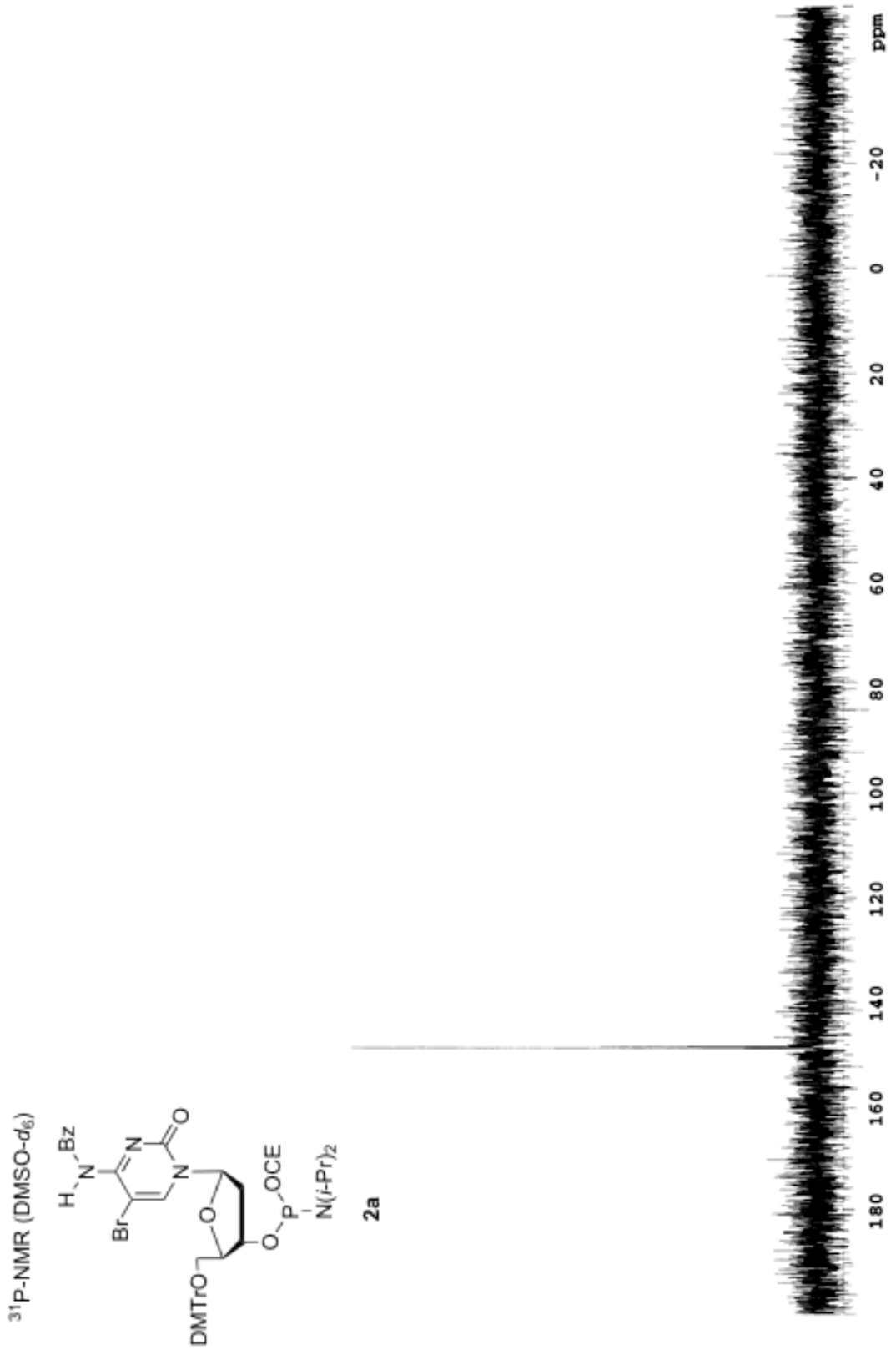


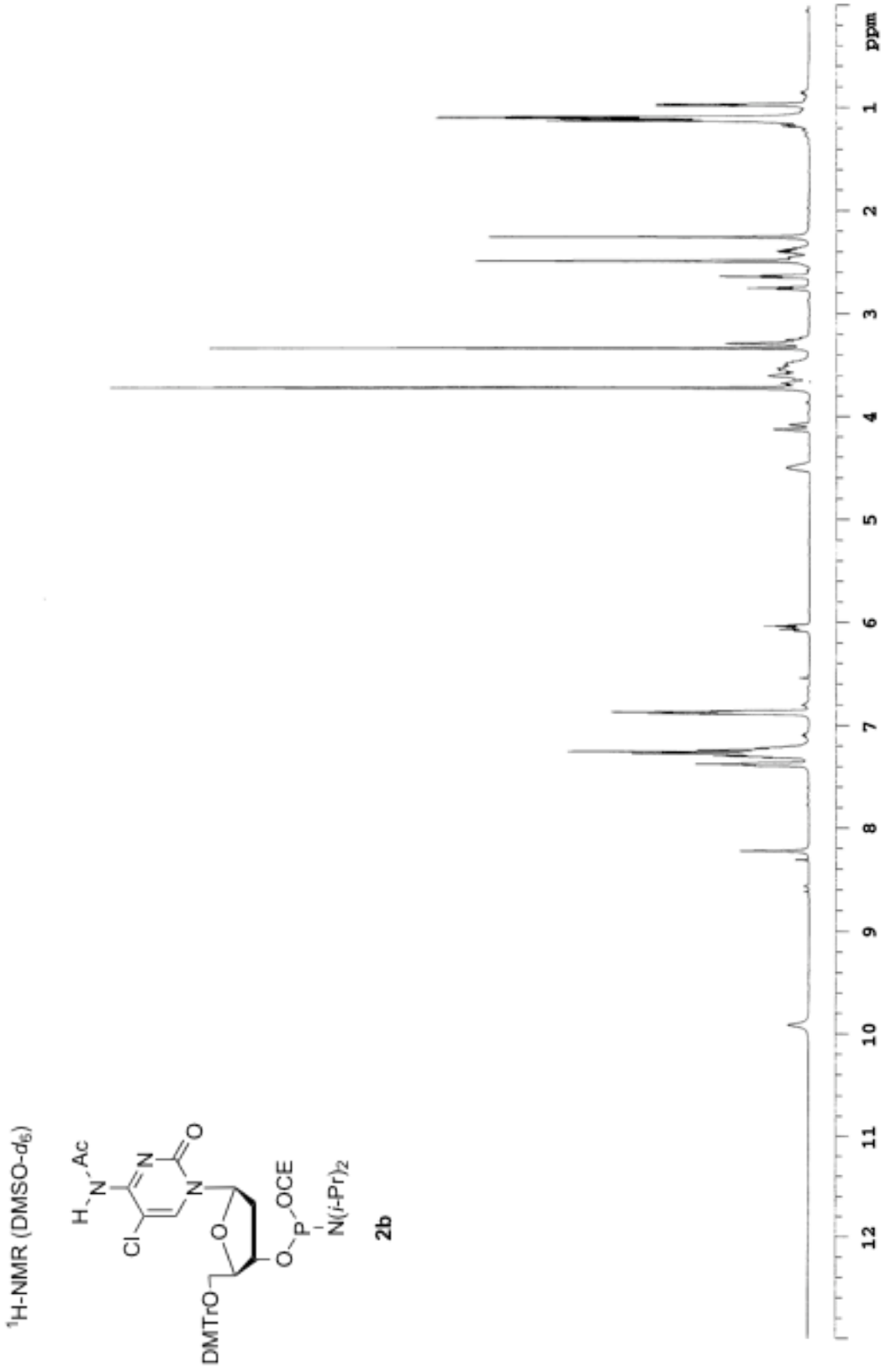


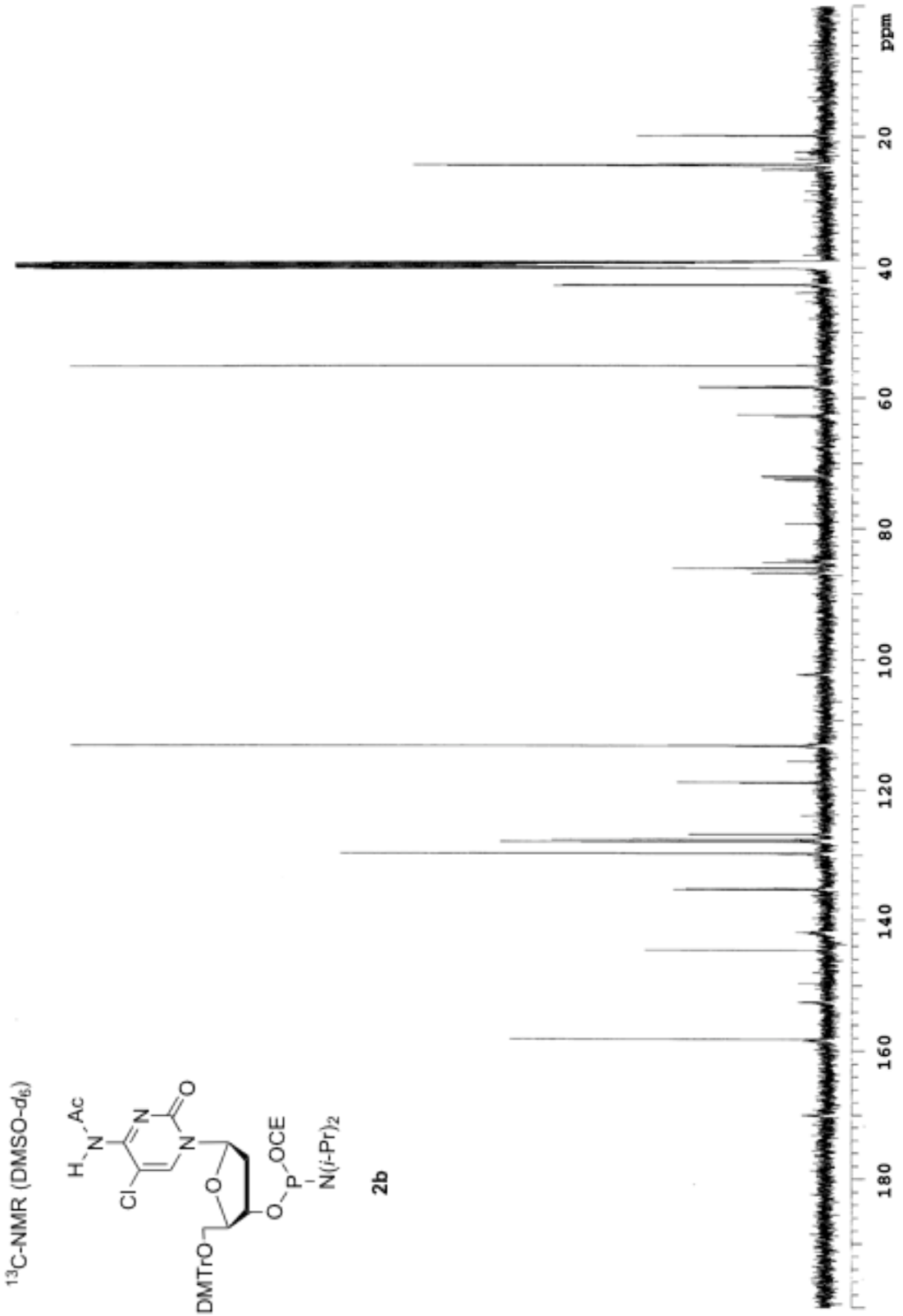
3c

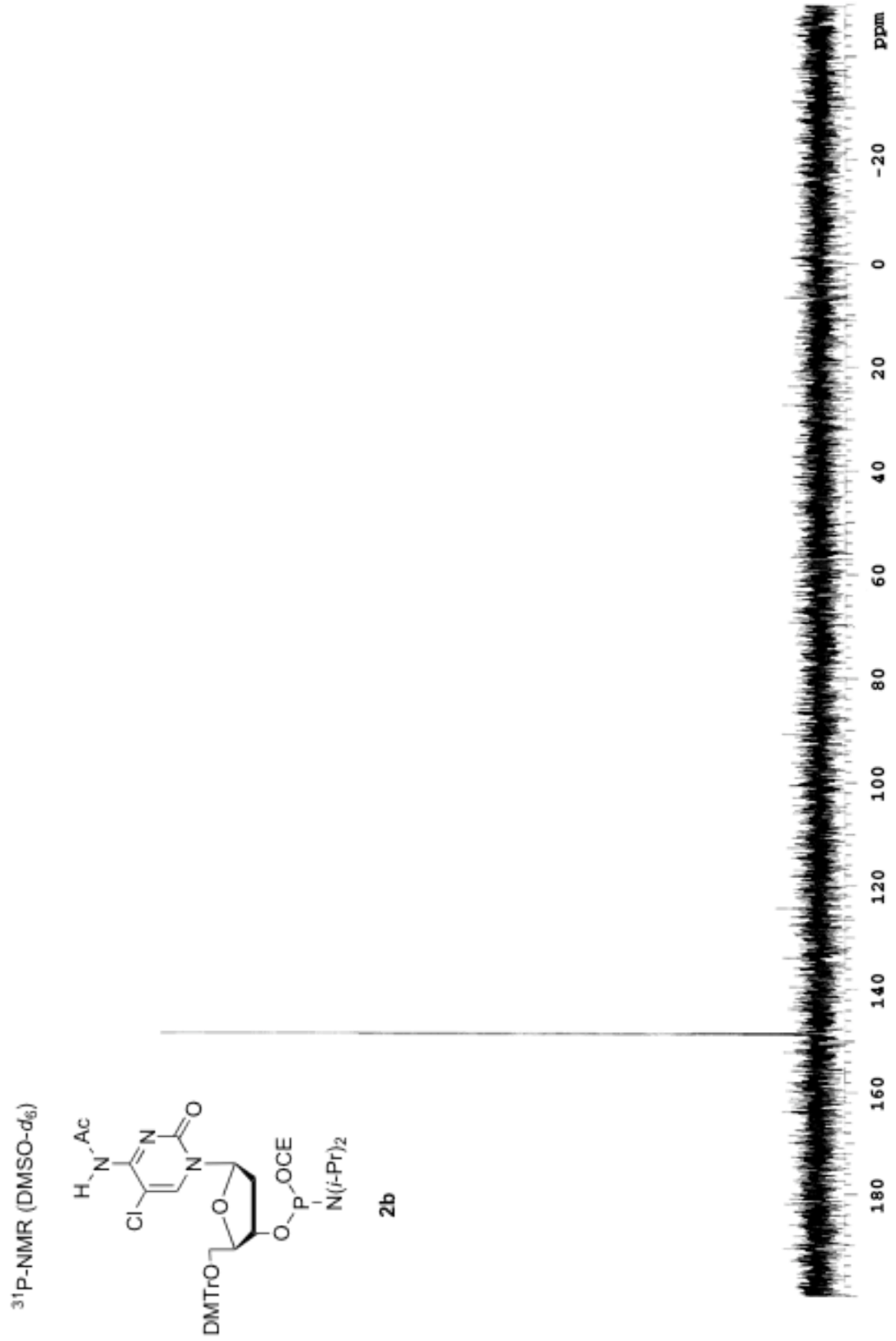






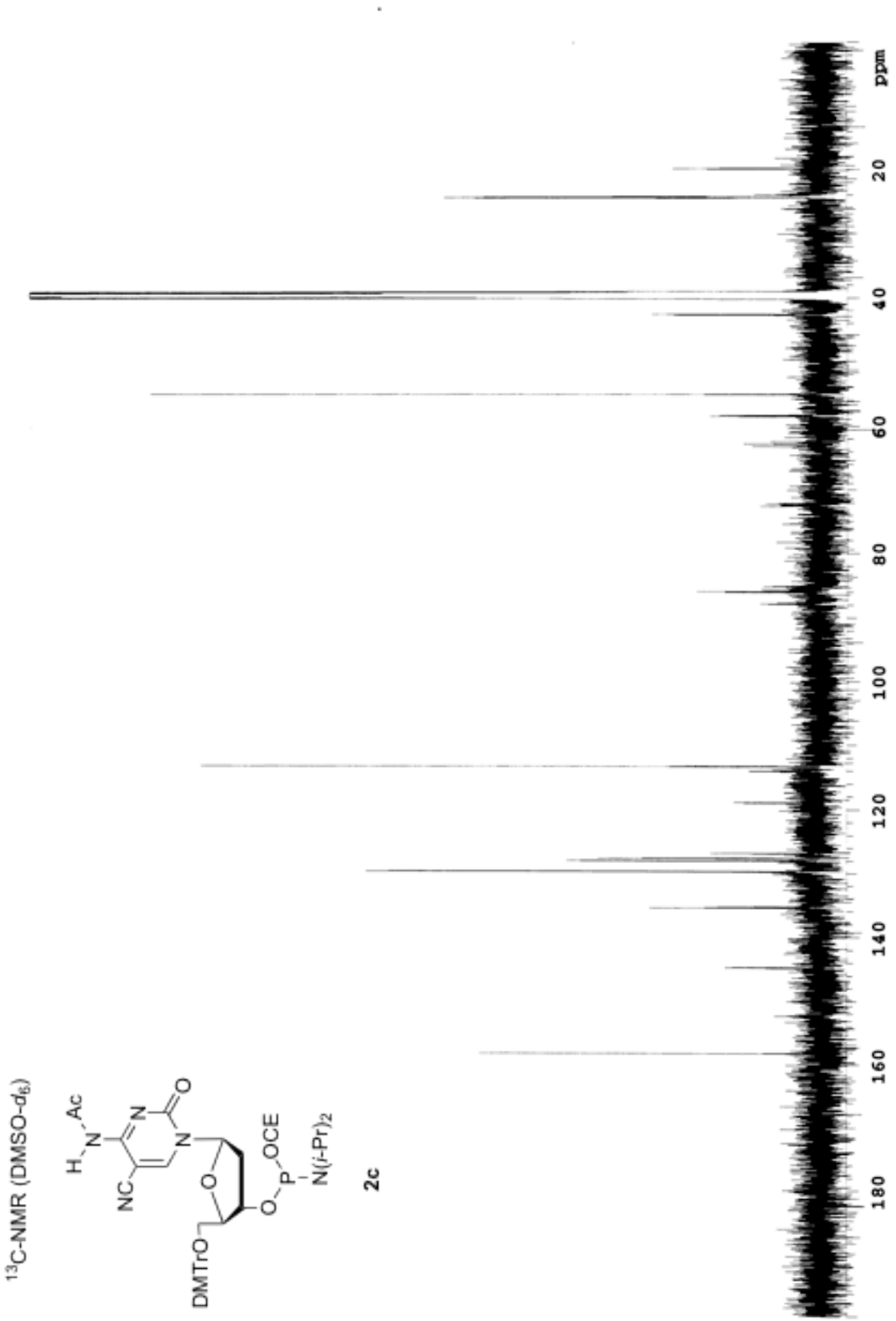


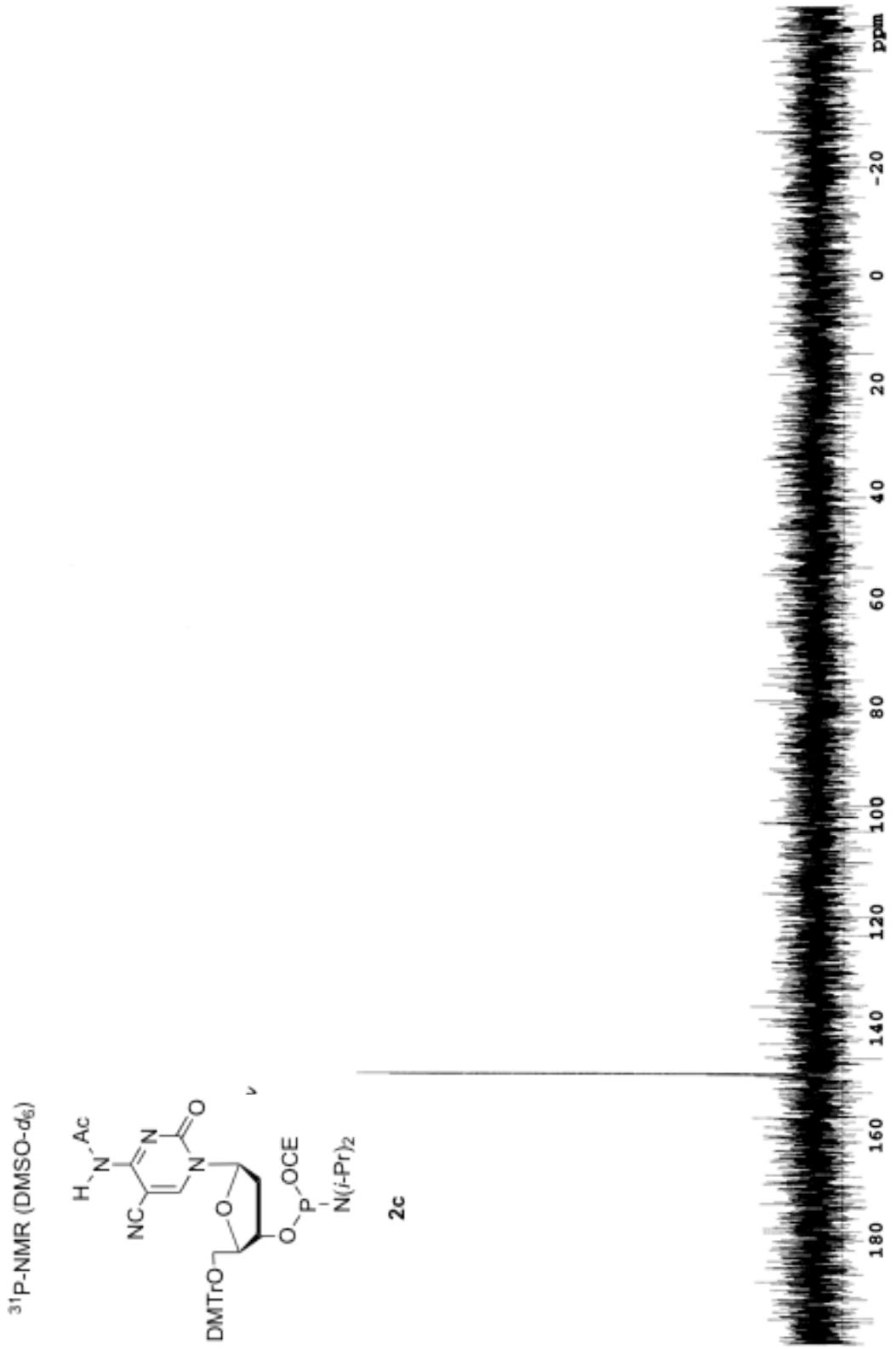


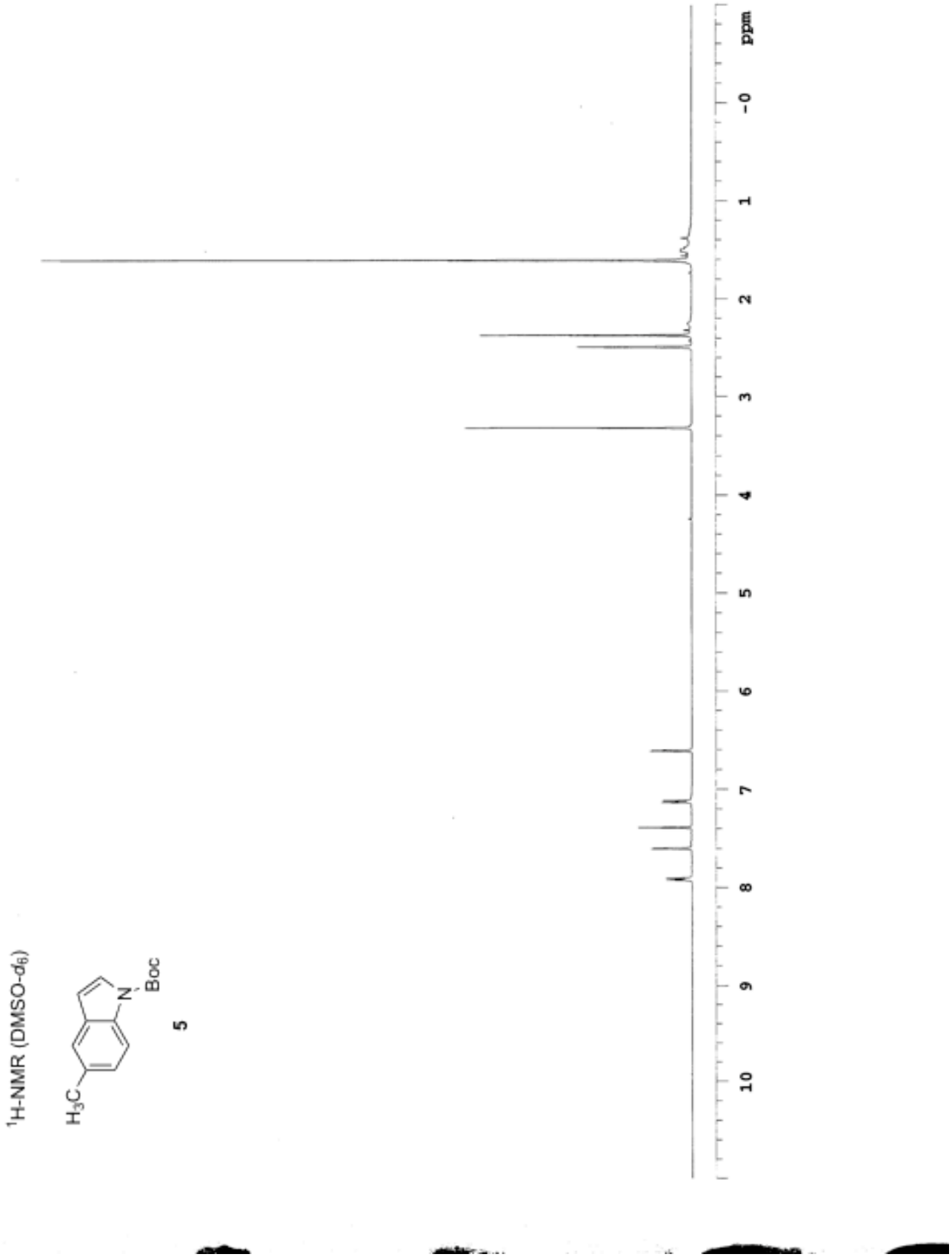

^{31}P-NMR (DMSO-d_6)
Ac
H
N
N
O
N
Cl
O
DMTrO
O
O
P
OCE
N(i-Pr)2
2b
180
160
140
120
100
80
60
40
20
0
-20
ppm

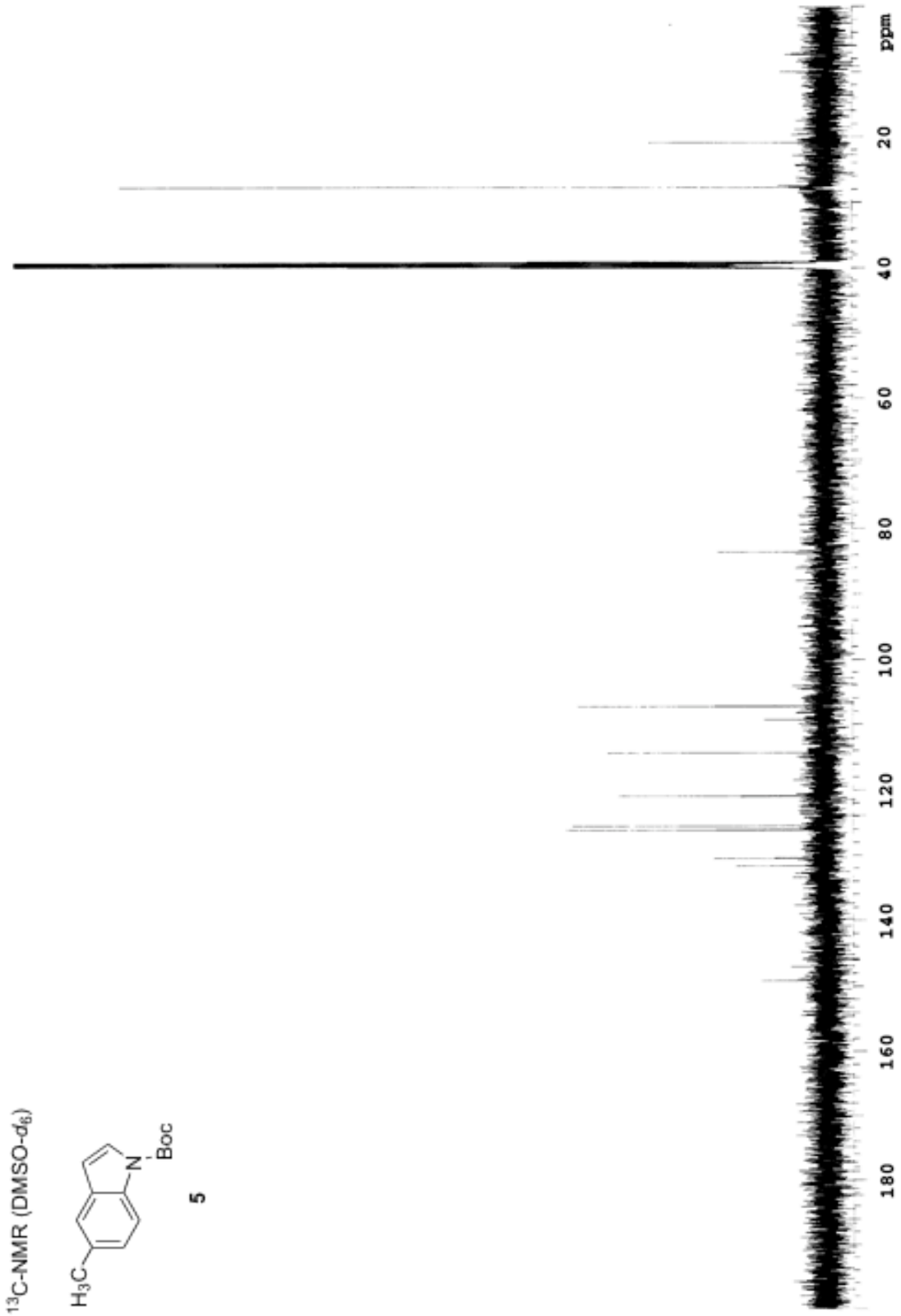
^{1}H-NMR (DMSO-d_6)

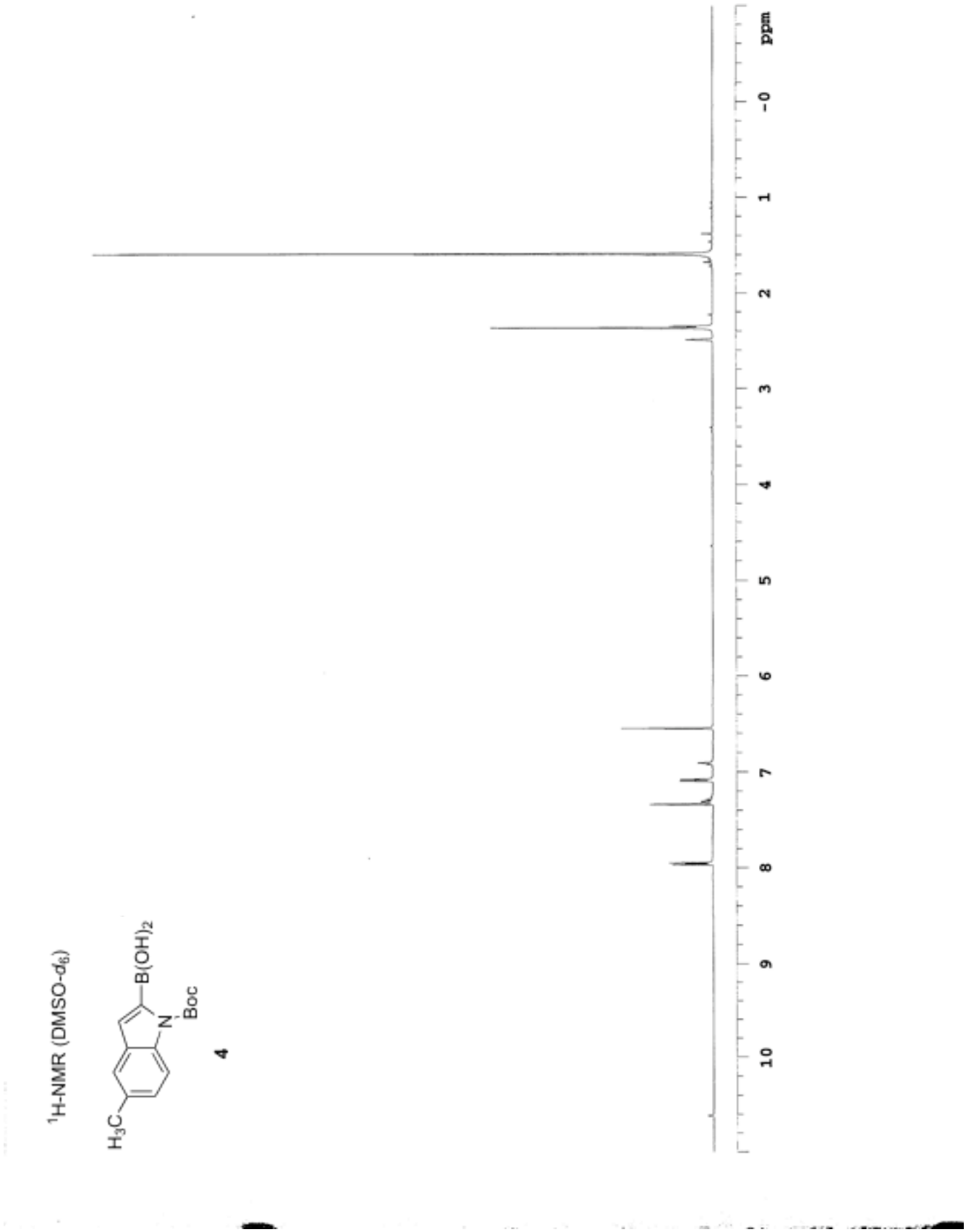
2c

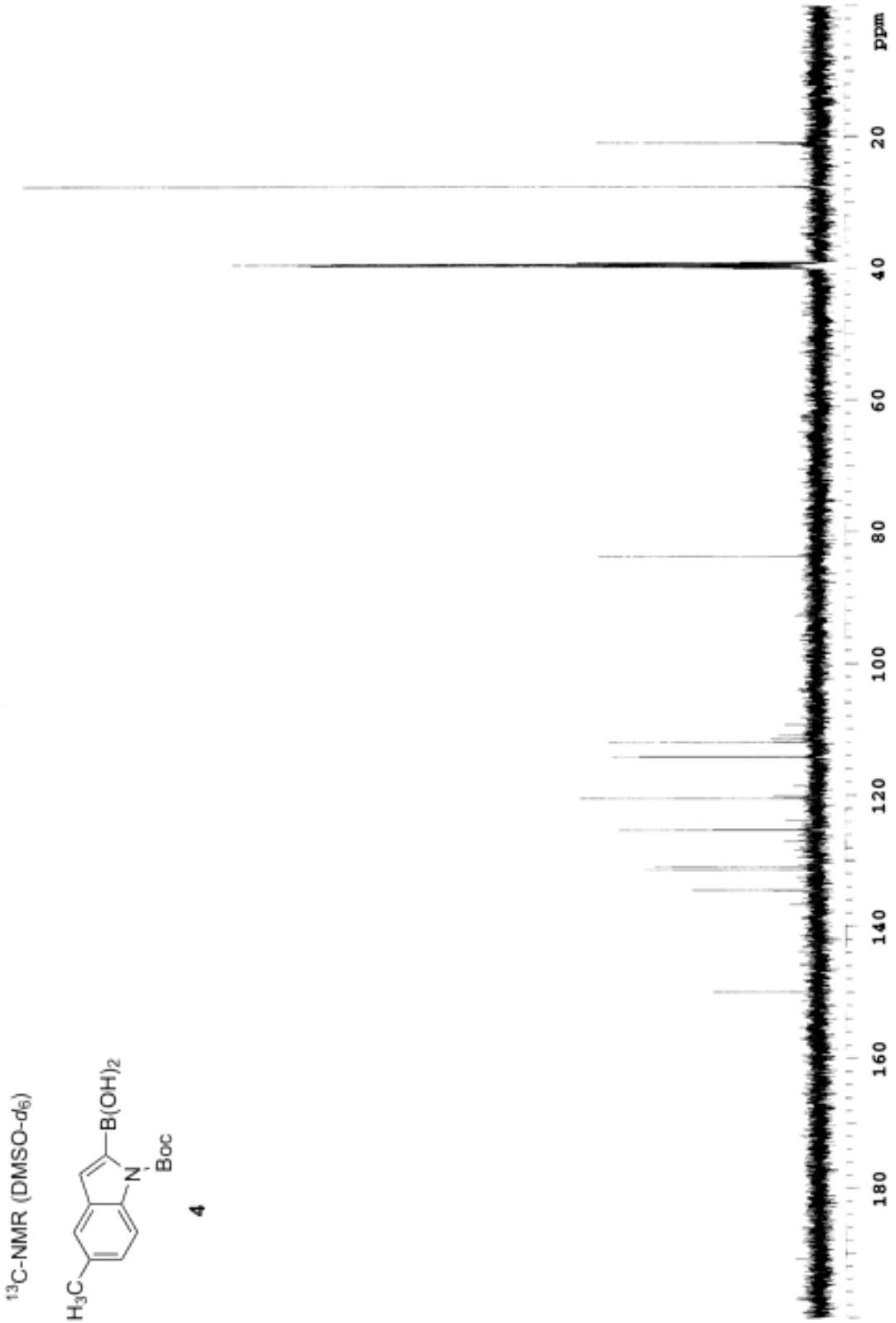


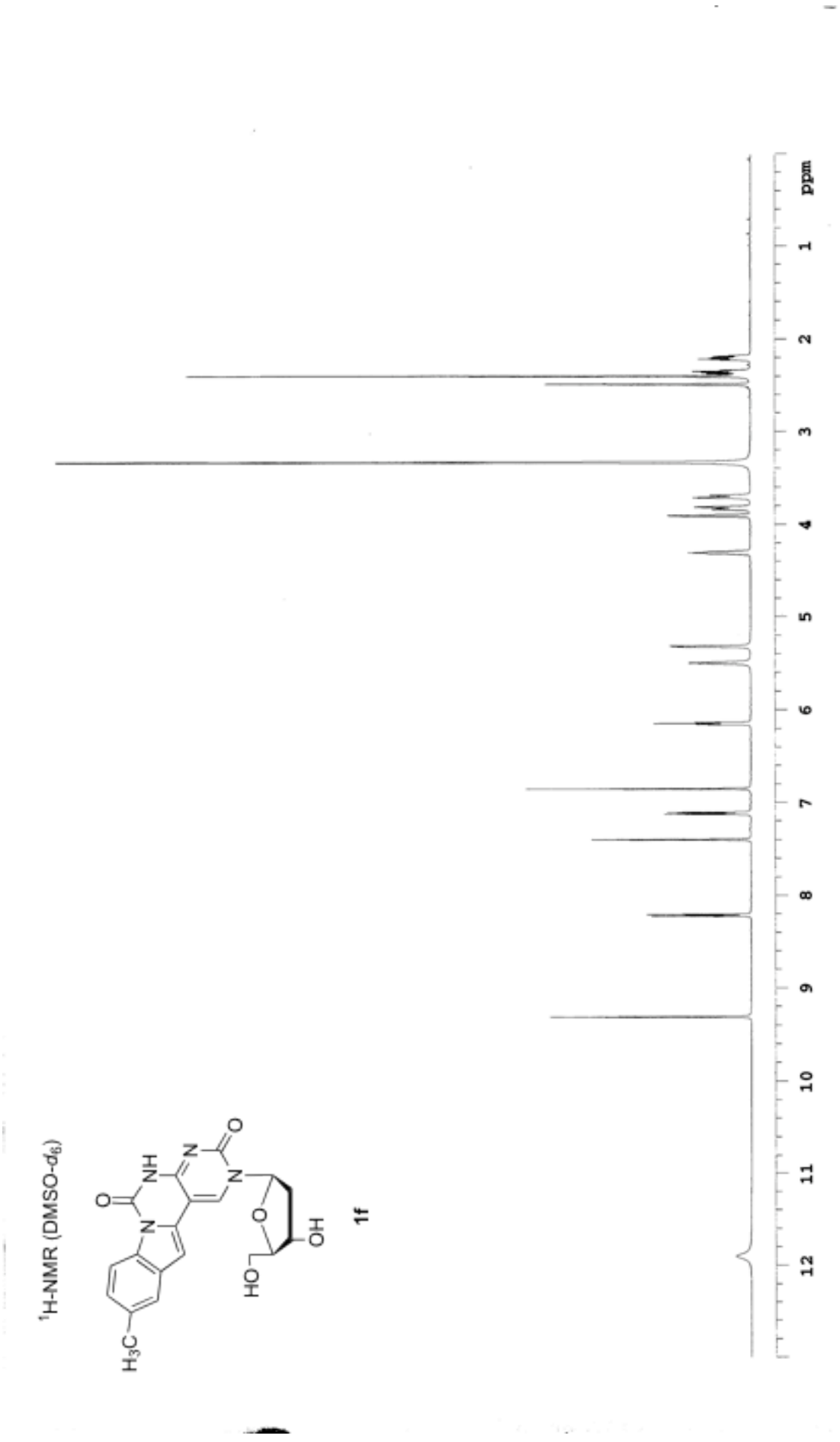




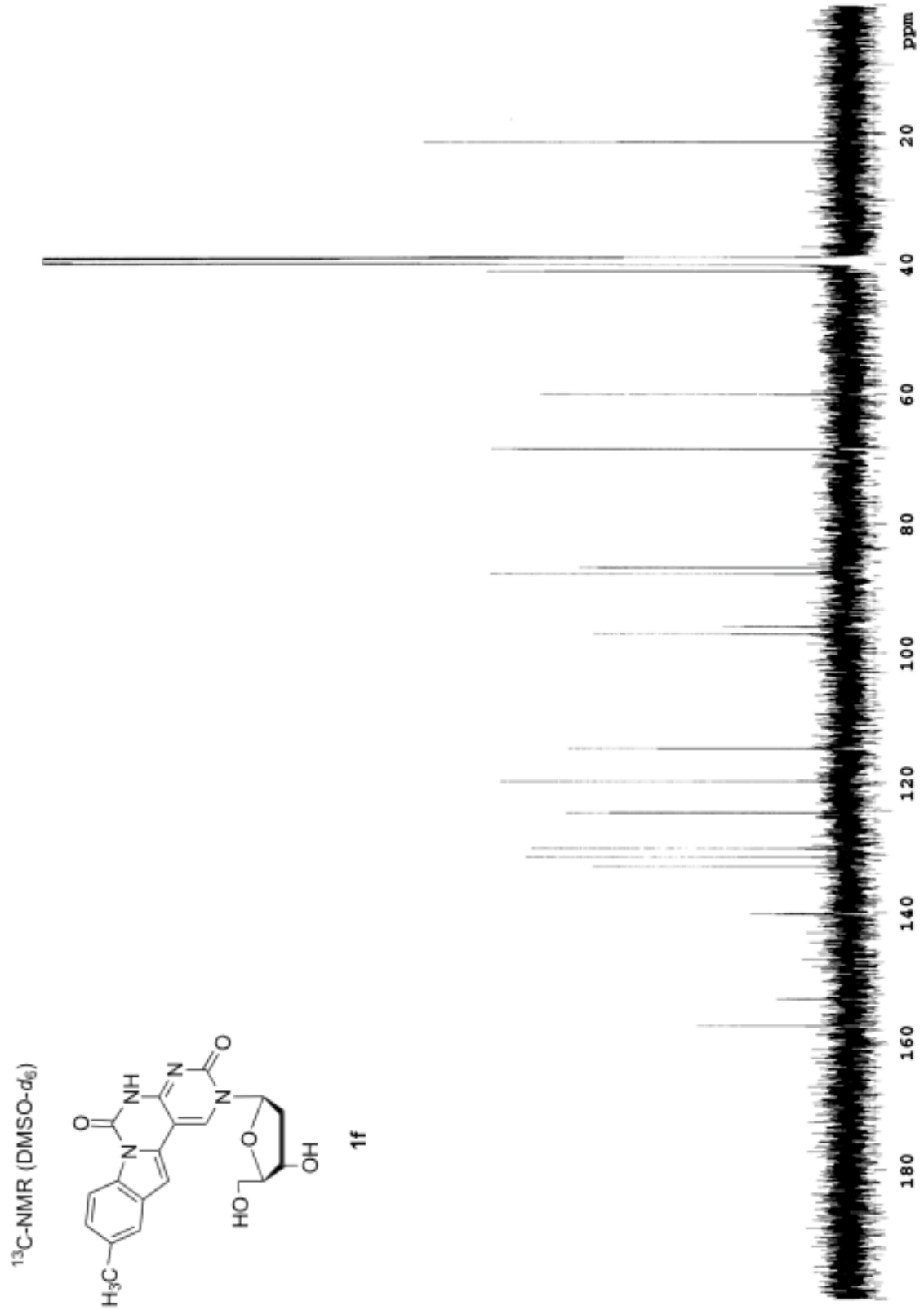


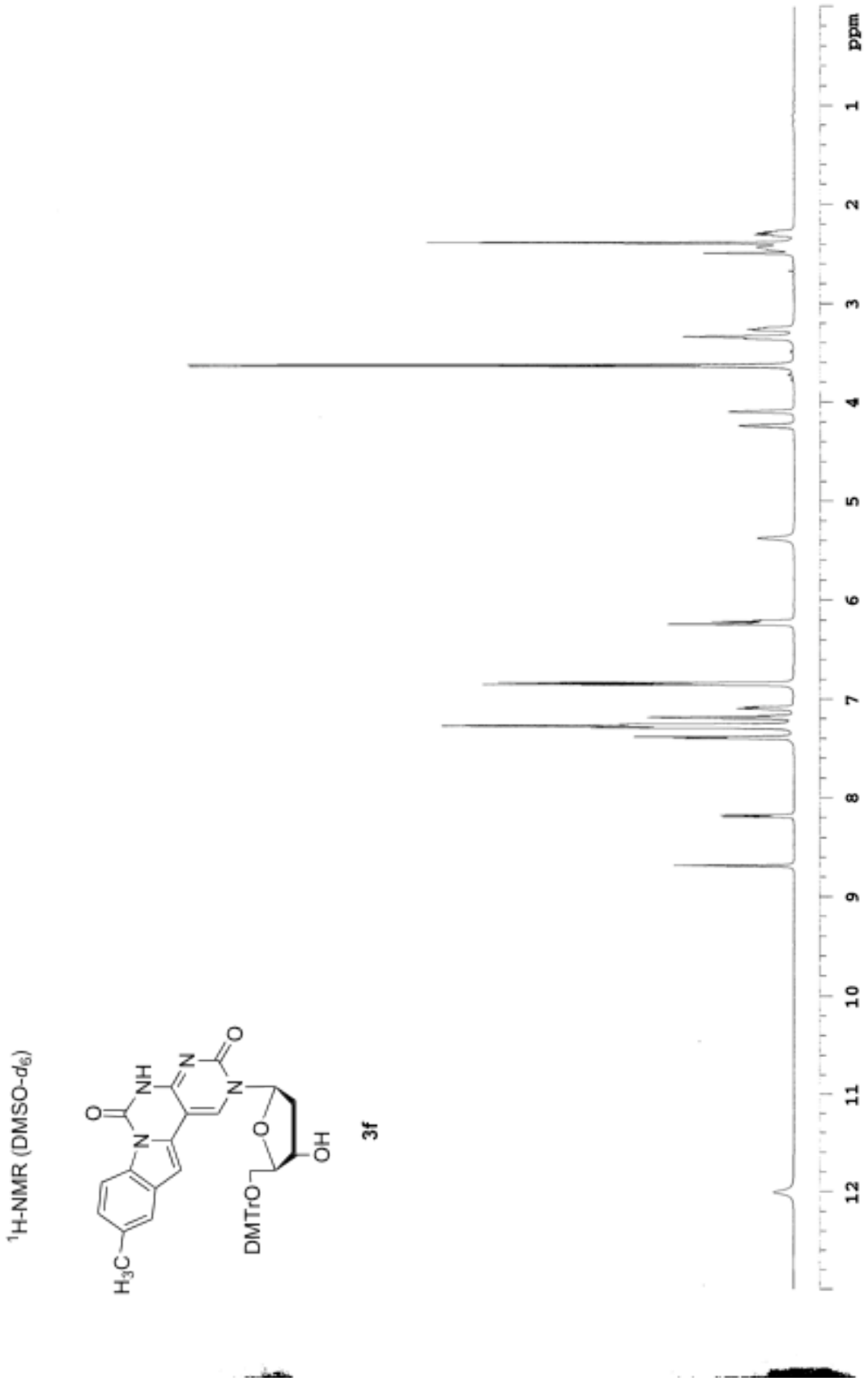


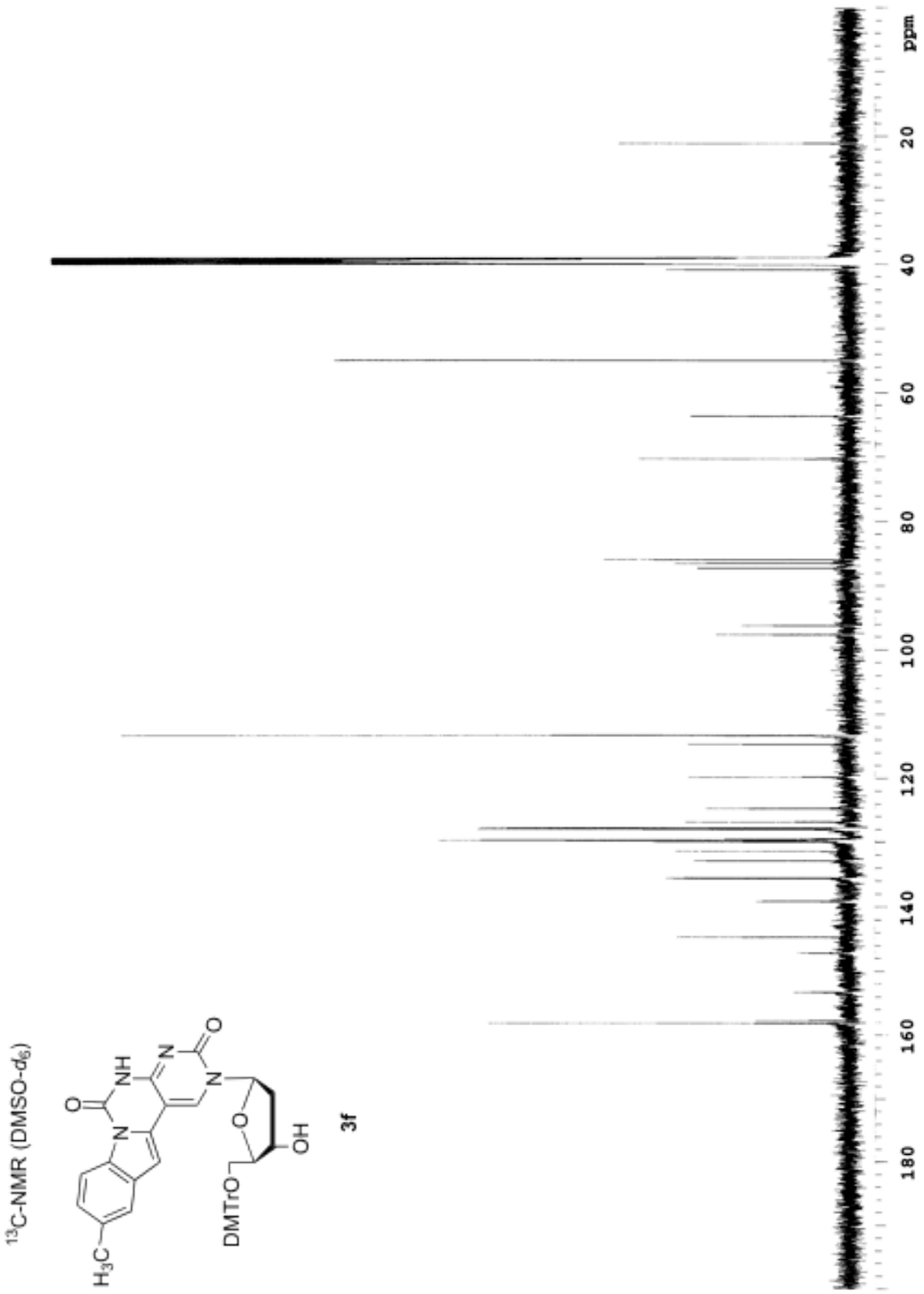


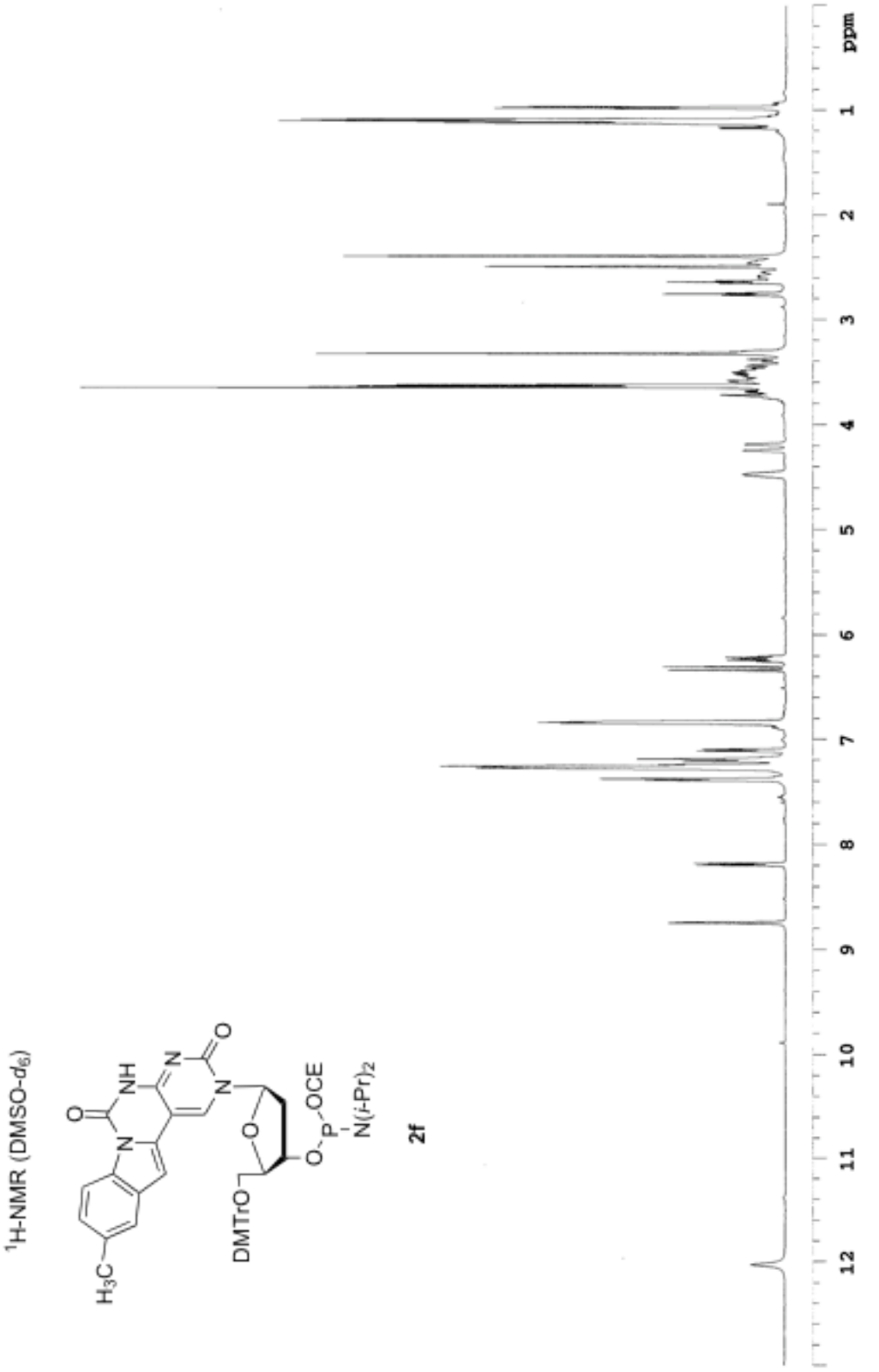


1f

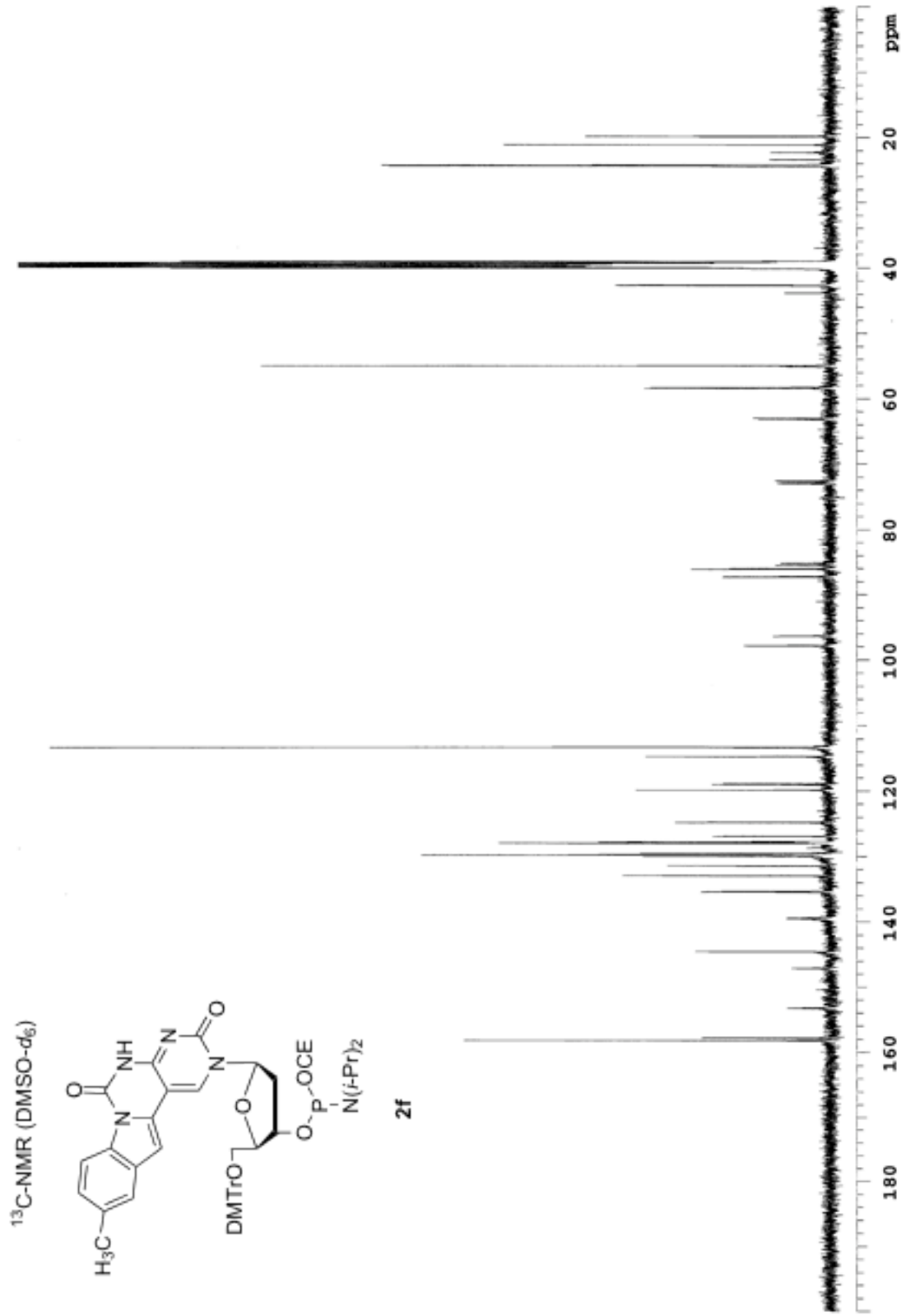




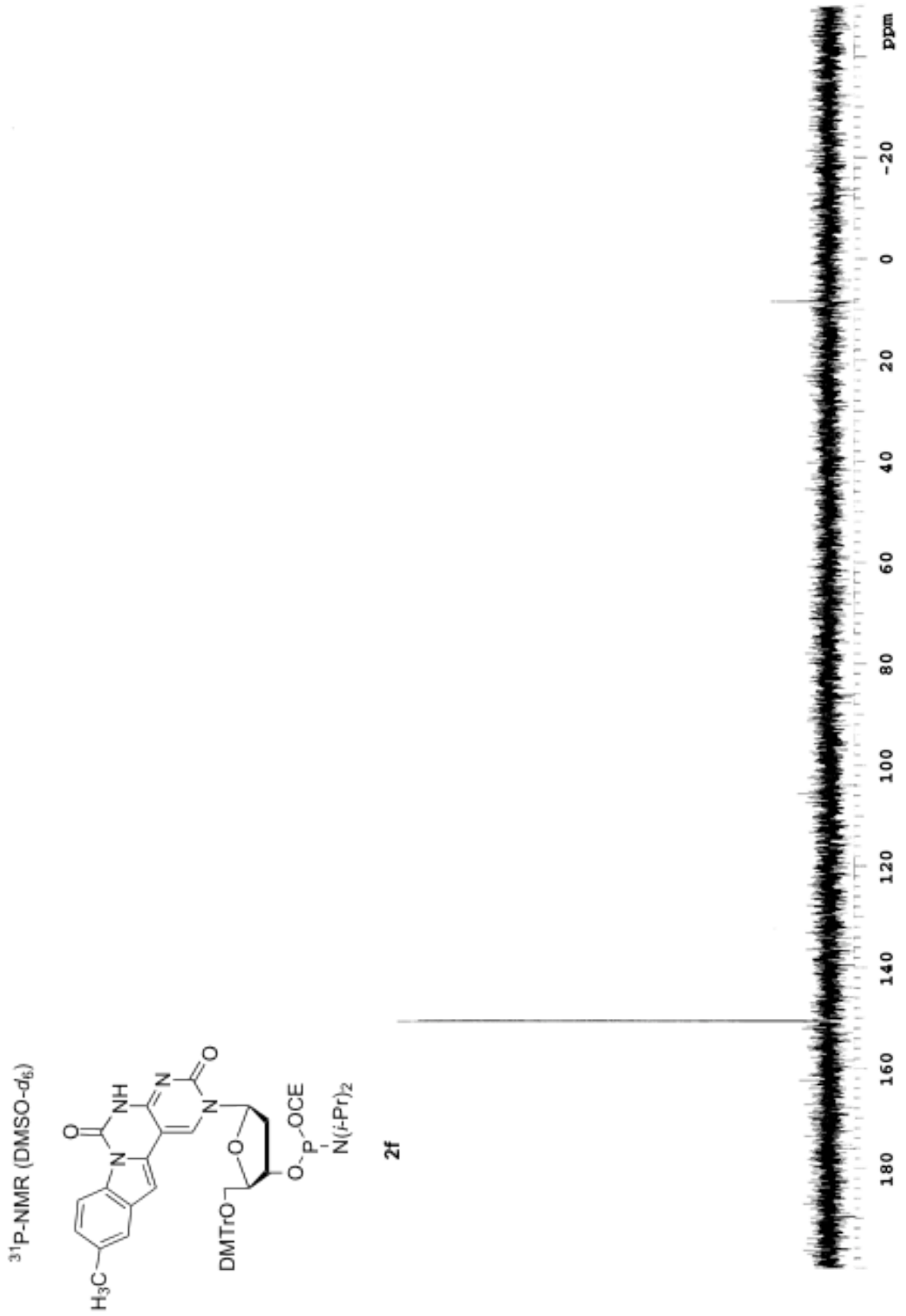




2f



2f


^{31}P-NMR (DMSO-d_6)
NH
O
N
O
N
N
O
H_3C
DMTrO
O
O
P
OCE
N(i-Pr)$_2$
2f
180
160
140
120
100
80
60
40
20
0
-20
ppm