Supplementary Information

Groove modification of siRNA duplex to elucidate siRNA–protein interactions using 7-bromo-7-deazaadenosine and 3-bromo-3-deazaadenosine as chemical probes

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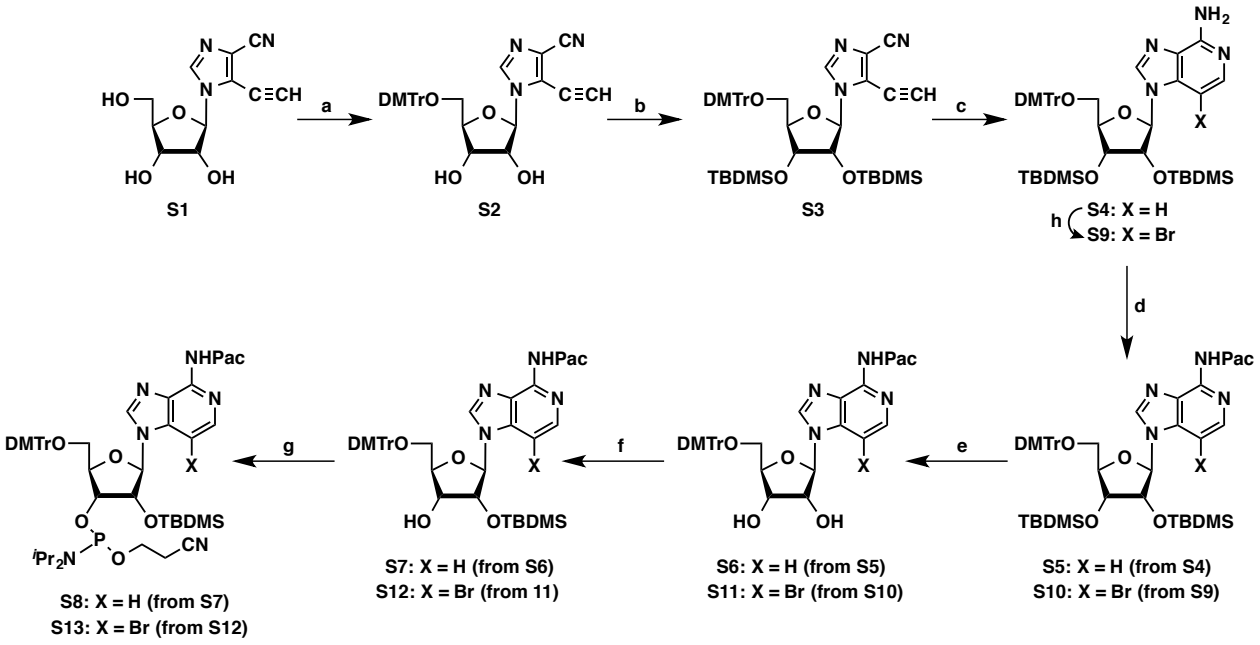
Chemistry

Scheme S1 Synthesis of Br^3C^3A and C^3A phosphoramidite units. Reagents and conditions; a) DMTrCl, pyridine; b) TBDMSCl, imidazole, DMF; c) NH_3/MeOH, 120 °C; d) PacCl, Et_3N, CH_3CN; e) TBAF, THF; f) TBDMSCl, $AgNO_3$, pyridine, THF; g) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, *i*Pr_2NEt, DMAP, CH_2Cl_2; h) NBS, CH_2Cl_2



5-Ethynyl-1-(5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazole-4-carbonitrile (S2). To a solution of **S1**[17d] (474 mg, 1.9 mmol) in pyridine (15 mL) was added DMTrCl (780 mg, 2.3 mmol), and the reaction mixture was stirred for 40 h at room temperature. The reaction was quenched by addition of ice, and the solvent was removed *in vacuo*. The residue was partitioned between AcOEt and H_2O, the organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. After being co-evaporated with toluene three times, the residue was purified by a neutralized silica gel column, eluted with MeOH in $CHCl_3$ (0–3%), to give **S2** (820 mg, 78%) as a yellow foam. FAB-LRMS m/z 522 (MH^+); FAB-HRMS calcd for $C_{32}H_{29}N_3O_6$ 551.20528, found 551.2023; ^{1}H NMR ($CDCl_3$) δ 7.77 (1 H, s), 7.39–7.18 (9 H, m), 6.84–6.82 (4 H, m), 5.80 (1 H, d, *J* = 4.0 Hz), 4.43 (1 H, dd, *J* = 4.0 and 4.8 Hz), 4.35 (1 H, dd, *J* = 4.8 and 8.3 Hz), 4.21 (1 H, dd, *J* = 3.5, 8.3 and 3.5 Hz), 3.78 (6 H, s), 3.76 (1 H, s), 3.50 (1 H, dd, *J* = 3.5 and 10.5 Hz), 3.20

(1 H, dd J = 3.5 and 10.5 Hz), 3.31 (1 H, br s, exchangeable with D_2O), 2.80 (1 H, br s, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$) δ 158.71, 144.19, 137.06, 135.40, 135.33, 130.04, 128.11, 128.06, 127.18, 121.70, 119.80, 113.36, 113.33, 91.54, 90.64, 86.99, 83.89, 75.89, 70.66, 68.16, 62.77, 55.30.

5-Ethynyl-1-(2,3-*O*-di-*tert*-butyldimethylsilyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazole-4-carbonitrile (S3). To a solution of **S2** (820 mg, 1.5 mmol) in DMF (15 mL) containing imidazole (613 mg, 9.0 mmol) was added TBDMSCl (678 mg, 4.5 mmol), and the whole mixture was stirred at room temperature. After 20 h, additional imidazole (204 mg, 3.0 mmol) and TBDMSCl (226 mg, 1.5 mmol) was added, and the whole mixture was stirred for further 10 h. The reaction was quenched by addition of ice, and the solvent was removed *in vacuo*. The residue was partitioned between AcOEt and H_2O the organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a neutralized silica gel column, eluted with hexane/AcOEt (1:0–7:1), to give **S3** (1.01 g, 87%) as a white foam. FABMS-LR *m*/*z* 802 (MNa^+); FABMS-HR calcd for $C_{44}H_{57}N_3NaO_6Si_2$ 802.3683, found 802.3682; ^1H NMR ($CDCl_3$) δ 7.84 (1 H, s), 7.41–7.22 (9 H, m), 6.85–6.83 (4 H, m), 5.75 (1 H, d, J = 6.5 Hz), 4.38 (1 H, dd, J = 6.5 and 4.5 Hz), 4.16 (1 H, ddd, J = 2.5, 3.5 and 4.0 Hz), 4.12 (1 H, dd, J = 2.5 and 4.0 Hz), 3.79 (6 H, s), 3.65 (1 H, s), 3.45 (1 H, dd, J = 4.0 and 10.8 Hz), 3.33 (1 H, dd J = 3.5 and 10.8 Hz), 0.88 and 0.83 (each 9 H, each s), 0.05, –0.01, –0.03, –0.21 (each 3 H, each s); ^{13}C NMR ($CDCl_3$) δ 158.77, 144.19, 137.33, 135.29, 135.26, 130.05, 128.09, 128.05, 127.19, 122.10, 120.07, 113.47, 113.35, 91.10, 89.33, 87.09, 85.64, 77.24, 72.82, 68.57, 63.06, 55.27, 25.76, 25.71, 17.99, 17.83, –4.50, –4.60,–4.64, –5.34.

4-Amino-1-(2,3-*O*-di-*tert*-butyldimethylsilyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (S4). A solution of **S3** (454 mg, 0.58 mmol) in NH_3/MeOH (saturated at 0 °C, 10 mL) was heated at 120 °C for 7 h in a sealed stainless tube. The solvent was removed *in vacuo*, and the residue was purified by a silica gel column, eluted with hexane/AcOEt (4:1–0:1), to give **S4** (405 mg, 87%) as a pale yellow foam. FABMS-LR *m*/*z* 797 (MH^+); FABMS-HR calcd for $C_{44}H_{61}N_4O_6Si_2$ 797.4130, found 797.4108; ^1H NMR ($CDCl_3$) δ 7.98 (1 H, s), 7.67 (1 H, d, J = 5.7

Hz), 7.48–7.22 (9 H, m), 6.96 (1 H, d, *J* = 5.7 Hz), 6.85–6.81 (4 H, m), 5.80 (1 H, d, *J* = 7.3 Hz), 5.13 (2 H, br s, exchangeable with D_2O), 4.51 (1 H, dd, *J* = 4.7 and 7.3 Hz), 4.21–4.19 (1 H, m), 4.17–4.15 (1 H, m), 3.79 (3 H, s), 3.78 (3 H, s), 3.48 (1 H, dd, *J* = 3.2 and 10.7 Hz), 3.41 (1 H, dd, *J* = 3.5 and 10.7 Hz), 0.89 and 0.75 (each 9 H, each s), 0.07, –0.02, –0.12, –0.50 (each 3 H, each s); ^{13}C NMR ($CDCl_3$) δ 158.72, 151.73, 144.33, 141.07, 139.80, 137.97, 135.37, 135.32, 130.12, 128.12, 128.03, 127.86, 127.11, 113.34, 99.39, 88.69, 87.13, 85.77, 75.65, 72.82, 63.33, 55.26, 25.81, 25.68, 18.01, 17.76, –4.55, –4.58, –4.66, –5.67.

4-*N*-Phenoxyacetylamino-1-(2,3-*O*-di-*tert*-butyldimethylsilyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (S5). To a solution of **S4** (880 mg, 1.1 mmol) containing Et_3N (0.39 mL, 2.8 mmol) in CH_3CN (10 mL) at 0 °C was added phenoxyacetyl chloride (PacCl) (0.39 mL, 2.8 mmol), and the mixture was stirred for 2 h at room temperature. Then, the reaction mixture was treated with NaOMe (28% solution in MeOH; 0.8 mL). After 10 min, the mixture was diluted with AcOEt and washed with H_2O, the organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a neutralized silica gel column, eluted with hexane/AcOEt (3:2–1:1), to give **S5** (933 mg, 91%) as a white foam. FAB-LRMS *m*/*z* 931 (MH^+); FAB-HRMS calcd for $C_{52}H_{67}N_4O_8Si_2$ 931.4499, found 931.4468; ^{1}H NMR ($CDCl_3$) δ 9.52 (1 H, br s, exchangeable with D_2O), 8.10 (1 H, s), 8.02 (1 H, d, *J* = 5.5 Hz), 7.47–6.84 (19 H, m), 5.86 (1 H, d, *J* = 7.8 Hz), 4.80 (2 H, br s), 4.58 (1 H, dd, *J* = 7.8 and 4.6 Hz), 4.27 (1 H, d, *J* = 4.6 Hz), 4.18–4.15 (1 H, m), 3.79 (6 H, s), 3.53 (1 H, dd, *J* = 2.8 and 11.0 Hz), 3.46 (1 H, dd, *J* = 3.2 and 11.0 Hz), 0.90 and 0.75 (each 9 H, each s), 0.09, –0.01, –0.11, –0.58 (each 3 H, each s); ^{13}C NMR ($CDCl_3$) δ 158.85, 157.31, 144.33, 143.40, 141.92, 141.21, 138.67, 135.28, 135.18, 130.24, 129.88, 128.21, 127.32, 122.32, 115.17, 113.46, 105.18, 89.00, 87.36, 86.31, 75.67, 72.82, 68.15, 63.31, 60.54, 55.38, 25.91, 25.77, 21.21, 18.14, 17.84, 14.33, –4.47, –5.59.

4-*N*-Phenoxyacetylamino-1-(5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (S6). To a solution of **S5** (354 mg, 0.38 mmol) in THF (6 mL) was added a 1 M THF solution of TBAF (1.0 mL, 1.0 mmol) at 0 °C, and the whole mixture was stirred for 15 min at room temperature. The reaction mixture was partitioned between AcOEt and H_2O, the organic

layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a neutralized silica gel column, eluted with MeOH in $CHCl_3$ (0–3%), to give **S6** (233 mg, 87%) as a pale yellow foam. FAB-LRMS *m*/*z* 703 (MH^+); FAB-HRMS calcd for $C_{40}H_{39}N_4O_8$ 703.2768, found 703.2768; ^{1}H NMR ($CDCl_3$) δ 9.43 (1 H, br s, exchangeable with D_2O), 7.98 (1 H, s), 7.52–6.76 (20 H, m), 5.82 (1 H, d, *J* = 6.9 Hz), 4.68 (2 H, br s), 4.63 (1 H, dd, *J* = 6.9 and 5.7 Hz), 4.50 (1 H, dd, *J* = 5.7 and 2.9 Hz), 4.30 (1 H, ddd, *J* = 2.9, 3.4 and 6.3 Hz), 3.74 and 3.73 (each 3 H, each s), 3.50–3.44 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 158.71, 157.16, 144.47, 142.21, 140.08, 138.69, 135.64, 135.47, 130.84, 130.32, 130.24, 129.95, 128.32, 128.11, 127.12, 122.51, 115.15, 113.38, 112.40, 104.90, 89.73, 86.90, 84.62, 74.26, 71.28, 67.96, 63.64, 58.63, 55.36, 18.58.

4-*N*-Phenoxyacetylamino-1-(2-*O*-*tert*-butyldimethylsilyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (S7). To a solution of **S6** (303 mg, 0.43 mmol) in pyridine (4 mL) was added $AgNO_3$ (146 mg, 0.86 mmol). After being stirred for 1 h at room temperature under exclusion of light, a solution of TBDMSCl (110 mg, 0.73 mmol) in THF (6 mL) was added, and the whole mixture was stirred at the same temperature. After being stirred for 24 h, insoluble materials of the reaction mixture were filtered off through a celite and the filtrate was diluted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$, followed by H_2O, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was co-eveporated with toluene three times, and the residue was purified by a neutralized silica gel column, eluted with hexane/AcOEt (5/1–3/1), to give **S7** (193 mg, 53%) as a white foam along with its 3'-*O*-TBDMS derivative. FAB-LRMS *m*/*z* 817 (MH^+); FAB-HRMS calcd for $C_{46}H_{53}N_4O_8Si$ 817.3633, found 817.3621; ^{1}H NMR ($CDCl_3$) δ 9.52 (1 H, br s, exchangeable with D_2O), 8.08 (1 H, s), 7.93 (1 H, d, *J* = 6.0 Hz), 7.43–6.82 (19 H, m), 5.83 (1 H, d, *J* = 7.3 Hz), 4.80 (2 H, br s), 4.76 (1 H, dd, *J* = 7.3 and 5.0 Hz), 4.39–4.37 (1 H, m), 4.35–4.32 (1 H, m), 3.79 and 3.78 (each 3 H, each s), 3.57 (1 H, dd, *J* = 2.3 and 11.0 Hz), 3.50 (1 H, dd, *J* = 2.8 and 11.0 Hz), 2.81 (1 H, d, *J* = 1.4 Hz), 0.82 (9 H, s), –0.12 and –0.37 (each 3 H, each s); ^{13}C NMR ($CDCl_3$) δ 158.86, 157.30, 144.37, 143.48, 141.75, 141.53, 138.62, 135.25, 135.14, 130.33, 129.92, 128.28, 128.19, 127.37, 122.37, 115.17, 113.45, 104.87, 89.19, 87.28, 84.75, 74.87, 71.69, 68.16, 63.58, 60.56, 55.39, 25.80, 25.64, 21.22, 17.95, 14.35, –5.17, –5.38.

4-*N*-Phenoxyacetylamino-1-[2-*O*-*tert*-butyldimethylsilyl-3-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphino)-5-*O*-dimethoxytrityl-β-D-ribofuranosyl]imidazo[4,5-*c*]pyridine (S8). To a solution of **S7** (580 mg, 0.71 mmol) in CH_2Cl_2 (10 mL) containing DMAP (catalytic) and *N*,*N*-diisopropylethylamine (0.49 mL, 2.84 mmol) was added 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.40 mL, 1.78 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of ice, and the mixture was diluted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$, followed by brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a neutralized silica gel column, eluted with hexane/AcOEt (3:1–2:1), to give **S8** (498 mg, 69%) as a white foam. FAB-LRMS *m*/*z* 1017 (MH^+); FAB-HRMS calcd for $C_{55}H_{70}N_6O_9PSi$ 1017.4705, found 1017.4705; ^{31}P NMR ($CDCl_3$) δ 152.64, 149.44.

4-Amino-7-bromo-1-(2,3-*O*-di-*tert*-butyldimethylsilyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (S9). To a solution of **S4** (398 mg, 0.5 mmol) in CH_2Cl_2 (10 mL) was added *N*-bromosuccinimide (NBS) (178 mg, 1.0 mmol) at –15 °C, and the reaction mixture was stirred for 15 min at the same temperature. The reaction was quenched by addition of cyclohexene (0.1 mL), and the solvent was removed *in vacuo*. The residue was partitioned between AcOEt and H_2O, the organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a neutralized silica gel column, eluted with hexane/AcOEt (3:1–1:2), to give **S9** (302 mg, 61%) as a pale yellow foam. FAB-LRMS *m*/*z* 875, 877 (MH^+); FAB-HRMS calcd for $C_{44}H_{60}BrN_4O_6Si_2$ 875.3235, found 875.3206; ^1H NMR (CDCl3) δ 8.24 (1 H, s), 7.90 (1 H, s), 7.45–7.22 (9 H, m), 6.95 (1 H, d, *J* = 4.6 Hz), 6.89–6.81 (4 H, m), 5.39 (2 H, br s, exchangeable with D_2O), 4.47 (1 H, dd, *J* = 4.6 and 7.5 Hz), 4.17–4.09 (2 H, m), 3.79 (6 H, s), 3.50 (1 H, dd, *J* = 3.4 and 10.9 Hz), 3.32 (1 H, dd, *J* = 2.9 and 10.9 Hz), 0.90 and 0.74 (each 9 H, each s), 0.06, –0.03, –0.11, –0.42 (each 3 H, each s); ^{13}C NMR ($CDCl_3$) δ 158.78, 151.15, 144.51, 143.82, 139.67, 136.07, 135.55, 135.48, 130.14, 128.83, 128.30, 128.19, 128.13, 127.23, 113.46, 90.71, 87.15, 85.91, 85.69, 78.29, 73.29, 63.42, 60.54, 55.39, 25.87, 25.72, 21.21, 18.07, 17.91, 14.34, –4.34, –4.55, –5.48.

7-Bromo-4-*N*-phenoxyacetylamino-1-(2,3-*O*-di-*tert*-butyldimethylsilyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (S10). In the similar manner as described for **S5**, **S9** (703 mg, 0.8 mmol) in CH_3CN was treated with Et_3N (0.25 mL, 2.8 mmol) and PacCl (0.25 mL, 1.8 mmol), followed by NaOMe (28% solution in MeOH; 0.5 mL) to give **S10** (735 mg, 91%) as a white foam. ESI-LRMS *m*/*z* 1031, 1033 (MNa^+); ESI-HRMS calcd for $C_{52}H_{65}BrN_4NaO_8Si_2$ 1031.3417, found 1031.3406; ^{1}H NMR ($CDCl_3$) δ 9.59 (1 H, br s, exchangeable with D_2O), 8.39 (1 H, s), 8.36 (1 H, s), 7.45–6.85 (19 H, m), 4.80 (2 H, br s), 4.47 (1 H, dd, *J* = 4.1 and 7.3 Hz), 4.18–4.04 (2 H, m), 3.79 (6 H, s), 3.55 (1 H, dd, *J* = 3.2 and 12.4 Hz), 3.32 (1 H, dd, *J* = 2.8 and 12.4 Hz), 0.90 and 0.72 (each 9 H, each 3), 0.06, –0.04, –0.12, –0.46 (each 3 H, each s); ^{13}C NMR ($CDCl_3$) δ 171.29, 166.19, 158.78, 157.21, 144.46, 143.98, 142.71, 141.69, 141.50, 137.03, 135.41, 135.33, 131.78, 130.11, 129.88, 129.62, 128.81, 128.29, 128.18, 128.06, 127.23, 122.36, 115.12, 113.59, 113.45, 111.37, 97.23, 87.17, 86.7, 86.11, 85.91, 78.41, 73.19, 68.10, 63.24, 60.50, 55.35, 29.81, 25.82, 25.65, 21.16, 18.02, 17.82, 14.29, 0.09, –4.38, –4.56, –4.59, –5.48.

7-Bromo-4-*N*-phenoxyacetylamino-1-(5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (S11). In the similar manner as described for **S6**, **S10** (735 mg, 0.73 mmol) in THF (10 mL) was treated with 1 M THF solution of TBAF (1.8 mL, 1.8 mmol) to give **S11** (583 mg, quant) as a pale yellow foam. ESI-LRMS *m*/*z* 803, 805 (MNa^+); ESI-HRMS calcd for $C_{40}H_{37}BrN_4NaO_8$ 803.1687, found 803.1688; ^{1}H NMR (DMSO-d_6) δ 8.63 (1 H, s), 8.30 (1 H, s), 7.46–6.80 (18 H, m), 6.57 (1 H, d, *J* = 3.7 Hz), 5.77 (1 H, d, *J* = 5.5 Hz), 5.30 (1 H, d, *J* = 6.0 Hz), 4.87 (2 H, br s), 4.61 (1 H, ddd, *J* = 3.7, 5.5 and 6.0 Hz), 4.27 (1 H, dt, *J* = 5.5 and 9.2 Hz), 4.16–4.08 (1 H, m), 3.70 (6 H, s), 3.21–3.17 (2 H, m); ^{13}C NMR (DMSO-d_6) δ 166.83, 158.09, 157.79, 144.78, 142.89, 142.59, 136.75, 135.46, 129.71, 129.56, 127.89, 127.67, 126.74, 121.21, 114.67, 113.21, 97.78, 88.34, 85.61, 82.98, 79.22, 74.14, 69.84, 67.04, 63.19, 55.04.

7-Bromo-4-*N*-phenoxyacetylamino-1-(2-*O*-*tert*-butyldimethylsilyl-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (S12). In the similar manner as described for **S7**, **S11** (583 mg, 0.75 mmol) in pyridine (4 mL) was treated with TBDMSCl (147 mg, 0.98 mmol) in the presence of $AgNO_3$ (253 mg, 1.5 mmol) in THF (6 mL) to give a mixture of **S12** and its

3'-*O*-TBDMS derivative. To a solution of resulting mixture in MeOH (20 mL) was added Et_3N (0.1 mL), and the whole mixture was stirred for 20 min at room temperature. Then, the reaction mixture was partitioned between AcOEt and H_2O, the organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a neutralized silica gel column, eluted with hexane/AcOEt (4:1–1:1), to give **S12** (409 mg, 61%) as a white foam. ESI-LRMS *m*/*z* 917, 919 (MNa^+); ESI-HRMS calcd for $C_{46}H_{51}BrN_4NaO_8Si$ 917.2552, found 917.2551; ^{1}H NMR ($CDCl_3$) δ 9.44 (1 H, br s, exchangeable with D_2O), 8.38 (1 H, s), 8.36 (1 H, s), 7.43–6.84 (19 H, m), 4.80 (2 H, br s), 4.68 (1 H, dd, *J* = 5.0 and 1.8 Hz), 4.33 (1 H, ddd, *J* = 4.6, 2.3 and 2.8 Hz), 4.30 (1 H, dd, *J* = 1.8 and 4.6 Hz), 3.80 (6 H, s), 3.57 (1 H, dd, *J* = 2.3 and 11.0 Hz), 3.39 (1 H, dd, *J* = 2.8 and 11.0 Hz), 2.85 (1 H, br s), 0.82 (9 H, s), –0.09 and –0.29 (each 3 H, each s); ^{13}C NMR ($CDCl_3$) δ 158.85, 157.22, 144.49, 144.10, 142.89, 141.79, 141.58, 136.81, 135.40, 135.32, 130.25, 130.02, 129.24, 128.36, 128.23, 128.09, 128.01, 127.89, 127.30, 122.42, 115.16, 113.61, 113.50, 113.40, 113.27, 87.16, 86.78, 84.58, 78.67, 72.24, 68.15, 63.74, 56.37, 25.70, 25.63, –4.65, –5.09, –5.23.

7-Bromo-4-*N*-phenoxyacetylamino-1-[2-*O*-*tert*-butyldimethylsilyl-3-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphino)-5-*O*-dimethoxytrityl-β-D-ribofuranosyl]imidazo[4,5-*c*]pyridine (S13). In the similar manner as described for **S8**, **S12** (309 mg, 0.35 mmol) in CH_2Cl_2 (4 mL) containing DMAP (catalytic) and *N*,*N*-diisopropylethylamine (240 mL, 1.4 mmol) was treated with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.24 mL, 0.86 mmol) to give **S13** (287 mg, 74%) as a white foam. ESI-LRMS *m*/*z* 1095, 1097 (MH^+); ESI-HRMS calcd for $C_{55}H_{69}BrN_6O_9PSi$ 1095.3811, found 1095.3811; ^{31}P NMR ($CDCl_3$) δ 152.72, 149.12.

Figure S1 Structural aspects of siRNA duplexes based on CD spectra. A series of **siR1** (A), **siR2** (B), and **siR3** (C).

CD spectra were obtained at 25 °C on a Jasco J1500. An aliquot containing annealed siRNA duplex (1.5 μM) in a buffer of 10 mM Na cacodylate (pH 7.0) containing 100 mM NaCl was prepared, and sample spectra were subtracted from the buffer spectrum. Molar ellipticity was calculated from equation $[\theta] = \theta/cl$, where θ is the relative intensity, c is sample concentration, and l is cell path length in centimeters.

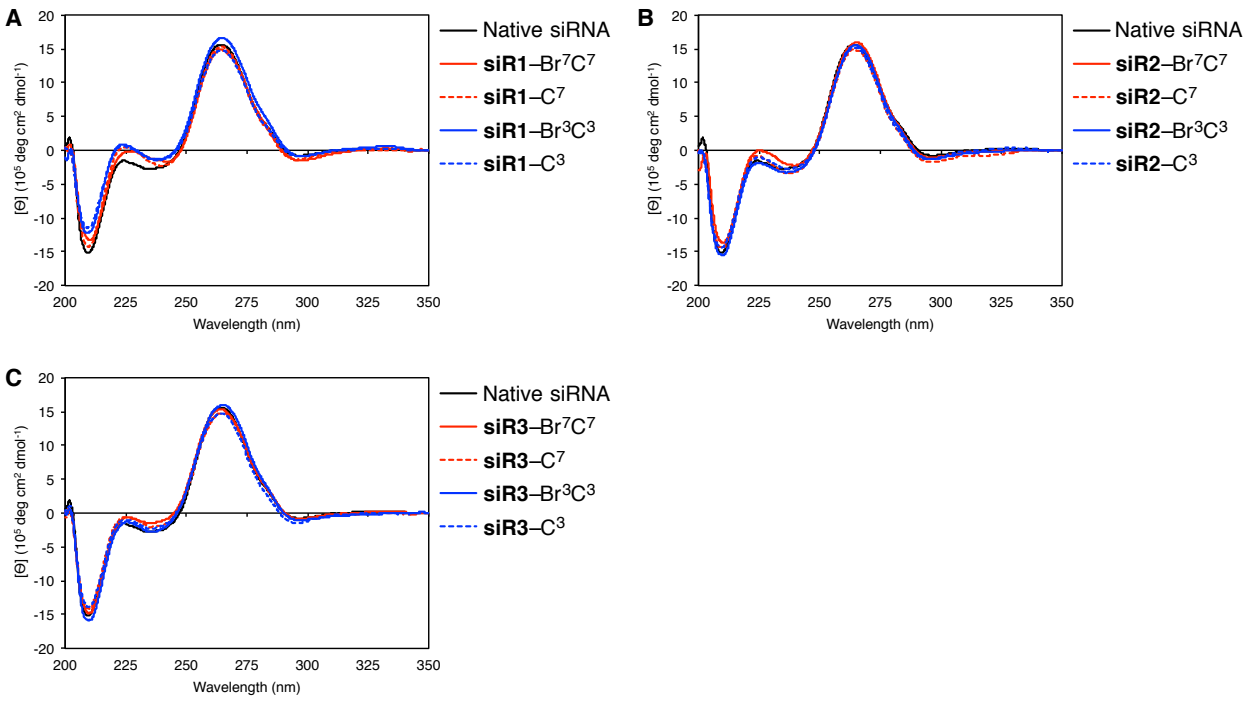


Figure S2 Schematic of our hypotheses that direction of RISC assembly might be determined from the interaction between Ago2 and siRNA in minor groove near 5'-end of passenger strand.

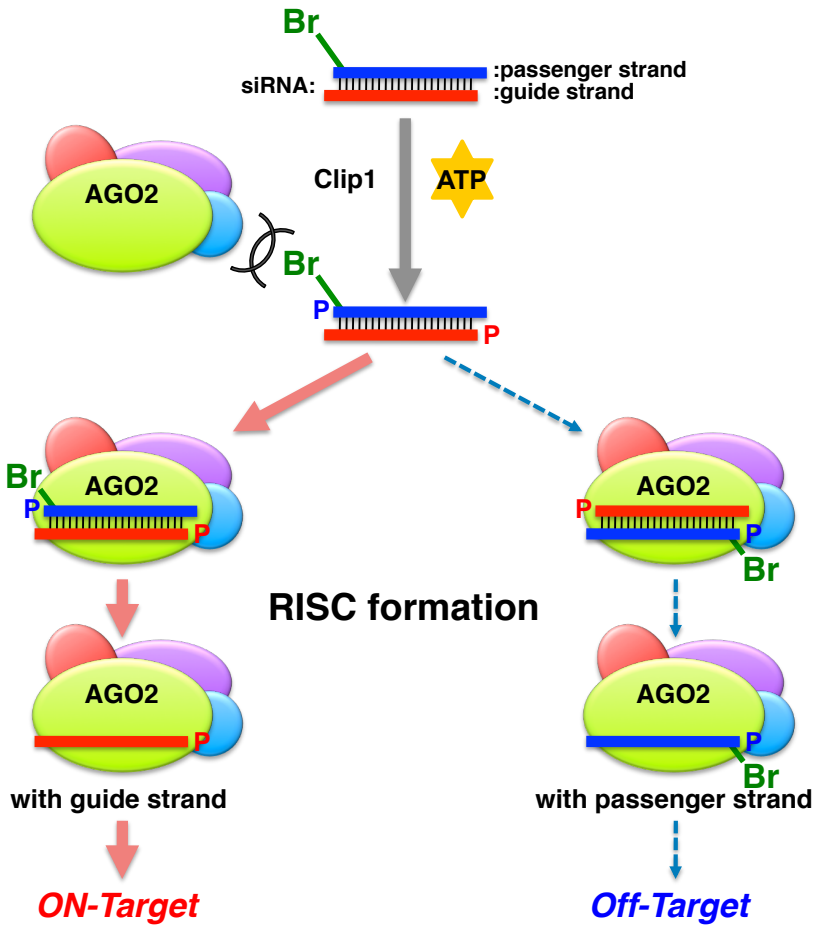


Figure S3 Effect of 5'-phosphorylation of siRNAs on strand selection in RNAi. Relative ON-target and Off-target potencies of 5'-phosphorylated **siR3**, **siR8**, **siR9**, and **siR10**, which have chemical probes at 5'-end in passenger strand. HeLa cells were transfected with all samples at 30 nM. After 24 h of incubation, the relative luciferase activities were analyzed. Error bars indicate standard deviations of three independent experiments. Level of luciferase activity in 5'-phosphorylated native siRNA was set to 1.0.

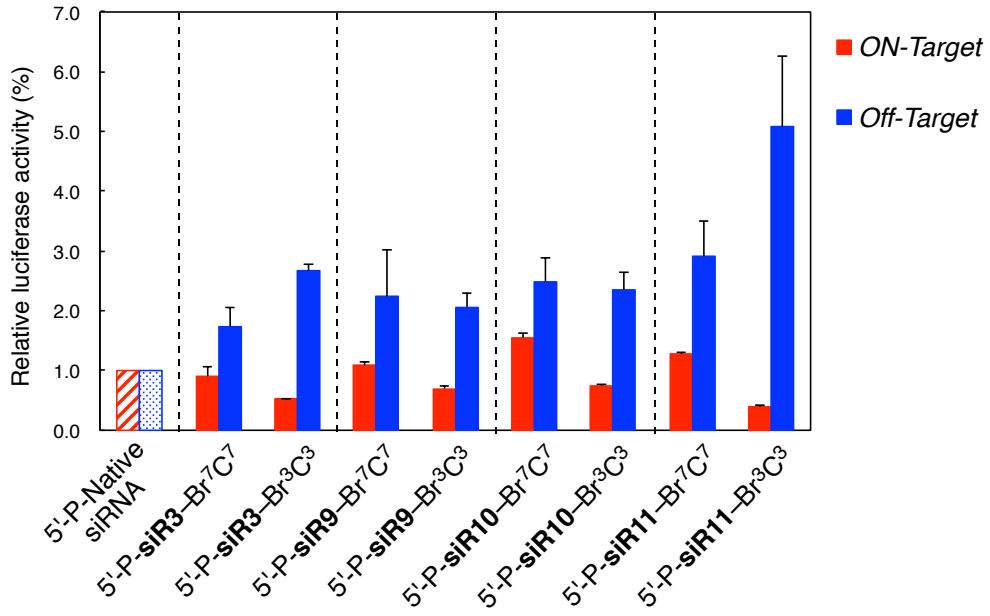
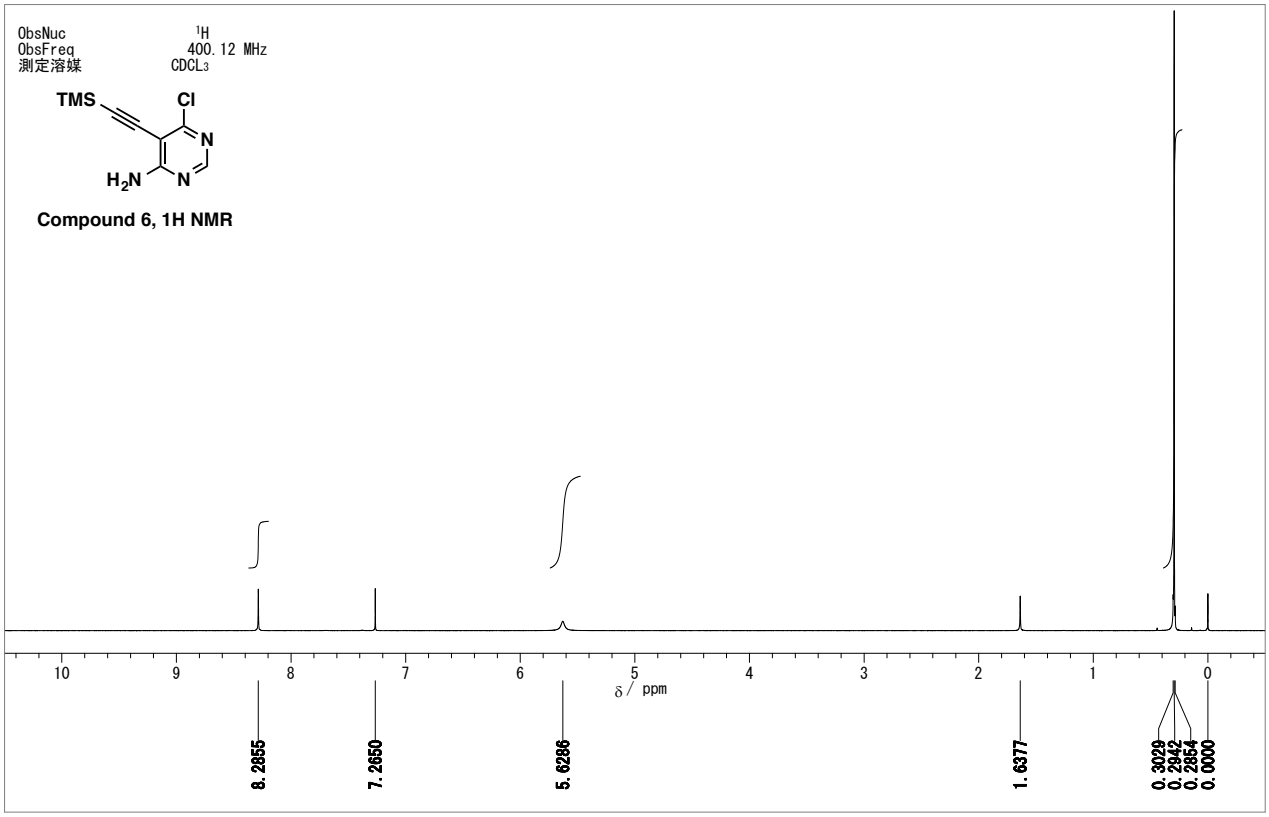
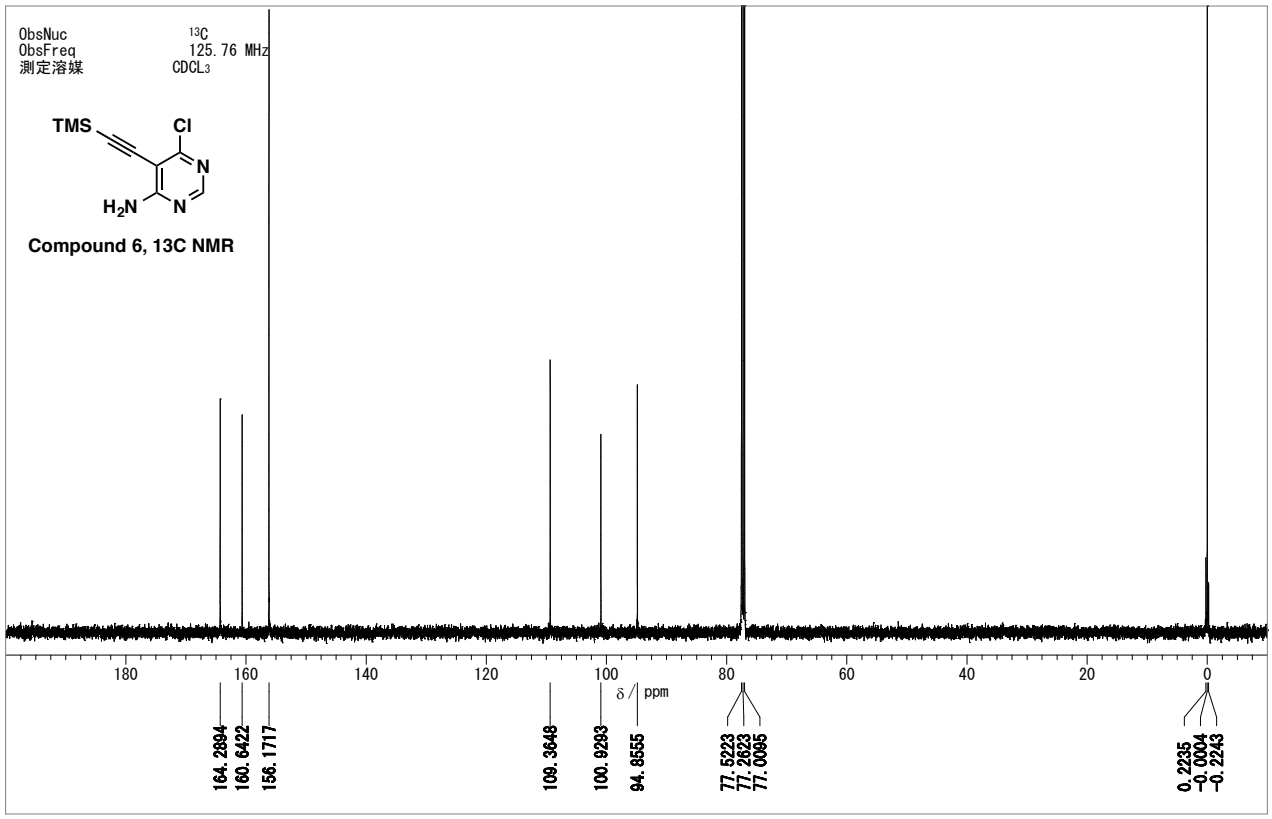
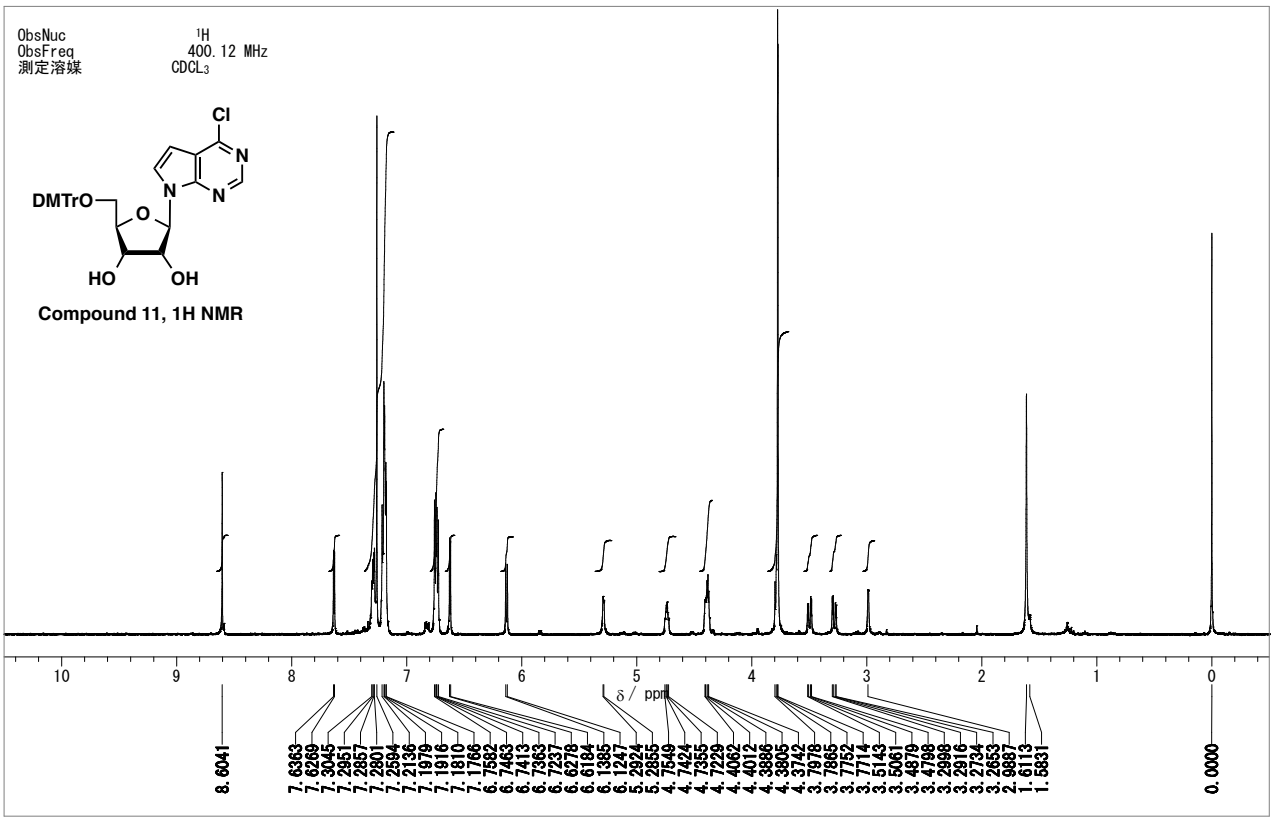


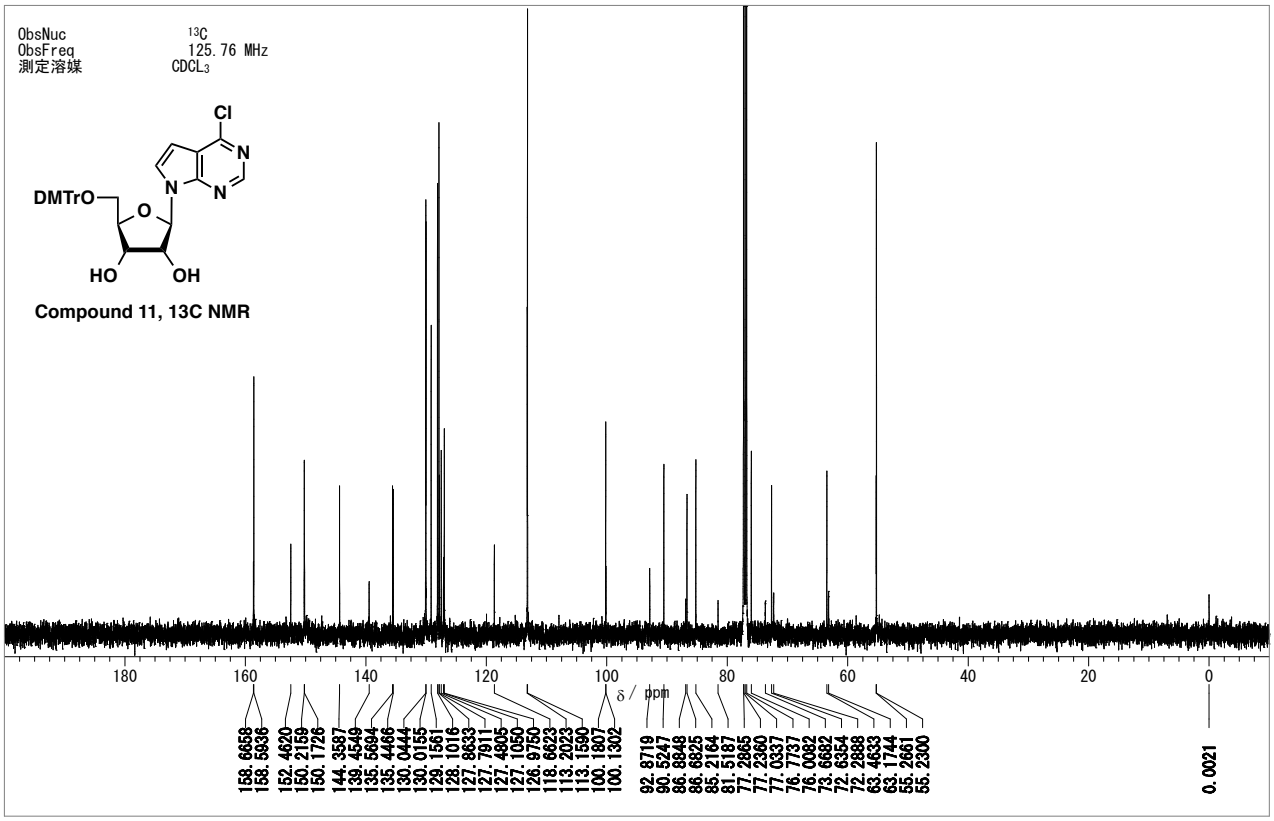
Table S1 Seaquence and MALDI-TOF/MASS analytical data of synthesized ssRNAs. Bold upper case letters represent nucleotide positions incorporating chemical probes; italics are 2'-doxynuclotides.

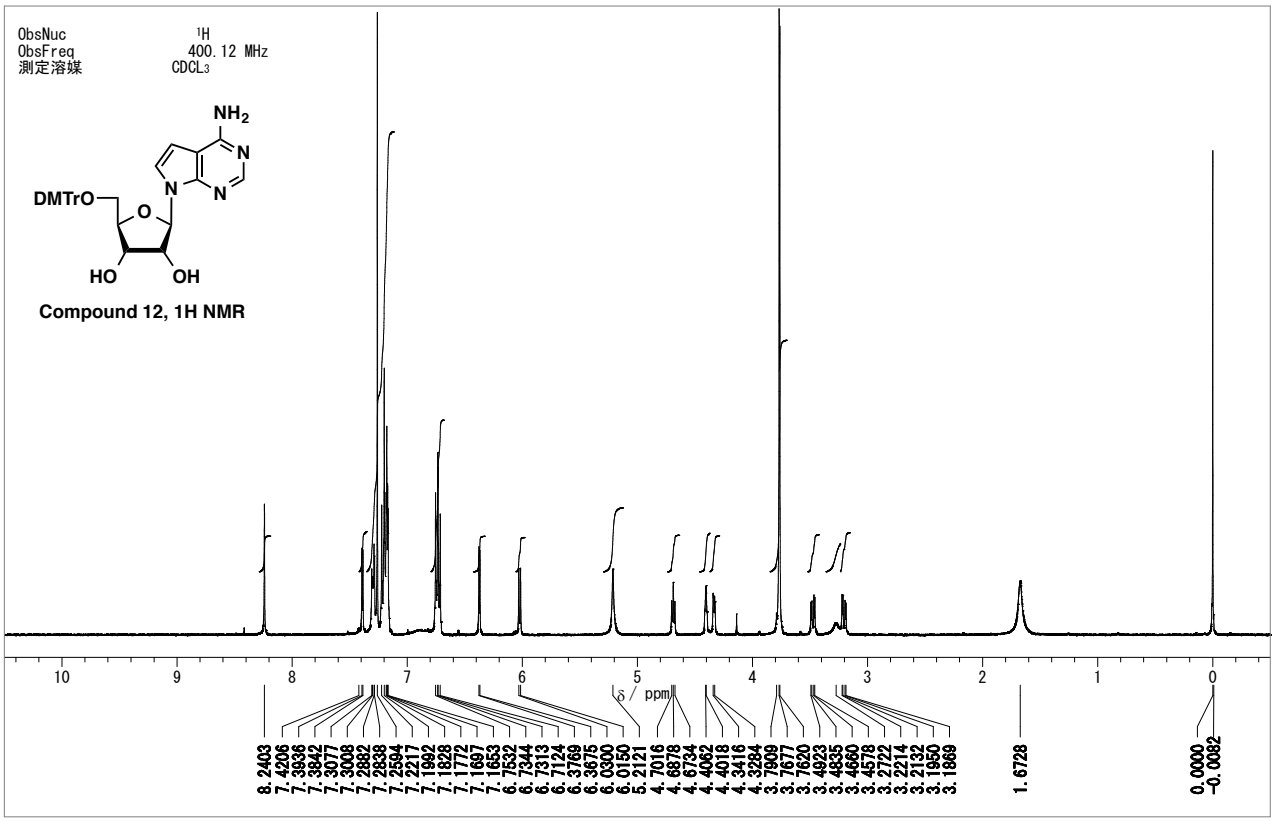
Sequence		Calcd (M–H)	Observed
ssRNA for siR1 5'-c**A**gc**A**uuuucugcauguuu*tt*-3'	$C_{199}H_{246}Br_2N_{62}O_{151}P_{20}$	6695.7	6699.8 (Br^7C^7) 6694.6 (Br^3C^3)
	$C_{199}H_{248}N_{62}O_{151}P_{20}$	6539.8	6538.9 (C^7) 6535.1 (C^3)
ssRNA for siR2 5'-aaacaugc**A**g**A**aaaugcug*tt*-3'	$C_{206}H_{252}Br_2N_{82}O_{139}P_{20}$	6873.8	6874.2 (Br^7C^7) 6871.2 (Br^3C^3)
	$C_{206}H_{254}N_{82}O_{139}P_{20}$	6718.0	6717.0 (C^7) 6714.0 (C^3)
ssRNA for siR3 5'-a**A**ac**A**ugcagaaaaugcug*tt*-3'	$C_{206}H_{252}Br_2N_{82}O_{139}P_{20}$	6873.8	6872.0 (Br^7C^7) 6871.5 (Br^3C^3)
	$C_{206}H_{254}N_{82}O_{139}P_{20}$	6718.0	6714.0 (C^7) 6716.8 (C^3)
ssRNA for siR4 5'-c**A**gcauuuucugcauguuu*tt*-3'	$C_{198}H_{246}BrN_{63}O_{151}P_{20}$	6618.7	6622.5 (Br^7C^7) 6621.7 (Br^3C^3)
ssRNA for siR5 5'-cagc**A**uuuucugcauguuu*tt*-3'	$C_{198}H_{246}BrN_{63}O_{151}P_{20}$	6618.7	6613.7 (Br^7C^7) 6615.4 (Br^3C^3)
ssRNA for siR6 5'-aaacaugc**A**gaaaaugcug*tt*-3'	$C_{205}H_{252}BrN_{83}O_{139}P_{20}$	6796.9	6800.5 (Br^7C^7) 6797.7 (Br^3C^3)
ssRNA for siR7 5'-aaac**A**ugcagaaaaugcug*tt*-3'	$C_{205}H_{252}BrN_{83}O_{139}P_{20}$	6796.9	6799.7 (Br^7C^7) 6799.4 (Br^3C^3)
ssRNA for siR8 5'-a**A**acaugcagaaaaugcug*tt*-3'	$C_{205}H_{252}BrN_{83}O_{139}P_{20}$	6796.9	6798.8 (Br^7C^7) 6799.5 (Br^3C^3)
ssRNA for siR9 5'-**A**aacaugcagaaaaugcug*tt*-3'	$C_{205}H_{252}BrN_{83}O_{139}P_{20}$	6796.9	6799.6 (Br^7C^7) 6800.2 (Br^3C^3)
ssRNA for siR10 5'-**AA**ac**A**ugcagaaaaugcug*tt*-3'	$C_{207}H_{252}Br_3N_{81}O_{139}P_{20}$	6950.7	6954.1 (Br^7C^7) 6953.0 (Br^3C^3)


ObsNuc 1H
ObsFreq 400.12 MHz
測定溶媒 CDCL3
TMS
Cl
N
H2N
N
Compound 6, 1H NMR
10
9
8
7
6
5
4
3
2
1
0
δ / ppm
8.2855
7.2650
5.6286
1.6377
0.3029
0.2942
0.2884
0.0000

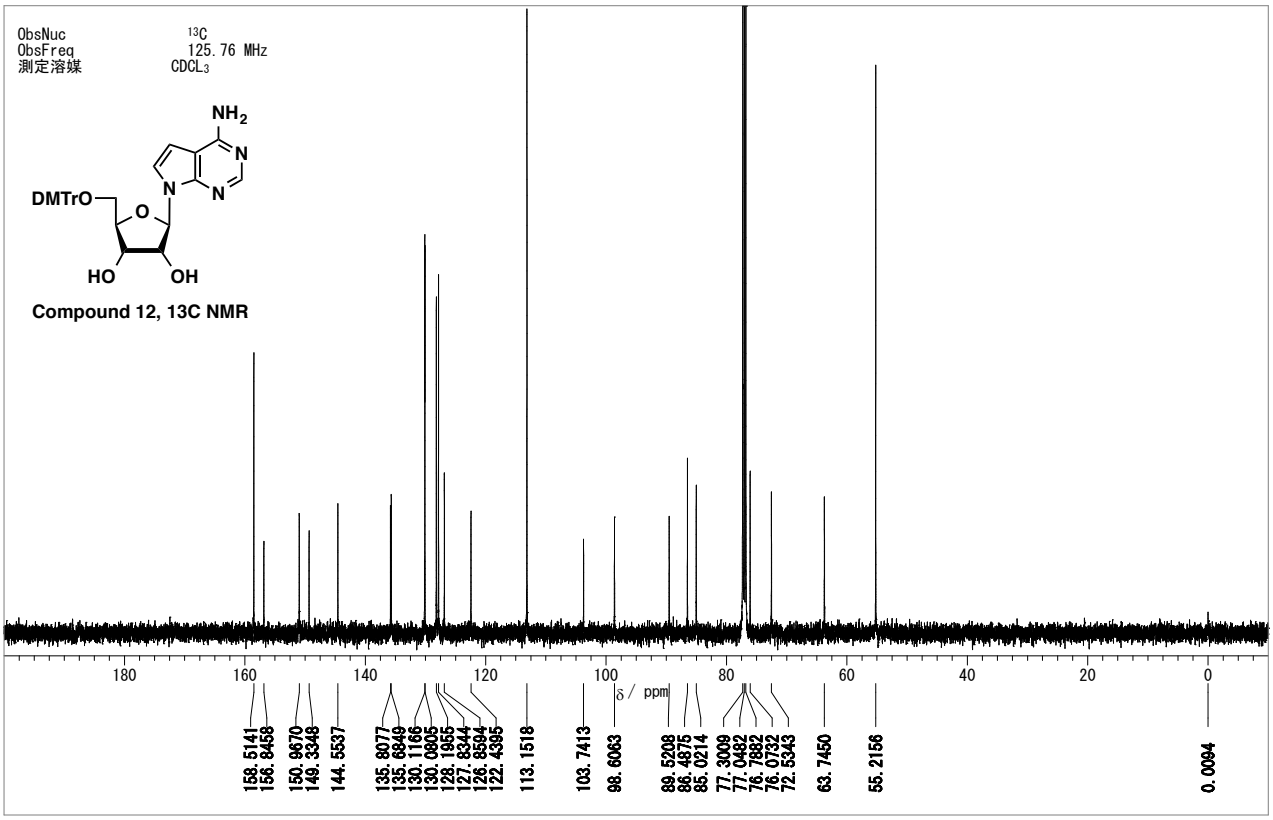

ObsNuc 13C
ObsFreq 125.76 MHz
測定溶媒 CDCL3
TMS
Cl
N
H2N
N
Compound 6, 13C NMR
180
160
140
120
100
80
60
40
20
0
δ / ppm
164.2894
160.6422
156.1717
109.3648
100.9293
94.8555
77.5223
77.2623
77.0085
0.2235
-0.0004
-0.2243


ObsNuc 1H
ObsFreq 400.12 MHz
測定溶媒 CDCl3
Cl
N
N
N
O
DMTrO
HO
OH
Compound 11, 1H NMR
δ / ppm
8.6041
7.6363
7.6269
7.3045
7.2951
7.2857
7.2801
7.2594
7.2136
7.1979
7.1916
7.1810
7.1786
6.7582
6.7463
6.7413
6.7363
6.7237
6.6278
6.6184
6.1385
6.1247
5.2924
5.2855
4.7549
4.7424
4.7355
4.7229
4.4062
4.4012
4.3986
4.3805
4.3742
3.7978
3.7865
3.7752
3.7714
3.5143
3.5061
3.4879
3.4798
3.2998
3.2916
3.2734
3.2653
2.9857
1.6113
1.5831
0.0000

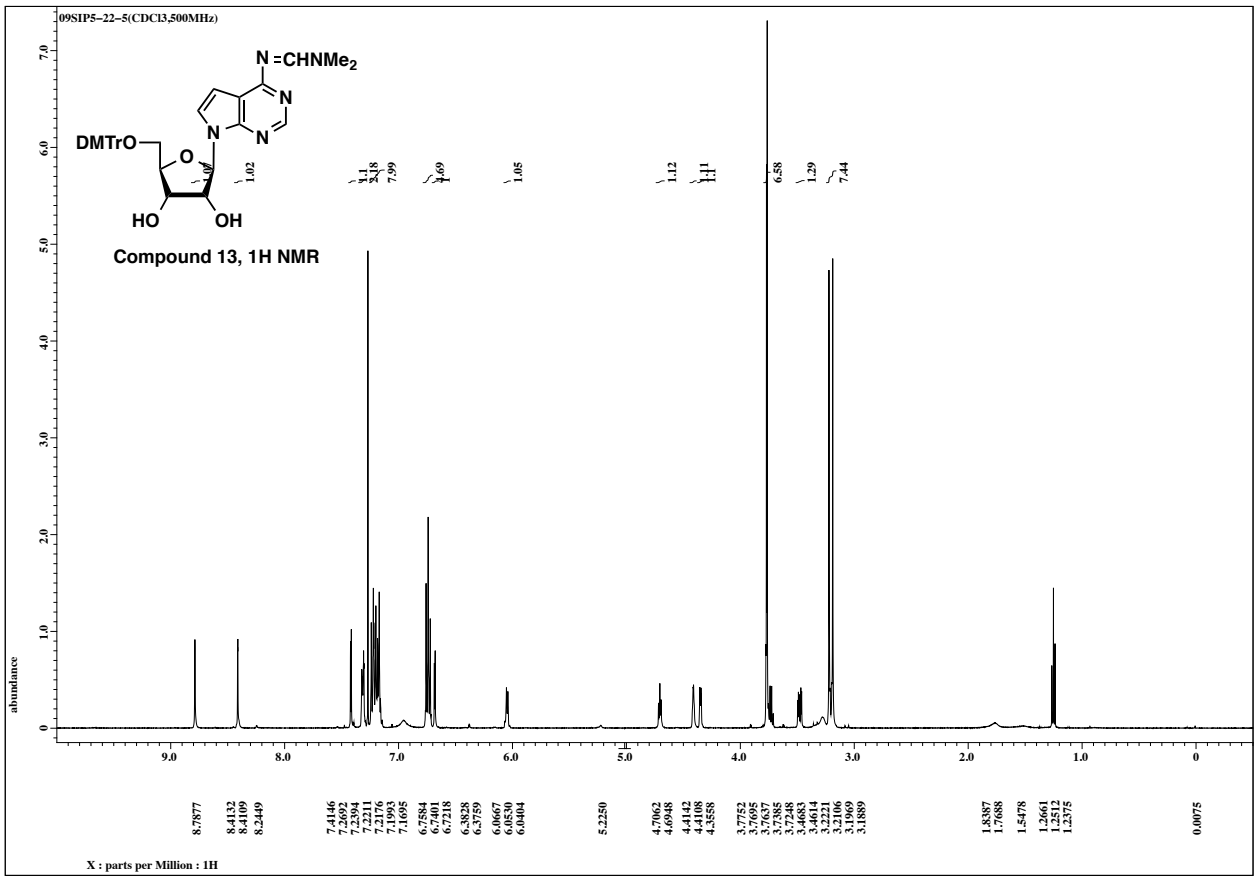

ObsNuc 13C
ObsFreq 125.76 MHz
測定溶媒 CDCl3
Cl
N
N
N
O
DMTrO
HO
OH
Compound 11, 13C NMR
δ / ppm
158.6658
158.5936
152.4620
150.2159
150.1726
144.3587
139.4549
135.5694
135.4466
130.0444
130.0155
129.1561
128.1016
127.8633
127.7911
127.4805
127.1050
126.9750
118.6823
113.2023
113.1590
100.1807
100.1302
92.8719
90.5247
86.8848
86.6825
85.2164
81.5187
77.2865
77.2360
77.0337
76.7737
76.0082
73.6682
72.6354
72.2888
63.4633
63.1744
55.2661
55.2300
0.0021



Compound 12, 1H NMR



Compound 12, 13C NMR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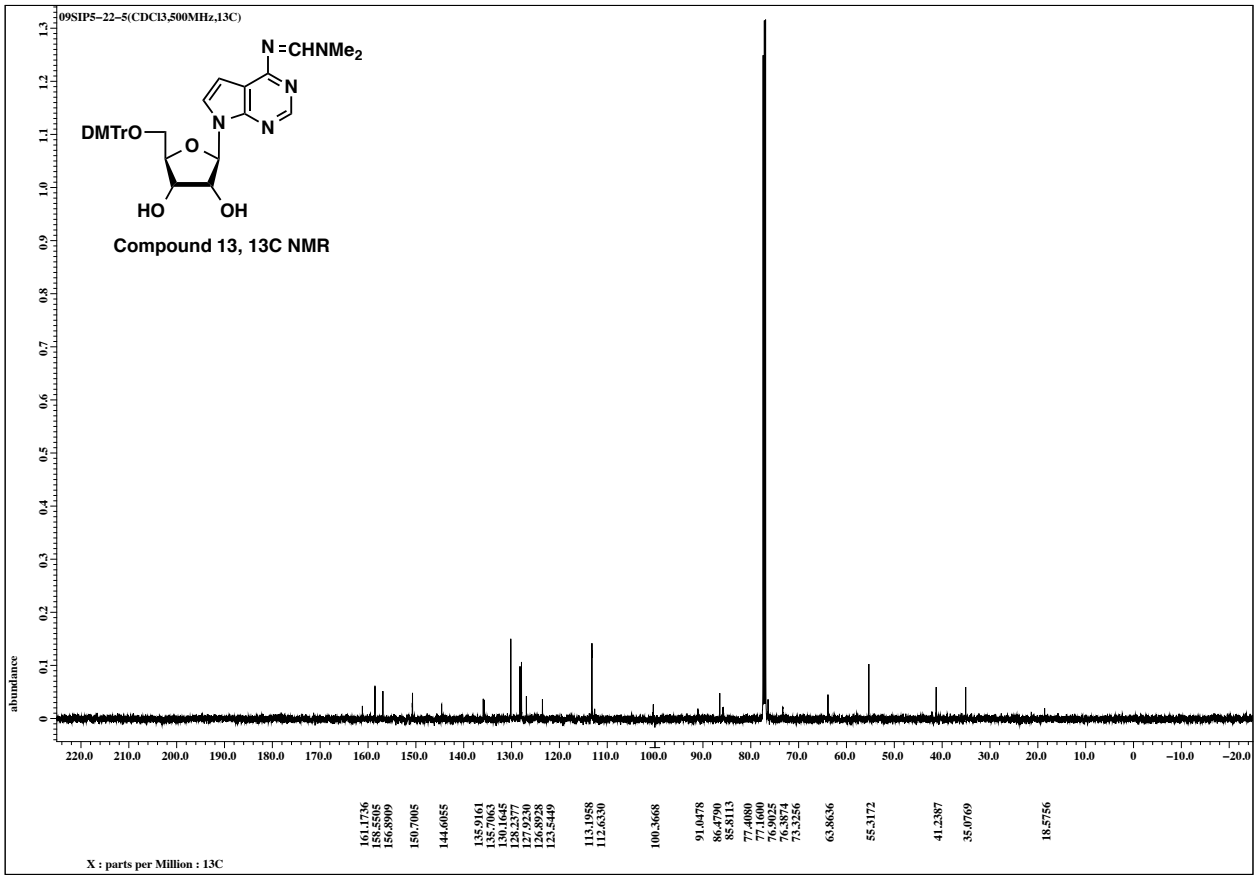
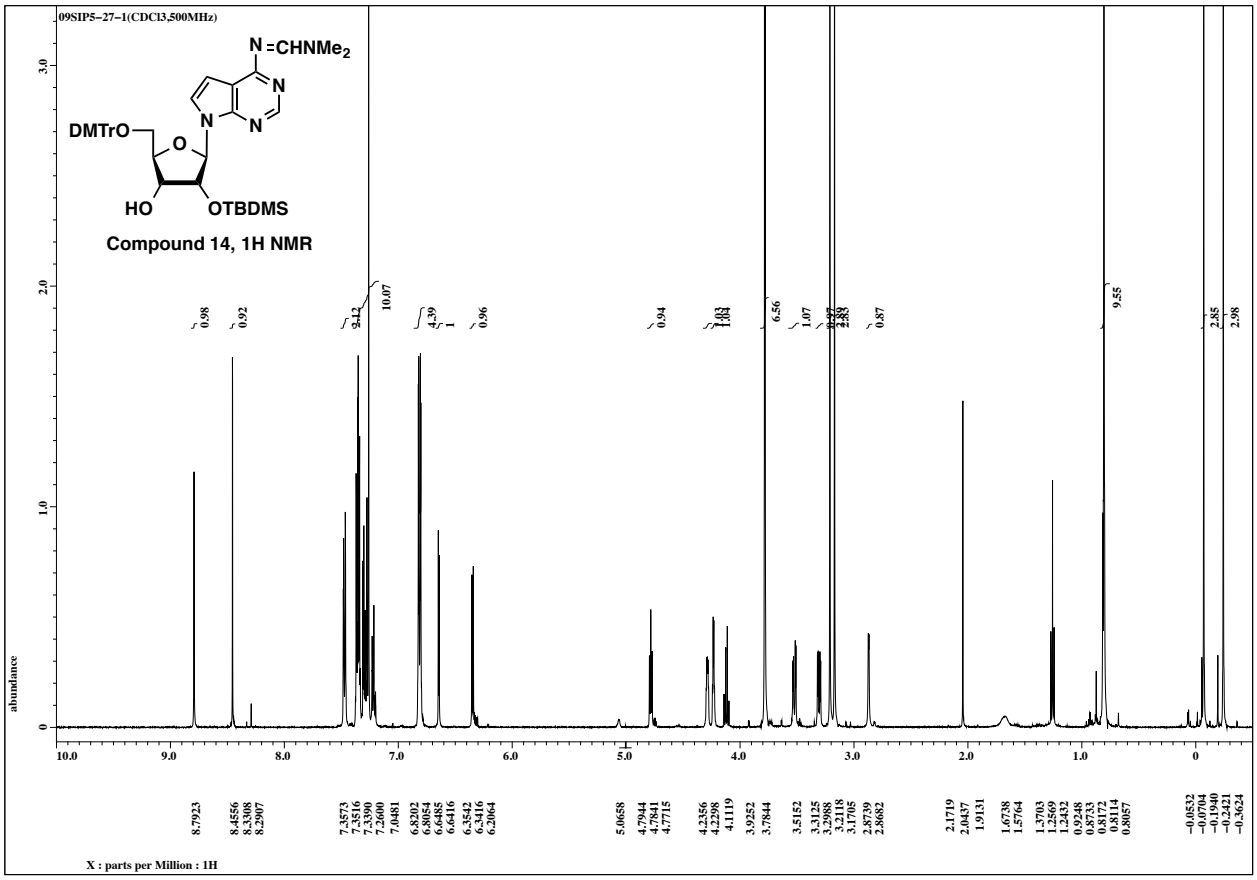
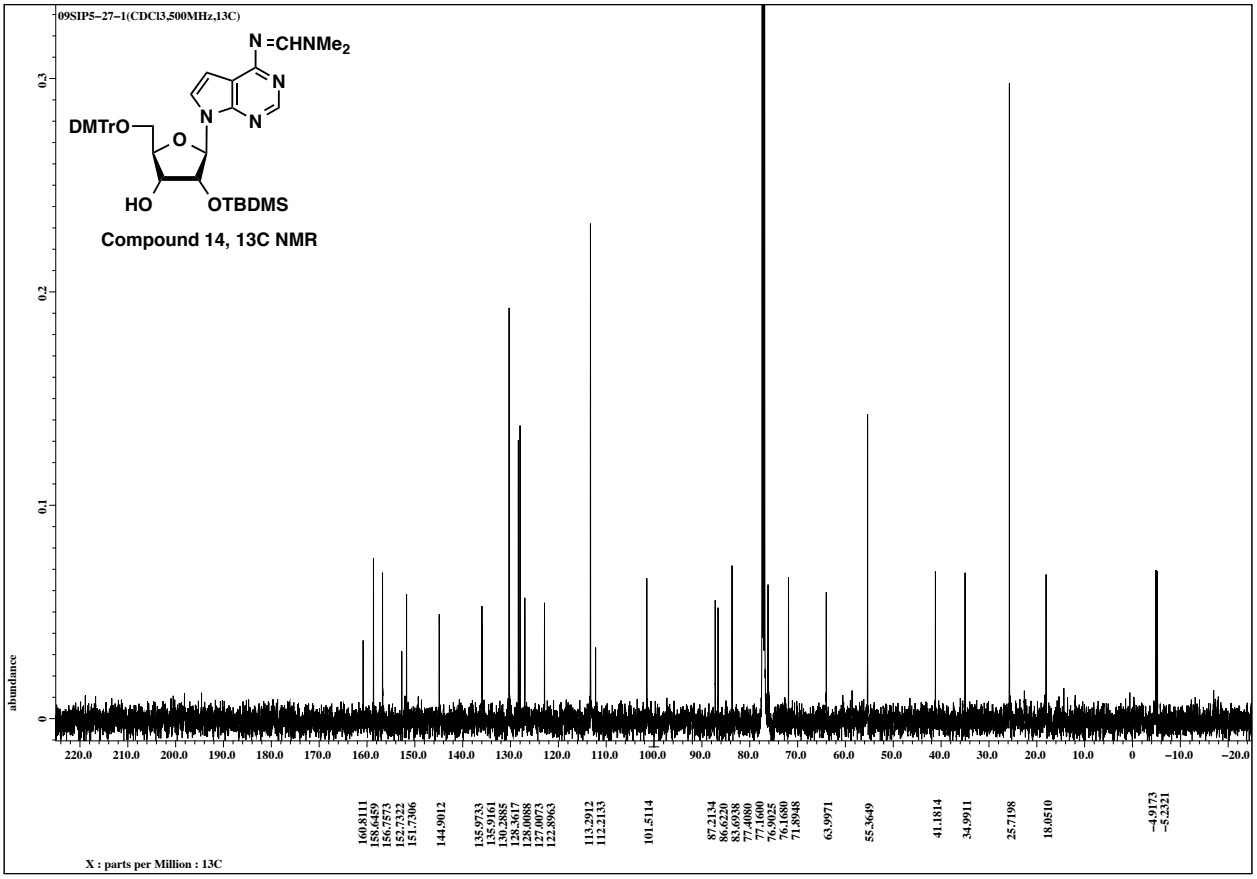
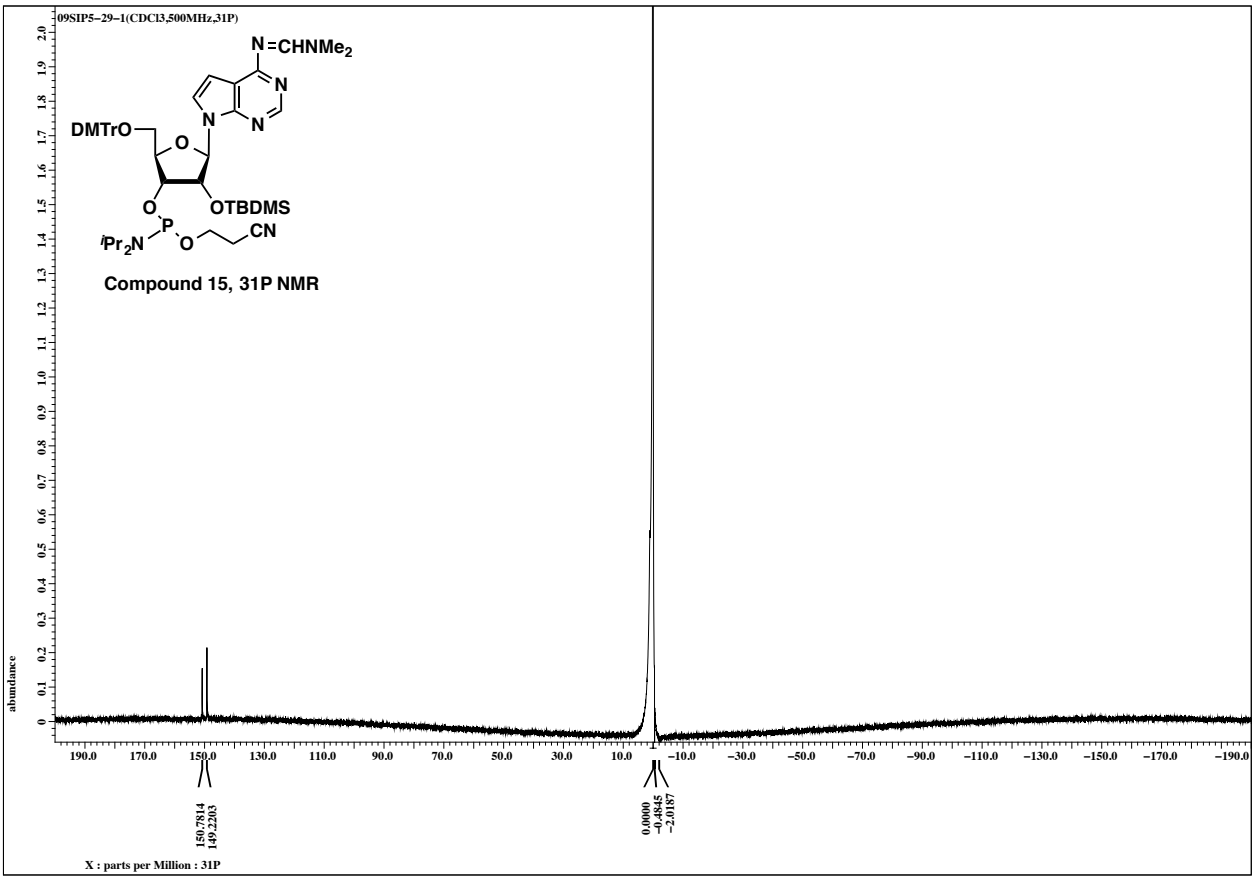
N=CHNMe2
DMTrO
HO
OH
Compound 13, 1H NMR
abundance
X : parts per Million : 1H


09SIP5-22-5(CDCl3,500MHz,13C)
N=CHNMe2
DMTrO
HO
OH
Compound 13, 13C NMR
abundance
X : parts per Million : 13C


09SIP5-27-1(CDCl3,500MHz)
N=CHNMe2
DMTrO
HO
OTBDMS
Compound 14, 1H NMR
abundance
X : parts per Million : 1H


09SIP5-27-1(CDCl3,500MHz,13C)
N=CHNMe2
DMTrO
HO
OTBDMS
Compound 14, 13C NMR
abundance
X : parts per Million : 13C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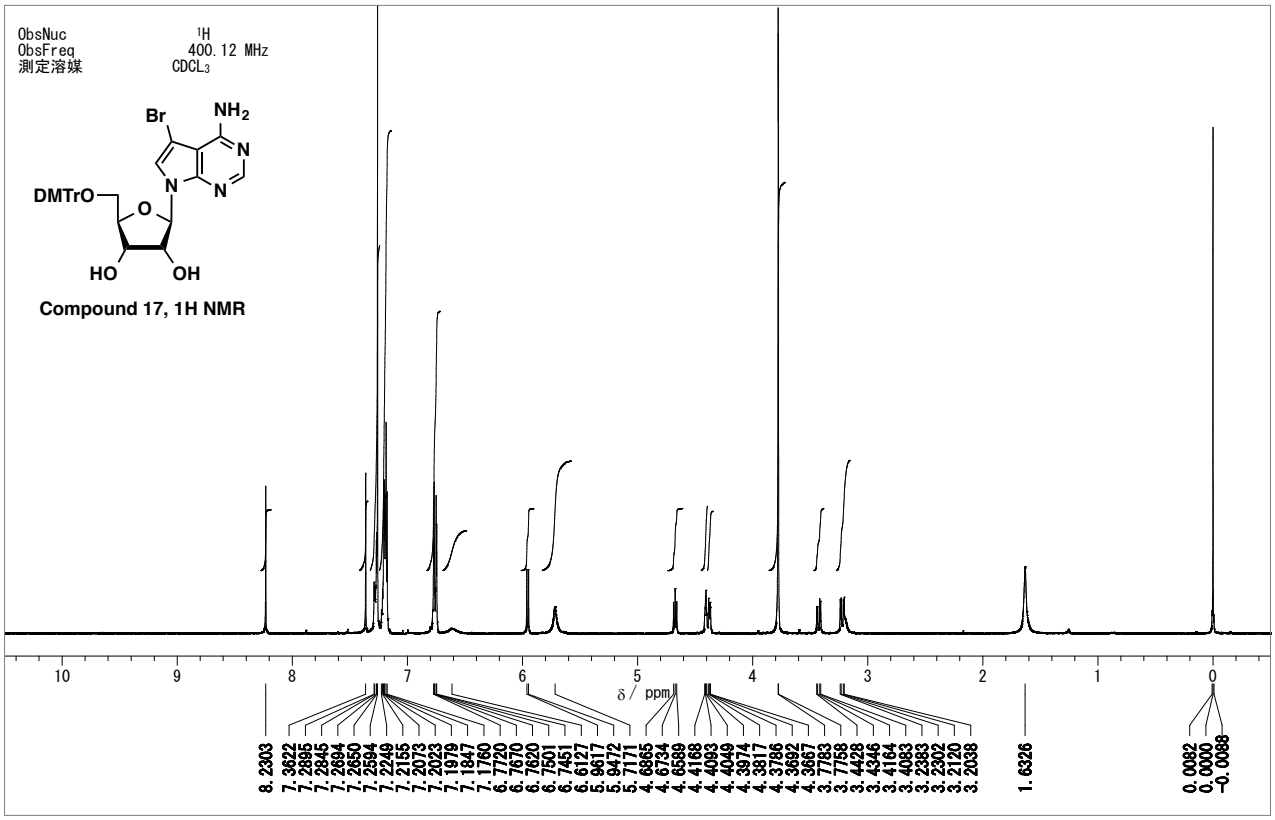
N=CHNMe2
DMTrO
OTBDMS
iPr2N
CN
Compound 15, 31P NMR
abundance
150.7814
149.2203
0.0000
-0.4845
-2.0187
X : parts per Million : 31P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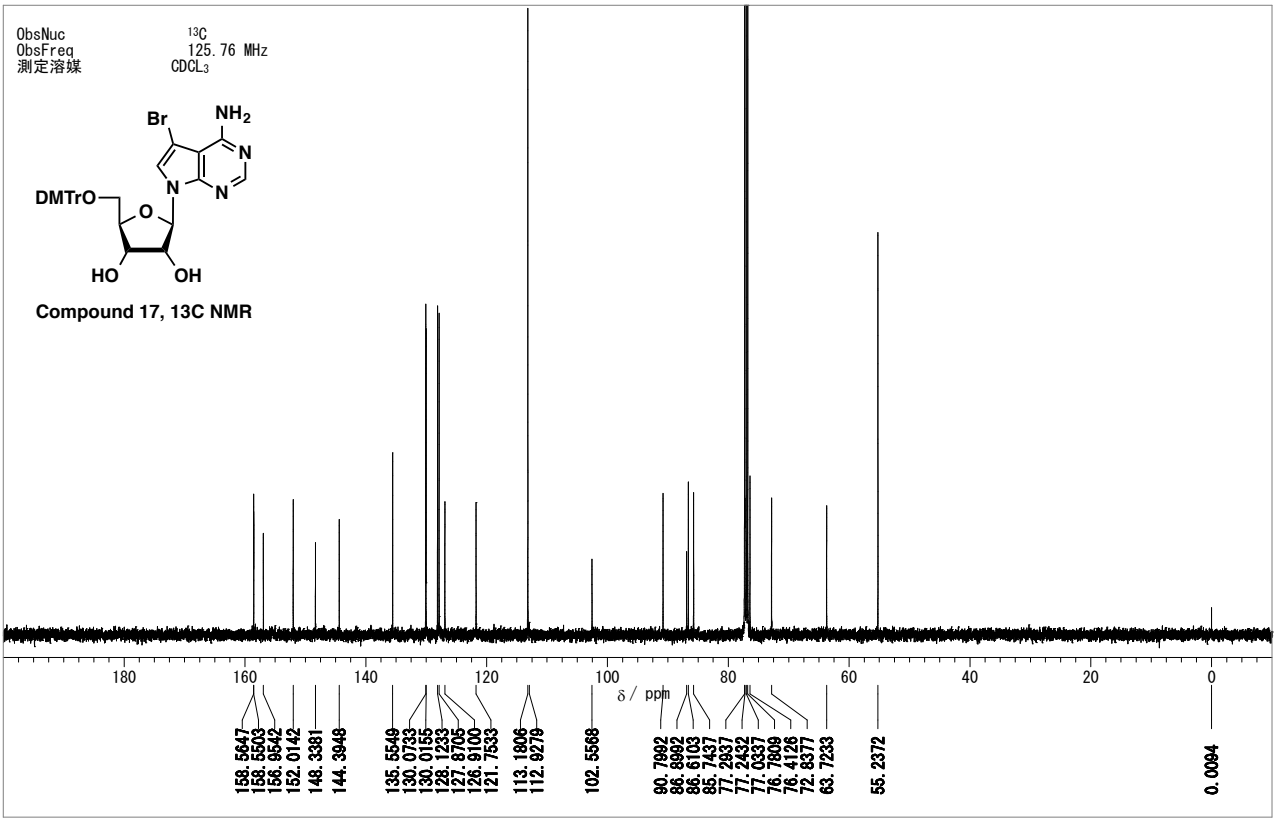
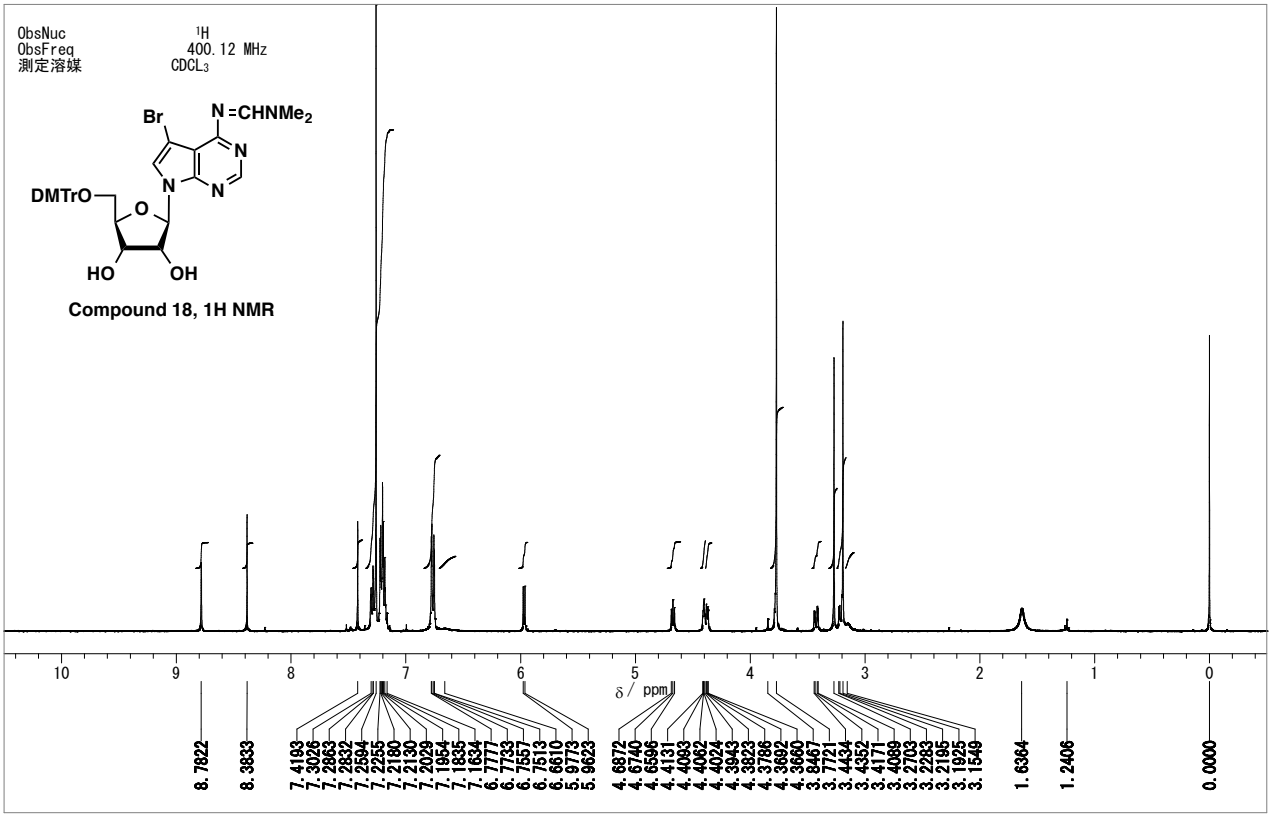
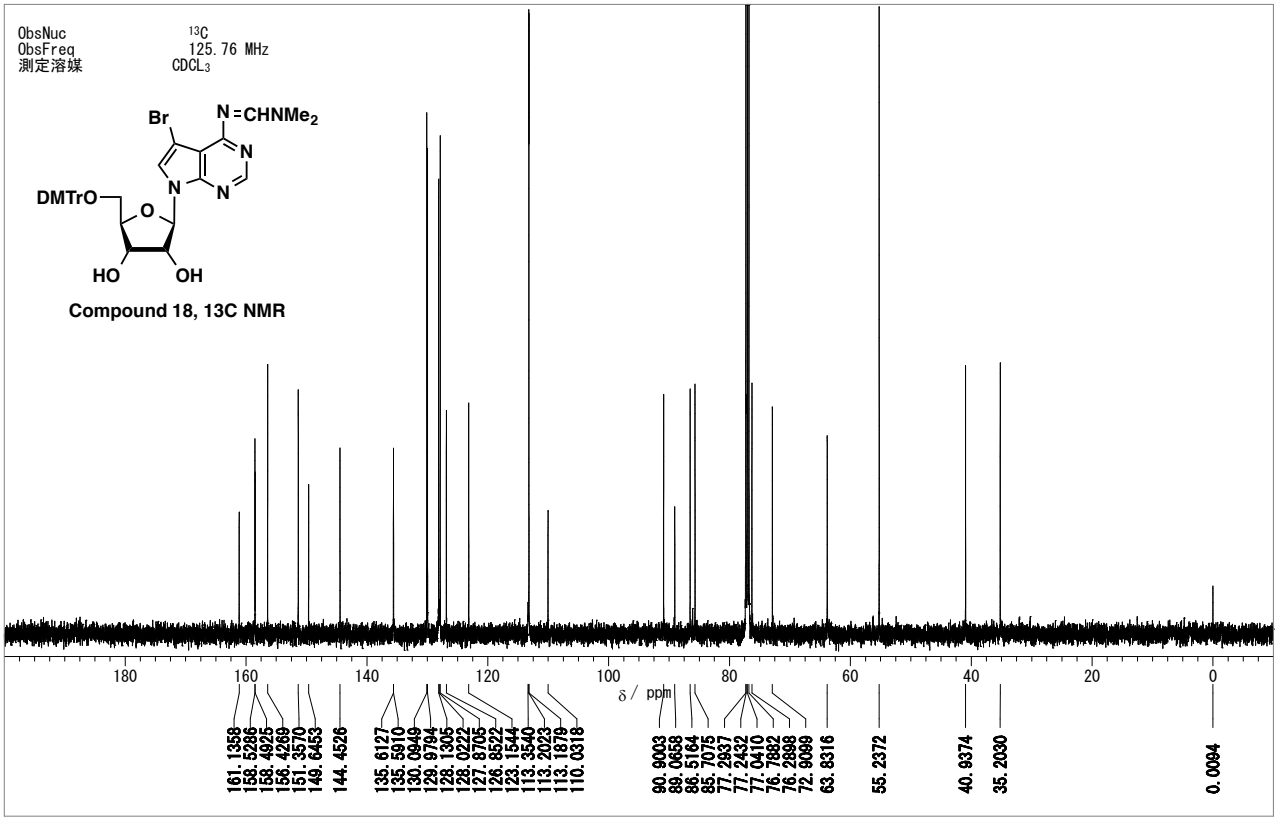
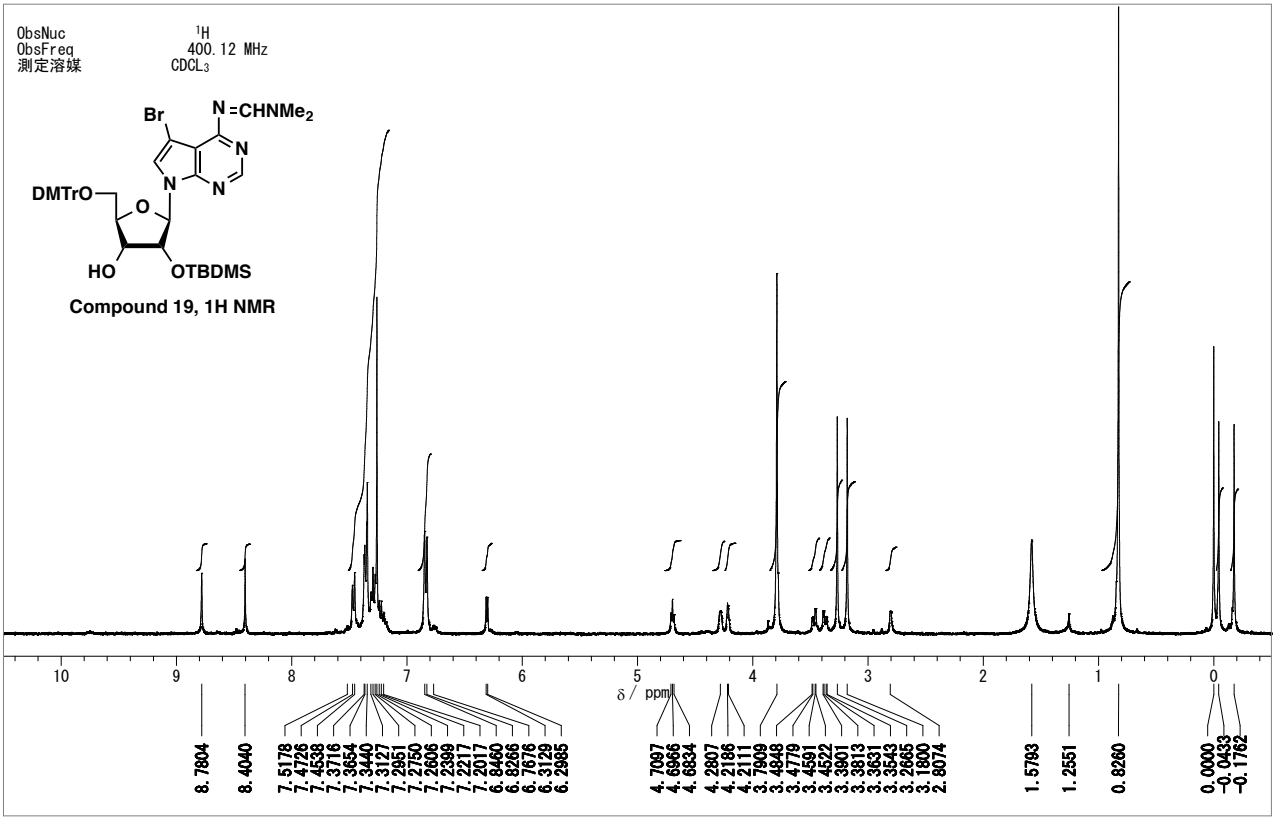
ObsFreq 400.12 MHz
測定溶媒 CDCL3
Br
NH2
DMTrO
HO
OH
Compound 17, 1H NMR
δ / ppm
8.2303
7.3622
7.2895
7.2845
7.2694
7.2650
7.2594
7.2249
7.2155
7.2073
7.2023
7.1979
7.1847
7.1760
6.7720
6.7670
6.7620
6.7501
6.7451
6.6127
5.9617
5.9472
5.7171
4.6865
4.6734
4.6589
4.4168
4.4093
4.4049
4.3974
4.3817
4.3786
4.3692
4.3667
3.7783
3.7758
3.4428
3.4346
3.4164
3.4083
3.2383
3.2302
3.2120
3.2038
1.6326
0.0082
0.0000
-0.0088


ObsNuc 13C
ObsFreq 125.76 MHz
測定溶媒 CDCL3
Br
NH2
N
N
N
N
DMTrO
O
HO
OH
Compound 17, 13C NMR
δ / ppm
158.5647
158.5503
156.9542
152.0142
148.3381
144.3948
135.5549
130.0733
130.0155
128.1233
127.8705
126.9100
121.7533
113.1806
112.9279
102.5568
90.7992
86.8892
86.6103
85.7437
77.2937
77.2432
77.0337
76.7809
76.4126
72.8377
63.7233
55.2372
0.0094


ObsNuc 1H
ObsFreq 400.12 MHz
測定溶媒 CDCL3
Br
N=CHNMe2
N
N
N
N
DMTrO
O
HO
OH
Compound 18, 1H NMR
δ / ppm
8.7822
8.3833
7.4193
7.3026
7.2863
7.2832
7.2594
7.2255
7.2180
7.2130
7.2029
7.1954
7.1835
7.1634
6.7777
6.7733
6.7557
6.7513
6.6610
5.9773
5.9623
4.6872
4.6740
4.6596
4.4131
4.4083
4.4062
4.4024
4.3943
4.3823
4.3786
4.3692
4.3660
3.8467
3.7721
3.4434
3.4352
3.4171
3.4089
3.2703
3.2283
3.2195
3.1925
3.1549
1.6384
1.2406
0.0000


ObsNuc 13C
ObsFreq 125.76 MHz
測定溶媒 CDCL3
Br
N=CHNMe2
N
N
N
DMTrO
O
HO
OH
Compound 18, 13C NMR
δ / ppm
161.1358
158.5286
158.4925
156.4269
151.3570
149.6453
144.4526
135.6127
135.5910
130.0949
129.9794
128.1305
128.0222
127.8705
126.8522
123.1544
113.3540
113.2023
113.1879
110.0318
90.9003
89.0658
86.5104
85.7075
77.2837
77.2432
77.0410
76.7882
76.2898
72.9099
63.8316
55.2372
40.9374
35.2030
0.0094


ObsNuc 1H
ObsFreq 400.12 MHz
測定溶媒 CDCL3
Br
N=CHNMe2
N
N
N
DMTrO
O
HO
OTBDMS
Compound 19, 1H NMR
δ / ppm
8.7804
8.4040
7.5178
7.4726
7.4538
7.3716
7.3654
7.3440
7.3127
7.2851
7.2750
7.2606
7.2399
7.2217
7.2017
6.8460
6.8266
6.7676
6.3129
6.2985
4.7097
4.6966
4.6834
4.2807
4.2186
4.2111
3.7909
3.4848
3.4779
3.4591
3.4522
3.3901
3.3813
3.3631
3.3543
3.2665
3.1800
2.8074
1.5783
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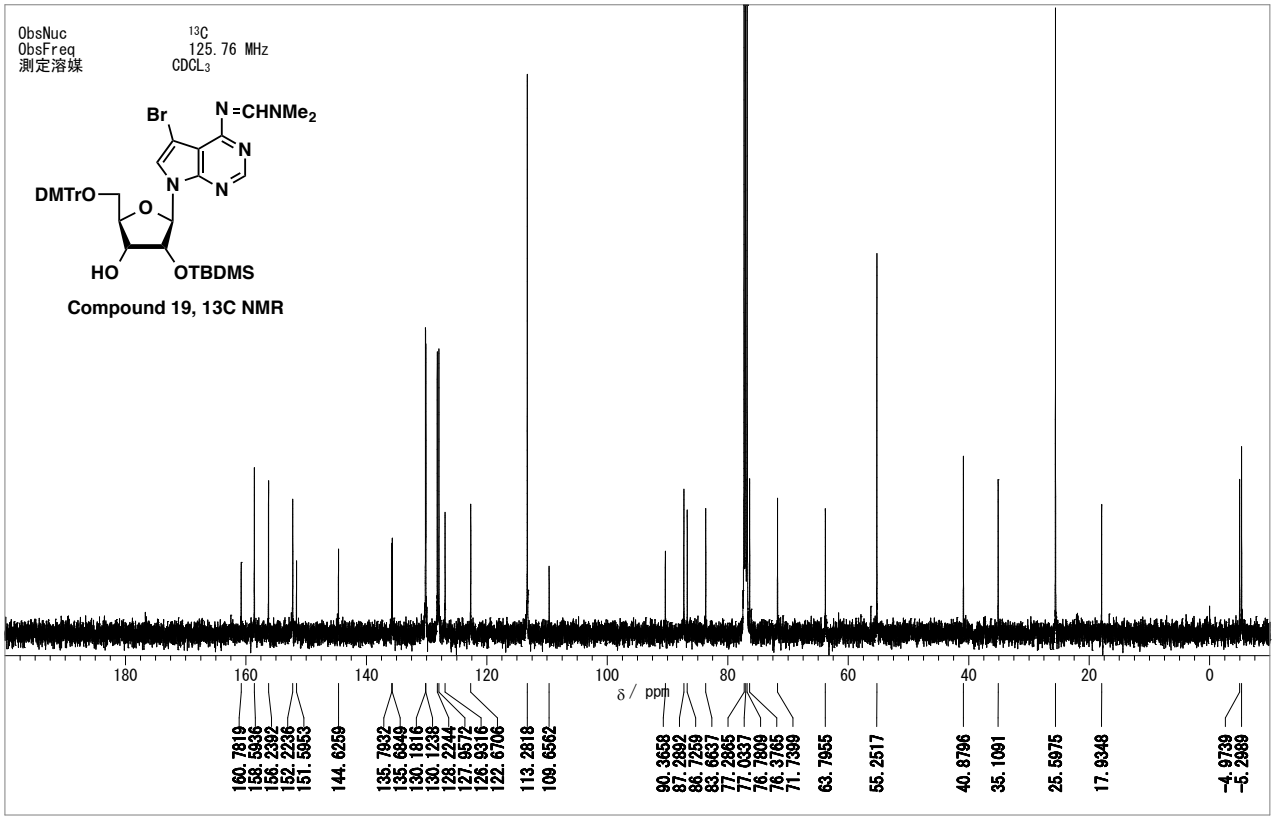
0.0000
-0.0433
-0.1782


ObsNuc 13C
ObsFreq 125.76 MHz
測定溶媒 CDCL3
Br
N=CHNMe2
N
N
N
DMTrO
O
HO
OTBDMS
Compound 19, 13C NMR
δ / ppm
160.7819
158.5936
156.2392
152.2236
151.5953
144.6259
135.7932
135.6849
130.1816
130.1238
128.2244
127.9572
126.9316
122.6706
113.2818
109.6562
90.3658
87.2892
86.7259
83.6637
77.2965
77.0337
76.7809
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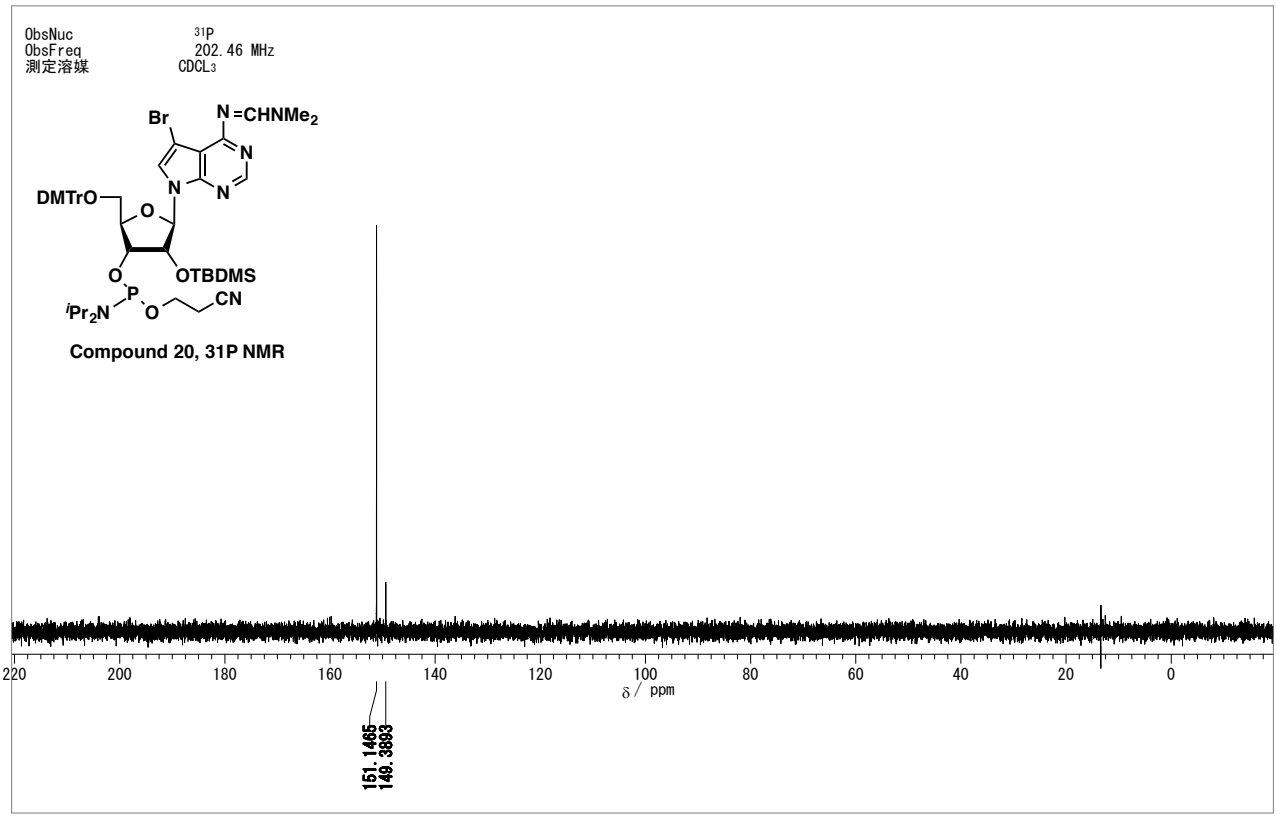
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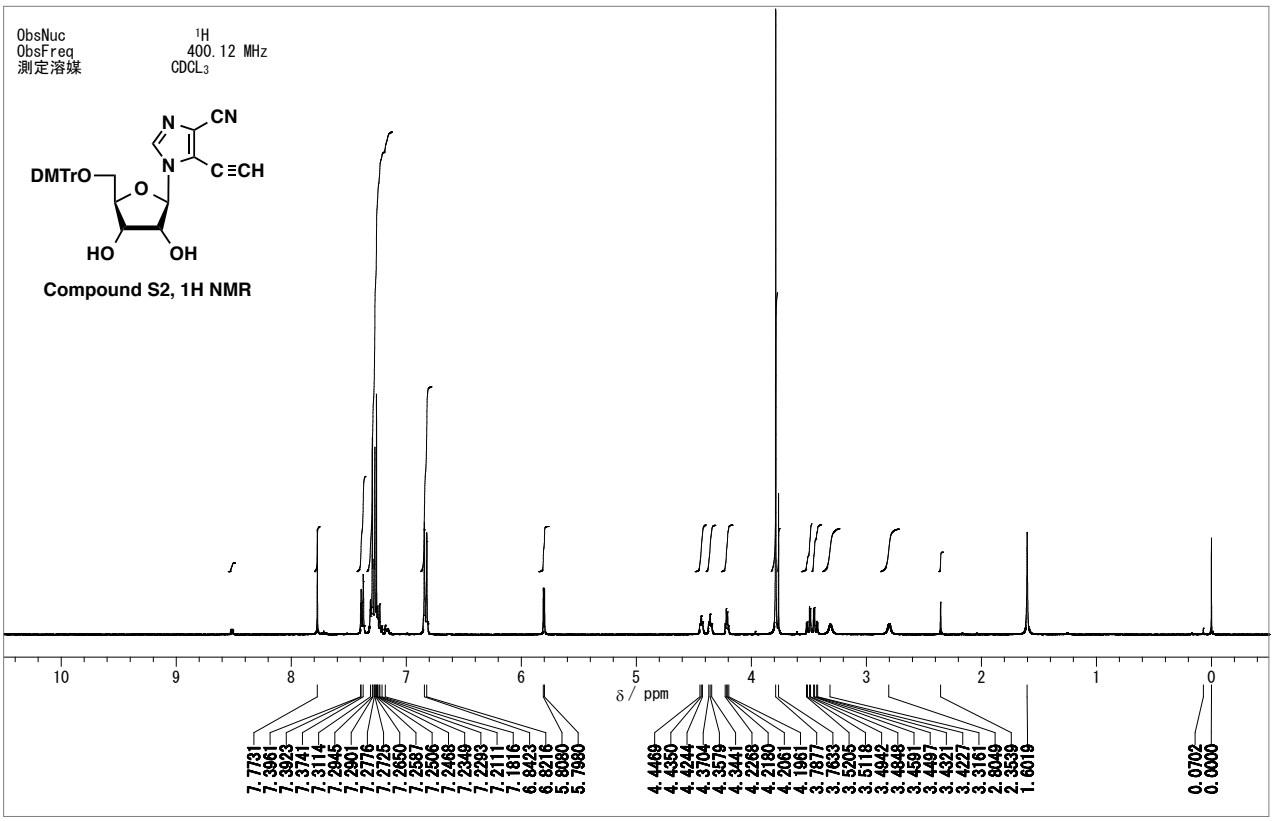
ObsFreq 202.46 MHz
測定溶媒 CDCL3
Br
N=CHNMe2
N
N
N
DMTrO
O
O
OTBDMS
P
iPr2N
O
CN
Compound 20, 31P NMR
δ / ppm
151.1465
149.3883


ObsNuc 1H
ObsFreq 400.12 MHz
測定溶媒 CDCL3
DMTrO
HO
OH
CN
C≡CH
Compound S2, 1H NMR
δ / ppm
7.7731
7.3961
7.3923
7.3741
7.3114
7.2945
7.2901
7.2776
7.2725
7.2650
7.2587
7.2506
7.2468
7.2349
7.2293
7.2111
7.1816
6.8423
6.8216
5.8080
5.7980
4.4469
4.4350
4.4244
4.3704
4.3579
4.3441
4.2268
4.2180
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3.4848
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2.3539
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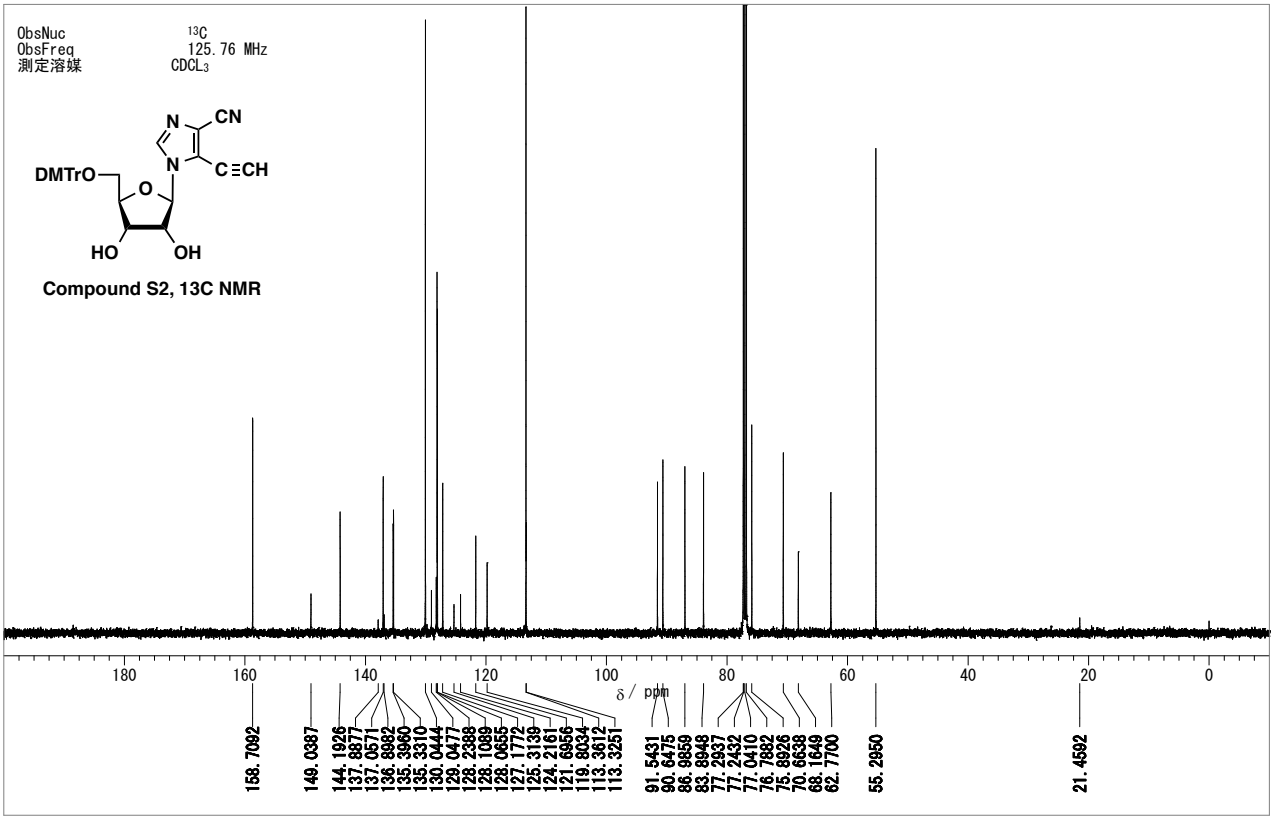
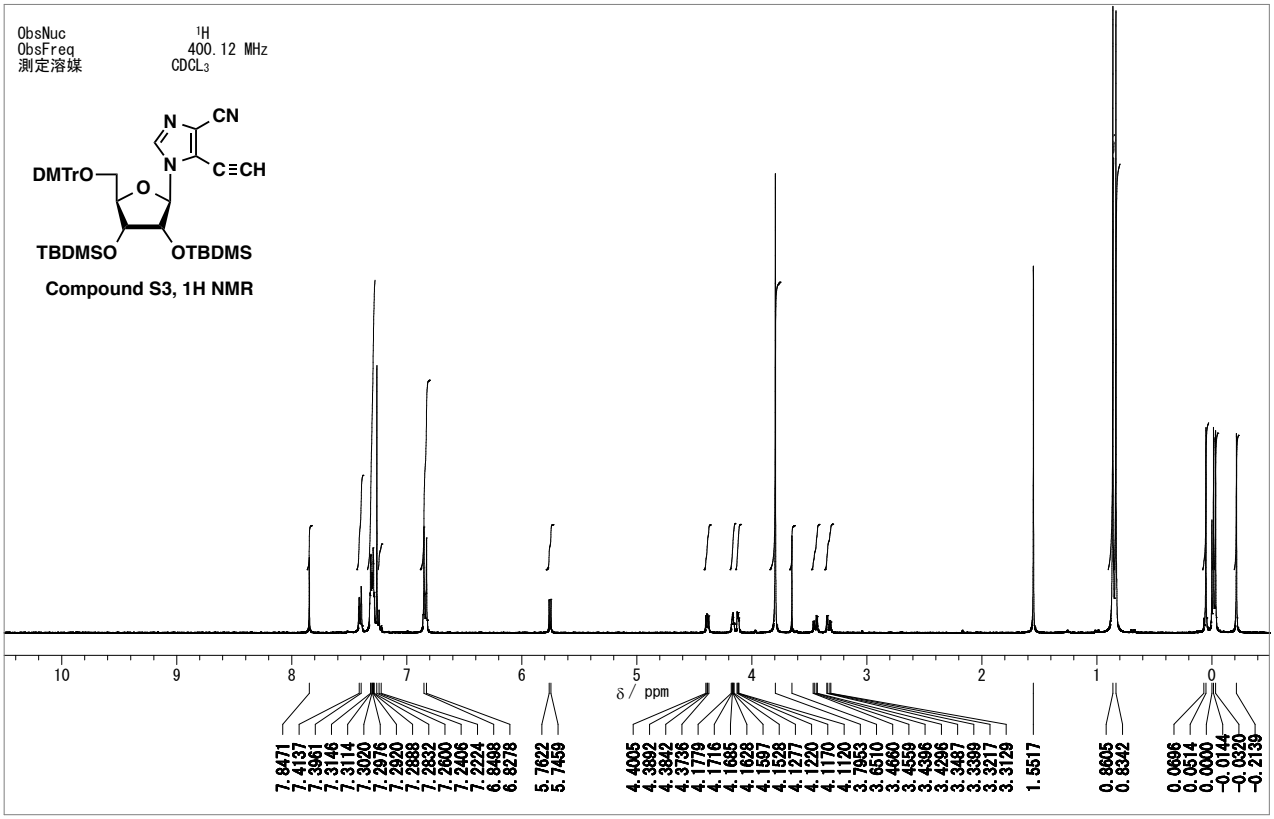
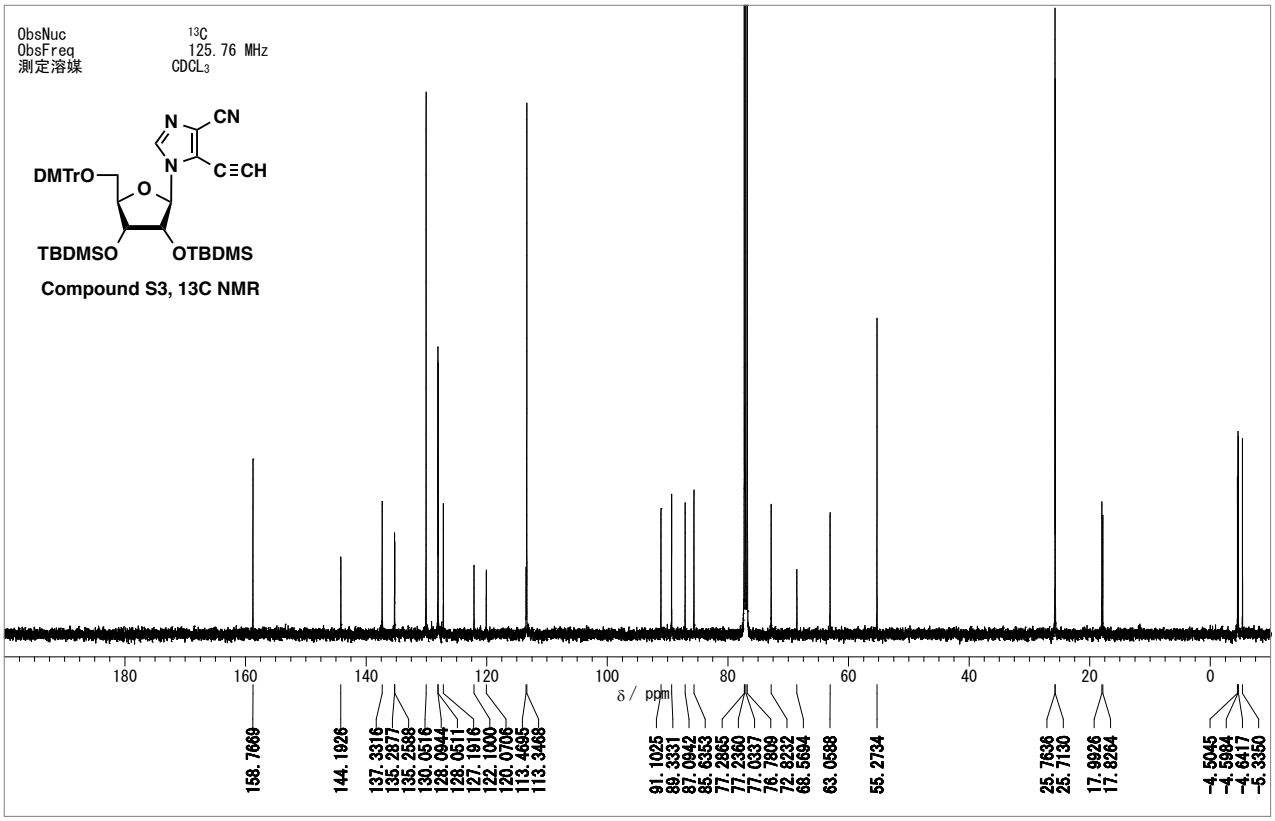
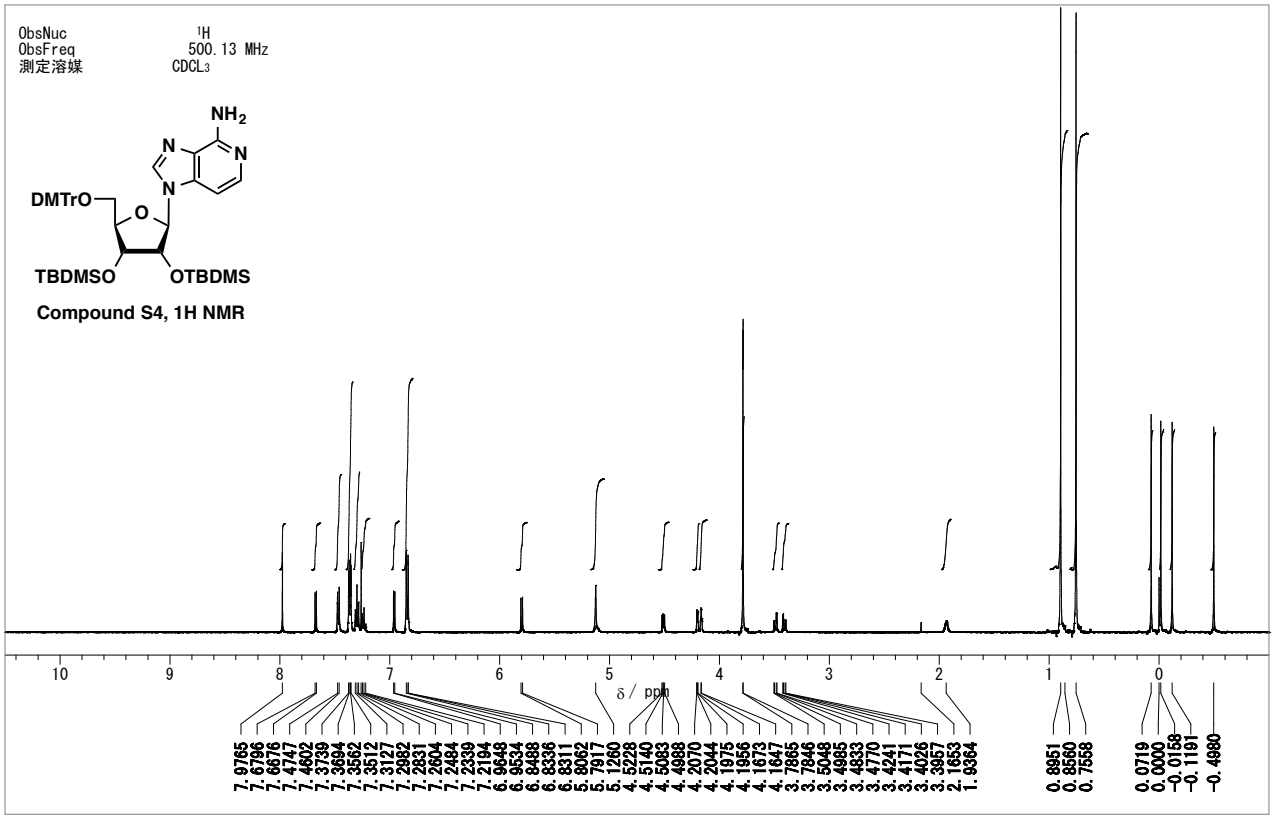
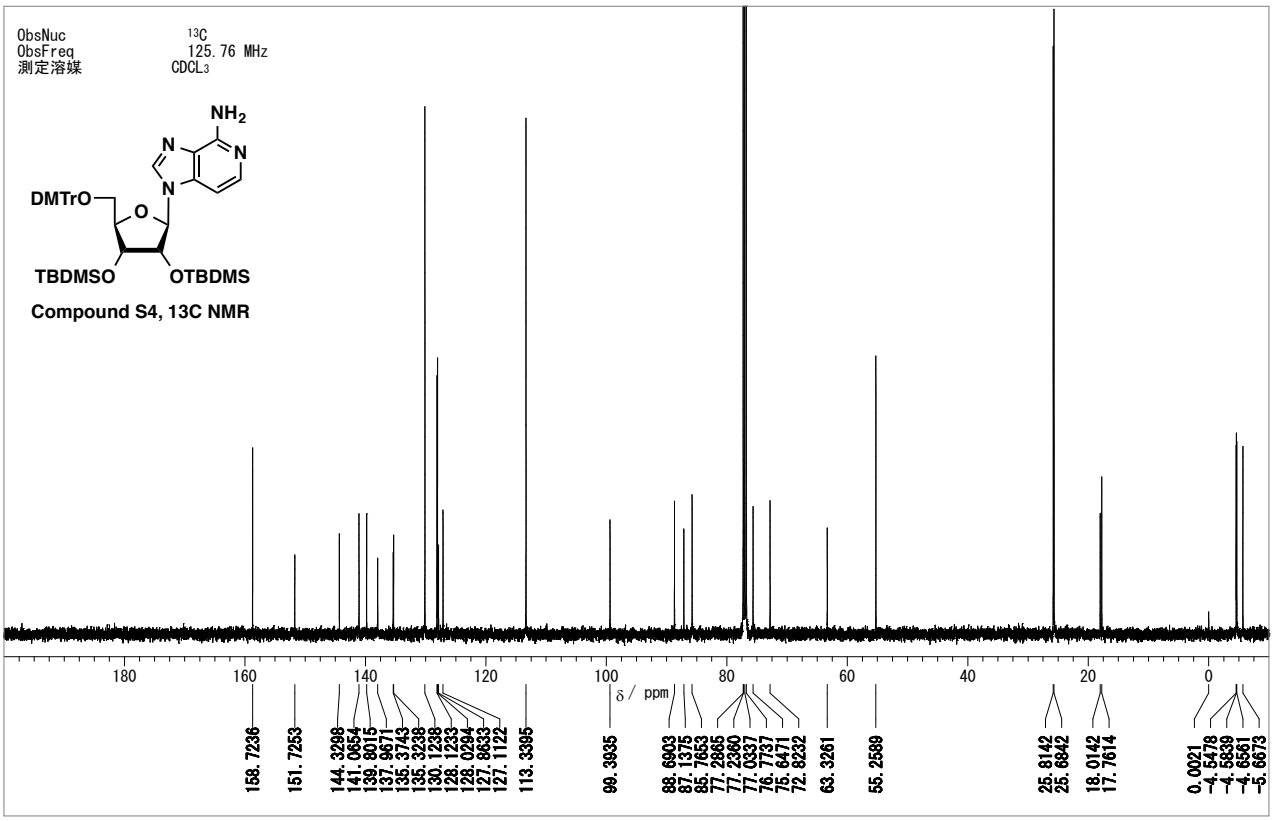
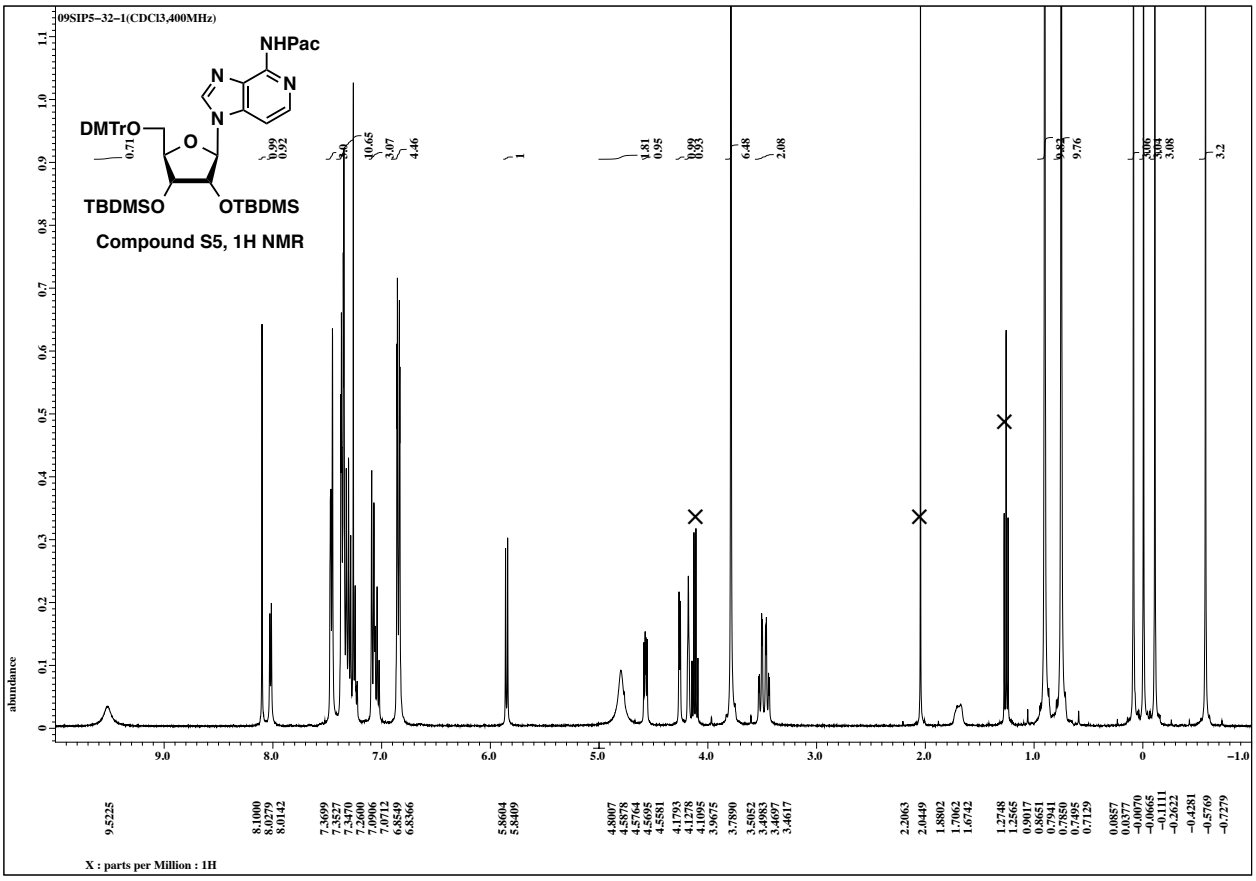

ObsNuc 13C
ObsFreq 125.76 MHz
測定溶媒 CDCL3
DMTrO
HO
OH
CN
C≡CH
Compound S2, 13C NMR
δ / ppm
158.7092
149.0387
144.1926
137.8877
137.0571
136.8982
135.3960
135.3310
130.0444
129.0477
128.2388
128.1089
128.0655
127.1772
126.3139
124.2161
121.6956
119.8034
113.3612
113.3251
91.5431
90.6475
86.9859
83.8948
77.2937
77.2432
77.0410
76.7882
75.8926
70.6638
68.1649
62.7700
55.2950
21.4592

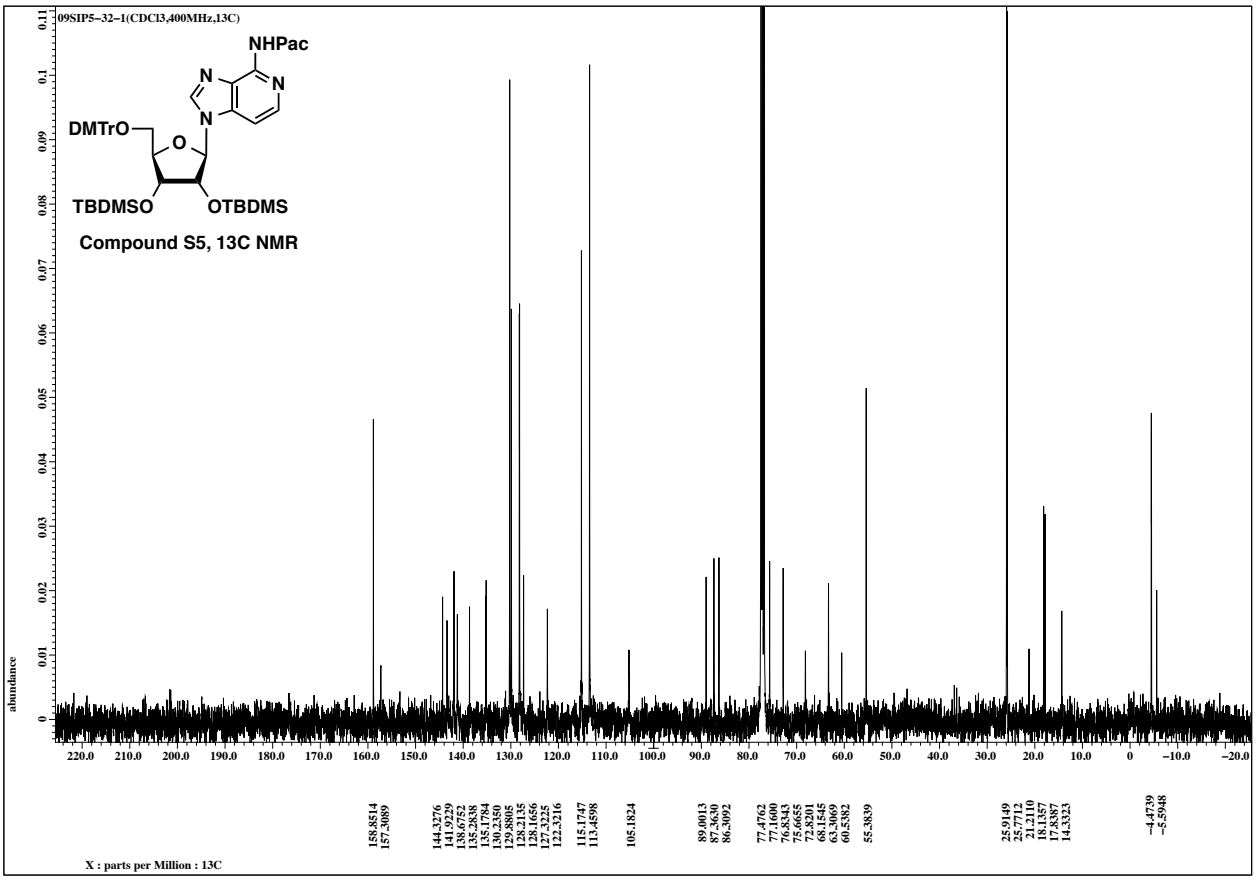

ObsNuc 1H
ObsFreq 400.12 MHz
測定溶媒 CDCL3
DMTrO
TBDMSO
OTBDMS
CN
C≡CH
Compound S3, 1H NMR
δ / ppm
7.8471
7.4137
7.3961
7.3146
7.3114
7.3020
7.2976
7.2920
7.2888
7.2832
7.2600
7.2406
7.2224
6.8498
6.8278
5.7622
5.7459
4.4005
4.3892
4.3842
4.3736
4.1779
4.1716
4.1685
4.1628
4.1597
4.1528
4.1277
4.1220
4.1170
4.1120
3.7953
3.6510
3.4660
3.4559
3.4396
3.4296
3.3487
3.3399
3.3217
3.3129
1.5517
0.8605
0.8342
0.0696
0.0514
0.0000
-0.0144
-0.0320
-0.2139

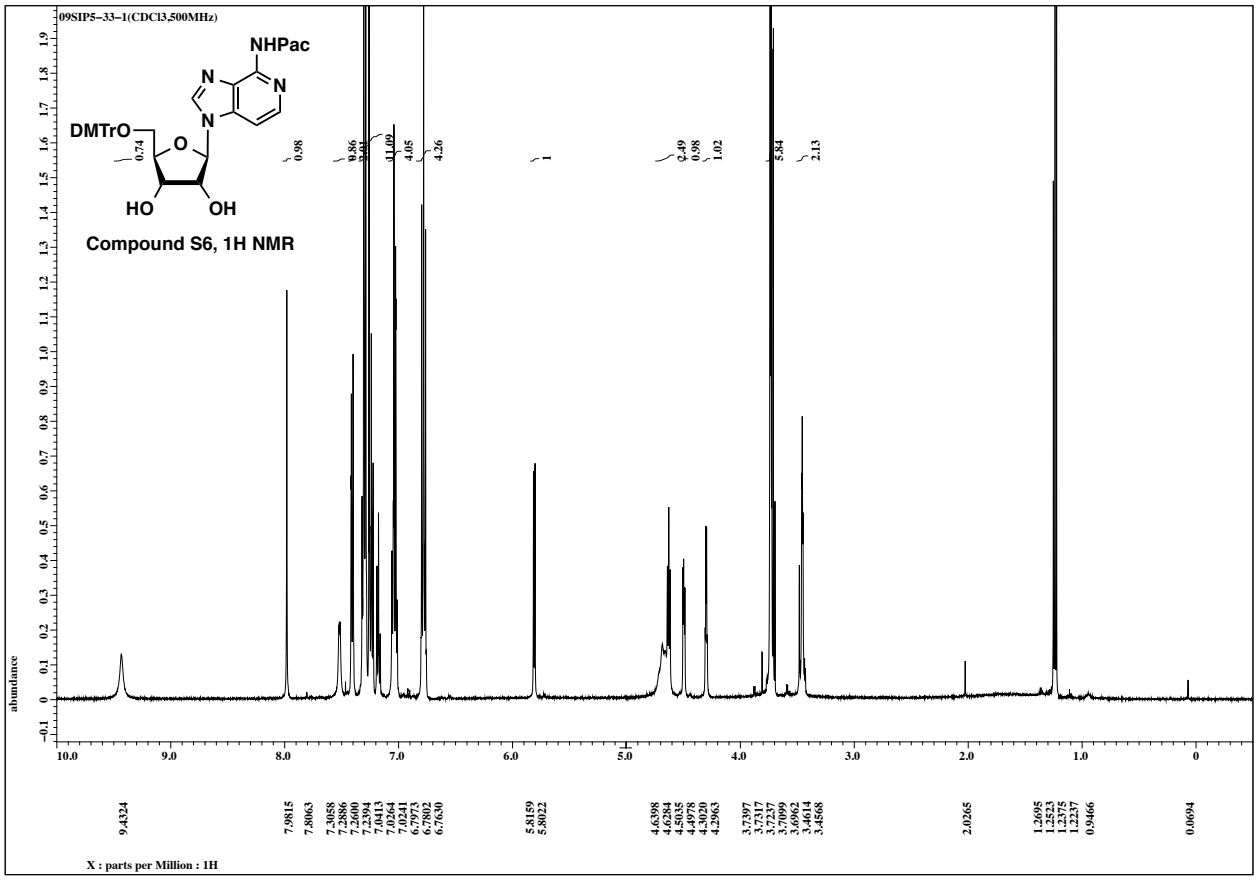

ObsNuc 13C
ObsFreq 125.76 MHz
測定溶媒 CDCL3
DMTrO
TBDMSO
OTBDMS
CN
C≡CH
Compound S3, 13C NMR
δ / ppm
158.7669
144.1926
137.3316
135.2877
135.2588
130.0516
128.0944
128.0511
127.1916
122.1000
120.0706
113.4695
113.3468
91.1025
89.3331
87.0942
85.6353
77.2865
77.2360
77.0337
76.7809
72.8232
68.5694
63.0588
55.2734
25.7636
25.7130
17.9926
17.8264
-4.5045
-4.5984
-4.6417
-5.3350

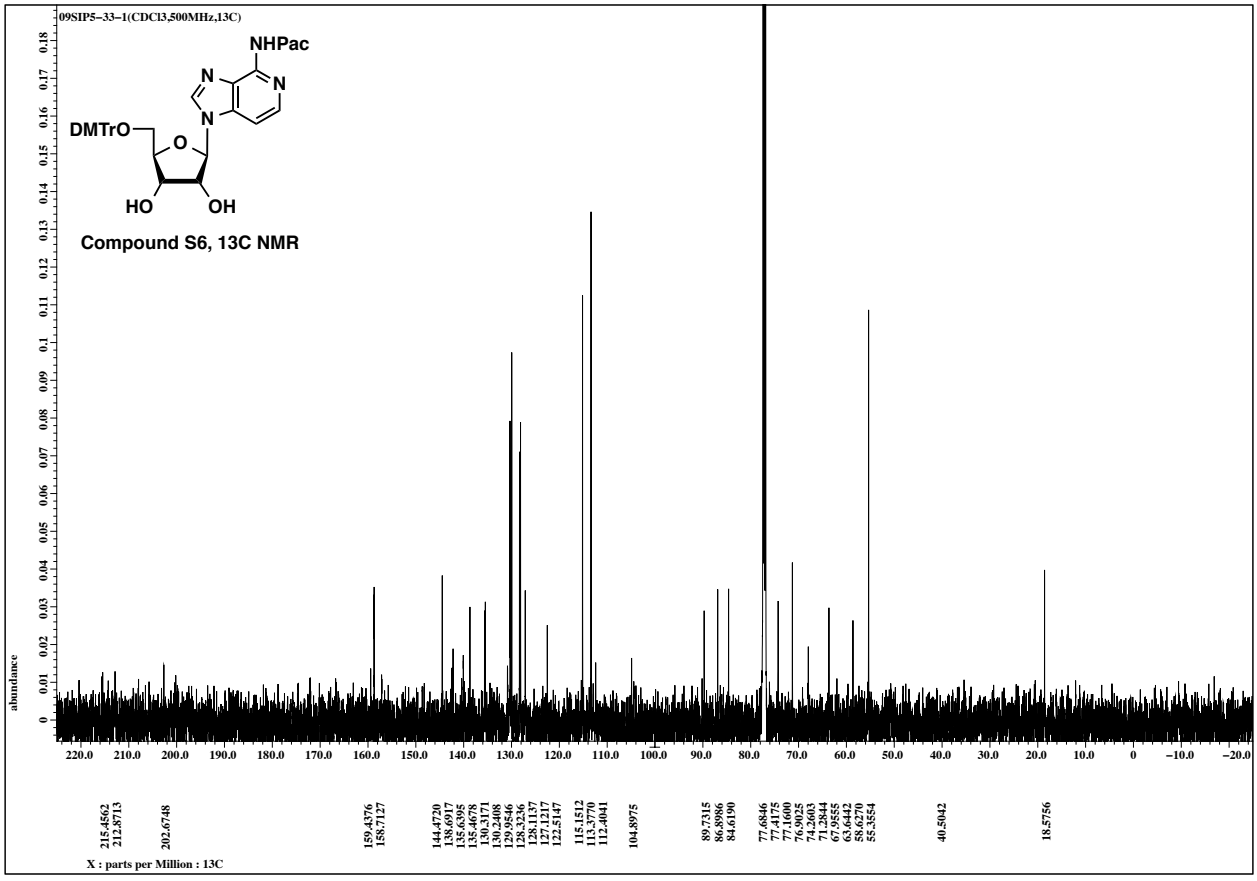

ObsNuc 1H
ObsFreq 500.13 MHz
測定溶媒 CDCL3
NH2
DMTrO
TBDMSO
OTBDMS
Compound S4, 1H NMR
δ / ppm
7.9765
7.6796
7.6676
7.4747
7.4602
7.3739
7.3694
7.3562
7.3512
7.3127
7.2982
7.2831
7.2604
7.2484
7.2339
7.2194
6.9648
6.9534
6.8488
6.8336
6.8311
6.8062
5.7917
5.1280
4.5228
4.5140
4.5083
4.4988
4.2070
4.2044
4.1975
4.1956
4.1673
4.1647
3.7865
3.7846
3.5048
3.4985
3.4833
3.4770
3.4241
3.4171
3.4026
3.3957
2.1653
1.9364
0.8951
0.8560
0.7558
0.0719
0.0000
-0.0158
-0.1191
-0.4980

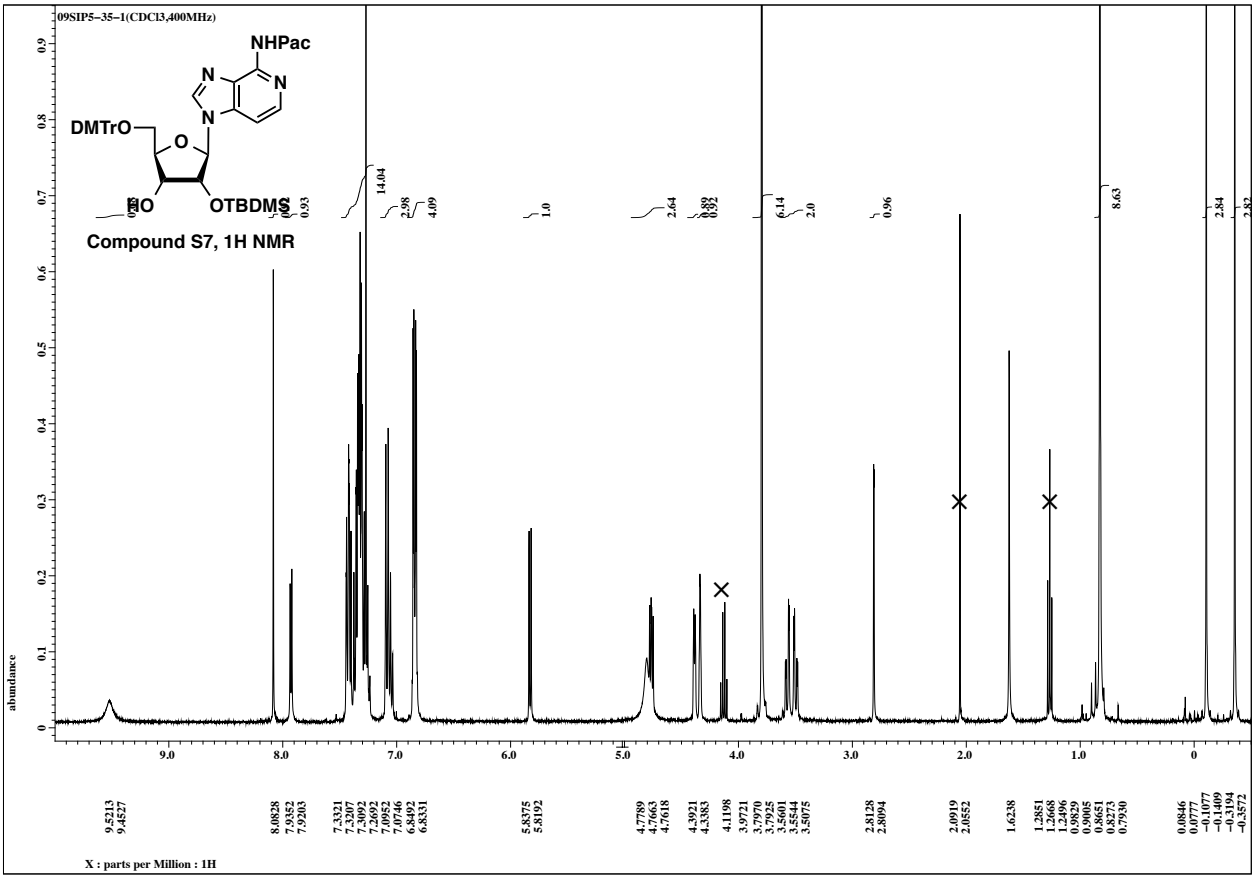

ObsNuc 13C
ObsFreq 125.76 MHz
測定溶媒 CDCL3
NH2
DMTrO
TBDMSO
OTBDMS
Compound S4, 13C NMR
δ / ppm
158.7236
151.7253
144.3298
141.0654
139.8015
137.9671
135.3743
135.3238
130.1238
128.1233
128.0294
127.8633
127.1122
113.3395
90.3935
88.6903
87.1375
85.7653
77.2885
77.2360
77.0337
76.7737
75.6471
72.8232
63.3261
55.2589
25.8142
25.6842
18.0142
17.7614
0.0021
-4.5478
-4.5839
-4.6561
-5.6673

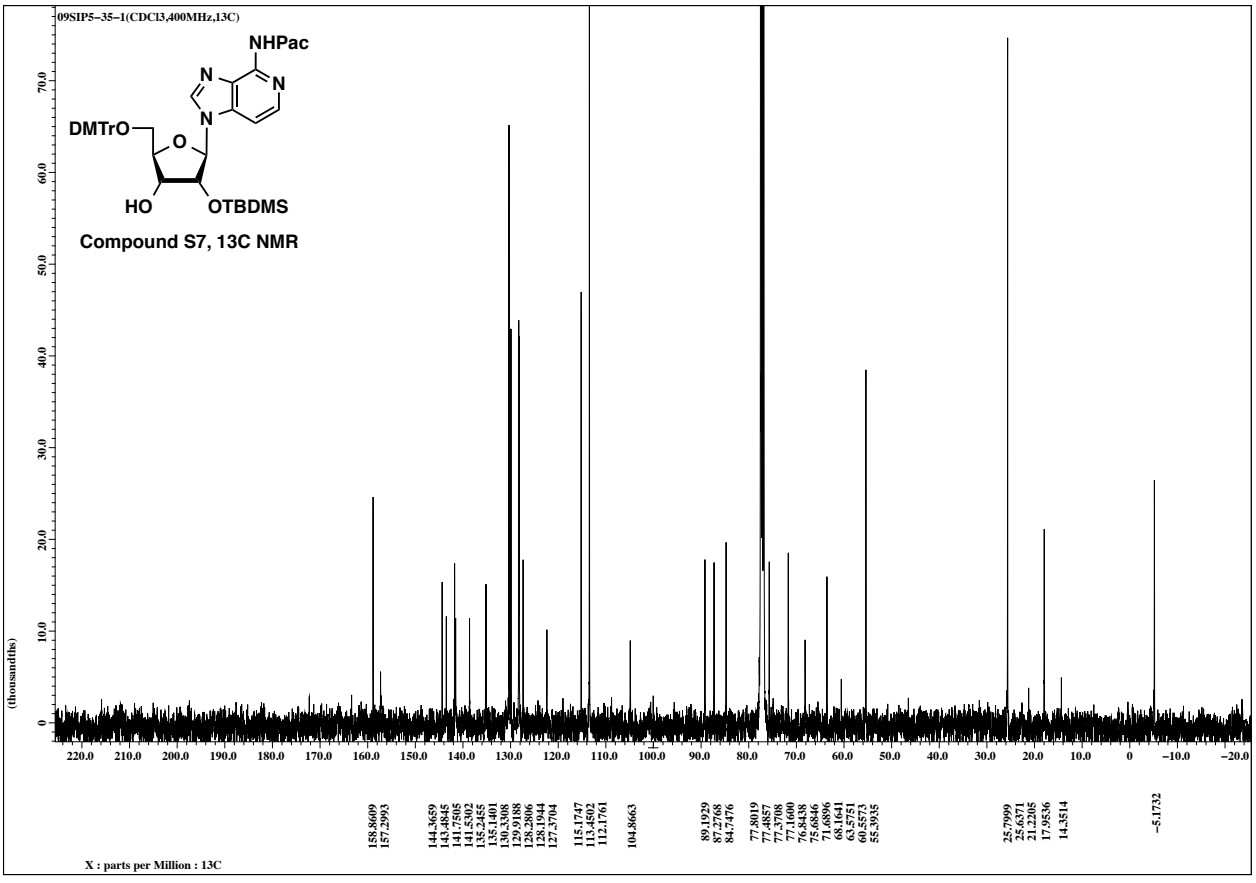

09SIP5-32-1(CDCl3,400MHz)
NHPac
DMTrO
TBDMSO
OTBDMS
Compound S5, 1H NMR
abundance
X : parts per Million : 1H

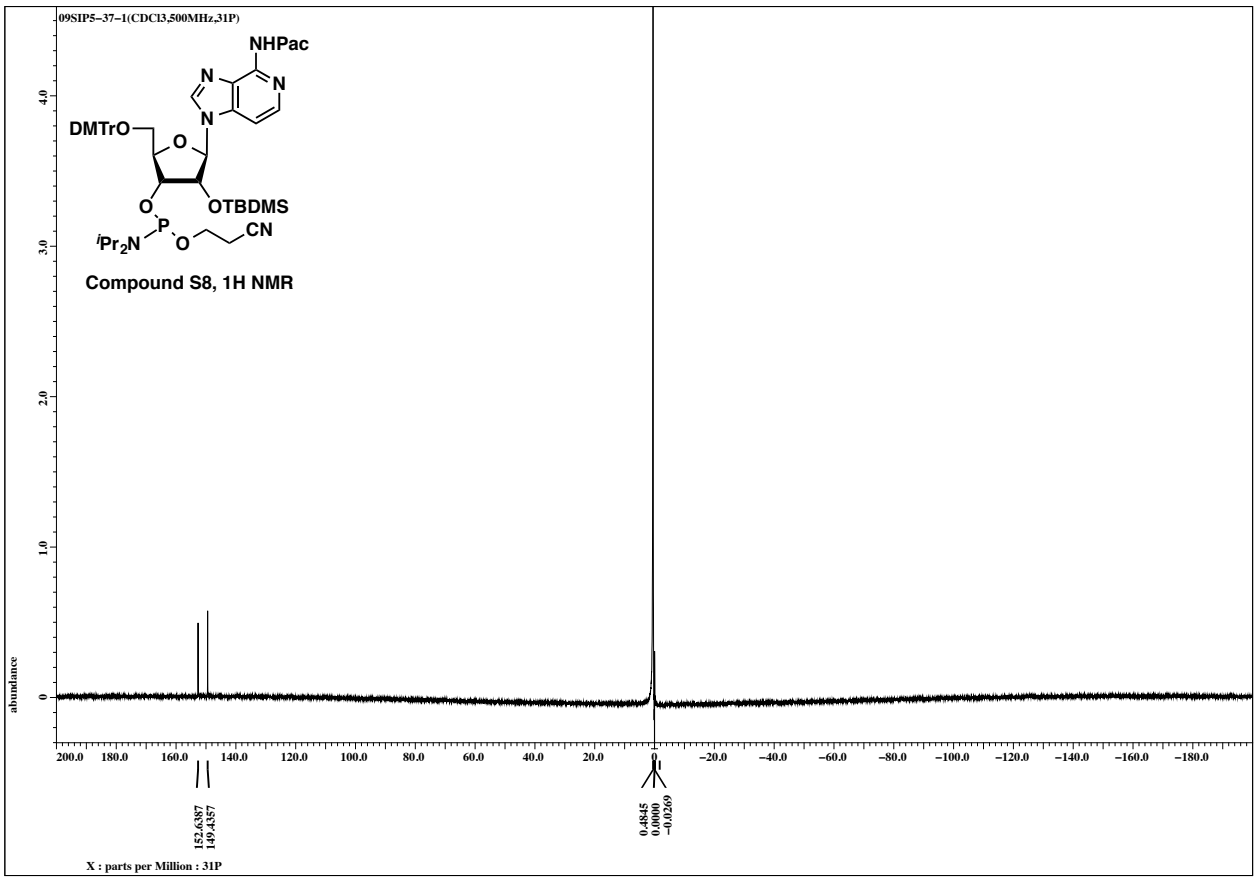

09SIP5-32-1(CDCl3,400MHz,13C)
NHPac
DMTrO
TBDMSO
OTBDMS
Compound S5, 13C NMR
abundance
X : parts per Million : 13C

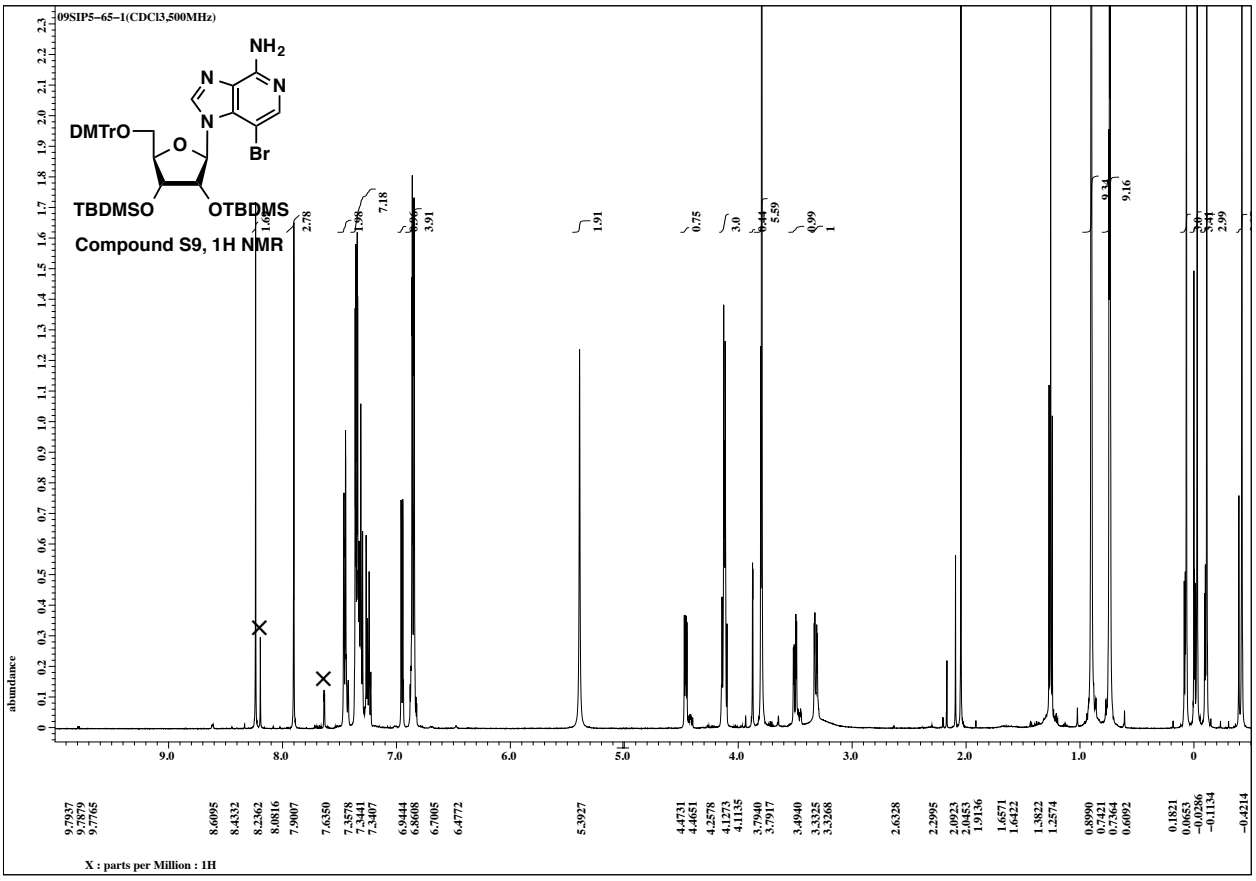

09SIP5-33-1(CDCl3,500MHz)
NHPac
DMTrO
HO
OH
Compound S6, 1H NMR
abundance
X : parts per Million : 1H

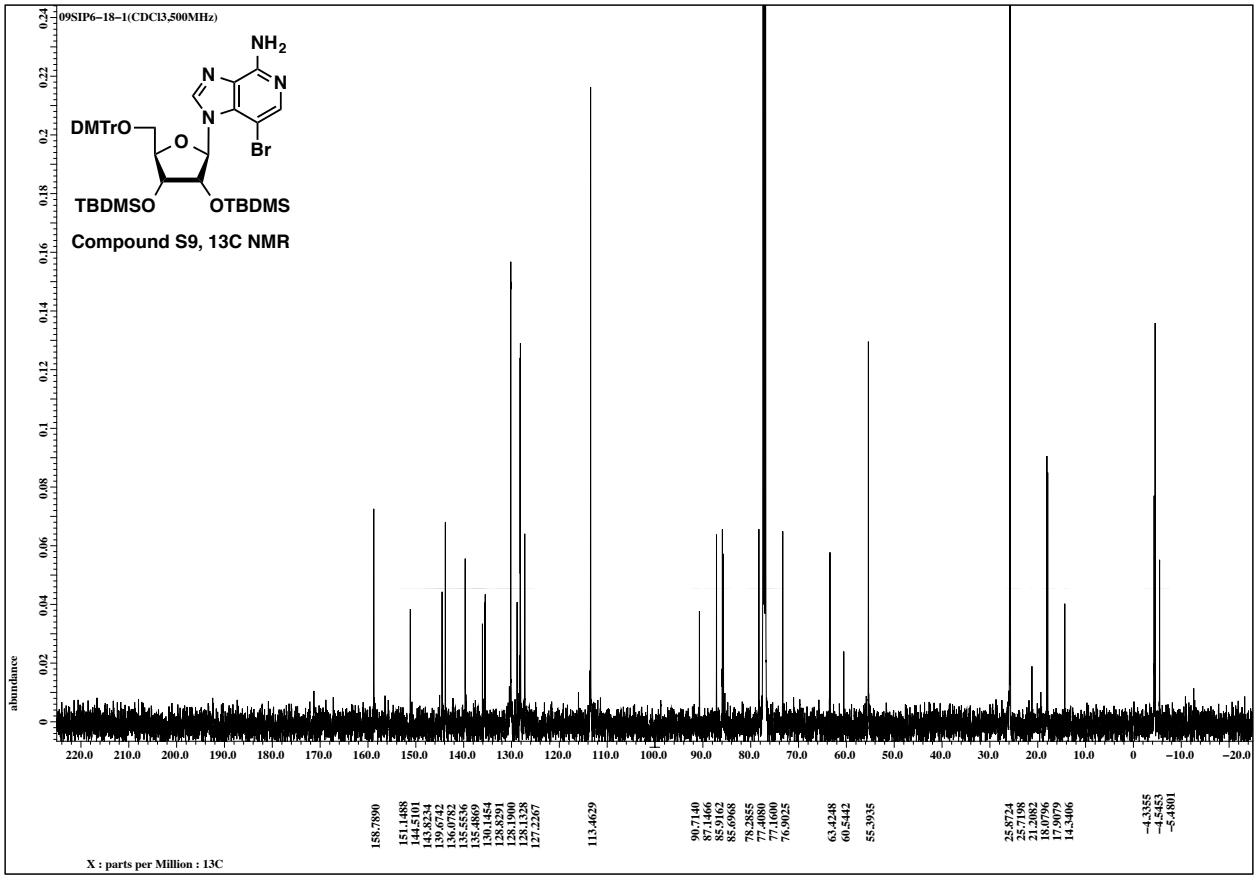

09SIP5-33-1(CDCl3,500MHz,13C)
NHPac
DMTrO
HO
OH
Compound S6, 13C NMR
abundance
X : parts per Million : 13C

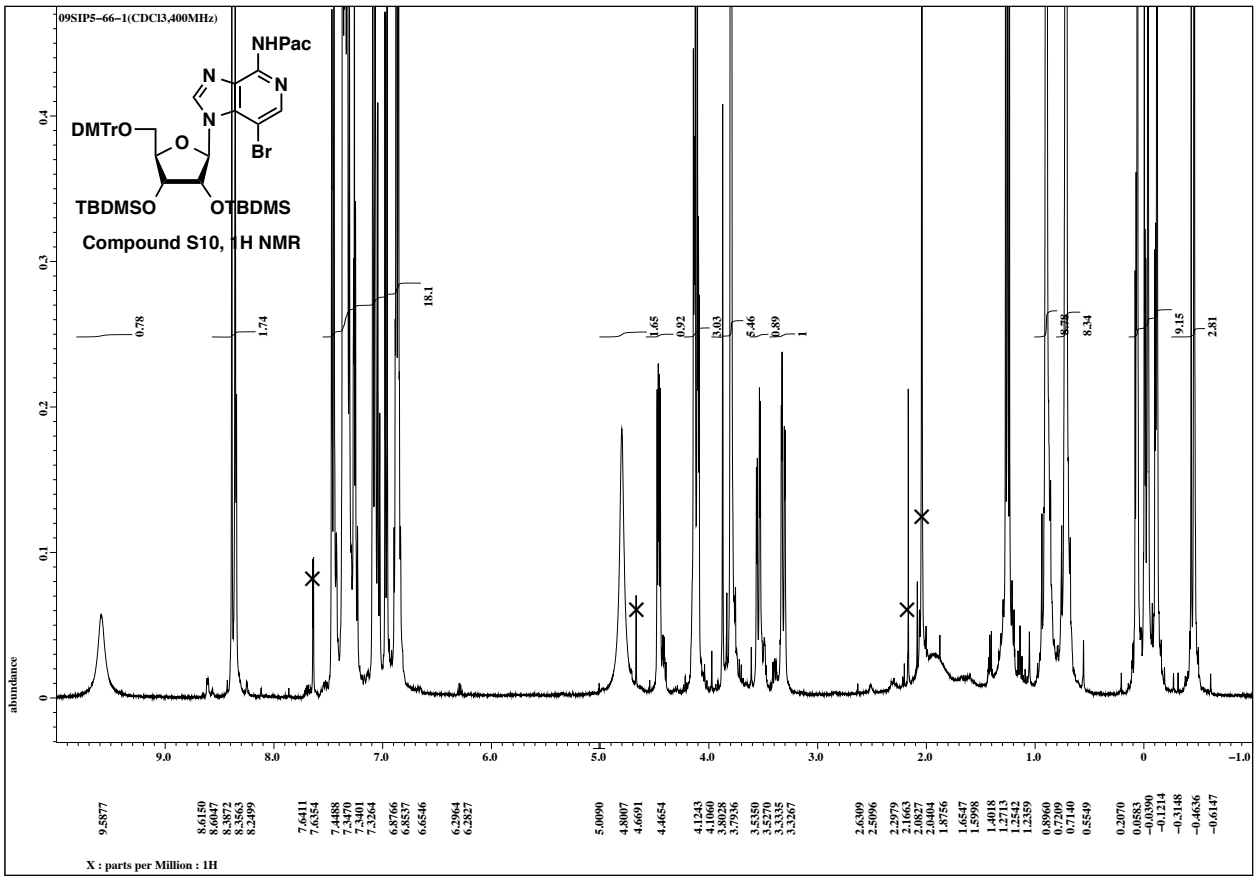

09SIP5-35-1(CDCl3,400MHz)
NHPac
DMTrO
HO
OTBDMS
Compound S7, 1H NMR
X : parts per Million : 1H

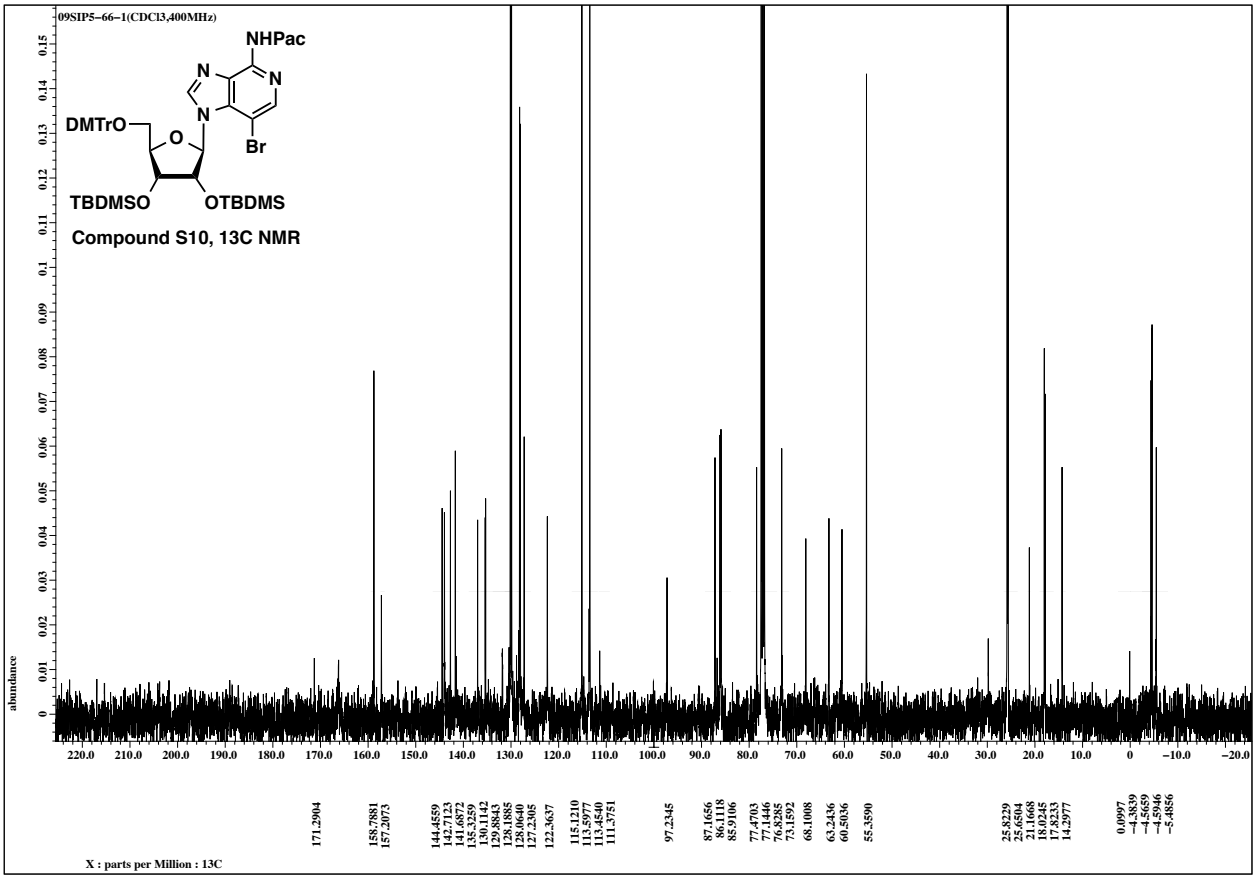

09SIP5-35-1(CDCl3,400MHz,13C)
NHPac
DMTrO
HO
OTBDMS
Compound S7, 13C NMR
X : parts per Million : 13C

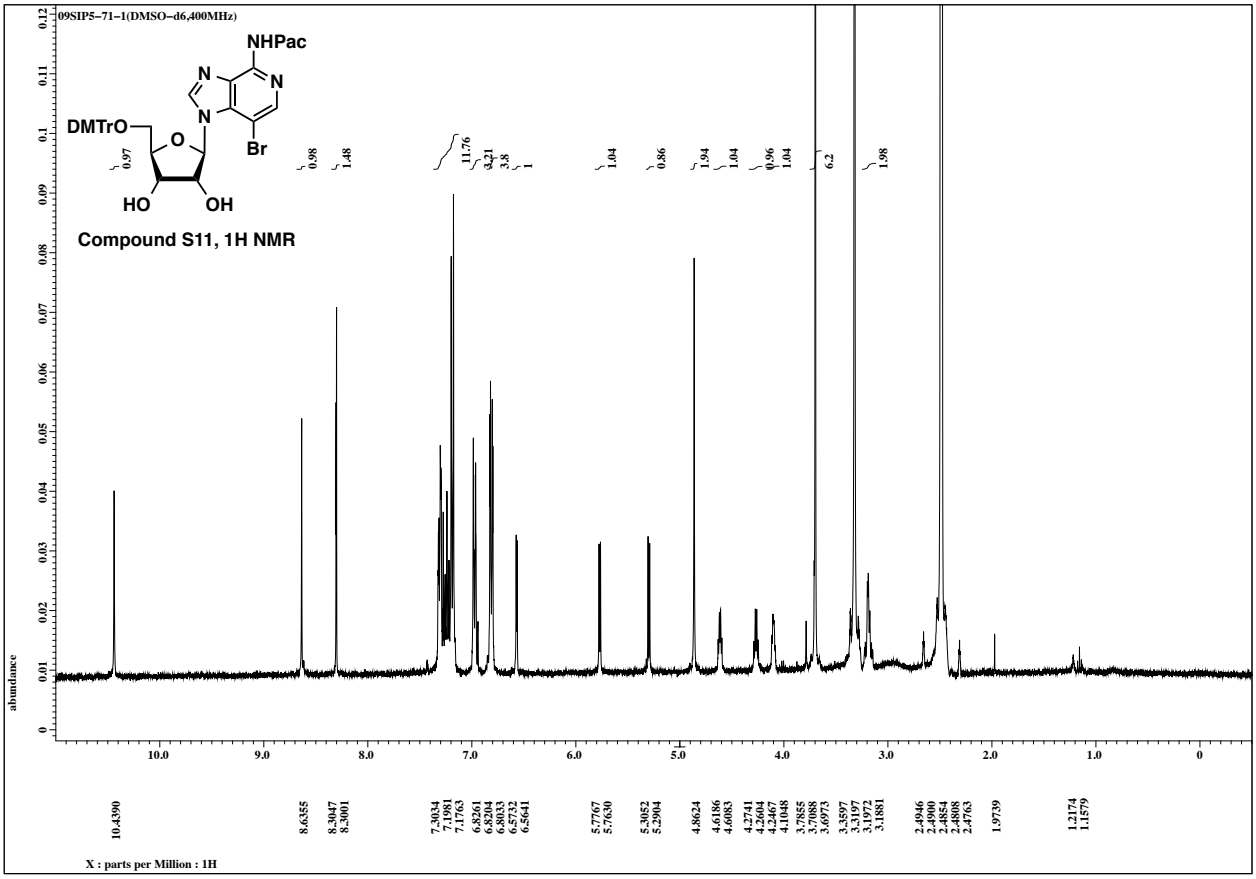

09SIP5-37-1(CDCl3,500MHz,31P)
NHPac
DMTrO
OTBDMS
CN
Compound S8, 1H NMR
abundance
X : parts per Million : 31P

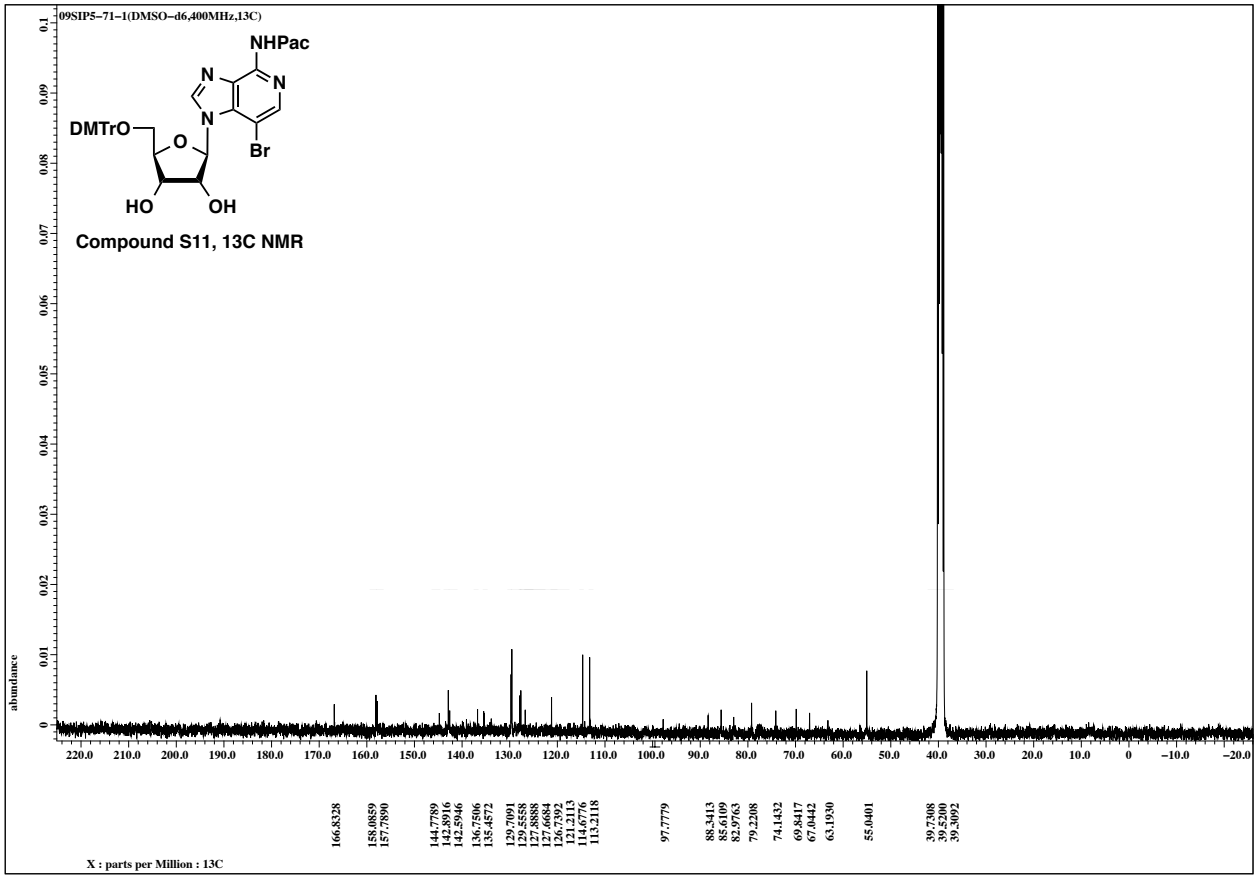

09SIP5-65-1(CDCl3,500MHz)
NH2
DMTrO
Br
TBDMSO
OTBDMS
Compound S9, 1H NMR
abundance
X : parts per Million : 1H

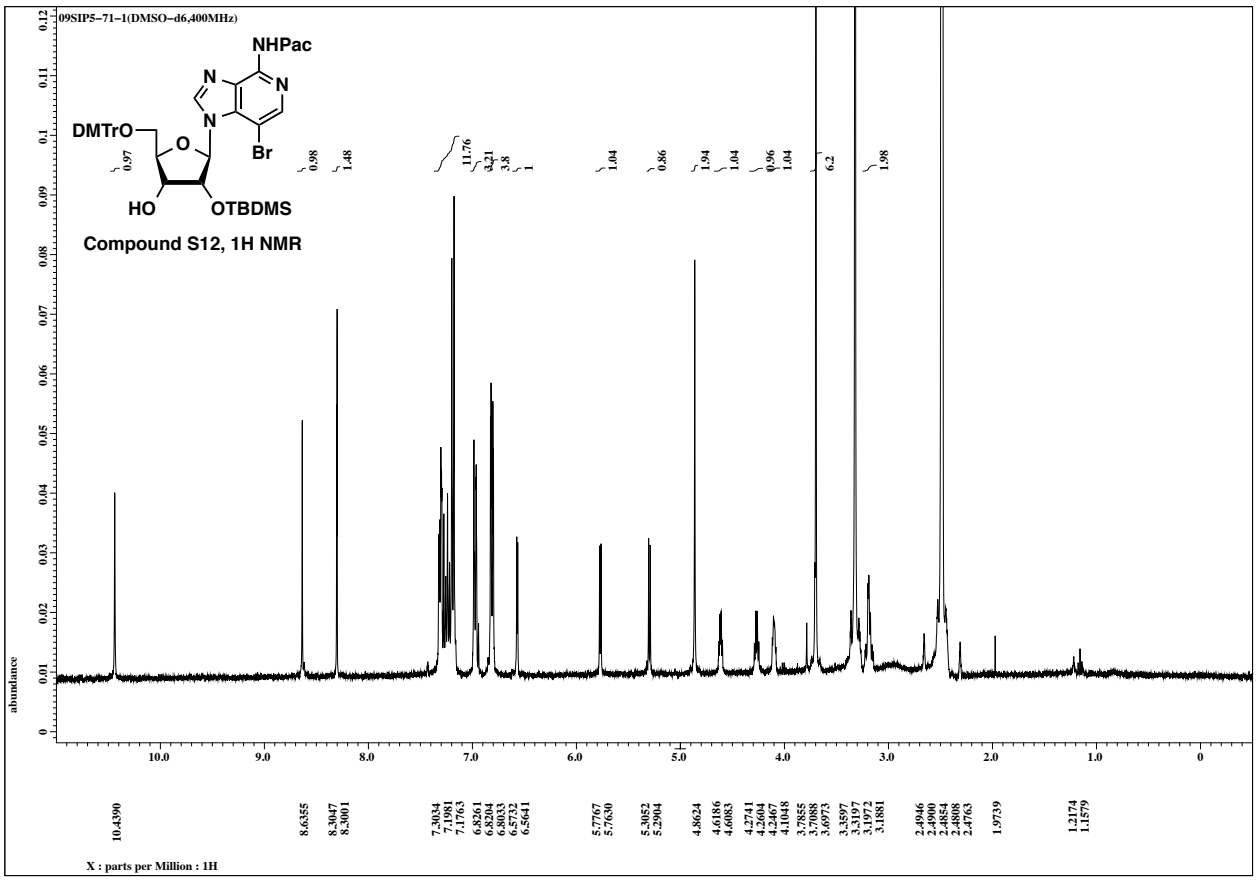

09SIP6-18-1(CDCl3,500MHz)
NH2
N
N
N
DMTrO
O
Br
TBDMSO
OTBDMS
Compound S9, 13C NMR
abundance
158.7890
151.1488
144.5101
143.8234
139.6742
136.0782
135.4556
135.3869
130.1454
128.8291
128.1900
128.1328
127.2267
113.4629
90.7140
87.1466
85.9162
85.6968
78.2855
77.4080
77.1600
76.9025
63.4248
60.5442
55.3935
25.8724
25.7198
21.2082
18.0796
17.9079
14.3406
-4.3355
-4.5453
-5.4801
X : parts per Million : 13C

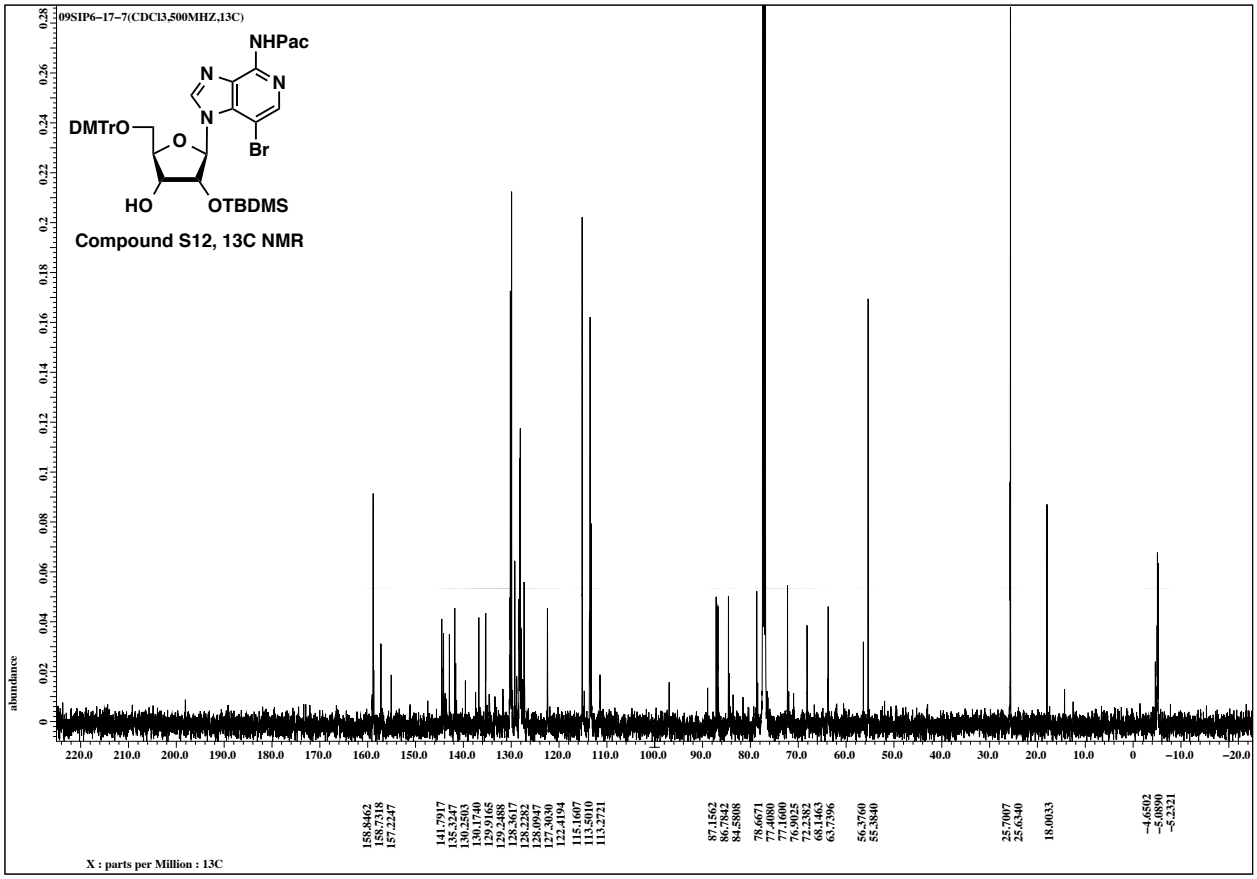

09SIP5-66-1(CDCl3,400MHz)
NHPac
N
N
N
DMTrO
O
Br
TBDMSO
OTBDMS
Compound S10, 1H NMR
abundance
0.78
1.74
18.1
1.65
0.92
3.03
5.46
0.89
1
9.15
2.81
9.5877
8.6150
8.6047
8.3872
8.3563
8.2499
7.6411
7.6354
7.4488
7.3470
7.3401
7.3264
6.8766
6.8537
6.6546
6.2964
6.2827
5.0090
4.8907
4.6691
4.4654
4.1243
4.1060
3.9038
3.7936
3.5350
3.5270
3.3335
3.3267
2.6309
2.5096
2.2979
2.1663
2.0827
2.0044
1.8756
1.6547
1.5998
1.4018
1.2713
1.2543
1.2359
0.8960
0.7709
0.7140
0.5549
0.2070
0.0583
-0.0390
-0.1214
-0.3148
-0.4636
-0.6147
X : parts per Million : 1H

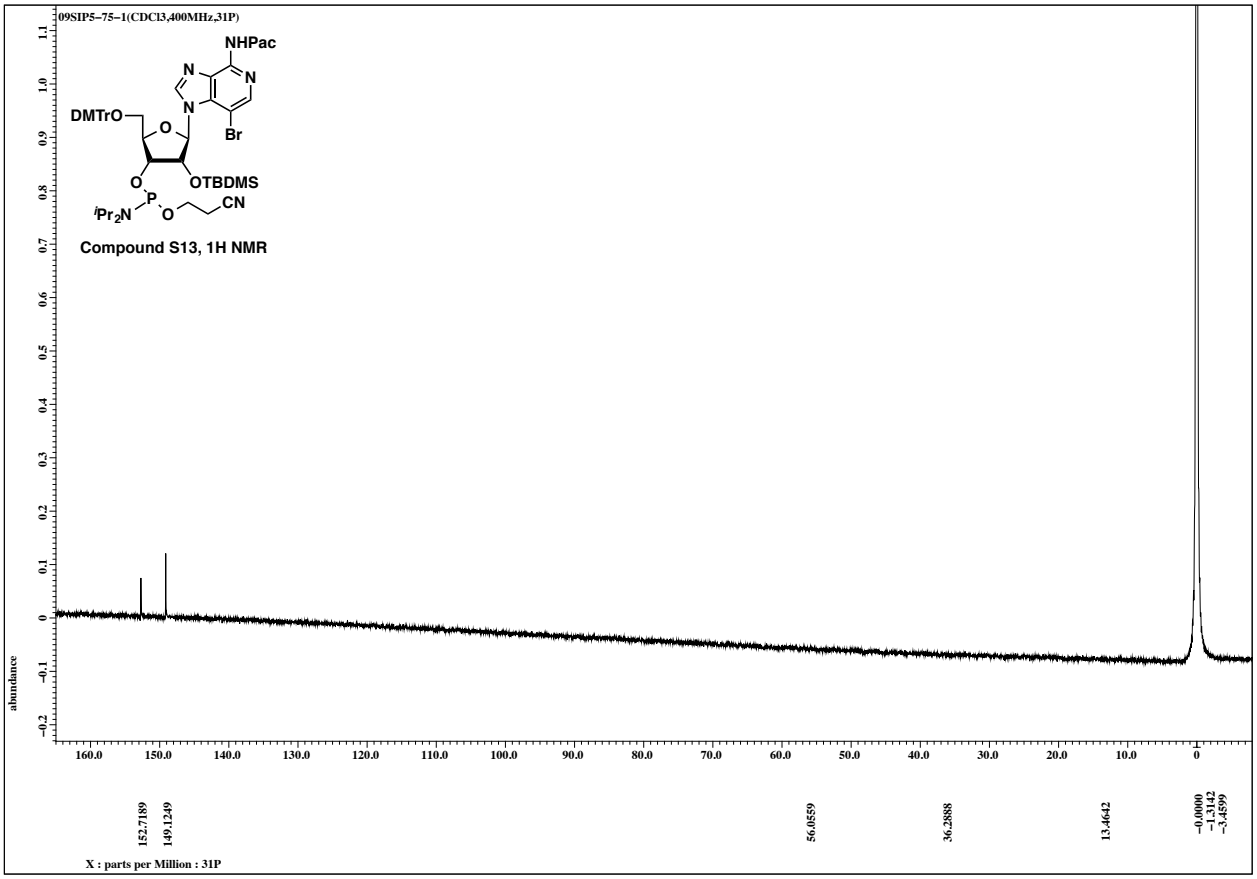

09SIP5-66-1(CDCl3,400MHz)
NHPac
DMTrO
Br
TBDMSO
OTBDMS
Compound S10, 13C NMR
abundance
X : parts per Million : 13C


09SIP5-71-1(DMSO-d6,400MHz)
NHPac
DMTrO
Br
HO
OH
Compound S11, 1H NMR
abundance
X : parts per Million : 1H


09SIP5-71-1(DMSO-d6,400MHz,13C)
NHPac
DMTrO
Br
HO
OH
Compound S11, 13C NMR
abundance
X : parts per Million : 13C


09SIP5-71-1(DMSO-d6,400MHz)
NHPac
DMTrO
Br
HO
OTBDMS
Compound S12, 1H NMR
abundance
X : parts per Million : 1H


09SIP6-17-7(CDCl3,500MHZ,13C)
NHPac
N
N
N
DMTrO
O
Br
HO
OTBDMS
Compound S12, 13C NMR
abundance
158.8462
158.7318
157.2247
141.7917
135.3247
130.2503
130.1740
129.9165
129.2488
128.3417
128.2252
128.0947
127.3030
122.4194
115.1607
113.5010
113.2721
87.1562
86.7842
84.5808
78.6671
77.4080
77.1600
76.9025
74.2353
68.1463
63.7396
56.3760
55.3840
25.7007
25.6340
18.0033
-4.6502
-5.0890
-5.2321
X : parts per Million : 13C


09SIP5-75-1(CDCl3,400MHz,31P)
NHPac
N
N
N
DMTrO
O
Br
O
OTBDMS
P
CN
iPr2N
O
Compound S13, 1H NMR
abundance
152.7189
149.1249
56.0559
36.2888
13.4642
-0.0000
-1.3142
-3.4599
X : parts per Million : 31P