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Supplementary information

Development of a new synthetic method for deoxyoligonucleotides using 3'-*H*-phosphonamidate derivatives

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1. Experimental section

General information

All reactions were conducted under an Ar atmosphere. Dry organic solvents were prepared by appropriate procedures. Additionally, ^{1}H NMR spectra were recorded at 400 or 600 MHz with tetramethylsilane (δ 0.0) as the internal standard in $CDCl_3$, CD_3CN, or pyridine-d_5 or CH_3CN (δ 2.06) as the internal standard in D_2O. Further, ^{13}C NMR spectra were recorded at 100 or 151 MHz with $CDCl_3$, which was used as the internal standard at δ 77.0 or CH_3CN (δ 1.47) as the internal standard in D_2O. Furthermore, ^{31}P NMR spectra were recorded at 162 MHz with H_3PO_4 (δ 0.0) as the external standard in $CDCl_3$, CD_3CN, or pyridine-d_5. Analytical thin-layer chromatography was performed on commercial glass plates with a 0.25 mm-thick silica gel layer. Silica gel column chromatography was performed on silica gel (Yamazen UNIVERSAL Premium column (30 μm 60 Å)) or ODS silica gel (Yamazen UNIVERSAL Premium column (30 μm 120 Å)) (Yamazen Corporation) using automated flash chromatography system W-prep 2XY (Yamazen Corporation). RP-HPLC for analysis and purification was performed using a μBondasphere 5 μm C18, 100 Å, 19 × 150 mm^2 (Waters).

Synthesis of compounds

5′-*O*-Dimethoxytritylthymidine 3′-morpholino *H*-phosphonamidate (2t)

Firstly, 1,8-diazabicyclo [5.4.0] undec-7-enium 5′-*O*-dimethoxytritylthymidine 3′-*H*-phosphonate monoester **1t**[1] (0.380 g, 0.5 mmol) and morpholine (0.044 mL, 0.50 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and dry pyridine (0.1 mL) with molecular sieves 4A (1.0 g). Afterward, bis (2-oxo-3-oxazolidinyl) phosphinic chloride (BOPCl) (0.32 g, 1.25 mmol) was added to the solution at rt. The mixture was stirred for 20 min at rt. Thereafter, morpholine (0.044 mL, 0.50 mmol) was added to the solution. After the mixture was stirred for 30 min at rt, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (2 × 30 mL). The aqueous layers were combined and back-extracted with CH_2Cl_2 (2 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was carried out on the Yamazen UNIVERSAL Premium column (M size) using the automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed three times with a linear gradient of 0%–100% acetone in $CHCl_3$ to afford **2t** as a colorless foam (0.144 g, 0.21 mmol, 43%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.01, 8.98 (br, 1H (H-3), diastereomers), 7.57, 7.56 (d, *J* = 1.2 Hz, 1H (H-6), diastereomers), 7.39–7.36 (m, 2H (Ar)), 7.33–7.22 (m, 7H (Ar)),

6.86–6.83 (m, 4H (Ar)), 6.81 (d, J = 657 Hz, 1H (P-H)), 6.47 (dd, J = 8.8, 5.5 Hz, 1H (H-1′), one of diastereomers), 6.44 (dd, J = 8.5, 5.6 Hz, 1H (H-1′), one of diastereomers), 5.15–5.08 (m, 1H (H-3′)), 4.25, 4.20 (dd, J = 4.7, 2.4, 4.7, 2.5 Hz, 1H (H-4′), diastereomers), 3.79 (s, 6H (-OC$\underline{H}_3$)), 3.67–3.52 (m, 5H (H-5′, -NC$\underline{H}_2$-)), 3.37 (dd, J = 11.8, 2.6 Hz, 1H (H-5′), diastereomers), 3.18–3.00 (m, 4H (-C$\underline{H}_2$O-)), 2.63–2.55 (m, 1H (H-2′)), 2.46–2.40 (m, 1H (H-1′)), 1.43, 1.42 (d, J = 1.1 Hz, 3H (5-C$\underline{H}_3$), diastereomers) ; ^{13}C {^{1}H} NMR (151 MHz, $CDCl_3$) δ163.6, 163.6 (C-4, diastereomers), 158.8, 158.8 (Ar), 150.4 (C-2), 144.0, 143.9 (Ar), 135.1, 135.0 (C-6, diastereomers), 134.9, 134.9 (Ar), 130.1, 129.1, 128.1, 128.1, 128.0, 128.0, 127.3, 127.2, 113.3, 113.3 (Ar), 111.7, 111.6 (C-5, diastereomers), 87.2, 87.2 (-O$\underline{C}$(Ar)$_3$), 84.8 (d, $^3J_{PC}$ = 5.3 Hz, C-4′, one of diastereomers), 84.7 (d, $^3J_{PC}$ = 5.9 Hz, C-4′, one of diastereomers), 84.3, 84.3 (C-1′, diastereomers), 75.0 (d, $^2J_{PC}$ = 5.7 Hz, C-3′, one of diastereomers), 74.7 (d, $^2J_{PC}$ = 5.9 Hz, C-3′, one of diastereomers), 66.9, 66.9 (d, $^2J_{PC}$ = 5.5 Hz, -N$\underline{C}$H$_2$-, diastereomers), 63.2, 62.9 (C-5′, diastereomers), 55.2 (-O$\underline{C}$H$_3$), 42.6 (d, $^3J_{PC}$ = 1.5 Hz, -O$\underline{C}$H$_2$-), 39.5 (d, $^3J_{PC}$ = 3.5, Hz, C-2′, one of diastereomers), 39.3 (d, $^3J_{PC}$ = 3.9 Hz, C-2′, one of diastereomers), 11.7, 11.6 (5-$\underline{C}$H$_3$, diastereomers) ; ^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 13.1 ($^1J_{PH}$ = 659 Hz), 12.7 ($^1J_{PH}$ = 655 Hz)

HRMS (ESI-TOF) *m/z* calcd for $C_{35}H_{44}N_4O_9P^+$ $[M+NH_4]^+$, 695.2840; found 695.2844.

5′-*O*-Dimethoxytritylthymidine 3′-thiomorpholino *H*-phosphonamidate (3t)

Firstly, 1,8-diazabicyclo [5.4.0] undec-7-enium 5′-*O*-dimethoxytritylthymidine 3′-*H*-phosphonate monoester **1t** (1.52 g, 2.0 mmol) and thiomorpholine (0.19 mL, 2.0 mmol) were dissolved in dry pyridine (20 mL) and dry MeCN (20 mL) with molecular sieves 3A (4.0 g). Afterward, BOPCl (1.27 g, 5.0 mmol) was added to the solution at 0 ℃. After the mixture was stirred for 20 min at 0 ℃, the mixture was diluted with CH_2Cl_2 (80 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (2 × 60 mL). The aqueous layers were combined and back-extracted with CH_2Cl_2 (2 × 60 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was carried out on the Yamazen UNIVERSAL Premium column (L size) using the automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed with a linear gradient of 0%–100% acetone in $CHCl_3$-pyridine (99:1, v/v) to afford **3t** as a colorless foam (0.94 g, 1.35 mmol, 68%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.2–9.1 (br, 1H (H-3)), 7.57 (d, J = 1.4 Hz, 0.5H (H-6) one of diastereomers), 7.56 (d, J = 0.9 Hz, 0.5H (H-6) one of diastereomers), 7.39–7.36 (m, 2H (Ar)), 7.33–7.23 (m, 7H (Ar)), 6.87–6.82 (m, 4H (Ar)), 6.78 (d, J = 655.9 Hz, (P-$\underline{H}$)

one of diastereomers), 6.73 (d, J = 653.2 Hz, (P-H) one of diastereomers), 6.49–6.42 (m, 1H (H-1′)), 5.12–5.05 (m, 1H (H-3′)), 4.22 (q, J = 2.3 Hz, 0.5H (4′), diastereomers), 4.20 (q, J = 1.8 Hz, 0.5H (4′), diastereomers), 3.79 (s, 6H (-OC$\underline{H}_3$)), 3.56–3.51 (m, 1H (H-5′)), 3.45–3.19 (m, 5H (H-5″, thiomorpholine-NC$\underline{H}_2$)), 2.62–2.39 (m, 6H (H-2′, thiomorpholine-SC$\underline{H}_2$)), 1.43 (d, J = 0.9 Hz, 1.5H (5-C$\underline{H}_3$), one of diastereomers), 1.40 (d, J = 1.4 Hz, 1.5H (5-C$\underline{H}_3$), diastereomers); ^{13}C {^{1}H} NMR (151 MHz, $CDCl_3$) δ 163.6, 163.6 (C-4, diastereomers), 158.8, 158.8, 158.8, 158.7 (Ar), 150.4 (C-2), 144.0, 143.9 (Ar), 135.1, 135.1 (C-6, diastereomers), 135.0, 134.9, 134.9, 130.1, 130.0, 130.0, 128.1, 128.1, 128.0, 128.0, 127.3, 127.2, 113.3, 113.3 (Ar), 111.7, 111.6 (C-5, diastereomers), 87.2, 87.2 (-$\underline{C}Ar_3$, diastereomers), 84.7, 84.7 ($^3J_{PC}$ = 5.7 Hz, C-4′, diastereomers), 84.3, 84.3 (C-1′, diastereomers), 74.9 ($^2J_{PC}$ = 5.7 Hz, C-3′, one of diastereomers), 74.7 ($^2J_{PC}$ = 5.9 Hz, C-3′, one of diastereomers), 63.2, 62.9 (C-5′, diastereomers), 55.2 (-O$\underline{C}H_3$)), 44.3 ($^2J_{PC}$ = 3.9 Hz, thiomorpholine-N$\underline{C}H_2$), 39.4 ($^3J_{PC}$ = 3.5 Hz, CH_2 (2′), one of diastereomers), 39.3 ($^3J_{PC}$ = 3.8 Hz, CH_2 (2′), one of diastereomers), 27.4, 27.3 (thiomorpholine-S$\underline{C}H_2$, diastereomers), 11.7, 11.6 (5-$\underline{C}H_3$), diastereomers); ^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 13.6 ($^1J_{PH}$ = 655 Hz), 13.2 ($^1J_{PH}$ = 655 Hz)

HRMS (ESI-TOF) *m/z* calcd for $C_{35}H_{44}N_4O_8PS^+$ $[M+H]^+$, 711.2612; found 711.2627.

5′-*O*-Dimethoxytritylthymidine 3′-*N*-methylpiperadino *H*-phosphonamidate (4t)

Firstly, 1,8-diazabicyclo [5.4.0] undec-7-enium 5′-*O*-dimethoxytritylthymidine 3′-*H*-phosphonate monoester **1t** (0.380 g, 0.5 mmol) and *N*-methylpiperadine (0.055 mL, 0.50 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and dry pyridine (0.1 mL) with molecular sieves 4A (1.0 g). Afterward, BOPCl (0.32 g, 1.25 mmol) was added to the solution at rt. The mixture was stirred for 20 min at rt. Thereafter, *N*-methylpiperadine (0.055 mL, 0.50 mmol) was added to the solution. After the mixture was stirred for 30 min at rt, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (2 × 30 mL). The aqueous layers were combined and back-extracted with CH_2Cl_2 (2 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford **4t** as a colorless foam. Compound **4t** was used for next reaction, without further purifications.

^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 13.1 ($^1J_{PH}$ = 650 Hz), 12.9 ($^1J_{PH}$ = 646 Hz)

HRMS (ESI-TOF) *m/z* calcd for $C_{36}H_{44}N_4O_8P^+$ $[M+H]^+$, 691.2891; found 691.2890.

5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyldeoxyadenosine 3′-thiomorpholino *H*-phosphonamidate (3a)

Firstly, 1,8-diazabicyclo [5.4.0] undec-7-enium 5′-*O*-dimethoxytrityl-*N*^{6}-

benzoyldeoxyadenosine 3′-*H*-phosphonate monoester **1a** (0.44 g, 0.50 mmol) and thiomorpholine (0.047 mL, 0.50 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and dry pyridine (0.1 mL) with molecular sieves 4A (1.0 g). Afterward, BOPCl (0.32 g, 1.25 mmol) was added to the solution at rt. The mixture was stirred for 20 min at rt. Thereafter, thiomorpholine (0.047 mL, 0.50 mmol) was added to the solution. After the mixture was stirred for 30 min at rt, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (2 × 30 mL). The aqueous layers were combined and back-extracted with CH_2Cl_2 (2 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was carried out on the Yamazen UNIVERSAL Premium column (L size) using the automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed three times with a linear gradient of 0%–100% acetone in $CHCl_3$ to afford **3a** as a colorless foam (0.21 g, 0.26 mmol, 53%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H (-CONH-)), 8.72 (d, J = 4.1 Hz, 1H (H-2)), 8.16 (d, J = 5.0 Hz, 1H (8-H)), 8.02 (d, J =7.3 Hz, 2H (Ar)), 7.60 (t, J = 6.6 Hz, 1H (Ar)), 7.51 (t, J = 7.6 Hz, 2H (Ar)), 7.40–7.36 (m, 2H (Ar)), 7.29−7.19 (m, 7H (Ar)), 6.82 (d, J = 657.7 Hz, 1H (P-H)), 6.82–6.79 (m, 4H (Ar)), 6.53–6.49 (m, 1H (H-1′)), 5.26–5.16 (m, 1H (H-3′)), 4.38–4.33 (m, 1H (H-4′)), 3.77 (s, 6H (-OCH_3)), 3.50–3.30 (m, 6H (H-5′, 5″, thiomorpholine-NCH_2)), 3.13–3.06 (m, 1H (H-2′)), 2.85–2.74 (m, 1H (H-2″)), 2.64–2.49 (m, 4H (thiomorpholine-SCH)); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) δ 164.5 (-CONH-), 158.5 (Ar), 152.6 (C-2), 151.4 (C-4), 149.5 (C (6)), 144.2 (Ar), 144.1 (Ar), 141.4, 141.4 (C-8, diastereomers), 135.3, 135.3, 135.2, 133.5, 132.7, 129.9, 129.9, 129.1, 128.8, 128.0, 127.9, 127.8, 127.0 (Ar), 123.5 (C-5), 113.2 (Ar), 86.7 (O-C(Ar)$_3$), 85.2, 85.1 (C-4″, diastereomers), 84.5 (C-1′), 75.0 (d, $^2J_{PC}$ = 4.8 Hz, C-3′, one of diastereomers), 74.6 (d, $^2J_{PC}$ = 5.8 Hz, C-3′, one of diastereomers), 63.1, 62.8 (C-5′, diastereomers), 55.2 (-OCH_3), 44.4, ($^2J_{PC}$ = 3.9 Hz, thiomorpholine-NCH_2, diastereomers), 44.3 (thiomorpholine-NCH_2, diastereomers), 38.9, 38.8 (C-2′, diastereomers), 27.4, 27.4 (thiomorpholine-SCH_2, diastereomers); ^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 13.9 ($^1J_{PH}$ = 650 Hz), 13.0 ($^1J_{PH}$ = 659 Hz)

HRMS (ESI-TOF) *m/z* calcd for $C_{42}H_{44}N_6O_7PS^+$ $[M+H]^+$, 807.2724; found 807.2726.

5′-*O*-Dimethoxytrityl-*N*^{4}-isobutyryldeoxycytidine 3′-thiomorpholino *H*-phosphonamidate (3c)

Firstly, 1,8-diazabicyclo [5.4.0] undec-7-enium 5′-*O*-dimethoxytrityl-*N*^{4}-isobutyryldeoxycytidine 3′-*H*-phosphonate monoester **1c** (0.41 g, 0.50 mmol) and

thiomorpholine (0.047 mL, 0.50 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and dry pyridine (0.1 mL) with molecular sieves 4A (1.0 g). Bis (2-oxo-3-oxazolidinyl) phosphinic chloride (BOPCl) (0.32 g, 1.25 mmol) was added to the solution at rt. The mixture was stirred for 20 min at rt. Thereafter, thiomorpholine (0.047 mL, 0.50 mmol) was added to the solution. After the mixture was stirred for 30 min at rt, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2 × 30 mL). The aqueous layers were combined and back-extracted with CH_2Cl_2 (2 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was carried out on the Yamazen UNIVERSAL Premium column (L size) using the automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed four times with a linear gradient of 0%–100% acetone in $CHCl_3$ to afford **3c** as a colorless foam (0.22 g, 0.29 mmol, 58%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (br, 1H (-CONH-)), 8.14 (d, J = 7.3 Hz, 0.5H (H-6), one of diastereomers), 8.09 (d, J = 7.8 Hz, 0.5H (H-6), one of diastereomers), 7.37–7.14 (m, 9H (Ar)), 6.87–6.84 (m, 4H (Ar)), 6.79 (d, J = 651.8 Hz, 0.5H (P-H) one of diastereomaers), 6.71 (d, J = 654.1 Hz, 0.5H (P-H) one of diastereomaers), 6.33–6.26 (m, 1H (H-1′)), 5.06–4.99 (m ,1H (H-3′)), 4.34–4.31 (m, 1H (H-4′)), 3.80 (s, 6H (-OCH_3)), 3.52–3.25 (m, 6H (H-5′, 5″, thiomorpholine-NCH_2)), 2.89–2.80 (m, 1H (H-2′)), 2.62–2.50 (m, 5H (-C$H(CH_3)_2$, thiomorpholine-SCH_2)), 2.36–2.27 (m, 1H (H-2′)), 1.24–1.21 (m, 6H (-CH(CH_3)); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) δ176.5 (-CONH-), 162.2 (C-4), 158.7 (Ar), 155.0 (C-2), 144.2 (C-6), 143.8 (Ar), 135.1, 135.0, 135.0, 134.9, 130.0, 130.0, 128.0, 127.2, 113.3 (Ar), 96.2 (C-5′), 87.1 (-CAr_3), 87.1, 86.9 (C-1′, diastereomers), 85.4, 85.3 (C-4′, diastereomers), 74.0, 73.4 (d, $^2J_{PC}$ = 5.8 Hz, C-3′, diastereomers), 62.5, 62.1 (C-5′, diastereomers), 55.2 (CH_3 (-OCH_3)), 44.3, 44.2 (d, $^2J_{PC}$ = 3.9 Hz, CH_2 (thiomorpholine-NCH_2, diastereomers), 40.7, 40.5 (d, $^3J_{PC}$ =3.8 Hz, C-2′, diastereomers), 36.8 (-C$H(CH_3)_2$), 27.4, 27.4 (d, $^3J_{PC}$ = 3.9 Hz, CH_2 (thiomorpholine-SCH)), 19.0, 19.0 (CH_3 (-CH(CH_3)$_2$, diastereomers) ; ^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 13.5 ($^1J_{PH}$ = 650 Hz)

HRMS (ESI-TOF) *m/z* calcd for $C_{38}H_{46}N_4O_8PS^+$ $[M+H]^+$, 749.2768; found 749.2766.

5′-*O*-Dimethoxytrityl-*N*^{2}-isobutyryldeoxyguanosine 3′-thiomorpholino *H*-phosphonamidate (3g)

Firstly, 1,8-diazabicyclo [5.4.0] undec-7-enium 5′-*O*-dimethoxytrityl-*N*^{2}-isobutyryldeoxyguanosine 3′-*H*-phosphonate monoester **1g** (0.43 g, 0.50 mmol) and thiomorpholine (0.047 mL, 0.50 mmol) were dissolved in dry pyridine (5 mL) and dry

MeCN (5 mL) with molecular sieves 3A (1.0 g). Afterward, BOPCl (0.32 g, 1.25 mmol) was added to the solution at 0 ℃. After the mixture was stirred for 20 min at 0 ℃, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (2 × 30 mL). The aqueous layers were combined and back-extracted with CH_2Cl_2 (2 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was carried out on the Yamazen UNIVERSAL Premium column (L size) using the automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed with a linear gradient of 0%–60% acetone in $CHCl_3$ for the first time and 0%–60% acetone in $CHCl_3$-pyridine (1:1, v/v) for the second time to afford **3g** as a colorless foam (0.21 g, 0.27 mmol, 53%)

^{1}H NMR (400 MHz, $CDCl_3$) δ12.0 (brs, 1H (NH-1)), 9.37 (s, 0.5H (-CO<u>H</u>N-), one of diastereomers), 8.98 (s, 0.5H (-CO<u>H</u>N-), one of diastereomers), 7.78 (s, 1H, H-8), 7.42–7.40 (m, 1H (Ar)), 7.36–7.34 (m, 1H (Ar)), 7.30–7.16 (m, 7H (Ar)), 6.83–6.73 (m, 4H (Ar)), 6.82 (d, J = 659.1 Hz, P-<u>H</u>), 6.71 (d, J = 656.4 Hz, P-<u>H</u>), 6.17–6.13 (m, 1H (H-1′)), 5.62–5.57 (m, 0.5H (H-3′), one of diastereomers), 5.46–5.41 (m, 0.5H (H-3′), one of diastereomers), 4.23–4.19 (m, 1H (H-4′)), 3.77, 3.76 (s, 6H (-OC<u>H</u>$_3$), 3.44–3.19 (m, 6H (H-5′, thiomorpholine-NC<u>H</u>$_2$)), 3.17–3.03 (m, 1H (H-2′)), 2.71–2.53 (m, 3H (H-2′, thiomorpholine-SC<u>H</u>$_2$)), 2.46 (t, J = 5.0 Hz, 2H (thiomorpholine-SC<u>H</u>$_2$)), 2.37 (quin, J = 6.9 Hz, 0.5H (-C<u>H</u>(CH$_3$)$_2$), one of diastereomers), 2.17 (quin, J = 6.9 Hz, 0.5H (-C<u>H</u>(CH$_3$)$_2$), one of diastereomers), 1.14-0.95 (m, 6H (-CH(C<u>H</u>$_3$)$_2$, diastereomers, rotamers) ; ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) δ 178.9, 178.8 (-<u>C</u>ONH-, diastereomers), 158.6, 158.6 (Ar) , 155.5, 155.5 (C-6, diastereomers), 147.8, 147.6 (C-2, diastereomers), 147.5, 147.4 (C-4, diastereomers), 144.4, 144.3 (Ar), 137.9 (C-8), 135.5, 135.2, 135.2, 129.8, 127.9, 127.9, 127.9, 127.1, 127.0 (Ar), 121.9, 121.8 (C-5, diastereomers), 113,2, 113.1 (Ar), 86.4, 86.4 (-CAr$_3$, diastereomers), 84.2（d, $^3J_{PC}$ = 6.7 Hz, C-4′, one of diastereomers）84.2 , 83.9 (C-1′, diastereomers) 83.8 (d, $^3J_{PC}$ = 6.7 Hz, C-4′, one of diastereomers), 73.9 (d, $^2J_{PC}$ = 4.8 Hz, C-3′, one of diastereomers), 73.8 (d, $^2J_{PC}$ = 5.8 Hz, C-3′, one of diastereomers), 62.9, 62.1 (C-5′, diasreteomers), 55.2 (-O<u>C</u>H$_3$)), 44.3, 44.2 (d, $^2J_{PC}$ = 3.9 Hz, CH$_2$ (thiomorpholine-N<u>C</u>H$_2$), diastereomers), 38.4, 38.2 (C-2′, diastereomers), 36.1, 36.1 (-<u>C</u>H(CH$_3$)$_2$), 27.3 (d, $^3J_{PC}$ = 5.9 Hz, CH$_2$ (thiomorpholine-S<u>C</u>H), one of diastereomers), 27.3 (d, $^3J_{PC}$ = 4.8 Hz, CH$_2$ (thiomorpholine-S<u>C</u>H), one of diastereomers), 18.7, 18.7 (CH$_3$ (-CH(<u>C</u>H$_3$)$_2$, diastereomers); ^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 14.2 ($^1J_{PH}$ = 652 Hz), 13.4 ($^1J_{PH}$ = 659 Hz)

HRMS (ESI-TOF) *m/z* calcd for $C_{39}H_{46}N_6O_8PS^+$ $[M+H]^+$, 789.2830; found 789.2831.

3′-*O*-*t*-Butyldiphenylsilyl-N^4-isobutyryldeoxycytidine (5c)

Firstly, 5′-*O*-dimethoxytrityl-N^4-isobutyryldeoxycytidine (6.00 g, 10 mmol) and imidazole (2.72 g, 40 mmol) were dissolved in dry DMF (50 mL). *t*-Butyldiphenylchlorosilane (5.14 mL, 20 mmol) was added to the mixture at rt. After the mixture was stirred for 1 h at rt, *t*-butyldiphenylchlorosilane (2.57 mL, 10 mmol) was added. The mixture was stirred for 30 min at rt. Thereafter, the reaction was quenched with MeOH (10 mL). The mixture was diluted with ethyl acetate (EtOAc) (100 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (6×100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (200 mL) and 6% dichloroacetic acid (DCA) solution in CH_2Cl_2 (200 mL) was added to the solution. After the mixture was stirred for 2 h at rt, MeOH (20 mL) was added to the mixture and the mixture was washed with saturated aqueous solutions of $NaHCO_3$ (3 × 200 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was carried out on Yamazen UNIVERSAL Premium column (L size) using automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed with a linear gradient of 0%–10% MeOH in $CHCl_3$ to afford **5c** as a colorless foam (4.95 g, 9.23 mmol, 92%)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.74 (s, 1H, -NHCO-), 8.17 (d, J = 7.5 Hz, 1H, H-6), 7.63 (dd, J = 8.0, 1.3 Hz, 4H, Ar), 7.46–7.33 (m, 7H, H-5, Ar), 6.25 (t, J = 6.4 Hz, 1H, H-1′), 4.44 (dt, J = 5.9, 3.3 Hz, 1H, H-3′), 4.04 (q, J = 2.9 Hz, 1H, H-4′), 3.65 (d, J = 12.0 Hz, 1H, H-5′), 3.24 (dt, J = 12.0, 3.4 Hz, 1H, H-5′), 2.71–2.69 (br, 1H, 5′-OH), 2.68–2.63 (m, 1H, -CH(CH$_3$)$_2$), 2.57 (dd, J = 6.1, 3.5 Hz, 1H, H-2′, diastereomers), 2.15 (t, J = 6.6 Hz, 1H, H-2′, diastereomers), 1.19, 1.18 (d, J = 2.4 Hz, 6H, -CH(CH$_3$)$_2$, rotamers), 1.08 (s, 9H, -C(CH$_3$)$_3$) ; ^{13}C {^{1}H} NMR (151 MHz, $CDCl_3$) δ177.6 (-NHCO-),162.4 (C-4), 155.3 (C-2), 145.6 (C-6), 135.5, 135.5, 133.2, 132.9, 129.9, 129.8, 127.7 (Ar), 96.7 (C-5), 88.5 (C-4′), 88.1 (C-1′), 73.0 (C-3′), 61.5 (C-5′), 41.7 (C-2′), 36.2 (-CH(CH$_3$)$_2$), 26.7(-C(CH$_3$)$_3$), 19.0, 18.9 (-CH(CH$_3$)$_2$, rotamers), 18.9 (-C(CH$_3$)$_3$)

HRMS (ESI-TOF) *m/z* calcd for $C_{29}H_{38}N_3O_5Si^+$ $[M+H]^+$, 536.2575; found 536.2573.

The synthesis of $T_{PH}T$ (6tt) dimer by azeotropic manipulation

Firstly, *H*-phosphonamidate monomer **2t** (0.0254 g, 0.0375 mmol), **3t** (0.0260 g, 0.0375 mmol), or **4t** (0.0259 g, 0.0375 mmol) and a thymidine derivative **5t** (0.0120 g, 0.025 mmol) were dried by coevaporation with dry pyridine (6 × 1 mL). Afterward, the residue was dissolved in $CDCl_3$ (0.5 mL) and ^{31}P NMR spectrum was recorded. The formation of **6tt** was confirmed by ^{31}P NMR spectra (δ 9.2, 7.6 ppm, $^1J_{PH}$ = 715, 724 Hz) (Fig. S4–S6)

and mass spectrometry analysis (high-resolution mass spectroscopy (electrospray ionization–time-of-flight) (HRMS (ESI-TOF)) *m/z* calcd for $C_{57}H_{63}N_4NaO_{13}Psi^+$ $[M+Na]^+$, 1093.3791; found 1093.3817.).

The synthesis of $T_{PH}T$ (6tt) dimer under conditions of constant temperature, concentration, and pressure. (Table 2)

Firstly, 5′-*O*-dimethoxytritylthymidine 3′-morpholino *H*-phosphonamidate **3t** (0.0173 g, 0.025 mmol) and a thymidine derivative **5t** (0.0144 g, 0.030 mmol) were dissolved in dry pyridine-d_5 (0.125 or 0.50 mL) with MS4A (0.4 g). After the mixture was stirred for 1 or 2 h at 25 or 40 °C, the reaction mixture was transferred into an NMR sample tube (5 mm × 180 mm) and a spectrum was recorded. The formation of **6tt** was confirmed by ^{31}P NMR spectra (δ 9.1, 7.6 ppm, $^1J_{PH}$ = 715, 724 Hz) (Fig. S7–S11)

Synthesis of dimers

$T_{PSCE}T$ dimer (9tt)

Firstly, 5′-*O*-dimethoxytritylthymidine 3′-morpholino *H*-phosphonamidate **3t** (0.0416 g, 0.060 mmol) and a thymidine derivative **5t** (0.0192 g, 0.040 mmol) were dissolved in dry pyridine (0.40 mL) with MS4A (0.8 g). After the mixture was stirred for 2 h at 40 °C, a sulfurizing reagent (**8**) in pyridine (0.8 mL, 0.048 mmol) was added to the mixture. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed by repeated coevaporations with toluene. The residue was dissolved in $CHCl_3$ (2 mL) and 2% TFA solution in $CHCl_3$ (2 mL) was added. After the mixture was stirred for 10 min at rt, MeOH (2 mL) and $CHCl_3$ (30 mL) were successively added to the mixture, and the mixture was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 20 mL). The aqueous layers were combined and back-extracted with $CHCl_3$ (1 × 30 mL) The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was carried out on Yamazen UNIVERSAL Premium column (M size) using automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed with a linear gradient of 0%–10% MeOH in $CHCl_3$ to afford **9tt** as a colorless foam (0.0252 g, 0.030 mmol, 74%)

^1H NMR (400 MHz, $CDCl_3$) δ 9.62, 9.48, 9.46 (s, 2H, H-3, diastereomers), 7.66–7.63 (m, 4H, Ar), 7.51–7.39 (m, 7H, H-6 (5′-upstream), Ar), 7.18, 7.15 (d, *J* = 0.9 Hz, 1H, H-6 (3′-downstream), diastereomers), 6.34, 6.31 (t, *J* = 6.9 Hz, , 1H, H-1′ (3′-downstream), diastereomers), 6.16 (t, *J* = 6.9 Hz, , 1H, H-1′ (5′-upstream), one of diastereomers), 6.13 (t, *J* = 7.1 Hz, , 1H, H-1′ (5′-upstream), one of diastereomers), 5.24–5.16 (m, 1H, H-3′

(5′-upstream)), 4.40–4.34 (m, 1H, H-3′ (3′-downstream)), 4.15–3.70 (m, 6H, H-4′, H-5′), 3.38 (br, 1H, 5′-OH), 3.12–2.92 (m, 2H, -SC$\underline{H_2}$-), 2.89–2.68 (m, 2H, -C$\underline{H_2}$CN), 2.54–2.28 (m, 3H, H-2′), 2.07–1.99 (m, 1H, H-2′ (3′-downstream)), 1.90–1.83 (m, 6H, 5-C$\underline{H_3}$), diastereomers), 1.09 (s, 9H, -C(C$\underline{H_3}$)$_3$) ; ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) δ164.1 (C-4 (3′-downstream)), 164.0, 163.9 (C-4 (5′-upstream), diastereomers), 150.5, 150.4 (C-2 (5′-upstream), diastereomers), 150.3 (C-2 (3′-downstream)), 136.6, 136.4 (C-6 (5′-upstream), diastereomers), 136.0, 135.9 (C-6 (3′-downstream), diastereomers), 135.7, 135.7, 132.8, 132.6, 130.3, 130.2, 128.1, 128.0 (Ar), 117.4 (-CN), 111.4, 111.3 (C-5 (3′-downstream), diastereomers), 111.2 (C-5 (5′-upstream)), 86.1 (C-1′ (5′-upstream)), 85.8 (d, $^3J_{PC}$ = 3.9 Hz, C-4′ (3′-downstream), one of diastereomers), 85.6 (C-1′ (3′-downstream)), 85.4 (d, $^3J_{PC}$ = 5.8 Hz, C-4′ (3′-downstream), one of diastereomers), 84.8 (d, $^3J_{PC}$ = 8.7 Hz, C-4′ (5′-upstream), one of diastereomers), 84.7 (d, $^3J_{PC}$ = 7.7 Hz, C-4′ (5′-upstream), one of diastereomers), 78.9 (d, $^2J_{PC}$ = 6.7 Hz, C-3′ (5′-upstream) one of diastereomers), 78.5 (d, $^2J_{PC}$ = 4.8 Hz, C-3′ (5′-upstream) one of diastereomers), 72.8, 72.6 (C-3′ (3′-downstream), diastereomers), 67.1 (d, $^2J_{PC}$ = 7.7 Hz, C-5′ (3′-downstream), one of diastereomers), 67.0 (d, $^2J_{PC}$ = 6.7 Hz, C-5′ (3′-downstream), one of diastereomers), 61.9, 61.7 (C-5′ (5′-upstream), diastereomers), 40.0, 39.9 (C-2′ (3′-downstream), diastereomers), 38.4 (d, $^3J_{PC}$ = 2.9 Hz, C-2′ (5′-upstream), one of diastereomers), 38.3 (d, $^3J_{PC}$ = 5.8 Hz, C-2′ (5′-upstream), one of diastereomers), 26.8 (-C(C$\underline{H_3}$)$_3$), 26.7, 26.4 (d, $^2J_{PC}$ = 3.9 Hz, -SC$\underline{H_2}$-, diastereomers), 19.8 (-C$\underline{H_2}$CN), 19.0 (-C(C$\underline{H_3}$)$_3$), 12.5, 12.4, 12.4 (5-C$\underline{H_3}$, diastereomers) ; ^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 27.8, 27.3

HRMS (ESI-TOF) m/z calcd for $C_{39}H_{49}N_5O_{11}PSSi^+$ $[M+H]^+$, 854.2651; found 854.2652.

A$_{PSCE}$A dimer (9aa)

Firstly, 5′-*O*-dimethoxytrityl-*N*^{6}-benzoyldeoxyadenosine 3′-thiomorpholino *H*-phosphonamidate **3a** (0.0484 g, 0.060 mmol) and a deoxyadenosine derivative **5a** (0.0484 g, 0.060 mmol) were dissolved in dry pyridine (0.40 mL) with MS4A (0.8 g). After the mixture was stirred for 2 h at 40 °C, a sulfurizing reagent (**8**) in pyridine (0.8 mL, 0.048 mmol) was added to the mixture. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed by repeated coevaporations with toluene. The residue was dissolved in $CHCl_3$ (2 mL), and 2% trifluoroacetic acid (TFA) solution in $CHCl_3$ (2 mL) was added. After the mixture was stirred for 10 min at rt, MeOH (2 mL) and $CHCl_3$ (30 mL) were successively added to the mixture, and the mixture was washed with saturated aqueous solutions of $NaHCO_3$ (3 × 20 mL). The aqueous layers were combined and back-extracted with $CHCl_3$ (1 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column

chromatography. Column chromatography was carried out on a Yamazen UNIVERSAL Premium column (M size) using the automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed with a linear gradient of 0%–10% MeOH in $CHCl_3$ to afford **9aa** as a colorless foam (0.0324 g, 0.030 mmol, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23–9.17 (m, 2H (-CONH-)), 8.72-8.70 (m, 2H, H-2), 8.17–8.14 (m, 2H, H-8), 8.02–7.94 (m, 4H (Ar)), 7.71–7.69 (m, 4H (Ar)), 7.61 (t, J = 7.8 Hz, 1H (Ar)), 7.54–7.42 (m, 11H (Ar)), 6.55 (dd, J = 12.4 Hz, 5.9 Hz, 1H, H-1′ (3′-downstream)), 6.44–6.30 (m, 1H, H-1′ (5′-upstream)), 5.9–5.8 (br, 1H (5′-OH)), 5.30–5.25 (m, 1H H-3′ (5′-upstream)), 4.75–4.71 (m, 1H, H-3′ (3′-downstream)), 4.28 (d, 2H, H-4′), 4.16–3.71 (m, 4H, H-5′), 3.22–2.90 (m, 3H, H-2′ (5′-upstream), -SCH_2-), 2.77–2.48 (m, 5H, H-2′, -CH_2CN)), 1.14 (s, 9H (-C(CH_3)$_3$) ; ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) δ 164.8, 164.5 (-CONH-, diastereomers), 152.5 (C-2 (3′-downstream)) 152.0 (C-2 (5′-upstream)), 151.6 (C-4 (3′-downstream)), 150.5 (C-4 (5′-upstream)), 150.2 (C-6) 3′-downstream)), 149.6 (C-6 (5′-upstream)), 142.7 (C-8 (5′-upstream)), 141.7, 141.6 (C-8 (3′-downstream), diastereomers), 135.7, 133.4, 133.2, 132.9, 132.8, 132.6, 130.3, 128.8, 128.7, 128.1, 128.0, 127.9 (Ar), 124.4 (C-5 (5′-upstream)), 123.6 (C-5 (3′-downstream)), 117.2 (-CN)), 87.6 (d, $^3J_{PC}$ = 2.9 Hz, C-4′ (3′-downstream), one of diastereomers), 87.3 (d, $^3J_{PC}$ = 4.8 Hz, C-4′ (3′-downstream), one of diastereomers), 87.1, 86.9 (C-1′ (5′-upstream), diastereomers), 85.5, 85.4, 85.4 (C-4′ (5′-upstream), diastereomers), 84.8, 84.6 (C-1′ (3′-downstream), diastereomers), 80.3 (d, $^2J_{PC}$ = 6.3 Hz, C-3′ (5′-upstream), one of diastereomers)), 80.2 (d, $^2J_{PC}$ = 5.9 Hz, C-3′ (5′-upstream), one of diastereomers)), 73.0, 72.9 (C-3′ (3′-downstream), diastereomers), 66.8 (d, $^2J_{PC}$ = 5.8 Hz, C-5′ (3′-downstream)), 62.8 (C-5′ (5′-upstream)), 40.0 (C-2′ (5′-upstream)), 38.9 (C-2′ (3′-downstream)), 26.8 (-C(CH_3)$_3$), 26.7, 26.5 (-SCH_2-, diastereomers), 19.7 (-CH_2CN)), 19.0 (-C(CH_3)$_3$); ^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 27.4, 26.5

HRMS (ESI-TOF) *m/z* calcd for $C_{53}H_{55}N_{11}O_9PSSi^+$ $[M+H]^+$, 1080.3406; found 1080.3390.

$C_{PSCE}C$ dimer (9cc)

Firstly, 5′-*O*-dimethoxytrityl-N^4-benzoyldeoxycytidine 3′-thiomorpholino *H*-phosphonamidate diester **3c** (0.0449 g, 0.060 mmol) and a deoxycytidine derivative **5c** (0.0214 g, 0.040 mmol) were dissolved in dry pyridine (0.40 mL) with MS4A (0.8 g). After the mixture was stirred for 2 h at 40 ℃, a sulfurizing reagent (**8**) in pyridine (0.8 mL, 0.048 mmol) was added to the mixture. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed by repeated coevaporations with toluene. The residue was dissolved in $CHCl_3$ (2 mL) and 2% TFA solution in $CHCl_3$ (2 mL) was added. After

the mixture was stirred for 10 min at rt, MeOH (2 mL) and $CHCl_3$ (30 mL) were successively added to the mixture, and the mixture was washed with saturated aqueous solutions of $NaHCO_3$ (3 × 20 mL). The aqueous layers were combined and back-extracted with $CHCl_3$ (1 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was carried out on a Yamazen UNIVERSAL Premium column (M size) using the automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed with a linear gradient of 0%–10% MeOH in $CHCl_3$ to afford **9cc** as a colorless foam (0.0288 g, 0.030 mmol, 75%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99–8.83 (m, 2H (-CONH-)), 8.25, 8.21 (d, *J* = 7.3 Hz, 1H, H-6 (5′-upstream), diastereomers), 7.93 (d, *J* = 7.3 Hz, 1H, H-6 (3′-downstream), one of diastereomers), 7.89 (d, *J* =7.8 Hz, 1H, H-6 (3′-downstream), one of diastereomers), 7.66–7.62 (m, 4H (Ar)), 7.50–7.38 (m, 8H, H-5, Ar), 6.28 (t, *J* = 5.5 Hz, 1H, H-1′ (3′-downstream)), 6.16 (t, *J* = 5.5 Hz, 1H, H-1′ (5′-upstream)), 5.31–5.22 (m, 1H, H-3′ (5′-upstream)), 4.46–4.36 (m, 1H, H-3′ (3′-downstream)), 4.23–4.13 (m, 2H, H-4′), 4.09–3.90 (m, 2H, H-5′), 3.86–3.60 (m, 2H, H-5′), 3.07–2.97 (m, 2H, -SCH_2-), 2.80–2.59 (m, 6H, H-2′, -CH_2CN, -COCH$(CH_3)_2$), 2.45–2.36 (m, 1H, H-2′ (5′-upstream)), 2.00–1.92 (m, 1H, H-2′ (3′-downstream)), 1.21, 1.18 (d, *J* = 6.9 Hz, 12H, -COCH(CH_3)$_2$), 1.08 (s, 9H, -C(CH_3)$_3$) ; ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) δ177.5, 177.4, 177.2, 177.2 (-CONH-, diastereomers, rotamers), 162.6 (C-4 (5′-upstream)), 162.5 (C-4 (3′-downstream)), 155.3 (C-2 (5′-upstream)), 155.2, 155.2 (C-2 (3′-downstream), diastereomers), 145.4 (C-6 (5′-upstream)), 144.4, 144.3 (C-6 (3′-downstream), diastereomers), 135.7, 135.6, 135.5, 132.9, 132.9, 132.5, 130.3, 130.2, 128.1, 128.0 (Ar), 117.4, 117.3 (-CN, diastereomers), 96.8 (C-5 (5′-upstream)), 96.6 (C-5 (3′-downstream)), 88.0 (C-1′), 86.5 (d, $^3J_{PC}$ = 3.9 Hz, C-4′ (5′-upstream), one of diastereomers), 86.4 (d, $^3J_{PC}$ = 5.8 Hz, C-4′ (5′-upstream), one of diastereomers), 85.6, 85.4 (d, $^3J_{PC}$ = 7.7 Hz, C-4′ (3′-downstream), diastereomers), 78.7, 78.4 (d, $^2J_{PC}$ = 5.8 Hz, C-3′ (5′-upstream), diastereomers), 72.7, 72.5 (C-3′ (3′-downstream), diastereomers), 66.5 (d, $^2J_{PC}$ = 7.7 Hz, C-5′ (3′-downstream)), 61.5, 61.4 (C-5′ (5′-upstream), diastereomers), 41.6, 41.3 (C-2′ (3′-downstream), diastereomers), 39.8, 39.6 (C-2′ (5′-upstream), diastereomers), 36.6, 36.5 (-COCH$(CH_3)_2$), 26.8 (-C(CH_3)$_3$), 26.6 (d, $^2J_{PC}$ = 3.9 Hz -SCH_2-), 19.8 (-CH_2CN), 19.0 (-C$(CH_3)_3$), 18.9 (-COCH(CH_3)$_2$) ; ^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 27.8, 27.2

HRMS (ESI-TOF) *m/z* calcd for $C_{45}H_{59}N_7O_{11}PSSi^+$ $[M+H]^+$, 964.3495; found 964.3484.

G$_{PSCE}$G dimer (9gg)

Firstly, 5′-*O*-dimethoxytrityl-*N*^{2}-isobutyryldeoxyguanosine 3′-thiomorpholino *H*-

phosphonamidate diester **3g** (0.0473 g, 0.060 mmol) and a deoxyguanosine derivative **5g** (0.0230 g, 0.040 mmol) were dissolved in dry pyridine (0.40 mL) with MS4A (0.8 g). After the mixture was stirred for 2 h at 40 ℃, a sulfurizing reagent (**8**) in pyridine (0.8 mL, 0.048 mmol) was added to the mixture. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed by repeated coevaporations with toluene. The residue was dissolved in $CHCl_3$ (2 mL) and 2% TFA solution in $CHCl_3$ (2 mL) was added. After the mixture was stirred for 10 min at rt, MeOH (2 mL) and $CHCl_3$ (30 mL) were successively added to the mixture, and the mixture was washed with saturated aqueous solutions of $NaHCO_3$ (3 × 20 mL). The aqueous layers were combined and back-extracted with $CHCl_3$ (1 × 30 mL) The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was carried out on Yamazen UNIVERSAL Premium column (M size) using automated flash chromatography system W-prep 2XY (Yamazen Corporation), which was performed with a linear gradient of 0%–10% MeOH in $CHCl_3$ to afford **9gg** as a colorless foam (0.0326 g, 0.031 mmol, 78%)

^{1}H NMR (400 MHz, $CDCl_3$) δ12.4–12.2 (m, 2H (1), diastereomers, rotamers), 10.9, 10.6 (s, 1H (-NHCO-), diastereomers), 10.0, 9.69 (s, 1H (-NHCO-), diastereomers), 7.96, 7.94 (s, 1H, H-8 (5′-upstream),diastereomers), 7.71–7.64 (m, 5H, H-8 (3′-downstream, Ar)), 7.53–7.40 (m, 6H (Ar)), 6.29–6.23 (m, 1H, H-1′ (3′-downstream)), 6.10 (dd, *J* = 8.6, 6.4 Hz, 0.3 H, H-1′ (5′-upstream), one of diastereomers), 5.76 (dd *J* = 8.9, 5.7 Hz, 0.7H, H-1′ (5′-upstream), one of diastereomers), 5.18 (t, *J* = 6.9 Hz, 0.3H, H-3′ (5′-upstream), one of diastereomers), 5.02 (t, *J* = 5.5 Hz, 0.7H, H-3′ (5′-upstream), one of diastereomers), 4.57 (m, 0.3H, H-3′ (3′-downstream), one of diastereomers), 4.45 (d, *J* = 4.6 Hz, 0.7H, H-3′ (3′-downstream), one of diastereomers), 4.24–4.09 (m, 3.3H, H-4′, H-5′, diastereomers), 3.90–3.83 (m, 0.7H, H-5′ (5′-upstream), one of diastereomers), 3.75–3.69 (m, 1.3H, H-5′, diastereomers), 3.59 (d, *J* = 11.0 Hz, 0.7H, H-5′ (5′-upstream), one of diastereomers), 3.14–2.61 (m, 8H, H-2′, -COC<u>H</u>$(CH_3)_2$, -SC<u>H</u>$_2$-, -C<u>H</u>$_2$CN), diastereomers), 2.49–2.44 (m, 1H, H-2′), 2.30–2.26 (m, 1H, H-2′), 1.32-1.10 (m, 21H, -COCH(C<u>H</u>$_3$)$_2$, -C(C<u>H</u>$_3$)$_3$, diastereomers, rotamers) ; ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) δ180.7, 180.2, 180.0, 179.4 (-NHCO-, diastereomers, rotamers), 156.5, 155.8, 155.6, 155.5 (C-6, diastereomers, rotamers), 148.6, 148.4, 148.3, 148.3 (C-4, diastereomers, rotamers), 148.2, 148.1, 147.7, 147.2 (C-2, diastereomers, rotamers), 139.8 (C-8 (5′-upstream)), 138.2, 132.8, 138.0, 137.8 (C-8 (3′-downstream), diastereomers, rotamers), 135.7, 135.6, 132.8, 132.6, 132.6, 130.4, 130.3, 128.1, 128.0 (Ar), 123.0 (C-5 (3′-downstream)), 121.7, 121.3, 121.2 (C-5 (5′-upstream), diastereomers), 117.4, 117.1 (-<u>C</u>N, diastereomers), 87.0 (C-1′ (3′-downstream)), 86.3 (d, $^3J_{PC}$ = 4.8 Hz, C-4′, one of

diastereomers), 86.2 (d, $^{3}J_{PC}$ = 6.7 Hz, C-4′, one of diastereomers), 85.4 (d, $^{3}J_{PC}$ = 7.7 Hz, C-4′, one of diastereomers), 85.1 (d, $^{3}J_{PC}$ = 8.7 Hz, C-4′, one of diastereomers), 84.6, 84.6 (C-1′, diastereomers), 80.6, 80.2 (d, $^{2}J_{PC}$ = 6.7 Hz, C-3′ (5′-upstream), diastereomers), 73.6, 72.8 (C-3′ (3′-downstream), diastereomers), 67.7, 66.4 (d, $^{2}J_{PC}$ = 6.7 Hz, C-5′ (3′-downstream), diastereomers), 62.1, 61.9 (C-5′ (5′-upstream), diastereomers), 38.6 (C-2′), 36.2, 36.1, 35.9, 35.9 (-$\underline{C}$H(CH_3)$_2$, diastereomers, rotamers), 26.8 (-C($\underline{C}H_3$)$_3$), 26.4, 26.0 (d, $^{2}J_{PC}$ = 3.9 Hz, -S$\underline{C}H_2$-, diastereomers) 19.7 (d, $^{3}J_{PC}$ = 2.9 Hz, -$\underline{C}H_2$CN), 19.2, 19.2 (-CH($\underline{C}H_3$)$_2$, diastereomers), 19.1 (-$\underline{C}$(CH_3)$_3$), 19.0, 18.9, 18.9, 18.6, 18.5 (-CH($\underline{C}H_3$)$_2$, diastereomers, rotamers) ; ^{31}P {^{1}H}NMR (162 MHz, $CDCl_3$) δ 27.9, 27.5

HRMS (ESI-TOF) *m/z* calcd for $C_{47}H_{59}N_{11}O_{11}PSSi^{+}$ $[M+H]^{+}$, 1044.3618; found 1044.3599.

T$_{PS}$T$_{PS}$T trimer (10)

Thymidine 5′-*O*-dimethoxytritylthymidine 3′-thiomorpholino *H*-phosphonamidate **3t** (0.0208 g, 0.030 mmol) and a thymidine derivative **5t** (0.0096 g, 0.02 mmol) were dissolved in dry pyridine (0.20 mL) with MS4A (0.4 g). After the mixture was stirred for 2 h at 40°C, the sulfurizing reagent (**8)** in pyridine (0.4 mL, 0.024 mmol) was added. The mixture was stirred for 1 h at rt, and dimethyl phosphonate (0.011 mL, 0.12 mmol) was added. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed from the mixture by repeated coevaporation with toluene. The residue was dissolved in $CHCl_3$ (1 mL), and 2% TFA solution in $CHCl_3$ (1 mL) was added. After the mixture was stirred for 10 min at rt, MeOH (1 mL) and $CHCl_3$ (30 mL) were successively added, and the mixture was washed with H_2O (20 mL) and a saturated aqueous solution of $NaHCO_3$ (5 × 30 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used for the next reaction without further purification. The residue was dried by repeated coevaporation with dry pyridine and dissolved in dry pyridine (0.2 mL) with MS4A (0.4 g). Afterward, 5′-*O*-dimethoxytritylthymidine 3′-thiomorpholino *H*-phosphonamidate **3t** (0.0208 g, 0.030 mmol) was added. After the mixture was stirred for 2 h at 40°C, the sulfurizing reagent (**8)** in pyridine (0.4 mL, 0.024 mmol) was added. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed from the mixture by repeated coevaporation with toluene. The residue was dissolved in $CHCl_3$ (1 mL), and 2% TFA solution in $CHCl_3$ (1 mL) was added to the mixture. After the mixture was stirred for 10 min at rt, MeOH (1 mL) and $CHCl_3$ (20 mL) were successively added, and the mixture was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 20 mL). The aqueous layers were combined and back-extracted with $CHCl_3$ (1 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in dry MeCN (0.32 mL).

BSA (0.04 mL, 0.16 mmol) and DBU (0.04 mL, 0.26 mmol) were added to the mixture. After the mixture was stirred for 10 min at rt, it was diluted with $CHCl_3$ (20 mL) and washed with 1.0 M triethylammonium bicarbonate aqueous solutions (pH 8, 3 × 20 mL). The aqueous layers were combined and back-extracted with $CHCl_3$ (1 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in dry THF (0.9 mL). Further, 1 M TBAF in THF solution (0.3 mL, 0.3 mmol) was added to the mixture. After the mixture was stirred for 2 h at rt, EtOH (3 mL) and 3% (w/w) NH_3 aqueous solution (15 mL) were successively added to the solution, and the mixture was washed with diethyl ether (2 × 20 mL). The organic layers were combined and back-extracted with a 3% (w/w) NH_3 aqueous solution (10 mL). The aqueous layers were combined and concentrated under reduced pressure to afford crude $T_{PS}T_{PS}T$. The crude $T_{PS}T_{PS}T$ was analyzed by RP-HPLC, which was performed with a linear gradient of 0%–30% MeCN in 0.1 M TEAA buffer (pH 7.0) over 40 min at 50°C at a rate of 0.5 mL min^{-1} using μBondasphere 5 μm C18, 100 Å, 19 × 150 mm^2 (Waters). The crude $T_{PS}T_{PS}T$ was purified by silica gel column chromatography. Column chromatography was carried out on the Yamazen UNIVERSAL Premium ODS column (M size) using the automated flash chromatography system W-prep 2XY (Yamazen Corporation). The chromatography was performed with a linear gradient of 0%–30% MeCN in 0.1 M TEAA buffer (pH 7.0) for the first time and 0%–30% MeCN in H_2O for the second time to afford $T_{PS}T_{PS}T$ as a terabutylammoniun salt and a colorless foam (8.7 mg, 6.4 μmol, 32%).

^1H NMR (400 MHz, D_2O) δ 7.80–7.64 (m, 3H, H-6), 6.32–6.24 (m, 3H, H-1′), 5.12-5.02 (m, 2H, H-3′), 4.58 (m, 1H, H-3′), 4.40 (d, J = 8.2 Hz, 1H, H-4′), 4.22–4.16 (m, 6H, H-4′, H-5′), 3.84–3.79 (m, 2H, H-5′), 3.20 (t, J = 4.4 Hz, 16H, -NC$\underline{H_2}$-), 2.54–2.35 (m, 6H, H-2′), 1.95–1.87 (m, 9H, 5-C$\underline{H_3}$)), 1.65 (br, 16H, -NCH$_2$C$\underline{H_2}$-), 1.38–1.34 (m, 16H, -CH$_2$C$\underline{H_2}$CH$_3$), 0.99–0.92 (m, 24H, -CH$_2$C$\underline{H_3}$) ; ^{13}C {^1H} NMR (100 MHz, D_2O) δ168.0 (C-4), 153.0, 152.9 (C-2), 137.9, 137.6 (C-6), 112.3, 112.2, 112.1 (C-5), 86.1, 85.6, 84.9, 84.6 (C-1′, C-4′), 76.8, 76.4, 75.8, 71.5 (C-3′), 66.1, 65.8, 61.6 (C-5′), 58.7 (-N$\underline{C}$H$_2$-), 39.5, 38.4, 38.2 (C-2′), 23.7 (-NCH$_2\underline{C}$H$_2$-), 19.8 (-CH$_2\underline{C}$H$_2$CH$_3$), 13.4 (-CH$_2$CH$_2\underline{C}$H$_3$), 12.5, 12.5, 12.3 (5-$\underline{C}$H$_3$); ^{31}P {^1H}NMR (162 MHz, D_2O) δ 56.1, 55.9, 55.8

HRMS (ESI-TOF) m/z calcd for $C_{30}H_{38}N_6O_{17}P_2S_2^{2-}$ $[M-2H]^{2-}$, 440.0611; found 440.0609.

References for SI

1. K. Sato, H. Imai, T. Shuto, R. I. Hara and T. Wada, *The Journal of Organic Chemistry*, 2019, **84**, 15032–15041.

2. ^{31}P NMR analysis of the synthesis of 3'-*H*-phosphonamidate monomers (Table 1)

Table 1, entry 1

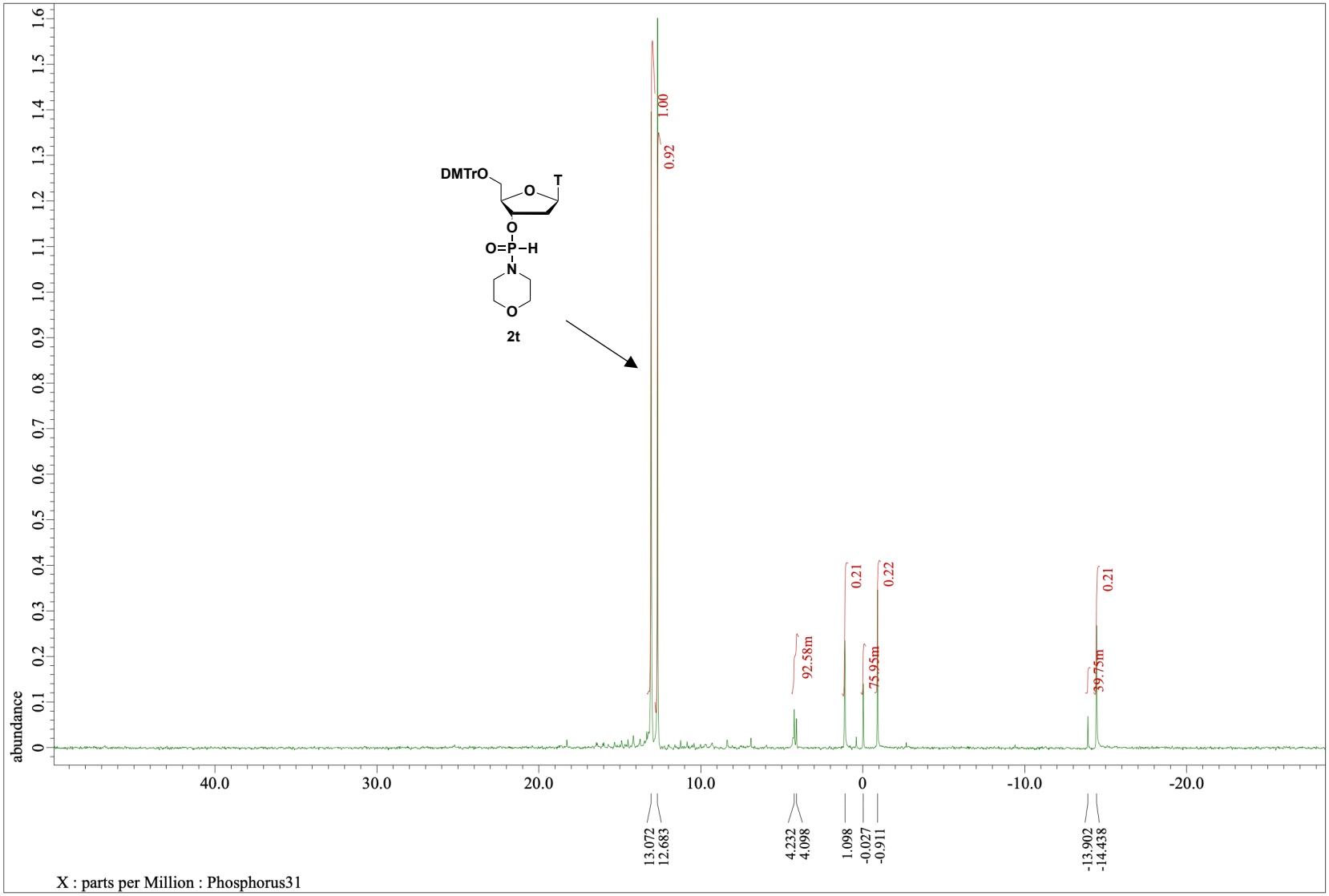


Fig. S1 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture of **2t** after extraction.

Table 1, entry 2

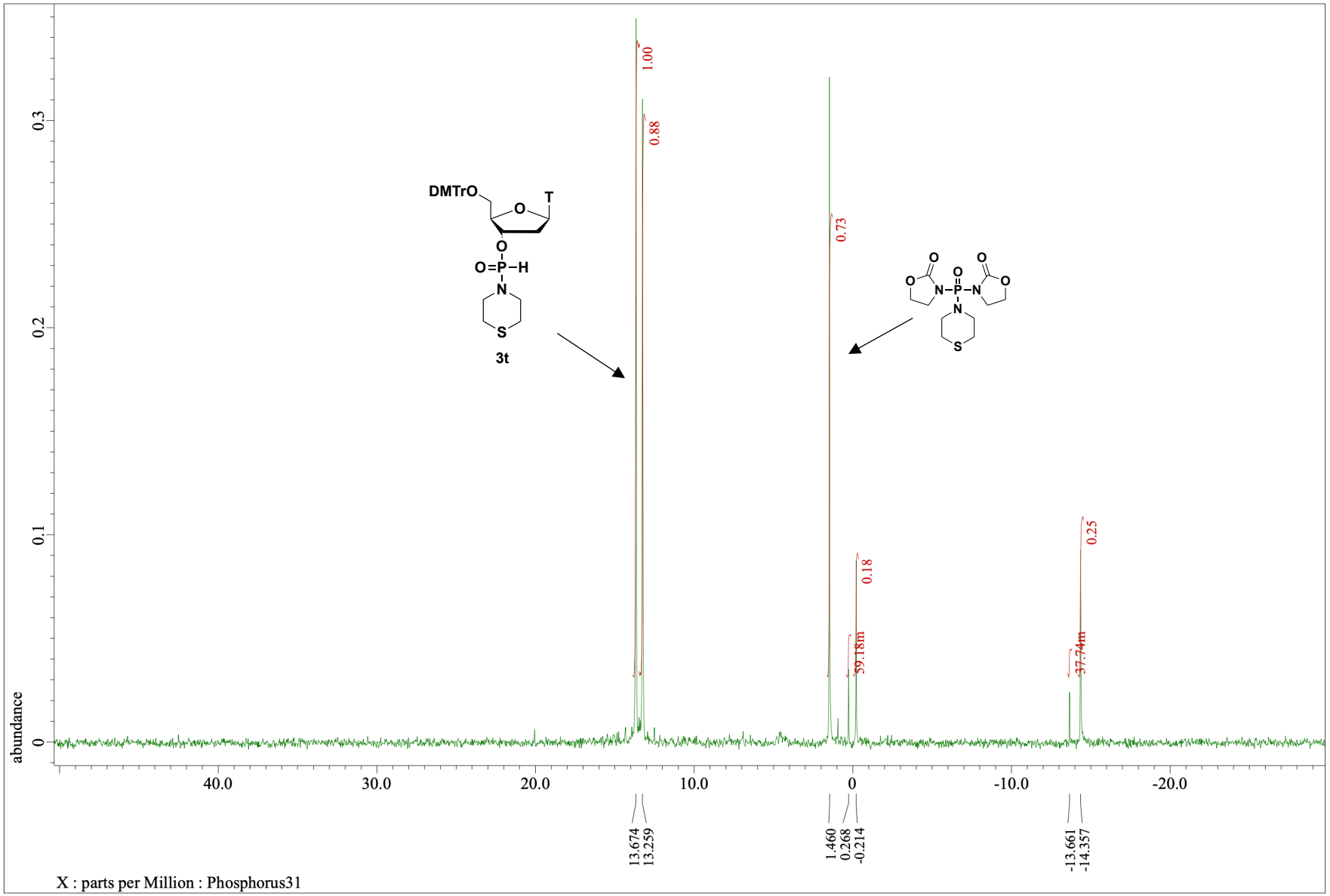


Fig. S2 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture of **3t** after extraction.

Table 1, entry 3

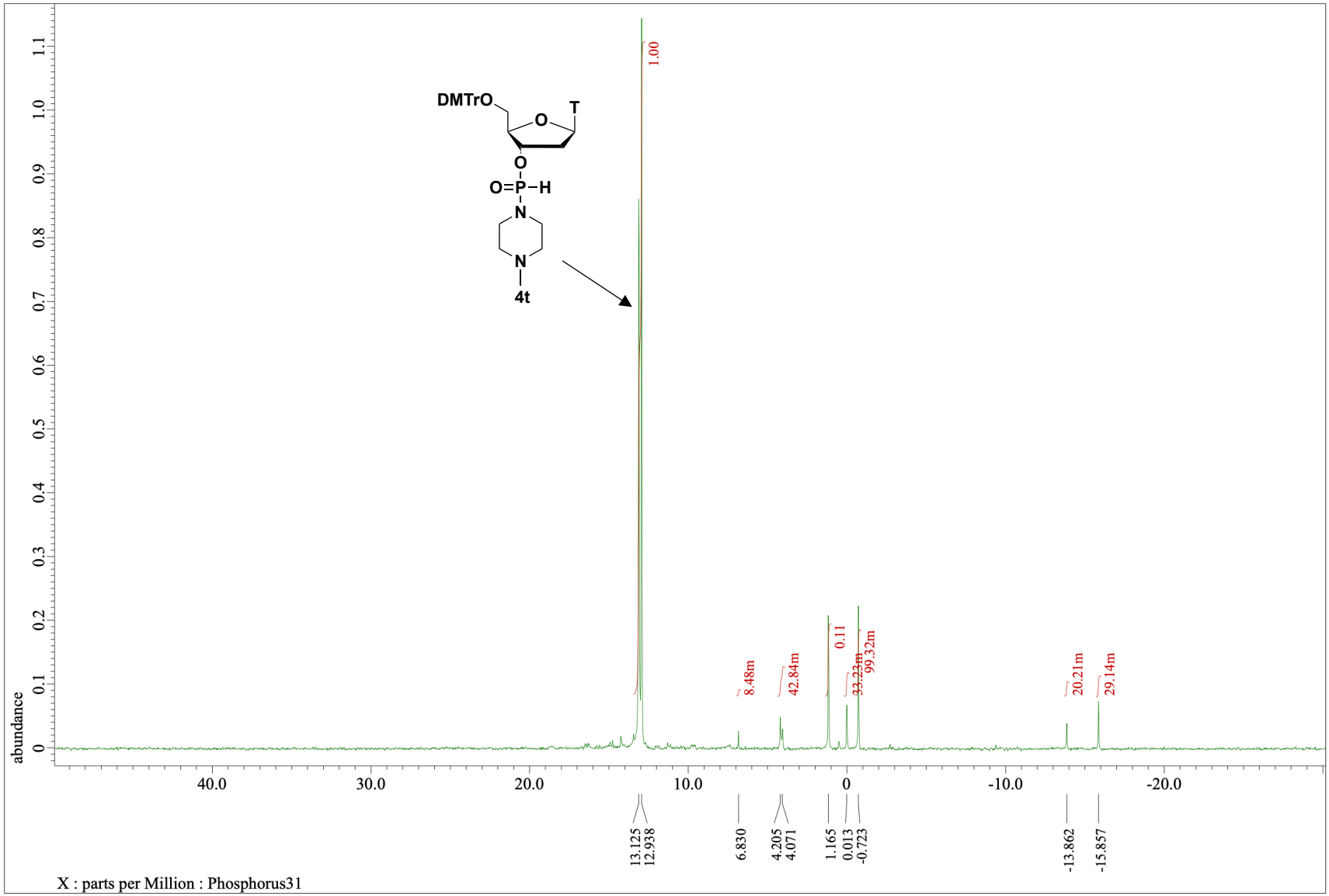


Fig. S3 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture of **4t** after extraction.

3. ^{31}P NMR analysis of the condensation of a 3'-*H*-phosphonamidate monomer 2t, 3t, or 4t with a thymidine derivative 5t during azeotropic manipulation.

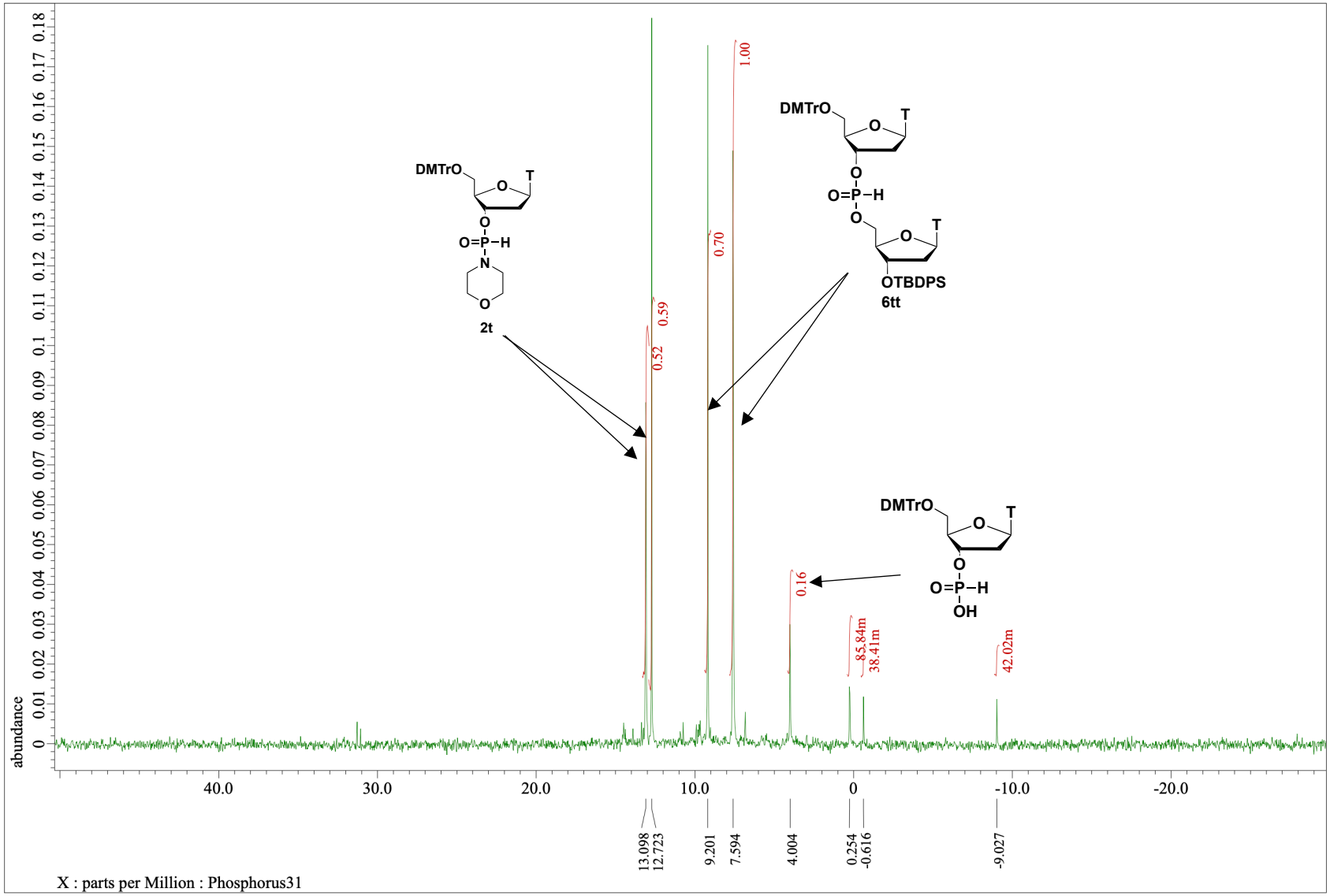


Fig. S4 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture after azeotropic manipulation. (*H*-phosphonamidate monomer **2t**)

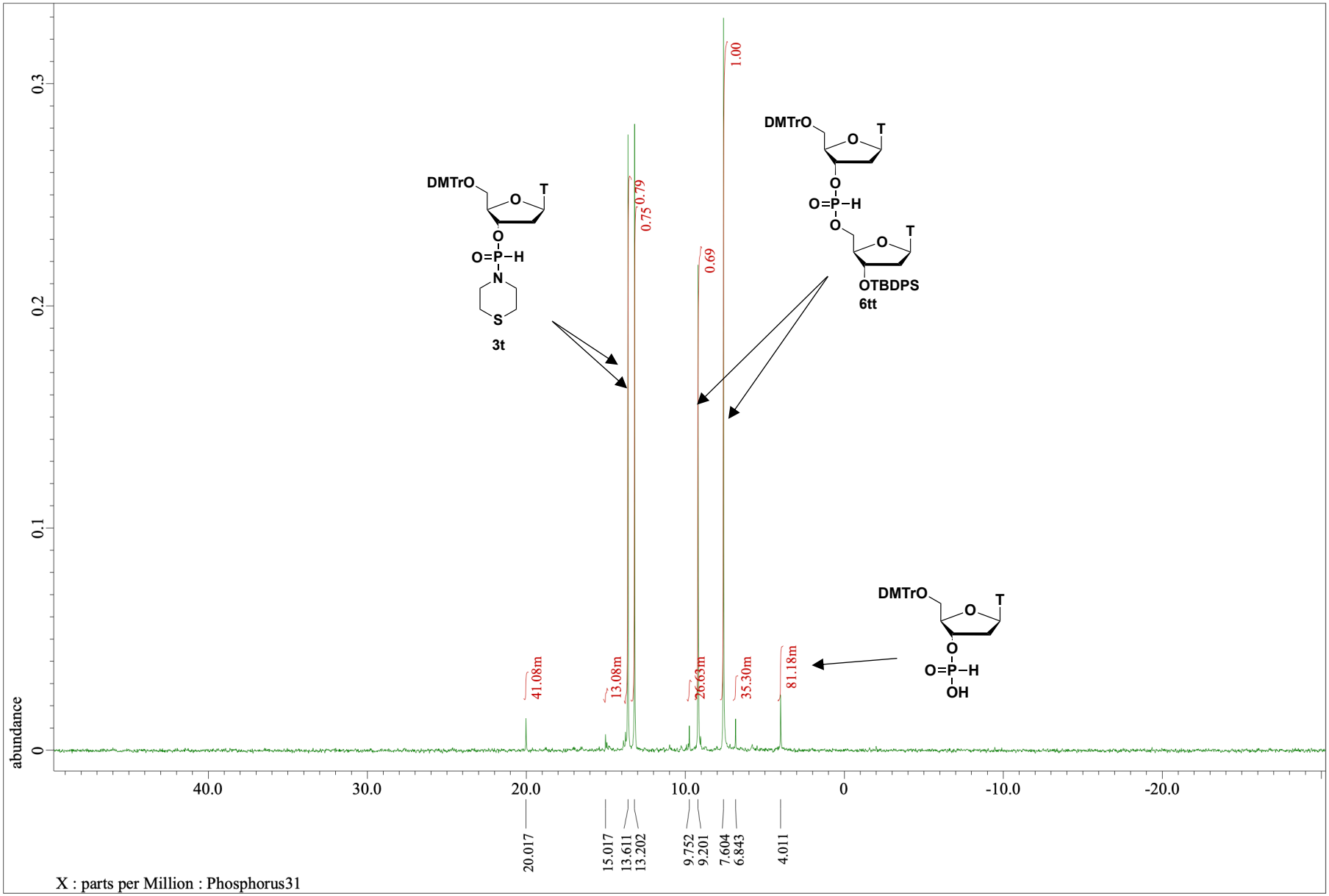


Fig. S5 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture after azeotropic manipulation. (*H*-phosphonamidate monomer **3t**)

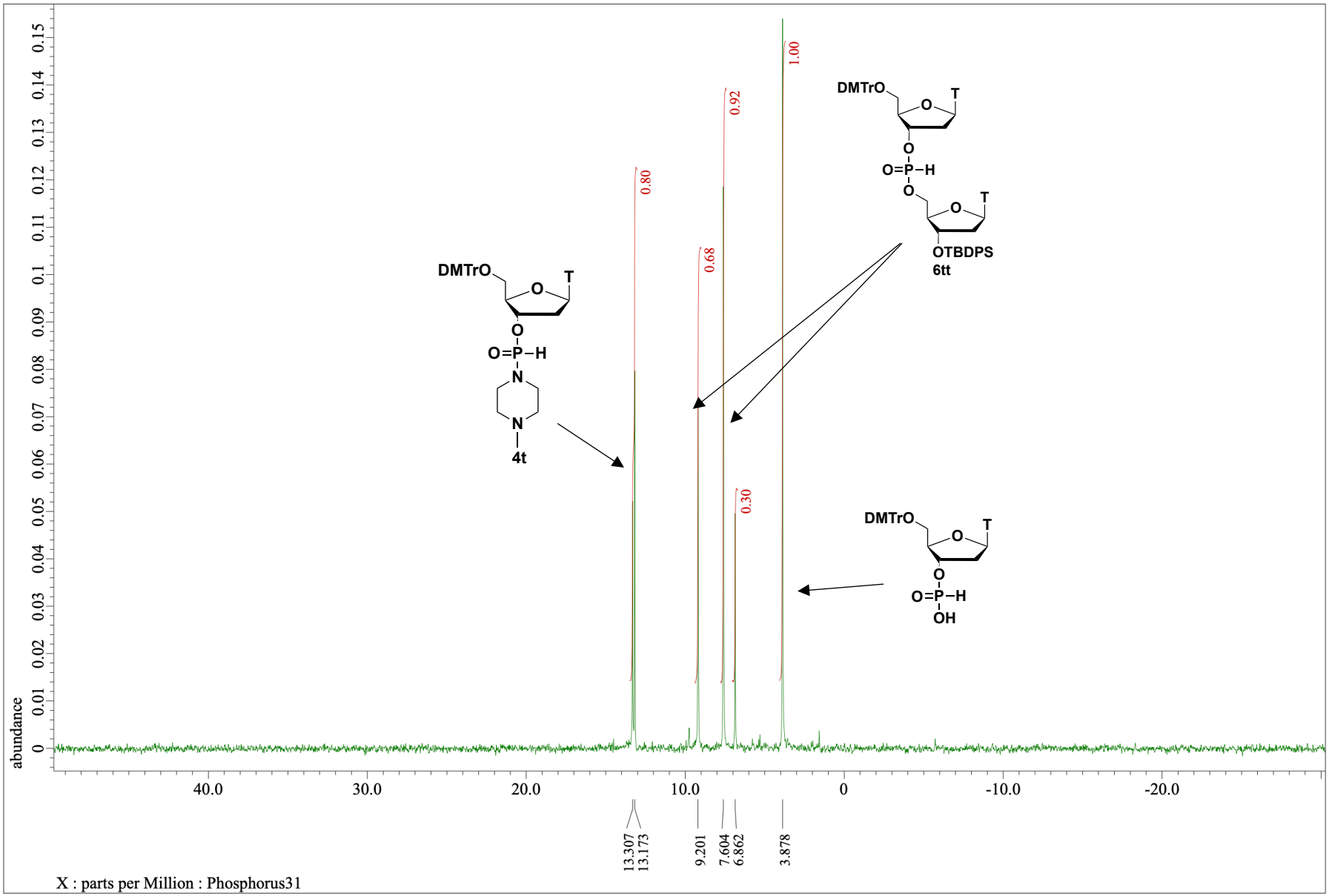


Fig. S6 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture after azeotropic manipulation. (*H*-phosphonamidate monomer **4t**)

4. ^{31}P NMR analysis of the condensation of a 3'-*H*-phosphonamidate monomer 3t with a thymidine derivative 5t (Table 2)

Table 2, entry 1

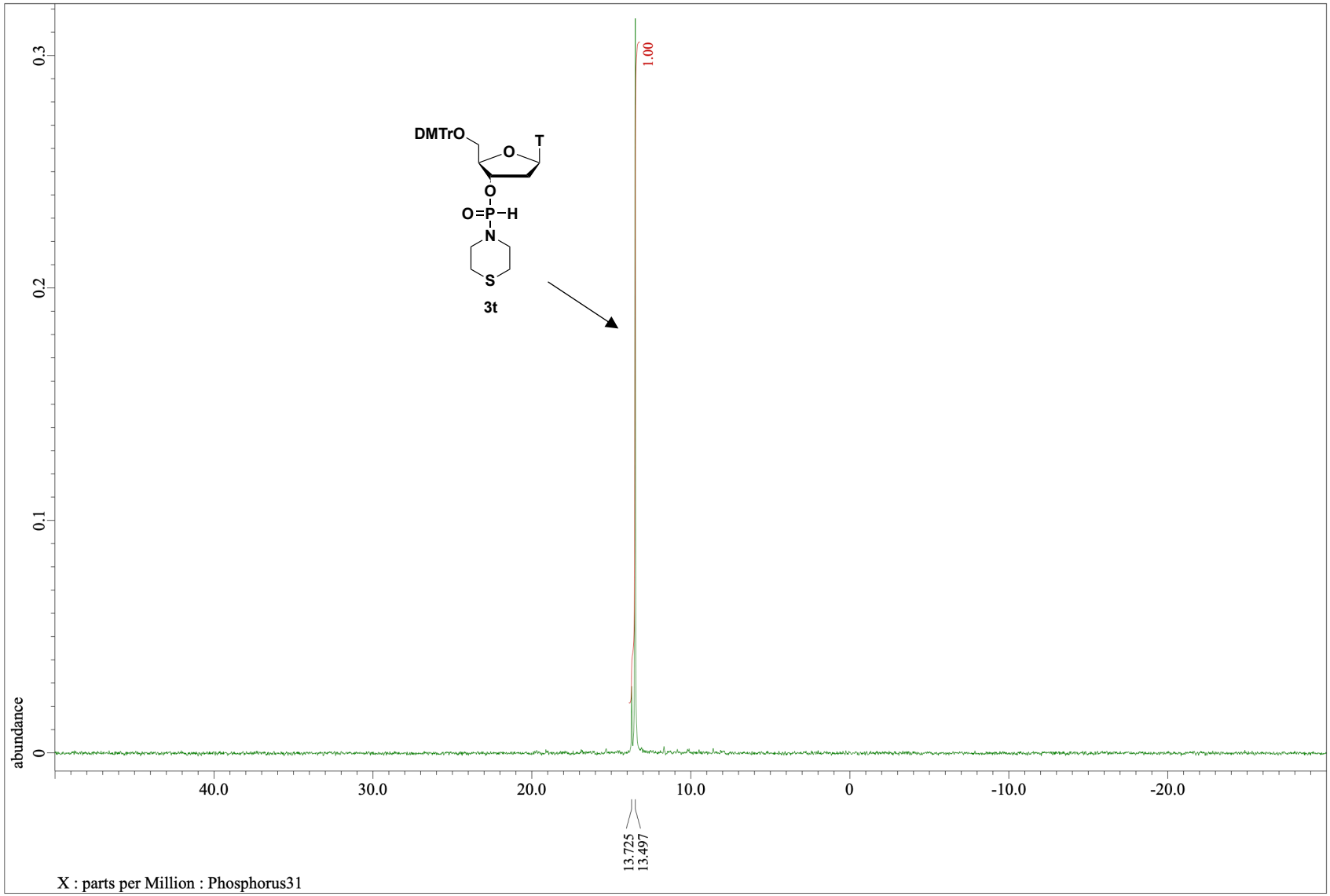


Fig. S7 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture (reaction conditions: 1 h at rt and 0.05 M).

Table 2, entry 2

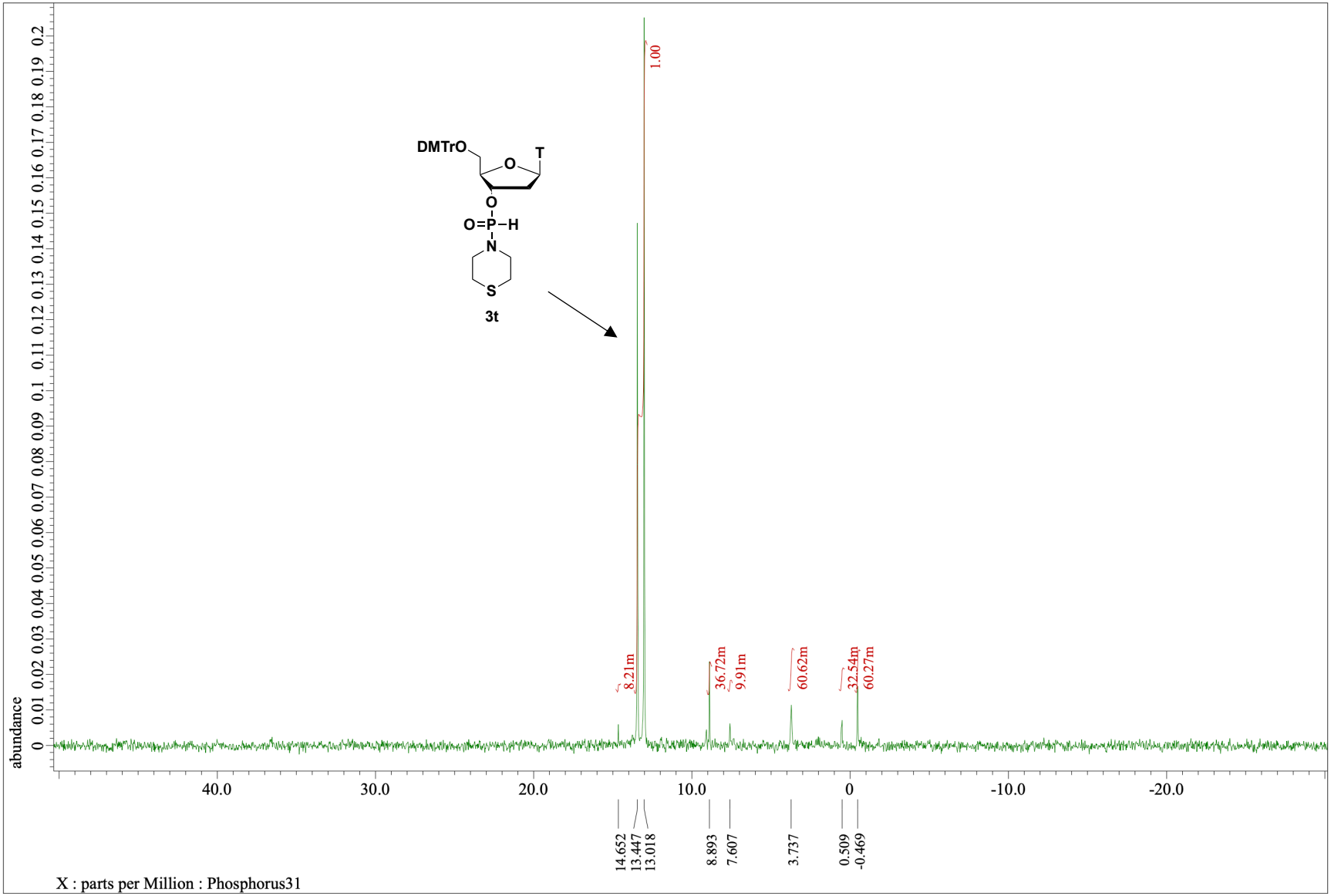


Fig. S8 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture (reaction conditions:1 h at 25 °C and 0.20 M).

Table 2, entry 3

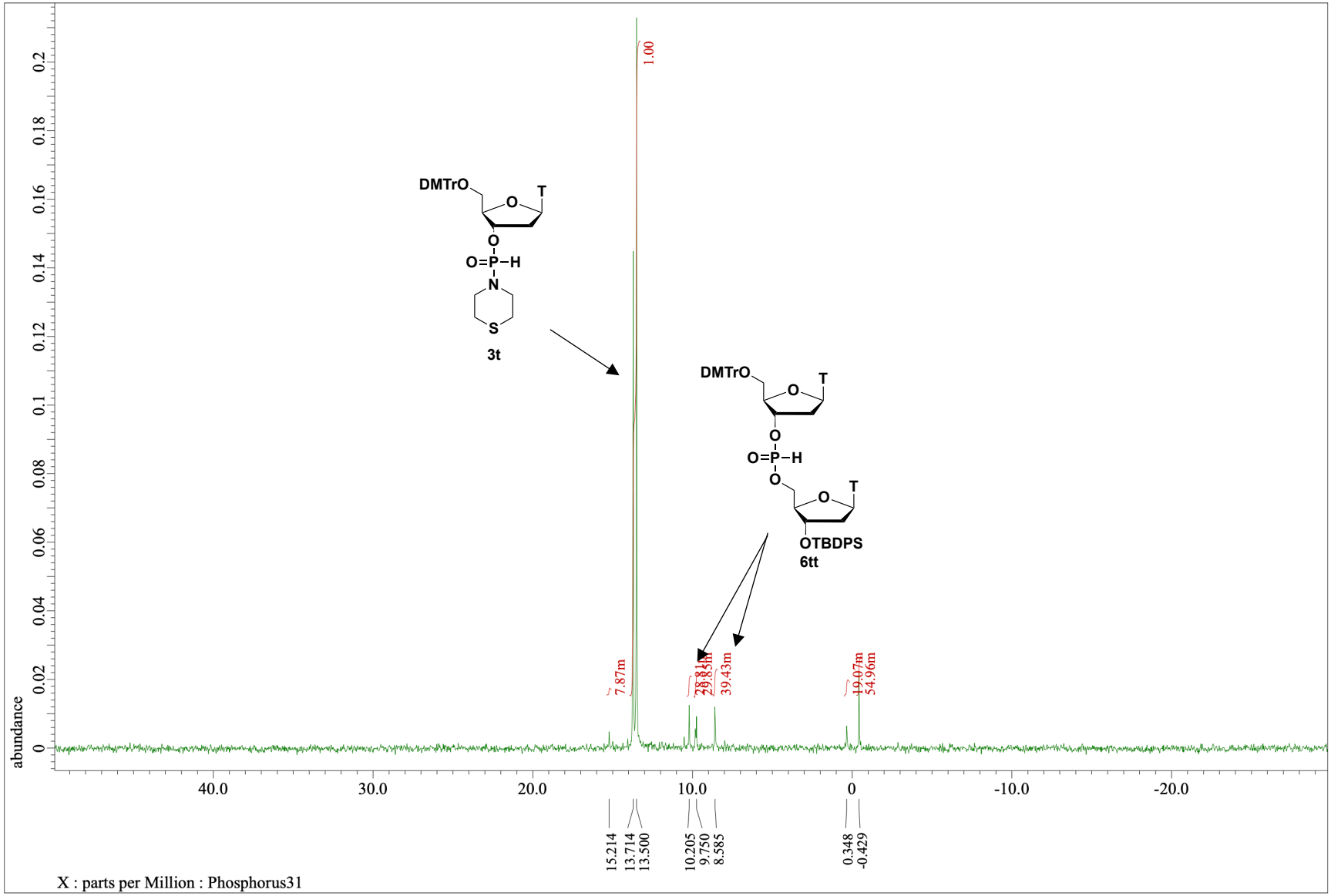


Fig. S9 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture (reaction conditions:1 h at 40 °C and 0.05 M).

Table 2, entry 4

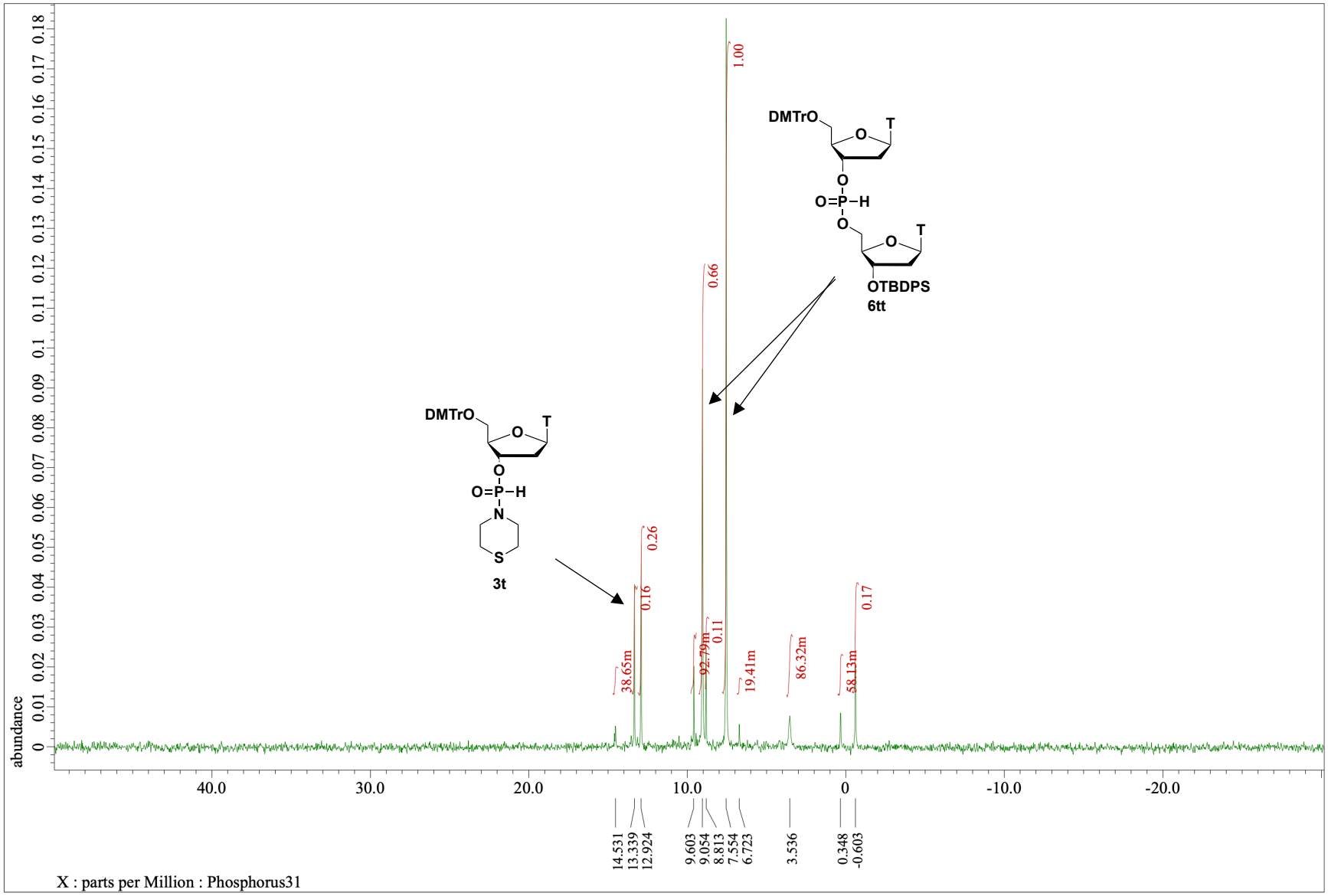


Fig. S10 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture (reaction conditions:1 h at 40 °C and 0.20 M).

Table 2, entry 5

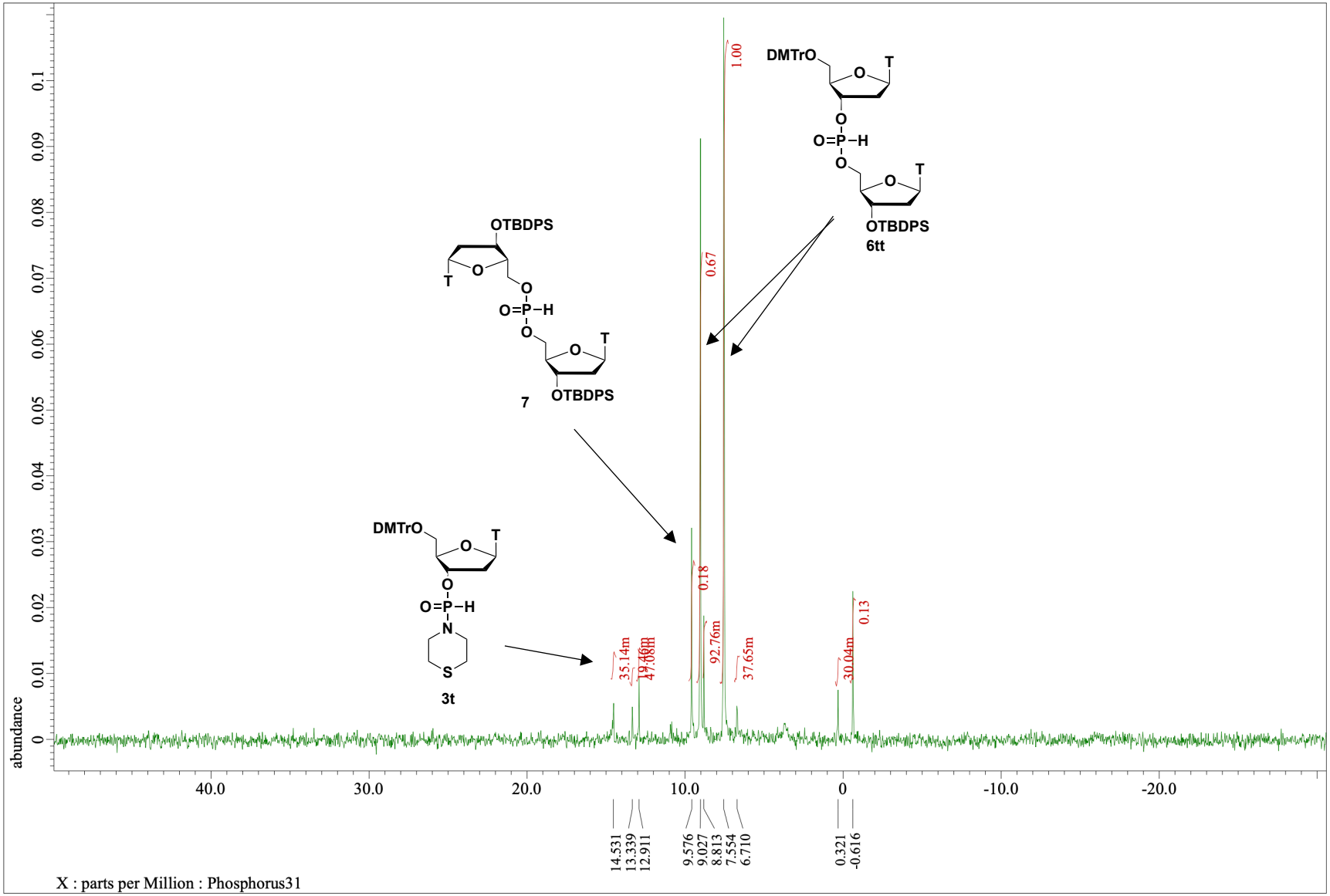


Fig. S11 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture (reaction conditions: 2 h at 40 °C and 0.20 M).

5. ^{31}P NMR analysis of the synthesis of *S*-cyanoethyl phosphorothioate diester ($T_{PSCE}T$) 9

Table 3, entry1

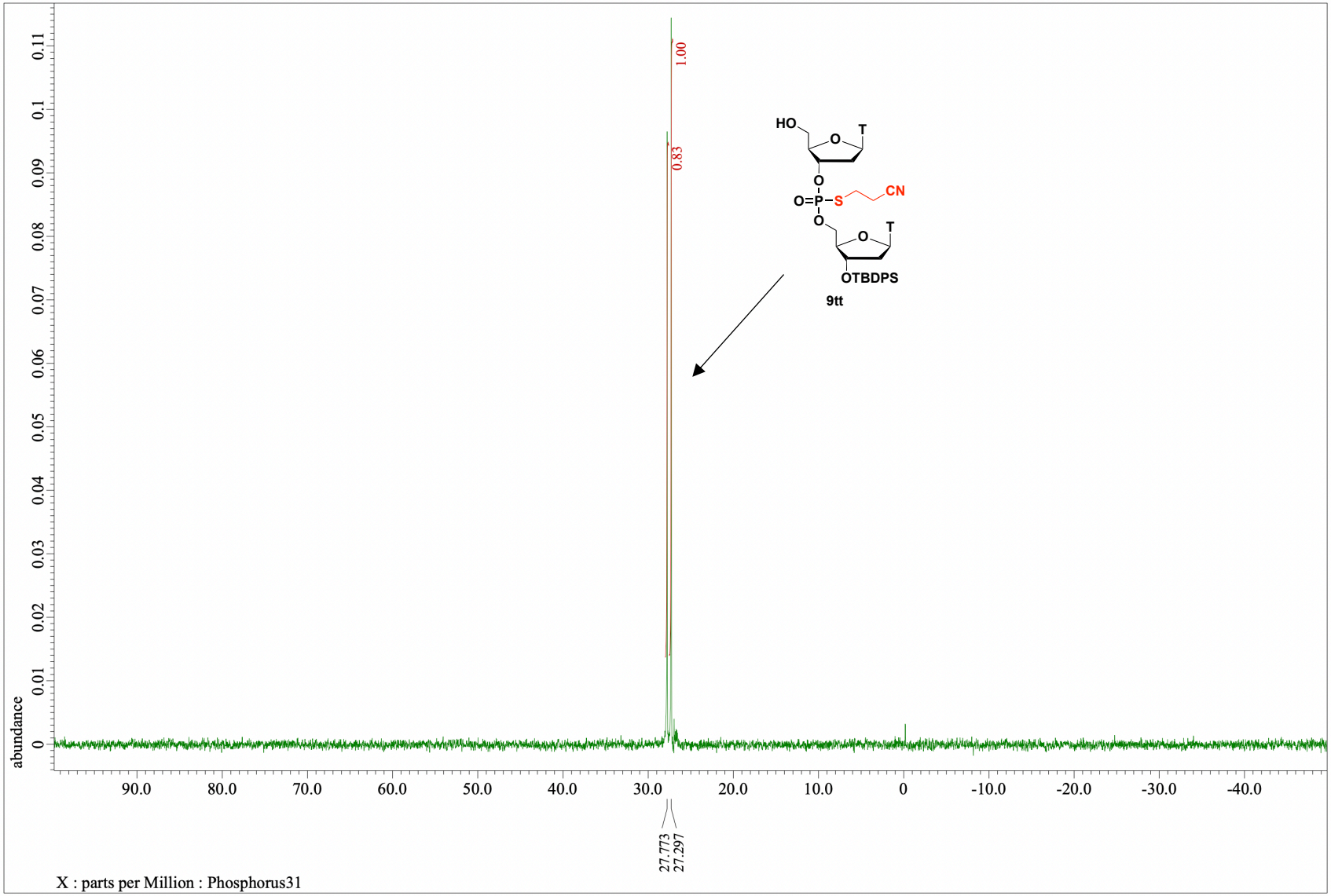


Fig. S12 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture of **9tt** after extraction.

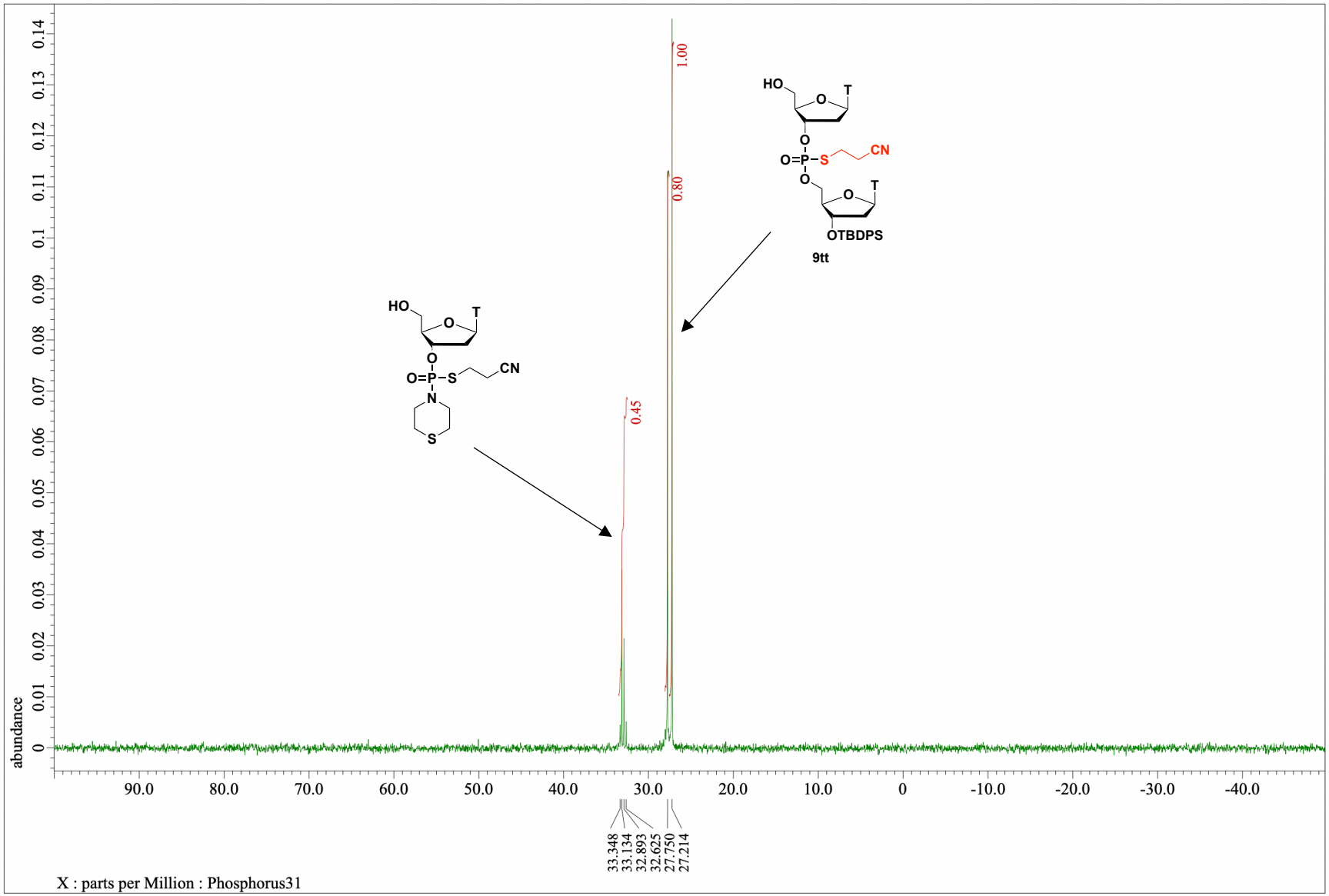
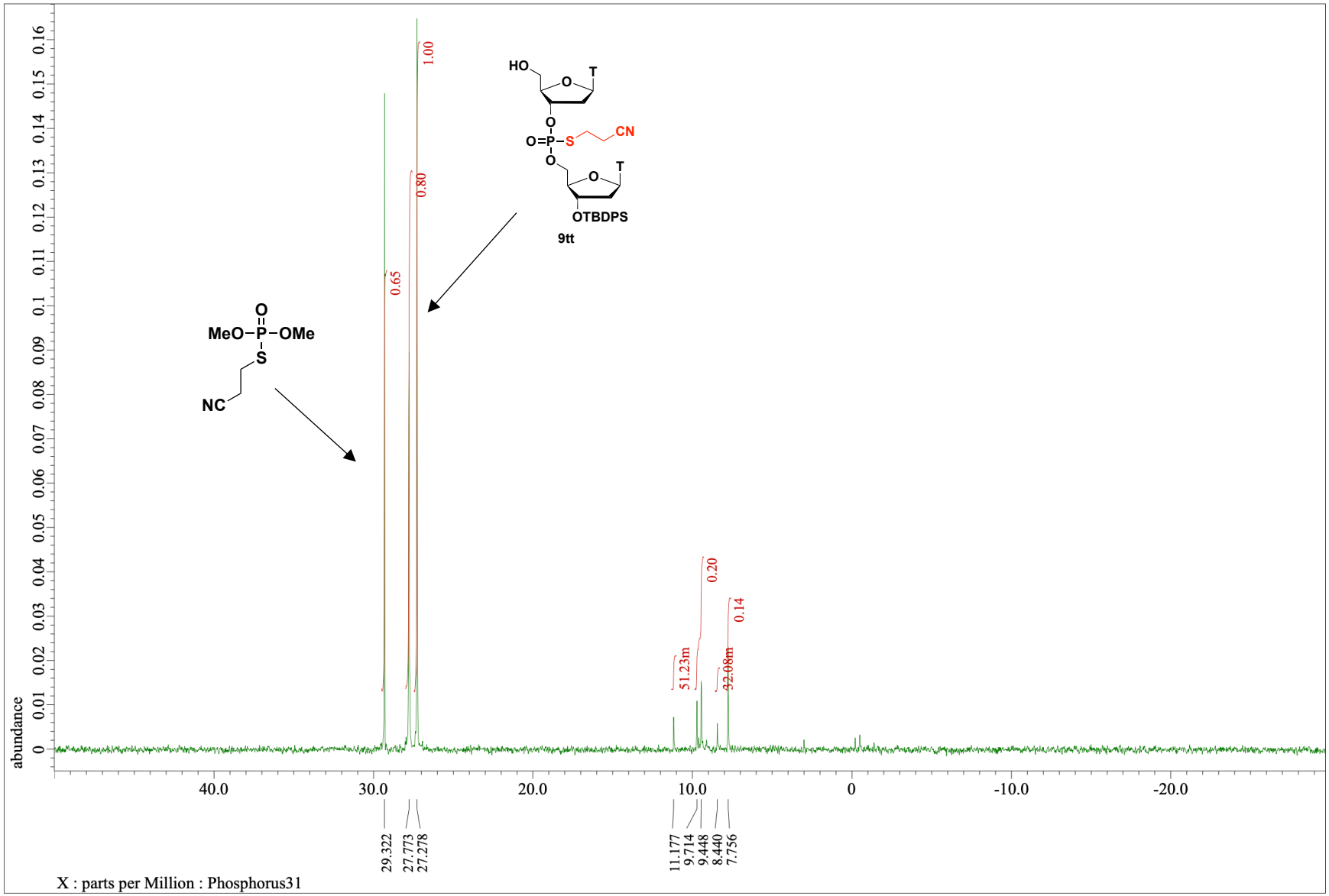


Fig. S13 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture of **9tt** after extraction. In the sulfurization reaction, BSA was added. The *H*-phosphonamidate monomer **3t** was also converted to the phosphorothioamidate monoester derivative which was not removed by extraction after detritylation.

6. ^{31}P NMR analysis of the synthesis of $T_{PS}T_{PS}T$ trimer 10 (Scheme 5)

A) ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture of **9tt** after extraction.



B) ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture of $T_{PSCE}T_{PSCE}T$ after extraction.

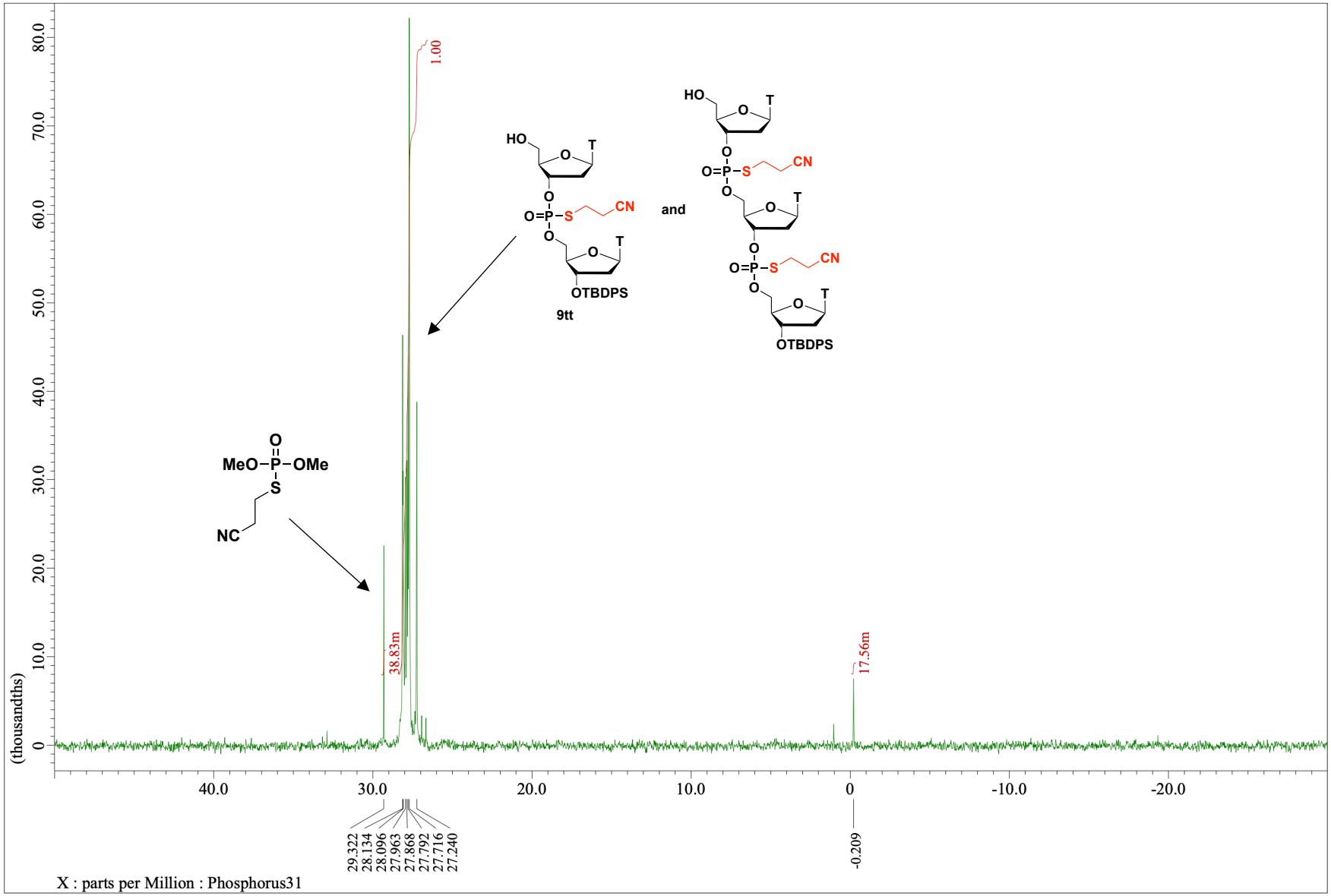
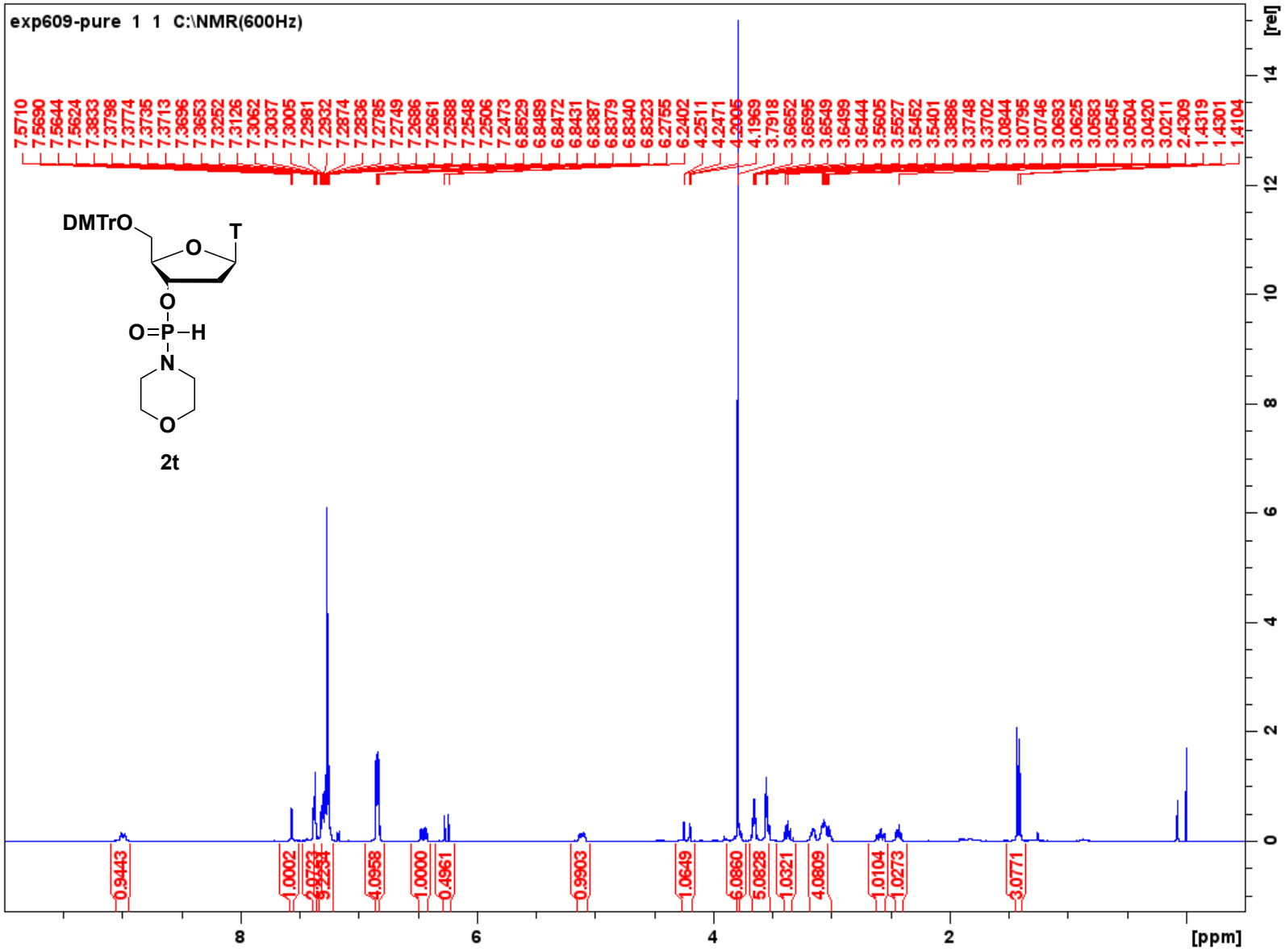


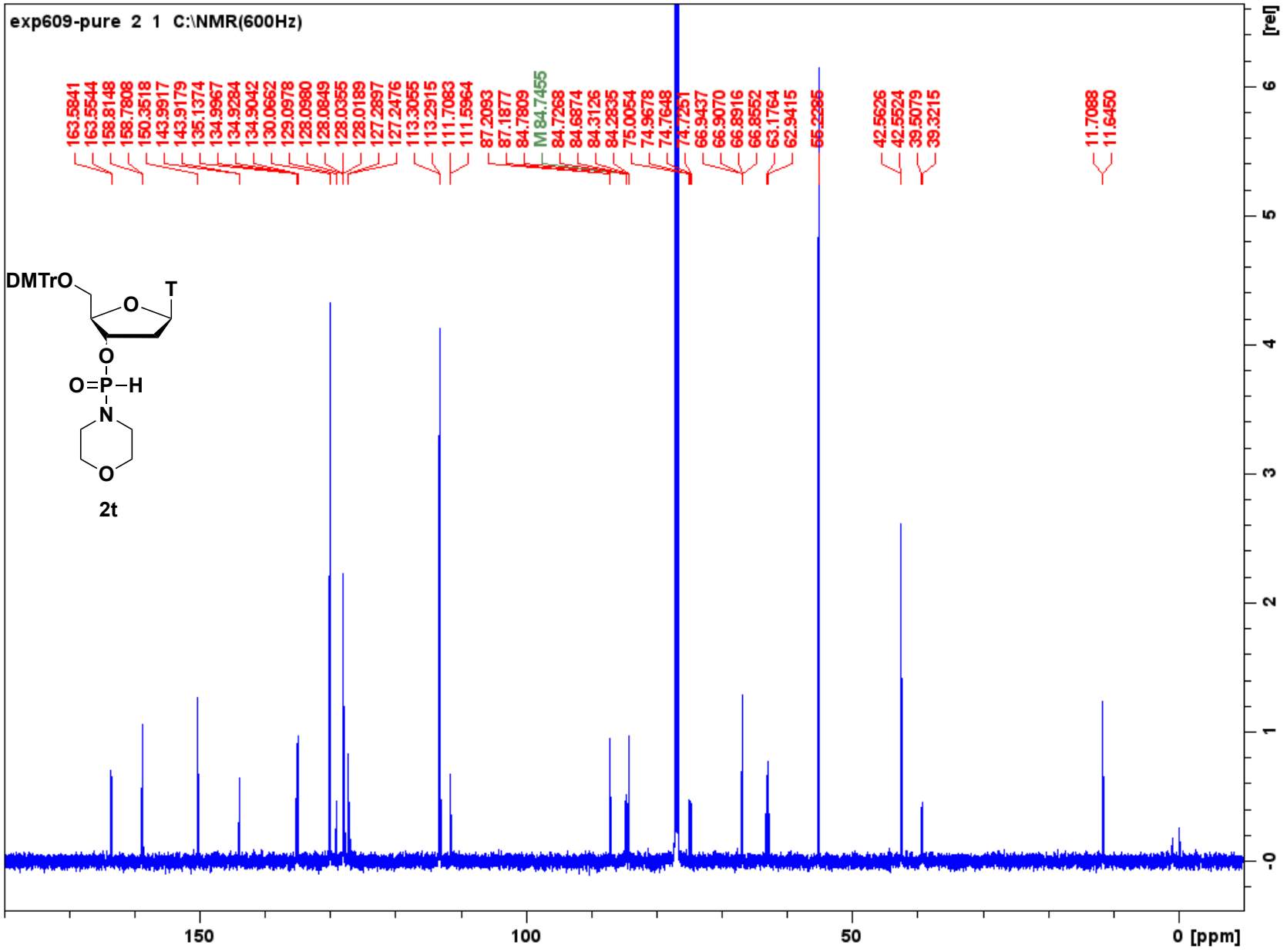
Fig. S14 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of the crude mixture after extraction. A) **9tt**, B) $T_{PSCE}T_{PSCE}T$

7. ^{1}H, ^{13}C, ^{31}P NMR spectra of isolated compounds

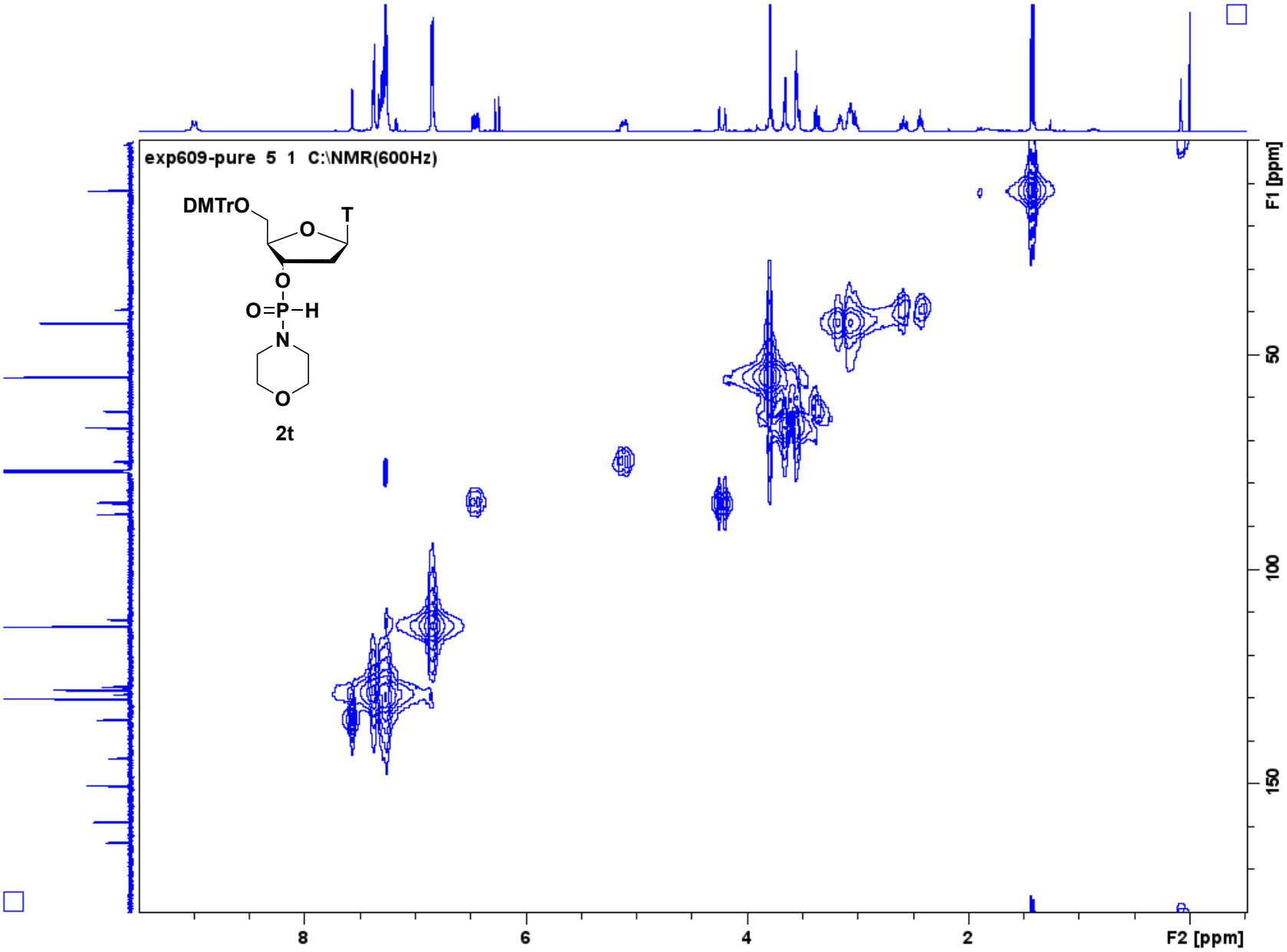
^{1}H-NMR (600 MHz, $CDCl_3$)



$^{13}C\{H\}$-NMR (151 MHz, $CDCl_3$)



HMQC ($CDCl_3$)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4
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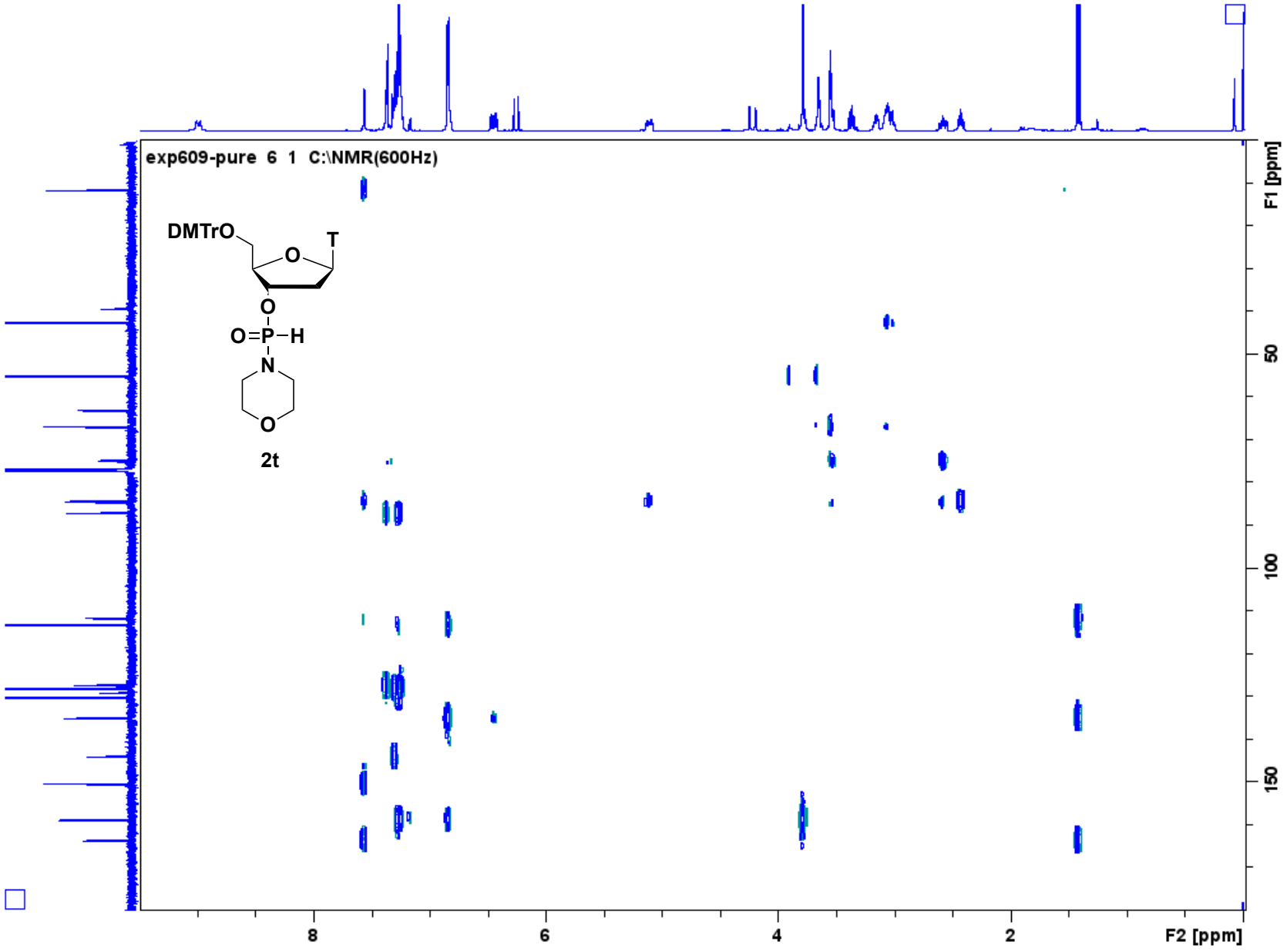
HMBC ($CDCl_3$)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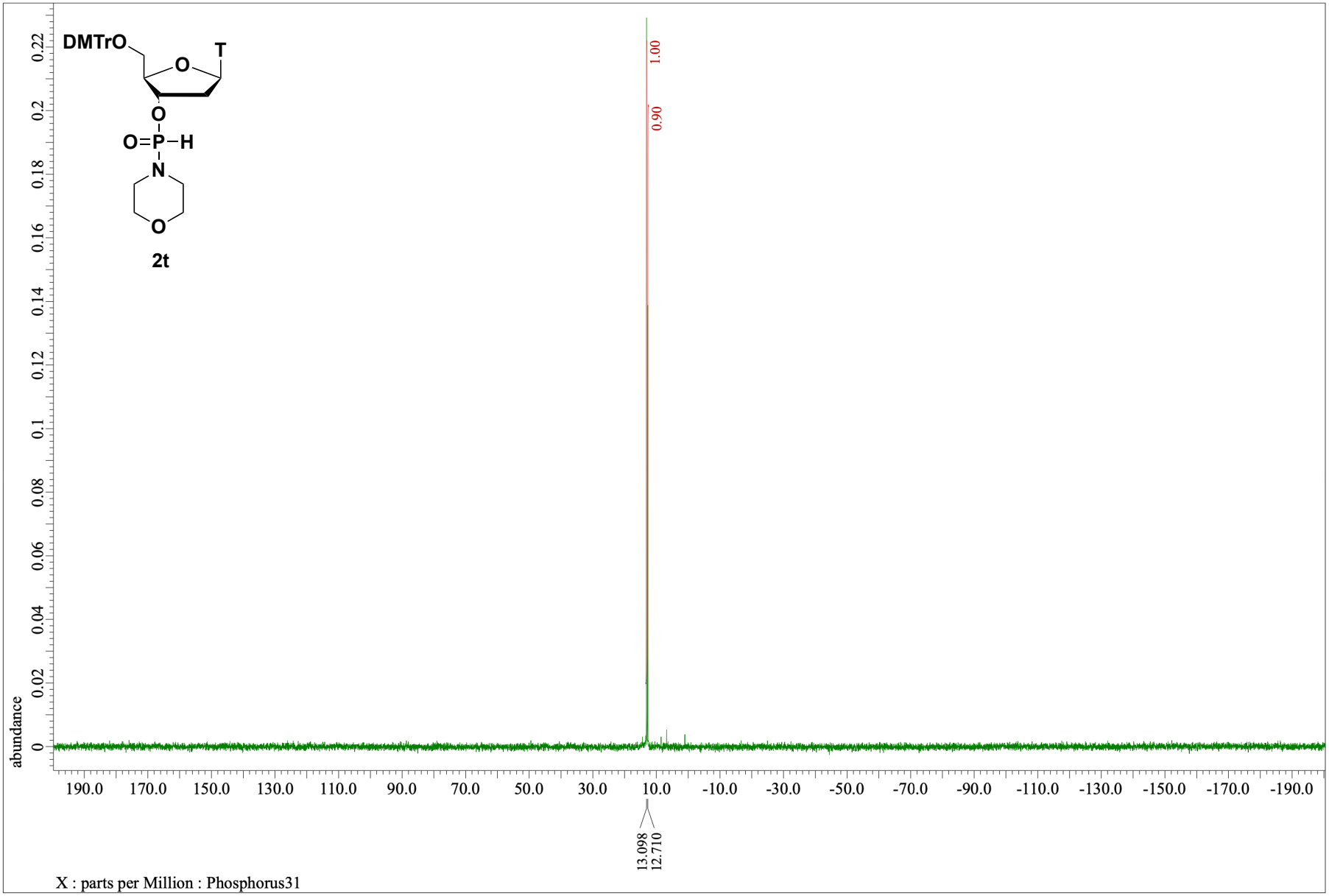
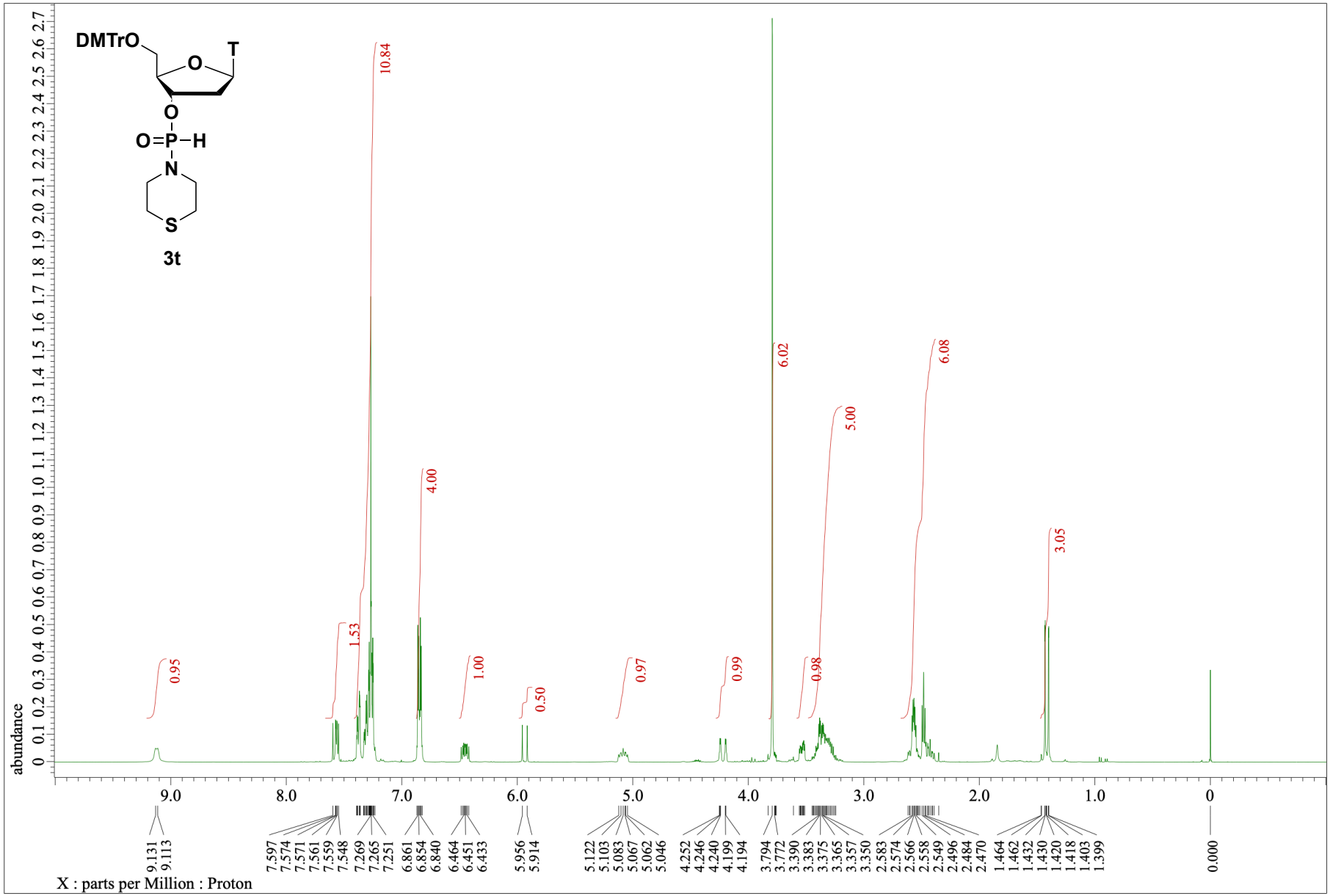
F2 [ppm]

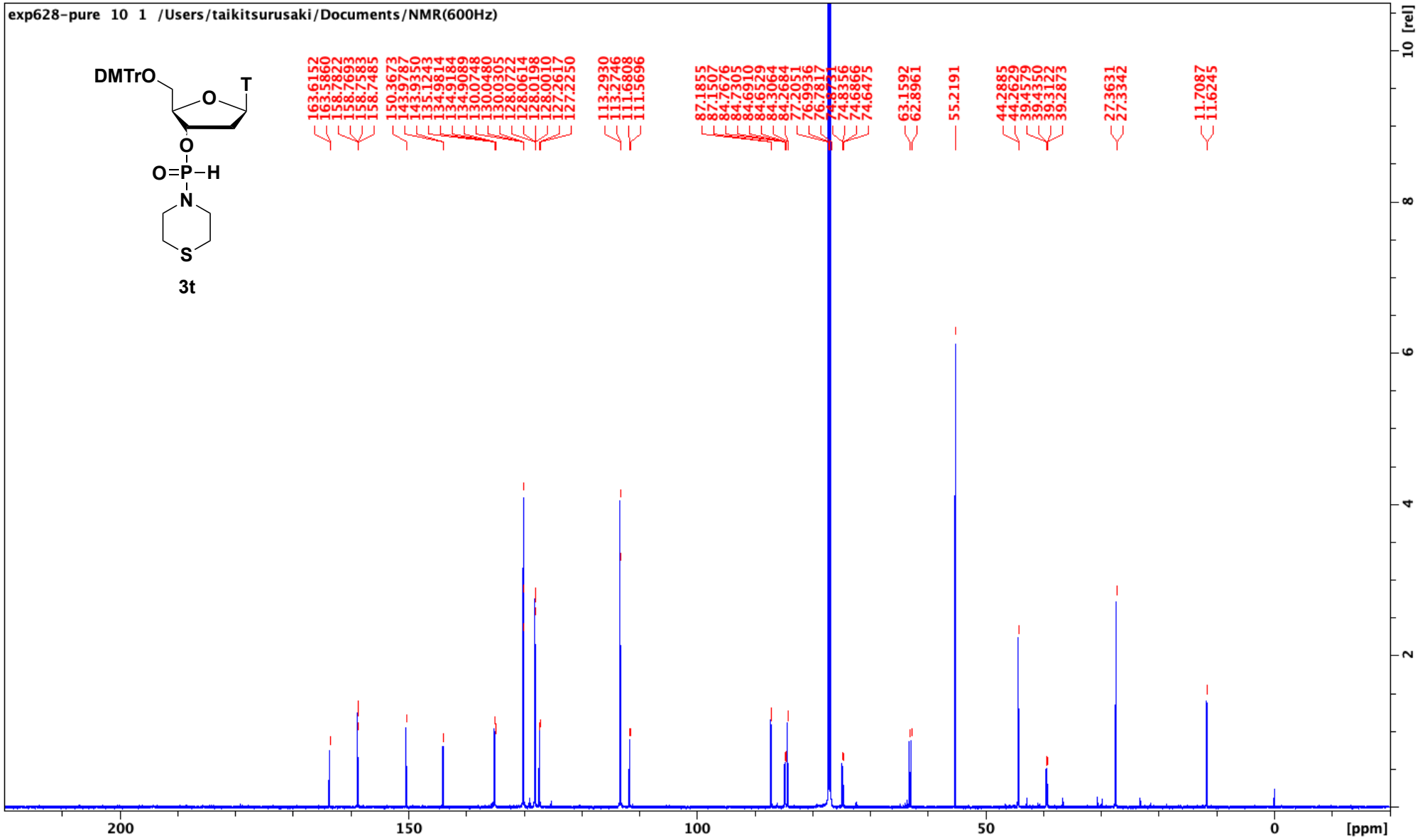
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



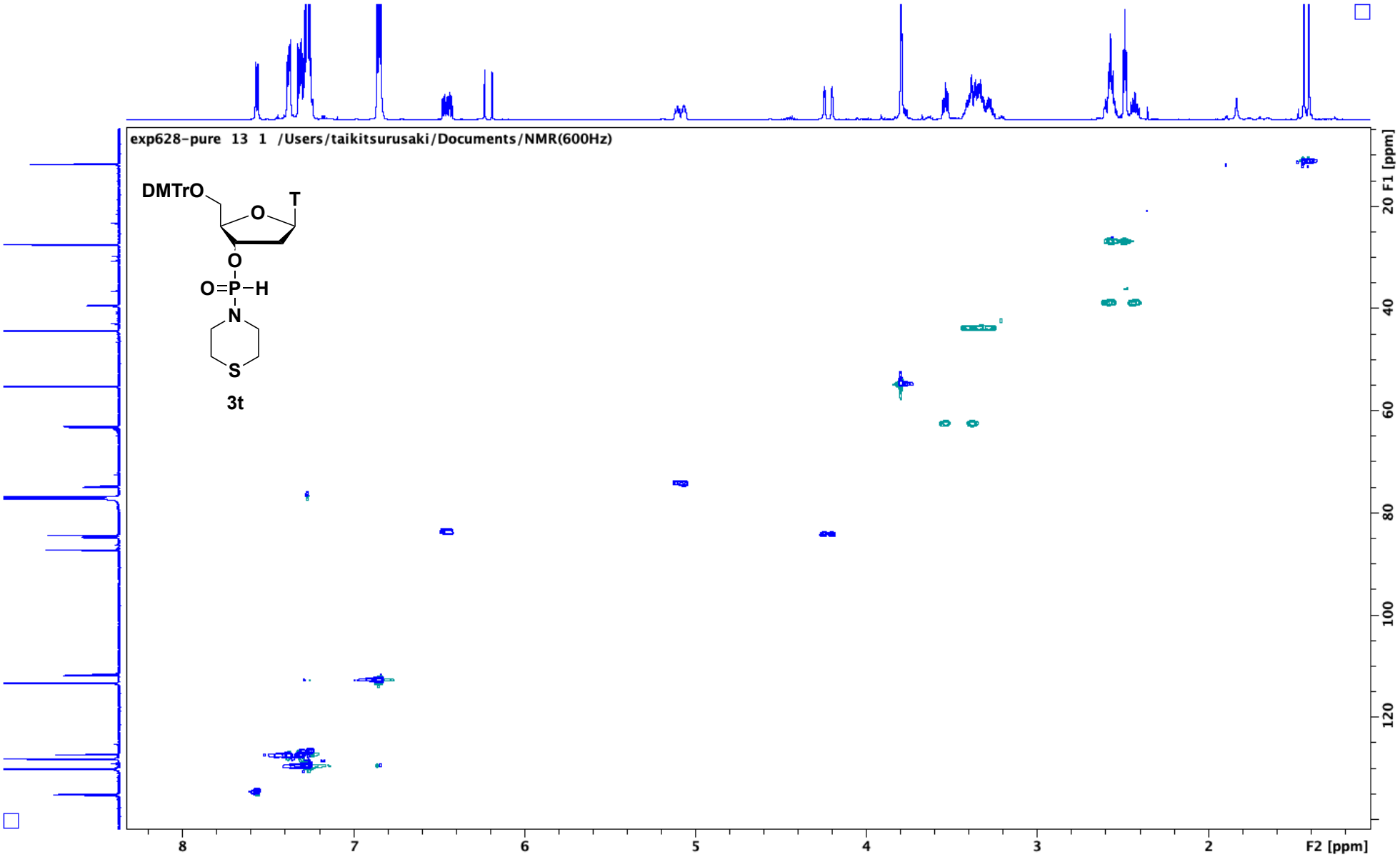
^{1}H-NMR (400 MHz, $CDCl_3$)



$^{13}C\{H\}$-NMR (151 MHz, $CDCl_3$)



HSQC ($CDCl_3$)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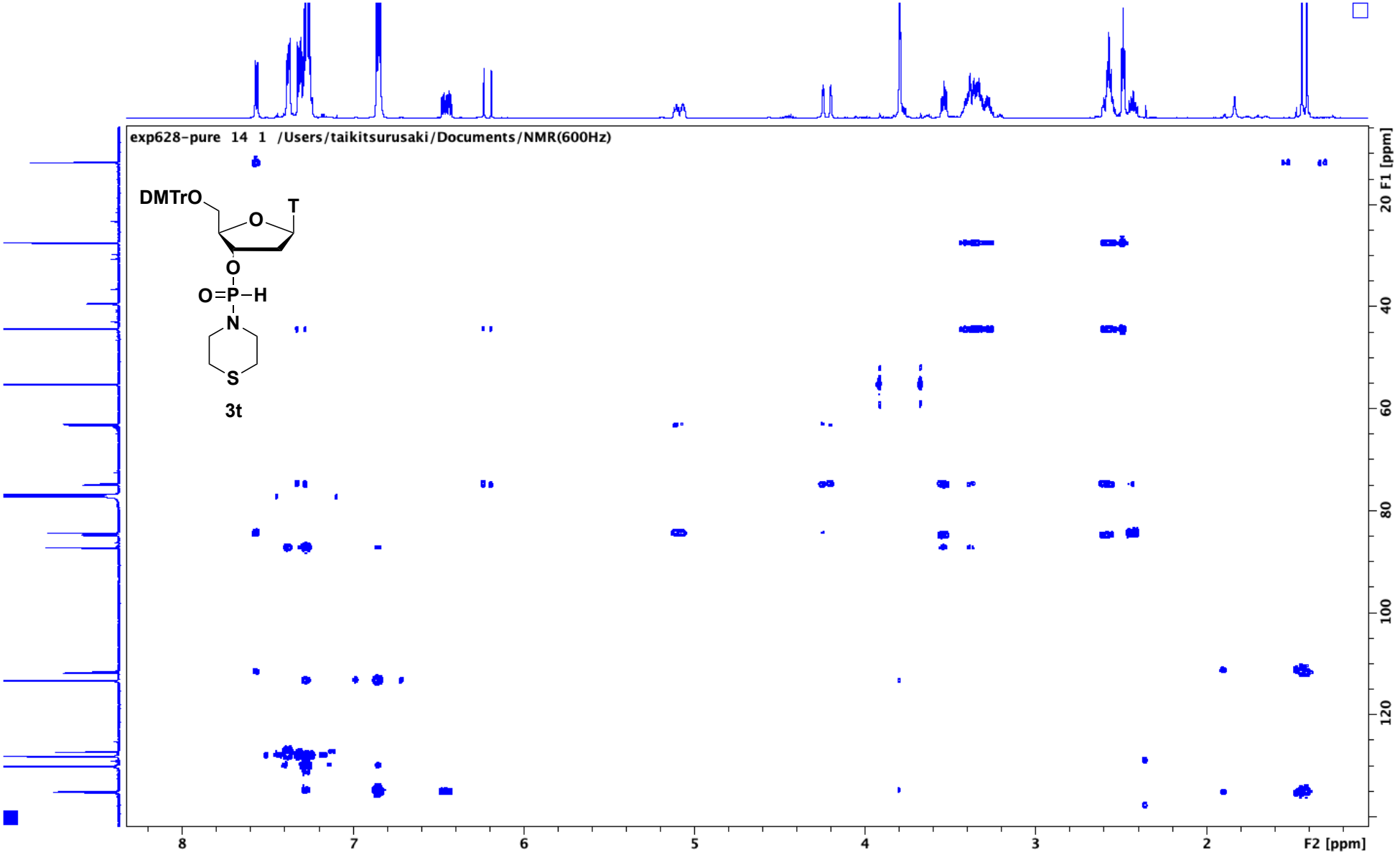
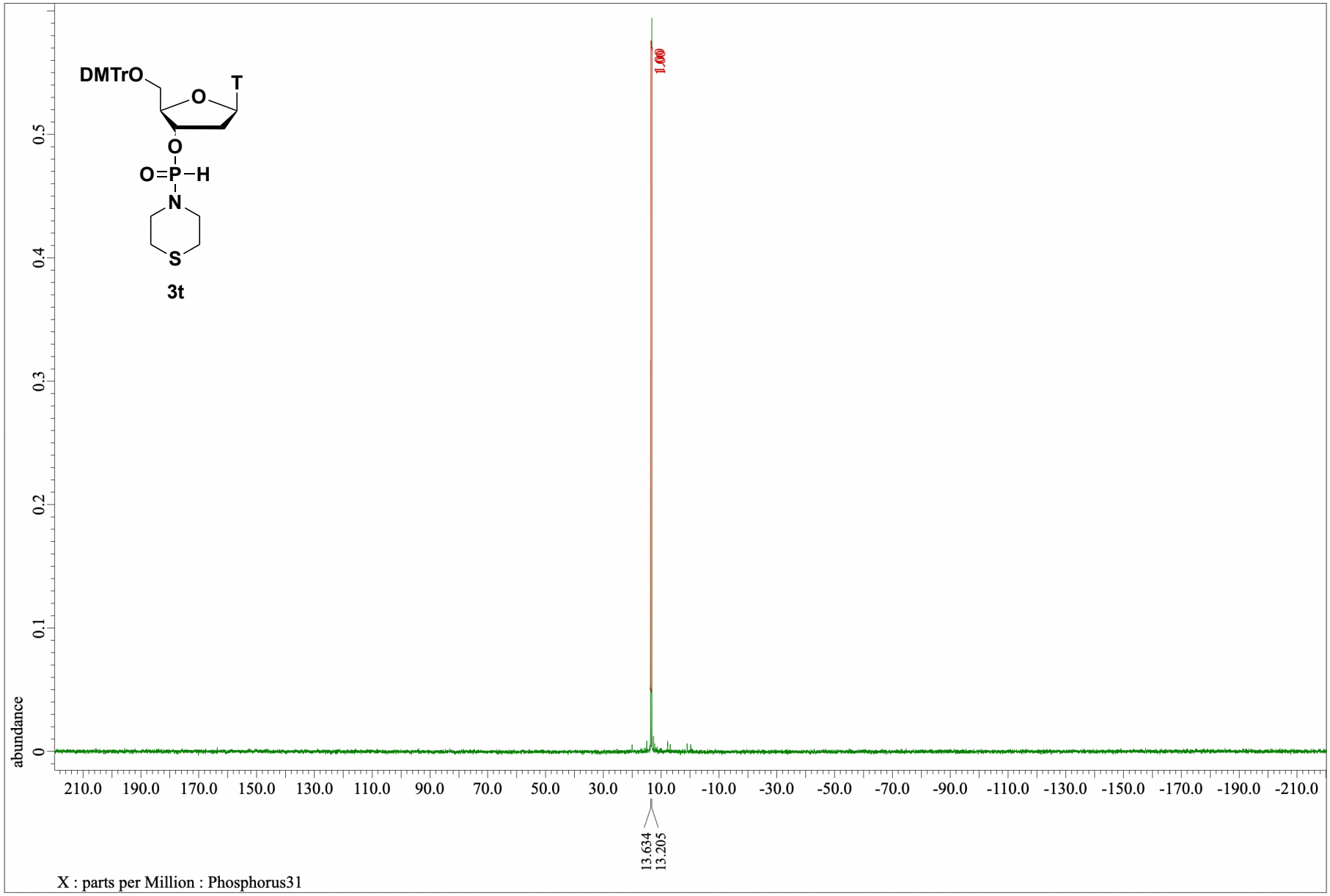
5
4
3
2
F2 [ppm]

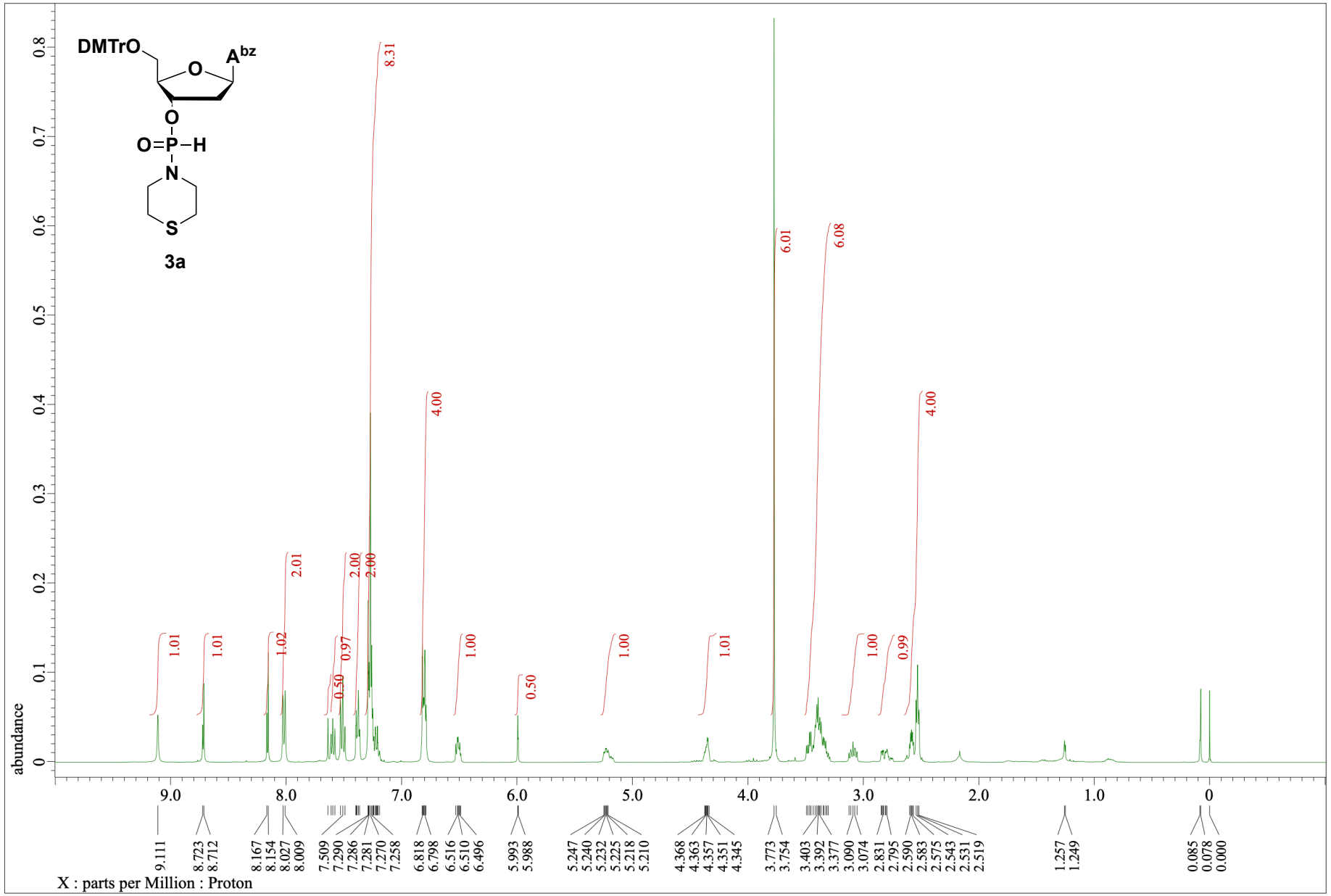
HMBC ($CDCl_3$)


exp628-pure 14 1 /Users/taikitsurusaki/Documents/NMR(600Hz)
DMTrO
O
T
O
O=P–H
N
S
3t
F2 [ppm]
F1 [ppm]

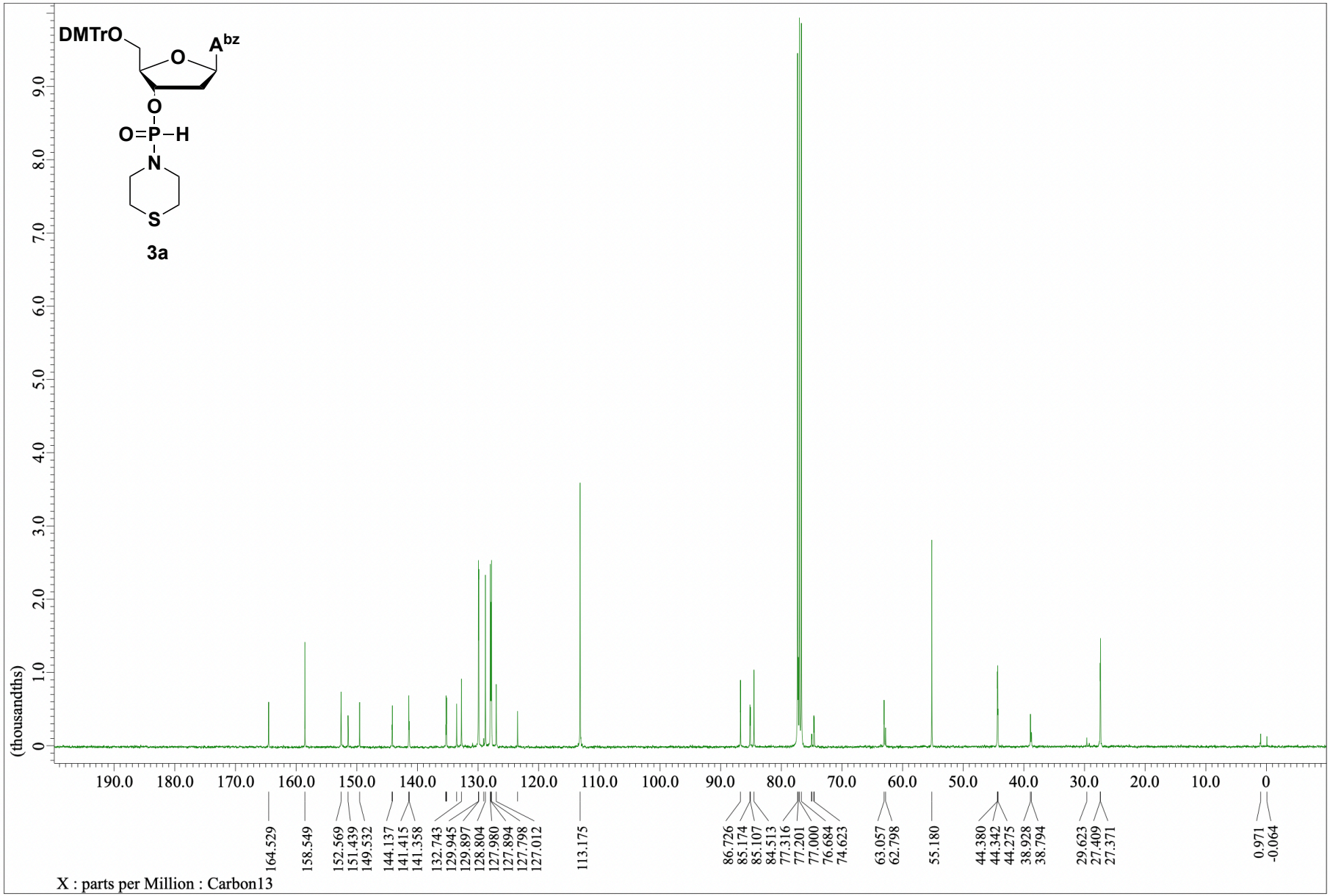
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



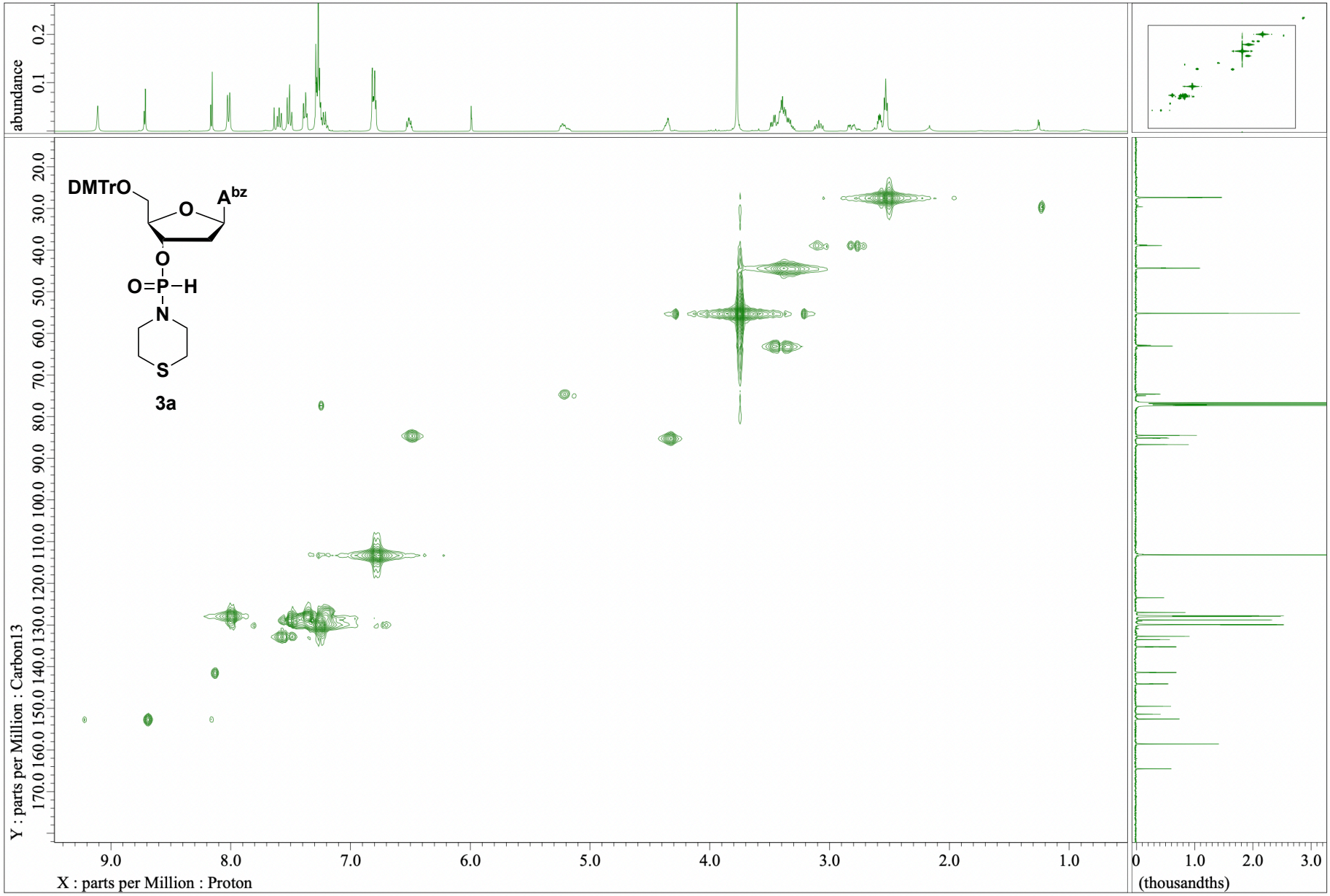
^{1}H-NMR (400 MHz, $CDCl_3$)



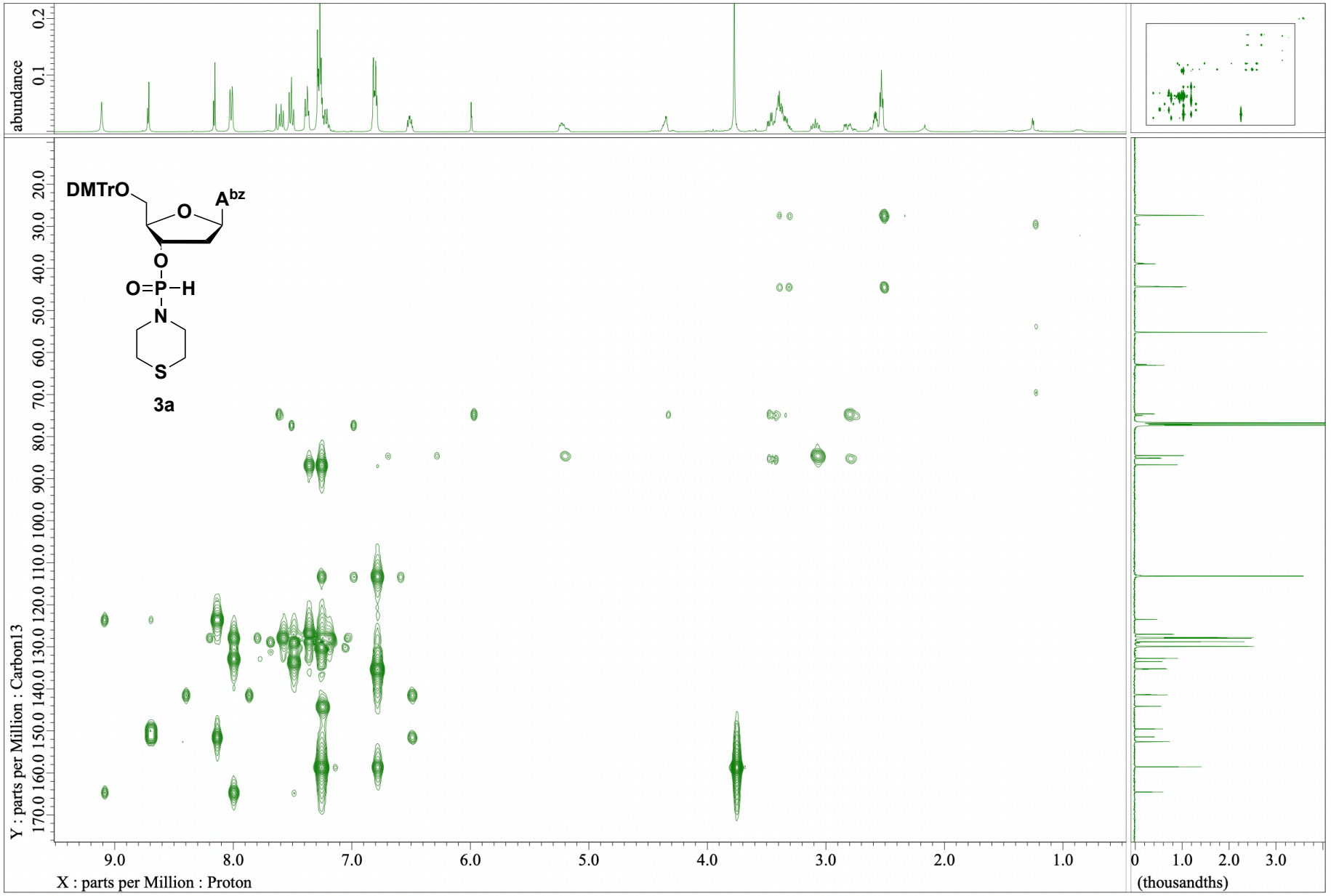
$^{13}C\{H\}$-NMR (100 MHz, $CDCl_3$)



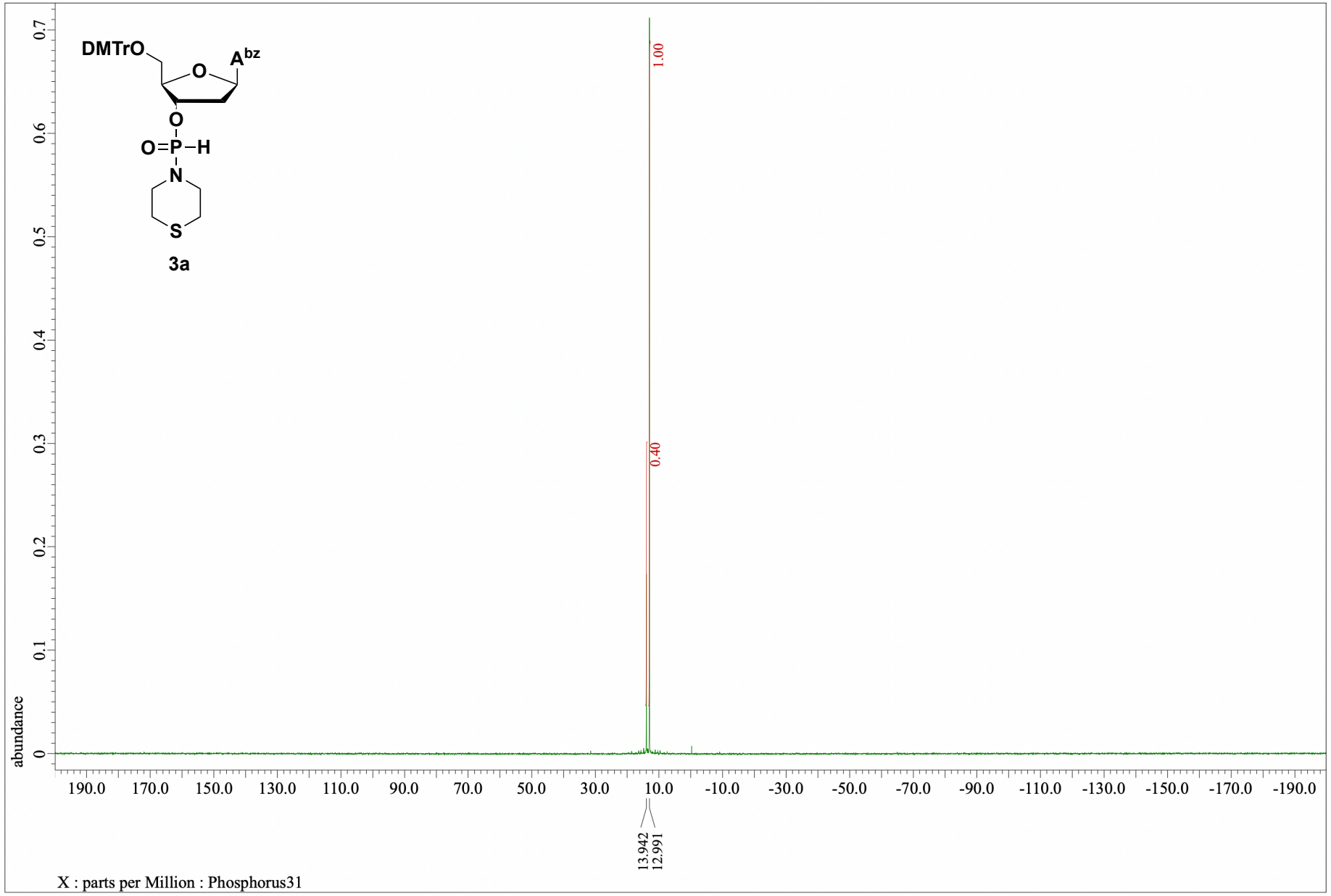
HMQC ($CDCl_3$)


abundance
0.2
0.1
DMTrO
O
A^bz
O
O=P–H
N
S
3a
Y : parts per Million : Carbon13
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
1.0
2.0
3.0
(thousandths)

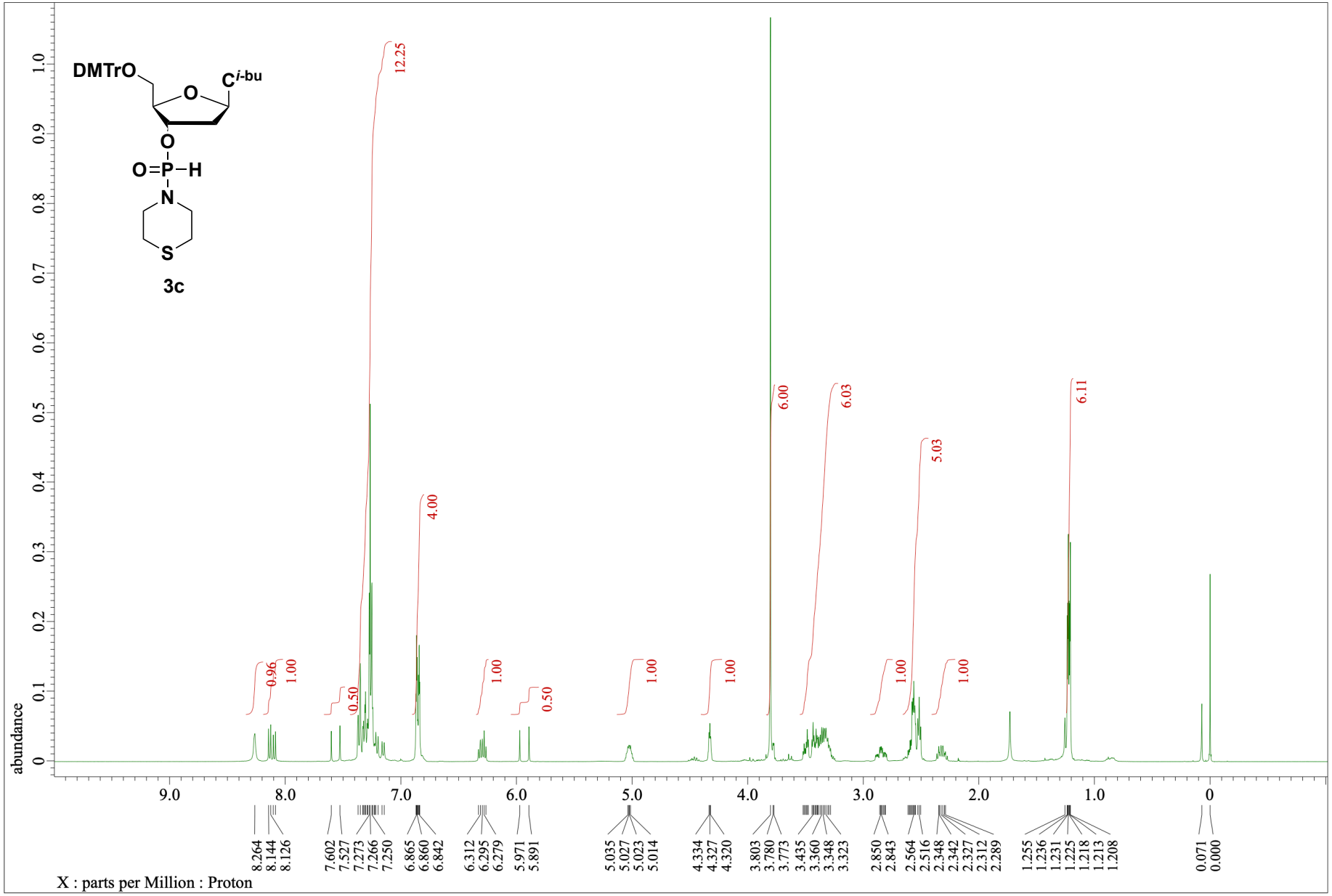
HMBC ($CDCl_3$)


abundance
0.2
0.1
DMTrO
O
A^bz
O
O=P–H
N
S
3a
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0
Y : parts per Million : Carbon13
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
1.0
2.0
3.0
(thousandths)

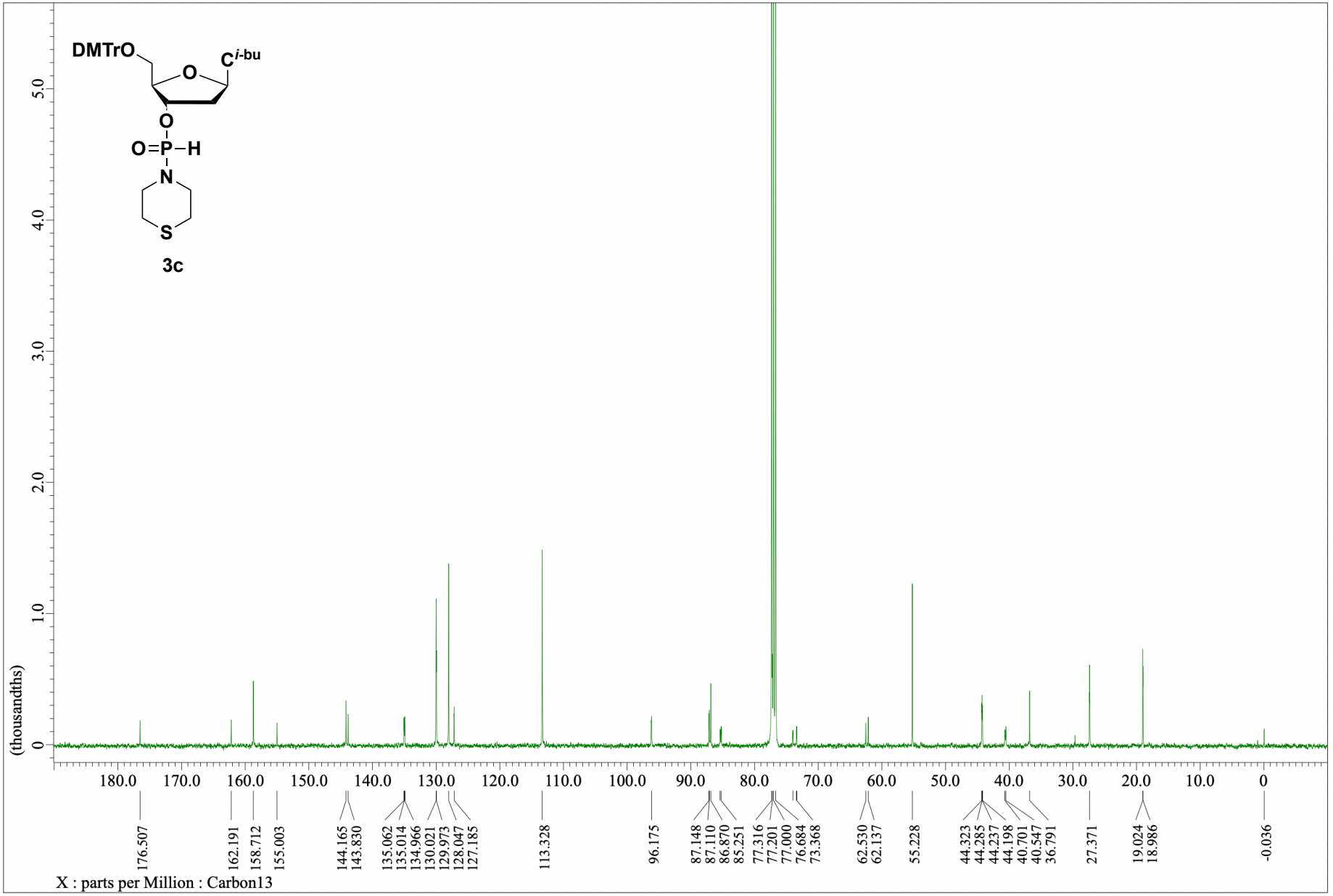
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



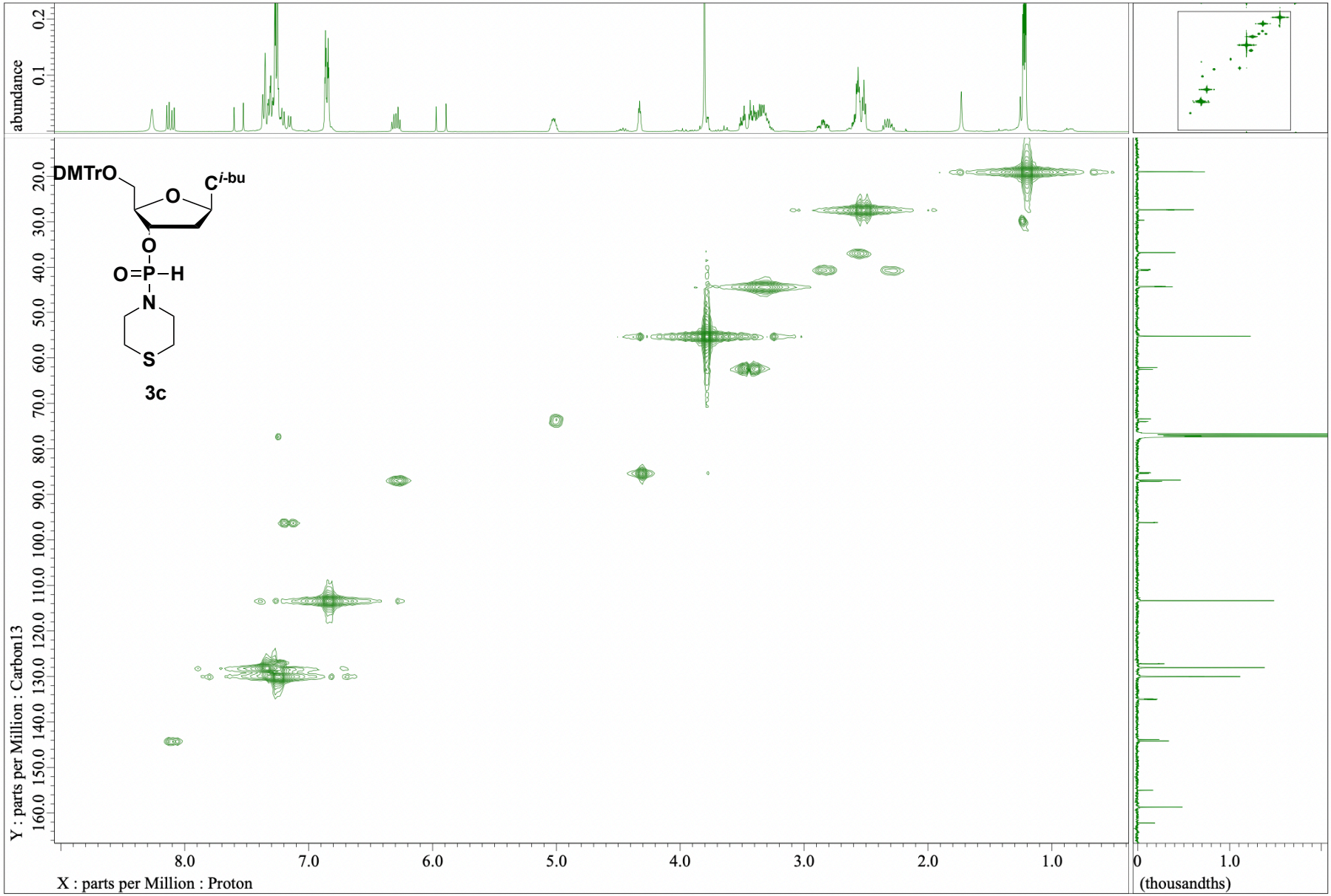
^{1}H-NMR (400 MHz, $CDCl_3$)



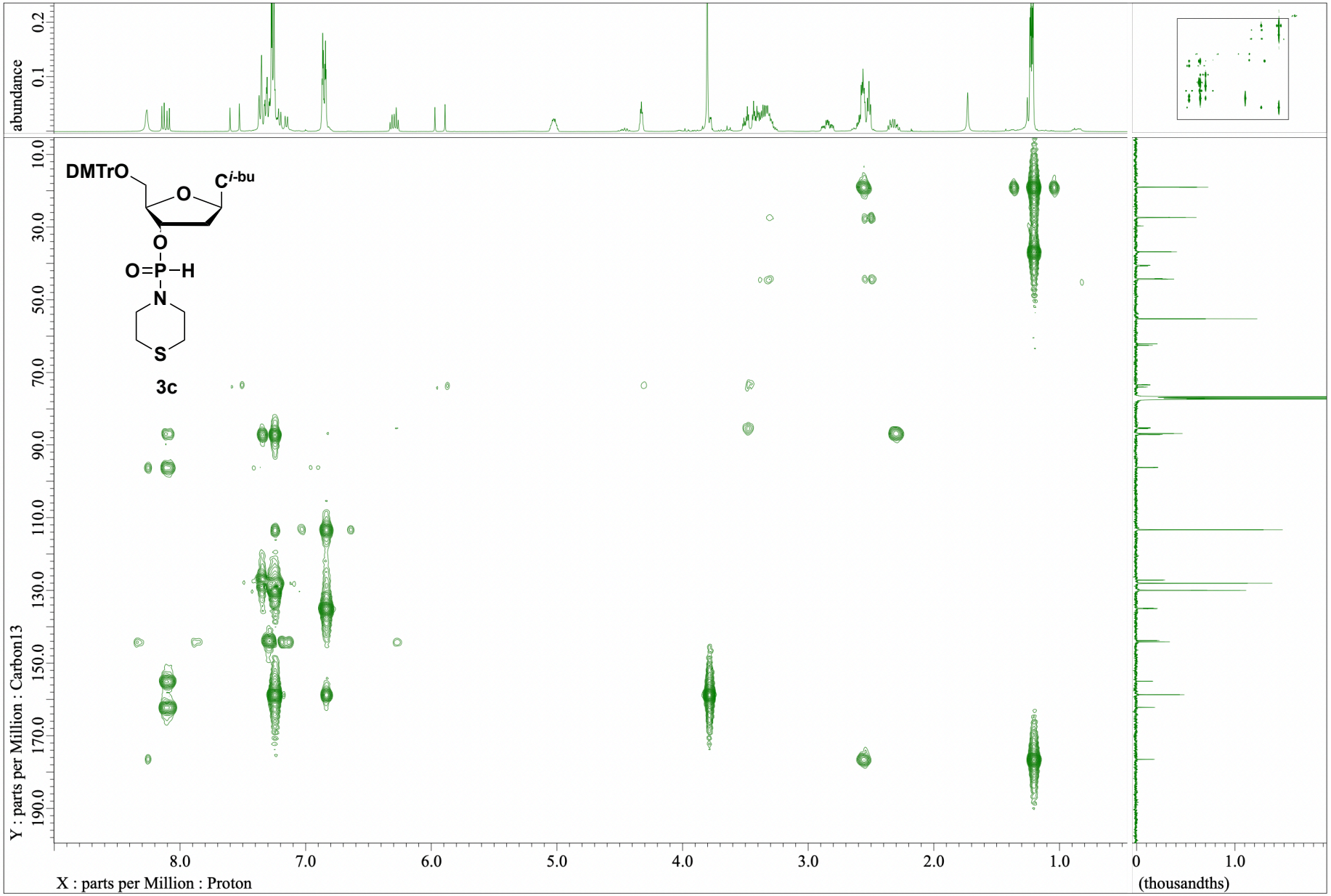
$^{13}C\{H\}$-NMR (100 MHz, $CDCl_3$)



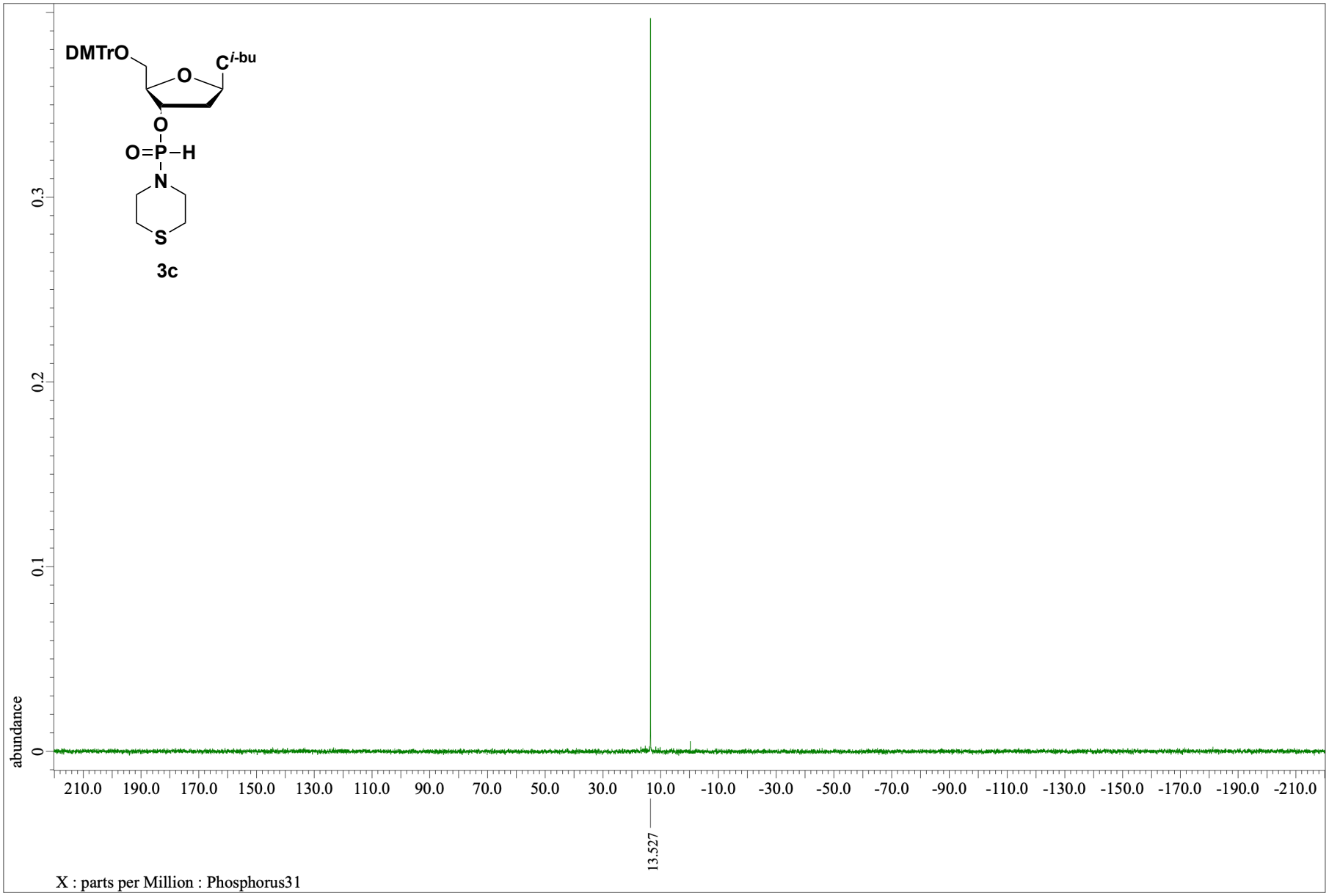
HMQC ($CDCl_3$)


abundance
0.2
0.1
DMTrO
O
C*i*-bu
O
O=P–H
N
S
3c
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
Y : parts per Million : Carbon13
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
1.0
(thousandths)

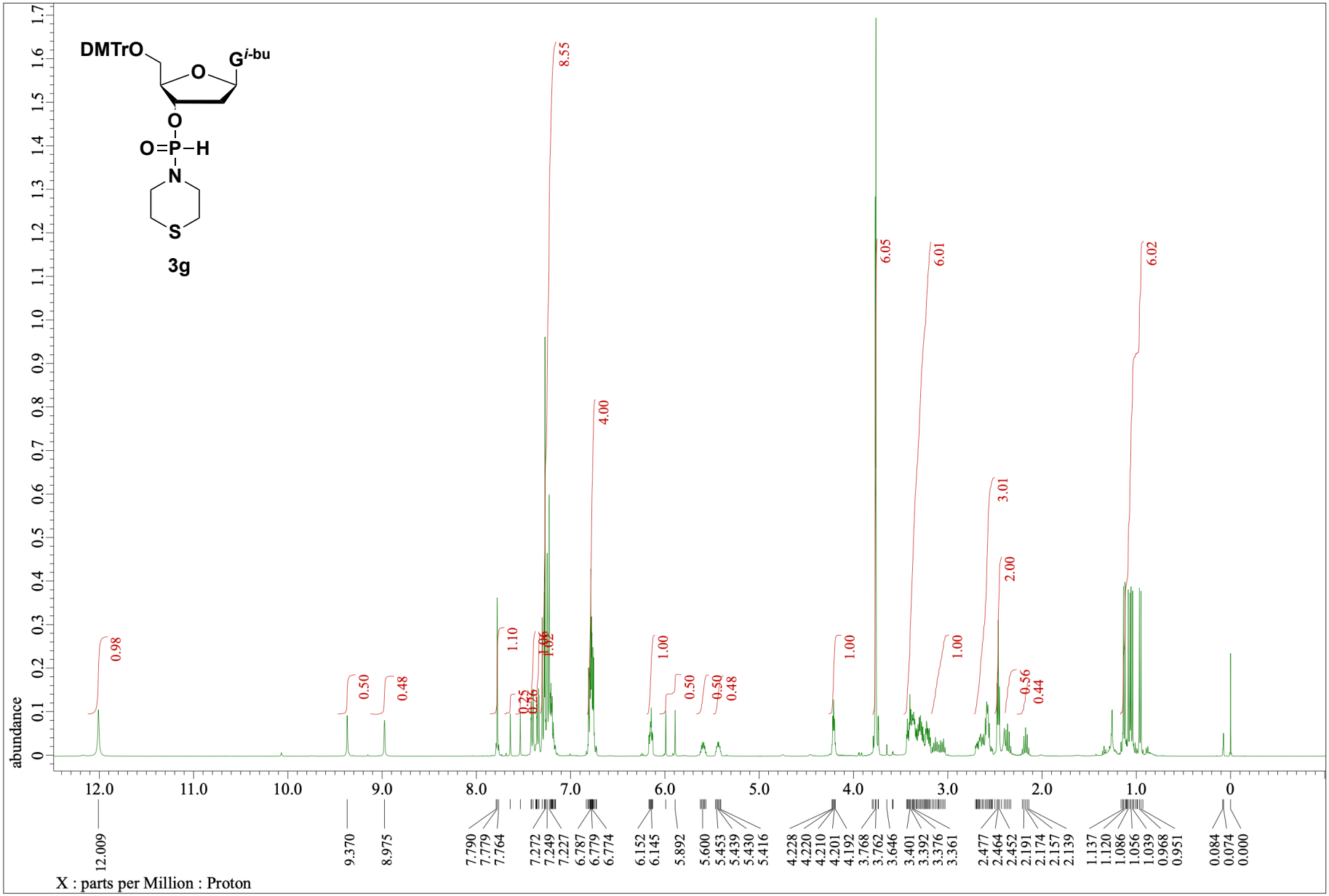
HMBC ($CDCl_3$)


abundance
0.2
0.1
DMTrO
O
C i-bu
O
O=P–H
N
S
3c
10.0
30.0
50.0
70.0
90.0
110.0
130.0
150.0
170.0
190.0
Y : parts per Million : Carbon13
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1.0
X : parts per Million : Proton
(thousandths)

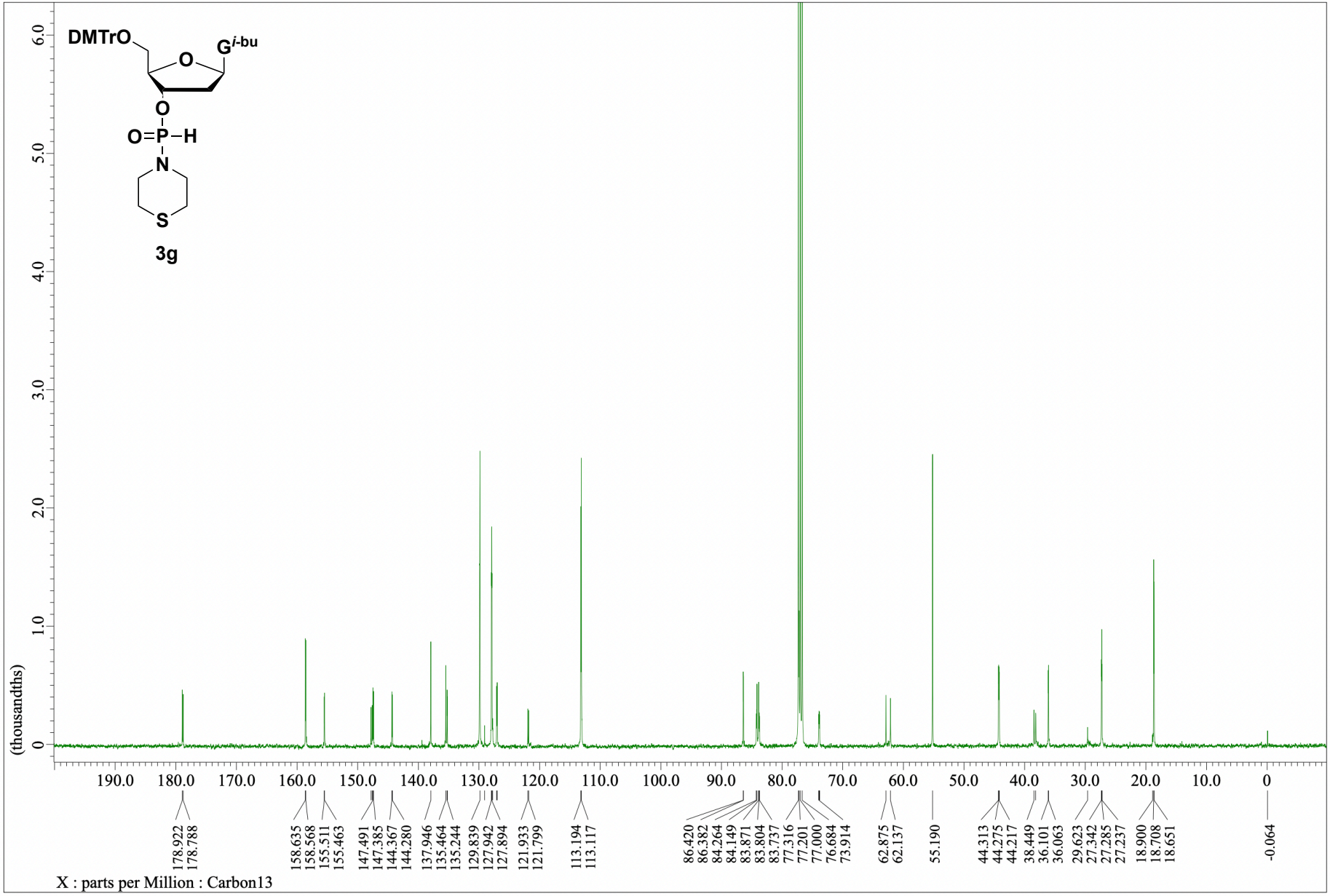
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



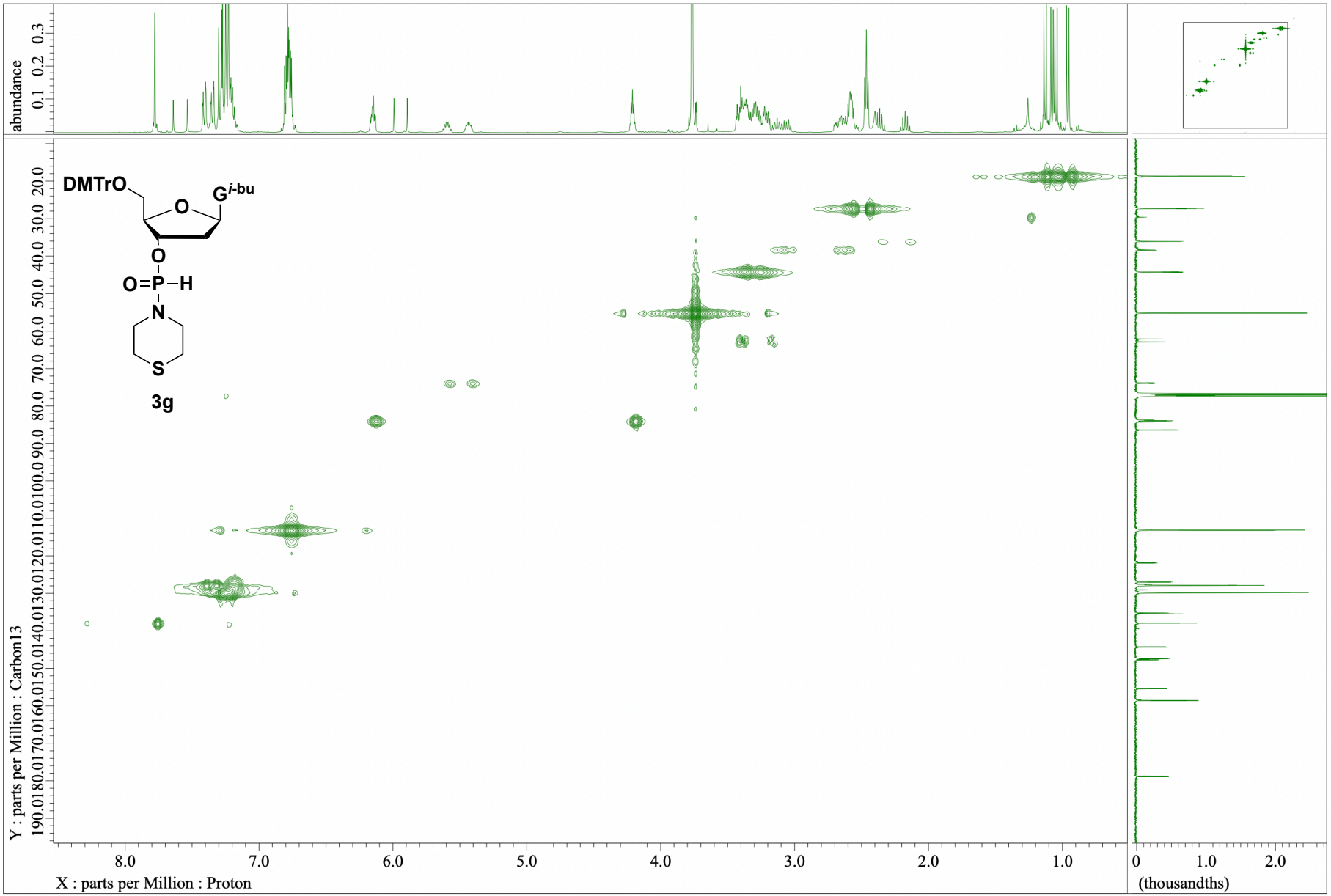
^{1}H-NMR (400 MHz, $CDCl_3$)



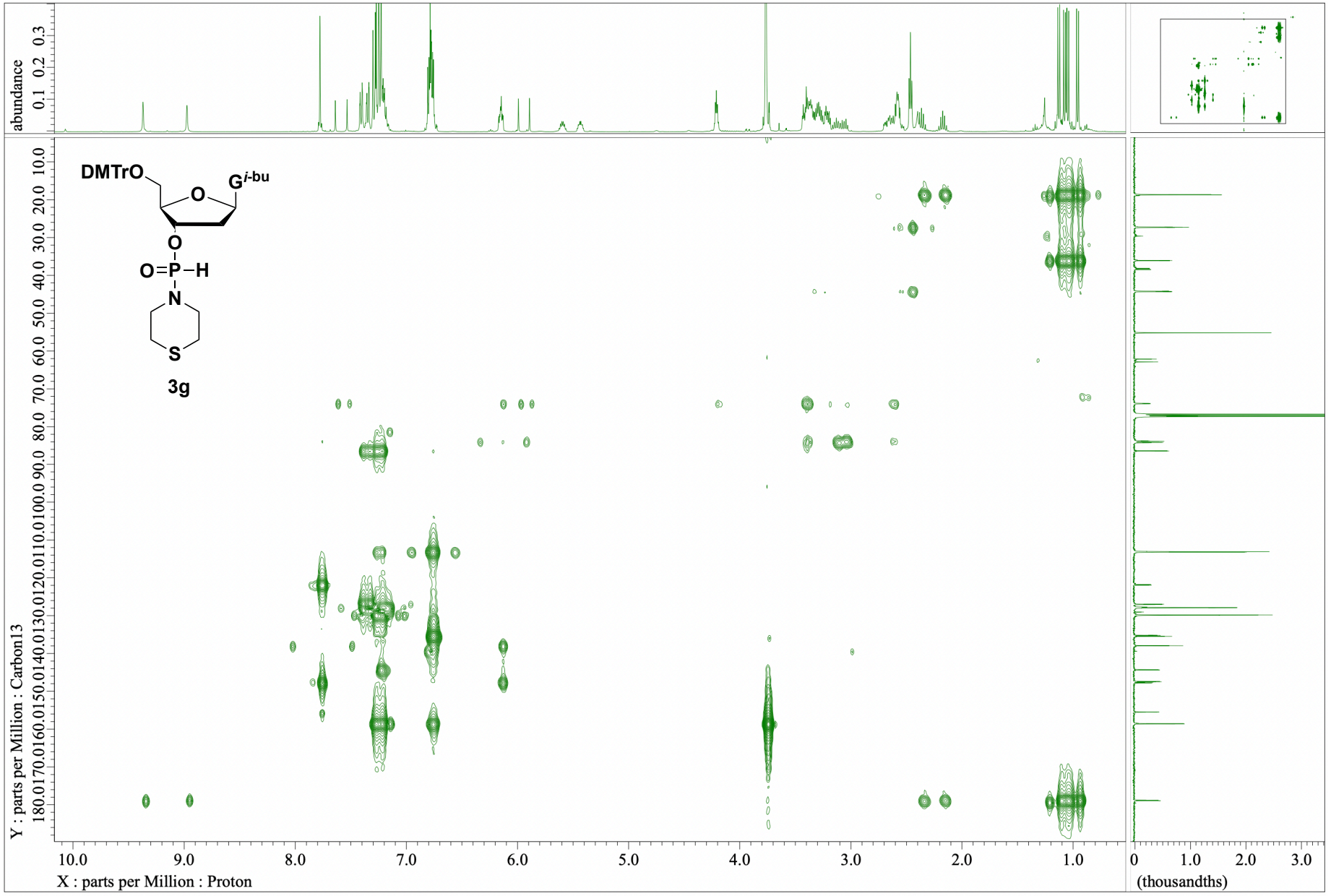
$^{13}C\{H\}$-NMR (100 MHz, $CDCl_3$)



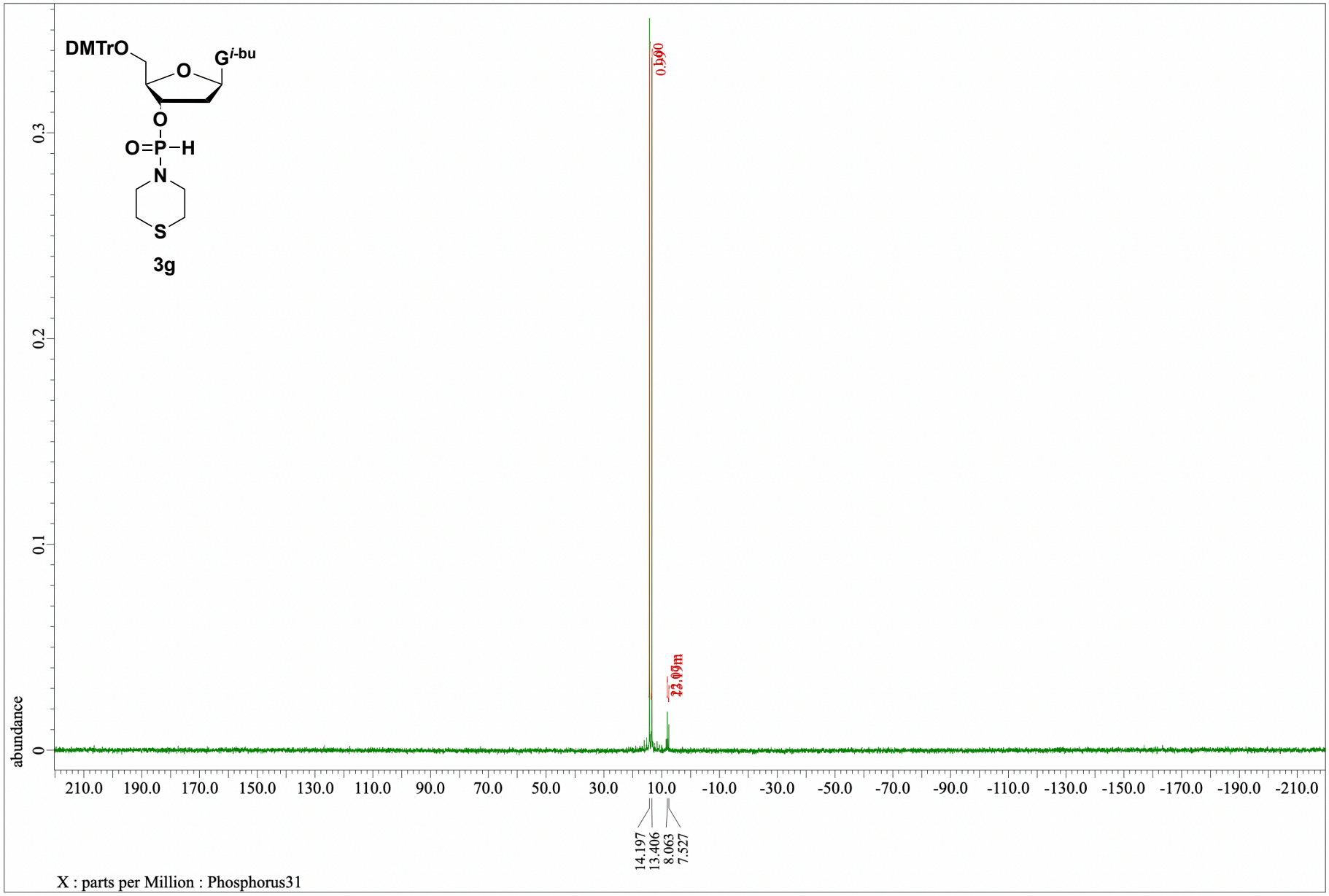
HMQC ($CDCl_3$)


abundance
0.1
0.2
0.3
DMTrO
O
G*i*-bu
O
O=P–H
N
S
3g
Y : parts per Million : Carbon13
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
1.0
2.0
(thousandths)

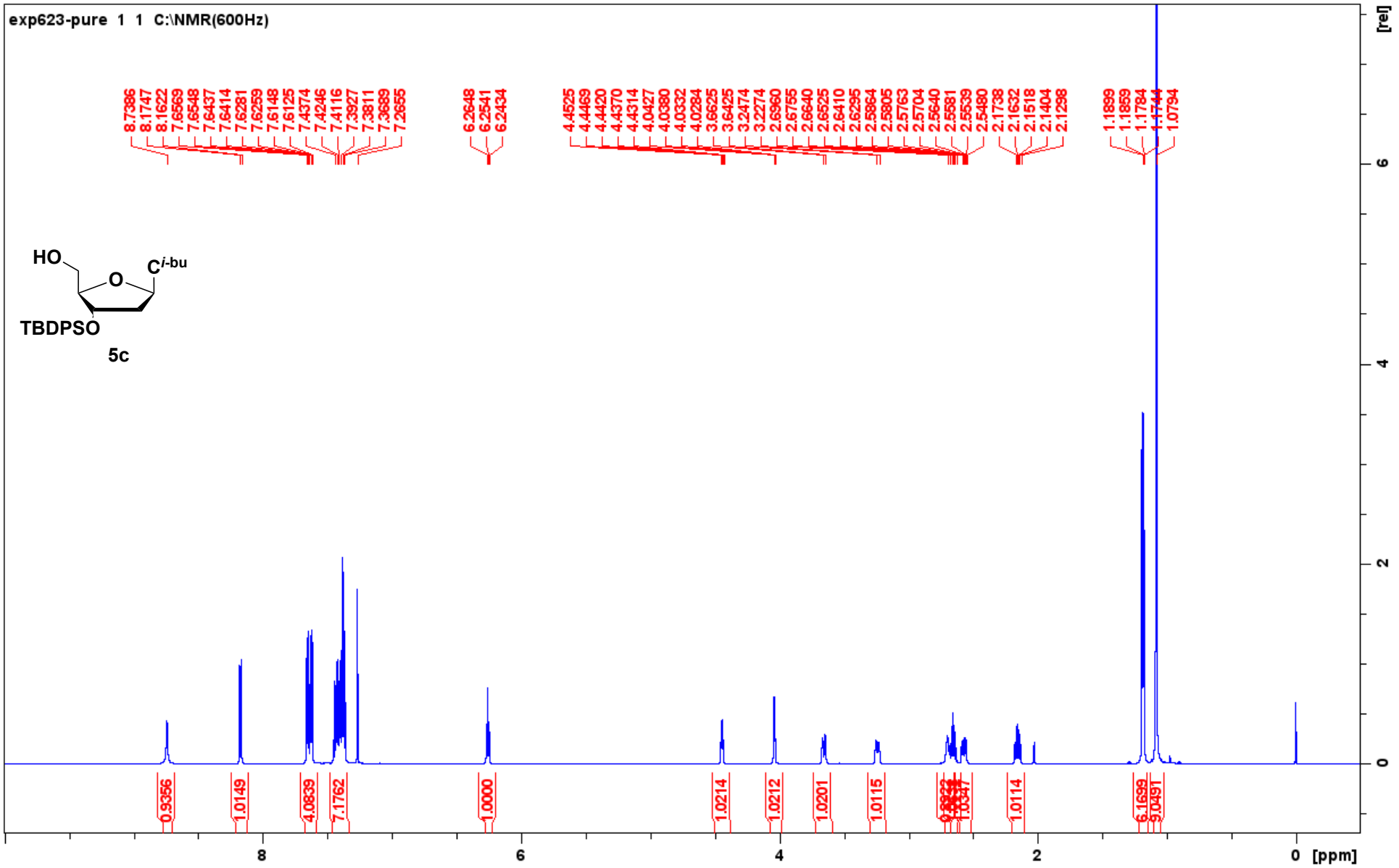
HMBC ($CDCl_3$)


abundance
0.1
0.2
0.3
DMTrO
O
G*i*-bu
O
O=P–H
N
S
3g
Y : parts per Million : Carbon13
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0
180.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
1.0
2.0
3.0
(thousandths)

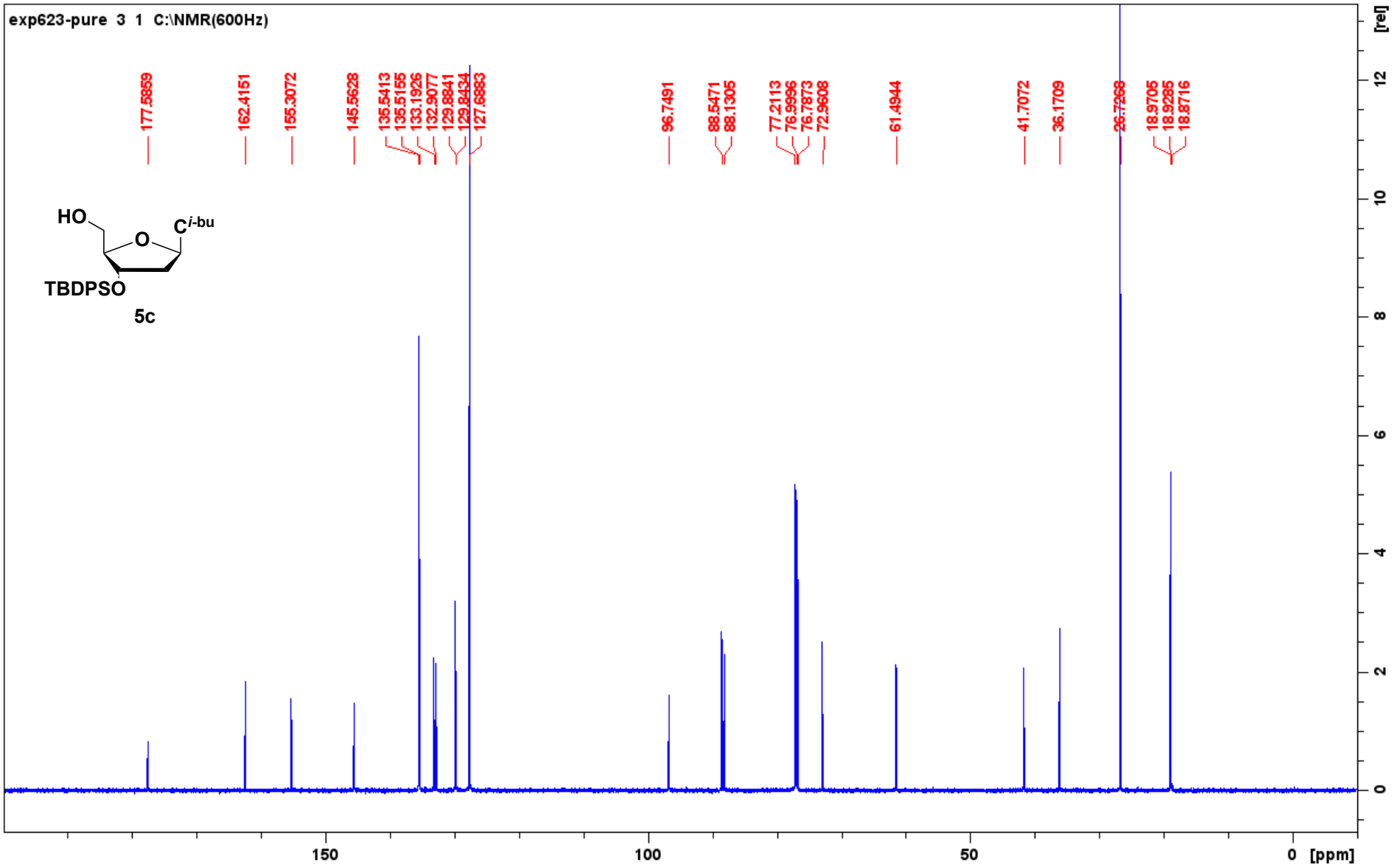
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



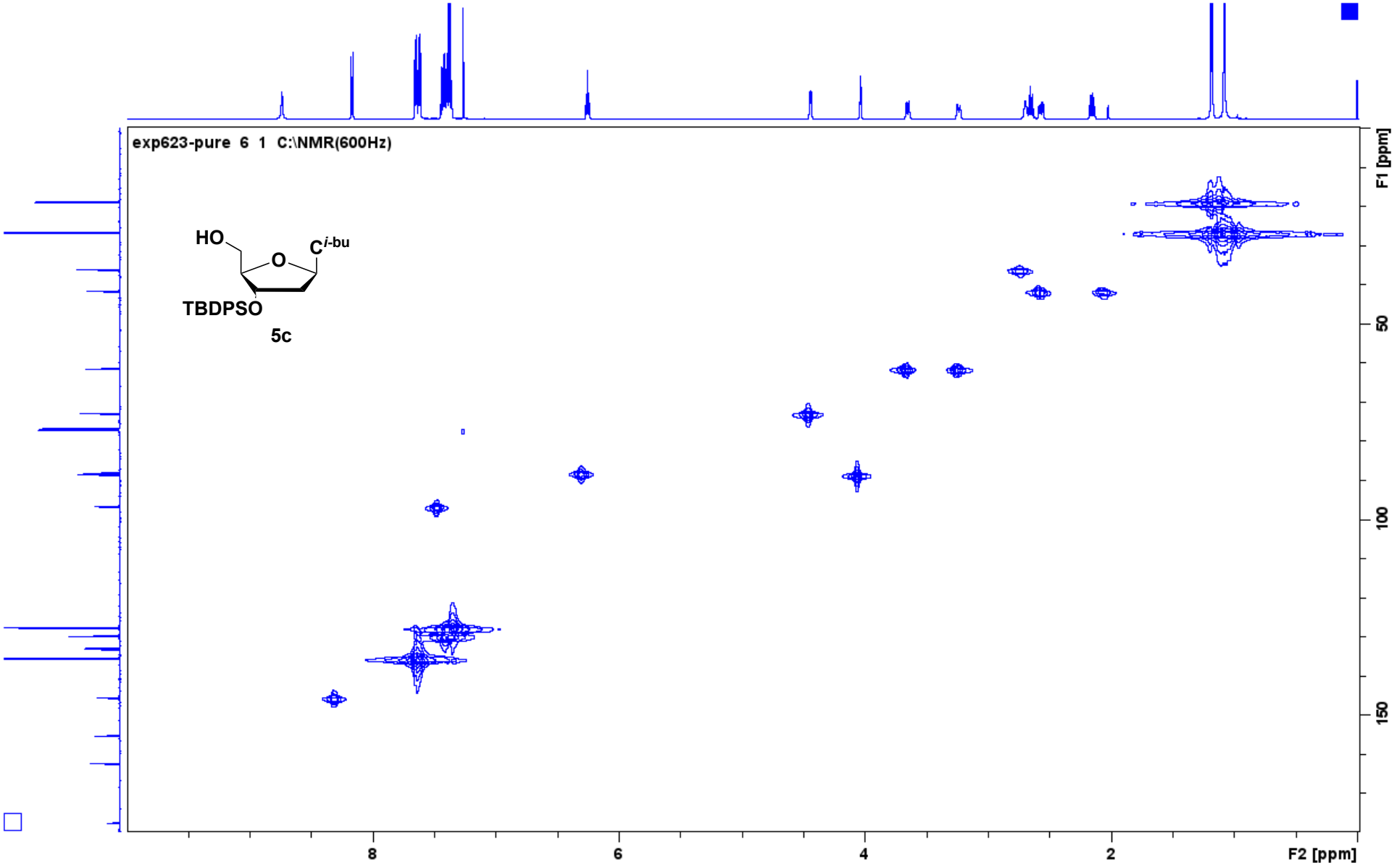
^{1}H-NMR (600 MHz, $CDCl_3$)



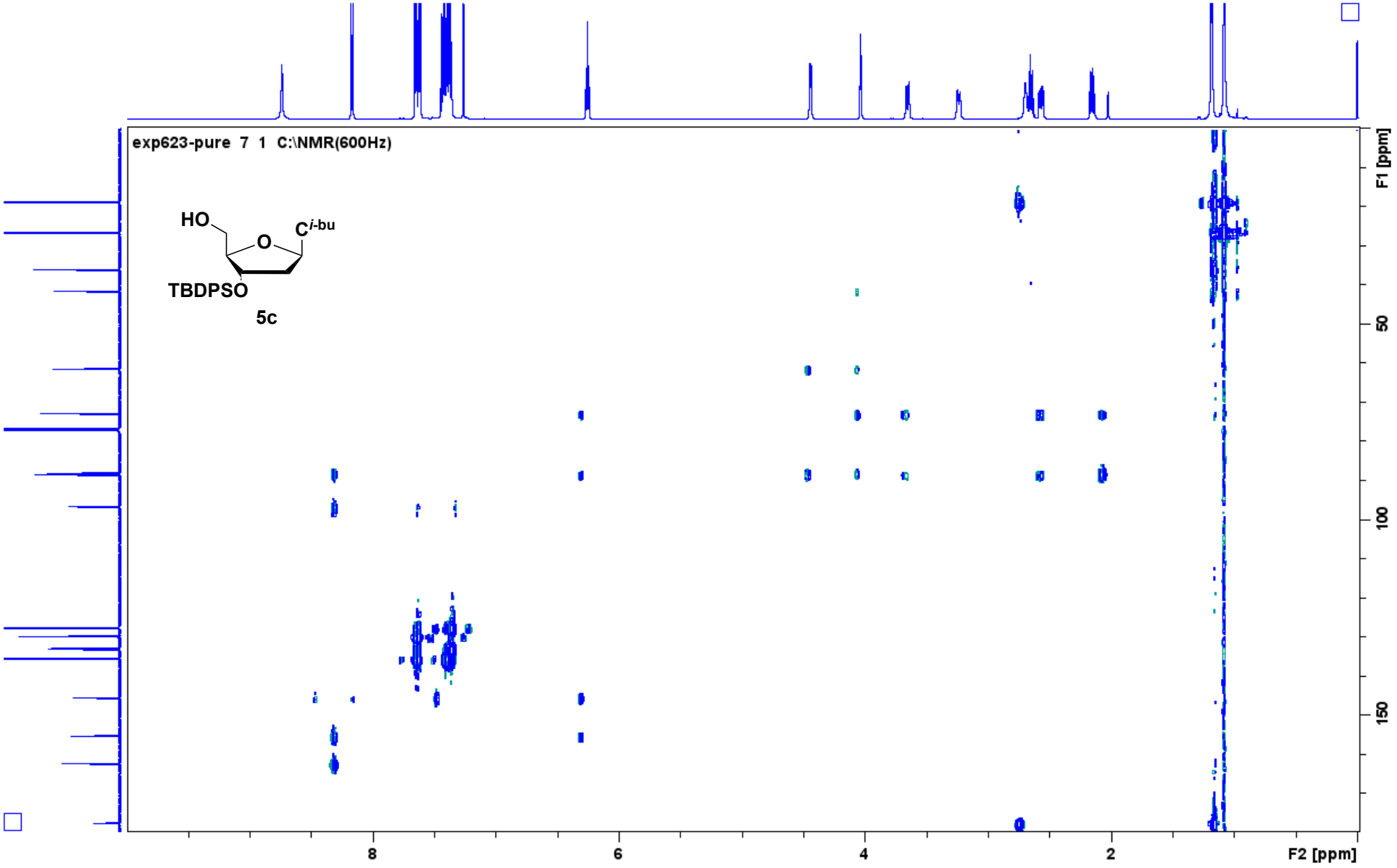
$^{13}C\{H\}$-NMR (151MHz, $CDCl_3$)



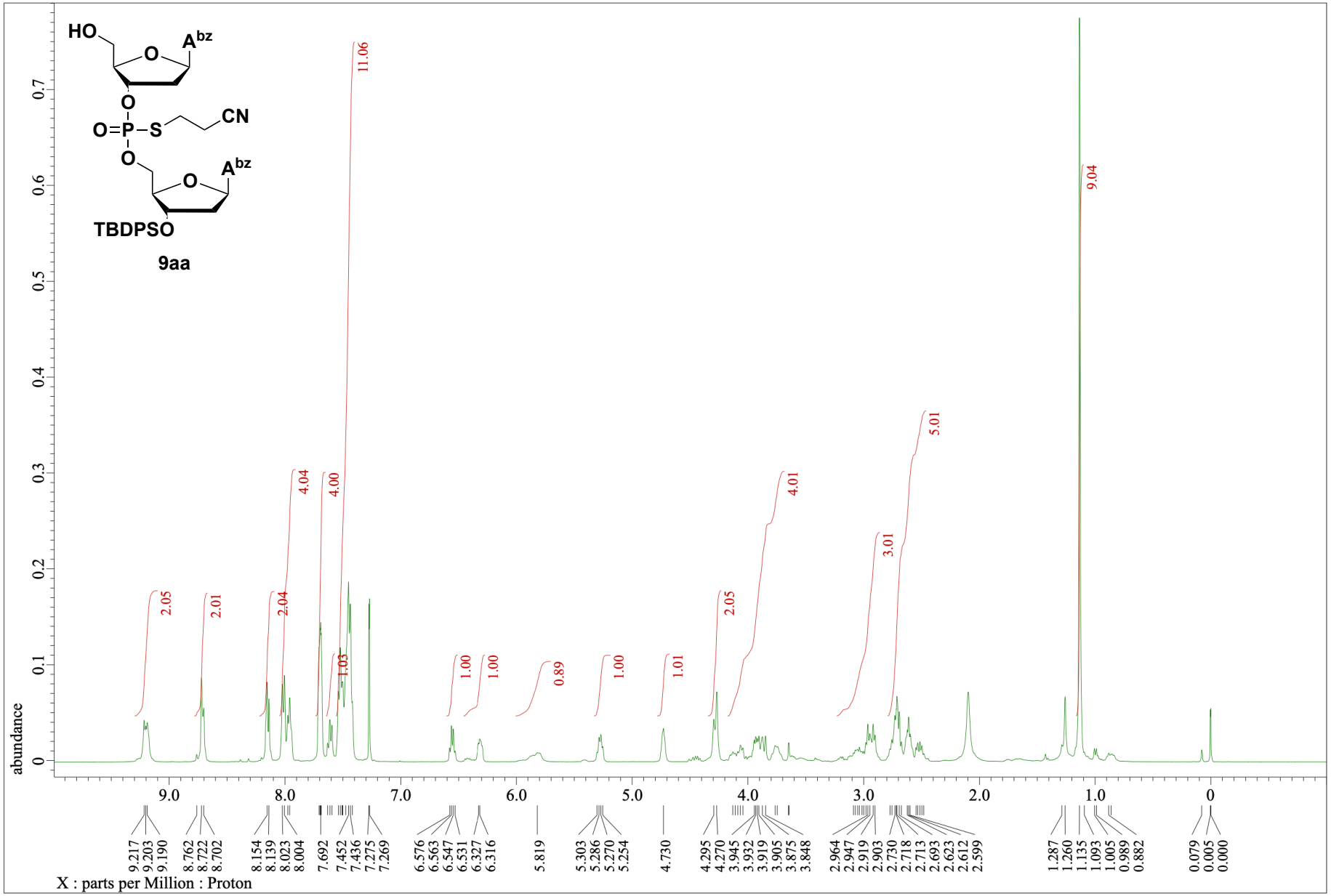
HMQC ($CDCl_3$)


exp623-pure 6 1 C:\NMR(600Hz)
HO
O
C^{i}-bu
TBDPSO
5c
F1 [ppm]
50
100
150
8
6
4
2
F2 [ppm]

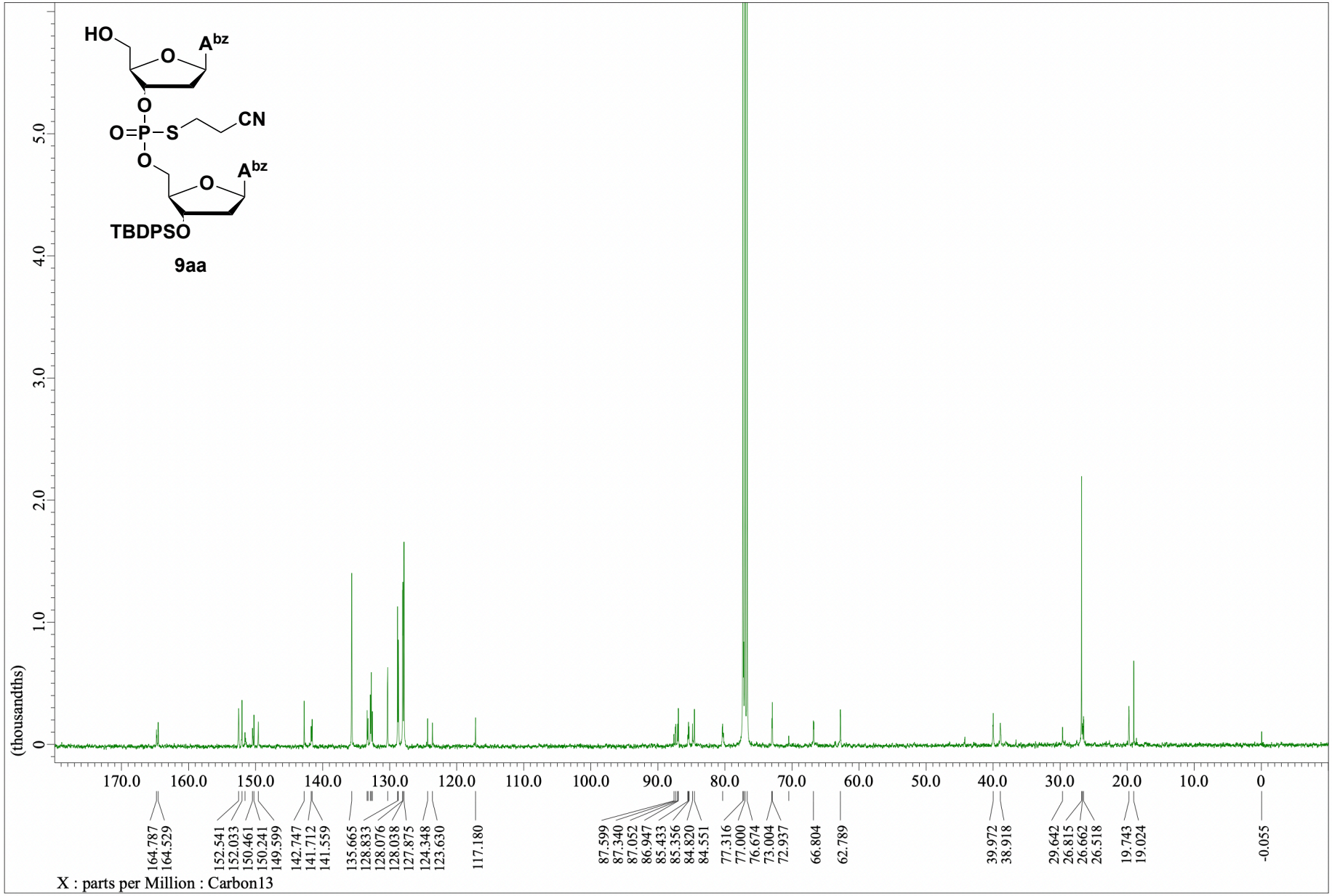
HMBC


exp623-pure 7 1 C:\NMR(600Hz)
HO
O
C i-bu
TBDPSO
5c
F1 [ppm]
50
100
150
8
6
4
2
F2 [ppm]

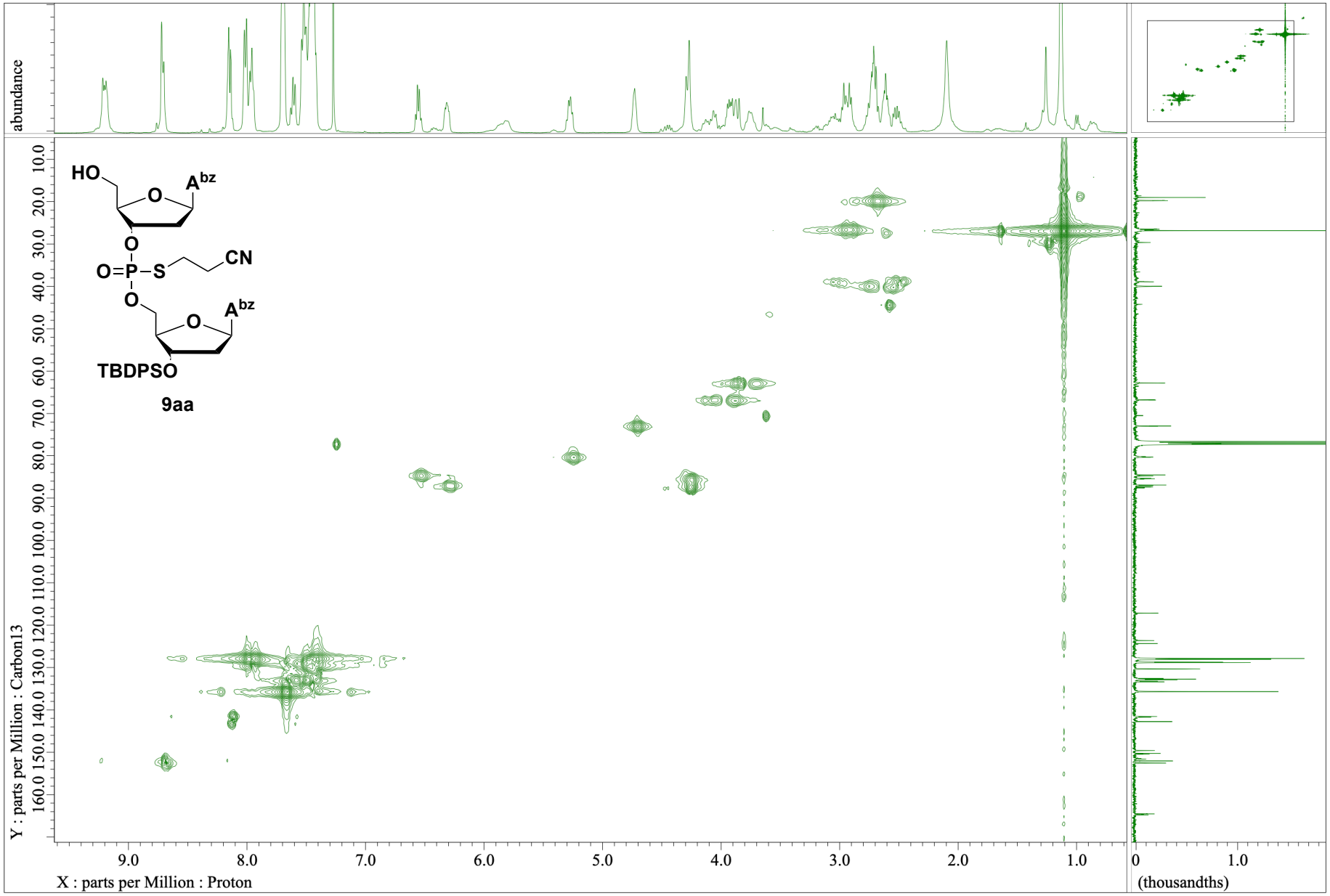
^{1}H-NMR (400 MHz, $CDCl_3$)



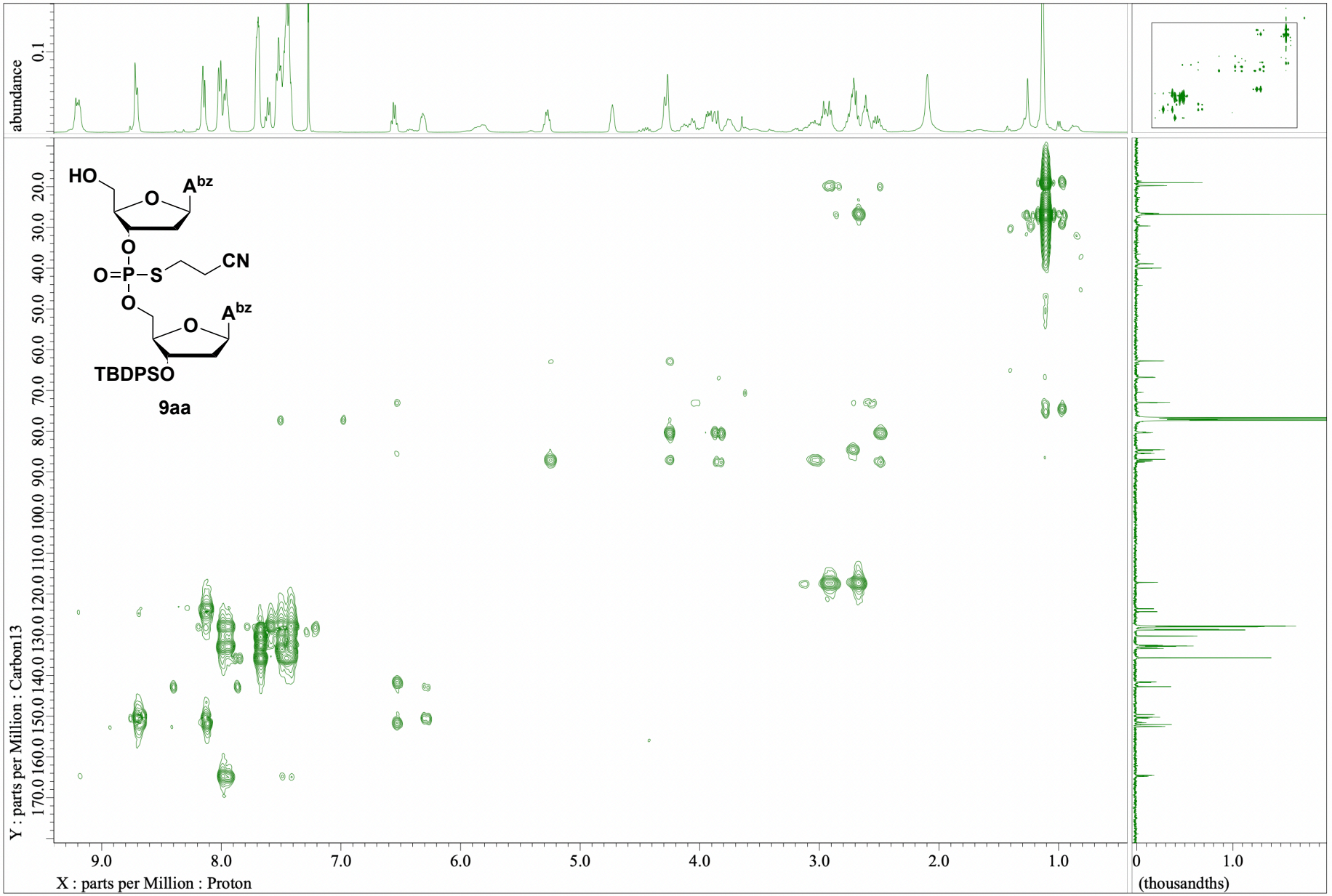
$^{13}C\{H\}$-NMR (100 MHz, $CDCl_3$)



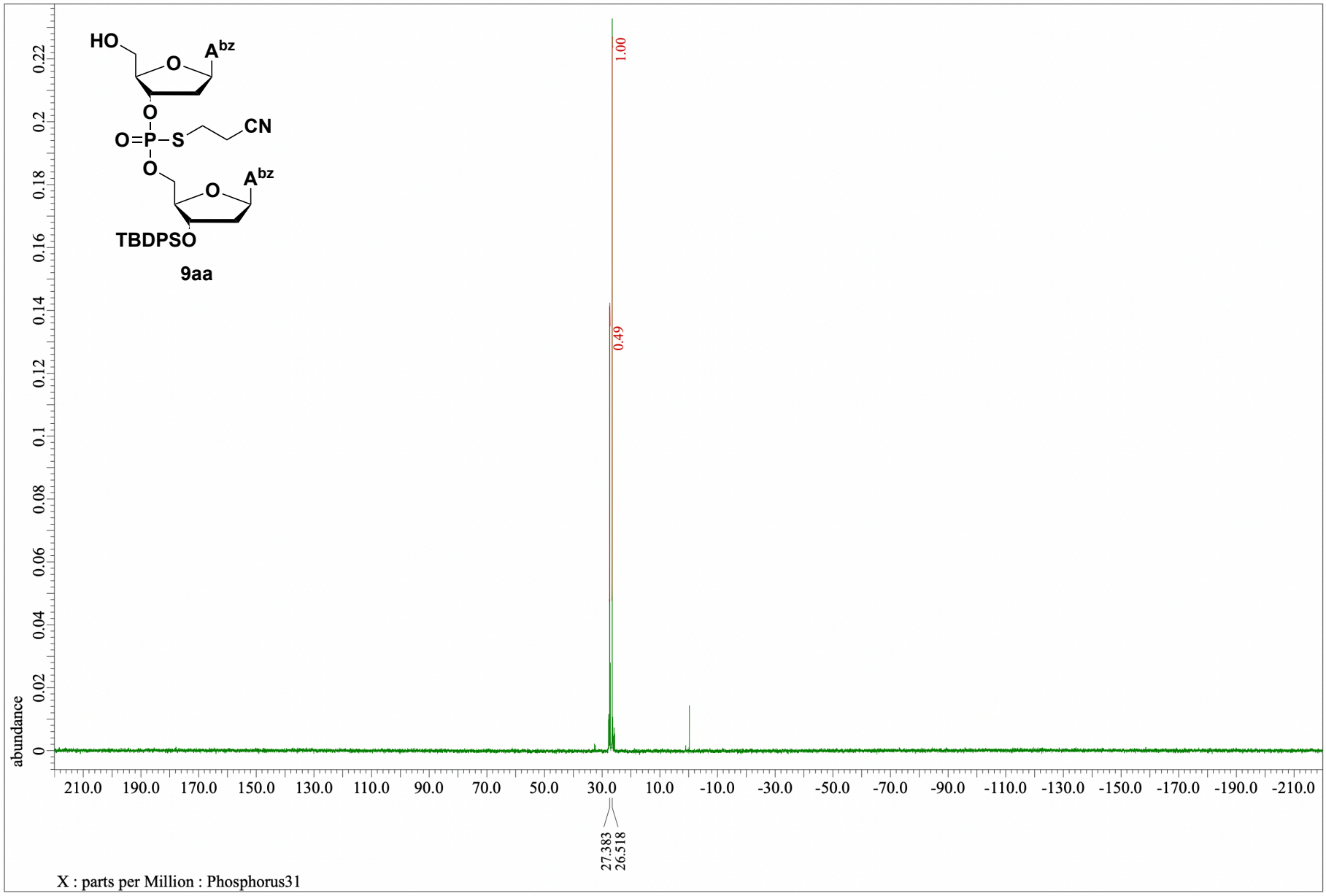
HMQC


abundance
HO
O
A^bz
O
O=P–S
CN
O
O
A^bz
TBDPSO
9aa
Y : parts per Million : Carbon13
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
1.0
(thousandths)

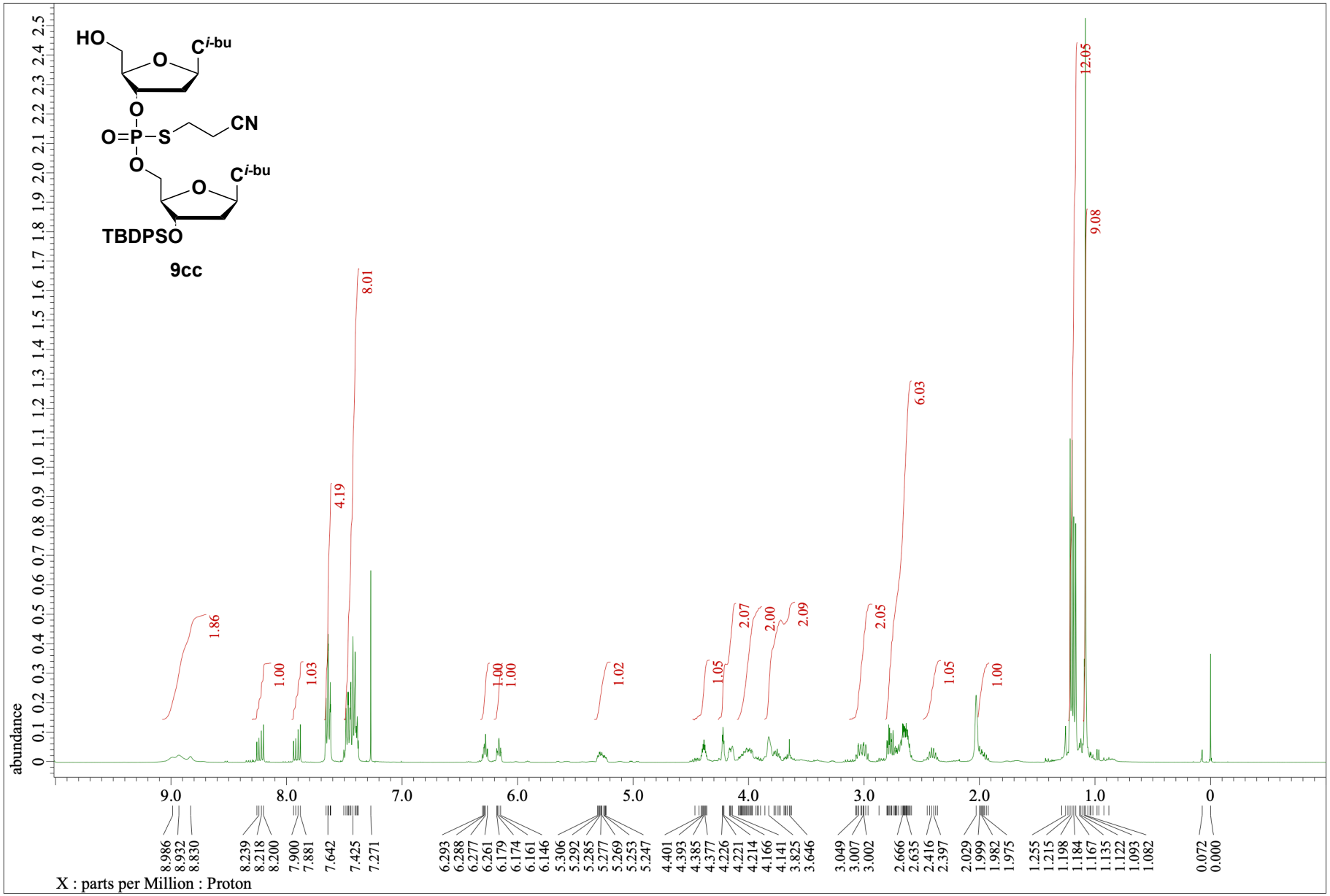
HMBC


abundance
0.1
HO
A^bz
O
O
O=P–S
CN
O
A^bz
O
TBDPSO
9aa
Y : parts per Million : Carbon13
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
1.0
(thousandths)

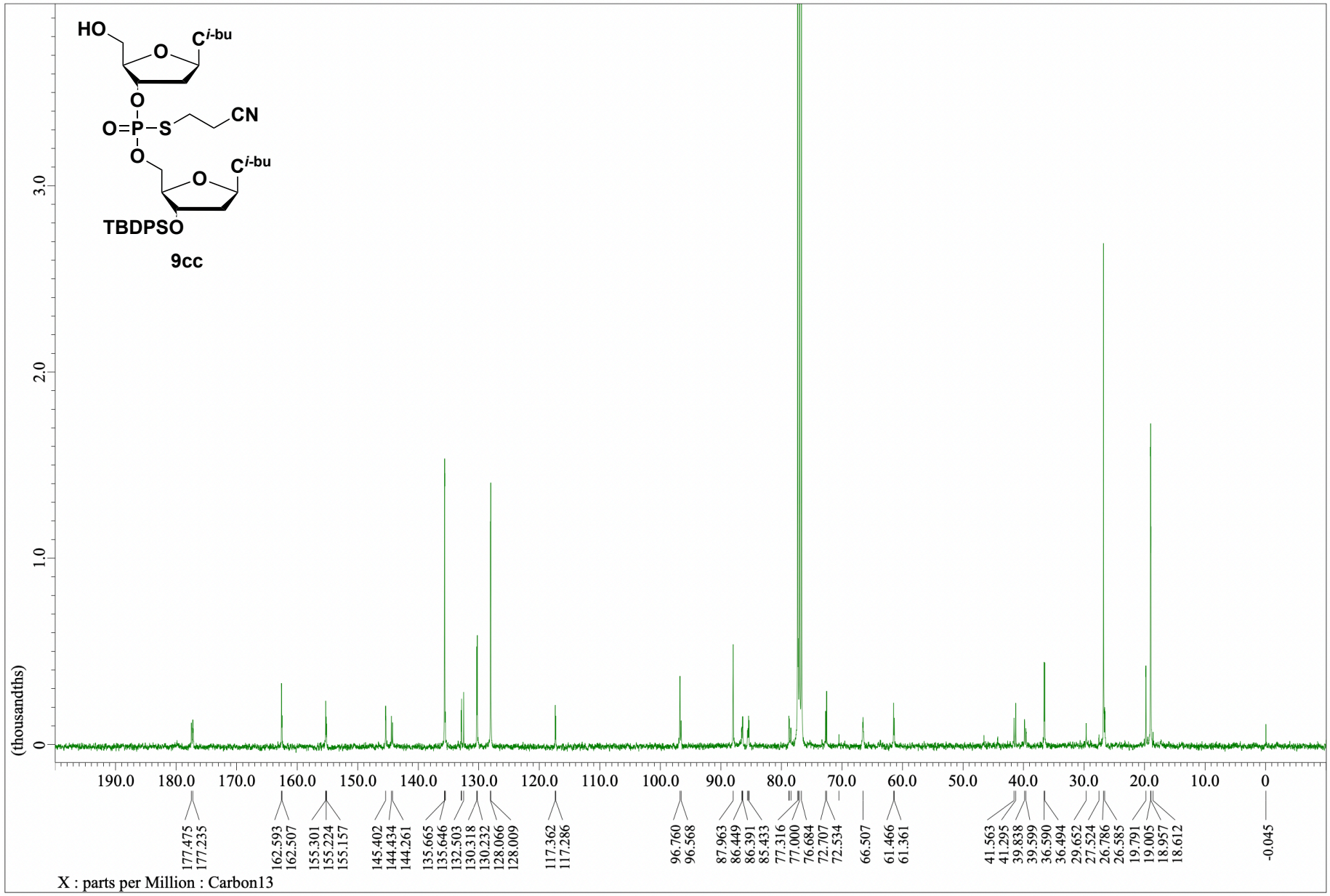
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



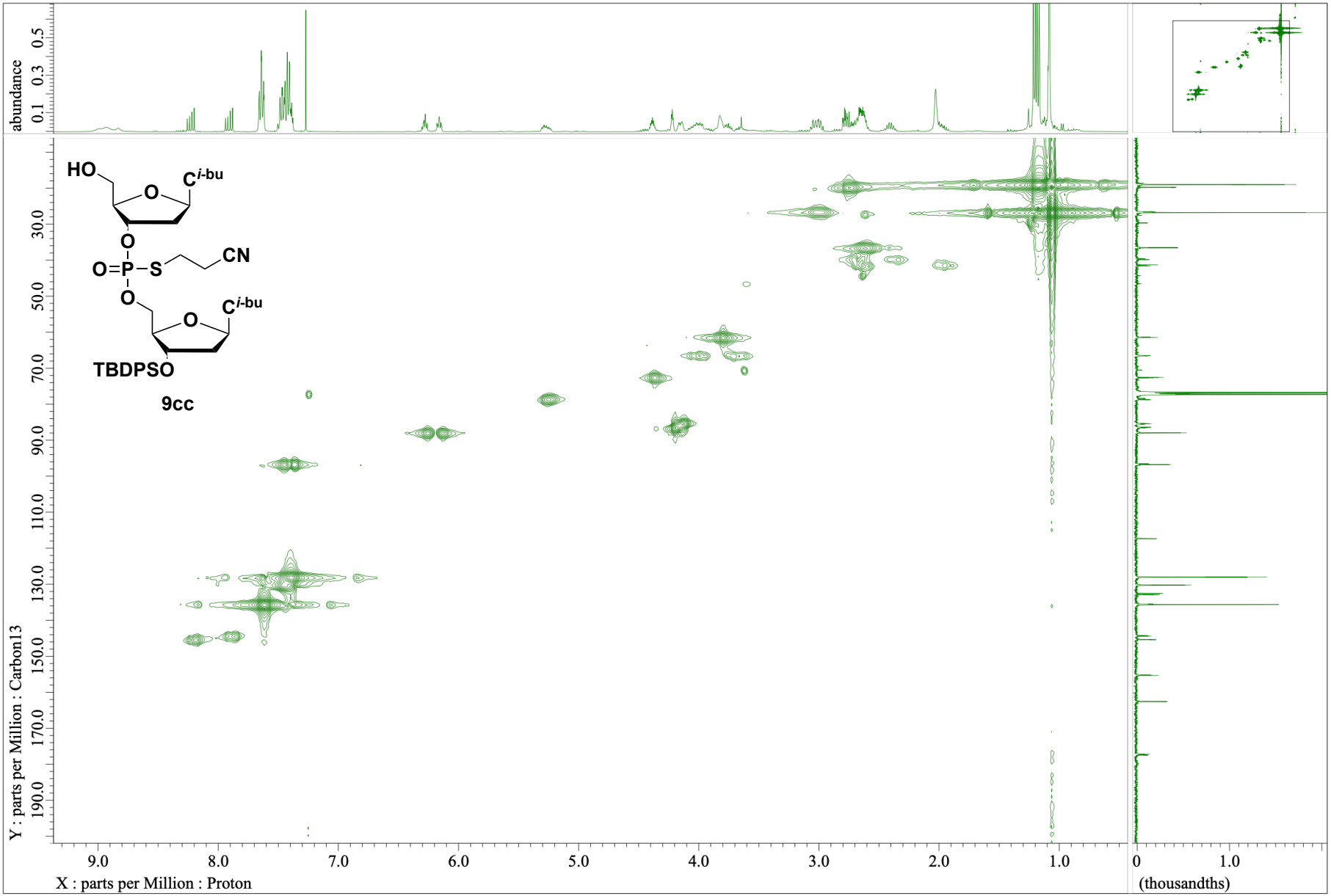
^{1}H-NMR (400 MHz, $CDCl_3$)



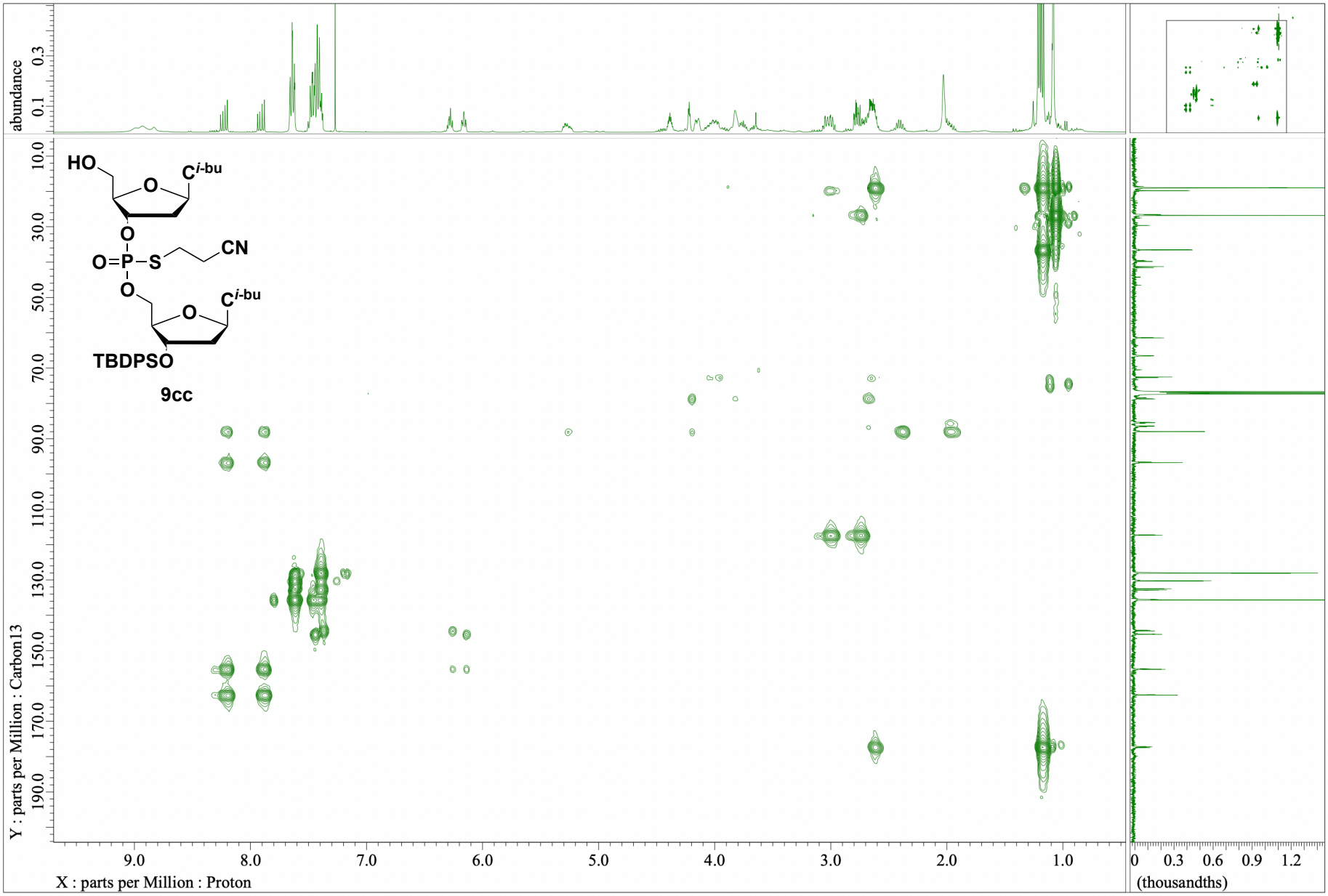
$^{13}C\{H\}$-NMR (100 MHz, $CDCl_3$)



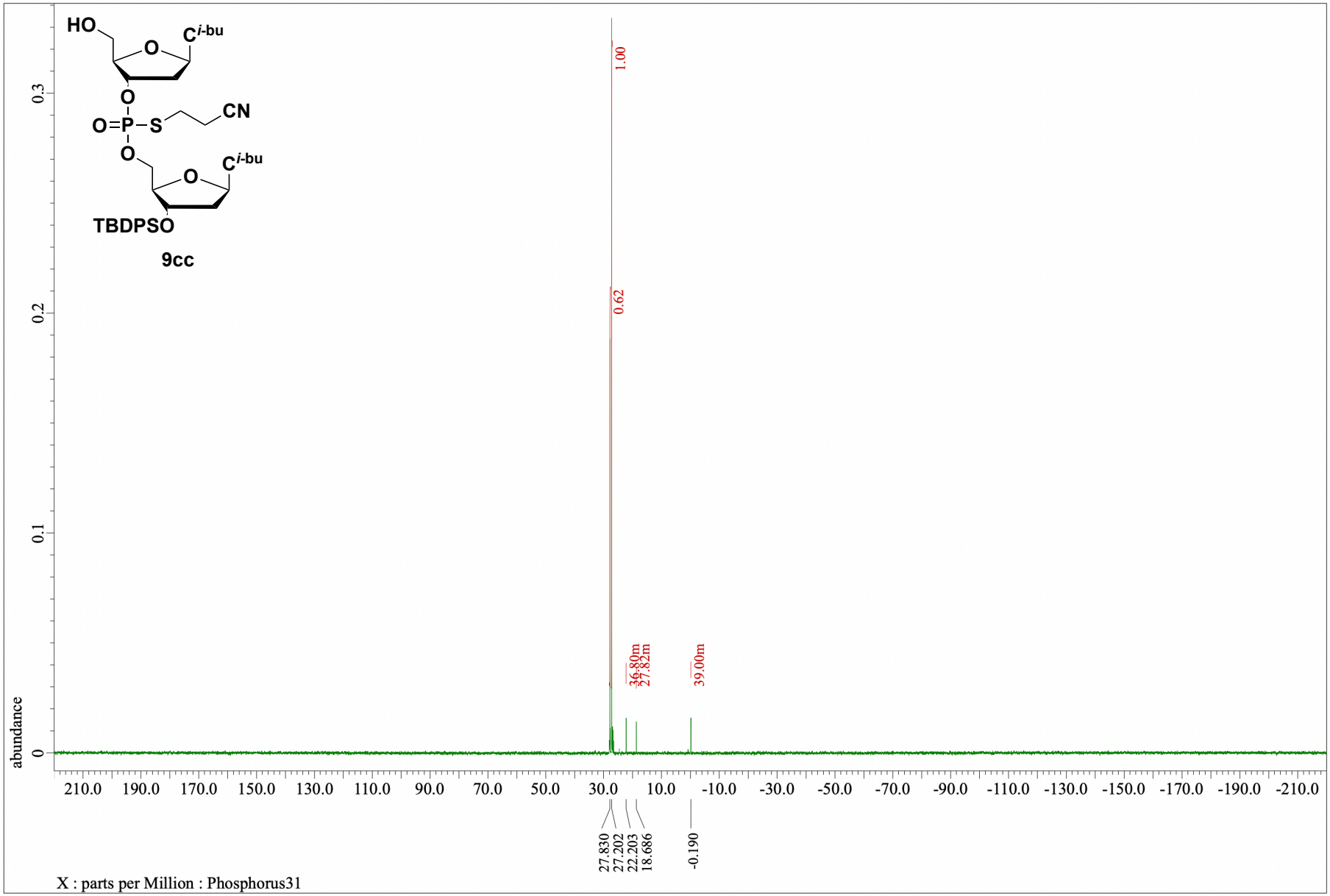
HMQC


abundance
0.1
0.3
0.5
HO
O
C i-bu
O
O=P–S
CN
O
O
C i-bu
TBDPSO
9cc
Y : parts per Million : Carbon13
30.0
50.0
70.0
90.0
110.0
130.0
150.0
170.0
190.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
1.0
(thousandths)

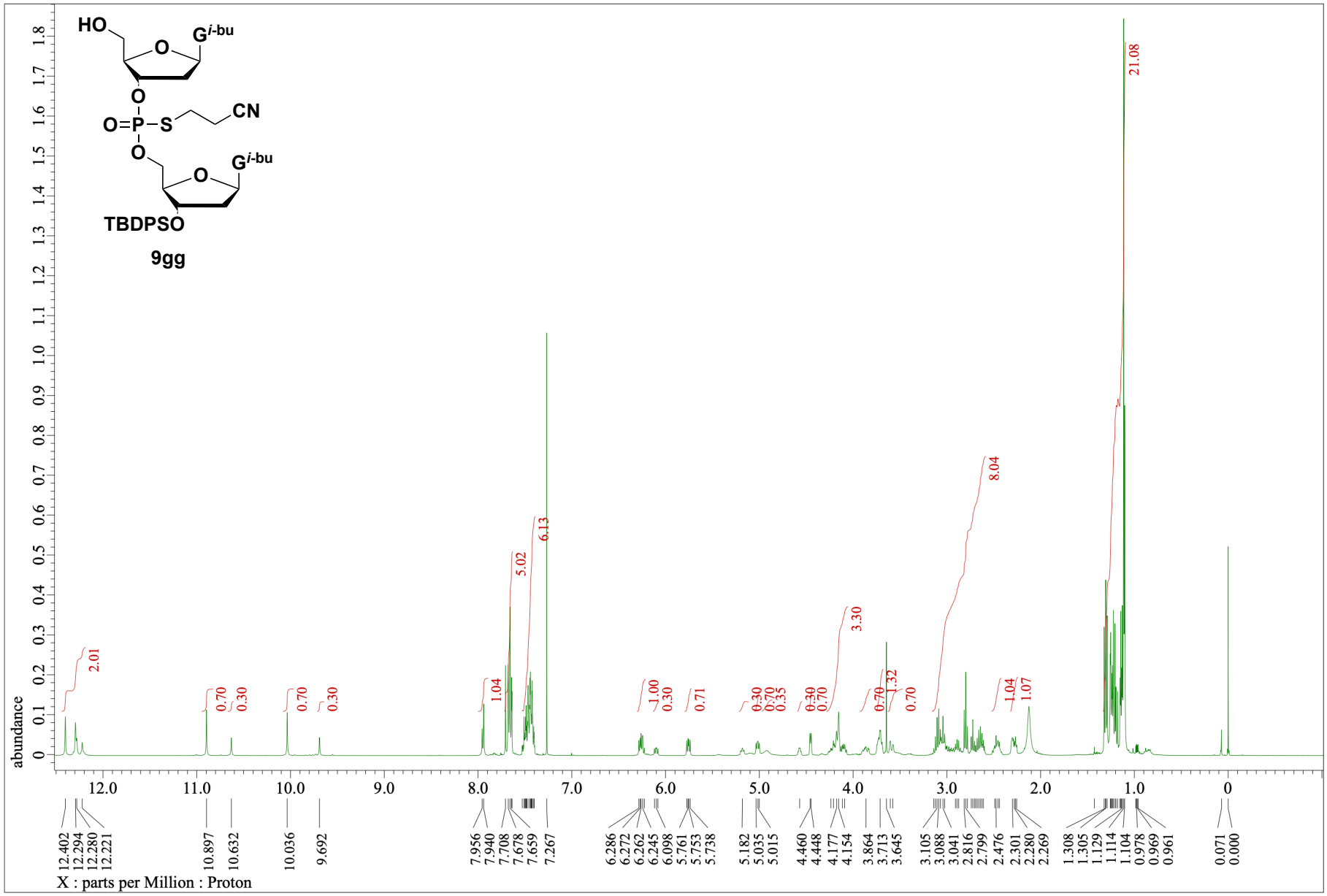
HMBC


abundance
0.1
0.3
HO
O
C*i*-bu
O
O=P–S
CN
O
O
C*i*-bu
TBDPSO
9cc
10.0
30.0
50.0
70.0
90.0
110.0
130.0
150.0
170.0
190.0
Y : parts per Million : Carbon13
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
0.3
0.6
0.9
1.2
(thousandths)

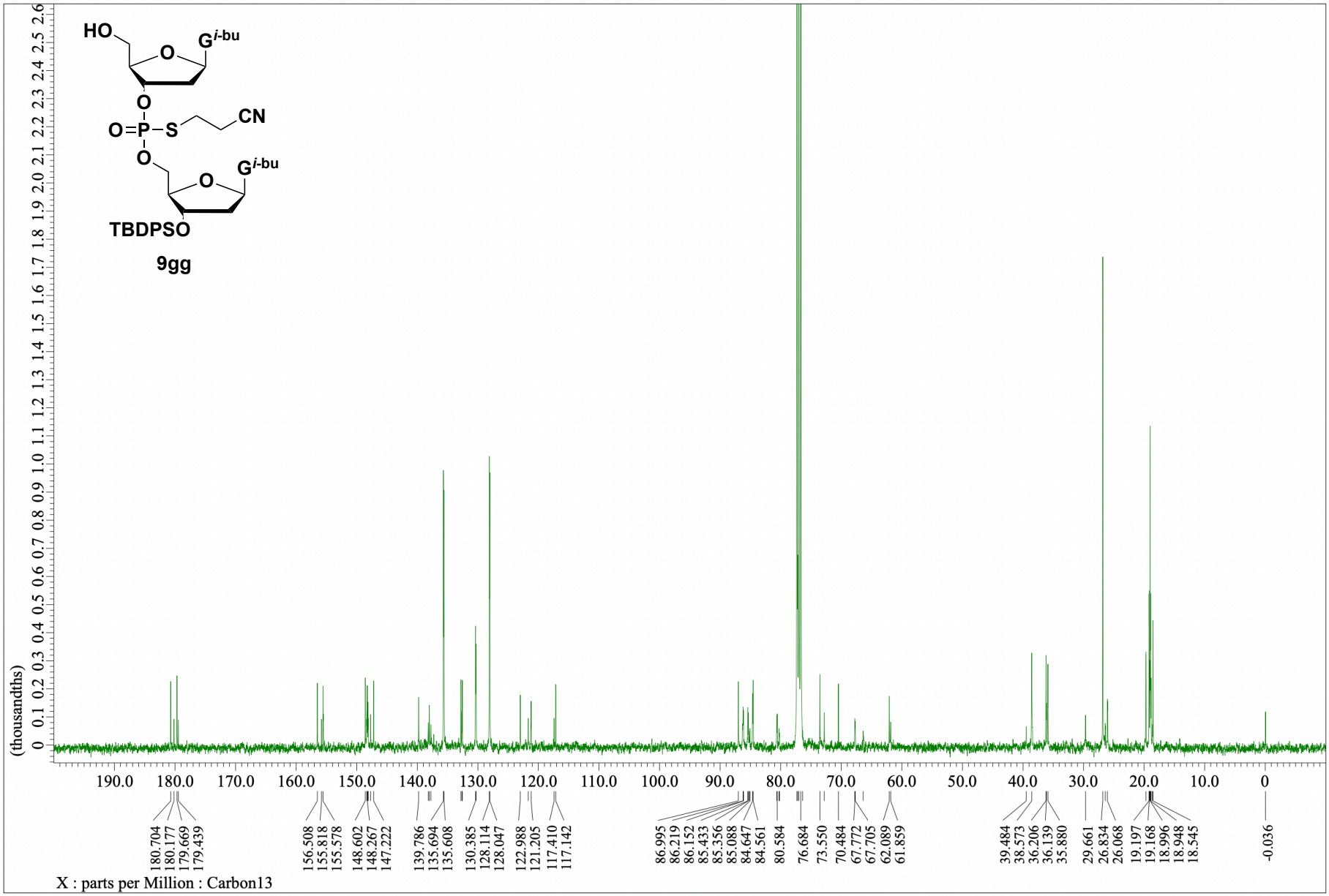
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



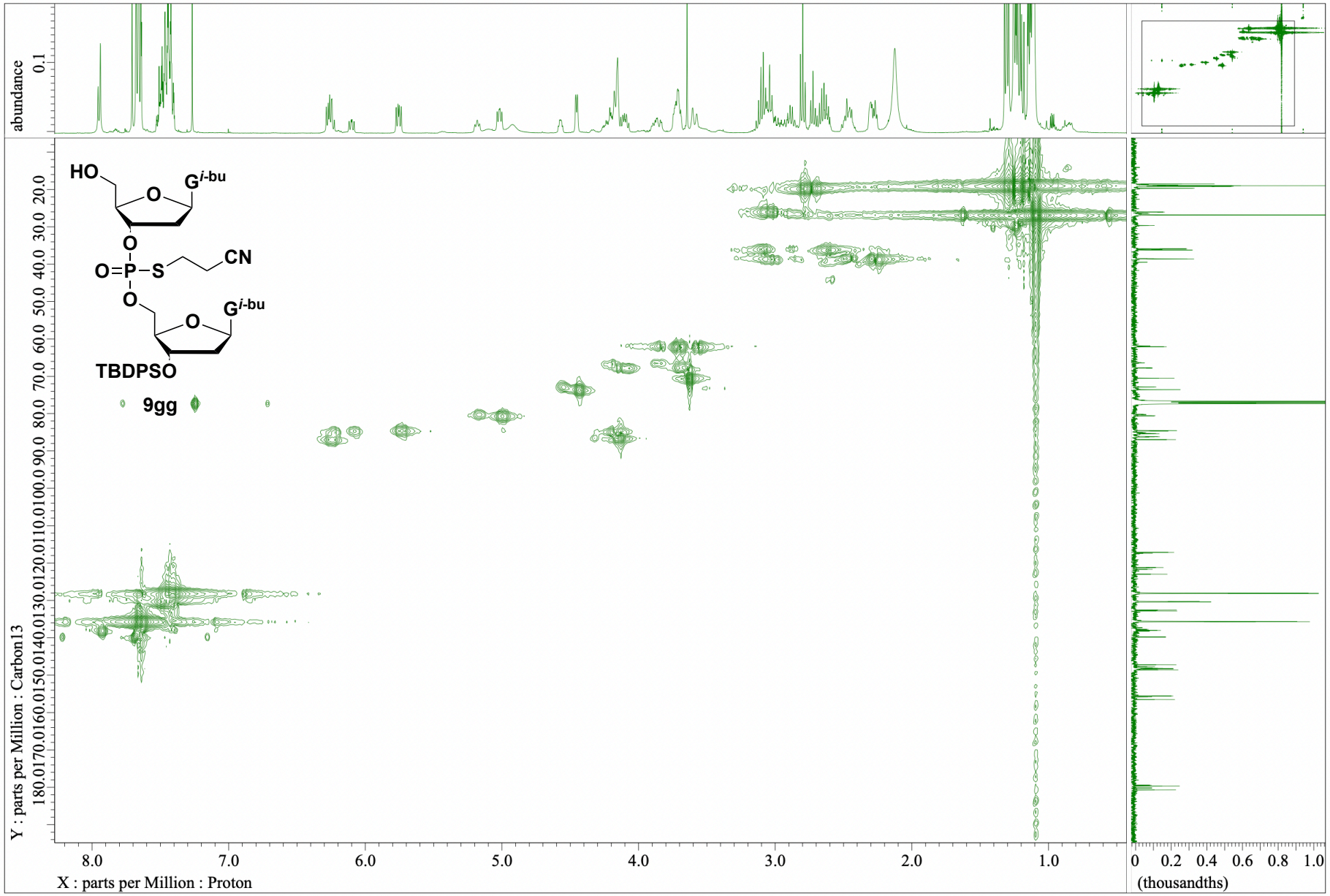
^{1}H-NMR (400 MHz, $CDCl_3$)



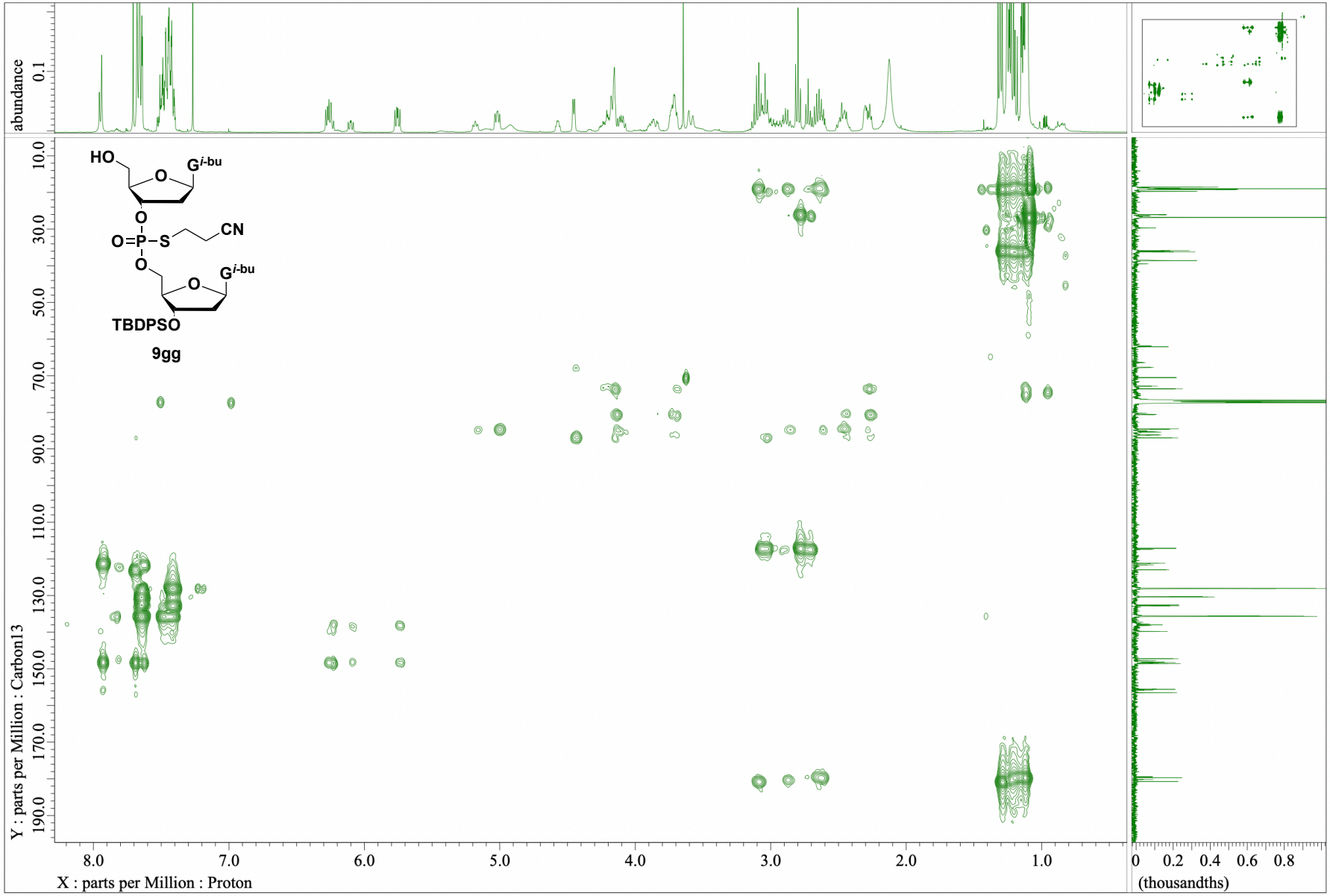
$^{13}C\{H\}$-NMR (100 MHz, $CDCl_3$)



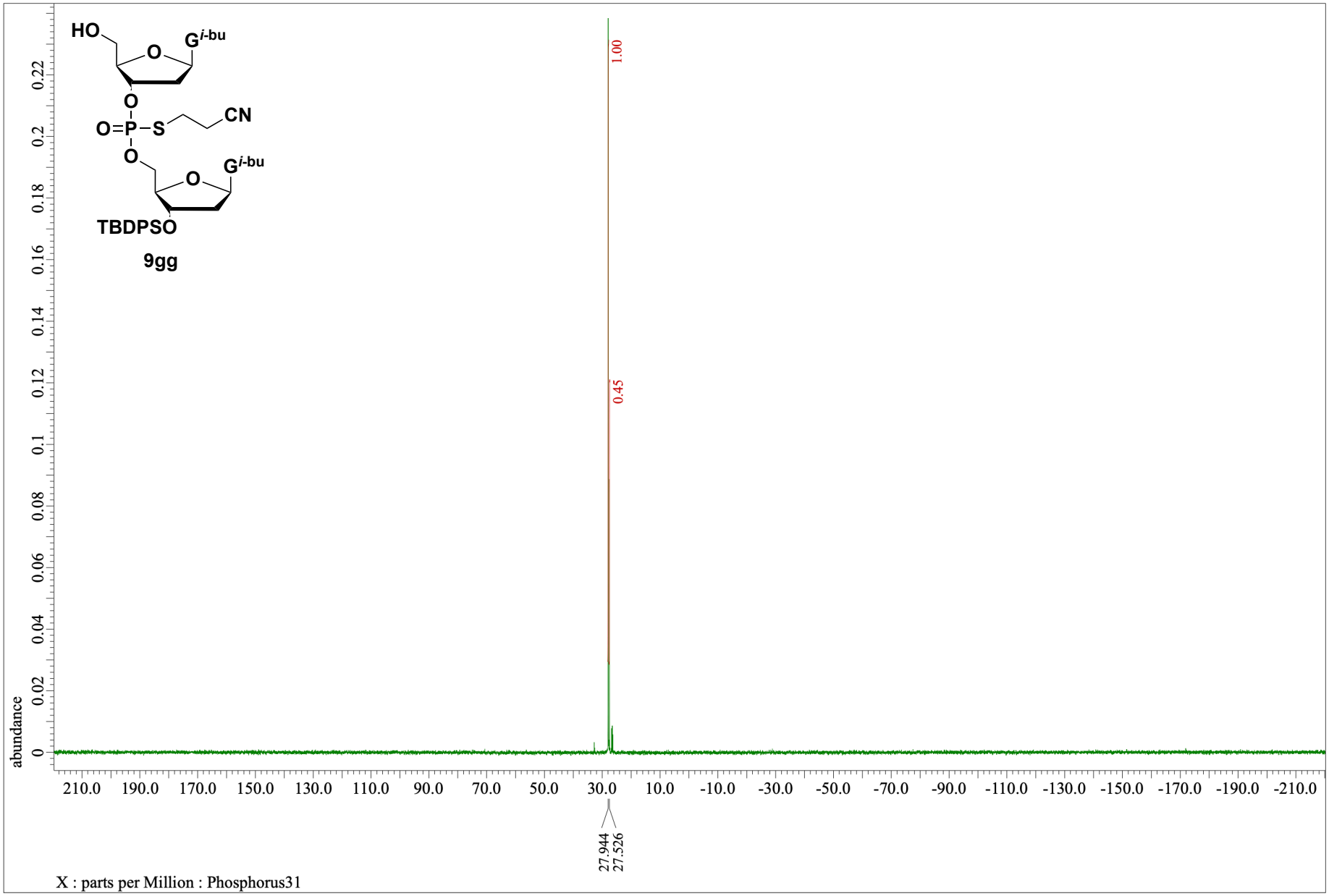
HMQC


abundance
0.1
HO
G*i*-bu
O
O
O=P–S
CN
O
G*i*-bu
O
TBDPSO
9gg
Y : parts per Million : Carbon13
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0
180.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
0.2
0.4
0.6
0.8
1.0
(thousandths)

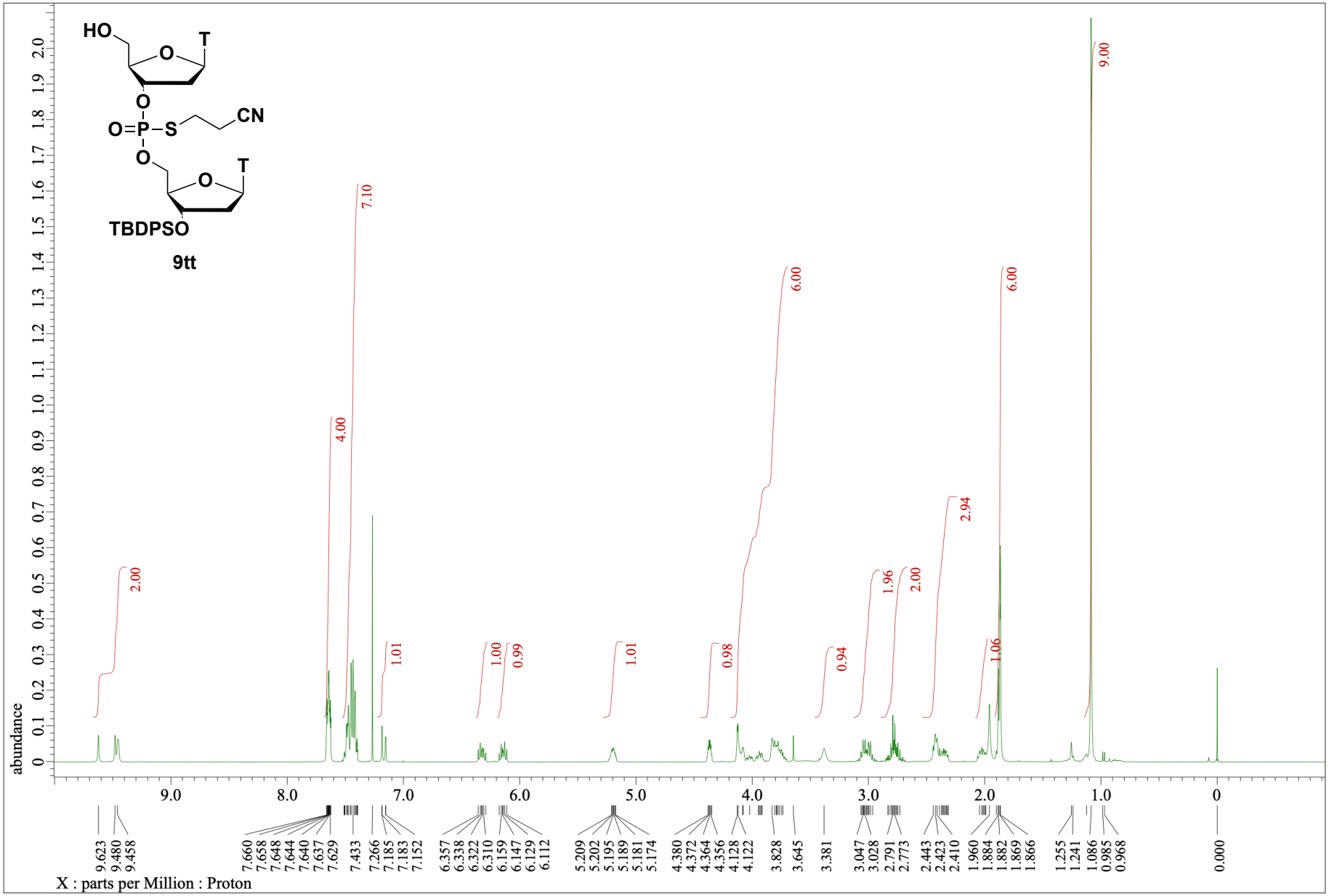
HMBC


abundance
0.1
HO
O
G*i*-bu
O
O=P–S
CN
O
O
G*i*-bu
TBDPSO
9gg
Y : parts per Million : Carbon13
10.0
30.0
50.0
70.0
90.0
110.0
130.0
150.0
170.0
190.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0
0.2
0.4
0.6
0.8
(thousandths)

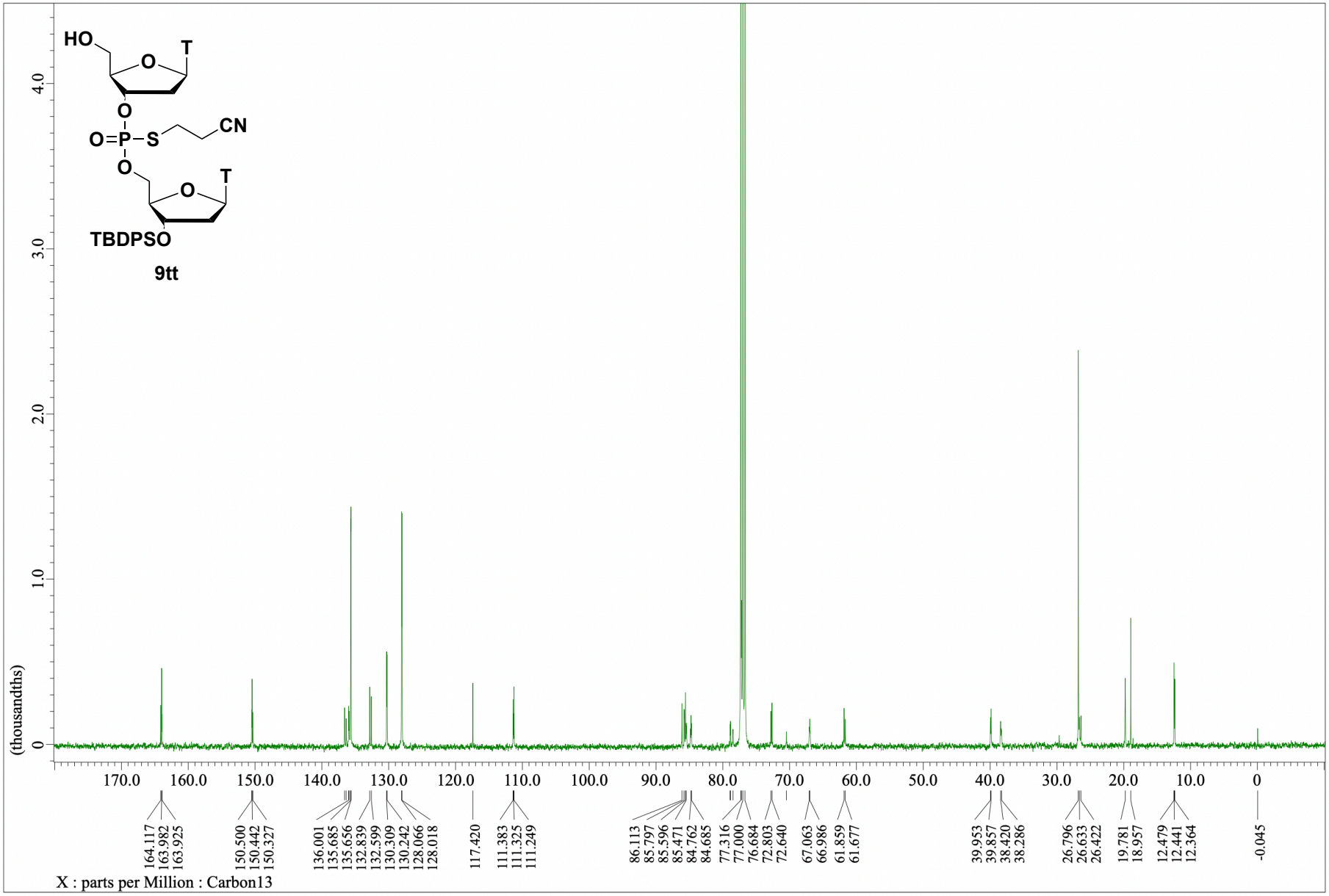
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



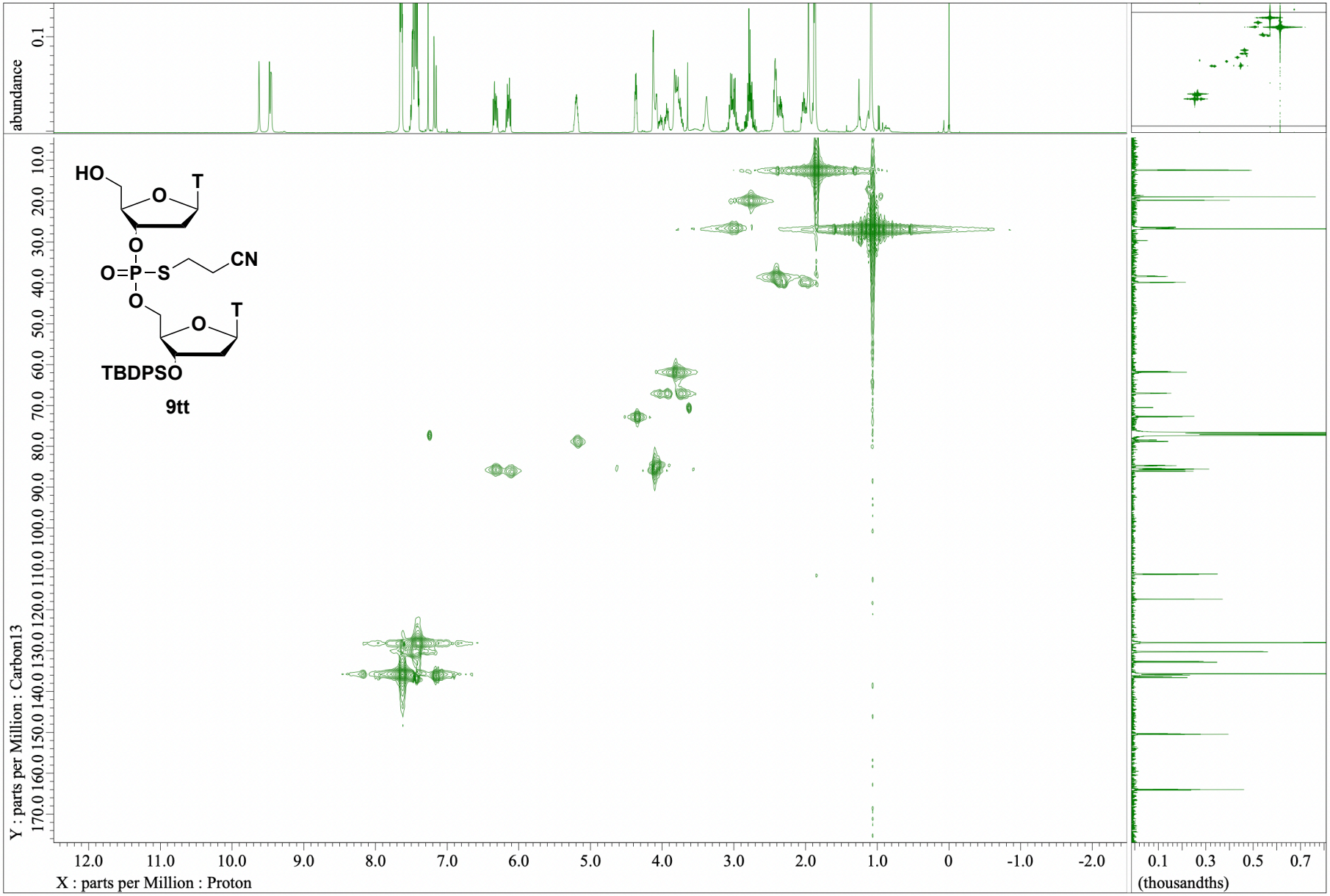
^{1}H-NMR (400 MHz, $CDCl_3$)



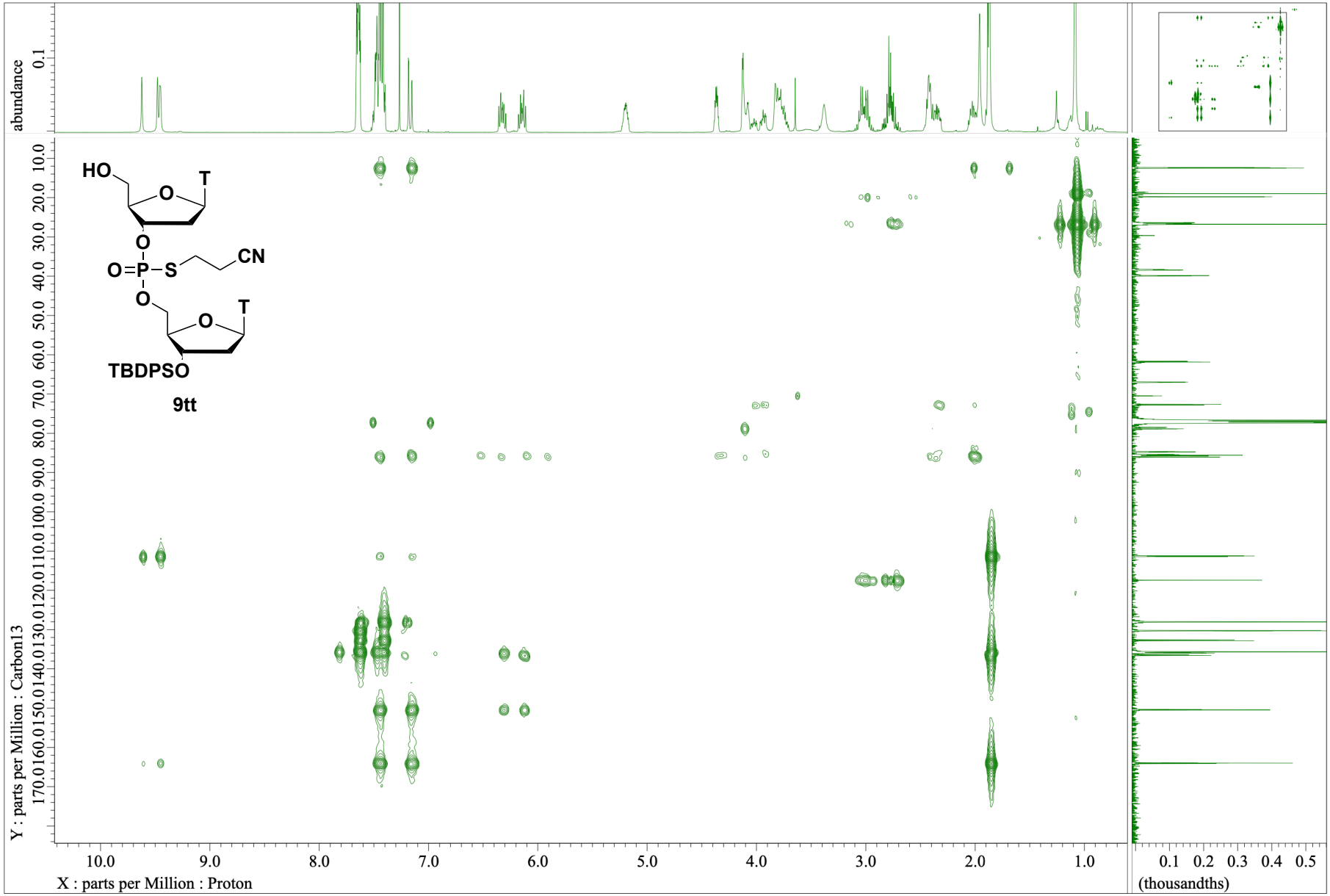
$^{13}C\{H\}$-NMR (100 MHz, $CDCl_3$)



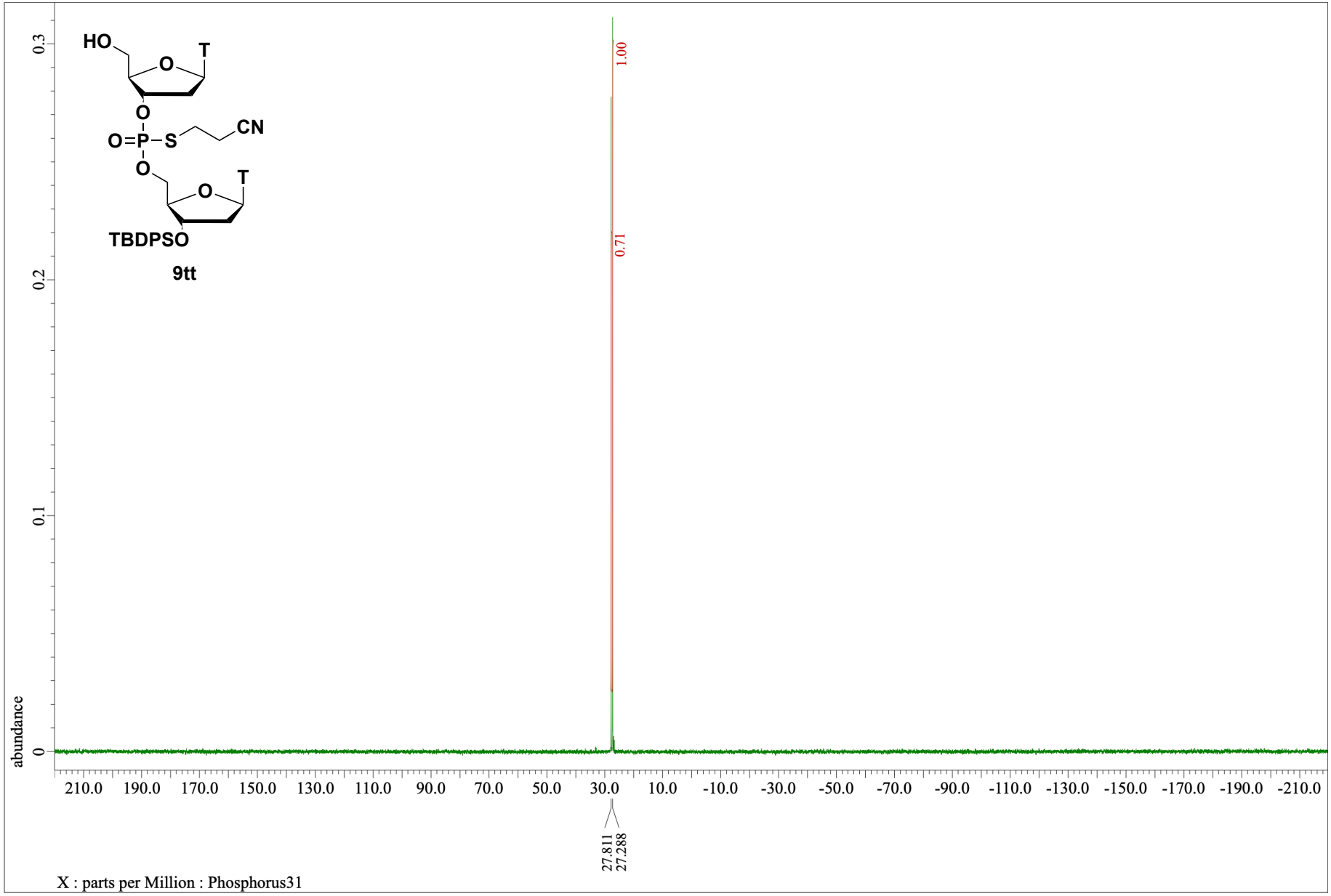
HMQC


abundance
0.1
HO
T
O
O
O=P–S
CN
O
T
O
TBDPSO
9tt
Y : parts per Million : Carbon13
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
X : parts per Million : Proton
0.1
0.3
0.5
0.7
(thousandths)

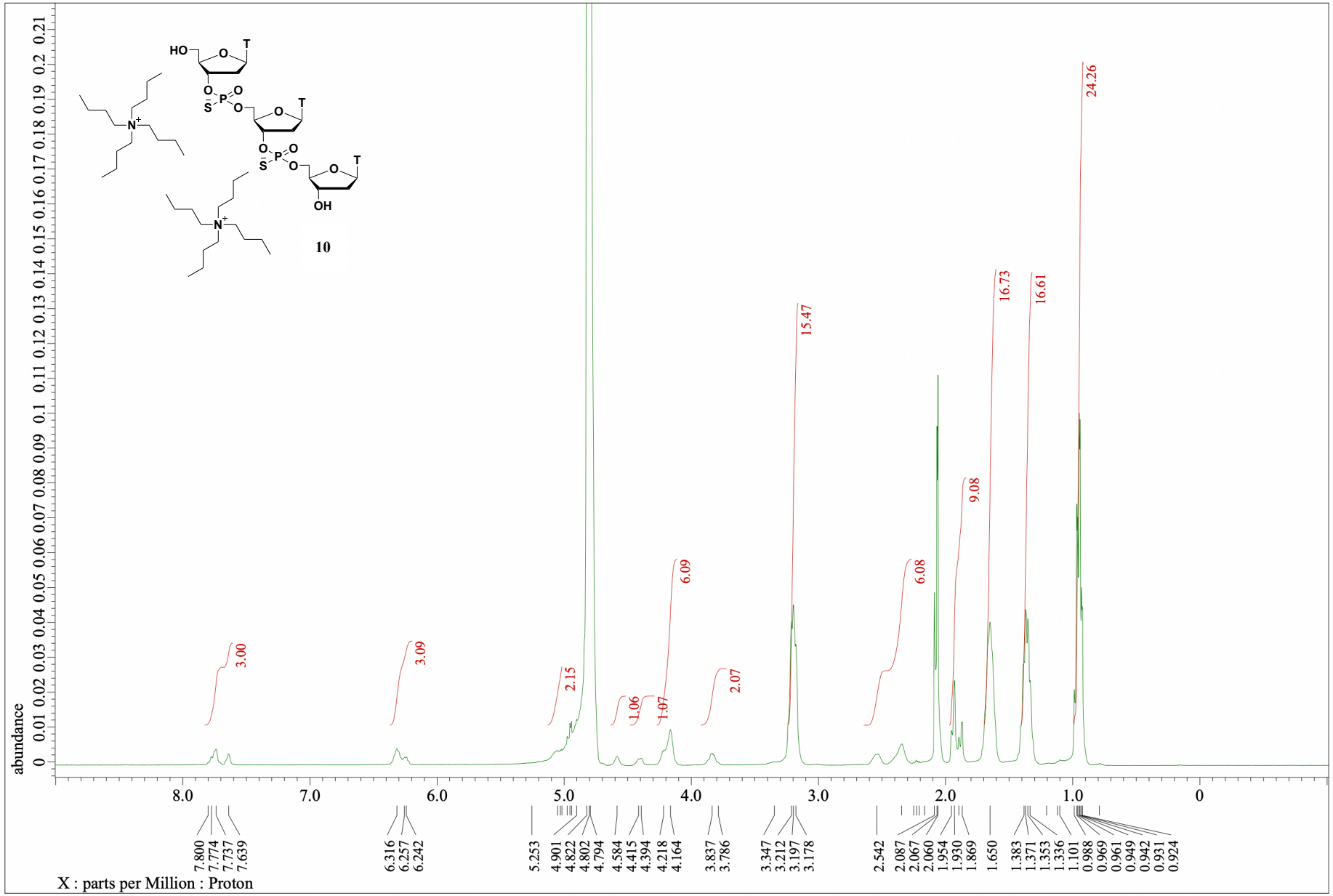
HMBC


abundance
0.1
HO
T
O
O
O=P–S
CN
O
T
O
TBDPSO
9tt
Y : parts per Million : Carbon13
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : Proton
0.1
0.2
0.3
0.4
0.5
(thousandths)

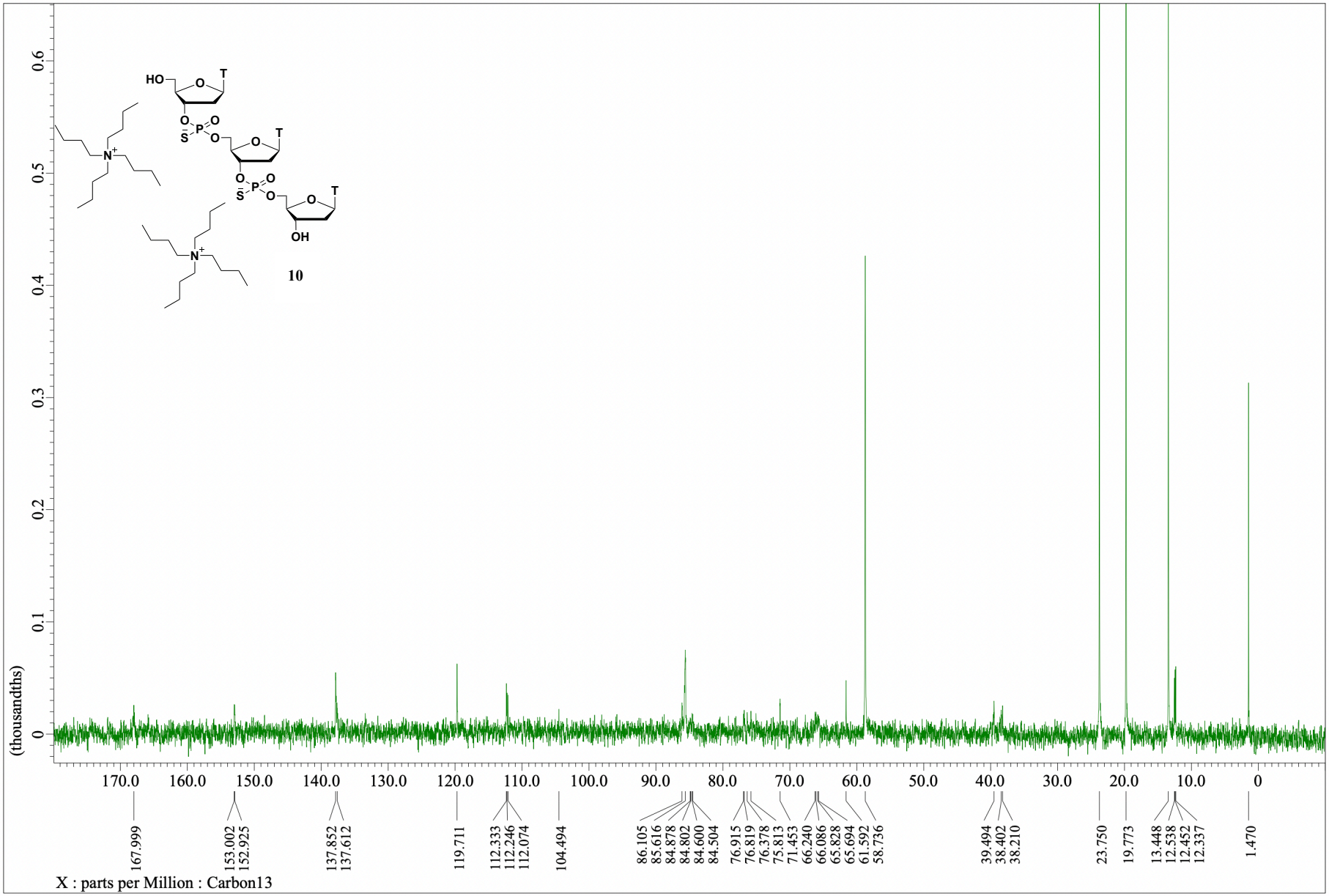
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



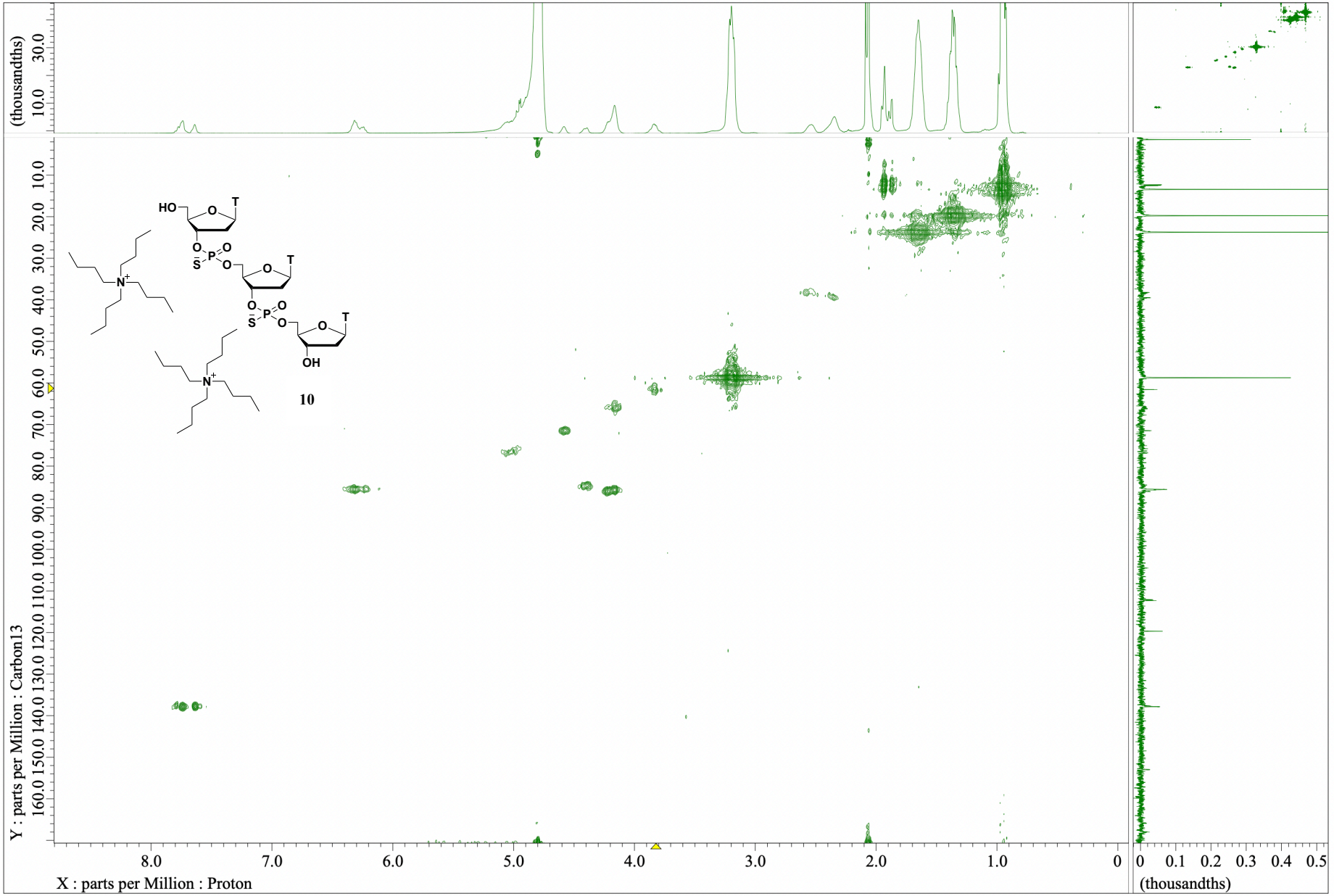
^{1}H-NMR (400 MHz, D_2O)



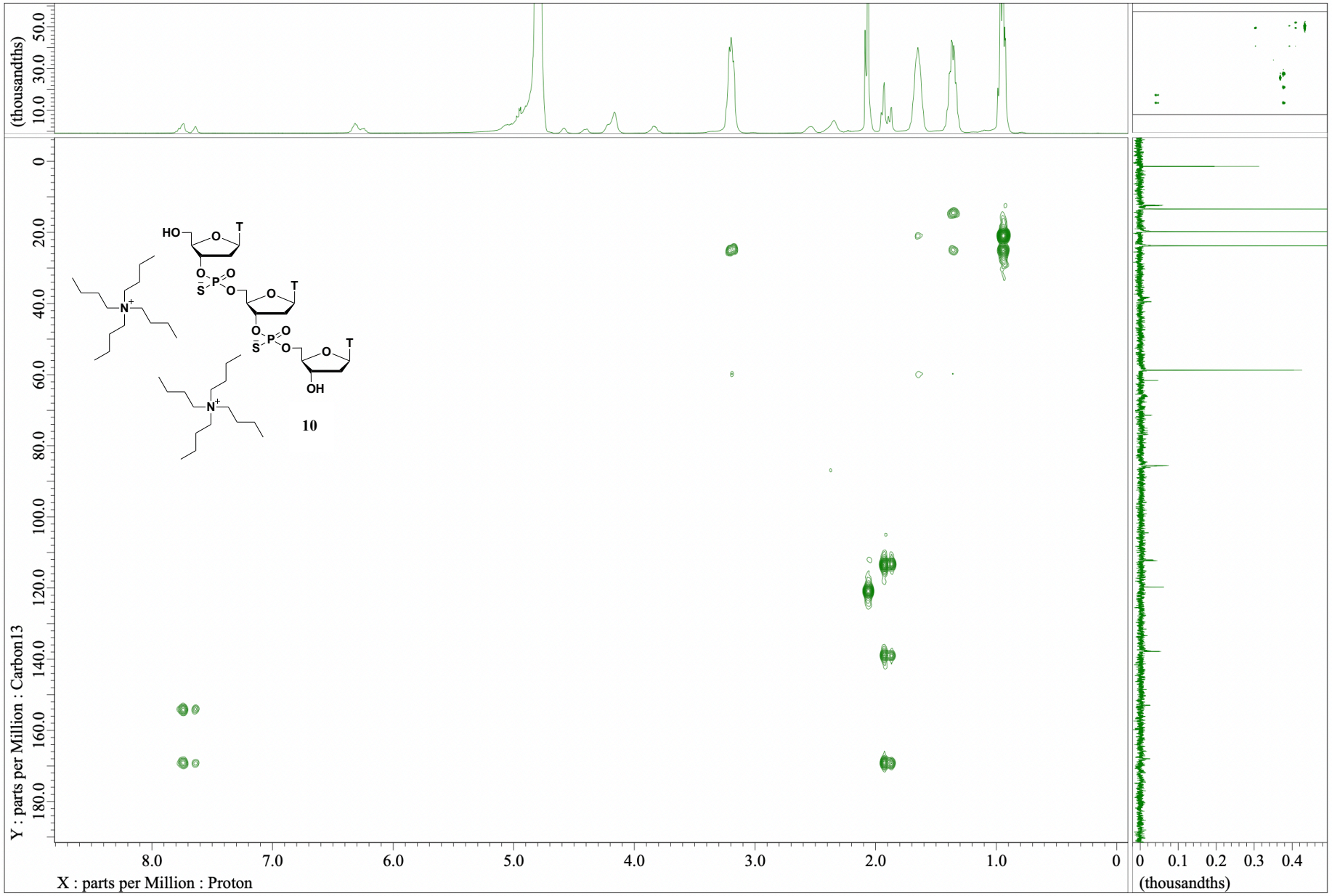
$^{13}C\{H\}$-NMR (100 MHz, D_2O)



HMQC


(thousandths)
10.0
30.0
HO
T
O
S
P
N+
OH
10
Y : parts per Million : Carbon13
X : parts per Million : Proton
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
0.1
0.2
0.3
0.4
0.5
(thousandths)

HMBC


(thousandths)
10.0
30.0
50.0
HO
T
O
S
P
N+
OH
10
Y : parts per Million : Carbon13
0
20.0
40.0
60.0
80.0
100.0
120.0
140.0
160.0
180.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
X : parts per Million : Proton
0
0.1
0.2
0.3
0.4
(thousandths)

$^{31}P\{^{1}H\}$ NMR (162 MHz, D_2O)