Introduction of a cyano group at the 2-position of an (*R*,*S*)-3-hydroxy-2-(phosphonomethoxy)propyl (HPMP) derivative of thymine elicits selective anti-HBV activity

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Supporting Information

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1. Synthetic Procedures

General Information. All reagents and solvents were obtained from commercial sources and used as received. Moisture sensitive reactions were performed using oven-dried glassware under an argon or nitrogen atmosphere. NMR spectra (^{1}H, ^{13}C, and ^{31}P) were acquired on Bruker Advance 300, 500, or 600 MHz spectrometers using tetramethylsilane as internal standard or referenced to the residual solvent signal. The final compounds were characterized by using 2D NMR (H-COSY, HSQC, and HMBC) spectroscopic techniques. High-resolution mass spectra (HRMS) were measured on a quadruple orthogonal accelerate time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min, and spectra were obtained in positive or negative ionization mode with a resolution of 15000 (fwhm) using leucine enkephalin as lock mass. Thin layer chromatography (TLC) was performed on precoated aluminum sheets (Fluka silica gel/TLC-cards, 254 nM). Column chromatography was performed on silica gel (60 Å, 0.035-0.070 mm, Acros Organics). Preparative RP-HPLC purification was performed on a Phenomenex Gemini 110A column (C18, μm, 21.2 mm × 250 mm) using CH_3CN/0.05 M TEAB buffer as eluent. Purities of all tested compounds were determined to be >95% by HPLC analysis.

1-(*tert*-Butyldiphenylsilyloxy)-3-hydroxypropan-2-one (6).[1] To a solution of dihydroxyacetone (0.708 g, 7.86 mmol) and imidazole (0.268 g, 3.93 mmol) in anhydrous DMF (5 mL) was added dropwise TBDPSCl (0.720 g, 2.62 mmol) at rt under N_2. The resulting mixture was stirred at rt for 18 h. The reaction was then quenched with water and extracted with diethyl ether. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane/EtOAc, 10:1, 8:1, 6:1) to afford compound **2** (0.450 g, 52% yield) as a colorless oil. HRMS for $C_{19}H_{24}O_3Si$ $[M + Na]^+$ calcd.: 351.1387; found, 351.1387.

1-(*tert*-Butyldiphenylsilyloxy)-3-methoxymethoxypropan-2-one (7).[1] *N*,*N*-diisopropylethylamine (15.7 g, 122 mmol) and chloromethyl methyl ether (5.88 g, 73.1 mmol) were added successively to a solution of hydroxyketone **6** (8.00 g, 24.4 mmol) in anhydrous DCM (140 mL) at rt under an Ar atmosphere. The resulting mixture was stirred at rt for 18 h. It was then quenched with saturated aq. NH_4Cl and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane/EtOAc, 30:1, 20:1) to afford compound **7** (6.10 g, 67%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (dd, J = 7.7, 1.5 Hz, 4H, Ar), 7.48–

7.37 (m, 6H, Ar), 4.66 (s, 2H, CH_2OSi), 4.48 (s, 2H, CCH_2O), 4.32 (s, 2H, OCH_2O), 3.36 (s, 3H, OCH_3), 1.10 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.6 (C=O), 135.6, 132.4, 130.2, 128.1 (Ar), 96.6 (O*C*H$_2$O), 70.7 (SiO*C*H$_2$), 68.8 (C*C*H$_2$O), 55.8 (O*C*H$_3$), 26.9 (*C*(CH$_3$)$_3$), 19.3 (C(*C*H$_3$)$_3$); HRMS: [M + Na]$^+$ calcd for $C_{21}H_{28}O_4Si$, 395.1649; found, 395.1644.

3-((*tert*-Butyldiphenylsilyl)oxy)-2-hydroxy-2((methoxymethoxy)methyl)propanenitrile (8). To a stirred solution of ketone **7** (4.30 g, 11.5 mmol) and NH_4Cl (0.802 g, 15.0 mmol) in a mixture of water (10 mL) and diethyl ether (10 mL) was added NaCN (0.622 g, 12.7 mmol). The reaction mixture was stirred at rt for 18 h. The organic and aqueous phases were separated, and the aqueous phase was extracted with diethyl ether. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane/EtOAc, 15:1, 10:1, 8:1) to afford compound **8** (3.34 g, 72%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80–7.59 (m, 4H, Ar), 7.50–7.37 (m, 6H, Ar), 4.71 (AB system, d, *J* = 6.8 Hz, 1H, OCH_2O), 4.67 (AB system, d, *J* = 6.8 Hz, 1H, OCH_2O), 4.23 (s, 1H, O*H*), 4.00 (AB system, d, *J* = 11.1 Hz, 1H, CH_2OSi), 3.82 (AB system, d, *J* = 10.2 Hz, 1H, CCH_2O), 3.74 (AB system, d, *J* = 10.2 Hz, 1H, CCH_2O), 3.66 (AB system, d, *J* = 11.1 Hz, 1H, CH_2OSi), 3.44 (s, 3H, OCH_3), 1.10 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.7, 132.2, 130.3, 128.1 (Ar), 119.6 (*C*N), 97.7 (O*C*H$_2$O), 72.3 (*C*OH), 72.0 (SiO*C*H$_2$), 66.1 (C*C*H$_2$O), 56.2 (O*C*H$_3$), 26.9 (*C*(CH$_3$)$_3$), 19.4 (C(*C*H$_3$)$_3$); HRMS: [M + Na]$^+$ calcd for $C_{22}H_{29}NO_4Si$, 422.1758; found, 422.1754.

Diisopropyl (((6-cyano-10,10-dimethyl-9,9-diphenyl-2,4,8-trioxa-9-silaundecan-6-yl)oxy)methyl)phosphonate (9). To a solution of alcohol **8** (2.70 g, 6.76 mmol) in dry THF (2 mL) at -78 °C was added a solution of 2.5 M *n*-BuLi (2.73 mL, 6.82 mmol). The reaction mixture was stirred at -78 °C for 10 min, and then (diisopropoxyphosphoryl)methyl triflate (2.44 g, 7.43 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. It was then quenched with saturated aq. NH_4Cl. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane/EtOAc,10:1, 4:1, 2:1) to afford compound **9** (2.81 g, 72% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68–7.66 (m, 4H, Ar), 7.46–7.38 (m, 6H, Ar), 4.75–4.69 (m, 2H, C*H*(CH$_3$)$_2$), 4.64 (s, 2H, OCH_2O), 4.04 (AB system, d, *J* = 4.2 Hz, 1H, CH_2OSi), 4.00 (AB system, d, *J* = 4.2 Hz, 1H, CH_2OSi), 3.91–3.78 (m, 4H, CCH_2O, OCH_2P), 3.36 (s, 3H, O*C*H$_3$), 1.33–1.26 (m, 12H, CH(CH_3)$_2$), 1.08 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.8, 132.3, 130.2, 128.1 (Ar), 116.5 (*C*N), 96.8 (O*C*H$_2$O), 80.0 and 79.8 (*C*CN), 71.6 (d, $^2J_{COP}$ = 6.3 Hz, PO*C*H), 67.5 (*C*H$_2$OSi),

64.2 (C*C*H$_2$O), 62.10 (d, $^1J_{C,P}$ = 171.8 Hz, P*C*H$_2$), 55.9 (O*C*H$_3$), 26.9 (*C*(CH$_3$)$_3$), 24.2 – 23.1 (m, CH(*C*H$_3$)$_2$), 19.36 (C(*C*H$_3$)$_3$); ^{31}P NMR (121 MHz, CDCl$_3$) δ 17.4; HRMS: [M + H]$^+$ calcd for $C_{29}H_{44}NO_7PSi$, 578.2697; found, 578.2708.

Diisopropyl (((2-cyano-1-hydroxy-3-(methoxymethoxy)propan-2-yl)oxy)methyl)phosphonate (10). To a solution of compound **9** (2.60 g, 4.50 mmol) in dry THF (5 mL) was added a solution of 1 M TBAF (9.0 mL, 9 mmol) at 0 °C. The reaction mixture was allowed to stir at rt for 1 h. The organic layer was then washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude residue was purified by silica gel column chromatography (hexane/EtOAc, 8:1, 4:1) to afford compound **10** (0.981 g, 64% yield) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.90 (brs, 1H, OH), 4.76–4.70 (m, 2H, C*H*(CH$_3$)$_2$), 4.65 (s, 2H, OC*H*$_2$O), 3.97–3.72 (m, 6H, SiOC*H*$_2$, C*H*$_2$MOM, OC*H*$_2$P), 3.37 (s, 3H, OC*H*$_3$), 1.31 (s, 12H, CH(C*H*$_3$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 116.9 (*C*N), 96.7 (O*C*H$_2$O), 80.0 and 79.9 (*C*CN), 72.8, 72.7, 72.3 and 72.2 (PO*C*H), 66.9 (*C*H$_2$OMOM), 61.6 (*C*H$_2$OH), 61.4 (d, $^1J_{C,P}$ = 171.2 Hz, P*C*H$_2$), 55.8 (O*C*H$_3$), 24.2–23.9 (m, CH(*C*H$_3$)$_2$).^{31}P NMR (121 MHz, $CDCl_3$) δ 19.6; HRMS: [M + H]$^+$ calcd for $C_{13}H_{26}NO_7P$, 340.1520; found, 340.1522.

2-Cyano-2-((diisopropoxyphosphoryl)methoxy)-3-(methoxymethoxy)propyl triflate (11). To a solution of alcohol **10** (6.80 g, 20.0 mmol) in dry Et_2O (40 mL) at -78 °C was added a solution of 2.5 M n-BuLi (8.42 mL, 21.0 mmol). The reaction mixture was stirred -78 °C for 10 min, and then (diisopropoxyphosphoryl)methyl triflate (3.55 g, 21.0 mmol) was added dropwise. The reaction mixture was further stirred at -78 °C for 2 h. It was then quenched with saturated aq. NH_4Cl. The organic and aqueous phases were separated, and the latest was extracted with diethyl ether. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* at rt to afford **11** (8.20 g, 87% yield) as a crude yellow oil, which was used in the next step without further purification.

Diisopropyl (((1-(3-benzoyl-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-cyano-3-(methoxymethoxy)propan-2-yl)oxy)methyl)phosphonate (12a). To a solution of triflate **11** (0.600 g, 1.27 mmol), *N*^{3}-benzoylthymine (0.440 g, 1.91 mmol) in dry DMF (5 mL) was added anhydrous Cs_2CO_3 (0.829 g, 2.55 mmol). The reaction mixture was stirred at rt for 20 h. It was then quenched with saturated aq. NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude residue was purified by silica gel column chromatography (DCM/MeOH, 100:1, 80:1, 70:1) to afford compound **12a** (0.303 g, 43% yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.2 Hz, 2H, Ar), 7.64 (t, *J* = 7.4 Hz, 1H, Ar), 7.48 (t, *J* = 7.6 Hz,

2H, Ar), 7.39 (s, 1H, H-6), 4.83–4.71 (m, 2H, C*H*(CH_3)$_2$), 4.65 (s, 2H, OCH_2O), 4.50 (AB system, d, *J* = 14.6 Hz, 1H, H-3'), 4.04–3.97 (m, 3H, OCH_2P, H-3'), 3.85–3.76 (m, 2H, H-1') 3.38 (s, 3H, OCH_3), 1.98 (s, 3H, CH_3-5), 1.37–1.33 (m, 12H, CH(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5 (Ar*C*=O), 162.9 (C-4), 150.2 (C-2), 140.8 (C-6), 135.1, 131.5, 130.6, 129.2 (Ar), 115.0 (*C*N), 111.2 (C-5), 96.9 (O*C*H_2O), 78.7 and 78.6 (C-2'), 72.0–71.8 (m, (PO*C*H)), 67.4 (C-3'), 62.3 (d, $^1J_{C,P}$ = 172.3 Hz, P*C*H_2), 56.2 (O*C*H_3), 50.2 (C-1'), 24.2–24.1 (m, CH(*C*H_3)$_2$), 12.5 (*C*H_3-5); ^{31}P NMR (121 MHz, $CDCl_3$) δ 17.0. HRMS: $[M + Na]^+$ calcd for $C_{25}H_{34}N_3O_9P$, 574.1925; found, 574.1940.

Diisopropyl (((1-(3-benzoyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-cyano-3-(methoxymethoxy)propan-2-yl)oxy)methyl)phosphonate (12b). Compound **12b** was obtained as a sticky white solid (0.898 g, 39% yield) according to the procedure used for the preparation of compound **12a**, starting from triflate **11** (2 g, 4.24 mmol), *N*^{3}-benzoyluracil (1.38 g, 6.36 mmol), and anhydrous Cs_2CO_3 (2.76 g, 8.49 mmol) in dry DMF (10 mL). The crude residue was purified by silica gel column chromatography (DCM/MeOH, 90:1, 80:1, 70:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, *J* = 7.7 Hz, 2H, Ar), 7.62 (t, *J* = 7.4 Hz, 1H, Ar), 7.53–7.42 (m, 3H, Ar, H-6'), 5.79 (d, *J* = 8.1 Hz, 1H, H-5'), 4.85–4.65 (m, 2H, C*H*(CH_3)$_2$), 4.62 (s, 2H, OCH_2O), 4.51 (AB system, d, *J* = 14.7 Hz, 1H, H-3'), 4.01–3.93 (m, 3H, OCH_2P, H-3'), 3.81 (AB system, d, *J* = 10.9 Hz, 1H, H-1'), 3.75 (AB system, d, *J* = 11.0 Hz, 1H, H-1'), 3.35 (s, 3H, OCH_3), 1.34–1.30 (m, 12H, CH(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3 (Ar*C*=O), 161.9 (C-4), 150.0 (C-2), 144.8 (C-6), 135.2, 131.3, 130.6, 129.2 (Ar), 114.8 (*C*N), 102.5 (C-5), 96.9 (O*C*H_2O), 78.5 and 78.3 (C-2'), 71.9 – 71.7 (m, PO*C*H), 67.2 (C-3'), 62.1 (d, $^1J_{C,P}$ = 172.1 Hz, P*C*H_2), 56.1 (O*C*H_3), 50.3 (C-1'), 24.1–24.0 (m, CH(*C*H_3)$_2$); ^{31}P NMR (121 MHz, $CDCl_3$) δ 16.8. HRMS: $[M + Na]^+$ calcd for $C_{24}H_{32}N_3O_9P$, 560.1769; found, 560.1774.

Diisopropyl (((1-(6-chloro-9H-purin-9-yl)-2-cyano-3-(methoxymethoxy)propan-2-yl)oxy)methyl)phosphonate (12c). Compound **12c** was obtained as a white foam (0.470 g, 36% yield) according to the procedure used for the preparation of compound **12a**, starting from triflate **11** (1.00 g, 2.12 mmol), 6-Cl-purine (0.492 g, 3.18 mmol), and anhydrous Cs_2CO_3 (1.38 g, 4.24 mmol) in dry DMF (10 mL). The crude residue was purified by silica gel column chromatography (DCM/MeOH, 90:1, 80:1, 70:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (s, 1H, H-8), 8.38 (s, 1H, H-2), 4.93 (AB system, d, *J* = 14.7 Hz, 1H, H-1'), 4.82–4.67 (m, 4H, C*H*(CH_3)$_2$, OCH_2O), 4.64 (d, *J* = 14.8 Hz, 1H, H-1'), 4.08–3.95 (m, 2H, OCH_2P), 3.87 (AB system, d, *J* = 10.7 Hz, 1H, H-3'), 3.71 (AB system, d, *J* = 10.7 Hz, 1H, H-3'), 3.42 (s, 3H, OCH_3), 1.29 (m, 12H, CH(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.5 (C-6), 152.4 (C-2), 151.6 (C-4), 146.2 (C-8), 131.2 (C-5), 115.0 (*C*N), 97.0 (O*C*H_2O), 78.0 and 77.8 (C-2'), 72.2

– 71.9 (m, PO*C*H), 67.0 (C-3'), 62.4 (d, $^{1}J_{C,P}$ = 171.9 Hz, P*C*H$_2$), 56.4 (O*C*H$_3$), 46.9 (C-1'), 24.2 – 24.0 (m, CH(*C*H$_3$)$_2$); ^{31}P NMR (121 MHz, $CDCl_3$) δ 16.4. HRMS: $[M + H]^+$ calcd for $C_{18}H_{27}ClN_5O_6P$, 476.1460; found, 476.1452.

Diisopropyl (((2-cyano-1-hydroxy-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propan-2-yl)oxy)methyl)phosphonate (13a). Compound **12a** (0.230 g, 0.42 mmol) was dissolved in 7 N methanolic ammonia (5 mL). After stirring at rt for 2 h, the volatiles were evaporated *in vacuo*. The residue was dissolved in THF (2 mL), and then 2 N HCl (2 mL) was added to this solution. The resulting solution was stirred at 50 °C for 1 h, it was then neutralized with $NaHCO_3$. After removal of the volatiles *in vacuo*, the resulting residue was purified by silica gel column chromatography (DCM/MeOH, 40:1, 30:1, 20:1) to afford compound **13a** (0.143 g, 85% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (s, 1H, N*H*), 7.16 (d, *J* = 1.1 Hz, 1H, H-6), 5.14 (brs, 1H, O*H*), 4.83–4.69 (m, 2H, C*H*(CH$_3$)$_2$), 4.23 (s, 2H, OC*H*$_2$P), 4.05–3.89 (m, 3H, H-3', H-1'), 3.75–3.68 (m, 1H, H-1'), 1.92 (d, *J* = 0.9 Hz, 3H, C*H*$_3$-5), 1.38–1.33 (m, 12H, CH(C*H*$_3$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.7 (C-4), 151.5 (C-2), 140.9 (C-6), 115.7 (*C*N), 111.5 (C-5), 79.4 and 79.3 (C-2'), 72.7–72.6 (m, PO*C*H), 61.6 (d, $^{1}J_{C,P}$ = 171.4 Hz, P*C*H$_2$), 61.6 (C-3'), 49.6 (C-1'), 24.1 (d, $^{3}J_{C,P}$ = 4.1 Hz, CH(*C*H$_3$)$_2$), 12.4 (*C*H$_3$-5); ^{31}P NMR (121 MHz, $CDCl_3$) δ 18.8. HRMS: $[M - H]^-$ calcd for $C_{16}H_{26}N_3O_7P$, 402.1435; found, 402.1438.

Diisopropyl (((2-cyano-1-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-hydroxypropan-2-yl)oxy)methyl)phosphonate (13b). Compound **13b** was obtained as a white solid (0.139 g, 77% yield) according to the procedure used for the preparation of compound **13a**, starting from compound **12b** (0.250 g, 0.47 mmol). The crude residue was purified by silica gel column chromatography (DCM/MeOH, 35:1, 25:1, 15:1). ^{1}H NMR (300 MHz, MeOD) δ 7.55 (d, *J* = 8.0 Hz, 1H, H-6), 5.65 (d, *J* = 7.9 Hz, 1H, H-5), 4.76–4.65 (m, 2H, C*H*(CH$_3$)$_2$), 4.49 (AB system, d, *J* = 14.7 Hz, 1H, H-3'), 4.15–3.99 (m, 3H, OC*H*$_2$P, H-3'), 3.82 (s, 2H, H-1'), 1.34–1.29 (m, 12H, CH(C*H*$_3$)$_2$); ^{13}C NMR (75 MHz, MeOD) δ 166.3 (C-4), 152.8 (C-2), 147.8 (C-6), 116.8 (*C*N), 102.5 (C-5), 81.1 and 80.9 (C-2'), 73.7–73.6 (m, PO*C*H), 63.3 (C-3'), 61.96 (d, $^{1}J_{C,P}$ = 171.7 Hz, C-2'), 50.4 (C-1'), 24.3–24.2 (m, CH(*C*H$_3$)$_2$); ^{31}P NMR (121 MHz, MeOD) δ 17.6. HRMS: $[M + H]^+$ calcd for $C_{15}H_{24}N_3O_7P$, 390.1426; found, 390.1426.

Diisopropyl (((1-(6-amino-9*H*-purin-9-yl)-2-cyano-3-hydroxypropan-2-yl)oxy)methyl)pho-sphonate (13c). Compound **12c** (0.270 g, 0.57 mmol) was dissolved in saturated ethanolic ammonia (5 mL). After stirred at 70 °C for 18 h, the volatiles were removed *in vacuo*. The residue was dissolved in THF (2 mL), and then 2 N HCl (2 mL) was added to

this solution. The resulting solution was stirred at 50 °C for 1 h, it was then neutralized with $NaHCO_3$. After removal of all the volatiles *in vacuo*, the resulting residue was purified by silica gel column chromatography (DCM/MeOH, 40:1, 30:1, 20:1) to afford compound **13c** (0.171 g, 73% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO) δ 8.16 (s, 1H, H-2), 8.03 (s, 1H, H-8), 7.33 (s, 2H, N*H*$_2$), 6.03 (t, *J* = 5.9 Hz, 1H, O*H*), 4.80 (AB system, d, *J* = 14.9 Hz, 1H, H-1'), 4.63–4.48 (m, 3H, C*H*(CH$_3$)$_2$, H-1'), 4.15–3.95 (m, 2H, OC*H*$_2$P), 3.82 (ABX system, dd, *J* = 12.0, 6.1 Hz, 1H, H-3'), 3.71 (ABX system, dd, *J* = 12.0, 5.7 Hz, 1H, H-3'), 1.25–1.14 (m, 12H, CH(C*H*$_3$)$_2$); ^{13}C NMR (75 MHz, DMSO) δ 156.0 (C-6), 152.5 (C-2), 149.8 (C-4), 141.1 (C-8), 117.9 (C-5), 116.3 (*C*N), 79.6 and 79.4 (C-2'), 70.8 and 70.7 (PO*C*H), 61.4 (C-3'), 60.7 (d, $^1J_{C,P}$ = 166.5 Hz, P*C*H$_2$), 44.7 (C-1'), 23.7–23.4 (m, CH(*C*H$_3$)$_2$); ^{31}P NMR (121 MHz, DMSO) δ 16.8. HRMS: [M + H]$^+$ calcd for $C_{24}H_{32}N_3O_9P$, 413.1697; found, 413.1690.

[2-Cyano-3-hydroxy-2-(phosphonomethoxy)propy]thymine triethylammonium salt (14a). TMSBr (0.425 g, 2.78 mmol) was added dropwise to a mixture of compound **13a** (0.070 g, 0.17 mmol) and 2,6-lutidine (0.298 g, 2.78 mmol) in dry acetonitrile (3 mL) at 0 °C. The resulting mixture was stirred at rt in the dark for 18 h. It was then quenched with 2 M TEAB, and all the volatiles were removed *in vacuo*. The crude residue was first purified by silica gel column chromatography (Acetone/Et_3N/H_2O, 15:1:1, 10:1:1, 5:1:1), followed by further RP-HPLC purification (linear gradient, 2–27% CH_3CN in 0.05 TEAB solution) to afford the desired phosphonate acid triethylammonium salt **14a** (0.040 g, 49% yield) as a white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.63 (s, 1H, H-6), 4.36 (AB system, d, *J* = 14.8 Hz, 1H, H-1'), 4.10 (AB system, d, *J* = 14.8 Hz, 1H, H-1'), 3.98 (AB system, d, *J* = 12.8 Hz, 1H, H-3'), 3.79 (AB system, d, *J* = 12.7 Hz, 1H, H-3'), 3.67 (AB system, d, *J* = 9.5 Hz, 2H, OC*H*$_2$P), 1.87 (s, 3H, C*H*$_3$); ^{13}C NMR (151 MHz, D_2O) δ 166.8 (C-4), 152.4 (C-2), 143.3 (C-6), 116.6 (*C*N), 110.9 (C-5), 79.1 (d, $^3J_{C,P}$ = 11.1 Hz, C-2'), 64.3 (d, $^1J_{C,P}$ = 148.9 Hz, *C*H$_2$P), 61.7 (C-3'), 49.3 (C-1'), 11.2 (*C*H$_3$); ^{31}P NMR (121 MHz, D_2O) δ 11.9. HRMS: [M - H]$^-$ calcd for $C_{10}H_{14}N_3O_7P$, 318.0497; found, 318.0493.

[2-Cyano-3-hydroxy-2-(phosphonomethoxy)propy]uracil triethylammonium salt (14b). Phosphonate acid triethylammonium salt **14b** was obtained as a white foam (0.033 g, 46% yield) according to the procedure used for the preparation of compound **14a**, starting from compound **13b** (0.070 g, 0.18 mmol), TMSBr (0.440 g, 2.88 mmol), and 2,6-lutidine (0.308 g, 2.88 mmol) in dry acetonitrile (3 mL). ^{1}H NMR (600 MHz, D_2O) δ 7.79 (d, *J* = 8.0 Hz, 1H, H-6), 5.87 (d, *J* = 8.0 Hz, 1H, H-5), 4.50 (AB system, d, *J* = 14.8 Hz, 1H, H-1'), 4.14 (AB system, d, *J* = 14.8 Hz, 1H, H-1'), 3.99 (AB system, d, *J* = 12.6 Hz, 1H, H-3'), 3.87 (AB system, d, *J* = 12.7 Hz, 1H, H-3'), 3.75 (m, 2H, OCH_2P); ^{13}C NMR (151 MHz, D_2O) δ 166.5

(C-4), 152.3 (C-2), 147.8 (C-6), 116.3 (*C*N), 101.8 (C-5), 79.1 (d, $^{3}J_{C,P}$ = 12.5 Hz, C-2'), 63.7 (d, $^{1}J_{C,P}$ = 152.9 Hz, *C*H_2P), 61.5 (C-3'), 49.6 (C-1'); ^{31}P NMR (121 MHz, D_2O) δ 12.3; HRMS: [M - H]$^-$ calcd for $C_9H_{12}N_3O_7P$, 304.0340; found, 304.0331.

[2-Cyano-3-hydroxy-2-(phosphonomethoxy)propy]adenine triethylammonium salt (14c). Phosphonate acid triethylammonium salt **14c** was obtained as a white foam (0.035 g, 48% yield) according to the procedure used for the preparation of compound **14a**, starting from compound **13c** (0.070 g, 0.18 mmol), TMSBr (0.416 g, 2.72 mmol), and 2,6-lutidine (0.291 g, 2.72 mmol) in dry acetonitrile (3 mL). ^{1}H NMR (600 MHz, D_2O) δ 8.27 (s, 1H, H-8), 8.13 (s, 1H, H-2), 4.74 (AB system, d, *J* = 15.1 Hz, 1H, H-1'), 4.64 (AB system, d, *J* = 15.1 Hz, 1H, H-1'), 3.97 (AB system, d, *J* = 12.7 Hz, 1H, H-3'), 3.86 (AB system, d, *J* = 12.7 Hz, 1H, H-3'), 3.82–3.69 (m, 2H, OC*H*$_2$P); ^{13}C NMR (151 MHz, D_2O) δ 155.2 (C-6), 152.3 (C-2), 149.0 (C-4), 143.2 (C-8), 117.6 (C-5), 116.1 (*C*N), 79.1 (d, $^{3}J_{C,P}$ = 12.8 Hz, C-2'), 63.6 (d, $^{1}J_{C,P}$ = 154.1 Hz, *C*H_2P), 61.2 (C-3'), 45.9 (C-1'); ^{31}P NMR (121 MHz, D_2O) δ 12.6; HRMS: [M - H]$^-$ calcd for $C_{10}H_{13}N_6O_5P$, 327.0612; found, 327.0611.

Diisopropyl (((2-cyano-1-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-(methoxymethoxy)propan-2-yl)oxy)methyl)phosphonate (15). Compound **12b** (0.250 g, 0.47 mmol) was dissolved in 7 N methanolic ammonia (10 mL). After stirring at rt for 2 h, all the volatiles were removed *in vacuo*. The resulting residue was purified by silica gel column chromatography (DCM/MeOH, 60:1, 40:1, 30:1) to afford compound **15** (0.174 g, 86% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.90 (s, 1H, N*H*), 7.43 (d, *J* = 8.1 Hz, 2H, H-6), 5.71 (d, *J* = 8.0 Hz, 1H, H-5), 4.79–4.71 (m, 2H, C*H*$(CH_3)_2$), 4.68 (s, 2H, OC*H*$_2$O), 4.47 (AB system, d, *J* = 14.6 Hz, 1H, H-3'), 4.04–3.99 (m, 3H, OC*H*$_2$P, H-3'), 3.85–3.77 (m, 2H, H-1'), 3.41 (s, 3H, OC*H*$_3$), 1.35–1.30 (m, 12H, CH(C*H*$_3$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.0 (C-6), 150.9 (C-2), 145.3 (C-4), 114.9 (*C*N), 102.7(C-5), 96.9 (O*C*H_2O), 78.4 and 78.2 (C-2'), 72.0–71.8 (m, PO*C*H), 67.4 (C-3'), 62.2 (d, $^{1}J_{C,P}$ = 172.3 Hz, *C*H_2P), 56.3 (O*C*H_3), 50.2 (C-1'), 24.2–24.1 (m, CH(*C*H_3)$_2$); ^{31}P NMR (121 MHz, $CDCl_3$) δ 16.9; HRMS: [M + H]$^+$ calcd for $C_{17}H_{28}N_3O_8P$, 434.1687; found, 434.1688.

Diisopropyl (((1-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-2-cyano-3-(methoxymethoxy)propan-2-yl)oxy)methyl)phosphonate (16). TPSCl (0.210 g, 0.70 mmol) was added to a solution of compound **15** (0.100 g, 0.23 mmol) and *N*,*N*-diisopropylethylamine (0.180 g, 1.39 mmol) in dry acetonitrile (5 mL). The resulting mixture was stirred at rt for 6 h, and then a 25% ammonia solution (5 mL) was added. The resulting mixture was further stirred at rt for 18 h. After removal of all the volatiles *in vacuo*, the residue was purified by silica gel column chromatography (DCM/MeOH, 40:1, 30:1, 20:1) to afford compound **16** (0.062 g, 62%

yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (brs, 1H, NH_2), 7.40 (d, *J* = 7.3 Hz, 1H, H-6), 6.38 (brs, 1H, N*H*$_2$), 5.85 (d, *J* = 7.3 Hz, 1H, H-5), 4.82–4.68 (m, 2H, C*H*$(CH_3)_2$), 4.66 (s, 2H, OC*H*$_2$O), 4.39 (AB system, d, *J* = 14.4 Hz, 1H, H-3'), 4.15 (AB system, d, *J* = 14.4 Hz, 1H, H-3'), 4.09–4.04 (m, 2H, OC*H*$_2$P), 3.85 (AB system, d, *J* = 11.1 Hz, 1H, H-1'), 3.77 (AB system, d, *J* = 11.1 Hz, 1H, H-1'), 3.39 (s, 3H, OC*H*$_3$), 1.34–1.29 (m, 12H, C*H*$(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.3 (C-4), 156.6 (C-2), 146.2 (C-6), 115.4 (*C*N), 96.8 (O*C*H$_2$O), 95.5 (C-5), 78.8 and 78.6 (C-2'), 71.9–71.7 (m, PO*C*H) 68.3 (C-3'), 62.3 (d, $^1J_{C,P}$ = 171.7 Hz, *C*H$_2$P), 56.1 (O*C*H$_3$), 50.9 (C-1'), 24.2–24.0 (m, CH(*C*H$_3$)$_2$); ^{31}P NMR (121 MHz, $CDCl_3$) δ 17.4; HRMS: $[M + H]^+$ calcd for $C_{17}H_{29}N_4O_7P$, 433.1846; found, 433.1843.

Diisopropyl (((1-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-2-cyano-3-hydroxypropan-2-yl)oxy)methyl)phosphonate (17). To a solution of compound **16** (0.105 g, 0.42 mmol) in THF (2 mL) was added 2 N HCl (2 mL). The resulting solution was stirred at 50 °C for 1 h, and then it was neutralized with $NaHCO_3$. After removal of all the volatiles *in vacuo*, the resulting residue was purified by silica gel column chromatography (DCM/MeOH, 25:1, 20:1, 15:1) to afford compound **17** (0.078 g, 83% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (brs, 1H, N*H*$_2$), 7.38 (d, *J* = 7.3 Hz, 1H, H-6), 6.82 (brs, 1H, N*H*$_2$), 5.95 (d, *J* = 7.3 Hz, 1H, H-5), 5.65 (brs, 1H, O*H*), 4.77–4.65 (m, 2H, C*H*$(CH_3)_2$), 4.31–4.20 (m, 2H, H-3'), 4.02–3.90 (m, 2H, OC*H*$_2$P), 3.78–3.68 (m, 2H, H-1'), 1.34–1.29 (m, 12H, C*H*$(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6 (C-4), 157.8 (C-2), 146.6 (C-6), 116.3 (*C*N), 96.3 (C-5), 79.7 and 79.6 (C-2'), 72.3–72.2 (m, PO*C*H), 61.5 (d, $^1J_{C,P}$ = 171.0 Hz, *C*H$_2$P), 61.2 (C-3'), 50.5 (C-1'), 24.2 and 24.1 (CH(*C*H$_3$)$_2$); ^{31}P NMR (121 MHz, $CDCl_3$) δ 17.6; HRMS: $[M + H]^+$ calcd for $C_{15}H_{25}N_4O_6P$, 389.1584; found, 389.1581.

[2-Cyano-3-hydroxy-2-(phosphonomethoxy)propy]cytosine triethylammonium salt (18). Phosphonate acid triethylammonium salt **18** was obtained as a white foam (0.025 g, 45% yield) according to the procedure used for the preparation of compound **14a**, starting from compound **17** (0.054 g, 0.14 mmol), TMSBr (0.341 g, 2.22 mmol), and 2,6-lutidine (0.238 g, 2.22 mmol) in dry acetonitrile (3 mL). ^{1}H NMR (600 MHz, D_2O) δ 7.71 (d, *J* = 7.4 Hz, 1H, H-6), 6.03 (d, *J* = 7.4 Hz, 1H, H-5), 4.51 (AB system, d, *J* = 14.7 Hz, 1H, H-1'), 4.12 (AB system, d, *J* = 14.7 Hz, H-1'), 3.96 (AB system, d, *J* = 12.7 Hz, 1H, H-3'), 3.86 (AB system, d, *J* = 12.7 Hz, 1H, H-3'), 3.82–3.74 (m, 2H, OC*H*$_2$P); ^{13}C NMR (151 MHz, D_2O) δ 166.4 (C-4), 158.1 (C-2), 147.6 (C-6), 116.2 (*C*N), 95.9 (C-5), 79.4 (d, $^3J_{C,P}$ = 13.2 Hz, C-2'), 63.3 (d, $^2J_{C,P}$ = 154.9 Hz, *C*H$_2$P), 61.7 (C-3'), 50.3 (C-1'); ^{31}P NMR (121 MHz, D_2O) δ 12.8; HRMS: $[M - H]^-$ calcd for $C_9H_{13}N_4O_6P$, 303.0500; found, 303.0501.

1,3-Bis(1,1-dimethylethyl)-2-(6-chloro-9-{2-Cyano-2-((diisopropoxyphosphoryl)methoxy)-3-(methoxymethoxy)propyl}-9*H*-purin-2-yl)imidodicarbonate (19). Compound **19** was obtained as a colorless oil (0.593 g, 41% yield) according to the procedure used for the preparation of compound **12a**, starting from triflate **11** (2.00 g, 4.24 mmol), 6-chloro-2-(di-Boc-amino)-9*H*-purine (1.38 g, 6.36 mmol), and anhydrous Cs_2CO_3 (2.76 g, 8.49 mmol) in dry DMF (10 mL). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H, H-8), 4.89 (AB system, d, *J* = 14.7 Hz, 1H, H-1'), 4.82–4.69 (m, 2H, C*H*$(CH_3)_2$), 4.68–4.62 (m, 2H, OC*H*$_2$P), 4.55 (d, *J* = 14.7 Hz, 1H, H-1'), 4.05–3.91 (m, 2H, OC*H*$_2$P), 3.85 (AB system, d, *J* = 10.7 Hz, 1H, H-3'), 3.70 (AB system, d, *J* = 10.7 Hz, 1H, H-3'), 3.39 (s, 3H, OC*H*$_3$), 1.41 (s, 18H, C(C*H*$_3$)$_3$), 1.37–1.24 (m, 12H, CH(C*H*$_3$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.1 (C-4), 152.3 (C-2), 151.6 (*C*=O), 150.5 (C-6), 147.2 (C-8), 129.5 (C-5), 114.5 (*C*N), 96.8 (O*C*H$_2$O), 83.9 (O*C*(CH$_3$)$_3$), 77.9 and 77.7 (C-2'), 72.2–71.9 (m, PO*C*H), 66.7 (C-3'), 62.3 (d, $^1J_{C,P}$ = 171.7 Hz, *C*H$_2$P), 56.3 (O*C*H$_3$), 47.2 (C-1'), 27.9 (C(*C*H$_3$)$_3$), 24.2–24.0 (m, CH(*C*H$_3$)$_2$); ^{31}P NMR (121 MHz, $CDCl_3$) δ 16.5; HRMS: $[M + Na]^+$ calcd for $C_{28}H_{44}ClN_6O_{10}P$, 713.2437; found, 713.2436.

[2-Cyano-3-hydroxy-2-(phosphonomethoxy)propy]guanine triethylammonium salt (20). TMSBr (0.425 g, 2.78 mmol) was added dropwise to a mixture of compound **19** (0.300 g, 0.17 mmol) in dry acetonitrile (3 mL) at 0 °C. The resulting mixture was stirred at rt in the dark for 18 h, and then 2 N HCl (5 mL) was added. The reaction mixture was stirred at 80 °C for 6 h, and it was then neutralized with $NaHCO_3$. After removal of all the volatiles *in vacuo*, the resulting residue was first purified by silica gel column chromatography (Acetone/Et_3N/H_2O, 15:1:1, 10:1:1, 4:1:1), followed by further RP-HPLC purification (linear gradient, 2–27% CH_3CN in 0.05 TEAB solution) to afford the desired phosphonate acid triethylammonium salt **20** (0.062 g, 30% yield) as a white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.98 (s, 1H, H-8), 4.60 (AB system, d, *J* = 15.0 Hz, 1H, H-1'), 4.52 (AB system, d, *J* = 15.0 Hz, 1H, H-1'), 3.94 (AB system, d, *J* = 12.8 Hz, 1H, H-3'), 3.83 (AB system, d, *J* = 12.7 Hz, 1H, H-3'), 3.78–3.76 (m, 2H, OC*H*$_2$P); ^{13}C NMR (151 MHz, D_2O) δ 158.8 (C-6), 153.8 (C-4), 151.9 (C-5), 140.6 (C-8), 116.3 (*C*N), 115.3 (C-2), 79.3 (C-2'), 63.9 (d, $^1J_{C,P}$ = 153.0 Hz, CH_2P), 61.3 (C-3'), 45.1 (C-1'); ^{31}P NMR (121 MHz, $CDCl_3$) δ 12.4; HRMS: $[M - H]^-$ calcd for $C_{10}H_{13}N_6O_6P$, 343.0561; found, 343.0565.

2. Antiviral Assays

Anti-Herpesviruses Activity Measurement. The antiviral activity against HCMV AD169 and VZV Ellen was evaluated using cytopathic effect (CPE) reduction assays according to standard methods established before.[2, 3] Briefly, monolayers of HFF cells were infected with either HCMV or VZV at a multiplicity of infection of approximately 0.01 plaque forming units per cell. At 14 d following infection CPE was evaluated in cells infected with HCMV and VZV. For each virus, concurrent cytotoxicity studies were performed using the same cells and compound exposure and cell number was determined using CellTiter-Glo (Promega). The control compounds acyclovir (ACV) and ganciclovir (GCV) were purchased from the University of Alabama Hospital Pharmacy. Data obtained were used to calculate concentrations of compounds sufficient to inhibit viral replication by 50% (EC_{50}) and cell number 50% (CC_{50}). Multiple assays were performed for each compound to obtain statistical data.

Anti-HBV Activity Measurement. Antiviral activity against HBV was measured by quantitating the virus associated extracellular HBV DNA (Virus yield assay) in cell culture supernatants of HepG2.2.2.15 cells using real-time qPCR (TaqMan) as previously reported.[4, 5] HepG2 2.2.15 cells seeded in 96-well plates were treated with serially diluted compounds (triplicate wells/compound dilution) next day and incubated at 37 °C with 5% CO_2 for three days, followed by replenishment with fresh compounds. After three more days (total six days post-treatment), cell culture supernatant was treated with pronase and virus associated DNA was measured using real time qPCR according to the standard PCR method. EC_{50} and EC_{90} were determined after normalizing extracellular HBV DNA copy numbers to untreated controls. Antiviral compound Lamivudine (3TC) was used as a positive control in each assay. Cytotoxicity was measured by CellTiter® 96 Reagent, (Promega) uptake assay to determine CC_{50}. Selectivity index 50% (SI_{50}) was calculated by the ratio of $CC5_0/EC_{50}$.

Anti-HIV-1 and -HIV-2 Activity Measurement. Evaluation of the antiviral activity of all compounds against HIV-1 (strain III_B) and HIV-2 (strain ROD) in MT-4 cells was performed using the MTT assay as previously described.[6, 7] Stock solutions (10 × final concentration) of test compounds were added in 25 μL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated HIV- and mock-infected cell samples were included as controls. HIV-1 (III_B) stock

(50 μL) at 100–300 $CCID_{50}$ (50% cell culture infectious doses) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effects of test compound on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells were centrifuged for 5 min at 220 g, and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50 μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically using the MTT assay. The MTT assay is based on the reduction of yellow-colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics) by mitochondrial dehydrogenase activity in metabolically active cells to a blue–purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Infinite M1000, Tecan) at two wavelengths (540 and 690 nm). All data were calculated using the median absorbance value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The concentration achieving 50% protection against the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

Table S1. Anti-HIV Activity and Cytotoxicity of CHPMP Derivatives

Compound	EC_{50}[a] (μM)		CC_{50}[b] (μM)	SI[c]	
	HIV-1 (III_B)	HIV-2 (ROD)		HIV-1	HIV-2
14a	> 391.6	> 391.6	> 391.6	1	1
14b	> 409.6	> 409.6	> 409.6	1	1
14c	308.9	> 380.8	> 380.8	> 1	1
18	> 410.9	> 410.9	> 410.9	1	1
20	51.0	33.7	300.2	6	9
3TC	2.5	9.9	> 87.2	> 34	> 9

[a]Effective concentration required to achieve 50% protection of MT-4 cells against HIV-induced cytopathicity. [b]Cytotoxic concentration required to reduce the viability of mock-infected cells by 50%. [c]Selectivity index (SI): CC_{50}/EC_{50}.

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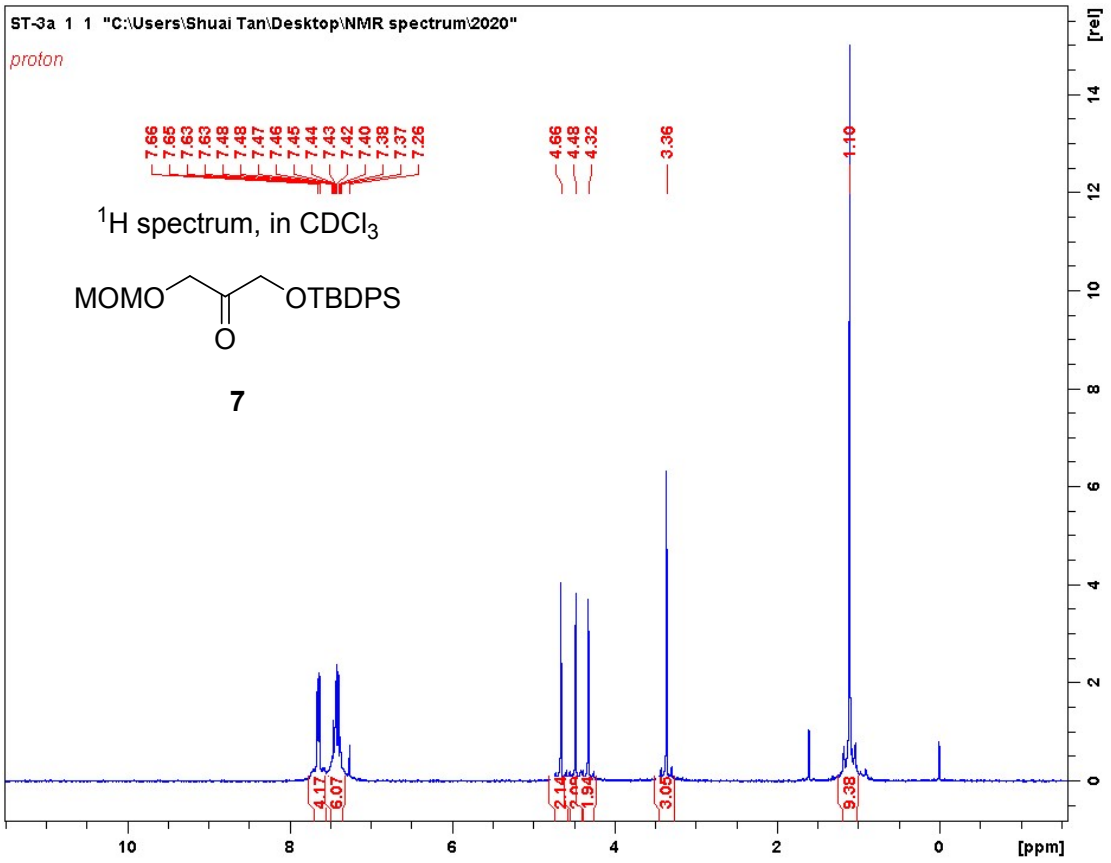
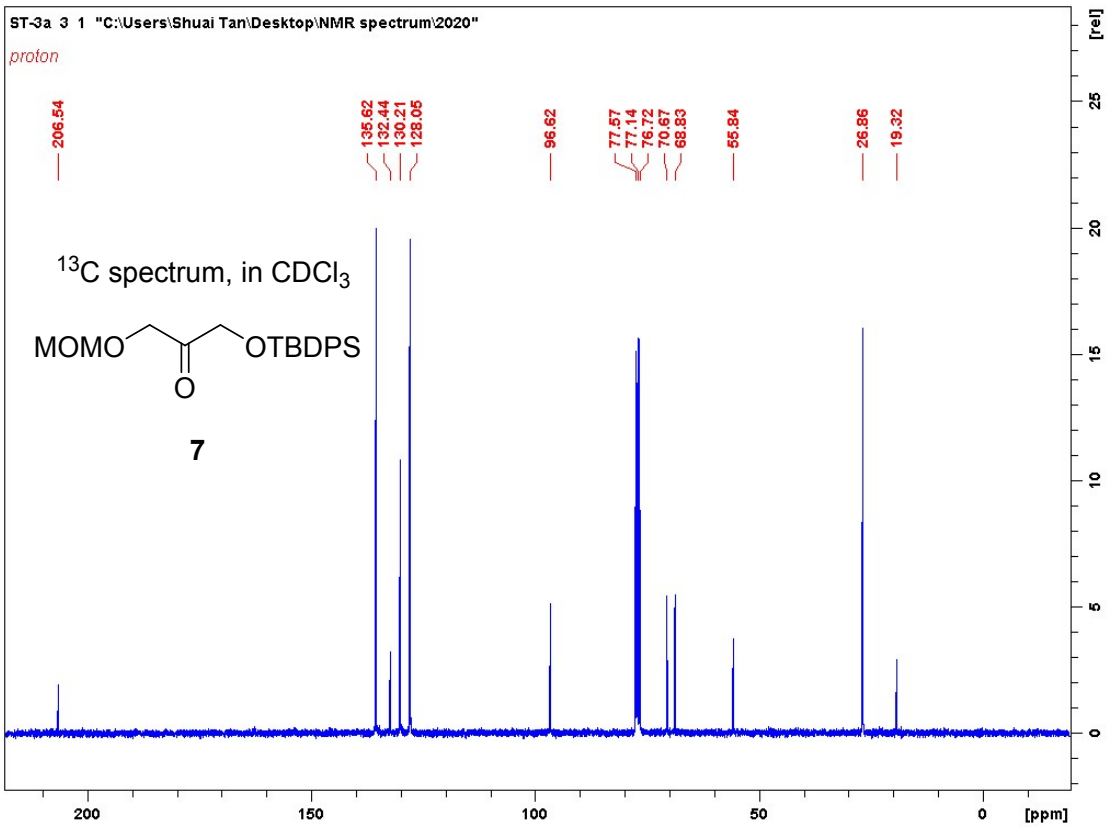
^{1}H , ^{13}C, and ^{31}P NMR spectra



^{1}H spectrum, in $CDCl_3$

7



^{13}C spectrum, in $CDCl_3$

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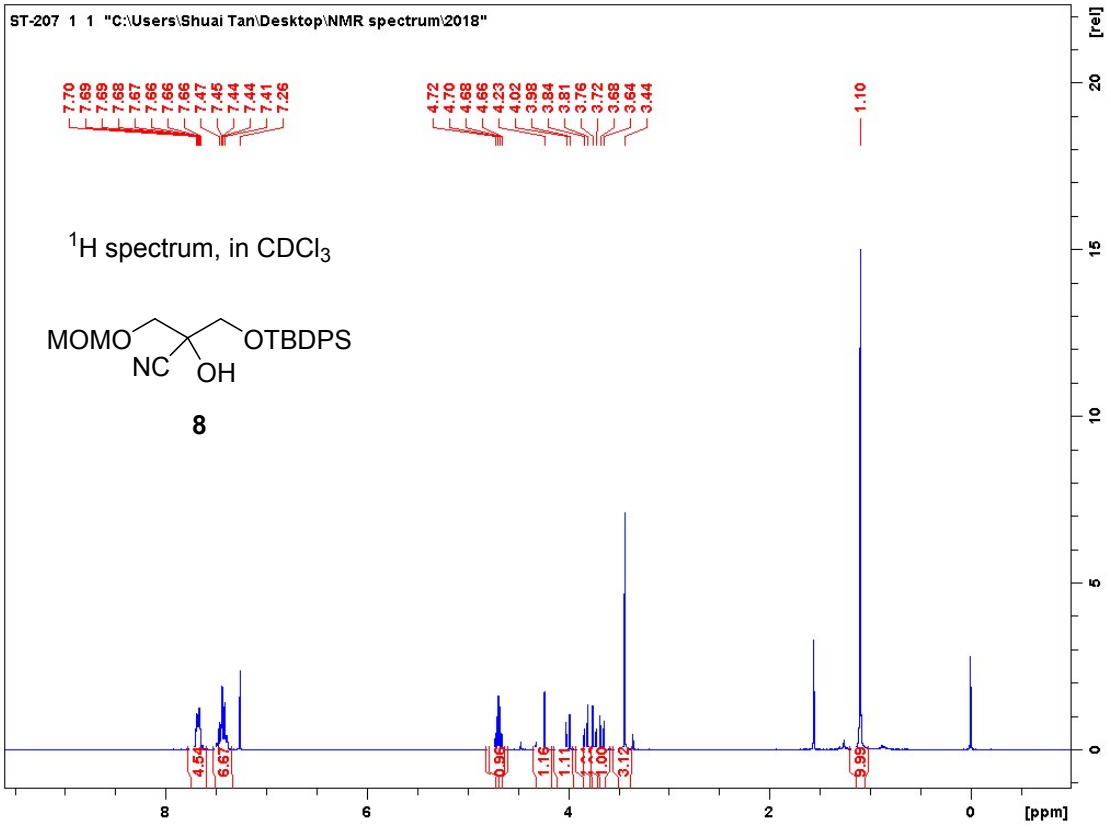
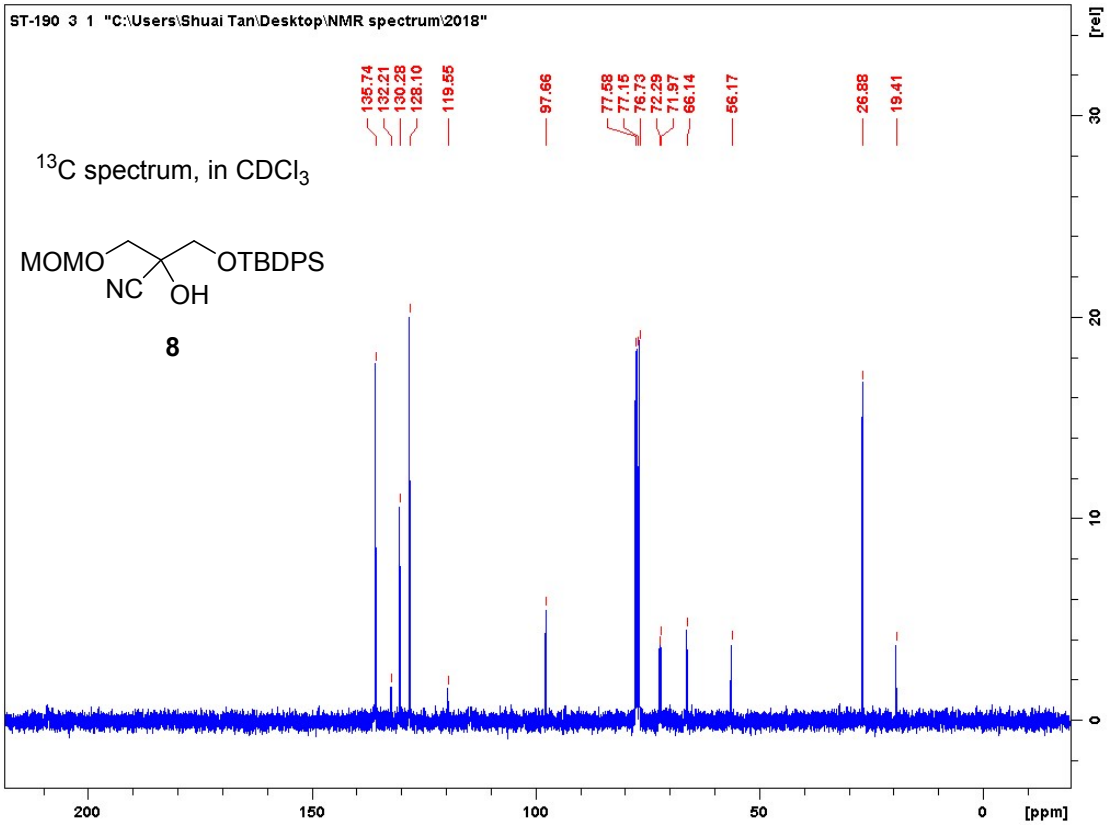


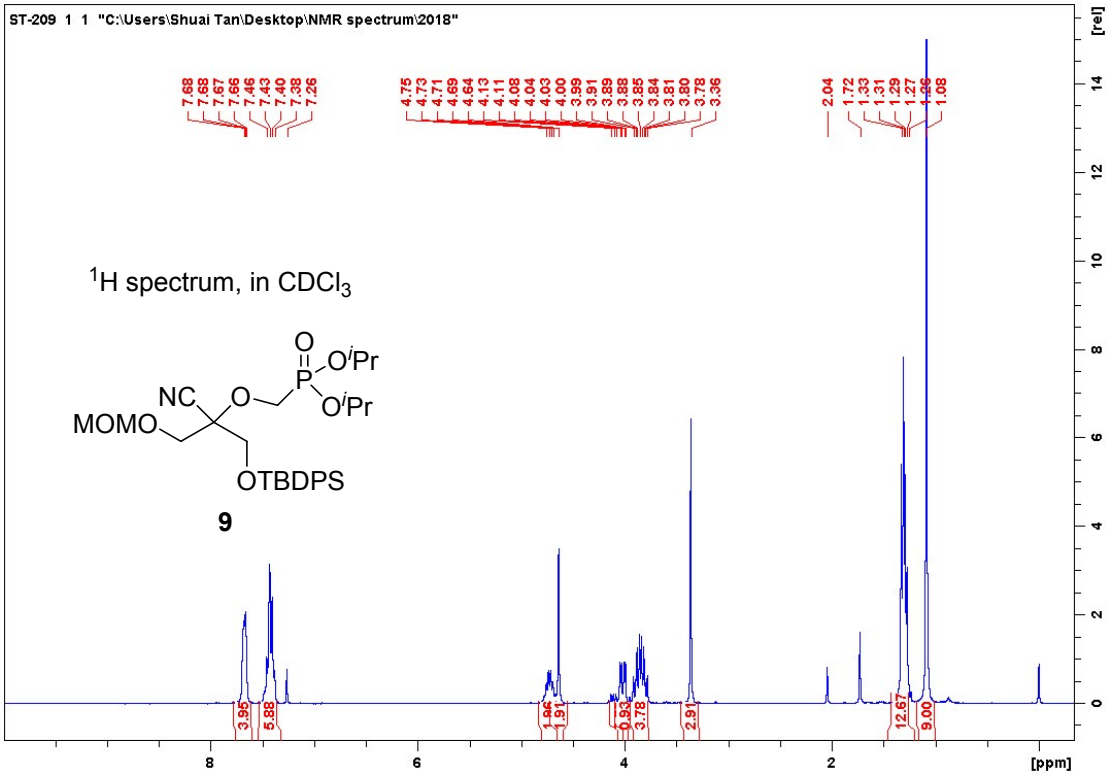
^{1}H spectrum, in $CDCl_3$

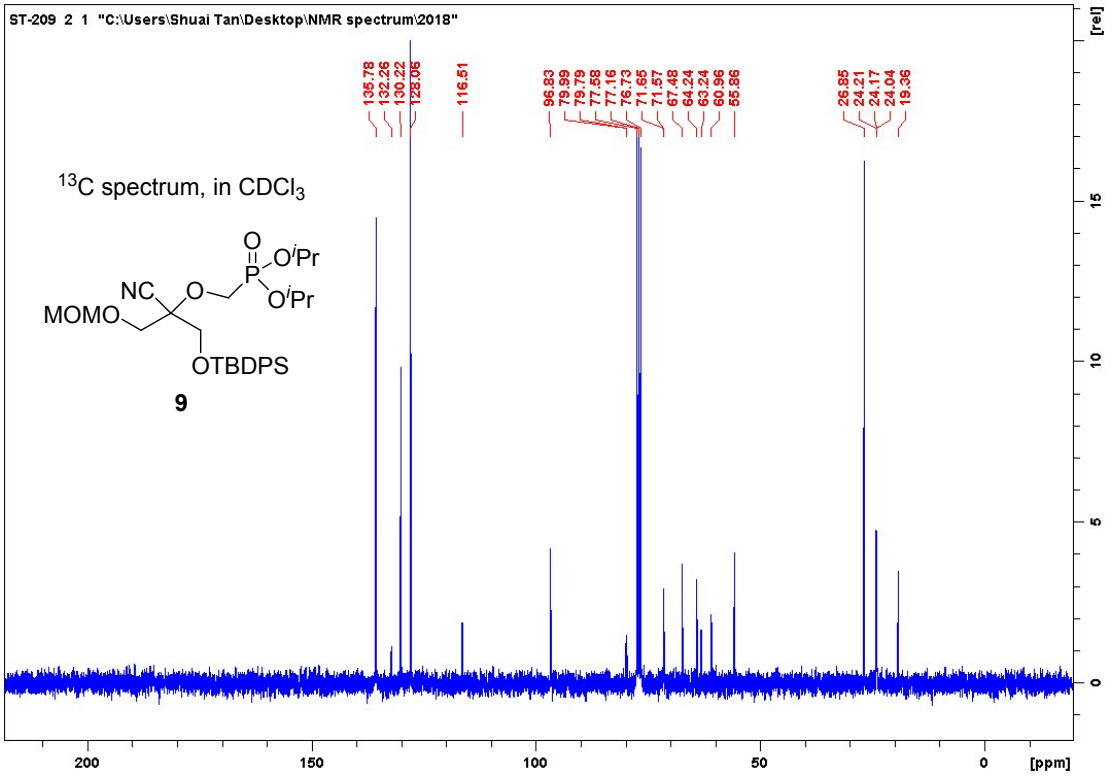
8

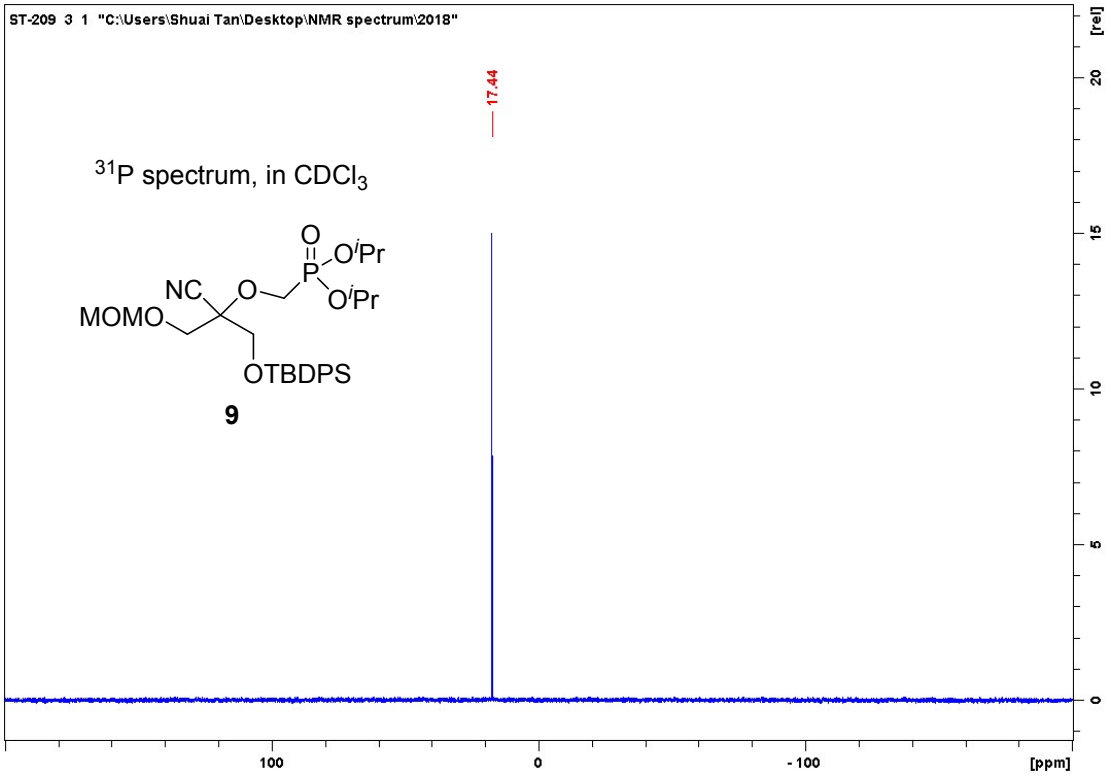


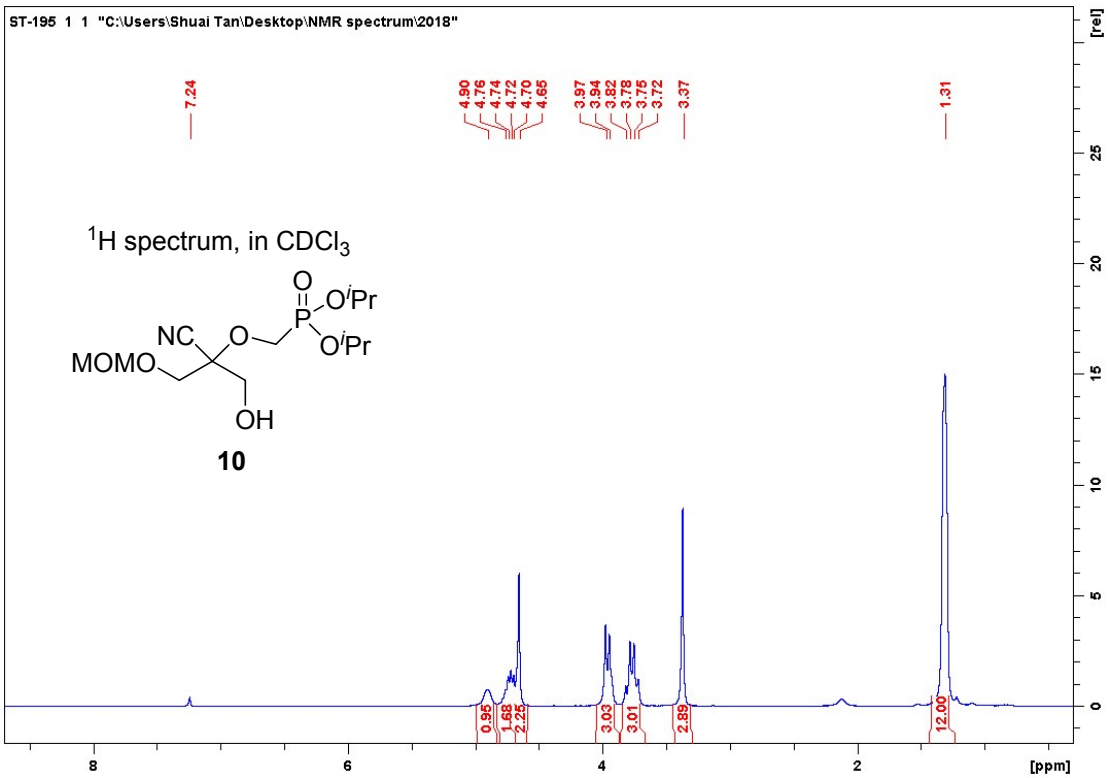
^{13}C spectrum, in $CDCl_3$

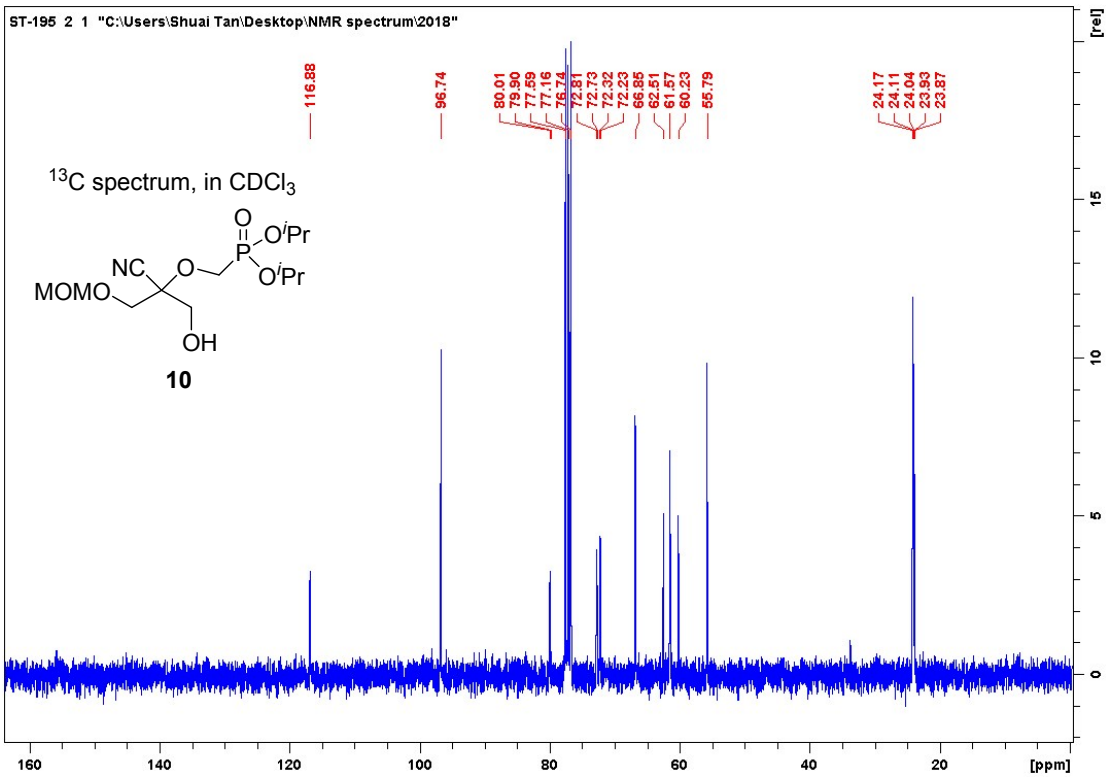
8

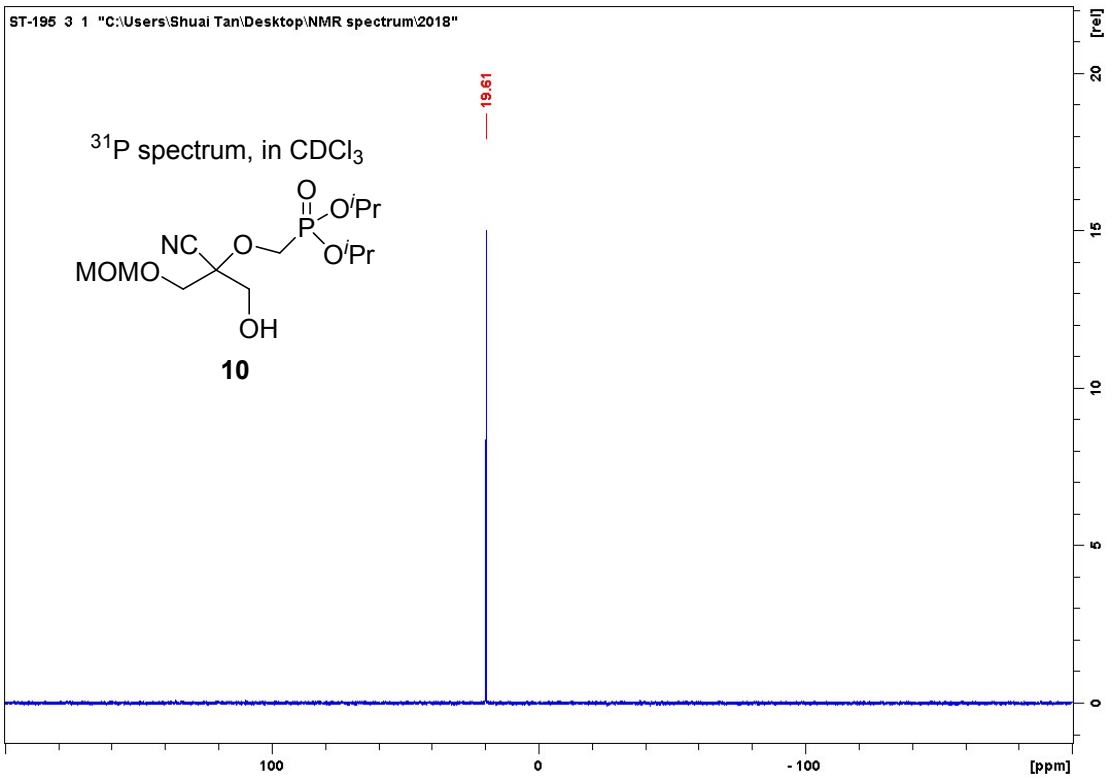

ST-209 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
7.68
7.68
7.67
7.66
7.46
7.43
7.40
7.38
7.26
4.75
4.73
4.71
4.69
4.64
4.13
4.11
4.08
4.04
4.03
4.00
3.99
3.91
3.89
3.88
3.85
3.84
3.81
3.80
3.78
3.36
2.04
1.72
1.33
1.31
1.29
1.27
1.08
[rel]
[ppm]
3.95
5.88
1.96
1.91
0.93
3.78
2.91
12.67
9.00
^{1}H spectrum, in CDCl$_3$
NC
O
O
P
O^{i}Pr
O^{i}Pr
MOMO
OTBDPS
9

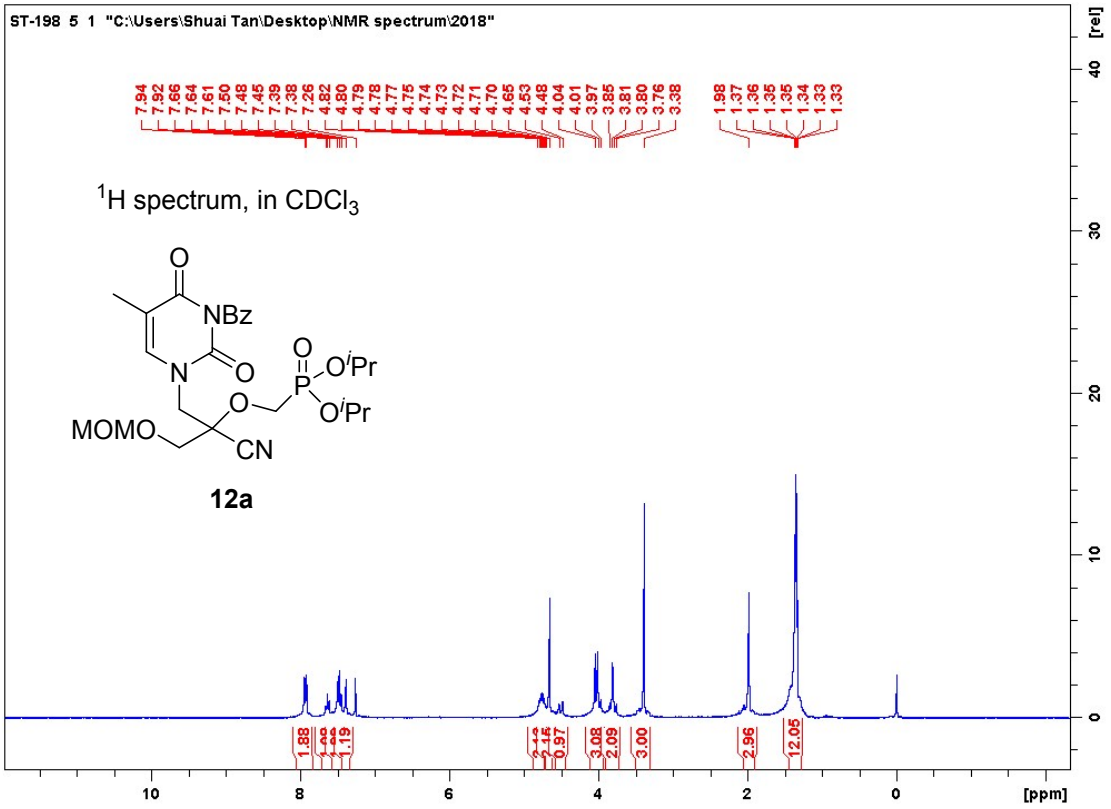

ST-209 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
135.78
132.26
130.22
128.06
116.51
96.83
79.99
79.79
77.58
77.16
76.73
71.65
71.57
67.48
64.24
63.24
60.96
55.86
26.85
24.21
24.17
24.04
19.36
[rel]
[ppm]
^{13}C spectrum, in CDCl$_3$
NC
O
O
P
O^{i}Pr
O^{i}Pr
MOMO
OTBDPS
9

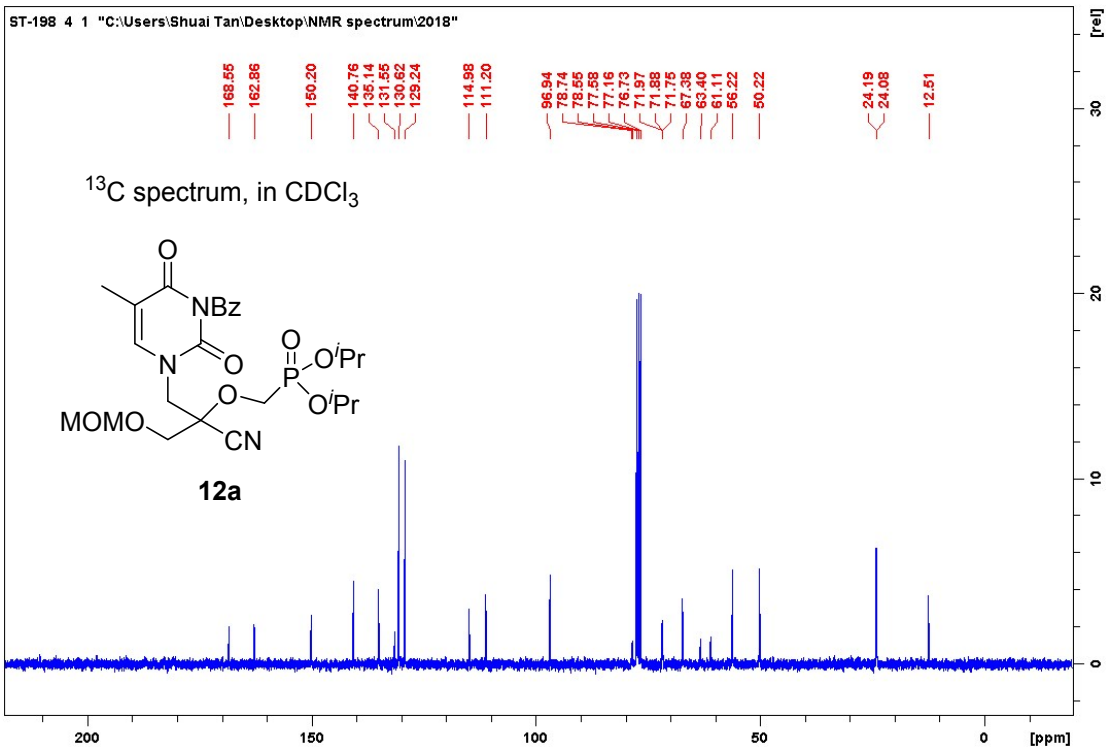

ST-209 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
17.44
31P spectrum, in CDCl3
O
O*i*Pr
P
O*i*Pr
NC
O
MOMO
OTBDPS
9
[rel]
20
15
10
5
0
100
0
-100
[ppm]

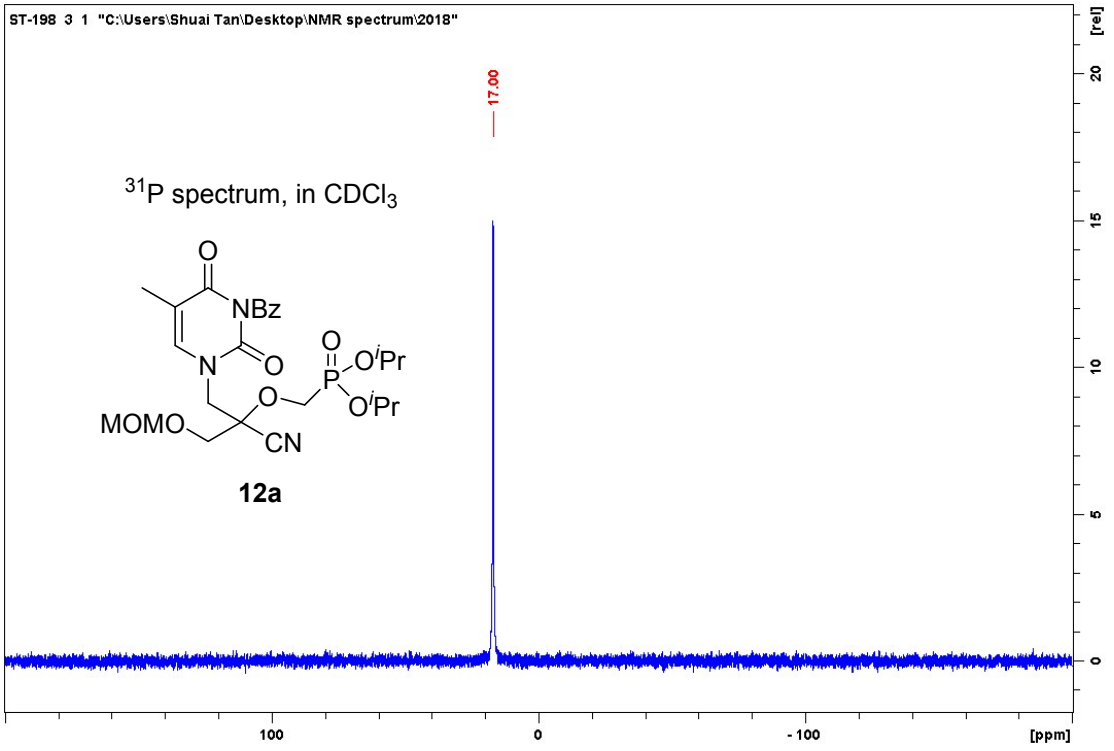

ST-195 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
7.24
4.90
4.76
4.74
4.72
4.70
4.66
3.97
3.94
3.82
3.78
3.75
3.72
3.37
1.31
1H spectrum, in CDCl3
O
O*i*Pr
P
O*i*Pr
NC
O
MOMO
OH
10
0.95
1.68
2.25
3.03
3.01
2.89
12.00
[rel]
25
20
15
10
5
0
8
6
4
2
[ppm]

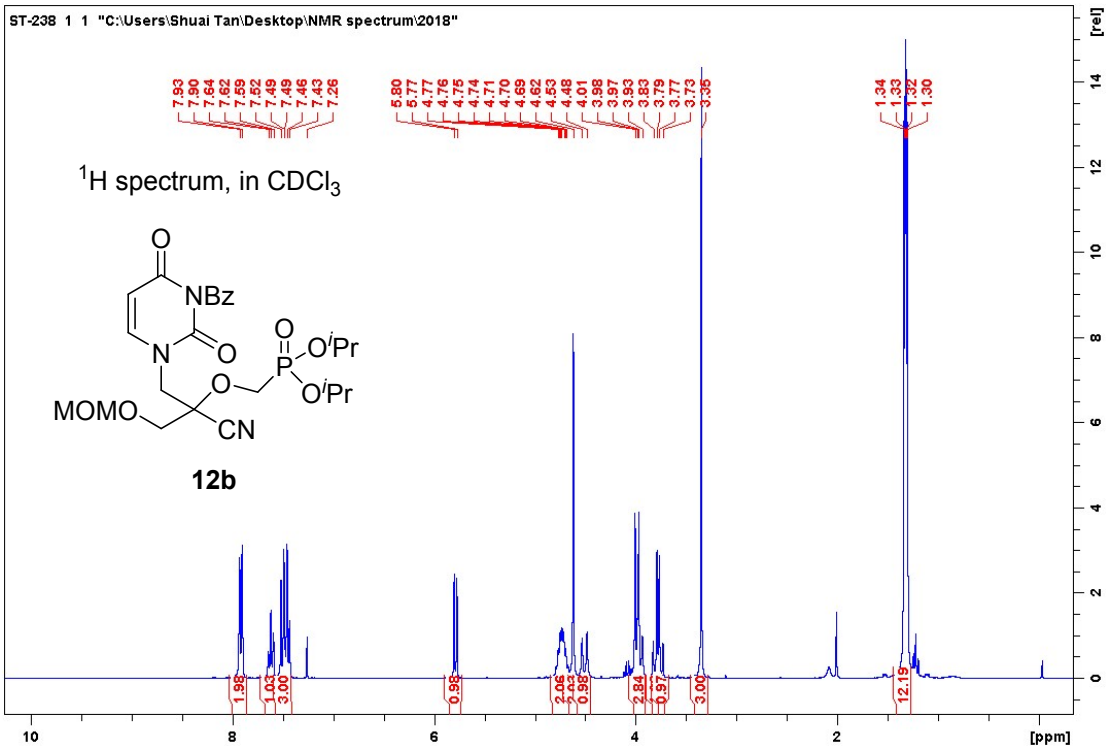

ST-195 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
116.88
96.74
80.01
79.90
77.59
77.16
76.74
72.81
72.73
72.32
72.23
66.85
62.51
61.57
60.23
55.79
24.17
24.11
24.04
23.93
23.87
13C spectrum, in CDCl3
NC
O
O
P
OiPr
OiPr
MOMO
OH
10
[rel]
[ppm]

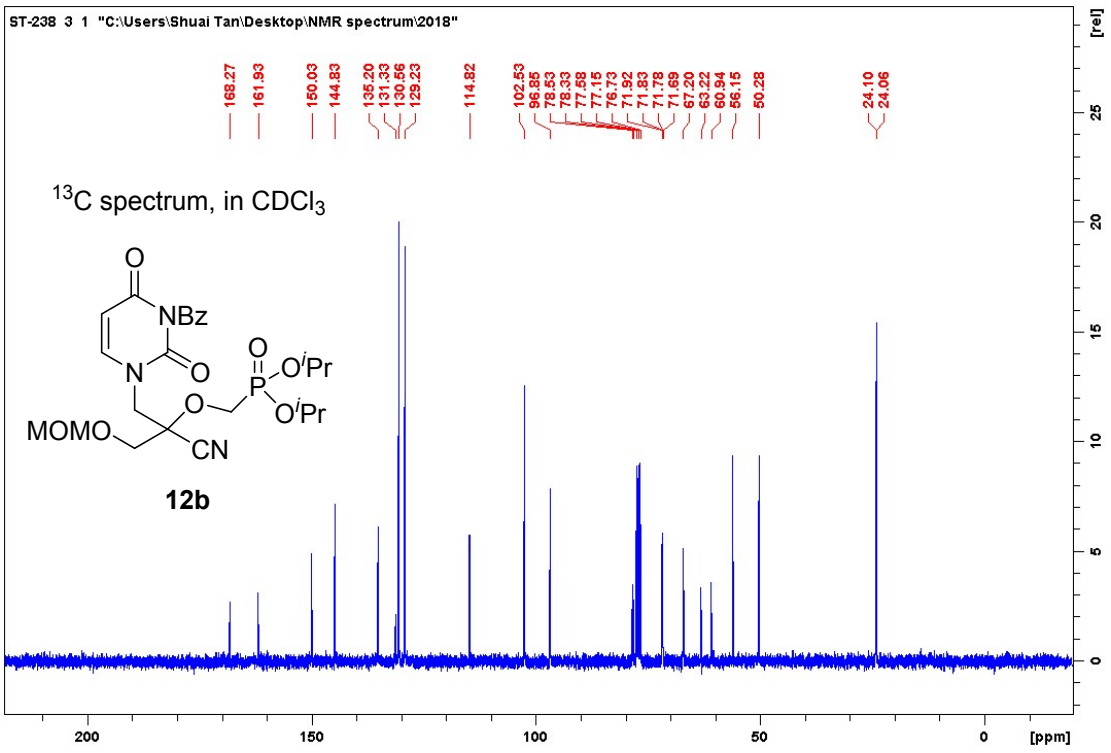

ST-195 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
19.61
31P spectrum, in CDCl3
NC
O
O
P
OiPr
OiPr
MOMO
OH
10
[rel]
[ppm]

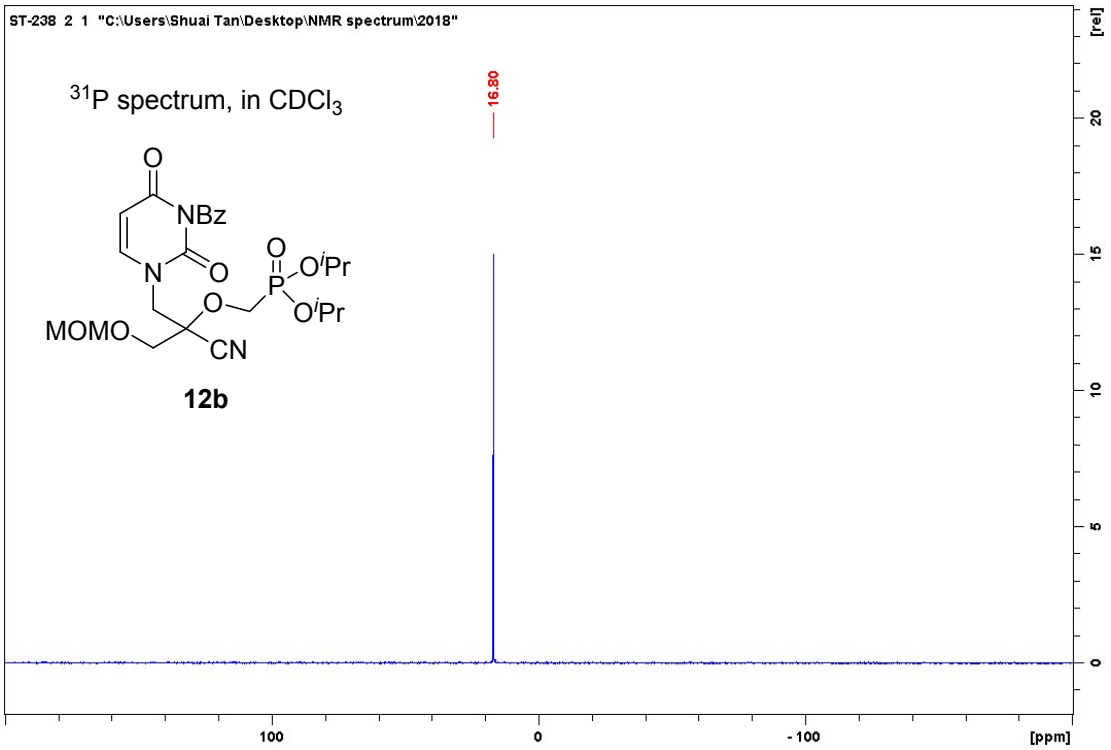

ST-198 5 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
7.94 7.92 7.66 7.64 7.61 7.50 7.48 7.45 7.39 7.38 7.26 4.82 4.80 4.79 4.78 4.77 4.75 4.74 4.73 4.72 4.71 4.70 4.65 4.53 4.48 4.04 4.01 3.97 3.85 3.81 3.80 3.76 3.38
1.98 1.37 1.36 1.35 1.35 1.34 1.33 1.33
1H spectrum, in CDCl3
NBz
O
N
O
O
P
OiPr
OiPr
MOMO
CN
12a
1.88 1.19 0.97 3.08 2.09 3.00 2.96 12.05
10 8 6 4 2 0 [ppm]
[rel]

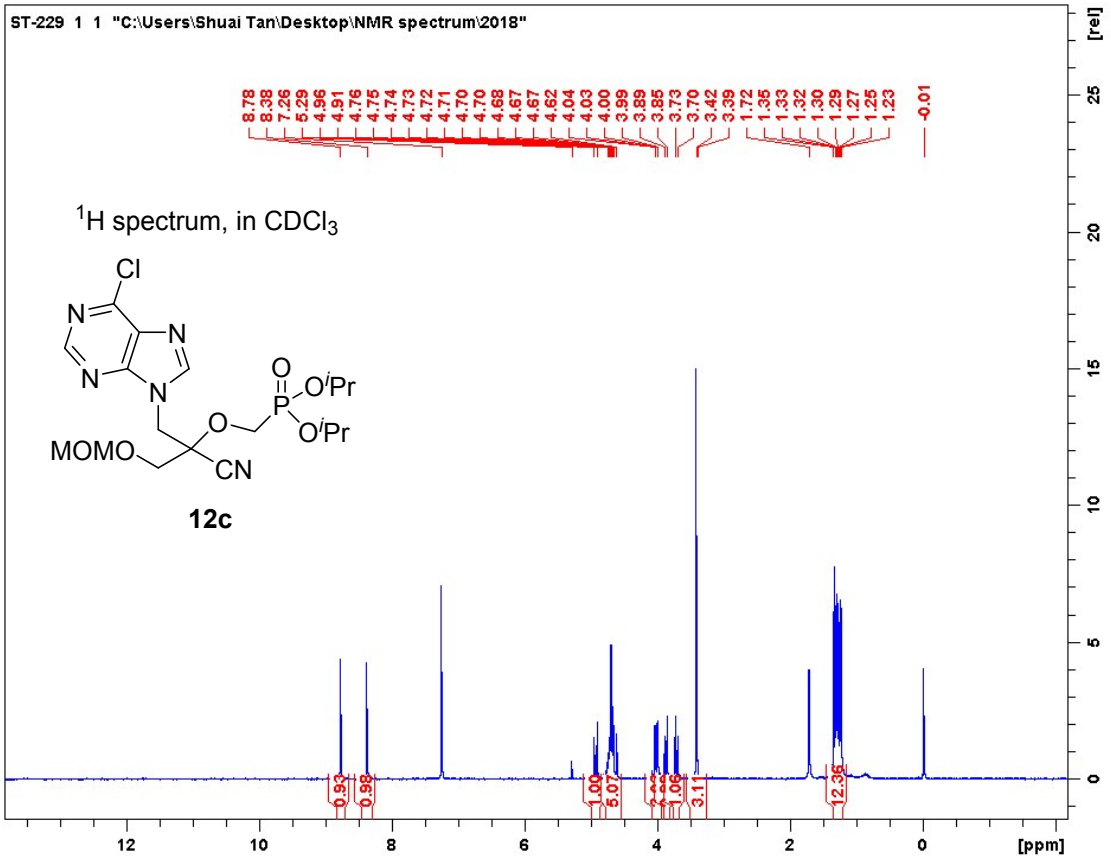

ST-198 4 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
168.55 162.86 150.20 140.76 135.14 131.55 130.62 129.24 114.98 111.20
96.94 78.74 78.55 77.58 77.16 76.73 71.97 71.88 71.75 67.38 63.40 61.11 56.22 50.22
24.19 24.08 12.51
13C spectrum, in CDCl3
NBz
O
N
O
O
P
OiPr
OiPr
MOMO
CN
12a
200 150 100 50 0 [ppm]
[rel]

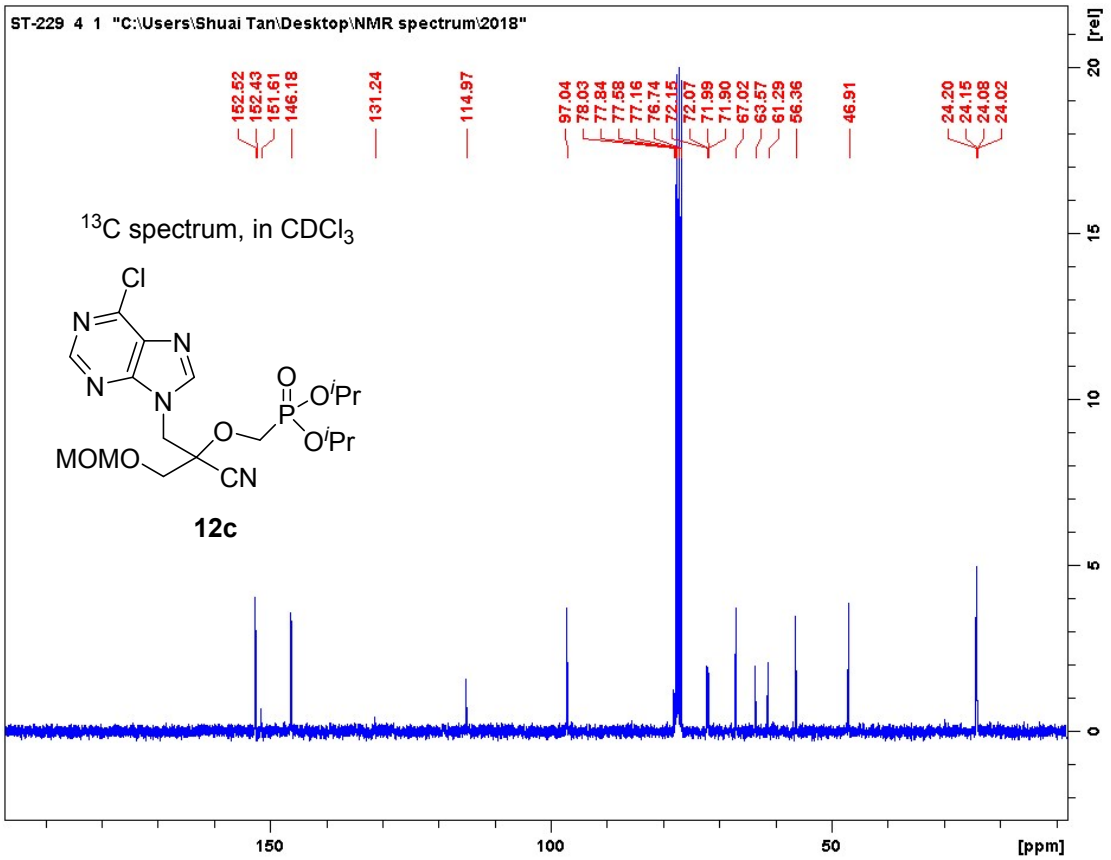

ST-198 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
17.00
31P spectrum, in CDCl3
O
NBz
N
O
O
P
OiPr
OiPr
MOMO
CN
12a
[rel]
[ppm]

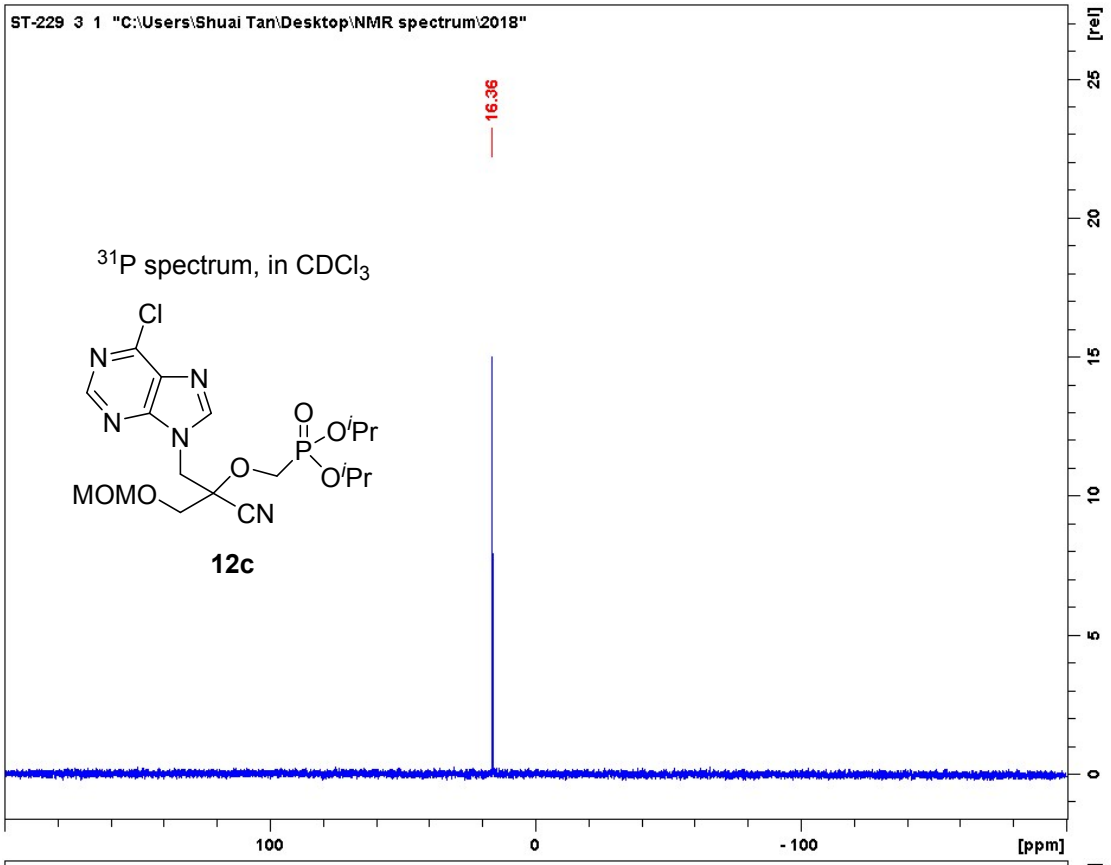

ST-238 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
7.93
7.90
7.64
7.62
7.59
7.52
7.49
7.46
7.43
7.26
5.80
5.77
4.77
4.76
4.75
4.74
4.71
4.70
4.69
4.62
4.53
4.48
4.01
3.98
3.97
3.93
3.83
3.79
3.77
3.73
3.35
1.34
1.33
1.32
1.30
1H spectrum, in CDCl3
O
NBz
N
O
O
P
OiPr
OiPr
MOMO
CN
12b
1.98
1.03
3.00
0.98
2.06
2.03
0.98
2.84
0.97
3.00
12.15
[rel]
[ppm]

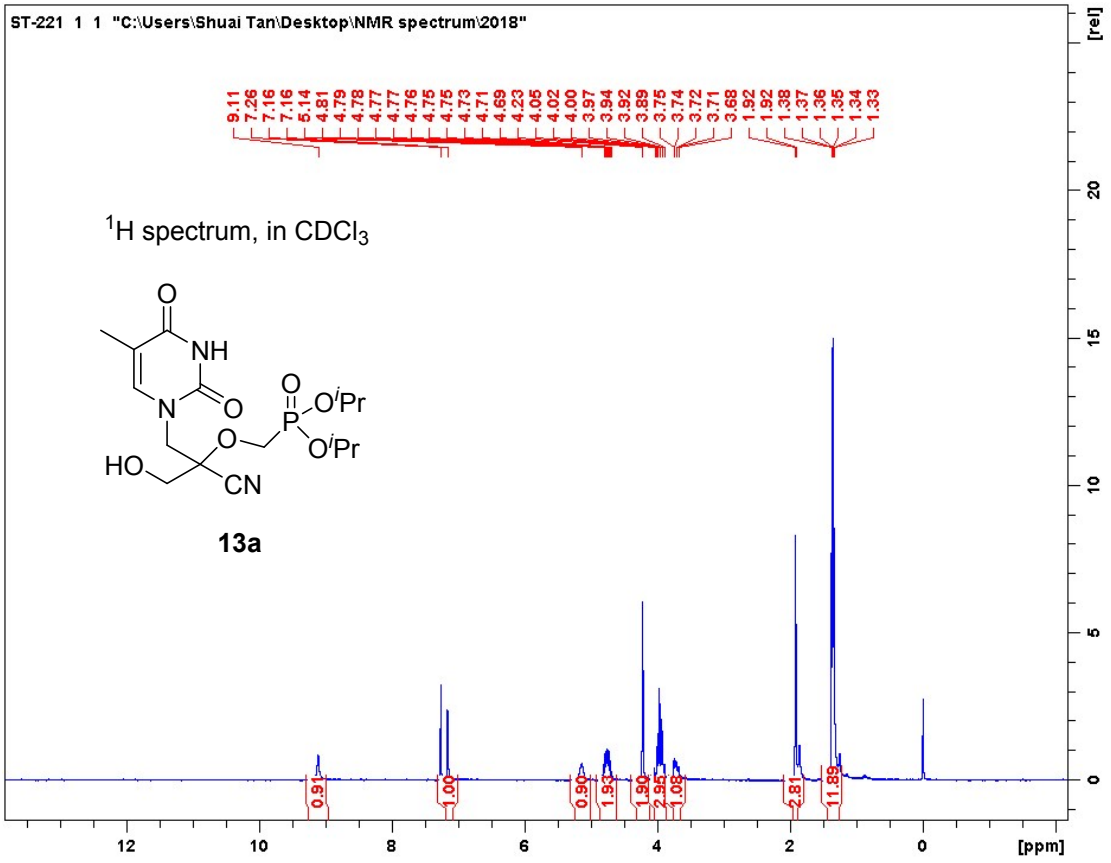

ST-238 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
168.27
161.93
150.03
144.83
135.20
131.33
130.66
129.23
114.82
102.53
96.85
78.53
78.33
77.58
77.15
76.73
71.92
71.83
71.78
71.69
67.20
63.22
60.94
56.15
50.28
24.10
24.06
13C spectrum, in CDCl3
NBz
MOMO
CN
O
iPr
12b
[rel]
[ppm]

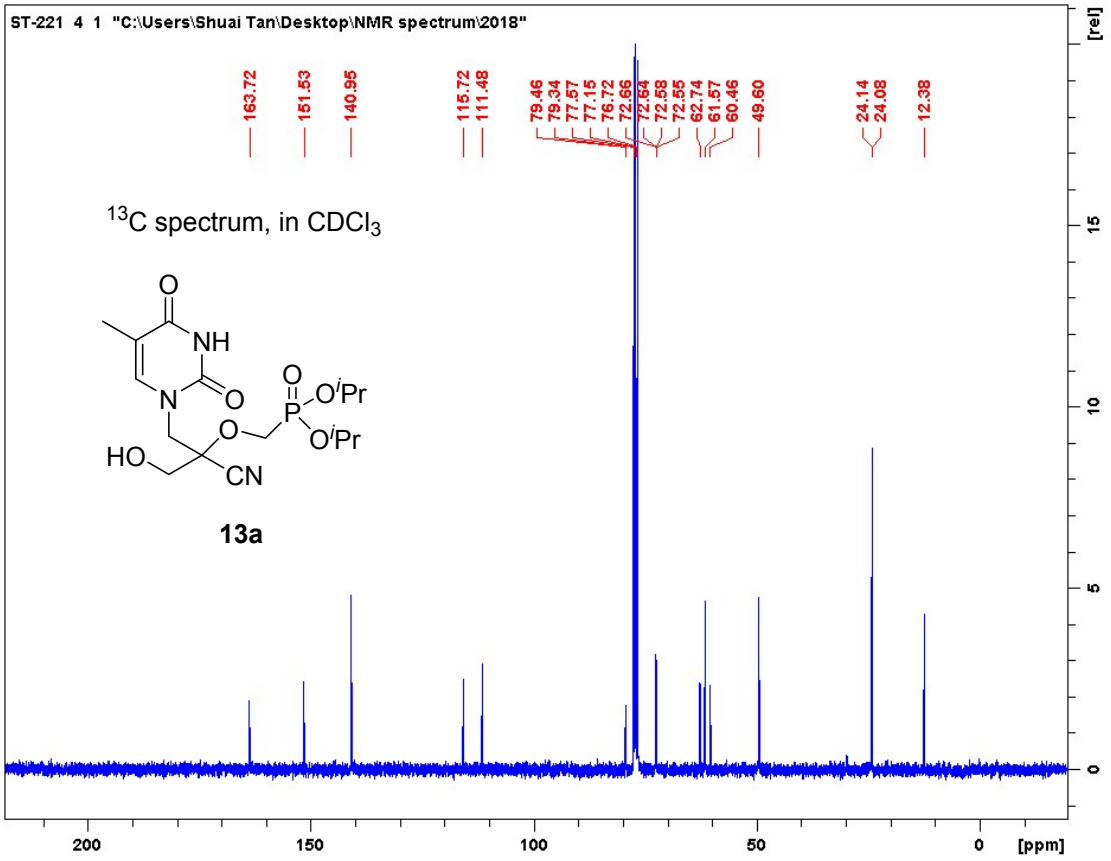

ST-238 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
16.80
31P spectrum, in CDCl3
NBz
MOMO
CN
O
iPr
12b
[rel]
[ppm]

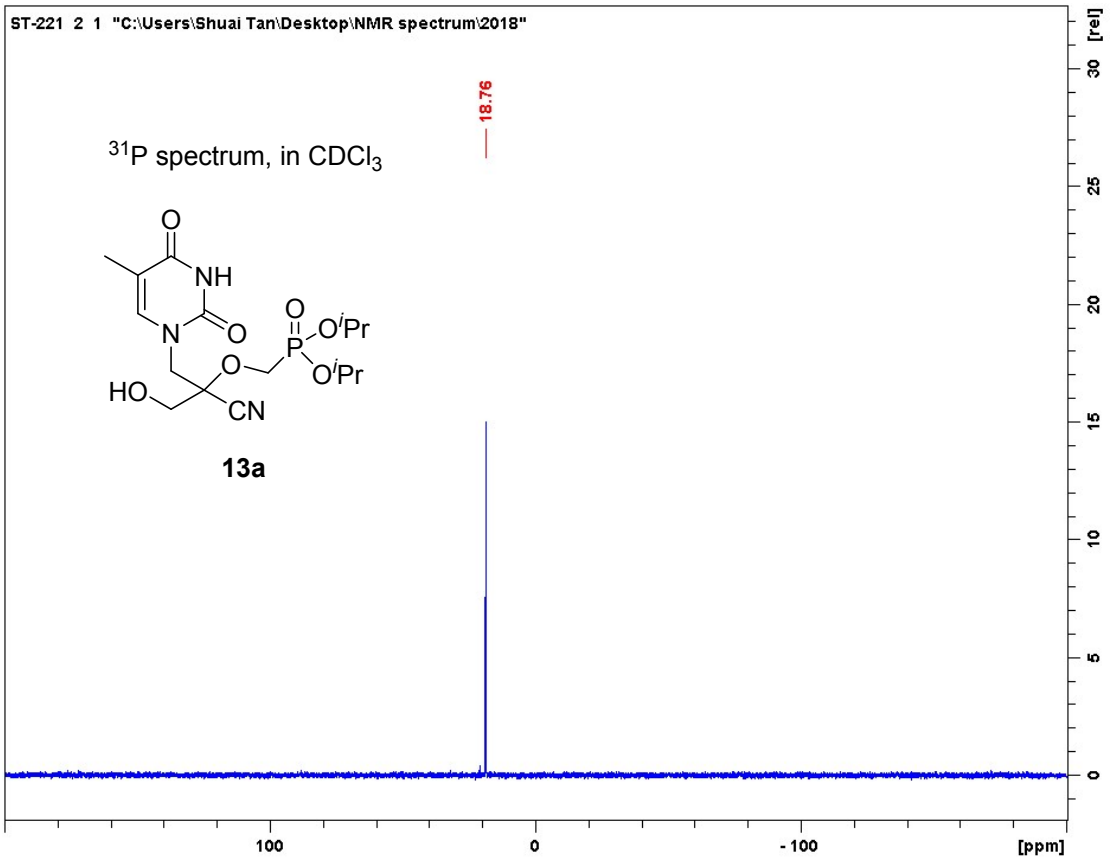

ST-229 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
8.78
8.38
7.26
5.29
4.96
4.91
4.76
4.75
4.74
4.73
4.72
4.71
4.70
4.70
4.68
4.67
4.67
4.62
4.04
4.03
4.00
3.99
3.89
3.85
3.73
3.70
3.42
3.39
1.72
1.35
1.33
1.32
1.30
1.29
1.27
1.25
1.23
-0.01
[rel]
25
20
15
10
5
0
^{1}H spectrum, in $CDCl_3$
Cl
N
N
N
N
O
P
O^{i}Pr
O^{i}Pr
O
MOMO
CN
12c
0.93
0.98
1.00
5.07
3.11
12.36
12
10
8
6
4
2
0
[ppm]

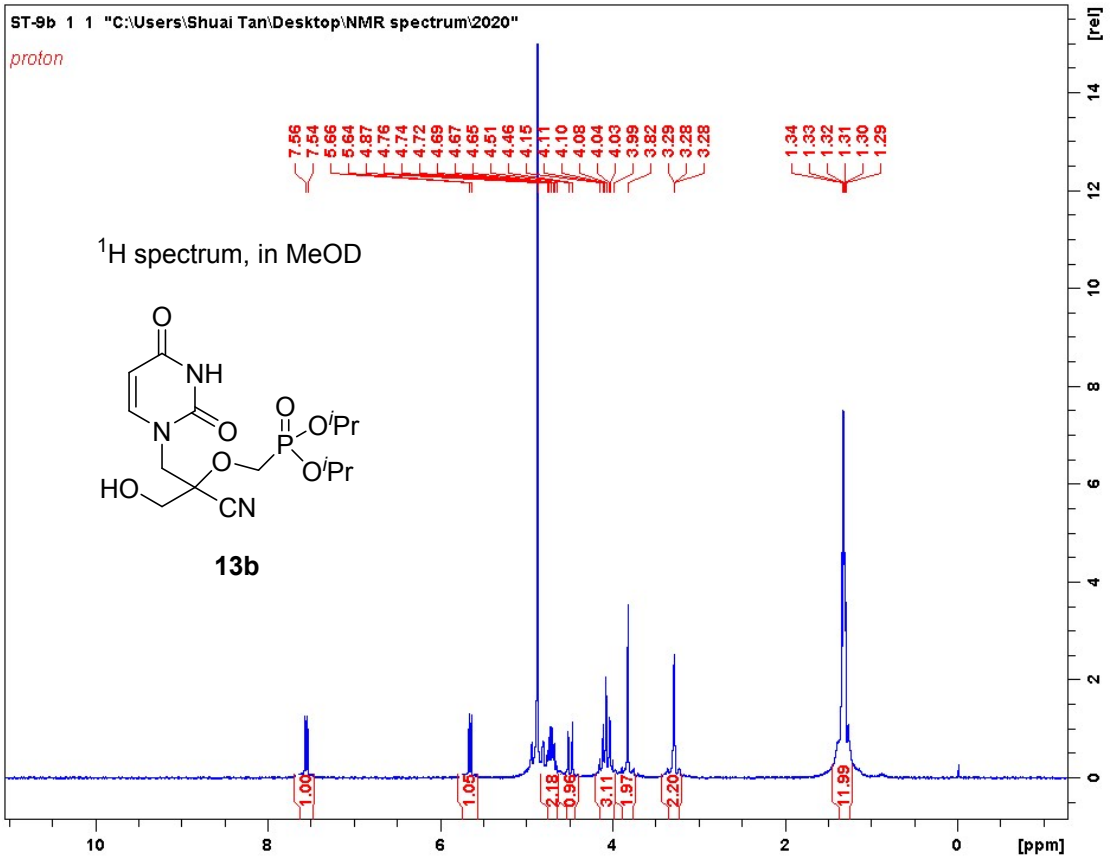

ST-229 4 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
152.52
152.43
151.61
146.18
131.24
114.97
97.04
78.03
77.84
77.58
77.16
76.74
72.15
72.07
71.99
71.90
67.02
63.57
61.29
56.36
46.91
24.20
24.15
24.08
24.02
[rel]
20
15
10
5
0
^{13}C spectrum, in $CDCl_3$
Cl
N
N
N
N
O
P
O^{i}Pr
O^{i}Pr
O
MOMO
CN
12c
150
100
50
[ppm]

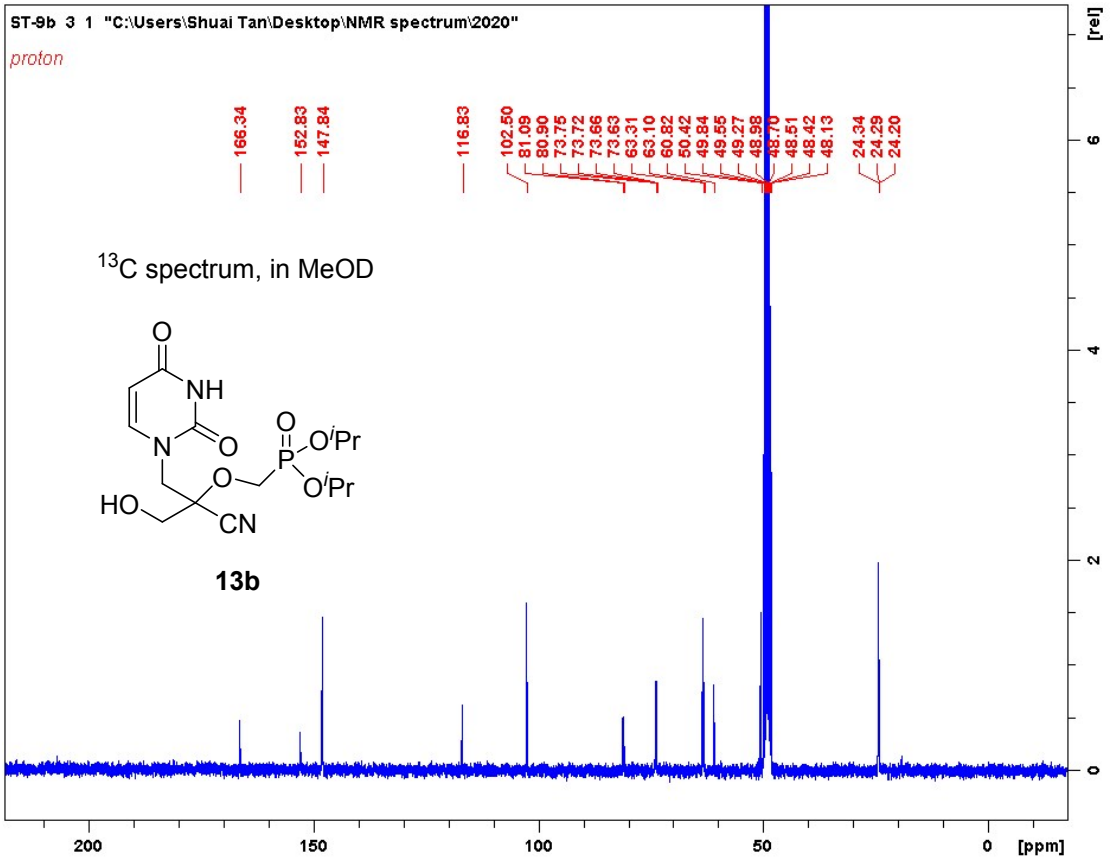

ST-229 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
16.36
31P spectrum, in CDCl3
Cl
N
N
N
N
O
O
iPr
P
O
iPr
MOMO
CN
12c
[rel]
25
20
15
10
5
0
100
0
- 100
[ppm]

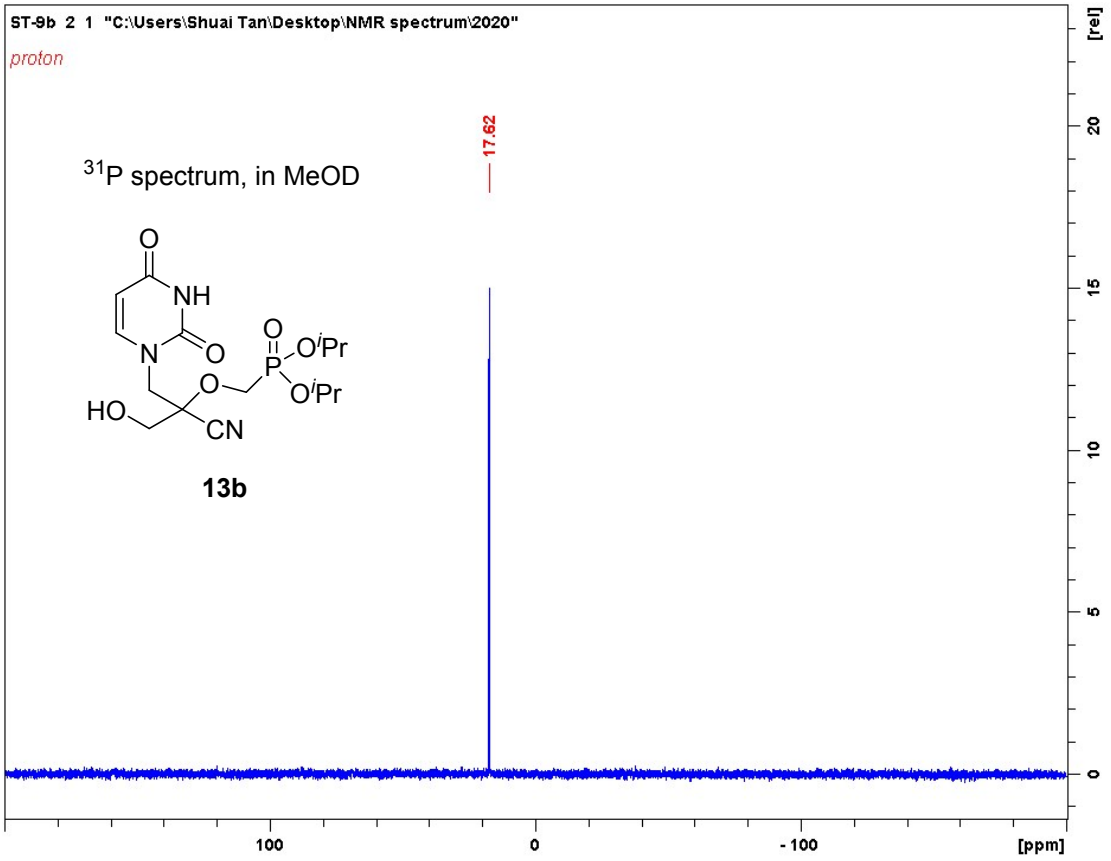

ST-221 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
9.11
7.26
7.16
7.16
5.14
4.81
4.79
4.78
4.77
4.77
4.76
4.75
4.75
4.73
4.71
4.69
4.23
4.05
4.02
4.00
3.97
3.94
3.92
3.89
3.75
3.74
3.72
3.71
3.68
1.92
1.92
1.38
1.37
1.36
1.35
1.34
1.33
1H spectrum, in CDCl3
O
NH
N
O
O
O
iPr
P
O
iPr
HO
CN
13a
0.91
1.00
0.90
1.93
1.90
2.95
1.08
2.81
11.89
[rel]
20
15
10
5
0
12
10
8
6
4
2
0
[ppm]

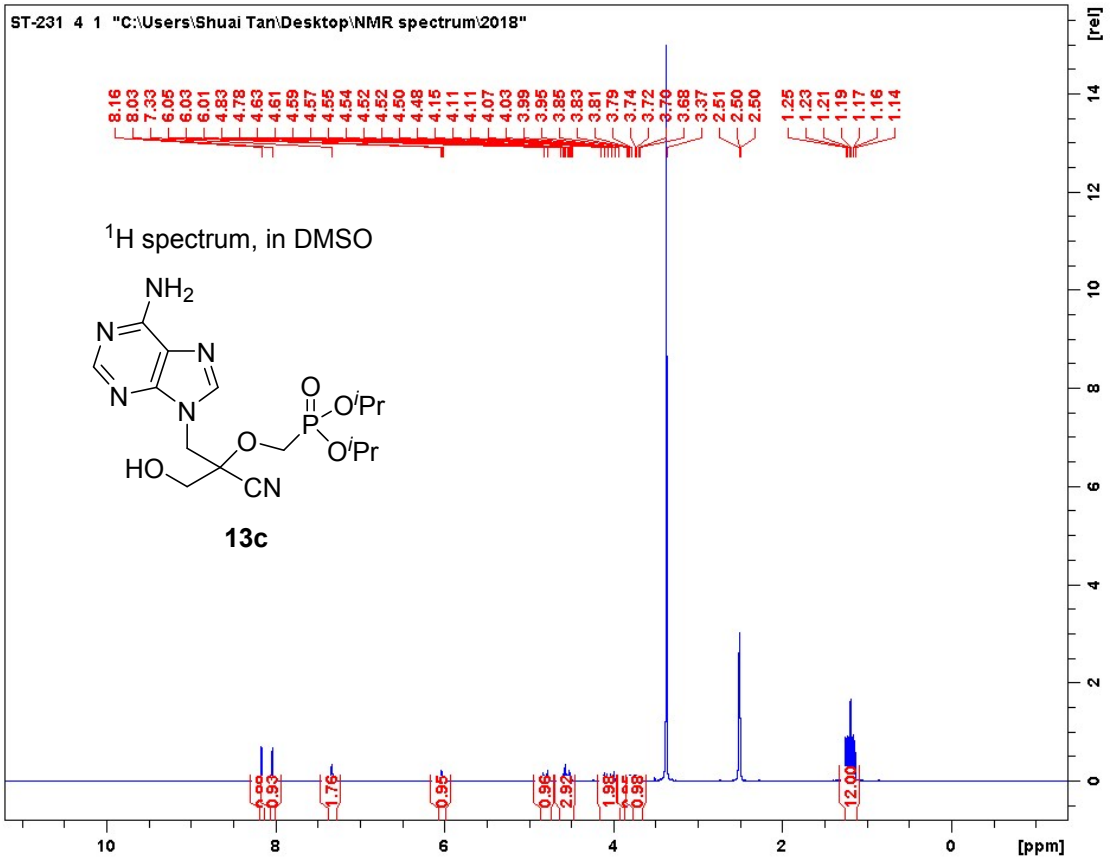

ST-221 4 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
163.72
151.53
140.95
115.72
111.48
79.46
79.34
77.57
77.15
76.72
72.66
72.64
72.58
72.55
62.74
61.57
60.46
49.60
24.14
24.08
12.38
[rel]
^{13}C spectrum, in CDCl$_3$
13a
200
150
100
50
0
[ppm]

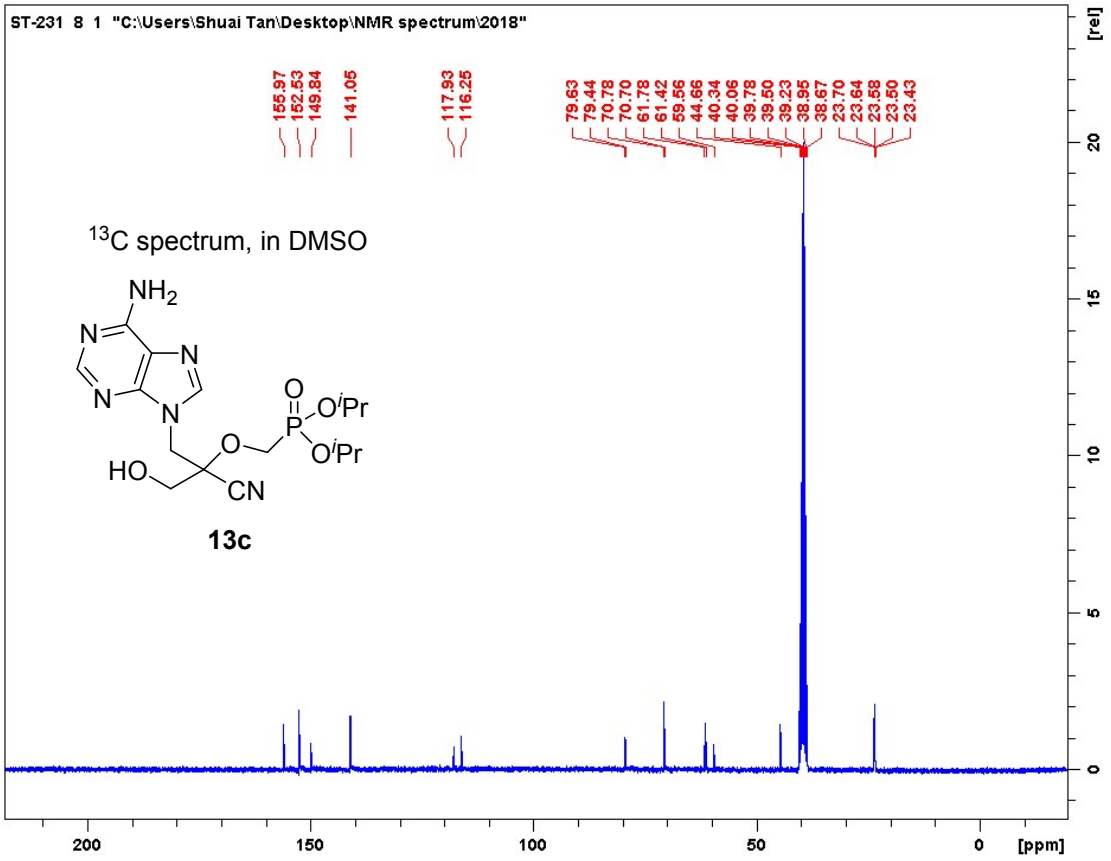

ST-221 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
18.76
[rel]
^{31}P spectrum, in CDCl$_3$
13a
100
0
- 100
[ppm]


ST-9b 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
7.56
7.54
5.66
5.64
4.87
4.76
4.74
4.72
4.69
4.67
4.65
4.51
4.46
4.15
4.11
4.10
4.08
4.04
4.03
3.99
3.82
3.29
3.28
3.28
1.34
1.33
1.32
1.31
1.30
1.29
1H spectrum, in MeOD
O
NH
N
O
O
P
OiPr
OiPr
O
HO
CN
13b
1.00
1.05
2.18
0.96
3.11
1.97
2.20
11.99
10
8
6
4
2
0
[ppm]
[rel]


ST-9b 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
166.34
152.83
147.84
116.83
102.50
81.09
80.90
73.75
73.72
73.66
73.63
63.31
63.10
60.82
50.42
49.84
49.55
49.27
48.98
48.70
48.51
48.42
48.13
24.34
24.29
24.20
13C spectrum, in MeOD
O
NH
N
O
O
P
OiPr
OiPr
O
HO
CN
13b
200
150
100
50
0
[ppm]
[rel]

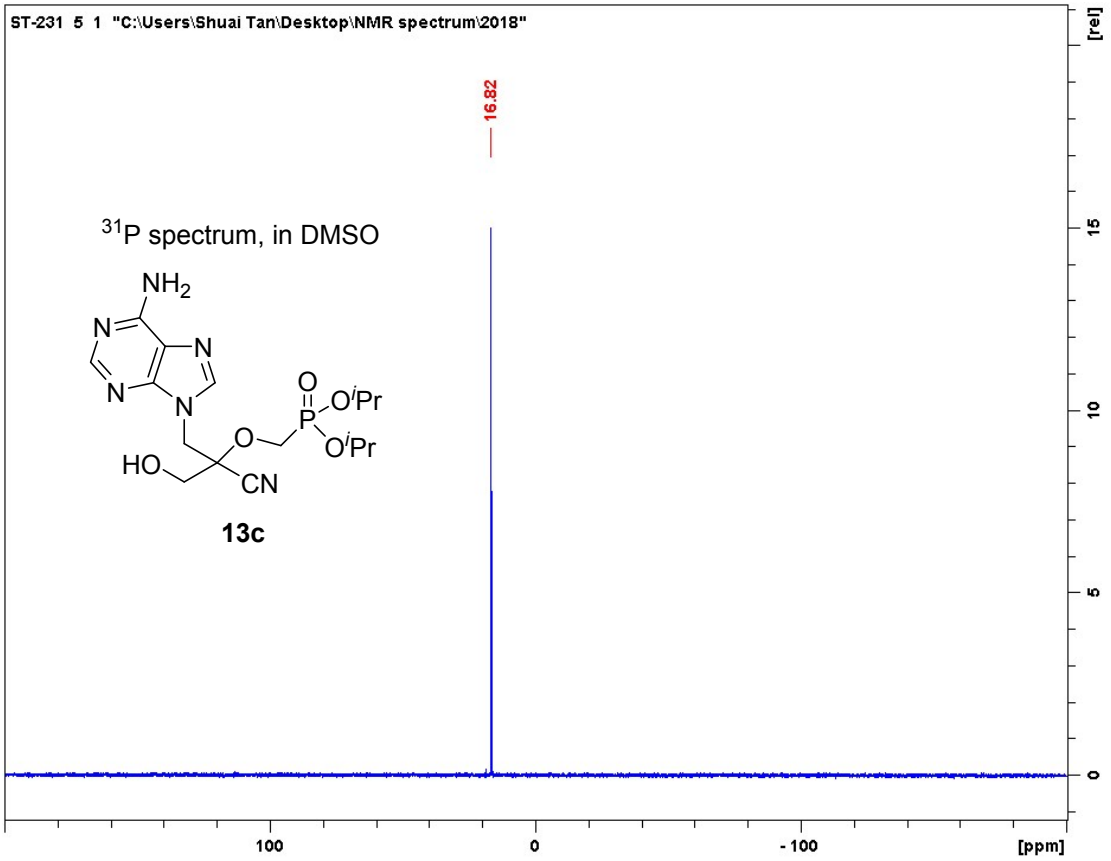

ST-9b 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
31P spectrum, in MeOD
17.62
13b
[rel]
[ppm]


ST-231 4 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
1H spectrum, in DMSO
13c
[rel]
[ppm]



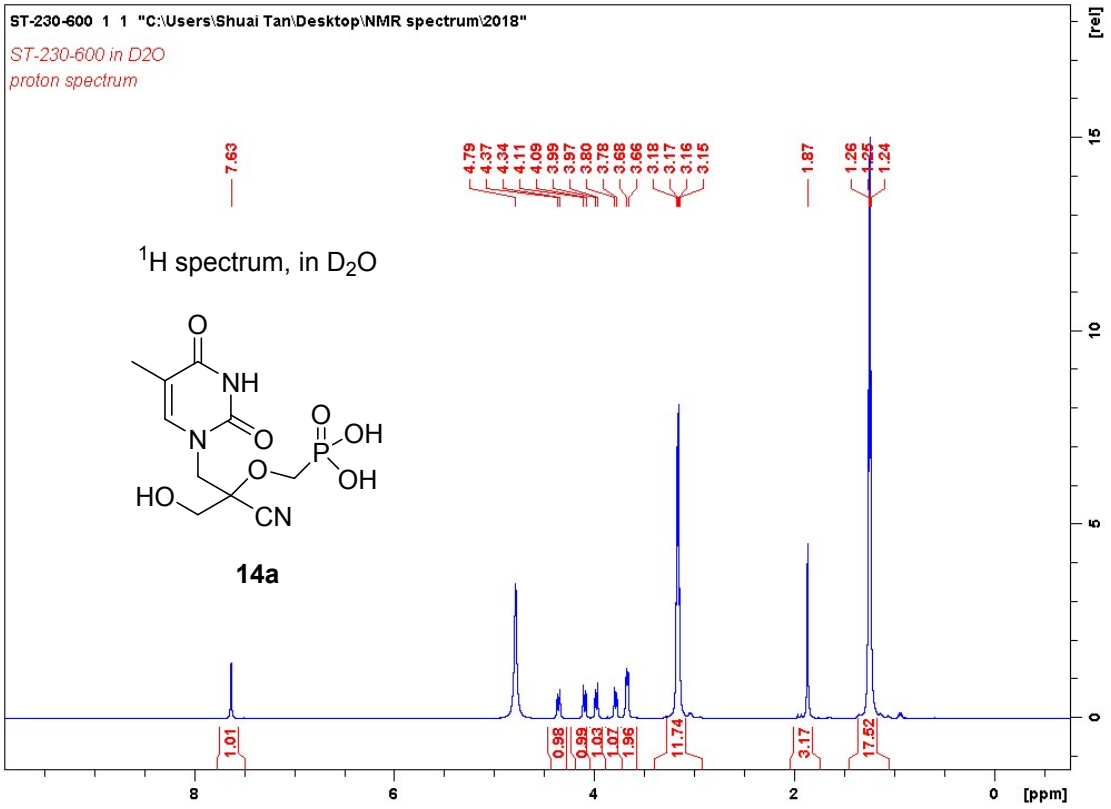
^{13}C spectrum, in DMSO

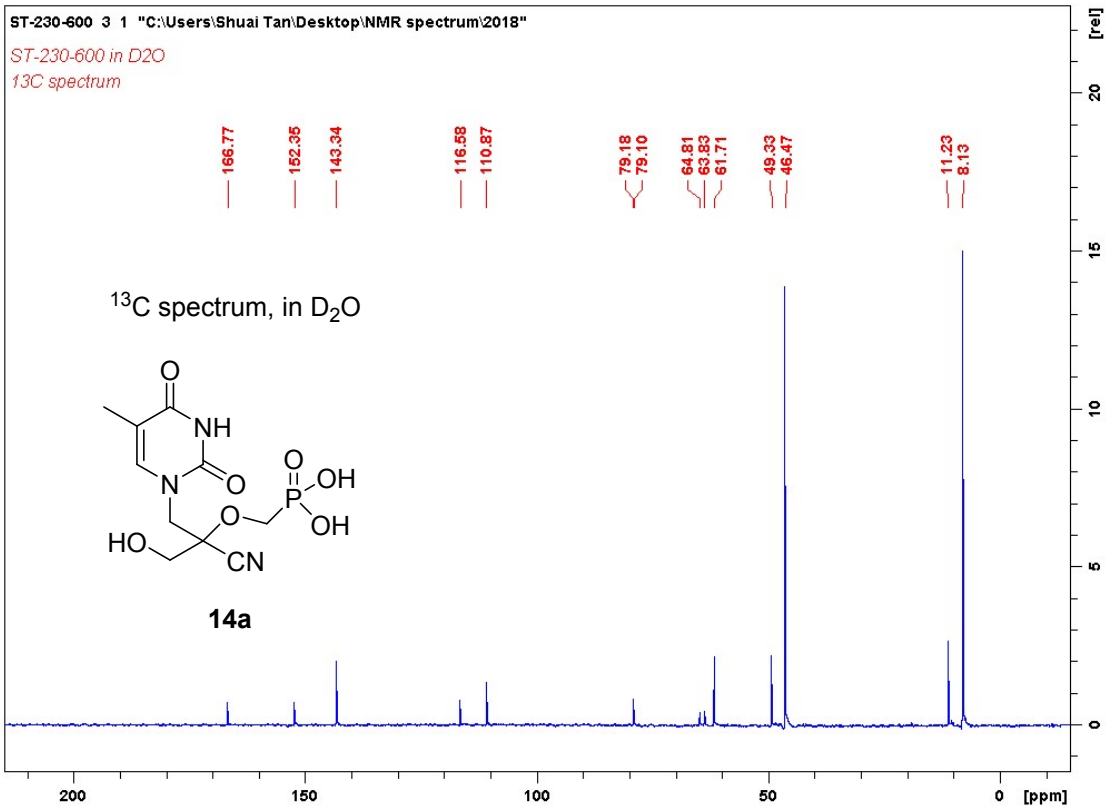
13c

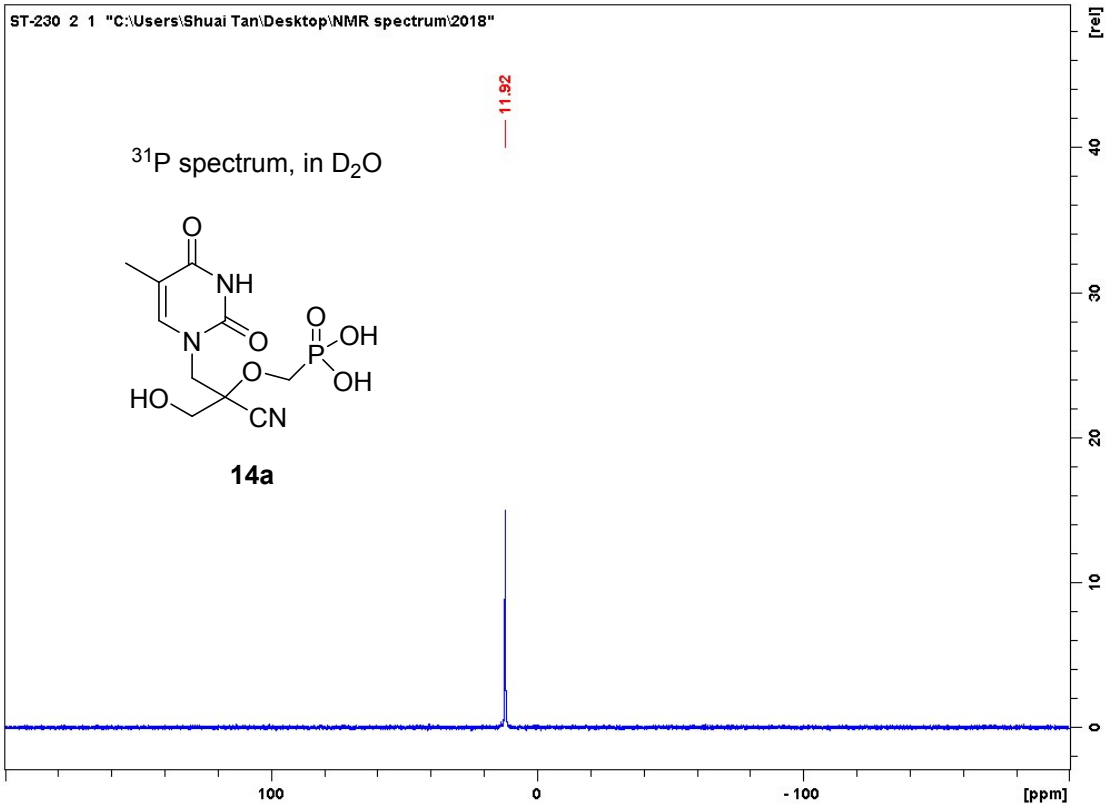


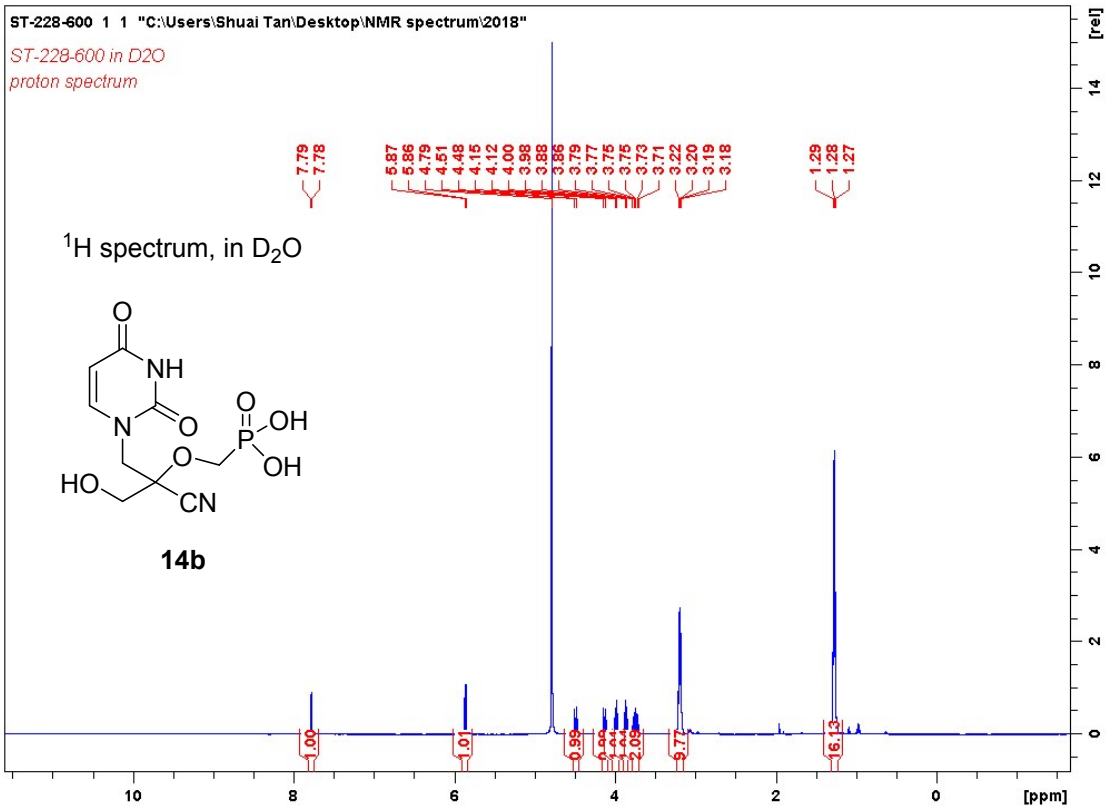
^{31}P spectrum, in DMSO

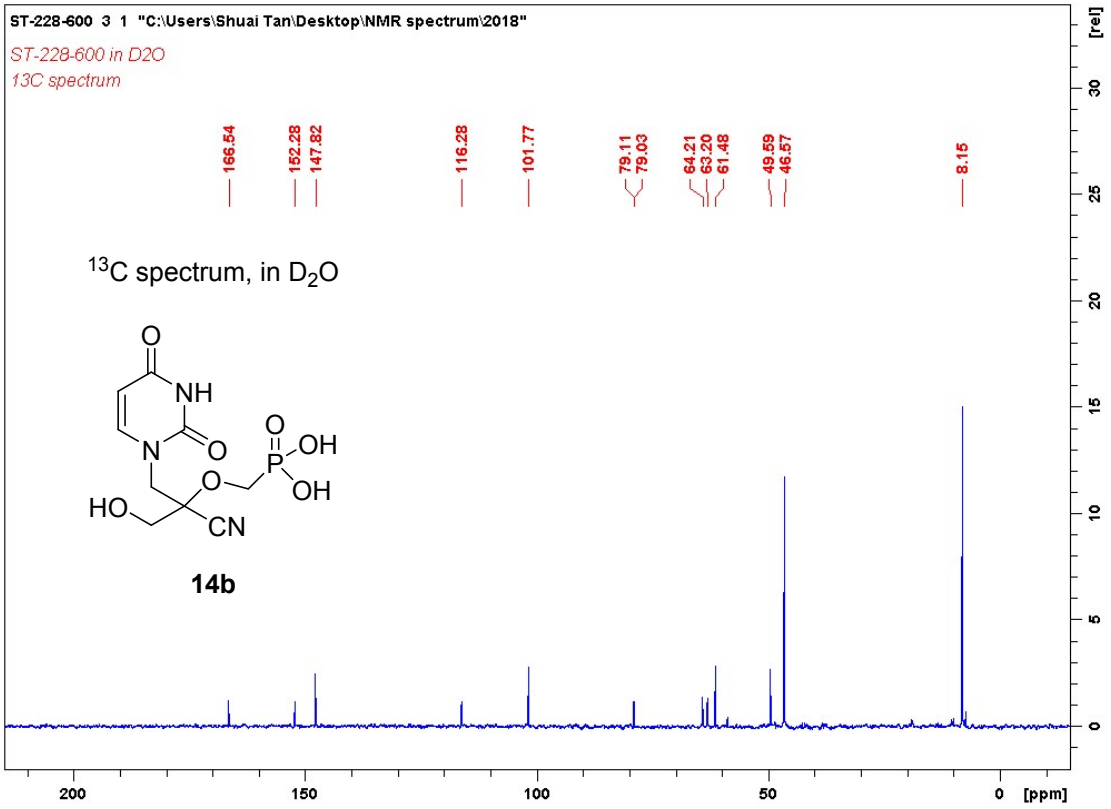
13c

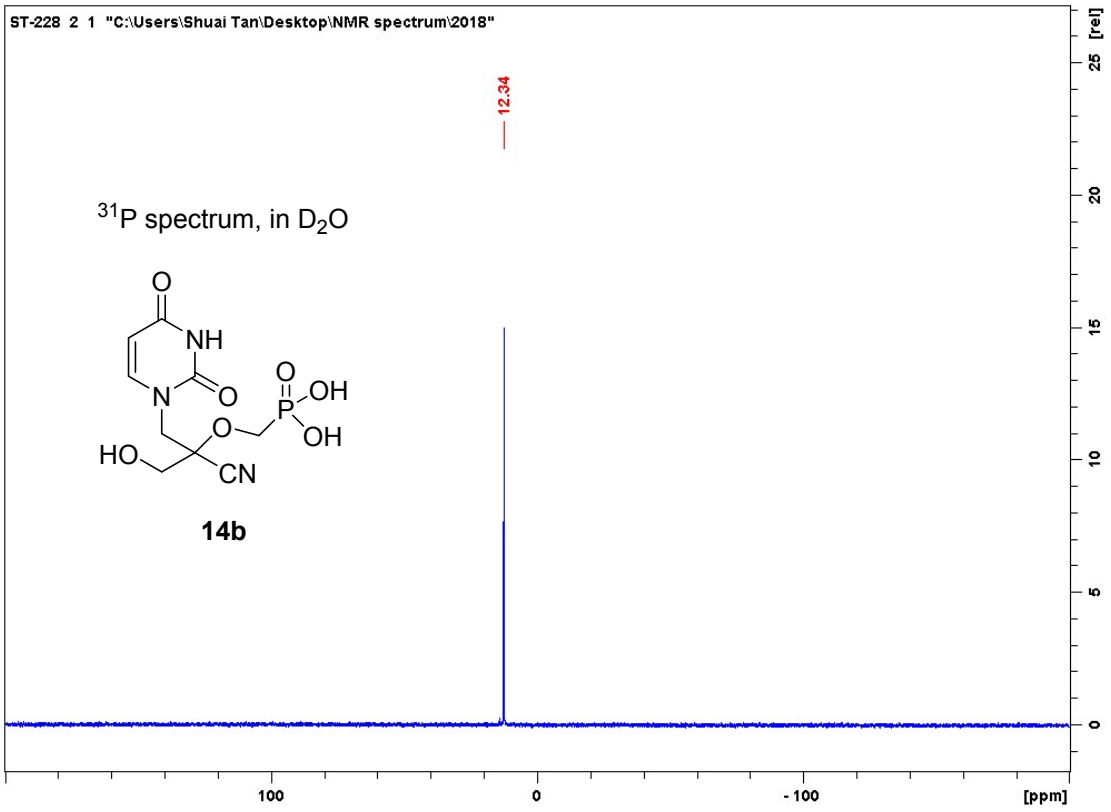

ST-230-600 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
ST-230-600 in D2O
proton spectrum
7.63
4.79
4.37
4.34
4.11
4.09
3.99
3.97
3.80
3.78
3.68
3.66
3.18
3.17
3.16
3.15
1.87
1.26
1.25
1.24
1H spectrum, in D2O
O
NH
N
O
O
P
OH
OH
O
HO
CN
14a
1.01
0.98
0.99
1.03
1.07
1.96
11.74
3.17
17.52
[rel]
[ppm]

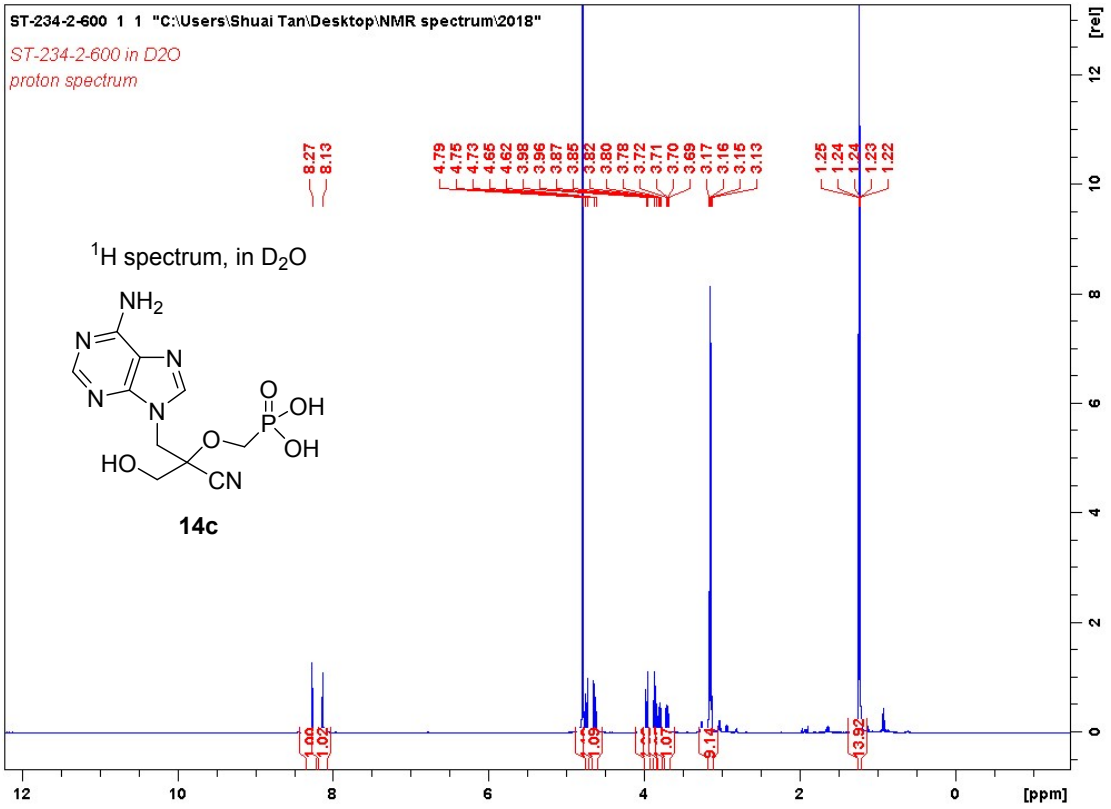

ST-230-600 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
ST-230-600 in D2O
13C spectrum
166.77
152.35
143.34
116.58
110.87
79.18
79.10
64.81
63.83
61.71
49.33
46.47
11.23
8.13
13C spectrum, in D2O
O
NH
N
O
O
P
OH
OH
O
HO
CN
14a
[rel]
[ppm]

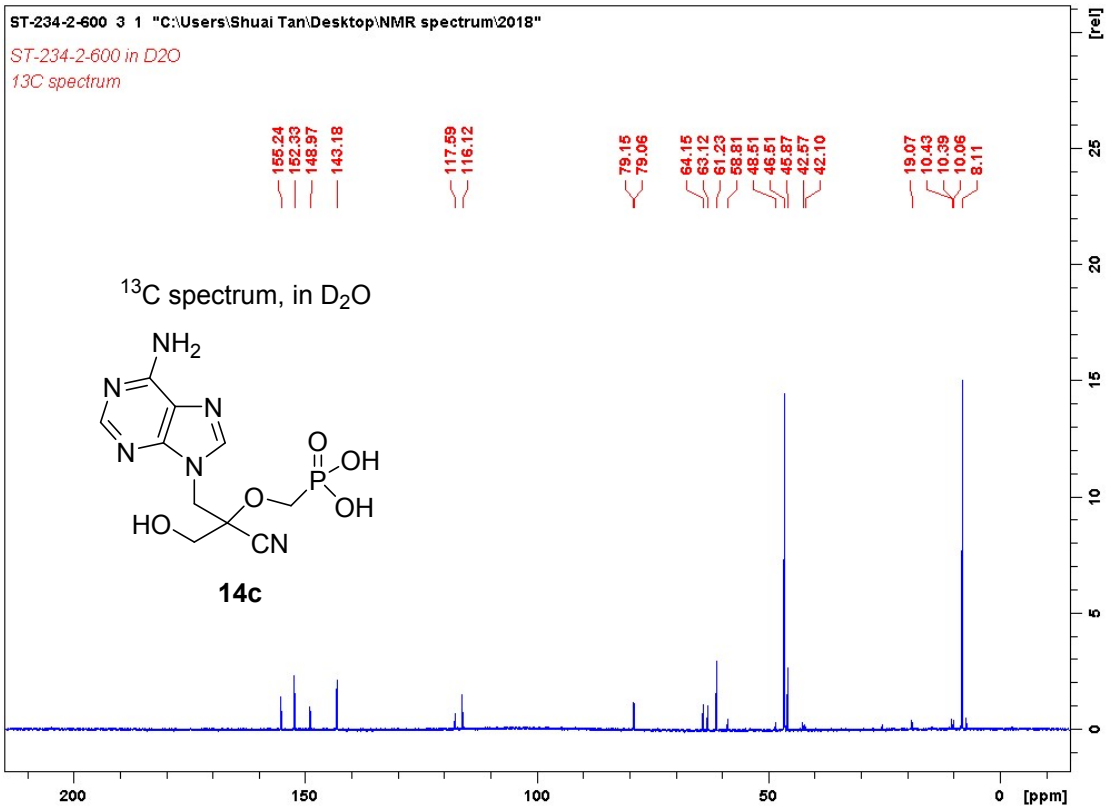

ST-230 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
11.92
31P spectrum, in D2O
14a
[rel]
[ppm]

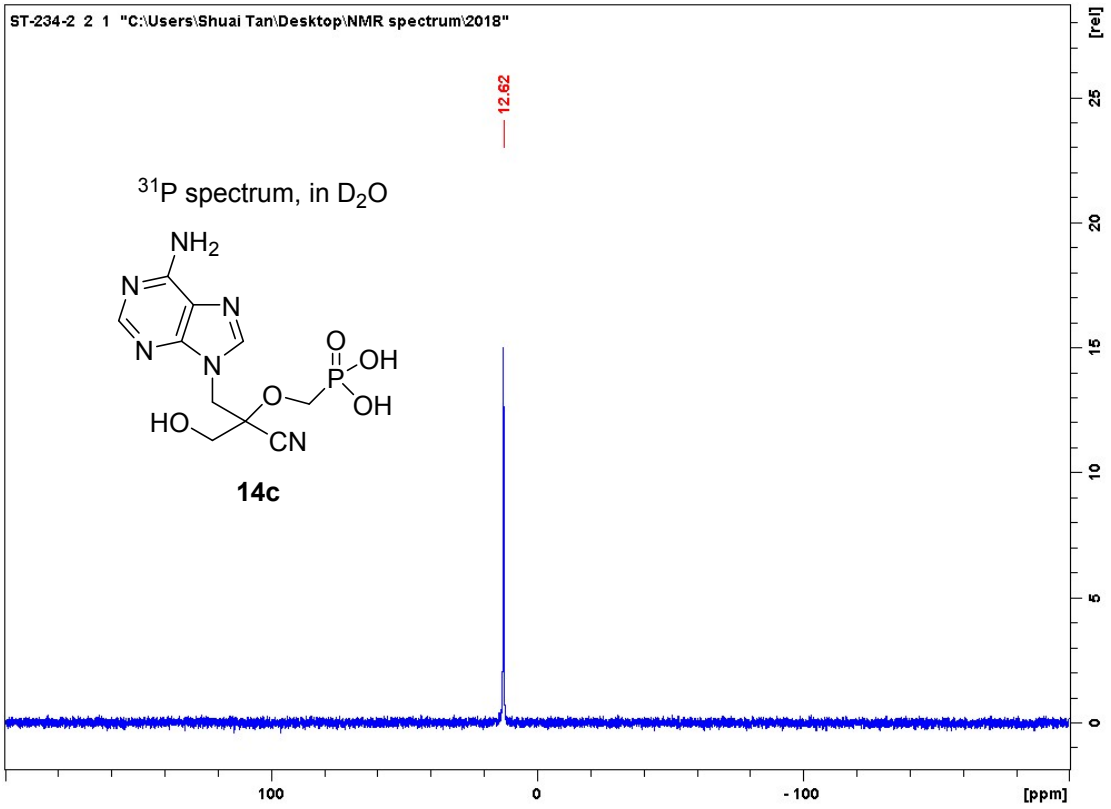

ST-228-600 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
ST-228-600 in D2O
proton spectrum
7.79
7.78
5.87
5.86
4.79
4.51
4.48
4.15
4.12
4.00
3.98
3.88
3.86
3.79
3.77
3.75
3.75
3.73
3.71
3.22
3.20
3.19
3.18
1.29
1.28
1.27
1H spectrum, in D2O
14b
1.00
1.01
0.99
9.77
16.13
[rel]
[ppm]

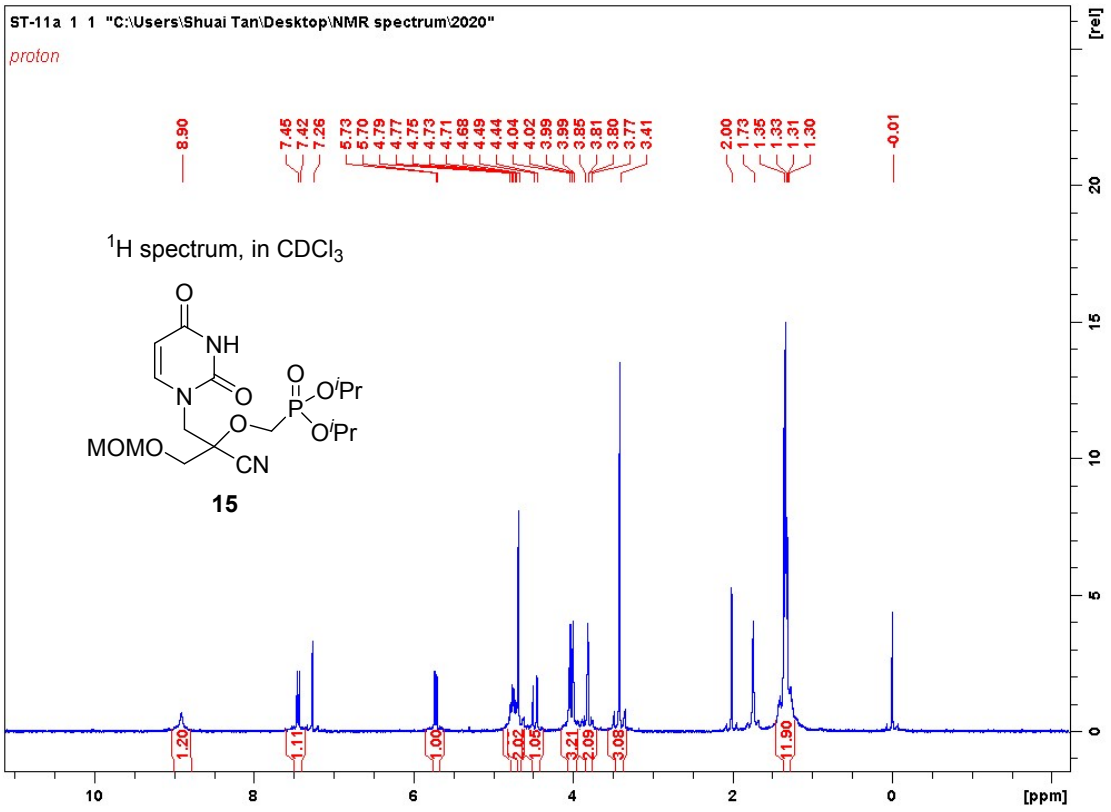

ST-228-600 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
ST-228-600 in D2O
13C spectrum
166.54
152.28
147.82
116.28
101.77
79.11
79.03
64.21
63.20
61.48
49.59
46.57
8.15
13C spectrum, in D2O
14b
[rel]
[ppm]

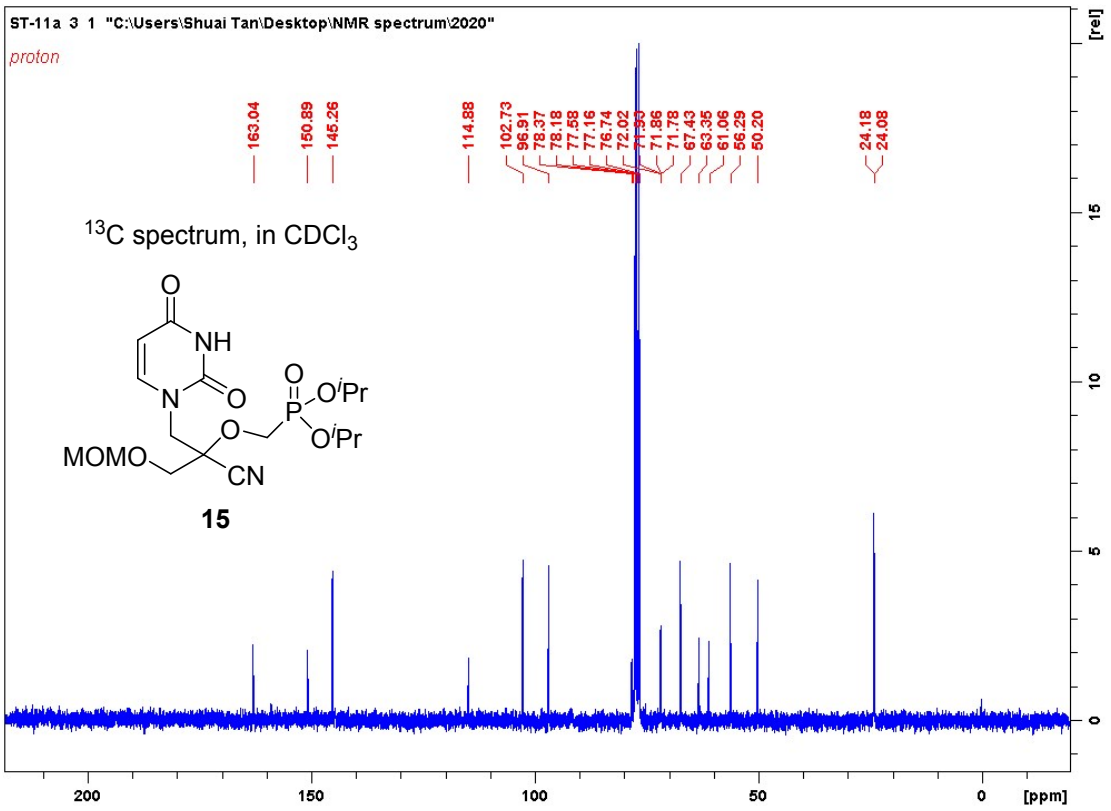

ST-228 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
12.34
31P spectrum, in D2O
14b
[rel]
[ppm]

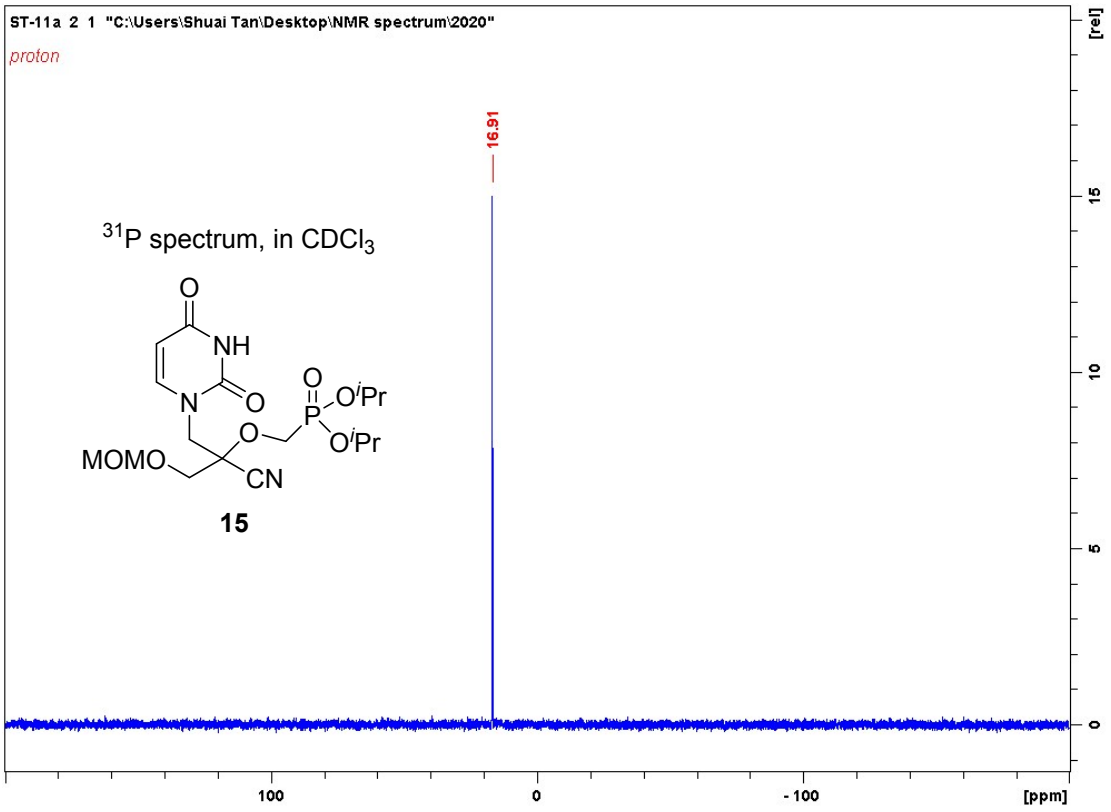

ST-234-2-600 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
ST-234-2-600 in D2O
proton spectrum
8.27
8.13
4.79
4.75
4.73
4.65
4.62
3.98
3.96
3.87
3.85
3.82
3.80
3.78
3.72
3.71
3.70
3.69
3.17
3.16
3.15
3.13
1.25
1.24
1.24
1.23
1.22
1H spectrum, in D2O
NH2
N
N
N
N
O
OH
P
OH
HO
CN
14c
1.00
1.02
1.08
1.07
9.14
13.92
[rel]
[ppm]

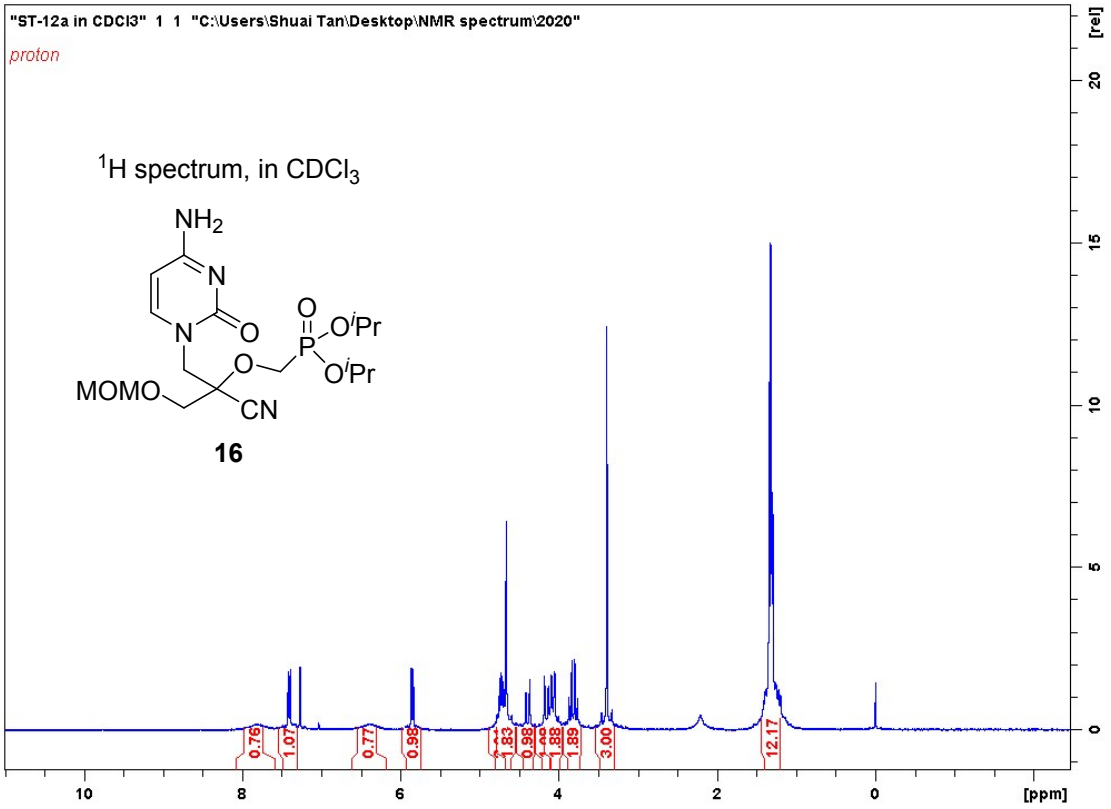

ST-234-2-600 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
ST-234-2-600 in D2O
13C spectrum
155.24
152.33
148.97
143.18
117.59
116.12
79.15
79.06
64.15
63.12
61.23
58.81
48.51
46.51
45.87
42.57
42.10
19.07
10.43
10.39
10.06
8.11
13C spectrum, in D2O
NH2
N
N
N
N
O
OH
P
OH
HO
CN
14c
[rel]
[ppm]

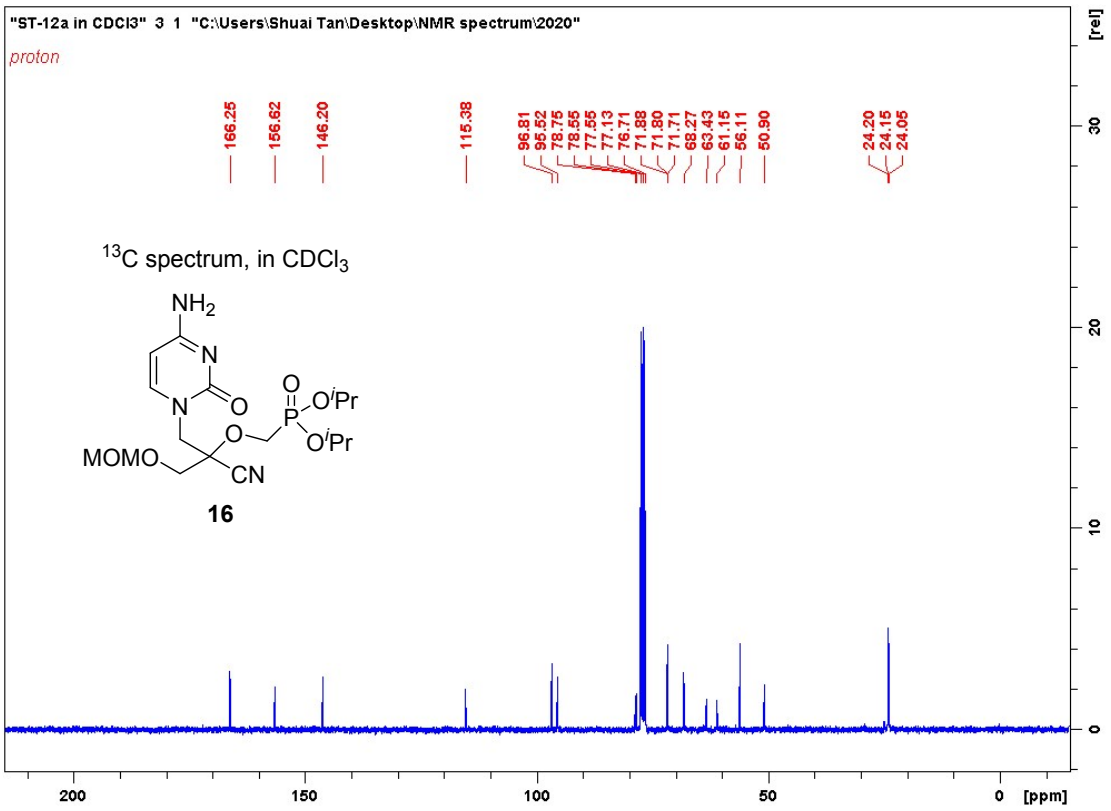

ST-234-2 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
12.62
31P spectrum, in D2O
NH2
N
N
N
N
O
OH
P
OH
O
HO
CN
14c
[rel]
25
20
15
10
5
0
100
0
- 100
[ppm]

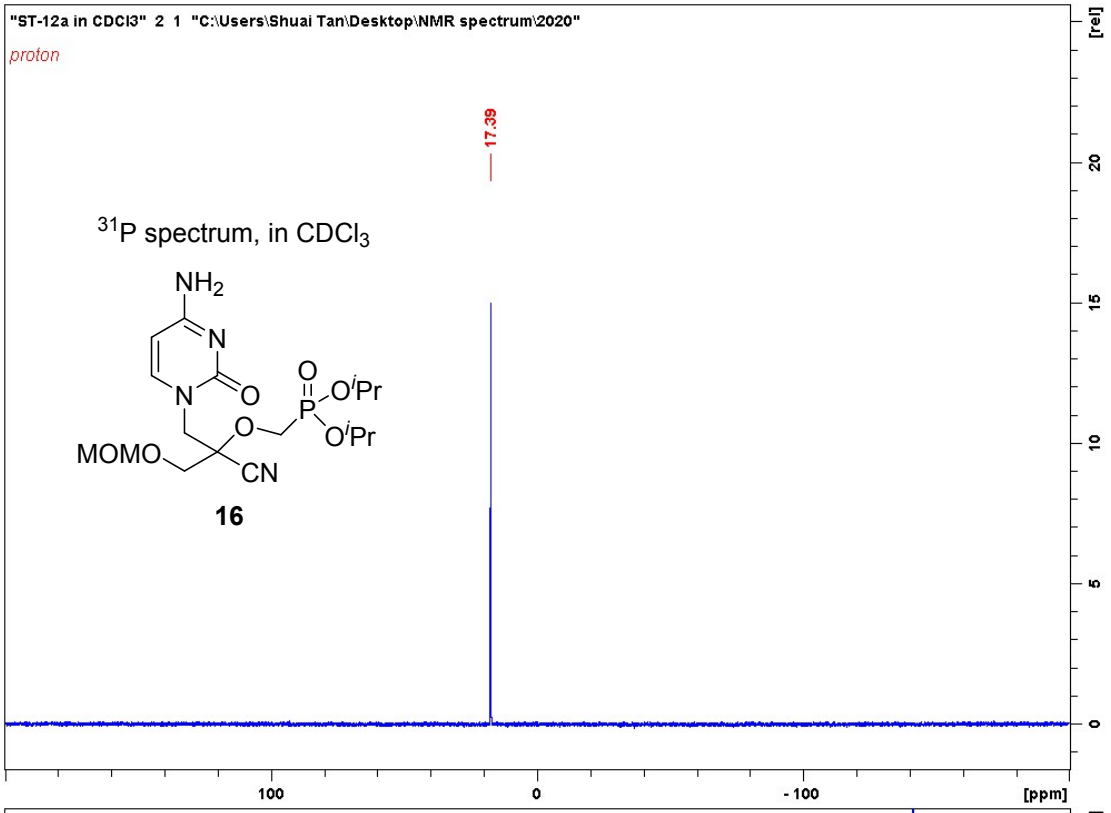

ST-11a 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
8.90
7.45
7.42
7.26
5.73
5.70
4.79
4.77
4.75
4.73
4.71
4.68
4.49
4.44
4.04
4.02
3.99
3.99
3.85
3.81
3.80
3.77
3.41
2.00
1.73
1.35
1.33
1.31
1.30
-0.01
1H spectrum, in CDCl3
O
NH
N
O
O
P
OiPr
O
OiPr
MOMO
CN
15
1.20
1.11
1.00
2.02
1.05
3.21
2.09
3.08
11.90
[rel]
20
15
10
5
0
10
8
6
4
2
0
[ppm]

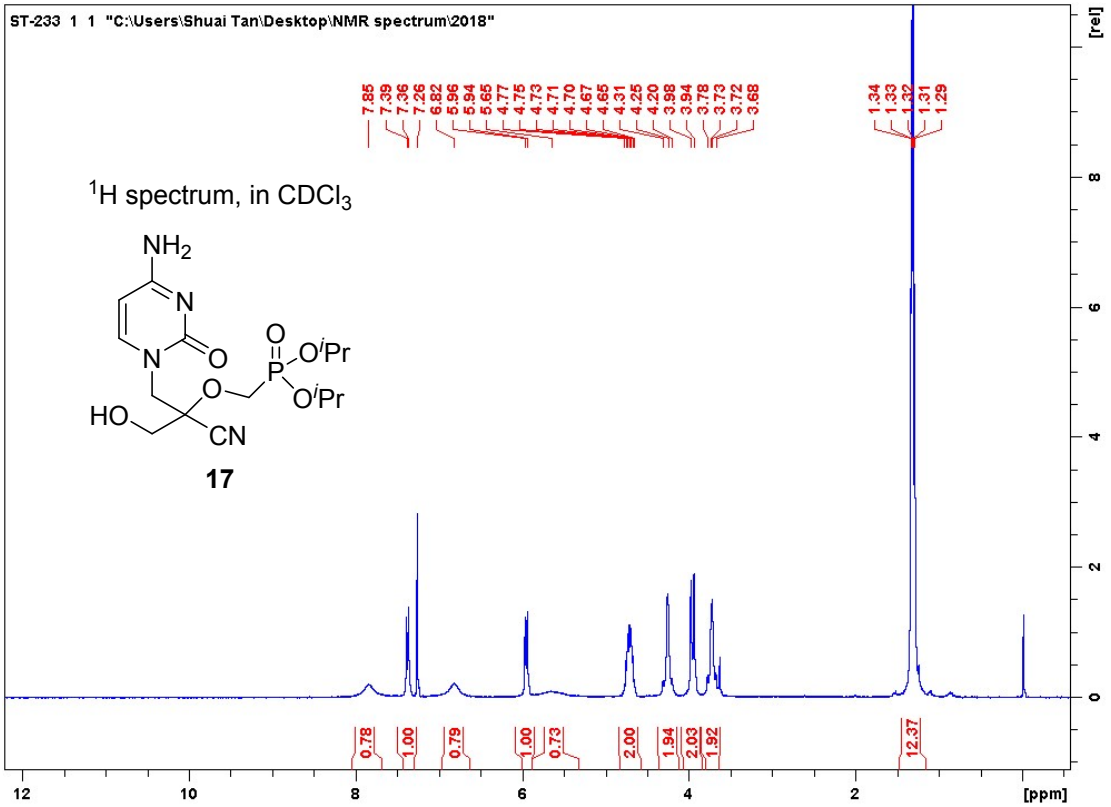

ST-11a 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
163.04
150.89
145.26
114.88
102.73
96.91
78.37
78.18
77.58
77.16
76.74
72.02
71.86
71.78
67.43
63.35
61.06
56.29
50.20
24.18
24.08
13C spectrum, in CDCl3
O
NH
N
O
O
P
OiPr
OiPr
O
MOMO
CN
15
[rel]
[ppm]
200
150
100
50
0

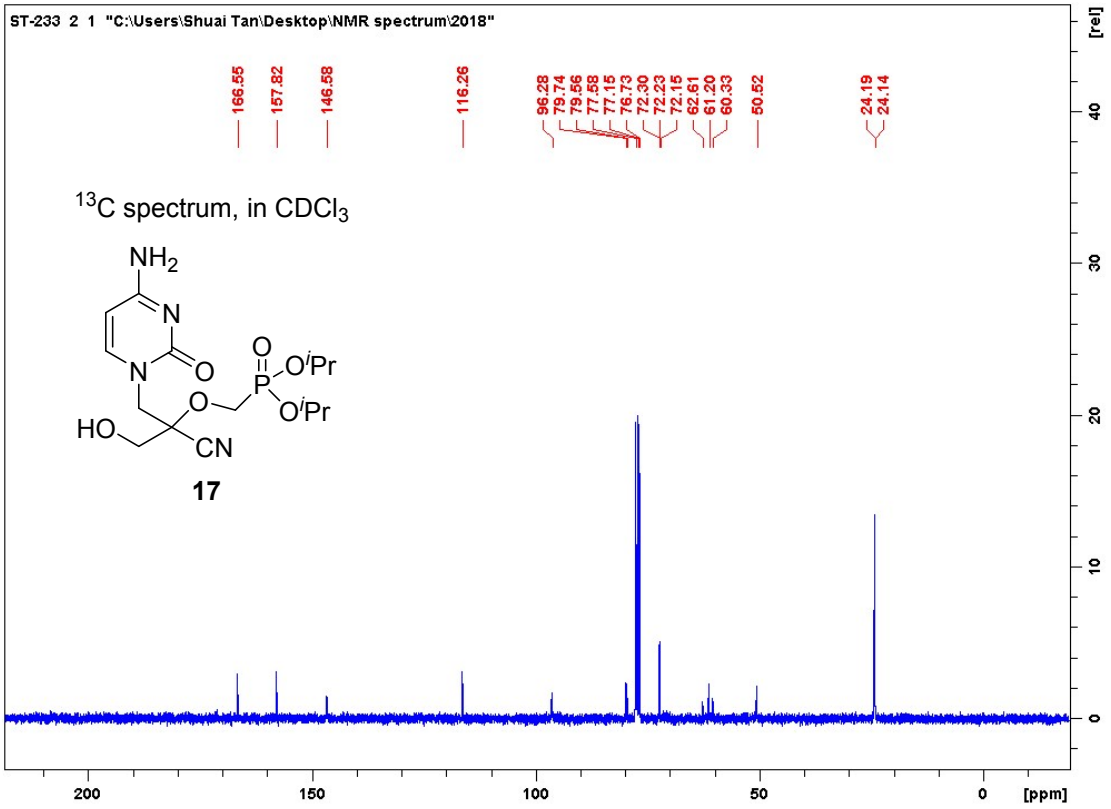

ST-11a 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
16.91
31P spectrum, in CDCl3
O
NH
N
O
O
P
OiPr
OiPr
O
MOMO
CN
15
[rel]
[ppm]
100
0
- 100


"ST-12a in CDCl3" 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
^{1}H spectrum, in $CDCl_3$
NH2
N
O
N
O
P
O^{i}Pr
O^{i}Pr
O
MOMO
CN
16
0.76
1.07
0.77
0.98
1.83
0.98
1.88
1.89
3.00
12.17
10
8
6
4
2
0
[ppm]
[rel]
20
15
10
5
0


"ST-12a in CDCl3" 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
166.25
156.62
146.20
115.38
96.81
95.52
78.75
78.55
77.55
77.13
76.71
71.88
71.80
71.71
68.27
63.43
61.15
56.11
50.90
24.20
24.15
24.05
^{13}C spectrum, in $CDCl_3$
NH2
N
O
N
O
P
O^{i}Pr
O^{i}Pr
O
MOMO
CN
16
200
150
100
50
0
[ppm]
[rel]
30
20
10
0

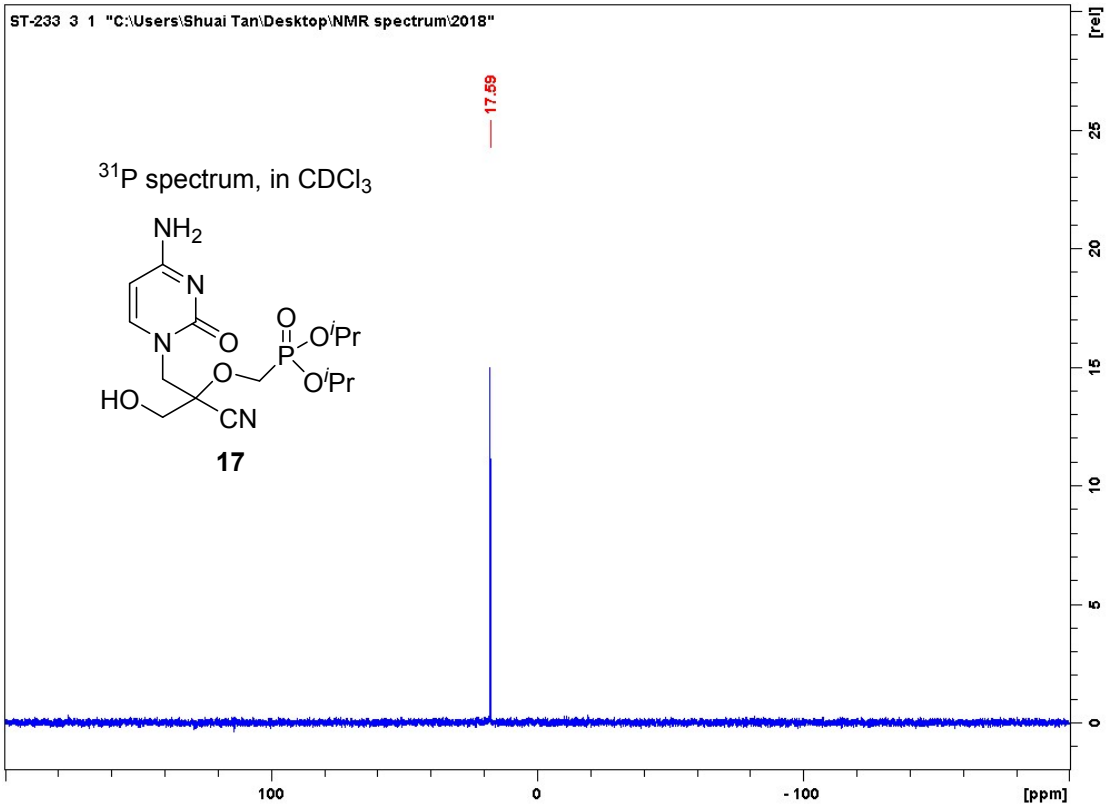

"ST-12a in CDCl3" 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
17.39
31P spectrum, in CDCl3
NH2
N
N
O
O
P
OiPr
OiPr
MOMO
CN
16
[rel]
20
15
10
5
0
100
0
- 100
[ppm]


ST-233 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
7.85
7.39
7.36
7.26
6.82
5.96
5.94
5.85
4.77
4.75
4.73
4.71
4.70
4.67
4.65
4.31
4.25
4.20
3.98
3.94
3.78
3.73
3.72
3.68
1.34
1.33
1.32
1.31
1.29
1H spectrum, in CDCl3
NH2
N
N
O
O
P
OiPr
OiPr
HO
CN
17
0.78
1.00
0.79
1.00
0.73
2.00
1.94
2.03
1.92
12.37
[rel]
8
6
4
2
0
12
10
8
6
4
2
[ppm]



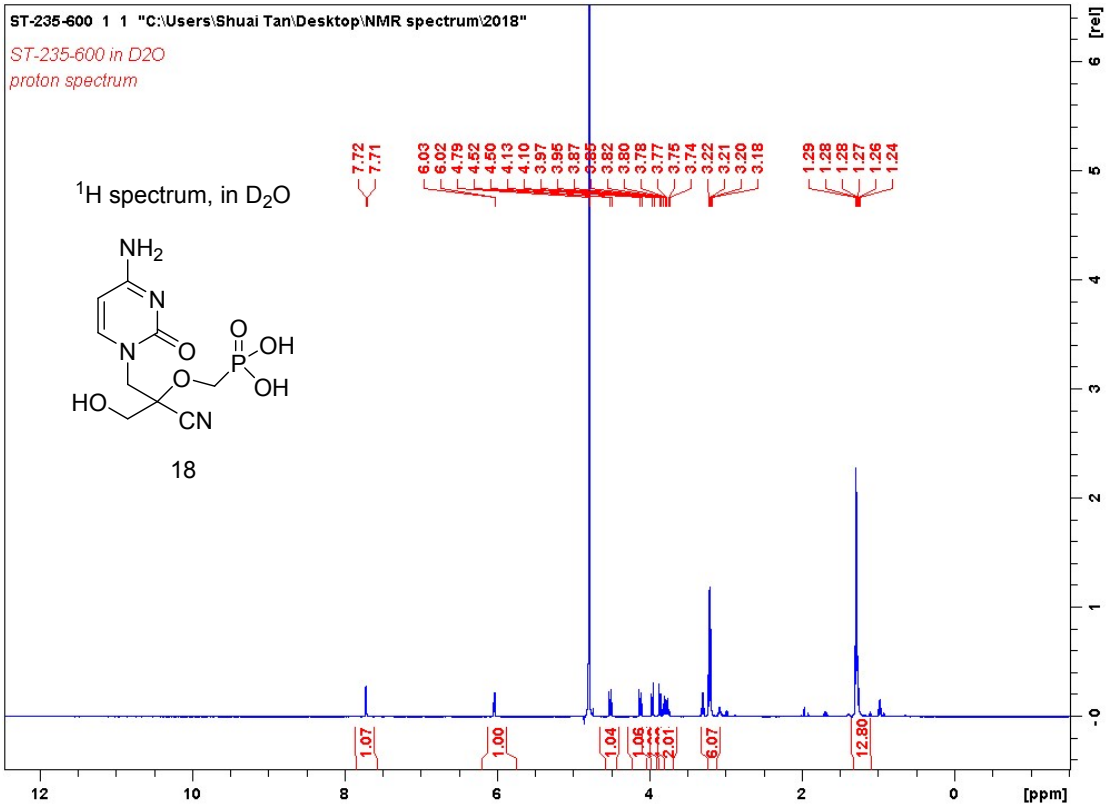
^{13}C spectrum, in $CDCl_3$

17



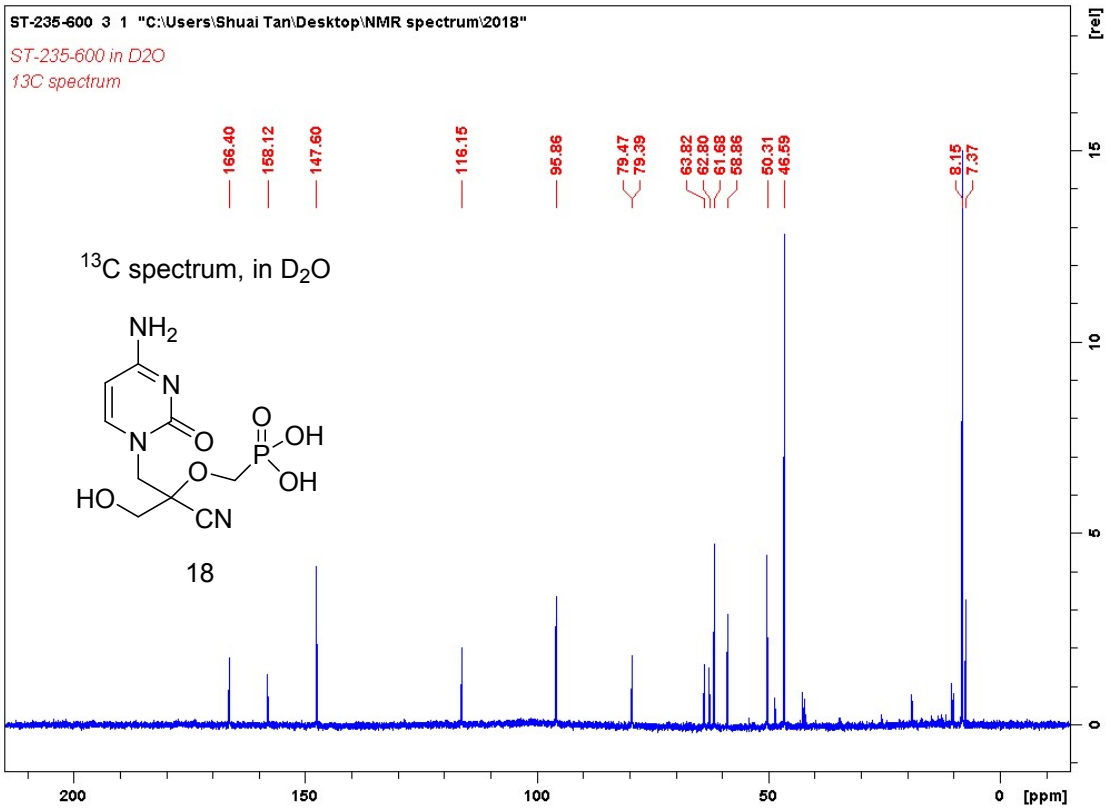
^{31}P spectrum, in $CDCl_3$

17



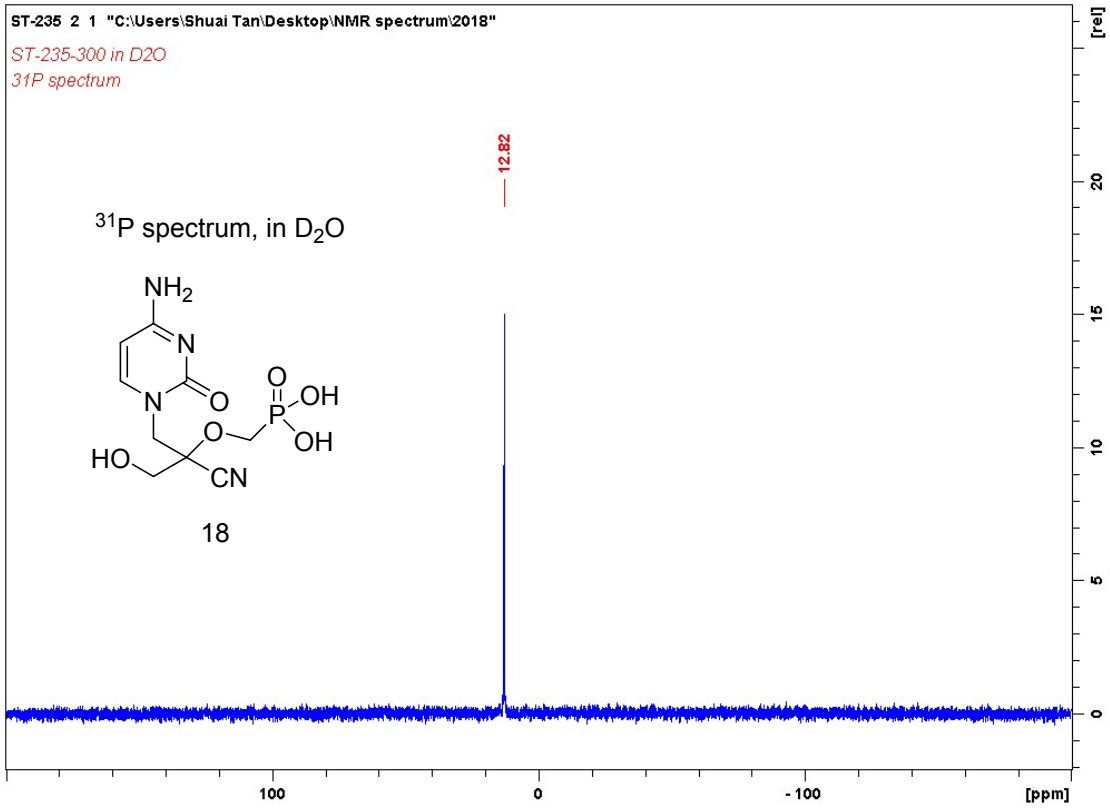
^{1}H spectrum, in D_2O

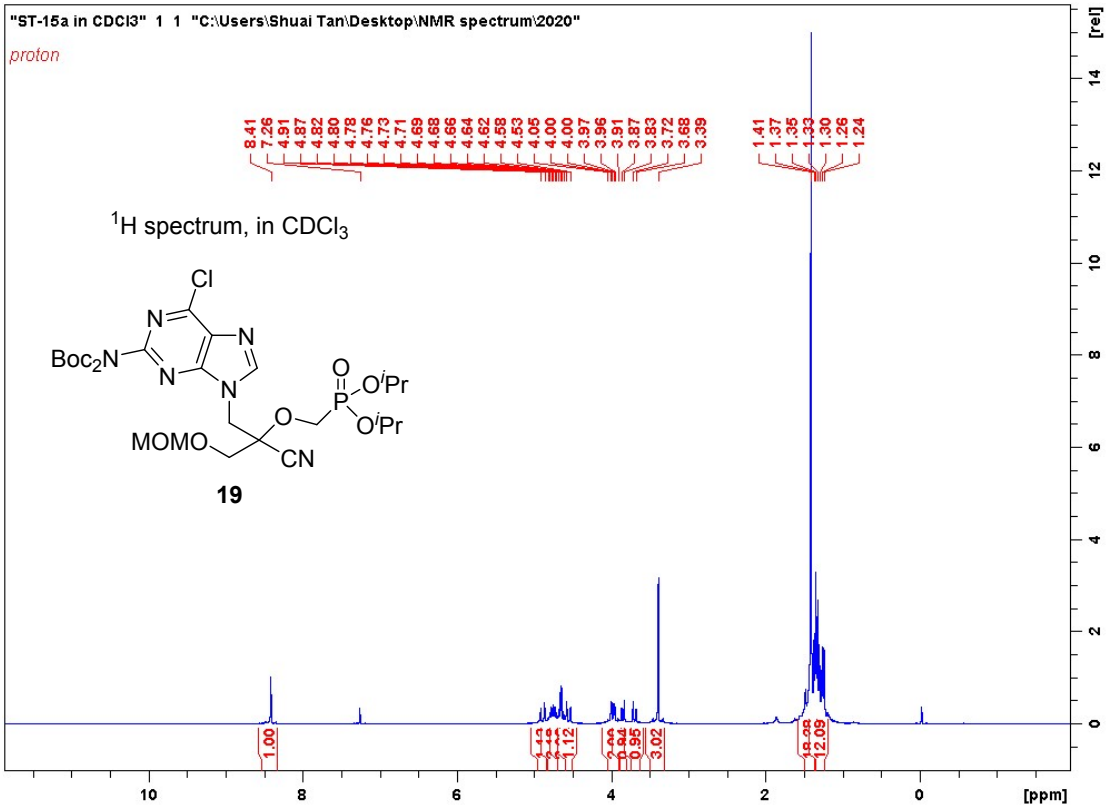
18

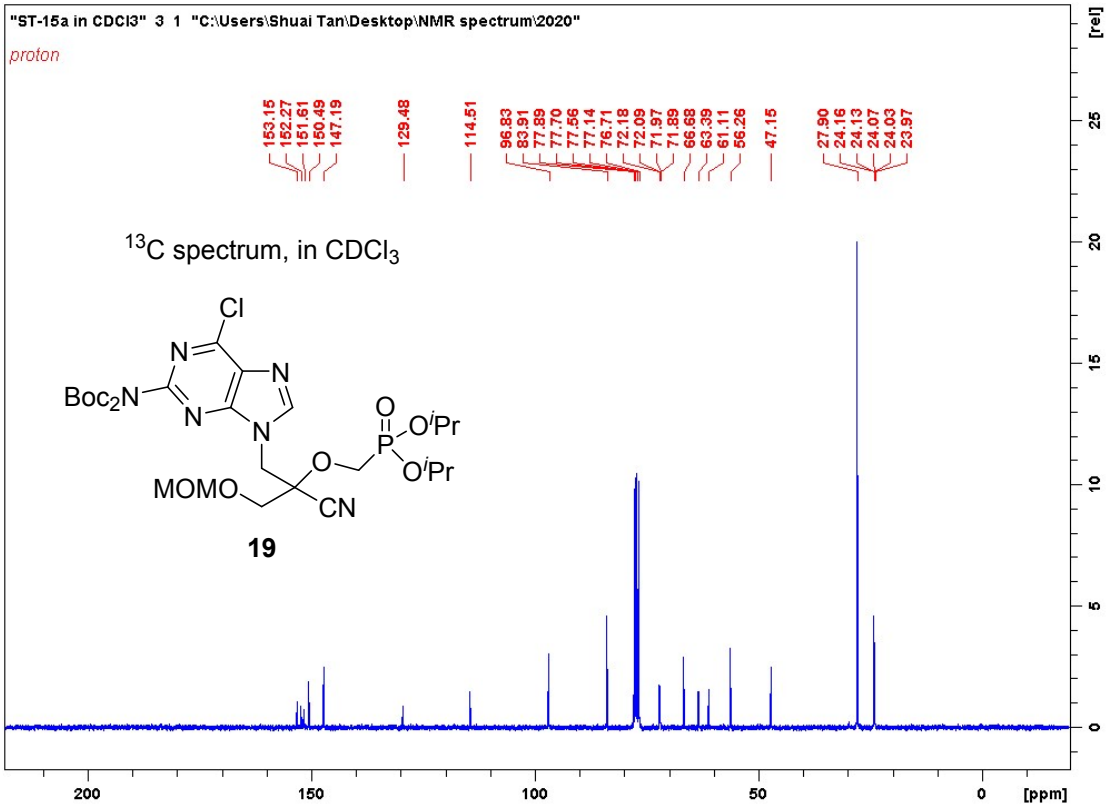


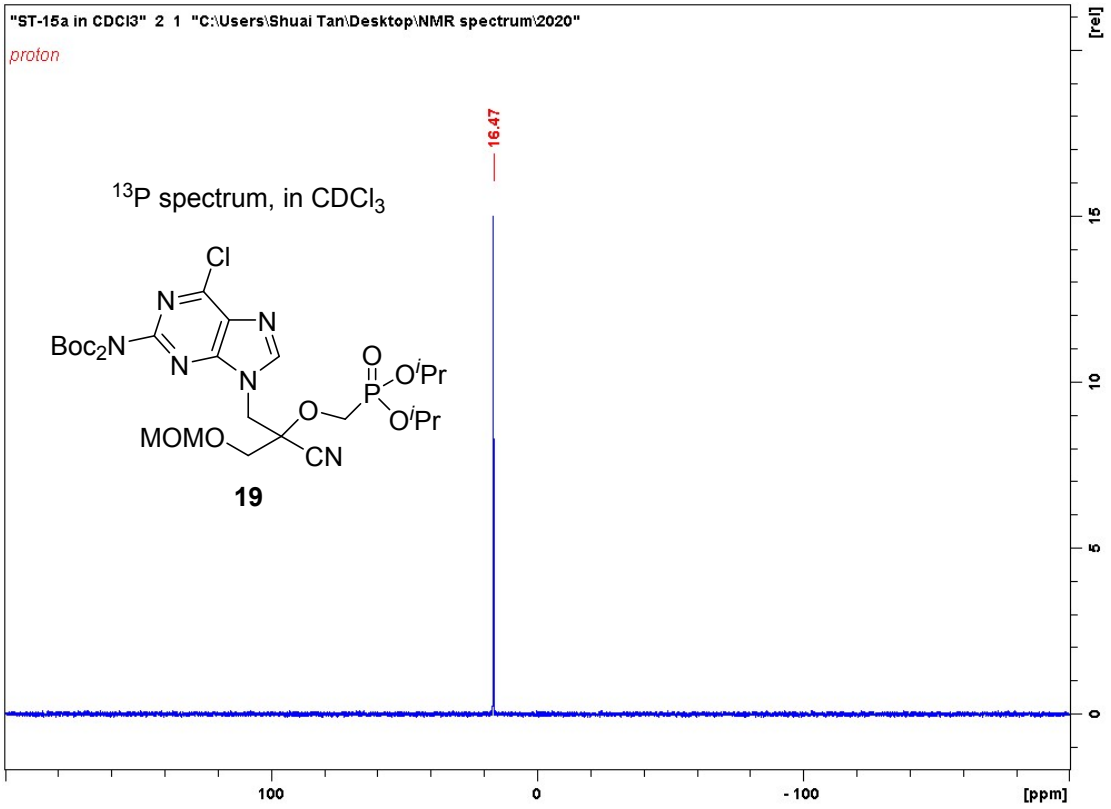
^{13}C spectrum, in D_2O

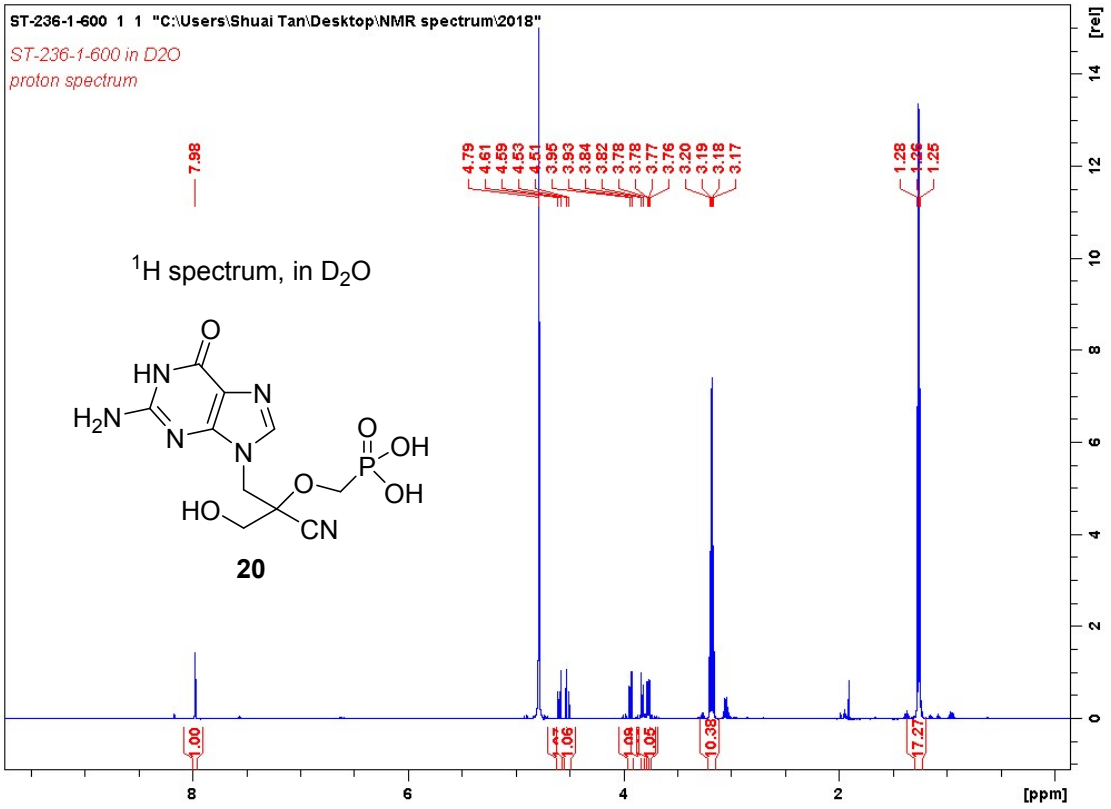
18

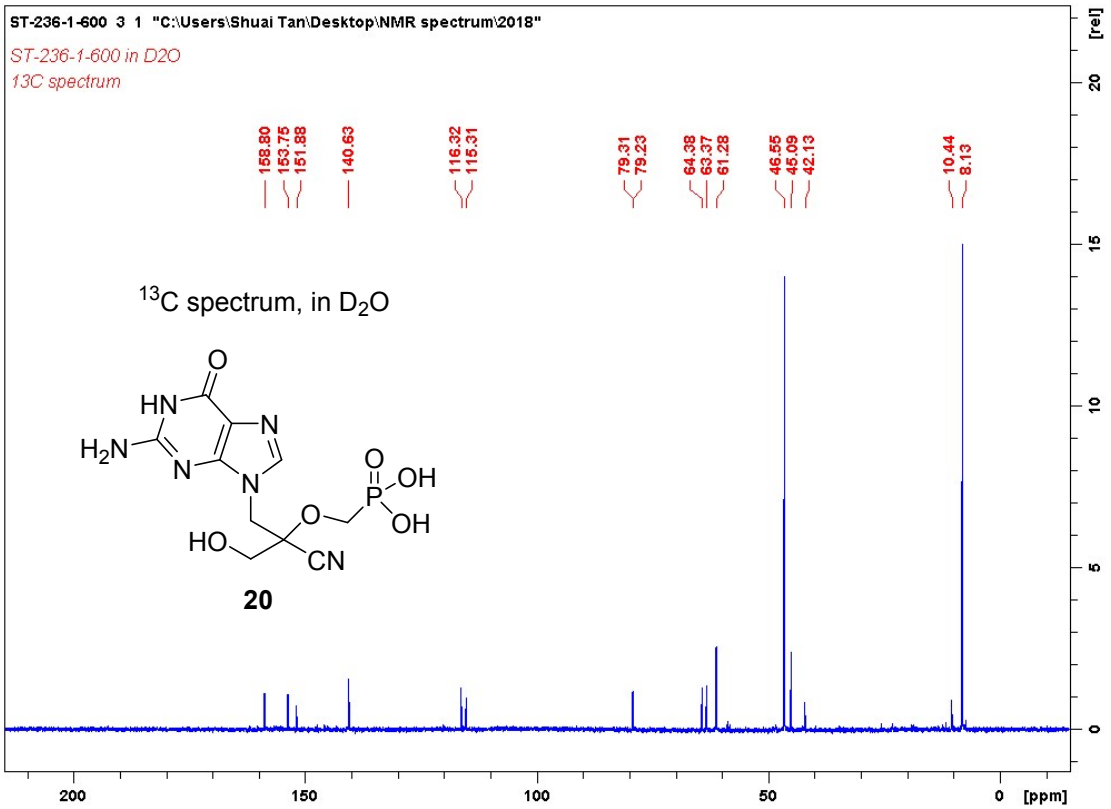

ST-235 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
ST-235-300 in D2O
31P spectrum
12.82
31P spectrum, in D2O
NH2
N
N
O
O
P
OH
OH
O
HO
CN
18
[rel]
20
15
10
5
0
100
0
- 100
[ppm]

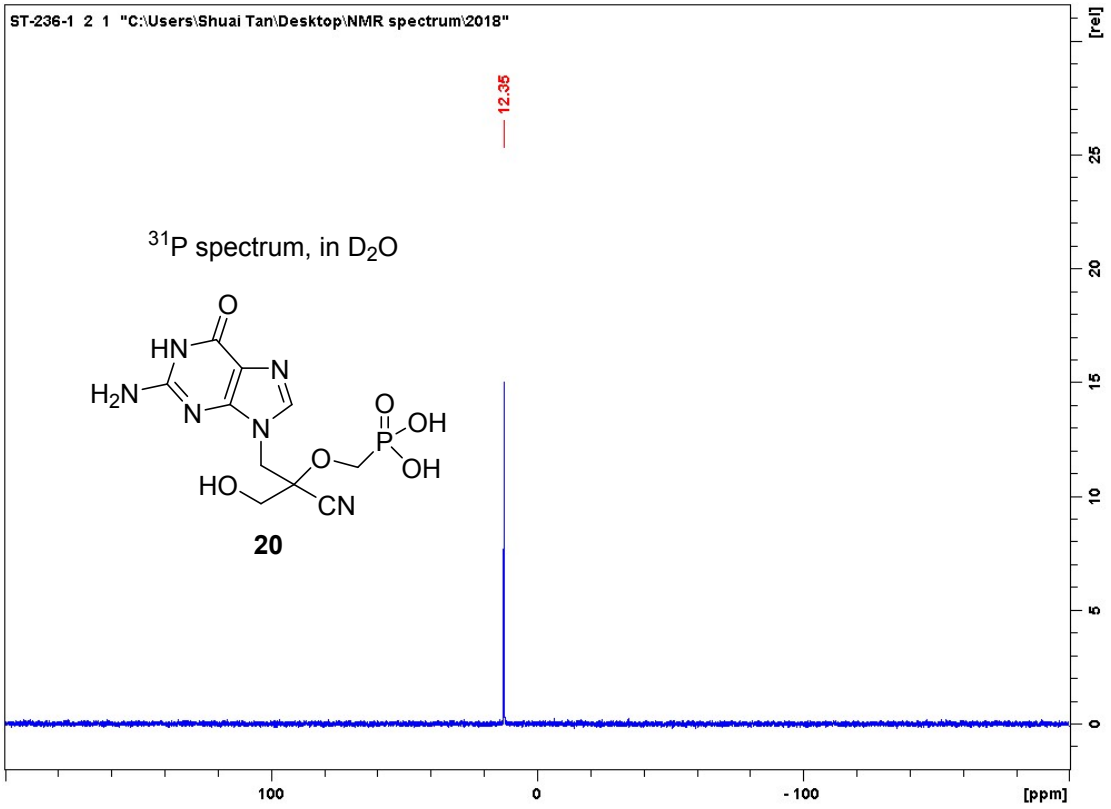

"ST-15a in CDCl3" 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
8.41
7.26
4.91
4.87
4.82
4.80
4.78
4.76
4.73
4.71
4.69
4.68
4.66
4.64
4.62
4.58
4.53
4.05
4.00
4.00
3.97
3.96
3.91
3.87
3.83
3.72
3.68
3.39
1.41
1.37
1.35
1.33
1.30
1.26
1.24
1H spectrum, in CDCl3
Cl
N
N
Boc2N
N
N
O
O
P
OiPr
OiPr
MOMO
CN
19
1.00
1.12
3.02
12.09
[rel]
14
12
10
8
6
4
2
0
10
8
6
4
2
0
[ppm]


"ST-15a in CDCl3" 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
153.15
152.27
151.61
150.49
147.19
129.48
114.51
96.83
83.91
77.89
77.70
77.56
77.14
76.71
72.18
72.09
71.97
71.89
66.68
63.39
61.11
56.26
47.15
27.90
24.16
24.13
24.07
24.03
23.97
13C spectrum, in CDCl3
Cl
N
N
Boc2N
N
N
O
OiPr
P
OiPr
O
MOMO
CN
19
[rel]
25
20
15
10
5
0
200
150
100
50
0
[ppm]


"ST-15a in CDCl3" 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2020"
proton
16.47
31P spectrum, in CDCl3
Cl
N
N
Boc2N
N
N
O
OiPr
P
OiPr
O
MOMO
CN
19
[rel]
15
10
5
0
100
0
-100
[ppm]


ST-236-1-600 1 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
ST-236-1-600 in D2O
proton spectrum
7.98
4.79
4.61
4.59
4.53
4.51
3.95
3.93
3.84
3.82
3.78
3.78
3.77
3.76
3.20
3.19
3.18
3.17
1.28
1.26
1.25
1H spectrum, in D2O
20
1.00
10.38
17.27
8
6
4
2
[ppm]
[rel]


ST-236-1-600 3 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
ST-236-1-600 in D2O
13C spectrum
158.80
153.75
151.88
140.63
116.32
115.31
79.31
79.23
64.38
63.37
61.28
46.55
45.09
42.13
10.44
8.13
13C spectrum, in D2O
20
200
150
100
50
0
[ppm]
[rel]


ST-236-1 2 1 "C:\Users\Shuai Tan\Desktop\NMR spectrum\2018"
12.35
^{31}P spectrum, in D_2O
O
HN
H_2N
N
N
N
O
OH
P
OH
O
HO
CN
20
[rel]
25
20
15
10
5
0
100
0
- 100
[ppm]