Influence of 4'-Substitution on the Activity of Gemcitabine and its ProTide Against VZV and SARS-CoV-2

(Supporting Information)

Zihua Zheng,[§] Elisabetta Groaz,[†] Robert Snoeck,[§] Steven de Jonghe,[§] Piet Herdewijn,[†] and Graciela Andrei*[,§]

[§] Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Herestraat 49 bus 1043, 3000 Leuven, Belgium

[†] Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, Herestraat 49, 3000 Leuven, Belgium

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EXPERIMENTAL SECTION

General information. All reagents and solvents were of analytical grade and used without further purification. All sensitive reactions were carried out in dry solvents under an argon or nitrogen atmosphere. ^{1}H, ^{13}C, and ^{31}P NMR spectra were obtained on a 300, 500 or 600 MHz Bruker Avance spectrometer using tetramethylsilane (TMS) as an internal standard or by referencing to the residual solvent signal. Coupling constants are reported in hertz (Hz) and were directly obtained from the spectra. The following abbreviations were used to denote the NMR splitting patterns: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), m (multiplet), and br (broad). High-resolution mass spectra (HRMS) were obtained on a quadrupole orthogonal acceleration 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 15 000 FWHM using leucine enkephalin as the lock mass. Pre-coated aluminum sheets (254 nm) were used for thin layer chromatography (TLC). Intermediate compounds were purified by silica gel column chromatography (60 Å, 0.060–0.200 mm, Acros Organics).

Chemical procedures

*(2R,3R,5R)-5-(4-Benzamido-2-oxopyrimidin-1(2H)-yl)-2-((benzoyloxy)methyl)-4,4-difluorotetrahydrofuran-3yl benzoate (**3**).* To a solution of gemcitabine (10.0 g, 38.0 mmol) and DMAP (14.0 g, 114.0 mmol) in anhydrous pyridine (150 mL) was added dropwise BzCl (17.6 mL, 152.0 mmol) at 0 °C under a N_2 atmosphere. The mixture was stirred for 3.5 h at room temperature and quenched with water. Then, it was concentrated *in vacuo* and the resulting residue was dissolved in CH_2Cl_2. The organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. To the residue was added EtOAc (70 mL) and the solution was heated at 80 °C, followed by the dropwise addition of heptane (100 mL). After stirring for 1 h, the mixture was cooled to 40 °C and filtered to afford **3** (20.4 g, 93%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.5 (1H, s), 8.23 (1H, d, J = 7.6 Hz), 8.08–7.95 (6H, m), 7.74 (1H, t, J = 7.4 Hz), 7.68–7.45 (8H, m), 7.41 (1H, d, J = 7.6 Hz), 6.50 (1H, t, J = 8.7 Hz), 5.89 (1H, q, J = 8.9 Hz), 4.90–4.72 (3H, m); ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.5, 165.3, 164.2, 163.8, 153.8, 145.7, 134.1, 133.5, 132.9, 132.7, 129.6, 129.1, 129.0, 128.8, 128.6, 128.4, 128.3, 127.9, 125.0, 121.6, 118.1, 97.0, 85.4, 76.3, 71.9, 71.6, 71.3, 63.2; HRMS (ESI^+): m/z, $[M + H]^+$ calcd for $C_{30}H_{24}F_2N_3O_7^+$, 576.1576; found, 576.1572.

*((2R,3R,5R)-3-(Benzoyloxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4,4-difluorotetrahydrofuran-2-yl)methyl benzoate (**4**).* Compound **3** (20.4 g, 35.4 mmol) was dissolved in 80% AcOH (275 mL) and the mixture was refluxed overnight. It was then concentrated *in vacuo* to leave a crude product, which was redissolved in CH_2Cl_2 and neutralized with saturated aq. $NaHCO_3$

(pH = 8–9). The aqueous layer separated and further extracted with CH_2Cl_2. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. To the residue was added EtOAc (60 mL) and the mixture was heated at 80 °C, followed by the dropwise addition of heptane (90 mL). After stirring for 1 h, the mixture was cooled to 40 °C and filtered to afford **4** (15.1 g, 90%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.7 (1H, s), 8.04 (2H, d, J = 7.7 Hz), 7.94 (2H, d, J = 7.7 Hz), 7.73 (2H, t, J = 7.6 Hz), 7.65 (1H, t, J = 7.3 Hz), 7.57 (2H, t, J = 7.6 Hz), 7.48 (2H, t, J = 7.6 Hz), 6.36 (1H, t, J = 9.2 Hz), 5.83 (1H, q, J = 9.0 Hz), 5.73 (1H, d, J = 8.2 Hz), 4.79–4.66 (3H, m); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.2, 164.2, 162.5, 149.9, 140.9, 134.1, 133.4, 129.5, 129.1, 129.0, 128.8, 128.6, 127.8, 124.9, 121.5, 118.0, 102.4, 84.0, 75.7, 71.7, 71.3, 71.0, 63.1; HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{23}H_{18}F_2N_2O_7Na^+$, 495.0974; found, 495.0975.

*1-((2R,4R,5R)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**5**).* Compound **4** (15.1 g, 32.0 mmol) was dissolved in 7 M NH_3 in methanol (350 mL) and the mixture was stirred at room temperature overnight. It was then concentrated *in vacuo* and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1 to 10:1) to afford **5** (7.2 g, 85%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.6 (1H, br), 7.78 (1H, d, J = 8.1 Hz), 6.30 (1H, d, J = 6.5 Hz), 6.06 (1H, t, J = 7.8 Hz), 5.72 (1H, d, J = 8.1 Hz), 5.26 (1H, t, J = 5.4 Hz), 4.21–4.14 (1H, m), 3.85-3.82 (1H, m), 3.78-3.75 (1H, m), 3.62 (1H, ddd, J_1 = 6.3 Hz, J_2 = 3.1 Hz, J_3 = 1.8 Hz); ^{13}C NMR (151 MHz, DMSO-d_6): δ 162.6, 150.1, 139.8, 126.3, 122.8, 119.4, 102.0, 83.5, 83.1, 82.6, 80.8, 68.7, 68.4, 68.1, 58.8. HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_9H_{11}F_2N_2O_5^+$, 265.0630; found, 265.0628.

*1-((2R,4R,5S)-3,3-Difluoro-4-hydroxy-5-(iodomethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**6**).* To a solution of **5** (5.00 g, 18.9 mmol), imidazole (2.57 g, 37.8 mmol), PPh_3 (7.45 g, 28.4 mmol), and pyridine (21 mL) in anhydrous THF (50 mL) was added dropwise a solution of I_2 (6.24 g, 24.6 mmol) in anhydrous THF (70 mL) at 0 °C over 1 h under a N_2 atmosphere. The mixture was stirred at room temperature for 3 h, quenched with MeOH (40 mL), and concentrated *in vacuo*. The residue was redissolved in EtOAc and washed with saturated aq. NaS_2O_3 and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 30:1) to afford **6** (6.00 g, 84%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.6 (1H, s), 7.62 (1H, d, J = 8.1 Hz), 6.55 (1H, d, J = 6.2 Hz), 6.14 (1H, t, J = 8.9 Hz), 5.75 (1H, d, J = 8.1 Hz), 4.08–3.98 (1H, m), 3.81 (1H, td, J_1 = 7.3 Hz, J_2 = 3.8 Hz), 3.63 (1H, dd, J_1 = 11.1 Hz, J_2 = 3.5 Hz), 3.50 (1H, dd, J_1 = 11.1 Hz, J_2 = 3.8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.5, 149.9, 140.5, 126.1, 122.7, 119.3, 102.4, 83.7, 83.4, 82.8, 78.7, 78.6, 74.1, 73.7, 73.5, 5.1; HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_9H_{10}F_2IN_2O_4^+$, 374.9649; found, 374.9634.

*1-((2R,4R)-3,3-Difluoro-4-hydroxy-5-methylenetetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**7**).* To a solution of **6** (12.0 g, 32.1 mmol) in anhydrous THF (400 mL) was added DBU (19.7 mL, 131.5 mmol) under a N_2 atmosphere. The mixture was then heated at 60 °C and stirred for 1.5 h. After reaction

completion, the mixture was quenched with water and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 30:1; Heptane/EtOAc = 2:1) to afford **7** (5.2 g, 65 %) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.6 (1H, s), 7.58 (1H, d, J = 8.0 Hz), 6.62 (1H, d, J = 7.0 Hz), 6.28 (1H, t, J = 8.0 Hz), 5.72 (1H, d, J = 8.0 Hz), 4.99 (1H, q, J = 9.7 Hz), 4.63 (1H, s), 4.42 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.5, 157.9, 157.7, 149.9, 141.0, 124.7, 121.3, 117.9, 102.6, 87.3, 85.3, 85.2, 84.9, 69.4, 69.1, 68.8; HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_9H_9F_2N_2O_4^+$, 247.0524; found, 247.0522.

*1-((2R,4R,5S)-3,3-Difluoro-4-hydroxy-5-(iodomethyl)-5-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**8a**).* To an ice cooled solution of **7** (1.50 g, 6.09 mmol) and $PbCO_3$ (3.25 g, 12.2 mmol) in anhydrous MeOH (30 mL) was added dropwise a solution of I_2 (3.09 g, 12.2 mmol) in anhydrous MeOH (10 mL) at 0 °C under a N_2 atmosphere. The mixture was stirred at room temperature overnight, quenched with saturated aq. NaS_2O_3, and filtered through Celite. The filtrate was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 50:1) to afford **8a** (1.50 g, 61%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.63 (1H, d, J = 8.1 Hz), 6.12 (1H, t, J = 7.8 Hz), 5.78 (1H, d, J = 8.1 Hz), 4.50 (1H, t, J = 13.0 Hz), 3.83 (1H, d, J = 11.6 Hz), 3.55 (1H, d, J = 11.7 Hz), 3.43 (3H, s); ^{13}C NMR (75 MHz, MeOH-d_4): δ 165.2, 151.7, 141.9, 125.9, 122.4, 119.0, 103.5, 102.6, 102.5, 85.5, 85.1, 84.6, 77.1, 76.8, 76.5, 49.6, 0.8; HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{10}H_{12}F_2IN_2O_5^+$,404.9755; found, 404.9738.

*1-((2R,4R,5R)-3,3,5-Trifluoro-4-hydroxy-5-(iodomethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**8b**).* To an ice cooled solution of **7** (1.50 g, 6.09 mmol) and AgF (3.09 g, 24.4 mmol) in anhydrous MeCN (60 mL) was added dropwise a solution of I_2 (3.09 g, 12.2 mmol) in anhydrous MeCN (90 mL) at -10 °C under a N_2 atmosphere, and the mixture was stirred for 30 min at -10 °C. It was then filtered over a small bed of silica gel using 50% MeOH/CH_2Cl_2 as eluent to remove the Ag salts. The collected fraction was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (Heptane/EtOAc = 2:1 to 3:2) to afford **8b** (0.85 g, 35%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.58 (1H, d, J = 8.1 Hz), 6.27 (1H, br), 5.76 (1H, d, J = 9.1 Hz), 4.81–4.66 (1H, m), 3.74–3.58 (2H, m); ^{13}C NMR (75 MHz, MeOH-d_4): δ 165.2, 151.5, 142.5, 125.6, 122.1, 118.7, 115.1, 114.9, 112.0, 111.8, 103.8, 87.7, 74.8, 74.5, 74.2, 73.8, 1.0, 0.6; HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_9H_9F_3IN_2O_4^+$, 392.9555; found, 392.9551.

*(2S,3R,5R)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4,4-difluoro-2-(iodomethyl)-2-methoxytetrahydrofuran-3-yl benzoate (**9a**).* To an ice cooled solution of **8a** (1.17 g, 2.89 mmol) in anhydrous pyridine (20 mL) was added dropwise BzCl (0.37 mL, 3.18 mmol) at 0 °C under a N_2 atmosphere. The mixture was stirred for 1 h at 0 °C, quenched with water, and concentrated *in vacuo*. The crude residue was dissolved in CH_2Cl_2 and washed with saturated aq. $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was

purified by silica gel column chromatography (Heptane/EtOAc = 5:1 to 2:1) to afford **9a** (1.38 g, 94%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 9.62 (1H, s), 8.15–8.11 (2H, m), 7.67-7.60 (1H, m), 7.56–7.46 (3H, m), 6.37 (1H, t, J = 8.3 Hz), 5.89–5.78 (2H, m), 3.77 (1H, d, J = 11.5 Hz), 3.53 (1H, d, J = 11.5 Hz), 3.44 (3H, s); ^{13}C NMR (75 MHz, MeOH-d_4): δ 165.1, 162.5, 150.2, 140.2, 140.1, 134.2, 130.4, 128.7, 128.3, 123.9, 120.4, 120.3, 116.9, 103.6, 101.6, 101.4, 83.6, 83.3, 83.1, 82.8, 75.0, 74.8, 74.6, 74.4, 49.6, 2.3; HRMS (ESI$^+$): *m*/*z*, [M + H]$^+$ calcd for $C_{17}H_{16}F_2IN_2O_6^+$, 509.0017; found, 509.0013.

*(2R,3R,5R)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,4,4-trifluoro-2-(iodomethyl)tetrahydrofuran-3-yl benzoate (**9b**)*. To an ice cooled solution of **8b** (650 mg, 1.66 mmol) in anhydrous pyridine (20 mL) was added dropwise BzCl (0.21 mL, 1.82 mmol) at 0 °C under a N_2 atmosphere. After stirring for 1 h at 0 °C, the mixture was quenched with water and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2, washed with saturated aq. $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (Heptane/EtOAc = 2:1) to afford **9b** (720 mg, 87%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.3 (1H, s), 8.13 (2H, d, J = 7.7 Hz), 7.65 (1H, t, J = 7.1 Hz), 7.50 (2H, t, J = 7.4 Hz), 7.42 (1H, d, J = 8.1 Hz), 6.42 (1H, br), 6.10–6.05 (1H, m), 5.92 (1H, d, J = 8.2 Hz), 3.77–3.63 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.7, 163.0, 149.9, 140.6, 134.5, 130.4, 128.8, 127.6, 123.4, 119.9, 116.4, 113.1, 109.9, 104.0, 86.4, 72.4, 72.1, 1.6, 1.2; HRMS (ESI$^+$): *m*/*z*, [M + H]$^+$ calcd for $C_{16}H_{13}F_3IN_2O_5^+$, 496.9817; found, 496.9813.

*((2R,3R,5R)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4,4-difluoro-3-hydroxy-2-methoxytetrahydrofuran-2-yl)methyl benzoate (**10a**)*. To an ice cooled solution of **9a** (870 mg, 1.71 mmol) in CH_2Cl_2 (47 mL) and H_2O (1.7 mL) was added m-CPBA (1.53 g, 6.85 mmol) under an Ar atmosphere. The mixture was heated at 40 °C and stirred for 5 h. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (Heptane/EtOAc = 2:1 to 3:2) to afford **10a** (496 mg, 72%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 8.08 (2H, d, J = 7.0 Hz), 7.65 (1H, t, J = 7.3 Hz), 7.54–7.45 (3H, m), 6.19 (1H, t, J = 7.4 Hz), 5.55 (1H, d, J = 8.1 Hz), 4.81 (1H, d, J = 12.2 Hz), 4.66–4.55 (2H, m), 3.54 (3H, s); ^{13}C NMR (75 MHz, MeOH-d_4): δ 167.0, 165.2, 151.6, 141.7, 134.7, 130.7, 130.6, 129.7, 125.9, 122.4, 119.0, 103.4, 103.3, 103.1, 86.2, 85.7, 85.3, 75.9, 75.6, 75.3, 62.0, 50.5; HRMS (ESI$^+$): *m*/*z*, [M + H]$^+$ calcd for $C_{17}H_{17}F_2N_2O_7^+$, 399.0998; found, 399.0989.

*((2S,3R,5R)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,4,4-trifluoro-3-hydroxytetrahydrofuran-2-yl)methyl benzoate (**10b**)*. To an ice cooled solution of **9b** (460 mg, 0.927 mmol) in CH_2Cl_2 (27 mL) and H_2O (1 mL) was added m-CPBA (1.66 g, 7.42 mmol) under an Ar atmosphere. The mixture was heated at 40 °C and stirred for 5 h. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (Heptane/EtOAc = 2:1 to 3:2) to afford **10b** (213 mg, 59%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 8.10–8.06 (2H, m), 7.67-7.61 (1H, m), 7.53–

7.47 (3H, m), 6.29 (1H, br), 5.62 (1H, d, J = 8.1 Hz), 4.92 (1H, br), 4.78–4.60 (2H, m).; ^{13}C NMR (75 MHz, MeOH-d_4): δ 166.9, 165.2, 151.3, 142.5, 134.7, 130.8, 130.4, 129.7, 125.0, 121.6, 118.1, 115.7, 115.5, 112.6, 112.4, 103.7, 88.6, 74.0, 73.7, 73.3, 73.0, 63.3, 62.8; HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{16}H_{14}F_3N_2O_6^+$, 387.0798; found, 387.0798.

*((2R,3R,5R)-3-(Benzoyloxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4,4-difluoro-2-methoxytetrahydrofuran-2-yl)methyl benzoate (**11a**)*. To a solution of **10a** (496 mg, 1.24 mmol), Et_3N (0.86 mL, 6.20 mmol), and DMAP (30.0 mg, 0.248 mmol) in anhydrous THF (25 mL) was added dropwise BzCl (144 µL, 1.24 mmol) under a N_2 atmosphere, and the mixture was stirred for 30 min. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (Heptane/EtOAc = 2:1 to 3:2) to afford **11a** (406 mg, 65%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.7 (1H, br), 7.99 (1H, d, J = 8.1 Hz), 7.94–7.91 (2H, m), 7.74–7.68 (3H, m), 7.55–7.47 (3H, m), 7.22–7.17 (2H, m), 6.34–6.24 (2H, m), 5.80 (1H, d, J = 8.1 Hz), 4.82 (2H, s), 3.51 (3H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.6, 164.0, 162.5, 150.0, 141.3, 134.0, 133.3, 129.4, 128.9, 128.8, 128.5, 128.3, 127.7, 124.2, 120.8, 117.3, 102.5, 101.2, 101.1, 101.0, 84.3, 73.8, 73.5, 73.2, 61.3, 49.6; HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{24}H_{21}F_2N_2O_8^+$, 503.1260; found, 503.1241.

*((2S,3R,5R)-3-(Benzoyloxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,4,4-trifluorotetrahydrofuran-2-yl)methyl benzoate (**11b**)*. To a solution of **10b** (250 mg, 0.647 mmol), Et_3N (0.45 mL, 3.23 mmol), and DMAP (16 mg, 0.129 mmol) in anhydrous THF (15 mL) was added dropwise BzCl (75 µL, 0.647 mmol) under a N_2 atmosphere, and the mixture was stirred for 45 min. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (CH_2Cl_2/Methanol = 100:1) to afford **11b** (240 mg, 75%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 8.03–8.00 (2H, m), 7.90–7.83 (2H, m), 7.68–7.62 (2H, m), 7.51–7.44 (3H, m), 7.22 (2H, t, J = 7.8 Hz), 6.52–6.41 (2H, m), 5.75 (1H, d, J = 8.0 Hz), 4.81–4.69 (2H, m); ^{13}C NMR (75 MHz, MeOH-d_4): δ 166.7, 165.7, 165.2, 151.3, 143.6, 135.3, 134.5, 131.1, 130.6, 130.0, 129.8, 129.4, 129.2, 124.7, 121.3, 117.8, 114.9, 114.8, 111.7, 111.6, 103.9, 89.9, 73.4, 73.1, 64.6, 64.2; HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{23}H_{18}F_3N_2O_7^+$, 491.1060; found, 491.1054.

*1-((2R,4R,5R)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)-5-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**12a**)*. Compound **10a** (28 mg, 0.070 mmol) was dissolved in 7 N NH_3 in MeOH (3 mL) under a N_2 atmosphere, and the mixture was stirred overnight. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) to afford **12a** (14 mg, 68%) as a white solid. ^{1}H NMR (600 MHz, MeOH-d_4): δ 7.73 (1H, d, J = 4.0 Hz), 6.21–6.18 (1H, m), 5.74 (1H, d, J = 4.0 Hz), 4.47 (1H, t, J = 6.9 Hz), 3.97 (1H, d, J = 6.0 Hz), 3.65 (1H, d, J = 6.0 Hz), 3.43 (3H, s); ^{13}C NMR (151 MHz, MeOH-d_4): δ 165.5, 151.9, 141.5, 124.6, 122.8, 121.1, 105.6, 105.5, 103.2, 85.3, 85.2, 85.0, 84.9, 72.7, 72.6, 72.4, 59.6, 50.2; HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{10}H_{13}F_2N_2O_6^+$, 295.0736; found, 295.0741.

1-((2R,4R,5S)-3,3,5-Trifluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-

*2,4(1H,3H)-dione (**12b**)*. Compound **10b** (40 mg, 0.103 mmol) was dissolved in 7 N NH_3 in MeOH (5 mL) under a N_2 atmosphere, and the mixture was then stirred overnight. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) to afford **12b** (15 mg, 51%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.68 (1H, d, J = 8.1 Hz), 6.38 (1H, d, J = 11.9 Hz), 5.76 (1H, d, J = 8.1 Hz), 4.67–4.52 (1H, m), 3.78 (1H, d, J = 1.8 Hz); ^{13}C NMR (75 MHz, MeOH-d_4): δ 165.3, 151.7, 141.1, 125.3, 121.9, 118.4, 117.6, 117.4, 114.5, 114.3, 103.7, 86.9, 86.6, 86.4, 86.0, 71.8, 71.5, 71.2, 70.9, 60.7, 60.1; HRMS (ESI$^+$): *m*/*z*, $[M + H]^+$ calcd for $C_9H_{10}F_3N_2O_5^+$, 283.0536; found, 283.0535.

*4-Amino-1-((2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)-5-methoxytetrahydrofuran-2-yl)pyrimidin-2(1H)-one (**1a**)*. To an ice cooled solution of **11a** (268 mg, 0.533 mmol), Et_3N (1.48 mL, 10.7 mmol), and 1,2,4-triazole (552 mg, 8.00 mmol) in anhydrous MeCN (20 mL) was added dropwise $POCl_3$ (0.20 mL, 2.13 mmol) at 0 °C under a N_2 atmosphere, and the mixture was stirred for 1 h at 0 °C. After removal of all the volatiles *in vacuo*, the crude residue was filtered through a small pad of silica gel (Heptane/EtOAc = 1:1). The collected filtrate was concentrated *in vacuo* and dissolved in MeCN (20 mL), followed by the addition of 25% aq. NH_4OH (20 mL). The mixture was stirred for 30 min, and then was concentrated *in vacuo*. The residue was dissolved in 7 M NH_3 in methanol (20 mL) and the mixture was stirred overnight. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10:1 to 5:1) to afford **1a** (50 mg, 38%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.74 (1H, d, J = 7.5 Hz), 6.35–6.25 (1H, m), 5.93 (1H, d, J = 7.5 Hz), 4.43 (1H, t, J = 13.8 Hz), 3.98 (1H, d, J = 12.1 Hz), 3.64 (1H, d, J = 12.1 Hz), 3.43 (3H, s); ^{13}C NMR (75 MHz, MeOH-d_4): δ 167.7, 157.8, 142.2, 122.9, 119.5, 105.3, 105.2, 96.5, 86.2, 85.9, 85.7, 85.4, 73.1, 72.8, 72.5, 59.7, 50.1; HRMS (ESI$^+$): *m*/*z*, $[M + H]^+$ calcd for $C_{10}H_{14}F_2N_3O_5^+$, 294.0895; found, 294.0900.

*4-Amino-1-((2R,4R,5S)-3,3,5-trifluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1H)-one (**1b**)*. To an ice cooled solution of **11b** (240 mg, 0.489 mmol), Et_3N (1.36 mL, 9.78 mmol), and 1,2,4-triazole (507 mg, 7.34 mmol) in anhydrous MeCN (15 mL) was added dropwise $POCl_3$ (0.18 mL, 1.96 mmol) at 0 °C under a N_2 atmosphere, and the mixture was stirred for 1 h at 0 °C. After removal of all the volatiles *in vacuo*, the crude residue was filtered through a small pad of silica gel (Heptane/EtOAc = 1:1). The collected filtrate was concentrated *in vacuo* and the residue was dissolved in MeCN (15 mL), followed by the addition of 25% aq. NH_4OH (15 mL). The mixture was stirred for 30 min, and then concentrated *in vacuo*. The residue was redissolved in 7 M NH_3 in methanol (15 mL) and the mixture was stirred overnight. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10:1 to 5:1) to afford **1b** (78 mg, 56%) as a white solid. ^{1}H NMR (600 MHz, MeOH-d_4): δ 7.68 (1H, d, J = 3.7 Hz), 6.45 (1H, br), 5.95 (1H, d, J = 3.8 Hz), 4.60–4.54 (1H, m), 3.82–3.76 (2H, m); ^{13}C NMR (126 MHz, MeOH-d_4): δ 167.7, 157.6, 141.6, 124.0, 121.9, 119.9, 116.9, 116.8, 115.0, 114.9, 97.0, 96.9, 86.9, 71.4, 71.2, 60.5, 60.2; HRMS

(ESI$^+$): *m*/*z*,. [M + H]$^+$ calcd for $C_9H_{11}F_3N_3O_4^+$, 282.06 96; found, 282.0703.

*(2R,3R,5R)-5-(4-Amino-2-oxopyrimidin-1(2H)-yl)-4,4-difluoro-2-(hydroxymethyl)-2-methoxytetrahydrofuran-3-yl tert-butyl carbonate (**13a**)*. To a solution of **1a** (50 mg, 0.170 mmol) and Na_2CO_3 (90 mg, 0.852 mmol) in dioxane/H_2O (4/1, 5 mL) was added DBDC (37 mg, 0.170 mmol) under an Ar atmosphere, and the mixture was then stirred for 72 h. Next, 2 mL of water was added, and the mixture was extracted with EtOAc. 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 (CH_2Cl_2/MeOH = 10:1) to afford **13a** (24 mg, 35%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.74 (1H, d, J = 7.5 Hz), 6.35 (1H, t, J = 7.8 Hz), 5.95 (1H, d, J = 7.5 Hz), 5.44 (1H, t, J = 11.9 Hz), 3.95 (1H, d, J = 12.2 Hz), 3.69 (1H, d, J = 12.1 Hz), 3.43 (3H, s), 1.50 (9H, s); ^{13}C NMR (75 MHz, MeOH-d_4): δ 167.7, 157.7, 153.3, 142.4, 125.5, 122.0, 118.6, 105.0, 96.7, 86.1, 85.7, 85.3, 84.9, 74.9, 74.6, 74.3, 60.7, 50.3, 27.8; HRMS (ESI$^+$): *m*/*z*, [M + H]$^+$ calcd for $C_{15}H_{22}F_2N_3O_7^+$, 394.1420; found, 394.1412.

*(2S,3R,5R)-5-(4-Amino-2-oxopyrimidin-1(2H)-yl)-2,4,4-trifluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl tert-butyl carbonate (**13b**)*. To a solution of **1b** (78 mg, 0.277 mmol) and Na_2CO_3 (147 mg, 1.39 mmol) in dioxane/water (4/1, 5 mL) was added DBDC (61 mg, 0.277 mmol) under an Ar atmosphere, and the mixture was stirred for 48 h. Next, 2 mL of water was added, and the mixture was extracted with EtOAc. 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 (CH_2Cl_2/MeOH = 20:1 to 10:1) to afford **13b** (25 mg, 23%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.67 (1H, d, J = 7.6 Hz), 6.48 (1H, br), 5.97 (1H, d, J = 7.7 Hz), 5.64 (1H, br), 3.87–3.78 (2H, m), 1.51 (9H, s); ^{13}C NMR (75 MHz, MeOH-d_4): δ 167.8, 157.3, 153.0, 142.3, 124.5, 121.0, 117.6, 116.4, 116.3, 116.2, 113.2, 113.1, 113.0, 97.1, 97.1, 87.8, 85.5, 73.7, 73.4, 73.1, 72.8, 61.7, 61.2, 27.7; HRMS (ESI$^+$): *m*/*z*, [M + H]$^+$ calcd for $C_{14}H_{19}F_3N_3O_6^+$, 382.1220; found, 382.1196.

*(2R,3R,5R)-5-(4-Amino-2-oxopyrimidin-1(2H)-yl)-4,4-difluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl tert-butyl carbonate (**13c**)*. To a solution of gemcitabine (44 mg, 0.167 mmol) and Na_2CO_3 (88 mg, 0.835 mmol) in dioxane (3 mL) and H_2O (0.75 mL) was added DBDC (37 mg, 0.167 mmol) under an Ar atmosphere, and the mixture was stirred for 72 h. Next, 2 mL of water was added, and the mixture was then extracted with EtOAc. 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 (CH_2Cl_2/MeOH = 20:1 to 10:1) to afford **13c** (36 mg, 59%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.80 (1H, d, J = 7.6 Hz), 6.28 (1H, t, J = 8.8 Hz), 5.94 (1H, d, J = 7.6 Hz), 5.26–5.18 (1H, m), 4.17–4.12 (1H, m), 3.93 (1H, dd, J_1 = 12.8 Hz, J_2 = 2.8 Hz), 3.77 (1H, dd, J_1 = 12.8 Hz, J_2 = 3.4 Hz), 1.49 (9H, s); ^{13}C NMR (75 MHz, MeOH-d_4): δ 167.6, 157.5, 153.2, 142.8, 126.1, 122.7, 119.2, 96.5, 86.2, 85.9, 85.7, 85.4, 84.9, 80.7, 80.6, 73.9, 73.7, 73.5, 73.3, 60.7, 27.8; HRMS (ESI$^+$): *m*/*z*, [M + H]$^+$ calcd for $C_{14}H_{20}F_2N_3O_6^+$, 364.1314; found, 364.1329.

*Benzyl (chloro(phenoxy)phosphoryl)-L-alaninate (**15**).* To an ice cooled solution of **14** (200 mg, 0.927 mmol) and aryl dichlorophosphate (138 µL, 0.927 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise anhydrous triethylamine (0.26 mL, 1.85 mmol) at -78 °C under an Ar atmosphere, and the mixture was stirred for 1 h at -78 °C. It was then allowed to slowly reach room temperature and further stirred overnight. After removal of all the volatiles *in vacuo*, the crude residue was redissolved in anhydrous Et_2O and filtered. The filtrate was dried under reduced pressure to afford **15** (330 mg) as an oil, which was used without further purification in the next step. ^{31}P NMR (121 MHz, $CDCl_3$) δ 8.60, 8.36 (int. 1:1).

*Benzyl ((((2R,3R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3-((tert-butoxycarbonyl)oxy)-4,4-difluoro-2-methoxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**16a**)*. To a solution of **13a** (24 mg, 0.061 mmol) in anhydrous THF (2.0 mL) was added dropwise 1.7 M tert-butylmagnesium chloride in THF (0.07 mL, 0.122 mmol) followed by a solution of **15** (65 mg) in anhydrous THF (1.0 mL) under an Ar atmosphere, and the mixture was then stirred overnight. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1 to 10:1) to afford **16a** (24 mg, 55%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.55–7.20 (11H, m), 6.32 (1H, br), 5.92, 5.83 (1H, 2d, J = 7.4 Hz), 5.49–5.37 (1H, m), 5.16 (2h, s), 4.48–4.41 (1H, m), 4.38–4.23 (1H, m), 4.08–4.02 (1H, m), 3.40, 3.39 (3H, 2s), 1.47, 1.45 (9H, 2s), 1.39 (3H, d, J = 7.1 Hz); ^{13}C NMR (75 MHz, MeOH-d_4): δ 174.9, 174.8, 174.7, 174.4, 167.6, 167.6, 157.4, 153.3, 152.0, 151.9, 142.7, 142.4, 137.2, 130.9, 130.8, 129.6, 129.3, 129.3, 129.2, 126.4, 126.3, 125.1, 125.0, 121.7, 121.5, 121.4, 118.0, 103.1, 103.0, 102.9, 102.8, 102.7, 96.9, 86.1, 85.7, 85.2, 75.9, 75.7, 75.6, 75.4, 75.3, 75.1, 68.1, 68.0, 64.9, 51.7, 51.6, 50.7, 50.6, 27.8, 20.4, 20.3, 20.2; ^{31}P NMR (121 MHz, MeOH-d_4): δ 3.48, 3.31 (int. 1:1); HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{31}H_{38}F_2N_4O_{11}P_1^+$, 711.2237; found, 711.2240.

*Benzyl ((((2S,3R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3-((tert-butoxycarbonyl)oxy)-2,4,4-trifluorotetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**16b**)*. To a solution of **13b** (25 mg, 0.065 mmol) in anhydrous THF (2.0 mL) was added dropwise 1.7 M tert-butylmagnesium chloride in THF (0.07 mL, 0.072 mmol) followed by a solution of **15** (66 mg) in anhydrous THF (1.5 mL) under an Ar atmosphere, and the mixture was then stirred overnight. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1 to 10:1) to afford **16b** (30 mg, 66%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.47, 7.44 (1H, 2d, J = 7.5 Hz), 7.36–7.29 (7H, m), 7.25–7.16 (3H, m), 6.39 (1H, brs), 5.91, 5.87 (1H, 2d, J = 7.5 Hz), 5.78 (1H, br), 5.14 (1H, s), 4.45–4.35 (2H, m), 4.08–3.98 (1H, m), 1.48, 1.46 (9H, 2s), 1.37 (3H, d, J = 7.1 Hz); ^{13}C NMR (75 MHz, MeOH-d_4): δ 174.8, 174.7, 174.5, 174.4, 167.9, 167.8, 157.1, 153.0, 151.9, 151.8, 143.2, 137.1, 130.8, 130.7, 129.5, 129.3, 129.2, 126.3, 124.3, 124.2, 121.5, 121.4, 121.4, 121.3, 120.84, 120.76, 117.32, 114.47, 114.41, 114.39, 114.34, 114.28, 111.4, 111.3, 111.2, 111.1, 97.3, 89.1, 85.8, 85.7, 74.7, 74.6, 74.4, 74.1, 73.8, 73.7, 68.1, 68.0, 65.9, 65.4, 51.7, 51.6, 27.8, 20.4,

20.3, 20.2; ^{31}P NMR (121 MHz, MeOH-d_4): δ 3.48, 3.31 (int. 1:1). HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{30}H_{35}F_3N_4O_{10}P_1^+$, 699.2037; found, 699.2013.

*Benzyl ((((2R,3R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3-((tert-butoxycarbonyl)oxy)-4,4-difluorotetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**16c**)*. To a solution of **13c** (34 mg, 0.093 mmol) in anhydrous THF (2.0 mL) was added dropwise 1.7 M tert-butylmagnesium chloride in THF (0.11 mL, 0.187 mmol) followed by a solution of **15** (99 mg) in anhydrous THF (1.0 mL) under an Ar atmosphere, and the mixture was then stirred overnight. After removal of all the volatiles *in vacuo*, the crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1 to 10:1) to afford **16c** (22 mg, 34%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.55, 7.42 (1H, 2d, J = 7.4 Hz), 7.43–7.30 (7H, m), 7.24–7.18 (3H, m), 6.28 (1H, q, J = 8.8 Hz), 5.89, 5.82 (1H, 2d, J = 7.5 Hz), 5.23–5.10 (3H, m), 4.43–4.26 (3H, m), 4.06–3.99 (1H, m), 1.49, 1.48 (9H, 2s, 1.37 (3H, t, J = 6.2 Hz); ^{13}C NMR (75 MHz, MeOH-d_4): δ 174.8, 174.7, 174.5, 174.4, 167.7, 167.6, 157.5, 157.4, 153.1, 153.0, 152.1, 152.0, 142.7, 137.2, 130.9, 130.8, 129.6, 129.4, 129.3, 126.3, 126.2, 125.8, 125.7, 122.3, 122.2, 121.5, 121.4, 121.4, 121.3, 118.8, 118.7, 96.8, 96.7, 86.2, 86.1, 85.9, 85.8, 85.4, 85.3, 85.2, 78.7, 78.6, 74.2, 74.0, 73.8, 73.5, 68.0, 65.7, 65.6, 51.8, 51.7, 27.8, 20.3, 20.2, 20.1; ^{31}P NMR (121 MHz, MeOH-d_4): δ 3.78, 3.57 (int. 1:1); HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{30}H_{36}F_2N_4O_{10}P_1^+$, 681.2131; found, 681.2123.

*Benzyl ((((2R,3R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-4,4-difluoro-3-hydroxy-2-methoxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**2a**)*. Compound **16a** (24 mg, 0.033 mmol) was dissolved in TFA/CH_2Cl_2 (1:1, 1 mL) at 0 °C under an Ar atmosphere, and the mixture was stirred for 1 h at 0 °C. It was then concentrated *in vacuo* and the residue was dissolved in CH_2Cl_2, washed with saturated aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1 to 10:1) to afford **2a** (10 mg, 49%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.47–7.30 (8H, m), 7.27–7.20 (3H, m), 6.27 (1H, br), 5.90, 5.81 (1H, d, J = 7.5 Hz), 5.16, 5.15 (1H, 2s), 4.54–4.43 (1H, m), 4.41–4.32 (1H, m), 4.29–4.17 (1H, m), 4.10–3.98 (1H, m), 3.43, 3.42 (3H, 2s), 1.37 (3H, t, J = 6.3 Hz); ^{13}C NMR (75 MHz, MeOH-d_4): δ 175.0, 174.9, 174.6, 174.5, 167.7, 167.6, 157.7, 157.6, 152.2, 152.1, 152.0, 151.9, 142.1, 137.3, 137.2, 130.9, 130.8, 129.6, 129.4, 129.3, 129.2, 126.3, 125.8, 125.7, 122.4, 122.3, 121.4, 121.3, 118.9, 118.8, 103.3, 103.2, 103.1, 102.9, 96.9, 96.8, 86.4, 86.3, 85.9, 85.8, 85.5, 85.4, 74.3, 74.0, 73.7, 68.1, 68.0, 64.2, 64.1, 64.0, 51.8, 51.7, 50.5, 20.4, 20.3, 20.2, 20.1; ^{31}P NMR (121 MHz, MeOH-d_4): δ 3.69, 3.44 (int. 1:1); HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{25}H_{27}F_3N_4O_8P_1^+$, 599.1512; found, 599.1513.

*Benzyl ((((2S,3R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-2,4,4-trifluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**2b**)*. Compound **16b** (30 mg, 0.042 mmol) was dissolved in TFA/CH_2Cl_2 (1:1, 1 mL) at 0 °C under an Ar atmosphere, and the mixture was stirred for 1 h at 0 °C. It was then concentrated *in vacuo* and the residue was dissolved in CH_2Cl_2, washed with

saturated aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1 to 10:1) to afford **2b** (14 mg, 54%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.40–7.17 (11H, m), 6.44 (1H, br), 5.90, 5.85 (1H, 2d, J = 7.6 Hz), 5.19–5.13 (2H, m), 4.61 (1H, br), 4.41–4.33 (2H, m), 4.09–3.98 (1H, m), 1.36 (3H, t, J = 6.3 Hz).; ^{13}C NMR (75 MHz, MeOH-d_4): δ 174.8, 174.7, 174.6, 174.5, 167.7, 157.4, 157.3, 152.0, 151.9, 142.1, 137.1, 130.8, 129.5, 129.3, 129.2, 126.4, 124.8, 121.4, 121.3, 115.5, 115.4, 115.2, 112.4, 112.3, 112.2, 97.2, 87.9, 73.1, 72.8, 72.5, 72.3, 71.9, 68.0, 64.8, 64.7, 64.2, 64.1, 51.8, 51.6, 20.4, 20.3, 20.2, 20.1; ^{31}P NMR (121 MHz, MeOH-d_4): δ 3.57, 3.36 (int. 1:1); HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{26}H_{30}F_2N_4O_9P_1^+$, 611.1712; found, 611.1703.

*Benzyl ((((2R,3R,5R)-5-(4-Amino-2-oxopyrimidin-1(2H)-yl)-4,4-difluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**2c**)*. Compound **16c** (22 mg, 0.032 mmol) was dissolved in TFA/CH_2Cl_2 (1:1, 1 mL) at 0 °C under an Ar atmosphere, and the mixture was stirred for 1.5 h at 0 °C. It was then concentrated *in vacuo*, dissolved in DCM, washed with saturated aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1 to 10:1) to afford **2c** (6 mg, 32%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 7.56, 7.50 (1H, 2d, J = 7.5 Hz), 7.37–7.31 (7H, m), 7.25–7.19 (3H, m), 6.27–6.20 (1H, m), 5.87, 5.83 (1H, 2d, J = 7.4 Hz), 5.15–5.13 (2H, m), 4.27–4.49 (2H, m), 4.25–4.13 (1H, m), 4.07–3.96 (2H, m), 1.35 (3H, t, J = 6.4 Hz); ^{13}C NMR (75 MHz, MeOH-d_4): δ 174.8, 174.7, 174.6, 174.5, 167.5, 157.6, 157.5, 152.1, 152.0, 142.5, 142.4, 137.2, 130.9, 130.8, 129.6, 129.4, 129.3, 129.2, 126.9, 126.8, 126.2, 123.4, 123.4, 121.4, 121.3, 120.0, 120.0, 96.6, 86.4, 86.0, 85.5, 80.4, 71.7, 71.4, 71.0, 70.8, 68.1, 65.8, 65.7, 65.6, 65.5, 51.8, 51.7, 20.4, 20.3, 20.2, 20.1; ^{31}P NMR (121 MHz, MeOH-d_4): δ 3.83, 3.66 (int. 1:1); HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{25}H_{28}F_2N_4O_8P_1^+$, 581.1607; found, 581.1609.

VZV and HCMV antiviral assay

The compounds were investigated against the following viruses: varicella-zoster virus (VZV) strain Oka, TK− VZV strain 07−1, cytomegalovirus (HCMV) strains AD-169 and Davis. The antiviral assays are based on the inhibition of virus-induced cytopathicity (CMV) or plaque formation (VZV) in human embryonic lung (HEL) fibroblasts. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) (VZV). After adsorption for 2 h, the viral inoculum was removed and the cultures were further incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation was recorded after 5 (VZV) or 6−7 (CMV) days post-infection. Antiviral activity was expressed as the EC_{50} or compound concentration required inhibiting virus-induced cytopathicity or viral plaque formation by 50%.

The cytostatic activity measurements were based on the inhibition of cell growth. HEL cells were

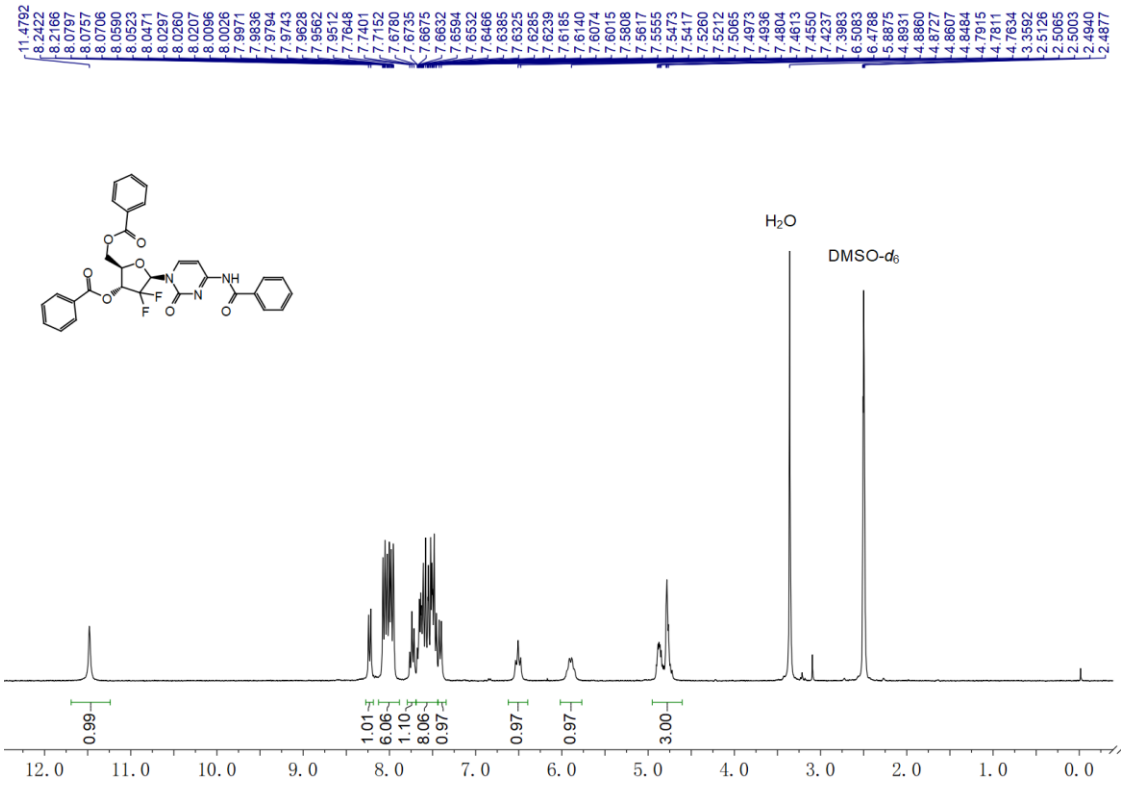
seeded into 96-well microtiter plates at a rate of 5×10^3 cells/well and proliferated for 24 h. Then, medium containing the test compounds at different concentrations was added. After 3 days of incubation at 37 °C, the cell number was determined using a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Alternatively, cytotoxicity of the test compounds was expressed as the minimum cytotoxic concentration (MCC) or compound concentration that causes a microscopically detectable alteration of cell morphology.

SARS-CoV-2 antiviral assay

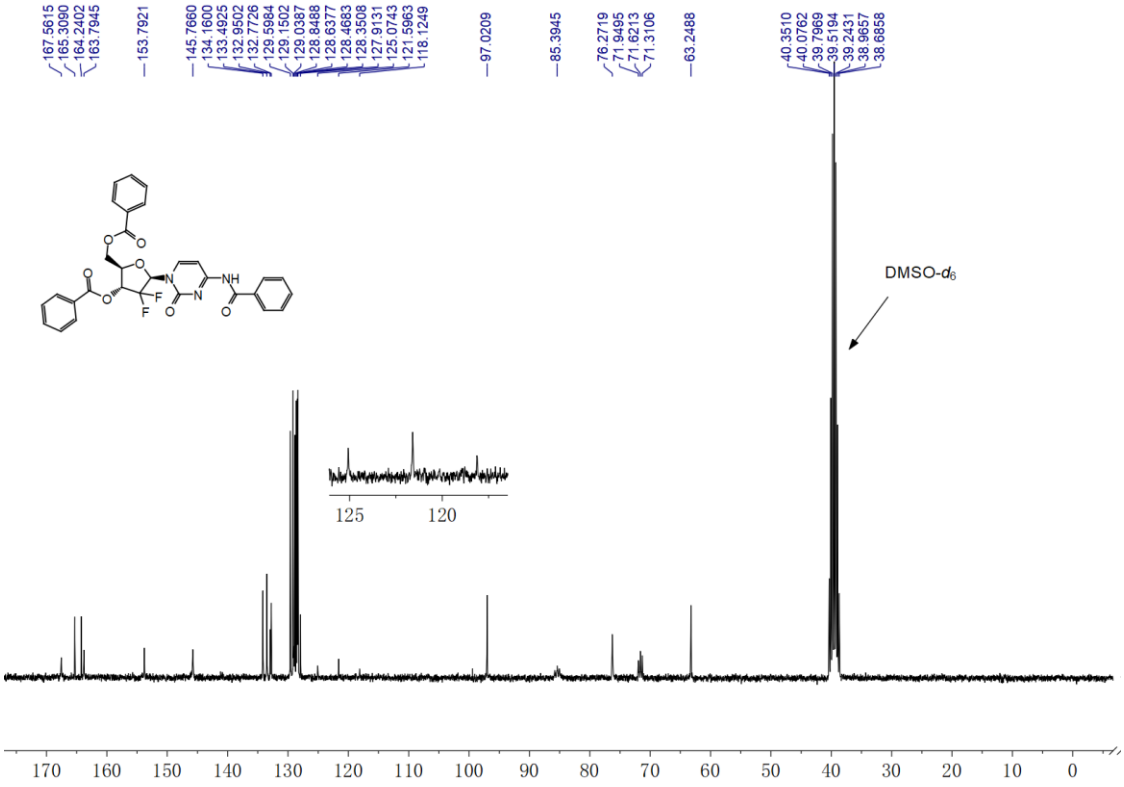
Vero cells (ATCC-CCL81) were grown in Dulbecco's Modified Eagle's Medium (DMEM, ThermoFisher, Belgium) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, and 10 mM HEPES at 37 °C in a 5% CO_2 humidified atmosphere. The SARS-CoV-2 strains UC-1074 and UC-1075 were isolated from the nasopharyngeal swabs of two COVID-19 patients that had a Ct of 19 and 22, respectively, for the detection of the SARS-CoV-2 E protein by real-time reverse transcription PCR (RT-qPCR). The infectious virus titer of the clinical isolates was determined in Vero cells and expressed as 50% tissue culture infectious dose ($TCID_{50}$) per mL. The titers of the viral stocks were 1.58E+06 (UC-1074) and 1.08E+04 (UC-1075) $TCID_{50}$/mL. For the antiviral assays, Vero cells were seeded in 96-well plates at a density of 1×10^4 cells per well in DMEM 10% FCS medium. After 24 h growth, the medium was removed and cells were treated with different compound concentrations and mocked-infected or SARS-CoV-2-infected with about 100 $TCID_{50}$/well (final volume 200 µL/well in DMEM 2% FCS). On day 5 post-infection, viral CPE was recorded microscopically and the 50% effective concentration (EC_{50}) was calculated. In parallel, the cytotoxic effects of the compounds were assessed by evaluating the MCC (minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology). The effects of the compounds on cell growth were determined by counting the number of cells with a Coulter counter in mock-infected cultures and expressed as the cytostatic concentration required to reduce cell growth by 50% (CC_{50}). All SARS-CoV-2-related work was conducted in the BSL3 facilities of the KU Leuven Rega Institute (3CAPS) according to institutional guidelines.

NMR Spectra

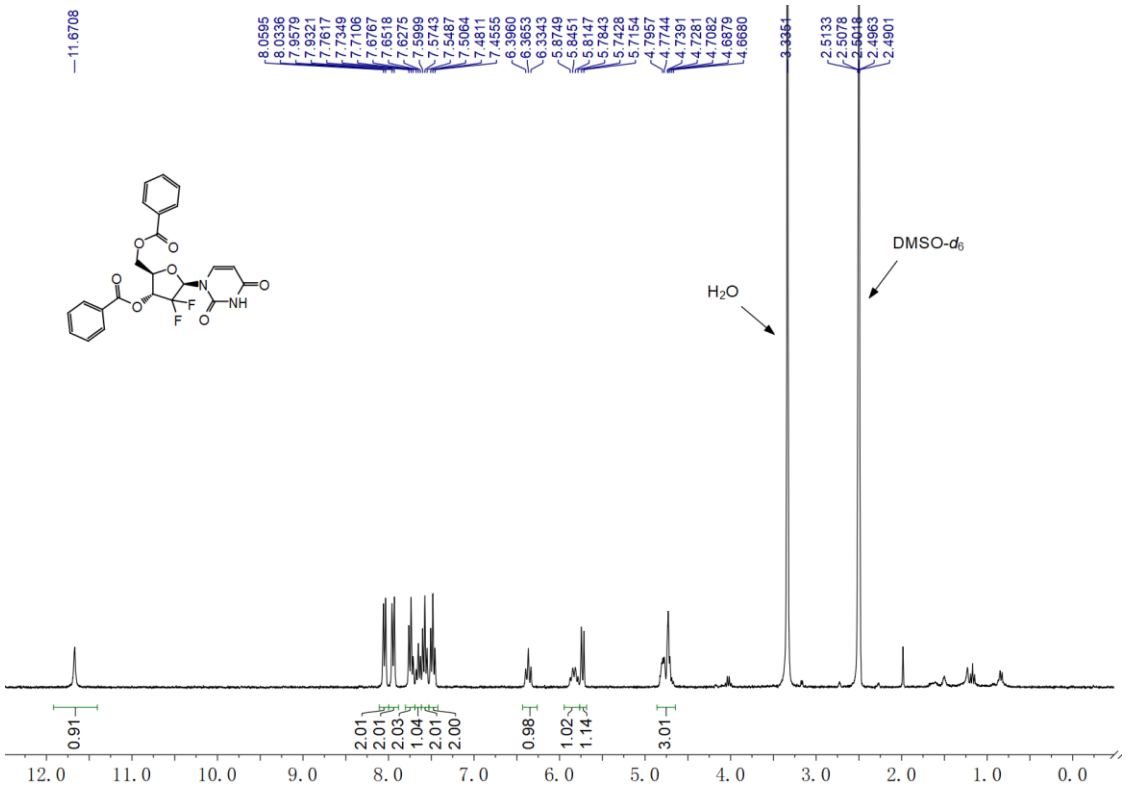
NMR spectra of compound **3** in DMSO-d_6, ^{1}H NMR spectrum, 300MHz



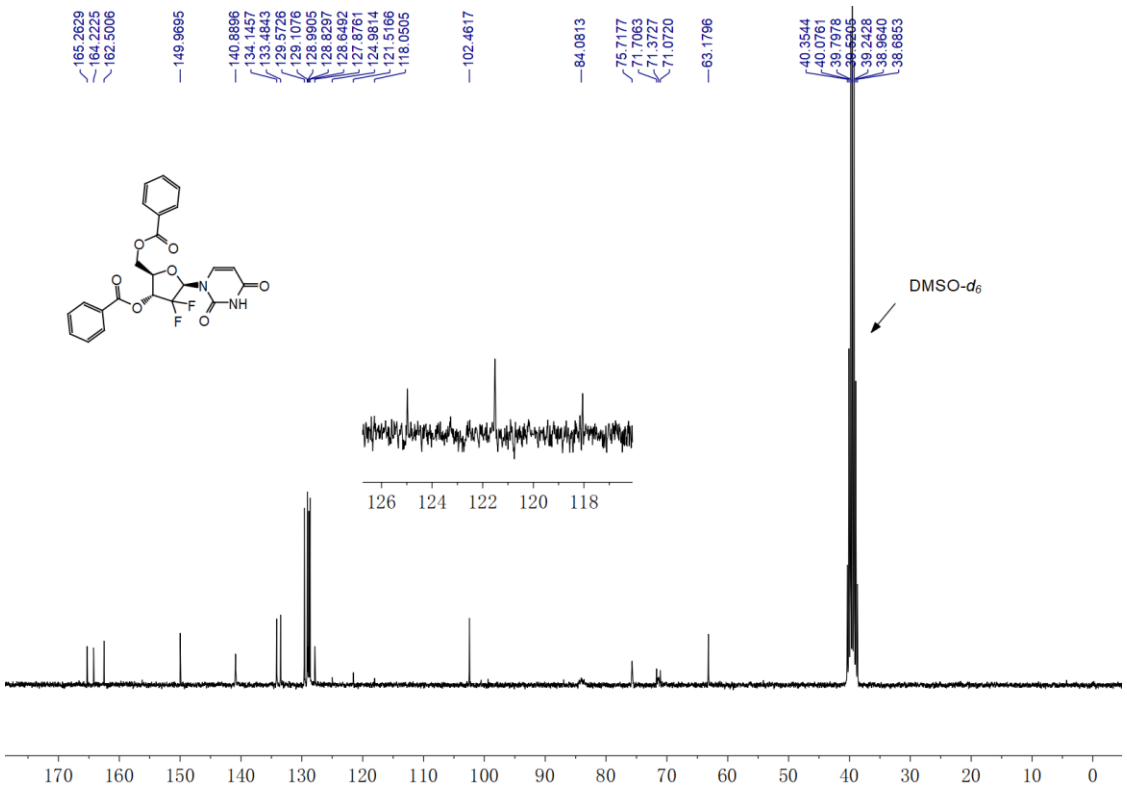
NMR spectra of compound **3** in DMSO-d_6, ^{13}C NMR spectrum, 75MHz



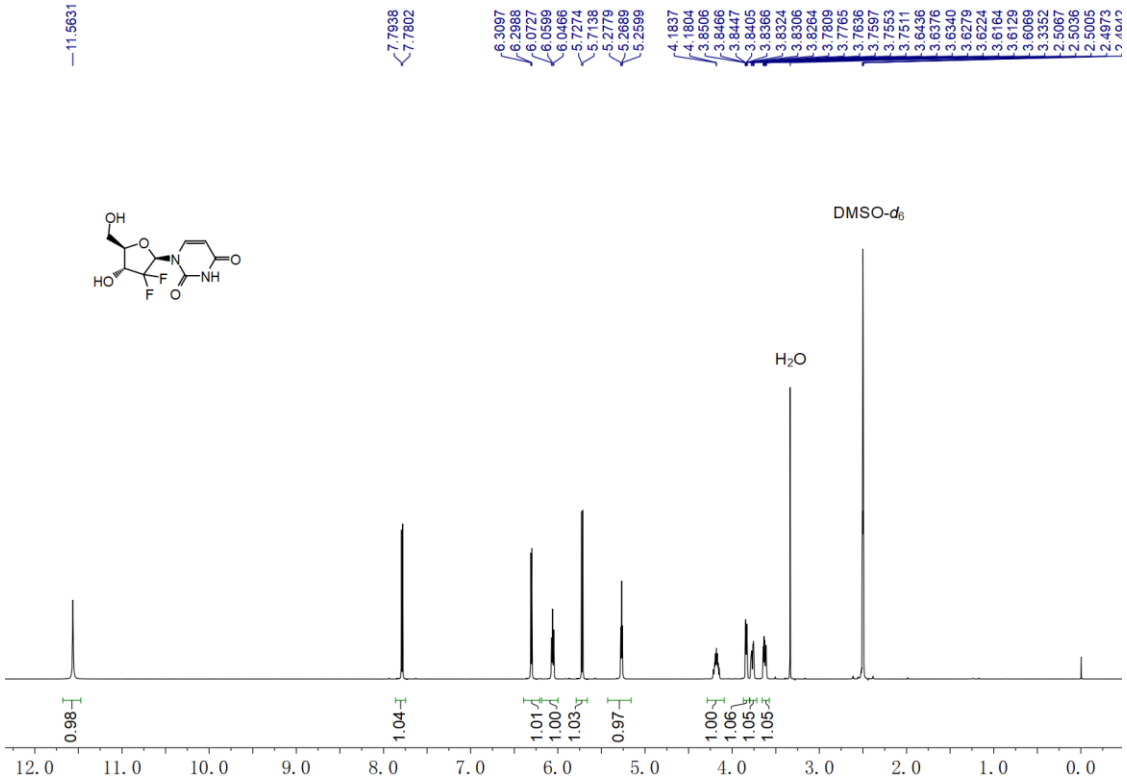
NMR spectra of compound **4** in DMSO-d_6, ^{1}H NMR spectrum, 300MHz



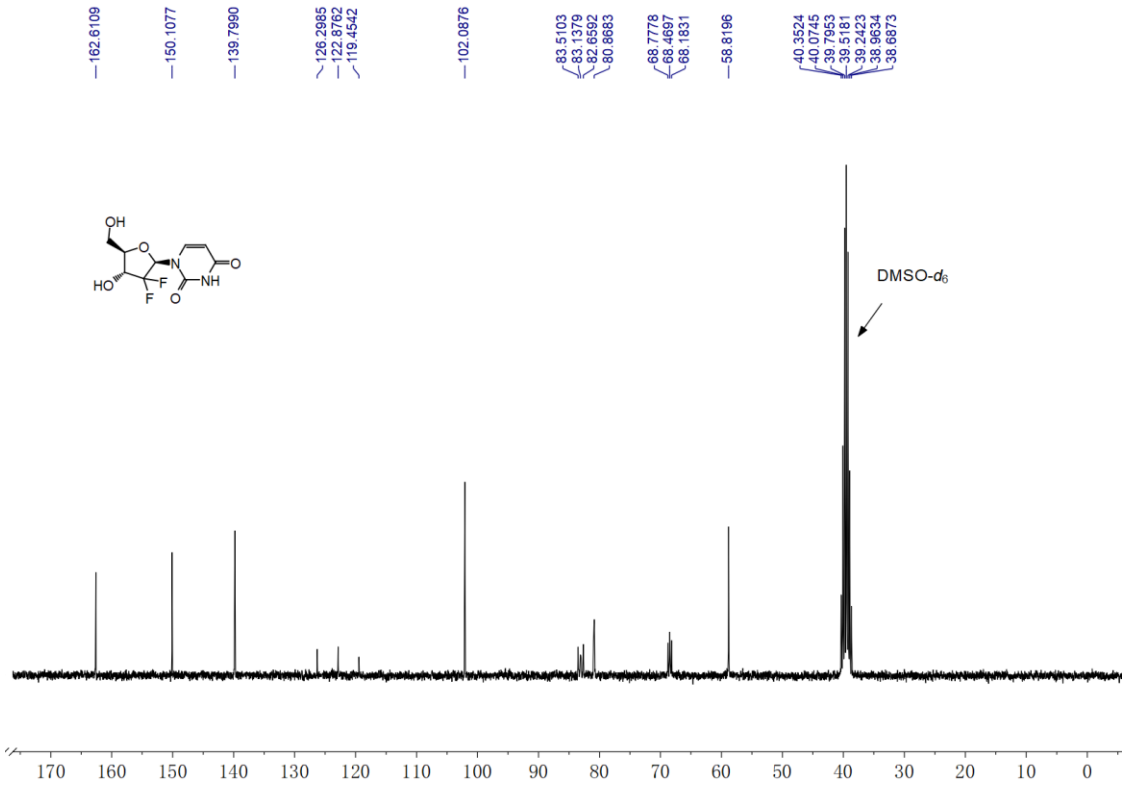
NMR spectra of compound **4** in DMSO-d_6, ^{13}C NMR spectrum, 75MHz



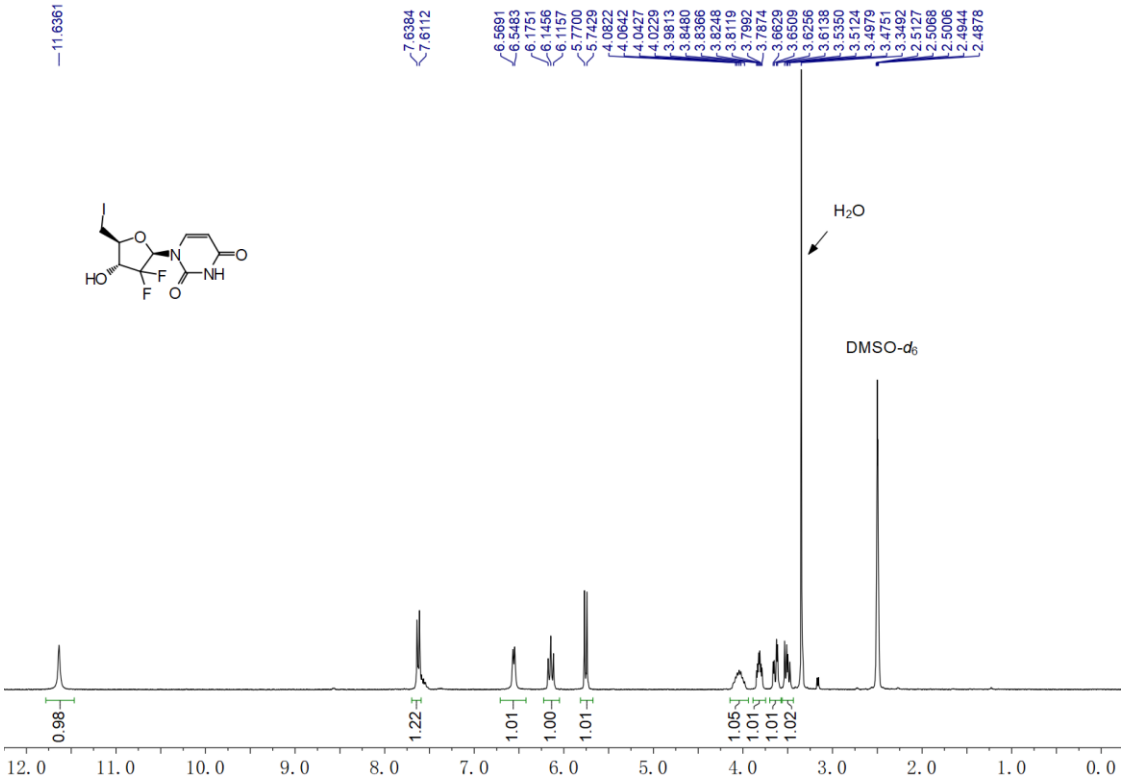
NMR spectra of compound **5** in DMSO-d_6, ^{1}H NMR spectrum, 600MHz



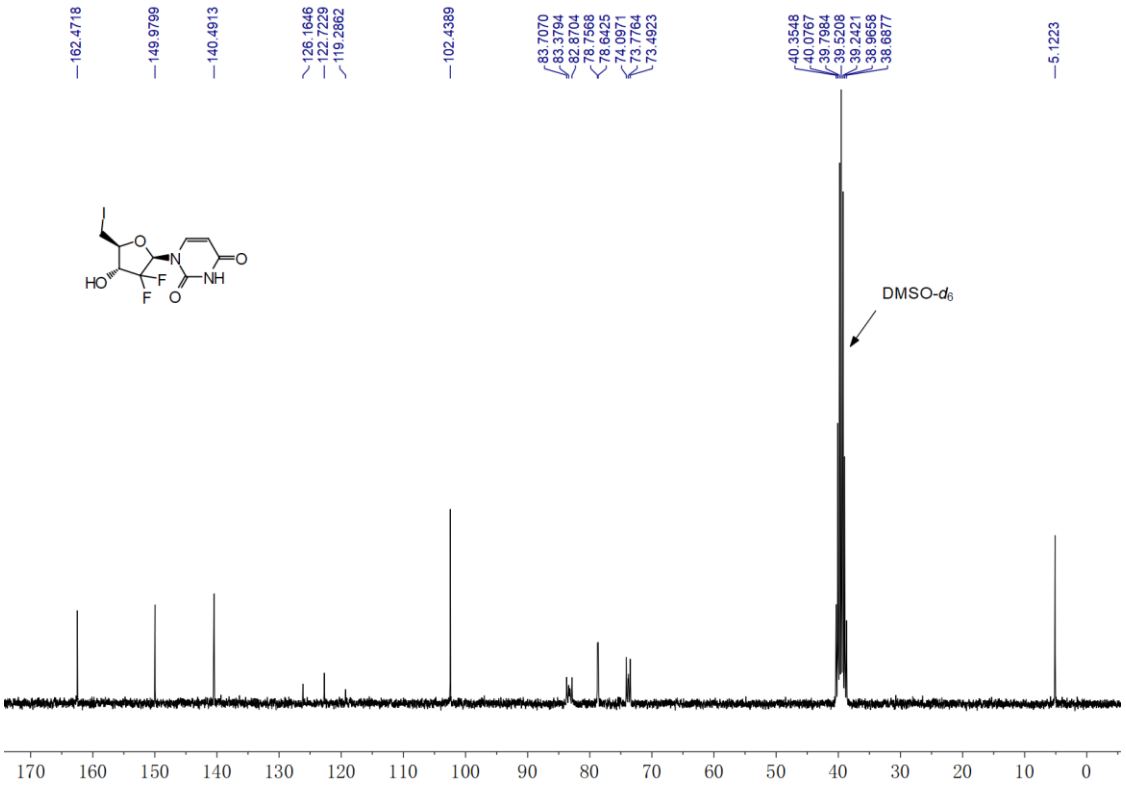
NMR spectra of compound **5** in DMSO-d_6, ^{13}C NMR spectrum, 151MHz



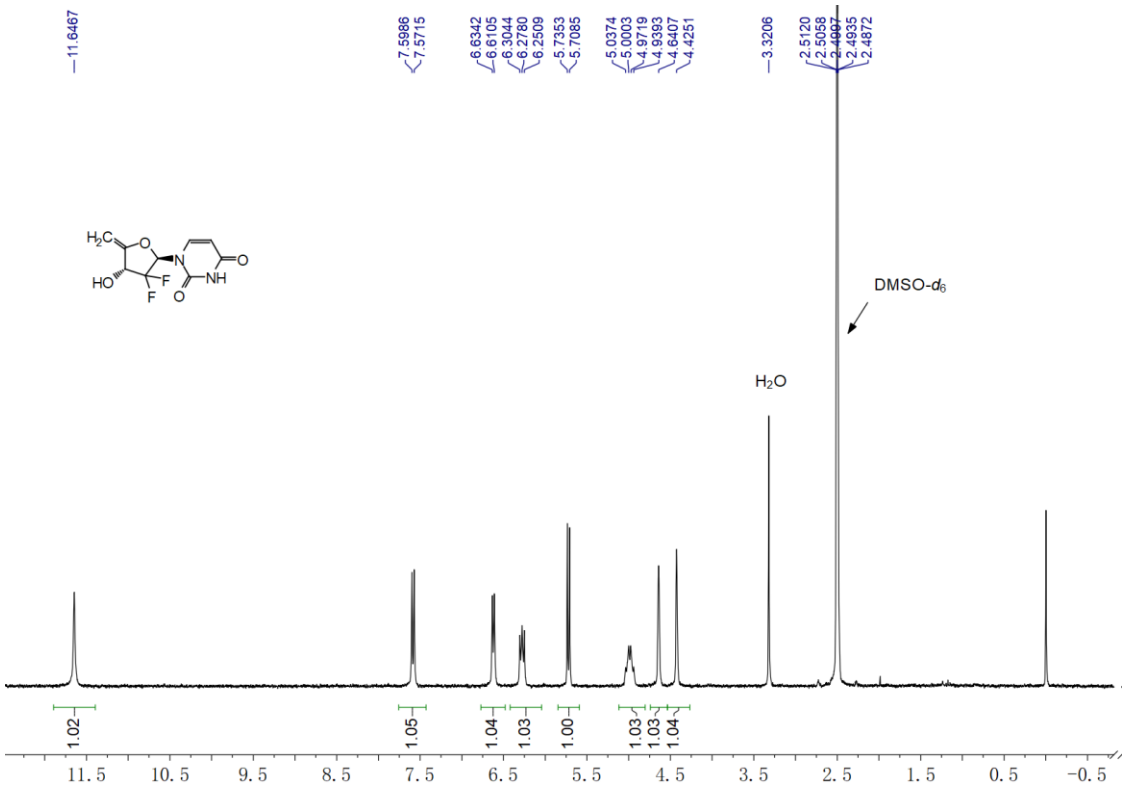
NMR spectra of compound **6** in DMSO-d_6, ^{1}H NMR spectrum, 300MHz



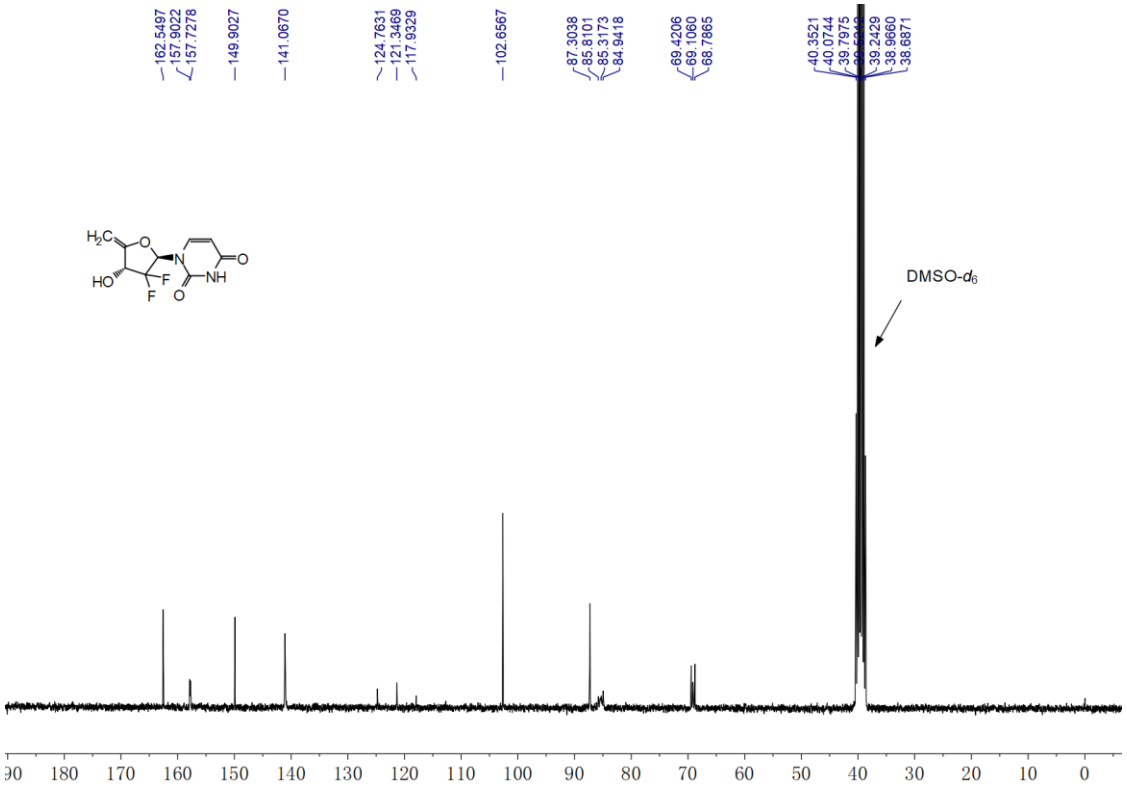
NMR spectra of compound **6** in DMSO-d_6, ^{13}C NMR spectrum, 75MHz



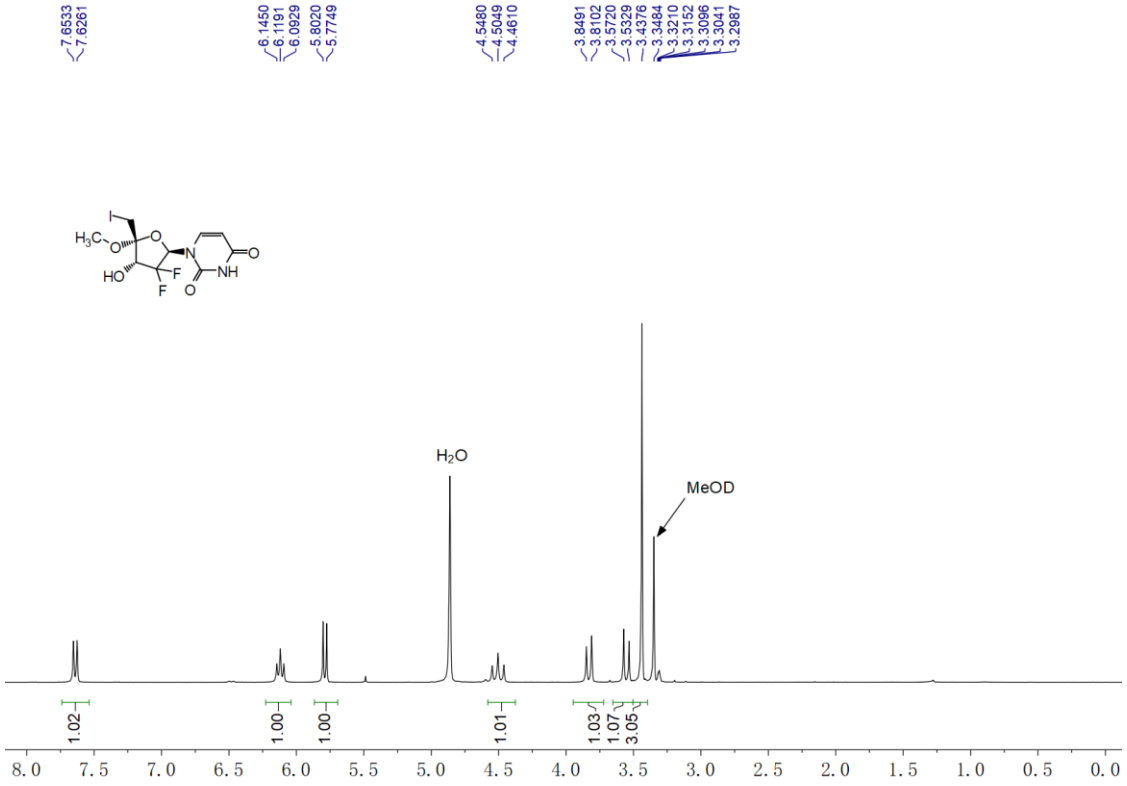
NMR spectra of compound **7** in DMSO-d_6, ^{1}H NMR spectrum, 300MHz



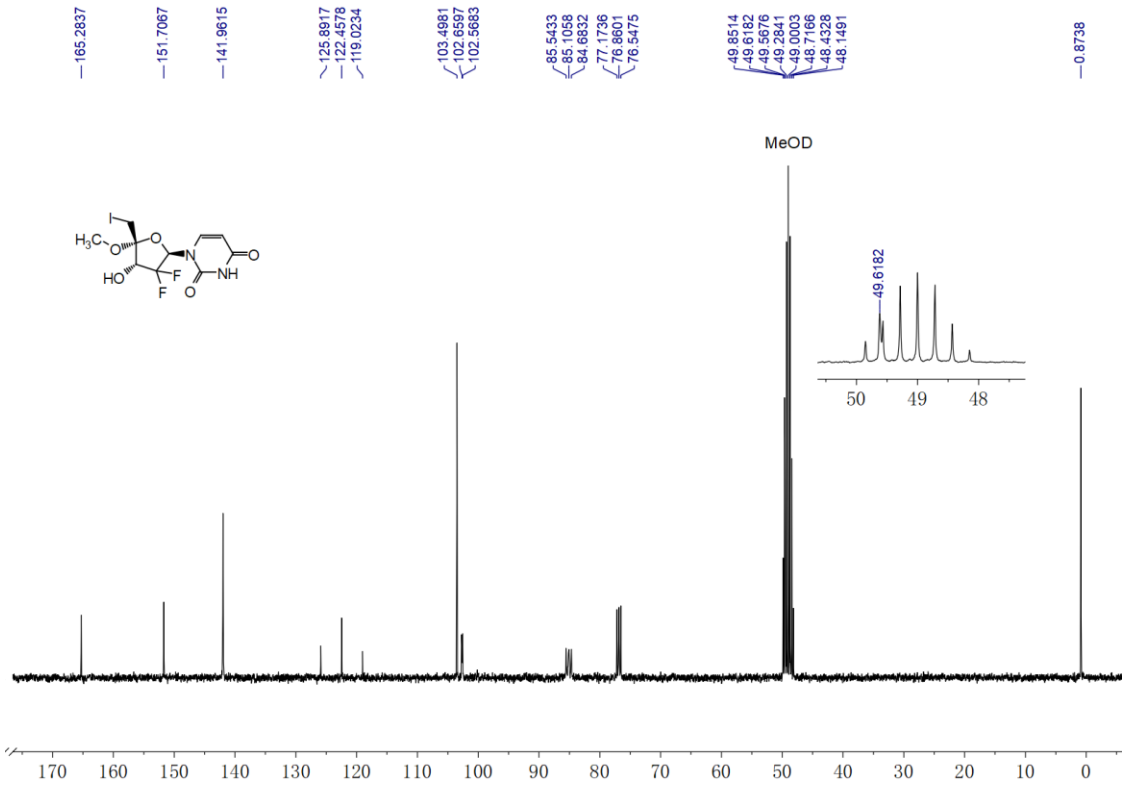
NMR spectra of compound **7** in DMSO-d_6, ^{13}C NMR spectrum, 75MHz



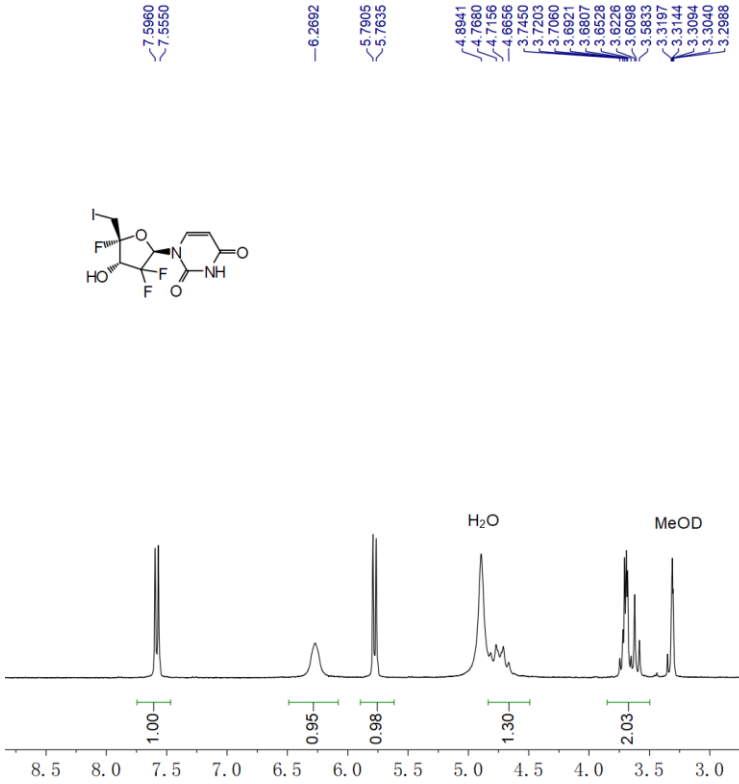
NMR spectra of compound **8a** in MeOD, ^{1}H NMR spectrum, 300MHz



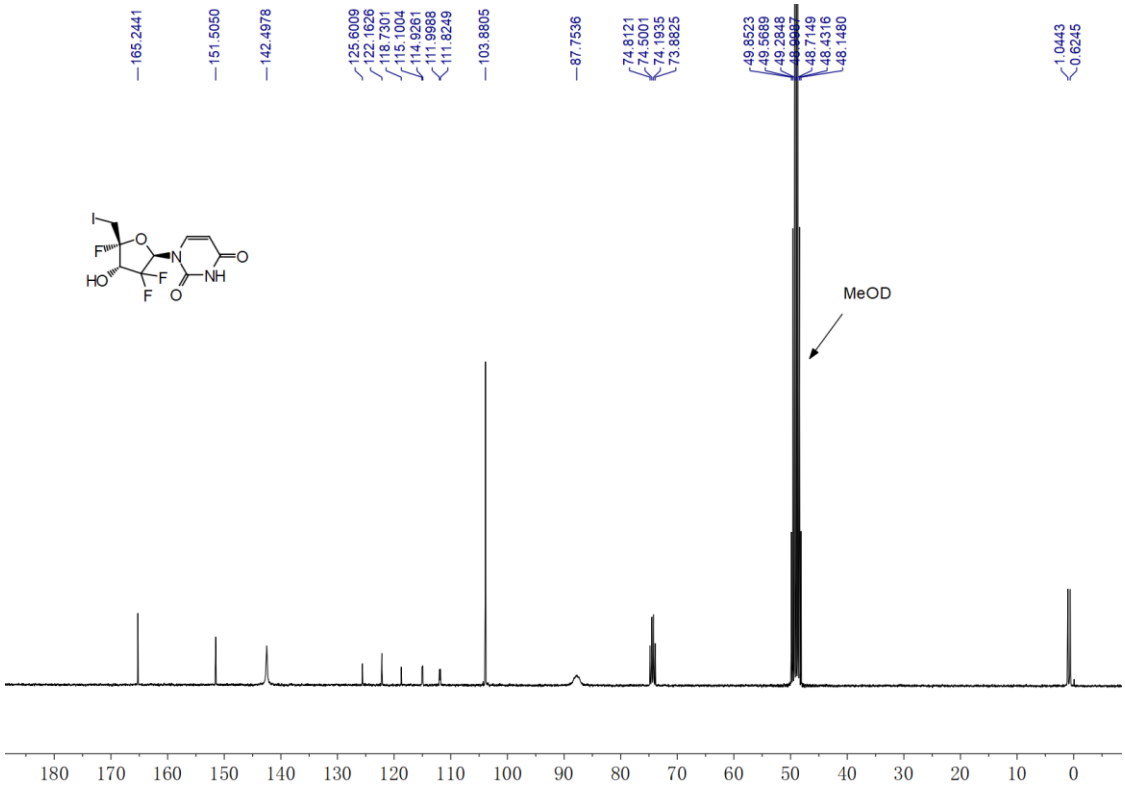
NMR spectra of compound **8a** in MeOD, ^{13}C NMR spectrum, 75MHz



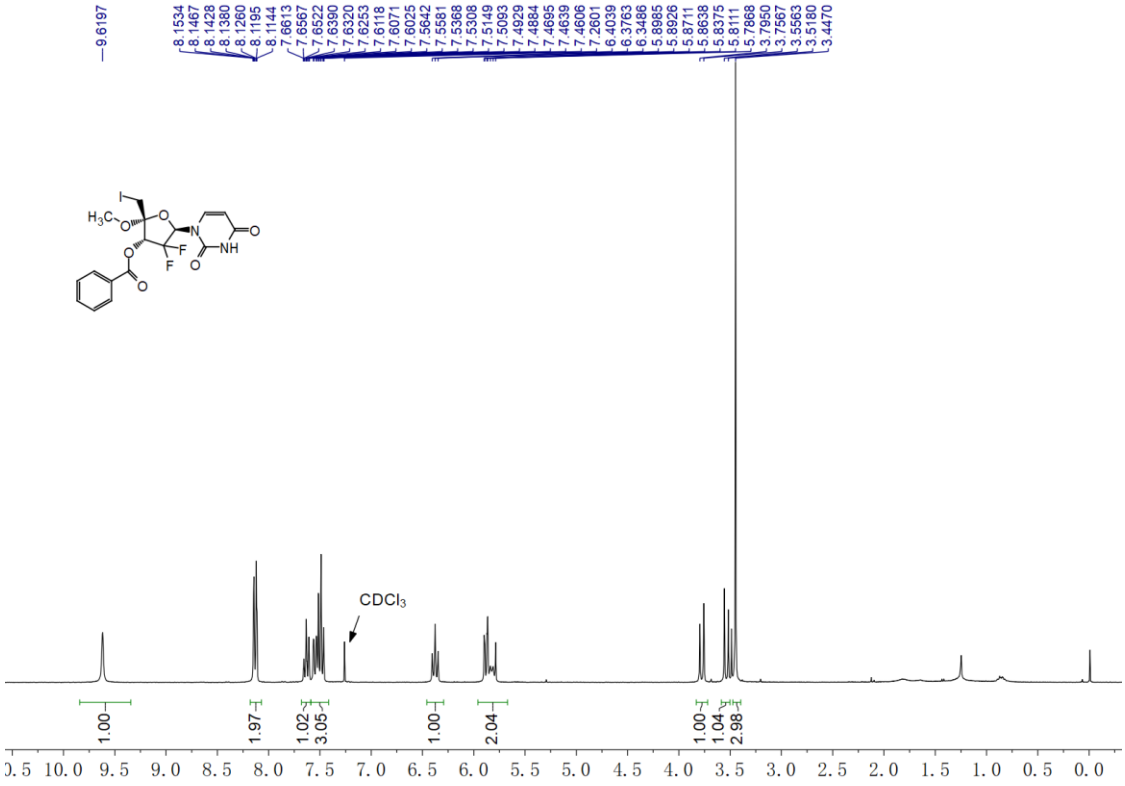
NMR spectra of compound **8b** in MeOD, ^{1}H NMR spectrum, 300MHz



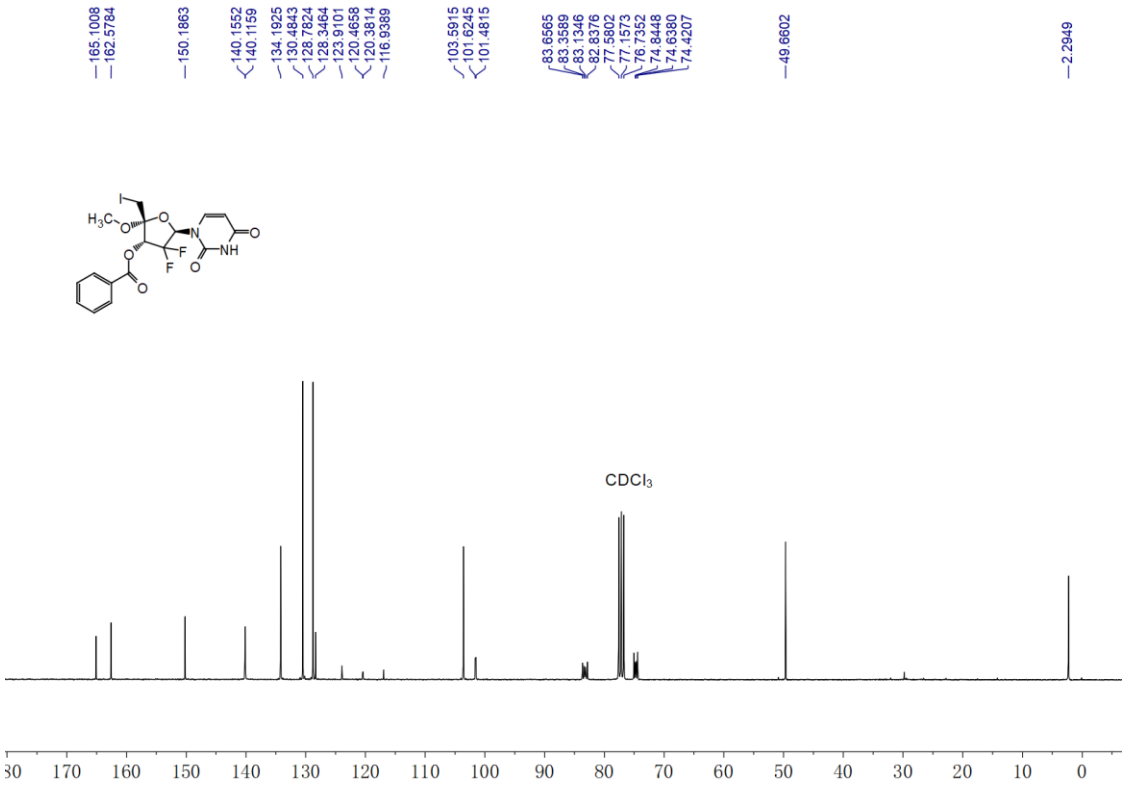
NMR spectra of compound **8b** in MeOD, ^{13}C NMR spectrum, 75MHz



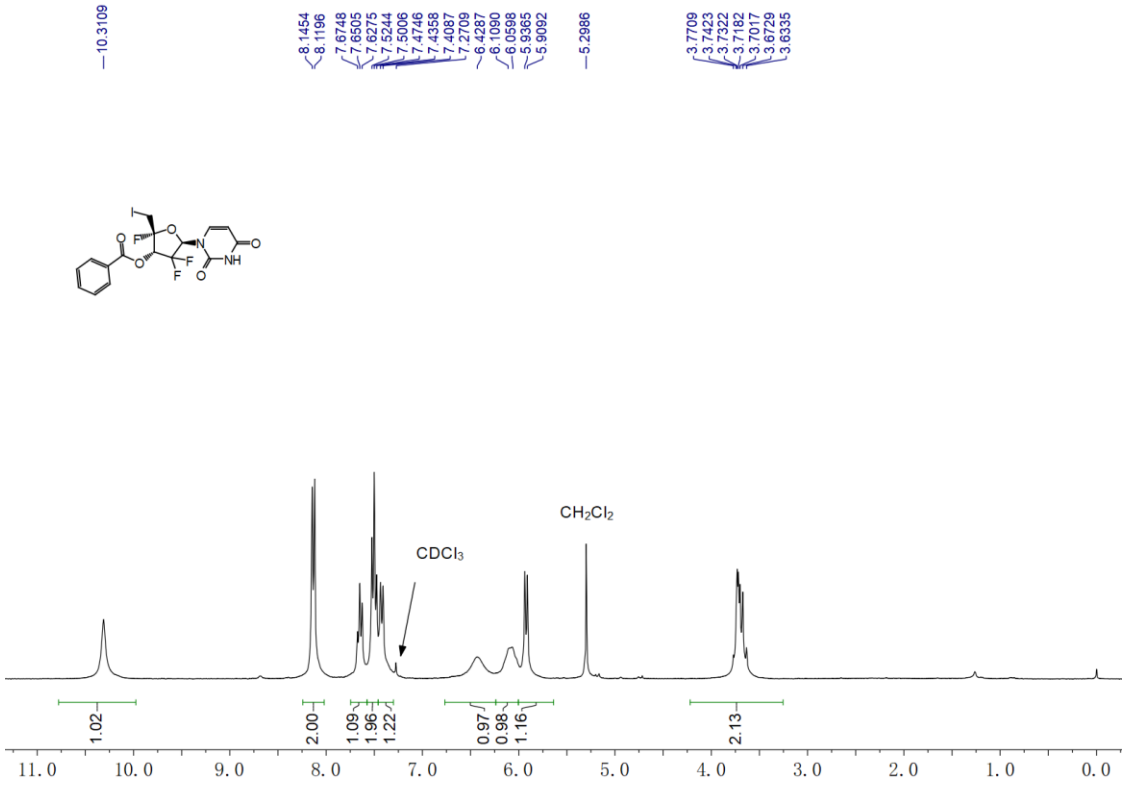
NMR spectra of compound **9a** in $CDCl_3$, ^1H NMR spectrum, 300MHz



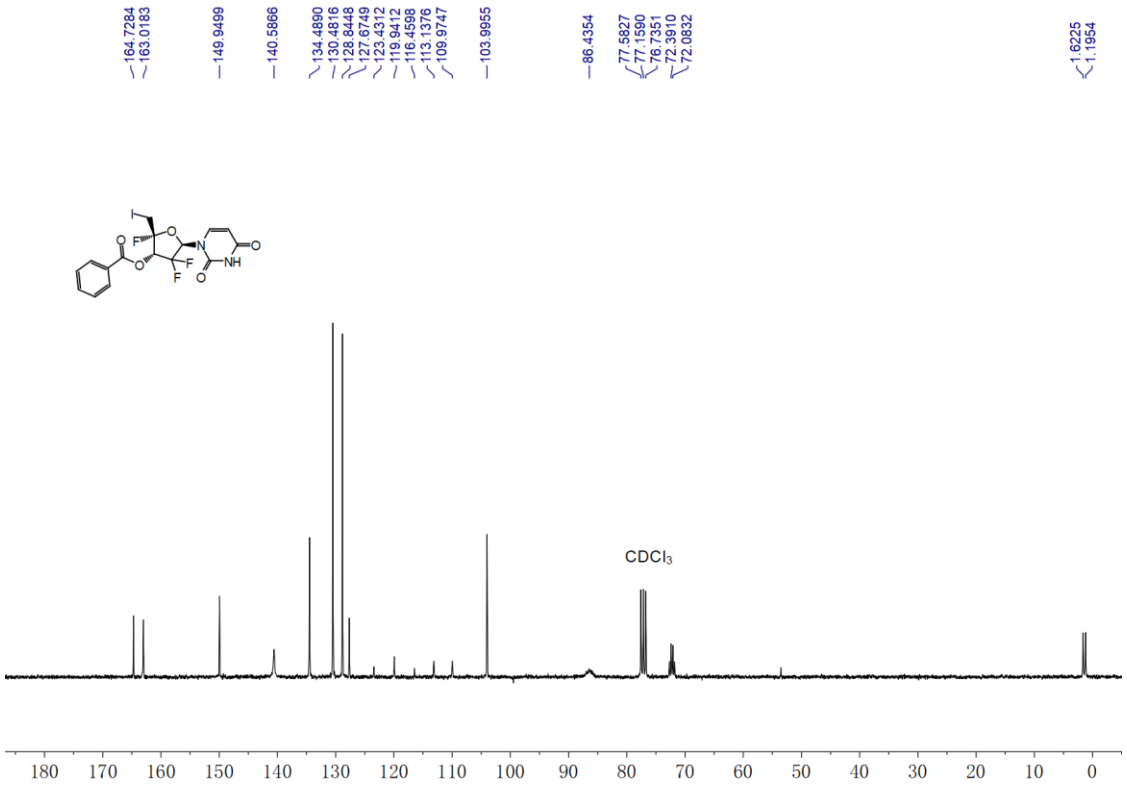
NMR spectra of compound **9a** in $CDCl_3$, ^{13}C NMR spectrum, 75MHz



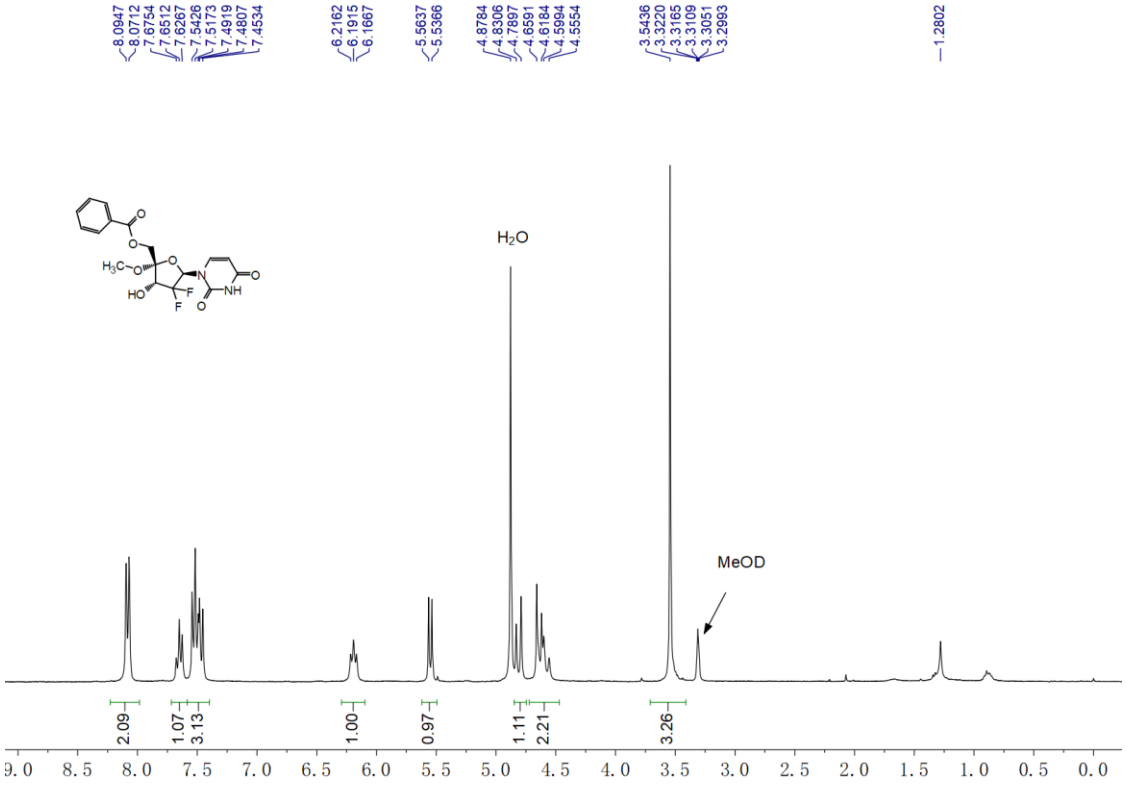
NMR spectra of compound **9b** in $CDCl_3$, ^1H NMR spectrum, 300MHz



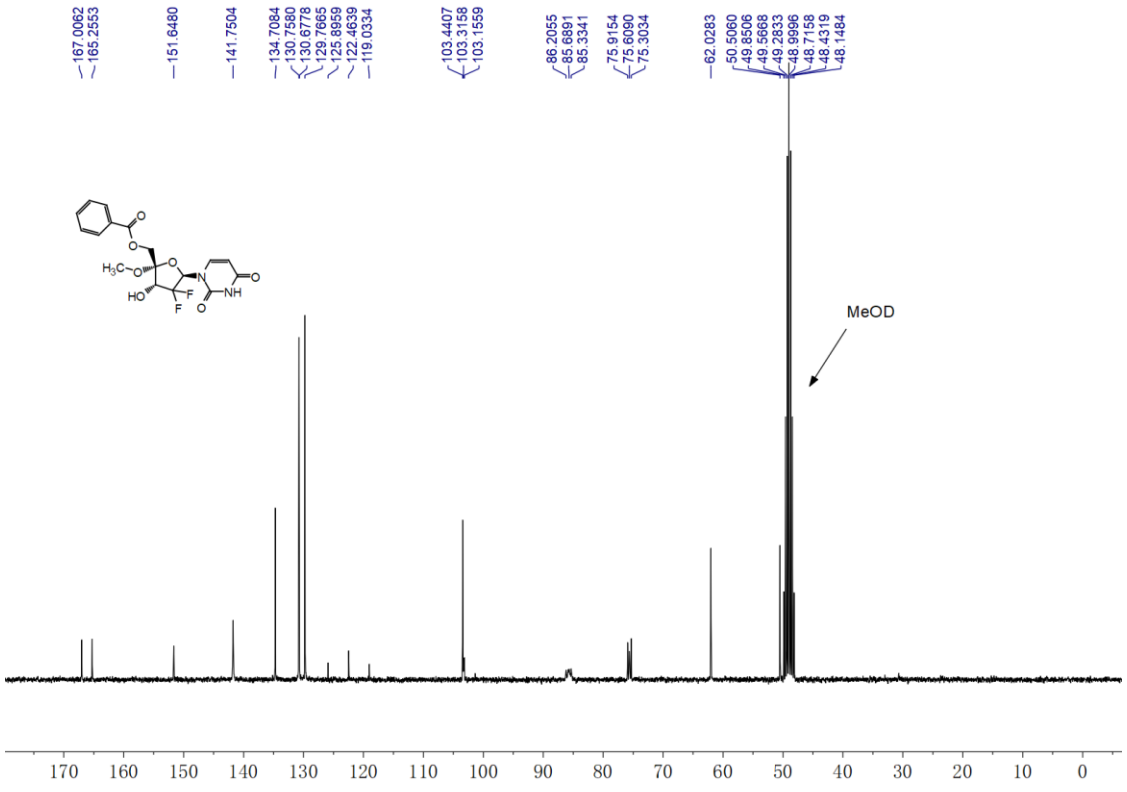
NMR spectra of compound **9b** in $CDCl_3$, ^{13}C NMR spectrum, 75MHz



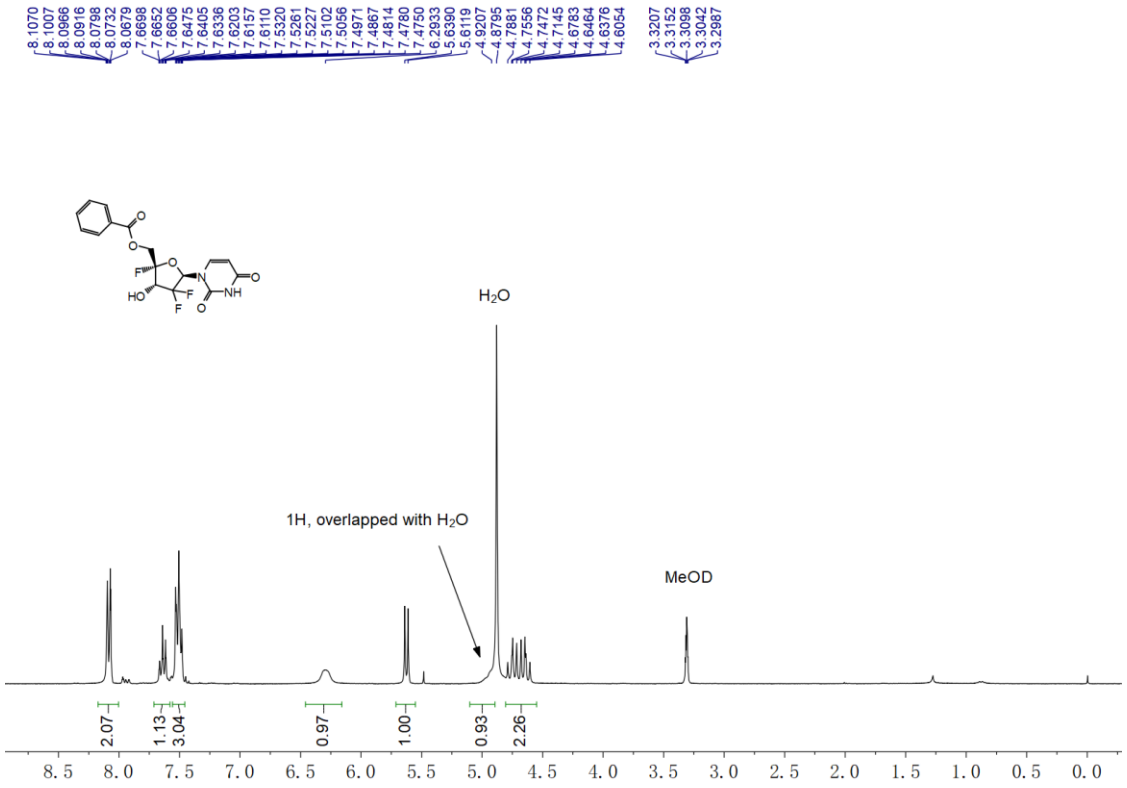
NMR spectra of compound **10a** in MeOD, ^{1}H NMR spectrum, 300MHz



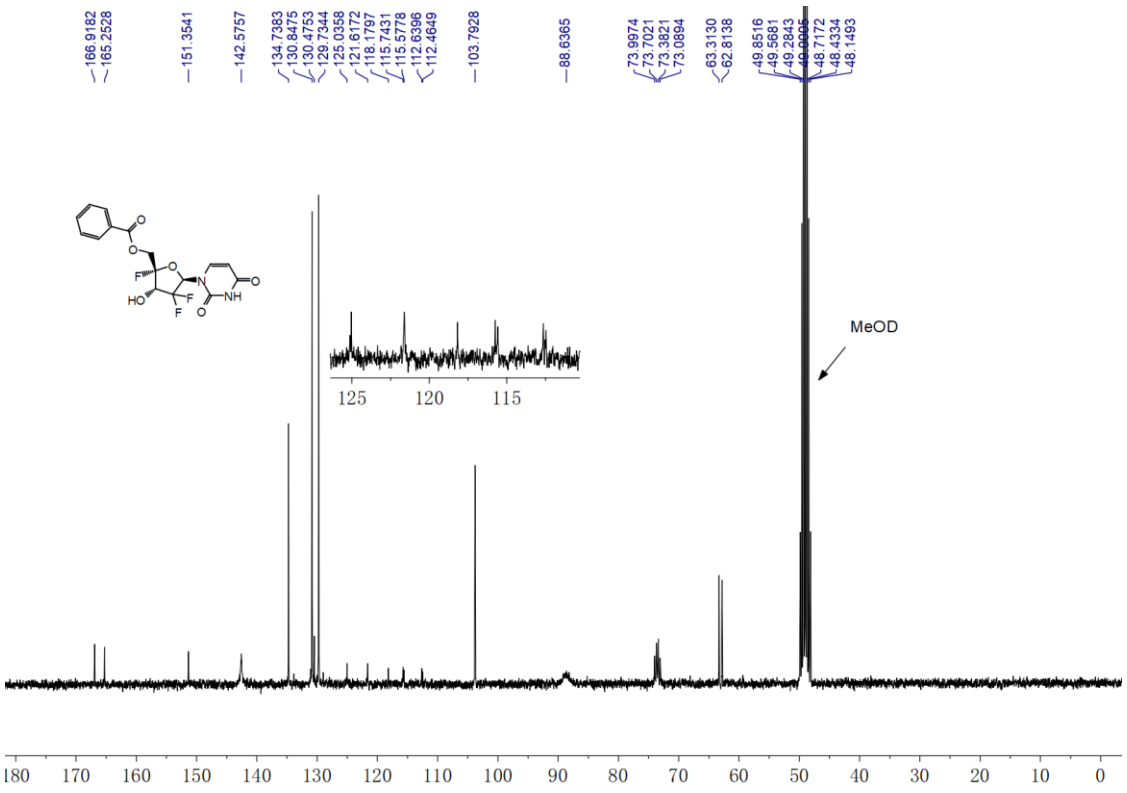
NMR spectra of compound **10a** in MeOD, ^{13}C NMR spectrum, 75MHz



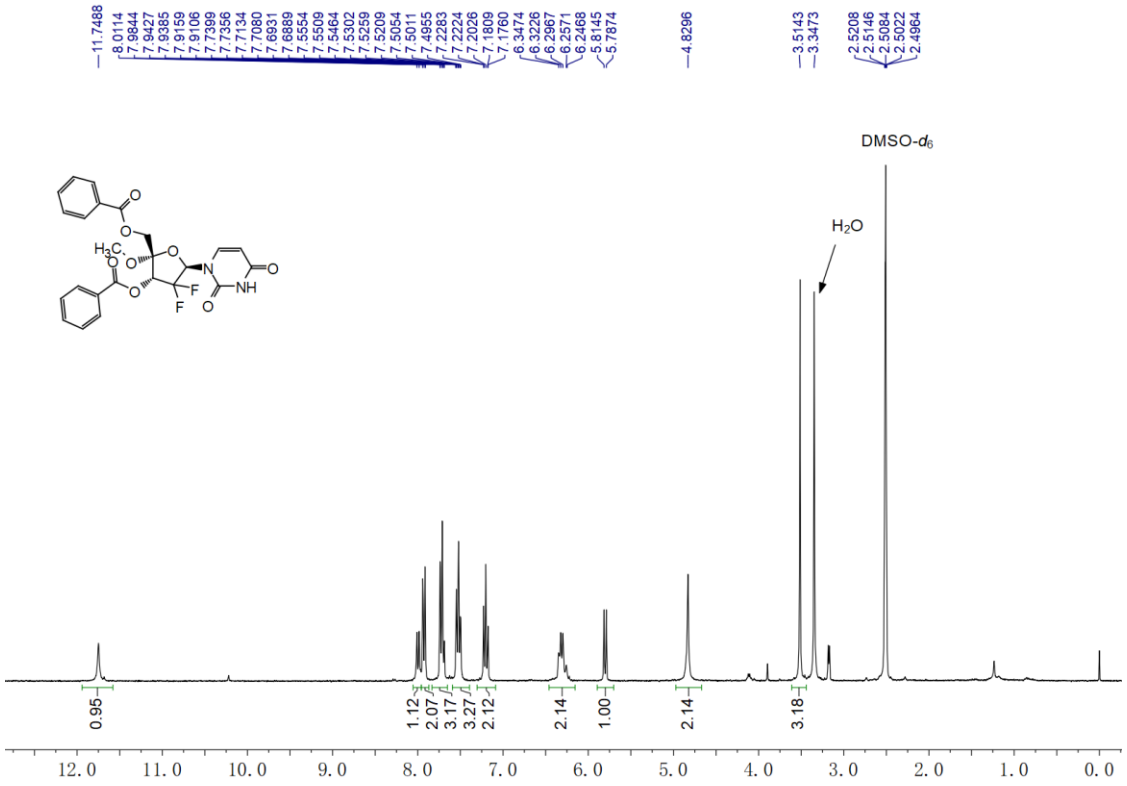
NMR spectra of compound **10b** in MeOD, ^{1}H NMR spectrum, 300MHz



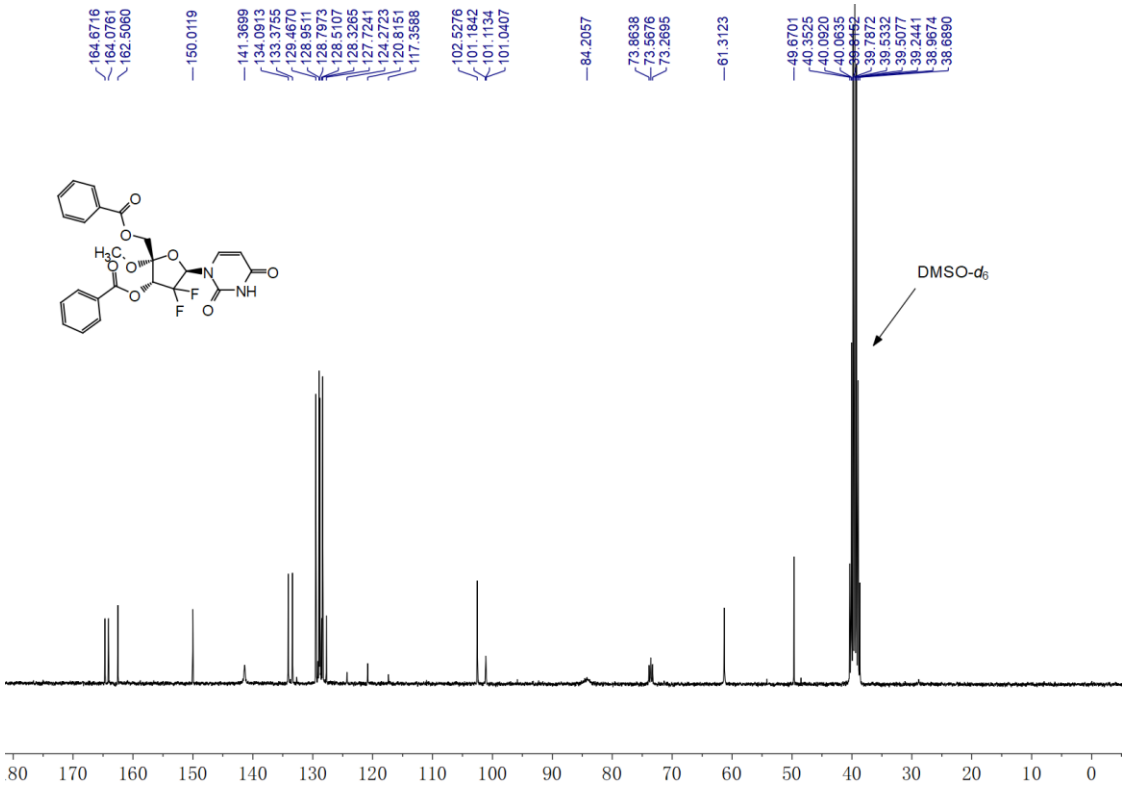
NMR spectra of compound **10b** in MeOD, ^{13}C NMR spectrum, 75MHz



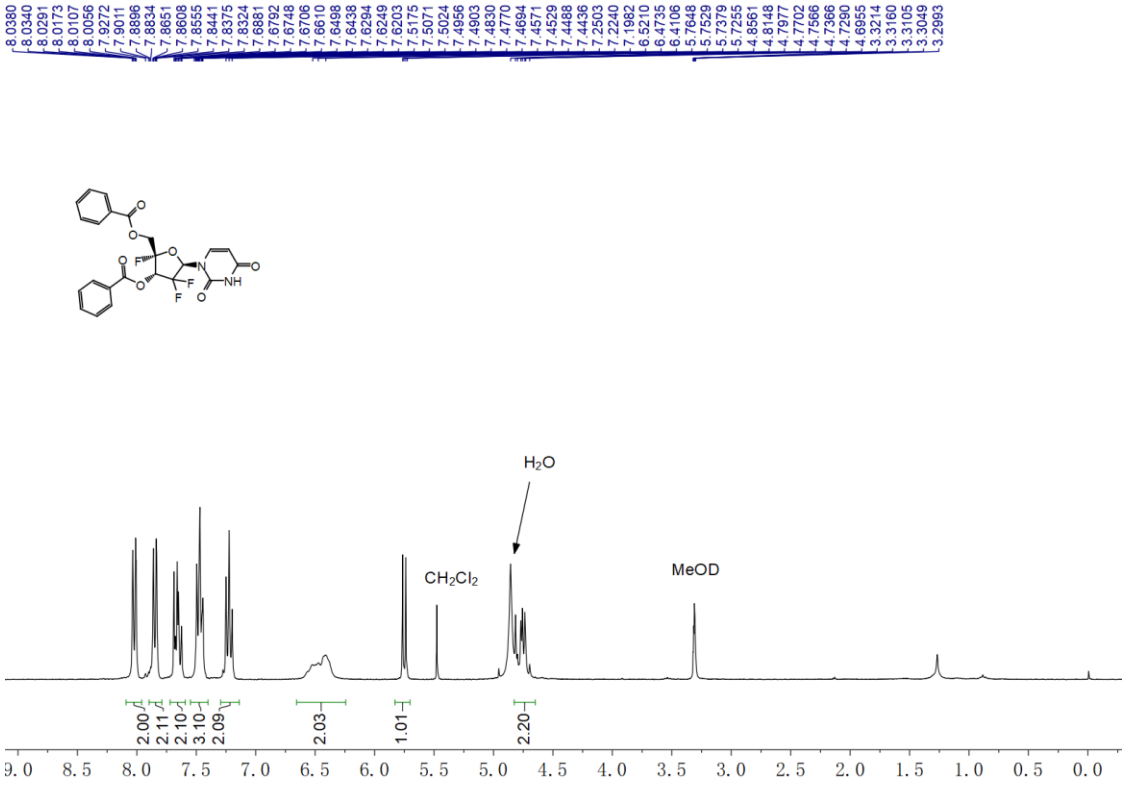
NMR spectra of compound **11a** in DMSO-d_6, ^{1}H NMR spectrum, 300MHz



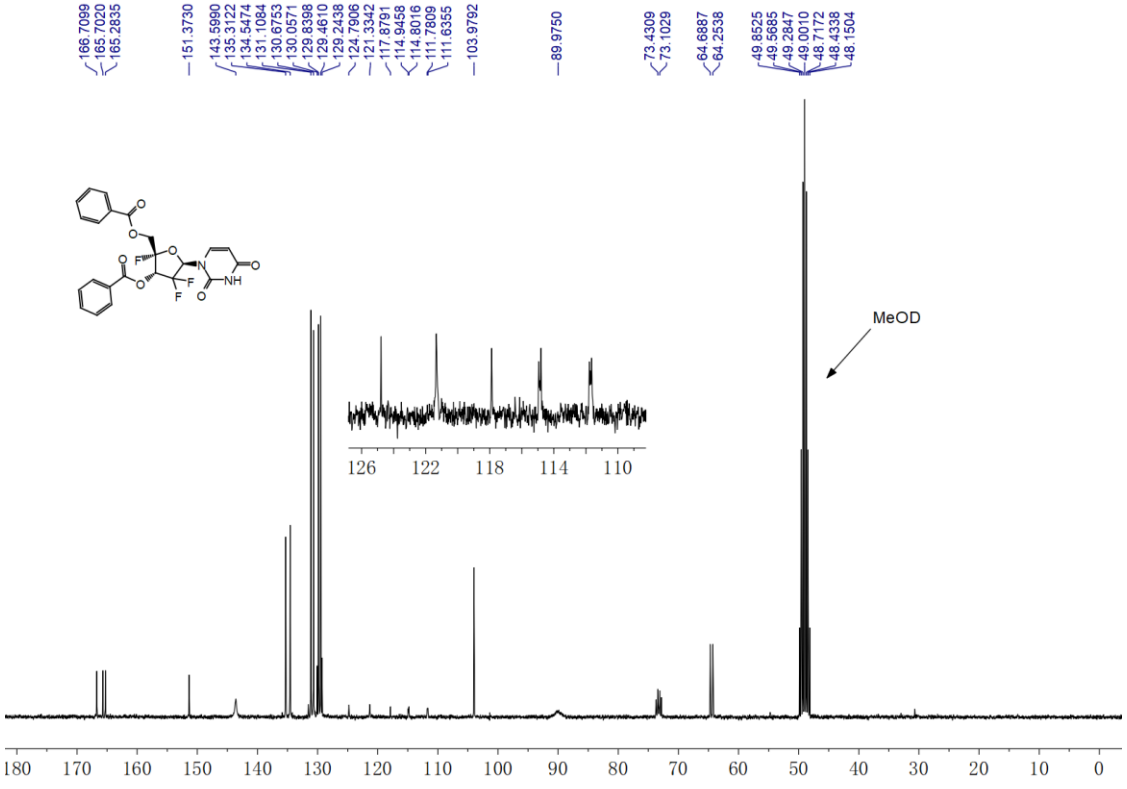
NMR spectra of compound **11a** in DMSO-d_6, ^{13}C NMR spectrum, 75MHz



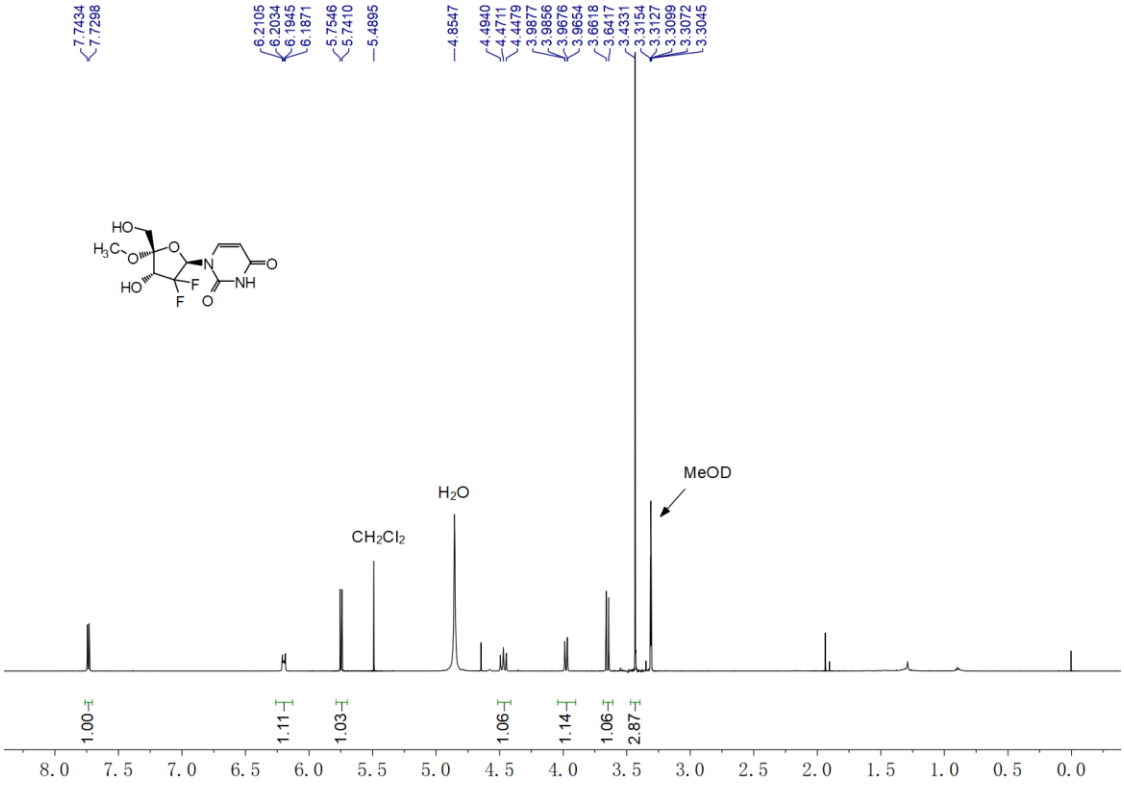
NMR spectra of compound **11b** in MeOD, ^{1}H NMR spectrum, 300MHz



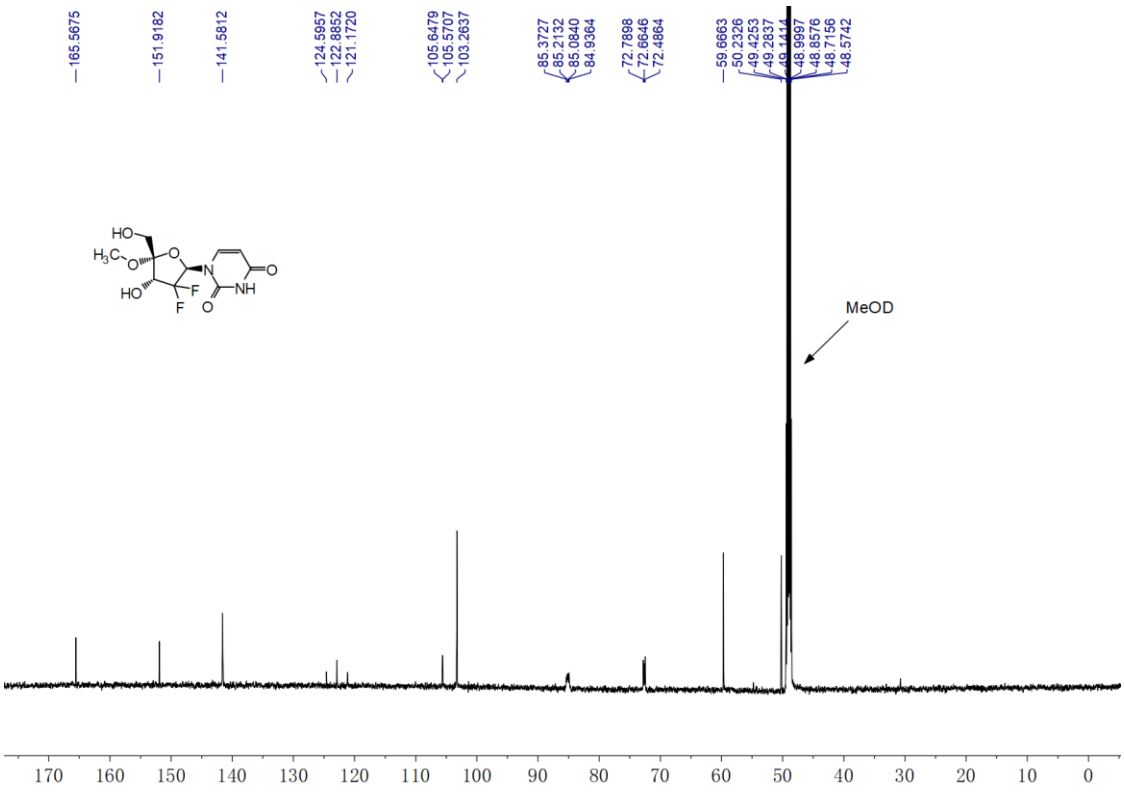
NMR spectra of compound **11b** in MeOD, ^{13}C NMR spectrum, 75MHz



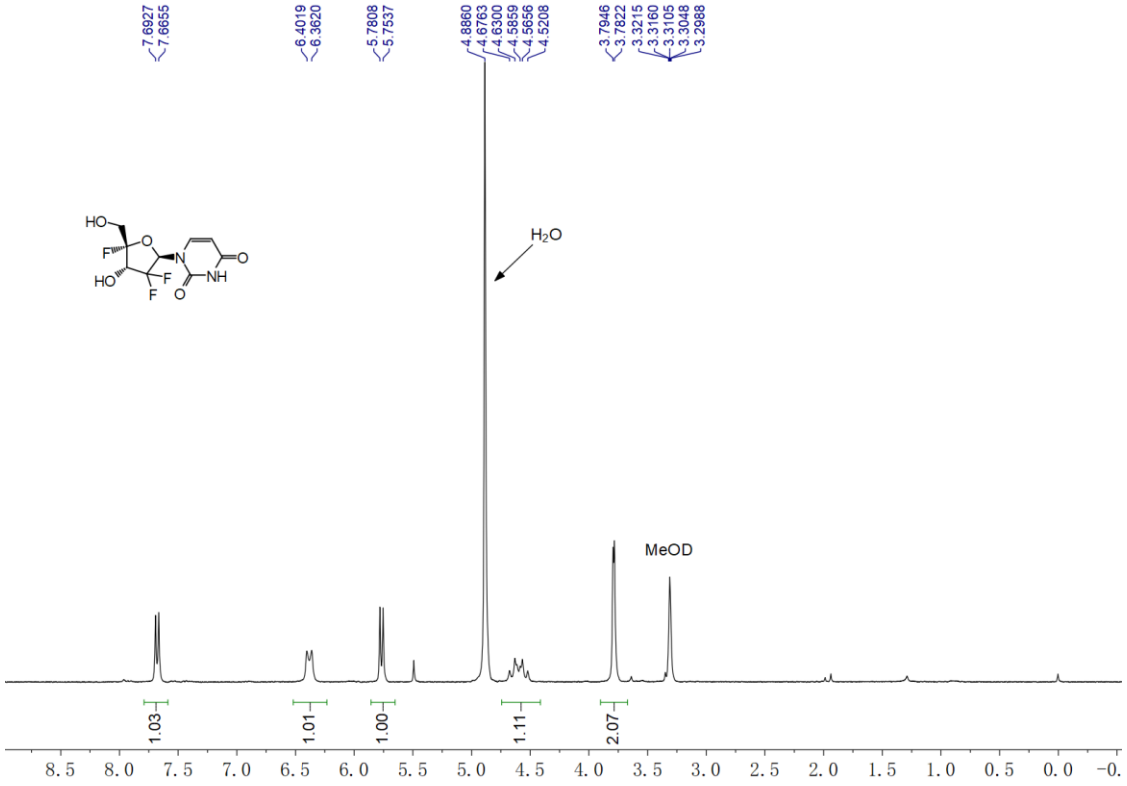
NMR spectra of compound **12a** in MeOD, ^{1}H NMR spectrum, 600MHz



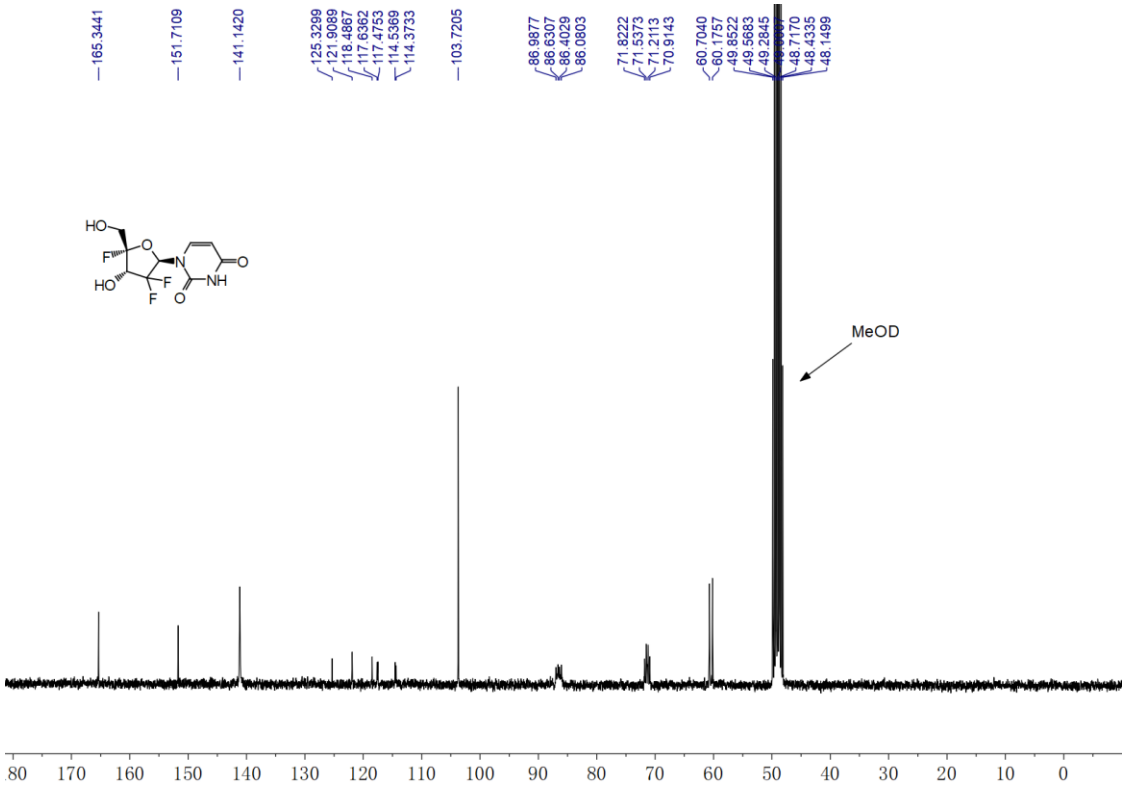
NMR spectra of compound **12a** in MeOD, ^{13}C NMR spectrum, 151MHz



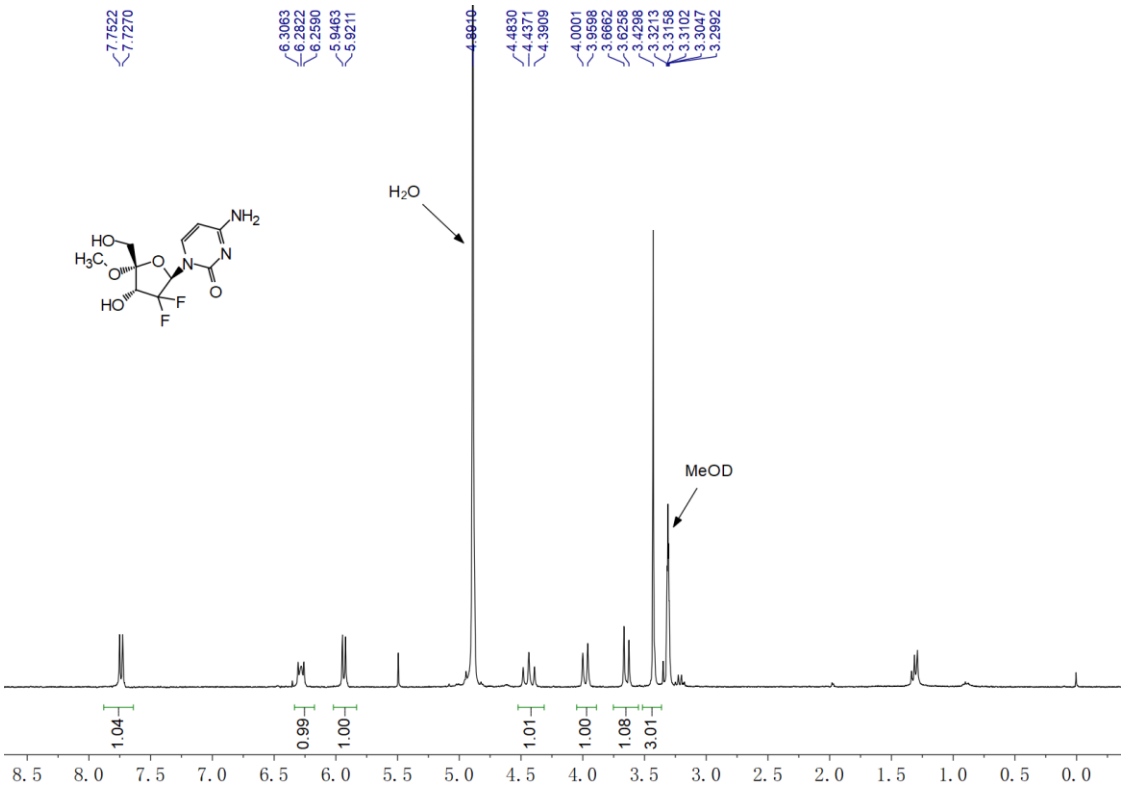
NMR spectra of compound **12b** in MeOD, ^{1}H NMR spectrum, 300MHz



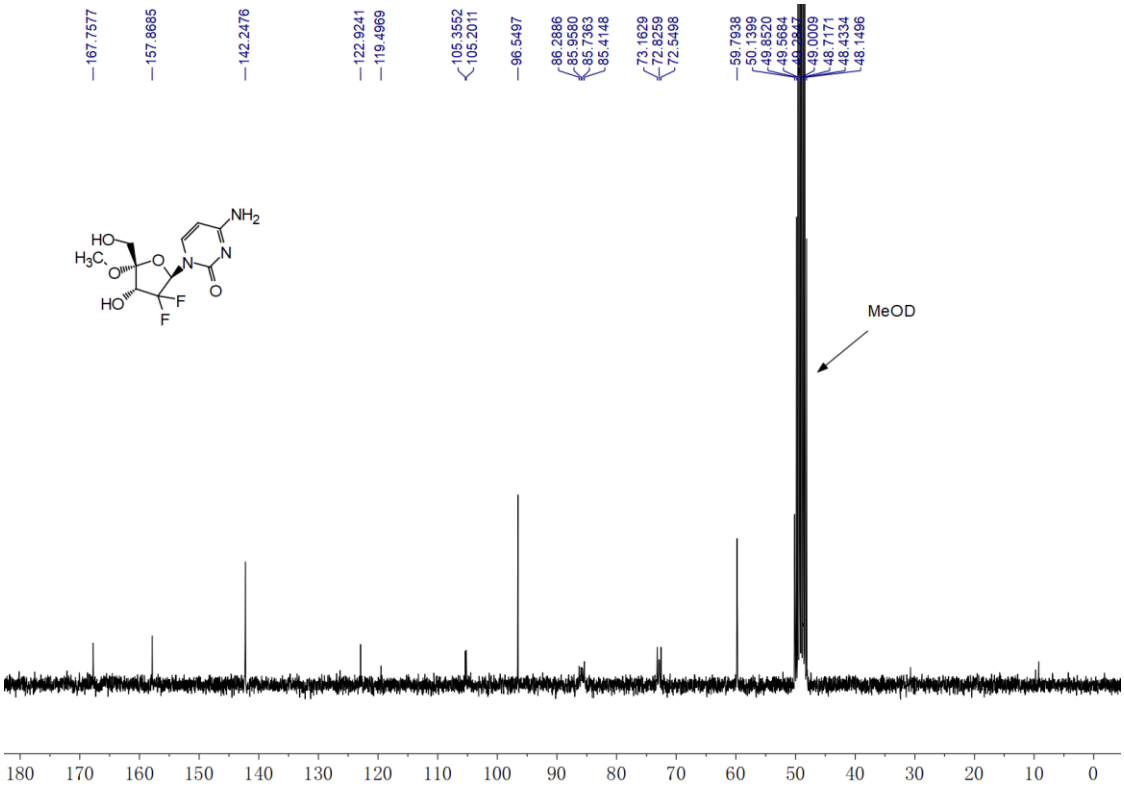
NMR spectra of compound **12b** in MeOD, ^{13}C NMR spectrum, 75MHz



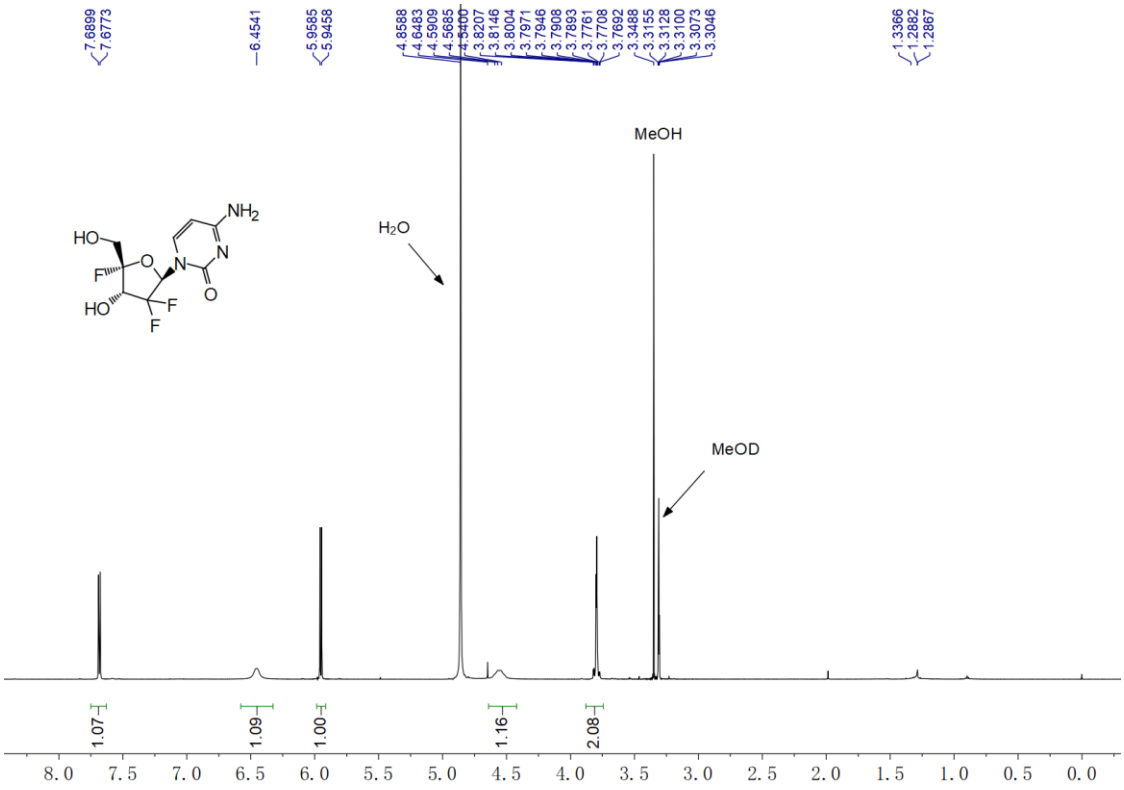
NMR spectra of compound **1a** in MeOD, ^{1}H NMR spectrum, 300MHz



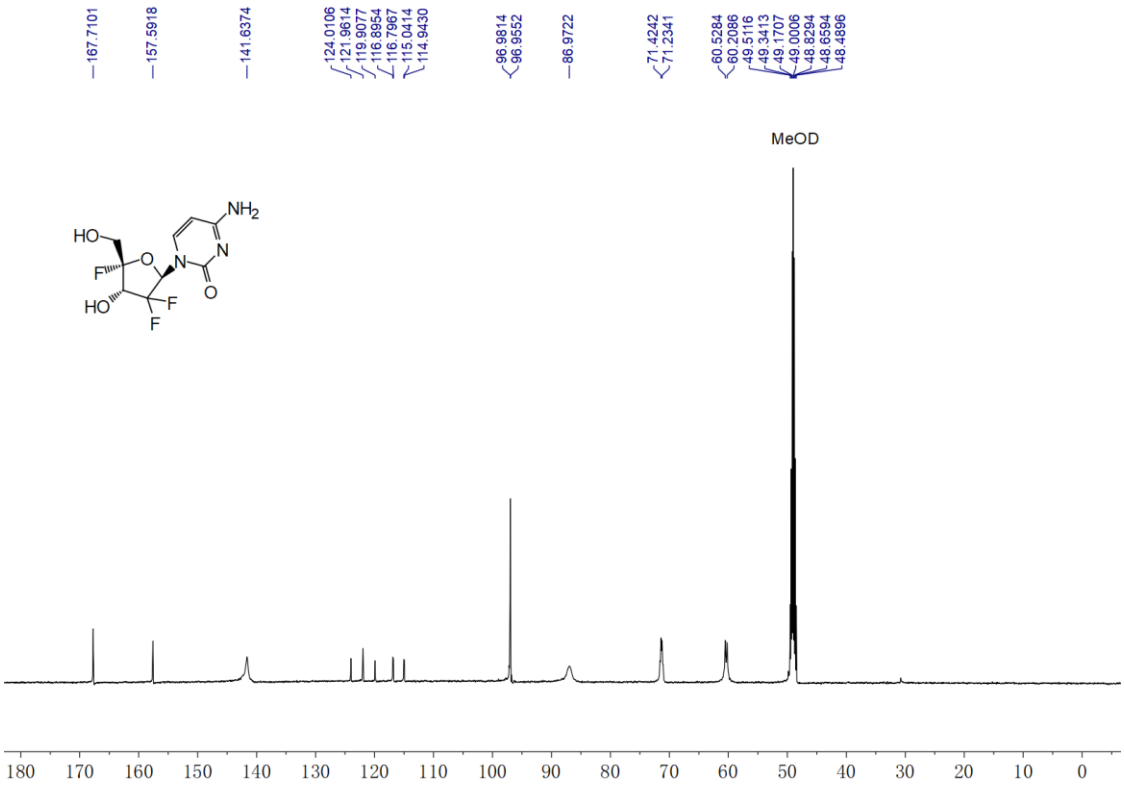
NMR spectra of compound **1a** in MeOD, ^{13}C NMR spectrum, 75MHz



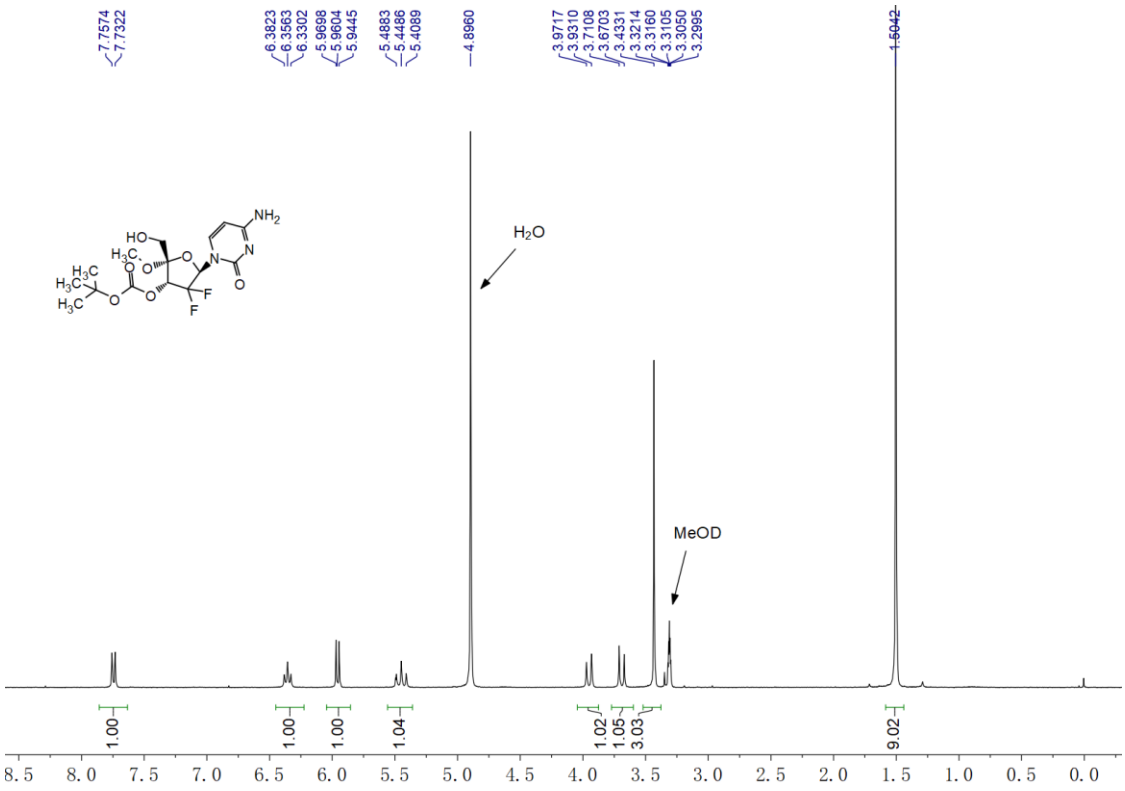
NMR spectra of compound **1b** in MeOD, ^{1}H NMR spectrum, 600MHz



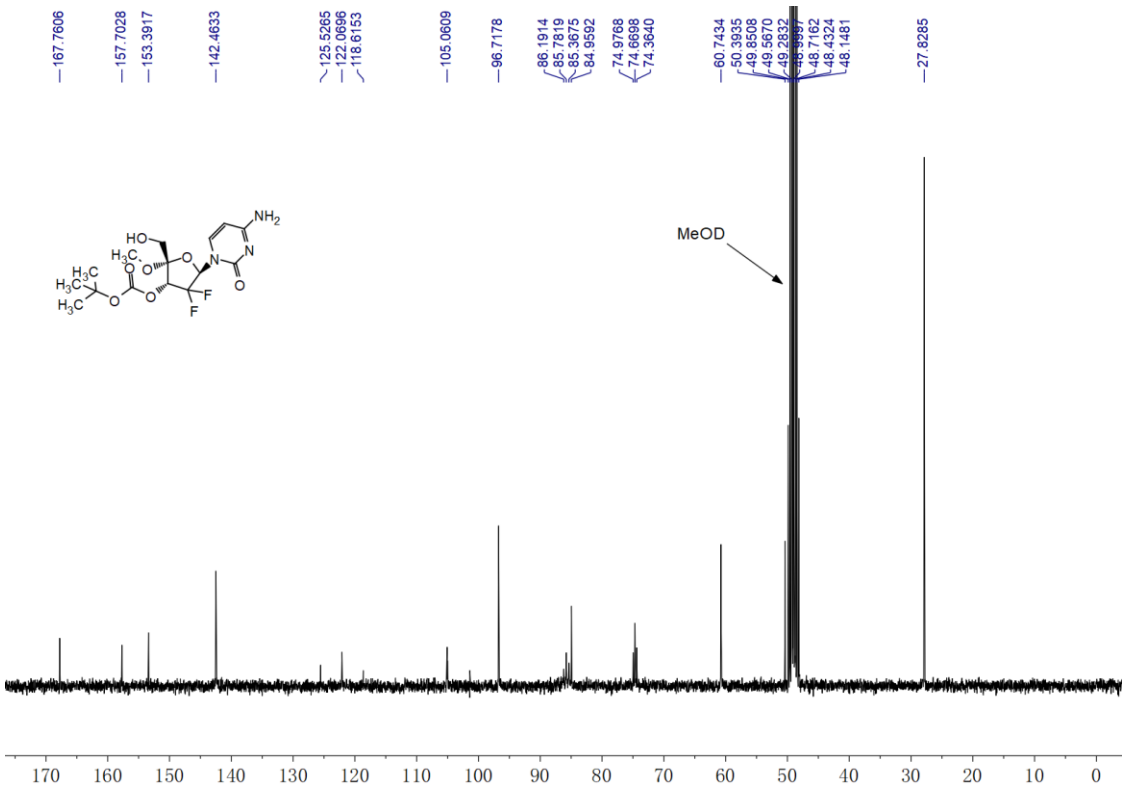
NMR spectra of compound **1b** in MeOD, ^{13}C NMR spectrum, 126MHz



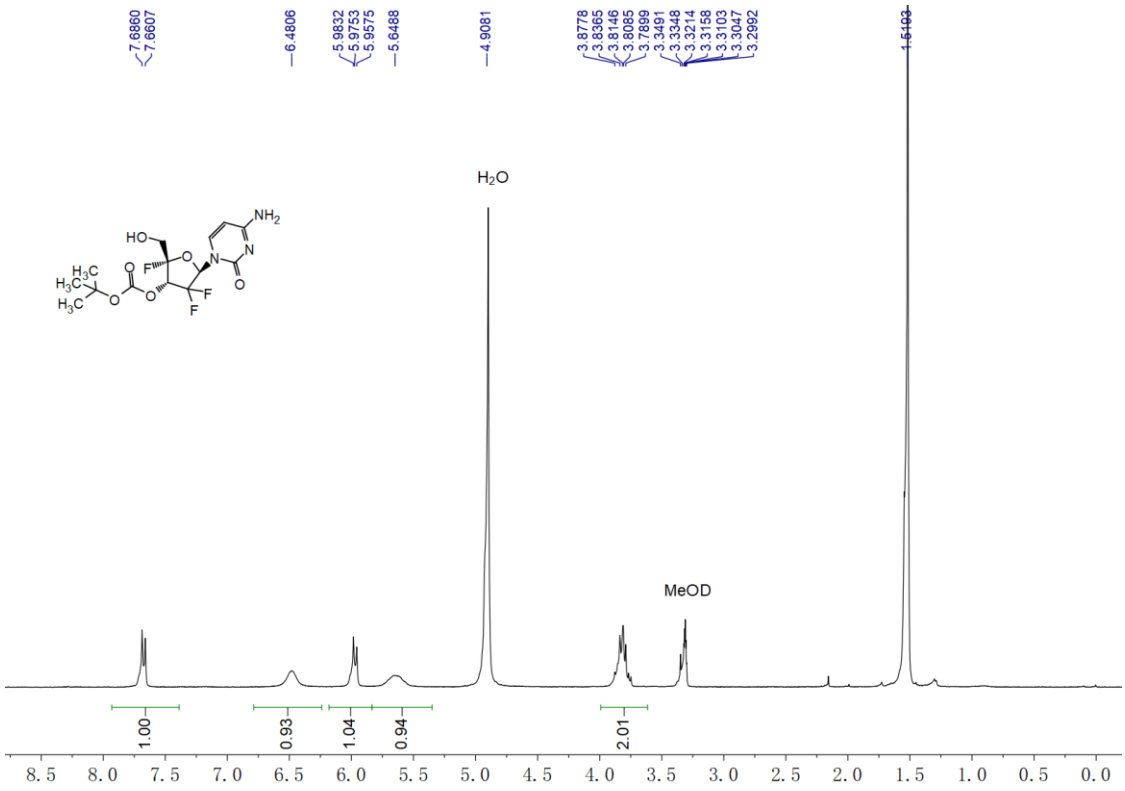
NMR spectra of compound **13a** in MeOD, ^{1}H NMR spectrum, 300MHz



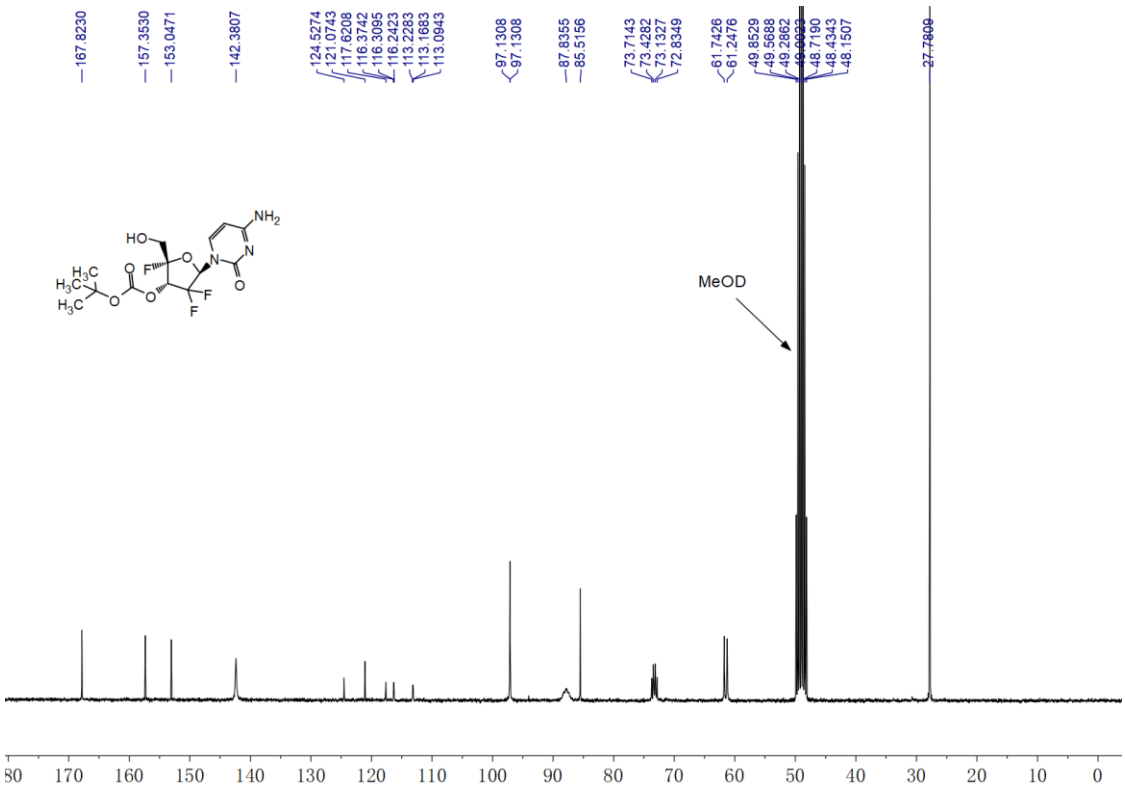
NMR spectra of compound **13a** in MeOD, ^{13}C NMR spectrum, 75MHz



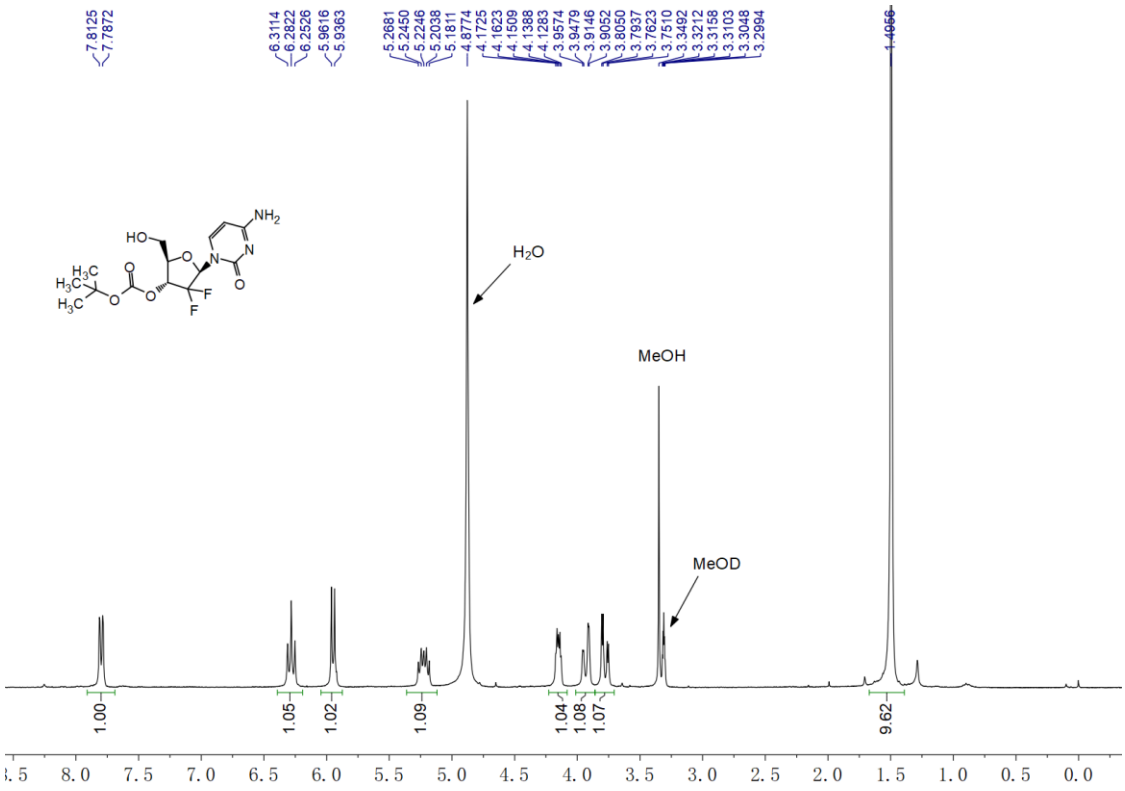
NMR spectra of compound **13b** in MeOD, ^{1}H NMR spectrum, 300MHz



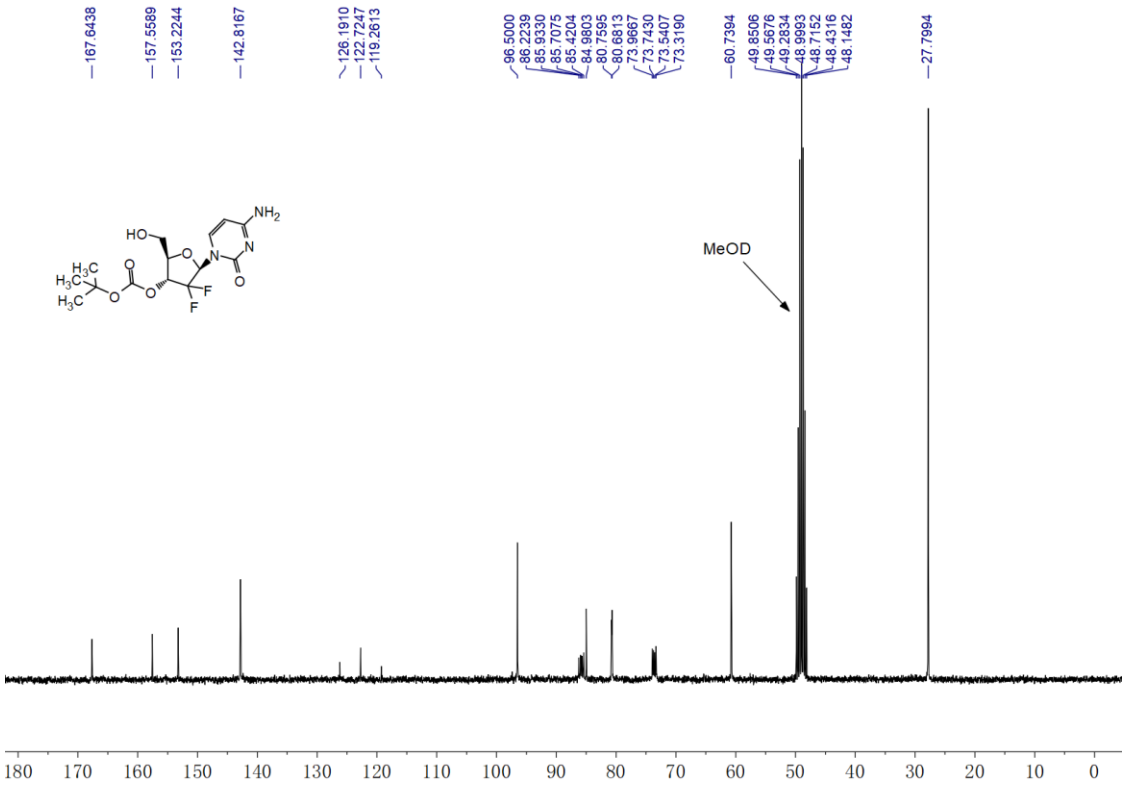
NMR spectra of compound **13b** in MeOD, ^{13}C NMR spectrum, 75MHz



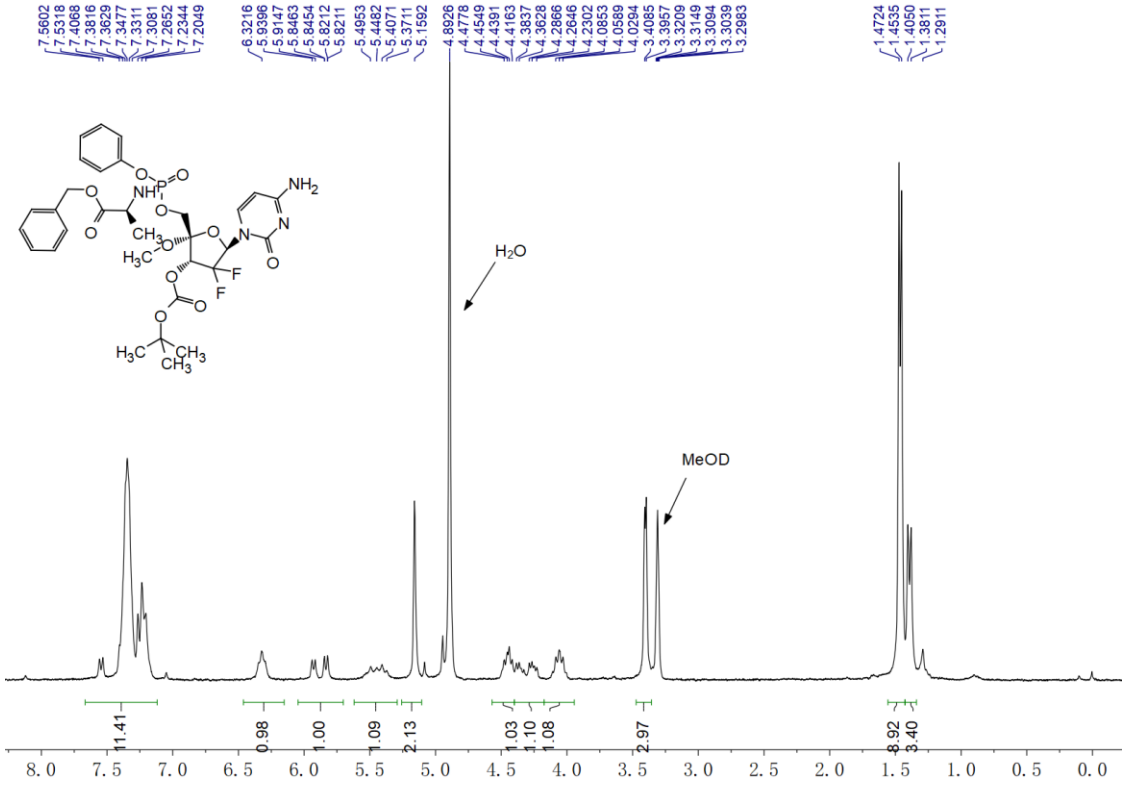
NMR spectra of compound **13c** in MeOD, ^{1}H NMR spectrum, 300MHz



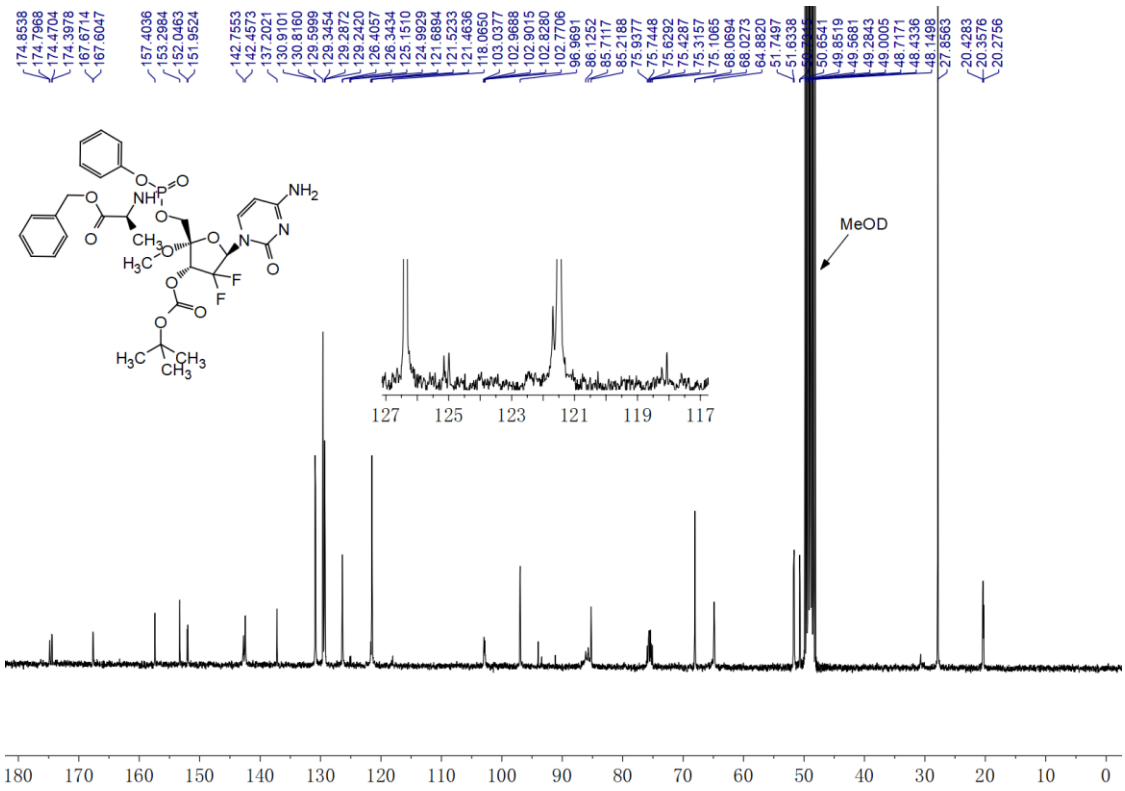
NMR spectra of compound **13c** in MeOD, ^{13}C NMR spectrum, 75MHz



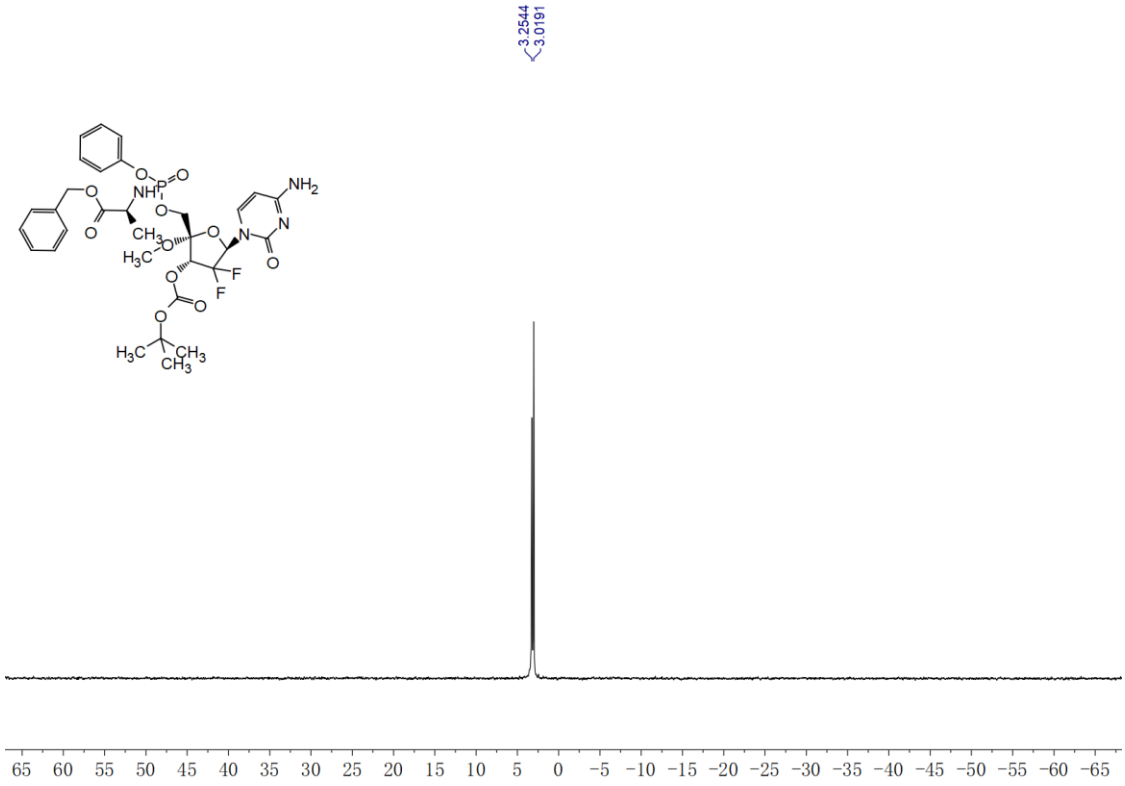
NMR spectra of compound **16a** in MeOD, ^{1}H NMR spectrum, 300MHz



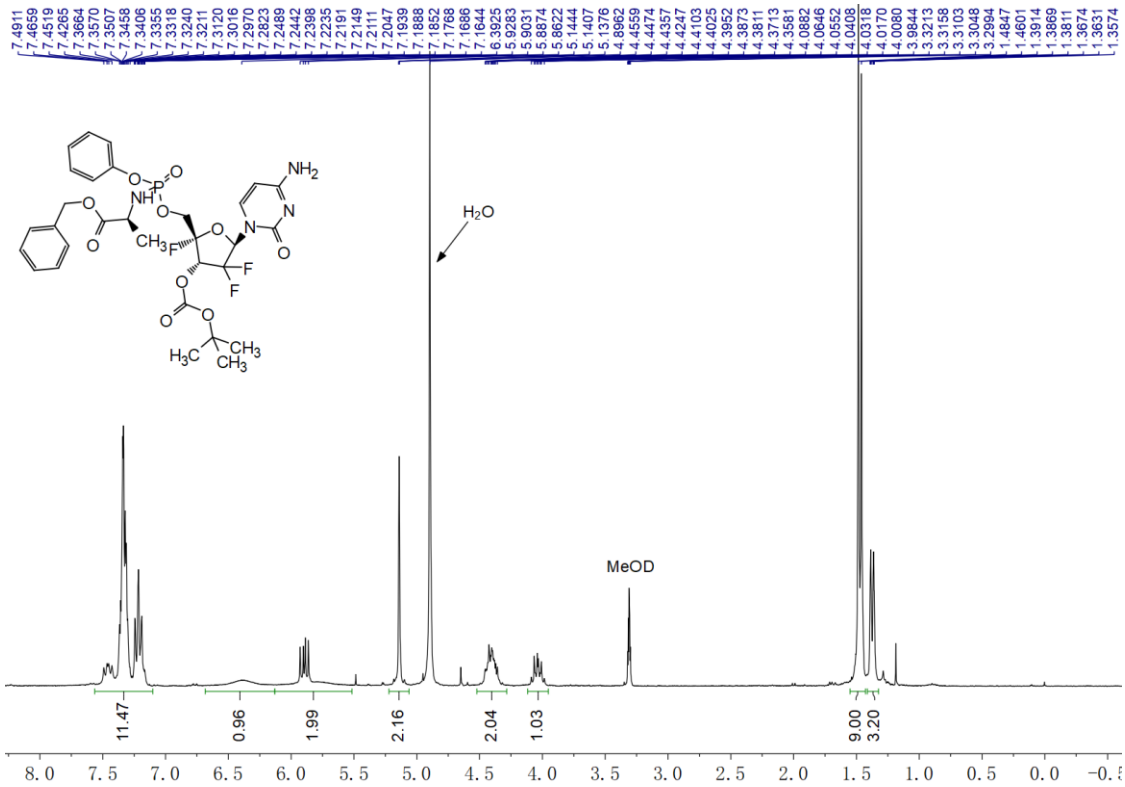
NMR spectra of compound **16a** in MeOD, ^{13}C NMR spectrum, 75MHz



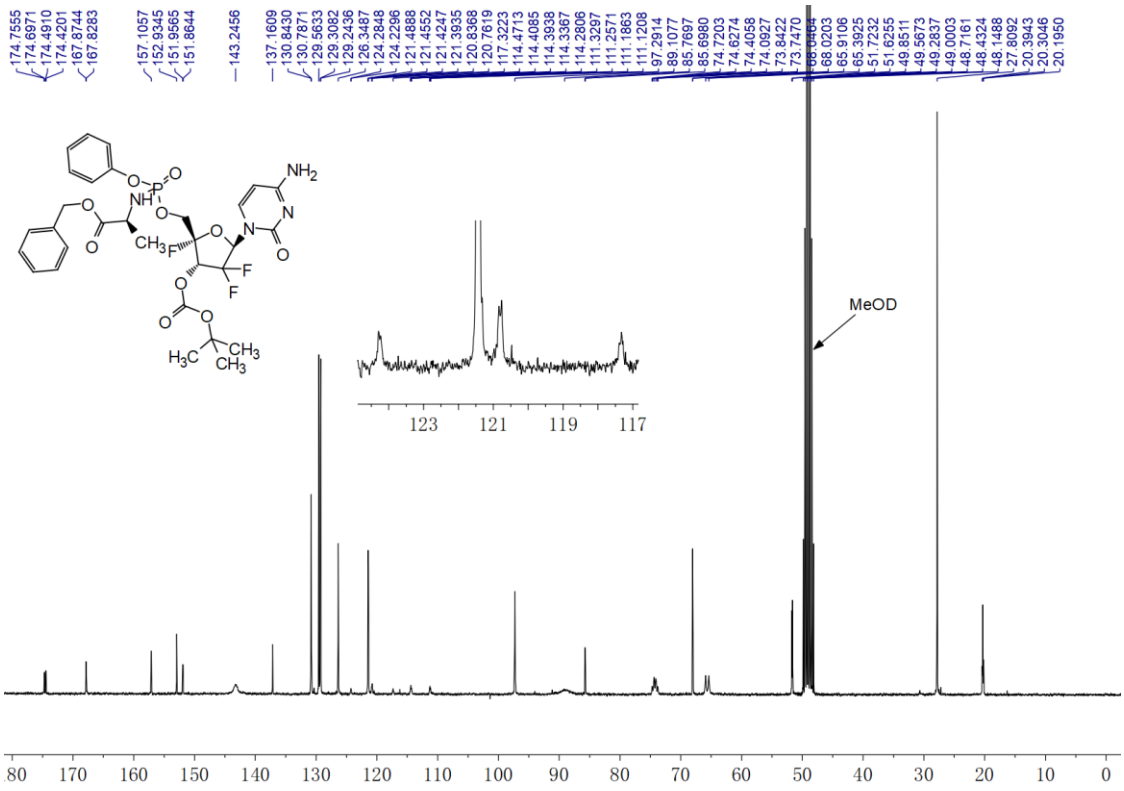
NMR spectra of compound **16a** in MeOD, ^{31}P NMR spectrum, 121MHz



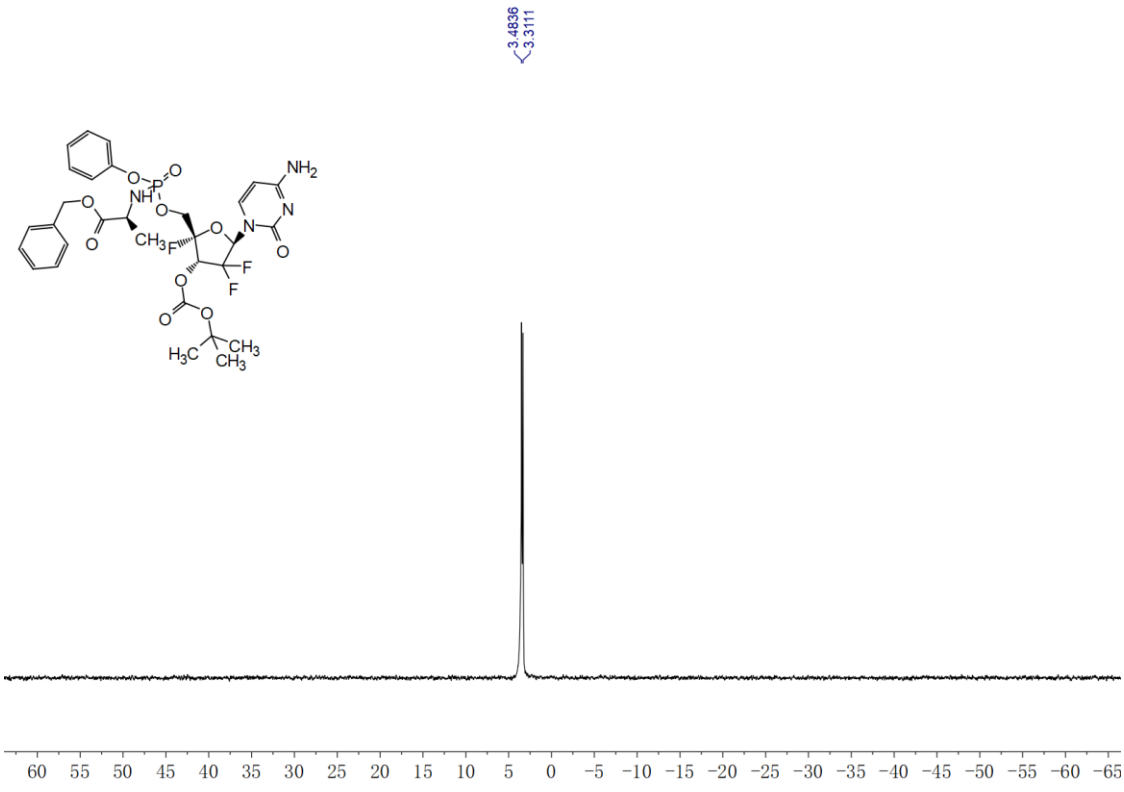
NMR spectra of compound **16b** in MeOD, ^{1}H NMR spectrum, 300MHz



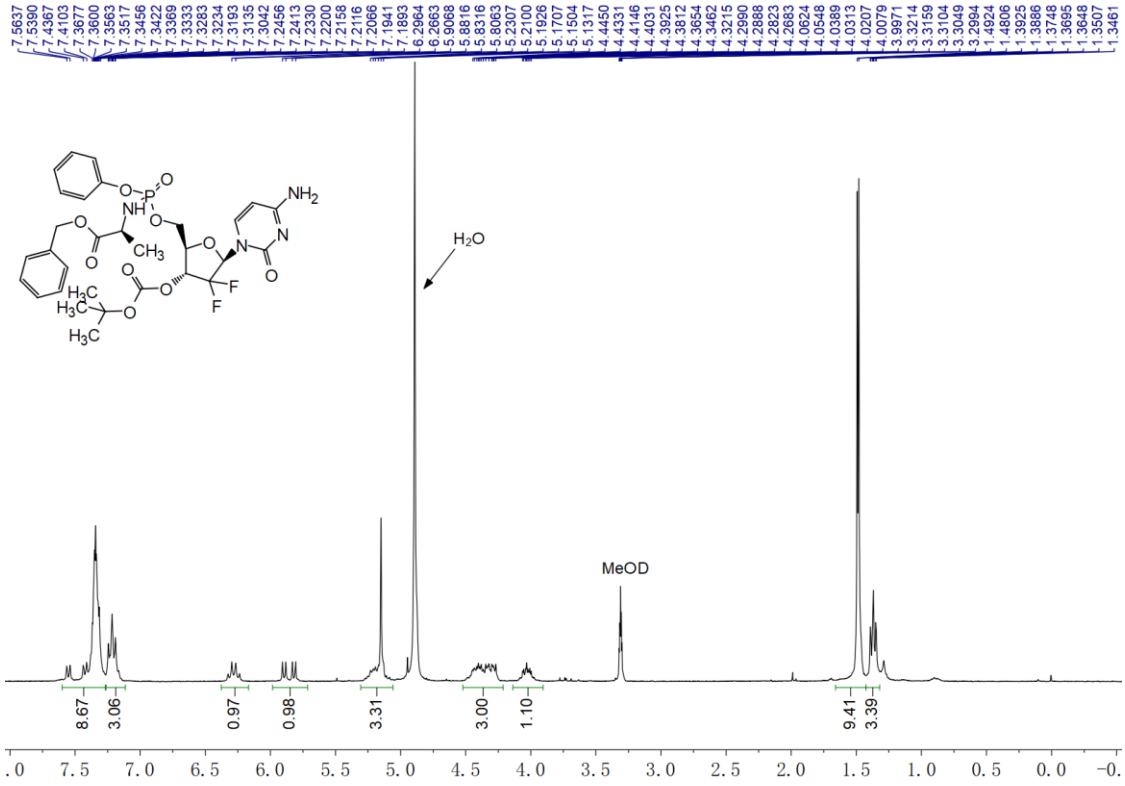
NMR spectra of compound **16b** in MeOD, ^{13}C NMR spectrum, 75MHz



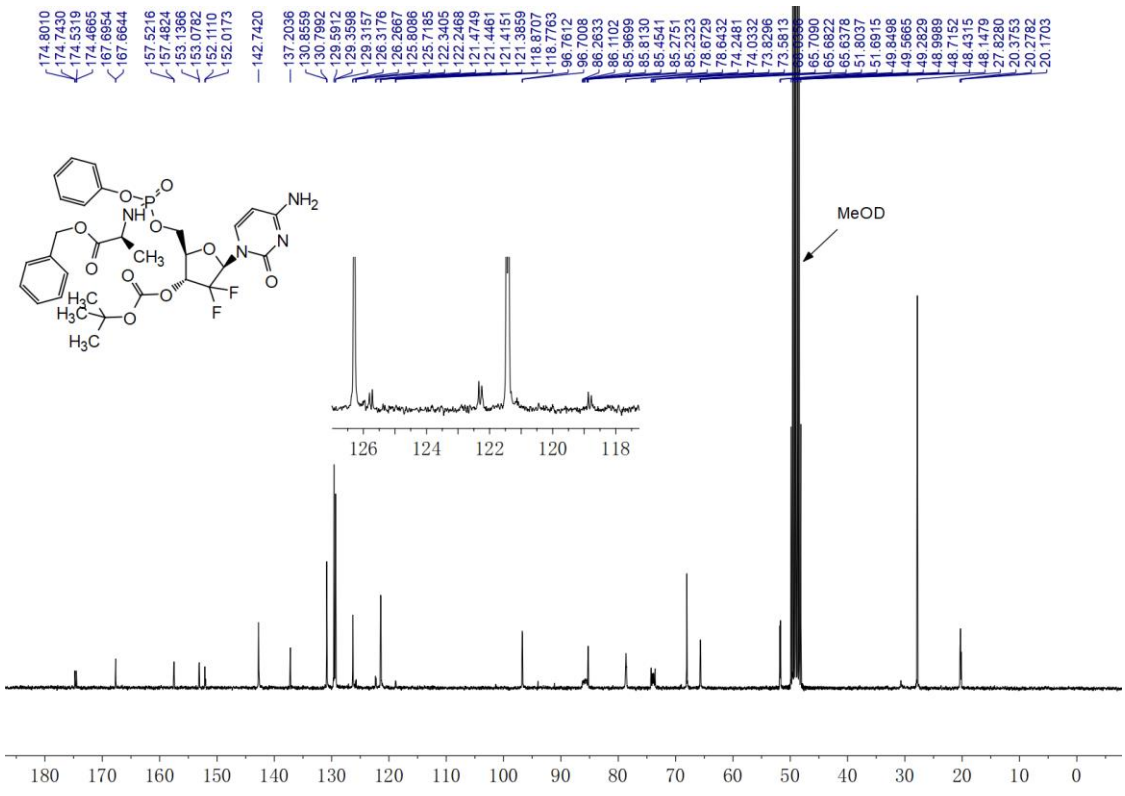
NMR spectra of compound **16b** in MeOD, ^{31}P NMR spectrum, 121MHz



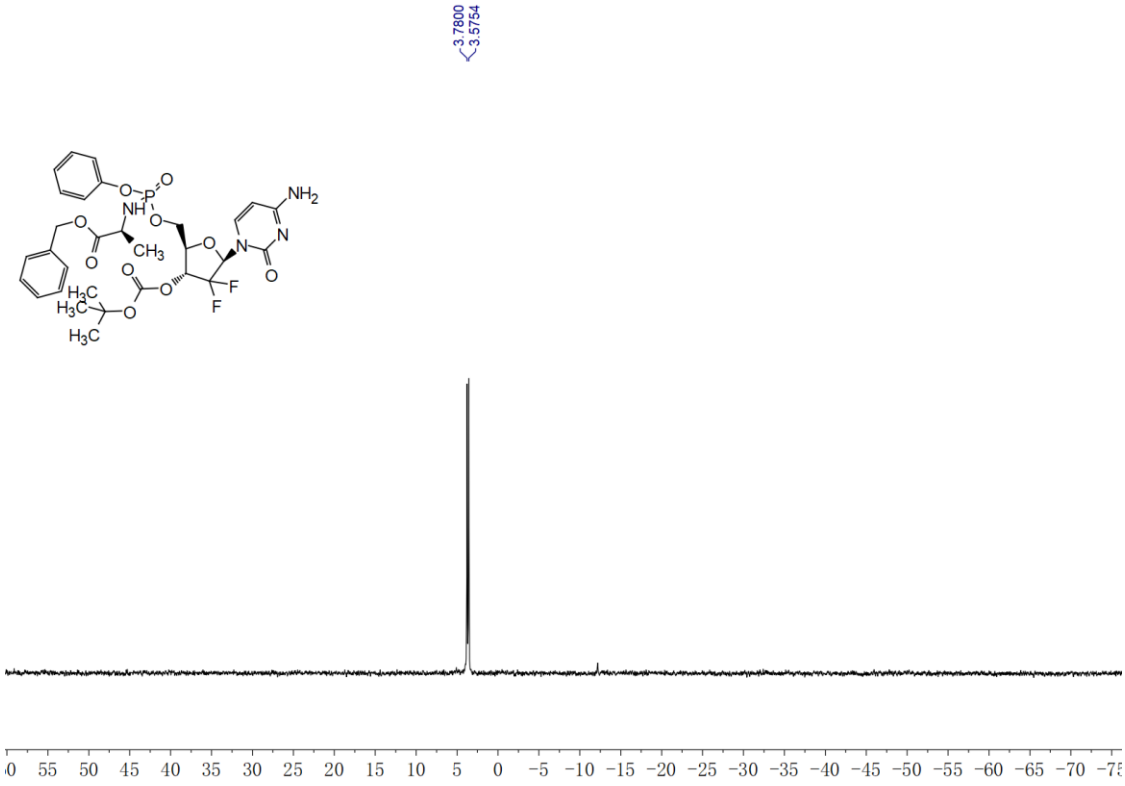
NMR spectra of compound **16c** in MeOD, ^{1}H NMR spectrum, 300MHz



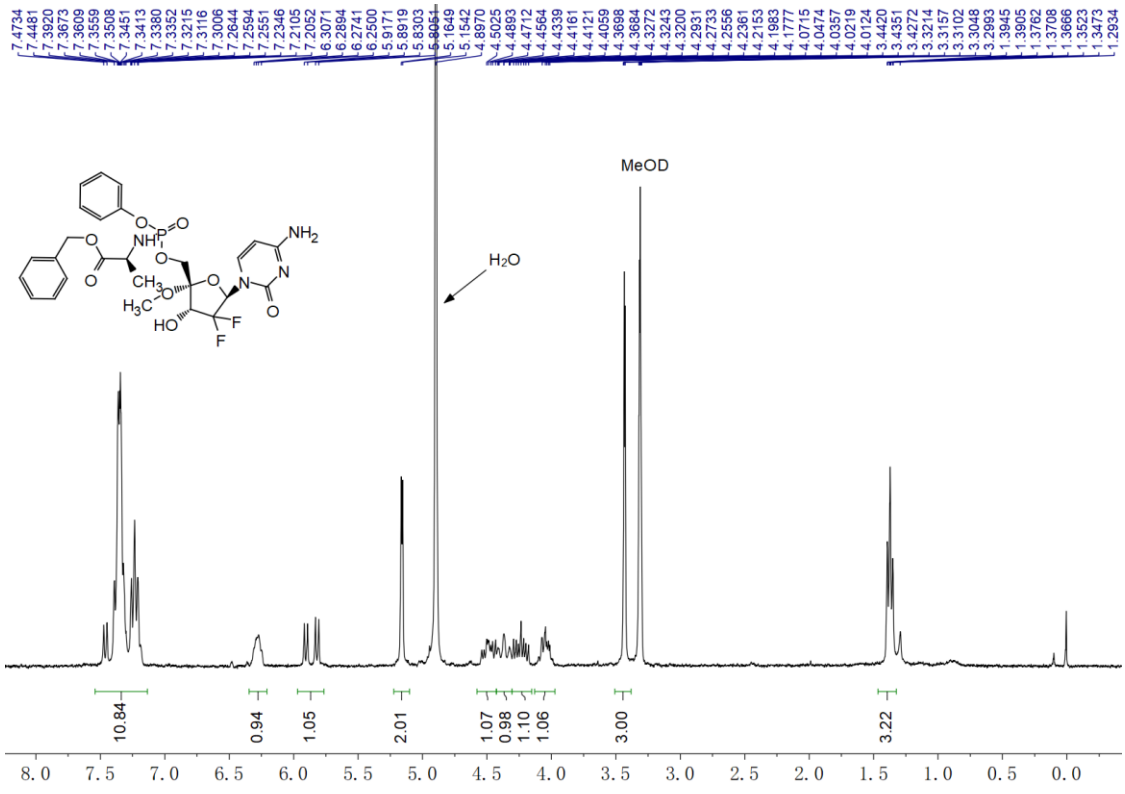
NMR spectra of compound **16c** in MeOD, ^{13}C NMR spectrum, 75MHz



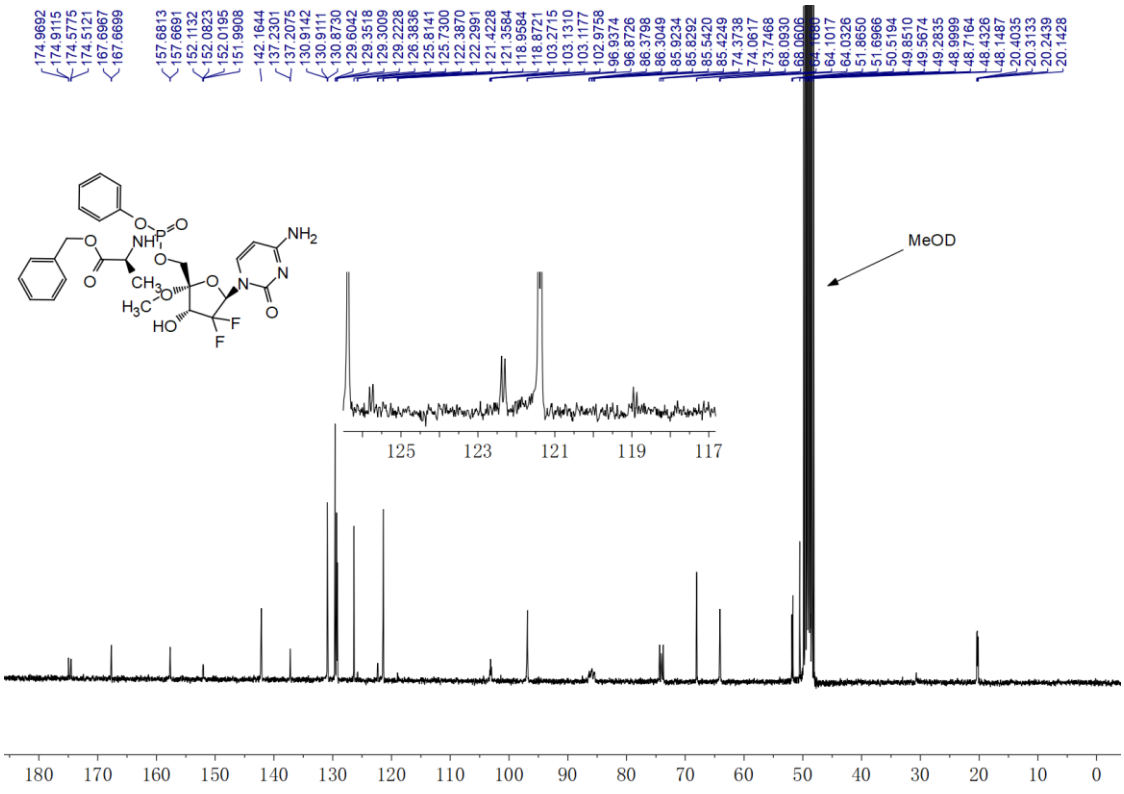
NMR spectra of compound **16c** in MeOD, ^{31}P NMR spectrum, 121MHz



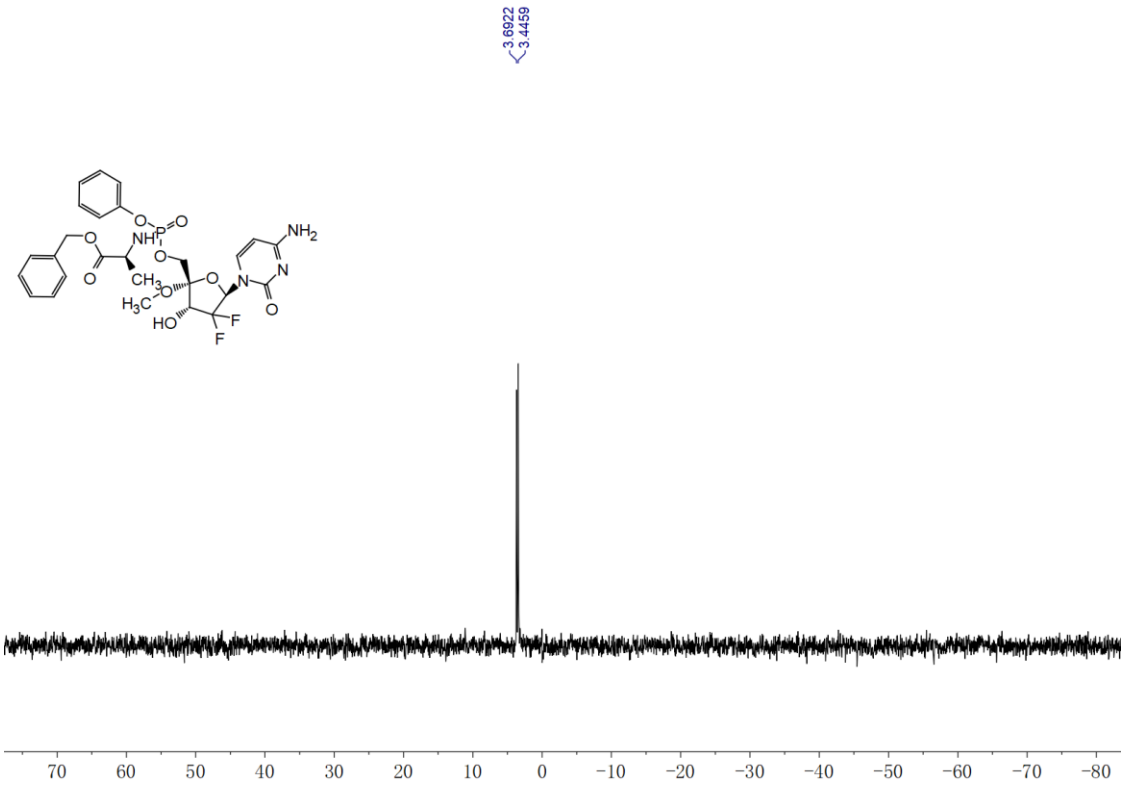
NMR spectra of compound **2a** in MeOD, ^{1}H NMR spectrum, 300MHz



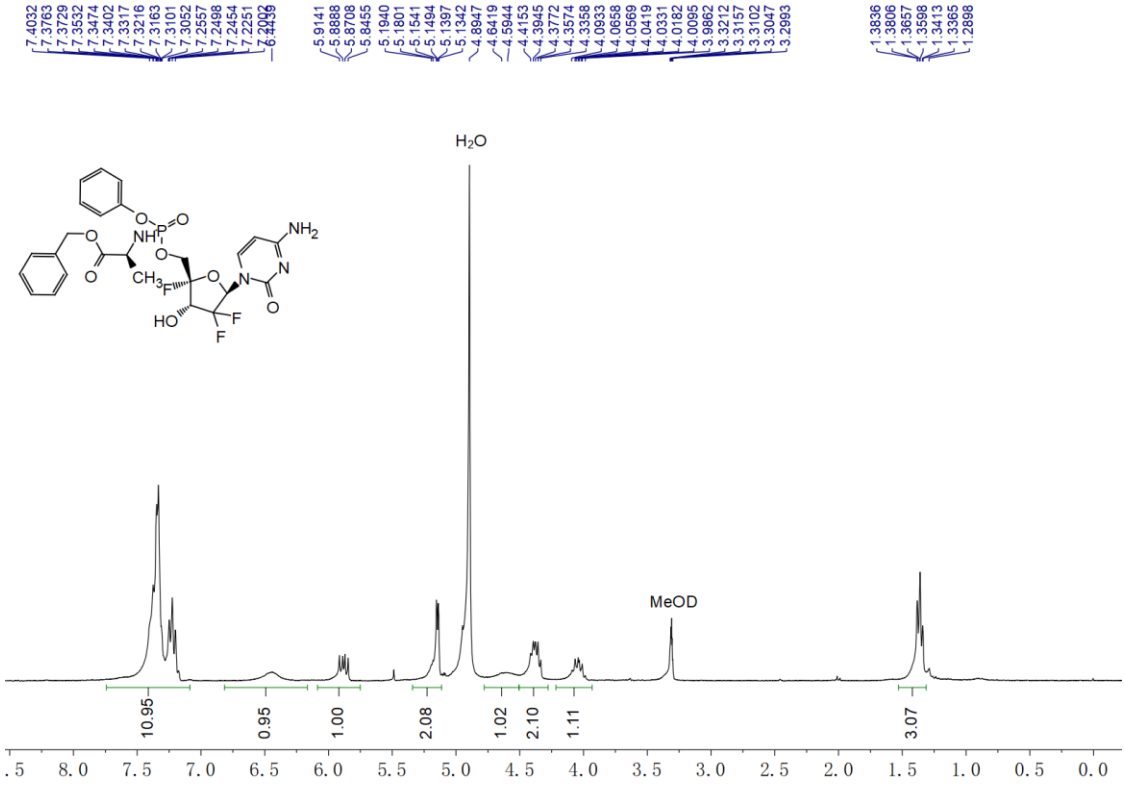
NMR spectra of compound **2a** in MeOD, ^{13}C NMR spectrum, 75MHz



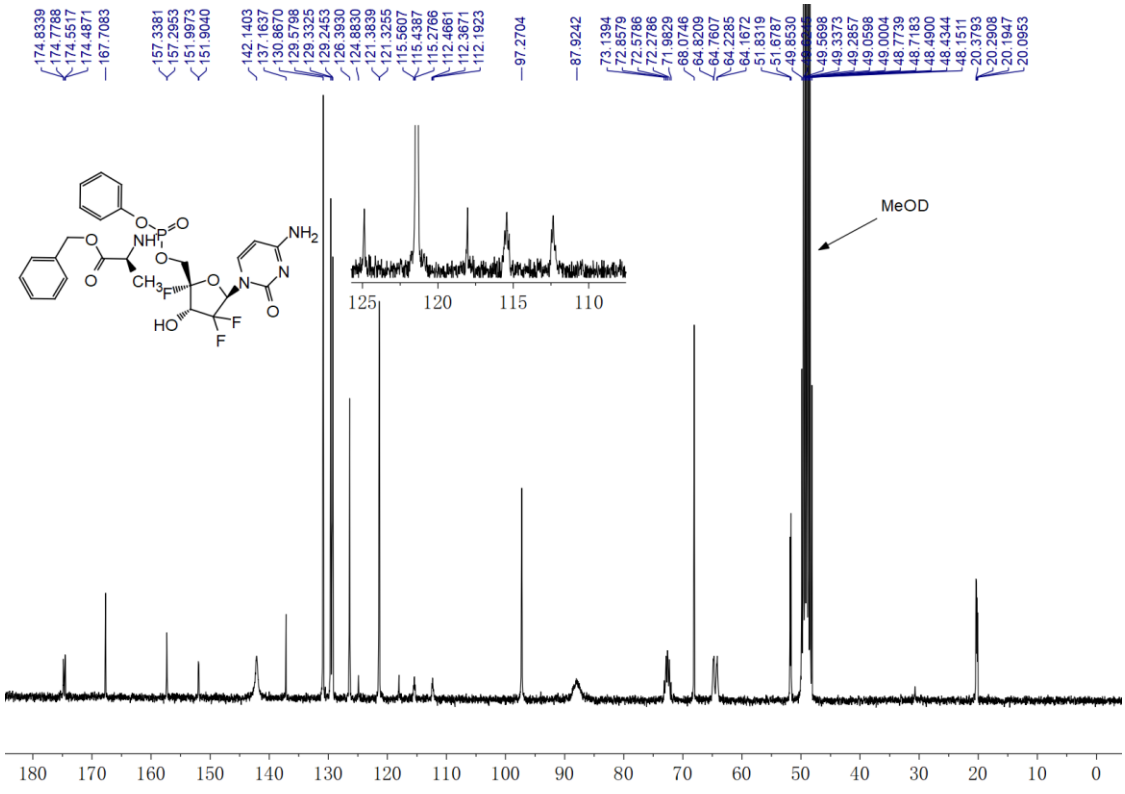
NMR spectra of compound **2a** in MeOD, ^{31}P NMR spectrum, 121MHz



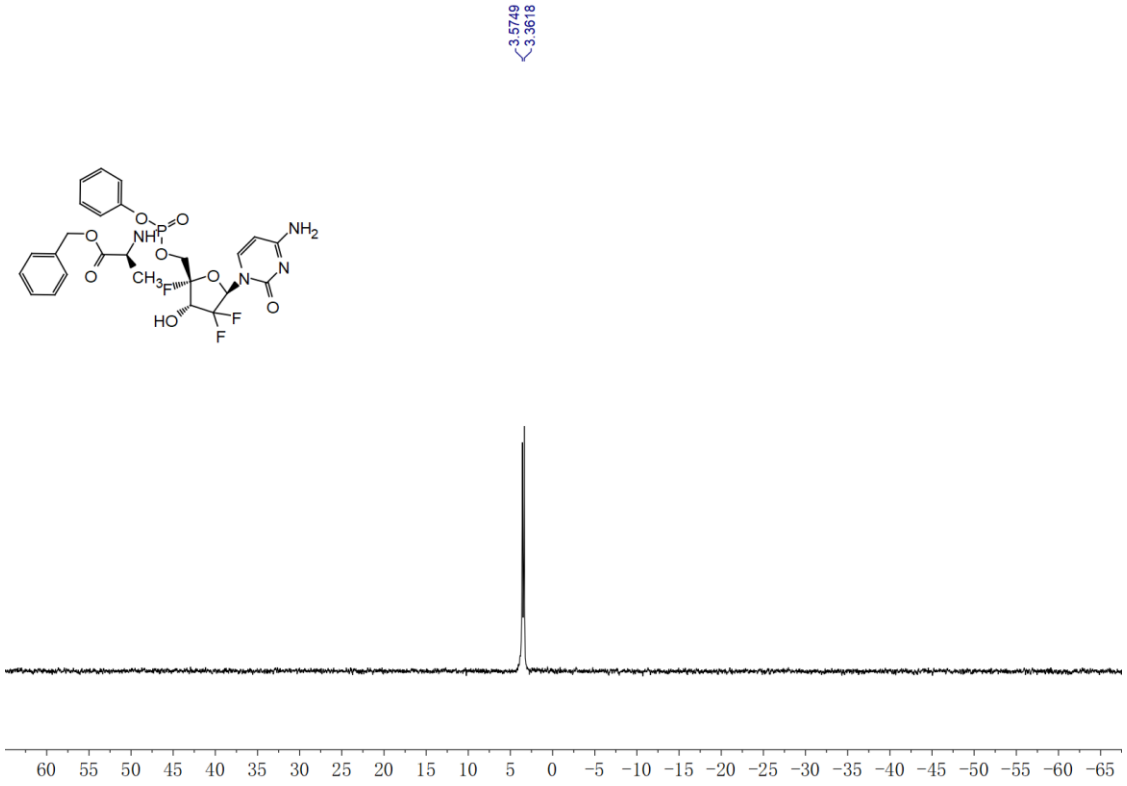
NMR spectra of compound **2b** in MeOD, ^{1}H NMR spectrum, 300MHz



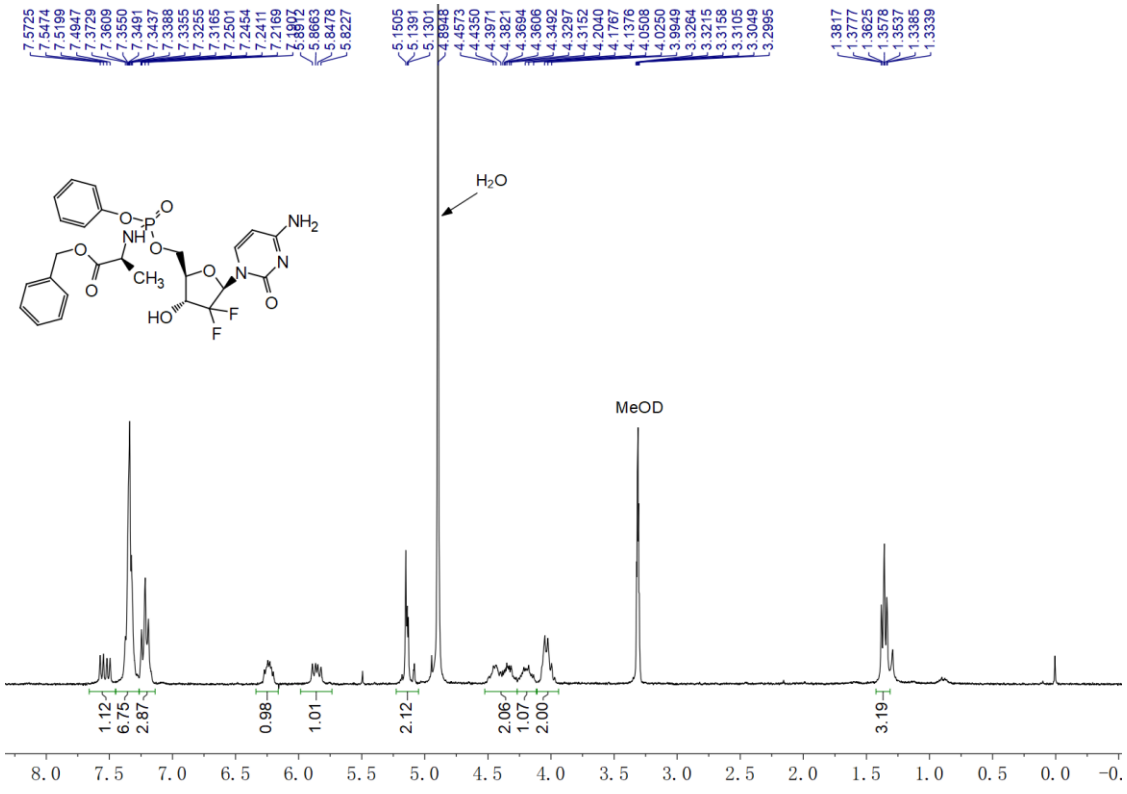
NMR spectra of compound **2b** in MeOD, ^{13}C NMR spectrum, 75MHz



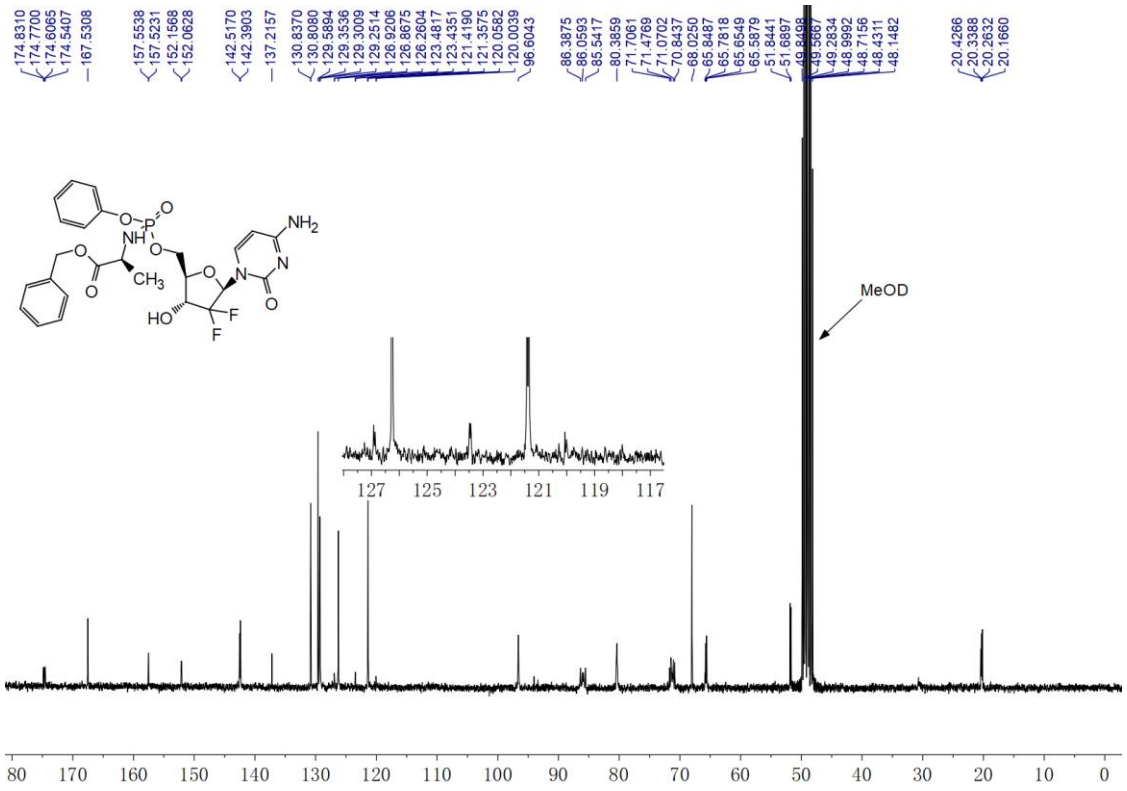
NMR spectra of compound **2b** in MeOD, ^{31}P NMR spectrum, 121MHz



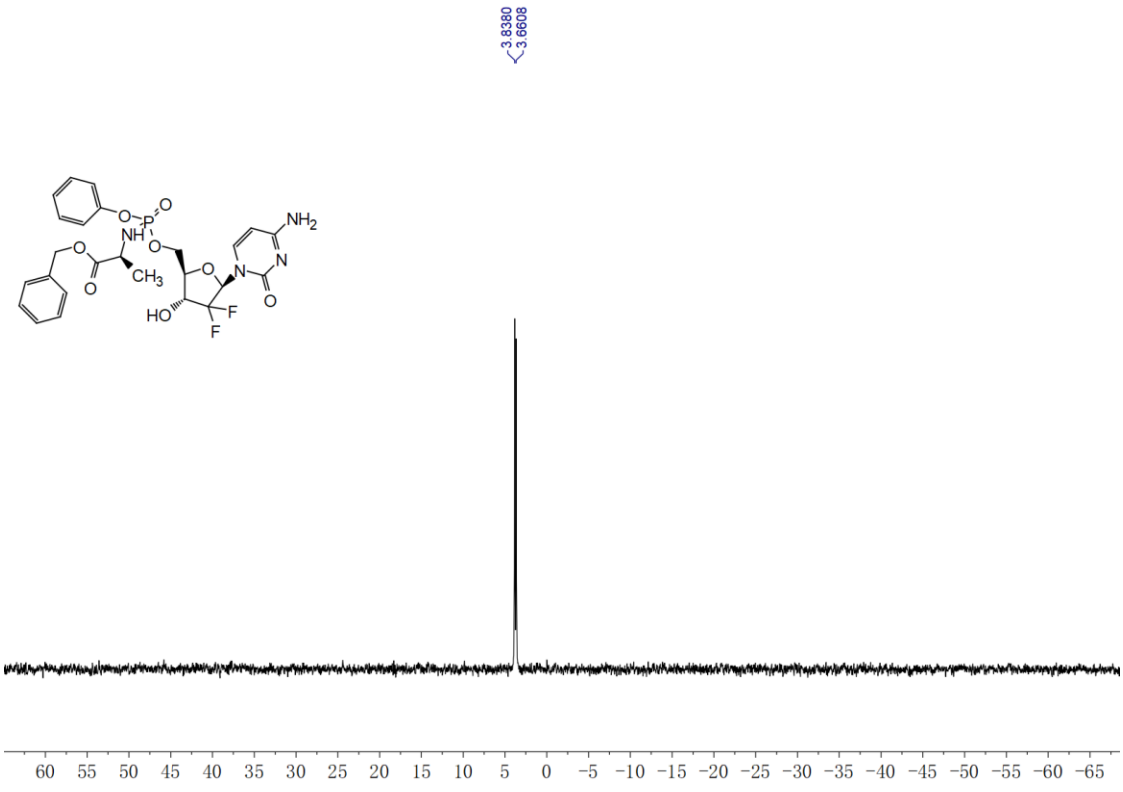
NMR spectra of compound **2c** in MeOD, ^{1}H NMR spectrum, 300MHz



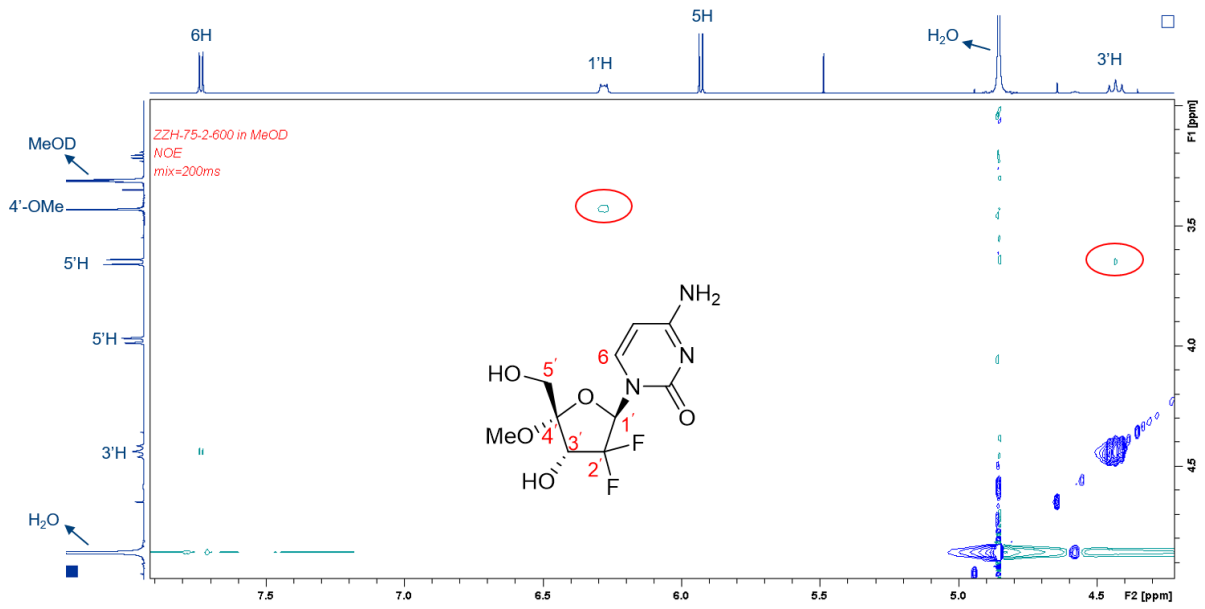
NMR spectra of compound **2c** in MeOD, ^{13}C NMR spectrum, 75MHz



NMR spectra of compound **2c** in MeOD, ^{31}P NMR spectrum, 121MHz



NMR spectra of compound **1a** in MeOD, NOESY NMR spectrum, 600MHz



NMR spectra of compound **1b** in MeOD, NOESY NMR spectrum, 600MHz