Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Supporting Information

Synthesis and Antiviral Evaluation of 4'-(1,2,3-Triazol-1-yl)thymidines

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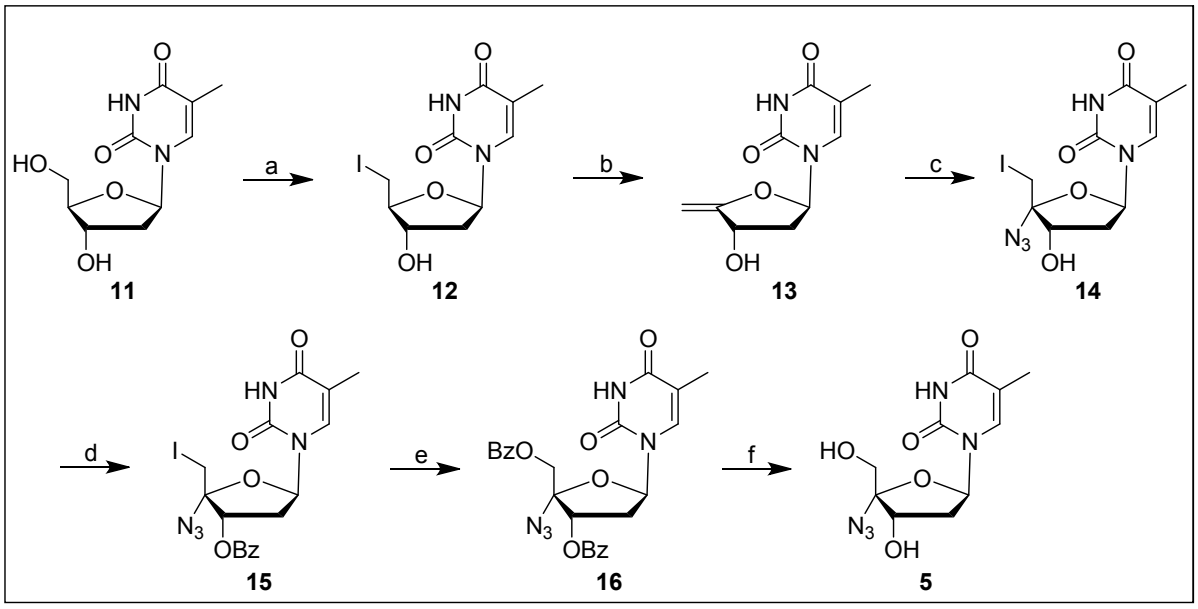
Table of contents

Chemistry Experimental

General Procedures. All commercial chemicals were used as supplied unless otherwise indicated. Dry solvents (THF, Et_2O, CH_2Cl_2 and DMF) were dispensed under argon from an anhydrous solvent system with two packed columns of neutral alumina or molecular sieves. Flash chromatography was performed on a Teledyne Combiflash RF-200 with RediSep columns (silica) and indicated mobile phase. All reactions were performed under an inert atmosphere of ultra-pure argon with oven-dried glassware. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 MHz spectrometer. Mass data were acquired on an Agilent TOF II TOS/MS spectrometer capable of ESI and APCI ion sources. Analysis of sample purity was performed on a Varian Prepstar SD-1 HPLC system with a Phenomenex Gemini, 5 micron C18 column (250mm x 4.6 mm). HPLC conditions**:** solvent A = H_2O, solvent B = MeCN; flow rate = 1.0 mL/min; compounds were eluted with a gradient of 5% MeCN/H_2O to 100% MeCN/H_2O for 25 min. Purity was determined by total absorbance at 254 nm. All tested compounds have a purity ≥ 96.



1-((2R,4S,5S)-4-Hydroxy-5-(iodomethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (12).[S1] Thymidine (5.0 g, 20 mmol), PPh_3 (6.5 g, 25 mmol) and imidazole (1.7 g, 25 mmol) were slurried in dry THF (50 mL). A solution of I_2 (5.7 g, 22 mmol) in dry THF (50 mL) was added slowly while the reaction temperature was maintained below 25 °C. The reaction was stirred at room temperature for 18 h. The reaction was stopped by adding water and the THF was removed in vacuo. The aqueous layer was extracted with EtOAc (3 x 150 mL) and the combined organic phases were washed with aqueous solution of $Na_2S_2O_3$, and water. The organic layer was dried over Na_2SO_4, filtered and concentrated to give the crude product. The crude product was purified by flash column chromatography (DCM/MeOH; 9:1) to afford **12** (5.6 g, 16 mmol, 77%) as a white solid. mp 180–183 ºC; ^{1}H NMR (CD_3OD, 600 MHz) δ 7.60 (s, 1H), 6.28 (t, J = 6.7 Hz, 1H), 4.31-4.29 (m, 1H), 3.86-3.83 (m, 1H), 3.51 (dd, J = 5.6 Hz, J = 10.2 Hz, 1H), 3.45 (dd, J = 5.4 Hz, J = 10.2 Hz, 1H), 2.34-2.24 (m, 2H), 1.90 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.7, 136.5, 110.4, 85.4, 84.8, 73.7, 38.6, 11.0, 5.2; HRMS-ESI(+) *m/z* calcd for $C_{10}H_{14}IN_2O_4$ 352.9998 $[M+H]^+$, found 352.9987.

1-((2R,4S)-4-Hydroxy-5-methylenetetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (13).[S1] To a solution of **12** (2.0 g, 5.7 mmol) in MeOH (30 mL) was added 0.5 M solution of NaOMe in MeOH (28.0 mL, 14.2 mmol) and stirred at 60 °C for 15 h. The reaction mixture was then added to a solution of *N*-methylmorpholinium mesylate in MeOH (prepared *in situ* by adding *N*-methylmorpholine (1.43 g, 14.2 mmol) to a solution of methanesulfonic acid (1.36 g, 14.2 mmol)). The reaction was concentrated in vacuo and the residue was extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with water, dried over Na_2SO_4, filtered and concentrated to give **13** (1.1 g, 4.6 mmol, 81%) as a white solid, without any further purification used for the next reaction. mp 189–191 ºC; ^{1}H NMR (CD_3OD, 600 MHz) δ 7.31 (s, 1H), 6.48 (t, J = 6.94 Hz, 1H), 4.78 (dd, J = 3.20 Hz, J =

6.3 Hz, 1H), 4.44 (s, 1H), 4.27 (s, 1H), 2.41-2.31 (m, 2H), 1.88 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.7, 149.5, 135.8, 116.1, 85.9, 82.6, 69.6, 38.3, 10.9; HRMS-ESI(+) *m/z* calcd for $C_{10}H_{13}N_2O_4$ 225.0875 $[M+H]^+$, found 225.0863.

1-((2R,4S,5S)-5-Azido-4-hydroxy-5-(iodomethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (14).[S1] Benzyltriethylammonium chloride (2.4 g, 10.1 mmol) and sodium azide (0.69 g, 10.1 mmol) were suspended in anhyd. CH_3CN (15 mL) and sonicated for a few minutes. The resulting suspension was filtered into anhyd. THF solution (15 mL) of compound **13** (1.2 g, 5.3 mmol). *N*-Methylmorpholine (0.1 g, 1 mmol) was added and the resulting solution was cooled on an ice-water bath. A solution of iodine (2.7 g, 10.1 mmol) in anhyd. THF (10 mL) was added dropwise over 30 min. The reaction mixture was stirred at 0–5 °C for another 2 h. The reaction was stopped by adding solution of $NaHCO_3$ and solution of $Na_2S_2O_3$. The THF was removed in vacuo and the residue was extracted with EtOAc (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated to give the crude product. The crude product was purified by flash column chromatography (DCM/MeOH; 9:1) to afford **14** (1.1 g, 2.8 mmol, 52%) as a 9:1 mixture of diastereoisomers. ^{1}H NMR (CD_3OD, 600 MHz) δ 7.48 (s, 1H), 6.34 (t, J = 6.5 Hz, 1H), 4.68 (t, J = 6.6 Hz, 1H), 3.66 (dd, J = 12.0 Hz, J = 24.0 Hz, 2H), 2.54-2.44 (m, 2H), 1.90 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 166.3, 152.2, 138.5, 112.4, 98.9, 86.4, 75.3, 38.2, 12.5, 7.2; HRMS-ESI(+) *m/z* calcd for $C_{10}H_{13}IN_5O_4$ 394.0012 $[M+H]^+$, found 394.0002.

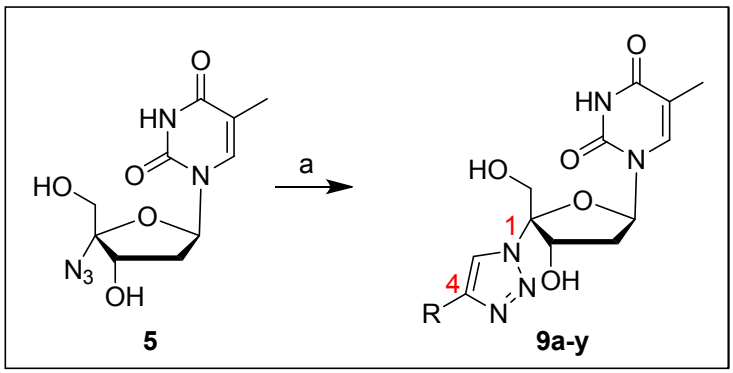
(2S,3S,5R)-2-Azido-2-(iodomethyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl benzoate (15).[S1] To a solution of **14** (1.1 g, 2.7 mmol) in dry pyridine (10 mL) was added catalytic amount of DMAP and followed by dropwise addition of benzoyl chloride (0.43 g, 3.1 mmol). The reaction mixture was stirred at room temperature for 3 h. The reaction was stopped by adding water and pyridine was removed under reduced pressure. The residue was extracted with EtOAc (3 x 50 mL) and the combined organic

phases were washed with dilute HCl, and $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated to give the crude product. The crude product was purified by flash column chromatography (DCM/MeOH; 9.5:0.5) to afford **15** (1.1 g, 2.2 mmol, 81%) as a solid. mp 170–172 ºC; ^{1}H NMR (CD_3OD, 600 MHz) δ 8.10 (d, J = 7.8 Hz, 2H), 7.66-7.64 (m, 1H), 7.57 (s, 1H), 7.51 (t, J = 8.4 Hz, 2H), 6.49 (dd, J = 6.1 Hz, J = 6.0 Hz, 1H), 5.90 (dd, J = 6.1 Hz, J = 6.0 Hz, 1H), 3.81 (s, 2H), 2.94-2.90 (m, 1H), 2.72-2.71 (m, 1H), 1.92 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 165.3, 164.7, 150.6, 137.3, 133.5, 129.5, 128.8, 128.3, 127.0, 110.9, 96.7, 85.8, 75.2, 35.0, 10.9, 5.7; HRMS-ESI(+) *m/z* calcd for $C_{17}H_{17}IN_5O_5$ 498.0274 $[M+H]^+$, found 498.0263.

(2R,3S,5R)-2-Azido-2-((benzoyloxy)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl benzoate (16).[S1] To a solution of compound **15** (1.45 g, 2.9 mmol) in DCM:H_2O (4:1, 30 mL), tetrabutylammonium hydrogensulfate (1.2g, 3.5 mmol), potassium phosphate dibasic (1.0 g, 5.8 mmol), *m*-chlorobenoic acid (5.0 g, 3.2 mmol) and *m*-chloroperbenzoic acid (2.0 g, 11.6 mmol) were added. The reaction mixture was stirred at room temperature for 15 h. The reaction was stopped by adding a solution of $Na_2S_2O_3$, and solution of $NaHCO_3$. The organic layer was separated and dried over Na_2SO_4, filtered and concentrated to give the crude product. The crude product was purified by flash column chromatography (DCM/MeOH; 9.5:0.5) to afford **16** (1.3 g, 16 mmol, 84%) as a foam. ^{1}H NMR (CD_3OD, 600 MHz) δ 8.02 (d, J = 7.6 Hz, 2H), 7.92-7.88 (m, 2H), 7.62-7.54 (m, 2H), 7.46-7.44 (m, 3H), 7.36 (t, J = 7.6 Hz, 1H), 6.42 (dd, J = 4.8 Hz, J = 7.8 Hz, 1H), 6.02 (t, J = 7.2 Hz, 1H), 4.78 (s, 2H), 2.92-2.87 (m, 1H), 2.76-2.72 (m, 1H), 1.79 (s, 3H).

4'-Azidothymidine (5).[S1] Compound **16** (1.2 g, 2.3 mmol) was dissolved in 7*N* NH_3 in methanol (110 mL) and stirred at room temperature for overnight. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography

(DCM/MeOH; 9:1) to afford **5** (0.51 g, 1.8 mmol, 79%) as a white solid. mp 164–165 ºC; ^{1}H NMR (CD_3OD, 600 MHz) δ 7.69 (s, 1H), 6.38 (t, J = 6.0 Hz, 1H), 4.56 (t, J = 7.2 Hz, 1H), 3.79 (d, J = 11.4Hz, 1H), 3.72 (d, J = 11.4 Hz, 1H), 2.42-2.40 (m, 2H), 1.87 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.7, 136.6, 110.5, 99.8, 84.4, 71.0, 62.4, 37.3, 10.9; HRMS-ESI(+) *m/z* calcd for $C_{10}H_{13}N_5O_5Na$ 306.0814 $[M+Na]^+$, found 306.0803.



General procedure for the synthesis of 4'-(4-substituted-1*H*-1,2,3-triazol-1-yl)thymidine derivatives via CuACC.[S2] To the mixture of 4'-azidothymidine **5** (1.0 equiv.) and alkyne (2.0 equiv.) in 4.0 mL of THF/H_2O (3:1) was added freshly prepared 1 M solution of sodium ascorbate (0.1 equiv.) in water, followed by the addition of freshly prepared 1 M solution of $CuSO_4$• $5H_2O$ (0.06 equiv.) in water. The heterogeneous reaction mixture was stirred at 60 ºC for 5-7 days and monitored by TLC and MS. It wss required to add every 24 h fresh catalyst and sodium ascorbate. After the completion, the reaction was evaporated to dryness. The crude product was purified by column chromatography, eluted with 5-10% MeOH in DCM, yielded the desired triazole.

1-((2R,4S,5R)-5-(4-Cyclopropyl-1*H*-1,2,3-triazol-1-yl)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9a). The reaction of 4'-azidothymidine **5** (35 mg, 0.12 mmol) with alkyne (16 mg, 0.24 mmol) yielded compound **9a** (28 mg, 65%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD) δ 7.69 (s, 2H), 6.56 (t, J = 6.8 Hz, 1H), 4.80 (t, J = 6.2 Hz, 1H), 4.25 (d, J = 12.0 Hz, 1H), 3.94 (d, J = 12.0 Hz, 1H), 2.51-2.47 (m, 1H), 2.34-2.27 (m, 1H), 1.87-1.82 (m, 1H), 1.80 (s, 3H), 0.85 (d, J =

7.8 Hz, 2H), 0.69 (d, *J* = 4.8 Hz, 2H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.8, 148.9, 137.2, 119.9, 110.5, 99.9, 86.1, 71.5, 63.1, 37.2, 11.0, 6.6, 5.8; HRMS-ESI(+) *m/z* calcd for $C_{15}H_{20}N_5O_5$ 350.1463 $[M+H]^+$, found 350.1460.

1-((2R,4S,5R)-5-(4-Cyclopentyl-1*H*-1,2,3-triazol-1-yl)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9b). The reaction of 4'-azidothymidine **5** (30 mg, 0.10 mmol) with alkyne (20 mg, 0.21 mmol) yielded compound **9b** (21 mg, 52%) as a white solid. mp 124–129 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 7.73 (s, 1H), 7.71 (s, 1H), 6.57 (t, *J* = 6.6 Hz, 1H), 4.81 (t, *J* = 6.6 Hz, 1H), 4.27 (d, *J* = 12.6 Hz, 1H), 3.97 (d, *J* = 12.6 Hz, 1H), 3.08-3.06 (m, 1H), 2.48-2.51 (m, 1H), 2.31-2.35 (m, 1H), 2.02-2.06 (m, 2H), 1.80 (s, 3H), 1.59-1.72 (m, 6H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.9, 137.2, 120.0, 110.5, 99.9, 86.1, 71.5, 63.1, 37.2, 36.4, 32.7, 24.6, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{17}H_{24}N_5O_5$ 378.1777 $[M+H]^+$, found 378.1765.

1-((2R,4S,5R)-5-(4-Cyclohexyl-1*H*-1,2,3-triazol-1-yl)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9c). The reaction of 4'-azidothymidine **5** (25 mg, 0.08 mmol) with alkyne (19 mg, 0.18 mmol) yielded compound **9c** (18 mg, 52%) as a white solid. mp 159–163 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 7.81 (s, 2H), 6.67 (t, *J* = 6.7 Hz, 1H), 4.90 (t, *J* = 6.6 Hz, 1H), 4.36 (d, *J* = 12.2 Hz, 1H), 4.06 (d, *J* = 12.2 Hz, 1H), 2.58-2.74 (m, 1H), 2.54-2.57 (m, 1H), 2.40-2.47 (m, 1H), 2.02-2.04 (m, 2H), 1.90 (s, 3H), 1.83-1.72 (m, 3H), 1.28-1.48 (m, 5H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.9, 137.2, 119.7, 110.5, 99.9, 86.1, 71.5, 63.1, 37.2, 35.1, 32.6, 32.6, 25.8, 25.6, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{18}H_{26}N_5O_5$ 392.1934 $[M+H]^+$, found 392.1922.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9d). The reaction of 4'-azidothymidine **5** (35 mg, 0.12 mmol) with alkyne (25 mg, 0.24 mmol) yielded compound **9d** (35 mg, 72%) as a white solid. mp 114–117 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.44 (s, 1H),

7.81-7.85 (m, 3H), 7.42 (t, *J* = 6.8 Hz, 2H), 7.33 (t, *J* = 7.2 Hz, 1H), 6.75 (t, *J* = 6.6 Hz, 1H), 4.96 (t, *J* = 6.1 Hz, 1H), 4.44 (d, *J* = 12.6 Hz, 1H), 4.16 (d, *J* = 12.6 Hz, 1H), 2.61-2.65 (m, 1H), 2.49-2.45 (m, 1H), 1.91 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.9, 146.5, 137.2, 130.4, 128.5, 127.8, 125.3, 120.2, 110.6, 100.3, 86.4, 71.6, 63.2, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{18}H_{20}N_5O_5$ 386.1464 $[M+H]^+$, found 386.1443.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(thiophen-3-yl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9e). The reaction of 4'-azidothymidine **5** (30 mg, 0.10 mmol) with alkyne (23 mg, 0.21 mmol) yielded compound **9e** (26 mg, 63%) as a white solid. mp 129–134 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.36 (s, 1H), 7.82 (s, 1H), 7.78 (s, 1H), 7.49-7.53 (m, 2H), 6.74 (t, *J* = 6.6 Hz, 1H), 4.96 (t, *J* = 6.1 Hz, 1H), 4.43 (d, *J* = 12.0 Hz, 1H), 4.15 (d, *J* = 12.0 Hz, 1H), 2.61-2.65 (m, 1H), 2.49-2.45 (m, 1H), 1.91 (s, 3H), 1.13-1.12 (m, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.9, 142.8, 137.2, 131.4, 126.1, 125.3, 120.7, 120.0, 110.6, 100.3, 86.3, 71.6, 63.1, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{16}H_{18}N_5O_5S$ 392.1030 $[M+H]^+$, found 392.1015.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9f). The reaction of 4'-azidothymidine **5** (35 mg, 0.12 mmol) with alkyne (29 mg, 0.24 mmol) yielded compound **9f** (30 mg, 61%) as a white solid. mp 108–114 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.37 (s, 1H), 7.82 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 6.74 (t, *J* = 6.6 Hz, 1H), 4.95 (t, *J* = 6.3 Hz, 1H), 4.43 (d, *J* = 12.4 Hz, 1H), 4.15 (d, *J* = 12.4 Hz, 1H), 2.60-2.64 (m, 1H), 2.47-2.44 (m, 1H), 2.36 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.9, 146.5, 137.2, 129.1, 127.5, 125.3, 119.8, 110.6, 100.2, 86.3, 71.6, 63.2, 37.2, 19.8, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{19}H_{22}N_5O_5$ 400.1621 $[M+H]^+$, found 400.1614.

1-((2R,4S,5R)-5-(4-(4-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9g). The

reaction of 4'-azidothymidine **5** (20 mg, 0.07 mmol) with alkyne (18 mg, 0.14 mmol) yielded compound **9g** (16 mg, 56%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD) δ 8.33 (s, 1H), 7.78 (t, J = 6.0 Hz, 2H), 7.72 (s, 1H), 7.07 (t, J = 8.4 Hz, 2H), 6.65 (t, J = 6.6 Hz, 1H), 4.85 (t, J = 6.4 Hz, 1H), 4.34 (d, J = 12.0 Hz, 1H), 4.06 (d, J = 12.0 Hz, 1H), 2.58-2.53 (m, 1H), 2.38-2.34 (m, 1H), 1.81 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.9, 145.6, 137.2, 127.2, 126.8, 120.1, 115.4, 115.2, 110.6, 100.3, 86.3, 71.6, 63.2, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{18}H_{19}FN_5O_5$ 404.1371 $[M+H]^+$, found 404.1356.

1-((2R,4S,5R)-5-(4-(4-Bromophenyl)-1*H*-1,2,3-triazol-1-yl)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9h). The reaction of 4'-azidothymidine **5** (30 mg, 0.10 mmol) with alkyne (38 mg, 0.21 mmol) yielded compound **9h** (26 mg, 54%) as a white solid. mp 136–142 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.48 (s, 1H), 7.82 (s, 1H), 7.78 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 6.74 (t, J = 6.8 Hz, 1H), 4.94 (t, J = 6.3 Hz, 1H), 4.43 (d, J = 11.4 Hz, 1H), 4.15 (d, J = 11.4 Hz, 1H), 2.61-2.64 (m, 1H), 2.47-2.44 (m, 1H), 1.91 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.9, 145.4, 137.2, 131.6, 129.6, 127.0, 121.4, 120.5, 110.6, 100.3, 86.3, 71.6, 63.2, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{18}H_{19}BrN_5O_5$ 464.0570 $[M+H]^+$, found 464.0554.

***N*-(4-(1-((2R,3S,5R)-3-Hydroxy-2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)phenyl)acetamide (9i)**. The reaction of 4'-azidothymidine **5** (20 mg, 0.07 mmol) with alkyne (22 mg, 0.14 mmol) yielded compound **9i** (19 mg, 63%) as a white solid. mp 138–144 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.31 (s, 1H), 7.73 (s, 1H), 7.71 (d, J = 7.8 Hz, 2H), 7.70 (d, J = 7.4 Hz, 2H), 6.65 (t, J = 6.4 Hz, 1H), 4.85 (t, J = 6.4 Hz, 1H), 4.33 (d, J = 12.6 Hz, 1H), 4.05 (d, J = 12.6 Hz, 1H), 2.58-2.52 (m, 1H), 2.38-2.34 (m, 1H), 2.04 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 172.7, 164.8, 156.1, 150.8, 137.2, 125.7, 119.9, 111.4,

100.2, 86.3, 71.6, 63.2, 37.2, 25.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{20}H_{23}N_6O_6$ 443.1679 $[M+H]^+$, found 443.1666.

1-((2R,4S,5R)-5-(4-(4-(Dimethylamino)phenyl)-1*H*-1,2,3-triazol-1-yl)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9j). The reaction of 4'-azidothymidine **5** (30 mg, 0.10 mmol) with alkyne (31 mg, 0.21 mmol) yielded compound **(9j)** (25 mg, 56%) as a pale yellow solid. mp 148–152 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.24 (s, 1H), 7.82 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.4 Hz, 2H), 6.72 (t, J = 6.6 Hz, 1H), 4.94 (t, J = 6.3 Hz, 1H), 4.42 (d, J = 12.0 Hz, 1H), 4.13 (d, J = 12.0 Hz, 1H), 2.96 (s, 6H), 2.60-2.64 (m, 1H), 2.48-2.44 (m, 1H), 1.91 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.9, 150.9, 147.1, 137.2, 126.2, 118.5, 112.4, 110.5, 100.1, 86.2, 71.6, 63.1, 39.3, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{20}H_{25}N_6O_5$ 429.1887 $[M+H]^+$, found 429.1868.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9k). The reaction of 4'-azidothymidine **5** (20 mg, 0.07 mmol) with alkyne (18 mg, 0.14 mmol) yielded compound **9k** (18 mg, 61%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD) δ 8.23 (s, 1H), 7.72 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 6.89 (d, J = 8.4 Hz, 2H), 6.64 (t, J = 6.8 Hz, 1H), 4.85 (t, J = 6.3 Hz, 1H), 4.33 (d, J = 12.6 Hz, 1H), 4.05 (d, J = 12.6 Hz, 1H), 3.72 (s, 3H), 2.50-2.55 (m, 1H), 2.37-2.33 (m, 1H), 1.81 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 159.8, 150.9, 137.2, 126.6, 122.8, 119.4, 113.9, 110.6, 100.2, 86.3, 71.6, 63.2, 54.3, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{19}H_{22}N_5O_6$ 416.1571 $[M+H]^+$, found 416.1559.

1-((2R,4S,5R)-4-Hydroxy-5-(4-(hydroxy(phenyl)methyl)-1*H*-1,2,3-triazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9l). The reaction of 4'-azidothymidine **5** (30 mg, 0.10 mmol) with alkyne (26 mg, 0.21 mmol) yielded compound **9l** (26 mg, 58%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD) δ 7.89 (s, 1H), 7.77 (d, J = 5.4 Hz, 1H), 7.44 (d, J = 7.8 Hz, 2H), 7.33 (t, J = 7.2 Hz, 2H), 7.26 (t, J = 6.0 Hz,

1H), 6.65 (t, *J* = 6.6 Hz, 1H), 5.94 (s, 1H), 4.89 (t, *J* = 6.6 Hz, 1H), 4.33 (dd, *J* = 3.6 Hz, *J* = 12.4 Hz, 1H), 4.07 (d, *J* = 12.6 Hz, 1H), 2.61-2.57 (m, 1H), 2.39-2.44 (m, 1H), 1.89 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.8, 142.7, 137.2, 128.0, 127.3, 127.2, 126.3, 110.6, 100.2, 86.3, 71.6, 68.7, 63.2, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{19}H_{22}N_5O_6$ 416.1563 $[M+H]^+$, found 416.1556.

1-((2R,4S,5R)-5-(4-([1,1'-Biphenyl]-4-yl)-1*H*-1,2,3-triazol-1-yl)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9m). The reaction of 4'-azidothymidine **5** (35 mg, 0.12 mmol) with alkyne (44 mg, 0.24 mmol) yielded compound **9m** (30 mg, 53%) as a white solid. mp 145–148 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.48 (s, 1H), 7.94 (d, *J* = 7.2 Hz, 2H), 7.83 (s, 1H), 7.69 (d, *J* = 7.1 Hz, 2H), 7.65 (d, *J* = 7.1 Hz, 2H), 7.43 (t, *J* = 7.8 Hz, 2H), 7.33 (t, *J* = 7.8 Hz, 1H), 6.76 (t, *J* = 6.6 Hz, 1H), 4.96 (t, *J* = 6.1 Hz, 1H), 4.45 (d, *J* = 12.0 Hz, 1H), 4.17 (d, *J* = 12.0 Hz, 1H), 2.66-2.62 (m, 1H), 2.50-2.47 (m, 1H), 1.91 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 166.4, 152.5, 147.7, 142.4, 138.7, 130.8, 129.9, 128.6, 128.5, 127.9, 127.2, 121.7, 112.0, 101.9, 87.8, 73.2, 64.8, 38.8, 12.6; HRMS-ESI(+) *m/z* calcd for $C_{24}H_{24}N_5O_5$ 462.1777 $[M+H]^+$, found 462.1769.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(4-phenoxyphenyl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9n). The reaction of 4'-azidothymidine **5** (30 mg, 0.10 mmol) with alkyne (41 mg, 0.21 mmol) yielded compound **9n** (28 mg, 55%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD) δ 8.29 (s, 1H), 7.73-7.71 (m, 3H), 7.26 (t, *J* = 6.8 Hz, 2H), 7.02 (t, *J* = 7.2 Hz, 1H), 6.93-6.91 (m, 4H), 6.64 (t, *J* = 6.6 Hz, 1H), 4.86 (t, *J* = 6.1 Hz, 1H), 4.33 (d, *J* = 12.4 Hz, 1H), 4.06 (d, *J* = 12.6 Hz, 1H), 2.51-2.55 (m, 1H), 2.39-2.36 (m, 1H), 1.81 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 157.5, 150.9, 146.0, 137.2, 129.5, 126.9, 125.4, 123.3, 119.8, 118.7, 118.4, 110.6, 100.3, 86.4, 71.6, 63.2, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{24}H_{24}N_5O_6$ 478.1727 $[M+H]^+$, found 478.1719.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(6-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9o). The reaction of 4'-azidothymidine **5** (50 mg, 0.18 mmol) with alkyne (64 mg, 0.35 mmol) yielded compound **9o** (27 mg, 33%) as a white solid. mp 164–169 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.52 (s, 1H), 8.28 (s, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.84-7.80 (m, 3H), 7.26 (s, 1H), 7.16 (d, J = 9.0 Hz, 1H), 6.78 (t, J = 9.0 Hz, 1H), 4.98 (t, J = 6.1 Hz, 1H), 4.47 (d, J = 12.0 Hz, 1H), 4.19 (d, J = 12.0 Hz, 1H), 3.92 (s, 3H), 2.63-2.67 (m, 1H), 2.51-2.46 (m, 1H), 1.93 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 158.1, 155.3, 146.7, 137.2, 134.6, 129.2, 127.2, 125.6, 123.8, 120.1, 118.9, 113.7, 110.5, 105.4, 86.3, 71.6, 63.2, 54.3, 48.4, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{23}H_{24}N_5O_6$ 466.1727 $[M+H]^+$, found 466.1723.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(6-methoxynaphthalen-2-yl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9p). The reaction of 4'-azidothymidine **5** (40 mg, 0.14 mmol) with alkyne (51 mg, 0.28 mmol) yielded compound **9p** (44 mg, 68%) as a white solid. mp 124–128 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.51 (s, 1H), 8.25 (s, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.82-7.77 (m, 3H), 7.23 (s, 1H), 7.14 (dd, J = 1.8 Hz, J = 9.0 Hz, 1H), 6.77 (t, J = 7.2 Hz, 1H), 4.97 (t, J = 6.2 Hz, 1H), 4.46 (d, J = 12.1 Hz, 1H), 4.19 (d, J = 12.1 Hz, 1H), 3.90 (s, 3H), 2.47-2.67 (m, 2H), 1.93 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.9, 158.1, 150.9, 137.2, 134.6, 129.2, 126.8, 123.8, 118.9, 110.1, 105.4, 100.3, 86.3, 73.4, 71.6, 63.2, 54.3, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{23}H_{24}N_5O_6$ 466.1727 $[M+H]^+$, found 466.1724.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(phenanthren-9-yl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (**9q**). The reaction of 4'-azidothymidine **5** (20 mg, 0.07 mmol) with alkyne (28 mg, 0.14 mmol) yielded compound **9q** (12 mg, 36%) as a white solid. mp 144–148 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.83 (d, J = 8.4 Hz, 1H), 8.77 (d, J = 8.4 Hz, 1H), 8.43 (s, 1H), 8.27 (d, J = 7.8 Hz, 1H), 7.97 (s, 1H),

7.94 (d, *J* = 7.4 Hz, 1H), 7.82 (s, 1H), 7.69-7.72 (m, 2H), 7.59-7.63 (m, 2H), 6.78 (t, *J* = 6.6 Hz, 1H), 5.02 (t, *J* = 6.2 Hz, 1H), 4.50 (d, *J* = 12.0 Hz, 1H), 4.26 (d, *J* = 12.0 Hz, 1H), 2.70-2.67 (m, 1H), 2.55-2.52 (m, 1H), 1.91 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.9, 150.9, 145.3, 137.3, 131.2, 130.6, 130.3, 130.1, 128.5, 128.2, 127.0, 126.6, 126.5, 125.9, 123.4, 122.7, 122.2, 110.5, 100.5, 86.6, 71.8, 63.3, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{26}H_{24}N_5O_5$ 486.1778 $[M+H]^+$, found 486.1765.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(ferrocen-1-yl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9r). The reaction of 4'-azidothymidine **5** (25 mg, 0.08 mmol) with alkyne (37 mg, 0.18 mmol) yielded compound **9r** (23 mg, 54%) as a brown solid. ^{1}H NMR (600 MHz, CD_3OD) δ 8.12 (s, 1H), 7.83 (s, 1H), 6.75 (t, *J* = 6.2 Hz, 1H), 4.93 (t, *J* = 6.4 Hz, 1H), 4.78 (s, 2H), 4.43 (d, *J* = 12.2 Hz, 1H), 4.32 (s, 2H), 4.17 (d, *J* = 12.2 Hz, 1H), 4.06 (s, 5H), 2.59-2.64 (m, 1H), 2.48-2.43 (m, 1H), 1.92 (s, 2H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 137.2, 119.4, 110.5, 100.2, 86.3, 74.6, 69.2, 68.3, 66.2, 61.2, 37.4, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{22}H_{22}FeN_5O_5$ 493.0970 $[M+H]^+$, found 493.1033.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9s). The reaction of 4'-azidothymidine **5** (50 mg, 0.17 mmol) with alkyne (20 mg, 0.35 mmol) yielded compound **9s** (35 mg, 55%) as a white solid. mp 171–174 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.04 (s, 1H), 7.80 (s, 1H), 6.69 (t, *J* = 6.8 Hz, 1H), 4.93 (t, *J* = 6.4 Hz, 1H), 4.70 (s, 2H), 4.37 (d, *J* = 12.0 Hz, 1H), 4.09 (d, *J* = 12.0 Hz, 1H), 2.64-2.59 (m, 1H), 2.41-2.45 (m, 1H), 1.91 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.9, 150.9, 137.2, 122.2, 110.6, 100.1, 86.2, 71.5, 63.2, 55.0, 37.2, 28.1, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{13}H_{17}N_5O_6Na$ 362.1077 $[M+Na]^+$, found 362.1058.

1-((2R,4S,5R)-4-Hydroxy-5-(4-(1-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9t). The reaction of 4'-azidothymidine **5** (30 mg, 0.10 mmol) with alkyne (15 mg, 0.21 mmol) yielded compound **9t** (28 mg, 70%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD) δ 7.96 (s, 1H), 7.70 (s, 1H), 6.65 (t, J = 6.4 Hz, 1H), 4.96 (br s, 1H), 4.88 (t, J = 6.6 Hz, 1H), 4.33 (d, J = 12.4 Hz, 1H), 4.04 (d, J = 12.4 Hz, 1H), 2.55-2.58 (m, 1H), 2.36-2.39 (m, 1H), 1.87 (s, 3H), 1.52 (br s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 137.2, 115.2, 100.1, 86.2, 80.3, 71.5, 63.1, 48.4, 37.2, 22.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{14}H_{19}N_5O_6Na$ 376.1233 $[M+Na]^+$, found 376.1226.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-(phenoxymethyl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9u). The reaction of The reaction of 4'-azidothymidine **5** (40 mg, 0.14 mmol) with alkyne (37 mg, 0.28 mmol) yielded compound **9u** (35 mg, 59%) as a white foam. ^{1}H NMR (600 MHz, CD_3OD) δ 8.20 (s, 1H), 7.80 (s, 1H), 7.28 (t, J = 7.1 Hz, 2H), 7.01 (d, J = 7.2 Hz, 2H), 6.95 (t, J = 7.2 Hz, 1H), 6.70 (t, J = 6.8 Hz, 1H), 5.18 (s, 2H), 4.93 (t, J = 6.4 Hz, 1H), 4.39 (d, J = 12.0 Hz, 1H), 4.12 (d, J = 12.0 Hz, 1H), 2.64-2.59 (m, 1H), 2.41-2.45 (m, 1H), 1.91 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 158.4, 150.8, 137.2, 129.1, 123.4, 120.8, 114.4, 110.6, 100.3, 86.4, 71.6, 63.2, 60.9, 32.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{19}H_{22}N_5O_6$ 416.1570 $[M+H]^+$, found 416.1608.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-((phenylthio)methyl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9v). The reaction of 4'-azidothymidine **5** (35 mg, 0.12 mmol) with alkyne (37 mg, 0.24 mmol) yielded compound **9v** (28 mg, 53%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD) δ 7.78 (s, 2H), 7.34 (d, J = 7.2 Hz, 2H), 7.28 (t, J = 7.1 Hz, 2H), 7.21 (t, J = 7.2 Hz, 1H), 6.60 (t, J = 6.8 Hz, 1H), 4.89 (t, J = 6.2 Hz, 1H), 4.31 (d, J = 12.0 Hz, 1H), 4.21 (s, 2H), 4.01 (d, J = 12.0 Hz,

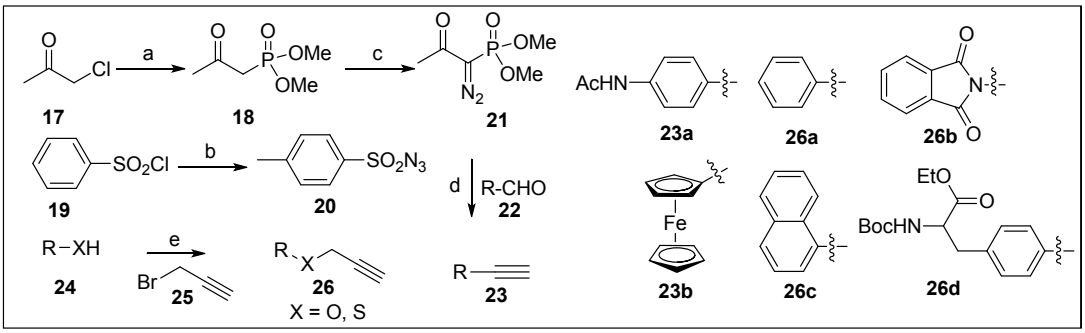
1H), 2.55-2.59 (m, 1H), 2.34-2.37 (m, 1H), 1.90 (s, 3H); HRMS-ESI(+) *m/z* calcd for $C_{19}H_{22}N_5O_5S$ 432.1342 $[M+H]^+$, found 432.1322.

Ethyl-2-((*tert*-butoxycarbonyl)amino)-3-(4-((1-((2R,3S,5R)-3-hydroxy-2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)propanoate (9w). The reaction of 4'-azidothymidine **5** (40 mg, 0.14 mmol) with alkyne (98 mg, 0.28 mmol) yielded compound **9w** (50 mg, 56%) as a white foam. ^{1}H NMR (600 MHz, CD_3OD) δ 8.18 (s, 1H), 7.80 (s, 1H), 7.13 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 6.69 (t, J = 7.2 Hz, 1H), 5.15 (s, 2H), 4.93 (t, J = 6.4 Hz, 1H), 4.39 (d, J = 12.0 Hz, 1H), 4.28 (t, J = 6.2 Hz, 1H), 4.09-4.13 (m, 3H), 2.87-3.09 (m, 2H), 2.60-2.69 (m, 1H), 2.41-2.44 (m, 1H), 1.91 (s, 3H), 1.39 (s, 9H), 1.19 (t, J = 7.2 Hz, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 172.4, 164.8, 157.3, 156.3, 150.8, 142.6, 137.2, 130.1, 129.5, 123.2, 114.3, 113.3, 110.5, 100.3, 86.4, 79.2, 63.2, 60.8, 55.3, 37.3, 36.4, 27.3, 13.1, 10.3; HRMS-ESI(+) *m/z* calcd for $C_{29}H_{39}N_6O_{10}$ 631.2728 $[M+H]^+$, found 631.2719.

2-((1-((2R,3S,5R)-3-Hydroxy-2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-3,4 dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)isoindoline-1,3-dione (9x). The reaction of 4'-azidothymidine **5** (30 mg, 0.10 mmol) with alkyne (42 mg, 0.21 mmol) yielded compound **9x** (32 mg, 62%) as a white solid. mp 145–149 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.33 (s, 1H), 7.81-7.79 (m, 5H), 6.65 (dd, J = 6.1 Hz, J = 7.2 Hz, 1H), 5.32 (s, 2H), 4.92 (t, J = 7.1 Hz, 1H), 4.34 (d, J = 12.0 Hz, 1H), 4.06 (d, J = 12.0 Hz, 1H), 2.60-2.56 (m, 1H), 2.40-2.35 (m, 1H), 1.90 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 163.8, 150.7, 140.3, 137.1, 134.4, 128.2, 125.2, 122.9, 110.5, 100.3, 86.2, 71.4, 69.2, 63.0, 37.1, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{21}H_{21}N_6O_8$ 485.1421 $[M+H]^+$, found 485.1411.

1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)-5-(4-((naphthalen-2-yloxy)methyl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9y). The

reaction of 4'-azidothymidine **5** (25 mg, 0.09 mmol) with alkyne (32 mg, 0.18 mmol) yielded compound **9y** (22 mg, 54%) as a white solid. mp 109–112 ºC; ^{1}H NMR (600 MHz, CD_3OD) δ 8.20 (s, 1H), 7.78-7.73 (m, 4H), 7.42-7.38 (m, 2H), 7.27 (t, J = 7.8 Hz, 1H), 7.12 (dd, J = 2.4 Hz, J = 9.0 Hz, 1H), 6.66 (dd, J = 5.4 Hz, J = 7.2 Hz, 1H), 5.25 (s, 2H), 4.89 (t, J = 6.1 Hz, 1H), 4.35 (d, J = 12.0 Hz, 1H), 4.08 (d, J = 12.0 Hz, 1H), 2.55-2.59 (m, 1H), 2.36-2.41 (m, 1H), 1.85 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 164.8, 150.8, 142.6, 137.2, 134.6, 129.3, 129.0, 127.2, 126.5, 125.9, 123.5, 123.4, 118.4, 110.5, 106.8, 100.3, 86.4, 71.6, 63.2, 60.1, 37.2, 11.0; HRMS-ESI(+) *m/z* calcd for $C_{23}H_{24}N_5O_6$ 466.1727 $[M+H]^+$, found 466.1726.



Dimethyl 2-Oxopropylphosphonate (18) To a stirred suspension of KI (82.0 g, 490 mmol) in acetone (100 mL) and MeCN (125 mL) was added chloroacetone **10** (45.0 g, 490 mmol). Stirring was continued for 1 h at room temperature. Trimethyl phosphite (58.0 mL, 490 mmol) was slowly added. After 12 h at room temperature, the mixture was heated to 50 °C to ensure complete conversion. Filtration through a pad of celite and evaporation of the solvents under reduced pressure yielded the crude product. The crude product was further purified by flash column chromatography (EtOAc/hexane 1:1) furnished the product **18 (**41.0 g, 353 mmol, 72%) as a colorless liquid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.78 (d, J = 11.0 Hz, 6H), 3.07 (d, J = 22.8 Hz, 2H), 2.29 (s, 3H).

Tosyl azide (20) Tosylchloride (5.72 g, 26.0 mmol) was dissolved in acetone (85 mL) and water (85 mL). The solution was cooled in ice-bath and NaN_3 (1.71 g, 26.0 mmol) was added. The reaction was stirred for 2 h at the same temperature, then 12 h at room temperature. The

acetone was removed under vacuum and the remaining water layer was extracted with EtOAc (2 x 50 mL). The EtOAc layer was dried over Na_2SO_4, filtered and concentrated. The intermediate tosyl azide was obtained as a white solid **20** (4.8 g, 24.3 mmol, 94%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 2.46 (s, 3H).

Dimethyl 1-diazo-2-oxopropylphosphonate (21).[S3] To a stirred suspension of NaH (0.56 g, 60 % w/w, 23.2 mmol) in THF/ benzene (1:4, 70 mL) was added a solution of dimethyl (2-oxopropyl)-phosphonate **18** (3.5 g, 21.1 mmol) in dry benzene (15 mL) at 0 °C. A white solid was formed and the stirring was continued for 1 h. A solution of tosylazide **20** (4.3 g, 21.8 mmol) in dry benzene (10 mL) was added. The mixture was stirred overnight at room temperature, and then filtered through a pad of celite and evaporation of the solvents under reduced pressure yielded the crude product. The crude product was further purified by flash column chromatography (EtOAc/hexane 1:1) furnished the product **21 (**2.6 g, 13.3 mmol, 65%) as a colorless liquid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.85 (d, J = 11.8 Hz, 6H), 2.28 (s, 3H).

General procedure 2 for the synthesis of aromatic alkyne from aldehyde.[S3] To a stirred solution of aldehyde (1.0 equiv.) in MeOH were added Bestmann reagent (1.1 equiv.) in MeOH and K_2CO_3 (3.0 equiv.) at room temperature. The mixture was stirred at room temperature for 12 h. Solvent was evaporated and saturated aqueous NH_4Cl solution was added and extracted with ethyl acetate. The combined extracts were dried over $MgSO_4$ and concentrated to get crude product. The crude product was purified by column chromatography, eluted with 10-20% EtOAc in hexane, yielded the desired aromatic alkyne.

***N*-(4-Ethynylphenyl)acetamide (23a).** Prepared from *N*-(4-formylphenyl) acetamide, yielded *N*-(4-ethynylphenyl) acetamide **23a** (72%) as a brown oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (bs, 1H), 7.50-7.41 (m, 4H), 3.05 (s, 1H), 2.16 (s, 3H).

1-Ethynylferrocene (23b). Prepared from ferrocenecarboxaldehyde, yielded 1-ethynylferrocene **23b** (67%) as a brown oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.87 (m, 2H), 4.50 (m, 2H), 4.21 (m, 5H), 3.33 (s, 1H).

General procedure 3 for the synthesis of propargyl aryl ethers from phenols. To a stirred solution of phenol (1.0 equiv.) in DMF were added K_2CO_3 (2.0 equiv.) and propargyl bromide (1.5 equiv.) in DMF at room temperature. The mixture was stirred at room temperature for 12-15h. Solvent was evaporated and water was added and extracted with ethyl acetate. The combined extracts were dried over Na_2SO_4 and concentrated to get crude product. The crude product was purified by column chromatography, eluted with 10-15% EtOAc in hexane, yielded the desired propargyl aryl ethers.

(Prop-2-yn-1-yloxy)benzene (26a). Prepared from phenol, yielded (prop-2-yn-1-yloxy)benzene **26a** (76%) as a oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28–7.39 (m, 2H), 6.85–6.98 (m, 3H), 4.65 (s, 2H), 2.48 (s, 1H).

2-(Prop-2-yn-1-yloxy)isoindoline-1,3-dione (26b). Prepared from N-hydroxyphthalimide, yielded 2-(Prop-2-yn-1-yloxy)isoindoline-1,3-dione **26b** (76%) as a oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87-7.78 (m, 4H), 4.82 (s, 2H), 2.41 (s, 1H).

1-(Prop-2-yn-1-yloxy)naphthalene (26c). Prepared from naphthalen-1-ol, yielded 1-(prop-2-yn-1-yloxy)naphthalene **26c** (75%) as a solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.34-8.26 (m, 1H), 7.82 (dt, J = 4.0, J = 3.0 Hz, 1H), 7.50-7.52 (m, 3H), 7.42 (t, J = 7.9 Hz, 1H), 6.96 (d, J = 7.6 Hz, 1H), 4.91 (d, J = 2.4 Hz, 2H), 2.56 (t, J = 2.3 Hz, 1H).

Ethyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoate (26d). Prepared from ethyl 2-((tert-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propanoate, yielded ethyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoate **26d** (63%) as a oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.01 (d, J = 9.0 Hz, 2H), 6.78 (d, J = 9.0 Hz, 2H),

4.97 (d, *J* = 8.0 Hz, 1H), 4.65 (d, *J* = 2.4 Hz, 2H), 4.52-4.57 (m, 1H), 4.23 (q, 2H), 2.98-3.10 (m, 1H), 2.49 (t, *J* = 2.6 Hz, 1H), 1.41 (s, 9H), 1.26 (t, 3H).

Biology

HIV Cytoprotection Antiviral Assay.[S4] The HIV Cytoprotection assay used CEM-SS cells and the IIIB strain of HIV-1. Briefly virus and cells were mixed in the presence of test compound and incubated for 6 days. The virus was pre-titered such that control wells exhibit 70 to 95% loss of cell viability due to virus replication. Therefore, antiviral effect or cytoprotection was observed when compounds prevent virus replication. Each assay plate contained cell control wells (cells only), virus control wells (cells plus virus), compound toxicity control wells (cells plus compound only), compound colorimetric control wells (compound only) as well as experimental wells (compound plus cells plus virus). Cytoprotection and compound cytotoxicity were assessed by MTS (CellTiter® 96 Reagent, Promega, Madison WI Each assay included the HIV RT inhibitor AZT as a positive control.

Influenza A Virus Cytoprotection Antiviral Assay. Compounds were evaluated for ability to prevent influenza A virus-mediated CPE and cell killing. MDCK and A549 cells were cultured in Dulbecco's Modified Eagle's (DME) medium supplemented with 10% fetal bovine serum (FBS) and 100 IU streptomycin/penicillin per ml. Cells were plated in 96-well plates (2.5 X 10^4 per well) and the next day washed twice in phosphate buffered saline (PBS). The cells were inoculated with a 1:1000 dilution of influenza A/WSN/33 virus stock in influenza cell medium (DME, 100 IU streptomycin/penicillin per ml, 4% bovine serum albumin w/v, 1.5 μg/ml TPCK-trypsin) plus compound dissolved in DMSO (final compound concentration 10 μM). The virus stock was pre-titered such that the virus inoculum in this assay results in at least a 90% loss of cell viability in control samples due to virus replication. Other samples added to each plate were those containing DMSO in medium, medium alone, and the compound nucleozin[S5] at 10 μM as a control influenza A inhibitor. The cells were

incubated at 37°C and 5% CO2 in a humidified incubator for 5 days. Cell viability was assessed by a neutral red retention assay.[S6] The medium was removed, cells washed once in PBS and neutral red medium was added (DME 10% FBS, 40 μg/ml neutral red). After a two hour incubation at 37°C, the neutral red medium was removed, cells washed once in PBS and 100 μl of 50% ethanol/0.1% acetic acid was added per well. The level of neutral red retained by cells was measured at absorbance 540 nm using a Molecular Devices M5e plate reader. Compounds were tested in triplicate wells and the mean value was determined. The mean value was compared to the mean value for cells treated with DMSO but no virus to calculate inhibition %.

Replicon Assays. Compounds were evaluated for antiviral properties using viral subgenomic replicon-containing cells. For West Nile Virus (WNV), the baby hamster kidney replicon cell line BHK-WII RepRen1B (obtained from Dr. T. Pierson, NIH/NIAID) was used and for hepatitis C virus (HCV), the human hepatoma replicon cell line Huh-7/HCV1b-Rluc[S7] was used. Viral subgenomic replicon RNAs are capable of self-replication in susceptible cells and are routinely used to identify potential inhibitors of viral or cellular replicative enzymes. Both the HCV and WNV replicon RNAs express renilla luciferase (Luc) thereby permitting the measurement of Luc activity as an indication of the level of replicon RNA in the respective cells. The BHK-WII RepRen1B cells were maintained in Dulbecco's modified Eagle's (DME) medium supplemented with 10% FBS, 100 IU streptomycin/penicillin per ml, 500 μg/ml G418 (Invitrogen) and 5 μg/ml plasmocin (InvivoGen). Three thousand WNV replicon-containing cells per well were plated in white opaque 96-well plates in the absence of G418 and the next day, compounds dissolved in DMSO were added in culture medium to triplicate wells. The compounds were tested at 10μM final concentration and each plate also contained DMSO alone, medium alone, and 1 μM lycorine was used as a control

inhibitory compound. Lycorine is a natural product that inhibits West Nile virus replication.[S8] Three days later, medium was replaced with DME lacking phenol red and 10% FBS supplemented with a 1:1000 dilution of ViVi-Ren Live Cell Substrate (Promega). Luminescence was measured in a Molecular Devices M5e plate reader. Mean values of triplicate wells were determined and compared to the mean value for the wells that received DMSO alone. The Huh-7/HCV1b-Rluc cells were maintained and the replicon assay conducted as described.[S7] For the HCV replicon assay, the compounds were tested at 10μM final concentration and each plate also contained DMSO alone, medium alone, and 0.5 μM 2'-*C*-methyl adenosine was used as a control inhibitory compound.[9] Mean values of triplicate wells were determined and compared to the mean value for the wells that received DMSO.

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