From oxetane to thietane: Extending the antiviral spectrum of 2'-spirocyclic uridines by substituting oxygen with sulfur

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Contents

1-Biological Assay description

1a-Dengue Virus Assay

The antiviral activity of several nucleoside analogs was determined against DENV-2/16681/eGFP in a phenotypic antiviral assay with eGFP readout as a measure for the amount of virus. The assay was performed on two different cell types (Vero and Huh-7). In brief, 2.5 × 103 Vero cells or Huh-7 cells were seeded in 384-well plates containing 9-fold serially diluted test compound. After incubating for 24 h at 37 °C, Vero and Huh-7 cells were infected with DENV-2/16681/eGFP at a multiplicity of infection (MOI.) of 1 and 5, respectively. After 3 days of incubation at 37 °C, viral replication was quantified by measuring eGFP expression in the cells with a laser microscope.

1b-Hepatitis C Virus Assay

The antiviral activity against HCV (*Flaviviridae*) was tested in a HCV replicon-containing cell culture system consisting of Huh7 cells that are stably transfected with a selectable self-replicating sub-genomic HCV Genotype 1b (Clone ET) RNA sequence harboring a luciferase reporter gene (Huh7-Luc cells).[7]

In brief, Huh7-Luc replicon-containing cells were seeded in 384-well plates (2,500 cells/well) and incubated for 3 days (37°C; humidified 5% CO_2 atmosphere) with a concentration range of serially diluted compound in a final DMSO concentration of 0.5% in cell culture medium without G418. HCV replicon RNA replication was determined by means of measuring the firefly luciferase reporter gene expression using the SteadyLite Plus™ assay kit (PerkinElmer) and luminescence measurement using a ViewLux™ reader (PerkinElmer).

1c-Chikungunya Virus Assay

The antiviral activity against CHIKV (S27), a mosquito-borne RNA virus that belongs to the *Alphavirus* genus (*Togaviridae*), was measured by using CPE as read-out.

Briefly, Huh7 cells (8,000 cells/well) were seeded in 384-well plates (Costar), containing serially diluted compound in cell culture DMEM medium supplemented with 2% FBS, and were infected with CHIKV strain S27 at a MOI of 0.25. Plates were incubated at 37°C and 5% CO_2 for 2 days until the viral CPE in the virus control wells reached ~100%. Then, ATPLite™ was added to all wells according to the supplier's instructions to assess the viability of the cells and thus the preventive effect of the antiviral test compound on CPE. Luminescence was measured using a Viewlux (PerkinElmer) apparatus.

1d-Sinbis Virus Assay

The antiviral activities of the nucleoside analogs against SINV were tested using a cell-based high-throughput CPE (cytopathic effect) inhibition assay. In this assay, SINV strain AR339 and Huh7 cells (a human hepatoma cell line) were employed. The test compounds were plated in a 384-well plate with 9-point fourfold serial dilutions and the highest concentrations tested were 50uM. The antiviral efficacy of test compounds was determined by measuring the ATP levels in live cells protected from viral CPE by a test compound. The readout is luminescence.

1e-Cytotoxicity assay

Cytotoxicity of the compounds was assessed in Huh-7 cells.

Briefly, Huh7 cells (8,000 cells/well) were seeded in 384-well plates (Costar), containing serially diluted compound in cell culture DMEM medium supplemented with 2% FBS. Plates were incubated at 37°C and 5% CO_2 for 2 days. Then, ATPLite™ was added to all wells according to the supplier's instructions to assess the viability of the cells. Luminescence was measured using a Viewlux (PerkinElmer) apparatus.

2-ADME assay description

2a-Pharmacokinetics study in mouse

All animal studies were performed with the approval of and under the guidelines of the ethics committee. The pharmacokinetics profile was evaluated in fed male C57bl/6 mice (n = 3 per group, 8 weeks old; Charles River Germany). Prodrug was administered by oral gavage at 50 mg per kg, formulated as a solution in Hydroxypropyl-beta-cyclodextrine 20% (w/v) pH3.9, and blood samples were collected at 0.5, 2 and 7h after dosing via heart puncture. Blood samples were immediately centrifuged at 4 °C and plasma was stored at –20 °C. Prodrug concentrations in the plasma samples were determined via a qualified research LC-MS/MS method using an API 4000 LC–MS/MS system mass spectrometer (Applied Biosystems). Tissue biopts were snap frozen in liquid nitrogen and homogenized immediately after sampling in 70% Methanol – 30% 20mM (EDTA-EGTA) solution. Triphosphate levels in liver biopts were determined using a qualified research LC-MS/MS method. The analysis was carried out on QExactive (HRMS) instrument. Prodrug concentrations were also checked in the liver biopts. Plasma and liver concentration–time profiles were subjected to a non-compartmental pharmacokinetics analysis using Phoenix WinNonlin v.6.3. (Certara). Tissue biopts were snap frozen in liquid nitrogen and homogenized immediately after sampling in 70% Methanol – 30% 20mM (EDTA-EGTA) solution.After homogenization, quantification is done on an ion-exchange LC-column (BioBasic AX 50x4.6mm 5µ, Thermo). Mobile phases consisted of AmmoniumAcetate 0.025M pH5/ 5% ACN (solvent A) and AmmoniumAcetate 0.025M pH10/ 5% ACN (solvent B) with post-column addition of methanol at 0.2ml/min. Starting conditions were 80% solvent A and 20% solvent B, an isocratic hold for 1.8 min followed by a step gradient to 10% solvent A and 90% solvent B with an isocratic hold for 3.2min, followed by a step gradient back to the original settings, at a flow rate of 0.6 ml/min.

LC-MS/MS analysis was carried out on a triple quad massspectrometer (Sciex, Toronto, Canada), which was coupled to a Shimadzu UHPLC-system. The massspec operated eighter in the negative ion mode using the TurboIonSprayÔ-interface (electrospray ionization), the MSMS method was optimized for the quantification of the compound. MRM transition was as follows: 542.9 > 158.8 (quantifier); 542.9 > 445 (qualifier). Samples were quantified against calibration curves prepared to cover the concentration range of the study samples. The curves were prepared in the same matrix as the study samples. For each analytical batch, spiked quality control samples were analyzed together with the study samples and calibration curve.

2b-Metabolic stability in liver microsomes

The test compound was incubated under the generic condition (0.5 mg/ml microsomal protein, 1 mM NADPH, 1 mM MgCl2, and 0.1 M phosphate buffer, pH 7.4, 37°C, n=1) at a defined substrate concentration (typically 1 µM) in liver microsomes of selected species across a time course (typically 0, 5, 10, 20, 40 and 60 minutes) at Cyprotex. The liver microsomes, buffer and test compound were pre-incubated for 5 min. The co-factor NADPH was added to initiates the reaction and the reaction was

terminated by addition of acetonitrile. The samples were centrifuged prior to analysis by LC-MS/MS analysis. The relative amount of parent compound remaining in the active incubations versus the control incubations (t=0 min) for each compound was measured by peak area comparison. The *in vitro* metabolic half-life ($t_{1/2}$) was calculated using the slope of the log-linear regression from the percentage parent compound remaining versus time relationship (k). The *in vitro* intrinsic clearance (CL_{int}) (µl/min/mg microsomal protein) was calculated using the *in vitro* metabolic half-life, incubation volume and the weight of microsomal protein in the incubation.

2c-Stability in simulated gastric fluid and fasted simulated intestinal fluid

2c.1. Preparation of Simulated Gastric Fluid (SGF)

2g NaCl and 3.2g pepsine (origine : porcine)(Acros,code 417070200 CAS:9001-75-6) were dissolve in 900ml MilliQ. 7ml HCl 37% (=11.97g/l) or 80ml 1M HCl were added Volume was adjusted to 1 L with MilliQ pH (SGF solution)=1.2.

2c.2. Preparation of Fasted simulated intestinal fluid (FaSSIF)

Step 1 :Preparation of phosphatebuffer for FaSSIF (1 Litre):

0.42 g NaOH (pellets), 3.95 g of $NaH_2PO_4*H_20$ (monohydrate), 6.19 g of NaCl, were dissolved in 0.9 L of purified water. The pH was adjusted to 6.5 with either 1 N NaOH or 1 N HCl and make up to volume (1 L) with purified water

Step 2 : 2.24 g of SIF Powder was added to 500 mL of buffer at room temperature. The mixture was stirred until SIF Powder has dissolved. The volume was adjusted to 1L with the Buffer. FaSSIF was let stand for 2 hours. FaSSIF will become slightly opalescent and is ready to use. pH (FassiF)=6.5.

Step 3 Incubation procedure FASSIF

1 mL FASSIF was supplemented with pancreatine (10mg/ml) and esterase 20 U/ml [Pancreatine : Sigma Aldrich P3292 CAS:8049-47-6 647-014-00-9, Esterase Sigma Aldrich E3019 CAS:9016-18-6 18U/mg]. pH was not measured after addition of the enzymes/

Step 4 : Test Compound (1 µM, n=2) was incubated for 6h in simulated gastric fluid and fasted simulated intestinal fluid at 37ºC. Samples were taken at several time points (0, 5min, 15 min, 30 min, 1h, 2h and 6h) to allow determination of the half-life of the test compound. Sample analysis was performed using LC-MS/MS analysis. The relative amount of parent compound remaining in the active incubations versus the control incubations (t=0 mins) for each compound was measured by peak area comparison.

2d-Stability in mouse and human plasma

Test Compound (1 µM, n=2) was incubated for 24h in mouse and human plasma at 37 ºC. Samples were taken at several time points (0, 1, 4, 7 and 24h) to allow determination of the half-life of the test compound. Sample analysis was performed using LC-MS/MS analysis. The relative amount of parent compound remaining in the active incubations versus the control incubations (t=0 mins) for each compound was measured by peak area comparison.

3-Sequence alignment

Multiple sequence alignments for the RNA-dependent RNA polymerase domains of HCV NS5B, DENV NS5, CHIKV NSP4 and SINV NSP4. Multiple examples of different strains were extracted from

GenBank at NCBI in order to strengthen block and motif information. 10 randomly selected sequences chosen for each of the 4 viruses were then aligned to obtain the cross-virus sequence alignment using CLUSTAL Omega website with standard settings[1] and subsequent manual adjustments. This sequence alignment is in agreement with the one recently published for the structural motifs GFABCDE.[2]

4-Synthetic procedures and data

All reactions were carried out by employing standard chemical techniques under an inert atmosphere. Solvents used for extraction, washing, and chromatography were HPLC grade. Unless otherwise noted, all reagents were purchased from commercial suppliers and were used without further purification. No unexpected or unusually high safety hazards were encountered. Reaction progress and purity determination was followed by HPLC analysis using either of the methods below. Condition A: system, Waters Alliance 2695; column, Waters XTerra 2.5 μm, 4.6 × 50 mm; column temp., 55 °C; flow, 2 mL/min; mobile phase A, 10 mM NH_4OOCH + 0.1% HCOOH in H_2O; mobile phase B, CH_3CN; gradient, 0 min, 85/15 A/B to 3.0 min, 5/95 A/B, to 4.20 min, 5/95 A/B. Condition B: column, Hypercar 3 μm, 4.6 × 50 mm; mobile phase C, 10 mM NH_4OAc in H_2O/CH_3CN 1/9; mobile phase D, 10 mM NH4OAc in H_2O/CH_3CN 9/1; column temp., 50 °C; flow gradient, 2 mL/min; 0 min, 0/100 C/D to 3.0 min, 100/0 C/D, to 4.20 min, 100/0 C/D. Condition C: column, SunFire C18 3.5 μm, 4.6 × 100 mm; mobile phase A, 10 mM NH_4OOCH + 0.1% HCOOH in H_2O; mobile phase B, MeOH operating at a column temperature of 50 °C using a flow rate of 1.5 mL/min. Gradient conditions: t = 0 min, 65% A, 35% B; t = 7 min, 5% A, 95% B; t = 9.6 min, 5% A, 95% B; t = 9.8 min, 65% A, 35% B; t = 12 min, 65% A, 35% B.

NMR spectra were recorded on a Bruker Avance 400 spectrometer, operating at 400 MHz for 1 H, 100 MHz for 13 C and 161 MHz for 31P. Chemical shifts are given in ppm and J values in Hz. Multiplicity is indicated using the following abbreviations: d for doublet, t for a triplet, m for a multiplet, etc.

Compound names were generated using ChemBioDraw Ultra, version 14.0.0.117. Using this system, the atom numbering can be somewhat different from the classical nucleoside numbering where the anomeric C atom is numbered “1”.

Liquid chromatography (LC) experiments for high resolution mass spectrometry (HR-MS) determinations were performed using an Ultimate 3000 RS UHPLC system (Thermo Fisher Scientific, Germering, Germany) composed of a gradient pump, an autosampler, a column oven, and a diode-array detector (DAD). DAD scanning wavelengths ranging from 200 to 400 nm were selected. Mobile phase A consisted of 0.1% ammonium bicarbonate in 95% H_2O + 5% CH_3CN, and mobile phase B consisted of CH_3CN. The chromatographic experiments were carried out at a flow rate of 0.6 mL/min. A linear gradient was applied from 95% A to 5% A in 2.10 min and held for 1.9 min. The column compartment was kept constant at 55 °C. A 2.1 mm i.d. x 100 mm Acquity UPLC BEH C18 column packed with 1.7 um particles was obtained from Waters Corporation (Milford, MA, USA). A 1:10 flow split from the column to the MS spectrometer was applied.

The high-resolution mass spectrometry (HR-MS) experiments were performed on a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) using electrospray ionization (ESI) and in

[1] Sievers, F.; Higgins, D. G. Clustal Omega for making accurate alignments of many protein sequences. *Protein Sci*. **2018**, 27, 135-145.

[2] Tan, Y.B.; Lello, L.S.; Liu X., Law Y.S.; Kang, C.; Lescar, J.; Zheng, J.; Merits, A.; Luo, D.*Nucleic Acids Res*. **2022**, *50,* 1000-1016.

Full MS scan type mode. Nitrogen was used as the nebulizer gas. The MS was operated both in positive and negative mode, and the ESI parameters were as follows: spray voltage: 4.00 kV; capillary temperature: 320 °C; S-lens RF level: 50.0. Masses in the *m/z* 150 to 1200 range were selected and the experiments were performed at a resolution of 140,000. Xcalibur (version 4.4, Thermo Fisher Scientific) was used as data acquisition software. The MS was calibrated in both modes according to the manufacturer instructions. The reported accurate masses correspond to the [M+H]+ (protonated monoisotopic molecular mass) and/or $[M-H]^-$ (deprotonated monoisotopic molecular mass).

Preparative SFC

The SFC separations were performed using a Supercritical fluid chromatography (SFC) system composed by a binary pump for delivering carbon dioxide (CO_2) and modifier a diode array detector, a column Chiralpak Diacel AS 20 x 250 mL Mobile phase: CO_2, (EtOH + 0.4 *iPr*NH_2)

Preparative HPLC

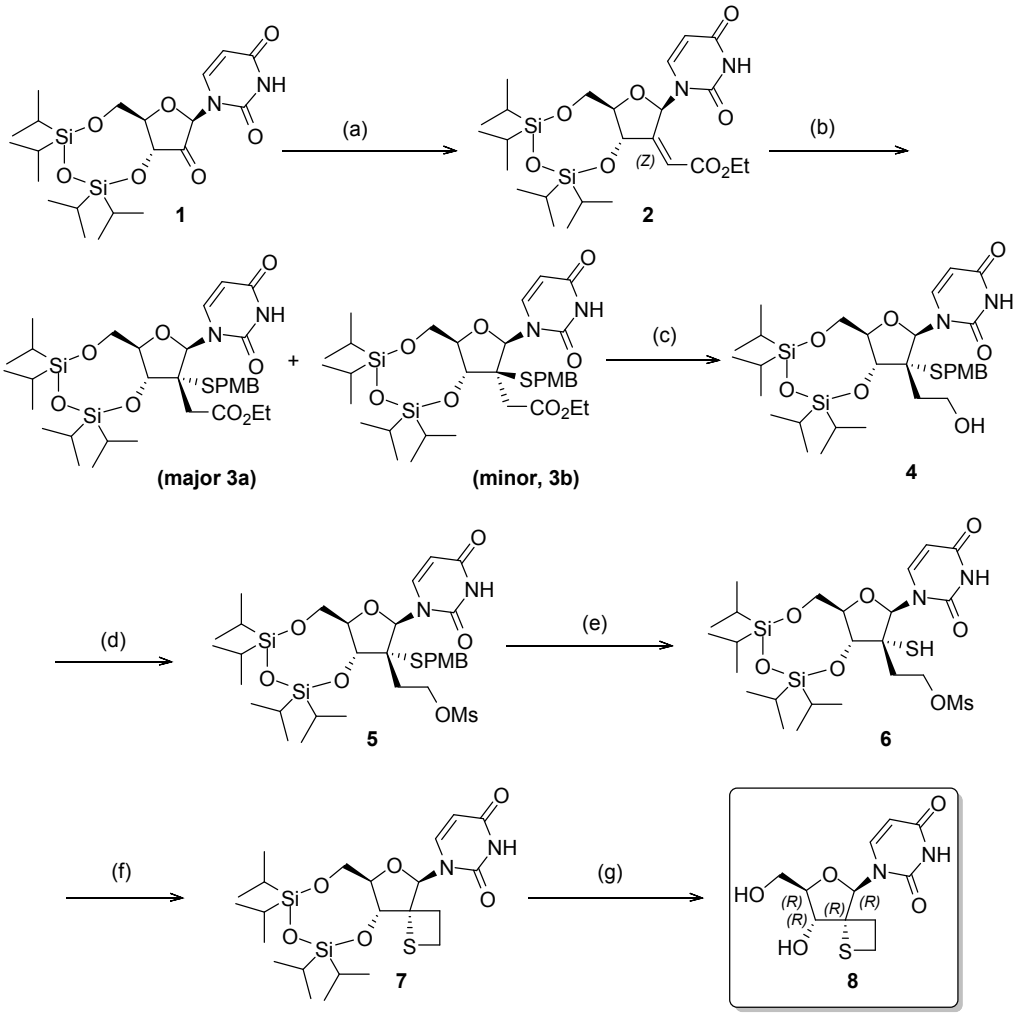
Method A: (Stationary phase: RP SunFire Prep C18 OBD-10µm,30x150mm, Mobile phase: 0.25% NH_4HCO_3 solution in water, MeOH)

Method B: (Stationary phase: RP SunFire Prep C18 OBD-10µm,30x150mm, Mobile phase: 0.25% NH_4HCO_3 solution in water, CH_3CN)

Method C: (Stationary phase: RP XBridge Prep C18 OBD-10µm,50x150mm, Mobile phase: 0.25% NH_4HCO_3 solution in water, CH_3CN)

Method D: Stationary phase: RP XBridge Prep C18 OBD-10µm,30x150mm, Mobile phase: 0.25% NH_4HCO_3 solution in water, CH_3CN

Experimental Procedures



Scheme S1. Synthesis access to first spirothietane nucleoside **8**. *Conditions :* (a) $(EtO)_2P(O)CH_2CO_2Et$, ^tBuOK, THF, -15°C to RT (1h), then **1** in THF 0°C -15°C (1h) then RT (5h) 83%. (b) (4-methoxyphenyl)methanethiol, KHMDS; THF, -40°C to 20°C (2 h), 50% (**3a**) 12% (**3b**). (c) $LiAlH_4$, Et_2O, 0°C to 20 °C (16 h) 60%. (d) MsCl, pyridine, 25°C (16 h), 78%. (e) $Hg(OAc)_2$, CF_3CO_2H, PhOH, 0°C (1 h), then DTT, 0°C (10 min).(f) NaH, THF, 0°C to 25°C (16 h), 46% over 2 steps. g) TBAF, THF, RT (2 h), 86%.

Synthesis of ethyl (Z)-2-((6aR,8R,9aS)-8-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2,4,4-tetraisopropyldihydro-6*H*-furo[3,2-f][1,3,5,2,4]trioxadisilocin-9(8*H*)-ylidene)acetate **2**

To a solution of ^tBuOK in dry THF (300 mL) was added under N_2 triethylphosphonoacetate [867-13-0] (16.6 g, 74 mmol) dropwise at -15 °C. The solution was stirred at room temperature for 1 h then cooled to -15 °C before adding a solution of intermediate **1** (30 g, 62 mmol) dissolved in THF (200 mL) dropwise. The reaction mixture was stirred at -15 °C for 1 h and at room temperature for 4 h before quenching with aq. NH_4Cl (100 mL) and water (100 mL). The phases were separated, and the aqueous layer was extracted with EtOAc (2x400 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and evaporated.
The residue was purified by flash column chromatography on silica gel using DCM/EtOAc 100:0 to 50:50. The product fractions were collected, and the organic solvent was evaporated to afford (28.5 g, 83%) of a pale-yellow viscous oil.

m/z $[M + H]^+$ calcd for $C_{25}H_{42}N_2O_8Si_2$: 555.79, found, 555.

Synthesis of ethyl 2-((6aR,8R,9R,9aR)-8-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2,4,4-tetraisopropyl-9-((4-methoxybenzyl)thio)tetrahydro-6*H*-furo[3,2-f][l,3,5,2,4]trioxadisilocin-9-yl)acetate **3a** and ethyl 2-((6aR,8R,9S,9aR)-8-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2,4,4-tetraisopropyl-9-((4-methoxybenzyl)thio)tetrahydro-6H-furo[3,2-f][1,3,5,2,4]trioxadisilocin-9-yl)acetate **3b**

(4-Methoxyphenyl)methanethiol CAS[258-60-22] (69.4 g, 450.6 mmol) in THF (5 L) was stirred at 20°C under nitrogen. The mixture was cooled to -40°C then KHMDS (1 M, 495 mL, 495.7 mmol) was added dropwise. The resulting white viscous liquid was stirred for 30 min then intermediate **2** (250 g, 451 mmol) in THF (1 L) was added at -40°C. The reaction mixture was allowed to warm slowly to 20°C and stirred for 2 h. The reaction mixture was quenched by addition of aqueous solution 1 N of HC1 (2L) then extracted with EtOAc (2x2L). The organic layer was successively washed with aqueous solution of sodium bicarbonate (2 L), brine (2 L), dried over Na_2SO_4 and evaporated. The resulting residue was purified by column chromatography (PE/EtOAc=20/l to 3/1) to yield :

Compound **3a** (159 g, 50%) as colourless oil.

m/z $[M + H]^+$ calcd for $C_{33}H_{52}N_2O_9SSi_2$: 710.01, found, 710.

^{1}H NMR (400 MHz, CDC1$_3$) δ ppm 8.26 (s, 1 H), 7.82 (d, 7=8.0 Hz, 1 H), 7.29 (d, 7=8.4 Hz, 2 H), 6.85 (d, 7=8.4 Hz, 2 H), 6.28 (s, 1 H), 5.66-5.63 (m, 1 H), 5.34-5.30 (m, 1 H), 4.41-4.23 (m, 1 H), 4.19-4.04 (m, 5 H), 3.80-3.78 (m, 4 H), 3.23- 3.19 (m, 1 H), 2.92 (d, 7=16.4 Hz, 1 H), 1.30-0.86 (m, 31 H).

Compound **3b** (38 g, 12%) as colourless oil.

m/z $[M + H]^+$ calcd for $C_{33}H_{52}N_2O_9SSi_2$: 710.01, found, 710.

^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ ppm 0.97 - 1.09 (m, 28 H) 1.24 (t, J=7.2 Hz, 3 H) 2.92 (d, J=16.3 Hz, 1 H) 3.15 (d, J=16.1 Hz, 1 H) 3.36 (br d, J=10.6 Hz, 1 H) 3.72 (s, 3 H) 3.84 - 3.89 (m, 1 H) 3.94 - 4.04 (m, 2 H) 4.04 - 4.17 (m, 4 H) 5.71 (dd, J=8.1, 2.0 Hz, 1 H) 6.76 (br s, 1 H) 6.83 - 6.88 (m, 2 H) 7.04 - 7.08 (m, 2 H) 7.68 (d, J=8.1 Hz, 1 H) 11.56 (br s, 1 H)

Synthesis of l-((6aR,8R,9R,9aR)-9-(2-hydroxyethyl)-2,2,4,4-tetraisopropyl-9-((4-methoxybenzyl)thio) tetrahydro-6H-furo[3,2-f] [l,3,5,2,4]trioxadisilocin-8- yl)pyrimidine-2,4(l*H*,3*H*)-dione **4**

Lithium aluminium hydride (4 g, 105 mml) was suspended in diethyl ether (1.5 L) under nitrogen at 0°C then intermediate **3a** (50 g, 70 mmol) in diethyl ether (200 mL) was added slowly at 0°C. The resulting white turbid solution was stirred at 20°C for 16 h. The reaction mixture was quenched by addition of aqueous solution 1 N of HCl (1L) then extracted with EtOAc (2 x 1 L). The organic layer was dried over Na_2SO_4 and evaporated. The resulting residue was purified by column chromatography (PE/EtOAc=10/l to 1/1) to yield compound **4** (27.8 g, 60%) as colourless oil.

m/z $[M + H]^+$ calcd for $C_{31}H_{50}N_2O_8SSi_2$: 667.98, found, 668.

^{1}HNMR: (400 MHz, CDC1$_3$) δ ppm 8.78 (s, 1 H), 7.89 (d, 7=8 Hz, 1 H), 7.30 (d, 7=8.4 Hz, 2 H), 6.85 (d, 7=8.8 Hz, 2 H), 6.35 (s, 1 H), 5.73 (d, 7=8 Hz, 1 H), 4.36-3.91 (m, 12 H), 3.79 (s, 3 H), 2.23-2.20 (m, 2 H), 1.78-1.73 (m, 1 H), 1.11-0.97 (m, 30 H)

Synthesis of 2-((6aR,8R,9R,9aR)-8-(2,4-dioxo-3,4-dihydropyrimidin-l(2H)-yl)- 2.2.4.4-tetraisopropyl-9-((4-methoxybenzyl)thio) tetrahydro-6H-furo[3,2- f][l,3,5,2,4]trioxadisilocin-9-yl)ethyl methanesulfonate **5**

Intermediate **4** (50 g, 75 mmol) was dissolved in pyridine (500 mL) under nitrogen at 25°C, then mesylchloride (12.8 g, 112.5 mmol) was slowly added at 25°C. The resulting yellow solution was stirred at 25°C for 16 h. The reaction mixture was quenched by addition of aqueous solution 1 N of HC1 (1 L) then extracted with EtOAc (2 x 1 L). The organic layer was dried over Na_2SO_4 and evaporated. The resulting residue was purified by column chromatography (PE/EtOAc=10/l to 1/1) to yield compound **5** (43 g, 78%) as colorless oil.

m/z $[M + H]^+$ calcd for $C_{32}H_{52}N_2O_{10}S_2Si_2$: 746.06, found, 746.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.56 (s, 1 H), 7.92 (d, 7=8.4 Hz, 1 H), 7.31 (d, 7=8.8 Hz, 2 H), 6.87-6.85 (m, 2 H), 6.27 (s, 1 H), 5.77-5.74 (m, 1 H), 4.55-4.53 (m, 2 H), 4.38-4.02 (m, 8 H), 3.79 (s, 3 H), 2.95 (s, 3 H), 2.28-2.21 (m, 1 H), 1.12-1.01 (m, 31 H)

Synthesis of 2-((6aR,8R,9R,9aR)-8-(2,4-dioxo-3,4-dihydropyrimidin-l(2H)-yl)- 2.2.4.4-tetraisopropyl-9-mercaptotetrahydro-6H-furo[3,2-f][l,3,5,2,4]trioxadisilocin-9- yl)ethyl methanesulfonate **6**

To intermediate **5** (62 g, 83.2 mmol) in TFA (250 mL) at 25°C, mercury acetate (53 g, 166.4 mmol) and phenol (39.1 g, 416 mmol) were added slowly at 0°C. The resulting dark red solution was stirred at 0°C for 1 h. The l,4-dimercaptobutane-2,3-diol (DTT) (25.6 g, 166.4 mmol) was added at 0°C. The resulting mixture was stirred 10 min then filtered over celite® and washed with ethyl acetate (1 L). The pH was adjusted to 7 by addition of an aqueous solution of sodium bicarbonate. The resulting mixture was filtered over celite® and extracted with EtOAc (2 x 1 L). The organic layer was dried over Na_2SO_4 and evaporated at 25°C to give the title intermediate **6** (64 g, crude) as brown oil.

Synthesis of l-((2'R,6aR,8R,9aR)-2,2,4,4- tetraisopropyltetrahydrospiro[furo[3,2-f][1,3,5,2,4]trioxadisilocine-9,2'-thietan]-8-yl) pyrimidine-2,4(l*H*,3*H*)-dione **7**

Intermediate **6** (57 g, 91 mmol) was dissolved in THF (500 mL) at 20°C under nitrogen. The resulting mixture was stirred at 0°C then sodium hydride (3.6 g, 135 mmol) was added slowly. The reaction mixture was stirred at 25°C for 16 h. The reaction mixture was quenched by addition of aqueous solution 1 N of HCI (1L) then extracted with EtOAc (2 x 1 L). The organic layer was dried over Na_2SO_4 and evaporated. The resulting residue was purified by column chromatography (PE/EtOAc=10/l to 5/1) to yield intermediate **7** (18.3 g, 46%, 2 steps) as colourless oil.

m/z $[M + H]^+$ calcd for $C_{23}H_{40}N_2O_6SSi_2$: 529.81, found, 529.

^{1}H NMR (400MHz, $CDCl_3$) δ 8.55 (s, 1 H), 7.93 (d, 7=8 Hz, 1 H), 6.58 (s, 1 H), 5.69 (d, 7=8 Hz, 1 H), 4.20-4.17 (m, 1 H), 4.05-3.96 (m, 2 H), 3.54-3.51 (m, 1 H), 3.33-3.32 (m, 1 H), 2.96-2.87 (m, 2 H), 2.85-2.69 (m, 1 H), 1.17-0.98 (m, 30 H).

Synthesis of l-((4R,5R,7R,8R)-8-hydroxy-7-(hydroxymethyl)-6-oxa-l-thiaspiro[3.4]octan-5-yl)pyrimidine-2,4(f*H*,3*H*)-dione **8**

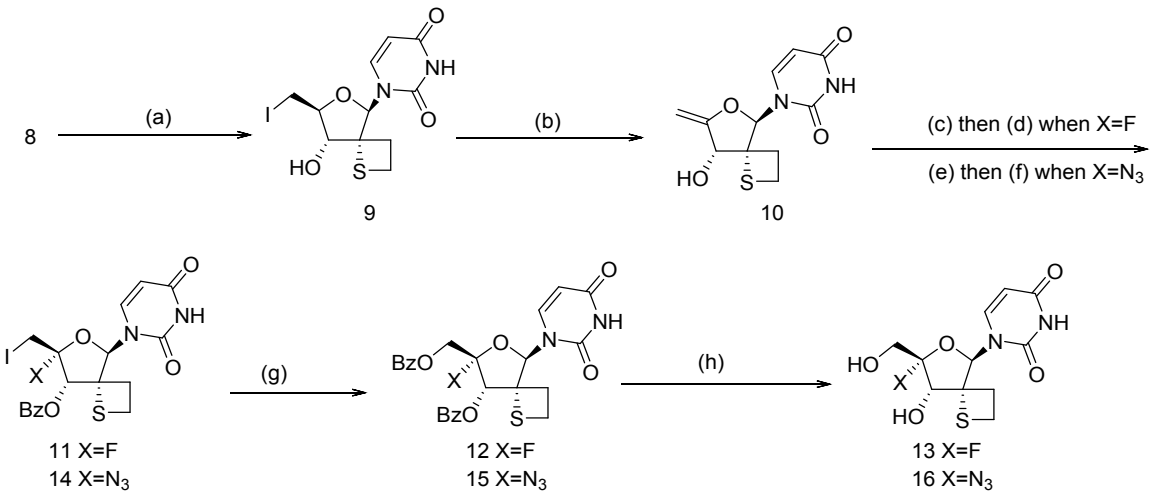
To a solution of intermediate **7** (15 g, 28.365 mmol) in THF (300 mL), TBAF (57 mL, 56.7 mmol, 1M in THF) was added. The resulting mixture was stirred under N_2 atmosphere at RT for 2 h. Afterwards, the solvent was evaporated, and the crude was purified by Prep HPLC using method A to yield intermediate **8** (7 g, 86%) as white powder.

m/z $[M - H]^-$ calcd for $C_{11}H_{14}N_2O_5S$: 285.30, found, 285.

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 2.44 - 2.49 (m, 1 H), 2.78 - 2.90 (m, 2 H), 2.99 - 3.09 (m, 1 H), 3.39 - 3.45 (m, 1 H), 3.59 (dd, J=12.4, 2.8 Hz, 1 H), 3.74 (dd, J=12.4, 2.1 Hz, 1 H), 3.92 (br d, J=8.1 Hz, 1 H), 5.23 (br s, 1 H), 5.62 (d, J=8.1 Hz, 1 H), 5.68 (br s, 1 H), 6.40 (s, 1 H), 8.00 (d, J=8.1 Hz, 1 H), 11.40 (br s, 1 H).

^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ ppm 19.99 (s, 1 C) 30.65 (s, 1 C) 59.07 (s, 1 C) 61.55 (s, 1 C) 71.90 (s, 1 C) 82.14 (s, 1 C) 92.17 (s, 1 C) 101.94 (s, 1 C) 140.74 (s, 1 C) 151.38 (s, 1 C) 163.43 (s, 1 C)

HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{11}H_{15}O_5N_2S^+$, 287.06962; found, 287.06979.



Scheme S2. Synthetic access to 4'-functionalized spirothietane nucleosides

Conditions :(a) I_2, $P(Ph)_3$, NMI, THF, 25°C (4h). (b) MeONa, MeOH, reflux (2 h), 55% over 2 steps. (c) $Et_3N(HF)_3$, THF/ACN, NIS, -15°C (1 h). (d) Et_3N, DMAP, THF, BzCl, 0°C to 25°C (3 h), 62% (e) 1) $BnEt_3NCl$, NaN_3, CH_3CN, RT (16h) then **10**, NMM, I_2, THF, 0°C to RT (5h), 99%. (f) BzCl, Et_3N, DMAP, THF, RT (2h), 81%. (g) BzONa, 15-crown-5, DMF, 120°C (18h), 59% when X=F, 47% when X=N_3, (h) NH_3, MeOH, RT, overnight, 65% when X=F, 84% when X=N_3.

Synthesis of l-((4R,5R,7S,8R)-8-hydroxy-7-(iodomethyl)-6-oxa-l-thiaspiro[3.4]octan-5-yl)pyrimidine-2,4(l*H*,3*H*)-dione **9**

Iodine (6.7 g, 26.2 mmol) and TPP (6.8 g, 26.2 mmol) were added to a suspension of intermediate **8** (5 g, 17.47 mmol) in NMI (7 mL mL, 1.03 g/mL, 87.3 mmol) and THF (200 mL, 0.886 g/mL, 2457.5 mmol) at RT. The reaction mixture was stirred for 4 h under N_2 atmosphere. The reaction mixture was quenched with a saturated solution of $Na_2S_2O_4$, concentrated, and diluted with EtOAc (100 mL). The organic layer was washed with brine (50 mL) dried over $MgSO_4$ and concentrated. The crude was purified by chromatography column using Heptane/EtOAc as eluent to afford a white solid (6 g) containing intermediate **9** 80% and triphenylphosphine oxide 20%.

m/z $[M - H]^-$ calcd for $C_{11}H_{13}IN_2O_4S$: 395.20, found, 395.

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 2.55 - 2.67 (m, 1 H), 2.68 - 2.80 (m, 1 H), 2.85 - 2.95 (m, 2 H), 3.35 - 3.47 (m, 2 H), 3.50 - 3.60 (m, 1 H), 3.81 (t, J=6.7 Hz, 1 H), 5.66 (d, J=8.1 Hz, 1 H), 5.97 (d, J=6.2 Hz, 1 H), 6.33 (s, 1 H), 7.53 (d, J=8.1 Hz, 1 H), 11.50 (s, 1 H).

l-((4R,5R,8R)-8-hydroxy-7-methylene-6-oxa-1 -thiaspiro[3,4]octan-5- yl)pyrimidine-2,4(l*H*,3*H*)-dione **10**

The mixture containing intermediate **9** (6 g) was suspended in MeOH (100 mL). NaOMe (30% in MeOH) (14 mL, 5.4 M, 75.7 mmol) was added to the suspension. The resulting mixture was stirred at reflux for 2 h. The reaction mixture was allowed to cool down to RT and filtrated over a small pad of Decalite®. The filtrate was purified by prep HPLC using method A. The fractions were freeze-dried to deliver intermediate **10** (2.6 g, 55% over two steps) as white solid.

m/z [M - H]$^-$ calcd for $C_{11}H_{12}N_2O_4S$: 267.29, found, 267.

^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ ppm 2.54 - 2.68 (m, 1 H), 2.72 - 2.84 (m, 1 H), 2.91 (td, J=8.5, 5.7 Hz, 1 H), 2.94 - 3.05 (m, 1 H), 4.26 (s, 1 H), 4.45 (t, J=1.8 Hz, 1 H), 4.56 (br d, J=6.2 Hz, 1 H), 5.66 (d, J=7.9 Hz, 1 H), 6.06 (d, J=6.4 Hz, 1 H), 6.51 (s, 1 H), 7.33 (d, J=8.1 Hz, 1 H), 11.54 (br s, 1 H)

Synthesis of (4R,5R,7R,8R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-fluoro-7-(iodomethyl)-6-oxa-1-thiaspiro[3.4]octan-8-yl benzoate **11**

Intermediate **10** (1 g, 3.7 mmol) was dissolved in ACN (20 mL) and THF (30 mL), the resulting mixture was cooled to -15°C under N_2 atmosphere then triethylamine trihydrofluoride (0.6 mL, 0.989 g/mL, 3.7 mmol) in 5 mL of ACN was added dropwise followed by the addition of NIS (1 g, 4.4 mmol). The resulting reaction mixture was stirred for 1h at -15°C under N_2 atmosphere. Afterwards, Et_3N (2.6 mL, 0.728 g/mL, 18.6 mmol) and DMAP (9.1 mg, 0.08 mmol) were added to the reaction mixture. The reaction mixture was diluted with 40 mL of THF followed by the dropwise addition of benzoyl chloride (0.433 mL, 1.211 g/mL, 3.7 mmol) at 0°C. The reaction mixture was allowed to warm up to RT and stirred for 3 h. The reaction mixture was diluted with EtOAc (30 mL) and successively washed with brine, a saturated solution of $Na_2S_2O_3$, dried over $MgSO_4$ and purified by column chromatography (heptane/EtOAc) to afford intermediate **11** (1.2 g, yield 62 %) as light yellow solid.

m/z [M - H]$^-$ calcd for $C_{18}H_{16}FIN_2O_5S$: 516.98, found, 516.8.

^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ ppm 2.80 (br s, 2 H), 2.93 (br d, 7=5.3 Hz, 1 H), 3.04 - 3.20 (m, 1 H), 3.50 - 3.77 (m, 2 H), 5.78 (br d, 7=7.7 Hz, 1 H), 6.04 (br s, 1 H), 6.59 (br s, 1 H), 7.63 (br t, 7=7.3 Hz, 2 H), 7.70 - 7.98 (m, 1 H), 8.18 (br d, 7=7.3 Hz, 2 H), 11.65 (br s, 1 H)

Synthesis of((4R,5R,7S,8R)-8-(benzoyloxy)-5-(2,4-dioxo-3,4- dihydropyrimidin-1 (2H)-yl)-7-fluoro-6-oxa-1 -thiaspiro[3,4]octan-7-yl)methyl benzoate **12**

Intermediate **11** (1.2 g, 2.3 mmol), sodium benzoate (1.7 g, 11.6 mmol) and 15-crown-5 (4.6 mL, 1.11 g/mL, 23.2 mmol) were suspended in DMF (50 mL) under N_2 atmosphere. The reaction mixture was stirred for 18 h at 120°C. Afterwards, the reaction mixture was allowed to cool down to 45-50°C then diluted with EtOAc (l00 mL) and filtrated. The organic layer was washed successively with brine, a saturated solution of $Na_2S_2O_3$, and dried over Na_2SO_4. The solvent was removed, the crude was purified by column chromatography (heptane/EtOAc: 100/100 to 50/50) to afford intermediate **12** (700 mg, 59 %) as light yellow solid.

m/z [M - H]$^-$ calcd for $C_{25}H_{21}FN_2O_7S$: 511.51, found, 511.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.76 (br s, 1 H), 2.89 - 2.95 (m, 1 H), 3.11 (br s, 1 H), 3.17 - 3.30 (m, 1 H), 4.54 (dd, 7=12.3, 5.7 Hz, 1 H), 4.72 (dd, 7=12.2, 8.7 Hz, 1 H), 5.53 - 5.64 (m, 1 H), 5.92 (s, 1 H), 6.58 - 6.79 (m, 1 H), 7.28 (s, 1 H), 7.33 - 7.42 (m, 2 H), 7.47 - 7.54 (m, 2 H), 7.54 - 7.59 (m, 1 H), 7.65 (t, 7=6.9 Hz, 1 H), 7.98 (d, 7=7.7 Hz, 2 H), 8.25 (d, 7=7.6 Hz, 2 H).

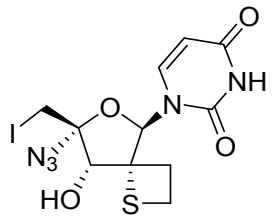
Synthesis of l-((4R,5R,7S,8R)-7-fluoro-8-hydroxy-7-(hydroxymethyl)-6-oxa-l-thiaspiro[3.4]octan-5-yl)pyrimidine-2,4(l*H*,3*H*)-dione **13**

Intermediate **12** (700 mg, 1.4 mmol) was solubilized in NH_3 (7M in MeOH) (200 mL) and stirred overnight at RT. The solvent was removed, and the solid was triturated in Et_2O to obtain compound **13** (269 mg, 65 %).

m/z [M - H]$^-$ calcd for $C_{11}H_{13}FN_2O_5S$: 303.29, found, 303.

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 2.32 - 2.45 (m, 1 H), 2.83 (br dd, >8.4, 4.0 Hz, 1 H), 2.88 - 3.03 (m, 1 H), 3.08 - 3.20 (m, 1 H), 3.51 - 3.67 (m, 2 H), 4.08 (br d, >19.4 Hz, 1 H), 5.67 (d, >7.9 Hz, 1 H), 5.75 (br s, 1 H), 5.93 (br s, 1 H), 6.71 (br s, 1 H), 7.65 (br d, >8.4 Hz, 1 H), 11.53 (br s, 1 H).

Synthesis of l-((4R,5R,7S,8R)-7-azido-8-hydroxy-7-(iodomethyl)-6-oxa-l-thiaspiro[3.4]octan-5-yl)pyrimidine-2,4(lH,3H)-dione **14a**



(14a)

N-benzyl-N,N-diethylethanaminium Chloride ($BnEt_3NCI$) (4.59 g, 20.13 mmol) and sodium azide (NaN_3) (1.31 g, 20.13 mmol) were suspended in MeCN (30 mL) and stirred for 16h. The mixture was filtrated into a solution of intermediate **10** (900 mg, 3.36 mmol) and NMM (5.4 mL, 0.917 g/mL, 48.97 mmol) in THF (60 mL). The reaction mixture was cooled to 0°C and Iodine (5.11 g, 20.13 mmol) in THF (18 mL) was added. The reaction mixture was stirred for 5h at RT. N-acetyl-cysteine (2g) was added to the mixture until no gas evolved. Saturated aqueous $Na_2S_2O_3$ was added to the mixture until a light-yellow solution developed. The solution was concentrated under reduced pressure then diluted in EtOAc (50 mL). The organic layer was washed with brine and dried over $MgSO_4$. Solvent was removed and the crude was purified by column chromatography using Heptane/EtOAc as eluent to afford intermediate **14a** (1.49 g, yield 99%).

m/z [M - H]$^-$ calcd for $C_{11}H_{12}IN_5O_4S$: 436.21, found, 436.

H NMR (400 MHz, DMSO-*d*$_6$)) δ ppm 2.52 - 2.61 (m, 1 H), 2.76 - 2.98 (m, 3 H), 3.75 (s, 2 H), 4.34 (br s, 1 H), 5.68 (d, J=8.1 Hz, 1 H), 6.47 (br d, J=6.2 Hz, 2 H), 7.43 - 7.57 (m, 1 H), 11.57 (s, 1 H).

Synthesis of(4R,5R,7S,8R)-7-azido-5-(2,4-dioxo-3,4-dihydropyrimidin-l(2*H*)- yl)-7-(iodomethyl)-6-oxa-l-thiaspiro[3.4]octan-8-yl benzoate **14**

Intermediate **14a** (1.49 g, 3.41 mmol) was dissolved in THF (45 mL) and the mixture was cooled to 0°C. Et_3N (2.368 mL, 0.728 g/mL, 17.04 mmol) and DMAP (8.33 mg, 0.07 mmol) were added to the mixture followed by the dropwise addition of benzoyl chloride (0.475 mL, 1.211 g/mL, 4.09 mmol). The reaction mixture was stirred for 2 h at RT. The reaction mixture was diluted in EtOAc (100 mL). The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude was purified by column chromatography using Heptane/EtOAc as eluent to afford intermediate **14** (1.5 g, yield 81%) as white foam.

m/z [M - H]$^-$ calcd for $C_{18}H_{16}IN_5O5S$: 539.99, found, 540.

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 2.73 - 2.84 (m, 2 H), 2.84 - 2.94 (m, 1 H), 3.02-3.12 (m, 1 H), 3.79 (brd, J=11.7 Hz, 1 H), 3.92 (br d, J=11.7 Hz, 1 H), 5.77 (dd, J=8.0, 2.1 Hz, 1 H), 6.02 (br s, 1 H), 6.50 (br s, 1 H), 7.63 (t, J=7.2 Hz, 2 H), 7.72 - 7.85 (m, 2 H), 8.18 (d, J=7.6 Hz, 2 H), 11.63 (s, 1 H)

Synthesis of[(4R,5R,6R,8R)-6-azido-5-benzoyloxy-8-(2,4-dioxopyrimidin-lyl)-7-oxa-l-thiaspiro[3.4]octan-6-yl]methyl benzoate **15**

Intermediate **14** (1.5 g, 2.77 mmol) and BzONa (2 g, 13.86 mmol) were suspended in DMF (80 mL) followed by the addition of 15-crown-5 (5.5 mL, 1.11 g/mL, 27.7 mmol). The reaction mixture was stirred overnight at 120°C. The reaction mixture was diluted in EtOAc (100 mL), filtrated over a small bed of decalite and washed with water. The organic layer was dried over $MgSO_4$ and the solvent was removed. The crude was purified by column chromatography using Heptane/EtOAc as eluent to afford intermediate **15** (700 mg, yield 47%) as light yellow solid 63% pure as determined by LC-MS. The compound was used as such in the next step.

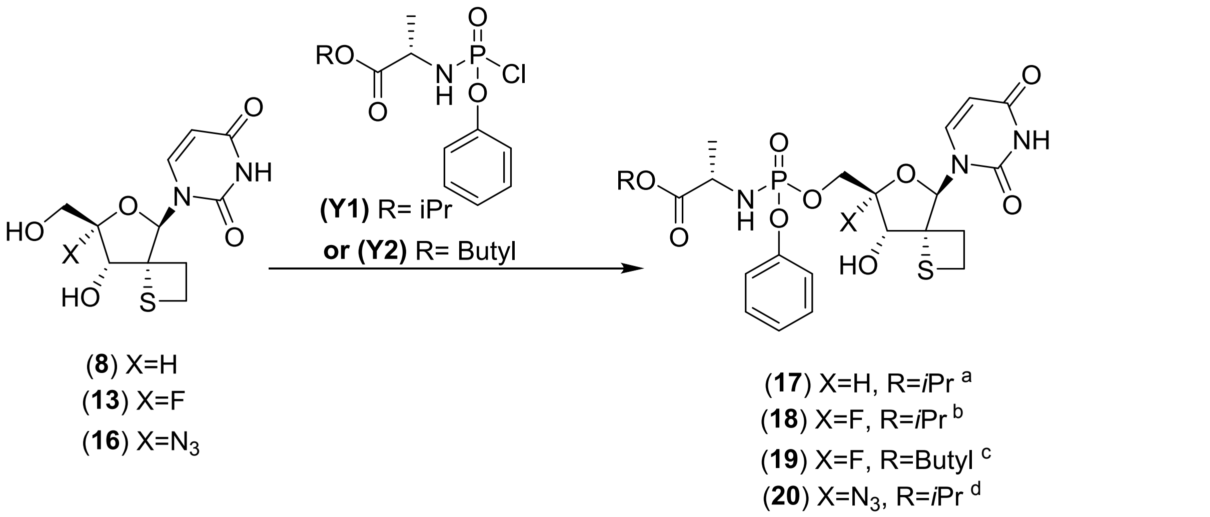
m/z $[M - H]^-$ calcd for $C_{25}H_{21}N_5O_7S$: 534.53, found, 534.1.

Synthesis of (4R,5R,7R,8R)-7-azido-7-((benzoyloxy)methyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-6-oxa-1-thiaspiro[3,4] octan-8-yl benzoate **16**

Intermediate **15** (700 mg, 1.307 mmol) was dissolved in ammoniac (7M in MeOH) (150 mL, 7 M, 1.05 mol) and the mixture was stirred overnight at RT. The reaction mixture was concentrated until dryness and the solid was triturated in Et_2O to afford 1- [(4R,5R,6R,8R)-6-azido-5-hydroxy-6-(hydroxymethyl)-7-oxa-l-thiaspiro[3.4]octan-8-yl]pyrimidine-2,4-dione **16** (360 mg, yield 84%) as light yellow solid.

m/z $[M - H]^-$ calcd for $C_{11}H_{13}N_5O_5S$: 326.31, found, 326.

^{1}H NMR (400 MHz, DMSO-*d₆*) δ ppm 2.38 - 2.48 (m, 1 H), 2.78 - 2.92 (m, 2 H), 3.02 - 3.10 (m, 1 H), 3.69 - 3.78 (m, 2 H), 4.11 (br d, J=5.3 Hz, 1 H), 5.67 (d, J=8.1 Hz, 1 H), 5.76 (br s, 1 H), 5.93 (br d, J=4.2 Hz, 1 H), 6.60 (br s, 1 H), 7.66 (d, J=8.1 Hz, 1 H), 11.31 (brs, 1 H).



Scheme S3. 5'-phosphoramidate nucleoside synthesis

Conditions : (a) **Y1**, DCM, NMI, 20°C (16 h), 12%.(b) ^{t}BuMgCl, THF, -5°C (45 min.) then **Y1**, -5°C (2 h) then RT (overnight), 38%. (c) **Y2**, NMI, DCM, (overnight), 17%. (d) **Y1**, NMI, DCM, RT (20 h), 22%.

Scheme S3 Synthesis of (2S)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate **Y1**

To a solution of (S)-isopropyl 2-aminopropanoate hydrochloride [39825-33-7] (5 g, 29.8 mmol) in dichloromethane (50 mL), phenyl phosphorodichloridate [770-12-7] (4.45 g, 29.8 mmol) was added at 20°C. The resulting mixture was cooled to -78°C then diisopropylethyl amine (10.4 mL, 59.6 mmol)

was added dropwise. The reaction mixture was stirred at -78°C for 1 h then the temperature of the reaction was allowed to rise to 20°C. After 1 h the solvent was removed under reduced pressure. Dry Et_2O (about 50 ml) was added, and the formed precipitate was filtered off and washed two times with dry Et_2O under nitrogen. The filtrate was evaporated to dryness to give yellow colourless oil **Y1** (8.32 g, 91%) which was stored as a 1 M solution in dry tetrahydrofuran (THF) in the freezer at -20°C.

^{1}H NMR (400 MHz, $CDC1_3$) δ ppm 1.24-1.31 (m, 6 H), 1.50 (dd, J=7.0, 2.1 Hz, 3 H), 4.06 - 4.20 (m, 1 H), 4.23 - 4.41 (m, 1 H), 5.02 - 5.14 (m, 1 H), 7.19 - 7.30 (m, 3 H), 7.34-7.41 (m,2H).

Synthesis of (2S)-butyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate Y2

(S)-l-Butoxy-l-oxopropan-2-aminium [81305-85-3] (2 g, 11 mmol) was solubilized in dichloromethane (50 mL) and cooled to -78°C. To this mixture phenyl phosphorodichloridate [770-12-7] (1.6 mL, 11 mmol) was added slowly followed by the dropwise addition of DIPEA (3.9 mL, 0.742 g/mL, 22 mmol) under N_2 atmosphere. The reaction mixture was stirred for 1h then allowed to warm up until room temperature and stirred for 2h. Afterwards, the solvent was removed. Dry Et2O (100 mL) was added under nitrogen the resulting mixture was filtrated under nitrogen flow and the filtrate was concentrated under reduced pressure to afford intermediate **Y2** (2.87 g, 82 %) as colourless oil. This oil was stored as a 1 M solution in dry tetrahydrofuran in the freezer at -20°C.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.94 (td, J=7.4, 5.4 Hz, 3 H), 1.33 - 1.45 (m, 2 H), 1.49 - 1.54 (m, 3 H), 1.59 - 1.70 (m, 2 H), 4.09 - 4.26 (m, 3 H), 4.53 - 4.67 (m, 1 H), 7.20 - 7.30 (m, 3 H), 7.33 - 7.41 (m, 2 H).

Synthesis of (2S)-isopropyl 2-(((((4R,5R,7R,8R)-5-(2,4-dioxo-3,4- dihydropyrimidin-1 (2*H*)-yl)-8-hydroxy-6-oxa-1-thiaspiro[3,4]octan-7-yl)methoxy)(phenoxy)phosphoryl)amino) propanoate **17**

Compound **8** (500 mg, 1.7 mmol) was dissolved in dry pyridine (15 mL) and stirred for 1 h at RT then evaporated to dryness. The resulting precipitate was suspended in dry dichloromethane (15 mL) and N-methyl imidazole (1.3 mL, 17.4 mmol) was added dropwise. The resulting solution was treated with phosphorochloridate **Y1** (2.62 mL, 2.62 mmol) 1 M solution in dry THF under nitrogen. The reaction mixture was stirred at 20°C for 16 h and was diluted with DCM (20 mL) and washed with aqueous solution of 1M HC1 (3 x 20 mL). The combined aqueous layers were extracted with DCM (30 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (gradient DCM/MeOH 1 to 10%) to yield (100 mg, 12%) of compound **17** as white foam.

m/z $[M + H]^+$ calcd for $C_{23}H_{30}N_3O_9PS$: 556.54, found, 556.

^{1}H NMR (400 MHz, $CDC1_3$) δ ppm 1.18 - 1.26 (m, 6 H), 1.30 - 1.38 (m, 2 H), 2.62 (s, 1 H), 2.66 - 2.90 (m, 2 H), 3.01 (td, J=8.7, 5.6 Hz, 1 H), 3.14 - 3.22 (m, 1 H), 3.46 - 3.63 (m, 1 H), 3.70 (s, 1 H), 3.85 - 4.04 (m, 3 H), 4.32 - 4.54 (m, 2 H), 4.97 - 5.07 (m, 1 H), 5.59 - 5.65 (m, 1 H), 6.50 - 6.55 (m, 1 H), 7.15 - 7.25 (m, 3 H), 7.30 - 7.37 (m, 2 H), 7.46 - 7.55 (m, 1 H), 9.07 (br s, 1 H).

^{13}C NMR (101 MHz, DMSO-*d_6*) d ppm 19.86 (s, 1 C) 19.90 (s, 1 C) 20.06 (s, 1 C) 20.14 (s, 1 C) 20.21 (s, 1 C) 20.27 (s, 1 C) 21.86 (d, 1 C) 31.19 (s, 1 C) 31.29 (s, 1 C) 40.89 (s, 1 C) 50.24 (s, 1 C) 50.39 (s, 1 C) 60.64 (s, 1 C) 60.75 (s, 1 C) 64.82 (br d, 1 C) 65.31 (br d, 1 C) 68.47 (s, 1 C) 73.12 (s, 1 C) 73.44 (s, 1 C) 79.87 (d, 1 C) 80.01 (s, 1 C) 80.09 (s, 1 C) 92.41 (s, 1 C) 92.45 (s, 1 C) 102.33 (s, 1 C) 102.33 (s, 1 C) 115.68 (s, 1 C) 119.24 (s, 1 C) 120.47 (br d, 1 C) 125.04 (s, 1 C) 128.46 (s, 1 C) 129.82 (s, 1 C) 130.12 (s, 1 C) 140.57 (s, 1 C) 140.84 (s, 1 C) 151.11 (br d, 1 C) 151.19 (s, 1 C) 151.19 (s, 1 C) 163.30 (s, 1 C) 163.30 (s, 1 C) 173.03 (s, 1 C) 173.09 (s, 1 C) 173.17 (d, 1 C).

HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{23}\ H_{31}\ O_9\ N_3\ P\ S^+$, 556,15131; found, 556,15189.

Synthesis of (2S)-isopropyl 2-(((((4R,5R,7S,8R)-5-(2,4-dioxo-3,4- dihydropyrimidin-1 (2H)-yl)-7-fluoro-8-hydroxy-6-oxa-1-thiaspiro[3,4]octan-7-yl)methoxy)(phenoxy)phosphoryl) amino) propanoate **18**

Compound **13** (250 mg, 0.8 mmol) was dissolved in dry pyridine (10 mL) and the solvent was removed under reduced pressure. The foam obtained was solubilized in THF (50 mL) the mixture was cooled to -5°C. The resulting mixture was stirred at -5°C for 10 minutes then *tert*-butylmagnesium chloride [677-22-5] (2.14 mL, 1 M, 2.14 mmol) was added at -5°C over 5 minutes. The reaction mixture was kept at -5°C for 45 minutes. To this mixture intermediate **Y1** (1.15 mL, 1 M, 1.15 mmol) was added dropwise under N_2 atmosphere at -5°C, the reaction mixture was kept at -5°C for 2 hours then left at RT for night. The reaction mixture was quenched with a mixture of 100 mL of cold water and 30 mL of EtOAc. The mixture was extracted with ethyl acetate (3 x 50 mL). The organic layer was dried over Na_2SO4, filtrated and the solvent was removed under reduced pressure to afford a foam containing the compound. A purification was performed by column chromatography to yield (450 mg, 58% pure) of a foam this was purified by Prep HPLC (method C) to yield compound **18** (170 mg, 38 %) as white powder.

The compound were separated by SFC method described above

Compound **18**

m/z $[M - H]^-$ calcd for $C_{23}H_{29}FN_3O_9PS$: 572.53, found, 572.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.15 (d, >6.2 Hz, 6 H), 1.21 (dd, >10.6, 7.3 Hz, 3 H), 2.53 - 2.64 (m, 1 H), 2.82 - 2.97 (m, 2 H), 3.05 (br s, 1 H), 3.72 - 3.85 (m, 1 H), 4.14 - 4.34 (m, 3 H), 4.85 (dt, >12.5, 6.3 Hz, 1 H), 5.58 (d, >8.1 Hz, 1 H), 6.02 - 6.19 (m, 2 H), 6.67 (br s, 1 H), 7.14 - 7.25 (m, 3 H), 7.37 (br t, >7.9 Hz, 3H), 10.86- 11.82 (m, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.16 (d, 1 C) 20.55 (s, 1 C) 21.82 (s, 1 C) 21.87 (s, 1 C) 31.79 (s, 1 C) 31.84 (s, 1 C) 50.29 (d, 1 C) 57.08 (s, 1 C) 62.80 (br d, 1 C) 63.19 (s, 1 C) 68.51 (s, 1 C) 68.54 (s, 1 C) 73.65 (s, 1 C) 73.65 (s, 1 C) 73.93 (br d, 1 C) 103.15 (s, 1 C) 114.85 (br d, 1 C) 113.81 (s, 1 C) 116.12 (d, 1 C) 120.51 (d, 1 C) 125.17 (s, 1 C) 128.67 (s, 1 C) 130.13 (s, 1 C) 150.91 (s, 1 C) 150.97 (br d, 1 C) 151.02 (s, 1 C) 163.19 (s, 1 C) 173.16 (d, 1 C).

HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{23}\ H_{30}\ O_9\ N_3\ F\ P\ S^+$, 574,14189 ; found, 574,14236.

Isopropyl ((S)-(((4R,5R,7S,8R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-fluoro-8-hydroxy-6-oxa-1-thiaspiro[3.4]octan-7-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate **18a**

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.13 - 1.17 (m, 6 H) 1.18 - 1.22 (m, 3 H) 2.52 - 2.61 (m, 1 H) 2.82 - 2.95 (m, 2 H) 2.99 - 3.10 (m, 1 H) 3.66 - 3.84 (m, 1 H) 4.13 - 4.34 (m, 3 H) 4.85 (spt, *J*=6.3 Hz, 1 H) 5.58 (d, *J*=8.1 Hz, 1 H) 6.03 - 6.21 (m, 2 H) 6.67 (br s, 1 H) 7.11 - 7.23 (m, 3 H) 7.31 - 7.43 (m, 3 H) 11.57 (br s, 1 H)
^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 18.90 (s, 1 C) 18.97 (s, 1 C) 19.47 (s, 1 C) 20.77 (d, 1 C) 30.70 (s, 1 C) 39.73 (s, 1 C) 39.94 (s, 1 C) 49.31 (s, 1 C) 56.01 (s, 1 C) 61.72 (br d, 1 C) 62.13 (s, 1 C) 67.45 (s, 1 C) 72.67 (br d, 1 C) 102.07 (s, 1 C) 113.91 (dd, 10.5 Hz, 1 C) 119.44 (d, 1 C) 124.08 (s, 1 C) 129.05 (s, 1 C) 149.87 (d, 1 C) 162.10 (s, 1 C) 172.08 (d, 1 C).

HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{23}\ H_{30}\ O_9\ N_3\ F\ P\ S^+$, 574,14189 ; found, 574,14221.

Isopropyl ((R)-(((4R,5R,7S,8R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-7-fluoro-8-hydroxy-6-oxa-1-thiaspiro[3.4]octan-7-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate **18b**

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 1.15 (d, *J*=6.2 Hz, 6 H) 1.23 (d, *J*=7.0 Hz, 3 H) 2.52 - 2.65 (m, 1 H) 2.82 - 2.97 (m, 2 H) 2.99 - 3.11 (m, 1 H) 3.73 - 3.89 (m, 1 H) 4.13 - 4.39 (m, 3 H) 4.85 (spt, *J*=6.2 Hz, 1 H) 5.58 (d, *J*=7.9 Hz, 1 H) 5.99 - 6.16 (m, 2 H) 6.66 (br s, 1 H) 7.21 (d, *J*=8.6 Hz, 3 H) 7.28 - 7.55 (m, 3 H) 11.54 (br s, 1 H)

^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ ppm 17.75 (s, 1 C) 17.82 (s, 1 C) 18.16 (s, 1 C) 19.48 (d, 1 C) 29.50 (br d, 1 C) 47.83 (s, 1 C) 54.72 (s, 1 C) 61.02 (br d, 1 C) 66.12 (s, 1 C) 71.56 (br d, 1 C) 100.73 (s, 1 C) 112.53 (dd, 1 C) 118.16 (d, 1 C) 122.77 (s, 1 C) 127.74 (s, 1 C) 148.51 (s, 1 C) 148.63 (d, 1 C) 160.83 (s, 1 C) 170.63 (d, 1 C).

HRMS (ESI$^+$): m/z, $[M + H]^+$ calcd for $C_{23}\ H_{30}\ O_9\ N_3\ F\ P\ S^+$, 574,14189; found, 574,14221.

Synthesis of(2R)-butyl 2-(((((4R,5R,7S,8R)-5-(2,4-dioxo-3,4- dihydropyrimidin-1 (2H)-yl)-7-fluoro-8-hydroxy-6-oxa-1-thiaspiro[3,4]octan-7-yl)methoxy)(phenoxy)phosphoryl)amino) propanoate **19**

Compound **13** (100 mg, 0.329 mmol) was dissolved in pyridine (5 mL) and the solvent was removed under reduced pressure. The foam obtained was solubilized in dichloromethane (5 mL) and N-methyl imidazole (0.13 mL, 1.64 mmol). To the resulting mixture, intermediate **Y2** (0.65 mL, 1 M, 0.66 mmol) was added dropwise under N_2 atmosphere at RT. After 5 h of stirring, another equivalent of intermediate **Y2** was added and the mixture was stirred overnight, the reaction mixture was quenched with a mixture of 20 mL of cold water and 20 mL of dichloromethane. The resulting mixture was acidified with HCl 1M until pH = 4 and extracted with dichloromethane (3 x 50 mL). The organic layer was dried over Na_2SO_4, filtrated and the solvent was removed under reduced pressure to afford 300 mg of foam containing the compound. Purification was performed by Prep HPLC using method B to yield compound **19** (32.6 mg, 17 %).

m/z $[M - H]^-$ calcd for $C_{24}H_{31}FN_3O_9PS$: 586.56, found, 586.1.

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 0.85 (td, J=7.4, 2.6 Hz, 3 H), 1.22 (dd, J=10.6, 7.3 Hz, 3 H), 1.25 - 1.33 (m, 2 H), 1.47 - 1.54 (m, 2 H), 2.52 - 2.62 (m, 1 H), 2.88 (br s, 2 H), 3.05 (br s, 1 H), 3.83 (br d, J=9.7 Hz, 1 H), 4.00 (qd, J=6.4, 2.5 Hz, 2 H), 4.22 (br d, J=9.2 Hz, 3 H), 5.57 (d, J=8.4 Hz, 1 H), 6.13 (br d, J=8.4 Hz, 2 H), 6.60 - 6.73 (m, 1 H), 7.20 (br t, J=8.2 Hz, 3 H), 7.33 - 7.40 (m, 3 H), 11.00 - 11.61 (m, 1 H).

Synthesis of isopropyl (2R)-2-[[[(4R,5R,6R,8R)-6-azido-8-(2,4- dioxopyrimidin-1 -yl)-5-hydroxy-7-oxa-1 -thiaspiro[3,4]octan-6-yl]methoxy-phenoxyphosphoryl]amino]propanoate **20**

Compound **16** (200 mg, 0.61 mmol) was dissolved in dry pyridine (5 mL) and the solvent was removed under reduced pressure to obtain a foam. The foam was dissolved in DCM (10 mL) and (0.24 mL, 1.03 g/mL, 3.06 mmol) of N-methylimidazole was added. The reaction mixture was stirred for 5 min at RT under N_2 atmosphere. Intermediate **Y1** (1M in THF) (0.92 mL, 1 M, 0.92 mmol) was added and the reaction mixture was stirred for 20 h at RT under N_2 atmosphere. The reaction mixture was poured in cold water (20 mL) and DCM (20 mL). The aqueous layer was extracted with DCM (3 x 50 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude obtained was purified by Prep HPLC using method D. The obtained fraction was freeze-dried to deliver compound **20** as white powder (80 mg, 22%)

m/z $[M - H]^-$ calcd for $C_{23}H_{29}N_6O_9PS$: 595.55, found, 595.

MS (ES-): 595

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.14 (dd, J=6.2, 2.4 Hz, 6 H), 1.22 (d, J=7.3 Hz, 3 H), 2.52 - 2.60 (m, 1 H), 2.80 - 2.90 (m, 2 H), 2.95 - 3.04 (m, 1 H), 3.72 - 3.84 (m, 1 H), 4.21 - 4.38 (m, 3 H), 4.84 (quind, J=6.3, 6.3, 6.3, 6.3, 4.0 Hz, 1 H), 5.60 (dd, J=7.9, 3.3 Hz, 1 H), 6.06 - 6.22 (m, 2 H), 6.49 - 6.62 (m, 1 H), 7.15 - 7.24 (m, 3 H), 7.33 - 7.40 (m, 2 H), 7.47 (br d, J=7.0 Hz, 1 H), 11.52 (br s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.05 (s, 1 C) 20.12 (s, 1 C) 20.19 (s, 1 C) 20.79 (s, 1 C) 20.79 (s, 1 C) 21.83 (d, 1 C) 21.92 (s, 1 C) 21.92 (s, 1 C) 31.92 (s, 1 C) 50.30 (d, 1 C) 58.03 (s, 1 C) 58.11 (s, 1 C) 66.34 (br d, 1 C) 66.69 (br d, 1 C) 68.45 (s, 1 C) 68.51 (s, 1 C) 75.53 (s, 1 C) 75.80 (s, 1 C) 95.48 (d, 1 C) 103.08 (s, 1 C) 120.53 (br d, 1 C) 120.53 (s, 1 C) 125.16 (s, 1 C) 130.04 (s, 1 C) 130.11 (d, 1 C) 150.97 (d, 1 C) 151.06 (s, 1 C) 163.21 (s, 1 C) 163.25 (s, 1 C) 173.09 (d, 1 C)