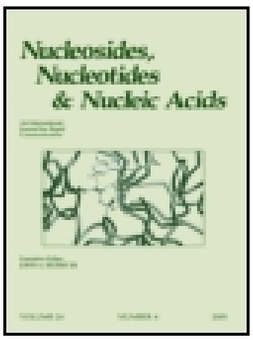

Nucleosides, Nucleotides and Nucleic Acids

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Synthesis and Evaluation of 2′-Deoxy-2′-Spirodiflurocyclopropyl Nucleoside Analogs

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ABSTRACT

The preparation of 2′-deoxy-2′-siprodifluorocyclopropany-lnucleoside analogs has been achieved from α-D-glucose in several steps. The key step in the synthesis was the introduction of the difluorocyclopropane through a difluorocarbene type reaction at the 2′-position. Then, a series of novel 2′-deoxy-2′-spirodifluorocyclopropanyl nucleoside analogs were synthesized using the Vorbrüggen method. All the synthesized nucleosides were characterized and subsequently evaluated against hepatitis C and influenza A virus strains *in vitro*.

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2′-deoxy-2′-spirodifluorocyclopropanyl nucleosides; hepatitis C; influenza A

Introduction

Structurally diverse nucleoside analogs which act as selective inhibitors of polymerases and other enzymes have long been implicated for the treatment of human viral diseases and cancer.[1] Of the nucleosides that are currently used in clinical settings, the majority have modified sugar moieties with naturally occurring nucleobases. In particular, the 2′-modified nucleoside derivatives, such as gemcitabine[2] and recently approved sofosbuvir[3] (Figure 1A and B), have acquired great commercial success. In this respect, fluorine[4,5] substitutions have been shown to affect the metabolic stability, lipophilicity, and the binding affinity of many 2′-modified nucleoside analogs[6].

In addition, 2′-deoxy-2′-spirocyclopropylcytidine[7] (Figure 1C) has been reported as a new member of the class of 2′-modified nucleoside derivatives. This compound exhibits potent antiviral activity by inhibiting the hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase. 2′-Deoxy-2′-spirocyclopropylcytidine has been shown to display an EC_{50} of 7.3 μM measured in the Huh7-Rep cell line and no associated cytotoxicity ($CC_{50} > 98.4$ μM). The

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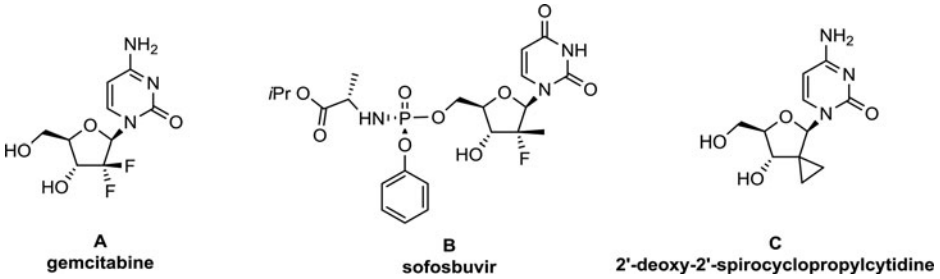


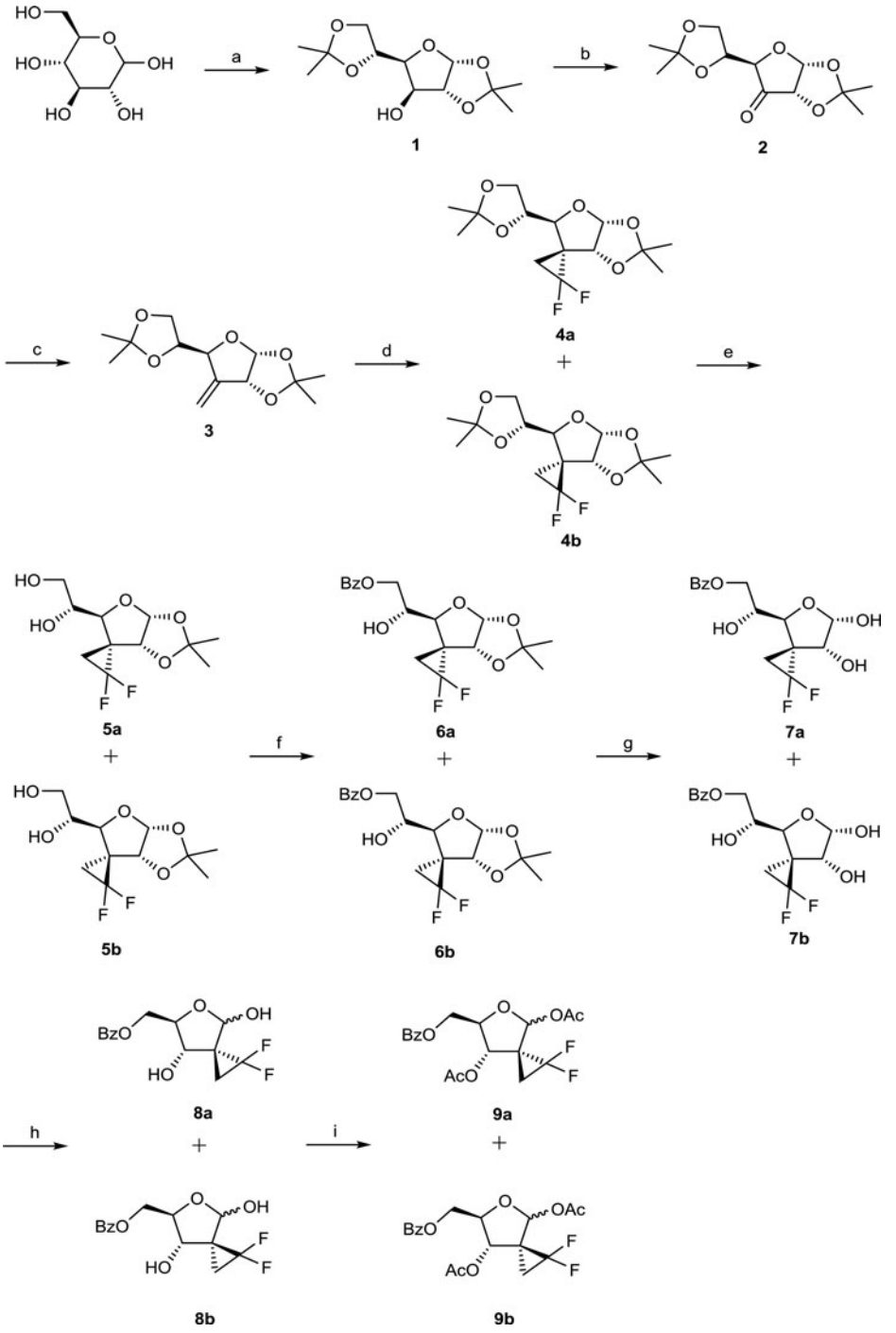
Figure 1. 2′-modified nucleosides.

key structural feature of the 2′-deoxy-2′-spirocyclopropylcytidine is the previously unreported 2′-cyclopropyl substitution, which makes it a useful surrogate in the series of anti-HCV nucleosides. As part of our effort to identify novel nucleoside derivatives for the treatment of viral infections, we sought a synthetic means to prepare the diastereomers of the 2-difluorocyclopropane sugar that could be further transformed to 2′-deoxy-2′-spirodifluorocyclopropanyl nucleosides. The results are described here.

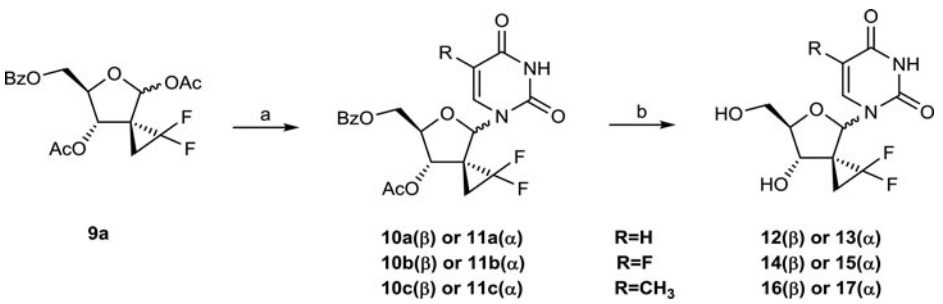
Chemistry

For the synthesis of the targeted nucleosides, we first designed and constructed a series of 2-difluorocyclopropane ribosugars. The 2-difluorocyclopropane sugar **9a** and **9b** were synthesized from α-D-glucose via **4a** and **4b**. The α-D-glucose was treated with conc. H_2SO_4 and acetone with recrystallization to give compound **1** in 65% yield. Compound **1** was subjected to oxidation and Wittig reaction to get the alkenes **3**. Then, different conditions were tried to generate difluocarbene, such as $TMSCF_3$,[8] $TMSCF_2Br$,[9] $ClF_2CCOONa$,[10] TMSCl/MDFA,[11] and TFDA[11,12]. The catalyst was chosen from NaI, NaF, KI, AIBN, and TBAB, and the solvent was chosen from DMF, THF, CH_3CN, toluene and diglyme. The reaction temperature ranged from room temperature to 180°C, and the reaction time varied from a few hours to several days. However, all of these condensation conditions failed to generate detectable amounts of the desired product, except $TMSCF_3$ with the catalyst NaI in THF at 100°C that yielded difluorocyclopropanes **4a**(R) and **4b**(S) in 30% (R:S = 5:1). **4a** was selectively deprotected and protected with a benzoyl group at the primary hydroxyl group to give **6a**, which was further deprotected to lead to **7a**. Compound **7a** was then treated with $NaIO_4$ and saturated sodium bicarbonate solution to give **8a**, which was then protected by treatment with acetic anhydride and trimethylamine in dichloromethane to give compound **9a**. Compound **7b** was also subjected to the same conditions to yield compound **9b** (Scheme 1).

For the preparation of targeted compounds, we first employed Vörbrügen[13] sugar-base condensations under various conditions. Two sugars **9a** and **9b** and five bases (uracil, cytosine, 5′-fluorouracil, thymidine and adenine) were used for the condensation reactions, while the silylating agent was either *N,O*-bis(trimethylsilyl)acetamide (BSA) or hexamethyldisilazane (HMDS).[14] Solvents



Scheme 1. Conditions: (a) acetone, conc. H_2SO_4, 0 °C; (b) Tempo, NaClO, $NaHCO_3$, TBAB, KBr, DCM; (c) Methyltriphenylphosphonium bromide, Potassium tert-butoxide, THF; (d) $TMSCF_3$, NaI, THF 100 °C; (e) 0.8% H_2SO_4, MeOH; (f) BzCl, TEA, −5 °C ∼ 0 °C; (g) 50% CF_3COOH, overnight; (h) $NaIO_4$, $NaHCO_3$, DCM; (i) Ac_2O, TEA, DMAP, DCM.



Scheme 2. Conditions: (a) persilylated base, TMSOTf, 1, 2-DCE, r.t.; (b) NH_3 in MeOH.

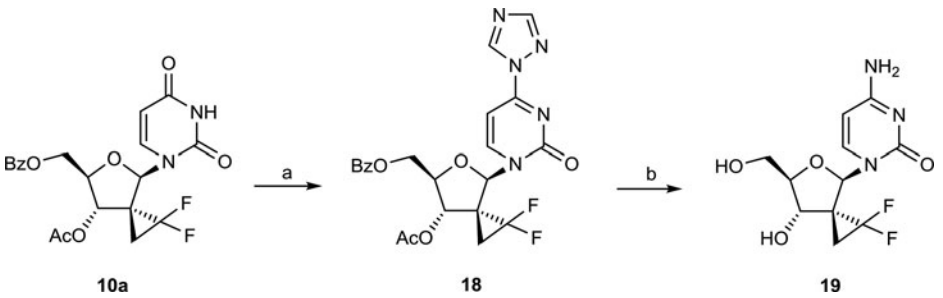
DCM, 1,2-DCE, and CH_3CN were analyzedin the presence or absence of a Lewis acid catalyst trimethylsilyltrifluoromethanesulfonate (TMSOTf) or $SnCl_4$[15]. The reaction temperature ranged from room temperature to 50°C,and the reaction time varied from a few hours to several days. Finally, it was found that dissolving the mixture of sugar and silylated base in 1,2-DCE that was followed by the slow addition of TMSOTf at room temperature can give the desired product in good yields.

The modified uridine, 5′-fluorouridine and thymidine were prepared as depicted in Scheme 2. Uridine **12**(β) and **13**(α) in 66% yield (over two steps) were obtained as a 2:1 β:α mixture of the anomers. The 5′-fluorouridine **14**(β) and **15**(α) in 49% yield (over two steps) were obtained as a 3:1 β:α mixture of anomers. The thymidine **16**(β) and **17**(α) in 69% yield (over two steps) were obtained as a 4:1 β:α mixture of anomers.

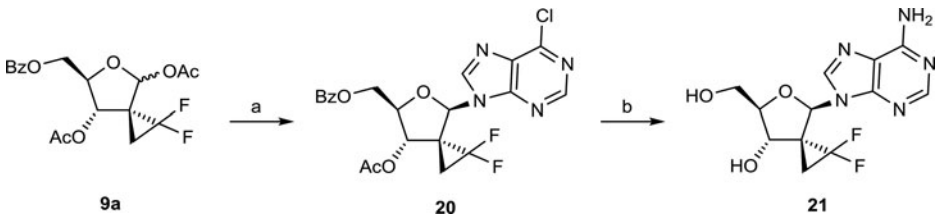
With the intermediate **10a**in hand, the synthesis of **19** was accomplished by conversion of the uracil moiety to the corresponding cytidine derivative, using phosphorus oxychloride and 1 H-1,2,4-triazole as the reagents to obtain **18**, which under went aminolysis to give the target compound (Scheme 3).

Purine nucleosides were obtained using a similar synthetic strategy as described in Scheme 2 to give intermediate **20**, which was then subjected toaminolysis with NH_4OH at 100°C to yield the adenosine nucleoside **21** (Scheme 4).

The other isomer **9b** was also subjected to a similar synthetic route to afford the corresponding target compounds. The desired uridines **22**(β) and **23**(α) were



Scheme 3. Conditions: (a) $POCl_3$, 1 H-1,2,4-triazole, triethylamine, DCM, r.t, 4 h; (b) (i) NH_4OH, THF, r.t., 12 h; (ii) NH_3 in MeOH, r.t., overnight.



Scheme 4 Conditions: (a) persilylated6-chloropurine, TMSOTf, 1,2-DCM, r.t; (b) NH_4OH, 1,4-dioxane, 100 °C, 12 h.

obtained in 44% yield (over two steps) as a 1.8:1 β:α mixture of anomers. The 5′-fluorouridine **24**(β) and **25**(α) were obtained in 43% yield (over two steps) as a 2.7:1 β:α mixture of anomers. The thymidines **26**(β) and **27**(α) were obtained in 54% yield (over two steps) as a 4:1 β:α mixture of anomers. The adenine nucleoside **30** was obtained in 24% yield (over two steps). These compounds are depicted in Figure 2.

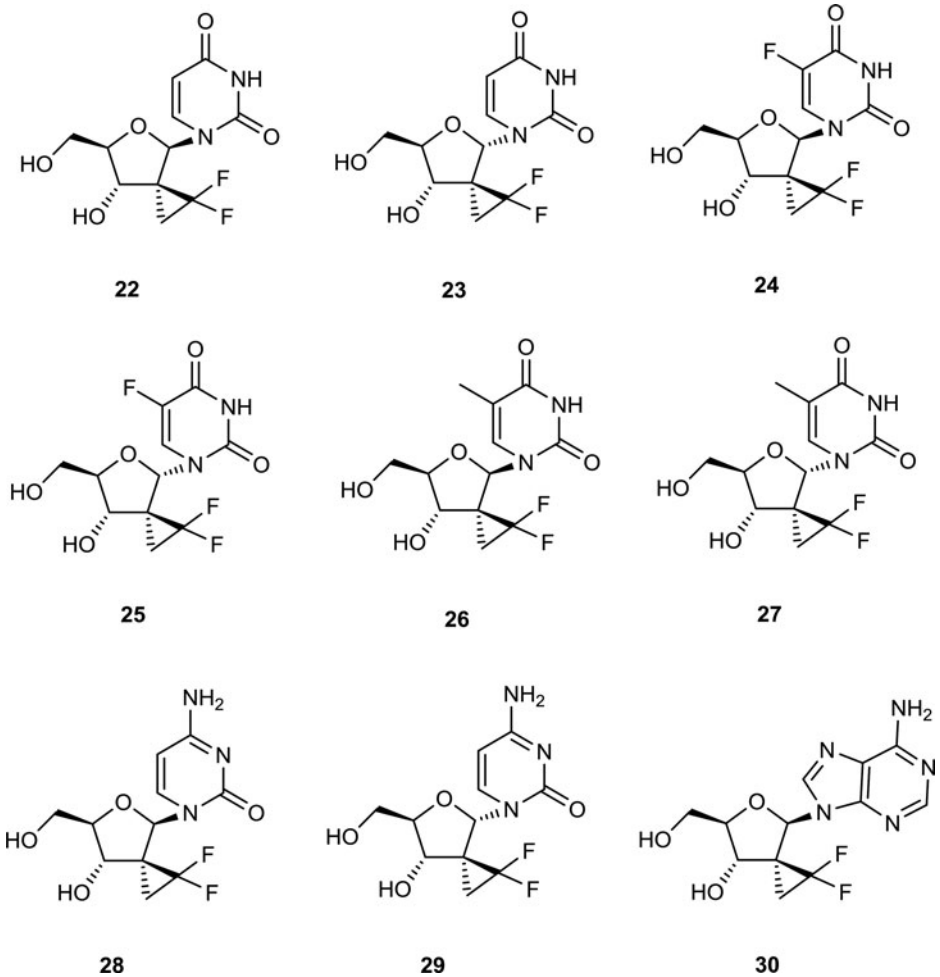


Figure 2. The chemical structures of compounds 22 to 30.

The synthesized compounds were assigned based on the presence of a cross-peak between H-1′,H-3′, H-4′ in 2D NOESY experiments to confirm the α and β anomers.

Antiviral results

To gain some measure of biological potential, all the synthesized nucleoside derivatives were evaluated *in vitro* against the HCV, and influenza A virus (H1N1, H3N2) strains. All of the compounds had EC_{50} and IC_{50} values of >100 μM, and were neither active nor cytotoxic in the performed assays.

Conclusion

We have designed and syntheses a novel type of nucleoside analogs which bear a difluorocyclopropane mioety at the C-2 position of the ribosugar. The 2-difluorocyclopropane precursors **9a** and **9b** can be obtained from glucose, and the nucleoside analogs were obtained through the conventional Vörbrügen method.

Experimental

Reactions requiring anhydrous conditions were conducted in oven-dried apparatus under a dry argon atmosphere, utilizing commercially available dry solvents and reagents. All common reagents were purchased from commercial sources and used without further purification. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on Bruker AV 400 MHz or 500 MHz Fourier transformation spectrometer. Spectra were obtained from samples in $CDCl_3$, CD_3OD, or DMSO-d_6. Multiplicities are as quoted: s = singlet,d = doublet, t = triplet, q = quartet, m = multiplet. Coupling constants (J) are reported in Hz. Signal assignments are based on COSY, DEPT, spectra. HRMS spectra were obtained using Agilent1200, MSD LC-MS.

(3aR,5S,6S,6aR)-5-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-6-ol(1)

Using a 1 L round bottom flask, 30 g (0.17 mol) of anhydrous α-D-glucose was added in 0.6 L of acetone. The mixture was stirred vigorously in an ice-water bath. Concentrated H_2SO_4 (30 mL) was added dropwise for 6 hours. After all the acid was added, the solution was cooled to 0°C and neutralized by 50% aqueous *potassium* hydroxide solution to maintain the pH of the solution near 7. After stirring overnight, the solution was filtered, and the solvent was removed under reduced pressure. The solid was dissolved in 100 mL of dichloromethane and the solution was washed with 100 mL of water. The aqueous phase was then extracted three times with 100 mL of dichloromethane. The organic layers were combined and then concentrated under reduced pressure. Recrystallization from petroleum ether gave 28 g of white crystals (64.7% yield).^{1}H NMR (500 MHz, Chloroform-*d*) δ: 5.94 (d, $J = 3.6$ Hz, 1 H), 4.53

(d, $J = 3.7$ Hz, 1 H), 4.39–4.29 (m, 2H), 4.16 (dd, $J = 8.7$, 6.2 Hz, 1 H), 4.06 (dd, $J = 7.6$, 2.8 Hz, 1 H), 3.98 (dd, $J = 8.6$, 5.4 Hz, 1 H), 2.65 (d, $J = 3.7$ Hz, 1 H), 1.49 (s, 3H), 1.44 (s, 3H), 1.36 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ: 111.96, 109.79, 105.42, 85.22, 81.27, 75.34, 73.61, 67.80, 26.99, 26.90, 26.32, 25.28.

(3aR,5R,6aS)-5-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyldihydrofuro[2,3-d][1,3]dioxol-6(5H)-one (2)

To a solution of diacetone glucose **1** (30 g, 1.24 mol) in DCM (300 mL) were added KBr (1.4 g, 11.5 mmol), TBAB (3,7 g, 11.5 mmol) and TEMPO (3.6 g, 23.1 mmol). The resulting mixture was vigorously stirred, and an aqueous solution of NaClO (1.6 mol/L, 150 mL, 0.24 mol; pH adjusted with $NaHCO_3$ to 9.5) was added dropwise. After addition of the bleach solution, the resulting reaction mixture was stirred for 5 min, and the layers were separated. The aqueous phase was then extracted three times with 100 mL of dichloromethane. The organic layers were combined, dried (Na_2SO_4), and concentrated under reduced pressure. Product **2** was obtained as a yellowish liquid and was used in the next step without additional purification.

(3aR,5S,6aR)-5-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-6-methylene-tetrahydrofuro[2,3-d][1,3]dioxole (3)

Methyl triphenylphosphonium bromide (49.4g, 0.14 mol) and potassiumtert-butoxide (15.5g, 0.14 mol) was added in anhydrous THF (300 mL) at 0°C. Then, a solution of ketone (4.6 g, 17.8 mmol) in anhydrous THF (200 mL) was added to the reaction mixture. After 1 hour of stirring at room temperature, the mixture was partitioned between EA and water. The aqueous layer was washed 3 times with EA, The combined organic layer was dried over $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (PE:EA = 8:1) to give compound **3** as a colorless oil (24.2g, two steps of 82% yield).^{1}H NMR (400 MHz, Chloroform-*d*) δ: 5.78 (d, $J = 4.2$ Hz, 1H), 5.48 (d, $J = 1.9$ Hz, 1H), 5.42 (d, $J = 2.2$ Hz, 1H), 4.86 (d, $J = 4.0$ Hz, 1H), 4.63 (d, $J = 5.8$ Hz, 1H), 4.07–3.99 (m, 2H), 3.95–3.86 (m, 1H), 1.49 (s, 3H), 1.41 (s, 3H), 1.34 (d, $J = 4.8$ Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ: 146.76, 113.31, 112.51, 109.69, 104.45, 82.08, 79.17, 77.25, 66.66, 27.30, 27.00, 26.51, 25.35.

(1R,3a′R,5′S,6a′R)-5′-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-difluoro-2′,2′-dimethyldihydro-5′H-spiro[cyclopropane-1,6′-furo[2,3-d][1,3]dioxole] (4a) and (1S,3a′R,5′S,6a′R)-5′-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-difluoro-2′,2′-dimethyldihydro-5′H-spiro[cyclopropane-1,6′-furo[2,3-d][1,3]dioxole] (4b)

Anhydrous NaI (0.909 g, 6 mmol, 0.2 eq), 30 mL of freshly distilled THF as solvent, and **3** (8 g, 31.2 mmol, 1 eq) were added sequentially in a 200 mL pressure tube under inert atmosphere. Then $TMSCF_3$ (24 mL, 156 mmol, 5 eq) was added. The reaction vessel was sealed and heated to 100 °C in an oil bath for a period of 10 hours, and then cooled to ambient temperature and was evaporated to give crude mixture

of **4a** and **4b**. With carefully conducted column chromatography, (PE/EA = 12:1), **4a** (3R) (2.486 g, 26%) and **4b** (3S) (0.478 g, 5%) were obtained as slightly yellow oils, while **3** (4.423g, 55%) was recovered. Compound **4a** [slightly more polar than **3** on TLC (PE/EA = 4:1)] had:^{1}H NMR (400 MHz, Chloroform-*d*) δ: 5.86 (d, J = 3.6 Hz, 1H), 4.41–4.34 (m, 2H), 4.09–3.96 (m, 2H), 3.86 (ddd, J = 7.8, 6.3, 4.8 Hz, 1H), 1.89 (ddd, J = 11.8, 8.1, 4.6 Hz, 1H), 1.57 (s, 3H), 1.35 (d, J = 8.7 Hz, 6H), 1.29 (s, 3H).^{13}C NMR (126 MHz, Chloroform-*d*) δ: 113.22, 109.92, 104.56, 87.00, 77.83, 75.99, 75.98, 67.11, 27.24, 26.87, 26.39, 25.27, 17.82, 17.66. ^{19}F NMR (471 MHz, Chloroform-*d*) δ: −128.81 (ddd, J = 164.2, 13.9, 4.5 Hz), −134.56 (dd, J = 164.7, 10.6 Hz). Compound **4b** [less polar than **3** on TLC (PE/EA = 4:1)] had: ^{1}H NMR (500 MHz, Chloroform-*d*) δ: 5.84 (d, J = 3.9 Hz, 1H), 4.55 (d, J = 3.7 Hz, 1H), 4.27 (t, J = 7.6 Hz, 1H), 4.22–4.14 (m, 1H), 4.08–4.01 (m, 1H), 3.90 (dd, J = 8.1, 5.0 Hz, 1H), 1.80 (dt, J = 11.4, 7.4 Hz, 1H), 1.74–1.66 (m, 1H), 1.53 (s, 3H), 1.39 (s, 3H), 1.30 (d, J = 15.2 Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ: 112.21, 111.44, 109.97, 104.79, 81.09, 79.34, 74.16, 68.04, 37.51, 27.11, 27.06, 26.94, 25.45, 15.55.^{19}F NMR (471 MHz, Chloroform-*d*) δ: −127.74 (ddt, J = 158.7, 13.8, 6.9 Hz), −135.67 (dd, J = 158.7, 11.4 Hz).

(R)-1-((1R,3a′R,5′S,6a′R)-2,2-difluoro-2′,2′-dimethyldihydro-5′H-spiro[cycloprop-ane-1,6′-furo[2,3-d][1,3]dioxol]-5′-yl)ethane-1,2-diol (5a)

A solution of **4a** (5 g, 16.33 mmol) in 75 mL methanol and 0.8% aqueous H_2SO_4 (75 mL) was added dropwise and stirred at room temperature. After the starting material disappeared, the mixture was neutralized with aqueous $NaHCO_3$ solution and extracted three times with 150 mL of EA. The organic layers were concentrated to give the crude product **5a** (3.956 g, 76%, pale-yellow syrup) that was used directly in the next step. A small portion was purified by column chromatography. ^{1}H NMR (500 MHz, Methanol-d_4) δ: 5.86 (s, 1H), 4.49–4.35 (m, 2H), 3.69 (d, J = 11.5 Hz, 1H), 3.58 (dd, J = 11.1, 6.6 Hz, 1H), 3.31 (s, 1H), 1.98 (t, J = 9.6 Hz, 1H), 1.62–1.54 (m, 1H), 1.52 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 113.76, 112.96, 105.66, 88.15, 78.41, 73.65, 64.63, 39.15, 27.43, 26.96, 18.05.^{19}F NMR (471 MHz, Methanol-d_4) δ: −129.59 (ddd, J = 165.2, 13.6, 4.0 Hz), −136.54 (dd, J = 164.9, 11.2 Hz).

(R)-1-((1S,3a′R,5′S,6a′R)-2,2-difluoro-2′,2′-dimethyldihydro-5′H-spiro[cycloprop-ane-1,6′-furo[2,3-d][1,3]dioxol]-5′-yl)ethane-1,2-diol (5b)

The compound **5b** (yield 75%) was obtained using a similar synthetic strategy as compound **5a**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ: 5.76 (d, J = 4.2 Hz, 1H), 4.47 (d, J = 4.2 Hz, 1H), 4.22 (t, J = 7.7 Hz, 1H), 3.79–3.63 (m, 4H), 3.50 (s, 1H), 1.85–1.77 (m, 1H), 1.66–1.58 (m, 1H), 1.43 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ: 113.92, 112.07, 104.40, 80.76, 78.62, 70.38, 64.45, 60.61, 37.39, 26.77, 16.19.^{19}F NMR (471 MHz, Chloroform-*d*) δ: −127.24 (ddt, J = 159.2, 13.7, 6.9 Hz), −136.05 (dd, J = 159.3, 11.3 Hz).

(R)-2-((1R,3a′R,5′S,6a′R)-2,2-difluoro-2′,2′-dimethyldihydro-5′H-spiro[cyclopropane-1,6′-furo[2,3-d][1,3]dioxol]-5′-yl)-2-hydroxyethyl benzoate (6a)

To a cold (−5°C) stirred solution of **5a** (3.956g, 14.86mmol) in dry DCM (70 mL) was added TEA (2.5 mL, 17.84 mmol, 1.2 eq). Then a mixture of benzoyl chloride (1.71 mL, 14.86 mmol, 1.0 eq) in dichloromethane (20 mL) was added dropwise. The reaction mixture was kept for an additional 4 h and stirred overnight at 0°C. The solution was washed with a saturated $NaHCO_3$ solution (3 ×150 mL) and water (2 ×150 mL), dried with sodium sulfate, and then evaporated to a colorless solid. Finally, column chromatography was conducted with PE:EA = 3:1 to give **6a** (3.22g, 59%, white solid). ^{1}H NMR (500 MHz, Chloroform-*d*) *δ*: 8.07–7.98 (m, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 2H), 5.92 (d, *J* = 3.6 Hz, 1H), 4.64 (dd, *J* = 12.0, 2.4 Hz, 1H), 4.59 (d, *J* = 6.4 Hz, 1H), 4.44–4.38 (m, 2H), 3.86 (s, 1H), 2.14 (ddd, *J* = 12.0, 8.5, 4.3 Hz, 1H), 1.58 (s, 3H), 1.51 (ddd, *J* = 12.4, 8.4, 3.4 Hz, 1H), 1.35 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) *δ*: 167.39, 133.69, 130.05, 129.91, 128.80, 113.59, 111.35, 104.66, 87.21, 77.56, 71.34, 67.09, 38.01, 27.50, 27.17, 18.11.^{19}F NMR (471 MHz, Chloroform-*d*) *δ*: −128.91 (ddd, *J* = 167.5, 13.9, 4.4 Hz), −134.53−−135.35 (m).

(R)-2-((1S,3a′R,5′S,6a′R)-2,2-difluoro-2′,2′-dimethyldihydro-5′H-spiro[cyclopropane-1,6′-furo[2,3-d][1,3]dioxol]-5′-yl)-2-hydroxyethyl benzoate (6b)

The compound **6b** (yield 56%) was obtained using a similar synthetic strategy as compound **6a**. ^{1}H NMR (500 MHz, Chloroform-*d*) *δ*: 8.01 (d, *J* = 8.3 Hz, 2H), 7.52 (t, *J* = 7.4 Hz, 1H), 7.38 (t, *J* = 7.8 Hz, 2H), 5.86 (d, *J* = 4.1 Hz, 1H), 4.60 (dd, *J* = 11.8, 2.3 Hz, 1H), 4.56 (d, *J* = 4.1 Hz, 1H), 4.43 (t, *J* = 7.8 Hz, 1H), 4.35 (dd, *J* = 11.8, 6.3 Hz, 1H), 4.21–4.15 (m, 1H), 1.94–1.87 (m, 1H), 1.72 (ddd, *J* = 11.5, 8.5, 4.4 Hz, 1H), 1.51 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) *δ*: 167.32, 133.42, 129.99, 129.95, 128.60, 113.93, 112.24, 104.51, 80.98, 78.23, 69.39, 67.59, 37.45, 26.99, 26.95, 16.39.^{19}F NMR (471 MHz, Chloroform-*d*) *δ*: −127.15 (ddt, *J* = 160.3, 13.9, 7.0 Hz), −136.03 (dd, *J* = 160.1, 11.2 Hz).

(3R,6R,7S)-6-((benzoyloxy)methyl)-1,1-difluoro-5-oxaspiro[2.4]heptane-4,7-diyl diacetate (9a)

The compound **6a** was dissolved in 50mL CF_3COOH (50% in water), and stirred overnight. After the starting material disappeared, the mixture was evaporated to give crude product **7a**, which was then used directly in the next step.

7a was dissolved in 50 mL DCM, followed by the addition of 50 mL water, $NaHCO_3$ (1.09 g, 13.1 mmol, 1.5 eq), and $NaIO_4$ (2.05 g, 9.6 mmol, 1.1 eq). The solution was stirred at room temperature. After the starting material disappeared, the reaction mixture was filtered. Organic phase was evaporated, and the water phase was extracted three times with 150 mL of EA. The organic layer was concentrated to give the crude product **8a** as slightly yellow oil.

8a was dissolved in 50 mL of dry DCM, which was followed by the addition of dry TEA (2.5 mL, 18.2 mmol, 2.1 eq) and catalytic amount of DMPA at 0°C. Then Ac_2O (1.7 mL, 17.3 mmol, 2.0 eq) was added dropwise, and reaction mixture was stirred at room temperature for 5 h. Then sat. aq. $NaHCO_3$ (50 mL) was added, the mixture was partitioned, and the aqueous layer was washed three times with DCM. The organic layers was concentrated, and recrystallization was performed to give **9a** (2.8 g, three steps of 84% yield) as white solid. Compound **9a**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ: 8.07 (d, $J = 7.5$ Hz, 2H), 7.58 (t, $J = 7.4$ Hz, 1H), 7.45 (t, $J = 7.5$ Hz, 2H), 6.41 (s, 1H), 5.39 (s, 1H), 4.55–4.44 (m, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.90–1.78 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ: 170.21, 169.43, 166.14, 133.50, 129.84, 129.72, 129.57, 129.59, 128.58, 110.28, 95.35, 84.46, 77.93, 63.40, 36.30, 20.97, 20.83, 18.74. ^{19}F NMR (471 MHz, Chloroform-*d*) δ: −132.77 (ddd, $J = 164.5, 13.2, 6.0$ Hz), −135.16 (ddd, $J = 164.5, 12.8, 5.5$ Hz).

(3S,6R,7S)-6-((benzoyloxy)methyl)-1,1-difluoro-5-oxaspiro[2.4]heptane-4,7-diyl diacetate (9b)

The compound **9b (**with three steps of 80% yield) was obtained using a similar synthetic strategy as compound **9a**.^{1}H NMR (500 MHz, Chloroform-*d*) δ: 8.06–8.01 (m, 2H), 7.55 (t, $J = 7.4$ Hz, 1H), 7.42 (t, $J = 7.7$ Hz, 2H), 6.28 (s, 1H), 5.26 (d, $J = 3.1$ Hz, 1H), 4.66 (p, $J = 3.8$ Hz, 1H), 4.58 (d, $J = 3.6$ Hz, 2H), 2.13 (d, $J = 5.3$ Hz, 6H), 1.76 (ddd, $J = 11.4, 9.1, 5.7$ Hz, 1H), 1.69 (ddd, $J = 12.0, 9.1, 6.0$ Hz, 1H).^{13}C NMR (126 MHz, Chloroform-*d*) δ: 170.87, 170.42, 166.51, 133.46, 130.03, 129.77, 128.62, 111.59, 96.95, 85.15, 72.97, 64.02, 38.78, 21.10, 15.28. ^{19}F NMR (471 MHz, Chloroform-*d*) δ: −134.65 (ddd, $J = 154.2, 11.4, 6.0$ Hz), −135.62 (ddd, $J = 154.2, 12.0, 5.9$ Hz).

1-((3R,4R,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)pyrimidine-2,4(1H,3H)-dione (12) and 1-((3R,4S,6R,7S)-1,1-difluoro-7hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)pyrimidine-2,4(1H,3H)-dione (13)

Compound **9a** (0.1g, 0.26 mmol) was dissolved in anhydrous 1,2-DCE (8 mL) and added to the silylated uracil (1.04 mmol; see the general method for silylation of uracil below), followed by the dropwise addition of TMSOTf (0.1 mL, 0.53 mmol, 2eq; ∼2 min addition) at room temperature. The mixture was stirred for 2 hours until the starting material disappeared, and then quenched with sat. aq. $NaHCO_3$ (10 mL). The aqueous layer was extracted with DCM (3 × 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuum. The crude reaction mixture was purified by column chromatography to give compound **10a** and **11a**77 mg in total (66% yield) of a 2:1 mixture of β:α anomers of the nucleosides.

Compound **10a** (50 mg, 0.11 mmol) was treated with 10 mL solution of NH_3 in MeOH, and stirred overnight. After the starting material disappeared, the reaction mixture was concentrated in vacuum and purified by column chromatography (DCM: isopropanol = 10:1) to give compound **12** as white solid (32 mg, 98%). The similar synthetic strategy was used to give compound **13** as white solid (15 mg, 87%). Compound **12**:^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.36 (s, 1H), 7.58 (d, J = 8.0 Hz, 1H), 6.26 (s, 1H), 5.55 (d, J = 7.7 Hz, 2H), 5.02 (s, 1H), 3.87–3.64 (m, 3H), 3.57 (s, 1H), 1.68 (d, J = 9.0 Hz, 1H), 1.46 (dd, J = 13.6, 9.1 Hz, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 165.80, 151.87, 143.34, 112.99, 103.25, 88.16, 84.72, 84.69, 76.32, 39.95, 19.32. ^{19}F NMR (471 MHz, Methanol-d_4) δ: −139.55 (dd, J = 170.8, 11.5 Hz), −142.59 (ddd, J = 171.0, 13.5, 5.5 Hz). MS (ESI) m/z: 291.1[M+H$^+$], 325.0[M + Cl$^-$]. Compound **13**: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.30 (s, 1H), 8.29 (d, J = 8.1 Hz, 1H), 6.19 (s, 1H), 6.14 (s, 1H), 5.75 (d, J = 8.1 Hz, 1H), 4.95 (t, J = 5.5 Hz, 1H), 4.22 (t, J = 5.5 Hz, 1H), 4.14 (s, 1H), 3.45 (d, J = 4.7 Hz, 2H), 2.09–1.99 (m, 1H), 1.99–1.88 (m, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 166.01, 152.53, 144.08, 113.51, 102.99, 91.79, 90.53, 78.42, 63.16, 39.68, 24.62.^{19}F NMR (471 MHz, Methanol-d_4) δ: −132.74–−133.70 (m), −137.54 (ddd, J = 168.3, 13.5, 5.0 Hz). MS (ESI) m/z: 291.1[M+H+], 325.0[M+Cl−].

General procedure for silylation of uracil

Uracil (0.233 g, 2.08 mmol) was treated with BSA (10 mL) and stirred at 80°C for 2.5 h. Excess BSA was evaporated under reduced pressure at 50°C to give a cloudy oil, which was directly used in the nucleoside glycosylation reaction.

1-((3R,4R,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)-5-fluoropyrimidine-2,4(1H,3H)-dione (14) and 1-((3R,4S,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)-5-fluoropyrimidine-2,4(1H,3H)-dione (15)

The compounds **14** and **15** were obtained using a similar synthetic strategy as compound **12** and **13** with compounds **14** (30 mg) and **15** (10 mg), two steps of 49% yield and a 3:1 mixture of β:α anomers. Compound **14**: ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.28 (d, J = 6.8 Hz, 1H), 6.49 (d, J = 1.8 Hz, 1H), 4.36 (td, J = 4.3, 1.8 Hz, 1H), 4.02 (dt, J = 4.7, 2.8 Hz, 1H), 3.87 (dd, J = 12.3, 2.6 Hz, 1H), 3.78 (dd, J = 12.2, 3.0 Hz, 1H), 1.78 (ddd, J = 13.0, 8.9, 6.3 Hz, 1H), 1.50 (ddd, J = 14.2, 8.9, 3.6 Hz, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 159.22, 150.70, 141.82, 126.78, 113.00, 88.41, 85.03, 76.26, 62.21, 39.97, 19.35.^{19}F NMR (471 MHz, Methanol-d_4) δ: −131.33 (dd, J = 165.1, 13.7 Hz), −137.64 (dd, J = 165.3, 12.9 Hz), −167.35 (d, J = 6.9 Hz).MS (ESI) m/z: 309.1[M+H$^+$], 343.0[M+Cl$^-$]. Compound **15**: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.91 (s, 1H), 8.57 (d, J = 7.2 Hz, 1H), 6.30 (d, J = 3.8 Hz, 1H), 6.18 (s, 1H), 4.95 (t, J = 5.9 Hz, 1H), 4.25 (t, J = 5.6 Hz, 1H), 4.15 (d, J = 3.5 Hz, 1H), 3.44 (d, J = 5.5 Hz, 2H), 2.13–2.02 (m, 1H), 2.00–1.91 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ: 157.97, 150.28, 140.84, 126.72, 113.44, 91.14, 89.35,

76.71, 62.25, 38.37, 24.06.^{19}F NMR (471 MHz, DMSO-d_6) δ: −129.78−−131.26 (m), −135.60 (ddd, J = 164.2, 13.9, 5.8 Hz), −166.53 (d, J = 7.2 Hz). MS (ESI) m/z: 309.1[M+H$^+$], 343.0[M+Cl$^-$].

1-((3R,4R,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (16) and 1-((3R,4S,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (17)

The compounds **16** and **17** were obtained using a similar synthetic strategy as compound **12** and **13** with compounds **16** (45 mg) and **17** (11 mg), two steps of 69% yield and a 4:1 mixture of β:α anomers. Compound **16**: ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.78 (d, J = 1.3 Hz, 1H), 6.48 (s, 1H), 4.38 (dt, J = 6.5, 3.1 Hz, 1H), 4.00 (dt, J = 4.7, 3.0 Hz, 1H), 3.86 (dd, J = 12.3, 2.8 Hz, 1H), 3.77 (dd, J = 12.3, 3.4 Hz, 1H), 1.88 (d, J = 1.2 Hz, 3H), 1.76 (ddd, J = 13.0, 8.9, 6.4 Hz, 1H), 1.39 (ddd, J = 13.3, 8.8, 3.6 Hz, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 166.10, 152.08, 138.76, 112.99, 112.00, 88.00, 84.65, 76.18, 62.24, 39.80, 19.31, 12.40.^{19}F NMR (471 MHz, Methanol-d_4) δ: −131.20 (ddd, J = 165.6, 13.5, 5.9 Hz), −137.77 (dd, J = 164.9, 12.9 Hz).MS (ESI) m/z: 305.0[M+H$^+$], 339.1[M+Cl$^-$]. Compound **17**: ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.23 (d, J = 1.4 Hz, 1H), 6.26 (d, J = 4.1 Hz, 1H), 4.35 (t, J = 5.2 Hz, 1H), 4.18 (s, 1H), 3.62 (d, J = 5.2 Hz, 2H), 1.93 (td, J = 8.5, 7.4, 3.2 Hz, 1H), 1.90 (d, J = 1.2 Hz, 3H), 1.85–1.75 (m, 1H).^{13}C NMR (126 MHz, Methanol-d_4) δ: 166.32, 152.74, 139.70, 111.52, 111.82, 91.55, 90.43, 78.46, 63.21, 39.52, 24.60, 12.53.^{19}F NMR (471 MHz, Methanol-d_4) δ: −132.67−−133.49 (m), −137.27−−137.85 (m). MS (ESI) m/z: 305.0[M+H$^+$], 339.1[M+Cl$^-$].

4-amino-1-((3R,4R,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)pyrimidin-2(1H)-one (19)

The compound **10a**(180 mg, 0.413 mmol) was dissolved in 8 mL dry DCM, followed by the addition of 1, 2, 4-triazole (313 mg, 4.54 mmol, 11eq), and TEA (0.63 mL, 4.54 mmol, 11eq). The reaction mixture was stirred at 0 °C, and the dry $POCl_3$ (0.1 mL, 1.1 mmol, 2.6 eq) was added. It was then placed at room temperature for 4 hours, until the starting material disappeared, and the mixture was quenched with cold water. The organic layer was partitioned and evaporated to give the crude product **18**.

The compound **18** was dissolved in THF (5 mL), followed by the addition of NH_4OH (1 mL), and stirred at room temperature overnight. After the starting material disappeared, it was evaporated and then a solution of NH_3 in MeOH (5 mL) was added. Stirred overnight, and then evaporated and purified by column chromatography (DCM:ir-propanol = 4:1) to give compound **19** (25 mg, three steps of 21% yield) as pale-yellow solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.10 (d, J = 7.7 Hz, 1H), 6.54 (s, 1H), 6.00 (d, J = 7.6 Hz, 1H), 4.37 (s, 1H), 4.02 (q, J = 3.6 Hz, 1H),

3.86 (dd, $J = 12.3, 2.9$ Hz, 1H), 3.77 (dd, $J = 12.2, 3.4$ Hz, 1H), 1.74 (dt, $J = 14.0, 8.1$ Hz, 1H), 1.47–1.33 (m, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 165.38, 154.90, 145.06, 112.92, 96.41, 88.15, 85.59, 75.98, 62.18, 40.26, 19.00.^{19}F NMR (471 MHz, Methanol-d_4) δ: −130.67−−131.40 (m), −137.56 (dd, $J = 165.2, 12.8$ Hz). MS (ESI) *m/z*: 291.1[M+H$^+$], 325.0[M+Cl$^-$].

(3R,4R,6R,7S)-4-(6-amino-9H-purin-9-yl)-1,1-difluoro-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-7-ol (21)

Compound **9a** (0.1 g, 0.26 mmol) was dissolved in anhydrous 1,2-DCE (8 mL), and the mixture was then added to the silylated 6-chloropurine (164 mg, 1.04 mmol, 4 eq; see the general method for silylation of uracil), followed by the dropwise addition of TMSOTf (0.1 mL, 0.53 mmol, 2eq; ∼2 min addition) at room temperature. The mixture was stirred for 2 h until the starting material disappeared, and then quenched with sat. aq. $NaHCO_3$ (10 mL). The aqueous layer was extracted with DCM (3 × 10 mL), and the combined organic layers were dried over Na_2SO_4, then filtered, and finally concentrated in vacuum. The reaction mixture was purified by column chromatography to give the crude product **20**, which was then dissolved in 2 mL of 1,4-dioxane, followed by the addition of 5 mL of conc. NH_4OH. The reaction vessel was sealed and heated to 100°C in an oil bath for 12 hours. It was then evaporated and purified by column chromatography (DCM : ir-propanol = 3:1) to give compound **21**(20 mg, three steps of 24% yield) as pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.50 (s, 1H), 8.23 (s, 1H), 6.86 (s, 2H), 6.42 (s, 1H), 6.17 (d, $J = 4.2$ Hz, 1H), 4.97 (t, $J = 5.9$ Hz, 1H), 4.26 (s, 1H), 4.13 (t, $J = 5.5$ Hz, 1H), 3.56–3.46 (m, 3H), 2.25–2.14 (m, 1H), 2.15–2.06 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 160.68, 153.38, 152.31, 145.15, 111.39, 91.63, 89.32, 76.53, 62.18, 52.97, 38.75, 23.81.^{19}F NMR (471 MHz, DMSO-d_6) δ: −137.46 (dd, $J = 162.2, 12.8$ Hz), −139.58 (dd, $J = 163.0, 12.3$ Hz). MS (ESI) *m/z*: 314.0[M+H$^+$], 348.1[M+Cl$^-$].

1-((3S,4R,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)pyrimidine-2,4(1H,3H)-dione(22) and 1-((3S,4S,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)pyrimidine-2,4(1H,3H)-dione (23)

The similar synthetic strategy with compounds **12**(β) and **13**(α) was used to give compounds **22**(β) (22 mg) and **23**(α) (12 mg) as white solids, two steps of 44% yield and a 1.8:1 mixture of β:α anomers. Compound **22**: ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.72 (d, $J = 8.1$ Hz, 1H), 6.14 (d, $J = 5.7$ Hz, 1H), 5.72 (d, $J = 8.1$ Hz, 1H), 4.49 (d, $J = 7.4$ Hz, 1H), 3.92 (dd, $J = 12.0, 2.2$ Hz, 1H), 3.85 (ddd, $J = 7.0, 4.3, 2.2$ Hz, 1H), 3.79 (dd, $J = 12.0, 4.3$ Hz, 1H), 1.99 (ddd, $J = 13.6, 8.3, 5.8$ Hz, 1H), 1.64 (ddd, $J = 14.0, 8.3, 5.3$ Hz, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 165.96, 152.10, 143.24, 130.84, 112.69, 103.11, 86.53, 68.61, 61.80, 38.57, 16.41.^{19}F NMR (471 MHz, Methanol-d_4) δ: −135.51 (d, $J = 164.0$ Hz), −138.83 (ddd, $J = 163.9, 14.7, 5.9$ Hz).MS (ESI) *m/z*: 291.1[M+H$^+$], 325.0[M+Cl$^-$]. Compound **23**:

^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.10 (d, J = 8.1 Hz, 1H), 6.34 (s, 1H), 5.74 (d, J = 8.1 Hz, 1H), 4.36 (t, J = 5.0 Hz, 1H), 4.20 (d, J = 1.7 Hz, 1H), 3.61 (d, J = 4.4 Hz, 2H), 1.90–1.81 (m, 1H), 1.47–1.39 (m, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 164.62, 151.35, 142.53, 111.82, 101.87, 89.24, 85.28, 70.59, 61.79, 40.05, 14.46.^{19}F NMR (471 MHz, Methanol-d_4) δ: −135.61−−136.08 (m), −136.10−−136.55 (m). MS (ESI) m/z: 291.1[M+H$^+$], 325.0[M+Cl$^-$].

1-((3S,4R,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)-5-fluoropyrimidine-2,4(1H,3H)-dione(24) and 1-((3S,4S,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)-5-fluoropyrimidine-2,4(1H,3H)-dione (25)

The similar synthetic strategy with compounds **12**(β) and **13**(α) was used to give compounds **24**(β) (27 mg) and **25**(α) (10 mg) as white solids, two steps of 43% yield and a 2.7:1 mixture of β:α anomers. Compound **24**: ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.99 (d, J = 6.5 Hz, 1H), 6.17 (s, 1H), 4.50 (d, J = 7.2 Hz, 1H), 3.96–3.90 (m, 1H), 3.89–3.84 (m, 1H), 3.81 (dd, J = 12.1, 3.7 Hz, 1H), 1.99 (ddd, J = 13.6, 8.0, 5.9 Hz, 1H), 1.66 (ddd, J = 14.0, 8.3, 5.5 Hz, 1H).

^{13}C NMR (126 MHz, Methanol-d_4) δ: 176.37, 159.39, 150.82, 141.97, 126.73, 112.75, 86.71, 68.40, 61.55, 38.70, 16.35.^{19}F NMR (471 MHz, Methanol-d_4) δ: −135.14 (d, J = 163.1 Hz), −138.75—139.58 (m), −168.42(s). MS (ESI) m/z: 309.1[M+H$^+$], 343.0[M+Cl$^-$]. Compound **25**: ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.30 (d, J = 6.8 Hz, 1H), 6.34 (d, J = 1.5 Hz, 1H), 4.38 (t, J = 3.9 Hz, 1H), 4.19 (d, J = 1.2 Hz, 1H), 3.60 (d, J = 4.5 Hz, 2H), 1.87 (ddd, J = 11.1, 9.1, 7.0 Hz, 1H), 1.48 (ddt, J = 11.7, 5.9, 3.6 Hz, 1H).^{13}C NMR (126 MHz, Methanol-d_4) δ: 159.51, 151.43, 141.92, 127.39, 113.17, 90.71, 86.97, 71.99, 63.18, 41.40, 15.84.^{19}F NMR (471 MHz, Methanol-d_4) δ: −135.64 (ddd, J = 153.6, 11.5, 5.7 Hz), −136.22 (ddd, J = 153.3, 12.2, 6.8 Hz), −167.74 (d, J = 5.9 Hz). MS (ESI) m/z: 309.1[M+H$^+$], 343.0[M+Cl$^-$].

1-((3S,4R,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione(26) and 1-((3S,4S,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (27)

The similar synthetic strategy with compounds **12**(β) and **13**(α) was used to give compounds **26**(β) (34 mg) and **27**(α) (9 mg) as white solids, two steps of 54% yield and a 4:1 mixture of β:α anomers. Compound**26**: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.32 (s, 1H), 7.52 (d, J = 1.4 Hz, 1H), 6.04 (s, 1H), 5.68 (d, J = 6.6 Hz, 1H), 5.01 (s, 1H), 4.36 (t, J = 7.1 Hz, 1H), 3.74 (qd, J = 5.6, 2.6 Hz, 2H), 3.63 (dt, J = 12.1, 5.4 Hz, 1H), 1.89 (dt, J = 13.7, 7.8 Hz, 1H), 1.75–1.61 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 164.57, 151.09, 138.57, 112.72, 110.47, 100.40, 86.08, 67.88, 61.30, 37.64, 16.32, 12.96.^{19}F NMR (471 MHz, Methanol-d_4) δ: −135.53 (d, J = 161.9 Hz), −139.01

(ddd, $J = 163.4, 14.5, 5.8$ Hz). MS (ESI) *m/z*: 305.0[M+H$^+$], 339.1[M+Cl$^-$]. Compound**27**: ^{1}H NMR (500 MHz, Methanol-d_4) δ: 7.93 (d, $J = 1.5$ Hz, 1H), 6.33 (s, 1H), 4.36 (td, $J = 4.5, 1.9$ Hz, 1H), 4.21 (d, $J = 2.0$ Hz, 1H), 3.61 (d, $J = 4.4$ Hz, 2H), 1.88 (d, $J = 1.4$ Hz, 3H), 1.87–1.82 (m, 1H), 1.37 (ddd, $J = 10.9, 9.1, 6.7$ Hz, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 166.24, 152.90, 139.50, 113.24, 112.08, 90.47, 86.58, 72.00, 63.25, 41.33, 15.95, 12.54.^{19}F NMR (471 MHz, Methanol-d_4) δ: −135.66—136.04 (m), −136.04—136.46 (m). MS (ESI) *m/z*: 305.0[M+H$^+$], 339.1[M+Cl$^-$].

4-amino-1-((3S,4R,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)pyrimidin-2(1H)-one (28)

The similar synthetic strategy with compound **19** was used to give compound **28** as white solid (10 mg, three steps of 32% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 7.70 (d, $J = 7.5$ Hz, 1H), 6.23 (s, 1H), 5.90 (d, $J = 7.5$ Hz, 1H), 4.47 (d, $J = 7.3$ Hz, 1H), 3.92 (dd, $J = 11.9, 2.2$ Hz, 1H), 3.91–3.73 (m, 2H), 1.98–1.87 (m, 1H), 1.68 (dt, $J = 14.1, 7.5$ Hz, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 167.68, 158.22, 143.60, 112.84, 96.50, 86.32, 68.96, 66.88, 61.87, 38.98, 16.21.^{19}F NMR (471 MHz, Methanol-d_4) δ: −134.77 (d, $J = 163.2$ Hz), −138.86 (ddd, $J = 162.7, 14.3, 5.6$ Hz). MS (ESI) *m/z*: 291.1[M+H$^+$], 325.0[M+Cl$^-$].

4-amino-1-((3S,4S,6R,7S)-1,1-difluoro-7-hydroxy-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-4-yl)pyrimidin-2(1H)-one (29)

The similar synthetic strategy with compound **19** was used to give compound **29** as white solid (8 mg, three steps of 29% yield).^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.04 (d, $J = 7.5$ Hz, 1H), 6.35 (s, 1H), 5.92 (d, $J = 7.5$ Hz, 1H), 4.37 (q, $J = 3.8$ Hz, 1H), 4.18 (d, $J = 1.9$ Hz, 1H), 3.61 (d, $J = 4.6$ Hz, 2H), 1.82 (q, $J = 9.0$ Hz, 1H), 1.37 (dd, $J = 16.3, 10.2$ Hz, 1H).^{13}C NMR (126 MHz, Methanol-d_4) δ: 167.46, 158.50, 144.70, 113.37, 96.64, 90.54, 72.15, 68.89, 63.13, 41.64, 15.76.^{19}F NMR (471 MHz, Methanol-d_4) δ: −134.78 (d, $J = 159.0$ Hz), −138.86 (dt, $J = 162.3, 9.0$ Hz). MS (ESI) *m/z*: 291.1[M+H$^+$], 325.0[M+Cl$^-$].

(3S,4R,6R,7S)-4-(6-amino-9H-purin-9-yl)-1,1-difluoro-6-(hydroxymethyl)-5-oxaspiro[2.4]heptan-7-ol (30)

The similar synthetic strategy with compound **21** was used to give compound **30** as pale-yellow solid (8 mg, three steps of 24% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.24 (s, 1H), 8.19 (s, 1H), 6.29 (d, $J = 4.6$ Hz, 1H), 4.94 (d, $J = 7.5$ Hz, 1H), 4.02–3.98 (m, 1H), 3.95 (dd, $J = 12.3, 2.4$ Hz, 1H), 3.84 (dd, $J = 12.3, 4.4$ Hz, 1H), 2.08 (dt, $J = 14.0, 7.3$ Hz, 1H), 1.78–1.71 (m, 1H). ^{13}C NMR (126 MHz, Methanol-d_4) δ: 157.36, 153.80, 150.29, 141.32, 120.23, 112.37, 87.64, 68.61, 62.26, 39.42, 16.39.^{19}F NMR (471 MHz, Methanol-d_4) δ: −137.07 (ddt, $J = 165.6, 11.6, 5.2$ Hz), −137.95 (ddd, $J = 165.4, 14.2, 6.3$ Hz). MS (ESI) *m/z*: 314.0[M+H$^+$], 348.1[M+Cl$^-$].

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