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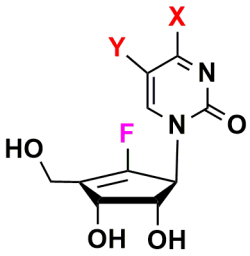
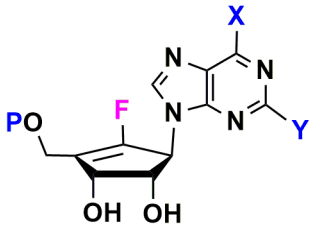


X = OH or NH_2
Y = H, F, Cl, Br, or I

Anticancer activity
X : NH_2 > OH
Y : H > halogen



X = NH_2, NHMe, NH-cyclopropyl or OH
Y = H or NH_2
P = H or phosphoramidite

Anticancer activity
X : NH_2 > NHMe > NHCP > OH
Y : H > NH_2
P : H > phosphormidite

A systematic structuire-activity relationship study of 6'-fluorocyclopentenyl-pyrimidines and -purines as anticancer agents is described

Design, Synthesis and Anticancer Activity of Fluorocyclopentenyl-purines and –pyrimidines

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Key words: Fluorocyclopentenyl nucleoside; Structure-activity relationship; Fluorination; Phosphorylation; Prodrug; Anticancer

Abstract

Based on the potent anticancer activity of 6'-fluorocyclopentenyl-cytosine **2b** in phase IIa clinical trials for the treatment of gemcitabine-resistant pancreatic cancer, we carried out a systematic structure-activity relationship study of 6'-fluorocyclopentenyl-pyrimidines **3a-i** and -purines **3j-o** to discover novel anticancer agents. We also synthesized the phosphoramidate prodrug **3p** of adenine derivative **1b** to determine if the anticancer activity depended on the inhibition of DNA and/or RNA polymerase in cancer cells and/or on the inhibition of *S*-adenosylhomocysteine (SAH) hydrolase. All of the synthesized pyrimidine nucleosides exhibited much less potent anticancer activity *in vitro* than the cytosine derivative **2b**, acting as RNA and/or DNA polymerase inhibitor, indicating that they could not be efficiently converted to their triphosphates for anticancer activity. Among all the synthesized purine nucleosides, adenine derivative **1b** and N^6-methyladenine derivative **3k** showed potent anticancer activity, showing equipotent inhibitory activity as the positive control, neplanocin A (**1a**) or Ara-C. However, the phosphoramidate prodrug **3p** showed less anticancer activity than **1b**, indicating that it did not act as a RNA and/or DNA polymerase inhibitor like **2b**. This result also demonstrates that the anticancer activity of **1b** largely depends on the inhibition of histone methyltransferase, resulting from strong inhibition of SAH hydrolase. The deamination of the N^6-amino group, the addition of the bulky alkyl group at the N^6-amino group, or the introduction of the amino group at the C2 position almost abolished the anticancer activity.

1. Introduction

Neplanocin A (**1a**) [1], a naturally occurring nucleoside exhibits potent antiviral and antitumor activities by inhibition of *S*-adenosylhomocysteine (SAH) hydrolase, which catalyzes the interconversion of SAH into adenosine and L-homocysteine (Figure 1) [2].

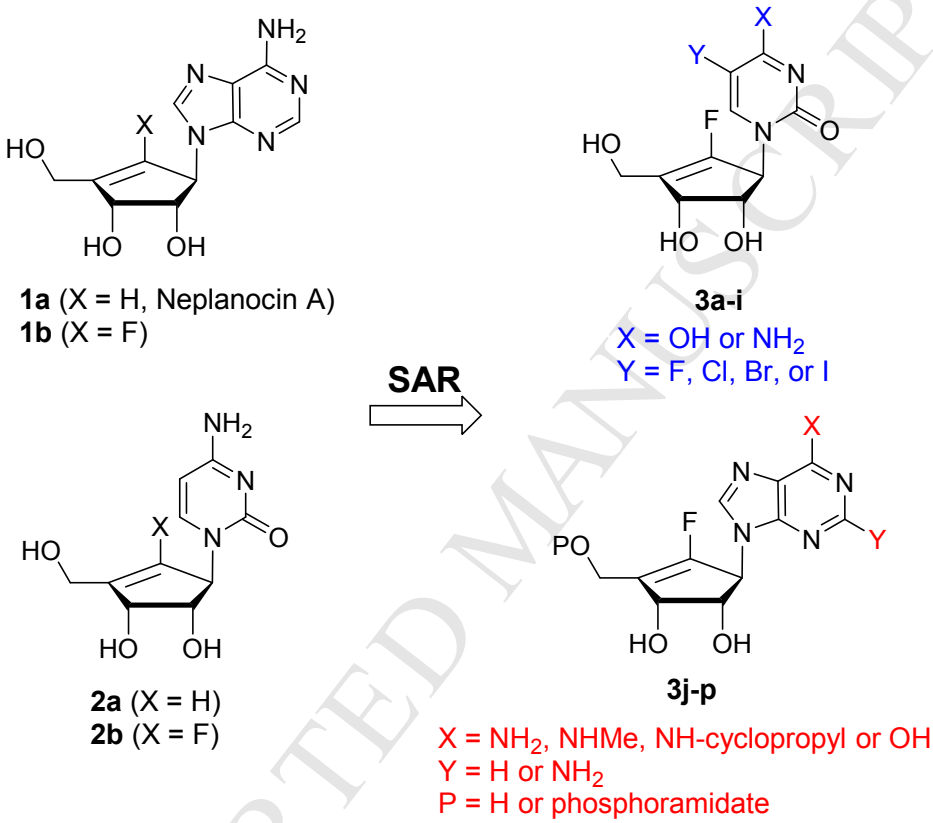


Figure 1. The rationale for the design of target nucleosides **3a-p**

Its 6'-fluoro analogue, fluoro-neplanocin A (**1b)** is also a potent inhibitor (IC_{50} = 0.48 μM) of SAH hydrolase [3] and exhibits potent antiviral activity [3] and anticancer activity in combination with 5-azacytidine [4]. On the other hand, the cyclopentenyl-cytosine (**2a**) [5] exhibits potent antiviral and/or antitumor activities by reducing the cytidine-5'-phosphate (CTP) pools and its 6'-fluoro analogue **2b** also displays highly potent antitumor effects in a broad range of tumor cell lines [6], but it shows different mechanisms of action [7] from that of **2a**. Compound **2b** is intracellularly taken by a human equilibrative nucleoside transporter

(hENT) and is phosphorylated by uridine-cytidine kinase (UCK) to its monophosphate and subsequently to its triphosphate, which can be incorporated into RNA. Compound **2b** is also incorporated into DNA by the action of ribonucleotide reductase and/or it acts as a DNA methyl transferase (DNMT) inhibitor [6] either by the incorporation of **2b** into DNA by the formation of a complex with the enzyme or directly by its triphosphate [7]. Compound **2b** is now undergoing phase IIa clinical trials for the treatment of gemcitabine-resistant pancreatic cancer.

Inspired by the potent anticancer activity of **1b** and **2b**, it was of great interest to synthesize novel 6'-fluorocyclopentenyl-pyrimidines **3a-i** and –purines **3j-o** for a systematic structure-activity relationship (SAR) study and to evaluate them for anticancer activity. We also synthesized the phosphoramidate prodrug **3p** from the adenine analog **1b** to determine if the anticancer activity depended on the inhibition of SAH hydrolase or DNA and/or RNA polymerase. Herein, we report the comprehensive structure-activity relationships of 6'-fluorocyclopentenyl-pyrimidines **3a-i** and -purines **3j-o** and the phosphoramidate prodrug **3p** as novel antitumor agents.

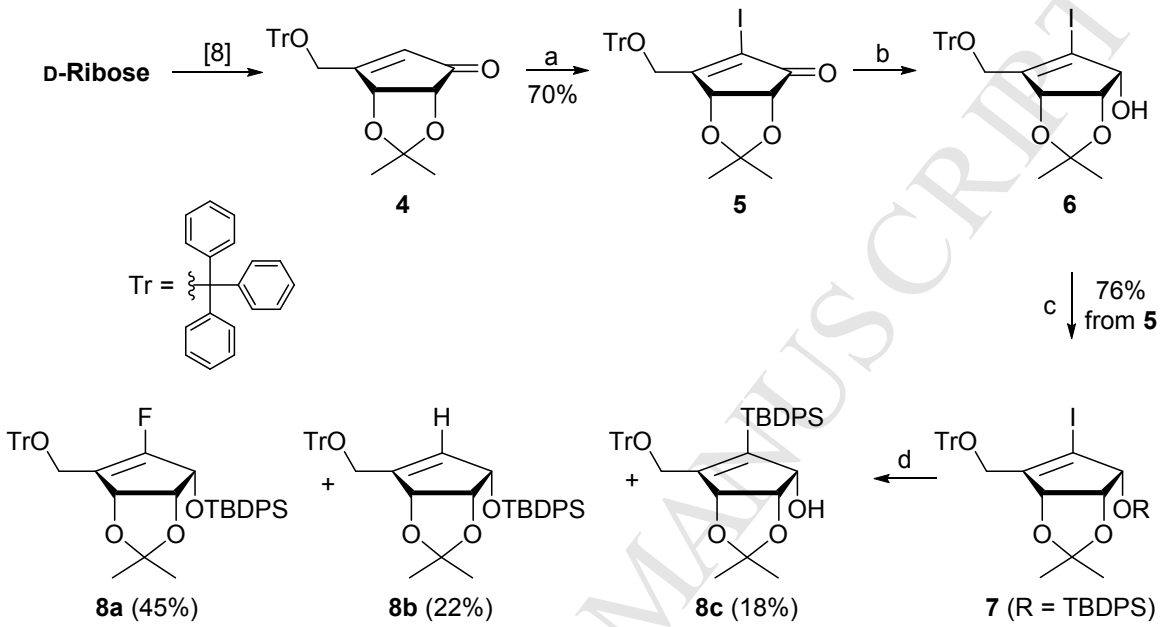
2. Results and discussion

2.1. Chemistry

For the synthesis of the target nucleosides **3**, the key fluoro intermediate **8a** was first synthesized from D-ribose, as shown in Scheme 1. D-Ribose was converted to the key intermediate **4**, according to our previously published procedure [8]. The iodination of **4** with iodine in the presence of pyridine afforded α-iodo compound **5 [**9], which was reduced with $NaBH_4$ followed by the protection of the resulting alcohol **6** with the TBDPS group to yield **7 [**9], which is the precursor for the α-fluorination. Treatment of **7** with *N*-fluorobenzenesulfonimide (NFSI) in the presence of *n*-BuLi in the mixed solvents (THF,

methyl *tert*-butyl ether (MTBE), heptane) at -78 °C yielded the desired 6-fluoro compound **8a** with concomitant formation of 6-H derivative **8b** and TBDPS-migrated derivative **8c** in a 2 : 1 : 1 ratio [3,9].

Scheme 1

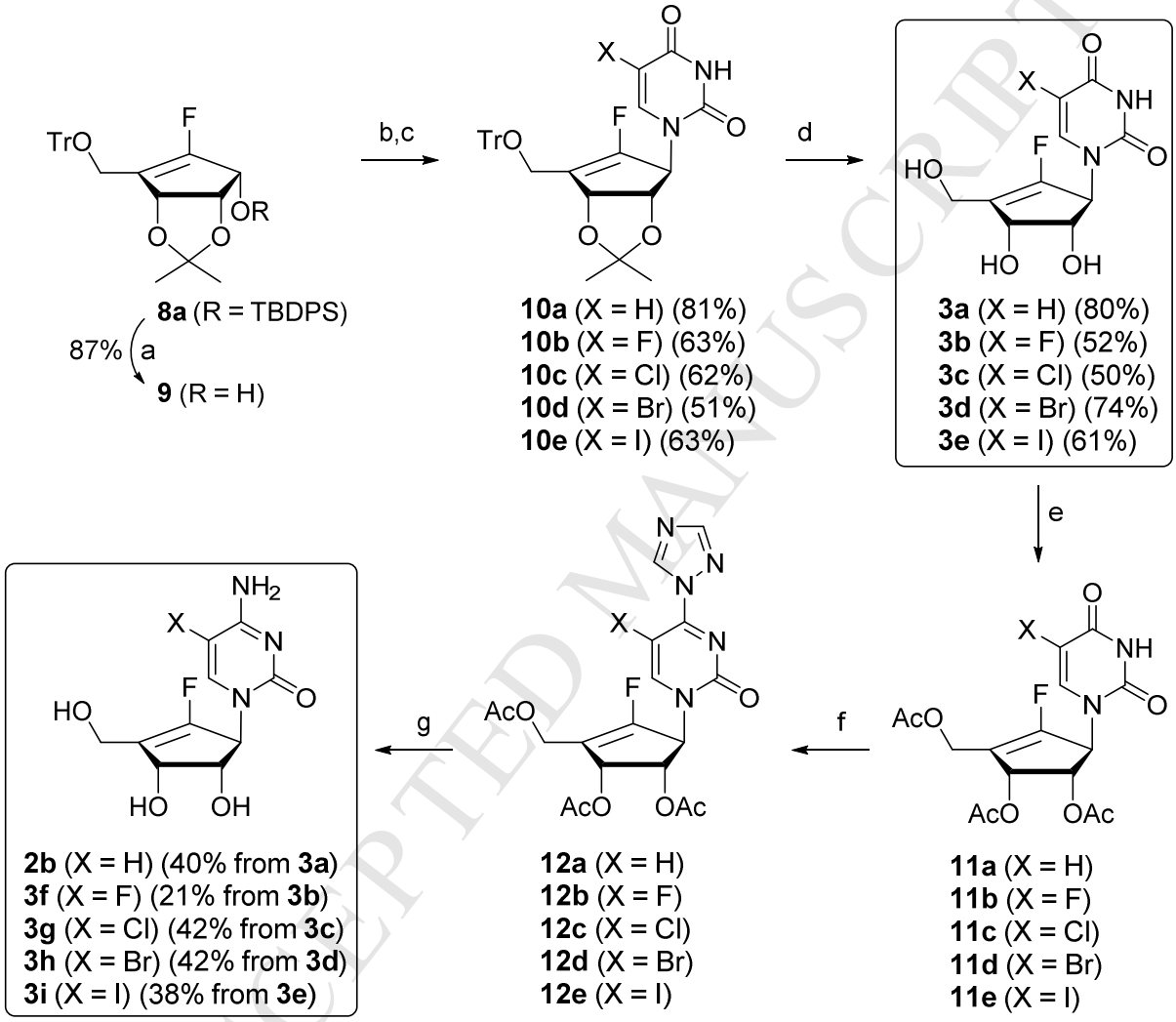


Reagents and Conditions: a) I_2, pyridine, THF, rt, 4 h; b) $NaBH_4$, $CeCl_3$-$7H_2O$, MeOH, 0 °C, 30 min; c) TBDPSCl, imidazole, DMF, 40 °C, 12 h; d) NFSI, *n*-BuLi, THF/MTBE/heptane, -78 °C, 30 min.

The key fluoro intermediate **8a** was converted to the glycosyl donor **9** by treating it with TBAF, which was subjected to the Mitsunobu condensation to synthesize the final pyrimidine nucleosides **2b** and **3a-i**, as illustrated in Scheme 2. The condensation of **9** with N^3-benzoyl-pyrmidines under the standard Mitsunobu conditions followed by the removal of the N^3-benzoyl group, yielded the condensed products **10a-e**. The treatment of **10a-e** with 2 *N* HCl afforded the final pyrimidine nucleosides **3a-e**, respectively [6]. For the conversion of the uracil derivatives **3a-e** into the cytosine derivatives **2b** and **3f-i**, compounds **3a-e** were peracetylated to give **11a-e**, which were treated with 1,2,4-triazole and $POCl_3$ in the presence of Et_3N to give the N^4-triazole derivatives **12a-e**, respectively. Treatment of **12a-e** with ammonia water in 1,4-dioxane followed by the successive treatment with methanolic

ammonia afforded the final cytosine derivatives **2b** and **3f-i** [6]. This conventional method [10a] turned out to be superior to other methods using 2,4,6-triisopropylbenzenesulfonyl chloride/NH_3 [10b] or $POCl_3$/NaN_3/Pd-C [10c] in terms of overall yield.

Scheme 2

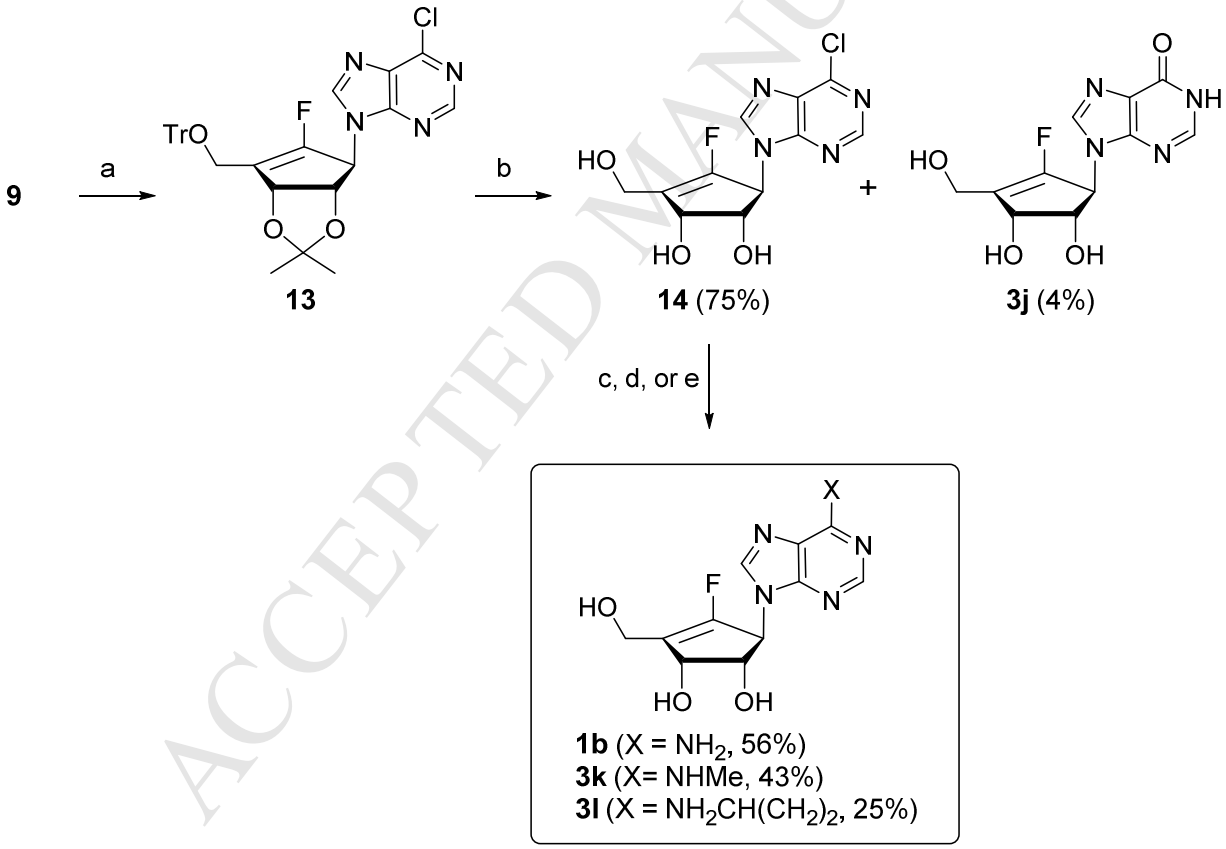


Reagents and Conditions: a) TBAF, THF, 0 °C to rt, 3 h; b) N^3-benzoyluracil or N^3-benzoyl-5-halouracil, PPh_3, DIAD, THF, 0 °C to rt, 16 h; c) NH_3, MeOH, rt, 15 h; d) 2 *N* HCl, MeOH, rt, 18 h; e) Ac_2O, pyridine, rt, 16 h; f) $POCl_3$, 1,2,4-triazole, Et_3N, MeCN, rt, 16 h; g) NH_4OH, H_2O, 1,4-dioxane, rt, 18 h, then NH_3, MeOH, rt, 3 h.

The synthesis of the fluorocyclopentenyl-N^6-substituted-purines **1b** and **3j-l** was accomplished by condensing the glycosyl donor **9** with 6-chloropurine, as shown in Scheme 3. The condensation of **9** with 6-chloropurine under the standard Mitsunobu conditions afforded the 6-chloropurine derivative **13**. To remove the acid-sensitive protecting groups such as

trityl (Tr) and 2,3-isopropylidene, compound **13** was treated with 50% aqueous trifluoroacetic acid (TFA) to yield the 6-chloropurine derivative **14** (75%), with the concomitant minor formation of the N^6-hydrolyzed hypoxanthine analog **3j** (4%). It should be noted that higher concentration of TFA, elevated temperature, and prolonged reaction time increased the formation of **3j** significantly and use of 2 *N* HCl instead of 50% TFA yielded the hypoxanthine analog **3j** as the major product. Treatment of **14** with ammonia, methylamine, and cyclopropylamine afforded the final adenine analog **1b** [3c], the N^6-methyladenine analog **3k**, and the N^6-cyclopropyladenine analog **3l**, respectively.

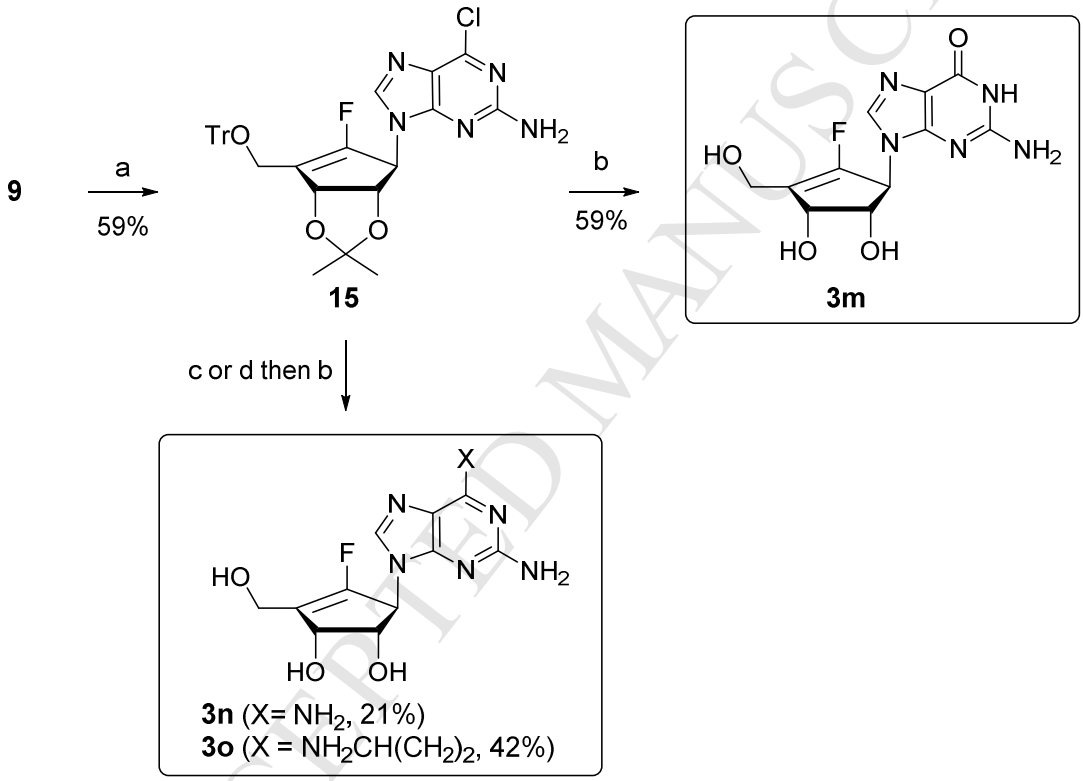
Scheme 3



Reagents and Conditions: a) 6-chloropurine, PPh_3, DIAD, THF, 0 °C to rt, 15 h; b) TFA/H_2O (1:1), rt, 15 h; c) NH_3, *t*-BuOH, steel bomb, 70-90 °C, 20 h; d) $MeNH_2/H_2O$ (40 wt%), EtOH, sealed tube, rt, 20 h; e) cyclopropylamine, Et_3N, EtOH, 60-67 °C, 24 h;

To prepare the 2,6-disubstituted-purine analogs **3m-o**, the glycosyl donor **9** was condensed with 2-amino-6-chloropurine under the standard Mitsunobu conditions to afford the 2-amino-6-chloropurine derivative **15** (Scheme 4). Compound **15** was treated with 50% aqueous TFA to yield the guanine analog **3m**. Treatment of **15** with ammonia and cyclopropylamine followed by treatment with 50% aqueous TFA, afforded the 2,6-diaminopurine derivative **3n** and the 2-amino-N^6-cyclopropylaminopurine **3o**, respectively.

Scheme 4

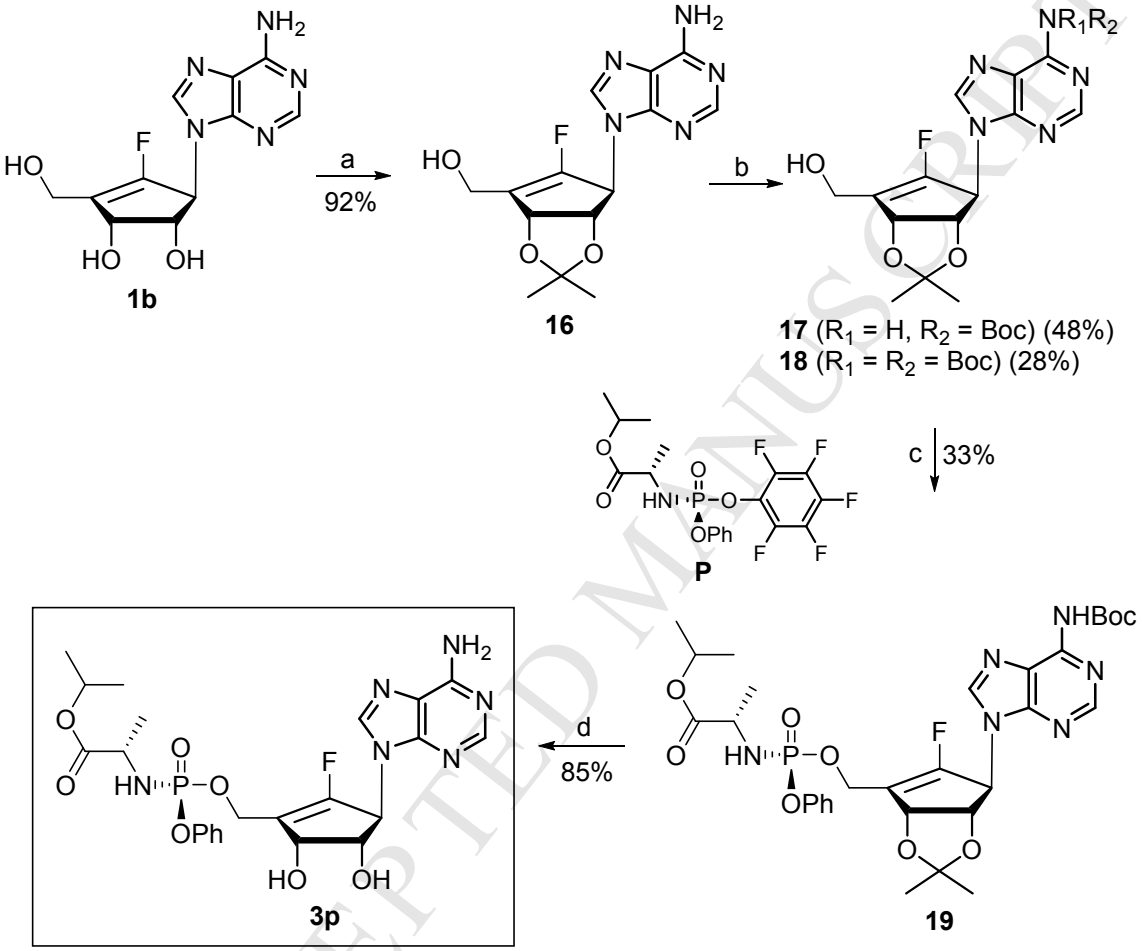


Reagents and Conditions: a) 2-amino-6-chloropurine, PPh_3, DIAD, THF, 0 ^oC to rt, 15 h; b) TFA/H_2O (1:1), rt, 15 h; c) NH_3, *t*-BuOH, steel bomb, 70-90 ^oC, 20 h; d) cyclopropylamine, Et_3N, EtOH, 60-67 ^oC, 24 h.

In order to determine if the anticancer activity depended on inhibiting DNA and/or RNA polymerase in cancer cells, we prepared the phosphoramidate prodrug **3p**, as shown in Scheme 5. The adenine derivative **1b** was protected as 2,3-acetonide **16**, which was treated with di-*tert*-butyl dicarbonate (Boc_2O) to give a mixture of mono-Boc- and di-Boc-adenine derivatives **17** and **18**. Treatment of the mixture of **17** and **18** with the phosphoramidate

reagent (**P**) [11] in the presence of *t*-butylmagnesium chloride afforded the phosphoramidate derivative **19**, which was treated with 50% HCOOH to yield the final phosphoramidate derivative **3p**.

Scheme 5



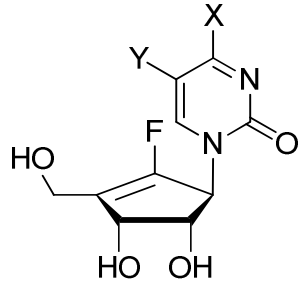
Reagents and Conditions: a) acetone, conc.H_2SO_4, 6 h; b) i. HMDS, DMAP, TMSOTf, 75 °C, 2 h; ii. Boc_2O, THF, rt, 4 h; iii. MeOH:Et_3N (5:1), 55 °C, 16 h; c) **P**, *t*-BuMgCl, molecular seives, THF, 0 °C to rt, 48 h; d) 50% HCOOH, rt, 8 h.

2.2. Biological evaluation

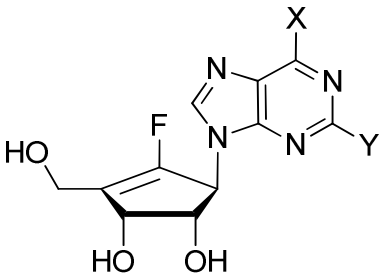
All of the synthesized final nucleosides **1b**, **2b**, and **3a-p** were assayed for the anticancer activity in various human cancer cell lines including A549 (lung cancer cells), HCT-116 (colon cancer cells), SNU-638 (stomach cancer cells), MDA-MB-231 (breast cancer cells),

SK-Hep-1 (liver cancer cells), and PC-3 (prostate cancer cells), using the SRB method (Table 1) [12,13].

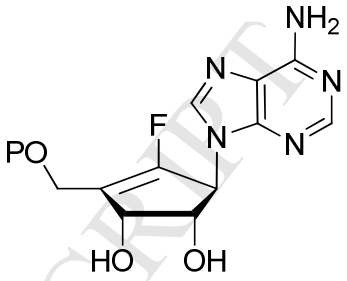
Table 1. Anticancer activity of the 6'-fluorocyclopentenyl-pyrimidines **2b**, **3a-i** and –purines **1b**, **3j-p** in human cancer cell lines



2b, **3a-i**



1b, **3j-o**



3p (P = phosphoramidate)

Compound No.	IC_{50} (μM)					
	A549	**HCT-116**	**SNU-638**	**MDA-MB-231**	**SK-Hep-1**	**PC-3**
2b (X = NH_2, Y = H)[a]	0.76	0.18	0.31	0.48	0.62	0.64
3a (X = OH, Y = H)[a]	>100	>100	>100	>100	>100	>100
3b (X = OH, Y = F)	>100	52.34	27.79	67.72	71.57	43.73
3c (X = OH, Y = Cl)	>100	>100	86.83	>100	>100	61.88
3d (X = OH, Y = Br)	>100	33.09	31.97	44.01	74.99	32.05
3e (X = OH, Y = I)	>100	84.5	70.24	55.15	>100	46.4
3f (X = NH_2, Y = F)	>100	>100	>100	>100	>100	>100
3g (X = NH_2, Y = Cl)	>100	>100	>100	>100	>100	>100
3h (X = NH_2, Y = Br)	>100	>100	>100	>100	>100	>100
3i (X= NH_2, Y = I)	>100	99.84	83.65	79.79	>100	82.89
1b (X= NH_2, Y = H)[c]	2.17	1.10	1.88	1.45	1.35	1.52
3j (X= OH, Y = H)	>100	>100	>100	>100	>100	>100
3k (X= NHMe, Y = H)	2.39	2.14	2.28	8.05	2.76	15.3
3l (X= NHCP[b], Y = H)	>100	>100	>100	>100	>100	>100
3m (X= OH, Y = NH_2)	>100	>100	>100	>100	>100	>100

3n (X= NH_2, Y = NH_2)	85.43	21.9	32.19	22.77	>100	>100
3o (X= NHCP[b], Y = NH_2)	>100	>100	>100	>100	>100	>100
3p (P = phosphoramidate)	18.61	4.57	3.05	18.37	23.96	10.54
Neplanocin A (**1a**)[d]	2.16	1.45	0.82	0.86	2.36	2.14
Ara-C[d]	1.75	4.4	1.08	10.49	1.8	48.4

[a]ref 6; [b]CP = cyclopropyl; [c]ref 3c; [d]positive control

As shown in Table 1, the cytosine analog **2b** exhibited the most potent anticancer activity among the pyrimidine nucleosides. The introduction of a halogen at the C-5 position of the pyrimidine nucleosides dramatically dropped the anticancer activity, indicating that they could not be efficiently converted to their triphosphates by cellular kinases, unlike the cytosine derivative **2b**, which was efficiently converted by UCK, resulting in potent anticancer activity. The 5-halouracil derivatives **3a-e** generally exhibited slightly better anticancer activity than the corresponding 5-halocytosine derivatives **3f-i**, demonstrating that the 5-halouracil moiety was a somewhat better substrate for cellular kinases than the 5-halocytosine moiety in this series, although they exhibited weak anticancer activity. This result was interesting in that the cytosine derivative **2b** was very potent, while the corresponding uracil derivative **3a** was inactive up to 100 μM. Among the synthesized purine nucleosides, the adenine derivative **1b** (X = NH_2, Y = H) exhibited potent anticancer activity, and it was as equipotent as the positive control, neplanocin A (**1a**) or 2'-β-arabinofuranosyl-cytosine (Ara-C), but its N^6-deaminated compound **3j** (X = OH, Y = H) was totally inactive up to 100 μM, indicating that the adenine derivative **1b** was not the substrate for adenosine deaminase. The addition of a methyl group at the N^6-amino group, giving **3k** (X = NHMe, Y = H), still maintained a similar anticancer activity, when compared to **1b**. However, the introduction of a bulkier group, like cyclopropyl, giving **3o**, totally abolished the anticancer activity. To our disappointment, the introduction of the amino group at the C2 position, such

as **3m-n**, resulted in abolished anticancer activity, indicating that no substitution at the C2 position favored anticancer activity. Surprisingly, the phosphoramidate analog **3p** exhibited less potent anticancer activity than the parent nucleoside **1b**. The phosphoamidate prodrug generally exhibits better biological activity than the parent drug because critical 5'-mono-phosphorylation by cellular kinase is skipped [11a], which indicates that the inhibition of DNA and/or RNA polymerase in cancer cells responsible for anticancer activity of **1b**, is a minor pathway, although it is converted to the triphosphate. It has been reported that inhibition of SAH hydrolase increases the intracellular SAH concentration and reduction in the level of S-adenosylmethionine (SAM), which results in the inhibition of enhancer of zeste homolog 2 (EZH2) histone methyltransferase [14]. Inhibition of EZH2 activity induces apotosis in different types of cancer cells [15]. The phosphoramidate prodrug **3p** is not active against SAH hydrolase up to 100 μM, but the parent compound **1b** is a strong inhibitor (IC_{50} = 0.48 μM) of SAH hydrolase [3], which in turns inhibits EZH2 histone methyltransferase [4]. Similarly, the anticancer activity of **3k** is attributed to the inhibition of SAH hydrolase (IC_{50} = 3.5 μM). Thus, it is suggested that the major pathway for the anticancer activity of **1b** is the inhibition of histone methyltransferase, resulting from strong inhibition (IC_{50} = 0.48 μM) of SAH hydrolase.

3. Conclusions

Based on the potent anticancer activity of the 6'-fluorocyclopentenyl-cytosine **2b**, we carried out a structure-activity relationship study of the 6'-fluorocyclopentenyl-pyrmidines **3a-i** and –purines **3j-p**, which were synthesized from D-ribose via a vinyl fluorination as a key step. From this study, the cytosine derivative **2b** exhibited the most potent anticancer activity among all of the tested compounds. The steric and electronic effects induced by halogen atoms, which were substituted at the C5 position, were detrimental to cellular

phosphorylation of the final nucleosides, resulting in weak anticancer activity. The adenine derivative **1b** and the N^6-methyladenine derivative **3k** demonstrated potent anticancer activity comparable to the positive control, neplanocin A (**1a**) or Ara-C. It was quite surprising in that only the cytosine derivative **2b** without any substitution at the C5 position was acceptable for anticancer activity in the pyrimidine series, whereas a small substituent such as H (**1b**) or Me (**3k**) in the N^6-substituted-aminopurine series was only tolerable for potent anticancer activity, demonstrating a very narrow structural requirement for biological activity. The phosphoramidate prodrug **3p** of **1b** did not improve the anticancer activity dramatically, indicating that **1b** did not seem to act as a DNA and/or RNA polymerase inhibitor after being converted to its triphosphate, but acted as a histone methyltransferase inhibitor, resulting from the inhibition of SAH hydrolase. All of these findings will contribute greatly to the design of biologically active fluoro-carbocyclic nucleosides.

4. Experimental section

4.1. Chemistry

The proton (^{1}H), carbon (^{13}C) NMR and fluorine (^{19}F) NMR spectra were obtained on a Jeol JNM-LA300 (300/75 MHz), Bruker AV 400 (400/376/100 MHz), Bruker AMX 500 (500/125 MHz), Jeol JNM-ECA600 (600/150 MHz), or Bruker AVANCE III 800 (800/200 MHz) spectrometer. The chemical shifts are reported in ppm units with Me_4Si or $CHCl_3$ as the internal standard. All reactions were routinely carried out under an inert atmosphere of dry nitrogen. The reactions were checked by thin layer chromatography (Kieselgel 60 F254, Merck). The spots were detected by viewing them under a UV light, and by colorizing them with charring after dipping them in a *p*-anisaldehyde solution or phosphomolybdic acid solution. In aqueous work-up, all organic solutions were dried over anhydrous magnesium sulfate and filtered prior to rotary evaporation at water pump pressure. The crude compounds were purified by column chromatography on a silica gel (Kieselgel 60, 70-230 mesh, Merck).

Unless otherwise noted, the materials were obtained from commercial suppliers and were used without purification. All of the solvents were purified and dried by standard techniques just before use. THF and Et_2O were freshly distilled from sodium and benzophenone. Methylene chloride, toluene, and benzene were purified by refluxing with CaH_2. The hexanes and ethyl acetate were purified by simple distillation. The mass spectra were obtained on a Jeol JMS-700 (FAB) or Agilent 6530 Accurate-Mass Q-TOF (ESI). The optical rotations were determined on JASCO P-2000 in the appropriate solvent. The UV spectra were recorded on a Perkin Elmer Lambda 25. The melting points were determined on a Barnstead Electrothermal IA9100.

*4.1.1. tert-Butyl((3aR,4R,6aR)-5-fluoro-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yloxy)diphenylsilane (**8a**).*

n-Butyllithium (11 mL, 17.6 mmol, 1.6 M solution in hexanes) was added dropwise to a cooled (–78 °C) solution of **7** [9] (5.58 g, 7.04 mmol) and *N*-fluorobenzenesulfonimide (3.33 g, 10.56 mmol) in anhydrous THF/*tert*-butyl methyl ether/*n*-heptane (3:1:1.5, 90 mL total, 0.08 M) over a period of 1 h under N_2. After stirring at the same temperature for 30 min, the reaction mixture was quenched with saturated aqueous NH_4Cl (100 mL) and was diluted with EtOAc (100 mL). Then the layers were separated and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed successively with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. Finally, the residue was purified by medium pressure liquid chromatography (silica gel, hexanes/EtOAc = 25/1) to give vinyl fluoride **8a** (2.17 g, 45%), **8b** (1.03 g, 22%) and **8c** (0.84 g, 18%).

8a: $[\alpha]_D^{25}$ = –8.42 (*c* 0.19, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 6.6 Hz, 2 H), 7.72 (d, *J* = 6.6 Hz, 2 H), 7.35-7.44 (m, 13 H), 7.16-7.27 (m, 8 H), 4.93 (t, *J* = 6.6 Hz, 1 H), 4.34 (s, 1 H), 3.22-3.25 (m, 1 H), 3.89 (d, *J* = 11.8 Hz, 1 H), 3.77 (d, *J* = 12.3 Hz, 1 H), 1.44 (s, 3 H), 1.38 (s, 3 H), 1.07 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.3 (d, *J* = 288.0 Hz),

143.8, 136.0 (d, *J* = 23.1 Hz), 133.5 (d, *J* = 21.2 Hz), 129.7 (d, *J* = 4.9 Hz), 127.7, 127.5 (d, *J* = 9.2 Hz), 127.0, 115.6 (d, *J* = 4.2 Hz), 111.8, 87.0, 78.6 (d, *J* = 8.8 Hz), 75.3 (d, *J* = 7.2 Hz), 71.0 (d, *J* = 18.9 Hz), 56.3, 29.5 (d, *J* = 29.8), 27.9, 27.2, 26.7, 19.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −128.2; MS (ESI+) found 707.2968 [calcd for $C_{44}H_{45}FNaO_4Si$ $(M+Na)^+$ 707.2969].

8b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73-7.81 (m, 4 H), 7.34-7.45 (m, 13 H), 7.16-7.29 (m, 8 H), 5.76 (s, 1 H), 4.66 (d, *J* = 5.6 Hz, 1 H), 4.53-4.58 (m, 1 H), 4.42 (t, *J* = 5.5 Hz, 1 H), 3.84 (d, *J* = 14.2 Hz, 1 H), 3.58 (d, *J* = 14.2 Hz, 1 H), 1.42 (s, 3 H), 1.32 (s, 3 H), 1.09 (s, 9 H).

8c: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72-7.81 (m, 4 H), 7.35-7.45 (m, 13 H), 7.22-7.30 (m, 8 H), 5.13 (m, 2 H), 4.80 (d, *J* = 13.6 Hz, 1 H), 4.19 (d, *J* = 14.4 Hz, 1 H), 3.73 (t, *J* = 5.2 Hz, 1 H), 1.31 (s, 3 H), 1.22 (s, 3 H), 1.12 (s, 9 H).

*4.1.2. (3aS,4R,6aR)-5-Fluoro-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-ol (**9**).*

Tetra-*n*-butylammonium fluoride (9.1 mL, 1.0 M solution in THF) was added dropwise to a cooled (0 °C) solution of **8a** (2.50 g, 3.65 mmol) in anhydrous THF (20 mL) under N_2. After stirring at room temperature for 2 h, the reaction mixture was evaporated. Finally, the residue was purified by medium pressure liquid chromatography (silica gel, hexanes/EtOAc = 4/1) to give **9** (1.417 g, 87%) as a white foam: $[\alpha]_D^{25}$ = +20.77 (*c* 0.39, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.48 (m, 6 H), 7.21-7.31 (m, 9 H), 5.13 (t, *J* = 6.6 Hz, 1 H), 4.66-4.69 (m, 1 H), 4.40 (t, *J* = 6.6 Hz, 1 H), 3.92 (d, *J* = 12.0 Hz, 1 H), 3.77 (dd, *J* = 1.6, 12.0 Hz, 1 H), 2.81 (d, *J* = 9.3 Hz, 1 H), 1.42 (s, 3 H), 1.41 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7 (d, *J* = 287.8 Hz), 143.7, 128.6, 127.8, 127.0, 115.7 (d, *J* = 4.5 Hz), 112.3, 87.1, 78.7 (d, *J* = 8.9 Hz), 73.8 (d, *J* = 7.7 Hz), 69.1 (d, *J* = 21.0 Hz), 56.2, 27.7, 26.6; ^{19}F NMR (376 MHz, $CDCl_3$) δ −130.1; MS (ESI+) found 469.1779 [calcd for $C_{28}H_{27}FNaO_4$ $(M+Na)^+$ 469.1791].

4.1.3. General Procedure for Mistunobu Condensation

Diisopropyl azodicarboxylate (2.5 equiv) was added dropwise to a cooled (0 ^{o}C) suspension of triphenylphosphine (2.5 equiv) and 5-halo-*N*^{3}-benzoyluracil (2.5 equiv) in anhydrous THF (0.2 M) under N_2. After stirring at room temperature for 15 min, the reaction mixture was re-cooled to 0 ^{o}C. Then, a solution of **9** (1.0 equiv) in anhydrous THF (0.2 M) was added dropwise to the mixture. After stirring at room temperature for 24 h, the reaction mixture was evaporated. Finally, the residue was purified by medium pressure liquid chromatography (silica gel, hexanes/EtOAc = 3/1) to give the base condensed derivatives.

4.1.4. General Procedure for Debenzoylation

Saturated methanolic ammonia (5 mL) was added to the above generated base condensed derivatives at room temperature. After stirring at room temperature for 15 h, the reaction mixture was evaporated. Finally, the residue was purified by column chromatography (silica gel, hexanes/EtOAc = 2/1) to give the debenzoylated derivatives **10a-e** as white foams.

*4.1.4.1. 1-((3aS,4S,6aR)-5-fluoro-2,2-dimethyl-6-((trityloxy)methyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)pyrimidine-2,4(1H,3H)-dione (**10a**).*

Yield: 81%; $[\alpha]_D^{25}$ = –37.04 (*c* 0.20, MeOH); UV (MeOH) λmax 263 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.43 (bs, 1 H), 7.46 (d, *J* = 7.4 Hz, 6 H), 7.46 (t, *J* = 7.2 Hz, 6 H), 7.22 (d, *J* = 7.3 Hz, 3 H, merged with $CDCl_3$), 6.90 (d, *J* = 8.0 Hz, 1 H), 5.73 (dd, *J* = 1.5, 8.0 Hz, 1 H), 5.34 (t, *J* = 5.4 Hz, 1 H), 5.27 (s, 1 H), 4.56 (t, *J* = 5.0 Hz, 1 H), 3.97 (d, *J* = 12.7 Hz, 1 H), 3.83 (d, *J* = 12.7 Hz, 1 H), 1.44 (s, 3 H), 1.35 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ

163.1, 150.3, 149.9 (d, *J* = 228.4 Hz), 143.6, 141.2, 128.6, 127.9, 127.1, 122.6, 112.3, 103.0, 87.4, 80.3 (d, *J* = 5.3 Hz), 79.7 (d, *J* = 7.3 Hz), 65.7 (d, *J* = 19.1 Hz), 56.9, 27.4, 25.7; ^{19}F NMR (378 MHz, $CDCl_3$) δ −130.5; HRMS (FAB+) found: 541.2132 [calcd for $C_{32}H_{30}FN_2O_5$ $(M+H)^+$ 541.5594].

*4.1.4.2. 5-Fluoro-1-((3aS,4S,6aR)-5-fluoro-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)pyrimidine-2,4(1H,3H)-dione (**10b**)*

Yield: 63%; $[\alpha]_D^{25}$ = −16.94 (*c* 0.36, MeOH); UV (MeOH) λ_{max} 268 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (bs, 1 H), 7.45-7.48 (m, 6 H), 7.22-7.32 (m, 9 H), 6.97 (d, *J* = 5.4 Hz, 1 H), 5.31 (m, 2 H), 4.52 (t, *J* = 5.2 Hz, 1 H), 4.01 (d, *J* = 12.6 Hz, 1 H), 3.86 (d, *J* = 12.8 Hz, 1 H), 1.35 (s, 3 H), 1.44 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3 (d, *J* = 26.8 Hz), 149.5 (d, *J* = 285.5 Hz), 148.9, 143.5, 140.8 (d, *J* = 238.7 Hz), 128.6, 127.9, 127.3, 125.2 (d, *J* = 32.8 Hz), 123.4 (d, *J* = 3.1 Hz), 112.6, 87.5, 80.3 (d, *J* = 6.8 Hz), 79.5 (d, *J* = 9.2 Hz), 65.6 (d, *J* = 19.6 Hz), 56.9, 27.3, 25.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −128.2, −165.4; MS (ESI–) found: 557.1891 [calcd for $C_{32}H_{27}F_2N_2O_5$ $(M-H)^-$ 557.1888].

*4.1.4.3. 5-Chloro-1-((3aS,4S,6aR)-5-fluoro-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)pyrimidine-2,4(1H,3H)-dione (**10c**).*

Yield: 62%; $[\alpha]_D^{25}$ = −55.26 (*c* 0.19, MeOH); UV (MeOH) λ_{max} 276 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (bs, 1 H), 7.46 (d, *J* = 7.4 Hz, 7 H), 7.19-7.32 (m, 9 H), 5.32-5.35 (m, 2 H), 4.55 (t, *J* = 5.3 Hz, 1 H), 3.98 (d, *J* = 12.8 Hz, 1 H), 3.82 (d, *J* = 12.8 Hz, 1 H), 1.44 (s, 3 H), 1.35(s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 149.6 (d, *J* = 285.7 Hz), 149.3, 143.5, 137.8, 128.6, 127.9, 127.2, 123.3 (d, *J* = 3.1 Hz), 112.6, 109.9, 87.5, 80.3 (d, *J* = 6.7

Hz), 79.6 (d, *J* = 9.2 Hz), 66.0 (d, *J* = 20.2 Hz), 56.9, 27.3, 25.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −128.2; MS (ESI+) found 597.1556 [calcd for $C_{32}H_{28}ClFN_2NaO_5$ $(M+Na)^+$ 597.1568].

*4.1.4.4. 5-Bromo-1-((3aS,4S,6aR)-5-fluoro-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)pyrimidine-2,4(1H, 3H)-dione (**10d**).*

Yield: 51%; $[\alpha]_D^{25}$ = −52.50 (*c* 0.20, MeOH); UV (MeOH) λ_{max} 279 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (bs, 1 H), 7.46-7.48 (m, 6 H), 7.22-7.32 (m, 10 H), 5.34 (t, *J* = 6 Hz, 1 H), 5.30 (s, 1 H), 4.57 (t, *J* = 5.1 Hz, 1 H), 3.98 (d, *J* = 12.6 Hz, 1 H), 3.80 (d, *J* = 12.6 Hz, 1 H), 1.44 (s, 3 H), 1.35 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 149.7 (d, *J* = 285.4 Hz), 149.6, 143.5, 140.4, 128.6, 127.9, 127.2, 123.2 (d, *J* = 2.9 Hz), 112.6, 97.6, 87.5, 80.3 (d, *J* = 6.6 Hz), 79.7 (d, *J* = 9.3 Hz), 66.2 (d, *J* = 20.2 Hz), 56.9, 27.3, 25.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −128.2; MS (ESI+) found 619.1073 [calcd for $C_{32}H_{29}BrFN_2O_5$ $(M+H)^+$ 619.1244].

*4.1.4.5. 1-((3aS,4S,6aR)-5-Fluoro-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta-[d][1,3] dioxol-4-yl)-5-iodopyrimidine-2,4(1H,3H)-dione (**10e**).*

Yield: 63%; $[\alpha]_D^{25}$ = −70.67 (*c* 0.15, MeOH); UV (MeOH) λ_{max} 284 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (bs, 1 H), 7.43-7.48 (m, 7 H), 7.22-7.33 (m, 9 H), 5.35 (t, *J* = 5.4 Hz, 1 H), 5.27 (s, 1 H), 4.58 (t, *J* = 5.1 Hz, 1 H), 3.96 (d, *J* = 12.5 Hz, 1 H), 3.78 (d, *J* = 12.5 Hz, 1 H), 1.43 (s, 3 H), 1.35(s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7, 149.9, 149.8 (d, *J* = 285.7 Hz), 145.6, 143.5, 128.6, 127.9, 127.2, 123.0 (d, *J* = 2.8 Hz), 112.5, 110.0, 87.4, 80.3 (d, *J* = 6.5 Hz), 79.7 (d, *J* = 9.2 Hz), 69.1, 66.3 (d, *J* = 21.2 Hz), 56.9, 27.3, 25.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −128.2; MS (ESI+) found: 689.0916 [calcd for $C_{32}H_{28}FIN_2NaO_5$ $(M+Na)^+$ 689.0925].

4.1.5. General Procedure for Trityl and Isopropylidene Group Deprotection

A 2 *N* HCl solution (0.6 M) was added dropwise to a cooled (0 °C) solution of **10a-e** (1.0 equiv) in MeOH (0.6 M). After stirring at room temperature for 18 h, the reaction mixture was co-evaporated with MeOH three times. The residue was diluted with MeOH and was neutralized with saturated methanolic ammonia. The resulting reaction mixture was allowed to stir at room temperature for 1 h, and was then evaporated. Finally, the crude residue was purified by column chromatography (^{18}C reverse-phase silica gel, H_2O/MeCN = 99/1) to give the 5-halouracil derivatives **3a-e** as white solids.

*4.1.5.1. 1-((1S,4R,5S)-2-Fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopent-2-en-1-yl)pyrimidine-2,4(1H,3H)-dione (**3a**)[6]*

Yield : 80%; mp 78-79 ^{o}C; $[\alpha]_D^{27.2}$ = –93.5 (*c* 1.2, MeOH); UV (MeOH) λ_{max} 264.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 4.12 (dt, *J* = 2.4, 13.2 Hz, 1 H), 4.19 (td, *J* = 1.2, 5.6 Hz 1 H), 4.37 (d, *J* = 12.8 Hz, 1 H), 4.68 (td, J = 1.2, 6.0 Hz, 1 H), 5.43 (bs, 1 H), 5.73 (d, *J* = 8.0 Hz, 1 H), 7.47 (dd, *J* = 1.2, 8.0 Hz, 1 H); ^{13}C (100 MHz, CD_3OD) δ 166.8, 155.4 (d, *J* = 284.6 Hz), 153.6, 144.7, 123.8 (d, *J* = 1.9 Hz), 104.1, 75.5 (d, *J* = 5.3 Hz), 71.6 (d, *J* = 8.8 Hz), 66.1 (d, *J* = 17.6 Hz), 55.1; ^{19}F (376 MHz, CD_3OD) δ -128.9; HRMS (ESI+) found 259.0722 [calculated for $C_{10}H_{12}FN_2O_5$ 259.065 $(M+H)^+$]; Anal. calcd for $C_{10}H_{11}FN_2O_5$: C, 46.52; H, 4.29; N, 10.85. Found: C, 46.55; H, 4.31; N, 10.91.

4.1.5.2. *5-Fluoro-1-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopent-2-enyl)pyrimidine-2,4(1H,3H)-dione (**3b**).* Yield: 52%; m.p: charred above 213 ^{o}C; $[\alpha]_D^{25}$ = –10.00 (c 0.21, MeOH); UV (MeOH) λ_{max} 272 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.74 (dd,

J = 0.8, 6.4 Hz, 1 H), 5.46 (bs, 1 H), 4.68 (td, J = 1.6, 6.0 Hz, 1 H), 4.37 (d, J = 13.2 Hz, 1 H), 4.19 (td, J = 1.6, 5.6 Hz, 1 H), 4.14 (t, J = 2.4 Hz, 1 H), 4.11 (t, J = 2.4 Hz, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5 (d, *J* = 25.8 Hz), 154.5 (d, *J* = 284.9), 151.7, 143.6 (d, *J* = 233.2 Hz), 127.8 (d, *J* = 34.2 Hz), 123.6, 74.8 (d, *J* = 5.3 Hz), 70.9 (d, *J* = 8.3 Hz), 65.4 (d, *J* = 18.2 Hz), 54.4; ^{19}F NMR (376 MHz, CD_3OD) δ –126.9, –162.6 (d, J = 6.4 Hz); MS (FAB) found: 277.0645 [calcd for $C_{10}H_{11}F_2N_2O_5$ $(M+H)^+$ 277.0636]; Anal. Calcd for $C_{10}H_{11}F_2N_2O_5$: C, 43.49; H, 3.65; N, 10.14. Found: C, 43.09; H, 3.61; N, 9.98.

4.1.5.3. *5-Chloro-1-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopent-2-enyl)pyrimidine-2,4(1H,3H)-dione (**3c**).* Yield: 50%; m.p: 124-126 ^oC; $[\alpha]_D^{25}$ = –121.33 (c 0.15, MeOH); UV (MeOH) λ_{max} 278 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (d, J = 0.8 Hz, 1 H), 5.46 (bs, 1 H), 4.69 (td, J = 1.6, 6.0 Hz, 1 H), 4.38 (d, J = 13.2 Hz, 1 H), 4.21 (td, J = 1.2, 6.0 Hz, 1 H), 4.14 (t, J = 2.4 Hz, 1 H), 4.11 (t, J = 2.4 Hz, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.5, 154.4 (d, *J* = 284.2 Hz), 152.1, 140.9, 123.5, 110.3, 74.9 (d, *J* = 5.3 Hz), 70.8 (d, *J* = 8.4 Hz), 65.8, 65.7, 54.4; ^{19}F NMR (376 MHz, CD_3OD) δ –126.9; MS (FAB+) found 293.0345 [calcd for $C_{10}H_{11}ClFN_2O_5$ $(M+H)^+$ 293.0341]; Anal. Calcd for $C_{10}H_{11}ClFN_2O_5$: C, 41.04; H, 3.44; N, 9.57. Found: C, 41.34; H, 3.04, N, 9.17.

4.1.5.4. *5-Bromo-1-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopent-2-enyl)pyrimidine-2,4(1H,3H)-dione (**3d**).* Yield: 74%; m.p: 68-70 ^oC; $[\alpha]_D^{25}$ = –163.68 (c 0.19, MeOH); UV (MeOH) λmax 281 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.89 (s, 1 H), 5.45 (bs, 1 H), 4.67 (t, J = 5.2 Hz, 1 H), 4.37 (d, J = 13.0 Hz, 1 H), 4.21 (t, J = 5.6 Hz, 1 H), 4.11 (d, J = 13.0 Hz, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.7, 154.5 (d, *J* = 284.2 Hz), 152.4, 143.5, 123.4, 98.0, 74.9 (d, *J* = 5.4 Hz), 70.9 (d, *J* = 8.4 Hz), 65.8 (d, *J* =17.4 Hz), 54.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ –126.9; MS (FAB+) found 337.1014 [calcd for $C_{10}H_{10}BrFN_2O_5$ $(M+H)^+$

336.9835]; Anal. Calcd for $C_{10}H_{10}BrFN_2O_5$: C, 35.63; H, 2.99; N, 8.31. Found: C, 35.34; H, 2.67; N, 8.32.

4.1.5.5. *1-((1S,4R,5S)-2-Fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopent-2-enyl)-5-iodopyrimidine-2,4(1H,3H)-dione (**3e**).* Yield: 61%; m.p: 87-89 ^{o}C; $[\alpha]_D^{25} = -153.24$ (c 0.37, MeOH); UV (MeOH) λ_{max} 287 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.90 (d, J = 0.8 Hz, 1 H), 5.43 (bs, 1 H), 4.68 (td, J = 1.2, 5.6 Hz, 1 H), 4.38 (d, J = 12.8 Hz, 1 H), 4.22 (td, J = 1.2, 5.6 Hz, 1 H), 4.12 (dt, J = 2.4, 12.8 Hz, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.9, 154.6 (d, *J* = 284.2 Hz), 152.7, 148.5, 123.2, 74.9 (d, *J* = 4.6 Hz), 70.8 (d, *J* = 8.3 Hz), 69.5, 65.7 (d, *J* = 17.5 Hz), 54.4; ^{19}F NMR (376 MHz CD_3OD) δ −126.8; MS (ESI+) found 406.9514 [calcd for $C_{10}H_{10}FIN_2NaO_5$ $(M+Na)^+$ 406.9516]; Anal. Calcd for $C_{10}H_{10}FIN_2NaO_5$: C, 31.27; H, 2.62; N, 7.29. Found: C, 31.45; H, 2.23; N, 7.08.

*4.1.6. General Procedure for the Conversion of the Uracil Derivatives **3a-e** to the Cytosine Derivatives **2b** and **3f-i**.*

[*Acetylation*] Acetic anhydride (4.0 equiv) was added dropwise to a stirred solution of the pyrimidine derivatives **3a-e** (1.0 equiv) in anhydrous pyridine (0.17 M) under N_2. After stirring at room temperature for 18 h, the reaction mixture was evaporated. Then, the residue was diluted with CH_2Cl_2 and H_2O, and the organic layer was separated, dried over $MgSO_4$, filtered, and evaporated. Finally, the residue was purified by medium pressure liquid chromatography (silica gel, hexanes/EtOAc = 9/1) to give the triacetates **11a-e** as a thick liquid.

[*Introduction of 1,2,4-triazole*] $POCl_3$ (10.0 equiv) was added dropwise- to a cooled (0 ^{o}C) suspension of 1,2,4-triazole (10.0 equiv) in anhydrous CH_3CN (1.1 M) under N_2. After stirring at room temperature for 1 h, the reaction mixture was cooled to 0 ^{o}C and Et_3N (10.0

equiv) was added, followed by **11a-e** (1.0 equiv) in anhydrous CH_3CN (0.1 M). After stirring for an additional 24 h at room temperature, the reaction mixture was evaporated to a half-volume, diluted with CH_2Cl_2, and washed with H_2O. The layers were separated, the organic layer was dried over $MgSO_4$, filtered, and evaporated to give **12a-e**, which were used for the next step without further purification.

[*Amination with Deacetylation*] Aqueous ammonia (0.2 M) was added to a stirred solution of **12a-e** in 1,4-dioxane (0.13 M). After stirring at room temperature for 16 h in glass-sealed tube, the reaction mixture was evaporated. A methanolic ammonia solution (5.0 mL) was added to the above-generated reaction mixture. After stirring for an additional 15 h at room temperature, the reaction mixture was evaporated. Finally, the residue was purified by column chromatography (^{18}C reverse-phase silica gel, H_2O/MeCN = 99/1) to give **2b** and **3f-i** as white solids.

4.1.6.1. *4-Amino-1-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopent-2-en 1-yl)pyrimidin-2(1H)-one (**2b**) [6]*. Yield : 40%; mp 101-102 ^{o}C; $[\alpha]_D^{27.2} = -135.47$ (c 5.3, MeOH); UV (MeOH) λmax 274.5 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 4.10 (dt, J = 2.4, 12.8 Hz, 1 H), 4.24-4.27 (m, 1 H), 4.36 (d, J = 12.8 Hz, 1 H), 4.68 (td, J = 1.2, 6.0 Hz 1H), 5.34 (bs, 1 H), 5.92 (d, J = 7.2 Hz, 1 H), 7.47 (J = 6.8 Hz, 1 H); ^{13}C (100 MHz, CD_3OD) δ 167.6, 158.8, 155.6 (d, *J* = 284.9 Hz), 145.0, 122.0, 96.6, 74.6 (d, *J* = 3.9 Hz), 70.9 (d, *J* = 8.9 Hz), 67.1 (d, *J* = 17.5 Hz), 54.3; ^{19}F (376 MHz, CD_3OD) δ −128.1; HRMS (ESI+) found 258.0822 [calculated for $C_{10}H_{13}FN_3O_4$ $(M+H)^+$ 258.081]; Anal. calcd for $C_{10}H_{12}FN_3O_4$: C, 46.59; H, 4.70; N, 16.34. Found: C, 46.63; H, 4.77; N, 16.40.

4.1.6.2. *4-Amino-5-fluoro-1-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl) cyclopent-2-enyl)pyrimidin-2(1H)-one (**3f**).* Yield: 21%; m.p: 121-123 ^{o}C; $[\alpha]_D^{25}$ = –60.00 (c 0.21, MeOH); UV (MeOH) λ_{max} 276 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (d, J = 3.0 Hz, 1 H), 5.82 (s, 1 H), 4.84 (bs, 2 H), 4.51 (s, 1 H), 4.02 (d, J = 7 Hz, 1 H), 3.87-3.93 (m, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.8, 154.9 (d, *J* = 13.7 Hz), 154.1 (d, *J* = 282.6 Hz), 143.4, 141.0, 139.7 (d, *J* = 20.2 Hz), 121.9, 79.8 (d, *J* = 18.2 Hz), 73.4 (d, *J* = 6.3 Hz), 68.5 (d, *J* = 9.3 Hz), 52.3; ^{19}F NMR (376 MHz, CD_3OD) δ –128.6, –157.3 (d, J = 8.6 Hz); MS (ESI+) found 276.0793 [calcd for $C_{10}H_{12}F_2N_3O_4$ $(M+H)^+$ 276.0796]; Anal. Calcd for $C_{10}H_{12}F_2N_3O_4$: C, 43.32; H, 4.73; N, 15.16. Found: C, 43.45; H, 4.44; N, 14.98.

*4.1.6.3. 4-Amino-5-chloro-1-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl) cyclopent-2-enyl)pyrimidin-2(1H)-one (**3g**).* Yield: 42%; m.p: 135-137 ^{o}C; $[\alpha]_D^{25}$ = –166.19 (c 0.21, MeOH); UV (MeOH) λ_{max} 289 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (s, 1 H, D_2O exchangeable), 7.80 (s, 1 H), 7.25 (s, 1 H, D_2O exchangeable), 5.31 (s, 1 H), 5.17 (d, J = 7.1 Hz, 1 H, D_2O exchangeable), 4.98 (d, J = 5.9 Hz, 1 H, D_2O exchangeable), 4.78 (dd, J = 6.2, 4.6 Hz, 1 H, D_2O exchangeable), 4.47 (dd, J = 10.2, 4.8 Hz , 1 H), 4.10-16 (m, 2 H), 3.87-91 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.3, 154.1, 152.5 (d, *J* = 281.9 Hz), 141.4, 121.4, 99.1, 72.7 (d, *J* = 4.8 Hz), 68.9 (d, *J* = 9.1 Hz), 64.5 (d, *J* = 20.4 Hz), 52.6; ^{19}F NMR (376 MHz, DMSO-d_6) δ –134.5; MS (ESI+) found 292.0492 [calcd for $C_{10}H_{12}ClFN_3O_4$ $(M+H)^+$ 292.0500]; Anal. Calcd for $C_{10}H_{12}ClFN_3O_4$: C, 40.90; H, 4.46; N, 14.31. Found: C, 40.98; H, 4.06; N, 13.98.

4.1.6.4. *4-Amino-5-bromo-1-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl) cyclopent-2-enyl)pyrimidin-2(1H)-one (**3h**).* Yield: 42%; m.p: 219-221 ^{o}C; $[\alpha]_D^{25}$ = –188.42 (c 0.19, MeOH); UV (MeOH) λ_{max} 290 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.85 (s, 1 H),

5.37 (bs, 1 H), 4.69 (t, J = 5.2 Hz, 1 H), 4.37 (d, J = 12.8 Hz, 1 H), 4.28 (td, J = 1.2, 5.6 Hz, 1 H), 4.11 (dt, J = 2.4, 12.8 Hz, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.9, 158.4, 157.4 (d, *J* = 284.4 Hz), 146.4, 123.1, 90.0, 75.5 (d, *J* = 5.2 Hz), 71.6 (d, *J* = 8,8 Hz), 68.3 (d, *J* = 16.8 Hz), 55.1; ^{19}F NMR (376 MHz, CD_3OD) δ −133.8; MS (ESI+) found 335.9994 [calcd for $C_{10}H_{12}BrFN_3O_4$ $(M+H)^+$ 335.9995]; Anal. Calcd for $C_{10}H_{12}BrFN_3O_4$: C, 35.52; H, 3.88; N, 12.43. Found: C, 35.13; H, 4.23; N, 12.04.

4.1.6.5. *4-Amino-1-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopent-2-enyl)-5-iodopyrimidin-2(1H)-one (**3i**).* Yield: 38%; m.p: 228-230 °C; $[\alpha]_D^{25}$ = −199.44 (c 0.18, MeOH); UV (MeOH) λ_{max} 297 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 7.88 (s, 1 H), 5.32 (bs, 1 H), 4.67 (t, J = 5.5 Hz, 1 H), 4.36 (d, J = 13.0 Hz, 1 H), 4.27 (t, J = 6.0 Hz, 1 H), 4.09 (dt, J = 2.0, 13.0 Hz, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.1, 154.7, 153.2 (d, *J* = 282.2 Hz), 149.8, 121.5, 73.1, 69.3 (d, *J* = 9.1 Hz), 65.0 (d, *J* = 17.9 Hz), 53.0, 49.0; ^{19}F NMR (376 MHz, CD_3OD) δ −133.7; MS (ESI+) found 384.1259 [calcd for $C_{10}H_{12}FIN_3O_4$ $(M+H)^+$ 383.9857]; Anal. Calcd for $C_{10}H_{12}FIN_3O_4$: C, 31.19; H, 3.40; N, 10.91. Found: C, 31.56; H, 3.80; N, 9.89.

4.1.7. *6-Chloro-9-((3aS,4S,6aR)-5-fluoro-2,2-dimethyl-6-((trityloxy)methyl)-3a,6a-dihydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-9H-purine (**13**).* Compound **9** was converted to **13**, using a procedure similar to the one used in the preparation of **10**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (s, 1 H), 8.01 (s, 1 H), 7.43-7.50 (m, 6 H), 7.20-7.35 (m, 9 H), 5.53-5.62 (m, 2 H), 4.78 (t, J = 5.7 Hz, 1 H), 4.02 (d, J = 12.6 Hz, 1 H), 3.83 (d, J = 12.6 Hz, 1 H), 1.48 (s, 3 H), 1.37 (s 3 H); The resulting **13**, contaminated with DIAD, was used in the next step.

*4.1.8. (1S,2R,5S)-5-(6-Chloro-9H-purin-9-yl)-4-fluoro-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (**14**) and 9-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopent-2-en-1-yl)-1H-purin-6(9H)-one (**3j**).*

A suspension of **13** (1.0 g, 1.72 mmol) in 50% TFA (10 mL, 0.17 M) was stirred at room temperature for 15 h. The mixture was evaporated and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9/1 to 4/1) to give the 6-chloropurine derivative **14** (387 mg, 75% from **9**) as a pale yellow solid and **3j** (19 mg, 4% from **9**) as a white solid.

Compound **14**: m.p: 103-105 ^oC; $[\alpha]_D^{20} = -12.52$ (*c* 0.24, MeOH); UV (MeOH) λ_{max} 265 nm; ^1H NMR (600 MHz, CD_3OD) δ 8.72 (s, 1 H), 8.62 (s, 1 H), 5.73 (s, 1 H), 4.83 (m, 1 H, merged with H_2O), 4.63 (t, *J* = 5.5 Hz, 1 H), 4.41 (d, *J* = 12.8 Hz, 1 H), 4.19-4.22 (m, 1 H); ^{13}C NMR (150 MHz, CD_3OD) δ 154.5 (d, *J* = 284.4 Hz), 154.1, 153.9, 152.4, 148.2, 133.6, 124.0, 76.1 (d, *J* = 4.3 Hz), 71.8 (d, *J* = 8.6 Hz), 64.8 (d, *J* = 18. Hz), 55.2; ^{19}F NMR (376 MHz, CD_3OD) δ −78.8, −134.7; MS (ESI+) found 301.0511 [calcd for $C_{11}H_{11}ClFN_4O_3$ $(M+H)^+$ 301.0504].

Compound **3j**: m.p: 280 ^oC (decomp.); $[\alpha]_D^{20} = -3.23$ (*c* 0.22, MeOH); UV (MeOH) λ_{max} 250 nm; ^1H NMR (800 MHz, CD_3OD) δ 8.11 (s, 1 H), 8.02 (s, 1 H), 5.59 (s, 1 H), 4.78 (t, *J* = 5.0 Hz, 1 H), 4.52 (t, *J* = 5.6 Hz, 1 H), 4.39 (d, *J* = 13.2 Hz, 1 H), 4.17 (dt, *J* = 12.8, 2.2 Hz, 1 H); ^{13}C NMR (200 MHz, CD_3OD) δ 159.7, 155.0 (d, *J* = 284.4 Hz), 151.3, 147.5, 141.8, 126.5, 123.5, 76.6 (d, *J* = 4.7 Hz), 71.7 (d, *J* = 8.5 Hz), 64.3 (d, *J* = 18.1 Hz), 55.1; ^{19}F NMR (376 MHz, CD_3OD) δ −78.7, −132.4, −134.7; MS (ESI+) found: 283.0842 [calcd for $C_{11}H_{11}FN_4O_4$ $(M+H)^+$ 283.0843]; Anal. Calcd for $C_{11}H_{11}FN_4O_4$: C, 46.81; H, 3.93; N, 19.85. Found: C, 46.98; H, 4.13; N, 19.65.

*4.1.9. (1S,2R,5S)-5-(6-Amino-9H-purin-9-yl)-4-fluoro-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (**1b**) [3c].*

Saturated ammonia in *tert*-butanol (10.0 mL) was added to a solution of **14** (69 mg, 0.23 mmol) in *tert*-butanol (1.0 mL, 0.23 M) in a steel bomb. After stirring at 70 ^{o}C for 24 h, the reaction mixture was evaporated. Finally, the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 4/1) to give **1b** (36 mg, 56%) as a white solid: mp 181-184 ^{o}C; $[\alpha]_D^{25} = -181.1$ (*c* 0.62, MeOH); UV (H_2O) λmax 260.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (s, 1 H), 8.15 (s, 1 H), 5.56 (bs, 1 H), 4.79 (t, *J* = 5.2, 6.0 Hz, 1 H), 4.56 (t, *J* = 5.6 Hz, 1 H), 4.41 (d, *J* = 13.2 Hz, 1 H), 4.16 (td, *J* =2.4, 13.2 Hz, 1 H); ^{13}C NMR(100 MHz, CD_3OD) δ 157.7, 154.7 (d, *J* = 285.3 Hz), 154.2, 151.2, 142.1, 122.8 (d, *J* = 2.3 Hz), 120.8, 75.7 (d, *J* = 4.6 Hz), 71.2 (d, *J* = 8.5 Hz), 63.6 (d, *J* = 18.4Hz), 54.7; ^{19}F NMR (376 MHz, CD_3OD) δ −133.1; MS (FAB+) found 282 [calcd for $C_{11}H_{13}FN_5O_3$ 282.2554 ($M+H^+$)]; Anal. Calcd for $C_{11}H_{12}FN_5O_3$: C, 46.98; H, 4.30; N, 24.90. Found: C, 46.99; H, 4.28; N, 25.20.

*4.1.10. (1S,2R,5S)-4-Fluoro-3-(hydroxymethyl)-5-(6-(methylamino)-1H-purin-9(6H)-yl)cyclopent-3-ene-1,2-diol (**3k**).*

Aqueous methylamine (40 wt%, 1 mL) was added to a stirred solution of **14** (57 mg, 0.19 mmol) in ethanol (2.0 mL, 0.1 M) in a glass-seal tube. After stirring for 20 h at room temperature, the mixture was evaporated. Finally, the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9/1) to give **3k** (24 mg, 43%) as a white solid: m.p: 157-160 ^{o}C; $[\alpha]_D^{20} = -14.95$ (*c* 0.36, MeOH); UV (MeOH) λ_{max} 266 nm; ^{1}H NMR (600 MHz, CD_3OD) δ 8.23 (s, 1 H), 8.08 (s, 1 H), 5.55 (s, 1 H), 4.79 (t, *J* = 5.9 Hz, 1 H), 4.55 (t, *J* = 6.0 Hz, 1 H), 4.41 (d, *J* = 12.8 Hz, 1 H), 4.14-4.19 (m, 1 H), 3.10 (bs, 3 H); ^{13}C NMR (150

MHz, CD_3OD) δ 157.6, 155.3 (d, *J* = 284.4 Hz), 154.6, 150.6, 142.0, 123.2, 121.9, 76.2 (d, *J* = 5.0 Hz), 71.7 (d, *J* = 8.7 Hz), 64.1 (d, *J* = 17.9 Hz), 55.2, 28.5; ^{19}F NMR (376 MHz, CD_3OD) δ −78.7, −134.3; MS (ESI+) found: 296.1159 [calcd for $C_{12}H_{15}FN_5O_3$ $(M+H)^+$ 296.1159]; Anal. Calcd for $C_{12}H_{15}FN_5O_3$: C, 48.81; H, 4.78; N, 23.72. Found: C, 48.90; H, 4.48; N, 23.43.

*4.1.11. (1S,2R,5S)-5-(6-(Cyclopropylamino)-9H-purin-9-yl)-4-fluoro-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (**3l**).*

Cyclopropylamine (82 μL, 1.18 mmol) and triethylamine (230 μL, 1.65 mmol) were successively added to a solution of **14** (71 mg, 0.24 mmol) in ethanol (1.5 mL, 0.16 M) in a steel bomb. The reaction mixture was stirred at 67 ^oC for 24 h, and was evaporated. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 7/1) to give **3l** (20 mg, 25%) as a white solid: m.p: 93-95 ^oC; $[\alpha]_D^{20} = -12.85$ (*c* 0.11, MeOH); UV (MeOH) λ_{max} 270 nm; ^1H NMR (600 MHz, CD_3OD) δ 8.27 (s, 1 H), 8.11 (s, 1 H), 4.79 (t, *J* = 6.0 Hz, 1 H), 4.56 (t, *J* = 5.9 Hz, 1 H), 4.41 (d, *J* = 12.7 Hz, 1 H), 4.14-4.20 (m, 1 H), 2.96 (bs, 1 H), 0.87-0.92 (m, 2 H), 0.62-0.68 (m, 2 H); ^{13}C NMR (150 MHz, CD_3OD) δ 157.9, 155.2 (d, *J* = 285.1 Hz), 151.0, 149.5, 142.4, 123.3, 121.9, 76.2 (d, *J* = 4.3 Hz), 71.7 (d, *J* = 4.6 Hz), 64.2 (d, *J* = 18.7 Hz), 55.2, 25.3, 8.36; ^{19}F NMR (376 MHz, CD_3OD) δ −78.8, −134.4; MS (ESI+) found 322.1326 [calcd for $C_{14}H_{17}FN_5O_3$ $(M+H)^+$ 322.1315]; Anal. Calcd for $C_{14}H_{17}FN_5O_3$: C, 52.33; H, 5.02; N, 21.80. Found: C, 52.76; H, 4.98; N, 21.99.

4.1.12. *6-Chloro-9-((3aS,4S,6aR)-5-fluoro-2,2-dimethyl-6-((trityloxy)methyl)-3a,6a-dihydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-9H-purin-2-amine (**15**).* Compound **9** (123 mg, 0.28 mmol) was converted to **15** (90 mg, 55%) as a white foam, using a procedure similar

to the preparation of **10**: $[\alpha]_D^{20}$ = –1.23 (*c* 0.47, MeOH); UV (MeOH) λ_{max} 255 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.65 (s, 1 H), 7.45-7.49 (m, 6 H), 7.22-7.31 (m, 9 H), 5.58 (t, *J* = 4.56 Hz, 1 H), 5.33 (s, 1 H), 5.00 (s, 2 H), 4.72 (t, *J* = 5.46 Hz, 1 H), 4.04 (d, *J* = 11.8 Hz, 1 H), 3.77-3.82 (m, 1 H), 1.46 (s, 3 H), 1.38 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.0, 153.3, 151.8, 150.2 (d, *J* = 285.1 Hz), 143.7, 140.8, 128.6, 127.9, 127.2, 125.7, 121.5, 112.4, 87.3, 80.5 (d, *J* = 5.8 Hz), 80.2 (d, *J* = 9.3 Hz), 62.4 (d, *J* = 20.1 Hz), 56.5, 27.5, 26.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ –129.0; MS (ESI+) found 598.2027 [cacld for $C_{33}H_{30}FN_5O_3$ $(M+H)^+$ 598.2021].

*4.1.13. 2-Amino-9-((1S,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopent-2-en-1-yl)-1H-purin-6(9H)-one (**3m**).*

A suspension of **15** (65 mg, 0.11 mmol) in 50% TFA (6 mL, 0.018 M) was stirred for 15 h at room temperature. The reaction mixture was evaporated and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 7/1) to give **3m** (19 mg, 59%) as a white solid: m.p: 223 ^{o}C (decomp.); $[\alpha]_D^{20}$ = –6.70 (*c* 0.29, MeOH); UV (MeOH) λ_{max} 255 nm; ^{1}H NMR (800 MHz, CD_3OD) δ 7.73 (s, 1 H), 5.38 (s, 1 H), 4.76 (t, *J* = 4.8 Hz, 1 H), 4.49 (t, *J* = 5.6 Hz, 1 H), 4.39 (d, *J* = 12.9 Hz, 1 H), 4.13-4.17 (m, 1 H); ^{13}C NMR (200 MHz, CD_3OD) δ 160.2, 156.1, 155.6 (d, *J* = 284.7 Hz), 154.3, 139.2, 123.0, 118.8, 76.2 (d, *J* = 4.9 Hz), 71.7 (d, *J* = 8.6 Hz), 63.7 (d, *J* = 18.1 Hz), 55.0; ^{19}F NMR (376 MHz, CD_3OD) δ –78.7, –134.3; MS (ESI+) found 298.0950 [calcd for $C_{11}H_{13}FN_5O_4$ $(M+H)^+$ 298.0952]; Anal. Calcd for $C_{11}H_{13}FN_5O_4$: C, 44.45; H, 4.07; N, 23.56. Found: C, 44.12; H, 3.89; N, 23.16.

*4.1.14. (1S,2R,5S)-5-(2,6-Diamino-9H-purin-9-yl)-4-fluoro-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (**3n**).*

[*Amination*] Saturated ammonia in *tert*-butanol (10.0 mL) was added to a solution of **15** (110 mg, 0.18 mmol) in *tert*-butanol (1.0 ml, 0.18 M) in a steel bomb. After heating at 90 ^{o}C for 20 h, the reaction mixture was evaporated. Finally, the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 19/1) to give a diamino intermediate (26 mg, 25 %) as a white foam: $[\alpha]_D^{20} = -2.07$ (*c* 0.08, MeOH); UV (MeOH) λ_{max} 258, 282 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45-7.51 (m, 7 H), 7.19-7.31 (m, 9 H), 5.67 (bs, 2 H), 5.57 (t, *J* = 5.52 Hz, 1 H), 5.27 (s, 1 H), 4.69-4.80 (m, 3 H), 4.00 (d, *J* = 12.4 Hz, 1 H), 3.76 (d, *J* = 11.9 Hz, 1 H), 1.44 (s, 3 H), 1.36 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.8, 155.8, 150.9 (d, *J* = 288.0 Hz), 143.8, 136.7, 128.7, 128.5, 127.9, 127.1, 120.8, 114.8, 112.2, 87.3, 80.7 (d, *J* = 7.2 Hz), 80.3 (d, *J* = 9.3 Hz), 62.0, 56.7, 27.6, 26.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ −129.0; MS (ESI+): found 579.2501 [Calcd for for $C_{33}H_{32}FN_6O_3$ $(M+H)^+$ 579.2520].

[*Deprotection*] A 50% TFA (2.0 mL) was added to a solution of the above generated diamino intermediate (26 mg, 0.045 mmol) in 1,4-dioxane (0.2 mL, 0.2 M), and it was stirred for 20 h at room temperature. Finally, the mixture was evaporated, and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 7/1) to give **3n** (11 mg, 83%) as a white solid: m.p: 219 ^{o}C (decomp.); $[\alpha]_D^{20} = -9.01$ (*c* 0.15, MeOH); UV (MeOH) λ_{max} 257, 283 nm; ^{1}H NMR (600 MHz, CD_3OD) δ 7.80 (s, 1 H), 5.39 (s, 1 H), 4.77 (t, *J* = 4.6 Hz, 1 H), 4.51 (t, *J* = 5.4 Hz, 1 H), 4.40 (d, *J* = 13.2 Hz, 1 H), 4.14-4.18 (m, 1 H); ^{13}C NMR (150 MHz, CD_3OD) δ 161.9, 157.8, 155.6 (d, *J* = 284.4 Hz), 154.1, 139.5, 123.1, 115.1, 76.1 (d, *J* = 5.0 Hz), 71.7 (d, *J* = 8.6 Hz), 63.6 (d, *J* = 18.0 Hz), 55.1; ^{19}F NMR (376 MHz, CD_3OD) δ −78.8, −134.0; MS (ESI+) found 297.1108 [calcd for $C_{11}H_{14}FN_6O_3$ $(M+H)^+$ 297.1111]; Anal. Calcd for $C_{11}H_{14}FN_6O_3$: C, 44.60; H, 4.42; N, 28.37. Found: C, 44.98; H, 4.13; N, 28.19.

*4.1.15. (1S,2R,5S)-5-(2-Amino-6-(cyclopropylamino)-9H-purin-9-yl)-4-fluoro-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (**3o**).*

[*Amination*] Cyclopropylamine (32 μL, 0.46 mmol, 5.0 equiv) and triethylamine (90 μL, 0.644 mmol, 7.0 equiv) were added to a solution of **15** (55 mg, 0.092 mmol, 1.0 equiv) in ethanol (1.0 mL, 0.1 M) in a steel bomb. The reaction mixture was stirred at 60 ^{o}C for 24 h, and was then evaporated. The residue was purified with column chromatography (silica gel, CH_2Cl_2/MeOH = 33/1) to give a cyclopropylamino intermediate (27 mg, 47%) as a white foam: $[\alpha]_D^{25} = -0.94$ (*c* 0.45, MeOH); UV (MeOH) λ_{max} 261, 286 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.15-7.50 (m, 6 H), 7.36 (s, 1 H), 7.20-7.30 (m, 9 H), 5.72 (bs, 1 H), 5.57 (t, *J* = 5.0 Hz, 1 H), 5.27 (s, 1 H), 4.73 (t, *J* = 5.0 Hz, 1 H), 4.68 (bs, 2 H), 3.99 (d, *J* = 11.8 Hz, 1 H), 3.75 (d, *J* = 12.4 Hz, 1 H), 2.97 (bs, 1 H), 1.43 (s, 3 H), 1.36 (s, 3 H), 0.81-0.85 (m, 2 H), 0.56-0.62 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.0, 156.3, 151.1 (d, *J* = 285.8 Hz), 143.7, 135.9, 128.7, 128.5, 127.9, 127.1, 120.7, 115.1, 112.1, 87.3, 80.7 (d, *J* = 5.7 Hz), 80.3 (d, *J* = 9.3 Hz), 61.8 (d, *J* = 20.8 Hz), 56.6, 27.6, 26.1, 22.6, 7.4; ^{19}F NMR (376 MHz, CD_3OD) δ −117.1, −128.9; MS (ESI+) found: 619.2828 [calcd for $C_{36}H_{36}FN_6O_3$ $(M+H)^+$ 619.2833].

[*Deprotection*] A 50% TFA (2 mL) was added to a solution of the cyclopropylamino intermediate (27 mg, 0.044 mmol) generated above, in 1,4-dioxane (0.2 mL, 0.2 M) and it was stirred for 20 h at room temperature. Finally, the mixture was evaporated and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 7/1) to give **3o** (13 mg, 89%) as a white solid: m.p: 57-60 ^{o}C; $[\alpha]_D^{20} = -16.27$ (*c* 0.07, MeOH); UV (MeOH) λ_{max} 260, 287 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (s, 1 H), 5.40 (s, 1 H), 4.77 (t, *J* = 5.0 Hz, 1 H), 4.49 (t, *J* = 5.0 Hz, 1 H), 4.39 (d, *J* = 12.8 Hz, 1 H), 4.14-4.18 (m, 1 H), 2.89 (bs, 1 H), 0.89-0.94 (m, 2 H), 0.67-0.73 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.2, 153.9, 153.3 (d, *J* = 284.4 Hz), 151.4, 138.1, 121.1, 114.2, 113.0, 74.0 (d, *J* = 5.0 Hz), 69.6 (d, *J* = 8.6 Hz), 61.5 (d, *J* = 18.0 Hz), 53.0, 22.9, 6.3; ^{19}F NMR (376 MHz, CD_3OD) δ −78.8, −134.2; MS (ESI+)

found: 337.1421 [calcd for $C_{14}H_{18}FN_6O_3$ $(M+H)^+$ 337.1424]; Anal. Calcd for $C_{14}H_{18}FN_6O_3$: C, 50.00; H, 5.09; N, 24.99. Found: C, 50.23; H, 4.98; N, 25.04.

*4.1.16. ((3aS,4S,6aR)-4-(6-Amino-9H-purin-9-yl)-5-fluoro-2,2-dimethyl-3a,6a-dihydro-4H-cyclopenta[d][1,3]dioxol-6-yl)methanol (**16**).*

cH_2SO_4 (1 drop) was added dropwise to a stirred suspension of **1b** (21 mg, 0.074 mmol) in acetone (40 mL), at 0 °C under N_2, and the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was then neutralized with solid $NaHCO_3$, filtered, and evaporated. Finally, the residue was further purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9/1) to give **16** (21 mg, 92%) as a colorless liquid: ^{1}H NMR (400 MHz, CD_3OD): δ 8.20 (s, 1 H), 8.13 (s, 1 H), 5.62 (br s, 1 H), 5.54 (td, *J* = 1.7, 6.3 Hz, 1 H), 4.85-4.81 (m, merged with signal due to H_2O in CD_3OD, 1 H), 4.43 (d, *J* = 13.3 Hz, 1 H), 4.17 (dt, *J* = 2.2, 13.3 Hz, 1 H), 1.48 (s, 3 H), 1.36 (s, 3 H); HRMS (ESI+) (m/z): found 322.1309 [calcd for $C_{14}H_{17}FN_5O_3$ $(M+H)^+$ 322.1310].

*4.1.17. Tert-butyl (9-((3aS,4S,6aR)-5-fluoro-6-(hydroxymethyl)-2,2-dimethyl-4,6a-dihydro 3aH-cyclopenta[d][1,3]dioxol-4-yl)-9H-purin-6-yl)carbamate (**17**) and ((3aS,4S,6aR)-4-(N6,N6-Bis(tert-Butoxycarbonyl)-6-amino-9H-purin-9-yl)-5-fluoro-2,2-dimethyl-3a,6a-dihydro-4H-cyclopenta[d][1,3]dioxol-6-yl)methanol (**18**).*

Trimethylsilyl trifluoromethanesulfonate (5 μL) was added dropwise to a suspension of **16** (19 mg, 0.059 mmol), hexamethyldisilazane (2 mL), and 4-dimethylaminopyridine (120 mg) and the reaction mixture was heated at 75 °C for 2 h. After concentration, anhydrous THF (10 mL) and di-*t*-butyl dicarbonate (64 mg, 0.29 mmol) were added to the reaction mixture at 0 ^oC under N_2. After stirring at room temperature for 4 h, the reaction mixture was evaporated and methanol/trimethylamine (5:1, total 12 mL) was added to the residue. Then, it was

heated with stirring at 55 °C for 16 h. Finally, the reaction mixture was evaporated, and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 19/1) to give **17** (11.5 mg, 48%) and **18** (6.7 mg, 28%) as colorless liquids.

Compound **17**: ^{1}H NMR (500 MHz, CD_3OD): δ 8.56 (s, 1 H), 8.31 (s, 1 H), 5.69 (br s, 1 H), 5.57 (m, 1 H), 4.89 (m, 1 H), 4.43 (d, *J* = 13.4 Hz, 1 H), 4.18 (d, *J* = 13.4 Hz, 1 H), 3.63 (br s, 1 H), 1.57 (s, 9 H), 1.49 (s, 3 H), 1.36 (s, 3 H); HRMS (ESI+) (m/z): found 422.1839 [calcd for $C_{19}H_{25}FN_5O_5$ $(M+H)^+$ 422.1834].

Compound **18**: ^{1}H NMR (500 MHz, CD_3OD): δ 8.85 (s, 1 H), 8.56 (s, 1 H), 5.78 (br s, 1 H), 5.62 (m, 1 H), 4.96 (m, 1 H), 4.43 (d, *J* = 13.5 Hz, 1 H), 4.19 (d, *J* = 13.5 Hz, 1 H), 1.50 (s, 3 H), 1.38 (s, 18 H), 1.37 (s, 3 H); HRMS (ESI+) (m/z): found 522.2366 [calcd for $C_{24}H_{33}FN_5O_7$ $(M+H)^+$ 522.2359].

*4.1.18. (S)-Isopropyl 2-(((S)-(((3aR,6S,6aS)-6-(6-((tert-butoxycarbonyl)amino)-9H-purin-9-yl)-5-fluoro-2,2-dimethyl-6,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (**19**).*

A *tert*-butylmagnesium chloride solution (0.15 mL, 1.0 M in THF, 0.15 mmol) was added to a cooled (0 ^{o}C) suspension of **17** (8 mg, 0.018 mmol), **18** (5 mg, 0.0095 mmol) and powdered molecular sieves (4 Å, 35 mg) in anhydrous THF (25 mL) under N_2. After 5 min, a solution of pentafluro-phosphoramidate reagent **P** (20 mg, 0.046 mmol) [10] in anhydrous THF (5 mL) was added dropwise and the reaction mixture was stirred at room temperature for 48 h. Next, the reaction mixture was cooled in an ice bath and then quenched by dropwise addition of methanol (10 mL). The reaction mixture was filtered through a Celite and was washed with methanol (50 mL) and evaporated. Finally, the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9/1) to give the phosphoramidate **19** (6.9 mg,

33%) as a colorless liquid: ^{1}H NMR (500 MHz, CD_3OD): δ 8.53 (s, 1 H), 8.33 (s, 1 H), 7.30 (m, 2 H), 7.22-7.13 (m, 3 H), 5.72 (br s, 1H), 5.53 (m, 1 H), 4.91-5.02 (m, 3 H), 4.65 (m, 1 H), 4.40 (br s, 1 H), 3.90-3.98 (m, 1 H), 1.58 (s, 9 H), 1.52 (s, 3 H), 1.32-1.38 (m, 6 H), 1.20-1.26 (m, 6 H); HRMS (ESI+) found 691.2671 [calcd for $C_{31}H_{41}FN_6O_9P$ $(M+H)^+$ 691.2651].

*4.1.19. (S)-Isopropyl 2-(((S)-(((3S,4S,5R)-3-(6-amino-9H-purin-9-yl)-2-fluoro-4,5-dihydroxycyclopent-1-en-1-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (**3p**).*

A solution of **19** (4 mg, 0.005 mmol) in 5 mL of formic acid/H_2O (1:1, 2 mL) was stirred at room temperature for 8 h. The reaction mixture was evaporated at 25 °C, and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 6/1) followed by lyophilization (H_2O/acetonitrile = 3:1, 3 mL) to give **3p** (2.7 mg, 85%) as a white solid: UV (MeOH) λ_{max} 259.5 nm; ^{1}H NMR (500 MHz, CD_3OD): δ 8.23 (s, 1 H), 8.19 (s, 1 H), 7.40 (m, 2 H), 7.24 (d, *J* = 8.5 Hz, 2 H), 7.18 (m, 1 H), 5.62 (br s, 1 H), 4.92-5.01 (m, 2 H), 4.75-4.80 (m, 1H), 4.70 (m, 1H), 4.56 (m, 1 H), 3.90-3.99 (m, 1 H), 1.35 (d, *J* = 7.0 Hz, 3 H), 1.22 (d, *J* = 6.3 Hz, 6 H); HRMS (ESI+) (m/z): found 551.1799 [calcd for $C_{23}H_{29}FN_6O_7P$ $(M+H)^+$ 551.1814]; Anal. Calcd for $C_{23}H_{28}FN_6O_7P$: C, 50.18; H, 5.13; N, 15.27; Found: C, 50.15; H, 5.11; N, 15.23.

4.2. Biology

4.2.1. Cell culture

The human lung cancer (A549), colon cancer (HCT-116), stomach cancer (SNU-638), breast cancer (MDA-MB-231), liver cancer (SK-Hep-1), and prostate cancer (PC-3) cell lines were provided by the Korean Cell Line Bank (Seoul, Korea). The cells were grown in medium (DMEM for the MDA-MB-231 and SK-Hep-1 cells and RPMI 1640 medium for the A549, HCT-116, SNU-638 and PC-3 cells) supplemented with 10% fetal bovine serum (FBS)

and antibiotics−antimycotics (PSF: 100 units/mL penicillin G sodium, 100 μg/mL streptomycin, and 250 ng/mL amphotericin B). All cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

4.2.2. Cytotoxicity assay (sulforhodamine B assay)[12,13]

The anticancer activity of the test compounds against various human cancer cells was determined using the sulforhodamine B (SRB) assay. The cells were seeded in 96-well plates at a density of 5×10^4 cells/mL and were treated with various concentrations of the test compounds for 72 h. the cells were fixed with 10% trichloroacetic acid solution, washed with tap water, and dried in air. The cells were stained with 0.4% SRB in 1% acetic acid solution. After washing the unbound dye and drying, the stained cells were dissolved in 10 mM Tris (pH 10.0), and the absorbance was measured at 515 nm. Cell viability was calculated by comparison with the absorbance of the vehicle-treated control group. The IC_{50} values were determined by nonlinear regression analysis using TableCurve software.

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· Eighteen 6'-fluorocyclopentenyl-pyrimidines and -purines were designed and synthesized.

· The mechanism of action of the cytosine analog **2b** is related to the inhibition of DNA/RNA polymerase.

· The mechanism of action of the adenosine analogs, **1b** and **3k** is related to the inhibition of SAH hydrolase, associated with the inhibition of histone methyltransferase.