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Design, Synthesis, and Anti-RNA Virus Activity of 6′-Fluorinated-Aristeromycin Analogues

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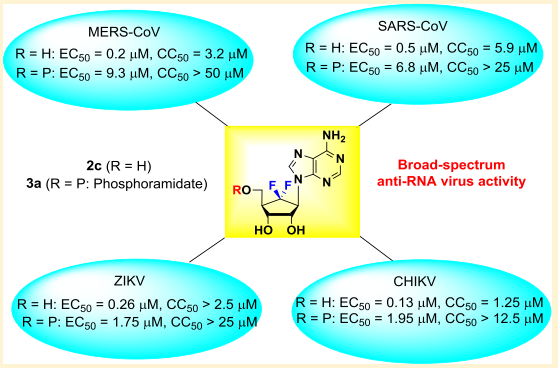
Supporting Information

ABSTRACT: The 6′-fluorinated aristeromycins were designed as dual-target antiviral compounds aimed at inhibiting both the viral RNA-dependent RNA polymerase (RdRp) and the host cell *S*-adenosyl-L-homocysteine (SAH) hydrolase, which would indirectly target capping of viral RNA. The introduction of a fluorine at the 6′-position enhanced the inhibition of SAH hydrolase and the activity against RNA viruses. The adenosine and N^6-methyladenosine analogues **2a−e** showed potent inhibition against SAH hydrolase, while only the adenosine derivatives **2a−c** exhibited potent antiviral activity against all tested RNA viruses such as Middle East respiratory syndrome-coronavirus (MERS-CoV), severe acute respiratory syndrome-coronavirus, chikungunya virus, and/or Zika virus. 6′,6′-Difluoroaristeromycin (**2c**) showed the strongest antiviral effect for MERS-CoV, with a ∼2.5 log reduction in infectious progeny titer in viral load reduction assay. The phosphoramidate prodrug **3a** also demonstrated potent broad-spectrum antiviral activity, possibly by inhibiting the viral RdRp. This study shows that 6′-fluorinated aristeromycins can serve as starting points for the development of broad-spectrum antiviral agents that target RNA viruses.



INTRODUCTION

Over the past 15 years, outbreaks of a number of emerging positive-stranded RNA (+RNA) viruses,[1] such as the severe acute respiratory syndrome coronavirus (SARS-CoV),[2] Middle East respiratory syndrome coronavirus (MERS-CoV),[3] chikungunya virus (CHIKV),[4] and Zika virus (ZIKV)[5] have seriously threatened human health and have had a substantial socio-economic impact. SARS-CoV and MERS-CoV cause serious respiratory diseases[6] that can be fatal in approximately 10 and 35% of cases, respectively. CHIKV is transmitted by mosquitoes and causes a painful arthritis that can persist for months.[7] ZIKV is also transmitted by mosquitoes,[8] although sexual transmission[8] occurs as well. This virus usually causes mild disease, but can cause neurological complications in adults and fetal death or severe complications, including microcephaly in infants when women are infected during pregnancy.[9] CHIKV and ZIKV have caused massive outbreaks, totaling millions of infections over the past decade. Currently, there are no effective chemotherapeutic agents or vaccines that can prevent or cure infections of any of these four serious pathogens.

The aforementioned viruses belong to the +RNA virus group (Baltimore class IV),[1] which indicates that their genomic RNA has the same polarity as mRNA and can be directly translated by host ribosomes upon release into the cytoplasm of a host cell. After infection, the genomes of these viruses are translated into polyproteins that are subsequently cleaved into individual proteins by viral and/or host proteases. The nonstructural proteins (nsps) of these viruses harbor a variety of enzymatic activities that are required for the replication of the viral RNA and invariably include a RNA-dependent RNA polymerase (RdRp),[10] an enzyme which is not present in uninfected cells. The RdRp transcribes the genomic RNA into a complementary

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negative-stranded RNA that subsequently serves as the template for the synthesis of new positive-stranded RNA.

Many +RNA viruses (including coronaviruses, CHIKV, and ZIKV) also encode methyltransferases (MTases)[11] that are required for methylations of viral mRNA cap structures.[12] Because this capping is crucial for stability and translation of the viral RNA, and evasion of the host innate immune response, the viral MTases are considered promising targets for the development of antiviral therapy.[12] Inhibition of MTases can be indirectly achieved by the inhibition of *S*-adenosyl-L-homocysteine (SAH) hydrolase.[13] The SAH hydrolase catalyzes the interconversion of SAH into adenosine and L-homocysteine. Inhibition of this enzyme leads to the accumulation of SAH in the cell, which in turn inhibits *S*-adenosyl-L-methionine (SAM)-dependent transmethylase reactions by feedback inhibition.[13,14] Most of the viral MTases are dependent on SAM as the only methyl donor. Compounds that target cellular proteins might exhibit a broader spectrum of activity, are less likely to lead to drug-resistance, but have a higher likelihood of toxicity. Compounds that are specifically aimed at viral proteins are expected to be less cytotoxic, but might have a narrower spectrum of antiviral activity and might have a lower barrier antiviral drug-resistance[14] Thus, the approach of targeting cellular proteins such as SAH hydrolase can be considered as a promising strategy for the development of broad-spectrum antiviral agents.[14] A number of compounds have been reported to act as SAH hydrolase inhibitors.[14] Type I inhibitors act through inactivation of the NAD^+ cofactor, and their inhibitory effect on the catalytic activity of the enzyme can be reversed by the addition of excess NAD^+.[14] Type II inhibitors are irreversible inhibitors of the SAH hydrolase that form covalent bonds with amino acid residues in the active site of the enzyme. This irreversible inhibition cannot be reversed by the addition of NAD^+ or adenosine or by dialysis.[14]

Because both the viral RdRp and host SAH hydrolase are critical for virus replication, we aimed to design broad-spectrum nucleoside analogue inhibitors that could directly target RdRp activity and/or indirectly inhibit the methylation of viral RNA through their effect on the host SAH hydrolase. Modified nucleosides are usually taken up by the cell via nucleoside transporters and can be successively converted into mono-, di-, and triphosphates by cellular kinases.[15] Then, these modified nucleoside triphosphates (NTPs) can compete with natural NTPs during RNA synthesis or can be incorporated into the nascent viral RNA, leading to chain termination or detrimental mutations.[15]

(−)-Aristeromycin (**1**) is a naturally occurring carbocyclic nucleoside that was originally identified as a metabolite of *Streptomyces citricolor* in 1967.[16a] The first synthesis of **1** as racemate was reported by Clayton and his co-worker,[16b−d] and its asymmetric syntheses have since been reported.[16e−h] It is a type I SAH hydrolase inhibitor and exhibits potent antiviral activity against many viruses.[14a] However, it could not be further advanced into clinical development because of its cytotoxicity.[17] Compound **1** was found to be toxic at low concentrations in both adenosine kinase-positive (AK^+) and AK^- cells. AK^+ cells were presumably killed by the 5′-phosphorylated form of **1**, while the toxicity in AK^- cells was caused by **1** itself.[17] However, this compound is also metabolized into a triphosphate form and has been observed to exert a variety of metabolic effects.[17] We aimed to use **1** as a prototype for the design of dual-target compounds intended at directly inhibiting the viral RdRp and indirectly inhibiting the capping process through targeting of cellular SAH hydrolase.

Since the introduction of a fluorine at the 6′-position of carbocyclic nucleosides has been known to affect biological activities to a significant extent,[18] we aimed to synthesize the 6′-fluorinated-aristeromycin analogues **2** by introducing fluorine at the 6′-position of **1** (Figure 1). Prisbe and his co-workers[18a] have

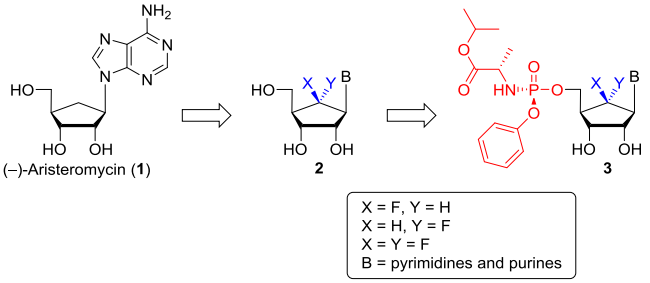

Figure 1. Rationale for the design of the target nucleosides **2** and **3**.

reported the synthesis of (±)-6′-*α*- and (±)-6′-*β*-fluorinated aristeromycins and their inhibitory activity on SAH hydrolase, but the synthesis and biological activity of (±)-6′,6′-difluoroaristeromycin was not reported, despite the fact that the structure was claimed in the patent.[18b] Thus, we set out to synthesize the 6′-fluorinated-aristeromycin analogues **2** in the optically pure D-forms because biological activity can generally be attributed to one enantiomer, the D-isomer. Yin and co-workers[18c] reported the elegant synthesis of optically pure (−)-6′-*β*-fluoro-aristeromycin, but its biological activity was not reported. Their synthetic route involved the 6-*β*-fluoroazide as the key intermediate, which was synthesized by employing S_N2 fluorination of the 6-*α*-triflic azide with tris(dimethylamino)-sulfur(trimethylsilyl)difluoride, whereas our current approach[19] included the stereoselective electrophilic fluorination of silyl enol ether with Selectfluor as the fluorine source. In addition to the adenosine analogues, aimed at inhibiting SAH hydrolase and/or RdRp, we have also synthesized 6′-fluorinated purine and pyrimidine nucleosides (changes in B of the structures in Figure 1), which could interfere with viral RNA synthesis by targeting the viral RdRp after their phosphorylation by cellular kinases.[15] To bypass the first and rate-limiting 5′-mono-phosphorylation step, we have also synthesized a phosphoramidate prodrug **3** of nucleoside **2**, using the McGuigan ProTides.[20] Herein, we report the synthesis of the 6′-fluoro-aristeromycin analogues **2** and **3** and a preliminary characterization of their effect on several +RNA viruses, which provided insight into structure−activity relationships (SARs).

RESULTS AND DISCUSSION

Chemistry. For the synthesis of the target nucleosides **2**, the key fluorosugars **8a−c** were synthesized from D-ribose via electrophilic fluorination, as shown in Scheme 1.

D-Ribose was converted to D-cyclopentenone **4** according to our previously published procedure.[21] The 1,4-conjugated addition of **4** with Gilman reagent yielded the D-cyclopentanone derivative **5**.[19,22] Treatment of **5** with lithium hexamethyldisilazide (LiHMDS) followed by trapping with triethylsilyl chloride (TESCl) gave silylenol ether **6**, which was treated with (1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]-octane bis(tetrafluoroborate): Selectfluor) in dimethylformamide (DMF) at 0 °C to yield a 5:1 ratio of 6-*β*-fluorosugar **7a** to

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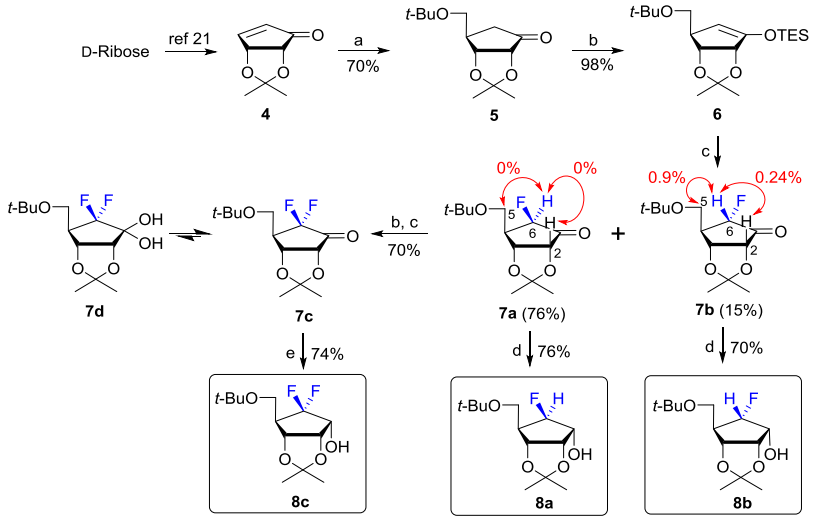
Scheme 1. Synthesis of 6-β-Fluoro-, 6-α-Fluoro-, and 6-Difluorosugar 8a−c[a]

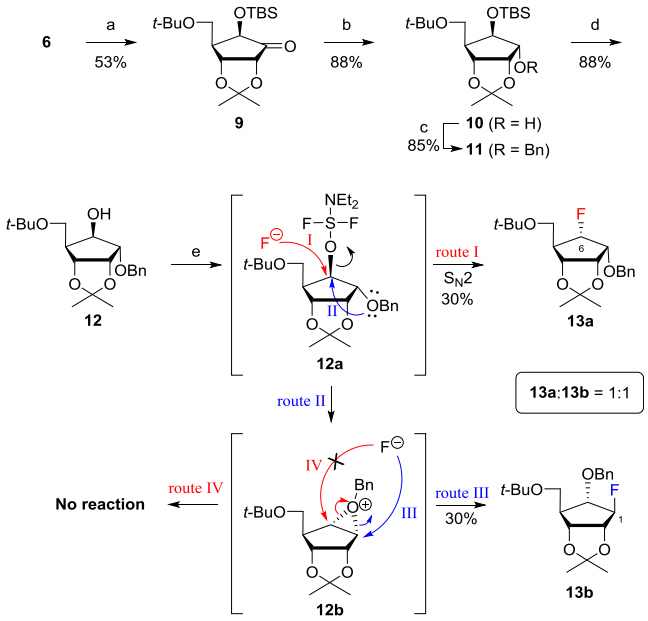


[a]Reagents and conditions: (a) $LiCu(CH_2Ot\text{-}Bu)_2$; (b) TESCl, LiHMDS, THF, −78 °C, 10 min; (c) Selectfluor, DMF, 0 °C, 12 h; (d) $NaBH_4$, MeOH, 0 °C, 30 min. (e) $LiBH_4$, MeOH, 0 °C, 30 min.

6-α-fluorosugar **7b**.[19] The stereochemistry of the fluorine in **7a** and **7b** was confirmed by ^{1}H NOE experiments. Irradiation of 6-H of **7b** gave NOE effects on its 2-H and 5-H, indicating the 6-α-fluoro configuration, but no NOE effects were observed on the same experiment in the case of **7a**, confirming the 6-β-fluoro configuration. The configuration of the fluorine in **7b** was further confirmed by the X-ray crystal structure obtained after it was converted to the final uracil derivative **2g** (Scheme 5). Further electrophilic fluorination of 6-β-fluorosugar **7a** or 6-α-fluorosugar **7b** under the same conditions yielded the 6,6-difluorosugar **7c**, which was equilibrated to form a geminal diol because of the presence of electronegative fluorine atoms. Electrophilic fluorinations with other electrophilic fluorines such as *N*-fluorobenzenesulfonimide (NFSI) or *N*-fluoro-*O*-benzenedisulfonimide (NFOBS) were problematic, resulting in low yields with many side spots. The reduction of **7a−c** with sodium borohydride ($NaBH_4$) or lithium borohydride ($LiBH_4$) in MeOH resulted in the production of the 1-hydroxyl derivatives **8a−c**.

As the α-fluoro derivative **8b** was obtained as the minor isomer, as shown in Scheme 1, we wanted to improve the stereoselective synthesis of **8b**, by using Rubottom[23] oxidation as the key step, as illustrated in Scheme 2. Rubottom oxidation of silylenol ether **6** with osmium tetroxide (OsO_4) and *N*-methylmorpholine-*N*-oxide (NMO) followed by trapping with *t*-butyldimethylsilyl chloride (TBSCl) produced 6-β-alkoxyketone **9** as a single stereoisomer in 53% yield. The reduction of ketone **9** with $NaBH_4$ gave alcohol **10**, which was protected with a benzyl group to give **11**. Removal of the *t*-butyldimethylsilyl (TBS) group in **11** with tetra-*n*-butylammonium fluoride (TBAF) yielded the 6-β-alcohol **12**. To our disappointment, the treatment of **12** with *N*,*N*-diethylaminosulfur trifluoride (DAST) gave the desired product, 6-α-fluoride **13a**, but also the undesired product 1-β-fluoride **13b** at a 1:1 ratio. The formation of **13a** (route I) resulted from the direct S_N2 reaction of **12a** with fluoride, while **12a** was readily converted into the oxonium ion **12b** (route II) via its participation of the neighboring benzyl group, which was attacked exclusively by the fluoride at the less sterically hindered 1-position to yield the undesired product **13b**

Scheme 2. Synthetic Approach to 6-α-Fluorosugar 8b via Rubottom Oxidation[a]



[a]Reagents and conditions: (a) (i) OsO_4, NMO·H_2O, THF, rt, 1 h, then $NaHCO_3$, MeOH, rt, 3 h; (ii) TBSCl, imidazole, DMF, rt, 3 h; (b) $NaBH_4$, MeOH, rt, 1 h; (c) BnBr, NaH, DMF, 0 °C to rt, 12 h; (d) TBAF, THF, rt, 12 h; (e) DAST, toluene, 0 °C to rt, 2 h.

(route III). However, the product via route IV was not formed because of the steric effect of the *t*-butyloxymethyl substituent.

To avoid the participation of the neighboring group, we considered using a cyclic sulfate substrate with electron-withdrawing property and conformational restraint to be the best choice. Furthermore, cyclic sulfate has the advantage that it can be utilized as a surrogate for epoxide during nucleobase condensation, as shown in Scheme 3. The regioselective cleavage of the 2,3-acetonide in **10** with trimethylaluminum ($AlMe_3$) followed by treatment of the resulting diol with thionyl chloride ($SOCl_2$) yielded the 6-β-hydroxyl cyclic sulfite **14** after

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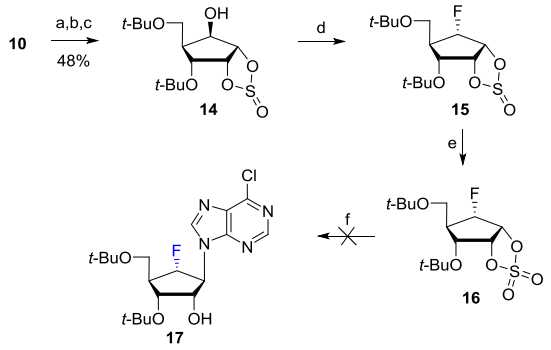
J. Med. Chem. 2019, 62, 6346−6362

Scheme 3. Synthetic Approach to 2b via Cyclic Sulfate[a]



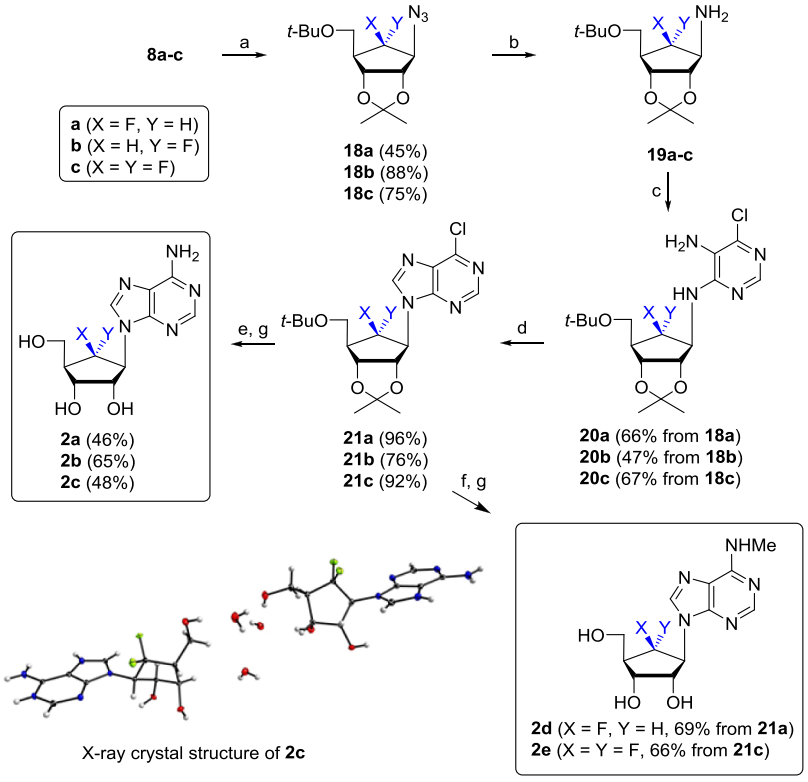
[a]Reagents and conditions: (a) $AlMe_3$, CH_2Cl_2, −78 °C to rt, 12 h; (b) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C, 10 min; (c) TBAF, AcOH, THF, rt, 12 h; (d) DAST, CH_2Cl_2, 0 °C to rt, 4 h; (e) $RuCl_3$, $NaIO_4$, CCl_4/CH_3CN/H_2O (1/1/1.5), rt, 20 min; (f) (i) 6-chloropurine, 18-crown-6, NaH, THF, 65 °C, 15 h; (ii) 20% H_2SO_4, rt, 1 h.

the removal of the TBS group. The treatment of **14** with DAST yielded the desired 6-α-fluoro cyclic sulfite **15** as a single stereoisomer. The cyclic sulfite **15** was oxidized to form cyclic sulfate **16**, which was subsequently condensed with 6-chloropurine anions; however, this resulted in decomposition.[19] Thus, we decided to synthesize the 6-α-fluoro derivative **8b** according to Scheme 1.

Scheme 4 depicts the synthesis of the aristeromycin analogues **2a−e** from the 6-β-fluoro-, 6-α-fluoro-, and 6,6-difluorosugars **8a−c**.[19] Compounds **8a−c** were treated with triflic anhydride (Tf_2O) followed by treatment with sodium azide to give azido derivatives **18a−c**. The catalytic hydrogenation of **18a−c** yielded the amino derivatives **19a−c**, respectively, which are starting compounds for the base-building process. The treatment of **19a−c** with 5-amino-4,6-dichloropyrimidine[18a−c,24] in the presence of *N*,*N*-diisopropylethylamine (DIPEA) under microwave radiation conditions yielded **20a−c**, which were cyclized with diethoxymethyl acetate[18a−c,24] in the presence of microwave radiation to produce the 6-chloropurine derivatives **21a−c**. The treatment of **21a−c** with *t*-butanolic ammonia followed by the removal of protective groups under acidic conditions yielded the 6′-β-fluoro-, 6′-α-fluoro-, and 6′,6′-difluoroaristeromycins **2a−c**, respectively. The structure of compound **2c** was confirmed by a single-crystal X-ray analysis (see the Supporting Information).[25] The treatment of **21a** and **21c** with 40% aqueous methylamine followed by aqueous trifluoroacetic acid (TFA) resulted in N^6-methyl-aristeromycin analogues **2d** and **2e**, respectively.

The amino derivatives **19a−c** were also converted into the pyrimidine nucleoside derivatives **2f−j**, as shown in Scheme 5. Treatment of **19a−c** with (*E*)-3-methoxy-2-propenoyl isocyanate, which was prepared by reacting 3-methoxyacryloyl chloride with silver cyanate,[26] in benzene produced **22a−c**, respectively, which were cyclized with 2 M H_2SO_4 to yield the uridine derivatives **2f−h**, respectively. The structures of **2g** and **2h** were confirmed by the X-ray crystallography (see the Supporting Information) (Scheme 5).[27] To synthesize the cytidine derivatives **2i** and **2j**, compounds **2f** and **2h** were benzoylated to give **23a** and **23b**, respectively, which were

Scheme 4. Synthesis of β-Fluoro-, α-Fluoro-, and Difluoro-Aristeromycin Analogues 2a−e[a]



[a]Reagents and conditions: (a) (i) Tf_2O, pyridine, 0 °C, 30 min; (ii) NaN_3, DMF, 60−100 °C, 4−15 h; (b) Pd/C, H_2, MeOH, rt, 18 h; (c) 5-amino-4,6-dichloropyrimidine, DIPEA, *n*-BuOH, 170−200 °C, 4−7 h, MW; (d) $CH_3C(O)OCH(OEt)_2$, 140 °C, 3 h, MW; (e) NH_3/*t*-BuOH, 120 °C, 15 h; (f) NH_2Me/H_2O, (40 wt %), EtOH, 30 °C, 2 h; (g) 67% aq TFA, 50 °C, 15 h.

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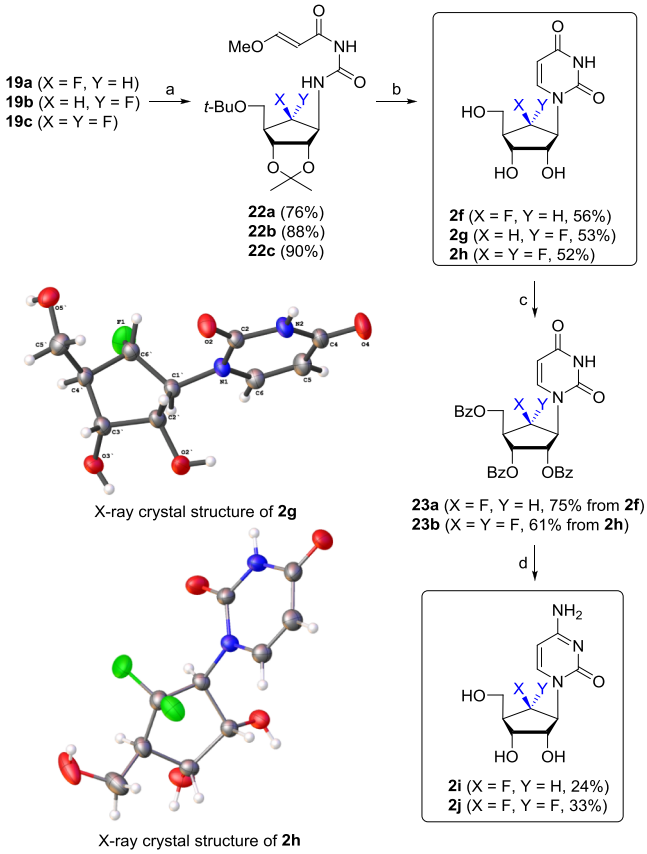
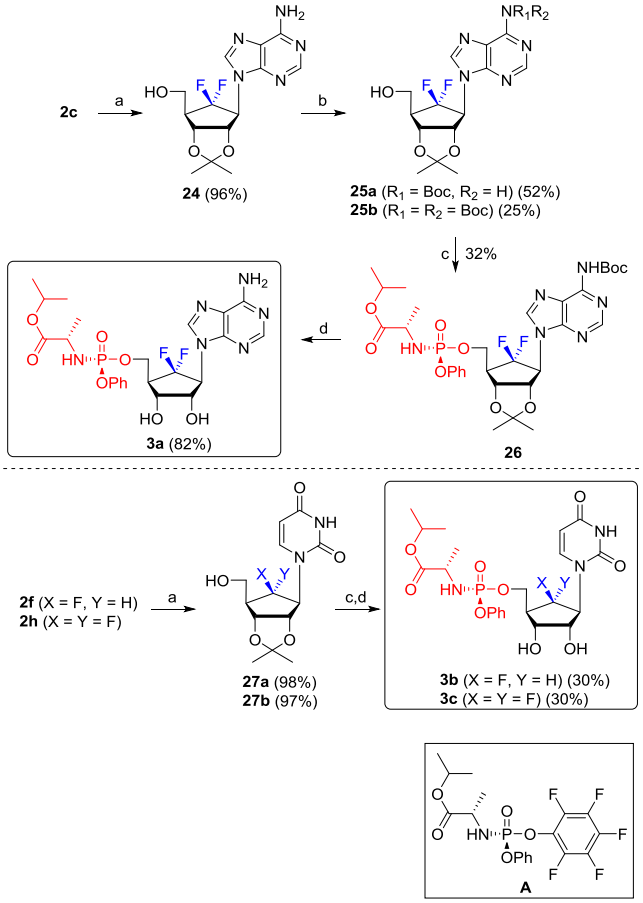
Scheme 5. Synthesis of Fluorinated Pyrimidine Nucleoside Analogues 2f–j[a]



[a]Reagents and conditions: (a) (*E*)-3-methoxy-2-propenoyl isocyanate, benzene, 4 Å-MS, DMF, −20 °C to rt, 15 h; (b) 2 M H_2SO_4, dioxane, reflux, 1.5 h; (c) BzCl, pyridine, CH_2Cl_2, rt, 15 h; (d) (i) 1,2,4-triazole, $POCl_3$, Et_3N, CH_3CN, rt, 15 h. (ii) NH_4OH, dioxane, rt, 15 h. (iii) NH_3/MeOH, rt, 15 h.

Scheme 6. Synthesis of Phosphoramidate Prodrugs 3a–c[a]



[a]Reagents and conditions: (a) cH_2SO_4, acetone, rt, 4 h; (b) (i) TMSOTf, DMAP, HMDS, 75 °C, 2 h; (ii) Boc_2O, THF, rt, 4 h; (iii) MeOH/Et_3N (5:1), 55 °C, 16 h; (c) **A**, *t*-BuMgCl, 4 Å-MS, THF, 0 °C to rt, 36 h; (d) 50% HCOOH, rt, 8 h.

converted to the cytidine derivatives **2i** and **2j** using conventional three-step procedures.[28]

The uracil phosphoramidate analogue Sofosbuvir[20] is used in the clinic as a powerful anti-hepatitis C virus agent. Therefore, we have also synthesized the uracil phosphoramidate prodrugs **3b–c** and the adenine phosphoramidate prodrug **3a** derived from the purine and pyrimidine nucleoside analogues **2a–j** by using McGuigan's ProTide prodrug methodology,[20] as shown in Scheme 6. 6′,6′-Difluoro-aristeromycin (**2c**) was treated with acetone under acidic conditions to give 2,3-acetonide **24**. The treatment of **24** with di-*tert*-butyl dicarbonate (Boc_2O) yielded a mixture of **25a** and **25b** in a 2:1 ratio, which was converted to the phosphoramidate prodrug **26** by treating with phosphoramiditing reagent (**A**)[29] in the presence of *t*-butylmagnesium chloride. The treatment of **26** with 50% formic acid produced the final product, prodrug **3a**. The monofluoro- and difluoropyrimidine derivatives **2f** and **2h** were similarly converted to the final prodrugs **3b** and **3c**.

Inhibition of SAH Hydrolase. All compounds **1**, **2a–j**, and **3a–c** were assayed for their ability to inhibit recombinant human SAH hydrolase protein, expressed in *Escherichia coli* JM109, using a 5,5′-dithiobis-2-nitrobenzoate (DTNB) coupled assay as described by Lozada-Ramírez et al.[30] As expected, all adenosine derivatives **2a–e** potently inhibited SAH hydrolase, but none of the pyrimidine analogues **2f–j** showed any inhibitory activity at concentrations up to 100 μM. None of the prodrugs **3a–c** exhibited inhibitory activity at concentrations up to 100 μM. This result is not surprising because adenosine is the substrate for SAH hydrolase. Among the adenosine analogues, 6′-β-fluoroaristeromycin (**2a**) exhibited the most potent inhibitory activity (IC_{50} = 0.37 μM), which was 3.6-fold more potent than the control **1** (IC_{50} = 1.32 μM). However, 6′-α-fluoroaristeromycin (**2b**, IC_{50} = 9.70 μM) was 26-fold less potent than the corresponding 6′-β-fluoro analogue **2a** and 7.4-fold less active than the 6′-unsubstituted compound **1**. This indicates that the stereochemistry at the 6′-position is important for inhibitory activity. Interestingly, the introduction of two fluorines at the 6′-position resulted in **2c** (IC_{50} = 1.06 μM), which was slightly more potent than the control **1**. The inhibitory activity of the 6′-fluoro-aristeromycin series can be ranked in the following order: 6′-β-F > 6′,6′-F,F > 6′-H > 6′-α-F. The introduction of a methyl group at the N^6-amino group of **2a**, resulting in **2d**, decreased the inhibitory activity (IC_{50} = 4.39 μM) by 11.9-fold, while the addition of a methyl group to the N^6-amino group of **2c**, resulting in **2e**, increased the inhibitory activity (IC_{50} = 0.76 μM) by 1.7-fold. These results demonstrate that the N^6-methyladenine and the adenine moieties do not lead to a decrease in inhibitory activity.

Antiviral Activity. The novel 6′-fluoro-aristeromycin analogues **2a–j** and **3a–c** were screened for antiviral activity

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Table 1. Inhibition of SAH Hydrolase and the Replication of Several +RNA Viruses by All Final Nucleoside Analogues 2a−j and 3a−c[a,b,c,d]

compound no.	SAH hydrolase IC_{50} (μM)	MERS-CoV			SARS-CoV			ZIKV			CHIKV		
		EC_{50} (μM)	CC_{50} (μM)	SI	EC_{50} (μM)	CC_{50} (μM)	SI	EC_{50} (μM)	CC_{50} (μM)	SI	EC_{50} (μM)	CC_{50} (μM)	SI
1	1.32	>50	2		>50	>5		0.64	2.4	3.8	0.8	6.3	7.9
2a	0.37	0.20	0.60	3	ND	ND		ND	ND		>100	>100	
2b	9.70	ND	ND		ND	ND		2.54	3.97	1.56	0.53	1.32	2.49
2c	1.06	0.2	3.2	16	0.5	5.9	11.8	0.26	>2.5	>9.6	0.13	>1.25	>9.6
2d	4.39	>50	>50		>100	>100		>100	>100		>100	>100	
2e	0.76	>50	12.5		>100	>100		>100	>100		>100	>100	
2f	>100	>100	>100		>100	>100		>100	>100		>100	>100	
2g	>100	>100	>100		>100	>100		>100	>100		>100	>100	
2h	>100	>50	>50		>100	>100		>100	>100		>100	>100	
2i	>100	>100	>100		>100	>100		>100	>100		>100	>100	
2j	>100	>50	>50		>100	>100		>100	>100		>100	>100	
3a	>100	9.3	>50		6.8	>25	>3.7	1.75	>25	>14.3	1.95	>12.5	>6.4
3b	>100	>50	>50		>100	>100		>100	>100		>100	>100	
3c	>100	>50	>50		>100	>100		>100	>100		>100	>100	

[a]ND: not determined; SI = CC_{50}/EC_{50}. [b]EC_{50}: effective concentration to inhibit the replication of the virus by 50%. [c]CC_{50}: cytotoxic concentration to inhibit the replication of normal cells by 50%. [d]EC_{50} > 100 indicates that no antiviral activity was observed at the highest concentration tested because either there was no protection or the compound was toxic.

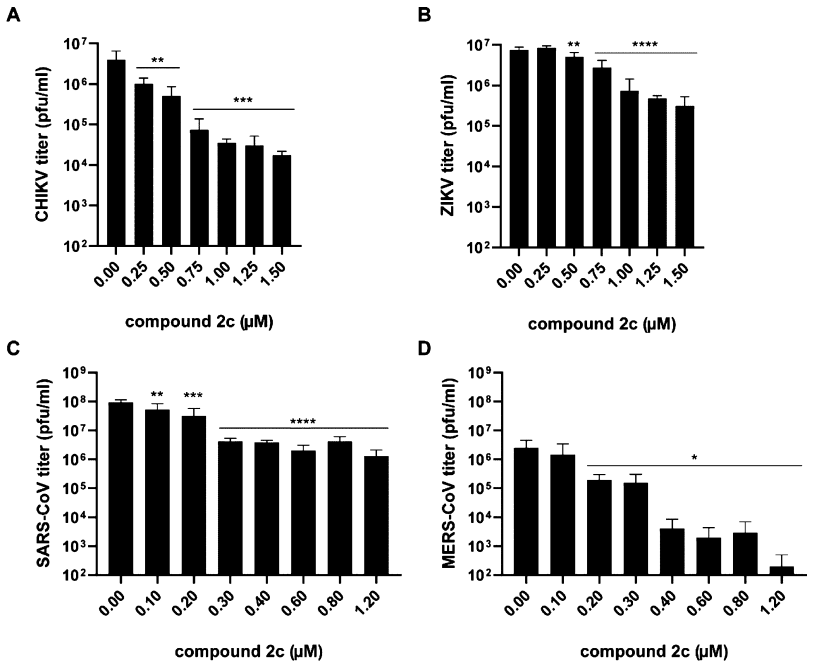

Figure 2. Effect of **2c** on the infectious progeny of CHIKV, ZIKV, SARS-CoV, and MERS-CoV. Cells were infected with the virus indicated on the y-axis of the graph in medium with various concentrations of **2c**. Infectious progeny titers were determined by plaque assay (n = 4) and viability of noninfected cells was monitored using the CellTiter 96AQueous Non-Radioactive Cell Proliferation Assay (Promega). Significant differences are indicated by *: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

against a variety of +RNA viruses. The compounds were tested for antiviral activity in cytopathic effect (CPE) reduction assays at 4 concentrations, that is, 150, 50, 16.7, and 5.6 μM by preparing 3-fold serial dilutions. Compounds that demonstrated antiviral activity in this primary screen were further tested more extensively in dose response experiments at 8 different concentrations to determine the EC_{50}. Cytotoxicity (CC_{50}) was determined in parallel in uninfected cells (Table 1).

As shown in Table 1, only the adenosine derivatives **2a−c** exhibited potent antiviral activities against +RNA viruses, while the other purine N^6-methyladenine derivatives **2d** and **2e** and pyrimidine derivatives **2f−j** did not show significant antiviral activities, not even at 100 μM. This result suggests that the antiviral activity might be due to an (indirect) effect on viral MTase activity through the inhibition of host SAH hydrolase. Inhibition of the viral RdRp appears not to be important. The mechanism of action of these compounds has been studied in more detail and results will be published elsewhere.

Compound **2a** inhibited MERS-CoV replication with an EC_{50} of 0.20 μM; however, it was also rather cytotoxic, resulting in a selectivity index (SI) of 3. Replacement of the remaining 6′-H in **2a** with F resulted in compound **2c**, which exhibited a > 5-fold

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J. Med. Chem. 2019, 62, 6346−6362

reduction in cytotoxicity, while its antiviral activity remained unchanged, with an EC_{50} of ~0.20 μM and an SI of 15 for MERS-CoV. This compound was also active against SARS-CoV with an SI of 12.5, suggesting that it may be a broad-spectrum coronavirus inhibitor. In addition, it also inhibited ZIKV replication with an EC_{50} of 0.26 μM (SI > 10) and was active against CHIKV with an EC_{50} of 0.13 μM. Compound **2b** showed some inhibitory effects on CHIKV and ZIKV replication, but this was likely due to pleiotropic cytotoxic effects, as the SI was <3. Among the phosphoramidate prodrugs **3a–c**, only the adenosine prodrug **3a** exhibited significant broad-spectrum antiviral activities, demonstrating that it may inhibit the RdRp of RNA viruses after conversion into the triphosphate form, although it remains to be determined in biochemical assays whether the triphosphate form affects RdRp activity.[20] Compound **3a** had an EC_{50} of 9.3 μM for MERS-CoV and 6.8 μM for SARS-CoV, but it also had an SI < 10, and it was therefore not considered a potent inhibitor of coronavirus replication. However, for CHIKV and ZIKV, **3a** had EC_{50} values of 1.95 and 1.75 μM, respectively, with good selectivity indices. Interestingly, the prodrug **3a** was less potent, but also much less cytotoxic than the parent compound **2c**, which is unusual as regularly the phosphoramidate is more potent than the parent drug.[20] The phosphoramidate **3a** might be slowly hydrolyzed to the 5′-monophosphate by metabolic enzymes, or to the parent drug **2c** by a phosphatase, which could inhibit SAH hydrolase, explaining the observed antiviral effect. Viral load reduction assays were performed with compound **2c** by infecting cells with CHIKV, ZIKV, SARS-CoV, and MERS-CoV, followed by treatment with different concentrations of **2c**. At 30 hpi (CHIKV) or 48 hpi (ZIKV, SARS- and MERS-CoV), infectious progeny titers in the medium were determined by plaque assay (Figure 2). Treatment with concentrations higher than 1 μM of **2c** reduced infectious CHIKV titers by more than 2 log. The effect on ZIKV infectious progeny titers was limited and showed a ~1 log reduction. For SARS-CoV, the reduction in infectious progeny titer was ~1.5 log at 2c concentrations above 0.3 μM. The strongest antiviral effect was observed for MERS-CoV, with a ~2.5 log reduction in infectious progeny titers when infected cells were treated with 2c concentrations above 0.3 μM. Follow-up studies to gain more insights into the mode of action of **2c** and **3a** and related compounds are currently ongoing, and results will be published elsewhere.

Finally, we measured the log *P* of the most active compound **2c** by the pH-metric method, using a T3 Sirius instrument, because the lipophilicity is a major determinant for compound absorption, distribution in the body, penetration across biological barriers, metabolism, and excretion. The measured log *P* was 0.02, indicating that it is almost equally partitioned between the lipid and aqueous phases. The relatively low log *P* of **2c** is expected to be overcome by converting it to the phosphoramidate **3a**.

CONCLUSIONS

We have synthesized the 6′-fluorinated aristeromycin analogues **2a–j**, which were designed as dual-target antiviral compounds aimed at inhibiting both the viral RdRp and the host SAH hydrolase. The electrophilic fluorination of silyl enol ether with Selectfluor was the key step in the synthesis. We have also synthesized the phosphoramidate prodrugs **3a–c** to determine whether these would inhibit virus replication through an effect on the viral RNA polymerase. Figure 3 depicts the summarized SAR of the synthesized 6′-fluorinated final nucleoside analogues

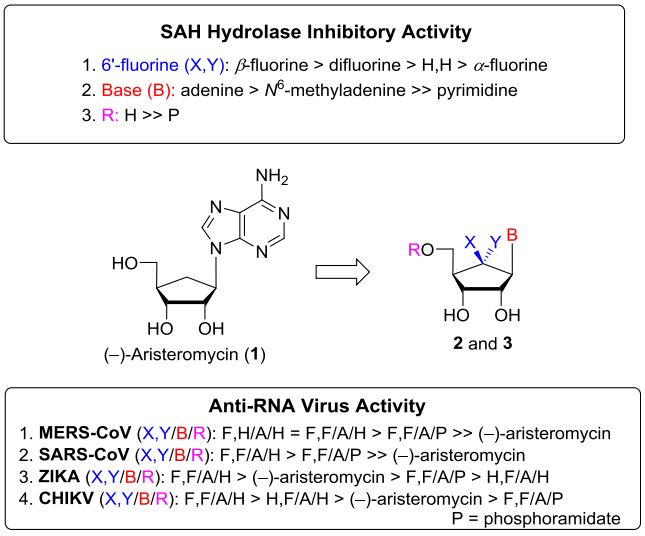

Figure 3. Summarized SAR of 6′-fluorinated aristeromycin analogues **2** and **3**.

2a–j and **3a–c** concerning the inhibition of human SAH hydrolase and the inhibition of the replication of various +RNA viruses with capped genomes. It was discovered that the introduction of fluorine at the 6′-position increases the inhibitory activity on SAH hydrolase and the replication of selected +RNA viruses. Compared to the 6′-unsubstituted compound **1**, the 6′-fluorinated aristeromycin analogues **2a** and **2c** more potently inhibited SAH hydrolase activity and the replication of MERS-CoV, SARS-CoV, ZIKV, and CHIKV. Among these compounds, 6′-β-fluoroaristeromycin (**2a**) was the most potent with an IC_{50} of 0.37 μM for SAH hydrolase activity and an EC_{50} of 0.20 μM for MERS-CoV replication. There was a correlation between the inhibition of SAH hydrolase and the antiviral activity of the compounds, suggesting that the latter was mainly due to indirect targeting of viral methylation reactions. The SAR studies and a lack of antiviral effects of several purine and pyrimidine analogues suggest that the antiviral effect of **1**, **2a**, and **2c** is unlikely due to targeting of the viral RdRp. Compound **2c** appears to be an interesting compound for further development and evaluation as a broad-spectrum antiviral agent, as it inhibited several coronaviruses, CHIKV, and ZIKV. More detailed biological studies on the efficacy of these compounds in virus-infected cells and into their mode of action are currently ongoing and will be published elsewhere.

EXPERIMENTAL SECTION

Chemical Synthesis. *General Methods.* Proton (^{1}H) and carbon (^{13}C) NMR spectra were obtained on a Bruker AV 400 (400/100 MHz), Bruker AMX 500 (500/125 MHz), JEOL JNM-ECA600 (600/150 MHz), or Bruker AVANCE III 800 (800/200 MHz) spectrometer. Chemical shifts are reported as parts per million (δ) relative to the solvent peak. Coupling constants (J) are reported in hertz. Mass spectra were recorded on a Thermo LCQ XP instrument. Optical rotations were determined on Jasco III in appropriate solvent. UV spectra were recorded on U-3000 made by Hitachi in methanol or water. Infrared spectra were recorded on FT-IR (FTS-135) made by Bio-Rad. Melting points were determined on a Buchan B-540 instrument and are uncorrected. The crude compounds were purified by column chromatography on a silica gel (Kieselgel 60, 70–230 mesh, Merck). Elemental analyses (C, H, and N) were used to determine the purity of all synthesized compounds, and the results were within ±0.4% of the calculated values, confirming ≥95% purity.

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*(((3aR,6R,6aR)-6-(tert-Butoxymethyl)-2,2-dimethyl-6,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)oxy)triethylsilane (**6**).* To a cooled (−78 °C) solution of **5** (1568.0 mg, 6.470 mmol) in anhydrous tetrahydrofuran (THF; 32.0 mL, 0.2 M) was dropwise added chlorotriethylsilane (5.4 mL, 32.355 mmol), followed by addition of LiHMDS (19.0 mL, 1.0 M solution in THF, 19.0 mmol) under N_2. After being stirred at the same temperature for 10 min, the reaction mixture was quenched with saturated aqueous NH_4Cl (80 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (EtOAc; 150 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 100/1 to 30/1) to give **6** (2267.0 mg, 98%) as colorless oil: $[\alpha]_D^{20}$ = +36.48 (*c* 1.23, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73 (dd, *J* = 1.1, 6.0 Hz, 1H), 4.58 (d, *J* = 2.1 Hz, 1H), 4.36 (d, *J* = 6.1 Hz, 1H), 3.27 (dd, *J* = 5.6, 8.6 Hz, 1H), 3.15 (dd, *J* = 6.6, 8.6 Hz, 1H), 2.72 (dd, *J* = 5.9, 5.9 Hz, 1H), 1.42 (s, 3H), 1.32 (s, 3H), 1.12 (s, 9H), 0.96 (t, *J* = 8.0 Hz, 9H), 0.66−0.72 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.1, 110.3, 104.4, 82.8, 79.7, 72.5, 63.9, 47.9, 27.4 (3 × CH_3-*tert*-butyl), 27.3, 25.8, 6.5 (3 × triethylsilyl), 4.6 (3 × triethylsilyl); IR (neat): 2973, 1648, 1363, 1262, 1204, 1056, 851, 748 cm^{-1}; HRMS (FAB): found, 356.2388 [calcd for $C_{19}H_{36}O_4Si^+$ $(M + H)^+$, 356.2383].

*(3aR,5R,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyldihydro-3aH-cyclopenta[d][1,3]dioxol-4(5H)-one (**7a**) and (3aR,5S,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyldihydro-3aH-cyclopenta[d][1,3]dioxol-4(5H)-one (**7b**).* To a cooled (0 °C) solution of silyl enol ether **6** (8.75 g, 24.548 mmol) in anhydrous DMF (123.0 mL, 0.20 M) was added 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (13.04 g, 36.824 mmol, Selectfluor) in one portion under N_2. After being stirred at the same temperature for 12 h, the reaction mixture was quenched with saturated aqueous NH_4Cl (130 mL), diluted with EtOAc (130 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 40/1 to 20/1) to give **7a** and **7b** (5.80 g, 91%, total yield, **7a**/**7b** = 5.2:1 by ^{1}H NMR analysis).

*Compound **7a**.* It was obtained as a white solid; $[\alpha]_D^{25}$ = −156.69 (*c* 0.735, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.29 (dd, *J* = 8.2, 49.5 Hz, 1H), 4.70 (t, *J* = 5.7 Hz, 1H), 4.20 (dd, *J* = 2.4, 6.1 Hz, 1H), 3.61 (dd, *J* = 1.6, 8.6 Hz, 1H) 3.38−3.41 (m, 1H), 2.75 (d, *J* = 8.2 Hz, 1H), 1.41 (s, 3H), 1.30 (s, 3H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.0 (d, *J* = 12.9 Hz), 111.4, 88.5 (d, *J* = 201.5 Hz), 78.2 (d, *J* = 6.9 Hz), 75.0 (d, *J* = 3.1 Hz), 74.3, 56.6 (d, *J* = 6.6 Hz), 40.5 (d, *J* = 15.5 Hz), 26.8 (3 × CH_3-*tert*-butyl), 26.2, 23.6; ^{19}F NMR (376 MHz, $CDCl_3$): δ −220.60 to −221.14 (m); LRMS (ESI$^+$): found, 283.13 [calcd for $C_{13}H_{21}FO_4Na^+$ $(M + Na)^+$, 283.1322]; Anal. Calcd for $C_{13}H_{21}FO_4$: C, 59.98; H, 8.13. Found: C, 59.99; H, 8.53.

*Compound **7b**.* It was obtained as a white solid; $[\alpha]_D^{25}$ = −83.72 (*c* 0.495, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.21−5.36 (ddd, *J* = 1.3, 4.5, 50.8 Hz, 1H), 4.55 (d, *J* = 5.9 Hz, 1H), 4.50 (d, *J* = 5.9 Hz, 1H), 3.63 (d, *J* = 2.2 Hz, 2H), 2.52−2.58 (m, 1H), 1.41 (s, 3H), 1.33 (s, 3H), 1.13 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 207.8 (d, *J* = 12.9 Hz), 112.2, 91.9 (d, *J* = 192.4 Hz), 78.78 (d, *J* = 3.5 Hz), 78.74, 73.6, 60.5 (d, *J* = 4.3 Hz), 45.0 (d, *J* = 17.9 Hz), 27.2 (3 × CH_3-*tert*-butyl), 26.8, 25.2; ^{19}F NMR (376 MHz, $CDCl_3$): δ −196.0 to −196.2 (m); HRMS (FAB): found, 262.1679 [calcd for $C_{13}H_{22}FO_4^+$ $(M + H)^+$, 261.1505]; Anal. Calcd for $C_{13}H_{21}FO_4$: C, 59.98; H, 8.13. Found: C, 59.77; H, 8.45.

*(3aR,6R,6aR)-6-(tert-Butoxymethyl)-5,5-difluoro-2,2-dimethyldihydro-3aH-cyclopenta[d][1,3]dioxol-4(5H)-one (**7c**).* It was obtained in 70% yield (mixture of **7c** and **7d**) as a white solid; $[\alpha]_D^{25}$ = −4.34 (*c* 0.21, MeOH); ^{1}H NMR (**7c** and **7d** mixture, 400 MHz, $CDCl_3$; **7c** and **7d** mixture): δ 4.82 (s, 1H), 4.72 (t, *J* = 6.1 Hz, 1H), 4.52−4.57 (m, 1H), 4.35−4.41 (m, 1H), 4.25 (dd, *J* = 8.0, 4.0 Hz, 1H), 3.74 (s, 1H), 3.69 (d, *J* = 8.0 Hz, 1H), 3.67−3.60 (m, 1H), 3.54−3.59 (m, 1H), 3.46 (d, *J* = 8.3 Hz, 1H), 2.68 (d, *J* = 17.4 Hz, 1H), 2.53−2.62 (m, 1H), 1.48 (s, 3H), 1.44 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H), 1.21 (s, 9H), 1.06 (s, 9H).

*General Procedure for the Synthesis of **8a**−**c**.* To a cooled (0 °C) solution of **7a**−**c** (1 equiv) in MeOH (0.18 M), sodium borohydride or lithium borohydride was added in a single portion in a N_2 atmosphere. After stirring for 30 min at the same temperature, the reaction mixture was neutralized with acetic acid (2 mL) and evaporated. The residue was diluted with saturated aqueous NH_4Cl, and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 20/1) to give **8a**−**c**.

*(3aS,4R,5R,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxol-4-ol (**8a**).* It was obtained in 71% yield as a colorless syrup; $[\alpha]_D^{25}$ = −47.46 (*c* 0.395, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.91 (td, *J* = 6.6, 52.5 Hz, 1H), 4.51−4.52 (m, 1H), 4.47 (ddd, *J* = 1.6, 6.3, 7.8 Hz, 1H), 4.26−4.34 (m, 1H), 3.52 (dd, *J* = 3.3, 8.8 Hz, 1H), 3.36−3.39 (m, 1H), 2.67 (d, *J* = 7.9 Hz, 1H), 2.46 (br s, 1H), 1.45 (s, 3H), 1.32 (s, 3H), 1.14 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 111.1, 99.5 (d, *J* = 185.9 Hz), 81.2 (d, *J* = 4.4 Hz), 76.3 (d, *J* = 9.0 Hz), 74.0 (d, *J* = 23.4 Hz), 73.0, 56.8 (d, *J* = 8.2 Hz), 44.6 (d, *J* = 18.1 Hz), 27.3 (3 × CH_3-*tert*-butyl), 26.1, 24.1; ^{19}F NMR (376 MHz, $CDCl_3$) −211.0 to −211.21 (m); HRMS (FAB): found, 263.1662 [calcd for $C_{13}H_{24}FO_4^+$ $(M + H)^+$, 263.1659]; Anal. Calcd for $C_{13}H_{23}FO_4$: C, 59.52; H, 8.84. Found: C, 59.32; H, 9.15.

*(3aS,4R,5S,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxol-4-ol (**8b**).* It was obtained in 67% yield as a colorless syrup; $[\alpha]_D^{25}$ = −40.42 (*c* 0.22, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.68 (dd, *J* = 4.1, 52.4 Hz, 1H), 4.46−4.53 (m, 2H), 4.13−4.24 (m, 1H), 3.33−3.40 (m, 1H), 2.81 (d, *J* = 11.4 Hz, 1H), 2.50 (dt, *J* = 2.9, 22.9 Hz, 1H), 1.46 (s, 3H), 1.30 (s, 3H), 1.08 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 111.4, 98.4 (d, *J* = 181.5 Hz), 82.8, 79.3, 73.8 (d, *J* = 16.3 Hz), 73.0, 60.6 (d, *J* = 12.1 Hz), 49.2 (d, *J* = 18.3 Hz), 27.1 (3 × CH_3-*tert*-butyl), 26.2, 24.2; HRMS (ESI$^+$): found, 285.1480 [calcd for $C_{13}H_{23}FNaO_4^+$ $(M + Na)^+$, 285.1478]; Anal. Calcd for $C_{13}H_{23}FO_4$: C, 55.70; H, 7.91. Found: C, 55.40; H, 7.75.

*(3aS,4R,6R,6aR)-6-(tert-Butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxol-4-ol (**8c**).* It was obtained in 74% yield as a colorless syrup; $[\alpha]_D^{25}$ = 22.37 (*c* 0.28, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.53 (t, *J* = 5.7 Hz, 1H), 4.44 (ddd, *J* = 2.6, 6.4, 8.9 Hz, 1H), 4.20−4.29 (m, 1H), 3.55 (d, *J* = 8.7 Hz, 1H), 3.39 (d, *J* = 8.8 Hz, 1H), 2.76 (d, *J* = 11.5 Hz, 1H), 2.43 (d, *J* = 17.2 Hz, 1H), 1.46 (s, 3H), 1.31 (s, 3H), 1.12 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 126.9 (dd, *J* = 252.3, 260.3 Hz), 110.9, 79.6 (d, *J* = 5.9 Hz), 75.5 (d, *J* = 11.3 Hz), 73.7 (dd, *J* = 18.5, 25.8 Hz), 73.4, 57.6 (dd, *J* = 4.6, 8.5 Hz), 48.7 (t, *J* = 20.8 Hz), 27.2 (3 × CH_3-*tert*-butyl), 25.9, 24.2; HRMS (ESI$^+$): found, 298.1834 [calcd for $C_{13}H_{26}F_2NO_4^+$ $(M + NH_4)^+$, 298.1830]; Anal. Calcd for $C_{13}H_{22}F_2O_4$: C, 55.70; H, 7.91. Found: C, 55.45; H, 7.56.

*(3aR,5R,6R,6aR)-6-(tert-Butoxymethyl)-5-((tert-butyldimethylsilyl)oxy)-2,2-dimethyldihydro-3aH-cyclopenta[d][1,3]dioxol-4(5H)-one (**9**).* To a cooled (0 °C) solution of **6** (1275 mg, 3.57 mmol) in anhydrous THF (12 mL, 0.3 M) were added 4-methylmorpholine *N*-oxide monohydrate (967 mg, 7.15 mmol, 2 equiv) and osmium tetroxide (1000 mg, 3.93 mmol, 1.1 equiv) under a N_2 atmosphere. After stirring for 30 min, to the reaction mixture were added sodium thiosulfate pentahydrate (300 mg), sodium sulfite (300 mg), and acetone (30 mL) and stirred for additional 1 h at the same temperature. The layers were separated, and the aqueous layer was extracted with EtOAc (100 mL). The combined organic layers were washed with H_2O followed by saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was used for the next step without further purification. To a solution of above generated intermediate in anhydrous DMF (18 mL, 0.19 M) were added TBSCl (1614 mg, 10.71 mmol) and imidazole (729 mg, 10.71 mmol) under a N_2 atmosphere. After stirring for 3 h at room temperature, the reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL) and diluted with EtOAc (50 mL). 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 saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 40/1

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to 20/1) to give **9** (705 mg, 53%) as a colorless syrup: $[\alpha]_D^{25} = -103.19$ (*c* 0.30, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.65 (d, *J* = 6.4 Hz, 1H), 4.53 (d, *J* = 8.0 Hz, 1H), 4.11 (d, *J* = 6.3 Hz, 1H), 3.61 (dd, *J* = 1.6, 8.0 Hz, 1H), 3.30 (dd, *J* = 2.4, 8.1 Hz, 1H), 2.41−2.46 (m, 1H), 1.42 (s, 3H), 1.30 (s, 3H), 1.03 (s, 9H), 0.88 (s, 9H), 0.13 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.2, 110.9, 78.1, 75.8, 73.7, 71.3, 56.9, 42.3, 27.0 (3 × CH_3-*tert*-butyl), 26.4, 25.7 (3 × CH_3-*tert*-butyl), 23.8, 18.3, −4.4, −5.6; HRMS (FAB$^+$) (*m*/*z*): found, 373.2398 [calcd for $C_{19}H_{37}O_5Si^+$ (M + H)$^+$, 373.2410]; Anal. Calcd for $C_{19}H_{36}O_5Si$: C, 61.25; H, 9.74. Found: C, 61.26; H, 9.75.

(3aS,4R,5R,6R,6aR)-6-(tert-Butoxymethyl)-5-((tert-butyldimethylsilyl)oxy)-2,2-dimethyltetrahydro-3aH-cyclopenta[d]-[1,3]dioxol-4-ol ***(10)****.* To a cooled (0 °C) solution of **9** (471 mg, 1.26 mmol) in methanol (6.3 mL, 0.2 M) was added sodium borohydride (144 mg, 3.79 mmol, 3 equiv) under a N_2 atmosphere. After being stirred at the same temperature for 1 h, the reaction mixture was diluted with H_2O (20 mL) and EtOAc (20 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 30/1 to 20/1) to give **10** (415 mg, 88%) as a colorless syrup: $[\alpha]_D^{25} = -40.39$ (*c* 0.32, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.49 (d, *J* = 6.1 Hz, 1H), 4.41 (t, *J* = 6.2 Hz, 1H), 4.07 (t, *J* = 6.9 Hz, 1H), 3.95 (dd, *J* = 6.8, 14.7 Hz, 1H), 3.48 (dd, *J* = 3.9, 8.5 Hz, 1H), 3.32 (dd, *J* = 4.6, 8.5 Hz, 1H), 2.43 (d, *J* = 8.4 Hz, 1H), 2.12−2.18 (m, 1H), 1.45 (s, 3H), 1.32 (s, 3H), 1.12 (s, 9H), 0.87 (s, 9H), 0.09 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 110.4, 81.0, 78.8, 77.0, 76.1, 72.6, 57.3, 46.0, 27.4 (3 × CH_3-*tert*-butyl), 26.2, 25.8 (3 × CH_3-*tert*-butyl), 24.0, 18.1, −4.5, −5.1; HRMS (FAB$^+$) (*m*/*z*): found, 375.2584 [calcd for $C_{19}H_{39}O_5Si^+$ (M + H)$^+$, 375.2567]; Anal. Calcd for $C_{19}H_{38}O_5Si$: C, 60.92; H, 10.23. Found: C, 60.91; H, 10.25.

(((3aR,4R,5R,6R,6aR)-4-(Benzyloxy)-6-(tert-butoxymethyl)-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-yl)oxy)(tert-butyl)dimethylsilane ***(11)****.* To a cooled (0 °C) solution of **10** (193 mg, 0.515 mmol) in DMF (5.2 mL, 0.1 M) was added benzyl chloride (0.12 mL, 1.030 mmol, 2.0 equiv) and sodium hydride (41 mg, 1.030 mmol, 2.0 equiv) under a N_2 atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was diluted with H_2O (20 mL) and EtOAc (20 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 50/1) to give **11** (204 mg, 85%) as a colorless syrup: $[\alpha]_D^{25} = -46.64$ (*c* 0.66, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22−7.39 (m, 5H), 4.76 (d, *J* = 12.4 Hz, 1H), 4.59 (d, *J* = 12.4 Hz, 1H), 4.45 (d, *J* = 6.0 Hz, 1H), 4.33−4.37 (m, 2H), 3.83 (dd, *J* = 5.6, 8.8 Hz, 1H), 3.39 (dd, *J* = 4.4, 8.8 Hz, 1H), 3.32 (dd, *J* = 4.0, 8.4 Hz, 1H), 2.05−2.11 (m, 1H), 1.48 (s, 3H), 1.29 (s, 3H), 1.03 (s, 9H), 0.88 (s, 9H), 0.09 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (200 MHz, $CDCl_3$): δ 138.9, 128.4, 128.1, 127.9, 127.7, 127.2, 110.0, 82.1, 80.2, 76.0, 75.6, 72.4, 71.7, 57.5, 45.7, 27.3 (3 × CH_3-*tert*-butyl), 26.4, 25.8 (3 × CH_3-*tert*-butyl), 24.2, −4.7, −4.9; HRMS (FAB$^+$) (*m*/*z*): found, 465.3001 [calcd for $C_{26}H_{45}O_5Si^+$ (M + H)$^+$, 465.3029]; Anal. Calcd for $C_{26}H_{44}O_5Si$: C, 67.20; H, 9.54. Found: C, 67.22; H, 9.55.

(3aR,4S,5R,6S,6aR)-4-(Benzyloxy)-6-(tert-butoxymethyl)-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-ol ***(12)****.* To a cooled (0 °C) solution of **11** (179 mg, 0.385 mmol) in anhydrous THF (3.8 mL, 0.1 M) was added TBAF solution (1.2 mL, 1.0 M solution in THF, 1.2 mmol, 3.0 equiv) under a N_2 atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was diluted with H_2O (30 mL) and EtOAc (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 8/1) to give **12** (129 mg, 88%) as a colorless syrup: $[\alpha]_D^{25} = -49.04$ (*c* 0.28, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 (d, *J* = 7.2 Hz, 2H), 7.29−7.35 (m, 2H), 7.23−7.28 (m, 1H), 4.85 (d, *J* = 12.4 Hz, 1H), 4.62 (d, *J* = 12.4 Hz, 1H), 4.51 (t, *J* = 6.0 Hz, 1H), 4.40−4.45 (m, 2H), 3.81 (dd, *J* = 4.8, 7.2 Hz, 1H), 3.58 (dd, *J* = 3.6, 8.8 Hz, 1H), 3.44 (dd, *J* = 4.4, 8.8 Hz, 1H), 2.70 (br s, 1H), 2.26−2.32 (m, 1H), 1.48 (s, 3H), 1.31 (s, 3H), 1.08 (s, 9H); ^{13}C NMR (200 MHz, $CDCl_3$): δ 138.5, 128.3 (2 × CH-benzene), 128.0 (2 × CH-benzene), 127.5, 111.1, 82.7, 80.6, 77.2, 76.7, 73.4, 71.9, 59.3, 45.4, 27.2 (3 × CH_3-*tert*-butyl), 26.5, 24.6; Anal. Calcd for $C_{20}H_{30}O_5$: C, 68.54; H, 8.63. Found: C, 68.52; H, 8.64.

(3aR,4R,5S,6R,6aR)-4-(Benzyloxy)-6-(tert-butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxole ***(13a)****.* To a cooled (0 °C) solution of **12** (20 mg, 0.052 mmol) in anhydrous toluene (2.0 mL, 0.026 M) was dropwise added diethylaminosulfur trifluoride (30 μL, 0.210 mmol, 4.0 equiv) under a N_2 atmosphere. After being stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous NH_4Cl (30 mL) and EtOAc (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 30/1) to give **13a** (5.6 mg, 30%) and **13b** (5.6 mg, 30%) as a colorless syrup.

Compound ***13a****.* $[\alpha]_D^{25} = -26.59$ (*c* 0.22, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.25−7.34 (m, 5H), 4.96 (ddd, *J* = 2.6, 6.8, 52.7 Hz, 1H), 4.72 (dd, *J* = 0.8, 11.6 Hz, 1H), 4.54 (d, *J* = 11.6 Hz, 1H), 4.44−4.52 (m, 2H), 4.02−4.09 (m, 1H), 3.41−3.47 (m, 2H), 2.15−2.18 (m, 1H), 1.47 (s, 3H), 1.28 (s, 3H), 1.12 (s, 9H); ^{13}C NMR (200 MHz, $CDCl_3$): δ 137.8, 128.3 (2 × CH-benzyl), 128.1 (2 × CH-benzyl), 127.8, 111.8, 96.0 (d, *J* = 187.1 Hz), 81.6, 79.3, 78.2 (d, *J* = 15.7 Hz), 72.6, 71.8, 60.6 (d, *J* = 11.0 Hz), 50.2 (d, *J* = 18.7 Hz), 27.0 (3 × CH_3-*tert*-butyl), 26.6, 24.4; HRMS (FAB$^+$) (*m*/*z*): found, 353.2121 [calcd for $C_{20}H_{30}FO_4^+$ (M + H)$^+$, 353.2128]; Anal. Calcd for $C_{20}H_{29}FO_4$: C, 68.16; H, 8.29. Found: C, 68.13; H, 8.27.

Compound ***13b****.* $[\alpha]_D^{25} = -61.72$ (*c* 0.42, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (t, *J* = 7.3 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 2H), 7.25 (d, *J* = 7.2 Hz, 1H), 5.18 (dt, *J* = 7.8, 53.7 Hz, 1H), 4.76 (d, *J* = 12.2 Hz, 1H), 4.66 (d, *J* = 12.2 Hz, 1H), 4.45−4.49 (m, 1H), 4.41−4.44 (m, 1H), 4.19 (ddd, *J* = 5.9, 7.7, 16.5 Hz, 1H), 3.45 (dd, *J* = 3.0, 8.8 Hz, 1H), 3.31−3.34 (m, 1H), 2.37−2.43 (m, 1H), 1.47 (s, 3H), 1.28 (s, 3H), 1.01 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.0, 128.3, 127.9 (2 × CH-benzyl), 127.7 (2 × CH-benzyl), 112.2, 103.5, 102.1, 81.5 (d, *J* = 27.5 Hz), 81.1 (d, *J* = 20.0 Hz), 72.6, 72.4, 57.6, 48.8 (d, *J* = 6.2 Hz), 27.4 (3 × CH_3-*tert*-butyl), 27.1, 25.0; HRMS (FAB$^+$) (*m*/*z*): found, 353.2131 [calcd for $C_{20}H_{30}FO_4^+$ (M + H)$^+$, 353.2128]; Anal. Calcd for $C_{20}H_{29}FO_4$: C, 68.16; H, 8.29. Found: C, 68.13; H, 8.27.

(3aR,4R,5S,6R,6aS)-4-(tert-Butoxy)-5-(tert-butoxymethyl)-6-hydroxytetrahydro-3aH-cyclopenta[d][1,3,2]dioxathiole 2-Oxide ***(14)****.* To perform regioselective cleavage, to a cooled (−78 °C) solution of **10** (420 mg, 1.121 mmol) in anhydrous CH_2Cl_2 (5.6 mL, 0.2 M) was dropwise added trimethylaluminum (3.4 mL, 2.0 M solution in hexane, 6.727 mmol, 6.0 equiv) under a N_2 atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was quenched with saturated aqueous NH_4Cl (30 mL) and EtOAc (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 10/1) to give diol intermediate (245 mg, 56%) 10a as a colorless syrup. For the introduction of cyclic sulfite, to a cooled (0 °C) solution of diol intermediate **10a** (250 mg, 0.639 mmol) in anhydrous CH_2Cl_2 (6.4 mL, 0.1 M) was dropwise added triethylamine (0.3 mL, 2.239 mmol, 3.5 equiv) followed by thionyl chloride (70 μL, 0.959 mmol) under a N_2 atmosphere. After being stirred at room temperature for 30 min, the reaction mixture was quenched with saturated aqueous NH_4Cl (30 mL) and diluted with EtOAc (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel, hexanes/EtOAc, 10/1) to give cyclic sulfite intermediate **10b** (249 mg, 89%) as a colorless syrup. For

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TBS deprotection, to a cooled (0 °C) solution of **10b** (286 mg, 0.654 mmol) in anhydrous THF (6.5 mL, 0.1 M) was added acetic acid (0.13 mL, 0.131 mmol, 0.2 equiv) followed by TBAF solution (2.6 mL, 1.0 M solution in THF, 2.6 mmol, 4.0 equiv) under a N_2 atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was quenched with H_2O (30 mL) and diluted with EtOAc (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 6/1) to give **14** (202 mg, 96%, two diastereomers **A** and **B** were generated from sulfoxide stereogenic center) as a colorless syrup: for **A**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.27 (t, J = 5.4 Hz, 1H), 5.02 (d, J = 5.9 Hz, 1H), 4.79 (s, 1H), 4.44 (dd, J = 4.8, 11.4 Hz, 1H), 4.19 (d, J = 3.9 Hz, 1H), 3.80 (dd, J = 2.6, 9.3 Hz, 1H), 1.90−1.94 (m, 1H), 1.27 (s, 9H), 1.21 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 86.9, 82.6, 74.9, 74.5, 74.1, 69.4, 58.2, 43.6, 28.3 (3 × CH_3-*tert*-butyl), 27.2 (3 × CH_3-*tert*-butyl); HRMS (FAB$^+$) (m/z): found, 323.1530 [calcd for $C_{14}H_{27}O_6S^+$ (M + H)$^+$, 323.1528]; for **B**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.98−5.07 (m, 2H), 4.79 (d, J = 6.4 Hz, 1H), 4.36 (dd, J = 4.6, 11.5 Hz, 1H), 4.31 (d, J = 4.1 Hz, 1H), 3.84 (d, J = 9.2 Hz, 1H), 3.77 (d, J = 9.3 Hz, 1H), 2.65 (d, J = 10.1 Hz, 1H), 1.25 (s, 9H), 1.21 (s, 9H).

*(3aR,4R,5R,6S,6aR)-4-(tert-Butoxy)-5-(tert-butoxymethyl)-6-fluorotetrahydro-3aH-cyclopenta[d][1,3,2]dioxathiole 2-Oxide (**15**).* To a cooled (0 °C) solution of **14** (33 mg, 0.102 mmol) in anhydrous CH_2Cl_2 (1.5 mL, 0.068 M) was dropwise added diethylaminosulfur trifluoride (60 μL, 0.434 mmol, 4.0 equiv) under a N_2 atmosphere. After being stirred at room temperature for 4 h, the reaction mixture was quenched with saturated aqueous NH_4Cl (30 mL) and diluted with EtOAc (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel, hexanes/EtOAc, 15/1) to give **15** (12 mg, 37%) as a colorless syrup: ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.17 (ddd, J = 4.6, 7.8, 52.7 Hz, 1H), 5.03 (t, J = 8.2 Hz, 1H), 4.92 (ddd, J = 5.0, 8.7, 17.8 Hz, 1H), 4.06 (ddd, J = 7.8, 11.0, 16.5 Hz, 1H), 3.53 (ddd, J = 2.7, 2.7, 6.8 Hz, 1H), 3.44 (dd, J = 2.2, 9.1 Hz, 1H), 2.54−2.58 (m, 1H), 1.17 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 102.1 (d, J = 191.2 Hz), 87.2 (d, J = 28.2 Hz), 81.9 (d, J = 5.8 Hz), 74.5, 72.8, 72.4 (d, J = 19.2 Hz), 55.5, 50.4 (d, J = 6.5 Hz), 28.6 (3 × CH_3-*tert*-butyl), 27.5 (3 × CH_3-*tert*-butyl).

*(3aR,4R,5R,6S,6aR)-4-(tert-Butoxy)-5-(tert-butoxymethyl)-6-fluorotetrahydro-3aH-cyclopenta[d][1,3,2]dioxathiole 2,2-Dioxide (**16**).* To a solution of cyclic sulfite **15** (13 mg, 0.040 mmol) in CCl_4/CH_3CN/H_2O (1:1:1.5, total 1.75 mL, 0.14 M) was added in one portion sodium periodate (26 mg, 0.120 mmol), followed by ruthenium(III) chloride trihydrate (2 mg, 0.008 mmol) at room temperature under a N_2 atmosphere. After being stirred at the same temperature for 20 min, the reaction mixture was quenched with H_2O (20 mL) and diluted with CH_2Cl_2 (20 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The crude product **16** was used for the next step without further purification.

*General Procedure for the Synthesis of **18a−c**. Triflation.* To a cooled (0 °C) solution of **8a−c** (1 equiv) in anhydrous pyridine (0.32 M), trifluoromethanesulfonic anhydride (2 equiv) was added dropwise in a N_2 atmosphere. After stirring at the same temperature for 30 min, the reaction mixture was quenched with H_2O (50 mL) and diluted with EtOAc (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with saturated aqueous $CuSO_4$ followed by water, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was used for the next step without further purification.

Azidation. To a solution of triflate intermediate (1 equiv) in anhydrous DMF (0.19 M), sodium azide (3 equiv) was added in a single portion at room temperature. After being heated to 60−100 °C and stirred for 4−15 h, the reaction mixture was cooled to room temperature, quenched with H_2O (50 mL), and diluted with EtOAc (50 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with H_2O followed by saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 10/1) to give **18a−c**.

*(3aS,4S,5R,6R,6aR)-4-Azido-6-(tert-butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxole (**18a**).* It was obtained in 45% yield as a colorless syrup; $[\alpha]_D^{25}$ = −24.42 (c 0.016, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.16 (td, J = 52.4, 3.1 Hz, 1H), 4.66 (t, J = 6.0 Hz, 1H), 4.41 (t, J = 6.5 Hz, 1H), 3.62−3.69 (m, 1H), 3.54 (s, 1H), 3.50 (s, 1H), 2.27−2.36 (m, 1H), 1.47 (s, 3H), 1.29 (s, 3H), 1.16 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 114.1, 96.9 (d, J = 182.6 Hz), 82.0, 80.2, 73.1, 67.9 (d, J = 15.7 Hz), 57.8 (d, J = 7.2 Hz), 49.4 (d, J = 17.6 Hz), 27.3 (3 × CH_3-*tert*-butyl), 27.1, 24.6; ^{19}F NMR (376 MHz, $CDCl_3$) −206.9 to −207.2 (m); IR (neat): 2108 cm^{-1}; LR-MS (ESI$^+$): 310.15 [calcd for $C_{13}H_{22}FN_2NaO_3^+$ (M + Na)$^+$, 310.1543]; Anal. Calcd for $C_{13}H_{22}FN_3O_3$: C, 54.34; H, 7.72; N, 14.62. Found: C, 54.35; H, 7.45; N, 14.23.

*(3aS,4S,5S,6R,6aR)-4-Azido-6-(tert-butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxole (**18b**).* It was obtained in 88% yield as a colorless syrup; $[\alpha]_D^{25}$ = 9.66 (c 0.51, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.75 (dt, J = 7.7, 53.0 Hz, 1H), 4.41 (dd, J = 4.5, 6.7 Hz, 1H), 4.22 (t, J = 5.7 Hz, 1H), 4.00 (ddd, J = 5.5, 7.4, 16.6 Hz, 1H), 3.43−3.50 (m, 2H), 2.33−2.44 (m, 1H), 1.50 (s, 3H), 1.27 (s, 3H), 1.15 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 112.7, 95.8 (d, J = 188.9 Hz), 81.0 (d, J = 8.6 Hz), 77.8 (d, J = 7.2 Hz), 73.0, 70.9 (d, J = 20.1 Hz), 57.9, 49.1 (d, J = 18.7 Hz), 27.3 (3 × CH_3-*tert*-butyl), 27.2, 25.0; IR (neat): 2111 cm^{-1}; Anal. Calcd for $C_{13}H_{22}FN_3O_3$: C, 54.34; H, 7.72; N, 14.62. Found: C, 54.12; H, 7.94; N, 14.33.

*(3aS,4S,6R,6aR)-4-Azido-6-(tert-butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxole (**18c**).* It was obtained in 75% yield as a colorless syrup; $[\alpha]_D^{25}$ = −43.39 (c 0.36, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.40−4.44 (m, 1H), 4.34−4.39 (m, 1H), 3.87−3.95 (m, 1H), 3.61 (dd, J = 6.5, 9.3 Hz, 1H), 3.48 (t, J = 7.6 Hz, 1H), 2.54−2.66 (m, 1H), 1.49 (s, 3H), 1.28 (s, 3H), 1.17 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 127.1 (dd, J = 255.9, 260.9 Hz), 113.0, 80.0 (d, J = 5.9 Hz), 78.4 (d, J = 5.6 Hz), 73.4, 69.1 (dd, J = 18.8, 25.1 Hz), 57.2 (d, J = 6.4 Hz), 50.8 (t, J = 20.0 Hz), 27.3 (3 × CH_3-*tert*-butyl), 26.9, 24.7; IR (neat): 2116 cm^{-1}; Anal. Calcd for $C_{13}H_{21}F_2N_3O_3$: C, 51.14; H, 6.93; N, 13.76. Found: C, 51.45; H, 7.21; N, 14.10.

*General Procedure for the Synthesis of **19a−c**.* To a suspension of **18a−c** (1 equiv) in methanol (0.2 M), 10% palladium on activated carbon (0.03 equiv) was added and stirred overnight at room temperature in a H_2 atmosphere. After filtration, the solvent was removed, and the residue was used for the next step without further purification.

*General Procedure for the Synthesis of **20a−c**.* To a solution of **19a−c** (1 equiv) in *n*-butanol (0.38 M), 5-amino-4,6-dichloro pyrimidine (3−10 equiv) and diisopropylamine (10 equiv) were added. The reaction mixture was placed under microwave irradiation at 170−200 °C for 4−7 h. The solvent was co-evaporated with MeOH, and the residue was purified with column chromatography (silica gel, hexane/EtOAc, 4/1) to give **20a−c**, respectively.

*N^4-((3aS,4S,5R,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-6-chloropyrimidine-4,5-diamine (**20a**).* It was obtained in 66% yield from **18a**; yellow foam; $[\alpha]_D^{25}$ = −53.8 (c 0.10, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08 (s, 1H), 5.27−5.33 (br s, 1H), 5.24 (td, J = 3.5, 52.9 Hz, 1H), 4.71−4.81 (m, 1H), 4.57 (t, J = 6.1 Hz, 1H), 4.44 (t, J = 6.3 Hz, 1H), 3.58−3.63 (m, 1H), 3.53 (t, J = 9.2 Hz, 1H), 3.39 (br s, 2H), 2.42−2.55 (m, 1H), 1.52 (s, 3H), 1.30 (s, 3H), 1.18 (s, 9H); ^{13}C NMR (200 MHz, $CDCl_3$): δ 154.4, 149.0, 122.4, 113.8, 95.9 (d, J = 178.7 Hz), 84.2, 80.1, 77.1, 73.3, 59.8 (d, J = 15.9 Hz), 58.0 (d, J = 7.0 Hz), 49.4 (d, J = 17.6 Hz), 27.4 (3 × CH_3-*tert*-butyl), 27.2, 24.8; ^{19}F NMR (376 MHz, $CDCl_3$) −212.8 to −213.1 (m); UV (CH_2Cl_2): λ_{max} 287 nm; LRMS (ESI$^+$): found, 388.17 [calcd for $C_{17}H_{27}ClFN_4O_3^+$ (M + H)$^+$,

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J. Med. Chem. 2019, 62, 6346−6362

389.1756]; Anal. Calcd for $C_{17}H_{26}ClFN_4O_3$: C, 52.51; H, 6.50; N, 14.45. Found: C, 52.45; H, 6.13; N, 14.15.

*N^4-((3aS,4S,5S,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-6-chloropyrimidine-4,5-diamine (**20b**).* It was obtained in 47% yield from **18b**; yellow foam; $[\alpha]_D^{25} = -11.79$ (c 0.36, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (s, 1H), 5.56 (d, J = 9.2 Hz, 1H), 4.89 (dt, J = 3.1, 51.0 Hz, 1H), 4.77 (dd, J = 9.1, 21.2 Hz, 1H), 4.61 (dd, J = 2.5, 5.0 Hz, 1H), 4.51 (dd, J = 2.4, 6.0 Hz, 1H), 3.60 (dd, J = 2.6, 9.2 Hz, 1H), 3.55 (dd, J = 2.5, 9.3 Hz, 1H), 3.39 (br s, 2H), 2.60 (d, J = 23.5 Hz, 1H), 1.54 (s, 3H), 1.29 (s, 3H), 1.21 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.2, 149.6, 143.4, 122.4, 111.7, 101.3 (d, J = 185.1 Hz), 85.5 (d, J = 3.3 Hz), 82.0 (d, J = 2.6 Hz), 74.0, 63.7 (d, J = 26.6 Hz), 60.6 (d, J = 7.1 Hz), 51.3 (d, J = 20.5 Hz), 27.5 (3 × CH_3-*tert*-butyl), 27.1, 24.9; UV (MeOH): λ_{max} 297.60, 265.07 nm; HRMS (ESI^+): found, 389.1762 [calcd for $C_{17}H_{27}ClFN_4O_3^+$ $(M + H)^+$, 389.1756]; Anal. Calcd for $C_{17}H_{26}IFN_4O_3$: C, 52.51; H, 6.50; N, 14.45. Found: C, 52.56; H, 6.51; N, 14.43.

*N^4-((3aS,4S,6R,6aR)-6-(tert-Butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-6-chloropyrimidine-4,5-diamine (**20c**).* It was obtained in 67% yield from **18c**; yellow foam; $[\alpha]_D^{25} = -61.76$ (c 0.23, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11 (s, 1H), 5.71 (d, J = 10.1 Hz, 1H), 5.03 (t, J = 12.7 Hz, 1H), 4.56 (t, J = 4.6 Hz, 1H), 4.40–4.45 (m, 1H), 3.69 (dd, J = 2.6, 9.5 Hz, 1H), 3.57 (dd, J = 4.4, 9.4 Hz, 1H), 3.38 (br s, 2H), 2.72 (d, J = 14.7 Hz, 1H), 1.53 (s, 3H), 1.44 (s, 3H), 1.25 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.5, 149.6, 143.9, 128.0 (dd, J = 257.3, 260.0 Hz), 122.3, 111.7, 84.5, 79.7 (d, J = 4.1 Hz), 74.5, 61.7 (dd, J = 18.1, 31.9 Hz), 58.3 (t, J = 5.8 Hz), 51.6 (t, J = 22.6 Hz), 27.5 (3 × CH_3-*tert*-butyl), 26.7, 24.6; UV (MeOH): λ_{max} 297.39, 263.29 nm; HRMS (ESI^+): found, 407.1658 [calcd for $C_{17}H_{26}ClF_2N_4O_3^+$ $(M + H)^+$, 407.1661]; Anal. Calcd for $C_{17}H_{25}ClF_2N_4O_3$: C, 50.19; H, 6.19; N, 13.77. Found: C, 50.11; H, 6.23; N, 13.65.

*General Procedure for the Synthesis of **21a–c**.* A solution of **20a–c** in diethoxymethyl acetate (0.15 M) was placed under microwave irradiation at 140 °C for 3 h. The mixture was then co-evaporated with MeOH three times, and the resulting residue was purified with column chromatography (silica gel, hexane/EtOAc, 7/1) to give **21a–c**.

*9-((3aS,4S,5R,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-6-chloro-9H-purine (**21a**).* It was obtained in 96% yield as yellow foam; $[\alpha]_D^{25} = -29.2$ (c 0.17, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H), 8.34 (d, J = 2.4 Hz, 1H), 5.28–5.43 (td, J = 2.8, 52.8 Hz, 1H), 5.12–5.23 (m, 2H), 4.61 (t, J = 5.0 Hz, 1H), 3.65–3.69 (m, 1H), 3.61 (t, J = 9.2 Hz, 1H), 2.56–2.71 (m, 1H), 1.56 (s, 3H), 1.32 (s, 3H), 1.17 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.3, 151.4, 144.2, 144.1, 131.4, 115.4, 97.7–95.9 (d, J = 181.2 Hz), 82.9, 80.1, 73.5, 63.1 (d, J = 16.1 Hz), 58.0 (d, J = 7.4 Hz), 50.0 (d, J = 17.5 Hz), 27.6 (3 × CH_3-*tert*-butyl), 27.5, 25.1; ^{19}F NMR (376 MHz, $CDCl_3$) −202.6–202.9 (m); UV (CH_2Cl_2): λ_{max} 271 nm; LRMS (ESI^+): found, 399.16 [calcd for $C_{18}H_{25}ClFN_4O_3^+$ $(M + H)^+$, 399.1599]; Anal. Calcd for $C_{18}H_{24}ClFN_4O_3$: C, 54.20; H, 6.06; N, 14.05. Found: C, 54.12; H, 6.34; N, 14.23.

*9-((3aS,4S,5S,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-6-chloro-9H-purine (**21b**).* It was obtained in 76% yield as yellow foam; $[\alpha]_D^{25} = -31.54$ (c 0.54, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.67 (s, 1H), 8.15 (s, 1H), 5.55 (dt, J = 8.4, 53.6 Hz, 1H), 5.02 (t, J = 6.4 Hz, 1H), 4.84–4.94 (m, 1H), 4.65 (t, J = 5.1 Hz, 1H), 3.53–3.63 (m, 2H), 2.47–2.57 (m, 1H), 1.54 (s, 3H), 1.25 (s, 3H), 1.17 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 151.7, 151.5, 151.3, 144.8, 132.3, 113.1, 93.9 (d, J = 191.0 Hz), 79.1 (d, J = 7.9 Hz), 77.6 (d, J = 7.9 Hz), 73.1, 67.8 (d, J = 20.8 Hz), 58.1, 48.7 (d, J = 18.7 Hz) 27.5 (3 × CH_3-*tert*-butyl), 27.3, 25.0; UV (MeOH): λ_{max} 264.36 nm; HRMS (ESI^+): found, 399.1589 [calcd for $C_{18}H_{25}ClFN_4O_3^+$ $(M + H)^+$, 399.1599]; Anal. Calcd for $C_{18}H_{24}ClFN_4O_3$: C, 54.20; H, 6.06; N, 14.05. Found: C, 54.34; H, 6.46; N, 13.99.

*9-((3aS,4S,6R,6aR)-6-(tert-Butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-6-chloro-9H-purine (**21c**).* It was obtained in 92% yield as yellow foam; $[\alpha]_D^{25} = -46.05$ (c 0.43, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.73 (s, 1H), 8.28 (d, J = 2.1 Hz, 1H), 5.30 (dt, J = 6.9, 20.1 Hz, 1H), 5.10 (t, J = 6.7 Hz, 1H), 4.57–4.62 (m, 1H), 3.63–3.73 (m, 2H), 2.81–2.93 (m, 1H), 1.56 (s, 3H), 1.30 (s, 3H), 1.18 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.4, 152.4, 151.3, 143.9 (d, J = 4.0 Hz), 131.2, 125.6 (dd, J = 253.4, 264.6 Hz), 114.0, 79.5 (d, J = 7.7 Hz), 77.9 (d, J = 7.5 Hz), 73.7, 64.6 (dd, J = 19.3, 24.3 Hz), 57.1 (d, J = 7.1 Hz), 50.3 (t, J = 19.8 Hz), 27.3 (3 × CH_3-*tert*-butyl), 27.2, 25.0; UV (MeOH): λ_{max} 263.74 nm; HRMS (ESI^+): found, 417.1500 [calcd for $C_{18}H_{24}ClF_2N_4O_3^+$ $(M + H)^+$, 417.1505]; Anal. Calcd for $C_{18}H_{23}ClF_2N_4O_3$: C, 51.86; H, 5.56; N, 13.44. Found: C, 51.56; H, 5.96; N, 13.13.

*General Procedure for the Synthesis of **2a–c**.* To a solution of **21a–c** in *tert*-butanol (2 mL, 0.27 M) contained in a stainless steel bomb reactor, saturated ammonia in *tert*-butanol (15 mL) was added and the reactor was locked. After being heated to 120 °C with stirring for 15 h, the mixture was cooled to room temperature and co-evaporated with MeOH. Without purification, the residue was added to a TFA/H_2O solution (2:1, v/v, total 15 mL) and heated to 50 °C with stirring for 15 h. After the reaction mixture was evaporated, the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 9/1) to give **2a–c**.

*(1R,2S,3S,4R,5R)-3-(6-Amino-9H-purin-9-yl)-4-fluoro-5-(hydroxymethyl)cyclopentane-1,2-diol (**2a**).* It was obtained in 43% yield as a white solid; mp 172–177 °C; $[\alpha]_D^{25} = -64.49$ (c 0.22, MeOH); ^{1}H NMR (800 MHz, CD_3OD-d_6): δ 8.26 (d, J = 2.0 Hz, 1H), 8.21 (s, 1H), 5.21 (dt, J = 4.0, 54.6, 1H), 4.99 (ddd, J = 3.4, 10.8, 29.5 Hz, 1H), 4.75 (dd, J = 6.7, 9.4 Hz, 1H), 4.02 (dd, J = 4.8, 6.4 Hz, 1H), 3.79–3.85 (m, 2H), 2.42–2.51 (m, 1H); ^{13}C NMR (200 MHz, CD_3OD): δ 158.1, 154.6, 152.2, 142.4 (d, J = 3.3 Hz), 120.5, 92.8 (d, J = 180.7 Hz), 74.3, 71.8, 64.0 (d, J = 17.0 Hz), 60.6 (d, J = 10.7 Hz), 54.3 (d, J = 17.9 Hz); ^{19}F NMR (376 MHz, CD_3OD): δ −204.7 to −205.4 (m); UV (MeOH): λ_{max} 259.90 nm; HRMS (ESI^+): found, 284.1161 [calcd for $C_{11}H_{15}FN_5O_3^+$ $(M + H)^+$, 284.1159]; Anal. Calcd for $C_{11}H_{14}FN_5O_3$: C, 46.64; H, 4.98; N, 24.72. Found: C, 46.65; H, 5.38; N, 25.10.

*(1R,2S,3S,4S,5R)-3-(6-Amino-9H-purin-9-yl)-4-fluoro-5-(hydroxymethyl)cyclopentane-1,2-diol (**2b**).* It was obtained in 71% yield as a white solid; mp 182–186 °C; $[\alpha]_D^{25} = -11.85$ (c 0.26, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 8.19 (s, 1H), 8.18 (s, 1H), 5.40 (ddd, J = 5.2, 7.3, 54.4 Hz, 1H), 5.03 (ddd, J = 7.5, 9.8, 20.7 Hz, 1H), 4.60 (dd, J = 5.1, 9.9 Hz, 1H), 4.05–4.09 (m, 1H), 3.80 (d, J = 5.8 Hz, 2H), 2.28–2.40 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ 158.0, 154.3, 151.9, 143.4, 121.6, 95.8 (d, J = 186.4 Hz), 74.2 (d, J = 7.4 Hz), 73.2 (d, J = 3.3 Hz), 68.6 (d, J = 21.1 Hz), 62.6, 54.6 (d, J = 19.0 Hz); ^{19}F NMR (378 MHz, CD_3OD): δ −185.244 (dt, J = 23.8, 53.7 Hz); UV (MeOH): λ_{max} 260.88 nm; HRMS (ESI^+): found, 284.1155 [calcd for $C_{11}H_{15}FN_5O_3^+$ $(M + H)^+$, 284.1159]; Anal. Calcd for $C_{11}H_{14}FN_5O_3$: C, 46.64; H, 4.98; N, 24.72. Found: C, 46.38; H, 5.12; N, 24.33.

*(1R,2S,3S,5R)-3-(6-Amino-9H-purin-9-yl)-4,4-difluoro-5-(hydroxymethyl)cyclopentane-1,2-diol (**2c**).* It was obtained in 61% yield as a white solid; mp 180–185 °C; $[\alpha]_D^{25} = -56.51$ (c 0.30, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 8.26 (d, J = 19.5 Hz, 1H), 8.20 (s, 1H), 5.33 (dt, J = 10.0, 17.0 Hz, 1H), 4.79 (dd, J = 5.2, 10.6 Hz, 1H, merged with solvent peak), 4.13–4.17 (m, 1H), 3.79–3.91 (m, 2H), 2.60–2.71 (m, 1H); ^{13}C NMR (200 MHz, CD_3OD): δ 158.2, 154.8, 152.6, 142.7 (d, J = 2.4 Hz), 125.9 (dd, J = 252.3, 258.4 Hz), 120.6, 73.7 (d, J = 7.3 Hz), 71.8 (d, J = 3.3 Hz), 64.8 (dd, J = 19.4, 23.8 Hz), 59.6 (d, J = 10.8 Hz), 56.4 (t, J = 19.9 Hz); ^{19}F NMR (378 MHz, CD_3OD): δ −97.5 (d, J = 238.5 Hz), −115.4 (dt, J = 15.9, 238.9 Hz); UV (MeOH): λ_{max} 259.92 nm; HRMS (ESI^+): found, 302.1066 [calcd for $C_{11}H_{14}F_2N_5O_3^+$ $(M + H)^+$, 302.1065]; Anal. Calcd for $C_{11}H_{13}F_2N_5O_3$: C, 43.86; H, 4.35; N, 23.25. Found: C, 44.17; H, 4.14; N, 23.05.

*General Procedure for the Synthesis of **2d** and **2e**.* To a solution of **21a** and **21c** (0.283 mmol) in EtOH (1.5 mL, 0.19 M) in a sealed glass tube, methylamine (40 wt % in H_2O, 10 mL) was added. After being stirred at room temperature for 2 h, the mixture was concentrated and added to a TFA/H_2O solution (2:1, v/v, total 15 mL) without purification. After being heated to 50 °C with stirring for 15 h, the reaction mixture was evaporated. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 9/1) to give **2d** and **2e**.

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*(1S,2R,3R,4R,5S)-4-Fluoro-3-(hydroxymethyl)-5-(6-(methylamino)-9H-purin-9-yl)cyclopentane-1,2-diol (**2d**).* It was obtained in 67% yield as a white solid; mp 197−201 °C; $[\alpha]_D^{25}$ = −61.46 (*c* 0.40, MeOH); ^{1}H NMR (800 MHz, CD_3OD): δ 8.27 (s, 1H), 8.20 (d, *J* = 18.4 Hz, 1H), 5.21 (dt, *J* = 4.0, 54.6 Hz, 1H), 4.98 (ddd, *J* = 3.4, 10.0, 29.6 Hz, 1H), 4.74 (dd, *J* = 6.7, 9.4 Hz, 1H), 4.01 (dd, *J* = 4.9, 6.4 Hz, 1H), 3.79−3.85 (m, 2H), 3.11 (br s, 3H), 2.42−2.51 (m, 1H); ^{13}C NMR (200 MHz, CD_3OD): δ 157.5, 154.6, 151.1, 141.8 (d, *J* = 3.7 Hz), 121.1, 92.9 (d, *J* = 180.8 Hz), 74.3, 71.8, 64.0 (d, *J* = 17.0 Hz), 60.6 (d, *J* = 10.5 Hz), 54.3 (d, *J* = 18.0 Hz), 28.5; ^{19}F NMR (376 MHz, CD_3OD): δ −206.3 (dt, *J* = 29.7, 53.4 Hz); UV (MeOH): λ_{max} 266.89 nm; HRMS (ESI^+): found, 298.1317 [calcd for $C_{12}H_{17}FN_5O_3^+$ $(M + H)^+$, 298.1315]; Anal. Calcd for $C_{12}H_{16}FN_5O_3$: C, 48.48; H, 5.42; N, 23.56. Found: C, 48.50; H, 5.22; N, 23.93.

*(1S,2R,3R,5S)-4,4-Difluoro-3-(hydroxymethyl)-5-(6-(methylamino)-9H-purin-9-yl)cyclopentane-1,2-diol (**2e**).* It was obtained in 76% yield as a white solid; mp 125−129 °C; $[\alpha]_D^{25}$ = −48.62 (*c* 0.25, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 8.24 (s, 1H), 8.20 (s, 1H), 5.33 (dt, *J* = 9.9, 18.4 Hz, 1H), 4.79 (dd, *J* = 10.3, 10.2 Hz, 1H), 4.17 (s, 1H), 3.81−3.90 (m, 2H), 3.10 (br s, 3H), 2.67 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ 157.5, 154.7, 151.5, 142.1, 125.9 (dd, *J* = 252.4, 258.1 Hz), 121.1, 73.7 (d, *J* = 7.25 Hz), 71.9 (d, *J* = 3.1 Hz), 64.7 (dd, *J* = 20.0, 24.3 Hz), 59.6 (d, *J* = 10.8 Hz), 56.4 (t, *J* = 19.9 Hz), 28.6; ^{19}F NMR (378 MHz, CD_3OD): δ −97.4 (d, *J* = 238.5 Hz), −115.3 (d, *J* = 238.9 Hz); UV (MeOH): λ_{max} 263.72 nm; HRMS (ESI^+): found, 316.1227 [calcd for $C_{12}H_{16}F_2N_5O_3^+$ $(M + H)^+$, 316.1221]; Anal. Calcd for $C_{12}H_{15}F_2N_5O_3$: C, 45.71; H, 4.80; N, 22.21. Found: C, 45.99; H, 4.47; N, 22.02.

*General Procedure for the Synthesis of **22a−c**.* To a cooled (−20 °C) solution of **19a−c** (1 equiv) in DMF (0.2 M), 3-methoxyacryloyl isocyanate (2 equiv) in benzene was added dropwise in a N_2 atmosphere. After the reaction mixture was slowly warmed to room temperature for 15 h with stirring, the reaction mixture was filtered with CH_2Cl_2 and co-evaporated with toluene and ethanol. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 1.5/1) to give **22a−c**.

*(E)-N-(((3aS,4S,5R,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)carbamoyl)-3-methoxyacrylamide (**22a**).* It was obtained in 76% yield as a colorless syrup; $[\alpha]_D^{25}$ = −19.41 (*c* 0.37, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.24 (s, 1H), 9.16 (d, *J* = 8.2 Hz, 1H), 7.61 (d, *J* = 12.4 Hz, 1H), 5.35 (d, *J* = 12.4 Hz, 1H), 5.06 (dt, *J* = 3.2, 52.7 Hz, 1H), 4.51 (t, *J* = 6.6 Hz, 1H), 4.29−4.38 (m, 2H), 3.64 (s, 3H), 3.45−3.52 (m, 2H), 2.21−2.31 (m, 1H), 1.41 (s, 3H), 1.21 (s, 3H), 1.10 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.0, 163.3, 155.4, 113.7, 97.5, 96.7 (d, *J* = 178.8 Hz), 84.4, 80.1, 72.9, 58.6 (d, *J* = 15.8 Hz), 57.8 (d, *J* = 6.5 Hz), 57.4, 49.8 (d, *J* = 17.2 Hz), 27.2 (3 × CH_3-*tert*-butyl), 27.1, 24.6; UV (MeOH): λ_{max} 243.14 nm; HRMS (ESI^+): found, 389.2088 [calcd for $C_{18}H_{30}FN_2O_6^+$ $(M + H)^+$, 389.2088].

*(E)-N-(((3aS,4S,5S,6R,6aR)-6-(tert-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)carbamoyl)-3-methoxyacrylamide (**22b**).* It was obtained in 88% yield as a colorless syrup; $[\alpha]_D^{25}$ = −20.47 (*c* 0.34, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.33 (s, 1H), 8.96 (d, *J* = 7.4 Hz, 1H), 7.63 (d, *J* = 12.3 Hz, 1H), 5.39 (d, *J* = 12.3 Hz, 1H), 4.80 (dt, *J* = 6.4, 52.5 Hz, 1H), 4.44 (t, *J* = 5.5 Hz, 1H), 4.33−4.41 (m, 2H), 3.67 (s, 3H), 3.46 (d, *J* = 32.5 Hz, 2H), 2.33−2.42 (m, 1H), 1.46 (s, 3H), 1.24 (s, 3H), 1.13 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.1, 163.2, 155.5, 111.9, 97.9 (d, *J* = 187.4 Hz), 97.5, 83.3 (d, *J* = 7.2 Hz), 79.0 (d, *J* = 6.5 Hz), 73.1, 61.9 (d, *J* = 23.7 Hz), 58.6 (d, *J* = 2.1 Hz), 57.4, 49.9 (d, *J* = 19.4 Hz), 27.3 (3 × CH_3-*tert*-butyl), 27.2, 25.0; UV (MeOH): λ_{max} 242.93 nm; HRMS (ESI^+): found, 389.2098 [calcd for $C_{18}H_{30}FN_2O_6^+$ $(M + H)^+$, 389.2088].

*(E)-N-(((3aS,4S,6R,6aR)-6-(tert-Butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)carbamoyl)-3-methoxyacrylamide (**22c**).* It was obtained in 90% yield as a colorless syrup; $[\alpha]_D^{25}$ = −40.41 (*c* 0.52, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.26 (s, 1 H), 9.11 (d, *J* = 8.7 Hz, 1H), 7.65 (d, *J* = 12.3 Hz, 1H), 5.37 (d, *J* = 12.4 Hz, 1H), 4.52−4.62 (m, 1H), 4.39 (s, 2H), 3.67 (s, 3H), 3.53−3.60 (m, 2H), 2.57−2.68 (m, 1H), 1.47 (s, 3H), 1.27 (s, 3H), 1.16 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.9, 163.4, 155.7, 126.9 (dd, *J* = 252.9, 261.3 Hz), 112.4, 97.4, 82.5 (d, *J* = 6.9 Hz), 78.6 (d, *J* = 4.9 Hz), 73.5, 60.6 (dd, *J* = 19.4, 29.2 Hz), 57.4 (d, *J* = 6.1 Hz), 57.3, 50.8 (t, *J* = 20.8 Hz), 27.1 (3 × CH_3-*tert*-butyl), 27.0, 24.9; UV (MeOH): λ_{max} 242.22 nm; HRMS (ESI^+): found, 407.1991 [calcd for $C_{18}H_{29}F_2N_2O_6^+$ $(M + H)^+$, 407.1994].

*General Procedure for the Synthesis of **2f−h**.* To a stirred solution of **22a−c** in 1,4-dioxane (3 mL, 2.5 M), 2 M sulfuric acid (0.3 mL) was dropwise added. After refluxing with stirring for 1 h, the reaction mixture was cooled to room temperature and neutralized with DOWEX 66 ion-exchange resin. The mixture was filtered and evaporated. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 9/1) to give **2f−h**.

*1-((1S,2R,3R,4R,5S)-2-Fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidine-2,4(1H,3H)-dione (**2f**).* It was obtained in 56% yield as a white solid; mp 112−118 °C; $[\alpha]_D^{25}$ = −77.11 (*c* 0.20, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 7.70 (dd, *J* = 1.1, 8.1 Hz, 1H), 5.69 (d, *J* = 8.0 Hz, 1H), 5.10 (dt, *J* = 4.1, 55.3 Hz, 1H), 4.91 (dd, *J* = 3.4, 10.2 Hz, 1H, merged with solvent peak), 4.46 (dd, *J* = 6.6, 10.1 Hz, 1H), 3.93 (t, *J* = 4.8 Hz, 1H), 3.70−3.80 (m, 2H), 3.69 (s, 1H), 2.29−2.41 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.9, 154.2, 145.5 (d, *J* = 3.8 Hz), 102.7, 93.0 (d, *J* = 180.1 Hz), 72.4, 71.7, 64.4 (d, *J* = 16.6 Hz), 60.6 (d, *J* = 11.4 Hz), 53.8 (d, *J* = 17.9 Hz); ^{19}F NMR (378 MHz, CD_3OD): δ −208.9 (dt, *J* = 29.9, 59.7 Hz); UV (MeOH): λ_{max} 264.11 nm; HRMS (ESI^+): found, 261.0886 [calcd for $C_{10}H_{14}FN_2O_5^+$ $(M + H)^+$, 261.0887]; Anal. Calcd for $C_{10}H_{13}FN_2O_5$: C, 46.16; H, 5.04; N, 10.77. Found: C, 45.98; H, 5.44; N, 10.98.

*1-((1S,2S,3R,4R,5S)-2-Fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidine-2,4(1H,3H)-dione (**2g**).* It was obtained in 53% yield as a white solid; mp 195−200 °C; $[\alpha]_D^{25}$ = −16.89 (*c* 0.35, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 7.60 (d, *J* = 7.9 Hz, 1H), 5.69 (d, *J* = 7.9 Hz, 1H), 5.07−5.21 (ddd, *J* = 5.10, 6.8, 55.2 Hz, 1H), 4.61−4.69 (ddd, *J* = 7.3, 8.7, 22.6 Hz, 1H), 4.32 (dd, *J* = 5.25, 9.0 Hz, 1H), 3.98 (t, *J* = 3.7 Hz, 1H), 3.70 (m, 2H), 2.24 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.1, 153.6, 147.4, 103.5, 94.8 (d, *J* = 183.9 Hz), 73.4 (d, *J* = 7.3 Hz), 73.1 (d, *J* = 22.0 Hz), 72.7 (d, *J* = 3.5 Hz), 62.3 (d, *J* = 1.8 Hz), 54.1 (d, *J* = 18.9 Hz); ^{19}F NMR (378 MHz, CD_3OD): δ −184.3 (dt, *J* = 23.8, 53.7 Hz); UV (MeOH): λ_{max} 265.33 nm; HRMS (ESI^+): found, 261.0894 [calcd for $C_{10}H_{14}FN_2O_5^+$ $(M + H)^+$, 261.0887]; Anal. Calcd for $C_{10}H_{13}FN_2O_5$: C, 46.16; H, 5.04; N, 10.77. Found: C, 46.24; H, 5.23; N, 10.78.

*1-((1S,3R,4R,5S)-2,2-Difluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidine-2,4(1H,3H)-dione (**2h**).* It was obtained in 52% yield as a white solid; mp 164−169 °C; $[\alpha]_D^{25}$ = −31.06 (*c* 0.30, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 7.67 (dd, *J* = 2.3, 8.1 Hz, 1H) 5.71 (d, *J* = 8.0 Hz, 1H), 5.36 (dt, *J* = 10.3, 17.7 Hz, 1H), 4.41 (dd, *J* = 5.15, 10.7 Hz, 1H), 4.07 (m, 1H), 3.73−3.82 (m, 2H), 2.53 (m, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 166.6, 154.1, 145.3 (d, *J* = 4.3 Hz), 126.8 (dd, *J* = 252.8, 258.5 Hz), 103.4, 72.5 (d, *J* = 7.9 Hz), 71.8 (d, *J* = 2.9 Hz), 64.4 (dd, *J* = 18.7, 25.1 Hz), 59.5 (d, *J* = 11.5 Hz), 56.3 (t, *J* = 20.1 Hz); ^{19}F NMR (378 MHz, CD_3OD): δ −96.6 (d, *J* = 238.9 Hz), −116.9 (dt, *J* = 15.1, 238.5 Hz); UV (MeOH): λ_{max} 262.41 nm; HRMS (ESI^+): found, 279.0801 [calcd for $C_{10}H_{13}F_2N_2O_5^+$ $(M + H)^+$, 279.0793]; Anal. Calcd for $C_{10}H_{12}F_2N_2O_5$: C, 43.17; H, 4.35; N, 10.07. Found: C, 43.34; H, 4.67; N, 9.94.

*General Procedure for the Synthesis of **2i** and **2j**. Benzoylation.* To a cooled (0 °C) solution of **2f** or **2h** (1 equiv) in CH_2Cl_2 (0.07 M), benzoyl chloride (6 equiv) and pyridine (6.7 equiv) were added in a N_2 atmosphere. After being stirred for 15 h at room temperature, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The organic layers were combined and washed with H_2O followed by brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified with column chromatography (silica gel, hexane/EtOAc, 1/1) to give the benzoylated intermediate.

Introduction of Triazole. To a cooled (0 °C) suspension of 1,2,4-triazole (10 equiv) in anhydrous MeCN (0.6 M), phosphoryl chloride (10 equiv) was added dropwise in a N_2 atmosphere. After stirring, the benzoylated intermediate (1 equiv) in MeCN (0.14 M) followed by trimethylamine (10 equiv) were added to the reaction mixture. After additional stirring at room temperature for 15 h, the reaction mixture was evaporated. The reaction mixture was diluted with CH_2Cl_2 and

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J. Med. Chem. 2019, 62, 6346−6362

H_2O. The layers were separated, and the organic layers were washed with H_2O, dried over $MgSO_4$, filtered, and evaporated.

Amination. In the sealed glass tube, the above-generated intermediate in 1,4-dioxane (0.06 M) was added to excess saturated aqueous ammonia at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was evaporated and purified with flash chromatography (silica gel, CH_2Cl_2/MeOH, 7/1) to give the benzoyl protected cytosine intermediate.

Benzoyl Deprotection. In a sealed glass tube, the above-generated benzoyl-protected cytosine intermediate in MeOH (0.2 M) was added to saturated ammonia in MeOH (0.2 M). After being stirred at the same temperature for 2 d, the reaction mixture was evaporated and diluted with H_2O and CH_2Cl_2. The layers were separated, and the H_2O layers were washed with CH_2Cl_2 10 times and evaporated to give **2i** and **2j**, respectively.

*4-Amino-1-((1S,2R,3R,4R,5S)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidin-2(1H)-one (**2i**).* It was obtained in 17% yield as a white solid; mp 230−233 °C; $[\alpha]_D^{25} = -84.26$ (*c* 0.20, MeOH); ^{1}H NMR (800 MHz, CD_3OD): δ 7.67 (dd, J = 1.3, 7.5 Hz, 1H), 5.88 (d, J = 7.4 Hz, 1H), 5.23 (dt, J = 3.7, 55.4 Hz, 1H), 4.93 (ddd, J = 3.4, 10.3, 30.4 Hz, 1H), 4.44 (dd, J = 6.6, 10.3 Hz, 1H), 3.92 (dd, J = 4.5, 6.3 Hz, 1H), 3.71−3.78 (m, 2H), 2.31−2.40 (m, 1H); ^{13}C NMR (200 MHz, $CDCl_3$): δ 168.3, 160.3, 145.7 (d, J = 3.1 Hz), 96.2, 93.0 (d, J = 179.9 Hz), 72.5, 71.8, 65.3 (d, J = 16.6 Hz), 60.7 (d, J = 11.3 Hz), 53.9 (d, J = 17.9 Hz); ^{19}F NMR (376 MHz, CD_3OD): δ −209.4 (dt, J = 29.3, 53.4 Hz); UV (MeOH): λ_{max} 274.67 nm; HRMS (ESI$^+$): found, 260.1041 [calcd for $C_{10}H_{15}FN_3O_4^+$ (M + H)$^+$, 260.1047]; Anal. Calcd for $C_{10}H_{14}FN_3O_4$: C, 46.33; H, 5.44; N, 16.21. Found: C, 46.71; H, 5.12; N, 15.99.

*4-Amino-1-((1S,3R,4R,5S)-2,2-difluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidin-2(1H)-one (**2j**).* It was obtained in 20% yield as a white solid; mp 242−245 °C; $[\alpha]_D^{25} = -39.85$ (*c* 0.30, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 7.62 (dd, J = 7.45, 2.35 Hz, 1H), 5.90 (d, J = 7.40 Hz, 1H), 5.51 (dt, J = 18.2, 10.0 Hz, 1H), 4.37 (dd, J = 10.6, 5.25 Hz, 1H), 4.06 (m, 1H), 3.73−3.83 (m, 2H), 2.54 (m, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 168.2, 160.1, 145.7 (d, J = 3.6 Hz), 126.9 (dd, J = 252.1, 259.2 Hz), 96.8, 72.9 (d, J = 8.6 Hz), 71.7 (d, J = 3.6 Hz), 65.1 (dd, J = 18.7, 23.0 Hz), 59.6 (d, J = 10.8 Hz), 56.3 (t, J = 20.1 Hz); ^{19}F NMR (378 MHz, CD_3OD): δ −97.4 (d, J = 235.9 Hz), −117.4 (dt, J = 14.7, 238.9 Hz); UV (MeOH): λ_{max} 272.27, 237.93 nm; HRMS (ESI$^+$): found, 278.0954 [calcd for $C_{10}H_{14}F_2N_3O_4^+$ (M + H)$^+$, 278.0952]; Anal. Calcd for $C_{10}H_{13}F_2N_3O_4$: C, 43.32; H, 4.73; N, 15.16. Found: C, 43.56; H, 4.56; N, 15.44.

*General Procedure for the Synthesis of **24**, **27a** and **27b**.* To a cooled (0 °C) suspension of **2c**, **2f**, and **2h** (1 equiv) in acetone (0.005 M) were added 1−2 drops of cH_2SO_4 in N_2 (g). After being stirred at room temperature for 4 h, the reaction mixture was neutralized with solid $NaHCO_3$, filtered, and evaporated under reduced pressure. The residue was further purified by silica gel column chromatography to give **24**, **27a**, and **27b**, respectively.

*((3aR,4R,6S,6aS)-6-(6-Amino-9H-purin-9-yl)-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)methanol (**24**).* It was obtained in 96% yield as a colorless syrup; ^{1}H NMR (500 MHz, CD_3OD): δ 8.31 (s, 1H), 8.21 (s, 1H), 5.30−5.40 (m, 2H), 4.70 (br s, 1H), 3.94 (dd, J = 6.8, 11.4 Hz, 1H), 3.86 (dd, J = 6.8, 11.4 Hz, 1H), 2.81−2.90 (m, 1H), 1.58 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 163.5 (dd, J = 33.1, 69.2 Hz), 156.5, 152.2, 152.0, 143.4, 128.0 (dd, J = 251.7, 263.6 Hz), 116.0, 80.7 (d, J = 7.3 Hz), 79.6 (d, J = 8.3 Hz), 66.1 (dd, J = 19.2, 22.8 Hz), 59.2 (d, J = 8.0 Hz), 53.8 (t, J = 19.4 Hz), 28.2, 25.9; HRMS (ESI$^+$) (m/z): found, 342.1370 [calcd for $C_{14}H_{18}F_2N_5O_3^+$ (M + H)$^+$, 342.1372]; Anal. Calcd for $C_{14}H_{17}F_2N_5O_3$: C, 49.27; H, 5.02; N, 20.52. Found: C, 49.28; H, 4.98; N, 20.91.

*1-((3aS,4S,5R,6R,6aR)-5-Fluoro-6-(hydroxymethyl)-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)pyrimidine-2,4-(1H,3H)-dione (**27a**).* It was obtained in 98% yield as a colorless syrup; ^{1}H NMR (500 MHz, CD_3OD): δ 7.75 (dd, J = 1.4, 8.1 Hz, 1H), 5.70 (d, J = 8.1 Hz, 1H), 5.20 (dt, J = 3.1, 54.1 Hz, 1H), 5.01−5.13 (m, 2H), 4.58 (d, J = 6.3 Hz, 1H), 3.73−3.83 (m, 2H), 2.42−2.56 (m, 1H), 1.50 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.7, 153.7, 145.3 (d, J = 5.9 Hz), 116.5, 103.2, 99.2 (d, J = 180.2 Hz), 82.2, 82.0, 65.0 (d, J = 15.7 Hz), 60.3 (d, J = 8.7 Hz), 53.2 (d, J = 17.7 Hz), 28.4, 25.9; HRMS (ESI$^+$) (m/z): found, 301.1185 [calcd for $C_{13}H_{18}FN_2O_5^+$ (M + H)$^+$, 301.1194]; Anal. Calcd for $C_{13}H_{17}FN_2O_5$: C, 52.00; H, 5.71; N, 9.33. Found: C, 52.15; H, 5.47; N, 9.15.

*1-((3aS,4S,6R,6aR)-5,5-Difluoro-6-(hydroxymethyl)-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)pyrimidine-2,4-(1H,3H)-dione (**27b**).* It was obtained in 97% yield; ^{1}H NMR (500 MHz, CD_3OD): δ 7.71 (dd, J = 2.0, 8.1 Hz, 1H), 5.73 (d, J = 8.1 Hz, 1H), 5.33 (dt, J = 6.8, 21.3 Hz, 1H), 4.94 (d, J = 6.8 Hz, 1H), 4.57−4.63 (m, 1H), 3.88 (dd, J = 6.7, 11.4 Hz, 1H), 3.81 (dd, J = 6.7, 11.4 Hz, 1H), 2.68−2.79 (m, 1H), 1.54 (s, 3H), 1.34 (s, 3H); HRMS (ESI$^+$) (m/z): found, 319.1104 [calcd for $C_{13}H_{17}F_2N_2O_5^+$ (M + H)$^+$, 319.1100]; Anal. Calcd for $C_{13}H_{16}F_2N_2O_5$: C, 49.06; H, 5.07; N, 8.80. Found: C, 49.43; H, 5.47; N, 8.43.

*Synthesis of tert-Butyl-(9-((3aS,4S,6R,6aR)-5,5-difluoro-6-(hydroxymethyl)-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-9H-purin-6-yl)carbamate (**25a**) and Its N^6-Di-Boc derivative (**25b**).* To a suspension of **24** (20 mg, 0.058 mmol) and 4-dimethylaminopyridine (1 mg, 0.0058 mmol) in hexamethyldisilazane (3 mL), trimethylsilyl trifluoromethanesulfonate (TMSOTf; 5 μL) was added dropwise at room temperature in a N_2 atmosphere (g). After being heated to 75 °C with stirring for 2 h, the reaction mixture was evaporated, and anhydrous THF (7 mL) was added. To a cooled (0 °C) reaction mixture, di-*t*-butyl dicarbonate (63 mg, 0.29 mmol) was added. After stirring for 4 h at room temperature, the reaction mixture was evaporated, and the residue was added to MeOH/trimethylamine (6 mL, 5:1 (v/v)). After heating to 55 °C with stirring for 16 h, the reaction mixture was evaporated, and the residue was purified with column chromatography (silica gel, CH_2Cl_2/MeOH, 50/1) to give **25a** (13 mg, 52%) and **25b** (8 mg, 25%) as a colorless syrup.

*Compound **25a**.* ^{1}H NMR (500 MHz, CD_3OD): δ 8.59 (s, 1H), 8.49 (s, 1H), 5.36−5.50 (m, 2H), 4.72 (d, J = 5.6 Hz, 1H), 3.95 (dd, J = 6.8, 11.4 Hz, 1H), 3.87 (dd, J = 6.8, 11.4 Hz, 1H), 2.83−2.95 (m, 1H), 1.57 (s, 12H), 1.34 (s, 3H); HRMS (ESI$^+$) (m/z): found, 442.1899 [calcd for $C_{19}H_{26}F_2N_5O_5^+$ (M + H)$^+$, 442.1897].

*Compound **25b**.* ^{1}H NMR (500 MHz, CD_3OD): δ 8.87 (s, 1H), 8.73 (d, J = 1.8 Hz, 1H), 5.46−5.57 (m, 2H), 4.75 (d, J = 5.4 Hz, 1H), 3.95 (dd, J = 6.8, 11.4 Hz, 1H), 3.88 (dd, J = 6.8, 11.4 Hz, 1H), 2.84−2.95 (m, 1H), 1.59 (s, 3H), 1.37 (s, 21H); ^{13}C NMR (125 MHz, CD_3OD): δ 156.2, 154.2, 152.2, 152.1 (2 × C(O)-Boc protection group), 147.8 (d, J = 2.4 Hz), 130.6, 128.1 (dd, J = 251.8, 263.3 Hz), 116.0, 86.1, 80.4 (d, J = 7.4 Hz), 79.7 (d, J = 8.2 Hz), 72.7, 66.5 (dd, J = 19.1, 23.1 Hz), 59.2 (d, J = 8.0 Hz), 53.8 (t, J = 19.2 Hz), 28.7 (6 × CH_3-*tert*-butyl), 28.3, 25.9; HRMS (ESI$^+$) (m/z): found, 542.2411 [calcd for $C_{24}H_{34}F_2N_5O_7^+$ (M + H)$^+$, 542.2421].

*Iso-propyl ((S)-(((3aR,4R,6S,6aS)-6-(6-((tert-Butoxycarbonyl)-amino)-9H-purin-9-yl)-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**26**).* To a stirred suspension of **25a** (16 mg, 0.036 mmol), **25b** (7 mg, 0.012 mmol), and powdered molecular sieves (4 Å, 62 mg) in anhydrous THF (20 mL), *tert*-butylmagnesium chloride solution (0.26 mL, 1.0 M in THF, 0.26 mmol) was added at 0 °C in a nitrogen atmosphere. After 10 min, a solution of pentafluorophosphoramidate reagent **A** (47 mg, 0.10 mmol) in THF (12 mL) was slowly added, and the reaction mixture was stirred at room temperature for 36 h. Then, it was quenched by the dropwise addition of methanol (10 mL), filtered, and evaporated. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 9/1) to give the phosphoramidate **26** as a colorless liquid (12 mg, 33%): ^{1}H NMR (500 MHz, CD_3OD): δ 8.59 (s, 1H), 8.45 (s, 1H), 7.37 (d, J = 7.8 Hz, 2H), 7.25 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 7.5 Hz, 1H), 5.50 (dt, J = 5.9, 22.3 Hz, 1H), 5.40−5.45 (m, 1H), 4.92−4.99 (m, 1H), 4.73−4.80 (m, 1H), 4.36−4.50 (m, 2H), 3.86−3.98 (m, 1H), 3.07−3.19 (m, 1H), 1.58 (s, 12H), 1.34 (s, 6H), 1.21 (d, J = 6.2 Hz, 3H), 1.17 (d, J = 6.2 Hz, 3H); HRMS (ESI$^+$) (m/z): found, 711.2716 [calcd for $C_{31}H_{42}F_2N_6O_9P^+$ (M + H)$^+$, 711.2713].

*Iso-propyl ((S)-(((1R,3S,4S,5R)-3-(6-Amino-9H-purin-9-yl)-2,2-difluoro-4,5-dihydroxycyclopentyl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**3a**).* A solution of **26** (15 mg, 0.021 mmol) in 10 mL of formic acid/H_2O (1:1, v/v) was stirred at room temperature for 8 h. After evaporation, the crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 6/1) to give **3a** (9.9 mg,

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J. Med. Chem. 2019, 62, 6346−6362

82%) as a colorless solid: mp 95−100 °C; UV (MeOH): λ_{max} 259.6 nm; $[\alpha]_D^{25}$ = −38.06 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 8.18 (s, 1H), 8.17 (d, *J* = 1.6 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 8.6 Hz, 2H), 7.18 (d, *J* = 8.0 Hz, 1H), 5.26−5.38 (m, 1H), 4.81−4.98 (m, merged with H_2O peak, 1H), 4.74 (dd, *J* = 4.8, 10.0 Hz, 1H), 4.29−4.43 (m, 2H), 7.17 (br s, 1H), 3.82−3.93 (m, 1H), 2.79−2.94 (m, 1H), 1.32 (d, *J* = 6.8 Hz, 3H), 1.19 (d, *J* = 6.2 Hz, 3H), 1.14 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD): δ 175.2 (d, *J* = 5.7 Hz), 158.1, 154.7, 153.0, 152.9, 152.6, 142.6, 131.6 (2 × CH-phenyl), 127.0, 124.4 (dd, *J* = 253.5, 260.6 Hz), 122.2 (d, *J* = 4.3 Hz), 120.6 (2 × CH-phenyl), 73.3 (d, *J* = 7.1 Hz), 71.2 (d, *J* = 5.0 Hz), 70.9, 64.6, 64.1 (dd, *J* = 5.0, 10.7 Hz), 52.4, 22.6 (d, *J* = 2.9 Hz, 2 × CH_3), 21.2 (d, *J* = 6.5 Hz); ^{19}F NMR (376 MHz, CD_3OD): δ −98.71 (d, *J* = 238.4 Hz), −115.13 (dt, *J* = 14.9, 236.4 Hz); HRMS (ESI^+) (*m*/*z*): found, 571.1889 [calcd for $C_{23}H_{30}F_2N_6O_7P^+$ (M + H)$^+$, 571.1876]; Anal. Calcd for $C_{23}H_{29}F_2N_6O_7P$: C, 48.42; H, 5.12; N, 14.73. Found: C, 48.74; H, 4.98; N, 14.54.

*Iso-propyl ((S)-(((1R,2R,3S,4S,5R)-3-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-fluoro-4,5-dihydroxycyclopentyl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**3b**). Introduction of Phosphoramidate.* To a cooled (0 °C) suspension of **27a** (21 mg, 0.069 mmol) and molecular sieves (4 Å, 35 mg) in anhydrous THF (15 mL, 0.005 M), *tert*-butylmagnesium chloride solution (0.34 mL, 1.0 M in THF, 0.34 mmol) was added dropwise in a N_2 atmosphere (g). After being stirred for 5 min, a solution of the phosphoramidate reagent **A** (31 mg, 0.069 mmol) in anhydrous THF (7 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 36 h, quenched with MeOH (5 mL), filtered, and evaporated, and the residue was purified by column chromatograph (silica gel, CH_2Cl_2/MeOH, 24/1) to give phosphoramidate as a colorless liquid (13 mg, 33%): ^{1}H NMR (500 MHz, CD_3OD): δ 7.73 (dd, *J* = 1.3, 8.1 Hz, 1H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.24 (d, *J* = 7.8 Hz, 2H), 7.19 (d, *J* = 7.4 Hz, 1H), 5.70 (d, *J* = 8.1 Hz, 1H), 5.02−5.22 (m, 3H), 4.93−5.01 (m, 1H), 4.66 (d, *J* = 6.3 Hz, 1H), 4.29 (d, *J* = 7.6 Hz, 2H), 3.87−3.95 (m, 1H), 2.62−2.73 (m, 1H), 1.51 (s, 3H), 1.34 (d, *J* = 7.7 Hz, 3H), 1.32 (s, 3H), 1.22 (d, *J* = 6.2 Hz, 6H); HRMS (ESI^+) (*m*/*z*): found, 570.2003 [calcd for $C_{25}H_{34}FN_3O_9P^+$ (M + H)$^+$, 570.2011].

Hydrolysis. A solution of phosphoramidate (13 mg, 0.022 mmol) in a formic acid/H_2O solution (1:1, v/v, 7 mL total) was stirred at room temperature for 8 h. The reaction mixture was evaporated and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 7/1) to give the phosphoramidate prodrug **3b** (10.8 mg, 90%) as a white solid: mp 107−110 °C; UV (MeOH): λ_{max} 262.8 nm; $[\alpha]_D^{25}$ = −59.40 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 7.64 (d, *J* = 8.1 Hz, 1H), 7.36 (d, *J* = 7.9 Hz, 2H), 7.23 (d, *J* = 7.9 Hz, 2H), 7.19 (d, *J* = 7.4 Hz, 1H), 5.68 (d, *J* = 8.1 Hz, 1H), 5.04 (dt, *J* = 4.1, 55.4 Hz, 1H), 4.87−4.98 (m, merged with H_2O peak, 2H), 4.45 (dd, *J* = 6.6, 9.7 Hz, 1H), 4.26 (d, *J* = 7.1 Hz, 2H), 3.99 (d, *J* = 5.4 Hz, 1H), 3.85−3.93 (m, 1H), 2.49−2.60 (m, 1H), 1.33 (d, *J* = 7.0 Hz, 3H), 1.21 (d, *J* = 6.1 Hz, 6H); ^{13}C NMR (125 MHz, CD_3OD): δ 175.2, 166.9, 154.0, 151.1, 145.4, 131.5 (2 × CH-phenyl), 126.9 (2 × CH-phenyl), 122.2 (d, *J* = 4.6 Hz), 102.8, 93.3 (d, *J* = 184.5 Hz), 80.3, 79.9 (d, *J* = 32.5 Hz), 72.3, 71.2, 70.9, 64.2 (d, *J* = 16.0 Hz), 52.4, 22.7 (d, *J* = 9.2 Hz, 2 × CH_3), 21.2 (d, *J* = 6.8 Hz); ^{19}F NMR (376 MHz, CD_3OD): δ −208.27 (dt, *J* = 29.7, 59.4 Hz); HRMS (ESI^+) (*m*/*z*): found, 530.1685 [calcd for $C_{22}H_{30}FN_3O_9P^+$ (M + H)$^+$, 530.1698]; Anal. Calcd for $C_{22}H_{29}FN_3O_9P$: C, 49.91; H, 5.52; N, 7.94. Found: C, 50.03; H, 5.32; N, 7.54.

*Iso-propyl ((S)-(((1R,3S,4S,5R)-3-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-difluoro-4,5-dihydroxycyclopentyl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**3c**).* Compound **3c** was synthesized according the same procedure used in the preparation of **3b**: yield = 30%; white solid; mp 174 °C (decomp); UV (MeOH): λ_{max} 262.8 nm; $[\alpha]_D^{25}$ = −19.40 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 7.53 (dd, *J* = 2.1, 8.1 Hz, 1H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.25 (d, *J* = 7.8 Hz, 2H), 7.20 (d, *J* = 7.6 Hz, 1H), 5.70 (d, *J* = 8.1 Hz, 1H), 5.29−5.39 (m, 1H), 4.93−5.02 (m, 1H), 4.30−4.39 (m, 2H), 4.23−4.29 (m, 1H), 4.08 (br s, 1H), 3.84−3.92 (m, 1H), 2.69−2.80 (m, 1H), 1.33 (d, *J* = 7.1 Hz, 3H), 1.22 (d, *J* = 6.2 Hz, 6H); ^{19}F NMR (376 MHz, CD_3OD): δ −98.47 (d, *J* = 237.2 Hz), −116.91 (dt, *J* = 17.6, 237.2 Hz); HRMS (ESI^+) (*m*/*z*): found, 548.1619 [calcd for $C_{22}H_{29}F_2N_3O_9P^+$ (M + H)$^+$, 548.1604]; Anal. Calcd for $C_{22}H_{28}F_2N_3O_9P$: C, 48.27; H, 5.16; N, 7.68. Found: C, 48.12; H, 4.98; 8.01.

SAH Hydrolase Assay.[18e−g,30] The gene encoding human placental SAH hydrolase was cloned into expression plasmid pPROKcd20. Recombinant SAH hydrolase protein was produced in *E. coli* JM109 in 50 mM Tris-HCl (pH 7.5) containing 2 mM ethylenediaminetetraacetic acid and was purified by DEAE-cellulose column (2.8 cm × 6 cm), ammonium sulfate fractionation (35−60%), Sephacryl S-300HR (1.0 cm × 105 cm), and DEAE cellulose (2.8 cm × 24 cm). The protein homogeneity was confirmed by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. The protein concentration was determined by using the Bradford method. Bovine serum albumin was a standard material for protein assay. Enzyme activity was determined in reaction mixtures (250 μL) that contain 50 mM sodium phosphate (pH 8.0), 2 μM SAH hydrolase (0.5 μM tetrameric form), and varying concentrations of compounds. The reaction mixtures were first preincubated with the compounds for 10 min at 37 °C, after which the reaction was initiated by adding 100 μM SAH. The reaction was allowed to proceed for 20 min, followed by the addition of 5,5′-dithiobis-2-nitrobenzoate (DNTB) to a final concentration of 200 μM. The absorbance of the product 5-thio-2-nitrobenzoic acid (TNB) was measured at 412 nm using a spectrophotometer (Varian, Cary 100). The molar extinction coefficient for TNB (ε_{412} = 13 700 M^{-1} cm^{-1}) was used in calculations to quantify TNB formation.

Cells, Viruses, and Compounds. Vero E6 and Vero CCL81 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Lonza), supplemented with 8% fetal calf serum (FCS; PAA), 2 mM L-glutamine, 100 IU/mL of penicillin and 100 μg/mL of streptomycin, and were grown at 37 °C in a humidified incubator with 5% CO_2. Vero cells were maintained in Eagle's minimum essential medium (EMEM; Lonza), supplemented with 8% FCS (FCS; PAA), 100 IU/mL of penicillin and 100 μg/mL of streptomycin, and were grown at 37 °C in a humidified incubator with 5% CO_2. Infections were performed in EMEM with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Lonza) supplemented with 2% FCS, L-glutamine, and antibiotics. Infectious clone-derived CHIKV(CHIKV-LS3) was generated as described by Scholte et al.[31] The ZIKV strain SL0612 was isolated from an infected traveler returning from Suriname as described by van Boheemen et al.[32] The Sindbis virus (SINV) strain HR and Semliki forest virus (SFV) strain SFV4 are part of the LUMC virus collection. The MERS-CoV strain EMC/2012 was isolated from patient material in the Dr. Soliman Fakeeh Hospital, Jeddah, Saudi Arabia, and was obtained from Erasmus Medical Center, Rotterdam.[33] The SARS-CoV strain Frankfurt 1 was provided by H. F. Rabenau and H. W. Doerr (Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany).[34] The compounds were dissolved in dimethylsulfoxide to obtain 20 mM stock solutions. All work with infectious CHIKV, MERS-CoV, SARS-CoV, and ZIKV was performed inside biosafety cabinets in the BSL-3 facilities of the Leiden University Medical Center.

Antiviral CPE-Reduction Assays. VeroE6 cells were seeded at a density of 5000 cells/well (CHIKV) and 10 000 cells/well (SARS-CoV, SFV and SINV) in a total volume of 100 μL per well in 96-well plates. Vero cells were seeded at a density of 20 000 cells/well when used for MERS-CoV infections, and Vero CCL81 cells were seeded at a density of 5000 cells/well for ZIKA infections under the same conditions as described for Vero E6. The following day, compound dilutions with concentrations of 150, 50, 16.7, and 5.6 μM were prepared in the infection medium by 3-fold serial dilution of the 150 μM solution. After replacing the culture medium with the respective dilutions of the compound, the cells were infected with CHIKV (MOI 0.005), SFV (MOI 0.025), SINV (MOI 0.025), ZIKV (MOI 0.05), MERS-CoV (MOI 0.005), or SARS-CoV (MOI 0.01). Viability assays were conducted in parallel. Each compound was tested at each concentration in quadruplicate (4 biological replicates per concentration). An MTS colorimetric assay was conducted 40 hpi for SFV, 76 hpi for SINV, 72 h hpi for MERS- and SARS-CoV, and 96 hpi for CHIKV and ZIKV by adding 20 μL/well of the CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS) reagent (Promega). The assay was stopped

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J. Med. Chem. 2019, 62, 6346−6362

after 2−2.5 h by fixing the cells with 37% formaldehyde. The absorbance was measured at 495 nm in a Berthold Mithras LB 940 plate reader, and the values were expressed relative to uninfected (infection) or untreated (viability) samples. The results represent the average of quadruplicate samples expressed as the mean ± SD. Compounds that were found to be protective were further evaluated in CPE reduction assays by testing 8 different concentrations to determine the EC_{50} as previously described.[31,34] The cytotoxicity (CC_{50}) of the compounds was determined in parallel, and all experiments were performed in quadruplicate. Graph-Pad Prism 8.0.1 was used for EC_{50} and CC_{50} determination by nonlinear regression.

Viral Load Reduction Assays. VeroE6 (CHIKV, ZIKV) cells were seeded at a density of 7.5×10^4 cells/well in 0.5 mL DMEM/8%FCS in 24-well cell culture plates and allowed to adhere overnight. For MERS-CoV and SARS-CoV, a cell density of or 6.0×10^4 cells/well of Vero E6 and Vero cells was used, respectively, under the same conditions as described above. The next day, compound dilutions (0−1.5 μM) were prepared in EMEM/2%FCS to which virus was added to yield inocula for infecting the cells with an MOI of 0.1 for CHIKV, MOI of 1 for ZIKV, and MOI of 0.01 for SARS- and MERS-CoV. Cells were incubated at 37 °C with 250 μL/well of the inoculum for 1 h (CHIKV and SARS- and MERS-CoV) or 2 h (ZIKV). After the infection, the cells were washed twice with 1 mL/well warm phosphate-buffered saline and 0.5 mL/well fresh EMEM/2%FCS with different concentrations of compound **2c** (0−1.5 μM) was added. The cells were incubated for 30 h (CHIKV) or 48 h (ZIKV, SARS- and MERS-CoV) at 37 °C, after which supernatants were harvested and stored at −80 °C for determination of the infectious virus titer by plaque assay. Viability assays were conducted in parallel as described in the previous paragraph. Plaque assays with CHIKV and SARS-CoV on VeroE6 cells, MERS-CoV on Vero cells, and ZIKV on Vero CCL81 cells were performed as described previously.[31,34a,35] Compound **2c** was tested at each concentration in duplicate in two independent experiments (n = 4). Graph-Pad Prism 8.0.1 was used for statistical analysis with a one-way ANOVA multiple comparison test.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.9b00781.

^{1}H and ^{13}C NMR copies of all final compounds **2a−j** and **3a−c** (PDF)

Crystallographic data for **2c**, **2g**, and **2h** (ZIP)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

RdRp, RNA-dependent RNA polymerase; SAH, *S*-adenosyl-L-homocysteine; SARS-CoV, severe acute respiratory syndrome coronavirus; CHIKV, chikungunya virus; ZIKV, Zika virus; nsps, nonstructural proteins; MTase, methyltransferase; NTP, nucleoside triphosphate; SAM, *S*-adenosyl-L-methionine; AK, adenosine kinase; LiHMDS, lithium hexamethyldisilazide; TESCl, triethylsilyl chloride; NFSI, *N*-fluorobenzenesulfonimide; NFOBS, *N*-fluoro-*O*-benzenedisulfonimide; $NaBH_4$, sodium borohydride; $LiBH_4$, lithium borohydride; NMO, *N*-methylmorpholine-*N*-oxide; TBS, *t*-butyldimethylsilyl; TBAF, tetra-*n*-butylammonium fluoride; DAST, *N*,*N*-diethylaminosulfur trifluoride; $AlMe_3$, trimethylaluminum; $SOCl_2$, thionyl chloride; DIPEA, *N*,*N*-diisopropylethylamine; TFA, trifluoroacetic acid; Boc_2O, di-*tert*-butyl dicarbonate; DNTB, 5,5′-dithiobis-2-nitrobenzoate; CPE, cytopathic effect; TMSOTf, trimethylsilyl trifluoromethanesulfonate; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; NEAA, non-essential amino acid; EMEM, Eagle's minimum essential medium; SINV, Sindbis virus; SFV, Semliki forest virus

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