Journal of Medicinal Chemistry

Article

Design, Synthesis and Anti-RNA Virus Activity of 6'-Fluorinated-aristeromycin Analogues

Ji-seong Yoon, Gyudong Kim, Dnyandev Jarhad, Hong-Rae Kim, Young-Sup Shin, Shuhao Qu, Pramod Sahu, Hea Ok Kim, Hyuk Woo Lee, Su Bin Wang, Yun Jeong Kong, Tong-Shin Chang, Natacha Ogando, Kristina Kovacikova, Eric J. Snijder, Clara Posthuma, Martijn van Hemert, and Lak Shin Jeong

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.9b00781 • Publication Date (Web): 07 Jun 2019

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

Ji-seong Yoon,[1,#] Gyudong Kim,[1,2,#] Dnyandev B. Jarhad,[1] Hong-Rae Kim,[1] Young-Sup Shin,[1] Shuhao Qu,[1] Pramod K. Sahu,[3] Hea Ok Kim,[3] Hyuk Woo Lee,[3] Su Bin Wang,[4] Yun Jeong Kong,[4] Tong-Shin Chang,[4] Natacha S. Ogando,[5] Kristina Kovacikova,[5] Eric J. Snijder,[5] Clara C. Posthuma,[5] Martijn J. van Hemert,[5] Lak Shin Jeong[1,*]

[1]Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Korea, [2]College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 500-757, Korea, [3]Future Medicine Co., Ltd, Seoul 06665, Korea, [4]College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea and [5]Department of Medical Microbiology, Leiden University Medical Center, Albinusdreef 2, 2333ZA Leiden, The Netherlands

lakjeong@snu.ac.kr

[#]Contributed equally to this work

Keywords: 6′-Fluorinated-ariseromycin, *S*-Adenosylhomocysteine hydrolase, Anti-RNA virus activity, Zika, MERS-CoV, Chikungunya, SARS-CoV

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-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 MERS-coronavirus, SARS-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 harbour 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 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] Since 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 methyltransferases 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 more narrow 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]

Since 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]

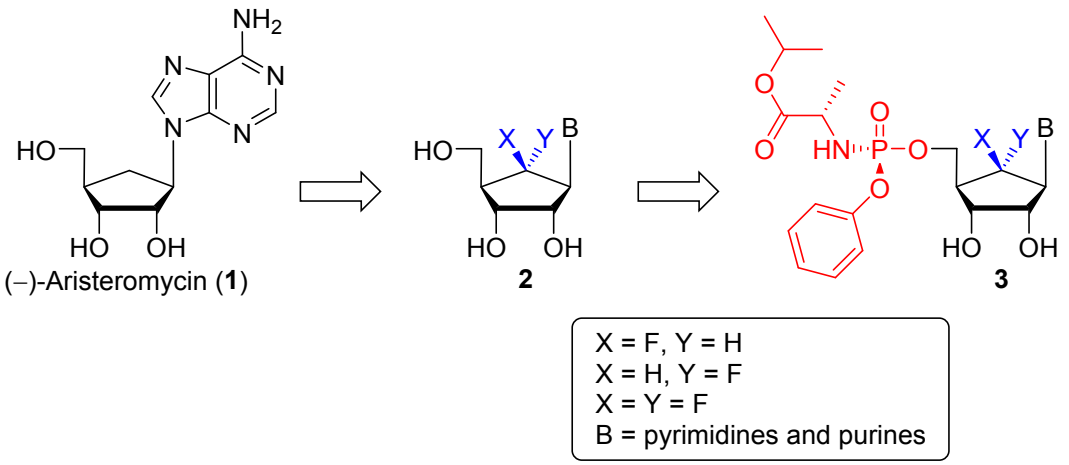


Figure 1. Rationale for the design of the target nucleosides **2** and **3**.

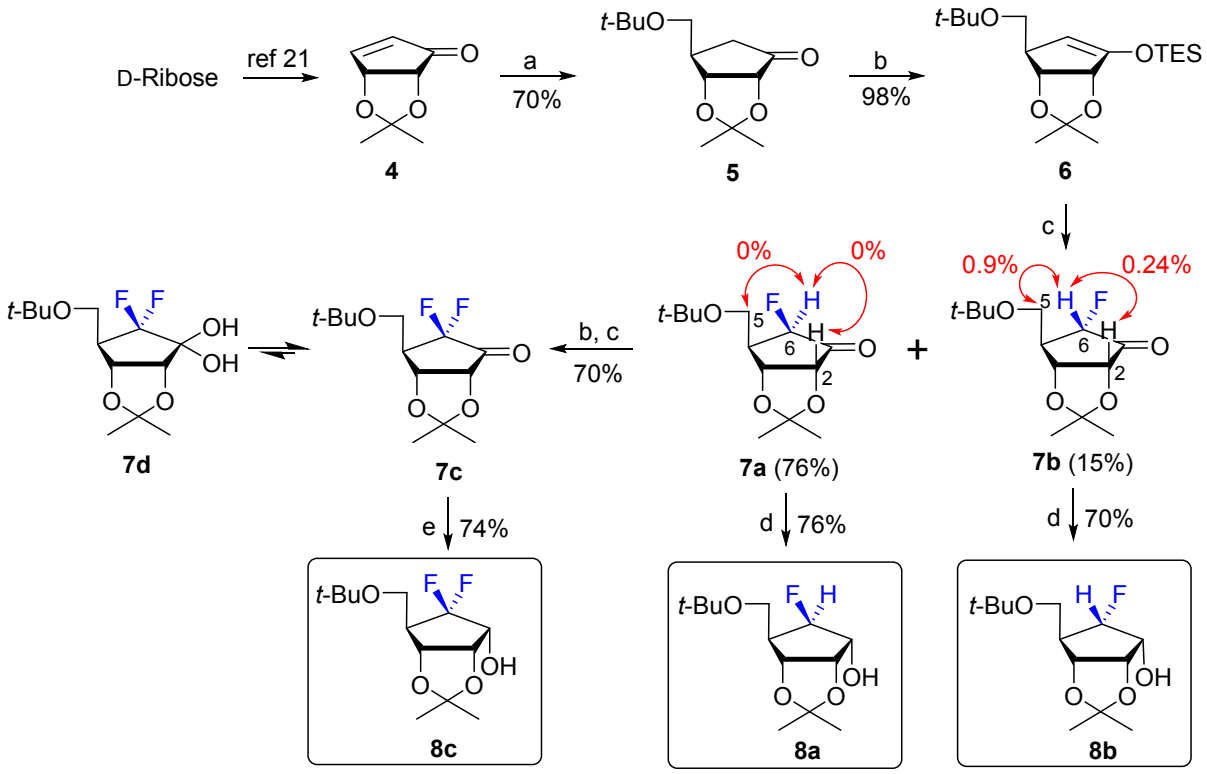
(–)-Aristeromycin (**1**) is a naturally occurring carbocyclic nucleoside, that was originally identified as a metabolite of *Streptomyces citricolor* in 1967.[16a] The first synthesis of **1** as racemate was reported by Clayton and his co-worker,[16b-d] and its asymmetric syntheses have since been 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 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 since biological activity can generally be attributed to one enantiomer, the D-isomer. Schneller 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 (TASF), 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 Fiugure 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′-monophosphorylation 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.

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



Reagents and conditions: a. LiCu(CH_2Ot-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.

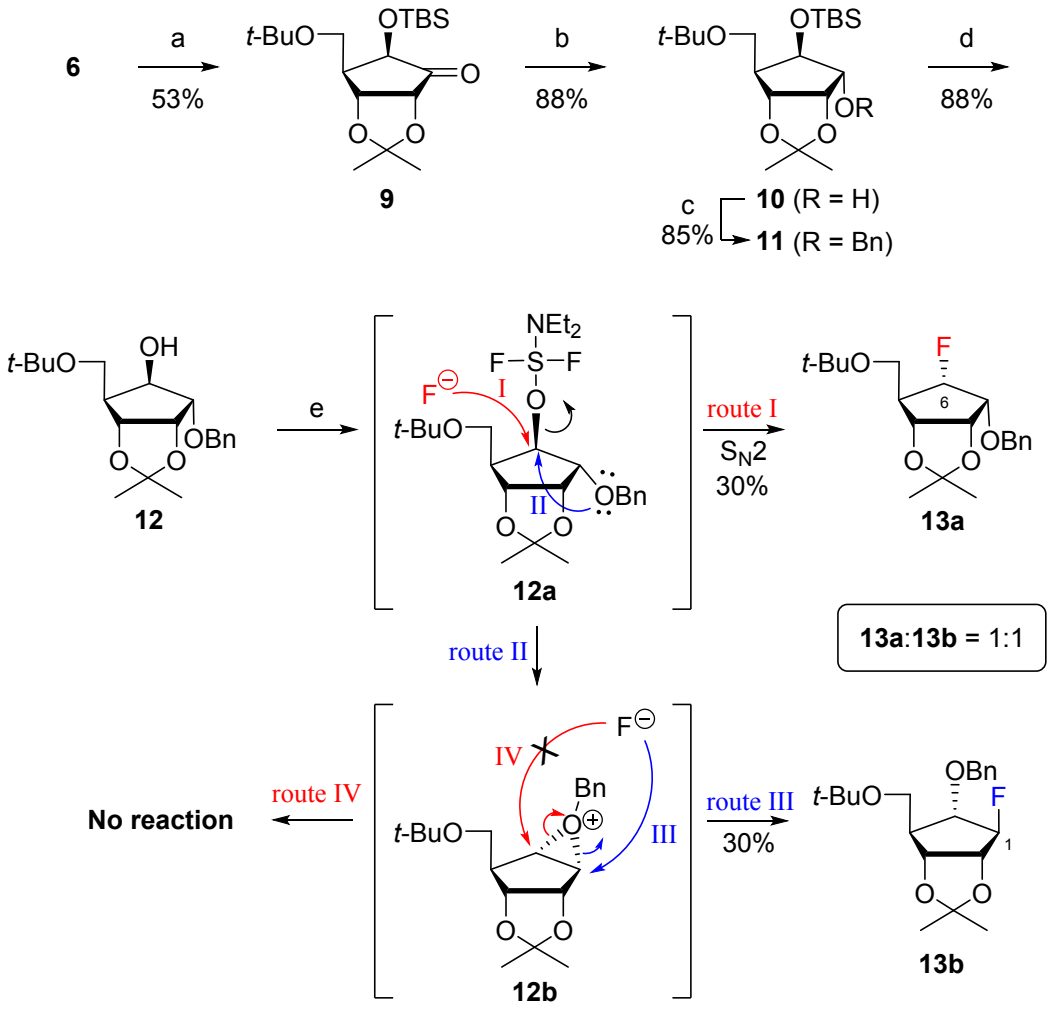
D-Ribose was converted to D-cyclopentenone **4** according to our previously published procedure.[21] The 1,4-conjugated addition of **4** with Gilman reagent yielded the D-cyclopentanone derivative **5**.[22] Treatment of **5** with lithium hexamethyldisilazide (LiHMDS) followed by trapping with triethylsilyl 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 DMF at 0 °C to yield a 5:1 ratio of 6-β-fluorosugar **7a** to 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 due to 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 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** (route III). However, the product via route IV was not formed because of the steric effect of *t*-butyloxymethyl substituent.

Scheme 2. Synthetic Approach to 6-α-Fluorosugar **8b** via Rubottom Oxidation

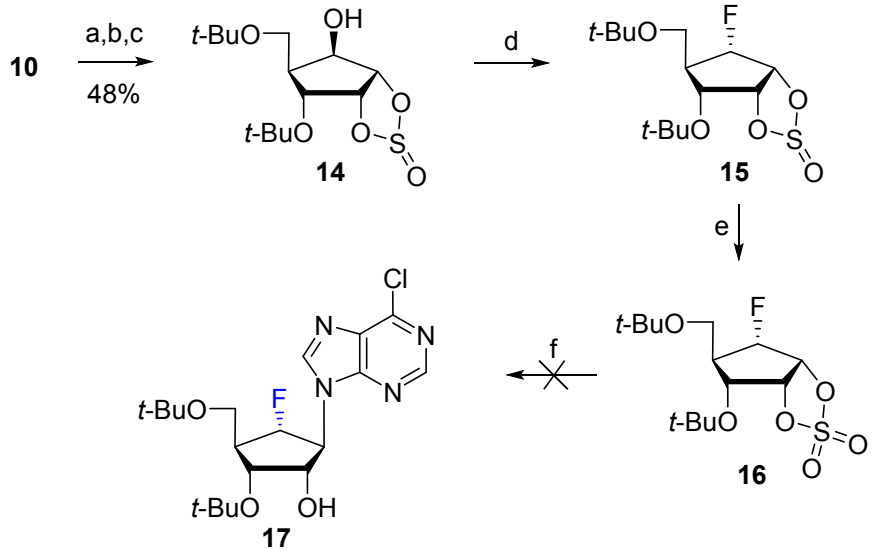


Reagents and conditions: a. i. OsO_4, NMO · H_2O, THF, rt, 1 h, then $NaHCO_3$, MeOH, rt, 3 h; ii. TBSCl, imidazole, DMF, rt, 3 h; b. $NaBH_4$, MeOH, rt, 1 h; c. BnBr, NaH, DMF, 0 °C to rt, 12 h; d. TBAF, THF, rt, 12 h; e. DAST, toluene, 0 °C to rt, 2 h.

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 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 anion; however, this resulted in decomposition.[19] Thus, we decided to synthesize the 6-α-fluoro derivative **8b** according to Scheme 1.

Scheme 3. Synthetic Approach to 6-α-Fluorosugar **8b** via Cyclic Sulfate

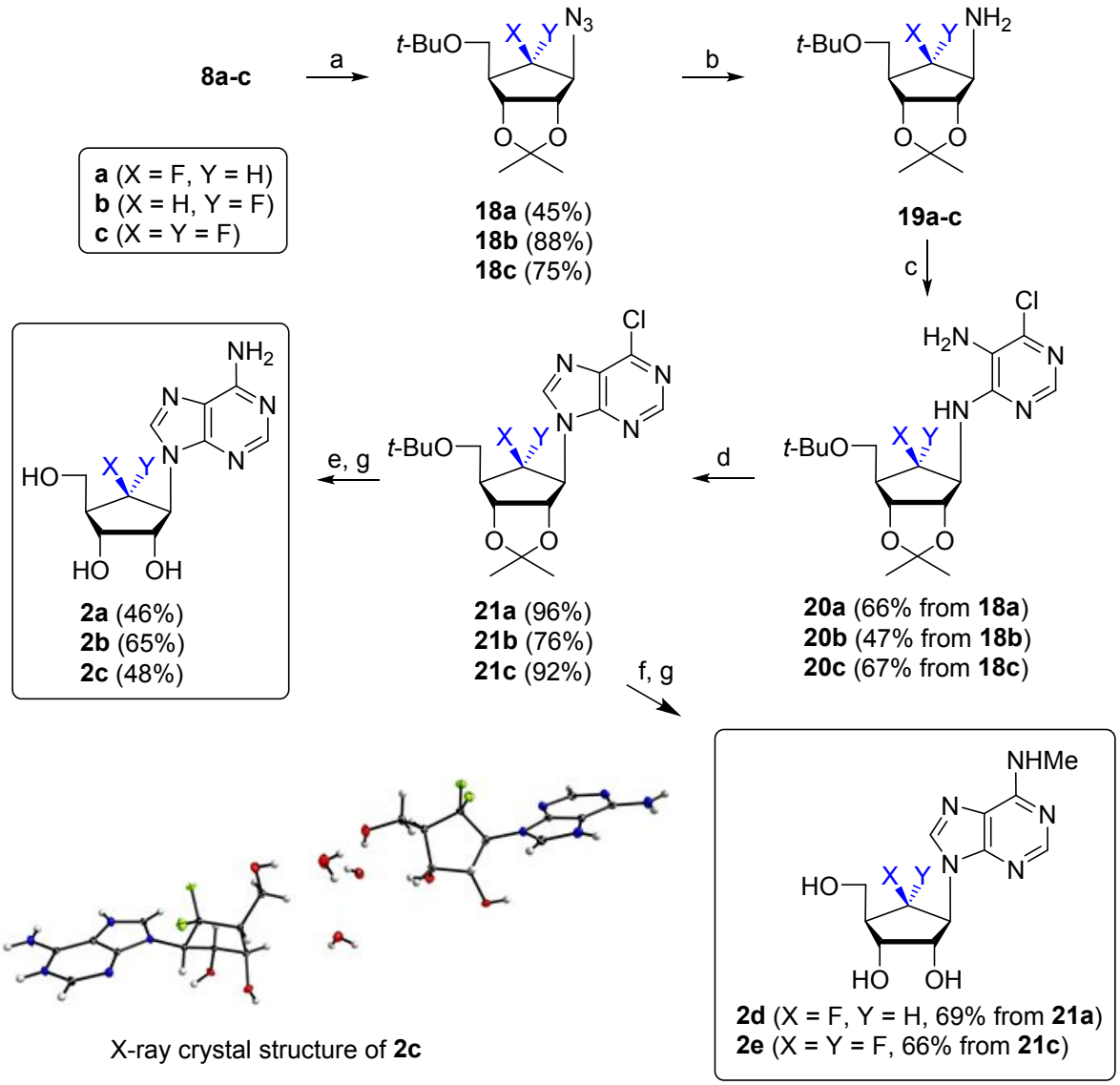


Reagents and conditions: a. $AlMe_3$, CH_2Cl_2, -78 °C to rt, 12 h; b. $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C, 10 min; c. TBAF, AcOH, THF, rt, 12 h; d. DAST, CH_2Cl_2, 0 °C to rt, 4 h; e. $RuCl_3$, $NaIO_4$, CCl_4:CH_3CN:H_2O (1/1/1.5), rt, 20 min; f. i. 6-chloropurine, 18-crown-6, NaH, THF, 65 °C, 15 h; ii.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

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.

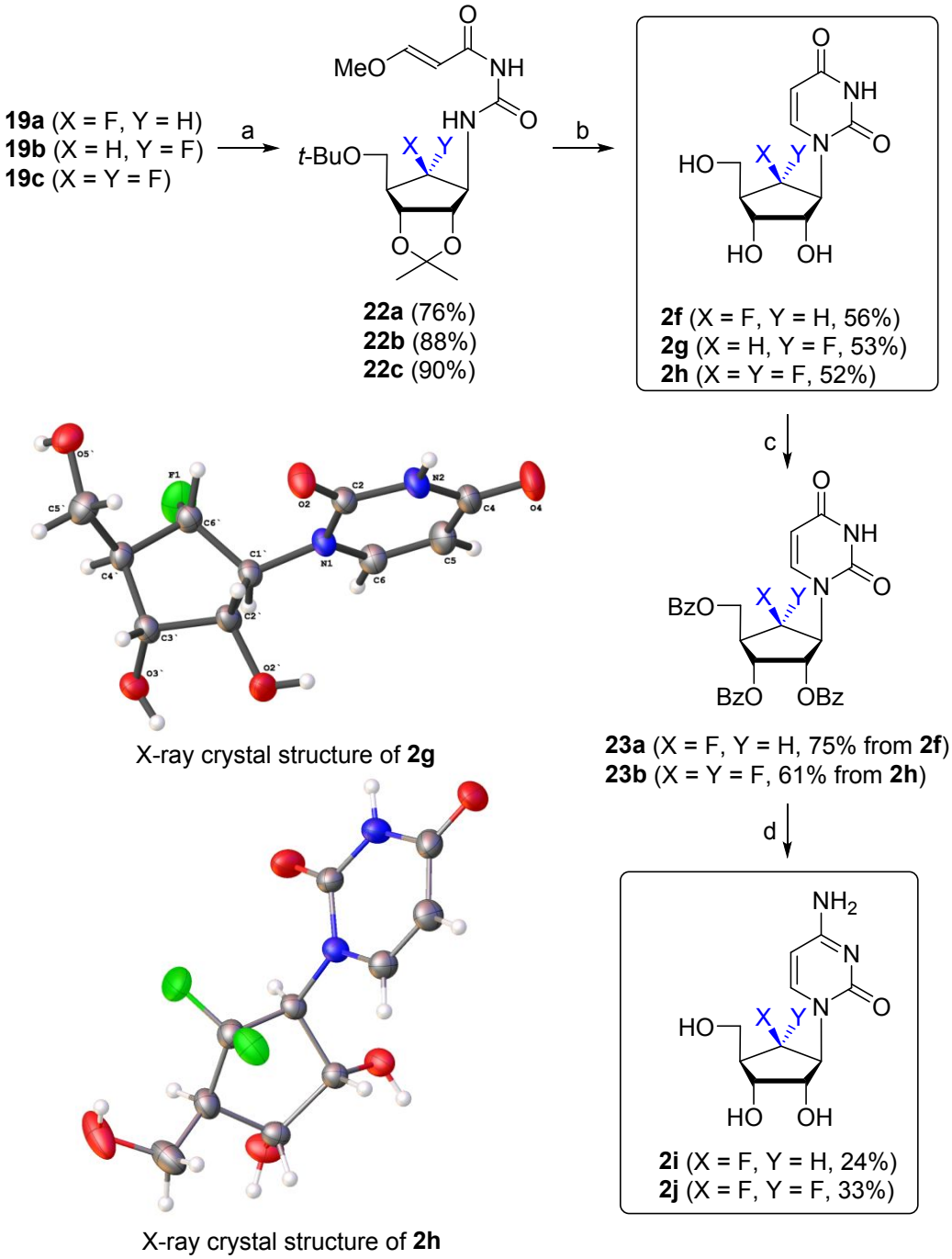
Scheme 4. Synthesis of β-Fluoro-, α-Fluoro-, and Difluoro-aristeromycin Analogues **2a-e**



X-ray crystal structure of **2c**

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.

Scheme 5. Synthesis of Fluorinated Pyrimidine Nucleoside Analogues **2f-j**

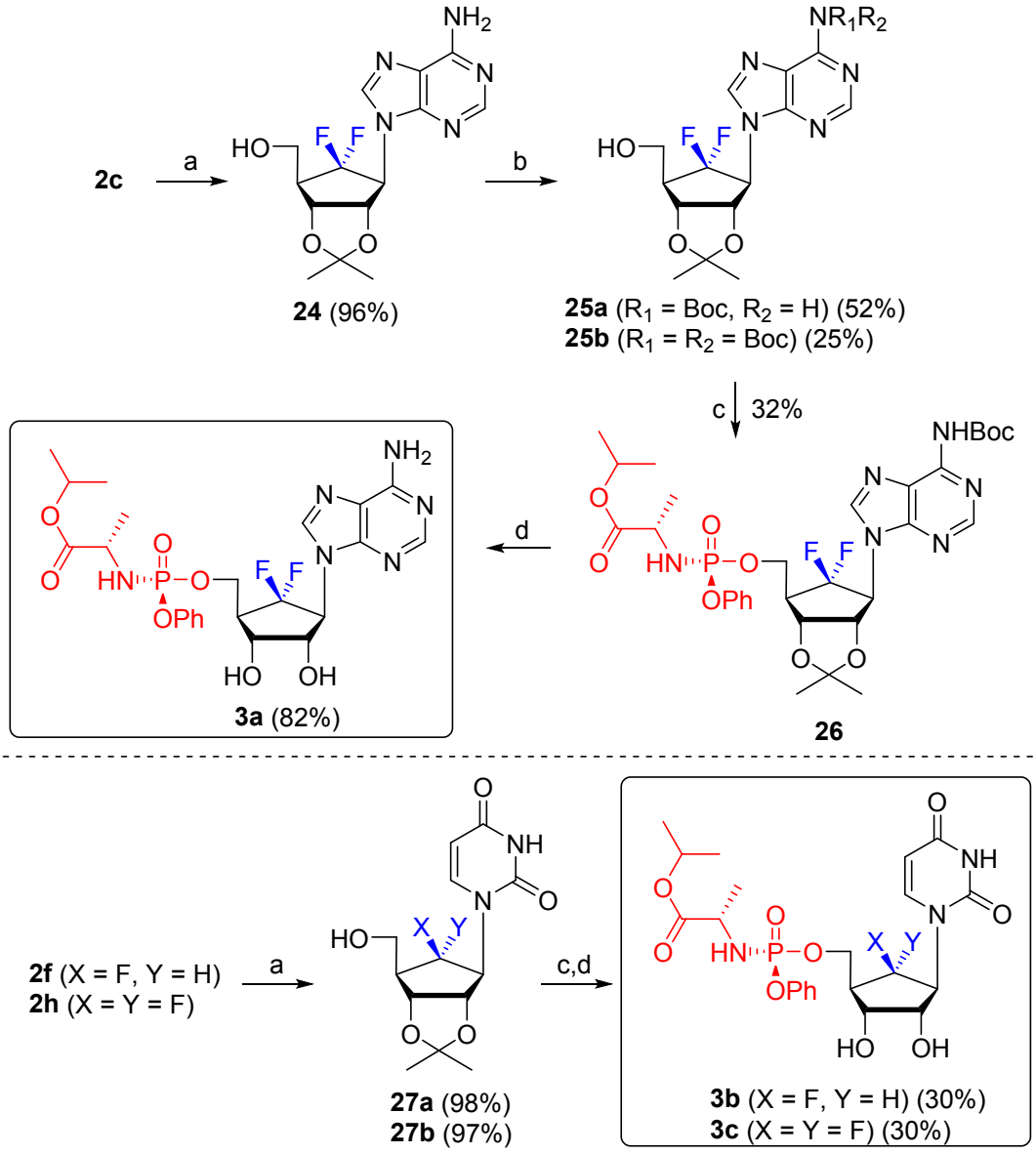


Reagents and conditions: a. (*E*)-3-methoxy-2-propenoyl isocyanate, benzene, 4Å-MS, DMF, -20 ^{o}C to rt, 15 h; b. 2 M H_2SO_4, dioxane, reflux, 1.5 h; c. BzCl, pyridine, CH_2Cl_2, rt, 15 h; d. i. 1,2,4-triazole, $POCl_3$, Et_3N, CH_3CN, rt, 15 h. ii. NH_4OH, dioxane, rt, 15 h. iii. NH_3/MeOH, rt, 15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

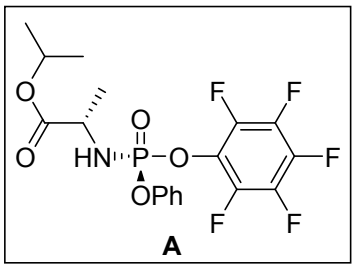
The amino derivatives **19a-c** were also converted into the pyrimidine nucleoside derivatives **2f-j**, as shown in Scheme 5. Treatment of **19a-c** with (*E*)-3-methoxy-2-propenoyl isocyanate,

which was prepared by reacting 3-methoxyacryloyl chloride with silver isocyanate,[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 converted to the cytidine derivatives **2i** and **2j** using a conventional three step procedures.[28]

Scheme 6. Synthesis of Phosphoamidate Prodrugs **3a-c**



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, 4Å-MS, THF, 0 °C to rt, 36 h; d. 50% HCOOH, rt, 8 h.



The uracil phosphoramidate analogue Sofosbuvir[20] is used in the clinic as a powerful anti-hepatitis C virus (HCV) 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**.

Table 1. Inhibition of SAH hydrolase and the replication of several +RNA viruses by all final nucleoside analogues **2a-j** and **3a-c**

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	

ND: Not Determined; Selectivity Index (SI) = CC_{50}/EC_{50}
EC_{50}: Effective concentration to inhibit the replication of the virus by 50%
CC_{50}: Cytotoxic concentration to inhibit the replication of normal cells by 50%
EC_{50}>100 indicates that no antiviral activity was observed at the highest concentration tested, either because there was no protection or the compound was toxic.

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 *E. coli* JM109, using a 5,5′-dithiobis-2-nitrobenzoate (DTNB) coupled assay as described by Lozada-Ramirez 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-mehyladenine 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 against a variety of +RNA viruses. The compounds were tested for antiviral activity in cytopathic effect (CPE) reduction assays at 4 concentrations, i.e. 150, 50, 16.7, and 5.6 μM by preaparing 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 100μM. 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 reduction in cytotoxicity, while its antiviral activity remained unchanged, with an EC_{50} of ~0.20 μM and a SI of 15 for MERS-CoV. This compound was also active against SARS-CoV with a 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 a 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 μM 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 phosphoamidate is more potent than the parent drug.[20] The phosphoamidate **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 insight into the mode of action of **2c** and **3a** and related compounds are currently ongoing and results will be published elsewhere.

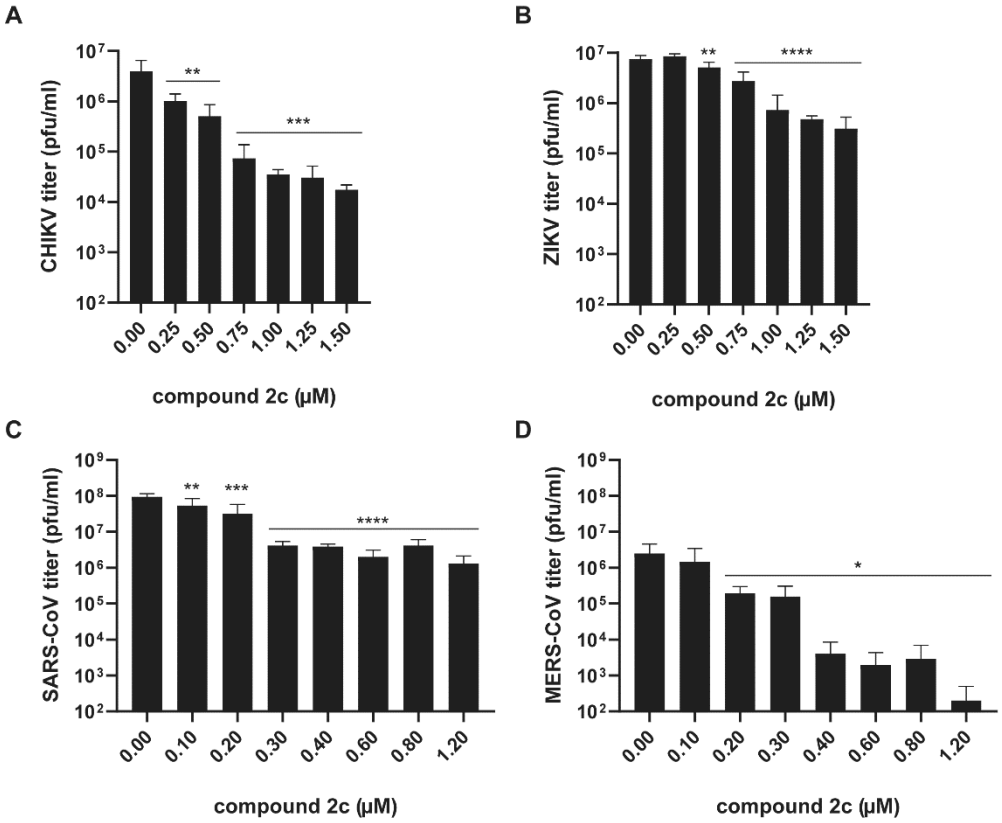


Figure 2: Effect of **2c** on the infectious progeny of CHIKV, ZIKV, SARS-CoV and MERS-CoV. Cells were infected with the virus indicated on the y-axis of the graph in medium with various concentrations of **2c**. Infectious progeny titers were determined by plaque assay (n = 4) and viability of non-infected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

Finally, we measured the log*P* of the most active compound **2c** by 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 phosphoamidate **3a**.

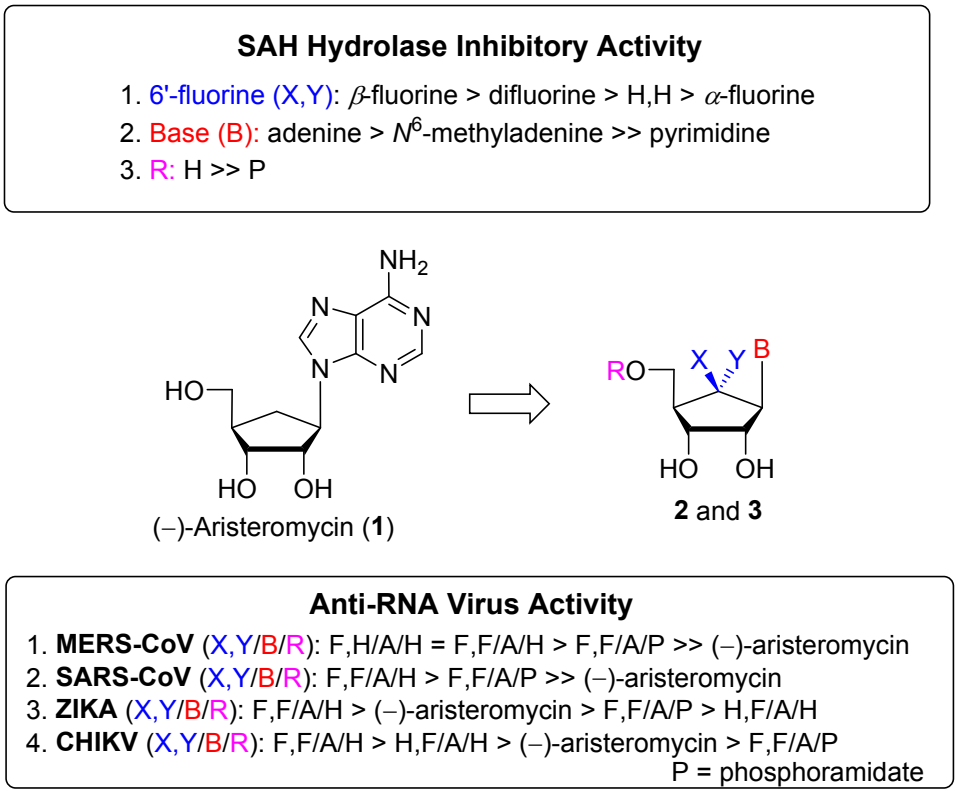


Figure 3. Summarized SAR of 6'-fluorinated aristeromycin analogues **2** and **3**.

■ CONCLUSION

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, **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 the latter was mainly due to indirect targeting of viral methylation reactions. The SAR studies and lack of antiviral effect of several purine and pyrimidine analogues suggests 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 (Hz). 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.

(((3a*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-2,2-dimethyl-6,6a-dihydro-3a*H*-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 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 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, 1 H), 4.58 (d, *J* = 2.1 Hz, 1 H), 4.36 (d, *J* = 6.1 Hz, 1 H), 3.27 (dd, *J* = 5.6, 8.6 Hz, 1 H), 3.15 (dd, *J* = 6.6, 8.6 Hz, 1 H), 2.72 (dd, *J* = 5.9, 5.9 Hz, 1 H), 1.42 (s, 3 H), 1.32 (s, 3 H), 1.12 (s, 9 H), 0.96 (t, *J* = 8.0 Hz, 9 H), 0.66-0.72 (m, 6 H); ^{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].

(3a*R*,5*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-fluoro-2,2-dimethyldihydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4(5*H*)-one (7a) and (3a*R*,5*S*,6*R*,6a*R*)-6-(*tert*-butoxymethyl)-5-fluoro-2,2-dimethyldihydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4(5*H*)-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: 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, 1 H), 4.70 (t, *J* = 5.7 Hz, 1 H), 4.20 (dd, *J* = 2.4, 6.1 Hz, 1 H), 3.61 (dd, *J* = 1.6, 8.6 Hz, 1 H) 3.38-3.41 (m, 1 H), 2.75 (d, *J* = 8.2 Hz, 1 H), 1.41 (s, 3 H), 1.30 (s, 3 H), 1.06 (s, 9 H); ^{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 –220.60~221.14 (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: 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, 1 H), 4.55 (d, *J* = 5.9 Hz, 1 H), 4.50 (d, *J* = 5.9 Hz, 1 H), 3.63 (d, *J* = 2.2 Hz, 2 H), 2.52-2.58 (m, 1 H), 1.41 (s, 3 H), 1.33 (s, 3 H), 1.13 (s, 9 H); ^{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 –196.0~196.2 (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.

(3a*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5,5-difluoro-2,2-dimethyldihydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4(5*H*)-one (7c). Yield = 70% (mixture of **7c** and **7d**); 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, 1 H), 4.72 (t, *J* = 6.1 Hz, 1 H), 4.52-4.57 (m, 1 H), 4.35-4.41 (m, 1 H), 4.25 (dd, *J* = 8.0, 4.0 Hz, 1 H), 3.74 (s, 1 H), 3.69 (d, *J* = 8.0 Hz, 1 H), 3.67-3.60 (m, 1 H), 3.54-3.59 (m, 1 H), 3.46 (d, *J* = 8.3 Hz, 1 H), 2.68 (d, *J* = 17.4 Hz, 1 H), 2.53-2.62 (m, 1 H), 1.48 (s, 3 H), 1.44 (s, 3 H), 1.34 (s, 3 H), 1.32 (s, 3 H), 1.21 (s, 9 H), 1.06 (s, 9 H).

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**.

(3a*S*,4*R*,5*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (8a). Yield = 71%; 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, 1 H), 4.51-4.52 (m, 1 H), 4.47 (ddd, *J* = 1.6, 6.3, 7.8 Hz, 1 H), 4.26-4.34 (m, 1 H), 3.52 (dd, *J* = 3.3, 8.8 Hz, 1 H), 3.36-3.39 (m, 1 H), 2.67 (d, *J* = 7.9 Hz, 1 H), 2.46 (bs, 1 H), 1.45 (s, 3 H), 1.32 (s, 3 H), 1.14 (s, 9 H); ^{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, $CDCl_3$) –211.0~211.21 (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.

(3a*S*,4*R*,5*S*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (8b). Yield = 67%; 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, 1 H), 4.46-4.53 (m, 2 H), 4.13-4.24 (m, 1 H), 3.33-3.40 (m, 1 H), 2.81 (d, *J* = 11.4 Hz, 1 H), 2.50 (dt, *J* = 2.9, 22.9 Hz, 1 H), 1.46 (s, 3 H), 1.30 (s, 3 H), 1.08 (s, 9 H); ^{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.

(3a*S*,4*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (8c). Yield = 74%; 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, 1 H), 4.44 (ddd, *J* = 2.6, 6.4, 8.9 Hz, 1 H), 4.20-4.29 (m, 1 H), 3.55 (d, *J* = 8.7 Hz, 1 H), 3.39 (d, *J* = 8.8 Hz, 1 H), 2.76 (d, *J* = 11.5 Hz, 1 H), 2.43 (d, *J* = 17.2 Hz, 1 H), 1.46 (s, 3 H), 1.31 (s, 3 H), 1.12 (s, 9 H); ^{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.

(3a*R*,5*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethyldihydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4(5*H*)-one (9). To a cooled (0 °C) solution of **6** (1275 mg, 3.57 mmol) in anhydrous THF (12 mL, 0.3 M) was 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 N_2 atmosphere. After stirring for 30 min, the reaction mixture was

added sodium thiosulfate pentahydrate (300 mg), sodium sulfite (300 mg) and acetone (30 mL) and stirred 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) was added *tert*-butyldimethylsilyl chloride (1614 mg, 10.71 mmol) and imidazole (729 mg, 10.71 mmol) under 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 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, 1 H), 4.53 (d, *J* = 8.0 Hz, 1 H), 4.11 (d, *J* = 6.3 Hz, 1 H), 3.61 (dd, *J* = 1.6, 8.0 Hz, 1 H), 3.30 (dd, *J* = 2.4, 8.1 Hz, 1 H), 2.41-2.46 (m, 1 H), 1.42 (s, 3 H), 1.30 (s, 3 H), 1.03 (s, 9 H), 0.88 (s, 9 H), 0.13 (s, 3 H), 0.05 (s, 3 H); ^{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.

(3a*S*,4*R*,5*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethyltetrahydro-3a*H*-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 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, 1 H), 4.41 (t, *J* = 6.2 Hz, 1 H), 4.07 (t, *J* = 6.9 Hz, 1 H), 3.95 (dd, *J* = 6.8, 14.7 Hz, 1 H), 3.48 (dd, *J* = 3.9, 8.5 Hz, 1 H), 3.32 (dd, *J* = 4.6, 8.5 Hz, 1 H), 2.43 (d, *J* = 8.4 Hz, 1 H), 2.12-2.18 (m, 1 H), 1.45 (s, 3 H), 1.32 (s, 3 H), 1.12 (s, 9 H), 0.87 (s, 9 H), 0.09 (s. 3 H), 0.05 (s, 3 H); ^{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.

(((3a*R*,4*R*,5*R*,6*R*,6a*R*)-4-(Benzyloxy)-6-(*tert*-butoxymethyl)-2,2-dimethyltetrahydro-3a*H*-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 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, 5 H), 4.76 (d, *J* = 12.4 Hz, 1 H), 4.59 (d, *J* = 12.4 Hz, 1 H), 4.45 (d, *J* = 6.0 Hz, 1 H), 4.33-4.37 (m, 2 H), 3.83 (dd, *J* = 5.6, 8.8 Hz, 1 H), 3.39 (dd, *J* = 4.4, 8.8 Hz, 1 H), 3.32 (dd, *J* = 4.0, 8.4 Hz, 1 H), 2.05-2.11 (m, 1 H), 1.48 (s, 3 H), 1.29 (s, 3 H), 1.03 (s, 9

H), 0.88 (s, 9 H), 0.09 (s, 3 H), 0.05 (s, 3 H); ^{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.

(3a*R*,4*S*,5*R*,6*S*,6a*R*)-4-(Benzyloxy)-6-(*tert*-butoxymethyl)-2,2-dimethyltetrahydro-3a*H*-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 tetra-*n*-butylammonium fluoride solution (1.2 mL, 1.0 M solution in THF, 1.2 mmol, 3.0 equiv) under 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, 2 H), 7.29-7.35 (m, 2 H), 7.23-7.28 (m, 1 H), 4.85 (d, *J* = 12.4 Hz, 1 H), 4.62 (d, *J* = 12.4 Hz, 1 H), 4.51 (t, *J* = 6.0 Hz, 1 H), 4.40-4.45 (m, 2 H), 3.81 (dd, *J* = 4.8, 7.2 Hz, 1 H), 3.58 (dd, *J* = 3.6, 8.8 Hz, 1 H), 3.44 (dd, *J* = 4.4, 8.8 Hz, 1 H), 2.70 (bs, 1 H), 2.26-2.32 (m, 1 H), 1.48 (s, 3 H), 1.31 (s, 3 H), 1.08 (s, 9 H); ^{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.

(3a*R*,4*R*,5*S*,6*R*,6a*R*)-4-(benzyloxy)-6-(*tert*-butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3a*H*-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 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, 5 H), 4.96 (ddd, *J* = 2.6, 6.8, 52.7 Hz, 1 H), 4.72 (dd, *J* = 0.8, 11.6 Hz, 1 H), 4.54 (d, *J* = 11.6 Hz, 1 H), 4.44-4.52 (m, 2 H), 4.02-4.09 (m, 1 H), 3.41-3.47 (m, 2 H), 2.15-2.18 (m, 1 H), 1.47 (s, 3 H), 1.28 (s, 3 H), 1.12 (s, 9 H); ^{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, 2 H), 7.31 (t, *J* = 7.2 Hz, 2 H), 7.25 (d, *J* = 7.2 Hz, 1 H), 5.18 (dt, *J* = 7.8, 53.7 Hz, 1 H), 4.76 (d, *J* = 12.2 Hz, 1 H), 4.66 (d, *J* = 12.2 Hz, 1 H), 4.45-4.49 (m, 1 H), 4.41-4.44 (m, 1 H), 4.19 (ddd, *J* = 5.9, 7.7, 16.5 Hz, 1 H), 3.45 (dd, *J* = 3.0, 8.8 Hz, 1 H), 3.31-3.34 (m, 1 H), 2.37-2.43 (m, 1 H), 1.47 (s, 3 H), 1.28 (s, 3 H), 1.01 (s, 9 H); ^{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.

(3a*R*,4*R*,5*S*,6*R*,6a*S*)-4-(*tert*-Butoxy)-5-(*tert*-butoxymethyl)-6-hydroxytetrahydro-3a*H*-cyclopenta[*d*][1,3,2]dioxathiole 2-oxide (14). *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 haxane, 6.727 mmol, 6.0 equiv) under 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. *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 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. *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 tetra-*n*-butylammonium fluoride solution (2.6 mL, 1.0 M solution in THF, 2.6 mmol, 4.0 equiv) under 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, 1 H), 5.02 (d, *J* = 5.9 Hz, 1 H), 4.79 (s, 1 H), 4.44 (dd, *J* = 4.8, 11.4 Hz, 1 H), 4.19 (d, *J* = 3.9 Hz, 1 H), 3.80 (dd, *J* = 2.6, 9.3 Hz, 1 H), 1.90-1.94 (m, 1 H), 1.27 (s, 9 H), 1.21 (s, 9 H); ^{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, 2 H), 4.79 (d, *J* = 6.4 Hz, 1 H), 4.36 (dd, *J* = 4.6, 11.5 Hz, 1 H), 4.31 (d, *J* = 4.1 Hz, 1 H), 3.84 (d, *J* = 9.2 Hz, 1 H), 3.77 (d, *J* = 9.3 Hz, 1 H), 2.65 (d, *J* = 10.1 Hz, 1 H), 1.25 (s, 9 H), 1.21 (s, 9 H).

(3a*R*,4*R*,5*R*,6*S*,6a*R*)-4-(*tert*-butoxy)-5-(*tert*-butoxymethyl)-6-fluorotetrahydro-3a*H*-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 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, 1 H), 5.03 (t, *J* = 8.2 Hz, 1 H), 4.92 (ddd, *J* = 5.0, 8.7, 17.8 Hz, 1 H), 4.06 (ddd, *J* = 7.8, 11.0, 16.5 Hz, 1 H), 3.53 (ddd, *J* = 2.7, 2.7, 6.8 Hz, 1 H), 3.44 (dd, *J* = 2.2, 9.1 Hz, 1 H), 2.54-2.58 (m, 1 H), 1.17 (s, 18 H); ^{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).

(3a*R*,4*R*,5*R*,6*S*,6a*R*)-4-(*tert*-butoxy)-5-(*tert*-butoxymethyl)-6-fluorotetrahydro-3a*H*-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 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**.

(3a*S*,4*S*,5*R*,6*R*,6a*R*)-4-Azido-6-(*tert*-butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole (18a). Yield = 45%; 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, 1 H), 4.66 (t, *J* = 6.0 Hz, 1 H), 4.41 (t, *J* = 6.5 Hz, 1 H), 3.62-3.69 (m, 1 H), 3.54 (s, 1 H), 3.50 (s, 1 H), 2.27-2.36 (m, 1 H), 1.47 (s, 3 H), 1.29 (s, 3 H), 1.16 (s, 9 H); ^{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, $CDCl_3$) –206.9~207.2 (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.

(3a*S*,4*S*,5*S*,6*R*,6a*R*)-4-Azido-6-(*tert*-butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole (18b). Yield = 88%; 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, 1 H), 4.41 (dd, *J* = 4.5, 6.7 Hz, 1 H), 4.22 (t, *J* = 5.7 Hz, 1 H), 4.00 (ddd, *J* = 5.5, 7.4, 16.6 Hz, 1 H), 3.43-3.50 (m, 2 H), 2.33-2.44 (m, 1 H),1.50 (s, 3 H), 1.27 (s, 3 H), 1.15 (s, 9 H); ^{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.

(3a*S*,4*S*,6*R*,6a*R*)-4-Azido-6-(*tert*-butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole (18c). Yield = 75%; colorless syrup; $[\alpha]_D^{25} = -43.39$ (*c* 0.36, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.40-4.44 (m, 1 H), 4.34-4.39 (m, 1 H), 3.87-3.95 (m, 1 H), 3.61 (dd, *J* = 6.5, 9.3 Hz, 1 H), 3.48 (t, *J* = 7.6 Hz, 1 H), 2.54-2.66 (m, 1 H), 1.49 (s, 3 H), 1.28 (s, 3 H), 1.17 (s, 9 H); ^{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}-((3a*S*,4*S*,5*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)-6-chloropyrimidine-4,5-diamine (20a).** Yield = 66% 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, 1 H), 5.27-5.33 (bs, 1 H,), 5.24 (td, J = 3.5, 52.9 Hz, 1 H), 4.71-4.81 (m, 1 H), 4.57 (t, J = 6.1 Hz, 1 H), 4.44 (t, J = 6.3 Hz, 1 H), 3.58-3.63 (m, 1 H), 3.53 (t, J = 9.2 Hz, 1 H), 3.39 (bs, 2 H), 2.42-2.55 (m, 1 H), 1.52 (s, 3 H), 1.30 (s, 3 H), 1.18 (s, 9 H); ^{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, $CDCl_3$) –212.8~213.1 (m); UV (CH_2Cl_2) λ_{max} 287 nm; LRMS (ESI^+) found 388.17 [calcd for $C_{17}H_{27}ClFN_4O_3^+$ $(M+H)^+$ 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}-((3a*S*,4*S*,5*S*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)-6-chloropyrimidine-4,5-diamine (20b).** Yield = 47% from

18b; yellow foam; $[\alpha]_D^{25} = -11.79$ (*c* 0.36, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1 H), 5.56 (d, *J* = 9.2 Hz, 1 H), 4.89 (dt, *J* = 3.1, 51.0 Hz, 1 H), 4.77 (dd, *J* = 9.1, 21.2 Hz, 1 H), 4.61 (dd, *J* = 2.5, 5.0 Hz, 1 H), 4.51 (dd, *J* = 2.4, 6.0 Hz, 1 H), 3.60 (dd, *J* = 2.6, 9.2 Hz, 1 H), 3.55 (dd, *J* = 2.5, 9.3 Hz, 1 H), 3.39 (bs, 2 H), 2.60 (d, *J* = 23.5 Hz, 1 H), 1.54 (s, 3 H), 1.29 (s, 3 H), 1.21 (s, 9 H); ^{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 $C_{17}H_{26}lFN_4O_3$: C, 52.51; H, 6.50; N, 14.45. Found: C, 52.56; H, 6.51; N, 14.43.

***N*^{4}-((3a*S*,4*S*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)-6-chloropyrimidine-4,5-diamine (20c).** Yield = 67% from **18c**; yellow foam; $[\alpha]_D^{25} = -61.76$ (*c* 0.23, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (s, 1 H), 5.71 (d, *J* = 10.1 Hz, 1 H), 5.03 (t, *J* = 12.7 Hz, 1 H), 4.56 (t, *J* = 4.6 Hz, 1 H), 4.40-4.45 (m, 1 H), 3.69 (dd, *J* = 2.6, 9.5 Hz, 1 H), 3.57 (dd, *J* = 4.4, 9.4 Hz, 1 H), 3.38 (bs, 2 H), 2.72 (d, *J* = 14.7 Hz, 1 H), 1.53 (s, 3 H), 1,44 (s, 3 H), 1.25 (s, 9 H); ^{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-((3a*S*,4*S*,5*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)-6-chloro-9*H*-purine (21a). Yield = 96%; yellow foam; $[\alpha]_D^{25}$ = –29.2 (*c* 0.17, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1 H), 8.34 (d, *J* = 2.4 Hz, 1 H), 5.28-5.43 (td, *J* = 2.8, 52.8 Hz, 1 H), 5.12-5.23 (m, 2 H), 4.61 (t, *J* = 5.0 Hz, 1 H), 3.65-3.69 (m, 1 H), 3.61 (t, *J* = 9.2 Hz, 1 H), 2.56-2.71 (m, 1 H), 1.56 (s, 3 H), 1.32 (s, 3 H), 1.17 (s, 9 H); ^{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-((3a*S*,4*S*,5*S*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)-6-chloro-9*H*-purine (21b). Yield = 76%; yellow foam; $[\alpha]_D^{25}$ = –31.54 (*c* 0.54, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1 H), 8.15 (s, 1 H), 5.55 (dt, *J* = 8.4, 53.6 Hz, 1 H), 5.02 (t, *J* = 6.4 Hz, 1 H), 4.84-4.94 (m, 1 H), 4.65 (t, *J* = 5.1 Hz, 1 H), 3.53-3.63 (m, 2 H), 2.47-2.57 (m, 1 H), 1.54 (s, 3 H), 1.25 (s, 3 H), 1.17 (s, 9 H); ^{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-((3a*S*,4*S*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)-6-chloro-9*H*-purine (21c). Yield = 92%; yellow foam; $[\alpha]_D^{25}$ = –46.05 (*c* 0.43, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (s, 1 H), 8.28 (d, *J* = 2.1 Hz, 1

H), 5.30 (dt, *J* = 6.9, 20.1 Hz, 1 H), 5.10 (t, *J* = 6.7 Hz, 1 H), 4.57-4.62 (m, 1 H), 3.63-3.73 (m, 2 H), 2.81-2.93 (m, 1 H), 1.56 (s, 3 H), 1.30 (s, 3 H), 1.18 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.4, 152.4, 151.3, 143.9 (d, *J* = 4.0 H), 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 trifluoroacetic acid/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**.

(1*R*,2*S*,3*S*,4*R*,5*R*)-3-(6-Amino-9*H*-purin-9-yl)-4-fluoro-5-(hydroxymethyl)cyclopentane-1,2-diol (2a). Yield = 43%; 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, 1 H), 8.21 (s, 1 H), 5.21 (dt, *J* = 4.0, 54.6, 1 H), 4.99 (ddd, *J* = 3.4, 10.8, 29.5 Hz, 1 H), 4.75 (dd, *J* = 6.7, 9.4 Hz, 1 H), 4.02 (dd, *J* = 4.8, 6.4 Hz, 1 H), 3.79-3.85 (m, 2 H), 2.42-2.51 (m, 1 H); ^{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 ~ 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.

(1*R*,2*S*,3*S*,4*S*,5*R*)-3-(6-Amino-9*H*-purin-9-yl)-4-fluoro-5-(hydroxymethyl)cyclopentane-1,2-diol (2b). Yield = 71%; 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, 1 H), 5.40 (ddd, *J* = 5.2, 7.3, 54.4 Hz, 1 H), 5.03 (ddd, *J* = 7.5, 9.8, 20.7 Hz, 1 H), 4.60 (dd, *J* = 5.1, 9.9 Hz, 1 H), 4.05-4.09 (m, 1 H), 3.80 (d, *J* = 5.8 Hz, 2 H), 2.28-2.40 (m, 1 H); ^{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.

(1*R*,2*S*,3*S*,5*R*)-3-(6-Amino-9*H*-purin-9-yl)-4,4-difluoro-5-(hydroxymethyl)cyclopentane-1,2-diol (2c). Yield = 61%; 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, 1 H), 8.20 (s, 1 H), 5.33 (dt, *J* = 10.0, 17.0 Hz, 1 H), 4.79 (dd, *J* = 5.2, 10.6 Hz, 1 H, merged with solvent peak), 4.13-4.17 (m, 1 H), 3.79-3.91 (m, 2 H), 2.60-2.71 (m, 1 H); ^{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 trifluoroacetic acid/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** respectively.

(1*S*,2*R*,3*R*,4*R*,5*S*)-4-Fluoro-3-(hydroxymethyl)-5-(6-(methylamino)-9*H*-purin-9-yl)cyclopentane-1,2-diol (2d). Yield = 67%; 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, 1 H), 8.20 (d, *J* = 18.4 Hz, 1 H), 5.21 (dt, *J* = 4.0, 54.6 Hz, 1 H), 4.98 (ddd, *J* = 3.4, 10.0, 29.6 Hz, 1 H), 4.74 (dd, *J* = 6.7, 9.4 Hz, 1 H), 4.01 (dd, *J* = 4.9, 6.4 Hz, 1 H), 3.79-3.85 (m, 2 H), 3.11 (bs, 3 H), 2.42-2.51 (m, 1 H); ^{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.

(1*S*,2*R*,3*R*,5*S*)-4,4-Difluoro-3-(hydroxymethyl)-5-(6-(methylamino)-9*H*-purin-9-yl)cyclopentane-1,2-diol (2e). Yield = 76%; 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, 1 H), 8.20 (s, 1 H), 5.33 (dt, *J* = 9.9, 18.4 1H), 4.79 (dd, *J* = 10.3, 10.2 Hz, 1 H), 4.17 (s, 1 H), 3.81-3.90 (m, 2 H), 3.10 (bs, 3 H), 2.67 (m, 1 H); ^{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*-(((3a*S*,4*S*,5*R*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)carbamoyl)-3-methoxyacrylamide (22a). Yield = 76%; colorless syrup; $[\alpha]_D^{25} = -19.41$ (*c* 0.37, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.24 (s, 1 H), 9.16 (d, *J* = 8.2 Hz, 1 H), 7.61 (d, *J* = 12.4 Hz, 1 H), 5.35 (d, *J* = 12.4 Hz, 1 H), 5.06 (dt, *J* = 3.2, 52.7 Hz, 1 H), 4.51 (t, *J* = 6.6 Hz, 1 H), 4.29-4.38 (m, 2 H), 3.64 (s, 3 H), 3.45-3.52 (m, 2 H), 2.21-2.31 (m, 1 H), 1.41 (s, 3 H), 1.21 (s, 3 H), 1.10 (s, 9 H); ^{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*-(((3a*S*,4*S*,5*S*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5-fluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)carbamoyl)-3-methoxyacrylamide (22b). Yield = 88%; colorless syrup; $[\alpha]_D^{25} = -20.47$ (*c* 0.34, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.33 (s, 1 H), 8.96 (d, *J* = 7.4 Hz, 1 H), 7.63 (d, *J* = 12.3 Hz, 1 H), 5.39 (d, *J* = 12.3 Hz, 1 H), 4.80 (dt, *J* = 6.4, 52.5 Hz, 1 H), 4.44 (t, *J* = 5.5 Hz, 1 H), 4.33-4.41 (m, 2 H), 3.67 (s, 3 H), 3.46 (d, *J* = 32.5 Hz, 2 H), 2.33-2.42 (m, 1 H), 1.46 (s, 3 H), 1.24 (s, 3 H), 1.13 (s, 9 H); ^{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*-(((3a*S*,4*S*,6*R*,6a*R*)-6-(*tert*-Butoxymethyl)-5,5-difluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)carbamoyl)-3-methoxyacrylamide (22c). Yield = 90%; colorless syrup; $[\alpha]_D^{25}$ = –40.41 (*c* 0.52, MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 10.26 (s, 1 H), 9.11 (d, *J* = 8.7 Hz, 1 H), 7.65 (d, *J* = 12.3 Hz, 1 H), 5.37 (d, *J* = 12.4 Hz, 1 H), 4.52-4.62 (m, 1 H), 4.39 (s, 2 H), 3.67 (s, 3 H), 3.53-3.60 (m, 2 H), 2.57-2.68 (m, 1 H), 1.47 (s, 3 H), 1.27 (s, 3 H), 1.16 (s, 9 H); ^{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**, respectively.

1-((1*S*,2*R*,3*R*,4*R*,5*S*)-2-Fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidine-2,4(1*H*,3*H*)-dione (2f). Yield = 56%; white solid; mp 112-118 °C; $[\alpha]_D^{25}$ = –77.11 (*c* 0.20, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 7.70 (dd, *J* = 1.1, 8.1 Hz, 1 H), 5.69 (d, *J* = 8.0 Hz, 1 H), 5.10 (dt, *J* = 4.1, 55.3 Hz, 1 H), 4.91 (dd, *J* = 3.4, 10.2 Hz, 1 H, merged with solvent peak), 4.46 (dd, *J* = 6.6, 10.1 Hz, 1 H), 3.93 (t, *J* = 4.8 Hz, 1 H), 3.70-3.80 (m, 2 H), 3.69 (s, 1 H),

2.29-2.41 (m, 1 H); ^{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-((1*S*,2*S*,3*R*,4*R*,5*S*)-2-Fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidine-2,4(1*H*,3*H*)-dione (2g). Yield = 53%; 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, 1 H), 5.69 (d, *J* = 7.9 Hz, 1 H), 5.07-5.21 (ddd, *J* = 5.10, 6.8, 55.2 Hz, 1 H), 4.61-4.69 (ddd, *J* = 7.3, 8.7, 22.6 Hz, 1 H), 4.32 (dd, *J* = 5.25, 9.0 Hz, 1 H), 3.98 (t, *J* = 3.7 Hz, 1 H), 3.70 (m, 2 H), 2.24 (m, 1 H); ^{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-((1*S*,3*R*,4*R*,5*S*)-2,2-Difluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidine-2,4(1*H*,3*H*)-dione (2h). Yield = 52%; 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, 1 H) 5.71 (d, *J* = 8.0 Hz, 1 H), 5.36, (dt, *J* = 10.3, 17.7 Hz, 1 H), 4.41 (dd, *J* = 5.15, 10.7 Hz, 1 H), 4.07 (m, 1 H), 3.73-3.82 (m, 2 H), 2.53 (m, 1 H); ^{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 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, above-generated intermediate in 1,4-dioxane (0.06 M) was added 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 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-((1*S*,2*R*,3*R*,4*R*,5*S*)-2-fluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidin-2(1*H*)-one (2i). Yield = 17%; 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, 1 H), 5.88 (d, *J* = 7.4 Hz, 1 H), 5.23 (dt, *J* = 3.7, 55.4 Hz, 1 H), 4.93 (ddd, *J* = 3.4, 10.3, 30.4 Hz, 1 H), 4.44 (dd, *J* = 6.6, 10.3 Hz, 1 H), 3.92 (dd, *J* = 4.5, 6.3 Hz, 1 H), 3.71-3.78 (m, 2 H), 2.31-2.40 (m, 1 H); ^{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-((1*S*,3*R*,4*R*,5*S*)-2,2-difluoro-4,5-dihydroxy-3-(hydroxymethyl)cyclopentyl)pyrimidin-2(1*H*)-one (2j). Yield = 20%; 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, 1 H), 5.90 (d, *J* = 7.40 Hz, 1 H), 5.51 (dt, *J* = 18.2, 10.0 Hz, 1 H), 4.37 (dd, *J* = 10.6, 5.25 Hz, 1 H), 4.06 (m, 1 H), 3.73-3.83 (m, 2 H), 2.54 (m, 1 H); ^{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) was 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.

((3a*R*,4*R*,6*S*,6a*S*)-6-(6-Amino-9*H*-purin-9-yl)-5,5-difluoro-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)methanol (24). Yield = 96%; colorless syrup; ^{1}H NMR (500 MHz, CD_3OD) δ 8.31 (s, 1 H), 8.21 (s, 1 H), 5.30-5.40 (m, 2 H), 4.70 (br s, 1 H), 3.94 (dd, *J* = 6.8, 11.4 Hz, 1 H), 3.86 (dd, *J* = 6.8, 11.4 Hz, 1 H), 2.81-2.90 (m, 1 H), 1.58 (s, 3 H), 1.35 (s, 3 H); ^{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-((3a*S*,4*S*,5*R*,6*R*,6a*R*)-5-Fluoro-6-(hydroxymethyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione (27a). Yield = 98%; colorless syrup; ^{1}H NMR (500 MHz, CD_3OD) δ 7.75 (dd, *J* = 1.4, 8.1 Hz, 1 H), 5.70 (d, *J* = 8.1 Hz, 1 H), 5.20 (dt, *J* = 3.1, 54.1 Hz, 1 H), 5.01-5.13 (m, 2 H), 4.58 (d, *J* = 6.3 Hz, 1 H), 3.73-3.83 (m, 2 H), 2.42-2.56 (m, 1 H), 1.50 (s, 3 H), 1.32 (s, 3 H); ^{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-((3a*S*,4*S*,6*R*,6a*R*)-5,5-Difluoro-6-(hydroxymethyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[d][1,3]dioxol-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione (27b). Yield = 97%; ^{1}H NMR

(500 MHz, CD_3OD) δ 7.71 (dd, *J* = 2.0, 8.1 Hz, 1 H), 5.73 (d, *J* = 8.1 Hz, 1 H), 5.33 (dt, *J* = 6.8, 21.3 Hz, 1 H), 4.94 (d, *J* = 6.8 Hz, 1 H), 4.57-4.63 (m, 1 H), 3.88 (dd, *J* = 6.7, 11.4 Hz, 1 H), 3.81 (dd, *J* = 6.7, 11.4 Hz, 1 H), 2.68-2.79 (m, 1 H), 1.54 (s, 3 H), 1.34 (s, 3 H); 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-((3a*S*,4*S*,6*R*,6a*R*)-5,5-difluoro-6-(hydroxymethyl)-2,2-dimethyltetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-yl)-9*H*-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 (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 colorless syrup.

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

Compound 25b: ^1H NMR (500 MHz, CD_3OD) δ 8.87 (s, 1 H), 8.73 (d, *J* = 1.8 Hz, 1 H), 5.46-5.57 (m, 2 H), 4.75 (d, *J* = 5.4 Hz, 1 H), 3.95 (dd, *J* = 6.8, 11.4 Hz, 1 H), 3.88 (dd, *J* = 6.8, 11.4 Hz, 1 H), 2.84-2.95 (m, 1 H), 1.59 (s, 3 H), 1.37 (s, 21 H); ^{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*)-(((3a*R*,4*R*,6*S*,6a*S*)-6-(6-((tert-butoxycarbonyl)amino)-9*H*-purin-9-yl)-5,5-difluoro-2,2-dimethyltetrahydro-4*H*-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 pentafluoro-phosphoramidate 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, 1 H), 8.45 (s, 1 H), 7.37 (d, J = 7.8 Hz, 2 H), 7.25 (d, J = 8.1 Hz, 2 H), 7.19 (d, J = 7.5 Hz, 1 H), 5.50 (dt, J = 5.9, 22.3 Hz, 1 H), 5.40-5.45 (m, 1 H), 4.92-4.99 (m, 1 H), 4.73-4.80 (m, 1 H), 4.36-4.50 (m, 2 H), 3.86-3.98 (m, 1 H), 3.07-3.19 (m, 1 H), 1.58 (s, 12 H), 1.34 (s, 6 H), 1.21 (d, J = 6.2 Hz, 3 H), 1.17 (d, J = 6.2 Hz, 3 H); HRMS (ESI^+) (m/z) found 711.2716, [calcd for $C_{31}H_{42}F_2N_6O_9P^+$ $(M+H)^+$ 711.2713].

***iso*-Propyl((*S*)-(((1*R*,3*S*,4*S*,5*R*)-3-(6-amino-9*H*-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, 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, 1 H), 8.17 (d, *J* = 1.6 Hz, 1 H), 7.35 (d, *J* = 8.4 Hz, 2 H), 7.23 (d, *J* = 8.6 Hz, 2 H), 7.18 (d, *J* = 8.0 Hz, 1 H), 5.26-5.38 (m, 1 H), 4.81-4.98 (m, merged with H_2O peak, 1 H), 4.74 (dd, *J* = 4.8, 10.0 Hz, 1 H), 4.29-4.43 (m, 2 H), 7.17 (br s, 1 H), 3.82-3.93 (m, 1 H), 2.79-2.94 (m, 1 H), 1.32 (d, *J* = 6.8 Hz, 3 H), 1.19 (d, *J* = 6.2 Hz, 3 H), 1.14 (d, *J* = 6.2 Hz, 3 H); ^{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*)-(((1*R*,2*R*,3*S*,4*S*,5*R*)-3-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-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, 1 H), 7.36 (d, *J* = 7.8 Hz, 2 H), 7.24 (d, *J* = 7.8 Hz, 2 H), 7.19 (d, *J* = 7.4 Hz, 1 H), 5.70 (d, *J* = 8.1 Hz, 1 H), 5.02-5.22 (m, 3 H), 4.93-5.01 (m, 1 H), 4.66 (d, *J* = 6.3 Hz, 1 H), 4.29 (d, *J* = 7.6 Hz, 2 H), 3.87-3.95 (m, 1 H), 2.62-2.73 (m, 1 H), 1.51 (s, 3 H), 1.34 (d, *J* = 7.7 Hz, 3 H), 1.32 (s, 3 H), 1.22 (d, *J* = 6.2 Hz, 6 H); 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, 1 H), 7.36 (d, *J* = 7.9 Hz, 2 H), 7.23 (d, *J* = 7.9 Hz, 2 H), 7.19 (d, *J* = 7.4 Hz, 1 H), 5.68 (d, *J* = 8.1 Hz, 1H), 5.04 (dt, *J* = 4.1, 55.4 Hz, 1 H), 4.87-4.98 (m, merged with H_2O peak, 2 H), 4.45 (dd, *J* = 6.6, 9.7 Hz, 1 H), 4.26 (d, *J* = 7.1 Hz, 2 H), 3.99 (d, *J* = 5.4 Hz, 1 H), 3.85-3.93 (m, 1 H), 2.49-2.60 (m, 1 H), 1.33 (d, *J* = 7.0 Hz, 3 H), 1.21 (d, *J* = 6.1 Hz, 6 H); ^{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*)-(((1*R*,3*S*,4*S*,5*R*)-3-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-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, 1 H), 7.36 (d, *J* = 7.8 Hz, 2 H), 7.25 (d, *J* = 7.8 Hz, 2 H), 7.20 (d, *J* = 7.6 Hz, 1 H), 5.70 (d, *J* = 8.1 Hz, 1 H), 5.29-5.39 (m, 1 H), 4.93-5.02 (m, 1 H), 4.30-4.39 (m, 2 H), 4.23-4.29 (m, 1 H), 4.08 (br s, 1 H), 3.84-3.92 (m, 1 H), 2.69-2.80 (m, 1 H), 1.33 (d, *J* = 7.1 Hz, 3 H), 1.22 (d, *J* = 6.2 Hz, 6 H);

^{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 EDTA and was purified by DEAE-cellulose column (2.8 cm x 6 cm), ammonium sulfate fractionation (35-60%), Sephacryl S-300HR (1.0 cm x 105 cm), and DEAE cellulose (2.8 cm x 24 cm). The protein homogeneity was confirmed by 10% SDS-PAGE. The protein concentration was determined by using 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 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, Cary100). The molar extinction coefficient for TNB (ε_{412} = 13700 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% fetal calf serum (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 HEPES (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 DMSO 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 5,000 cells/well (CHIKV), 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 5,000 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 h post-

infection (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 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 non-linear regression.

Viral load reduction assays

VeroE6 (CHIKV, ZIKV) cells were seeded at a density of 7.5 x 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 x 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 a MOI of 0.1 for CHIKV, MOI of 1 for ZIKV and a 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, SARS- and MERS-CoV) or 2 h (ZIKV). After the infection, the cells were washed twice with 1 mL/well warm PBS 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 one-way ANOVA multiple comparison test.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge via the Internet at http://pubs.acs.org.

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

Molecular formula strings (CSV)

■ AUTHOR INFORMATION

Corresponding Author

Lak Shin Jeong, Ph.D., Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Korea. Phone: 82-2-880-7850. E-mail: lakjeong@snu.ac.kr

Author Contributions

#J. Y. and G.K. contributed equally to this work.

All authors have contributed to the manuscript and given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ Acknowledgments

This research was supported by grants from Mid-career Research Program (2016R1A2B3010164) and the Ministry of Science, ICT & Future Planning (2017M3A9A8032086) of the National Research Foundation (NRF), Korea. The work in Leiden (NSO and KK) was supported by the EU Marie Skłodowska-Curie ETN 'ANTIVIRALS' (Grant Agreement No. 642434).

■ ABBREVIATIONS USED

RdRp, RNA-dependent RNA polymerase; SAH, *S*-adenosyl-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; 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.

References

(1) (a) Baltimore, D. Expression of Animal Virus Genomes. *Bacteriological Rev.* **1971**, *35*, 235-241. (b) Modrow, S.; Falke, D.; Truyen, U.; Schätzl, H. Viruses with Single-Stranded, Positive-Sense RNA Genomes. *Molecular Virology*, Berlin, Heidelberg: Springer Berlin Heidelberg, 2013, pp. 185–349.

(2) Thiel, V. *Coronaviruses: Molecular and Cellular Biology*, 1st ed.; Caister Academic Press: Poole, UK, 2007.

(3*)* Zumla, A.; Hui, D. S.; Perlman, S. Middle East Respiratory Syndrome. *Lancet* **2015**, *386*, 995–1007.

(4) Caglioti, C; Lalle, E.; Castilletti, C.; Carletti, F.; Capobianchi, M. R.; Bordi, L. Chikungunya Virus Infection: An Overview. *New Microbiol.* **2013**, *36*, 211–227.

(5) (a) Musso, D.; Gubler, D. J. Zika Virus. *Clin. Microbiol. Rev.* **2016**, *29*, 487–524. (b) Agumadu, V. C.; Ramphul, K. Zika Virus: A Review of Literature. *Cureus* **2018**, *10*, e3025.

(6) (a) Forgie, S.; Marrie, T. J. Healthcare-Associated Atypical Pneumonia. *Semin. Respir. Crit. Care Med.* **2009**, *30*, 67–85. (b) Chan-Yeung, M.; Xu, R. H. SARS: Epidemiology. *Respirology* **2003**, *8* (Suppl), S9–S14. (c) Hui, D. S.; Azhar, E. I.; Kim, Y.-J.; Memish, Z. A.; Oh, M.-d.; Zumla, A. Middle East Respiratory Syndrome Coronavirus: Risk Factors and Determinants of Primary, Household, and Nosocomial Transmission. *Lancet Infect. Dis.* **2018**, *18*, e217-e227.

(7) (a) Schwartz, O.; Albert, M. L. Biology and Pathogenesis of Chikungunya Virus. *Nat. Rev. Microbiol.* **2010**, *8*, 491–500. (b) Presti, A. L.; Lai, A.; Cella, E.; Zehender, G.; Ciccozzi, M. Chikungunya Virus, Epidermiology, Clinics, and Pathogenesis: A Review. *Asian Pac. J. Trop. Med.* **2014**, *7*, 925–932. (c) Ng, L. F. P. Immunopathology of Chikungunya Virus Infection: Lessons Learned from Patients and Animal Models. *Ann. Rev. Virol.* **2017**, *4*, 413–427.

(8) Abushouk, A. I.; Negida, A.; Ahmed, H. An Updated Review of Zika Virus. *J. Clin. Virol.*

2016, *84*, 53–58.

(9) (a) Musso, D.; Roche, C.; Robin, E.; Nhan, T.; Teissier, A.; Cao-Lormeau, V. M. Potential Sexual Transmission of Zika Virus. *Emerg. Infect. Dis.* **2015**, *21*, 359-361. (b) Oster, A. M.; Russell, K. Stryker, J. E.; Friedman, A.; Kachur, R. E.; Peterson, E. E.; Jamieson, D. J.; Cohn, A. C.; Brooks, J. T. Update: Interim Guidance for Prevention of Sexual Transmission of Zika Virus. *Morb. Mortal.Wkly Rep.* **2016**, *65*, 323–325.

(10) Ahlquist, P. RNA-Dependent RNA Polymerases, Viruses, and RNA Silencing. *Science* **2002**, *296*, 1270-1273.

(11) (a) Coutard, B.; Barral, K.; Lichiere, J.; Selisko, B.; Martin, B.; Aouadi, W.; Lombardia, M. O.; Debart, F.; Vasseur, J.-J.; Guillemot, J. C.; Canard, B.; Decroly, E. Zika Virus Methyltransferase: Structure and Functions for Drug Design Perspectives. *J. Virol.* **2017**, *91*, e02202-e02216. (b) Case, J. B.; Ashbrook, A. W.; Dermody, T. S.; Denison, M. R. Mutagenesis of *S*-Adenosyl-L-Methionine-Binding Residues in Coronavirus Nsp14 N7-Methyltransferase Demonstrates Differing Requirements for Genome Translation and Resistance to Innate Immunity. *J. Virol*. **2016**, *90*, 7248-7256.

(12) (a) Decroly, E.; Ferron, F.; Lescar, J.; Canard, B. Conventional and Unconventional Mechanisms for Capping Viral mRNA. *Nat. Rev. Microbiol.* **2011**, *10*, 51–65. (b) Cougot, N.; Van Dijk, E.; Babajko, S.; Seraphin, B. Cap-Tabolism. *Trends Biochem. Sci.* **2004**, *29*, 436-444. (c) Ferron, F.; Decroly, E.; Selisko, B.; Canard, B. The Viral RNA Capping Machinery as a Target for Antiviral Drugs. *Antiviral Res.* **2012**, *96*, 21-31.

(13) (a) Turner, M. A.; Yang, X.; Yin, D.; Kuczera, K.; Borchardt, R. T.; Howell, P. L. Structure and Function of *S*-Adenosylhomocysteine Hydrolase. *Cell Biochem. Biophys.* **2000**, *33*, 101–125. (b) Cantoni, G. L. The Centrality of *S*-Adenosylhomocysteinase in the Regulation of the Biological Utilization of *S*-Adenosylmethionine. In *Biological Methylation and Drug*

Design; Borchardt, R. T., Creveling, C. R., Ueland, P. M., Eds.; Humana Press: Clifton, NJ, 1986; pp 227-238.

(14) (a) Wolfe, M. S.; Borchardt, R. T. *S*-Adenosyl-L-Homocysteine Hydrolase as a Target for Antiviral Chemotherapy. *J. Med. Chem.* **1991**, *34*, 1521–1530. (b) De Clercq, E. Strategies in the Design of Antiviral Drugs. *Nat. Rev. Drug Discov.* **2002**, *1*, 13-25.

(15) Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. Advances in the Development of Nucleoside and Nucleotide Analogues for Cancer and Viral Diseases. *Nat. Rev. Drug Discov.* **2013**, *12*, 447-464.

(16) (a) Kusaka, T.; Yamamoto, H.; Shibata, M.; Muro, M.; Kishi, T.; Mizuno, K. Streptomyces Citricolor Nov. Sp. and a New Antibiotic Aristeromycin. *J. Antibiot. (Tokyo)* **1968**, *21*, 255-263. (b) Shealy, Y. F.; Clayton, J. D. 9-[β-DL-2α,3α-Dihydroxy-4β-(hydroxymethyl)-cyclopentyl]adenine, the Carbocyclic Analog of Adenosine. *J. Am. Chem. Soc.* **1966**, *88*, 3885-3887. (c) Shealy, Y. F.; Clayton, J. D. Synthesis of Carbocyclic Analogs of Purine Ribonucleosides. *J. Am. Chem. Soc.* **1969**, *91*, 3075-3083. (d) Shealy, Y. F.; Thorpe, M. C.; Coburn, Jr. W. C.; Clayton, J. D. Identity of the Synthetic Carbocylic Analog of Adenosine and Aristeromycin. *Chem. Pharm. Bull.* **1980**, *28*, 3114-3117. (e) Arita, M.; Adachi, K.; Ito, Y.; Sawai, H.; Ohno, M. Enantioselective Synthesis of the Carbocyclic Nucleosides (−)-Aristeromycin and (−)-Neplanocin A by a Chemicoenzymatic Approach. *J. Am. Chem. Soc.* **1983**, *105*, 4049-4055. (f) Yoshikawa, M.; Okaichi, Y.; Cha, B. C.; Kitagawa, I. Synthesis of (−)-Aristeromycin from D-Glucose. *Tetrahedron*, **1990**, *46*, 7459-7470. (g) Wolfe, M. S.; Lee, Y.; Bartlett, W. J.; Borcherding, D. R.; Borchardt, R. T. 4′-Modified Analogs of Aristeromycin and Neplanocin A: Synthesis and Inhibitory Activity Toward *S*-Adenosyl-L-Homocysteine Hydrolase. *J. Med. Chem.* **1992**, *35*, 1782-1791. (h) Madhavan, G. V.; Martin, J. C. A Novel

and Stereospecific Synthesis of (±)- and (−)-Aristeromycin. *J. Org. Chem.* **1986**, *51*, 1287-1293.

(17) (a) Bennett, L. L., Jr.; Allan, P. W.; Rose, L. M.; Comber, R. N.; Secrist, J. A. III. Differences in the Metabolism and Metabolic Effects of the Carbocyclic Adenosine Analogs, Neplanocin A and Aristeromycin. *Mol. Pharmacol.* **1986**, *29*, 383-390. (b) Bennett, L. L.; Bowdon, B. J.; Allan, P. W.; Rose, L. M. Evidence that the Carbocyclic Analog of Adenosine Has Different Mechanisms of Cytotoxicity to Cells with Adenosine Kinase Activity and to Cells Lacking this Enzyme. *Biochem. Pharmacol.* **1986**, *35*, 4106-4109.

(18) (a) Madhavan, G. V. B.; McGee, D. P. C.; Rydzewski, R. M.; Boehme, R.; Martin, J. C.; Prisbe, E. J. Synthesis and Antiviral Evaluation of 6′-Substituted Aristeromycins: Potential Mechanism-Based Inhibitors of *S*-Adenosylhomocysteine Hydrolase. *J. Med. Chem.* **1988**, *31*, 1798−1804. (b) Verheyden, J. P.; Martin, J. C.; Madhaven, B.; McGee, D. P. C.; Prisbe, E. J. Purinyl or Pyrimidinyl Substituted Hydroxycyclopentane Compounds Useful as Antivirals. U.S. Patent 4,605,659, Aug 12, 1986. (c) Yin, X. –Q.; Schneller, S. W. Chiral Syntheses of 6′-β-Fluoroaristeromycin, 6′-β-Fluoro-5′-Noraristeromycin and Aristeromycin. *Tetrahedron Lett.* **2005**, *46*, 7535–7538. (d) Jeong, L. S.; Moon, H. R.; Park, J. G.; Shin, D. H.; Choi, W. J.; Lee, K. M.; Kim, H. O.; Chun, M. W.; Kim, H. D.; Kim, J. H. Synthesis and Biological Evaluation of Halo-Neplanocin A as Novel Mechanism-Based Inhibitors of *S*-Adenosylhomocysteine Hydrolase. *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22*, 589−592. (e) Jeong, L. S.; Yoo, S. J.; Lee, K. M.; Koo, M. J.; Choi, W. J.; Kim, H. O.; Moon, H. R.; Lee, M. Y.; Park, J. G.; Lee, S. K.; Chun, M. W. Design, Synthesis, and Biological Evaluation of Fluoroneplanocin A as the Novel Mechanism-Based Inhibitor of *S*-Adenosylhomocysteine Hydrolase. *J. Med. Chem.* **2003**, *46*, 201−203. (f) Lee, K. M.; Choi, W. J.; Lee, Y.; Lee, H. J.; Zhao, L. X.; Lee, H. W.; Park, J. G.; Kim, H. O.; Hwang, K. Y.; Heo, Y. X-ray Crystal Structure

and Binding Mode Analysis of Human *S*-Adenosylhomocysteine Hydrolase Complexed with Novel Mechanism-Based Inhibitors, Haloneplanocin A Analogues. *J. Med. Chem.* **2011**, *54*, 930−938. (g) Chandra, G.; Moon, Y. W.; Lee, Y.; Jang, J. Y.; Song, J.; Nayak, A.; Oh, G.; Mulamoottil, V. A.; Sahu, P. K.; Kim, G.; Chang, T.-S.; Noh, N.; Lee, S. K.; Choi, S.; Jeong, L. S. Structure−Activity Relationships of Neplanocin A Analogues as *S*-Adenosylhomocysteine Hydrolase Inhibitors and Their Antiviral and Antitumor Activities. *J. Med. Chem.* **2015**, *58*, 5108−5120.

(19) Kim, G.; Yoon, J.-S.; Jarhad, D. B.; Shin, Y. S.; Majik, M. S.; Mulamoottil, V. A.; Hou, X.; Qu, S.; Park, J.; Baik, M.-H.; Jeong, L. S. Asymmetric Synthesis of (−)-6′-Fluoro-Aristeromycin via Stereoselective Electrophilic Fluorination. *Org. Lett.* **2017**, *19*, 5732−5735.

(20) (a) Siddiqui, A. Q.; Balatore, C.; McGuigan, C.; Pathirana, T. N.; Balzarini, J.; De Clercq, E. The Presence of Substituents on the Aryl Moiety of the Aryl Phosphoramidate Derivative of d4T Enhances Anti-HIV Efficacy in Cell Culture: A Structure−Activity Relationship. *J. Med. Chem.* **1999**, *42*, 393−399. (b) Mehellou, Y.; Rattan, H. S.; Balzarini, J. The ProTide Prodrug Technology: From the Concept to the Clinic. *J. Med. Chem.* **2018**, *61*, 2211–2226. (c) Mehellou, Y.; Balzarini, J.; McGuigan C. Aryloxy Phosphoramidate Triesters: A Technology for Delivering Monophosphorylated Nucleosides and Sugars into Cells. *Chem. Med. Chem.* **2009**, *4*, 1779–1791. (d) McGuigan, C.; Pathirana, R. N.; Balzarini, J.; De Clercq, E. Intracellular Delivery of Bioactive AZT Nucleotides by Aryl Phosphate Derivatives of AZT. *J. Med. Chem.* **1993**, *36*, 1048–1052. (e) McGuigan, C.; Harris, S. A.; Daluge, S. M.; Gudmundsson, K. S.; McLean, E. W.; Burnette, T. C.; Marr, H.; Hazen, R.; Condreay, L. D.; Johnson, L.; De Clercq, E.; Balzarini, J. Application of Phosphoramidate Pronucleotide Technology to Abacavir Leads to a Significant Enhancement of Antiviral Potency. *J. Med. Chem.* **2005**, *48*, 3504–3515. (f) McGuigan, C.; Hassan-Abdallah, A.; Srinivasan, S.; Wang, Y.; Siddiqui, A.; Daluge, S. M.; Gudmundsson, K. S.; Zhou, H.; McLean, E. W.; Peckham, J.

P.; Burnette, T. C.; Marr, H.; Hazen, R.; Condreay, L. D.; Johnson, L.; Balzarini, J. Application of Phosphoramidate ProTide Technology Significantly Improves Antiviral Potency of Carbocyclic Adenosine Derivatives. *J. Med. Chem.* **2006**, *49*, 7215–7226. (g) Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H.-R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Steuer, H. M. M.; Niu, C.; Otto, M. J.; Furman, P. A. Discovery of a β-D-2′-Deoxy-2′-α-fluoro-2′-β-*C*-methyluridine Nucleotide Prodrug (PSI-7977) for the Treatment of Hepatitis C Virus. *J. Med. Chem.* **2010**, *53*, 7202–7218. (h) Slusarczyk, M.; Lopez, M. H.; Balzarini, J.; Mason, M.; Jiang, W. G.; Blagden, S.; Thompson, E.; Ghazaly, E.; McGuigan, C. Application of ProTide Technology to Gemcitabine: A Successful Approach to Overcome the Key Cancer Resistance Mechanisms Leads to a New Agent (NUC-1031) in Clinical Development. *J. Med. Chem.* **2014**, *57*, 1531–1542.

(21) (a) Choi, W. J.; Park, J. G.; Yoo, S. J.; Kim, H. O.; Moon, H. R.; Chun, M. W.; Jung, Y. H.; Jeong, L. S. Syntheses of D- and L-Cyclopentenone Derivatives Using Ring-Closing Metathesis: Versatile Intermediates for the Synthesis of D- and L-Carbocyclic Nucleosides. *J. Org. Chem.* **2001**, *66*, 6490−6494. (b) Moon, H. R.; Choi, W. J.; Kim, H. O.; Jeong, L. S. Improved and Alternative Synthesis of D- and L-Cyclopentenone Derivatives, the Versatile Intermediates for the Synthesis of Carbocyclic Nucleosides. *Tetrahedron:Asymmetry* **2002**, *13*, 1189−1193. (c) Mulamoottil, V. A.; Nayak, A.; Jeong, L. S. Recent Advances in the Synthesis of Carbocyclic Nucleosides via Ring Closing Metathesis. *Asian J. Org. Chem.* **2014**, *3*, 748−761.

(22) (a) Gilman, H.; Jones, R. G.; Woods, L. A. The Preparation of Methylcopper and Some Observations on the Decomposition of Organocopper Compounds. *J. Org. Chem.* **1952**, *17*, 1630−1634. (b) Song, G. Y.; Paul, V.; Choo, H.; Morrey, J.; Sidwell, R. W.; Schinazi, R. F.; Chu, C. K. Enantiomeric Synthesis of D- and L-Cyclopentenyl Nucleosides and Their Antiviral

Activity Against HIV and West Nile Virus. *J. Med. Chem.* **2001**, *44*, 3985−3993.

(23) Rubottom, G. M.; Gruber, J. M.; Boeckman, R. K., Jr; Ramaiah, M.; Medwid, J. B. Clarification of the Mechanism of Rearrangement of Enol Silyl Ether Epoxides. *Tetrahedron Lett.* **1978**, *19*, 4603–4606.

(24) Montgomery, J. A.; Temple, Jr., C. Synthesis of Potential Anticancer Agents. IX. 9-Ethyl-6-substituted-purines. *J. Am. Chem. Soc.*, **1957**, *79*, 5238–5242.

(25) The X-ray CIF File for **2c** Has Been Deposited at the Cambridge Crystallographic Data Centre (Deposition Number: CCDC 1914672).

(26) (a) Shaw, G.; Warrener, R. N. Purines, Pyrimidines, and Glyoxalines. Part VIII. New Synthesis of Uracil and Thymine. *J. Chem. Soc.* **1958**, 157–159. (b) Jeong, L. S.; Buenger, G.; McCormack, J. J.; Cooney, D. A.; Hao, Z.; Marquez, V. E. Carbocyclic Analogues of the Potent Cytidine Deaminase Inhibitor 1-(β-D-Ribofuranosyl)-1,2-dihydropyrimidin-2-one (Zebularine). *J. Med. Chem.* **1998**, *41*, 2572-2578.

(27) The X-ray CIF Files for **2g** and **2h** Have Been Deposited at the Cambridge Crystallographic Data Centre (Deposition Numbers: CCDC 1871331 for **2g** and CCDC 1871332 for **2h**).

(28) Divaker, K. J.; Reese, C. B. 4-(1,2,4-Triazol-1-yl)- and 4-(3-nitro-1,2,4-triazol-1-yl)-1-(β-D-2,3,5-tri-*O*-acetylarabinofuranosyl)pyrimidin-2(1*H*)-ones. Valuable Intermediates in the Synthesis of Derivatives of 1-β-D-Arabinofuranosyl)cytosine (Ara-C). *J. Chem. Soc. Perkin I* **1982**, 1171−1176.

(29) Ross, B. S.; Reddy, P. G.; Zhang, H.-R.; Rachakonda, S.; Sofia, M. J. Synthesis of Diastereomerically Pure Nucleotide Phosphoramidates. *J. Org. Chem.* **2011**, *76*, 8311−8319.

(30) Lozada-Ramírez, J. D.; Martínez-Martínez, I.; Sánchez-Ferrer, A.; García-Carmona, F. A Colorimetric Assay for *S*-Adenosylhomocysteine Hydrolase. *J. Biochem. Biophys. Methods*

2006, *67*, 131−140.

(31) Scholte, F. E. M.; Tas, A.; Martina, B. E. E.; Cordioli, P.; Narayanan, K.; Makino, S.; Snijder, E. J.; van Hemert, M. J. Characterization of Synthetic Chikungunya Viruses Based on the Consensus Sequence of Recent E1-226V Isolates. *PLoS One.* **2013**, *8*, e71047.

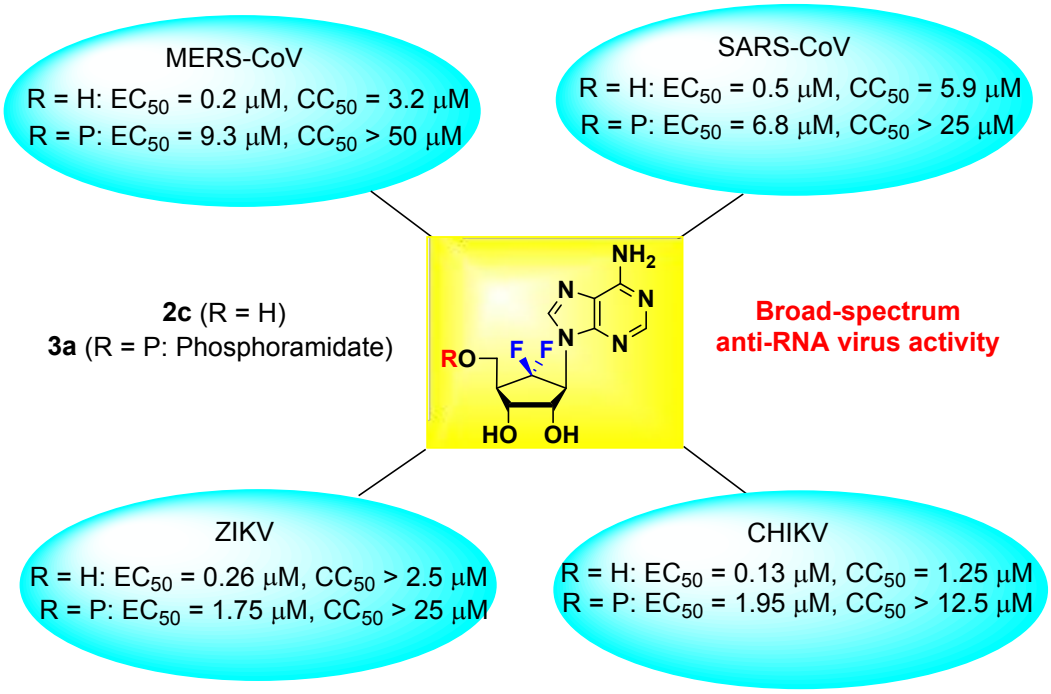
(32) van Boheemen, S.; Tas, A.; Anvar, S. Y.; van Grootveld, R.; Albulescu, I. C.; Bauer, M. P.; Feltkamp, M. C.; Bredenbeek, P. J.; van Hemert, M. J. Quasispecies Composition and Evolution of a Typical Zika Virus Clinical Isolate from Suriname. *Sci Rep.* **2017**, *7*, 2368.

(33) van Boheemen, S.; de Graaf, M.; Lauber, C.; Bestebroer, T. M.; Raj, V.S.; Zaki, A. M.; Osterhaus, A. D.; Haagmans, B. L.; Gorbalenya, A. E.; Snijder, E. J.; Fouchier, R. A. Genomic Characterization of a Newly Discovered Coronavirus Associated with Acute Respiratory Distress Syndrome in Humans. *mBio* **2012**, *3*, e00473−12.

(34) (a) Albulescu, I. C.; Kovacikova, K.; Tas, A.; Snijder, E. J.; van Hemert, M. J. Suramin Inhibits Zika Virus Replication by Interfering with Virus Attachment and Release of Infectious Particles. *Antiviral. Res.* **2017**, *143*, 230−236. (b) de Wilde, A. H.; Jochmans, D.; Posthuma, C. C.; Zevenhoven-Dobbe, J. C.; van Nieuwkoop, S.; Bestebroer, T. M.; van den Hoogen, B. G.; Neyts, J.; Snijder, E. J. Screening of a FDA-Approved Compound Library Identifies Four Small-Molecule Inhibitors of Middle East Respiratory Syndrome Coronavirus Replication in Cell Culture. *Antimicrob. Agents Chemother.* **2014**, *58*, 4875−4884.

(35) van den Worm, S. H. E.; Eriksson, K. K.; Zevenhoven, J. C.; Weber, F.; Zust, R.; Kuri, T.; Dijkman, R.; Chang, G.; Siddell, S. G.; Snijder, E. J.; Thiel, V.; Davidson, A. D. Reverse Genetics of SARS-Related Coronavirus Using Vaccinia Virus-Based Recombination. *PLoS One* **2012**, *7*, e32857.

Table of Contents graphic


MERS-CoV
R = H: EC50 = 0.2 μM, CC50 = 3.2 μM
R = P: EC50 = 9.3 μM, CC50 > 50 μM
SARS-CoV
R = H: EC50 = 0.5 μM, CC50 = 5.9 μM
R = P: EC50 = 6.8 μM, CC50 > 25 μM
2c (R = H)
3a (R = P: Phosphoramidate)
NH2
N
N
N
N
RO
F
F
HO
OH
Broad-spectrum
anti-RNA virus activity
ZIKV
R = H: EC50 = 0.26 μM, CC50 > 2.5 μM
R = P: EC50 = 1.75 μM, CC50 > 25 μM
CHIKV
R = H: EC50 = 0.13 μM, CC50 = 1.25 μM
R = P: EC50 = 1.95 μM, CC50 > 12.5 μM