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Article

Synthesis and Modification of Cordycepin-Phosphoramidate ProTide Derivatives for Antiviral Activity and Metabolic Stability

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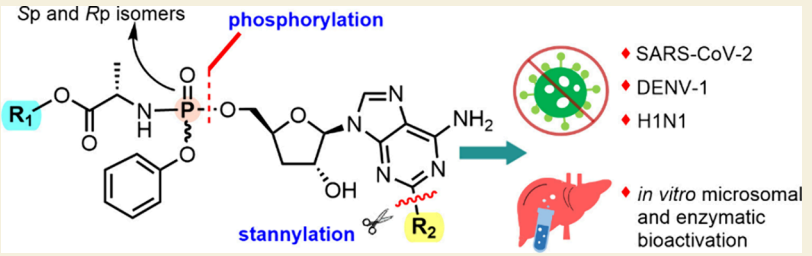

ABSTRACT: In our study, Prodrug nucleoTide (ProTide) technology was applied to cordycepin to enhance its antiviral activities and metabolic stability. Using cordycepin as starting material, we developed a synthetic method to access a series of stereospecific-phosphoramidate derivatives with various ester groups. We also successfully synthesized halogenated cordycepin derivatives via stannylation. Our 17 ProTide-cordycepin derivatives were pharmacologically evaluated for their antiviral activities. Phosphorus diastereomers **22***S* and **22***R* showed moderate inhibitory activity against corona and influenza viruses, while these compounds and derivatives (**25***S*, **27***S*, and **27***R*) demonstrated promising antiviral efficacy against dengue virus. Pharmacological screening indicated that *Sp*-isomers generally exhibited slightly greater inhibitory activity than their *Rp*-isomer counterparts against the dengue virus. The selected ProTides were assessed for their metabolic mechanism and stability via carboxypeptidase and microsomes. The hydrolysis rate of the *Rp*-isomers was observed to be slightly higher than that of the *Sp*-isomers, and the addition of a fluorine group also modestly increased this rate and fluorinated **39***S* extended its half-life compared to nonfluorinated counterparts. These findings suggested not only structure−activity relationships of cordycepin ProTide but also the comprehensive synthetic route to access cordycepin derivatives for further antiviral development.

KEYWORDS: *cordycepin, ProTide, antiviral, halogenation, metabolism*

INTRODUCTION

Cordycepin (3′-deoxyadenosine, **1**) is a drug-like natural product produced from fungi in the family *Cordycipitaceae*.[1,2] Cordycepin **1** exhibits a wide variety of pharmacological activities, for example, anti-inflammatory, anticancer, antimicrobial, antibacterial, antimalarial, antiprotozoal, antioxidant, immunomodulatory/immunoregulatory, etc.[3−6] Furthermore, cordycepin has demonstrated promising antiviral properties, especially virulent RNA viruses such as influenza viruses, dengue virus, hepatitis C virus, human immunodeficiency virus, and Zika virus.[7−10] Recently, cordycepin was discovered to inhibit SARS-CoV-2 replication *in vitro* (VOC-202012/01 strain), demonstrating superior efficacy compared to the drug remdesivir.[11] Several targets of cordycepin were proposed in silico, including RNA-dependent RNA polymerase.[12]

Given its promising antiviral activities and possible drug target, enhancing cordycepin's drug-like properties could significantly amplify its therapeutic potential. ProTide technology, already successful in developing antiviral drugs like remdesivir (**2**) and sofosbuvir (**3**), can achieve this by increasing the lipophilicity of nucleoside compounds. This enhancement improves cellular uptake and leads to the formation of triphosphate active metabolites that inhibit targeted polymerase (Figure 1). For example, in the current anticancer drug

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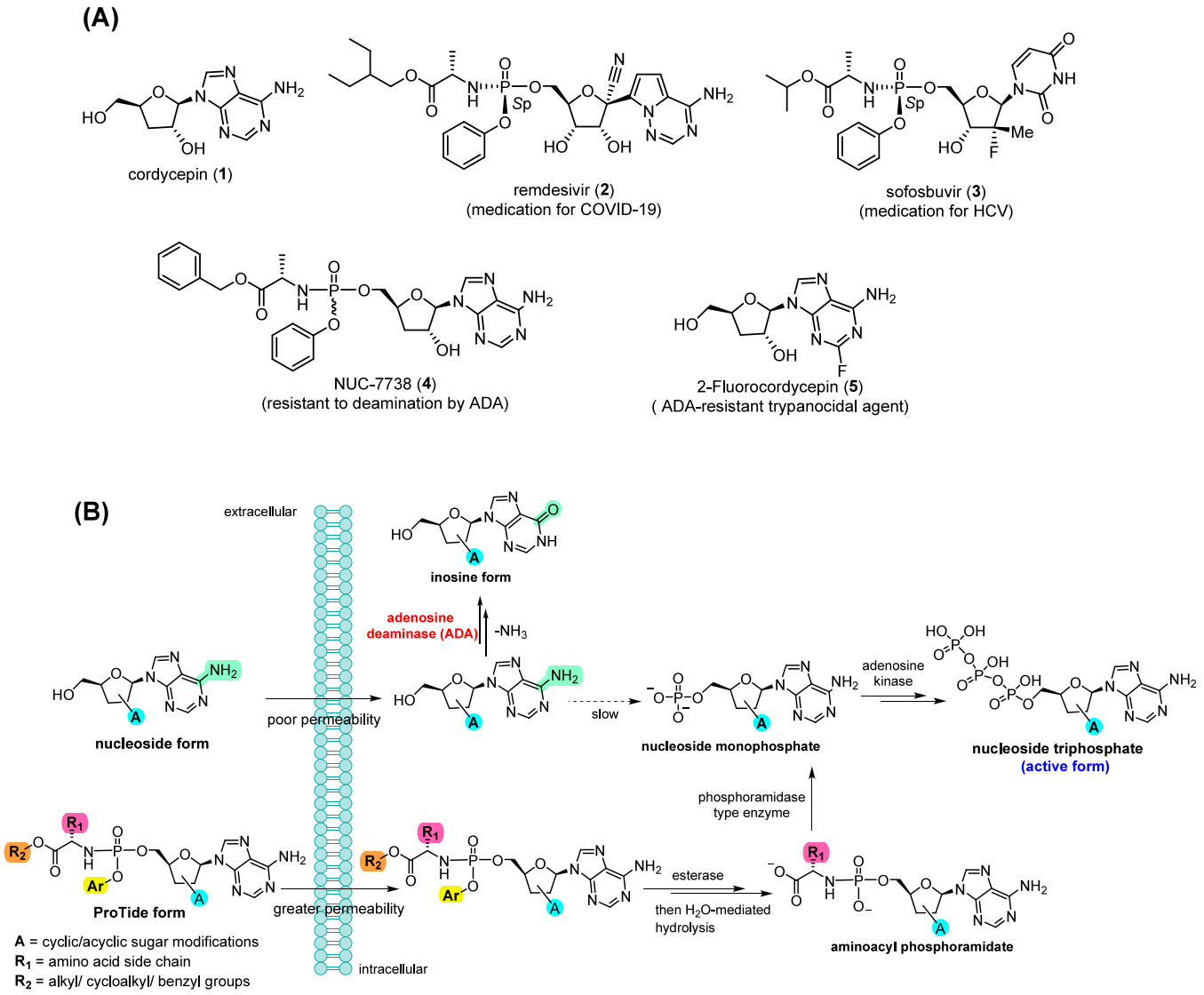

Figure 1. (A) Chemical structures of cordycepin (**1**), remdesivir (**2**), sofosbuvir (**3**), NUC-7738 (**4**), and 2-fluorocordycepin (**5**). (B) Intracellular conversion of nucleoside and ProTides forms: Upon entering the cell, ProTide derivatives are metabolized into the aminoacyl phosphoramidate form through esterase and H_2O-mediated hydrolysis of the amino acid ester. Phosphoramidase-type enzymes then hydrolyze the P−N bond, releasing the nucleoside monophosphate. This compound undergoes further phosphorylation to produce an active nucleoside triphosphate analogue. The nucleoside form has poor cell permeability and generates nucleoside monophosphate at a slower rate compared with the ProTide form.

discovery program (ClinicalTrials.gov ID NCT03829254), cordycepin (**1**) underwent structural modifications into NUC-7738, a racemic 5′-aryloxy phosphoramidate ProTide (**4**).[14] This ProTide compound was discovered to be tolerant to adenosine deaminase (ADA) metabolic deactivation, resulting in increased levels of intracellular nucleoside triphosphate analogue as the active metabolite, whereas the parent cordycepin was subject to ADA simultaneously (Figure 1B).[14−17] In contrast, another trypanocidal discovery project found that 2-fluorocordycepin (**5**), which lacks a phosphoramidate part, was also tolerant to ADA.[18] Taken together, the racemic phosphoramidate ProTide-cordycepin and the presence of a fluorine group at the C2 position of cordycepin derivatives may contribute to enhancing their biological activities and stability.

To explore the relationship between the antiviral and stereospecific activities of ProTide-cordycepin, we developed the synthesis of distinct *Sp*- and *Rp*-phosphoramidate ProTide-cordycepin derivatives with various ester functionalities. Different substituent groups in the ester moieties of phosphoramidates might influence the hydrolysis rate by esterase or carboxypeptidase-like enzymes upon entering the cell, potentially affecting the production rate of the active nucleoside triphosphate analogue. Additionally, we established a novel synthetic pathway for C-2 halogenated cordycepin ProTides. Our study also involved the preparation of 2′-deoxyadeonsine-phosphoramidate and 2′, 3′-dideoxyadenosine-phosphoramidate to compare their antiviral properties to the cordycepin (**1**)-phosphoramidate counterpart. These compounds have been evaluated against a spectrum of viruses, including coronavirus (SARS-CoV-2 delta and omicron strain), dengue virus (DENV1), and influenza (H1N1) virus. Moreover, we also investigated their metabolic stability using NMR analysis and fluorescence measurement of hydrolysis reactions catalyzed by mimicking carboxypeptidase Y as well as *in vitro* microsomal stability assays.

RESULTS AND DISCUSSION

In order to prepare our series of phosphoramidate ProTide analogues, the retrosynthetic analysis toward cordycepin **1** as the

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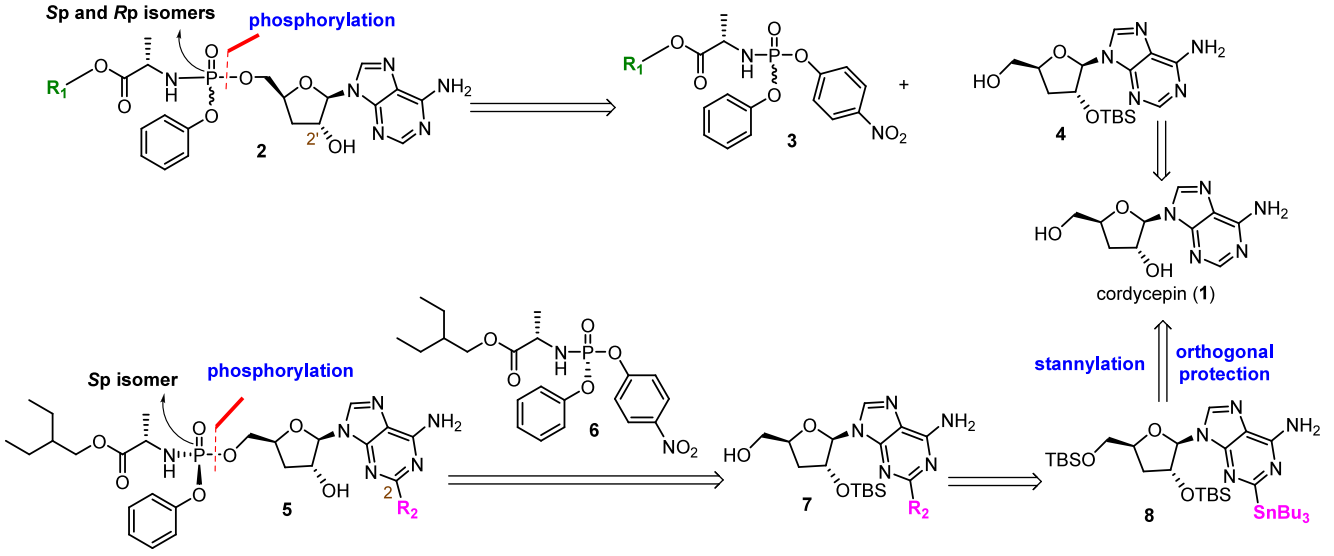
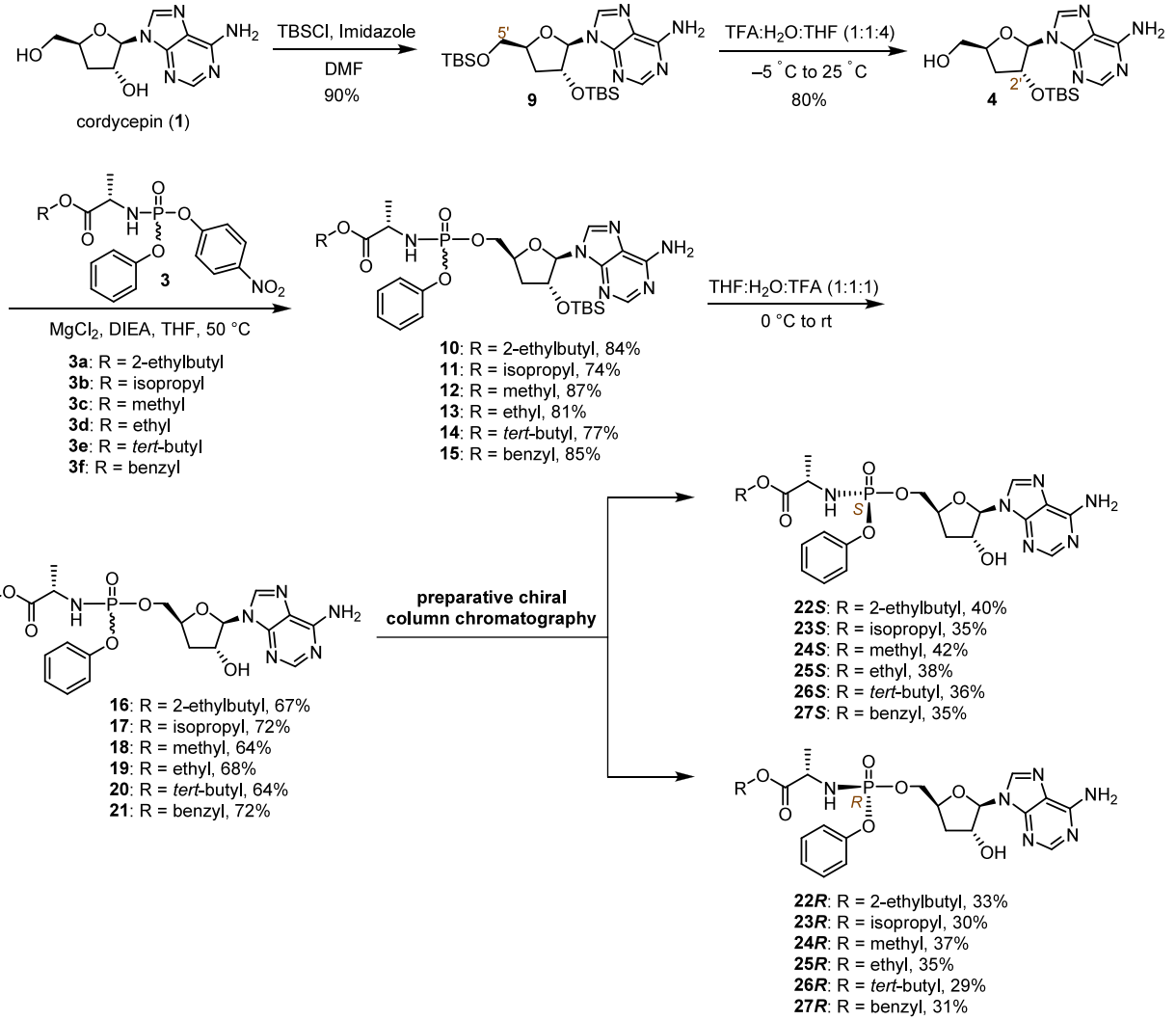
Scheme 1. Retrosynthetic Analysis of Cordycepin-Phosphoramidate ProTide Derivatives Using Cordycepin (1) as the Starting Material



Scheme 2. Synthesis and Isolation of Single Diastereomer Cordycepin-Phosphoramidate Derivatives (Compounds 22–27)



starting material was illustrated in Scheme 1. *Sp*- and *Rp*-Phosphoramidate ProTide-cordycepin **2** with diverse ester functionalities (R_1 including methyl, ethyl, isopropyl, *tert*-butyl, 2-ethylbutyl, and benzyl esters) would be coupled from

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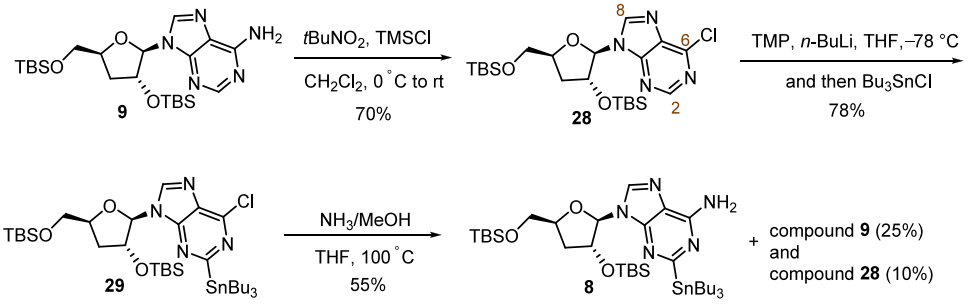
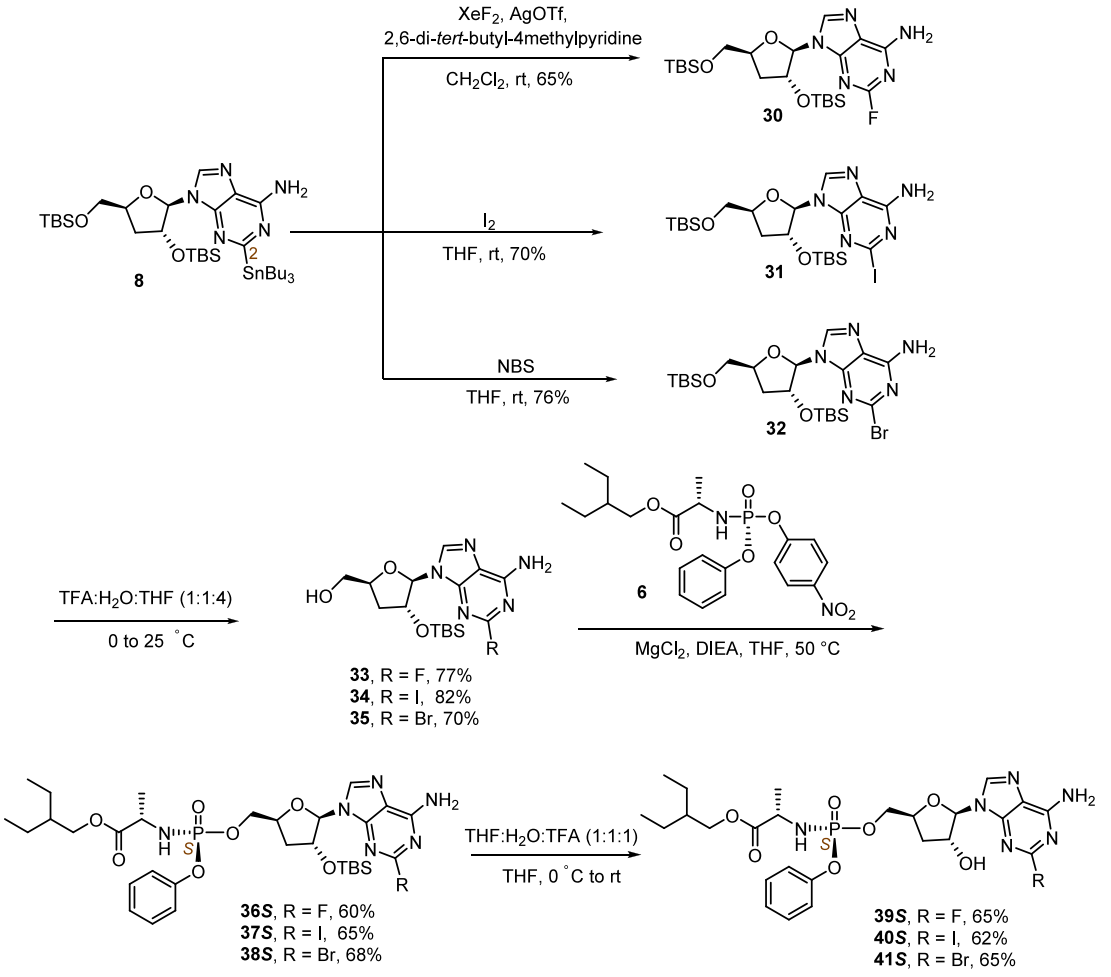
Scheme 3. Synthesis of 2-Stannylated Cordycepin Intermediate 8



Scheme 4. Synthesis of C2-Halogenated Cordycepin-Phosphoramidate (39*S*–41*S*)



known racemic phosphoramidate reagent **3**[19] and protected nucleoside **4**. On the other hand, the coupling between commercially available *Sp*-phosphoramidate reagent **6** and protected halogenated nucleoside **7** (R_2 including fluorine, bromine, and iodine groups) was carried out to provide *Sp*-halogenated cordycepin-phosphoramidate ProTides. The halogenated nucleoside at the C2-position (**7**) could be synthesized from 2-stannylated cordycepin (**8**), which was prepared from cordycepin (**1**) via orthogonal protection and stannylation using the modified method by Tanaka and several research groups.[20−24]

In our experiment, the syntheses of *Sp*- and *Rp*-phosphoramidate ProTide-cordycepin **22**–**27** were prepared from cordycepin (**1**) (Scheme 2). Initially, the aim was to synthesize the primary alcohol nucleoside **4** as a key intermediate from **1**. The TBS group was chosen as a protecting group for the 2′-hydroxy group as it could presumably be removed under mild acidic conditions in the final step. Selective protection of the secondary alcohol moiety of diol **1** was achieved in two steps: protection of both the 2′ and 5′ hydroxyl groups with TBSCl and removal of the 5′ TBS protecting group of **9** using a solution of trifluoroacetic acid (TFA) in water and THF in a ratio of (1:1:4) at 0 °C to give the corresponding primary alcohol nucleoside **4** in 72% over two steps.[25] Intermediate **4** was subsequently coupled with corresponding racemic phosphonate reagent **3**[19] to provide racemic intermediate **10**–**15** in the presence of $MgCl_2$ and base in a good yield.[26] Subsequently, the racemic **10**–**15** were desilylated in TFA, water, and THF in a ratio of 1:1:1 at 0

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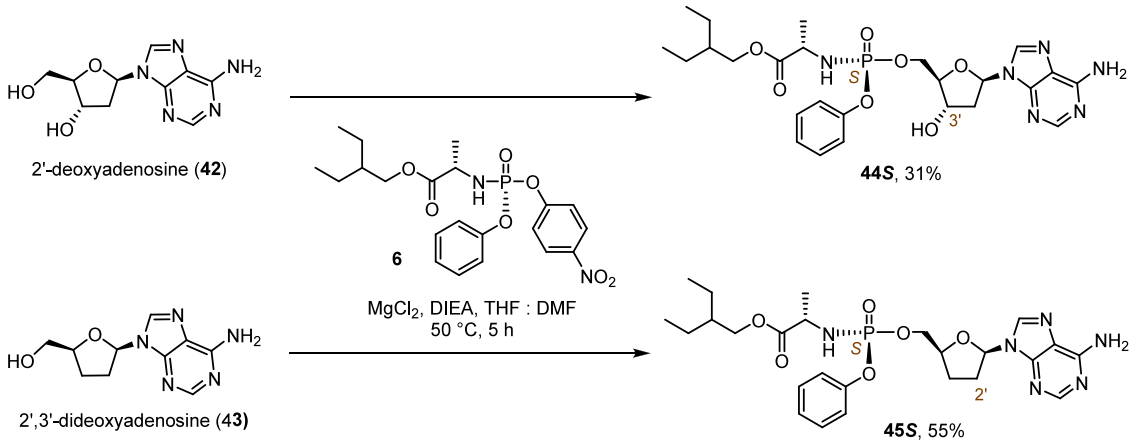
Scheme 5. Synthesis of 2′-Deoxyadeonsine-Phosphoramidate (44*S*) and 2′,3′-Dideoxyadenosine-Phosphoramidate (45*S*)



°C to room temperature for 24 h, providing racemic phosphoramidate ProTide **16**−**21**. Lastly, all racemic mixtures were isolated to obtain single isomers of *Sp* and *Rp*-**22**−**27** in 29−42% yields using preparative chiral column chromatography with a Lux 5 μm Cellulose-2 LC Column. Chirality at the phosphorus atom was confirmed by ^{31}P NMR spectroscopy, with the *Sp*-isomer resonating upfield and the *Rp*-isomer downfield, consistent with trends in similar nucleoside analog ProTides.[27−30] Moreover, the diastereomeric purity of these compounds was found to exceed 95% de by chiral HPLC analysis.

Next, to investigate the effect of C-2 substitution, the syntheses of *Sp*-halogenated cordycepin-phosphoramidate derivatives were performed using 2-stannylated cordycepin intermediate **8** as the key intermediate. Slightly modified from previous reports,[20,24] the preparation of intermediate **8** was achieved in three steps from silyl ether **9** (Scheme 3). First, a chlorodediazoniation reaction was performed using intermediate **9** in the presence of *tert*-butyl nitrite and TMSCl in order to introduce an electronegative chlorine atom at the C6 position to afford 6-chloropurine derivative **28**. This step enhances the electron deficiency in the purine ring, facilitating functionalization at the C2 position of 6-chloropurine in **28**. To achieve this, the lithiation-mediated stannyl transfer of 6-chloropurine nucleoside **28**, as reported by Tanaka and co-workers, was employed.[21] Upon treatment of **28** with freshly prepared lithium 2,2,6,6-tetramethylpiperidide (LiTMP) derived from *n*-BuLi and 2,2,6,6-tetramethylpiperidine (TMP), the C8-lithiated species was initially formed due to the higher acidity of the C8-hydrogen compared to the C2-hydrogen. Subsequent reaction of this C8-lithiated intermediate with tributyltin chloride ($SnBu_3Cl$) smoothly produced 6-chloro-2-(tributylstannyl)purine (**29**) as the sole product with a 78% yield. This result arose from the anionic transfer of the stannyl group from the C8 to the C2 position, which is stable under lithiation conditions, leading to its accumulation.[21,24] Finally, stannyl compound **29** was subjected to nucleophilic substitution in the presence of methanolic ammonia in a sealed tube at 100 °C, yielding the desired intermediate **8** in a moderate yield (55%).[21] The yield of intermediate **8** was compromised due to the instability of the tributylstannyl group under these conditions, resulting in a reversion to silyl ethers **9** and **28** with yields of 25% and 10%, respectively. Nevertheless, these compounds could be reclaimed for further use.

Upon obtaining the 2-stannylated cordycepin intermediate (**8**), the subsequent step was to introduce a halogen group at the C2 position. The synthesis of fluoro-, iodo-, and bromocordycepins is shown in Scheme 4. Modified from a previous report,[21] electrophilic fluorination, iodination, and bromination of compound **8** were executed utilizing XeF_2/AgTf, iodine (I_2), and *N*-bromosuccinimide (NBS) to yield compounds **30**, **31**, and **32** in 65%, 70%, and 76%, respectively. In contrast, the installation of a chlorine group at the C2 position using *N*-chlorosuccinimide (NCS) was not successful due to decomposition. Then, these obtained halogenated compounds **30**−**32** were treated with TFA/H_2O/THF (1:1:4) at 0−25 °C, providing compounds **33**−**35** in 77%, 82%, and 70% yield, respectively. The syntheses of C2-substituted cordycepin-phosphoramidate ProTides (**39***S*−**41***S*) were accomplished in two steps from nucleoside **33**−**35**, employing a synthetic procedure similar to that described for *Sp*- and *Rp*-Phosphoramidate ProTide-cordycepin, as illustrated in Scheme 2. Compounds **33**−**35** were treated with commercially available phosphate reagent **6** followed by removal of the remaining TBS group to obtain the desired phosphoramidated and halogenated ProTide-cordycepin with yields of 65%, 62%, and 65%, respectively. The diastereomeric purity of final compounds **39***S*−**41***S* was also found to exceed 95% de by chiral HPLC analysis.

In addition, the preparation of 2′-deoxyadeonsine-phosphoramidate (**44***S*) and 2′,3′-dideoxyadenosine-phosphoramidate (**45***S*) was performed in order to investigate SAR of hydroxy group at 2′ and 3′ (Scheme 5). The syntheses of both compounds were achieved by coupling commercially available 2′-deoxyadenosine (**42**) and 2′,3′-dideoxyadenosine (**43**) with phosphate reagent **6**, providing the desired compound with yields of 31% and 55%, respectively.

Our synthetic phosphoramidate ProTides were evaluated for their *in vitro* antiviral activity against three RNA viruses, including SARS-CoV-2 virus (Delta and Omicron-XBB.1.16 variants), dengue virus serotype 1 (DENV-1), and influenza virus (H1N1). All compounds were screened for preliminary antiviral activity at 25 μM, revealing that most of the compounds showed no antiviral effects. However, only **22***S*, **22***R*, **25***S*, **27***S*, **27***R*, and **39***S* exhibited promising inhibition against one of these three viruses. Hence, these active and parent compounds were selected for further IC_{50} measurement and cytotoxicity testing against the African green monkey kidney fibroblast (Vero) cells (Table 1). Initial findings demonstrated that the presence of a

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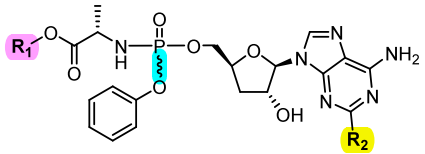
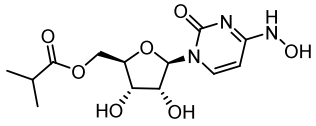
Table 1. Selected Antiviral and Cytotoxic Activities of Synthetic ProTides against Three Viruses (SARS-CoV-2 Virus (Delta and Omicron-XBB.1.16 variants), Dengue Virus Serotype 1 (DENV-1), and Influenza Virus (H1N1)[e]



22*S*: R_1 = 2-ethylbutyl, R_2 = H
22*R*: R_1 = 2-ethylbutyl, R_2 = H
25*S*: R_1 = ethyl, R_2 = H
25*R*: R_1 = ethyl, R_2 = H
27*S*: R_1 = benzyl, R_2 = H
27*R*: R_1 = benzyl, R_2 = H
39*S*: R_1 = 2-ethylbutyl, R_2 = F



Molnupiravir (**46**)

	antiviral activities, IC_{50} (μM)				cytotoxicity, IC_{50} (μM)
compounds	SARS-CoV-2 (Delta)[a]	SARS-CoV-2 (Omicron-XBB.1.16)[b]	dengue serotype 1 (DENV-1)[c]	influenza (H1N1)[d]	Vero cells
cordycepin (1)	>200	N/A	87.50 ± 29.60	5.73 ± 0.36	N/A
22*S*	69.8 ± 9.91	N/A	1.76 ± 0.77	43.7 ± 3.67	N/A
22*R*	49.4 ± 8.62	N/A	5.53 ± 1.41	23.4 ± 1.80	65.38 ± 8.85
25*S*		N/A	13.50 ± 5.91	N/A	N/A
25*R*		N/A	N/A	N/A	N/A
27*S*		N/A	2.80 ± 0.90	N/A	N/A
27*R*		N/A	3.07 ± 0.14	N/A	N/A
39*S*		N/A	71.20 ± 32.30	N/A	15.35 ± 6.99
remdesivir (2)		4.99 ± 0.07	0.06 ± 0.01	N/A	N/A
molnupiravir (46)	2.32 ± 0.45	8.60 ± 1.92	N/A	20.2 ± 1.49	N/A

[a]The virus and the test substances are added to CaLu-3 cells. [b]The virus and the test substances are added to Vero-E6 cells. [c]The virus and the test substances are added to K562-CD209 cells. [d]The virus and the test substances are added to Madin-Darby Canine Kidney (MDCK) cells. [e]Antiviral activity N/A: the dose−response experiment was not performed due to no inhibitory properties against corresponding virus at 25 μM in our preliminary screening (see Supporting Information); Cytotoxicity N/A: no toxicity was observed at 50 μg/mL or approximately 90 μM.

hydroxy group at the 2′ position, as observed in these active compounds, is crucial for antiviral inhibition. Notably, **44*S*** and **45*S*** did not exhibit any inhibitory effects against the tested viruses, whereas **22*S*** showed inhibitory activities against all three viruses. Therefore, the absence of this hydroxy group at 2′ or its presence at 3′ or neither of them significantly reduces antiviral efficacy. Furthermore, we also found that most of these active compounds were noncytotoxic to Vero cells at 50 μg/mL; however, **22*R*** and **39*S*** showed sign of toxicity, with IC_{50} values of 65.38 and 15.35 μM, respectively. These results suggest that differences between *Sp*- and *Rp*-isomers as well as the presence of a fluorine group in ProTide-cordycepin derivatives might influence their toxicity to Vero cells.

In our assay evaluating the anti-SARS-CoV-2 activity against the Delta and Omicron strains, we observed that cordycepin (**1**) exhibited no inhibitory effects on these variants, despite demonstrating potent inhibitory activity against the VOC-202012/01 strain in a previous reported.[11] Nevertheless, compared to compound **1** as a parent compound, compounds **22*S*** and **22*R*** exhibited marginal inhibitory properties against the Delta variant of the SARS-CoV-2 virus, with IC_{50} values of 69.8 and 49.4 μM, respectively, albeit with much lower efficacy in inhibiting this virus variant compared to the standard drug molnupiravir (**46**) (IC_{50} = 2.32 μM). For the Delta variant, a limited number of compounds were screened, placing greater focus on the new Omicron variant. In the case of the SARS-CoV-2 Omicron-XBB.1.16 variant, none of our synthetic compounds displayed inhibitory activity at 25 μM, resulting in no further testing for the dose−response assay. Similarly, only the standard drugs remdesivir (**2**) and molnupiravir (**46**) demonstrated strong anti-SARS-CoV-2 activity against this variant, with IC_{50} values of 4.99 and 8.60 μM, respectively. It should be noted that our synthetic cordycepin-ProTides did not exhibit enhanced antiviral efficacy against the SARS-CoV-2 virus of both variants. We anticipated that the difference in inhibition activity of compounds targeting the RdRp between Delta and Omicron variants of SARS-CoV-2 despite no mutations at the target binding site, might be due to the influence of other factors, including replication rate and virulence. The Omicron variant favors an endocytic entry path into host cells, which is different from the Delta variant. This suggests that the mutations in the Omicron spike protein affect its mechanism of cell entry, potentially influencing RdRp function once the virus is inside the host cell.[31] Another finding was that the ProTide technology promoted the inhibitory activities, so the polymerase could be a target of cordycepin.

On the other hand, preliminary screening results indicated that **22*S***, **22*R***, **25*S***, **27*S***, **27*R***, and **39*S*** possessed promising inhibitory activity against dengue virus serotype 1 (DENV-1) without inducing cytotoxicity in their host cells (K562). Although parent cordycepin (**1**) showed low activities against dengue virus with an IC_{50} value of 87.5 μM, the ProTides **22*S***, **22*R***, **27*S***, and **27*R***, bearing a large ester group (2-ethylbutyl and benzyl moiety, respectively), demonstrated the high level of inhibition with IC_{50} values of 1.76, 5.53, 2.80, and 3.07 μM, respectively. For the small ester group, compound **25*S***, an ethyl-substituted phosphoramidate derivative, also showed relatively strong anti-DENV-1 activity with an IC_{50} value of 13.5 μM, whereas the **25*R*** isomer exhibited no anti-DENV-1 activity. Moreover, other halogenated compounds showed no activity at 25 μM and fluorinated compound **39*S*** exhibited sign of cytotoxicity against Vero cells as aforementioned. Notably, these

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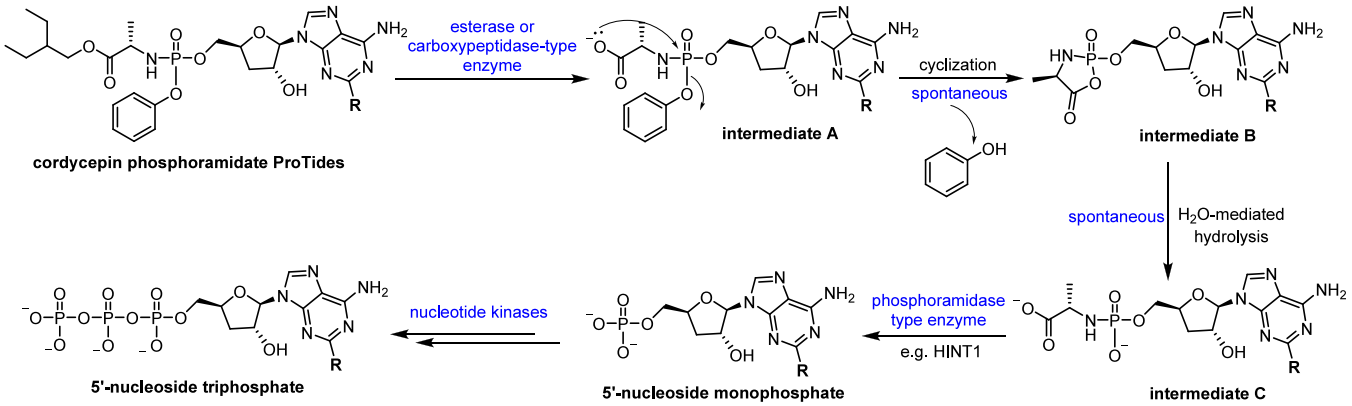
Scheme 6. Proposed Mechanism for the Metabolic Activation Pathway of Cordycepin ProTides



results indicated that *S*-forms of **22**, **25**, and **27** showed significantly greater inhibitory activities compared to *R*-forms isomers. It might be attributed to differences in biotransformation into the active form, similar to those observed with *S*- and *R*-sofosbuvir.[29] However, all compounds were less potent in inhibiting dengue virus serotype 1 compared with remdesivir (**2**), which demonstrated impressive efficacy with an IC_{50} value of 0.058 μM.

Likewise, all compounds were primarily screened at 25 μM against influenza (H1N1) in MDCK cells. None of the cordycepin ProTides were active, whereas cordycepin (**1**), as a nucleoside, showed good inhibitory activity with an IC_{50} value of 5.73 μM, stronger than that of molnupiravir (**46**) (IC_{50} = 20.2 μM). However, we selected present ProTides **22***S* and **22***R* for the dose−response assay (IC_{50} = 43.7 and 23.4 μM, respectively). These results indicated that ProTide compounds **22***S* and **22***R* exhibited inferior efficacy compared to that of nucleoside **1**. Evaluating the anti-influenza activity of these synthetic ProTides necessitates further investigation as these results were obtained from canine kidney cells, which might exhibit differences in cellular uptake and biotransformation compared to human cells. Alternatively, the target of cordycepin against influenza might not be polymerase.

To investigate further on the enzymatic activation process, the efficiency of ProTides depends on their active nucleotide forms mediated by host esterases, including carboxypeptidases. The proposed mechanism for metabolic activation pathway of cordycepin ProTides is shown in Scheme 6.[13,32] These enzymes hydrolyze the ester bond of the amino acid moiety, leading to the generation of intermediate A. Subsequently, five-membered cyclic intermediate B is formed followed by another round of spontaneous hydrolysis reactions. Consequently, the P−N bond undergoes cleavage, converting the compound to the corresponding aminoacyl phosphoramidate (intermediate C). This compound is critically important as it leads to the formation of the bioactive compound (5′-nucleoside triphosphate).

To explore the mechanism of bioactivation and assess the metabolic stability of our ProTides, enzymatic hydrolysis studies of ester bonds were performed using the ^{31}P NMR technique and fluorescence measurement at the characteristic wavelength of phenol in the presence of yeast carboxypeptidase Y (CPY) as a representative model of human carboxypeptidase. This approach specifically highlighted the catalytic role of Cathepsin A in ProTide metabolism in the human liver. Only the active compounds *Sp*-**22**, *Rp*-**22**, and *Sp*-**39** were chosen for these studies because their interesting antiviral activities as well as core structures include both isomers and a halogen group at the C2 position.

First, the kinetic study of **22***S*, **22***R*, and **39***S* hydrolysis with yeast CPY, determined by ^{31}P NMR spectroscopy, was conducted to examine intermediates A and C. In the ^{31}P NMR experiments of **22***S* and **22***R* compounds, chemical shifts at 3.95 and 3.56 ppm, respectively, were observed (Figure 2A,B). When carboxypeptidase Y enzyme was added, it was observed that the signal intensity corresponding to **22***S* gradually diminished and completely disappeared within 5 h. For **22***R*, it vanished within 3 h. The emergence of new signal intensities of these compounds was found at approximately 4.70 and 7.12 ppm, corresponding to intermediate A and intermediate C, respectively, according to the previous reported pattern.[32] Therefore, **22***S* undergoes hydrolysis by the carboxypeptidase Y enzyme more slowly than does its stereoisomer **22***R*. In the case of 2-fluorocordycepin phosphoramidate ProTide (**39***S*), as illustrated in Figure 2C, the experiment revealed a chemical shift of the starting material at 3.74 ppm. Upon the addition of carboxypeptidase Y enzyme, **39***S* underwent hydrolysis to form intermediate C at 7.13 ppm within 3 h, but detection of intermediate A of **39***S* was not clear because of noise background under the similar condition. Although compound **39***S* contains the same *Sp* form as **22***S*, the presence of a fluorine group might lead to an increased rate of hydrolysis by CPY. Notably, these NMR results were obtained using a representative enzyme at high concentration, and the hydrolysis rates of these compounds showed minimal variation.

In order to monitor compound hydrolysis in a time-resolved and kinetic manner, we developed a new fluorescence-based assay. Unlike the NMR assay, this assay exploits the release of the phenol group upon spontaneous cyclization of intermediate A into intermediate B (Scheme 6). Test compounds were diluted in PBS buffer at pH 7.4, and CPY was added to initiate the reaction, leading to a rapid increase of fluorescence at 305 nm (Figure 3A). We noted that a lag phase is observed in the first 2 min, which corresponds to the accumulation of intermediate A. Following this, Michaelis−Menten curves were recorded for compounds **22***S*, **22***R*, and **39***S* (Figure 3A−C), and V_{max} and K_M values were extracted. These values encompassed two reactions: ester hydrolysis by CPY and the spontaneous cyclization of intermediate A (which is intramolecular and independent of substrate concentration).

From this experiment, **22***R* appears to be hydrolyzed slightly faster than **22***S* (V_{max} of 0.019 mM min^{-1} vs 0.0122 mM min^{-1}, p = 0.017) but shows weaker binding (K_M of 0.46 mM vs 0.17 mM,

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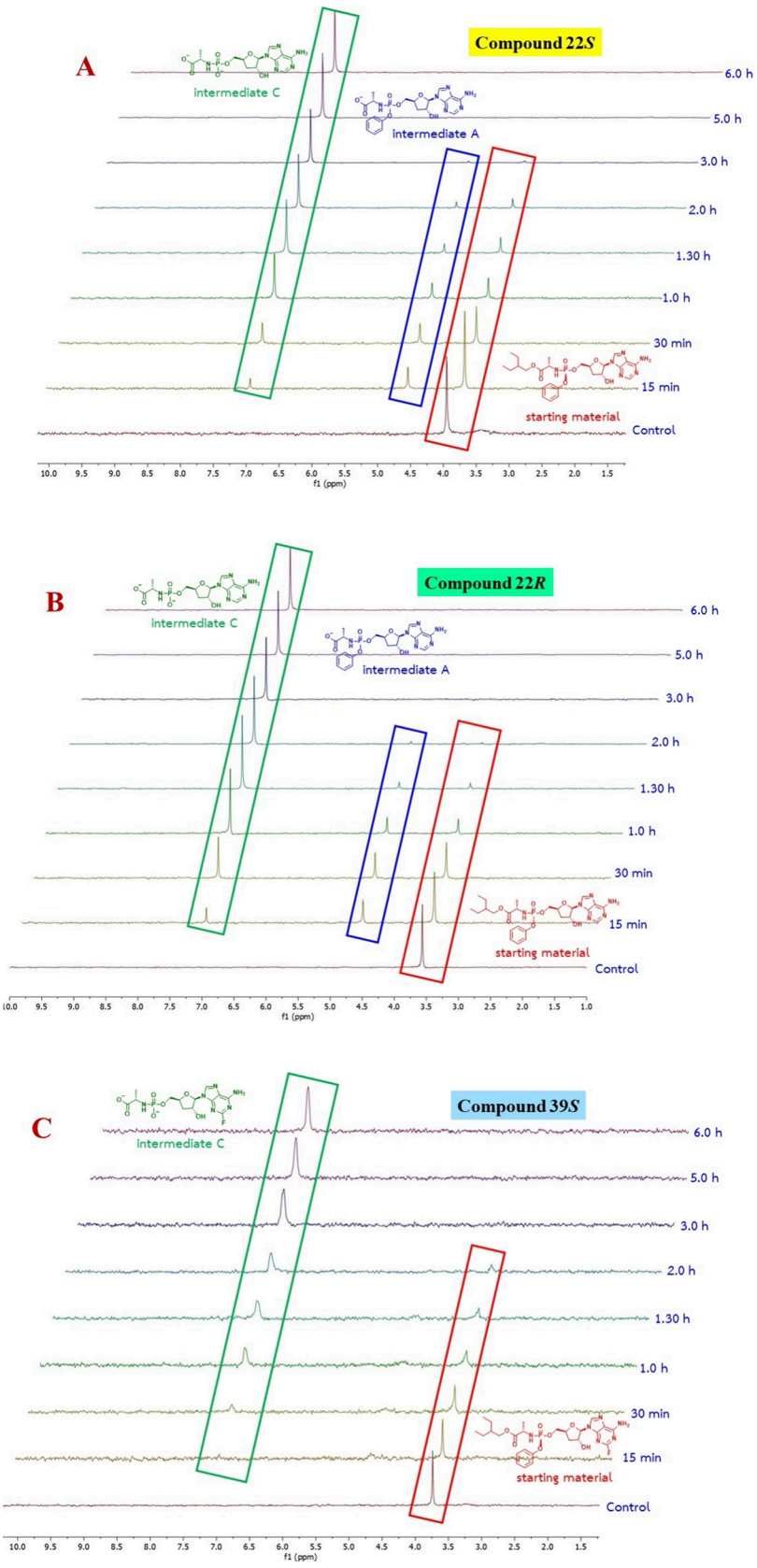

Figure 2. ^{31}P NMR spectra showing the time course of metabolic transformation for (A) compound **22***S*, (B) compound **22***R*, and (C) compound **39***S*, catalyzed by carboxypeptidase Y (CYP).

$p = 0.0055$). If one considers k_{cat}/K_M parameters, **22***S* is a more efficient substrate for CPY than **22***R*. If comparing **22***S* with **39***S*, which have the same stereochemistry but differs by one fluorine atom, compounds do not show any significant difference in

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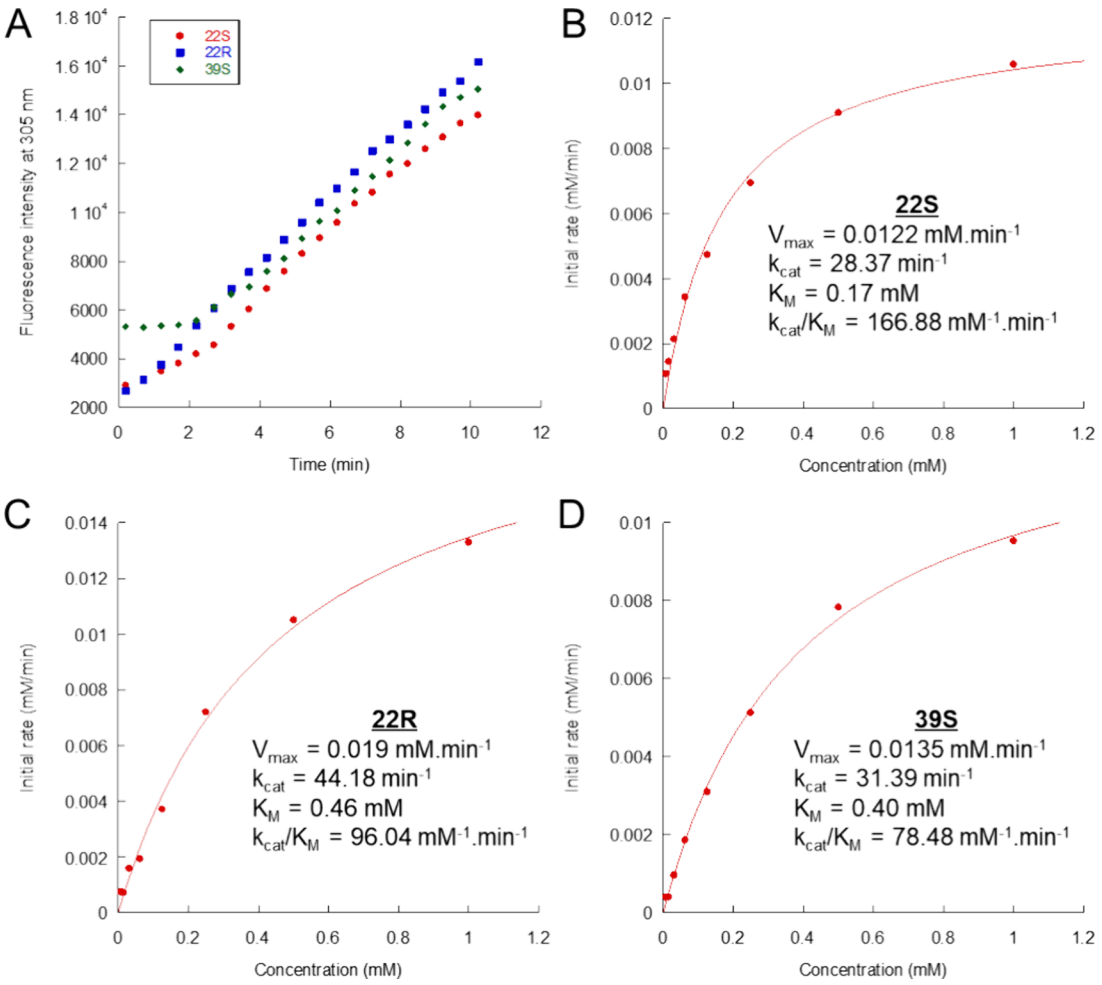

Figure 3. Activation of ProTides compounds by CPY monitored by fluorescence. (A) Representative curves obtained for **22***S*, **22***R*, and **39***S* at 1 mM, (B) Michaelis–Menten curve for **22***S*, (C) Michaelis–Menten curve for **22***R*, and (D) Michaelis–Menten curve for **22***S*.

reaction rate (V_{max} of 0.0122 mM min^{-1} vs 0.0135 mM min^{-1}, p = 0.46). Yet, the presence of the fluorine atom results in a significantly weaker enzyme affinity (K_M of 0.40 mM vs 0.17 mM, p = 0.041), making **22***S* an overall more efficient substrate.

Lastly, to further explore metabolic mimicry, human microsomal stability assays for cordycepin and its ProTide were carried out for the same derivatives, including **22***S*, **22***R*, and **39***S*, as shown in Table 2. Comparing to standard drugs, including carbamazepine and propranolol, our compounds were rapidly metabolized in the presence of human liver microsomes (HLM) supplemented with and without NADPH (see Supporting Information Figure 4). Therefore, the fast metabolic labile could stem from phase I metabolism (simple ester hydrolysis) and metabolic stability. Notably, 2-fluorocordycepin phosphoramidate (**39***S*) has a significantly extended half-life, approximately 2-fold that of **22***S*, indicating greater stability. These findings align with the research reported by Vodnala and co-workers[18] as well as the effect on fluorination of cordycepin.

Table 2. Metabolic Stability of Cordycepin and Its Derivatives by a Microsomal Stability Assay

	human liver microsomes NADPH (n = 3)		
test compounds	Cl_{int} (μL/min/mg protein)	$t_{1/2}$ (min)	Cl_{int} class
carbamazepine	<3	>180	low
propranolol	32.12 ± 4.89	54.86 ± 9.00	moderate
cordycepin (1)	141.78 ± 10.20	12.27 ± 0.88	high
22*S*	2117.29 ± 89.73	0.82 ± 0.04	high
22*R*	2613.26 ± 245.39	0.67 ± 0.06	high
39*S*	1228.96 ± 101.93	1.42 ± 0.12	high

CONCLUSIONS

In conclusion, we have successfully developed synthetic methods from cordycepin and obtained both *Sp*- and *Rp*-phosphoramidate ProTide **22**–**27** with diverse ester functionalities. In addition, the C2 halogenated *Sp*-**39**, **40**, and **41** were efficiently accessed via the stannylated intermediate. These novel synthetic ProTides were evaluated for their antiviral activity against three RNA viruses. In contrast to previous research, cordycepin and our ProTides did not show inhibitory activities against SARS-CoV-2, but cordycepin showed greater activity against influenza in comparison to its ProTides. Notably, **22***S*, **27***S*, and **27***R* displayed intriguing activity against dengue virus without cytotoxicity to Vero cells, and the *Sp*-isomer generally demonstrated enhanced efficacy. These ProTides are promising candidates for further dengue drug development. To understand the effect of the newly synthesized ProTide compound on biotransformation, *in vitro* metabolic studies were performed. As a result, the addition of *Sp*-cordycepin ProTide and fluorine could delay the intrinsic metabolic effect.

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These findings underscore the importance of structural modifications and chirality of ProTides in biological activity and metabolic stability, informing the design of next-generation antiviral agents.

EXPERIMENTAL SECTION

General Information

All reactions were conducted under an argon or nitrogen atmosphere in oven- or flamed-dried glassware. Solvents were used as received from the suppliers. All other reagents were obtained from commercial sources and used without further purification. Racemic phosphoramidate reagent **3** was stock chemical and made in our research group.[19] Column chromatography was performed on Silica gel 60 (0.063−0.200 mm, Merck) and Ultra-Performance Flash Purification Chromatography (UPFP), Interchim Generation 5-PF-5.125. Preparative chiral column chromatography using Lux 5 μm Cellulose-2, Column size 250 mm × 21.2 mm was used to separate all racemic mixture. Thin-layer chromatography (TLC) was performed on Silica gel 60 F_{254} (Merck). ^{1}H, ^{13}C, ^{31}P and ^{19}F NMR Spectroscopic data were reported in ppm on the δ scale and recorded on 400 and 500 MHz Bruker Mercury-400 plus or Bruker/Advance 400 NMR spectrometer. The data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad, and app = apparent), coupling constant (s) in hertz (Hz). High-resolution mass spectra (HRMS) were obtained on a micrOTOF mass spectrometer (Bruker Daltonics) using the ESI technique. Diastereomeric purity was determined by ultrahigh-performance liquid chromatography (UHPLC) on Vanquish with Chromeleon 7 software using CHIRALPAK IG-U 1.6 μm 100 × 3.0 mm column and a gradient mobile phase consisting of methanol (phase A) and acetonitrile (phase B). The gradient was as follows: isocratic for 3 min at 100% A, from 3 to 12 min decreased to 5% A, from 12 to 13 min raised to 95% A, and finally column equilibrated at 100% A until 15 min. Flow rate was 0.4 mL/min, and temperature was 40 °C.

General Procedure for Silyl Deprotection at the 5′-Position

To a solution of silyl ether derivatives (1.0 equiv) in anhydrous THF (0.16 M) at 0 °C under an argon atmosphere was added a solution of TFA:H_2O (1:1, 0.33 M). The reaction was allowed to react at 25 °C for 4−8 h, monitored by TLC. Then, the reaction was neutralized with saturated aqueous $NaHCO_3$ and extracted with EtOAc (×3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by column chromatography to give the primary alcohol derivative.

General Procedure for Phosphorylation

To a solution of primary alcohol derivative (1 equiv) in anhydrous THF (0.065 M) were added phosphate reagent (1.5 equiv) and $MgCl_2$ (1.5 equiv) and stirred at 50 °C. After stirring at 50 °C for 10 min, *N*,*N*-diisopropylethylamine (DIEA) (2.5 equiv) was added and stirred at this temperature for 2−6 h monitored by TLC. The reaction mixture was then cooled to room temperature and diluted with H_2O. The organic phase was extracted with EtOAc (×4), washed with saturated aqueous $NaHCO_3$ and brine, dried over with anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by column chromatography to afford the phosphoramidate ProTide-cordycepin derivative.

General Procedure for Silyl Deprotection at the 2′-Position

To a solution of phosphoramidate ProTide-cordycepin derivative (1 equiv) in anhydrous THF (0.22 M) at 0 °C under an argon atmosphere was added a solution of TFA:H_2O (1:1, 0.1 M). The reaction was stirred at 0 °C to room temperature for 18−24 h, monitored by TLC, and then concentrated under reduced pressure. Purification of the crude residue by column chromatography yielded the desired product.

9-((2*R*,3*R*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-9*H*-purin-6-amine (9). To a solution of cordycepin (**1**) (5.00 g, 19.9 mmol) in anhydrous DMF (46 mL, 0.43 M) at room temperature under an argon atmosphere were added imidazole (8.94 g, 131.34 mmol) and *tert*-butyldimethylsilyl chloride, TBSCl) (9.90 g, 65.67 mmol). The reaction was stirred at room temperature for 20 h, then quenched with saturated aqueous NH_4Cl (150 mL), and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The crude residue was purified by column chromatography (10−30% EtOAc/hexanes) to afford **4** (8.63 g, 90%) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.30 (s, 1H), 8.13 (s, 1H), 7.29 (br s, 2H), 5.88 (d, J = 2.3 Hz, 1H), 4.73−4.67 (m, 1H), 4.43−4.35 (m, 1H), 3.94 (dd, J = 11.5, 3.0 Hz, 1H), 3.76 (dd, J = 11.5, 3.6 Hz, 1H), 2.33−2.25 (m, 1H), 1.96−1.89 (m, 1H), 0.88 (s, 9H), 0.82 (s, 9H), 0.06 (s, 6H), 0.00 (s, 3H), −0.00 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 155.9, 152.6, 148.9, 138.2, 118.9, 90.3, 80.0, 76.5, 63.9, 33.9, 25.8 (3C), 25.5 (3C), 18.0, 17.6, −5.0, −5.1, −5.4, −5.4; HRMS (ESI) m/z calcd for $C_{22}H_{42}N_5O_3Si_2$ $[M + H]^+$ 480.2821, found 480.2825.

((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methanol (4). Alcohol **4** was prepared from **9** (6.52 g, 13.6 mmol) using the general procedure for silyl deprotection at the 5′-position. The crude residue was purified by column chromatography (40% EtOAc/hexanes−100% EtOAc) to give **4** (3.98 g, 80%) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.38 (s, 1H), 8.14 (s, 1H), 7.32 (br s, 2H), 5.84 (d, J = 3.0 Hz, 1H), 5.30−5.23 (m, 1H), 4.79−4.73 (m, 1H), 4.39−4.31 (m, 1H), 3.72 (d, J = 12.0 Hz, 1H), 3.53 (d, J = 12.0 Hz, 1H), 2.32−2.24 (m, 1H), 1.97−1.90 (m, 1H), 0.81 (s, 9H), −0.03 (s, 3H), −0.05 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.0, 152.4, 148.8, 139.0, 119.1, 90.6, 80.3, 75.8, 62.5, 34.3, 25.5 (3C), 17.6, −5.1, −5.1; HRMS (ESI) m/z calcd for $C_{16}H_{27}N_5NaO_3Si$ $[M + Na]^+$ 388.1775, found 388.1771.

2-Ethylbutyl ((((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy) tetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (10). Compound **10** was prepared from alcohol **4** (1.10 g, 3 mmol) and phosphate reagent **3a** (2.02 g, 4.5 mmol) using the general procedure for phosphorylation. The crude residue was purified by column chromatography (70% EtOAc/hexanes−100% EtOAc) to afford **10** as a white solid (1.70 g, 84%): ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.27−8.25 (m, 1H), 8.14 (s, 1H), 7.37−7.26 (m, 4H), 7.22−7.13 (m, 3H), 6.09−5.98 (m, 1H), 5.89 (dd, J = 7.5, 2.3 Hz, 1H), 4.85−4.77 (m, 1H), 4.58−4.45 (m, 1H), 4.33−4.10 (m, 2H), 3.97−3.76 (m, 3H), 2.35−2.26 (m, 1H), 2.04−1.94 (m, 1H), 1.46−1.36 (m, 1H), 1.30−1.15 (m, 7H), 0.86−0.72 (m, 15H), 0.02 to −0.07 (m, 6H); The ratio of two diastereomers *Sp*:*Rp* was 1.6:1 based on ^{31}P NMR (202 MHz, DMSO-d_6, δ 3.91 (*Rp*), 3.62 (*Sp*)); HRMS (ESI) m/z calcd for $C_{31}H_{49}N_6NaO7PSi$ $[M + Na]^+$ 699.3062, found 699.3064.

Isopropyl ((((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy) tetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (11). Compound **11** was prepared from alcohol **4** (0.3565 g, 1 mmol) and phosphate reagent **3b** (0.612 g, 1.5 mmol) using the general procedure for phosphorylation. The crude residue was purified by column chromatography (70% EtOAc/hexanes−100% EtOAc) to provide **11** as a white solid (0.471 g, 74%): ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.26 (s, 1H), 8.14 (s, 1H), 7.38−7.25 (m, 4H), 7.22−7.12 (m, 3H), 6.04−5.94 (m, 1H), 5.92−5.87 (m, 1H), 4.87−4.77 (m, 2H), 4.59−4.46 (m, 1H), 4.32−4.10 (m, 2H), 3.82−3.67 (m, 1H), 2.36−2.28 (m, 1H), 2.04−1.95 (m, 1H), 1.22−1.15 (m, 3H), 1.14−1.06 (m, 6H), 0.82 (s, 9H), − 0.01− −0.05 (m, 6H); the ratio of two diastereomers *Sp*:*Rp* was 1.4:1 based on ^{31}P NMR (202 MHz, DMSO-d_6, δ 3.90 (*Rp*), 3.66 (*Sp*)); HRMS (ESI) m/z calcd for $C_{28}H_{43}N_6NaO7PSi$ $[M + Na]^+$ 657.2592, found 657.2595.

Methyl ((((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (12). Compound **12** was prepared from alcohol **4** (0.73 g, 2 mmol) and phosphate reagent **3c** (1.14 g, 3 mmol) using the general procedure for phosphorylation. The crude residue was purified by column chromatography (70% EtOAc/hexanes−100% EtOAc) to give **12** as a white solid (1.05 g, 87%): ^{1}H NMR (500 MHz, CD_3OD) δ 8.31−8.27 (m, 1H), 8.21−8.18 (m, 1H), 7.37−7.29 (m, 2H), 7.25−7.14 (m, 3H), 6.00−5.96 (m, 1H), 4.92−

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4.87 (m, 1H), 4.71−4.61 (m, 1H), 4.49−4.37 (m, 1H), 4.33−4.25 (m, 1H), 3.97−3.84 (m, 1H), 3.67−3.60 (m, 3H), 2.41−2.32 (m, 1H), 2.11−2.04 (m, 1H), 1.34−1.24 (m, 3H), 0.91−0.85 (m, 9H), 0.08−0.01 (m, 6H); the ratio of two diastereomers *Sp*:*Rp* was 1.4:1 based on ^{31}P NMR (202 MHz, DMSO-d_6, δ 4.03 (*Rp*), 3.79 (*Sp*)); HRMS (ESI) m/z calcd for $C_{26}H_{39}N_6NaO_7PSi$ $[M + Na]^+$ 629.2279, found 629.2276.

Ethyl ((((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (13). Compound **13** was prepared from alcohol **4** (0.73 g, 2 mmol) and phosphate reagent **3d** (1.18 g, 3 mmol) using a general procedure for phosphorylation. The crude residue was purified by column chromatography (70% EtOAc/hexanes−100% EtOAc) to give **13** as a white solid (1.01 g, 81%): ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 8.32−8.27 (m, 1H), 8.22−8.19 (m, 1H), 7.37−7.30 (m, 2H), 7.25−7.14 (m, 3H), 5.98−5.97 (m, 1H), 4.93−4.87 (m, 1H), 4.70−4.61 (m, 1H), 4.49−4.38(m, 1H), 4.33−4.26 (m, 1H), 4.14−4.06 (m, 2H), 3.96−3.84 (m, 1H), 2.41−2.32 (m, 1H), 2.14−2.05 (m, 1H), 1.32−1.17 (m, 6H), 0.89−0.87 (m, 9H), 0.09−0.00 (m, 6H); the ratio of two diastereomers *Sp*:*Rp* was 1.3:1 based on ^{31}P NMR (202 MHz, DMSO-d_6, δ 4.06 (*Rp*), 3.81 (*Sp*)); HRMS (ESI) m/z calcd for $C_{27}H_{41}N_6NaO_7PSi$ $[M + Na]^+$ 643.2436, found 643.2421.

***tert*-Butyl ((((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy) tetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (14).** Compound **14** was prepared from alcohol **4** (0.73 g, 2 mmol) and phosphate reagent **3e** (1.27 g, 3 mmol) using a general procedure for phosphorylation. The crude residue was purified by column chromatography (70% EtOAc/hexanes−100% EtOAc) to give **14** as a white solid (1.00 g, 77%): ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 8.31−8.26 (m, 1H), 8.22−8.18 (m, 1H), 7.37−7.29 (m, 2H), 7.26−7.13 (m, 3H), 5.99−5.95 (m, 1H), 4.91−4.87 (m, 1H), 4.71−4.61 (m, 1H), 4.48−4.38 (m, 1H), 4.34−4.26 (m, 1H), 3.84−3.74 (m, 1H), 2.40−2.31 (m, 1H), 2.11−2.05 (m, 1H), 1.43−1.39 (m, 9H), 1.30−1.22 (m, 3H), 0.89−0.86 (m, 9H), 0.08−0.01 (m, 6H); the ratio of two diastereomers *Sp*:*Rp* was 1.4:1 based on ^{31}P NMR (202 MHz, DMSO-d_6, δ 4.10 (*Rp*), 3.90 (*Sp*)); HRMS (ESI) m/z calcd for $C_{29}H_{45}N_6NaO_7PSi$ $[M + Na]^+$ 671.2749, found 671.2750.

Benzyl ((((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy) tetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (15). Compound **15** was prepared from alcohol **4** (0.73 g, 2 mmol) and phosphate reagent **3e** (1.37 g, 3 mmol) using a general procedure for phosphorylation. The crude residue was purified by column chromatography (70% EtOAc/hexanes−100% EtOAc) to give **15** as a white solid (1.17 g, 85%): ^{1}H NMR (500 MHz, CD_3OD) δ 8.32−8.24 (m, 1H), 8.19 (s, 1H), 7.36−7.24 (m, 7H), 7.23−7.13 (m, 3H), 5.97−5.93 (m, 1H), 5.15−5.04 (m, 2H), 4.88−4.80 (m, 1H), 4.64−4.55 (m, 1H), 4.43−4.34 (m, 1H), 4.27−4.18 (m, 1H), 4.02−3.90 (m, 1H), 2.33−2.23 (m, 1H), 2.05−1.96 (m, 1H), 1.33−1.25 (m, 3H), 0.88 (s, 9H), 0.08−0.00 (m, 6H); the ratio of two diastereomers *Sp*:*Rp* was 1.5:1 based on ^{31}P NMR (202 MHz, DMSO-d_6, δ 4.11 (*Rp*), 3.71 (*Sp*)); HRMS (ESI) m/z calcd for $C_{32}H_{43}N_6NaO_7PSi$ $[M + Na]^+$ 705.2592, found 705.2596.

2-Ethylbutyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)-phosphoryl)-*L*-alaninate (22*S*) and 2-Ethylbutyl ((*R*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (22*R*). Compounds **22***S* and **22***R* were synthesized from **10** (1.51 g, 2.23 mmol) using the general procedure for silyl deprotection at the 2′-position to give racemic product **16** (0.845 g, 67%), which was purified by preparative chiral column chromatography (5−7% MeOH/ACN; flow rate 6 mL/min) to afford two separable diastereomers: **22***R* (*Rp*-isomer, white solid, 140 mg, 33%): ^{1}H NMR (500 MHz, CD_3OD): δ 8.28 (s, 1H), 8.21 (s, 1H), 7.34−7.28 (m, 2H), 7.22−7.13 (m, 3H), 6.02 (d, J = 1.8 Hz, 1H), 4.74−4.66 (m, 2H), 4.51−4.43 (m, 1H), 4.34−4.27 (m, 1H), 4.07−3.95 (m, 2H), 3.94−3.84 (m, 1H), 2.40−2.29 (m, 1H), 2.14−2.04 (m, 1H), 1.53−1.42 (m, 1H), 1.37−1.25 (m, 7H), 0.86 (t, J = 7.5 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.1 (d, $^3J_{C-P}$ = 4.4 Hz), 157.2, 153.7, 152.1 (d, $^2J_{C-P}$ = 6.7 Hz), 150.1, 140.5, 130.7 (2C), 126.1, 121.4 (2C, d, $^3J_{C-P}$ = 4.7 Hz), 120.4, 93.1, 80.4 (d, $^3J_{C-P}$ = 8.7 Hz), 76.6, 68.3 (d, $^2J_{C-P}$ = 5.3 Hz), 68.0, 51.7, 41.7, 34.6, 24.2, 24.2, 20.4 (d, $^3J_{C-P}$ = 7.4 Hz), 11.3, 11.2; ^{31}P NMR (202 MHz, CD_3OD): δ 3.98; HRMS (ESI) m/z calcd for $C_{25}H_{35}N_6NaO7P$ $[M + Na]^+$ 585.2197, found 585.2195. The diastereopurity of **22***R* was determined to be 99% de: retention time = 3.723 min; **22***S* (*Sp*-isomer, white solid, 170 mg, 40%): ^{1}H NMR (500 MHz, CD_3OD): δ 8.27 (s, 1H), 8.21 (s, 1H), 7.36−7.29 (m, 2H), 7.22 (d, J = 8.4 Hz, 2H), 7.17 (t, J = 7.4 Hz, 1H), 6.01 (d, J = 1.6 Hz, 1H), 4.75−4.70 (m, 1H), 4.69−4.62 (m, 1H), 4.43−4.36 (m, 1H), 4.31−4.24 (m, 1H), 4.00 (dd, J = 10.9, 5.8 Hz, 1H), 3.96−3.86 (m, 2H), 2.41−2.32 (m, 1H), 2.13−2.05 (m, 1H), 1.49−1.39 (m, 1H), 1.36−1.25 (m, 7H), 0.93−0.76 (m, 6H); ^{13}C NMR(126 MHz, CD_3OD): δ 174.9 (d, $^3J_{C-P}$ = 5.2 Hz), 157.3, 153.8, 152.1 (d, $^2J_{C-P}$ = 7.2 Hz), 150.1, 140.5, 130.7 (2C), 126.1, 121.3 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.5, 93.2, 80.4 (d, $^3J_{C-P}$ = 8.5 Hz), 76.6, 68.6 (d, $^2J_{C-P}$ = 5.3 Hz), 68.0, 51.5, 41.7, 34.9, 24.2, 24.2, 20.6 (d, $^3J_{C-P}$ = 6.6 Hz), 11.3, 11.2; ^{31}P NMR (202 MHz, CD_3OD): δ 3.78; HRMS (ESI) m/z calcd for $C_{25}H_{35}N_6NaO7P$ $[M + Na]^+$ 585.2197, found 585.2191. The diastereopurity of **22***S* was determined to be 98% de: retention time = 6.203 min.

Isopropyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (23*S*) and Isopropyl ((*R*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (23*R*). Compounds **23***S* and **23***R* were synthesized from **11** (0.980 g, 1.45 mmol) using the general procedure for silyl deprotection at the 2′-position to give racemic product **17** (0.542 g, 72%), which was purified by preparative chiral column chromatography (3% MeOH/ACN; flow rate 6 mL/min) to afford two separable diastereomers: **23***R* (*Rp*-isomer, white solid, 81 mg, 30%): ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 8.28 (s, 1H), 8.21 (s, 1H), 7.33−7.28 (m, 2H), 7.21−7.13 (m, 3H), 6.02 (d, J = 1.9 Hz, 1H), 4.98−4.90 (m, 1H), 4.75−4.67 (m, 2H), 4.49−4.44 (m, 1H), 4.34−4.27 (m, 1H), 3.86−3.78 (m, 1H), 2.40−2.32 (m, 1H), 2.13−2.06 (m, 1H), 1.25 (dd, J = 7.2, 1.1 Hz, 3H), 1.21−1.18 (m, 6H); ^{13}C NMR (126 MHz, CD_3OD): δ (ppm) 174.5 (d, $^3J_{C-P}$ = 4.7 Hz), 157.2, 153.6, 152.1 (d, $^2J_{C-P}$ = 6.6 Hz), 150.2, 140.5, 130.7 (2C), 126.1, 121.4 (2C, d, $^3J_{C-P}$ = 4.7 Hz), 120.4, 93.1, 80.4 (d, $^3J_{C-P}$ = 8.7 Hz), 76.6, 70.1, 68.3 (d, $^2J_{C-P}$ = 5.2 Hz), 51.7, 34.7, 21.9, 21.8, 20.3 (d, $^3J_{C-P}$ = 7.2 Hz); ^{31}P NMR (202 MHz, CD_3OD): δ 4.02; HRMS (ESI) m/z calcd for $C_{22}H_{29}N_6NaO7P$ $[M + Na]^+$ 543.1728, found 543.1727. The diastereopurity of **23***R* was determined to be 100% de: retention time = 2.449 min; **23***S* (*Sp*-isomer, white solid, 95 mg, 35%): ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 8.32 (s, 1H), 8.25 (s, 1H), 7.35−7.30 (m, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.17 (t, J = 7.3 Hz, 1H), 6.03 (d, J = 1.6 Hz, 1H), 4.95−4.88 (m, 1H), 4.76−4.73 (m, 1H), 4.71−4.65 (m, 1H), 4.44−4.37 (m, 1H), 4.31−4.24 (m, 1H), 3.91−3.82 (m, 1H), 2.43−2.34 (m, 1H), 2.14−2.06 (m, 1H), 1.29 (d, J = 7.1 Hz, 3H), 1.21−1.14 (m, 6H); ^{13}C NMR (126 MHz, CD_3OD): δ 174.3 (d, $^3J_{C-P}$ = 5.4 Hz), 157.3, 153.8, 152.1 (d, $^2J_{C-P}$ = 6.8 Hz), 150.2, 140.5, 130.7 (2C), 126.1, 121.3 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.5, 93.2, 80.3 (d, $^3J_{C-P}$ = 8.5 Hz), 76.5, 70.0, 68.6 (d, $^2J_{C-P}$ = 5.3 Hz), 51.6, 34.9, 21.9, 21.8, 20.5 (d, $^3J_{C-P}$ = 6.5 Hz); ^{31}P NMR (202 MHz, CD_3OD): δ (ppm) 3.84; HRMS (ESI) m/z calcd for $C_{22}H_{29}N_6NaO7P$ $[M + Na]^+$ 543.1728, found 543.1727. The diastereopurity of **23***S* was determined to be 97% de: retention time = 7.810 min.

Methyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (24*S*) and methyl ((*R*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (24*R*). Compounds **24***S* and **24***R* were synthesized from **12** (0.910 g, 1.5 mmol) using the general procedure for silyl deprotection at the 2′-position to give racemic product **18** (0.474 g, 64%), which was purified by preparative chiral column chromatography (3% MeOH/ACN; flow rate 6 mL/min) to afford two separable diastereomers: **24***R* (*Rp*-isomer, white solid, 90 mg, 37%): ^{1}H NMR (500 MHz, CD_3OD): δ 8.28 (s, 1H), 8.21 (s, 1H), 7.34−7.29 (m, 2H), 7.21−7.14 (m, 3H), 6.02 (d, J = 1.8 Hz, 1H), 4.76−4.67 (m, 2H), 4.46 (ddd, J = 11.4, 5.6, 2.7 Hz, 1H), 4.29 (ddd, J = 11.4, 5.9, 3.7 Hz, 1H), 3.87−3.78 (m, 1H), 3.64 (s, 3H), 2.38 (ddd, J =

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13.5, 9.4, 5.7 Hz, 1H), 2.09 (ddd, J = 13.4, 6.1, 2.5 Hz, 1H), 1.25 (dd, J = 7.2, 1.1 Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD): 175.5 (d, $^3J_{C-P}$ = 4.0 Hz), 157.3, 153.8, 152.1 (d, $^2J_{C-P}$ = 6.6 Hz), 150.2, 140.4, 130.7 (2C), 126.1, 121.4 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.4, 93.1, 80.4 (d, $^3J_{C-P}$ = 8.7 Hz), 76.6, 68.1 (d, $^2J_{C-P}$ = 5.2 Hz), 52.7, 51.4, 34.6, 20.2 (d, $^3J_{C-P}$ = 7.4 Hz); ^{31}P NMR (202 MHz, CD_3OD): δ 3.98; HRMS (ESI) m/z calcd for $C_{20}H_{25}N_6NaO_7P$ $[M + Na]^+$ 515.1415, found 515.1416. The diastereopurity of **24***R* was determined to be 100% de: retention time = 2.782 min; **24***S* (*S*p-isomer, white solid, 101 mg, 42%): ^{1}H NMR (500 MHz, CD_3OD): δ 8.26 (s, 1H), 8.21 (s, 1H), 7.35−7.30 (m, 2H), 7.24−7.14 (m, 3H), 6.01 (d, J = 1.6 Hz, 1H), 4.75−4.72 (m, 1H), 4.70−4.64 (m, 1H), 4.42−4.36 (m, 1H), 4.31−4.24 (m, 1H), 3.96−3.88 (m, 1H), 3.61 (s, 3H), 2.42−2.34 (m, 1H), 2.13−2.07 (m, 1H), 1.29 (d, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD): 175.2 (d, $^3J_{C-P}$ = 5.2 Hz), 157.3, 153.8, 152.1 (d, $^2J_{C-P}$ = 6.7 Hz), 150.2, 140.5, 130.7 (2C), 126.1, 121.3 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.5, 93.2, 80.3 (d, $^3J_{C-P}$ = 8.5 Hz), 76.6, 68.6 (d, $^2J_{C-P}$ = 5.3 Hz), 52.7, 51.4, 34.8, 20.4 (d, $^3J_{C-P}$ = 6.5 Hz); ^{31}P NMR (202 MHz, CD_3OD): δ 3.81; HRMS (ESI) m/z calcd for $C_{20}H_{25}N_6NaO_7P$ $[M + Na]^+$ 515.1415, found 515.1414. The diastereopurity of **24***S* was determined to be 98% de: retention time = 5.692 min.

Ethyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (25*S*) and ethyl ((*R*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)-phosphoryl)-*L*-alaninate (25*R*). Compounds **25***S* and **25***R* were synthesized from **13** (0.931 g, 1.5 mmol) using the general procedure for silyl deprotection at the 2′-position to give racemic product **19** (0.513 g, 68%), which was purified by preparative chiral column chromatography (3−5% MeOH/ACN; flow rate 6 mL/min) to afford two separable diastereomers: **25***R* (*R*p-isomer, white solid, 89 mg, 35%): ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 8.28 (s, 1H), 8.21 (s, 1H), 7.34−7.28 (m, 2H), 7.21−7.13 (m, 3H), 6.02 (d, J = 1.5 Hz, 1H), 4.76−4.67 (m, 2H), 4.49−4.43 (m, 1H), 4.32−4.26 (m, 1H), 4.14−4.06 (m, 2H), 3.87−3.79 (m, 1H), 2.41−2.33 (m, 1H), 2.12−2.06 (m, 1H), 1.26 (d, J = 7.2 Hz, 3H), 1.20 (t, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 175.0 (d, $^3J_{C-P}$ = 4.4 Hz), 157.3, 153.8, 152.1 (d, $^2J_{C-P}$ = 6.7 Hz), 150.2, 140.4, 130.7 (2C), 126.1, 121.4 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.4, 93.1, 80.4 (d, $^3J_{C-P}$ = 8.6 Hz), 76.6, 68.2 (d, $^2J_{C-P}$ = 5.1 Hz), 62.3, 51.6, 34.7, 20.3 (d, $^3J_{C-P}$ = 7.3 Hz), 14.4; ^{31}P NMR (202 MHz, CD_3OD): δ 4.01; HRMS (ESI) m/z calcd for $C_{21}H_{27}N_6NaO_7P$ $[M + Na]^+$ 529.1571, found 529.1570. The diastereopurity of **25***R* was determined to be 99% de: retention time = 2.708 min; **25***S* (*S*p-isomer, white solid, 98 mg, 38%): ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 8.26 (s, 1H), 8.21 (s, 1H), 7.35−7.29 (m, 2H), 7.24−7.13 (m, 3H), 6.01 (d, J = 1.6 Hz, 1H), 4.76−4.71 (m, 1H), 4.71−4.64 (m, 1H), 4.43−4.37 (m, 1H), 4.31−4.24 (m, 1H), 4.13−4.01 (m, 2H), 3.94−3.86 (m, 1H), 2.42−2.35 (m, 1H), 2.12−2.06 (m, 1H), 1.30 (d, J = 7.1 Hz, 3H), 1.18 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 174.8 (d, $^3J_{C-P}$ = 5.4 Hz), 157.3, 153.8, 152.1 (d, $^2J_{C-P}$ = 6.9 Hz), 150.1, 140.5, 130.7 (2C), 126.1, 121.3 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.4, 93.2, 80.3 (d, $^3J_{C-P}$ = 8.5 Hz), 76.6, 68.6 (d, $^2J_{C-P}$ = 5.5 Hz), 62.3, 51.4, 34.9, 20.4 (d, $^3J_{C-P}$ = 6.5 Hz), 14.4; ^{31}P NMR (202 MHz, CD_3OD): δ 3.83; HRMS (ESI) m/z calcd for $C_{21}H_{27}N_6NaO_7P$ $[M + Na]^+$ 529.1571, found 529.1577. The diastereopurity of **25***S* was determined to be 99% de: retention time = 7.271 min.

tert*-Butyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (26*S*) and *tert*-Butyl ((*R*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (26*R*).** Compounds **26S* and **26***R* were synthesized from **14** (0.973 g, 1.5 mmol) using the general procedure for silyl deprotection at the 2′-position to give racemic product **20** (0.510 g, 64%), which was purified by preparative chiral column chromatography (2% MeOH/ACN; flow rate 10 mL/min) to give two separable diastereomers: **26***R* (*R*p-isomer, white solid, 74 mg, 29%): ^{1}H NMR (500 MHz, CD_3OD): δ 8.27 (s, 1H), 8.21 (s, J = 4.0 Hz, 1H), 7.35−7.27 (m, 2H), 7.23−7.13 (m, 3H), 6.01 (d, J = 1.8 Hz, 1H), 4.75−4.67 (m, 2H), 4.49−4.44 (m, 1H), 4.35−4.28 (m, 1H), 3.80−3.69 (m, 1H), 2.40−2.32 (m, 1H), 2.12−2.05 (m, 1H), 1.41 (s, 9H), 1.24 (d, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 174.3 (d, $^3J_{C-P}$ = 4.8 Hz), 157.3, 153.8, 152.1 (d, $^2J_{C-P}$ = 6.8 Hz), 150.2, 140.4, 130.7 (2C), 126.1, 121.4 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.4, 93.1, 82.5, 80.4 (d, $^3J_{C-P}$ = 8.6 Hz), 76.6, 68.3 (d, $^2J_{C-P}$ = 5.2 Hz), 52.2, 34.7, 28.1 (3C), 20.4 (d, $^3J_{C-P}$ = 7.2 Hz); ^{31}P NMR (202 MHz, CD_3OD): δ 4.06; HRMS (ESI) m/z calcd for $C_{23}H_{31}N_6NaO_7P$ $[M + Na]^+$ 557.1884, found 557.1874. The diastereopurity of **26***R* was determined to be 100% de: retention time = 2.229 min; **26***S* (*S*p-isomer, white solid, 92 mg, 36%): ^{1}H NMR (500 MHz, CD_3OD): δ 8.27 (s, 1H), 8.21 (s, 1H), 7.35−7.30 (m, 2H), 7.24−7.15 (m, 3H), 6.01 (d, J = 1.8 Hz, 1H), 4.75−4.71 (m, 1H), 4.70−4.64 (m, 1H), 4.43−4.38 (m, 1H), 4.30−4.25 (m, 1H), 3.84−3.75 (m, 1H), 2.44−2.37 (m, 1H), 2.14−2.06 (m, 1H), 1.40 (s, J = 8.0 Hz, 9H), 1.27 (d, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 174.0 (d, $^3J_{C-P}$ = 5.6 Hz), 157.3, 153.8, 152.1 (d, $^2J_{C-P}$ = 7.0 Hz), 150.2, 140.5, 130.7 (2C), 126.1, 121.3 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.5, 93.2, 82.6, 80.3 (d, $^3J_{C-P}$ = 8.4 Hz), 76.5, 68.6 (d, $^2J_{C-P}$ = 5.4 Hz), 52.1, 34.9, 28.1 (3C), 20.7 (d, $^3J_{C-P}$ = 6.4 Hz); ^{31}P NMR (202 MHz, CD_3OD): δ 3.95; HRMS (ESI) m/z calcd for $C_{23}H_{31}N_6NaO_7P$ $[M + Na]^+$ 557.1884, found 557.1882. The diastereopurity of **26***S* was determined to be 95% de: retention time = 5.608 min.

Benzyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (27*S*) and Benzyl ((*R*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)-(phenoxy)phosphoryl)-*L*-alaninate (27*R*). Compounds **27***S* and **27***R* were synthesized from **15** (1.02 g, 1.5 mmol) using the general procedure for silyl deprotection at the 2′-position to give racemic product **21** (0.614 g, 72%), which was purified by preparative chiral column chromatography (3% MeOH/ACN; flow rate 15 mL/min) to give two separable diastereomers: **27***R* (*R*p-isomer, white solid, 95 mg, 31%): ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 8.23 (s, 1H), 8.20 (s, 1H), 7.35−7.24 (m, 7H), 7.21−7.12 (m, 3H), 5.98 (d, J = 1.6 Hz, 1H), 5.10−5.03 (m, 2H), 4.69−4.57 (m, 2H), 4.40−4.32 (m, 1H), 4.25−4.18 (m, 1H), 4.01−3.92 (m, 1H), 2.34−2.26 (m, 1H), 2.06−2.00 (m, 1H), 1.30 (d, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 174.8 (d, $^3J_{C-P}$ = 4.1 Hz), 157.3, 153.8, 152.1, 150.1, 140.4, 137.2, 130.7 (2C), 129.5 (2C), 129.3 (3C), 126.1, 121.4 (2C, d, $^3J_{C-P}$ = 4.7 Hz), 120.4, 93.1, 80.4 (d, $^3J_{C-P}$ = 8.6 Hz), 76.6, 68.2 (d, $^2J_{C-P}$ = 5.1 Hz), 67.9, 51.7, 34.5, 20.2 (d, $^3J_{C-P}$ = 7.4 Hz); ^{31}P NMR (202 MHz, CD_3OD): δ 4.03; HRMS (ESI) m/z calcd for $C_{26}H_{29}N_6NaO_7P$ $[M + Na]^+$ 591.1728, found 591.1731. The diastereopurity of **27***R* was determined to be 99% de: retention time = 4.358 min; **27***S* (*S*p-isomer, white solid, 108 mg, 35%): ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 8.25 (s, 1H), 8.20 (s, 1H), 7.34−7.25 (m, 7H), 7.19−7.12 (m, 3H), 5.99 (d, J = 1.3 Hz, 1H), 5.14−5.07 (m, 2H), 4.72−4.68 (m, 1H), 4.66−4.60 (m, 1H), 4.42−4.36 (m, 1H), 4.25−4.20 (m, 1H), 3.96−3.88 (m, 1H), 2.34−2.26 (m, 1H), 2.08−2.01 (m, 1H), 1.27 (d, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 174.6 (d, $^3J_{C-P}$ = 5.0 Hz), 157.3, 153.8, 152.0 (d, $^2J_{C-P}$ = 6.7 Hz), 150.1, 140.4, 137.2, 130.7 (2C), 129.5 (2C), 129.3, 129.2 (2C), 126.1, 121.3 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.5, 93.2, 80.3 (d, $^3J_{C-P}$ = 8.5 Hz), 76.6, 68.5 (d, $^2J_{C-P}$ = 5.3 Hz), 67.8, 51.5, 34.8, 20.3 (d, $^3J_{C-P}$ = 6.6 Hz); ^{31}P NMR (202 MHz, CD_3OD): δ 3.71; HRMS (ESI) m/z calcd for $C_{26}H_{29}N_6NaO_7P$ $[M + Na]^+$ 591.1728, found 591.1725. The diastereopurity of **27***S* was determined to be 96% de: retention time = 6.214 min.

9-((2*R*,3*R*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-6-chloro-9*H*-purine (28). To a solution of silyl ether **9** (1.0 g, 2.08 mmol) in anhydrous CH_2Cl_2 (20 mL, 0.1 M) were added *tert*-butyl nitrite (1.0 mL, 8.33 mmol, 4 equiv) and chlorotrimethylsilane (530 μL, 4.17 mmol, 2 equiv) at 0 °C to room temperature under an argon atmosphere for 5 h. The reaction mixture was quenched with aqueous $NaHCO_3$ (25 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification of the crude residue by column chromatography (10−20% EtOAc/hexanes) yielded **28** as a white solid (728 mg, 70%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.78 (s, 1H), 6.02 (d, J = 1.4 Hz, 1H), 4.79−4.77 (m, 1H), 4.48−4.44 (m, 1H), 4.00 (dd, J = 11.7, 2.9 Hz, 1H), 3.79 (dd, J = 11.7, 2.9 Hz, 1H), 2.27 (ddd, J = 13.2, 9.7, 5.0 Hz, 1H), 1.90

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(ddd, J = 13.2, 5.7, 2.2 Hz, 1H), 0.85 (s, 9H), 0.84 (s, 9H), 0.05 (s, 3H), 0.04 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.6, 151.0, 149.1, 144.6, 131.4, 91.3, 81.1, 76.6, 63.5, 33.5, 25.8 (3C), 25.5 (3C), 18.1, 17.6, − 4.9, − 5.0, − 5.4 (2C); HRMS (ESI) m/z calcd for $C_{22}H_{39}ClN_4NaO_3Si_2$ $[M + Na]^+$ 521.2147, found 521.2145.

9-((2*R*,3*R*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-6-chloro-2-(tributylstannyl)-9*H*-purine (29). To a solution of 2,2,6,6-tetramethylpiperidide (TMP) (1.3 mL, 7.97 mmol, 5 equiv) in anhydrous THF (8 mL, 0.1 M) was slowly added *n*-BuLi (1.6 M in hexanes) (5 mL, 7.95 mmol, 5 equiv) at −78 °C under an argon atmosphere. The reaction mixture was stirred at this temperature for 1 h before the addition of a solution of compound **28** (800 mg, 1.59 mmol) in anhydrous THF (5.5 mL, 0.3 M). After stirring for 1 h, to the reaction mixture was added Bu_3SnCl (2.2 mL, 7.97 mmol, 5 equiv), and the mixture was stirred at −78 °C for 30 min. The reaction was warmed to room temperature, quenched with aqueous NH_4Cl (20 mL), and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The crude residue was purified by column chromatography (5−10% EtOAc/hexanes) yielded 6-chloro-2-(tributylstannyl) purine (**29**) as a light yellow oil (980 mg, 78%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 6.13 (d, J = 1.2 Hz, 1H), 4.63−4.57 (m, 2H), 4.16 (dd, J = 11.7, 2.4 Hz, 1H), 3.80 (dd, J = 11.7, 2.4 Hz, 1H), 2.32 (ddd, J = 13.0, 10.0, 4.6 Hz, 1H), 1.88 (ddd, J = 13.0, 5.4, 1.9 Hz, 1H), 1.69−1.56 (m, 6H), 1.38−1.19 (m, 12H), 0.95 (s, 9H), 0.91 (s, 9H), 0.91−0.87 (m, 9H), 0.15−0.14 (m, 9H), 0.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.4, 150.1, 148.9, 142.3, 130.7, 91.7, 81.4, 77.4, 63.5, 33.4, 28.8 (3C), 27.1 (3C), 25.9 (3C), 25.5 (3C), 18.5, 17.8, 13.6 (3C), 10.5 (3C), −4.6, −5.0, −5.3, −5.4; HRMS (ESI) m/z calcd for $C_{34}H_{65}ClN_4NaO_3Si_2Sn$ $[M + Na]^+$ 811.3203, found 811.3200.

9-((2*R*,3*R*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-2-(tributylstannyl)-9*H*-purin-6-amine (8). To a solution of **29** (300 mg, 0.38 mmol) in THF (2 mL, 0.1 M) was added ammonia 7 N in MeOH (35 mL, 1.9 mmol, 5 equiv) at room temperature under an argon atmosphere. The reaction mixture was heated at 100 °C for 9 h. The reaction was warmed to room temperature and concentrated under reduced pressure, and purification of the crude residue by column chromatography (20−40% EtOAc/hexanes) yielded **8** as a light yellow oil (165 mg, 55%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (s, 1H), 7.03 (brs, 2H), 5.89 (d, J = 2.2 Hz, 1H), 4.85−4.83 (m, 1H), 4.37−4.34 (m, 1H), 3.89 (dd, J = 11.4, 3.4 Hz, 1H), 3.74 (dd, J = 11.4, 4.1 Hz, 1H), 2.38−2.32 (m, 1H), 1.94−1.88 (m, 1H), 1.61−1.40 (m, 6H), 1.32−1.23 (m, 6H), 1.16−0.95 (m, 6H), 0.85 (s, 9H), 0.82−0.68 (m, 18H), 0.01 (s, 6H), −0.02 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ178.5, 154.3, 148.1, 138.1, 118.4, 90.7, 80.0, 76.1, 64.2, 34.4, 28.6 (3C), 26.7 (3C), 25.8 (3C), 25.5 (3C), 18.0, 17.6, 13.5 (3C), 9.8 (3C), −5.0, −5.1, −5.4 (2C); HRMS (ESI) m/z calcd for $C_{34}H_{67}N_5NaO_3Si_2Sn$ $[M + Na]^+$ 792.3702, found 792.3701.

9-((2*R*,3*R*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-2-fluoro-9*H*-purin-6-amine (30). To a solution of 2-stannylated cordycepin intermediate **8** (150 mg, 0.195 mmol) in anhydrous CH_2Cl_2 (9 mL, 0.022 M) were added 2,6-di-*tert*-butyl-4-methylpyridine (40.2 mg, 0.195 mmol, 1.0 equiv), XeF_2 (82.7 mg, 0.49 mmol, 2.5 equiv), and silver triflate (125.5 mg, 0.49 mmol, 2.5 equiv), and the mixture was stirred at room temperature for 30 min under an argon atmosphere. The reaction mixture was quenched with $NaHCO_3$ (15 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. Purification of the crude residue by column chromatography (5−10% EtOAc/hexanes) afforded **30** as a white solid (64 mg, 65%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 6.56 (br s, 2H), 5.90 (d, J = 1.5 Hz, 1H), 4.60−4.53 (m, 1H), 4.12 (dd, J = 11.6, 2.6 Hz, 1H), 3.76 (dd, J = 11.6, 2.6 Hz, 1H), 2.27−2.20 (m, 1H), 1.84 (ddd, J = 13.0, 5.6, 2.2 Hz, 1H), 0.93 (s, 9H), 0.89 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H), 0.11 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1 (d, $^1J_{C-F}$ = 210.2 Hz), 157.2 (d, $^2J_{C-F}$ = 20.1 Hz), 150.7 (d, $^2J_{C-F}$ = 19.8 Hz), 139.3 (d, $^4J_{C-F}$ = 3.0 Hz), 118.2 (d, $^3J_{C-F}$ = 4.0 Hz), 92.0, 81.5, 77.4, 63.7, 33.6, 26.1 (3C), 25.8 (3C), 18.6, 18.0, −4.6, −4.9, −5.1, −5.3. ^{19}F NMR (470 MHz, $CDCl_3$) δ − 51.57; HRMS (ESI) m/z calcd for $C_{22}H_{40}FN_5NaO_3Si_2$ $[M + Na]^+$ 520.2551, found 520.2550.

9-((2*R*,3*R*,5*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-2-iodo-9*H*-purin-6-amine (31). To a solution of 2-stannylated cordycepin intermediate **8** (100 mg, 0.13 mmol) in anhydrous THF (4.5 mL, 0.030 M) was added iodine (50 mg, 0.195 mmol, 1.5 equiv) at room temperature under an argon atmosphere, and the mixture was stirred for 30 min. The reaction mixture was quenched with 10% $Na_2S_2O_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the crude residue by column chromatography (5−10% EtOAc/hexanes) yielded **31** as a light yellow oil (56 mg, 70%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 6.33 (brs, 2H), 5.91 (d, J = 1.0 Hz, 1H), 4.59−4.54 (m, 2H), 4.15 (dd, J = 11.6, 2.5 Hz, 1H), 3.77 (dd, J = 11.6, 2.5 Hz, 1H), 2.16 (ddd, J = 13.0, 10.2, 4.6 Hz, 1H), 1.79 (ddd, J = 13.0, 5.3, 1.8 Hz, 1H), 0.93 (s, 9H), 0.92 (s, 9H), 0.21 (s, 3H), 0.14 (s, 3H), 0.13 (s, 3H), 0.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.2, 149.5, 138.9, 120.0, 119.4, 92.3, 81.8, 77.6, 63.4, 33.2, 26.1 (3C), 25.8 (3C), 18.7, 18.1, −4.4, −4.9, −5.1, −5.3; HRMS (ESI) m/z calcd for $C_{22}H_{40}IN_5NaO_3Si_2$ $[M + Na]^+$ 628.1612, found 628.1607.

2-Bromo-9-((2*R*,3*R*,5*S*)-3-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-9*H*-purin-6-amine (32). To a solution of 2-stannylated cordycepin intermediate **8** (290 mg, 0.375 mmol) in anhydrous THF (17 mL, 0.022 M) was added NBS (167 mg, 0.9375 mmol, 2.5 equiv) at room temperature under an argon atmosphere, and the mixture was stirred for 1 h. The reaction mixture was quenched with aqueous $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. Purification of the crude residue by column chromatography (10−20% EtOAc/hexanes) yielded **32** as a light yellow oil (160 mg, 76%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 6.45 (brs, 2H), 5.94 (d, J = 1.1 Hz, 1H), 4.62−4.56 (m, 2H), 4.16 (dd, J = 11.7, 2.5 Hz, 1H), 3.78 (dd, J = 11.7, 2.5 Hz, 1H), 2.20 (ddd, J = 13.0, 10.0, 4.6 Hz, 1H), 1.82 (ddd, J = 13.0, 5.4, 1.9 Hz, 1H), 0.94 (s, 9H), 0.93 (s, 9H), 0.21 (s, 3H), 0.15 (s, 3H), 0.14 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 150.0, 144.6, 139.2, 119.3, 92.3, 81.8, 77.6, 63.5, 33.2, 26.1 (3C), 25.8 (3C), 18.6, 18.1, −4.5, −4.9, −5.1, −5.3; HRMS (ESI) m/z calcd for $C_{22}H_{40}BrN_5NaO_3Si_2$ $[M + Na]^+$ 580.1751, found 580.1748.

((2*S*,4*R*,5*R*)-5-(6-Amino-2-fluoro-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methanol (33). Alcohol **33** was prepared from **30** (750 mg, 1.50 mmol) using the general procedure for silyl deprotection at the 5′-position. The crude residue was purified by column chromatography (40% EtOAc/hexanes−100% EtOAc) to give **33** (440 mg, 77%) as a white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (s, 1H), 5.83 (d, J = 3.0 Hz, 1H), 4.81−4.77 (m, 1H), 4.52−4.47 (m, 1H), 3.93 (dd, J = 12.4, 2.7 Hz, 1H), 3.68 (dd, J = 12.4, 3.4 Hz, 1H), 2.35−2.28 (m, 1H), 2.06−2.00 (m, 1H), 0.87 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 160.4 (d, $^1J_{C-F}$ = 209.4 Hz), 159.0 (d, $^2J_{C-F}$ = 20.4 Hz), 151.5 (d, $^2J_{C-F}$ = 19.7 Hz), 141.1 (d, $^4J_{C-F}$ = 3.1 Hz), 118.8 (d, $^3J_{C-F}$ = 4.2 Hz), 93.3, 82.3, 77.5, 64.2, 35.3, 26.1 (3C), 18.7, −4.8, −4.9. ^{19}F NMR (470 MHz, CD_3OD) δ −53.45; HRMS (ESI) m/z calcd for $C_{16}H_{26}FN_5NaO_3Si$ $[M + Na]^+$ 406.1687, found 406.1681.

((2*S*,4*R*,5*R*)-5-(6-Amino-2-iodo-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methanol (34). Alcohol **34** was prepared from **31** (75 mg, 0.124 mmol) using the general procedure for silyl deprotection at the 5′-position. The crude residue was purified by column chromatography (40% EtOAc/hexanes−100% EtOAc) to give **34** (49 mg, 82%) as a white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (s, 1H), 5.84 (d, J = 2.7 Hz, 1H), 4.80−4.77 (m, 1H), 4.53−4.48 (m, 1H), 3.94 (dd, J = 12.4, 2.7 Hz, 1H), 3.67 (dd, J = 12.4, 3.4 Hz, 1H), 2.25 (ddd, J = 13.5, 8.1, 5.6 Hz, 1H), 2.02−1.96 (m, 1H), 0.89 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 157.0, 150.3, 140.6, 120.4, 120.4, 120.4,

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93.5, 82.5, 77.6, 64.0, 35.0, 26.2 (3C), 18.8, − 4.7, − 4.8; HRMS (ESI) m/z calcd for $C_{16}H_{26}IN_5NaO_3Si$ $[M + Na]^+$ 514.0747, found 514.0742.

((2*S*,4*R*,5*R*)-5-(6-Amino-2-bromo-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methanol (35). Alcohol **35** was prepared from **32** (160 mg, 0.283 mmol) using the general procedure for silyl deprotection at the 5′-position. The crude residue was purified by column chromatography (40% EtOAc/hexanes−100% EtOAc) to give **35** (86 mg, 70%) as a white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (s, 1H), 5.85 (d, J = 2.9 Hz, 1H), 4.81−4.78 (m, 1H), 4.53−4.48 (m, 1H), 3.93 (dd, J = 12.4, 2.7 Hz, 1H), 3.67 (dd, J = 12.4, 3.4 Hz, 1H), 2.27 (ddd, J = 13.4, 7.9, 5.7 Hz, 1H), 2.01 (ddd, J = 12.9, 6.6, 4.0 Hz, 1H), 0.89 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 157.8, 151.0, 145.8, 141.1, 119.9, 93.5, 82.5, 77.6, 64.1, 35.1, 26.1 (3C), 18.8, −4.7, −4.9; HRMS (ESI) m/z calcd for $C_{16}H_{26}BrN_5NaO_3Si$ $[M + Na]^+$ 466.0886, found 466.0880.

(*S*)-2-Ethylbutyl 2-(((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-2-fluoro-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (36*S*). ProTide product **36***S* was prepared from alcohol **33** (250 mg, 0.65 mmol) and phosphate reagent ((*S*)-2-ethylbutyl 2-(((*S*)-(4-nitrophenoxy)(phenoxy)phosphoryl)amino)propanoate (**6**)) (468 mg, 1.06 mmol) using a general procedure for phosphorylation. The crude residue was purified by column chromatography (70% EtOAc/hexanes−100% EtOAc) to afford **36***S* (270 mg, 60%) as a white solid; ^{1}H NMR (400 MHz, CD_3OD): δ 8.21 (s, 1H), 7.36−7.32 (m, 2H), 7.26−7.23 (m, 2H), 7.19−7.16 (m, 1H), 5.86 (d, J = 2.2 Hz, 1H), 4.83−4.80 (m, 1H), 4.66−4.61 (m, 1H), 4.44−4.39 (m, 1H), 4.32−4.26 (m, 1H), 4.03−3.99 (m, 1H), 3.96−3.90 (m, 2H), 2.32−2.25 (m, 1H), 2.05−1.99 (m, 1H), 1.47−1.39 (m, 1H), 1.35−1.27 (m, 7H), 0.89 (s, 9H), 0.87−0.83 (m, 6H), 0.09 (s, 3H), 0.08 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 174.9 (d, $^3J_{C-P}$ = 5.2 Hz), 160.5 (d, $^1J_{C-F}$ = 208.9 Hz), 159.0 (d, $^2J_{C-F}$ = 20.5 Hz), 152.1 (d, $^2J_{C-P}$ = 6.6 Hz), 151.7 (d, $^2J_{C-F}$ = 19.9 Hz), 140.5 (d, $^4J_{C-F}$ = 2.8 Hz), 130.7 (2C), 126.15, 121.3 (2C, d, $^3J_{C-P}$ = 4.9 Hz), 118.7, 93.1, 80.2 (d, $^3J_{C-P}$ = 8.5 Hz), 77.7, 68.5 (d, $^2J_{C-P}$ = 5.3 Hz), 68.0, 51.5, 41.6, 35.5, 26.1 (3C), 24.2, 24.1, 20.6 (d, $^3J_{C-P}$ = 6.5 Hz), 18.7, 11.3, 11.2, − 4.7, − 4.8; ^{31}P NMR (162 MHz, CD_3OD): δ 3.77. ^{19}F NMR (470 MHz, CD_3OD) δ − 53.06; HRMS (ESI) m/z calcd for $C_{31}H_{48}FN_6NaO_7PSi$ $[M + Na]^+$ 717.2973, found 717.2968.

2-Ethylbutyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-2-iodo-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (37*S*). ProTide product **37***S* was prepared from alcohol **34** (60 mg, 0.127 mmol) and phosphate reagent ((*S*)-2-ethylbutyl 2-(((*S*)-(4-nitrophenoxy)(phenoxy)phosphoryl)amino)propanoate (**6**)) (90 mg, 0.20 mmol) using the general procedure for phosphorylation. The crude residue was purified by column chromatography (70% EtOAc/hexanes−100% EtOAc) to afford **37***S* (63 mg, 65%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD): δ 8.16 (s, 1H), 7.34 (dd, J = 8.7, 7.0 Hz, 2H), 7.27−7.24 (m, 2H), 7.20−7.16 (m, 1H), 5.88 (d, J = 1.6 Hz, 1H), 4.83−4.81 (m, 1H), 4.70−4.63 (m, 1H), 4.44 (ddd, J = 11.4, 5.9, 2.8 Hz, 1H), 4.31 (ddd, J = 11.4, 6.2, 4.4 Hz, 1H), 4.00 (dd, J = 10.9, 5.8 Hz, 1H), 3.96−3.88 (m, 2H), 3.32−3.30 (m, 1H), 2.20 (ddd, J = 13.2, 9.7, 5.2 Hz, 1H), 1.99 (ddd, J = 13.2, 5.9, 2.4 Hz, 1H), 1.46−1.40 (m, 1H), 1.34−1.28 (m, 7H), 0.92 (s, 9H), 0.87−0.83 (m, 6H), 0.15 (s, 3H), 0.13 (s, 3H);^{13}C NMR (100 MHz, CD_3OD) δ 174.9 (d, $^3J_{C-P}$ = 5.1 Hz), 157.0, 152.1 (d, $^2J_{C-P}$ = 6.6 Hz), 150.4, 139.9, 130.7 (2C), 126.1, 121.3 (2C, d, $^3J_{C-P}$ = 5.1 Hz), 120.6, 120.3, 93.6, 80.6 (d, $^3J_{C-P}$ = 8.4 Hz), 77.9, 68.4 (d, $^2J_{C-P}$ = 5.3 Hz), 68.1, 51.5, 41.6, 35.3, 26.2 (3C), 24.2, 24.1, 20.6 (d, $^3J_{C-P}$ = 6.6 Hz), 18.8, 11.3, 11.2, − 4.5, − 4.7; ^{31}P NMR (162 MHz, CD_3OD): δ 3.77; HRMS (ESI) m/z calcd for $C_{31}H_{48}IN_6NaO_7PSi$ $[M + Na]^+$ 825.2034, found 825.2028.

2-Ethylbutyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-2-bromo-9*H*-purin-9-yl)-4-((*tert*-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (38*S*). ProTide product **38***S* was prepared from alcohol **35** (70 mg, 0.160 mmol) and phosphate reagent ((*S*)-2-ethylbutyl 2-(((*S*)-(4-nitrophenoxy)(phenoxy)phosphoryl)amino)propanoate (**6**)) (109 mg, 0.24 mmol) using a general procedure for phosphorylation. The crude residue was purified by column chromatography (70% EtOAc/hexanes−100% EtOAc) to afford **38***S* (80 mg, 68%) as a colorless solid; ^{1}H NMR (400 MHz, CD_3OD): δ 8.22 (s, 1H), 7.36−7.32 (m, 2H), 7.25−7.23 (m, 2H), 7.20−7.16 (m, 1H), 5.88 (d, J = 2.0 Hz, 1H), 4.82−4.81 (m, 1H), 4.46−4.41 (m, 1H), 4.33−4.27 (m, 1H), 3.99 (dd, J = 10.9, 5.8 Hz, 1H), 3.95−3.88 (m, 2H), 2.26−2.19 (m, 1H), 2.01 (ddd, J = 13.2, 6.0, 2.7 Hz, 1H), 1.45−1.39 (m, 1H), 1.33−1.25 (m, 7H), 0.89 (s, 9H), 0.86−0.82 (m, 6H), 0.12 (s, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 175.1 (d, $^3J_{C-P}$ = 5.2 Hz), 157.7, 152.0 (d, $^2J_{C-P}$ = 6.8 Hz), 151.0, 145.9, 140.4, 130.8 (2C), 126.2, 121.2 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 119.7, 93.4, 80.4 (d, $^3J_{C-P}$ = 8.5 Hz), 77.8, 68.5 (d, $^2J_{C-P}$ = 5.3 Hz), 68.1, 51.5, 41.5, 35.3, 26.2 (3C), 24.1, 24.1, 20.6 (d, $^3J_{C-P}$ = 6.5 Hz), 18.8, 11.3, 11.2, − 4.6, − 4.7; ^{31}P NMR (162 MHz, CD_3OD): δ 3.74; HRMS (ESI) m/z calcd for $C_{31}H_{48}BrN_6NaO_7PSi$ $[M + Na]^+$ 777.2172, found 777.2170.

(*S*)-2-Ethylbutyl 2-(((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-2-fluoro-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (39*S*). Compound **39***S* was prepared from compound **36***S* (200 mg, 0.29 mmol) using the general procedure for silyl deprotection at the 2′-position. The crude residue was purified by column chromatography (5% MeOH/CH_2Cl_2) to give **39***S* (*Sp*-isomer, white solid, 110 mg, 65%); ^{1}H NMR (400 MHz, CD_3OD): δ 8.20 (s, 1H), 7.34−7.29 (m, 2H), 7.21−7.19 (m, 2H), 7.17−7.13 (m, 1H), 5.90 (s, 1H), 4.72−4.69 (m, 1H), 4.67−4.63 (m, 1H), 4.42−4.37 (m, 1H), 4.30−4.24 (m, 1H), 4.02−3.97 (m, 1H), 3.95−3.88 (m, 2H), 2.39−2.31 (m, 1H), 2.09−2.04 (m, 1H), 1.46−1.40 (m, 1H), 1.32−1.27 (m, 7H), 0.86−0.82 (m, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 174.9 (d, $^3J_{C-P}$ = 5.3 Hz), 160.5 (d, $^1J_{C-F}$ = 208.9 Hz), 159.0 (d, $^2J_{C-F}$ = 20.4 Hz), 152.1 (d, $^2J_{C-P}$ = 6.7 Hz), 151.6 (d, $^2J_{C-F}$ = 19.7 Hz), 140.6 (d, $^4J_{C-F}$ = 3.0 Hz), 130.7 (2C), 126.1, 121.3 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 118.7, 93.3, 80.4 (d, $^3J_{C-P}$ = 8.5 Hz), 76.5, 68.6 (d, $^2J_{C-P}$ = 5.3 Hz), 68.0, 51.5, 41.7, 34.9, 24.2, 24.1, 20.6 (d, $^3J_{C-P}$ = 6.6 Hz), 11.3, 11.2; ^{31}P NMR (162 MHz, CD_3OD): δ 3.76; ^{19}F NMR (470 MHz, CD_3OD): δ − 53.18; HRMS (ESI) m/z calcd for $C_{25}H_{34}FN_6NaO_7P$ $[M + Na]^+$ 603.2108, found 603.2108. The diastereopurity of **39***S* was determined to be 98% de: retention time = 2.657 min.

2-Ethylbutyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-2-iodo-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)-phosphoryl)-*L*-alaninate (40*S*). Compound **40***S* was prepared from compound **37***S* (56 mg, 0.07 mmol) using the general procedure for silyl deprotection at the 2′-position. The crude residue was purified by column chromatography (5% MeOH/CH_2Cl_2) to give **40***S* (*Sp*-isomer, colorless solid, 28 mg, 62%); ^{1}H NMR (400 MHz, CD_3OD): δ 8.14 (s, 1H), 7.34−7.30 (m, 2H), 7.23−7.20 (m, 2H) 7.18−7.14 (m, 1H), 5.94 (d, J = 1.5 Hz, 1H), 4.68−4.65 (m, 2H), 4.40 (ddd, J = 11.3, 5.9, 2.8 Hz, 1H), 4.29 (ddd, J = 11.3, 6.2, 4.7 Hz, 1H), 4.00 (dd, J = 10.9, 5.8 Hz, 1H), 3.96−3.88 (m, 2H), 2.35 (ddd, J = 13.4, 9.8, 5.6 Hz, 1H), 2.06 (ddd, J = 13.4, 6.0, 2.2 Hz, 1H), 1.47−1.40 (m, 1H), 1.34−1.27 (m, 7H), 0.87−0.83 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.9 (d, $^3J_{C-P}$ = 5.0 Hz), 157.0, 152.0 (d, $^2J_{C-P}$ = 7.0 Hz), 150.5, 140.2, 130.7 (2C), 126.1, 121.3 (2C, d, $^3J_{C-P}$ = 4.9 Hz), 120.6, 120.3, 93.4, 80.6 (d, $^3J_{C-P}$ = 8.5 Hz), 76.7, 68.5 (d, $^2J_{C-P}$ = 5.5 Hz), 68.0, 51.5, 41.6, 34.8, 24.2, 24.1, 20.6 (d, $^3J_{C-P}$ = 6.6 Hz), 11.3, 11.3; ^{31}P NMR (162 MHz, CD_3OD): δ 3.71; HRMS (ESI) m/z calcd for $C_{25}H_{34}IN_6NaO_7P$ $[M + Na]^+$ 711.1169, found 711.1162. The diastereopurity of **40***S* was determined to be 97% de: retention time = 3.936 min.

2-Ethylbutyl ((*S*)-(((2*S*,4*R*,5*R*)-5-(6-Amino-2-bromo-9*H*-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (41*S*). Compound **41***S* was prepared from compound **38***S* (74 mg, 0.098 mmol) using the general procedure for silyl deprotection at the 2′-position. The crude residue was purified by column chromatography (5% MeOH/CH_2Cl_2) to give **41***S* (*Sp*-isomer, colorless solid, 37 mg, 65%); ^{1}H NMR (400 MHz, CD_3OD): δ 8.19 (s, 1H), 7.34−7.30 (m, 2H), 7.23−7.20 (m, 2H), 7.18−7.14 (m, 1H), 5.94 (d, J = 1.6 Hz, 1H), 4.69−4.64 (m, 2H), 4.41 (ddd, J = 11.3, 5.9, 2.8 Hz, 1H), 4.33−4.25 (m, 1H), 4.00 (dd, J = 10.9, 5.8 Hz, 1H), 3.96−3.89 (m, 2H), 2.38−2.31 (m, 1H), 2.06 (ddd, J = 13.4, 5.9, 2.2 Hz, 1H), 1.47−1.41 (m, 1H), 1.34−1.27 (m, 7H), 0.87−0.83 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.9 (d, $^3J_{C-P}$ = 5.2 Hz), 157.8, 152.0 (d, $^2J_{C-P}$ = 6.7 Hz), 151.0, 145.9, 140.5, 130.7 (2C),

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126.1, 121.3 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 119.8, 93.4, 80.6 (d, $^3J_{C-P}$ = 8.5 Hz), 76.6, 68.5 (d, $^2J_{C-P}$ = 5.3 Hz), 68.0, 51.5, 41.6, 34.7, 24.2, 24.1, 20.6 (d, $^3J_{C-P}$ = 6.5 Hz), 11.3, 11.2; ^{31}P NMR (162 MHz, CD_3OD): δ 3.73; HRMS (ESI) m/z calcd for $C_{25}H_{34}BrN_6NaO_7P$ $[M + Na]^+$ 663.1308, found 663.1308. The diastereopurity of **41***S* was determined to be 95% de: retention time = 3.728 min.

2-Ethylbutyl ((*S*)-(((2*R*,3*S*,5*R*)-5-(6-Amino-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)methoxy) (phenoxy)-phosphoryl)-L-alaninate (44*S*). The mixture of 2′-deoxyadeonsine (**42**) (0.200 g, 0.796 mmol), 2-(((*S*)-(4-nitrophenoxy)(phenoxy)-phosphoryl)amino)propanoate (**6**)) (0.538 g, 1.19 mmol), and $MgCl_2$ (0.114 g, 1.19 mmol) in THF (8.0 mL) and DMF (4.0 mL) were stirred at 50 °C for 10 min. Then, *N*,*N*-diisopropylethylamine or DIEA (0.35 mL, 1.99 mmol) was added to the reaction mixture and stirred for 5 h. After removal of THF, the residue was extracted with EtOAc and water and washed with brine solution and sat. $NaHCO_3$. The combined organic layers were evaporated and purified by column chromatography (hexane:EtOAc:MeOH (50:50:0 to 0:100:0 to 0:97:3)) to give **44***S* (*Sp*-isomer, white solid, 0.140 g, 31%); ^{1}H NMR (500 MHz, DMSO) δ 8.28 (s, 1H), 8.13 (s, 1H), 7.37−7.30 (m, 2H), 7.27 (s, 2H), 7.22−7.12 (m, 3H), 6.36 (t, J = 6.9 Hz, 1H), 6.01 (dd, J = 13.0, 10.1 Hz, 1H), 5.47 (d, J = 4.3 Hz, 1H), 4.49−4.41 (m, 1H), 4.26−4.19 (m, 1H), 4.09−3.99 (m, 2H), 3.93 (dd, J = 10.9, 5.8 Hz, 1H), 3.89−3.77 (m, 2H), 2.80−2.72 (m, 1H), 2.30 (ddd, J = 13.3, 6.3, 3.4 Hz, 1H), 1.47−1.36 (m, 1H), 1.30−1.17 (m, 7H), 0.83−0.75 (m, 6H); ^{13}C NMR (126 MHz, CD_3OD) δ 173.5 (d, $^3J_{C-P}$ = 5.2 Hz), 155.9, 152.4, 150.7 (d, $^2J_{C-P}$ = 6.9 Hz), 148.9, 139.4, 129.3, 124.7, 119.9 (d, $^3J_{C-P}$ = 4.8 Hz), 119.1, 85.4 (d, $^3J_{C-P}$ = 8.2 Hz), 84.3, 70.9, 66.7, 66.3 (d, $^2J_{C-P}$ = 5.5 Hz), 50.1, 40.2, 39.5, 22.8, 22.8, 19.2 (d, $^3J_{C-P}$ = 6.5 Hz), 9.9, 9.9; ^{31}P NMR (202 MHz, DMSO) δ 3.68; HRMS (ESI) m/z calcd for $C_{25}H_{35}N_6NaO_7P$ $[M + Na]^+$ 585.2203, found 585.2208. The diastereopurity of **44***S* was determined to be 90% de: retention time = 5.554 min.

2-Ethylbutyl ((*S*)-(((2*S*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-tetrahydrofuran-2-yl)methoxy)(phenoxy) phosphoryl)-L-alaninate (45*S*). The mixture of 2′,3′-dideoxyadenosine (**43**) (50 mg, 0.213 mmol), 2-(((*S*)-(4-nitrophenoxy)(phenoxy)phosphoryl)amino)-propanoate (**6**)) (144 mg, 0.320 mmol), and $MgCl_2$ (30 mg, 0.320 mmol) in THF (2.0 mL) and DMF (1.0 mL) were stirred at 50 °C for 10 min. Then, DIEA (93 μL, 0.533 mmol) was added to the reaction mixture, and the mixture was stirred for 5 h. After removal of THF, the residue was extracted with EtOAc and water and washed with brine solution and sat. $NaHCO_3$. The combined organic layers were evaporated and purified by column chromatography (hexane:EtOAc:-MeOH (50:50:0 to 0:100:0 to 0:97:3)) to provide **45***S* (*Sp*-isomer, white solid, 0.140 g, 31%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.29 (s, 1H), 8.20 (s, 1H), 7.35−7.28 (m, 2H), 7.22−7.12 (m, 3H), 6.30 (dd, J = 6.8, 3.7 Hz, 1H), 4.44−4.37 (m, 1H), 4.34 (ddd, J = 11.1, 6.1, 3.2 Hz, 1H), 4.24 (ddd, J = 11.1, 6.2, 5.0 Hz, 1H), 4.00 (dd, J = 10.9, 5.8 Hz, 1H), 3.97−3.83 (m, 2H), 2.64−2.46 (m, 2H), 2.25−2.14 (m, 2H), 1.48−1.40 (m, 1H), 1.36−1.25 (m, 7H), 0.90−0.82 (m, 6H); ^{13}C NMR (126 MHz, CD_3OD) δ 174.9 (d, $^3J_{C-P}$ = 5.3 Hz), 157.2, 153.7, 152.1 (d, $^2J_{C-P}$ = 6.9 Hz), 150.0, 140.6, 130.7 (2C), 126.0, 121.3 (2C, d, $^3J_{C-P}$ = 4.8 Hz), 120.4, 86.8, 81.1 (d, $^3J_{C-P}$ = 8.4 Hz), 68.9 (d, $^2J_{C-P}$ = 5.5 Hz), 68.0, 51.5, 41.7, 33.0, 26.8, 24.2, 24.2, 20.5 (d, $^3J_{C-P}$ = 6.5 Hz), 11.3, 11.2; ^{31}P NMR (202 MHz, CD_3OD) δ 3.73; HRMS (ESI) m/z calcd for $C_{25}H_{35}N_6NaO_6P$ $[M + Na]^+$ 569.2253, found 569.2243. The diastereopurity of **45***S* was determined to be 86% de: retention time = 10.072 min.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsbiomedchemau.4c00071.

^{1}H, ^{13}C, ^{31}P and ^{19}F NMR spectroscopic data for all compounds; preliminary screening of antiviral activities at a concentration of 25 μM; detailed cytotoxicity, antiviral activity, kinetic study of an enzymatic hydrolysis and phenol fluorescence assays; material and methods of *in vitro* pharmacokinetic study; and the remaining percentage of the test compounds from microsomal stability assay (PDF)

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Notes

The authors declare no competing financial interest.

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