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Synthesis and Anti-Influenza Activity of Pyridine, Pyridazine, and Pyrimidine *C*-Nucleosides as Favipiravir (T-705) Analogues

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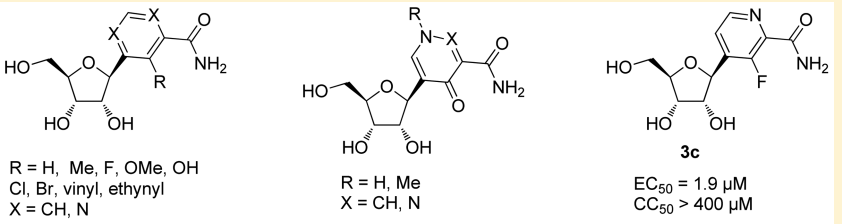
Supporting Information



ABSTRACT: Influenza viruses are responsible for seasonal epidemics and occasional pandemics which cause significant morbidity and mortality. Despite available vaccines, only partial protection is achieved. Currently, there are two classes of widely approved anti-influenza drugs: M2 ion channel blockers and neuraminidase inhibitors. However, the worldwide spread of drug-resistant influenza strains poses an urgent need for novel antiviral drugs, particularly with a different mechanism of action. Favipiravir (T-705), a broad-spectrum antiviral agent, has shown potent anti-influenza activity in cell-based assays, and its riboside (**2**) triphosphate inhibited influenza polymerase. In one of our approaches to treat influenza infection, we designed, prepared, and tested a series of *C*-nucleoside analogues, which have an analogy to **2** and were expected to act by a similar antiviral mechanism as favipiravir. Compound **3c** of this report exhibited potent inhibition of influenza virus replication in MDCK cells, and its triphosphate was a substrate of and demonstrated inhibitory activity against influenza A polymerase. Metabolites of **3c** are also presented.

Influenza viruses are responsible for seasonal epidemics and occasional pandemics, which cause significant morbidity and mortality. Influenza occurs globally with an annual attack rate estimated at 5%−10% in adults and 20%−30% in children.[1] Worldwide, these annual epidemics are estimated to result in about 3 to 5 million cases of severe illness, and about 250,000 to 500,000 deaths. Although safe and effective influenza vaccines are available, they may be less effective among the elderly in preventing illness.[1] Moreover, vaccines need to be reformulated each year due to the genetic variability of the virus, and they are not always protective; in addition, a rapidly emerging influenza pandemic cannot be contained by vaccination.[2] There are currently two classes of widely approved anti-influenza drugs: M2 channel blockers (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir, zanamivir, laninamivir, and peramivir).[2−5] Due to widespread resistance to the class, the M2 ion channel inhibitors are not recommended currently for therapy.[5−7] Resistance to the only orally bioavailable drug oseltamivir was dominant in the 2007−2008 influenza season.[8,9] Low bioavailability and their nasal route of administration limit the use of zanamivir and laninamivir in severely ill patients, while peramivir is used by IV route only.[5] Thus, the worldwide spread of drug-resistant influenza strains poses an urgent need for novel antiviral drugs, particularly with a different mechanism of action. Current drug discovery continues NA and M2 inhibitor optimization via structure based drug design to solve the drug resistance issue.[10] Recently, VX-787, a potent inhibitor of the influenza virus replication complex PB2 subunit, was disclosed, which is currently in phase II clinical development.[11] Inhibitors targeting the influenza endonuclease have also been reported.[12] From the drug discovery standpoint, it would be ideal to develop broad-spectrum anti-influenza drugs that are active against all strains of influenza, thus circumventing the need for combination therapy. Among this class are ribavirin and T-705 (favipiravir).[13] The latter, in the brand name of Avigan, has been approved in Japan as an oral anti-influenza drug. Favipiravir was found to have potent anti-influenza activity in cell-based assays, and its riboside (**2**) triphosphate inhibited the influenza polymerase.[13,14] Additional studies suggested that favipiravir acts via lethal viral mutagenesis.[15,17,18] As part of

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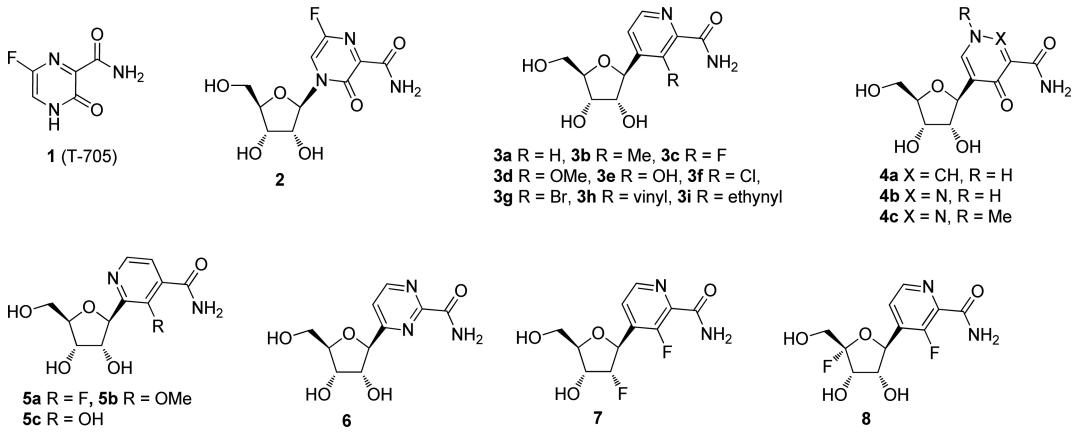
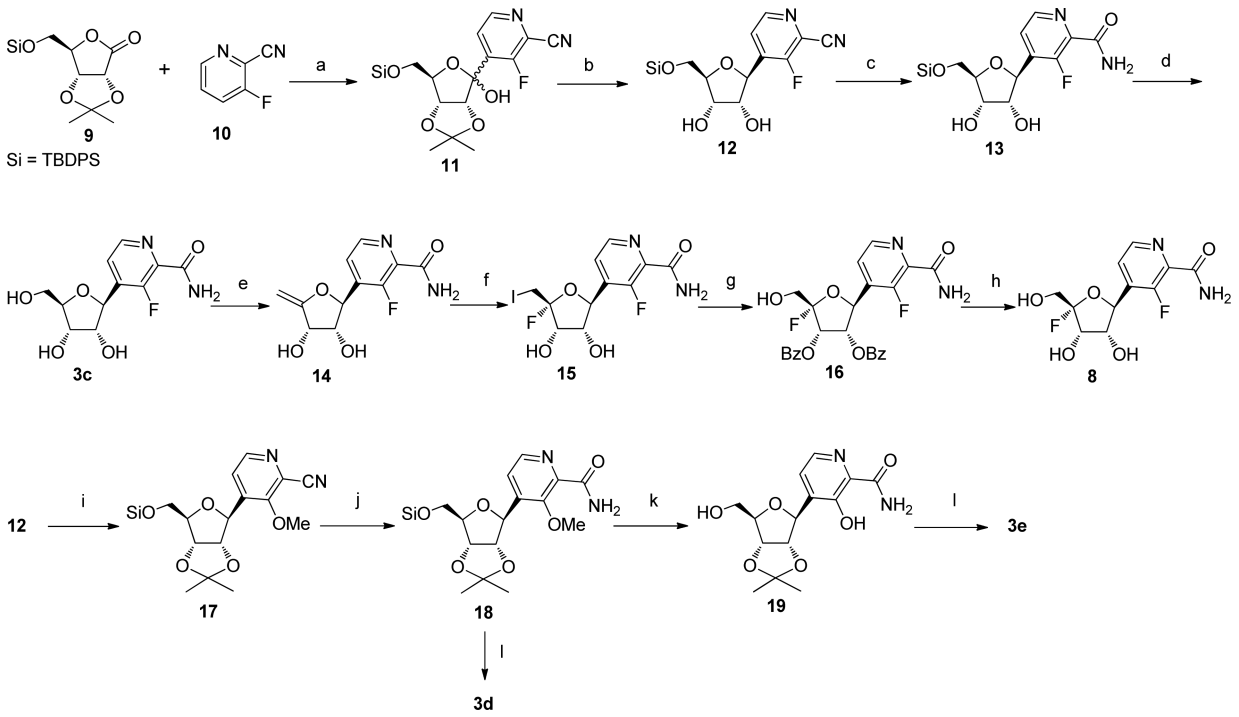


Figure 1. Favipiravir and *C*-nucleoside analogues.

Scheme 1[a]



[a]Regents and conditions: (a) LDA, THF, −78 °C, 54%; (b) BF_3-Et_2O, Et_3SiH, 0 °C to rt, 24%; (c) NH_4OH, H_2O_2, MeOH, 50%; (d) NH_4F, MeOH, 67%; (e) i. I_2, PPh_3, py, overnight, 51%; ii. DBU, THF, 60 °C, overnight, 89%; (f) NIS, TEA-3HF, MeCN, 67%; (g) i. BzCl, py, 75%; ii. mCPBA, Bu_4NOH-TFA, 67%; (h) NH_3/MeOH, 72%; (i) i. 2,2-dimethoxypropane, TsOH, acetone, 84%; ii. NaOMe, dioxane, 28%; (j) H_2O_2, NH_3, MeOH/H_2O, 73%; (k) EtSNa, DMF, 40 °C, 55%; (l) HCl, MeOH, 24% for both **3d** and **3e**.

broad influenza programs at Alios, we investigated a variety of nucleoside analogues as potential anti-influenza agents. One series of the analogues investigated in our laboratories are pyridine, pyridazine, and pyrimidine *C*-nucleoside, which retain the carboxamide moiety of favipiravir. Like ribavirin and favipiravir, the *C*-nucleosides are expected to mimic both adenosine and guanosine via its two orientations of the carboxamide when they base-paired with an RNA template. Therefore, lethal viral mutagenesis may result from this series of compounds. The goal of this drug discovery program is to identify new chemical entities which can possess broad-spectrum antiviral activity via a similar mechanism of action to those of favipiravir and ribavirin. In this article, we report the synthesis and in vitro anti-influenza activity of pyridine, pyridazine, and pyrimidine *C*-nucleosides **3**-**8** (Figure 1). The inhibitory effects of the 5′-triphosphate of **3c** on viral polymerases as well as the metabolic properties of **3c** are also presented.

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Synthesis of **3c**, **3d**, **3e**, and **8** is shown in Scheme 1. Compound **9**[19] was coupled with the pyridine derivative **10** via a nucleophilic addition in the presence of LDA to give **11**. Removal of the 1′-hydroxyl was achieved by reduction with triethylsilane in the presence of a strong Lewis acid, which also removed isopropylidene concomitantly. The isolated yield of **12** was relatively low, partly due to the generation of both α- and β-1′-epimers. Conversion of the β-isomer **12** to the amide **13** was effected by an oxidative hydrolysis, and the latter was subjected to desilylation to give **3c**. Compound **3c** was

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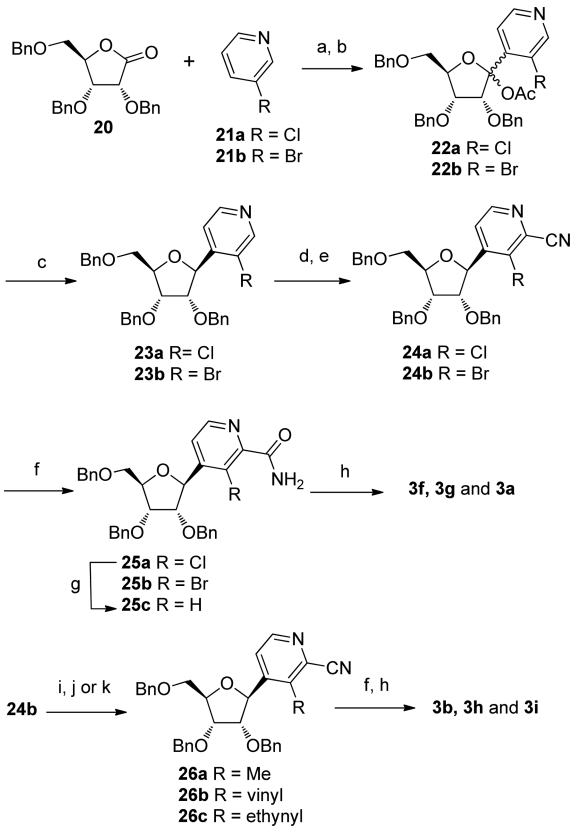
converted to the 4′,5′-olefin **14** by iodination at the 5′-position and subsequent HI elimination in the presence of a base. Introducing 4′-fluorine on a nucleoside was first reported by Moffatt and co-workers using iodine and silver fluoride.[20] Thus far, most of the known 4′-fluoronucleosides were synthesized using the same reagents. In addition to iodine and silver fluoride, we also used NIS and TEA-3HF for introducing 4′-fluoro to nucleosides, usually with better yields. Thus, treatment of **14** with NIS and TEA-3HF yielded the 4′-fluoro-5′-iodo intermediate **15** in good yield. Benzoylation of **15** and subsequent oxidative hydrolysis[21] yielded **16**, which was treated with ammonia to give **8** in good yield. Replacement of the 3-fluorine of **12** with methoxy was complicated by 5′-*O*,*C*-3-cyclization under strong basic condition, under which the 5′-*O*-TBDPS group could be partially removed, therefore resulting in a 5′-*O*,*C*-3-cyclized byproduct (not shown) and yielding **17** in only moderate yield. Oxidative hydrolysis of **17** yielded **18**, which was converted to **19** via demethylation and concomitant desilylation by NaSEt. Treatment of **18** and **19** with HCl in MeOH yielded **3d** and **3e**, respectively.

Synthesis of **3a**, **3b**, **3f**, **3g**, **3h**, and **3i** is shown in Scheme 2. Compounds **22a**–**b** were prepared, respectively, by condensation of the lactone **20**[22] with 3-halopyridines **21a**–**b** by a similar procedure as described in Scheme 1. The 1′-OH of **22a** was converted to the 1′-*O*-acetate, which was reduced by Et_3SiH more effectively than the 1′-OH. The resulting **23a** was oxidized to an N-oxide, which was treated with TMSCN[23] to give the nitrile **24a** in good yield. Oxidative hydrolysis of **24a** yielded the amide, which was debenzylated with boron trichloride to give **3f**. Compound **3g** was prepared from **22b** via the same sequence of reactions as described for **3f**. Compound **25a** was reduced by catalytic hydrogenation to **25c**, which was debenzylated to give **3a**. Compound **24b** was subjected to palladium-mediated coupling reactions to afford, after oxidative hydrolysis and debenzylation, the methyl derivative **3b**, the vinyl derivative **3h**, and the ethynyl derivative **3i**, respectively, in good yields.

Synthesis of **4a** is shown in Scheme 3. The lactone **27**[24] was reduced to the 1-OH product, which was treated with a phosphonium ylide and yielded the ethyl ester **28** according to a procedure for a similar compound.[25] Reaction of **28** with acetonitrile anion yielded the cyano derivative **29**. Compound **30** was prepared by reaction of **29** with DMF-DMA/*tert*-butoxybis(dimethylamino)methane and subsequent heating with ammonium acetate by a procedure for a similar pyridone compound.[26] Compound **30** was converted to **31** by successive removal of isopropylidene and benzyl groups. Compound **4a** was obtained by treatment with ammonia via amidine formation and hydrolysis.

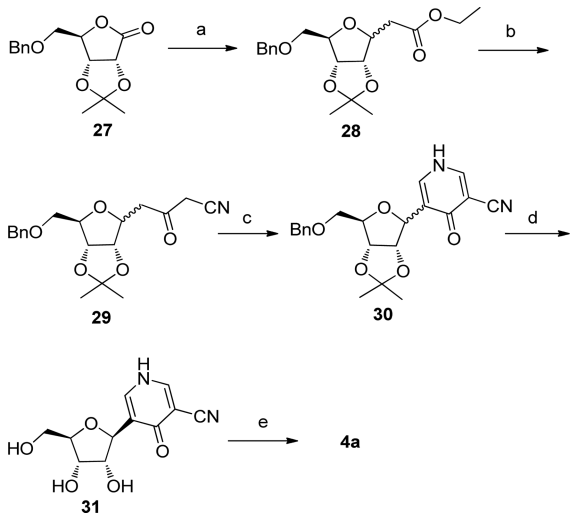
Synthesis of **4b** and **4c** is shown in Scheme 4. Compound **32**[27] was converted to the nitrile **33** in the same manner as **28**. Treatment of **33** with TfN_3 in the presence of triethylamine[28] yielded the diazo intermediate **34**, which was subjected to a reduction by PMe_3. After protection of **35** with Boc, the resulting hydrazone **36** reacted with DMF-DMA to give a mixture of the expected pyridazine derivative **37** and unintended **38**, which were separated by flash chromatography. Compounds **37** and **38** were converted to the amides and then

Scheme 2[a]



[a]Reagents and conditions: (a) LDA, THF, −78 °C, 1 h; (b) LiHMDS, Ac_2O, 70% (**22a**, 2 steps); (c) Et_3SiH, BF_3-Et_2O, DCM, 1 h, 51% (**23a**); (d) mCPBA, DCM, 2 h; (e) TMSCN, Et_3N, MeCN, reflux, 5 h, 58% (**24a**, 2 steps); (f) H_2O_2, K_2CO_3, DMSO, 1 h, 72% (**25a**); (g) H_2, 10% Pd/C, 5 h, 36%; (h) BCl_3, DCM, −78 °C, 1 h, 62% (**3f**); (i) $AlMe_3$, $Pd(PPh_3)_4$, reflux, 10 h, 88%; (j) $Bu_3SnCH{=}CH_2$, Pd(dppf)-Cl_2, KF, dioxane, MW 120 °C, 0.5 h, 79%; (k) i. ethynyl-TMS, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, MW 135 °C, 2 h, 51%; ii. TBAF, THF, 5 h, 86%.

Scheme 3[a]



[a]Reagents and conditions: (a) i. DIBAL-H, toluene, −78 °C, 30 min, 94%; ii. $Ph_3P{=}CHCOOEt$, CH_3CN, reflux, 16 h, 93%; (b) BuLi, −78 °C, CH_3CN, 30 min, 69% ; (c) i. DMF-DMA, *t*-BuOCH$(NMe_2)_2$, 100 °C; ii. NH_4Ac, EtOH, reflux, 1 d, 56%; (d) i. 80% TFA/H_2O, 0 °C, 3 h, 50%; ii. 4 M HCl/MeOH, 60 °C, 2 d, 20%; (e) NH_3, THF, 100 °C, 15 h, 6%.

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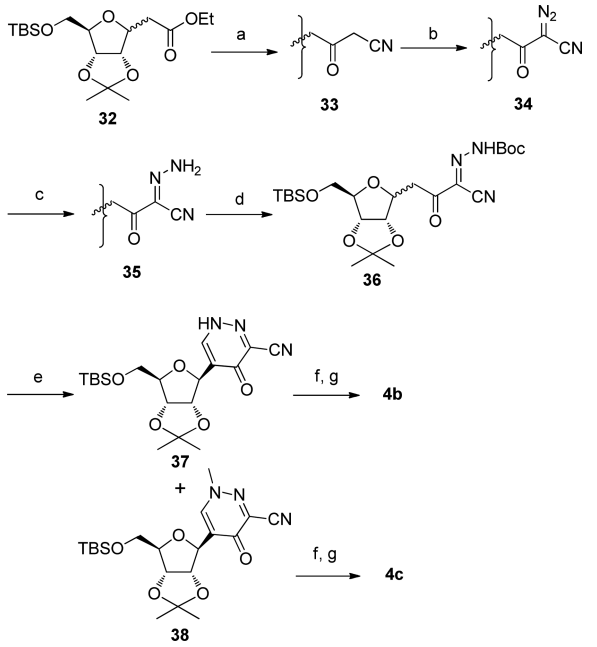
Scheme 4[a]



[a]Reagents and conditions: (a) nBuLi, CH_3CN, 61%; (b) TfN_3, TEA, MeCN, 36%; (c) PMe_3, THF, H_2O, 86%; (d) Boc_2O, py, 66%; (e) DMF-DMA, THF, 12 h; (f) TEA, H_2O_2, MeOH, 83%; (g) 80% aq TFA, 55% for **4b** and 51% for **4c**

desilylated to give **4b** and **4c**, respectively, in the same manner as described for **3c**.

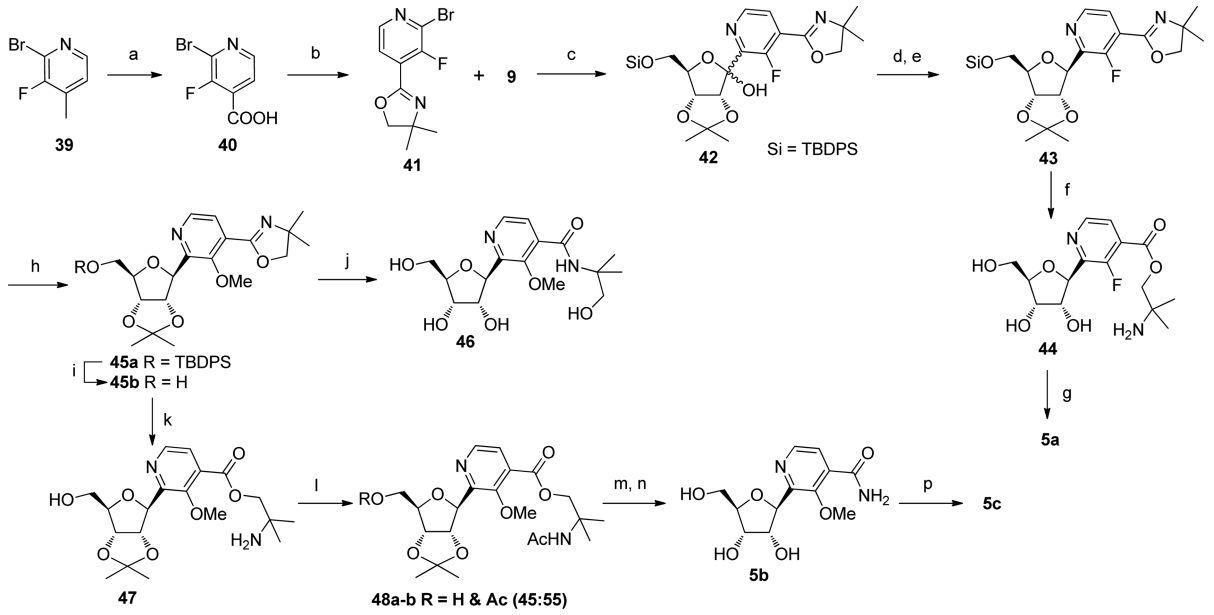
Synthesis of compounds **5a, 5b**, and **5c** is shown in Scheme 5. Commercially available **39** was oxidized to the acid **40**, which was converted to an amide by reaction of its anhydride form with 2-amino-2-methylpropanol according to a procedure for a known compound[29] and then heated with concentrated sulfuric acid to yield the dihydrooxazole **41**. Treatment of **41** with BuLi and subsequent reaction with the lactone **9** yielded the 1′-OH product **42**, which was reduced, via its 1′-OAc, by Et_3SiH. Deprotection by acidic hydrolysis of the resulting **43** also opened the dihydrooxazole ring and yielded the ester **44**, which was treated with ammonia to give **5a**. Compound **43** was readily converted to the 3-OMe product **45a**, which was then desilylated to **45b**. The acidic deprotection of **45b** yielded an undesired byproduct **46**, presumably via an intramolecular amination of an intermediate similar to **47**. Transamination of **46** with NH_3 did not readily proceed as expected. Alternatively, a controlled acidic hydrolysis of **45b** and careful purification on silica gel could provide **47**, which was subjected to acetylation under nonbasic conditions to yield a mixture of **48a** and **48b**. Acetylation of NH_2 could prevent the intramolecular amination. Then, treatment of **48a** and **48b** with ammonia afforded, after sugar deprotection, the desired **5b** in good yield, which was converted to **5c** by a SN2 reaction.

Synthesis of **6** started with acid **49**[30] (Scheme 6), which was converted to the Weinreb amide **50** and then reacted with TMS-ethylnyllithium to give **51**. Cyclization of **51** with methyl carbamimidothioate yielded the pyrimidine **52**. The methylthio was replaced, after oxidation, by a cyano group. The resulting nitrile **53** was readily converted to **6** in the same manner as described for **3f**.

Synthesis of **7** is shown in Scheme 7. The lactone **56**, prepared from **55**,[31] was coupled with **10** to afford the 1′-OH product **57**. Further treatment as described for **3f** yielded **7**.

To investigate the feasibility to bypass the first cellular phosphorylation of **3c**, well-known bis-POM prodrug[32] and phosphoramidate prodrug[33] were employed. Thus, the prodrug **59** and the prodrug **60** were prepared. For biochemical and DMPK studies, nucleoside triphosphates **61** and **62** as well as

Scheme 5[a]



[a]Reagents and conditions: (a) $KMnO_4$, H_2O, 12 h, 60%; (b) i. *i*-BuOC(O)Cl, $NH_2CMe_2CH_2OH$, 2 h, 38%; ii. H_2SO_4, 120 °C, 30 min, 55%; (c) BuLi, THF, −78 °C, 2 h, 59%; (d) LiHMDS, Ac_2O, 88%; (e) BF_3-Et_2O, Et_3SiH, hexanes, 30 min, 36%; (f) 80% aq TFA, 30 min; (g) NH_4OH, 50 °C, 12 h, 23% (2 steps); (h) NaOMe, dioxane, 6 h, 68%; (i) TBAF, THF, 1h; (j) 80% aq HCOOH, 4 h; 40%; (k) 2% TFA/DCM, 84%; (l) Ac_2O, 40 °C, 73%; (m) NH_3, MeOH, 80%; (n) TFA/H_2O (1:5), 80%; (p) EtSNa, DMF, 60 °C, 41%.

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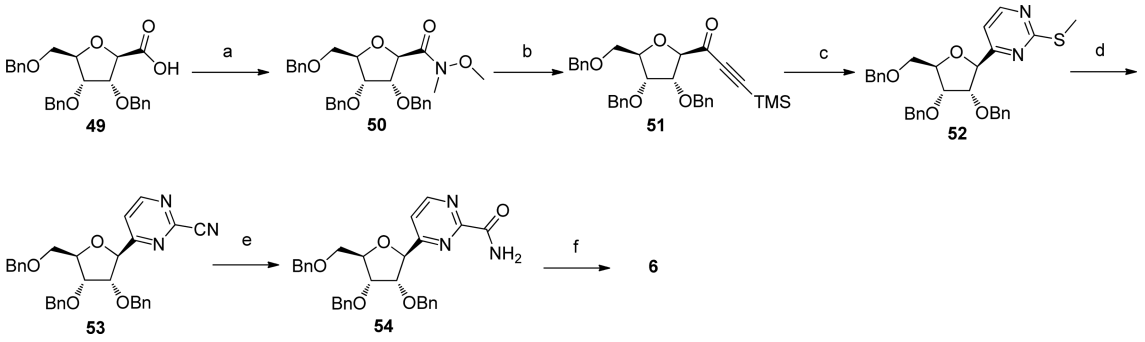
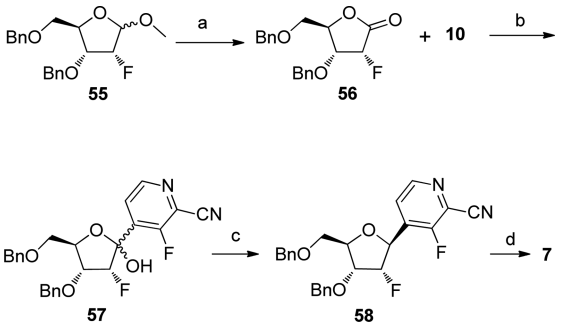
Scheme 6[a]



[a]Reagents and conditions: (a) HATU, DIPEA, *N*,*O*-dimethylhydroxylamine, DCM, 8 h, 82%; (b) BuLi, TMS-acetylene, THF, −78 °C, then rt 3 h; (c) methyl carbamimidothioate-HCl, MeCN, 80 °C, 3 h, 32% (2 steps); (d) i. mCPBA, DCM, 3 h, 92%; ii. KCN, DMSO, overnight, 63%; (e) H_2O_2, NH_4OH, MeOH, 1 h, 57%; (f) BCl_3, DCM, 0 °C, 4 h, 41%.

Scheme 7[a]

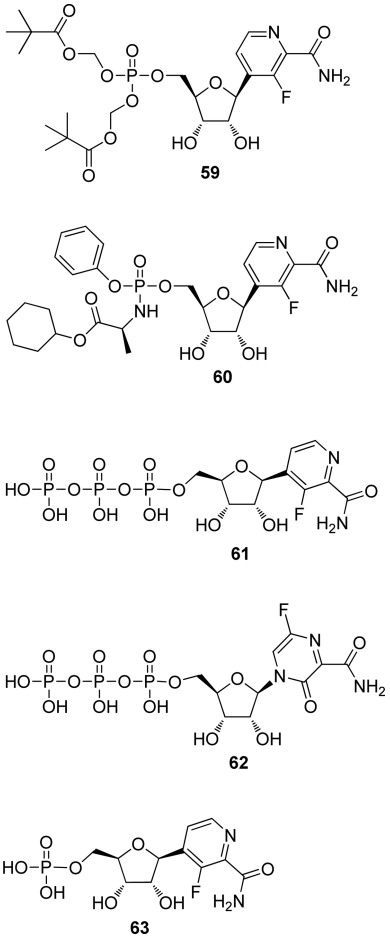


[a]Reagents and conditions: (a) i. 80% TFA, 12 h, 90%; ii. PDC, DCM, 95/%; (b) LDA, THF −78 °C, 1 h, 37%; (c) i. LiHMDS, Ac_2O, rt, 65%; ii. Et_3SiH, BF_3−EtO, 1 h, 71%; (d) i. H_2O_2, K_2CO_3, DMSO, 1 h, 87%; ii. BCl_3, DCM, −78 °C, 1 h, 45%.

nucleoside monophosphate **63** were prepared from **3c** and **2**, respectively, by general procedures.

IN VITRO ANTI-INFLUENZA ACTIVITY

Anti-influenza activity was obtained by using a neuraminidase activity-based assay as described by Eichelberger et al.[34] Madin-Darby canine kidney (MDCK) epithelial cells were infected with influenza strain A/WSN/33 (H1N1). All the compounds in this report were tested for their inhibitory effects. Table 1 shows the compounds that have at least weak anti-influenza activity while compounds with EC_{50} > 100 μM are not listed in the table. As shown in Table 1, **3c** has comparable activity to favipiravir (**1**).[27] The EC_{50} value of 1.9 μM for **3c** is the average of multiple repeats in the same assay. Compound **3c** did not exhibit cytotoxicity up to 400 μM. Although compound **3e** exhibited potent anti-influenza activity, it was almost equally cytotoxic, which excludes the possibility for further development as an anti-influenza agent. Compounds **3h** and **3i**, having the same pyridine scaffold as **3c**, showed only moderate inhibition. Compounds **4a** and **5a**−**c**, in which the nitrogen of the pyridine shifted position, showed little inhibition, with **5b** being weakly active. Compounds **4b** and **4c**, having a pyridazine scaffold, showed little inhibitory effect. However, the pyrimidine compound **6** showed significant activity. Compounds **7** and **8**, in which the sugars were modified at the 2′- and 4′-positions by fluorination, had only weak activity. Although **59**, the bis-POM-prodrug of **3c**, exhibited a good activity, its potency did not exceed its parent compound **3c**,



which suggested that **3c** might be efficiently converted to its 5′-monophosphate in cells. In contrast, **60**, the phosphoramidate prodrug of **3c**, showed little inhibition (EC_{50} = 129 μM), which might be ascribed to its lack of efficient conversion to the monophosphate of **3c** in MDCK cells.

INHIBITION OF INFLUENZA A POLYMERASE BY THE 5′-TRIPHOSPHATE OF 3c (61)

The inhibitory effect of **61** on RNA synthesis catalyzed by the recombinant influenza A polymerase (PA/PB1/PB2) complex was performed as described previously.[18] In the RNA synthesis assay, the polymerase complex makes RNA products of ∼50 nucleotides (nt) in length when a 50-nt RNA template is used

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Table 1. Anti-Influenza Activity of 1, 2, and C-Nucleosides[a,b]

Compound	EC_{50}, μM	CC_{50}, μM
1	2.7	>400
2	2.2	>400
3c	1.9	>400
3e	1.3	2.0
3h	58	152
3i	36	98
4c	91	291
5b	88	207
6	8.7	>200
7	87	306
8	98	185
59	5.8	>400

[a]Cell-based assay was performed in MDCK cells infected with A/WSN/33 (H1N1). [b]Each EC_{50} value is an average of $n \geq 2$ determinations.

in the assay. As shown in Figure 2, **61** effectively inhibited the RNA synthesis by the polymerase with an IC_{50} of 4.8 μM, a

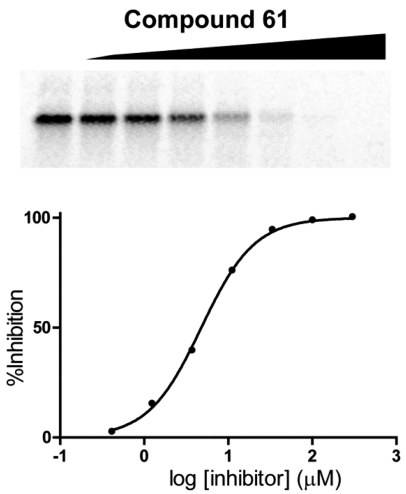

Figure 2. Polyacrylamide gel electrophoresis (PAGE) gel image and the plot that demonstrates the inhibition of RNA synthesis by **61**.

similar level of inhibition as the 5′-triphosphate of **2** (**62**) (IC_{50} = 2.8 μM in our assay). As shown in Figure 3, **61** can be incorporated into RNA by the polymerase complex. As the sugar moiety of **3c** is an unmodified ribose with both 2′- and 3′-hydroxyls, a complete chain termination was unlikely. However, factors such as the unconventional base-pairing of **3c** with the RNA template and the orientation of the sugar moiety of **3c** as well as other unknown factors might significantly reduce the incorporation efficiency of **3c** and the next incoming nucleotides, leading to the inhibition of full length RNA synthesis in this *in vitro* assay. Previously, **62** was shown to effectively terminate chain elongation when **62** was incorporated into two consecutive positions in a growing RNA.[18] Similarly, two consecutive incorporations of **3c** might play a role in the inhibition of the chain elongation in a similar manner.

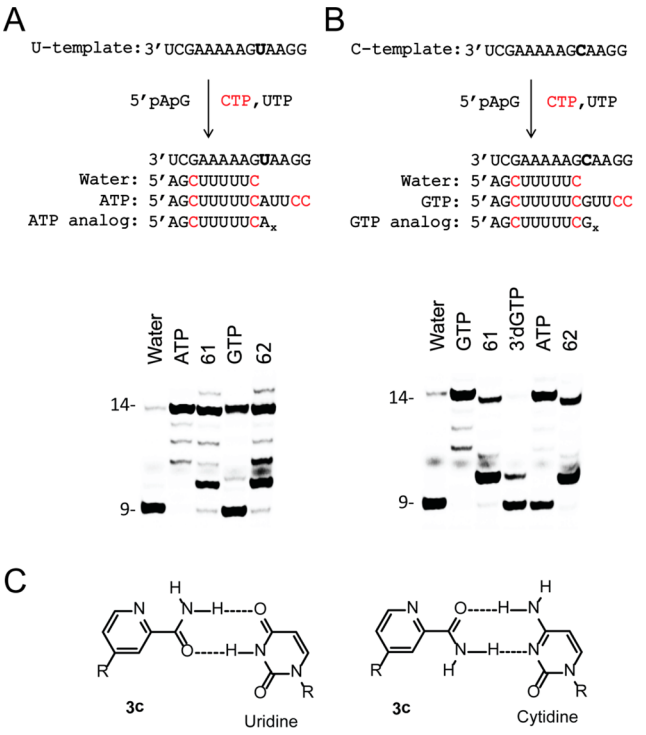

Figure 3. Template directed nucleotide incorporation catalyzed by influenza A polymerase. (A) Top, the reaction scheme using the U-template to test the incorporation of **61** as an ATP analogue. Bottom, the gel image of the reactions. (B) Top, the reaction scheme using the C-template to test the incorporation of **61** as a GTP analogue. Bottom, the gel image of the reactions. (C) **3c** appears capable of base-pairing with both cytidine and uridine.

INCORPORATION OF 61 INTO RNA AS THE A/G ANALOGUE BY INFLUENZA POLYMERASE

Although **61** exhibited the inhibition of RNA synthesis in the influenza polymerase assay, the inhibition was not necessarily the only mechanism of action that contributed to the cell-based anti-influenza activity. Similar to **2**, compound **3c** appears capable of base-pairing with both cytidine and uridine (Figure 3C); therefore, we tested whether **61** could be incorporated into RNA by the influenza polymerase as an A or a G analogue using a template-directed nucleotide incorporation assay.[18] As shown in Figure 3, A and B, **61** as substrate was indeed incorporated into RNA with either U or C on the template. When U was the nucleotide on the RNA template (Figure 3A), **61** was not only incorporated into the RNA, but also allowed the incorporation of further nucleotides to form full length products. As controls, the reactions with water and ATP yielded a 9-mer and a 14-mer product, respectively. For the reaction with water, the faint bands above the 9-mer were most likely misincorporation products. For the reaction with **61**, the 9-mer was converted to longer RNA products, the majority of which was 14-mer product, indicating that **61** was incorporated into the RNA and the oligomer was further extended after its incorporation. Its incorporation and extension profile was similar to that of **62**.[18] The reaction with GTP served as a control to indicate the level of GTP misincorporation opposite U on the template. By comparison, **61** and **62** were completely incorporated while GTP was only partially incorporated.

When C was the nucleotide on the template (Figure 3B), the control reactions with water and GTP generated the expected products. For **61**, the 9-mer was completely converted to longer

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RNA products, the majority of which was 10-mer product, indicating the incorporation of **61** into the RNA. The 14-mer product was also formed, indicating further extension after the incorporation of **3c**. Again, the **61** incorporation and extension profile was similar to that of **62**. By comparison, misincorporation of ATP was incomplete. The reaction with 3′dGTP showed the incorporation profile of an obligated chain terminator: no further extension after the incorporation of 3′dGTP.

Incorporation of **61** and **62** into RNA sequence opposite both U and C on the templates indicates that they can serve as analogues of both ATP and GTP. Further extension after their incorporation indicates that they can be incorporated and exist in the RNA molecule. Such incorporation of an ambiguous base-pairing nucleoside into the influenza viral RNA sequence may introduce mutations to the genomic RNA during the next rounds of viral RNA transcription/replication. It has been shown that favipiravir exerted strong mutagenic effects on influenza virus and norovirus that generated nonviable viruses,[15−17] and the molecular basis of this effect was linked to its ambiguous base-paring property.[14,18] Similarly, lethal viral mutagenesis combined with polymerase inhibition could be the primary mechanism for the observed antiviral activity of **3c** in the cell-based assay.

ANTIVIRAL ACTIVITY SPECTRUM OF 61

The inhibitory effect of **61** on RNA synthesis catalyzed by other viral RNA polymerases was also measured, using standard internal procedures.[14,35] As shown in Table 2, the RdRp activity of hepatitis C virus, rhinovirus, and norovirus was almost equally inhibited by **61** with an IC_{50} of 5.3, 4.0, and 3.5 μM, respectively. These results indicate that **3c** may have the potential to be a broad-spectrum antiviral agent if it can be phosphorylated to **61** in infected cells.

Table 2. Inhibition of Various RNA Polymerases

Viral polymerase[a]	IAV pol	HCV NS5B	HRV16 pol	NV pol
IC_{50} (μM)	4.8	5.3	4.0	3.5

[a]IAV pol stands for influenza RNA polymerase, HCV NS5B is for hepatitis C virus RNA polymerase; HRV16 pol is human rhinovirus type 16 RNA polymerase; NV pol is for human norovirus RNA polymerase.

METABOLIC PROPERTIES OF 3c AND ITS PRODRUGS

In vivo nucleoside 5′-triphosphate (NTP) levels in the target tissue are critical to in vivo efficacy of an antiviral nucleoside polymerase inhibitor. In vitro NTP formation in appropriate cell lines may provide the first assessment for predicting in vivo NTP level. Thus, we measured the in vitro NTP level of **3c** and its prodrugs **59** and **60** in three cell types: A549, normal human bronchiolar epithelial (NHBE), and MDCK cells according to a standard procedure.[36] In vitro NTP levels were obtained by incubating each compound with each cell type for 24 h and analyzing the cell extracts for the corresponding NTP concentration. As shown in Table 3, a much higher level of **61** was observed in MDCK cells than in the other two cell types for **3c** while there were moderate to high levels of 5′-monophosphate of **3c** (**63**) in all three types of cells. The results suggested that conversion of **63** to **61** was efficient only in MDCK cells. The levels of **61** are correlated well with the observed in vitro anti-influenza activity, indicating that **61** was the active metabolite. Compounds **59** and **60**, the prodrugs of **3c**, were also tested for their nucleotide formation in the cells. The bis-POM prodrug **59** yielded similar level of **61** in MDCK cells as **3c** and moderate to high level of **63** in all three types of cells while the phosphoramidate prodrug **60** yielded low to moderate level of **63** and below-detection level of **61**. Compared to metabolism of **3c** to **63**, conversion of **63** to **61** appears to be rate-limiting in NHBE and A549 cells. Other five *C*-nucleosides (**3a**, **3f**, **4c**, **6** and **8**) in Figure 1 were also subjected to the same testing in A549 cells and they generated either very low or below-detection levels of NTPs.

To assess in vivo nucleotide formation, female Balb/c mice were dosed with compound **59** by IV route. The studies were conformed to regulations and guidelines regarding animal care and welfare.[37] In vivo, high level of **3c** was observed in blood and high level of **63** was observed in the lung (Table 4). However, the level of **61** was below the limit of quantitation in the lung (60 nM), consistent with the low levels of **61** and the weak anti-influenza activity in NHBE and A549 cells. Conversion of **63** to **61**, therefore, appeared to be a rate-limiting step in the lung.

Table 4. Formation of NTPs from 59 in Mouse

	Conc after IV dose of **59**	
Metabolites of **59**	0.25 h	2 h
3c (nM) in blood	1436	422
63 (nM) in lung	1764	792
61 (nM) in lung	BQL	BQL

CONCLUSION

A series of novel pyridine, pyridazine, and pyrimidine *C*-nucleosides as favipiravir analogues were synthesized, and **3c** demonstrated potent anti-influenza activity and an excellent selectivity window in MDCK cells. The 5′-triphosphate of **3c** exhibited potent inhibition of RNA synthesis by influenza A polymerase in an enzyme assay. Both influenza polymerase inhibition and lethal viral mutagenesis are assumed to be responsible for the potent anti-influenza activity. Compound **3c**

Table 3. Formation of NTPs from 3c and Its Prodrugs in Three Types of Cells and in Vitro Activity in Different Cells

	NTP (pmol/million cells)			NMP (pmol/million cells)			Anti-influenza EC_{50} (μM)		
Compd	NHBE	A549	MDCK	NHBE	A549	MDCK	NHBE[a]	A549[b]	MDCK
3c	21.6	11.6	192	262	41.0	48.5	29.5	52.0	1.9
59	7.22	5.00	179	113	74.0	248	57.5	34.7	5.8
60	BLQ	BLQ	BLQ	42.6	59.5	44.7	NT[c]	74.1	129

[a]Cell-based assay was performed in NHBE cells infected with A/WSN/33 (H1N1). [b]Cell-based assay was performed in A549 cells infected with A/WSN/33 (H1N1). [c]NT: not tested.

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could be phosphorylated to its monophosphate **63** without the need of monophosphate prodrugs in all three types of cells used. The levels of its triphosphate **61** were good only in MDCK cells while they were below levels of quantitation in A549 and NHBE cells. Consistent with the in vitro results in NHBE cells, conversion of **63** to **61** was identified as a rate limiting step in the mouse lung. As **61** also showed inhibition of other RNA viral polymerases, investigation into the potential of **3c** as antiviral against various RNA viruses is underway.

EXPERIMENTAL SECTION

Favipiravir (**1**) and its riboside (**2**) were prepared according to patent applications from Toyama Chemical Co.[38,39] All commercially obtained solvents and reagents were used as received. All solvents used for chemical reactions were anhydrous grade, unless specifically indicated. Structures of the target compounds in this work were assigned by use of NMR spectroscopy and MS spectrometry. The purities of all compounds were >95% as determined on an Agilent 1200 HPLC, XTerra 3.5 μm 4.6 × 150 mm MS C18 column, using 0.04 (v/v) TFA in water and 0.02 (v/v) TFA in acetonitrile as mobile phase. ^{1}H NMR spectra were recorded on a Bruker Advance III (400 MHz) or a Varian 400MR (400 MHz) NMR spectrometer. Chemical shifts are reported in parts per million (ppm, δ) using residual solvent line as an internal reference. Splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Coupling constants (J) are reported in herz (Hz). Mass spectrometric analyses for nucleosides were performed on an Agilent 1200 HPLC with Agilent 6110/6140/1956C MSD mass spectrometer using ESI as ionization, Phenomenex Luna C18 5 μm 5.0 × 20 mm column; mobile phase: 0.04% (v/v) TFA in water and 0.02%(v/v) TFA in acetonitrile, 40 °C, flow rate 0.4 mL/min. Mass spectrometric analyses for nucleotides were performed on an Agilent 1100 HPLC with API 2000 LC-MS/MS System using ESI as ionization, Synergi 75 × 2.0 mm, 4 μm Hydro-RP80 Å column, 50 mM TEAA in water and 50 mM in acetonitrile, flow rate 0.4 mL/min. Workup procedures for most of chemical reactions are the same or similar, therefore, unless specifically indicated, the workup refers to the following procedure: the reaction mixture at 0 °C is quenched with water or 5% $NaHCO_3$, diluted with ethyl acetate or dichloromethane, washed with 5% sodium bicarbonate and then with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. Unless specifically indicated, chromatography refers to a flash chromatography on a silica gel column.

Compound 11. To a solution of **10** (5.72 g, 46.9 mmol) in THF (50 mL) at −78 °C was added LDA in THF (2.0 M, 23.5 mL). The mixture was stirred at −78 °C for 30 min, and then **9** (20.0 g, 46.9 mmol) in THF (150 mL) was added. The resulting mixture was stirred at −78 °C for 6 h, quenched with Saturated NH_4Cl, and extracted with ethyl acetate. After workup, chromatography with 20% EA in PE gave 14.0 g (54%) of **11** as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.25−8.36 (m, 1H), 7.45−7.87 (m, 11H), 5.15−5.20 (m, 1H), 3.90−4.25 (m, 5H), 2.75−2.84 (m, 1H), 2.29−2.41 (m, 1H), 1.07 (s, 9H).

Compound 12. To a mixture of **11** (14 g, 25.5 mmol), Et_3SiH (118 g, 101.7 mmol) in DCM (140 mL) at 0 °C was added BF_3-Et_2O (4.2 mL, 33.6 mmol). The mixture was stirred at 0 °C for 1 h, and then more BF_3-Et_2O (11.2 mL, 90.7 mmol) was added. The mixture was stirred at rt for 16 h. After workup, chromatography with 20−50% EA in PE gave **12** (3.0 g, 24%) as a white solid.

Compound 13. To a mixture of **12** (2.7 g, 5.5 mmol) in MeOH/H_2O (10:1, 8 mL) was added aqueous ammonia (10%, 20 mL), followed by H_2O_2 (30%, 8 mL) at rt. The mixture was stirred for 30 min, then quenched with saturated Na_2SO_3. After workup, chromatography with 3% MeOH in DCM gave **13** (1.4 g, 50%) as a white solid. ^{1}H NMR (CD_3OD) δ 8.15 (d, J = 5.2 Hz, 1H), 7.82 (m, 1H), 7.73 (m, 4H), 7.38−746 (m, 6H), 5.18 (d, J = 4.4 Hz, 1H), 4.20 (m, 1H), 4.04−4.08 (m, 3H), 3.90 (m, 1H), 1.08 (s, 9H).

3-Fluoro-4-(β-D-ribofuranosyl)-2-pyridinecarboxamide (**3c**). A solution of **13** (1.4 g, 2.75 mmol) and NH_4F (4.2 g, 11.3 mmol) in MeOH (100 mL) was refluxed for 16 h and then concentrated. Chromatography with 0.5% NH_4OH and 5% MeOH in DCM gave **3c** (500 mg, 67%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.38 (d, J = 4.4 Hz, 1H, H-6), 7.98 (br s, 1H, NH), 7.80 (t, J = 4.4 Hz, 1H, H-5), 7.63 (br s, 1H, NH), 5.25 (d, J = 6.0 Hz, 1H, H-1′), 5.01 (d, J = 4.8 Hz, 1H, 2′- or 3′−OH), 4.97 (d, J = 5.2 Hz, 1H, 2′- or 3′−OH), 4.89 (t, J = 5.2 Hz, 1H, 5′−OH), 3.82−3.88 (m, 3H, H-2′, H-3′, H-4′), 3.53−3.66 (m, 2H, H-5′). ^{19}F NMR (DMSO-d_6) δ −128.35 (d, J = 4.9 Hz). MS, m/z 272.9 $(M + H)^+$.

Compound 14. A solution of **3c** (0.83 g, 3.05 mmol), PPh_3 (1.29 g, 4.88 mmol) and iodine (1.162 g, 4.57 mmol) in pyridine (10 mL) was stirred overnight. The reaction was quenched with saturated $Na_2S_2O_3$ (30 mL). After workup, the residue was purified on silica gel with 1% MeOH in DCM to give the 5′-iodo intermediate (590 mg, 51%), which was dissolved in THF (10 mL), followed by addition of DBU (1.17 g, 7.7 mmol). The resulting mixture was stirred at 60 °C overnight, then quenched with 1 N HCl. After workup, chromatography with 80% EA in PE gave **14** (350 mg, 89%) as a white solid. ^{1}H NMR (CD_3OD) δ 8.44 (m, 1H), 7.56 (m, 1H), 5.42 (d, J = 3.6 Hz, 1H), 4.51 (m, 1H), 4.42 (m, 1H), 4.28 (m, 1H), 4.10 (m, 1H).

Compound 15. To an ice-cold solution of **14** (360 mg, 1.42 mmol) in MeCN (5 mL) was added TEA-3HF (343 mg, 2.12 mmol), followed by NIS (400 mg, 1.77 mmol). The resulting mixture was stirred at rt overnight, then the precipitate was filtered and washed with DCM to give the crude **15** (380 mg, 67%) as a white solid.

3-Fluoro-4-(4-fluoro-β-D-ribofuranosyl)-2-pyridinecarboxamide (**8**). To a solution of **15** (380 mg, 0.95 mmol) in pyridine (4 mL) was added BzCl (320 mg, 2.28 mmol) at 0 °C. The resulting mixture was stirred at rt for 30 min, then quenched with water. After workup, chromatography with 2% MeOH in DCM gave the 2′,3′-dibenzoyl intermediate (433 mg, 75%). An aqueous solution of Bu_4NOH (55%, 3.95 mL, 7.9 mmol) was acidified with TFA to pH ∼ 4. To this solution at 0 °C was added the dibenzoyl intermediate (200 mg, 0.33 mmol), followed by mCPBA (480 mg, 2.8 mmol). The resulting mixture was stirred at rt overnight, then quenched with saturated $Na_2S_2O_3$ (30 mL). After workup, chromatography with 90% EA in PE gave **16** (110 mg, 67%) as a white solid. A solution of **16** (120 mg, 0.24 mmol) in NH_3/MeOH (7 N, 10 mL) was stirred for 5 h and then concentrated. The crude product was washed thoroughly with DCM to give **8** (50 mg, 73%) as a white solid. ^{1}H NMR (CD_3OD) δ 8.45 (br s, 1H, H-6), 7.80 (br s, 1H, H-5), 5.50 (br s, 1H, H-1′), 4.11−4.29 (m, 2H, H-2′, H-3′), 3.83 (m, 2H, H-5′a, H-5′b). ^{19}F-NMR (CD_3OD) δ −124.7, −127.4. MS, m/z 312.9 $(M + Na)^+$.

Compound 17. A suspension of **12** (3.0 g, 6.1 mmol), 2,2-dimethoxypropane (4.2 g, 40.3 mmol) and TsOH-H_2O (0.3 g, 1.6 mmol) in acetone (50 mL) was stirred overnight, then quenched with solid $NaHCO_3$ to pH ∼ 7, and filtered to remove the salt. After workup, chromatography with 2% MeOH in DCM gave the 2′,3′-isopropylidene intermediate (3.2 g). A solution of the intermediate (450 mg, 0.84 mmol) and NaOMe (177 mg, 3.2 mmol) in 1,4-dioxane (5 mL) was stirred for 3 h, then quenched with aqueous HCl. The crude product was purified on a preparative TLC (silica gel, 40% EA in PE) to give **17** (130 mg, 28%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.24 (m, 1H), 7.63−7.68 (m, 5H), 7.38−7.41 (m, 6H), 5.18 (d, J = 4.8 Hz, 1H), 4.74 (dd, J = 3.6 Hz, 6.4 Hz, 1H), 4.47 (m, 1H), 4.22 (m, 1H), 4.14 (s, 3H), 4.00 (dd, J = 3.2 Hz, 11.2 Hz, 1H), 3.86 (dd, J = 3.6 Hz, 11.6 Hz, 1H) 1.62 (s, 3H), 1.34 (s, 3H), 1.05 (s, 9H).

Compound 19. To a solution of **17** (820 mg, 1.5 mmol) in MeOH and water (10:1, 4 mL) was added aqueous ammonia (10%, 13 mL), followed by H_2O_2 (30%, 13 mL) at rt, and the resulting mixture was stirred for 8 h, then quenched with Na_2SO_3. After workup, chromatography with 80% EA in PE gave **18** (618 mg, 73%) as a white solid. To a solution of EtSH (713 mg, 11.5 mmol) in DMF (18 mL) was added NaH (60% in mineral oil, 460 mg, 11.5 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and a solution of **18** (920 mg, 1.63 mmol) in DMF (10 mL) was added. The reaction mixture was stirred at 40 °C for 4 h, then quenched with water. The crude product was purified by RP-HPLC (0.1% HCOOH in water and MeCN) to give **19** (282 mg, 55%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.29 (s, 1H), 8.57 (s, 1H), 8.22 (s, 1H), 8.12 (d, J = 4.0 Hz, 1H), 7.65 (d, J

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= 4.0 Hz, 1H), 5.12 (d, J = 2.8 Hz, 1H), 4.95 (t, J = 5.4 Hz, 1H), 4.63 (m, 2H), 4.02 (m, 1H), 3.57 (m, 2H), 1.51 (s, 3H), 1.27 (s, 3H).

*3-Methoxy-4-(β-D-ribofuranosyl)-2-pyridinecarboxamide (**3d**).* A solution of **18** (230 mg, 0.41 mmol) and concentrated hydrochloric acid (0.02 mL) in MeOH (2 mL) was stirred at 40 °C for 5 h, then neutralized to pH ∼ 7 with NH_4OH and concentrated. The residue was purified by RP-HPLC (NH_4HCO_3 in water and MeCN) to give **3d** (28 mg, 24%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.28 (d, J = 4.8 Hz, 1H, H-6), 7.87 (s, 1H, NH), 7.69 (d, J = 4.4 Hz, 1H, H-5), 7.51 (s, 1H, NH), 5.05 (br, 3H, 2′−OH, 3′−OH, 5′−OH), 4.98 (d, J = 4.4 Hz, 1H, H-1′), 3.81−3.92 (m, 6H, H-2′, H-3′, H-4′, OMe), 3.79 (dd, J = 3.0 Hz, 11.8 Hz, 1H, H-5′a), 3.57 (dd, J = 3.6 Hz, 11.6 Hz, 1H, H-5′b). MS, m/z 306.9 $(M + Na)^+$.

*3-Hydroxy-4-(β-D-ribofuranosyl)-2-pyridinecarboxamide (**3e**).* By the same procedure as described for **3d**, **19** (49 mg, 0.16 mmol) was converted to **3e** (10 mg, 24%) as a white solid. ^{1}H NMR (CD_3OD) δ 8.01 (d, J = 3.6 Hz, 1H, H-6), 7.65 (d, J = 3.6 Hz, 1H, H-5), 5.08 (d, J = 3.6 Hz, 1H, H-1′), 3.96 (m, 1H, H2′), 3.80−3.93 (m, 3H, H-3′, H-4′, H-5′a), 3.67 (dd, J = 4.4 Hz, 12.0 Hz, 1H, H-5′b). MS, m/z 270.9 $(M + H)^+$.

Compound 23a. To a stirred solution of **21a** (2.0 g, 17.7 mmol) in THF (50 mL) at −78 °C was added dropwise LDA in THF (2 M, 8.85 mL). The resulting mixture was stirred at this temperature for 30 min, then a solution of **20**[14] (3.7 g, 8.85 mmol) in THF (10 mL) added dropwise. After stirring for one more hour at −78 °C, the reaction mixture was quenched with aqueous NH_4Cl. After workup, chromatography with 20% EA in PE gave a 1′-hydroxy nucleoside (4.1 g, 87%) as colorless syrup. To a stirred solution of the 1′-hydroxy nucleoside (2.1 g, 3.95 mmol) in THF (10 mL) was added dropwise LiHMDS (1.0 M in THF, 5.9 mL) at rt, then stirring continued for 10 min. To the above mixture was added dropwise Ac_2O (610 mg, 6.0 mmol), then stirring continued for 20 min. More LiHMDS and Ac_2O were added until the reaction was completed. After workup, chromatography with 25% EA in PE gave the 1′-*O*-acetyl intermediate **22a** (1.8 g, 80%). To a stirred solution of **22a** (2.0 g, 3.5 mmol) in DCM (20 mL) was added dropwise Et_3SiH (12.1 g, 104.7 mmol) and BF_3-Et_2O (743 mg, 5.23 mmol) at rt, and the mixture was stirred for 1 h. The reaction was quenched with aqueous $NaHCO_3$. After workup, chromatography with 25% EA in PE gave **23a** (910 mg, 56%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.48 (s, 1H), 8.19 (d, J = 4.8 Hz, 1H), 7.75 (d, J = 5.2 Hz, 1H), 7.25−7.33 (m, 15H), 5.41 (d, J = 2.4 Hz, 1H), 4.75 (d, J = 12.0 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 4.40−4.60 (m, 3H), 4.39 (m, 2H), 4.05 (dd, J = 4.6 Hz, 7.8 Hz, 1H), 3.89−3.98 (m, 2H), 3.69 (dd, J = 2.8 Hz, 10.8 Hz, 1H). MS, m/z 516.1 $(M + H)^+$.

Compound 24a. To a stirred solution of **23a** (1.0 g, 1.94 mmol) in anhydrous DCM (10 mL) was added mCPBA (666 mg, 3.9 mmol) at rt under nitrogen. The reaction mixture was stirred for 2 h, then quenched with aqueous Na_2SO_3. After workup, chromatography gave a crude product, which was dissolved in acetonitrile (10 mL). TMSCN (1.62 g, 16.6 mmol) was added, followed by addition of TEA (1.65 g, 16.3 mmol). The resulting mixture was refluxed for 5 h, then cooled and quenched with $NaHCO_3$. After workup, chromatography with 30% EA in PE gave **24a** (0.6 g, 58%) as a white solid.

Compound 25a. To a stirred solution of **24a** (735 mg, 1.36 mmol) in DMSO (20 mL) was added K_2CO_3 (750 mg, 5.4 mmol) and H_2O_2 (30%, 12.3 mL) at rt. The resulting mixture was stirred for 1 h, then quenched with Na_2SO_3. After workup, chromatography with 50% EA in PE gave **25a** (550 mg, 72%) as a white solid.

*3-Chloro-4-(β-D-ribofuranosyl)-2-pyridinecarboxamide (**3f**).* To a stirred solution of **25a** (220 mg, 0.39 mmol) in DCM (6 mL) at −78 °C under nitrogen was added BCl_3 (1.0 M in DCM, 4.7 mL). The mixture was stirred at this temperature for 1 h, then quenched with pyridine in MeOH. The solvent was removed and the residue purified by RP-HPLC (NH_4HCO_3 in water and MeCN) to give **3f** (70 mg, 62%) as a white solid. ^{1}H NMR (CD_3OD) δ 8.45 (d, J = 5.2 Hz, 1H, H-6), 7.92 (d, J = 4.8 Hz, 1H, H-5), 5.26 (d, J = 2.8 Hz, 1H, H-1′), 3.92−4.01 (m, 4H, H-2′, H-3′, H-4′, H-5′a), 3.79 (dd, J = 12.0, 4.0 Hz, 1H, H-5′b). MS, m/z 289.0 $(M + H)^+$.

*4-(β-D-Ribofuranosyl)-2-pyridinecarboxamide (**3a**).* A mixture of **25a** (300 mg, 0.54 mol) and Pd−C (10%, 150 mg) in MeOH (10 mL) was stirred under H_2 balloon for 5 h. The catalyst was filtered through silica gel pad and washed with MeOH. The filtrate was concentrated, the residue was purified on silica gel (50% EA in PE) to give **25c** (100 mg, 36%) as a white solid. MS, m/z 525.1 $(M + H)^+$. Compound **25c** (100 mg, 0.19 mmol) was converted to **3a** (30 mg, 62%) as a white solid by the same procedure as described for **3f**. ^{1}H NMR (CD_3OD) δ 8.57 (d, J = 5.2 Hz, 1H, H-6), 8.20 (t, J = 0.8 Hz, 1H, H-3), 7.65 (dd, J = 5.2, 1.2 Hz, 1H, H-5), 4.78 (d, J = 2.8 Hz, 1H, H-1′), 4.01−4.05 (m, 2H, H-2′, H-3′), 3.70−3.83 (m, 3H, H-4′, H-5′a, H-5′b). MS, m/z 255.0 $(M + H)^+$.

Compound 22b. Compound **22b** (1.2 g, 20%) as a colorless syrup was prepared from **21** (3.0 g, 19.1 mmol) and **20** (4.0 g, 9.6 mmol) by the same procedure as described for **22a**.

Compound 23b. Compound **23b** (650 mg, 34%) as a white solid was prepared from **22b** (2.1 g, 3.4 mmol) by the same procedure as described for **23a**. ^{1}H NMR ($CDCl_3$) δ 8.56 (s, 1H), 8.14 (d, J = 5.2 Hz, 1H), 7.69 (d, J = 5.2 Hz, 1H), 7.19−7.31 (m, 15H), 5.29 (d, J = 2.4 Hz, 1H), 4.70 (s, 2H), 4.45−4.56 (m, 3H), 4.31−4.39 (m, 2H), 4.03 (dd, J = 4.4 Hz, 7.6 Hz, 1H), 3.85−3.90 (m, 2H), 3.65 (dd, J = 2.8 Hz, 10.8 Hz, 1H).

Compound 24b. Compound **23b** (0.9 g, 1.6 mmol) was converted to **24b** (500 mg, 54%) as a white solid by the same procedure as described for **24a**. ^{1}H NMR ($CDCl_3$) δ 8.12 (d, J = 4.4 Hz, 1H), 7.95 (d, J = 4.8 Hz, 1H), 7.26 (m, 15H), 5.25 (s, 1H), 4.34−4.77 (m, 7H), 4.04 (m, 1H), 3.89 (m, 2H), 3.62 (m, 1H). MS, m/z 585.0 $(M + H)^+$.

Compound 25b. Compound **24b** (250 mg, 0.43 mmol) was converted to **25b** (150 mg, 58%) as a white solid by the same procedure as described for **25a**. MS, m/z 604.7 $(M + H)^+$.

*3-Bromo-4-(β-D-ribofuranosyl)-2-pyridinecarboxamide (**3g**).* Compound **25b** (150 mg, 0.25 mmol) was converted to **3g** (50 mg, 61%) as a white solid by the same procedure as described for **3f**. ^{1}H NMR (CD_3OD) δ 8.46 (d, J = 5.2 Hz, 1H, H-6), 7.89 (d, J = 4.8 Hz, 1H, H-5), 5.23 (d, J = 2.8 Hz, 1H, H-1′), 3.93−4.04 (m, 4H, H-2′, H-3′, H-4′, H-5′a), 3.79 (dd, J = 12.4, 4.0 Hz, 1H, H-5′b). MS, m/z 333.0 $(M + H)^+$.

Compound 26a. To a vigorously stirred solution of **24b** (320 mg, 0.55 mmol) and $Pd(PPh_3)_4$ (31 mg, 0.027 mmol) in THF (5 mL) under nitrogen at rt was added Me_3Al (1 M in toluene, 1.1 mL, 1.1 mmol). The resulting mixture was refluxed for 10 h, then cooled, poured into saturated NaH_2PO_4 and extracted with EA. Chromatography with 30% EA in PE gave **26a** (250 mg, 88%) as a white solid. MS, m/z 521.1 $(M + H)^+$.

*3-Methyl-4-(β-D-ribofuranosyl)-2-pyridinecarboxamide (**3b**).* To a stirred solution of **26a** (210 mg, 0.40 mmol) in DMSO (10 mL) were added K_2CO_3 (221 mg, 1.61 mmol) and H_2O_2 (30%, 3.6 mL) at rt, and the mixture was stirred for 1 h. Then the reaction was quenched with Na_2SO_3. After workup, chromatography with 50% EA in PE gave the amide product (150 mg, 69%) as white solid. The amide (135 mg, 0.25 mmol) was converted to **3b** (50 mg, 74%) as a white solid by the same procedure as described for **3f**. ^{1}H NMR (CD_3OD) δ 8.35 (d, J = 5.2 Hz, 1H, H-6), 7.78 (d, J = 5.2 Hz, 1H, H-5), 5.13 (d, J = 4.8 Hz, 1H, H-1′), 3.96−3.99 (m, 2H, H-2′, H-3′), 3.87−3.91 (m, 2H, H-4′, H-5′a), 3.77 (dd, J = 4.4 Hz, 12.0 Hz, 1H, H-5′b), 2.54 (s, 3H, Me). MS, m/z 269.0 $(M + H)^+$.

Compound 26b. To a stirred solution of **24b** (700 mg, 1.19 mmol) in dioxane (2 mL) under nitrogen were added tributyl(vinyl)-stannane (3.79 g, 12.0 mmol), $Pd(dppf)Cl_2$ (40 mg) and KF (30 mg, 0.52 mmol). The reaction vessel was sealed and heated under microwave at 120 °C for 30 min. After workup, chromatography with 30% EA in PE gave **26b** (501 mg, 79%) as a white solid. MS, m/z 533.0 $(M + H)^+$.

*4-(β-D-Ribofuranosyl)-3-vinyl-2-pyridinecarboxamide (**3h**).* Compound **26b** (600 mg, 1.12 mmol) was converted to the amide product (550 mg, 89%) as a white solid by the same procedure as described in preparation of **3b**.The amide (250 mg, 0.45 mmol) was converted to **3h** (81 mg, 64%) as a white solid by the same procedure as described for **3b**. ^{1}H NMR (CD_3OD) δ 8.42 (d, J = 5.2 Hz, 1H, H-6), 7.82 (d, J

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= 5.2 Hz, 1H, H-5), 7.06 (dd, J = 17.6, 11.6 Hz, 1H, CH=CH_2), 5.55 (dd, J = 1.6 Hz, 11.6 Hz, 1H, CH=CHaHb), 5.49 (dd, J = 1.4 Hz, 17.8 Hz, 1H, CH=CHaHb), 5.14 (d, J = 5.2 Hz, 1H, H-1′), 4.03 (t, J = 5.2 Hz, 1H, H-2′), 3.94−3.97 (m, 2H, H-3′, H-4′), 3.87 (dd, J = 12.0, 2.8 Hz, 1H, H-5′a), 3.76 (dd, J = 12.0, 4.4 Hz, 1H, H-5′b). MS, m/z 280.9 $(M + H)^+$.

Compound 26c. To a stirred solution of **24b** (200 mg, 0.342 mmol) in TEA (1.0 mL) under nitrogen were added ethynyltrimethylsilane (334 mg, 3.42 mmol), CuI (10 mg) and $Pd(PPh_3)_2Cl_2$ (20 mg). The mixture was heated under microwave at 135 °C for 1 h. After workup, chromatography with 30% EA in PE gave the 3-(TMS-ethynyl)pyridine (105 mg, 51%) as a white solid. A solution of this product (110 mg, 0.18 mmol) and TBAF (72 mg, 0.27 mmol) in THF (3 mL) was stirred at rt for 5 h. After workup, chromatography with 20% EA in PE gave **26c** (80 mg, 86%) as a white solid. MS, m/z 531.1 $(M + H)^+$.

*3-Ethynyl-4-(β-D-ribofuranosyl)-2-pyridinecarboxamide (**3i**).* Compound **26c** (160 mg, 0.30 mmol) was converted to **3i** (31 mg, 37%) as a white solid by the same procedure as described for **3b**. ^1H NMR (CD_3OD) δ 8.51 (d, J = 4.8 Hz, 1H, H-6), 7.93 (d, J = 4.8 Hz, 1H, H-5), 5.36 (d, J = 3.2 Hz, 1H, H-1′), 4.23 (s, 1H, CCH), 3.92−4.03 (m, 4H, H-2′, H-3′, H-4′, H-5′a), 3.79 (dd, J = 12.0 Hz, 4.0 Hz, 1H, H-5′b). MS, m/z 279.0 $(M + H)^+$.

Compound 28. To a solution of **27** (13.5 g, 48.5 mmol) in toluene (90 mL) at −78 °C was added DIBAL-H (1 M in toluene, 73 mL). The mixture was stirred at −70 °C for 30 min, then quenched with acetone. After workup, chromatography (10% EA in PE) gave the desired 1-OH product (12.8 g, 94%) as yellow oil. A mixture of the 1-OH intermediate (15.4 g, 55 mmol) and ethyl (triphenylphosphoranylidene)acetate (38.3 g, 110 mmol) in MeCN (250 mL) was refluxed for 16 h. The solvent was removed *in vacuo* and the residue was purified by chromatography (10% EA in PE) to give **28** (17.9 g, 93%) as a white solid.

Compound 30. To a solution of MeCN (5.0 g, 122 mmol) in THF (65 mL) at −78 °C was added *n*-BuLi (2.5M, 44.8 mL), then the solution was stirred at −78 °C for 30 min. To the above solution was added **28** (17.9 g, 51 mmol) in THF (15 mL). The mixture was stirred at −78 °C for 30 min. The reaction was quenched with AcOH (7.2 mL, 125 mmol). After workup, chromatography (20% EA in PE) gave **29** (12.2 g, 69%) as a white solid. A solution of **29** (10.1 g, 29.24 mmol) and *tert*-butoxybis(dimethylamino)methane (5.1 g, 29.3 mmol) in DMF-DMA (25 mL) was stirred at 100 °C for 16 h, then concentared. The residue was dissolved in EtOH (60 mL) and ammonium acetate (6.76 g, 87.7 mmol) was added. The mixture was refluxed for 24 h and then concentrated. After workup, chromatography (40% EA in PE) gave **30** (6.25 g, 56%) as a white solid.

*3-Carbamoyl-5-(β-D-ribofuranosyl)-4−1H-pyridinone (**4a**).* A solution of **30** (3.0 g, 7.08 mmol) in 80% TFA (30 mL) was stirring at 0 °C for 3 h, then concentrated to dryness. The residue was purified by chromatography (2% MeOH in DCM) to give the 2′,3′−OH intermediate (1.3 g, one isomer), which was dissolved in 4 M HCl/MeOH (20 mL). The solution was stirred at 60 °C for 2 days and concentrated to dryness. The residue was purified by RP-HPLC (NH_4HCO_3 in water and MeCN) to give **31** (200 mg, 10%) as a white solid. Compound **31** (150 mg, 0.6 mmol) in THF (10 mL) saturated with NH_3 in a sealed tube was stirred at 100 °C for 15 h. The solution was concentrated and the residue was purified by RP- HPLC (NH_4HCO_3 in water and MeCN) to give **4a** (9.2 mg, 6%) as a white solid. ^1H NMR (CD_3OD) δ 8.54 (d, J = 1.6 Hz, 1H, H-2), 8.08 (t, J = 3.4 Hz, 1H, H-6), 4.93 (dd, J = 0.8 Hz, 4.8 Hz, 1H, H-1′), 4.09 (m, 1H, H-2′), 4.06 (t, J = 5.2 Hz, 1H, H-3′), 4.02 (m, 1H, H-4′), 3.84 (dd, J = 3.0 Hz, 12.2 Hz, 1H, H-5′a), 3.70 (dd, J = 4.2 Hz, 12.2 Hz, 1H, H-5′b). MS, m/z 271.1 $(M + H)^+$.

Compound 35. Compound **32**[19] (54.0 g, 144.4 mmol) was converted to **33** (32.6 g, 61%) as a white solid by the same procedure as described for **29**. A solution of **33** (32.0 g, 80.7 mmol), TfN_3 (22.6 g, 130.0 mmol) and py (13.7 g, 173 mmol) in MeCN (40 mL) was stirred at rt for 3 h. The solvent was removed and the residue was purified by chromatography (10% EA in PE) to give **34** (12.4 g, 36%) as colorless oil. To a solution of **34** (12.0 g, 30.4 mmol) in THF (20 mL) at rt was added $P(CH_3)_3$ (1.0 M in THF, 32.0 mL). The mixture was stirred for 15 min, then quenched with water. After concentration, chromatography (15% EA in PE) gave **35** (10.4 g, 86.2%) as a white solid. ^1H NMR ($CDCl_3$) δ 7.72−7.74 (m, 2H), 4.64−4.66 (m, 1H), 4.37−4.46 (m, 2H), 4.04−4.06 (m, 1H), 3.68−3.69 (m, 2H), 2.98−3.20 (m, 2H), 1.53 (s,3H), 1.33 (s,3H), 0.89 (s, 3H), 0.05 (s, 6H).

Compound 37 and 38. A solution of **35** (10.0 g, 25.2 mmol) and $(Boc)_2O$ (5.6 g, 25.6 mmol) in pyridine (20 mL) was stirred at room temperature for 3 h. After workup, chromatography (10% EA in PE) gave **36** (8.3 g, 66%) as a white solid. A solution **36** (3.8 g, 7.6 mmol) and DMF-DMA (4.1 g, 34.2 mmol) in THF (10 mL) was stirred at rt for 12 h, treated with water, and stirred for 30 min. The solvent was evaporated and the residue purified by chromatography (50% EA in PE) to give **37** (385 mg) and **38** (238 mg), both as a white solid. Compound **37**: ^1H NMR ($CDCl_3$) δ 8.65 (s, 1H), 5.09 (d, J = 3.2 Hz, 1H) 4.67 (m, 2H), 4.38 (d, J = 2.0 Hz, 1H), 3.94 (dd, J = 2.2 Hz, 11.0 Hz, 1H), 3.76 (dd, J = 3.0 Hz, 11.4 Hz, 1H), 1.58 (s, 3H), 1.39 (1s, 3H), 0.79 (s, 9H), 0.02 (s, 3H), −0.02 (s, 3H). MS, m/z 408.1 $(M + H)^+$. Compound **38:** ^1H NMR ($CDCl_3$): δ 8.05 (s, 1H), 5.07 (s, 1H), 4.59 (m, 2H), 4.18 (m, 1H), 3.97 (s, 3H), 3.88 (dd, J = 2.6 Hz, 11.4 Hz, 1H), 3.72 (dd, J = 3.6 Hz, 11.6 Hz, 1H) 1.58 (s, 3H), 1.32 (s, 3H), 0.87 (s, 9H), 0.05 (s, 6H). MS, m/z 421.9 $(M + H)^+$.

*3-Carbamoyl-5-(β-D-ribofuranosyl)-4−1H-pyridazinone (**4b**).* To a stirred solution of **37** (370 mg, 0.9 mmol) in anhydrous MeOH (3 mL) at rt was added H_2O_2 (30%, 10 mL) and TEA (400 mg, 4.0 mmol). The resulting mixture was stirred for 12 h and then quenched with aq. Na_2SO_3. After workup, chromatography (50% EA in PE) gave the desired amide (320 mg, 83%), which was dissolved in 80% aq. TFA (5 mL) and stirred at rt for 1 h. After removal of volatiles, the residue was purified by RP-HPLC (NH_4HCO_3 in water and MeCN) to give **4b** (112 mg, 55%, containing ∼10% of another isomer) as a white solid. ^1H NMR (D_2O) δ 8.37 (s, 1H, H-6), 4.87 (d, J = 3.6 Hz, 1H, H-1′), 4.02 (m, 1H, H-2′), 3.88 (m, 2H, H-3′, H-4′), 3.75 (m, 1H, H-5′a), 3.60 (m, 1H, H-5′b). MS, m/z 272.1 $(M + H)^+$.

*3-Carbamoyl-1-methyl-5-(β-D-ribofuranosyl)-4-pyridazinone (**4c**).* Compound **38** (220 mg, 0.52 mmol) was converted to **4c** (76 mg, 51%) as a white solid by the same procedure as described for **4b**. ^1H NMR (D_2O): δ 8.54 (s, 1H, H-6), 5.01 (d, J = 3.2 Hz, 1H, H-1′), 4.16, 4.06 (2 m, 1H, 2H, H-2′, H-3′, H-4′), 4.09 (s, 3H, Me), 3.93 (dd, J = 1.6 Hz, 13.2 Hz, 1H, H-5′a), 3.77 (m, 1H, H-5′b). MS, m/z 286.0 $(M + H)^+$.

Compound 41. A solution of **39** (40.0 g, 0.2 mol) and $KMnO_4$ (100 g, 0.6 mol) was refluxed for 12 h, then cooled to rt, and the resulting precipitate filtered. The filtrate was concentrated to about 200 mL, then acidified with hydrochloric acid. The resulting white precipitate was filtered to give **40** (28.0 g, 60%). To a solution of **40** (28.0 g, 128 mmol) in DCM (500 mL) at 0 °C were added TEA (19.3 g, 192 mmol) and isobutyl chloroformate (20 g, 147 mmol), followed by 2-amino-2-methylpropanol (13.1 g, 147.0 mmol). The reaction mixture was stirred at rt for 2 h, then quenched with water. After workup, chromatography (20% EA in PE) gave an intermediate (12.0 g, 38%) as colorless oil, which was dissolved in concentrated H_2SO_4 (60 mL) and stirred at 120 °C for 30 min. The reaction mixture was cooled to rt, and poured into NH_4OH (50 mL) at 0 °C. After workup, chromatography (10% EA in EA) gave **41** (7.3 g, 55%) as a white solid. ^1H NMR ($CDCl_3$) δ 8.23 (d, J = 5.2 Hz, 1H), 7.70 (t, J = 5.2 Hz, 1H), 4.13 (s, 2H), 1.39 (s, 6H).

*3-Fluoro-2-(β-D-ribofuranosyl)-4-pyridinecarboxamide (**5a**).* To a stirred solution of **41** (7.0 g, 25.7 mmol) in anhydrous THF (50 mL) at −78 °C was added *n*-BuLi (1.3 M in hexane, 19.7 mL). The resulting mixture was stirred at −78 °C for 30 min, then **9** (21.9 g, 51.4 mmol) in THF (20 mL) was added. The reaction mixture was stirred at −78 °C for 2 h, then quenched with aqueous NH_4Cl. After workup, chromatography (30% EA in PE) gave **42** (9.4 g, 59%) as a white solid. Compound **42** (6.0 g, 9.7 mmol) was converted to **43** (1.8 g, 36%) as a white solid by the same procedure as described for **23a**. A solution of **43** (800 mg, 1.3 mmol) in aqueous TFA (80%, 5 mL) was stirred at rt for 30 min, then concentrated. The residue was dissolved in water, washed with DCM, and then concentrated to get **44** as a syrup, which was dissolved in concentrated aqueous ammonia (50

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mL) in a sealed tube. The solution was stirred at 50 °C for 12 h and then concentrated. The residue was purified by RP-HPLC (NH_4HCO_3 in water and MeCN) to give **5a** (83 mg, 23%) as a white solid. ^{1}H NMR (D_2O) δ 8.52 (d, J = 4.8 Hz, 1H, H-6), 7.73 (t, J = 5.2 Hz, 1H, H-5), 5.34 (dd, J = 1.4 Hz, 5.8 Hz, 1H, H-1′), 4.42 (t, J = 5.6 Hz, 1H, H-2′), 4.31 (t, J = 5.6 Hz, 1H, H-3′), 4.18 (dd, J = 3.2 Hz, 4.6 Hz, 1H, H-4′), 3.94 (dd, J = 3.2 Hz, 12.4 Hz, 1H, H-5′a), 3.80 (dd, J = 4.6 Hz, 12.6 Hz, 1H, H-5′b). MS, m/z 273.1 $(M + H)^+$.

Compound 45a. A solution of compound **43** (1.0 g, 1.7 mmol) and NaOMe (918.0 mg, 17.0 mmol) in anhydrous 1,4-dioxane (50 mL) was stirred at rt for 6 h. After workup, chromatography (20% EA in PE) gave **45a** (710 mg, 68%) as colorless oil. ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H), 7.76−7.63, 7.31−7.39 (2m, 11H), 5.45 (d, J = 3.6 Hz, 1H), 5.26 (dd, J = 3.6 Hz, 6.4 Hz, 1H), 4.84 (dd, J = 3.4 Hz, 6.2 Hz, 1H), 4.13 (s, 2H), 3.86 (s, 3H), 3.69 (m, 2H), 1.60 (s, 3H), 1.37 (s, 6H), 1.36 (s, 3H), 1.01 (s, 9H). MS, m/z 634.1 $(M + 18)^+$.

Compound 46. To a solution of **45a** (0.61 g, 1.0 mmol) in THF (10 mL) at 0 °C was added TBAF (1 M in THF; 2 mL, 2 mmol) and the resulting mixture stirred at rt for 1 h. The reaction was quenched with silica and evaporated to dryness. Chromatography on silica gel with 4−10% MeOH in DMC gave 0.33 g (90%) of **45b**. A solution of **45b** (38 mg, 0.1 mmol) in 80% aq. HCOOH was stirred at rt for 4 h, then concentrated and purified by RP-HPLC (A: 50 mM TEAA in water, pH 7; B: 50 mM TEAA in MeCN, pH 7) to yield **46** (21 mg, 40%). ^{1}H NMR (DMSO-d_6) δ 8.35 (d, J = 4.8 Hz, 1H, H-6), 7.99 (br, 1H, NH), 7.38 (d, J = 4.8 Hz, 1H, H-5), 5.06 (d, J = 5.2 Hz, 1H, H-1′), 4.23 (dd, J = 4.8 Hz, 5.2 Hz, 1H, H-2′), 4.01 (dd, J = 4.8 Hz, 5.2 Hz, 1H, H-3′), 3.84 (m, 1H, H-4′), 3.80 (s, 3H, OMe), 3.58 (dd, J = 4.0 Hz, 11.6 Hz, 1H, H-5′a), 3.44 (s, 2H, $C\underline{H}_2OH$), 3.40−3.44 (m, 1H, H-5′b), 1.28 (s, 6H, CMe_2). MS, m/z 357.3 $(M+1)^+$.

Compound 47. A solution of **45b** (0.23 g, 0.6 mmol) in 2% TFA in DCM (6 mL) stood at rt overnight, then concentrated and coevaporated with toluene several times. Chromatography on silica gel with 4−15% MeOH in DCM yielded 0.24 g (84%) of **47**. ^{1}H NMR (DMSO-d_6) δ 8.48 (d, J = 4.8 Hz, 1H, H-6), 7.76 (d, J = 4.8 Hz, 1H, H-5), 5.22 (d, J = 8.0 Hz, 1H, H-1′), 5.05 (dd, J = 4.0 Hz, 6.4 Hz, 1H, H-2′), 4.98 (t, J = 5.6 Hz, 1H, 5′−OH), 4.73 (dd, J = 3.2 Hz, 6.4 Hz, 1H, H-3′), 4.19 (s, 2H, $C(O)OCH_2$), 4.02 (m, 1H, H-4′), 3.81 (s, 3H, OMe), 3.32 (m, 2H, H-5′a, H-5′b), 1.48, 1.27 (2s, 6H, CMe_2), 1.21 (s, 6H, CMe_2). MS, m/z 397.3 $(M+1)^+$.

3-Methoxy-2-(β-D-ribofuranosyl)-4-pyridinecarboxamide ***5b***. A mixture of **47** (0.14 g, 0.35 mmol) in MeCN (1.5 mL) and acetic anhydride (1.5 mL) was stirred at rt for 1 day, then concentrated and purified on silica gel with 4−15% *i*-PrOH in DCM to give the N-acetate **48a** (68 mg, 40%) and the 5′-O, N-diacetate **48b** (50 mg, 33%), which were separately treated with methanolic ammonia (7 N, 3 mL) for 20 h. After evaporation of volatiles and coevaporation with methanol, the residues were separately dissolved in 17% aqueous TFA and stood for 2 h. After evaporation the residue was purified by RP-HPLC (A: 50 mM TEAA in water; B: 50 mM TEAA in MeCN) to yield **5b** as a white solid (85 mg, 80%). ^{1}H NMR (DMSO-d_6): δ 8.35 (d, J = 4.8 Hz, 1H, H-6), 7.94, 7.76 (2 br, 2H, NH_2), 7.35 (d, J = 4.8 Hz, 1H, H-5), 5.07 (d, J = 5.2 Hz, 1H, H-1′), 4.25 (m, 1H, H-2′), 4.02 (t, J = 5.2 Hz, 1H, H-3′), 3.85 (m, 1H, H-4′), 3.80 (s, 3H, OMe), 3.58 (dd, J = 4.2 Hz, 11.8 Hz, 1H, H-5′a), 3.43 (dd, J = 4.6 Hz, 11.8 Hz, 1H, H-5′b). MS, m/z 283.4 $(M-1)^-$.

3-Hydroxy-2-(β-D-ribofuranosyl)-4-pyridinecarboxamide ***5c***. A mixture of **5b** (45 mg, 0.11 mmol) and NaSEt (64 mg, 0.77 mmol) in DMF (0. Six mL) were stirred at 60 °C for 2 h, then cooled down and purified by RP-HPLC, gradient 0−60% B (A: 50 mM aqueous TEAA, B: 50 mM TEAA in MeCN) to yield triethylammonium salt of **5c** as a white solid (16 mg, 41%). ^{1}H NMR (DMSO-d_6) δ 8.90, 8.28 (2 br, 2H, NH_2), 8.04 (d, J = 4.8 Hz, 1H, H-6), 7.71 (d, J = 5.1 Hz, 1H, H-5), 5.12 (d, J = 4.0 Hz, 1H, H-1′), 5.07 (br, 1H, 3-OH), 4.84 (d, J = 5.6 Hz, 3′−OH), 4.18 (dd, J = 4.4 Hz, 4.8 Hz, H-2′), 4.00 (dd, J = 5.6 Hz, 11.2 Hz, 1H, H-3′), 3.84 (m, 1H, H-4′), 3.63 (dd, J = 3.6 Hz, 11.6 Hz, 1H, H-5′a), 3.45 (dd, J = 4.0 Hz, 11.6 Hz, 1H, H-5′b). MS, m/z 269.0 $(M-1)^-$.

Compound 52. To a suspension of **49**[22] (2 g, 4.5 mmol) in DCM (20 mL) were added DIPEA (1.7 g, 13.5 mmol) and HATU (2.1 g, 5.5 mmol), followed by N,O-dimethylhydroxylamine (0.5 g, 5 mmol). The reaction mixture was stirred at rt for 8 h. After workup, chromatography (50% EA in PE) gave **50** (1.8 g, 82%) as a white solid. To a stirred solution of trimethylsilylacetylene (234 mg, 2.4 mmol) in THF (8 mL) at −78 °C was added dropwise *n*-BuLi (2.5 M in hexane, 1 mL). The solution was stirred at −78 °C for 30 min, then **50** (900 mg, 1.8 mmol) in THF (5 mL) added. The reaction mixture was stirred at rt for 3 h, then quenched with aqueous NH_4Cl. Usual workup yielded the crude **51**, which was dissolved in MeCN (10 mL) and H_2O (5 mL), followed by addition of methyl carbamimidothioate hydrochloride (464 mg, 3.7 mmol). The reaction mixture was stirred at 80 °C for 3 h. After workup, chromatography (20% EA in PE) gave **52** (400 mg, 32%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.28 (d, J = 4.8 Hz, 1H), 7.23−7.41 (m, 16H), 5.08 (m, 1H), 4.82 (d, J = 12.0 Hz, 1H), 4.70 (d, J = 12.4 Hz, 1H), 4.49−4.59 (m, 3H), 4.32−4.14 (m, 2H), 4.11 (m, 1H), 3.91 (m, 1H), 3.85 (m, 1H), 3.64 (m, 1H), 2.52 (s, 3H).

*4-(β-D-Ribofuranosyl)-2-pyrimidinecarboxamide (****6****).* A solution of **52** (400 mg, 0.76 mmol) and mCPBA (400 mg, 2.3 mmol) in DCM (10 mL) was stirred at rt for 3 h. After workup, chromatography (50% EA in PE) gave an oxidized intermediate (390 mg, 92%) as a white solid. A solution of the intermediate (350 mg, 0.625 mmol) and KCN (60 mg, 0.92 mmol) in anhydrous DMSO (3 mL) was stirred at rt overnight, then diluted with EA (15 mL). After workup, chromatography (50% EA in PE) gave **53** (200 mg, 63%) as a white solid. Compound **53** (200 mg, 0.4 mmol) was converted to **54** (120 mg, 57%) as a white solid by the same procedure as described for **13**. A solution of **54** (100 mg, 0.19 mmol) and BCl_3 (1 M in DCM, 1.9 mL) in anhydrous DCM (5 mL) was stirred at 0 °C for 4 h, then quenched with pyridine and MeOH. The solvent was removed and the residue was purified by RP-HPLC ($AcONH_4$ in water and MeCN) to give **6** (20 mg, 41%) as a white solid. ^{1}H NMR (CD_3OD): δ 8.86 (d, J = 5.1 Hz, 1H, H-6), 8.11 (br s, 1H, NH), 7.81 (d, J = 5.1 Hz, 1H, H-5), 7.77 (br s, 1H, NH), 5.28 (d, J = 5.5 Hz, 1H, H-1′), 4.89 (d, J = 5.5 Hz, 1H, 3′−OH), 5.85 (t, J = 5.5 Hz, 1H, 5′−OH), 4.72 (d, J = 4.3 Hz, 1H, 2′−OH), 3.99 (m, 1H, H-2′), 3.85 (m, 2H, H-3′, H-4′), 3.65 (m, 1H, H-5′a), 3.51 (m, 1H, H-5′b). MS, m/z 256.1 $(M + H)^+$.

Compound 58. A solution of **55**[23] (10.6 g, 30 mmol) in aqueous TFA (80%, 100 mL) was stirred at rt for 12 h. Volatiles were evaporated and the residue was purified by chromatography (20% EA in PE) to give the 1-OH intermediate (9.0 g, 90%) as colorless oil. To a solution of the intermediate (9.0 g, 27.0 mmol) in DMSO (60 mL) was added Ac_2O (40 mL) at rt, and the mixture was stirred at rt. for 12 h. After workup, the residue was purified by chromatography (10% EA in PE) to give **56** (8.5 g, 95%) as a colorless syrup. To a stirred solution of 3-fluoropicolinonitrile (2.9 g, 24.2 mmol) in THF (50 mL) at −78 °C was added dropwise LDA (12.0 mL, 24.2 mmol). The mixture was stirred at this temperature for 30 min. To the above mixture was added a solution of **56** (8.0 g, 24.0 mmol) in THF (10 mL) at −78 °C, and stirring continued for 1h. The reaction was quenched with aqueous NH_4Cl. After workup, chromatography (20% EA in PE) gave **57** (4.0 g, 37%) as a white solid. Compound **57** (4.0 g, 8.8 mmol) was converted to **58** (1.7 g, 44%) as a white solid by the same procedure as described for **23a**. ^{1}H NMR (CD_3OD) δ 8.20 (d, J = 4.8 Hz, 1H), 8.01 (dd, J = 5.2 Hz, 5.6 Hz, 1H), 7.27−7.34 (m, 10H), 5.47 (d, J = 23.2 Hz, 1H), 5.16 (dm, J = 54.4 Hz, 1H), 4.69 (d, J = 12.0 Hz, 1H), 4.57 (d, J = 11.2 Hz, 1H), 4.49 (m, 2H), 4.24 (m, 1H), 4.12 (ddd, J = 3.8 Hz, 8.4 Hz, 10.8 Hz, 1H), 3.94 (dd, J = 2.0 Hz, 10.8 Hz, 1H), 3.69 (dd, J = 2.8 Hz, 11.2 Hz, 1H).

*3-Fluoro-4-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-2-pyridinecarboxamide (****7****).* Compound **58** (1.7 g, 3.8 mmol) was converted to the amide (1.5 g, 87%) as described for compound **25a**.The amide intermediate (300 mg, 0.66 mmol) was converted to **7** (82 mg, 45%) as a white solid by the same procedure as described for **3f**. ^{1}H NMR (D_2O) δ 8.46 (br s, 1H, H-6), 7.85 (br s, 1H, H-5), 5.60 (d, J = 25.6 Hz, 1H, H-1′), 5.12 (dd, J = 3.6 Hz, 54.4 Hz, 1H, H-2′), 4.16−4.24 (m, 2H, H-3′, H-4′), 4.05 (dd, J = 1.6 Hz, 12.8 Hz, 1H, H-5′a), 3.86 (dd, J = 4.4 Hz, 12.8 Hz, 1H, H-5′b). MS, m/z 275.1 $(M + H)^+$.

Compound 59. A mixture of **3c** (100 mg, 0.37 mmol), TsOH·H_2O (7 mg, 0.037 mmol), and trimethoxymethane (390 mg, 3.7

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mmol) in MeCN (2 mL) was heated at 70 °C overnight. The mixture was diluted with MeOH (5 mL) and aqueous NH_3 (0.2 mL) and stood at rt for 2 h. After evaporation, the residue was purified on silica gel (5% MeOH in DCM) to give the 2′,3′-methoxymethylidene derivative of **3c** (68 mg, yield: 59%) as white foam. A mixture of triethylammonium bis(pivaloxymethyl)phosphate (0.35 mmol, prepared from 112 mg of bis(POM)phosphate and Et_3N) and the 2′,3′-methoxymethylidene derivative of **3c** (72 mg; 0.23 mmol) was coevaporated with anhydrous toluene and then dissolved in THF (3 mL). Diisopropylethylamine (0.2 mL, 1.2 mmol), BOP-Cl (146 mg, 0.57 mmol) and 3-nitro-1,2,4-triazole (66 mg, 0.57 mmol) were added, and the resulting mixture stirred at rt for 1 h. After workup, the residue was purified on silica gel with 3−10% *i*-PrOH in DCM. Thus, obtained nucleotide prodrug was treated with 80% aqueous HCOOH (1 mL) at rt for 15 min. The reaction mixture was concentrated and coevaporated with a mixture of toluene and methanol containing one drop of Et_3N. The residue was purified on silica gel with 4−10% MeOH in DCM to yield **59** as white foam (62 mg, 47% for 2 steps). ^{1}H NMR (DMSO-d_6) δ 8.37 (d, J = 5.7 Hz, 1H, H-6), 8.0 (brs, 1H, NH), 7.65 (br s, 1H, NH), 7.63 (d, J = 5.7 Hz, 1H, H-5), 5.58 (m, 4H, 2 x OCH_2O), 5.43 (d, J = 5.5 Hz, 1H, H-1′), 5.22 (d, J = 5.5 Hz, 1H, 3′−OH), 4.98 (d, J = 4.7 Hz, 1H, 2′−OH), 4.27 (m, 1H, H-5′a), 4.18 (m, 1H, H-5′b), 4.03 (m, 1H, H-4′), 3.82 (m, 2H, H-2′, H-3′), 1.13, 1.12 (2s, 18H, 2 x $C(CH_3)_3$). ^{19}F-NMR (DMSO-d_6) δ −127.73 (d, J = 5.5 Hz). ^{31}P NMR ($CDCl_3$) δ −4.15 (s). MS, m/z 581.4 $(M+1)^+$.

Compound 60. To a stirred solution of the 2′,3′-*O*-methoxymethylidene derivative of **3c** (32 mg, 0.1 mmol) in NMI (0.2 mL) and MeCN (0.3 mL) at 0 °C was added (2S)-cyclohexyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate (1.0 M in THF, 0.5 mL). The resulting solution was stirred at 30 °C for 5 h. After workup, the crude product was dissolved in 80% formic acid and stirred for 3 h. Evaporation and coevaporation with toluene gave a residue, which was dissolved in MeOH containing a few drops of triethyamine and stood at 35 °C for 2 h. After evaporation, the residue was purified on silica gel with 5−10% MeOH in DCM to give 33 mg (57%) of **60** as a white solid. ^{1}H NMR (CD_3OD, two P-isomers) δ 8.38, 8.32 (2d, J = 4.8 Hz, 1H, H-6), 7.80, 7.73 (2t, J = 4.8 Hz, 1H, H-5), 7.14−7.36 (m, 5H, Ph), 5.15 (m, 1H, H-1′), 4.71 (m, 1H, CH of Ala), 4.29−4.49 (m, 2 H, H-2′, H-3′), 4.18 (m, 1H, H-1 of cyclohexyl), 3.91−4.06 (m, 3H, H-4′, H-5′a, H-5′b), 3.75−3.88 (m, 1H, NH of Ala), 1.22−1.84 (m, 10 H, 5xCH_2 of cyclohexyl), 1.35, 1.32 (2d, J = 7.2 Hz, Me of Ala). MS, m/z 582.6 $(M + H)^+$, 604.9 $(M + Na)^+$. ^{19}F-NMR (CD_3OD, two P-isomers) δ −127.81 (d J = 4.5 Hz), −127.86 (d, J = 4.1 Hz). ^{31}P NMR (CD_3OD, two P-isomers) δ 3.88 (s), 3.75 (s).

Preparation of NTPs 61 and 62. Compound **61** and **62** were prepared from **3c** and **2**, respectively, by a general method described in our previous publiocation.[36] The purities of **61** and **62** were >95%, determined on an Agilent 1100 HPLC, 50 mM TEAA in water and 50 mM TEAA in acetonitrile as mobile phase.

61: ^{1}H NMR (D_2O) δ 8.35 (d, J = 4.8 Hz, 1H, H-6), 7.83(dd, J = 4.8, 5.2 Hz, 1H, H-5), 5.15 (d, J = 5.2 Hz, H-1′), 4.08−4.23 (m, 5H, H-2′, H-3′, H-4′, H-5′). ^{31}P NMR (D_2O) δ −4.15 (d, J = 19.1 Hz,), −10.77 (d, J = 18.8 Hz,), −20.60 (dd, J = 19.1, 18.8 Hz,). MS, m/z 511.0 $(M-1)^-$.

62: ^{1}H NMR (D_2O) δ 8.30 (d, J = 5.2 Hz, 1H, H-5), 5.95 (d, J = 1.2 Hz, 1H, H-1′), 4.21−4.30 (m, 5H, H-2′, H-3′, H-4′, H-5′a, H-5′b). ^{31}P NMR (D_2O) δ −5.47 (d, J = 18.9 Hz,), −11.27 (d, J = 19.4 Hz,), −21.00 (dd, J = 19.4, 18.9 Hz,). ^{19}F-NMR (D_2O) δ −102.94 (d, J = 4.9 Hz,). MS, m/z 528.1 $(M-1)^-$.

Antiviral and cytotoxicity assay. The antiviral assay is based on a neuraminidase activity assay as described by Eichelberger et al.[26] In brief, Madin-Darby canine kidney epithelial cells (MDCK, ATCC) were plated at a density of 1×10^5 cells/ml (1×10^4 cells/well) in assay media (DMEM supplemented with 0.3% FBS, 1% penicillin/streptomycin and 1% DMSO) in 96-weel plates. After 24 h, serially diluted compounds were added to cells and incubated for an additional 24 h. Cells were infected with 250 IU/well of Influenza strains A/WSN/33 (H1N1) (Virapur, San Diego CA) and incubated for 20 h at 37 °C, 5% CO_2. The cell culture supernatant was aspirated off and 50 μL 25 μM 2′-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid (Sigma-Aldrich) dissolved in 33 mM MES, pH 6.5 (Emerald Biosystems, Bainbridge Island, WA) was added to the cells. After incubation for 45 min at 30 °C, reactions were stopped by addition of 150 μL stop solution (100 mM glycine, pH 10.5, 25% ethanol, all Sigma-Aldrich). Fluorescence was measured with excitation and emission filters of 355 and 460 nm, respectively, on a Victor X3 multilabel plate reader (PerkinElmer, Waltham, MA). Cytotoxicity of uninfected parallel cultures was determined by addition of 100 μL CellTiter-Gloreagent (Promega, Madison, WI), and incubation for 10 min at room temperature. Luminescence was measured on a Victor X3 multilabel plate reader.

Inhibition of recombinant influenza A polymerase complex. The determination of the half maximal inhibitory concentration, IC_{50}, of the compounds against the recombinant influenza polymerase complex, PA/PB1/PB2, was performed as described previously.[18] In summary, each 10-μL reaction was performed at 37 °C for 40 min in a reaction mixture containing reaction buffer (25 mM Tris-Cl, pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 2 mM DTT, 5% glycerol), 0.15 μM polymerase complex, 0.4 mM 5′-ApG primer, 1.5 μM 50-nt 3′vRNA template, 1.6 μM 15-nt 5′vRNA, 0.20 U/μL RNaseIn, 500 μM UTP, ATP, and CTP, 1 μM GTP, 2.5 μCi [α-^{33}P]GTP and compounds at various concentrations. The reactions were stopped by a quench solution containing formamide with 50 mM EDTA. The samples were incubated at 95 °C for 5 min. The samples were run on a 15% denaturing PAGE gel (Invitrogen) at 190 V for 50 min. The gel was exposed to a storage phosphor screen and visualized by a Typhoon scanner (GE healthcare). The amount of RNA products was proportional to the intensity of the bands on the gel that were quantified using the ImageQuant software (GE Healthcare).

The compound concentration at which the RNA products was reduced by 50% (IC_{50}) was calculated by fitting the data to the equation,

$$Y = \min + \frac{\max - \min}{1 + 10^{(\log IC50 - X)\cdot h}}$$

where Y corresponds to the percentage of inhibition (percentage of RNA products reduced), *Min* is the minimal percentage of inhibition, *Max* is the maximal inhibition at high concentration of compound, X corresponds to the log of compound concentration, and h is the Hillslope.

Nucleotide incorporation assay using recombinant influenza A polymerase complex. The recombinant polymerase complex catalyzed the primer extension reaction using 5′-pApG as primer, a 14-nt synthetic RNA oligonucleotide as template and the 15-nt 5′vRNA as promoter. The 14-nt RNA template and the 15-nt 5′vRNA promoter form a panhandle structure that was required for RNA replication activity of the influenza polymerase complex.[18] To study if **61** can be incorporated opposite U or C on the template, nucleotide incorporation assays using specific templates were performed (sequences shown in Figure 3). A typical reaction was performed at 37 °C in a reaction mixture containing reaction buffer (40 mM Tris-HCl, pH7, 20 mM NaCl, 5 mM $MgCl_2$, and 2 mM DTT), 0.15 μM polymerase complex, 1 μM 15-nt 5′vRNA promoter, 2.5 μM 14-nt RNA template, 0.4 mM 5′-pApG primer, 0.2 U/μL RNaseIn, 25 μM UTP, 25 μM CTP, 0.033 μM [α-^{33}P]CTP, and the testing NTPs or NTP analogues at 100 μM. The replication reactions were stopped after 35 min by mixing with 2x volume of the formamide quench solution containing 50 mM EDTA. The quenched reactions were denatured at 95 °C for 3 min and then were loaded onto a 22.5% denaturing polyacrylamide gel with 7 M urea (National Diagnostics, Atlanta, GA). The electrophoresis was performed at 80 W using a Sequi-Gen GT system from Bio-Rad (Hercules, CA).

In vivo nucleotide formation of 3c. The studies were conducted at WuXi AppTec and conformed to the regulation and guidelines[37] regarding animal care and welfare: The study protocols were reviewed by and approved by WuXi AppTec's Institutional Animal Care and Use Committee (IAUCUC) prior to the study initiation. Female Balb/c mice (N = 2) were fasted overnight before administrated intravenously with the prodrug **59**. The prodrug was formulated as a

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solution of in 10% DMSO/60% PEG400/30%water and administrated at 2 mg/kg. While the animals were under anesthesia with isoflurane, blood samples were collected at 0.25 and 2 h post dose into tubes containing K_2EDTA as the anticoagulant. Following an immediate plasma protein precipitation treatment, the supernatant of the blood samples was analyzed for **3c** concentrations using a LC/MS/MS method. After the terminal blood collection, lung tissue was removed immediately and flash-frozen into liquid nitrogen to prevent any ex vivo degradation of the phosphorylated metabolites. The frozen tissue was homogenized in an extraction solution in a sample tube set in a dry ice/ethanol bath to maintain cold temperature. The supernatant of the lung extracts was subjected to LC/MS/MS analysis for the concentrations of **63** and **61**.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01933.

A table with molecular formula strings and the associated biochemical and biological data (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

IBX, 2-iodoxybenzoic acid; DMTrCl, 4,4′-dimethoxytrityl, chloride; MMTrCl, 4-methoxytrityl chloride; TBDPSCl, *tert*-butyldiphenylchlorosilane; DMP, Dess−Martin periodinane; TEA, triethylamine; TPSCl, 2,4,6-triisopropylbenzenesulfonyl chloride; EDCI, 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride; PE, petroleum ether; EA, ethyl acetate

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