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Bicyclic and Tricyclic "Expanded" Nucleobase Analogues of Sofosbuvir: New Scaffolds for Hepatitis C Therapies

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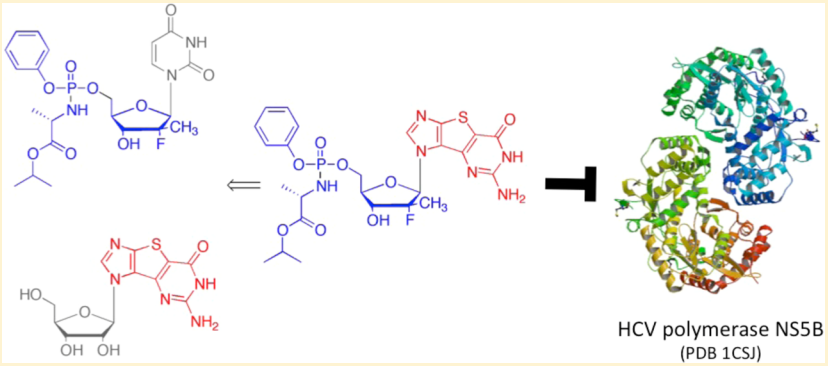

ABSTRACT: Given the impressive success of Gilead's Sofosbuvir, many laboratories, including ours, have explored the unique 2′-sugar modification (2′-Me, 2′-F) of nucleoside analogues in the hopes of exploiting the biological activity that this unique modification has imparted to the nucleoside scaffold. In that regard, we have combined our tricyclic "expanded" purine base motif with the 2′-Me, 2′-F sugar modification. Although the synthesis of these complex molecules proved to be nontrivial, with the best results coming from a linear approach, the overall strategy resulted in highly promising biological results for several of the target compounds, including their corresponding McGuigan ProTides. Modest activity against HCV was observed with inhibitory concentrations of as low as 20 μM.

KEYWORDS: *nucleoside scaffold, hepatitis C, Sofosbuvir, purines, antiviral, Huh 5.2, luminescence, antireplicon effect*

The impact that Gilead's Sofosbuvir (Figure 1), a 2′-Me, 2′-F-sugar modified nucleoside analogue, has had on the hepatitis C virus (HCV) is unprecedented. The fact that just a year ago there was no available treatment that was effective for many patients, much less a cure, points to the importance of this drug.[1,2] Sofosbuvir's mechanism of action works by inhibiting the RNA polymerase that HCV uses to replicate.[3,4] In combination with other therapies, Sofosbuvir can effectively cure hepatitis in 90% of patients.[1,2]

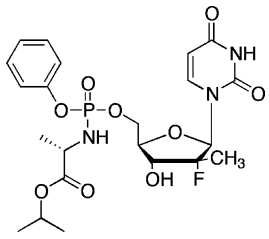

Figure 1. Sofosbuvir.

Several years ago our group developed a series of tricyclic "expanded" purines (Figure 2) related to Nelson Leonard's lin-benzoadenosine nucleosides that were designed to explore their effects on viral replication.[5−13] The thiophene spacer ring was designed to impart increased polarizability and stacking effects.[14] Notably, the parent tricyclic nucleosides and their

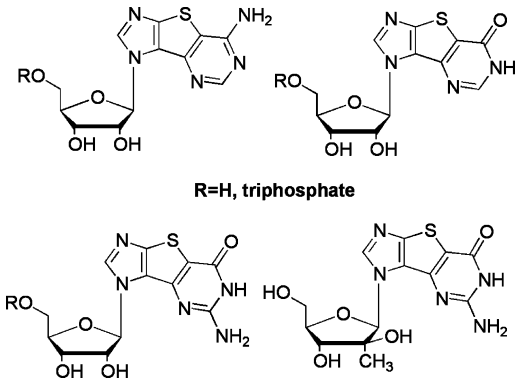

Figure 2. Tricyclic expanded purines.

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corresponding triphosphates exhibited promising activity against HCV.[11]

In an effort to further explore the potential of this interesting scaffold, we decided to combine our unique bases with the 2′-modified sugar found in Sofosbuvir to give corresponding tricyclic Sofosbuvir analogues **1**, **3**, and **4** as depicted in Figure 3. We also decided to pursue bicyclic intermediate **2** because it

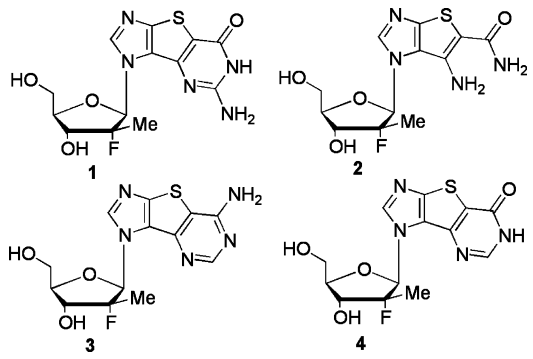


Figure 3. Tricyclic and bicyclic targets.

is facile to obtain during the synthesis and it also resembles Ribavirin, AICAR, and other structurally related nucleosides that have shown promising antiviral activity.[15,16] In short, we felt that combining the potent activity exhibited by Sofosbuvir's 2′-Me, 2′-F sugar with our unique bases could prove interesting biologically.

CHEMISTRY

Two approaches were attempted to realize the target compounds. Initially we attempted the more straightforward method of oxidizing the 2′-hydroxyl group and then stereospecifically functionalizing it as has been reported in the literature.[17] Unfortunately the yields were disappointingly low, so a linear approach was taken instead. As shown in Scheme 1, the stereospecific synthesis begins with the construction of 2′-difunctionalized sugar **5**, which can be obtained from D-mannitol in eight steps with an overall yield of 9.3% using previously reported literature procedures.[18]

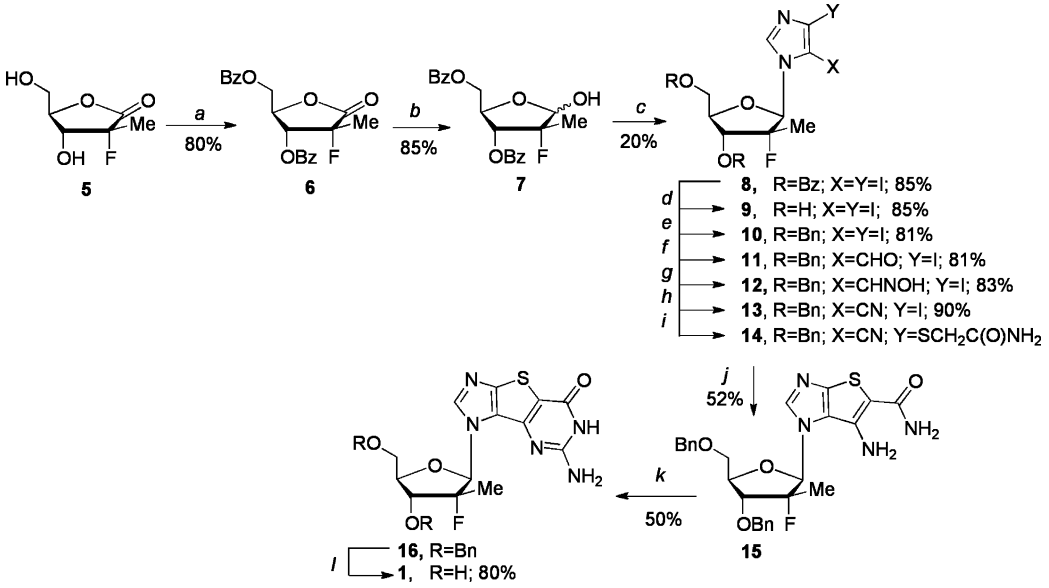
Once in hand, the hydroxyl groups were protected with BzCl using standard procedures to give **6**, followed by reduction of the carbonyl to provide **7** and Mitsunobu coupling with 4,5-diiodoimidazole to afford **8**.[18,19] The Bz groups were then removed and replaced with the more robust Bn groups to provide **10** because of the use of Grignard conditions in subsequent steps.[20] The tricyclic ring system of **16** was then constructed stepwise using our previously reported route.[11,13] Deprotection of the Bn groups of **16** using standard procedures afforded **1**.[20]

It should be noted that we initially approached this route by installing the Bn protecting groups first (as would be logical); however, the yields proved to be significantly lower and the workups more tedious, which fortunately turned out not to be the case with this protecting group "switch" approach using Bz and then Bn. Overall, the yields were much better and the workups quite facile, so ultimately we now use this route instead.

As mentioned earlier, the bicyclic intermediate was also of interest because of its similarity to Ribavirin and AICAR and other related nucleosides.[15,16] Although this was not as straightforward as initially envisioned because of the presence of the exocyclic amine and amide functionalities attached to the thiophene ring, desired bicyclic target **2** was finally obtained by manipulating the synthesis route to employ a different protecting group strategy as outlined in Scheme 2.

The key difference in the approach involves using the Markiewicz reagent to bis-protect the 3′- and 5′-hydroxyls

Scheme 1 Synthesis Conditions below[a]



[a]Reagents and conditions: (a) BzCl, Py, DCM, 0 °C, 10 min, then rt, 30 min. (b) $LiAl(OtBu)_3H$, THF, −20 °C, 6 h. (c) 4,5-Diiodoimidazole, Ph_3P, DIAD, THF, 0 °C to rt, 24 h. (d) MeONa, MeOH, 0 °C, 1 h. (e) (i) NaH, 0 °C, 2 h; (ii) BnBr, TBAI, DMF, rt, 4 h. (f) (i) EtMgBr, THF, 0 °C, 30 min; (ii) anhydrous DMF, rt, overnight. (g) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH, reflux, 5 h. (h) CDI, THF, reflux, 8 h. (i) $NH_2C(O)CH_2SH$, K_2CO_3, DMF, 65 °C, 24 h. (j) EtONa, EtOH, reflux, 2 h. (k) (i) NaOH, CS_2, MeOH, 150 °C, 18 h. (ii) H_2O_2, MeOH, 0 °C, 2 h. (iii) NH_3/MeOH, 130 °C,12 h. (l) $BF_3{\cdot}Et_2O$, EtSH, DCM, rt, 72 h.

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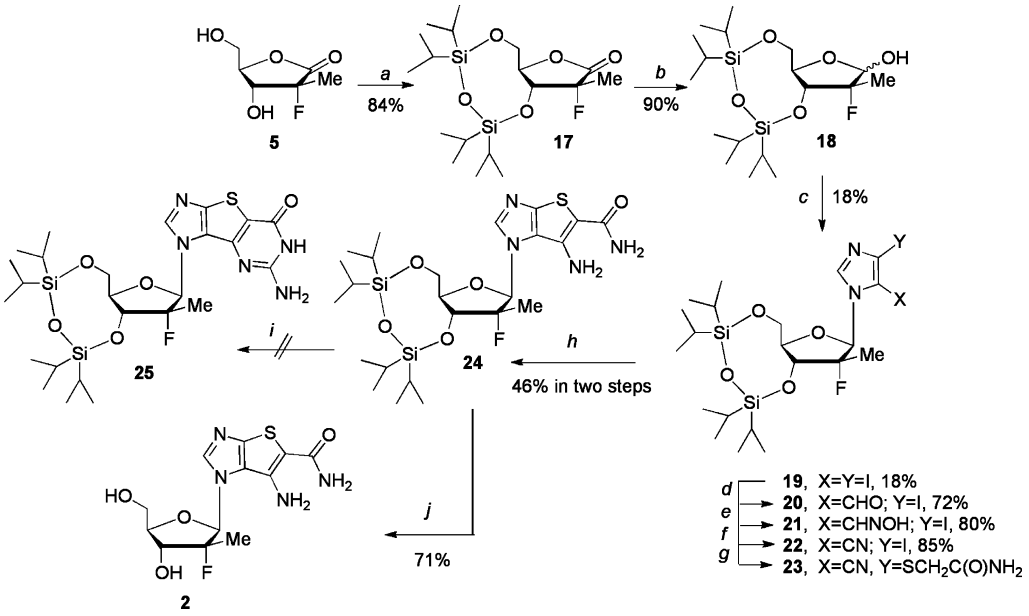
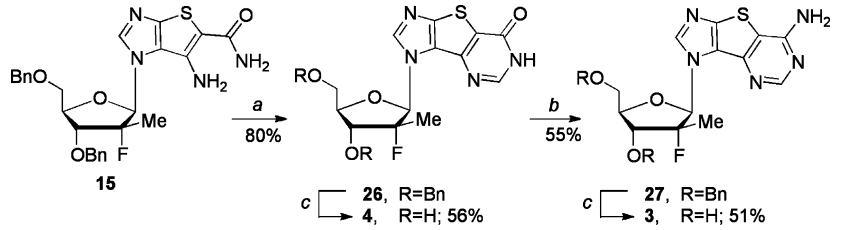
Scheme 2 Synthesis Conditions below[a]



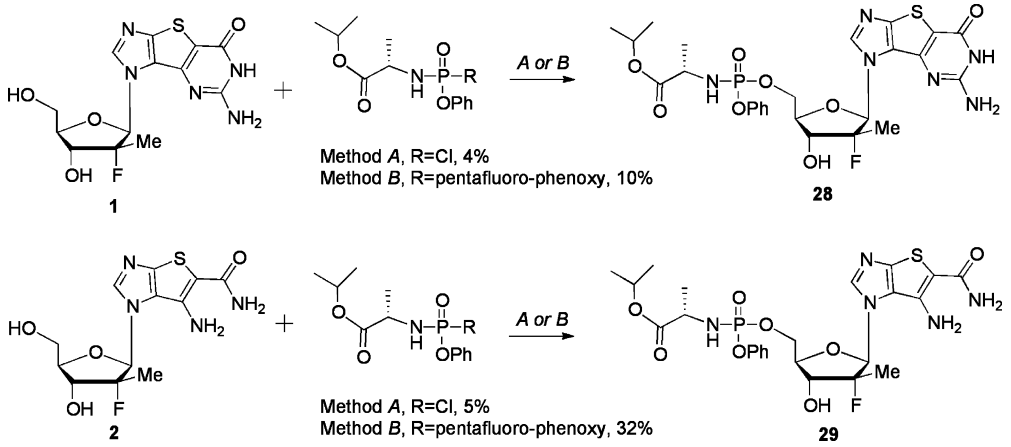
[a]Reagents and conditions: (a) $TIPDSCl_2$, Py, rt, 16 h. (b) $LiAl(OtBu)_3H$, THF, −20 to 10 °C, 3 h. (c) 4,5-Diiodoimidazole, Ph_3P, DIAD, THF, 0 °C to rt, 24 h. (d) (i) EtMgBr, THF, 0 °C, 15 min. (ii) Anhydrous DMF, rt, 16 h. (e) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH, reflux, 5 h. (f) CDI, THF, reflux, 16 h. (g) $NH_2C(O)CH_2SH$, K_2CO_3, DMF, 65 °C, 24 h. (h) EtONa, EtOH, reflux, 2 h. (i) (i) NaOH, CS_2, MeOH, 145 °C, 18 h; (ii) H_2O_2, 0 °C, 2 h; (iii) NH_3/MeOH, 130 °C, 16 h. (j) TBAF, THF, rt, 2 h.

Scheme 3 Synthesis Conditions below[a]



[a]Reagents and conditions: (a) $CH(OEt)_3$, 4Å molecular sieves, reflux, 6 h. (b) (i) TPSCl, DMAP, TEA, MeCN, rt, 3 h; (ii) NH_3, 24 h, rt. (c) $BF_3{\cdot}Et_2O$, EtSH, CH_2CI_2, 72 h.

Scheme 4 Synthesis Conditions below[a]



[a]Reagents and conditions: (A) ^{t}BuMgCl, DMF, −78 °C, 1 h, then rt, 4 h. (B) ^{t}BuMgCl, DMF, 0 °C, 1 h, then rt, overnight.

instead of the previously employed Bn groups.[20] This was necessary because the conditions for removal (unlike for the Bn group) would be amenable to the sensitive amide and amine functionalities present. Interestingly, despite all efforts to the contrary, we were unable to cyclize the bicyclic to the tricyclic ring system of **25** because of the harsh conditions. This was

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unfortunate because it would have also provided **1** following deblocking of the silyl groups.

The tricylic adenosine and inosine analogues were also of interest, and their syntheses proceeded in a similar manner as shown in Scheme 3.

Once the parent compounds were in hand, the corresponding McGuigan ProTides of tricyclic **1**, **28**, and bicyclic **2**, **29**, were sought.[21,22] The two approaches to realize the prodrugs are outlined in Scheme 4 and follow literature procedures.[23−27] Although the yields were disappointingly (but typically) low, enough compound was obtained to allow for limited biological testing.

ANTIVIRAL TESTING

The anti-HCV activity of the target compounds was investigated in Huh 5.2 cells carrying a subgenomic genotype 1b replicon.[28] The 50% effective concentration (EC_{50}) is the compound concentration that inhibits viral RNA replication by 50%. Similarly, we quantified the toxicity of the compound in the same cell line, and the 50% cytotoxic concentration (CC_{50}) is the compound concentration that reduces cell viability by 50%. Both numbers allow the calculation of a selectivity index ($SI = CC_{50}/EC_{50}$) which is a measure of the therapeutic potential of a compound. These values for the different compounds can be found in Table 1.

Table 1. HCV Assay Results for Compounds 1−4, 28, and 29

replicon 1b, con 1 strain, Huh 5−2 cells (μM)			
compound	EC_{50} replicon (μM)[a]	CC_{50} MTS (μM)[b]	SI[c]
1[d]	32	54	1.7
2[e]	42 ± 4	>100	>2.4
3[e]	20 ± 6	161 ± 5	8.1
4[e]	76 ± 28	>200	>2.6
28[e]	110 ± 1	145 ± 0.6	1.3
29[e]	84 ± 16	>100	>1.2

[a]EC_{50}: effective compound concentration that inhibits the viral RNA replication by 50% (luciferase-based assay). [b]CC_{50}: concentration of compound that reduces the overall cellular metabolic activity by 50% (MTS-based assay). [c]SI: selectivity index, CC_{50}/EC_{50} ratio. [d]The data represent the measurement from a single experiment [e]The data represent an average of at least two independent measurements in duplicate ± SD.

Only compounds that produce a significant inhibition of viral replication at concentrations where no antimetabolic effect (cell viability >90%) on the host cell can be observed are selective inhibitors of HCV replication. For most molecules shown in Table 1 the antiviral activity found was low (EC_{50} > 50 μM) and/or not significantly different from the antimetabolic activity (SI < 3). For one of the target compounds, **3**, we found selective antiviral activity with an SI of 8.1. A more detailed look at the dose−response curves (Figure 4) showed that this molecule at 30 μM inhibits viral RNA replication by 70% without a significant antimetabolic effect. At 100 μM we observed 92% inhibition of viral RNA replication but also cell viability was significantly reduced (cell viability 77% as compared to that of an untreated control). Therefore, we conclude that this molecule can inhibit HCV viral RNA replication but only at high concentrations and with limited selectivity. Also, typically the ProTide approach has resulted in a significant increase in activity compared to the parent nucleosides,[23−26] as well as for Sofosbuvir as compared to its parent nucleoside;[3] however, in our case, this did not prove to be the case. Although we have no immediate explanation for this, we speculate that perhaps the tricyclic and bicyclic ring system may somehow be interfering with the cleavage of the prodrug so perhaps the reaction is occurring more slowly, thus leading to the decrease in activity.

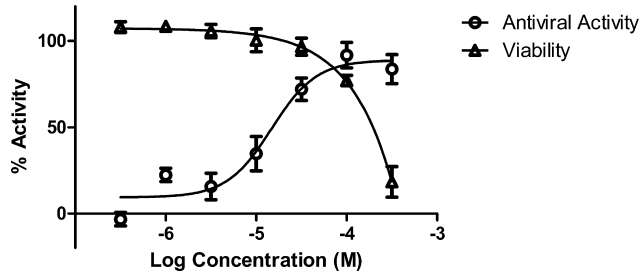

Figure 4. Dose−response curves of the effect of **3** on HCV viral RNA replication and cell viability. Huh 5.2 cells, containing the hepatitis C virus genotype 1b replicon, where grown for 72 h in the presence of different concentrations of **3**. Antiviral activity was then determined by the quantification of lucerferase activity (reporter gene in viral RNA replicon). Cell viability was measured by quantification of the conversion of MTS by the mitochondria of living cells.

DISCUSSION

The nontrivial synthesis of the two structurally unique nucleobase scaffolds combined with Gilead's 2′-Me, 2′-F sugar as well as the corresponding McGuigan ProTides was successful, albeit in very poor overall yield due to the many steps and low yields. Although the compounds' activity against HCV proved not to be superior to that of Sofosbuvir, the activity observed should still be considered promising, particularly for tricyclic analogue **3**. As a result, current efforts are focused on ways to improve this activity, including the pursuit of the diamino, 3-deaza, and other heterocyclic scaffolds. In addition, the triphosphates of these compounds are of interest to help elucidate the mechanism of action, and we will be interested to see if they inhibit the HCV RdRp. Finally, the routes to realize these compounds clearly need optimization if these are to be studied in more detail. The results of those efforts will be reported elsewhere as they are obtained.

EXPERIMENTAL SECTION

All chemicals were obtained from commercial sources and used without further purification unless otherwise noted. Anhydrous DMF, MeOH, DMSO, and EtOH were purchased from Fisher Scientific. Anhydrous THF, acetone, CH_2Cl_2, CH_3CN, and ether were obtained using a solvent purification system (mBraun Labmaster 130). NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). Purification was performed on a Teledyne Isco CombiFlash Rf 200. All ^1H, ^{13}C, ^{19}F, and NMR spectra were obtained either on a JEOL ECX 400 MHz NMR, operated at 400 and 100 MHz, respectively, or on a Bruker AVANCE III HD 500 MHz NMR operated at 500 and 125 MHz, respectively, and referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman Diamond silica gel 60-F_{254} precoated plates. Column

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chromatography was performed using silica gel (63−200 μ) from Dynamic Adsorbtions Inc. (Norcross, GA) and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials. Mass spectra were recorded at the Johns Hopkins Mass Spectrometry Facility.

Synthesis of 3,5-Dibenzoyloxy-2-deoxy-2-fluoro-2-*C*-methyl-D-ribono-γ-lactone (6). To a stirred solution of diol **5** (31.5 g, 0.192 mol) in anhydrous Py (400 mL) at 0 °C under nitrogen was added BzCl (108 g, 0.768 mol) slowly. The resulting mixture was allowed to warm to room temperature and was stirred for 30 min. Then cold water (120 mL) was added, and the mixture was stirred for 5 min to form a suspension. The precipitated product was collected by filtration. The filter cake was suspended in cold water (400 mL), and the solid was collected. This was repeated three times, followed by Combi-Flash chromatography (PE/EtOAc = 100:1−1:2) to give **6** as a white solid (57.2 g, 80%); R_f = 0.5, (PE/EtOAc = 4:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.66 (d, 3H, J = 24.3 Hz, CH_3), 4.60−4.72 (m, 2H, H-5, 5′), 5.10−5.14 (m, 1H, H-4), 5.74 (dd, 1H, J = 6.8, 18.3 Hz, H-3), 7.43−7.56 (m, 4H, Ar), 7.60−7.71 (m, 2H, Ar), 7.91−7.93 (m, 2H, Ar), 7.99−8.01 (m, 2H, Ar). These results agree with the literature.[18]

Synthesis of 3,5-Dibenzoyloxy-2-deoxy-2-fluoro-2-*C*-methyl-D-ribono-γ-lactol (7). To a stirred solution of protected lactone **6** (54.0 g, 0.145 mol) in anhydrous THF (600 mL) was added lithium tri-*tert*-butoxyaluminum hydride (42.5 g, 0.167 mol) in several batches at −20 °C under nitrogen and then slowly warmed to room temperature. After 6 h, the reaction was complete on the basis of TLC analysis. Cold saturated NH_4Cl was added slowly to quench the reaction. The solvent was removed, and the residue was purified by silica gel column chromatography to give compound **7** (46.5 g, 85%) as a white solid. R_f = 0.45, (PE/EtOAc = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52−1.60 (m, 3H, Me), 3.56−3.58 (d, 1H, OH), 4.43−4.69 (m, 3H, H-5, H-5′ and H-4), 5.27−5.35 (m, 1H, H-3), 5.37−5.68 (m, 1H, H-1), 7.33−7.62 (m, 6H, Ar), 7.97−8.01 (m, 4H, Ar).

Synthesis of 2′-Deoxy-2′-fluoro-2′-*C*-methyl-1′-(4,5-diiodoimidazol-3-yl)-α/β-D-ribofuranose (9). To a stirred solution of triphenylphosphine (40.0 g, 0.153 mol), diiodoimidazole (45.1 g, 0.141 mol), and compound **7** (44.0 g, 0.118 mol) in anhydrous THF (4.0 L) at 0 °C was added DIAD (30.9 g, 0.153 mol) dropwise. The resulting mixture was stirred at this temperature for 30 min, and then the reaction mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (PE/EtOAc = 3:1) to give mixture product **8** (53.0 g) as an off-white crude foam. The above mixture, **8** (53.0 g, 78.4 mmol), was dissolved in reagent methanol (1.2 L), and MeONa (8.70 g, 0.161 mol) was added in five batches at 0 °C for 15 min. The resulting solution was stirred at this temperature for 45 min, and then HOAc was added to neutralize the solution. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (DCM/MeOH = 50:1−10:1) twice to give a mixture of two isomers **9** (α/β = 2.2:1). α isomer (20.1 g, 37%) as an off-white foam: R_f = 0.24 (DCM/MeOH = 20:1). ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.47 (d, 3H, J = 22.0 Hz, Me), 3.71 (dd, 1H, J = 3.6 Hz, J = 12.4 Hz, H-5), 3.89 (dd, 1H, J = 1.8 Hz, J = 12.4 Hz, H-5′), 4.14−4.28 (m, 2H, H-3 and H-4), 5.93 (d, 1H, J = 19.2 Hz, H-1), 7.94 (d, 1H, J = 3.7 Hz, Ar). ^{13}C NMR (100 MHz, MeOH-d_4) δ 20.5 (d, J = 24.8 Hz) 64.2, 76.8 (d, J = 18.1 Hz), 87.0, 95.8, 98.5, 101.7 (d, J = 189.7 Hz), 102.6, 146.2. β isomer (9.1 g, 17%) as an off-white solid: R_f = 0.25 (DCM/MeOH = 20:1). ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.15 (d, 3H, J = 22.0 Hz, Me), 3.79 (dd, 1H, J = 1.8 Hz, J = 12.4 Hz, H-5), 3.96−4.03 (m, 2H, H-5′ and H-4), 4.13 (dd, 1H, J = 9.2 Hz, J = 23.8 Hz, H-3), 5.90 (d, 1H, J = 17.0 Hz, H-1), 8.38 (d, 1H, Ar). ^{13}C NMR (100 MHz, MeOH-d_4) δ 16.2 (d, J = 24.8 Hz), 58.6, 70.4 (d, J = 17.2 Hz), 82.0, 93.7, 94.1, 95.3, 100.7 (d, J = 181.2 Hz), 140.7.

Synthesis of 3′,5′-Dibenzyloxy-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-(4,5-diiodoimidazol-3-yl)-β-D-ribofuranose (10). To a solution of **9** (2.50 g, 5.34 mmol) in anhydrous DMF (60 mL) at 0 °C was added a suspension of NaH (95%, 322 mg, 13.4 mmol) in anhydrous DMF (20 mL) dropwise over a period of 10 min. The resulting mixture was stirred at 0 °C for 2 h, and then BnBr (2.53 mL, 21.3 mmol) and TBAI (197 mg, 0.533 mmol) were added slowly. The mixture was stirred at room temperature for an additional 4 h. The reaction mixture was quenched with cold water and neutralized with HOAc at 0 °C. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (PE/EtOAc = 5:1) to give **10** (2.82 g, 81%) as an off-white syrup. R_f = 0.50, (PE/EtOAc = 5:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.12 (d, 3H, J = 22.2 Hz, Me), 3.63 (dd, 1H, J = 2.0, 13.4 Hz, H-5), 3.93 (dd, 1H, J = 1.8, 11.4 Hz, H-5′), 4.13−4.26 (m, 2H, H-3 and H-4), 4.52−4.66 (m, 4H, CH_2), 5.86 (d, 1H, J = 16.4 Hz, H-1), 7.29−7.42 (m, 10H, Ar), 8.25 (s, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9 (d, J = 24.9 Hz), 66.8, 73.6, 74.3, 76.9, 79.7, 80.7, 93.7 (d, J = 38.1 Hz), 97.3, 101.7 (d, J = 184.0 Hz), 127.8, 128.3 (m), 137.2, 140.5. HRMS [FAB] calculated for $C_{23}H_{23}FI_2N_2O_3$ [M + H$^+$], 648.9860; found, 648.9863.

Synthesis of 3′,5′-Dibenzyloxy-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-[(5-iodo-4-carbaldehyde)-imidazole-3-yl]-β-D-ribofuranose (11). Compound **10** (1.60 g, 2.47 mmol) was dissolved in anhydrous THF (50 mL) at 0 °C, and then EtMgBr (1 M in THF, 2.72 mL, 2.72 mmol) was added dropwise under nitrogen. The reaction mixture was stirred at 0 °C for 30 min. Anhydrous DMF (2 mL) was added, and the reaction mixture was stirred overnight and quenched with water. The solvent was removed, and the residue was purified by silica gel column chromatography (PE/EtOAc = 2:1) to give **11** (1.10 g, 81%) as a colorless syrup. R_f = 0.45, (PE/EtOAc = 5:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (d, 3H, J = 22.0 Hz, Me), 3.59 (dd, 1H, J = 1.8, 13.3 Hz, H-5), 3.93 (dd, 1H, J = 1.8, 13.3 Hz, H-5′), 4.06−4.41 (m, 2H, H-3 and H-4), 4.47−4.61 (m, 4H, CH_2), 6.66 (d, 1H, J = 15.6 Hz, H-1), 7.29−7.43 (m, 10H, Ar), 8.62 (s, 1H, Ar), 9.61 (s, 1H, CHO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.6 (d, J = 24.9 Hz), 66.8, 73.6, 74.3, 79.7, 89.7 (d, J = 39.3 Hz), 99.4, 101.7 (d, J = 93.9 Hz), 126.7, 127.8, 128.3 (m), 137.1 (d, J = 17.2 Hz), 143.1, 181.3. MS [ESI, pos] m/z: 551.1 [M + H$^+$].

Synthesis of 3′,5′-Dibenzyloxy-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-[(5-iodo-4-carbonitrile)-imidazole-3-yl]-β-D-ribofuranose (13). To a stirred solution of **11** (1.10 g, 2.00 mmol) in anhydrous EtOH (30 mL) was added hydroxylamine hydrochloride (556 mg, 8.00 mmol) and $NaHCO_3$ (672 mg, 8.00 mmol). The resulting mixture was refluxed for 5 h. The solvent was removed under reduced pressure, and the crude product was extracted in EtOAc (2 × 25 mL) and excess water. The combined organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to give crude oxime intermediate **12** as a syrup (1.20 g), which was then used

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for the next step without further purification. Oxime intermediate **12** (1.20 g) was dissolved in anhydrous THF (25 mL) and 1, 1′-carbonyldiimidazole (973 mg, 6.0 mmol) was added to the mixture. The resulting solution was stirred under reflux for 8 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (PE/EtOAc = 8:1) to provide cyano nucleoside **13** as a light-yellow syrup (820 mg, 75% in two steps). R_f = 0.75 (PE/EtOAc = 3:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 (d, 3H, J = 22.0 Hz, Me), 3.60 (dd, 1H J = 2.3, 11.5 Hz, H-5), 3.91 (dd, 1H, J = 1.8, 11.4 Hz, H-5′), 4.07−4.15 (m, 1H, H-4), 4.26−4.28 (m, 1H, H-3), 4.47−4.63 (m, 4H, CH_2), 5.83 (d, 1H, J = 14.7 Hz, H-1), 7.21−7.39 (m, 10H, Ar), 8.25 (s, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.0 (d, J = 24.8 Hz), 66.8, 73.7, 74.3, 80.5, 91.2 (d, J = 38.1 Hz), 96.5, 100.0 (d, J = 189.9 Hz), 110.6, 128.3, 128.4, 128.5, 128.6, 128.8, 128.9, 136.9, 140.7. HRMS [FAB] calculated for $C_{24}H_{23}FIN_3O_3$ [M + H^+], 548.0846; found, 548.0850.

Synthesis of 3′,5′-Dibenzyloxy-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-[(5-carboxamide)[2,3-*d*]imidazole-3-yl]-β-D-ribofuranose (15). To a solution of **13** (3.00 g, 5.48 mmol) in anhydrous DMF (600 mL) was added thioglycolamide (3.00 g, 32.9 mmol) and K_2CO_3 (3.80 g, 27.5 mmol). The resulting mixture was held at 65 °C for 24 h under nitrogen. Crude mixture **14** was filtered over a pad of Celite, and the solid was washed with DMF. The solvent was removed under reduced pressure to give a crude mixture, which was then refluxed in a NaOEt (21%, 12 mL and anhydrous EtOH, 200 mL) mixture for 2 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (DCM/MeOH = 50:1−10:1) to afford compound **15** (1.46 g, 52%) as a yellow foam. R_f = 0.50, (DCM/MeOH = 15:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 (d, 3H, J = 22.9 Hz, Me), 3.63 (d, 1H, J = 11 Hz, H-5), 3.90 (d, 1H, J = 11.0 Hz, H-5′), 4.09−4.12 (m, 2H, H-3 and H-4), 4.43−4.58 (m, 3H, CH_2), 4.76 (d, 1H, J = 11.9 Hz, CH_2), 5.26 (s, 2H, NH_2), 5.89 (d, 1H, J = 18.3 Hz, H-1), 6.45 (s, 2H, $CONH_2$), 7.25−7.41 (m, 10H, Ar), 7.84 (s, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.6 (d, J = 25.7 Hz), 66.1, 73.7, 77.9(d, J = 15.3 Hz), 79.8, 93.0 (d, J = 40.0 Hz), 97.9 (d, J = 189.7 Hz), 99.0, 128.2, 128.3, 128.5(m), 128.7, 128.9, 136.6, 137.1, 139.4, 142.8, 146.0, 168.2. HRMS (FAB) calculated for $C_{26}H_{27}FN_4O_4S$ [M + H^+], 511.1815; found, 511.1796.

Synthesis of 3′,5′-Dibenzyloxy-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-[(2-amino-imidazo-[4′,5′:4,5]-thieno-[3,2-*d*]-pyrimidin-3-yl-7-one)]-β-D-ribofuranose (16). To a stirred solution of **15** (1.50 g, 2.94 mmol) in anhydrous MeOH (45 mL) was added NaOH (588 mg, 14.7 mmol). The resulting mixture was stirred at room temperature until the mixture was homogeneous. Carbon disulfide (1.34 g, 17.6 mmol) was added, and the resulting mixture was held in a steel bomb for 18 h at 150 °C. The mixture was cooled to 0 °C. H_2O_2 (8 mL, 30%) was then added dropwise and allowed to stir at 0 °C for 2 h. The resulting suspension was added to a steel bomb, and anhydrous ammonia was bubbled in at −40 °C for 20 min. The bomb was then held at 130 °C for 12 h. The solvent was removed under reduced pressure, and the crude yellow residue was purified by silica gel column chromatography to afford **15** (787 mg, 50%) as a yellow foam. R_f = 0.35, (DCM/MeOH = 15:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (d, 3H, J = 22.7 Hz, Me), 3.75 (dd, 1 H, J = 2.7, 9.2 Hz, H-5), 3.95 (dd, 1 H, J = 1.6, 9.3 Hz, H-5′), 4.21 (d, 1H, J = 7.4 Hz, H-4), 4.47 (dd, 1 H, J = 11.9, 19.4 Hz, H-3), 4.58 (dd, 2 H, J = 9.5, 25.4 Hz, CH_2), 4.72 (dd, 2 H, J = 12.8, 22.1 Hz, CH_2), 6.45 (d, 1H, J = 13.4 Hz, H-1), 6.59 (s, 2H, NH_2), 8.43 (s, 1H, Ar), 7.32−7.39 (m, 10H, Ar), 11.07 (s, 1H, NH). HRMS (FAB) calculated for $C_{27}H_{26}FN_5O_4S$ [M + H^+] 536.1768; found, 536.1767.

Synthesis of 2′-Deoxy-2′-fluoro-2′-*C*-methyl-1′-[(2-amino-imidazo-[4′,5′:4,5]-thieno-[3,2-*d*]-pyrimidin-3-yl-7-one)]-β-D-ribofuranose (1). To a solution of **16** (150 mg, 0.280 mmol) in anhydrous DCM (30 mL) was added EtSH (622 mL, 8.40 mmol) and $BF_3{\cdot}Et_2O$ (48%, 2.22 mL, 8.40 mmol) at 0 °C. The reaction was allowed to proceed for 72 h at room temperature before TLC analysis confirmed complete product formation. The solvent was removed under reduced pressure, and the residue was purified by Combi-flash silica column chromatography ($CHCl_3$/MeOH =100:1−1:1) to afford target nucleoside **1** (80.0 mg, 80%) as a white powder. R_f = 0.15, (DCM/MeOH = 5:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.11 (d, 3H, J = 22.6 Hz, Me), 3.72 (d, 1H, J = 11.9 Hz, H-5), 3.90−3.96 (m, 2H, H-5′ and H-4), 4.20−4.25 (m, 1H, H-3), 5.43 (br, 2H, OH), 6.45 (d, 1H, J = 16.3 Hz, H-1), 6.59 (s, 2H, NH_2), 8.62 (s, 1H, Ar), 11.08 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.7 (d, J = 24.9 Hz), 59.3, 70.3 (d, J = 17.2 Hz), 82.6, 90.0 (d, J = 39.3 Hz), 101.7 (d, J = 180.1 Hz), 110.3, 128.0, 143.0, 145.9, 148.4, 155.4, 158.9. HRMS (FAB) calculated for $C_{13}H_{14}FN_5O_4S$ [M + H^+], 356.0829; found, 356.0826.

Synthesis of 3,5-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2-deoxy-2-fluoro-2-*C*-methyl-D-ribono-γ-lactone (17). To a stirred solution of diol **5** (620 mg, 3.78 mmol) in anhydrous Py (15 mL) was added $TIPDSCl_2$ (1.31 g, 4.15 mmol) slowly at 0 °C under nitrogen. The mixture was then stirred at room temperature for 16 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (hexanes/EtOAc = 20:1−5:1) to give **16** as a colorless syrup (1.29 g, 84%). R_f = 0.55, (hexanes/EtOAc = 15:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03−1.10 (m, 28H), 1.60 (d, 3H, J = 22.9 Hz), 4.06−4.21 (m, 3H), 4.37−4.40 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 12.7, 13.0, 13.8, 17.0−17.4(m), 59.1, 72.5 (d, J = 17.2 Hz), 81.2, 92.5 (d, J = 184.0 Hz), 169.8 (d, J = 20.0 Hz). MS (ESI, pos) m/z: 429.1 (M + Na^+).

Synthesis of 3,5-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2-deoxy-2-fluoro-2-*C*-methyl-D-ribono-γ-lactol (18). To a stirred solution of protected lactone **17** (1.30 g, 3.20 mmol) in anhydrous THF (30 mL) was added lithium tri-*tert*-butoxyaluminum hydride (980 mg, 3.85 mmol) at −20 °C under nitrogen, and then the solution was warmed to 10 °C. After 3 h, the mixture was quenched with saturated NH_4Cl solution. THF was removed under reduced pressure, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with water and brine and dried over Na_2SO_4. The organic layer was removed under reduced pressure, and the residue was purified by silica gel column chromatography (hexanes/EtOAc = 10:1−5:1) to give **18** as a white solid (1.15 g, 90%). R_f = 0.7 (hexanes/EtOAc = 8:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04−1.10 (m, 28H, $CH(CH_3)$), 1.44−1.51 (m, 3H, Me), 3.56−3.61 (m, 1H, OH), 3.94−4.05 (m, 3H, H-5, H-5′, and H-4), 4.16−4.25 (m, 1H, H-3), 5.07−5.20 (m, 1H, H-1). MS (ESI, pos) m/z: 431.1 (M + Na^+).

Synthesis of 3′,5′-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-(4,5-diiodoimidazol-3-yl]-α/β-D-ribofuranose (19). To a mixture of diiodoimidazole (646 mg, 2.02 mmol), triphenylphosphine

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(530 mg, 2.02 mmol), and lactol **18** (550 mg, 1.35 mmol) in THF (150 mL) was added DIAD (408 mg, 2.02 mmol) dropwise at 0 °C. The resulting mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (hexanes/EtOAc = 20:1−1:1) to give a mixture of two isomers **19**. α isomer (516 mg, 54%) as a colorless syrup: R_f = 0.60 (PE/EtOAc = 10:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.05−1.09 (m, 28H), 1.51 (d, 3H, J = 21.4 Hz), 4.00−4.10 (m, 2H), 4.24−4.30 (m, 2H), 5.76 (d, 1H, J = 18.8 Hz), 7.79 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7, 12.8, 13.1, 13.8, 17.0, 17.1, 17.2, 17.3, 17.3, 17.4, 18.2 (d, J = 25.7 Hz), 60.4, 73.7 (d, J = 17.2 Hz), 80.9, 82.4, 91.1 (d, J = 14.3 Hz), 96.4, 97.3 (d, J = 195.5 Hz), 141.8 (d, J = 7.6 Hz). HRMS (FAB) calculated for $C_{21}H_{37}FI_2N_2O_4Si_2$ [M + H$^+$], 711.0444; found, 711.0437. β isomer (172 mg, 18%) as colorless syrup: R_f = 0.80 (PE/EtOAc = 10:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03−1.14 (m, 28H), 1.24 (d, 3H, J = 22.0 Hz), 4.02−4.10 (m, 2H), 4.15−4.28 (m, 2H), 5.84 (d, 1H, J = 16.8 Hz), 8.01 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 13.0, 13.1, 13.8, 17.0(m), 17.4, 17.5, 17.6, 17.7, 59.5, 71.0 (d, J = 17.2 Hz), 80.3, 80.9, 93.3 (d, J = 38.1 Hz), 97.4, 100.3 (d, J = 185.9 Hz), 139.9. HRMS (FAB) calculated for $C_{21}H_{37}FI_2N_2O_4Si_2$ [M + H$^+$], 711.0444; found, 711.0439.

Synthesis of 3′,5′-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-[(5-iodo-4-carbaldehyde)-imidazole-3-yl]-β-D-ribofuranose (20). To a stirred solution of **19** (1.60 g, 2.25 mmol) in anhydrous THF (40 mL) at 0 °C was added EtMgBr (900 μL, 2.70 mmol, 3 M in THF) dropwise under nitrogen. The reaction mixture was stirred for 0.5 h at 0 °C. Anhydrous DMF (2 mL) was added, and the reaction mixture was stirred overnight, quenched with water, and extracted with EtOAc (50 mL × 2). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed, and the residue was purified by silica gel column chromatography (PE/EtOAc = 10:1) to give **20** (993 mg, 72%) as a colorless syrup. R_f = 0.60 (PE/EtOAc = 9:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 1.01−1.24 (m, 31H), 4.12−4.34 (m, 4H), 6.59 (d, 1H, J = 15.6 Hz), 8.30 (s, 1H), 9.65 (d, 1H, J = 0.9 Hz). ^{13}C NMR (100 MHz, acetone-d_6) δ 12.6, 12.8, 12.9, 13.5, 15.6, 15.9, 16.5, 16.6, 16.7, 16.8, 16.9, 17.0, 17.1, 60.0, 71.0 (d, J = 17.2 Hz), 80.9, 89.4 (d, J = 38.1 Hz), 100.4 (d, J = 185.9 Hz), 102.1, 129.5, 141.9, 181.2. HRMS (FAB) calculated for $C_{22}H_{38}FIN_2O_5Si_2$ [M + H$^+$], 613.1426; found, 613.1442.

Synthesis of 3′,5′-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-[(5-iodo-4-carbonitrile)-imidazole-3-yl]-β-D-ribofuranose (22). To a stirred mixture of nucleoside **20** (900 mg, 1.47 mmol) in anhydrous EtOH (40 mL) were added hydroxylamine hydrochloride (511 mg, 7.35 mmol) and $NaHCO_3$ (617 mg, 7.35 mmol). The resulting mixture was refluxed for 5 h. The solvent was removed under reduced pressure, and the crude product was extracted in EtOAc (2 × 30 mL) and excess water. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give crude oxime intermediate **21** as a syrup (1.10 g). This was dissolved in anhydrous THF (30 mL), and 1,1′-carbonyldiimidazole (574 mg, 3.54 mmol) was added to the mixture. The resulting solution was stirred under reflux for 16 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (PE/EtOAc = 8:1) to provide cyano nucleoside **22** as a yellow syrup (625 mg, 70% in two steps). R_f = 0.60 (PE/EtOAc = 10:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01−1.10 (m, 28H), 1.29 (d, 3H, J = 22.0 Hz), 4.01−4.05 (m, 1H), 4.10−4.19 (m, 2H), 4.25−4.28 (m, 1H), 5.84 (d, 1H, J = 14.6 Hz), 8.04 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 12.9, 13.0, 13.8, 16.5, 16.7, 16.9 (m), 17.1, 17.3, 17.4, 17.5, 59.6, 70.6 (d, J = 17.2 Hz), 81.6, 90.8 (d, J = 37.2 Hz), 96.7, 99.9 (d, J = 186.8 Hz), 110.6 (d, J = 16.2 Hz), 140.0. HRMS (FAB) calculated for $C_{22}H_{37}FIN_3O_4Si_2$ [M + H$^+$], 610.1430; found, 610.1435.

Synthesis of 3′,5′-(1,1,3, 3-Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-[(5-carboxamide)-[2,3-*d*]-imidazole-3-yl]-β-D-ribofuranose (24). To a stirred mixture of **22** (500 mg, 0.820 mmol) and thioglycolamide (299 mg, 3.28 mmol) in anhydrous DMF (100 mL) was added anhydrous K_2CO_3 (453 mg, 3.28 mmol). The reaction was held at 65 °C for 24 h under nitrogen. The crude mixture was filtered over a pad of Celite, and the solid was washed with a little DMF. The solvent was removed under reduced pressure to give crude mixture **23**, which was refluxed in a EtONa (21%, 500 μL) and EtOH (20 mL) mixture for 2 h before the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (PE/EtOAc = 1:2) to give intermediate **24** as a yellow syrup (215 mg, 46% in two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01−1.17 (m, 31H, Me and $CH(Me)_2$), 3.98−4.10 (m, 2H, H-5 and H-5′), 4.26−4.35 (m, 2H, H-4 and H-3), 5.43 (s, 2H, NH_2), 5.94 (d, 1H, J = 21.0 Hz, H-1), 6.35 (s, 2H, $CONH_2$), 7.93 (s, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 13.0, 13.6, 13.7, 17.0, 17.1, 17.2, 17.3, 17.5, 17.7, 58.9, 71.3 (d, J = 16.2 Hz), 80.6, 93.2 (d, J = 40.0 Hz), 98.9 (d, J = 187.8 Hz), 100.1, 126.4, 139.1, 142.5, 146.0, 168.3. MS (ESI, pos) m/z: 573.3 (M + H$^+$), 595.3 (M + Na$^+$).

Synthesis of 2′-Deoxy-2′-fluoro-2′-*C*-methyl-1′-[(5-carboxamide)-[2,3-*d*]-imidazole-3-yl]-β-D-ribofuranose (2). To a solution of compound **24** (240 mg, 0.419 mmol) in anhydrous THF (5 mL) was added TBAF (1 M in THF, 4.20 mL, 4.20 mmol). The reaction mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (DCM/MeOH = 30:1−10:1) to give **2** (99 mg, 71%) as a colorless powder. R_f = 0.15, (DCM/MeOH = 25:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96 (d, 3H, J = 22.4 Hz, Me), 3.69−3.71 (m, 1H, H-5), 3.86−3.88 (m, 2H, H-5′ and H-4), 4.06−4.08 (m, 1H, H-3), 5.44−5.43 (m, 1H, OH), 5.68 (d, 1H, J = 6.9 Hz, H-1, OH), 6.42 (d, 1H, J = 17.4 Hz, H-1), 6.72 (s, 2H, NH_2), 6.92 (s, 2H, $CONH_2$), 8.44 (s, 1H, Ar). ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.1 (d, J = 24.8 Hz), 58.6, 70.2 (d, J = 17.2 Hz), 82.0, 90.0 (d, J = 39.3 Hz), 100.8, 100.9 (d, J = 182.1 Hz), 126.9, 139.0, 143.4, 144.5, 168.2. HRMS (FAB) calculated for $C_{12}H_{15}FN_4O_4S$ [M + H$^+$], 331.0876; found, 331.0863.

Synthesis of 3′,5′-Dibenzyloxy-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-(imidazo[4′,5′:4,5]-thieno-[3,2-*d*]-pyrimidin-3-yl-7-one]-β-D-ribofuranose (26). A mixture of **15** (200 mg, 0.39 mmol), triethylorthoformate (25 mL), and 4 Å molecular sieves (oven-dried before use) was refluxed for 6 h. The reaction mixture was filtered, the excess solvent was evaporated, and the residue was purified by silica gel column chromatography (DCM/MeOH, 50:1−20:1) to afford compound **26** (164 mg, 80.4%) as a hygroscopic white foam. R_f = 0.40 (DCM/MeOH = 20:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (d, 3H, J = 22.0 Hz, Me), 3.66−3.69 (m, 1H, H-5), 3.98−4.01 (m, 1H, H-5′), 4.27−4.34(m, 2H, H-2 and H-3), 4.51−

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4.68 (m, 4H, CH_2), 6.55 (d, 1H, J = 15.6 Hz, H-1), 7.26−7.43 (m, 11H, Ar), 8.16 (s, 1H, Ar), 8.59 (s, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.0 (d, J = 24.8 Hz), 67.4, 73.7, 74.3, 76.8, 80.0, 90.5 (d, J = 38.1 Hz), 100.6 (d, J = 183.1 Hz), 122.1, 128.7 (m), 137.2, 143.6, 145.3, 150.9, 160.4. HRMS calculated for $C_{27}H_{25}FN_4O_4S$ [M + H^+], 520.1580; found, 520.1662.

Synthesis of 3′,5′-Dibenzyloxy-2′-deoxy-2′-fluoro-2′-*C*-methyl-1′-[(7-aminoimidazo-[4′,5′:4,5]-thieno-[3,2-*d*]-pyrimidin-3-yl]-1-β-D-ribofuranose (27). To a mixture of **26** (150 mg, 0.29 mmol), DMAP (141 mg, 1.15 mmol), and TEA (2 mL) in MeCN (6 mL) was added TPSCl (349 mg, 1.15 mmol) portionwise over 5 min. The mixture was stirred at room temperature for 3 h, and the solvent was removed under reduced pressure. THF (5 mL) was added, the resulting mixture was transferred to a bomb and cooled to −78 °C, and ammonia was bubbled in for 10 min. The bomb was sealed and left to stir overnight at room temperature. Then the solvent was removed and the residue was purified by silica gel column chromatography (DCM/MeOH = 25:1) to afford compound **27** (82 mg, 55%) as a yellow foam. R_f = 0.35 (DCM/MeOH = 20:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 (d, 3H, J = 22.0 Hz), 3.67−3.70 (m, 1H), 3.97−4.00 (m, 1H), 4.33−4.40 (m, 2H), 4.51−4.68 (m, 4H), 5.32 (s, 2H), 6.63 (d, 1H, J = 15.6 Hz), 7.26−7.42 (m, 10H), 8.53 (s, 1H), 8.57 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.0 (d, J = 24.8 Hz), 67.4, 73.7, 74.3, 79.9, 90.7 (d, J = 38.1 Hz), 100.6 (d, J = 183.1 Hz), 114.0, 114.4, 127.9, 128.2, 128.2, 128.4, 128.5, 128.7, 128.8, 137.3, 142.9, 146.4, 148.5, 154.7, 158.0. MS (ESI, pos) m/z: 520.1 (M + H^+).

Synthesis of 2′-Deoxy-2′-fluoro-2′-*C*-methyl-1′-[(7-aminoimidazo-[4′,5′:4,5]-thieno-[3,2-*d*]-pyrimidin-3-yl]-1-β-D-ribofuranose (3). To a solution of **27** (150 mg, 0.29 mmol) in anhydrous DCM (8 mL) was added EtSH (0.6 mL, 8.1 mmol) and BF_3·OEt_2 (2.1 mL, 8.0 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 72 h. The solvent was removed, and the residue was purified by silica gel column chromatography (DCM/MeOH = 25:1) to afford compound **3** (50 mg, 51%) as a white powder. R_f = 0.15, (DCM: MeOH = 5:1). ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.14 (d, 3H, J = 22.5 Hz), 3.90 (dd, 1H, J = 2.8 Hz, J = 13.3 Hz, H-5), 4.08−4.11 (m, 2H, H-5′ and H-4), 4.35 (dd, 2H, J = 9.2 Hz, J = 23.4 Hz, H-3), 6.63 (d, 1H, J = 16.0 Hz, H-1), 8.43 (s, 1H, Ar). ^{13}C NMR (100 MHz, MeOH-d_4) δ 59.0, 15.4 (d, J = 24.8 Hz), 82.3, 70.4 (d, J = 17.2 Hz), 91.0 (d, J = 39.1 Hz), 100.7 (d, J = 180.2 Hz), 113.9, 119.2, 134.5, 145.4, 148.1, 154.3, 159.0. ^{19}F NMR (376 MHz, MeOH-d_4) δ −155.0. HRMS (FAB) calculated for $C_{13}H_{14}FN_5O_3S$ [M + H^+], 340.0880; found, 340.0877.

Synthesis of 2′-Deoxy-2′-fluoro-2′-*C*-methyl-1′-(imidazo-[4′,5′:4,5]-thieno-[3,2-*d*]-pyrimidin-3-yl-7-one]-β-D-ribofuranose (4). To a solution of **26** (120 mg, 0.23 mmol) in anhydrous DCM (8 mL) was added EtSH (0.34 mL, 4.6 mmol) and BF_3·OEt_2 (1.2 mL, 4.6 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred for 72 h. The solvent was removed, and the residue was purified by silica gel column chromatography (DCM/MeOH = 5:1) to afford compound **4** (44 mg, 56%) as an off-white powder. R_f = 0.20 (DCM/MeOH = 5:1). ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.14 (d, 3H, J = 22.5 Hz, Ar), 3.87−3.92 (m, 1H, H-5), 4.11−4.19 (m, 2H, H-5′ and H-4), 4.31−4.39 (m, 1H, H-3), 6.62 (d, 1H, J = 16.0 Hz, H-1), 6.76 (s, 1H, Ar), 8.34 (s, 1H, Ar). ^{13}C NMR (100 MHz, MeOH-d_4) δ 14.9 (d, J = 24.8 Hz), 58.5, 69.5 (d, J = 18.1 Hz), 83.2, 91.7 (d, J = 42.0 Hz), 100.8 (d, J = 187.8 Hz), 114.1, 124.6, 132.8, 143.8, 148.1, 152.2, 158.2. ^{19}F NMR (376 MHz, MeOH-d_4) δ −156.2. HRMS (FAB) calculated for $C_{13}H_{13}FN_4O_4S$ [M + H^+], 341.0720; found, 341.0717.

General Procedure for the Preparation of Compounds 28 and 29. *Method A.* To a stirred solution of free nucleoside **1** and **2** (10−20 mg, 1 equiv) in anhydrous DMF (2−4 mL) was added *tert*-butyl magnesium chloride (1.20 equiv, 1 M solution in THF) slowly at −78 °C. After the completion of the addition, the mixture was stirred at room temperature for 30 min. To the above mixture was added freshly prepared phosphorus reagent *N*-(chlorophenoxyphosphinyl)-L-alanine-1-methylethyl ester (1.20 equiv, 1 M solution in THF) dropwise at −78 °C, and the resulting mixture was then slowly allowed to warm to room temperature for 4 h. The mixture was quenched with cold water, and the aqueous phase was extracted with EtOAc (20 mL × 3). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The residue was purified by Combi-flash silica gel column chromatography (DCM/MeOH = 100:1−10:1) twice to give target nucleoside prodrugs **28** and **29**, respectively.

Method B. To a stirred solution of free nucleoside **1** and **2** (10−20 mg, 1.00 equiv) in anhydrous DMF (3−6 mL) was added *tert*-butyl magnesium chloride (1.50 equiv, 1 M solution in THF) slowly at 0 °C. After the completion of the addition, the mixture was stirred at 0 °C for 1 h. To the above mixture was added freshly prepared phosphorus reagent (*S*)-2-[(2,3,4,5,6-pentafluoro-phenoxy)-phenoxy-phosphorylamino] propionic acid isopropyl ester (1.15 equiv, 1 M solution in THF) dropwise, and the resulting mixture was stirred at 0 °C for 1 h and then slowly allowed to warm to room temperature overnight. The mixture was quenched with cold water, and the aqueous phase was extracted with EtOAc (20 mL × 3). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The residue was purified by Combi-Flash silica gel column chromatography (DCM/MeOH = 100:1−10:1) twice to give target nucleoside prodrugs **28** and **29**, respectively.

Synthesis of a Tricyclic Nucleoside Prodrug (28). Off-white powder. R_f = 0.75, (DCM/MeOH = 10:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78−1.54 (m, 12H, Me), 3.98−4.06 (m, 2H), 4.37−4.45 (m, 1H), 4.71 (s, 1H), 5.12−5.25 (m, 3H), 5.89−6.14 (m, 2H), 6.71−6.84 (m, 2H), 7.14 (s, 1H, Ar), 7.22−7.30 (m, 4H, Ar), 7.66 (s, 1H, Ar), 11.10−11.31 (m, 1H, NH). ^{19}F NMR (376 MHz, $CDCl_3$) δ −157.5. ^{31}P NMR (400 MHz, $CDCl_3$) δ 5.76, 5.97, two isomers: S_p/R_p or R_p/S_p = 4:3. HRMS (FAB) calculated for $C_{25}H_{30}FN_6O_8PS$ [M + H^+], 625.1646; found, 625.1645.

Synthesis of a Bicyclic Nucleoside Prodrug (29). Colorless syrup. R_f = 0.45 (DCM/MeOH = 25:1). ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.07−1.20 (m, 9H, Me), 1.27−1.34 (m, 3H, Me), 3.85−3.98 (m, 2H), 4.04−4.22 (m, 3H), 4.92−4.96 (m, 1H), 6.29 (d, 1H, J = 17.8 Hz, H-1), 7.15−7.18 (m, 1H, Ar), 7.25−7.35 (m, 4H, Ar), 8.47 (s, 1H, Ar). ^{19}F NMR (400 MHz, $CDCl_3$) δ −161.3. ^{31}P NMR (162 MHz, MeOH-d_4) δ 3.4, 4.3, two isomers: S_p/R_p or R_p/S_p = 1:1. HRMS calculated for $C_{24}H_{30}FN_5O_8PS$ [M + H^+], 600.1693; found, 600.1687.

Cell-Based Inhibition and Cytotoxicity Assays.[28] The Huh 5.2 HCV subgenomic replicon-containing cells were provided by Prof. R. Bartenschlager (University of Heidelberg, Heidelberg, Germany).

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The inhibitory potency (EC_{50} values) and cytotoxicity (CC_{50} values) of the compounds were evaluated in Huh 5.2 cells as described previously.[29]

In brief, Huh 5.2 cells, containing the hepatitis C virus genotype 1b I389luc-ubi-neo/NS3-3′/5.1 replicon,[30] were subcultured in DMEM supplemented with 10% FCS, 1% nonessential amino acids, 1% penicillin/streptomycin, and 2% Geneticin at a ratio of 1:3 to 1:4 and grown for 3 to 4 days in 75 cm^2 tissue culture flasks. One day before addition of the compound, cells were harvested and seeded in an assay medium (DMEM, 10% FCS, 1% nonessential amino acids, 1% penicillin/streptomycin) at a density of 6500 cells/well (100 μL/well) in 96-well tissue culture microtiter plates for the evaluation of the antimetabolic effect and in a CulturPlate (PerkinElmer) for the evaluation of the antiviral effect. The microtiter plates were incubated overnight (37 °C, 5% CO_2, 95−99% relative humidity), yielding a nonconfluent cell monolayer. Compounds were subsequently added to the plates at the indicated concentrations. Following assay setup, the microtiter plates were incubated for 72 h (37 °C, 5% CO_2, 95−99% relative humidity). For the evaluation of antimetabolic effects, the assay medium was aspirated, replaced with 75 μL of a 5% MTS solution in phenol red-free medium, and incubated for 1.5 h (37 °C, 5% CO_2, 95−99% relative humidity). Absorbance was measured at a wavelength of 498 nm (Safire2, Tecan), and optical densities (OD values) were converted to the percentage of untreated controls. For the evaluation of antiviral effects, the assay medium was aspirated and the cell monolayers were washed with PBS. The wash buffer was aspirated, and 25 μL of Glo lysis buffer (Promega) was added, allowing cell lysis to proceed for 5 min at room temperature. Subsequently, 50 μL of the luciferase assay system (Promega) was added, and the luciferase luminescence signal was quantified immediately (1000 ms integration time/well, Safire2, Tecan). Relative luminescence units were converted to the percentage of untreated controls.

EC_{50} and EC_{90} (values calculated from the dose−response curve) represent the concentrations at which 50 and 90% inhibition, respectively, of viral replication are achieved. CC_{50} (value calculated from the dose−response curve) represents the concentration at which the metabolic activity of the cells is reduced by 50% as compared to that of untreated cells.

The concentration of the compound is considered to elicit a genuine antiviral effect in the HCV replicon system when the antireplicon effect is significant at concentrations where no antimetabolic activity is observed.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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