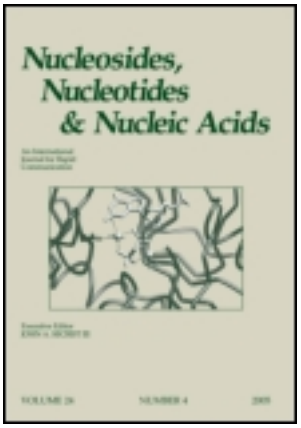

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Synthesis of Stable Isotope Labeled Analogs of the Anti-Hepatitis C Virus Nucleotide Prodrugs PSI-7977 and PSI-352938

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SYNTHESIS OF STABLE ISOTOPE LABELED ANALOGS OF THE ANTI-HEPATITIS C VIRUS NUCLEOTIDE PRODRUGS PSI-7977 AND PSI-352938

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□ *In order to support bioanalytical LC/MS method development and plasma sample analysis in preclinical and clinical studies of the anti-hepatitis C-virus nucleotides, PSI-7977 and PSI-352938, the corresponding stable isotope labeled forms were prepared. These labeled compounds were prepared by addition reaction of the freshly prepared Grignard reagent $^{13}CD_3MgI$ to the corresponding 2′-ketone nucleosides followed by fluorination of the resulting carbinol with DAST. As expected, these 2′-C-(trideuterated-^{13}C-methyl) nucleotide prodrugs showed similar anti-HCV activity to that of the corresponding unlabeled ones.*

Keywords PSI-7851; PSI-7977; PSI-352938; stable isotope label; hepatitis C virus; DAST; fluorination; $^{13}CD_3MgI$

INTRODUCTION

β-D-2′-Deoxy-2′-α-fluoro-2′-β-*C*-methyl nucleotides, PSI-7851 (**1a**),[1,2] PSI-7977 (**1b**),[1] and PSI-352938 (**2**)[3,4] have demonstrated potent anti-HCV activity. Currently, PSI-7977 (**1b**), a single diastereomer of PSI-7851 (**1a**), and PSI-352938 (**2**) are in Phase II clinical development. In order to support bioanalytical quantitative LC/MS analysis of plasma samples from preclinical and clinical studies of these agents, it was necessary to prepare stable isotope labeled versions to be used as internal standards. These standards were required to have similar physicochemical characteristics to those of the nucleotides of interest and have a molecular weight different enough so as not to overlap with the analytes of interest (PSI-7977 or PSI-352938) in quantitative mass spectroscopic analysis. In order to fulfill these requirements, preparation of nucleotides **3** and **4** each having a 4 amu difference relative

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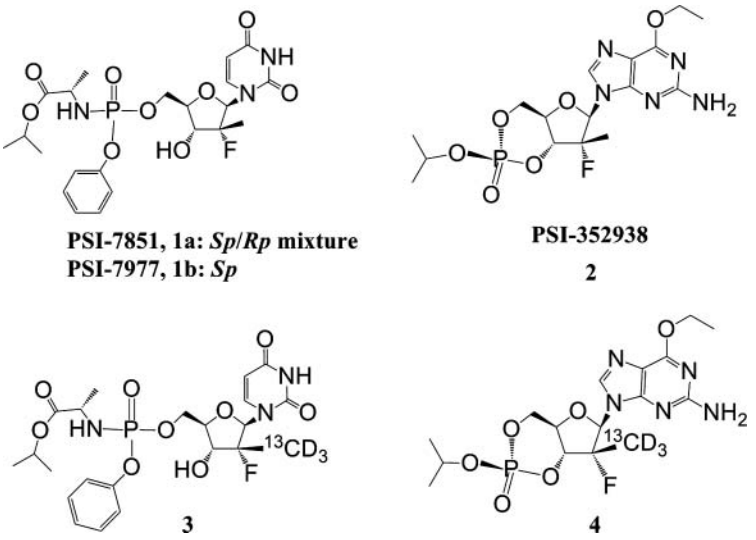
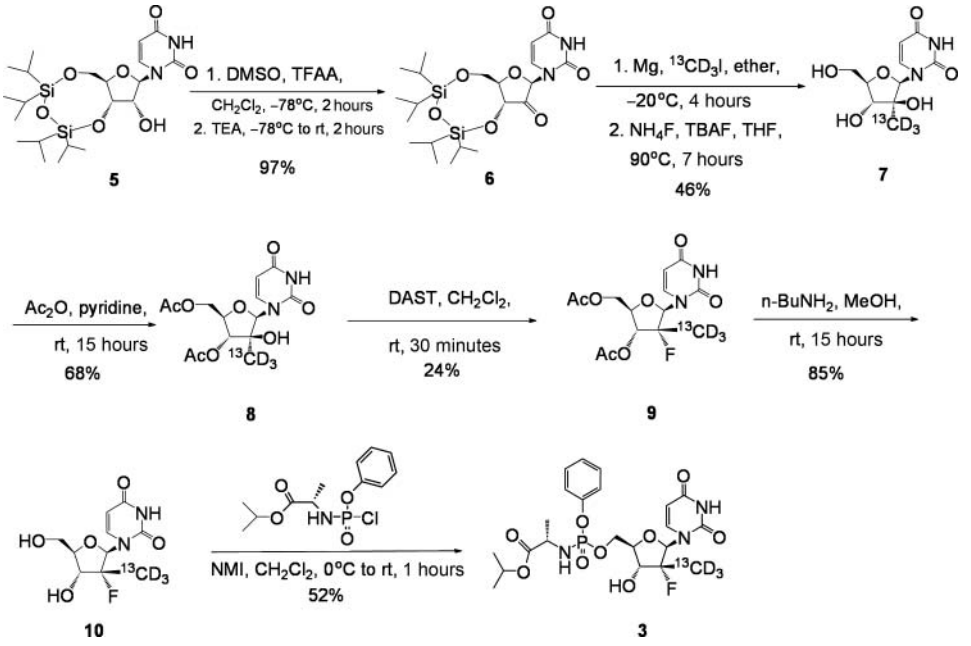


FIGURE 1 Structures of the nucleotide prodrugs and their labeled versions.

to their parent drugs, PSI-7977 and PSI-352938, was undertaken. We decided to label the 2′-methyl group using Grignard reaction with $^{13}CD_3MgBr$ because of the easy synthesis using the same chemistry developed for the unlabeled versions.[5,6] Otherwise, the labeling would have involved additional difficult multistep reactions to introduce 4 amu as shown in the reported examples.[7–10] Accordingly, compounds **3** and **4** each replaced the 2′-*C*-methyl group of the parent prodrugs **1a** and **2** with a $^{13}CD_3$ group.

RESULTS AND DISCUSSION

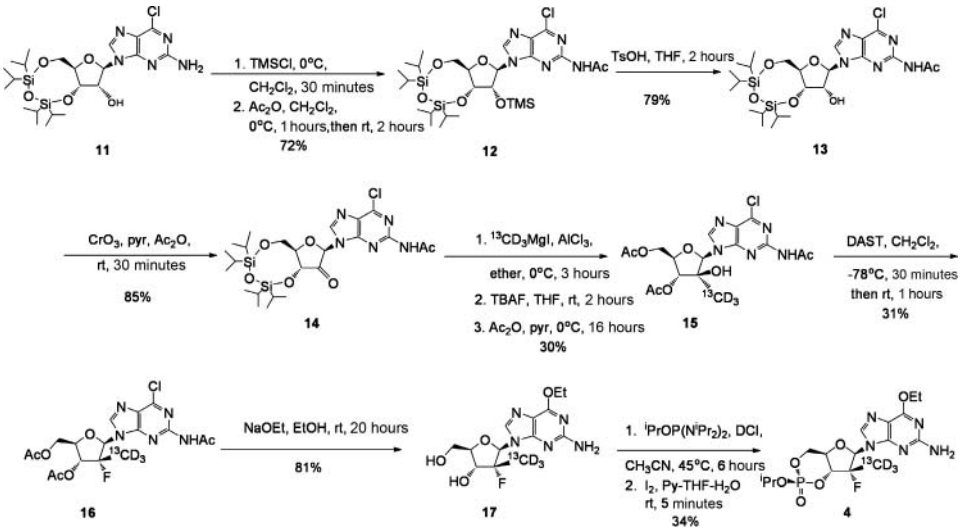
In order to prepare the target nucleotide prodrug **3**, 3′,5′-TIPDS-protected uridine (**5**) was readily prepared from uridine and then subjected to Swern oxidation to afford 2′-ketone nucleoside **6** in 97% yield.[5] The labeled Grignard reagent was prepared by reacting commercially available $^{13}CD_3I$ with magnesium metal in dry ether. The freshly prepared $^{13}CD_3MgI$ was then reacted with ketone **6** at low temperature to give carbinol **7** as the major product in 46% yield. A large excess (ca 5 eq) of $^{13}CD_3MgI$ was required to force the reaction to completion. Compound **7** was then deprotected with ammonium fluoride and reprotected with acetic anhydride to give diacetate **8** in 68% yield. Fluorination of compound **8** was effected by treating with DAST[5] to afford 2-α- fluorinated compound **9** in 74% yield, which was then deacetylated with *n*-$BuNH_2$ to give compound **10** in 85% yield. Finally, stable isotope labeled phosphoramidate **3** was



SCHEME 1 Synthesis of the labeled phosphoramidate nucleotide prodrug.

prepared as a *Sp/Rp* diastereomeric mixture by reacting **10** with (2*S*)-isopropyl-[(chloro(phenoxy)phosphoryl)amino]-propanoate in the presence of *N*-methylimidazole in 52% yield (Scheme 1).[1] Mass spectroscopic analysis of compound **3** showed greater than 99% isotopic purity and molecular weight increase by 4 amu as expected.

In order to prepare labeled purine nucleotide **4**, protected purine nucleoside **11**[6] was further protected at the 2-amino group through a transient protection of the 2′-hydroxyl with TMSCl followed by acetylation with Ac_2O, and then selective hydrolysis of the 2′-O-TMS by treatment with TsOH to provide the N^2-acetyl-nucleoside **13** in 57% yield. The oxidation of compound **13** was best accomplished by $CrO_3/Py/Ac_2O$ to give 2′-ketone nucleoside **14** in 85% yield. In order to prepare the labeled carbinol intermediate, ketone **14** was treated with $^{13}CD_3MgI$ to give the addition product in low yield. However, treatment with $^{13}CD_3MgI/AlCl_3$ gave an improved yield, probably due to in situ production of more reactive $Al(^{13}CD_3)_3$.[11] Desilylation of the carbinol intermediate with TBAF followed by reprotection with Ac_2O gave compound **15** in 30% yield. Fluorination of **15** with DAST afforded 2′-a-fluorinated compound **16** in 31% yield,[5,6] which, in turn, was treated with NaOEt to give labeled 6-OEt-nucleoside **17** in 81% yield. Treatment of compound **17** with 1-chloro-*N,N,N′,N′*-tetraisopropyl-phosphinediamine in the presence of 4,5-dicyanoimidazole formed a cyclic phosphite triester that



SCHEME 2 Synthesis of the labeled cyclic phosphate nucleotide prodrug.

was then directly oxidized with iodine to afford cyclic phosphate triester target compound **4** in 34% yield as a single *Rp* diastereomer as determined by ^{31}P- and ^{1}H-NMR comparison with the unlabeled parent structure (Scheme 2).[3,12] Mass spectroscopic analysis of compound **4** showed greater than 99% isotopic purity and molecular weight increase by 4 amu as expected.

Although it was not a main objective to evaluate labeled compounds **3** and **4** for antiviral activity, we were interested in evaluating whether labeling affects anti-HCV activity of the 2′-fluoro-*C*-methyl class of nucleosides. In fact, the anti-HCV activity of labeled nucleotides **3** and **4** was not significantly different in an in vitro replicon assay[2] from that of their corresponding unlabeled clinical agents **1a** and **2** (Table 1).

In summary, we successfully prepared stable isotope labeled nucleotides **3** and **4** through 2′-ketone nucleoside intermediates using freshly prepared Grignard reagent $^{13}CD_3MgI$ and evaluated their anti-HCV activity. These products were used as internal standards to support the quantitative mass

TABLE 1 Anti-HCV activity[a]: unlabeled versus labeled

	Phosphoramidate		Cyclic phosphate	
Compound	Unlabeled **1a**	Labeled **3**	Unlabeled **2**	Labeled **4**
EC_{50}	0.17 μM	0.14 μM	0.07 μM	0.03 μM
EC_{90}	0.73 μM	0.27 μM	0.26 μM	0.22 μM
CC_{50}	>100 μM	>100 μM	>100 μM	>50 μM

[a]Antiviral activity was indirectly measured through luciferase activity in a replicon system and shown as an average value of three tests for the unlabeled compounds and a result of a single test for the labeled.[2]

spectroscopic analysis of samples for preclinical and clinical studies of PSI-7851 (**1a**), PSI-7977 (**1b**), and PSI-352938 (**2**).

EXPERIMENTAL

General

Nuclear magnetic resonance spectra were recorded on a Varian AS 400 spectrometer at room temperature with tetramethylsilane as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Values given for coupling constants are first order. UV spectra were recorded on a Spectronic Genesis 10 Bio from Thermo Eclectron Corporation. Electron Spray Mass Spectrometry was recorded on Acquity Ultra Performance LC from Waters. Flash silica gel chromatography was performed on Intelli Flash 280 from AnaLogix.

[3-^{13}C]-1-((2R,3S,4R,5R)-3,4-Dihydroxy-5-(Hydroxymethyl)-3-Trideu-teromethyltetrahydrofuran-2-yl)Pyrimidine-2,4(1H,3H)-Dione (7)

To a dry three-necked round flask were added anhydrous CH_2Cl_2 (600 mL) and DMSO (30.82 g, 394.5 mmol). The solution was cooled to -78°C under an atmosphere of nitrogen. Trifluoroacetic anhydride (77.7 g, 369.8 mmol) was added via a syringe over 40 minutes to give a cloudy mixture. A solution of the protected uridine (**5**) (38.30 g, 78.70 mmol) in CH_2Cl_2 (600 mL) was added dropwise over 75 minutes at -78°C via an addition funnel. The resulting heterogeneous mixture was stirred for 2 hours at -78 to -65°C and then dry triethylamine (92 mL) was added via a syringe quickly to form a clear light yellow solution. After 1 hour at that temperature, the cooling bath was removed and the reaction mixture was warmed up slowly to room temperature over 1 hour. The reaction was quenched by addition of saturated NH_4Cl (180 mL). Water (200 mL) was added and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (300 mL). The combined organic layer was washed with water (3×400 mL), brine (150 mL), and dried over Na_2SO_4. Removal of solvent afforded a sticky brown residue. The crude oil residue was stored overnight in the freezer. After overnight storage, some crystal solid was observed in the oil. The oil was dissolved in 500 mL hexanes at room temperature. The solution was stored in the freezer for 24 hours, resulting in the formation of a solid. Solid (**6**) was collected via filtration and rinsed with cold 10% CH_2Cl_2 (1 L) in hexanes to remove most of the orange color. The filtrate was concentrated and the residue was purified by flash silica gel column chromatography (EtOAc 10%–70% in hexanes) to afford an additional amount of compound **6** as a

light orange solid. The combined solid was dried under vacuum at 50°C for 15 hours before use in the next reaction (37 g, 97%).

In order to prepare the Grignard reagent, magnesium metal (3.53 g, 160.45 mmol) was washed with d-HCl and dried in a hot oven, put into an argon-filled two-neck round-bottomed flask equipped with a magnetic stirrer and a condenser; then anhydrous ether (80 mL) was added. To the magnesium in ether was added trideuterated [^{13}C]-methyliodide (15.06 g, 103.16 mmol) slowly, which generated an exothermic reaction. After the reaction mixture cooled, the supernatant was transferred to a solution of compound **6** (10.0 g, 20.63 mmol) in anhydrous THF (1 L) at −50°C over 20 minutes. The temperature was allowed to rise to −20°C and the mixture was stirred at −20°C for 4 hours. Upon completion of the reaction, the mixture was diluted with EtOAc (1 L) and brine (300 mL) was added slowly with stirring. Then, the organic layer was separated, washed with ammonium chloride solution (300 mL × 2), and dried with sodium sulfate. After filtration and concentration, the residue was dissolved in MeOH (250 mL) to give a solution to which ammonium fluoride (12 g, 323.97 mmol) and TBAF (400 mg, 1.53 mmol) were added. The resulting mixture was stirred at 90°C for 7 hours and concentrated in vacuo and purified by silica gel column chromatography (MeOH:CH_2Cl_2 = 1:20 to 1:10) to give compound **7** (5 g, 46%) as a white solid. ^{1}H NMR (DMSO-d_6) δ (ppm) 11.26 (s, 1H, NH), 7.65 (d, 1H, J = 8.4 Hz), 5.77 (d, 1H, J = 2.4 Hz,), 5.57 (d, 1H, J = 8.0 Hz), 5.46 (d, 1H, J = 5.2 Hz), 5.24 (d, 1H, J = 2.4 Hz), 5.14 (t, 1H, J = 5.6 Hz), 3.74–3.56 (m, 4H).

[4-^{13}C]-((2R,3R,4R,5R)-3-Acetoxy-5-(2,4-Dioxo-3,4-Dihydropyrimidin-1(2H)-yl)-4-Fluoro-4-Trideuteromethyltetrahydrofuran-2-yl)Methyl Acetate (9)

To a solution of compound **7** (5.0 g, 19.07 mmol) in anhydrous pyridine (100 mL) was added acetic anhydride (3 mL) at room temperature. The resulting mixture was stirred at room temperature for 15 hours, diluted with EtOAc (250 mL), washed with water (50 mL × 3), and dried with sodium sulfate. After filtration and concentration, the residue was purified by flash column chromatography (MeOH 0%–5% in CH_2Cl_2) to give compound **8** (4.0 g, 68%) as a gray solid. To a solution of compound **8** (2.33 g, 6.73 mmol) in anhydrous CH_2Cl_2 (60 mL) was added DAST (1.33 mL, 10.09 mmol) at −78°C slowly. The resulting mixture was stirred for 30 minutes after being exposed to room temperature, diluted with CH_2Cl_2 (100 mL), washed with ice water (30 mL × 2) and then cold aqueous $NaHCO_3$ solution (30 mL × 2), and finally, dried with sodium sulfate. Upon filtration and concentration, the residue was purified by flash silica gel column chromatography (EtOAc 0%–50% in hexanes) to give compound **9** (580 mg, 24%) as a white solid. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.27 (s, 1H), 7.55 (d, 1H, J = 8.4 Hz), 6.17 (d,

1H, $J = 18.8$ Hz), 5.78 (dd, 1H, $J = 1.2, 8.4$ Hz), 5.12 (dd, 1H, $J = 9.6, 21.6$ Hz), 4.40–4.31 (m, 3H), 2.19 (s, 3H), 2.15 (s, 3H).

[3-^{13}C]-1-((2R,3R,4R,5R)-3-Fluoro-4-Hydroxy-5-(Hydroxymethyl)-3-Trideuteromethyltetrahydrofuran-2-yl)Pyrimidine-2,4(1H,3H)-Dione (10)

To a solution of compound **9** (2.0 g, 5.74 mmol) in methanol (20 mL) was added *n*-butylamine (6 mL). The resulting mixture was stirred at room temperature for 15 hours and concentrated in vacuo. The obtained residue was purified by flash silica gel column chromatography (MeOH 0%–10% in CH_2Cl_2) to give compound **10** (1.3 g, 85%) as a white solid. ^{1}H NMR (CD_3OD) δ (ppm) 8.08 (d, 1H, $J = 8.0$ Hz), 6.13 (d, 1H, $J = 18.4$ Hz), 5.70 (d, 1H, $J = 8.0$ Hz), 3.99 (d, 1H, $J = 13.6$ Hz), 3.97–3.91 (m, 2H), 3.80 (dd, 1H, $J = 2.0, 12.8$ Hz), LRMS (ESI) [M + H] calculated for $C_9{}^{13}CH_{11}D_3FN_2O_5$ 265, found 265.

[4-^{13}C]-(2S)-Isopropyl 2-(((((2R,3R,4R,5R)-5-(2,4-Dioxo-3,4-Dihydropyrimidin-1(2H)-yl)-4-Fluoro-3-Hydroxy-4-Trideuteromethyltetrahydrofuran-2-yl)Methoxy)(Phenoxy)Phosphoryl)Amino)Propanoate (3)

To a solution of compound **10** (207 mg, 0.783 mmol) and *N*-methylimidazole (0.4 mL, 5.0 mmol) in THF (4 mL) was added dropwise (2S)-isopropyl-[(chloro(phenoxy)phosphoryl)amino]-propanoate in THF (1.0 M, 2.35 ml, 2.35 mmol) at 0°C. The reaction was slowly warmed to room temperature over 1 hour, and then, water (1 mL) and EtOAc (5 mL) were added. The organic solution was washed with saturated aqueous sodium citrate mono basic (2 × 2 mL), saturated aqueous $NaHCO_3$ (1 × 2 mL), dried over magnesium sulfate, and concentrated in vacuo. The residue obtained was purified by flash silica gel column chromatography (iPrOH 0–5% in CH_2Cl_2) to give compound **3** (216 mg, 52%, 1:1 mixture of P-diastereomers) as a white solid: ^{1}H NMR (DMSO-d_6) δ 11.54 (s, 1H), 7.56 (d, 1H, $J = 6.8$ Hz), 7.40–7.35 (m, 2H), 7.23–7.18 (m, 3 H), 6.14–5.96 (m, 2H), 5.89 (dd, 1H, $J = 5.6, 25.6$ Hz), 5.55 (t, 1H, $J = 8.4$ Hz), 4.85 (dq, 1H, $J = 1.6, 6.0$ Hz), 4.44–4.32 (m, 1H), 4.25 (m, 1H), 4.06–3.98 (m, 1H), 3.86–3.70 (m, 2H), 1.30–1.08 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 173.3, 163.1, 150.6, 139.5, 130.2, 125.6, 120.0, 103.2, 100.6, 89.5, 80.0, 77.5, 71.8, 70.0, 64.2, 50.1, 21.8, 21.0, 15.9; ^{31}P NMR (DMSO-d_6) δ 4.90, 4.77; HRMS (ESI) $[M + H]^+$ calculated for $C_{21}{}^{13}CH_{27}D_3FN_3O_9P$ 534.1925, found 534.1924.

N-(6-Chloro-9-((6aR,8R,9R,9aR)-2,2,4,4-Tetraisopropyl-9-((Trimethylsilyl)Oxy)Tetrahydro-6H-Furo[3,2-f][1,3,5,2,4] Trioxadisilocin-8-yl)-9H-Purin-2-yl)Acetamide (12)

To a solution of compound **11** (5.70 g, 10.5 mmol) in pyridine (10 mL) and CH_2Cl_2 (90 mL) was added TMSCl (3.42 g, 31.5 mmol) at 0°C. The reaction mixture was stirred at 0°C for 30 minutes and Ac_2O (3.21 g, 31.5 mmol) was added. The resulting mixture was then stirred at 0°C for 1 hour and then at room temperature for 2 hours. EtOAc (200 mL) was added and the mixture was washed with brine and dried over Na_2SO_4. Solvent was evaporated in vacuo and the residue was purified by flash silica gel column chromatography (EtOAc 10%–60% in hexanes) to give compound **12** (5.0 g, 72%). ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1H), 7.95 (s, 1H), 5.93 (s, 1H), 4.00–4.41 (m, 5H), 2.60 (s, 3H), 0.91–1.11 (m, 28H), 0.21 (s, 9H).

N-(6-Chloro-9-((6aR,8R,9R,9aS)-9-Hydroxy-2,2,4,4-Tetraisopropyltetrahydro-6H-Furo[3,2-f][1,3,5,2,4] Trioxadisilocin-8-yl)-9H-Purin-2-yl)Acetamide (13)

To a solution of **12** (5.0 g, 7.6 mmol) in THF (100 mL) was added $TsOH.H_2O$ (1 g) and the solution was stirred at room temperature for 3 hours and neutralized with triethylamine. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (EtOAc 10%–80% in hexanes) to give product **13** (3.5 g, 79%). ^{1}H NMR ($CDCl_3$) δ 8.21 (d, 1H, $J = 1.2$ Hz), 8.00, 8.02 (ds, 1H), 6.00 (s, 1H), 4.68 (t, 1H, $J = 7.2$ Hz), 4.45 (d, 1H, $J = 4.8$ Hz), 4.05–4.19 (m, 3H), 3.17 (s, 1H), 2.54 (s, 3H), 1.03–1.10 (m, 28H).

N-(6-Chloro-9-((6aR,8R,9aR)-2,2,4,4-Tetraisopropyl-9-Oxotetrahydro-6H-Furo[3,2-f][1,3,5,2,4] Trioxadisilocin-8-yl)-9H-Purin-2-yl)Acetamide (14)

To a mixture of CrO_3 (2.2 g, 21.99 mmol) in CH_2Cl_2 (30 mL) were sequentially added pyridine (1.74 g, 22.0 mmol), Ac_2O (2.24 g, 22 mmol), and then a solution of **13** (4.3 g, 7.33 mmol) in CH_2Cl_2 (10 mL). The resulting mixture was stirred at room temperature for 30 minutes and EtOAc (200 mL) was added slowly. After stirring at room temperature for 1 hour, the solid generated was filtered off through a silica gel pad. The filtrate was concentrated to dryness and the residue was co-evaporated with toluene. EtOAc (100 mL) was added to the residue, which was then filtered through a silica gel pad. The filtrate was evaporated and the residue co-evaporated with toluene to give a white solid. The residue was purified by flash silica gel column chromatography (EtOAc 5 to 80% in hexane) to give compound **14** as a syrup, which was co-evaporated with toluene (2 × 50 mL) and dried under vacuum overnight before use (3.65 g, 85%). ^{1}H NMR ($CDCl_3$) δ 8.00

(s, 1H), 7.86 (s, 1H), 5.77 (s, 1H), 5.25 (d, 1H, $J = 9.2$ Hz), 4.07–4.28 (m, 3H), 2.41 (s, 3H), 1.06–1.16 (m, 28H).

[4-^{13}C]-(2R,3R,4S,5R)-5-(2-Acetamido-6-Chloro-9H-Purin-9-yl)-2-(Acetoxymethyl)-4-Hydroxy-4-Trideuteromethyltetrahydrofuran-3-yl Acetate (15)

To a mixture of magnesium metal (1.68 g, 69.14 mmol) in dry ethyl ether (40 mL) was added 1/3 of $^{13}CD_3I$ (8.70 g, 60.00 mmol) and the mixture was stirred at room temperature until reflux. To the mixture was added the rest of $^{13}CD_3I$ at the rate that kept the reaction under gentle reflux. After being cooled down to room temperature, the solution was transferred to another flask in an ice bath, to which was added $AlCl_3$ (2.67 g, 20.00 mmol). The resulting mixture was stirred at 0°C for 1 hour and concentrated at room temperature. The obtained syrup was then dissolved in CH_2Cl_2 (100 mL) to give a solution, to which was added a solution of compound **14** (5.84 g, 10.00 mmol) in CH_2Cl_2 (30 mL) slowly at 0°C. The mixture was stirred at 0°C for 3 hours and after adding saturated NH_4Cl (10 mL) and EtOAc (300 mL), the mixture was stirred at room temperature for 10 minutes. After removal of solid by filtration, the filtrate was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue obtained was dissolved in THF (100 mL) and 1 M TBAF in THF (30 mL, 30 mmol) was added. The resulting mixture was stirred at room temperature for 2 hours, concentrated in vacuo, and purified by flash silica gel column chromatography (MeOH 0%–15% in CH_2Cl_2) to give a labeled nucleoside intermediate, which was dissolved in pyridine–CH_2Cl_2 (30:100 mL). After addition of Ac_2O (3 mL, excess), the resulting mixture was stirred at 0°C for 16 hours. After adding water (10 mL), the mixture was stirred at room temperature for 10 minutes, diluted with EtOAc (200 mL), washed with water and brine, and dried over Na_2SO_4. Solvent was evaporated in vacuo and the residue was purified by flash silica gel column chromatography (MeOH 0%–8% in CH_2Cl_2) to give compound **15** as a white solid (1.33 g, 29.9%). ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 1H), 8.10 (s, 1H), 6.03 (d, 1H, $J = 2.4$ Hz), 5.22 (d, 1H, $J = 3.2$ Hz), 4.50 (m, 2H), 4.24 (m, 1H), 4.00 (s, 1H), 2.45 (s, 3H), 2.20 (s, 3H), 2.12 (s, 3H).

[4-^{13}C]-(2R,3R,4R,5R)-5-(2-Acetamido-6-Chloro-9H-Purin-9-yl)-2-(Acetoxymethyl)-4-Fluoro-4-Trideuteromethyltetrahydrofuran-3-yl Acetate (16)

To a solution of **15** (0.68 g, 1.53 mmol) in CH_2Cl_2 (68 mL) precooled at −78°C was added DAST (0.74 g, 4.59 mmol) over 20 minutes and the resulting mixture was stirred at the same temperature for 30 minutes and then at room temperature for 1 hour. EtOAc (200 mL) was added and the mixture was washed with aq. $NaHCO_3$ and brine, and dried over Na_2SO_4.

Solvent was evaporated and the residue was purified by flash silica gel column chromatography (EtOAc 0%–90% in hexanes) to give compound **16** (0.21 g, 30.6%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.26 (s, 1H), 8.11 (s, 1H), 6.23 (d, 1H, $J = 17.2$ Hz), 5.75 (m, 1H), 4.39, 4.56 (m, 3H), 2.49 (s, 3H), 2.21 (s, 3H), 2.17 (s, 3H).

[3-^{13}C]-N-(6-Ethoxy-9-((2R,3R,4R,5R)-3-Fluoro-4-Hydroxy-5-(Hydroxymethyl)-3-Trideuteromethyltetrahydrofuran-2-yl)-9H-Purin-2-yl)Acetamide (17)

To the solution of **16** (0.35 g, 0.78 mmol) in EtOH (36 mL) was added 21% NaOEt in EtOH (3.1 mL, 7.83 mmol). The resulting mixture was stirred at room temperature for 20 hours and neutralized with AcOH. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (MeOH 0%–10% in CH_2Cl_2) to give compound **17** (0.21 g, 81%). ^{1}H NMR (CD_3OD) δ 8.22 (s, 1H), 6.15 (d, 1H, $J = 18.0$ Hz), 4.52 (q, 2H, $J = 6.8$ Hz), 4.38 (dd, 1H, $J = 5.8$, 23.6 Hz), 4.03 (m, 2H), 3.86 (m, 1H), 1.42 (t, 3H, $J = 7.20$ Hz). LRMS (ESI) [M + H$^+$] calculated for $C_{12}{}^{13}CH_{16}D_3FN_5O_4$ 332, found 332.

[7-^{13}C]-(4aR,6R,7R,7aR)-6-(2-Amino-6-Ethoxy-9H-Purin-9-yl)-7-Fluoro-2-Isopropoxy-7-Trideuteromethyltetrahydro-4H-Furo[3,2-d][1,3,2]Dioxaphosphinine-2-Oxide (4)

To a dry 50 mL round-bottomed flask was charged compound **17** (205 mg, 0.62 mmol). Anhydrous aceotonitrile (4 mL) was added and the suspension was cooled in an ice bath. To the mixture were added 4,5-dicyanoimidazole (183 mg, 1.52 mmol) and isopropyl tetraisopropylphosphorodiamidite (209 mg, 0.72 mmol). The resulting clear solution was then heated at 45°C for 6 hours, concentrated, and dissolved in ethyl acetate (20 mL). White solid was removed by filtration and the filtrate was concentrated and cooled in an ice bath. A solution of iodine (ca. 0.1 M in a mixture of THF (2 mL), pyridine (2 mL), and water (4 mL)) was added and the brown solution was stirred at room temperature for 5 minutes. An aqueous solution of sodium thiosulfate (10%) was added dropwise until a light brown solution was formed. After removal of solvents, the residue was dissolved in ethyl acetate (20 mL) and solid was removed by filtration. The filtrate was washed with saturated sodium bicarbonate (2 × 15 mL) and brine (5 mL), and dried over Na_2SO_4. Solvent was removed in vacuo and the residue obtained was purified by flash silica gel column chromatography (40% EtOAc in hexanes) to afford product **4** (98.7 mg, 34%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 1 H), 6.01 (d, 1 H, $J = 18.0$ Hz), 5.41 (br, 1H), 4.88 (s, 2 H), 4.85 (m, 1H), 4.64–4.46 (m, 4H), 4.38–4.32 (m, 1H), 1.45–1.41 (m, 9 H); ^{13}C NMR ($CDCl_3$) δ 161.8, 159.2, 152.6, 138.0, 117.3, 92.6, 79.8,

77.5, 73.8, 70.5, 69.5, 63.1, 23.9, 23.5, 15.3, 14.5; ^{31}P NMR ($CDCl_3$): δ −5.96; HRMS (ESI) $[M + H]^+$ calculated for $C_{15}{}^{13}CH_{21}D_3FN_5O_6P$ 436.1670, found 436.1666.

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