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Synthesis of [$^{13}C_4$]Baraclude® (entecavir)

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Entecavir, labeled as 1H-[$^{13}C_4$]purin-6(9H)-one, was prepared from commercially available [^{13}C]guanidine HCl, 1 and diethyl [1,2,3-$^{13}C_3$]malonate, 2. The reagents were condensed together to give 2-amino-4,6-dichloro[2,4,5,6-$^{13}C_4$]pyrimidine 3, which in turn was coupled to an optically active amino cyclopentanol derivative, 9. A further sequence of eight reaction steps completed the constructions of the purine ring system and the exocyclic olefin attachment on the cyclic pentyl portion, 18. The removal of the methoxide and benzyl protecting groups gave [$^{13}C_4$]entecavir, 20 in an overall yield of 6.8%. The chemical purity of the title compound was determined by HPLC to be 99.23%. The percent isotopic [$^{13}C_4$] abundance was found by mass spectral analysis to be 96.7%. No detectable level of the unlabeled entecavir was found by LC-MS analysis.

Keywords: carbon-13; entecavir; Baraclude®; diethyl [1, 2, 3-$^{13}C_3$]malonate; antiviral; nucleotide

Introduction

Hepatitis B virus (HBV) represents one of the most prevalent viral diseases in the world and is a cause of serious liver disorders. About 400 million people are thought to be infected with HBV, which left untreated can lead to cirrhosis and hepatocellular carcinoma. HBV infection causes an alarming 0.5–1.2 million deaths per year.[1] Entecavir was approved under the trade name Baraclude® by the US Food and Drug Administration in March 2005 for the treatment of chronic HBV infection in adults. Entecavir is an orally active, carbocyclic guanosine nucleoside analog, with potent selectivity against HBV DNA polymerase.[2] Prior to this preparation, our laboratories used the structural guanosine analog Lobucavir, 21 as an internal reference standard in LC-MS quantification analyses of drug material in clinical samples.[3] The failure rates in the bioanalytical assay gave rise to concerns about the reliability of the internal standard. Isotope labeled versions of a drug are usually superior as reference standards for LC-MS quantization and qualification analyses. The confirmatory assurance that comes from the co-elution of drug with its stable isotope labeled form is in part accountable for the superior quality of the reference standard.[4] Hence, we became interested in the synthesis of [$^{13}C_4$]entecavir.

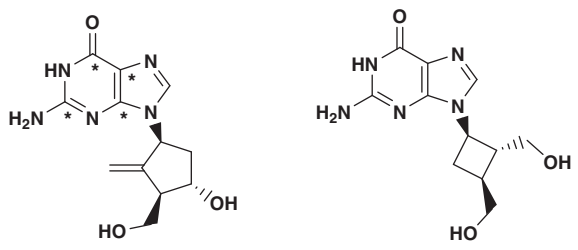

Figure 1. [$^{13}C_4$]entecavir, 20 and Lobucavir, 21.

We report here the synthesis and characterization of [$^{13}C_4$]-entecavir 20 (Figure 1).

Results and discussion

Among the many methods for making purine nucleosides, the strategy that goes through a pyrimidine intermediate was practical to prepare [$^{13}C_4$]entecavir, 20.[5] In such a synthetic sequence, 2-amino-4,6-dichloro[2,4,5,6-$^{13}C_4$]pyrimidine, 3 could be coupled with (1*S*,2*S*,3*S*,5*S*)-5-amino-3-(benzyloxy)-2-(benzyloxymethyl)cyclopentanol, 9 to form the pyrimidinylamino derivative 10. If a requisite 5-amino function could be introduced by reduction of 5-[(*p*-chlorophenyl)azo derivative 11, a ring-closure reaction thereafter with triethyl orthoformate would lead to the protected nucleoside 14. Although the proposed sequence contains discouraging problematic transformations, it has been extensively used in the literature to access purine nucleosides and carbocyclic analogs.[6] Fortunately, [^{13}C]guanidine HCl, 1 and diethyl [1,2,3-$^{13}C_3$]malonate, 2 are useful labeled reagents and readily available. These reagents provided 2-amino-4,6-dichloro[2,4,5,6-$^{13}C_4$]pyrimidine, 3 in a two-step sequence.[7] By this approach we condensed 1 and 2 in absolute ethanol containing sodium ethoxide to afford 2-amino[2,4,5,6-$^{13}C_4$]pyrimidine-4,6-diol. Subsequent chlorination using phosphorus oxychloride and *N*,*N*-dimethylaniline gave 3 in 74% yield (Scheme 1).

The next phase of the synthesis required the preparation of (1*S*,2*S*,3*S*,5*S*)-5-amino-3-(benzyloxy)-2-(benzyloxymethyl)cyclopentanol, 9.[8,9] A sequence of steps in which Bom-Cl alkylation

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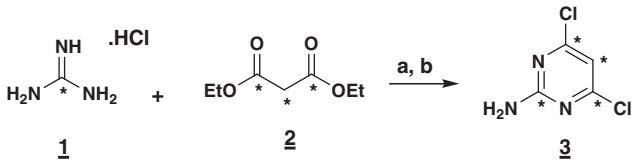


1 2 3

Scheme 1. Synthesis of 2-amino-4,6-dichloro[2,4,5,6-$^{13}C_4$]pyrimidine, 3: (a) NaOEt, EtOH, reflux, 1 h; (b) $POCl_3$, *N*,*N*-dimethylaniline, 60°C, 6 h, 74%.

of sodium cyclopentadiene 4, followed by (−)-IPC$_2$BH hydroboration, alkaline peroxide oxidation, and stereospecific epoxidation with *tert*-BuOOH/VO(acac)$_2$ furnished (1*S*,2*R*,3*S*,5*R*)-2-(benzyloxymethyl)-6-oxabicyclo[3.1.0]hexan-3-ol, 6. Benzylation of 6 gave the protected alcohol 7 on which sequential azide opening of the epoxide ring and reduction of the azide group to the amino compound 9 were completed (Scheme 2). Coupling of 9 with 3 (Scheme 3) was conducted in hot butanol in the presence of triethylamine to give 10 in 68%. In this synthesis, we chose to introduce the required 5-amino group via 5-[(*p*-chlorophenyl)azo]-pyrimidine 11 prepared from 10 by its reaction with *p*-chlorobenzenediazonium chloride. However, the chloride substituent on the pyrimidine ring had to be replaced with a methoxy group that would tolerate subsequent reaction conditions. It was likely that dehalogenation would accompany the Zn reduction of the azo functionality 11 to the amine and potentially complicate the unmasking of the carbonyl of guanosine down stream. Following the Zn reduction, compound 14 was made by a ring closure reaction by reacting tetra-substituted pyrimidine 13 with triethyl orthoformate in the presence of an acid catalyst. Labeled [$^{13}C_4$]intermediates 11, 12, 13, 15, 16, and 17 were prepared as described.[8]

To complete the synthesis of [$^{13}C_4$]entecavir, the exocyclic olefin was installed. In preparation for this transformation, the amino group of 14 was selectively protected by reaction with 4-monomethoxytrityl chloride (MMTrCl). The alcohol functionality in 15 was oxidized with Dess-martin periodinane to give the ketone 16.[9] Reaction of 16 with Nysted reagent installed the requisite olefin group, and in the process afforded 17, fully protected [$^{13}C_4$]entecavir. Removal of MMTr group was accomplished with 2 N HCl at room temperature to give 18 in 69% yield. Demethylation was achieved with 3 N HCl at 60°C cleanly unmasking the carbonyl to afford the dibenzyl guanoside 19 in 75% yield. Debenzylation of 19 with excess BCl_3 afforded 20 after crystallization from methanol. An analytical sample was obtained by recrystallization from warm water in 89% recovery. A total of 86 mg of [$^{13}C_4$]entecavir, 20 was synthesized in an overall yield of 6.8% from 10. Mass spectrometric analysis of [$^{13}C_4$]entecavir was performed using positive ion electrospray on a Q-Tof Ultima mass spectrometer giving a molecular ion $[M+H]^+$ at 282.14 Da. This is consistent with the molecular formula $^{13}C_4C_8H_{15}N_5O_3$ for the free base. Isotopic purity was calculated to be 96.7%. A molecular ion of 281.13 Da, corresponding to the empirical formula $^{13}C_3C_9H_{15}N_5O_3$, was 3.3%. Unlabeled entecavir was below levels of detection.

In short, we have achieved the synthesis of entecavir bearing $^{13}C_4$-labeled purine base from commonly available diethyl [1,2,3-$^{13}C_3$]malonate and [^{13}C]guanidine HCl. At the start, these compounds were transformed into 2-amino-4,6-dichloro-[2,4,5,6-$^{13}C_4$]pyrimidine. Coupling with substituted amino cyclopentanol followed by additional steps to ultimately install the exocyclic olefin on the carbocycle completed the construction of the target labeled guanosine nucleoside. [$^{13}C_4$]Entecavir, 20 met our analytical specifications for use as an internal reference standard.

Experimental

Isotopic labeled diethyl [1,2,3-$^{13}C_3$]malonate with a ^{13}C enrichment of 99% was purchased from Cambridge Isotope Laboratories, Inc. and [^{13}C]guanidine HCl with a ^{13}C enrichment of 99.4% was purchased from Isotec.

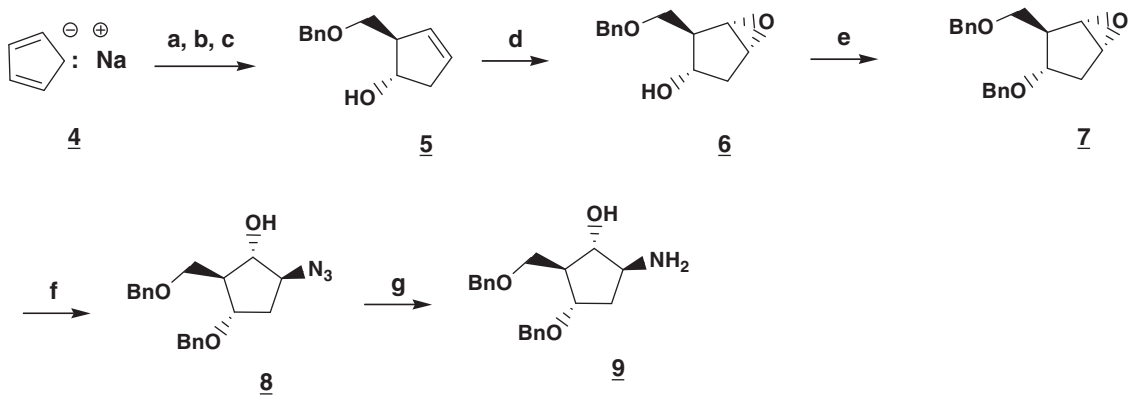
High Performance Liquid Chromatography: HPLC methods described below were used for in process and final product analyses. Co-injections with authentic samples were used when possible. All HPLC purities were measurements of UV purity and performed on a Shimadzu SLC-10A system controller, dual LC-10AT pumps, SPD-10A UV-VIS detector at 254 nm and CLASS VP 5.0 integration software. Method: YMC-ODS-AQ C18, 3 μm, 4.6 × 150 mm^2, detected at 254 nm. Mobile phase A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile. Gradient: 0 min 5% B, 7 min 50% B, 35 min 95% B, 40 min 95%B, 45 min 5% B, flow rate = 1 mL/min. LCMS Method: Phenomenex C18, 5 μm, 4.6 × 50 mm, detected at 220 nm. Mobile phase A: 0.1% TFA in 10% MeOH, 90% water, B: 0.1% TFA in 90% MeOH, 10% water; oven temperature: 40°C. Gradient: 0 min 0% B, 4 min 100% B, flow rate = 4 mL/min. NMR Spectra were recorded on Bruker Avance NMR spectrometer. Only carbon-13 enriched positions are reported for C-13 characterization purposes. Optical rotation was measured with a polarimeter by use of a 50 mm cell at 589 nm. 8.16 mg of the sample was dissolved in 1.0 mL of MeOH. The reported specific rotation is the average of ten individual measurements taken at ten-second intervals.

2-Amino-4,6-dichloro[2,4,5,6-$^{13}C_4$]pyrimidine, 3

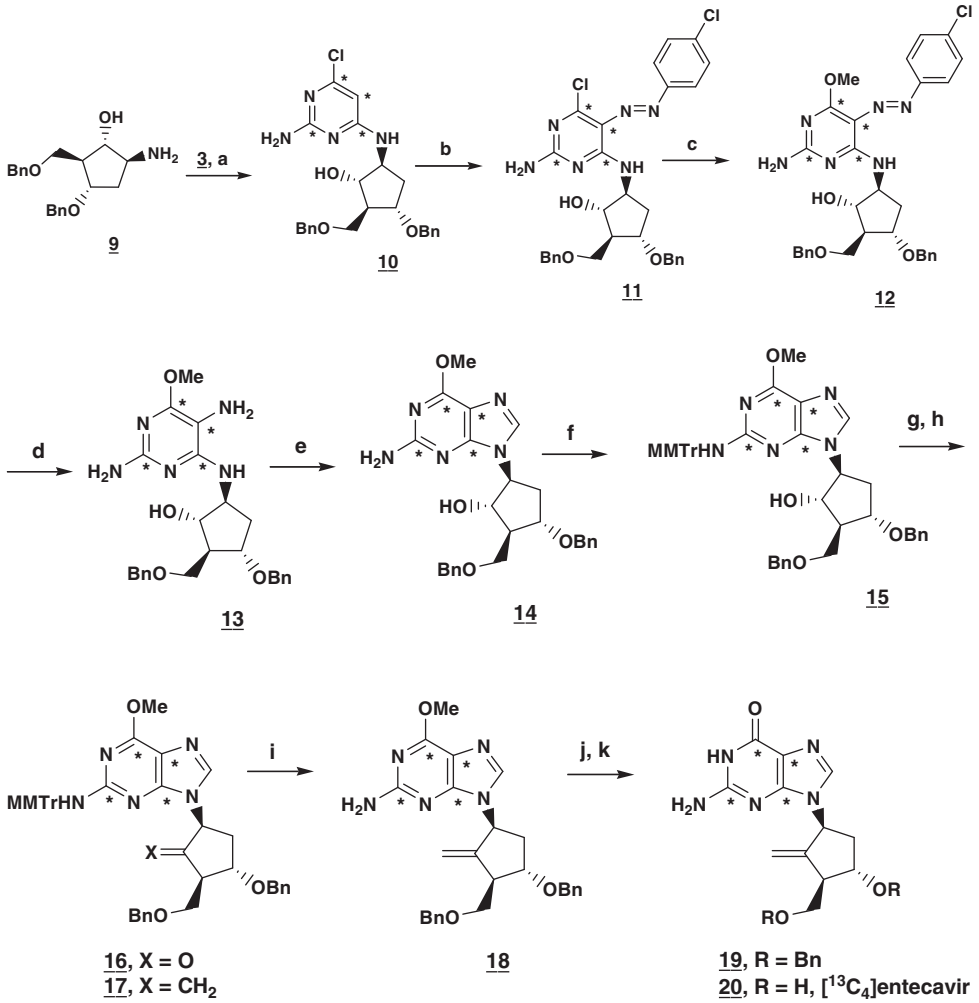
NaOEt (3.75 g, 55.2 mmol) was slowly added to a flask equipped with a water cooled condenser containing a mixture of diethyl [1,2,3-$^{13}C_3$]malonate (3 g, 18.4 mmol) and [^{13}C]guanidine HCl (1.77 g, 18.4 mmol) in EtOH (25 mL). The reaction was heated to reflux for 1 h. The mixture was cooled to rt and filtered. A white cake was obtained, and then dissolved in water (20 mL) and the pH adjusted to 4 with 5% aqueous acetic acid. The resulting solid was collected by vacuum filtration and washed with additional water. The solid was dried under high vacuum for 16 h. The solid was placed in a dry flask, which was charged with phosphorus oxychloride (11.3 g, 73.6 mmol). The mixture was heated to 60°C for 4 h. While maintaining this temperature, *N*,*N*-dimethylaniline (4.46 g, 36.8 mmol) was added over 1 h. The reaction mixture was stirred for an additional 1 h, then cooled to 0°C and quenched with water (25 mL). The precipitated product was collected by vacuum filtration. The filter cake was washed with water and dried under vacuum to give a pale yellow solid (2.29 g, 74%). This product was used in the next step without further purification. MS $[M+H]^+ = 167.99$, $^{37}Cl^{35}Cl$ $[M+H]^+ = 169.98$, $^{37}Cl_2$ $[M+H]^+ = 171.98$. ^{1}H NMR (400 MHz, $CDCl_3$, δ):6.93, 6.47 (d, $J^{13}CH = 183$ Hz), 5.35 (bs, 2H).

(1*S*,2*S*,3*S*,5*S*)-5-Amino-3-(benzyloxy)-2-(benzyloxymethyl)-cyclopentanol, 9

Product 9[8] (clear oil, 3.10 g). TLC (10% MeOH/CH_2Cl_2, Product $R_f = 0.05$), HPLC (Product, $R_t = 7.77$ min), LCMS (LCMS Method, Product $R_t = 2.89$ min). MS $[M+H]^+ = 328.3$. ^{1}H NMR (400 MHz,



Scheme 2. Synthesis of amino cyclopentanol intermediate, 9: (a) Bom-Cl, DMF, −40°C, 15 min; (b) (−)-IPC$_2$BH, THF, −78 to −10°C, 48 h; (c) NaOH/H_2O_2, rt, 12 h; 75% over three steps; (d) VO(acac)$_2$, *tert*-BuOOH, DCM, rt, 2.5 h; (e) NaH, 60% dispersion, BnBr, TBAI, DMF, rt then −50°C, 18 h; 83% over two steps; (f) NaN_3, NH_4Cl, EtOH, 85°C, 22 h, 99%; (g) Ph_3P, water, THF, rt, 5 h, 84%.



Scheme 3. Synthesis of [$^{13}C_4$]entecavir, 20: (a) Et_3N, *n*-BuOH, 115°C, 18 h, 68%; (b) $NaNO_2$, water, 4-chloroaniline, 3 N HCl, 0°C, 30 min then 3, KOAc, acetic acid, CH_3CN/water (2:1), 24 h, 78%; (c) 5 N NaOH, MeOH, 80°C, 2 h, 95%; (d) Zn, AcOH, water, MeOH, 80°C, 2 h, 95%; (e) HC(OEt)$_3$, TsOH, CH_3CN, 70%; (f) MMTrCl, DMAP, Et_3N, CH_2Cl_2, 88%; (g) Dess-martin periodinane, *t*-BuOH, CH_2Cl_2, 84%; (h) Nysted reagent, $TiCl_4$, CH_2Cl_2, THF, 60%; (i) 2 N HCl, MeOH, THF, rt, 4 h, 69%; (j) 3 N HCl, MeOH, THF, 60°C, 22 h, 75%; (k) 1M BCl_3, CH_2Cl_2, −78 to −20°C, 1.5 h, 89%.

DMSO-d_6, δ): 7.36–7.23 (m, 10H), 4.88 (bs, 1H), 4.47 (s, 2H), 4.41 (d, 2H, J = 4.0 Hz), 3.74–3.71 (m, 1H), 3.54 (dd, 1H, J = 4.0, 8.0 Hz), 3.43 (dd, 1H, J = 4.0, 8.0 Hz), 3.33 (bs, 2H), 3.18 (t, 1H, J = 8.0 Hz), 3.03–2.97 (m, 1H), 1.92–1.81 (m, 2H), 1.45–1.37 (m, 1H). $[\alpha]_D^{20}$ +32.30°.

(1*S*,2*S*,3*S*,5*S*)-5-(2-Amino-6-chloro[$^{13}C_4$]pyrimidin-4-ylamino)-3-(benzyloxy)-2-(benzyloxymethyl)cyclopentanol, 10

Triethylamine (2.04 g, 20.21 mmol) was added to a solution of 9 and 2-amino-4,6-dichloro[2,4,5,6-$^{13}C_4$]pyrimidine, 3 (0.70 g,

4.16 mmol) in *n*-butanol (15 mL). The solution was heated to 115°C for 18 h. The reaction was monitored by TLC (35% EtOAc/Hex, Product $R_f = 0.2$). The solution was evaporated to dryness, and the residue dissolved in EtOAc (3 × 10 mL) and washed with water (15 mL). The layers were partitioned and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 5−50% EtOAc/Hexane to afford a white solid 10 (1.31 g, 68%). LCMS (LCMS Method, Product $R_t = 3.07$ min). MS $[M+H]^+ = 459.2$, $^{37}Cl^{35}Cl$ $[M+H]^+ = 461.2$. ^1H NMR (400 MHz, $CDCl_3$, δ 7.39–7.28 (m, 10H), d, $J^{13}CH = 173$ Hz), 5.00 (s, 2H), 4.55 (m, 3H), 4.25 (s, 1H), 3.95(s, 1H), 3.84 (t, 1H, $J = 7.2$ Hz), 3.70–3.60 (m, 2H), 2.35–2.31 (m, 2H), 1.81–1.74 (m, 1H). ^{13}C NMR ($CDCl_3$, δ 164.31 (d, $J = 60.37$ Hz), 161.83 (m), 160.31 (d, $J = 72.94$ Hz), 94.81 (m).

(1*S*,2*S*,3*S*,5*S*)-5-(2-Amino-6-chloro-5-((*E*)-(4-chlorophenyl)-diazenyl)[$^{13}C_4$]pyrimidin-4-ylamino)-3-(benzyloxy)-2-(benzyloxymethyl)cyclopentanol, 11

Product 11[8] (yellow solid, 1.32 g, 78%), LCMS (LCMS Method, Product $R_t = 4.40$ min). MS $[M+H]^+ = 597.20$. ^1H NMR (400 MHz, $CDCl_3$, δ 10.72 (s, 1H), 7.69–7.66 (dd, 2H, 4.49, 9.56 Hz), 7.47–7.41 (dd, 2H, 4.49, 9.56 Hz), 7.38–7.27 (m, 9H), 5.59 (bs, 2H), 4.58–4.50 (m, 5H), 4.0 (m, 1H), 3.99 (t, 1H, $J = 4.2$ Hz), 3.94–3.90 (t, 1 H, $J = 7.6$ Hz), 3.69–3.62 (m, 2H), 2.44–2.34 (m, 2H), 1.94–1.86 (m, 1H), 1.60 (bs, 2H). ^{13}C NMR ($CDCl_3$, δ 165.86 (m), 159.39 (m), 156.50 (m), 119.47 (m).

(1*S*,2*S*,3*S*,5*S*)-5-(2-Amino-5-((*E*)-(4-chlorophenyl)diazenyl)-6-methoxy[$^{13}C_4$]pyrimidin-4-ylamino)-3-(benzyloxy)-2-(benzyloxymethyl)cyclopentanol, 12

Product 12[8] (orange solid, 1.25 g, 95%), LCMS (LCMS Method, Product $R_t = 4.10$ min). MS $[M+H]^+ = 593.30$, $^{37}Cl^{35}Cl$ $[M+H]^+ = 595.3$. ^1H NMR (400 MHz, $CDCl_3$, δ 10.95 (s, 1H), 7.62–7.58 (dd, 2H, $J = 4.7$, 9.5 Hz), 7.42–7.26 (m, 12H), 6.20 (bs, 1H), 5.16 (s, 2H), 4.55 (s, 3H), 4.45 (s, 1H), 4.06 (d, 2H, $J = 3.8$ Hz), 4.02 (m, 1H), 3.90 (t, 1H, $J = 7.8$ Hz), 3.70–3.59 (m, 2H), 2.41–2.33 (m, 2H), 1.96–1.88 (m, 1H), 1.57 (s, 2H). ^{13}C NMR ($CDCl_3$, δ 169.58–168.66 (dd, $J = 8.25$ Hz, 83.81 Hz), 161.32 (m), 156.50 (m), 113.0 (m).

(1*S*,2*S*,3*S*,5*S*)-3-(Benzyloxy)-2-(benzyloxymethyl)-5-(2,5-diamino-6-methoxy[$^{13}C_4$]pyrimidin-4-ylamino)cyclopentanol, 13

Product 13[8] (off-white foam, 0.94 g, 95%), LCMS (LCMS Method, Product $R_t = 2.79$ min). MS $[M+H]^+ = 470.20$. ^1H NMR (500 MHz, $CDCl_3$, δ 7.28–7.37 (10H, m), 5.26 (1H, br. s.), 4.38–4.61 (4H, m), 4.18 (1H, m), 3.95 (2H, m), 3.81–3.89 (3H, m), 3.76 (2H, t, $J = 7.97$ Hz), 3.63–3.71 (2H, m), 3.58 (2H, m), 2.20–2.40 (3H, m), 1.83 (1H, ddd, $J = 13.33$, 10.58, 6.87 Hz).

(1*S*,2*S*,3*S*,5*S*)-5-(2-Amino-6-methoxy-9H-[$^{13}C_4$]purin-9-yl)-3-(benzyloxy)-2-(benzyloxymethyl)cyclopentanol, 14

p-Toluenesulfonic acid monohydrate (20 mg, 0.1 mmol) was added to a solution of 13 (0.93 g, 1.98 mmol) and anhydrous triethyl orthoformate (0.44 g, 2.98 mmol) in acetonitrile (20 mL) under nitrogen. The solution was heated to 90°C for 1 h. The reaction was monitored by TLC (75% EtOAc/Hex, Product $R_f = 0.2$). The solution was evaporated to dryness, dissolved in EtOAc (3 × 10 mL), and washed with water (15 mL). After the layers were separated, the organic layer was washed with brine, dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 25% EtOAc/Hexane to 100% EtOAc to 0.25% MeOH/EtOAc to afford a white foam 14 (0.66 g, 70%). LCMS (LCMS Method, Product $R_t = 3.13$ min). MS $[M+H]^+ = 480.2$. ^1H NMR (500 MHz, $CDCl_3$, δ 7.54–7.50 (dd, 1H, $J = 4.9$, 6.5 Hz), 7.31–7.24 (m, 10H), 4.88 (bs, 2H), 4.64–4.55 (m, 1H), 4.52–4.49 (m, 5H), 4.23 (t, 1H, $J = 8.3$ Hz), 4.12–4.01 (m, 4H), 3.67–3.60 (m, 2H), 2.53 (m, 1H), 2.43 (m, 1H), 2.32–2.25 (m, 1H). ^{13}C NMR ($CDCl_3$, δ 162.27 (dd, $J = 7.6$, 86.5 Hz), 158.28 (d, $J = 7.6$ Hz), 153.65 (d, $J = 66.1$ Hz), 115.98 (m).

(1*S*,2*S*,3*S*,5*S*)-3-(Benzyloxy)-2-(benzyloxymethyl)-5-(6-methoxy-2-((4-methoxyphenyl)diphenylmethylamino)-9H-[$^{13}C_4$]purin-9-yl)cyclopentanol, 15

Product 15[8] (light-yellow solid, 0.9 g, 88%), LCMS (LCMS Method), MS $[M+H]^+$ = undetectable. ^1H NMR (500 MHz, $CDCl_3$, δ 7.55–6.70 (m, 25H), 6.25 (s, 1H), 4.53 (m, 5H), 4.11 (bs, 1H), 3.77 (m, 5H), 3.67–3.60 (m, 3H), 2.34 (bs, 2H), 2.11 (bs, 1H), 1.64 (bs, 2H). ^{13}C NMR ($CDCl_3$, δ 161.27 (dd, $J = 86.0$ Hz), 157.00 (m), 153.65 (m), 115.00 (m).

(2*R*,3*S*,5*S*)-3-(Benzyloxy)-2-(benzyloxymethyl)-5-(6-methoxy-2-((4-methoxyphenyl)diphenylmethylamino)-9H-[$^{13}C_4$]purin-9-yl)cyclopentanone, 16

Product 16[8] (light-yellow foam, 0.90 g, 84%), LCMS (LCMS Method), MS (observed mol. ion of the unprotected, $R_t = 3.36$ min) $[M+H]^+ = 478.20$. ^1H NMR (400 MHz, $CDCl_3$, δdd, 1H, $J = 1.26$, 8.31 Hz), 7.39–7.17 (m, 24H), 6.77–6.75 (d, 2H, $J = 8.81$ Hz), 6.21 (bs, 1H), 4.53 (bs, 2H), 4.41 (m, 2H), 4.15–4.25 (bs, 1H), 3.76 (m, 4H), 3.58 (bs, 2H), 2.71 (s, 1H), 1.59 (s, 3H).

9-((1*S*,3*R*,4*S*)-4-(Benzyloxy)-3-(benzyloxymethyl)-2-methylenecyclopentyl)-6-methoxy-*N*-((4-methoxyphenyl)diphenylmethyl)-9H-[$^{13}C_4$]purin-2-amine, 17

Product 17[8] (off-white solid, 0.54 g, 60%). MS $[M+H]^+ = 748.36$. ^1H NMR (500 MHz, $CDCl_3$, δ 7.60 (bs, 1H), 7.38–7.12 (m, 22H), 6.73 (d, 2H, $J = 8.8$ Hz), 6.25 (s, 1H), 5.15 (s, 2H), 4.72 (s, 1H), 4.45 (m, 4H), 3.98 (s, 1H), 3.72 (s, 3H), 3.65–3.50 (m, 3H), 2.90 (s, 1H), 1.65–1.45 (m, 2H). ^{13}C NMR ($CDCl_3$, δ 162.27 (m), 158.28 (m), 153.50 (m), 114.0 (s).

9-((1*S*,3*R*,4*S*)-4-(Benzyloxy)-3-(benzyloxymethyl)-2-methylenecyclopentyl)-6-methoxy-9H-[$^{13}C_4$]purin-2-amine, 18

Compound 17 (505 mg, 0.67 mmol) was dissolved in THF/MeOH (1:1) (6 mL). To this solution, 2 N HCl (1.7 mL, 3.38 mmol) was slowly added. The reaction was stirred at rt for 9 h. The reaction progress was monitored by TLC (70% EtOAc/Hexane, R_f SM = 0.8, R_f Product = 0.35). The solution was concentrated to dryness. The pH was adjusted to 8 with 2.5 N KOH and extracted with EtOAc (3 × 15 mL). After the layers were separated, the organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 15–75% EtOAc/Hex to afford a colorless oil 18 (221 mg, 69%). MS $[M+H]^+ = 476.18$. ^1H NMR (500 MHz, $CDCl_3$, δ 7.65 (m, 1H), 7.35–7.28 (m, 10H), 5.54 (bm, 1H), 5.18 (s, 1H), 4.80 (m, 3H), 4.57–4.51 (m, 4H), 4.17 (m, 1H), 4.08 (m, 3H), 3.67–3.65 (m, 2H), 3.03 (s, 1H), 2.41–2.34 (m, 2H).

2-Amino-9-((1*S*,3*R*,4*S*)-4-(benzyloxy)-3-(benzyloxymethyl)-2-methylenecyclopentyl)-1H-[$^{13}C_4$]purin-6(9H)-one, 19

Of total, 0.5 mL of 3 N HCl was added to a solution of 18 (73.5 mg, 0.154 mmol) in THF/MeOH (1:1) (3 mL) under N_2. The reaction was heated to 60°C for 18 h. The reaction was monitored by TLC (70% EtOAc/Hexane, SM $R_f = 0.8$ and 10% MeOH/CH_2Cl_2, Product $R_f = 0.2$) and HPLC. The solution was evaporated to dryness and the residue was dissolved in EtOAc (15 mL) and sat $NaHCO_3$ (5 mL). The pH was adjusted with 5 N KOH to 7.5–8. After the layers were separated, the organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 0–5% MeOH/$CHCl_3$ to afford a pure white solid 19 (53.5 mg, 75%). LCMS (LCMS Method, Product $R_t = 3.16$ min). MS $[M+H]^+ = 462.1$. ^{1}H NMR (400 MHz, $CDCl_3$, δ 12.11 (s, 1H), 7.80 (s, 1H), 7.55–7.20 (m, 10H), 6.02 (s, 2H), 5.52 (m, 1H), 5.23 (s, 1H), 4.87 (s, 1H), 4.63–5.52 (m, 6H), 4.20 (s, 1 H), 3.69 (m, 2H), 3.06 (s, 1H), 2.41 (m, 2H).

[$^{13}C_4$]Entecavir, 20

Compound 19 (158 mg, 0.34 mmol) was placed in a 25 mL reaction flask and dissolved in dichloromethane (3 mL). The flask was cooled to −78°C, and BCl_3 (1 M in CH_2Cl_2, 2.05 mL, 2.05 mmol) was added slowly. The solution was stirred at −78°C for 0.5 h, then at −20°C for 1 h with monitoring by HPLC (HPLC Method, Product $R_t = 23.33$ min). The reaction flask was cooled back to −78°C and MeOH (6 mL) was slowly added. The resulting clear solution was warmed to rt, then evaporated to dryness, and the crude product was crystallized from MeOH (1 mL). The white solid was collected by vacuum filtration and washed with CH_2Cl_2 (1 mL) to give 74 mg of product. The mother liquor was dissolved in 50:50 saturated $NaHCO_3$/water (20 mL) and washed with CH_2Cl_2 (3 × 10 mL) (the product had low solubility in CH_2Cl_2). After the two layers were separated, the aqueous layer was concentrated to dryness under high vacuum to give a crude product (HPLC purity = 93.8%, 28 mg). This crude product was crystallized from warm water (4 mL) to give an additional 12 mg of product (total amount synthesized, 20: 86 mg, yield: 89%; overall yield: 6.8% from 10). HPLC chemical purity = 99.23%. HPLC coinjected with the authentic unlabeled entecavir, $R_t = 23.03$ min. ^{1}H NMR (500 MHz, DMSO-d_6, δ 10.53 (d, 2H, $J = 3.9$ Hz), 7.65 (dd, 1H, $J = 4.9$, 11.0 Hz), 6.38 (s, 2H), 5.34 (m, 1H), 5.09 (s, 1H), 4.85–4.79 (m, 2H), 4.54 (s, 1H), 4.22 (s, 1H), 3.52 (t, 2 H, $J = 6$ Hz), 2.19 (m, 1H), 2.04 (m, 1H). ^{13}C NMR (DMSO-d_6, δ 156.82 (dd, $J = 7.7$, 86.5 Hz), 153.18 (s), 150.91 (dd, $J = 7.7$, 61.0 Hz), 115.81 (m). Elemental analysis for $^{13}C_4C_8H_{15}N_5O_3$.1.5 equiv. water, calculated (%) $C = 48.05$; $H = 4.86$; $N = 22.71$, found (%) $C = 47.56$; $H = 5.17$; $N = 22.96$.

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