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Research Article

Synthesis of [^{14}C]-radiolabelled entecavir

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Summary

Radiolabelled [^{14}C]entecavir, (**1**), was prepared in 12 steps from (1S,2R,3S,5R)-3-(benzyloxy)-2-(benzyloxymethyl)-6-oxa-bicyclo[3.1.0]hexane **2**. The chemical yield of [^{14}C]entecavir was 14% from the epoxide **2**. Introduction of [^{14}C] radiolabel was achieved by elaboration of 4,5-diaminopyrimidine **8** with triethyl[^{14}C]orthoformate to purine derivative **9**. The radiochemical yield of [^{14}C]entecavir from triethyl[^{14}C]orthoformate was 11.3%. Radiochemical purity of [^{14}C]entecavir determined by HPLC was 99.8%. The specific activity of [^{14}C]entecavir was 108 μCi/mg (29.9 mCi/mmol).

Key Words: [^{14}C]; triethyl[^{14}C]orthoformate; entecavir; antiviral; nucleoside

Introduction

Available oral antiviral agents offer the potential for improved management of chronic hepatitis B virus (HBV) infection which, left untreated, can lead to cirrhosis and hepatocellular carcinoma.[1,2] Entecavir is a carbocyclic guanosine nucleoside analogue which is a potent selective inhibitor of hepatitis B virus.[3–5] Preparation of [^{14}C]entecavir (Figure 1) was required for clinical study of absorption, distribution, metabolism and elimination (ADME).

The synthetic strategy for preparation of [^{14}C]entecavir involved the elaboration of the pyrimidine **8** to purine **9** by introducing the [^{14}C] isotope at the C-8 carbon. The radiolabelled precursor for the imidazole ring closure is the readily available formate or orthoformate. This general strategy has been achieved on the base as well as the nucleoside, and it is widely applicable in the preparation of labelled nucleosides.[6,7] This procedure has previously been used

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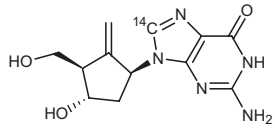


1, [^{14}C] entecavir

Figure 1. 1, [^{14}C]entecavir

within our laboratory to prepare [^{14}C]lobucavir.[8] This report details the preparation and characterization of [^{14}C]entecavir.

Results and discussion

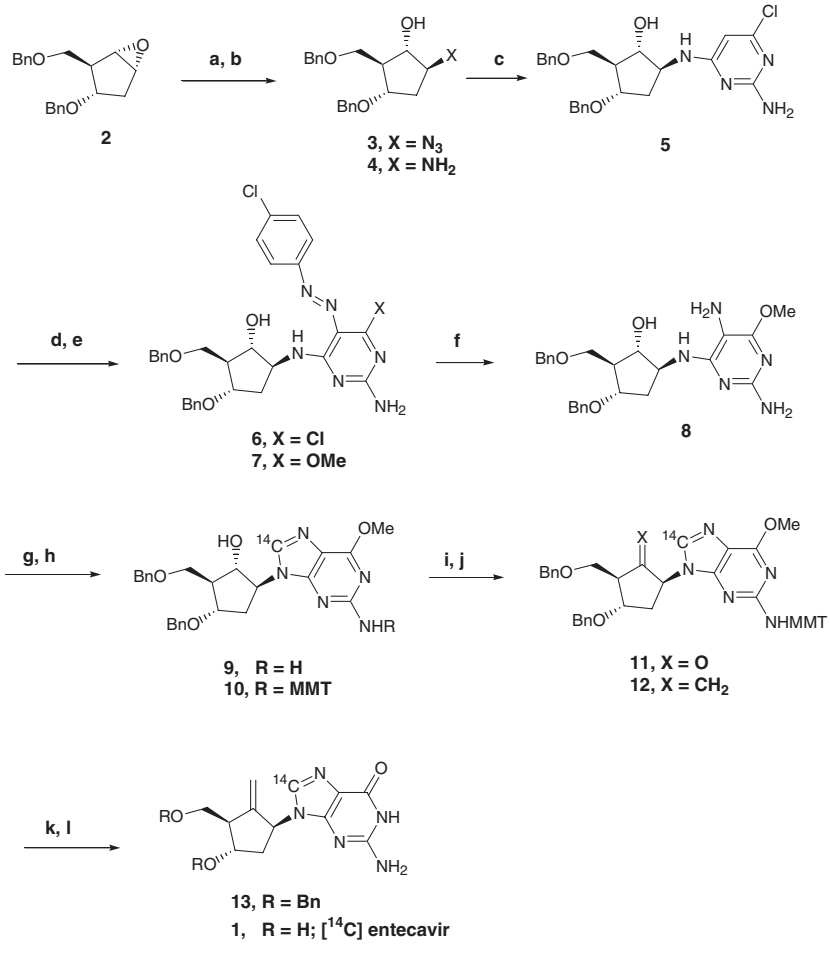
The synthesis of [^{14}C]entecavir was achieved starting with the chiral epoxide **2**[9] as shown in Scheme 1. The chiral epoxide was reacted with sodium azide in the presence of NH_4Cl to afford the *trans*-azidoalcohol **3** in quantitative yield. The azide **3** was reduced using triphenylphosphine in wet THF to afford amine **4** as an oil in 84% yield. The amine **4** readily reacted with 2-amino-4,6-dichloropyrimidine at 115°C to furnish 6-chloro diaminopyrimidine **5** in 82% yield.[10,†]

The pyrimidine **5** was reacted with *p*-chlorobenzenediazonium chloride, generated *in situ*, to afford the bright yellow 5-diazopyrimidine **6** in 91% yield. Reaction of **6** with aqueous potassium hydroxide in MeOH gave 4-methoxy-5-(*p*-chlorophenylazo)pyrimidine **7** as an orange solid in 78% yield. Cleavage of the diazo linkage of **7** with zinc in acetic acid gave the triaminopyrimidine **8** in 77% yield.[†,‡]

Introduction of the [^{14}C]radiolabel was achieved by elaboration of the triaminopyrimidine **8** to purine **9**. Reaction of a slight excess of **8** with high specific activity triethyl[^{14}C]orthoformate and catalytic toluenesulphonic acid in dry acetonitrile afforded the purine **9**. The volatile radioactive by-products were isolated by distillation and **9** was then isolated in 86% yield after chromatography. The specific activity of this isolated solid was 116 μCi/mg (53 mCi/mmol).

The 2-amino group of the guanine **9** was protected in the quantitative yield as the monomethoxytritylamine to afford **10** and then was oxidized with Dess–Martin periodinate[11,12] to give the labile ketone **11**.[§] The crude ketone **11** was converted to the exocyclic methylenic compound **12** by the reaction with excess Nysted reagent in the presence of titanium tetrachloride in 75% yield. The radiochemical purity of isolated **12** was 92%.

[†] MS and elemental analysis was consistent for the expected product.
[‡] Manipulation of the diamine **8**, including filtration and chromatography, were conducted under a blanket of argon.
[§] Ketone **11** is a very labile compound prone to *β*-elimination of the 3′-benzyloxy group to give the corresponding cyclopentenone.



Reagents: a. NaN_3, NH_4Cl, EtOH, 99%; b. Ph_3P, H_2O, THF, 84%; c. 2-amino-4,6-dichloropyrimidine/Et_3N/n-BuOH, 82%; d. $NaNO_2/H_2O$/*p*-chloroaniline, CH_3CN, 91%; e. KOH, MeOH, 78%; f. Zn, HOAc, H_2O, MeOH, 77%; g. H[^{14}C]C$(OEt)_3$, TsOH, CH_3CN, 86%; h. 4-methoxytrityl chloride, Et_3N, DMAP, CH_2Cl, 104%; i. Dess-Martin periodinate, t-BuOH, CH_2Cl_2, 113%; j. Nysted reagent, $TiCl_4$, CH_2Cl_2, THF, 75%; k. 2N HCl, MeOH, THF, 88%; l. BCl_3, CH_2Cl_2, 67%

Scheme 1. Synthesis of [^{14}C]entecavir (1)

Conversion of **12** to [^{14}C]entecavir was achieved by two consecutive deprotection steps. The 2-monomethoxytritylamine and 6-methoxy protecting groups were removed concurrently in good yield by reaction treatment with 2 N hydrochloric acid in methanol-tetrahydrofuran. The isolated penultimate intermediate **13** had a radiochemical purity of 96%.¶

¶Structural identity of **13** was confirmed by high resolution mass spectrometry and by comparison to authentic standard.

Debenzylation of the penultimate **13** was achieved with boron trichloride in dichloromethane. The crude product was isolated by extractive work up and then further purified and desalted by chromatography on HP20 resin. The [^{14}C]entecavir obtained from the HP20 chromatography was crystallized from water to afford crystalline [^{14}C]entecavir. The specific activity of **1** was diluted by addition of unlabelled entecavir followed by final crystallization from water to afford [^{14}C]entecavir in 67% yield from **13**. The specific activity of **1** was 108 μCi/mg and the radiochemical purity was 99.8%.

Experimental

General: Radioactivity was determined with a Wallac Model 1409 liquid scintillation counter (Wallac-LKB Instruments, Inc.). Counting efficiency was determined by the channels ratio method. Analytical samples were weighed on a Sartorius Model MC-5 microbalance. Mass spectra were obtained with a Finnigan TSQ or a Finnigan LCQ mass spectrometer. Exact mass spectra were obtained on a Micromass LCT TOF mass spectrometer. Proton NMR spectra were recorded on a Varian Unity/Inova 500 MHz spectrometer. RadioTLC was detected with a Bioscan System 200 Imager. TLC was performed on 60 F_{254} silica gel plates (Merck). Flash chromatography was conducted on KP-Sil silica gel (Biotage). HP-20 resin was obtained from Mitsubishi Chemical Company.

Specific activity was determined gravimetrically by dissolution of two weighed samples (ca 0.5 mg ea.) into DMF in 10- and 25-ml volumetric flasks. Aliquots of 25 and 50 μl for each sample were diluted into Ecolite cocktail (10 ml) and counted.

Materials: Triethyl [^{14}C]orthoformate was purchased from Amersham Pharmacia Biotech (radiochemical purity 98.4%, specific activity 58 mCi/mmol). The chiral epoxide **2**, penultimate standard **13** and unlabelled entecavir were prepared by reported methods (**9**). MilliPore water was used for all reactions except the final conversion and crystallization where B/Braun USP water was employed.

High Performance Liquid Chromatography: HPLC was performed on a YMC-ODS-A, 4.6 × 150 mm, S3 column using a Rainin Model SD-200 HPLC, a Rainin model UV-1 UV detector and a Beta-Ram radiometric detector with a 0.5 ml flow cell (IN/US Systems Inc). Method A consisted of isocratic elution using a mobile phase of CH_3CN/H_2O (60:40) and a flow rate 1.0 ml/min. UV detection was monitored at 254 nm. Method B consisted of mobile phases A: H_2O and B: CH_3CN and a flow rate of 1.0 ml/min. A gradient of 40% B from 0–10 min, 40–90% B from 10–20 min, and 90% B from 20–30 min was used. UV detection was monitored at 254 nm. Method C consisted of mobile phases A: 0.2% H_3PO_4/H_2O and B: 0.2% H_3PO_4/CH_3CN and a flow rate of 1.0 ml/min. A gradient of 0–10% B from 0–15 min, 10% B

from 15–20 min, and 10–90% B from 20–30 min was used. UV detection was monitored at 254 nm.

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

A solution of epoxide **2** (19.9 g, 64.1 mmol) in anhydrous EtOH (50 ml) was added dropwise over 10 min to a suspension of NH_4Cl (10.3 g, 0.19 mol) and NaN_3 (12.6 g, 0.19 mol) in anhydrous EtOH (100 ml). The reaction was carried out at 85°C under argon for 22 h when TLC (EtOAc/hexanes 1:1) showed that the reaction was complete, and evaporated under vacuum. The residue was dissolved in CH_2Cl_2 (75 ml) and extracted with H_2O (3 × 50 ml). The combined aqueous layer was back-extracted with CH_2Cl_2 (2 × 25 ml). The combined CH_2Cl_2 layer was dried over $MgSO_4$, filtered and evaporated under vacuum to afford 22.4 g (99% yield) of compound **3** as a light brown oil. TLC (EtOAc/hexanes 1:1) R_f 0.55. HPLC (Method A) RT 12.3 min, AP 95.6%. Mass spec: 352.3 $(M-H)^-$. ^{1}H and ^{13}C NMR ($CDCl_3$) were consistent with structure.

(1S,2S,3S,5S)-5-amino-3-(benzyloxy)-2-(benzyloxymethyl)cyclopentanol (**4**)

Solid triphenylphosphine (20.0 g, 76.2 mmol) was added in small portions over 30 min to a solution of the azide **3** (22.4 g, 63.4 mmol) and H_2O (1.7 ml) dissolved in THF (100 ml). The resulting solution was stirred under argon at room temperature for 5 h when TLC (EtOAc/hexanes 1:1) showed that the reaction was complete. The solution was evaporated under vacuum and the residue was chromatographed on silica gel (150 g) packed in CH_2Cl_2. The column was eluted with CH_2Cl_2 (1900 ml) and then with a stepwise gradient of CH_2Cl_2/MeOH (9:1) (600 ml), CH_2Cl_2/MeOH (7:3) (300 ml), and CH_2Cl_2/MeOH (1:1) (300 ml) to afford 9.28 g of **4** as an oil. Impure fractions were rechromatographed as described to afford an additional 8.16 g of product. Total yield of the amine product **4** was 17.4 g (84%). This amine **4** solidified upon storage at −20°C. TLC (CH_2Cl_2/MeOH 9:1) R_f 0.2, on spot by UV. Mass spec: 328.1 $(M+H)^+$. ^{1}H and ^{13}C NMR ($CDCl_3$) were consistent with structure.

(1S,2S,3S,5S)-5-(2-amino-6-chloropyrimidin-4-yl)amino-3-(benzyloxy)-2-(benzyloxymethyl)cyclopentanol (**5**)

A solution of the cyclopentylamine **4** (19.2 g, 58.6 mmol), 2-amino-4,6-dichloropyrimidine (11.1 g, 67.7 mmol) and Et_3N (40 ml, 0.29 mol) dissolved in *n*-BuOH (100 ml) were heated at 115°C under argon overnight. The solvent was evaporated under vacuum. The residue was dissolved in EtOAc, extracted with H_2O (2 × 50 ml), dried over $MgSO_4$, filtered and evaporated under vacuum to afford crude product. This crude material was chromatographed on silica gel (170 g) eluting with EtOAc/hexanes (1:1) (750 ml) followed by EtOAc

(750 ml). Fractions containing the desired product were combined and evaporated under vacuum to afford 22.0 g (82% yield) of compound **5**. TLC (EtOAc/hexanes 1:1) R_f 0.2, one spot by UV. Mass spec: 455.2 $(M+H)^+$, 453.4 $(M-H)^-$. Analytical Calculated for $C_{24}H_{27}ClN_4O_3$0.25EtOAc: C, 62.95; H, 6.13; N, 11.75; Cl, 7.43. Found: C, 62.87; H, 5.96; N, 12.03; Cl, 7.68.

2-Amino-4-chloro-6-[(1α,2β,3α,4β)-2-hydroxy-4-(phenylmethoxy)-3-[(phenylmethoxy)methyl]cyclopentyl]amino-5-(4-chlorophenyl)diazopyrimidine (**6**)

A solution of $NaNO_2$ (3.44 g, 49.9 mmol) dissolved in H_2O (20 ml) was added dropwise over 10 min to an ice cold solution of 4-chloroaniline (6.08 g, 47.7 mmol) in 3 M HCl (80 ml). The resulting solution was stirred at 0°C for 30 min. This solution of *p*-chlorobenzenediazonium chloride was added dropwise, over 20 min, to a solution of pyrimidine **5** (19.6 g, 43.1 mmol), KOAc (65.0 g, 0.66 mol) and HOAc (150 ml, 2.4 mol) dissolved in CH_3CN/H_2O (2:1) (450 ml). The reaction was stirred at room temperature for 24 h. The suspension was then diluted with H_2O (300 ml) and stirred for 1 h in an ice bath. The solid was isolated by filtration, washed 3×50 ml H_2O, and dried under vacuum to afford 23.3 g (91% yield) of **6** as a solid. TLC ($CHCl_3$/MeOH/HOAc 95:5:0.1) R_f 0.8. HPLC (Method B) RT 33.4 min. Mass spec: 593.1 $(M+H)^+$, 591.3 $(M-H)^-$.

2-Amino-4-methoxy-6-[(1α,2β,3α,4β)-2-hydroxy-4-(phenylmethoxy)-3-[(phenylmethoxy)methyl]cyclopentyl]amino-5-(4-chlorophenyl)diazopyrimidine (**7**)

A solution of 5 M KOH (3.4 ml, 17.0 mmol) was added to a suspension of 5-diazopyrimidine **6** (5.00 g, 8.43 mmol) in MeOH (100 ml). The suspension was stirred at 80°C under a blanket of nitrogen. After 2 h, TLC (CH_2Cl_2/MeOH/HOAc 95:5:0.1) showed the reaction was complete. The suspension was cooled to room temperature and evaporated to dryness under vacuum. The solid was dissolved in CH_2Cl_2 (100 ml) and extracted with half-saturated brine (3×50 ml). The combined aqueous phase was back-extracted with CH_2Cl_2 (2×20 ml). The combined CH_2Cl_2 phase was dried over $MgSO_4$, filtered and evaporated under vacuum to afford an orange foam. The foam was dissolved in CH_3CN and evaporated under vacuum to a solid, redissolved in CH_3CN (70 ml) and allowed to stand at room temperature overnight. The crystalline solid was collected by filtration, washed with CH_3CN (5 ml), and air dried under suction to afford 3.88 g (78% yield) of compound **7** as an orange solid. TLC (CH_2Cl_2/MeOH 95:5) R_f 0.9, one spot by UV.

2-Amino-4-methoxy-6-[(1α,2β,3α,4β)-2-hydroxy-4-(phenylmethoxy)-3-[(phenylmethoxy)methyl]cyclopentyl]amino-5-aminopyrimidine (**8**)

The diazopyrimidine **7** (2.27 g, 3.86 mmol) was suspended in MeOH/H_2O/HOAc (40 ml:2.8 ml:1.65 ml) and heated to 80°C under a blanket of argon.

Solid Zn (2.52 g, 38.5 mmol) was added over 1 min via a solid addition funnel maintained under argon. After 2 h, TLC (EtOAc/hexanes 4:1) showed the reaction was complete. The reaction was cooled to room temperature and the supernatant solution was transferred under argon via cannula to a dry recovery flask. The solution was evaporated under vacuum to afford a yellow residue. The residue was suspended in $CHCl_3$ containing 0.1% HOAc (30 ml) and the insoluble Zn salts were removed by filtration. The solid was washed with 2×10 ml of $CHCl_3$ containing 0.1% HOAc and the combined filtrate was evaporated under vacuum to afford a residue. The residue was chromatographed on a Biotage 40M column. The column was eluted with EtOAc/hexanes (4:1) (500 ml) and then with EtOAc (900 ml). Fractions containing the product were combined and evaporated under vacuum, redissolved in CH_3CN (20 ml) and evaporated under vacuum to afford 1.39 g (77% yield) of **8** as a solid. TLC (EtOAc/hexanes 4:1) R_f 0.2, one spot by UV. ^{1}H NMR was consistent with the structure.

2-Amino-6-methoxy-1,9-dihydro-9-[(1α,2β,3α,4β)-2-hydroxy-4-(phenylmethoxy)-3-[(phenylmethoxy)methyl]cyclopentyl]-6H-purine-8-[^{14}C] (**9**)

A solution of the triaminopyrimidine **8** (933.1 mg, 2.00 mmol) and TsOH (30.4 mg, 0.16 mmol) dissolved in CH_3CN (2 ml) was added to a solution of triethyl [^{14}C]orthoformate (100 mCi, 1.75 mmol) dissolved in CH_3CN (5 ml). The resulting solution was heated at 90°C under a blanket of argon. HPLC showed the reaction to be near completion after 2 h. The reaction was cooled to room temperature and the solvent evaporated via a closed system vacuum transfer to recover 6 mCi of volatile radioactivity. The solid residue was chromatographed on a Biotage 40M column. The column was eluted with EtOAc/hexanes (8:2) (1000 ml) followed by EtOAc:hexanes (9:1) (1000 ml). Fractions containing the desired product were combined and evaporated under a stream of nitrogen at 40°C to afford 716.9 mg (86% yield) of **9** as a light pink solid. HPLC (Method B) RT 16.1 min, Radiochemical purity 99.3%. TLC (EtOAc/hexanes 4:1) R_f 0.15, one spot by radiometric detection. Specific activity was determined to be 116.3 μCi/mg (53.3 mCi/mmol).

[1S-(1α,2β,3α,4β)]-9-[2-hydroxy-4-(phenylmethoxy)-3-[(phenylmethoxy)methyl]-cyclopentyl]-2-[[(4-methoxyphenyl)diphenylmethyl]amino]-6-(methoxy)-9H-purine-8-[^{14}C] (**10**)

DMAP (32.2 mg, 0.26 mmol), Et_3N (312.3 mg, 3.09 mmol) and *p*-anisyl chlorodiphenylmethane (611.6 mg, 1.98 mmol) were added to a solution of purine **9** (716.9 mg, 1.51 mmol, 83.4 mCi) dissolved in CH_2Cl_2 (8 ml). The resulting solution was protected from light and stirred under argon at room temperature. After 3.5 h, HPLC (Method B) and TLC ($CHCl_3$/MeOH/ 95:5) showed the reaction was complete. The solution was evaporated under a

stream of argon to afford a residue. The residue was chromatographed on a Biotage 40L column eluting with $CHCl_3$/MeOH (97:3). Fractions containing the desired product were combined and evaporated under a stream of nitrogen. The resulting solid was dissolved in CH_3CN (30 ml), evaporated under a stream of nitrogen, and dried under high vacuum to afford 1.17 g (104% yield) of **10** as an orange foam.

[1S-(1α,2β,3α,4β)]-9-[2-oxo-4-(phenylmethoxy)-3-[(phenylmethoxy)methyl]cyclopentyl]-2-[[(4-methoxyphenyl)diphenylmethyl]amino]-6-(methoxy)-9H-purine-8-[^{14}C] (**11**)

The alcohol **10** (1.17 g, 1.57 mmol) was dissolved in anhydrous CH_2Cl_2 (10 ml) and placed under argon. To this solution was added *tert*-BuOH (220.1 mg, 2.98 mmol) followed by solid Dess–Martin periodinate (1.34 g, 3.16 mmol). The resultant light green solution was stirred at room temperature under argon. After 1 h, HPLC (Method B) showed the reaction was complete. A solution of 10% Na_2SO_3/10% $NaHCO_3$/brine (5 ml:10 ml:5 ml) was added and the reaction was stirred under argon for 15 min. The aqueous layer was removed and the CH_2Cl_2 layer was further extracted with H_2O (15 ml) and brine (15 ml). The combined aqueous layer was back-extracted with CH_2Cl_2 (5 ml). The combined CH_2Cl_2 layer was dried over Na_2SO_4, filtered, and evaporated under a stream of nitrogen. The residue was dried under high vacuum to afford 1.32 g (113% yield) of crude **11** as a brown foam having a radiochemical purity of 84%. This crude product was used without further purification.

[1S-(1α,3α,4β)]-9-[2-methylene-4-(phenylmethoxy)-3-[(phenylmethoxy)methyl]-2-[[(4-methoxyphenyl)(diphenyl)methyl]amino]-6-(methoxy)-9H-purine-8-[^{14}C] (**12**)

The ketone **11** (1.32 g, 1.57 mmol) was dissolved in CH_2Cl_2 (18 ml), placed under a blanket of argon and cooled to −78°C. Nysted reagent (6.0 ml, 1.42 g, 3.12 mmol) was added to the reaction dropwise over 5 min. To the resultant suspension was added a solution of 1 M $TiCl_4$ in CH_2Cl_2 (3 ml, 3 mmol) dropwise over 5 min. The reaction was allowed to warm to room temperature and stirred under argon. After 1 h, HPLC (Method B) showed the reaction was complete. The reaction was cooled in an ice bath and saturated $NaHCO_3$ (2.5 ml) was added dropwise over 15 min (Caution-gas evolution). The suspension was filtered and the zinc colloid was washed with CH_2Cl_2 (3×10 ml). The combined filtrate was extracted with H_2O (2×25 ml). The combined aqueous layer was back-extracted with CH_2Cl_2 (10 ml). The combined CH_2Cl_2 layer was dried over Na_2SO_4, filtered and the Na_2SO_4 was washed with CH_2Cl_2 (2×5 ml). The combined filtrate and washings were evaporated under a stream of nitrogen and dried under high vacuum to afford

1.15 g of crude **12** as an orange foam. The crude product was chromatographed on a Biotage 40M column eluting with EtOAc/hexanes (3:7) (1000 ml) and then with EtOAc/hexanes (1:1) (1000 ml) to afford 670.6 mg (67%) of **12** with a radiochemical purity of 91%. Mixed chromatography fractions were combined and rechromatographed as described to afford an additional 75.5 mg (7.6%) of compound **12** with a radiochemical purity of 98.5%.

2-Amino-1,9-dihydro-9-[(1S,3R,4S)-2-methylene-4-(phenylmethoxy)-3-[(phenylmethoxy)methyl]cyclopentyl]-6H-purin-6-one-8-[^{14}C] (**13**)

A solution of the compound **12** (670.6 mg, 0.90 mmol) in MeOH/THF (1:1) (6 ml) was heated under a blanket of argon at 60°C and then a solution of 2 M HCl (2 ml, 4 mmol) was added. The reaction was stirred at 60°C overnight at which time HPLC (Method C) showed 10% of the starting material **12** remained. The organic solvent was evaporated under a stream of nitrogen and the resultant aqueous suspension was neutralized to pH 8 by addition of 5 M KOH (0.75 ml). The aqueous phase was extracted with EtOAc (3×10 ml). The combined EtOAc phase was evaporated under a stream of nitrogen and the residue dried under high vacuum to afford 688.8 mg of a foam. This crude material was chromatographed on Biotage 40M eluting with $CHCl_3$ (300 ml) followed by $CHCl_3$/MeOH (93:7). Fractions containing the purified product were combined and evaporated under a stream of nitrogen to afford 361.3 mg (88% yield) of **13** as an orange solid. HPLC (Method B) RT 15.2 min, radiochemical purity 95.8%. Mass spec (exact mass) 460.2199 $(M+H)^+$ consistent for $^{14}C_1^{12}C_{25}H_{27}N_5O_3$.

[^{14}C]Entecavir (**1**)

A solution of **13** (57.9 mg, 0.13 mmol) dissolved in CH_2Cl_2 (1.5 ml) was placed under nitrogen and cooled to −65°C. A 1 M solution of BCl_3 (2.0 ml, 2.0 mmol) dissolved in CH_2Cl_2 was added dropwise over 2 min and the solution was stirred at −65°C for 5 min and then at −20°C for 1 h. HPLC and TLC showed the reaction was complete. The solution was cooled to −78°C and quenched by dropwise addition of MeOH (20 ml) over 2 min. The clear solution was stirred at 0°C for 45 min and then the solvent was evaporated under a stream of nitrogen. The residue was dissolved in H_2O (10 ml) and extracted with $CHCl_3$ (2×5 ml). The combined $CHCl_3$ layer was back-extracted with H_2O (5 ml). The aqueous layer was neutralized by the addition of 1 M NaOH and reduced in volume under a stream of nitrogen. This aqueous solution was chromatographed in two batches on a column (1.5×3 cm) of HP-20 resin eluting with H_2O (10 ml), 2.5% CH_3CN/H_2O (10 ml), 5% CH_3CN/H_2O (10 ml) and 10% CH_3CN/H_2O (55 ml). The product which eluted in 10% CH_3CN/H_2O was evaporated under a stream of nitrogen

to afford 33.9 mg (97%) of [^{14}C]entecavir as a white solid. This was combined with an additional 45.5 mg of [^{14}C]entecavir prepared similar to that described above. HPLC radiochemical purity (Method C) of the combined product was 98.4%.

HP20 purified [^{14}C]entecavir (79.4 mg) was dissolved in hot H_2O (15 ml). The solution was filtered hot (Whatman Autovial, 0.45 μm) and the filter washed with hot H_2O (3 × 2 ml) and CH_3CN (5 ml). The combined filtrate and washings were evaporated under a stream of nitrogen and the solid was crystallized from hot H_2O (2.5 ml). Following crystallization, the solvent was reduced in volume to 0.5 ml under a stream of nitrogen. The crystals were isolated, washed with CH_3CN (0.5 ml), and dried under vacuum to afford 63.2 mg of [^{14}C]entecavir as a white solid (77% yield from **13**). This crystalline[^{14}C]entecavir (63.2 mg) and unlabelled entecavir (61.7 mg) were dissolved in hot H_2O (5 ml) and then evaporated to a solid under a stream of nitrogen. A portion of this solid (107.5 mg) was dissolved in hot MeOH/H_2O (6:4) (5 ml). The hot solution was decolorized with charcoal (4.4 mg). The charcoal was removed by hot filtration (Whatman Autovial, 0.45 μm) and washed with hot MeOH/H_2O (6:4) (3 × 1.5 ml). The combined filtrate and washings were reduced in volume to 2 ml under a stream of nitrogen, heated to afford a solution and allowed to crystallize at room temperature and then at 0°C. The crystals were isolated, washed with CH_3CN (0.5 ml), and dried under vacuum to afford 96.8 mg of [^{14}C]entecavir as a white solid (90% yield). HPLC (Method C) RT 10.3 min, radiochemical purity 99.8%. Specific activity was determined to be 108 μCi/mg.

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