The Synthesis of HIV Reverse Transcriptase Inhibitors [^{14}C]L-697,661, [^{14}C]L-697,639, [^{14}C]L-702,007

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Summary

The synthesis of three carbon-14 labeled reverse transcriptase inhibitors has been accomplished by elaboration of a common intermediate, 5-ethyl-6-methyl-3-amino-2-(1H)-[4-^{14}C]-pyridinone (**7**). Ethyl [1-^{14}C]formate, prepared by esterification of sodium [^{14}C]formate, was combined with 2-pentanone under basic conditions to afford 3-[^{14}C]carboxaldehyde-2-pentanone sodium salt (**2**). The pyridinone ring was constructed by condensation of **2** with nitro acetamide **4**. Reduction of the nitro group afforded 5-ethyl-6-methyl-3-amino-2-(1H)-[4-^{14}C]-pyridinone (**7**)(specific activity 54 mCi/mmol). Subsequent alkylation of **7** provided the desired reverse transcriptase inhibitors [^{14}C]L-697,661, [^{14}C]L-697,639, and [^{14}C]L-702,007.

Key words: HIV reverse transcriptase inhibitor, ethyl [1-^{14}C]formate, amino pyridinone

Results and Discussion

In the course of developing new therapeutic candidates targeted at the HIV reverse transcriptase enzyme, metabolism and distribution studies necessitated the synthesis of three structurally related carbon-14 labeled reverse transcriptase inhibitors: [^{14}C]L-697,661, [^{14}C]L-697,639, and [^{14}C]L-702,007. The synthesis, biological activity, and structure activity relationship data of these compounds have been previously reported.[1] Herein we report the synthesis of 5-ethyl-6-methyl-3-amino-2-(1H)-[4-^{14}C]-pyridinone **7** (Scheme 1), and the elaboration of this common intermediate into the aforementioned reverse transcriptase inhibitors.

Anhydrous ethyl [1-^{14}C]formate (**1**) was conveniently prepared by heating solid sodium [^{14}C]formate with triethylphosphate at 220 °C. Addition of a rigorously dried ethereal solution[2] of **1** to a mixture of 2-pentanone and sodium ethoxide in ethanol afforded 3-[^{14}C]-carboxaldehyde-2-pentanone sodium salt (**2**) in 60% yield along with the isomeric 3-keto-[1-^{14}C]hexenal (**3**) in 26% yield. Condensation of the crude mixture with the ammonium salt of the nitro acetamide (**4**) in water and two equivalents of glacial acetic acid[3] produced 5-ethyl-6-methyl-3-nitro-2-(1H)-[4-^{14}C]-pyridinone (**5**) in 50% yield. Subsequent reduction of the nitro group with 5% Pd/C in methanol under 40 psi H_2 afforded 5-ethyl-6-methyl-3-amino-2-(1H)-[4-^{14}C]-pyridinone (**7**) in 98% yield.

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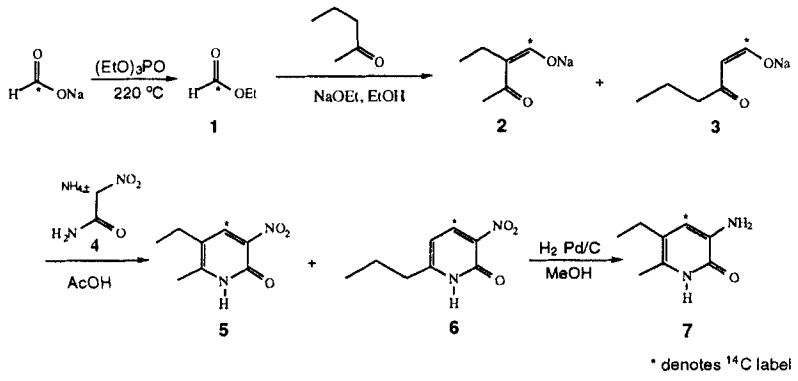
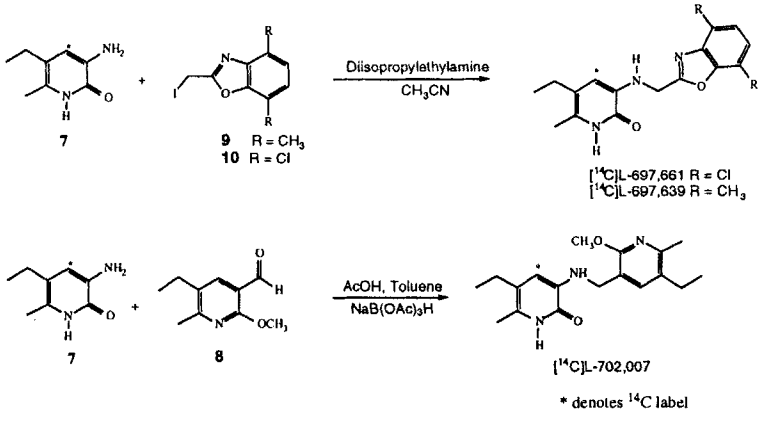
Scheme 1



[^{14}C]L-697,661 and [^{14}C]L-697,639 were prepared by coupling aminopyridinone **7** to the appropriately substituted iodobenzoxazole [1c] (**9** and **10**) in 33 and 41% yield, respectively. [^{14}C]L-702,007 was obtained in a 65% yield through reductive amination of aldehyde **8** with **7** in the presence of sodium triacetoxyborohydride.[4] (Scheme 2) Purification of the crude products by preparative HPLC afforded the final tracers of greater than 97% radiochemical purity.

Scheme 2



In summary, amino pyridinone **7** has proven to be a versitile labeled intermediate in the synthesis of several carbon-14 labeled HIV reverse transcriptase inhibitors. Furthermore, the synthetic route used to prepare **7** can easily be modified to accommodate a variety of different ketone and acetamide components, and thus provide access to several highly functionalized labeled pyridinones.

Experimental

Radioactivity measurments were carried out using a Packard Tri-carb 1000 TR liquid scintillation spectrometer and Scintiverse I^{TM} as scintillation medium. Analytical TLC was performed using silica gel 60 F-254 (E. Merck) with the radioactivity measurements carried out with a Berthold Model LB2760 Scanner.

Analytical HPLC analyses were performed using a Dupont Zorbax RX C-8 column (4.6 mm x 25 cm), Rainin UV-1 detector at 254 nm, Berthold LB-506-C radioactivity monitor, Spectra-Physics SP8810 LC pump and controller, and software run on an IBM PS/2 computer. Preparative HPLC was carried out using Altex pumps with a Beckman UV detector at 254 nm and either a Whatman M9 (25 mm x 25 cm) Partisil or Dupont (25 mm x 25 cm) Zorbax RX C8 column. NMR was obtained on a Bruker EM300 NMR. The identities of the labeled intermediates as well as the final product were established by co-elution via HPLC or TLC with authentic material.

Ethyl [1-^{14}C]Formate

An aqueous solution of sodium [^{14}C]formate in a 1-piece distillation apparatus was concentrated at 100 °C by nitrogen stream, then for 2 h at 100 °C and 0.05 mm Hg to afford dry, solid sodium [^{14}C]formate (164 mg, 127 mCi, 2.34 mmol, S.A. 54.3 mCi/mmol). Triethylphosphate (6 mL) was added and the mixture was heated to 210 °C for 30 min. The volatile ethyl [1-^{14}C]formate distillate was trapped in a flask cooled by a dry ice/2-propanol bath. The refluxing phosphate solution was cooled to room temperature and ether (1.2 mL) was added and distilled at 45 °C to co-distill any residual ethyl [1-^{14}C]formate from the apparatus. The ethereal solution of ethyl [1-^{14}C]formate (98 mCi, 77% yield) was dried over activated 4A molecular sieves (116 mg) for a minimum of 2 h before use in the condensation with 2-pentanone.

3-[^{14}C]-Carboxaldehyde-2-pentanone (2)

Sodium ethoxide in ethanol (2.07 mL of 0.93M, 1.93 mmol) was evaporated to dryness with a nitrogen stream and redissolved in absolute ethanol (230 uL). To the dried solution of ethyl [1-^{14}C]formate (98 mCi, 1.8 mmol) in ether (1.2 mL) was added 2-pentanone (600 uL, 5.79 mmol, 3 equivalents). This solution was then added at 0 °C to the ethoxide solution over 5 min. The mixture was aged for 18 h at room temperature, then concentrated with a nitrogen stream to afford crude 3-[^{14}C]-carboxaldehyde-2-pentanone (**2**) as a white solid. HPLC analysis (Zorbax RX C-8, 80/20 A/B, A = 0.05M K_2HPO_4 pH 8.0, B = CH_3OH) showed the solid contained sodium [^{14}C]formate (14%), desired 3-[^{14}C]-carboxaldehyde-2-pentanone sodium salt (**2**) (60%), and undesired isomer 3-keto[1-^{14}C]-hexenal sodium salt (**3**, 26%).

5-Ethyl-6-methyl-3-nitro-2-(1H)-[4-^{14}C]-pyridinone (5)

To a solution of crude **2** (in 2.3 mL of H_2O) was added nitro acetamide **4** (145 mg, 1.2 mmol) and glacial acetic acid (240 mg, 2 equivalents). The mixture was aged for 64 h at room temperature during which time a yellow precipitate formed. The mixture was cooled to 0 °C, aged for 30 minutes, and filtered to yield 96.7 mg (30 mCi, 53% yield) of the desired [^{14}C]nitropyridone **5** as a yellow crystalline solid. An additional 48 mCi containing a mixture of **5** and isomeric [^{14}C] nitropyridones **6** was obtained by treating the with mother liquors with 1.0 N NaOH, followed by extraction with ethyl acetate. The basic aqueous layer contained 14 mCi of recoverable sodium [^{14}C]formate.

5-Ethyl-6-methyl-3-amino-2-(1H)-[4-^{14}C]-pyridinone (7)

5-Ethyl-6-methyl-3-nitro-2-(1H)-[4-^{14}C]-pyridinone **5** (96.7 mg, 0.53 mmol, 30 mCi) was slurried in methanol (2.5 mL) and 5% Pd/C (10 mg) was added. The mixture was shaken under 40 psi H_2 for 1 h after which all yellow color had disappeared and HPLC analysis (Zorbax RX C-8, 55/45 A/B, A = CH_3CN, B = H_2O, flow 1.0 mL/min) indicated complete consumption of **5**. The mixture was filtered through a pad of Celite and the methanol solution concentrated to yield 29.6 mCi (98% yield) of 5-ethyl-6-methyl-3-amino-2-(1H)-[4-^{14}C]-pyridinone (**7**). NMR (^{1}H, $CDCl_3$): 6.5 (s, 1H, H), 2.3 (m, 2H, CH_2), 2.2 (s, 3H, CH_3), 1.1 (m, 3H, CH_3)

3-[2-(4,7-Dimethylbenzoxazoyl)methyl]amino-5-ethyl-6-methyl-1H-pyridin-2-one [^{14}C]L-697,639

A mixture consisting of [^{14}C]aminopyridinone **7** (15.9 mCi, 0.325 mmol), 2-iodomethyl-4,7-dimethylbenzoxazole **9** (100 mg, 0.326 mmol), diisopropylethyl amine (89 mg, 0.652 mmol) and 2.1 mL of CH_3CN was heated at 35 °C for 5 h, then aged at room temperature for 14 h. HPLC (Zorbax RX C-8, 55/45 A/B, A = CH_3CN, B = H_2O, flow 1.0 mL/min) analysis indicated the reaction was only 50% complete. An additional charge of iodobenzoxazole (75 mg) and diisopropylethyl amine (121 uL) was added and the mixture heated to 35 °C for 18 h. HPLC analysis indicated the reaction to be 95% complete by radioactivity. The solids were filtered and recrystallized from ethanol. Final purification was effected by preparative HPLC (Zorbax RX C8 25mm x 25 cm, 60/40 A/B, A = CH_3CN, B = H_2O). The pure fractions were concentrated in vacuo, then lyophilized to afford 5.2 mCi (33% yield) of 97.9% radiochemically pure [^{14}C]L-697,639. The tracer was redissolved in 20 mL of ethanol for use in the study.

3-[2-(4,7-Dichlorobenzoxazoyl)methyl]amino-5-ethyl-6-methyl-1H-pyridin-2-one [^{14}C]L-697,661

[^{14}C]Aminopyridinone **7** (14.0 mCi, 0.309 mmol), 2-iodomethyl-4,7-dichlorobenzoxazole **10** (102 mg, 0.309 mmol) and diisopropylethyl amine (80 mg, 0.618 mmol) were slurried in 1.6 mL of CH_3CN. The mixture was heated at 35 °C for 5 h, then aged at room temperature for 36 h. HPLC (Zorbax RX C8, 55/45 A/B, A = CH_3CN, B = H_2O, flow 1.0 mL/min) analysis showed that the reaction was only 50% complete. An additional charge of iodobenzoxazole (51 mg) and diisopropylethyl amine (62 uL) were added and the mixture heated to 35 °C for 12 hours. HPLC analysis indicated the reaction to be 70% complete by radioactivity. The solid product was filtered and recrystallized from ethanol. Final purification was effected by preparative HPLC (Whatman Partisil 25 mm x 25 cm, CH_2Cl_2/Ethanol (99/1 to 95/5 gradient over 2h). The pure fractions were concentrated in vacuo to afford 5.8 mCi (41% yield) of [^{14}C]L-697,661. The tracer was redissolved in 20 mL of ethanol for delivery and the radiochemical purity determined by HPLC analysis (as above) was 97.3%.

3-[3-(5-ethyl-2-methoxy-6-methylpyridyl)methyl] amino-5-ethyl-6-methyl-[4-14C]-pyridine-2(1H)-one [^{14}C]L-702,007

To a solution of **7** (6.2 mCi, 16.9 mg, 0.111 mmol) in toluene (0.700 mL) was added aldehyde **8** (20 mg, 0.111 mmol, 1.0 equivalent), followed by acetic acid (9.2 uL, 0.166 mmol, 1.5 equivalents). The mixture was stirred for 20 min and then sodium triacetoxyborohydride (35.2 mg, 0.166 mmol, 1.5 equivalents) was added to the bright yellow solution. The mixture was aged at 25 °C for 2.5 h after which HPLC analysis (Zorbax RX C8, 55/45 A/B, A = CH_3CN, B = H_2O, flow 1.0 mL/min.) indicated complete reaction.

The mixture was diluted with toluene (1.0 mL) and this solution was extracted with saturated aqueous $NaHCO_3$ (2 x 0.5 mL). The organic layer was washed with additional saturated aqueous $NaHCO_3$ (1 x 0.5 mL), filtered, and concentrated to yield 5.7 mCi (92%) of crude [^{14}C]L-702,007. Purification was effected by preparative HPLC (Zorbax RX C-8 column, 70/30 A/B, A= H_2O, B = CH_3CN). The pure fractions were combined and concentrated to afford 3.5 mCi of [^{14}C]L-702,007 as a solid. The radiochemical purity as determined by HPLC analysis (Zorbax RX C8, 65% A, 35% B, A = CH_3CN, B = 0.05 K_2HPO_4 pH 8.0 buffer) was 98.9%.

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2. The ethereal solution of **1** was dried over oven dried 4A molecular sieves for 2h before condensation. Failure to rigorously dry the solution results in extensive hydrolysis of the ethyl [1-^{14}C]formate to sodium [^{14}C]formate.

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