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Synthesis of [^{14}C]- and [$^{13}C_6$]-labeled potent HIV non-nucleoside reverse transcriptase inhibitor

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5,11-Dihydro-11-ethyl-5-methyl-8-{2-{(1-oxido-4-quinolinyl)oxy}ethyl}-6*H*-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one, (1), labeled with carbon-14 in the quinoline–benzene ring, in one of the pyridine rings of the dipyridodiazepinone tricyclic moiety, and in the side chain, was prepared in three different syntheses with specific activities ranging from 44 to 47 mCi/mmol (1.63–1.75 GBq/mmol). In the first synthesis, 5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-6*H*-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (2) was coupled to 4-hydroxyquinoline, [benzene-^{14}C(U)]-, using Mitsunobu's reaction conditions, followed by the oxidation of the quinoline nitrogen with 3-chloroperoxybenzoic acid to give ([^{14}C]-(1a)) in 43% radiochemical yield. Second, 3-amino-2-chloropyridine, [2,6-^{14}C]-, was used to prepare 8-bromo-5,11-dihydro-11-ethyl-5-methyl-6*H*-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (8), and then Stille coupled to allyl(tributyl)tin followed by ozonolysis of the terminal double bond and *in situ* reduction of the resulting aldehyde to alcohol (10). Mitsunobu etherification and oxidation as seen before gave ([^{14}C]-(1b)) in eight steps and in 11% radiochemical yield. Finally, carbon-14 potassium cyanide was used to prepare isopropyl cyanoacetate (12), which was used to transform bromide (8) to labeled aryl acetic acid (13) under palladium catalysis. Trihydroborane reduction of the acid gave alcohol (14) labeled in the side chain, which was used as described above to prepare ([^{14}C]-(1c)) in 4.3% radiochemical yield. The radiochemical purities of these compounds were determined by radio-HPLC and radio-TLC to be more than 98%. To prepare [$^{13}C_6$]-(1), [$^{13}C_6$]-4-hydroxyquinoline was prepared from [$^{13}C_6$]-aniline and then coupled to (2) and oxidized as seen before.

Keywords: NNRT inhibitor; HIV; radiosynthesis; carbon-14; carbon-13

Introduction

Since 1981 more than 25 million people died of AIDS and the number of infections with the HIV has continued to rise at more than 40 000 infections each year.[1] The number of people living with HIV has risen from around 8 million in 1990 to more than 33 millions today.[2] With the availability of highly active antiretroviral therapy in the developed countries, AIDS is complacently being viewed as another chronic disease.[3] The effective drugs usually target the viral protease or reverse transcriptase and to a lesser extent the viral integrase enzymes.[4] However, a single mutation in one of those enzymes may render the drugs ineffective.[5] To combat resistance, a combination of two or three drugs is now the standard therapy. This has made the cost of the treatment prohibitive in most of the developing countries, where in Africa, for example, more than 180 million people are chronically undernourished and too destitute to afford these therapies.

Nevirapine (Figure 1), the first non-nucleoside reverse transcriptase inhibitor ever reported,[6–9] is also effective in reducing mother-to-child viral transmission.[10] Resistance to nevirapine was traced to a single mutation in the reverse transcriptase.[5,11] To overcome this resistance, it was necessary to design and develop protease inhibitors that pick up potency from interaction with amino-acid residues located on the side chain surrounding the binding pocket of nevirapine. Thus, by introducing appropriate substituents on the tricyclic moiety of nevirapine multiple interactions with this enzyme were achieved.[12,13] It was found that introducing an arylethyl substituent at the 8-position of the tricyclic dipyridodiazepinone skeleton increases the potency against resistance.[14] The 8-hetroarylthiomethyldipyridodiazepinone derivatives, for example, were found to have a good potency and a good pharmacokinetic profile albeit poor metabolic stability.[15] The search for compounds with similar potency, but with improved metabolic stability, has lead to the synthesis of (**1**).[16] It has a similar tricyclic structure of nevirapine.[6] The main differences are the introduction at carbon-8 of an ethoxy-4-quinoline-*N*-oxide, the migration of the methyl group from the 4- to the 5-position, and the replacement of the *N*-cyclopropyl with *N*-ethyl. These changes enhance the potency of this compound specifically in patients who have developed resistance to drugs targeting reverse transcriptase. See Figure 1.

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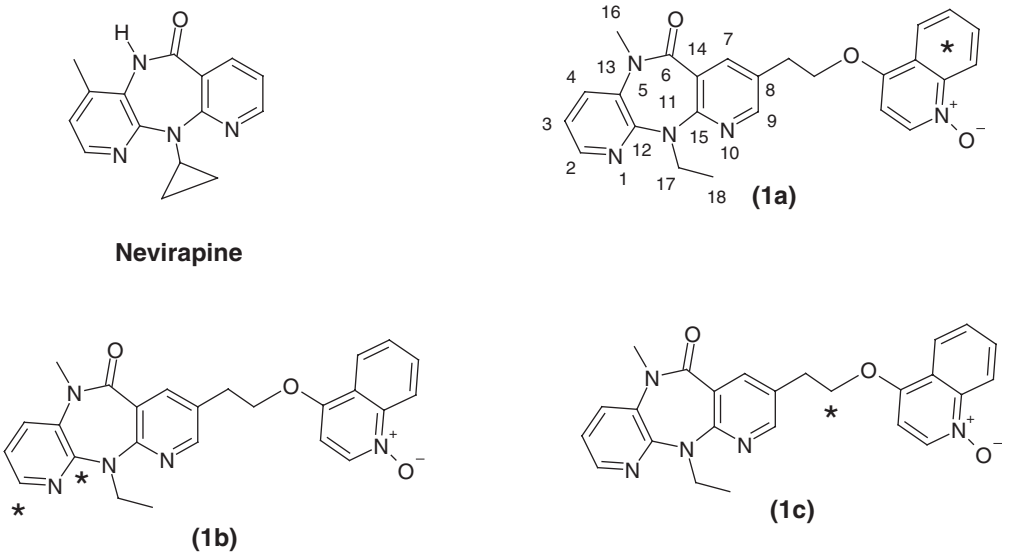
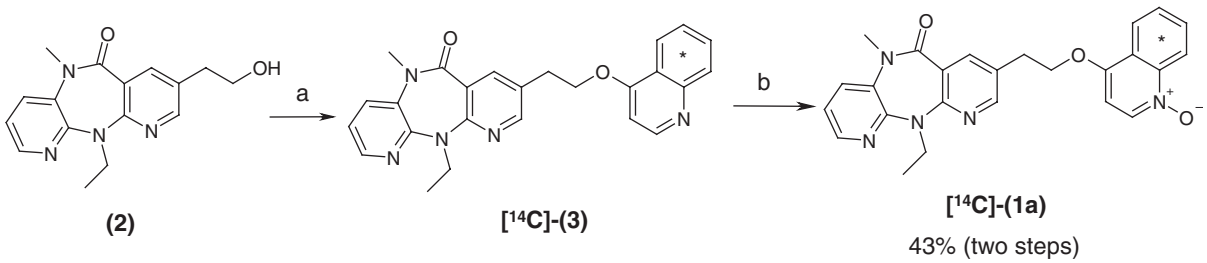


Figure 1. Nevirapine (viramune®) and its analogue (**1**), asterisks indicate the position of carbon-14 atom(s).



Scheme 1. (a) DEAD, Ph_3P, 4-hydroxyquinoline, [benzene-^{14}C(U)]-, THF and (b) *m*-CPBA, CH_2Cl_2.

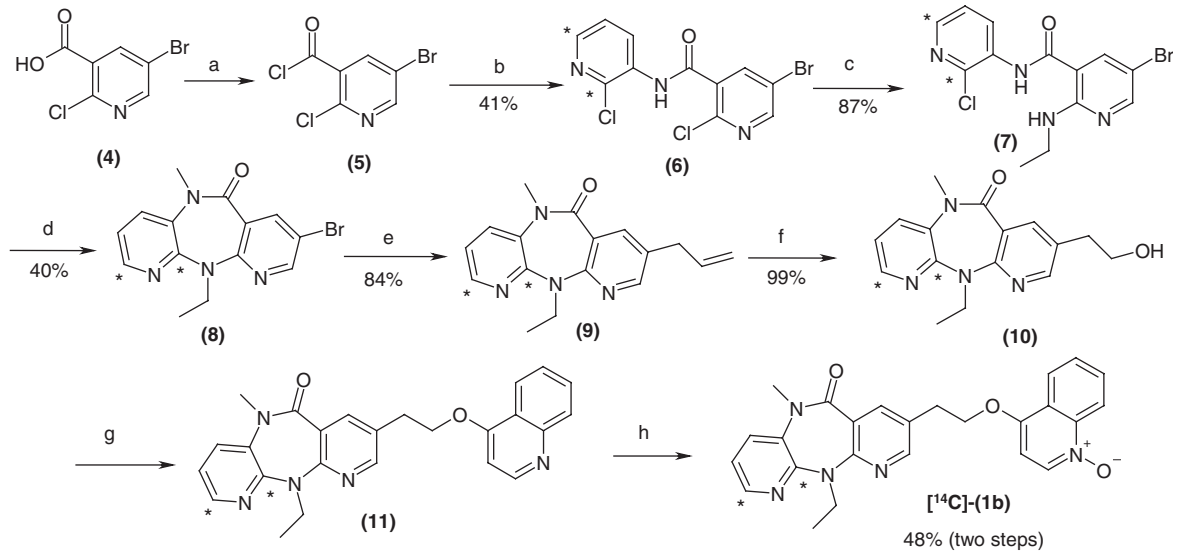
The goal of this work was to prepare carbon-14 labeled (**1**) in different positions of the molecule and [$^{13}C_6$]-(**1**) to support research, drug metabolism, and pharmacokinetic (DMPK) studies.

Results and discussion

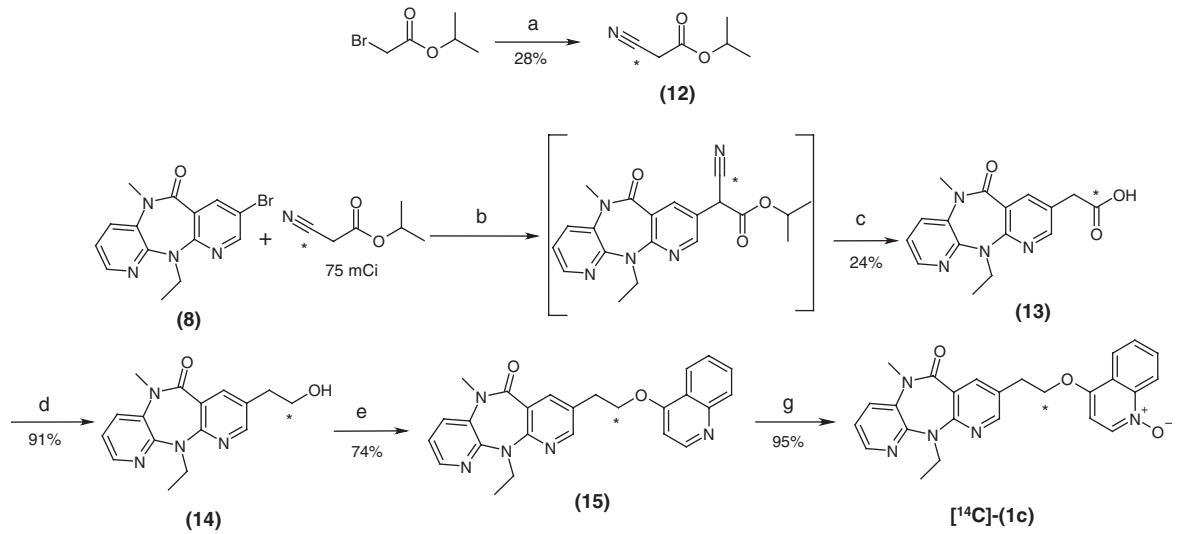
The preparation of **[^{14}C]-(1a)** (Scheme 1) was accomplished in two steps by first coupling [^{14}C]-labeled 4-hydroxyquinoline, uniformly labeled with carbon-14 at the benzene ring and with a specific activity of 51 mCi/mmol, to 5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-6*H*-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**2**) via a Mitsunobu reaction and then oxidizing the quinoline nitrogen with 3-chloroperoxybenzoic acid. The pure [^{14}C]-(**1a**) was obtained in 43% radiochemical yield. In the Mitsunobu coupling reaction, we found that using a slight excess of alcohol (**2**) was necessary to consume all the labeled 4-hydroxyquinoline. The unreacted alcohol and the product of the coupling (**3**) eluted close to each other on a silica gel TLC plate. No efforts were made to separate these materials; they were used as a mixture in the following oxidation with 3-chloroperoxybenzoic acid. The [^{14}C]-(**1a**) is more polar than (**2**) and was easily separated by flash chromatography. The desired product (**1a**) with a specific activity of 46.68 mCi/mmol (1.73 GBq/mmol) was thus obtained. HPLC and radio-TLC showed that the purity of [^{14}C]-(**1a**) exceeded 98%. The purity was nearly unchanged after 3 weeks of storage at −80°C.

Methyl and ethyl groups were not considered to incorporate the radioactive isotopes because of their metabolic cleavage in animal studies. However, (**1**) was found to undergo a non-enzymatic cleavage of the quinoline moiety. This prompted us to consider introducing the radioactive carbon either in the dipyridodiazepinone skeleton or on the C8 side chain. With the availability of 3-amino-2-chloropyridine, [2,6-^{14}C]-, we followed the route developed by Boehringer's chemists to prepare (**1**) on a large scale to introduce the C14 atoms in the tricyclic moiety.[16,17] Thus, 5-bromo-2-chloronicotinoyl chloride (**5**) was reacted with 3-amino-2-chloropyridine, [2,6,-^{14}C]-, in acetonitrile and a saturated solution of sodium bicarbonate to give the amide (**6**) in 41% yield. Treatment with a THF solution of ethylamine gave the amine (**7**) in 87% yield. The bromide (**8**) was prepared in one pot via intramolecular S_NAR followed by anion trapping with methyl iodide. Stille's reaction gave the C8 allyl substituted (**9**). The double bond was oxidized with ozone and the resulting aldehyde was reduced *in situ* to the alcohol (**10**) in 99% yield. Mitsunobu etherification with 4-hydroxyquinoline and oxidation with 3-chloroperoxybenzoic acid in methylene chloride as described above gave [^{14}C]-(**1b**) in 11% yield in eight steps and a specific activity of 44 mCi/mmol (1.63 GBq/mmol), Scheme 2.

Isopropyl cyanoacetate was found to undergo arylation by bromide (**8**) under palladium catalysis using triphenylphosphine as ligand. This reaction was followed by hydrolysis and decarboxylation to the carboxylic acid in one pot.[17] This one

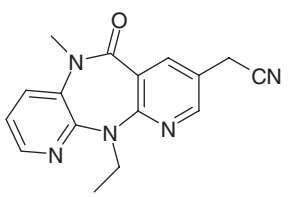


Scheme 2. (a) $SOCl_2$; (b) 3-amino-2-chloropyridine, [2,6-^{14}C]-, $NaHCO_3$, MeCN; (c) $EtNH_2$, THF; (d) NaHMDS, pyridine, MeI, THF; (e) allyl(tributyl)tin, $(Ph_3P)_4Pd$, DMF; (f) O_3, 10% $MeOH/CH_2Cl_2$, $NaBH_4$; (g) 4-hydroxyquinoline, DEAD, Ph_3P, THF; and (h) *m*-CPBA, CH_2Cl_2.

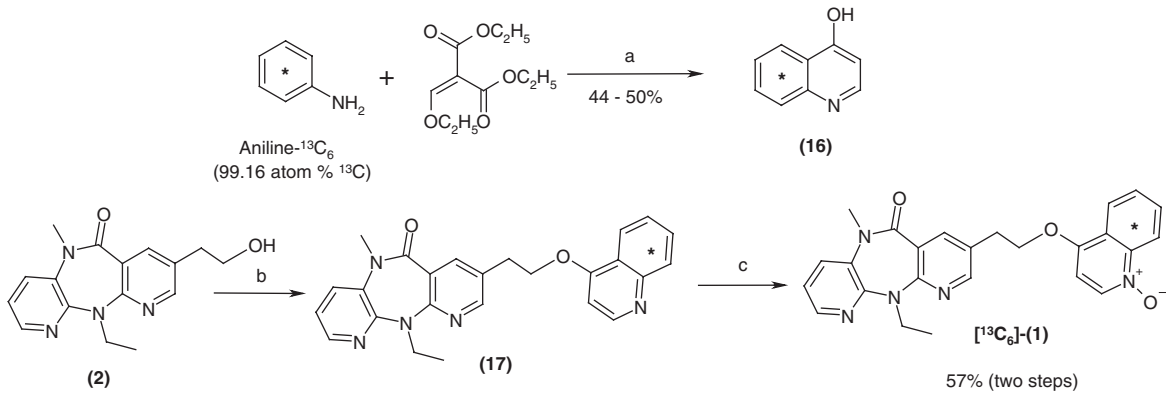


Scheme 3. (a) KCN, [^{14}C]-, 25% $MeOH/H_2O$; (b) $Pd(OAc)_2$, Ph_3P, NaH, $PhCH_3$; (c) NaOH, 80°C; (d) BH_3–THF; (e) 4-hydroxyquinoline, DIAD, Ph_3P, THF; and (f) *m*-CPBA, CH_2Cl_2.

pot transformation of the bromide (**8**) to carboxylic acid (**13**), Scheme 3, was shown to proceed via ester hydrolysis first and then there is loss of carbon dioxide before the cyano group gets hydrolyzed to the acid. Therefore, when carbon-13 labeled at the cyano carbon of isopropyl cyanoacetate was used in this procedure, it gave the carboxylic acid with more than 95% of the carbon-13 retained in the product.[17] The arylacetonitrile intermediate was isolated and characterized.[18]



Arylcyanoacetates have been reported to undergo hydrolysis to arylacetonitriles using diluted hydrochloric acid.[19] Arylacetonitriles can be obtained also from arylbromides and trimethylsilylacetonitrile as described by Wu and Hartwig.[20] The carbon-14 isopropyl cyanoacetate was prepared from the reaction of isopropyl bromoacetate and potassium cyanide.[21] While the non-radioactive synthesis gave yields comparable to the literature, the radioactive synthesis gave the labeled cyanoacetate in only 28% yield.[22] This was probably due to the use of partially degraded carbon-14 potassium cyanide. Reduction of acid (**13**) to alcohol (**14**) was accomplished using BH_3/THF in 91% yield.[17] Mitsunobu's etherification with 4-hydroxyquinoline derivative gave (**15**) in 74% yield. Oxidation with 3-chloroperoxybenzoic acid in methylene chloride as seen before gave [^{14}C]-(**1c**) in 95% yield and a specific activity of 47.29 mCi/mmol (1.75 GBq/mmol) and a radiopurity of 99%. This material was



Scheme 4. (a) Ph_2O, NaOH, heat; (b) DIAD, Ph_3P, THF, (**16**); and (c) *m*-CPBA, CH_2Cl_2.

prepared to continue DMPK studies after [^{14}C]-(**1b**) was consumed.

To prepare [$^{13}C_6$]-(**1**), Scheme 4, the commercially available [$^{13}C_6$]-aniline was condensed with diethyl ethoxymethylene malonate first at 110°C neat and then in phenyl ether at 260°C. Saponification, followed by decarboxylation, gave [$^{13}C_6$]4-hydroxyquinoline in 44% overall yield.[23–26] Mitsunobu's reaction with (**2**) and oxidation with *m*-CPBA as seen before gave [$^{13}C_6$]-(**1**) in 57% in two steps. The product was more than 99 at% ^{13}C.

Materials and methods

General

Liquid scintillation counting was accomplished using a Beckman LS5000TA and ready safe™ cocktail (Beckman, Fullerton, CA). Radio-TLC was carried out on a Bioscan System 200 imaging scanner using an auto change 1000 and WinScan software version 2.1a (Bioscan Inc., Washington, DC). The quantification of the HPLC chromatograms was carried out using an HPLC system composed of a radiomatic A515 Flo-oneradioactivity flow detector (Packard Instrument Company, Meriden, CT), two pumps (HITACHI L-6200A intelligent pump), a linear UVIS 200, Ultima Flo™ AP cocktail (Packard), and radiomatic 500TR V 3.60 for data evaluation. Mass spectra for non-radioactive compounds were acquired by a Hewlett-Packard auto sampler Series 1150 connected to a Micromass Platform LCZ in the ES mode. NMR spectra were recorded with a Bruker 400 MHz DPXB spectrometer using deuterated chloroform as a solvent and tetramethyl silane as the internal standard. Double encapsulation was used for carbon-14-labeled compounds. Pre-coated TLC sheets (silica gel 60 F_{254}) and silica gel 60–200 mesh (nominal, ID, grade 62) for flash chromatography were obtained from EM Science (Gibbstown, NJ). Most of the reagents were purchased from Aldrich Chemicals. 4-Hydroxyquinoline, [benzene-^{14}C(U)]-, was purchased from Moravek Biochemicals Inc. (Brea, CA) with a specific activity of 51 mCi/mmol. 3-Amino-2-chloropyridine, [2,6-^{14}C]-, with a specific activity of 49 mCi/mmol was obtained from GE Healthcare (Piscataway, NJ). Potassium cyanide, [^{14}C]-, with a specific activity of 54 mCi/mmol was purchased from American Radiolabeled Compounds (St Louis, MO). Specific activities were determined by weight assays. [$^{13}C_6$]-aniline was purchased from Isotec (Miamisburg, OH). The analytical HPLC purity verification was carried out on a Zorbax SB-C18 column, particle size 5 μm, 4.6 × 150 mm, and the column was fitted with an OPTIGuard C18 column guard (1.0 mm) and a gradient mobile phase from 10 to 100% water in acetonitrile (both solvents contain 10 mM TFA) and UV detection at 220 nm and radioactivity flow detector described above.

Synthesis

Synthesis of [^{14}C]-(1a)

*5,11-Dihydro-11-ethyl-5-methyl-8-{2-(4-quinolinyloxy)[benzene-^{14}C(U)]ethyl}-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**3**)*

To a solution of 4-hydroxyquinoline, [benzene-^{14}C(U)]- (25 mCi, 0.49 mmol), triphenylphosphine (196.7 mg, 0.75 mmol), and 5,11-dihydro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-6*H*-dipyrido-[3,2-b:2′,3′-e][1,4]diazepin-6-one (223.8 mg, 0.75 mmol) in anhydrous THF (10 mL) was added diethyl azodicarboxylate (DEAD, 118 μL, 0.75 mmol) and the resulting solution was stirred overnight at room temperature under nitrogen atmosphere. The solution was then concentrated *in vacuo* and the residue was purified by flash chromatography using methanol/chloroform (1–10%). Vials containing the excess of alcohol (**2**) and the desired product (**3**) (290 mg, 75% pure) were combined and concentrated under reduced pressure. This mixture was used in the next step without further purification.

*5,11-Dihydro-11-ethyl-5-methyl-8-{2-{(1-oxido-4-quinolinyl)[benzene–^{14}C(U)]oxy}-ethyl}-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one, [^{14}C]-(**1a**)*

To a solution of the above mixture in chloroform (15 mL) was added *m*-CPBA (3-chloroperoxybenzoic acid, 375 mg) in one portion and the resulting solution was stirred at room temperature for 2 h. A saturated solution of $Na_2S_2O_3$ (2 × 10 mL) was then added and the mixture was stirred vigorously. The aqueous phase was pipetted out. A saturated solution of $NaHCO_3$ (2 × 10 mL) was added next and after stirring vigorously the aqueous phase was pipetted out. The chloroform solution was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography using first 50% ethyl acetate:chloroform to

remove non-polar impurities and then 5–25% methanol/chloroform was used. Vials containing the pure product were combined and concentrated *in vacuo*. The residue was dissolved in methylene chloride and precipitated by adding hexane. The white solid was dried to give 102 mg of pure product. The total activity of the pure product was 10.73 mCi and the specific activity was determined to be 46.68 mCi/mmol or 74.8% isotopic enrichment and more than 98% radiochemical purity. ^{1}H NMR in $CDCl_3$ was identical to an unlabeled standard.[17]

Synthesis of [^{14}C]-(1b)

5-Bromo-2-chloro-N-*(2-chloro-[2,6-^{14}C]-pyridin-3-yl)-nicotinamide (**6**)*

5-Bromo-2-chloro-nicotinic acid (**4**) (307 mg, 1.3 mmol) in $SOCl_2$ (5 mL) was refluxed for 2.5 h. The solution was cooled to room temperature and concentrated *in vacuo*. Hexane was added to the liquid residue and then evaporated *in vacuo*. The residue was diluted with MeCN (20 mL), $NaHCO_3$ (420 mg, 5 mmol) and 3-amino-2-chloropyridine, [2,6-^{14}C]- (141 mg, 1.064 mmol, 53.7 mCi at 47 mCi/mmol), were added and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated, diluted with H_2O, extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated giving **[^{14}C]-6** (330 mg, 72% chemical yield, 41.3 mCi at 43.9 mCi/mmol) as an off-white solid. TLC (50% EtOAc/hexane) was identical to an authentic unlabeled sample.

5-Bromo-N-*(2-chloro-[2,6-^{14}C]-pyridin-3-yl)-2-ethylamino-nicotinamide (**7**)*

A solution of the above compound (330 mg, 0.940 mmol) and 2 M $EtNH_2$ in THF (10 mL) was heated to 100°C and stirred overnight. The resulting yellow solution was then cooled to room temperature and excess ethylamine and THF were removed under a stream of nitrogen. The residue was diluted with CH_2Cl_2, washed with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and concentrated giving a yellow solid. The crude solid was re-crystalized from MeOH to give 160 mg of the product in 47% chemical yield (19.5 mCi at 43.9 mCi/mmol) as yellow crystals in 98% radiochemical purity. The mother liquor was concentrated giving 140 mg, 17.1 mCi as a red brown solid. TLC (50% EtOAc/hexane) analysis of the product was consistent with an authentic unlabeled sample.

*8-Bromo-[2,11a-^{14}C]-5,11-dihydro-11-ethyl-5-methyl-6H-dipyrido-[3,2-b:2′,3′-e][1,4]diazepin-6-one (**[^{14}C]-8**)*

NaHMDS (1 mL, 1.0 mmol, 1.0 M in THF) was added to a solution of (**[^{14}C]-7**) (160 mg, 0.445 mmol) in pyridine (5 mL) at room temperature. The resulting dark solution was heated to 50°C and stirred for 0.5 h. The reaction was cooled to room temperature. Methyl iodide (142 mg, 1.0 mmol) was added and stirred overnight. The dark mixture was diluted with ethyl acetate and washed twice with 0.5 M HCl. The organic material was dried over Na_2SO_4 and concentrated giving a brown solid. The residue was purified by flash chromatography (25% EtOAc/hexane) to give (**[^{14}C]-8**) (114 mg, 76% chemical yield, 14.8 mCi at 43.9 mCi/mmol) as a yellow solid. TLC (50% EtOAc/hexane) of the product was consistent with an authentic unlabeled sample.

*8-(2-Propenyl)-[2,11a-^{14}C]-5,11-dihydro-11-ethyl-5-methyl-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**9**)*

$Pd(PPh_3)_4$ (20 mg, 0.017 mmol), (**[^{14}C]-8**) (114 mg, 0.338 mmol), and $Bu_3SnCH_2CH=CH_2$ (125 mg, 0.378 mmol) in DMF (5 mL) were sealed under N_2 and heated at 85°C for 2.5 h. The reaction mixture was concentrated under high vacuum. The residue was then diluted with EtOAc (30 mL) and 1 M KF (25 mL) and stirred vigorously for 1.5 h. The mixture was filtered, then the organic layer was separated, dried over Na_2SO_4, and concentrated giving a white semi-solid. TLC showed only partial conversion; therefore, the material was re-subjected to the above reaction conditions and stirred overnight. The reaction was worked up as above and the crude residue was purified by flash chromatography (25% EtOAc/hexane) to give (**[^{14}C]-9**) (84 mg, 83.3% chemical yield, 12.4 mCi at 43.9 mCi/mmol) as a colorless oil. TLC (25% EtOAc/hexane) of the product was consistent with an authentic unlabeled sample.

*8-(2-Hydroxy-ethyl)-[2,11a-^{14}C]-5,11-dihydro-11-ethyl-5-methyl-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**10**)*

A stream of O_3 in O_2 was bubbled through a solution of the above alkene (85 mg, 0.285 mmol) in 10% MeOH/CH_2Cl_2 (15 mL) at −78°C until a blue color persisted in the solution (15 min). Nitrogen was bubbled through the solution to remove excess O_3 (15 min), then $NaBH_4$ (80 mg, 2.13 mmol) was added. The mixture was stirred at −78°C for 0.5 h, then warmed to room temperature and stirred for 2 h. The reaction was quenched by the addition of saturated NH_4Cl, extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated giving the alcohol (**[^{14}C]-10**) (86 mg, 99% chemical yield, 12.5 mCi at 43.9 mCi/mmol) as a light yellow solid. TLC (5% MeOH/CH_2Cl_2) of the product was similar to an authentic unlabeled sample.

*8-[2-(Quinolin-4-yloxy)-ethyl]-[2,11a-^{14}C]-5,11-dihydro-11-ethyl-5-methyl-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**11**)*

DEAD (77 mg, 0.442 mmol) was added to a solution of (**[^{14}C]-10**) (86 mg, 0.284 mmol), 4- hydroxyquinoline (64 mg, 0.441 mmol), and Ph_3P (115 mg, 0.439 mmol) in THF (7 mL) at room temperature. The mixture was stirred at room temperature for 16 h and concentrated giving a yellow oil. The oil was purified by flash chromatography (5%MeOH/EtOAc) to give 65 mg, 53% chemical yield, 6.6 mCi at 43.9 mCi/mmol of a white solid. TLC (10% MeOH/CH_2Cl_2) was consistent with an authentic unlabeled sample.

*8-(1-Oxy-quinolin-4-yloxy)-ethyl]-[2,11a-^{14}C]-5,11-dihydro-11-ethyl-5-methyl-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**[^{14}C]-1b**)*

m-CPBA (75 mg, 0.435 mmol) was added to a solution of the above compound (65 mg, 0.151 mmol) in CH_2Cl_2 (10 mL) at room temperature. After stirring for 1.5 h, the reaction was diluted with EtOAc and quenched by the addition of saturated solution of $Na_2S_2O_3$. The organic material was washed sequentially with saturated $Na_2S_2O_3$, saturated $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated giving an off-white solid. The residue was purified by flash chromatography (5–7.5% MeOH/CH_2Cl_2) to give **[^{14}C]-(1b)** (59 mg, 88% chemical yield, 5.9 mCi at 43.9 mCi/mmol) as a white solid. HPLC: retention time 12.0 min; UV 220 nm 99%; radio-LSC flow detection 98%. TLC:

(20% MeOH/$CHCl_3$) $R_f = 0.67$; 98%. Authentic (1) sample TLC: (20% MeOH/$CHCl_3$) $R_f = 0.67$. ^1H NMR in $CDCl_3$ was identical to the unlabeled standard.[17]

Synthesis of ([^{14}C]-1c)

*[^{14}C]-isopropyl cyanoacetate (**12**)*

To a solution of $K^{14}CN$ (340 mg, specific activity = 54.1 mCi/mmol, 5.22 mmol) in water (2 mL) and methanol (0.5 mL) was added isopropyl bromoacetate dropwise at room temperature. The mixture was stirred for 48 h before a saturated solution of $NaHCO_3$ was added. The mixture was extracted with ethyl acetate (3 × 10 mL) and the combined extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a yellowish oil. Purification by flash chromatography using a 12 g RediSep™ disposable column and a gradient ethyl acetate/hexane (0–50% EtOAc) gave 176 mg of a colorless oil of pure product in 28% yield. The low yield is maybe due to using a batch of $K^{14}CN$ that has been stored at −20°C for more than 15 years. In the unlabeled run the yield was comparable to the literature. $R_f = 0.16$ in 15% EtOAc:hexane.

*5,11-Dihydro-11-ethyl-8-(2-[^{14}C]-carboxyethyl)-5-methyl-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**[^{14}C]-13**)*

To a mixture of bromide (**8**) (458.5 mg, 1.38 mmol), palladium acetate (3.1 mg, 0.0138 mmol), and triphenylphosphine (14.52 mg, 0.055 mmol) in toluene (4 mL) was added sodium hydride (60% oil dispersion, 138.4 mg, 3.46 mmol). The resulting mixture was heated to 60°C in an oil bath and [^{14}C]-isopropyl cyanoacetate (**12**) in toluene (3 mL) was added dropwise slowly. After the addition was complete, the reaction was heated to 100°C and stirred overnight. The mixture was cooled to room temperature and treated with isopropanol to quench the excess sodium hydride. A solution of 1.0 M NaOH (5 mL) was then added and the mixture was heated to 80°C and stirred overnight. The reaction was then cooled to room temperature and the organic phase was removed. The aqueous phase was extracted with ethyl acetate (5 mL) before it was treated with a solution of sulfuric acid (6.0 N) at 0°C until pH = 3. The resulting mixture was stirred for 45 min at this temperature and then extracted with ethyl acetate. The combined extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to a solid residue. Purification by flash chromatography gave 107 mg of the acid in 24% yield or 18.3 mCi.

*5,11-Dihydro-11-ethyl-8-(2-[^{14}C]-hydroxyethyl)-5-methyl-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**[^{14}C]-14**)*

To a solution of the above carboxylic acid (107 mg, 0.34 mmol, 18.3 mCi) in dry THF (6 mL) was added a solution of borane–THF (0.8 mL, 1.0 M) dropwise at 0°C. The reaction was warmed to room temperature and stirred for 6 h. The reaction was quenched with 0.5 mL of 1:1 acetic acid:water and most of the solvent was removed *in vacuo*. The residue was diluted with ethyl acetate and washed with a saturated solution of $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was purified by flash chromatography using a gradient 0–10% methanol in methylene chloride to give 93 mg of material in 91% chemical yield.

*5,11-Dihydro-11-ethyl-5-methyl-8-{2-(4-quinolinyloxy)[^{14}C]ethyl}-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**[^{14}C]-15**)*

To a mixture of the above alcohol (93 mg, 0.312 mmol), triphenylphosphine (123 mg, 0.47 mmol), and 4-hydroxyquinoline (50 mg, 0.34 mmol) in dry THF (10 mL) was added diisopropyl azodicarboxylate (DIAD, 93 μL, 0.45 mmol) dropwise at room temperature under nitrogen atmosphere. The reaction was stirred overnight before it was concentrated *in vacuo* and the product was isolated by flash chromatography in 74% yield or 11.4 mCi and with a specific activity of 49.43 mCi/mmol.

*5,11-Dihydro-11-ethyl-5-methyl-8-{2-{(1-oxido-4-quinolinyl)[^{14}C]-oxy}ethyl}-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one, [^{14}C]-(**1c**)*

m-CPBA (90 mg, 77% max.) was added in one portion to a solution of (**15**) (80 mg, 0.188 mmol, 9.3 mCi) in methylene chloride (5 mL) at room temperature. The reaction was stirred for 2 h and then it was treated with a 10% solution of $Na_2S_2O_3$ (2 × 5 mL) and with a saturated solution of $NaHCO_3$ (3 × 5 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The pure product was isolated after flash chromatography in 95% yield and with a specific activity of 47.29 mCi/mmol. The radiochemical purity was more than 98% by HPLC. TLC of this material co-migrated with the unlabeled standard and ^1H NMR in $CDCl_3$ (500 MHz) was identical to the unlabeled standard.[17]

Synthesis of [$^{13}C_6$]-(1)

*[$^{13}C_6$]-4-hydroxyquinoline (**16**)*

A solution of aniline-$^{13}C_6$ (2.0 g, 20 mmol, 99.16 atom % ^{13}C) and diethyl ethoxymethylene malonate (5.678 g, 26.26 mmol) was heated to 110°C for 1 h in a 50 mL round-bottom flask in the open to remove ethanol. To this was added phenyl ether (15 mL) and the solution was heated to 260°C for 1 h. After cooling the reaction to 100°C, a solution of 10% aqueous NaOH (21 mL) was added and stirred at this temperature until all the solid was dissolved in about 1–2 h. After cooling to room temperature, the organic layer was removed and the aqueous was extracted with ether (2 × 25 mL). The aqueous layer was then treated with concentrated aqueous HCl (3.5 mL) to give a precipitate that was filtered, washed with water, and dried in open air in the frit. The solid was further dried at 50°C under reduced pressure to give 1.2 g of a cream-colored solid. The unreacted aniline-$^{13}C_6$ was extracted from the organic phase by aqueous HCl (2 N, 30 mL). LC-MS: $M^- = 194.41$ as the only peak at 0.91 min. The aqueous extracted was combined and treated with aqueous NaOH (4 N) until pH = 12, and then extracted with methylene chloride. The extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 0.7 g of a pale yellow oil. Thus, the yield of 3-carboxy-4-hydroyquinoline based on reacted aniline was 47%. This acid (1.1 g, 5.61 mmol) was suspended in phenyl ether (15 mL) and heated to 282°C for 1 h. After cooling to room temperature, the resulting suspension was treated with anhydrous ether, filtered, and dried in the air in the frit to give 0.8 g of an off-white solid in 94% crude yield. ^1H NMR (MeOH-d_4) δ: 8.30 (dm, $J_{H-C} = 1$ Hz), 7.97 (t, $J = 7.66$ Hz, 1H), 7.71 (dm, $J_{H-C} = 158.73$ Hz, 1H), 7.75 (dm, $J_{H-C} = 162.11$ Hz, 1H), 7.40 (dm, $J_{H-C} = 163.72$ Hz, 1H), 6.34 (dt, $J = 1.68$, 5.33 Hz, 1H). ^{13}C NMR (MeOH-d_4) δ: 142.30 (m), 134.5 (enhanced m), 124–128 (m), 119.40 (m). LC-MS: $R_t = 0.36$ min, $MH^+ = 152.46$ as the only peak.

*[$^{13}C_6$]-5,11-dihydro-11-ethyl-5-methyl-8-{2-(4-quinolinyloxy)ethyl}-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (**17**)*

To a mixture of (**2**) (0.9 g, 3.0 mmol), triphenylphosphine (1.311 g, 5.0 mmol), and the above 4-hydroxyquinoline (0.5 g, 3.31 mmol) in anhydrous THF (50 mL) was added DIAD (1.036 mL, 5.0 mmol) dropwise at room temperature under nitrogen to give a solution by the end of the addition. After stirring for 12 h, the solution was concentrated under reduced pressure to a yellow foam. Purification by flash chromatography using 0–10% methanol in methylene chloride gave 0.95 g of the product in 73% yield and 100 mg of unreacted 4-hydroxyquinoline. LC-MS: $R_t = 1.10$ min, $MH^+ = 432.23$ as the only peak.

*[$^{13}C_6$]-5,11-dihydro-11-ethyl-5-methyl-8-{2-{(1-oxido-4-quinolinyl)-oxy}ethyl}-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one [$^{13}C_6$]-(**1**)*

To a solution of the above compound (0.75 g, 1.74 mmol) in CH_2Cl_2 (25 mL) was added *m*-CPBA (0.5 g, 77% max.). The resulting solution was stirred for 2 h, then treated with a solution of $Na_2S_2O_3$ (100 mL), a saturated solution of $NaHCO_3$ (100 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 0.95 g of a yellow solid. Purification by flash chromatography using 5% MeOH/CH_2Cl_2 to remove non-polar impurities and then with 30–50% MeOH/CH_2Cl_2 gave the pure product in 77% yield or 0.6 g. HPLC: ^{1}H NMR ($CDCl_3$) δ: 8.71 (dm, $J_{H-C} = 170.35$ Hz, 1H), 8.36–8.43 (m, 2H), 8.20 (dm, $J_{H-C} = 165.21$ Hz, 1H), 8.19 (dd, $J = 1.63$, 4.68 Hz, 1H), 8.10 (d, $J = 2.45$ Hz, 1H), 7.85 (dm, $J_{H-C} = 164.31$ Hz, 1H), 7.71 (dm, $J_{H-C} = 162.35$ Hz, 1H), 7.48 (dd, $J = 1.59$, 7.93 Hz, 1H), 7.08 (dd, $J = 4.66$, 7.94 Hz, 1H), 6.57 (t, $J = 6.99$ Hz, 1H), 4.34 (t, $J = 6.31$ Hz, 2H), 4.19 (q, $J = 7.05$ Hz, 2H), 3.51 (s, 3H), 3.22 (t, $J = 6.31$ Hz, 2H), 1.26 (t, $J = 7.05$ Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 149.27, 142.57, 138.85–139.94 (m), 128.68–129.59 (m), 125.52–127.10 (m), 120.11–121.28 (m), 117.51–118.57 (m), 67.06, 39.30, 35.45, 29.83, 11.71. LC-MS: $R_t = 1.19$ min, $MH^+ = 448.45$ as the only peak. HPLC: 8.35 min, 99%.

Conclusion

A potent non-nucleoside reverse transcriptase inhibitor was prepared with the radioactive carbon atom(s) in different locations of the molecule to perform metabolism, pharmacokinetics, and other studies. The specific activities ranged from 44 to 47 mCi/mmol. This inhibitor labeled with carbon-13 was also synthesized to support analytical studies.

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