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Asymmetric Synthesis of *(R)*-(+)-[[(2-Bromoethyl)amino]methyl]-2-nitro-1H-imidazole-[1-^{14}C]-ethanol monohydrobromide

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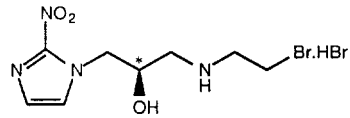
SUMMARY

The enantioselective synthesis of *(R)*-(+)-2-(*tert*-butyldiphenylsiloxylmethyl)[^{14}C]oxirane from ^{14}C-labeled barium carbonate was accomplished. This labeled oxirane facilitated an asymmetric synthesis *(R)*-(+)-[[(2-bromoethyl)amino]methyl]-2-nitro-1H-imidazole-[1-^{14}C]-ethanol monohydrobromide (CI-1010), a potent hypoxic cell selective radiosensizing anti-cancer agent. From labeled barium carbonate the labeled oxirane was prepared in 26.2% yield, and this gave the target labeled CI-1010 in 17% yield.

INTRODUCTION

(R)-(+)-α-[[2-Bromoethyl)amino]methyl]-2-nitro-1H-imidazole-[1-14C]ethanol monohydrobromide (CI-1010) (12) is one of the more potent hypoxic cell selective cytotoxic 2-nitro-imidazole derivatives to have emerged from our program to develop these compounds as radiosensitizing agents for cancer therapy.[1] Carbon-14 labeled (12) was needed for pharmacokinetics and metabolic studies.



(12), CI-1010

Originally, the unlabeled (12) was prepared starting from commercially obtained enantiopure *(R)*-epichlorohydrin, *(S)*-glycidyl tosylate or 1-glycidyl-2-oxazolidinone derived from the foregoing compound.[2] In these earlier work, epichlorohydrin or glycidyl tosylate reacted with 2-nitro-imidazole to make the adduct that was transformed into target compound (12). The alternative preparation whereby 1-glycidyl-2-oxazolidinone was condensed with 2-nitro-imidazole and deprotected to target compound was also been reported. Making the labeled CI-1010 by anyone of these strategies presented the challenge of

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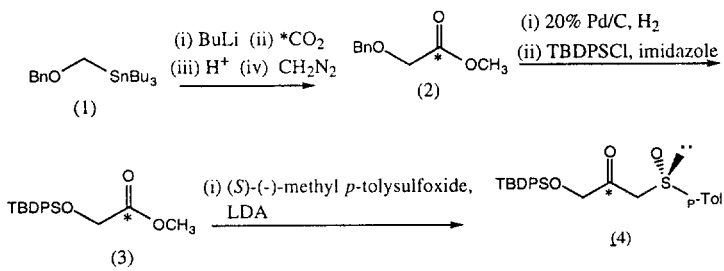
asymmetric synthesis of the starting radiolabeled *(R)*-oxirane. Since carbon-14 labeled *(R)*-epichlorohydrin or *(S)*-glycidyl tosylate may only be accessible at very high cost by custom synthesis, a method for preparing either compound or other substitute oxirane in the enantiomerically pure form had to be found. *(R)*-(+)-2-(*tert*-Butyldiphenylsiloxylmethyl)oxirane (8)[3] was the preferred starting oxirane, and methods were sought that would provide the labeled form. The new procedure that had been developed in our laboratory to make *(R)*- and *(S)*- oxirane (8)[4] was applied, and it facilitated overall, a cost effective asymmetric radio-synthesis of the target CI-1010. The alternative methods examined were either less enantioselective or afforded oxiranes in yields that could not be acceptable in radiosynthesis.

RESULTS AND DISCUSSION

The present synthesis of *(R)*-(+)-α-[[2-bromoethyl)amino]methyl]-2-nitro-1H-imidazole-[1-^{14}C]ethanol monohydrobromide (12) began with the preparation of [1-^{14}C]glycolic acid. Thus the sequence of transmetallation of tributyl[(benzyloxyl)methyl]stannane (1)[5] with *n*-butyllithium to benzyloxymethyllithium, reaction of benzyloxymethyllithium with the [^{14}C]carbon dioxide that was generated from barium [1-^{14}C]carbonate, and acidification gave 2-benzyloxy[1-^{14}C]acetic acid. This free acid was immediately converted to methyl 2-(benzyloxy)-[1^{14}C]acetate (2) in 66% yield from barium [^{14}C]carbonate by treatment with a solution of diazomethane in ether. The compound (2) was debenzylated by catalytic hydrogenolysis to methyl [1-^{14}C]glycolate and converted to methyl 2-*tert*-butyldiphenysiloxy[^{14}C]glycolate (3) in 85% yield by reaction with *tert*-butyldiphenylchlorosilane in DMF containing imidazole.

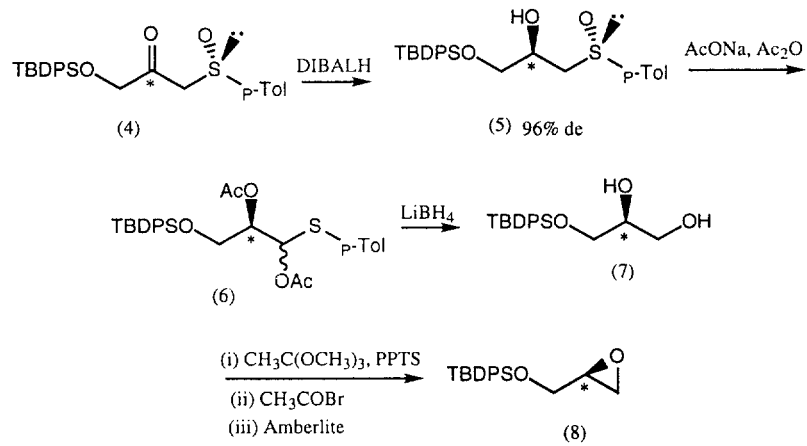
Scheme 1



The enantioselective preparation of *(R)* and *(S)* -*tert*-butyldiphenylsiloxylmethyl)oxirane from methyl 2-*tert*-butyldiphenysiloxyglycolate was recently achieved in our laboratory and has been descibed elsewhere.[4] By this method, (8) was derived from the compound (3) as shown in scheme II. The third

carbon required to complete this targeted oxirane skeleton was introduced from S**(S)*-(-)-methyl *p*-tolylsulfoxide as follows. S**(S)*-(-)-Methyl *p*-tolylsulfoxide was deprotonated with lithium diisopropylamide (LDA) and reacted with (3) to make S**(S)*-3-*tert*-butyldiphenylsiloxyl-1-tolylsulfinyl-2-[2-^{14}C]propanone (4) in 68% yield. As expected on the basis of literature precedence,[6] the chiral sulfoxide group directed a diastereofacially selective DIBAL-H reduction of S**(S)*-3-*tert*-butyldiphenylsiloxyl-1-tolylsulfinyl-2-[2-^{14}C]propanone (4), and furnished 2*(R)*, S*(S)-3-*tert*-butyldiphenylsiloxyl-1-tolylsulfinyl-2-[2-^{14}C]propanol (5), 96% de (by HPLC) in 98% yield. The rearrangement of compound (5) in a Pummerer process followed by reductive deprotection with lithium borohydride in THF furnished 2*(R)*-3-(*tert*-butyldiphenylsiloxyl)-1,2-[2-^{14}C]propane diol (7) in 78% isolated yield.

Scheme II

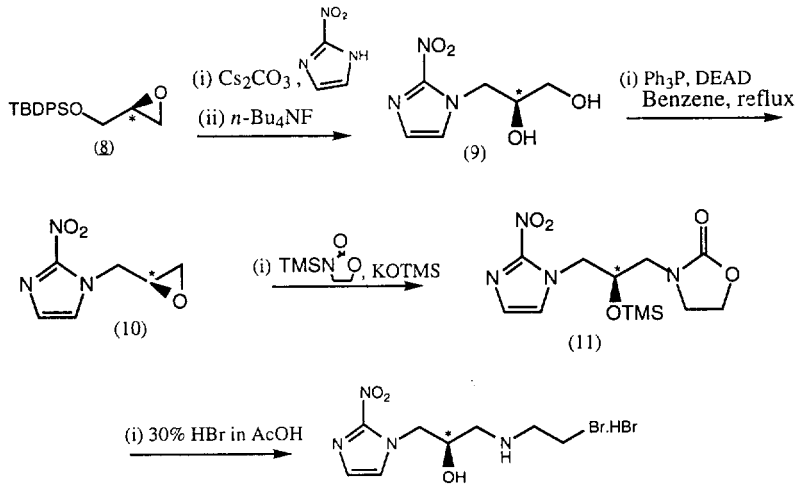


By combining this highly diastereoselective synthesis of alcohol and the stereospecific method for converting diol to epoxide,[3] *(R)*-(+)-2-(*tert*-butyldiphenylsiloxylmethyl)[2-^{14}C]oxirane (8) was prepared in a overall yield of 27%.

The compound (8) rather than epichlorohydrin, was selected as the target synthon because of the concerned that the chirality of the final product (12) could be compromised at the step where 2-nitro-imidazole is reacted with the epoxide. Only the epoxide ring opening reaction may occur in compound (8). The troublesome displacement of chloride that may occur with epichlorohydrin is effectively eliminated, and the optical purity of the reaction product would therefore not deteriorate during the coupling reaction. The best coupling reaction, scheme lll, was accomplished in refluxing absolute ethanol containing excess 2-nitro-imidazole and one mole equivalent of cesium carbonate. After desilylation, the compound *(S)*-α-(hydroxymethyl)-2-nitro-1H-imidazole-1-[2-^{14}C]ethanol (9) was made in 72% yield.

By refluxing compound (9) in benzene containing triphenylphosphane and diisopropylazodicarboxylate (DIAD), a modified Mitsunobu reaction,[7] *(S)*-(-)-2-nitro-1-(2-[2-^{14}C]oxiranylmethyl)-1H-imidazole (10) was made in 95.7% yield. The method of Sharpless *et al* [3] was less effective in this case, probably due to solubility difficulties.

Scheme III



Upon obtaining compound (10), the sequence of reactions reported by earlier workers was applied to make CI-1010.[1] Accordingly, (10) was condensed with 3-trimethylsilyl-2-oxazolidinone in a reaction catalyzed with potassium trimethylsilanoate. A subsequent one pot deprotection of silyl ether group, cleavage of the oxazolidinone ring and the substitution with bromide ion followed by crystallization gave compound (12) in radioenantiomeric purity of 99.7%, enantiomeric purity of 99.6%, and chemical purity of 99.6%

In summary, methyl 2-*tert*-butyldiphenylsiloxyl[1-^{14}C]glycolate was prepared from barium [^{14}C]carbonate, and by subsequent reaction with deprotonated *(S)*-(-)-methyl *p*-tolylsulfoxide the required oxirane skeleton in *(R)*-(+)-2-(tert-butyldiphenylsiloxylmethyl)[2-^{14}C]oxirane (8) was set. The β-keto sulfoxide group was used to provide a powerful stereo-directing influence in the metal hydride reduction of the carbonyl to chiral alcohol. The sequence of Pummerer rearrangement, reductive deprotection, and the Sharpless' stereospecific diol-epoxide conversion method gave (8). From the epoxide (8) the C-14 labeled CI-1010 was made by sequential coupling to 2-nitro-imidazole, conversion to *(S)*-(-)-2-nitro-1-(2-[2-^{14}C]oxiranylmethyl)-1H-imidazole (10), and condensation with 3-trimethylsilyl-2-oxazolidinone, followed by deprotection.

EXPERIMENTAL

General Methods.

All reactions were carried out under an atmosphere of argon unless otherwise stated. ^{1}H-NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer. Radiochemical purity of all the labeled compounds were determined by TLC radiochromatogram with a Bioscan 200 imaging scanner. Carbon-14 labeled barium carbonate was purchased from American Radiolabelled Chemicals, Inc., St. Louis, MO, and was used undiluted. Radiochemical counting was performed on a Packard 574 liquid scintillation counter using Beckman Readi-Solv MP cocktail. HPLC analyses of the final products were performed on a Water Associates 600E system with on line Applied Biosystems 1000S diode array detector and either a β-RAM radioactivity detector or Radiomatic series A-200 radioactivity flow detector. Column chromatography was carried out on Merck Kieselgel 60 (230μ) silica gel.

Methyl 2-(benzyloxyl)[1-^{14}C]acetate (2)

n-Butyllithium (1.6 M in THF, 5.75 mL, 9.2 mmol) was added dropwise in a few minutes to a solution of tributyl[(benzyloxyl)methyl]stannane[5] (3. 77 g, 9. 22 mmol) in THF (50 mL) at -78 °C under an atmosphere of argon. It was stirred for 10 min, and frozen in a liquid nitrogen bath. The reaction flask was transferred to a vacuum manifold connected to a carbon dioxide generator. Carbon dioxide was generated from barium carbonate (1.8057 g, 9.14 mmol, 54.9 mCi/mmol) by the dropwise addition of excess sulfuric acid and stored in a cold finger portion of the manifold. It was subsequently transferred into the reaction vessel following a freeze-thaw sequence to degas the reaction. The liquid nitrogen bath was replaced with dry ice-acetone bath, and after the reaction mixture was stirred for 15 min at -78 °C it was allowed to warm to room temperature in 1 hr. Saturated NH_4Cl solution (5.0 ml) was added followed by acidification with 6.0 M HCl (3.0 mL). The reaction was partitioned between methylene chloride and water. The aqueous phase was extracted with methylene chloride (4 X 60 mL), and the combined organic fractions was dried on $MgSO_4$. The solvent was removed on a rotary, and the residue was dried under high vacuum for 2 hr. A solution of the dried residue in anhydrous ether (120 mL) was treated with freshly prepared diazomethane which was added until solution attained a permanent yellow coloration. The excess diazomethane was destroyed after 10 min reaction time by the dropwise addition of acetic acid. TLC Analysis (10% acetone in hexane) showed a radioactive slow running spot, the desired compound and a faster running non-radioactive spot. The solution was concentrated and the residue was purified by column chromatography on silica gel with 10% acetone in hexane to yield methyl

2-(benzyloxyl)[1-^{14}C]acetate (1.085 g, 66%): ^{1}H-NMR ($CDCl_3$) δ 7.35 ($C_6\underline{H}_5CH_2O$-, 5H), 4.63 (s, $C_6H_5C\underline{H}_2O$-, 2H), 4.10 ($OC\underline{H}_2COOCH_3$, 2H), 3.75 (s, $OCH_2COOC\underline{H}_3$, 3H).

Methyl 2-*tert*-butyldiphenylsiloxyl[1-^{14}C]glycolate (3)

Methyl 2-(benzyloxyl)[1-^{14}C]acetate (1.085mg, 6.32 mmol) in anhydrous THF (40 mL) and 20% Pd/C (300 mg) was degassed, connected to a balloon of hydrogen and stirred overnight under an atmosphere of hydrogen. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated on a rotary. The product (methyl [1-^{14}C]glycolate) was dissolved in anhydrous DMF (15 mL); imidazole (516 mg, 7.58 mmol) and *tert*-butyldiphenylchlorosilane (2.08 g, 1.97 mL, 7.58 mmol) were added. After 18 hr the reaction was concentrated on a high vacuum pump at room temperature, and the residue was transferred to a separatory flask with chloroform (80 mL), washed with ice-cold water (6 X 20 mL) and dried. Filtration through a short column of silica gel with 10% ethyl acetate in hexane gave methyl-2-*tert*-butyldiphenylsiloxyl[1-^{14}C]glycolate (1.68 g, 5.1 mmol, 85.2%): ^{1}H-NMR ($CDCl_3$) δ 7.70 and 7.41 (m, aromatic, 10H), 4.24 (s, -$OC\underline{H}_2COOCH_3$, 2H), 3.67 (s, -$OCH_2COOC\underline{H}_3$, 3H), 1.09 (s, $(C\underline{H}_3)_3CSi$-, 9H).

S**(S)*-3-*tert*-Butyldiphenylsiloxyl-1-tolylsulfinyl-2-[2-^{14}C]propanone (4)

To a solution of diisopropylamine (1.254 g, 1.73 mL, 12.4 mmol) in dry THF (30 mL) at - 40 °C was added *n*-BuLi (1.6 M solution in THF, 7.04 mL, 11.3 mmol). After 30 min at - 40 °C under inert atmosphere, a solution of *(S)*-(-)-methyl *p*-tolylsulfoxide (1.57 g, 10.2 mmol) in dry THF (10 mL) was added slowly. The reaction temperature was lowered to -78 °C after another 30 min, and a solution of methyl 2-*tert*-butyldiphenylsiloxyl[1-^{14}C]glycolate (1.68 g, 5.1 mmol) in dry THF (10 mL) was added rapidly. The reaction was stirred for a further 1 hr and poured into a sat'd NH_4Cl solution (15 mL). The organic phase was separated and the aqueous phase was acidified with 10% HCl and extracted (3 X 50 mL) of dichloromethane. The combined organic extract was dried, evaporated and chromatographed on silica gel column with 10% acetone in hexane to give S**(S)*-3-*tert*-butyldiphenylsiloxyl-1-tolylsulfinyl-2-[2-^{14}C]propanone (1.56 g , 68%): ^{1}H-NMR ($CDCl_3$) δ 7.54-7.26 (m, aromatic, 14H), 4.15 (s, -$OC\underline{H}_2CO$, 2H), 3.96 (q, $OC\underline{H}_2SO$-, 2H), 2.41 (s, *p*-$C\underline{H}_3C_6H_5SO$, 3H), 1.10 (s, $(CH_3)_3CSi$, 9H). ^{13}C-NMR 201.39, 142.73, 140.55, 135.99, 132.65, 130.52, 128.43, 124.68, 71.02, 65.51, 27.23, 21.99 and 19.69.

2*(R)*, S**(S)*-3-*tert*-Butyldiphenylsiloxyl-1-tolylsulfinyl-2-[2-^{14}C]propanol (5)

To a stirred solution of S**(S)*-3-*tert*-butyldiphenylsiloxyl-1-tolylsulfinyl-2-[2-^{14}C]propanone (1.56 g, 3.5 mmol in dry THF (15 mL) at -78 °C was added diisobutylaluminum hydride (DIBAL-H) (1.5 M solution in toluene, 2.8 mL, 4.2 mmol) dropwise. It was stirred at this temperature for 1 hr and poured into sat'd sodium tartrate solution (8.0 mL). The organic phase was separated and the aqueous portion was extracted with ethyl acetate (5 X 20 mL). The combined organic extract was washed with brine (4 X 10 mL) and dried. The solvent was removed to give 2*(R)*, S**(S)*-3-*tert*-butyldiphenylsiloxyl-1-tolylsulfinyl-2-[2-^{14}C]propanol (1.53 g, 98% yield), and shown by chiral HPLC to be 96% de. ^{1}H-NMR ($CDCl_3$) δ 7.61-7.28 (m, aromatic, 14H), 4.23 (m, -OCH_2C<u>H</u>O<u>H</u>CH_2SO-, 1H), 3.70 (br d, -C<u>H</u>O<u>H</u>-, exch. D_2O), 3.61 (m, -O<u>C</u>H_2CHOHC<u>H</u>$_2$SO-, 2H), 3.01 (dd, -O<u>C</u>H_2SO-, 1H, J = 9.50, 13.5 Hz), 2.78 (dd, -O<u>C</u>H_2SO-, 1H, J = 2.3, 13.5 Hz), 2.42 (s, *p*-C<u>H</u>$_3C_6H_5$SO, 3H), 1.01 (s, ($C\underline{H}_3$)$_3$CSi, 9H). ^{13}C-NMR 142.05, 140.30, 135.85, 133.38, 133.33, 130.53, 130.37, 128.25, 124.48, 67.73, 67.22, 58.30, 27.28, 21.88 and 19.68. It was used in the Pummerer rearrangement reaction as such.

2*(R)*, 3-(*tert*-Butyldiphenylsiloxyl)-1,2-[2-^{14}C]propane diol (7)

2*(R)*, S**(S)*-3-*tert*-Butyldiphenylsiloxyl-1-tolylsulfinyl-2-[2-^{14}C]propanol (1.53 g, 3.5 mmol) in acetic anhydride (25 mL) containing sodium acetate (2.5 g, 30.4 mmol) was refluxed for 6 hr and the solvent was removed on a rotary. Ether (200 mL) was added to the residue obtained after azeotrop with toluene, and filtered. The solid cake was washed with additional ether (100 mL) and the combined ethereal filtrate was concentrated to yield an oil 1.75 g. Lithium borohydride (2.0 M solution in THF, 66.0 mL, 132 mmol) was added to a solution of this product (1.75 g, 3.3 mmol) in THF (10 mL) and stirred at room temperature overnight. After the reaction was cooled to -40 °C, 1 M HCl was cautiously added making sure the internal temperature did not rise above -40 °C. The solid precipitate was removed by fitration, and the cake was washed with additional methylene chloride (200 mL). The combined organic filtrate was dried on anhyd. Na_2SO_4, filtered and concentrated to an oil. Purification by column chromatography on silica gel column with 35% ethyl acetate in hexane gave 2*(R)*,3-(*tert*-butyldiphenylsiloxyl)-1,2-[2-^{14}C]propane diol (854 mg, 78.5%): ^{1}H-NMR ($CDCl_3$) δ 7.67 - 7.63 (m, aromatic, 5H), 7.42 - 7.36 (m, aromatic, 5H), 3.72 - 3.65 (m, TBDPSOC<u>H</u>$_2$C<u>H</u>OHC<u>H</u>$_2$OH, 5H), 2.65 (d, -O<u>H</u>-, exch. D_2O, 1H), 2.08 (triplet, -O<u>H</u>-, exch. D_2O, 1H), 1.06 (s, ($C\underline{H}_3$)$_3$C-, 9H); ^{13}C-NMR 136.01, 133.50, 130.47, 128.34, 72.32, 65.75, 64.35, 27.34 and 19.71.

(R)-(+)-2-(*tert*-Butyldiphenylsiloxylmethyl)[2-^{14}C]oxirane (8)

Trimethylorthoacetate (422.5 μL, 3.3 mmol) was added to a solution of 2*(R)*,3-(*tert*-butyldiphenylsiloxyl)-1,2-[2-^{14}C]propane diol (854.7 mg, 2.6 mmol) in CH_2Cl_2 (4.0 mL), followed by pyridinium *p*-toluenesulfonate (PPTS) (6.5 mg, 0.025 mmol). After 15 min, the volatiles were evaporated and residual methanol was removed under high vacuum for 2 min. Triethylamine (39.0 μL, 0.279 mmol) in CH_2Cl_2 (5.2 mL) was added. The solution was cooled to 0 °C and acetyl bromide (234 μL, 3.2 mmol) was introduced dropwise via a syringe. After 90 min, saturated $NaHCO_3$ (2.6 mL) was added at 0 °C and stirred vigorously. A further amount of sat'd $NaHCO_3$ (26 mL) was added to the mixture in a separatory funnel, and extracted with methylene chloride (3 X 30 mL). After drying over $MgSO_4$, the solution was concentrated to an oil which was applied to a pad of silica gel and eluted with 10% ether in pentane to give a mixture of acetoxy bromides (743 mg). This mixture of acetoxy bromides (743 mg, 2.4 mmol), Amberlite IRA 410 (1.8 g, OH^- form; prepared from the Cl^- form by washing with 2.0 M NaOH, water and MeOH, then dried under vacuum and used immediately) in MeOH (3.0 mL) was stirred vigorously at room temperature for 5 hr. After the solid materials were removed by filtration, the filtrate was concentrated at room temperature under reduced pressure to a viscous liquid. The liquid was purified on a silica gel column with 10% ether in pentane to give *(R)*-(+)-2-(*tert*-butyldiphenylsiloxylmethyl)[2-^{14}C]oxirane (734 mg, 2.4 mmol, 92.3%): ^{1}H-NMR ($CDCl_3$) δ 7.67 - 7.71 (m, aromatic, 5H), 7.35 - 7.45 (m, aromatic, 5H), 3.86 (dd, TBDPSO-C<u>H</u>$_2$-, J = 2.9, 12.5 Hz, 1H), 3.70 (dd, TBDPSO-C<u>H</u>$_2$-, J = 4.4, 12.5 Hz, 1H), 3.10 - 3.13 (m, -OCH$_2$C<u>H</u>OCH$_2$-, 1H), 2.73 (dd, -OCH$_2$CHOC<u>H</u>$_2$-, J = 3.3, 5.8 Hz, 1H), 2.60 (dd, -OCH$_2$CHOC<u>H</u>$_2$-, J = 3.3, 5.1 Hz, 1H), 1.06 (s, (C<u>H</u>$_3$)$_3$C-, 9H).

(S)-α-(Hydroxymethyl)-2-nitro-1H-imidazole-1-[2-^{14}C]ethanol (9)

2-Nitro-imidazole (1.33 g 11.8 mmol), Cs_2CO_3 (919 mg, 2.8 mmol) and (R)-(+)-2-(tert-butyldiphenylsiloxylmethyl)[2-^{14}C]oxirane (734 mg, 2.4 mmol) in absolute ethanol (15 mL) was refluxed under nitrogen atmosphere while the reaction was monitored by TLC (10% ethanol in $CHCl_3$). After 3 hr the solvent was removed on a rotary, and the solid residue was re-suspended in chloroform. Insoluble 2-nitro-imidazole was filtered off by passage through a pad of celite, and the filtrate was evaporated to give *(S)*-(*tert*-butyldiphenylsiloxylmethyl)-2-nitro-1H-imidazole-1-[2-^{14}C]ethanol

contaminated by a minor amount of 2-nitro-imidazole. The crude *(S)*-(*tert*-butyldiphenylsiloxylmethyl)-2-nitro-1H-imidazole-1-[2-^{14}C]ethanol was dissolved in dry THF (15 mL) and treated with tetrabutyl ammonium fluoride (1 M solution in THF, 2.5 mL) added in one portion. After 45 min at room temperature the solvent was removed under reduced pressure and the residue was applied to a column of silica gel. Elution of the silica gel column with 10% methanol in ethyl acetate first gave 2-nitro-imidazole followed by *(S)*-α-(hydroxymethyl)-2-nitro-1H-imidazole-1-[2-^{14}C]ethanol (326.2 mg, 72.5%). ^{1}H-NMR (DMSO-d_6) δ 7.00 (s, -NC<u>H</u>=CHN-, 1H), 6.71 (s, -NCH=C<u>H</u>N-, 1H), 4.48 (d, -O<u>H</u>, exch. D_2O), 4.30 (dd, -C<u>H</u>$_2$NCH=CH-, 1H), 4.08 (br, -O<u>H</u>, exch. D_2O), 3.96 (dd, -C<u>H</u>$_2$NCH=CH-, 1H), 3.50 (m, -OCH$_2$C<u>H</u>OHCH$_2$N-, 1H), 3.15 (dd, HOC<u>H</u>$_2$CHOH-, 2H); ^{13}C-NMR 128.70, 127.73, 70.48, 63.70, and 52.69.

<u>*(S)*-(-)-2-Nitro-1-(2-[2-^{14}C]oxiranylmethyl)-1H-imidazole (10)</u>

(S)-α-(Hydroxymethyl)-2-nitro-1H-imidazole-1-[2-^{14}C]ethanol (326.2 mg, 1.744 mmol), triphenylphosphine (498 mg, 1.9 mmol), and diisopropylazodicarboxylate (DIAD) (388 mg, 378 µL, 1.91 mmol) was refluxed in dry benzene for 2 hr. The solvent was removed and the residue was applied to a column of silica gel, and eluted first with [chloroform:hexane:ethanol] (35:60:5, v/v/v) to remove unknown impurities and triphenylphosphine, and then followed by elution with a (40:50:10, v/v/v) ratio of same solvent combination to give *(S)*-(-)-2-nitro-1-(2-[2-^{14}C]oxiranylmethyl)-1H-imidazole (282.8 mg, 95.7%): ^{1}H-NMR ($CDCl_3$) δ 7.15 (overlapping singlets, -NC<u>H</u>=C<u>H</u>-N-, 2H), 5.05 (dd, -OCHC<u>H</u>$_2$N-, 1H), 4.18 (dd, -OCHC<u>H</u>$_2$N-, 1H), 3.39 (m, -OCH$_2$C<u>H</u>OCH$_2$N-, 1H), 2.91 (triplet. -OC<u>H</u>$_2$CHOCH$_2$N-, 1H), 2.57 (dd, -OC<u>H</u>$_2$CHOCH$_2$N-, 1H); ^{13}C-NMR 128.00, 127.05, 52.06, 50.42, and 46.06.

<u>*(S)*-3-[3-(2-Nitro-1H-imidazol-1-yl)-2-[(trimethylsilyl)oxy]-propyl]-2-oxazolidinone (11)</u>

To *(S)*-(-)-2-nitro-1-(2-[2-^{14}C]oxiranylmethyl)-1H-imidazole (282.8 mg, 1.67 mmol) in dry THF (10 mL) was added potassium trimethylsilanoate (5 mg, 0.04 mmol), and 3-trimethylsilyl-2-oxazolidinone (470 µL, 3.07 mmol). After the THF was slowly removed by distillation, the reaction was heated for a further 2 hr at 100 °C and allowed to cool to room temperature. It was transferred onto a column of silica gel with minimum methylene chloride, and eluted with 20% cyclohexane in ethyl acetate to give fast running impurities followed by *(S)*-3-[3-(2-nitro-1H-imidazol-1-yl)-2-[(trimethylsilyl)oxy]-propyl]-2-oxazolidinone (353 mg, 1.07 mmol, 64.3%): ^{1}H-NMR ($CDCl_3$) δ 7.29 (

s, 1H), 7.20 (s, 1H), 4.78 (d, 1H), 4.50 (m, 2H), 4.31 (d, 1H), 4.28 (m, 1H), 3.73 (m, 2H), 3.50 (dd, 1H), 3.38 (dd, 1H), 0 (s, 9H); ^{13}C-NMR 159.51, 128.51, 128.12, 77.72, 70.20, 62.63, 53.85, 48.39, and 47.07.

(R)-(+)-α-[[2-Bromoethyl)amino]methyl]-2-nitro-1H-imidazole-[1-^{14}C]ethanol monohydrobromide (12)

(S)-3-[3-(2-Nitro-1H-imidazol-1-yl)-2-[(trimethylsilyl)oxy]-propyl]-2-oxazolidinone (353 mg, 1.07 mmol) in 30% HBr in acetic acid (3.0 mL) was stirred at room temperature overnight and isopropyl alcohol (3.0 mL) was added. It was stirred for another 48 hr at room temperature. It was frozen in liquid nitrogen and evaporated to dryness. After it was again azeotroped with isopropyl alcohol, the residue crystallized from methanol to give CI-1010 (153 mg, 0.409 mmol, 38.2%). The compound was purified by a sequence of crystallization from isopropanol, ethyl acetate, methanol, isopropanol and finally ethyl acetate to give *(R)*-(+)-α-[[2-bromoethyl)amino]methyl]-2-nitro-1H-imidazole-[1-14C]ethanol monohydrobromide: 7.5 mCi, sp. act. 55 mCi/mmol. HPLC Analysis using a Beckman Ultrasphere ODS 5μ, 4.66 mm ID X 250 column, 0.001 M octanesulfonic acid, Na salt adjusted to pH 2.2 with H_3PO_4:CH_3OH (70:30 v/v) mobile phase, showed the radiochemical purity to be 99.58%, and the chemcal purity (UV detection wavelength of 313 nm) to be 99.82% at a retention time of 9 min (flow rate 1.0 mL/min) which matched reference standard Lot B. A chiral analysis on a Diacel Chiralpak AS 10μ, 4.6 mm ID x 250 column, using hexane:ethanol:diethylamine (90:10:0.1v/v/v) mobile phase, gave the radioenantiomeric purity of 99.70%, and enantiomeric purity (detection by UV) of 99.62% at a retention time of 18.8 min (flow rate 0.8 mL/min) which matched reference standard Lot B. ^{1}H-NMR (DMSO-d_6) δ 8.78 (brs, 2H), 7.53 (s, 1H), 7.21 (s, 1H); 5.93 (d, 1 H); 4.55 (m, 1H); 4.39 (m, 1H); 4.23 (m, 1H); 3.69 (triplet, 2H); 3.42 (m, 2H); 3.29 (m, 1H); 2.98 (m, 1H). Similarly, a second batch 9.152 mCi, specific activity 26.3 mCi/mmol, analyzed to give a radiochem. purity of 99.68%, chem. purity of 99.66%, and identical retention time which matched reference standard Lot B. Radioenantiomeric purity was determined, as with the first batch, by HPLC to be 99.9%, and enantiomeric purity was found to be 99.49%.

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