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SYNTHESIS OF [^{14}C]CI-980, ETHYL [5-AMINO-1,2-DIHYDRO-2(*S*)-METHYL-3-[^{14}C]PHENYLPYRIDO[3,4-*b*]PYRAZIN-7-YL]CARBAMATE ISETHIONATE SALT, A TUBULIN-BINDING, ANTIMITOTIC, BROAD-SPECTRUM ANTITUMOR AGENT

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SUMMARY

[^{14}C]CI-980 (**14b**) was synthesized in eight steps starting from [U-^{14}C]benzene (**5**), which was converted to bromo[^{14}C]benzene (**6**) in the presence of tetrabutylammonium bromide as catalyst. Reaction of **6** with the anion of *N*-ethoxycarbonyl-*N'*-methoxy-*N'*-methyl-L-alaninamide (**4a**) gave the chiral (*S*)-ketone **8** with ee exceeding 96%. Sodium borohydride reduction of **8**, followed by sequential condensation with ethyl 6-amino-4-chloro-5-nitro-2-pyridine carbamate (**11**), chromium trioxide oxidation, and catalytic hydrogenation over Raney nickel gave the free base form of [^{14}C]CI-980 (**14a**), which was extremely unstable and readily aromatized to **15**. The free base **14a** was, however, isolated under specially developed conditions and converted to the crystalline isethionate salt **14b** in pure form.

keywords: [^{14}C]CI-980, bromo[^{14}C]benzene, chiral α-aminoketone synthon, antitumor agent.

INTRODUCTION

Ethyl [5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (racemate of **14**) has shown broad-spectrum activity against experimental tumors in vitro including those that are resistant to drugs in clinical use. It retains full activity against vincristine- and multidrug-resistant tumors. It shows synergistic activity with vincristine. Both *rac*-**14** and vincristine arrest mitosis and are believed to derive their cytotoxic effects from binding at different sites on cellular tubulin, with the former binding at the colchicine site. The 2-(*S*) enantiomer **14**, prepared more recently, has been shown to the significantly more active component of the racemate.[1]

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The 2-(*S*) enantiomer is being developed clinically in its isethionate salt form as an antitumor agent named CI-980 (**14b**). For the purpose of pharmacokinetics and drug metabolism studies, both the ^{14}C and ^{3}H labeled forms of CI-980 were synthesized. This paper will present the ^{14}C-labeled synthesis, while the ^{3}H-labeled synthesis will be the subject of an accompanying publication which follows.

RESULTS AND DISCUSSION

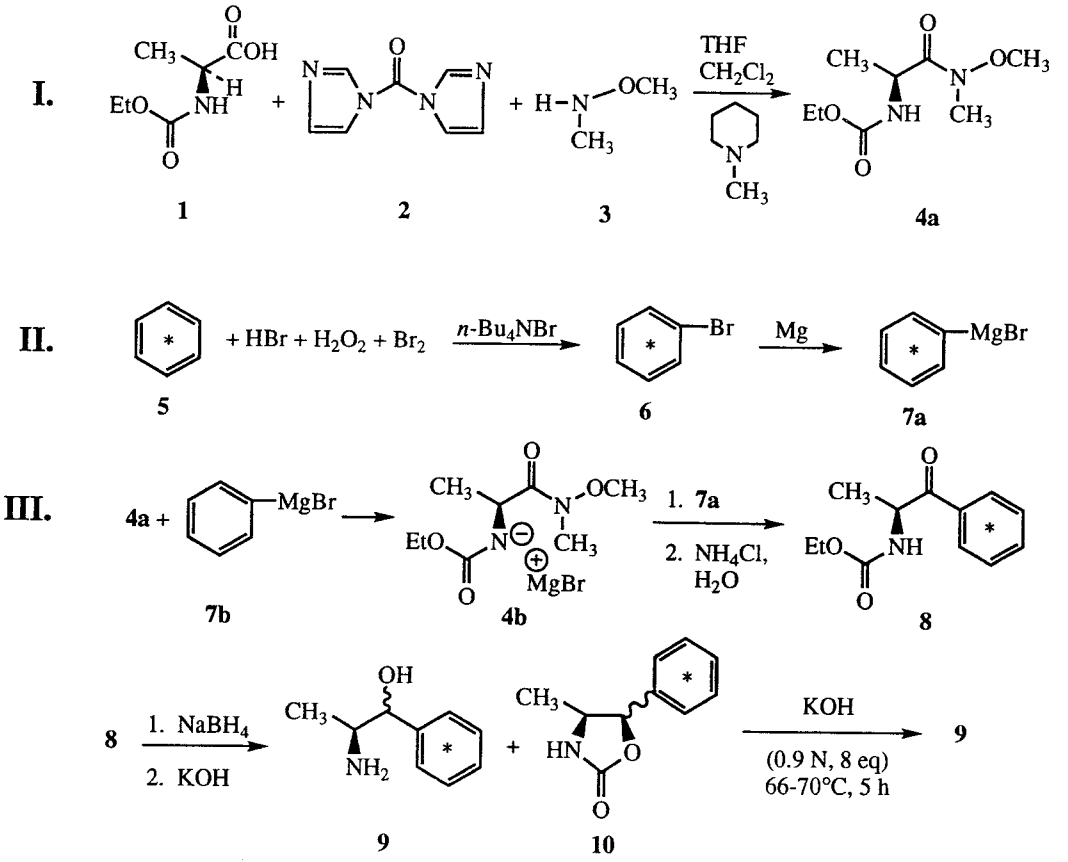
[^{14}C]CI-980 was synthesized through four series of reactions (Scheme I). Labeled benzene (**5**) was brominated in the presence of a phase-transfer agent[2] to [^{14}C]bromobenzene (**6**) in 55-71% yield;[3] **6** was then converted to the Grignard reagent **7a**. *N*-Ethoxycarbonyl-*N'*-methoxy-*N'*-methyl-L-alaninamide (**4a**),[4,5] deprotonated with one equivalent of the corresponding unlabeled Grignard reagent **7b**, was then treated with one equivalent of the labeled reagent **7a** to give the key labeled chiral α-aminoketone synthon **8**, obtained in crystalline form after chromatography (overall yield **6** to **8**, ca. 55%). As demonstrated in cold runs, the conversion of **4a** to **8** proceeded with chiral integrity (96-97% ee); apparently the alpha-carbon of **4b** was protected from isomerization by the presence of the negative charge on the adjacent nitrogen.[5]

Two alternative approaches[5] for the conversion of **1** to **8** were examined in unlabeled experiments using a variety of conditions but found to be unsuitable for labeled synthesis. Thus reaction of the acid chloride of **1** with one, two, and four equivalents of benzene in the presence of aluminum chloride gave unacceptably poor yield of **8** (4 to <16%, based on benzene used), accompanied by numerous side products, and the reaction of **1** with one equivalent of n-butyl lithium followed by two equivalents of phenyl lithium gave only 21% of **8**.

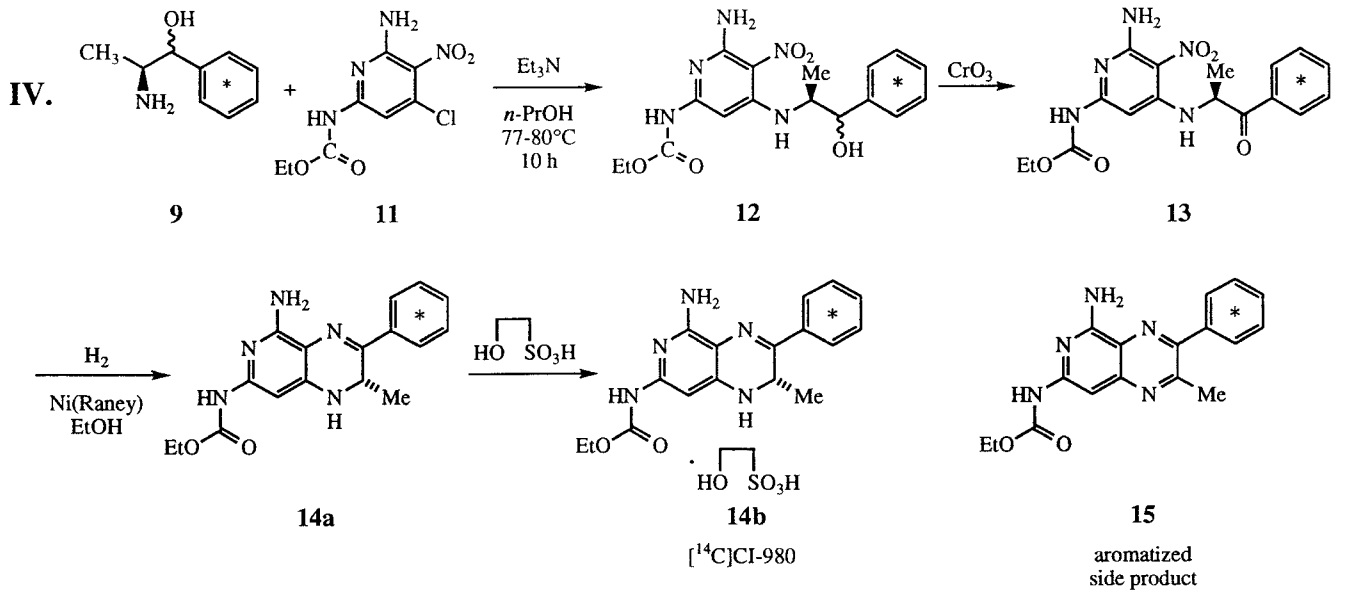
Reduction of **8** followed by alkaline hydrolysis gave the expected product **9** (29% yield), together with the cyclic carbamate **10**. However, the cyclic compound could be further hydrolyzed to **9**. The total yield of **9** was 75%.

The labeled compound **9** was converted to ^{14}C-labeled CI-980 by known procedures,[1] with some modifications. The products (**14a** and **14b**), being extremely air-sensitive,

Scheme I. Synthesis of [^{14}C]CI-980



Scheme I. Continued



aromatized to the side product **15** so readily that attempted chromatographic purification led to extensive decomposition. However, by conducting the hydrogenation and cyclization precisely to 100% completion and working up under argon atmosphere at low temperature, side product formation was suppressed, and the desired product was isolated as the isethionate salt (**14b**) (78% yield), in high purity and in crystalline form. The overall yield from **5** to **14b** was 9.6-12.4%.[3]

EXPERIMENTAL

Radioactivity was determined with a Packard Tri-Carb 4530 liquid scintillation counter, using Beckman Ready-Gel as the counting medium. TLC plates, E. Merck silica gel 60 F_{254}, were scanned on a Berthold LB2832 automatic TLC linear analyzer. Column chromatography was performed with E. Merck Silica gel, 230-400 mesh, packed in hexane. HPLC was conducted with a Waters 600 E system controller for solvent delivery, Applied Biosystems 1000S Diode Array Detector for UV detection, and Radiomatic Beta Flow 1 radioactivity flow detector, and, unless otherwise specified, Altech Econosil columns, C18, 10 μ, 4.7 mm x 25 cm. All labeled compounds synthesized were identified by TLC or HPLC comparison, or both, with the corresponding authentic unlabeled compounds.

Ethyl 2-(Methoxymethylamino)-1(*S*)-methyl-2-oxoethylcarbamate (4); *N*-Ethoxycarbonyl-*N'*-methoxy-*N'*-methyl-L-alaninamide. 1,1'-Carbonyl diimidazole, 2.22 g, was added in 15 min to a solution of 2.01 g of *N*-ethoxycarbonyl-L-alanine (**1**) in 25 mL of tetrahydrofuran and 25 mL of dichloromethane at -16°C. After 2 h, when temperature had gradually warmed up to -3°C, a cold solution of 1.34 g of *N,O*-dimethylhydroxylamine hydrochloride and 1.375 g of 1-methylpiperidine in 15 mL of dichloromethane was added during 10 min. After 52 h without external cooling, the reaction mixture was evaporated in vacuo to a syrup (7.5 g), dissolved in 60 mL of ethyl acetate, and washed with 3 mL, then several 1-mL portions of 1 M citric acid until the washing had a pH of 2.8. The organic phase was then washed twice with 2-mL portions of saturated sodium bicarbonate, followed by two 1-mL portions of brine, dried with magnesium sulfate, and evaporated to 2.32 g of colorless **4** as a liquid, which was used without further purification. NMR

($CDCl_3$): δ 1.241 (3H, t), 1.333 (3H, d), 3.215 (3H, s), 3.777 (3H, s), 4.110 (2H, q), 4.727 (1H, q), 5.417 (1H, d).

Bromo[^{14}C]benzene (6). [U-^{14}C]Benzene (**5**) (10 mmoles at 100 mci/mmole according to vendor) was vacuum-transferred into a 20-mL conical flask; however, some radioactivity was retained in the brown and non-volatile residue. The flask was cooled in liquid nitrogen, and a solution of 452 mg of tetra-*n*-butylammonium chloride in 2.90 g of 48% hydrogen bromide was added. The flask was then fitted with efficient condenser cooled with ice-cold water and a small addition funnel. The flask was heated at an oil bath maintained at 62-64°C while 1.754 g of 30% hydrogen peroxide was added in portions during 2.7 h. The reaction was monitored by ^{1}H-NMR spectroscopy (200 MHz) on samples of about 10 to 15 mg in $CDCl_3$. The signals of bromobenzene (m, δ 7.45-7.58, prominent at 7.495, 7.506, 7.536, and 7.544, 2H's) can be differentiated from the unresolved signals of benzene and *p*-dibromobenzene (s, δ 7.377), and those of *o*-dibromobenzene (m, δ 7.489, 7.500, 7.530, and 7.538, 2H's) and *m*-dibromobenzene (7.087, 7.127, 7.167, 1H; 7.689, 7.696, 1H). The reaction was continued with additional amounts of bromine added, heating time, and possible product distribution as follows: 0 mg of bromine, 30 min, 66% **6**; 671 mg added in portions, 4.5 h, 78% **6**; 287 mg in one portion, then 5 h, 81% **6** and 17% *p*-, 2% *o*-, and 0% *m*-dibromobenzene; 142 mg, then 2 h, no analyses.

The mixture, consisting of a light orange upper phase and a deep red lower organic phase, was partially decolorized by gradual addition of 605 mg of sodium bisulfite, with good mixing and cooling at -10°C. The lower red layer was further washed with a solution of 187 mg potassium carbonate, 49 mg of sodium hydroxide and 0.5 mL of water. The upper phase (0.15 mCi) was discarded; the lower phase (649 mCi) was dried with 127 mg of magnesium sulfate and clarified through a syringe filter. Additional product was recovered by washing the drying agent with ether and pentane and slowly distilling off the solvent. The product fractions were combined and vacuum-transferred to a receiver through a tube containing 2.88 g of Molecular Sieves 4A previously dried at 350°C for 4 h. The gain in weight of Molecular Sieve was 159 mg, and 1.22 g of distillate, which according to NMR consisted of 92 mol% of bromobenzene and 8 mol% of n-pentane, was collected. The yield was 7.14 mmol of bromobenzene with an activity of 549 mCi (ca. 77 mCi/mmol; 55-71%[3] radiochemical yield).

Ethyl (1(*S*)-methyl-2-oxo-2-[^{14}C]phenylethyl)carbamate (8). To 47.5 mg of magnesium turnings in a 15-mL 2-neck flask under argon was added 0.15 mL of ether, followed by 5 μL of 1,2-dibromoethane. Upon stirring, the mixture became cloudy, and gas evolution was observed momentarily. A dilute solution of [^{14}C]bromobenzene (**6**) (234 mg; 1.58 mmol; 122 mCi) in 4.8 mL of ether was then slowly added dropwise in about 2 h to give labeled phenyl magnesium bromide. Separately a solution of 301 mg of **4a** in 4.5 mL of dried THF in a 25-mL 2-neck flask under argon was cooled with vigorously stirring in a Dry Ice-acetone bath. A solution of 1.52 mmol of 3.24 M unlabeled phenyl magnesium bromide in diethyl ether was added (the normality was determined by sequential treatment with excess 0.1N HCl and back-titration with sodium hydroxide). The gel and solid which formed were allowed to dissolve by slightly raising the temperature so as to obtain the deprotonated **4b** in a clear solution but at the lowest possible temperature. The labeled phenyl magnesium bromide was then added. The resulting purple solution was stirred overnight to give a clear light yellow solution, while the temperature was allowed to warm up to room temperature. TLC (chloroform) showed the presence of product **8** at Rf 0.31 (89.2% of the radioactivity) and impurities at Rf 0.03 (3.5%) and 0.98 (5.3%, probably biphenyl). After three days, the reaction was slowly added to 3.5 mL of cold saturated ammonium chloride solution. The product was isolated by extraction with 10-, 5-, and 5-mL portions of ether, washed with 1 mL saturated sodium chloride and dried with 310 mg of magnesium sulfate. Evaporation gave 322 mg of an oil which slowly turned to a gummy solid on standing after seeding and trituration and was chromatographed over a column of 2.2 g of silica gel packed in hexanes. Appropriate fractions obtained from elution with 18 mL of 1:2 chloroform-hexane followed by 16 mL of 2:1 chloroform-hexane gave, after evaporation, 179 mg of crystals (66.7 mCi) with a specific activity of 83 mCi/mmol (55% radiochemical yield, 51% chemical yield). Crystallization of 79 mg of the product with 29 mg dichloromethane and 290 mg of hexane resulted in 60 mg of crystals. Parallel reactions using unlabeled bromobenzene gave unlabeled **8** (mp 57-62°C, hot stage), which consisted of 98.3% of the (*S*)-isomer (6.00 min) and 1.7% of the (*R*)-isomer (7.32 min) according to HPLC (Chiralcel OD, a carbamated-cellulose derived silica gel, from Chiral Technologies, Inc., Exton, PA, 0.46 x 25 cm, 5 μ, 90:10 hexane-2-propanol, 1 mL/min).

NMR ($CDCl_3$): δ 1.269 (3H, t), 1.434 (3H, d), 4.153 (2H, q), 5.345 (1H, q), 5.75 (1H, d), 6.879-8.013 (>5H, m).

α(*RS*)-(1(*S*)-Aminoethyl)[^{14}C]benzenemethanol (9); (1*RS*,2*S*)-[^{14}C]norephedrine. 4(*S*)-Methyl-5(*RS*)-[^{14}C]phenyl-2-oxazolidinones (10). To a stirred, ice-cold solution of 258 mg (1.16 mmol; 96.5 mCi) of **8** in 5 mL of methanol was added, in portions, 69 mg of sodium borohydride during 1.5 h. The reaction was stirred for 20 min at room temperature, then cooled to -10°C, and 259 mg of glacial acetic acid was added dropwise. Evaporation in vacuo gave a residue which was then partitioned between 6 mL of dichloromethane and 5.5 mL of saturated aqueous sodium bicarbonate. The aqueous phase was extracted twice with 4-mL portions of dichloromethane. The extracts were evaporated to 239 mg of a mixture of the desired products **9** and the 2-oxazolidinones **10** (TLC in 83:15:2 chloroform-methanol triethylamine, Rf, 0.25 and 0.86, respectively). The mixture was then treated with 5 mL of a 0.45N potassium hydroxide in 3:1 methanol-water and heated under reflux for 2.3 h. Addition of 5.5 mL of cold water, followed by acidification to pH 2.0 with 43% phosphoric acid at 0°C and four extractions with about 3.6-mL portions of ether removed the unreacted **10** (49 mCi, 106 mg) (TLC in 96:3:1 chloroform-methanol-triethylamine, Rf approx. 0.34). The aqueous phase was adjusted with stirring and ice cooling to pH 10.8 with 1.8 mL of 10N sodium hydroxide and extracted four times with 6-mL portions of chloroform. Evaporation of the chloroform extracts gave 28 mCi of **9**. The unreacted **10** was further heated with about 5.4 mL of 0.9N potassium hydroxide in 3:1 ethanol-water at 66-70°C for 5.2 h. TLC showed the presence of **9** and **10** (83% and 9.6%, respectively), together with two spots at Rf 0.47 and 0.57 (3.5 and 4.0%, respectively). Differential extractions at acidic and basic conditions as described above gave an additional 44 mCi of **9**. The total radiochemical yield was 74.6%.

Ethyl 6-Amino-4-[1(*RS*)-hydroxy-1-[^{14}C]phenyl-2(*S*)-propyl)amino]-5-nitropyridine-2-ylcarbamate (12). A mixture of 68 mg of **9** (0.44 mmol, 38 mCi), 109.5 mg (0.48 mmol) of the slightly soluble **11**, and 52.2 mg of triethylamine in 1.5 mL of isopropyl alcohol was heated with stirring in a closed 2-dram vial at 78-80°C for 6.5 h. TLC (97:3:1 chloroform-methanol-triethylamine) showed the presence of product (83.8%, Rf 0.4) and starting material (14.5%, Rf 0.03). After further addition of triethylamine (10 μL), the solution was

heated for 3.5 h, when the ratio of product to starting material increased to 87:10. Another 85-mg lot of **9** (0.55 mmol, 49 mCi) was similarly reacted with **11**. The combined products from the two runs were evaporated and then co-evaporated with toluene in vacuo to a foam. This was dissolved in portions of dichloromethane totalling 11 mL and added to a column containing 4.1 g of silica gel packed in hexane. The column was washed with 50 mL of 6:4 dichloromethane-hexane containing a trace of triethylamine, then 250 mL of 7:3 dichloromethane-hexane. Further elution with 50 mL of the latter mixture, followed by 45 mL of chloroform and 15 mL of 99:1 chloroform-methanol gave, after evaporation, 386 mg of purified **12** (65 mCi, 74.7% radiochemical yield).

Ethyl [6-Amino-4-(1(*S*)-methyl-2-oxo-2-[^{14}C]phenylethyl)-5-nitro-2-pyridinyl]carbamate (13). To 430 mg of chromium trioxide (4.3 mmol) in a 50-mL 2-neck cone-shape flask under argon was added 10 mL of dried dichloromethane, followed by 704 mg of pyridine added dropwise in 5-10 min with magnetic stirring. To the resulting mixture of dark brown solution and sticky dark solid, a solution of **12** (357 mg, 60.3 mCi) in 10 mL of dichloromethane was added in 5-10 min. After stirring overnight, TLC (96:3:1 chloroform-methanol-triethylamine; Rf 0.58) showed that reaction was complete. The reaction mixture was filtered through a 0.5-μ filter, evaporated and chromatographed over 2.5 g of silica gel packed in hexane. The column was washed with dichloromethane until the yellow band reached the end of the column. The product was then mainly collected by further elution with about 10 mL of dichloromethane, followed by 11 mL of 99:1 dichloromethane-methanol, and 5 mL of 98:1:1 chloroform-methanol-triethylamine. Appropriate fractions were combined and evaporated to give 234 mg of **13** (51.6 mCi). The product was crystallized by concentrating a 7.5 mL solution in ethanol, diluting with hexane, and cooling at 0°C. Additional crystals were similarly obtained from the mother liquor and combined to give a total of 44 mCi of purified **13** (73% radiochemical yield).

Ethyl [5-Amino-1,2-dihydro-2(*S*)-methyl-3-[^{14}C]phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamates (14a) and Isethionate Salt (14b). A solution of 42 mg (9.24 mCi) of **13** in 3.7 g of ethyl alcohol (Aldrich, HPLC grade) was placed in a 50-mL flask fitted with a hydrogen inlet and an outlet connected to a Firestone valve. Raney nickel (Davison Chemical, washed 10 times with water; 271 mg, centrifuged wet weight) was slurried with

10 mL of the alcohol and added. Hydrogen was bubbled into the stirred solution for 2.25 h, when TLC indicated the presence of 5.7% of unreacted **13** (1:1 ethyl acetate-heptane). The reaction was found complete after one additional hour of hydrogenation. To avoid aromatization through facile air-oxidation, subsequent isolation and purification steps were carried out under argon as much as possible, and solvents were saturated with argon before use. Thus argon gas was bubbled through the reaction mixture, which was then filtered through a 5-μ syringe-filter and washed with ethanol. A solution of 0.63 mL of 0.153N isethionic acid in methanol was added. The solution was evaporated and dried in high vacuum for 5 h to 88 mg of residue, which dissolved in 0.5 g of acetonitrile but later crystallized. After standing in the dark, the supernatant (1 mCi) was removed, and the crystals were washed with a little acetonitrile to give the isethionate salt **14b** (40.0 mg; 7.21 mCi; 82 mCi/mmol; 78% radiochemical yield). The purity according to TLC (Rf 0.28, 1:1 ethyl-hexane) was greater than 98.1% and, according to HPLC (55% 0.05 M triethylamine adjusted to pH 3.1 with formic acid, 45% acetonitrile, 1 mL/min, retention time 10.9 min), was 98.2% radiochemical and 98.5% chemical (267 nm).

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