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SYNTHESIS OF 2-METHYL-2-[[(7-METHYL-7*H*-BENZO[*c*]CARBAZOL-10-YL)[^{14}C]METHYL]AMINO]-1,3-PROPANEDIOL MESYLATE - A POTENTIAL ANTITUMOR AGENT

JOHN A. HILL and JOHN F. EADDY

Chemical Development Laboratories

Burroughs Wellcome Co.

Research Triangle Park, North Carolina 27709

SUMMARY

2-Methyl-2-[[(7-methyl-7*H*-benzo[*c*]carbazol-10-yl)methyl]amino]-1,3-propanediol (1, 7U85) was synthesized as the mesylate salt in the [^{14}C]-labelled form with specific activity 48.4 mCi/mmol suitable for metabolism and tissue distribution studies in animals. The synthetic sequence involved the regiospecific synthesis of a bromobenzo[*c*]carbazole by a modified Fischer indole synthesis, formylation by a Bouveault reaction, and reductive amination. The radiochemical purity was >97.3%.

Key Words: 2-methyl-2-[[(7-methyl-7*H*-benzo[*c*]carbazol-10-yl)methyl]amino]-1,3-propanediol, 7U85, AMAP, Fischer, antitumor agent.

INTRODUCTION

Although it has been shown that a wide variety of planar ring systems can intercalate with DNA, and that the chemotherapeutic activity of many drugs can be related to intercalation with DNA, the relationship between chemotherapeutic activity, DNA interaction and molecular structure is unclear (1).

The AMAPs (2-[(arylmethyl)amino]-1,3-propanediols) are a rationally designed class of planar polycyclic aromatic derivatives containing polar side chains that are DNA intercalators, and which possess a broad spectrum of antitumor activity (2, 3, 4, 5).

The novel heterocyclic AMAP 2-methyl-2-[[(7-methyl-7*H*-benzo[*c*]carbazol-10-yl)methyl]amino]-1,3-propanediol (1, 7U85) (6,7) was designed to minimize the CNS toxicity seen with the earlier AMAPs, and has been investigated in Phase I clinical trials.

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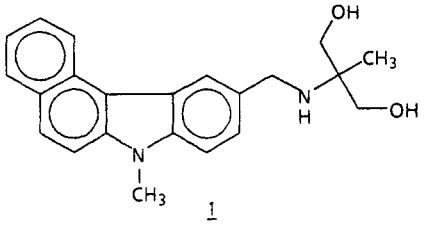

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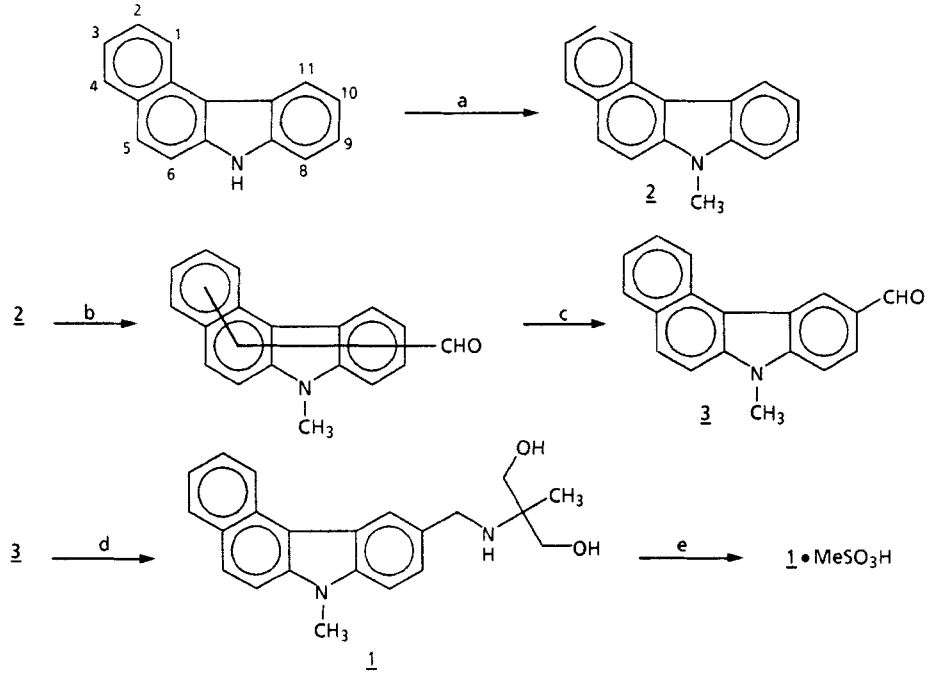
To facilitate metabolism and tissue distribution studies of 1 in animals, a carbon-14 labelled version was required. These studies required that the [^{14}C]-1 should have a high chemical and radiochemical purity, and a minimum specific activity of 40 mCi/mmol. This paper describes the preparation of [^{14}C]-labelled 1.Mesylate with specific activity 48.4 mCi/mmol.

RESULTS AND DISCUSSION

The original synthesis of 1 was accomplished using the route (6,7) outlined in Scheme I.

Scheme I



a) KO*t*-Bu, Me_2SO_4, THF; NaOH
b) $CHCl_2OMe$, CH_2Cl_2, $SnCl_4$
c) SiO_2, toluene; CH_2Cl_2, pentane
d) 2-Amino-2-methyl-1,3-propanediol, *p*-TsOH, toluene, reflux (-H_2O) EtOH, $NaBH_4$
e) EtOH, $MeSO_3H$

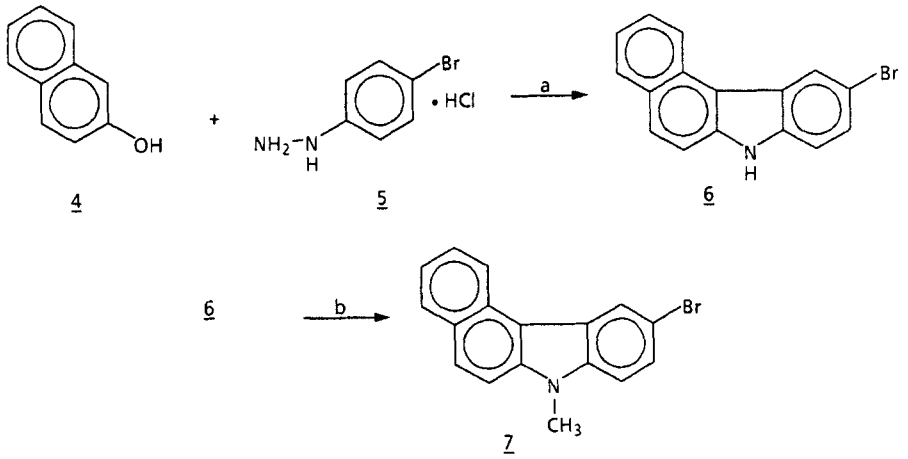
This route is not suitable, however, for the efficient preparation of [^{14}C]-labelled 1. The Friedel-Crafts formylation of 7-methyl-7*H*-benzo[c]carbazole 2 is not regiospecific and produces several isomeric

aldehydes; 50-60% 10-carbaldehyde 3, 10-20% 5-, and not insignificant amounts of 3-, 8- and 9-carbaldehydes. This necessitates a very difficult separation in order to isolate the desired 7-methyl-7*H*-benzo[c]carbazole-10-carbaldehyde 3.

A regiospecific synthesis of aldehyde 3 was required, and synthesis of 10-bromo-7-methyl-7*H*-benzo[c]carbazole 7 followed by metal-halogen exchange and quenching of the organolithium intermediate with *N,N*-dimethylformamide (or [^{14}C]-DMF) was seen as a potentially straightforward regiospecific route to 3 (or [^{14}C]-3).

Attempts were made to regiospecifically synthesize the 10-bromo-compound 6 by a Bucherer reaction (8, 9) as shown in Scheme II.

Scheme II

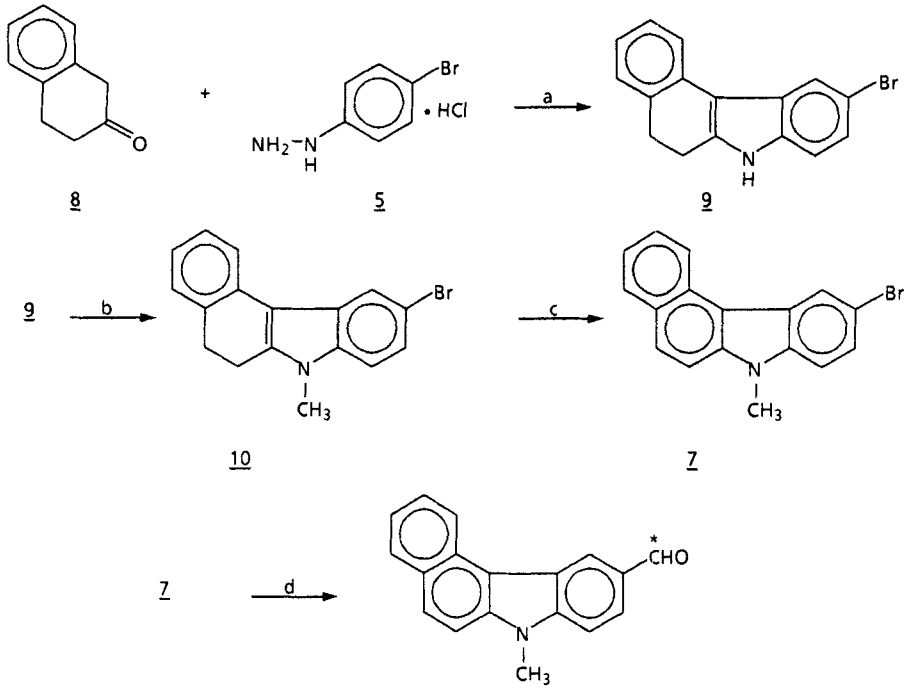


a) $Na_2S_2O_5$, HCl, reflux
b) NaH, MeI, THF

2-Naphthol 4 was reacted with 4-bromophenylhydrazine hydrochloride 5 in the presence of sodium metabisulphite and 12*N* HCl for several days at reflux. Work-up gave a black solid which could not be purified by recrystallization, but extensive chromatography eventually gave reasonably pure 6 in 27.5% yield. *N*-Methylation and recrystallization from EtOH-H_2O gave 7 as a tan solid in 14.4% yield from 6 and, therefore, in 3.9% overall yield from 4. Although this material was 98.4% pure by HPLC analysis, not enough remained to be useful for our purposes!

An alternative strategy for the regiospecific synthesis of 7, and a method for the formylation of 7 to 3, have now been successfully developed as shown in Scheme III.

Scheme III



a) H_2O, HCl, reflux
b) NaH, MeI, THF
c) Tetrachloro-1,4-benzoquinone, toluene, reflux
d) DME, BuLi, -60°C, $H\overset{*}{C}ONMe_2$

The Fischer indole synthesis has been used for the preparation of a wide variety of C-alkylbenzocarbazoles (10), and the methodology has now been adapted to the synthesis of the 10-bromo-5,6-dihydro-7*H*-benzo[*c*]carbazole 9 from 4-bromophenylhydrazine hydrochloride 5.

Reaction of 2-tetralone 8 with 5 in aqueous HCl at reflux for 5h followed by work-up and recrystallization from 1,2-dichloroethane gave the dihydrobenzocarbazole 9 in 74.3% yield (three crops).

Methylation of 9 was carried out in THF solution overnight with MeI in the presence of NaH. Following quench and work-up, recrystallization from EtOAc-hexane resulted in the isolation of a 91.0% yield of white crystalline 10-bromo-5,6-dihydro-7-methyl-7*H*-benzo[c]carbazole 10 shown to be of excellent purity by TLC, HPLC and 300 MHz ^{1}H NMR.

The dehydrogenation of 10 was performed with tetrachloro-1,4-benzoquinone in refluxing toluene. After removal of the hydroquinone, base extraction and evaporation of the toluene filtrate, crude 7 was isolated in 97.8% yield. Recrystallization from EtOAc-hexane gave a 95.1% yield (two crops)

of light yellow crystalline 10-bromo-7-methyl-7*H*-benzo[c]carbazole 7 shown to be of excellent purity by TLC, HPLC and 300 MHz ^{1}H NMR.

The Bouveault reaction of 7 with butyllithium and *N*,*N*-dimethylformamide in 1,2-dimethoxyethane was investigated extensively. It was found that metallation in DME at -55°C to -60°C followed by DMF addition, warming from -60°C to 10°C, and work-up resulted in the production of clean 3 with minimal by-product formation. Precipitation from an aqueous acidic mixture resulted in the isolation of a 94.7% yield of light yellow crystalline 7-methyl-7*H*-benzo[c]carbazole-10-carbaldehyde 3 shown to be of good purity by TLC and HPLC when compared with an authentic sample.

Following the successful synthesis of 3, the typical AMAP reductive amination method (2, 6, 7) was successfully adapted to semi-micro scale. A mixture of 3, 2-amino-2-methyl-1,3-propanediol (2 equiv), *p*-toluenesulphonic acid and toluene was distilled for 2 h with removal of H_2O, cooled, and treated with excess $NaBH_4$ in EtOH. Work-up and isolation gave an 88.3% yield of crude base shown by HPLC to be >95% pure 1. After treatment of an EtOH slurry of the base with methanesulphonic acid and toluene, the product crystallized from the medium to give cream crystalline 1.Mesylate in an overall 80.6% yield. The product was shown to be of excellent purity by TLC (single-spot identical with authentic material) and HPLC (99.8% AUC). Elemental analysis showed the not unexpected presence of 0.25 mol H_2O in the product.

The radiolabelled synthesis of [^{14}C]-1.Mesylate was carried out using essentially the same reaction conditions as described above, and the details are included in the experimental section.

The preparation of [carbonyl-^{14}C]-3 from [^{14}C]-DMF and 10-bromo-7-methyl-7*H*-benzo[c]carbazole 7 was performed by Sigma Radiochemicals, St. Louis, Missouri. Elaboration of this material as described above provided a 91.0% yield of crude [^{14}C]-1.

Crystallization of the [^{14}C]-1.Mesylate from the EtOH/toluene medium gave an 89.0% yield (based on [^{14}C]-3) of 2-methyl-2-[[(7-methyl-7*H*-benzo[c]carbazol-10-yl)[^{14}C]methyl]amino]-1,3-propanediol mesylate (1.CH_3SO_3H.0.25H_2O) with a specific activity of 48.4 mCi/mmol. The radiolabelled material was identical with an authentic sample of 1.Mesylate by TLC (single-spot material) and HPLC (99.2% AUC). The radiochemical purity was >97.3% by plate-scanning (no impurities detected).

[^{14}C]-1 has been used successfully in numerous studies including disposition and metabolism in dogs (11).

EXPERIMENTAL

[Carbonyl-^{14}C]-7-methyl-7*H*-benzo[c]carbazole-10-carbaldehyde was obtained from Sigma Radiochemicals, St. Louis, Missouri. 2-Tetralone, 4-bromophenylhydrazine hydrochloride, tetrachloro-

1,4-benzoquinone and 2.5*M* butyllithium in hexane were purchased from Aldrich Chemical Company. Sodium hydride, 50% dispersion in oil, and methanesulphonic acid (99.5%) were purchased from Alfa products. 2-Amino-2-methyl-1,3-propanediol was purchased from Angus Chemical Company. All other solvents and reagents were of reagent purity and were obtained from readily available commercial sources. Tetrahydrofuran, 1,2-dimethoxyethane and 100% EtOH were dried over type 3Å Molecular Sieves. Toluene was dried over type 4Å Molecular Sieves.

High pressure liquid chromatography (HPLC) was performed using an LDC/Milton Roy mini-Pump, a Waters Lambda-Max Model 481 LC Spectrophotometer, a Hewlett-Packard 3392A Integrator, and two sets of conditions: System A - Merck LiChrosorb Si 60 5μ 4.6 mm x 25 cm column, mobile phase hexane/EtOAc (4/1, v/v), and flow rate 1.0 mL/min with UV detection at 280 nm; System B - Alltech Spherisorb phenyl 5μ 4.6 mm x 25 cm column, mobile phase MeCN/H_2O/H_2SO_4 (700/300/1, v/v), and flow rate 1.0 mL/min with UV detection at 254 nm. Thin layer chromatography (TLC) was performed on 5 x 20 cm glass plates pre-coated with 0.25 mm silica gel 60 (E. Merck). Proton NMR spectra were obtained in $CDCl_3$ using a Varian XL-300 spectrometer (300 MHz). Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Radiochemical purity was determined by radiochromatogram scanning of a TLC plate using a Bioscan System 200 Imaging Scanner. The specific activity was determined on an accurately weighed sample using a Packard Tri-Carb 460 CD liquid scintillation counter and Optifluor (Packard) liquid scintillation cocktail.

10-Bromo-5,6-dihydro-7*H*-benzo[c]carbazole 9

To a vigorously stirred mixture of 2-tetralone 8 (123.68 g) in H_2O (450 mL) under nitrogen was added in portions 4-bromophenylhydrazine hydrochloride 5 (190.00 g) and then 12*N* aqueous HCl (150 mL). The mixture was refluxed for 5 h, cooled and stirred at 25°C overnight. The mixture was filtered and the solid was washed with H_2O. The filtrate was extracted with CH_2Cl_2, and the CH_2Cl_2 solution and the solid were recombined. CH_2Cl_2 was added to the refluxing mixture under nitrogen until complete solution was attained (in approximately 2200 mL CH_2Cl_2). After cooling, the CH_2Cl_2 solution was washed with 1*N* aqueous HCl (500 mL), and H_2O (500 mL), dried and evaporated to dryness under reduced pressure. The crude solid was crystallized from 1,2-dichloroethane (350 mL) to give 156.17 g (61.9%) of off-white solid 9; m.p. 153-155°C [lit. (12) m.p. 158-160°C]; TLC: hexane/EtOAc (3/2, v/v), single-spot material R_f = 0.43; HPLC (System A): 100.0% (t_R = 14.14 min); ^1H NMR: δ 2.98 (d of t, 4 H, CH_2CH_2), 7.09-7.35 (m, 5 H, Ar-H-2, -3, -4, -8 and -9), 7.77 (d, 1 H, Ar-H-1), 8.00 (bs, 1 H, N-H), 8.14 (d, 1 H, Ar-H-11). Anal. Calcd. for $C_{16}H_{12}BrN$: C, 64.45; H, 4.06; N, 4.70%. Found: C, 64.53; H, 4.08; N, 4.67%.

Concentration of the 1,2-dichloroethane filtrate resulted in the isolation of two further crops of 9 - 28.56 g (11.3%) and 2.75 g (1.1%), for a total yield of 187.48 g (74.3%).

10-Bromo-5,6-dihydro-7-methyl-7*H*-benzo[c]carbazole 10

To a stirred suspension of sodium hydride (38.99 g of 50% dispersion in oil) in dry THF (1 L) under nitrogen was added dropwise a solution of 9 (186.33 g) in dry THF (1 L). The yellow suspension was stirred for 45 min. Methyl iodide (115.31 g) was added dropwise and the mixture stirred overnight. The reaction was monitored by TLC (hexane/EtOAc (3/2, v/v)) H_2O was added to quench any unreacted NaH and the mixture evaporated to near dryness. The residue was partitioned between CH_2Cl_2 (900 mL) and H_2O, and the CH_2Cl_2 solution was washed with H_2O (3 x 250 mL), dried over $MgSO_4$, filtered and evaporated to give a yellow solid. This was dissolved in refluxing EtOAc (250 mL) and diluted with hexane (1 L) to give a white precipitate. After cooling at 0°C overnight, the solid was filtered and washed with hexane (250 mL). The white solid was dried at 50°C *in vacuo* to give 177.5 g (91.0%) of white crystalline solid 10; m.p. 120-122°C; TLC: hexane/EtOAc (3/2, v/v), one major spot $R_f = 0.55$; HPLC (System A): 100% ($t_R = 10.38$ min); ^1H NMR: δ 2.99 (d of t, 4 H, CH_2CH_2), 3.67 (s, 3 H, N-CH_3), 7.07-7.35 (m, 5 H, Ar-H-2, -3, -4, -8 and -9), 7.77 (d, 1 H, Ar-H-1), 8.15 (d, 1 H, Ar-H-11). Anal. Calcd. for $C_{17}H_{14}BrN$: C, 65.40; H, 4.52; N, 4.49%. Found: C, 65.42; H, 4.57; N, 4.46%.

10-Bromo-7-methyl-7*H*-benzo[c]carbazole 7

A stirred mixture of the dihydro-compound 10 (83.74 g) and tetrachloro-1,4-benzoquinone (79.14 g) in toluene (400 mL) was heated under reflux for 3.5 h. The reaction was monitored by TLC (hexane/EtOAc (3/2, v/v)). After cooling to room temperature, the solid was filtered and washed with toluene (~600 mL) until the cake was yellow-brown in color. The combined toluene filtrate was washed with a 1:1 (v/v) mixture of 10% aqueous sodium hydrosulphite solution and 1*N* aqueous NaOH solution (3 x 200 mL) and H_2O (2 x 200 mL). The yellow solution was dried over $MgSO_4$, filtered and evaporated to give a yellow solid which was dried *in vacuo* at 55°C to give 81.37 g (97.8%) of crude 7. Recrystallization from EtOAc-hexane (1:2, 600 mL) resulted in the isolation of 71.47 g (85.9%) of light yellow crystalline 7; m.p. 127-129°C; TLC: hexane/EtOAc (3/2, v/v), one major spot $R_f = 0.71$; HPLC (System A): 98.4% ($t_R = 8.52$ min); ^1H NMR: δ 3.95 (s, 3 H, N-CH_3), 7.39-8.02 (m, 7 H, Ar-H-2, -3, -4, -5, -6, -8 and -9), 8.66-8.68 (m, 2 H, Ar-H-1 and -11). Anal. Calcd. for $C_{17}H_{12}BrN$: C, 65.83; H, 3.90; N, 4.52%. Found: C, 65.90; H, 3.91; N, 4.49%. Concentration of the filtrate gave a second crop of 7.68 g (9.2%), for a total yield of 79.15 g (95.1%).

7-Methyl-7*H*-benzo[c]carbazole-10-carbaldehyde 3

Dry 1,2-dimethoxyethane (275 mL) was stirred under nitrogen and cooled to -60°C in a dry ice/acetone bath. 2.5*M* Butyllithium in hexane (2.6 mL; 6.5 mmol BuLi) was added and the mixture stirred for 60 min. Bromo-compound 7 (20.16 g; 65 mmol) was added in small portions and then 2.5*M* butyllithium in hexane (28.6 mL; 71.5 mmol BuLi) was added dropwise at a rate to keep the internal temperature at <-55°C. After stirring for 45 min, *N,N*-dimethylformamide (9.50 g; 130 mmol) was added dropwise and the mixture was allowed to warm to 10°C. The progress of the reaction was monitored by HPLC using System A.

The mixture was then chilled to -10°C and treated with 6*N* aqueous HCl (13 mL). The resulting mixture was concentrated to about half of its original volume on the rotary evaporator, chilled in an ice-bath and H_2O (100 mL) was added. After filtration, the solid was washed with H_2O and dried *in vacuo* at 50°C overnight to give 15.97 g (94.7%) of yellow solid 3; TLC: hexane/EtOAc (3/2, v/v), one major spot $R_f = 0.30$ corresponding to authentic 3; HPLC (System A): 98.4% ($t_R = 18.75$ min, corresponding to authentic 3).

2-Methyl-2-[[(7-methyl-7*H*-benzo[c]carbazol-10-yl)methyl]amino]-1,3-propanediol mesylate 1.Mesylate

The procedure for preparation of 1 from 3 was essentially identical to that used below in the preparation of [^{14}C]-1. From 363.0 mg of 3 and 302.8 mg of 2-amino-2-methyl-1,3-propanediol was obtained 430.8 mg (88.3%) of white solid 1 free base; TLC: EtOAc/MeOH/Et_3N (75/25/1, v/v), one major spot $R_f = 0.21$; HPLC (System B): 95.5% ($t_R = 4.36$ min). Conversion to the salt resulted in the isolation of 506.6 mg (80.6% based on 3) of cream solid 1.$MeSO_3H$.0.25H_2O; TLC: EtOAc/MeOH/Et_3N (75/25/1, v/v), single-spot material with $R_f = 0.21$ corresponding to authentic 1; HPLC (System B): 99.8% ($t_R = 4.25$ min, corresponding to authentic 1). Anal. Calcd. for $C_{22}H_{24}N_2O_2.CH_3SO_3H.0.25H_2O$: C, 61.52; H, 6.40; N, 6.24; S, 7.14%. Found: C, 61.54; H, 6.44; N, 6.17; S, 7.02%.

2-Methyl-2-[[(7-methyl-7*H*-benzo[c]carbazol-10-yl)[^{14}C]methyl]amino]-1,3-propanediol mesylate 1.Mesylate

To a mixture of 2-amino-2-methyl-1,3-propanediol (365.2 mg, 3.47 mmol) and *p*-toluenesulphonic acid hydrate (35.6 mg) were added [carbonyl-^{14}C]-3 (440.7 mg, 1.69 mmol, with specific activity ~50 mCi/mmol) and dry toluene (7.5 mL). The mixture was stirred and heated to reflux in an oil-bath under argon during 40 min, and toluene and H_2O were distilled out for 2.5 h while dry toluene was added to maintain constant volume. The mixture was allowed to cool, stirred at 25-28°C and treated with dry

100% EtOH (1.2 mL). A mixture of $NaBH_4$ (67.8 mg) and dry 100% EtOH (7.6 mL) was added dropwise during 3 min, and the resulting pale yellow cloudy solution was stirred under argon overnight at 25°C. After evaporation to dryness, the yellowish foam was treated with H_2O (18.1 mL) and the mixture stirred and cooled to 0°C. The solid was filtered, washed with ice-cold H_2O (3.6 mL), and dried *in vacuo* at 42°C overnight. The yield of white solid [^{14}C]-1 free base was 538.1 mg (91.0% yield); TLC: EtOAc/MeOH/Et_3N (75/25/1, v/v), one major spot with $R_f = 0.20$ corresponding to authentic 1.

The [^{14}C]-1 base was stirred in dry 100% EtOH (4.0 mL) under argon, treated with CH_3SO_3H (99.5%, 156 mg, 1.05 equiv) and the mixture stirred vigorously for 15 min. Dry toluene (8.0 mL) was added and the mixture stirred at 25°C for 2.75 h and at 0°C for 1.25 h. The crystals were filtered, washed with ice-cold toluene (2.6 mL), and dried *in vacuo* at 42°C overnight. The yield of cream crystalline solid [^{14}C]-1.CH_3SO_3H.0.25H_2O was 677.5 mg (72.75 mCi; 89.0% yield from [^{14}C]-3) with specific activity 48.4 mCi/mmol.

TLC: EtOAc/MeOH/Et_3N (75/25/1, v/v) showed single-spot material with $R_f = 0.21$ corresponding to authentic 1. No impurities were detected by radioactive scanning of the TLC plate, and the radiochemical purity was 97.3%.

HPLC (System B): 99.2% ($t_R = 4.36$ min, corresponding to authentic 1).

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