Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 1095-1105 (2000)

Carbon-14 Labeling of a Potential New Immunoregulant Agent

M. A. McEvoy Egan*, D. C. Dean, T. M. Marks,
Zhiguo Song, D. G. Melillo
Merck Research Laboratories, P. O. Box 2000, Rahway, N. J. 07065

Summary

A carbon-14 labeled version of the ascomycin analog **1**, a potential new immunosuppressant agent, was synthesized for utilization in animal and human drug metabolism studies. In order to place the carbon-14 label at a metabolically stable position, it was necessary to modify the established synthesis of a key intermediate. [^{14}C]**1** is prepared by a highly chemoselective alkylation of ascomycin at the C-32 hydroxy position with a carbon-14 labeled imidazolyl trichloroimidate side chain **2**. Carbon-14 was efficiently incorporated in **2** through carboxylation of an imidazole C-2 lithiate with [^{14}C]carbon dioxide.

Keywords: carbon-14, immunoregulant, imidazole, lithiation, coupling

Introduction

The development of improved immunosuppressants for the treatment of autoimmune disease and prevention of organ transplant rejection has been an important, yet elusive, research objective over the last decade. The isolation of a 23-member macrolactam designated FK-506 in 1984 by Fujisawa scientists from *Streptomyces tsukubaensis* initiated the most significant advance in immunosuppression discovery since the advent of cyclosporins.[1] FK-506, and its C21-ethyl analog ascomycin[1,2] (see Figure 1), were found to be 50-100 fold more potent than cyclosporin A. Although these naturally occurring 21-membered macrocyclic lactones possess exceptional immunosuppressive activity, their poor bioavailability and unacceptably high toxicity has sparked an ongoing search for less toxic derivatives with similar efficacy to FK-506. Compound **1** is a promising ascomycin analog developed at Merck containing an aryl imidazole side chain attached via an ether linkage at the C-32 hydroxyl.[3] Herein, we report the synthesis of [^{14}C]**1**, which was required for animal and human ADME studies, through the preparation and subsequent attachment of a ^{14}C-labeled imidazole side chain.

* To whom correspondence should be addressed. Ph. (732) 594-2695, FAX (732) 594-7090, e-mail: marjorie_egan@merck.com

Received 8 March 2000
Revised 12 June 2000
Accepted 26 June 2000

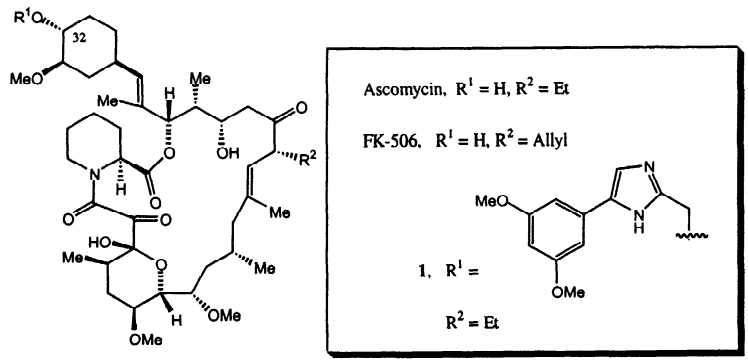


Figure 1: FK-506, Ascomycin, and Analog **1**

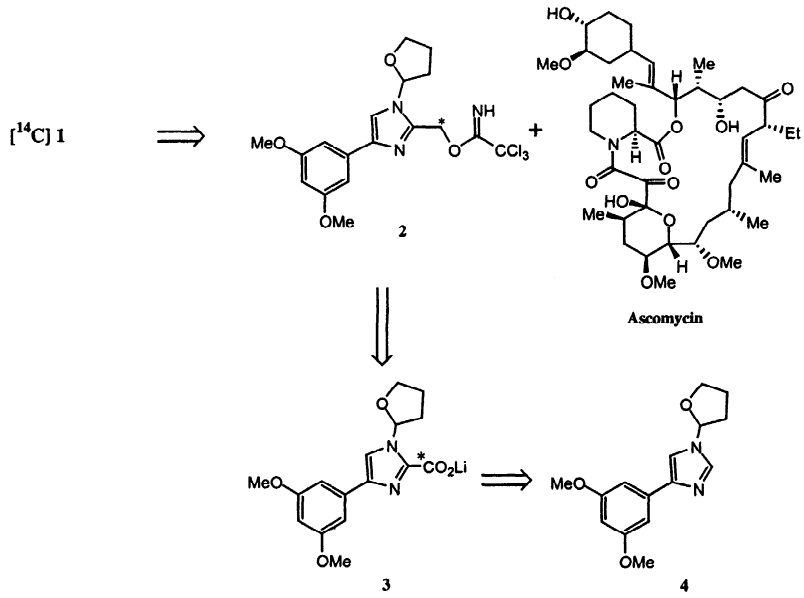
Carbon-14 labeling of the FK-506 macrocycle via fermentation using sodium [1-^{14}C] propionate has been previously reported[1c]. However, the extremely low radiochemical yield (0.59%) obtained through fermentation was a severe hurdle for generating significant quantities of [^{14}C]ascomycin. A convergent synthesis of **1** was recently reported by Song and coworkers of Merck Process Research which employs a highly chemoselective alkylation of ascomycin at the C-32 hydroxyl with an imidazoyltrichloroimidate (Scheme 1).[3c] Fortunately, the apparent metabolic stability of the ether linkage at C-32[4] allowed label incorporation in the imidazole fragment **2** to be pursued as a more efficient alternative to labeled fermentation. Synthesis of [methylene-^{14}C]imidazole side chain **2** appeared accessible from the imidazole 2-[^{14}C]carboxylate **3** obtained via [^{14}C]carboxylation of the 2-lithiate of **4**.

Results and Discussion

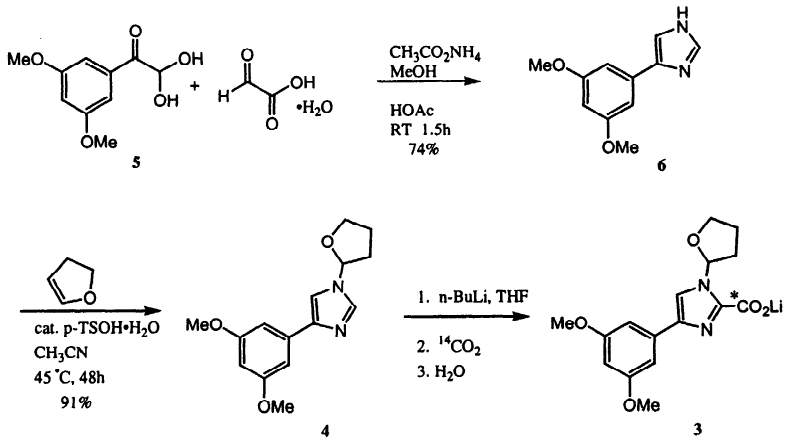
Preparation of unlabeled imidazole precursor **4** was accomplished through reaction of 3,5-dimethoxyphenylglyoxal (**5**)[3c,5] with glyoxylic acid monohydrate and ammonium acetate to give 4-aryl imidazole **6** followed by protection of the amine group with dihydrofuran in the presence of catalytic p-toluene sulfonic acid[6] to provide **4** in an overall yield of 67% after silica gel chromatography. Regioselective C-2 lithiation[7] of **4** was effected by treatment with 1.2 equivalents of n-BuLi at –20 °C followed by addition of 1.5 equivalents of [^{14}C]carbon dioxide to provide 2-[^{14}C]carboxylate **3** in 69% radiochemical yield. Due to the propensity of the protonated acid of imidazole 2-carboxylates to undergo decarboxylation,[8] **3** was isolated as the

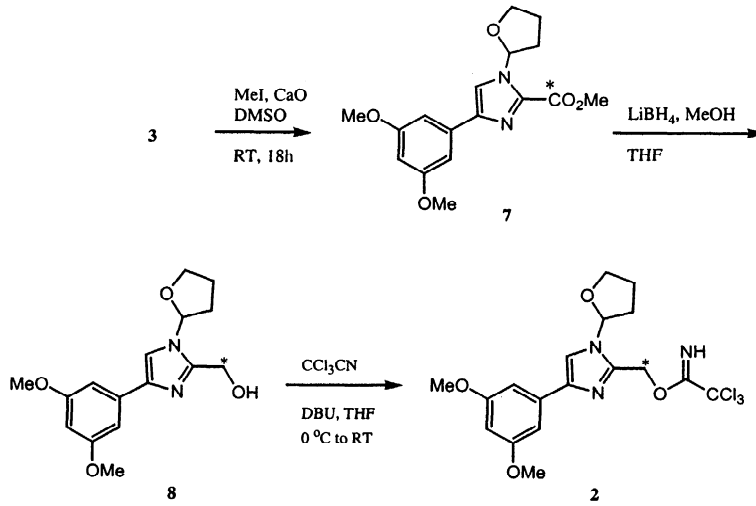
lithium salt and immediately converted to the methyl ester **7** by alkylation with methyl iodide in DMSO (68% yield).[9]

Scheme 1



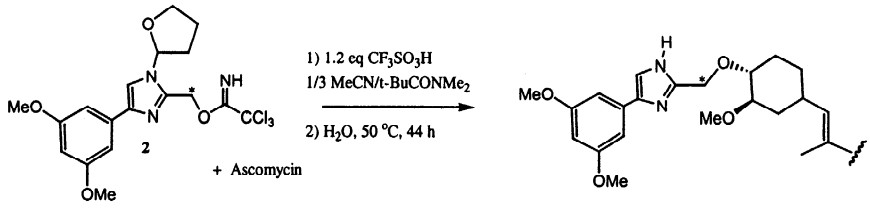
Reduction of **7** with lithium borohydride gave alcohol **8** in 71% radiochemical yield.[10] Reaction of **8** with trichloroacetonitrile in the presence of a catalytic amount of DBU gave [^{14}C]trichloroimidate **2** in 92% yield, completing the synthesis of the desired side-chain.[11]

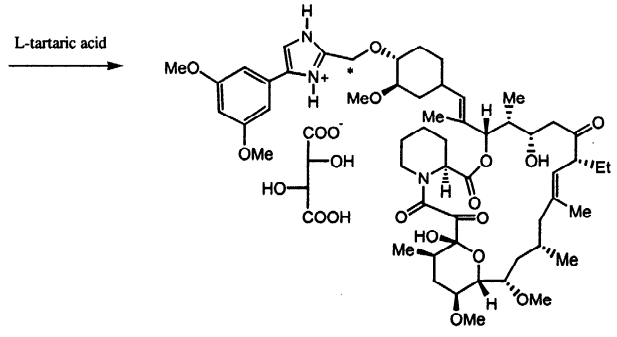




Chemoselective coupling of **2** to the macrocycle ascomycin was conducted according to the highly optimized conditions established by Merck Process Research.[3c] Because ascomycin is unstable toward strong bases, the ether linkage between the side chain and the C-32 hydroxyl of the macrocycle must be made under nearly neutral or acidic conditions. Exposure of the trichloroacetimidate **2** to a strong acid such as trifluoromethanesulfonic acid presumably produces a carbonium ion intermediate which can be stabilized by the basic N,N-dimethyl pivalamide cosolvent. This stabilization effectively suppresses oligimerzation byproduct (formed via Friedel-Crafts reaction with the electron-rich dimethoxyphenyl ring of **2**) and was found to be essential for efficient selective capture by ascomycin. Accordingly, treatment of **2** with 1.5 equivalents of ascomycin and 1.2 equivalents triflic acid in a 3:1 mixture of N,N-dimethylpivalamide:acetonitrile was followed by hydrolytic removal of the THF group (water, 50 °C, 44 h). Initial purification of the resulting crude [^{14}C]**1** free base was effected by flash silica chromatography system to provide product in an overall 19% radiochemical yield (8.8 mCi) for the coupling process at 83% radiochemical purity.

Crystallization of the free base from ethyl acetate/acetonitrile/water in the presence of 1.25 equivalents of L-tartaric acid generated the corresponding tartrate salt at 94.4% radiochemical purity. Dilution of specific activity through addition of unlabeled drug followed by recrystallization in a similar manner increased the radiochemical purity to 97.9% providing 4.27 mCi of tracer (49% radiochemical yield from the free base) suitable for human ADME studies.





Experimental

Silica chromatography was carried out using the Biotage Flash 40 system. Radioactivity measurements were determined using a Packard Tri-carb 1000 TR liquid scintillation spectrometer and Scintiverse I^{TM} as scintillation medium. Analytical TLC was performed using silica gel 60 F-254 (E. Merck). Unless otherwise noted, analytical HPLC analyses were performed using a Dupont SB-phenyl column (4.6 mm x 25 cm), Kratos Analytical Spectraflow 757 UV detector, Berthold LB-506-C radioactivity monitor, Thermo Separations Products SpectraSYSTEM P4000 LC pump and controller, and software run on a Dell Masterpiece computer. ^{1}H NMR spectra were recorded on a Bruker EM300 NMR. Melting points were determined on a Laboratory Devices, USA Mel-temp II with a Fluke 51 K/J thermometer. The identities of the labeled intermediates **2**, **6**, **7**, and **8**, as well as the final product **1**, were established by co-elution via HPLC or TLC with authentic material from Merck Process Research.[3c]

4-(3′,5′-dimethoxyphenyl)imidazole (6)

A 500 mL round bottom flask was charged with glyoxylic acid monohydrate (6.1g, 66.0 mmol), ammonium acetate (10.2 g, 132 mmol), methanol (135 mL) and glacial acetic acid (65

mL). The mixture was cooled to 0 °C and a solution of 3,5-dimethoxyphenylglyoxal (**4**) (7.0 g, 33 mmol) in methanol (150 mL) was added over 15 minutes via an addition funnel. The solution was stirred at 0 °C for 3.5 h then warmed to room temperature and stirred for an additional 16 h. The solvent was concentrated to approximately 60 mL, transferred to a 400 mL beaker, and water (70 mL) was added. 5N NaOH was added until the pH reached 9, and the aqueous solution was then extracted with ethyl acetate (2 x 70 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (2 x 50 mL) and water (2 x 50 mL), and dried over anhydrous magnesium sulfate. The solvent was removed on a rotary evaporator and the resulting orange solid was dissolved in 60 mL of hot ethyl acetate, cooled to room temperature, and 70 mL of hexanes added over 5 min. The suspension was stirred at room temperature for 16 h. The solids were filtered and dried under vacuum for 2 h at 40 °C to yield 4.05g (74% yield) of 4-(3′,5′-dimethoxyphenyl)imidazole (**6**) at 93.4% chemical purity by HPLC (Zorbax SB-phenyl, 1 mL/min, 40A:60B over 30 min, A = acetonitrile, B = 20 mM phosphate buffer pH = 6 containing 5% acetonitrile, t_R = 5.12 min): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.91 (broad s, 1H), 7.77 (s, 1H), 7.34 (s, 1H), 6.89 (d, 2H, J = 2 Hz), 6.38-6.37 (m, 1H), 3.78 (s, 6H).

4-(3′,5′-dimethoxyphenyl)-1-(1″-tetrahydrofuranyl)imidazole (4)

A 250 mL round bottom flask was charged with 4-(3′,5′-dimethoxyphenyl)imidazole (**6**) (4.0 g, 19.4 mmol), 2,3-dihydrofuran (4.08 g, 4.4 mL, 58.3 mmol), and CH_3CN (110 mL). The mixture was heated at 50 °C until all the solid had dissolved, at which time p-toluenesulfonic acid (1.1 g, 5.83 mmol) and 4Å molecular sieves (1 g) were added. The mixture was stirred at 50 °C for 26 h at which point another 0.5 mL of 2,3-dihydrofuran was added. The reaction was stirred at 50 °C until the reaction was complete (a total of 43 h) as determined by HPLC analysis [SB-Phenyl, 1 mL/min, 40A:60B over 30 min, A = acetonitrile, B = 20 mM phosphate buffer pH = 6 containing 5% acetonitrile, t_R = 8.10 min]. The mixture was cooled to room temperature, filtered to remove the molecular sieves, and concentrated in vacuo. The residue was dissolved in methylene chloride (50 mL) , extracted with 5% sodium carbonate solution (50 mL), the aqueous re-extracted with methylene chloride (50 mL), and the combined organics dried over anhydrous magnesium sulfate. The solvent was removed in vacuo and the residue dissolved in 20 mL of 98:2 ethyl acetate:methanol . The solution was split into 10 mL portions, each of which was purified using a Biotage Flash 40 SiO_2 chromatography system (90g cartridge; elution solvent = 98:2 ethyl acetate:methanol) to yield a total of 4.51 g of 4-(3′,5′-dimethoxyphenyl)-1-(1″-tetrahydrofuranyl)-imidazole (**4**) with a purity of 94% as determined by HPLC analysis (conditions as above): mp 94-95 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.67 (d, 1H, J = 0.95 Hz), 7.25 (d, 1H, J = 1.20 Hz), 6.95 (d, 2H, J = 2.2 Hz), 6.37 (t, 1H, J = 2.2 Hz), 5.92-5.89 (dd, 1H, J = 6.23, 2.9 Hz), 4.18-4.02 (m, 2H), 3.83 (s, 6H), 2.43-2.28 (m, 2H), 2.15-2.04 (m, 2H).

4-(3′,5′-dimethoxyphenyl)-1-(1″-tetrahydrofuranyl)imidazole lithium [^{14}C]carboxylate (3)

An oven dried, two-necked, 100 mL pear-shaped flask equipped with a magnetic stirrer was attached to a gas transfer manifold and the system purged with nitrogen. The flask was then charged with pure 4-(3′,5′-dimethoxyphenyl)-1-(1″-tetrahydrofuranyl)-imidazole (**4**) (842 mg, 3.07 mmol) and anhydrous tetrahydrofuran (42 mL). The solution was cooled to -20 °C and n-butyllithium (1.6M in hexanes; 2.3 mL, 3.70 mmol) added. This solution was stirred 30 min, then frozen with a liquid nitrogen bath. [^{14}C]CO_2 (250 mCi, 4.55 mmol, 55 mCi/mmol) was transferred under vacuum via a break-seal flask previously attached to the manifold. The reaction flask was warmed to -78 °C and stirred at this temperature for 30 min during which time a bright pink color developed. The solution was warmed to -10 °C and stirred for an additional 45 min. Water (6 mL) was added, and the reaction mixture stirred 48 h at ambient temperature. Additional water (50 mL) and methylene chloride (50 mL) were added, and the layers separated. The aqueous layer was washed with methylene chloride (3 x 40 mL), concentrated to approximately 10 mL, and lyophilized to give 4-(3′,5′-dimethoxyphenyl)-1-(1″-tetrahydro-furanyl)-imidazole lithium [^{14}C]carboxylate (**3**) (192 mCi) in 69% radiochemical yield with a purity of 60% as determined by HPLC [SB-phenyl, 1 mL/min, 40A:60B over 30 min, A = methanol, B = 20 mM phosphate buffer pH = 6 containing 5% acetonitrile]. This material was taken on without further purification.

[^{14}C]-2-carbomethoxy-4-(3′,5′-dimethoxyphenyl)-1-(1″-tetrahydro-furanyl)imidazole (7)

Crude [^{14}C]carboxylate **3** (256 mCi; 55 mCi/mmol; 65 % radiochemical purity) in dimethylsulfoxide (40 mL) was placed in a 250 mL round bottom flask equipped with a magnetic stirrer. Methyl iodide (4.35 g, 30.68 mmol), calcium oxide (3.99 g, 71.18 mmol), and calcium sulfate (6.4 g, 20-40 mesh) were added and the mixture stirred at room temperature for 16 h. The solids were removed by filtration, and rinsed with ethyl acetate (30 mL). Additional ethyl acetate (50 mL) and water (50 mL) were added, the layers separated, and the aqueous layer extracted with ethyl acetate (2 x 30 mL). The combined organic layers were washed with 10% sodium thiosulfate (3 x 30 mL), 5% sodium carbonate (30 mL), and brine (30 mL). The solution was dried over magnesium sulfate, and the solvent was concentrated in vacuo. A solvent mixture of 1:1 ethyl acetate:hexanes (12 mL) was added to the residual solid, and the mixture was heated to boiling with vigorous stirring. Much of the solid remained undissolved. After cooling to room temperature, the solids were collected by filtration, washed with ice-cold 1:1 ethyl acetate:hexanes (25 mL), and dried under vacuum for 16 h to give [^{14}C]-2-carbomethoxy-4-(3′,5′-dimethoxyphenyl)-1-(1″-tetrahydrofuranyl)imidazole (**7**) (112.7 mCi, 593 mg), in 68% yield, with a radiochemical purity of 87% by HPLC [SB-Phenyl, 1 mL/min, 40A:60B over 30 min, A = acetonitrile, B = 20 mM phosphate buffer pH = 6 containing 5% acetonitrile, t_R = 11.54 min)]. This off-white solid was taken on without further purification.

[^{14}C]4-(3′,5′-dimethoxyphenyl)-2-hydroxymethyl-1-(1″-tetrahydrofuranyl)imidazole (8)

A 100 mL recovery flask equipped with a magnetic stirrer was charged with [^{14}C]-2-carbomethoxy-4-(3′,5′-dimethoxyphenyl)-1-(1″-tetrahydrofuranyl)imidazole (7) (696 mg, 2.09 mmol, 137 mCi) and tetrahydrofuran (30 mL). The solution was cooled to -5 °C, purged with nitrogen, and methanol (90 μL, 2.09 mmol) added. Lithium borohydride (51 mg, 2.30 mmol) was added, and the solution warmed to 10 °C over 45 min. After the reaction mixture was stirred an additional 1.25 h at 10 °C, saturated ammonium chloride (7 mL) was added over 5 min, and the mixture stirred for 50 min at room temperature. The reaction mixture was dissolved in ethyl acetate (20 mL) and water (20 mL), the organic layer washed with brine (2 x 10 mL), and dried over anhydrous magnesium sulfate. The solvent was concentrated in vacuo and the residue purified using a Biotage Flash 40 SiO_2 chromatography system (40 g cartridge, elution solvent = 98:2 ethyl acetate:methanol) to give a 71% radiochemical yield of [^{14}C]4-(3′,5′-dimethoxyphenyl)-2-hydroxymethyl-1-(1″-tetrahydrofuranyl)imidazole (8) (97.3 mCi, 518 mg), 99% radiochemically pure by HPLC [SB-Phenyl, 1 mL/min, 40A:60B over 30 min, A = acetonitrile, B = 20 mM phosphate buffer pH = 6 containing 5% acetonitrile, t_R = 5.36 min)].

[^{14}C]1-(1″-Tetrahydrofuranyl)-2-trichloroacetimidoxymethyl-4-(3′,5′-dimethoxyphenyl)-imidazole (2)

A 50 mL round bottom flask equipped with a magnetic stirrer was charged with [^{14}C]4-(3′,5′-dimethoxyphenyl)-2-hydroxymethyl-1-(1″-tetrahydrofuranyl)imidazole (8) (97.3 mCi, 1.77 mmol) and tetrahydrofuran (16 mL), and the solution cooled to 0 °C. Trichloroacetonitrile (382 mg, 265 μL, 2.65 mmol) and 1,8-diazobicyclo[5,4,0]undec-7-ene (DBU) (12 mg, 12μL, 0.08 mmol) were added, and the reaction aged 16 h at room temperature. The solution was filtered through celite, concentrated in vacuo, and ethyl acetate (4 mL) added which produced a thick slurry. Hexanes (6 mL) was added, and the slurry stirred 3 h at room temperature. The solids were filtered, washed with ice-cold 1:1.5 ethyl acetate:hexanes (6 mL), and hexanes (3 x 5 mL). The solid was dried under vacuum for 16 h to give [^{14}C]1-(1″-tetrahydrofuranyl)-2-trichloroacetimidoxymethyl-4-(3′,5′-dimethoxyphenyl)imidazole (2) (89.5 mCi, 622 mg, 92% radiochemical yield) of 96% radiochemical purity by HPLC [SB-phenyl, 1 mL/min, 55A:45B over 30 min, A = acetonitrile, B = 20 mM phosphate buffer pH = 6 containing 5% acetonitrile, t_R = 8.42 min]. This material was degassed and stored in acetonitrile at 0 °C.

[32C-O-methylene-^{14}C]1 Free Base

A 25 mL round bottom flask was charged with [^{14}C]1-(1″-tetrahydrofuranyl)-2-trichloroacetimidoxymethyl-4-(3′,5′-dimethoxyphenyl)imidazole (2) (39.6 mCi, 0.727 mmol),

ascomycin (864 mg, 1.091 mmol) and N,N-dimethyltrimethylacetamide[4] (DTA, 4.2 mL). Acetonitrile (15 mL) was added, and the solution was concentrated at 25 °C/28 mm Hg. The acetonitrile flush was repeated 3 times. Anhydrous acetonitrile (1.45 mL) was added and the solution was cooled to -33 °C. Triflic acid (77 μL, 131 mg, 0.872 mmol) was added under positive nitrogen pressure. The mixture was warmed to 0 °C and stirred for 3 h. Water (2.4 mL) and triflic acid (25 μL) were added, and the reaction mixture stirred for 50 h at 50 °C. The mixture was diluted with ethyl acetate (8 mL) and saturated sodium bicarbonate (5 mL), the layers separated, and the organic portion washed with brine (5 mL). The aqueous layer was back-extracted with ethyl acetate (5 mL). The combined organic layers were concentrated in vacuo. Acetonitrile (10 mL) was added, the solution concentrated at 25 °C/28 mm Hg, and this azeotropic drying was repeated twice. The residual oil was flushed with isopropanol (2 x 10 mL), followed by additional acetonitrile (2 x 10 mL). The crude product was dissolved in acetonitrile (9 mL) and hexanes (6 mL), the layers separated, and the acetonitrile layer extracted four times with hexanes (6 mL portions) to remove all dimethyl pivalamide which had formed during the reaction.

A portion (21.4 mCi) of the crude [^{14}C]**1** free base in acetonitrile was concentrated in vacuo to a thick oil, dissolved in 3 mL ethyl acetate, and loaded onto the Biotage Flash 40 SiO_2 chromatography system (90g cartridge). The column was initially eluted with 50:50 ethyl acetate:n-heptane (1100 mL). The solvent was changed to 100% ethyl acetate and 2000 mL collected which contained 9.49 mCi. The solvent was changed to 2% methanol in ethyl acetate and 760 mL were collected which contained 3.2 mCi. The ethyl acetate and 2% methanol in ethyl acetate fractions were combined (12.7 mCi) and concentrated in vacuo. In order to further increase the radiochemical purity of this material, the residual oil was dissolved in 2 mL ethyl acetate, and loaded onto the Flash 40 system (90 g cartridge). The column was sequentially eluted with 50:50 ethyl acetate:n-heptane (600 mL), ethyl acetate (970 mL), and 2% methanol in ethyl acetate. The eluent from the latter was collected in 40 mL fractions. The 2% methanol in ethyl acetate fractions were analyzed by thin-layer chromatography (SiO_2, 2% methanol in ethyl acetate) and by HPLC. The purest fractions were combined, and the solvent removed on the rotary evaporator to give [32C-O-methylene-^{14}C]**1** free base (8.83 mCi, 176 mg) of 83% radiochemical purity by HPLC (YMC ODS-AM, 1.2 mL/min, 50 °C, 90A:10B to 60A:40B over 10 min, then to 30A:70B over 50 min, then to 0A:100B over 1min, A = 0.1% H_3PO_4, B = acetonitrile).

[32C-O-methylene-^{14}C]1 tartrate salt

A 25 mL pear-shaped flask, equipped with a magnetic stirrer was charged with [32C-O-methylene-^{14}C]**1** free base (8.83 mCi, 0.16 mmol, 176 mg), ethyl acetate (1.2 mL), acetonitrile

(0.97 mL), and water (19 μL). L-tartaric acid (30 mg, 0.20 mmol) was added. Upon heating to 50 °C the solid dissolved, and the solution was seeded with unlabeled **1** tartrate (0.3 mg). The slurry was aged 16 h at room temperature. The solids were filtered, washed with ice-cold ethyl acetate (3 x 0.3 mL), and dried under vacuum for 3 h to give [32C-O-methylene-^{14}C]**1** tartrate salt (5.73 mCi, 52.7 mCi/mmol, 126 mg) of 94.4% radiochemical purity by HPLC analysis (YMC ODS-AM, 1.2 mL/min, 50 °C, 90A:10B to 60A:40B over 10 min, then to 30A:70B over 50 min, then to 0A:100B over 1min, A = 0.1% H_3PO_4, B = acetonitrile). The solid was dissolved in acetonitrile (4.4 mL), ethyl acetate (5.3 mL), and water (98 μL). Unlabeled **1** tartrate (693 mg, 0.60 mmol) was added, and the mixture heated to 50 °C until the solid dissolved. The solution was cooled to ambient temperature, seeded with 0.7 mg unlabeled **1** tartrate, and stirred for 16 h, under nitrogen. The solid was collected by filtration, washed with ice-cold ethyl acetate (1.5 mL), and dried 20 h under vacuum to give tracer (633.2 mg, 4.27 mCi, 6.74 μCi/mg) of 99.3% UV purity and 97.9% radiochemical purity by HPLC analysis (YMC ODS-AM 5μ 4.6 x 250 mm, 1.2 mL/min, 50 °C, UV at 215 nm, 55A:45B to 35A:65B in 40 min, then to 100B in 20 min, A = 0.1% aqueous H_2SO_4, B = 90:10 acetonitrile:tetrahydrofuran).

References/Notes

1. (a) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto,T.; Hashimoto, M.; Taga, T.; *J. Am. Chem. Soc.* **1987**, *109*, 5031. (b) Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *Science*, **1991**, *252*, 839. (c) O'Connor, S. P.; Ellsworth, R. L.; Omstead, M. N.; Jenkins, R. G.; Kaplan, L. *J. Labelled Compd. Radiopharm.* **1992**, *31*, 103-108. (d) Goulet, M.; Sinclair, P. J.; Wong, F.; Wyvratt, M. J. US Patent 5247076, **1993**. (e) Goulet, M.; Hodkey, D. W.; Hung, S. H. Y.; Siekierka, J. J.; Wyvratt, M. J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 921. (f) Goulet, M.; Rupprecht, K. M.; Sinclair, P. J.; Wyvratt, M. J.; Parsons, W. H. *Perspect. Drug Discovery Des.* **1994**, *2*, 145. (g) Jones, T.; Reamer, R. A.; Desmond, R.; Mills, S. G. J. Am. Chem. Soc. 1990, 112, 2998
2. (a) Byrne, K. M.; Shafiee, A.; Nielsen, J. B.; Arison, B.; Monaghan, R. L.; Kaplan, L. in "Microbial Metabolites: vol. 32, p. 29. Nash, C.; Hunter-Cevera, J; Cooper, R.; Eveleigh, D. E.; Hamill, R. Editor, Wm. C. Brown Publishers, 1992. (b)Ok, H. O.; Szumiloski, J. L.; Beattie, T. R.; Goulet, M. T.; Staruch, M. J.; Dumont, F. J., Wyvratt, M. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2199.
3. (a) Goulet, M. T.; McAlpine, S. R.; Staruch, M. J.; Koprak, S.; Dumont, F. J.; Cryan, J. G.; Wiederrecht, G. J.; Rosa, R.; Wilusz, M. B.; Peterson, L. B.; Wyvratt, M. J.; Parsons, W. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2253. (b) Mathre, D. J.; Shuman, R. F.; Sohar, P.; Song, Z. US Patent 5777105, **1998**. (c) Song, Z.; DeMarco, A.; Zhao, M.; Corley, E. G.; Thompson,

A. S.; McNamara, J.; Li, Y.; Rieger, D.; Sohar, P.; Mathre, D. J.; Tschaen, D. M.; Reamer, R. A.; Huntington, M. F.; Ho, G.-J.; Tsay, F.-R.; Emerson, K.; Shuman, R.; Grabowski, E. J. J.; Reider, P. J. *J. Org. Chem.* **1999**, *64*, 1859-1867.

4. Unpublished results.
5. Grimmett, M. R. in "Comprehensive Heterocyclic Chemistry", vol. 5, pp. 457-497; Katritzky, A. R., Rees, C. W., Potte, K. T. Editors, Pergamon Press, **1984**.
6. Wu, D. C. J.; Cheer, C. J.; Panzica, R. P.; Abushannab, E. *J. Org. Chem.* **1982**, *47*, 2661.
7. Shapiro, G.; Gomez-Lor, B. *J. Org. Chem.* **1994**, *59*, 5524.
8. (a) Jones, R. G. *J. Am. Chem. Soc.* **1949**, *71*, 383. (b) Curtis, N. J.; Brown, R. S. *J. Org. Chem.* **1980**, *45*, 4038. (c) Iddon, B. *Heterocycles*, **1985**, *23*, 417.
9. Mehta, G. *Synthesis*, **1972**, 262.
10. Soai, K.; Ookawa, A. *J. Org. Chem.* **1986**, *51*, 4000.
11. (a) Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrehedron Lett.* **1988**, *29*, 4139. (b) Schmidt, U.; Respondek, M.; Liebernecht, A.; Werner, J.; Fisher, P. *Synthesis* **1989**, 256.

