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SYNTHESIS OF CARBON-14 AND TRITIUM LABELED ANALOGS OF MANOALIDE

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ABSTRACT

Several Manoalide analogs, 4-(1-acetyloxyalkyl)-5-hydroxy-2(5H)-furanones labeled with carbon-14 in the alkyl side chain, were synthesized. The key step in these syntheses was singlet oxygenation of 2-trialkylsilyl-4-alkylfurans to give the corresponding 4-alkyl-5-hydroxy-2(5H)-furanones. The carbon-14 label in the side chain was introduced earlier in the synthesis by reaction of the desired labeled alkyl Grignard reagent with the appropriate 2-trialkylsilyl-4-furancarboxaldehyde and trapping of the resulting magnesium alkoxide with acetic anhydride. Labeled n-alkyl Grignard reagents were obtained by carbonation of the corresponding nor-alkyl bromides using ultrapure magnesium metal and barium carbonate-^{14}C followed by reduction of the resulting acid to the alcohol. Bromination followed by metallation gave the desired reagents. Manoalide analogs bearing a carbon-14 or tritium label in the acetyl moiety of the side chain were synthesized by acetylation of the desired sidechain alcohol with acetyl chloride-1-^{14}C, and by acetylation of the desired alcohol with dibromoacetic acid-DCC followed by catalytic halogen-tritium exchange and completion of the synthesis.

INTRODUCTION

Manoalide (A, Figure 1) was isolated by Scheuer from Luffariella variabilis.[1] It was shown to inhibit phospholipase A_2 and possess topical anti-inflammatory activity.[2] Total syntheses of A have been reported by Katsumura[3] and Garst[4]. A series of 4-alkyl substituted-5-hydroxy-2(5H)-furanones and corresponding des-hydroxy compounds has been synthesized and evaluated as analogs of manoalide.[5]

Key Words: Manoalide, 5-hydroxy-2(5H)-furanones, Grignard carbonation, singlet oxygenation

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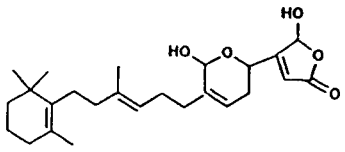
Figure 1



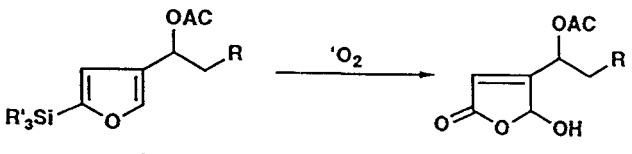
Manoalide, A

Some of these and related compounds were desired in carbon-14 and tritium labeled form for biological studies. The syntheses of analogs labeled with carbon-14 in the 2-position of the 4-alkyl sidechain (2 a-c, Scheme 1), with carbon-14 in the 1-position of the acetyl group of the 4-alkyl sidechain (2f, Scheme 4), and with tritium in the acetoxy group of the 4-alkyl sidechain (2g, Scheme 4) are described herein.

DISCUSSION

Modification of the non-isotopic syntheses[5] of the analogs was sufficient to obtain suitable radiochemical synthetic routes, obviating the need for de novo radiosynthesis development. The key step in the synthesis of these compounds is the construction of the 4-alkyl-5-hydroxy-2(5H)-furanone moiety 2 by singlet oxygenation of 2-trialkylsilyl-4-alkylfurans of the general structure 3[6] (Scheme 1). Deoxygenation of the resulting 4-alkyl-5-hydroxy-2(5H)-furanones is accomplished by sodium borohydride reduction (Scheme 1).

Scheme 1



3a-c

R'=CH_3 or C_2H_5

2a-c

3a R=$(CH_2)_6CH_3$ 2a R=$(CH_2)_6CH_3$

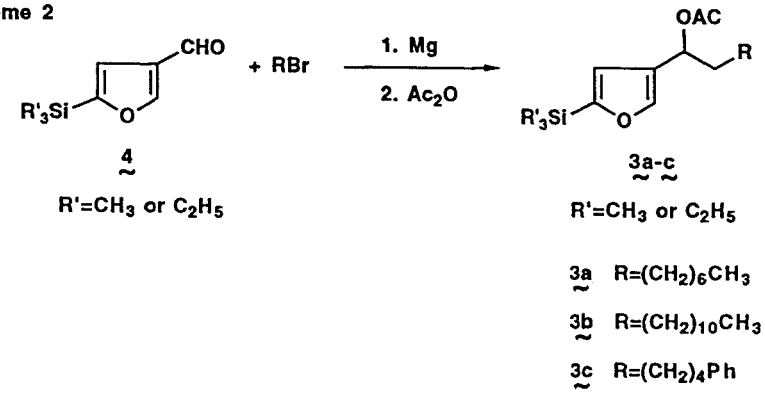
3b R=$(CH_2)_{10}CH_3$ 2b R=$(CH_2)_{10}CH_3$

3c R=$(CH_2)_4Ph$ 2c R=$(CH_2)_4Ph$

The substrates for the singlet oxygenations were obtained by Grignard reaction of the desired alkyl bromide 5a-c (Scheme 3) with the appropriate 2-trialkylsilyl-4-furancarboxaldehyde 4. In-situ quenching of the resulting magnesium alkoxide addition product with acetic anhydride gave the desired 4-(1-acetyloxyalkyl)-2-trialkylsilylfurans 3a-c (Scheme 2). The necessary trialkylsilylfuran carboxaldehydes 4 were prepared by literature methods from commercially available starting materials.[7]

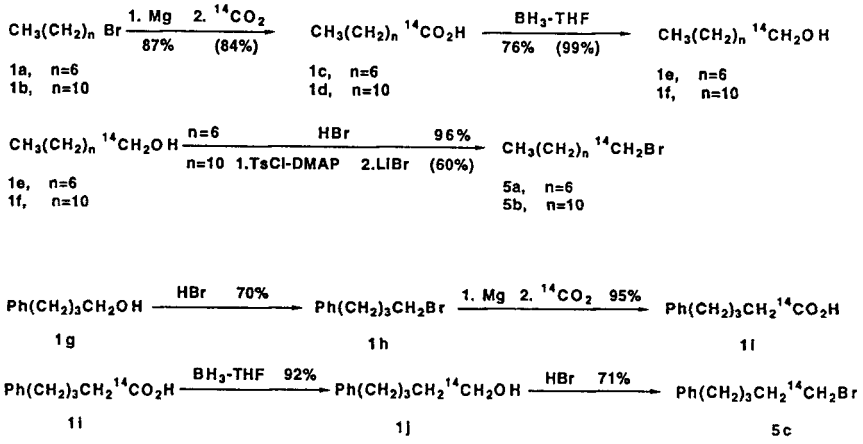
Radiolabeled alkyl halides 5a-c were prepared by standard Grignard reaction-based homologation techniques using barium carbonate-^{14}C as the source of the label (Scheme 3).[8]

Scheme 2



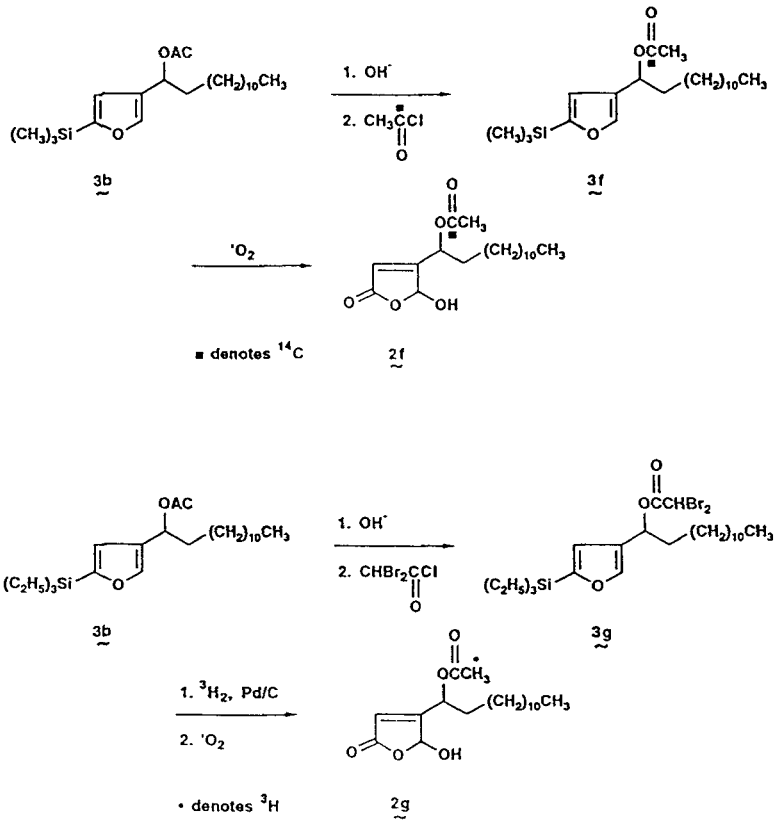
The required precursor nor-alkyl halides 1a,b,h (Scheme 3) were commercially available or synthesized by bromination of the corresponding primary alcohol.

Scheme 3



The carbon-14 label in the acetyl moiety of 2f (Scheme 4) was introduced by basic hydrolysis of 4-(1-acetyloxytridecyl)-2-trimethylsilylfuran 3b, (R' = methyl), re-acetylation of the resulting alcohol with acetyl-1-^{14}C-chloride, and subsequent singlet oxygenation. The tritium label in the acetyl moiety of 2g was introduced by hydrolysis of 4-(1-acetyloxytridecyl)-2-triethylsilylfuran 3b (R' = ethyl), reacetylation with dibromoacetic acid, and subsequent catalytic halogen-tritium exchange followed by singlet oxygenation (Scheme 4).

Scheme 4



RESULTS

A. Synthesis of Carbon-14 Labeled Alkyl Halides

1-Bromooctane-1-^{14}C 5a, 1-bromododecane-1-^{14}C 5b, and 1-bromo-5-phenylpentane-1-^{14}C 5c were synthesized as shown in Scheme 3. Grignard reagent formation from 1-bromoheptane 1a or 1-bromoundecane 1b and ultrapure magnesium metal in ether was followed by treatment

of the alkylmagnesium bromide with carbon dioxide-^{14}C gas (generated from sulfuric acid treatment of barium carbonate-^{14}C at a calculated specific activity of 20 mCi/mmol and 25 mCi/mmol, respectively) using vacuum line techniques. The resulting octanoic-1-^{14}C (1c) and dodecanoic-1-^{14}C (1d) acids (obtained in 87% and 84% radiochemical yield, respectively) were treated with borane-tetrahydrofuran to give octan-1-ol-1-^{14}C and dodecan-1-ol-1-^{14}C (1e,f) in 76% and 99% yield, respectively. 1-Bromo-4-phenylbutane 1h (prepared in 70% yield by treatment of 1-hydroxy-4-phenylbutane 1g with concentrated hydrobromic acid) was converted to the corresponding Grignard reagent and carbonated in the same fashion (barium carbonate-^{14}C at a calculated specific activity of 19.7 mCi/mmol) to give a 95% radiochemical yield of 5-phenyl-1- pentanoic acid-1-^{14}C 1i. Borane-tetrahydrofuran reduction as before gave a 92% radiochemical yield of 1-hydroxy-5-phenylpentane-1-^{14}C 1j. Bromination of these three 1-^{14}C-alcohols was accomplished directly by treatment with concentrated hydrobromic acid (in the case of 1-hydroxy-5-phenylpentane-1-^{14}C) or by a two step sequence (in the case of octan-1-ol-1-^{14}C and dodecan-1-ol-1-^{14}C) involving tosylate formation followed by lithium bromide displacement. The radiochemical yields of 5a, 5b and 5c were 61%, 96%, and 71%, respectively after flash chromatography over Silica Gel.

B. Synthesis of 2-Trialkylsilyl-4-(1-acetyloxyalkyl)furans

The carbon-14 labeled alkyl bromides described above were converted to the corresponding Grignard reagents (Scheme 2) by slow addition of the alkyl bromide to a suspension of dibromoethane-etched ultrapure magnesium in ether with care to exclude oxygen. These precautions decreased the severity of Grignard reagent coupling (dimerization) and oxygenation side reactions. If these experimental proceduress were not followed exactly, the dimerization reaction occurred to an extent of nearly 60%. Addition of 2-triethylsilyl-4-furancarboxaldehyde 4 in ether to 1-bromomagnesiooctane-1-^{14}C or 1-bromomagnesio-5-phenylpentane-1-^{14}C at 0° was followed by addition of acetic anhydride. The radiochemical yields of 4-(1-acetyloxynonyl-2-^{14}C)-2-triethylsilylfuran 3a and 4-(1-acetyloxy-6-phenylhexyl-2-^{14}C)-2-triethylsilylfuran 3c were 35% and 53%, respectively after flash chromatography. Analogous treatment of 1-bromomagnesioundecane-1-^{14}C with 2-trimethylsilyl-4-fruancarboxaldehyde 4 and acetic anhydride provided 4-(1-acetyloxytridecyl-2-^{14}C)-2-trimethylsilylfuran 3b in 52% radiochemical yield after flash chromatography. There was no

apparent difference between the trimethylsilyl and triethylsilyl furaldehyde substrates with respect to the efficiency of this transformation.

C. Formation of 4-(1-Acetoxylalkyl)-5-hydroxy-2(5H)-furanones by Singlet Oxygenation of 2-Trialkylsilyl-4-(1-acetyloxyalkyl)furans

Irradiation (Scheme 1) of 3a or 3c with a 650 watt incandescent lamp at 0° for 30-90 min in tetrahydrofuran-water under an oxygen atmosphere using Rose Bengal as a sensitizer gave 4-(1-acetyloxynonyl-2-^{14}C)-5-hydroxy-2(5H)-furanone 2a and 4-(1-acetyloxy-6-phenylhexyl-2-^{14}C)-5-hydroxy-2(5H)-furanone 2c in 90% and 88% radiochemical yield, respectively after preparative TLC purification. Irradiation of 4-(1-acetyloxytridecyl-2-^{14}C)-2-trimethylsilylfuran 3b with a 250 watt incandescent lamp at -78° for 60 min in tetrahydrofuran under an oxygen atmosphere using Rose Bengal as a sensitizer was followed by treatment with water for 75 min at room temperature. An 86% radiochemical yield of 4-(1-acetyloxytridecyl-2-^{14}C)-5-hydroxy-2(5H)-furanone 2b was obtained after chromatography. The similarity of the yields of final products indicates that the differences in the photolysis-hydrolysis conditions are not significant. However, cursory experiments indicated that photolysis at temperatures above 0° resulted in lower yields.

D. Synthesis of 1-Acetyloxy Carbon-14 and Tritium Labeled Manoalide Analogs

Synthesis of these compounds is shown in Scheme 4. Hydrolytic de-acetylation of 3b (R'-methyl) with potassium hydroxide (quantitative yield) was followed by re-acetylation with acetyl-1-^{14}C chloride (no carrier added, 57 mCi/mmol). Unlabeled acetyl chloride was added after addition of the labeled material to drive the reaction to completion. A 66% radiochemical yield and 91% chemical yield of 4-(1-acetyloxy-1-^{14}C-tridecyl)-2-trimethylsilylfuran 3f were realized. Singlet oxygenation as before at -78° provided a 71% radiochemical yield of 4-(1-acetyloxy-1-^{14}C-tridecyl)-5-hydroxy-2(5H)-furanone 2f after purification. The tritiated compound was synthesized by acetylation of 3b (R'=ethyl) with dibromoacetic acid (84% yield after purification) and subsequent catalytic tritium-halogen exchange using tritium gas over 10% Pd/C in ethyl acetate-triethylamine. Although catalytic tritium-halogen exchanges of this type

are not reported in the literature to our knowledge, preliminary studies with deuterium gas gave promising results. The total deuterium incorporation was 51% of theory, with regiospecific deuteration accounting for most of the products (33% D_1 and 9% D_2). Use of 10 Ci no-carrier-added tritium gas provided 551 mCi of 4-[(1-acetyloxy-t)-tridecyl]-2-triethylsilylfuran 3g after purification. Photooxygenation as before provided 4-[(1-acetyloxy-t)-tridecyl]-5-hydroxy-2(5H)-furanone 2g in 19% radiochemical yield after two stages of chromatographic purification at a specific activity of 18.3 Ci/mmol. This specific acitvity is consistent with the deuterium incorporation results.

SUMMARY

The following Table shows overall radiochemical yields, specific activities, and radiochemical purities for the final products.

Compound	RC Yield[1]	Specific Activity[2]	RC Purity[3]
2a	13%	21.7 mCi/mmol	98.1%[4]
2b	36%	30.0 mCi/mmol	98.9%
2c	29%[5]	19.0 mCi/mmol[6]	99.1%
2f	52%[5]	20.5 mCi/mmol[6]	98.8%
2g	n.a.[7]	18.3 Ci/mmol	97.2%

1. overall yield from barium carbonate-^{14}C
2. determined by CI-MS unless otherwise noted
3. determined by HPLC-radiodetection unless otherwise noted
4. determined by TLC-radioscanning
5. overall yield from acetyl chloride-1-^{14}C
6. determined by LSC
7. starting activity was 10 Ci tritium gas

EXPERIMENTAL SECTION

General: Solvents, mineral acids, and common inorganic reagents were obtained from Aldrich or Baker. Magnesium metal (Puratronic Grade, 99.9999%) was obtained from Johnson Matthey. Sodium borohydride, borane-tetrahydrofuran, lithium bromide, barium carbonate, dibromoacetic acid, 1-bromoheptane, 1-bromododecane, 1,2-dibromoethane, 1-hydroxy-4-phenylbutane, Rose Bengal, oxygen gas, toluenesulfonyl chloride, N,N-dimethyl-4-aminopyridine and lactic acid (80% tech.)were obtained from Aldrich, as were chromatographic

standards as indicated. Dicyclohexylcarbodiimide was obtained from Fluka. 2-Trimethylsilyl-4-furancarboxaldehyde and 2-triethylsilyl-4-furancarboxaldehyde were provided by Dr. G. Lee, Allergan Pharmaceuticals. 10% Palladium on carbon catalyst was obtained from Englehard.

Barium carbonate-^{14}C (59 mCi/mmol) was obtained from Chemsyn Science Laboratories, Lenexa, KS. Acetyl-1-^{14}C chloride (57 mCi/mmol) and carrier-free tritium gas were obtained from Dupont-NEN, Boston, MA.

Chromatographic purification was accomplished by using preparative thin layer chromatography (E Merck Silica Gel, 2 mm adsorbent thickness, 20 cm x 20 cm), flash chromatography (Baker Flash Chromatography Silica Gel), or semi-preparative HPLC. Details of the purifications are given in the individual preparations. High pressure liquid chromatography employed a Beckman Model 110A pump, a Rheodyne 7130 injection valve, a Kratos SF 770 UV detector, and the column described in each preparation.
Analytical high pressure liquid radiochromatography employed a similar solvent delivery system, injection system, and a RAMONA radioactive flow detector or Radiomatic Flo-I radioactive flow detector with a 0.50 mL flow cell and Tru-Count™ LS cocktail at 5 mL/min. The column is specified in the individual preparation. Analytical thin layer radiochromatography used 5 x 20 cm Silica Gel plates (0.25 mm adsorbent thickness, Analtech) and were scanned with a Berthold LB 2230 Linear Analyzer. Mass was visualized by iodine stain or UV light. Proton nuclear magnetic resonance spectra were acquired on a Bruker AM400 instrument at 400 MHz in the indicated solvent. Liquid scintillation counting was done on a Tracor Model 8000 instrument using Beckman ReadySafe™ LS cocktail. Mass spectrometry was done on a VG instrument using the indicated conditions.

PREPARATIONS

1-Bromo-4-phenylbutane 1h To 1-hydroxy-4-phenylbutane 1g (1.2 g, 8 mmol) was added concentrated hydrobromic acid (47% aqueous solution, 10 mL). The mixture was heated at 90° for 1.5 hr. The yellow solution was poured into water and extracted three times with diethyl

ether. The combined ethereal extracts were washed with saturated aqueous sodium bicarbonate solution and dried over magnesium sulfate. Removal of the drying agent by filtration and evaporation of solvent in vacuo gave a yellow oil. Flash chromatography over Silica Gel (5:95 v/v diethyl ether:hexane) gave a colorless oil (1.1 g, 70%).

400 MHz ^{1}H NMR ($CDCl_3$, TMS):

1.65 (2H, m, $-C\underline{H}_2CH_2-$), 1.80 (2H, m, $-CH_2C\underline{H}_2$), 2.55 (2H, t, J=7.0 Hz, $PhC\underline{H}_2CH_2$), 3.35 (2H, t, J=7.0 Hz, $-CH_2C\underline{H}_2Br$), 7.15 (5H, m, aromatic).

General Procedure for Grignard Carbonations of Alkyl Bromides with Carbon Dioxide-^{14}C Generated from Barium Carbonate-^{14}C Using a Vacuum Line.

No-carrier added barium carbonate-^{14}C was diluted with carrier barium carbonate. To the solid was added concentrated sulfuric acid at room temperature and 0.01 torr on a vacuum manifold. The resulting carbon dioxide-^{14}C gas was trapped at liquid nitrogen temperature over phosphorous pentoxide. Back-expansion of the gas into a calibrated portion of the manifold allowed calculation of the yield. In another part of the manifold, under helium at room temperature and atmospheric pressure, magnesium metal in dry diethylether (10 mL) was treated with a catalytic amount (ca 10 uL) of dibromoethane for 15 min. To the mixture was added at room temperature the desired alkyl halide in diethylether (2 mL) in one portion. Upon completion of the reaction (ca 30-45 min), the mixture was frozen at liquid nitrogen temperature and evacuated to 0.01 torr. Carbon dioxide-^{14}C was condensed on the frozen solution, the reaction vessel was isolated from the manifold, and the mixture allowed to warm to room temperature with rapid stirring for 2 hr. To the mixture was added saturated aqueous ammonium chloride solution (5 mL) and 5% aqueous hydrochloric acid solution (15 mL). The mixture was extracted with diethyl ether. The combined ethereal extracts were extracted with saturated aqueous sodium bicarbonate solution. The combined aqueous extracts were acidified with 5% aqueous hydrochloric acid solution and back-extracted with diethyl ether. The ethereal extracts were dried over magnesium sulfate, filtered, and solvent removed at reduced pressure to give the desired 1-^{14}C-acid.

Octanoic acid-1-^{14}C 1c. Barium carbonate: 750 mg, 3.8 mmol, calculated specific activity 20 mCi/mmol. Magnesium: 203 mg, 6.08 mmol. 1-Bromoheptane 1a: 1.36 g, 5.32 mmol. Radiochemical Yield: 67 mCi (87%). Chemical Yield: 483 mg clear oil, (88%).

400 MHz ^{1}H NMR ($CDCl_3$, TMS):

0.88 (3H, t, J=7.0 Hz, 8-CH_3), 1.255 (8h, br s, methylene envelope), 1.62 (2H, quintet, J=7.4 Hz, 3-CH_2), 2.35 (2H, t, J=7.5 Hz, 2-CH_2).

Dodecanoic Acid-1-^{14}C 1d. Barium carbonate: 1.19 g, 6 mmol, calculated specific activity 28 mCi/mmol. Magnesium: 240 mg, 10 mmol. 1-Bromoundecane 1b: 1.66 g, 7.1 mmol. Radiochemical Yield: 126 mCi (84%). Chemical yield: 1.03 g (clear oil, 86%).

400MHz ^{1}H NMR (CDCl3, TMS):

0.877 (3H, t, J=7.0 Hz, 12-CH_3), 1.256 (16H, br s, methylene envelope), 1.628 (2H, quintet, J=7.4 Hz, 3-CH_2), 2.346 (2H, t, J=7.5 Hz, 2-CH_2).

5-Phenylpentanoic acid-1-^{14}C 1i. Barium carbonate: 660 mg, 3.32 mmol, calculated specific activity 19.7 mCi/mmol. Magnesium: 195 mg, 8.36 mmol. 1-Bromo-4-phenylbutane 1h: 1.42 g, 6.69 mmol. Radiochemical yield: 63 mCi (95%). Chemical yield: 566 mg (white crystalline solid, 95%).

400 MHz ^{1}HNMR ($CDCl_3$, TMS):

1.68 (4H, m, 3,4-CH_2), 2.39 (2H, t, J=7.0 Hz, 5-CH_2), 2.64 (2H, t, J=7.0 Hz, 2-CH_2), 7.18-7.3 (5H, m. aromatic).

General Procedure for Borane-Tetrahydrofuran Reduction of 1-^{14}C Carboxylic Acids to the Corresponding Primary 1-^{14}C Alcohols.

To a solution of the desired 1-^{14}C-carboxylic acid in dry tetrahydrofuran (5 mL) under argon at 0^o was added borane tetrahydrofuran complex (1.0 M). The solution was allowed to warm to room temperature over 1.5 hr. To the solution was added 1:1 v/v tetrahydrofuran:water (10 mL) and 5% aqueous hydrochloric acid solution (20 mL). The mixture was extracted with diethyl ether and the combined ethereal extracts were dried over magnesium sulfate. Filtration and solvent removal at reduced pressure gave the desired 1-^{14}C-alcohols.

1-Octanol-1-^{14}C 1e. Octanoic acid-1-^{14}C 1c: 483 mg, 67 mCi, 3.34 mmol). Borane-tetrahydrofuran (1M): 5 mmol. Radiochemical yield: 51 mCi (76%). Chemical yield: 429 mg (98%). TLC analysis (40:60 v/v ethylacetate:hexane, Silica Gel, $R_f = 0.42$, 98.6% radiochemical purity) revealed a single component co-migrating with authentic standard (Aldrich).

1-Hydroxy-5-phenylpentane-1-^{14}C 1j. 5-Phenylpentanoic acid-1-^{14}C 1i: 566 mg, 63 mCi, 3.18 mmol). Borane-tetrahydrofuran (1.0 M): 4.8 mmol. Radiochemical yield: 58 mCi (92%). Chemical yield: 476 mg (91%). TLC analysis (40:60 v/v ethylacetate:hexane, Silica Gel, $R_f = 0.5$, 98.9% radiochemical purity) revealed a single component co-migrating with synthetic standard.

1-Dodecanol-1-^{14}C 1f. Dodecanoic acid-1-^{14}C 1b: 126 mCi, 841 mg, 4.2 mmol). Borane-tetrahydrofuran (1.0 M): 10mmol. Radiochemical yield: 125 mCi (99%). Chemical yield 767 mg (98%). TLC analysis (30:70 v/v ethylacetate:hexane, Silica Gel, $R_f = 0.38$, 99.1% radiochemical purity) revealed a single component co-migrating with synthetic standard.

400 MHz ^{1}HNMR ($CDCl_3$, TMS):

0.876 (3H, t, J = 7.0 Hz, 12-CH_3), 1.256 (16 H, br s, methylene envelope), 1.462 (2H, br s, 3-CH_2), 1.563 (2H, quintet, J = 7.4 Hz, 2-CH_2), 3.637 (2H, td, J = 6.6, 1.8 Hz, 1-CH_2).

General Procedure for Bromination of Primary 1-^{14}C Alcohols

Method A: To a solution of the desired 1-^{14}C-alcohol in pyridine containing a catalytic amount of N,N-dimethyl-4-aminopyridine was added at 0° p-toluenesulfonylchloride. The reaction progress at 0° was monitored by TLC analysis (30:70 ethylacetate:hexane, Silica Gel). Upon reaching ca 90% completion, lactic acid (80% tech) was added to the solution at 0°. The mixture was stirred at 0° for 30 min, quenched with water, and the aqueous mixture was extracted with ethyl acetate. The combined organic extracts were washed with saturated aqueous copper sulfate solution, water, and dried over magnesium sulfate. The drying agent was removed by filtration, and solvent removal at reduced pressure gave the crude tosylate. This material was dissolved in acetone and treated with lithium bromide at room temperature. Monitoring the reaction progress by TLC was done as described above. The mixture was quenched with water. The aqueous mixture was extracted with hexane, and the combined

organic extracts were dried over magnesium sulfate. Filtration and solvent evaporation at reduced pressure was followed by flash chromatography (Silica Gel, hexane) to give the final 1-^{14}C-primary bromide.

1-Bromooctane-1-^{14}C 5a. Tosylation: 1-Octanol-1-^{14}C 1e: 429 mg, 51 mCi, 3.29 mmol). Pyridine: 10 mL. N,N-Dimethyl-4-aminopyridine: 30 mg. p-Toluenesulfonyl chloride: 1.25 g, 6.58 mmol. Reaction time to ca 90% completion by TLC analysis: 3 d. Lactic acid: 2 mL. Crude radiochemical yield of tosylate: 45 mCi. Crude chemical yield of tosylate: 607 mg. Bromination: Acetone: 10 mL. Lithium bromide: 860 mg, 1 mmol. Radiochemical yield of bromide after chromatography: 31 mCi (61% from 1e. Chemical yield: 384 mg (60% from 1-octanol-1-^{14}C). TLC analysis (20:80 v/v ethylacetate:hexane, Silica Gel, $R_f = 0.81$, Radiochemical Purity 99.9%) revealed a single component that co-migrated with authentic standard (Aldrich).

1-Bromododecane-1-^{14}C 5b. Tosylation: 1-Dodecanol-1-^{14}C 1f: 782 mg, 125 mCi, 4.2 mmol. Pyridine: 20 mL. N,N-Dimethyl-4-aminopyridine: 50 mg. p-Toluenesulfonyl chloride:6 g, 26.5 mmol. Reaction time to ca 90% completion by TLC analysis: 12 h. Lactic acid: 4 mL. Bromination: Acetone: 30 mL. Lithium bromide: 5 g, 17 mmol. Radiochemical yield of 5b after chromatography: 120 mCi, 96.2%. Chemical yield: 1.0 g (96%). TLC analysis (20:80 v/v ethylacetate:hexane, Silica gel, $R_f = 0.7$, Radiochemical Purity 99.8%) revealed a single component co-migrating with authentic standard (Aldrich).
400 MHz ^{1}HNMR ($CDCl_3$, TMS): 0.878 (3H, t, J=7.0 Hz, 12-CH_3), 1.258 (16 H, br s, methylene envelope), 1.851 (2H, quintet, J=7.6 Hz, 2-CH_2), 3.405 (2H, td, J=6.9, 1.7 Hz, 1-CH_2).

Method B: 1-Bromo-5-phenylpentane 5c. A mixture of 1-hydroxy-5-phenylpentane-1-^{14}C 1j (476 mg, 58 mCi, 2.89 mmol) and concentrated aqueous hydrobromic acid (47%, 10 mL) was heated to 90° for 2.5 h. The mixture was cooled to room temperature, quenched with water, and the resulting mixture was extracted with diethyl ether. The combined ethereal extracts were washed with aqueous saturated sodium bicarbonate and dried over magnesium sulfate. The drying agent was removed by filtration, and solvent was evaporated at reduced pressure. The

resulting oil was purified by flash chromatography (Silica Gel, 5:95 v/v/ ether:hexane) to give a clear oil. The radiochemical yield of 5c was 41 mCi (71%). The chemical yield was 488 mg (74%). TLC analysis (40:60 v/v ethylacetate:hexane, Silica Gel, $R_f = 0.7$, Radiochemical Purity 99.4%) revealed a single component co-migrating with synthetic standard.

General Procedure for Grignard Addition of 1-^{14}C-Alkyl Bromides to 2-Trialkylsilyl-4-furancarboxaldehydes and Subsequent Acetylation.

The desired alkyl bromide-1-^{14}C in diethylether (10 mL) was added over 45 min to a suspension of magnesium metal in diethyl ether (3 mL, metal etched with 50 uL dibromoethane prior to addition of bromide) at room temperature. The mixture was stirred at room temperature for an additional 30 min, and chilled to 0° on an ice-water bath. To the mixture was added the appropriate 2-trialkylsilyl-4-furancarboxaldehyde 4 in ether (3 mL). The mixture was allowed to warm to room temperature over 2.5 h, and treated with acetic anhydride, and stirred at room temperature for 18 h. The mixture was cooled to 0°, quenched with saturated aqueous ammonium chloride solution (ca 15-20 mL), and the resulting aqueous mixture was extracted with diethyl ether. The combined ethereal extracts were washed with water, 1% aqueous sodium bicarbonate solution, water, and dried over magnesium sulfate. The drying agent was removed by filtration, the solvent was evaporated at reduced pressure, and the residue subjected to flash chromatography (Silica Gel, 5:95 ethyl v/v acetate:hexane) to give a clear oil.

4-(1-Acetyloxynonyl-2-^{14}C)-2-triethylsilylfuran 3a. 1-Bromooctane-1-^{14}C 5a: 384 mg, 31 mCi, 1.9 mmol. Magnesium: 47 mg, 1.9 mmol. 2-Triethylsilyl-4-furancarboxaldehyde 4 (R' = ethyl): 400 mg, 1.9 mmol). Acetic anhydride: 0.6 mL. Radiochemical yield: 11 mCi (35%). Chemical yield: 213 mg (35%). TLC analysis (40:60 v/v ethylacetate:hexane, Silica Gel, $R_f = 0.5$, Radiochemical purity 99.9%) revealed a single component co-migrating with synthetic standard.

4-(1-Acetyloxy-6-phenylhexyl-2-^{14}C)-2-triethylsilylfuran 3c. 1-Bromo-5-phenylpentane-1-^{14}C 5c: 488 mg, 41 mCi, 2.15 mmol. Magnesium: 66 mg, 2.71 mmol. 2-Triethylsilyl-4-furancarboxaldehyde 4 (R' = ethyl): 543 mg, 2.58 mmol. Acetic anhydride: 0.5 mL.

Radiochemical yield: 21.6 mCi (53%). Chemical yield: 444 mg (52%). TLC analysis (10:90 v/v ether:hexane, Silica Gel, $R_f = 0.4$, Radiochemical purity 98.3%) revealed a single component that co-migrated with unlabeled synthetic standard.

4-(1-Acetyloxytridecyl-2-^{14}C)-2-trimethylsilylfuran 3b. 1-Bromododecane-1-^{14}C 5b: 500 mg, 120 mCi, 2.0 mmol. Magnesium: 200 mg, 8 mmol. 2-Trimethylsilyl-4-furancarboxaldehyde 4 (R' = methyl): 350 mg, 2.1 mmol. Acetic anhydride: 0.3 mL. Radiochemical yield: 64 mCi, (53%). Chemical yield: 418 mg, (55 %). TLC analysis (5:95 v/v ethylacetate:hexane, Silica Gel, $R_f = 0.34$, Radiochemical purity 99%) revealed a single component that co-migrated with synthetic standard.

400MHz ^{1}H NMR ($CDCl_3$, TMS):

0.247 (9H, s, Si-(CH_3)$_3$), 0.877 (3H, t, J = 7.0 Hz, sidechain CH_3), 1.246 (2H, br s, sidechain methylene), 2.045 (3H, s, CH_3CO_2), 5.759 (1H, t, J = 7.4 Hz, CHOAc), 6.579 (1H, s, 3-H), 7.587 (1H, s, 5-H).

General Procedure for Photooxygenation of 4-(1-Acetyloxyalkyl-2-^{14}C)-2-trialkylfurans

Method A: To a solution of freshly distilled tetrahydrofuran and water under an oxygen atmosphere was added Rose Bengal (2 mg) and a solution of the desired triethylsilylfuran in tetrahydrofuran (0.5 mL). The solution was cooled to 0° on an ice-water bath and irradiated with a 650 watt incandescent lamp for 90 min. The volatiles were removed at reduced pressure, and the residue was purified by chromatography.

4-(1-Acetyloxynonyl-2-^{14}C)-5-hydroxy-2(5H)-furanone 2a. Tetrahydrofuran: 10 mL. Water: 0.15 mL. 4-(1-Acetyloxynonyl-2-^{14}C)-2-triethylsilylfuran 3a: 213 mg, 11 mCi, 0.58 mmol. Chromatography: Preparative TLC, 4:96 v/v ethylacetate:hexane, Silica Gel. Radiochemical yield: 9.9 mCi (90%). Chemical yield 144 mg (88%). TLC analysis (40:60 v/v ethyl acetate:hexane, Silica Gel, $R_f = 0.35$, Radiochemical purity 98.1%) revealed a single component co-migrating with synthetic standard.

400 MHz 1H NMR ($CDCl_3$, TMS):

0.88 (3H, t, J = 7.0 Hz, sidechain CH_3), 1.30 (12H, br s, methylene envelope), 1.83 (2H, m, sidechain methylene), 2.11 (1H, s, CH_3CO_2 minor epimer), 2.13 (2H, s, CH_3CO major epimer),

5.30 (0.3H, t, J=7.2 Hz, CHOAc minor epimer), 5.48 (1H,t, J=7.5 Hz, CHOAc major epimer), 6.01 (1H, s, C-5 H), 6.19 (1H, s, C-3 H)

4-(1-Acetyloxy-6-phenylhexyl)-2-^{14}C-5-hydroxy-2(5H)-furanone 2c. Tetrahydrofuran: 20 mL. Water: 0.61 mL). 4-(1-Acetyloxy-6-phenylhexyl-2-^{14}C)-2-triethylsilylfuran 3c: 444 mg, 21.6 mCi, 1.11 mmol. Chromatography: Flash chromatography, Silica Gel, 50:50 v/v ethyl acetate:hexane. Radiochemical yield 19 mCi (88%). Chemical yield: 337 mg (95%).

Analysis of Final Product:

Radiochemical Purity by TLC (50:50 v/v ethylacetate:hexane, Silica Gel, R_f=0.4): 98.2%. Radiochemical Purity by HPLC (Beckman Ultrasphere C-8 column, 0.46 x 25 cm, 5 micron packing, 55:45 v/v acetonitrile, 1.0 mL/min, UV at 215 nm, radiodetection described above, R_t 8 min, co-migrating with synthetic standard): 99.1%. Chemical purity by HPLC UV peak area (identical analytical system): 98%. Specific Activity (LSC): 19.0 mCi/mmol.

400 MHz ^{1}HNMR ($CDCl_3$, TMS):

1.42 (4H, m, C-3,4 CH_2), 1.65 (2H, m, C-5 CH_2), 1.85 (2H, m, C-2 CH_2), 2.09, (1H, s, CH_3CO_2 minor epimer), 2.12 (2H total, s, CH_3CO^2 major epimer), 2.61 (2H, t, J=7.0 Hz, $PhCH_2$), 3.46 (0.3H, d, J=7.8 Hz, C-5 CHOH minor epimer), 4.44 (0.7H, d, J=10 Hz, C-5 CHOH major epimer), 5.34 (0.3H, t, J=5.1 Hz, CHOAc minor epimer), 5.93 (0.7H, t, J=5.5 Hz, CHOAc major epimer), 5.47 (0.3 H, d, J-5.8 Hz C1 H minor epimer), 6.17 (0.7H, d, J=6.2 Hz, C1 H major epimer).

Method B: 4-(1-Acetyloxytridecyl-2-^{14}C)-5-hydroxy-2(5H)-furanone 2b. A solution of 4-(1-acetyloxytridecyl-2-^{14}C)-2-trimethylsilylfuran 3b (500 mg, 63.8 mCi, 2.0 mmol) and Rose Bengal (1.8 mg) in freshly distilled tetrahydrofuran (15 mL) under an oxygen atmosphere was cooled to -78° on a Dry Ice-acetone bath. The solution was irradiated with a 250 watt incandescent lamp for 1 hr. The cooling bath was removed, water 90.3 mL) was added, and the oxygen atmosphere was replaced with argon. The mixture was stirred at room temperature for 1.75 hr. Volatiles were removed at reduced pressure, and the residue was purified by flash chromatography (0-95% v/v diethylether:hexane step gradient). The resulting oil was crystallized from methylenechloride:hexane. The radiochemical yield was 47.5 mCi (74%). The chemical yield was 538 mg (79%). Analysis by HPLC (Altex Ultrasphere C-8 column, 5 micron packing, 0.46 x 25 cm, 10:90 water:acetonitrile, 1.0 mL/min, UV at 210 nm, radioactive

detection as described above, R_t 7.3 min, radiochemical purity 98.9%) revealed a single peak that co-migrated with synthetic standard.

400MHz ^{1}H NMR ($CDCl_3$, TMS):

0.88 (3H, s, sidechain CH_3), 1.26, (20H, br s, methylene envelope), 1.81 (2H, m, sidechain methylene), 2.11 (0.3 H, s, CH_3CO_2 minor epimer), 2.12 (0.7H, s, CH_3CO_2 major epimer) 3.55 (0.3H, d, J=7.2 Hz, OH minor epimer), 4.58 (0.7H, d, J=10.3 Hz, OH major epimer), 5.31 (0.7 Hz, t, J=6.5 Hz, CHOAc major epimer), 5.4 (0.3H, t, J=5.2 Hz, CHOAc minor epimer), 6.00 (0.7 H, d, J=9.3 Hz, C5-H major epimer), 6.01 (0.7 H, s, C3-H major epimer), 6.03 (0.3H, s, C3-H minor epimer), 6.19 (1H, d, J=7.3 Hz, C5-H).

General Procedure for Alkaline Hydrolysis of 4-(-1-Acetyloxytridecyl)-2-trialkylsilylfurans

The desired trialkylsilylfuran was stirred in 5% aqueous potassium hydroxide solution for 1 hr. The mixture was chilled to 0°, acidified with 5% aqueous hydrochloric acid solution, and the aqueous mixture was extracted with ethyl acetate. The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution, brine, and dried over sodium sulfate. Filtration was followed by solvent evaporation at reduced pressure to give a clear oil.

4-(1-Hydroxytridecyl)-2-trimethylsilylfuran 3b'. 4-(1-Acetyloxytridecyl)-2-trimethylsilylfuran 3b (R'=methyl): 700 mg, 1.8 mmol. Potassium hydroxide solution: 15 mL. Chemical yield: 0.65 g, 100%.

400 MHz ^{1}HNMR ($CDCl_3$, TMS):

0.25 (9H, s, $Si(CH_3)_3$), 0.88 (3H, t, J=6.5hz, sidechain CH_3), 1.25 (20H, br s, methylene envelope), 1.61 (2H, m, sidechain CH_2), 4.64 (1H, q, J=4.3 Hz, CHOAc), 6.62 (1H, s, C3-H), 7.56 (1H, s, C5-H).

4-(1-Hydroxytridecyl)-2-triethylsilylfuran 3b''. 4-(1-Acetyloxytridecyl)-2-triethylsilylfuran 3b, (R'=ethyl): 2.08 g, 4.9 mmol. Potassium hydroxide solution: 50 mL. Chemical yield: 2.48 g (89%).

400 MHz ^{1}HNMR ($CDCl_3$, TMS):

0.75 (6H, q, J=7.9 Hz, $SiCH_2CH_3$), 0.88 (3H, t, J=6.7 Hz, sidechain CH_3,), 0.98 (9H, t, J=7.8 Hz, $SiCH_2CH_3$), 1.27 (18H, br s, methylene envelope), 4.65 (1H, t, J=7.0 Hz, CHOH), 6.63 (1H, s, C3 or C5 H).

CIMS (NF_3, m/z, % intensity):

379 ($(M-H)^-$, 13.5), 309 (0.9), 361 ($(M-H-H_2O)^-$, 0.6), 285 (3.4), 265 ($(M-SiEt_3)^-$, 100%), 247,($(M-SiEt_3-H_20)^-$, 7.6), 197 (8.9), 131 (18.9).

4-(1-Acetyloxy-1-^{14}C-tridecyl)-2-trimethylsilylfuran 3f. A solution of 4-(1-hydroxytridecyl)-2-trimethylsilylfuran 3b' (480 mg, 1.4 mmol) in pyridine (12 mL) was frozen at liquid nitrogen temperature and evacuated to 0.01 torr on a vacuum manifold. Acetyl-1-^{14}C chloride (40 mCi, 57 mCi/mmol, 0.70 mmol) was condensed on the solution by static vacuum transfer, the flask isolated from the manifold, and the mixture was allowed to warm to room temperature over 1hr. The mixture was frozen at liquid nitrogen temperature for a second time, and allowed to warm to room temperature over 30 min. The reaction flask was charged with helium gas to atmospheric pressure, and to the solution was added unlabeled acetyl chloride (200 ul, 220 mg, 2.8 mmol). The mixture was stirred at room temperature for an additional 2hr. The reaction was quenched with methanol (1.0 mL), and volatiles were removed by static vacuum distillation. The resulting residue was purified by flash chromatography (Silica Gel, 0-50% methylenechloride:hexane, 5% step gradient). Evaporation of solvent gave the title compound (radiochemical yield: 26 mCi, 66%; chemical yield: 490 mg, 91%.). Analysis by TLC (Silica Gel, 20:80 ethylacetate:hexane, $R_f = 0.69$, Radiochemical purity 99%) revealed a single component that co-migrated with synthetic standard.

400 MHz ^{1}HNMR ($CDCl_3$, TMS):

0.25 (9H, s, Si(CH3)3), 0.88 (3H, t, J=6.8 Hz, sidechain CH_3), 1.25 (20H, br s, methylene envelope), 2.04 (3H, s, CH_3C0_2), 5.76 (2H, t, J=6.4 Hz, CHOAc), 6.58 (1H, s, C3-H), 7.59 (1H, s, C5-H).

4-(1-Acetyloxy-1-^{14}C-tridecyl)-5-hydroxy-2(5H)-furanone 2f. The General Procedure, Method B, for photooxygenation of 2-trialkylsilyl-4-alkylfurans was employed. 4-(1-Acetyloxy-1-^{14}C-tridecyl)-2-trimethylsilylfuran 3f: 490 mg, 1.28 mmol, 26 mCi. Tetrahydrofuran: 10 mL. Rose Bengal: 3.2 mg. Water: 1.0 mL. Purification by flash chromatography (Silica Gel, 0-65% diethylether:hexane, 5% step gradient) to give a clear oil. The oil was crystallized from methylenechloride:hexane. Radiochemical yield: 18.8 mCi, (72%). Chemical yield: 311 mg, (71%).

Analysis of Final Product: Radiochemical Purity by HPLC (Beckman C-8 column, 0.46 x 25 cm, 5 micron packing, 80:20 v/v acetonitrile:water, 1.0 mL/min, UV at 210 nm, radiodetection as described above, co-migrating with synthetic standard, R_t 9 min): 98.8%. Specific activity: 20.5 mCi/mmol, determined by LSC.

400MHz ^{1}H NMR ($CDCl_3$, TMS):

0.88 (3H, t, J=7.0 Hz, sidechain CH_3), 1.26 (20H, br s, methylene envelope), 1.80 (2H, m, sidechain methylene), 2.11 (0.3H, s, CH_3CO_2 minor epimer), 2.13 (0.7H, s, CH_3CO_2 major epimer), 3.69 (0.3H, d, J=7.3 Hz, OH, minor epimer), 4.68 (0.7H, d, J=10.4 Hz, OH, major epimer), 5.32 (0.7H, t, J=6.4 Hz, CHOAc major epimer), 5.48 (0.3H, t, J=6.3 Hz, CHOAc, minor epimer), 5.99 (0.7H, s, C3-H major epimer), 6.00 (0.7H, d, J=9.7 Hz, C5-H major epimer), 6.03 (0.3H, s, C3-H minor epiemer), 6.18 (0.3H, d, J=7.1 Hz, C5-H minor epimer).

4-[1-(Dibromoacetyloxy)-tridecyl]-2-triethylsilylfuran 3g. To a stirred solution of 4-(1-hydroxytridecyl)-2-triethylsilylfuran 3b'' (350 mg, 0.9 mmol) and dibromoacetic acid (500 mg, 2.3 mmol) in diethyl ether (8 mL) was added at room temperature a solution of dicyclohexylcarbodiimide (500 mg, 2.4 mg) in diethyl ether (2 mL) over a period of 2 min. The mixture was stirred at room temperature for 2 hr, filtered, and the filtrate was concentrated at reduced pressure. The residue was purified by flash chromatography (Silica Gel, 0-8% ether:hexane, 4% step gradient) to give a clear oil (450 mg, 84%).

400 MHz ^{1}H NMR ($CDCl_3$, TMS):

0.76 (6H, q, J=7.9 Hz, $SiCH_2CH_3$), 0.89 (3H, t, J=6.7 Hz, sidechain CH_3), 0.97 (9H, t, J=7.8 Hz, $SiCH_2CH_3$), 1.27 (18H, br s, methylene envelope), 5.83 (1H, t, J=5.9 Hz, CHOAc), 6.26 (1H, s, $COCHBr_2$), 6.65 (1H, s, C3 or C5 H), 7.67 (1H, s, C5 or C3 H).

CIMS (CH_4, m/z, % intensity):

580 ($(M+H)^+$, 0.4), 551 ($(M-C_2H_5)^+$, 0.9), 391 (3.5), 363 (100.0), 333, 4.2 303 (14.3), 277 (7.8), 259 (20.8), 219 (9.0).

4-[1-(Acetyloxy-t)-tridecyl]-2-triethylsilylfuran 3g'. A mixture of 4-[1-(Dibromoacetyloxy)-tridecyl]-2-triethylsilylfuran 3g (15 mg, 0.026 mmol) and 10% Pd/C catalyst (8 mg) in ethyl acetate (1 mL) was attached to a Toepler vacuum pump. The mixture was frozen at liquid nitrogen temperature, evacuated, and tritium gas was transferred from the shipping ampoule to the reaction flask. The mixture was stirred under a tritium atmosphere at room temperature for 5 hr.

The mixture was frozen at liquid nitrogen temperature, and excess tritium gas was back-transferred onto platinum oxide. The reaction flask was filled with helium and allowed to warm to room temperature at atmosphere pressure. The catalyst was removed by filtration through a cotton plug, and the plug was washed with ethyl acetate. Solvent was removed by static vacuum distillation. Exchangeable tritium was removed by repeated addition of methanol to the resulting residue and static vacuum distillation. The final residue (698 mCi) was dissolved in hexane (1.5 mL) and purified by semi-preparative HPLC (Lichrosorb Si-60 Silica Gel, 1 cm x 25 cm, 7 micron packing, 100:0.6 hexane:isopropanol, 2.0 mL/min, UV at 230 nm, 0.5 mL injections). The combined desired fractions were concentrated at reduced pressure and dissolved in ethyl acetate (ca 10 mL). The radiochemical yield was 551 mCi. HPLC analysis (Lichrosorb Silica Gel column, Si-60, 5 micron packing, 0.46 cm x 25 cm, 1000:0.6 hexane:isopropanol, 1.0 mL/min, radiodetection as described above, $R_t = 11.2$ min, co-migration with synthetic standard) revealed the radiochemical purity to be 97.5%.

4[1-(Acetyloxy-t)-tridecyl]-5-hydroxy-2(5H)-furanone 2g. 4-[1-(Acetyloxy-t)-tridecyl]-2-triethylsilylfuran 3g'was photooxygenated using the General Procedure, Method B. 4-[1-(Acetyloxy-t)-tridecyl]-2-triethylsilylfuran: 551 mCi. Tetrahydrofuran: 2 mL. Rose Bengal: 0.3 mg. Water: 200 uL. Purification by semi-preparative HPLC (Altex Ultrasphere C-18 column, 5 micron packing, 1 cm x 25 cm, 20:80 water:acetonitrile, 2.5 mL/min, UV at 210 nm) provided 218 mCi of desired product. HPLC solvents were removed by lyophilization, and the residue was dissolved in toluene (ca. 6 mL) for storage at -60°.

Analysis of Final Product: Radiochemical purity by HPLC (Beckman C-8 column, 5 micron packing, 0.46 x 25 cm, 20:80 v/v water:acetonitrile, 1.0 mL/min, UV at 210 nm, radiodetection as described, $R_t = 10.3$ min, co-migrates with synthetic standard): 97.4%. Specific Activity: 18.3 Ci/mmol, determined by CI-MS.

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