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NOTE

SYNTHESIS OF CARBON-14 AND TRITIUM LABELED DOPAMINE BETA-HYDROXYLASE INHIBITORS OF THE IMIDAZOLINETHIONE TYPE

S.G. Senderoff*, S.C. Shilcrat, S.H. Levinson, and J.R. Heys

Department of Synthetic Chemistry, L-830

Smith Kline & French Laboratories

709 Swedeland Road

King of Prussia, PA 19406

SUMMARY

1-[(3,5-Difluoro-4-hydroxyphenyl)methyl]-1,3-dihydro-2H-imidazole-2-thione, 1-[(3,5-difluoro-4-methoxyphenyl)methyl]-1,3-dihydro-2H-imidazole-2-thione, and 1-[(3,5-difluorophenyl)methyl]-1,3-dihydro-2H-imidazole-2-thione (SK&F 102048, SK&F 102055 and SK&F 102698, respectively) were synthesized in carbon-14 and tritium labeled forms. Carbon-14 from potassium [^{14}C]thiocyanate was incorporated into the imidazolethione ring of SK&F 102055 and SK&F 102698 at C-2 by condensation with N-(2,2-dimethoxyethyl)-3,5-difluoro-4-methoxybenzenemethanamine or N-(2,2-dimethoxyethyl)-3,5-difluorobenzenemethanamine. Treatment of SK&F [2-^{14}C]102055 with boron tribromide gave SK&F [2-^{14}C]102048. Tritiated SK&F 102048 was synthesized by sodium [^{3}H]borohydride reduction of N-(3,5-difluoro-4-methoxyphenylmethylene)-2,2-dimethoxyethanamine, followed by condensation with potassium thiocyanate and demethylation with boron tribromide.

Key Words: Carbon-14, tritium, imidazolinethione, benzylimidazole

INTRODUCTION

1-[(3,5-Difluoro-4-hydroxyphenyl)methyl]-1,3-dihydro-2H-imidazole-2-thione, 1-[(3,5-difluoro-4-methoxyphenyl)methyl]-1,3-dihydro-2H-imidazole-2-thione, and 1-[(3,5-difluorophenyl)methyl]-1,3-dihydro-2H-imidazole-2-thione (SK&F 102048, SK&F 102055, and SK&F 102698, 1-3, Figure 1) are potent inhibitors of dopamine beta-hydroxylase[1], which

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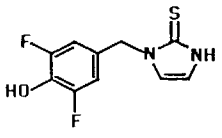
catalyses the benzylic oxidation of dopamine to norepinephrine.[2] Elevated levels of norepinephrine have been correlated with increased blood pressure in humans.[3] Selective inhibition of dopamine beta-hydroxylase may be an approach to the treatment of cardiovascular disorders such as hypertension and congestive heart failure.[4,5]

Compounds 1-3 (Figure 1) were required in radiolabeled form for biological and pharmacological studies. This paper describes the synthesis of 1-3 labeled with carbon-14 in the imidazolethione ring (C-2) and the synthesis of 1 via 2 labeled with tritium at the benzylic methylene carbon.

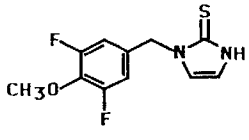
DISCUSSION

The preparation of unlabeled precursors of compounds 1 and 2 is shown in Scheme 1. The previously reported synthesis[5] was modified by substitution of a one step conversion of 4 to 5 which provided a 96% yield. The synthesis of aminoacetal 8 (Scheme 2) has been reported previously.[3]

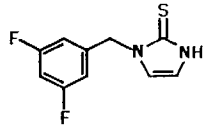
Figure 1



1



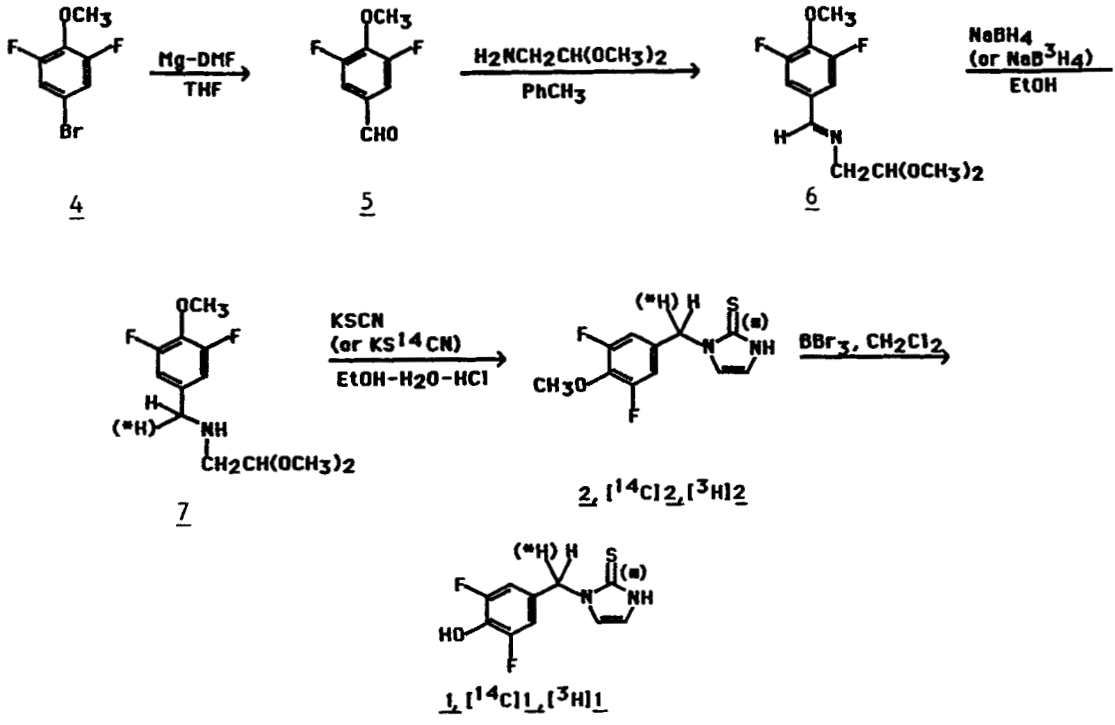
2



3

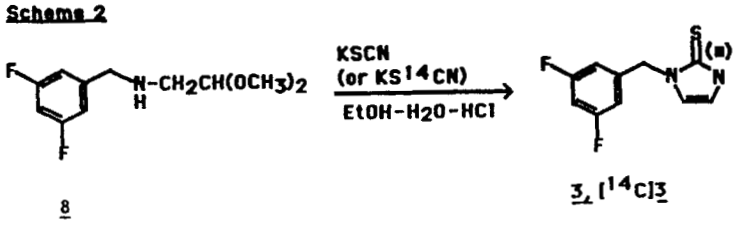
The key step in the preparation of the carbon-14 labeled compounds, paralleling the original syntheses,[1,3,5] was the condensation of potassium [^{14}C]thiocyanate with the appropriately substituted N-(2,2-dimethoxyethyl)benzenemethanamine (7 or 8) in aqueous hydrochloric acid (Schemes I and II). In the synthesis of [^{14}C]3, dilution of specific activity to the desired 20 mCi/mmol and maximization of the radiochemical yield were accomplished by beginning the condensation with a molar deficiency of commercial KS^{14}CN (58 mCi/mmol), then, after a suitable time, adding excess unlabeled KSCN. The radiochemical yield of [^{14}C]3 obtained in this way was 90%; its radiochemical purity was greater than 98% by HPLC and TLC analysis. Application of the same procedure to the preparation of [^{14}C]2 resulted in a low yield

Scheme 1



* denotes ^{14}C
* denotes ^{3}H

Scheme 2



* denotes ^{14}C

of impure product. This outcome may reflect the lesser stability of acetal 7 or the corresponding aldehyde, as compared to 8 under these reaction conditions. In this case, the best results were obtained when equimolar quantities of acetal 7 and $KS^{14}CN$ of the appropriate specific activity were combined at the beginning of the reaction. A 90% radiochemical yield of [^{14}C]2 (at 98% radiochemical purity and specific activity of 22.4 mCi/mmol) was realized by use of this procedure. The O-methyl group of [^{14}C]2 was removed by treatment with boron tribromide in methylene chloride followed by semi-preparative HPLC to give a 31% yield of [^{14}C]1 at a radiochemical purity of 98% by HPLC and TLC analysis.

Preparation of 1 in tritium labeled form followed the route shown in Scheme 1. Sodium [^{3}H]borohydride was substituted for sodium borohydride in the conversion of N-(3,5-difluoro-4-methoxyphenylmethylene)-2,2-dimethoxyethanamine 6 to N-(2,2-dimethoxyethyl)-3,5-difluoro-4-methoxybenzene[^{3}H]methanamine, [^{3}H]7. Condensation of [^{3}H]7 with potassium thiocyanate and demethylation with boron tribromide completed the synthesis, giving 2.4 mCi of [^{3}H]1 with a radiochemical purity of 98% and a specific activity of 7.1-7.8 Ci/mmol.

EXPERIMENTAL SECTION

General. Silica Gel GF plates (Analtech, 5 x 20 cm, 250 micron adsorbent thickness) were used for thin layer chromatography (TLC) and were developed 15 cm. Mass was detected by UV light and radioactivity was detected with a Berthold LB 2832 TLC Linear Analyzer or a Berthold LB 2772 TLC Scanner. Liquid scintillation counting was done on a Tracor Analytic Mark III instrument with Biofluor (New England Nuclear) scintillation cocktail. High pressure liquid chromatography (HPLC) was performed with an Altex 110A pump, a Rheodyne 7130 injector, and the column described in each analysis or preparation. Mass was detected with a Kratos SF 775 UV detector. Radioactivity was detected with a Ramona Radioactive Flow Monitor using TruCount scintillation cocktail at 5 mL/min in a 750 microliter cell. Proton nuclear magnetic resonance (NMR) spectra were taken at 60 MHz on a Varian EM-360 instrument, and IR spectra were taken on a Perkin-Elmer 283 spectrophotometer. Potassium [^{14}C]thiocyanate (58 mCi/mmol) was obtained from Amersham. Sodium [^{3}H]borohydride (79.1 Ci/mmol) was obtained from New England Nuclear. All other organic and inorganic reagents were obtained from Apache Chemical, Aldrich, Fisher, or Baker Chemicals.

3,5-Difluoro-4-methoxybenzaldehyde 5. A suspension of magnesium metal turnings (4.37 g, 0.18 g-atom) in dry tetrahydrofuran (150 mL) was treated dropwise at room temperature with 4-bromo-2,6-difluoro-1-methoxybenzene 4 (33.45 g, 0.15 mol) over 30 minutes. The mixture was stirred at room temperature for 2 hours. The solution was cooled on an ice bath to 5° C and dimethylformamide (14.25 g, 0.20 mol) was added dropwise. The mixture was stirred for one hour and allowed to warm to room temperature. The reaction was quenched by careful addition of 10% hydrochloric acid and the resulting mixture was extracted with ethyl acetate. The combined organic extracts were washed with 10% hydrochloric acid, water, brine, and dried over magnesium sulfate. The drying agent was removed by filtration and concentration at reduced pressure gave 7 as an oily solid (24.75 g, 96% yield) that was used without further purification.

mp: 38-40° C.

NMR ($CDCl_3$): 9.82, s, 1H, (H-C=O), 7.13-7.70, m, 2H, aromatic, 4.13, s, 3H, (Ph-OCH_3).

IR (neat): 2840, 1685, 1580, 1330, 995 cm^{-1}.

N-(3,5-Difluoro-4-methoxyphenylmethylene)-2,2-dimethoxyethanamine 6. A solution of 3,5-difluoro-4-methoxybenzaldehyde 5 (24.75 g, 0.14 mol) and aminoacetaldehyde dimethyl acetal (Aldrich, 16.62 g, 0.16 mol) in toluene (125 mL) was heated at reflux for 1 hour. Continuous removal of water was effected with a Dean-Stark trap. The solution was cooled to room temperature and concentrated at reduced pressure to give the crude product 6 as an oil (35.24 g, 95% yield) which was used without further purification.

NMR ($CDCl_3$): 8.12, s, 1H, (HRC=N-R') 7.03-7.57, m, 2H, (aromatic), 4.65, t, 1H, J=5.5 Hz, (=N-$CH_2CH(OCH_3)_2$), 4.05, s, 3H, (Ph-OCH_3) 3.75, d, 2H, J=5.5 Hz, (-$CH_2CH(OCH_3)_2$, 3.43, s, 6H, ($CH(OCH_3)_2$).

IR (neat): 2940, 1510, 1435, 1330, 1025, 855 cm^{-1}

N-(2,2-Dimethoxyethyl)-3,5-difluoro-4-methoxybenzenemethanamine 7. A solution of N-(3,5-difluoro-4-methoxyphenylmethylene)-2,2-dimethoxyethanamine 6 (35.24 g, 0.14 mol) in ethanol (235 mL) was treated with sodium borohydride (5.71 g, 0.15 mol). The mixture was stirred for 18 hours at room temperature and concentrated to a residue at reduced pressure. The residue was partitioned between ethyl acetate and water. The organic portion was washed

with water, brine, and dried over magnesium sulfate. The drying agent was removed by filtration. Concentration of the solution at reduced pressure gave crude product as an oil. Vacuum distillation (190-200° C, 0.2 mm, Kugelrohr) gave pure product 9 (32.17 g, 92% yield).

NMR ($CDCl_3$): 6.62-7.12, m, 2H, (aromatic) 4.43, t, 1H, J=5.2 Hz, ($-CH_2CH(OCH_3)_2$) 3.93, s, 3H, ($Ph-OCH_3$) 3.70, s, 2H, ($PhCH_2NHR$) 3.38, s, 6H, ($RCH(OCH_3)_2$) 2.70, d, 2H J=5.2 Hz, ($CH_2CH(OCH_3)_2$) 1.58, s, 1H (NH).

IR (neat): 3340, 2945, 1580, 1440, 1330, 1030 cm^{-1}

1-[(3,5-Difluoro-4-methoxyphenyl)methyl]-1,3-dihydro-[2-^{14}C]2H-imidazole-2-thione (SK&F [2-^{14}C]102055, [^{14}C]2). To N-(2,2-dimethoxyethyl)-3,5-difluoro-4-methoxybenzenemethanamine 7 (337 mg, 1.29 mmol) was added 5 mL of a solution of potassium [^{14}C]thiocyanate in ethanol-water (1.73 mL ethanol-3.28 mL water, potassium [^{14}C]thiocyanate: 42 mg, 0.43 mmol, 25 mCi at 58 mCi/mmol). To the resulting mixture was added unlabeled potassium thiocyanate (84 mg, 0.86 mmol) and concentrated hydrochloric acid (0.3 mL). The mixture was heated at reflux for 4 hours, cooled to room temperature, and carefully poured into ice water. The resulting white crystalline solid was collected by filtration and dried in vacuo. The chemical yield of [2-^{14}C]2 was 265 mg (80%). The specific activity was determined to be 22.4 mCi/mmol. Therefore, the radiochemical yield was 22.6 mCi (90%) The radiochemical purity by TLC (SiO_2, 98:2 (v/v) methylene chloride:methanol, R_f=0.55) was 97.8%. This material co-migrated with authentic unlabeled SK&F 102055.

1-[(3,5-Difluoro-4-hydroxyphenyl)methyl]-1,3-dihydro-[2-^{14}C]2H-imidazole-2-thione (SK&F [2-^{14}C]102048, [^{14}C]1). To a solution of SK&F [2-^{14}C]102055 ([^{14}C]2, 154 mg, 0.64 mmol, 14 mCi at 22.4 mCi/mmol) in dry methylene chloride (2 mL) at 0° C was added a solution of boron tribromide in methylene chloride (BBr_3: 0.5 g/mL, 11 mL, 5.5 g, 22 mmol). The solution was stirred at 0° C for 1 hour, then allowed to warm to room temperature over 2 hours. The solution was re-chilled to 0° C and methanol was slowly added until solution was complete and gas evolution had ceased. The solution was evaporated to dryness at reduced pressure and a minimum amount of water was added to the resulting residue. The resulting brown crystalline solid was collected by filtration and dried in vacuo. The solid was dissolved in a minimum amount of methanol and the volume of the solution was increased to 9 mL by addition of HPLC mobile phase solvent (97.5:2.5 (v/v) methylene chloride:methanol). The

material was purified by semi-preparative HPLC (IBM silica gel column, 10 mm x 25 cm, 10 micron particle size, mobile phase at 4.0 mL/min, UV detection at 235 nm, 10 mg injections). The pooled eluate fractions containing [^{14}C]1 were evaporated in vacuo. The resulting crystalline solid was recrystallized from methylene chloride-methanol (trace)-hexane to give 44 mg (31% chemical yield) of SK&F [2-^{14}C]102048 ([^{14}C]1). The specific activity was determined to be 22.9 mCi/mmol. Therefore, the radiochemical yield was 4 mCi (28%). The radiochemical purity by TLC was 98.1-98.3% (System I: 98:2 (v/v) methylene chloride:methanol, $R_f = 0.55$; System II: 90:10:0.1 (v/v/v) chloroform:methanol:formic acid, $R_f = 0.45$). The radiochemical purity by HPLC (Waters uBondapak C-18 column, 3.9 mm x 30 cm, 75:25 (v/v) water:95% ethanol at 1.0 mL/min, UV detection at 228 nm, radioactivity detection by Ramona Flow Monitor, $R_t = 11.2$ minutes) was 98.6%. Co-elution of activity with authentic unlabeled SK&F 102048 was seen in all chromatographic assays.

1-[(3,5-Difluorophenyl)methyl]-1,3-dihydro-[2-^{14}C]2H-imidazole-2-thione (SK&F [2-^{14}C]102698, [^{14}C]3). To N-(2,2-dimethoxyethyl)-3,5-difluorobenzenemethanamine 8[6] (300 mg, 1.31 mmol) was added at room temperature a solution of potassium [^{14}C]thiocyanate in water-ethanol (3.13 mL water-1.83 mL ethanol, potassium [^{14}C]thiocyanate: 42 mg, 0.42 mmol, 25 mCi at 59 mCi/mmol). To the resulting solution was added concentrated hydrochloric acid (0.30 mL). The mixture was heated at reflux for 2.6 hours. Unlabeled potassium thiocyanate (30 mg, 0.31 mmol) was added and the reaction mixture was heated at reflux for an additional 2.2 hours. The mixture was allowed to cool to room temperature over 1 hour and the volume of the mixture was reduced gradually by rotary evaporation for 1 hour. The resulting crystalline product was collected by filtration, washed with 1:2 (v/v) ethanol:water, and dried in vacuo for 15 hours. The chemical yield was 256 mg (86.2%). The specific activity was determined to be 20.0 mCi/mmol. Therefore, the radiochemical yield was 22.6 mCi (90%). The radiochemical purity by TLC was 97.8-98.2% (System 1: Whatman $KC_{18}F$ reverse phase adsorbent, 5 x 20 cm, 250 micron adsorbent thickness, 3:2 (v/v) methanol:water, $R_f = 0.25$; System 2: Baker Silica Gel GF, 5 x 20 cm, 250 micron adsorbent thickness, 4:1 (v/v) methylene chloride:acetonitrile, $R_f = 0.42$). The radiochemical purity by HPLC was 99.0-99.5% (System 1: Lichrosorb Si-60 Silica Gel column, 4.6 mm x 25 cm, 5 micron particle size, 4:1 (v/v) hexane:isopropanol at 1.0 mL/min, UV detection at 220 nm, radioactivity detection by Ramona flow monitor $R_t = 10.3$ minutes); System 2: Waters uBondapak C-18 column, 3.9 mm x 30 cm,

1:1 (v/v) 95% ethanol:water at 0.8 mL/min, detection as above, $R_t = 6.9$ minutes). Co-elution of activity with authentic non-labeled standards was observed in all chromatographic assays.

1-[(3,5-Difluoro-4-hydroxyphenyl)[^{3}H]methyl]-1,3-dihydro-2H-imidazole-2-thione (SK&F [^{3}H]102048, [^{3}H]1). To solid sodium [^{3}H]borohydride (500 mCi, 79 Ci/mmol, 0.3 mg, 0.0065 mmol) was added N-(3,5-difluoro-4-methoxy-phenylmethylene)-2,2-dimethoxyethanamine 6 (1.68 mg, 0.0063 mmol) in ethanol (26 uL) at 0° C. To the chilled solution was added additional ethanol (75 uL). The clear solution was stirred at 0° C for 15 minutes and allowed to warm to room temperature over 1 hour. The solution was added carefully to a solution of potassium thiocyanate (0.92 mg, 0.0095 mmol) in 2.4 M aqueous hydrochloric acid (40 uL) in a Reacti-Vial™ (Aldrich, 2.0 mL). The vial was sealed, and the solution was heated at 60-70° C for 20 hours. The solution was cooled to room temperature and subjected to preparative HPLC (Waters u-Bondapak C-18 column, 3.9 x 30 cm, mobile phase 65:35 v/v water:95% ethanol at 1.5 mL/min, UV at 228 nm). The radiochemical purity of the combined eluate fractions containing SK&F [^{3}H]102055 ([^{3}H]2) by TLC (silica gel, mobile phase 90:10 v/v chloroform/methanol, $R_f = 0.63$) was 97%. This solution was concentrated to dryness in vacuo and the residue was dissolved in methylene chloride (0.3 mL). The solution was transferred to a Reacti-Vial™ (Aldrich, 2 mL), and to the solution was added at room temperature a 1.0 M solution of boron tribromide in methylene chloride (0.32 mL, 0.32 mmol). The solution was stirred for 4 hours. TLC analysis (silica gel, mobile phase 90:10 chloroform:methanol, $R_f = 0.51$) indicated complete consumption of starting material. The solution was chilled to 0° C on an ice bath and methanol (0.24 mL) was added slowly. The brown mixture was heated to 50° C for 15 minutes, cooled to room temperature, and then the volatiles were removed in vacuo. The resulting red-brown oil was dissolved in ethanol (0.25 mL), and subjected to preparative HPLC (Waters u-Bondapak C-18 column 3.9 mm x 30 cm, mobile phase 65:35 v/v water:95% ethanol, flow rate 0.8 mL/min, UV at 254 nm). The main fraction (12.7 mCi) and a side fraction (1.5 mCi) were concentrated and subjected separately to a second identical preparative HPLC procedure. The total radiochemical yield was 2.4 mCi in two batches of 1.5 and 0.9 mCi. The material was stored as a solution in 65:35 v/v water:95% ethanol at 0° C. The radiochemical purity by HPLC was 98.2-99.0% (Waters u-Bondapak C-18 column, mobile phase 65:35 v/v water:95% ethanol, flow rate 1.0 mL/min, UV at 228 nm, activity quantitated by fraction collection and liquid scintillation counting). The

radiochemical purity by TLC was 96.5-98.0% (silica gel, 90:10 v/v chloroform:methanol, $R_f = 0.52$). The specific activity was 7.1-7.8 Ci/mmol.

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