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SYNTHESIS OF CARBON-14 LABELED 6-(4-FLUOROPHENYL)-5-(4-PYRIDYL)-2,3-DIHYDROIMIDAZO[2,1-b]THIAZOLE (SK&F 86002)

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SUMMARY

SK&F 86002, 6-(4-fluorophenyl)-5-(4-pyridyl)-2,3-dihydroimidazo[2,1-b]thiazole, was synthesized labeled with carbon-14 at C-1 or C-2,3 using either [^{14}C]thiourea or 1,2-dibromo[$^{14}C_2$]ethane, respectively. The synthetic route, involving the condensation of an asymmetric benzoin with thiourea followed by alkylation, gave a mixture of SK&F 86002 and structural isomer SK&F 86055, 5-(4-fluorophenyl)-6-(4-pyridyl)-2,3-dihydroimidazo-[2,1-b]thiazole. The two products were separated easily by flash chromatography. The use of 1,2-dibromo[$^{14}C_2$]ethane as the labeling reagent was superior to the use of [^{14}C]thiourea in this sequence, for the radiolabel was incorporated in the final step of the synthesis by an alkylation reaction, thus providing greatly increased overall radiochemical yields.

Key Words: Carbon-14, imidazothiazole

INTRODUCTION

SK&F 86002 may be a useful non-steroidal anti-inflammatory agent for the treatment of arthritic conditions, notably rheumatoid arthritis. It also appears to have the ability to act as an immunomodulatory agent.[1,2] This compound was needed in radiolabeled form for drug metabolic and pharmacokinetic studies. A requirement for moderate specific activity and the need for a radiolabel amenable to longer-term biological studies suggested the use of carbon-14 as the labeling isotope.

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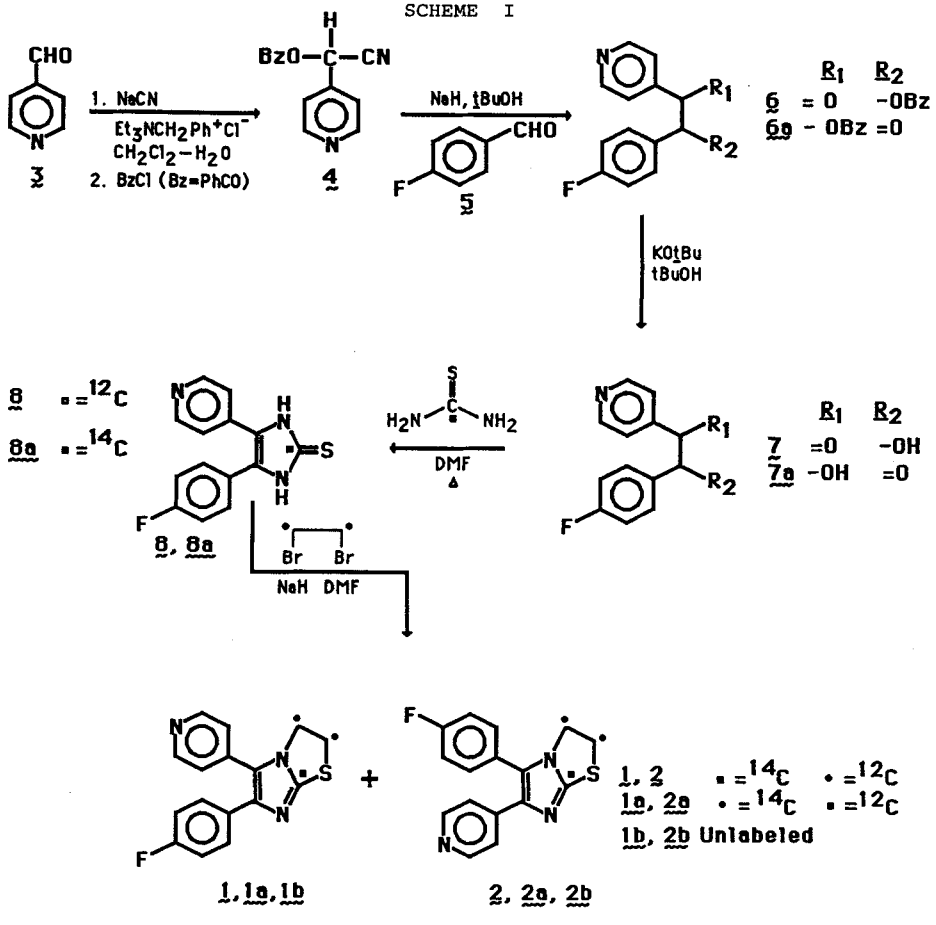
RESULTS AND DISCUSSION

A previously developed[2] non-isotopic synthesis of SK&F 86002 (1b) and SK&F 86055 (2b) (Scheme I) was selected for modification for radiosynthesis. The starting material was pyridine 4-carboxaldehyde (3). Formation of the benzoyl protected cyanohydrin 4 under phase transfer conditions was followed by benzoin condensation with p-fluorobenzaldehyde 5. The resulting mixture of stable benzoyl benzoin isomers 6 and 6a was purified but not separated by flash chromatography over silica gel. The benzoyl protecting group of 6, 6a was cleaved with potassium t-butoxide, and the resulting unstable benzoin isomers 7 and 7a were condensed with thiourea at elevated temperature to yield imidazo-thione intermediate 8. The non-isotopic synthesis was completed by alkylation of thione (8) with 1,2-dibromoethane to give isomeric 1b and 2b. Typical overall yields of the non-isotopic isomer mixture ranged from 40-50%. Flash chromatography of the mixture over silica gel gave the pure structural isomers. The desired SK&F 86002 (1b) (10-20% purified yield) eluted well after the undesired structural isomer SK&F 86055 (2b).

Previous workers[2] effected the transformation of 4 to 8 in one pot on 10-500 g scale without isolation and purification of intermediates. However, this approach failed to give radiolabeled 1 and 2 when applied in a small scale (0.09 mmol) trial radiochemical synthesis with the use of one equivalent of [^{14}C]thiourea at 57 mCi/mmol. Increasing the mass scale of the reaction by use of 23.6 mmol of [^{14}C]thiourea (250 mCi at 11 mCi/mmol) in 1.5-fold molar excess over the benzoins 7, 7a resulted in the isolation of 1.65 g of crude [^{14}C]thione 8a. This material was alkylated with 1,2-dibromoethane to give 9.5 mCi of the mixture of [1-^{14}C]1 and [1-^{14}C]2. Flash chromatographic separation followed by recrystallization of [1-^{14}C]1 from ethyl acetate gave 1.8 mCi (0.7% overall radiochemical yield) of final product at 11.0 mCi/mmol with chemical and radiochemical purities greater than 97%.

An alternative labeled starting material was utilized in order to obtain improved radiochemical yields in subsequent syntheses. 1,2-Dibromo-[$^{14}C_2$]ethane (specific activity 47 and 56 mCi/mmol) was diluted to 16

SCHEME I



mCi/mmol, and highly purified unlabeled thione 8 was alkylated with 1.01 equivalents (100 mCi) of this material. The crude radiochemical yield of the isomeric mixture of [2,3-$^{14}C_2$]1a and [2,3-$^{14}C_2$]2a was 50%. Flash chromatography and recrystallization gave the desired SK&F [2,3-$^{14}C_2$]86002 (1a) in 17% final purified radiochemical yield as a white crystalline solid at a specific activity of 16.0 mCi/mmol with radiochemical and chemical purities of greater than 99%. A second run with 140 mCi of 1,2 dibromo[$^{14}C_2$]ethane (specific activity 14 mCi/mmol) provided the isomer mixture in 40% yield. Purification as before gave a 10% overall radiochemical yield of SK&F [2,3$^{14}C_2$]86002 (1a) at a specific activity of 14.0 mCi/mmol with radiochemical and chemical purities comparable to the first run. Storage of the radiochemical as a solid under argon at -25°C for ca. 6 months resulted in no change in radiopurity.

EXPERIMENTAL

Pyridine 4-carboxaldehyde, p-fluorobenzaldehyde, thiourea, 1,2-dibromoethane, sodium hydride, potassium t-butoxide, sodium cyanide, benzoyl chloride, and benzyltriethylammonium chloride were obtained from Aldrich. All solvents were obtained from Mallinckrodt or Burdick and Jackson.

[^{14}C]Thiourea at 58 mCi/mmol was obtained from Amersham. 1,2-dibromo-[$^{14}C_2$]ethane with specific activity of 47 or 56 mCi/mmol (stored at -78°C in methylene chloride or ether) was obtained from Imperial Chemical Industries Physics and Radioisotope Services Group, Billingham, England. The storage solvent was removed by vacuum transfer prior to use.

Thin layer chromatography employed Analtech plates (silica gel 60 with indicator) developed 15 cm and visualized under UV light for mass detection. A Berthold Linear Analyzer (LB 2832) or TLC Scanner (LB 2760) was used to detect radioactivity. Flash chromatography was done with Baker components and Baker flash chromatography silica gel.

High pressure liquid chromatography was performed on the following isocratic system: Altex 110A pump, Rheodyne 7130 injector, Waters uBondapak C-18 column, Kratos Model SF 769-Z UV detector, and Hewlett-Packard Model 3380S recording integrator. Radioactivity was detected with either a Radiomatic Flow-One or Ramona radioactive flow detector. Details of the HPLC conditions are given in the individual preparations.

Liquid scintillation counting was performed on a TM Analytic Mark III 6881 instrument using Biofluor cocktail (DuPont-NEN Products).

2-(4-Pyridyl)-2-benzoyloxyacetonitrile (4): Pyridine-4-carboxaldehyde (40 g, 373 mmol) was dissolved in methylene chloride (400 mL) and added slowly at 0°C to a solution of sodium cyanide (78.4 g, 1600 mmol) and benzyltriethylammonium chloride (12 g, 53 mmol) in water (300 mL). The orange mixture was stirred at 0°C for 30 min. To the solution was added benzoyl chloride (56 g, 398 mmol) in methylene chloride (200 mL). The solution turned cherry red and was stirred at 0°C. for 30 min. The aqueous layer was removed, and the organic layer was washed with saturated sodium carbonate solution until the washings were basic.

The solution was dried over magnesium sulfate. The drying agent was removed and the solution was treated with Darco. The decolorizing agent was removed by filtration through Celite, and the solution was filtered through a short column of silica gel. The filtrate was concentrated to a solid residue, dissolved in chloroform, and purified by flash chromatography over silica gel (chloroform) to give 30.75 g (66%) purified benzoylcyanohydrin 4.

TLC (SiO_2, ether), R_f 0.4, co-migrates with standard.

M.P. 85-87°C. (Lit.[2] 86-87°C.).

2-(4-Fluorophenyl)-1-(4-pyridyl)-2-(benzoyloxy)-ethanone (6) and 1-(4-fluorophenyl)-2-(4-pyridyl)-2-(benzoyloxy)-ethanone (6a): Benzoylcyanohydrin 4 (5.0 g, 21 mmol) was suspended in t-butanol (50 mL) at room temperature. To the mixture was added 4-fluorobenzaldehyde (5) (2.60 g, 21 mmol, freshly distilled) followed by sodium hydride (60% oil dispersion, 0.84 g, 21 mmol). The reaction was stirred efficiently at room temperature for 2 hr. The resulting mixture was poured into water and extracted with chloroform. The combined organic extracts were washed with water and brine, and dried over magnesium sulfate. Filtration and concentration gave a residue that was taken up in ether-chloroform and subjected to flash chromatography (silica gel, ether). A mixture consisting exclusively of the benzoylbenzoin isomers 6 and 6a was obtained (7.54 g, 98%). The mixture was carried to the next reaction without further purification.

TLC (C-18 silica gel, 70:30 methanol:water), Two close-running components R_f ca. 0.8 corresponding to authentic standards.[2]

6-(4-Fluorophenyl)-5-(4-pyridyl)-2,3-dihydrioimidazo[2,1-b]-[1-^{14}C]thiazole and 5-(4-fluorophenyl)-6-(4-pyridyl)-2,3-dihydroimidazo[2,1-b]-[1-^{14}C]thiazole (1 and 2): The isomeric benzoyl benzoins 6 and 6a (4.9 g, 14.6 mmol) were dissolved in t-butanol (50 mL). To the solution was added potassium t-butoxide (4.92 g, 43.8 mmol). The cherry red solution was stirred at room temperature for 2.5 hr upon which TLC analysis (silica gel, ether) showed complete consumption of starting material. The mixture was poured into water and extracted into methylene chloride. The organic layer was washed with water

until the washings were neutral and dried briefly over magnesium sulfate. Filtration and concentration gave a residue (isomeric benzoins 7 and 7a) that was immediately carried to the next reaction without further characterization due to the marked instability of the material.

The isomeric benzoins (3.7 g, 14.6 mmol) were dissolved in dry dimethylformamide (DMF, 5 mL) and added slowly to a solution of [^{14}C]thiourea (250 mCi, 1.67 g, 11.6 mCi/mmol, 21.89 mmol) in dry DMF at reflux. The reaction was heated at reflux overnight. The volume of the reaction was reduced by one-half by distillation, and the solution was poured into ice water. A yellow solid precipitated which was collected, dried, and triturated with acetone to give 1.65 g of crude [^{14}C]thione 8a.

The [^{14}C]thione 8a was suspended in DMF and treated with sodium hydride (60% oil dispersion, 0.37 g, 9.20 mmol) at room temperature. After 1 hr, 1,2-dibromoethane (0.79 mL, 1.72 g, 9.20 mmol) was added to the deep red solution, and the mixture was stirred at room temperature overnight. The resulting clear reaction mixture was poured into water and extracted with methylene chloride. The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated to a dark yellow oil. The oil was dissolved in ether. The ethereal solution was washed with water and brine, dried over magnesium sulfate, filtered, and concentrated to a clear oil (0.84 g, 34.2 mCi, 14% radiochemical yield based on [^{14}C]thiourea). TLC analysis (silica gel, 2:1 ether:isopropanol) showed two UV-active radioactive products corresponding to authentic unlabeled 1 (R_f 0.2) and 2 (R_f 0.4).

The oil was dissolved in ether-chloroform and subjected to flash chromatography (silica gel, 25% isopropanol in ether) to give two radioactive components, SK&F [1-^{14}C]86055 (2) (6 mCi, earlier eluting component) and SK&F [1-^{14}C]86002 (1) (3.5 mCi, later eluting component). The combined SK&F [1-^{14}C]86002 fractions were concentrated to a solid and recrystallized from ethyl acetate to give two crops of final product [1-^{14}C]1 (0.027 g, 1 mCi and 0.020 g, 0.8 mCi, purified radiochemical yield 0.7 % based on [^{14}C]thiourea). Analytical data are given for crop I.

Radiochemical Purity: HPLC, 97.6% (Waters uBondapak C-18 column, 4.7

mm x 25 cm, 60:40 0.1N ammonium acetate:acetonitrile, 1.0 mL/min, UV at 250 nm). TLC (Silica Gel GF) System I, 98.5%; System II, 98.1% (System I: 60:40:10 toluene:chloroform:methanol, ammonia saturated atmosphere; System II: 90:10 chloroform:methanol, ammonia saturated atmosphere).

Chemical Purity: HPLC-UV (external standard), 98.4% (HPLC system described above).

Specific Activity: 11.9 mCi/mmol.

6-(4-fluorophenyl)-5-(4-pyridyl)-[2,3-$^{14}C_2$]2,3-dihydroimidazo[2,1-b]thiazole and 5-(4-fluorophenyl)-6-(4-pyridyl)-[2,3-$^{14}C_2$]2,3-dihydroimidazo[2,1-b]thiazoles (1a and 2a): Unlabeled thione 8 (purified by repeated trituration with acetone, 2.47 g, 9.82 mmol) was suspended in dry dimethylforamide (40 mL) at room temperature. To the suspension was added sodium hydride (460 mg of a 60% oil dispersion, 11.5 mmol). After 30 minutes, 1,2-dibromo[$^{14}C_2$]ethane (1.88 g at 14 mCi/mmol, 140 mCi, 10 mmol) was added to the cherry red solution. The solution became clear, and the reaction was stirred overnight. The clear solution was quenched with water and extracted with chloroform. The combined organic extracts were concentrated to an oil which was dissolved in ether. The ethereal solution was washed with water, brine, and then dried over magnesium sulfate. TLC analysis as before revealed the presence of SK&F [2,3-$^{14}C_2$]86002 (1a) and SK&F [2,3-$^{14}C_2$]86055 (2a). The crude radiochemical yield was 65 mCi (40%). The mixture was evaporated to dryness at reduced pressure, taken up in a minimum amount of 2:1 ether:isopropanol, and subjected to flash chromatography (silica gel, 2:1 ether:isopropanol). The SK&F [2,3-$^{14}C_2$]86002 fractions were combined, concentrated to an oily solid, and recrystallized from ethyl acetate. Two crops were collected: 324 mg (13.4 mCi) and 19 mg (0.8 mCi). The overall purified radiochemical yield was 10%. Analytical data are given for the first crop.

Radiochemical Purity: HPLC, 99.4%. TLC System I, 99.3%; System II, 99.3%. All systems are described above.

Chemical Purity: HPLC-UV, 102.5% (external standard).

Specific Activity: 12.4 mCi/mmol.

NMR (270 MHz, $CHCl_3$): identical to reference material.[2]

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