SYNTHESIS OF [BENZYL-7-^{3}H] AND [BENZOYL-7-^{14}C] METHYL 4-(2,5-DIHYDROXYBENZYLAMINO)BENZOATE

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SUMMARY

4-Amino[7-^{14}C]benzoic acid (**3**) is prepared by carbonation of the lithium salt of N,N-bis(trimethylsilyl)aniline. The methyl ester (**4**) of **3** is generated with trimethylsilyldiazomethane, and **4** is coupled with 2,5-dihydroxybenzaldehyde to yield methyl 4-(2,5-dihydroxybenzylimino)[7-^{14}C]benzoate (**5**). **5** is reduced with sodium cyanoborohydride to give methyl 4-(2,5-dihydroxybenzylamino)[7-^{14}C]-benzoate with a specific activity of 38.9 mCi/mmol. Unlabeled **5** is reduced with tritium gas to give methyl 4-(2,5-dihydroxy[7-^{3}H]-benzylamino)benzoate with specific activity of 7.6 Ci/mmol.

Key words: methyl 4-(2,5-dihydroxybenzylamino)benzoate, AG957, tritium, carbon-14

INTRODUCTION

Methyl 4-(2,5-dihydroxybenzylamino)benzoate or AG957 (**6**), a synthetic analog of the natural product erbstatin, inhibits DNA and RNA synthesis in chronic myelogenous leukemia cells by interfering with p210$^{bcr\text{-}abl}$ protein tyrosine kinase activity.[1] In order to facilitate pharmacological studies, we have prepared AG957 labeled with carbon-14 and with tritium.

RESULTS AND DISCUSSION

4-Amino[7-^{14}C]benzoic acid (**3**) was prepared after the method of Ellsworth et al.[2] The synthetic scheme is outlined in Chart 1. 4-Bromo-N,N-bis(trimethylsilyl)aniline (**1**) was treated with *n*-butyllithium to form lithium salt **2**. Reaction of **2** with [^{14}C]carbon dioxide gave 4-amino[7-^{14}C]benzoic acid (**3**) in 19% yield after hydrolysis of the silyl

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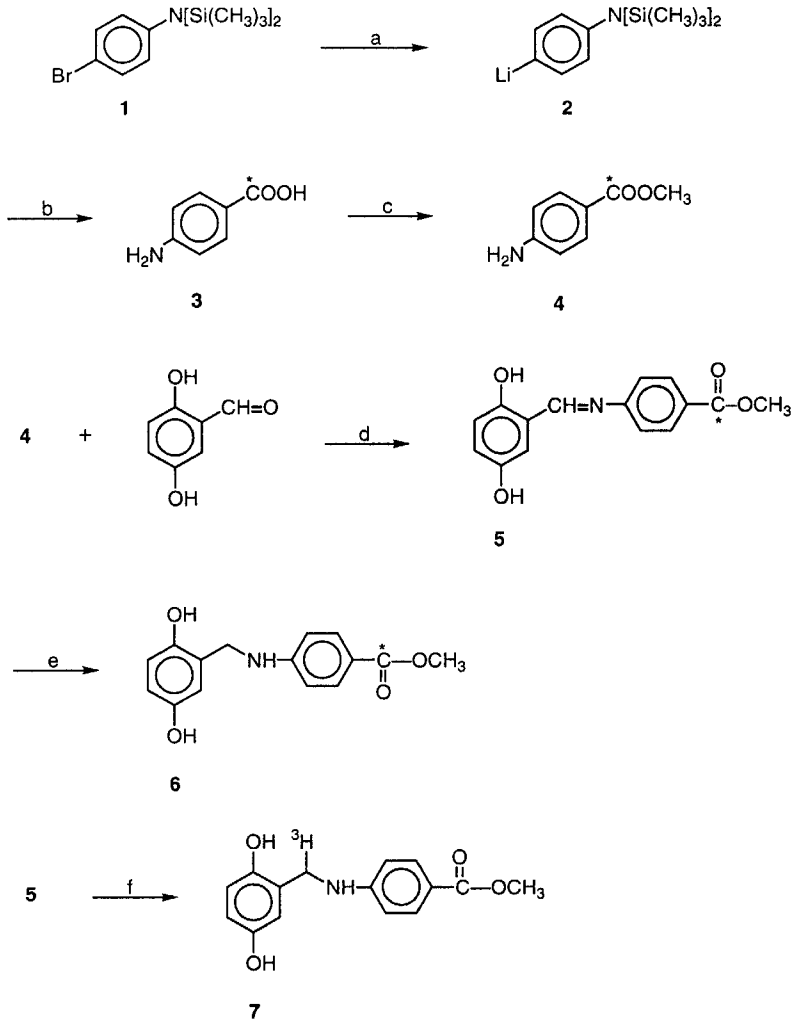
Chart 1



a) n-butyl Li
b) $^*CO_2$
c) $(CH_3)_3SiCHN_2$
d) CH_3OH
e) $NaCNBH_3$
f) $^3H_2$, Pd(C)

groups. Methyl ester **4** was prepared in 65% yield by reaction of **3** with trimethylsilyldiazomethane. **4** was coupled with 2,5-dihydroxybenzaldehyde to give imine **5** in 80% yield. Reduction of **5** with sodium cyanoborohydride[3] gave the desired [benzoyl-7-^{14}C]AG957 (**6**) in 21% yield with a specific activity of 38.9 mCi/mmol after purification by preparative HPLC.

The labeled compound proved to be very unstable at room temperature when exposed to air and light. Two dimensional TLC showed that it was also unstable on silica. It was necessary to run TLC analyses in the dark at reduced temperature (~ 5 °C). HPLC analysis on most octadecylsilane columns also failed because of decomposition on the column. HPLC using an acidic eluant and a highly end-capped column with a high carbon load gave satisfactory results.

Imine **5** offered a convenient intermediate for the preparation of tritium labeled material. A preliminary reduction of **5** with 99.9% deuterium gas in THF with palladium on carbon catalyst was done to determine reaction conditions. Mass spectral analysis of the bistrimethylsilyl derivative of the product showed only 24% deuterium incorporation. Deuterium reduction of the bistrimethylsiyl derivative of **5** where the active phenolic hydrogens are replaced with trimethylsilyl groups gave the same 24% incorporation, and this level of incorporation was also obtained with carrier-free tritium. Reduction of **5** with 4.9 Ci of tritium gas gave 137 mCi of [benzyl-7-^{3}H]AG957 (**7**) with specific activity of 7.60 Ci/mmol. The ^{3}H-NMR spectrum of the product confirmed that at least 98% of the tritium was in the benzylic position. Less than 0.5% of the tritium was lost to back exchange in pH 7 phosphate buffer in 24 hours at room temperature.

EXPERIMENTAL

NMR spectra were recorded on a Bruker AMX-500 multinuclear Fourier transform spectrometer equipped with a tritium/proton dual probe, a tritium E coupler, and a selective preamplifier for tritium. Mass spectra were run on a Hewlett Packard GC/MS system comprised of a 5890 series II gas chromatograph, a 5989A MS Engine and a Chemstation (HP-UX) for system control. A J & W Scientific DB-1 capillary column (30 M, 0.25 mm film) was used with a helium carrier flow of 1.5 mL/min. The injection port temperature was held at 175 °C; the initial oven temperature 50 °C (0.5 min) was increased at 30 °C/min to 250 °C (3 min) and then 40 °C/min to 300 °C for 7 min. The temperature of the transfer line to the mass spectrometer was 250 °C, and the source

and analyzer temperatures were maintained at 200 °C and 100 °C, respectively. Silyl derivatives were made using bis(trimethylsilyl)trifluoroacetamide with 1% chlorotrimethyl-silane at 60 °C for 15 min. Radioactive samples were counted on a Packard Tricarb 4000 liquid scintillation counter. Analytical TLC were performed using E. Merck silica-gel 60F-254 or C_{18} F plates. Developed TLC plates were scanned on a Berthold Model LB 285 Linear Analyzer system. HPLC-RAM was done using a Waters Associates Model 6000A dual pump system with a Model U6K septumless injector, and a IN/US systems; Inc. Model 1B β-RAM Flow-Through Radioactivity Monitor. Preparative HPLC was done on Waters, Assoc. 25 × 100 μ-bondapak RCM C_{18} columns. Analytical HPLC was done on a Beckman ODS ultrasphere (4.6 mm x 25 cm) column. Barium [^{14}C]carbonate was obtained from American Radiochemicals, Inc. Tritium gas was generated by the thermal decomposition of uranium tritide. Solvents were removed from solutions on a rotary evaporator under water aspirator vacuum at ambient temperature unless otherwise noted. A standard sample of AG957 was provided by The National Cancer Institute.

4-Amino[7-^{14}C]benzoic Acid (3)[2]

All glassware was oven dried. *n*-Butyllithium (2.5 mL, 4.0 mmol, 1.6 M in hexane) was added to a solution of 4-bromo-N,N-bis(trimethylsilyl)aniline (1.580 g, 5.0 mmol) in 40 mL of dry ether. The mixture was refluxed for 15 min under nitrogen. After cooling to room temperature, the flask was attached to a vacuum manifold and cooled in liquid nitrogen . [^{14}C]Carbon dioxide, generated by addition of barium [^{14}C]carbonate (737 mg, 3.7 mmol, 149 mCi) to 10 mL of conc. sulfuric acid, was vacuum transferred to the reaction flask. The mixture was warmed to -78 °C and stirred for 0.5 h. Water (5.0 mL) was added, and the mixture adjusted to pH 1 at 0-5 °C with 6 N HCl. Removal of the trimethylsilyl groups was achieved by heating the two phase mixture at 35 °C for 0.5 h. The mixture was adjusted to pH 9.5 with 2.5 N NaOH, and the ether layer was removed. The aqueous layer was adjusted to pH 2.5-3.0 with 6 N HCl and extracted with ether (3 × 20 mL). The extracts were dried (Na_2SO_4) and evaporated to give 29 mCi (19% radiochemical yield) of **3** which was 98.9% radiochemically pure by TLC-RAM (silica gel, $CHCl_3$-MeOH, 4:1, R_f 0.56).

Methyl 4-amino[7-^{14}C]benzoate (4)

Trimethylsilyldiazomethane (0.6 mL of a 2.0 M hexane solution) was added to a solution of 146 mg (1.0 mmol) of **3** in 3 mL of MeOH. After 30 min another 1.6 mL was added. TLC analysis (silica gel, hexane-EtOAc, 4:1, R_f 0.39) 30 min after the last addition indicated that the reaction was complete. The solvent was removed, and the residue was dissolved on 20 mL of EtOAc. The EtOAc solution was extracted twice with brine and dried (Na_2SO_4). Removal of the solvent gave 95 mg (20 mCi, 65% chemical and radiochemical yield) of **4** which was pure by TLC-RAM (same system as above).

Methyl 4-(2,5-dihydroxybenzylimino)[7-^{14}C]benzoate (5)

A solution of 2,5-dihydroxybenzaldehyde (85 mg, 0.62 mmol) and methyl 4-amino[7-^{14}C]benzoate (95 mg, 0.52 mmol) in 5 mL of anhydrous MeOH was refluxed for 24 h under nitrogen. Removal of the solvent gave 135 mg (0.5 mmol) of **5,** which was carried directly to the next step..

Methyl 4-(2,5-dihydroxybenzylamino)[7-^{14}C]benzoate (6)[3]

Sodium cyanoborohydride (62 mg, 1.0 mmol) and 0.01 mL of HOAc were added to a solution of 135 mg (0.5 mmol) of **5** in 5 mL of anhydrous MeOH and stirred at ambient temperature for 2 h. Another 62 mg of $NaBH_3CN$ and 0.01 mL of AcOH were added and stirring continued another 0.5 h. Removal of the solvent gave a dark red residue which was dissolved in 20 mL of EtOAc. The EtOAc solution was washed with water (2 × 10 mL) and brine (10 mL) and dried ($MgSO_4$). The solvent was removed, and the residue was chromatographed on two 20 x 20 preparative TLC plates ($CHCl_3$-CH_3OH, 85:15). The product was washed from the silica gel with $CHCl_3$-EtOH (1:1). This product was further purified by preparative-HPLC (methanol-water 2:3, 10 mL/min) to give 56 mg (8.01 mCi) of **6** which was >95% pure by TLC-RAM [silica gel, $CHCl_3$-CH_3OH-HCOOH (90:10:0.1), R_f O.32 and C_{18}-F, CH_3CN-H_2O (1:1), R_f 0.52] with R_f values the same as a standard sample. Mp 158-160 °C dec. (standard sample mp 160-161 °C dec); UV max (CH_3OH) 306 nm (30,000), 224 (11,900) the same as the standard. ^{1}H NMR was identical to the standard. The specific activity was 142 mCi/mg (38.9 mCi/mmol).

Methyl 4-(2,5-dihydroxy[7-^{3}H]benzylamino)benzoate (7)

A solution of 6.7 mg (0.0247 mmol) of imine **5** in 0.5 mL of THF with 2.5 mg of 10% palladium on carbon catalyst was exposed to carrier-free tritium gas (4.9 Ci, 658 torr) for

1 h at ambient temperature. The catalyst was filtered away, and the filtrate was exchanged 3 times on a vacuum line with 5 mL of methanol. The crude product was purified by preparative HPLC (methanol-water 1:1, 10 mL/min, UV-254) to give 137 mCi of **7** that was 98% pure by HPLC-RAM (methanol-water 40:60 0.05 M with formic acid linear gradient in 30 min to methanol 0.05 M with formic acid, 1 mL/min). TLC-RAM (silica gel, $CHCl_3$-CH_3OH-HCOOH 90:10:0.1 ,R_f 0.40, run at 5 °C in the dark in a pre-chilled tank) showed 97% purity. The specific activity was determined using UV absorbance at 306 nm (methanol) to be 7.6 Ci/mmol (27.8 mCi/mg). ^3H NMR (533 MHz-acetone-d_6) showed a four-peak multiplet at δ 4.34 which collapsed to a doublet, J = 17 Hz, in methanol-d_4 in the proton coupled spectrum and was a singlet in the proton decoupled spectrum. No other tritium signals were observed, indicating that at least 98% of the tritium was at the 7-benzyl position. Back-exchange in pH 7 phosphate buffer for 24 h at room temperature showed 0.5% exchange.

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