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SYNTHESIS OF RADIOLABELED RACEMIC AND ENANTIOMERIC ANTIARRHYTHMIC AGENTS

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SUMMARY

Ibutilide fumarate, racemic N-[4-[4-(ethyl-n-heptylamino)-1-hydroxybutyl]phenyl]methanesulfonamide hemifumarate, and artilide, the R-(+)-enantiomer of N-[4-[4-(di-n-butylalmino)-1-hydroxybutyl]-phenyl]methanesulfonamide hemifumerate, are under clinical investigation as Class III antiarrhythmic agents. For conducting drug disposition studies, we synthesized carbon-14 labeled ibutilide, as well as its two enantiomeric forms. In addition, high specific activity tritium labeled ibutilide was also prepared to facilitate development of a radioimmunoassay and for studying receptor site characteristics of this agent. Results of metabolism studies with [^{14}C]ibutilide led us to prepare tritium labeled artilide, which is more readily accessible than the C-14 labeled drug. The optical antipode of artilide was also labeled with tritium for comparative drug disposition investigations on the two enantiomers.

Key Words: Carbon-14, Tritium, Ibutilide, Artilide, Antiarrhythmics, Enantiomers

INTRODUCTION

A leading cause of fatality in patients suffering from cardiovascular disorder is ventricular fibrillation. Several approaches to treating cardiac dysrhythmia with various medicinal agents have been developed.[1-5] One approach utilizes compounds categorized as "Class III" antiarrhythmic agents which prolong cardiac action potential and provide a uniform increase in the refractoriness of cardiac tissue, without affecting the sodium current.[6-8] Investigations into structural requirements necessary for such biological activities led to the synthesis of a series of compounds of which ibutilide fumarate[9] and artilide fumarate are representative members. These compounds share structural features found in known Class III antiarrhythmics such as clofilium[10] and sotalol[11,12]. For conducting drug disposition studies in test animals and human subjects, we synthesized several radioisotope labeled versions of ibutilide fumarate and artilide fumarate.

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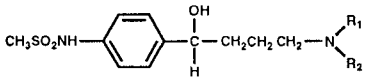


• 1/2 Fumaric Acid

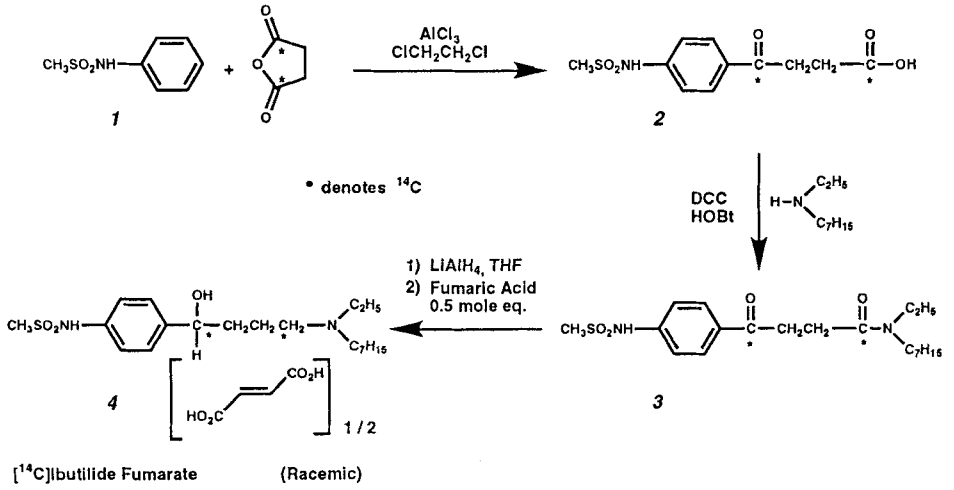
$R_1 = C_2H_5$, $R_2 = C_7H_{15}$ Ibutilide Fumarate (Racemic)

$R_1 = C_4H_9$, $R_2 = C_4H_9$ Artilide Fumarate (R - (+) -Enantiomer)

RESULTS AND DISCUSSION

The first compound of the series chosen for development as a potential new antiarrhythmic drug was the racemic ibutilide fumarate. The synthesis of carbon-14 labeled ibutilide fumarate (*4*) is shown in Scheme 1. N-phenyl methanesulfonamide (*1*) was acylated with [1,4-^{14}C]succinic anhydride in the presence of aluminum chloride to give the ketoacid *2* labeled with carbon-14 at both the carbonyl and carboxy carbons. The ketoacid was converted to the labeled ketoamide *3* with ethyl-n-heptylamine. Reduction of *3* with lithium aluminum hydride (LAH) produced the corresponding racemic hydroxylamine which was isolated as the crystalline hemifumarate salt *4*. This material had a specific activity (SA) of 56.3 μCi/mg and was 99% radiochemically pure (RCP) as analyzed by high performance liquid chromatography (HPLC).

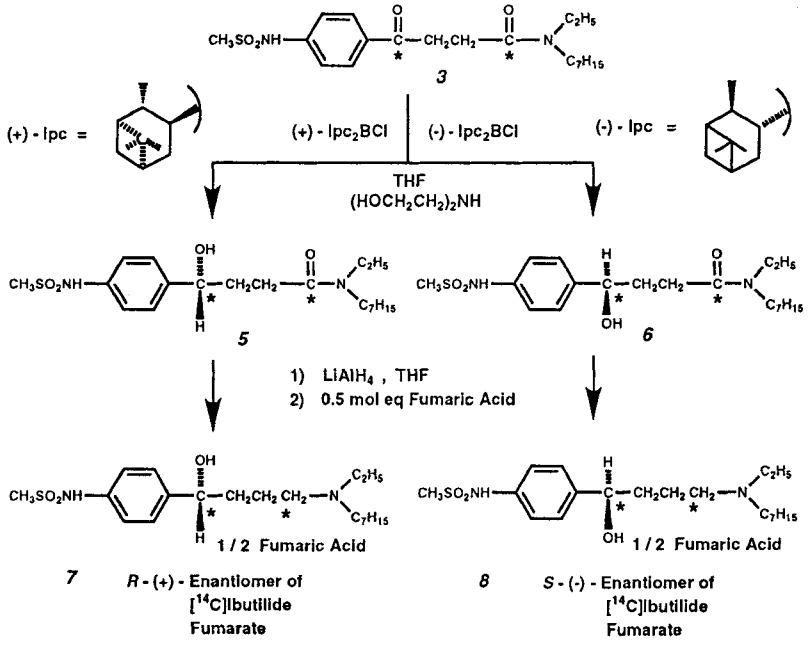
SCHEME 1.



[^{14}C]Ibutilide Fumarate (Racemic)

To facilitate investigations into potential kinetic and chemical differences in the biotransformations of the optical antipodes of ibutilide, we also synthesized the enantiomers of ***4*** as shown in Scheme 2. The ketoamide ***3*** was reduced with (+)- and (-)B-chlorodiisopinocampheylborane (Ipc_2BCl)[13,14] to give, respectively, the chiral hydroxyamides ***5*** and ***6***. Further reduction of the amides ***5*** and ***6*** with lithium aluminum hydride in tetrahydrofuran afforded the enantiomeric forms ***7*** and ***8*** of [^{14}C]ibutilide, which were also isolated as their hemifumarate salts. Their enantiomeric purity was ascertained by means of HPLC analysis[15] on a covalently bound D-phenylglycine Pirkle analytical column. The R-(+)-enantiomer ***7*** had SA of 28.8 μCi/mg with 97.5% RCP by HPLC, and was found to have 99% enantiomeric purity (EP). The optical antipode ***8*** has SA of 32.1 μC/mg with 98.1% RCP and 99.6% EP.

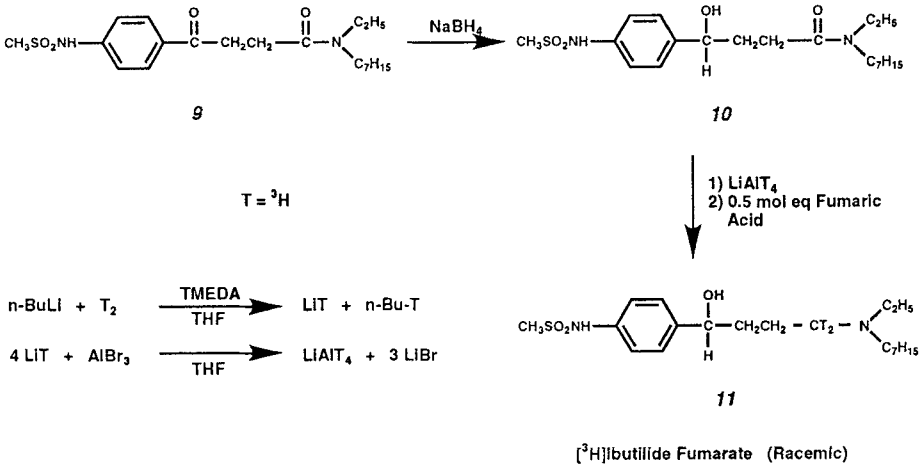
SCHEME 2. SYNTHESIS OF ENANTIOMERS OF [^{14}C]IBUTILIDE FUMARATE



To provide a radiolabeled ligand for developing a radioimmunoassay and for conducting receptor site characterization studies, radioactive ibutilide fumarate with high specific activity was needed. We therefore also synthesized tritium labeled ibutilide fumarate

(*11*), as shown in Scheme 3. The unlabeled ketoamide *9*, prepared in the analogous manner as *3*, was reduced with sodium borohydride to give the racemic hydroxyamide *10*. Further reduction of *10* with lithium aluminum tritide (LAT) afforded racemic [^{3}H]ibutilide, which was isolated as the hemifumarate salt *11*. The radiolytic instability of high specific activity LAT[16] required its preparation immediately prior to its use, as shown in Scheme 3. The LAT generated from carrier-free tritium gas led to [^{3}H]ibutilide of SA 136 mCi/mg (52.3 Ci/mmol) with 98% RCP by HPLC.

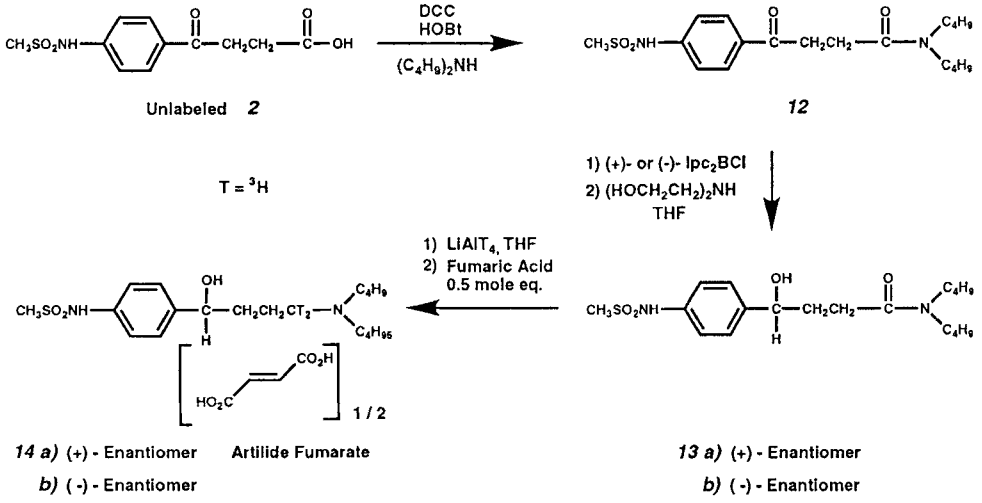
SCHEME 3.



Preliminary studies in test animals showed that although ibutilide fumarate was well absorbed, it was found to undergo extensive first-pass metabolism[17] following oral administration. Artilide fumarate, the (R)-(+)-enantiomer of the ibutilide fumarate analog with the di-n-butylamino group in the side-chain, has been shown to possess improved bioavailability, and was therefore also developed as a potential new drug. Studies with ibutilide fumarate had also shown that there were apparently no metabolic changes at the side-chain carbon atom adjacent to the amino group. That position is readily accessible for tritium incorporation as shown in the synthesis of *11*, and the introduction of radioisotope

can be delayed until after the chirality at the carbon atom adjacent to the phenyl ring has been established. We therefore elected to prepare tritium labeled artilide fumarate (***14a***), as shown in Scheme 4, for conducting metabolism studies with this compound. The unlabeled ketoacid ***2*** was converted to its N,N-di-n-butyl amide ***12***. Reduction of ***12*** with (+)-Ipc_2BCl afforded the chiral hydroxyamide ***13a***, which on further reduction with LAT, followed by salt formation with fumaric acid, gave [^{3}H]artilide fumarate (***14a***) of SA 15.0 mCi/mg with 98.6% RCP (HPLC) and 98.2% EP. Similarly, reduction of ***12*** with (-)-Ipc_2BCl produced the hydroxyamide ***13b***, which upon further reduction with LAT led to ***14b***, the S-(-)-enantiomer of artilide fumarate, SA 471 μCi/mg with 99% RCP (HPLC) and 90% EP.

SCHEME 4.



EXPERIMENTAL

Thin layer chromatography (TLC) analysis was performed with 2.5 x 10 cm glass plates precoated with a 250 μm layer of silica gel (Analtech). Developed zones were visualized by exposure to iodine vapor. TLC radiochromatograms were obtained with a Bioscan System 200 Imaging Scanner. HPLC analyses were carried out with a Spectra Physics Model 8700 solvent delivery system and a LDC/Milton Roy SpectroMonitor D variable wavelength detector set at 230 nm, in series with an IN/US Beta-Ram flow through

radioactivity detector using Flo-Scint II as the liquid scintillation cocktail. Beta-Ram software was used to perform both UV and radioactivity chromatogram integrations. Non-chiral HPLC radiochemical purity was determined using a Supelcosil LC-18 5 μ (250 x 4.6 mm I.D.) analytical column with a mobile phase of 45:55 v/v acetonitrile (CH_3CN):buffer A. Buffer A was prepared from 6.9 g of $NaH_2PO_4 \cdot H_2O$ and 1 mL of triethylamine (TEA) in 1 L of water, the pH adjusted to 3.0 with H_3PO_4. HPLC analyses to determine enantiomeric purity** of ***5*** and ***6*** were carried out on a Regis covalently bound D-phenylglycine Pirkle column (4.6 mm ID x 250 mm) after derivatization of the sample with 1-naphthylisocyanate (Regis Chemical Co.). A 1 mg sample of ***5*** or ***6*** was dissolved in 200 μl of CH_3CN and derivatized with 185 μl of 1-naphthylisocyanate solution (240 mg in 25 mL of CH_3CN) for 1 h at room temperature. The mixture was quenched with 0.5 mL of MeOH for 5 min, the solvents removed under a nitrogen stream, and the residue dissolved in 0.5 mL of 650:350:0.5:0.5 v/v ethanol:hexane:TEA:trifluoroacetic acid (TFA), which served as the mobile phase in this assay. For the enantiomeric HPLC assay of ***7*** or ***8*** and ***14a*** or ***14b***, a 1 mg sample was dissolved in 126 μl of TEA solution (126 μl of TEA in 10 mL of CH_3CN) and derivatized with 62 μl of 1-naphthylisocyanate solution (240 mg in 25 mL of CH_3CN) at room temperature for 10 min. The mixture was quenched with 260 μl of 800:200:0.5:0.5 v/v methanol:isopropanol:TEA:TFA, which was also the mobile phase of this assay. This method was also used to determine the enantiomeric purity of ***15*** and ***16***, except that these compounds required a longer derivatization time of 1 h.

Preparative HPLC purifications were performed on a Waters Delta Prep unit using a Supelcosil LC-18 5 μ (250 x 21.2 mm I.D.) preparative column. Mobile phase (45:55 v/v, acetonitrile:buffer A) was pumped isocratically at 15 mL/min. Detection by UV at 230 nm was done with a LDC/Milton Roy SpectroMonitor D with a semi prep cell. The elution time of the collected peak was from 6-9 min. Tritium nuclear magnetic resonance (NMR) spectra were obtained on a 300 MHz IBM AF-300 instrument in methanol-d_4 with TMS ghost referencing. Radioactivity determinations were carried out on a Wallac Model 1410 liquid

**HPLC analyses for determining enantiomeric purity of radiactive compounds were based on radioactivity as detection endpoint.

scintillation spectrometer using the external standard method. Ultima Gold (Packard) was used as the liquid scintillation cocktail. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen immediately prior to use. All other solvents were HPLC grade.

4-[4-(Methanesulfonamide)phenyl]-4-oxol[1,4^{14}C]butanoic Acid (*2*)

A mixture of [1,4^{14}C]succinic anhydride (179 mg, 1.75 mmol, American Radiolabeled Chemicals Inc., nominally 93.4 mCi, 53.13 mCi/mmol) and methanesulfonanilide (*1*, 306 mg, 1.79 mmol) in 4 mL of 1,2-dichloroethane was stirred for 30 min at room temperature. This mixture was added dropwise to a vigorously stirred slurry of aluminum chloride (1.91 g, 14.3 mmol) in 3 mL of 1,2-dichloroethane under a nitrogen atmosphere at 0°C (ice bath). After the addition was complete, the ice bath was removed and stirring was continued for 1.25 h. The reaction was quenched by the addition of 15 g of ice. The resulting solids were collected by filtration, washed with cold water, and air dried. The dry solids were dissolved in 15 mL of 0.5 N NaOH solution and filtered to remove a dark insoluble material. The filtrate was adjusted to pH 1.5 with the dropwise addition of 12 N HCl. The precipitated solids were collected by filtration, washed with water and dried under high vacuum at room temperature to give 267 mg (71.4%) of *2*, SA 182 μCi/mg, identical to a reference sample of unlabeled *2* by TLC (95:5 v/v, CH_2Cl_2:MeOH,Rf 0.2).

N-[4-[4-(Ethyl-n-heptylamino)-1,4-dioxo[1,4-^{14}C]-butyl[phenyl]methanesulfonamide (*3*)

A solution of dicyclohexylcarbodiimide (DCC, 295 mg, 1.43 mmol) and 1-hydroxybenzotriazole (HOBt, 193 mg, 1.43 mmol) in 2 mL of N,N-dimethylformamide (DMF) was added with stirring under a nitrogen atmosphere to a cold (0°C) solution of *2* (367 mg, 1.34 mmol, 66.7 mCi). After 1 h of stirring, a solution of ethyl-n-heptylamine (204 mg, 1.43 mmol) in 2 mL of DMF was added. Stirring was continued for 20 min at 0°C, then at room temperature overnight. The reaction mixture was added to 40 mL of ethyl acetate (EtOAc) and washed with 2 x 30 mL of 0.5 N HCl, 2 x 30 of 0.5 M $NaHCO_3$, and 20 mL of brine in that order, dried over sodium sulfate, and concentrated. The residue was

chromatographed on a column of 36 g of silica gel packed and eluted with 6:4 v/v EtOAc:hexane. Fractions of 15 mL each were collected at a rate of 1.9 mL/min, and were analyzed by TLC (6:4 v/v, EtOAc:hexane). The pooled fractions 17-40 were concentrated and the residue crystallized from 1.2 mL of EtOAc and 10 mL of hexane to give 382 mg (76%) of ***3*** of SA 132.8 μCi/mg, with 99% RCP by TLC (8:2 v/v EtOAc:hexane, Rf 0.36, identical to an authentic sample of unlabeled ***3***.

N-[4-[4-(Ethyl-n-heptylamino)-1-hydroxy[1,4-^{14}C]butyl]phenyl]methanesulfonamide Hemifumarate, (***4***) (^{14}C]Ibutilide Fumarate)

To a stirred, ice cold slurry of 158 mg of LAH (4.18 mmol) in 5 mL of anhydrous THF was added dropwise under N_2 a solution of 552 mg of ***3*** (1.39 mmol, 34.1 mCi) in 10 mL of anhydrous THF. The reaction mixture was stirred at 0°C for 2 h, treated with 10 mL of 0.5 N potassium sodium tartrate tetrahydrate and partitioned with 10 mL of 50% brine and 30 mL of EtOAc. The aqueous phase was extracted with 5 x 20 mL of EtOAc, and the combined organic layers were washed with 25 mL of brine and dried over Na_2SO_4. The concentrated residue was chromatographed on 60 g of silica gel packed in and eluted with 0.5:5:95 v/v NH_4OH:MeOH:CH_2Cl_2 to give 24.2 mCi of oil, which was dissolved in 3 mL of absolute ethanol and treated with 2.85 mL of 0.17 M fumaric acid in the same solvent. The mixture was crystallized from 3.5 mL of acetone to give 346 mg of ***4***, SA 56.2 μCi/mg with 98.8% RCP by TLC (85:15:1.5 CH_2Cl_2:MeOH:NH_4OH, Rf 0.64, identical to an authentic sample of ibutilide fumarate) and >99% RCP by HPLC. From the mother liquor there was obtained a second crop of ***4*** with isotopic dilution, 75 mg, SA 14.6 μCi/mg with >99% RCP by HPLC. The total radiochemical yield was 61% from ***3***.

(+)-N-[4-[4-(Ethyl-n-heptylamino)-1-hydroxy-4-oxo[1,4-^{14}C]butyl]phenyl]-methanesulfonamide (***5***)

A solution of (+)-Ipc_2BCl (449 mg, 1.4 mmol, Aldrich Chemical Co.) in 1.5 mL of dry THF was added dropwise over 5 min to a solution of ***3*** (184.7 mg, 0.466 mmol, 24.5 mCi) in 2.0 mL of THF under a nitrogen atmosphere at -50°C (CH_3CN/dry ice bath). The reaction

mixture was stirred at -50° to -40°C for 1 h. The progress of the reaction was checked by TLC (95:5 v/v, CH_2Cl_2:MeOH) until no starting material remained. The mixture was diluted with 12 mL of EtOAc and stirred with diethanolamine (294 mg, 2.8 mmol) for 1.5 h at room temperature. The quenched mixture which contained gummy solids was concentrated, and the residue was triturated with 15 mL of 95:5 v/v CH_2Cl_2:MeOH. The mixture was filtered to remove some white solids and the filtrate was concentrated at 15 torr and 20°C. The residue was chromatographed on a column of 60 g of silica gel packed in and eluted with 95:5 v/v CH_2Cl_2:MeOH to give, after isotopic dilution with 150 mg of unlabeled material, 15.2 mCi of ***5***, 232 mg, SA 68.3 μCi/mg with 99% RCP by HPLC and 98.7% EP. Further isotopic dilution of the mother liquor with 200 mg of unlabeled compound afforded 4.5 mCi (224 mg) of ***5*** with SA of 20.1 μCi/mg and 96.4% EP. The total radiochemical yield was 19.7 mCi or 80.2% from ***3***.

(-)-N-[4-[4-(Ethyl-n-heptylamino)-1-hydroxy-4-oxo[1,4-^{14}C]butyl]phenyl]-methanesulfonamide (***6***)

In a similar manner, reduction of 184.2 mg of ***3*** (0.465 mmol, 24.5 mCi) with 449 mg of (-)-Ipc_2BCl(1.4 mmol) produced 14.6 mCi of ***6*** with SA of 68.3 μCi/mg, 98.8% and 98.9% RCP by HPLC and TLC, respectively, and 99.2% EP. A second crop of ***6*** was also obtained, 5.4 mCi (206 mg) with SA of 26.2 μCi/mg, >99% RCP by HPLC, and 97.7% EP.

(+)-N-[4-[4-(Ethyl-n-heptylamino)-1-hydroxy[1,4-^{14}C]butyl]phenyl]methanesulfonamide. (***7***)

A solution of 220 mg of ***5*** (0.55 mmol, 15.0 mCi) in 3.0 mL of dry THF was added dropwise over 10 min from an addition funnel into a solution of 1.4 mL of 1.0 M $LiAlH_4$•THF (Aldrich Chemical Co.) in 1.1 mL of dry THF which had been cooled to -23°C (CCl_4/dry ice bath) under a nitrogen atmosphere. After the addition, stirring was continued at -20°C for 45 min. The progress of the reaction was checked by TLC (95:5 v/v CH_2Cl_2:MeOH, Rf 0.05 for ***7***, 0.3 for ***5***) until only a trace of starting material remained. Stirring was continued for 45 min and the reaction was quenched by the dropwise addition of 15.0 mL of 0.5 M sodium potassium tartrate at -20°C. the mixture was extracted with 2 x 15 mL of EtOAc. The

extract was washed with 20 mL of brine, dried over sodium sulfate and concentrated under high vacuum at room temperature. The residue (215 mg, 14.5 mCi) was dissolved in 2 mL of absolute ethanol and mixed with 2 mL of a freshly prepared fumaric acid solution (1.47 g in 100 mL of absolute ethanol). The solution was stirred for 5 min and concentrated under high vacuum at room temperature. The residue was mixed with 200 mg of unlabeled **7** and recrystallized by dissolving in 2 mL of hot acetone, concentrating the solution to 1 mL, seeding at room temperature and cooling to -15°C for 18 h. The resulting mixture was quickly mixed with 4.0 mL of dry ether while cold, filtered, washed with 2.0 mL of dry ether, and dried under high vacuum at room temperature to give 408 mg of **7**, SA 28.8 μCi/mg, 11.8 mCi, 98.1% RCP by TLC, 97.5% RCP by HPLC, and 99% EP.

A second crop of **7** was obtained with further isotopic dilution from the mother liquor, SA 7.7 μCi/mg, 169 mg, 1.3 mCi, 96.8% RCP by TLC, 96.7% RCP by HPLC, and 98.5% EP.

(-)-N-[4-[4-(Ethyl-n-heptylamino)-1-hydroxy[1,4-^{14}C]butyl]phenyl]methanesulfonamide fumarate (2:1 salt) (*8*)

Similarly, 210 mg of ***6*** (14.3 mCi, 0.53 mmol) was reduced with 1.3 mL of 1.0 M $LialH_4$•THF in THF to give, after isotopic dilution, 370 mg of ***8***, SA 32.1 μCi/mg, 11.9 mCi, 98.8% RCP by TLC, 98.1% RCP by HPLC, and 99.6% enantiomerically pure. A second crop obtained from the mother liquor with further isotopic dilution was found to have SA of 6.64 μCi/mg, 179mg, 1.19 mCi, 98.0% RCP by TLC, 97.9% RCP by HPLC.

Lithium Aluminum Tritide (LAT)[18]

A 3 mL round-bottom flask with side-arm was charged with ~1 atm of tritium gas, and a solution of 340 μl of 1 M n-butyllithium (Aldrich) in hexane was added with a syringe to the flask. With vigorous stirring, 70 μl of tetramethylethylenediamine (TMEDA, 0.46 mmol, Aldrich) was added at room temperature. A white precipitate of lithium tritide formed immediately. The mixture was stirred for 1 h, and frozen with a liquid nitrogen bath. The excess tritium gas and [^{3}H]butane produced as a by-product were removed and trapped for disposal. In a separate flask, 200 μl of 1 M aluminum bromide (Aldrich) in methylene

bromide was evaporated under reduced pressure, the residue was cooled to -23°C (CCl_4/dry ice), and dissolved in 1 mL of slowly added dry THF under a nitrogen atmosphere. The resulting dark solution was added dropwise by syringe to the flask containing the thawed mixture of lithium tritide and hexane. The resulting homogeneous tan solution of LAT was used immediately in the next reaction.

N-[4-[4-(Ethyl-n-heptylamino)-1-hydroxy[4-^{3}H]butyl]phenyl]methanesulfonamide, [^{3}H]Ibutilide (*11*).

The above solution of LAT was cooled to -23°C (CCl_4/dry ice bath). A solution of 26 mg of the racemic hydroxyamide ***10****** in 0.5 mL of dry THF was added dropwise to the cold LAT solution. Stirring was continued for 3 h at room temperature under a nitrogen atmosphere. The reaction was quenched by the addition of 0.5 mL of 0.5 M sodium potassium tartrate solution and 2 mL of methanol. The methanol was evaporated under vacuum to remove exchangeable tritium. The reaction mixture was transferred to a 15 mL centrifuge tube with 8 mL of 0.5 M sodium potassium tartrate solution and 6 mL of EtOAc. After partitioning, the organic layer was separated and the aqueous layer extracted with an additional 6 mL of EtOAc. The combined organic layer was washed with 3 mL of brine and dried over anhydrous sodium sulfate. The dry extract was concentrated under vacuum. The residue was dissolved in 2 mL of perdeuterated methanol, 2.67 Ci, 93% RCP by HPLC. Tritium NMR spectrum of this material showed exclusive tritium labeling at the 4-position of the hydroxybutyl moiety. Ditritiated ibutilide was the predominant species (~95%) while monotritiated material made up the remainder (~5%). The crude ***11*** was purified by preparative HPLC on a Phenomenex ODS-30 7 μ column (22.5 mm ID x 250 mm) eluted with 1:1 v/v AcCN:buffer (20.5 g NaOAc in 1 L of water, adjusted to pH 5.5 with acetic acid) at 15 mL/min. The sample was dissolved in 1 mL of CH_3CN and 0.5 mL of mobile phase. Eight injections were made and the peaks corresponding to ibutilide as detected by UV at 230 nm

The racemic hydroxyamide ***10 can be readily prepared by reduction of the ketoamide ***9*** with sodium borohydride. However, because of greater availability of unlabeled ***5*** and ***6*** at the time of this experiment, the sample of ***10*** used was actually prepared by mixing equal amounts of the enantiomers.

were collection. The pooled collection was concentrated to remove the CH_3CN, and the remaining aqueous residue was adjusted to pH 9.4 with the addition of 1N NaOH. The basified solution was extracted with 2 x 50 mL of EtOAc. The combined extract was washed with 25 mL of water, 25 mL of brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give 2.27 Ci of ***11***. This material was stored as a solution in methanol and had a radiochemical purity of 98.4% by HPLC, but only 91% by TLC. A sample of 227 mCi of this material was further purified by preparative TLC to afford 181 mCi of ***11*** with SA of 136 mCi/mg (52.3 Ci/mmol). Specific activity of this purified sample of ***11*** was determined by relating the radioactivity of an injected HPLC sample to its mass, which was determined using a standard UV curve constructed with the use of a reference sample of ibutilide. The radiochemical purity was shown to be 98.7% by HPLC and 99.2% by TLC. The [^{3}H]ibutilide was stored as a solution of 2.26 mCi/ml in methanol at -70°C.

(+)-(R)-N-[4-[4-(Di-n-butylamino)-1-hydroxy-[4-^{3}H]butyl]phenyl]methanesulfonamide Hemifumarate ([^{3}H]Artilide Fumarate) (***14a***)

The unlabeled ketoacid ***2*** was converted to the di-n-butylamide ***12***[9] in a similar manner as the preparation of the ketoamide ***3*** and ***2***, substituting di-n-butylamine for ethyl-n-heptylamine. Reduction of ***12*** with (+)- or (-)- Ipc_2BCl afforded the enantiomeric (+)- or (-)-hydroxyamide ***13a*** or ***13b***[19], respectively. LAT was prepared according to the procedure described earlier in this report. Tritium gas of lower specific activity (~ 6.6 Ci/mmol, isotopically diluted with hydrogen gas) was used in this experiment, since compound ***14a*** of only modest specific activity was required. A freshly prepared solution of nominally 0.1 mmol of LAT in THF was cooled to -23°C (CCl_4\dry ice bath), and a solution of 27 mg of ***14a*** (0.07 mmol) in dry THF was added dropwise with stirring under a nitrogen atmosphere over 5 min. The cooling bath was removed and the reaction mixture was allowed to warm to room temperature for 2.5 h. The reaction was then quenched by the addition of 1.0 mL of methanol and 0.2 mL of 1N HCL (aq). The mixture was concentrated under reduced pressure to remove the methanol. One mL of methanol was again added and the concentration repeated to remove any remaining exchangeable tritium from the reaction mixture. The

aqueous remainder was transferred to a 15 mL centrifuge tube with 5 mL of ethyl acetate and 5 mL of 0.25 M Na_2HPO_4. After partitioning, the aqueous layer was extracted with an additional 5 mL of ethyl acetate. The combined extract was washed with 5 mL of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give 402 mCi of crude product with a radiochemical purity of 89.2% by HPLC. A tritium NMR spectrum was obtained which indicated tritium incorporation at 2.5 ppm as expected. The material was purified by preparative HPLC using a Supelcosil LC-18 column. The pooled peak collections were concentrated to remove the acetonitrile. A solution of 12 mL of 0.3 M K_2HPO_4 was added and the pH was adjusted to 9.2 with 1N sodium hydroxide. This solution was then extracted 2 x 30 mL with ethyl acetate. The combined extracts were washed with 25 mL of water, 25 mL of brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give 19 mg (337 mCi) of free base, which was dissolved in 0.2 mL of absolute ethanol and 3.1 mg of fumaric acid (0.027 mmol) in 0.4 mL of warm absolute ethanol was added. The solution was cooled to room temperature, and diluted with 2.4 mL of ether. The resulting crystals were collected by filtration, washed with 2 mL of ether and dried under high vacuum at room temperature to give 18.8 mg (282 mCi) of ***14a***, SA 15.0 mCi/mg (6.43 Ci/mmol), >98% RCP by HPLC and TLC, and 98.2% EP. The crystalline product was stored in a liquid nitrogen freezer, while a portion, 220 mCi, was dissolved in 9:1 v/v ethanol:water (550 μCi/ml) and stored at -70°C.

(-)-(S)-N-[4[4-(Di-n-butylamino)-1-hydroxy-[4-^{3}H]butyl]phenyl]methanesulfonamide Fumarate (2:1 salt) (***14b***)

The procedure described above for preparing ***14a*** was followed. A dry THF solution of freshly prepared LAT (nominally 0.1 mmol) was used to reduce 0.07 mmol of the hydroxyamide ***13b*** to give 65 mCi of free base, which was converted to the fumarate salt ***14b***, 61 mCi (129 mg) of SA 471 μCi/mg (202 mCi/mmol) after isotopic dilution with unlabeled ***14b***, 99% RCP by HPLC and TLC. The enantiomeric purity of this material was found to be 90%, in agreement with the enantiomeric purity of the available sample of starting material ***13b***. The crystalline product was stored in a liquid nitrogen freezer, while a portion of the material was stored at -70°C as a solution of 519 μCi/ml in 9:1 v/v ethanol:water.

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REFERENCES

1. Morgan P.H., Mathison I.W. - J. Pharm. Sci. 65:467 (1976).
2. Mason D.T., De Maria A.N., Amsterdam E.A., Vismara L.A., Miller R.R., Vera E., Lee G., Zelis R., and Massumi R.A. - Cardiovascular Drugs, ADIS Press, Sydney 1977 Vol. 1, Chapter III.
3. Vaughan Williams E.M. - Symposium on Cardiac Arrhythmias, Sandoe E., Flensted-Jansen E., and Olesen K.H., Eds., AB Astra, Sodertalje, Sweden, 1970. p.449.
4. Vaughan Williams E.M. - J. Clin. Pharmacol. 24:129 (1984).
5. Cardiac Arrhythmia Pilot Study (CAPS) Investigators - Amer. J. Cardiol. 61:501 (1988).
6. Singh B.N. and Vaughan Williams E.M. - Br. J. Pharmacol. 39:657 (1970).
7. Singh B.N. and Vaughan Williams E.M. - Br. J. Pharmacol. 39:675 (1970).
8. Singh B.N. and Nademanee K. - Amer. Heart J., 109:421 (1985).
9. Hester J.B., Gibson J.K., Cimini M.G., Emmert D.E., Locker P.K., Perricone S.C., Skaletzky L.L., Sykes J.K., and West B.E. - J. Med. Chem. 34:308 (1991).
10. Platia E.V. and Reid P.R. - Clin. Pharmacol. Ther. 35:193 (1984).
11. Kienzle M.G., Martins J.B., Wendt D.J., Constantin L., Hopson R., and McCue M.L. - Amer. J. Cardiol. 61:1012 (1988).
12. Singh B.N., Cohen A., Chen Y., Wish N., Thoben-O'Grady L., Peralba J., Gottdiener J., and Fletcher R.D. - Amer. J. Cardio. 62:399 (1988).
13. Chandrasekharan J., Ramachandran P.V., and Brown H.C. - J. Org. Chem. 50:5448 (1986).
14. Brown H.C., Chandrasekharan J., and Ramachandran P.V. - J. Org. Chem. 51:3396 (1986).
15. Hsu C.L. and Walters R.R. - J. Chromatography 550:621 (1991).
16. Andres H., Morimoto H., and Williams P.G. - J. Chem. Soc., Chem. Commun. 1990:627.
17. Wickrema Sinha A.J., unpublished results.
18. High specific activity lithium aluminum tritide was prepared and used in reduction reactions at the National Tritium Labeling Facility of the Lawrence Berkeley Laboratory in collaboration with H. Morimoto and P.G. Williams.
19. Details for the preparation of ***12, 13a,*** and ***13b*** will be published elsewhere by J.B. Hester and his co-workers.