SYNTHESIS OF THE ENANTIOMERS AND THREE RACEMIC METABOLITES OF CARVEDILOL LABELED TO HIGH SPECIFIC ACTIVITY WITH TRITIUM

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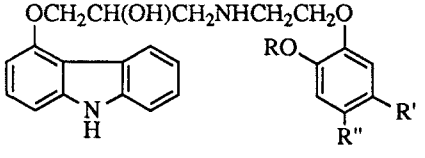
ABSTRACT

Carvedilol (SK&F 105517) possesses unique cardiovascular activity, and is under development for indications such as angina and hypertension. Tritium labeled enantiomers of Carvedilol and racemates of three metabolites were needed for pharmacologic and drug metabolic studies. These compounds were synthesized by catalytic tritium-halogen exchange using tritium gas and 10% palladium-on-carbon catalyst. The precursors were polyhalogenated in the carbazole ring. Direct electrophilic bromination of the enantiomers of Carvedilol gave precursors that were converted to the corresponding tritiated final products by catalytic tritium halogen exchange. Bromination of 4-(2,3-epoxypropyloxy)-9H-carbazole gave an intermediate that was converted to the halogenated precursors of the racemic metabolites. Elaboration of this intermediate, 1,3,6-tribromo-4-(2,3-epoxypropyloxy)-9H-carbazole, to the desired metabolite precursors was achieved by nucleophilic epoxide opening with suitably functionalized N-benzyl aryloxyethylamines. Catalytic tritium-halogen exchange upon the brominated metabolite precursors was accompanied by cleavage of N- and O-benzyl protecting groups. Radiochemical purities of all tritiated final products were greater than 98% after preparative HPLC. Specific activities of the final products, determined by mass spectrometry, ranged from 35 to 76 Ci/mmol. Optical purity of the Carvedilol enantiomers, determined by chiral HPLC, was greater than 99%

INTRODUCTION

Carvedilol (SK&F 105517) is under development for indications such as angina and hypertension.[1] High specific activity tritium labeled enantiomers and three racemic metabolites of Carvedilol (Figure 1) were required for

FIGURE 1



Carvedilol	R=Me, R'=R"=H
M2	R=H, R'=R"=H
M4	R=Me, R'=H, R"=OH
M5	R=Me, R'=OH, R"=H

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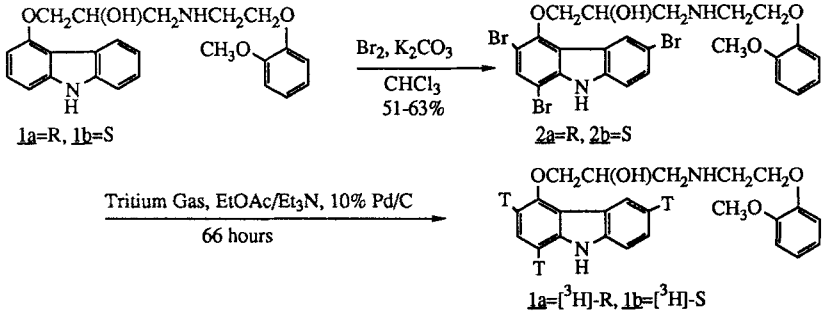
pharmacologic and drug metabolic studies. The strategy for the synthesis of the compounds involved catalytic tritiodehalogenation of tribrominated precursors with tritium gas in the final step. Described herein is the synthesis of the enantiomeric Carvedilol precursors by direct electrophilic bromination, the multistep synthesis of the brominated precursors to the metabolites, and the tritiation of the brominated precursors to give the title compounds.

DISCUSSION

Synthesis of the tritium labeled enantiomers of Carvedilol involved bromination of each enantiomer (1a, 1b) as shown in Scheme 1. This gave

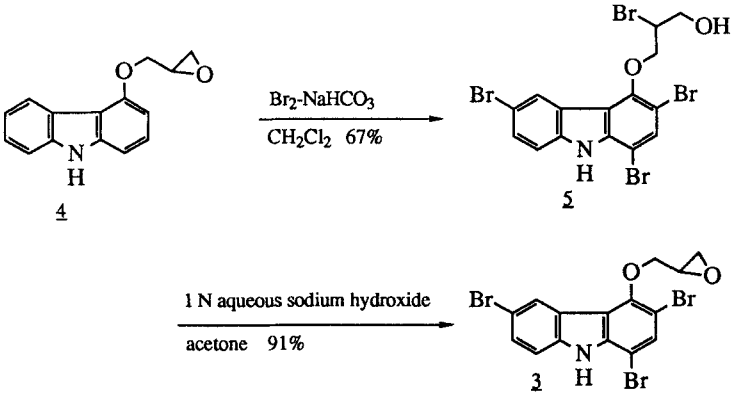
SCHEME 1



the desired tribromo compounds (2a, 2b) without racemization in 51-63% yield after semi-preparative HPLC purification. Catalytic tritium-halogen exchange over 10% Pd/C catalyst gave the desired tritiated products after HPLC purification. Optical purity was greater than 99%, as determined by chiral HPLC assay.

The synthesis of the three racemic metabolites involved elaboration of a common intermediate to the desired tribrominated precursors. Synthesis of the common intermediate, 1,3,6-tribromo-4-(2,3-epoxypropyloxy)-9H-carbazole 3, is shown in Scheme 2. Bromination of epoxy carbazole 4 under

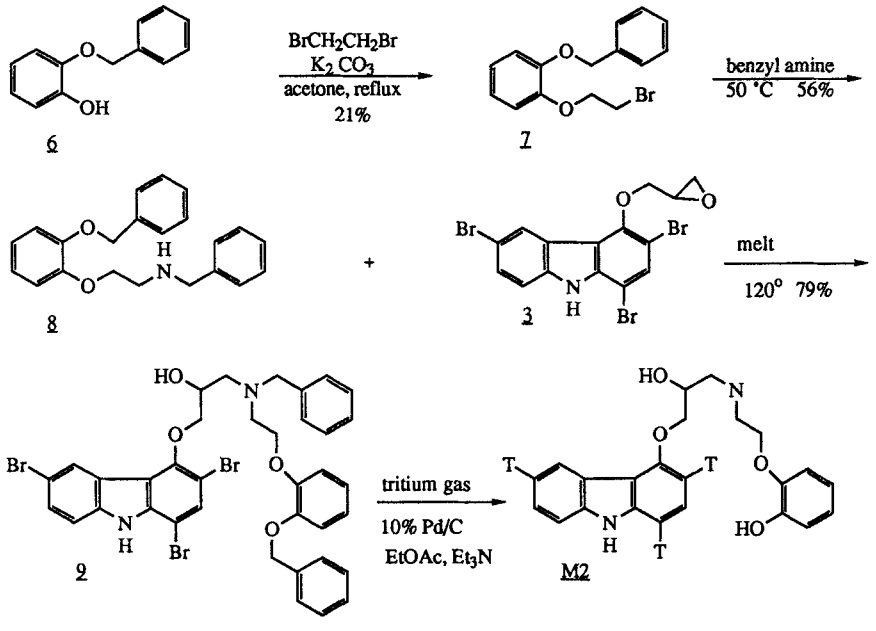
SCHEME 2



basic conditions gave tribromocarbazole bromohydrin 5 (67%), presumably by concurrent electrophilic bromination and nucleophilic epoxide opening. Closure of the bromohydrin to the desired epoxide 3 (91%) was effected by treatment.with base.

Elaboration of tribromocarbazole epoxide 3 to metabolite M2 is shown in Scheme 3. Commercially available monobenzylated catechol 6 was

SCHEME 3



alkylated with dibromoethane in the presence of base to give bromoethoxy benzene 7 in 21% yield after chromatographic purification. Reaction of 7 with neat benzylamine at 50° gave N-[2-[2-(phenylmethoxy)phenoxy]-ethyl] benzenemethanamine 8 in 56% yield after purification. Nucleophilic epoxide opening of 3 with 8 as a melt gave 3-[(1,3,6,-tribromo-9H-carbazol-4-yl)oxy]-1-[[2-[2-(phenylmethoxy)phenoxy]ethyl](phenylmethyl) amino]-2-propanol 9 in 79% yield after preparative HPLC. Catalytic tritium-halogen exchange with concomitant debenzylation of 9 over 10% Pd/C catalyst gave the desired metabolite M2.

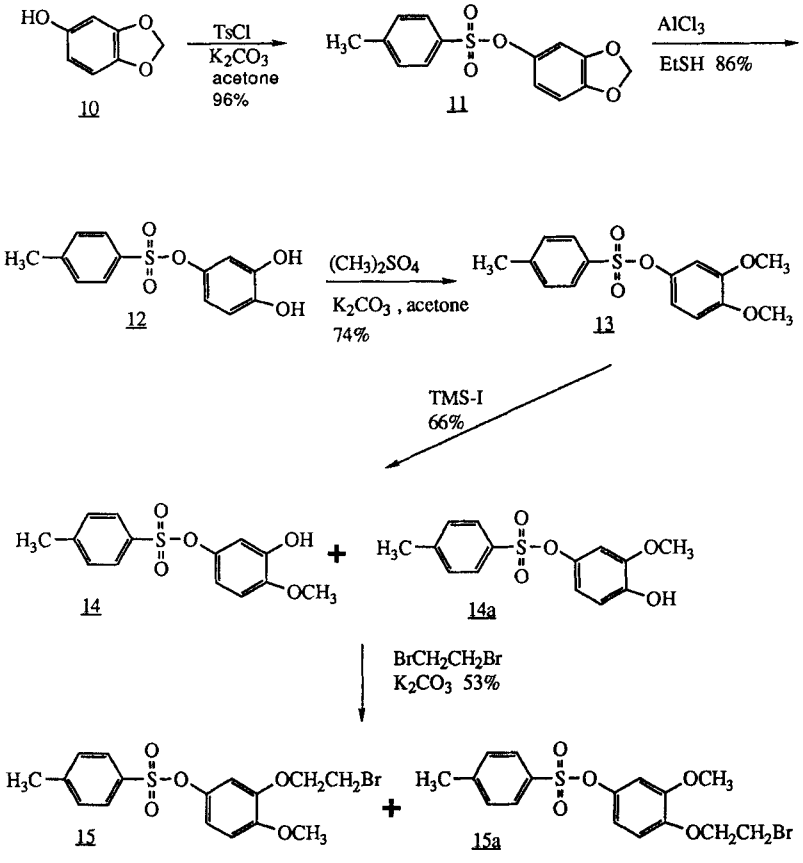
The tribromocarbazole epoxide 3 was elaborated into regioisomeric metabolites M4 and M5 as shown in Scheme 4. Preparation of these metabolites required the synthesis of regioisomeric monomethoxy phenols 14 and 14a. Although metabolites M4 and M5 are separable by chromatography, it was our intention to prepare and isolate 14 and 14a, which would then be elaborated into metabolites M4 and M5, respectively.

Tosylation of commercially available sesamol 10 afforded the tosyloxy derivative 11 in 96% yield. Dealkylation of 11 with aluminum trichloride[2] gave the corresponding tosyloxy catechol 12 in 89% yield. However,

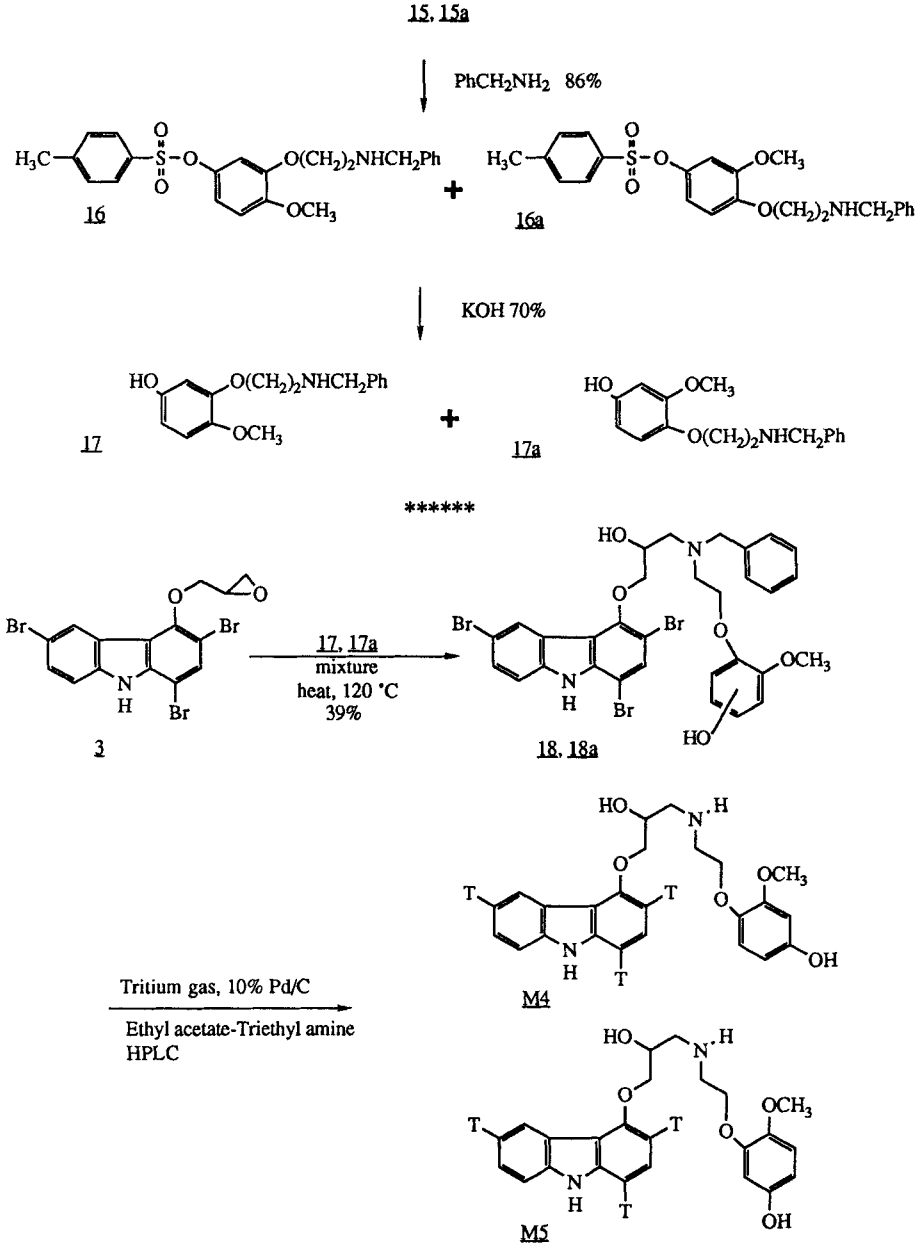
selective monomethylation of catechol 12 with either one equivalent of methyl iodide/potassium carbonate or dimethyl sulfate failed. Reaction with methyl iodide gave only a 20% yield of a mixture of monomethylated products along with a trace amount of dimethylated product. Reaction with dimethyl sulfate gave a 50% yield of the dimethyl compound 13, with only an 8% yield of monomethylated products being obtained. In each case the remainder of the reaction mixture was unreacted starting material.

The failure of the selective methylation route necessitated a permethylation (dimethyl sulfate, 79% yield)-nonselective demethylation (trimethylsilyl iodide,[3] 66% yield) sequence which gave a 63:33:1 ratio (by ^{1}H-NMR) of regioisomeric monomethoxytosyloxy phenols 14, 14a and starting material. A pure sample of the slightly less polar phenol 14a could be obtained by flash chromatography and recrystallization. However, a pure sample of 14 could not be obtained by a similar procedure. Therefore, we opted to carry the mixture of regioisomers through to the final metabolites and separate the desired products by HPLC. Thus, alkylation of the mixture of 14 and 14a with dibromoethane and base gave a 62:38 mixture of the regioisomeric bromides 15 and 15a in 53% yield. Reaction of this mixture with neat benzylamine at elevated temperature gave a 60:40 regioisomeric

SCHEME 4



SCHEME 4, CONTINUED



mixture of the corresponding secondary amines (16 and 16a) in 86% yield. Detosylation of 16 and 16a with aqueous potassium hydroxide gave a 55:45 mixture of the phenols 17 and 17a in 70% yield after chromatography.

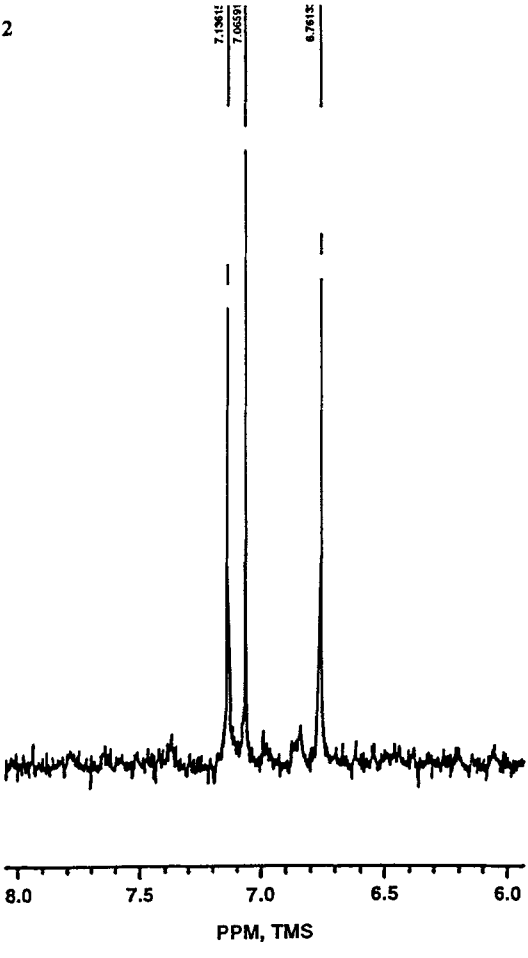
Subsequent reaction of 17 and 17a with the tribromocarbazole epoxide 3 as a melt gave a 55:45 mixture of tertiary amines 1-(1,3,6-tribromo-9H-carbazol-4-yloxy)-3-[[2-(2-methoxy-4(5)-hydroxyphenoxy)ethyl](phenylmethyl)amino]-2-propanol (18 and 18a) in 39% yield after preparative HPLC.

Catalytic tritiodehalogenation with concomitant debenzylation of 18, 18a over 10% Pd/C catalyst gave the desired metabolites M4 and M5 after HPLC separation and purification.

CONCLUSION

The specific activities and radiochemical purities of the tritium labeled Carvedilol enantiomers and three racemic metabolites are shown in the Table. Proton decoupled tritium NMR spectra (400 MHz, methanol d_4) of the labeled Carvedilol enantiomers and metabolite M2 gave spectra exhibiting three singlets of equal intensity in the aromatic region. This is consistent with tritons at carbons 1, 3 and 6 on the carbazole nucleus.[4] A representative spectrum (tritiated (S)-Carvedilol) is shown is Figure 2.

FIGURE 2



In summary, a high yield high specific activity synthesis of the tritiated enantiomers of Carvedilol has been accomplished. The conditions developed for these tritiations have been applied to the synthesis of three tritiated metabolites. The precursors for the tritiated metabolites were synthesized by a concise, convergent approach.

TABLE

Compound	Radiochemical Purity[1]	Specific Activity[2]
R-Carvedilol-[^{3}H]	99.5%	35.2 Ci/mmol
S-Carvedilol-[^{3}H]	99.4%	61.0 Ci/mmol
Metabolite M2-[^{3}H]	97.8%	48.2 Ci/mmol
Metabolite M4-[^{3}H]	99.5%	76.4 Ci/mmol[3]
Metabolite M5-[^{3}H]	98.2%	61.7 Ci/mmol[3]

1. Determined by HPLC-radiodetection
2. Determined by CI-MS
3. Determined by LSC

EXPERIMENTAL SECTION

General

All organic reagents were obtained from Aldrich and were used without further purification. Molecular bromine and inorganic salts were obtained from Aldrich or Baker. All reactions (with the exception of melts and tritiations) were run under an inert atmosphere. Silica Gel for flash chromatography was obtained from Baker. Aldrich glassware was used for flash chromatographic separations. HPLC conditions are given in the individual preparations. Tritium gas was obtained from DuPont-NEN. Proton and triton nuclear magnetic resonance spectra were obtained on a Bruker WM-400 instrument. The solvents are indicated in the individual preparations. Chemical shifts (δ) are reported downfield from tetramethylsilane.

Precursor Preparation

A. Precursors of Tritiated Carvedilol Enantiomers

R(+)-1-(1,3,6-Tribromo-9H-carbazol-4-yloxy)-3-[2-(2-methoxy-phenoxy)ethyl]amino-2-propanol 2a: A solution of R(+)-Carvedilol 1a (20 mg, 0.050 mmol) in chloroform (5 mL) was treated with sodium carbonate (28 mg, 0.2 mmol) at room temperature. To the suspension was added dropwise at room temperature bromine (28 mg, 0.16 mmol) in chloroform (1 mL). The mixture was stirred at room temperature for 18 hr. To the mixture was added water (10 mL) and sodium thiosulfate (50 mg). The mixture was stirred for 15 min. The organic phase was separated and reserved. The aqueous phase was extracted with ethyl acetate (2 x 10 mL). The organic extracts were combined with the reserved organic phase, and dried over magnesium sulfate. The drying agent was removed by filtration, and the solvent evaporated under reduced pressure to give a crystalline solid (19.7 mg, 62%). The crystalline solid was dissolved in mobile phase (50:45:5:0.1 v/v/v/v methylene chloride:hexane:

methanol:diethyl amine, 1 mL) and methanol (0.1 mL). The solution was subjected to preparative HPLC (2 x 500 µL injections, two Dynamax^{TM} Silica Gel columns in series, 21.4 mm x 25 cm, 8 micron particle size, flow rate 20 mL/min, UV at 254 nm). The desired fractions were collected, and solvent was removed at reduced pressure by rotary evaporation to give a crystalline solid (10 mg, 51% recovery). Analytical data were obtained on racemic material prepared and purified using an identical procedure.

400 MHz ^{1}H-NMR Spectrum (methanol d4,TMS)
3.05 (1H, dd, J=3 Hz, 8 Hz; sidechain 3H), 3.15 (2H, m; sidechain 1'H), 3.20 (1H, dd, J=3 Hz, 8 Hz; sidechain 3H), 3.78 (3H, s; OCH3), 4.25 (4H, m; sidechain 1H, 2'H), 4.35 (1H, m; sidechain 2H), 6.80-7.15 (4H, o-subst arom.; catechol-H), 7.43 (1H, d, J=7 Hz; carbazole 8H), 7.55 (1H, d, J=7 Hz; carbazole 7H), 7.70 (1H, s; carbazole 2H), 8.60 (1H, s; carbazole 5H).

Mass Spectrum (DCI, methane), m/e (%base))
648 (3), 647 (8), 646 (9), 645 (19), 644 (9), 643 (20), 642 (4), 641[9, (M^++H)], 424 (6), 423 (11), 422 (16), 421 (24), 420 (18), 419 (23), 418 (8), 417 [8, (M^++H-$C_{12}H_{18}O_3N$)], 222 (53), 180 [100, ($C_{10}H_{14}O_2N$)], 124 (50), 74 (22).

S(-)-1-(1,3,6-Tribromo-9H-carbazol-4-yloxy)-3-[2-(2-methoxy-phenoxy)ethylamino-2-propanol 2b: A procedure identical to the one described for the bromination of the corresponding R(+) isomer was used. The yield after HPLC purification was 63% (11 mg). Analytical data were obtained on racemic material prepared and purified using an identical procedure, and are presented in the previous experimental description.

B. Precursors of Tritiated Metabolites M2, M4, and M5

1-(2-Bromoethoxy)-2-(phenylmethoxy)benzene 7: 2-(Benzyloxy)phenol 6 (2.00 g, 10.0 mmol) was dissolved in 20 mL of acetone. 1,2-Dibromoethane (5.62 g, 30 mmol) was added along with 1.52 g (11 mmol) of potassium carbonate. The mixture was heated at reflux for 16 hours. The mixture was filtered, and the solvent was removed in vacuo. The crude product was purified by flash chromatography (Silica Gel, 1:1 v/v methylene chloride:hexane) to give 0.65 g (21%) of the desired product as a colorless oil. The oil crystallized upon cooling to -30° (mp 45-47°).

400 MHz ^{1}H-NMR Spectrum (chloroform-d, TMS): 3.65 (2H, t, J=6.7 Hz; H1), 4.35 (2H, t, J=6.7 Hz; H2), 5.14 (2H, s; H3, H4), 6.92-6.97 (4H, m; H3, H4, H5, H6), 7.31-7.46 (5H, m; H8, H9, H10, H11, H12).

N-[2-[2-(Phenylmethoxy)phenoxy]ethyl]benzenemethanamine 8: Bromoethoxybenzene 7 (0.4 g, 1.3 mmol) was dissolved in benzylamine (0.42 g, 3.9 mmol). The solution was warmed to 50° for 5 hours, allowed to cool to room temperature, and partitioned between 10% aqueous sodium hydroxide solution and chloroform. The organic extract was dried over magnesium sulfate, filtered, and solvent was removed in vacuo. The resulting oil was

purified by flash chromatography (Silica Gel, 96:4 v/v methylene chloride:methanol). The yield was 0.24 g (56%) as a pale yellow oil. The oil crystallized at -60° (mp 40-41°).

400 MHz ^{1}H-NMR Spectrum (chloroform-d, TMS): 2.02 (1H, s; NH), 2.95 (2H, t, J=5.2 Hz; H1), 3.76 (2H, s; H11), 4.09 (2H, t, J=5.2 Hz; H2), 5.02 (2H, s; H7), 6.80-6.88 (4H, m, H3-6), 7.15-7.35 (10H, m; H8-10, H12-14)

Calcd. for $C_{22}H_{23}NO_2$: C, 79.25; H, 6.95; N, 4.20
Found: C, 78.65; H, 6.99; N, 4.23

2-Bromo-3-[(1,3,6-tribromo-9H-carbazol-4-yl)oxy]propanol 5: A methylene chloride solution (100 mL) of 4-oxiranylmethoxy-9H-carbazole 4 (2.4 g, 10 mmol) was treated with 8.4 g (100 mmol) of sodium bicarbonate. The mixture was cooled to 0° C, and a solution of bromine (4.8 g, 30 mmol) in methylene chloride (80 mL) was added over 15 minutes. Upon completion of the addition, the cooling bath was removed and the reaction was allowed to warm to room temperature over 15 minutes. The mixture was poured into ice-water, and saturated aqueous sodium bisulfite was added (1 mL). Upon decolorization, the organic layer was separated, washed with brine, dried over magnesium sulfate, filtered, and evaporated in vacuo to a crystalline solid. The material was recrystallized from benzene to give 3.74 g (67%) of polybrominated product, mp 132-35°.

400 MHz ^{1}H-NMR Spectrum (chloroform-d, TMS): 3.78 (1H, q, J= 16.2, 5.7 Hz; H12), 3.84 (1H, q, J=16.2, 5.7 Hz; H12'), 4.30 (2H, d, J=4.6 Hz; H 10, 10'), 4.39 (1H, m; H11), 7.38 (1H, D, J=7.8 Hz; H8), 7.58 (1H, dd, J=7.8, 1.8 Hz; H7), 7.73 (1H, s; H2), 8.35 (1H, s; NH), 8.46 (1H, d, J=1.8 Hz; H5)

Mass Spectrum (DCI, ammonia), m/e (%base)) 493 (10), 495 (12), 571 (20, 573 (71), 575 (100), 577 (69), 579 (19)

Calcd. for $C_{15}H_{11}Br_4NO_2$: C, 32.35; H, 1.99; Br, 57.39; N, 2.52
Found: C, 32.47; H, 1.81; Br, 57.96; N, 2.40

1,3,6-Tribromo-4-(2,3-epoxypropyloxy)-9H-carbazole 3: To a solution of tetrabromide 5 (2.8 g, 5 mmol) in 65 mL of acetone was added 65 mL of water. To the resulting suspension was added at room temperature 1N aqueous sodium hydroxide (5 mL). The mixture was stirred at room temperature for 1 hour, and then poured into ice-water. The mixture was extracted with ethyl acetate, and the combined organic extracts were washed with brine and dried over magnesium sulfate. Drying agent was removed by filtration, and solvent was evaporated in vacuo to give a white crystalline solid. The material was triturated with 35:1 v/v petroleum ether:benzene to give 2.19 g (91%) of the desired epoxide, mp 178-80°.

400 MHz ^{1}H-NMR Spectrum (chloroform-d, TMS): 2.81 (1H, dd, J gem=7.4 Hz, J cis=2.6 Hz; H12), 2.97 (1H, t, J gem=8.2 Hz, J trans=4.6 Hz; H12'), 3.56 (1H, m; H11), 4.15 (1H, dd, J gem=15.6 Hz, J trans=6.3 Hz; H10), 4.48 (1H, dd,

J gem=15.6 Hz, J cis=3.1 Hz; H10'), 7.37 (1H, d, J=7.7 H; H8), 7.58 (1H, dd, J=6.8Hz, 2.0 Hz; H7), 7.73 (1H, s; H2), 8.32 (1H, S; NH), 8.48 (1H, d, J=2.0 Hz; H5)

Mass Spectrum (DCI, ammonia), m/e (%base)): 393(5), 472(33) 476(38), 552(20), 556(100)

Calcd. for $C_{15}H_{10}Br_3NO_2$: C, 37.85; H, 2.12; Br, 50.37; N, 2.94
Found C, 38.06; H, 2.02; Br, 50.74, N, 2.67

3-[(1,3,6,-Tribromo-9H-carbazol-4-yl)oxy]-1-[[2-[2-(phenylmethoxy)-phenoxy]ethyl](phenylmethyl)amino]-2-propanol 9: To a 75.8 mg (0.16 mmol) portion of epoxide 3 in a 1 mL conical vial was added 182 mg (0.55 mmol) of N-[2-[2-(phenylmethoxy)phenoxy]ethyl]benzenemethanamine 8. The mixture was heated at 120° for 1 hr, and then allowed to cool to room temperature. The melt was dissolved in ethyl acetate (1 mL), and the product was isolated by preparative HPLC (Rainin Dynamax^{TM} Si, 8 micron, 41.4 mm I.D. x 25 cm, 70:30 v/v hexane: ethyl acetate, 40 mL/min, UV detection at 254 nm). The eluate was collected and concentrated in vacuo to a white crystalline solid. The yield was 103 mg (79%), mp 44-46°.

400 MHz ^{1}H-NMR Spectrum (chloroform-d, TMS): 2.98-3.12 (4H, m; H 12,12',13, 13'), 3.80 (1H, d, J=13.6 Hz; H21), 3.97 (2, J=13.6 Hz; H21'), 4.05 (1H, dd, J=5.5, 5.6Hz; H10), 4.11 (1H, m; H10'), 4.13 (2H, m; H14), 4.25 (1H, m; H11), 5.06 (2H, s; OCH2Ph), 6.87 (4H, s; H17, 18, 19, 20), 7.14-7.28 (5H, m; C6H5-CH2O), 7.32-7.34 (5H, m; H23, 24, 25, 26, 27), 7.36 (1H, D, J=7.2 Hz; H8), 7.54 (1H, dd, J=6.8, 1.9 Hz; H7), 7.69 (1H, s; H2), 8.29, (1H, s; NH), 8.61 (1H, s; H5)

Mass Spectrum (DCI, ammonia), m/e (%base)) 108(30), 218(88), 244(30), 334(64), 390(100), 607(4), 807(13, M^++H)

Calcd. for $C_{37}H_{33}Br_3N_2O_4$: C, 54.91; H, 4.11; Br, 29.62; N, 3.46
Found: C, 54.96; H, 4.09; Br, 29.32; N, 3.24

(3,4-Methylenedioxy)phenyl-4-methylbenzenesulfonate 11: 3,4-Methylenedioxyphenol 10 (2.00g, 14.5 mmol) was dissolved in 15 mL of acetone. To this solution was added p-toluenesulfonyl chloride (2.76 g, 14.5 mmol) and powdered potassium carbonate (2.00 g 14.5 mmol). The mixture was heated at reflux for 16 hours. The solution was filtered through 10 g of Silica Gel, and the filtrate was concentrated in vacuo to give 4.07 g (96%) of tosylate 11 as a tan crystalline solid. The material was used without further purification.

400 MHz ^{1}H-NMR Spectrum (chloroform-d, TMS): 2.45 (3H, s; $ArCH_3$), 5.96 (2H, s; OCH2), 6.38 (1H, dd, J=8.4, 2.4 Hz; H6), 6.52 (1H, d, J=2.4 Hz; H2), 6.63 (1H, d, J=8.4 Hz, H5), 7.32 (2H, d, J-8.4 Hz; ArH ortho to methyl), 7.71 (2H, d, J=8.4 Hz; ArH ortho to sulfonyl)

Mass Spectrum (DCI, ammonia), m/e (%base)) 310 (100, M+ NH_4)

Calcd. for $C_{14}H_{12}O_5S$: C, 57.53; H, 4.14
Found: C, 57.44; H, 4.02

(3,4-Dihydroxy)phenyl-4-methylbenzenesulfonate 12: To a stirred solution of aluminum chloride (4.0 g) in ethanethiol (15 mL) was added a solution of tosylate 11 in 15 mL of ethanethiol. The mixture was stirred at 0° for 45 minutes, and then poured into water. The mixture was acidified to pH=2 with 3N aqueous hydrochloric acid and extracted with diethyl ether. The organic extracts were washed with brine, dried over sodium sulfate for 30 minutes, filtered, and concentrated in vacuo to an oil. Addition of hexane (20 mL) gave a white precipitate. The solid was collected by filtration, washed with cold hexane and dried in vacuo. The yield was 2.49 g (89%). The material was used without further purification.

400 MHz ^{1}H-NMR Spectrum (chloroform-d,TMS): 2.45 (3H, s; $ArCH_3$), 5.32 (1H, s; OH meta to sulfonyl), 5.52 (1H, s; OH para to sulfonyl), 6.37 (1H, dd, J=8.6, 2.6 Hz; H6), 6.60 (1H, d, J=2.6 Hz; H2), 6.71 (1H, d, J=8.6 Hz; H5), 7.31 (2H, d, J=8.2 Hz; ArH ortho to methyl), 7.70 (2H, d, J=8.2 Hz; ArH ortho to sulfonyl).

Calcd. for $C_{13}H_{12}O_5S$: C, 55.71; H, 4.32
Found: C, 55.79; H, 4.17

(3,4-Dimethoxy)phenyl-4-methylbenzenesulfonate 13: A solution of tosylate-diol 12 (1.25 g, 4.5 mmol) in acetone (7.5 mL) was treated with dimethyl sulfate (1.23 g, 9.8 mmol) and potassium carbonate (2.70 g, 9.8 mmol). The mixture was stirred at room temperature for 5 hours. The mixture was poured onto a bed of Silica Gel (30 g), and the product was eluted with methylene chloride. The eluate was concentrated in vacuo to a residue, and the material was purified by flash chromatography (Silica Gel, methylene chloride) to give a clear oil. The yield was 1.02 g (74%).

400 MHz ^{1}HNMR Spectrum (chloroform-d, TMS): 2.45 (3H, s; $ArCH_3$), 3.73 (3H, s; OCH_3, meta to sulfonyl), 3.83 (3H, s, OCH3 para to sulfonyl), 6.48 (1H, dd, J=8.6, 2.6 Hz; H6) 6.51 (1H, d, J=2.6 Hz; H2), 6.70 (1H, d, J=8.6 Hz; H5), 7.31 (2H, d, J=8.2 Hz; ArH ortho to methyl), 7.71 (2H, d, J=8.2 Hz, ArH ortho to sulfonyl).

(4-Hydroxy-3-methoxy)phenyl-4-methylbenzenesulfonate and (3-Hydroxy-4-methoxy)phenyl-4-methylbenzenesulfonate 14, 14a: (3,4-Dimethoxy)phenyl-4-methylbenzensulfonate 13 (1.02 g, 3.31 mmol) was dissolved in methylene chloride (10 mL). To the solution was added trimethylsilyl iodide (4.23 mL, 5.96 g, 29.77 mmol). The solution was heated at reflux for 3 hours. The reaction was allowed to cool to room temperature, quenched with methanol, and concentrated in vacuo to a residue. The crude product was purified by flash chromatography (Silica Gel, methylene chloride). A product fraction was isolated (395 mg) containing a 63:33:1 mixture of (4-hydroxy-3-methoxy)phenyl-4-methylbenzenesulfonate:(3-hydroxy-4-methoxy)-phenyl-4-methylbenzenesulfonate:(3,4-Dimethoxy)phenyl-4-methylbenzen-sulfonate, as determined by NMR analysis. This mixture was carried to the next step without further purification.

4-(2-Bromoethyl)oxy-3-methoxyphenyl-4-methylbenzenesulfonate and 3-(2-Bromoethyl)oxy-4-methoxyphenyl-4-methylbenzenesulfonate 15, 15a: The mixture 14, 14a (395 mg, 1.34 mmol) was dissolved in acetone (5 mL). Dibromoethane (1.14 g, 6.05 mmol) was added along with potassium carbonate (833 mg, 6.1 mmol). The reaction was heated at reflux for 40 hours. The mixture was filtered, and the filtrate was concentrated in vacuo to a tan oil. The material was purified by flash chromatography (Silica Gel, methylene chloride) to give 287 mg (53%) of a 62:38 mixture of 4-(2-bromoethyl)oxy-3-methoxyphenyl-4-methylbenzenesulfonate:3-(2-bromoethyl)-oxy-4-methoxyphenyl-4-methylbenzenesulfonate, as determined by NMR analysis. The material was carried to the next step without further purification.

4-[2-[(Phenylmethyl)amino]ethyl]oxy-3-methoxyphenyl-4- methylbenzenesulfonate and 3-[2-[(Phenylmethyl)amino]ethyl]oxy-4-methoxyphenyl-4-methylbenzenesulfonate 16, 16a: The mixture 15, 15a (246 mg, 0.613 mmol) was dissolved in 335 uL of benzylamine (332 mg, 3.10 mmol). The mixture was stirred at 55° for 16 hours. The mixture was diluted with 25 mL of methylene chloride and extracted with 10 mL of 5% aqueous sodium bicarbonate. The organic layer was concentrated in vacuo. The residue was purified by flash chromatography (Silica Gel, 9:1 v/v methylene chloride:methanol to give 226 mg (86%) of a 60:40 mixture of 4-[2-[(phenylmethyl)amino]ethyl]oxy-3-methoxyphenyl- 4-methylbenzenesulfonate:3-[2-[(phenylmethyl)amino]ethyl]oxy-4-methoxyphenyl-4-methylbenzenesulfonate, as determined by NMR. The material was used in the next step without further purification.

4-[2-[(Phenylmethyl)amino]ethyl]oxy-3-methoxyphenol and 3-[2-[(Phenylmethyl)amino]ethyl]oxy-4-methoxyphenol 17, 17a: The mixture 16, 16a (170 mg, 0.4 mmol) was dissolved in 1 ml of ethanol. To this solution was added 7.5 mL of 0.53 M potassium hydroxide in 9:1 v/v ethanol water. The mixture was stirred for 1 hour at 95°. The mixture was adjusted to pH=8 by careful addition of 3N aqueous hydrochloric acid, and the resulting solution was extracted with chloroform. The combined organic layers were dried over magnesium sulfate for 20 minutes, filtered, and the filtrate was concentrated in vacuo. The resulting oil was purified by flash chromatography (Silica Gel, 9:1 v/v methylene chloride:methanol to provide 75.6 mg (70%) of a 55:45 mixture of 4-[2-[(phenylmethyl)amino]ethyl]oxy-3-methoxyphenol:3-[2-[(phenylmethyl)amino]ethyl]oxy-4-methoxyphenol, as determined by NMR. The mixture was carried to the next step without further purification.

1-(1,3,6,-Tribromo-9H-carbazole-4-yloxy)-3-[[2-(2-methoxy-4(5)-hydroxyphenoxy)ethyl](phenylmethyl)amino]-2-propanol 18, 18a: To a 26 mg portion of epoxide 3 (54.4 umol) in a 1 mL screwcap vial was added 59.4 mg (217.6 μmol) of mixture 17, 17a. The mixture was heated at 120° for 40 minutes. The melt was allowed to cool to room temperature, dissolved in 0.5 mL of ethyl acetate, and the product isolated by preparative HPLC (Rainin Dynamax™ Si 8 micron, 21.4 x 250 mm, 60:40 v/v hexane:ethyl acetate, 20 mL/min, UV detection at 300 nm). The yield was 16 mg (39%) of a 55:45 mixture of 18, 18a.

400 MHz ^{1}H-NMR Spectrum (chloroform-d,TMS): 3.03 (3H, m; H12,13,13'), 3.12 (1H, m; H12'), 3.78 (3H, s; OCH3), 4.04 (2H, m; H21), 4.14 (2H, m; H14), 4.22 (2H, m; H10), 4.29 (1H, m; H11), 6.26 (1H, dd, J=5.8, 2.8 Hz; H18'), 6.33 (1H, dd, J=5.8, 2.8 Hz; H19), 6.38, (1H, d, J=2.8 Hz; H17), 6.41 (1H, d, J=2.8 Hz; H20'), 6.68 (1H, d, J=4.0 Hz; H17'), 6.70 (1H, d, J=4.1 Hz; H20), 7.29-7.36 (5H, m; H23-27), 7.39 (1H, d, J=7.2 Hz; H8), 7.55 1H, dd, J=8.6, 2.2 Hz; H7), 7.70 (1H, s; H2), 8.33 (1H, s; NH), 8.63 (1H, s; H5)

Mass Spectrum (DCI, ammonia), m/e (%base)) 274 (100), 288 (35), 330 (31), 364 (39), 410 (54), 412 (50), 747 (0.9,M+H), 749 (2.4, M^++H)

Calcd. for $C_{31}H_{29}Br_3N_2O_5$:	C, 49.69; H, 3.90; N, 3.74
Found:	C, 50.54; H, 4.20; N, 3.22

Tritiations

The experimental description for the synthesis of R(+)-Carvedilol-[^{3}H] is representative of all tritiations described in this paper. Only pertinent data are given for the other tritiations.

R(+)-1-(9H-Carbazol-4-yloxy-1,3-6-t$_3$)-3-[2-(2- methoxyphenoxy)]-ethylamino-2-propanol, SK&F R(+)-105517-[^{3}H] (R(+)-Carvedilol-[^{3}H]): A solution of tribromocarbazole 2a (4.0 mg, 0.0063 mmol) in DMF (1.4 mL) and triethylamine (100 µL) in a roundbottom flask (2.5 mL, O-ring joint) was treated with 10% Pd/C catalyst (Engelhard Lot 17659, 4 mg). The mixture was attached to the steel tritiation manifold and frozen at liquid nitrogen temperature. The mixture was subjected to three freeze-pump-thaw cycles. Tritium gas (5.78 Ci, 0.098 mmol, 59 Ci/mmol) was introduced into the reaction flask. The flask was isolated from the manifold, and the mixture was allowed to warm to room temperature. The mixture was stirred rapidly for 66 hr at room temperature, and then frozen at liquid nitrogen temperature. Gaseous tritium was removed. An uptake of 0.034 mmol tritium gas was noted. The mixture was allowed to warm to room temperature under helium, and the flask was removed from the manifold. The mixture was filtered through a nylon syringe filter and the filter was washed with 5 mL methanol. To the filtrate was added methanol (20 mL). Solvents and labile tritium were removed by static vacuum transfer. To the residue was added methanol (20 mL), and static vacuum transfer was repeated. A final exchange-static vacuum transfer with ethanol (3 mL) gave a residue. The residue was dissolved in ethanol (20 mL). A radioactivity assay showed that 226 mCi of tritium were present. Solvent was removed by rotary evaporation at reduced pressure, and the crude material was dissolved in mobile phase (60:40:0.1 v/v/v water:acetonitrile:TFA, 1 mL). The crude material was partially purified by semi-preparative HPLC (10 x 100 µL injections, Beckman C-18 semipreparative column, 10 mm x 25 cm, 7 micron particle size, 3 mL/min, UV detection at 254 nm). The desired fractions were collected, and mobile phase was removed by rotary evaporation at reduced pressure until a volume of 15 mL remained. To the solution was added ethanol (20 mL) to give a volume of 35 mL. Total activity was 145 mCi (4.14 µCi/µL). The partially purified material was stored at -78°. HPLC assay showed a

radiochemical purity of 80%. A portion of the partially purified material (20 mCi) was reduced to dryness by rotary evaporation at reduced pressure and dissolved in mobile phase (300 μL, 60:40:0.1 v/v/v water:acetonitrile:TFA)-methanol (100 μL). This material was purified by HPLC (Beckman C-18 analytical column, 4.6 x 250 mm, 5 micron particle size, 2 x 200 μL injections, 1 mL/min). The heart of the desired peak was collected by discarding the leading 10% and trailing 10% of the peak height. Collection was followed by rotary evaporation of mobile phase at reduced pressure to a volume of 1 mL. Ethanol (19 mL) was added to the solution to give a volume of 20 mL. Total activity was 12.8 mCi. The material was stored at liquid nitrogen temperature.

S(-)-1-(9H-Carbazol-4-yloxy-1,3-6-t3)-3-[2-(2- methoxyphenoxy) ethyl]amino-2-propanol, SK&F S(-)-105517-[^{3}H] (S-(-)-Carvedilol-[^{3}H])

Substrate: 1b
Reaction Solvent: DMF-triethylamine
Starting Activity: 6.4 Ci
Tritium Uptake: 0.058 mmol
Crude yield after removal of exchangeables: 411 mCi
Yield after final purification of 20 mCi batch: 13.2 mCi

1-(9H-Carbazol-4-yloxy-1,3-6-t3)-3-[2-(2-hydroxyphenoxy) ethyl]amino-2-propanol, SK&F 105652-[^{3}H] (M2-[^{3}H])

Substrate: 9
Reaction Solvent: Ethyl acetate-triethylamine
Starting Activity: 8.0 Ci
Tritium Uptake: 0.085 mmol
Crude yield after removal of exchangeables: 523 mCi
Yield after final purification of 20 mCi batch: 11.8 mCi

1-(9H-Carbazol-4-yloxy-1,3-6-t3)-3-[2-(2-methoxy-4-hydroxy-phenoxy)ethyl]amino-2-propanol, SB 203231-[^{3}H] (M4-[^{3}H]) and 1-(9H-Carbazol-4-yloxy-1,3-6-t3)-3-[2-(2-methoxy-5-hydroxy-phenoxy)ethyl]amino-2-propanol, SB 203232-[^{3}H], (M5-[^{3}H])

Substrate: 18, 18a
Reaction solvent: Ethyl acetate-triethylamine
Starting Activity: 7.6 Ci
Reaction time: 23 hours
Tritium uptake: 0.06 mmol
Crude yield after removal of exchangeables: 533 mCi
HPLC Purification Method: Zorbax™ RX C-8, 5 micron, 9.4 x 250 mm, 60:40 v/v 0.04 M aqueous ammonium acetate (pH=4):methanol, 7.5 mL/min, UV detection at 254 nm
Yield after final purification: M4-[^{3}H], 6.2 mCi; M5-[^{3}H], 9.9 mCi

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REFERENCES

1. a. Anonymous, Drugs. Fut. 16: 949 (1991)
   b. Nichols, A.J., Gellai, M., and Ruffolo, R. R., Fundam. Clin. Pharmacol. 5: 25, (1991)

2. Node, M, Nishide, K., Sai, K., Ichikawa, K., Fuji, K., and Fujita, E., Chem Lett. 97 (1979).

3. Jung, M. E.and Lyster, M. A., J. Org. Chem. 42: 3761 (1977).

4. Black, P.J. and Heffernan, D.E., Aust. J. Chem. 18: 353 (1965).