Carbostyril Derivatives Having Potent β-Adrenergic Agonist Properties

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Derivatives carrying a substituent in the para position of the phenyl group of 8-hydroxy-5-[2-[(1-phenyl-2-methylprop-2-yl)amino]-1-hydroxyethyl]carbostyril (**10**) were prepared and their effects on β-adrenoceptors evaluated in vitro. Unsubstituted compound **10**, iodo **11**, amino **12**, and bromoacetamido **13** derivatives (all racemic) bound to the receptor with 15–100-fold lower dissociation constants than that of (−)-isoproterenol. All the above compounds stimulated adenylate cyclase more potently than (−)-isoproterenol. The instrinsic activities of compounds **10** and **12** were equal to that of (−)-isoproterenol. The intrinsic activities of compounds **11** and **13** were 1.3 and 1.2 times that of (−)-isoproterenol, respectively. Treatment of membrane preparations with bromoacetamido derivative **13** resulted in an irreversible loss of binding sites, and thus, **13** seems to be an alkylating affinity label for β-adrenoceptors.

It is well established that there are major differences between the interaction of agonists and antagonists with the β-adrenoceptor. For example, agonist binding shows several affinity states[1] and is modulated by guanine nucleotides[2], magnesium[3], and *N*-ethylmaleimide.[4] In contrast, antagonist binding usually shows only a single affinity state and is not affected by these modulators. To date, most agonist–receptor interaction studies have been performed indirectly by competing unlabeled agonists for labeled antagonists. Although some studies have used labeled agonists, these compounds are generally unstable, have low specific activities, and have large nonspecific binding components.[5–7] In addition, over the past few years, several irreversible antagonists for the β-adrenoceptor have been developed.[8–10] However, because of the differences between agonist and antagonist interaction with the receptor, there is a need for better agonist receptor probes. Recently, an alkylating catecholamine was shown to have some novel receptor interactions.[11] This compound was only a weak partial agonist and, like catecholamines in general, was unstable. We therefore initiated a program to develop some potent and stable β-adrenergic agonists with the potential of being radiolabeled for direct agonist–receptor studies or irreversibly binding to the receptor. The great biopotency of catecholamine congeners from the class of carbostyrils,[12–14] indole-2-carboxylic acid,[15] and catecholamines carrying amido groups[16] gave indications that such compounds could be obtained. Eventually we selected carbostyril congeners, since the chemistry of compounds of this class has received some additional recent attention.[17,18] The substituents on the amino group selected are the alkyl–aromatic part of an anorexic phentermine, 1-phenyl-2-amino-2-methylpropane, which has been proven effective in catecholamine derivatives[19] and β-blockers.[20]

Results

Chemistry. The method of synthesis of the carbostyril derivatives (Scheme I) was based on Goodman's approach to the synthesis of catecholamine derivatives.[11,16] Commercial 8-hydroxyquinoline was converted by aqueous formaldehyde–hydrogen chloride to the hydrochloride of 5-(chloromethyl)-8-hydroxyquinoline, which, by treatment with aqueous ammonia, yielded 8-hydroxy-5-(hydroxymethyl)quinoline (**1**). Both of these steps were described previously.[21] After protection of the phenolic group of compound **1** with a benzyl group introduced by benzyl bromide and giving compound **2**, oxidation by 3-chloroperbenzoic acid yielded *N*-oxide **3**, which in turn was further oxidized by pyridinium chlorochromate to the aldehyde **4**. In this step some care is required since the

Scheme I

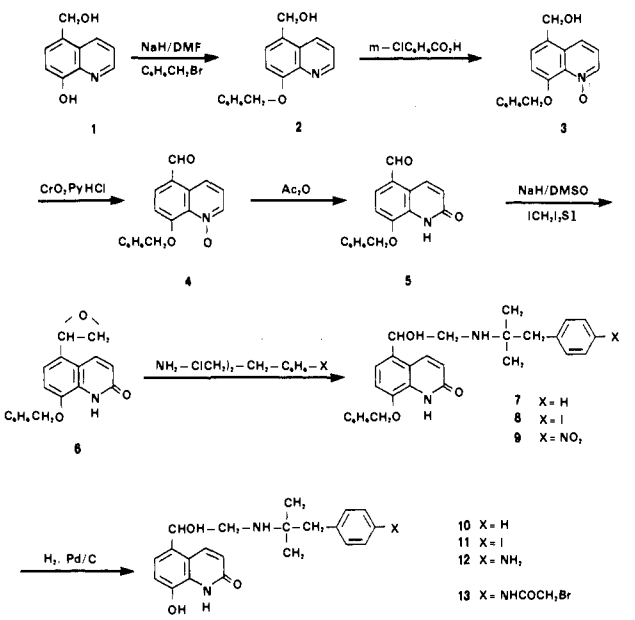

Table I. Ability of (−)-Isoproterenol and Carbostyril Derivatives To Stimulate Adenylate Cyclase Activity and To Inhibit (−)-[^{125}I]Iodocyanopindolol Binding in Rat Reticulocyte Membranes

	adenylate cyclase		
ligand	EC_{50},[a] nM	intrinsic activity[b]	[^{125}I]cyanopindolol binding: IC_{50},[c] nM
(−)-isoproterenol	226 ± 37	1.0	325 ± 17
10	6.9 ± 1.5[d]	1.0	3.1 ± 0.3[d]
11	5.6 ± 1.4[d]	1.3	7.6 ± 1.3[d]
12	14.2 ± 4.7[d]	1.0	22 ± 3[d]
13	8.5 ± 3.5[d]	1.2	5.3 ± 0.8[d]

[a] Concentration that produced half-maximal enzyme activation. [b] As compared to (−)-isoproterenol which was set to 1.0. [c] Concentration that inhibited [^{125}I]cyanopindolol binding by 50%. [d] Significantly different from the (−)-isoproterenol group ($P < 0.001$) by ANOVA. All data are the mean ± SE, n = 3–6.

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loss of *N*-oxide can occur, leading to 8-(benzyloxy)-quinoline-5-carboxaldehyde. Polonovski's reaction of *N*-oxide **4** with acetic anhydride yielded carbostyril **5**. The aldehydic function of this compound (**5**) was converted by dimethylsulfonium methylide to oxirane **6**, which, by reaction with 2-amino-2-methyl-1-phenylpropane and its derivatives, yielded amino alcohols **7–9**. Catalytic hydrogenation of compounds **7–9** resulted in debenzylation, yielding compounds **10–12**, with the nitro group in **9** being concurrently reduced. By reaction with bromoacetyl bromide, amine **12** was converted to compound **13**.

Pharmacology. In Table I, the ability of several carbostyril derivatives to activate adenylate cyclase activity is compared to that of (−)-isoproterenol. The four derivatives (**10–13**) were all substantially more potent than (−)-isoproterenol with EC_{50} values ranging from 5.6 to 14.2 nM, whereas the EC_{50} value for (−)-isoproterenol was 226 nM. Furthermore, compounds **10** and **12** showed the same intrinsic activity (efficacy) as (−)-isoproterenol. However, compounds **11** and **13** showed intrinsic activities of 1.3 and 1.2, respectively, which were higher than that of (−)-isoproterenol. The initial rate of enzyme activation by 1 μM of all the compounds was blocked by 10 μM of the β-blocker propranolol (data not shown). Table I also shows the ability of the compounds to inhibit [^{125}I]cyanopindolol binding to the β-adrenoceptor. As with the cyclase activation, all of the carbostyril derivatives were substantially more potent (15–104-fold) than (−)-isoproterenol for inhibiting cyanopindolol binding.

Ireversible binding of compound **13** to the reticulocyte β-adrenoceptor was indicated by washout experiments. After membrane pretreatment with 20 nM of compound **13** followed by four wash cycles, there was a 71% decrease in the receptor concentration (control, 576 ± 22; compound **13** treated, 168 ± 15 fmol/mg of protein, mean ± SD, n = 3) with no change in the K_D value for (−)-[^{125}I]iodocyanopindolol binding to the remaining receptors (control, 13 ± 3; compound **13** treated, 11 ± 2 pM). In addition, when nadolol (10 μM), a β-antagonist, was added to the preincubation in the presence of compound **13**, the loss of specific binding sites was completely prevented.

Discussion

Compounds that have pure agonist or antagonist activity on the β-adrenoceptor have no obvious advantage as drugs. On the other hand, pure agonist or antagonist activity is highly desired in the probes used for molecular characterization of β-adrenoceptors. Compounds **11–13** obviously have pure agonist activity on the β-adrenoceptor since they have intrinsic activities equal to or greater than that of (−)-isoproterenol. Furthermore, since compound **11** containing the iodo substituent retained a high potency and intrinsic activity, then synthesis of compound **11** containing radioiodine may yield a ligand for direct agonist–receptor interaction studies. Alternatively, it may be possible to radioiodinate compound **12** by procedures developed for other adrenergic probes.[20] Finally, the present data indicate that compound **13** irreversibly binds to the β-adrenoceptor, as preincubation followed by washing results in a decreased binding maximum with no change in the K_D value for the receptors left. Thus, compound **13** may be a useful alkylating probe for the β-adrenoceptor.

Experimental Section

Melting points were determined on a hot-stage melting point apparatus (Fisher-Johns) and were not corrected. ^{1}H NMR spectra were measured on a JEOL PMX-60SI (60-MHz) instrument; internal tetramethylsilane standard was used. Precoated silica gel sheets (60F-254, 0.2 mm, EM Reagents) were used for thin-layer chromatography. Silica gel (grade 60, 230–400 mesh, Merck Co.) was used for flash chromatography and silica gel H was used for short-column chromatography. Hydrogenations were performed in a low-pressure Parr hydrogenator at room temperature.

5-(Hydroxymethyl)-8-hydroxyquinoline (1). 5-(Chloromethyl-8-hydroxyquinoline was prepared as described and converted into 5-(hydroxymethyl)-8-hydroxyquinoline (**1**):[21] R_f 0.28 (chloroform–methanol, 9:1); NMR (Me_2SO-d_6) δ 8.68 (dd, J_1 = 2 Hz, J_2 = 4 Hz, 1 H, H-2), 8.37 (dd, J_1 = 2 Hz, J_2 = 9 Hz, 1 H, H-4), 7.41 (dd, J_1 = 4 Hz, J_2 = 9 Hz, 1 H, H-3), 7.32 (d, J = 8 Hz, 1 H, H-6), 6.92 (d, J = 8 Hz, 1 H, H-7), 4.78 (s, 2 H, CH_2OH).

8-(Benzyloxy)-5-(hydroxymethyl)quinoline (2). 5-(Hydroxymethyl)-8-hydroxyquinoline (**1**) (6.44 g, 36.75 mmol) was dissolved in dry dimethylformamide (40 mL) and sodium hydride (1.3 g of 80% suspension in mineral oil, corresponding to 1.04 g, 43.3 mmol) was added. The mixture was stirred until hydrogen evolution ceased (~45 min). Benzyl bromide (4.5 mL, 6.47 g, 37.8 mmol) was added and the mixture was stirred for 4 h. The solution was poured into water (350 mL) and stirred for 20 min, and the precipitate was filtered and washed with water. After crystallization from benzene, 5.4 g of product **2** was obtained; mp 192–194 °C. An analytical sample was recrystallized from toluene; R_f 0.68 (chloroform–methanol, 9:1); NMR (Me_2SO-d_6) δ 8.73 (dd, J_1 = 2 Hz, J_2 = 4 Hz, 1 H, H-2), 8.37 (dd, J_1 = 2 Hz, J_2 = 9 Hz, 1 H, H-4), 7.60–7.01 (m, 8 H, benzyl aromatic protons and H-3, H-6, H-7), 5.23 (s, 2 H, $C_6H_5CH_2$), 4.8 (s, 2 H, CH_2OH), 3.27 (s, 1 H, OH, disappears on addition of D_2O). Anal. ($C_{17}H_{15}NO_2$) C, H, N.

8-(Benzyloxy)-5-(hydroxymethyl)quinoline *N*-Oxide (3). 8-(Benzyloxy)-5-(hydroxymethyl)quinoline (**2**) (5.37 g, 20.24 mmol) was suspended in methylene chloride (500 mL). *m*-Chloroperbenzoic acid (7 g of 85% preparation, corresponding to 34 mmol) was added and the mixture was stirred at room temperature. After 10 min the solution turned dark blue and then almost black. After 1.5 h the color began to fade and after 4 h, when no starting material could be detected by TLC, the solution turned light green. The reaction mixture was then extracted with aqueous sodium bicarbonate and the organic phase was separated and evaporated. The residue was stirred wih acetone (150 mL) and after 30 min the product was filtered (3.45 g). The filtrate was evaporated and again treated with acetone (30 mL), giving another crop of crystals (0.5 g). The product **3** has the following: mp 155–161 °C dec; R_f 0.44 (chloroform–methanol, 9:1); NMR (CD_3OD) δ 8.40 (br d, J = 6 Hz, 1 H, H-2), 8.13 (br d, J = 9 Hz, 1 H, H-4), 7.65–7.05

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(m, 8 H, benzyl aromatic protons and H-3, H-6, H-7), 5.23 (s, 2 H, $C_6H_5CH_2$), 4.87 (s, 2 H, CH_2OH). Anal. ($C_{17}H_{15}O_3N$) C, H; N: calcd, 4.98; found, 4.54.

8-(Benzyloxy)quinoline-5-carboxaldehyde *N*-Oxide (4). 8-(Benzyloxy)-5-(hydroxymethyl)quinoline *N*-oxide (**3**) (0.5 g, 1.78 mmol) was added to a solution of pyridinium chlorochromate (0.42 g, 1.95 mmol) in dichloromethane (10 mL) and the solution was stirred for 1 h, when TLC indicated that the reaction was completed. The solution was then washed with water, dried over Na_2SO_4, and evaporated. The residue was passed through a short silica gel column using dichloromethane–methanol mixture (100:3) to elute the product. Evaporation gave a yellow solid 4 (0.27 g, 55%): mp 118–121 °C; R_f 0.64 (chloroform–methanol, 9:1). NMR ($CDCl_3$) δ 9.93 (s, 1 H, CHO), 9.07 (d, J = 9 Hz, 1 H, H-4), 8.35 (d, J = 5 Hz, 1 H, H-2), 7.80 (d, J = 8 Hz, 1 H, H-6), 7.73–7.20 (m, 6 H, benzyl aromatic protons and H-3), 7.07 (d, J = 8 Hz, 1 H, H-7), 5.31 (s, 2 H, $C_6H_5CH_2$). Anal. ($C_{17}H_{15}NO_3$) C, H, N.

8-(Benzyloxy)carbostyril-5-carboxaldehyde (5). 8-(Benzyloxy)-5-(hydroxymethyl)quinoline-5-carboxaldehyde *N*-oxide (**4**) (2.52 g, 9.02 mmol) in acetic anhydride (6 mL) was heated to 50 °C for 2.5 h. The solution was cooled, diluted with methanol (25 mL), and poured into diluted ammonia (1:10, 100 mL). After 5 min of stirring, the mixture was extracted with methylene chloride (2 × 75 mL), the extracts were dried with Na_2SO_4 and evaporated, and the residue was separated on silica gel column (40 g), eluting with chloroform–methanol (99:1). The product (**5**) was obtained as yellow cyrstals (1.98 g): mp 147–149 °C; R_f 0.9 (chloroform–methanol, 9:1), 0.43 (chloroform–methanol, 19:1); NMR ($CDCl_3$) δ 9.93 (s, 1 H, CHO), 9.40 (br s, 1 H, NH, disappears on addition of D_2O), 9.02 (d, J = 10 Hz, 1 H, H-4), 7.50 (d, J = 8 Hz, 1 H, H-6), 7.35 (s, 5 H, benzyl aromatic protons), 7.07 (d, J = 8 Hz, 1 H, H-7), 6.70 (d, J = 10 Hz, 1 H, H-3), 5.23 (s, 2 H, $C_6H_5CH_2$). Anal. ($C_{17}H_{13}NO_3$) C, H, N.

8-(Benzyloxy)-5-oxiranylcarbostyril (6). Sodium hydride (0.14 g of 80% suspension in mineral oil, corresponding to 0.112 g, 4.65 mmol of hydride) was placed in a flask equipped with a magnetic stirrer, a pressure-equalizing dropping funnel, and a rubber septum with a gas-escape port. Rigorously dry reaction conditions were maintained. Argon was introduced via a hypodermic needle placed in the septum and dry dimethyl sulfoxide (6 mL) was introduced through another needle. With stirring, the flask was immersed into a 70 °C bath for 1 h. Then the solution was cooled, tetrahydrofuran (19 mL) was added, and the flask was immersed in an ice bath. A solution of trimethylsulfonium iodide (0.955 g, 4.68 mmol) in dimethyl sulfoxide (8 mL) was added dropwise during a 10-min period and the reaction was allowed to proceed for 15 min. Next a solution of 8-(benzyloxy)carbostyril-5-carboxaldehyde (**5**) (0.585 g, 2.09 mmol) in dimethyl sulfoxide–tetrahydrofuran (1:1, 14 mL) was added dropwise and the brown solution was stirred for 15 min. Then the contents of the flask were poured into water (150 mL) and extracted with dichloromethane (3 × 50 mL). The combined extracts were washed with aqueous NaCl, dried (Na_2SO_4), and evaporated. The residue was recrystallized from benzene–isooctane (2:3), giving shiny yellowish crystals of **6** (0.49 g): mp 114–116 °C; R_f 0.38 (chloroform–ethyl acetate, 5:3); NMR ($CDCl_3$) δ 9.10 (br s, 1 H, NH, disappears on addition of D_2O), 7.98 (d, J = 10 Hz, 1 H, H-4), 7.32 (s, 5 H, benzyl aromatic protons), 6.93 (s, 2 H, H-6, H-7), 6.63 (d, J = 10 Hz, 1 H, H-3), 5.12 (s, 2 H, $C_6H_5CH_2$), 4.15 (dd, J_1 = 2 Hz, J_2 = 4 Hz, 1 H, oxirane CH), 3.17 (dd, J_1 = 4 Hz, J_2 = 6 Hz, 1 H, oxirane CH_2), 2.75 (dd, J_1 = 6 Hz, J_2 = 2 Hz, 1 H, oxirane CH_2). Anal ($C_{18}H_{15}NO_3$) C, H, N.

8-(Benzyloxy)-5-[2-[(1-phenyl-2-methylprop-2-yl)-amino]-1-hydroxyethyl]carbostyril (7). The oxirane **6** (40 mg, 0.136 mmol) and 2-amino-2-methyl-1-phenylpropane (free base of commercial phentermine) (100 mg, 0.67 mmol) were heated under reflux in 2-propanol (2 mL) for 24 h. The solvent was evaporated and the residue was dissolved in chloroform (0.5 mL) and chromatographed on a silica gel column eluted with chloroform–methanol–concentrated ammonia (96:4:0.1). The product **7**, still slightly contaminated with the starting amine, was obtained as a yellow foam (40 mg): R_f 0.92 (chloroform–methanol–concentrated ammonia, 4:1:0.05); NMR ($CDCl_3$) δ 8.04 (d, J = 9 Hz, 1 H, H-4), 7.54–7.06 (m, 11 H, phenyl aromatic protons, H-6), 7.00 (d, J = 8 Hz, 1 H, H-7), 6.64 (d, J = 9 Hz, 1 H, H-3), 5.21 (s, 2 H, $C_6H_5CH_2O$), 5.24–4.97 (m, 1 H, CHOH), 4.04 (br, 3 H, OH + NH, disappears on addition of D_2O), 3.0 (m, 2 H, CH_2NH), 2.80 (s, 2 H, $C_6H_5CH_2C$), 1.28 (s, 6 H, CH_3).

8-Hydroxy-5-[2-[(1-phenyl-2-methylprop-2-yl)amino]-1-hydroxyethyl]carbostyril (10). Crude benzyloxy derivative **7** from the previous reaction (40 mg, 0.09 mmol) was dissolved in methanol (2.5 mL) and ammonium formate (30 mg, 0.47 mmol) was added. After 5 min of stirring, palladium on carbon (10%, 20 mg) was added. The mixture was stirred for 15 min at room temperature and then 1 h at 50 °C at which time TLC indicated completion. Product **10**: R_f 0.37, chloroform–methanol–concentrated ammonia, 4:1:0.05; identical with the compound prepared according to Yoshizaki et al.;[13] isolated by silica gel column chromatography.

2-Amino-2-methyl-1-(4-nitrophenyl)propane Sulfate. A slight modification in the procedure described previously[20] enables direct isolation of the product instead of column chromatographic separation of ortho (20%) and para (80%) isomers, both of which are products of the nitration. 2-Amino-2-methyl-1-phenylpropane hydrochloride (97 g, 0.41 mol) was dissolved in concentrated H_2SO_4 (195 mL) and the solution was cooled in an ice bath. Nitric acid (70%, 40 mL, 56 g, 0.6 mol) was added with stirring while the temperature was kept under 10 °C and then the solution was stirred for an additional 20 min. Next the mixture was poured on ice (4 L), and after 16 h white crystals were filtered, washed with cold water and methanol, and dried in air. Yield: 71.3 g (56%); mp 293–298 °C dec; R_f 0.24 (dichloromethane–methanol–ammonia, 4:1:0.03).

2-Amino-2-methyl-1-(4-aminophenyl)propane. 2-Amino-2-methyl-1-(4-nitrophenyl)propane sulfate (6.07 g, 25 mmol) was suspended in ethanol (50 mL) and 6% sodium hydroxide in ethanol (33 mL, 50 mmol) was added with stirring. Hydrazine (2.5 g, 78 mmol) was added, followed by Raney nickel (0.2 mL of 50% slurry). After 1 min gas evolution began, and the mixture turned yellow and was warmed to about 40 °C. After 20 min gas evolution had almost ceased. Fresh catalyst was added and the mixture was boiled with stirring for 5 min to decompose excess hydrazine. After cooling, the suspension was filtered, the catalyst was washed with ethanol, and the filtrates were evaporated. The residue was extracted with diethyl ether (3 × 40 mL), and the extracts were dried (K_2CO_3) and evaporated, leaving a yellow oil, which solidified (3.87 g); mp 82–84 °C.

2-Amino-2-methyl-1-(4-iodophenyl)propane. 2-Amino-2-methyl-1-(4-aminophenyl)propane (0.72 g, 4.33 mmol) was stirred with 5% H_2SO_4 (35 mL) and cooled to 0 °C. Sodium nitrite (0.38 g, 5.5 mmol) was added to the fine suspension of sulfate in water (1 mL) while the temperature was held under 5 °C. The mixture was stirred for 30 min. A clear solution formed after about 10 min. Sodium iodide (7 g, 46.7 mmol) in water (5 mL) was added and the mixture was stirred for 30 min. A dark brown solution was made alkaline with Na_2CO_3 and extracted with ether (3 × 25 mL). The combined extracts were washed with 10% sodium thiosulfate, dried (Na_2SO_4), and evaporated. A yellow oil was separated on a silica gel column using chloroform–methanol (96:4). The product was obtained as a yellow oil (0.41 g); R_f 0.36 (chloroform–methanol, 9:1).

5-[2-[[1-(4-Iodophenyl)-2-methylprop-2-yl]amino]-1-hydroxyethyl]-8-hydroxycarbostyril (11). Oxirane **6** (0.2 g, 0.68 mmol) and 2-amino-2-methyl-1-(4-iodophenyl)propane (0.21 g, 0.763 mmol) in 1-butanol (4 mL) were heated under reflux for 24 h. The solvent was evaporated under reduced pressure and the remaining clear oil was separated on a silica gel column (20 g) using chloroform–methanol (97:3) as an eluent. Compound **8** was obtained in the form of a cream-colored foam (0.132 g, 35%); R_f 0.27 (chloroform–methanol, 9:1).

Compound **8** (0.033 g, 0.058 mmol) was dissolved in methanol (2 mL), palladium on carbon (5%, 0.05 g) was added, and the mixture was hydrogenated at 50 psi for 5 h. Then the mixture was filtered and washed with methanol (15 mL), and the filtrates were acidified with methanolic HCl (1%, 0.5 mL) and evaporated, yielding **11** as a yellow amorphous solid (0.023 g, 76%): TLC R_f 0.62 (chloroform–methanol–concentrated ammonia, 4:1:0.03); NMR (CD_3OD) δ 8.77 (d, J = 9 Hz, H-4), 7.65–7.0 (m, 6 H, iodophenyl moiety and H-6, H-7), 6.90 (d, J = 9 Hz, 1 H, H-3), 5.60 (m, 1 H, CHOH), 3.33–3.20 (m, 2 H, CH_2NH), 3.10 (s, 2 H, $CH_2C(CH_3)_2$), 1.40 (s, 6 H, CH_3). Anal. ($C_{21}H_{23}IN_2O_3 \cdot HCl$) C, N; H: calcd, 4.70; found, 5.57.

8-(Benzyloxy)-5-[2-[[1-(4-nitrophenyl)-2-methylprop-2-yl]amino]-1-hydroxyethyl]carbostyril (9). The oxirane **6** (275 mg, 0.94 mmol) and 2-amino-2-methyl-1-(4-nitrophenyl)propane (0.3 mL, 1.6 mmol) were heated in 1-butanol (3 mL) under reflux for 20 h. The solvent was evaporated and the residue was dissolved in dichloromethane (30 mL) and washed with hydrochloric acid (1%, 20 mL). Then the residue was washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica gel column (10 g) using chloroform–methanol (97:3) as an eluent, giving 245 mg of product **9** in the form of a yellow foam: R_f 0.92 (chloroform–methanol–ammonia, 4:1:0.03); NMR ($CDCl_3$) δ 7.97 (two superimposed doublets, J_1 = 8 Hz, J_2 = 10 Hz, 3 H, H-4 of carbostyril and two protons in ortho to NO_2 group), 7.40–7.00 (m, 9 H, remaining aromatic protons and NH), 6.90 (d, J = 8 Hz, 1 H, H-7), 6.51 (d, J = 10 Hz, 1 H, H-3), 5.10 (s, 2 H, $C_6H_5CH_2$), 5.2–4.7 (m, 1 H, CHOH), 2.90 (m, 2 H, CH_2NH), 2.75 (s, 2 H, phentermine moiety CH_2), 1.08 (s, 6 H, CH_3). Anal. ($C_{28}H_{29}N_3O_5 \cdot 0.5H_2O$) C, H, N.

5-[2-[[3-[4-(Bromoacetamido)phenyl]-2-methylprop-2-yl]amino]-1-hydroxyethyl]-8-hydroxycarbostyril (13). The nitro derivative **9** (0.18 g) was dissolved in methanol (30 mL), palladium on carbon (5%, 100 mg) was added, and the mixture was hydrogenated (25 psi). The reaction was monitored by TLC (chloroform–methanol–ammonia, 4:1:0.04). The starting material (R_f 0.9) disappeared after about 35 min, giving rise to two other compounds of R_f 0.6 (presumably a hydroxy nitro derivative) and R_f 0.17 (the desired hydroxy amino derivative). When hydrogenolysis was carried out longer, a byproduct of high R_f (0.95) started to form; after 2 h it amounted to about 20%. Optimal hydrogenolysis time was 75 min, but reaction preferably should be monitored. The catalyst was filtered off, the product was washed with methanol, and the solutions were evaporated. The residue was dissolved in chloroform–methanol (3:1) and chromatographed on a silica gel column (chloroform–methanol, 7:2). Fractions containing the product were pooled, evaporated, dissolved in methanol (20 mL), and centrifuged to remove colloidal silica gel from chromatography. After evaporation and coevaporation with 2-propanol, the product **12** was obtained as a light yellow powder. Yield: 33 mg of pure product and 35 mg of slightly contaminated product; R_f 0.11 (chloroform–methanol–concentrated ammonia, 4:1:0.03). When the above reduction was attempted with the ammonium formate procedure, the formation of several products occurred.

Free amine **12** (obtained by hydrogenation of 83 mg of compound **9** and used without purification, 0.17 mmol) was dissolved in dimethylformamide (2 mL). The solution was cooled in an ice bath and bromoacetyl bromide (39 mg, 0.19 mmol) was added. After 1.5 h the mixture was added dropwise to diethyl ether (100 mL) with vigorous stirring. After 5 min the precipitate was filtered and washed with ether. On exposure to air the solid quickly liquified to a viscous oil, which was again dissolved in methanol and evaporated. The residue (93 mg) was dissolved in methanol (0.2 mL), methylene chloride (0.8 mL) was added, and the resulting suspension was applied to a silica gel column (10 g). It was eluted with dichloromethane–methanol (9:1, 100 mL) and then with a 5:1 mixture. Fractions of 40 mL were collected; the product was contained in the 9th and 10th fractions. After evaporation and trituration with isooctane, these fractions gave a light yellow solid (45 mg; 46% yield): R_f 0.29 (chloroform–methanol–concentrated ammonia, 4:1:0.03); NMR ($CDCl_3$–CD_3OD, 2:1) δ 8.37 (d, J = 10 Hz, 1 H, H-4), 7.67–6.95 (m, 6 H, aromatic protons, C_6H_4 and carbostyril), 6.65 (d, J = 10 Hz, 1 H, H-3), 5.70–5.40 (m, 1 H, CHOH), 3.93 (s, 2 H, CH_2Br), 3.33–2.80 (m, 4 H, CH_2NH and $-CH_2C(CH_3)_2-$), 1.33 (s, 6 H, CH_3). Anal. ($C_{23}H_{26}BrN_3O_4 \cdot HBr \cdot 2.5H_2O$) H, N; C: calcd, 44.96; found, 44.52.

Biochemical Methods. Adenylate Cyclase Activity. Rat reticulocytes were induced and membranes prepared as described previously.[11] Enzyme activity was determined by incubation in a total volume of 0.1 mL containing 50 mM Tris·HCl buffer at pH 7.4, membrane protein (0.01–0.05 mg), 1.6 mM ATP, 5 mM $MgCl_2$, 1 mM EGTA, 10 mM theophylline, 0.01% bovine serum albumin, 10 units of creatine phosphokinase, and 2.5 mM phosphocreatine for 10 min at 32 °C. These are the basal conditions. When stimulation of activity was measured, the assay also contained 0.5 mM GTP and varying concentrations of (–)-isoproterenol or the carbostyril derivatives. At the end of the incubation, 0.3 mL of 10 mM Tris·HCl buffer at pH 7.0 containing 5 mM EDTA was added to each tube and the tubes were placed in a boiling water bath for 5 min. After cooling to room temperature, the tubes were centrifuged for 5 min at 10000*g* and the supernatant saved. The cAMP content of the supernatant was determined by a competitive protein binding assay as previously reported.[11] The amount of cAMP present was calculated from a standard curve determined with unlabeled cAMP. Enzyme activity was linear with time and protein through 10 min and 0.1 mg, respectively. Basal and GTP-stimulated activity values were subtracted from the values obtained in the presence of ligands and the concentration of ligands that produced half-maximal activated (EC_{50}) were determined by using a dose–effect analysis with an Apple IIe computer. Membrane protein content was determined by the Lowry method[22] with bovine serum albumin as standard.

β-Adrenoceptor Binding. The ability of (–)-isoproterenol and the carbostyril derivatives to interact with the β-adrenoceptor was determined by competition assays. Reticulocyte membrane protein (0.003–0.008 mg) was incubated in a total volume of 0.25 mL containing 50 mM Tris·HCl buffer at pH 7.4, 30 pM of (–)-[^{125}I]iodocyanopindolol, without (for controls) and with varying concentrations of ligands, 0.1 mM 5′-guanylyl imidodiphosphate (Gpp(NH)p) and without and with 0.001 mM alprenolol for 60 min at 36 °C. At the end of the incubation, the suspensions were diluted with 4 mL of ice-cold incubation buffer and poured onto Whatman GF/B glass fiber filters under reduced pressure. Each filter was washed with a further 8 mL of ice-cold buffer, placed in a scintillation vial, and counted. Specific binding to the receptor was calculated as the difference between total binding determined in the absence of alprenolol and the nonspecific binding determined in the presence of alprenolol. Specific binding of [^{125}I]-iodocyanopindolol was about 95% of the total bound and was saturable with an apparent dissociation constant of 10 pM. The concentration of ligands that inhibited [^{125}I]idodcyanopindolol binding by 50% (IC_{50}) was determined from Hill plots. All data was statistically analyzed by using a one-way analysis of variance (ANOVA).

In the pretreatment experiments, reticulocyte membrane protein (3 mg/mL) was incubated in 50 mM Tris·HCl buffer at pH 7.4, containing 5 mM $MgCl_2$ without and with 20 nM compound **13** for 30 min at 32 °C. A parallel incubation was also performed containing 20 nM compound **13** plus 10 μM nadolol. At the end of the incubation the tubes were filled (30 mL) with ice-cold incubation buffer and centrifuged at 48000*g* for 15 min. The pellets were washed three more times by centrifugation and resuspension and the final pellets were resuspended in 1 mL of incubation buffer for assay. The membrane content of β-adrenoceptors was determined with (–)-[^{125}I]iodocyanopindolol (10–100 pM) as described above. The binding maximums and dissociation constants (K_D) were determined from linear regression analysis of Scatchard plots.

Source of Materials. (–)-[^{125}I]iodocyanopindolol (2200 Ci/mmol) and [^{3}H]cAMP (31 Ci/mmol) were purchased from Amersham Radiochemical Center (Arlington Heights, IL) and New England Nuclear (Boston, MA), respectively. ATP, GTP, Gpp(NH)p, theophylline, alprenolol, creatine phosphokinase, phosphocreatine, and (–)-isoproterenol bitartrate were obtained from Sigma Chemical Co. (St. Louis, MO).

Registry No. **1**, 4053-44-5; **2**, 108835-25-2; **3**, 108835-26-3; **4**, 108835-27-4; **5**, 66546-38-1; **6**, 108835-28-5; **7**, 108835-29-6; **8**, 108835-30-9; **9**, 108835-31-0; **10**, 108835-32-1; **11**·HCl, 108835-33-2; **12**, 108835-34-3; **13**, 108835-35-4; **13**·HBr, 108835-37-6; 5-(chloromethyl)-8-hydroxyquinoline, 10136-57-9; 2-amino-2-methyl-1-phenylpropane, 122-09-8; 2-amino-2-methyl-1-(4-nitrophenyl)-propane sulfate, 108835-36-5; 2-amino-2-methyl-1-(-nitrophenyl)propane, 82408-64-8; 2-amino-2-methyl-1-phenylpropane hydrochloride, 1197-21-3; 2-amino-2-methyl-1-(4-aminophenyl)-propane, 51131-55-6; 2-amino-2-methyl-1-(4-iodophenyl)propane, 96684-29-6.

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