Affinity Labels for β-Adrenoceptors: Preparation and Properties of Alkylating β-Blockers Derived from Indole

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New alkylating ligands derived from indole with high affinity for β-adrenoceptors were synthesized and their properties examined. N^8-(Bromoacetyl)-N^1-[3-(4-indolyloxy)-2-hydroxypropyl]-(*Z*)-1,8-diamino-*p*-menthane (**8**) and its N^1,N^8 isomer (**9**) were prepared by the reaction of bromoacetyl bromide with a product of the condensation of 4-indolyl glycidyl ether with (*Z*)-1,8-diamino-*p*-menthane. A similar reaction employing 2-cyano-4-indolyl glycidyl ether yielded the respective cyano derivatives **10** and **11**. Apparent affinities (K_i, M) for β-adrenoceptors on membrane preparations from rat heart and lung were 4.6×10^{-10} and 1.34×10^{-9} for **8**, 2.3×10^{-8} and 4.5×10^{-9} for **9**, 6.1×10^{-10} and 1.49×10^{-9} for **10**, and 1.83×10^{-9} and 2.78×10^{-9} for **11**, respectively. When membranes were preincubated with the above ligands (1×10^{-8} M, 30 min, 30 °C) and then washed extensively, reduction in the concentration of specific binding sites of [^{3}H]dihydroalprenolol ranged from 7% to 76% and there was no change in K_D of the remaining binding sites. (±)-Alprenolol and (−)-isoproterenol, but not (+)-isoproterenol, when included with the alkylating ligands in the preincubation mixtures, prevented the reduction in concentration of [^{3}H]dihydroalprenolol binding sites. Compounds **8**–**11** alone did not stimulate adenylate cyclase activity in rat heart homogenates. However, these compounds inhibited (−)-isoproterenol-stimulated adenylate cyclase activity with K_i values ranging between 5×10^{-9} and 60×10^{-9} M. These results suggest that high-affinity irreversible β-adrenergic antagonists were obtained that may be useful for in vivo studies of β-adrenoceptors.

Affinity labeling of β-adrenoceptors has been extensively used in their biochemical characterization and in studying their physiological function. For the former studies photoreactive labels are preferred, whereas the latter studies have to be performed with less selective chemoreactive labels. Existing chemoreactive labels contain a pharmacophore of an established β-blocker and a reactive bromoacetyl group joined together by a variety of linkers. Ethylenediamine was used as a linker, but the claims of specific alkylation achieved by that probe (NBE-propranolol) and also by the probe carrying a bromoacetamido group directly on the pharmacophore (Ro 03-7894) were refuted.[1-3] On the other hand, ligands containing 1,8-diamino-*p*-menthane and its aromatic analogue 4-(2-aminoisobutyl)aniline as linkers reproducibly alkylated β-adrenoceptors in good yields.[1,4-6] Recently, two alkylating ligands based on 1,2-diamino-2-methylpropane have been described;[7,8] this linker is quite similar to the above-mentioned ethylenediamine. In this paper we describe the use of 1,8-diamino-*p*-menthane in conjunction with the pharmacophores of pindolol and cyanopindolol. The aim was to combine the proven receptor alkylating potency of the *p*-menthane linker with the high affinity for β-adrenoceptors and low lipophilicity of pindolol and cyanopindolol.

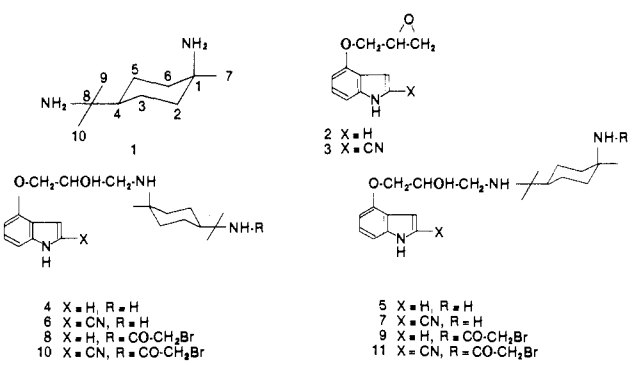
Results

Chemistry. Reaction of (*Z*)-1,8-diamino-*p*-menthane (**1**) with epoxides **2** or **3** yielded mixtures from which the desired monosubstituted products were isolated; these obviously were mixtures of isomers (pair **4** and **5** and pair **6** and **7**), but no suitable separation system could be found for them. Consequently these mixtures were allowed to react with bromoacetyl bromide to yield monobromoacetyl derivatives, which were easily separated into compounds **8**–**11**.

Table I. Apparent Affinity of Alkylating β-Blockers at β-Adrenoceptors from Rat Tissues (K_i, nM)[a]

compound	tissue: heart	tissue: lung
8	0.46 ± 0.21[b]	1.34 ± 0.52
9	22.6 ± 1.5	4.46 ± 0.56
10	0.61 ± 0.13	1.49 ± 0.56
11	1.83 ± 0.29	2.78 ± 0.45
BAAM(Z-1)[c,e]	31.2	19.8
BAAM(Z-8)[d,e]	391.0	126.0

[a] IC_{50} concentrations were estimated by computer-fitted logistic curves and apparent inhibition dissociation constants were calculated by using the equation of Cheng and Prusoff;[14] as daily controls measurements of specific and nonspecific binding of dihydroalprenolol to the membrane preparations were used. [b] Standard error calculated from three independent experiments. [c] N^8-(Bromoacetyl)-N^1-[3-[(*o*-allylphenyl)oxy]-2-hydroxypropyl]-(*Z*)-1,8-diamino-*p*-menthane. [d] N^1-(Bromoacetyl)-N^8-[3-[(*o*-allylphenyl)oxy]-2-hydroxypropyl](*Z*)-1,8-diamino-*p*-menthane. [e] Data from ref 4.



The structures of compounds **8**–**11** were suggested on the basis of electron-impact mass spectra. In structures **9** and **11** the fragmentation between carbons 4 and 8 of the *p*-menthane residue is favored by the proximity of the nitrogen atom with the lone electron pair providing resonance stabilization and by the high substitution of carbons 4 and 8. Such fragmentation results in a loss of C_9H_{15}-BrNO from the respective molecules. In structures **8** and

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Table II. Binding Parameters[a] of [^{3}H]Dihydroalprenolol to Membrane Preparations Pretreated[b] with Alkylating β-Blockers

	heart			lung		
alkylating β-blocker	B_{max}, fmol/mg of protein	K_D, nM	% blockade	B_{max}, fmol/mg of protein	K_D, nM	% blockade
none (control)	43.2 ± 1.2	2.7 ± 0.34		418 ± 28	1.2 ± 0.11	
8	23.3 ± 3.3	2.4 ± 0.22	47[c]	214 ± 8	1.6 ± 0.06	49
9	34.1 ± 5.1	2.0 ± 0.05	21	329 ± 21	1.40 ± 0.01	21
10	11.0 ± 0.53	1.6 ± 0.09	74[d]	99.2 ± 14	1.0 ± 0.15	76
11	40.2 ± 2.8	1.6 ± 0.25	7	232 ± 25	1.2 ± 0.27	45

[a] Values given are the averages of three to five indepedent experiments, ±1 standard error of the mean. [b] Preincubation for 30 min, 30 °C; all derivatives were tested at 1×10^{-8} M. [c] At 1×10^{-7} M, this compound blocked approximately 70% of the [^{3}H]dihydroalprenolol binidng sites on membrane preparations from rat reticulocytes ($n = 2$). [d] At 1×10^{-7} M, this compound blocked 89% of the [^{3}H]dihydroalprenolol binding sites on membrane preparations from rat reticulocytes ($n = 2$). At 1×10^{-6} M, this compound blocked 95% of the sites ($n = 2$).

10, where the nitrogen is amidic, the resonance stabilization should be lower; ion C_5H_9BrNO is formed in this case. The second analytically useful fragmentation occurs across the cyclohexane ring, i.e., simultaneously across the carbon 2–carbon 3 and carbon 5–carbon 6 bonds; fragments $C_8H_{14}BrNO$ or $C_6H_{10}BrNO$ may be eliminated from **8**/**10** or **9**/**11**, respectively. These and related ions were observed in the mass spectra (Experimental Section) and we concluded consequently that isomers that have a higher R_f have structures **8** and **10**, respectively, and isomers of lower R_f values have structures **9** and **11**, respectively.

Pharmacology. Compounds **8** and **10** inhibited the specific binding of [^{3}H]dihydroalprenolol to rat heart or lung β-adrenoceptors with high affinity; the K_i values were in the nanomolar range (Table I). Isomeric compounds **9** and **11** at these same receptor sites had affinities that were 4–50 times weaker. Compounds **8**–**11** appeared to be irreversible (Table II). After preincubation with compounds **8**–**11**, followed by extensive washing of membranes and assaying for specific [^{3}H]dihydroalprenolol binding, Scatchard analysis of the saturation data indicated a reduction in the concentration of binding sites (B_{max}) without appreciable change in affinities (K_D) of the remaining sites (Table II). The potency for irreversible blockade of β-adrenoceptors decreased in the order **10** > **8** > **11** ≅ **9**.

The effects of compounds **8** and **10** appeared to be at β-adrenergic binding sites, since (±)-alprenolol and to a lesser extent (−)-isoproterenol, but not (+)-isoproterenol, when included in the preincubation with **8** and **10**, prevented the reduction in binding sites (Table III). Furthermore, when brain membranes were preincubated with **8** and **10** at 1×10^{-8} M, a concentration causing about 40–50% reduction in [^{3}H]dihydroalprenolol binding sites, there was no reduction in the concentration of [^{3}H]prazosin or [^{3}H]yohimbine binding sites in this tissue (data not shown).

Compouds **8**–**11** were not agonists at heart β-adrenoceptors. They did not stimulate adenylate cyclase activity above basal levels [10.8 pmol min^{-1} (mg of protein^{-1})] as compared with approximately a 3-fold increase above basal level induced by 1×10^{-6} M (−)-isoproterenol, a full β-adrenergic agonist. All bromoacetylated derivatives were antagonists in this system, inhibiting (−)-isoproterenol-stimulated adenylate cyclase activity. The apparent K_i values (M) for this inhibition were 5.1×10^{-9}, 5.7×10^{-8}, 5.9×10^{-9}, and 1.6×10^{-8} for **8**, **9**, **10**, and **11**, respectively ($n = 2$), which are values 2- to 10-fold larger than the apparent K_i values for inhibition of [^{3}H]dihydroalprenolol binding.

Discussion

Compounds **8**–**11** have some features of useful alkylating β-blockers. The affinity of compounds **8** and **10** toward the β-adrenoceptor is about 2 orders of magnitude better than that of (bromoacetyl)alprenololmenthane, a compound that has been extensively used (ref 4 and references therein). The reaction is irreversible as documented by parallel lines in Scatchard plots. The labeling is covalent as documented by sodium dodecyl sulfate electrophoresis of a homogeneous β-adrenoceptor preparation labeled by an ^{125}I derivative of compound **8** (Dohlman, H.; Caron, M. G., private communication); the results on the labeling of specially pretreated crude membrane preparations suggest the same (Nye, J., private communication). The β-adrenoceptor can be alkylated in yields that are as high as previously obtained with about 50 times higher concentrations of (bromoacetyl)alprenololmenthane and the alkylation may be brought to a virtual completion (manuscript in preparation). Particularly, the above data and results of in vivo experiments (not shown here) suggest that compound **10** may be one of the more potent alkylating β-blockers synthesized. This compound may find extensive applications for in vivo experiments; our preliminary results indicate that even a small dose (0.01 mg/kg, ip, in rats) leads to nearly a 50% loss of β-adrenoceptors in lung, heart, and red blood cells.

Furthermore, compounds **8** and **10** appear to be quite specific for β-adrenoceptors since (±)-alprenolol and (−)-isoproterenol, both β-adrenergic ligands, but not (+)-isoproterenol, prevent the irreversible blockade of receptors by **8** and **10** and since neither compounds **8** nor **10** interact with α_1- or α_2-adrenergic receptor sites in vitro as evidenced by a lack of inhibition of [^{3}H]prazosin and [^{3}H]yohimbine binding. Thus, these compounds may be well-suited for selective inactivation of β-adrenergic regulatory systems, an approach desired in many in vivo studies. Although, for example, pindolol and other indole derivatives are mixed agonist–antagonists on β-adrenoceptors, compounds **8**–**11** are pure antagonists; these compounds even slightly inhibit basal activity in β-adreno-

Table III. Protection by Adrenergic Ligands of [^{3}H]Dihydroalprenolol Binding Sites from Alkylation by Compounds **8** and **10**

	% of control B_{max} values[a]	
compd	heart	lung
8[b]	53.3[c]	54.3[c]
8 + (−)-isoproterenol	82	80.0
8 + (+)-isoproterenol	64	68
8 + (±)-alprenolol	100	95
10	32[d]	41[d]
10 + (−)-isoproterenol	75	97
10 + (+)-isoproterenol	43	59
10 + (±)-alprenolol	99	99

[a] Control B_{max} values for heart, 29.1 ± 1.4 fmol/mg of protein and for lung, 268 ± 30.0 fmol/mg of protein, $n = 5$. [b] Concentrations used were as follows: **8** and **10**, 1×10^{-8} M; (−)-isoproterenol and (+)-isoproterenol, both 1×10^{-5} M; (±)-alprenolol, 1×10^{-6} M. [c] All values are the average of two independent sets of experiments for the series utilizing compound **8**. [d] All values are the average of three independent sets of experiments for the series utilizing compound **10**.

ceptor systems, a phenomenon that is probably due to displacement of endogenous agonists present in rat heart homogenates.

Experimental Section

Chemistry. Commercial reagent grade chemicals were used in the syntheses. Thin-layer chromatography was performed on EM Reagents precoated silica gel sheets (60F-254, 0.2 mm, EM Reagents). Silica gel grade H60, 230–400 mesh (Merck Co.), was used for column chromatography. Nuclear magnetic resonance spectra were measured at 60 MHz on a JEOL PMX-60 spectrometer in deuteriochloroform or deuterioacetone with tetramethylsilane as the standard. Elemental analytical results were within 0.4% of theoretical values except where indicated. Compounds 4–11 were amorphous and occluded solvents tenaciously. Compounds 8–11 had a tendency to decompose, which further complicated their identification by mass spectrometry. Molecular ion peaks were present only in some of the MS (70 eV) spectra of the same samples. Ions, which were obviously formed through the decomposition of dimerized species, were detected in MS (PD, Cf-252) and in MS (FEB, Xe8KeV) spectra and these were again absent in MS (CI, NH_3) spectra of the same samples.

Glycidyl 4-Indolyl Ether (2) and Glycidyl 2-Cyano-4-indolyl Ether (3). Samples of these compounds were obtained from Dr. Berthold (Sandoz, Ltd.); later these compounds were resynthesized. Biopotency of the products prepared from compound 3 is well-appreciated,[9-11] but the preparation of this epoxide has not been described, and since we experienced some difficulties, we include an abbreviated description of the synthesis of compound 3 and a somewhat improved synthesis of compound 2. Commercial 4-hydroxyindole was converted to compound 2 by reflux (2 h) in epichlorohydrin (17 molar excess) with a catalytic amount of 1-methylpiperazine. Compound 2 was purified by chromatography on silica gel with chloroform–methanol (98:2) as eluent, yield 65%, mp 63 °C.

Compound 3 was prepared from commercial 2-methyl-3-nitrophenol by conversion to 4-(benzyloxy)indole-2-carboxylic acid in three steps.[12] Treatment of this acid with $SOCl_2$ in dichloromethane containing a catalytic amount of dimethylformamide for 3 h at room temperature converted it to the acid chloride, which, after dissolution in dichloromethane, was treated with anhydrous NH_3 in diethyl ether at −20 °C and thus converted to an amide, mp 185–187 °C (ethanol–water), in 79% overall yield. The amide was converted to nitrile by treatment with trifluoroacetic acid anhydride in pyridine for 2 h at 0 °C with a yield of 83%; mp 125-128 °C (benzene–hexane). Debenzylation was difficult to achieve; in the final procedure 2-cyano-4-(benzyloxy)indole (248 mg, 1 mmol), ammonium formate (315 mg, 5 mmol), and Pd (5%) on carbon (200 mg) in methanol (20 mL) were stirred for 1 h at room temperature and then refluxed until the starting material was undetectable (TLC, chloroform–methanol, 9:1). Column chromatography on silica gel with chloroform–methanol (95:5) as the eluent was used to purify the product; yield (129 mg) 82%; mp 188–190 °C (benzene–methanol). 2-Cyano-4-hydroxyindole was refluxed in epichlorohydrin (10 molar excess) with a catalytic amount of morpholine until the starting material was consumed (about 2.5 h), yielding, after silica gel chromatography with chloroform as an eluent, compound 3 (175 mg, 75%); mp 152–153 °C. The same reaction, when attempted in the presence of equivalent amounts of alkali, yielded complex mixtures.

***N*-[3-(4-Indolyloxy)-2-hydroxypropyl]-(*Z*)-1,8-diamino-*p*-menthane (4, 5).** *Z* diastereomer 1 was isolated from commercial 1,8-diamino-*p*-menthane (5 g) by flash chromatography;[4] the column (300 g of silica gel) was eluted with ethanol–concentrated NH_4OH (8:2). Upon thin-layer chromatography the *Z* diastereomer had an R_f of 0.60, while the *E* diastereomer had an R_f of 0.39 in the same solvent system as above. Compounds were detected with iodine vapor. Alternatively, *Z* diastereomer 1 was isolated by dissolution of commercial 1,8-diamino-*p*-menthane (23 mL) in a mixture of diethyl ether (30 mL) and *n*-hexane (50 mL) and cooling the solution to about −40 °C. *Z* diastereomer 1 crystallized and was separated by filtration at low temperatures, yielding about 3 g of product. A mixture of (*Z*)-1,8-diamino-*p*-menthane (1; 1.022 g, 6 mmol) and 4-[(2,3-epoxypropyl)oxy]indole (2; 189 mg, 1 mmol) was stirred in an oil bath at 70 °C for 5 h. Excess diamine was removed by distillation [80 °C (2 mmHg)] and the residue was purified by column chromatography with ethanol–NH_4OH (95:5) as the eluent. The main product (mixture of 4 and 5; 234 mg, 65% yield) had an R_f of 0.39 (the same eluent as used for the column) and formed a white foam; mp 55–62 °C. Analysis indicated that the product is the carbonate of 4 and 5. Anal. ($C_{43}H_{68}N_6O_7$) C, H, N. Subsequently, the sample (100 mg) was evaporated with 1% HCl in 70% ethanol (5 mL), and the residue was dried in vacuo, leaving dihydrochloride dihydrate. Anal. ($C_{21}H_{39}Cl_2N_3O_4$) C, H, N. Results of integration of nuclear magnetic resonance signals confirmed that this product has a 1:1 ratio of indole and *p*-menthane moieties. NMR ($CDCl_3$) δ 8.7 (br s, 1 H, indole N-*H*), 7.2–6.3 (m, 5 H, aromatic protons), 4.1 (m, 3 H, C*H*$_2$-O, C*H*OH), 2.8 (m, 2 H, C*H*$_2$NH), 2.1–0.9 (m, 21 H, *p*-menthane protons, -CH$_2$-N*H*-). The side product had an R_f of 0.70 and the above ratio of indole and *p*-menthane moieties was 2:1. For further identification, the desired compounds 4, 5 were derivatized. The amine (0.085 g, 0.24 mmol) and 2,4-dinitrofluorobenzene (0.05 g, 0.27 mmol) were heated under reflux in acetonitrile (10 mL) in the presence of sodium bicarbonate (0.2 g). After 5 h the solution was cooled and filtered, the precipitated salts were washed with methylene chloride, and the combined filtrates were evaporated. The remaining yellow oil was separated on a column of silica gel (10 g) with methylene chloride–methanol (96:4). Two isomers were obtained as amorphous solids. One isomer had an R_f of 0.49 in chloroform–methanol (9:1) and altogether 0.043 g (35%) was obtained. MS (CI, NH_3) $(M + H)^+$ at *m/e* 526. The other isomer had an R_f of 0.33 in the same system and altogether 0.037 g (30%) was obtained. MS (CI, NH_3) $(M + H)^+$ at *m/e* 526.

***N*-[3-[(2-Cyano-4-indolyl)oxy]-2-hydroxypropyl]-(*Z*)-1,8-diamino-*p*-menthane (6, 7).** The above procedure was repeated with 2-cyano-4-[(2,3-epoxypropyl)oxy]indole (3; 0.745 g, 3.48 mmol) and (*Z*)-1,8-diamino-*p*-menthane (3 mL, 2.742 g, 16.71 mmol), yielding a mixture of 6 and 7 (1.1 g, 77%) in the form of an off-white foam, R_f 0.94 in the same system as above. The corresponding hydrochloride was prepared by evaporation of a solution of base 6/7 in methanol with hydrochloric acid (5%) and drying in vacuo, 120 °C, 0.5 h. Anal. ($C_{22}H_{34}Cl_2N_4O_2$) C, H; N: calcd, 12.25; found, 11.83. NMR spectra were used to confirm 1:1 stoichiometry in the products. NMR ($CDCl_3$) δ 7.21–6.70 (m, 3 H, aromatic protons on carbons 3, 5, and 6), 6.36 (d, J = 7 Hz, 1 H, aromatic proton on carbon 7), 4.05 (br s, 4 H, C*H*$_2$O, C*H*-O*H*, reduced to 3 H upon addition of D_2O), 2.73 (m, 2 H, C*H*$_2$-NH), 1.9–0.83 (m, 21 H, *p*-menthane protons, -CH$_2$-N*H*). The side product had an R_f of 0.67 and, as was obvious from NMR spectra, arose from the reaction of two epoxide molecules with one of diamine. Dinitrophenyl derivatives of the amines 6, 7 were obtained by the same procedure as above. One of them (yield 37%) had an R_f of 0.53 in the same solvent system. MS (CI, NH_3) $(M + H)^+$ at *m/e* 551. The other (yield 34%) had an R_f of 0.42. MS (CI, NH_3) $(M + H)^+$ at *m/e* 551.

***N*^{8}-(Bromoacetyl)-*N*^{1}-[3-(4-indolyloxy)-2-hydroxypropyl]-(*Z*)-1,8-diamino-*p*-menthane (8) and *N*^{1}-(Bromoacetyl)-*N*^{8}-[3-(4-indolyloxy)-2-hydroxypropyl]-(*Z*)-1,8-diamino-*p*-menthane (9).** The mixture of compounds 4 and 5 (1.56 g, 4.39 mmol) was dissolved in anhydrous tetrahydrofuran (70 mL) and cooled in an ice bath. Bromoacetyl bromide (0.39 mL, 0.91 g, 4.5 mmol) was then added dropwise and the mixture was stirred for an additional 25 min. Solvent was partially evaporated in vacuo to about a fifth of the original amount, ethyl acetate (120 mL) was added, and the solution was washed with aqueous sodium bicarbonate (20 mL, 8%), water (twice × 20 mL), and dried over anhydrous sodium sulfate. Evaporation of the solution yielded an off-white foam (1.95 g, 93%) containing, according to thin-layer chromatography, two main components. These were separated on a silica gel column (60 g) eluted with chloroform–methanol

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(85:15). The compound eluting first, **8**, formed a white foam (520 mg, 25%), R_f 0.50 (chloroform–methanol, 75:25). To obtain an acceptable analysis the foam was dissolved in methanol and this solution, with stirring, was added to distilled water. The resulting suspension was freeze-dried. Anal. ($C_{23}H_{36}BrN_3O_4$) C, H, Br, N. MS (70 eV) *m/e* 479 (<2, M), 466 (4, M – CH_3), 464 (4, M – CH_3), 301 (36, M – C_5H_9BrNO), 259 (100, M – $C_8H_{14}BrNO$ – H), 219 (<1), 190 (1), 180 (10, C_5H_9BrNO), 178 (10, C_5H_9BrNO). MS (CI, NH_3), *m/e* 482, 480 (MH). NMR ($CDCl_3$) δ 8.3 (br s, 1 H, indole N-H), 7.1–6.3 (m, 5 H, aromatic protons), 6.1 (br s, 1 H, N*H*CO), 4.6–4.0 (m, 4 H, CH_2-O, C*H*OH), 3.8 (s, 2 H, CH_2Br), 3.8–2.8 (m, 2 H, CH_2-NH), 2.7–0.9 (m, 19 H, *p*-menthane protons, -CH_2-N*H*). Compound **9** was also a white foam (560 mg, 27%), R_f 0.36 in the same system. After processing by freeze-drying as above anal. ($C_{23}H_{36}BrN_3O_4$) C, H; N: calcd, 8.43; found, 7.92. MS (70 eV), *m/e* 466 (4, M – CH_3), 464 (4, M – CH_3), 287 (<2, M – $C_6H_{10}BrNO$–H), 247 (100, M – $C_9H_{15}BrNO$), 192 (5, $C_6H_{10}BrNO$ – H), 190 (5, $C_6H_{10}BrNO$–H). MS (CI, NH_3), *m/e* 482, 480 (MH). NMR ($CDCl_3$) δ 8.3 (br s, 1 H, indole N-*H*), 7.1–6.3 (m, 5 H, aromatic protons), 6.1 (br s, 1 H, N*H*CO), 4.6–4.0 (m, 4 H, CH_2O, C*H*OH), 3.8 (s, 2 H, CH_2Br), 3.8–2.8 (m, 2 H, CH_2-NH), 2.7–0.9 (m, 19 H, *p*-menthane protons, -CH_2-N*H*-).

N^8-(Bromoacetyl)-N^1-[3-[(2-cyano-4-indolyl)oxy]-2-hydroxypropyl]-(*Z*)-1,8-diamino-*p*-menthane (10) and N^1-(Bromoacetyl)-N^8-[3-[(2-cyano-4-indolyl)oxy]-2-hydroxypropyl]-(*Z*)-1,8-diamino-*p*-menthane (11). The procedure for synthesizing compounds **8** and **9** was repeated with a mixture of **6** and **7** (1.025 g, 2.67 mmol), tetrahydrofuran (45 mL), and bromoacetyl bromide (0.255 mL, 0.592 g, 2.93 mmol) as starting material and yielded a mixture of **10** and **11** (1.37 g, 100%) in the form of an off-white foam, which was fractionated on a silica gel column (60 g), chloroform–methanol (92:8). Compound **10** was obtained, 0.42 g (31%), R_f 0.48. Anal. ($C_{24}H_{33}BrN_4O_3$) C, H; N: calcd, 11.07; found, 10.64. MS (70 eV), *m/e* 326 (3, M – C_5H_9BrNO), 284 (20, M – $C_8H_{14}BrNO$ – H), 180 (26, C_5H_9BrNO), 178 (27%, C_5H_9BrNO). MS (CI, NH_3), *m/e* 507, 505 (MH). NMR ($CDCl_3$) δ 7.26–6.73 (m, 3 H, aromatic protons on carbons 3, 5, 6), 6.36 (*J* = 7 Hz, 1 H, aromatic proton on carbon 7), 6.16 (s, 1 H, -N*H*CO-, slowly disappeared on addition of D_2O), 4.06 (br s, 4 H, -O-CH_2-C*H*OH-, reduced to 3 H on addition of D_2O), 3.77 (s, 2 H, CH_2Br), 2.77 (m, 2 H, CH_2N), 2.10–0.83 (m, 19 H, *p*-menthane protons, -CH_2-N*H*-). Compound **11** had an R_f of 0.31 and was obtained in the form of an off-white foam (0.49 g, 36.4%) and after processing through freeze-drying as above gave **11** as hydrate (3.5 H_2O). Anal. ($C_{48}H_{80}Br_2N_8O_{13}$) C, N; H: calcd, 7.09; found, 6.49. MS (70 eV), *m/e* 312 (6, M – $C_6H_{10}BrNO$ – H), 284 (8), 272 (100, M – $C_9H_{15}BrNO$), 234 (3), 221 (1), 219 (<1). MS (CI, NH_3), no MH ion detected. NMR ($CDCl_3$) δ 7.26–6.73 (m, 3 H, aromatic protons on carbons 3, 5, 6), 6.40 (d, *J* = 7 Hz, 1 H, aromatic proton on carbon 7), 6.10 (s, N*H*CO, 1 H, disappeared slowly upon addition of D_2O), 4.06 (br s, 4 H, -OCH_2-C*H*OH-, reduced to 3 H on addition of D_2O), 3.75 (s, 2 H, CH_2Br), 2.80 (m, 2 H, CH_2N), 2.03–0.80 (m, 19 H, *p*-menthane protons, -CH_2-N*H*-).

Stability of Compounds 8–11. In the solid state, compounds 8–11 showed traces of decomposition after about 1 month of storage at –20 °C unless rigorously dry; the decomposition seemed to be an autocatalytic process. Methanolic solutions (10 mg/mL) are suitable storage forms. When these were kept at –20 °C for 3 months, no decomposition was detected; at 4 °C for 2 months decomposition was barely detectable; at 20 °C for 2 months decomposition was observable but probably less than 5%.

Pharmacology. Membrane Preparations. Membranes were prepared as described previously from heart and lung tissues of rats (250–300 g, male, Sprague–Dawley).[4] Final resuspension of membrane pellets of all preparations was in 50 mM Tris, 10 mM $MgCl_2$ buffer, pH 7.4 buffer, approximately 15 mL/gram wet weight of tissue.

To test for irreversibility of the bromoacetylated ligands, membrane preparations (approximately 5-mL protions) were preincubated for 30 min at 30 °C with indicated concentrations of the compounds and then diluted to 40 mL with the same buffer and centrifuged (48000*g*, 10 min, 4 °C). The membrane pellets were resuspended in 40 mL of buffer by hand homogenization with a Teflon and glass homogenizer and recentrifuged. This procedure was repeated twice more, and then the membranes were resuspended in their original volumes for assay (apporoximately 300 μg of protein/assay for heart and 100 μg for lung) of saturation of [^{3}H]dihydroalprenolol binding. Where indicated the preincubation mixtures also contained (±)-alprenolol, (–)-isoproterenol, or (+)-isoproterenol to assess the specificity of the reaction of the bromoacetylated compounds.

Measurement of [^{3}H]Dihydroalprenolol Binding. Binding of [^{3}H]dihydroalprenolol (90 Ci/mmol, New England Nuclear Corp., Boston, MA) to membrane preparations was performed as described previously.[4] Saturation experiments were performed by using 12 different concentrations of [^{3}H]dihydroalprenolol ranging from 0.3 to 6.0 nM; to measure nonspecific binding, (±)-alprenolol (10 μM) was included in the assays. For affinity studies, 3 nM [^{3}H]dihydroalprenolol was used and displacement of specific binding by the ligands was measured. Duplicate tubes were used for each assay condition, and all experiments were performed three separate times except where noted.

Measurement of Adenylate Cyclase Activity. Adenylate cyclase activity in homogenates from rat hearts was measured as described previously.[4,13] Assays included the bromoacetylated derivatives alone (1×10^{-9} to 1×10^{-3} M) when agonist activity was measured or (–)-isoproterenol (1×10^{-6} M) in the presence of varying concentrations of the compounds (1×10^{-9} to 1×10^{-4} M) when the antagonist activity was measured.

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Registry No. (*Z*)-**1**, 54166-24-4; **2**, 35308-87-3; **3**, 73907-82-1; **4**, 106469-48-1; **4**·2HCl, 106469-59-4; **4**·1/2H_2CO_3, 106469-60-7; **5**, 106469-49-2; **5**·2HCl, 106469-58-3; **5**·1/2H_2CO_3, 106502-45-8; **6**, 106502-44-7; **6**·2HCl, 106469-61-8; **7**, 106469-50-5; **7**·2HCl, 106469-62-9; **8**, 106469-51-6; **9**, 106469-52-7; **10**, 106469-53-8; **11**, 106469-54-9; $BrCH_2COBr$, 598-21-0; 4-hydroxyindole, 2380-94-1; epichlorohydrin, 106-89-8; 4-(benzyloxy)indole-2-carboxylic acid, 39731-09-4; 2-amino-4-(benzyloxy)indole, 106469-55-0; 2-cyano-4-(benzyloxy)indole, 106469-56-1; 2-cyano-4-hydroxyindole, 106469-57-2; 1,8-diamino-*p*-menthane, 80-52-4.

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