Synthesis of Iodine-125 Labeled (±)-15-(4-Azidobenzyl)carazolol: A Potent β-Adrenergic Photoaffinity Probe

Sarah L. Heald,† Peter W. Jeffs,*,† Thomas N. Lavin,‡ Ponnal Nambi,‡ Robert J. Lefkowitz,‡ and Marc G. Caron*,‡

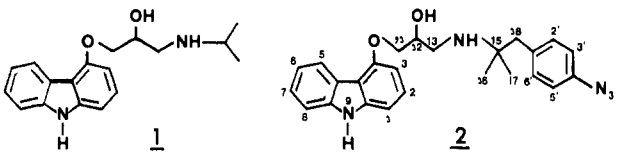
Department of Chemistry, Duke University, Durham, North Carolina 27706, and Howard Hughes Medical Institute Laboratories and Departments of Medicine (Cardiology) and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710. *Received May 5, 1982*

(±)-15-(4-Azidobenzyl)carazolol (2), a potent β-adrenergic photoaffinity ligand developed in our laboratories, has been radioiodinated to theoretical specific activity (2175 Ci/mmol) and shown to label covalently β-adrenergic receptor peptides in avian and amphibian erythrocyte membrane preparations. The radioiodinated analogues of the desired compound (2) were optimally prepared by two synthetic steps from (±)-15-(4-aminobenzyl)carazolol (8). The latter was iodinated with carrier-free $Na^{125}I$ and chloramine T to yield two major isotopomers (the monoiodinated derivatives 9 and 10), which were separated by thin-layer chromatography and converted via diazonium salt formation to their respective 4-azides, 12 and 6. These azides can be used interchangeably in ligand binding or photoaffinity labeling experiments. Compound 8 was obtained by catalytic reduction of the nitro derivative (7), which was arrived at by direct reaction of 1,1-dimethyl-2-(4-nitrophenyl)ethylamine (3) with 4-(2,3-epoxypropoxy)carbazole (5). Of the desired isomers, (±)-15-(4-azido-3-iodobenzyl)carazolol (6) could be synthesized from 1,1-dimethyl-2-(4-azido-3-iodophenyl)ethylamine (4) by direct reaction with 5. This and the preceding sequence of reactions were carried out by using nonradioactive materials, and separation and purification of products were accomplished by high-performance liquid chromatography. The compounds described have been shown to be potent β-adrenergic antagonists by virtue of their ability to inhibit β-adrenergic stimulation of adenylate cyclase or to compete for the binding of another β-adrenergic ligand, [^{125}I]cyanopindolol, to the β-adrenergic receptors of frog erythrocytes. The photoactive azide derivatives of these compounds (6 and 12) have been shown to covalently incorporate into the β-adrenergic receptor binding subunit of frog and turkey erythrocyte membrane preparations. Incorporation of the ligands into these polypeptides can be blocked specifically by both β-adrenergic agonists and antagonists.

The characterization of hormone and drug receptors at the molecular level involves their purification and identification of the structure of their ligand binding subunit(s). Because most of these proteins exist in cells in extremely small amounts, biospecific techniques such as affinity chromatography for purification and affinity or photoaffinity labeling for biochemical characterization have proven to be of great importance. Very recently, the β-adrenergic receptor has been purified to apparent homogeneity[1] by the use of affinity chromatography and high-performance liquid chromatography. The same polypeptide identified by purification as the ligand binding subunit of the receptor in this system has been labeled covalently in purified preparations by the photoactive antagonist [^{3}H]-15-(4-azidobenzyl)carazolol.[2]

Although many attempts to develop specific affinity probes for the β-adrenergic receptor have been reported,[2-8] only two of the ligands recently described appear to possess the critical properties of such a reagent. An effective affinity ligand for a receptor binding site should meet two major requirements: (1) the ligand should possess high affinity for the receptors, thus providing great specificity of labeling; (2) it should be possible to label the ligand to high specific radioactivity, thus enhancing the experimental sensitivity. Thus, ligands radiolabeled with tritium[2,3,6] all possess the same drawback of low specific radioactivity, affording only low levels of experimental sensitivity. Nonetheless, under certain circumstances, such as extremely long times of exposure[3] or with the use of purified receptor preparations,[2] these ligands have been shown to incorporate covalently into specific receptor peptides. However, the need has continued to exist for ligands of high affinity and specific radioactivity that might be used to label the receptors in crude membrane preparations. Recently, ^{125}I-labeled *p*-azidobenzylpindolol[7] and two iodinated azide derivatives of cyanopindolol[8] have been introduced as useful photoaffinity probes of the β-adrenergic receptor. Full details of the characterization[8] and radiosynthetic[9] procedures used for these compounds have yet to be reported.

Our strategy was to functionalize the potent β-adrenergic antagonist carazolol[10] (1, K_D = 100 pM). The model compound, containing a photoactive azide group, is 15-(4-azidobenzyl)carazolol (pABC,[11] 2). The synthesis of this compound in radiolabeled form with tritium (specific activity = 26–40 Ci/mmol) has been previously described from this laboratory.[2] Although biochemical investigations indicated that this compound could covalently label the binding subunit of the β-adrenergic receptor, its relatively low specific radioactivity limited its use to partially purified receptor preparations.[2] This paper describes the synthesis and chemical characterization of iodinated derivatives of 2 that can be prepared at theoretical specific radioactivities of 2175 Ci/mmol. These compounds can specifically label



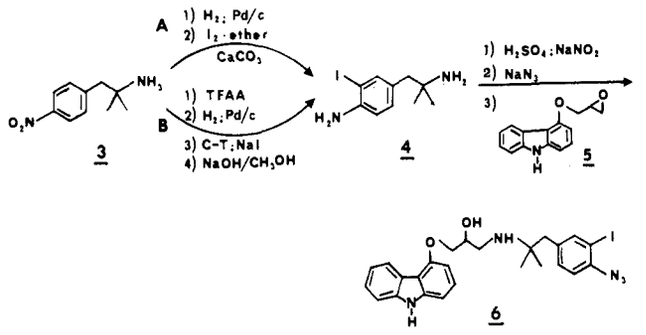
1 2

† Department of Chemistry, Duke University.
‡ Duke University Medical Center.

(1) Shorr, R. G. L.; Lefkowitz, R. J.; Caron, M. G. *J. Biol. Chem.* **1981**, *256*, 5820–5826.
(2) Lavin, T. N.; Heald, S. L.; Jeffs, P. W.; Shorr, R. G. L.; Lefkowitz, R. J.; Caron, M. G. *J. Biol. Chem.* **1981**, *256*, 11944–11950.
(3) Atlas, D.; Levitzki, A. *Nature (London)* **1978**, *272*, 370–371.
(4) Darfler, F. V.; Marinetti, G. V. *Biochem. Biophys. Res. Commun.* **1977**, *79*, 1–7.
(5) Wrenn, S. W.; Homcy, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 44490–4453.
(6) Pitha, J.; Zjawiony, J.; Nasrin, N.; Lefkowitz, R. J.; Caron, M. G. *Life Sci.* **1980**, *27*, 1791–1798.
(7) Rashidbaigi, A.; Ruoho, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1609–1613.
(8) Burgermeister, W.; Hekman, M.; Helmreich, E. J. M. *J. Biol. Chem.* **1982** *257*, 5306–5311.
(9) Rashidbaigi, A.; Ruoho, A. E. *J. Pharm. Sci.* **1982**, *71*, 305–307.
(10) Bartsch, W.; Dietman, K.; Leinert, H.; Sponer, G. *Arzneim.-Forsch.* **1977**, *27*, 1022.
(11) Abbreviation used for 15-(4-azidobenzyl)carazolol in biochemical publications was pABC.

0022-2623/83/1826-0832$01.50/0

Scheme I



the β-adrenergic receptor in crude membrane preparations from frog and turkey erythrocyte membranes, as well as several other tissues.[12]

Recently, the ^{125}I-labeled (±)-15-(4-aminobenzyl)carazolols have been demonstrated to irreversibly incorporate into the β-adrenergic receptor by using the photoactive cross-linker *N*-succinimidyl-6-[(4-azido-2-nitrophenyl)-amino]hexanoate.[13] Though this direct use of the radioiodinated amines had the advantage of involving only one radioactive synthetic step in their preparation, the biochemical methodology required was distinctly more complex.

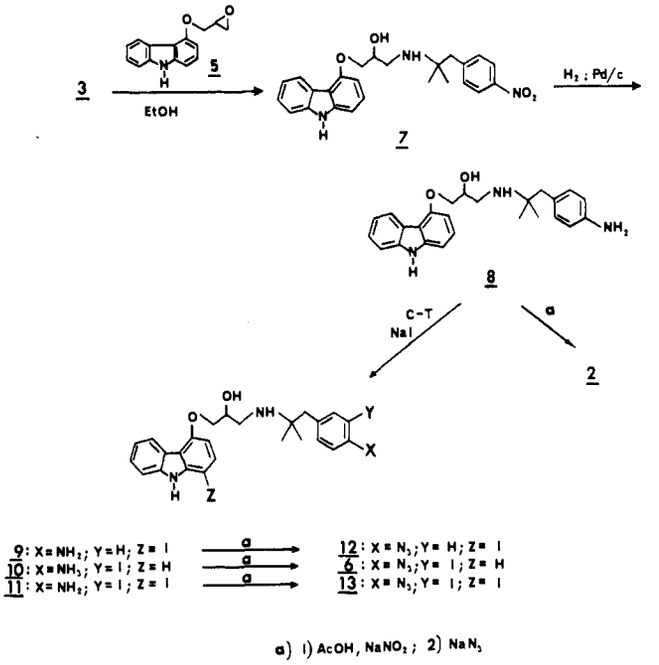
The biological activity of the iodinated and radioiodinated **2** derivatives has been clearly demonstrated by (1) their ability to potently inhibit β-adrenergic stimulation of adenylate cyclase activity (cf. Figure 2); (2) their ability to bind specifically and reversibly in the absence of light to β-adrenergic receptors of several tissues,[12] and (3) their ability to covalently label the binding subunit(s) of both β_1 and β_2 subtypes of adrenergic receptors in a specific and photodependent manner (cf. Figure 3). The full details of these biochemical studies are to be published elsewhere.[12]

Synthesis. The synthesis of (±)-15-(4-azido-3-iodobenzyl)carazolol (**6**), which was accomplished in this work and formed the basis for the development and execution of the synthesis of ^{125}I-labeled analogues, is summarized in Schemes I and II.

In Scheme I, the diamine, which was obtained by reduction of 1,1-dimethyl-2-(4-nitrophenyl)ethylamine (**3**) and has been utilized in a previous synthesis in this series,[2] was iodinated under mild conditions in a biphasic mixture of aqueous calcium carbonate and ether containing 1 equiv of iodine.[14] The iodo amine **4**, obtained as the hydrochloride in only 25% yield, as converted quantitatively to the corresponding azide by diazotization and reaction with sodium azide. Reaction of the azide with 4-(2,3-epoxypropoxy)carbazole (**5**) in ethanol at 65 °C for 7 days[10] gave a crude product, which afforded the required compound **6** (22% yield).

The low yields in two steps of this sequence led us to explore an alternate approach. Considerable improvement in the conversion of **3** to **4** was accomplished by iodination of the trifluoroacetyl derivative of **4**, which was obtained from the corresponding aromatic nitro compound **3** by standard methods. With the protection of the aliphatic amino group, it was possible to employ the more reactive iodination reagent, *N*-chlorotoluene-*p*-sulfonamide (chloramine T) and sodium iodide,[15] to effect the introduction of iodine into the aromatic ring. Under these conditions, the iodination product **4** was obtained in greater than 90% yield. While the latter reaction sequence seemed to offer an attractive method for adaptation to the synthesis of [^{125}I]**6**, the reactions proved highly capricious when carried out on a submicrogram scale. There was no evidence for the formation of any [^{125}I]**6** in these reactions. Evidently, the 100-fold decrease in concentration under which these experiments are necessarily carried out with high specific activity Na^{125}I (2175 Ci/mmol; 10 mCi ≡ 0.67 μg) has an adverse effect on the course of one or the other of the reactions.[16]

Scheme II



The radioiodination of (±)-15-(4-aminobenzyl)carazolol (**8**) yielded ^{125}I-labeled carazolol derivatives (Scheme II). The synthesis of **8** was affected from **3**. The intermediate (±)-15-(4-nitrobenzyl)carazolol (**7**) was obtained in 72% yield after removal of the 2-nitrobenzyl isomer. Catalytic reduction of **7** provided **8** in 82% yield.

The reservations concerning the presence of multiple reaction sites in **8**, which had deterred us earlier from considering direct iodination of this compound, were confirmed by formation of three major iodinated compounds from the reaction of **8** with chloramine T and sodium iodide. Three important factors led us to pursue this seemingly undesirable reaction: (1) the three major iodo compounds could be readily separated by HPLC and TLC, (2) the reaction was quite reproducible at the micromolar level with ^{125}I-carrier-free sodium iodide, and (3) each of the iodo derivatives could potentially meet the need, following a one-step conversion to the corresponding azide (see Scheme II), in providing a highly specific radiochemically labeled photoaffinity ligand.

The identity of these iodinated products was established as (±)-15-(4-amino-3-iodobenzyl)carazolol (**9**), (±)-15-(4-aminobenzyl)-1-iodocarazolol (**10**), and (±)-15-(4-amino-

(12) Lavin, T. N.; Nambi, P.; Heald, S. L.; Jeffs, P. W.; Lefkowitz, R. J.; Caron, M. G. *J. Biol. Chem.*, **1982**, *257*, 12332–12340.
(13) Shorr, R. G. L.; Heald, S. L.; Jeffs, P. W.; Lavin, T. N.; Strohsacker, M. W.; Lefkowitz, R. J.; Caron, M. G. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 2879–2882.
(14) Wheeler, H. L.; Liddle, L. M. *Am. Chem. J.* **1909**, *42*, 441.
(15) (a) Brown, E. M.; Aurbach, G. D.; Hauser, D.; Troxler, F. *J. Biol. Chem.* **1976**, *251*, 1232. (b) Campbell, M. M.; Johnson, G. *Chem. Rev.* **1978**, *78* (1), 65.
(16) The problem is most reasonably attributed to the slow nucleophilic ring opening of the epoxide, which in the nonradioactive model studies occurs over the course of 1 week.

Table I. Effect of the Concentration of Reactants on the Yield of Iodination Products of 8

molar ratio[a] of NaI/C-T/8	product ratio of 9/10/11	overall % yield
1.0:3.9:1.6[b]	1.0:1.0:1.2	64
1.0:1.5:1.1	1.0	36
1.0:9.3:1.2	1.1:1.0:2.5	61
1.0:3.1:1.6	1.6:1.0:1.2	76
1.0:9.3:3.2	1.8:1.7:1.0	83
1.0:9.3:6.4	2.9:1.0	92

[a] Reaction conditions were identical with those described under Experimental Section under radioiodination. The reactant ratio was based on a 2.5 mCi NaI scale. [b] pH maintained at 5.6 (first set of conditions only).

3-iodobenzyl)-1-iodocarazolol (11) from their spectral properties, which are discussed subsequently. The ^{125}I-labeled compound corresponding to compounds 9–11 were obtained under closely parallel reaction conditions as described for the preparative scale synthesis. The isotopomer distribution was directly controlled by the ratio of NaI, chloramine T, and 8 (Table I). Reaction conditions were not found where only one product formed in good yield. The success of this procedure therefore hinged upon the development of the HPLC separation of these compounds.[17] The radioiodinated amines could be completely isolated and purified by TLC. Due to the clean separation of the radioiodinated products from the unsubstituted starting material 8 and the use of carrier-free Na^{125}I, a specific activity of 2175 Ci/mmol could be assumed for compounds 9 and 10.

Conversion of the 4-aminobenzyl group in the carazolol derivatives to the corresponding 4-azidobenzyl moiety via an intermediate diazonium salt was studied with the parent (±)-15-(4-aminobenzyl)carazolol (8). After an unsuccessful attempt to diazotize 8 in 4.5 N H_2SO_4, which lead to *N*-nitrosocarbazole, the transformation of 8 to the required azide 2 proceeded readily via the diazonium salt prepared in 3 N acetic acid. When this procedure was applied to the two monoiodinated *p*-aminocarazolols 9 and 10 and to the corresponding ^{125}I-labeled compounds, they were converted quantitatively to the azides 12 and 6 and to their radioiodinated isotopomers. Again, radioactive azide reaction mixtures were applied directly to silica TLC for complete isolation and purification, allowing the assumption of a specific activity of 2175 Ci/mmol for products 12 and 6.

Spectral Characterization of the Various Benzylcarazolol Derivatives. Characterization of the carazolol derivatives described in the previous section was most readily obtained from analysis of their 250-MHz ^{1}H NMR spectra.

In the parent benzylcarazolol (14), only the hydrogen signals at positions 1, 3, and 5 are clearly distinguishable at δ 7.04, 6.62, and 8.30, respectively, and are essentially unaffected in compounds where substituents are introduced in the remote benzyl group. The effect of introduction of a substituent in the 4-position of the benzyl group leads to a clearly recognizable symmetrial pattern of an AA′BB′ system with a pronounced upfield shift of the benzyl 3,5-proton signals occurring when the substituent is an amino group (8: δ 6.94 and 6.55) in comparison to the shifts observed for these proton signals for a 4-azido (2: δ 7.07 and 6.78) or a 4-nitro (7: δ 7.22 and 7.91) group. With the introduction of iodine to the 3-position of the benzyl group, the AA′BB′ patterns are clearly modified to an AMX system with a marked downfield shift of the benzyl 2-position proton (10 and 11: δ 7.44, 7.06, 6.59; 6: δ 7.47, 7.06, 6.76).[18]

In locating the position of the iodine in the carbazole nucleus in the iodo compounds 9 and 12 and in the diiodo product 11, the ^{1}H NMR spectra narrowed the possibilities to a 1-iodo or 3-iodo substituent. In the spectra of each these compounds, a pair of doublets were present at δ 7.61 and 6.53 (J = 8.45 Hz) in accord with an ortho coupling.

Two independent experiments were conducted to clearly identify the position of the iodo substituent. The model, 4-(2,3-epoxypropoxy)carbazole (5), was iodinated. The product, 4-(2,3-epoxypropoxy)-1-iodocarbazole (15), obtained showed excellent correlation of its ^{1}H NMR chemical shifts of the protons in the carbazole nucleus with compounds 9, 11, and 12, indicating that the iodine substituent was located at the same site in all four compounds.

A clear indication for favoring the location of the iodine in the carbazole at the 1-position was obtained by the ^{13}C NMR spectral data of 5 and its iodination product. An observed 38.5-Hz upfield shift was exhibited by the iodinated carbon from δ 104.2 to 65.7 ppm. The strong effect of the iodine on the ortho, meta, and para carbon positions was somewhat attenuated across the large carbazole ring system, but comparison of observed and calculated chemical shifts supported iodination at the 1-position rather than the isomeric 3-position.[19]

Convincing evidence for the assignment of the structure of 15 was obtained by a ^{1}H NOE experiment. Irradiation at δ 4.31 corresponding to the resonance from the 11-methylene protons resulted in a 15% increase in the signal at δ 6.48. This result indicates the presence of a hydrogen at C-3[20] and thus confirms the assignment of the structure, 4-(2,3-epoxypropoxy)-1-iodocarbazole, to the iodination product (15). It should be noted that this result is not that expected from previous studies of electrophilic substitution in simple carbazoles, which occur at the C-3 and C-6 positions.[21]

The mass spectral fragmentation patterns of 15-benzylcarazolol and its analogues described in this paper were most important in originally recognizing the existence of iodine in the carbazole ring of 9, 11, and 12. This simplified the interpretation of the complex NMR data.

All of the compounds show a common dominant cleavage pathway in their EI mass spectra, which is represented by a nitrogen-directed β-scission of the C15–C18 bond[22] with predominant charge retention on the carbazole-containing fragment. This ion appeared as the parent peak at m/e 297 in the MS of benzylcarazolol, 2, 6–8, and 10 and at m/e 423 in the MS of 9, 11, and 12. Although other ion fragments were in low abundance, the allocation of an iodo substituent to the benzyl group or to the carbazole portion of the carazolol is readily apparent.

Biological Studies. 15-(4-Azido-3-iodobenzyl)carazolol (6) was examined for its ability to inhibit the β-adrenergic stimulation of the enzyme adenylate cyclase in the frog

(17) Bidlingmeyer, B. A.; Del Rios, J. K.; Korpi, J. *Anal. Chem.* **1982**, *54*, 442.
(18) (a) Allinger, N. L.; Youngdale, G. A. *J. Am. Chem. Soc.* **1982**, *84*, 1020. (b) Sadtler Standard ^{1}H NMR Spectra, 6688 (1980).
(19) (a) Mester, I.; Bergenthal, D.; Reisch, J. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1979**, *34B* (4), 650. (b) Levy, G. C.; Nelson, G. L.; Cargioli, J. D. *Chem. Commun.* **1971**, 506. (c) Sadtler Standard Carbon-13 Spectra, 6418 (1980).
(20) Phillipsborn, W. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 472.
(21) (a) Longuet-Higgins, H. C.; Coulson, C. A. *Trans. Faraday Soc.* **1947**, *43*, 87. (b) Since in the spectra of 9, 11, and 12 the ^{1}H resonance from the C-5 proton was the same as the unsubstituted derivatives, iodination at C-6 was not considered.
(22) Collin, J. *Bull. Soc. Chim. Belg.* **1954**, *63*, 500.
(23) Wyllie, S. G.; Djerassi, C. *J. Org. Chem.* **1968**, *33* (1), 305.

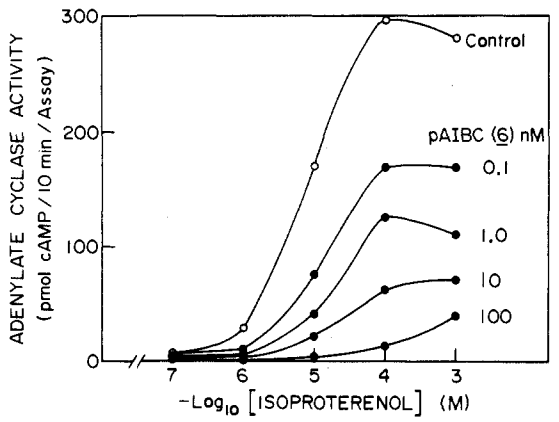


Figure 1. Inhibition of the β-adrenergic stimulation of frog erythrocyte adenylate cyclase by **6**. Frog erythrocyte membranes resuspended in 75 mM Tris-HCl, 25 mM $MgCl_2$, pH 7.4, were incubated at 25 °C with increasing concentrations of **6**. After a 45-min incubation, each membrane aliquot was assayed for adenylate cyclase activity at 37 °C for 15 min in the presence of increasing concentrations of (−)-isoproterenol. Results shown are representative of two experiments performed in triplicate.

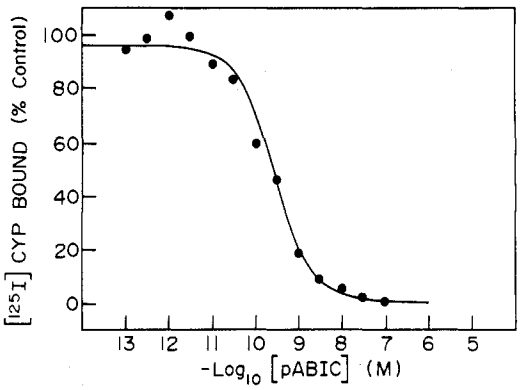


Figure 2. Inhibition of [^{125}I]cyanopindolol binding to frog erythrocyte membranes by **6**. Frog erythrocyte membranes in 25 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.4, were incubated at a receptor concentration of ~20 pM in a total volume of 0.5 mL with ~20–30 pM [^{125}I]cyanopindolol in the presence of increasing concentrations of **6** for a period of 90 min at 25 °C. Bound ligand was determined by rapid vacuum filtration through GF/C filters and counting. In separate binding isotherm experiments, the K_D or [^{125}I]cyanopindolol for binding to the receptor was estimated to be 11 pM. Data were analyzed by nonlinear least-squares curve fitting.[26] The data shown represent the mean of two experiments determined in duplicate.

erythrocyte membrane system. As shown in Figure 1, the dose–response curve for stimulation of adenylate cyclase by isoproterenol was progressively shifted to the right in the presence of increasing concentrations of compound **6**, indicating that **6** acts as a potent β-adrenergic antagonist in this system. The reason for the apparent decrease in the maximal stimulation of adenylate cyclase by isoproterenol is likely the following. Membranes were incubated for 45 min with compound **6** prior to the addition of isoproterenol and [^{32}P]ATP and the 15-min subsequent incubation at 37 °C for enzyme assay. Since, as shown by direct ligand binding experiments,[12] the azidoiodocarazolol **6** dissociates from the receptor-binding site with a $t_{1/2}$ of about 6 h at 25 °C, it is expected to behave to an extent as an irreversible blocker during the relatively brief incubation period with isoproterenol.

Figure 2 depicts the ability of **12** to compete for the binding of a reversible radioligand, ^{125}I-labeled cyanopindolol,[24] to the β-adrenergic receptor of frog erythrocyte membranes. An apparent dissociation constant (K_D) of 71 pM can be calculated from these data. The value is in the same range as the dissociation constant (10–20 pM) obtained by direct binding isotherms in the same system.[12] 15-(4-Azidobenzyl)carazolol and its ^{3}H-labeled derivative have previously been reported to display K_D values of 100–200 pM for binding to the β-adrenergic receptors in this system. It appears therefore that iodination of the azido compound **2** leads to an increase in the affinity of the receptor for both the 1-iodo and 3-iodobenzyl derivatives.

In the absence of light, these high-affinity ligands labeled to high specific radioactivity can be used as tools to reversibly label receptors. In frog and turkey erythrocyte membranes, both ^{125}I-labeled **6** and **12** bind in a saturable fashion with high affinity (K_D = 10–20 pM) and display appropriate β_1 and β_2 subtype adrenergic specificity and stereoselectivity[12] (data not shown). However, their greatest utility is for the covalent labeling of the receptor binding subunit of the β-adrenergic receptors. As shown in Figure 3, incubation of frog (A) and turkey (B) erythrocyte membranes with [^{125}I]**6** leads to the covalent incorporation of the radioactive probe into a polypeptide of M_r = 58 000 for the β_2 receptor system of the frog erythrocyte (A) and two polypeptides of M_r = 40 000 and 45 000 for the β_1 receptor system of the turkey erythrocyte. Covalent incorporation in both systems was blocked by both the β-adrenergic antagonist (−)-alprenolol and the agonist (−)-isoproterenol. Moreover, incorporation can be blocked more potently by the (−) isomers of these agents as opposed to their (+) isomers, and an appropriate β_1 and β_2 selectivity of labeling can be demonstrated (data not shown). The same peptides can also be labeled with the isotopomer [^{125}I]**12**. The peptides (M_r = 58 000 and M_r = 40 000, 45 000) labeled in these two systems by photoaffinity labeling are identical with the peptides that have been identified in both systems as containing the ligand binding subunit of the receptor by purification of the protein.[13,25]

Experimental Section

General Methodology and Procedures. Thin-layer chromatography (TLC) was carried out on Scientific Products silica gel 60 F-254 plates (5 × 20 cm) with fluorescent indicator with a chloroform/methanol/phenol (80:20:0.001, v/v) solvent system, except where noted. Developed plates were visualized with short-wavelength UV light, and radiolabeled compounds were detected by autoradiography and/or with a Berthold Series 6000 Radio Scanner. High-performance liquid chromatography (HPLC) was carried out on a Waters Model 6000 chromatograph coupled with a Schoeffel UV Spectroflow monitor SF770 at 300 nm. ^{1}H NMR spectra of the products were obtained at 100 MHz with a JEOL MH-100 continuous-wave system and at 250 MHz with a Bruker WM 250 Fourier-transform system. Samples were run as 0.1–10%, w/w, solutions in deuterated chloroform, internally referenced with tetramethylsilane and $CHCl_3$. ^{13}C NMR spectra were obtained at 14.1 MHz with a JOEL FX-60 FT NMR spectrometer. Mass spectra were obtained on an AEI MS904 spectrometer. Infrared spectra were recorded on a Perkin-Elmer Model 297 spectrometer. Elemental analyses were performed by MHW Laboratories.

Radioiodination Procedures. In general, where possible, synthesis of ^{125}I-labeled compounds followed iodination procedures that had been carried out successfully on a preparative scale to allow characterization of the products and then subsequently on a microscale level, which approximated the quantities used in the

(24) Engel, G.; Hoyer, D.; Berthold, R.; Wagner, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1981**, *317*, 277–285.

(25) Shorr, R. G. L.; Strohsacker, M. W.; Lavin, T. N.; Lefkowitz, R. J.; Caron, M. G. *J. Biol. Chem.* **1982**, *257*, 12341–12350.

(26) Hancock, A. A.; De Lean, A.; Lefkowitz, R. J. *Mol. Pharmacol.* **1979**, *16*, 1–9.

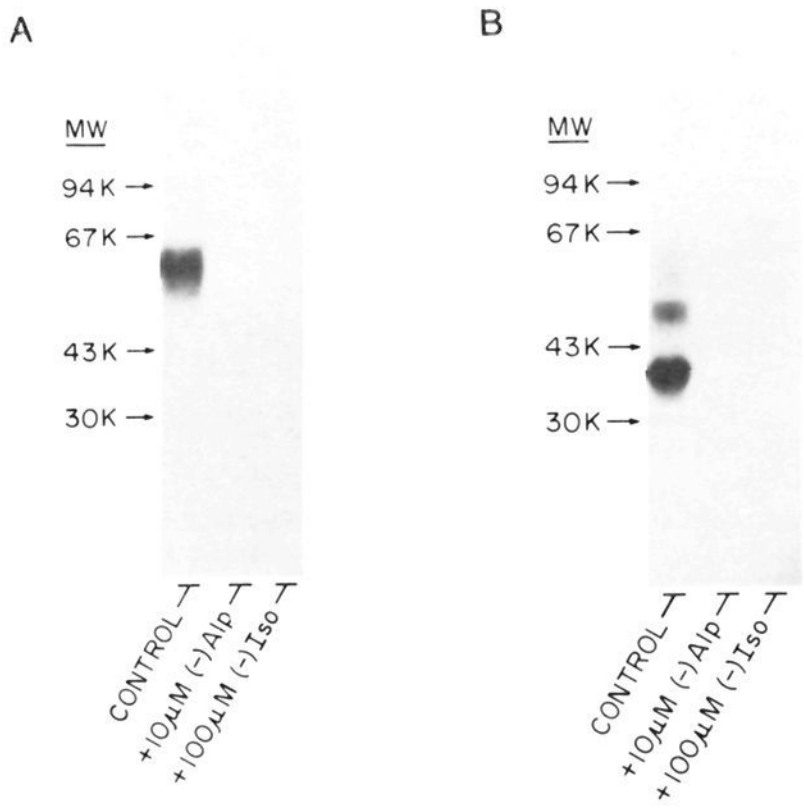


Figure 3. Photoaffinity labeling pattern of β_2- and β_1-adrenergic receptor from frog and turkey erythrocyte membranes. (A) Frog erythrocyte membranes (25–30 pM receptor concentration) were incubated with [^{125}I]**6** (25–30 pM) for 90 min at 25 °C in the absence (lane 1) and presence of 10^{-5} M (–)-alprenolol (lane 2) or 10^{-4} M (–)-isoproterenol (lane 3). At the end of the incubation, the samples were washed three times with 0.5% BSA in incubation buffer (Tris/Mg^{2+}, 25:2, pH 7.4) and once with incubation buffer alone. Photolysis was carried out for 90 s (Hanovia 450-W, high-pressure mercury lamp—12 cm from light source—through 6 mm of Pyrex[12]), after which the samples were solubilized in 10% $NaDodSO_4$, 10% glycerol, 5% β-mercaptoethanol, and 50 mM Tris-HCl, pH 6.8, and denatured at room temperature for 30–60 min. Electrophoresis was performed on an 8% polyacrylamide slab gel according to the method of Leammli.[29] The gel was then dried and exposed on Kodak XAR-5 film for 2–3 days with lightning plus (Dupont) intensifying screens. Molecular weight standards iodinated by chloramine T and Na^{125}I were from Pharmacia: phosphorylase *b*, $M_r \approx 94\,000$; albumin, $M_r = 67\,000$; ovalbumin, $M_r = 43\,000$; carbonic anhydrase, $M_r = 30\,000$; soybean trypsin inhibitor, $M_r = 20\,100$. Relative mobility of protein standards is indicated by the arrows. (B) Turkey erythrocyte membranes (25–30 pM receptor concentration) were incubated with [^{125}I]**6** (25–30 pM) for 90 min at 25 °C in the absence (lane 1) and presence of 10^{-5} M (–)-alprenolol (lane 2) or 10^{-4} M (–)-isoproterenol (lane 3). At the end of the incubation, the samples were washed three times with 0.5% BSA in Tris/Mg^{2+} (75:25, pH 7.4) and once with Tris buffer alone. Photolysis was carried out for 90 s, after which the samples were solubilized in $NaDodSO_4$ sample buffer. Electrophoresis and autoradiogram were developed as above.

^{125}I-labeled experiments, consistent with the objective of maximizing yield and obtaining products of high specific activity. A detailed procedure to obtain [^{125}I]**6** and **-12** from **8** is described elsewhere.[12]

In the specific cases where the microscale radiosynthesis of a compound differs in any important respects from the preparative scale procedures, a separate description of the experimental method is provided (see below).

Biochemical Procedures. Erythrocyte membranes, ~3 mg of protein/mL, were prepared from grass frogs (*Rana pipiens*) as described previously.[27] Membranes were stored frozen at –70 °C in 25 mM Tris-HCl, 2 mM $MgCl_2$, 100 μM phenylmethylsulfonyl fluoride, 250 mM sucrose, pH 7.4, at 4 °C. Membranes were washed with 25 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.4, or 75 mM Tris-HCl, 25 mM $MgCl_2$, pH 7.4, prior to use. Turkey erythrocyte membranes were prepared as described previously.[12] ^{125}I-labeled cyanopindolol[24] assays were carried out as described previously.[13] Adenylate cyclase activity was assayed as previously described.[28]

Synthesis. 1,1-Dimethyl-2-(4-amino-3-iodophenyl)ethylamine (4). Method A. 1,1-Dimethyl-2-(4-aminophenyl)ethylamine (1.33 g, 5.6 mmol), obtained from reduction of the corresponding nitro compound **3** as previously described,[2] was refluxed in ether (30 mL) containing a mixture of $CaCO_3$ (1.27 g, 2.2 equiv)/30 mL of H_2O and I_2 (1.43 g, 1.0 equiv) for 48 h under N_2. The reaction was halted with Na_2HSO_3 (1.2 g, 2 equiv), and **4** was isolated by extraction with ethyl acetate (4 × 15 mL). After the solvent was removed, the amine was converted to the hydrochloride (amorphous) (0.5 g, 24% yield): ^{1}H NMR (D_2O) δ 7.30 (m, 3.2, arom), 2.80 (d, 2, CH_2), 1.10 (s, 6, CH_3) (HOD at δ 4.6).

Method B. 1,1-Dimethyl-2-(4-nitrophenyl)ethylamine[2] (**3**; 2.00 g, 8.7 mmol), as the free base in THF, was reacted at 0 °C with trifluoroacetic anhydride (1 mL; freshly distilled from TFA/P_2O_5) for 15 min at room temperature. The product was crystallized with 50% hexane/ether from the concentrated reaction mixture (2.396 g, 96% yield): mp 110–112 °C; ^{1}H NMR (D_2O) δ 7.60 (m, 4, arom), 3.10 (s, 2, CH_2), 1.30 (s, 6, CH_3). Anal. ($C_{12}H_{13}N_2O_3F_3$) C, H. It was then (2.00 g, 7.0 mmol) reduced in 1% concentrated HCl/CH_3OH (30 mL) with 5% Pd/C (50 mg) under 53 psi of H_2 in a Parr low-pressure hydrogenator until hydrogen uptake was complete (1 h). The reaction mixture was filtered, and the filtrate was concentrated and crystallized with EtOAc (1.43 g, 70% yield): mp 192–194 °C; ^{1}H NMR (D_2O) δ 7.30 (m, 4, arom), 3.00 (s, 2, CH_2), 1.30 (s, 6, CH_3). Anal. ($C_{12}H_{15}N_2OF_3$·HCl) C, H. This amine (100 mg, 0.34 mmol) was dissolved in 10 mL of dioxane and added to 100 mL of 1.0 M K_2HPO_4 buffer (pH 7.5; adjusted with 1.0 M KH_2PO_4). NaI (180 mg, 1.2 equiv/0.1 NaOH (10 mL) and chloramine T (150 mg, 1.5 equiv) were added sequentially. The reaction was halted with $Na_2S_2O_5$ (200 mg, 3 equiv) after 15 min. The product was isolated by extraction with CH_2Cl_2 (4 × 30 mL). The concentrated extract was left in NaOH/H_2O/CH_3OH (1:4:20) overnight to hydrolyze the trifluoroacetamide. The unprotected iodo amine **4** (88 mg, 90% yield) was isolated by extraction with CH_2Cl_2 (4 × 20 mL).

(±)-15-(4-Azido-3-iodobenzyl)carazolol (6). Method A. Compound **4** (0.50 g, 1.4 mmol) was reacted with $NaNO_2$ (0.113 g, 1.1 equiv)/10 mL of 4.5 N H_2SO_4 and NaN_3 (0.113 g, 1.2 equiv)/1 mL of H_2O under identical reaction conditions and workup as described earlier[1] to give the crude 1,1-dimethyl-2-(4-azido-3-iodophenyl)ethylamine: ^{1}H NMR ($CDCl_3$) δ 7.00 (m, 3, arom), 2.60 (s, 2, CH_2), 1.70 (s, 2, NH_2), 1.15 (s, 6, CH_3); IR 2120 (N_3) cm^{-1}. The iodo azide was combined with 4-(2,3-epoxypropoxy)carbazole (**5**; 0.336 g, 1.0 equiv) in absolute EtOH (20 mL) and left at 65 °C under N_2 and in the dark for 1 week. The reaction mixture was then concentrated to dryness, and the residue was chromatographed on silica gel (2 × 70 cm column; 200 g of Fisher grade, 100–200 mesh; packed in CH_2Cl_2; eluted with 500 mL of CH_2Cl_2 and then 1000 mL of 0.5% CH_3OH/CH_2Cl_2; flow rate = 2 mL/min). The 925–1175-mL fraction contained **6** (amorphous solid; 0.170 g, 22% yield): TLC R_f 0.48; ^{1}H NMR ($CDCl_3$) δ 7.42 (m, 8, carbazole), 7.10 (m, 3, arom), 4.66 (m, 1, CHOH), 4.28 (m, 2, RO-CH_2), 3.28 (m, 2, CH_2NHR), 2.92 (s, 2, CH_2), 1.25 (s, 6, CH_3); IR 2120 (N_3) cm^{-1}; MS, *m/e* 555 (M^+, <1), 297 (100), 298, 232, 222, 183, 154, 114, 112, 110. Anal. Calcd for $C_{18}H_{21}N_2O_2$: 297.1602. Found: 297.1605. In the 1200–1500-mL fraction was isolated **2** (0.130 g): TLC R_f 0.42, characterized previously[1]; MS, *m/e* 429 (<1), 297 (100), 298, 222, 196, 183, 166, 154, 148.5, 114. Anal. Calcd for $C_{18}H_{21}N_2O_2$: 297.1602. Found: 297.1605. Anal. ($C_{25}H_{27}N_5O_2$ picrate) C, H.

Method B. The iodoaminocarazolol **10** (1 mg, 2.5 μmol) was treated by addition of 0.2 mg of $NaNO_2$ (0.2 mg, 1.2 equiv)/50 μL of H_2O and then (2-min interval) NaN_3 (0.2 mg, 1.3 equiv)/50 μL of H_2O in 1.0 mL of 3 N acetic acid at 0 °C. After 5 min, the reaction was halted by neutralization with concentrated NH_4OH (0.4 mL) and extraction with CH_2Cl_2 (4 × 1 mL). Compound **6** was isolated from the concentrated CH_2Cl_2 extract by HPLC (94% yield by HPLC). HPLC conditions were the same as for the

(27) Caron, M. G.; Srinivasan, Y.; Pitha, J.; Kociolek, K.; Lefkowitz, R. J. *J. Biol. Chem.* **1979**, *254*, 2923–2927.
(28) Mukherjee, C.; Caron, M. G.; Coverstone, M.; Lefkowitz, R. J. *J. Biol. Chem.* **1975**, *250*, 4869–4876.
(29) Laemmli, U. K. *Nature (London)* **1970**, *222*, 680–686.

iodoaminocarazolol, except that flow rate was 1.0 mL/min and retention time was 6.2 min, where **2** HPLC retention time was 7.3 min. Compared **6** was identical in all respects with that obtained in method A.

(±)-15-(4-Nitrobenzyl)carazolol (7). 2-(4-Nitrophenyl)-1,1-dimethylethylamine hydrochloride (1.0 g, 4.3 mmol) containing some of the 2-nitrophenyl isomer was partitioned between 10 mL of CH_2Cl_2 and 5 mL of 1 N NaOH to give, after standard operations, the free base, which was then combined with **5** (1.03 g, 1.0 equiv) in 20 mL of absolute EtOH and left at 65 °C under N_2 for 72 h. The reaction mixture was concentrated to dryness and left at <1 mmHg for 24 h. The residue was then dissolved in 5 mL of 1% CH_3OH/CH_2Cl_2 and applied to a silica gel column (2 × 70 cm; 200 g of Fisher grade, 100–200 mesh; packed in CH_2Cl_2; eluted with 200 mL of CH_2Cl_2, 500 mL of 1% CH_3OH/CH_2Cl_2, and then 500 mL of 2% CH_2OH/CH_2Cl_2; flow rate = 2 mL/min). 15-(2-Nitrobenzyl)carazolol was generally isolated in the 650–800-mL fraction: (amorphous solid; 0.34 g, 18% yield): TLC R_f 0.52 (20% $CH_3OH/CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.45 (m, 8, carbazole), 7.53 (m, 4, arom), 4.26 (m, 2, $ROCH_2$) 4.17 (m, 1, CHOH), 3.17 (s, 2, CH_2), 2.95 (m, 2, $RNHCH_2$), 1.06 (s, 6, CH_3). Compound **7** was isolated in the 850–1200-mL fraction (amorphous solid; 1.35 g, 72% yield): TLC R_f 0.50 (20% $CH_3OH/CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.45 (m, 8, carbazole), 7.91 (d, 2, arom), 7.22 (d, 2, arom), 4.25 (m, 3, $ROCH_2CHOH$), 3.07 (m, 2, $RNHCH_2$), 2.78 (s, 2, CH_2), 1.12 (s, 6, CH_3); MS, *m/e* 433 (<1), 297 (100), 298, 222, 183, 154, 114. Anal. Calcd for $C_{18}H_{21}N_2O_2$: 297.1602. Found: 297.1605. Anal. ($C_{25}H_{25}N_3O_4$ picrate) C, H.

(±)-15-(4-Aminobenzyl)carazolol (8). Compound **7** (1.00 g, 2.3 mmol) was dissolved in 25 mL of 2% concentrated HCl/CH_3OH (v/v), and ~50 mg of 5% Pd/C was left under 55 psi of H_2 pressure in a Parr low-pressure hydrogenator until hydrogen uptake was complete (1.5 h). The crude product was purified by chromatography on silica gel (1 × 70 cm; 100 g of Fisher grade, 100–200 mesh, packed in 5% CH_3OH/CH_2Cl_2; eluted with 200 mL of 5% CH_3OH/CH_2Cl_2 and then 500 mL of 10% CH_3OH/CH_2Cl_2; flow rate = 2 mL/min)., The 250–450-mL fraction contained the pure amine **8** (amorphous solid; 0.763 g, 82% yield): TLC R_f 0.20 (20% $CH_3OH/CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.47 (m, 8, carbazole), 6.94 (d, 2, arom), 6.55 (d, 2, arom), 4.25 (m, 3, $ROCH_2CHOH$), 3.03 (m, 2, $RNHCH_2$), 2.63 (s, 2, CH_2), 1.11 (s, 3, CH_3); MS, *m/e* 403 (<1), 297 (100) 298, 222, 183, 154, 148.5, 114. Anal. Calcd for $C_{18}H_{21}N_2O_2$: 297.1602. Found: 297.1605. Anal. ($C_{25}H_{29}N_3O_2$ picrate) C, H.

(±)-15-(4-Azidobenzyl)carazolol (2). The aminocarazolol **8** (10 mg, 25 mmol) was dissolved in 1 mL of 3 N acetic acid and treated with 2 mg of $NaNO_2$/20 mL of H_2O (1 equiv) at 0 °C for 20 min. $NaNO_2$ (1.8 mg, 1 equiv)/10 mL of H_2O) was then added, and the reaction was warmed at room temperature for 20 min. The reaction was halted by neutralization with concentrated NH_4OH (0.4 mL) and extracted with CH_2Cl_2 (4 × 5 mL). The crude product was purified by preparative TLC (Analtech silica gel GF; 1500 μm; 20 × 20 cm; developed in 20% $CH_3OH/CHCl_3$; R_f 0.55) to give **2** (7.5 mg, 70% yield; TLC R_f 0.42) identical with previously described pABC.[2]

Iodination of (±)-15-(4-Aminobenzyl)carazolol. The amine **8** (50 mg, 0.15 mmol) in 10 mL of 10% dioxane/0.1 N HCl was added to 100 mL of 0.5 N sodium acetate buffer (pH 5.6). To this was added, in rapid succession, 28 mg of NaI (1.5 equiv) in 85 mL 0.1 N NaOH and then 54 mg of chloramine T (1.5 equiv) in 25 mL of H_2O. A slight orange suspension evolved almost immediately (pH 10). At 5 min, the reaction was halted by the addition of 70 mg of $Na_2S_2O_5$ (3.0 equiv) in 25 mL of H_2O, and the aqueous mixture was then extracted with 4 × 50 mL of CH_2Cl_2. The crude product was subjected to preparative TLC on silica gel GF (20 × 20 cm; 1500 μm; applied in 1 mL of 20% $CH_3OH/CHCl_3$; eluted with the same solvent). A broad band between R_f 0.28 and 0.47 was eluted with 20% $CH_3OH/CHCl_3$ (2 × 30 mL), and the product mixture was subjected to HPLC [Alltech 0.46 × 25 cm silica column; 10 μm; eluted with 0.01% TEA/5% CH_3OH/CH_2Cl_2, v/v; flow rate = 1.5 mL/min; monitored by a Schoeffel UV monochromator (λ 300 nm)] to give the diiodo compound **11** [HPLC t_R = 6.00 min; TLC R_f = 0.38; ^1H NMR ($CDCl_3$) δ 7.39 (m, 7, carbazole), 6.97 (m, 3, arom), 4.22 (m, 3, $ROCH_2CHOH$), 3.00 (m, 2, $RNHCH_2$), 2.56 (s, 2, CH_2), 1.08 (s, 6, CH_3); MS, *m/e* 423 (100), 297 (69) 424, 369, 309, 232, 222, 183, 154, 116, 114), the 3-iodobenzyl compound **10** [HPLC t_R = 6.80 min; TLC R_f = 0.30; ^1H NMR ($CHCl_3$) δ 7.47 (m, 8, carbazole), 6.98 (m, 3, arom), 4.26 (m, 3, $ROCH_2CHOH$), 3.08 (m, 2, $RNHCH_2$), 2.63 (s, 2, CH_2), 1.14 (s, 6, CH_3); MS, *m/e* 529 (M^+ <5), 297 (100), 514, 298, 232, 222, 183, 154, 114. Anal. Calcd for ($M^+ - CH_3$) $C_{24}H_{25}N_3O_2I$: 514.0992. Found: 514.0997], and the 1-iodocarazolol (**9**) [HPLC t_R = 10.30 min; TLC R_f = 0.25; ^1H NMR ($CDCl_3$) δ 7.39 (m, 7, carbazole), 6.93 (d, 2, arom), 6.54 (d, 2, arom), 4.22 (m, 3, $ROCH_2CHOH$), 3.00 (m, 2, $RNHCH_2$), 2.59 (s, 2, CH_2), 1.04 (s, 6, CH_3); MS, *m/e* 529 (M^+, <5), 423 (100), 514, 424, 348, 309, 287, 222, 183, 154, 114, 106. Anal. Calcd for ($M^+ - CH_3$) $C_{24}H_{25}N_3O_2I$: 514.0992. Found: 514.0997]. Some starting material was also recovered: HPLC t_R = 13.30 min.

(±)-15-(4-Azidobenzyl)-1-iodocarazolol (12). By following exactly the second procedure described for the preparation of **2**, **9** (2 mg, 5.0 mmol) was converted into **12** (99% yield by HPLC): TLC R_f 0.50; HPLC t_R = 6.5 min; ^1H NMR ($CDCl_3$), 7.47 (m, 7, carbazole), 7.11 (d, 2, arom), 6.82 (d, 2, arom), 4.25 (m, 3, $ROCH_2CHOH$), 3.10 (m, 2, $RNHCH_2$), 2.71 (s, 2, CH_2), 1.25 (s, 6, CH_3); IR 2120 (N_3) cm^{-1}; MS, *m/e* 555 (M^+, <1), 423 (100), 424, 368, 348, 309, 297, 280, 222, 183, 154, 148.5, 114, 106. Anal. Calcd for $C_{18}H_{20}N_2O_2I$: 423.0570. Found: 423.0576.

(±)-15-Benzylcarazolol (14). 1,1-Dimethyl-2-phenylethylamine hydrochloride[2] (0.50 g, 2.7 mmol) was reacted, as the free base, with **5** (0.65 g, 1.0 equiv) in EtOH (20 mL) at 65 °C for 72 h. The reaction mixture was concentrated to dryness, and the residue was purified by chromatography (silica; 200 g of Fisher grade, 100–200 mesh; packed in CH_2Cl_2, eluted with 500 mL of CH_2Cl_2, 500 mL of 1% CH_3OH/CH_2Cl_2, and then 500 mL 5% CH_3OH/CH_2Cl_2; flow rate = 2 mL/min). Purified **14** was obtained in 84% yield (amorphous solid, 0.88 g): TLC R_f 0.35 (20% $CH_3OH/CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.47 (m, 13, carbazole and arom), 4.38 (m, 1, CHOH), 4.20 (m, 2, $ROCH_2$), 3.18 (m, 2, $RNHCH_3$), 2.86 (s, 2, CH_2), 1.21 (s, 6, CH_3); MS, *m/e* 388 (<1), 297 (100), 373, 298, 222, 196, 183, 154, 148.5, 114, 91. Anal. Calcd for ($M^+ - CH_3$) $C_{24}H_{25}N_2O_2$: 373.1915. Found: 373.1914. Anal. ($C_{25}H_{28}N_2O_2$ picrate) C, H.

4-(2,3-Epoxypropoxy)-1-iodocarbazole (15). Compound **5** (300 mg, 1.3 mmol) in CH_3OH (100 mL) was added to a freshly prepared mixture of chloramine T (707 mg, 2 equiv) and NaI (380 mg, 2 equiv) in 0.1 M K_2HPO_4 (100 mL; buffered to pH 7.5 with 0.1 M $KHPO_4$). The reaction color changed from orange to green immediately. The addition of $Na_2S_2O_5$ (150 mg, 2 equiv) at 20 min halted the reaction. The crude product was purified by chromatography on silica gel (2 × 25 cm; 100 g of Fisher grade, 100–200 mesh; packed and eluted with CH_2Cl_2; flow rate = 1 mL/min). The 20–55-mL fraction contained **15** (0.220 g, 48% yield) [mp 144–146 °C (crystallized from 50% hexane/ether); TLC R_f 0.74 (2% $CH_3OH/CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.38 (m, 7, carbazole), 4.31 (m, 2, $ROCH_2$), 3.52 (m, 1, CHO), 2.92 (m, 2, CH_2O); ^{13}C NMR ($CDCl_3$) δ 142.5 (C-1a), 65.7 (C-1), 134.3 (C-2), 103.5 (C-3), 155.2(C-4), 113.2 (C-4a), 122.9 (C-5a), 125.5 (C-5), 119.9 (C-6), 123.3 (C-7), 110.9 (C-8), 138.5 (C-8a), 68.9 (C-11), 50.2 (C-12), 44.5 (C-13); MS, *m/e* 366 (M^+ + 1), 365 (M^+, 100), 309, 308, 280, 195, 181, 154, 153, 126, and 81. Anal. Calcd for $C_{15}H_{12}IO_2N$: 364.9915. Found: 364.9911. Anal. ($C_{15}H_{12}IO_2N$) C, H, N] and unreacted **5** (140 mg) [TLC R_f 0.67; ^{13}C NMR ($CDCl_3$) δ 141.2 (C-1a), 104.2 (C-1), 126.6 (C-2), 101.2 (C-3), 155.0 (C-4), 112.8 (C-4a), 122.5 (C-5a), 125.0 (C-5), 119.6 (C-6), 123.1 (C-7), 110.1 (C-8), 138.9 (C-8a), 68.8 (C-11), 50.4 (C-12), 44.8 (C-13).

Radioiodination. The 4-aminobenzyl compound **8** (6 μg, 15 nmol, 1 μg/μL of 20 mM HCl) in 0.5 M sodium acetate buffer (24 μL, pH 5.6) was treated by sequential addition with $Na^{125}I$ (10 mCi, 4.5 nmol, >350 mCi/mL 0.1 N NaOH) and chloramine T (6 μg, 21 nmol) at room temperature. The reaction was halted at 1 min with $Na_2S_2O_5$ (8 μg, 42 nmol) and analyzed by TLC (20% $CH_3OH/CHCl_3$/1 mM phenol) cospotting with **8**. With a radioscanner, product distribution was determined to be **9** (60%, R_f 0.25), **10** (30%, R_f 0.30), and **11** (10%, R_f 0.38), with **8** observed by UV at R_f 0.20. The iodo amines were purified by TLC with autoradiography to locate the bands of product. Each band was eluted with $CHCl_3/CH_3CN$/TEA (85:35:5, v/v), filtered, concentrated, and immediately reconstituted in 1 mM phenol/ethyl acetate.[15a] Samples were stored under N_2 in the dark at −4 °C. Each isolated product was demonstrated to comigrate on TLC with the known parent compound. Since the radioiodinated

products cleanly separated from the unreacted 8, a specific activity of 2200 Ci/mol was assumed.

For conversion into azides, each sample of [^{125}I]**9**, **-10**, or **-11** (~1 mCi) was concentrated to dryness and reconstituted in 10 μL of 6 N acetic acid and then diluted with 10 μL of H_2O. At 0 °C, $NaNO_2$ (5 μg/mL of H_2O) and NaN_3 (5 μg/1 μL of H_2O) were added (2 min interval), and the reaction was warmed at room temperature for 5 min before neutralization with NH_4OH (8 μL). The reaction mixture was analyzed, and the products were isolated by the same procedure as described in the radioiodination above. Greater than 90% yield of **12** (R_f 0.50), **6** (R_f 0.48), and **13** (R_f 0.56) was obtained from **9**, **10**, and **11**, respectively. Each isolated product was demonstrated to comigrate with the known parent compound on TLC.

Acknowledgment. This work was supported in part by Grant HL16023. The authors thank Drs. W. Bartsch and K. Koch of Boehringer Mannheim GmbH for their generous gift of 4-(2,3-epoxypropoxy)carbazole, Marc Zdankiewicz, Rodney Seaforth, and Dr. Ludek Jirousek of New England Nuclear Corp. for their assistance with some of the radiolabeling experiments, Fred Williams of Research Triangle Institute for performing mass spectroscopy experiments, and Donna Addison and Lynn Tilley for expert secretarial assistance.

Registry No. **2**, 85135-25-7; **2** picrate, 85135-37-1; **3**, 82408-64-8; **4**, 81530-23-6; **4**·HCl, 85135-34-8; **5**, 51997-51-4; **6**, 85135-26-8; [^{125}I]**6**, 85135-45-1; **7**, 85135-27-9; **7** picrate, 85135-39-3; **8**, 82408-63-7; **8** picrate, 85150-62-5; **9**, 85135-28-0; [^{125}I]**9**, 85135-41-7; **10**, 85135-29-1; [^{125}I]**10**, 85135-42-8; **11**, 85135-30-4; [$^{125}I_2$]**11**, 85135-43-9; **12**, 85135-31-5; [^{125}I]**12**, 85135-44-0; [$^{125}I_2$]**13**, 85135-46-2; **14**, 85135-32-6; **14** picrate, 85135-40-6; **15**, 85135-33-7; 1,1-dimethyl-2-(4-aminophenyl)ethylamine, 51131-55-6; trifluoroacetic anhydride, 407-25-0; *N*-[1,1-dimethyl-2-(4-nitrophenyl)ethyl]-trifluoroacetamide, 85135-35-9; *N*-[1,1-dimethyl-2-(4-aminophenyl)ethyl]trifluoroacetamide hydrochloride, 85135-36-0; 1,1-dimethyl-2-(4-azido-3-iodophenyl)ethylamine, 81530-24-7; 2-(4-nitrophenyl)-1,1-dimethylethylamine hydrochloride, 79886-11-6; 2-(2-nitrophenyl)-1,1-dimethylethylamine hydrochloride, 79886-18-3; (±)-15-(2-nitrobenzyl)carazolol, 85135-38-2; 1,1-dimethyl-2-phenylethylamine, 122-09-8.

Supplementary Material Available: ^{1}H NMR spectra (250 MHz) of the aromatic region of all carazolol derivatives described in this paper (Figure 1), tabulated ^{1}H NMR chemical shifts (δ) of all carazolol derivatives described in this paper (Table 1), tabulated ^{13}C NMR chemical shifts (δ) of 4-(2,3-epoxypropoxy)carbazole (**5**) and 4-(2,3-epoxypropoxy)-1-iodocarbazole (**15**) (Table 2), and MS fragmentation pattern of carazolol derivatives (Scheme 1) (4 pages). Ordering information is given on any current masthead page.

Quantitative Aspects of the Receptor Binding of Cytokinin Agonists and Antagonists

Hajime Iwamura,*[†] Noboru Masuda,[‡] Koichi Koshimizu,[‡] and Satoshi Matsubara[§]

Department of Agricultural Chemistry, Faculty of Agriculture, and Department of Food Science and Technology, Kyoto University, Kyoto 606, Japan, and Laboratory of Applied Biology, Kyoto Prefectural University, Shimogamo, Kyoto 606, Japan. *Received August 23, 1982*

Congeneric 4-anilino- and 4-(alkylamino)-2-methylpyrrolo[2,3-*d*]pyrimidines showed cytokinin and anticytokinin activities, depending on the structure of their 4-substituents, and the antagonistic nature of the latter was established kinetically. The effect of the substituent on these activities was analyzed quantitatively by using physicochemical parameters and regression analysis to give a single, common equation for both the agonists and antagonists. The results indicated that the maximum width of the N^4 substituents is an important factor both for binding to the receptor, thus the extent of activity, and for the quality of activity, agonistic or antagonistic. The electron-withdrawing effect and hydrophobicity of the substituents further enhance binding and, thus, activity, irrespective of the quality of the activity. These results coincide with and/or provide evidence for the hypothesis that in hormonal action, agonist binding causes a conformational change of an otherwise inactive receptor to the active form and that antagonists are species that bind similarly to the receptor but do not cause the effective conformational change.

The agonists and antagonists of a biologically active compound play an important role in studying its bioregulatory mechanisms and its mode of action. In a field of a class of plant hormones, cytokinins, five structural classes of antagonists, anticytokinins, have been developed in the past 10 years.[1-7] All of them possess similarities in structure to naturally occurring N^6-adenylate cytokinins, like zeatin [(*E*)-6-[(4-hydroxy-3-methyl-2-butenyl)-amino]purine] and 6-(3-methyl-2-butenylamino)purine. Among these, the 4-substituted 2-methylpyrrolo[2,3-*d*]-pyrimidines[6] are interesting because their activity varies from agonistic to antagonistic with the transformation of the side chain at the 4-position. In the previous paper,[6] we have shown that a steric substituent parameter, W_{max}, which represents the maximum width of substituents from the bond axis between the exocyclic nitrogen atom and its α-carbon atom, governs the variation of the activity; i.e., the W_{max} values of the cytokinin agonists in this series are within the range of 4.7–6 Å, and those in which the W_{max} values are smaller or larger than this range are anticytokinins. It is thus obvious that the steric dimension of compounds is one of the main factors that determines the intrinsic activity, i.e., agonistic or antagonistic. The question that immediately arises is how they interact with the receptor. Quantitative structure–activity relationship

[†] Kyoto University, Department of Agricultural Chemistry.
[‡] Kyoto University, Department of Food Science and Technology.
[§] Kyoto Prefectural University.

(1) Hecht, S. M.; Bock, R. M.; Schmitz, R. Y.; Skoog, F.; Leonard, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 2608.
(2) Skoog, F.; Schmitz, R. Y.; Bock, R. M.; Hecht, S. M. *Phytochemistry* **1973**, *12*, 25.
(3) Iwamura, H.; Ito, T.; Kumazawa, Z.; Ogawa, Y. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 412.
(4) Iwamura, H.; Ito, T.; Kumazawa, Z.; Ogawa, Y. *Phytochemistry* **1975**, *14*, 2317.
(5) Skoog, F.; Schmitz, R. Y.; Hecht, S. M.; Frye, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 3508.
(6) Iwamura, H.; Masuda, N.; Koshimizu, K.; Matsubara, S. *Phytochemistry* **1979**, *18*, 217.
(7) Iwamura, H.; Murakami, S.; Koga, J.; Matsubara, S.; Koshimizu, K. *Phytochemistry* **1979**, *18*, 1265.

0022-2623/83/1826-0838$01.50/0