Development of a High Affinity and Stereoselective Photoaffinity Label for the D-1 Dopamine Receptor: Synthesis and Resolution of 7-[^{125}I]Iodo-8-hydroxy-3-methyl-1-(4'-azidophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine

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In an earlier paper, we reported the development of (±)-7-iodo-8-hydroxy-3-methyl-1-(4'-azidophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (I-MAB) and its ^{125}I analogue ([^{125}I]I-MAB) as selective, high affinity photoaffinity labels for the D-1 dopamine receptor. In this report, we now describe the complete synthesis and resolution of I-MAB and the pharmacological characterization of the stereoisomers in canine striatal membranes. *R*-(+)-I-MAB showed highly specific dopamine D-1 receptor binding (K_D = 0.28 nM) and binds selectively and stereoselectively to the D-1 receptor. These results further confirm the previous suggestion that, in the benzazepine series of DA agonists and antagonists, the activity principally resides in the *R*-(+) enantiomer, the *S*-(−) enantiomer being considerably less potent or inactive. Moreover, *R*-(+)-[^{125}I]I-MAB, upon photolysis, identifies the ligand-binding subunits of the neuronal D-1 receptor, with an apparent M_r of 74 000, 62 000, and 51 000 as assessed by autoradiography following sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Photoincorporation of *R*-(+)-[^{125}I]I-MAB into these polypeptides was stereoselectively blocked by D-1 dopaminergic ligands with an appropriate pharmacologic profile for the receptor. *R*-(+)-[^{125}I]I-MAB should thus prove to be a useful stereoselective photoaffinity label for the further characterization of the D-1 receptors.

Among the catecholamine neurotransmitters, dopamine (DA) occupies a unique position, owing to its extensive innervation in the central nervous system (CNS) as well as the periphery.[1,2] Disorders associated with these dopaminergic neurons have thus been implicated as the cause of several neurological, endocrinological, and cardiovascular diseases.[3] DA agonists are of clinical utility in Parkinsons disease[6] and neuroendocrine[4,5] disorders while DA antagonists have clinical utility in treatment of neuropsychiatric diseases[4,6] including schizophrenia and Huntington's disease.

The recent classification of DA receptors into D-1 and D-2 subtypes[7,8] has stimulated the development of potent, selective agonists and antagonists for potential clinical use. In order to fully realize the therapeutic potential of DA agonists and antagonists, it will be necessary to first understand the molecular nature and structure of the DA receptors and the nature of their interaction with ligands (agonists and antagonists), transducer units (G proteins), effectors (adenyl cyclase), and second messengers (cAMP) leading to a characteristic pharmacological response in a particular tissue or organ. The first step in this direction would be to characterize the receptor or more appropriately its ligand-binding subunit in terms of its molecular nature and size. This can usually be achieved by photoaffinity labeling[9,10] of either the crude (membrane bound) receptors or after purification by affinity chromatography using an immobilized affinity ligand, possessing an inherent selective affinity for the receptor, as the matrix.

Recent developments in the design and use of selective photoaffinity labels for the DA receptors have been critically reviewed.[11] The use of probes such as [^{125}I]N_3-NAPS,[12,13] [^{3}H]AMS,[14,15] and [^{125}I]IAC[16] has resulted in the preliminary identification and characterization of the D-2 receptor ligand binding subunit (M_r 94 000) in a variety of tissues and species. Progress in the development of photoaffinity labels for the D-1 receptor has been much slower, owing to a paucity of available ligands. A *p*-amino analogue of the potent and selective D-1 receptor antagonist, SCH 23390, was photoaffinity cross-linked to the D-1 receptor via a cross-linking agent, SANPAH.[17] The two obvious drawbacks here were the low level of photoincorporation via the cross-linking process and the lack of chemical characterization of the ligand, which could preclude commercial utility. In order to obtain a directly acting photoaffinity probe which could be made available commercially, we embarked on a program for the development of a selective, high-affinity and high specific activity photoaffinity probe for the D-1 receptor. This

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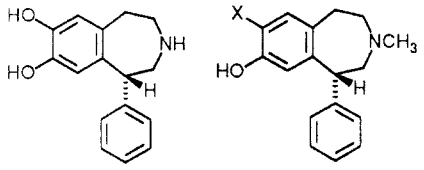
Scheme I



1, SKF 38393

2a: X = Cl, SCH 23390
b: X = Br, SKF 83566
c: X = I, SKF 103108A

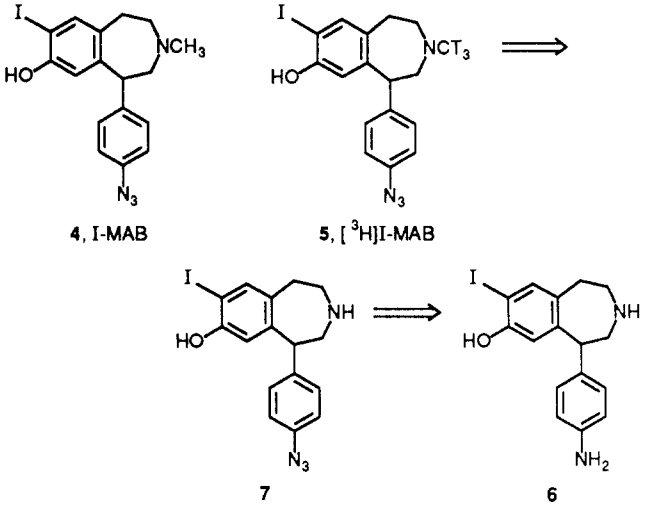
program has resulted in the successful development of (±)-[^{125}I]I-MAB,[18] which photolabeled the canine, bovine, and porcine neuronal D-1 receptors, identifying in the process a major polypeptide of an apparent M_r 74 000 and two minor polypeptides of apparent M_r 62 000 and 51 000 as the ligand-binding subunits of the receptors.[18,19]

Previous work with benzazepine DA receptor agonists and antagonists has led to the suggestion that the D-1 receptor binding affinity and agonist activity of these compounds resides principally in the *R*-(+) enantiomer with the *S*-(−) enantiomer being considerably less potent or inactive.[20-22] It would thus be reasonable to assume that only one of the enantiomers of (±)-I-MAB, possessing a relatively high affinity, is covalently bound to the D-1 receptor following photolysis (exhibiting a relatively high degree of specific photoincorporation). The other enantiomer, possessing relatively lower affinity, could be expected to exhibit a relatively low degree of specific photoincorporation accompanied by undesirable nonspecific covalent binding. Thus in order to preclude interference from the less potent enantiomer and thereby reduce the degree of nonspecific covalent binding and also obtain a photoaffinity label with a higher affinity and selectivity, we embarked on the development of a stereoselective photoaffinity probe, R-(+)-I-MAB. The complete synthesis and resolution of I-MAB will be discussed in this report along with the preliminary photoaffinity labeling and pharmacological binding results obtained with the enantiomers of I-MAB.

Design Considerations and Chemistry

The 7-substituted 8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines form a family of compounds possessing a potent, selective affinity for the D-1 subclass of DA receptors.[20,21] When the 7-substituent is hydroxyl, the resulting catechol benzazepines are potent, selective D-1 receptor agonists as in SKF 38393 (1).[20,21] Other 7-substituents such as the halogens (Cl = Br > I ≫ F), alkyl groups (methyl), or H (unsubstituted) provide potent, selective antagonists.[22] Since the available dopamine D-1 antagonists usually possess a higher affinity and selectivity than the D-1 agonists, they could serve as the prototypes for the development of molecular probes such as photoaffinity labels. We thus considered using SCH 23390 [7-Cl, **2a**, the most potent and selective D-1 receptor antagonist] as our prototype and carrying out two types of

Scheme II



4, I-MAB

5, [^{3}H]I-MAB

7

6

modifications (Scheme I): (1) replacement of the 7-Cl with a 7-I substituent, a modification which does not significantly affect the affinity or selectivity of the compound [SKF 103108A (7-I substituent, **2c**) retains the high affinity and selectivity of SCH 23390 (7-Cl substituent)],[23] and (2) introduction of an amino and subsequently the photolyzable azido substituent at the 4-position of the previously unsubstituted 1-aryl ring.

The initial strategy was to synthesize a tritiated photoaffinity ligand such as **5**. An approach to **5** was considered which involved an initial synthesis of **6** followed by conversion to the corresponding azide **7** and finally tritiation with CT_3I to obtain **5**. The synthesis of **6** was achieved by the route depicted in Scheme II. The 1-aryl-2,3,4,5-tetrahydro-1*H*-3-benzazepine skeleton was constructed by the method of Walter and Chang[24] (Scheme III). Condensation of *p*-nitrostyrene oxide[25] (**8**) with 4-methoxyphenethylamine (**9**) yielded **10**. Cyclization of **10** to **11** required the use of a more powerful dehydrating agent such as PPA instead of the usual mild and convenient sulfuric acid–TFA procedure. O-Demethylation of **11** with BBr_3 yielded **12**, which was selectively iodinated with ICl in AcOH to yield the 7-iodo analogue **13**. A number of reducing agents were used in an attempt to selectively reduce the nitro group without any significant deiodination. These, including hydrogenation over various catalysts and catalytic transfer hydrogenation (Raney Ni/hydrazine hydrate), were generally unsuccessful. However, reduction with Fe powder in a mixture of $AcOH/H_2O/EtOH$[26] led to the isolation of **6**, albeit with low overall yields (Scheme III). These low yields, together with the added attractiveness of a radioiodinated photoprobe as compared to a tritiated photoprobe (a radioiodinated probe can be obtained with a specific activity several orders of magnitude higher than that obtainable with a tritiated probe), prompted us to devise an alternative strategy for the synthesis of **4** (I-MAB), which could yield **4** in high specific activity by permitting radioiodination at the terminal step. This route, depicted in Scheme IV, has been discussed in our earlier paper.[18]

Resolution of I-MAB was carried out by repetitive recrystallizations of the diastereomeric di-*p*-toluoyltartaric acid salts of **14a** until the optical rotations reached a

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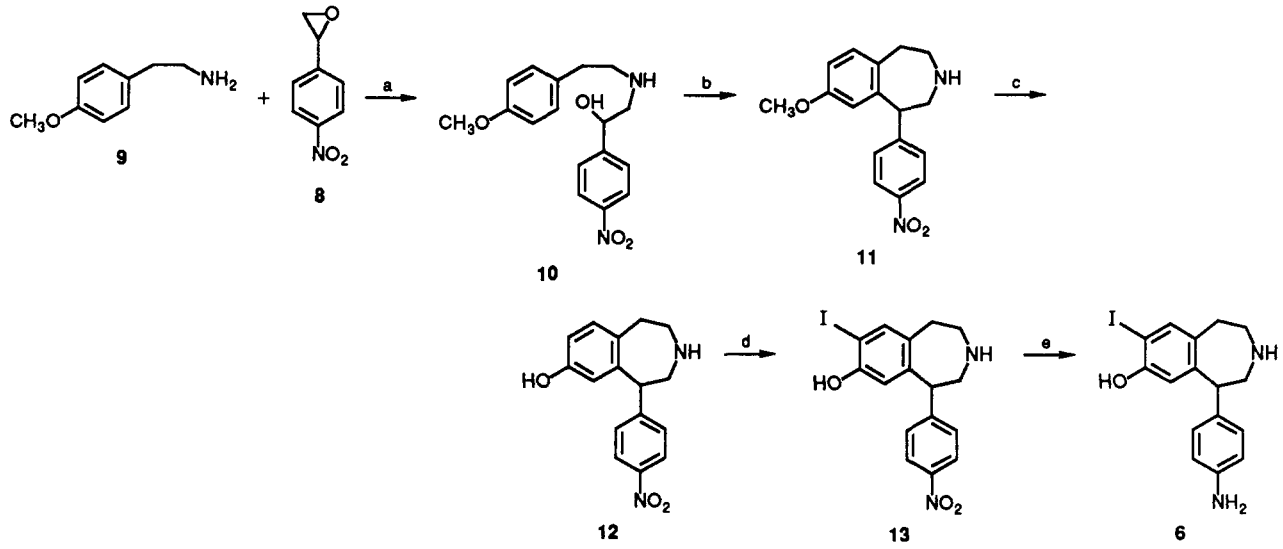
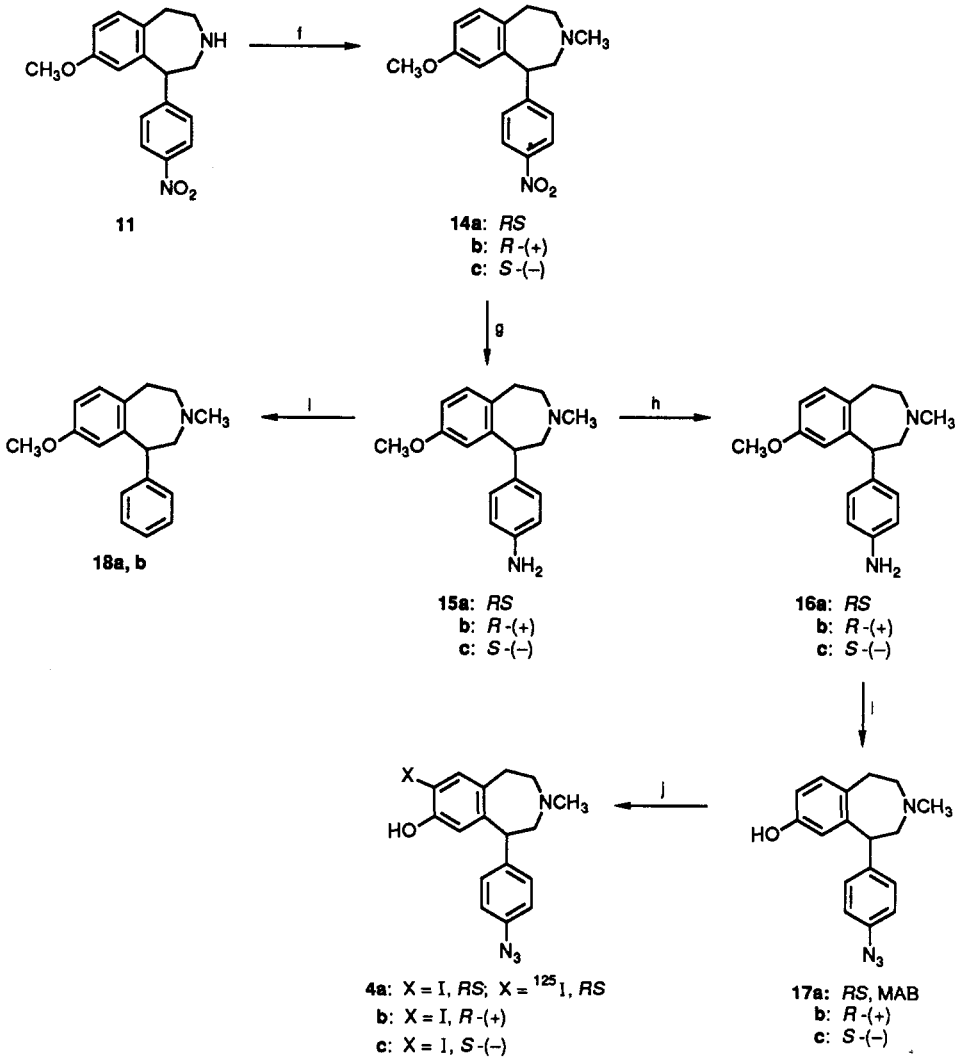
Scheme III[a]



[a] (a) THF, Δ; (b) PPA, 100 °C; (c) BBr_3, −70 °C; (d) ICl/AcOH; (e) Fe–EtOH/H_2O/AcOH (10:9:1).

Scheme IV[a]



[a] (f) HCHO/HCOOH; (g) Raney Ni/N_2H_4, H_2O; (h) BBr_3, −70 °C or 48% HBr; (i) $NaNO_2/H^+$, NaN_3; (j) ICl/AcOH or Na [^{125}I]chloramine T; (l) conc H_2SO_4, $NaNO_2$, H_3PO_2.

constant value. The optically pure enantiomers were converted to their free bases before successive Raney Ni reduction, HBr O-demethylation, and diazotization followed by treatment with sodium azide and finally iodination with ICl in AcOH, in a similar manner as described for the racemic (±)-I-MAB (**4a**), to obtain optically pure *R*-(+)-I-MAB (**4b**) and *S*-(−)-I-MAB (**4c**). The optical purity and absolute configuration of the enantiomers were

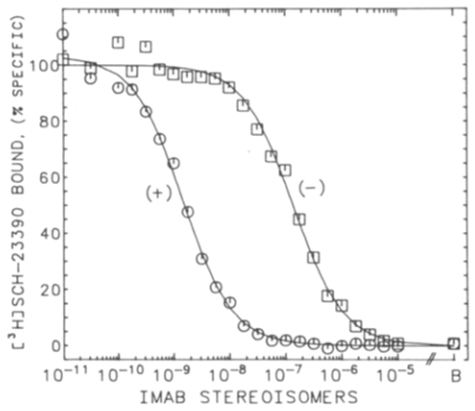


Figure 1. Competition of [^{3}H]SCH-23390 binding to canine striatal D1 receptors by (+)-IMAB and (−)-IMAB. Membranes were prepared and incubated with 150 pM [^{3}H]SCH-23390 and the indicated concentrations of competing ligands in the dark for 120 min at 22 °C and assayed for D-1-receptor activity. Data were analyzed by the nonlinear least-square curve-fitting program LIGAND with estimated K_D values listed in the text. Results are representative of three independent experiments.

determined by deamination of the optically pure enantiomers of **15a** by diazotization followed by treatment with hypophosphorous acid, to obtain the respective enantiomers of the known 8-methoxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine.[27] Values of the specific rotations of **18a** and **18b** were identical with the values reported in the literature.[27] Radioiodination of both MAB and *R*-(+)-MAB was carried out in an essentially similar manner, as previously reported,[19] by using a modified Na[^{125}I]chloramine T procedure. the respective radioiodinated analogues [^{125}I]I-MAB and *R*-(+)-[^{125}I]I-MAB were obtained, after purification by reverse-phase HPLC, in high radiochemical yields (50%) and high specific activity (2200 Ci/mmol).

Pharmacological Results

Figure 1 depicts the ability of the stereoisomers (+)- and (−)-I-MAB to compete for [^{3}H]SCH 23390 binding to canine striatal membranes. As predicted from previous structure–activity relationships of various benzazepine-like compounds on the D-1 dopamine receptor,[20-22] (+)-I-MAB was 100-fold more potent than the (−)-enantiomer at inhibiting [^{3}H]SCH 23390 binding with an estimated K_i of 280 pM, vs 28 nM, respectively. These K_i values correspond exceedingly well to the K_i estimates of the stereoisomers (+)-SCH 23390 and SCH 23388 (the (−)-isomer of SCH 23390) for [^{3}H]SCH 23390 binding to dopamine D-1 receptors of both the brain (150 pM vs 24 nM) and calf parathyroid gland (160 pM vs 18 nM) and with the estimated K_i values of these compounds at inhibiting dopamine-stimulated adenylate-cyclase activity (100 pM vs 37 nM) in dispersed cells of the bovine parathyroid gland.[28]

Figure 2 depicts the results obtained when striatal membranes were incubated with varying concentrations for (+)-[^{125}I]I-MAB and photolyzed, and samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. As with (±)-[^{125}I]I-MAB,[18,19] three distinct bands of apparent M_r 74 000, 62 000, and 51 000 were labeled in canine striatal membranes. Photoincorporation of *R*-(+)-[^{125}I]I-MAB into these polypeptides was concentration dependent and was completely inhibited by 100 nM (+)-butaclamol during

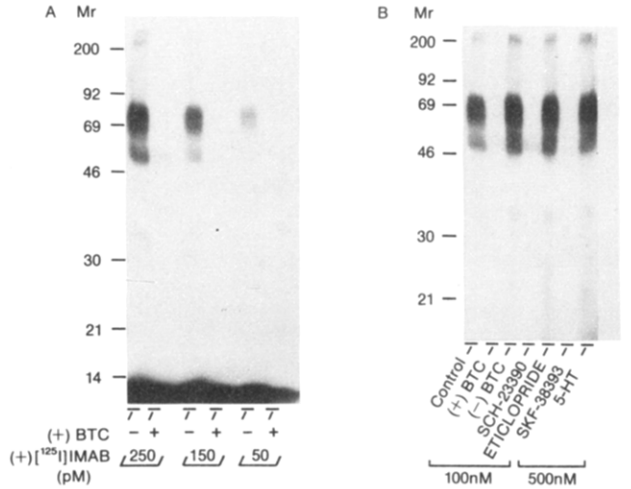


Figure 2. (A and B) Photoaffinity labeling and pharmacological specificity of (+)-[^{125}I]IMAB photoincorporation into canine striatal membranes. Membranes were prepared, incubated with the indicated concentrations of (+)-[^{125}I]IMAB in the presence or absence of 1 μM (+)-butaclamol (A) or with 250 pM (+)-[^{125}I]IMAB and the indicated concentrations of competing ligands (B), and photolyzed, and samples were subjected to SDS-PAGE using a 12% acrylamide gel and autoradiography. The M_r of known protein standards are shown × 1000. The results shown are representative of two similar experiments. Abbreviations used are (+)BTC, butaclamol and 5-HT, serotonin.

photoaffinity labeling. The pharmacological specificity of (+)-[^{125}I]I-MAB photoincorporation was assessed further by examining the ability of various dopaminergic agents to block the labeling of these subunits. As shown in Figure 2B, the photolysis-dependent labeling of the M_r 74 000, 62 000, and 51 000 polypeptides was stereoselectively antagonized by (+)- and not (−)-butaclamol, and by the D-1 receptor agonist SKF 38393 and antagonist SCH 23390, but not by the selective D-2 receptor antagonist eticlopride or the neurotransmitter serotonin. The data appear consistent with the contention that the labeled M_r 74 000, 62 000, and 51 000 polypeptides represent the ligand-binding subunits of the D-1 dopamine receptor. Recent work using *R*-(+)-[^{125}I]I-MAB has indicated that the existence of multiple labeled D-1 receptor polypeptides seen with SDS-PAGE is due to the microheterogeneity in the deglycosylation patterns of the polypeptides and upon complete deglycolyzation only one labeled polypeptide is observed.[29] Taken together, these data suggest that *R*-(+)-[^{125}I]I-MAB is a potent photoaffinity probe for D-1 dopamine receptors and will undoubtedly aid in the subsequent molecular characterization of these proteins.

Experimental Section

Melting points were determined on a Thomas Hoover apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab Co., Atlanta, GA and were ±0.4% of the theoretical values. ^{1}H NMR spectra were determined with a Varian XL-300 (300 Hz) spectrometer. Mass spectra were determined with a nuclide 12–90-G mass spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter.

***p*-Nitrostyrene Oxide (8).** To a solution of 30.0 g (0.196 mol) of 4-nitrobenzaldehyde in 700 mL of methylene chloride were added 0.4 g of tetrabutylammonium iodide, 40.8 g (0.2 mol) of trimethylsulfonium iodide, and 150 mL of a 50% aqueous solution of sodium hydroxide. The reaction mixture was stirred under reflux for 24 h and then poured into 700 mL of cold water, and

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the organic layer was drawn off. the aqueous layer was further extracted with methylene chloride, and the combined organic extracts were washed well with water, saturated aqueous sodium metabisulfite solution, water, and brine. The extract was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Recrystallization of the residue from methanol afforded 22 g (67%) of yellow crystals, mp 84–85 °C (lit.[25] mp 85–86 °C). Anal. ($C_8H_7NO_3$) C, H, N.

α-[2-[*N*-(4-Methoxyphenethyl)amino]methyl]benzyl Alcohol (10). A solution of 11.3 g (0.075 mol) of 4-nitrostyrene oxide (8) and 11.3 g (0.0927 mol) of 4-methoxyphenethylamine (9) in 110 mL of dry THF was stirred and allowed to reflux under nitrogen for 24 h. The reaction mixture was cooled and concentrated in vacuo. Anhydrous ether (250 mL) was added and the precipitated product was filtered, washed with ether until colorless, and dried in vacuo to yield 14 g (65%) of a white, fluffy powder, mp 120–122 °C. Anal. ($C_{17}H_{20}N_2O_4$) C, H, N.

8-Methoxy-1-(4′-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (11). Product 10 (11.0 g, 35 mmol) was triturated with 100 g of PPA and the mixture was heated at 100 °C for 2 h. The mixture was poured into boiling water, made alkaline with aqueous sodium hydroxide solution, and cooled. Extraction with methylene chloride followed by washing with water, drying over anhydrous magnesium sulfate, filtering, and concentration in vacuo yielded a pale orange oil, which crystallized on standing overnight at 0 °C to orange-red crystals 9.5 g (91%), mp 132–134 °C. Anal. ($C_{17}H_{18}N_2O_3$) C, H, N.

8-Hydroxy-1-(4′-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrobromide (12). A solution of 0.7 g (25 mmol) of 11 in 70 mL of dry methylene chloride was stirred under N_2 at −70 °C and a solution of 1.25 g (5 mL, 1.0 M solution) of BBr_3 in hexane was added dropwise to it. The reaction mixture was stirred at −70 °C for 1 h and then at room temperature for 2 h and was quenched by cooling to −70 °C and adding 25 mL of anhydrous MeOH dropwise. The solution was allowed to reflux for 30 min, cooled, and concentrated in vacuo to a tan foam. Recrystallization from MeOH/Et_2O gave 0.66 g of a tan powder (80%), mp >250 °C. Anal. ($C_{16}H_{17}N_2O_3Br\cdot 2H_2O$) C, H, N.

7-Iodo-8-hydroxy-1-(4′-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrochloride (13). To a stirred solution of 0.6 g (2.1 mmol) of 12 in 30 mL of AcOH was added 0.35 g (2.1 mmol) of ICl. The reaction mixture was stirred at room temperature for 3 h and poured into water, and the resulting yellow precipitate was filtered, washed with cold water until free of acid, and dried in vacuo to yield 0.5 g of yellow powder. The crude product was reasonably pure by TLC (silica gel, CH_2Cl_2/MeOH 9:1) and ^1H NMR spectra (300 Hz, CD_3OD) was consistent with the assigned structure of the product.

7-Iodo-8-hydroxy-1-(4′-aminophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine Dihydrochloride (6). To 0.3 g (0.75 mmol) of 13 dissolved in 40 mL of a mixture containing 20 mL of EtOH, 18 mL of water, and 2 mL of AcOH was added 0.3 g of Fe powder, and the suspension was stirred under reflux for 6 h. The mixture was then poured into water, made alkaline with aqueous ammonia, and extracted with ethyl acetate. The extracts were washed well with water and with brine and then were dried over anhydrous magnesium sulfate and filtered. Concentration of the filtrate in vacuo gave a pale yellow residue, which was crystallized from MeOH/Et_2O·HCl to yield 60 mg (20%) of a yellow crystalline powder: mp 235–238 °C; MS m/z = 380 (M^+). Anal. ($C_{16}H_{17}N_2OI\cdot 2HCl\cdot 1.25H_2O$) C, H, N.

8-Methoxy-3-methyl-1-(4′-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (14a). A solution of 0.65 g (2.2 mmol) of 11 in 0.36 g (0.008 M) of 88% formic acid and 0.55 g (6.7 mmol) of 36% formaldehyde was stirred and allowed to reflux for 24 h. The reaction mixture was then poured into ice-cold water and made alkaline with aqueous ammonia and extracted with methylene chloride. The extracts were washed well with water and with brine and then were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to a yellow oil, which crystallized on standing overnight at 0 °C. The crystals were filtered, washed with a little cold ethanol, and dried in vacuo to yield 0.63 g (92%), mp 116–118 °C. Anal. ($C_{18}H_{20}N_2O_3$) C, H, N.

(*R*)-(+)-8-Methoxy-3-methyl-1-(4′-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrochloride (14b). To 9.0 g (0.29 mol) of 14a were added 11.7 g (0.29 mol) of (−)-di-toluoyl-L-tartaric acid and 300 mL of methanol. The mixture was allowed to reflux until all the solids dissolved. After standing at room temperature overnight, the crystals were collected and washed with methanol followed by ether. The crystals were dried in vacuo and the specific rotation was determined. This process was repeated until a constant rotation was obtained: $[\alpha]_D$ −52.1° (*c* 0.5, MeOH); mp 184–186 °C. Anal. ($C_{18}H_{20}N_2O_3\cdot C_{20}H_{18}O_8$) C, H, N.

The optically pure salt (4.3 g), obtained in the preceding step, was suspended in water, aqueous $NaHCO_3$ was added, and the mixture was extracted with ether. The extracts were dried and concentrated to an oil. The HCl salt was crystallized from EtOH·HCl/Et_2O to yield 2.0 g of a white, crystalline powder: mp 258–260 °C; $[\alpha]_D$ +26.9° (*c* 1, MeOH). Anal. ($C_{18}H_{20}N_2O_3$·HCl) C, H, N.

(*S*)-(−)-8-Methoxy-3-methyl-1-(4′-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrochloride (14c). The combined mother liquors from the resolution of 14b were evaporated in vacuo and the residue was converted to free base by making it alkaline with aqueous $NaHCO_3$ and extracting with ether. The free base was allowed to reflux with 1 equiv of (+)-ditoluoyl-D-tartaric acid in 300 mL of MeOH. The same resolution procedure as before for the *R*-(+)-stereoisomer was repeated until a constant rotation was obtained: $[\alpha]_D$ +52.0° (*c* 0.5, MeOH); mp 184–186 °C.

The crystalline salts were converted to the oily free base in the same way as before and the HCl salt was crystallized from EtOH/Et_2O to yield 1.8 g of a white crystalline powder: mp 258–260 °C; $[\alpha]_D$ −26.5° (*c* 1, MeOH). Anal. ($C_{18}H_{20}N_2O_3$·HCl) C, H, N.

8-Methoxy-3-methyl-1-(4′-aminophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (15a). To a stirred solution of 1.2 g (3.85 mmol) of 14a in 25 mL of absolute ethanol at 50–55 °C was added 1.2 mL (24.2 mmol) of hydrazine hydrate followed by small portions of Raney Ni until all gas evolution ceased. The reaction mixture was filtered to remove the catalyst and was concentrated in vacuo to yield 0.8 g (92%) of a yellow solid, mp 190–192 °C. Anal. ($C_{18}H_{22}N_2O$) C, H, N.

(*R*)-(+)-8-Methoxy-3-methyl-1-(4′-aminophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine Dihydrochloride (15b). A similar procedure was applied to 0.4 g of 14b to obtain 0.38 g of the HCl salt as a white, crystalline powder (93%): mp 265–268 °C; $[\alpha]_D$ +18.3° (*c* 1, MeOH). Anal. ($C_{18}H_{22}N_2O\cdot 2HCl$) C, H; N: calcd, 7.88; found, 7.30.

(*S*)-(−)-8-Methoxy-3-methyl-1-(4′-aminophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine Dihydrochloride (15c). A similar procedure was applied to 0.6 g of 14c to yield 0.575 g (94%) of 15c: mp 265–268 °C; $[\alpha]_D$ −17.8° (*c* 1, MeOH). Anal. ($C_{18}H_{22}N_2O\cdot 2HCl$) C, H, N.

8-Hydroxy-3-methyl-1-(4′-aminophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (16a). To a solution of 1.0 g (12.2 mmol) of 15a in 20 mL of dry methylene chloride at −70 °C under N_2 was added a solution of 5 g (20 mmol) of boron tribromide in 20 mL of hexane (1.0 M solution). The reaction mixture was stirred at −70 °C for 1 h and then at room temperature for 3 h. It was then quenched with methanol at −70 °C and concentrated in vacuo to an oil. A suspension of this oil in methylene chloride was washed with 10% aqueous sodium bicarbonate solution, and the organic layer was drawn off and washed with water and with brine. It was then dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to a pale yellow oil, which was purified by flash chromatography (silica gel, CH_2Cl_2/CH_3OH 9:1) to yield 780 mg of a yellow solid (80%), mp 154–156 °C. A small sample was crystallized as the methanesulfonate salt from ethanol/ether: mp 230–232 °C; MS m/z = 268 (M^+). Anal. ($C_{17}H_{20}N_2O\cdot 2CH_3SO_3H\cdot 2H_2O$) C, H, N.

(*R*)-(+)-8-Hydroxy-3-methyl-1-(4′-aminophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine Dihydrochloride (16b). A solution of 0.3 g (0.84 mmol) of 15b in 10 mL of 48% hydrobromic acid was heated with stirring at 110 °C for 10 h. The reaction mixture was poured into water, made alkaline with aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The extract was dried over anhydrous $MgSO_4$ and concentrated in vacuo to yield 0.223 g (86%) of a pale yellow solid, mp 224–226 °C. The free base was converted to the dihydrochloride 16b: mp >270 °C; $[\alpha]_D$ +21.5° (*c* 0.5, MeOH).

(S)-(-)-8-Hydroxy-3-methyl-1-(4'-aminophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine Dihydrochloride (16c). A similar procedure was applied to 0.2 g of **15c** to yield 0.12 g of a pale yellow solid (62%), mp 224–226 °C. The free base was converted to the dihydrochloride **16c**: mp >270 °C; $[\alpha]_D$ −22.8° (*c* 0.5, MeOH).

8-Hydroxy-3-methyl-1-(4'-azidophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (17a, MAB). Compound **16a** (125 mg, 0.47 mmol) was dissolved in a solution of 6 mL of concentrated H_2SO_4 in 30 mL of water and cooled to 0 °C. Sodium nitrite (69 mg, 1.0 mmol) in 2 mL of water was added to it and the reaction mixture was stirred at 0 °C for 15 min before being quenched with a slight excess of sulfamic acid. Sodium azide (65 mg, 1.0 mmol) in 2 mL of water was added to the reaction mixture at 0 °C and it was stirred for 2 h. Then it was poured over crushed ice and made alkaline with aqueous ammonia and extracted with methylene chloride. The extracts were washed well with water and with brine and then were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to yield a yellow residue: yield 110 mg (80%); mp 95–100 °C dec; MS *m/z* = 294 (M^+), 266 ($M - N_2$)$^+$. Anal. ($C_{17}H_{18}N_4O \cdot 0.5H_2O$) C, H; N: calcd, 18.47; found, 19.99.

(R)-(+)-8-Hydroxy-3-methyl-1-(4'-azidophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (17b). A similar procedure was applied to 0.19 g of **16b** to yield 60 mg of **17b** as yellow crystals: mp 125–127 °C; $[\alpha]_D$ +69° (*c* 0.5, MeOH); MS *m/z* = 294 (M^+). Anal. ($C_{17}H_{18}N_4O$) C, H, N.

(S)-(-)-8-Hydroxy-3-methyl-1-(4'-azidophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (17c). A similar procedure was applied to 0.14 g of **16c** to yield 64 mg of **17c** as yellow crystals: mp 125–127 °C; $[\alpha]_D$ −68° (*c* 0.5, MeOH); MS *m/z* = 294 (M^+). Anal. ($C_{17}H_{18}N_4O$) C, H; N: calcd, 18.85; found, 18.08.

8-Hydroxy-7-iodo-3-methyl-1-(4'-azidophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine Hydrochloride (4a, I-MAB). To a stirred solution of 40 mg (0.136 mmol) of **17a** in 35 mL of glacial acetic acid was slowly added a solution of 22 mg (0.135 mmol) of iodine monochloride in 15 mL of glacial acetic acid. The reaction mixture was stirred at room temperature for 1 h, then poured over crushed ice and made alkaline with aqueous ammonia. The mixture was then extracted with CH_2Cl_2; the extracts were washed well with water and with brine and were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to a brown oil. Purification by flash chromatography on a silica gel column, eluting with CH_2Cl_2/MeOH (9:1), yielded a pale yellow oil, pure by TLC. The HCl salt was crystallized from EtOH·HCl/Et_2O to yield 30 mg (50%) of a white, crystalline powder: mp 175–180 °C dec; MS *m/z* = 420 (M^+), 392 ($M - N_2$)$^+$. Anal. ($C_{17}H_{18}N_4OICl \cdot 1.25H_2O$) C, H, N.

(R)-(+)-8-Hydroxy-7-iodo-3-methyl-1-(4'-azidophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine Hydrochloride (4b). A similar procedure was applied to 40 mg of **17b** to yield 32 mg of a white, crystalline powder: mp 179–182 °C dec; $[\alpha]_D$ +16.7° (*c* 0.5, MeOH); MS *m/z* = 420 (M^+). Anal. ($C_{17}H_{18}N_4OICl$) C, H; N calcd, 12.27; found, 11.48.

(S)-(-)-8-Hydroxy-7-iodo-3-methyl-1-(4'-azidophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine Hydrochloride (4c). A similar procedure was applied to 50 mg of **17c** to yield 30 mg of a white, crystalline powder: mp 180–182 °C dec; $[\alpha]_D$ −16.2° (*c* 0.5, MeOH); MS *m/z* = 420 (M^+). Anal. ($C_{17}H_{18}N_4OICl$) C: calcd, 44.71; found, 44.05; H: calcd, 3.91; found, 4.08; N: calcd, 12.27; found, 11.34.

(R)-(+)-7-[^{125}I]Iodo-8-hydroxy-3-methyl-1-(4'-azidophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine Hydrochloride ([^{125}I]-4b). The same procedure as previously reported[19] for the synthesis of *RS*[^{125}I]-**4a** was applied to the synthesis of *R*-(+)-[^{125}I]-**4b**. A product with a specific activity of 2200 Ci/mmol was obtained, after purification by reverse-phase HPLC.

Determination of Configuration and Optical Purity of 14b and 14c. The configuration and optical purity of **14b** and **14c** were determined by conversion of **15b** and **15c** to the corresponding enantiomers of 8-methoxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, which are known compounds.[27] Deamination was performed as follows.

Compound **15b** (200 mg, 0.56 mmol) was dissolved in a mixture of 4 mL of concentrated H_2SO_4 in 20 mL of water. The solution was cooled to 0 °C and 60 mg (0.87 mmol) of sodium nitrite in 2 mL of water was added. After 20 min, 1 g (7.6 mmol) of 50% aqueous H_3PO_2 was added to the reaction mixture, which was stirred at 0 °C for 4 h. The mixture was then made alkaline with aqueous $NaHCO_3$; the product extracted was with ether. The extract was dried over $MgSO_4$ and evaporated to yield an oil, which crystallized on standing at 0 °C to give 126 mg of **18a**: mp 50–51 °C; $[\alpha]_D$ +51.3° (*c* 1, EtOH). [lit[29] $[\alpha]_D$ +51.8° (*c* 1, EtOH)].

The same procedure was applied to 150 mg of **14c** to yield **18b**, $[\alpha]^{25}_D$ −50.6° (*c* 1, EtOH).

[^{3}H]SCH 23390 Binding Assays. Canine striatal membranes were prepared and incubated with [^{3}H]SCH 23390 (150 pM) and the indicated concentrations of (+)- and (−)-I-MAB in the dark for 120 min at 22 °C as previously described.[19]

Photoaffinity Labeling and SDS-PAGE. Canine striatal membranes were photolyzed with the indicated concentrations of *R*-(+)-[^{125}I]I-MAB (2200 Ci/mmol), followed by SDS-PAGE and autoradiography as previously described.[19]

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Registry No. **4a**, 116234-50-5; **4a**·HCl, 116351-51-0; **4b**, 123356-68-3; **4b**·HCl, 123406-40-6; (^{125}I)-**4b**, 123356-70-7; (^{125}I)-**4b**·HCl, 123406-39-3; **4c**, 123356-69-4; **4c**·HCl, 123406-41-7; (±)-**6**, 123291-60-1; (±)-**6**·2HCl, 123291-59-8; **8**, 6388-74-5; **9**, 55-81-2; (±)-**10**, 116351-49-6; (±)-**11**, 116234-46-9; (±)-**12**, 123291-57-6; (±)-**12**·HBr, 123291-61-2; (±)-**13**, 123291-58-7; (±)-**13**·HCl, 123291-62-3; **14a**, 116234-47-0; **14b**, 123356-53-6; **14b**·HCl, 123356-55-8; **14b**·L-tartrate salt, 123356-54-7; **14c**, 123356-56-9; **14c**·HCl, 123356-58-1; **14c**·D-tartrate salt, 123356-57-0; **15a**, 116263-67-3; **15b**, 123356-59-2; **15b**·2HCl, 123406-55-3; **15c**, 123356-60-5; **15c**·2HCl, 123356-61-6; **16a**, 116234-48-1; **16a**·2$MeSO_3H$, 116351-50-9; **16b**, 123356-62-7; **16b**·HCl, 123356-63-8; **16c**, 123356-64-9; **16c**·HCl, 123356-65-0; **17a**, 116234-45-8; **17b**, 123356-66-1; **17c**, 123356-67-2; *p*-$NO_2C_6H_4CHO$, 555-16-8; dopamine, 51-61-6.