base), 103923-50-8; **11**, 100227-05-2; **11** (free base), 103923-27-9; **12**, 103923-33-7; **12** (free base), 103923-51-9; **13**, 103923-28-0; **13** (free base), 103923-53-1; **14**, 115561-85-8; **14** (free base), 115561-91-6; **15**, 103923-39-3; **15** (free base), 103923-57-5; **16**, 103923-40-6; **16** (free base), 103923-58-6; **17**, 103923-31-5; **17** (free base), 103923-49-5; **18**, 115561-86-9; **18** (free base), 115561-92-7; **19**, 103923-36-0; **19** (free base), 103923-54-2; **20**, 103923-41-7; **20** (free base), 103923-59-7; **21**, 103923-43-9; **21** (free base), 103944-26-9; **22**, 103923-46-2; **22** (free base), 103923-63-3; **23**, 103923-44-0; **23** (free base), 103923-61-1; **24**, 103923-45-1; **24** (free base), 103923-62-2; **25**, 96287-46-6; **25** (free base), 102635-81-4; **26**, 103923-47-3; **26** (free base), 103923-64-4; $CH_3(CH_2)_6Cl$, 629-06-1; $CH_3(CH_2)_5Cl$, 544-10-5; $CH_3(CH_2)_7Br$, 111-83-1; $CH_3(CH_2)_8Cl$, 2473-01-0; $CH_3(CH_2)_6Br$, 629-04-9; $PhCH_2Cl$, 100-44-7; p-$ClC_6H_4CH_2Cl$, 104-83-6; 4-(*n*-octylamino)pyridine, 64690-19-3; 1-chlorooctane, 111-85-3; 4-(*n*-hexylamino)pyridine, 64690-14-8; *n*-hexyl bromide, 111-25-1; sodium saccharinate, I28-44-9; 4-(*n*-decylamino)pyridine, 64690-61-5; 1-iodopentane, 628-17-1; *N*-heptyl-4-pyridinamine, 35036-87-4; *N*-nonyl-4-pyridinamine, 64690-27-3; *N*-dodecyl-4-pyridinamine, 64690-59-1; *N*-tetradecyl-4-pyridinamine, 115561-94-9; *N*-(2-ethylhexyl)-4-pyridinamine, 64690-39-7; 1-bromo-2-ethylhexane, 18908-66-2.

(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-[^{125}I]iodo-2-methoxybenzamide Hydrochloride, a New Selective Radioligand for Dopamine D-2 Receptors

Tomas de Paulis,*[†] Aaron Janowsky,[‡] Robert M. Kessler,[§] Jeffrey A. Clanton,[§] and Howard E. Smith[†]

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235, and Departments of Psychiatry and Radiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232. *Received February 2, 1988*

From salicyclic acid, the two enantiomers of *N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2-methoxybenzamide (**6b**) were prepared in a five-step synthesis. With use of Heindel's triazene method for introduction of the radionuclide, the iodine-125-labeled substituted benzamide was obtained with a calculated specific activity of 136 Ci/mmol and 14% radiochemical yield. For the preparation of the iodine-125-labeled benzamide with higher specific activity, this method was unsuccessful and utilization of the corresponding tri-*n*-butyltin derivative was required. Treatment of the latter in dilute hydrochloric acid with sodium iodide-125 and chloramine-T gave [^{125}I](*S*)-**6b** in 56% radiochemical yield and at least 97% radiochemical purity. The displacement of [^{125}I](*S*)-**6b** and [^{3}H](*S*)-sulpiride from their respective binding sites in striatal rat brain homogenates using various neuroleptic agents showed that (*S*)-**6b** has the same binding profile but more potent binding for dopamine D-2 receptors than has sulpiride. These experiments also indicate that the *S* enantiomer of **6b** is a specific ligand (K_D = 1.2 nM) for the D-2 receptor. Further, the octanol–water partition coefficient of (*S*)-**6b** as determined by reverse-phase high-performance liquid chromatography was found to be 40 times greater than that for sulpiride. Thus (*S*)-**6b** has a lipophilicity that will allow a relatively higher uptake into the brain compared to sulpiride. In vivo experiments with rats show that [^{125}I](*S*)-**6b** penetrates readily into the brain and is preferentially localized in the striatum as compared to the cerebellum, the ratio of uptake being 7.2 to 1, 60 min after injection. These observations of good brain penetration and high affinity and selectivity for D-2 receptors indicate that the corresponding iodine-123-labeled benzamide may be a useful ligand for the noninvasive visualization study of dopamine D-2 receptor sites in vivo by single photon emission computed tomography.

Selective, radioiodinated ligands for brain neurotransmitter binding sites may serve as pharmacological tools for the elucidation of the mechanism of action of psychotropic agents and as diagnostic agents in the investigation of patients suffering from neuropsychiatric disorders. New radioiodination techniques for small organic molecules have made this possible.[1] Several radionuclides for labeling of central dopamine receptors using various γ or positron emitting isotopes have been described: ^{125}I in autoradiography, ^{77}Br and ^{123}I in single photon emission computed tomography (SPECT),[1,2] and ^{76}Br, ^{18}F, and ^{11}C in positron emission tomography (PET).[2]

Recently the binding of the selective dopamine D-1 receptor ligand SCH 23390 [(*R*)-**1a**][3] (Chart I) and its equally selective iodinated analogue, SKF 103108 [(*RS*)-**1b**], were reported.[4] Thus, the iodine-125 form of (*R*)-**1b**, [^{125}I]SCH 23982, is considered the ligand of choice for the identification of the dopamine D-1 receptor.[4] Similarly, the butyrophenone spiperone (**2a**) has been radiolabeled in the 2′- and 4-positions (**2b** and **2c**), and these radiolabled analogues have been used for the localization of the dopamine D-2 receptor,[5,6] the receptor having been implicated in the antipsychotic action of neuroleptic agents. 4-[^{125}I]Iodospiperone (**2c**) in comparison with tritiated spiperone, however, was shown not to be a useful ligand for D-2 receptors in the central nervous system (CNS) due to aberrant binding kinetics.[7]

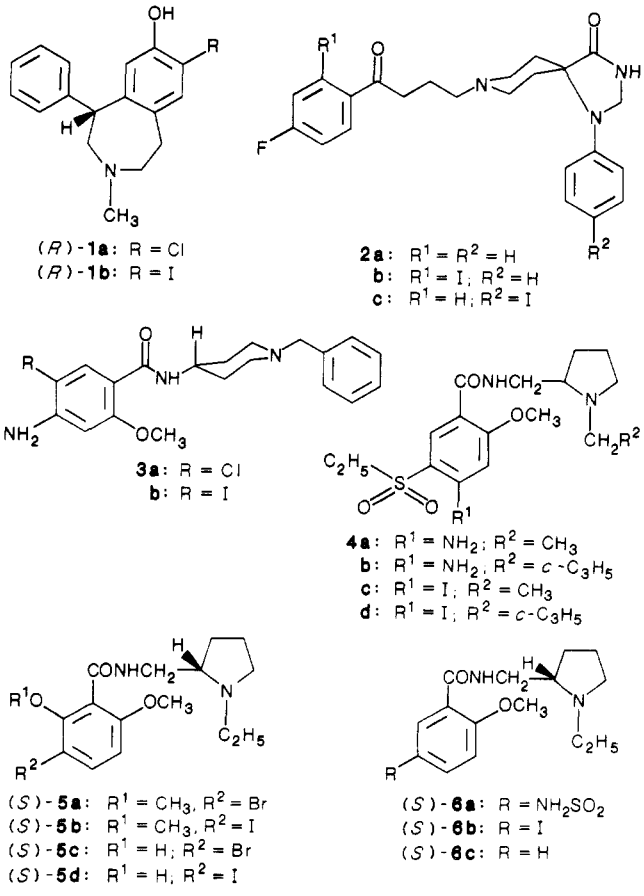
Substituted benzamides are also potent dopamine antagonists. Neumeyer and co-workers[8] have shown that the replacement of the chlorine atom in the substituted benzamide clebopride (**3a**) with an iodine atom (**3b**) retains binding characteristics similar to those of the butyrophenone haloperidol.[8] However, the in vivo ratio of striatal to cerebellar uptake of [^{125}I]-**3b** was 1.9 to 1,[8] which is less than optimally required for in vivo imaging. In addition, no substituted benzamide structurally related to clebopride seems to possess sufficient separation of antipsychotic activity from extrapyramidal side effects to be an effective therapeutic agent,[9] and thus, the relevance of such an agent

[†] Department of Chemistry.
[‡] Department of Psychiatry.
[§] Department of Radiology.

(1) Seevers, R. H.; Counsell, R. E. *Chem. Rev.* **1982**, *82*, 575–590.
(2) Sedvall, G.; Farde, L.; Persson, A; Wiesel, F.-A. *Arch. Gen. Psychiat.* **1986**, *43*, 995–1005.
(3) Iorio, L. C.; Barnett, A.; Leitz, F. H.; Houser, V. P.; Korduba, C. A. *J. Pharmacol. Exp. Ther.* **1983**, *226*, 462–468.
(4) Sidhu, A.; van Oene, J. C.; Dandridge, P.; Kaiser, C.; Kebabian, J. W. *Eur. J. Pharmacol.* **1986**, *128*, 213–220.
(5) Nakatsuka, I.; Saji, H.; Shiba, K.; Shimizu, H.; Okuno, M.; Yoshitake, A.; Yokoyama, A. *Life Sci.* **1987**, *41*, 1989–1997.
(6) Gundlach, A. L.; Largent, B. L.; Synder, S. H. *Life Sci.* **1984**, *35*, 1981–1988.
(7) Kilpatrick, G. J.; Jenner, P.; Marsden, C. D. *J. Pharm. Pharmacol.* **1986**, *38*, 406–408.
(8) Neumeyer, J. L.; Guan, J.-H.; Niznik, H. B.; Dumbrille-Ross, A.; Seeman, P.; Padmanabhan, S.; Elmaleh, D. R. *J. Med. Chem.* **1985**, *28*, 405–407.

0022-2623/88/1831-2027$01.50/0

Chart I



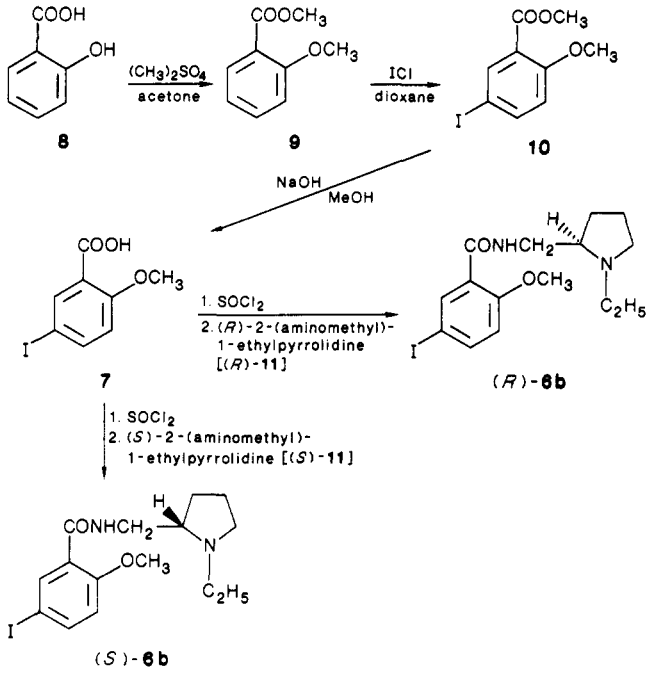
for the study of the D-2 receptor sites in connection with antipsychotic activity is unclear. Other substituted benzamides are potent dopamine D-2 antagonists, as well as antipsychotic agents, and exhibit weak or no extrapyramidal side effects. Amisulpride (**4a**) and its *N*-cyclopropylmethyl analogue, LUR 2366 (**4b**), are substituted benzamides with a pyrrolidinylmethyl moiety as the amide substituent similar to that of remoxipride [(*S*)-**5a**] and sulpiride (**6a**), both of which have demonstrated antipsychotic activity.[9] The ^{125}I-labeled derivatives (**4c** and **4d**) of **4a** and **4b** are also described as potent pharmacological equivalents of sulpiride (**6a**),[10,11] and [^{125}I]iodosulpride ([^{125}I]-**4d**) has also been investigated for in vivo labeling of D-2 receptors.[11] The wide-spread distribution of [^{125}I]iodosulpride in rat cerebellum and cortex[12] and its low penetration into the mouse brain after systemic administration[13] seem to preclude its use as a selective imaging agent. Both remoxipride [(*S*)-**5a**] and its iodinated analogue [(*S*)-**5b**] have weak affinity for spiperone binding sites[14] and therefore are unsuitable as imaging agents. In contrast, the des-*O*-methyl derivative (*S*)-**5c** of remoxipride and (*S*)-**5d** are equipotent with haloperidol in their ability to displace spiperone from dopamine D-2 binding sites in in vitro.[14] In fact, the iodine-123 derivative of (*S*)-**5d** [[^{123}I]-I-FLA 981, [^{123}I](*S*)-**5d**][15] and the iodine-125 derivative of (*S*)-**5d** [IBZM, [^{125}I](*S*)-**5d**][16] have recently been used for the in vivo study of the distribution of dopamine receptors in human brain and rodent brain, respectively. The radioiodination procedure used to prepare [^{123}I]-(*S*)-**5d**[15] and [^{125}I](*S*)-**5d**,[16] however, possibly produces a mixture of the 3-iodo- and the 5-iodo-substituted 6-methoxysalicyl amides, and the 5-iodo isomer is predicted to have a low affinity to the D-2 receptor.[14] Its presence may explain the poor image quality reported for [^{123}I]-(*S*)-**5d**.[15]

In summary, although all of the neuroleptics discussed above are blocking agents for dopamine receptors, only a few have strong and selective affinity for the D-2 receptor and have been shown clinically to have antipsychotic action. Thus, the use of radiolabeled iodospiperones (**2b** and **2c**), and iodoclebopride (**3b**), or iodosulpride (**4d**) in the study of D-2 receptors in schizophrenic patients seems questionable. The most relevent approach for brain imaging of D-2 receptors would be the use of iodinated derivatives of the proven antipsychotic agent sulpiride (**6a**).

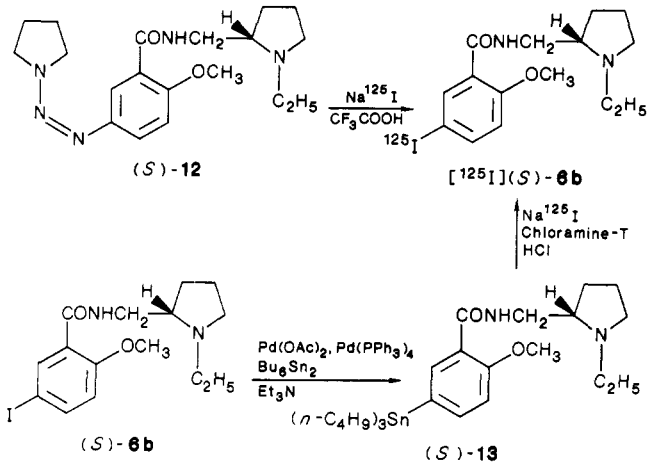
Sulpiride is used clinically as a racemic mixture of the optical antipodes. The central antidopaminergic activity of sulpiride resides in the *S* enantiomer[17] whereas the *R* isomer is slightly more toxic in the mouse.[18] It has not been established whether the antipsychotic action of sulpiride also resides exclusively in the *S* isomer or requires the presence of the *R* enantiomer.[9] In a behavioral experiment, (*S*)-sulpiride when injected bilaterally into the nucleus accumbens of the rat brain was found to be 10 times more active than the low dose neuroleptic fluphenazine in blocking dopamine agonist-induced hyperactivity.[19] In contrast, intraperitoneal administration of sulpiride yielded different results,[19] confirming the suggestion that sulpiride penetrates poorly into the brain when taken orally or injected systemically. Since sulpiride in all other respects fulfills the criteria as a selective D-2 ligand,[17] a derivative of the biologically active enantiomer (*S*)-sulpiride with improved bioavailability would be desirable as a tool for the study of central D-2 receptors. Therefore, we have prepared both enantiomers of a new iodine-substituted benzamide, *N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2-methoxybenzamide (**6b**), containing the pyrrolidinyl moiety of sulpiride. The more active enantiomer (*S*)-**6b** retains all of the structural features of (*S*)-sulpiride except that the hydrophilic aminosulfonyl substitutent on the benzene ring is replaced by an hydrophobic iodine atom, rendering the molecule more lipophilic but not interfering with its selectivity for dopamine D-2 receptors. In fact, an iodine atom at this position would be expected to enhance the drug's bioavailability due to increased lipophilicity.[20] We have also prepared the iodine-125-labeled derivative of (*S*)-**6b**

(9) de Paulis, T. In *Proceedings of the VIIIth International Symposium on Medicinal Chemistry*; Dahlbom, R., Nilsson, J. L. G.; Eds.; Swedish Pharmaceutical: Stockholm, 1985; Vol. 1, pp 405–425.
(10) Cardoso, M. T.; Pradelles, P. J. *Labelled Compd. Radiopharm.* **1982**, *19*, 1103–1109.
(11) Martres, M.-P.; Sales, N.; Bouthenet, M.-L.; Schwartz, J.-C. *Eur. J. Pharmacol.* **1985**, *118*, 211–219.
(12) Martres, M.-P.; Bouthenet, M.-L.; Sales, N.; Sokoloff, P.; Schwartz, J.-C. *Science (Washington, D.C.)* **1985**, *228*, 752–755.
(13) Martres, M.-P., Department of Neurobiology, Paul Broca Center, Paris, personal communication, 1985.
(14) de Paulis, T.; Kumar, Y.; Johansson, L.; Rämsby, S.; Florvall, L.; Hall, H.; Ängeby-Möller, K.; Ögren, S.-O. *J. Med. Chem.* **1985**, *28*, 1263-1269.
(15) Crawley, J. C. W.; Smith, T.; Zanelli, G. D.; Oldland, S. R. D.; Owen, F.; Veall, N. *Clin. Sci.* **1986**, *70*, Suppl 13, (Abstr. 145).
(16) Kung, H. F.; Guo, Y.-Z.; Billings, J.; Xu, X.-J.; Mach, R. H.; Blau, M.; Ackerhalt, R. E. *Nucl. Med. Biol.* **1988**, *15*, 195–201.
(17) Zahniser, N. R.; Dubocovich, M. L. *J. Pharmacol. Exp. Ther.* **1983**, *227*, 592–599.
(18) Miller, C. S.; Engelhardt, E. L.; Thominet, M. L. Fr. Pat. M 5916, 1968; *Chem. Abstr.* **1969**, *71*, 70484a.
(19) Andrews, C. D.; Woodruff, G. N. *Br. J. Pharmacol.* **1978**, *64*, 434P.
(20) de Paulis, T.; Hall, H. In *QSAR in Design of Bioactive Compounds*; Kuchar, M., Ed.; Prous: Barcelona, 1984; pp 199–208.

Scheme I



Scheme II



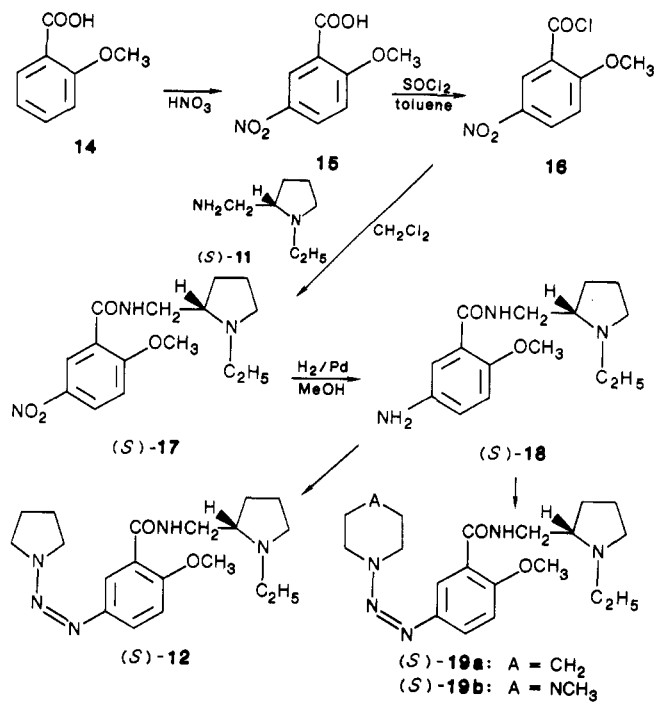
[[^{125}I](*S*)-**6b**] for use in the in vitro localization of dopamine D-2 receptors. By use of the same synthetic scheme, [^{123}I](*S*)-**6b** could be prepared for the noninvasive visualization study of dopamine D-2 receptors in vivo by SPECT.

Results and Discussion

Synthesis. As shown in Scheme I, (*R*)-**6b** and (*S*)-**6b** were prepared from 5-iodo-2-methoxybenzoic acid (**7**). The latter was obtained from salicyclic acid (**8**) via its dimethyl ether ester **9** and the iodinated compound **10** by using the procedure of Bergman and co-workers.[21] Reaction of the acid chloride of **7** with either enantiomer of 2-(aminomethyl)-1-ethylpyrrolidine, (*R*)-**11** or (*S*)-**11**, produced the two new substituted benzamides (*R*)-**6b** and (*S*)-**6b**, respectively. Racemic **11** was resolved by precipitation of the ditartrate salts from 50% aqueous ethanol and subsequent leaching with hot methanol according to Bulteau.[22]

Introduction of the radiolabel (Scheme II) as the last step in the preparation of [^{125}I](*S*)-**6b** was achieved either

Scheme III



by Heindel's modification of the Sandmeyer–Wallach reaction[23] with the triazene (*S*)-**12** or by iododestannylation[24] of the organotin intermediate (*S*)-**13**. The Sandmeyer–Wallach reaction was useful for preparation of [^{125}I](*S*)-**6b** with low to moderate specific radioactivity (16-fold iodide carrier-added). When the triazene method was used in the attempted preparation of no carrier-added iodine-125-labeled compound, the radiochemical yield was very low (2%). The organotin reagent (*S*)-**13** was prepared from the corresponding iodobenzamide (*S*)-**6b**, and when (*S*)-**13** was treated with chloramine-T in the presence of high specific activity sodium iodide-125 (2.2 Ci/μmol) in hydrochloride acid, the radiochemical yields of [^{125}I](*S*)-**6b** (no carrier-added) was much improved (63%). The tri-*n*-butyltin reagent (*S*)-**13** was prepared from (*S*)-**6b** in triethylamine by using bis(tri-*n*-butyltin) with 10% palladium(II) acetate and 5% tetrakis(triphenylphosphine)-palladium(0) as catalysts.[25] The organotin compound (*S*)-**13** was a stable, colorless, viscous oil, which resisted all attempts at crystallization.

As shown in Scheme III, the triazene (*S*)-**12** was prepared in a five-step synthesis from 2-methoxybenzoic acid (**14**). For the first step, the literature methods for nitration of **14** and of its methyl ester **9** were compared. Method A, as described by Lumbroso and Rumpf[26] using methyl 2-methoxybenzoate, gave pure methyl 2-methoxy-5-nitrobenzoate but in poor yield. Method B as shown in Scheme III utilizes 2-methoxybenzoic acid (**14**) but involves the separation of two structural isomers. Thus, nitration of **14** with fuming nitric acid according to Simonsen and Rau[27] gave a mixture of the 3- and 5-nitro isomers in a 1:6 ratio. Separation of the potassium 3- and 5-nitro-2-methoxybenzoates by fractional crystallization following the modification of Hirwe and Gavanker[28] gave the desired

(21) Bergman, J. J.; Griffith, E. A. H.; Robertson, B. E.; Chandler, W. D. *Can. J. Chem.* **1973**, *51*, 162–170.
(22) Bulteau, G. S. African Pat. 6802593, 1968; *Chem. Abstr.* **1969**, *71*, 30354*b*.
(23) Heindel, N. D.; Van Dorf, M. *J. Org. Chem.* **1985**, *50*, 1988–1990.
(24) Moerlein, S. M.; Coenen, H. H. *J. Chem. Soc., Perkin Trans. 1*, **1985**, 1941–1947.
(25) Azizian, H.; Eaborn, C.; Pidcock, A. *J. Organomet. Chem.* **1981**, *215*, 49–58.
(26) Lumbroso, H.; Rumpf, P. *Bull Soc. Chim. Fr.* **1951**, 628–632.
(27) Simonsen, J. L.; Rau, M. G. *J. Chem. Soc.* **1917**, *111*, 220–236.

benzoic acid **15** in 57% yield. The latter was transformed to its acid chloride **16** and condensed with (*S*)-2-(aminomethyl)-1-ethylpyrrolidine [(*S*)-**11**] to form the 5-nitro-substituted benzamide derivative (*S*)-**17**. The racemic form of **17** is known from the patent literature.[18]

Catalytic hydrogenation of (*S*)-**17** gave the corresponding 5-amino derivative (*S*)-**18**. The latter was transformed into its diazonium chloride with nitrous acid generated in situ, and addition of an excess of pyrrolidine gave the corresponding 5-[(3,3-(1,4-butanediyl)triazeno]benzamide (*S*)-**12**. Addition of piperidine or *N*-methylpiperazine instead of pyrrolidine produced the corresponding 5-[3,3-(1,5-pentanediyl)triazeno]triazeno] and 5-[3,3-(3-methyl-3-aza-1,5-pentanediyl)triazeno] derivatives (*S*)-**19a** and (*S*)-**19b**, respectively. None of the triazenes was a crystalline compound, but the molecular ion of each of the triazenes (*S*)-**12**, (*S*)-**19a**, and (*S*)-**19b** was detected by fast atom bombardment mass spectroscopy. The absolute mass number of each molecular ion was determined by electron-impact mass spectroscopy and compared with that calculated from the elemental compositions. The proton nuclear magnetic resonance (^{1}H NMR) and mass spectra of the triazene derivatives were consistent with their structures.

Iodine-125 Labeling. The acid-catalyzed regeneration of the diazonium salt from (*S*)-**12** was found to be dependent on the solvent used. In hydrochloric acid, no reaction of sodium iodide took place due to the poor solubility of the triazene in water. In acetone and using trifluoroacetic acid, the reaction proceeded rapidly, but radiolabeled side products rendered this solvent unsuitable. In benzene, the formation of some desiodo compound (*S*)-**6c** was detected when the reaction was run on a micromolar scale. The possible presence of (*S*)-**6c** in [^{125}I](*S*)-**6b** could be detected only by ^{1}H NMR spectroscopy since neither thin-layer chromatography nor high-performance liquid chromatography gave sufficient separation of the two compounds. Complete suppression of this side reaction is possible by addition of 0.5 equiv of free iodine to the reaction mixture.[29] However, for radioiodination purposes this was not practical since it would have lowered the specific radioactivity in an unpredictible manner. Attempted iodinations of the diazonium intermediates generated from triazenes (*S*)-**19a** and (*S*)-**19b** gave lower yields of (*S*)-**6b** than that from (*S*)-**12**. The attempted preparation of no carrier-added [^{125}I](*S*)-**6b** gave poor yields, presumably because of the requirement of a relatively high concentration of diazonium–iodide complex formed in situ.

A more suitable method for preparation of [^{125}I](*S*)-**6b** especially with high specific activity is the iododestannylation of the tri-*n*-butyltinbenzamide (*S*)-**13** with sodium iodide-125 and chloramine-T in hydrochloric acid according to the method of Moerlein and Coenen.[24] Thus, [^{125}I](*S*)-**6b** was prepared in at least 97% radiochemical purity in 56% radiochemical yield.

Lipophilicity. The apparent lipophilicity found for **6b** is substantially higher than that of sulpiride (**6a**). In this respect, the iodobenzamide **6b** behaves like the dichlorobenzamide raclopride (**20**), which shows an efficient penetration into the brain.[2] Similarly, Van Damme and coworkers[30] reported for the octanol–water partition coefficient (*P*) of the iodo analogue of metoclopramide (**22**) (log *P* = 3.09) and the corresponding aminosulfonyl-substituted benzamide (4-aminosulpiride) (log *P* = 0.66), a difference of 2.43 log *P* units. From Hansch substituent π-values, the difference in lipophilic contribution of an iodine atom (π 1.12) and an aminosulfonyl group (π −1.82) is calculated to be 2.94 log *P* units.[31] By use of the experimentally determined reversed-phase HPLC capacity factors (log k_w), the partition coefficients values for compound **6b** (log *P* = 2.85) and sulpiride (**6a**) (log *P* = 1.28) were found to differ by 1.57 log *P* units. Thus, **6b** is at least 40 times more lipophilic than is sulpiride. This should result in a relatively higher concentration of **6b** in the CNS compared to that of the same dose of sulpiride.[32]

Table I. Inhibition of [^{125}I](*S*)-**6b** and [^{3}H](*S*)-Sulpiride Binding in Striatal Homogenates of Rat Brain

compound	IC_{50} ± SEM, nM[a]	
	[^{125}I](*S*)-**6b**[b]	[^{3}H](*S*)-sulpiride[c]
(*R*)-**6b**	113 ± 15	145 ± 5
(*S*)-**6b**	1.5 ± 0.1	3.3 ± 0.9
sulpiride [(*RS*]-**6a**]	33 ± 0.5	36 ± 3.6
raclopride (**20**)	1.3 ± 0.1	14 ± 3.5
haloperidol (**21**)	1.2 ± 0.4	6.0 ± 2.4
SCH 23390 [(*R*)-**1a**]	600 ± 100	1900 ± 100
dopamine	1860 ± 700	1700 ± 600

[a] An IC_{50} value designates the concentration of a particular compound that displaces specific binding of the radiolabeled ligand by 50%. SEM is the standard error of the mean for three independent experiments conducted with triplicate determinations. [b] Concentration of [^{125}I](*S*)-**6b** was 1.0 nM, and nonspecific binding was determined in the presence of 10 μM of (*S*)-**6b**. [c] Concentration of [^{3}H](*S*)-sulpiride was 5.0 nM, and nonspecific binding was determined in the presence of 10 μM sulpiride.

Table II. Regional Uptake of [^{125}I](*S*)-**6b** in Rat Brain 60 min after Intravenous Injection

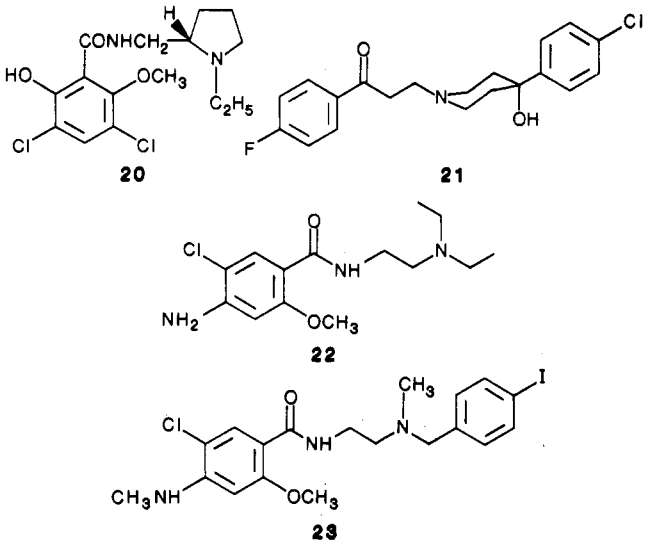
tissue	radioactivity, nCi/g ± SD[a]	ratio, striatum:tissue
striatum	56.2 ± 5.6	1.0:1
cerebellum	7.8 ± 1.2	7.2:1
frontal cortex	11.9 ± 4.8	4.7:1

[a] SD is the standard deviation.

Receptor Binding Assay. The binding of [^{125}I](*S*)-**6b** to rat striatal membranes was saturable, reversable, and stereospecific under the present experimental conditions and resembling that of sulpiride.[33] Binding reached equilibrium at 37 °C within 20 min and remained stable for at least 60 min. Scatchard analysis of the saturation isotherm revealed a single set of binding sites with a K_D of 1.2 ± 0.3 nM and a maximal number of binding sites of 305 ± 8 fmol/mg of protein. As shown in Table I, sulpiride displaced [^{125}I](*S*)-**6b** with an IC_{50} of approximately 33 nM, while the dopamine D-1 receptor antagonist SCH 23390 [(*R*)-**1a**] had an IC_{50} of approximately 600 nM. In displacement experiments with [^{3}H](*S*)-sulpiride as the radioligand, the *R* and *S* enantiomers of **6b** had IC_{50} values of 145 and 3.3 nM, respectively. Racemic sulpiride had an IC_{50} of 36 nM. The enantioselectivity in receptor binding, favoring the *S* isomer by a factor of about 40, indicates a high degree of stereospecificity of D-2 receptor binding site. The desiodo derivative (*S*)-**6c** had $IC_{50} > 600$ nM (data not shown), and thus it is virtually inactive. Both raclopride (**20**) and haloperidol (**21**) were potent displacers of [^{125}I](*S*)-**6b**, thus demonstrating the quali-

(28) Hirwe, N. W.; Gavanker, K. D. *Proc. Indian Acad. Sci.* **1937**, *5A*, 377–380; *Chem. Abstr.* **1937**, *31*, 6216.
(29) Coffen, D. L.; Schaer, B.; Bizzarro, F. T.; Cheung, J. B. *J. Org. Chem.* **1984**, *49*, 296–300.
(30) Van Damme, M.; Hanocq, M.; Molle, L. *Pharm. Acta Helv.* **1984**, *59*, 235–240.
(31) Craig, P. N. *J. Med. Chem.* **1971**, *14*, 680–684.
(32) Mizuchi, A.; Kitagawa, N.; Miyachi, Y. *Psychopharmacology* **1983**, *81*, 195–198.
(33) Imafuku, J. *Brain Res.* **1987**, *402*, 331–338.

Chart II



tative and quantitative similarities between these neuroleptic agents and (*S*)-**6b**.

In Vivo Studies. Injection of [^{125}I](*S*)-**6b** into rats shows that it penetrates rapidly into the brain, peak uptake in the striatum being observed after 20 min and was 0.9% of injected dose per gram of striatum. As shown in Table II, after 60 min, the ratio of radioactivity in the striatum to that in the cerebellum was 7.2 to 1, the former tissue being richer in dopamine D-2 receptor than is the latter.[2]

Conclusions

In vitro receptor binding studies of substituted benzamides have shown several of these to be selective antagonists of central dopamine D-2 receptors. Their potencies seem to be governed, at least in part, by their lipophilic character and particularly by the lipophilicity of the substituent in the 5 position of the aromatic ring.[14] In agreement with this observation, the introduction of an iodine atom in place of the aminosulfonyl group of sulpiride enhances the potency of the benzamide (*S*)-**6b** to displace both [^{125}I](*S*)-**6b** and [^{3}H]sulpiride by factors of 22 and 11, respectively. The respective rank order of potencies of substituted benzamide neuroleptics, including raclopride (**20**) and the butyrophenone neuroleptic haloperidol (**21**) (Chart II) in displacing these radioligands are identical, indicating that the binding profile of sulpiride is retained in (*S*)-**6b**. The enhanced lipophilicity of **6b** as compared to sulpiride predicts that (*S*)-**6b** would more readily cross the blood–brain barrier than sulpiride[34] and therefore will achieve a relatively higher CNS concentration than that of the same dose of sulpiride. This prediction is in fact confirmed by in vivo studies showing that [^{125}I](*S*)-**6b** penetrates rapidly into rat brain and is preferably localized in a brain area rich in dopamine D-2 receptors. Thus, (*S*)-**6b** is a highly selective and potent antagonist of central dopamine D-2 receptors. Compound [^{123}I](*S*)-**6b** then may be a useful radioligand for the in vivo vizualization of dopamine D-2 receptors of the human brain by SPECT. The 40-fold enantioselectivity of (*S*)-**6b** in blocking the striatal D-2 receptors indicates that the less potent [^{123}I](*R*)-**6b** could be valuable in quantitative visualization of the D-2 receptor distribution by digital subtraction technique[35] in which the specific binding of [^{123}I](*R*)-**6b** is substracted from that of [^{123}I](*S*)-**6b**.

The question of the clinical significance of using (*S*)-**6b** in the localization of dopamine D-2 receptors relevant for antischizophrenic action cannot be answered by receptor binding studies alone. Nonantipsychotic benzamides such as clebopride (**3a**) and metoclopramide (**22**) fulfill the same criteria as selective D-2 receptor antagonists as do the new antipsychotic sulpiride analogues, amisulpride (**4a**) and raclopride (**20**). Thus, [^{125}I]iodoclebopride ([^{125}I]-**3b**) and the [^{125}I]iodobenzyl derivative of metoclopramide ([^{125}I]-**23**) are potent antagonists of [^{3}H]spiperone ([^{3}H-**2a**) and haloperidol (**21**), respectively,[8,36] but presumably lack antipsychotic activity. It might therefore be speculated that the blockade of only a subpopulation of D-2 receptors in striatal areas of the brain is correlated to antipsychotic activity while general D-2 receptor blockade is correlated to extrapyramidal side effects.[37] This speculation may possibly be confirmed with SPECT by using [^{123}I](*S*)-**6b**.

Experimental Section

Melting points were taken in open capillary tubes and are corrected. Rotatory powers at the sodium D line were measured with an Autopol III automatic polarimeter and a 1-dm sample tube. All chemicals and solvent were analytical grade and were used without further purification. Iodine-125 was supplied by Medi-Physics, Inc., Emeryville, CA, and Du Pont-NEN, North Billerica, MA, and in the form of a solution of carrier-free Na^{125}I in NaOH (10 mCi/20 μL, pH 9.5). (*RS*)-Sulpiride (**6a**) was a gift from Delagrange Laboratories, Paris, France. [^{3}H](*S*)-Sulpride ([^{3}H]-**6a**) was purchased from Du Pont-NEN. Raclopride (**20**) [(*S*)-3,5-dichloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicyclamide L-(+)-tartrate] was a gift from Astra Alab, Södertälje, Sweden. Compound SCH 23390 [(*R*)-**1a**] was a gift from Schering-Plough, Corp., Bloomfield, NJ. Haloperidol (**21**) and dopamine were purchased from Sigma Chemical Co., St. Louis, MO. Fast atom bombardment mass spectra were determined in a glycerol matrix on a VG Micromass 70-250 HF instrument with 8-kV xenon atoms and were used to identify molecular radical ions in mass spectroscopy. For accurate mass determination of these ions, high-resolution mass spectroscopy (HRMS) using a 40-eV electron impact mode (ion source 200 °C) and perfluoroalkanes as mass reference were used (resolving power 5000). Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on an IBM NR 300 instrument, operating at 300 MHz. Samples (30–40 mg) were dissolved in $CDCl_3$, and the chemical shifts (δ) are reported in ppm downfield from internal tetramethylsilane. Significant ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), number of protons, proton assignment, and coupling constant (*J*) in hertz. Combustion analyses and determination of the dissociation constant (K_a) for (*R*)-**6b**·HCl were done by Galbraith Laboratories, Inc., Knoxville, TN. Combustion analyses gave elemental compositions within ±0.4% of the calculated values unless otherwise noted.

(*R*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-iodo-2-methoxybenzamide Hydrochloride Hydrate [(*R*)-6b·HCl·$^{1}/_{2}H_2O$]. The di-L-(+)-tartrate salt of (*R*)-2-(aminomethyl)-1-ethylpyrrolidine [(*R*)-**11**][22] (2.0 g, 4.7 mmol) was dissolved in 4 N NaOH (10 mL), and the free amine was extracted with CH_2Cl_2 (2 × 20 mL). The volume of the combined extracts was reduced to 20 mL, and the solution was added dropwise to a solution of 2-methoxy-5-iodobenzoyl chloride (1.1 g, 3.7 mmol), prepared from 2-methoxy-5-iodobenzoic acid (**7**) according to the method of Bergman et al.,[21] in CH_2Cl_2 (20 mL) at 30 °C. Stirring was continued for 35 min. The solvent was evaporated, and the residue was treated with 1 N NaOH (50 mL). Extraction with ether (3 × 50 mL), extraction of the combined ether solutions with 1 N HCl (2 × 50 mL), neutralization of the combined aqueous layer

(34) Benakis, A.; Brown, J. P. H.; Benard, P. *Eur. J. Drug Metab. Pharmacokin.* **1984**, *9*, 365–370.
(35) Sedvall, G.; Ehrin, E.; Farde, L. *Human Psychopharmacol.* **1987**, *2*, 23–30.
(36) Wilson, A. A.; Dannals, R. F.; Ravert, H. T.; Gilbart, F. W.; Wong, D. F.; Wagner, H. N., Jr. *J. Nucl. Med.* **1987**, *28*, Abstr. 718.
(37) Farde, L.; Wiesel, F.-A.; Jansson, P.; Uppfeldt, G.; Wahlen, A.; Sedvall, G. *Psychopharmacology* **1988**, *94*, 1–7.

with 4 N NaOH (30 mL), extraction with CH_2Cl_2 (3 × 30 mL), drying ($MgSO_4$), and evaporation of the CH_2Cl_2 gave 1.3 g (81%) of (*R*)-**6b** as an oil: $[\alpha]^{20}_D$ +63° (*c* 1.63, acetone). The oil was dissolved in acetone (12 mL) and 3 N HCl–ether (1.5 mL) and water (one drop) were added. Cooling to 0 °C precipitated 1.2 g (75%) of the benzamide hydrochloride hydrate. Recrystallization from 98% aqueous acetone (50 mL) gave 0.72 g (45%) of (*R*)-**6b**·HCl·$^1/_2H_2O$: mp 181–182 °C; pK_a = 8.89 in water. Anal. ($C_{15}H_{22}ClIN_2O_2 \cdot {}^1/_2H_2O$) C, H, Cl, I, N.

(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-iodo-2-methoxybenzamide hydrochloride hydrate [(*S*)-6b·HCl·H_2O] was prepared from the di-D-(−)-tartrate salt of (*S*)-2-(aminomethyl)-1-ethylpyrrolidine [(*S*)-**11**][22] and 2-methoxy-5-iodobenzoyl chloride as described for the preparation of (*R*)-**6b**·HCl·$^1/_2H_2O$. After recrystallization from 98% aqueous acetone, the yield of pure product was 0.63 g (39%): mp 179–181 °C. Anal. ($C_{15}H_{22}ClIN_2O_2 \cdot H_2O$) C, H, I, N; Cl: calcd, 8.01; found, 8.91. As described for compound (*R*)-**6b**, the free base (*S*)-**6b** was isolated as an oil: $[\alpha]^{20}_D$ −62° (*c* 2.01, acetone); ^1H NMR δ 8.43 (d, 1, C(6)-H, *J* = 2.6 Hz), 7.71 (dd, 1, C(4)-H), 6.76 (d, 1, C(3)-H, *J* = 8.7 Hz), 3.97 (s, 3, OCH_3), 1.5–3.8 (m, 11), 1.13 (t, 3, CH_3).

(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide [(*S*)-6c]. *o*-Anisic acid (**14**) (3.0 g, 0.020 mol) was converted to its acid chloride, and the latter was mixed with (*S*)-2-(aminomethyl)-1-ethylpyrrolidine [(*S*)-**11**] (3.8 g, 0.030 mol) as described for compound (*R*)-**6b**. The yield of (*S*)-**6c** was 5.1 g (96%) of a colorless oil: ^1H NMR of aromatic protons δ 8.20 (dd, 1, C(6)-H), 7.42 (dt, 1, C(4)-H) 7.08 (d, 1 C(5)-H), 7.0 (br d, 1, C(3)-H). Attempted crystallizations of the hydrochloride, oxalate, tartrate, and mesylate failed.

[^{125}I](*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-iodo-2-methoxybenzamide ([^{125}I](*S*)-6b). A. From the Triazene (*S*)-12. The reaction conditions were adapted from Heindel and Van Dort.[23] Sodium iodide (33.4 nmol) in acetone (25 μL) was added to 4.86 mCi $Na^{125}I$ (2.24 nmol) in aqueous NaOH at pH 8–10 (8 μL), and the mixture was diluted with benzene (140 μL). The solvent was removed by a stream of nitrogen, and to the residue were added the triazene (*S*)-**12** (21 μg, 58 nmol) in CH_2Cl_2 (7 μL) and CF_3COOH (60 μg, 0.52 μmol) in acetone (15 μL). The reaction mixture was shaken for 40 min at 20 °C, and 1 N NaOH (300 μL) was added. Extraction with CH_2Cl_2 (3 × 100 μL) gave [^{125}I](*S*)-**6b**, possibly contaminated by an unknown amount of the corresponding desiodo compound (*S*)-**6c**. Thin-layer chromatography (TLC) (Merck F_{254} SiO_2 with EtOAc–EtOH–25% NH_4OH, 100:10:1) of 2 μL of the extract showed radioactivity at R_f 0.25 identical with that of authentic (*S*)-**6b**. The solvent was removed, and 1 N HCl (100 μL) was added. The solution contained 0.66 mCi (14% radiochemical yield) of [^{125}I](*S*)-**6b**·HCl, calculated specific activity of 136 Ci/mmol based on the specific activity of the $Na^{125}I$ used.

B. From the (Tri-*n*-butylstannyl)benzamide (*S*)-13. The organotin compound (*S*)-**13** was dissolved in ether (2.15 mmol/L). Of this solution, 5 μL (6.1 μg, 11 nmol) was mixed with 14.0 mCi of no carrier-added $Na^{125}I$ in 29 μL NaOH (pH ~9.5) (0.95 μg, 6.4 nmol). Hydrochloric acid (0.1 N, 40 μL) was added followed by the addition of 0.014 N aqueous chloramine-T (5 μL, 70 nmol). The reaction mixture was stirred for 0.5 h at 20 °C. Ether (100 μL) was added to extract impurities. The ether layer contained 1.3 mCi (9.3%) of radioactivity. The aqueous layer was made basic by addition of NaOH (2 N, 20 μL). Extraction with ether (2 × 150 μL) gave 8.9 mCi of product with a radiochemical purity of 97% as determined by TLC. The combined ether layer was extracted with 0.1 N HCl (3 × 100 μL). The combined aqueous layer contained 7.8 mCi (56% radiochemical yield) of [^{125}I]-(*S*)-**6b**·HCl.

Di-D-(−)-tartrate Salt of (*S*)-2-(Aminoethyl)-1-ethylpyrrolidine [(*S*)-11]. Racemic 2-(aminomethyl)-1-ethylpyrrolidine (**11**) (84 mL, d^{20}_4 0.887, 0.58 mol) was slowly added with stirring at 20 °C with external cooling to D-(−)-tartaric acid (125 g, 0.83 mol) in water (220 mL). Ethanol (95%, 220 mL) was added, and the mixture was cooled to 8 °C and allowed to stand for 18 h. The solid precipitate was collected by filtration and washed with 50% aqueous ethanol (50 mL). Repeated (5×) trituration of the solid with boiling methanol (600 mL) for 15 min and filtration at 35 °C gave 55.6 g (44%) of the di-D-tartrate salt of (*S*)-**11**: mp 159–161 °C; $[\alpha]^{23}_D$ −38° (*c* 8.0, H_2O) [lit.[22] mp 161–162 °C; $[\alpha]^{20}_D$ + 39° (H_2O) for the enantiomer].

Di-L-(+)-tartrate Salt of (*R*)-2-(Aminoethyl)-1-ethylpyrrolidine [(*R*)-11]. The combined mother liquors from the preparation of the di-D-(−)-tartrate salt of (*S*)-**11** above were evaporated, and the residue was treated with 5 N potassium hydroxide (300 mL). The free amine was extracted into chloroform (3 × 200 mL). Evaporation of the solvent and distillation gave partially racemic (*R*)-**11** as an oil: bp 74–78 °C (20 mmHg). This oil was slowly added to a stirred solution of L-(+)-tartaric acid (80 g, 0.53 mol) in water (150 mL) at 20 °C with external cooling. Cooling at 5 °C for 20 h gave a precipitate. Repeated (4×) trituration of this solid with boiling methanol (200 mL) and filtration at 35 °C gave 43.5 g (35%) of the di-D-(−)-tartrate salt of (*R*)-**11**: mp 160–161 °C; $[\alpha]^{23}_D$ +38° (*c* 12.1, H_2O) [lit.[22] mp 161–162 °C; $[\alpha]^{20}_D$ +39° (H_2O)].

(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2-methoxy-5-[3,3-(1,4-butanediyl)triazeno]benzamide [(*S*)-12]. The hydrochloride of the aminobenzamide (*S*)-**18** (2.2 g, 7.0 mmol) was dissolved in a mixture of 1 N HCl (20 mL) and acetone (10 mL). Sodium nitrite (0.7 g, 0.01 mol) was slowly added at 0 °C. After 15 min, a solution of pyrrolidine (1.5 mL, 0.018 mol) in 50% aqueous acetone (10 mL) was added, and the mixture was stirred at 10 °C for 1.5 h. Dilute NaOH (60 mL) was added, and the product was extracted with CH_2Cl_2 (3 × 50 mL). Washing of the combined extracts with water (50 mL), drying (Na_2SO_4), and evaporation of the solvent gave 2.5 g of a tan residue. Column chromatography (180 g of Davisil 62 SiO_2 with EtOAc–EtOH–25% NH_4OH, 100:10:1) and evaporation of the fractions showing a spot at R_f 0.24 on TLC with the same solvent system gave 1.88 g (75%) of (*S*)-**12** as a viscous oil: ^1H NMR of aromatic protons δ 8.23 (d, 1, C(6)-H), 7.44 (dd, 1, C(4)-H), 6.93 (d, 1, C(3)-H); HRMS, *m/e* 359.2322 ($C_{19}H_{29}N_5O_2$ requires 359.2321).

(*S*)-(−)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2-methoxy-5-(tributylstannyl)benzamide [(*S*)-13]. The iodobenzamide (*S*)-**6b** (0.52 g, 1.3 mmol) was dissolved in triethylamine (2.0 mL, freshly distilled over CaH_2). Palladium(II) acetate (0.022 g, 0.098 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.06 g, 0.05 mmol) were added under nitrogen. The mixture was heated to 50 °C and bis(tributyltin)[25] (1.2 mL, *d* 1.15 g/mL, 2.4 mmol) was added and heated to reflux temperature (bath 100 °C) for 2.5 h. After cooling, the black reaction mixture was filtered, the insoluble material was washed with triethylamine (5 mL), and the filtrate was evaporated at reduced pressure at 50 °C. The yellow residual oil was dissolved in isopropyl ether (3 mL) and subjected to column chromatography (30 g of 0.063–0.200–mm SiO_2 with isopropyl ether–hexane, 1:1). The first fractions (200 mL) removed all reaction solvent residues and organotin compounds. Elution with ethyl acetate–ethanol (10:1) gave fractions containing the desired product as detected by TLC with R_f 0.35 (isopropyl ether–methanol–concentrated NH_4OH, 100:10:1). Evaporation of the solvent gave 0.24 g (40%) of (*S*)-**13** as an oil: ^1H NMR δ 8.26 (d, 1), 8.20 (br, NH). 7.51 (dd, 1), 6.94 (d, 1), 3.93 (s, OCH_3), 2.0–3.9 (m, 7), 0.7–2.0 (m, 34).

2-Methoxy-5-nitrobenzoic Acid (15). Method A. Methyl 2-methoxybenzoate (31 g, 0.19 mol) was slowly mixed with an ice-cooled solution of concentrated H_2SO_4 (100 mL), and a mixture of 70% HNO_3 (12 mL, d 1.41 g/mL, 0.19 mol) and concentrated H_2SO_4 (50 mL) was added dropwise over 1 h at 5 °C. After being stirred for an additional hour, the reaction mixture was poured into ice water (1 kg) and left over night. The crystalline precipitate was dissolved in ether (600 mL), and the solution was washed with 1 N NaOH (2 × 50 mL). Drying (Na_2SO_4) and evaporation of the solvent gave 12.8 g of residue. Recrystallization from MeOH (140 mL) gave 7.1 g (18%) of methyl 2-methoxy-5-nitrobenzoate: mp 100–101 °C (lit.[21] mp 100–101 °C). An additional 3.2 g (8%) of ester was recovered from the mother liquour. The ester (7.0 g, 0.033 mol) was dissolved in hot MeOH (100 mL), and 2 N NaOH (60 mL) was added. The mixture was heated under reflux for 2 h. Water (600 mL) was added, and the solution was extracted with ether (150 mL). The aqueous layer was neutralized by addition of concentrated HCl (15 mL), and the precipitated solid was recrystallized from water (700 mL). Filtration gave 6.1 g (95%, 24% overall) of **15**: mp 163–164 °C (lit.[21] mp 161–164 °C).

Method B. 2-Methoxybenzoic acid (**14**) (30 g, 0.20 mol) was added in small portions over 1 h to a stirred solution of fuming nitric acid (60 mL, *d* 1.59 g/mL) at 5–10 °C. After 2 h the reaction

mixture was poured into of ice water (400 mL). Filtration and recrystallization of the crude product from of water (1.25 L) gave 32 g (82%) of a 1:6 mixture of 3-nitro- and 5-nitro-2-methoxybenzoic acid, the ratio estimated by ^{1}H NMR: mp 148–154 °C (lit.[27] mp 147 °C). The mixture (30 g, 0.15 mol) was separated by careful addition of K_2CO_3 (11.2 g, 0.081 mol) to a stirred suspension of the nitrobenzoic acids in hot water (250 mL) (foaming). Cooling to 0 °C overnight gave a first crop of the potassium benzoate salt of the pure 5-nitro isomer. A second crop was obtained by evaporation of the solvent volume to 140 mL, its ^{1}H NMR spectrum showing no contamination of the 3-nitro isomer. The combined crops were dissolved in water (300 mL) and acidified by addition of 2 N HCl (100 mL). The precipitate was collected by filtration and recrystallized from water (500 mL). The yield was 21 g (70%, 57% from **14**) of **15**, mp 160–162 °C. The ^{1}H NMR spectrum of the product was identical with that of the product prepared by method A.

2-Methoxy-5-nitrobenzoyl Chloride (16). To a suspension of 2-methoxy-5-nitrobenzoic acid (**15**) (17.6 g, 0.0893 mol) in toluene (350 mL) was added thionyl chloride (15 mL, *d* 1.66 g/mL, 0.21 mol) followed by dimethylformamide (0.5 mL). The mixture was heated with stirring for 2 h at 75 °C. The solvent was evaporated until about 50 mL remained as residue. This was poured into hot hexane (800 mL), and cooling gave 18.6 g (97%) of pure **16**: mp 82–83 °C.

(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2-methoxy-5-nitrobenzamide [(*S*)-17]. The di-D-(−)-tartrate salt of (*S*)-2-(aminomethyl)-1-ethylpyrrolidine[22] [(*S*)-**11**] (35.5 g, 0.083 mol) was dissolved in of 20% NaOH (100 mL), and the free amine was extracted into CH_2Cl_2 (2 × 150 mL). The volume of the extract was reduced to 100 mL to remove trace of water, and the solution was added dropwise to a stirred solution of 2-methoxy-5-nitrobenzoyl chloride (**16**) (17.6 g, 0.082 mol) in CH_2Cl_2 (300 mL) at 25 °C. After 2 h the solvent was removed, and the residue was crystallized from acetone (400 mL). Filtration yielded 15.7 g (55%) of (*S*)-**17**·HCl·$^3/_4H_2O$: mp 152–154 °C. Anal. ($C_{15}H_{22}ClN_3O_4 \cdot {}^3/_4H_2O$) C, H, Cl, N. Addition of ether (100 mL) to the filtrate gave a second crop of 5.0 g (17%, total yield 72%). The free base from the second crop was extracted into ether from 1 N NaOH solution and crystallized from isopropyl ether (120 mL). This gave 1.8 g of (*S*)-**17**: mp 97–98 °C (lit.[18] mp 108.5–109.5 °C for the racemate); $[\alpha]^{20}_D$ −50° (*c* 1.23, acetone). Anal. ($C_{15}H_{21}N_3O_4$) C, H, N.

(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-amino-2-methoxybenzamide Hydrochloride [(*S*)-18·HCl]. The nitrobenzamide (*S*)-**17**·HCl·$^3/_4H_2O$ (3.7 g, 0.010 mol) was dissolved in methanol (120 mL) and under nitrogen, and 10% Pd on carbon (0.5 g) was added. Hydrogenation at room temperature and atmospheric pressure consumed the theoretical amount of H_2 (760 mL, 0.031 mol) over 1.5 h. The catalyst was removed by filtration through Celite, and the solvent was evaporated. The residue was dissolved in hot ethanol (65 mL), and concentrated HCl (1.0 mL) was added. Cooling and addition of ether (25 mL) gave 2.5 g (78%) of (*S*)-**18**·HCl: mp 217–218 °C (lit.[18] mp 121–122 °C for the racemic base); $[\alpha]^{20}_D$ −55° (*c* 0.22, MeOH); ^{1}H NMR δ 8.46 (br s, 1, CONH), 7.56 (dd, 1, C(6)-H), 6.77 (m, 2, C(3) and C(4)-H), 3.85 (s, 3, OCH_3), 3.60 (s, 2, NH_2) 3.7–1.5 (m, 11), 1.11 (t, 3, CH_3). Anal. ($C_{15}H_{24}ClN_3O_2$) C, H, Cl, N.

(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2-methoxy-5-[3,3-(1,5-pentanediyl)triazeno]benzamide [(*S*)-19a] and (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxy-5-[3,3-(3-methyl-3-aza-1,5-pentanediyl)triazeno]benzamide [(*S*)-19b] were prepared as described for the prepartion of (*S*)-**12** by using piperidine and N-methylpiperazine, respectively, instead of pyrrolidine: HRMS of (*S*)-**19a**, *m/e* 373.2472 ($C_{20}H_{31}N_5O_2$ requires 373.2477); HRMS of (*S*)-**19b**, *m/e* 388.2583 ($C_{20}H_{32}N_6O_2$ requires 388.2587).

Lipophilicity Measurements. The lipophilic properties of the iodobenzamide **6b** and of sulpiride **6a** were determined by reversed-phase HPLC according to the method of El Tayar and co-workers.[38] The retention time (*t*) was measured in 0.02 M 3-morpholinopropanesulfonic acid (MOPS) buffer and 0.2% (v/v) *n*-decylamine to deactivate the column (Merck Lobar Lichroprep RP8 40–63 μm). The buffer was adjusted to pH 7.50 with sodium hydroxide. Apparent capacity factors (*k*) were calculated using the formula $k = (t - t_o)/t_o$ for three different concentrations of methanol in the range of 40–70% of methanol in aqueous buffer and linearly extrapolated to 0% methanol (k_w). Retention time for the solvent (t_o) was found by addition of sodium nitrate (0.3%) to the 0.5-mL sample. The partion coefficient (*P*) was calculated from the equation:

$$\log P = \log k_w + \log (1 + 10^{pK_a - pH})$$

The dissociation constant (K_a) for (*R*)-**6b** was determined by NaOH titration of (*R*)-**6b**·HCl in water (pK_a = 8.89) and in 50% aqueous ethanol (pK_a = 8.80). By use of the equation above and the pK_a in water, the iodobenzamide **6b** has log *P* = 2.85. With use of the same method, sulpiride (**6a**) has log *P* = 1.28. The K_a for sulpiride (pK_a = 9.12) was taken from El Tayar and co-workers.[38]

Receptor Binding Assay. Male Sprague–Dawley rats (150–200 g) were killed by decapitation, and their brains were quickly removed and dissected on ice. Striata were removed and homogenized in 40 volumes of ice-cold Tris·HCl buffer (50 mM) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ (pH 7.4) with a Brinkman Polytron.[39] The suspension was centrifuged at 30000 *g* for 10 min at 4 °C, and the pellet was resuspended in 40 volumes of fresh buffer. The suspension was incubated at 37 °C for 10 min and centrifuged again at 30000 *g* for 10 min at 4 °C, and the final pellet was resuspended with the polytron in 200 volumes of ice-cold buffer. Assay tubes contained 25 μL of 20 nM [^{125}I](*S*)-**6b** (1.0 nM final concentration) or 0.10 μM [^{3}H](*S*)-sulpiride (5.0 nM final concentration), 25 μL of various concentrations of drug, 50 μL of buffer, and 400 μL of the striatal membrane preparation. Each test compound was dissolved in acetic acid (200 μL, 0.1 N) and diluted with water. Nonspecific binding was determined in the presence of 10 μM iodobenzamide (*S*)-**6b** or sulpiride, respectively. Tubes were incubated for 30 min at 37 °C and poured over Whatman GF/B filters (soaked in 0.3% polyethylenimine and 10 μM of the respective radiolabeled benzamide) under vacuum. Filters were rinsed three times with 4 mL of ice-cold buffer. Tritium radioactivity remaining on the filters was measured by conventional liquid scintillation spectrometry (40% efficiency), and iodine-125 radioactivity was measured by γ-well counting (93% efficiency).

In Vivo Testing. Male Sprague–Dawley rats (200–250 g) were each injected with 25 μCi of [^{125}I](*S*)-**6b** (250 Ci/mmol) in saline by tail vein. Groups of three rats were killed at various times after injection, and their brains were rapidly removed and rinsed in iced saline. The cerebellum, striatum, and frontal cortex were then dissected and placed in counting tubes, and the radioactivity per gram of tissue was measured by γ-well counting (93% efficiency).

Acknowledgment. We thank William J. McBride and Ronald M. Baldwin, Medi-Physics, Emeryville, CA, for helpful suggestions in the use of the organotin reagent and for providing some of the sodium iodide-125 used in this study. We also thank Delagrange, Astra Alab, and Schering for gifts of test substances.

Registry No. (*R*)-**6b**, 115860-70-3; (*S*)-**6b**, 115655-34-0; (*R*)-**6b**·HCl, 115860-71-4; (*S*)-**6b**·HCl, 115860-72-5; [^{125}I](*S*)-**6b**, 115655-33-9; [^{125}I](*S*)-**6b**·HCl, 115860-76-9; (*S*)-**6c**, 96382-88-6; (*R*)-**11**, 22795-97-7; (*S*)-**11**, 22795-99-9; (±)-**11**, 36845-22-4; (*R*)-**11**·2 L-tartrate, 113310-91-1; (*R*)-**11**·2 D-tartrate, 113624-01-4; (*S*)-**11**·2 D-tartrate, 84277-13-4; (*S*)-**12**, 115860-73-6; (*S*)-**13**, 115860-74-7; **14**, 579-75-9; **14** (acid chloride), 21615-34-9; **15**, 40751-89-1; **16**, 90763-46-5; (*S*)-**17**, 115860-75-8; (*S*)-**17**·HCl, 115860-78-1; (*S*)-**18**·HCl, 115860-77-0; (*S*)-**19a**, 115860-79-2; (*S*)-**19b**, 115860-80-5; 2-methoxy-5-iodobenzoyl chloride, 115860-69-0; D-(−)-tartaric acid, 147-71-7; pyrrolidine, 123-75-1; methyl 2-methoxybenzoate, 606-45-1; methyl 2-methoxy-5-nitrobenzoate, 34841-11-7; 3-nitro-2-methoxybenzoic acid, 40751-88-0; piperidine, 110-89-4; *N*-methylpiperazine, 109-01-3.

(38) El Tayar, N.; van de Waterbeemd, H.; Testa, B. *J. Chromatogr.* **1985**, *320*, 293–304 and 305–312.

(39) De Vreis, D. J.; Beart, P. M. *Mol. Brain Res.* **1986**, *1*, 29–35.