Fluorescent Probes for Dopamine Receptors: Synthesis and Characterization of Fluorescein and 7-Nitrobenz-2-oxa-1,3-diazol-4-yl Conjugates of D-1 and D-2 Receptor Ligands

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Fluorescent probes have been designed and developed for dopamine D-1 and D-2 receptors. Flourescein and/or NBD (7-nitrobenz-2-oxa-1,3-diazol-4-yl) derivatives of PPHT (D-2 agonist), spiperone (D-2 antagonist), SKF 38393 (D-1 agonist), and SKF 83566 (D-1 antagonist) were synthesized via their amino-functionalized analogues and all ligands were pharmacologically evaluated by measuring their ability to displace [^{3}H]SCH 23390 and [^{3}H]spiperone from D-1 and D-2 receptor sites in caudate putamen of monkeys (*Macaca fascicularis*). The fluorescein derivatives of PPHT and SKF 83566 and the NBD derivatives of spiperone and SKF 83566 retained the high affinity and selectivity of the parent ligands. The NBD derivatives of PPHT showed higher D-2 receptor affinity and selectivity than their parent ligands. The enantiomers of the fluorescent derivatives of PPHT were also synthesized and were found to exhibit stereoselectivity in binding to the D-2 receptor, with the *S* enantiomers having a considerably higher affinity than their *R* analogues. In contrast to these results, the fluorescein derivative of SKF 38393 showed only a low affinity for the D-1 receptor. These fluorescein- and NBD-coupled D-1 and D-2 receptor ligands have considerable significance as potential probes in the study of distribution of the receptors at the cellular/subcellular level and of their mobility in membranes in normal/diseased states by use of fluorescence microscopic and fluorescence photobleaching recovery techniques, respectively. The development of these novel fluorescent probes should also provide new leads for the design and synthesis of additional fluorescent ligands with better fluorescent properties and/or higher affinity/selectivity for the DA receptors.

Until recently, dopamine (DA) receptors were classified into two subtypes entitled D-1 and D-2, respectively, and extensive biochemical studies had implicated their roles in several neurological, psychiatric, and cardiovascular disorders. Thus, DA receptor agonists and antagonists were considered to have profound clinical utility in the treatment of such diseases.[1-4] These studies of the physiology, pathophysiology, and pharmacology of DA receptors have received a resurgence following the recent discovery of new DA receptors[5-7] which should stimulate the further demarkation of the localization and regulatory functions of each DA receptor subtype in different species and tissues both in normal and diseased states. Molecular studies of the D-1 and D-2 receptors with photoaffinity ligands have led to their isolation and molecular characterization.[8,9] The development of [^{125}I]N_3-NAPS[10,11] and [^{125}I]I-MAB[12-14] as selective high-affinity photoaffinity ligands has enabled the identification of the ligand binding subunits of the D-2 and D-1 receptors (M_r 94K and 74K), respectively. The regional distribution of brain D-1 and D-2 receptors has been characterized by autoradiographic techniques using high-affinity radioligands specific for each receptor subtype.[15-17] However, in order to more fully understand the nature and functioning of these receptors as well as their interactions with other membrane components, notably their transducers/effectors, it will be necessary to localize the distribution of these receptors at the cellular/subcellular level and also determine their mobility in the membranes in normal and diseased states.[18]

One approach, which has been increasingly successful in this direction, involves the light microscopic study of the membrane-bound receptors using fluorescently labeled ligands, at a resolution higher than that permitted by autoradiographic techniques.[19] This method has been fruitful in varying degrees in the study of receptors for insulin, epidermal growth factor and gonadotropin releasing hormone,[20] cholinergic,[21] opioid,[22] adrenergic,[23,24] and glucagon[25,26] receptors. More recently, the use of a fluorescent β-adrenergic receptor antagonist, carazolol, has enabled the localization of the ligand-binding region of the

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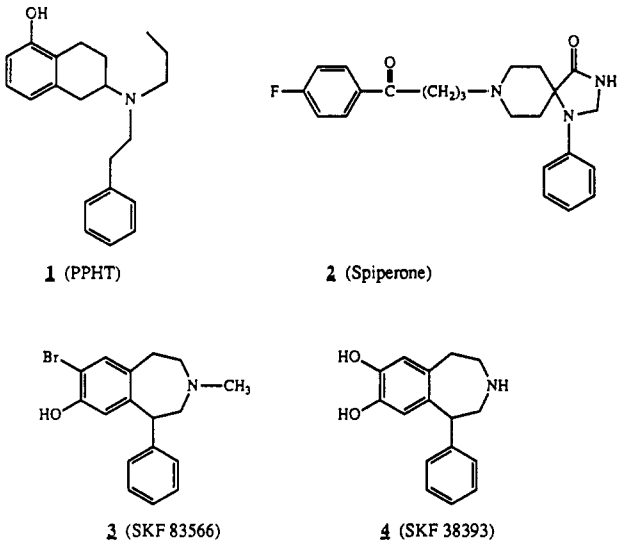
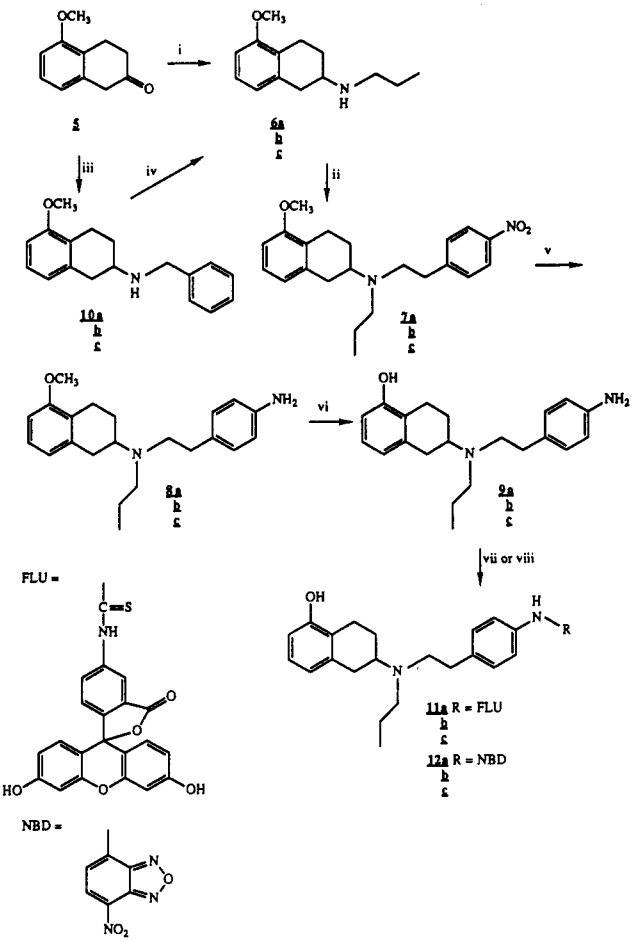
Chart I. Structures of D-1 and D-2, Receptor Agonist and Antagonist Ligands Used as Lead Compounds for the Design of Fluorescent Probes



receptor in its native membrane.[27] Fluorescent polarization studies have suggested that the ligand-binding domain of the β-adrenergic receptor is buried deep inside the membrane with a depth of ca. 11 Å.[27] However this approach has not so far been attempted in the study of DA receptors due to nonavailability of high-affinity fluorescent ligands for these receptors. In the course of our work on the development of fluorescent probes for the DA receptors,[28] Monsma et al.[29,30] reported the pharmacological characterization of some fluorescent ligands for labeling the DA receptors. In this report, we describe the synthesis and pharmacological characterization of a series of fluorescent probes with high affinity and selectivity for dopamine D-1 and D-2 receptors.

Scheme I[a]



[a] Series a is racemic, and b and c are the *R* and *S* enantiomers, respectively. (i) *n*-Propylamine, H_2/PtO_2; (ii) *p*-nitrophenacyl chloride, BH_3–THF; (iii) Benzylamine, H_2/PtO_2; (iv) D- or L-ditoluoyltartaric acid; propionic acid, $NaBH_4$; $H_2/Pd(OH)_2$; (v) Raney Ni, hydrazine hydrate; (vi) BBr_3; (vii) FITC; (viii) NDB-Cl.

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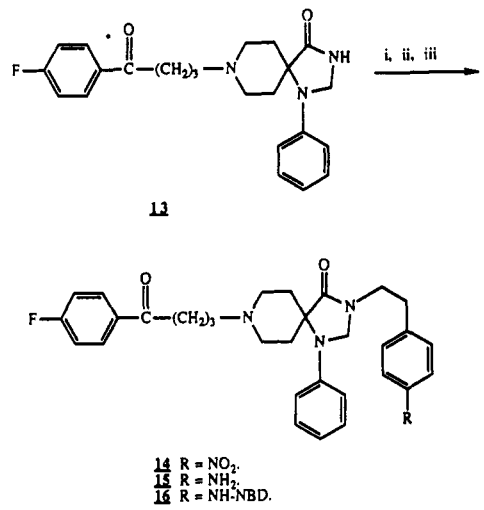
Design Aspects and Chemistry

The design and development of fluorescent probes for D-1 and D-2 receptors were undertaken in a stepwise manner: choice of high-affinity ligands (agonists and antagonists) selective for each receptor subtype; choice of a position in the ligand, where steric tolerance is indicated by previous structure–activity relationship (SAR) studies; introduction of a reactive functional group at that position; choice of fluorescent moieties possessing the desirable fluorescent properties; and reaction of the appropriate fluorescent reagent with the reactive group of the ligand.

The ligands were chosen for functionalization on the basis of their high affinity and selectivity for each receptor subtype and included 2-(*N*-phenethyl-*N*-propylamino)-5-hydroxytetralin (PPHT, **1**, D-2 agonist), 8-(4-(4-fluorophenyl)-4-oxobutyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (spiperone, **2**, D-2 antagonist), 7-bromo-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SKF 83566, **3**, D-1 antagonist), and 7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SKF 38393, **4**, D-1 agonist), which are depicted in Chart I. All these ligands have an unsubstituted phenyl ring which would provide a convenient location for the introduction of a reactive functionality. Since it is known that the unsubstituted aryl rings in PPHT, SKF 38393, and SKF 83566 are bound to the receptor in a region of steric tolerance,[31] the para positions of the aryl rings were felt to be the optimal positions for the introduction of a reactive amino group followed by coupling to a bulky fluorescent moiety without appreciable deleterious effects on the inherent affinity and selectivity of the ligands for their respective receptor subtypes. It has been previously observed that the *S* enantiomer of PPHT binds to the D-2 receptor with a ~30-fold higher affinity than its *R* enantiomer though their D-2/D-1 selectivity is similar.[32] Thus with the aim of developing fluorescent PPHT derivatives with higher affinities which exhibit a stereoselectivity at the receptor, we also synthesized the *R* and *S* enantiomers of fluorescent derivatives of PPHT. Though spiperone has an available unsubstituted phenyl ring, we choose instead to introduce the desired reactive amino functionality on a different aryl ring, to obtain a *N*-(*p*-aminophenethyl) derivative (NAPS) which has been previously synthesized and shown to retain the high affinity and selectivity of the parent ligand.[10,11] The fluorescent moieties chosen were fluorescein and NBD (7-nitrobenz-2-oxa-1,3-diazol-4-yl), the respective reagents being fluorescein isothiocyanate (FITC) and 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl).

The PPHT analogues were synthesized as depicted in Scheme I. 2-(*N*-propylamino)-5-methoxytetralin (**6a**) was prepared from 5-methoxy-2-tetralone (**5**) by reductive amination following the procedure of Hacksell et al.[32] Treatment of **6a** with *p*-nitrophenylacetyl chloride gave the intermediate amide which was reduced with diborane to tertiary amine **7a**. Catalytic transfer hydrogenation of the nitro group of **7a** with Raney Ni and hydrazine hydrate yielded the corresponding aryl amine **8a**. O-Demethylation of **8a** with BBr_3 gave the desired *p*-amino-functionalized derivative of PPHT (**9a**). The enantiomers of **9a** were prepared by resolution of the intermediate 2-(*N*-benzylamino)-5-methoxytetralin (**10a**) with D- and L-ditoluoyltartaric acids. Benzylamine **10a** itself was prepared from **5** by following the procedure of McDermed et al.[33] The stereochemical assignment of *R* and *S* enantiomers **10b** and **10c** was made by the comparison of the optical rotations of the respective HCl salts with the published values.[33] Reductive alkylation of the enantiomers of **10a** with propionic acid and $NaBH_4$ followed by debenzylation yielded the respective enantiomers **6b** and **6c**, which were then transformed into the desired enantiomeric precursors **9b** and **9c** by the same procedure as for the racemic compound. The amino-functionalized PPHT derivatives **9a–c** were then treated with FITC to obtain the fluorescent thioureas **11a–c**. Conjugation of the amines to NBD was carried out by nucleophilic reaction with NBD-Cl, in the presence of a catalytic amount of KI, to yield the fluorescent NBD derivatives **12a–c**. All the fluorescein and NBD derivatives were obtained as orange and red solids, respectively, and were characterized by their ^1H NMR spectra and elemental analysis.

Scheme II.[a]



[a] (i) *p*-Nitrophenethyl bromide, K_2CO_3, KOH, $(n\text{-Bu})_4NHSO_4$, toluene; (ii) Fe/HCl; (iii) NBD-Cl.

The synthesis of NAPS–NBD is depicted in Scheme II. NAPS (**15**) itself was prepared by treatment of spiperone (**13**) with *p*-nitrophenylethyl bromide followed by reduction of the nitro derivative **14** with Fe/HCl, in 40% overall yield. Conjugation of **15** to NBD was carried out essentially as before for the PPHT-based probes, to obtain the fluorescent NBD derivative **16**.

The substituted 1-phenyl-3-benzazepine-based D-1 agonist and antagonist probes were synthesized by the methods analogous to the recently described synthesis of IMAB in our laboratory.[14] The SKF 83566-based probes were synthesized as depicted in Scheme III.

8-Methoxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (**17**) was selectively brominated at the 7-position to afford **18**. Reduction of **18** to amine **19** was achieved without significant debromination, by catalytic transfer hydrogenation with Raney Ni and hydrazine hydrate. O-Demethylation of **19** with BBr_3 then gave the required amino-functionalized analogue of SKF 83566 (**20**). Conjugation of **20** to fluorescein and NBD was carried out in a similar fashion as for the PPHT-based probes to yield the fluorescein (**21**) and NBD (**22**) derivatives of SKF 83566.

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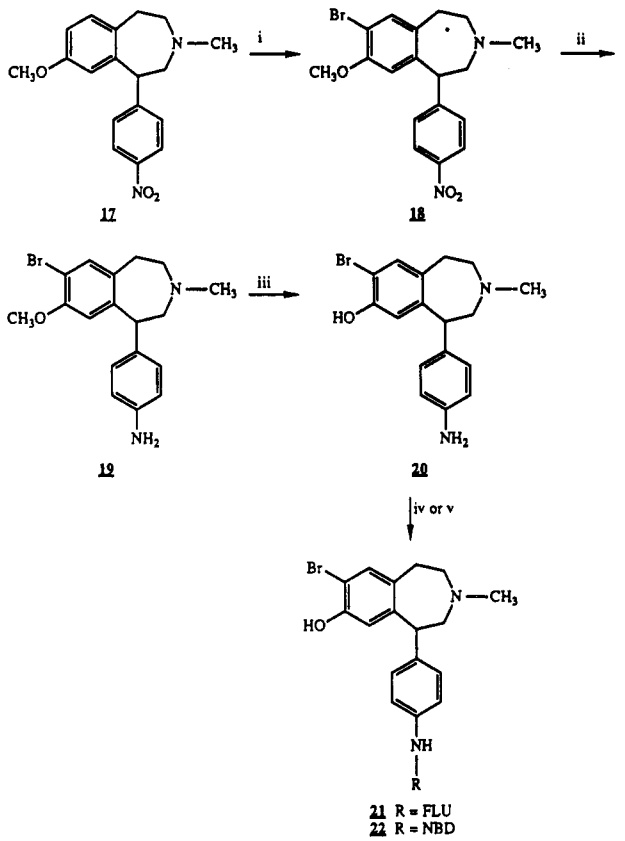
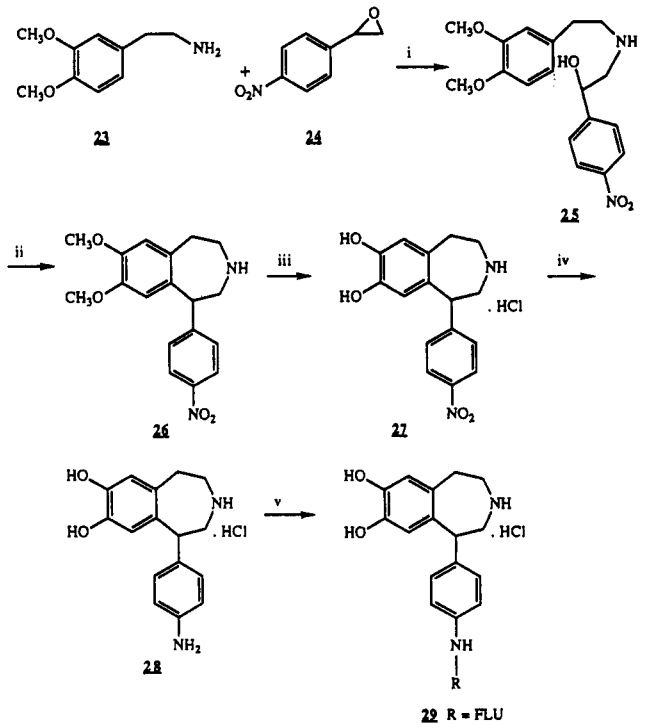
Scheme III.[a]



[a] (i) Br_2/AcOH; (ii) Raney Ni, hydrazine hydrate; (iii) BBr_3, CH_2Cl_2, −70 °C; (iv) FITC; (v) NBD-Cl.

Scheme IV.[a]



[a] (i) THF, reflux; (ii) PPA, (iii) HCl (12 N), reflux; (iv) H_2/Pd/C; (v) FITC.

A similar strategy was employed for the synthesis of the SKF 38393 based probes (Scheme IV). However, since the secondary amine function present in SKF 38393 can react with fluorescent reagents (FITC and NBD-Cl) to give unwanted 3-substituted derivatives, we considered either protecting the secondary amine with an acetyl group or using a monohydrochloride salt of amino-functionalized SKF 38393 with the more basic secondary amino group forming a salt while leaving the aromatic primary amino group in its free-base form. We decided on the latter approach since it would be considerably more difficult to deprotect the acylated secondary amine, without any adverse effects on the thiouriedo–fluorescent tag. Condensation of homoveratrylamine (**23**) with *p*-nitrostyrene oxide (**24**)[34] yielded benzyl alcohol **25**. Cyclization of **25** was carried out by heating with PPA to obtain benzazepine **26**. O-Demethylation of **26** with refluxing concentrated HCl gave catechol **27** as the monohydrochloride salt which was then catalytically hydrogenated to yield the required amino-functionalized derivative **28**. Conjugation of **28** to fluorescein was carried out as described before to obtain the fluorescent derivative of SKF 38393 (**29**).[36]

The fluorescein derivative of NAPS is not described in the present study as it was independently reported by Monsma et al.[26]

Table I. Affinity and Selectivity of Ligands for D-1 and D-2 Receptors

compound	affinity[a] D-1	affinity[a] D-2	selectivity[b]
1 (*RS*-PPHT)	804	13.3	60
1 (*R*-PPHT)	6500	60.0	110
1 (*S*-PPHT)	230	2.1	110
9a (*RS*)	710	6.8	100
9b (*R*)	4900	170	30
9c (*S*)	270	6.7	40
11a (*RS*)	340	7.0	50
11b (*R*)	24000	110	220
11c (*S*)	260	4.8	55
12a (*RS*)	170	0.45	380
12b (*R*)	3700	3.2	1200
12c (*S*)	130	0.3	430
2 (spiperone)	323	0.06	5600
16	99	0.66	150
3 (SKF 83566)	0.3	1360	4500
20	2.3	1600	700
21	16	>5000	>300
22	5.3	710	130
4 (SKF 38393)	130	6500	50
29	~10000		

[a] Affinities of the compounds for D-1 and D-2 receptors are listed in the form of their K_i values (nM) which were computed from the corresponding IC_{50} values using the LIGAND program. The IC_{50} values of the ligands were themselves determined from competition binding experiments with [^{3}H]SCH 23390 (0.2 nM, D-1) or [^{3}H]spiperone (0.1 nM, D-2) in monkey caudate–putamen membranes as described (including statistical limits) in detail by Madras et al.[35] [b] Selectivity values listed, were calculated as ratios (D-1/D-2 or D-2/D-1) of the corresponding K_i values of the compounds.

Pharmacology

The pharmacological characterization of the fluorescent ligands was carried out by measuring the ability of these

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(36) It is assumed that the monohydrochloride salt **28** on reaction with FLU-NCS gives **29**. The point of conjugation of the fluorescein group, however has not been unambiguously established.

ligands to displace [^{3}H]SCH 23390 and [^{3}H]spiperone from D-1 and D-2 receptor sites in the caudate–putamen of monkeys (*M. fascicularis*).[35] The affinity (K_i) and selectivity (D-1/D-2 or D-2/D-1 as the case may be) were determined for all the ligands and are shown in Table I. Also included are data for the parent ligands. Coupling of the selective D-1 antagonist SKF 83566 to fluorescein (**21**) and NBD (**22**) results in lowering of both the affinity (~18–50-fold) and the D-1/D-2 selectivity (~6–30-fold) but the ligands still retained high D-1 receptor affinity and selectivity. Coupling of the selective D-1 agonist SKF 38393 to fluorescein (**29**) resulted in a considerable loss of affinity, making this fluorescent derivative unsuitable for labeling the D-1 receptor. Since SKF 38393 itself has only a moderate affinity for the D-1 receptor (~100 nM), this loss of affinity is similar to that seen with the antagonist SKF 83566-based fluorescent probe. On account of the high affinity of the parent ligand (SKF 83566, 0.3 nM), it still retains the high affinity and receptor selectivity necessary for the ligand to be useful for fluorescent labeling studies. Coupling of NAPS, the *p*-aminophenethyl derivative of the selective D-2 antagonist spiperone, to NBD gave NAPS-NBD (**16**), which binds with high affinity and selectivity to the D-2 receptor, though once again there is a lowering of the affinity (~10-fold) and selectivity (~20-fold) when compared to those of spiperone. When the selective D-2 agonist PPHT and its enantiomers were coupled to fluorescein (**11**) the resulting ligands showed only a slight (~2-fold) lowering of the affinity and selectivity for the D-2 receptor. However when they were coupled to NBD, very interesting and unexpected results were seen. (*RS*)-PPHT-NBD (**12a**) binds with a ~10-fold higher affinity and selectivity to the D-2 receptor than (*RS*)-PPHT while (*R*)-PPHT-NBD (**12b**) exhibits a 30-fold and (*S*)-PPHT-NBD (**12c**) a ~10-fold higher D-2 receptor affinity and selectivity than (*R*)- and (*S*)-PPHT, respectively. Thus coupling of PPHT to the NBD fluorophore resulted in a considerable (~10–30 fold) increase in D-2 receptor affinity and selectivity. The lower affinity of the fluorescein and NBD derivatives of SKF 83566, of the fluorescein derivatives of SKF 38393 and PPHT, and of the NBD derivative of NAPS probably results from the adverse steric interactions of the larger fluorescent tags. On the other hand, the high affinity and selectivity of the NBD derivative of PPHT is probably due to the fact that lipophilic moieties like NBD, when coupled to PPHT, tend to interact with previously inaccessible secondary binding sites on the D-2 receptor.

In conclusion, at the very least, the development of these novel selective high-affinity and highly fluorescent ligands will provide new leads for the synthesis of additional fluorescent ligands with better fluorescent properties and/or higher affinity/selectivity for the DA receptors. More significantly, the use of these highly fluorescent ligands should enable the cellular/subcellular localization of brain DA receptors and the study of their mobility in membranes in normal/diseased states, work which is now underway and the results of which will be communicated at a later stage.

Experimental Section

Analytical thin-layer chromatography was performed using E. Merck F-254 plastic-backed thin-layer silica gel plates. Medium-pressure column chromatography was performed using Baker flash silica gel. The organic extracts were dried with anhydrous $MgSO_4$ unless indicated otherwise. Melting points were obtained with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian XL-300 (300 Hz) NMR spectrometer using TMS as the internal standard. Chemical shifts are reported down-field from TMS. Mass spectra were determined by Finnigan 4021 mass spectrometer under EI conditions and operated by the Department of Chemistry of Northeastern University. Microanalyses were performed by Atlantic Microlab Inc., Atlanta, GA, and were within ±0.4% of the calculated values.

(*R*)-(+)- and (*S*)-(−)-2-(*N*-Benzylamino)-5-methoxytetralin (10b and 10c). A solution of 5-methoxy-2-tetralone (**5**, 11.3 g, 64 mmol), benzylamine (8.6 g, 81 mmol), and *p*-TsOH (400 mg) in 100 mL of toluene was refluxed under nitrogen with continuous removal of water for 2 h. The toluene was distilled off under reduced pressure; the residual oil was taken up in EtOH, treated with 100 mg of PtO_2, and hydrogenated at 32 psi for 1 h. The catalyst was removed, the solvent and the excess benzylamine were evaporated, and the crude amine was converted into the HCl salt with ethanolic HCl. The salt was purified by recrystallization from EtOH–Et_2O to yield 14.5 g (75%); mp 246–248 °C (lit.[33] mp 246–248 °C).

The benzylaminotetralin free base (2.4 g, 9 mmol) in acetone (40 mL) was treated with D-di-*p*-toluoyltartaric acid (4.0 g, 9.9 mmol). The precipitated salt was collected and was recrystallized from acetone three times to give the (−)-amine·(−)-tartrate: 1.9 g (32%); mp 158–160 °C; $[\alpha]_D$ = −113.2° (*c* = 1, MeOH). The tartrate salt was then converted into the HCl salt to give 0.8 g: mp 246–248 °C; $[\alpha]_D$ = −60.3° (*c* = 1, MeOH) (lit.[32] mp 246–247 °C); $[\alpha]_D$ = −61°).

The mother liquor from the resolution was converted to the free base and was treated with equimolar amount of L-di-*p*-toluoyltartaric acid. The salt was recrystallized from acetone three times and then was converted into the HCl salt: 600 mg; mp 245–247 °C; $[\alpha]_D$ = +58.6° (*c* = 1, MeOH) (lit.[33] mp 246–247 °C; $[\alpha]_D$ = +61°).

(*RS*)-2-(*N*-Propylamino)-5-methoxytetralin Hydrochloride (6a). A mixture of 5-methoxy-2-tetralone (**5**, 9.0 g, 50 mmol), *n*-propylamine (8.9 g, 150 mmol), glacial HOAc (9.2 g), absolute EtOH (125 mL), and molecular sieves (4A, 20 g) was stirred at ambient temperature and under an atmosphere of nitrogen for 2 h. The molecular sieves were filtered off, and the filtrate was hydrogenated over 300 mg of PtO_2 at 42 psi until the uptake of H_2 was complete. The mixture was filtered and evaporated under reduced pressure to an oil. The oil was taken up in absolute EtOH and was treated with ethanolic HCl. The precipitated hydrochloride salt was collected and recrystallized from EtOH: 8.9 g (70%); mp 260–261 °C (lit.[32] mp 260–261 °C).

(*R*)-(+)-2-(*N*-Propylamino)-5-methoxytetralin (6b). To a solution of propionic acid (3.4 g, 45 mmol) in toluene stirred under N_2 at 10–20 °C was added $NaBH_4$ (580 mg, 15 mmol) in small portions. The mixture was stirred for 3 h. To this a solution of (*R*)-(+)-2-(*N*-benzylamino)-5-methoxytetralin (**10b**) (free base from 900 mg of the HCl salt, 3 mmol) in 10 mL of dry toluene was added and the resulting mixture was heated at reflux for 6 h, cooled, and treated with 20 mL of 10% NaOH. The organic layer was separated, dried, and evaporated. The residual oil was taken up in ethanol and was hydrogenated over $Pd(OH)_2$ for 3 h. The catalyst was removed, the solvent was evaporated, and the amine was converted into the HCl salt and recrystallized from EtOH–Et_2O to give 580 mg (76%): mp 275–276 °C; $[\alpha]_D$ = +71.8° (*c* = 1, MeOH).

(*S*)-(−)-2-(*N*-Propylamino)-5-methoxytetralin (6c) was prepared as described above from (*S*)-(−)-2-(*N*-benzylamino)-tetralin **10c**: 70% yield; mp 274–276 °C; $[\alpha]_D$ = −70.4° (*c* = 0.5, MeOH).

(*RS*)-2-[*N*-(*p*-Nitrophenethyl)-*N*-propylamino]-5-methoxytetralin Hydrochloride (7a). To a vigorously stirred mixture of propylaminotetralin hydrochloride **6a** (2.6 g, 10 mmol), 50 mL of CH_2Cl_2, and 100 mL of 5% NaOH at 25 °C was added a solution of *p*-nitrophenylacetyl chloride (prepared from 4-nitrophenylacetic acid and $SOCl_2$) in CH_2Cl_2 (2.0 g/25 mL). The resulting mixture was stirred for an additional period of 10 min; the organic layer was separated, washed with water, dried, and evaporated to give the amide as a yellow oil (3.7 g, 97%).

A solution of the amide in 50 mL of dry THF was added slowly to a solution of BH_3 in THF (30 mmol) stirred at 0 °C. The resulting orange-yellow solution was heated at reflux for 2 h. The mixture was cooled, treated with 10 mL of 6 N HCl, concentrated to 10 mL in vacuo, heated at reflux for 10 min, cooled, and basified with NaOH pellets. This was extracted with CH_2Cl_2, dried,

evaporated, and chromatographed on silica gel (CH_2Cl_2–MeOH, 9:1). The nitro amine was collected, converted to the HCl salt, and recrystallized from EtOH–Et_2O to yield 2.1 g (51%): mp 199–200 °C; ^1H NMR (CD_3OD) δ 1.0–1.1 (t, 3, CH_3), 1.85–2.0 (m, 4), 2.35–2.45 (m, 2), 2.6–2.75 (m, 2), 3.0–3.4 (m, 5), 3.5–3.6 (m, 2) 3.8 (s, 3, OCH_3), 6.75–6.8 (d, 2, ArH), 7.1–7.2 (t, 1, ArH), 7.6–7.65 (d, 2, ArH), 8.2–8.25 (d, 2, ArH); mass spectrum, *m/e* (relative intensity) 368 (25), 232 (100), 202, 161. Anal. Calcd for $C_{22}H_{29}ClN_2O_3$: C, H, N.

(*R*)-(+)-2-[*N*-(*p*-Nitrophenethyl)-*N*-propylamino]-5-methoxytetralin (7b). This compound was prepared from (*R*)-(+)-2-(*N*-propylamino)tetralin **6b**, by the method described above for the racemic compound. Product was found to be identical to the racemic compound by TLC and IR and ^1H NMR spectra: mp 196–198 °C; $[\alpha]_D$ = +42.2° (*c* = 0.5, MeOH).

(*S*)-(−)-2-[*N*-(*p*-Nitrophenethyl)-*N*-propylamino]-5-methoxytetralin (7c). This compound was prepared from (*S*)-(−)-2-(*N*-propylamino)tetralin **6c**, by the method described above for the racemic compound. Product was found to be identical to the racemic compound by TLC and IR and ^1H NMR spectra: mp 197–200 °C; $[\alpha]_D$ = −42.8° (*c* = 0.5, MeOH).

(*RS*)-2-[*N*-(*p*-Aminophenethyl)-*N*-propylamino]-5-hydroxytetralin Dihydrochloride (9a). A mixture of nitro compound **7a** (810 mg, 2 mmol) and hydrazine hydrate (1 mL) in 30 mL of absolute EtOH was stirred at 50 °C until the solution became clear. To this was added 250 mg of Raney Ni and the mixture was stirred until no more gas evolved. The mixture was cooled, filtered, and evaporated to a foamy solid (**8a**).

The product was converted to the free base and was treated with BBr_3 in CH_2Cl_2 at −78 °C to give demethylated compound **9a**, which was isolated and characterized as the dihydrochloride salt: yield, 640 mg, 78%; mp 200–205 °C; ^1H NMR (CD_3OD) δ 1.0–1.1 (t, 3, CH_3), 1.85–2.0 (m, 4), 2.4–2.5 (m, 2), 2.65–2.8 (m, 2), 3.0–3.6 (m, 6), 3.8–3.9 (m, 1), 6.6–6.7 (m, 2, ArH), 6.95–7.05 (dd, 1, ArH), 7.4–7.45 (d, 2, ArH), 7.6–7.65 (d, 2, ArH); mass spectrum, *m/e* 218 (100), 147 (55). Anal. Calcd for $C_{21}H_{30}Cl_2N_2O \cdot {}^3/_4H_2O$: C, H, N.

(*R*)-(+)-2-[*N*-(*p*-Aminophenethyl)amino]-5-hydroxytetralin (9b). This compound was prepared from (*R*)-(+)-2-(*N*-propylamino)tetralin **7b** by the method described above for the racemic compound. Product was found to be identical to the racemic compound by TLC and IR and ^1H NMR spectra; $[\alpha]_D$ = +41.1° (*c* = 0.5, MeOH).

(*S*)-(−)-2-[*N*-(*p*-Aminophenethyl)amino]-5-hydroxytetralin (9c). This compound was prepared from (*S*)-(−)-2-(*N*-propylamino)tetralin **7c** by the method described above for the racemic compound. Product was found to be identical to the racemic compound by TLC and IR and ^1H NMR spectra; $[\alpha]_D$ = −43.8° (*c* = 0.5, MeOH).

General Procedure for the Preparation of PPHT–Fluorescein–Thiourea Derivatives. A solution of amine (0.5 mmol) and fluorescein isothiocyanate (0.5 mmol) in 1 mL of dry DMF was left in the dark at 25 °C for 3 days. The solution was diluted with MeOH and Et_2O to precipitate the fluorescein derivatives as orange solids. The compounds are purified by recrystallization from DMF–MeOH–Et_2O.

General Procedure for the Preparation of PPHT–NBD Analogues. A mixture of NBD–Cl (0.5 mmol), the amine (0.3 mmol), KI (5 mg), and *n*-BuOH (10 mL) was heated at reflux under N_2 for 3 h. The reaction mixture on dilution with Et_2O precipitated the NBD derivative as a dark red solid. Recrystallization is done from MeOH–Et_2O.

[Fluoresceinyl(thioureido)]-PPHT (11a) was purified by recrystallization from DMF–MeOH–Et_2O: yield, 200 mg, 56%; mp 213–215 °C; IR (KBr) (cm^{-1}) 3500, 3000, 1750, 1650, 1620, 1550; ^1H NMR (DMSO-d_6) δ 0.9–1.0 (t, 3, CH_3), 1.85–2.0 (m, 4), 2.4–2.5 (m, 2), 2.65–2.8 (m, 2), 3.0–3.6 (m, 6), 3.8–3.9 (m, 1), 6.4–7.0 (m, 8, ArH), 6.85–6.9 (dd, 1, ArH), 7.15–7.2 (m, 3, ArH), 7.4–7.45 (d, 2, ArH), 7.75–7.8 (dd, 1, ArH), 8.2 (s, 1, ArH), 9.1–9.15 (br s, 1), 10.1–10.2 (br s, 2); mass spectrum, *m/e* 218 (100). Anal. Calcd for $C_{42}H_{39}N_3O_6S \cdot {}^3/_2H_2O$: C, H, N.

Similarly prepared were the *R* and *S* enantiomers **11b,c**. Optical rotations of the fluorescein derivatives could not be measured because of the intense absorptions and/or emissions.

NBD-NH-PPHT (12a). A mixture of PPHT-NH_2 (**9a**, 100 mg, 0.3 mmol), NBD-Cl (120 mg, 0.5 mmol), KI (5 mg), and 10 mL of *n*-BuOH was heated at reflux for 2 h. The reaction mixture was diluted with 10 mL of Et_2O and the precipitated dark red solid was filtered and washed with Et_2O. The solid was recrystallized from MeOH–Et_2O: yield, 130 mg; mp 153–155 °C dec; IR (KBr) 3400, 2900, 1710, 1610, 1600, 1550, 1500; ^1H NMR (DMSO-d_6) δ 1.0–1.05 (t, 3, CH_3), 1.3–1.4 (m, 2), 1.8–1.9 (m, 4), 2.4–2.6 (m, 4), 2.8–3.4 (m, 4), 3.75–3.8 (m, 1), 6.6 (d, 1, ArH), 6.7–6.75 (m, 2, ArH), 6.95–7.0 (t, 1, ArH), 7.5–7.6 (m, 4, ArH), 8.55–8.60 (d, 1, ArH), 9.4 (br s, ex), 10.4 (br s, ex), 11.1 (br s, ex); mass spectrum, *m/e* 218 (100), 147, 72. Anal. Calcd for $C_{27}H_{30}ClN_5O_4 \cdot H_2O$: C, H, N.

Similarly prepared were the *R* and *S* enantiomers **12b,c**. The products were identified by TLC, NMR, and elemental analysis.

3-[2′-(*p*-Aminophenyl)ethyl]-8-[3′-(*p*-fluorobenzoyl)-propyl]-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane (15). To a mixture of spiperone **13** (1.3 g, 3.3 mmol), powdered KOH (30 mg, 0.56 mmol), powdered K_2CO_3 (740 mg, 13.2 mmol), and tetra-*n*-butylammonium hydrogen sulfate (280 mg, 0.8 mmol) in anhydrous toluene (120 mL) stirred at 90 °C was added a solution of *p*-(nitrophenyl)ethyl bromide (1.5 g, 6.6 mmol) in 20 mL of anhydrous toluene over a period of 30 min. The resulting mixture was stirred at 90 °C for 2 h and then cooled and diluted with water. It was then extracted with EtOAc (2 × 50 mL), and the combined organic layers were washed with brine and then dried and evaporated in vacuo. The residue was purified by column chromatography (silica gel; CH_2Cl_2–MeOH, 9:1). The product (**14**, 1.3 g) in EtOH (25 mL) and concentrated HCl (25 mL) was treated with Fe powder and the mixture was refluxed for 30 min. After removal of EtOH, the reaction mixture was basified to pH 12 and was extracted with EtOAc. Evaporation of the solvent and column chromatography of the residue gave 900 mg of a foamy solid (53%). The product was found to be identical to the material prepared by the method of Amlaiky and Caron[10] by TLC and ^1H NMR.

3-[2′-[4-[(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-phenyl]ethyl]-8-[3′-(*p*-fluorobenzoyl)propyl]-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane (NBD-APE-SPI, 16). A mixture of (aminophenethyl)spiperone **15** (100 mg, 0.2 mmol), NBD-Cl (50 mg, 0.25 mmol), KI (2 mg), and 20 mL of *n*-BuOH was heated at reflux for 3 h. The reaction mixture was cooled, diluted with Et_2O, treated with ethereal HCl, and left at 0 °C overnight. The precipitated deep red solid was collected and dried: yield, 80 mg; mp 146–149 °C; ^1H NMR (DMSO-d_6) δ 1.65–1.7 (m, 2, CH_2), 2.0–2.1 (m, 2, CH_2), 2.45–2.5 (m, 2, CH_2), 2.9–3.0 (m, 4, CH_2s), 3.15–3.25 (m, 4, CH_2s), 3.5–3.7 (m, 4, CH_2s), 4.6 (s, 2, NCH_2N), 6.6–6.65 (d, 1, ArH), 6.8–6.9 (m, 1, ArH), 7.05–7.15 (m, 2, ArH), 7.25–7.3 (m, 2, ArH), 7.4–7.5 (m, 4, ArH), 8.0–8.1 (m, 2, ArH), 8.5–8.55 (d, 1, ArH), 10.9 (br s, 1, NH), 11.1 (br s, 1). Anal. Calcd for $C_{37}H_{38}Cl_2FN_7O_5$: C, H, N, Cl.

7-Bromo-8-methoxy-3-methyl-1-(4′-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (18). Compound **17**[14] (0.9 g, 2.88 mmol) was dissolved in 50 mL of acetic acid and stirred at 60 °C. A solution of bromine (0.6 g, 0.2 mL) in 10 mL of glacial HOAc was added dropwise. After stirring for 3 h, it was poured onto crushed ice and made alkaline with aqueous NH_3, extracted with CH_2Cl_2, and concentrated in vacuo to a deep red residue which was purified by flash chromatography (silica gel; $CHCl_3$) to give 0.52 g (46%) of crystals; mp 161–163 °C. Anal. Calcd for $C_{18}H_{19}BrN_2O_3$: C, H, N.

7-Bromo-8-methoxy-3-methyl-1-(4′-aminophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (19). Compound **18** (0.5 g, 1.28 mmol) was dissolved in 25 mL of absolute EtOH, 0.3 mL (6.0 mmol) of hydrazine hydrate added and the solution stirred and heated at 50 °C. Raney Ni was added to the mixture in small portions until gas evolution ceased. The catalyst was removed and the product was isolated after flash chromatography (silica gel; CH_2Cl_2–MeOH, 5%) to yield 0.340 g (70%). Anal. Calcd for $C_{18}H_{21}BrN_2O \cdot 0.5H_2O$: C, H, N.

7-Bromo-8-hydroxy-3-methyl-1-(4′-aminophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (20). Compound **19** (75 mg, 0.21 mmol) was dissolved in 25 mL of dry CH_2Cl_2 and stirred at −70 °C under N_2. To this was added dropwise a solution of BBr_3 in hexane (1.0 M solution, 1.25 g, 5 mmol). After the addition was complete, the reaction mixture was stirred at −70 °C for 1 h and then at room temperature for 3 h. It was then cooled to −70 °C and quenched with 50 mL of MeOH. The mixture was concen-

trated in vacuo and the residue was crystallized from MeOH/Et_2O to yield 65 mg of an off-white crystalline solid (62%): mp 230 °C; mass spectrum, *m/e* 347 (M^+). Anal. Calcd for $C_{17}H_{21}BrN_2O_3 \cdot 3H_2O$: C, H, N (H within 0.45).

7-Bromo-8-hydroxy-3-methyl-1-[4′-[*N*′-fluoresceinyl-(thiouriedo)]phenyl]-2,3,4,5-tetrahydro-1*H*-3-benzazepine (21). Compound **20** (35 mg, 0.1 mmol) and fluorescein isothiocyanate (40 mg, 0.11 mmol) were mixed in 2 mL of dry DMF and allowed to stand for 3 days at room temperature in the dark. The reaction mixture was diluted with Et_2O; the resulting orange precipitate was filtered, washed with Et_2O containing MeOH (10%), and dried in vacuo to yield 40 mg (54%); mp >300 °C. Anal. Calcd for $C_{38}H_{30}BrN_3O_6S \cdot 2H_2O \cdot 0.5DMF$: C, H, N.

7-Bromo-8-hydroxy-3-methyl-1-[4′-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]phenyl]-2,3,4,5-tetrahydro-1*H*-3-benzazepine (22). Compound **20** (70 mg, 0.2 mmol), NBD-Cl (60 mg, 0.3 mmol), and 10 mg of KI were dissolved in 10 mL of *n*-BuOH and stirred under reflux for 3 h. The reaction mixture was cooled, concentrated in vacuo to a dark red residue which was suspended in $CHCl_3$, and washed with a 5% aqueous solution of $NaHCO_3$. The organic layer was dried, evaporated and the residue was purified by flash chromatography (silica gel; CH_2Cl_2–MeOH, 5%). The product was isolated as the HCl salt from EtOH·HCl/Et_2O to yield 60 mg (50%); mp 215–218 °C. Anal. Calcd for $C_{23}H_{20}BrN_5O_4 \cdot 2HCl \cdot 0.5H_2O$: C, H, N (calcd, 11.82; found, 11.31).

α-[2-[*N*-[2-(3,4-Dimethoxyphenyl)ethyl]amino]ethyl]-*p*-nitrobenzyl Alcohol (25). Homoveratrylamine (**23**, 5 g, 0.03 mol) and *p*-nitrostyrene oxide (**24**, 4.5 g, 0.027 mol) were dissolved in freshly dry-distilled THF and stirred and refluxed overnight. The reaction mixture was cooled and then concentrated in vacuo to a dark residue. It was crystallized from EtOAc–hexane to yield 3.2 g of off-white powder (35%); mp 92–95 °C. Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, H, N.

7,8-Dimethoxy-1-(4′-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (26). Compound **26** (1.0 g, 0.003 mol) was triturated with 15 g of PPA and then heated at 100 °C for 1 h. The reaction mixture was poured onto crushed ice, the solution made alkaline with aqueous NH_3, and the resulting precipitate filtered, washed with water, and dried over P_2O_5 overnight. Conversion to the HCl salt with EtOH·HCl followed by recrystallization from MeOH–Et_2O gave colorless needles (700 mg, 66.5%); mp 170–172 °C. Anal. Calcd for $C_{18}H_{21}ClN_2O_4 \cdot 2H_2O$: C, H, N.

7,8-Dihydroxy-1-(4′-aminophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrochloride (28). Compound **27** (700 mg, 1.92 mmol) was dissolved in 20 mL of 12 N HCl and the solution stirred under reflux for 24 h. It was then cooled and concentrated in vacuo to dryness on a hot water bath. Crystallization from MeOH–Et_2O gave 525 mg (81%) of demethylated compound **27** as a tan crystalline powder; mp 198–200 °C.

A 250 mg (0.75 mmol) portion of tan solid **27** was dissolved in 100 mL of absolute EtOH, 25 mg of 10% Pd/C added to it, and the mixture was hydrogenated at 45 psi and room temperature for 24 h. As TLC indicated that reaction was not complete, a fresh 25 mg of 10% Pd/C was added and the reaction mixture was rehydrogenated at 45 psi and room temperature for another 24 h. The mixture was filtered to remove the catalyst and the filterate concentrated in vacuo to a pale yellow residue which was crystallized from MeOH–Et_2O to yield 135 mg (60%); mp 210–212 °C. Anal. Calcd for $C_{16}H_{19}ClN_2O_2 \cdot 1.5H_2O$: C, H, N (calcd, 8.38; found, 7.82).

7,8-Dihydroxy-1-[4′-[*N*′-fluorescienyl(thiouriedo)]-phenyl]-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrochloride (29). Compound **28** (30 mg, 0.1 mmol) and fluorescein isothiocyanate (39 mg, 0.1 mmol) were mixed in 2 mL of DMF and allowed to stand at room temperature in the dark for 3 days. The reaction mixture was diluted with water; the resulting precipitate was filtered, washed well with water, and dried in vacuo over P_2O_5 overnight to yield 30 mg (54%) of orange-red precipitate; mp >250 °C. Anal. Calcd for $C_{37}H_{30}ClN_3O_6S \cdot 2H_2O$: C, H, N.

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