SYNTHESIS, RESOLUTION AND RADIOIODINATION OF *S*(-)*trans*-5-HYDROXY-2-[N-n-PROPYL-N-(3'-IODO-2'-PROPENYL)AMINO]TETRALIN-*S*(-)*trans*-5-OH-PIPAT: A NEW DOPAMINE D2-LIKE RECEPTOR LIGAND

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SUMMARY

A new dopamine D2-like receptor ligand, (*R,S*)*trans*-5-hydroxy-2-[N-n-propyl-N-(3'-iodo-2'-propenyl)amino]tetralin ((*R,S*)*trans*-5-OH-PIPAT, **3**), based on high affinity dopamine receptor agonist 5-hydroxy-2-[N,N-(di-n-propyl)-2-amino]tetralin (5-OH-DPAT, **1**), was prepared. The synthesis was achieved by a reductive amination of 5-methoxy-2-tetralone with n-propylamine, followed by N-alkylation, to afford 5-methoxy-N-propyl-N-2'-propynyl-2-aminotetralin, **7**. Reduction of **7** with tributyltin hydride gave the tri-n-butyl tin derivative, **8**, which was converted to **9** by an iododemetalation reaction. Demethylation of **9** gave the desired compound, (*R,S*)*trans*-5-OH-PIPAT, **3**. The resolved (*R*) and (*S*)*trans*-5-OH-PIPAT, **3**, were also quantitatively prepared. *In vitro* binding studies showed the stereoselectivity of this new compound for binding to dopamine D2-like receptors. *S*(-)-**3** displayed high binding affinity, with inhibition constants (K_i) of 0.38, 0.09 and 0.67 nM for dopamine D2H (expressed in HEK293 cells), D3 (expressed in Sf9 cells) and D4H receptors (expressed in CHO cells), respectively. Using the same binding assays, the less active *R*(+) isomer displayed K_i values of 7.29, 4.87 and 16.44 nM for D2H, D3 and D4H receptors, respectively. In addition, radiolabeling was successfully performed, either with the racemic tin derivative, (*R,S*)-**11**, or using the optically resolved tin derivatives *R*(+)- or *S*(-)-**11**, to give the final radiolabeled product, [^{125}I]*R*(+) or *S*(-)-**3**.

Key Words: Dopamine D2, D3 and D4 Receptors, Iodine-125, Radioiodination, 5-OH-DPAT, 5-OH-PIPAT

INTRODUCTION

In recent years, the application of molecular biology techniques of expressing receptors in cloned cells has dramatically expanded our understanding of the complexity of dopamine receptors in the central nervous system (CNS). Cloning of dopamine receptors has produced at least five different dopamine receptor subtypes: D1, D2, D3, D4 and D5 (1-3). Currently, the dopamine receptors are generally categorized in two subtypes, D1-like receptors (D1 and D5) and D2-like receptors (D2, D3 and D4).

Initially, [^{3}H]7-OH-DPAT (7-hydroxy-N,N-(di-n-propyl)-2-aminotetralin) was identified as a selective ligand for the dopamine D3 receptor expressed in CHO cells, with a

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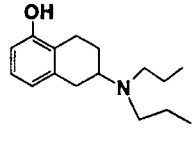
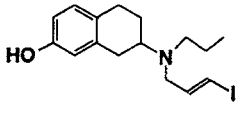
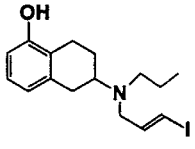
K_d value of 0.67 nM (4). We have reported the synthesis and binding study of an iodinated derivative based on 7-OH-DPAT, (*R,S*)*trans*-7-hydroxy-2-[N-n-propyl-N-(3'-iodo-2'-propenyl)amino]tetralin, ((*R,S*)*trans*-7-OH-PIPAT), **2**, which was formed by placing the iodine atom on the N-propenyl side chain (5). This unique feature produced a stable iodinated derivative with highly desirable properties: higher specific activity, more potent binding affinity and lower non-specific binding. Competition binding data exhibited the pharmacological profile of dopamine D3 receptors (5). This compound also displays stereoselective binding to dopamine D3 receptors; *R(+)trans*-7-OH-PIPAT is the active isomer, displaying the desired high binding affinity towards dopamine D3 receptors (6). The *S(-)trans*-7-OH-PIPAT exhibits low binding affinity to dopamine D3 receptors, but high affinity to sigma sites (7). Further binding studies showed that *R(+)trans*-7-OH-PIPAT not only exhibits high affinity to dopamine receptors; it also displays moderate affinity to CNS sigma binding sites (K_d = 10.8 nM, rat cerebellum) and high affinity to 5-HT_{1A} receptors (K_d = 0.38 nM, rat hippocampus) (8). The non-selective nature of [^{125}I]*R(+)trans*-7-OH-PIPAT, displayed by *in vitro* binding and autoradiography studies in rat brain, suggest that there is a need to develop compounds with higher selectivity for dopamine receptors (8). Based on the structure-activity relationship study of tetralin series of compounds, a new ligand, (*R,S*)*trans*-5-OH-PIPAT, **3**, was prepared and found to be highly selective for dopamine D2-like receptors, with low affinity to 5-HT_{1A} receptors and sigma sites (9). In order to further characterize the different binding profiles of the enantiomers of this new compound, we report herein the synthesis, optical resolution and stereoselectivity of each resolved isomer at dopamine D2-like receptors. In addition, radioiodination of each enantiomer was carried out and evaluated.



(*R,S*)-5-OH-DPAT, **1**



(*R,S*)*trans*-7-OH-PIPAT, **2**



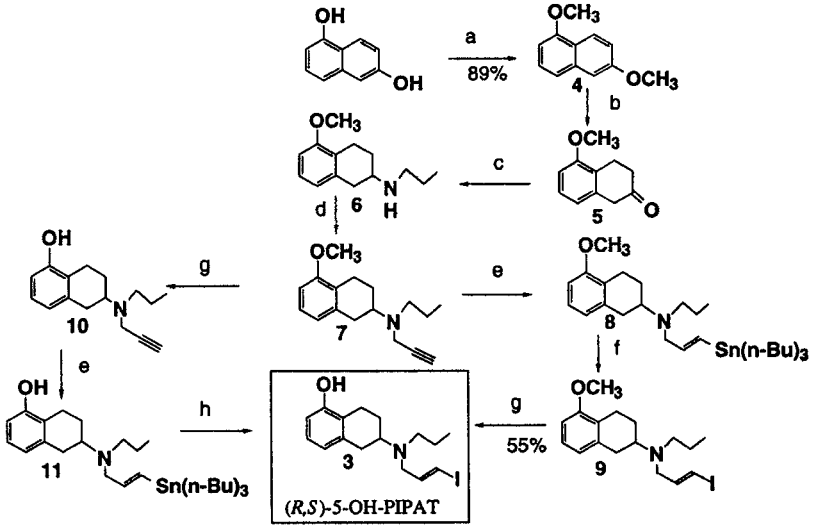
(*R,S*)*trans*-5-OH-PIPAT, **3**

RESULTS AND DISCUSSION

The starting material, 5-methoxy-2-tetralone, **5**, was synthesized according to a Birch reduction reaction described previously (Scheme 1; 10). Reductive amination of **5** with n-propylamine and sodium cyanoborohydride gave **6** (40-60%). N-Alkylation of **6** with

propargyl chloride resulted in 7 (85%). The ethynyl derivative 7 was then treated with tri-n-butyltin hydride in the presence of AIBN to give 8 (57%). The best solvent for preparing the desired compound 8 was THF. Along with the desired product 8, other related tin analogs, including one product without vinyl and the other with a tributyltin group at the 2'-position of vinyl moiety, were formed. These undesired side products can be readily separated by column chromatography. Iododemetalation of the tin derivative 8, followed by demethylation, gave the desired product *(R,S)trans*-5-OH-PIPAT, 3. The corresponding tin precursor for radioiodination to prepare 3 was synthesized by converting the intermediate 7 to 10 by demethylation reaction (98%). Reduction of 10 with n-tributyltin hydride produced the desired tin precursor 11, with low yield (20-22%). The major side product was the tin compound without vinyl group (based on ^{1}H NMR spectra). Apparently, under the reaction conditions, the tin hydride acted as an effective reducing agent and completely reduced the double bond.

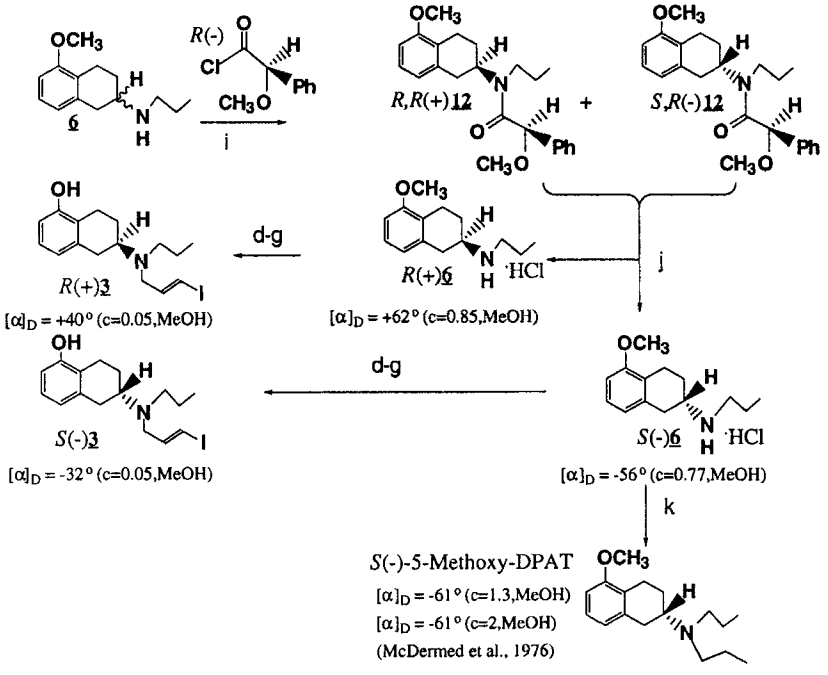
Scheme 1. Synthesis of *(R, S)trans*-5-OH-PIPAT



a.) $(CH_3)_2SO_4$, KOH; b.) Na, EtOH; c.) $CH_3CH_2CH_2NH_2$, $NaBH_3CN$; d.) $CH{\equiv}C\text{-}CH_2Cl$, K_2CO_3; e.) $(n\text{-}Bu)_3SnH$, AIBN, THF; f.) I_2, $CHCl_3$; g.) BBr_3, CH_2Cl_2; h.) H_2O_2, $Na^{125}I$

In a previous study, *(R,S)trans*-7-OH-PIPAT displayed stereoselective binding to dopamine D3 receptors, with only the *R*(+) isomer being active (6). To further investigate a closely related tetralin derivative, the racemic 3 was resolved in the same manner as reported

Scheme 2. Confirmation of the configuration of (*R*) and (*S*)*trans*-5-OH-PIPAT, **3**



d.) $CH{\equiv}C\text{-}CH_2Cl$, K_2CO_3; e.) $(n\text{-}Bu)_3SnH$, AIBN, THF; f.) I_2, $CHCl_3$; g.) BBr_3, CH_2Cl_2; i.) NaOH; j.) i. Column Chromatography, ii. $(CH_3)_3COK$, THF, iii. HCl; k.) $CH_3CH_2CH_2I$, K_2CO_3

previously for **2** (Scheme 2) (6). Since the configuration of the key intermediates (*R*) or (*S*)-**6** has not yet been reported, the following steps were taken to confirm the absolute configuration of the final product, (*R*) or (*S*)-**3**. The relative configurations of new compounds were determined based on reports of the configuration of **3**, in which the *S*-isomer was reported to have a levorotatory, (-) rotation and high dopamine receptor affinity (11, 12). *S*(-)-5-methoxy-DPAT was synthesized by N-alkylation of intermediate *S*(-)-**6** (a common intermediate for the preparation of *S*(-)-5-methoxy-DPAT) and *S*(-)-**3**. Therefore, *S*(-)-5-methoxy-DPAT, *S*(-)-**3**, and intermediate *S*(-)-**6** share the same configuration, (*S*). The optical rotation measured in methanol for all three of the *S*(-) isomers displayed the same levorotatory, (-) rotation (Scheme 2). The known compound, *S*(-)-5-methoxy-DPAT, prepared from intermediate *S*(-)-**6**, showed an identical optical rotation reading to that reported in the literature for the *S*-isomer ($[\alpha]_D$ = -61°; C = 1.3, MeOH) (12). Therefore, it is feasible to conclude that the desired product (*S*)-**3** is indeed an *S*-isomer ($[\alpha]_D$ = -32°; C = 0.05, MeOH). As expected, the corresponding *R*-isomer, (*R*)-**3**, displayed a dextrorotatory,

(+) rotation ($[\alpha]_D$ = +40°; C = 0.05, MeOH). The higher binding affinity to dopamine D2, D3 and D4 receptors of *S*(-)-5-OH-DPAT and *S*(-)**3** (see results below), further confirms that they are in the same configuration, which displays stereoselective binding affinity (Table 1).

The stereoselective binding affinity of these compounds can be evaluated using genetically transfected cells as test systems for pharmacological assessment. The binding affinities of resolved **3** were compared by determining the inhibition constants (K_i, nM) in various cell lines expressing different dopamine receptors (Table 1). Similar to the congener, 5-OH-DPAT, *S*(-)-**3** is the active isomer, displaying higher dopamine D3 receptor binding affinity expressed in Sf9 cells (K_i = 0.09 nM), as well as higher affinity to the high affinity states of dopamine D2 receptors (D2H), expressed in either HEK293 or A9L cells (K_i = 0.38 nM), and D4 receptors (D4H), expressed in CHO cells (K_i = 0.67 nM). The K_i values for dopamine D2H and D4H receptors with **3** were similar, further confirming the previously reported lack of subtype selectivity of this tetralin ligand series (8, 9). Contrary to the high affinity states of D2 and D4 receptors measured with the labeled agonist ligand, [^{125}I]*S*(-)-**3**, its lower binding affinity (K_i = 60.9 nM) in Sf9-D2 cells (D2 receptors at low affinity states) with [^{125}I]NCQ298 is consistent with the data reported previously for *R*(+)-**2** (9). The affinities of the *R*(+)-**3** toward D3, D2H and D4H receptors are approximately twenty to fiftyfold lower than those of the *S*(-)isomer, with K_i values of 4.87 nM, 7.29 nM and 16.44 nM, respectively. These findings are consistent with the previously reported stereoselectivity of 5-OH-DPAT (13), with a twenty-fivefold preference fo *S*(-)5-OH-DPAT in dopamine D2 receptor binding. The K_i values determined for the resolved isomers in the present study are consistently lower than values obtained previously with the racemic compounds (9), due to the addition of BSA to the buffer. The presence of 0.1% BSA in the dilution buffer apparently prevents the loss of compounds from adhesion to test tubes; therefore, under this condition, a more accurate estimation of the compound concentration is obtained.

To further test the new ligand, radioiodinated [^{125}I]*(R,S)*-**3** was prepared by radiolabeling the racemic tin derivative, **11**, using [^{125}]NaI in the presence of hydrogen peroxide. The purified product, [^{125}I]*(R,S)*-**3**, was resolved into two peaks, peak A and peak B, using HPLC with a chiral column, with retention times of 10.3 and 11.9 min, respectively. Peak A, which was coeluted with authentic cold *S(-)*-**3**, showed similar high affinity to dopamine D3 receptors (in HEK293 cells), D2 receptors (in HEK293 or A9L cells) and D4 receptors (in CHO cells), with a K_d of 0.4 nM (data not shown). The data suggest

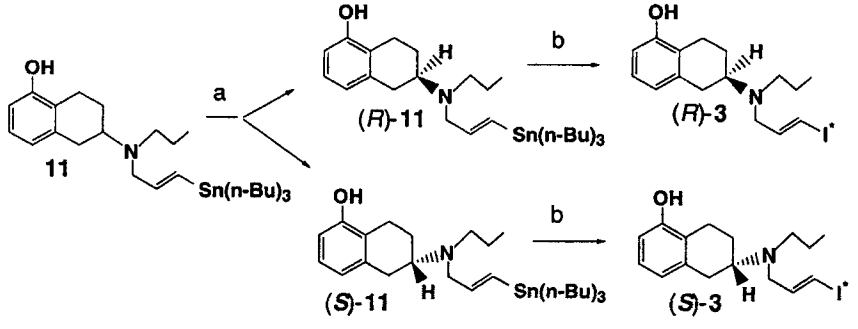
Table 1. Comparison of inhibition constants (K_i, nM) of *R-(+)trans*-5-OH-PIPAT, *R*-**3**, and *S-(-)trans*-5-OH-PIPAT, *S*-**3**, with dopamine D2H, D3 and D4H receptors.

Compound	D3 (Sf9)	D2 (Sf9)	D2(HEK293 or A9L)	D4 (CHO cells)
R(+)trans-5-OH-PIPAT, *R*-**3**	4.87±2.14	77.1±8.9	7.29±2.33	16.44±5.35
S(-)trans-5-OH-PIPAT, *S*-**3**	0.09±0.04	60.9±8.8	0.38±0.12	0.67±0.16

The compounds were diluted in 50 mM Tris-HCl buffer (pH 7.4) with 0.1% BSA. All values are the mean ± SD of triplicate determinations for at least two separate experiments. [^{125}I]NCQ298 was used as the labeled ligand for Sf9-D2 (low affinity state) and Sf9-D3 assays. [^{125}I]*S*(-)5-OH-PIPAT was used to label the high affinity state of D2 receptors in HEK293 or A9L cells and D4 receptors in CHO cells.

that the new ligand, similar to the corresponding 7-OH analog, shows excellent affinities to dopamine D3, D2 and D4 (G-protein coupled) receptors. However, the resolution of [^{125}I]*(R,S)*-**3** by chiral column was time consuming and gave low yields (20-23%). In addition, there were other concerns, particularly possible contamination between the two isomers. In order to avoid this potential problem of optical impurity, the method that employed initial radioiodination to produce the racemic [^{125}I]*(R,S)*-**3** racemate, followed by chiral column separation of the isomer, was abandoned. Instead, the racemic tin precursor **11** was optically resolved using a semi-preparative chiral column (Chiracel OD, eluted with isopropanol/hexanes = 2/98; flow rate = 2 mL/min); retention times for *R*-(+)- and *S*-(-)-**11** were 19.0 and 22.0 min, respectively. The optical purity of the resolved isomer was >99%. The optically pure *R(+)*-**11** and *S(-)*-**11** were subsequently radioiodinated as described above

Scheme 3.



a) chiral column Chiracel OD; b) H_2O_2, Na^{125}I

for the racemic mixture, affording [^{125}I]*R(+)*-**3** and *S(-)*-**3**, respectively (radiochemical yield 50-60%; radiochemical purity >98%). The [^{125}I]*S*(-)-**3** thus obtained displayed, as expected, the same binding properties as peak A (resolved in chiral column) described above.

In conclusion, the synthesis, optical resolution, radiolabeling and *in vitro* binding study of a new dopamine D2-like receptor ligand **3** are reported. It is demonstrated that **3**, which displays high affinity to dopamine D2H, D3 and D4H receptors expressed in different cells, is the active isomer.

EXPERIMENTAL SECTION

General Methods. NMR were recorded on a Varian EM 360A, a Bruker WM-250 (250 MHz) or a Bruker AM 500 (500 MHz) spectrometer. The chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. Infrared spectra were obtained with a Mattson Polaris FT-IR spectrophotometer. Melting points were determined on a Meltemp apparatus and are reported uncorrected. HPLC (Rabbit HP with dual pumps; Rainin Instrument Co. Inc., Emeryville, CA) was performed on a chiral column (Chiracel-OD, 4.1 x 250 mm; Chiral Technologies Inc., Exton, PA) or a silica column (Hamilton Co., Reno, Nevada). The HPLC system was equipped with a UV and a gamma detector. The data were collected and analyzed with Dynamax software on Macintosh computers. Optical rotation values were measured on a Perkin-Elmer 243B polarimeter. Mass spectra were performed on a VG 70-70 HS mass spectrometer with chemical ionization (CI), using methane or ammonia gas. Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA), and values were within 0.4% of the theoretical values. All chemicals were obtained from commercial sources. The starting material 5-methoxy-2 tetralone was synthesized according to the literature (10). The reductive amination step was carried out as described in the literature (14).

(*R,S*)-5-Methoxy-2-(*N*-*n*-propyl-N-propynylamino)tetralin, (*R,S*)-7. 2-Propynyl chloride (3.5 mL, 48.4 mmol) was added dropwise to a mixture of (*R,S*)-**6** (1.22 g, 5.57 mmol) and anhydrous potassium carbonate (0.88 g, 6.33 mmol) in acetone (10 mL) under nitrogen at 0°C, and the mixture was refluxed overnight. The acetone solution was evaporated, and the residue was dissolved in CH_2Cl_2. The methylene chloride solution was washed twice with water and once with saturated NaCl solution, then dried over anhydrous sodium sulfate. The solvent was evaporated and the crude product was purified by column

chromatography (silica gel, hexanes/ethyl acetate = 3/1) to give 1.59 g of (*R,S*)-**7** (yield 85%). ^{1}H NMR ($CDCl_3$) δ 7.09 (t, *J* = 7.8 Hz, 1H, ArH), 6.72 (d, *J* = 7.6 Hz, 1H, ArH), 6.66 (d, *J* = 8.1 Hz, 1H, ArH), 3.81 (s, 3H, OCH_3), 3.54 (d, *J* = 2.3 Hz, 2H, $CH_2C{\equiv}C$), 3.04-2.90 and 2.66-2.54 (m, 5H, CH_2ArCH_2 and CHN), 2.79 (t, 2H, NCH_2), 2.18 (t, 1H, C≡CH), 2.23-2.14 (m, 1H), 1.60-1.56 (m, 1H), 1.61-1.46 (hex, 2H, CH_2), 0.92 (t, 3H, CH_3). Anal. ($C_{17}H_{23}NO$) C = 79.33, H = 9.01, N = 5.44. Found C = 79.44, H = 9.08, N = 5.40.

(*R,S*)*trans*-5-Methoxy-2-[*N*-*n*-propyl-*N*-(3'-tributylstannyl-2'-propenyl)amino] tetralin, (*R,S*)-**8**. A mixture of (*R,S*)-**7** (0.50 g, 1.94 mmol) in dry THF (13 mL), tri-n-butyltin hydride (1.6 mL, 3.7 mmol) and AIBN (63 mg, 0.43 mmol) was heated at 95-100°C for 2 h under a nitrogen atmosphere. The solvent was evaporated, and the residue was purified by column chromatography (silica gel, hexanes/ethyl acetate = 3/1) to give 0.602 g of tin derivative as an oil (yield 57%). This oil residue was a mixture of (*R,S*)-**8**, cis-isomer, reduced compound without the vinyl group and compound with butyltin in a secondary position. Only a small sample of pure (*R,S*)-**8** was separated for NMR and elemental analysis. The crude mixture was used directly for the next reaction. ^{1}H NMR ($CDCl_3$) δ 8.07 (t, *J* = 8.7 Hz, 1H, ArH), 6.71 (d, *J* = 7.5 Hz, 1H, ArH), 6.65 (d, *J* = 8.1 Hz, 1H, ArH), 6.09 (d, *J* = 18.9 Hz, C=CHSn), 6.01 (dt, *J* = 18.9 Hz, 1H, -C=CHC) 3.80 (s, 3H, OCH_3), 3.30 (m, 2H, CH_2C=C), 3.24-3.15, 3.03-2.69 and 2.56-2.44 (m, 7H, CH_2 $ArCH_2$, CHN and NCH_2), 1.56-1.23 and 0.96-0.76 (m, 29, Bu_3Sn and CH_2), 0.83 (t, 3H, CH_3). Anal. ($C_{29}H_{51}SnNO$) C = 63.51, H = 9.40, N = 2.67. Found C = 63.27, H = 9.33, N = 2.65.

(*R,S*)*trans*-5-Hydroxy-2-[N-n-propyl-N-(3'-iodo-2'-propenyl) amino]tetralin, (*R,S*)-**3**. A 0.1 M solution of iodine in chloroform was gradually added to the mixture of (*R,S*)-**8** and its analog (0.5 g) in chloroform (30 mL) at room temperature until the color of iodine persisted. The mixture was stirred overnight. The KF solution (1 M; in methanol, 2 mL) and 5% aqueous $NaHSO_3$ (2 mL) were sequentially added to the reaction mixture. The chloroform layer was separated, and the aqueous layer was extracted once with chloroform. The combined chloroform layers were dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The crude product was purified by column chromatography (silica gel, hexanes/ethyl acetate/ammonium hydroxide = 80/20/1) to obtain 133 mg of the desired (*R,S*)*trans*-5-methoxy-2-[*N*-*n*-propyl-*N*-(3'-iodo-2'-propenyl)amino]tetralin, (*R,S*)-**9**. ^{1}H NMR ($CDCl_3$) δ 7.09 (t, *J* = 7.8 Hz, 1H, ArH), 6.70

(d, J = 5.3 Hz, 1H, ArH), 6.65 (d, J = 8.0, 1H, ArH), 6.61 (dt, J = 14.3 Hz, 1H, C=CH), 6.22 (dt, J = 14.3 Hz, 1H, C=CHI), 3.80 (s, 3H, OCH_3), 3.19 (dd, J = 6.1 Hz, 2H, CH_2C=C), 3.02-2.72 (m, 4H, CH_2ArCH_2 and CHN), 2.56-2.44 (m, 1H of CH_2) 2.48 (t, 2H, NCH_2), 2.07-2.00 and 1.64-1.49 (m, 2H, CH_2), 1.49-1.39 (hex, 2H, CH_2), 0.88 (t, 3H, CH_3). The deblocking of the O-methyl group was carried out using the following steps. BBr_3 (1.38 mL, 1.38 mmol) was added to the solution of (*R,S*)-**9** (0.13 g, 0.34 mmol) in dry dichloromethane (15 mL) at -12°C. The reaction mixture was stirred overnight at room temperature. After quenching the reaction with water, the volatile material was evaporated. The residue was taken up in more water (5 mL), basicified with 10% NaOH to pH 9, and adjusted to pH 8 with 1M HCl. The mixture was then extracted several times with dichloromethane. The combined extracts were dried over anhydrous sodium sulfate. After the solvent was evaporated, the desired product was purified by column chromatography (silica gel, hexanes/ethyl acetate/ammonium hydroxide =80/20/1) to give 70 mg of (*R,S*)-**3** (yield 55%). ^{1}H NMR ($CDCl_3$) δ 6.99 (t, J = 7.7 Hz, 1H, ArH), 6.68 (d, J = 7.6 Hz, 1H, ArH), 6.59 (d, J = 8.2, 1H, ArH), 6.62 (dt, J = 14.2 Hz, 1H, C=CH), 6.24 (dt, J = 14.4, 1H, C=CHI), 3.20 (dd, J = 6.3 Hz, 2H, CH_2C=C), 2.98-2.72 and 2.62-2.55 (m, 5H, CH_2ArCH_2 and CHN), 2.49 (t, 2H, NCH_2), 2.11-2.00 and 1.66-1.56 (m, 2H, CH_2), 1.54-1.43 (hex, 2H, CH_2), 0.88 (t, 3H, CH_3). Anal. ($C_{16}H_{23}NOICl$) C = 47.13, H = 5.69, N = 3.44. Found C = 47.12, H = 5.79, N = 3.45.

(*R,S*)-7-Hydroxy-2-(*N*-propyl-*N*-propynylamino)tetralin, (*R,S*)-10. The demethylation of (*R,S*)-**7** with BBr_3 was performed in the same manner as in the synthesis of (*R,S*)-**3** (98% yield). FT-IR (neat) 3300 cm^{-1}(strong, OH); ^{1}H NMR ($CDCl_3$) δ 6.98 (t, J =7.7 Hz, 1H, ArH), 6.68 (d, J = 7.6 Hz, 1H, ArH), 6.58 (d, J = 7.8 Hz, 1H, ArH), 3.55 (S, 2H, C≡CCH_2), 2.03-2.80 and 2.64-2.57 (m, 5H, CH_2ArCH_2 and CHN), 2.66 (t, 2H, NCH_2) 2.19 (S, 1H, C≡CH), 2.24-2.18 and 1.70-1.61 (m, 2H, CH_2), 1.59-1.47 (hex, 2H, CH_2), 0.92 (t, 3H, CH_3). Anal. ($C_{16}H_{21}NO$) C = 78.97, H = 8.70, N = 5.76. Found C = 79.03, H = 8.75, N = 5.72.

(*R,S*)*trans*-5-Hydroxy-2-[*N*-*n*-propyl-*N* -(3'-tributylstannyl-2'-propenyl)amino] tetralin, (*R,S*)-11. Compound (*R,S*)-**11** was synthesized in the same manner as (*R,S*)-**3**, using THF as the solvent (24% yield). ^{1}H NMR ($CDCl_3$) δ 6.97 (t J = 7.8 Hz, 1H ArH), 6.79 (d, J = 8.2 Hz, 1H, ArH), 6.57 (d, J = 7.8 Hz, 1H, ArH), 6.10 (d, J = 18.9 Hz, 1H, C=CHSn), 6.08 (dt, J = 18.9 , 1H, C=CHC), 3.32 (d, J - 4.6 Hz, 2H,

$CH_2C{=}C$), 3.05-2.68 and 2.64-2.47 (m, 5H, CH_2ArCH_2 and CHN), 2.53 (t, 2H, NCH_2), 2.16-1.89 (m, 2H, CH_2) 1.53-1.22 and 0.95-0.76 (m, 28H, Bu_3Sn and 1H of CH_2), 0.86 (t, 3H, CH_3). Anal. ($C_{28}H_{49}SnNO$). C = 62.93, H = 9.24, N = 2.62. Found C = 62.65, H = 9.23, N = 5.57.

(*S*)-(-)*trans*-5-Methoxy-2-*N*,*N*-dipropylamino tetralin ((*S*)-5-OH-DPAT). Iodopropane (0,132 g, 0.770 mmol) was added to a mixture of (*S*)-(-)**6** (57 mg, 26 mmol) and K_2CO_3 in ethanol (3 mL). The mixture was refluxed overnight, and the solvent was evaporated under reduced pressure. The residue was redissolved in dichloromethane (15 mL) and washed several times with water. The organic layer was dried over anhydrous Na_2SO_4. The product was purified by silica gel preparative plate to obtain 29 mg (43%) of the desired product and some starting material. ^1H NMR ($CDCl_3$) δ 7.09 (t, *J* = 7.9 Hz, 1H, ArH), 6.72 (d, *J* = 7.6 Hz, 1H, ArH), 6.65 (d, *J* = 8.1 Hz, 1H, ArH), 2.98-2.75 and 2.51-2.46 (m, 5H, CH_2ArCH_2 and CHN), 2.49 (t, 4H, NCH_2), 2.09-2.03 and 1.63-1.52 (m, 2H, CH_2) 1.53-1.42 (hex, 4H, CH_2) 0.89 (t, 6H, CH_3), $[\alpha]_D$ = -61° (C = 1.3, MeOH).

Radioiodination. No carrier-added [^{125}I]*S*(-)*trans*-5-OH-PIPAT was prepared using an iododestannylation reaction, with hydrogen peroxide as the oxidant, as described previously (6). The racemic ligand was first obtained using the tri-n-butyltin derivative, (*R*,*S*)-**11**, and subsequently subjected to chiral-HPLC separation (Chiracel-AD, size: 4.1 mm x 250 mm), eluting with a solvent of hexane/2-propanol (98/2, 0.5 ml/min). The identity of each peak, observed in chiral column, was confirmed with the authentic cold isomer. Alternately, radioiodinated stereoisomers were prepared directly from the enantiomerically pure corresponding tin derivatives *R*(+)-**11** or *S*(-)-**11** without a further chiral separation.

Binding studies. The membrane preparations (100 µl containing 2-4 µg protein), from *Spodoptera frugiperda* (Sf9) insect cell membranes expressing dopamine D2 or D3 receptors, were incubated with 50 µl [^{125}I] *S*(-)-NCQ298 (2200 Ci/mmol; at a concentration of 0.1-0.15 nM) in a final volume of 200 µl. The buffer used contained 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, the protease inhibitors mixture and BSA (1 mg/ml). The compounds, diluted with Tris-HCl buffer containing 0.1% BSA, at concentrations up to 10^{-5} M, were examined. Nonspecific binding was determined in the presence of 10 µM spiperone. After incubation for 30 min at 37°C, 5 ml of wash buffer (25 mM Tris-HCl, pH

7.4) were added, to stop the reaction, and a Brandel (M24-R) cell harvester was used to separate bound from free ligand by filtration through glass fiber filters (pretreated with 1% polyethylenimine). The filters were washed three times with wash buffer, then counted in a gamma counter (Packard 5000) at an efficiency of 70%. Binding assays for D2 receptors expressed in HEK293 cells were carried out as described (9). D2 and D4 receptors expressed in A9L and CHO cells were obtained from Receptor Biology (Glen Echo, MO). The assay procedures were carried out as recommended. [^{125}I]*S*(-)5-OH-PIPAT displayed a K_d value of 0.4 nM, in all the cell lines (data not shown); detailed studies will be published elsewhere. When [^{125}I]*S*(-)5-OH-PIPAT (2200 Ci/mmol) was used as the radiolabeled ligand for the competition studies, the assay buffer included 50 mM Tris-HCl (pH 7.4) and 2 mM $MgCl_2$ to facilitate the binding of [^{125}I]*S*(-)5-OH-PIPAT to the high affinity states of D2 and D4 receptors. The data were analyzed using the iterative nonlinear least-square curve fitting program LIGAND (15).

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