High Affinity Hydroxypiperidine Analogues of 4-(2-Benzhydryloxyethyl)-1-(4-fluorobenzyl)piperidine for the Dopamine Transporter: Stereospecific Interactions in Vitro and in Vivo

Sujit K. Ghorai,[†] Charles Cook,[‡] Matthew Davis,[†] Sylesh K. Venkataraman,[†] Clifford George,[§] Patrick M. Beardsley,[‡] Maarten E. A. Reith,[||] and Aloke K. Dutta*,[†]

Wayne State University, Department of Pharmaceutical Sciences, Detroit, Michigan 48202, Virginia Commonwealth University, Department of Pharmacology and Toxicology, Richmond, Virginia 23298, Laboratory for the Structure of matter, Code 6030, Naval Research Laboratory, 4555 Overlook Avenue, SW, Washington, DC 20735, and University of Illinois, College of Medicine, Department of Biomedical and Therapeutic Sciences, Peoria, Illinois 61605

Received June 27, 2002

In our effort to develop high-affinity ligands for the dopamine transporter which might find potential use as cocaine medication, a polar hydroxy substituent was introduced into the piperidine ring of one of our disubstituted lead analogues derived from 1-[2-(diphenylmethoxy)-ethyl]-4-(3-phenylpropyl)piperazine (GBR 12935). Both *cis*- and *trans*-3-hydroxy derivatives were synthesized and the racemic trans isomer, (±)-**5**, was further resolved into two enantiomers. Newly synthesized compounds were characterized for their binding affinity at the dopamine, serotonin, and norepinephrine transporter systems in rat brain. The two enantiomers (+)-**5** and (−)-**5** exhibited marked differential affinities at the dopamine transporter with (+)-**5** being 122-fold more potent than (−)-**5** in inhibiting radiolabeled cocaine analogue binding (IC_{50}; 0.46 vs 56.7 nM) and 9-fold more active for inhibiting dopamine uptake (IC_{50}; 4.05 vs 38.0 nM). Furthermore, the most active (+)-**5** was 22-fold more potent at the dopamine transporter compared to the standard GBR 12909. Absolute configuration of one of the enantiomers was determined unambiguously by X-ray structural analysis. In in vivo locomotor activity studies, the enantiomer (+)-**5** and the racemic (±)-**5**, but not (−)-**5**, exhibited stimulant activity with a long duration of effect. All three compounds, (+)-**5**, (−)-**5**, and (±)-**5**, within the dose range tested, partially (50%) but incompletely (80%) produced cocaine-like responses in mice trained to discriminate 10 mg/kg ip cocaine from vehicle. Compound (−)-**5** was distinctive in this regard in that, unlike (+)-**5** and (±)-**5**, it did not affect locomotor activity yet, but similar to them, was able to engender (albeit incompletely) cocaine-like responses.

Introduction

Cocaine is a powerful drug of abuse with strong reinforcing activity. Addiction to cocaine is a major problem in our society today which has greatly impacted the nation in terms of its economy and securing law and order.[1] Furthermore, it has also contributed to the spreading of HIV infection, as needle sharing is a pervasive problem among drug abusers. At present no effective medication is available for the treatment of cocaine addiction, and there is an urgent need for the development of an effective medication.[2] Cocaine acts on several sites in the brain which include all three monoamine neurotransporter systems for dopamine, serotonin, and norepinephrine.[3] Mechanistically cocaine's reinforcing effect is believed to originate mainly from its binding to the dopamine transporter (DAT).[4−6] Additionally, recent experiments involving knockout mice indicated that other systems, e.g., serotonin, might contribute to some extent to the central effects of cocaine.[7,8]

Since DAT has been implicated strongly in cocaine's reinforcing effect, many efforts have been directed toward developing drugs for this transporter in an effort to develop medication for cocaine addiction. Structurally different classes of compounds have been developed for DAT, and they are classified mainly into tropane, benztropine, methylphenidate, mazindol, and GBR derivatives.[9,10] In our structure−activity relationship (SAR) study with piperidine analogues of GBR 12935, we have developed many potent and selective analogues for the DAT molecule. We have looked at various aspects of aromatic substitutions, influence of chain lengths, and bioisosteric replacements with different isosteric aromatic moieties in these molecules.[11−13] In this respect, many analogues of conventional piperazine derivatives of GBR 12935 and the benztropine molecule have been made and characterized.[14−16] A wealth of SAR data thus generated has been very useful in the development of an understanding of the mode of interaction of these molecules with the DAT.

Hydroxy-functionalized GBR compounds have been synthesized in the past and recently. Rice et al. investigated introduction of a hydroxy group in GBR 12935 derivatives. In their derivatives, hydroxyl group was introduced on the phenyl propyl side chain and also in the phenyl ring.[15,17] In this regard, one of the earlier

* Corresponding author: Aloke K. Dutta, Ph.D., Department of Pharmaceutical Sciences, Applebaum College of Pharmacy & Health Sciences, Rm# 3128, Detroit, MI 48202, Tel: 1-313-577-1064, Fax: 1-313-577-2033, e-mail: adutta@ea.cphs.wayne.edu.
[†] Wayne State University.
[‡] Virginia Commonwealth University.
[§] Naval Research Laboratory.
[||] University of Illinois.

10.1021/jm020275k CCC: $25.00
Published on Web 03/04/2003

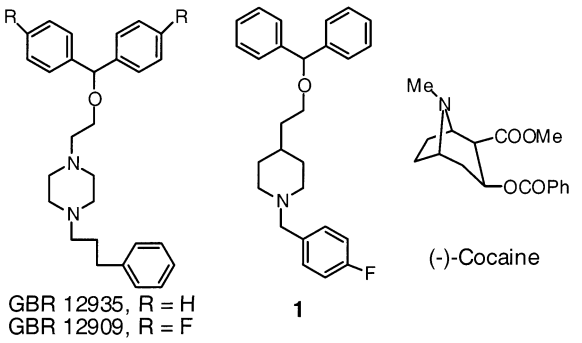


Figure 1. Molecular structures of DAT blockers.

hydroxy derivatives developed from this group was converted into a decanoate ester prodrug which could attenuate self-administration of cocaine for an extended period of time.[18] In our own work with piperidine analogues of GBR 12935, we have shown the introduction of hydroxy and methoxy groups in the pendant phenyl ring of the N-substituted benzyl group resulted in production of potent and selective derivatives.[13] A suitable hydroxy compound, when developed from these studies, can be converted into a lipophilic prodrug such as decanoate ester. Such a prodrug will have the potential to be a long-acting treatment agent in substitution therapy for cocaine addiction.

In our SAR series of piperidine analogues so far, introduction of a third substituent in the central piperidine ring had not yet been explored. As the central piperidine/piperazine ring could play an important role in the interaction of these molecules with CNS recognition sites, we decided to explore the influence of introduction of a polar group in the piperidine ring of our previous lead compound **1**. Polar groups such as hydroxy or amino may give rise to additional interactions through H-bonding or ionic interaction with the DAT which can potentially change their functional activity in addition to their binding interaction. Moreover, the presence of such functional group(s) will also be helpful in making suitable long-acting prodrugs for medication purpose.[19] In this report, we are describing the design, synthesis, and biological characterization of hydroxy substituted piperidine analogues of **1** (Figure 1).

Chemistry

Synthesis of our target compounds is shown in the Schemes 1 and 2. We wanted to introduce a hydroxyl functionality at the 3-position in the piperidine ring by chemically modifying a double bond which was introduced in the ring for this purpose. Our intention was to synthesize both *cis*- and *trans*-hydroxy compounds from this precursor. To generate a double bond in the correct position of the piperidine ring, we planned to exploit a well-known reaction involving reduction of a quaternary pyridinium salt by sodium borohydride in methanol to produce tetrahydropyridine intermediate **4**.[20] Thus, in Scheme 1, the intermediate **3** was first made by heating 2-pyridin-4-ylethanol and 4-fluorobenzyl chloride together in methanol which was followed by the reduction with sodium borohydride to provide the tetrahydro intermediate **3**. Conversion of the intermediate **3** into diphenylmethoxy derivative **4** was conducted by following our published procedure.[11] Hydroboration

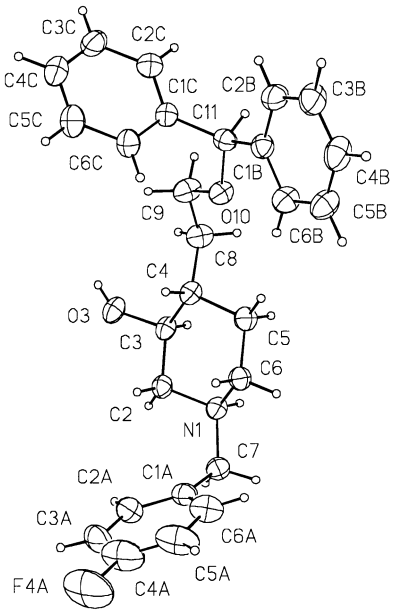


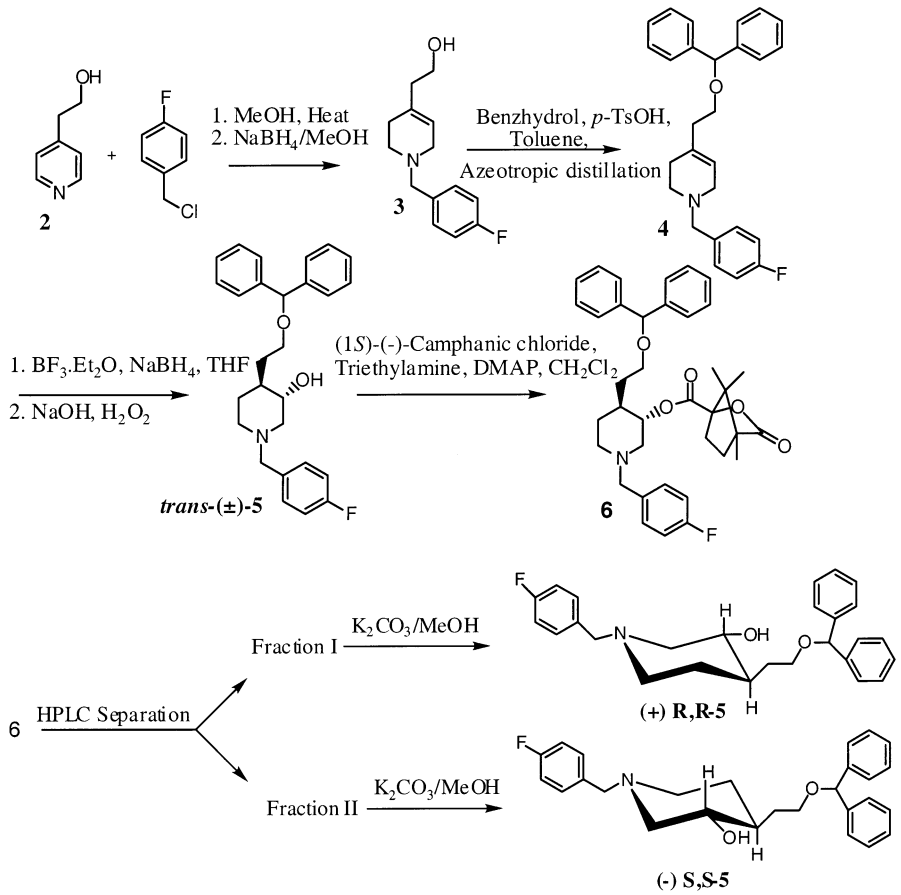
Figure 2. The molecular structure and numbering scheme for one of the two molecules in the asymmetric unit of compound (−)-**5** with displacement ellipsoids drawn at the 30% probability level.

of the olefinic double bond in **4** was carried out by a borane reagent generated by reaction of sodium borohydride with anhydrous boron trifluoride. The resulting borane complex was hydrolyzed by sodium hydroxide solution to yield racemic *trans*-hydroxy compound (±)-**5** in good yield. The trans structure of the product was confirmed by both X-ray structure of (−)-**5** (Figure 2) and ^{1}H NMR data analysis. Resolution of the racemic trans compound was carried out by converting racemic (±)-**5** into diastereoisomeric ester mixture **6** by treating with optically active *S*-(−)-camphanic chloride in the presence of a base. Separation of the diastereomeric mixture was carried out by semipreparative HPLC, and the respective optically pure hydroxy enantiomer was liberated by treating each pure diastereomer with potassium carbonate in methanol. One of the crystallized pure enantiomers, (−)-**5**, underwent X-ray structural analysis to establish the absolute stereochemistry of both the enantiomers.

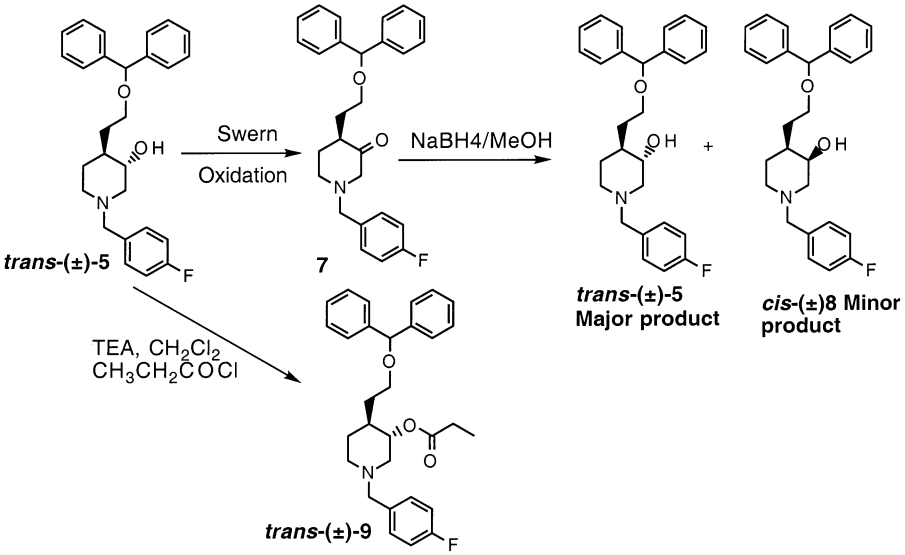
Synthesis of *cis*-hydroxy compound **8** was achieved by first oxidizing the hydroxy compound **5** to a keto derivative **7** followed by reduction with sodium borohydride. Thus, racemic hydroxy compound **5** was oxidized under Swern oxidation condition to the keto compound **7** in good yield. Reduction of this keto compound **7** by sodium borohydride in methanol produced a mixture of *trans*-(±)-**5** and cis-(±)-**8** hydroxy molecules which were separated by column chromatography. Finally, the racemic *trans*-hydroxy (±)-**5** was converted into an ester derivative **9**.

The stereochemical assignment of the structures **5** and **8** was determined by 1-D and 2-D NMR (400 MHz) experiments (see Supporting Information for detail) and by X-ray crystal structure. In these compounds the assumption that the benzhydrloxyethyl moiety would assume the equatorial position was borne out by the X-ray structure of one of the enantiomers of **5** and was also confirmed by the ^{1}H NMR data. The ^{1}H NMR spectra of compound **5** was taken in both $CDCl_3$ and

Scheme 1



Scheme 2



CD_3OD solvents. The spectra taken in CD_3OD was chosen for analysis as it gave clearer signals.

Finally, the absolute configuration of (−)-**5** was determined unambiguously from X-ray structural analysis (Figure 2) which designates its stereocenters as (3*S*,4*S*). Consequently, its enantiomer (+)-**5** must have the (3*R*,4*R*) configuration.

Results and Discussion

Our effort to introduce a polar hydroxy group at the 3-position of the piperidine ring in one of our lead piperidine analogues **1** (Figure 1) resulted in the design and synthesis of *trans*- and *cis*-hydroxy compounds **5** and **8**. Biological studies of these novel compounds were carried out to evaluate their binding affinity at the dopamine, serotonin (SERT), and norepinephrine (NET) transporter systems in the rat brain by measuring competition for the binding of [^{3}H]WIN 35 428, [^{3}H]-citalopram, and [^{3}H]nisoxetine, respectively. Selected compounds were also evaluated for their activity in inhibiting the uptake of [^{3}H]dopamine.

Binding results demonstrated that introduction of a hydroxy functionality in the piperidine ring of compound **1** resulted in the development of potent racemic trans

Table 1. Affinity of Drugs at the Dopamine, Serotonin, and Norepinephrine Transporters in Rat Striatum and in Inhibition of Dopamine (DA) Reuptake

compd	DAT binding, IC_{50}, nM, [^{3}H]WIN 35, 428[a]	SERT binding, IC_{50}, nM, [^{3}H]citalopram[a]	NET binding, IC_{50}, nM, [^{3}H]nisoxetine[a]	DAT uptake, IC_{50}, nM, [^{3}H]DA[a]
cocaine	266 ± 37	737 ± 160	3530 ± 550	
GBR 12909	10.6 ± 1.9	132 ± 0	496 ± 22	6.63 ± 0.43
1	17.2 ± 4.7[b]	1920 ± 230	NT[c]	2.48 ± 0.59
4	37.8 ± 3.4	1110 ± 120	NT	NT
(±)-**5**	4.14 ± 0.77	2360 ± 500	1030 ± 80	3.22 ± 1.0
(+)-*R,R*-**5**	0.46 ± 0.05	3600 ± 270	1880 ± 230	4.05 ± 0.73
(−)-*S,S*-**5**	56.7 ± 6.5	1830 ± 80	1550 ± 190	38.0 ± 6.0
7	99 ± 18	7750 ± 1620	6300 ± 690	
(±)-**8**	11.2 ± 1.0	3310 ± 270	2150 ± 660	10.1 ± 0.7
9	583 ± 81	178000 ± 53000	35800 ± 7700	NT

[a] For binding, the DAT was labeled with [^{3}H]WIN 35, 428, the SERT with [^{3}H]citalopram and the NET with [^{3}H]nisoxetine. For uptake by DAT, [^{3}H]DA accumulation was measured. Results are average ± SEM of three to eight independent experiments assayed in triplicate. Concentrations of radioligands used in binding 4.0, 3.0, and 1.1 nM for DAT, SERT, and NET. For uptake experiment 50 nM of [^{3}H]DA was used. [b] See reference no. 12. [c] Not tested.

compound (±)-**5**. Clearly, the presence of a hydroxyl functionality in compound **5** enhanced the interaction with the DAT compared with the parent molecule **1**, as racemic (±)-**5** displayed an approximately 3-fold lower IC_{50} value. This result perhaps indicates generation of additional binding interaction(s) mediated by the newly introduced hydroxyl group, possibly via H-bonding. In addition, compound (±)-**5** exhibited very high selectivity for the DAT when its binding was compared with that to the SERT and NET. When (±)-**5** was further resolved into its enantiomers (Table 1), an appreciable separation of activity was noted between the two enantiomers (+)-**5** and (−)-**5** with (+)-**5** being much more potent than (−)-**5** (0.46 nM vs 56.7 nM), consonant with stereospecific interaction of these molecules with the recognition sites. The most active (+)-**5** was 38-fold more active than the parent **1** (0.46 vs 17.2 nM).

We next wanted to evaluate the importance of contribution of the hydroxyl functionality in (±)-**5** in its high-affinity binding to the DAT. Thus, we converted the hydroxy group into its ester as in **9** and also oxidized it to the keto compound **7**. The keto compound **7** showed moderate activity while the ester derivative **9** exhibited relatively weak binding activity at the DAT (99 nM and 583 nM, respectively). In addition, compound **9** became essentially inactive at the SERT and NET. These results show the importance of a hydroxyl group for high affinity binding of compound (±)-**5** to the DAT probably due to H-bonding with the receptor. In consonance, this high potency was lost when the hydroxyl group was converted into an ester group as in **9**.

Next we evaluated another stereoisomeric form of *trans*-(±)-**5**, compound *cis*-(±)-**8**. The results showed cis derivative (±)-**8** to be three times less potent at DAT than trans compound (±)-**5** (11.4 vs 4.1 nM), illustrating the importance of stereochemistry of the hydroxyl moiety at the 3-position of the piperidine ring in binding to DAT; thus, the equatorial orientation of the hydroxyl group in the trans isomer was more favored for interaction than the axial orientation in the cis compound.

Selected potent compounds were tested for their potency in inhibiting dopamine uptake by DAT. Uptake activity of most compounds corresponded very well with their binding activity except for the compound (+)-**5** (IC_{50} of 4.05 nM for uptake inhibition vs 0.464 nM for binding inhibition). Following characterization of in vitro transporter activities, we subjected compounds (±)-**5**, (+)-**5**, and (−)-**5** to in vivo locomotor and drug discrimination studies.

Table 2. Selectivity of Various Ligands for Their Activity at Monoamine Transporters

compound	SERT binding/ DAT binding	NET binding/ DAT binding	DAT uptake/ DAT binding
GBR 12909	12	47	0.62
1	110		0.14
4	29		
(±)-**5**	570	250	1.7
(+)-*R,R*-**5**	7800	4100	8.7
(−)-*S,S*-**5**	32	27	0.67
7	78	64	
(±)-**8**	300	190	0.90
9	300	61	

Effects of Cocaine, (±)-5, (−)-5, and (+)-5 on Total Distance Traveled. Figure 3 shows the time-course effects of cocaine, (±)-**5**, (−)-**5**, and (+)-**5** during the first 60 min of testing as well as the summed effects across the remaining 3 h. Doses of 10 and 30 mg/kg cocaine produced peak effects on total distance traveled during the first 10 min following administration with near-vehicle levels of activity obtained by 60 min (Figure 3A). When the data were summed across the remaining 3 h, cocaine did not significantly increase total distance traveled ($F(3,27) = 2.52$, $P > 0.05$). During the first 60 min, doses of 30 and 56 mg/kg (±)-**5** produced peak levels of activity between 20 and 30 min postadministration and significantly increased distance traveled across the remaining 3 h ($F(3,28) = 7.76$, $P \leq 0.05$) (Figure 3B). (−)-**5**, up to a dose of 100 mg/kg, had little effect on total distance traveled during the first 60 min or across the remaining three h of the experimental sessions ($F(3,28) = 1.88$, $P > 0.05$) (Figure 3C). Doses of 30 and 56 mg/kg of (+)-**5** produced increases in total distance traveled with peak effects occurring within the first 20 min (Figure 3D). Locomotor activity effects of 56 mg/kg of (+)-**5** decreased to, and remained near, vehicle levels following 20 min postinjection, but activity levels following 30 mg/kg remained elevated throughout this period. Activity levels following administration of 56 mg/kg of (+)-**5**, which were near-vehicle levels from 20 to 60 min, increased to levels similar to that of 30 mg/kg such that when the data were summed over the remaining 3 h of the test session both doses produced significant increases in total distance traveled ($F(4,35) = P \leq 0.05$). As a comparison, a dose of 30 mg/kg cocaine results in producing peak levels of activity (approxi-

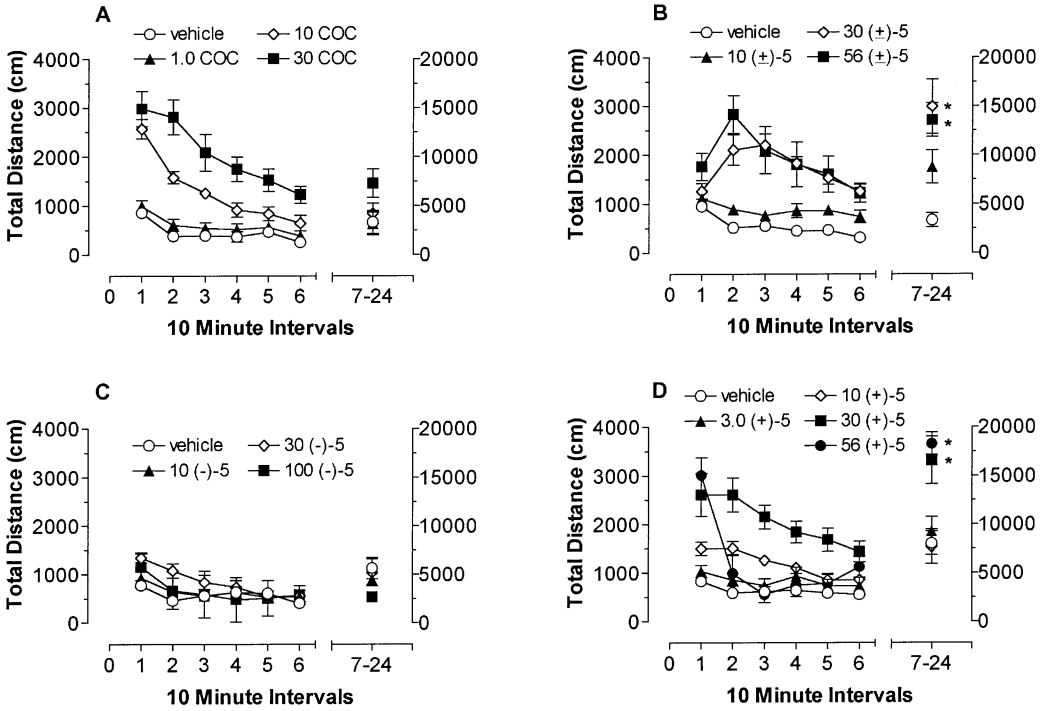


Figure 3. Effects of cocaine, (±)-**5**, (−)-**5**, and (+)-**5** on total distance traveled (cm) across the first 60 min of the test session as well as the effects of cocaine, (±)-**5**, (−)-**5**, and (+)-**5** when the data were summed across the remaining 3 h of the test session (10 min intervals 7−24). * Indicates significant differences compared to vehicle control based on Dunnett's posthoc tests.

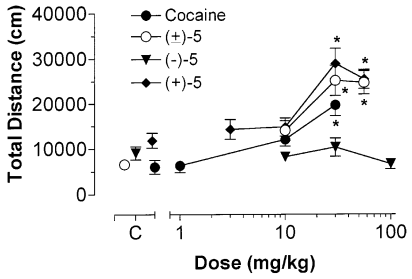


Figure 4. Effects of cocaine, (±)-**5**, (−)-**5**, and (+)-**5** on total distance traveled (cm) when the data were summed across the entire 4 h test session. * Indicates significant differences compared to vehicle control based on Dunnett's posthoc tests.

mately 2800 cm in total distance traveled) at 10 and 20 min postadministration, with total distance traveled values returning to near vehicle control levels by approximately 2 h.[13]

Figure 4 shows the effects of cocaine, (±)-**5**, (−)-**5**, and (+)-**5** at each dose when averaged over the entire 4-h test session. There was a main effect of dose for cocaine ($F(3,27) = 12.87$, $P \leq 0.05$) such that a dose of 30 mg/kg increased total distance traveled relative to vehicle control. There was a main effect of dose for (±)-**5** ($F(3,28) = 13.55$, $P \leq 0.05$) and (+)-**5** ($F(4,35) = 10.49$, $P \leq 0.05$) such that doses of 30 and 56 mg/kg increased total distance traveled relative to vehicle control. At the doses tested, (−)-**5** failed to alter total distance traveled relative to vehicle control ($F(3,28) = 1.17$, $P > 0.05$).

Effects of Cocaine, (±)-5, (−)-5, (+)-5 in Cocaine-Discriminating Mice. As shown in Figure 5, cocaine produced dose-dependent increases in cocaine lever responding with an ED_{50} value (± 95% C.I.) of 3.35 mg/kg (2.76−4.07). At the highest dose of cocaine tested (30 mg/kg) rate of responding was decreased by over 50% relative to saline levels. (±)-**5** produced dose-dependent increases in cocaine-lever responding with maximal effects (63%) obtained at a dose of 56 mg/kg resulting in an ED_{50} value of 27.66 (11.13−68.72). Two mice failed to respond at the 56 mg/kg dose of (±)-**5**, and the group mean rate of responding was reduced to levels similar to that produced by 30 mg/kg cocaine. (+)-**5** produced maximal cocaine lever responding (67%) at a dose of 30 mg/kg resulting in an ED_{50} value of 13.59 (4.90−37.71). At a dose of 30 mg/kg of (+)-**5**, rate of responding was suppressed with one mouse not responding. (−)-**5** produced dose-dependent increases in cocaine lever pressing. At a dose of 56 mg/kg, which resulted in 50% cocaine lever pressing, one of four rats failed to respond. At a dose of 100 mg/kg only three mice were tested, and this dose produced 99% cocaine lever pressing in the one mouse that responded. Because only one mouse responded at a dose of 100 mg/kg, an ED_{50} value was not calculated.

Conclusion

In this report we describe development of 3-hydroxy-substituted piperidine derivatives of GBR 12935 which exhibited low to subnanomolar activity for the DAT. Trans isomers displayed somewhat more activity compared to cis isomers. Enantiomers of the trans isomer showed differential activity with *R,R*-(+)-**5** exhibiting much higher activity than the *S,S*-(−)-**5** isomer. Separation of activity between the two enantiomers was very high, but such differential activity is not unprecedented.[15] This may be due to complex interaction of this racemic mixture with the recognition sites of the transporter molecule causing reduction in affinity of the most active enantiomer. A 9-fold separation between cocaine analogue binding and dopamine uptake inhibitory activity was obtained for *R,R*-(+)-**5**. As in the in vitro dopamine uptake assays, *R,R*-(+)-**5** was more potent than *S,S*-(−)-**5** in the in vivo behavioral assays, and it was more potent than, or similar to, racemic (±)-

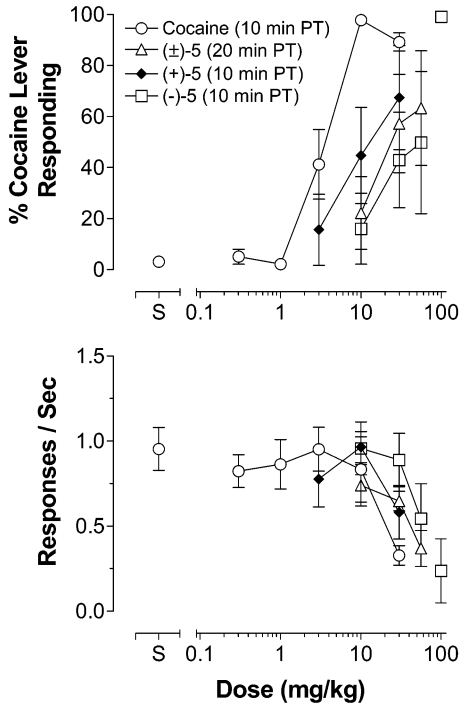


Figure 5. Effects of cocaine (10 min pretreatment interval (PT); $n = 11$), (±)-**5** (20 min PT); $n = 7$), (+)-**5** (20 min PT; $n = 7$), and (−)-**5** (20 min PT, $n = 7$) on the percentage of cocaine-lever responding (top panel) and rate of responding (bottom panel) in mice trained to discriminate 10 mg/kg cocaine from saline. At a dose of 56 mg/kg of (−)-**5** only five mice were tested as two mice had died from nondrug related causes. At a dose of 100 mg/kg (−)-**5**, only three mice were tested and two of those failed to respond. Therefore, the % cocaine lever responding data point for 100 mg/kg (−)-**5** represents the data from one mouse and the % control responding represents the mean data from three mice. Additional mice were not tested as convulsions were observed in the two mice that did not respond. The data points above "S" indicate the mean percentage of cocaine-lever responding and rate of responding following the administration of saline.

5. In locomotor activity studies, compounds *R*,*R*-(+)-**5** and (±)-**5** stimulated activity during the 4-h test session and for hours longer compared to cocaine. All three compounds, (+)-**5**, (−)-**5**, and (±)-**5**, within the dose range tested partially (50%) but incompletely (80%) produced cocaine-like responses in mice trained to discriminate 10 mg/kg ip cocaine from vehicle. Compound (−)-**5** was distinctive in this regard in that, unlike (+)-**5** and (±)-**5**, it did not affect locomotor activity yet, but similar to them, was able to engender (albeit incompletely) cocaine-like responding. The inactivity of (−)-**5** in locomotor activity tests is unlikely attributable to poor entry into the CNS because it reduced response rates and produced (incompletely) cocaine-like responding at similar doses to (±)-**5** during cocaine discrimination tests. Thus, a close correlation exists between in vitro and in vivo activities of the racemic hydroxy compound and its enantiomers.

Experimental Section

Analytical silica gel-coated TLC plates (Silica Gel 60 F_{254}) were purchased from EM Science and were visualized with UV light or by treatment with phosphomolybdic acid (PMA). Flash chromatography was carried out on Baker Silica Gel 40 mM. ^{1}H NMR spectra were routinely obtained on GE−300 MHz and Varian 400 MHz FT NMR. The NMR solvent used was either $CDCl_3$ or CD_3OD as indicated. TMS was used as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc. and were within ± 0.4% of the theoretical value. Optical rotation were recorded on a Perkin-Elmer 241 polarimeter.

[^{3}H]WIN 35,428 (86.0 Ci/mmol), [^{3}H]nisoxetine (80.0 Ci/mmol), and [^{3}H]dopamine (48.2 Ci/mmol) were obtained from Dupont-New England Nuclear (Boston, MA). [^{3}H]Citalopram (85.0 Ci/mmol) was from Amersham Pharmacia Biotech Inc. (Piscataway, NJ). WIN 35,428 naphthalene sulfonate was purchased from Research Biochemicals, Inc. (Natick, MA). (−)-Cocaine HCl was obtained from the National Institute on Drug Abuse. GBR 12909 dihydrochloride (1-[2-[bis(4-fluorophenyl)-methoxy]ethyl]-4-[3-phenylpropyl]piperazine) was purchased from Sigma-Aldrich (St. Louis, MO).

2-[1-(4-Fluorobenzyl)-1,2,3,6-tetrahydropyridin-4-yl]-ethanol (3). 2-Pyridin-4-ylethanol **2** (3.0 g, 25 mmol) and 4-fluorobenzyl chloride (3.89 g, 26.8 mmol) were dissolved in 15 mL of dry methanol and refluxed for 24 h. Methanol was removed in vacuo and dried for 2 h under high vacuum. The residue was dissolved in 50 mL of dry methanol and cooled in an ice bath. $NaBH_4$ (1.57 g, 40.0 mmol) was then added very slowly portionwise over a period of 1.5 h, and the solution was gradually brought to room temperature. The reaction was quenched with water after stirring for 4 h, and the methanol was removed in vacuo. The residual product was dissolved in ethyl acetate, washed with water, dried (Na_2SO_4), and concentrated. The crude product was purified by column chromatography over silica gel (Hex/EtOAc/MeOH = 20/10/2.5) to produce **3**: 2.87 g (50.0% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.09 (2H, brm, H-3), 2.23 (2H, t, $J = 5.6$ Hz, CH_2CH_2OH), 2.54 (2H, t, $J = 5.6$ Hz, H-2), 2.93 (2H, brm, H-6), 3.51 (2H, s, CH_2Ar) 3.66 (2H, t, $J = 5.6$ Hz, CH_2OH), 5.46 (1H, brm, CH=), 6.98 (2H, t, $J = 9.2$ Hz, ArH, ortho to F), 7.25−7.29 (2H, m, ArH).

4-(2-Benzhydryloxyethyl)-1-(4-fluorobenzyl)-1,2,3,6-tetrahydropyridine (4). Benzhydrol (4.04 g, 22.0 mmol), 2-[1-(4-fluorobenzyl)-1,2,3,6-tetrahydropyridin-4-yl]ethanol **3** (2.87 g, 12.2 mmol), and *p*-toluenesulfonic acid (2.90 g, 15.3 mmol) were mixed together in 160 mL of toluene, and the solution was heated to reflux under azeotropic distillation conditions for 3 h under nitrogen. Toluene was removed in vacuo, and the residue was partitioned between ether and saturated $NaHCO_3$ solution. The ether layer was separated, and the aqueous layer was further extracted thrice with ether. Combined organic layers were dried (Na_2SO_4) and concentrated to give the crude product which was chromatographed over silica gel (EtOAc/hexane = 1/5) to give **4**, 2.26 g (46% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.12 (2H, bs, H-3), 2.37 (2H, t, $J = 6.4$ Hz, CH_2CH_2O), 2.55 (2H, t, $J = 5.6$ Hz, H-2), 2.95 (2H, bs, H-6), 3.53−3.59 (4H, m, CH_2Ar and CH_2O), 5.38 (1H, s, CH(Ph)$_2$), 5.45 (1H, s, CH=), 7.02 (2H, t, $J = 8.8$ Hz, ArH, ortho to F), 7.24−7.38 (12H, m, ArH).

Racemic *trans*-4-(2-Benzhydryloxyethyl)-1-(4-fluorobenzyl)piperidin-3-ol (5). Into a stirred solution of $NaBH_4$ (0.57 g, 15 mmol) in 150 mL of dry THF at 0 °C under N_2 was added dropwise 48% w/w BF_3−ether complex (2.00 mL, 15.9 mmol). The cooling bath was removed, and the solution was allowed to stir for 1 h at room temperature (RT). The mixture was then cooled in an ice bath. Into the cooled solution was added dropwise a solution of 4-(2-benzhydryloxyethyl)-1-(4-fluorobenzyl)-1,2,3,6-tetrahydropyridine **4** (3.00 g, 7.48 mmol) dissolved in THF (15 mL). The solution was brought back to room temperature and stirred for an additional 2 h. The solution was again cooled to 0 °C and H_2O (8.5 mL), EtOH (8.5 mL), and 3 N NaOH solution(6 mL) were added followed by dropwise addition of 30% H_2O_2 (4 mL). The reaction mixture was stirred at 55 °C overnight and then cooled to RT, and THF was removed in vacuo. The product was partitioned between water and ethyl acetate, and the organic layer was collected. Water layer was extracted with ethyl acetate. Combined organic layers were dried (Na_2SO_4) and concentrated to give

a crude product which was chromatographed over silica gel (EtOAc/hexane = 3/1) to give **5** as a viscous liquid: 2.6 g (83% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.34−1.39 (2H, m, H-4, H-5ax), 1.59−1.66 (2H, m, H-5eq, CH_2CH_2O), 1.83 (1H, t, J = 10.4 Hz, H-2ax), 1.89−1.95 (2H, m, CH_2CH_2O, H-6ax), 2.55 (1H, brs, OH), 2.72 (1H, d, J = 9.9 Hz, H-6eq), 2.98 (1H, dd, J = 10.4, 4.0 Hz, H-2eq), 3.42−3.63 (5H, m, H-3ax, CH_2O, CH_2-Ar), 5.37 (1H, s, CH(Ph)$_2$), 6.98 (2H, t, J = 9.3 Hz, ArH, ortho to F), 7.22−7.35 (12H, m, ArH).

^{1}H NMR in (CD_3OD/D_2O, 400 MHz): δ 1.18−1.24 (1H, m, H-5ax), 1.32−1.41 (2H, m, H-4, CH_2CH_2O), 1.70−1.76 (1H, m, H-5eq), 1.79 (1H, t, J = 10.8 Hz, H-2ax), 1.92 (1H, t, J = 10.4 Hz, H-6ax), 2.14−2.21 (1H, m, CH_2CH_2O), 2.77 (1H, bd, J = 10.4 Hz, H-6eq), 2.95 (1H, ddd, J = 10.8, 3.2, 1.6 Hz, H-2eq), 3.22−3.32 (1H, m, H-3ax), 3.43−3.55 (4H, m, CH_2O, CH_2Ar), 5.36 (1H, s, CH(Ph)$_2$), 7.03(2H, t, J = 9.2 Hz, ArH, ortho to F), 7.19−7.23 (2H, m, ArH), 7.25−7.34 (10H, m, ArH). The free base was converted into its oxalate salt, mp 166−172 °C. Analysis calculated for ($C_{27}H_{30}FNO_2$·(COOH)$_2$·0.3H_2O) C, H, N.

4,7,7-Trimethyl-3-oxo-2-oxa-bicyclo[2.2.1]heptane-1-carboxylic Acid 4-(2- Benzhydryloxyethyl)-1-(4-fluorobenzyl)piperidin-3-yl Ester (6). Into a stirred solution of 4-(2-benzhydryloxyethyl)-1-(4-fluorobenzyl)piperidin-3-ol **5** (3.0 g, 7.2 mmol), Et_3N (1.40 g, 14.4 mmol), and DMAP (10 mg) in 75 mL of dry CH_2Cl_2 under nitrogen at 0 °C was added a solution of (1*S*)-(−)-camphanic chloride (2.30 g, 10.8 mmol). The temperature was allowed to rise to RT, and the mixture was stirred for 3 h. The reaction was quenched with water, and the product was partitioned between organic and aqueous layers. The organic layer was collected, and the aqueous layer was extracted with additional dichloromethane. The combined organic phase was dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography over silica gel (EtOAc/hexane = 1/3) to produce **6**: (3.6 g, 84% yield) as a colorless semisolid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.94 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.25−1.34 (1H, m, H-5$_{ax}$), 1.43−1.48 (1H, m, CH_2CH_2O), 1.63−1.81 (3H, m, H-5$_{eq}$, H-4, Camp-CH_2), 1.85−2.05 (5H, m), 2.33−2.40 (1H, m, Camp-CH_2,), 2.70 (1H, d, J = 10.8 Hz, H-6eq), 2.99−3.06 (1H, m, H-2eq), 3.39−3.53 (4H, m, CH_2−O, CH_2Ar), 4.48−4.86 (1H, brm, H-3), 5.29−5.40 (1H, s, CH(Ph)$_2$), 6.99 (2H, t, J = 9.2 Hz, ArH, ortho to F), 7.20−7.50 (12H, m, ArH).

Separation of diastereoisomers **6**: The diastereoisomers were separated by semipreparative HPLC using a normal phase column (Nova-Pack Silica 6 μm). The mobile phase used was 0.4% 2-propanol in hexane with a flow rate of 18 mL/min. The two fractions were eluted with retention time of 28.64 min for (+)-isomer and 31.36 min for (−)-isomer. Final purity of the separated diastereoisomers was checked by an analytical normal phase column (Nova-Pack Silica 60 Å 4 μm) using the same mobile phase with a flow rate of 1 mL/min. Pure diastereomers were eluted at 9.56 and 10.73 min, respectively.

Synthesis of (+)-5. The camphanic ester fraction I (0.41 g, 0.67 mmol) from HPLC separation of diastereomeric mixture of **6** was hydrolyzed with 50 mg of K_2CO_3 in methanol (5 mL) at room temprature for 12 h. Methanol was removed in vacuo, and the residue was partitioned between ethyl acetate and water. The organic layer was separated, and the water layer was extracted twice with ethyl acetate. Combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was chromatographed over silica gel (EtOAc/hexane = 3/1) to give **(+)5**, 0.3 g (76% yield). Optical rotation $[\alpha]^{25}_D$ = +19.8 (*c* 1, MeOH). The free base was converted to its oxalate salt, mp 167−171 °C. Analysis calculated for ($C_{27}H_{30}FNO_2$·(COOH)$_2$) C, H, N.

Synthesis of (−)-5. Fraction II (0.58 g, 0.96 mmol) from HPLC was treated with 75 mg of K_2CO_3 in MeOH to produce **(−)-5**, 0.3 g (76% yield), as mentioned above. $[\alpha]^{25}_D$ = −20.3 (*c* 1, MeOH). The free base was converted to the corresponding oxalate salt. mp 168−172 °C Analysis calculated for ($C_{27}H_{30}FNO_2$·(COOH)$_2$) C, H, N.

Synthesis of 4-(2-Benzhydryloxyethyl)-1-(4-fluorobenzyl)piperidin-3-one (7). To a stirred solution of oxalyl chloride 0.50 mL (5.72 mmol) in dry CH_2Cl_2 (10 mL) at −70 °C was added dropwise dimethyl sulfoxide 0.81 mL (11.45 mmol) in CH_2Cl_2 (3 mL). The mixture was stirred for 15 min, and a solution of racemic *trans*-4-(2-Benzhydryloxyethyl)-1-(4-fluorobenzyl)-piperidin-3-ol **5** (0.6 g, 1.43 mmol) dissolved in CH_2Cl_2 (4 mL) was added dropwise. After stirring for 15 min, triethylamine 3.21 mL (22.91 mmol) was added, and the reaction mixture was stirred for 10 min and then allowed to warm to room temperature. Water was added, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The crude product was purified by column chromatography over silica gel (EtOAc/hexane = 1/2) to produce **7**: 0.29 g (48% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.47−1.63 (2H, m, H-5, CH_2-CH_2O), 2.01−2.07 (1H, m, H-4), 2.22−2.30 (1H, m, CH_2CH_2O), 2.38−2.44 (1H, m, H-6), 2.47−2.55 (1H, m, H-5), 2.75 (1H, d, J = 12.8 Hz, H-2). 2.90 (1H, bd, J = 11.2 Hz, H-6), 3.19 (1H, d, J = 12.8 Hz, H-2), 3.46−3.56 (4H, m, CH_2O, CH_2Ar), 5.30 (1H, s, CH(Ph)$_2$), 6.99 (2H, t, J = 8.8 Hz, ArH, ortho to F), 7.20−7.39 (12, m, ArH). The free base was converted into its oxalate salt. Elemental analysis calculated for ($C_{27}H_{28}NO_2F$·(COOH)$_2$) C, H, N.

Racemic *cis*-4-(2-Benzhydryloxyethyl)-1-(4-fluorobenzyl)piperidin-3-ol (8). To a stirred solution of 4-(2-benzhydryloxyethyl)-1-(4-fluorobenzyl)piperidin-3-one **7** (0.16 g, 0.38 mmol) in 4 mL of dry methanol at 0 °C was added $NaBH_4$ portionwise and allowed to stir for 1 h. After quenching, methanol was removed under vacuo. The residue was dissolved in ethyl acetate and washed with water. The water layer was extracted with ethyl acetate twice. The combined organic phase was dried over Na_2SO_4 and concentrated. The crude product was purified by chromatography over silica gel (Hex/EtOAc/Et_3N = 80/40/1.2) to give a mixture of **5** and **8**: 0.11 g (66% yield). Eluting first: racemic *cis*-4-(2-benzhydryloxyethyl)-1-(4-fluorobenzyl)piperidin-3-ol **8:** 0.03 g, (26.4% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.43−1.49 (2H, m, H-5), 1.57−1.65 (2H, m, H-4, CH_2CH_2O-), 1.77−1.84 (1H, m, CH_2-CH_2O), 1.92−1.99 (1H, m, H-6ax), 2.12 (1H, bd, J = 11.2 Hz, H-2ax), 2.62 (1H, brs, OH), 2.78 (1H, dd, J = 10.8, 2.4 Hz, H-6eq), 2.92 (1H, ddd, J = 11.2, 3.2, 1.6 Hz H-2eq), 3.45−3.56 (4H, m, CH_2O, CH_2Ar), 3.63 (1H, brm, H-3eq), 5.32 (1H, s, CH-(Ph)$_2$), 6.99 (2H, t, J = 9.2 Hz, ArH, ortho to F), 7.21−7.33 (12H, m, ArH). Free base was converted into its oxalate salt, mp 152−155 °C. Analysis calculated for ($C_{27}H_{30}FNO_2$·(COOH)$_2$).0.7 H_2O) C, H, N.

Eluting second: racemic *trans*-**5**: 0.08 g (73.6% yield).

Racemic *trans*-3-Propionyl-4-[2-(diphenylmethoxy)-ethyl]-1-[(4-fluorophenyl)methyl]piperidine (9). To a solution of 3-hydroxy-4-[2-(diphenylmethoxy)ethyl]-1-[(4-fluorophenyl)methyl]piperidine (±)-**5** (0.06 g, 0.15 mmol) and triethylamine (0.02 g, 0.23 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added dropwise propionyl chloride (0.02 g, 0.19 mmol), and the reaction was allowed to warm to RT. After 1 h, the same quantities of triethylamine and propionyl chloride were added. One hour later water was added to quench the reaction, and the organic layer was separated, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel to yield the title compound as the free base 0.05 g (77% yield).

^{1}H NMR ($CDCl_3$) δ 1.1 (3H, t, J = 8.1 Hz), 1.26−1.95 (7H, m), 2.26 (2H, q, J = 7.2 and 7.5 Hz), 2.71 (1H, d, J = 11.7 Hz), 3.02 (1H, d, J = 10.5 Hz), 3.41−3.52 (4H, m), 4.67 (1H, t, J = 9.6 Hz), 5.31 (1H, s), 6.98 (2H, t, J = 9.3 Hz), 7.2−7.4 (12H, m). The free base was converted into its oxalate salt. Elemental analysis calculated for ($C_{30}H_{34}NO_3F$·(COOH)$_2$·0.5H_2O) C, H, N.

Transporter Binding Assays. The affinity of test compounds for the rat DAT, SERT, and NET was assessed by measuring inhibition of binding of [^{3}H]WIN 35,428, [^{3}H]-citalopram, and [^{3}H]nisoxetine, respectively, exactly as described by us previously.[13] Briefly, rat striatum was the source

for DAT, and cerebral cortex for SERT and NET. Final [Na^+] was 30 mM for DAT and SERT assays, and 225 mM for NET assays. All binding assays were conducted at 0−4°C, for a period of 2 h for [^{3}H]WIN 35,428 and [^{3}H]citalopram binding, and 3 h for [^{3}H]nisoxetine binding. Nonspecific binding of [^{3}H]-WIN 35,428 and [^{3}H]citalopram binding was defined with 100 μM cocaine, and that of [^{3}H]nisoxetine binding with 1 μM desipramine. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted out in 10% (v/v) DMSO. Additions from the latter stocks resulted in a final concentration of DMSO of 0.5%, which by itself did not interfere with radioligand binding. At least five triplicate concentrations of each test compound were studied, spaced evenly around the IC_{50} value. Radioligand concentrations in DAT, SERT, and NET assays were 4.0, 3.0, and 1.1 nM, respectively, as compared with observed K_d values of 21, 3.2, and 2.2 nM.

Dopamine Transporter Uptake Assays. Uptake of [^{3}H]-DA into rat striatal synaptosomes was measured exactly as described by us previously.[13] Briefly, rat striatal P_2 membrane fractions were incubated with test drug for 8 min followed by the additional presence of [^{3}H]DA for 4 min at 25 °C. Nonspecific uptake was defined with 100 μM cocaine. Construction of inhibition curves and dissolution of test compounds were as described above. The concentration of [^{3}H]DA was 50 nM compared with an observed K_m of uptake of 0.2 μM.

Locomotor Activity. Subjects. Adult male Swiss Webster mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) weighing 30−35 g were used. Mice were housed five per cage with continuous access to food and water and were allowed to acclimate to the vivarium environment one week prior to the start of any experiment. The mice were housed in an AALAC-accredited animal facility with a controlled temperature (22−24 °C) on a 12 h light-dark cycle. All testing occurred during the light component.

Apparatus Procedure and Analyses. The locomotor activity chambers and procedure have been described in detail elsewhere.[13] Briefly, four commercially obtained, automated activity monitoring devices each enclosed in sound- and light-attenuating chambers were used (AccuScan Instruments, Inc., Columbus, OH). The interior of each device was divided into two separate 20 × 20 × 30-cm arenas, permitting the independent and simultaneous measurement of two mice. Sixteen photobeam sensors were spaced 2.5 cm apart along the walls of the chamber. Nonhabituated mice were administered a dose of a test compound or vehicle and immediately placed into automated activity chambers where distance traveled (cm) was recorded for 24, 10 min periods. A one-factor (dose) ANOVA was conducted on each drug and its respective vehicle followed by Dunnett's posthoc tests when the overall ANOVA was significant. The alpha level for all comparisons was set at 0.05.

Drug Discrimination. Subjects. Adult male Swiss Webster mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were used. Mice were individually housed and maintained on a 12 h light−dark cycle with continuous access to water. Training and testing occurred during the light component. Mice were maintained at 35 ± 5 g by supplemental post-session feedings of laboratory chow (Harlan Tekled, Madison, WI).

Apparatus and Procedure. The drug discrimination chambers and procedure have been described in detail elsewhere.[13] Briefly, eight standard, light- and sound-attenuated mouse operant conditioning chambers were used (Med Associates, Inc., St. Albans, VT; model ENV-307A). Each chamber was equipped with two response levers separated by a trough into which a 0.01 mL dipper cup could be presented. A house light was centered at the top of the front panel and three cue lights were located above each lever. Control of lights, dipper presentations, and recording of lever presses were accomplished by a microcomputer system (Med-PC software, Med Associates, Inc., St. Albans, VT).

Mice were initially trained to press one of the two levers at a fixed-ratio 1 (FR1) schedule of reinforcement in which each lever press resulted in a 0.01 mL delivery of sweetened condensed milk. The response requirement was gradually increased to FR20. Subsequently, the mice were reinforced for pressing the opposite lever until reliable responding was obtained under FR20 conditions. Discrimination training occurred during daily (Mon−Fri) 15 min experimental sessions. Mice were injected with cocaine (10 mg/kg) or saline ip 10 min prior to the start of the session start. The cocaine- and saline-associated levers were counterbalanced across the mice. Responses on the injection-appropriate lever resulted in delivery of the sweetened milk solution. Responses on the injection-inappropriate lever resulted in resetting the response requirement on the correct lever. A schedule was used to determine which injection was administered, with the restriction that the same injection was not given on more than two consecutive sessions, and over 30 training sessions the number of saline and cocaine injections were approximately equal.

Testing commenced when (1) a mouse completed the first fixed-ratio (FFR) on the correct lever on at least 8 or 10 consecutive days; and (2) at least 80% of the total responses were made on the correct lever during those eight sessions. Tests were conducted on Tuesdays and Fridays provided that the mouse completed the FFR on the correct lever during the most recent cocaine and saline training sessions, otherwise, a training session was administered. During test days, responding on either lever was reinforced with milk.

Analyses. The percentage of responses on the cocaine-lever was calculated for each mouse during training and test sessions by dividing the number of lever presses emitted upon the cocaine-lever by the total number of lever presses emitted on both levers, and then this quotient was multiplied by 100. Additionally, rate of responding was calculated for each mouse by dividing the total number of responses emitted on both levers by 900 s. Individual cocaine-lever responding percentages and responses per second were then averaged (± SEM). If a mouse failed to complete a FFR, then its data were excluded from calculations of mean cocaine-lever responding, but were included for mean response rate calculations. ED_{50} values (95% confidence limit) (mg/kg) were calculated for percent cocaine-lever responding using a sigmoidal dose−response (variable slope) curve fitting procedure (GraphPad Prism; San Diego, CA). A log transformation on dose was used.

Drugs. Cocaine (U.S. National Institute Drug Abuse) was dissolved in 0.09% sterile saline. Compounds (±)-**5**, (+)-**5**, and (−)-**5** were dissolved in 20% w/v hydroxypropyl-β-cyclodextrin (Cavitron 82003, Cerestar USA, Inc., Hammond, IN). All drugs were administered by the ip route in a volume of 10 mL/kg. During drug discrimination testing the following pretreatment intervals were used: cocaine: 10 min, (±)-**5**: 20 min, (+)-**5**: 10 min and (−)-**5**: 10 min.

Single-Crystal X-ray Diffraction Analysis of (−)-5. $C_{27}H_{31}FN^+O_2 \cdot 1/2(C_4H_4O_6^{2-}) \cdot H_2O \cdot 0.106(CH_3OH)$, FW = 516.49, orthorhombic space group $P2_12_12_1$, a = 9.265(1), b = 18.010(1), c = 32.932(1) Å, V = 5495.1(4) Å^3, Z = 8, r_{calcd} = 1.249 mg mm^{-3}, l(Cu Kα) = 1.54178 Å, m = 0.752 mm^{-1}, $F(000)$ = 2203, T = 293 K.

A clear colorless 0.72 × 0.16 × 0.015 mm crystal grown from MeOH nitromethane and water was used for data collection with a Bruker SMART[21] 6K CCD detector on a Platform goniometer. The Rigaku rotating Cu anode source was equipped with incident beam Gobel mirrors. Lattice parameters were determined using SAINT[21] from 5078 reflections within 5.37< $2q$ < 127.38. Data were collected to $2q$ = 133.7°. A set of 25423 reflections was collected in the ω scan mode. There were 9345 unique reflections. Corrections were applied for Lorentz, polarization, and absorption effects. The structure was solved with SHELXTL[22] and refined with the aid of the SHELX97 system of programs. The full-matrix least-squares refinement on F^2 used eight restraints and varied 706 parameters: atom coordinates and anisotropic thermal parameters for all non-H atoms except the lower occupancy solvate atoms. H atoms were included using a riding model [coordinate shifts of C applied to attached H atoms, C−H distances set to 0.96 to 0.93 Å, H angles idealized, U_{iso}(H) were set to 1.2 to 1.5 U_{eq}(C). Final residuals were $R1$ = 0.070 for the 6300 observed data with F_o > 4s(F_o) and 0.93 for all data. Final difference Fourier excursions of 0.37 and −0.30 eÅ^{-3}. The asymmetric unit

contains two molecules of the title compound,. an l-(+)-tartrate anion, two molecules of water and a methanol solvate present at a partial occupancy of 0.216. The chirality of the title compound was based on the known chirality of the L-(+)-tartrate. The primary differences between the two molecules in the asymmetric unit are the orientations of the (4-fluorophenyl)methyl and the diphenylmethoxyethane moiety with respect to the piperidine ring where the respective torsion angles about the bond to the ring varied by 110.0 and 54.2°, respectively. Tables of coordinates, bond distances and bond angles, and anisotropic thermal parameters have been deposited with the Crystallographic Data Centre, Cambridge, CB2, 1EW, England.

Acknowledgment. This work was supported by the National Institute on Drug Abuse, Grant No. DA 12449 (A.K.D.). We thank Mrs. Janet Berfield and Li Juan Wang for their help with the binding and uptake assays.

Supporting Information Available: Crystal structure data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) *Substance Abuse and Mental Health Services Administration National Household Survey on Drug Abuse: Main Findings 1997*; Department of Health and Human Services: Washington, DC, 1998. (b) Mathias, R. Cocaine, marijuana and heroin abuse up, methamphetamine abuse down. *NIDA Notes* **2000**, *15* (3), 4−5.

(2) Carroll, F. I.; Howell, L. L.; Kuhar, M. J. Pharmacotherapies for Treatment of Cocaine Abuse: Preclinical Aspects. *J. Med. Chem*. **1999**, *42*, 2721−2736.

(3) Ritz, M. C.; Cone, E. J.; Kuhar, M. J. Cocaine inhibition of ligand binding at dopamine, norpinephrine and serotonin transporters: A structure−activity study. *Life Sci*. **1990**, *46*, 635−645.

(4) Ritz, M. C.; Lamb, R. J.; Goldberg, R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* **1987**, *237*, 1219−1223.

(5) Wilcox, K. M.; Paul, I. A.; Woolverton, W. L. Comparison between dopamine transporter affinity and self-administration potency of local anesthetics in rhesus monkeys. *Eur. J. Pharmacol.* **1999**, *367*, 175−181.

(6) Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The dopamine hypothesis of the reinforcing properties of cocaine. *Trends. Neurosci.* **1991**, *14*, 299−302.

(7) Rocha, B. A., Fumagalli, F., Gainetdinov, R. R., Jones, S., Ator, R., Giros, B., Miller G. W. and Caron, M. Cocaine self-administration in dopamine transporter knockout mice. *Nature Neurosci*. **1998**, *1*, 132−137.

(8) Walsh, S. L.; Cunningham, K. A. Serotonergic mechanisms involved in the discriminative stimulus, reinforcing and subjective effects of cocaine. *Psychopharmacology* **1997**, *130*, 41−58.

(9) Carrol, F. I.; Lewin, A. H.; Kuhar, M. J. Dopamine transporter uptake blockers: Structure activity relationships. In *Neurotransmitter transporters: Structure and function*; Reith, M. E. A., Eds.; Human Press: Totowa, NJ, 1997; pp 263−295.

(10) Singh, S. Chemistry, design, and structure−activity relationship of cocaine antagonists. Chem. Rev. **2000**, *100*, 925−1024.

(11) Dutta, A. K.; Xu, C.; Reith, M. E. A. Structure−Activity Relationship Studies of Novel 4-[2-[Bis(4-fluorophenyl)methoxy]-ethyl]-1-(3-phenylpropyl)piperidine Analogues: Synthesis and Biological Evaluation at the Dopamine and Serotonin Transporter Sites. *J. Med. Chem*. **1996**, *39*, 749−756.

(12) Dutta, A. K.; Coffey, L. L.; Reith, M. E. A. Highly selective, novel analogues of 4-[2-(diphenylmethoxy)ethyl]-1-benzylpiperidine for the dopamine transporter: Effect of different aromatic substitutions on their affinity and selectivity. *J. Med. Chem*. **1997**, *40*, 35−43.

(13) Dutta, A. K.; Davis, M. C.; Fei, X.-S.; Beardsley, P. M.; Cook, C. D.; Reith, M. E. A. Expansion of Structure−Activity Studies in piperidine analogues of 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine (GBR 12935) compounds by altering substitutions in the N-benzyl moiety and behavioral pharmacology of selected molecules. *J. Med. Chem*. **2002**, *45*, 654−662.

(14) Newman, A. H.; Allen, A. C.; Izenwasser, S.; Katz, J. L. Novel 3α-(diphenylmethoxy)tropane analogues: potent dopamine uptake inhibitors without cocaine-like behavioral profiles. *J. Med. Chem*. **1994**, *37*, 2258−2261.

(15) Hsin, L.-W.; Dersch, C. M.; Baumann, M. H.; Stafford, D.; Glowa, J. R.; Rothman, R. B.; Jacobson, A. E.; Rice, K. C. Development of long-acting dopamine transporter ligands as potential cocaine-abuse therapeutic agents. Chiral hydroxyl-containing derivatives of 1-[2-[bis(4-fluorophenyl)methoxy]ehtyl]-4-(3-phenylpropyl)piperazine and 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine. *J. Med. Chem*. **2002**, *45*, 1321−1329.

(16) Agoston, G. E.; Wu, J. H.; Izenwasser, S.; George, C.; Katz, J.; Kline, R. H.; Newman, A. H. Novel *N*-substituted 3α-[bis(4′-fluorophenyl)methoxy]tropane analogues: selective ligands for the dopamine transporter. *J. Med. Chem*. **1997**, *40*, 4329−4339.

(17) Lewis, D. B., Matecka, D., Zhang, Y., Hsin, L.-W., Dersch, C. M., Stafford, D., Glowa, J. R., Rothman, R. B., Rice, K. C. Oxygenated analogues of 1-[2-(diphenylmethoxy)ethyl]- and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazines (GBR 12935) and GBR 12909) as potential extended-action cocaine-abuse therapeutic agents. *J. Med. Chem*. **1999**, *42*, 5029−5042.

(18) Glowa, J. R., Fantegrossi, W. E., Lewis, D. B., Matecka, D., Rice, K. C., Rothman, R. B. Sustained decrease in cocaine-maintained responding in rhesus monkeys with 1-[2-[bis(4-fluorophenyl)-methoxy]ethyl]4-(3-hydroxy-3-phenylpropyl)piperazinyl decanoate, a long-acting ester derivative of GBR 12909. *J. Med. Chem*. **1996**, *39*, 4689−4691.

(19) Gorelick, D. A.; The rate hypothesis and agonist substitution approaches to cocaine abuse treatment. *Adv. Pharmacol. (NY)* **1998**, *42*, 995−997.

(20) Efange, S. M. N.; Michelson, R. H.; Remmel, R. P.; Boudreau, R. J.; Dutta, A. K.; Freshler, A. Flexible *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine analogues: Synthesis and monoamine oxidase catalyzed bioactivation. *J. Med. Chem*. **1990**, *33*, 3133−3138.

(21) *Bruker 1995 SMART and SAINT Data Collection and Reduction Software for the SMART system*; Bruker-AXS: Madison, WI.

(22) Sheldrick, G. M. 1997. *SHELXTL* Version 5.1, Bruker Analytical X-ray Instruments: Madison, WI.

JM020275K