Synthesis and Evaluation of Dopamine and Serotonin Transporter Inhibition by Oxacyclic and Carbacyclic Analogues of Methylphenidate

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Methylphenidate (Ritalin) binds stereoselectively and enantioselectively to the dopamine transporter (DAT) and inhibits dopamine reuptake with in vitro and in vivo potency similar to that of cocaine. Unlike cocaine, it manifests little, if any, tolerance or addiction liability. Since this compound has a substantial clinical history, it provides an excellent template from which to design potential medications for cocaine abuse. It has long been assumed that a nitrogen, such as exists in cocaine and methylphenidate, is essential for interaction with monoamine transporters. We previously demonstrated that an amine nitrogen in phenyltropane analogues of cocaine is not necessary for conferring high DAT binding affinity. We now report the synthesis of oxacyclic and carbacyclic analogues of methylphenidate, including the four enantiomerically pure isomers of 2-(3,4-dichlorophenyl)-2-(tetrahydropyran-2-yl)acetic acid methyl ester. The *threo* isomers are potent and selective inhibitors of the DAT. This is the first generalization of the principle that the presence of nitrogen is not a necessity for DAT inhibition.

Introduction

Methylphenidate[1] (Figure 1), marketed as Ritalin (DL-*threo*-methylphenidate) for the treatment of attention-deficit hyperactivity disorder (ADHD), binds stereoselectively and enantioselectively to the dopamine transporter (DAT) (IC_{50} = 34−83 nM).[2−4] It inhibits dopamine reuptake with in vitro and in vivo potency similar to that of cocaine[5] yet, unlike cocaine, manifests little, if any, tolerance or addiction liability.[5,6] Patrick et al.[7] have shown the D-*threo*-methylphenidate to be the pharmacologically active isomer. Ding et al.[8] reported a positron emission tomography (PET) study in which they confirmed that the biological activity of methylphenidate resides in the (2*R*,2′*R*)-D-*threo* isomer, which is distributed to sites in the basal ganglia. In contrast, they showed that the (2*S*,2′*S*)-L-*threo* isomer is distributed nonspecifically in both baboon and human brains. In view of this biological enantioselectivity, there has been a considerable effort devoted to the development of enantioselective synthetic pathways to (2*R*,2′*R*)-*threo*-methylphenidate.[9−12]

Methylphenidate has a mode of action (inhibition of the DAT) similar to that of cocaine and the 3-aryl-8-azabicyclo[3.2.1]octanes (3-aryltropanes).[3,13] Methylphenidate's substantial clinical history, combined with minimal, if any, addiction liability, suggested that this might provide an excellent template from which to design potential medications for cocaine abuse. This has been recognized by researchers, and Gatley et al.[4,14] have reported aromatic ring brominated analogues that manifest greater potency at the DAT than the parent methylphenidate, while Deutsch et al.[2] have evaluated a series of ring-substituted analogues of methylphenidate. Inhibition of binding to the dopamine transporter is most often measured by displacement studies in which either [^{3}H]RTI55 or [^{3}H]WIN 35,428 are used as radioligands. A desirable attribute of a potential antagonist-based cocaine medication is an ability to inhibit DAT binding of cocaine while allowing dopamine to transport intracellularly. Deutsch et al. have reported[2] a methylphenidate analogue that manifests a difference in inhibition of [^{3}H]WIN 35,428 versus dopamine reuptake at the DAT, and they postulated that this compound may provide a lead for a dopamine-sparing cocaine antagonist.

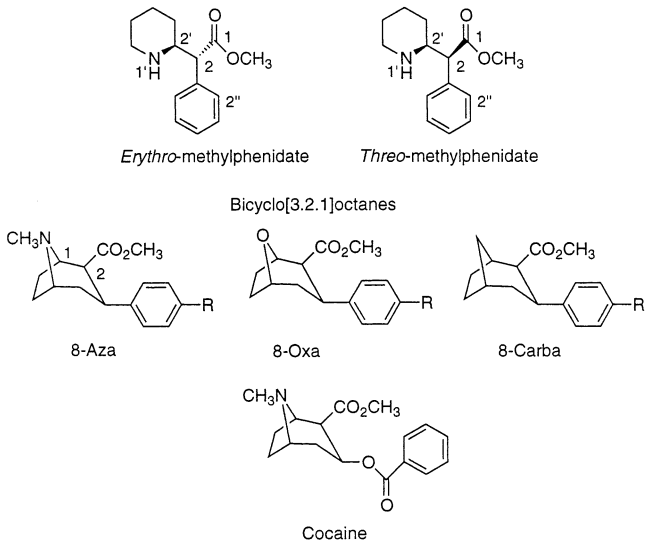

Figure 1.

Prior to our work on 8-oxabicyclo[3.2.1]octanes[15,16] and 8-carbabicyclo[3.2.1]octanes (Figure 1),[17] it had been assumed that a nitrogen, such as exists in cocaine, the 3-aryltropanes, and methylphenidate, was essential for interaction with monoamine transporters. Since we have shown unequivocally that the nitrogen "anchor"

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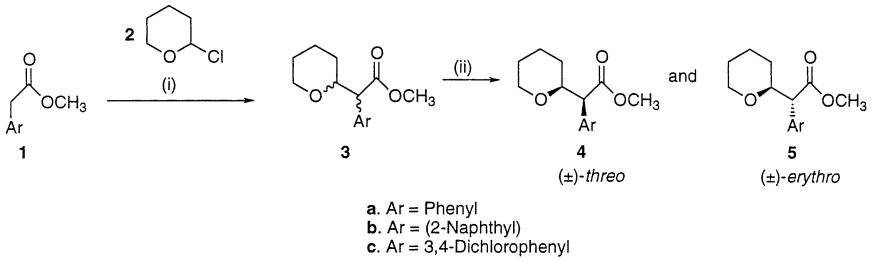
Scheme 1. Synthesis of Aryltetrahydropyranyl Methyl Esters[a]



[a] Reagents: (i) LDA, Et_2O; (ii) column chromatography.

Table 1. Inhibition of [^{3}H]WIN 35,428 Binding to the Dopamine Transporter and [^{3}H]Citalopram Binding to the Serotonin Transporter in Rhesus (*Macaca mulatta*) or Cynomolgus Monkey (*Macaca fascicularis*) Caudate-Putamen[a]

compd	Organix no.	DAT IC_{50} (nM)	SERT
DL-*threo* methylphenidate[b]		17 ± 2.0	>100000
4a	O-2602	3000	>100000
4b	O-2618	430	1000
4c	O-1730	29.1 ± 5.05	2180 ± 226
5a	O-2601	13000	>100000
5b	O-2617	4000	10000
5c	O-1731	286 ± 10.5	7795 ± 1840
12	O-1794	34 ± 8.6	1655
13	O-1783	17 ± 1.3	>10000
14	O-1793	736 ± 59	7800
15	O-1792	193 ± 3.5	>10000
16	O-2170	127	8000
17	O-2169	146	12000
18	O-2171	128	10000
19	O-2172	47	7000

[a] Each value is the mean of three or more independent experiments each conducted in different brains and in triplicate. Errors generally do not exceed 15% between replicate experiments. Highest doses tested were generally 10−100 μM. [b] Schweri et al.[3] reported D-*threo*-(2*R*,2′*R*)- and L-*threo*-(2*S*,2′*S*)-methylphenidate IC_{50} values of 88 nM and 1.2 μM, respectively.

point is not needed for potent biological activity in the bicyclo[3.2.1]octane series,[18] we wished to probe the generalizability of exchange of oxygen, or carbon, for nitrogen in monoamine uptake inhibitors. We therefore explored the substitution of the nitrogen in the piperidine ring of methylphenidate for an oxygen and a carbon. Herein, we describe the synthesis of 10 oxygen and 4 carbon analogues of methylphenidate. We report that the 3,4-dichlorophenyloxamethylphenidate analogues prove to be quite potent inhibitors of the dopamine transporter. The synthesis and binding of the four enantiomerically pure isomers of 2-(3,4-dichlorophenyl)-2-(tetrahydropyran-2-yl)acetic acid methyl ester are also described.

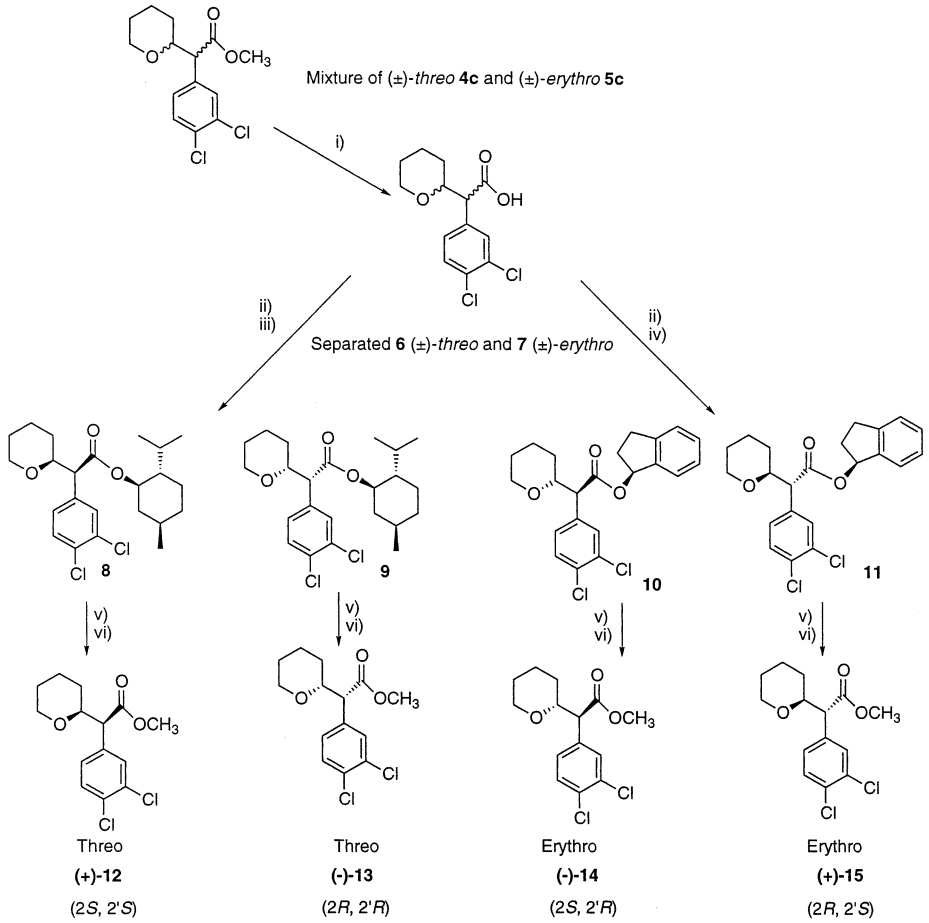
Chemistry

The synthesis of the racemic diastereomers **4** and **5** is presented in Scheme 1. 2-Chlorotetrahydropyran was reacted with the enolate of the appropriate methylaryl acetate. The mixture of diastereomers **4** and **5** thus obtained was separated by column chromatography to provide the racemic *threo* isomers **4a**−**c** and the racemic erythro isomers **5a**−**c**. The 3,4-dichloroaryl compounds **4c** and **5c** manifested nanomolar inhibition of the DAT (Table 1) and were therefore selected for enantiomeric resolution. The pairs of separated diastereomers **4c** and **5c** were then resolved into their (+)- and (−)-enantiomers as shown in Scheme 2. The (±)-*threo*-**4c** was hydrolyzed to the (±)-*threo*-**6**. Hydrolysis under basic conditions (LiOH) leads to racemization because the benzylic proton is labile. Therefore, nonbasic conditions were needed for the cleavage of the methyl ester. Hydrolysis was readily effected with trimethylsilyl iodide (TMSI).[19] The enantiomeric pair **6** was then transformed into diastereomeric menthyl esters **8** and **9** by treatment of their acid chlorides with optically pure L-menthol. Column chromatography then allowed separation of the two diastereomeric menthyl esters **8** and **9**. Separation was confirmed by ^{1}H NMR. The 2′-H doublet for **8** appeared at δ 7.465 and δ 7.459 and that for **9** at δ 7.508 and δ 7.502. On an expanded spectrum of a mixture of **8** and **9**, these sharp resonances can be clearly distinguished. The ^{1}H NMR spectrum for pure **8** shows no resonances due to **9**. Correspondingly, the spectrum for pure **9** shows no resonances attributable to **8**. Therefore, the enantiomeric purity of **8** and **9** is estimated at >98% ee. These two menthyl esters were each separately hydrolyzed, again with TMSI, to give the two optically pure acids (2*S*,2′*S*)-**6′** and (2*R*,2′*R*)-**6′** in good yield (74% and 77%, respectively). Acid (2*S*,2′*S*)-**6′** was crystallized, and its absolute configuration was confirmed by X-ray crystallography. The configuration of (2*S*,2′*S*)-**6′** was thus proved. Therefore (2*R*,2′*R*)-**6′** was confirmed as the 2*R*,2′*R* enantiomer. These acids were then methylated with trimethylsilyl diazomethane to furnish optically pure target molecules (2*S*,2′*S*)-**12′** and (2*R*,2′*R*)-**13′**. The compounds had equal and opposite optical rotations.

Conversion of (±)-*erythro*-**5c**, as described above, provided the (±)-erythro acids **7**. The menthyl esters of the pair of acids **7** proved to be difficult to separate by column chromatography. Instead, the racemic acids **7** were converted to the diastereomeric indanyl esters **10** and **11** by reaction with (*S*)-(+)-1-indanol, which were then resolved by medium-pressure flash column chromatography. Separation was again confirmed by ^{1}H NMR. The doublet for **10** (δ 3.544 and 3.515) and that for **11** (δ 3.557 and 3.528) were distinguishable on an expanded ^{1}H NMR spectrum. Each purified diastereomer showed less than 2% contribution from the other diastereomer. Therefore, the enantiomeric purity of each of **10** and **11** was estimated at >96% ee. The esters were hydrolyzed (TMSI) and methylated (as above) to provide the two enantiomerically pure methyl esters (2*S*,2′*R*)-**14′** and (2*R*,2′*S*)-**15′,** which again, had equal and opposite optical rotations.

A sample of the optically pure acid that provided **15** was also allowed to react with 4-nitrophenol to obtain the 4-nitrophenyl ester (Figure 2). This compound was recrystallized, and X-ray crystallographic analysis con-

Scheme 2. Resolution of *threo*- and *erythro*-3,4-Dichlorophenyltetrahydropyranyl Acetic Acid Methyl Esters **12**−**15**[a]



[a] Reagents: (i) TMSI, $CHCl_3$; (ii) $(COCl)_2$, DMF, CH_2Cl_2; (iii) pyridine, L-menthol, THF; (iv) pyridine, (*S*)-(+)1-indanol, THF; (v) TMSI, CCl_4; (vi) $TMSCHN_2$.

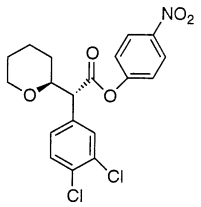
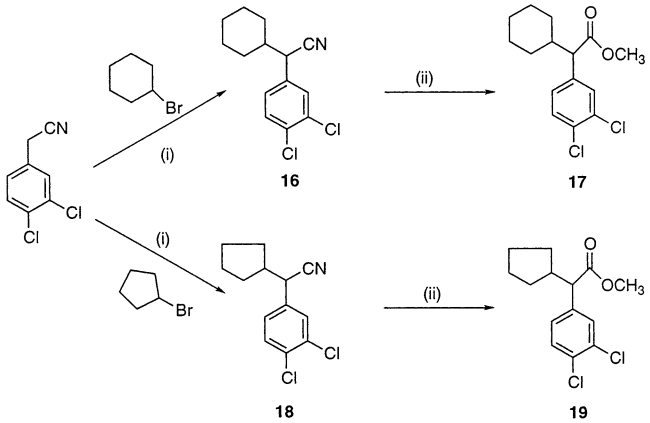


Figure 2. Absolute configuration of the 4-nitrophenyl ester of (2*R*,2′*S*)-**15**.

firmed its configuration as 2*R*,2′*S*. Therefore, the methyl ester derived from that acid was compound (2*R*,2′*S*)-**15′**. Therefore, the remaining enantiomer was confirmed as (2*S*,2′*R*)-**14′**.

The 3,4-dichlorophenyl carbacyclic analogues of methylphenidate were prepared (Scheme 3) by reaction of either cyclohexyl bromide or cyclopentyl bromide with 3,4-dichlorophenylacetonitrile to provide **16** and **18**. Hydrolysis and esterification then gave the desired carbacyclic analogues **17** and **19**, respectively.

Scheme 3. Synthesis of 3,4-Dichlorophenylcycloalkylacetic Acid Methyl Esters **17** and **19**[a]



[a] Reagents: (i) t-BuOK, cycloalkyl bromide; (ii) HCl, MeOH.

Biology

The affinities (IC_{50}) for inhibition of the dopamine and serotonin transporters were determined in competition studies and are presented in Table 1. The dopamine transporter was labeled with [^{3}H]3β-(4-fluorophenyl)-tropane-2β-carboxylic acid methyl ester ([^{3}H]WIN 35,428 or [^{3}H]CFT (1 nM)), and nonspecific binding was measured with (−)-cocaine (30 μM).[20] [^{3}H]Citalopram was used to label the serotonin transporter, and nonspecific binding was measured with fluoxetine (10 μM).[16] Studies were conducted in monkey striatum because this tissue is used in an ongoing investigation of structure−activity relationships at the DAT and meaningful comparisons with an extensive database can be made.[18] Competition studies were conducted with a fixed concentration of radioligand and a range of concentrations of the test drug. All drugs inhibited [^{3}H]WIN 35,428 and [^{3}H]citalopram binding in a concentration-dependent manner.

The (±)-*threo* isomers **4a**−**c** inhibited [^{3}H]WIN 35,428 binding to the DAT with a potency of IC_{50} = 29 nM to 3 μM, while the (±)-erythro isomers manifested an inhibitory potency range of 0.28−13 μM. The 3,4-dichlorophenyl compounds were most potent, and **4c** (3,4-dichlorophenyltetrahydrofuran methyl ester) had DAT (IC_{50} = 29.1 nM) with poor inhibition of the SERT (IC_{50} = 2.2 μM). The carbacyclic analogues **16**−**19** manifested DAT potency in the nanomolar range (IC_{50} = 47−146 nM) with considerable selectivity (SERT IC_{50} = 7−12 μM).

Discussion

Methylphenidate inhibits [^{3}H]WIN 35,428 binding to the DAT with substantial potency (IC_{50} = 17 nM) and selectivity (SERT IC_{50} ≥ 100 mM). In particular, the (2*R*,2′*R*)-*threo* isomer is about 14-fold more potent than its (2*S*,2′*S*)-*threo* counterpart.[3] We wished to investigate whether similar potency and biological enantioselectivity would be evidenced by analogues in which the amine had been exchanged for an ether. To further investigate whether a heteroatom was required for binding of these compounds to the DAT, we planned to synthesize and evaluate carbacyclic analogues of our most potent oxamethylphenidates, namely, the 3,4-dichlorophenyl compounds. The investigation was conducted in two phases. In the first phase, we prepared the oxa analogues with three different aromatic substitutions [phenyl (**4a** and **5a**), 2-naphthyl (**4b** and **5b**), and 3,4-dichlorophenyl (**4c** and **5c**)] and evaluated them as pairs of enantiomers. On the basis of the binding potencies (Table 1) of each of the (±)-*threo*-(**4**) and (±)-*erythro*-(**5**) racemates, we selected the most potent of these (**4c** and **5c**) to determine the biological enantio- and stereoselectivity in this new class of non-nitrogen DAT inhibitors. The asymmetric synthesis of an oxacyclic furanyl analogue of methylphenidate has been reported;[21] however, our general synthetic route (Scheme 1) provided access to all isomers simultaneously, and we judged that the most expeditious route to each isomer would be by subsequent separations, as already described. Thus, the enantiopure 3,4-dichlorophenyl analogues **12**−**15** were obtained.

Synthesis of the enantiomeric pairs of the carbacyclic analogues **17** and **19** was achieved through the intermediate nitriles **16** and **18**. This afforded us the opportunity to evaluate a 2-cyano carbacyclic analogue of methylphenidate as well as the C2 methyl esters. Indeed, we prepared both the six-membered (**16** and **17**) and the five-membered (**18** and **19**) analogues with some surprising biological results.

The exchange of the nitrogen for an oxygen or a carbon in these analogues of methylphenidate has proved to be extremely interesting. These compounds retain biological activity. The unsubstituted phenyl compounds **4a** and **5a** manifest micromolar potency at the DAT. For comparison, DL-*threo*-methylphenidate has an IC_{50} of 17 nM at the DAT. Therefore the DL-*threo*-oxa analogue **4a** is about 175-fold less potent. The naphthyl analogues **4b** and **5b** are slightly more potent than the unsubstituted compounds **4a** and **5a**, respectively. The 3,4-dichlorophenyl compounds **4c** and **5c** prove to be the most potent in this oxacyclic series. Indeed, the racemic *threo*-dichlorophenyl analogue **4c** manifests an IC_{50} of 29 nM. It should be noted that since **4c** is a 3,4-dichlorophenyl analogue, it cannot be compared directly with DL-*threo*-methylphenidate itself (IC_{50} = 17 nM). However, the 3,4-dichlorophenyl analogue of methylphenidate has been reported[2] to manifest an IC_{50} of 5.3 nM at the DAT. These IC_{50} values are obtained in different laboratories and therefore are not directly comparable; however, it is apparent that the oxa analogue **4c** is at least about 6-fold less potent than its aza counterpart. Notwithstanding this apparent loss in inhibitory potency, the dichlorophenyl oxamethylphenidate remains an extremely potent inhibitor of the DAT and is certainly as potent as methylphenidate itself.

It is notable that in all cases the *threo* isomers (**4**) are, as with methylphenidate itself, more potent diastereomers than the erythro compounds (**5**). As evidenced in the 3,4-dichlorophenyl series, the biological diastereoselectivity for these oxa analogues is similar to that of the aza counterparts. Thus, the (±)-*threo*-3,4-dichlorophenyl analogues **4c** are about an order of magnitude more potent at the DAT than their (±)-erythro counterparts **5c** (IC_{50}((±)-*threo*) = 29.1 ± 5.1 nM vs IC_{50}((±)-erythro) = 286 ± 10.5 nM). Also, the (2*R*,2′*R*)-(−)-*threo*-**13** (IC_{50} = 17 ± 1.3 nM) is about twice as potent as the (2*S*,2′*S*)-(+)-*threo*-**12** (IC_{50} = 34 ± 8.6 nM). Thus, it is interesting to note that the biological enantioselectivity seen in the (2*R*,2′*R*)-*threo*- versus (2*S*,2′*S*)-*threo*-methylphenidate is about 13.6-fold,[3] whereas for the oxygen analogues presented here, that ratio is reduced to about 2-fold. Finally, both (−)-**13** and (+)-**12** *threo* analogues exhibit substantial selectivity for the DAT over the SERT.

The carbacyclic analogues show surprising potency. The cyclohexyl analogues, as either a methyl ester **17** or a nitrile **16**, exhibit similar binding potency at the DAT (IC_{50} = 127−146 nM) and SERT (IC_{50} = 8−12 μM). While the cyclopentylnitrile analogue **18** is similarly potent (IC_{50} = 128 nM), the methyl ester **19** manifests substantial binding potency at the DAT (IC_{50} = 47 nM) with substantial selectivity (150-fold) over the SERT. It remains to be determined whether biological enantioselectivity is also manifested by this compound.

Conclusion

Oxacyclic and carbacyclic analogues of methylphenidate have been synthesized. Evaluation of their binding potency at both DAT and SERT has shown that these compounds manifest substantial inhibitory potency. We had reported that 3,4-dichlorophenyl substitution in 8-aza-, 8-oxa-, and 8-carbatropanes had provided especially potent inhibitors,[18] and this is evident in this new series as well. The four isomers of (3,4-dichlorophenyl)-(tetrahydropyran-2-yl)acetic acid methyl ester were prepared, and their absolute stereochemistry was established. It was shown that the *threo* isomers are about 1 order of magnitude more potent as DAT inhibitors than the erythro compounds. Furthermore, as with methylphenidate, the (2*R*,2′*R*)-*threo* isomer is more potent than its (2*S*,2′*S*)-*threo* counterpart.

Experimental Section

NMR spectra were recorded in $CDCl_3$, unless otherwise mentioned, on a JEOL 300 NMR spectrometer operating at 300.53 MHz for ^{1}H and at 75.58 MHz for ^{13}C. TMS was used as the internal standard. Melting points are uncorrected and

were measured on a Gallenkamp melting point apparatus. Thin-layer chromatography (TLC) was carried out on Baker Si250F plates. Visualization was accomplished with either UV exposure or treatment with phosphomolybdic acid (PMA). Flash chromatography was carried out on Baker silica gel, 40 μM. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. All reactions were conducted under an inert (N_2) atmosphere. [^{3}H]WIN 35,428 (2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-[^{3}H]methyltropane, 79.4−87.0 Ci/mmol) and [^{3}H]citalopram (86.8 Ci/mmol) were purchased from DuPont-New England Nuclear (Boston, MA). A Beckman 1801 scintillation counter was used for scintillation spectrometry. Bovine serum albumin (0.1%) was purchased from Sigma Chemicals. (*R*)-(−)-Cocaine hydrochloride for the pharmacological studies was donated by the National Institute on Drug Abuse [NIDA]. Room temperature is ca. 22 °C. TMSI is trimethylsilyl iodide. Yields have not been optimized.

2-Chlorotetrahydropyran. This compound was prepared by a modification of the method of Ficini.[22] Dry HCl gas was bubbled for a period of 2 h through a solution of 3,4-dihydro-2*H*-pyran (34.1 g, 0.41 mol) in anhydrous ether (150 mL), and the mixture was cooled in a dry ice/acetone bath. The solution was sparged with N_2 to remove free HCl. Ether was removed by evaporation. Fractional distillation of the residue under reduced pressure (bp 36−39 °C, 18 mmHg) furnished a colorless oil (36.7 g; 75%): ^{1}H NMR δ 6.27 (t, J = 0.54 Hz, 1H), 3.9−4.1 (m, 1H), 3.7−3.8 (m, 1H), 1.9−2.2 (m, 3H), 1.4−1.8 (m, 3H). This compound is not stable and is best used immediately.

(±)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid Methyl Esters (4c and 5c). *n*-Butyllithium (20.8 mL, 52 mmol, 2.5 M in hexane) was added dropwise to a solution of diisopropylamine (4.8 g, 48 mmol) in anhydrous THF (100 mL) at 0 °C. The yellow solution was stirred at 0 °C for 1.5 h. Methyl 3,4-dichlorophenylacetate (9.6 g, 44 mmol) in THF (20 mL) was added dropwise over 30 min. After the addition, the black solution was stirred for 2 h at 0 °C and then cooled to −78 °C and stirred for a further 20 min. A solution of 2-chlorotetrahydropyran (5.3 g, 44 mmol) in THF (40 mL) was then added dropwise over 1 h. The mixture was warmed slowly to 22 °C and stirred overnight. Cold (0 °C), aqueous HCl (104 mL, 0.5 N) was added, followed by EtOAc (400 mL). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. TLC showed two major spots (20% EtOAc in hexane; R_f = 0.59, 0.50) in a ratio of 1:1.5 (^{1}H NMR). The crude product was purified by gradient column chromatography (5−15% EtOAc in hexane). Compounds **5c** eluted first (1.6 g, oil), followed by **4c** (3.4 g, solid, mp 65 °C) (total yield, 75%). **5c**: ^{1}H NMR δ 7.47 (d, 1H), 7.38 (d, 1H), 7.21 (dd, 1H), 3.93−3.80 (m, 2H), 3.67 (s, 3H), 3.55 (d, J = 11.5 Hz, 1H), 3.40−3.26 (m, 1H), 1.90−1.20 (m, 6H). ^{13}C NMR δ 171.7, 136.6, 132.4, 131.6, 130.9, 130.3, 128.5, 78.2, 68.95, 56.9, 52.3, 29.9, 25.7, 23.2. Anal. ($C_{14}H_{16}O_3Cl_2$) C, H, Cl. For **4c**: ^{1}H NMR δ 7.47 (d, 1H), 7.38 (d, 1H), 7.19 (dd, 1H), 3.99 (m, 1H), 3.91 (m, 1H), 3.71 (s, 3H), 3.50 (d, 1H), 3.47 (m, 1H) 1.9−1.0 (m, 6H). ^{13}C NMR δ 172.7, 135. 5, 132.8, 132.1, 130.6, 128.2, 79.2, 68.9, 57.4, 52.4, 29.2, 25.7, 23.1. Anal. ($C_{14}H_{16}O_3Cl_2$) C, H, Cl.

(±)-(Phenyl)(tetrahydropyran-2-yl)acetic Acid Methyl Ester (4a and 5a). The procedure described above was followed to obtain **4a** and **5a**. **4a**: ^{1}H NMR δ 7.20−7.40 (m, 5H), 3.97−4.06 (m, 1H), 3.92 (td, J = 10.4, 3.45 Hz, 1H), 3.68 (s, 3H), 3.54 (d, J = 10.1 Hz, 1H), 3.52 (td, J = 11.5, 3.0 Hz, 1H), 1.0−1.8 (m, 6H). Anal. ($C_{14}H_{18}O_3$) C, H. **5a**: ^{1}H NMR δ 7.20−7.50 (m, 5H), 3.84−3.95 (m, 2H), 3.63 (s, 3H), 3.65 (t, J = 8.3 Hz, 1H), 3.25−3.40 (m, 1H), 1.30−2.00 (m, 6H). Anal. ($C_{14}H_{18}O_3$) C, H.

(±)-(2-Naphthyl)(tetrahydropyran-2-yl)acetic Acid Methyl Esters (4b and 5b). 2-Naphthylacetic acid (24.5 g, 0.132 mol) was dissolved in methanol (180 mL), and concentrated H_2SO_4 (2 mL) was added. The mixture was warmed to 45 °C and stirred for 18 h. It was then cooled to 22 °C and neutralized with $NaHCO_3$ to pH 7. Methanol was removed by evaporation, and H_2O (150 mL) was added. It was extracted with EtOAc (300 mL × 3). The combined organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. Distillation (130 °C, 0.5 mmHg) furnished a white solid (22.0 g, 84%). ^{1}H NMR δ 7.88−7.78 (m, 3H), 7.72 (s, 1H), 7.53−7.39 (m, 3H), 3.80 (s, 2H), 3.71 (s, 3H).

LDA (0.054 mol, 1.2 equiv, 2.0 M in heptane) was introduced into a 250 mL round-bottom flask and was cooled to 0 °C in an ice/water bath. The 2-naphthylacetic acid methyl ester prepared above (9.0 g, 0.045 mol) in anhydrous THF (20 mL) was added dropwise to the LDA solution, and the mixture was stirred at 0 °C for 2 h. It was then cooled to −78 °C, and 2-chlorotetrahydropyran (6.5 g, 0.054 mol) in THF (20 mL) was added dropwise. Stirring was continued for 1 h at −78 °C. The mixture was then allowed to warm to room temperature, and stirring was continued overnight. THF was removed in vacuo, and water (400 mL) was added. The mixture was extracted with EtOAc (3 × 300 mL), and the combined organic phases were dried (Na_2SO_4), filtered, and evaporated. The residue was then dried in vacuo to provide an oil (10.0 g). The crude product was purified by column chromatography (1% EtOAc in hexanes) to provide **5b** (1.2 g) and **4b** (2.8 g) in a total yield of 32%. **4b**: ^{1}H NMR δ 7.70−7.90 (m, 4H), 7.40−7.60 (m, 3H), 3.95−4.20 (m, 2H), 3.69 (s, 3H), 3.45−3.80 (m, 2H), 1.10−1.80 (m, 6H). Anal. ($C_{18}H_{20}O_3$) C, H. **5b**: ^{1}H NMR δ 7.75−7.90 (m, 4H), 7.50−7.60 (m, 1H), 7.35−7.50 (m, 2H), 3.25−4.15 (m, 4H), 3.66 (s, 3H), 1.30−2.00 (m, 6H). Anal. ($C_{18}H_{20}O_3 \cdot 0.1H_2O$) C, H.

(±)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid (6). The racemic methyl ester **4c** (8.5 g, 28 mmol) obtained from column chromatography was dissolved in anhydrous $CHCl_3$ (10 mL) at 22 °C. TMSI (14 g, 70 mmol, 2.5 equiv) was added dropwise with stirring. The solution was heated to 80 °C and stirred at that temperature overnight. It was cooled to 22 °C, and volatiles were removed in vacuo. Aqueous Na_2SO_3 (10 mL, 1%) and Et_2O (25 mL) were added to the residue. The Et_2O phase was concentrated and purified by gradient column chromatography (30−50% EtOAc in hexane). The racemates **6** were obtained (5.2 g. 65% yield). Mp 139.1−140.1 °C. ^{1}H NMR δ 7.45 (d, 1H), 7.40 (d, 1H), 7.18 (dd, 1H), 4.05 (m, 1H), 3.83 (m, 1H), 3.51 (d, 1H), 3.49 (m, 1H), 1.9−1.1 (m, 6H). ^{13}C NMR δ 177.8, 134.8, 132.9, 132.2, 130.6, 128.2, 78.9, 68.9, 57.2, 29.1, 25.5, 22.9.

(±)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid (7). The combined racemates of **7** were obtained from **5c** as described above for **6** (36%). Mp 124.1−125.1 °C. ^{1}H NMR δ 7.48 (d, 1H), 7.38 (d, 1H), 7.20 (dd, 1H), 4.0−3.8 (m, 2H), 3.60 (d, J = 7.41 Hz, 1H), 3.39 (m, 1H), 1.9−1.2 (m, 6H). ^{13}C NMR δ 176.7, 135.5, 132.4, 131.8, 131.2, 130.2, 128.7, 77.8, 68.9, 56.6, 29.6, 25.5, 23.0.

(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid Menthyl Esters (8 and 9). The racemic mixture of (3,4-dichlorophenyl)(tetrahydropyran-2-yl)acetic acid **6** (0.86 g, 3.0 mmol) was dissolved in anhydrous CH_2Cl_2 (80 mL). Three drops of DMF were added, followed by the dropwise addition of oxalyl chloride (0.58 g, 4.8 mmol, 1.6 equiv) at 22 °C. After 3 h, volatiles were removed and anhydrous THF (75 mL) was introduced, followed by pyridine (0.5 g). l-Menthol (0.47 g, 3.0 mmol) in THF (5 mL) was added dropwise. The reaction mixture was stirred overnight and then poured into water (100 mL). Et_2O (150 mL) was added. The layers were separated, and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with brine and dried over Na_2SO_4. TLC showed two major components (R_f = 0.54 and 0.50, 10% EtOAc in hexane). After column chromatography (hexane 800 mL, 1% EtOAc in hexane 800 mL, and finally 3% EtOAc in hexane 800 mL), 400 mg of the first product **9** and 425 mg of the second product **8** were obtained (65% yield). **8**: ^{1}H NMR δ 7.46 (d, 1H), 7.38 (d, 1H), 7.20 (dd, 1H), 4.69 (td, J = 4.4, 10.9 Hz, 1H), 3.94 (dt, J = 2.2, 11.2 Hz, 1H), 3.83 (td, J = 2.2, 10.2 Hz, 1H), 3.44 (td, J = 3.0, 12.1 Hz, 1H), 3.45 (d, J = 9.9 Hz, 1H), 2.0−0.9 (m, 15H), 0.88 (d, J = 6.7 Hz, 3H), 0.78 (d, J = 6.9 Hz, 3H), 0.61 (d, J = 6.9 Hz, 3H). ^{13}C NMR δ 171.2,

135.8, 132.6, 131.7, 130.5, 130.4, 128.1, 78.9, 75.0, 68.5, 57.3, 47.2, 40.7, 34.2, 31.4, 29.0, 25.8, 25.6, 23.1, 22.0, 20.7, 15.9. **9**: ^{1}H NMR δ 7.50 (d, 1H), 7.38 (d, 1H), 7.22 (dd, 1H), 4.70 (td, J = 4.4, 11.0 Hz, 1H), 3.94 (dt, J = 2.2, 11.3 Hz, 1H), 3.79 (td, J = 2.2, 10.4 Hz, 1H), 3.45 (td, J = 2.7, 9.1 Hz, 1H), 3.46 (d, J = 9.8 Hz, 1H), 2.0−0.9 (m, 15H), 0.88 (d, J = 7.7 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.72 (d, J = 6.9 Hz, 3H). ^{13}C NMR δ 171.6, 135.7, 132.6, 131.7, 130.5, 130.4, 128.2, 79.4, 74.9, 68.5, 57.8, 47.0, 40.5, 34.2, 31.4, 28.9, 25.7, 25.6, 23.0, 22.0, 20.9, 15.9.

2-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid Indanyl Esters (10 and 11). The racemic mixture of 2-(3,4-dichlorophenyl)(tetrahydropyran-2-yl)acetic acid **7** (4.0 g, 13.8 mmol) was dissolved in anhydrous CH_2Cl_2 (80 mL), and 4 drops of DMF were added. Oxalyl chloride (3.5 g, 27.7 mmol, 2 equiv) was added dropwise while the solution was vigorously stirred. Evolution of bubbles was observed. After completion of addition, the light-yellow solution was stirred at room temperature for a further 2.5 h.

Solvent was removed by evaporation, and the residue was dried in vacuo. (*S*)-(+)-1-Indanol (1.87 g, 13.9 mmol) was dissolved in anhydrous THF (25 mL), and dry pyridine (25 mL) was added. The solution was cooled to 0 °C. The acid chloride, prepared as above, in THF (50 mL) was added dropwise. The mixture was stirred at 0 °C for 2 h, and then it was warmed to room temperature and stirred overnight. ^{1}H NMR showed that the ratio of **10** (R_f = 0.71 in 10% EtOAc, 90% hexane, developed 3 times) to **11** (R_f = 0.67) was 4:5 based on resonances at δ 3.53 and δ 3.54. The mixture was evaporated to remove most of the solvent. The residue was taken up in a mixture of hexane/ethyl acetate (10:5) (80 mL) to provide a light-yellow suspension. The mixture was loaded on a short silica gel column and chromatographed with hexane/ethyl acetate (10:1). The product fractions were combined, evaporated, and dried. A yellow oil was obtained (4.0 g, 71%). It was further purified by column chromatography (300 g of silica gel, 0.4% of ethyl acetate, 99.6% of hexane, 4 L, then 0.8% ethyl acetate in hexane, 5 L). A total of 0.6 g of **10**, 1.0 g of a mixture of **10** and **11**, and 0.5 g of **11** were obtained. **10**: ^{1}H NMR δ 7.49 (d, J = 2.2 Hz, 1H), 7.39−7.10 (m, 6H), 6.20 (m, 1H), 3.92−3.80 (m, 2H), 3.544 and 3.515 (d, J = 8.8 Hz, 1H), 3.34−3.25 (m, 1H), 3.1−3.0 (m, 1H), 2.9−2.8 (m, 1H), 2.5−2.4 (m, 1H), 2.0−1.1 (m, 6H). ^{13}C NMR δ 171.07, 144.31, 140.56, 136.56, 132.19, 131.39, 130.90, 130.08, 129.04, 128.50, 126.73, 125.34, 124.85, 79.09, 78.16, 68.80, 57.08, 31.99, 30.13, 29.81. **11**: ^{1}H NMR δ 7.47 (d, J = 2.2 Hz, 1H), 7.37 (d, J = 8.3 Hz, 1H), 7.31−7.18 (m, 5H), 6.17 (m, 1H), 3.92−3.80 (m, 2H), 3.557 and 3.528 (d, J = 8.5 Hz, 1H), 3.40−3.27 (m, 1H), 3.14−3.03 (m, 1H), 2.95−2.82 (m, 1H), 2.59−2.40 (m, 1H), 2.2−1.2 (m, 6H). ^{13}C NMR δ 171.1, 144.3, 140.4, 136.5, 132.2, 131.4, 130.9, 130.1, 129.0, 128.5, 126.7, 125.3, 124.8, 79.2, 78.2, 68.8, 57.0, 32.2, 30.1, 29.8, 25.6, 23.1.

General Procedure for the Hydrolysis of Menthyl and Indanyl Esters (8−11) to the Corresponding Acids. The following experiment is representative (yields 38−55%). Indanyl ester **10** (1.65 g, 4.07 mmol) was dissolved in anhydrous CCl_4 (25 mL). Trimethylsilyl iodide (2.4 g, 12 mmol, 3 equiv) was added. The mixture was heated to 90 °C and stirred for 18 h, then cooled to 0 °C. Cold water (20 mL) and CH_2Cl_2 (50 mL) were added, and the layers were separated. The aqueous phase was washed with CH_2Cl_2 (50 mL × 2). The combined organic phases were dried (Na_2SO_4) and filtered. The filtrate was evaporated to dryness. The residue was purified by column chromatography (10% CH_3OH in CH_2Cl_2) to obtain (2*S*,2′*R*)-**7′** (600 mg, 55%).

(2*S*,2′*S*)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)-acetic Acid ((2*S*,2′*S*)-6′). ^{1}H NMR δ 7.45 (d, 1H), 7.40 (d, 1H), 7.18 (dd, 1H), 4.06 (dt, J = 11.2, 1.9 Hz, 1H), 3.9−3.7 (m, 1H), 3.52 (d, J = 9.3 Hz, 1H), 3.50 (td, J = 11.2, 3.3 Hz, 1H), 1.9−1.1 (m, 6H). Mp 138.9−139.9 °C. $[\alpha]^{20}_D$ 18.8° (*c* 1.0, $CHCl_3$).

(2*R*,2′*R*)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)-acetic Acid ((2*R*,2′*R*)-6′). NMR data are identical to those of (2*S*,2′*S*)-**6′**. Mp 139.1−140.1 °C. $[\alpha]^{20}_D$ −19.1° (*c* 1, $CHCl_3$)

(2*S*,2′*R*)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)-acetic Acid ((2*S*,2′*R*)-7′). ^{1}H NMR δ 7.47 (d, 1H), 7.40 (d, 1H), 7.21 (dd, 1H), 3.97 (dd, J = 10.8, 2.2 Hz, 1H), 3.92−3.83 (m, 1H), 3.60 (d, J = 7.1 Hz, 1H), 3.45−3.36 (m, 1H), 1.9−1.2 (m, 6H). ^{13}C NMR δ 176.7, 135.5, 132.4, 131.8, 131.2, 130.2, 128.7, 77.8, 68.9, 56.6, 29.6, 25.5, 23.0. Mp 124.2−125.2 °C. $[\alpha]^{20}_D$ 14.0° (*c* 1.0, $CHCl_3$).

(2*R*,2′*S*)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)-acetic Acid ((2*R*,2′*S*)-7′). NMR data are identical to acid (2*S*,2′*R*)-**7′**. Mp 124.1−125.1 °C. $[\alpha]^{20}_D$ −13.9° (*c* 1.0, $CHCl_3$).

General Procedure for the Methylation of Acids To Provide Methyl Esters (12−15). The following procedure is representative. (2*S*,2′*R*)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic acid (2*S*,2′*R*)-**7′** (90 mg, 0.31 mmol) was dissolved in anhydrous toluene (4 mL) and anhydrous CH_3OH (1 mL). Trimethylsilyldiazomethane (0.63 mL, 2.0 M in hexane, 4 equiv) was slowly added at 22 °C, and the mixture was stirred for 5 h. Volatiles were removed in vacuo. The residue (100 mg) was purified by column chromatography (2% EtOAc in hexane) to provide **14** as an oil (61 mg, 64%).

(2*S*,2′*S*)-(+)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid Methyl Ester (12). ^{1}H NMR δ 7.48 (d, 1H), 7.39 (d, 1H), 7.25 (dd, 1H), 4.03−3.95 (m, 1H), 3.84 (td, J = 10.7, 2.2 Hz, 1H), 3.70 (s, 3H), 3.50 (d, J = 9.9 Hz, 1H), 3.47 (td, J = 11.3, 3.3 Hz, 1H), 1.9−1.0 (m, 6H). $[\alpha]^{20}_D$ +29.8° (*c* 1.0, $CHCl_3$). Anal. ($C_{14}H_{16}Cl_2O_3$) C, H, Cl.

(2*R*,2′*R*)-(−)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid Methyl Ester (13). ^{1}H NMR data are identical to **12**. $[\alpha]^{20}_D$ −29.1° (*c* 1.0, $CHCl_3$). Anal. ($C_{14}H_{16}Cl_2O_3$) C, H, Cl.

(2*S*,2′*R*)-(−)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid Methyl Ester (14). ^{1}H NMR δ 7.47 (d, 1H), 7.39 (d, 1H), 7.22 (dd, 1H), 3.92−3.81 (m, 1H), 3.68 (s, 3H), 3.56 (d, J = 8.5 Hz, 1H), 3.39−3.20 (m, 1H), 1.9−1.1 (m, 6H). $[\alpha]^{20}_D$ −1.8° (*c* 1.0, $CHCl_3$). Anal. ($C_{14}H_{16}Cl_2O_3$) C, H, Cl.

(2*R*,2′*S*)-(+)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid Methyl Ester (15). ^{1}H NMR identical to **14**. $[\alpha]^{20}_D$ +2.0° (*c* 1.0, $CHCl_3$). Anal. ($C_{14}H_{16}Cl_2O_3$) C, H, Cl.

(±)-2,2-(3,4-Dichlorophenyl)cycloalkylacetic Acid Methyl Esters (17 and 19). The following procedure is representative. To a stirred solution of t-BuOK (11.0 mL, 1.0 M in THF), a solution of 3,4-dichlorophenylacetonitrile (1.86 g, 10.0 mmol) in THF (20 mL) was slowly added. The mixture was stirred for 0.5 h, and cyclopentyl bromide (1.57 g, 10.5 mmol) in THF (10 mL) was added. The dark-brown solution was stirred at 22 °C for 1 h and then heated to reflux overnight. After cooling, the solution was transferred to a separatory funnel and EtOAc (200 mL) and water (150 mL) were added. The organic phase was separated and washed consecutively with H_2O and brine, dried (Na_2SO_4), concentrated, and purified by column chromatography (1−2% EtOAc in hexane) to yield 2,2-(3,4-dichlorophenyl)cyclopentylacetonitrile **18** as an oil (1.78 g, 70%). ^{1}H NMR δ 7.45 (d, 1H), 7.43 (d, 1H), 7.18 (dd, 1H), 3.69 (d, J = 7.7 Hz, 1H), 2.4−1.2 (m, 9H). Anal. ($C_{13}H_{13}Cl_2N$) C, H, N, Cl.

The nitrile **18** (1.2 g, 4.7 mmol) was dissolved in HCl/methanol solution (65 mL, 10.3 M), sealed with a stopper, and stirred at 22 °C for 2 days. A 6 N hydrochloric acid (30 mL, exothermic!) solution was slowly added to the mixture, which was stirred for 10 min and evaporated to dryness. A further 50 mL of HCl/methanol solution (10.3 M) was added, and stirring continued for 4 days. An additional 30 mL of 6 N hydrochloric acid was added, and the mixture was brought to reflux for 2 days. After the mixture was cooled, EtOAc (200 mL) and water (150 mL) were added. The organic phase was washed with water followed by brine, dried (Na_2SO_4), and concentrated. The oil obtained (R_f = 0.45, 10% EtOAc in hexane,) was purified by column chromatography (0.5−1% EtOAc in hexane) to provide **19** as a colorless oil (1.1 g, 82%). ^{1}H NMR δ 7.45 (d, 1H), 7.35 (d, 1H), 7.19 (dd, 1H), 3.66 (s, 3H), 3.23 (d, J = 11.3 Hz, 1H), 2.6−2.4, m, 1H), 2.0−1.8 (m, 1H), 1.8−0.8 (m, 7H). Anal. ($C_{14}H_{16}O_2Cl_2$) C, H, Cl.

2,2-(3,4-Dichlorophenyl)cyclhexylacetonitrile (16). Yield, 46%. ^{1}H NMR δ 7.45 (d, 1H), 7.39 (d, 1H), 7.42 (dd, 1H), 3.60 (d, J = 6.3 Hz, 1H), 1.9−1.1 (m, 11H). Anal. ($C_{14}H_{15}Cl_2N$) C, H, N, Cl.

2,2-(3,4-Dichlorophenyl)cyclohexylacetic Acid Methyl Ester (17). Yield, 40%. ^{1}H NMR δ 7.43 (d, 1H), 7.38 (d, 1H), 7.28 (dd, 1H), 3.66 (s, 3H), 3.19 (d, J = 10.7 Hz, 1H), 2.1−0.7 (m, 11H). Anal. ($C_{14}H_{18}Cl_2O_2$) C, H, Cl.

(2*R*,2′*S*)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)-acetic Acid 4-Nitrophenyl Ester [(4-Nitrophenyl Ester of (2*R*,2′*S*)-7]. Compound (2*R*,2′*S*)-**7** obtained from **15** (100 mg, 0.33 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL). One drop of DMF was added. Oxalyl chloride (0.14 g) was slowly added, and the reaction mixture was stirred at room temperature for 3 h. Solvent was then removed by evaporation.

4-Nitrophenol (56 mg, 0.40 mmol) was dissolved in anhydrous THF (3 mL), and pyridine (85 mg) was added. The mixture was cooled to 0 °C in an ice/water bath. To this cooled, stirred solution was added the acid chloride prepared above in 2 mL of THF over 10 min. The mixture was warmed to room temperature and stirred overnight. The crude product was purified by column chromatography (0.6% EtOAc in hexane). The product (0.105 g) was obtained as a gummy material (75%). R_f = 0.46 (20% ethyl acetate in hexane). ^{1}H NMR δ 8.3−8.2 (m, 2H), 7.55 (d, J = 2.2 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.32−7.18 (m, 3H), 4.07−3.91 (m, 2H), 3.83 (d, J = 7.4 Hz, 1H), 3.46−3.37 (m, 1H), 2.0−1.2 (m, 6H). Crystals were obtained from pentane. X-ray structural analysis showed the enantiomerically pure *p*-nitrophenyl ester to be (2*R*,2′*S*)-**7**.

Single-Crystal X-ray Analysis of (2*S*,2′*S*)-(3,4-Dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid (6). Monoclinic crystals of (2*S*,2′*S*)-**6**, obtained from (+)-**12**, were obtained from pentane. A representative crystal was selected, and a data set was collected at room temperature. Pertinent crystal, data collection, and refinement parameters are the following: crystal size, 0.56 mm × 0.40 mm × 0.22 mm; cell dimensions, a = 11.988(1) Å, b = 8.222(1) Å, c = 14.539(1) Å, α = 90°, β = 104.35(1)°, γ = 90°; formula, $C_{13}H_{14}Cl_2O_3$; formula weight = 289.14; volume = 1388.4(2) Å^3; calculated density = 1383 Mg/m^{-3}; space group = $P2(1)$; number of reflections = 2231, of which 2048 were considered independent (R_{int} = 0.0185). Refinement method was full-matrix least-squares on F^2. The final R indices were [$I > 2\sigma(I)$] R1 = 0.0465 and wR2 = 0.1263.

Single-Crystal X-ray Analysis of (2*R*,2′*S*)-(3,4-dichlorophenyl)(tetrahydropyran-2-yl)acetic Acid (7) 4-Nitrophenyl Ester. Orthorhombic crystals of the purified *p*-nitrophenyl ester of **7** were obtained from 90% hexane/10% EtOAc. A representative crystal was selected, and a data set was collected at room temperature. Pertinent crystal, data collection, and refinement parameters are the following: crystal size, 0.49 mm × 0.08 mm × 0.06 mm; cell dimensions, a = 6.844 (1) Å, b = 11.516(2) Å, c = 23.922(6) Å, α = 90°, β = 90°, γ = 90°; formula, $C_{19}H_{17}Cl_2NO_5$; formula weight = 410.24; volume = 1885.3(7) Å^3; calculated density = 1.445 Mg/m^{-3}; space group = $P2_12_12_1$; number of reflections = 1580, of which 1529 were considered independent (R_{int} = 0.0215). Refinement method was full-matrix least-squares on F^2. The final R indices were [$I > 2\sigma(I)$] R1 = 0.0504 and wR2 = 0.1190.

Tissue Sources and Preparation. Brain tissue from adult male and female cynomolgus monkeys (*Macaca fascicularis*) and rhesus monkeys (*Macaca mulatta*) was stored at −85 °C in the primate brain bank at the New England Regional Primate Research Center. We recently cloned the DAT and SERT from both species and found them to have virtually identical protein sequences. The caudate-putamen was dissected from coronal slices and yielded 1.4 ± 0.4 g of tissue. Membranes were prepared as described previously. Briefly, the caudate-putamen was homogenized in 10 volumes (w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4, at 4 °C) and centrifuged at 38000*g* for 20 min in the cold. The resulting pellet was suspended in 40 volumes of buffer, and the entire procedure was repeated twice. The membrane suspension (25 mg of original wet weight of tissue/mL) was diluted to 12 mL/mL for [^{3}H]WIN 35,428 or [^{3}H]citalopram assay in buffer just before assay and was dispersed with a Brinkmann Polytron homogenizer (setting no. 5) for 15 s. All experiments were conducted in triplicate, and each experiment was repeated in each of two to three preparations from individual brains.

Dopamine Transporter Assay. The dopamine transporter was labeled with [^{3}H]WIN 35,428 ([^{3}H]CFT, (1*R*)-2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-[^{3}H]methyltropane, 81−84 Ci/mmol, DuPont-NEN). The affinity of [^{3}H]WIN 35,428 for the dopamine transporter was determined in experiments by incubating tissue with a fixed concentration of [^{3}H]WIN 35,428 and a range of concentrations of unlabeled WIN 35,428. The assay tubes received, in Tris-HCl buffer (50 mM, pH 7.4 at 0−4 °C, NaCl 100 mM), the following constituents at a final assay concentration: WIN 35,428, 0.2 mL (1 pM to 100 or 300 nM), [^{3}H]WIN 35,428 (0.3 nM); membrane preparation 0.2 mL (4 mg of original wet weight of tissue/mL). The 2 h incubation (0−4 °C) was initiated by addition of membranes and terminated by rapid filtration over Whatman GF/B glass fiber filters presoaked in 0.1% bovine serum albumin (Sigma Chemical Co.). The filters were washed twice with 5 mL of Tris-HCl buffer (50 mM) and were incubated overnight at 0−4 °C in scintillation fluor (Beckman Ready-Value, 5 mL). Radioactivity was measured by liquid scintillation spectrometry (Beckman 1801). The cpm data were converted to dpm following determination of the counting efficiency (>45%) of each vial by external standardization.

Total binding was defined as [^{3}H]WIN 35,428 bound in the presence of ineffective concentrations of unlabeled WIN 35,428 (1 or 10 pM). Nonspecific binding was defined as [^{3}H]WIN 35,428 bound in the presence of an excess (30 μM) of (−)-cocaine. Specific binding was the difference between the two values. Competition experiments to determine the affinities of other drugs at [^{3}H]WIN 35,428 binding sites were conducted using procedures similar to those outlined above. Stock solutions of water-soluble drugs were dissolved in water or buffer. Stock solutions of other drugs were made as follows. Oils were drawn up in preweighed capillary tubes and dissolved in ethanol. Stock solutions of relatively insoluble drugs were made in >95% ethanol or an ethanol/HCl combination. Several of the drugs were sonicated to promote solubility. The stock solutions were diluted serially in the assay buffer and added (0.2 mL) to the assay medium as described above. IC_{50} values were computed by the EBDA computer program and are the mean values of experiments conducted in triplicate.

Serotonin Transporter Assay. The serotonin transporter was assayed in caudate-putamen membranes using conditions similar to those for the dopamine transporter. The affinity of [^{3}H]citalopram (specific activity of 82 Ci/mmol, DuPont-NEN) for the serotonin transporter was determined in experiments by incubating tissue with a fixed concentration of [^{3}H]citalopram and a range of concentrations of unlabeled citalopram. The assay tubes received, in Tris-HCl buffer (50 mM, pH 7.4, at 0−4 °C, NaCl 100 mM), the following constituents at a final assay concentration: citalopram, 0.2 mL (1 pM to 100 or 300 nM), [^{3}H]citalopram (1 nM); membrane preparation 0.2 mL (4 mg of original wet weight of tissue/mL). The 2 h incubation (0−4 °C) was initiated by addition of membranes and terminated by rapid filtration over Whatman GF/B glass fiber filters presoaked in 0.1% polyethyleneimine. The filters were washed twice with 5 mL of Tris-HCl buffer (50 mM) and incubated overnight at 0−4 °C in scintillation fluor (Beckman Ready-Value, 5 mL). Radioactivity was measured by liquid scintillation spectrometry (Beckman 1801). The cpm data were converted to dpm following determination of the counting efficiency (>45%) of each vial by external standardization. Total binding was defined as [^{3}H]citalopram bound in the presence of ineffective concentrations of unlabeled citalopram (1 or 10 pM). Nonspecific binding was defined as [^{3}H]citalopram bound in the presence of an excess (10 μM) of fluoxetine. Specific binding was the difference between the two values. Competition experiments to determine the affinities of other drugs at [^{3}H]citalopram binding sites were conducted using procedures similar to those outlined above. IC_{50} values were

computed by the EBDA computer program and are the mean values of experiments conducted in triplicate.

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Supporting Information Available: ORTEP drawings of (2*S*,2′*S*)-(3,4-dichlorophenyl)(tetrahydropyran-2-yl)acetic acid (**6**) and (2*R*,2′*S*)-(3,4-dichlorophenyl)(tetrahydropyran-2-yl)-acetic acid (**7**) 4-nitrophenyl ester, crystal data and refinement parameters, coordinates, anisotropic temperature factors, distances, and angles This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Panizzon, L. The preparation of pyridine- and piperidine-arylacetonitriles and the alkylation products of transformation. *Helv. Chim. Acta* **1944**, *27*, 1748−1757.
(2) Deutsch, H. M.; Shi, Q.; Gruszecka-Kowalik, E.; Schweri, M. M. Synthesis and pharmacology of potential cocaine antagonists. 2. Structure−Activity relationship studies of aromatic ring-substituted methylphenidate analogs. *J. Med. Chem.* **1996**, *39*, 1201−1209.
(3) Schweri, M. M.; Skolnick, P.; Rafferty, M. F.; Rice, K. C.; Janowsky, A. J.; Paul, S. M. [^{3}H]Threo-(±)-methylphenidate binding to 3,4-dihydroxyphenylethylamine uptake sites in corpus striatum: correlation with the stimulant properties of ritalinic acid esters. *J. Neurochem.* **1985**, *45*, 1062−1070.
(4) Pan, D.; Gatley, S. J.; Dewey, S. L.; Chen, R.; Alexoff, D. A.; Ding, Y.-S.; Fowler, J. S. Binding of bromine-substituted analogs of methylphenidate to monoamine transporters. *Eur. J. Pharmacol.* **1994**, *264*, 177−182.
(5) Volkow, N. D.; Wang, G. J.; Fowler, J. S.; Fischman, M.; Foltin, R.; Abumrad, N. N.; Gatley, S. J.; Logan, J.; Wong, C.; Gifford, A.; Ding, Y. S.; Hitzemann, R.; Pappas, N. Methylphenidate and cocaine have a similar in vivo potency to block dopamine transporters in the human brain. *Life Sci.* **1999**, *65*, 7−12.
(6) Wilens, T. E.; Biederman, J. The Stimulants. *Pediatr. Psychopharmacol.* **1992**, *15*, 191−222.
(7) Patrick, K. S.; Caldwell, R. W.; Ferris, R. M.; Breese, G. R. Pharmacology of the enantiomers of *threo*-methylphenidate. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 152−158.
(8) Ding, Y. S.; Fowler, J. S.; Volkow, N. D.; Dewey, S. L.; Wang, G. J.; Logan, J.; Gatlet, S. J.; Pappas, N. Chiral drugs: comparison of the pharmacokinetics of [^{11}C]D-*threo* and L-*threo*-methylphenidate in the human and baboon brain. *Psychopharmacology* (*Berlin*) **1997**, *131*, 71−78.
(9) Matsumura, Y.; Kanda, Y.; Shirai, K.; Onomura, O.; Maki, T. A convenient method for synthesis of optically active methylphenidate from *N*-methoxycarbonylpiperidine by utilizing electrochemical oxidation and Evans aldol-type reaction. *Tetrahedron* **2000**, *56*, 7411−7422.
(10) Axten, J. M.; Ivy, R.; Krim, L.; Winkler, J. D. Enantioselective synthesis of D-*threo*-methylphenidate. *J. Am. Chem. Soc.* **1999**, *121*, 6511−6512.
(11) Axten, J. M.; Krim, L.; Kung, H. F.; Winkler, J. D. A stereoselective synthesis of DL-*threo*-methylphenidate: preparation and biological evaluation of novel analogues. *J. Org. Chem.* **1998**, *63*, 9628−9629.
(12) Prashad, M.; Kim, H.-Y.; Lu, Y.; Liu, Y.; Har, D.; Repic, O.; Blacklock, T. J.; Giannousis, P. The first enantioselective synthesis of (2*R*,2′*R*)-*threo*-(+)-methylphenidate hydrochloride. *J. Org. Chem.* **1999**, *64*, 1750−1753.
(13) Janowsky, A.; Schweri, M. M.; Berger, P.; Long, R.; Skolnick, P.; Paul, S. M. [^{3}H]threo-(±)-Methylphenidate binding: correlation with [^{3}H]dopamine uptake. *Eur. J. Pharmacol.* **1985**, *108*, 187−191.
(14) Gatley, S. J.; Pan, D.; Chen, R.; Chaturverdi, G.; Ding, Y. S. Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sci.* **1996**, *58*, 231−239.
(15) Meltzer, P. C.; Liang, A. Y.; Blundell, P.; Gonzalez, M. D.; Chen, Z.; George, C.; Madras, B. K. 2-Carbomethoxy-3-aryl-8-oxabicyclo-[3.2.1]octanes: Potent non-nitrogen inhibitors of monoamine transporters. *J. Med. Chem.* **1997**, *40*, 2661−2673.
(16) Madras, B. K.; Pristupa, Z. B.; Niznik, H. B.; Liang, A. Y.; Blundell, P.; Gonzalez, M. D.; Meltzer, P. C. Nitrogen-based drugs are not essential for blockade of monoamine transporters. *Synapse* **1996**, *24*, 340−348.
(17) Meltzer, P. C.; Blundell, P.; Yong, Y. F.; Chen, Z.; George, C.; Gonzalez, M. D.; Madras, B. K. 2-Carbomethoxy-3-aryl-8-bicyclo-[3.2.1]octanes: Potent non-nitrogen inhibitors of monoamine transporters. *J. Med. Chem.* **2000**, *43*, 2982−2991.
(18) Meltzer, P. C.; Blundell, P.; Madras, B. K. Structure activity relationships of inhibition of the dopamine transporter by 3-arylbicyclo[3.2.1]octanes. *Med. Chem. Res.* **1998**, *8*, 12−34.
(19) Weber, W. P. *Silicon Reagents for Organic Synthesis*; Springer-Verlag: Berlin, 1983; pp 30−32.
(20) Madras, B. K.; Fahey, M. A.; Bergman, J.; Canfield, D. R.; Spealman, R. D. Effects of cocaine and related drugs in nonhuman primates: I. [^{3}H]Cocaine binding sites in caudate-putamen. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 131−141.
(21) Davies, H. M. L.; Hansen, T.; Churchill, M. R. Catalytic asymmetric C−H activation of alkanes and tetrahydrofuran. *J. Am. Chem. Soc.* **2000**, *122*, 3063−3070.
(22) Ficini, J. *Bull. Soc. Chim. Fr.* **1956**, 119−124.

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