region from 20 to 30 male Sprague–Dawley rats (200–250 g) was rapidly removed over ice. Tissue was homogenized in 4 volumes of 0.32 M sucrose in a Brinkman Polytron (setting 6 for 20 s). The homogenate was centrifuged (36500*g* for 10 min), and the pellet was rehomogenized in the same volume of buffer. Aliquots of 4.5 mL were removed and stored at −70 °C for up to 3 weeks.

On the day of the experiment, a tissue aliquot was thawed on ice and diluted 1 to 20 with 50 mM Tris·HCl (pH 7.7). The homogenate was centrifuged two more times at 36500*g* for 10 min. Between the second and third centrifugations, the tissue homogenate was incubated at 37.5 °C for 10 min to allow degradation of residual neurotransmitter by MAO. The final pellet was resuspended in 50 mM Tris·HCl with 0.1% ascorbic acid, 5.7 mM $CaCl_2$, and 10 μM pargyline (pH 7.7) and preincubated for 10 min at 37.5 °C.

Assays were performed in triplicate with the buffer described above to which 200–400 μg of protein was added. Saturation experiments were performed with varying concentrations of [$^3$H]serotonin to label 5-$HT_{1B}$ binding sites and [$^3$H]ketanserin to label 5-$HT_2$ binding sites. Nonspecific binding was defined with 10 μM serotonin and 10 μM cinanserin for 5-$HT_{1B}$ and 5-$HT_2$ sites, respectively. The amount of protein added to each tube was determined by the method of Bradford.[27] The ability of the test compounds to displace 1 nM [$^3$H]-5-HT and 0.75 nM [$^3$H]-ketanserin was examined. Tubes were incubated at 37.5 °C for 15 min and then filtered with a Brandel cell harvestor, modified for receptor binding studies, through Whatman GF/C filters, followed by two 3-s washes with ice-cold buffer. The filters were counted by liquid scintillation spectrometry at 45% efficiency.

Scatchard and competition binding data for each experiment were analyzed by a nonlinear least-squares curve-fitting procedure, as embodied in the EBDA and LIGAND software, adapted for the IBM PC by McPherson.[28] The $K_d$, $B_{max}$, and $K_i$ values reported represent the average of two or three separate experiments, with 8–10 concentrations of displacing ligand per experiment. Analysis of variance and contrast comparisons were employed to analyze the results.

**Acknowledgment.** Support for this work was provided by PHS Grant DA-02189 from the National Institute on Drug Abuse. A special thanks to Don Richmond and Dena Wehr for technical assistance.

**Registry No.** (*R*)-**1a**, 7795-52-0; (*S*)-**1a**, 7795-51-9; (*R*)-**1b**, 113997-82-3; (*S*)-**1b**, 113997-83-4; (*R*)-**1c**, 86138-18-3; (*S*)-**1c**, 86138-19-4; (*R*)-**1d**, 114127-44-5; (*S*)-**1d**, 114127-45-6; (*R*)-**1e**, 113997-84-5; (*S*)-**1e**, 113997-85-6; (*R*)-**1f**, 95114-42-4; (*S*)-**1f**, 95114-43-5; **2a**, 120-72-9; **2b**, 4237-90-5; **2c**, 1006-94-6; **2d**, 3189-13-7; **2e**, 20289-26-3; **2f**, 1215-59-4; (±)-**3a**, 113997-48-1; (±)-**3b**, 113997-49-2; (±)-**3c**, 113997-50-5; (±)-**3d**, 113997-51-6; (±)-**3e**, 113997-52-7; (±)-**3f**, 113997-53-8; **4a**, 1201-26-9; **4b**, 113997-54-9; **4c**, 4761-29-9; **4d**, 65109-60-6; **4e**, 113997-55-0; **4f**, 113997-56-1; **5**, 113997-57-2; (*R,R*)-**7a**, 113997-58-3; (*S,S*)-**7a**, 113997-59-4; (*R,S*)-**7a**, 113997-60-7; (*S,R*)-**7a**, 113997-61-8; (*R,R*)-**7b**, 113997-62-9; (*S,S*)-**7b**, 113997-63-0; (*R,S*)-**7b**, 113997-64-1; (*S,R*)-**7b**, 113997-65-2; (*R,R*)-**7c**, 113997-66-3; (*S,S*)-**7c**, 113997-67-4; (*R,S*)-**7c**, 113997-68-5; (*S,R*)-**7c**, 113997-69-6; (*R,R*)-**7d**, 113997-70-9; (*S,S*)-**7d**, 113997-71-0; (*R,S*)-**7d**, 113997-72-1; (*S,R*)-**7d**, 113997-73-2; (*R,R*)-**7e**, 113997-74-3; (*S,S*)-**7e**, 113997-75-4; (*R,S*)-**7e**, 113997-76-5; (*S,R*)-**7e**, 113997-77-6; (*R,R*)-**7f**, 113997-78-7; (*S,S*)-**7f**, 113997-79-8; (*R,S*)-**7f**, 113997-80-1; (*S,R*)-**7f**, 113997-81-2; (±)-$CH_3CH(NO_2)$-$CH_2OH$, 62742-29-4; $CH_3C(NO_2)$=$CH_2$, 4749-28-4; (*R*)-PhCH-$(CH_3)NH_2$, 3886-69-9; (*S*)-PhCH$(CH_3)NH_2$, 2627-86-3; phthalic anhydride, 85-44-9.

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# Absolute Configurations and Pharmacological Activities of the Optical Isomers of Fluoxetine, a Selective Serotonin-Uptake Inhibitor

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Fluoxetine is a potent and selective inhibitor of the neuronal serotonin-uptake carrier and is a clinically effective antidepressant. Although fluoxetine is used therapeutically as the racemate, there appears to be a small but demonstrable stereospecificity associated with its interactions with the serotonin-uptake carrier. The goals of this study were to determine the absolute configurations of the enantiomers of fluoxetine and to examine whether the actions of fluoxetine in behavioral tests were enantiospecific. (*S*)-Fluoxetine was synthesized from (*S*)-(−)-3-chloro-1-phenylpropanol by sequential reaction with sodium iodide, methylamine, sodium hydride, and 4-fluorobenzotrifluoride. (*S*)-Fluoxetine is dextrorotatory (+1.60) in methanol, but is levorotatory (−10.85) in water. Fluoxetine enantiomers were derivatized with (*R*)-1-(1-naphthyl)ethyl isocyanate, and the resulting ureas were assayed by $^1$H NMR or HPLC to determine optical purities of the fluoxetine samples. Both enantiomers antagonized writhing in mice; following sc administration of (*R*)- and (*S*)-fluoxetine, $ED_{50}$ values were 15.3 and 25.7 mg/kg, respectively. Moreover, both enantiomers potentiated a subthreshold analgesic dose (0.25 mg/kg) of morphine, and $ED_{50}$ values were 3.6 and 5.7 mg/kg, respectively. Following ip administration to mice, the two stereoisomers antagonized *p*-chloroamphetamine-induced depletion of whole brain serotonin concentrations. $ED_{50}$ values for (*S*)- and (*R*)-fluoxetine were 1.2 and 2.1 mg/kg, respectively. The two enantiomers decreased palatability-induced ingestion following ip administration to rats; (*R*)- and (*S*)-fluoxetine reduced saccharin-induced drinking with $ED_{50}$ values of 6.1 and 4.9 mg/kg, respectively. Thus, in all biochemical and pharmacological studies to date, the eudismic ratio for the fluoxetine enantiomers is near unity.

Drugs that modulate the physiological and pathophysiological actions of serotonin are useful or potentially useful in the treatment of a variety of human diseases, including depression, anxiety, alcoholism, chronic pain, emesis, and eating disorders such as obesity and bulimia.[1] One can manipulate actions of serotonin by using drugs that interfere with its biosynthesis, stimulate its release from presynaptic storage vesicles, occupy one or more of the serotonin receptor subtypes, antagonize enzymes responsible for catabolism of serotonin, or inhibit presynaptic reuptake of serotonin. All of these avenues have been explored in attempts to develop novel therapeutic agents and pharmacological tools.[2]

Fluoxetine (LY110140; *N*-methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine) was one of the first

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compounds that allowed selective enhancement of serotonin neurotransmission.[3,4] This compound produces its pharmacological effects via inhibition of the presynaptic serotonin-uptake carrier of neurons.[5,6] The selectivity of fluoxetine is manifest from several perspectives: The compound has little affinity for the norepinephrine- or dopamine-uptake carriers; both in vitro and in vivo its selectivity for the serotonin-uptake carrier appears to be approximately 100-fold. For example, fluoxetine's $IC_{50}$ values for inhibition of the serotonin-, norepinephrine-, and dopamine-uptake carriers in rat brain synaptosomes were 0.07, 7, and 6 $\mu$M, respectively.[5,6] Another facet of the selectivity of fluoxetine is its lack of affinity for various neuronal receptors such as muscarinic, $\alpha$-adrenergic, histamine $H_1$, and serotonin receptors.[7] This contributes, at least in part, to the advantageous side-effect profile of fluoxetine compared to the tricyclic antidepressants.[8] Fluoxetine and other selective serotonin-uptake inhibitors such as zimelidine, femoxetine, and sertraline have been important in advancing our understanding of the central role of serotonin in mediation of a variety of physiological signals and in the etiology of several disease states.[9]

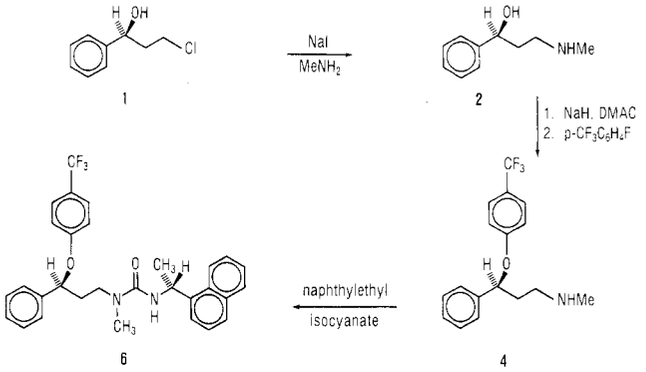
Fluoxetine is used therapeutically as the racemate, and most published pharmacology studies were conducted with the racemate. The two enantiomers have been obtained in optically pure form, and some of the pharmacological features of these two compounds have been studied. For example, Wong and co-workers showed that the (+) isomer was slightly more potent than the (−) isomer as a serotonin-uptake inhibitor in rat cortical synaptosomes.[10] Fuller and Snoddy demonstrated that the dextrorotatory isomer of fluoxetine was slightly more potent than the levorotatory isomer as an antagonist of *p*-chloroamphetamine-induced depletion of whole brain serotonin concentrations in rats.[11] Thus, there appears to be a surprisingly small but demonstrable stereospecificity associated with the interactions of fluoxetine with the serotonin-uptake carrier.

The goals of the present study were to determine the absolute configurations of the enantiomers of fluoxetine to assist our serotonin-uptake inhibitor drug design studies. Moreover, we wanted to determine if there is enantiospecificity with regard to the ability of fluoxetine to inhibit ingestive behavior in rats, potentiate morphine-induced analgesia in mice, or antagonize *p*-chloroamphetamine depletion of whole brain serotonin concentrations in mice.

## Results and Discussion

**Preparation of (*S*)-Fluoxetine from Optically Active Starting Material.** Recent advances in asymmetric reduction chemistry have led to the ready availability of chiral alcohols with enantiomeric excesses approaching 100%.[12-16] Particularly noteworthy is the progress in borane-mediated asymmetric induction,[12-14] and the recent report by Srebnik, Ramachandran, and Brown that prochiral alkyl aromatic ketones bearing a chlorine at the $\omega$-carbon are reduced with diisopinocampheylchloroborane to furnish the corresponding secondary alcohols, including 3-chloro-1-phenylpropanol, in high optical and chemical purities.[17] Our goal was to synthesize one of the fluoxetine enantiomers from one of the enantiomers of 3-chloro-1-phenylpropanol, the absolute configurations of which are known,[18] and thereby determine the absolute configurations of the fluoxetine enantiomers.

**Scheme I**




The synthesis began with (*S*)-(−)-3-chloro-1-phenylpropanol (**1**, Scheme I). We initially attempted to react this material with methylamine to provide amino alcohol **2** directly. However, this reaction proved to be slow and did not proceed to sole formation of **2**. Therefore, a Finkelstein reaction was performed on **1**, which yielded the corresponding iodo derivative **3**. This reaction was essentially quantitative as judged by disappearance of the starting material's methine resonance at 4.96 ppm in the $^1$H NMR spectrum and appearance of the product resonance at 4.83 ppm. Surprisingly, the sign of the rotation changed, and the magnitude of the rotation plummeted when the chlorine was replaced with an iodine. For example, whereas the $[\alpha]^{19}_D$ of starting material **1** was −24.7°, the $[\alpha]^{23}_D$ of iodo intermediate **3** was +3.14° (*c* 1%, $CHCl_3$). Reaction of this iodide with aqueous methylamine resulted in efficient formation of (*S*)-3-(methylamino)-1-phenylpropanol (**2**). This material was then reacted with sodium hydride in dimethylacetamide to generate the alkoxide. Addition of 4-fluorobenzotrifluoride led to a facile nucleophilic aromatic substitution. Recrystallization of the hydrochloride salt of the resulting material completed the preparation of (*S*)-fluoxetine hydrochloride (**4**). The $[\alpha]^{23}_D$ of this material was +1.60 (*c* 1%, methanol); interestingly, the sign of the rotation changed and the magnitude increased when water was used as a solvent ($[\alpha]^{23}_D$ −10.85°).

**Classical Resolution of Fluoxetine.** In order to determine the relationship of (*S*)-fluoxetine prepared from (*S*)-3-chloro-1-phenyl-1-propanol to the enantiomers of

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**Table I.** HPLC and NMR Analysis of (*R*)-1-(1-Naphthyl)ethyl Ureas Derived from Fluoxetine and Its Enantiomers

| amine component | NMR resonances,[a] ppm | | HPLC retention times[b] | ratio[c] |
|---|---|---|---|---|
| | $CHCH_3$ | $NCH_3$ | | |
| (*R,S*)-fluoxetine | 1.50, 1.64 | 2.84, 2.89 | 6.69, 7.52 | 48:52 |
| (*S*)-fluoxetine[d] | 1.64 | 2.84 | 6.74, 7.81 | 96:4 |
| (*S*)-fluoxetine[e] | 1.64 | 2.84 | 6.81, 7.82 | 99:1 |
| (*R*)-fluoxetine[e] | 1.50 | 2.89 | 6.86, 7.40 | 1.5:98.5 |

[a] Data were recorded with a GE 300-MHz instrument. The samples were dissolved in chloroform. The $CHCH_3$ represents the methyl derived from naphthylethyl isocyanate. The $NCH_3$ represents the methyl derived from fluoxetine. [b] Data were obtained using an IBM silica column eluted with 0.25% methanol in methylene chloride. For additional details, consult the Experimental Section. [c] Data were obtained by electronic integration of the HPLC peaks. [d] Material derived by synthesis from the chiral alcohol. [e] Material derived from fractional recrystallization of the mandelic acid salt.

fluoxetine derived from classical resolution, the resolution of fluoxetine was performed by fractional recrystallization of the D- and L-mandelic acid salts. After recrystallization to constant optical rotations and melting points, these salts were converted to the corresponding hydrochloride salts. From the physical constants of these enantiomers (see the Experimental Section) it is apparent that the *S* enantiomer of fluoxetine is obtained by recrystallization of the L-(+)-mandelic acid salt. (*R*)-Fluoxetine hydrochloride (**5**) is levorotatory in methanol, but in water it is dextrorotatory.

**Determination of Optical Purities by $^1$H NMR and HPLC.** Although the physical constants of the (*R*)- and (*S*)-fluoxetine hydrochloride samples obtained by fractional recrystallization suggested that the (*S*)-fluoxetine derived from (*S*)-3-chloro-1-phenyl-1-propanol was enantiomerically homogeneous, more definitive data were required. Consequently, we developed HPLC and $^1$H NMR methods to estimate optical purities of enantiomerically enriched fluoxetine samples. Both methods were based on reaction of fluoxetine with the *R* enantiomer of 1-(1-naphthyl)ethyl isocyanate (Scheme I), followed by analysis of the resulting diastereomeric ureas.

Reaction of (*R,S*)-fluoxetine with (*R*)-1-(1-naphthyl)ethyl isocyanate resulted in formation of *N*-methyl-*N'*-(*R*)-[1-(1-naphthyl)ethyl]-*N*-(*R,S*)-3-[4-(trifluoromethyl)phenoxy]-3-phenylpropylurea as an oil. Analytical HPLC analysis (normal phase; 0.25% methanol in methylene chloride; isocratic elution) provided separation of the two diastereomeric ureas; retention times were approximately 6.69 and 7.52 min. Reaction of (*S*)-fluoxetine (**4**) with the chiral isocyanate provided urea **6** (Scheme I). HPLC analysis revealed two peaks with retention times of 6.74 and 7.81 min and a ratio of 96:4, respectively (Table I). Analysis of (*R*)-fluoxetine gave peaks at 6.86 and 7.40 min with a ratio of 1.5:98.5. The enantiomeric purity of (*S*)-fluoxetine obtained by classical resolution was 99%. These data revealed that the urea that elutes more quickly was the *R,S* diastereomer, whereas the more slowly eluting diastereomer was the *R,R* urea. Moreover, the HPLC data indicated that the enantiomers of fluoxetine derived by resolution were essentially homogeneous in a stereochemical sense and that little, if any, racemization occurred during synthesis of (*S*)-fluoxetine from alcohol **1**.

$^1$H NMR analysis revealed significant chemical shift differences between portions of the NMR spectra of the two diastereomeric ureas (Table I). The $CH_3$ doublets from the naphthylethyl isocyanate portion resonate at 1.50 and 1.64 ppm in the two diastereomeric ureas. The $NCH_3$ singlets from the fluoxetine moiety resonate at 2.84 and 2.89 ppm. Examination of the $^1$H NMR spectra of the ureas derived from the homochiral fluoxetine samples indicated that the (*R*)-fluoxetine-derived methyl singlet resonates at 2.89 ppm, whereas the methyl doublet of this urea resonates at 1.50 ppm. Importantly, no contaminating diastereomer could be detected in any of these diastereomeric ureas.

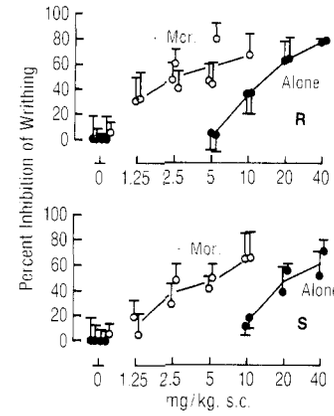


**Figure 1.** Effects of (*R*)-fluoxetine (top) and (*S*)-fluoxetine (bottom) on acetic acid induced writhing in mice either alone (filled circles) or when administered simultaneously with 0.25 mg/kg of morphine (unfilled circles). Each point is the mean ± SE of five mice. Each data point (except the morphine alone) was determined at least twice, and the lines connect the mean effect of the different determinations. The number of writhes under control conditions averaged 36 for the 10-min observation period.

**Pharmacology.** Previous studies have shown that the eudismic ratio[19] of the fluoxetine enantiomers is near unity. Biochemical investigations using rat cortical synaptosomes indicated that $K_i$ values of (+)- and (−)-fluoxetine as inhibitors of the serotonin-uptake carrier were 21 and 33 nM, respectively.[10] In vivo neurochemical evidence in rats also suggested rather modest differences in the potencies of the fluoxetine enantiomers.[11] We have now explored the activities of the fluoxetine enantiomers as serotonin-uptake inhibitors in two behavioral tests. One test examined the antinociceptive activities of the fluoxetine enantiomers in mice, while the other determined their abilities to decrease ingestive behavior in rats.

Extensive data suggests that serotonin may be involved in endogenous pain control and in opiate-induced antinociception.[20,21] Serotonin-uptake inhibitors potentiate morphine-induced antinociception in mice,[22-24] and several antidepressants appear to be effective in management of chronic pain in humans.[25] Consequently, we studied the abilities of the fluoxetine enantiomers either alone or in combination with morphine to antagonize acetic acid induced abdominal constriction (writhing) in mice. Both enantiomers, when injected alone, blocked writhing in a dose-dependent fashion, and $ED_{50}$ values (95% confidence intervals) of 15.3 (12.1–19.7) and 25.7 (19.9–37.7) mg/kg were obtained for the *R* and *S* stereoisomers, respectively (Figure 1). When administered in combination with 0.25 mg/kg of morphine, a subthreshold analgesic dose of this

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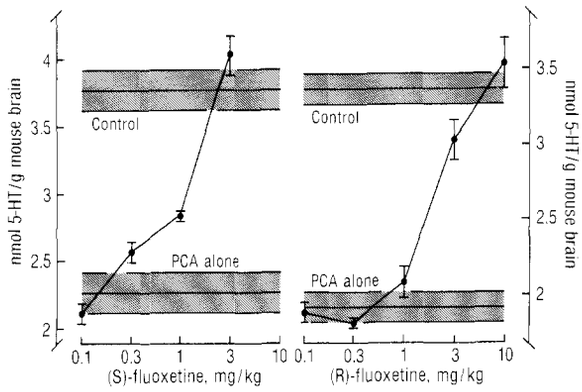


**Figure 2.** Antagonism of *p*-chloroamphetamine-induced depletion of mouse brain serotonin concentrations by (*R*)- or (*S*)-fluoxetine. *p*-Chloroamphetamine hydrochloride was injected 6 h before the mice were sacrificed and 1 h after the fluoxetine enantiomers were injected at the doses indicated. The upper heavy lines and shaded areas represent mean values ± SE, respectively, for control mice. The lower heavy lines and shaded areas represent mean values ± SE, respectively, for mice treated with *p*-chloroamphetamine alone. The data points indicate mean ± SE for mice treated with (*R*)- or (*S*)-fluoxetine in addition to *p*-chloroamphetamine.

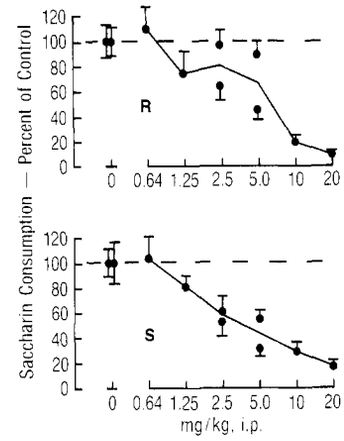


**Figure 3.** Effects of (*R*)-fluoxetine (top) and (*S*)-fluoxetine (bottom) on consumption of 0.01 M sodium saccharin solution. Each point is the mean ± SE of four to six rats. Control animals ingested approximately 22 mL of the saccharin solution.

opioid, the dose–response relationship was shifted markedly to the left (Figure 1). In these morphine potentiation studies, the $ED_{50}$ values for (*R*)- and (*S*)-fluoxetine were 3.6 (2.3–5.8) and 5.7 (3.7–13.0) mg/kg, respectively.

To aid in interpretation of data obtained from these mouse antinociception studies, the ability of the two fluoxetine enantiomers to inhibit *p*-chloroamphetamine-induced depletion of mouse brain serotonin concentrations was investigated as a measure of serotonin-uptake inhibition. *p*-Chloroamphetamine depletes serotonin from serotonergic neurons in a carrier-dependent fashion, and antagonism of this effect represents in vivo neurochemical evidence for inhibition of serotonin uptake. Various doses of the fluoxetine enantiomers were injected ip, and 1 h later 20.6 mg/kg of *p*-chloroamphetamine was administered ip. Six hours later concentrations of serotonin in whole brain were determined and results are shown in Figure 2. In both experiments, *p*-chloroamphetamine produced a marked decline in brain serotonin concentrations. This effect was antagonized dose dependently by each enantiomer of fluoxetine, and in this study (*S*)-fluoxetine was slightly more potent than (*R*)-fluoxetine; $ED_{50}$ values were 1.2 and 2.1 mg/kg, respectively. These data demonstrate that the eudismic ratio for the fluoxetine enantiomers is near unity in terms of their ability to antagonize *p*-chloroamphetamine-induced depletion of mouse brain serotonin and are consistent with similar studies previously performed in rats.[11] Moreover, the data indicate that both enantiomers potentiated morphine-induced antinociception in approximately the same dose ranges that they function as inhibitors of serotonin uptake in mice.

Considerable evidence indicates that serotonin is a physiological modulator of ingestive behaviors.[26] Direct serotonin agonists such as *m*-chlorophenylpiperazine and indirect serotonin agonists such as fenfluramine decrease food consumption in a variety of paradigms.[27,28] Fluoxetine and several other selective serotonin uptake inhibitors inhibit food intake in laboratory models,[29,30] and this effect appears to be manifest in humans.[31–34] We have previously demonstrated that racemic fluoxetine depresses saccharin-induced drinking (palatability-induced ingestion) in rats while having no effect on control water consumption.[35] It was therefore of interest to study effects of (*R*)- and (*S*)-fluoxetine in this model. Both enantiomers antagonized palatability-induced ingestion dose dependently, and $ED_{50}$ values were 4.9 (2.9–10.5) and 6.1 (3.3–20.5) mg/kg for (*S*)- and (*R*)-fluoxetine, respectively (Figure 3). Onset of activity began at 1.25 mg/kg, and a dose of 20 mg/kg ip essentially abolished saccharin-solution consumption during the 2-h observation period. Importantly, this is the dose range in which fluoxetine functions as a selective serotonin-uptake inhibitor in rats.[5,6]

## Conclusions

We have synthesized (*S*)-fluoxetine from (*S*)-(−)-3-chloro-1-phenylpropanol and have shown that it corresponds to the enantiomer that is most potent biochemically as a serotonin-uptake inhibitor.[10,11] Previous biochemical pharmacology experiments with enantiomers of fluoxetine suggested that the eudismic ratio is near unity in rats, and we have determined that this low eudismic ratio is also manifest in behavioral tests such as suppression of palatability-induced ingestion in rats and in the mouse abdominal constriction test. Thus, regardless of the species or pharmacological test, there is little enantiospecificity regarding interactions of fluoxetine with the serotonin-uptake carrier. This low eudismic ratio is surprising since addition of the 3-phenyl group (which is responsible for fluoxetine's chirality) to *N*-methyl-3-[4-(trifluoromethyl)phenoxy]propanamine leads to a 62-fold increase in potency as a serotonin-uptake inhibitor.[36] Although the 3-phenyl group of fluoxetine is mandatory for potent inhibition of serotonin uptake, from X-ray crystallographic

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and structure–activity relationship data we have hypothesized previously that the phenyl group serves primarily as a conformational anchor to enforce a folded three-dimensional orientation between the propanamine and (trifluoromethyl)phenoxy moieties of fluoxetine.[36] This conformational effect of the 3-phenyl group would be stereoindependent and is consistent with all the in vitro and in vivo data that indicate that the eudismic ratio of the fluoxetine enantiomers is near unity.[37]

## Experimental Section

**Methods.** Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected. Proton magnetic resonance ($^1$H NMR) spectra were taken on GE QE300 or JOEL FX90Q spectrometers. Chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard ($\delta$ scale). The $^1$H NMR data are presented in the form: (solvent in which spectra were taken), $\delta$ value of signal (peak multiplicity, integrated number of protons and assignment). Mass spectra were recorded from a Varian MAT CH-5 spectrometer, at the ionization voltage expressed in parentheses. Only the peaks of high relative intensity or of diagnostic importance are presented in the form: $m/e$ (intensity relative to base peak). Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories; only symbols of elements analyzed are given and they were within 0.4% of theoretical values unless indicated otherwise.

Except where noted, a standard procedure was used for product isolation. This involved quenching by addition to water, filtration or exhaustive extraction with a solvent (washing of extract with aqueous solutions, on occasion), drying over an anhydrous salt, and evaporation of solvent under reduced pressure. Particular solvents, aqueous washes (if needed), and drying agents are mentioned in parentheses after the phrase "product isolation".

(*S*)-(−)-3-Chloro-1-phenyl-1-propanol was obtained from Aldrich. The melting point was 60–62 °C, and the optical rotation was $[\alpha]^{19}_D$ −24.7° (*c* 1%, $CHCl_3$).

**Preparation of (*S*)-Fluoxetine from (*S*)-(−)-3-Chloro-1-phenyl-1-propanol: (*S*)-(+)-3-Iodo-1-phenyl-1-propanol (3).** A solution of (*S*)-(−)-3-chloro-1-phenyl-1-propanol (1.01 g, 5.9 mmol) in 200 mL of acetone that had been saturated with sodium iodide was refluxed overnight. The acetone was removed under reduced pressure. Product isolation (water, ether, water, brine, $Na_2SO_4$) provided 1.6 g (100%) of nearly homogeneous product as a light red oil that was used without further purification. An analytical sample was prepared by recrystallization from hexane/ether to provide white crystals with mp 54–55 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.22 (br m, 2, $CH_2$ $\beta$ to I), 3.20 and 3.31 (each m, each 1, $CH_2$I), 4.83 (m, 1, CH), 7.27–7.41 (m, 5, Ar H); mass spectrum (70 eV), $m/e$ (relative intensity) 262 (3, $M^+$), 107 (100); $[\alpha]^{23}_D$ +3.14° (*c* 1%, $CHCl_3$). Anal. ($C_9H_{11}IO$) C, H.

**(*S*)-(−)-3-(Methylamino)-1-phenyl-1-propanol (2).** A solution of (*S*)-(+)-3-iodo-1-phenyl-1-propanol (1.5 g, 5.7 mmol) and methylamine (4.4 mL of a 40% solution in water, 57.2 mmol) in 10 mL of THF was stirred at room temperature overnight. Solvent was removed under reduced pressure and then brine and 2 mL of 2 N sodium hydroxide was added. Product isolation (ether, brine, $Na_2SO_4$) provided 800 mg (85%) of homogeneous (*S*)-(−)-3-(methylamino)-1-phenyl-1-propanol as an oil: $^1$H NMR ($CDCl_3$) $\delta$ 1.82 (br m, 2, $CH_2$ $\beta$ to N), 2.45 (s, 3, $NCH_3$), 2.89 (m, 2, $CH_2$N), 4.94 (dd, 1, CH), 7.21–7.42 (m, 5, Ar H); mass spectrum (70 eV), $m/e$ (relative intensity) 165 (11, $M^+$), 104 (56), 77 (84), 59 (98), 45 (100); $[\alpha]^{23}_D$ −37.37° (*c* 1%, $CHCl_3$). An analytical sample was prepared by formation of the oxalate salt of **2** and recrystallization from ethyl acetate/ethanol: mp 154–156 °C; $[\alpha]^{23}_D$ −35.53° (*c* 1%, MeOH). Anal. ($C_{12}H_{17}NO_5$) C, H, N.

**(*S*)-Fluoxetine (4).** A mixture of (*S*)-(−)-3-(methylamino)-1-phenyl-1-propanol (800 mg, 4.86 mmol) and sodium hydride (208 mg of a 60% dispersion in oil, 5.2 mmol; oil removed with hexane) in 10 mL of dimethylacetamide was heated to 70 °C for 30 min. *p*-Fluorobenzotrifluoride (660 $\mu$L, 5.2 mmol) was added and the reaction was heated at 93 °C for 2.25 h. After the mixture cooled to room temperature, product isolation (water, ether, water, brine, $Na_2SO_4$) and flash chromatography [silica gel, methanol/methylene chloride/ammonium hydroxide (10/100/1)] provided 970 mg of homogeneous (*S*)-fluoxetine (free base) as an oil. HPLC analysis (vide infra) revealed that this material had an enantiomeric excess of 92%: $^1$H NMR ($CDCl_3$) $\delta$ 2.16 and 2.29 (each br m, each 1, $CH_2$ $\beta$ to N), 2.50 (s, 3, $NCH_3$), 2.88 (br t, 2, $CH_2$N), 5.36 (dd, 1, CH), 6.90 (d, 2, Ar H ortho to O), 7.23–7.38 (m, 5, $C_6H_5$), 7.43 (d, 2, Ar H ortho to $CF_3$); mass spectrum (70 eV), $m/e$ (relative intensity) 309 (8, $M^+$), 104 (53), 45 (100).

Generation of the hydrochloride salt and recrystallization from ether/hexane provided (*S*)-fluoxetine hydrochloride: mp 135–137 °C; $[\alpha]^{23}_D$ +1.60° (*c* 1%, MeOH); $[\alpha]^{23}_D$ −10.85° (*c* 1%, $H_2O$). Anal. ($C_{17}H_{19}ClF_3NO$) C, H, N.

**Classical Resolution of Fluoxetine.** A mixture of fluoxetine free base (34.6 g, 112 mmol) and D-(−)-mandelic acid (17.04 g, 112 mmol) was dissolved in 400 mL of hot benzene. After 3 days the precipitate was collected by filtration to provide 16.83 g (65%) of white crystals with mp 150–152 °C; $[\alpha]^{23}_D$ −32.36° (*c* 1%, MeOH). A second crystallization from 150 mL of benzene provided 14.26 g (55%) of material with mp 154–155 °C; $[\alpha]^{23}_D$ −33.88° (*c* 1%, MeOH); $^1$H NMR and mass spectra were consistent with the desired product and the material had an enantiomeric excess of 97% as judged by HPLC analysis (vide infra). Anal. ($C_{25}H_{26}F_3NO_4$) C, H, N. The hydrochloride salt was generated and gave a melting point of 140.0–141.5 °C; $[\alpha]^{23}_D$ −1.97° (*c* 1%, MeOH), $[\alpha]^{23}_D$ +10.32 (*c* 1%, $H_2O$). $^1$H NMR and mass spectra were identical with those reported for (*S*)-fluoxetine hydrochloride derived from (*S*)-(−)-3-chloro-1-phenyl-1-propanol. Anal. ($C_{17}H_{19}ClF_3NO$) C, H, N.

The filtrate of the first crystallization (vide supra) was converted to 22.8 g (73.7 mmol) of free base. A mixture of this substance and L-(+)-mandelic acid (11.2 g, 73.6 mmol) was dissolved in 330 mL of hot benzene. After the mixture was allowed to stand overnight, 19.63 g of product was obtained with a melting point of 150–153 °C. A second crystallization from 300 mL of benzene provided 16.17 g (63%) of product with a melting point of 154–155 °C; $[\alpha]^{23}_D$ +32.76° (*c* 1%, MeOH); $^1$H NMR and mass spectra were consistent with the desired product, and the material had an enantiomeric excess of 98% as judged by HPLC analysis (vide infra). Anal. ($C_{25}H_{26}F_3NO_4$) C, H, N. The hydrochloride salt was generated and gave a melting point of 140–141 °C; $[\alpha]^{23}_D$ +1.39° (*c* 1%, MeOH), $[\alpha]^{23}_D$ −9.23° (*c* 1%, $H_2O$). $^1$H NMR and mass spectra were identical with those reported for (*S*)-fluoxetine hydrochloride derived from (*S*)-(−)-3-chloro-1-phenyl-1-propanol. Anal. ($C_{17}H_{19}ClF_3NO$) C, H, N.

**Derivatization of Fluoxetine: Determination of Optical Purity by $^1$H NMR and HPLC.** (*R*,*S*)-Fluoxetine free base (1.23 g, 3.98 mmol) and (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (788 mg, 3.99 mmol) were refluxed in 25 mL of toluene for 2 h. TLC analysis revealed no starting material and a single product spot. Solvent was removed in vacuo to provide *N*-methyl-*N'*-(*R*)-[1-(1-naphthyl)ethyl]-*N*-(*R*,*S*)-3-[4-(trifluoromethyl)phenoxy]-3-phenylpropylurea as an oil; FD mass spectrum, $m/e$ (relative intensity) 505 (100, $M^+$). HPLC analysis was performed on a Waters 6000A chromatograph using a 4.6 mm × 25 cm IBM silica column eluted with 0.25% methanol in methylene chloride at 1000 psi and at a flow rate of 2 mL/min; the detector was a Waters 440 operated at 254 nm; integration was performed electronically. Under these conditions two peaks were observed with retention times of 6.69 and 7.52 min. The peaks represented 48% and 52% of the material, respectively. The $^1$H NMR spectrum clearly showed an equal mixture of diastereomers (figure in the supplementary material): $^1$H NMR ($CDCl_3$) $\delta$ 1.50 and 1.64 (each d, each 1.5, $CHCH_3$), 2.02–2.21 (br m, 2, $CH_2$ $\beta$ to ether O), 2.84 and 2.89 (each s, each 1.5, $NCH_3$), 3.44 (m, 2, $CH_2$N), 4.67 (br t, 1, NH), 5.15 (m, 1, OCH), 5.77 (m, 1, CHN), 6.74 (dd, 2, Ar H ortho to O), 7.12–8.22 (m, 14, Ar H).

**Determination of Optical Purity of (*R*)-Fluoxetine Prepared by Resolution with D-(−)-Mandelic Acid.** The urea was prepared as described above for the racemate; mass spectrum (70 eV), $m/e$ (relative intensity) 506 (2, $M^+$), 345 (61), 155 (100).

(37) Professor H. C. Brown and Professor Barry Sharpless and co-workers have recently completed asymmetric syntheses of fluoxetine enantiomers. For Brown's work, see ref 17. For Sharpless' work, see: Gao, Y.; Sharpless, K. B., manuscript in preparation.

HPLC analysis as described above revealed a major peak with a retention time of 7.40 min. A smaller peak eluted at 6.86 min, which represented the (*S*)-fluoxetine urea. The ratio of the two peaks was 98.5:1.5, respectively.

In the $^1$H NMR spectrum, the naphthylethyl $CH_3$ doublet resonated at 1.50 ppm, and the $NCH_3$ singlet resonated at 2.89 ppm. No peaks from the other diastereomer could be detected.

**Determination of Optical Purity of (*S*)-Fluoxetine Prepared by Resolution with L-(+)-Mandelic Acid.** The urea was prepared as described above for the racemate; mass spectrum (70 eV), *m/e* (relative intensity) 506 (3, $M^+$), 345 (41%), 155 (100). HPLC analysis as described above revealed a major peak with a retention time of 6.81 min; a smaller peak eluted at 7.82 min, which represented the (*R*)-fluoxetine urea. The ratio of the two peaks was 99:1, respectively.

In the $^1$H NMR spectrum, the naphthylethyl $CH_3$ doublet resonated at 1.64 ppm, and the $NCH_3$ singlet resonated at 2.84 ppm. No peaks from the other diastereomer could be detected.

**Determination of Optical Purity of (*S*)-Fluoxetine Synthesized from (*S*)-(−)-3-Chloro-1-phenyl-1-propanol.** The urea was prepared as described above for the racemate; mass spectrum (70 eV), *m/e* (relative intensity) 506 (2, $M^+$), 345 (33), 155 (100). HPLC analysis as described above revealed two peaks with retention times of 6.74 and 7.81 min and a ratio of 96:4, respectively.

In the $^1$H NMR spectrum, the naphthylethyl $CH_3$ doublet resonated at 1.64 ppm, and the $NCH_3$ singlet resonated at 2.84 ppm. No peaks from the other diastereomer could be detected.

**Pharmacological Methods. Antinociception Test in Mice.** The mouse abdominal constriction response ("writhe") was used as the antinociception test and was defined as contraction of the abdominal musculature followed by the extension of the hind limbs. This response was induced by intraperitoneal administration (10 mL/kg) of 0.6% acetic acid. Five CF-1 albino, male mice (Charles River Breeding Laboratories, Portage, MI), weighing approximately 20 g after being fasted overnight, were observed simultaneously for the writhing response. The observation period was 10 min in duration, beginning 5 min after injection of acetic acid. The percent inhibition of writhing was calculated from the average number of writhes in the control group for the day. Each data point is the mean ± standard error for five mice. The $ED_{50}$ was defined as the dose that inhibited writhing by 50% and was calculated by computer analysis of log dose vs percent inhibition on a probit scale using the method of least squares. Each mouse was used only once.

In determining the dose–effect data, (*R*)- or (*S*)-fluoxetine hydrochloride was administered subcutaneously in a volume of 10 mL/kg, and doses were expressed as mg/kg. The vehicle was distilled water. The injection of test drug was 20 min before injection of acetic acid. For the test of morphine potentiation, a small dose (0.25 mg/kg, sc) of morphine, which does not produce a significant inhibition of writhing, was administered simultaneously with various doses of (*R*)- or (*S*)-fluoxetine.

**Antagonism of *p*-Chloroamphetamine-Induced Serotonin Depletion.** (*R*)- or (*S*)-fluoxetine was injected ip as an aqueous solution into groups of five male albino mice (16–20 g). One hour later, *p*-chloroamphetamine hydrochloride (20.6 mg/kg) was injected ip. Six hours later, mice were decapitated, and whole brains were excised, frozen on dry ice, and stored at −15 °C until assayed. Serotonin concentrations in brain were measured by HPLC with electrochemical detection.

**Palatability-Induced Ingestion.** A group of 24 Long-Evans hooded male rats, weighing 400–600 g, were allowed access for 2 h each weekday to bottles containing 0.01 M sodium saccharin. Rats were individually housed in standard wire mesh cages and had free access to tap water and rodent lab chow at all times except the 0.5 h before and the 2 h of saccharin solution availability. The access period began around 10:30 a.m. Lights were on in the colony room from 6:00 a.m. to 6:00 p.m.

Injections of (*R*)- or (*S*)-fluoxetine hydrochloride were administered by the intraperitoneal route in a volume of 1 mL/kg body weight 0.5 h before saccharin solution availability. Drug injections were administered usually on Tuesdays and Fridays. Data obtained on Thursdays were used as noninjection control data against which to evaluate drug effects. Effects after drug or vehicle injection for each animal were calculated as a ratio of that animal's nearest noninjection control data, and then data were normalized such that the effect in the group of animals receiving distilled water was considered as 100% of control.

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**Registry No.** **1**, 100306-34-1; **2**, 114133-37-8; **2** oxalate, 114133-38-9; **3**, 114133-36-7; (*S*)-**4**, 100568-02-3; (*S*)-**4**·HCl, 114247-06-2; **5**, 114247-09-5; **6** (isomer 1), 114133-39-0; **6** (isomer 2), 114133-40-3; methylamine, 74-89-5; *p*-fluorobenzotrifluoride, 402-44-8; (±)-fluoxetine, 54910-89-3; (*R*)-fluoxetine D-(−)-mandelic acid salt, 114247-07-3; (*S*)-fluoretine L-(+)-mandelic acid salt, 114247-08-4; (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate, 42340-98-7; (*R*)-fluoxetine, 100568-03-4.

**Supplementary Material Available:** Partial $^1$H NMR spectra of diastereomeric ureas obtained by reaction of fluoxetine or its enantiomers with (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (1 page). Ordering information is given on any current masthead page.