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SYNTHESIS OF ^{14}C- AND ^{3}H-LABELED FLUOXETINE, A SELECTIVE SEROTONIN UPTAKE INHIBITOR

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SUMMARY

Fluoxetine (N-methyl-γ-(4-(trifluoromethyl)phenoxy)benzenepropanamine) is a potent, highly selective serotonin uptake inhibitor that is useful in treating a variety of major psychiatric derangements. We have synthesized this compound in ^{14}C- and ^{3}H-labeled forms. The tritium label was introduced in the final step by catalytic dehalogenation of the brominated fluoxetine precursor 6. Reaction conditions could be controlled such that catalytic hydrogenolysis of the labile C-O benzylic bond was minimized. Following HPLC purification, [^{3}H]-fluoxetine was obtained in a state of high radiochemical purity (98%) and specific activity (20.4 Ci/mmol). The ^{14}C-label was introduced in the final step *via* a nucleophilic aromatic substitution reaction between the sodium salt of α-(2-(methylamino)ethyl)benzenemethanol and uniformly ring-labeled p-chlorobenzotrifluoride. Following purification by flash chromatography, [^{14}C]-fluoxetine was obtained in 98.3% radiochemical purity with a specific activity of 5.52 mCi/mmol.

Key Words: fluoxetine, carbon-14, tritium, nucleophilic aromatic substitution, antidepressant.

INTRODUCTION

The hypothesis that inadequate serotonergic neurotransmission is etiologically involved in development of depression and other affective disorders has been studied for two decades.[1,2] However, conclusive evidence for this hypothesis required selective serotonergic agents. One mechanism whereby serotonin neurotransmission may be enhanced is through inhibition of the presynaptic serotonin uptake carrier; this leads to an increase in the concentration and/or half-life of serotonin in the synaptic cleft and thereby enhances post-synaptic receptor occupancy.[3] Although high concentrations of tricyclic antidepressants inhibit serotonin uptake, their predominant pharmacological action *in vivo* is inhibition of the uptake of the catecholamine neurotransmitter norepinephrine.[4]

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Fluoxetine was the earliest selective inhibitor of serotonin uptake, and has been invaluable as a pharmacological tool in elucidating the central role of serotonin in a variety of physiological systems and pathophysiological states.[5,6] Perhaps more importantly, fluoxetine is useful in the clinical management of depression[7] and obesity,[8] and accumulating evidence suggests that fluoxetine may be effective in the treatment of alcoholism, pain, anxiety, and obsessive-compulsive behavior.[3]

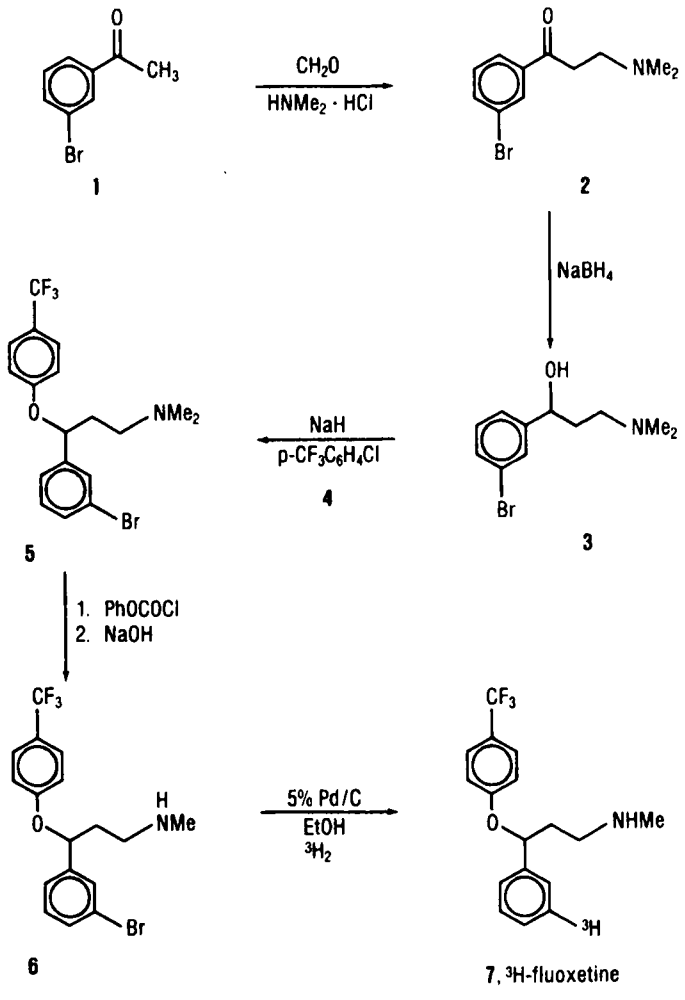
Research into the mechanism of action of fluoxetine at the molecular level, and studies on the human disposition and metabolism of fluoxetine required ^{3}H- and ^{14}C-labeled fluoxetine. In this report we detail the synthesis of these isotopomers of this important pharmacological tool and drug.

RESULTS AND DISCUSSION

We chose to tritiate the monosubstituted phenyl ring of fluoxetine in the meta position. Previous animal experiments indicated that the ultimate metabolic product of this ring of fluoxetine is hippuric acid; surprisingly, there is no evidence for phenyl ring hydroxylation of fluoxetine or any of the metabolites leading to hippuric acid.[9] Thus, a tritium placed on this ring, particularly in the meta position, should be metabolically stable. Synthesis of the brominated precursor 6 was straight forward and is depicted in Scheme 1. Reaction of 3-bromoacetophenone 1 with paraformaldehyde and dimethylamine in the presence of hydrochloric acid provided Mannich base 2. Reduction of this ketone with sodium borohydride furnished analytically pure alcohol 3 in essentially quantitative yield. The alkoxide of 3 was generated using sodium hydride in dimethylacetamide, and then the trifluoromethylphenyl moiety was introduced with p-chlorobenzotrifluoride (4). This nucleophilic aromatic substitution is facile and high yielding; a 68% yield of purified product (5) was obtained after heating the reaction 4 h at 130°C. Demethylation was effected *via* a two-step sequence: reaction of 5 with phenylchloroformate in refluxing toluene to form the intermediate carbamate, followed by sodium hydroxide hydrolysis to afford 6, the brominated precursor of [^{3}H]-fluoxetine. The yield for this two-step sequence was 67%.

Catalytic debromination of 6 was performed under carefully controlled conditions since hydrogenolysis of the secondary and benzylic carbon-oxygen bond also occurs readily. Preliminary experiments were conducted with deuterium at atmospheric pressure, and when deuterium uptake was carefully monitored, complete debromination occurred before significant quantities of C-O hydrogenolysis occurred. Ethanol and 5% palladium on carbon proved to be a suitable solvent and catalyst, respectively. In a preparative deuterium experiment, [^{2}H]-fluoxetine was produced in 65% yield after formation of the oxalate salt and recrystallization from ethyl acetate/methanol. Analytical

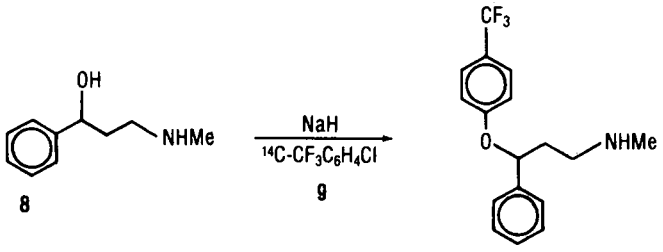
Scheme 1: Synthesis of ^{3}H-Labeled Fluoxetine



HPLC analysis of the crude product and the recrystallized material indicated purities of 83.3% and 99.1%, respectively.

Catalytic debromination of 6 in the presence of tritium, using experimental conditions previously described for production of [^{2}H]-fluoxetine, led to the formation of tritium-labeled fluoxetine, 7. Following purification by reverse-phase HPLC, the radiochemical purity of the material was 98% as assessed by TLC using three different systems. The TLC characteristics and UV spectrum of [^{3}H]-fluoxetine were identical with those of unlabeled material, and mass spectral analysis of fluoxetine and [^{3}H]-fluoxetine revealed parent ions at 310 and 312 mass units, respectively. The specific activity of 7 was found to be 20.4 Ci/mmol.

Scheme 2: Synthesis of ^{14}C-Labeled Fluoxetine



8 9

10, ^{14}C-fluoxetine

While the high specific activity, tritium-labeled form of fluoxetine is useful for in vitro and in vivo animal experiments, we desired [^{14}C]-fluoxetine for drug disposition and metabolism experiments in human subjects. This material was prepared as shown in Scheme 2. To permit introduction of the ^{14}C-label in the final synthetic step, the secondary amine α-(2-(methylamino) ethyl)benzenemethanol (8)[10] was used as the nucleophilic aromatic substitution substrate. Reaction of 8 with sodium hydride, followed by addition of [^{14}C]-p-chlorobenzotrifluoride (9) that was uniformly ring-labeled (5.90 mCi/mmol), yielded [^{14}C]-fluoxetine, 10. The product was purified by flash chromatography and recrystallization of the hydrochloride salt, and the specific activity of this isotopomer was 5.52 mCi/mmol. Radiochemical purity as determined by reverse-phase HPLC was 98.3%, while TLC indicated a radiochemical purity of 99.6%.

CONCLUSIONS

In this report we have detailed the efficient preparation of ^{3}H- and ^{14}C-labeled fluoxetine, a potent and selective inhibitor of serotonin uptake that is useful for clinical management of depression and other affective disorders. [^{3}H]-Fluoxetine was obtained in a state of high specific activity and radiochemical purity, and has been used as a ligand to selectively label the serotonin uptake carrier in rat brain synaptosomal preparations. The ^{14}C-labeled fluoxetine is currently being used to study the human disposition and metabolism of the drug. Synthetic methods were devised which enabled introduction of either the ^{14}C- or ^{3}H-label in the final synthetic step, and both isotopomers were readily purified.

EXPERIMENTAL

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 recorded employing a GE QE-300 spectrometer. Chemical shifts are

reported in ppm downfield from a tetramethylsilane internal standard (δ scale). ^{1}H-NMR data are presented in the form: (solvent in which spectra were taken), δ value of signal (peak multiplicity, integrated number of protons, coupling constant (if any), 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). High resolution exact mass determinations were recorded from a VG Analytical VG-ZAB3F spectrometer. Microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories.

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."

Radiochemical purity was measured by autoradiography employing E. Merck Silica Gel 60 F-254 TLC plates and Kodak X-ray film BB-5, and by HPLC performed on a Waters 6000A chromatograph using a 4.5 mm x 25 cm Alltech Spherisorb S-5-005 ODS column eluted with 0.05 M ammonium acetate/methanol (15/85, v/v) at 3700 psi and at a flow rate of 1 ml/min; the detector was a Waters 440 operated at 227 nm. For the HPLC determinations, equal fractions from the column were collected in vials containing PCS scintillation fluid (Amersham), and radioactivity was measured in a Packard Model 3375 Liquid Scintillation Spectrometer.

1-(3-Bromophenyl)-3-dimethylamino-1-propanone hydrochloride, 2

Paraformaldehyde (15.8 g, 175 mmol), dimethylamine hydrochloride (37.1 g, 455 mmol), and 1 mL of concentrated hydrochloric acid were added to a solution of 3-bromoacetophenone in ethanol, and the mixture was heated at reflux for 3.25 h. The reaction was slowly cooled to 0°C, and the precipitated product was filtered to afford 13.4 g (13%) of 2 as white crystals: mp 194-196°C (lit.[11] mp 202.5-203.5°C); ^{1}H-NMR (DMSO-d_6) δ 2.80 (s, 6H, NMe_2), 3.40 and 3.62 (each t, each 2H, $-CH_2CH_2-$), 7.54 (t, 1H, ArH meta to carbonyl), 7.90 and 8.01 (each d, each 1H, ArH), 8.18 (s, 1H, ArH ortho to bromo and carbonyl); mass spectrum (70ev) m/e (rel intensity) 257 (1, M^+), 255 (1, M^+), 58 (100).

Anal. Calcd for $C_{11}H_{15}NBrClO$: C, 45.15; H, 5.17; N, 4.79. Found: C, 45.20; H, 5.30; N, 4.77.

α-(2-(Dimethylamino)ethyl)-3-bromobenzenemethanol, 3

A saturated aqueous solution of potassium carbonate was added to a 0°C solution of 1-(3-bromophenyl)-3-dimethylamino-1-propanone hydrochloride (12.81 g, 43.8 mmol) in 250 mL of methanol and 100 mL of water until the pH was 10.

Sodium borohydride (1.66 g, 43.8 mmol) was added in portions, whereupon the reaction was warmed to room temperature and stirred overnight. Solvents were removed under reduced pressure and product isolation (water, ether, water, brine, Na_2SO_4) afforded 11.2 g (99%) of analytically pure 3 as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 1.80 (m, 2H, CH_2 β to N), 2.32 (s, 6H, NMe_2), 2.48 and 2.66 (m, each 1H, CH_2 α to N), 4.91 (dd, 1H, CH), 7.14-7.56 (m, 4H, ArH); mass spectrum (70 eV) m/e (rel intensity) 257 (10, M^+).

Anal. Calcd for $C_{11}H_{16}NBrO$: C, 51.18; H, 6.25; N, 5.43. Found: C, 51.20; H, 6.42; N, 5.38.

3-Bromo-N,N-dimethyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine ethanedioate, 5

A solution of α-(2-(dimethylamino)ethyl)-3-bromobenzenemethanol (22.2 g, 86.0 mmol) in 200 mL of N,N-dimethylacetamide was added dropwise to a suspension of sodium hydride (3.6 g of a 60% dispersion in oil, 90.3 mmol) in 100 mL of N,N-dimethylacetamide. The reaction was slowly heated to 70°C to complete formation of the alkoxide, and then 4-chlorobenzotrifluoride (12.6 mL, 94.7 mmol) was added in one portion. The reaction was heated to 130°C for 4 h and then poured into an ice/water mixture. Product isolation (ethyl acetate, water, brine, Na_2SO_4) and preparative HPLC [silica gel; 0-5% gradient of ammonium hydroxide/methanol (1/99) in methylene chloride] afforded 23.5 g (68%) of 5 as an oil. A small portion was converted to the oxalate salt and recrystallized from methanol/ethyl acetate to afford white crystals: mp 144-145.5°C; ^{1}H-NMR (DMSO-d_6) δ 2.26 (m, 2H, CH_2 β to N), 2.73 (s, 6H, NMe_2), 3.13 (m, 2H, CH_2 α to N), 5.60 (dd, 1H, CH), 7.09 (d, 2H, ArH ortho to O), 7.33-7.69 (m, 6H, ArH); mass spectrum (70 eV) m/e (rel intensity) 403 (20, M^+), 401 (21, M^+), 58 (100).

Anal. Calcd for $C_{20}H_{21}NBrF_3O_5$: C, 48.80; H, 4.30; N, 2.85. Found: C, 48.78; H, 4.21; N, 2.91.

3-Bromo-N-methyl-γ-(4-(trifluoromethyl)phenoxy)benzenepropanamine ethanedioate, 6

Phenylchloroformate (7.6 mL, 60.5 mmol) was added dropwise to a refluxing solution of 5 (22.1 g, 55 mmol) in 400 mL toluene. The reaction was refluxed 3 h and cooled. Product isolation (1N sodium hydroxide, water, 1N hydrochloric acid, brine, Na_2SO_4) afforded 29.92 g of the intermediate carbamate which was used in the following reaction without purification.

Sodium hydroxide (117.8 mL of a 5N solution, 589 mmol) was added to a solution of the carbamate (29.92 g) in 400 mL of propylene glycol and 50 mL of ethanol. The reaction was heated to 110°C for 3 h, cooled to room temperature, and diluted with water. Product isolation (ethyl acetate, water, brine, Na_2SO_4) and preparative HPLC [silica gel; 0-7.5% gradient of ammonium

hydroxide/methanol (0.5/99.5) in methylene chloride] afforded 14.2 g (67% over two steps) of homogeneous, analytically pure material as a colorless oil. Formation of the oxalate salt and recrystallization from ethyl acetate/methanol provided 6 as white crystals with mp 168-169.5°C; data for free base: ^{1}H-NMR ($CDCl_3$) δ 1.98 and 2.18 (m, each 1H, CH_2 β to N), 2.45 (s, 3H, NMe), 2.73 (m, 2H, CH_2 α to N), 5.29 (dd, 1H, CH), 6.89 (d, 2H, ArH ortho to O), 7.18-7.56 (m, 6H, ArH); mass spectrum (70 eV) m/e (rel intensity) 389 (26, M^+), 387 (29, M^+).

Anal. Calcd for $C_{17}H_{17}NBrF_3O$: C, 52.59; H, 4.41; N, 3.61. Found: C, 52.37; H, 4.34; N, 3.70.

3-[^{2}H]-N-methyl-γ-(4-(trifluoromethyl)phenoxy)benzenepropanamine ethanedioate

A suspension of 5% Pd/C in 10 mL of ethanol was exposed to deuterium at atmospheric pressure for 1.5 h. A solution of 6 (480 mg, 1.0 mmol) in 25 mL ethanol was added, and the reaction was stirred under deuterium at atmospheric pressure until the theoretical quantity had been consumed. Catalyst was removed by filtration through celite and solvent was removed under reduced pressure to afford product as an oil. Analytical HPLC analysis was performed employing a 4 x 300 mm Waters microbonded pack C-18 column eluted with 1% ammonium acetate/acetonitrile (3/7, v/v) at 2800 psi and at a flow rate of 2 ml/min, and revealed a purity of 83.3% for [^{2}H]-fluoxetine. Formation of the oxalate salt and recrystallization from ethyl acetate/methanol provided 260 mg (65%) of product as a white powder. Analytical HPLC revealed a purity of 99.1% for [^{2}H]-fluoxetine: mp 179-180.5°C; ^{1}H-NMR (DMSO-d_6) 2.10-2.36 (m, 2H, CH_2 β to N), 2.59 (s, 3H, NMe), 3.03 (m, 2H, CH_2 α to N), 5.62 (dd, 1H, CH), 7.07 (d, 2H, ArH ortho to O), 7.28-7.46 (m, 4H, ArH), 7.59 (d, 2H, ArH ortho to CF_3); mass spectrum (70 eV) m/e (rel intensity) 309 (6, M^+).

Anal. Calcd for $C_{19}H_{19}DNF_3O_5$: C, 57.29; H, 4.81; N, 3.52. Found: C, 57.44; H, 5.04; N, 3.77.

3-[^{3}H]-N-methyl-γ-(4-(trifluoromethyl)phenoxy)benzenepropanamine, 7

[^{3}H]-Fluoxetine was prepared in Amersham Laboratories by catalytic debromination of 6 in the presence of tritium gas, using experimental conditions previously described for the production of [^{2}H]-fluoxetine (vide supra). After purification by reverse-phase HPLC, the radiochemical purity was 98% as assessed by the following TLC systems: 1) silica gel TLC using chloroform/methanol/triethylamine (60/20/1); 2) silica gel TLC using methanol/water/acetic acid (90/10/0.25); 3) reverse-phase TLC on Whatman KC_{18} using methanol/water/acetic acid (90/10/0.25). The specific activity was 20.4 Ci/mmol and mass spectral analysis revealed a mass ion of 312.

N-Methyl-γ-(4-(trifluoromethyl)phenoxy-[ring-UL-^{14}C])benzenepropanamine hydrochloride, 10

N,N-Dimethylacetamide (1 mL) was added to a mixture of α-(2-(methylamino)

ethyl)benzenemethanol[10] (140 mg, 0.847 mmol) and sodium hydride (37 mg of a 60% dispersion in oil, 0.925 mmol), and the mixture was heated at 70°C for 15 min to ensure formation of the alkoxide. A solution of 9 [p-chlorobenzotrifluoride-[ring-UL-^{14}C], (Pathfinder Laboratories, 5 mCi, 0.847 mmol, 5.90 mCi/mmol)] in 1 mL N,N-dimethylacetamide was added dropwise, and the reaction was heated at 90-95°C for 2 h. After cooling to room temperature, product isolation (brine, ethyl acetate, Na_2SO_4) and flash chromatography [1.5 x 13.2 cm bed of 230-400 mesh silica gel eluted with chloroform/methanol/concentrated ammonium hydroxide (400/20/1)] afforded homogeneous product as an oil. Formation of the hydrochloride salt and recrystallization from ethyl acetate provided 57 mg of 10 with mp 154-155°C and a specific activity of 5.52 mCi/mmol. Radiochemical purity as assessed by reverse-phase HPLC (see Methods Section for details) was 98.3%, while TLC [silica gel, chloroform/methanol/concentrated ammonium hydroxide (100/10/1)] indicated a radiochemical purity of 99.6%.

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