Rapid Synthesis of [^{18}F]SR46349B, A Potent and Selective 5-HT$_2$ Receptor Antagonist

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Summary

F-18 labeled SR46349B, a highly potent and selective 5-HT$_2$ receptor antagonist, was synthesized as a potential radioligand for PET studies of brain 5-HT$_2$ receptors. Nucleophilic aromatic substitution of *trans*-1-(2-nitrophenyl)-3-(4-methoxymethoxyphenyl)-2-propenone (**10**) with NCA [^{18}F]fluoride in the presence of potassium carbonate and kryptofix-222, followed by HCl hydrolysis, gave F-18 labeled **12**, which was purified by a novel combination of C-18 Sep-Pak and silica column chromatography. Subsequent condensation of [^{18}F]ketone **12** with $Me_2NCH_2CH_2ONH_2$ gave a mixture of [^{18}F]SR46349B and its geometric isomer, which was separated by high performance liquid chromatography. The three step hot synthesis of [^{18}F]SR46349B required 170 min. and gave a specific activity of 1140 Ci/mmol, 5% radiochemical yield (EOB) and 96% radiochemical purity.

Key words: serotonin, receptor, PET, fluorine-18, SR46349B, isotope labeling, 5-HT$_2$.

Introduction

The brain serotonin system is involved in a number of physiological [1] and pathological [2] processes. It is also an important molecular target in the design and development of antidepressant drugs [3]. For these reasons the development of positron emitter labeled radiotracers to probe specific elements of the serotonin system has been the subject of a number of studies. For example, several ^{11}C- or ^{18}F-labeled radioligands have been synthesized for *in vivo* imaging of brain 5-HT$_2$ receptors with PET. These include [^{11}C]ketanserin [4], [^{18}F]spiperone [5], [^{11}C]methyl spiperone [6], [^{18}F]setoperone [7], [^{11}C]methyl bromo-LSD [8], [^{18}F]ritanserin [9] and [^{18}F]altanserin [10].

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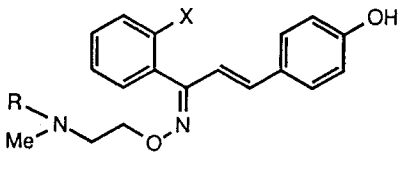
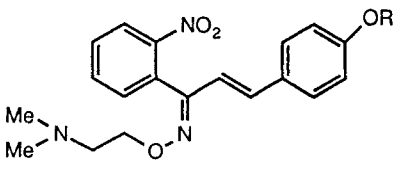
SR46349B is a highly potent and selective 5-HT_2 receptor antagonist with Kd =1.2 nM which contains fluorine and is being evaluated as an antidepressant [11]. In order to evaluate the use of SR46349B as a selective radioligand for the 5-HT_2 receptor and also to examine its pharmacokinetics *in vivo*, we recently synthesized [^{11}C]methyl SR46349B (4) by *N*-methylation of the des-methyl precusor 3 with [^{11}C]methyl iodide (**Figure 1**)[12]. We report here the synthesis of F-18 labeled SR46349B which, because of the longer half-life of fluorine-18, would allow an examination of its pharmacokinetics over a longer time period.

Results and Discussion

The ideal approach to the synthesis of [^{18}F]SR46349B (2) would be the one-step nucleophilic aromatic displacement of the corresponding nitro-precusor 5 with [^{18}F]fluoride (**Figure 1**)[13]. This direct approach has proved successful in labeling of large molecules like altanserin with [^{18}F]fluoride[10]. However, 5 has several structural features that are unfavorable to nucleophilic aromatic substitution. First, the oxime ether functionality of 5 has weaker electron withdrawing power as an activating group than acyl groups and there is no reported example available that utilizes oxime ether as an activating group in the nucleophilic aromatic substitution reaction[14]. Also, the ortho activating group in some cases does not work as well as the corresponding para-substituted compound[15]. Lastly, a free phenol hydroxyl group was known to hinder the nucleophilic aromatic substitution reaction and usually protected before substitution [16]. Our results were in accordance with these predictions. The reaction of the nitro- 5 with [^{18}F]fluoride in the presence of kryptofix-222 and potassium carbonate at 120 °C did not provide

Figure 1





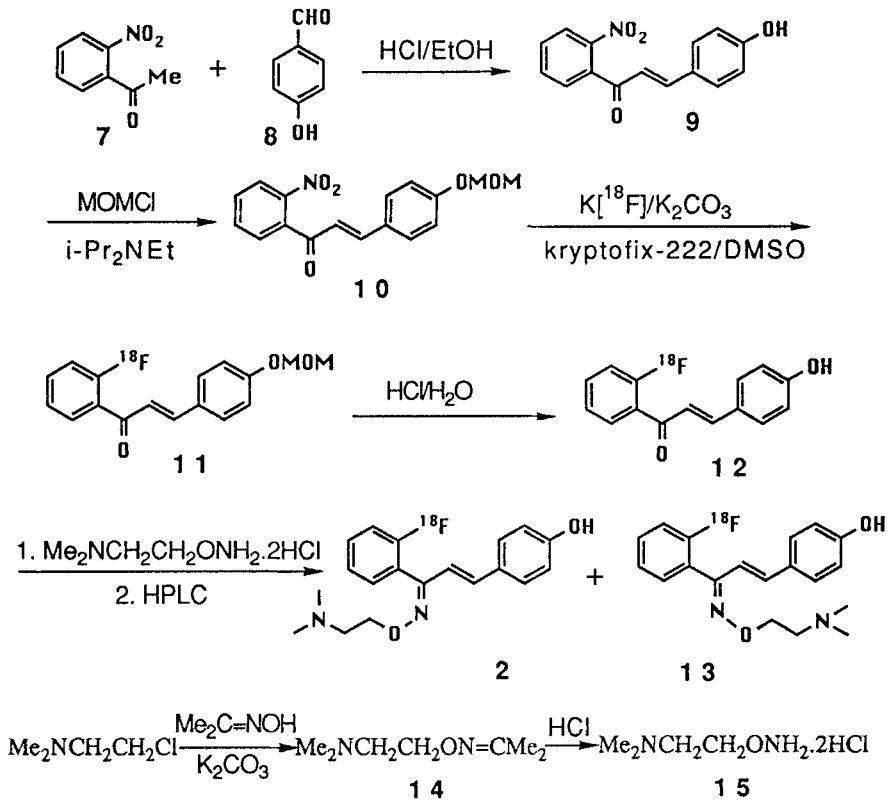
1 X = F, R = Me SR46349B
2 X = ^{18}F, R = Me [^{18}F]SR46349B
3 X = F, R = H
4 X = F, R = $^{11}CH_3$ [^{11}C]SR46349B

5 R = H
6 R = Bn

any detectable radioactive product. In order to avoid the interference of the free hydroxyl group in the reaction, the phenol hydroxyl group of **5** was protected with a benzyl group [17]. However, **6** did not undergo the nitro-fluorine exchange reaction under similar conditions. This indicated the ortho-oxime ether is not a good activating group and an alternative approach was examined.

Three potential precursors of [^{18}F]SR46349B, **7**, **9** and **10**, were also examined for their ability to undergo the ^{18}F-for-nitro substitution reaction (Figure 2). The reaction of 2'-nitroacetophenone (**7**) with [^{18}F]fluoride gave very low yield, consistent with a previous report [15b]. We found that **7** was thermally unstable, especially in the presence of base, presumably due to both intra- (nitro interacts with the methyl) and inter- (aldol condensation) molecular side reactions. Compound **9** did not undergo the substitution reaction, however, the hydroxyl protected compound **10** underwent the reaction well to afford F-18 labeled product **11** in 36.4±14.3% (n=19) labeling yield, based on radio-TLC analysis (silica, 2:1 hexane/ether). **11** was not isolated at this stage but was used directly in the next step without further purification.

Figure 2 Synthesis of [^{18}F]SR46349B



Compound **9** was prepared through the mixed aldol condensation [18] and the *trans* stereochemistry of the double bond was verified by the large coupling constant (J = 16.2 Hz) of the vinyl protons in ^{1}H NMR spectrum. The methoxymethyl (MOM) protecting group was chosen because of its facile preparation and removal [19, 20].

The successful nucleophilic aromatic displacement of **10** was a key step for the F-18 labeling of SR46349B. Removal of the MOM protecting group with 6.0 N HCl in the same pot gave **12** in quantitative yield [20]. The separation of ^{18}F-labeled **12** from its corresponding nitro-compound **9** was critical to the successful purification of the final compounds **2** and **13**, since **9** could also be converted to **5** in the step for condensation, which would severely interfere with the HPLC purification. We designed a device that combined the key features of a C-18 Sep-Pak and a silica column chromatography to accomplish this task. This system is composed of a C-18 Sep-Pak, a small silica gel column (100 x 10 mm) with a built-in 3-way stopcock at the top, a radiodetector connected to an HPLC recorder, and a test tube equipped with a heating/stirring unit. The C-18 Sep-Pak was used to concentrate the sample and remove the inorganic salt in the mixture by washing with water. Subsequently, organic solvent (2:1 hexane/ethyl ether) was applied to the C-18 Sep-Pak to elute the labeled compound, and the eluting solution was directed through the silica-column by turning the 3-way stopcock to the appropriate position. The more polar nitro-product **9** stayed on the column and the less polar fluorine-18 labeled intermediate **12** was eluted. The extent and efficiency could be conveniently monitored through the radiodetector that was connected to a HPLC recorder. The eluting solvent was collected in a test tube and evaporated simultaneously under a stream of nitrogen at 100 °C. This device is efficient enough to remove the majority of the nitro-product **9**. The use of silica gel column was necessary and could not be replaced with silica Sep-Pak(s) which contain(s) much less silica and was not efficient enough to separate the fluorine-18 compound **12** from the nitro-compound **9**. The entire process took *ca.* 20 min.

The condensation of the ketone **12** with 2-dimethylaminoethoxy amine **15** proceeded smoothly in the presence of an acid catalyst, *p*-toluenesulfonic acid (*p*-TsOH)[21]. The reaction time depends strongly on the temperature and solvent. The reaction mixture required heating under reflux overnight when ethanol was used as a solvent [22]. However, in 2-(2-methoxyethoxy)ethanol, the reaction was completed in 10 min. at 165°C and quantitative yield was obtained. Molecular sieves were used in the reaction to remove the water generated *in situ*. The condensation gave *cis* and *trans* isomers in approximately 1:1 ratio. Efforts were made to enrich

the desired *cis*-isomer by alteration of the reaction solvent, temperature and the concentration of *p*-TsOH, but in all cases there was no preference to the formation of either one of the isomers. The reaction mixture was pre-purified by using a C-18 Sep-Pak to remove alkoxyamine **15** and *p*-TsOH. The free bases of the two fluorine-18 labeled geometric isomers **2** and **13** were poorly soluble in water and HPLC solvent, and thus it was necessary to make the HCl salts of the free bases so they could be completely transferred to the HPLC loop.

HPLC conditions were optimized to resolve [^{18}F]SR46349B (**2**) from its geometric isomer **13**. It was determined the ternary solvent system composed of MeCN, MeOH and 0.1 M K_2HPO_4 in a ratio of 27.5/27.5/45 was optimal for the desired separation. Thus, when a semi-prep Econosil C-18 column (250 x 10 mm) was used with this solvent system at the flow rate of 5.0 ml/min., high resolution of **2** from **13** was obtained. A typical HPLC result is shown in **Figure 3**, [^{18}F]SR46349B (**2**) was cleanly separated from its geometric isomer **13** and other UV-sensitive impurities. The retention times for **2** and **13** were 46 and 35 min. respectively at flow rate of 5.0 ml/min.

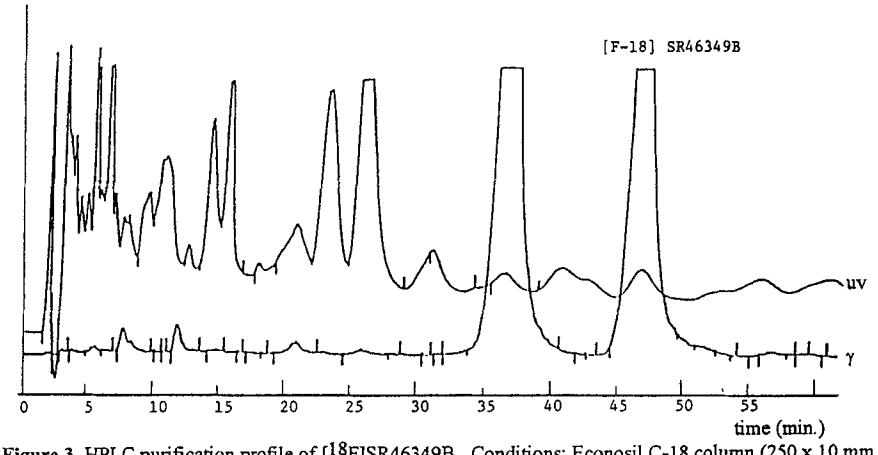


Figure 3 HPLC purification profile of [^{18}F]SR46349B. Conditions: Econosil C-18 column (250 x 10 mm, 10 micron); solvent: MeOH:MeCN:0.1 M K_2HPO_4 = 27.5:27.5:45; flow rate = 5.0 ml/min.

The HPLC fraction containing **2** was evaporated to dryness *in vacuo* at <30°C water bath. The resulting residue was dissolved in normal saline and filtered through a syringe filter to give a solution available for PET studies. The specific activity of [^{18}F]SR46349B (**2**) was 1140 Ci/mmol, which was determined by comparison of the HPLC peak area with the areas of known concentrations of authentic sample. The total synthesis time of **2** was *ca.* 170 min. and the radiochemical purity was 96%, which was determined by radio-TLC and analytical radio-HPLC

(Econosil C-18 analytical column, 250 x 4.6 mm, 5 micron, flow rate = 1.0 ml/min.). The *trans*-isomer **13** is known to be much less potent than the *cis*-isomer **2** for 5-HT_2 receptor binding and therefore the HPLC fraction of **13** was discarded. [^{18}F]SR46349B was reasonably stable in aqueous K_2HPO_4 at room temperature. However, prolonged heating may result in isomerization and probably degradation.

Experimental

Kryptofix-222, dimethylsulfoxide, 2-(2'-methoxyethoxy)ethanol, 4-hydroxybenzaldehyde, 2'-nitroacetophenone, 2-dimethylaminoethyl chloride hydrochloride, acetone oxime, methoxymethyl chloride and silica gel (flash-chromatography grade, 230-400 mesh) were purchased from Aldrich Chemical Co., Milwaukee, WI. Hexane, *p*-TsOH.H_2O, $CDCl_3$ and potassium phosphate (dibasic) were purchased from J. T. Baker Inc., Phillipsburg, NJ. Potassium carbonate and methanol were from Mallinckrodt, Paris, KY. Ethyl ether was obtained from Fisher Scientific, Pittsburgh, PA. [^{18}F]Fluoride ion was made by 17.4 MeV proton irradiation of [^{18}O]H_2O in a silver target or a [^{18}O]CO_2 [23]. ^{1}H NMR were conducted on a Brucker 300 MHz NMR spectrometer and the chemical shifts are reported in parts per million downfield from tetramethylsilane and the coupling constants, in Hertz. Mass spectroscopy were recorded with a Finnegan-Mat GC-MS 5100 mass spectrometer using electron impact ionization. HPLC analyses were carried out on a Knauer HPLC system with a UV- and radiodetector. Econosil C-18 semi-prep (250 x 10, 10 micron) and analytical (250 x 4.6, 5 micron) HPLC columns were purchased from Alltech Assoc. Inc., Deerfield, IL. TLC experiment was conducted on silica pre-coated alumina plates (0.2 mm, F254) purchased from EM Laboratories Inc., Elmsford, NY.

***trans*-1-(2-Nitrophenyl)-3-(4-hydroxyphenyl)-2-propenone (9)** Anhydrous HCl gas was purged into a mixture of 2'-nitroacetophenone (1.25 g, 7.2 mmol), 4-hydroxylbenzaldehyde (0.744 g, 6.0 mmol) and anhydrous ethanol (30 ml) for 14 min. The resulting reddish solution was kept at room temperature for 24 h and concentrated *in vacuo*. The residue was flash-chromatographed (2:1 ethyl ether/hexane) to give the product as a yellow solid (1.05 g, 65%), which was crystallized from chloroform to provide with the analytical sample as yellow needles. MP 171-171.5°C (lit. 167 °C [18]). ^{1}H NMR ($CDCl_3$) δ 6.84 (d, 2 H, ArH, J = 8.6), 6.88 (d, 1 H, =CH-, J = 16.2), 7.19 (d, 1 H, =CH-, J = 16.2), 7.40 (d, 2 H, ArH, J =

8.6), 7.50 (dd, 1 H, ArH, J = 1.3, 7.5), 7.65 (ddd, 1 H, J = 1.5, 7.8, 7.8), 7.73 (ddd, 1 H, ArH, J = 1.2, 7.5, 7.5), 8.18 (dd, 1 H, ArH, J = 0.99, 8.2). MS, m/e (rel intensity): 269.1 (M^+, 15.35), 165.1 (9.4), 148.2 (50), 134.1 (29.1), 121.1 (38.3), 107.1 (100).

***trans*-1-(2-Nitrophenyl)-3-(4-methoxymethoxyphenyl)-2-propenone (10)** Diisopropylethylamine (1.0 ml) was added to the light yellow solution of **9** (286 mg, 1.1 mmol) and methylene chloride (30 ml). The resulting reddish brown solution was cooled to -70 °C and methoxymethyl chloride (0.5 ml) was added. The resulting reaction mixture was allowed to warm up and kept at room temperature for 2 h. After TLC (1:1 hexane/ethyl ether) indicated completion of the reaction, the mixture was concentrated *in vacuo*. The residue was purified with flash chromatography [silica gel, ethyl ether:hexane (1:1)] to afford the product as a yellow oil (263 mg, 76%), which solidified during storage. ^{1}H NMR ($CDCl_3$) δ 3.47 (s, 3 H, Me), 5.20 (s, 2 H, CH_2), 6.90 (d, 1 H, =CH-, J = 16.2), 7.04 (d, 2 H, ArH, J = 8.8), 7.20 (d, 1 H, =CH-, J = 16.2), 7.45 (d, 2 H, ArH, J = 8.8), 7.50 (dd, 1 H, ArH, J = 1.5, 7.5), 7.65 (ddd, 1 H, ArH, J = 1.5, 7.5, 8.2), 7.76 (ddd, 1 H, ArH, J = 1.1, 7.5, 7.5), 8.18 (dd, 1 H, ArH, J = 1.1, 8.2). ^{13}C NMR ($CDCl_3$) δ 56.241, 94.148, 116.56, 124.484, 124.568, 127.655, 128.879, 130.337, 130.475, 133.992, 136.529, 146.185, 159.650, 192.979. MS, m/e (rel intensity) 313.2 (M^+, 3.74), 192.1 (1.88), 166.2 (2.32), 147.0 (9.79).

***O*-(2-Dimethylaminoethyl) acetone oxime (14).** Synthesized following literature procedure [21] as a colorless oil, b. p. 67-68°C/20 mm Hg. ^{1}H NMR ($CDCl_3$) δ 1.86 (s, 3 H, Me), 1.87 (s, 3 H, Me), 2.30 (s, 6 H, Me), 2.61 (t, 2 H, CH_2, J = 5.8), 4.14 (t, 2 H, CH_2, J = 5.8). MS, m/e (rel. intensity) 144.1 (M+, 1.96), 122.1 (1.05), 86.0 (0.92), 71.1 (32.8), 58.1 (100).

2-Dimethylaminoethoxylamine dihydrochloride (15). Synthesized following literature procedure as a white solid, MP 182-183°C (lit. 180-182°C)[21]. ^{1}H NMR (MeOH-d4) δ 2.94 (s, 6 H, Me), 3.57 (t, 2 H, CH_2, J = 4.8), 4.46 (t, 2 H, CH_2, J = 4.8), 4.87 (br, 3 H, NH_3).

[^{18}F]trans,4-[(3Z)3-(dimethylaminoethyl)oxyimino-3-(2-fluorophenyl) propen-1-yl]-phenol (2) ([^{18}F]SR46349B). A solution of kryptofix-222 (20 mg), anhydrous potassium carbonate (2.5 mg) and aqueous [^{18}F]KF was azeotropically distilled with MeCN at 120-155 °C. A solution of the nitro-precusor **10** (10 mg) and dimethyl sulfoxide (0.5 ml) was added. The resulting solution was shaken on a vortex mixer and heated at 120 °C for 6 min. The progress of the nitro-for-[^{18}F]fluoride exchange reaction was monitored by radio-TLC

(1:1 hexane:ethyl ether). Aqueous HCl solution (6.0 N, 5.0 ml) was added to the reaction mixture and heated at 100 °C for 8 min. to hydrolyze the MOM protecting group. The solution was diluted with water (5.0 ml) and loaded on a C-18 Sep-Pak that was attached to a small silica column (100 x 10 mm) with a 3-way stopcock on the top and pre-activated with methanol and water. The Sep-Pak was washed with water (30 ml) to remove the inorganic substances and dried by blowing anhydrous nitrogen gas through. The 3-way stopcock was then turned to the column, the Sep-Pak was further eluted with hexane-ethyl ether (2:1). The eluate was passed sequentially through the silica gel column and a radiodetector that was connected to a HPLC recorder, and collected in a test tube that contained a magnetic bar, *p*-TsOH.H_2O (10 mg) and 2-dimethylaminoethoxylamine dihydrochloride (**15**) (10 mg). The eluate was simultaneously distilled at 100 °C with stirring under a stream of nitrogen. The elution rate was controlled so that it was compatible with the rate of solvent evaporation. The elution was stopped when the radioactivity detector indicated completion. The resulting residue in the test tube was dried at 165 °C to remove the solvent.

To the labeled intermediate **12** was added 2-(2'-methoxyethoxy)ethanol (0.5 ml) and 4 Å molecular sieves (*ca.* 100 mg). The mixture was shaken on a vortex mixer for a few seconds and heated at 165 °C oil bath for 10 min. with stirring. Water was added (10 ml) and the mixture was loaded on another pre-activated C-18 Sep-Pak. The Sep-Pak was sequentially washed with water (30 ml), 0.1 N HCl (20 ml), water (40 ml), 5% potassium carbonate (20 ml) and water (40 ml). The product was eluted with methanol (3.0 ml) and collected in a test tube that contained 0.1 N HCl (0.5 ml). The mixture was concentrated to *ca.* 0.4 ml under a stream of nitrogen at 100 °C and diluted with water to 1.5 ml.

The HPLC purification was carried out with an Econosil C-18 semi-prep 10 micron column (250 x 10 mm) eluted with MeCN:MeOH:0.1 M K_2HPO_4 (27.5:27.5:45) at a flow rate of 5.0 ml/min. The retention times of *cis*- ([^{18}F]SR46349B) and *trans*- (compound **13**) isomers were 46 and 35 min. respectively (see **Figure 3**). The desired radioactive fraction (*ca.* 30 ml) was collected and evaporated *in vacuo* at <30°C water bath. The resulting residue was dissolved in saline (4.0 ml) and filtered through a syringe filter to give a formulation ready for PET studies.

The decay corrected yields for **2** and **13** were *ca.* 5%, respectively with a total synthesis time of 170 min. Radio-TLC of the final formulation of **2** was conducted on a silica gel coated alumina plate developed with methanol (Rf = 0.35). Analytical HPLC was carried out with an Econosil C-18 column (5 micron) at a flow rate of 1.0 ml/min. With both analytical methods, the radiochemical purity was 96%.

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13. Note: quaternary ammonium salt could be an alternative choice of leaving group. The authors did not try to use this approach because the ortho-substituted phenyl quaternary ammonium salts were known to be a poor substrate for nucleophilic aromatic substitution. For example, the reaction of 2-acetyl-N,N,N-trimethylanilium triflate with [^{18}F]fluoride gave low yield of [^{18}F]2-fluoroacetophenone with [^{18}F]MeF as the predominant product [15].

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17. Note: **6** was prepared as a geometric isomeric mixture by (1) protection of the phenol hydroxyl group of **9** with benzyl bromide; (2) condensation with **15** in the presence of *p*-TsOH.

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