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# AN EXPEDIENT SYNTHESIS OF HIGH SPECIFIC ACTIVITY TRITIUM LABELLED 4-FLUORO-1-[1-(2-THIENYL)]CYCLOHEXYLPIPERIDINE ([$^3$H]FTCP), A LIGAND FOR FURTHER CHARACTERIZATION OF THE PHENCYCLIDINE/NMDA RECEPTOR COMPLEX

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## SUMMARY

[$^{18}$F]4-Fluoro-1-[1-(2-thienyl)cyclohexyl)]piperidine is a ligand potentially useful for positron emission tomography of the PCP/NMDA receptor complex in the mammalian brain. The tritium-labelled title compound was synthesized in 5 steps starting with cyclohexanone. Catalytic tritiolysis in the final step afforded the title compound in high radiochemical yield and specific activity. This method represents a more efficient approach than previously published syntheses.

Key Words: PCP, TCP, Tritiation, FluoroTCP, NMDA Receptor

## INTRODUCTION

The elucidation of the precise mechanism of action of phencyclidine (1-(1-phenylcyclohexyl)piperidine, PCP) continues to be the subject of intense multidisciplinary study although the widespread abuse of this drug seen in some urban settings does not appear to be increasing at this time. Saturable, high affinity (1), enantioselective (2) binding sites for PCP have been identified in anatomically well-defined regions of the CNS and the binding of drugs from diverse structural classes to these sites is well correlated with some of the in vivo actions of PCP. This and other evidence strongly supports the pharmacologic relevance of these sites in the mediation of PCP effects. Other studies have shown that ligands which act on these sites are functional antagonists of *N*-methyl-D-aspartate (NMDA) excitatory amino acid receptors (3). These ligands are thought to exert their effects by blockade of ion channels regulated by competitive NMDA receptor agonists such as glutamate. Non-

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competitive NMDA channel blockers can function as potent anticonvulsants and neuroprotective agents in a variety of seizure and CNS injury paradigms, respectively.

In order to gain further insight into the structure and function of the PCP/NMDA receptor complex, it is necessary to develop compounds that are amenable to radiolabelling for both in vivo and in vitro studies. One such compound, 1-[1-(2-thienyl)cyclohexyl]-4-fluoropiperidine (4-fluoroTCP, FTCP, **7b**), exhibits a high affinity for the phencyclidine binding site (4). We recently described an 8-step synthesis of [$^3$H]FTCP ([$^3$H]4-fluoro-1-[1-(2-thienyl)]cyclohexylpiperidine) as a ligand to probe the utility of [$^{18}$F]FTCP as a potential ligand in PET studies of the PCP/NMDA receptor complex in living subjects (5). We also recently reported successful labelling of FTCP with [$^{18}$F] for positron emission tomography (PET) studies of the mammalian brain (4). Initial PET imaging studies with [$^{18}$F]FTCP in the monkey in the presence and absence of exogenous glutamate indicates [$^{18}$F]FTCP is a promising tool for study of the NMDA receptor-ion channel complex (6). Currently, [$^3$H]FTCP is being used in successful extensions of our studies of the biodistribution, pharmacokinetics, metabolism, autoradiographic localization and in vitro binding parameters of FTCP. However, with the substantial quantities of [$^3$H]FTCP required for in vivo studies, a more efficient synthesis of this compound was necessary.

In this study, we report a 5-step synthesis of [$^3$H]FTCP in which a [$^3$H] label is introduced on the thiophene ring. In the procedure previously described (5), two [$^3$H] labels were incorporated with low efficiency into the cyclohexane ring. Our new synthesis has the advantage of fewer synthetic steps and higher overall chemical and radiochemical yields than our previous method. In addition, the present route avoids the hazards associated with the use of liquid hydrogen fluoride required for the synthesis of the intermediate 4-fluoropiperidine used earlier.

## SYNTHESIS

The starting compound **1**, 1-[1-(4-hydroxypiperidinyl)cyclohexyl]carbonitrile, was obtained using the method of Lin, et al. (7) (Figure 1). Bruylants reaction (8) between **1** and excess 2-bromothiophene-5-magnesium bromide in diethyl ether proceeded smoothly to produce the thienyl derivative **2** in 41% yield. The methanesulfonate ester **3** was formed by reaction of **2** with methanesulfonyl chloride in the presence of triethylamine. Reaction of **3** with tetrabutylammonium fluoride afforded a mixture ($^1$H NMR) of the desired product **4** and the elimination product **5**. Separation of **4** and **5** could not be accomplished easily because they exhibit similar Rf values on silica gel chromatography. Hydroboration of the mixture of products converted **5** into its hydroxy derivative, which was easily separated from **4** by chromatography. Catalytic tritiolysis of **4** with carrier-free tritium gas (9) in the presence of 10% Pd/C afforded [$^3$H]FTCP (**6**) in 30% radiochemical yield with a specific activity of 29 Ci/mmol (100% isotopic incorporation).

Alternate attempts to prepare intermediates **8a,b** by direct bromination of 4-hydroxyTCP (**7a**) or FTCP (**7b**) were foiled by the facile elimination of the piperidine

ring (Figure 2). Initial strategies included bromination of **7a** using bromine in acetic acid at elevated temperature, a method successfully used to brominate TCP (10). Other unsuccessful attempts at brominating **7a** utilized milder bromination methods such as bromine in methanol at -20 °C, and bromine in methanol at low temperature in the presence of 1,8-diazabicyclo[5.4.0]undec-1-ene. In all cases, CIMS and TLC confirmed a quantitative formation of the 5-monobrominated elimination product **9**.

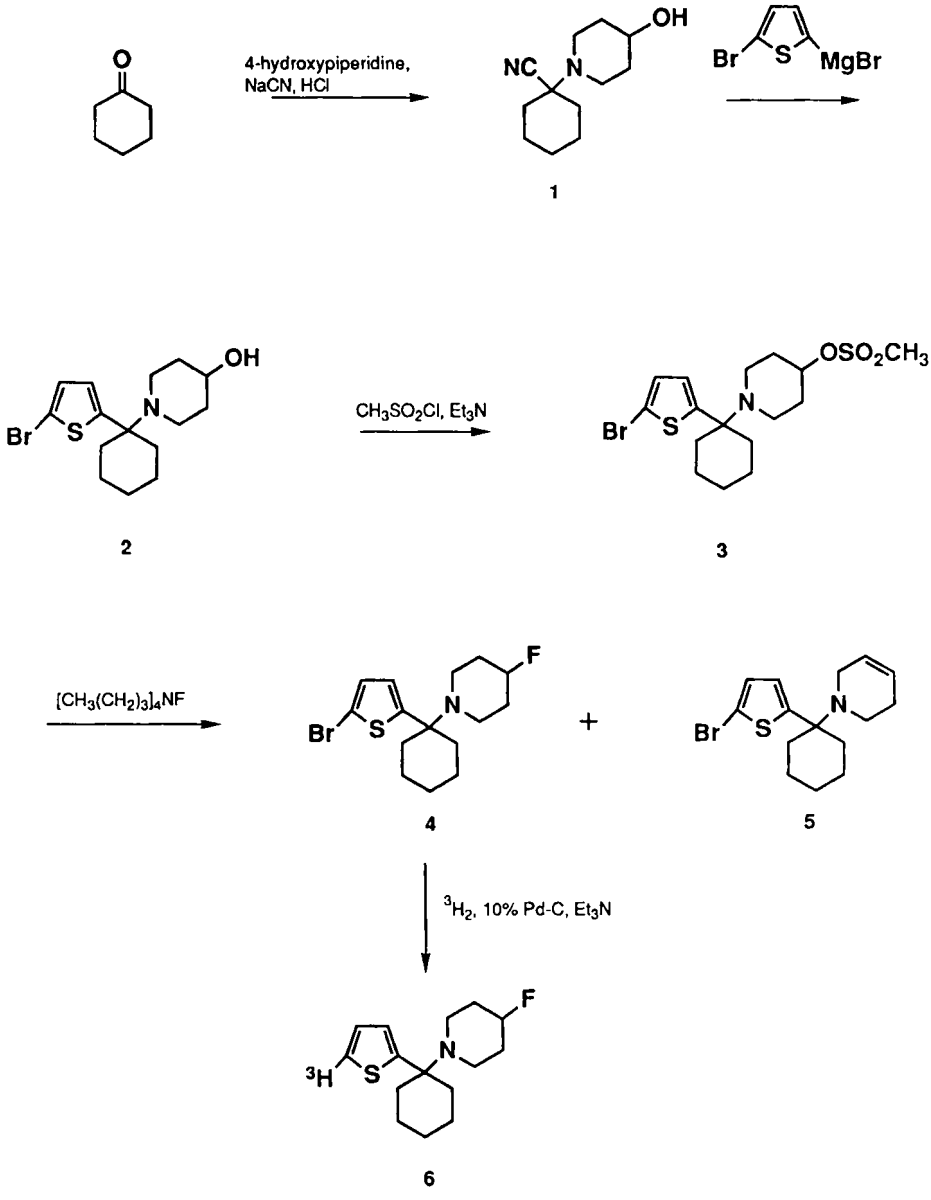
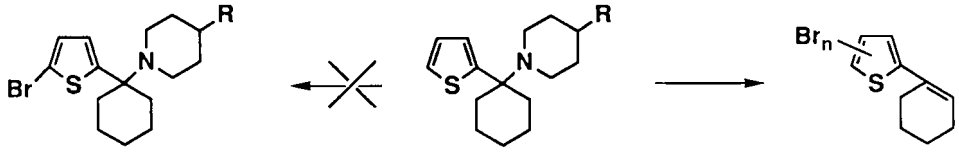



**Fig.1. Reaction Sequence**

Lowering the reaction temperature below -30 °C in each of these methods precluded any reaction. Bromination of **7a** or **7b** with *N*-bromoacetamide, *N*-bromosuccinimide or 1,3-dibromo-5,5-dimethylhydantoin in the presence of 2,2'-azobis(2-methylpropionitrile), and dibromoisocyanuric acid under a variety of conditions was also futile, producing a series of mono-, di- and tribrominated derivatives of the thienylcyclohexene **9**.




**8a** R = OH
**8b** R = F

**7a** R = OH
**7b** R = F

**9**

**Fig.2. Attempts at Direct Bromination of 4-HydroxyTCP (7a) and FTCP (7b).**

## DISCUSSION

Tritiolysis of **4** to **6** ([$^3$H]FTCP) was accomplished very efficiently, without any loss of fluorine. The high specific activity of **6** (29 Ci/mmol) is twice that reported in previous syntheses (14 Ci/mmol), although **6** contains only one [$^3$H] label. A quantitative incorporation of tritium was achieved, unlike that described in a previous synthesis (5) of [$^3$H]FTCP, where 23% incorporation was obtained. Apparently, tritiolysis of the bromine on the thiophene ring occurs more quickly and efficiently than does reductive tritiation of a double bond in the cyclohexane ring of FTCP. Therefore, the radiochemical yield of **6** (30%) is much higher than the 1.2% we obtained earlier. These results constitute a facile and high-yield synthesis of the target compound.

## EXPERIMENTAL

### GENERAL

Melting points were determined on a Thomas-Hoover capillary apparatus and are corrected. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA. Chemical-ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. High-resolution mass spectra (HRMS) and electron-impact mass spectra (EIMS) were obtained using a VG 7070F mass spectrometer. $^1$HNMR spectra were obtained using a Varian XL-300 spectrometer. Thin-layer chromatography (TLC) was performed on 250 μ Analtech GHLF silica gel plates. TLC plates were analyzed for radioactivity with a Bertold model LB 2760 TLC scanner. Radioactivity

determinations were carried out using a Packard model 2200 CA "Tri-Carb" liquid scintillation counter; tritium labelled compounds were counted in a Hydrofluor scintillation cocktail (National Diagnostics) with a counting efficiency of 45%. All synthetic and analytical operations were initially performed with unlabelled compounds and the structures were confirmed spectroscopically.

**4-Hydroxy-1-[1-(5-bromo-2-thienyl)cyclohexyl]piperidine (2)**

A solution of 2,5-dibromothiophene (2.6 mL, 23.3 mmol) in 45 mL of anhydrous diethyl ether was stirred at reflux under inert atmosphere with 0.56 g (23 mmol) of magnesium for 1 h. The Grignard reagent thus formed appears as an insoluble oil on the sides of the flask. A solution of intermediate cyanopiperidine **1** (7) (1.5 g, 7.7 mmol) in 20 mL of anhydrous THF was added dropwise to the reaction mixture. The resulting heterogeneous mixture was stirred at reflux for 3 h, then at ambient temperature for 24 h. The reaction mixture was quenched with an excess of saturated aqueous ammonium chloride solution, the two resulting layers were separated and the upper organic layer was extracted four times with 10 mL of an aqueous citric acid solution (pH 3-4). The combined citric acid washes were made basic with $NH_4OH$ and extracted with benzene (3 x 10 mL). The combined organic layers were washed with 10 mL of brine, dried ($Na_2SO_4$) and concentrated to obtain 1.27 g of crude product as an oil which crystallized upon standing. The oil was purified by chromatography ($SiO_2$, THF/hexanes/$NH_4OH$, 2 : 0.9 : 0.1) to obtain 1.08 g (41%) of cream-colored solid which was recrystallized from isopropanol: mp 135-136 $^oC$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.38-2.17 (complex m, 16H + OH), 2.79 (br m, 2H), 3.52 (m, 1H, CHOH), 6.56 (d, 1H, ArH, J = 3.78 Hz), 6.92 (d, 1H, ArH, J = 3.76 Hz); CIMS ($NH_3$) m/z 345 (M + 1). Anal. Calcd for $C_{15}H_{22}BrNOS$: C, 52.33, H, 6.44, N, 4.07%. Found: C, 52.39, H, 6.45, N, 4.03%.

**4-Methanesulfonyloxy-1-[1-(5-bromo-2-thienyl)-cyclohexyl]piperidine (3)**

A solution of thienylpiperidine **2** (1.1 g, 3.1 mmol) and triethylamine (0.86 mL, 6.2 mmol) in 10 mL of anhydrous THF was stirred under an inert atmosphere at 0 $^oC$. Freshly distilled methanesulfonyl chloride (0.47 mL, 6.1 mmol) was added dropwise to the solution and the flask was allowed to warm to room temperature over a 15-min period after the addition was complete. The mixture was filtered through celite to remove precipitated triethylamine hydrochloride, the precipitate was washed once with THF, and the combined filtrates and washings were concentrated in vacuo. The yellow, oily residue was dissolved in 10 mL of diethyl ether and the resulting solution was washed with a saturated aqueous $NaHCO_3$ solution (1 x 10 mL) and brine (2 x 10 mL). The brine layers were extracted with 10 mL of ether and the combined organic layers were dried ($Na_2SO_4$) and concentrated to obtain 1.23 g (93%) of a yellow oil that could not be further purified satisfactorily: CIMS ($NH_3$) m/z 423 (M + 1), 344 (M - $CH_3SO_3H$); $^1H$ NMR ($CDCl_3$) $\delta$ 1.35-2.20 (complex m, 16H), 2.75 (br m, 2H),

2.95 (s, 3H, $CH_3SO_3$), 4.55 (m, 1H, CHSO3Me), 6.55 (d, 1H, ArH, J = 3.76 Hz), 6.95 (d, 1H, ArH, J = 3.77 Hz). HRMS: Calcd for $C_{16}H_{24}NO_3SBr$ 421.0381. Found: (m/z) 421.0366.

**4-Fluoro-1-[1-(5-bromo-2-thienyl)cyclohexyl]piperidine (4)**

Tetrabutylammonium fluoride trihydrate (1.4 g, 4.5 mmol) was added to anhydrous acetonitrile (5 mL) and approximately 2 mL of the solvent were removed by distillation. The solvent was replaced and the procedure was repeated twice in order to assure anhydrous reaction conditions. The methanesulfonate ester **3** (0.64 g, 1.5 mmol) was added and the resulting solution was heated to 40 °C and stirred under inert atmosphere for 48 h. The solvent was removed in vacuo, 5 mL of $NH_4OH$ were added and the mixture was partitioned between diethyl ether and water. The ether layer was separated, washed with 5 mL of brine, dried ($Na_2SO_4$) and concentrated to obtain 0.50 g (96%) of a yellow oil. TLC (hexanes: ethyl acetate, 10 : 1) showed two products with similar Rf values. $^1H$ NMR ($CDCl_3$) of the products indicated a mixture of compounds **4** and **5**.

The mixture of products was dissolved in 10 mL of anhydrous diethyl ether and 3.6 mL of borane-THF complex (1.0 M solution in THF) was added to the stirred solution at room temperature. After 5 min, the solvent was removed from the cloudy solution in vacuo. The residual oil was dissolved in 10 mL of diethyl ether and 10 mL of an aqueous solution of 15% NaOH was added. The resulting biphasic solution was stirred vigorously while 0.6 mL of $H_2O_2$ (30%) was added dropwise. The solution was stirred for 5 min at ambient temperature and the two layers were separated. The aqueous layer was extracted with 5 mL of ether, the combined organic layers were washed once with 5 mL of brine, dried ($Na_2SO_4$) and concentrated to obtain 0.43 g of crude product as a yellow oil. The oil was purified by chromatography ($SiO_2$, hexanes/ethyl acetate, 10 : 1) to obtain 60 mg (12%) of **4**. Compound **4** formed a crystalline HCl salt from diethyl ether: mp 172-173 °C; EIMS m/z 346 (M); $^1H$ NMR ($CDCl_3$, free base) δ 1.39-2.18 (complex m, 16 H), 2.79 (br m, 2H), 4.47 (dm, 1H, CHF, J = 48 Hz), 6.55 (d, 1H, ArH, J = 3.76 Hz), 6.95 (d, 1H, ArH, J = 3.77 Hz), 6.55 (d, 1H, ArH, J = 3.76 Hz), 6.95 (d, 1H, ArH, J = 3.77 Hz). Anal. Calcd for $C_{15}H_{21}BrFNS.HCl$: C, 47.04; H, 5.79; N, 3.66%. Found: C, 47.02; H, 5.75; N, 3.63%.

**[$^3$H]4-Fluoro-1-[1-(2-thienyl)]cyclohexylpiperidine (6)**

To a solution of **4** (10 mg, 0.037 mmol) in 1 mL methanol was added 20 μL triethylamine and 10 mg of 10% Pd/C. The mixture was stirred for 30 min under an atmosphere of tritium gas (10 Ci, 0.17 mmol). The reaction mixture was filtered to remove catalyst and the solvent was evaporated under a stream of $N_2$ gas to remove labile tritium. The residue was reconstituted to a volume of 10 mL with methanol for storage. The solution was evaporated under a stream of $N_2$ gas and the residue was taken up in 2 mL of $CHCl_3$/MeOH/$NH_4OH$ (90 : 9 : 1) and applied to a 0.5 mm preparative TLC plate (20 x 20 cm). The plate was eluted with $CHCl_3$/MeOH/$NH_4OH$ (99 : 0.9 : 0.1) and the band comigrating with reference **7b** was scraped off and extracted

with 10 mL of $CHCl_3/MeOH/NH_4OH$ (90 : 10 : 1). After stirring for 10 min, the extract was filtered through glass wool and the filtrate was evaporated under a stream of $N_2$ gas. The residue was dissolved in 10 mL of $CH_2Cl_2$ and transferred to a flask for storage. The solvent was again evaporated under a stream of $N_2$ gas and the residue reconstituted to a total volume of 200 mL with absolute ethanol: yield of 6 313 mCi (30%); radiochemical purity >99.5% (by TLC analysis); specific activity 29 Ci/mmol (from $\varepsilon_{238}$ = 8660 $M^{-1}cm^{-1}$ for **7b** free base); percentage incorporation of tritium = 100%.

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