A Comparison of the Incorporation of ^{123}I and ^{18}F into 1-[1-(3-Hydroxyphenyl) cyclohexyl]-4-(methanesulfonyloxy)piperidine by Nucleophilic Displacement with $^{123}I^-$ and $^{18}F^-$

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SUMMARY

Unlabelled and labelled 4-fluoro- and 4-iodo-1-[1-(3-hydroxyphenyl)cyclohexyl]piperidines (**3** and **4**, respectively), derivatives of phencyclidine, were synthesized in 3-steps via nucleophilic displacement reaction of 1-[1-(3-hydroxyphenyl) cyclohexyl]-4-(methanesulfonyloxy)piperidine (**8**). The displacement reaction with 3 molar equivalents of $Bu_4N^+I^-$ gave 58% of **4** with no detectable methanesulfonate elimination. Reaction of a large excess of **8** with $Na^{123}I^-$ under similar conditions gave up to 40% yield of [^{123}I]**8**. In contrast reaction of **8** with 3 molar equivalents of unlabelled $Bu_4N^+F^-$ in refluxing acetonitrile yielded only 3.1% of **3** and 97% of elimination product. Similarly, reaction of a large excess of **8** with $Bu_4N^+{}^{18}F^-$ at 80 °C in acetonitrile failed to yield detectable quantities of [^{18}F]**3**. However, low (0-4%) yields of [^{18}F]**3** were obtained using $^{18}F^-$ in the presence of varying proportions of inorganic base and Krytofix in acetonitrile.

Key Words: 4-fluoro- and 4-iodo-1-[1-(3-hydroxyphenyl)cyclohexyl] piperidines, 1-[1-(3-hydroxyphenyl)cyclohexyl]-4-(methanesulfonyloxy) piperidine (**8**), phencyclidine, nucleophilic displacement, $Bu_4N^+I^-$, $Bu_4N^+F^-$ $Na^{123}I^-$, $Bu_4N^+{}^{18}F^-$, elimination, Kryptofix.

INTRODUCTION

The drug of abuse, phencyclidine (PCP, **1**) has elicited considerable

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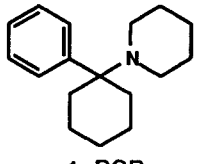
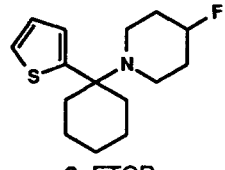
interest in the past decade [1] due to its wide spectrum of activity ranging from psychotomimetic effects in humans [2] to cerebroprotective effects in animal models [3]. Although a putative PCP binding site has been identified [4], and numerous studies have been aimed at determining its mode of action, the exact functional role of the PCP binding site is not completely clear. PCP has been found to elicit neuroprotective activity through its non-competitive inhibition of the excitotoxic stimulatory effects caused by elevated L-glutamic acid levels in the brain [3]. Raised levels of this neurotransmitter in the central nervous system (CNS) have been identified as a major factor in the neuropathophysiological cascade ultimately leading to cell death associated with anoxia, hypoxia and traumatic injury [5].

PCP and glutamate sites have been shown to have the same regional distribution in the brain [6]. In order to further understand the role of the PCP binding site in CNS injury, use of PET and SPECT ligands specific for PCP binding sites would allow correlation of the regional distribution of PCP binding sites in normal versus disease states and provide further insights into the function of the PCP/glutamate receptor complex in neuronal injury.



1, PCP



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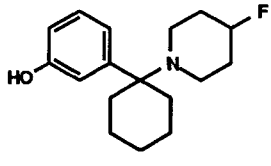
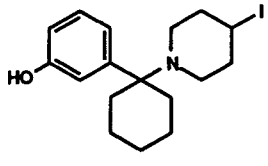


3, FOHPCP



4, IOHPCP

As part of our program to further delineate the structure and function of PCP binding sites in the CNS, we recently reported the synthesis of the PET ligands **2** [7] and **3** [8] which are structurally related to PCP (**1**). In the present study, we compare and contrast the formation of fluorinated ligand **3** and iodinated ligand **4** via treatment of a phenolic methanesulfonate ester precursor **8** with labelled and unlabelled F^- and I^-.

CHEMISTRY

Synthesis of **4** utilized aminonitrile **5** [8] as the starting material (Scheme 1). Treatment of **5** with the Grignard reagent generated from treatment of 2-(3-bromophenoxy)diethylether (**6**) [9] with Mg^o, afforded intermediate **7** (via the Bruylants reaction [10]). No attempt was made to

isolate or purify **7** because of its lability to hydrolysis. The sequence of treatment of **7** with $MeSO_2Cl/Et_3N$ and subsequent hydrolysis of the ethoxyethoxy protecting group during the isolation step (involving extraction of the reaction mixture with 10% aqueous citric acid) afforded crystalline phenolic methanesulfonate ester **8** in 53% overall yield from **5**. Treatment of **8** with 3 molar equivalents of tetrabutylammonium iodide in acetonitrile furnished the target compound **4** in 58% yield. Similarly, treatment of excess **8** with no carrier added [^{123}I]NaI [generated by the Xe(p, 2n) reaction] in acetonitrile (Scheme 1) gave [^{123}I]**4** in 30-40% yield.

Table 1: *Investigation of the yield of* [^{18}F]**3** *or unlabelled* **3** following treatment of **8** with $^{18}F^-$ or F^- under various conditions.

Expt Number	Conditions (as in Experimental section with the following modifications)	% Transfer (see Experimental)	%Yield of [^{18}F]**3**
1	K222 (4.5 mM), K_2CO_3 (4.5 mM)	50	3
2	Bu_4N^+ $^{18}F^-$ (10, mM)	89	0
3	K222 (4.5 mM), $KHCO_3$ (4.5 mM)	77	2
4	K222 (4.5 mM), $KOSO_2Me$ 4.5 mM)	15	0
5	K222 (4.5 mM), $KHCO_3$ (3.0 mM), K_2CO_3 (1.5 mM)	49	4
6	same as **3** except using 4 mg of methanesulfonate ester **8**	28	3

Expt Number	Conditions	O-Protected ?	%Yield of **3** or O-benzyl protected **3**
7	$Bu_4N^+F^-$ (3 eq), acetonitrile, reflux	no	3.1 (See Experimental)
8	$Bu_4N^+F^-$ (3 eq), acetonitrile, reflux	O-Benzyl	12 (see ref 8)

In contrast to the reaction with iodide, treatment of methanesulfonate ester **8** with excess (3 molar equivalents) anhydrous $Bu_4N^+F^-$ in refluxing acetonitrile (Scheme 1) afforded only low yields of **3** (3.1%) and high yields of elimination product **9** (97%). Compounds **3** and **9**

were identical spectroscopically (^{1}H-NMR and MS) and chromatographically (TLC) to authentic samples of **3** and **9** synthesized by different routes [refs 8 and 9, respectively]. As for unlabelled F^-, reaction of excess **8** with $^{18}F^-$ under a variety of conditions (Table 1) gave low (0-3%) yields of [^{18}F]**3** together with **9** as the major side product. It is possible that hydrolysis of the methanesulfonate ester in the presence of inorganic bases (Table 1) also contributed to the lowered yields. Significant amounts of starting material were recovered from all of the reactions with $^{18}F^-$ but not the reaction using excess unlabelled $Bu_4N^+F^-$. This is because the reactions with $^{18}F^-$ had precursor **8** in excess whereas the reactions with unlabelled F^- used excess $Bu_4N^+F^-$.

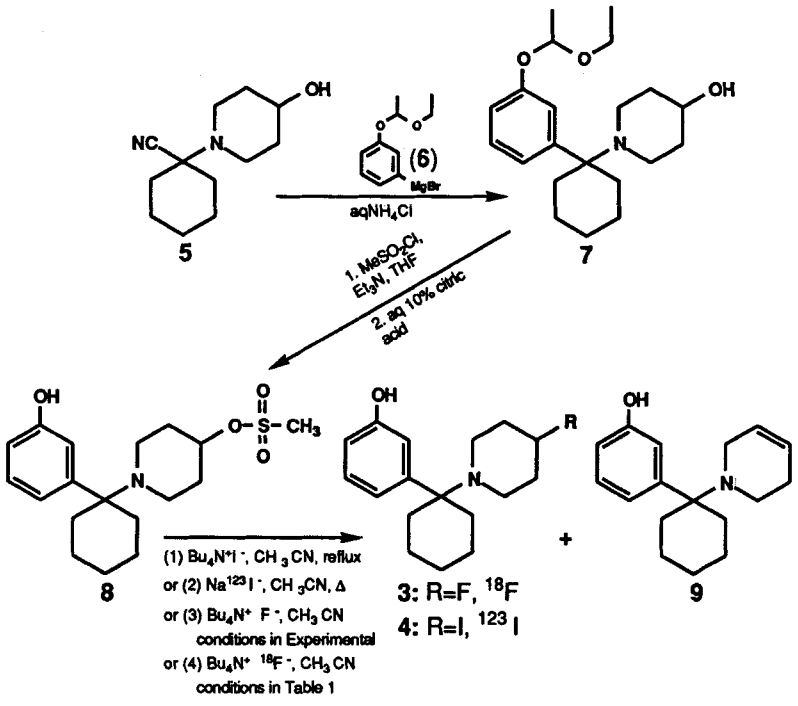
It is unlikely that lowered yields of [^{18}F]**3** are due to contamination of the $^{18}F^-$ by metal ions or other halides since the ^{18}F used in these reactions was aliquoted from a stock solution; the remaining activity was successfully used in the high yield (ca 50%) clinical production of [^{18}F] fluorodeoxyglucose ([^{18}F]FDG). The reactions shown in Table 1 each used 2 mg (5.7 mM) of **8**. Because **9** was the major product in each case, it is likely that the methanesulfonic acid generated from the excess **8** formed H^{18}F which reacted with the walls of the glass tube. This, as opposed to the presence of contaminating metal ions, would account for the low percent transfer of radioactivity.

DISCUSSION

Bruylants reaction [10] between aminonitrile **5** [8] and Grignard reagent **6** [9] gave **7** (Scheme 1). The 3-ethoxyethoxy protecting group of **7** proved important since it allowed selective methanesulfonylation of the 4-hydroxypiperidinyl moiety by masking the phenolic group. Precursor **8** is unique in that it permits nucleophilic displacement of the methanesulfonyloxy group in the presence of its unprotected phenol. This allowed introduction of the radiolabel in the final step of the reaction sequence without the need for deprotection.

The low yields of fluorinated product compared with iodinated product can be attributed to the greater basicity of F^- compared with I^-. As expected, the condition using $^{18}F^-$ in the presence of $Bu_4N^+OH^-$ gave the highest proportion of elimination product **9**. It is also possible that H-bonding interaction of F^- with the phenolic OH results in diminished reactivity of the F^- anion since in our previous study [8], the O-benzyl protected derivative of **8** gave a significantly higher (12-20%) yield of fluorinated product.

Scheme 1: Synthesis of unlabelled and ^{18}F-3 and ^{123}I-4



EXPERIMENTAL METHODS

Melting points were determined on a Thomas Hoover Capillary melting point apparatus and are uncorrected. Elemental analyses were determined at Atlantic Microlabs, Atlanta, Georgia. ^{1}H-Nuclear magnetic resonance spectra were determined using a Varian XL300 spectrometer. Analytical thin layer chromatography of unlabelled compounds was performed on Analtech 250 μM (GHLF) TLC plates. TLC of ^{123}I-labelled compounds was performed on 250 μM analytical TLC plates (GTLC, Macherey-Nagel, Germany). Radioactivity was detected on the TLC plates using a Bioscan system 200 imaging scanner. Radioactivity (^{123}I) determinations were made using a Radcal model 4050 Radionuclide calibrator (Radcal corporation) [22]. Na^{123}I [no carrier added) was purchased from Nordion International Inc., Vancouver, Canada and used immediately upon receipt. Reported radiochemical yields are optimized. No carrier added ^{18}F was produced at the Cyclotron facility of the Positron Emission Tomography Dept. using the ^{18}O(n.p)^{18}F reaction.

1-[1-(3-Hydroxyphenyl)cyclohexyl]-4-(methanesulfonyloxy) piperidine (8).

A mixture of magnesium turnings (3.17 g, 130 mmol, 4.4 eq), 3-(ethoxyethoxy) bromobenzene [9] (21.2 g, 65.2 mmol, 2.2 eq) and THF (200

mL) were placed in a flame-dried flask and the mixture was rapidly stirred under a nitrogen atmosphere. Vigorous refluxing occurred after ca. 5 min. The reaction was controlled by periodic immersion of the flask in an ice-bath. After the reaction had subsided, the reaction mixture was cooled to ambient temperature and a solution of aminonitrile **5** (6.0 g, 28.9 mmol) in dry THF (50 mL) was added dropwise. The solution was stirred overnight at ambient temperature after which time TLC (1:9:90 concentrated aqueous ammonia-MeOH-$CHCl_3$) indicated complete reaction. The mixture was quenched into ice (200 g) and saturated aqueous NH_4Cl (50 mL) and extracted with ether (2 x 200 mL). The combined organic extract was dried (anhydrous K_2CO_3) and the solvent was evaporated in vacuo to give crude **7** (see Scheme 1) as a colorless oil. This was dissolved in dry THF (200 mL) and treated with Et_3N (14.6 g, 144 mmol, 5eq based on aminonitrile **5**), followed by methanesulfonyl chloride (4.9 mL, 58 mmol, 2 eq based on aminonitrile **5**). After 10 min, TLC (1:9:90 concentrated aqueous ammonia-MeOH-$CHCl_3$) indicated complete reaction. The precipitated $Et_3N{\bullet}HCl$ was removed by filtration and washed with a little cold THF. The combined filtrate and washings were evaporated in vacuo and the residue was dissolved in 10% aqueous citric acid (500 mL), stirred for 5 minutes at ambient temperature, and then washed with ether (3 x 200 mL) and the combined ether extract was discarded. Addition of excess concentrated aqueous ammonia solution resulted in precipitation of the product. The basified solution was extracted with CH_2Cl_2 (2 x 200 mL) and the extract was dried (Na_2SO_4) and evaporated in vacuo to give **8** (5.40 g, 53% overall yield) as a white crystalline solid. Recrystallization from hot 2-propanol afforded an analytically pure sample: mp 137-138 °C (dec); ^{1}H-NMR ($CDCl_3$) δ 7.20 (t, J_{app}=7.9 Hz, 1H), 6.85 (d, J=7.4 Hz, 1H), 6.79 (m, 1H), 6.72 (dm, J=6.7 Hz, 1H), 4.52 (m, 1H), 2.94 (s, 3H), 2.72-2.85 (m, 2H), 1.89-2.13 (m, 6H), 1.22-1.89 (complex m, 11H); CIMS (MH^+ calcd for $C_{18}H_{27}NO_4S$): 354. Found: 354 (MH^+), 258 (MH^+-$MeSO_3H$), 180 (MH^+-SO_2Me-C_6H_5O); Anal (calcd for $C_{18}H_{27}NO_4S$): C 61.16, H 7.70, N 3.96%. Found: C 61.09, H 7.77, N 3.90%.

1-[1-(3-Hydroxyphenyl)cyclohexyl]-4-fluoropiperidine (3).

Tributylammonium fluoride•trihydrate (307.3 mg, 0.97 mmol, 3.0 eq) was azeotropically dried by addition and evaporation of toluene (3 x 5 mL). To the residue was added dry acetonitrile (5 mL) followed by **8** (113 mg, 0.32 mmol). The mixture was refluxed for 14h under a nitrogen atmosphere or until TLC (1:9:90 concentrated aqueous ammonia-MeOH-$CHCl_3$) indicated the absence of starting material. The reaction was cooled to ambient temperature and the solvent was evaporated in vacuo. The residue was partitioned between saturated aqueous $NaHCO_3$ (10 mL) and ether (10 mL). The aqueous layer was discarded and the ethereal layer was diluted to 20

mL with fresh ether, washed with water (2 x 10 mL) and evaporated in vacuo to give the crude product (80 mg, 89%) as an oil. Analysis of this product by ^{1}H-NMR ($CDCl_3$) indicated the presence of **3** [3.1% of product mixture: 4.45 (dm, J=49 Hz, CHF); CIMS (MH^+ calcd for $C_{17}H_{24}NOF$): 278. Found: 278 (MH^+). Treatment of the product mixture with HCl to protonate it followed by ^{1}H-NMR showed **3**•HCl at δ 4.87 (dm, J=47 Hz, CHF). Lit [8] ^{1}H-NMR (**3**•HCl, $CDCl_3$): δ 4.87 (dm, J=47 Hz, CHF)]. The ^{1}H-NMR also showed the presence of elimination product. See Table 1 for comparison with O-benzyl protected **8** under experiments 7 and 8.

Radiochemical synthesis of [^{18}F]1-[1-(3-Hydroxyphenyl)cyclo hexyl]-4-fluoro piperidine ([^{18}F]3) under different conditions (see Table 1 for six different conditions).

The synthesis of unlabelled **3** [8] and elimination product **9** [9] have been reported previously. These compounds were used as internal references in the identification of [^{18}F]**3** and **9**. Using a modification of previously described conditions [7], a 13x100 mm test tube was charged with K_2CO_3-Kryptofix, K222 containing ($^{18}F^-$, 2 mCi, no carrier added) together with Bu_4N^+ OH^- or some other pseudo carrier. The reaction mixture was placed in a hot block at 80 °C and then taken to dryness with a stream of nitrogen. Three portions of CH_3CN were added and each in turn was evaporated. Then, CH_3CN (200 µL) was added and the methanesulfonate ester precursor (**8**, 2 mg, 5.7 mmole) was added as the crystalline powder. The reaction was heated at 80 °C for 15 min. TLC aliquots were taken at 5 min intervals. The bulk of the activity was found to stick to the baseline of the TLC plate as $^{18}F^-$. It proved impossible to detect the product by TLC. After 15 min, the solution was transferred out of the reaction tube and the tube was rinsed with CH_3CN (300 µL). The amount of transferred radioactivity was found to vary with the conditions and is a measure of the amount of $^{18}F^-$ in the solution at the end of the reaction. The combined reaction solution and washings were purified by HPLC (flow rate 1.0 mL/min) using a Beckman Ultrasil ODS column (4.6x250 mm, 5 µm particle size). A solvent system consisting of a mixture of CH_3CN (70%) and aqueous buffer (5 mM Et_3N, 5mM NaH_2PO_4, pH 8) (30%) was used for the separation. The retention times under these conditions were found to be: **8** (5.08 min), **3** (7.75 min), and elimination product **9** (8.95 min). The three compounds exhibited similar UV spectra (UV-flow detector) under these HPLC conditions. The mass trace on the HPLC chromatogram always showed two major components. Some starting material **8** survived the reaction conditions and the second (slower) component migrated with a retention time corresponding to authentic elimination product [9]. The radiochemical yields for [^{18}F]**3** were calculated by multiplying the percent area under the HPLC trace by the percent transfer.

1-[1-(3-Hydroxyphenyl)cyclohexyl]-4-iodopiperidine (4).

A mixture of **8** (300 mg, 0.85 mmol) and $Bu_4N^+I^-$ (942 mg, 2.55 mmol, 3.0 eq) in dry acetonitrile (30 mL) was boiled under reflux for 5 h when TLC (1:9:90 concentrated aqueous ammonia-MeOH-$CHCl_3$) indicated the reaction to be complete. The solvent was evaporated in vacuo and the residue was partitioned between ether (100 mL) and water (50 mL). The ether layer was dried (Na_2SO_4) and the solvent was evaporated in vacuo to give an oily residue (270 mg). The crude product was purified by column chromatography on silica gel eluting with hexane-ethyl acetate (3:1) to give pure **4** (190 mg, 58%) as a colorless solid. Crystallization from hot 2-propanol afforded **4** as microcrystals: mp 149-150 °C (dec); ^{1}H-NMR ($CDCl_3$) δ 7.20 (t, J_{app}=7.9 Hz, 1H), 6.85 (d, J=8Hz, 1H), 6.78 (m, 1H), 6.71 (dd, J=2.2,7.9 Hz, 1H), 4.06 (m, 1H). 2.72 (m, 2H), 2.13 (m, 4H), 1.99 (m, 4H), 1.18-1.80 (complex m, 9H); CIMS (MH^+ calcd for $C_{17}H_{24}INO$): 386. Found: 386 (MH^+); Anal (calcd for $C_{17}H_{24}INO \cdot 0.5C_3H_8O$): C 53.56, H 6.68, N 3.38%. Found: C 53.53, H 6.56, N 3.62%.

[^{123}I]1-[1-(3-Hydroxyphenyl)cyclohexyl]-4-iodopiperidine ([I^{123}]4).

A solution of precursor **8** (100 mg, 0.000389 mmol) in dry acetonitrile (100 μL) was added to a V-shaped vial containing anhydrous Na^{123}I (1.0 mCi, no carrier added[11]). The reaction was heated at 75 °C for 45 min, cooled, and then applied to a silica gel TLC plate. The plate was eluted with 1:9:90 concentrated aqueous ammonia-MeOH-$CHCl_3$, and the product comigrating with unlabelled **4** was removed and extracted with the same solvent used for elution. The solvent was evaporated under a stream of argon and the residue was dissolved in ethanol prior to storage at -78 °C. The radiochemical yield was found to range from 30 to 40% with >98% radiochemical purity.

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11. The supplier of $Na^{123}I$ (Nordion International, Vancouver, Canada) manufactures no carrier added material which has the theoretical specific activity of 240000 Ci/mmol. Since ^{123}I decays directly to ^{123}Te, there is no effective change in the specific activity either of the $Na^{123}I$ or iodinated compound derived from it.

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