Synthesis and Evaluation of [^{3}H]-4-Fluoro-1-[1-(3-hydroxyphenyl)cyclohexyl]piperidine, a Potential Tool for Autoradiographic Study of the Phencyclidine Receptor.

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SUMMARY

The synthesis and *in vitro* binding of high specific activity tritium labelled 4-fluoro-1-[1-(3-hydroxyphenyl)cyclohexyl] piperidine ([^{3}H]FOH-PCP), a potential probe for autoradiographic study of the phencyclidine (PCP) receptor is described. [^{3}H]FOH-PCP will allow evaluation of [^{18}F]FOH-PCP as a PET scanning ligand for PCP receptors.

Key Words: Tritium Labelled 4-Fluoro-1-[1-(3-hydroxyphenyl)cyclohexyl]piperidine, Autoradiographic, Phencyclidine (PCP) Receptor, PET Scanning Ligand

INTRODUCTION

Phencyclidine [1-{1-Phenylcyclohexyl}piperidine] (PCP) was originally developed as an anaesthetic agent for human use, but was withdrawn from the market after the observation of bizarre dissociative effects in patients after their emergence from the anaesthesia. Today, phencyclidine has become a major drug of abuse that is rivaled only by drugs such as cocaine and heroin. Although a putative phencyclidine receptor has been discussed [1,2] and a great deal of research has been carried out to delineate the mode of action of this drug, the exact mechanisms through which it acts are still unclear. In order to more fully understand the physiological effects produced by PCP and related compounds, knowledge of the regional distribution of the binding sites for PCP-like ligands in the central nervous system is essential.

The location of phencyclidine binding sites in brain has been identified in brain slice experiments using [^{3}H]TCP, a high specific

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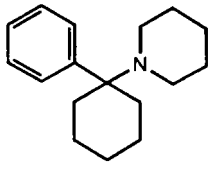
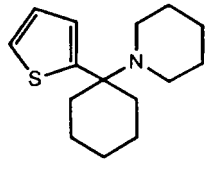
activity tritium labelled analog of PCP [3]. The main excitatory neurotransmitters in the brain, L-aspartic acid and L-glutamic acid exhibit neurotoxic activity through excitatory synaptic receptors known as N-methyl-D-aspartate (NMDA) receptors; these excitatory amino acids have been implicated in brain damage associated with anoxia, hypoxia, epilepsy and hypoglycemia [4]. PCP [5] and (+)-5-methyl-10,11-dihydro-5H-dibenzocycloheptene-5,10-imine (MK801) [6] have been shown to be potent non-competitive antagonists at the NMDA receptor. Furthermore, a high degree of correlation has been observed between the regional distribution of PCP binding sites and NMDA receptors [7] and there is evidence that at least some of the effects of PCP are mediated through non-competitive interaction with NMDA sites [8].

Thus a positron emitting [^{18}F] labelled PCP/NMDA ligand would provide valuable information as to the localization of NMDA receptors in living brain by a technique known as positron emission tomography (PET). We have previously employed this technique with great success in the PET visualization of opioid receptors in primates using 6-β-^{18}F-labelled 6-desoxynaltrexone ([^{18}F]cyclofoxy) [9]. More recently, N-(3-([^{18}F]fluoropropyl)-N-nordiprenorphine has also been characterized as an agent for PET scanning of opioid receptors [10].

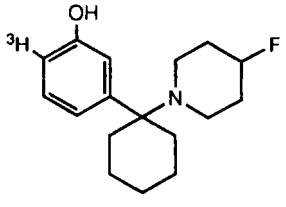
In order to evaluate the utility of an [^{18}F]-labelled receptor probe as a PET ligand, it is initially desirable to study the binding and *in vivo* biodistribution of the tritium labelled form. In light of this, we recently reported the synthesis of [^{3}H]4-fluoro-1-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]FTCP), an analog of the highly potent and selective PCP receptor ligand 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) [11]. Binding experiments indicated that FTCP (K_i=42 nM against [^{3}H]TCP) was twice as potent as PCP (Table 1) and preliminary experiments with [^{3}H]FTCP indicated that it localized in discreet brain regions known to contain PCP binding sites [12].



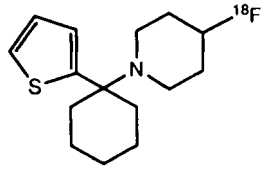
PCP



TCP



[^{3}H]-FOH-PCP



[^{18}F]-FTCP

In this study, we report the synthesis of [^{3}H]-4-fluoro-1-[1-(3-hydroxyphenyl)cyclohexyl]piperidine ([^{3}H]FOH-PCP), a compound which exhibits similar *in vitro* receptor affinity to FTCP, but which differs by virtue of its amphoteric character. Thus, FOH-PCP displaced [^{3}H]TCP with a K_i of 38 nM (Table I) and showed a degree of polarity comparable to our opiate based PET ligand, cyclofoxy. [^{3}H]FOH-PCP is presently being evaluated for its ability to localize in brain areas rich in PCP binding sites

CHEMISTRY

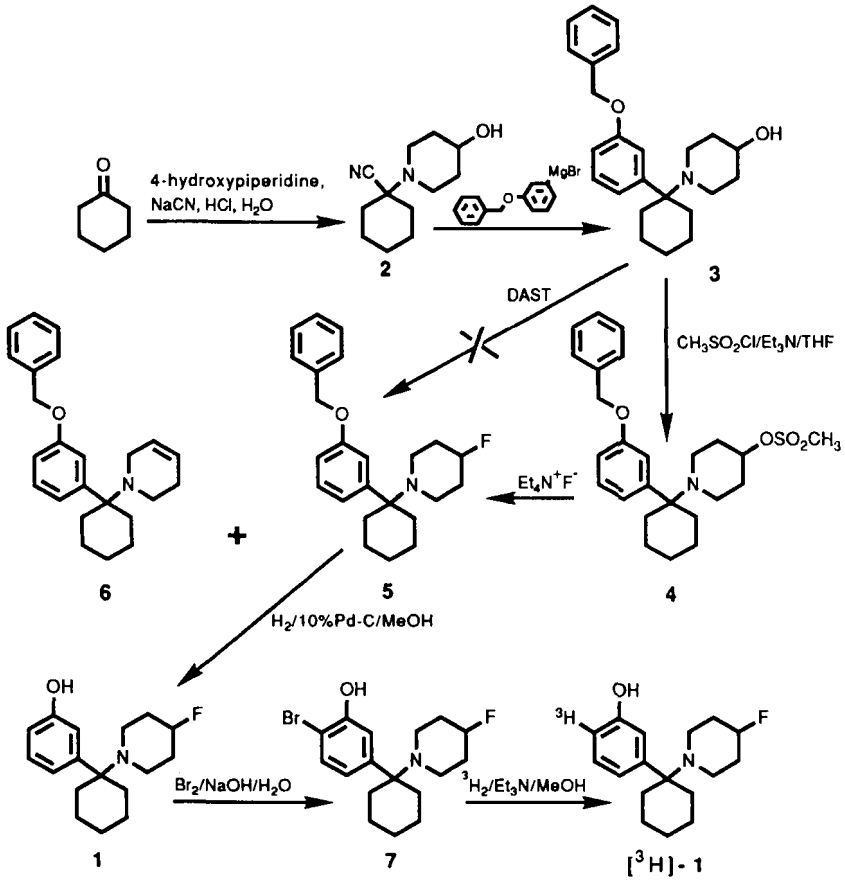
The synthetic route to both unlabelled and tritium labelled **1** utilized the Bruylants reaction [13] as the key reaction step. Thus, condensation of cyclohexanone with a mixture of 4-hydroxypiperidine and sodium cyanide at pH 3 gave an 84% yield of pure nitrile **2**. Reaction of this nitrile with 3 molar equivalents of the Grignard reagent prepared from 3-benzyloxy-bromobenzene afforded 4-hydroxy-1-[1-{3-(benzyloxy)phenyl}cyclohexyl]piperidine (**3**) in 29% recrystallized yield based on the amount of starting material **2**. Treatment of **3** with methanesulfonyl chloride in the presence of triethylamine afforded methanesulfonate ester **4** in quantitative yield. No attempt was made to further purify this material because of its instability. Reaction of **4** with excess tetraethylammonium fluoride at 55 °C for 48 h resulted in complete consumption of the starting material and a 12:88 ratio (^{1}H-NMR) of 4-fluoro-1-[1-{3-(benzyloxy)phenyl}cyclohexyl]piperidine **5** to the corresponding elimination product **6**. Complete separation of **5** and **6** was achieved by hydroboration of the product mixture followed by chromatographic separation. Attempts to replace directly the hydroxy group of **4** with fluorine by treatment with diethylaminosulfur trifluoride (DAST) resulted in decomposition of the starting material to a mixture of products. The desired 4-fluoro-1-[1-(3-hydroxyphenyl)cyclohexyl]piperidine (**1**) was synthesized in 88% recrystallized yield by catalytic hydrogenation of the HCl salt of **5** in MeOH in the presence of 10% Pd/C. Bromination of a solution of **1** in aqueous NaOH afforded a quantitative yield of monobromide **7**. Attempts to di- or tri-brominate **1** either by addition of excess bromine or by use of the brominating reagent N,N'-dibromoisocyanuric acid [14] resulted in complete decomposition of the starting material to give largely neutral products. Catalytic hydrogenolysis of **7** in MeOH in the presence of 10% Pd/C over 1 h resulted in regeneration of **1**. Attempts to overhydrogenate **7** by leaving the reaction overnight resulted in formation of 3-cyclohexylphenol as the only product. Thus, catalytic tritiolysis of **7** during 1 h with carrier free tritium gas afforded an 8.84% radiochemical yield of radiochemically pure (>99.5%) [^{3}H]-**1** with a specific activity of

23 Ci/mmol. This specific activity corresponds to 79.4% isotopic incorporation. [^{3}H]-**1** showed no significant decomposition after several months when stored at -30 °C in 100% ethanol at a dilution of 0.72 mCi/mL.

DISCUSSION

The synthetic route employed (Scheme I) indicated that the methanesulfonate ester **4** served successfully as a precursor for the introduction of a fluorine atom in the 4-position of the piperidine ring of **5**. The low percentage yield in the formation of **5** from **4** resulted from a competing fluoride promoted E_2-elimination of $MeSO_3H$ resulting in **6** as the major product. Attempts to directly convert alcohol **3** to the fluoride **5** with DAST resulted in decomposition. Hydroboration served admirably as a method to separate the olefin **6** from **5** [15].

Scheme 1: Synthesis of [^{3}H]4-fluoro-1-[1-(3-hydroxyphenyl) cyclohexyl]piperidine {[^{3}H]FOH-PCP}



Hydrogenolysis of **5** to the desired **1** was effected without loss of the fluorine atom because of the high stability of the F-C bond compared with typical carbon-halogen bonds. In order to maximize the specific activity of the final product, it was necessary to maximize the number of bromine atoms in our tritiation precursor. Unfortunately, attempts to di- or tribrominate **1** were unsuccessful since the 'activated' 3- and 6-positions on the aromatic ring are sterically hindered by their ortho location to the bulky tertiary carbon atom. The low (8.84%) radiochemical yield of [^3H]-**1** from catalytic tritiolysis of **7** can be accounted for by formation of 3-cyclohexylphenol as a competing side-reaction. The side reaction could be observed in model experiments even after relatively short hydrogenation times (< 1h) for the precursor, **7**. A less than theoretical incorporation of tritium into the product can be rationalized on the basis of catalytic dehydrogenation of the MeOH solvent [16].

Examination of **1** for affinity at the PCP receptor (Table I) indicated that it displaced the PCP receptor ligand [^3H]TCP with a Ki of 38 nM while FTCP exhibited a K_i of 45 nM. This compound should complement FTCP and provide valuable information on the effect of lipophilicity on receptor localization of radioligands for the PCP receptor from the same structural class.

BIOLOGICAL EVALUATION

Analysis of phencyclidine binding (Table I) by displacement of [^3H]1-[1-(2-thienyl)cyclohexyl]piperidine ([^3H]TCP) (SA=42.2 Ci/mmol) was performed in rat brain membranes as previously described [17].The inhibition constant (K_i) for determination of the affinity of compounds for the PCP receptor was calculated from the Cheng-Prusoff equation [18] by use of our K_d for TCP (16.5 nM) determined by Scatchard analysis. K_i determinations are the average of two experiments, each performed in triplicate.

Table I. Displacement of ([^3H]TCP) by FOH-PCP and related PCP receptor ligands

ligand	K_i, nM
FOH-PCP	38
PCP	70
TCP	16.8
FTCP	42

GENERAL EXPERIMENTAL DETAILS

Melting points were determined on a Thomas Hoover capillary apparatus and are uncorrected. Combustion analyses were determined at Atlantic Microlabs, Atlanta, GA. Chemical ionisation mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were taken from $CDCl_3$ solutions of compounds using a Varian XL-300 spectrometer. Infra-Red (IR) spectra were obtained from $CHCl_3$ solutions of compounds using a Beckman 4230 IR spectrometer. Ultra violet (UV) spectra were recorded from methanol solutions (unless otherwise stated) using a Hewlett-Packard 8450A UV/VIS spectrophotometer. Analytical thin layer chromatography (TLC) was performed on 250 μM Analtech GHLF silica gel plates. TLC plates were analysed 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 labeled compounds were counted in Hydrofluor scintillation cocktail (National Diagnostics) with a counting efficiency of 45%. All synthetic and analytical operations were initially performed with unlabeled compounds and the structures were confirmed spectroscopically.

1-[1-(4-Hydroxypiperidinyl)cyclohexyl]nitrile (2)

To a stirred mixture of 4-hydroxypiperidine (24.9 g, 246.2 mmol) in distilled water (100 mL) was added, dropwise, concentrated aqueous HCl (20.7 mL, 246.2 mmol) and the solution was adjusted to pH 3 by addition of a few drops of concentrated aqueous HCl. Cyclohexanone (24.2 g, 246.2 mmol) was added followed by a solution of NaCN (12.67g, 1.05 eq) in distilled water (100 mL). The mixture was stirred overnight resulting in a crystalline suspension of the product. The solution was filtered and the filter cake was washed until it gave a negative silver nitrate test (for Cl^-). The product was air dried for 2 days at room temperature, yield 43.2 g (84%): mp 108-109 °C; ^{1}H-NMR δ 3.73 (m, 1H, CHOH), 2.97-3.03 (m, 2H), 2.32 (m, 2H), 2.13 (m, 2H), 1.93-2.08 (m, 2H), 1.73-1.82 (m, 2H), 1.79-1.25 (complex m, 9H); EI (M^+=208, $C_{12}H_{20}N_2O$ requires 208). Anal (calc for $C_{12}H_{20}N_2O.0.75H_2O$): C 64.98, H 9.77, N 12.63%; Found: C 64.90, H 9.76, N 12.76%.

4-Hydroxy-1-[1-{3-(benzyloxy)phenyl}cyclohexyl]piperidine (3)

A stirred solution of *m*-(phenoxy)phenyl bromide (57g, 166.2 mmol) in dry THF (100 mL) was added dropwise at room temperature to a stirred suspension of Mg turnings (excess) in dry THF (50 mL) at such a rate as to maintain a controlled rate of reflux. After the solution had cooled to room temperature, it was placed in an ice-bath and and a solution of nitrile **2** (15 g, 72.1 mmol) in 50 mL of dry THF was added,

dropwise. The ice-bath was removed after the addition was complete, and the solution was allowed to stand overnight at room temperature. The reaction was quenched into a mixture of ice (200 g) and NH_4Cl (50 g). The aqueous mixture was extracted with (2 x 200 mL) of CH_2Cl_2 and the combined organic extract was back washed with 100 mL of water and evaporated to give impure crystalline product **3** (contaminated with unreacted nitrile **2**). The crude residue was dissolved in 200 mL of 10% aqueous acetic acid and washed with 4 x 200 mL of ether. The ether extract was discarded and the acetic acid layer was basified by addition of excess NH_4OH. Extraction of the basified solution with 2 x 200 mL of CH_2Cl_2 followed back washing with 200 mL of water and evaporation of the solvent afforded 20.4 g (78.5%) of crystalline product. Recrystallization from hot 2-propanol/isooctane (1 : 4) (200 mL) afforded analytically pure **3**: mp 129-130 °C; ^{1}H-NMR δ 7.22-7.46 (complex m, 6H), 6.85-6.90 (m, 3H), 5.07 (s, 2H, Ph$\underline{CH_2}$), 3.41 (m, 1H, $\underline{CH}$OH), 2.75-2.79 (m, 2H), 2.00 (m, 4H), 1.93-1.26 (complex m, 13H); EI (M^+=365, $C_{24}H_{31}NO_2$ requires 365). Anal (calc for $C_{24}H_{31}NO_2$.0.25 H_2O): C 77.91, H 8.58, N 3.79%; Found: C 77.93, H8.60, N3.87%.

4-Methanesulfonyloxy-1-[1-{3-(benzyloxy)phenyl}cyclohexyl] piperidine (4)

Methanesulfonyl chloride (0.51 mL, 6.59 mmol) was added dropwise (via syringe) at room temperature to a stirred solution of **3** (2.0 g, 5.48 mmol) and triethylamine (3.05 mL, 21.8 mmol) in dry THF (20 mL). After 10 min, the reaction was complete by TLC ($CHCl_3$/MeOH/NH_4OH 90 : 9 : 1). The reaction mixture was filtered and the filter-cake was washed with 50 mL of cold THF. The filtrate and washings were evaporated *in vacuo* to give **4** as a yellow oil (2.43 g, quantitative). No attempt was made to further purify this material because of the inherent lability: ^{1}H-NMR: δ 7.22-7.46 (complex m, 6H), 6.86-6.89 (m, 3H), 5.07 (s, 3H, Ph$\underline{CH_2}$), 4.45 (m, 1H, $\underline{CH}OSO_2Me$), 2.93 (s, 3H, $\underline{CH_3}SO_3$), 2.76 (br m, 2H), 2.64-1.23 (complex m, 15H); EI (M^+=443, $C_{25}H_{33}NO_4S$ requires 443).

4-Fluoro-1-[1-{3-(benzyloxy)phenyl}cyclohexyl]piperidine (5) and 1-[1-{3-(Benzyloxy)phenyl}cyclohexyl]-1,2,5,6-tetrahydropyridine (6)

To a stirred solution of methanesulfonate ester **4** (2.30 g, 5.19 mmol) in acetonitrile (20 mL) was added tetraethylammonium fluoride hydrate (5.45 g, 32.6 mmol), and the solution was heated to 55 °C and stirred under a nitrogen atmosphere for 2 d. TLC analysis ($CHCl_3$/MeOH/NH_4OH 98 : 1.8 : 0.2) of the reaction mixture revealed two products migrating very closely. The slower migrating product corresponded with the elimination product **6**. The solvent was

evaporated, excess NH_4OH (10 mL) was added, and the mixture was partitioned between ether (200 mL) and water (200 mL). The aqueous layer was discarded, and the ethereal layer was washed with water (100 mL) and evaporated *in vacuo*. The oily product was dried by azeotroping with ethanol (3 x 20 mL) and evaporated under high vacuum to give 1.70 g, 94% of an 88:12 ratio of elimination product **6** to fluoro substituted product **5**. Because of difficulty experienced in chromatographically separating **5** from **6**, the procedure described below was followed.

Separation of 5 and 6

Crude mixture **5/6** (2.08 g, 4.90 mmol) was dissolved in ether (20 mL), cooled to 0 °C and treated dropwise with 11.5 mL of a 1.0 M solution of borane-THF complex in THF (2 mol. eq. based on all of the mixture being in the form of **6**). After the addition was complete, the solvent was evaporated (*in vacuo*), and the residue was taken up in a mixture of ether (50 mL) and 15% aqueous NaOH (50 mL). The 2 phase mixture was stirred at room temperature and treated dropwise with 30% H_2O_2 (2.0 mL, 17.6 mmol). Vigorous effervescence occurred during the addition and TLC indicated that all of the elimination product had been hydroborated. The ether layer was separated, diluted to 200 mL with ether, and washed with 2 x 50 mL of water. Evaporation of the ether gave the crude product as a pale yellow oil. The crude product was dried by azeotropic distillation with 3 x 20 mL of ethanol. Purification by column chromatography, eluting with $CHCl_3$/MeOH/NH_4OH (95 : 4.5 : 0.5) afforded 300 mg (15.7%) of pure **5**. Crystallization from MeOH afforded an analytically pure sample: mp 104-105 °C; ^{1}H-NMR δ 7.27-7.46 (m, 6H), 6.85-6.90 (m, 3H), 5.07 (s, 2H, Ph$\underline{CH_2}$), 4.47 (dm, J=49 Hz, 1H, $\underline{CH}$F), 2.54 (br m, 2H), 2.04-1.25 (complex m, 16H); EI (M+=367, $C_{24}H_{30}FNO$ requires 367); Anal (calc for $C_{24}H_{30}FNO$): C 78.44, H 8.23, N 3.81%; Found: C 78.36, H 8.25, N 3.84%.

In order to characterize the elimination product **6**, a pure sample of **6** could be obtained by peak shaving of mixture **5/6** by partial chromatographic separation on silica-gel, eluting with $CHCl_3$/MeOH/NH_4OH (95 : 4.5 : 0.5). Crystallization from MeOH afforded **6** base: mp 75-76 °C; ^{1}H-NMR δ 7.47-7.24 (complex m, 6H), 6.95-6.85 (m, 3H), 5.61 (m, 2H, olefinic), 5.07 (s, 2H, Ph$\underline{CH_2}$), 2.94 (m, 2H), 2.35-2.22 (complex m, 4H), 2.05 (m, 2H), 1.89 (m, 2H), 1.69-1.21 (complex m, 6H); CIMS (M+H=348, $C_{24}H_{29}NO$ requires 347); Anal (calc for $C_{24}H_{29}NO$): C 82.95, H 8.41, N 4.03%; Found: C 83.04, H 8.43, N 4.00%.

4-Fluoro-1-[1-(3-hydroxyphenyl)cyclohexyl]piperidine (1)

5 (121.4 mg, 0.331 mmol) was suspended in MeOH (5 mL) and the suspension was acidified with conc. HCl (4-drops). Addition of the HCl

resulted in a homogeneous solution. The solution was flushed with N_2 and 12 mg of 10% Pd/C was added followed by an atmosphere of hydrogen. The reaction mixture was stirred overnight and the catalyst was removed by filtration of the solution through a bed of celite. The celite was washed with 5 mL of MeOH and the combined filtrate and washings were evaporated *in vacuo* to give a quantitative yield of **1**. Crystallization of the crude product from 5 mL of EtOAc afforded 87.6 mg (96%) of **1**.HCl: mp 193.5-194.5 °C (dec); ^{1}H-NMR (**1**.HCl) δ 7.57 (br s, 1H), 7.36-7.27 (complex m, 2H), 7.03-6.95 (m, 2H), 4.87 (dm, J=47 Hz, 1H, CHF), 3.50-3.36 (m, 2H), 2.91-2.67 (complex m, 4H), 2.30 (m, 2H), 2.05 (m, 2H), 1.81-1.18 (complex m, 8H); CIMS (M+H=278, $C_{17}H_{24}NOF$ requires 277); Anal (calc for $C_{17}H_{25}ClNOF.0.5H_2O$): C 63.25, H 8.12, N 4.34%; Found: C 63.21,H 8.18, N 4.10%.

4-Fluoro-1-[1-(4-bromo-3-hydroxyphenyl)cyclohexyl] piperidine (7)

1.HCl (68 mg, 0.24 mmol) was dissolved in water (5 mL) and treated with 10 mL of 10 % aq. NaOH to form a clear solution. The solution was treated dropwise at room temperature with a saturated solution of bromine in distilled water. Progress of the reaction was monitored continuously by TLC eluting with $CHCl_3$/MeOH/NH_4OH (90 : 9 : 1) until the reaction was found to be complete. Additional amounts of bromine water failed to effect any further change in the product. The reaction was poured into 50 mL of 10% aqueous NH_4Cl and extracted with $CHCl_3$ (3 x 20 mL). The organic layer was evaporated and the residue was applied to two 20 cm x 20 cm x 1 mm preparative TLC plates and chromatographed with $CHCl_3$/MeOH/NH_4OH (90 : 9 : 1). The major band was removed and the product was extracted with $CHCl_3$/MeOH/NH_4OH (80 : 18 : 2) (20 mL) and the solvent evaporated to give **7** (77 mg, 88%) as a colorless crystalline solid. Recrystallization from 2 mL of ethyl acetate-hexane (1:3) afforded an analytically pure sample: mp 154-156 °C (dec); ^{1}H-NMR δ 7.39 (d, J=8.4 Hz, 1H), 6.96 (d, J=2.2 Hz, 1H), 7.50 (dd, J=8.4 Hz, J=2.2 Hz, 1H), 4.46 (dm, J=49 Hz, 1H, CHF), 2.53 (br m, 2H), 2.15 (br m, 2H), 2.12-1.24 (complex m, 15H); CIMS (M+H=356, $C_{17}H_{23}{}^{79}BrFNO$ requires 355). Anal (calc for $C_{17}H_{23}BrFNO.0.25H_2O$): C 56.60, H 6.57, N 3.88%; Found: C 56.70, H 6.65,N 3.63%.

Trial Hydrogenolysis of 7 to 1

To a stirred solution of **7** base (5.0 mg, 0.014 mmol) and triethylamine (TEA) (10 μL) in MeOH (1 mL) was added 10% Pd/C (5 mg) and the solution was stirred at room temperature under an H_2 atmosphere. TLC analysis [$CHCl_3$/MeOH/NH_4OH (90 : 9 : 1)] revealed that the reaction was complete after 1 h at room temperature. The catalyst

was removed by filtration of the reaction mixture through a short column of celite. The celite was washed with a further 1 mL of MeOH and the combined filtrate and washings were evaporated *in vacuo* to give **1** identical to an authentic sample as synthesized above. Evaluation of the reaction mixture by GCMS revealed a side-product corresponding to 3-cyclohexylphenol. Attempts to overhydrogenate **7** by leaving the reaction overnight resulted in total loss of the fluoropiperidine moiety and formation of 3-cyclohexylphenol as the only product.

[^{3}H]-4-Fluoro-1-[1-(3-hydroxyphenyl)cyclohexyl]piperidine {[^{3}H]-1}

To a stirred solution of **7** base (10 mg, 0.0281 mmol) in methanol (1.0 mL) was added 20 μL of TEA and 10 mg of 10% Pd/C. The reaction mixture was stirred for 1 h at room temperature under an atmosphere of carrier-free tritium gas (10 Curies, 0.1724 mmol) and the solution was filtered, evaporated down under a gentle stream of N_2 gas to remove labile tritium and then reconstituted to 25 mL with MeOH for storage. Addition of 1 mL of glacial acetic acid acted as a suitable stabilizer. The solution was evaporated under a stream of N_2 and the residue was taken up in 5 mL of MeOH and treated with 2 drops of concentrated aqueous NH_4OH and evaporated under a stream of N_2. The residue was applied to one 20 cm x 20 cm x 1 mm preparative TLC plate and the plate was eluted with $CHCl_3$/MeOH/NH_4OH (95 : 4.5 : 0.5). The band co-migrating with the unlabelled reference **1** was scraped off and extracted with 10 mL of CH_2Cl_2/MeOH/NH_4OH (85 : 13.5 : 1.5). The extract was filtered and evaporated under a stream of N_2. The residue was taken up in CH_2Cl_2 (10 mL) and transferred to a new vial. The solvent was evaporated under a stream of N_2 and the residue was reconstituted to a volume of 100 mL with 100% ethanol for storage: yield of [^{3}H]-**1** 72 mCi (8.84%); radiochemical purity > 99.5% (by TLC analysis); specific activity 23 Ci/mmol (from ε_{281}=2799 $mol^{-1}.liter.cm^{-1}$); percentage incorporation of tritium=79.4%.

REFERENCES

1. Vincent, J. P., Kartalovsky, B., Geneste, P., Kamenka, J. M., Lazdunski, M., Proc. Natl. Acad. Sci. USA., 76: 4678 (1979).

2. Zukin, S. R., Zukin, R. S., Proc. Natl. Acad. Sci. USA., 76: 5372 (1979).

3. Contreras, P. C., Quiron, R., O'Donohue, T. L., Neurosci. Let., 67: 101 (1986).

4. McDonald, J. W., Silverstein, F. S., Johnston, M. V., Eur. J. Pharmacol., 141: 357 (1987).

5. Wong, E. H. F., Kemp, J. A., Priestley, J., Knight, A. R., Woodruff, G. N., Iversen, L. L., Proc. Natl. Acad. Sci. USA., 83: 7104 (1986).

6. Anis, N. A., Berry, S. C., Burton, N. R., Lodge, D., Br. J. Pharmacol., 79: 565 (1983).

7. Maragos, W. F., Chu, D. C. M., Greenamyre, J. T., Penney, J. B., Young, A. B., Eur. J. Pharmacol., 123: 173 (1986).

8. Contreras, P. C., Monahan, J. B., Lanthorn, J. H., Pullan, L. M., Di Maggio, D. A., Handelmann, N. M., Gray, N. M., O'Donohue, T. L., Mol. Neurobiol., 1: 191 (1987).

9. Burke, T. R., Jr., Rice, K. C., Pert, C. B., Heterocycles, 23: 99 (1985).

Pert, C. B., Danks, J. A., Channing, M. A., Eckelman, W. C., Larson, S. M., Bennett, J. M., Burke, T. R., Jr., Rice, K. C., FEBS. Let., 177: 281 (1984).

10. Chesis, P. L., Hwang, D. -R., Welch, M. J., J. Med. Chem., 33: 1482 (1990).

11. Thurkauf, A., de Costa, B. R., Kiesewetter, D. O., Jacobson, A. E., Rice, K. C., J. Labelled Comp. Radiopharm., 27: 653 (1988).

12. Ferrarese, C., Guidotti, A., Costa, E., Rice, K. C ., De Costa, B., Miletich, R. S., Di Chiro, G., Soc. Neurosci. Abs., in press.

13. Bruylants, P., Bull. Soc. Chim. Belges., 33: 467 (1924).

Yoshimura, J., Ohgo, Y., Sato, T., J. Am Chem. Soc., 86: 3858 (1964).

Yoshimura, J., Ohgo, Y., Sato, T., Bull. Chem. Soc. Japan, 38: 1809 (1965).

Hermans, B., van Deale, P., van de Westeringh, C., van der Eycken, C., Boey, J., Janssen, P. A. J., J. Med. Chem., 8: 851 (1965).

14. Gottardi, W., Monatsh. Chem., 98: 507 (1967).

15. Rice, K. C., personal communication.

16. de Costa, B. R., Lessor, R. A., Thurkauf, A., Highet, R. J., Jacobson, A. E., Rice, K. C., J. Labelled Comp. Radiopharm., 27: 1015 (1988).

17. Jacobson, A. E., Harrison, E. A., Jr., Mattson, M. V., Rafferty, M. G., Rice, K. C., Woods, J. H., Winger, G., Solomon, R. E., Lessor, R. A., Silverton, J. V., J. Pharmacol. Exp. Ther., 243: 110 (1987).

18. Cheng, Y.-C., Prusoff, W. H., Biochem. Pharmacol., 22: 3099 (1973).