SYNTHESIS AND EVALUATION OF FLUORINATED DERIVATIVES OF FENTANYL AS CANDIDATES FOR OPIATE RECEPTOR STUDIES USING POSITRON EMISSION TOMOGRAPHY

Dah-Ren Hwang[a], Anthony L. Feliu[b], Alfred P. Wolf, Robert R. MacGregor, Joanna S. Fowler*, Carroll D. Arnett
Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973 USA

M. Jean Holland, Kenneth Carr and Eric J. Simon
Department of Psychiatry, New York University Medical Center, 550 First Avenue, New York, NY 10016 USA

[a]Present Address: Department of Radiology, Washington University School of Medicine, St. Louis, MO 63110

[b]Present Address: Department of Neurology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021

SUMMARY

Three fluorinated derivatives of fentanyl, fluorofentanyl (3), keto-fluorofentanyl (5), and fluorofentanol (6), were synthesized and their abilities to compete with ^{3}H-diprenorphine for binding sites in guinea pig brain membranes were determined. The relative potencies were fentanyl > 3 ≅ 6 >> 5. On the basis of its apparent affinity for opiate receptors and its relative ease of synthesis, 6 was selected for further study. Fentanyl was slightly better than 6 in its ability to compete with [^{3}H]naltrexone for binding sites in rat brain membranes. Both fentanyl and 6 exhibited a similar high "sodium ratio" (quotient of the IC_{50}'s against [^{3}H]naltrexone in the presence and absence of sodium chloride) generally characteristic of opiate agonists. The analgesic potencies of fentanyl and 6 were determined in rats by measuring suppression of locomotion and vocalization responses to footshock. 6 appeared slightly less potent than fentanyl, but produced a similar analgesia and catalepsy which was entirely blocked by pretreatment of rats with naloxone, an opiate antagonist. A rapid synthesis of [^{18}F]-6 was developed and the tissue distribution of [^{18}F]-6 in mice was determined 5, 60, and 120 minutes after intravenous injection. The use of this general route to ^{18}F-labeled derivatives of fentanyl for studies of the opiate receptor using positron emission tomography is planned.

INTRODUCTION

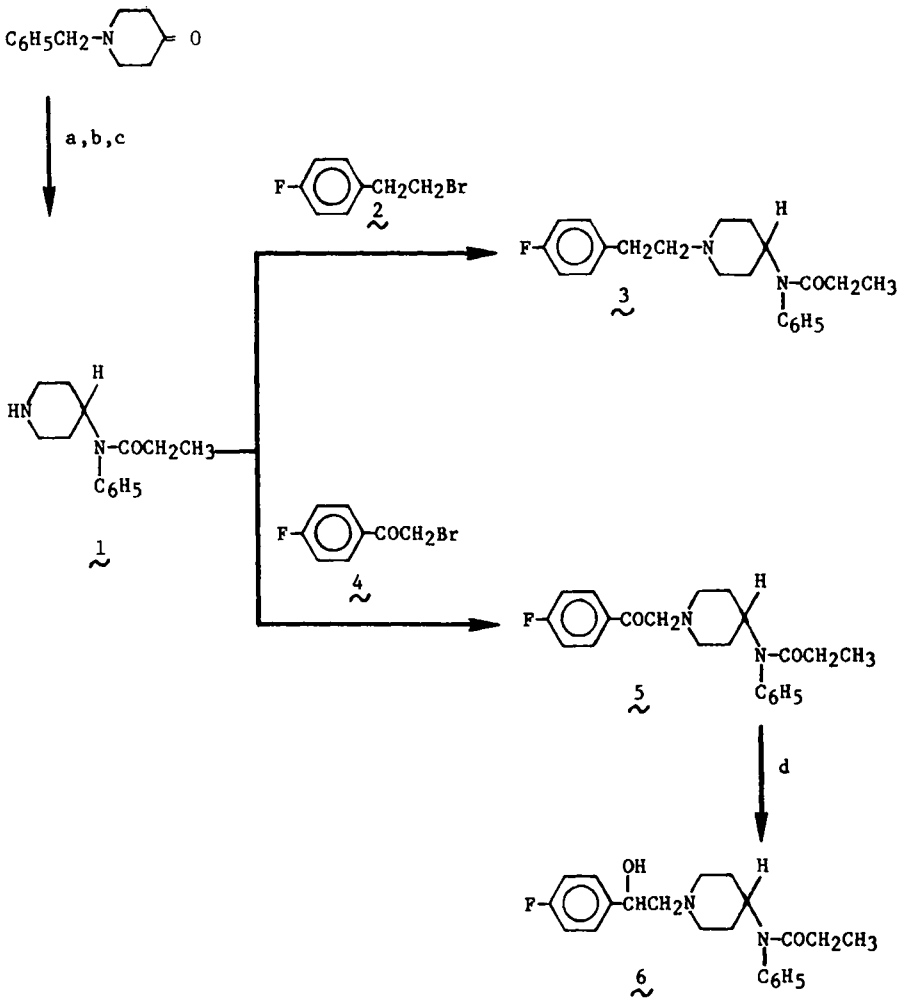
The development of rapid synthetic methods for introducing carbon-11 and fluorine-18 into opiate ligands has been undertaken by a number of investigators who plan to use the resulting radiotracers for the *in vivo* study of the opiate receptor using positron emission tomography (PET). Already a

Key Words: Opiate radioligands, fluorine-18 fentanyl derivatives

* Author to whom correspondence should be mailed.

0362-4803/86/030277-18$09.00 Received October 4, 1985

SCHEME 1



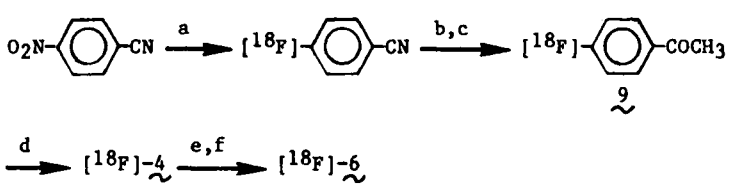
(a) $NaCNBH_3$, $C_6H_5NH_2$; (b) $(CH_3CH_2CO)_2O$; (c) H_2/Pd-C; (d) $NaBH_4$.

[^{18}F]Fluorofentanol and Related Compounds: Synthesis and Evaluation as Candidates for Opiate Receptor Studies Using Positron Emission Tomography

number of position-emitter labeled radioligands for the opiate receptor have been developed[1-5] and recently PET studies of the distribution of ^{11}C-labeled opiate ligands active tracers in the human[6] and baboon brain[7] were reported. Although both carbon-11 and fluorine-18 have been used in labeling neurotransmitter receptor ligands for positron emission tomography, recent PET studies of the dopamine receptor have shown the advantage of studying

radioligand - neurotransmitter receptor interactions for time periods of several hours in order to allow time for clearance of non-specifically bound radioactivity and to provide high contrast in the neurotransmitter rich areas.[8] Since studies of several hours or more are precluded by the half-life of carbon-11 (20.4 min) we are investigating the binding properties of fluorinated derivatives of opiate ligands which may be candidates for labeling with fluorine-18. We report here the synthesis of three fluorinated derivatives of fentanyl (Scheme 1), an opiate agonist with analgesic potency considerably greater that of morphine.[9] The affinities of these fentanyl derivatives for opiate receptors was assessed by competitive binding assays and their analgesic potencies were determined in rats by measuring thresholds for locomotion and vocalization in response to footshock. On the basis of these measurements and the rapid synthetic strategies currently available for labeling with fluorine-18 at high specific activity, one of these compounds, fluorofentanol (6), was selected for labeling with fluorine-18 and a rapid synthesis was developed (Scheme 2). The tissue distribution of [^{18}F]-6 in mice is reported.

SCHEME 2



(a) Cs[^{18}F], DMSO, 160°; (b) CH_3Li; (c) H_3O^+; (d) Br_2, HCl, HOAc, $CHCl_3$; (e) 1; (f) $NaBH_4$, CH_3OH

MATERIALS AND METHODS

All melting points are uncorrected. NMR spectra were run on a Varian HFT 80, mass spectra were run on a Finnigan 5100 and IR spectra were run on a Perkin Elmer Model 735B. Enriched water $H_2{}^{18}O$ (95-99%) was obtained from Mound Laboratory. [^{3}H]Naltrexone and [^{3}H]diprenorphine were obtained from

Dr. Richard Hawks (National Institute of Drug Abuse, Washington, D.C.). Carbon and hydrogen analyses were performed by Schwarzkopf Microanalytical Laboratory. Fluorine analyses were performed by ion chromatographic analysis of fluoride following sodium fusion.

Synthesis of N-(4-Piperidyl)Propionanilide (1): Compound 1 was synthesized in three steps from 1-benzyl-4-piperidone by the following modification of the literature procedure.[10] A solution of 1-benzyl-4-piperidone (Aldrich, 2 mL, 10.8 mmol), aniline (2 mL, 22 mmol) and glacial acetic acid (2 mL, 34 mmol) in methanol (50 mL) was stirred at room temperature for 30 min. To the mixture was added sodium cyanoborohydride (1.01 g, 15.9 mmol) in small portions. The mixture was stirred at room temperature for 10 h, an aqueous solution of sodium hydroxide (2N, 20 mL) was added and the resulting solution was stirred at room temperature for 15 min and concentrated *in vacuo*. The aqueous portion was extracted with ether and the ether extracts were combined, dried and concentrated *in vacuo* to leave an oil, which was recrystallized from ethanol and water to give N-(1-benzyl-4-piperidyl)aniline as a white solid (2.44 g, 85%):mp 84 - 84.5°C (lit.[10] 84.8 - 86°); IR (KBr) 3325, 1580 cm^{-1}; ^1H NMR ($CDCl_3$)δ 7.3 - 6.9 (a strong singlet at 7.3 overlaps with a m, 7H), 6.6 (m, 3H), 3.6 - 3.0 (a strong singlet at 3.52 overlaps with a m, 4H), 3.0 - 2.6 (m, 2H), 2.3 - 1.8 (m, 4H), 1.75 - 1.1 (m, 2H).

To a solution of N-(1-benzyl-4-piperidyl)aniline (0.4 g, 1.5 mmol) in benzene (5 mL) was added propionic anhydride (0.2 mL, 1.6 mmol) and potassium carbonate (0.7 g). The resulting mixture was refluxed for 8 h, 2 mL of NaOH solution (1N) was added and the mixture extracted with ether. The ether was dried and concentrated to produce an oil which dissolved in hot petroleum ether to produce a solution which was allowed to stand at 0° and then filtered. The filtrate was concentrated *in vacuo* to leave an oil which was purified by flash column chromatography (silica gel; methanol:ether:chloroform (1:20:20)) to produce N-(1-benzyl-4-piperidyl)propionanilide as an oil which was recrystallized from methanol and water to give a colorless solid (0.35 g,

73%): mp 72-73°C (lit[10] 74-76°); IR (KBr) 1650 cm^{-1}; ^1H NMR ($CDCl_3$)δ 7.5 - 6.8 (m, 10H), 4.9 - 4.3 (m, 1H), 3.4 (s, 2H), 3.0 - 2.65 (m, 2H), 2.3 - 1.5 (m, 6H), 1.5 - 1.1 (m, 2H), 1.0 (t, J=8 Hz, 3H).

A mixture of N-(1-benzyl-4-piperidyl)propionanilide (0.5 g, 1.6 mmol), Pd/C (0.1 g), glacial acetic acid (3 mL) and methanol (3 mL) was hydrogenated at 48 psi at 70°C for 11 h. The mixture was filtered, the catalyst was washed with methanol and the methanol solution was concentrated in vacuo. The residue was dissolved in ether and the ether layer was washed with aqueous sodium hydroxide (1N, 20 mL) then dried and concentrated in vacuo. The residue was dissolved in hot petroleum ether, and the solution was quickly cooled in a dry ice-acetone bath. The N-debenzylated amine (1) was obtained as colorless powder (0.34 g, 95%); mp 84-85°C (lit[10] 83-85°) IR (KBr) 3275, 1640 cm^{-1}; ^1H NMR ($CDCl_3$)δ 7.4-6.8 (m, 5H), 4.8-4.4 (m, 1H), 3.2-2.4 (m,4H), 2.2 (br. s, 1H) 2.1-1.55 (m, 4H), 1.5-1.1 (m, 2H), 0.9 (t, J=8 Hz, 3H).

N-(1-(β-(p-Fluorophenyl)ethyl)-4-piperidyl)propionanilide (fluorofentanyl) (3): The following modification of the literature method[11] was used. To a solution of 1 (0.22 g, 0.95 mmol) in tetrahydrofuran (THF, 5 mL) was added a mixture of β-(p-fluorophenyl)ethyl bromide[12] (2) (0.23 g, 1.13 mmol) in THF (5 mL) and potassium carbonate (0.2 g). The mixture was heated at reflux for 20 h. The mixture was filtered, and the filtrate was concentrated in vacuo. To the residue was added methanol and aqueous solution of sodium hydroxide (1N, 1 mL). The mixture was stirred well, and an aqueous solution of HCl (2N, 2 mL) was then introduced. Methanol was then removed in vacuo. To the remaining aqueous portion and white solids was added ether (30 mL) and water (10 mL). The mixture was cooled in an ice bath for 0.5 h and the solid was collected by filtration. (The solid was the HCl salt of the desired product, as indicated by ^1H NMR.) The solid was dissolved in methanol, and aqueous sodium carbonate was added until the aqueous solution was basic. The mixture was concentrated in vacuo to remove methanol. The remaining aqueous solution was extracted with ether (10 mL x 3). The ether extracts were

combined, dried over potassium carbonate and concentrated in vacuo to leave an oil, which was recrystallized in methanol and water to give a light yellow solid (0.30 g, 91%):mp 103-105°C (lit[11] 104-105°); IR (KBr) 1640 cm^{-1}; ^1H NMR ($CDCl_3$)δ 7.5-6.75 (m, 9H), 4.7 (tt, J=12.0, 4.0 Hz, 1H), 3.0 (br. d, 2H), 2.8-1.2 (m, 12H), 1.0 (t, J=8 Hz, 3H).

N-(1-(β-(p-Fluorophenyl)-β-oxoethyl)-4-piperidyl)propionanilide (Ketofluorofentanyl) (5): To a solution of 1 (0.26 g, 1.12 mmol) in ethanol (10 mL) was added a solution of p-fluorophenacyl bromide (4)[13] (0.11 g, 5.3 mmol) in ethanol (5 mL) and potassium carbonate (0.2 g). The mixture was stirred at 0-4°C for 0.5 h and at room temperature for 2 h. After filtration, the yellow filtrate was concentrated in vacuo to give a red oil, which was purified by flash column chromatography (silica gel; methanol:chloroform:ether (1:20:20). The desired fraction was collected and concentrated in vacuo. The residue was recrystallized from ethanol and water to give a yellow solid (0.37 g, 89%): mp 106-108°C; IR (KBr) 1680, 1620 cm^{-1}; ^1H NMR ($CDCl_3$)δ 7.9 (dd, J=6 Hz, 2H), 7.2 (m, 7H), 4.7 (tt, J=12, 5.0 Hz, 1H), 3.7 (s, 2H), 2.95 (m, 2H), 2.25 (dt, J=12.0, 4.0 Hz, 2H), 2.05 - 1.15 (m, 6H), 1.0 (t, J=8.0 Hz, 3H). Anal. Calcd. for $C_{22}H_{25}FN_2O_2$: C, 71.71; H, 6.84; F, 5.15. Found: C, 71.08; H, 7.18; F, 5.2.

N-(1-(β-(p-Fluorophenyl)-β-hydroxyethyl)-4-piperidyl)propionanilide (fluorofentanol) (6): To a solution of 5 (0.19 g, 0.52 mmol) in methanol (15 mL) was added sodium borohydride (0.16 mmol). The mixture was heated at 50-60°C for 2 h, an aqueous solution of HCl (2N, 10 mL) was added and the mixture was stirred at room temperature for 30 min. The methanol was then removed in vacuo and the white solid residue was washed with ether and water, and filtered. The solid thus collected was dissolved in methanol (20 mL), and an aqueous solution of sodium hydroxide (2N, 3 mL) was added. Methanol was then removed in vacuo, and the aqueous residue was extracted with ether (10 mL x 3). The ether extracts were combined, dried and concentrated in vacuo. The residue was recrystallized from methanol and water to give white

plates (0.18 g, 93%):mp 112-114°C; IR (KBr) 3400, 1630 cm^{-1}; ^1H NMR ($CDCl_3$)δ 7.6-6.2 (m, 9H), 4.9-4.3 (m,2H), 3.1 (m, 2H), 2.95 - 1.2 (m, 11H), 1.0 (t, J=8 Hz, 3H). Anal Calcd. for $C_{22}H_{27}FN_2O_2$: C, 71.32; H, 7.35, F, 5.12. Found: C, 71.43; H, 7.42; F, 5.2.

Synthesis of [^{18}F]Fluorofentanol ([^{18}F]-6): Fluorine-18 produced using the $^{18}O(p,n)^{18}F$ reaction[14] on ^{18}O enriched water (95-99%) in a titanium target[15] was added to 2 mg of Cs_2CO_3 in a borosilicate glass crucible. The solution was evaporated to dryness at 140° under a stream of nitrogen, and further dried by coevaporation with acetonitrile (5 mL).

p-Nitrobenzonitrile (2 mg) in dry dimethylsulfoxide (0.2 mL) was added to the dried Cs[^{18}F] crucible and heating was continued for ten minutes. After cooling, the reaction mixture was diluted with water (3 mL) and passed through a C-18 Sep-pak cartridge and rinsed with water (2 mL). p-[^{18}F]Fluorobenzonitrile was eluted from the cartridge with pentane (5 mL) which was passed through a K_2CO_3 drying tube.

Methyllithium (0.4 mL, 1.4 M in ether) was added, followed by hydrochloric acid (0.6 mL, 2 M in 50% aqueous methanol). The pentane was removed using an oil bath heated to 80° and the hydrolysis was allowed to continue at 80°C for nine minutes. After cooling, the mixture was diluted with water (5 mL) and passed through a C-18 Sep-pak cartridge which was washed with water (2 mL). p-[^{18}F]Fluoroacetophenone was recovered by elution with chloroform (5 mL) which was dried with potassium carbonate.

Bromine (0.8 M in 0.2 mL HOAc containing 5% HCl) was added to the $CHCl_3$ solution and the resulting solution heated at reflux for nine minutes. Unconsumed bromine was decomposed with propene. The reaction mixture was diluted with methanol (4 mL) and concentrated to 0.5 mL at 80°C under a stream of nitrogen to remove chloroform. Sodium bicarbonate (5 mL, saturated solution) was added to the residue and this was passed through a C-18 Sep-pak cartridge which was then rinsed with water (2 mL). p-[^{18}F]Fluorophenacyl

bromide ([^{18}F]-4) (radio-HPLC solvent system assay 85%) was eluted from the cartridge with chloroform (5 mL) and dried with potassium carbonate.

N-(4-Piperidyl)propionanilide (1) (8 mg) in methanol (4 mL) was added to the chloroform eluant and the solution was concentrated to 0.5 mL at 80°C under a stream of nitrogen. The alkylation reaction was then allowed to continue under gentle reflux for ten minutes. Reduction of the ketone of the resulting ^{18}F-labeled amine ([^{18}F]-5) was accomplished by adding $NaBH_4$ (30 mg) to the reaction mixture and warming (60°C) for an additional ten minutes. Residual $NaBH_4$ was decomposed with HCl (0.6 mL, 6N); $NaHCO_3$ (200 mg) was added to make the solution alkaline and it was then passed through a C_{18} Sep-pak cartridge with water (7 mL). [^{18}F]Fluorofentanol ([^{18}F]-6) was eluted from the cartridge with chloroform (6 mL), and after drying with potassium carbonate, was adsorbed onto a silica gel Sep-pak. Elution with 3.8 M ammonia-methanol: chloroform (4 mL, 1:9) gave the crude product, which was then purified by preparative HPLC (20 x 250 mm Spherisorb-5μ ODS column, HPLC Technology, methanol:0.01 M pH 4 ammonium phosphate buffer (65:35), 4 mL/min) to afford radiochemically pure (by analytical radio-HPLC) [^{18}F]-6 in 15% radiochemical yield (calculated to end of cyclotron bombardment and based on the total amount of ^{18}F produced by the $H_2{}^{18}O$ target). Synthesis time: 2.5 h, total mass associated with the radioactivity measured by UV spectroscopy, calculated as fluorofentanol: 15 micrograms (40.5 nmol). Thus from 200 mCi of [^{18}F]fluoride, 30 mCi (decay corrected to EOB) of [^{18}F]-6 is produced with a specific activity of 0.74 Ci/μmol (EOB).

Competitive Binding Assays: Assays were performed using [^{3}H]naltrexone (27 Ci/mmol) and [^{3}H]diprenorphine (27 Ci/mmol). Stock solutions of the test compounds were prepared by dissolving each in a small amount of 0.1 N HCl and diluting to volume with distilled water. Crude membrane fractions were prepared from either rat or guinea pig brains as previously described.[16] The membrane preparations were stored in 0.32 M sucrose at -70°C until needed.

Duplicate samples (2 mL) of membrane containing 1.0 ● 0.1 mg/mL protein in 0.05 M Tris-HCl at pH 7.4 with 1 mM dipotassium EDTA were first incubated with 1 nM tritiated ligand at 22°C for 1.5 hours. Competition experiments were then carried out with six concentrations of each test drug ranging from 10,000 nM to 0.1 nM. To estimate nonspecific binding, some samples also contained 1000 nM unlabelled ligand. After incubation, samples were filtered through Whatman GF/B filters, which were rinsed twice with buffer (4 mL) and then dried. The radioactivity remaining on the filters was determined by liquid scintillation.

Analgesic Activity In Vivo: Footshock intensity thresholds for eliciting locomotion and vocalization in rats (male Sprague-Dawley, 200 grams) were determined. Each rat received an ascending series of 200 msec duration scrambled footshocks delivered through a grid floor. Shock intensity was varied in steps of 0.25 mA over trials which were separated by 30 sec intertrial intervals. Thresholds for locomotion and vocalization responses were taken as the first of two consecutive intensities to elicit such responses. Rats were then injected (s.c.) with one of three doses of fentanyl, one of four doses of fluorofentanol (6), or saline vehicle. Compounds were prepared for injection by dissolving in 2 or 3 drops of 0.1 N HCl and diluting with saline. The injection volume for all dosages was 2 mL/kg body weight. Twenty minutes after injection thresholds were again determined.

Tissue Distribution of [^{18}F]-6 in Mice: [^{18}F]-6 in saline (~ 150 μCi/mouse) was injected intravenously into male mice and animals (3/time point) were sacrificed at 5, 60 and 120 minutes post injection. Tissues were removed, blotted free of blood, weighed, and counted in an automatic gamma counter (Packard, Model 5230). The radioactivity in each sample was corrected to a common time and normalized to the total injected dose.

RESULTS AND DISCUSSION

Synthesis, Competitive Binding Assay and Analgesic Potency of Fluorinated Derivatives of Fentanyl: Fluorofentanyl (3), a compound which has been mentioned in the patent literature[11], was initially a target compound for labeling with fluorine-18.[17] It was prepared for competitive binding assays via the alkylation of N-(4-piperidyl)propionanilide (1) with p-fluorophenethyl bromide (2). Anticipating the time constraints imposed by the 110 min half-life of fluorine-18, a synthetic strategy for 3 based on the use of a more reactive alkylating agent, p-fluorophenacyl bromide (4) was proposed and was tested using the unlabeled compounds (Scheme 1). As predicted, the alkylation of 4 with 1 proceeded rapidly and in high yield to produce 5. Unfortunately, all efforts to reduce the p-fluorophenacyl group to the p-fluorophenethyl group were unsuccessful and either produced the benzylic alcohol or unreacted starting material. For example, using a model substrate, p-fluorophenacyl piperidine, catalytic reduction with palladium invariably returned starting material. Under forcing conditions as described by Rosenmund to reduce secondary phenacyl amines[18], slow decomposition of the starting material was observed. Other reduction strategies also failed. For example, although ketones have also been deoxygenated via the corresponding tosylhydrazone[19] by exposure to borohydride, the model substrate resisted tosylhydrazone formation. Furthermore, although triethylsilane-boron trifluoride[20] reduced p-fluoroacetophenone to p-fluoroethylbenzene in quantitative yield, p-fluorophenacyl piperidine afforded only the corresponding amino alcohol, even after prolonged reaction. Since nearly all known methods to reduce aryl ketones and alcohols rely on a protic or Lewis acid to complex with the departing oxygen and thereby increase the electrophilicity of the benzylic center, the resistance of this α-amino substituted aryl ketone can probably be attributed to quaternization of the tertiary amine making formation of the required vicinal incipient benzyl cation unfavorable.

Although 3 could not be synthesized using this route which was amenable to ^{18}F-labeling, the alcohol (6) and keto derivative (5) were readily prepared. Therefore they were candidates for ^{18}F-labeling with the provision that their potency relative to fentanyl was not reduced by the presence of a keto or hydroxyl group α to the aromatic ring.

The potency of the fluorinated analogs of fentanyl was evaluated *in vitro* with competitive binding assays, results of which are given in Table 1.

Table 1. Stereospecific Competitive Binding Assay (IC_{50}, nM)

Brain Membrane:	Rat			Guinea Pig
Ligand (1 nM):	[^{3}H]Naltrexone			[^{3}H]Diphenorphine
Additives:	---	100 mM NaCl	Sodium Ratio	
Fentanyl	30	630	21	110
Fluorofentanyl, 3				400
Keto-Fluorofentanyl, 5				20000
Fluorofentanol, 6	60	1400	23	400
Levorphanol	6	100	17	
Naloxone	16	11	<1	

For comparison, fentanyl, levorphanol (a typical agonist), and naloxone (a typical antagonist) were also assayed. Analogs 3 and 6 were found approximately one-half as potent as fentanyl, with keto compound 5 at least two orders of magnitude less potent. The sodium ratio, a powerful predictor of agonist-antagonist properties[16,21], remained essentially unchanged among the four compounds and falls in the range characteristic of "pure agonists."

The analgesic effects of fluorofentanol (6) and fentanyl were compared *in vivo* by measuring footshock intensity thresholds for eliciting locomotion and vocalization responses in male Sprague-Dawley rats (see Table 2).
Both compounds produce clear analgesic and, at high doses, cataleptic effects although fluorofentanol (6) is somewhat less potent. In an additional experiment, administration of cataleptic doses of each compound was preceded (3 minutes) by injection of naloxone (1.0 mg/kg, s.c.). Rats treated in this

Table 2. Analgesic Effects* of Fentanyl and Fluorofentanol (6)

Dose	Locomotion		Vocalization	
	Fentanyl	Fluorofentanol	Fentanyl	Fluorofentanol
Vehicle	+0.5 (±5.3)		-2.0 (±5.8)	
0.01 mg/kg	+36 (±7.2)	+9 (±3.1)	+27 (±11.5)	+17 (±7.7)
0.05 mg/kg	+39 (±7.6)	+25 (±11.9)	+82 (±7.3)	+37 (±9.9)
0.10 mg/kg	profound catalepsy	+92 (±5.9)	profound catalepsy	+108 (±2.6)
0.50 mg/kg		profound catalepsy		profound catalepsy

*% change (+ s.e.m.) in threshold following injection.

manner, not only failed to display catalepsy, but also failed to display any changes in thresholds for eliciting the two pain responses. This antagonistic effect of naloxone confirms that the analgesia induced by both fentanyl and fluorofentanol is mediated by opiate receptors.

Based on these competitive binding assays and on measurements of analgesic activity, fluorofentanol (6) was determined to be of similar potency to the original target molecule fluorofentanyl (3) and was therefore selected for development of a rapid synthetic strategy for labeling with fluorine-18.

Synthesis of ^{18}F-Labeled 6: Since it was essential that the specific activity of the receptor active radiotracer be very high in order to avoid pharmacological effects, the labeling strategy was based on the use of ^{18}F-labeled fluoride which is available in very high specific activity up to (30 Ci/μmol) from an enriched water ($H_2{}^{18}O$) target.[15] Accordingly, the nucleophilic aromatic substitution reaction using no-carrier-added Cs[^{18}F][22] was chosen in a sequence which begins with the synthesis of *p*-[^{18}F]fluorobenzonitrile in 60-70% yield (Scheme 2). Elaboration of the nitrile group into a methyl ketone was then performed with methyllithium in the usual way.[23] Selective monobromination of the *p*-[^{18}F]fluoroacetophenone was accomplished using a solution of bromine-acetic acid containing a catalytic amount of hydrochloric acid[24] in refluxing chloroform. Chloroform proved to be the

Table 3. Biodistribution of [^{18}F]Fluorofentanol (^{18}F-6) in Mice at 5, 60 and 120 Minutes

	Time After Injection (Min)					
	5 (n=3)		60 (n=4)		120 (n=3)	
Tissue	%/g	%/organ	%/g	%/organ	%/g	%/organ
Blood	2.3±0.4		0.5 ±0.1		0.14±0.03	
Brain	3.3±0.1	1.5 ±0.1	0.23±0.04	0.11 ±0.02	0.07±0.02	0.03 ±0.01
Heart	4.1±0.1	0.53±0.10	0.5 ±0.1	0.07 ±0.01	0.13±0.03	0.015±0.00
Lungs	12 ±1	1.5 ±0.1	1.1 ±0.3	0.17 ±0.06	0.31±0.08	0.043±0.00
Liver	4.5±0.9	6.3 ±0.4	2.0 ±0.4	2.7 ±0.6	0.93±0.08	1.2 ±0.02
Spleen	7.2±0.8	0.78±0.08	0.9 ±0.2	0.10 ±0.02	0.24±0.09	0.024±0.00
Kidneys	16 ±2	6.3 ±0.4	2.6 ±0.7	1.1 ±0.4	2.6 ±2.0	1.1 ±0.9
Small Intestines	4.6±0.6	5.3 ±0.8	2.1 ±0.5	2.4 ±0.9	1.0 ±0.2	1.0 ±0.04
Testes	2.1±0.3	0.27±0.01	2.1 ±0.7	0.3 ±0.1	0.7 ±0.3	0.09 ±0.03
Muscle	2.7±0.3		0.53±0.17		0.19±0.05	

solvent of choice for avoiding overbromination of the NCA p-[^{18}F]fluoroacetophenone and consistently gave less than 5% dibromo, and greater than 85% monobromo ([^{18}F]-4) with the remaining 10% being unreacted starting material as determined by radio HPLC. Pure 4'-fluoro-2-bromoacetophenone (4), although stable in solution, even at elevated temperature, was particularly sensitive to decomposition by catalytic quantities of weak Lewis bases such as potassium carbonate and sodium acetate and therefore workup of the bromination reaction mixture was specifically designed to afford a salt free solution of p-[^{18}F]fluorophenacyl bromide, [^{18}F]-4. The alkylation of 1 with [^{18}F]-4 proceeded readily in methanol under gentle reflux to give [^{18}F]-5 which was reduced with sodium borohydride *in situ* to give [^{18}F]-6.

Crude [^{18}F]fluorofentanol ([^{18}F]-6) was purified by preparative reversed-phase HPLC to afford a radiochemically pure product in 15% overall yield. For injection, the radioactive fraction was evaporated to dryness with one drop of dilute hydrochloric acid, dissolved in isotonic saline, and sterilized by millipore filtration.

Tissue Distribution of [^{18}F]-6 in Mice: The time course of the tissue distribution of [^{18}F]-6 was measured at 5, 60 and 120 minutes post injection. Within one hour, the concentration in virtually all tissues had decreased dramatically (Table 3). These results are similar to those reported for the distribution of [^{3}H]fentanyl in rabbits.[25] In particular, brain levels of [^{3}H]fentanyl declined quickly, a finding consistent with the relatively short duration of pharmacological effects produced by fentanyl.[25] The similarly rapid egress of [^{18}F]-6 from mouse brain reported here appears to limit the usefulness of this radiotracer for PET studies of the opiate receptor in brain.

CONCLUSION

In summary, the introduction of a hydroxyl group α to the phenyl ring and a fluorine atom in the 4 position of the phenethyl group of fentanyl results in a small decrease in potency of the parent molecule as measured by competitive binding assays. The synthesis of the ^{18}F-labeled compound, although multistep, proceeds in relatively high overall yield and, since it proceeds through [^{18}F]-4, should provide a route to other fentanyl derivatives by varying the structure of the amine used in the alkylation step. Work is in progress to prepare a similar derivative of lofentanil, an opiate agonist with greater potency and far longer duration of pharmacological activity than fentanyl, to label it with fluorine-18 using the rapid synthesis reported here and to evaluate its suitability as a radiotracer for PET studies of the opiate receptor *in vivo*.

ACKNOWLEDGEMENT

This research was carried out at Brookhaven National Laboratory under contract DE-AC02-76CH00016 with the U. S. Department of Energy and supported by its Office of Health and Environmental Research and in part at New York University Medical Center and also supported by the National Institutes of Health, Grant NS-15638 and NS-15380. The authors thank Chyng-Yann Shiue for helpful discussions.

REFERENCES

1. Kloster G., Roder E., Machulla H.J. J. Label. Cmpds. Radiopharm. XVI: 441-448 (1979).

2. Moerlein S.M. and Stöcklin G. J. Label. Cmpds. Radiopharm. XXI: 875-887 (1984).

3. Frost J.J., Dannals R.F., Duelfer T., Burns H.D., Ravert H.T., Langström B., Balasubramanian V., Wagner H.N. Ann. Neurol. 15: S85-S92 (1984).

4. Pert C.B., Danks J.A., Channing M.A., Eckelman W.C., Larson S.M., Bennett J.M., Burke T.R. and Rice K.C. FEBS Letters 177 281-286 (1984).

5. Dannals R.F., Ravert H.T., Frost J.J., Wilson A.A., Burns H.D. and Wagner H.N. Int. J. Appl. Radiat. Isot. 36: 303-306 (1985).

6. Frost J.J., Wagner H.N., Jr., Dannals R.F., Ravert H.T., Links J.M., Wilson A.A., Burns H.D., Wong D.F., McPherson R.W., Rosenbaum A.E., Kuhar M.J. and Snyder S.H. J. Comput. Assist. Tomogr. 9: 231-236 (1985).

7. Hartvig P., Bergstrom K., Lindberg B., Lundberg P.O., Lundqvist H., Langstrom B., Svard H., Rane A. J. Pharm. Exp. Ther. 230: 250-255 (1984).

8. Arnett C.D., Shiue C.-Y., Wolf A.P., Fowler J.S., Logan J. and Watanabe M. J. Neurochem. 44: 835-844 (1985); Arnett C.D., Fowler J.S., Wolf A.P., Shiue C.-Y. and McPherson D.W. Life Sci. 36: 1359-1366 (1985).

9. Stahl K.D., VanBener W., Janssen D. and Simon E.J. Eur. J. Pharmacol. 46: 199-205 (1977).

10. Janssen P.A.J. French Patent 1,517,670, March 22, 1968; Chem. Abs. 71: 13026d (1969).

11. Janssen C. (N. V. Research Laboratorium). French Patent 1,517,671, 1968 (Chem. Abstr. 70 115015w (1969).

12. Compound 2 is prepared from p-fluorophenylacetic acid by lithium aluminum hydride reduction followed by phosphorus tribromide.

13. Lutz R.E., Allison R.K., Ashburn G., Bailey P.S., Clark M.T., Codington J.F., Deinet A.J., Freek J.A., Jordan R.H., Leake N.H., Martin T.A., Nicodemus K.C., Rowlett R.J., Shearer N.H., Smith J.D. and Wilson J.W. J. Org. Chem. 12: 617-703 (1947).

14. Ruth T.J. and Wolf A.P. Radiochim. Acta 26: 21-24 (1979).

15. Wieland B.W. and Wolf A.P. J. Nucl. Med. 24: P122 (1983).

16. Simon E.J., Hiller J.M., Groth J., Edelman I. J. Pharmacol. Exper. Therap. 192: 531-537 (1975).

17. Other derivatives of fentanyl having a fluorine atom on the N-phenyl ring have also been suggested as candidate structures for labeling with ^{18}F for PETT (Dannals R.F., Burns H.D., Pogun S., Frost J.J. and Wagner H.N. J. Label. Cmpds. Radiopharm. XIX: 1493-1494 (1982).

18. Rosenmund K. W., Karg E. Chem. Ber. 75: 1850-1859 (1942).

19. Hutchins R. O., Milewski C. A. and Maryanoff B. E. J. Amer. Chem. Soc. 95: 3662-68 (1973); Kabalka G. W. and Baker J. D., Jr. J. Org. Chem. 40: 1834-35 (1975); Rosini G. and Medici A. Synthesis 530-532 (1976).

20. Fry J. L., Orfanopoulos M., Adlington M. G., Dittman W. R., Jr., Silverman S.B. J. Org. Chem. 43: 374-375 (1978); Cf. West C. T., Donnelly S. J., Kooistra D. A., Doyle M. P. J. Org. Chem. 38 2675-2681 (1973).

21. Pert C. B. and Snyder S. H. Molec. Pharmacol. 10: 868-879 (1974).

22. Attina M., Cacace F. and Wolf A.P. J. Chem. Soc. Chem. Comm. 108-109 (1983); Attina M., Cacace F. and Wolf A.P. J. Label.Cmpds. Radiopharm. XX: 501-514 (1983); Shiue C.-Y., Watanabe M., Wolf A.P., Fowler J.S. and Salvadori P. J. Label. Cmpds. Radiopharm. XXI: 533-547 (1984); Shiue C.-Y., Fowler J.S., Wolf A.P., Watanabe M. and Arnett C.D. J. Nucl. Med. 26: 181-186 (1985).

23. Although [^{18}F]fluoride displacement on p-nitroacetophenone would have been a more direct route to p-[^{18}F]fluoroacetophenone, this reaction proceeded in unacceptably low yield.

24. Pinkus A.G. and Gopalan R. J. Amer. Chem. Soc. 106, 2630-2636 (1984); Cieciuch R.F.W. and Westheimer F.H. J. Amer. Chem. Soc. 85: 2591-2596 (1963).

25. Hess R., Herz A., Friedel K. J. Pharmacol. Exp. Therap. 179: 474-484 (1971).