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An Efficient Synthesis of N^{ω}-[^{18}F]Fluoroacetylserotonin (N^{ω}-[^{18}F]Fluoroacetyl-5-hydroxytryptamine)

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SUMMARY

A rapid synthesis of N^{ω}-[^{18}F]fluoroacetylserotonin (N^{ω}-[^{18}F]fluoroacetyl-5-hydroxytryptamine) starting from [^{18}F]fluoride and ethyl *p*-toluensulfonyloxyacetate is described. The total time required for its synthesis is *ca.* 90 min. The radiochemical yield, purity, and specific activity (end of bombardment) of the desired hormone are 13.5%, >98%, and 600 mCi/μmol, respectively.

Key Words: N^{ω}-[^{18}F]Fluoroacetylserotonin, N^{ω}-[^{18}F]fluoroacetyl-5-hydroxytryptamine, [^{18}F]fluoride, ethyl *p*-toluenesulfonyloxyacetate, N^{ω}-fluoroacetylserotonin.

INTRODUCTION

From tryptophan, serotonin(5-hydroxytryptamine) (1), a neurohumor and vasocontrictor in vertebrates, is formed, and its N^{ω}-acetyl derivative (2) is an important precursor for the biosynthesis of melatonin (N^{ω}-acetyl-5-methoxytryptamine) (3) (1). In a previous paper (2), we reported the labelling of this hormone (3) with fluorine-18 so providing a potential diagnostic imaging agent, [*fluoroactyl*-^{18}F]fluoromelatonin (4). We also reported the concise syntheses of [*carbonyl*-^{11}C]melatonin and N^{ω}-[*carbonyl*-^{11}C]acetylserotonin as imaging agents (3). The introduction of ^{18}F (β^{+} decay, $t_{\frac{1}{2}}$=110 min) at the terminal position of (2), metabolite of (1), is attractive for a diagnostic imaging agent in a positron emission tomography (PET) study.

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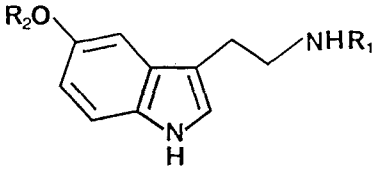
As part of the investigation of the synthesis of positron emitting compounds for PET study, this paper describes the rapid synthesis of N^{ω}-[^{18}F]fluoroacetylserotonin (N^{ω}-[^{18}F]fluoroacetyl-5-hydroxytryptamine) (5) from ethyl *p*-toluenesulfonyloxyacetate and [^{18}F]fluoride.



(1): R_1=R_2=H
(2): R_1=$COCH_3$, R_2=H
(3): R_1=$COCH_3$, R_2=CH_3
(4): R_1=$COCH_2{}^{18}F$, R_2=CH_3
(5): R_1=$COCH_2{}^{18}F$, R_2=H
(6): R_1=$COCH_2F$, R_2=H

RESULTS AND DISCUSSION

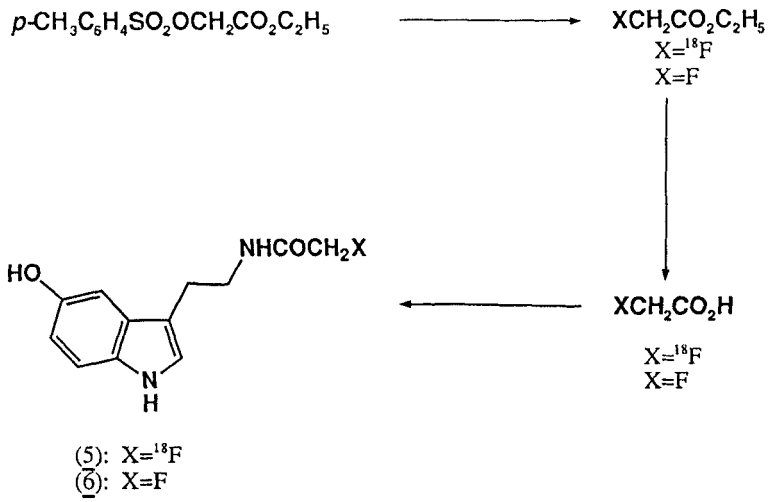
Either radioactive or unlabelled fluoro derivatives of indole ethylamine reported are ring-fluorinated compounds, such as 4-[^{18}F]fluoro- and 6-[^{18}F]fluoromelatonin (4), 6-fluoro[*carbonyl*-^{11}C]melatonin (5), 4-[^{18}F]fluoro- and 6-[^{18}F]fluoro-5-hydroxytryptophan (4), and 6-fluoro- and 4,6-difluoroserotonin (6), except for [*fluoroacetyl*-^{18}F]fluoromelatonin (4) and its unlabelled compound reported by us (2). Unlabelled N^{ω}-fluoroacetylserotonin (6), substituted with fluorine in the terminal position of the side-chain of (2), was synthesized from 5-hydroxytryptamine (1) with fluoroacetic acid by the ordinary method using dicyclohexylcarbodiimide (DCC). The yield of (6) based on (1) was 71.5%.

[^{18}F]Fluoride was produced by the ^{18}O(p, n)^{18}F nuclear reaction from a circulating 20%-enriched [^{18}O]water target using the Tohoku University Cyclotron (7). The ^{18}F nuclide thereby prepared was converted to potassium [^{18}F]fluoride with potassium carbonate. After addition of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosan (Kryptfix 222), the resulting mixture was evaporated to dryness. The residue and ethyl *p*-toluenesulfonyloxyacetate (derived from ethyl bromoacetate and silver *p*-toluenesulfonate) in anhydrous acetonitrile were heated at 82 °C for 10 min with stirring to give ethyl [^{18}F]fluoroacetate. The ester was then hydrolyzed with alkali, condensed with (1) in the presence of DCC, and subjected to preparative high performance liquid chromatography (HPLC) to afford the title compound (5) in a 13.5% radiochemical yield. The total synthesis time, radiochemical purity, and specific activity (end of bombardment) are *ca.* 90 min, >98%, and 600 mCi/μmol, respectively. The synthetic pathways of (5) and its radio-preparative HPLC chromatogram are shown in Figs. 1 and 2, respectively.

The medical use of (5) as a diagnostic imaging agent is being investigated and the result will be reported elsewhere.

Table 1. Retention Times of N^{ω}-Fluoroacetyl-5-hydroxytryptamine (6) in Various HPLC Systems

Run	Column (Size, mm)	Mobil Phase (Ratio, v/v)	Flow Rate (ml/min)	Retention Time (min)
1	Hiber Lichrosorb RP-18-7μm (4.0×250)	CH_3CN/H_2O (75/15)	1.0	2.40
2		CH_3CN/H_2O (60/40)	1.0	2.64
3	Hiber Lichrosorb RP-18-7μm (10.0×250)	CH_3OH/H_2O (80/20)	3.0	4.88
4		CH_3OH/H_2O (70/30)	3.0	5.10
5	Hiber Lichrosorb RP-8-7μm (4.0×250)	CH_3CN/H_2O (80/20)	1.0	2.70
6		CH_3CN/H_2O (75/25)	1.0	2.82
7		CH_3CN/H_2O (60/40)	1.0	2.88
8		CH_3CN/10 mM KH_2PO_4 aq. (60/40), pH=5.2	1.0	3.00
9		CH_3CN/0.05% CH_3CO_2H aq. (60/40), pH=4.5	1.0	2.94
10		CH_3CN/0.1% CH_3CO_2H aq. (60/40), pH=4.2	1.0	2.98
11		CH_3CN/0.05% CF_3CO_2H aq. (60/40), pH=2.3	1.0	2.98
12	Hiber Lichrosorb RP-8-7μm (10.0×250)	CH_3OH/H_2O (80/20)	3.0	5.52
13		CH_3OH/H_2O (70/30)	3.0	5.72
14		CH_3OH/H_2O (60/40)	3.0	6.30
15	YMC-Pak A-324 (10.0×300)	CH_3OH/H_2O (70/30)	3.0	5.30



(5): X=^{18}F
(6): X=F

Fig. 1. Synthetic Pathways of (5) and (6) from Ethyl *p*-Toluenesulfonyloxyacetate

EXPERIMENTAL

Silica gel 60, Kryptofix 222, Extrelut-3 columns, and TLC plates were purchased from E. Merck AG, Ger. Silver *p*-toluenesulfonate was from Sigma Chem. Co., USA. Ethyl bromoacetate was from Wako Chem. Ltd. Japan and distilled under reduced pressure. The other reagents were obtained commercially (Wako) and used without further purification. HPLC analyses were carried out either with a Waters Assoc. USA model 6000 equipped with a UV(254 nm) detector and a refractive index detector or with a Waters Assoc. model 4500 equipped with a UV detector and a radioactivity monitor. The packed columns [Cica-Merck Hiber Lichrosorb RP-18-7 μm and RP-8-7 μm (Kanto Chem. Co. Inc. Japan) and YMC-Pack A-324 (Yamamura Chem. Lab. Co. Japan)] were used in HPLC. Retention times of (6) in various HPLC systems are shown in Table 1. Absorption and ^{1}H NMR (90 MHz) spectra were recorded with a Hitachi Japan model U-3210 spectrophotometer and a JEOL Japan model FX90Q spetrometer, respectively. TLC analysis was carried out over a pre-coated silica gel $60F_{254}$ plate and its mobile phase was dichloromethane/ethanol (9/1, v/v). The detection was made with a UV lamp.

N^{ω}-Fluoroacetyl-5-hydroxytryptamine (6).

To a suspension of 5-hydroxytryptamine (1) hydrochloride (2.56 g, 12 mmol) in tetrahydrofuran(THF) (300 ml), was added sodium fluoroacetate (1.8 g, 18 mmol) in water (12 ml) and 3N hydrochloric acid (3 ml). DCC (5.15 g, 25 mmol) in THF (25 ml) was added to the resulting clear solution. The mixture was stirred overnight at room temperature and filtered. The filtrate was evaporated to dryness under reduced pressure and the residue was dissolved in a mixture of dichloromethane and ethanol (9/1, v/v). The resulting solution was chromatographed over a silica gel column (3×20 cm) to give oil of (6) (2.01 g) in a 71.5% yield. The oil is further purified by the use of preparative HPLC to give colorless glassy mass of (6).

Anal. Found C, 60.84; H, 5.64; N, 11.96%. Calcd. for $C_{12}H_{13}FN_2O_2$: C, 61.01; H, 5.55; N, 11.86%. UV(CH_3OH): λ_{max} nm(log ε) 222(4.38), 278(3.79), and 300(3.66). ^{1}H NMR [$CDCl_3$, internal $(CH_3)_4Si$]: δ 1.71(1H, broad s, -OH), 2.94(2H, t, J=7.0 Hz, side chain), 3.69(2H, t, J=7.0 Hz, side chain), 4.78(2H, d, J_{HF}=47.4 Hz, -CH_2F), 6.44(1H, broad s, =NH), 6.80(1H, dd, $J_{6,7}$=8.5 Hz, $J_{4,6}$=2.2 Hz, H-6),

7.02(1H, d, $J_{4,6}$=2.2 Hz, H-4), 7.23(1H, d, $J_{6,7}$=8.5 Hz, H-7), 7.26(1H, s, H-2), and 7.96(1H, broad s, =NH). TLC: R_f 0.58.

N^{ω}-[^{18}F]Fluoroacetyl-5-hydroxytryptamine (5).

[^{18}F]Fluoride was produced from the proton bombardment of 20% enriched [^{18}O]-water (5). To the aqueous solution of [^{18}F]fluoride, was added a mixture of aqueous potassium carbonate (33 μmol/0.2 ml) and Kryptofix 222 (27 mg, 72 μmol). The resulting solution was dried at 90 °C in a stream of dry nitrogen gas. To the residue, a solution of ethyl *p*-toluenesulfonyloxyacetate (5.4 mg, 21 μmol) in acetonitrile (1 ml) was added. The mixture was heated at 82 °C for 10 min with stirring and cooled. After addition of 1N aqueous potassium hydroxide (0.5 ml), the reaction mixture was heated for an additional 10 min and acidified with 2N hydrochloric acid (1 ml). The mixture was then charged on an Extrelut-3 column and eluted with ethyl ether. The effluent was evaporated to dryness and the residue was added to a mixture of (1) hydrochloride (5.3 mg, 25 μmol) in acetonitrile (1 ml) and DCC (103 mg, 0.5 mmol) in acetonitrile (1 ml). The mixture was heated at 82 °C for 10 min with stirring and cooled. After addition of water (2 ml), the mixture was filtered. The filtrate was passed through an Extrelut-3 column and eluted with a

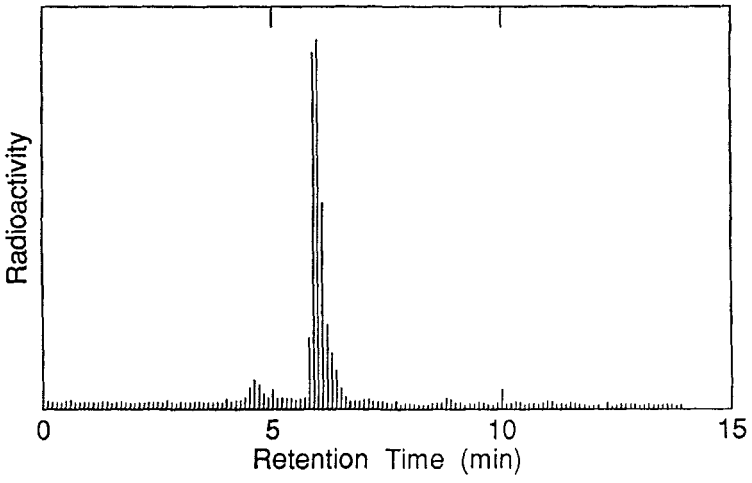


Fig. 2. Radio-preparative HPLC Chromatogram of Reaction Mixture.
The peak indicated with asterisk corresponds to the title compound (5), and chromatographic conditions are shown as Run 15 in Table 1.

mixture of dichloromethane and ethanol (25/1, v/v). The effluent was then passed through a Sep-Pak C_{18} cartridge (Waters Assoc. USA) and eluted with the same solvent. The eluting solution was evaporated to dryness under reduced pressure and the residue was dissolved in methanol/water (70/30, v/v)(0.5 ml). The solution was then subjected to preparative HPLC (Run 15 in Table 1). The radio-chromatogram is shown in Fig. 2. A radioactivity peak corresponding to (5) was then collected and the identity of the peak was confirmed by analytical HPLC (Run 7 in Table 1). The total synthesis time, the radiochemical yield and purity, and the specific activity (EOB) are *ca.* 90 min, 13.5%, >98%, and 600 mCi/μmol, respectively.

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