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A Rapid Synthesis of [*fluoroacetyl*-^{18}F]Fluoromelatonin (N^{ω}-[^{18}F]Fluoroacetyl-5-methoxytryptamine), a Potential Diagnostic Imaging Agent

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SUMMARY

An efficient synthesis of [*fluoroacetyl*-^{18}F]fluoromelatonin (N^{ω}-[^{18}F]Fluoroacetyl-5-methoxytryptamine) 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 compound are 14.2%, >98%, and 540 mCi/μmol, respectively.

Key Words: [*fluoroacetyl*-^{18}F]Fluoromelatonin, N^{ω}-[^{18}F]fluoroacetyl-5-methoxytryptamine, [^{18}F]fluoride, ethyl *p*-toluensulfonyloxyacetate, ethyl bromoacetate, N^{ω}-fluoroacetyl-5-methoxytryptamine.

INTRODUCTION

In previous papers (1,2), we reported the rapid and efficient one-pot syntheses of 2-deoxy-2-[^{18}F]fluoroacetamido-D-glucopyranose, -D-mannopyranose, and -D-galactopyranose, respectively, starting from [^{18}F]fluoride and ethyl bromoacetate. The D-glucopyranose derivative has potential as a tumor imaging agent, since mice bearing spontaneous hepatoma showed a high enough concentration of the sugar in the tumor for external detection and the rabbit with VX-2 tumor demonstrated clear positron emission tomography (PET) images (3).

The pineal gland hormone melatonin (N^{ω}-acetyl-5-methoxytryptamine)(1) has recently received much attention because it is an important neuroendocrine component of animal physiology (4). Melatonin (1), a metabolite of serotonin, is produced *in vivo* by *N*-acetylation and *O*-methylation. Recently we reported the concise

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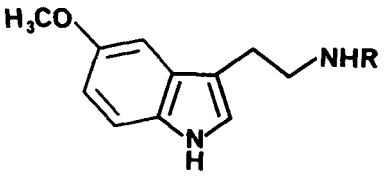


(4): R=H
(1): R=$COCH_3$
(3): R=$COCH_2F$
(2): R=$COCH_2{}^{18}F$

syntheses of [*carbonyl*-^{11}C]melatonin and *N*-[*carbonyl*-^{11}C]acetylserotonin as imaging agents (5). The introduction of ^{18}F (β^+ decay, $t_{\frac{1}{2}}$=110min) at the terminal position of (1) is attractive for a diagnostic imaging agent in a PET study.

As part of the investigation of the synthesis of positron emitting compounds for PET study, this paper describes the rapid synthesis of [*fluoroacetyl*-^{18}F]fluoromelatonin (N^{ω}-[^{18}F]fluoroacetyl-5-methoxytryptamine)(2) from [^{18}F]fluoride.

RESULTS AND DISCUSSION

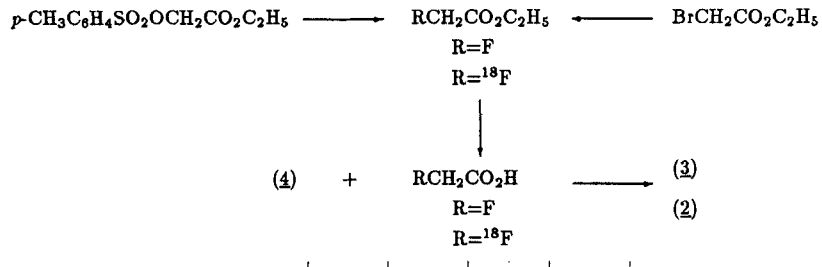
Radioactive halogen derivatives of (1), such as 4-[^{18}F]fluoro- (6), 6-[^{18}F]fluoro- (6), 2-[^{125}I]iodo- (7), and 6-fluoro[*carbonyl*-^{11}C]-melatonin (8), have been recently synthesized as radioactive probes for the elucidation of the mechanism of action of (1). The halogen in each compound is substituted in the indole ring of (1). One route for the metabolism of (1) is initiated by hydroxylation at the 6-position (9).

Unlabelled fluoromelatonin (N^{ω}-fluoroacetyl-5-methoxytryptamine) (3), substituted in the side-chain of (1), was prepared from 5-methoxytryptamine (4) with fluoroacetic acid by the ordinary method using dicyclohexylcarbodiimide (DCC). The yield of (3) based on (4) was 25%. We recently established the one-pot synthetic method for the introduction of a [^{18}F]fluoroacetyl group into aminosugar (1,2). The method is a combination of halogen exchange, alkaline hydrolysis, and condensation. This was applied to the syntheses of (3) and the title hormone (2) with some modifications. The one-pot synthesis from potassium fluoride and ethyl bromoacetate gave (3) in a 10% yield based on (4). The preparative HPLC chromatogram of (3) is shown in Fig. 1.

[^{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 (10). The ^{18}F nuclide thereby formed 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 (Kryptofix 222), the resulting mixture was submitted to the one-pot synthesis to afford the title hormone (2) in a 6.9% radiochemical yield (decay-corrected, based on [^{18}F]fluoride). The total time required for synthesis of (2) is *ca.* 90 min. The radiochemical purity is >95%.

As the reproducibility of the yield was invariably poor, the improvement was obtained by change of the substrate. The reaction of [^{18}F]fluoride with ethyl *p*-toluensulfonyloxyacetate derived from ethyl bromoacetate with silver *p*-toluensulfonate afforded ethyl [^{18}F]fluoroacetate, which was then hydrolyzed with alkali and condensed with (4) in the presence of DCC to give (2) in a 14.2% radiochemical yield. The purity and the specific activity(EOB) are >98% and 540 mCi/μmol, respectively.

Additionally, the latter method for the preparation of (2) could be adapted for automated synthesis. The medical use of (2) as a diagnostic imaging agent is being investigated and the result will be reported elsewhere.



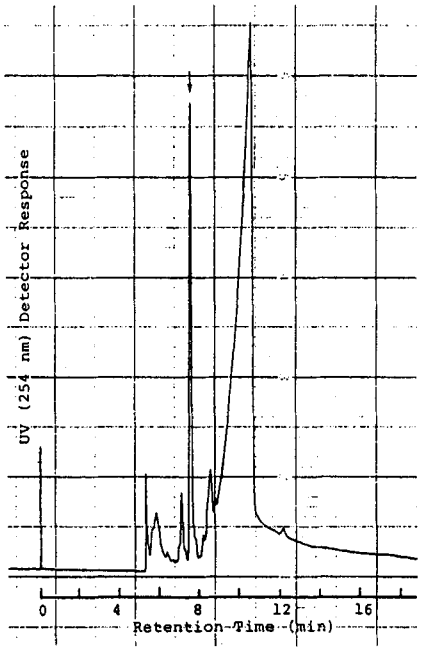


Fig. 1. Preparative HPLC Chromatogram of (3). Arrow indicates the peak corresponging to (3). The HPLC was carried out under the condition of Run 13 shown in Table 1.

EXPERIMENTAL

Kryptofix 222, silica gel 60, Extrelut-3 columns, and TLC plates were purchased from E. Merck AG, Ger. Ethyl bromoacetate was from Wako Chemical 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(254nm) 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-18-7μm and RP-8-7μm (Kanto Chem. Co. Inc., Japan), YMC-Pack A-303 and A-324 (Yamamura Chem. Lab. Co., Japan), and μ-Bondapack C_{18} and Nova-Pak Cartridge C_{18}(Waters)] were used in HPLC. Retention times of (3) 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 spectrometer, respectively. TLC analysis was carried out over a pre-coated silica gel 60 F_{254} plate and its mobile phase was dichloromethane/ethanol (25/1, v/v). The detection was made with a UV lamp.

N^{ω}-Fluoroacetyl-5-methoxytryptamine (3).

a) From Fluoroacetic Acid. To a solution of (4) (1.52 g, 8 mmol) in tetrahydrofuran (THF) (45 ml), was added fluoroacetic acid (0.78 g, 10 mmol). After addition of DCC (1.13 g, 5.5 mmol) in THF (10 ml), 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 (25/1, v/v). The resulting solution was chromatographed over a silica gel column (3×12 cm) to give oil of (3) (0.5 g) in a 25% yield. The oil is further purified by the use of preparative HPLC under the similar conditions of Run 13 shown in Table 1 to give colorless oil of (3).

Table 1. Retention Times of N^{ω}-Fluoroacetyl-5-methoxytryptamine (3) in Various HPLC Systems

Run	Column (Size, mm)	Mobil Phase Phase (Ratio, v/v)	Flow Rate (ml/min)	Retention Time (min)
1	Hiber Lichrosorb RP-8-7μm(4.0×250)	CH_3CN/H_2O (80/20)	1.0	2.92
2		(75/25)	1.0	3.00
3		(60/40)	1.0	3.54
4	Hiber Lichrosorb RP-8-7μm(10.0×250)	CH_3OH/H_2O (80/20)	3.0	6.06
5		(70/30)	3.0	6.74
6		(60/40)	3.0	8.24
7	Hiber Lichrosorb RP-18-7μm(4.0×250)	CH_3CN/H_2O (80/20)	1.0	2.60
8		(75/25)	1.0	2.66
9		(60/40)	1.0	3.08
10	Hiber Lichrosorb RP-18-7μm(10.0×250)	CH_3OH/H_2O (80/20)	3.0	5.52
11		(70/30)	3.0	6.18
12	YMC-Pak A-303 (4.6×250)	CH_3OH/H_2O (70/30)	1.0	3.54
13	YMC-Pak A-324 (10.0×250)	CH_3OH/H_2O (70/30)	2.5	7.62
14	μ-Bondapak C_{18} C_{18} (3.9×300)	CH_3CN/H_2O (70/30)	1.0	3.16
15	Nova-Pak Cartridge C_{18} (8.0×100)	CH_3CN/ $(C_2H_5)_3N$-H_3PO_4aq,pH2.0 (30/70)	3.0	3.13

Anal. Found: C, 62.16; H, 6.28; N, 10.96%. Calcd. for $C_{13}H_{15}FN_2O_2$: C, 62.39; H, 6.04; N, 11.19%. UV (CH_3OH): λ_{max} nm (log ε) 222 (4.39), 276 (3.73), 297 (3.63), and 309 (3.47). ^1H NMR [$CDCl_3$, internal $(CH_3)_4Si$]: δ 2.99 (2H, t, J=6.8 Hz, side chain), 3.64 (2H, t, J=6.3 Hz, side chain), 3.87 (3H, s, $-CH_3$), 4.77 (2H, d, J_{HF}=47.3 Hz, $-CH_2F$), 6.38 (1H, broad, =NH), 6.88 (1H, dd, $J_{6,7}$=8.4 Hz, $J_{4,6}$=2.2 Hz, H-6), 7.04 (1H, d, $J_{4,6}$=2.2 Hz, H-4), 7.26 (1H, s, H-2), 7.27 (1H, d, $J_{6,7}$=8.4 Hz, H-7), and 7.98 (1H, broad, =NH). TLC: R_f 0.42.

b) From Potassium Fluoride. To a mixture of potassium fluoride (12.8 mg, 0.22 mmol) and Kryptofix 222 (30 mg), was added ethyl bromoacetate (33.4 mg, 0.2 mmol) in acetonitrile (2 ml). The resulting mixture was heated at 82 °C for 10 min with stirring and cooled. After addition of 1N aqueous potassium hydroxide (0.4 ml), the reaction mixture was heated for an additional 5 min. To the mixture, were added DCC (103 mg, 0.5 mmol) in acetonitrile (0.5 ml) and (4) (38 mg, 0.2 mmol) in acetonitrile (1 ml). The solution was made acid with 2N hydrochloric

acid (0.3 ml), heated at 82 °C for 10 min with stirring, diluted with 1N aqueous potassium hydroxide (5 ml), and filtered. The filtrate was extracted with ethyl acetate, dried with anhydrous sodium sulfate, and then evaporated to dryness under reduced pressure. The residue gave (3) in a 10% yield based on (4).

[*fluoroacetyl*-^{18}F]Fluoromelatonin (2).

a) From Ethyl p-Toluenesulfonyloxyacetate. [^{18}F]Fluoride was produced from the proton bombardment of 20% enriched [^{18}O]water (10). To the aqueous solution of [^{18}F]fluoride, a mixture of aqueous potassium carbonate (33 μmol/0.2 ml) and Kryptofix 222 (27 mg, 72 μmol) was added. 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 resulting 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. To the effluent, a mixture of (4) (5.0 mg, 26 μmol) in acetonitrile (1 ml) and DCC (103 mg, 0.5 mmol) in acetonitrile (1 ml) was added. The mixture was heated at 82 °C for 10 min with stirring, cooled and made basic with 1N aqueous potassium hydroxide. The suspension was passed through an Extrelut-3 column and eluted with a 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 (30/30, v/v) (0.5ml). The solution was then subjected to preparative HPLC. The radio-chromatogram is shown in Fig. 2. A radioactivity peak corresponding to (2) was then collected and the identity of the peak was confirmed by analytical HPLC (Run 12 shown in Table 1). The total synthesis time, the radiochemical yield and purity, and the specific activity (EOB) are *ca.* 90 min, 14.2%, >98%, and 540 mCi/μmol (by HPLC analysis; Run 15 in Table 1), respectively.

b) From Ethyl Bromoacetate. Ethyl bromoacetate (16.7mg, 0.1 mmol) was used in place of ethyl *p*-toluensulfonyloxyacetate in *a)*. A mixture of [^{18}F]fluoride, the ester, and Kryptofix 222 was heated at 82 °C for 10 min with stirring and cooled. After addition of 1N aqueous potassium hydroxide (0.1 ml), the reaction mixture

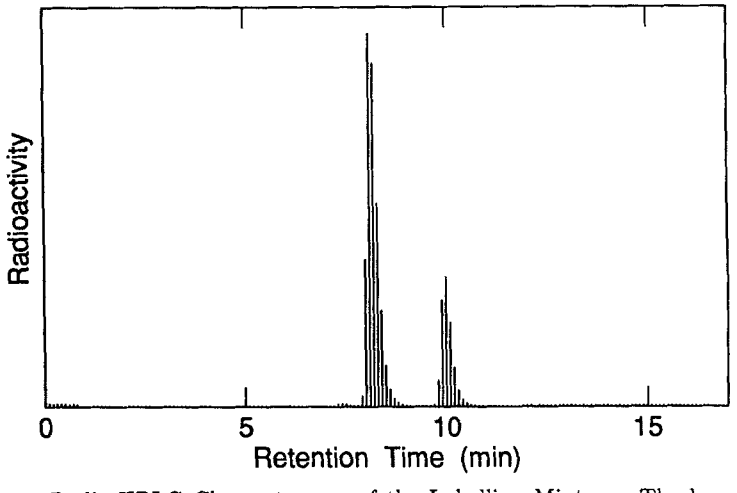


Fig. 2. Radio-HPLC Chromatogram of the Labelling Mixture. The large peak is the title compound (2), and chromatographic conditions are shown as Run 13 in Table 1.

was heated for an additional 5 min. To the resulting mixture, a mixture of (4) (22.8 mg, 0.12 mmol) in 2N hydrochloric acid (0.1 ml) and DCC (103 mg, 0.5 mmol) in acetonitrile (0.5 ml) was added. The mixture was heated at 82 °C for 10 min with stirring, diluted with water (2 ml), and filtered. The filtrate was evaporated to dryness under reduced pressure and the residue was extracted with a mixture of dichloromethane and ethanol (25/1, v/v). The organic layer was then passed through a Sep-Pak C_{18} cartridge, and eluted with the same solvent. The effluent 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 to give (2) in a 6.9 % radiochemical yield.

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