

III. 4. Synthesis of 17-[¹⁸F]Fluoro-5-methylheptadecanoic Acid

Takahashi T., Ido T. and Iwata R.

Division of Radiopharmaceutical Chemistry, Cyclotron and Radioisotope Center, Tohoku University

Abstract

5-Methyl-branched fatty acid, which is designed for less hindrance of the methyl group compared with the 3-methyl-branched fatty acid, was labeled with fluorine-18 at the ω -position. The total time of synthesis, including HPLC purification was 75-90 min from the start of reactive ¹⁸F- production. The decay-corrected isolated yields of 17-[¹⁸F]fluoro-5-methylheptadecanoic acid after HPLC purification was 13-19 % and the radiochemical purities were better than 97 %.

Introduction

Carbon-11 labeled fatty acids have been used for evaluation of both regional myocardial perfusion and regional fatty acid metabolism with a PET imaging system.¹⁾ Further, the more favorable fatty acid analogues such as methyl-branched [¹¹C]labeled fatty acids -- 3-methyl [1-¹¹C]heptadecanoic acid, 3,3-dimethyl [1-¹¹C]heptadecanoic acid -- have been prepared.^{2,3)} These methyl-branched fatty acids showed higher heart-to-blood ratios and longer myocardial retention than their straight chain analogues, suggesting that methyl-branching is an important structural characteristic that inhibits metabolism of the fatty acid in the cardiac muscle. While, for the elimination kinetic study of the radioactivity from the myocardium, a longer lived positron emitter such as fluorine-18 ($t_{1/2} = 109.8$ min) seems to be suitable. From this viewpoint, 3-methyl [17-¹⁸F]fluoroheptadecanoic acid and 3, 3-dimethyl [17-¹⁸F]fluoroheptadecanoic acid have been developed.⁴⁾ However, the initial uptakes of these 3-methyl-branched fatty acids were lower than those of the straight-chain fatty acids. This may be due to the steric effect of the methyl group at the 3-position. Therefore, we have proposed a 5-methyl-branched fluorofatty acid, which is designed for inhibition of the β -oxidation process and further less hindrance of the methyl group compared to the 3-methyl-branched fatty acid. In this paper, we have described the ¹⁸F-labeling of 5-methylheptadecanoic acid at the ω -position.

Materials and Methods

Chromatography

Analytical HPLC was performed using a reversed phase column (Fatty acid analysis (Waters), 8-10 μm , 3.9 mm i.d. \times 30 cm long) with THF/acetonitrile/water=5/9/7 (V/V) as the solvent (flow rate : 1.0 mL/min) and preparative HPLC using a reversed phase column (μ BONDAPAK C18 (Waters), 8-10 μm , 19 mm i.d. \times 15 cm long) with MeOH/H₂O/AcOH = 896/100/4 (V/V) as the solvent (flow rate : 7.0 mL/min).

Preparation of the starting materials and the standard compounds

Diethyl methylmalonate and 1, 12-dibromododecane (1) were purchased from Aldrich Chem. Co. Inc., 1M tetra n-butylammonium fluoride in THF solution was from Tokyo Kasei Kogyo Co., Ltd., diethyl malonate was from Wako Pure Chem. Ind. Ltd. and Kryptofix 2, 2, 2 was from Merck.

Synthesis of methyl 17-bromo-5-methylheptadecanoate : The synthetic scheme is shown in Fig. 1.

12-Benzyloxy-1-bromododecane (2) : 2 was prepared by a method similar to that described in the literature.⁵⁾ Yield : 33 % (from 1). b.p. : 203-208 °C/1.5 torr.

14-Benzyloxy-2-methyltetradecanoic acid (3) : 3 was synthesized by elongation of two carbon chains with malonic ester synthesis.^{6,7)} In this case, diethyl methylmalonate was used for the introduction of the methyl-branch at the 2-position. Yield : 36 % (from 2). m.p. : 41-43 °C. IR (CHCl₃) : 1715 cm⁻¹ (COOH).

14-Benzyloxy-1-bromo-2-methyltetradecane(4) : 4 was synthesized *via* 14-benzyloxy-2-methyl-1-tetradecanol by a method similar to that described in the literature.^{2,8)} Yield : 61 % (from 3). MS (m/z) : 382, 384 (M⁺), 303 (M⁺-Br).

16-Benzyloxy-4-methylhexadecanoic acid (5) : 5 was synthesized in a manner similar to the synthesis of 3. Yield : 40 % (from 4). m.p. : 35-39 °C. IR (KBr) : 1720 cm⁻¹ (COOH).

16-Benzyloxy-1-bromo-4-methylhexadecane (6) : 6 was synthesized in a manner similar to the synthesis of 4. Yield : 68 % (from 5). MS (m/z) : 424, 426 (M⁺).

17-Benzyloxy-5-methylheptadecanoic acid (7) : 6 was converted to 17-benzyloxy-5-methylheptadecanonitrile and subsequently 7 was synthesized from the nitrile by hydrolysis with 6N NaOH and DMSO.⁹⁾ Yield : 60 % (from 6). m.p. : 34-37 °C. IR (KBr) : 1720 cm⁻¹ (COOH).

17-Bromo-5-methylheptadecanoic acid (8) and Methyl 17-Bromo-5-methylheptadecanoate (9) : 8 was prepared in the usual way using 7 and 25 % HBr/AcOH. m.p. : 43-45 °C. IR (KBr) : 1720 cm⁻¹ (COOH). 9 was prepared in the usual way using 8, thionyl chloride and methanol. Yield : 21 % (from 7). m.p. : < 30 °C. IR (KBr) : 1725 cm⁻¹ (COOMe). MS (m/z) : 376, 378 (M⁺), 345, 347 (M⁺-OMe), 297 (M⁺-Br). ¹H-NMR (CDCl₃) : δ 0.85 (3H, d, 5-CH₃), 0.92-2.00 (27H, m), 2.28 (2H, t, -CH₂-COOMe), 3.40 (2H, t, Br-CH₂-), 3.67 (3H,

s, -COOCH₃).

Synthesis of the standard compound : Methyl 17-O-Tosyl-5-methylheptadecanoate (10) : 10 was prepared from 7 via methyl 17-benzyloxy-5-methylheptadecanoate and methyl 17-hydroxy-5-methylheptadecanoate by a method similar to that described in the literature.¹²⁾ Yield : 32 % (from 7). m.p. : 41-42 °C. IR (KBr) : 1730 cm⁻¹ (COOMe). MS (m/z) : 468 (M⁺), 437 (M⁺-OMe). ¹H-NMR (CDCl₃) : δ 0.85 (3H, d, -CH(CH₃)-), 0.94-1.86 (27H, m), 2.30 (2H, t, -CH₂-COOMe), 2.44 (2H, s, p-CH₃ (tosyl)), 3.66 (3H, s, -COOCH₃), 4.00 (2H, t, Tos-O-CH₂-), 7.38 (arom.2H (tosyl), d ; J=9 Hz), 7.77 (arom.2H (tosyl), d ; J=9 Hz).

17-fluoro-5-methylheptadecanoic acid (11) : 11 was synthesized via methyl 17-fluoro-5-methylheptadecanoate by a method similar to that described in the literature¹²⁾ using 10 and 1M tetra n- butylammonium fluoride in THF solution. Yield : 15 % (from 10). m.p. : 43-45 °C. IR (CHCl₃) : 1710 cm⁻¹ (COOH). ¹H-NMR (CDCl₃) : δ 0.86 (3H, d, -CH(CH₃)-), 0.96-1.80 (27H, m), 2.32 (2H, d, -CH₂- COOH), 4.44 (2H, d-t, F-CH₂-; J_{FH}=48 Hz, J_{HH}=7 Hz).

¹⁸F-Labeling

Production of [¹⁸F]fluoride : [¹⁸F]Fluoride is produced via the ¹⁸O(p,n)¹⁸F reaction by proton bombardment (18 MeV, 10 μA) of a circulating 20 % enriched [¹⁸O]water target using the CGR-MeV model 680 Cyclotron located at Tohoku University.¹⁰⁾

Synthesis of 17-[¹⁸F]fluoro-5-methylheptadecanoic acid and emulsification for injection : The substitution reaction was carried out according to a literature procedure¹¹⁾, as follows : i) Production of reactive ¹⁸F- using ¹⁸F-water (100-400 μL), 0.15M K₂CO₃ aq. sol. (200 μL) and Kryptofix 2, 2, 2 (26 mg, 69 μmol) ii) Br-for-¹⁸F exchange reaction using methyl 17-bromo-5- methylheptadecanoate (10 mg, 27 μmol) iii) Saponification with 0.5N methanolic KOH (1 mL) iv) Extraction with ether (15 mL × 3) v) Purification on preparative HPLC. The subsequent emulsification in water was carried out according to the literature⁹⁾ using Tween 20 and albumin (bovine).

Quality control : The radiochemical purity of the final product was determined by analytical HPLC. Retention times of 17-bromo-5-methylheptadecanoic acid and 17-fluoro-5-methylheptadecanoic acid are shown in Table 1.

Results and Discussion

For this ¹⁸F-fluorination, methyl 17-bromo-5-methylheptadecanoate was chosen as the starting material according to the result that the compounds combining bromine or O-tosyl as the leaving group and the methylester as the carboxylic acid - protecting group were the best starting materials for [ω-¹⁸F]labeling of fatty acids using [¹⁸F]fluoride substitution

reaction.¹²⁾ The time required for ^{18}F -labeling was 60-70 min from the start of reactive ^{18}F -production. After removal of salts with ether extraction, the subsequent HPLC purification was done on the preparative HPLC. The radiochemical analyses before and after HPLC separation are shown in Fig. 1 and the retention times of 17-bromo- and 17-fluoro-5-methylheptadecanoic acid are shown in Table 1. The time required for HPLC separation was 15-20 min and the recovery of radioactivity ranged from 80 to 85 %. Starting with 150-260 MBq (4-7 mCi) of ^{18}F fluoride, the decay-uncorrected isolated radioactivity of 5-methyl-branched ^{18}F fluorofatty acid after HPLC purification was 12-21 MBq (320- 560 μCi). The obtained radiochemical purities were better than 97 % and ca. 2 mg of bromofatty acid was usually contained in the final product. Due to the very low UV-absorption properties of the fluorofatty acid, the amount of radiotracer obtained under these no-carrier-added conditions was too small for direct determination of the specific activity with the equipment available.

In the emulsification, the addition of a small amount of Tween 20 to the albumin aqueous solution resulted in a good recovery of radioactivity (80-90 %). During the emulsification, complete removal of ethanol and the emulsifying temperature were important in order to prevent the precipitation of denatured albumin. The time required for emulsification was about 10 min.

By this synthesis, 17- ^{18}F fluoro-5-methylheptadecanoic acid has been already prepared more than eight times for medical studies.

References

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Table 1. HPLC retention times of 17-bromo-and 17-fluoro-5-methylheptadecanoic acid

Compound ^{a)}	Retention time (min.)	
	Analytical HPLC ^{b)}	Preparative HPLC ^{b)}
Br-(CH ₂) ₁₂ CH(CH ₂) ₃ COOH CH ₃	9.1	10.4
F-(CH ₂) ₁₂ CH(CH ₂) ₃ COOH CH ₃	7.7	8.8

a) detected by Refractive Index Detector

b) Conditions : See text

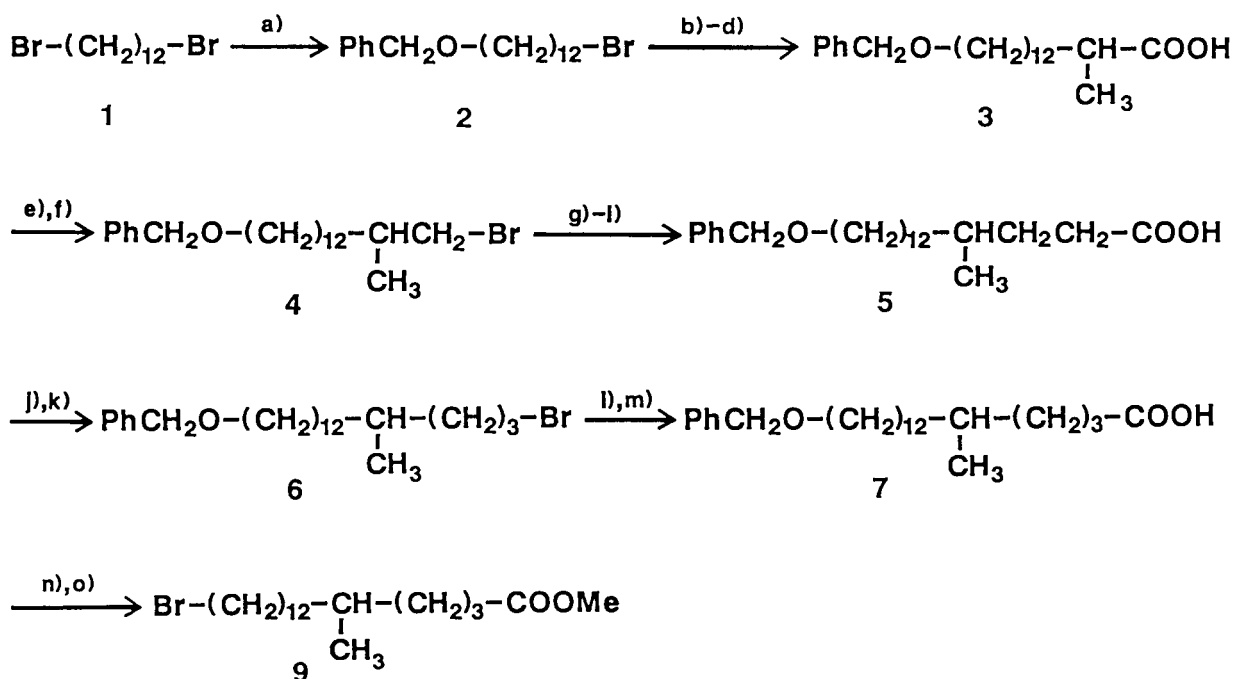
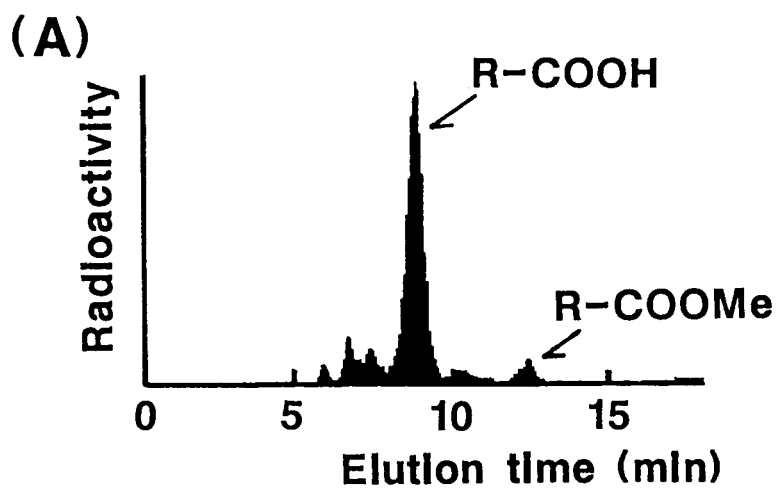
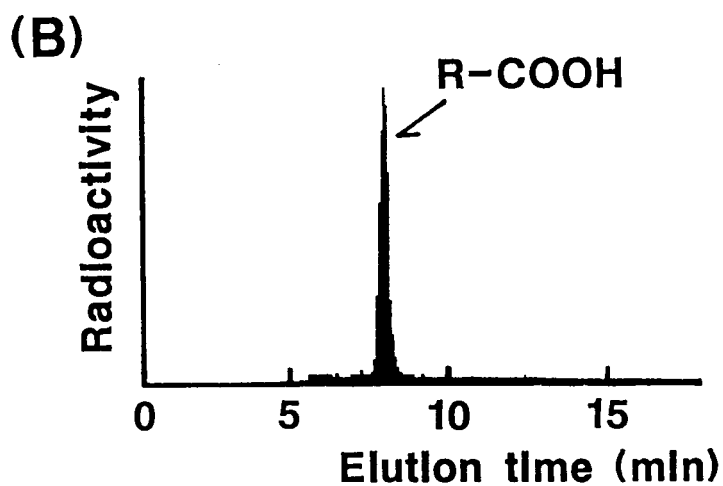


Fig. 1. Preparation of methyl 17-bromo-5-methylheptadecanoate.

- PhCH₂ONa
- MeCH(COOEt)₂/EtONa
- KOH/EtOH
- Δ
- LiAlH₄/abs. THF
- PPh₃, CBr₄/abs. benzene
- CH₂(COOEt)₂/EtONa
- KOH/EtOH
- Δ
- LiAlH₄/abs. THF
- PPh₃, CBr₄/abs. benzene
- 6CN/DMSO
- 6N NaOH/DMSO - c. HCl/DMSO
- 25% HBr/AcOH
- SOCl₂, MeOH.



Before HPLC purification



After HPLC purification

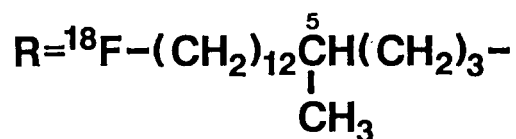


Fig. 2. Radiochemical analysis of 17- $[^{18}\text{F}]$ fluoro-3-methylheptadecanoic acid. (A) before HPLC purification with preparative HPLC ; (B) after HPLC purification with analytical HPLC.