III. 9 Effect of Leaving Groups on the Preparation of \( \omega^{-18}_F \)Fluorofatty Acid via Nucleophilic Fluorination

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Introduction

Fatty acids constitute the major energy source of heart tissue through \( \beta \)-oxidation catabolism.\(^1\) Carbon-11 labeled fatty acids have been commonly used for evaluation of both regional myocardial perfusion and regional fatty acid metabolism in conjunction with tomographic techniques.\(^2\text{-}10\)\) The carbon-11 labeled has the advantages such as no induction of any biochemical changes and also makes serial studies convenient. On the other hand, its shorter half-life has limited the longer lasting studies. For the elimination kinetic study of the radioactivity from the myocardium, a longer lived positron emitter such as fluorine-18 seems to be more suitable. For introduction of fluorine into fatty acids, several nucleophilic methods have been reported such as non-isotopic bromine-for-fluorine exchange in \( \omega \)-bromofatty acid in an acetamide melt,\(^11\) nucleophilic substitution with fluoride using neutral phase transfer catalysis,\(^12\text{-}13\) and so on. While the effect of leaving groups has been little reported. For the synthesis of starting materials, it is at first necessary to study which compounds are effective for labeling. We then examined \( 18_F \)-labeling of fatty acids by the nucleophilic substitution reaction on fatty acid methylester containing various leaving groups with reactive \( 18_F^- \). For the fluorination, we used the method previously reported by Coenen et al.\(^13\), that is, replacement of Br in \( \omega \)-bromofatty acid methylester by \( 18_F^- \), followed by saponification of \([\omega^{-18}_F \]fluorofatty acid methylester. In this paper, we describe the effect of leaving groups on the preparation of \([\omega^{-18}_F \]fluorofatty acids via nucleophilic fluorination.

Materials and Methods

Chromatography

Column chromatography\(^a\), thin layer chromatography (TLC)\(^b\) and preparative TLC\(^c\) was done on silica gel using the solvent indicated just below. High-performance liquid chromatography (HPLC) was carried out using a reversed phase column\(^d\) with THF/MeCN/H\(_2\)O (5/9/7) as the solvent (flow rate: 1.0 mL/min).

Preparation of the starting materials

11-Bromoundecanoic acid (1)\(^e\), 16-hydroxyhexadecanoic acid (2)\(^f\), undecanoic acid\(^g\), palmitic acid\(^g\), and kryptofix 2,2,2\(^h\) were purchased commercially. The acetonitrile used as reaction solvent was additionally
dried from commercially available special grade
g) by distillation from phosphorous pentoxide, followed by further distillation from calcium
hydride. 14

**Synthesis of 11-benzyloxyundecanoic acid methylester (3):**

11-benzyloxyundecanoic acid was synthesized from 1 by the similar method
previously reported. 15 Yield: 93% (crude). TLC Rf = 0.22 (CH₂Cl₂/MeOH = 10
mL/10 drops). IR(CHCl₃):1715 cm⁻¹(COOH). It was used for the next esterified
step without further purification. To thionyl chloride (15 mL), the crude
11-benzyloxyundecanoic acid (2.91 g) was added slowly at room temperature.
The reaction mixture was stirred at room temperature over night. After
removal of excess thionyl chloride in vacuo, methanol (70 mL) was added. The
reaction mixture was stirred at room temperature for 3 hr. After removal of
the solvent, the residue was purified by column chromatography (hexane/CHCl₃ =
3/7) to give 1.98 g of 3. Yield: 60% (from 1). TLC Rf = 0.70 (CH₂Cl₂). IR
(neat): 1740 cm⁻¹ (COOMe). Mass: 306 (M⁺).

**Synthesis of 11-hydroxyundecanoic acid methylester (4):**

To a suspension of 5% Pd-C (700 mg) in MeOH (30 mL), a solution of 3 (1.02 g,
3.33 mmol) dissolved in MeOH (30 mL) was added and then the mixture was
hydrogenated with H₂ at atmospheric pressure with stirring. The reduction
took 1 day. After filtration of the catalyst and concentration of the
filtrate, 471 mg of 4 was obtained. Yield: 65%. m.p.: < 30°C. TLC Rf= 0.41
(CH₂Cl₂/MeOH=10 mL/30 drops). IR (KBr): 3350 cm⁻¹ (OH). 1740 cm⁻¹ (COOMe).
Mass: 217 (M⁺ + 1), 198 (M⁺-H₂O), 186 ((M⁺ + 1)-OMe).

**Synthesis of 11-bromo undecanoic acid methylester (5):**

To thionyl chloride (6.5 mL), compound 1 (1.01 g, 3.81 mmol) was added and
treated in a manner similar to 3. The residue was purified by column
chromatography (hexane/CH₂Cl₂ = 1/1) to give 701 mg of 5. Yield: 66%. TLC
Rf=0.60 (hexane/CH₂Cl₂ = 1/1). IR (neat): 1740 cm⁻¹ (COOMe). Mass: 278,
280 (M⁺), 247, 249 (M⁺-OMe), 199 (M⁺-Br).

**Synthesis of 11-cyano undecanoic acid methylester (6):**

Compound 6 was synthesized by the similar method previously reported. 16 To a
solution of 5 (143 mg, 0.51 mmol) dissolved in DMSO (10 mL), KCN (63 mg, 0.97
mmol) was added. The mixture was heated at 140°C for 3 hr. After cooling to
room temperature, water (150 mL) was added, followed by extraction with ether
(60 mL × 3). The ether was dried over anhydrous Na₂SO₄ and evaporated. The
residue was purified by column chromatography (hexane/CHCl₃ = 1/1) to give 23
mg of 6. Yield: 20%. TLC Rf=0.41 (CHCl₃/MeOH=10 mL/10 drops). IR (neat):
2250 cm⁻¹ (CN), 1740 cm⁻¹ (COOMe). Mass: 225 (M⁺).
Synthesis of 11-nitro undecanoic acid methyl ester (7):

To a solution of 5 (105 mg, 0.38 mmol) dissolved in DMSO (5 mL), NaNO₂ (50 mg, 0.72 mmol) was added and treated in a manner similar to 6. The residue was purified by column chromatography (CH₂Cl₂) to give 9 mg of 7. Yield: 10%. TLC Rf=0.54 (CH₂Cl₂/MeOH=10 mL/10 drops). IR (neat): 1740 cm⁻¹ (COOMe). Mass 245 (M⁺).

Synthesis of 11-chloro undecanoic acid methyl ester (8):

To a solution of 4 (28 mg, 0.13 mmol) dissolved in pyridine (2 mL), thionyl chloride (4 mL) was added slowly in an ice-water bath and then the reaction mixture was heated at 80°C for 2 hr. After removal of excess thionyl chloride in vacuo, 1N-HCl (25 mL) was added, followed by ether extraction (20 mL x 3). The ether was dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by column chromatography (hexane/CH₂Cl₂ =3/7) to give 18 mg of 8. Yield: 59%. TLC Rf=0.73 (CH₂Cl₂). IR (neat): 1740 cm⁻¹ (COOMe).

Synthesis of 11-O-tosylundecanoic acid methyl ester (9):

To a solution of 4 (60 mg, 0.28 mmol) dissolved in abs. CH₂Cl₂ (5 mL), a solution of tosyl chloride (147 mg, 0.77 mmol) dissolved in abs. CH₂Cl₂ (5 mL) and triethylamine (150 μL) were added. The mixture was stirred at room temperature for 3 days. After removal of the solvent, 1N-HCl (12 mL) was added, followed by ether extraction (20 mL x 3). The ether was dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by preparative TLC (CH₂Cl₂) to give 68 mg of 9. Yield: 66%. TLC Rf=0.52(CH₂Cl₂). m.p.: <30°C. IR (CHCl₃): 1730 cm⁻¹ (COOMe). Mass: 370 (M⁺).

Synthesis of 11-O-mesylundecanoic acid methyl ester (10):

To a solution of 4 (30 mg, 0.14 mmol) dissolved in pyridine (2 mL), mesyl chloride (1 mL) was added dropwise in an ice-water bath and then the mixture was stirred at room temperature for 2 days. After water (20 mL) and 1N-HCl (30 mL) was added, the reaction mixture was extracted with ether (50 mL x 3). The ether was dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by preparative TLC (CH₂Cl₂) to give 14 mg of 10. Yield: 34%. TLC Rf=0.62 (CH₂Cl₂). IR (neat): 1740 cm⁻¹ (COOMe). Mass: 294 (M⁺).

Synthesis of 16-bromohexadecanoic acid methyl ester (11):

To 25% HBr/AcOH (5 mL), compound 2 (103 mg, 0.38 mmol) was added slowly and then the mixture was heated at 100°C for 1 day. After cooling to room temperature, excess HBr/AcOH was evaporated in vacuo. The residue (crude 16-bromohexadecanoic acid, TLC Rf=0.32 (CH₂Cl₂/MeOH=10 mL/20 drops)) was used for the next esterification step without further purification. To the residue thionyl chloride (5 mL) was added slowly at room temperature and treated in a manner similar to 3. The residue was purified by column chromatography (hexane/CH₂Cl₂=7/3) to give 92 mg of 11. Yield: 73%. m.p.: 30-31°C. TLC Rf=0.75 (CH₂Cl₂). IR (KBr): 1740 cm⁻¹ (COOMe). Mass: 348, 350 (M⁺), 317, 319 (M⁺ - OMe), 269 (M⁺ - Br).
Synthesis of 16-O-tosylhexadecanoic acid methylester (12):
To a mixture of 2 (104 mg, 0.38 mmol) and abs. MeOH (10 mL), thionyl chloride (0.6 mL) was added dropwise at 0°C and the mixture stirred for 15 min. It was then heated at reflux for 30 min. The pale yellow solution was evaporated to dryness and pumped under vacuum for several minutes. The residue (crude 16-hydroxyhexadecanoic acid methylester, TLC Rf=0.40 (CH₂Cl₂/MeOH=10 mL/30 drops)) was used for the next step without further purification. To a solution of the residue dissolved in abs CH₂Cl₂ (10 mL) and triethylamine (300 μL) were added and treated in a manner similar to 2. The residue was purified by preparative TLC (hexane/CH₂Cl₂ = 3/7) to give 64 mg of 13. Yield: 38% (from 2). m.p.: 56-58°C. TLC Rf= 0.46 (CH₂Cl₂). IR (CHCl₃): 1730 cm⁻¹ (COOMe). Mass: 440 (M⁺), 409 (M⁺-OME).

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. A 60 min irradiation gave [18F]fluoride in 390 mCi at the end of bombardment.

General nucleophilic 18F-for-X (X: leaving groups) substitution reaction:
Substitution reaction was carried out by the similar method previously reported. As the typical example, the synthetic procedure of 11-[18F]fluoro undecanoic acid was shown below. To the [18F]-water (100-400 μL), 0.15M K₂CO₃ aq. sol. (200 μL) and a solution of Kryptofix 2,2,2 (K 2,2,2) (26 mg, 69 μmol) dissolved in dry MeCN (1 mL) were added. While purging with He flow, the solvent was evaporated to dryness at 110°C. In order to remove water completely, dry MeCN (1 mL) was added, followed by removal of the solvent with He flow again. To the residue (dry K 2,2,2)¹⁺[18F⁻], a solution of 11-bromo undecanoic acid methylester (10 mg, 36μmol) dissolved in dry MeCN (1 mL) was added and then the mixture was refluxed for 10 min at 110°C. Subsequently 0.5 N methanolic KOH (1 mL) was added and the solution was refluxed again for further 15 min at the same temperature. After removal of the solvent in vacuo, water (15 mL) and 2N-HCl (1 mL) were added. The mixture was extracted with ether (10 mL x 3) and the ether was evaporated in vacuo.

Purity determination:
The radiochemical purity was determined by radio-HPLC. Undecanoic acid and palmitic acid were used as authentic samples of 11-[18F]fluoro undecanoic acid and 16-[18F]fluorohexadecanoic acid respectively.
Results and Discussion

The starting materials were prepared as shown in Fig. 1. These compounds were characterized by IR spectra and Mass spectra. Several attempts to prepare the 11- (O-trifluoromethanesulfonyl)undecanoic acid methylester were unsuccessful. It may be due to the instability of the triflate.

In a preliminary study, we reacted free bromofatty acid with the reactive $^{18}F^—$($^2K_{2,2,2}$, +$^{18}F$) for introduction of fluorine. It resulted in the low radiochemical yield and the production of many radioactive by-products (Fig. 2). On the basis of the replacement of the carboxylic acid function with fluorine reported by Patrick et al.\textsuperscript{18}, the formation of $^{18}$F-fluoroalkane has been suggested. For fluorination, we then used the $^{18}$F-labeling method reported by Coenen et al.\textsuperscript{13} (Fig. 3). Among several nucleophilic radiofluorination process, this procedure is the best method to prepare $^{18}$F-fluorofatty acids in high yields. In this fluorination, the use of well dried acetonitrile was necessary. Using the acetonitrile only dried with Molecular Sieves (3A), the radiochemical yields fell to half. The time of synthesis was 70-85 min.

As shown in Table 1, TosO- and Br- prove to be most reactive among the six leaving groups. It has been also shown that the leaving groups such as CN and NO$_2$ are not effective in the nucleophile displacement reaction with $^{18}$F- on aliphatic compounds. Between halogens, Br is more reactive than Cl.

The purification of these $^{18}$F-labeled compounds using a short silica gel or reversed phase column was effective in removing reagents and salts, but HPLC separation was needed for removing the bromo- from the fluoro fatty acid. The conditions of HPLC separation were the same as those of purity determination. Reversed phase chromatography used in the present study allows elution of the radiofluorinated product in front of the starting material. The time required for HPLC separation was 10-15 min.

On the basis of the result of this research, 3-methyl 17-bromoheptadecanoic acid methylester was synthesized for the preparation of 3-methyl 17-$^{18}$F-fluoro-heptadecanoic acid, which is expected to show longer myocardial retention. This $^{18}$F-labeled compound has been prepared in a radiochemical yield of 36-58 % with radiochemical purities of 99 %. The total time of synthesis was about 120 min.

Notes

a; Wakogel C-200 (Wako Pure Chemical Industries Ltd)
b; DC-Alufolien Kieselgel 60 F$_{254}$ (Art 5554) (Merck, Darmstadt, FRG)
c; DC-Fertigplatten Kieselgel 60 F$_{254}$ , Schichtdicke 0.5 mm (Art 5744) (Merck, Darmstadt, FRG)
d; Fatty acid analysis (WATERS), 8-10 µm, 3.9 mm I.D. x 30 cm long
e; Tokyo Kasei Kogyo Co., Ltd, Japan
f; Aldrich Chemical Company Inc, USA
References

Table 1. Experimental data on $[^\omega^{18}F]$fluorofatty acid synthesis

$$X-(\text{CH}_2)_n-\text{COOMe} \rightarrow \text{^{18}_F-(CH}_2)_n-\text{COOH}$$

<table>
<thead>
<tr>
<th>$X$</th>
<th>$n$</th>
<th>Radiochemical Yield (%)</th>
<th>Radiochemical purity (%)</th>
</tr>
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<tbody>
<tr>
<td>CN</td>
<td>10</td>
<td>15</td>
<td>98</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>10</td>
<td>6</td>
<td>&gt; 99</td>
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<tr>
<td>Cl</td>
<td>10</td>
<td>32</td>
<td>99</td>
</tr>
<tr>
<td>Br</td>
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<td>85, 90</td>
<td>99, &gt; 99</td>
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<tr>
<td>Br</td>
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<td>73, 64</td>
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<tr>
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<td>10</td>
<td>34</td>
<td>99</td>
</tr>
<tr>
<td>Tos-O-</td>
<td>10</td>
<td>81</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>Tos-O-</td>
<td>15</td>
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Fig. 1. Synthesis of starting materials.
Fig. 2. Reaction of ω-bromofatty acid or ω-bromofatty acid methylester with $^{18}\text{F}^-$. 

Fig. 3. Synthesis of $[\omega-^{18}\text{F}]$fluorofatty acid.