III. 2. $^{18}$F-Labeling of 1, 2-Diacylglycerol

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Introduction

The past several years have witnessed increasing interest in the study of the phosphatidylinositol (PI) turnover, newly defined as a second messenger system (Fig. 1). The system may play a central role in cellular signals in the central nervous system. From this viewpoint, second messenger imaging in vivo studies using PET has been suggested to be effective for the observation of neuronal functions manifested by the synaptic transmission process. As shown in Fig. 1, 2-diacylglycerol is one component in the PI turnover system and therefore Imahori et al. synthesized $^{11}$C-labeled 1, 2-diacylglycerol (1-Palmitoyl- sn-2-[1-$^{11}$C]butyrylglycerol) by $[^{11}$C]ethyl ketene $^1$ as a tracer for the PI turnover measurement by PET. However, the short half-life of $^{11}$C is disadvantageous for the metabolic studies and the longer PET studies and the labeling with a longer lived positron emitter such as $^{18}$F seems to be more suitable. In this paper, we report the synthesis of $^{18}$F-labeled 1, 2-diacylglycerols such as 1-(16-$^{18}$FFluorohexadecanoyl)-2-hexadecanoylglycerol (1) and 2-(16-$^{18}$FFluorohexadecanoyl)-1-hexadecanoylglycerol (2) (Fig. 2).

Experimental

Chromatography

Column chromatography and preparative TLC were carried out on silica gel (column chromatography : Wakogel C-200 (Wako Pure Chem. Ind. Ltd.), preparative TLC : DC-Fertig platten Kieselgel 60 F$_{254}$, Art 5744 (Merck)) using the solvent indicated below. Preparative HPLC was performed using a silica column (YMC-023-5 06 S-5 60A Sil, 10 mm i.d. $\times$ 25 cm long) with hexane/ether/isoproOH = 400/80/1.5 (V/V) as the solvent (flow rate: 7 mL/min) and analytical HPLC using a silica column (Nova Pak Silica, 8 mm i.d. $\times$ 10 cm long) with hexane/ether/isoproOH = 400/80/1.5 as the solvent (flow rate: 3 mL/min).

Preparation of the starting materials and the standard compounds

1-Monopalmitin, 2-monopalmitin, 1, 2-dipalmitin and 1, 3-dipalmitin were purchased from Funakoshi Co. Ltd., 1, 2- (3) was from Tokyo Kasei Kogyo Co. Ltd, sodium amide
and palmitoyl chloride were from Wako Pure Chem. Int. Ltd., 16-hydroxyhexadecanoic acid was from Aldrich Chem. Co. Inc. and Kryptofix 2, 2, 2 (K 2, 2, 2) was from Merck. 

**Synthesis of Methyl 16-O-Tosylhexadecanoate:** Methyl 16-O-tosylhexadecanoate was synthesized from 16-hydroxyhexadecanoic acid according to a literature procedure 2).

**Synthesis of 3-O-Benzyl-1,2-dihexadecanoylglycerol (6) and 3-O-Benzyl-2-(16-bromohexadecanoyl)-1-hexadecanoylglycerol (7):** The synthetic scheme is shown in Fig. 3.

**16-Bromohexadecanoyl chloride:** 16-Bromohexadecanoic acid was synthesized from 16-hydroxyhexadecanoic acid according to a literature procedure 2). To the obtained 16-bromohexadecanoic acid (102 mg, 0.3 mmol), thionyl chloride (5 mL) was added and the mixture was stirred at room temperature for 1 hr. After removal of excess thionyl chloride in vacuo, the residue was used for the next reaction without further purification.

**3-O-Benzyl-1,2-isopropylideneglycerol (4):** 4 was prepared by a method similar to that described in the literature 3). To a solution of 1, 2-isopropylideneglycerol (44 g, 0.33 mol) dissolved in benzene (83 mL), sodium amide (13 g, 0.33 mol) was added and the mixture was refluxed for 1 hr under Ar atmosphere. After cooling to room temperature, benzyl chloride (61 mL, 0.53 mol) was added dropwise and the mixture was refluxed for 1 day under Ar atmosphere. After cooling to room temperature, the benzene layer was washed with water (100 mL x 3) and the residue obtained by the evaporation was distilled twice under reduced pressure to give 4 (42 g). Yield: 57 %. b.p.: 120-124 °C/3 torr.

**3-O-Benzyl-1-hexadecanoylglycerol (5):** To the compound 4 (2.2 g, 9.9 mmol), isopropanol (2.2 mL), acetic acid (0.2 mL) and water (1 mL) were added and the mixture was refluxed for 6 hr. After removal of the solvent, the residue (crude 3-O-benzylglycerol, colorless oil) was used for the next synthesis without further purification. To a solution of the crude 3-O-benzylglycerol dissolved in dry chloroform (2 mL), a solution of palmitoyl chloride (2.76 g, 10 mmol) dissolved in dry chloroform (2 mL) and a solution of pyridine (787 mg, 10 mmol) dissolved in dry chloroform (1 mL) were added and the mixture was stirred at room temperature for 2 days. After removal of the solvent, 1N HCl (15 mL) was added, followed by ether extraction (15 mL x 3). The residue obtained by the evaporation was purified by column chromatography (hexane/CH₂Cl₂ = 1/1) to give 5 (1.25 g). Yield: 30 % (from 4).

A small amount of the compound 5 was characterized after further purification by preparative TLC (hexane/CH₂Cl₂ = 1/1). m.p.: < 30 °C. IR (KBr): 3470 cm⁻¹ (-OH), 1740 cm⁻¹ (-OCO-). MS (m/z): 421 (M⁺+1).

**3-O-Benzyl-1,2-dihexadecanoylglycerol (6):** To a solution of 5 (141 mg, 0.34 mmol) dissolved in dry benzene (4 mL), a solution of palmitoyl chloride (250 mg, 0.91 mmol) dissolved in dry benzene (3 mL) and triethylamine (130 µL, 0.93 mmol) were added and the mixture was refluxed for 1 hr. After removal of the solvent, water (10 mL) and conc. HCl (2 mL) were added, followed by ether extraction (15 mL x 3). The residue obtained by the evaporation was purified by preparative TLC (hexane/CH₂Cl₂ = 1/1) to give 6 (174 mg). Yield: 79 %. m.p.: 40-43 °C. IR (CHCl₃): 1735, 1730 cm⁻¹ (-OCO- x 2). MS (m/z): 658
(M⁺), 551 (M⁺-OCH₂Ph). ¹H-NMR (CDCl₃) : δ 0.42 - 1.76 (58H, m, -OCOCH₂-C₁₄H₂₉ × 2), 2.04 - 2.42 (4H, m, -OCO-CH₂-C₇H₉ × 2), 3.58 (2H, d, -OCH₂-CH(Opa)-CH₂O-CH₂Ph), 4.26 (1H, d-d, Pa-OCH₃/CH₃-CH(Opa)-CH₂O-), 4.34 (1H, d-d, Pa-OCH₃/CH₃-CH(Opa)-CH₂O-), 4.52 (2H, S, -OCH₂Ph), 5.22 (1H, m, -OCH₂-CH(Opa)-CH₂O-), 7.32 (arom. 5H). (Pa: Palmitoyl C₁₅H₃₁CO⁻)

3-O-Benzyl-2-(16-bromohexadecanoyl)-1-hexadecanoylglycerol (7) : 16-Bromohexadecanoyl chloride was reacted with 5 and treated in a manner similar to 6. The obtained residue was purified by preparative TLC (hexane/CH₂Cl₂ = 2/8) to give 7. Yield : 37 %. m.p. : < 36 °C. IR (CHCl₃) : 1740, 1735 cm⁻¹ (-OCO⁻ × 2). MS (m/z) : 629, 631 (M⁺-OCH₂Ph). ¹H-NMR (CDCl₃) : Fig. 4.

¹⁸F-Labeling

Production of [¹⁸F]fluoride : [¹⁸F]Fluoride was 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 ⁴). Synthesis of 1,2-[¹⁸F]FDAG(1-(16-[¹⁸F]Fluorohexadecanoyl)-2-hexadecanoylglycerol) (1) : The ¹⁸F-labeling scheme is shown in Fig. 5. 16-[¹⁸F]Fluorohexadecanoic acid was prepared using K 2, 2, 2 (25 mg, 66 µmol) and methyl 16-O-tosylhexadecanoate (6 mg, 14 µmol) by a method similar to that described in the literature⁵), ²). To the obtained 16-[¹⁸F]fluorohexadecanoic acid, thionyl chloride (500 µL) was added and the mixture was stirred at room temperature for 5 min. After complete removal of excess thionyl chloride in vacuo, a solution of 2-monopalmitin (6 mg, 18 µmol) dissolved in dry CH₂Cl₂ (1 mL) and 4-(N,N-dimethylamino)pyridine (1-2 pieces) were added and the mixture was stirred again at room temperature for a further 5 min. After removal of the solvent, water (10 mL) and 2N HCl (1 mL) were added, followed by ether extraction (10 mL × 3). The residue obtained by the evaporation was redissolved in preparative HPLC solvent (Ca. 1 mL) and the subsequent purification was done by preparative HPLC.

Synthesis of 1, 2-[¹⁸F]FDAG(2-(16-[¹⁸F]Fluorohexadecanoyl)-1-hexadecanoylglycerol) (2) : (Method A) 16-[¹⁸F]Fluorohexadecanoyl chloride was reacted with 1-monopalmitin and treated in a manner similar to 1, 2-[¹⁸F]FDAG.

(Method B) The ¹⁸F-labeling scheme is shown in Fig. 6. ¹⁸F was introduced in a manner similar to the synthesis of 16-[¹⁸F]fluorohexadecanoic acid ⁵), ²). To the [¹⁸F]water (100-400 µL), 0.15M K₂CO₃ aq. sol. (200 µL) and a solution of K 2, 2, 2 (25 mg, 66 µmol) dissolved in dry acetonitrile (1 mL) were added. While purging with flowing N₂, the solvent was completely evaporated to dryness at 120 °C. To the residue, a solution of compound 7 (7.5 mg, 12 µmol) dissolved in dry acetonitrile (1 mL) was added and then the mixture was refluxed for 10 min at 120 °C. After removal of the solvent, water (10 mL) and 2N HCl (1 mL) were added, followed by ether extraction (10 mL × 3). The crude 3-O-
benzyl-2-(16-[\(^{18}\)F]fluorohexadecanoyl)-1-hexadecanoylglycerol (8) obtained by the evaporation was used for the next reaction without further purification. To a suspension of 5% Pd-C (20 mg) in ethanol (2 mL), a solution of compound 8 dissolved in dioxane (2 mL) was added and the mixture was hydrogenated at atmospheric pressure with stirring at 45 °C for 30 min. After removal of the catalyst by filtration, the filtrate was concentrated. The residue was redissolved in preparative HPLC solvent (Ca. 1 mL) and the purification was performed in a same preparative HPLC system as that of 1, 2-[\(^{18}\)F]FDAG.

*Emulsification for injection*: Emulsification was performed using Tween 20 and albumin (bovine) by a similar method to that described in the literature 6).

*Quality control*: The radiochemical purities were controlled on analytical HPLC. 1, 2-Dipalmitin, 1, 3-dipalmitin and 3-O-benzyl-1, 2-dihexadecanoylglycerol(6) were used for the authentic samples of 1, 2-[\(^{18}\)F]FDAG, 1, 3-[\(^{18}\)F]FDAG and 3-O-benzyl-2-(16-[\(^{18}\)F]fluorohexadecanoyl)-1-hexadecanoylglycerol (8), respectively. Retention times of 1, 2-dipalmitin, 1, 3-dipalmitin and compound 6 are shown in Table 1.

Results and Discussion

*In the synthesis of 1, 2-[\(^{18}\)F]FDAG, the omission of ether extraction for 16-[\(^{18}\)F]fluorohexadecanoic acid purification resulted in very low radiochemical yields of 1, 2-[\(^{18}\)F]FDAG. This may be due to the degradation of 16-[\(^{18}\)F]fluorohexadecanoyl chloride by the unremoved K 2, 2, 2. Then, it has been well known that acyl group at 2-position in 1, 2-diacylglycerol readily undergoes rearrangement to 3-position 7). Therefore, 1,3-[\(^{18}\)F]FDAG was formed from 1, 2-[\(^{18}\)F]FDAG (Fig. 7) and the further HPLC purification was needed for removing 1, 3-isomer.

In the synthesis of 1, 2-[\(^{18}\)F]FDAG, the reaction of 16-[\(^{18}\)F]fluorohexadecanoyl chloride with 1-monopalmitin (Method A) was disadvantageous because 1, 3-[\(^{18}\)F]FDAG was a main product (Fig. 8). This dominant acylation at 3-position is due to the steric effect of palmitoyl group at 1-position. Therefore, for the synthesis of 1, 2-[\(^{18}\)F]FDAG, we planned to the another route using 3-O-benzyl-2-(16-bromohexadecanoyl)-1-hexadecanoylglycerol (7) (Method B). The compound 7 is stable and able to be stored for a long time at room temperature. In Method B, the removal of benzyl group by hydrogenolysis at room temperature was not effective and a large amount of compound 8 (3-O-benzyl-2-(16-[\(^{18}\)F]fluorohexadecanoyl)-1-hexadecanoylglycerol) were still remained after hydrogenolysis at room temperature for 1 hr. However, as shown in Fig. 9, the hydrogenolysis at 45 °C for 30 min gave the good yields of 1, 2-[\(^{18}\)F]FDAG. In this case, 1, 3-[\(^{18}\)F]FDAG was also
formed and the removal of 1, 3-isomer was needed by HPLC purification. The experimental data for $^{18}$F-labeling of 1, 2-diacylglycerol are listed in Table 2.

HPLC (preparative) retention times of acylglycerol derivatives are shown in Table 1. 1,2- and 1,3-$^{18}$FFDAG could be well separated by preparative HPLC. The time required for HPLC purification was 15-20 min and the recovery of radioactivity ranged from 47-58 %. In the emulsification, the required time was 10 min and the recovery of radioactivity was 75-92 %.

This synthesis has been already used for more than 10 preparations of 1, 2-$^{18}$FFDAG and 1, 2-$^{18}$FFDAG.

References

7) Fischer E., Ber., 53 (1920) 1621.

Table 1. HPLC retention times of acylglycerol derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention times (min)</th>
<th>Analytical HPLC&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>Preparative HPLC&lt;sup&gt;a)&lt;/sup&gt;</th>
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<tr>
<td>1,2-Dipalmitin</td>
<td>6.0</td>
<td>8.4</td>
<td></td>
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<tr>
<td>1,3-Dipalmitin</td>
<td>4.2</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>3-O-Benzyl-1,2-dihexadecanoylglycerol (6)</td>
<td>1.8</td>
<td>2.2</td>
<td></td>
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<sup>a)</sup> Conditions: See text
<sup>b)</sup> Detected by Refractive Index Detector

Table 2. Experimental data of 1,2-$^{18}$FFDAG synthesis

<table>
<thead>
<tr>
<th>1,2-$^{18}$FFDAG (Method B)</th>
<th>1,2-$^{18}$FFDAG</th>
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<tr>
<td>Radiochemical yield (%)&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>20 - 30</td>
</tr>
<tr>
<td>Radiochemical purity (%)&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>&gt; 97</td>
</tr>
<tr>
<td>Synthesis time (min)&lt;sup&gt;c)&lt;/sup&gt;</td>
<td>120 - 150</td>
</tr>
</tbody>
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<sup>a)</sup> based on $^{18}$F-
<sup>b)</sup> determined by analytical HPLC
<sup>c)</sup> including the time required for HPLC purification
Fig. 1. Inositol phospholipid turnover and signal transduction

PtdIns: phosphatidylinositol
PtdIns4P: Phosphatidylinositol-4-phosphate
PtdIns4,5P2: phosphatidylinositol-4, 5-bisphosphate
R1 and R2: acyl groups
I: inositol
P: phosphoryl group
Fig. 2. Radiopharmaceuticals

Fig. 3. Preparation of 3-O-benzyl-1,2-dihexadecanoylglycerol and 3-O-benzyl-2-(16-bromohexadecanoyl)-1-hexadecanoyl glycerol
Fig. 4. $^1$H-NMR spectra of 3-O-benzyl-2-(16-bromohexadecanoyl)-1-hexadecanoylglycerol

$^{18}$F-(CH$_2$)$_{15}$-COOH $\xrightarrow{SOCl_2}$ $^{18}$F-(CH$_2$)$_{15}$-COCl $\xrightarrow{}$ 2-Palmitoylglycerol

$^{18}$F-(CH$_2$)$_{15}$-COO$^-$ $\xrightarrow{}$ 2-Palmitoylglycerol

HPLC Purification $\xrightarrow{}$ $^{1,2-}_1$, $^{1,3-[18F]}$FDAG mix.

$^{18}$F-(CH$_2$)$_{15}$-COO$^-$ $\xrightarrow{}$ 2-Palmitoylglycerol

Fig. 5. Synthetic scheme of $^{1,2-[18F]}$FDAG
Fig. 6. Synthetic scheme of 1,2-[18F]FDAG

Fig. 7. Radiochromatogram synthesis of 1,2-[18F]FDAG
Fig. 8. Radiochromatogram synthesis of 1,2-[\textsuperscript{18}F]FDAG (Method A).

Fig. 9. Radiochromatogram synthesis of 1,2-[\textsuperscript{18}F]FDAG (Method B).