18F-Labeling of 1, 2-Diacylglycerol

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 phosphatidyl-inositol (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, 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]butyrylglycetrol) 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}F]Fluorohexadecanoyl)-2-hexadecanoylglycerol (1) and 2-(16-[^{18}F]fluorohexadecanoyl)-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. × 25 cm long) with hexane/ether/isoPrOH = 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. × 10 cm long) with hexane/ether/isoPrOH = 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 × 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 × 3). The residue obtained by the evaporation was purified by column chromatography (hexane/CH_2Cl_2 = 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/CH_2Cl_2 = 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 × 3). The residue obtained by the evaporation was purified by preparative TLC (hexane/CH_2Cl_2 = 1/1) to give **6** (174 mg). Yield : 79 %. m.p. : 40-43 °C. IR ($CHCl_3$) : 1735, 1730 cm^{-1} (-OCO- × 2). MS (m/z) : 658

(M^+), 551 (M^+-OCH_2Ph). ^{1}H-NMR ($CDCl_3$) : δ 0.42 - 1.76 (58H, m, -OCOCH_2-$C_{14}H_{29}$ × 2), 2.04 - 2.42(4H, m, -OCO-CH_2-$C_{14}H_{29}$ X 2), 3.58(2H, d, -OCH_2-CH(OPa)-CH_2O-CH_2Ph), 4.26(1H, d-d, Pa-OCH_AH_B-CH(Opa)-CH_2O-), 4.34(1H, d-d, Pa-OCH_AH_B-CH(OPa)-CH_2O-), 4.52(2H, S, -OCH_2Ph), 5.22(1H, m, -OCH_2-CH(OPa)-CH_2O-), 7.32(arom. 5H). (Pa: Palmitoyl $C_{15}H_{31}CO$-)

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/CH_2Cl_2 = 2/8) to give **7**. Yield : 37 %. m.p. : < 36 °C. IR ($CHCl_3$) : 1740, 1735 cm^{-1} (-OCO- × 2). MS (m/z) : 629, 631 (M^+-OCH_2Ph). ^{1}H-NMR ($CDCl_3$) : Fig. 4.

^{18}F-Labeling

Production of [^{18}F]fluoride : [^{18}F]Fluoride was produced *via* the $^{18}O(p,n)^{18}F$ reaction by proton bombardment (18 MeV, 10 μA) of a circulating 20 % enriched [^{18}O]water target using the CGR-MeV model 680 Cyclotron located at Tohoku University [4].

Synthesis of 1*,2 *-[^{18}F]FDAG(1-(16-[^{18}F]Fluorohexadecanoyl)-2-hexadecanoylglycerol) (1)* : The ^{18}F-labeling scheme is shown in Fig. 5. 16-[^{18}F]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 literature[5),2)]. To the obtained 16-[^{18}F]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 CH_2Cl_2 (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*-[^{18}F]FDAG(2-(16-[^{18}F]Fluorohexadecanoyl)-1-hexadecanoylglycerol) (2)* : (Method A) 16-[^{18}F]Fluorohexadecanoyl chloride was reacted with 1-monopalmitin and treated in a manner similar to 1*, 2-[^{18}F]FDAG.

(Method B) The ^{18}F-labeling scheme is shown in Fig. 6. ^{18}F was introduced in a manner similar to the synthesis of 16-[^{18}F]fluorohexadecanoic acid [5),2)]. To the [^{18}F]water (100-400 μL), 0.15M K_2CO_3 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 N_2, 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}F]FDAG 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}F]FDAG and 1, 2-[^{18}F]FDAG.

References

1) Imahori Y., et al., J. Nucl. Med., **32** (1991)1622.
2) Takahashi T., Ido T. and Iwata R., Appl. Radiat. Isot., **42**(1991) 801.
3) Eibl von H., et al., Liebigs Ann. Chem., **709** (1967) 226.
4) Iwata R., et al., Appl. Radiat. Isot., **38**(1987) 979.
5) Coenen H. H., et al., J. Labelled Compd. Radiopharm., **23**(1986)455.
6) Takahashi T., et al., Appl. Radiat. Isot., **41** (1990) 649.
7) Fischer E, Ber, **53** (1920) 1621.

Table 1. HPLC retention times of acylglycerol derivatives

Compound	Retention times (min) Analytical HPLC[a]	Preparative HPLC[a]
1,2-Dipalmitin	6.0	8.4
1,3-Dipalmitin	4.2	6.1
3-O-Benzyl-1,2- dihexadecanoylglycerol (6)	1.8	2.2

a) Conditions : See text
b) Detected by Refractive Index Detector

Table 2. Experimental data of 1,2-[^{18}F]FDAG synthesis

	1,2-[^{18}F]FDAG	1,2-[^{18}F]FDAG (Method B)
Radiochemical yield (%)[a]	20 - 30	15 - 20
Radiochemical purity (%)[b]	> 97	> 98
Synthesis time (min)[c]	120 - 150	120 - 150

a) based on ^{18}F$^-$
b) determined by analytical HPLC
c) including the time required for HPLC purification

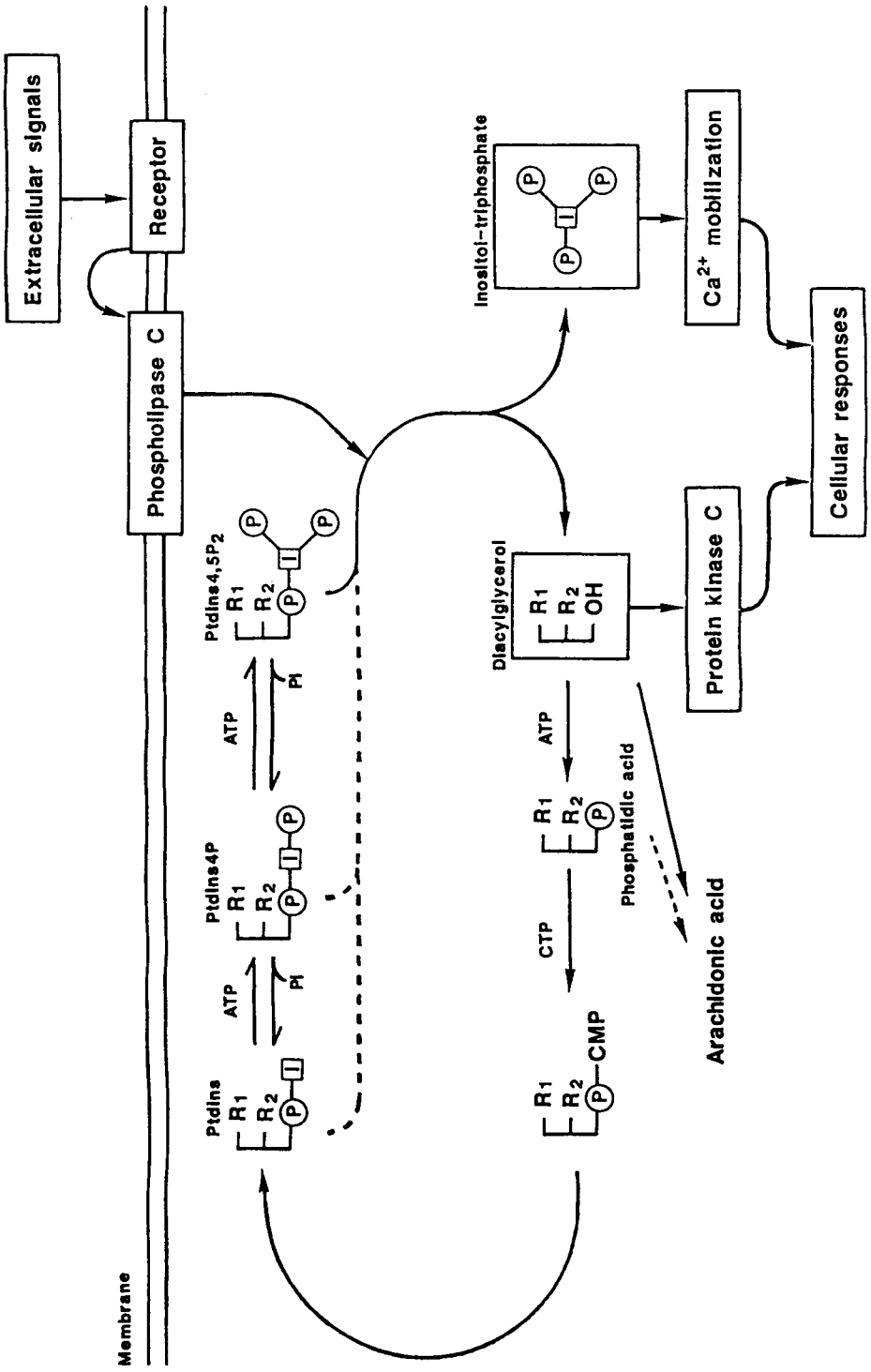

Fig. 1. Inositol phospholipid turnover and signal transduction

PtdIns:	phosphatidylinositol
PtdIns4P:	Phosphatidylinositol-4-phosphate
PtdIns4,5P_2 :	phosphatidylinositol-4, 5-bisphosphate
R_1 and R_2:	acyl groups
I:	inositol
P:	phosphoryl group

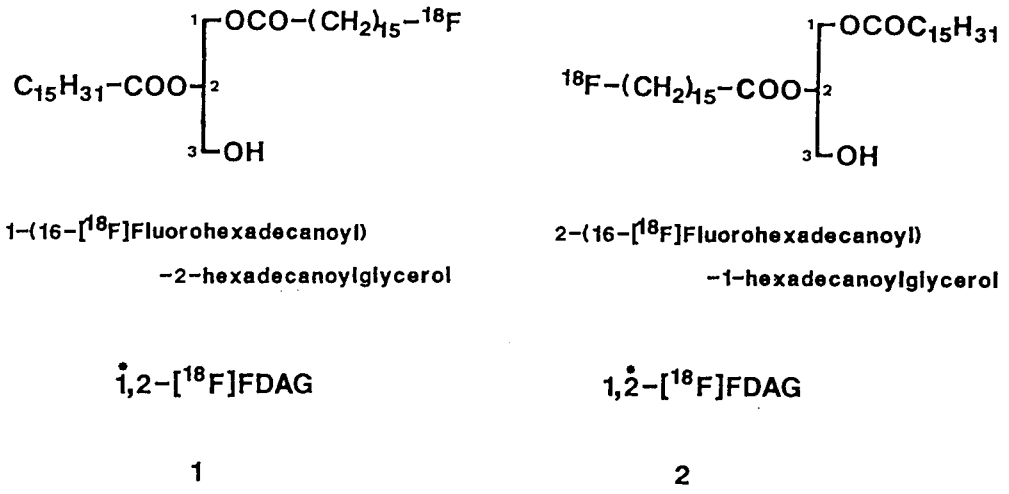

Fig. 2. Radiopharmaceuticals

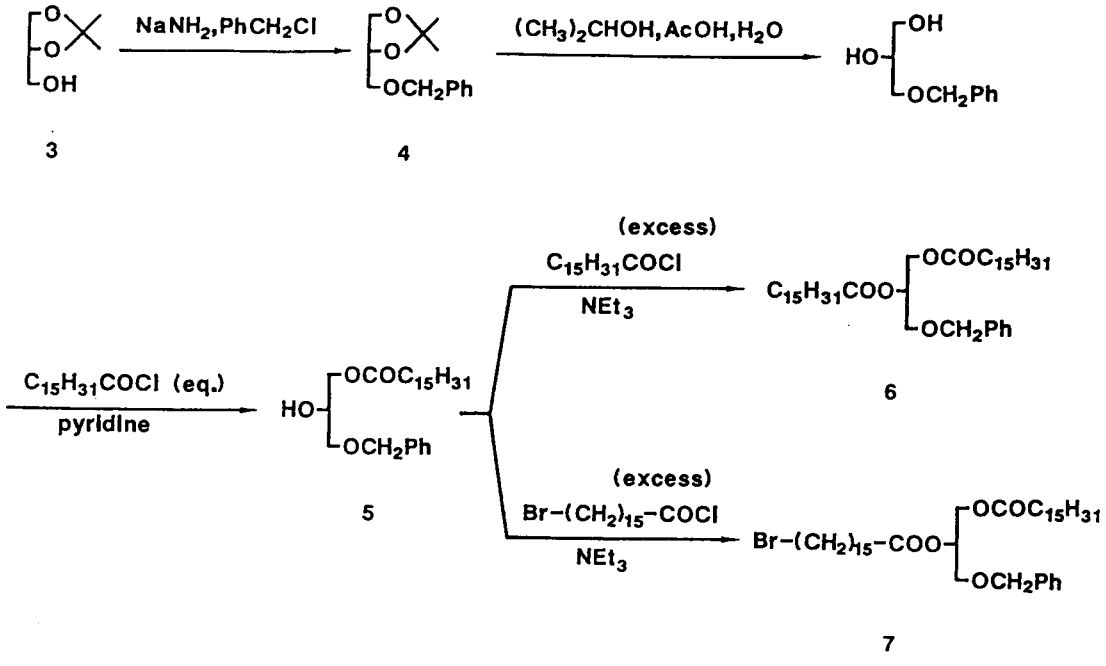

Fig. 3. Preparation of 3-O-benzyl-1,2-dihexadecanoylglycerol and 3-O-benzyl-2-(16-bromohexadecanoyl)-1-hexadecanoyl glycerol

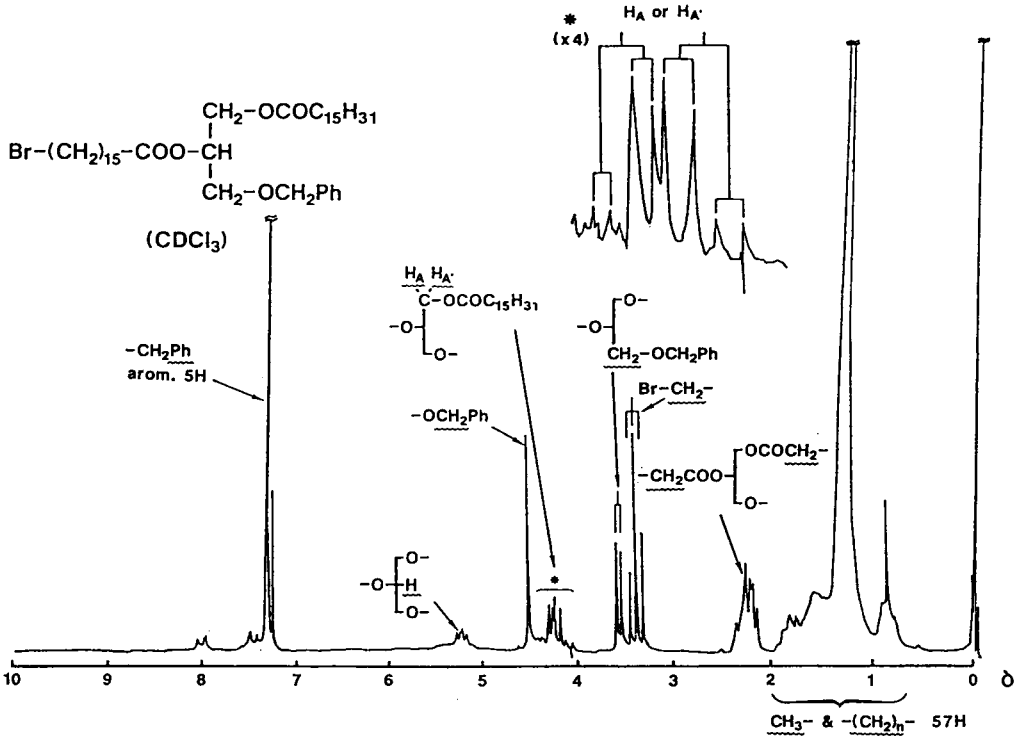

Fig. 4. ^{1}H-NMR spectra of 3-O-benzyl-2-(16-bromohexadecanoyl)-1- hexadecanoylglycerol

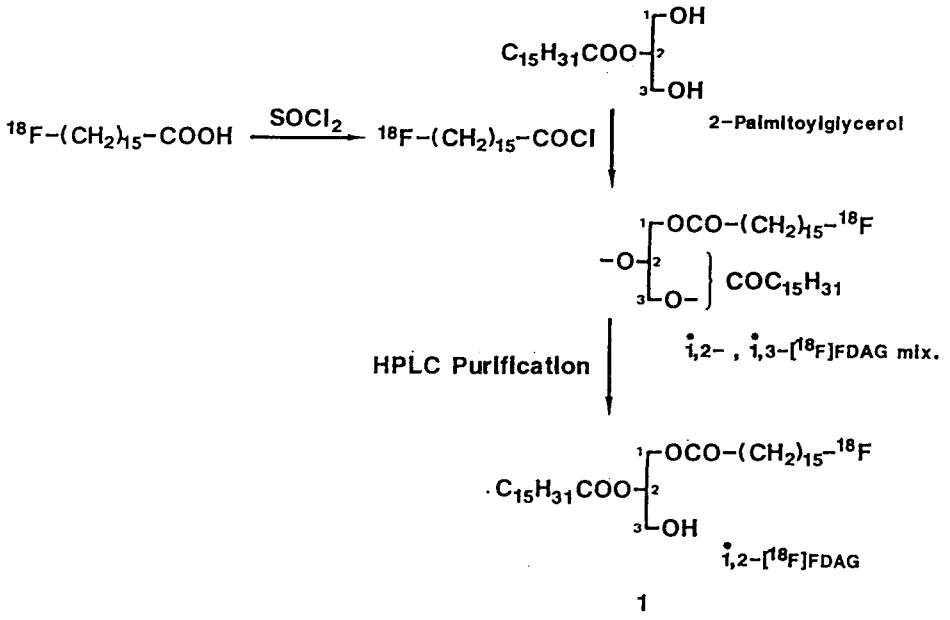

Fig. 5. Synthetic scheme of 1,2-[^{18}F]FDAG

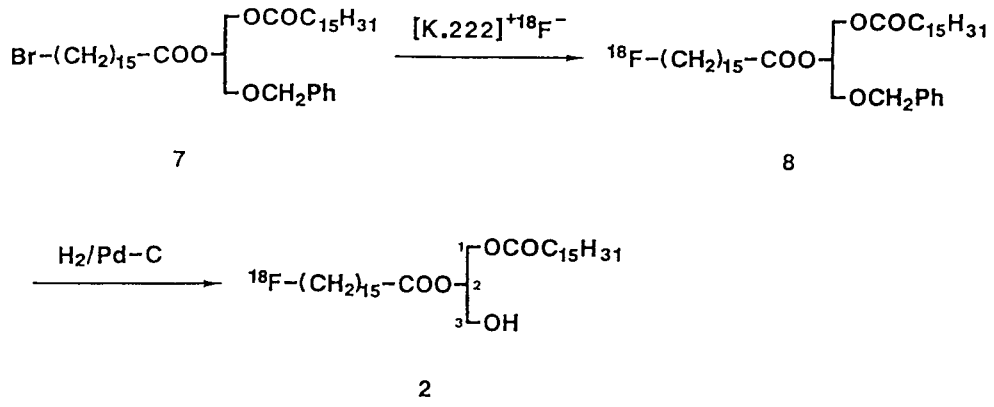

Fig. 6. Synthetic scheme of 1,2-[^{18}F]FDAG

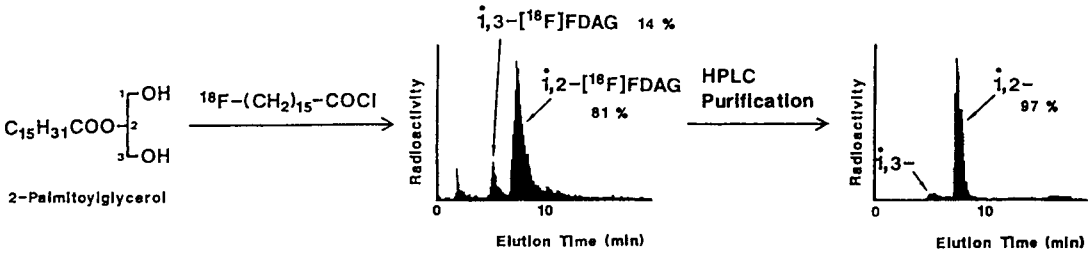

Fig. 7. Radiochromatogram synthesis of 1,2-[^{18}F]FDAG -

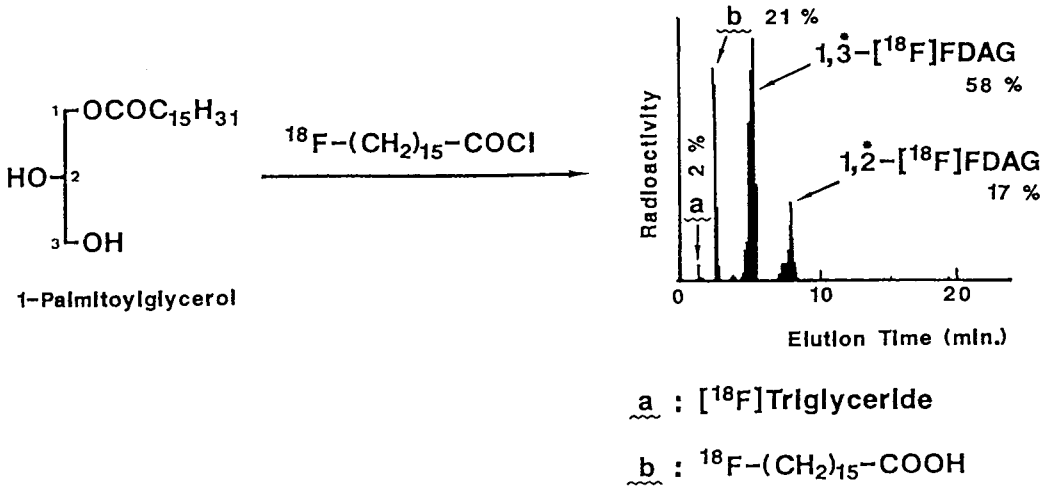

Fig. 8. Radiochromatogram synthesis of 1,2-[^{18}F]FDAG (Method A) -

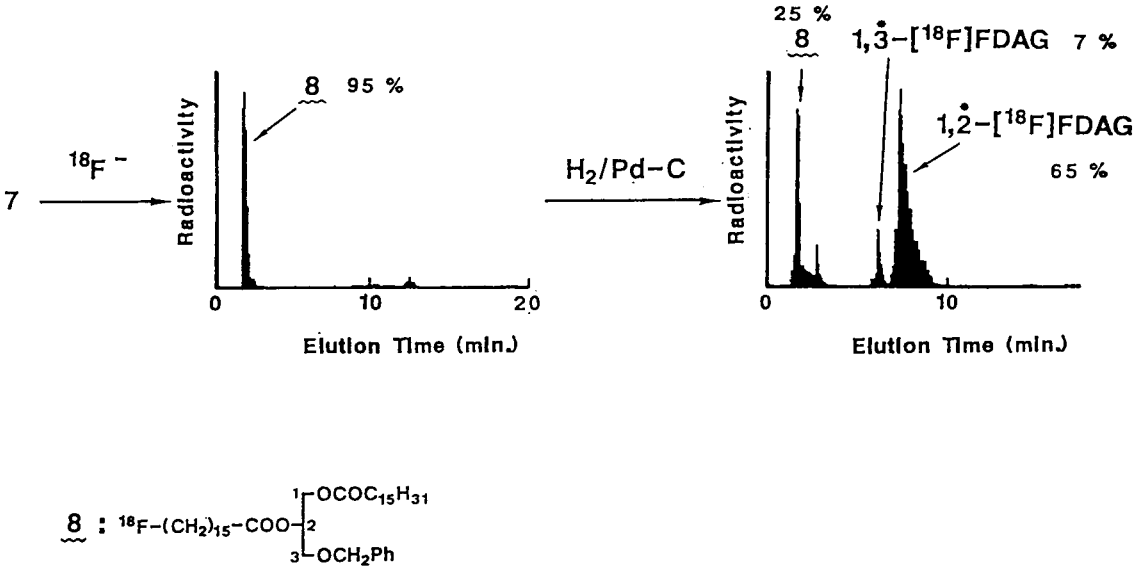

Fig. 9. Radiochromatogram synthesis of 1,2-[^{18}F]FDAG (Method B) -