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Synthesis of 1-*O*-(8-[^{18}F]fluorooctanoyl)-2-*O*-palmitoyl-*rac*-glycerol for Imaging Intracellular Signal Transduction

Shozo Furumoto*, Ren Iwata and Tatsuo Ido

Cyclotron and Radioisotope Center, Tohoku University
Sendai 980-8578, Japan

Summary

An improved approach to the synthesis of 1-*O*-(8-[^{18}F]fluorooctanoyl)-2-*O*-palmitoyl-*rac*-glycerol (*rac*-1,2-[^{18}F]FDAG) has been developed. We designed and synthesized two new types of precursors for the radiosynthesis and investigated their general utility. Each precursor was smoothly radiofluorinated (5 min) and the protecting group at the 3-*O*-position was rapidly removed (1 min). The resulting *rac*-1,2-[^{18}F]FDAG was obtained in overall radiochemical yields of 20-30% (EOB) within 70 min including final preparative HPLC separation.

Key words: Intracellular signal transduction, Second messenger, Diacylglycerols, Fluorine-18

Introduction

Neural imaging by positron emission tomography (PET) is one of the most useful approaches for *in vivo* studies of human brain functions. In general, the neural imaging is performed by measuring cerebral blood flow, metabolism, or the status of neurotransmitter-receptor systems. Although PET images derived from these studies are known to be associated with neural activity, it is also widely accepted that they provide little direct information on neuronal activities. For direct imaging of neuronal activities by PET, Imahori *et al.* focused on 1,2-*O*-diacylglycerols (DAGs) which play an important role in the postsynaptic intracellular signal transduction system as

* Corresponding author.
Fax: +81-22-217-3485; E-mail: furumoto@cyric.tohoku.ac.jp

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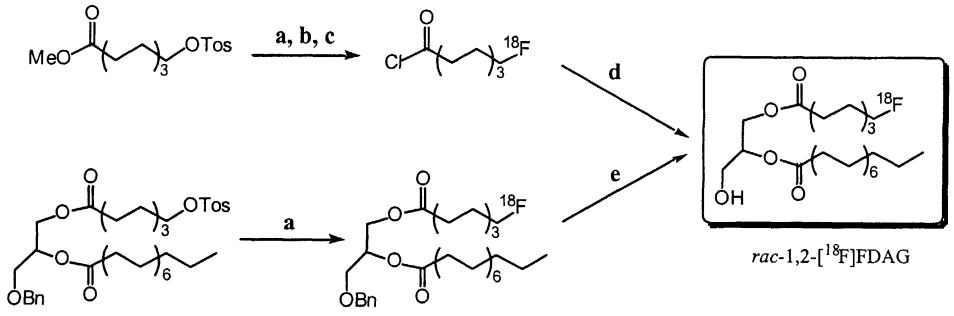


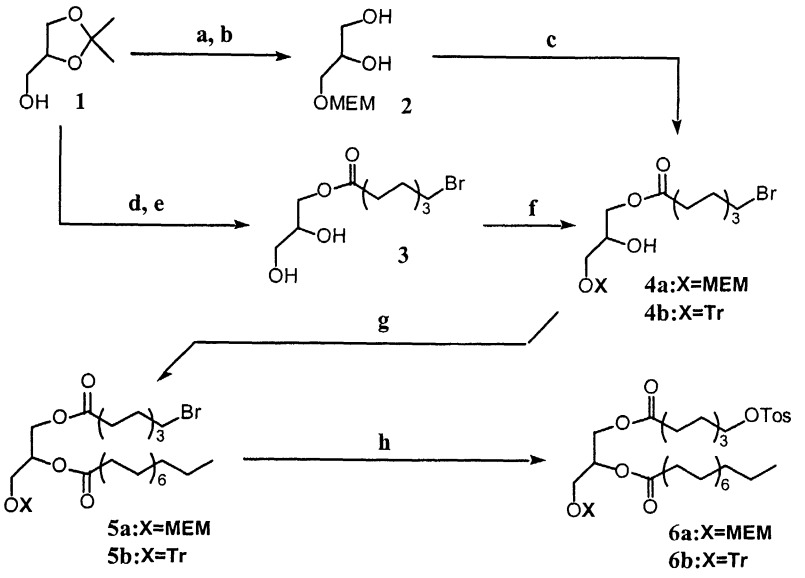
a: $K^{18}F$, Kryptofix 2.2.2, CH_3CN; **b**: Hydrosis of ester with aq. KOH, then neutralized with aq. HCl; **c**: $SOCl_2$; **d**: 2-palmitoylglycerol, DMAP, CH_2Cl_2; **e**: H_2, Pd/C, EtOH, aq. HCl.

Scheme 1. Original synthetic pathways for *rac*-1,2-[^{18}F]FDAG. The upper is an acylation method and the lower is a deprotection method.

second messengers. They also developed a carbon-11 labeled DAG analogue, 1-*O*-[1-^{11}C]butyryl-2-*O*-palmitoyl-*ras*-glycerol (1,2-[^{11}C]DAG) (1). They have shown that 1,2-[^{11}C]DAG is useful for imaging postreceptor signaling by demonstrating that 1,2-[^{11}C]DAG accumulation increases with neuronal activation (2).

DAG analogues labeled with fluorine-18 were also developed for imaging postsynaptic neuronal activities (3-5). Among these, 1-*O*-(8-[^{18}F]fluorooctanoyl) -2-*O*-palmitoyl-*rac*-glycerol (*rac*-1,2-[^{18}F]FDAG), a DAG analogue we originally designed, shows a unique characteristic. When *rac*-1,2-[^{18}F]FDAG is administered to rat, it is mainly metabolized into phosphatidylethanolamine (PE) (6), whereas [^{11}C]DAG is metabolized into phosphoinositides (PIs) (7). PE metabolic turnover is considered to be modulated by phospholipase D (PLD) (8) and is very closely related to the second messenger system in the same way that PI metabolic turnover is controlled by phospholipase C. Therefore, *rac*-1,2-[^{18}F]FDAG is expected to be a potential PET tracer for imaging and evaluating the intracellular signal transduction system.

We previously reported two procedures for the radiosynthesis of *rac*-1,2-[^{18}F]FDAG: one is an acylation method and the other is a deprotection method (Scheme 1) (3). However, there are some difficulties associated with these radiosyntheses. The acylation method consists of a multi-step radiosynthesis and also



a: MEMCl, DIEA, CH_2Cl_2; **b**: 10% aq.AcOH, EtOH; **c**: $BrC_7H_{14}COOH$, DCC, DMAP, CH_2Cl_2; **d**: $BrC_7H_{14}COCl$, TEA, CH_2Cl_2; **e**: Amberite 120(+), EtOH; **f**: TrCl, DIEA, CH_2Cl_2; **g**: $C_{15}H_{31}COCl$, DMAP, CH_2Cl_2; **h**: AgOTos, CH_3CN.

Scheme 2. Synthetic pathways for the precursors of *rac*-1,2-[^{18}F]FDAG.

requires the use of a very corrosive and toxic chlorination reagent, thionyl chloride. In addition, the deprotection method, in which the benzyl group is removed with H_2 and Pd/C, is a laborious, time-consuming (about 30 min) and low yielding reaction. Since the method consists of only two chemical steps, however, the deprotection method is still the shortest route for the synthesis of *rac*-1,2-[^{18}F]FDAG. It was therefore attractive for us from the viewpoint of simplification and improvement of the radiosynthesis. Consequently, we have continued to develop new precursors for the deprotection method. We report herein an improved deprotection method using newly designed precursors.

Results and Discussion

Synthesis of the New Precursors for [^{18}F]FDAG

Two types of new precursor **6a** and **6b** were synthesized from isopropylidene-*rac*-glycerol **1** following general routes to construct the 1,2-DAG structure with two different acyl chains (Scheme 2).

The intermediate **4a** was prepared according to a modification of the procedure described by Watts *et. al* (9). The methoxyethoxymethyl (MEM) protecting group was introduced into the glycerol **1** by treatment with MEM chloride and diisopropylethylamine (DIEA). Subsequent hydrolysis of the isopropylidene group in 10% aqueous acetic acid provided the diol **2**. Treatment of the diol **2** with one equivalent of 8-bromooctanoic acid in the presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) at 0°C afforded the intermediate **4a**. The intermediate **4b**, on the other hand, was synthesized according to a modification of the procedure described by Kodali *et. al* (10). Acylation of glycerol **1** with 8-bromooctanoyl chloride, followed by acid-catalyzed ethanolysis of the isopropylidene group using a cation exchange resin (11), gave the monoacylglycerol **3**, which was converted into the intermediate **4b** by treatment with trityl (Tr) chloride in the presence of DIEA.

Treatment of the intermediates **4a** and **4b** with palmitoyl chloride and DMAP gave the protected diacylglycerols **5a** and **5b**. Transforming the bromides of **5a** and **5b** into the *p*-toluenesulfonates by reacting with silver *p*-toluenesulfonate in refluxing

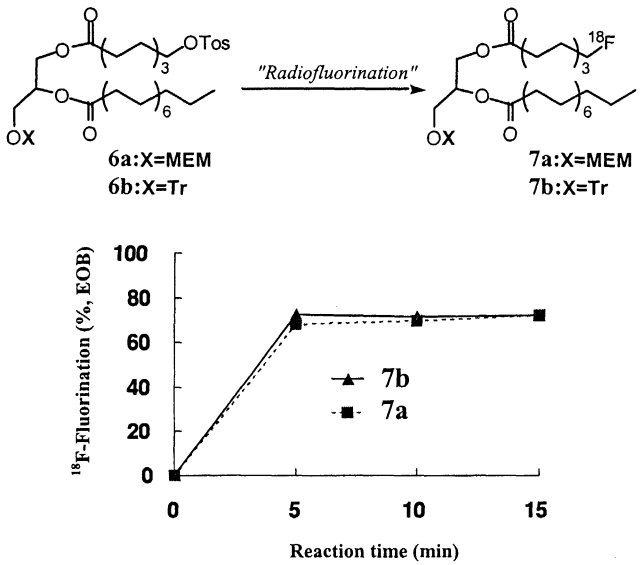


Figure 1. Scheme for the radiofluorination of the precursors **6a** and **6b** and the graph of yield against time.

acetonitrile (12) accomplished the synthesis of the corresponding precursors **6a** and **6b**. FDAG was synthesized from precursors **6a** or **6b** through general fluorination procedures with potassium fluoride activated by 18-crown-6 and subsequent deprotection procedures already reported in the literature (13, 14).

Radiosynthesis

The new method for radiosynthesis of *rac*-1,2-[^{18}F]FDAG consists of two chemical processes, radiofluorination and deprotection. To optimize the reaction times, the radiochemical reaction in each process was monitored by radio-thin layer chromatography.

^{18}F-Fluorinations of the precursors (**6a** and **6b**) were performed by treating them with NCA [^{18}F]KF and Kryptofix 2.2.2 in refluxing acetonitrile, and the changes

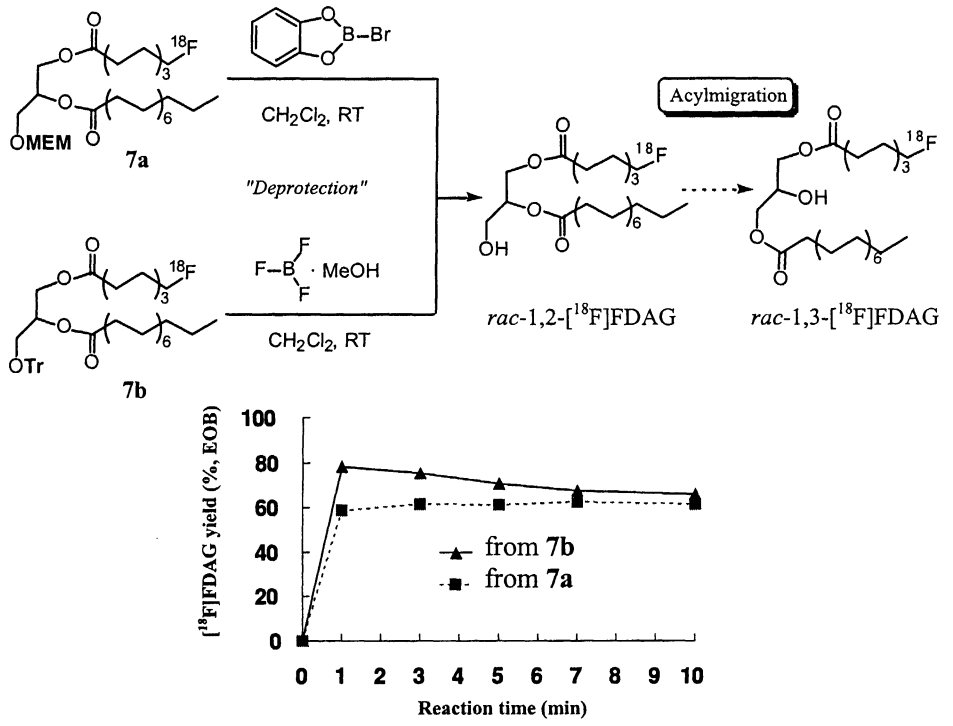


Figure 2. Scheme for the deprotection of **7a** and **7b** and the graph of yield against time.

in radiofluorination yield with time were monitored (Figure 1). Radiofluorination of each precursor proceeded smoothly in good radiochemical yield (about 70%) within 5 min and thereafter the yields did not increase. The optimum time for the radiofluorination was concluded to be 5 min under the above conditions.

The deprotections of **7a** and **7b** were carried out at room temperature by treatment with *B*-bromocatecholborane (13) and boron trifluoride-methanol complex (14) respectively as deprotection reagents. The deprotection method must generally be used with caution for the synthesis of 1,2-*O*-diacylglycerols, because they easily isomerize under acidic, basic or thermal conditions to 1,3-*O*-diacylglycerols (15). Therefore, we carefully investigated the changes in the yield with time (1, 3, 5, 7, 10 min) monitoring not only *rac*-1,2-[^{18}F]FDAG production but also production of the isomerized compound, *rac*-1,3-[^{18}F]FDAG (Figure 2).

Removal of the MEM protecting group proceeded rapidly in a yield of 59 % after 1 min. The yields did not change up to 10 min. On the other hand, the Tr protecting group was also readily removed giving a yield of 78 % in 1 min, but thereafter the yield decreased gradually to 66 % after 10 min. In this case, the yield of the isomerized product, *rac*-1,3-[^{18}F]FDAG increased from 6 % to 19 % with time as the yield of *rac*-1,2-[^{18}F]FDAG decreased. Even so, these deprotection conditions and reaction times were far milder and shorter than those used previously for the removal of the benzyl group (5).

Considering these results, the Tr group appears more suitable as the protecting group for the precursor than the MEM group, though the deprotection needs to be quenched at one minute to obtain a better yield of *rac*-1,2-[^{18}F]FDAG. We have repeatedly synthesized *rac*-1,2-[^{18}F]FDAG using precursor **6b** and always obtained superior yields (overall yields of 20-30%) than with the previously reported precursors (overall yields of 10-15%) (3). Furthermore, the total synthesis time was considerably shortened to about 70 min, compared to 120-150 min for the previously reported route (3) including final preparative HPLC separation (see also Figure 3 in the experimental section).

Conclusion

We have developed an improved method for the synthesis of *rac*-1,2-[^{18}F]FDAG using two novel protected precursors . Compared to the previously reported synthesis of *rac*-1,2-[^{18}F]FDAG the method is superior in terms of operational simplicity, improved radiochemical yield and reduced time for radiosynthesis.

The synthetic strategy used for the radiosynthesis of *rac*-1,2-[^{18}F]FDAG using a precursor protected with the Tr group was also applied to the synthesis of precursors for radiosyntheses of (*S*)- or (*R*)-1,2-[^{18}F]FDAG starting from (*R*)- or (*S*)-1,2-*O*-isopropylideneglycerol respectively. Biological evaluation of these radiolabeled compounds is currently under investigation and the results will be reported elsewhere.

Experimental

General

All starting material and reagents were obtained from commercial suppliers and were used without further purification. Reaction involving air- or/and moisture-sensitive reagents were executed using anhydrous organic solvents and under an inert atmosphere of dry N_2 gas. Amberite IR 120(+) was purchased from Aldrich, Sep-Pak cartridges of C18 and Dry (Na_2SO_4) from Waters, and Kryptofix 2.2.2 and *Extrelut* from Merck. Analytical radio thin layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} precoated aluminium sheets. Column chromatography was performed using Silica Gel 60 (spherical, 40-100 μm) purchased from Kanto Chemical Co., Inc. ^{1}H NMR spectra were recorded on a Varian Gemini 2000 (300 MHz) or a JEOL GX-400 (400 MHz). Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS-DX303 or -DX500. Radio TLC was analyzed with a BASS 5000 system (Fuji film, Japan). Preparative radio HPLC separation was performed using YMC-Pack Sil-06 (RCM 10.0 mm x 250 mm).

1-O-methoxyethoxymethyl-rac-glycerol (2)

7.0 mL of diisopropylethylamine (DIEA, 39 mmol) was added dropwise with stirring to a solution of 1,2-*O*-isopropylidene-*rac*-glycerol **1** (4.00 mL, 31.0 mmol) and MEM chloride (4.0 mL, 34 mmol) in CH_2Cl_2 (40 mL). After stirring for 4 hours at room temperature, the organic layer was successively washed with H_2O (1 x 40 mL) and 2*M* HCl (2 x 40 mL) and the combined water layers were extracted with $CHCl_3$ (1 x 100 mL). The combined organic layers were washed with saturated aqueous NaCl (1 x 100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The oil residue was purified by column chromatography eluting with hexane/AcOEt (2:1) to give 4.67 g (22.7 mmol, 71 %) of 1, 2-*O*-isopropylidene-3-*O*-methoxyethoxymethyl-*rac*-glycerol as colorless oil. 300 MHz ^{1}H NMR ($CDCl_3$/TMS) δ (ppm): 1.37 (3H, s), 1.43 (3H, s), 3.40 (3H, s), 3.55-3.58 (2H, m), 3.66 (2H, d, J=5.5), 3.70-3.75 (3H, m), 4.04-4.09 (1H, m), 4.26-4.32 (1H, m), 4.76 (1H, s).

A solution of 1, 2-*O*-isopropylidene-3-*O*-methoxyethoxymethyl-*rac*-glycerol (2.00 g, 9.10 mmol) in 10% aqueous acetic acid (10 mL) was stirred at 60°C for 90 min, and then the reaction was quenched by saturated aqueous $NaHCO_3$. After cooling to room temperature, acetonitrile was added and the solvent was removed under reduced pressure. The residue was purified by column chromatography eluting with AcOEt/methanol (4:1) to give 1.17 g (6.50 mmol, 72%) of **2** as colorless oil. 300 MHz ^{1}H NMR ($CDCl_3$/TMS) δ (ppm): 2.30 (1H, t, J=5.5), 3.05 (1H, d, J=5.0), 3.41 (3H, s), 3.56-3.59 (2H, m), 3.63-3.75 (6H, m), 3.84-3.91 (1H, m), 4.75 (2H, s). MS (EI): 181 (MH^+).

1-O-(8-bromooctanoyl)-3-O-methoxyethoxymethyl-rac-glycerol (4a)

A solution of dicyclohexylcarbodiimide (DCC, 3.70 g, 17.0 mmol) in CH_2Cl_2 (40 mL) was added dropwise over 15 min at 0°C to a solution of **2** (2.85 g, 15.8 mmol), 8-bromooctanoic acid(3.90 g, 17.0 mmol) and dimethylaminopyridine (DMAP, 2.96 g, 23.7 mmol). A precipitate began to form gradually upon the addition of the DCC solution. The resulting suspension was allowed to warm slowly to room temperature and stirred for 10 hours. The mixture was filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography eluting with

hexane/AcOEt/methanol (9:5:3) to give 3.70 g (9.60 mmol, 61 %) of **4a** as colorless oil. 300 MHz ^{1}H NMR ($CDCl_3$/TMS) δ (ppm): 1.32-1.39 (4H, m), 1.39-1.46 (2H, m), 1.57-1.70 (2H, m), 1.80-1.90 (2H, m), 2.35 (2h, t, J=7.4), 2.99 (1H, d, J=4.8), 2.99-3.43 (5H, m), 3.55-3.59 (2H, m), 3.62-3.78 (5H, m), 3.97-4.04 (1H, m), 4.15 (2H, dd, J=2.2, 5.2), 4.76 (2H, s). MS (FAB): 385 (MH^+).

1-O-(8-bromooctanoyl)-rac-glycerol (3)

$SOCl_2$ (10.0 mL) was added dropwise to 8-bromooctanoic acid (4.74 g, 20.0 mmol) and the mixture was stirred for 4 hours, followed by evaporation under reduced pressure to remove excess $SOCl_2$. The obtained brown oil residue was dissolved in CH_2Cl_2 (50 mL) followed by addition of 3.80 mL (30.0 mmol) of isopropylidene-*rac*-glycerol and 4.0 mL of triethylamine (30 mmol). After stirring for 1 day, the precipitate triethylamine hydrochloride was filtered off and the solvent was removed under reduced pressure. The oil residue was dissolved in Et_2O (30 mL) and the additional precipitate was filtered off. The Et_2O solution was washed with H_2O (2 x 30 mL) and the combined water solution was back-extracted with Et_2O (1 x 30 mL). The combined organic solution was dried over $MgSO_4$ and evaporated *in vacuo*, and the oil residue was purified by column chromatography eluting with hexane/AcOEt (3:1) to give 5.95 g (17.6 mmol, 89 %) of 1-*O*-(8-bromooctanoyl) -isopropylidene-*rac*-glycerol as colorless oil. 300 MHz ^{1}H NMR ($CDCl_3$/TMS) δ (ppm): 1.26-1.40(8H, m), 1.40-1.48 (4H, m), 1.59-1.69 (2H, m), 1.79-1.90 (2h, m), 2.34 (2H, t, J=6.6) 3.40 (2H, t, J=6.6), 3.69-3.78(1H, m), 4.05-4.20(3H, m), 4.26-4.35 (1H, m).

Amberite IR 120(+) (9.5 g) was added to a solution of 1-*O*-(8-bromooctanoyl)-isopropylidene-*rac*-glycerol (9.08 g, 26.9 mmol) in ethanol (60 mL) and heated at reflux for 45 min. The resin was filtered off quickly and the solvent was removed under reduced pressure. The oil residue was purified by column chromatography eluting with hexane/AcOEt/methanol (3:4:1) to give 6.68 g (22.4 mmol, 83%) of **3** as colorless oil. 300 MHz ^{1}H NMR ($CDCl_3$/TMS) δ (ppm): 1.30-1.38 (4H, m), 1.38-1.50 (2H, m), 1.59-1.70 (2H, m), 1.80-1.90 (2H, m), 2.08 (1H, br), 2.36 (2H, t, J=7.1), 3.45-3.75 (2H, m), 3.90-3.97 (1H, m), 4.10-4.25 (2H, m). HRMS (EI): calcd for $C_{11}H_{22}O_4Br$ 297.0701 (MH^+), found 297.0735.

*1-O-(8-bromooctanoyl)-3-O-trityl-rac-glycerol (**4b**)*

2.56 mL of DIEA (14.1 mmol) was added to a solution of **3** (2.88 g, 9.42 mmol) and trityl chloride (2.98 g, 10.7 mmol) in CH_2Cl_2(50 mL) and stirred for 30 h. The solvent was evaporated under reduced pressure and $CHCl_3$ (40 mL) was added to the residue. The solution was washed with saturated aqueous $NaHCO_3$ (1 x 40 mL) and H_2O (1 x 40 mL) successively. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*, and the oil residue was purified by column chromatography eluting with hexane/AcOEt (2:1) to give 4.28 g (7.91 mmol, 84 %) of **4b** as colorless oil. 400 MHz ^{1}H NMR ($CDCl_3$/TMS) δ (ppm): 1.29-1.33 (4H, m), 1.39-1.46 (2H, m), 1.55-1.62 (2H, m), 1.80-1.88 (2H, m), 2.29 (2H, t, J=7.6), 2.38 (1H, d, J=5.2), 3.20-3.33 (2H, m) 3.39 (2H, t, J=6.8), 3.96-4.03 (1H, m), 4.14-4.23 (2H, m), 7.22-7.33 (9H, m), 7.40-7.44 (6H, m). HRMS (EI): calcd for $C_{30}H_{35}O_4Br$ 538.1717 (M^+), found 538.1743.

*1-O-(8-bromooctanoyl)-2-O-palmitoyl-3-O-trityl-rac-glycerol (**5b**)*

Palmitoyl chloride (4.10 mL, 13.0 mmol) was added dropwise with stirring to a solution of **4b** (5.4 g, 10 mmol) and DMAP (2.20 g, 15.0 mmol) in CH_2Cl_2(40 mL). After stirring for 24 h at room temperature, the solvent was evaporated under reduced pressure and Et_2O (30 mL) was added to the residue followed by filtering the precipitate. The solvent was evaporated *in vacuo* and the oil residue was purified by column chromatography eluting with hexane/AcOEt (7:1) to give 5.96 g (7.66 mmol, 77%) of **5b** as colorless oil. 400 MHz ^{1}H NMR ($CDCl_3$/TMS) δ (ppm): 0.88 (3H, t, J=6.4), 1.20-1.34 (30H, m), 1.48-1.68 (4H, m), 1.80-1.88 (2H, m), 2.23 (2H, t, J=7.6), 2.33 (2H, t, J=7.4), 3.20-3.23 (2H, m), 3.39 (2H, t, J=6.8), 4.20-4.36(2H, m), 5.20-5.38 (1H, m), 7.22-7.33 (9H, m), 7.40-7.44 (6H, m). MS (EI): 776 (M^+).

Compound **5a** was synthesized from **4a** in a similar manner.

*1-O-(8-O-tosyloctanoyl)-2-O-palmitoyl-3-O-trityl-rac-glycerol (**6b**)*

A solution of **5b** (8.89 g, 11.4 mmol) and silver *p*-toluenesulfonate (6.55 g, 23.4 mmol) in acetonitrile (40 mL) was heated at reflux for 4 days. The reaction mixture

was filtered and evaporated *in vacuo*, and then $CHCl_3$ (70 mL) was added to the residue followed by washing with H_2O (1 x 100 mL) and saturated aqueous NaCl (1 x 100 mL) successively. The organic solution was dried over $MgSO_4$ and evaporated *in vacuo*, and the crude mixture was purified by column chromatography eluting with hexane/AcOEt (17:3) to give 7.85 g (9.01 mmol, 79 %) of **6b** as colorless oil. 300 MHz ^{1}H NMR ($CDCl_3$/TMS) δ (ppm): 0.87 (3H, t, J=6.8), 1.20-1.34 (30H, m), 1.48-1.68 (4H, m), 2.20 (2H, t, J=7.6), 2.33 (2H, t, J=7.4), 2.44 (3H, s), 3.20-3.23 (2H, m), 4.00 (2H, t, J=6.6), 4.20-4.36(2H, m), 5.20-5.38 (1H, m), 7.20-7.40 (17H, m), 7.78 (2H, d, J=8.3). MS (EI): 868 (M^+).

Compound **6a** was synthesized from **5a** in a similar manner.

1-O-(8-fluorooctanoyl)-2-O-palmitoyl-rac-glycerol (FDAG)

A solution of 18-crown-6 in acetonitrile (10 mL) was added with stirring to a suspension of **6b** (5.70 g, 6.50 mmol) and KF (444 mg, 7.50 mmol) in acetonitrile (35 mL). After stirring for 2 days with heating at reflux, the solution was concentrated under reduced pressure and added $CHCl_3$ (50 mL) followed by washing with H_2O (2 x 50 mL) and saturated aqueous NaCl (1 x 50 mL) successively. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*, and the crude mixture was purified by column chromatography eluting with hexane/EtOAc (2:1) to give 3.81 g (5.31 mmol, 82 %) of 1-*O*-(8-fluorooctanoyl)-2-*O*-palmitoyl-3-*O*-trityl-*rac*-glycerol as colorless oil. 400 MHz ^{1}H NMR ($CDCl_3$/TMS) δ (ppm): 0.86 (3H, t, J=7.1), 1.20-1.40 (34H, m), 1.45-1.80 (6H, m), 2.21 (2H, t, J=7.8), 2.33 (2H, t, J=7.23), 3.20-3.23 (2H, m), 4.00 (2H, t, J=6.6), 4.20-4.39 (3H, m), 4.45-4.51 (1H, m), 5.20-5.30 (1H, m), 7.20-7.45 (15H, m).

A solution of 14% BF_3 methanol (87.4 mg, 0.180 mmol) was added with stirring to a solution of 1-*O*-(8-bromooctanoyl)-isopropylidene-*rac*-glycerol (96.5 mg, 0.130 mmol) in CH_2Cl_2 (4.5 ml) at 2-3 °C. After stirring for 60 min, the solution was washed with H_2O (3 x 10 mL), and then the organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The oil residue was purified by column chromatography eluting with hexane/AcOEt (2:1) to give 480 mg (0.101 mmol, 78 %) of the titled compound as colorless oil. 400 MHz ^{1}H NMR ($CDCl_3$/TMS) δ

(ppm): 0.88 (3H, t, J=6.8), 1.20-1.42 (34H, m), 1.55-1.80 (6H, m), 2.20 (1H, brs), 2.30-2.38 (4H, m), 3.73 (2H, t, J=5.8), 4.23 (1H, dd, J=12, 5.6), 4.32 (1H, dd, J=12, 4.4), 4.43 (2H, dd, J=47, 6.1), 5.05-5.11 (1H, m). MS (EI): 474 (M^+).

Radiosynthesis of rac-1, 2-[^{18}F]FDAG

[^{18}F]Fluoride was produced by the ^{18}O(p, n)^{18}F nuclear reaction on a [^{18}O]water target using a HM-12 cyclotron (Sumitomo Heavy Industries, Japan) and the fluoride was converted to potassium [^{18}F]fluoride with potassium carbonate. To this [^{18}F]fluoride solution were added Kryptofix 2.2.2 (15-20 mg) and acetonitrile followed by azeotropic evaporation to dryness. To the radioactive residue was added **6a** or **6b** (15-20 mg) dissolved in dry acetonitrile (1.5 mL) and the radiofluorination was carried out at reflux. After quenching with water, the mixture was passed through a Sep-Pak C18 cartridge and the crude product was trapped on it. The column was then washed with 0.5*N* HCl (3.0 mL) and H_2O (2 x 3.0 mL) successively. The crude product was eluted with ethyl ether (3 x 3.0 mL) and the ether solution was dried by passing through *Extrelut* and Sep-Pak Dry cartridges. The dried solution was evaporated in vacuo and the following deprotection procedure was applied to the crude residue.

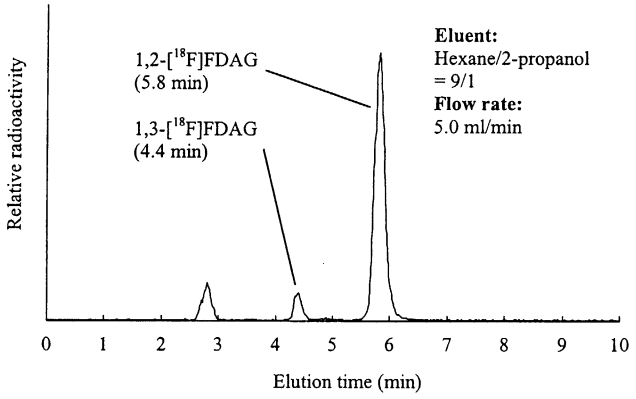


Figure 3. Radio-HPLC separation of *rac*-1,2-[^{18}F] FDAG synthesized from precursor **6b**.

The crude residue was dissolved in CH_2Cl_2 (1.0 mL) and the deprotection was carried out at room temperature using a 0.2 M CH_2Cl_2 solution of *B*-bromocatecholborane (4 eq. to **6a**) to **7a** or a solution of 14% BF_3 methanol (4 eq. to **6b**) to **7b**. After quenching with water, the mixture was passed through *Extrelut* and Sep-Pak Dry cartridge and the resulting organic solution was evaporated *in vacuo*. Separation of the product was performed by radio-HPLC with *n*-hexane/isopropanol (9 : 1) at flow rate of 5.0 mL/min. A typical preparative radio-HPLC chart of *rac*-1,2-[^{18}F]FDAG synthesis using **6b** is indicated in Figure 3. Peaks eluting at 5.8 min and 4.4 min were assigned to *rac*-1,2-[^{18}F]FDAG and *rac*-1,3-[^{18}F]FDAG respectively by comparing their retention times with those of authentic non radioactive standards.

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