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F-18 Labeling of Ether-Linked Analogs of Diacylglycerol

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Summary

Ether-linked analogs of fluorine-18 labeled diacylglycerol were synthesized in order to improve their bioavailability *in vivo*. Compounds were designed to have long chain (having sixteen carbons) alkyl substituent on 1-*O*-position and short alkyl or acyl group (eight carbons) on 2-*O*-position of glycerol. Introduction of ^{18}F into each chain resulted in four labeled tracers. Precursors having tosyloxy or bromo moiety as leaving group were obtained step wise alkylations and/or acylations of 3-*O*-benzylglycerol. Nucleophilic radiofluorination of the precursors thus obtained gave labeled intermediates in up to 40% radiochemical yield. Ether-linked diacylglycerol analogs were afforded through their deprotection with H_2 and Pd/C. Catalytic transfer hydrogenation was also attempted, however, insufficient result was achieved in spite of its usefulness as labeling procedure.

Key Words: Positron emission tomography, Second messenger, Diacylglycerol, Protein kinase C, Fluorine-18

Introduction

The potency of intracellular signal transduction measurements in nuclear medicine was well established by Imahori *et al.* (1, 2). Membrane trapping of carbon-11 labeled 1,2-*O*-diacylglycerol ([^{11}C]DAG) was shown to represent the rate of protein kinase C driven phosphoinositide turnover and the post-sigaling neuronal activity. Some approaches to extend their success to fluorine-18 labeled compounds were also presented (3, 4). The tracers of this class should be incorporated into

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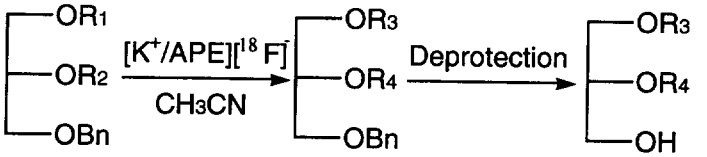
phopshoinositides, however, multiple metabolic pathways were also considered. Defluorination followed by accumulation of radioactivity to bone which is a major drawback of fluorine-18 labeled compounds was accompanied by hydrolysis of fatty acid chain after intravenous injection of [^{18}F]DAG. This study aimed to reduce this hydrolysis and therefore defluorination by substituting the ester links to ethers. There are many examples of 1-*O*-alkyl-2-*O*-acylglycerol that occur naturally including platelet activating factor and plasminogen. Another example of ether-linked glycerol analog is an artificial 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine (ET-16-OMe) which exhibited anti tumor activity (5). Ether-linked analogs of fluorine-18 labeled DAG were designed and the reaction conditions were examined (Figure 1).

Figure 1 Reaction Scheme



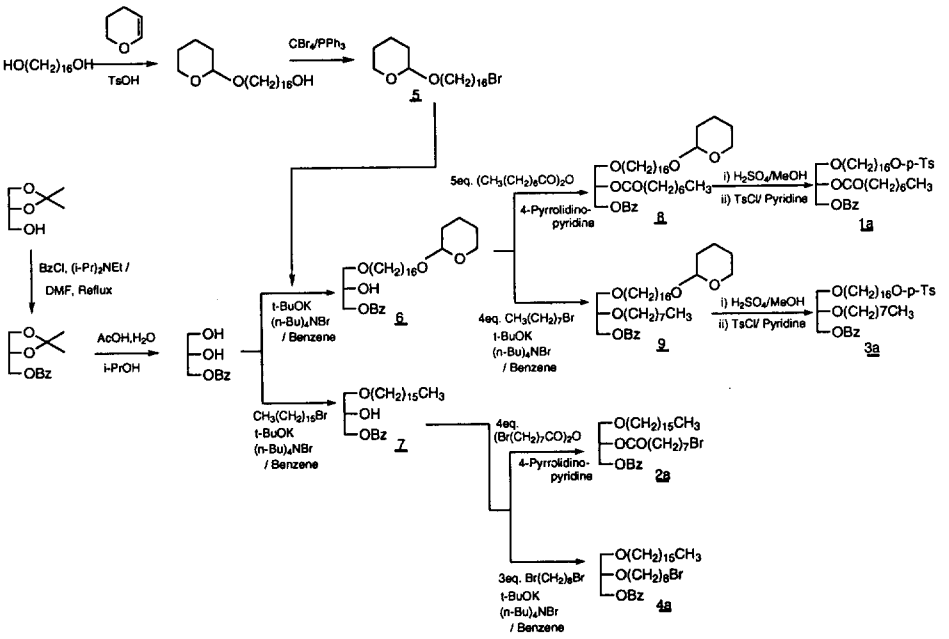
1a	R_1=$(CH_2)_{16}$O-p-Ts R_2=$CO(CH_2)_6CH_3$	1b	R_3=$(CH_2)_{16}{}^{18}F$ R_4=$CO(CH_2)_6CH_3$	1c	R_3=$(CH_2)_{16}{}^{18}F$ R_4=$CO(CH_2)_6CH_3$
2a	R_1=$(CH_2)_{15}CH_3$ R_2=$CO(CH_2)_7$Br	2b	R_3=$(CH_2)_{15}CH_3$ R_4=$CO(CH_2)_7{}^{18}F$	2c	R_3=$(CH_2)_{15}CH_3$ R_4=$CO(CH_2)_7{}^{18}F$
3a	R_1=$(CH_2)_{16}$O-p-Ts R_2=$(CH_2)_7CH_3$	3b	R_3=$(CH_2)_{16}{}^{18}F$ R_4=$(CH_2)_7CH_3$	3c	R_3=$(CH_2)_{16}{}^{18}F$ R_4=$(CH_2)_7CH_3$
4a	R_1=$(CH_2)_{15}CH_3$ R_2=$(CH_2)_8$Br	4b	R_3=$(CH_2)_{15}CH_3$ R_4=$(CH_2)_8{}^{18}F$	4c	R_3=$(CH_2)_{15}CH_3$ R_4=$(CH_2)_8{}^{18}F$

Results and Discussion

Drug Design and Precursor Synthesis

Naturally occurring 1-*O*-alkyl-2-*O*-acylglycerol was selected as a first candidate of ether-linked analog of DAG. The long chain acyl substituent was found to increase the liver uptake of ^{18}F label possibly by its hydrolysis followed by β-oxidation and/or defluorination of fatty acid thus obtained. So chain length of acyl group on 2-*O*-position was confined to eight carbons and hexadecyl group was to be introduced on 1-*O*-position. 1,2-*O*-Dialkyl congener having same chain length was also picked up as a reference compound. Either long or short chain was designed to be labeled with fluorine-18 (Figure 1). Racemic [^{11}C]DAG (1-*O*-[1-^{11}C]butyryl-2-*O*-palmitoyl-*rac*-glycerol) was reported to show similar uptake and metabolism as 1-*O*-palmytoyl-2-*O*-[1-^{11}C]butyryl-*sn*-glycerol (1). As they concluded that racemic mixture of DAG could serve as a tracer for receptor-linked PI turnover, we also employed the racemic isopropylidene-protected glycerol as a starting material of the preparations of labeling precursors.

Figure 2 Precursor Synthesis



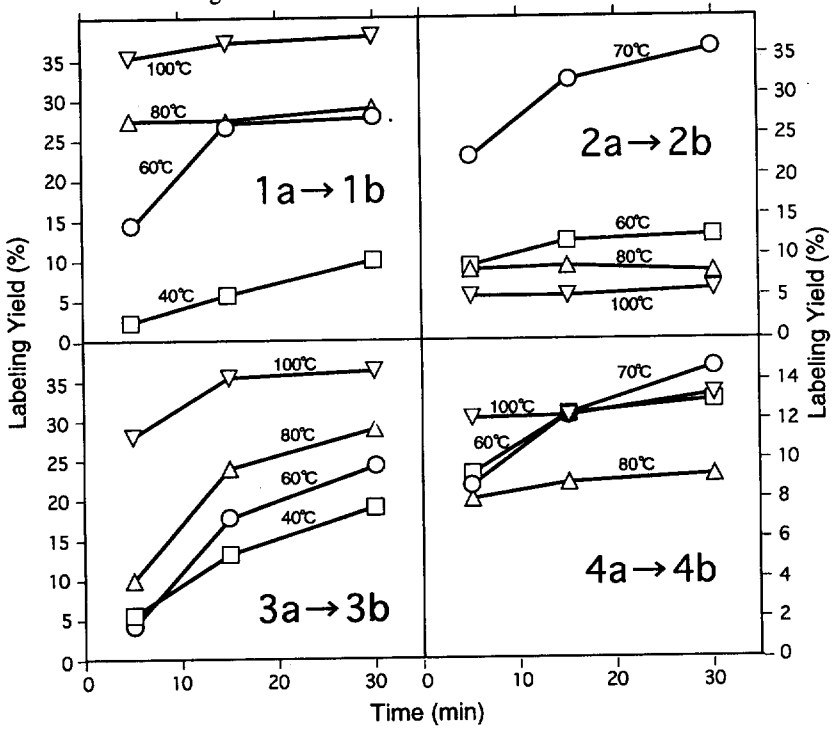
The step wise alkylation and acylation were carried out in order to obtain the glycerols with leaving group following the method of Duclos (6, Figure 2). Regioselective 1-*O*-alkylation of 3-*O*-benzylglycerol was carried out with 1 eq. of alkyl bromide in the presence of *t*-BuOK and *n*-Bu4NBr. Subsequent alkylation or acylation on 2-*O*- position was accomplished by excess amount (from 3 to 5 eq.) of alkylbromide or acid anhydride, respectively.

For the compounds having fluorine label on short chain acyl or alkyl substituent on 2-*O*-position reagent availability allowed us to prepare bromo precursors, 2a and 4a. But for derivatives labeled at the terminal of long alkyl chain on 1-*O*-position we had to prepare the p-toluenesulfonyloxyl precursors from protected alcohols. It became necessary to discriminate the terminal hydroxyl groups from that on 3-*O*-position of glycerol using different protecting groups. We employed the strategy of Takahashi (3) which utilizes acid labile tetrahydropyranyl (THP) group to protect the terminal hydroxyl group and benzyl group which is resistant to mild acid treatment (i.e. trifluoroacetic acid) for the protection of the residual OH of glycerol.

Radiofluorination

Radiosyntheses of ether linked analogs of DAG were carried out in two step reaction, radiofluorination and deprotection. The labeling precursors obtained as above were allowed to react with aminopolyether (APE, Kryptofix 222 (Merck, Germany)) activated [^{18}F]fluoride in acetonitrile following the usual manner (see experimental section). Results were shown in Figure 3.

Figure 3 Radiofluorination with NCA[^{18}F]fluoride



[^{18}F]Fluorination was carried out in CH_3CN (0.5mL) in the presence of K_2CO_3 and APE (final concentration was 40mM). Final concentration of K_2CO_3 was 10mM for [^{18}F]fluorination of 2a, 3a, or 4a and 2mM for that of 1a. Conditions were describe in detail in the text.

Precursors having p-toluenesulfonyloxyl group, 1a and 3a, showed temperature dependent improvement of radiochemical yield. In contrast, precursors having bromo group, 2a and 4a, showed highest yield at 70°C under the present reaction condition.

Compounds 2b, 3b and 4b were readily obtained with K_2CO_3 at final concentration of 10mM. But in the reaction mixture of [^{18}F]-fluorination of 1a under the same condition a radioactivity other than 1b and fluoride was observed. This byproduct increased with time resulting in the decrement of 1b when the reaction was carried out at 80°C and 100°C. The byproduction was almost disappeared (less than 2% of total radioactivity) when the base concentration was reduced to 2mM and the further reduction resulted in decline of fluorination yield. Base catalyzed hydrolysis of the acyl link of 1b could be expected despite the anhydrous reaction condition. Trace amount of water remained from the dry up process might contributed to this hydrolysis. It is noteworthy that no byproduction was observed during the synthesis of 2b although it also contain the ester link.

Debenzylation

The present synthetic strategy required deprotection of *O*-benzyl group which is resistant to mild acid treatment following [^{18}F]fluorination. Catalytic hydrogenation is considered to be the first choice for this step. But utilization of hydrogen gas in hot reaction is not convenient. Catalytic transfer hydrogenation (CTH) which utilizes cyclohexenone, formic acid or ammonium formate as hydrogen donor instead of gaseous hydrogen was reported to be fast, convenient and mild (7). We compared the desbenzylation by CTH with the result by the conventional hydrogenolysis.

Results are summarized in Table 1. Unfortunately CTH with ammonium formate was less effective than conventional hydrogenolysis in the every case tested. Deprotection of 3b and 4b were successfully carried out with conventional hydrogenation. Small amount of rearrangement of acyl group from 2-*O*- to 3-*O*- position which reduced yield of 1c was observed. No rearrangement was observed for the deprotection of 2b in spite it contained 1-*O*-alkyl-2-*O*-acyl structure as 1b. The yield of 2c would be improved along with more optimization of the reaction condition..

Conclusion

We reported syntheses of four ether-linked analogs of DAG. Compounds 1c, 3c and 4c were obtained in moderate radiochemical yield. Overall yield of compound 2c was less than 10% due to its low deprotection efficiency, however, this step would be improved. The ether-linked DAGs are considered to be more resistant to *in vivo* hydrolysis. Their incorporation into phosphoinositide turnover as [^{11}C]DAG should be elucidated.

Table 1 Deprotection of Labeled Intermediates

Product	Method	Temperature	Reaction Time	Conversion
1c	Conventional	70°C	30min	41.6%*
		100°C	30min	72.0%*
2c	Conventional	80°C	30min	37.3%
3c	CTH	50°C	30min	0%
		70°C	30min	16.5%
		100°C	30min	35.7%
	Conventional	70°C	30min	37.6%
		100°C	30min	90.4%
4c	CTH	60°C	210min	31.2%
	Conventional	80°C	30min	96.0%

Conventional hydrogenolysis was carried out with H_2 and 10% Pd/C. CTH was with $HCOONH_4$ as hydrogen donor and the same catalysis. Conditions were describe in detail in the text. *Rearrangement was also observed.

Experimental

General

Proton nuclear magnetic resonance (NMR) spectra were recorded on a JNM-EX90A spectrometer (JEOL, Japan) at 89.503633MHz. Chemical shifts (δ) were expressed as ppm referred to internal TMS standard. Mass spectra were measured by gas chromatography - mass spectrometer (QP-5000, Shimadzu, Japan) using its direct injection mode.

Radioactivity was determined with dose calibrator (Atomlab 3000, Biodex Medical Systems, U.S.A.) except for the following cases. Radiochemical reaction was monitored with radio-TLC using Kieselgel 60 plate (Merck, Germany) developed by diethylether:n:hexane (1:1) followed by being cut into pieces and counted with auto-well γ counter (Cobra, Packard Instrument, U.S.A.). Radio HPLC was carried out using RLC-700 radioanalyzer (Aloka, Japan). All reagents were commercially available. Enriched $H_2{}^{18}O$ (95%) was purchased from Enritech (Israel)

1-Bromo-16-(2'-tetrahydropyranyloxy)hexadecane (5)

1,16-Dihydroxyhexadecane (0.982g, 3.8mmol) and p-toluenesufonic acid (57mg, 0.3mmol) were dissolved in THF (20mL). THF (10mL) solution of dihydropyan (0.32g, 3.8mmol) was added drop wise to the above mixture at 0°C. The solution was stirred at room temperature for two days followed by direct purification by silica gel column chromatography. 1-Hydroxy-16-(2'-tetrahydropyranyloxy)-hexadecane (434mg, 33%) was obtained and 57% of the diol was recovered.

Mixture of the alcohol (556mg, 1.62mmol), carbon tetrabromide (806mg, 2.4mmol) and triphenyl phosphine (637mg, 2.4mmol) in benzene (10mL) was stirred at room temperature for a hour. Following evaporation bromide (5, 294mg, 45%) was eluted from silica gel column by mixture of diethyl ether : n-hexane (1:99). ^{1}H-NMR ($CDCl_3$) δ 1.125-1.935 (m, 32H), 3.327-3.787 (m, 8H). 4.563 (br, 1H), MS (m/z) 403, 405 (M^+)

3-Benzyl-1-(16-(2'-tetrahydropyranyloxy)hexadecyl)-rac-glycerol (6)

Compound 6 was prepared from 2,2-dimethyl-1,3-dioxolane-4-methanol by the method of Duclos (6). Compound 5 (1.1eq), potassium t-butoxide (1.1eq) and 0.1eq.of tetrabutylammonium bromide were added to benzene solution of mono benzyl glycerol that was obtained by hydrolysis of 1-benzyl-2,3-isopropylidene-rac-glycerol. The mixture was refluxed for 42 hrs. Silica gel column chromatography of $CHCl_3$ extract gave 6 in 18% overall yield from 2,2-dimethyl-1,3-dioxolane-4-methanol. ^{1}H-NMR ($CDCl_3$) δ 1.017-1.972 (m, 34H), 3.374-3.998 (m, 12H), 4.558 (s, 2H), 7.261-7.396 (m, 5H), MS(m/z) 507 (M^+)

3-Benzyl-1-hexadecyl-rac-glycerol (7)

The same synthetic work-up as for 6 gave title compound in overall yield of 19% from 2,2-dimethyl-1,3-dioxolane-4-methanol. ^{1}H-NMR ($CDCl_3$) δ 0.879-1.616 (m, 31H), 2.463-4.076 (m, 7H), 4.558 (s, 2H), 7.254-7.325 (m, 5H), MS(m/z) 507 (M^+)

3-Benzyl-2-n-capryl-1-(16-(2'-tetrahydropyranyloxy)hexadecyl)-rac-glycerol (8)

A solution of 6 (107mg, 0.212mmol), caprylic anhydride (270mg, 1mmol) and 4-pyrrolidinopyridine (148mg, 1mmol) in $CHCl_3$ was stirred at room temperature for one night. The compound 8 was quantitatively obtained through silica gel column chromatography (5% diethylether in n-hexane). ^{1}H-NMR ($CDCl_3$) δ 0.810-2.410 (m, 47H), 3.357-3.787 (m, 13H), 4.541 (s, 2H), 5.179 (m, 1H), 7.264-7.310 (m, 5H), MS(m/z) 633 (M^+)

3-benzyl-2-n-capryl-1-(16-p-toluenesulfonyloxy)hexadecyl)-rac-glycerol (1a)

cHCl (25μL) was added to ice cooled methanolic solution of 8 (74mg, 0.12mmol). The mixture was stirred for 150 min at room temperature. Water and $CHCl_3$ were added and separated organic layer was dried by Na_2SO_4 and evaporated *in vacuo*. To ice cooled pyridine solution of the residue p-toluenesufonylchloride (32mg, 0.17mmol) was added. This solution was stirred for 150min at 0°C followed by keeping this solution in refrigerator. The mixture was applied to silica gel column chromatography to afford compound 1a (35mg, 42%). ^{1}H-NMR ($CDCl_3$) δ 0.874-1.900 (m, 45H), 2.443 (s, 3H), 3.354-4.089 (m, 9H), 4.550 (s, 2H), 7.256-7.838 (m, 9H), MS(m/z) 516 (M^+ - $OSO_2C_6H_4CH_3$)

3-Benzyl-2-n-ccty1-(16-(2'-tetrahydropyranyloxy)hexadecyl)-rac-glycerol (9)

A benzene solution (10mL) of 6 (60mg, 0.12mmol), octylbromide (77mg, 0.4mmol), tetrabutylammonium bromide (13mg, 0.04mmol) and potassium t-butoxide (30mg, 0.18mmol) was refluxed for 3 days. Silica gel column chromatography (10% diethylether in n-hexane) gave 9 in 72% yield. ^{1}H-NMR ($CDCl_3$) δ 0.810-1.694 (m, 49H), 3.320-3.823 (m, 13H), 4.553 (s, 2H), 7.259-7.318 (m, 5H), MS(m/z) 619 (M^+)

3-Benzyl-2-n-octy1-(16-p-toluenesulfonyloxy)hexadecyl)-rac-glycerol (3a)

The same synthesis work-up as for 1a converted 9 into 3a. Yield was 48%. ^{1}H-NMR ($CDCl_3$) δ 1.208-2.740 (m, 43H), 2.851 (s, 3H), 3.762-4.495 (m, 8H), 4.948 (s, 2H), 5.584 (m, 1H), 7.667-8.246 (m, 9H), MS(m/z) 597 (M^+ - $OCH_2C_6H_5$)

3-Benzyl-2-(8'-bromo-n-capryl)-1-hexadecyl-rac-glycerol(2a)

8-Bromo-n-ocatanoic acid was converted to its anhydride by dicyclohexylcarbodiimide following the method of Duclos (6). About 4mmol of the obtained anhydride was added to a

solution of 7 (379mg, 0.93mmol) and 4-pyrrolidinopyridine (593mg, 4mmol) in $CHCl_3$. This mixture was stirred for 21 hrs at room temperature. Silica gel column chromatography (5% diethylether in n-hexane) gave title compound in 30% yield. ^{1}H-NMR ($CDCl_3$) δ 0.808-2.419 (m, 41H), 3.305-4.192 (m, 10H), 4.538 (s, 2H), 5.179 (m, 1H), 7.256-7.374 (m, 5H), MS(m/z) 504, 506 (M^+-$OCH_2C_6H_5$)

3-Benzyl-2-(8'-bromo-n-octyl)-1-hexadecyl-rac-glycerol (4a)

The same synthesis work-up as for 9 converted 7 into 4a by the use of 1,8-dibromooctane. Yield was 54%. ^{1}H-NMR ($CDCl_3$) δ 0.882-1.586 (m, 43H), 3.318-3.568 (m, 11H), 4.553 (s, 2H), 7.256-7.320 (m, 5H), MS(m/z) 597, 599 (M^+)

General Procedure for Radiofluorinations

Aqueous [^{18}F]fluoride solution was produced by proton irradiation of O-18 water using HM-18 cyclotron (Sumitomo Heavy Industries, Japan). Following collection of fluorine-18 from target water onto AG 1-X8 anion exchanging resin (Bio-Rad Laboratories, U.S.A.) potassium [^{18}F]fluoride was eluted from the resin along with small amount of aqueous potassium carbonate (2.8mg, 0.3mL). APE (Kryptofix 222, Merck, Germany) which improves the reactivity of [^{18}F]fluoride was added to this solution and the mixture was dried at 90°C under stream of nitrogen. After cooling the solution of a precursor (1mg) in acetonitrile (0.5mL) was added to the dried residue. Radiochemical yield was obtained by radio TLC analysis and summarized in Fig. 1. Each reaction mixture was diluted with 5mL of water and the solution was applied to SEPPAK C_{18} (Millipore, U.S.A.). The radioactive intermediate was eluted by methanol following the washing of the column by water.

Procedures for Reductive Debenzylations

CTH and conventional hydrogenation were carried out in pressure resistant test tube (Hyper Glassster THG-A4, Taiatu Glass Kogyou, Japan) connected to 3-way valve and separable connector (Swagelok, U.S.A.). For conventional hydrogenation 10% Pd/C (2.5mg, Nakarai Tesque, Japan) and cHCl (2μL) were added to methanolic solution of ^{18}F labeled intermediates. Air within the test tube was substituted by hydrogen gas using rotary pump for three times. Final pressure was 1.5 atm (gauge). For CTH ammoniumformate (30mg, 0.5mmol) was also added to the tube and the valve was closed. The both test tubes were heated under magnetic stirring. Radiochemical yield was obtained by HPLC analysis and summarized in Table 1. Finepak SIL (4.8 mm I.D. X 250 mm, JASCO, Tokyo, Japan) column was eluted with n-hexane : diethylether : i-propylalcohol (400:8:1.5) at flow rate of 3mL/min.

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