An Improved Procedure for the Detritylation of 1-Alkyl 2-Acyl 3-Trityl-*sn*-Glycerols

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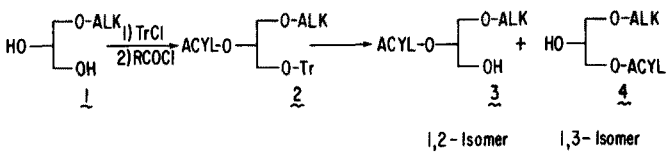
The effects of experimental conditions and acid catalysts on the removal of the trityl group of 1-alkyl-2-acyl-3-trityl-*sn*-glycerols to yield 1-alkyl-2-acyl-*sn*-glycerols have been investigated. Removal of the trityl protecting group was complicated by the concomitant migration of the 2-acyl moiety to yield the 1-alkyl-3-acyl-*sn*-glycerol isomer. The course of detritylation as well as the extent of the 2-acyl to 3-acyl migration under the various conditions used were followed by high performance liquid chromatography (HPLC). Optimum yields of the desired 1-alkyl-2-acyl-*sn*-glycerol (~90%) were obtained with a molar equivalent of boron trifluoride-methanol in methylene chloride at 22 C for five min.

1-Octadecyl-2-acetyl-*sn*-glycerol-3-phosphocholine, commonly referred to as platelet activating factor (PAF), and its homologues have received considerable attention because of their important biological properties (1). For example, ether lipids are known to play important roles in diverse biochemical processes such as platelet aggregation (2), neutrophil activation (3,4), selective tumor cytotoxicity (5) and hypotensive activity (6,7). More recently, neutral ether lipids, 1-alkyl-2-acetyl-*sn*-glycerols, also have been shown to exhibit hypotensive activity (8). In order to study further the biological functions of ether phospholipids several methods for their chemical synthesis have been developed. One common route to their preparation employs a 1-alkyl-*sn*-glycerol as the starting point (9). In this sequence the primary hydroxyl group of the 1-alkyl-*sn*-glycerol *1* (Equation 1) is protected by tritylation, followed by acylation of the 2-position to give the 1-alkyl-2-acyl-3-trityl-*sn*-glycerol *2*. Subsequent removal of the trityl group from the latter then gives as key products 1-alkyl-2-acyl-*sn*-glycerols *3*. The last step of this sequence, the acid-catalyzed detritylation reaction, however, is complicated by the concurrent migration of the 2-acyl moiety of *3* to yield the isomeric 3-acyl derivative *4*. Removal of the trityl protective group from 1,2-diacyl-, 1-alkyl-2-acyl-, and 1,2-dialkyl-3-trityl-*sn*-glycerols has been studied using a number of acid catalysts and procedures such as: (i) hydrogenolysis over palladium (10); (ii) hydrochloric acid in methanol (11); (iii) boric acid in ether (12); (iv) boric acid on silica (13); (v) trifluoracetic anhydride in trifluoracetic acid (14), and (vi) boron trifluoride in methanol (15). Each of these procedures suffers at least one disadvantage, such as low yields for methods (i) and (vi), limited application for method (ii) and time consuming steps for methods (iii)–(v). Another disadvantage of these methods has been the lack of an effective method for the separation of 1,2 and 1,3-isomers which in the past has been accomplished by time consuming column and/or thin layer chromatographic procedures. Accordingly, most of the emphasis in these methods was directed to suppressing the formation of the 1,3-isomers *4*, rather than in obtaining the maximum yield of the required 1,2-isomers *3*.

EQUATION I



ALK = *n*-hexadecyl; R=Acyl=oleoyl; Tr = triphenylmethyl

In the present work, we have reexamined the acid-catalyzed removal of the trityl protecting group from *1* (Equation 1) with the aid of a previously developed high performance liquid chromatographic (HPLC) method (16). This HPLC method allows for both the quantitation and isolation of isomeric 1,2 and 1,3 alkyl acyl glycerols. In this study we have focused our attention on the manipulation of reaction conditions and choice of acid catalyst to optimize the yields of the desired 1-alkyl-2-acyl-*sn*-glycerols *3*.

EXPERIMENTAL PROCEDURES

Materials. The model glyceride used for the detritylation studies was 1-hexadecyl-2-oleoyl-3-trityl-*sn*-glycerol which was synthesized according to literature procedures (17–19). The detritylation catalysts, boron trifluoride etherate, boron trichloride (in methylene chloride) and fluoroboric acid (in diethyl ether) were obtained from Eastman Kodak Co., (Rochester, New York), Aldrich Chem. Co., (Milwaukee, Wisconsin), and Alfa Products (Danvers, Massachusetts), respectively. Isooctane, isopropanol and other solvents used for HPLC were obtained from American Burdick & Jackson (Muskegon, Michigan). Cholesterol (> 99%) standard was obtained from Nu-Check-Prep Inc., (Elysian, Minnesota).

Analytical system. The HPLC system employed for the studies consisted of a Beckman Model 110 A solvent delivery module equipped with a Waters Differential Refractometer, Model R 401, detector (Waters Associates, Inc., Milford, Massachusetts) and an Altex Model 210 loop injector. The HPLC column used was a 4.6 mm i.d. × 25 cm stainless steel column prepacked with 5 μm silica (Zorbax SIL, Dupont Co., Wilmington, Delaware) for analytical HPLC. Semipreparative isolation of reaction products was carried out on a 10 mm i.d. × 25 cm Dynamac Silica Column 8 μm (Rainin Instrument Co., Wilburn, Massachusetts). The

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RI detector output was routed to a Chromatopac Model C-R3A integrator (Shimadzu Co., Columbia, Maryland) to determine retention times and peak areas. The samples were eluted isocratically with isooctane:isopropanol (98:2, v/v) at a flow rate of one ml/min (analytical HPLC) or three ml/min (semi-preparative HPLC). Samples were dissolved in the eluant and 20 μl (analytical) or 100 μl (semi-preparative) were injected via the loop injector.

Preparation of detritylation catalysts. a) BF_3/CH_3OH catalyst: To prepare the BF_3/CH_3OH catalyst accurately, boron trifluoride etherate was used. The etherate was distilled from calcium hydride in an all glass apparatus, b.p. 46 C/10 mm Hg, prior to use. The freshly distilled boron trifluoride etherate (246 μl) was dissolved in anhydrous methanol (1.754 ml) under a stream of nitrogen for a catalyst conc. of one mM/ml. b) BCl_3/CH_3OH catalyst: one ml of boron trichloride in methylene chloride (1 mM/ml) was dissolved in anhydrous methanol under nitrogen to give a catalyst conc. of one mM/1.877 ml. c) HBF_4/CH_3OH catalyst: 149 μl of tetrafluoroboric acid in diethylether (6.734 mM/ml) was dissolved in anhydrous methanol (851 μl) under nitrogen to give a catalyst conc. of one mM/ml.

Detritylation procedure. 1-Hexadecyl-2-oleoyl-3-trityl-*sn*-glycerol (16.4 mg, 20 μmol), BF_3/CH_3OH catalyst (5 to 40 μmol in methanol) and anhydrous methylene chloride (1 ml) were placed into a two-ml glass vial equipped with a magnetic stirring bar. The vial was sealed under nitrogen with a viton seal, and the mixture was stirred for four hr at 22 C. Similar reactions also were performed in the solvents hexane, carbon tetrachloride, benzene, chloroform, 1,2-dichloroethane and diethylether and at various reaction temperatures over the range of 0 to 22 C. All solvents used in the above reactions were dried over 4Å molecular sieves prior to use.

Quantitative analyses. In each detritylation reaction described above 100 μl samples were withdrawn at specified reaction times, between 0 and 240 min, and poured into a mixture of water (1 ml) and methylene chloride (1 ml) in eight-ml vials cooled to 0 C. The organic layer was separated, washed with cold water (4 × 1 ml), dried over anhydrous sodium sulfate and solvent evaporated under a stream of nitrogen. The residue was then dissolved in 300 μl of isooctane:isopropanol (98:2, v/v). To this was added 100 μl of 10% cholesterol in the same solvent, and 20 μl of each of the solutions were analyzed by HPLC.

RESULTS AND DISCUSSION

In a previous report we described an HPLC method that allows for the separation and quantitation of isomeric 1-alkyl-2-acyl and 1-alkyl-3-acyl-*sn*-glycerols (16). In that study a good linear RI detector response was obtained and detector response factors for each glyceride class could be obtained. Using these calculated factors, we have reexamined the acid-catalyzed detritylation of 1-hexadecyl-2-oleoyl-3-trityl-*sn*-glycerol in detail.

The effect of acid catalysts on the course of the detritylation reaction of *2* (Equation 1) was determined in methylene chloride solvent at a reaction temperature of 22 C. The amount of acid catalyst (20 μmol) was held constant for a series of experiments using a catalyst to glyceride molar ratio of 1:1. As shown in Table 1, both BF_3 and HBF_4 in methanol have excellent activity for removal of the trityl protective group of *2* in that for either reagent a > 98% mole conversion of *2* to *3* and *4* was obtained within 15 min reaction time. The other acids listed, namely BCl_3-methanol and HBF_4-ether, reacted more slowly, requiring a one-hr reaction time to attain a > 90% molar conversion. The lower reactivity of the HBF_4-ether compared to HBF_4-methanol is attributed to the absence of methanol because for this reaction triphenylcarbinol was the coproduct of detritylation, whereas for the methanol complexed acids triphenylmethyl methyl ether is the coproduct. More importantly, as shown in Table 1, with the BF_3-methanol reagent the yield of the 1-alkyl-2-acyl-glycerol isomer *3* was > 85 mole %, whereas for the other acid reagents listed the yield of *3* varied from 15 to 50 mole %. With increased reaction time to one hr all of the acids listed were equally effective in removal of the trityl group of *2*. However, under this condition the major product was the thermodynamically more stable 1-alkyl-3-acyl glycerol isomer *4*. From these results it was concluded that BF_3-methanol is the pre-

TABLE 1

Effect of Acid on Detritylation of 1-Hexadecyl-2-oleoyl-3-trityl-*sn*-glycerol[a]

Catalyst	15 min reaction[b] mole fraction			60 min reaction[b] mole fraction		
	2[c]	*4*[d]	*3*[e]	*2*[c]	*4*[d]	*3*[e]
BF_3/CH_3OH	0.020	0.086	0.894	0.004	0.443	0.553
BCl_3/CH_3OH	0.115	0.729	0.156	0.102	0.784	0.115
HBF_4/CH_3OH	0.009	0.430	0.501	0.000	0.866	0.134
HBF_4/Et_2O	0.140	0.443	0.417	0.076	0.796	0.127

[a]Reaction temperature 22 C, CH_2Cl_2 solvent, molar ratio of acid to glyceride 1:1.

[b]Average of 2 osbservations.

[c]*2*, 1-hexadecyl-2-oleoyl-3-trityl-*sn*-glycerol.

[d]*4*, 1-hexadecyl-3-oleoyl-*sn*-glycerol.

[e]*3*, 1-hexadecyl-2-oleoyl-*sn*-glycerol.

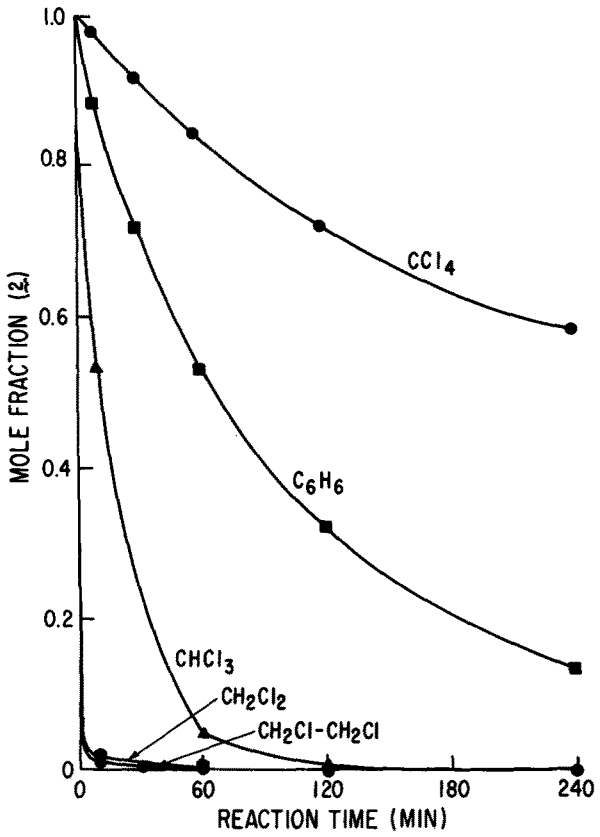

FIG. 1. Effect of solvent on the detritylation of 1-hexadecyl-2-oleoyl-3-trityl-*sn*-glycerol (*2*). Reaction temperature 22 C, BF_3-CH_3OH to glyceride rate 1:1.

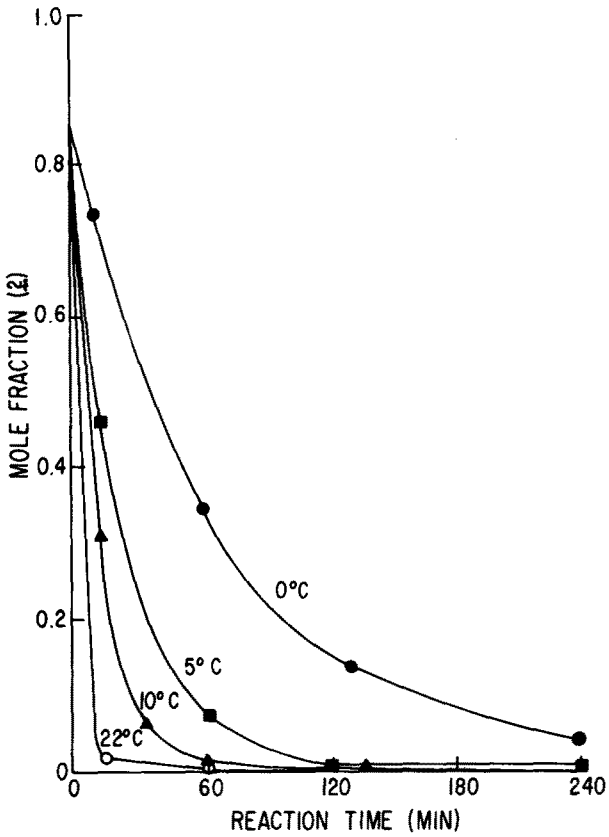

FIG. 2. Effect of temperature on the detritylation of 1-hexadecyl-2-oleoyl-3-trityl-*sn*-glycerol (*2*). Reactions carried out in CH_2Cl_2, BF_3-CH_3OH to glyceride ratio 1:1.

ferred reagent for the acid catalyzed removal of the trityl protecting group from glycerides such as *2*.

To assess the effect of solvent on the detritylation reaction the following solvents were used: methylene chloride, 1,2-dichloroethane, chloroform, carbon tetrachloride, benzene, hexane and diethyl ether. The results obtained for the detritylation of 1-hexadecyl-2-oleoyl-3-trityl-*sn*-glycerol (*2*) in these solvents are shown in Figure 1. Methylene chloride and 1,2-dichloroethane gave comparable results in that > 95% of *2* was detritylated within 30 min reaction time. With chloroform as solvent a slight decrease in the rate of detritylation of *2* was noted while a marked decrease in rate of reaction was observed with carbon tetrachloride and benzene as solvents. For the solvents hexane and ether no cleavage of the trityl group of *2* was observed even after four hr reaction time. These results with the latter solvents probably occurred because BF_3-methanol reagent has limited solubility in hexane and BF_3 forms a strong Lewis acid-base complex with oxygen-containing compounds such as diethyl ether. Accordingly in both instances the BF_3-methanol reagent is not available for reaction with *2*.

The effect of temperature on the course of the detritylation of *2* was studied over the range of 0 to 22 C. Temperature was an important consideration because this variable would affect not only the extent of reaction but also the rate of isomerization of the initally formed 1,2-isomer *3* to the 1,3-isomer *4*. As expected, the rate of detritylation of *2* decreased with a decrease in reaction temperature (Fig. 2). On the other hand, isomerization of the 1,2-isomer *3* to the 1,3-isomer *4* increased with an increase in temperature (Fig. 3). For example, at 0 C the detritylation of *2* required a reaction time of four hr to reach > 90% mole conversion (Fig. 2), to give an 80% mole yield of the 1,2-isomer *3* (Fig. 3). This result is to be contrasted with a previous report by Hermetter et al. (15) in which a 76% yield of 1,2-diacylglycerol, reportedly free of any 1,3-isomer, was obtained under similar conditions at 0 C for 30 min reaction. In this sutdy only a 50% yield of the 1,2-isomer *3* was realized under the same conditions (Fig. 3). From the data given in Figures 2 and 3, it can be seen that the best conversions of *2* and highest yields of the 1-alkyl-2-acyl-glycerol *3* were obtained at a reaction temperature of 22 C, provided that the reaction is carried out for short time periods, < 20 min.

Figure 4 shows the effect of the amount of BF_3 methanol reagent used in the detritylation reaction. In this part of the study the mole ratio of BF_3-methanol reagent to glyceride *2* was varied from 0.25 to 2.00. In each example the molar conversion of *2* to the glycerol derivatives *3* and *4* exceeded 98 mole%. However, the data shows there is a reduction in yield of the 1,2-isomer *3* with time with a corresponding increase of the 1,3-isomer *4* at the higher acid concentrations. From these results it is better to select a

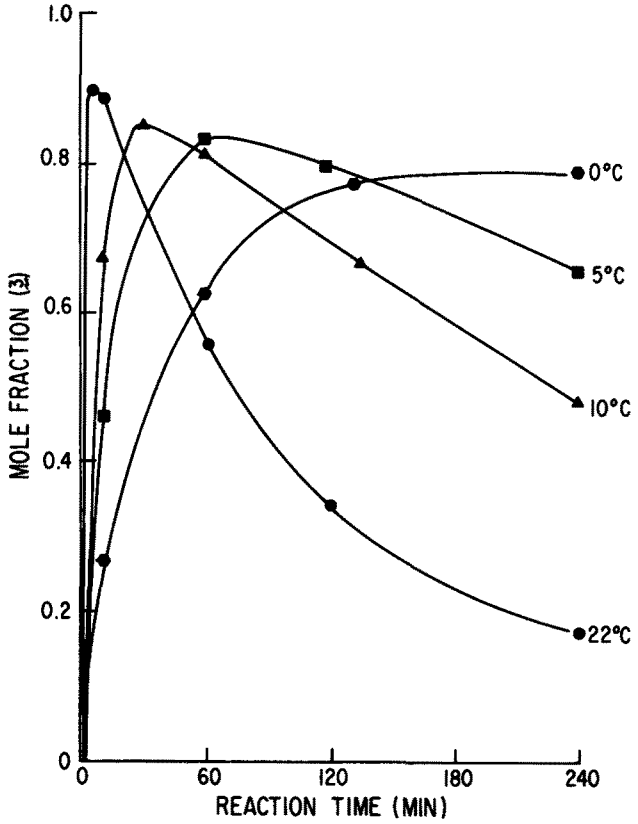

FIG. 3. Effect of temperature on yield of 1-hexadecyl-2-oleoyl-*sn*-glycerol (*3*). The reactions carried out in CH_2Cl_2, at a BF_3-CH_3OH to glyceride ratio of 1:1.

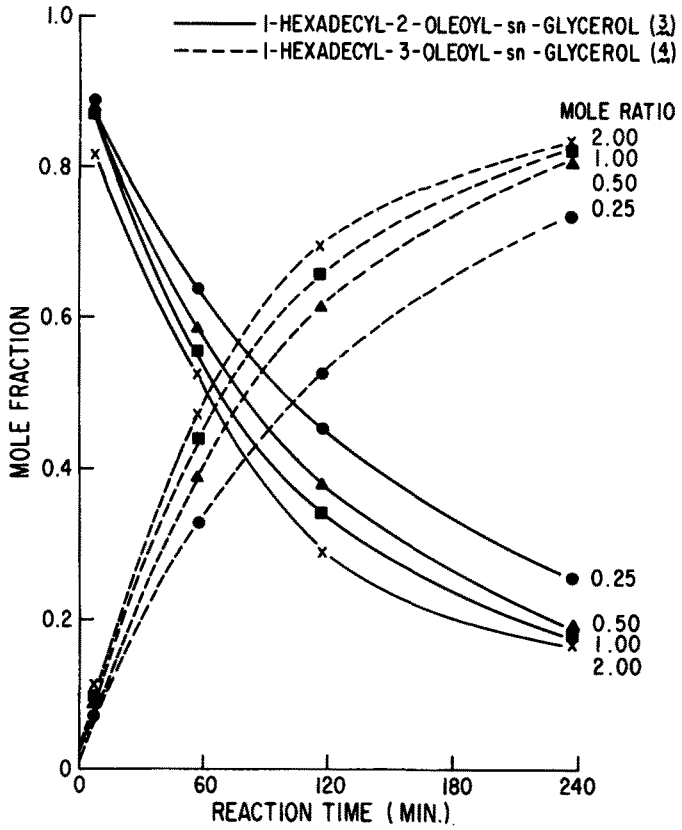

FIG. 4. Effect of BF_3-CH_3OH reagent on the isomerization of 1-hexadecyl-2-oleoyl-*sn*-glycerol (*3*) to 1-hexadecyl-3-oleoyl-*sn*-glycerol (*4*). Reaction temperature 22 C, CH_2Cl_2 solvent.

lower molar ratio of BF_3-methanol so as to decrease the extent of acyl migration even though the conversion rate is somewhat slower. A good compromise between the two effects would be a molar ratio of 1:1.

Up to this point our results indicated that detritylation of *2* proceeds very rapidly with the best yields of 1-alkyl-2-acyl glycerols obtained within 15 min reaction time. Figure 5 examines this reaction in greater detail over this time course. In Figure 5, sampling and analyses were done over the range of 0 to 8 min at one-min intervals. The experimental data show that the maximum yield of 1-alkyl-2-acyl glycerol *3* was 89.5%, with 8.5% of the 1-alkyl-3-acyl isomer *4* and 2% of starting glyceride *2* remaining at five min reaction time (Fig. 5). Accordingly, we can establish this time, five min, as optimum because longer times cause a loss of 1,2-isomer *3* due to isomerization to the 1,3-isomer *4*.

From all of the preceding we have established that the optimal conditions for removal of the trityl protecting group from glycerides are as follows: catalyst BF_3-methanol at a molar ratio of 1:1 to glyceride, methylene chloride as solvent, reaction temperature of 22 C and reaction time of five min. Under these conditions, yields of the desired 1-alkyl-2-acyl-*sn*-glycerols are > 90% compared to previously reported yield of ~75% (15). Moreover, preparative HPLC isolation of larger scale reactions under similar conditions gave comparable isolated yields of *3*, suggesting the general applica-

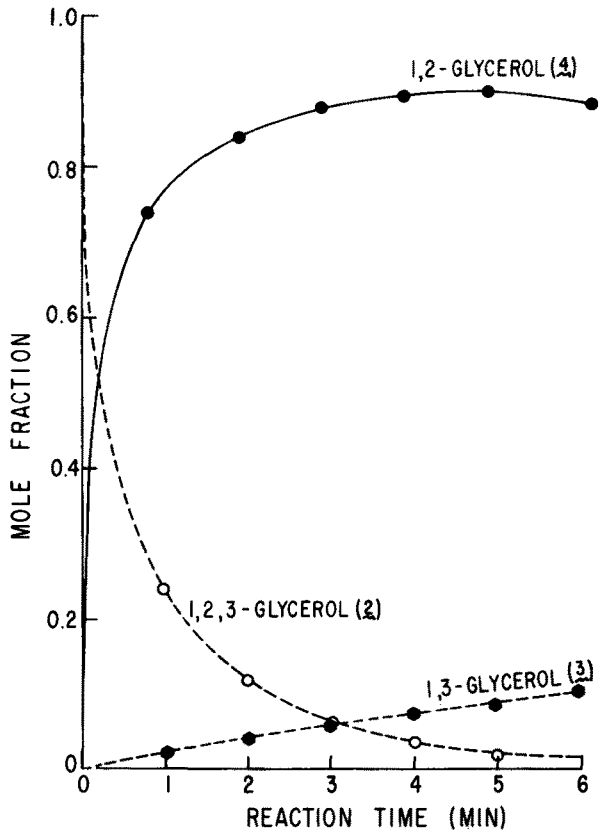

FIG. 5. Detritylation of 1-hexadecyl-2-oleoyl-3-trityl-*sn*-glycerol *2*) with BF_3-CH_3OH reagent, molar ratio 1:1, CH_2Cl_2 solvent at 22 C. 1,2 glycerol is 1-hexadecyl-2-oleoyl-*sn*-glycerol (*3*); 1,3 glycerol is 1-hexadecyl-3-oleoyl-*sn*-glycerol (*4*).

bility and utility of this procedure for the preparation of these important glyceride intermediates (16).

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