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# Synthesis of 1,2-Di-*O*-alkyl-*sn*-glycero-3-phosphatidylcholine Using 2-Methoxyethoxymethyl and 2-(Trimethylsilyl)ethoxymethyl Protective Groups[1)]

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2-Methoxyethoxymethyl (MEM) and 2-(trimethylsilyl)ethoxymethyl (SEM) groups were used to protect the *sn*-3-OH of optically active glycerols in the synthesis of 1,2-di-*O*-octadecyl-*sn*-glycero-3-phosphatidylcholine. Both MEM and SEM protective groups had advantages of a classical benzyl group by virtue of (i) the facile preparation of 1,2-*O*-isopropylidene-3-*O*-(2-methoxyethoxymethyl)-*sn*-glycerol and its *sn*-3-*O*-SEM analog as starting materials and (ii) the rapid demasking of the MEM and SEM moieties in lipid precursors, especially by means of titanium tetrachloride.

With increasing physicochemical and biological interests in biomembranes, various glycerophospholipids are in great demand now.[2,3)] Of representative glycerophospholipids, 1,2-di-*O*-acyl-*sn*-glycero-3-phosphatidylcholine is prepared conveniently by direct acylation of *sn*-glycero-3-phosphatidylcholine (GPC)[4)] which is derived from egg lecithin.[5)] Another representative glycerophospholipid, 1,2-di-*O*-alkyl-*sn*-glycero-3-phosphatidylcholine, can not be produced from GPC but has been obtained via the multistep synthesis which begins generally with long-chain alkylation of the *sn*-1- and *sn*-2-OH groups of 3-*O*-benzyl-*sn*-glycerol (**1**) followed by demasking of the benzyl group of the resulting 1,2-di-*O*-alkyl-3-*O*-benzyl-*sn*-glycerol by means of catalytic hydrogenolysis and the subsequent attachment of phosphorylcholine unit or the analog to the naked *sn*-3-OH group.[6-8)]

However, one would often encounter difficulty in the hydrogenolysis because of extreme sensitivity of palladium catalyst to poisoning by a trace of contaminants in the substrate or on the appratus used; thus, a large scale preparation being often rendered unpractical. Furthermore, to our experience, the hydrogenolysis tends to become inefficient with increasing alkyl-chain length in the lipid precursors.

In view of these inconveniences of the benzyl group in the glycerolipid synthesis, we attempted to protect the *sn*-3-OH groups of optically active glycerols with 2-methoxyethoxymethyl (MEM) and 2-(trimethylsilyl)-ethoxymethyl (SEM) groups, choosing natural 1,2-di-*O*-octadecyl-*sn*-glycero-3-phosphatidylcholine (**10**) as a synthetic target. The MEM and SEM groups are acid-labile protective groups and have been utilized in the preparation of carbohydrates and nucleic acid-related compounds.[9)]

## Results and Discussion

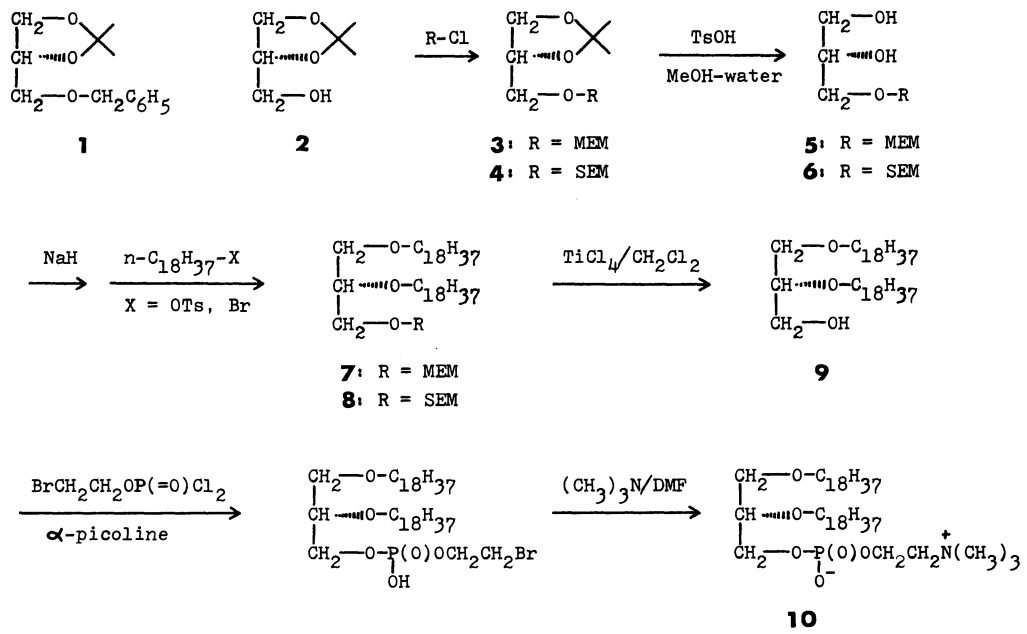
D-Mannitol was transformed into 1,2-*O*-isopropylidene-*sn*-glycerol (**2**) in a routine yield of 65—82% upon modifing a literature procedure.[10,11)] The compound **2** in THF was treated with sodium hydride, and the resulting sodium alkoxide was allowed to react with 2-methoxyethoxymethyl chloride or 2-(trimethylsilyl)ethoxymethyl chloride at ca. 0 °C for 2h. Distillation of the reaction mixtures furnished the corresponding 3-*O*-MEM and 3-*O*-SEM glycerols (**3** and **4**, respectively) in the yields of >80%.

Now, although the *O*-isopropylidene moiety and the *O*-MEM or *O*-SEM moiety of **3** and **4** are acid-labile, a treatment with 0.4 equiv of *p*-toluenesulfonic acid in 90% aqueous methanol at 25 °C for 2—3h cleaved the acetal moiety only to give 3-*O*-[(2-methoxyethoxymethyl)]- and 3-*O*-[2-(trimethylsilyl)-ethoxymethyl]-*sn*-glycerols (**4** and **5**, respectively) in the yields of 90—95% (NMR). Thus, in comparison with an analogous preparation of 3-*O*-benzyl-*sn*-glycerol (**1**) from **2**,[6)] **5**, and **6** were synthesized much readily with the yields comparable with or better than that of **1**.

Next, the sodium salts of **5** and **6** were allowed to react with octadecyl *p*-toluenesulfonate at ca. 190 °C for 0.5 h in the absence of any solvent. The modified procedure for the Williamson ether synthesis took place rapidly to give the corresponding 1,2-di-*O*-octadecylglycerols (**7** and **8**) in the yields of ca. 60%, which were higher than the values (45—47%) obtained in an ordinary procedure-reactions of the sodium alkoxide with octadecyl bromide with reflux in THF for 30h. No cleavage of the MEM and SEM protective groups was observed during the reactions.

Deprotection of the MEM and SEM-ether linkages could be performed by means of diphosphorus tetraiodide, triphenylmethyl tetrafluoroborate and zinc bromide.[9)] But, titanium tetrachloride ($TiCl_4$) was the most effective reagent of converting **7** and **8** into 1,2-di-*O*-octadecyl-*sn*-glycerol (**9**); i.e., an action of 0.6—1 equiv of $TiCl_4$ to a dichloromethane solution of **7** or **8** at ca. 0 °C for 0.5—1 h resulted in a quantitative demasking of the *sn*-3-*O*-ether moiety. No racemization occurred during the demasking reaction as judged from an optical activity of the resulting **9**.

Transformation of **9** into the phospholipid **10** was achieved as usual;[7,12)] i.e., a reaction of **9** with 2-bromoethyl phosphorodichloridate and the subsequent treatment of the reaction mixture with trimethy-




1 2 3: R = MEM 4: R = SEM 5: R = MEM 6: R = SEM 7: R = MEM 8: R = SEM 9 10

lamine afforded **10** in the yield of 35—51%.

In conclusion, the MEM and SEM groups were very useful to protect the *sn*-3-OH groups of the optically active glycerols (**2**—**8**). The protective groups were superior to the classical benzyl group with regard to (i) the facile preparation of **3** and **4** and (ii) the rapid demasking of the *sn*-3-*O*-MEM and -SEM moieties from **7** and **8**, especially by means of $TiCl_4$. Both MEM and SEM groups were similar in reactivity in the masking and demasking reactions.

## Experimental

1,2-*O*-Isopropylidene-*sn*-glycerol (**2**) was derived from D-mannitol as described previously.[11] 2-Methoxyethoxymethyl chloride, 2-(trimethylsilyl)ethoxymethyl chloride, and deprotecting reagents including $TiCl_4$ were used without further purification. Column chromatography was carried out using silica gel (Merck 7734, 70—230 mesh). Mobility ($R_f$) in thin-layer chromatography was measured on precoated silica-gel sheet (Merck 17345, $60F_{254}$) using the following solvent systems: A, hexane-ethyl acetate (10 : 1 v/v); B, hexane-ethanol (7 : 1 v/v); C, chloroform-methanol-concentrated ammonia (65 : 35 : 5 v/v/v). Compound spots in TLC were visualized under ultraviolet light spraying with 0.0012% aqueous Rhodamine 6G (for compounds having long-chain alkyl groups) and the Dittmer-Lester reagent[13] (for phosphoric acid esters). Mp's were measured using a Yanagimoto hot stage appratus and uncorrected. Optical rotations were recorded by means of a JASCO DIY-360 polarimeter. $^1$H NMR spectra were measured on a JEOL PS-100 using dilute solution in $CDCl_3$ or $CDCl_3$-methanol-$d_4$ (2 : 1 v/v) with $SiMe_4$ as an internal standard.

**1,2-*O*-Isopropylidene-3-*O*-(2-methoxyethoxymethyl)-*sn*-glycerol (3).** Sodium hydride (60% in oil, 1.4 g, 35 mmol) was added to a stirred THF solution (200 ml) of **2** (3.5 g, 26.5 mmol) at room temperature. After 30 min, (2-methoxyethoxy)-methyl chloride (3.6 ml, 31.5 mmol) was added dropwise to the sodium alkoxide solution cooled in an ice-water bath, and the mixture was swirled at ca. 0 °C for 2 h. The solution was then concentrated and the residue was extracted with chloroform (300 ml). The organic layer was filtered and concentrated to afford the liquid, which was distilled under reduced pressure to give **3** as a colorless substance, 4.8 g (82%); bp 92—94 °C/2 mmHg (1 mmHg=133.322 Pa); $[\alpha]_D^{25}$ +11.3° (neat); $^1$H NMR ($CDCl_3$) $\delta$=1.34 and 1.40 (3H, s, $CH_3$), 3.38 (3H, s, $OCH_3$), 3.47—4.16 (8H, complex m, $CH_2CHCH_2$ and $OCH_2CH_2O$), 4.28 (1H, m, CH), and 4.76 (2H, s, $OCH_2O$). Anal. ($C_{10}H_{20}O_5$) C, H.

**1,2-*O*-Isopropylidene-3-*O*-[2-(trimethylsilyl)ethoxymethyl]-*sn*-glycerol (4).** Using sodium hydride (60% in oil, 1.5 g, 37.5 mmol), **2** (4.1 g, 31 mmol) was converted into the sodium alkoxide, which was then allowed to react with 2-(trimethylsilyl)ethoxymethyl chloride (5.6 ml, 32 mmol) in a manner similar to that mentioned in the preparation of **3**, 6.5 g (80%); bp 118—120 °C/8 mmHg; $[\alpha]_D^{18}$ +10.0° (neat); $^1$H NMR ($CDCl_3$) $\delta$=0.0 [9H, s, $Si(CH_3)_3$], 0.93 (2H, t, $J$=16.4 Hz, $CH_2Si$), 1.30 and 1.37 (3H, s, $C(CH_3)_2$), 3.3—4.3 (7H, complex m, $CH_2CHCH_2$ and $OCH_2CH_2Si$), and 4.56 (2H, s, $OCH_2O$). Anal. ($C_{12}H_{26}O_4Si$) C, H.

**3-*O*-(2-Methoxyethoxymethyl)-*sn*-glycerol (5).** *p*-Toluenesulfonic acid monohydrate (1.5 g, 7.9 mmol) was added to a methanol-water (10 : 1 v/v) solution (55 ml) of **3** (4.7 g, 21 mmol) at 25 °C. After stirring the mixture at the same temperature for 2 h, the solution was neutralized with powdery sodium hydrogencarbonate. The supernatant was concentrated to give the residue which was extracted with chloroform. Evaporation of the solvent from a dried organic extract on anhydrous sodium sulfate furnished **5** as a colorless liquid, 3.4 g (89%); the $^1$H NMR and IR spectra were almost identical with those of an authentic sample obtained by distillation, 1.2 g (31%); bp 120—125 °C/1 mmHg; $[\alpha]_D^{26}$ −1.58° (*c* 9.2, methanol); $^1$H NMR ($CDCl_3$) $\delta$=3.33 (3H, s, $OCH_3$), 3.1—4.5 (9H, complex m, $CH_2CHCH_2$ and $OCH_2CH_2O$), and 4.68 (2H, s, $OCH_2O$). Anal. ($C_7H_{16}O_5$) C, H.

**3-*O*-[2-(Trimethylsilyl)ethoxymethyl]-*sn*-glycerol (6).** Compound (**4**, 5.1 g, 19.4 mmol) was processed with *p*-toluenesulfonic acid monohydrate (1.5 g, 7.9 mmol) in 90% aqueous methanol (55 ml) in a manner similar to that men-

tioned in the preparation of **5**. The reaction mixture was neutralized with sodium hydrogencarbonate, concentrated and extracted with chloroform. Evaporation of the solvent from the dried organic extract on anhydrous sodium sulfate gave crude **6** (3.9 g, 91%), which that the IR and $^1$H NMR spectra almost identical with those of the distilled material, 1.9 g (43%); bp 129—131 °C/3 mmHg; $[\alpha]_D^{24}$ +4.1° (neat); $^1$H NMR ($CDCl_3$) $\delta$=0.0 [9H, s, $Si(CH_3)_3$], 0.93 (2H, t, $J$=16.4 Hz, $CH_2Si$), 3.46—4.21 (7H, complex m, $CH_2CHCH_2$ and $OCH_2CH_2Si$), and 4.54 (2H, s, $OCH_2O$). Anal. ($C_9H_{22}O_4Si$) C, H.

**1,2-Di-*O*-octadecyl-3-*O*-(2-methoxyethoxymethyl)-*sn*-glycerol (7).** Compound **5**, (1.5 g, 8.3 mmol) in THF (100 ml) was stirred with sodium hydride (60% in oil, 0.7 g, 18 mmol) at room temperature for 30 min. Octadecyl *p*-toluenesulfonate (8.5 g, 20.0 mmol) was added to the alkoxide solution, and the mixture was heated to remove the solvent. The resulting semisolid residue was further heated in an oil bath at 190—200 °C for 30 min. A chloroform solution of the cooled reaction mixture was washed with water and concentrated to provide a residue which was dissolved in ethanol (150 ml) and allowed to stand in a refrigerator of ca. 10 °C overnight. The resulting solid was collected by suction-filtration, then applied to a silica-gel column. Elution with hexane-ethyl acetate (10 : 1 v/v) gave **7**, which was purified by recrystallization from acetone, 3.1 g, (54%). The mother liquior provided another crop of **7** through a similar purification process, 0.8 g (14%); mp 32—33 °C; $[\alpha]_D^{22}$ −2.2° (*c* 2.68, chloroform); $R_f$=0.54 (solvent B); $^1$H NMR ($CDCl_3$) $\delta$=0.89 (6H, two t separated by ca. $\delta$ 0.002, $2CH_3$), 1.25 [64H, coherent peak, $2(CH_2)_{16}$], ca. 1.53 (4H, m, $2OCH_2$), 3.38 (3H, s, $OCH_3$), 3.38—3.77 (9H, complex m, $CH_2CHCH_2$ and $OCH_2CH_2O$), and 4.73 (2H, s, $OCH_2O$). Anal. ($C_{43}H_{88}O_5$) C, H.

**1,2-Di-*O*-octadecyl-3-*O*-[2-(trimethylsilyl)ethoxymethyl]-*sn*-glycerol (8).** In a manner similar to that mentioned above, **8** was prepared from **6** in a yield of 65%. It was also obtained by the next Williamson synthesis. Compound (**6**, 2.4 g, 10.8 mmol) in THF (100 ml) was stirred with sodium hydride (60% in oil, 1.0 g, 25 mmol) at room temperature for 30 min. Octadecyl bromide (9.0 ml, 26.4 mmol) was added to the alkoxide solution, and the mixture was heated with reflux for 30 h. The cooled reaction mixture was concentrated to afford the residue which was then extracted with chloroform. The organic solution was washed with water, dried over anhydrous sodium sulfate, concentrated and applied to a silica-gel column. Elution with hexane-ethyl acetate (10 : 1 v/v) gave crude **8**, which was purified by recrystallization from acetone, 3.6 g (46%); mp 29—30 °C; $[\alpha]_D^{18}$ −1.5° (*c* 5.6, chloroform); $R_f$=0.54 (solvent A); $^1$H NMR ($CDCl_3$) $\delta$=0.0 [9H, s, $Si(CH_3)_3$], 0.80—1.07 (8H, complex m, $2CH_3$ and $CH_2Si$), 3.25—3.79 (9H, complex m, $CH_2CHCH_2$ and two $OCH_2C_{17}H_{35}$), and 3.64 (2H, t, $J$=16.4 Hz, $CH_2CH_2Si$); $m/z$ (rel intensity, %) (a JEOL JMS-06 spectrometer at 75 eV at 70 °C ion source temperature) 727 ($M^+$, 18), and 578 ($M^+$ −HO-SEM, 7). Found: C, 73.92; H, 13.50%. Calcd for $C_{45}H_{94}O_4Si$: C, 74.31; H, 13.03%.

**1,2-Di-*O*-octadecyl-*sn*-glycerol (9).** Titanium tetrachloride (0.4 ml, 3.64 mmol) was added to a cooled dichloromethane solution of **7** (0.74 g, 1.1 mmol) in an ice-water bath. After swirling 1 h, the reaction mixture was stirred vigorously with 1 M (1 M=1 mol $dm^{-3}$) sodium hydrogencarbonate, then extracted with chloroform. The organic solution was washed with water, dried over anhydrous sodium sulfate and concentrated to give **9**, 0.64 g (≈100%) which was purified by a silica-gel column chromatography using a mixture of hexane and ethyl acetate (8 : 1 v/v), 0.39 g (61%); mp 57—58 °C (lit,[6] 53.5—54.5 °C); $[\alpha]_D^{25}$ −7.47° (*c* 1.6, chloroform) (lit,[6] −6.85°); $R_f$=0.43 (solvent B); the IR and $^1$H NMR spectra as well as the $R_f$ of the product were identical with those of an authentic sample, which was prepared by hydrogenolysis of 1,2-di-*O*-octadecyl-3-*O*-benzyl-*sn*-glycerol.[11]

Similarly, **8** was demasked into **9** in a yield of 72% after column chromatographic purification. The mp and the $^1$H NMR spectrum were identical with those of **9** obtained from **7**.

**1,2-Di-*O*-octadecyl-*sn*-glycero-3-phosphatidylcholine (10).** The literature procedure[6] was slightly modified. A mixture of **9** (0.5 g, 0.83 mmol), 2-bromoethyl phosphorodichloridate (0.31 g, 1.3 mmol) and α-picoline (2 ml) in chloroform (30 ml) was stirred at ca. 40 °C overnight. The reaction mixture was then processed in a manner similar to that reported to give rise crude 2-bromoethyl 1,2-di-*O*-octadecyl-*sn*-glyceryl hydrogenphosphate, which was subsequently heated with 6.25 M DMF solution of trimethylamine (25 ml) at 55 °C overnight. The resulting solution was concentrated to give the residue which in 90% aqueous methanol (30 ml) was stirred vigorously with silver acetate (1.1 g, 6.6 mmol) for 1 h. The resulting precipitate was removed by filtration and the filtrate was concentrated and applied to a silica-gel column. Elution with a mixture of chloroform, methanol and water (65 : 35 : 5 v/v/v) furnished **10** as amorphous solid which was recrystallized from ethyl acetate, 0.26 g (42%); mp 205 (softening)—210 °C (melting) (lit,[8] 201—202 °C); $[\alpha]_D^{25}$ +2.1° [*c* 0.5, chloroform-methanol (2 : 1 v/v)] (lit,[8] +1.3°); $R_f$=0.30 (solvent C). The IR and $^1$H NMR spectra as well as the $R_f$ were identical with those of an authentic sample.

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