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Preparation of 2-*O*-Arachidonoyl-1-*O*-stearoyl-*sn*-glycerol and Other Di-*O*-Acyl Glycerol Derivatives

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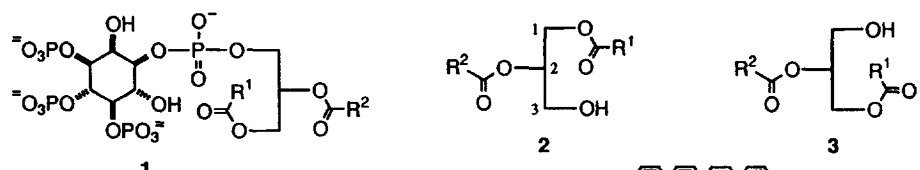
Abstract: *R*(-)-2,3- and *S*(+)-1,2-*O*-Isopropylidene-*sn*-glycerols (**4** and **9**) are converted into 2-*O*-arachidonoyl-1-*O*-stearoyl and 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerols (**2** and **3**, respectively); glycerol is also converted into its racemic 1,2- and its symmetrical 1,3-di-*O*-linoleoyl derivatives (**14** and **17**, respectively). © 1997 Elsevier Science Ltd.

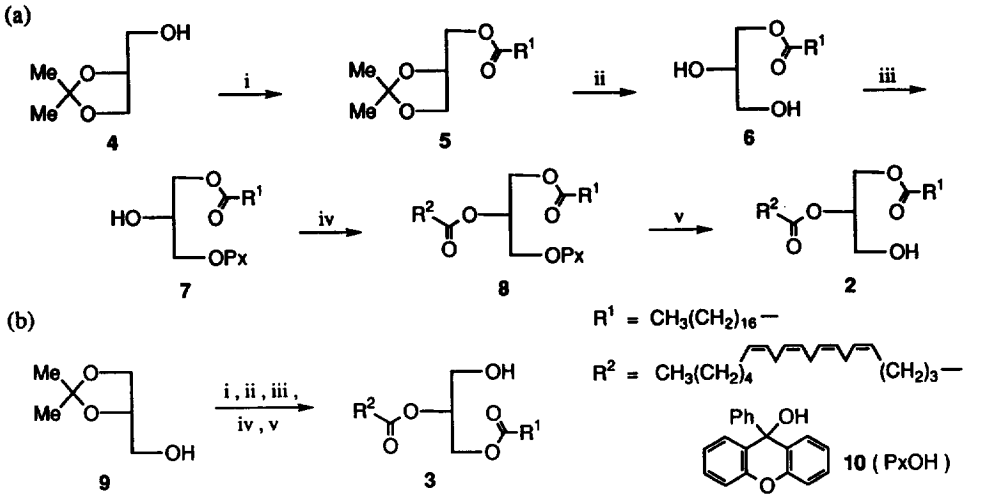
In connection with our studies on the synthesis of phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate (PIP_3)[1] **1**, we required a synthetic source of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol[2] **2**, and of the enantiomeric 3-*O*-stearoyl derivative **3**. We now report the synthesis of both of these enantiomers.



1 **2** **3**

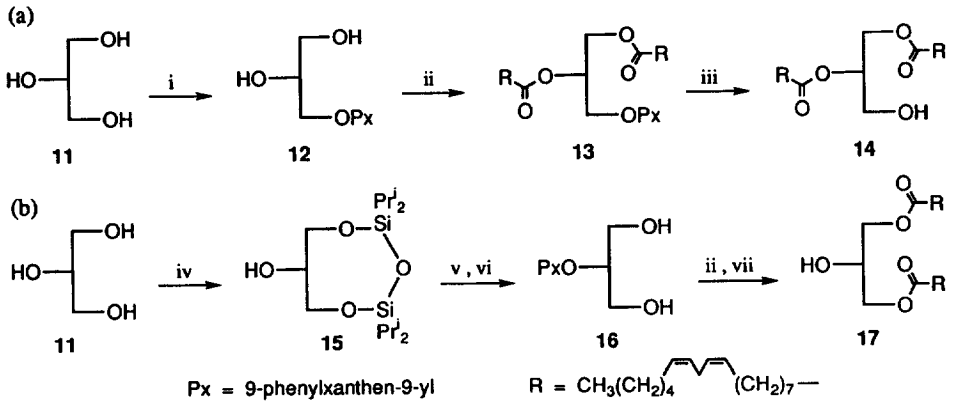
$R^1 = CH_3(CH_2)_{16}$— $R^2 = CH_3(CH_2)_4$...$(CH_2)_3$—

R(-)-2,3-*O*-Isopropylidene-*sn*-glycerol[3] **4** was allowed to react (Scheme 1a) with a slight excess of stearoyl chloride in the presence of a twofold excess of triethylamine and a catalytic quantity of 4-dimethylaminopyridine (DMAP) in dichloromethane solution to give its 1-*O*-stearoyl derivative **5** in virtually quantitative yield. A solution of the product **5** was treated with trifluoracetic acid in triethyl borate - 2,2,2-trifluoroethanol (8:1 v/v) at room temperature to give 1-*O*-stearoyl-*sn*-glycerol **6** as waxy needles[4] in 74% isolated yield. Despite the relatively drastic acidic conditions required to remove the isopropylidene protecting group, acyl migration, leading to 2-*O*-stearoylglycerol, occurred only to a minor extent. A solution of compound **6** and a slight excess (*ca.* 1.1 mol equiv.) of 9-phenylxanthen-9-ol[6] (PxOH) **10** in glacial acetic acid was evaporated under reduced pressure (bath temp. < 35°C) to give 3-*O*-(9-phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol **7** as an oil in 72% isolated yield[7]. The 2-*O*-arachidonoyl group was introduced in a very economical and effective way by allowing compound **7** to react with *ca.* 1.25 mol equiv. of arachidonic acid and *ca.* 2.5 mol equiv. of 2,6-dichlorobenzoyl chloride[8] in the presence of 1-methylimidazole in dichloromethane solution at room temperature[9]. The product **8**, which was obtained in nearly quantitative



Scheme 1 *Reagents and conditions* : i , $CH_3(CH_2)_{16}$-COCl , Et_3N , DMAP , CH_2Cl_2 , 0 °C to room temp., 50 min ; ii , CF_3CO_2H , CF_3CH_2OH , $B(OEt)_3$, room temp. , 5.5 h ; iii , PxOH **10** , AcOH , room temp. to < 35 °C , *ca.* 15 mmHg ; iv , arachidonic acid , 2,6-$Cl_2C_6H_3$-COCl , 1-methylimidazole , CH_2Cl_2 , room temp. ; v , Cl_2CHCO_2H , pyrrole , CH_2Cl_2 , room temp. , 5 min .

yield[10], was treated with dichloroacetic acid and pyrrole[11] in dichloromethane solution at room temperature for 5 min. The required 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **2** was thereby obtained under these very mild reaction conditions as a colourless oil, in high enantiomeric excess (96-97%) and in high yield[12]. *S*(+)-1,2-*O*-Isopropylidene-*sn*-glycerol[14] **9** was converted into 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **3** by the same five step procedure (Scheme 1b). Compound **3** was isolated in relatively high (*ca.* 90%) enantiomeric excess[12], and its overall yield was closely similar to that of its enantiomer **2** (Scheme 1a).



Scheme 2 *Reagents and conditions* : i , PxOH **10** , TsOH , DMF , room temp. ; ii , linoleic acid , 2,6-$Cl_2C_6H_3$-COCl , 1-methylimidazole , CH_2Cl_2 , room temp. ; iii , acetic acid , pyrrole , room temp. , 1 - 2 h ; iv , $Pr^i_2Si(Cl)OSi(Cl)Pr^i_2$, C_5H_5N , room temp. , 4 h ; v , 9-chloro-9-phenylxanthene (PxCl) , C_5H_5N , room temp. , 1 h ; vi , Et_4NF , MeCN , 60 °C , 5 min ; vii , Cl_2CHCO_2H , pyrrole , CH_2Cl_2 , room temp. , 5 min.

Glycerol can very easily be converted (Scheme 2a) into its crystalline racemic 1-*O*-(9-phenylxanthen-9-yl) derivative **12**[15] and, *via* its 1,3-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl) derivative **15**[16] (Scheme 2b), into its crystalline 2-*O*-(9-phenylxanthen-9-yl) derivative **16**[17]. These two compounds **12** and **16** are valuable intermediates in the preparation of racemic 1,2-di-*O*-acyl- and symmetrical 1,3-di-*O*-acyl-glycerol derivatives **14** and **17**, respectively; their two step conversions into racemic 1,2-di-*O*-linoleoylglycerol **14**[18] and symmetrical 1,3-di-*O*-linoleoylglycerol **17**[19] in 87 and 63% overall yields, respectively, is indicated in outline in Scheme 2. The conditions required[11] for the removal of the 9-phenylxanthen-9-yl protecting group in the presence of pyrrole are very mild indeed.

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4. 1-*O*-Stearoyl-*sn*-glycerol **6** (Found: C, 69.70; H, 11.97. Calc. for $C_{21}H_{42}O_4 \cdot 0.2\ H_2O$: C, 69.65; H, 11.8%) has m.p. 67.5 - 68.5°C and $[\alpha]_D^{20} = +3.66°$ (*c* 4, C_5H_5N) [lit.[5] $[\alpha]_D^{25} = +3.55°$ (*c* 5.24, C_5H_5N)]; δ_H [$CDCl_3$] includes the following signals: 3.57 (1 H, dd, *J* 5.9 and 11.5), 3.69 (1 H, dd, *J* 3.7 and 11.5), 3.92 (1 H, m), 4.16 (2 H, m); δ_C[$CDCl_3$] includes the following signals: 63.42, 65.18 and 70.33. The enantiomeric 3-*O*-stearoyl-*sn*-glycerol has m.p. 68.5 - 69.5°C and $[\alpha]_D^{20} = -3.41°$ (*c* 4, C_5H_5N).

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7. A mixture of 1-*O*-stearoyl-*sn*-glycerol **6** (8.0 mmol) and 9-phenylxanthen-9-ol **10** (8.8 mmol) was evaporated from acetic acid (3 x 100 ml) solution under reduced pressure (water-pump). The products were chromatographed on silica gel with hexane - ethyl acetate mixtures containing 0.5% pyridine as the eluting solvent. In addition to 3-*O*-(9-phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol **7** ($[\alpha]_D^{20} = +4.1°$ (*c* 2, toluene); R_f 0.48 [diethyl ether - hexane (1:1 v/v)]; δ_H [$CDCl_3$] includes the following signals: 2.94 (2 H, m), 3.85 (1 H, m), 4.07 (2 H, m); δ_C [$CDCl_3$] includes the following signals: 64.17, 65.92, 69.49), an appreciable quantity (*ca.* 15%) of 2,3-di-*O*-(9-phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol (R_f 0.75) was also isolated from the products. The enantiomeric 1-*O*-(9-phenylxanthen-9-yl)-3-*O*-stearoyl-*sn*-glycerol has $[\alpha]_D^{20} = -4.1°$ (*c* 3, toluene). The high enantiomeric excess of both **7** and its enantiomer was confirmed by converting each of them into its 2-*O*-[(1-*S*)-camphanate] ester. ^{13}C NMR spectroscopic analysis of the resulting products suggested that both of them were obtained in a diastereoisomerically-pure state.

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9. No migration of the stearoyl residue could be detected after a solution of racemic 3-*O*-(9-phenylxanthen-9-yl)-1-*O*-stearoylglycerol in 1-methylimidazole - $CDCl_3$ solution (6 : 94 v/v) had been allowed to stand at 22°C for 4 days.

10. 2-*O*-Arachidonoyl-3-*O*-(phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol **8** has $[\alpha]_D^{20} = +6.9°$ (*c* 2.5, toluene); δ_H [$CDCl_3$] includes the following signals: 3.09 (2 H, m), 4.22 (1 H, dd, *J* 6.5 and 11.8), 4.35 (1 H, dd, *J* 3.7 and 11.8), 5.19 (1 H, m); δ_C [$CDCl_3$] includes the following signals: 61.65,

62.67, and 70.22. The enantiomeric 2-*O*-arachidonoyl-1-*O*-(9-phenylxanthen-9-yl)-3-*O*-stearoyl-*sn*-glycerol has $[\alpha]_D^{20}$ = - 7.1° (c 2.5, toluene).

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12. 2-*O*-Arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **2** (Found: M+, 644.5360. $^{12}C_{41}{}^{1}H_{72}{}^{16}O_5$ requires: 644.5380) was purified by chromatography on silica gel with hexane - ethyl acetate mixtures containing 0.5% acetic acid[13] as the eluting solvent; it was isolated in *ca.* 90% overall yield for the two steps starting from 3-*O*-(9-phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol **7**; δ_H [$CDCl_3$] 0.88 (6 H, m), 1.25 - 1.45 (35 H, m), 1.61 (2 H, m), 1.72 (2 H, m), 2.06 (2 H, dd, *J* 6.9 and 13.8), 2.13 (2 H, dd, *J* 7.2 and 13.6), 2.34 (4 H, dt, *J* 16.6 and 7.6), 2.82 (6 H, m), 3.72 (2 H, d, *J* 5.1), 4.22 (1 H, dd, *J* 5.8 and 11.9), 4.33 (1 H, dd, *J* 4.3 and 12.0), 5.09 (1 H, m), 5.37 (8 H, m); δ_C [$CDCl_3$] includes the following signals: 61.58, 62.33, 72.41, 127.75, 128.05, 128.30, 128.51, 128.82, 128.99, 129.23, 130.71, 173.46, 174.08. The specific rotation of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **2** was too low to measure accurately; however, its high enantiomeric excess (96 - 97%) was confirmed by converting it into its 3-*O*-[(1-*S*)-camphanate]. Examination of the ^{13}C NMR spectrum (in $CDCl_3$) of the latter derivative revealed a very strong signal at δ 68.58 and a very weak signal at δ 68.72. These signals were assigned to the *C*-2 resonances of the camphanyl derivatives of **2** and **3**, respectively. Using the same analytical procedure, the enantiomeric excess of 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **3** was estimated to be *ca* . 90%. This preparation has not been optimized.

13. In order to minimize acyl migration, it is generally advisable to add a small quantity of acetic (or equivalent) acid to the eluting solvent in the chromatography of mono- and di-esters of glycerol.

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15. 1-*O*-(9-Phenylxanthen-9-yl)-glycerol **12** was crystallized from dichloromethane - cyclohexane and isolated in 92% yield (Found: C 75.61; H, 5.70. $C_{22}H_{20}O_4$ requires: C, 75.84; H, 5.79%), m.p. 118 - 119°C; R_f 0.46 [CH_2Cl_2 - EtOH (19:1 v/v)]; δ_H [$(CD_3)_2SO$] 2.87 (1 H, dd, *J* 5.9 and 8.8), 2.93 (1 H, dd, *J* 5.5 and 8.8), 3.33 (1 H, m), 3.42 (1 H, m), 3.63 (1 H, m), 4.45 (1 H, t, *J* 5.6), 4.69 (1 H, d, *J* 5.2), 7.1 - 7.4 (13 H, m); δ_C [$(CD_3)_2SO$] includes the following signals: 63.73, 65.52, 70.94.

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17. 2-*O*-(9-Phenylxanthen-9-yl)glycerol **16** was crystallized from cyclohexane and isolated in 73% overall yield for the three steps starting from glycerol **11** (Scheme 2b) (Found: C, 75.86; H, 5.70. $C_{22}H_{20}O_4$ requires: C, 75.84; H, 5.79%), m.p. 117 - 120°C; R_f 0.50 [CH_2Cl_2 - EtOH (19:1 v/v)]; δ_H [$(CD_3)_2SO$] 2.99 (2 H, m), 3.11 (3 H, m), 4.22 (2 H, m), 7.07 (2 H, m), 7.17 (3 H, m), 7.27 (4 H, m), 7.36 (4 H, m); δ_C [$CDCl_3$] 62.81, 73.00, 76.06, 116.55, 123.25, 123.60, 126.85, 127.14, 127.77, 129.73, 130.44, 148.13, 151.15.

18. 1,2-Di-*O*-linoleoylglycerol **14** was obtained as a colourless oil in 87% overall yield for the two steps starting from 1-*O*-(9-phenylxanthen-9-yl)glycerol **12**; R_f 0.34 [ether - hexane (1:1 v/v)]; δ_H [$CDCl_3$] 0.85 (6 H, t, *J* 6.9), 1.28 (28 H, m), 1.57 (4 H, m), 2.01 (8 H, m), 2.28 (4 H, m), 2.72 (4 H, m), 2.88 (1 H, br), 3.66 (2 H, d, *J* 5.2), 4.16 (1 H, dd, *J* 6.1 and 12.0), 4.29 (1 H, dd, *J* 4.1 and 12.0), 5.05 (1 H, m), 5.30 (8 H, m); δ_C [$CDCl_3$] includes the following signals: 61.11, 62.15, 71.95, 127.77, 127.94, 129.80, 130.02, 173.32, 173.62.

19. 1,3-Di-*O*-linoleoylglycerol **17** was obtained as a colourless oil in 63% overall yield for the two steps starting from 2-*O*-(9-phenylxanthen-9-yl)glycerol **16**; R_f 0.48 [ether - hexane (1:1 v/v)]; δ_H [$CDCl_3$] 0.89 (6 H, t, *J* 6.9), 1.30 (28 H, m), 1.62 (4 H, m), 2.06 (8 H, m), 2.35 (4 H, m), 2.77 (4 H, m), 4.05 - 4.20 (5 H, m), 5.35 (8 H, m); δ_C [$CDCl_3$] 14.11, 22.61, 24.89, 25.65, 27.22, 29.12, 29.19, 29.38, 29.62, 31.55, 34.11, 65.05, 68.32, 127.92, 128.09, 130.03, 130.24, 173.97.

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