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Synthesis of 1-*O*-alkyl-*sn*-glycerols and fluorescently labeled analogs from 2,5-*O*-methylene-D-mannitol as precursor

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Abstract

A new approach employing 2,5-*O*-methylene-D-mannitol as precursor for the synthesis of naturally occurring and fluorescently labeled alkyl glycerols is described. From 2,5-*O*-methylene-D-mannitol, which was prepared according to the method of Ness et al. [1], 2,2-*O*-methylene-bis-(3-*O*-trityl)-*sn*-glycerol, the central product, was synthesized to an enantiomerically pure molecule in four steps. 1-*O*-hexadecyl-*sn*-glycerol was prepared by condensation of hexadecyl methanesulfonate with the central product and by subsequent detritylation and cleavage of the methylene bridge. From the alkyl glycerol the different lipid classes can be synthesized by well known strategies. To form fluorescently labeled analogs of alkyl glycerols the fluorescence marker 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) was linked with the ω-amino group of 1-*O*-(12-aminododecyl)-*sn*-glycerol. Therefore the central product was condensed with 12-*N*-*t*-BOC-aminododecyl methanesulfonate. The methylene bridge, the trityl groups and the BOC protection group were cleaved with boron trifluoride/methanol in one step. 1-*O*-(12-NBD-aminododecyl)-*sn*-glycerol is useful as a biochemical substrate to build up fluorescently labeled neutral lipids and phospholipids.

Key words: Glyceryl ethers; Fluorescently labeled ether lipids; 2,5-*O*-methylene-D-mannitol; 2,2′-*O*-methylene-bis-(3-*O*-trityl-*sn*-glycerol); 1-*O*-hexadecyl-*sn*-glycerol; 1-*O*-(12-NBD-aminododecyl)-*sn*-glycerol

Introduction

In addition to their occurrence as components of membranes, ether lipids are molecules with important biological functions in the living cell. The isolation of defined glyceryl ethers in pure and sufficient quantities from natural sources is a complicated process. So there is an increasing demand for synthetic methods leading to ether lipids with defined 1-*O*-alkyl- or 1-*O*-alkenyl and acyl moieties. For investigations of ether lipid metabolism and lipid topogenesis the use of fluorescently labeled ether lipids is favourable. These analogs should resemble the naturally occurring glyceryl ethers as closely as possible. In most instances, the chemical total synthesis represents the only way to produce fluorescent analogs with the desired properties.

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The most applied procedure for the stereoselective synthesis of glyceryl ethers involves condensation of 1,2-isopropylidene-*sn*-glycerol with alkyl methanesulfonates [2]. The alkylation of 1,2-isopropylidene-*sn*-glycerol yields 3-*O*-alkyl-*sn*-glycerol, which can be isomerized to the natural *sn*-1 isomer via Walden inversion [3]. Since the isomerization at C-2 of glycerol via Walden inversion is incomplete [2], 1-*O*-alkyl-*sn*-glycerols free of isomers should be prepared from 2,3-isopropylidene-*sn*-glycerol, which could be directly synthesized from L-mannitol. However, L-mannitol does not occur in nature and it has to be prepared by tedious synthesis routes from L-mannose and ultimately from L-arabinose [4] or L-inositol [5]. Furthermore, 2,3-isopropylidene-*sn*-glycerol can be obtained from L-erythrulose [6], L-serine [7,8], L-arabinose [9–11], L-ascorbic acid [12,13] and L-dimethyltartrate [14]. Most of these synthesis routes are laborious, and the enantiomerical purity is insufficient, except the route described by Kodali [11], who found a similar enantiomerical purity for 2,3-isopropylidene-*sn*-glycerol as Eibl [2] for 1,2-isopropylidene-*sn*-glycerol.

Recent studies have shown that D-mannitol (**1**) can also be used for the synthesis of differentially blocked glycerol derivatives (Fig. 1).

The advantage of the intermediates 2,5-*O*-methllene-D-mannitol (**VII**), 3,4-isopropylidene-D-mannitol (**III**) and 1,3-4,6-bis-*O*-(*p*-methoxybenzylidene)-D-mannitol (**IV**) in comparison with 1,2-5,6-di-*O*-isopropylidene-D-mannitol (**II**) is that the different reactivity of the primary and secondary hydroxyl groups can be used at the beginning of the synthesis. Eibl and Woolley [15] have published a study for the synthesis of enantiomerically pure glyceryl esters and ethers employing 3,4-isopropylidene-D-mannitol (**III**) as precursor.

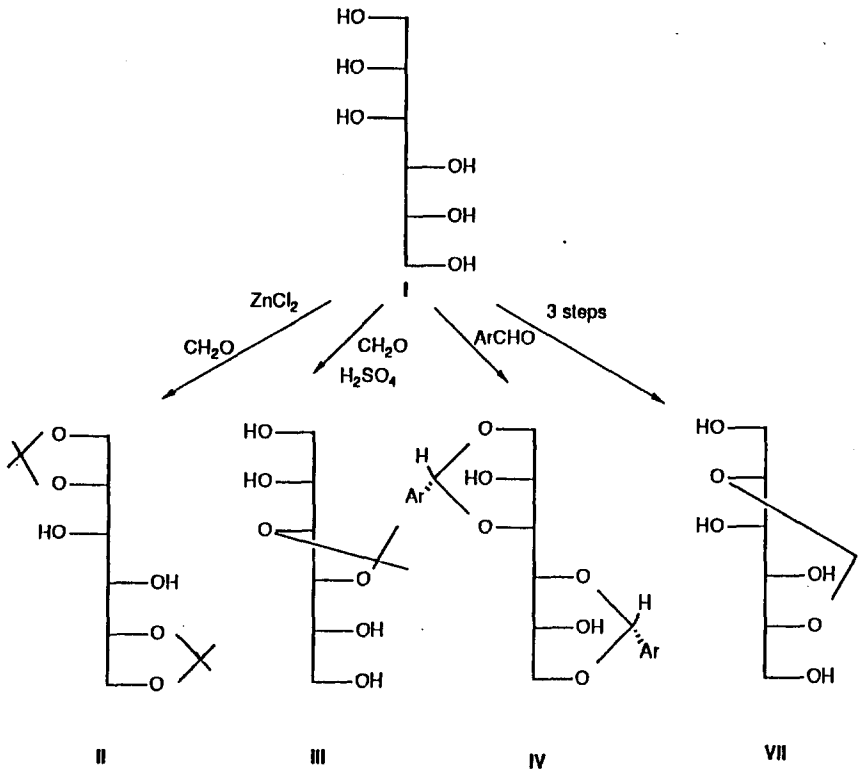

Fig. 1. Differently blocked D-mannitol derivatives for the synthesis of asymmetrically substituted glycerol compounds.

Also starting from 2,5-*O*-methylene-D-mannitol (**VII**), the preparation of 1-*O*-galactosyl-*sn*-glycerol [16] and 1,2-diacyl-*sn*-glycerols [17] was described. In this paper we will present a method for the synthesis of ether lipids using 2,5-*O*-methylene-D-mannitol (**VII**) as precursor [18].

2. Material and methods

2.1. Chemicals and solvents

11-Bromo-1-undecanol, hexadecyl methanesulfonate, di-*t*-butyl pyrocarbonate and bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) were purchased from Sigma (Deisenhofen, Germany); all other chemicals and solvents, p.a. grade, were obtained from Merck (Darmstadt, Germany).

2.2. Thin layer chromatography (TLC)

TLC was carried out on plates coated with a 0.25 mm layer of silica gel 60. Products were detected by charring after spraying with sulfuric acid (50%).

Preparative TLC was carried out on plates coated with 2 mm silica gel 60.

2.3. Gas chromatography and mass spectrometry of 2,2'-O-methylene-bis-(pivaloyl-sn-glycerol)

For derivatization 2,2'-*O*-methylene-bis-(pivaloyl-*sn*-glycerol) (1 mg) was dissolved in 75 μl of acetonitrile. Then BSTFA (75 μl) was added. After 10 min the mixture was directly used for gas chromatography/mass spectrometry (GC/MS).

GC was carried out on a 25 m 5% Phe-Me silicone column in a Hewlett-Packard 5890 Series II instrument linked to a Hewlett-Packard mass spectrometer 5971. Injections were made with a split of 1:50. As carrier gas, helium at 2×10^5 Pa inlet pressure was used. The temperature program ran from 170 to 250°C at 2°C/min.

2.4. 1,3-2,5-4,6-Tri-O-methylene-D-mannitol (**V**)

A mixture of D-mannitol (10 g, 55 mmol), 20 ml concentrated hydrochloric acid and 20 ml formaldehyde (37%) was allowed to stand at 50°C for 5 days in a closed flask. The reaction mixture was cooled at 5°C and the precipitate was filtered off and washed several times with ice-cold water. The crude product was recrystallized from dichloromethane/methanol. The yield was 11.1 g (93%), m.p. 234°C, $[\alpha]_D^{20}$ −103.6° (c 2.19, $CHCl_3$). E.a.: calc. for $C_9H_{14}O_6$ C 49.54, H 6.46; found: C 49.59, H 6.54. Lit. (1): m.p. 232–233°C, $[\alpha]_D^{20}$ −104.2°(c 2.19, $CHCl_3$).

2.5. 1,6-Diacetyl-3,4-di-(acetoxymethyl)-2,5-O-methylene-D-mannitol (**VI**)

Powdered 1,3-2,5-4,6-tri-*O*-methylene-D-mannitol (10 g, 46 mmol) was added in portions to a stirred mixture of 35 ml acetic anhydrid, 15 ml acetic acid and 0.4 ml concentrated sulfuric acid. Within 15 min the reaction mixture set to a magma of needle-like crystals. The mass was broken up, poured into 300 ml ice-cold water and stirred for 2 h. Then the precipitate was collected on a Büchner funnel and washed with 100 ml ice-cold water. The resultant product was dissolved in 60 ml dichloromethane and the arising water layer was separated and extracted twice with 20 ml portions of dichloromethane. The combined organic phases were dried over anhydrous sodium sulfate. The solvent was removed by evaporation under reduced pressure. The crude product was recrystallized from ethanol with a final yield of 15.7 g (81%), m.p. 128°C, $[\alpha]_D^{20}$ +58.7° (c 1.1, $CHCl_3$). E.a.: calc. for $C_{17}H_{26}O_{12}$ C 48.34, H 6.21; found: C 48.50, H 6.29. Lit. (1): m.p. 129–130°C, $[\alpha]_D^{20}$ +57.6° (c 1.1, $CHCl_3$).

2.6. 2,5-O-methylene-D-mannitol (**VII**)

1,6-Diacetyl-3,4-di-(acetoxymethyl)-2.5-*O*-methylene-D-mannitol (15 g, 36 mmol) was dissolved in 60 ml anhydrous dichloromethane and 20 ml methanol containing sodium methylate (0.2 M). The mixture was stirred for 3 h at room temperature and subsequently cooled at 4°C. The precipitated white crystalline product was filtered off and recrystallized from ethanol yielding 5.9 g (86%), m.p. 174°C, $[\alpha]_D^{20}$ −60.0° (c 1.2, H_2O). E.a.: calc. for $C_7H_{14}O_6$ C 43.29, H 7.27; found C 43.34, H 7.28. Lit. (1): m.p. 173–174°C, $[\alpha]_D^{20}$ −51.4° (c 1.2, H_2O).

2.7. 1,6-Dipivaloyl-2,5-O-methylene-D-mannitol (**VIII**)

Pivaloyl chloride (6.8 ml, 54 mmol) was added

dropwise with stirring to an ice-cold solution of 2,5-*O*-methylene-D-mannitol (5.0 g, 25.75 mmol) in anhydrous pyridine. After 1 h stirring at room temperature 10 ml methanol was added to the mixture. The solvent was evaporated at 40°C under reduced pressure and traces of pyridine were removed by several evaporations in the pressure of toluene. The residue was dissolved in 60 ml diethylether and the ether extract was washed twice with 10 ml portions of a solution of sodium chloride (5%) and dried over anhydrous sodium sulfate. The solvent was evaporated and the crude product was recrystallized from ethanol/water. The yield of the white crystalline product was 9.1 g (96%), m.p. 106°C. TLC showed a single spot at R_f 0.54 with diethylether as solvent. $[\alpha]_D^{20}$ -79.1° (c 1.0, $CHCl_3$).

IR spectrum (KBr): ν = 2530/2500 (O—H), 2990/2930/2880/2845/1485/1415 (CH, CH_2, CH_3), 1725/1710 (C=O), 1395/1365/1260($(CH_3)_3$), 1305/1285 (—C—O—CO—), 1195/1180/1145/1130/1095/1075/1045/1030 (C—O) cm^{-1}. ^{1}H-NMR spectrum (270 MHz, $CDCl_3$): δ = 1.22 (s, 18 H, $-(CH_3)_3$, 3.31–3.40 (m, 2H), 3.79 (m, 4 H), 4.32–4.44 (m, 4 H), 4.77 (s, 4 H, —O—CH_2—O—). E.a.: calc. for $C_{17}H_{30}O_8$ C 56.34, H 8.34; found C 56.52, H 8.37.

2.8. 2,2′-O-Methylene-bis-(1-pivaloyl-sn-glycerol) (X)

Sodium periodate (4.95 g, 23 mmol), dissolved in 50 ml of water, was added with stirring to an ice-cold solution of 1,6-dipivaloyl-2,5-*O*-methylene-D-mannitol (8 g, 22 mmol) in 60 ml ethanol. The mixture was stirred vigorously at room temperature for 3 h in the dark. Then it was cooled at 10°C and a solution of 7.8 ml barium chloride (1.5 M) was added to remove the periodate iones as insoluble barium periodate. The solid was collected on a Büchner funnel and washed twice with 10 ml portions of ethanol. Two drops of phenolphthalein (1% in ethanol) were added to the combined filtrates. The mixture was cooled in an ice bath and sodium borohydride (2 g, 50 mmol) was added in small portions. The reaction mixture was vigorously stirred at 0°C for 2 h under pH controlled conditions. To achieve pH control, acetic acid was added dropwise whenever the solution changed to a red stain, to avoid pH values greater than 7.5. The mixture was extracted three times with 40 ml portions of dichloromethane, and the combined organic phases were washed several times with 20 ml portions of a solution of sodium chloride (5%) and dried over anhydrous sodium sulfate. The solvent was evaporated and the crude product was directly used for the next modification. The yield was 7.0 g (86%). A probe was recrystallized from diethylether/hexane. TLC of the analytical probe showed a single spot at R_f 0.76 with diethylether as solvent, m.p. 48°C, $[\alpha]_D^{20}$ −73.4° (c 1.0, $CHCl_3$).

IR (KBr): ν = 3400 (O—H), 2990/2940/2920/2900/1475/1460, (CH, CH_2, CH_3), 1730/1715 (C=O), 1395/1365/1230 ($(CH_3)_3$), 1290 (—C—O—CO), 1175/1160/1065/1035/1020/(C—O) cm^{-1}.

^{1}H-NMR spectrum (270 MHz, $CDCl_3$): δ = 1.20 (s, 18H, $-(CH_3)_3$), 3.63 (dd, 2 H, ^2J = 6.7 Hz, ^3J = 11.8 Hz, H_A, $C\underline{H}_2$—OH), 3.70 (bs, 2H, —OH), 3.76 (dd, 2H, ^2J = 2.6 Hz, ^3J = 11.9 Hz, H_B, $-C\underline{H}_2$—OH), 3.90 (m, 2H, —CH—), 4.07–4.18 (m, 4H, CH_2—O—CO), 4.91 (s, 2H, —O—CH_2—O—). E.a.: calc. for $C_{17}H_{32}O_8$ C 56.34, H 8.34; found 56.52, H 8.37.

2.9. 2,2′-O-Methylene-bis-(3-O-trityl-sn-glycerol) (XII)

2,2′-*O*-Methylene-bis-(1-pivaloyl-*sn*-glycerol) (7 g, 19 mmol), 80 ml anhydrous butanol, 8.4 ml triethylamine (60 mmol) and 12.5 g tritylchloride (45 mmol) were placed in a 300 ml flask with reflux condenser and magnetic stirrer. The mixture was refluxed for 3 h and then half of the solvent was evaporated. The residue was boiled up with 100 ml petroleum ether and the warm precipitate of trityl alcohol and triethylamine hydrochloride was filtered off. The solvent of the filtrate was reduced by half and then 50 ml methanol containing sodium methylate (0.2 M) was added. The mixture was refluxed for 2 h and then treated with 50 ml water. The solution was extracted three times with 30 ml portions of dichloromethane, washed twice with 20 ml portions of water and dried over sodium sulfate. The solvent was evaporated and the oily residue was recrystallized from diethylether/petroleum ether and dried under vacuum. The

yield of 2,2'-*O*-methylene-bis-(3-*O*-trityl-*sn*-glycerol) was 10.5 g (82%), m.p. 143°C. TLC showed a spot at R_f 0.63 with diethylether as solvent. $[\alpha]^{20}_D$ +99.5° (c 2.0, $CHCl_3$).

IR (KBr): ν = 3400 (O—H), 3080/3050/790/770/760/710 (C—H, aromatic), 2980/2940/2885/1470 (CH, CH_2), 1960/1600/1500/1455 (C=C), 1220/1180/1090/1070/1040/1030 (C—O), 1130 (C—O—C) cm^{-1}.

^{1}H-NMR spectrum (270 MHz, $CDCl_3$): δ = 3.08–3.19 (m, 4 H, —C<u>H</u>$_2$—O—trityl), 3.34 (bs, 2H, —OH), 3.60 (dd, 2H, ^2J = 7.2 Hz, ^3J = 15.3 Hz, H_A, —C<u>H</u>$_2$—OH), 3.71 (dd, 2H, ^2J = 2.2 Hz, ^3J = 11.7 Hz, H_b, —C<u>H</u>$_2$—OH), 3.82 (m, 2H, —CH—), 4.88 (s, 2H, —O—CH_2—O—), 7.17-7.42 (m, 30 H, trityl). E.a.: calc. for $C_{45}H_{44}O_6$ C 79.39, H 6.51; found C 79.50, H 6.47. Lit. (16): m.p. 146-147.5°C, $[\alpha]^{20}_D$ +58.0° (c 2.0; $CHCl_3$).

2.10. 12-Hydroxydodecannitrile (**XIII**)

Powdered sodium cyanide (6.5 g, 0.133 mmol) in 35 ml anhydrous dimethylsulfoxide was stirred and heated at 90°C until the sodium cyanide was dissolved. Then the mixture was cooled at 60°C and 11-bromo-1-undecanol (25 g, 0.1 mol) in 15 ml anhydrous dimethylsulfoxide was added dropwise. The reaction mixture was heated at 90°C for 30 min. After cooling in an ice bath and the addition of 150 ml water and 50 ml diethylether, the water layer was separated and extracted twice with 25 ml portions of ether. The combined ether phases were shaken with a solution of 80 ml ice-cold hydrochloric acid (6 M) for 5 min, and then washed twice with 40 ml portions of a saturated solution of sodium chloride. The organic phase was dried over potassium carbonate and the solvent was evaporated. The crude product was recrystallized from diethylether/hexane at 4°C. The yield of the white crystalline product was 18.4 g (93%), m.p. 37°C. TLC showed a spot at R_f 0.36 with chloroform as solvent.

IR (KBr): ν = 3400 (O—H), 2930/2850/1465/720 (CH_2), 2255 (—C≡N), 1420 (—CH_2—C≡N), 1050 (C—O)cm^{-1}. E.a.: calc. for $C_{12}H_{23}NO$ C 73.04, H 11.75, N 7.10; found: C 73.11, H 11.77, N 7.18.

2.11. 12-Amino-1-dodecanol (**XIV**)

12-Hydroxydodecannitrile (16.8 g, 85 mmol) in 40 ml anhydrous diethylether was carefully added to a solution of 80 ml Vitrid (sodium aluminum bis-(2-methoxyethoxo)-dihydride), 50 ml anhydrous toluene and 120 ml diethylether. The mixture was stirred and refluxed for 2 h. After cooling, the excess of Vitrid was destroyed by the dropwise addition of water, and the solution was filtered off. The residue was extracted twice with diethylether at 50°C and the combined filtrates were dried over potassium carbonate. After evaporation of the solvent, the crude product was recrystallized from diethylether. Then the product was purified by sublimation at 0.15 mbar and 100°C, yielding 12.5 g (74%), m.p. 80°C. TLC showed a spot at R_f 0.67 with chloroform/methanol/ammonia (25%) (60:30:5, v/v/v) as solvent.

IR (KBr): ν = 3200/1370 (O—H), 2930/2850/1470/720 (CH_2), 3270/3240/1615/820 (NH_2), 1030 (C—N), 1060 (C—O)cm^{-1}. E.a.: calc. for $C_{12}H_{27}$-NO C 71.58, H 13.52, N 6.96; found: C 71.65, H 13.47, N 7.01.

2.12. 12-N-t-BOC-amino-1-dodecanol (**XV**)

A solution of di-*t*-butyl pyrocarbonate (14.8 g, 66 mmol) in 50 ml anhydrous tetrahydrofuran was added dropwise with stirring to a mixture of 12-amino-dodecanol (12.0 g, 60 mmol), 150 ml tetrahydrofuran and 9.5 ml freshly destilled triethylamine (66 mmol). The mixture was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure and the residue was dissolved in 300 ml ethylacetate. The solution was washed in each case with 70 ml of a solution of hydrochloric acid (0.01 M) and sodium hydrogencarbonate (5%) and finally with a saturated solution of sodium chloride. The organic phase was dried over sodium sulfate, the solvent was evaporated and the crude product was recrystallized with diethylether/petroleum ether. The yield of the white crystalline product was 18.0 g (99%), m.p. 78°C. TLC showed a single spot at R_f 0.39 with diethylether/petroleum ether (50:50 v/v) as solvent.

IR (KBr): ν = 3420 (O—H), 2970/2930/2850 (CH_2, CH_3), 3370 (NH), 1690 (C=O, amide I), 1515 (R—O—CO—NH—R, amide II), 1390/1365/1240 ($C(CH_3)_3$), 1165/1060 (C—O), 600 (NH, amide IV) cm^{-1}. E.a.: calc. for $C_{17}H_{35}NO_3$

C 67.73, H 11.70, N 4.65; found: C 67.78, H 11.74, N 4.65.

2.13. 12-N-t-BOC-aminododecyl methanesulfonate (**XVI**)

13-*N*-*t*-BOC-aminododecanol (16.0 g, 53 mmol) was dissolved in 250 ml anhydrous dichloromethane, containing 11.2 ml triethylamine (80 mmol). The mixture was cooled at 0°C, and freshly distilled methanesulfonyl chloride (4.4 ml, 56 mmol) was added dropwise. The solution was stirred continuously for 30 min at room temperature, then washed twice with 60 ml of a half-saturated ice-cold solution of sodium chloride and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was dissolved in 250 ml 2-propanol. The crude product precipitated at −20°C and the precipitate was recrystallized with diethylether/hexane. The yield was 18 g (89%), m.p. 61°C. TLC showed a spot at R_f 0.47 with diethylether/petroleum ether (50:50, v/v) as solvent, $[\alpha]_D^{20} \pm 0.2$ (c 1.0; MeOH).

IR (KBr): ν = 3370 (NH), 2970/2930/2850/1470/720 (CH_2, CH_3), 3055/1680 (C=O, amide I), 1525 (R—O—CO—NH—R, amide II), 1390/1365 ($C(CH_3)_3$), 1340/980/940/850 (R—O—SO_2—OR), 1165 (C—O), 600 (NH, amide IV) cm^{-1}. E.a.: calc. for $C_{18}H_{37}NO_5S$ C 56.96, H 9.83, N 3.69; found: C 57.05, H 9.88, N 3.76.

2.14. 1-O-Hexadecyl-sn-glycerol (**XVIII**)

2,2-*O*-methylene-bis-(3-*O*-trityl-*sn*-glycerol) (681 mg, 1 mmol) was dissolved in 10 ml anhydrous tetrahydrofuran containing 135 mg potassium *t*-butylate. Then hexadecyl methanesulfonate (705 mg, 2.2 mmol) dissolved in 5 ml anhydrous tetrahydrofuran was added and the mixture was stirred and refluxed for 2 h. Subsequently each 80 ml of water and diisopropylether were added. The organic phase was separated and dried over anhydrous sodium sulfate and evaporated under reduced pressure. The dialkylated product was dissolved in 2 ml anhydrous dichloromethane and a fresh solution of boron trifluoride/methanol (2 ml) was added. The cleavage of the methylene bridge and the trityl protection groups was carried out in an atmosphere of purified nitrogen. The flask was closed and heated at 40°C for 10 min with stirring. The mixture was cooled in an ice bath and the solvent was evaporated under reduced pressure. The residue was dissolved in a solution of sodium chloride (5%) and the suspension was neutralized. The suspension was extracted twice with 10 ml diethylether, the extracts were dried over sodium sulfate, and the solvent was evaporated. The crude product was purified by preparative TLC on plates coated with 2 mm silica gel 60. Diethylether and petroleum ether (50:50 v/v) were employed as solvents. The yield was 537 mg (85%), m.p. 65°C. The compound showed a single spot at R_f 0.69 with petroleum ether/diethylether (50:50, v/v) as solvent. The product was derivatized with 7-methoxycoumarin-4-acetic acid and compared with a standard according to the method of Mita et al. [19]. E.a.: calc. for $C_{19}H_{40}O_3$ C 72.09, H 12.73; found: C 71.84, H 12.84.

2.15. 1-O-(12-Aminododecyl)-sn-glycerol (**XX**)

2,2′-*O*-Methylene-bis-(3-*O*-trityl-*sn*-glycerol) (2 mmol, 681 mg) was dissolved in 15 ml freshly destilled dimethylformamide under purified nitrogen. Sodium hydride (106 mg, 4.5 mmol) was added and the mixture was stirred for 30 min until the end of gas production. Then 12-*t*-BOC-aminododecyl methanesulfonate (1.6 g, 42 mmol) was added in portions to the mixture. After stirring for 5 h at room temperature, 2 ml methanol and 30 min later 20 ml water were added. The solution was extracted twice with 30 ml diisopropylether and the combined extracts were washed several times with water and then dried over sodium sulfate. The solvent was evaporated and the dialkylated crude product (TLC shows the dialkylated product at R_f 0.77 and the monoalkylated product at R_f 0.57 with diethylether/petroleum ether (50:50, v/v) as solvent) was purified by column chromatography on silica gel KG 60. The sample dissolved in 10 ml chloroform was applied to the column and eluted with petroleum ether/diethylether (50:50, v/v). The fractions containing the dialkylated product were combined and the solvent was evaporated. The residue was dissolved in 2 ml dichloromethane, and 2 ml boron trifluoride/methanol was added. The flask was closed under purified nitrogen and heated at 40°C for 10

min with stirring. The mixture was cooled in an ice bath and the solvent was evaporated under reduced pressure. The residue was dissolved in a solution of sodium sulfate (5%) and the suspension was adjusted with sodium hydroxide at pH 10.5. The solution was extracted twice with 10 ml *n*-butanol, and the solvent was evaporated and the product was purified by recrystallization with ethanol, yielding 680 mg (62%), m.p. 79°C.

IR (KBr): ν = 3380 (O—H), 2940/2870/1470/720 (CH_2), 3360/3305/1615 (NH_2), 1130 (C—O—C), 1110 (C—N) cm^{-1}. ^{1}H-NMR (250 MHz, DMSO-D_6): δ = 1.25 (bs, 16 H, —$(CH_2)_8$—), 1.42–1.50 (m, 4 H, —$\underline{CH_2}$—$(CH_2)_8$—), 2.64 (bs, 2H, —OH), 3.21–3.38 (m, 8 H, —CH_2—O—, —$\underline{CH_2}$—OH, $\underline{CH_2}$—NH_2), 3.49–3.58 (m, 1 H, —CH—), 3.80 (s, 2H, NH_2). E.a.: calc. for $C_{15}H_{33}NO_3$ C 65.41, H 12.08, N 5.09; found: C 65.51, H 12.14, N 5.05.

2.16. 1-O-(12-NBD-aminododecyl)-sn-glycerol (XXI)

1-*O*-(12-aminododecyl)-*sn*-glycerol (0.25 mmol, 69 mg) was dissolved in 5 ml ethanol. Then 10 ml ethanol, containing 100 mg NBD-Cl and 70 μl freshly distilled triethylamine, was added dropwise and stirred for 3 h in the dark. After the addition of 30 ml water and 20 ml ethyl acetate, the water layer was separated and extracted twice with 10 ml portions of ethyl acetate. The solvent was removed and the brown oily residue was purified by column chromatography. The material was dissolved in 5 ml ethyl acetate and applied to a silica gel column. The column was eluted with the following solvents: first with chloroform, then with chloroform/ethanol (9:1, v/v). The fractions with the fluorescent products were collected, the solvent was removed and a preparative TCL on plates coated with 2 mm silica gel 60 was accomplished with chloroform/ethanol/ammonia (25%) (80:20:1, v/v/v) as solvent. After elution of the orange band (R_f 0.67) with acetone the solvent was removed and the product was precipitated with water/acetone at 4°C. The yield was 42 mg (38%), m.p. 78°C.

IR (KBr): ν = 3420 (O—H), 3340 (NH), 3100/3160 (C—H, aromatic), 2940/2860/1465/720 (CH_2), 1625/1515/1455/1275/1266/1195/1005/900/850/740 (C=C, C—H, C—Cl, benzofurazane), 1560/1305 (NO_2), 1330 (C—N), 1110 (C—O—C) cm^{-1}. E.a.: calc. for $C_{21}H_{34}N_4O_6$ C 57.52, H 7.81, N 12.78; found: C 57.32, H 7.89, N 12.58.

3. Results

The preparation of 2,2′-*O*-methylene-bis-(3-*O*-trityl-*sn*-glycerol) (**XII**) is shown in Fig. 2. This molecule is the central product in the described synthesis route of 1-*O*-hexadecyl-*sn*-glycerol (**XVIII**) or its fluorescently labeled analog (**XXI**). As precursor 2,5-*O*-methylene-D-mannitol (**VII**) was applied, which can be synthezised from D-mannitol (**I**) with a yield of 65%.

The primary hydroxyl groups of 2,5-*O*-methylene-D-mannitol (**VII**) were selectively acylated with pivaloyl chloride. In comparison with an acylation of 2,5-*O*-methylene-D-mannitol (**VII**) with benzyl chloride, the advantage of the acylation with pivaloyl chloride is the increased solubility of the product in a solution of ethanol/water (50:50, v/v). As a consequence of this, the subsequent periodate oxidation and the reduction of the resultant dialdehyde could be accomplished in aqueous conditions under pH control. The periodate oxidation was carried out by preservation of the methylene bridge.

The obtained dialdehyde 2,2′-*O*-methylene-bis-(1-pivaloyl-*sn*-glyceraldehyde) (**IX**), a symmetric molecule with two chiral centres, can be converted under slightly alkaline conditions from the *S,S*-product (*threo*-product) on the one hand into the *R,S*-diastereomer (*meso*-product) and on the other hand into a cyclic aldehyde by an aldol condensation [1,20]. The probability of obtaining the *R,R*-enantiomer is statistically very low [21/22]. To prevent a racemization and an aldol condensation, it must be guaranteed that the pH does not exceed 7.5 during the reduction of the dialdehyde with sodium borohydride. If racemization unexpectedly occurred during the reduction, the resultant *R,S*-diastereomer can be removed from the *S,S*-enantiomer by physical methods such as crystallization. Furthermore, it is possible to prove the enantiomeric purity of 2,2′-*O*-methylene-bis-(1-pivaloyl-*sn*-glycerol) (**X**) by the quantitative analysis of the *meso* product which was achieved by derivatization of the reduction products with trimethyl silyl and subsequent analysis by GC/MS.

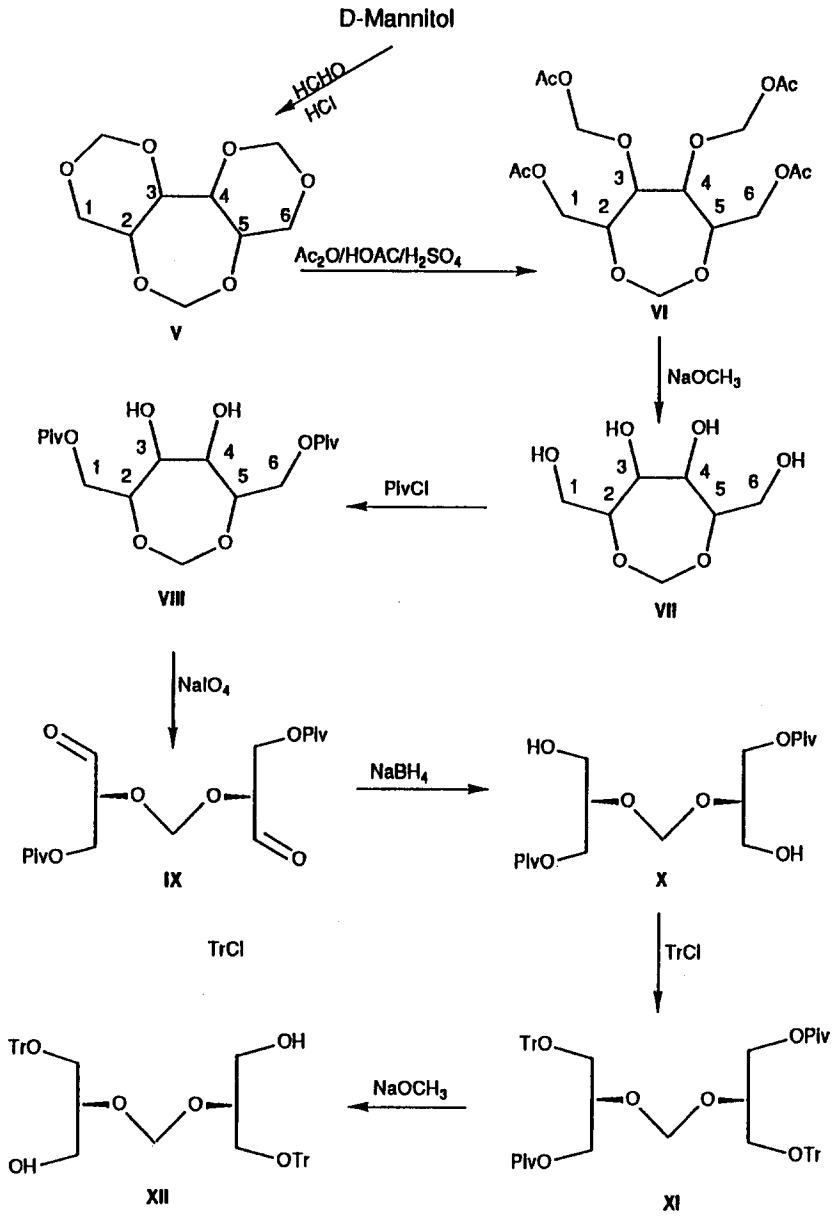

Fig. 2. Synthesis of the central product 2,2′-*O*-methylene-bis-(3-*O*-trityl-*sn*-glycerol).

Fig. 3a,b show the chromatograms of the products after the reduction of the dialdehyde with sodium borohydride under non-pH-controlled and under pH controlled conditions, respectively. Without pH control two peaks were observed, one of the *meso* and another of the *threo* product. These peaks were identified by mass spectrometry and showed a similar fragmentation pattern and the characteristic fragment ions of 2,2′-*O*-methylene-bis-(pivaloyl-*sn*-glycerol) (**X**). In the second

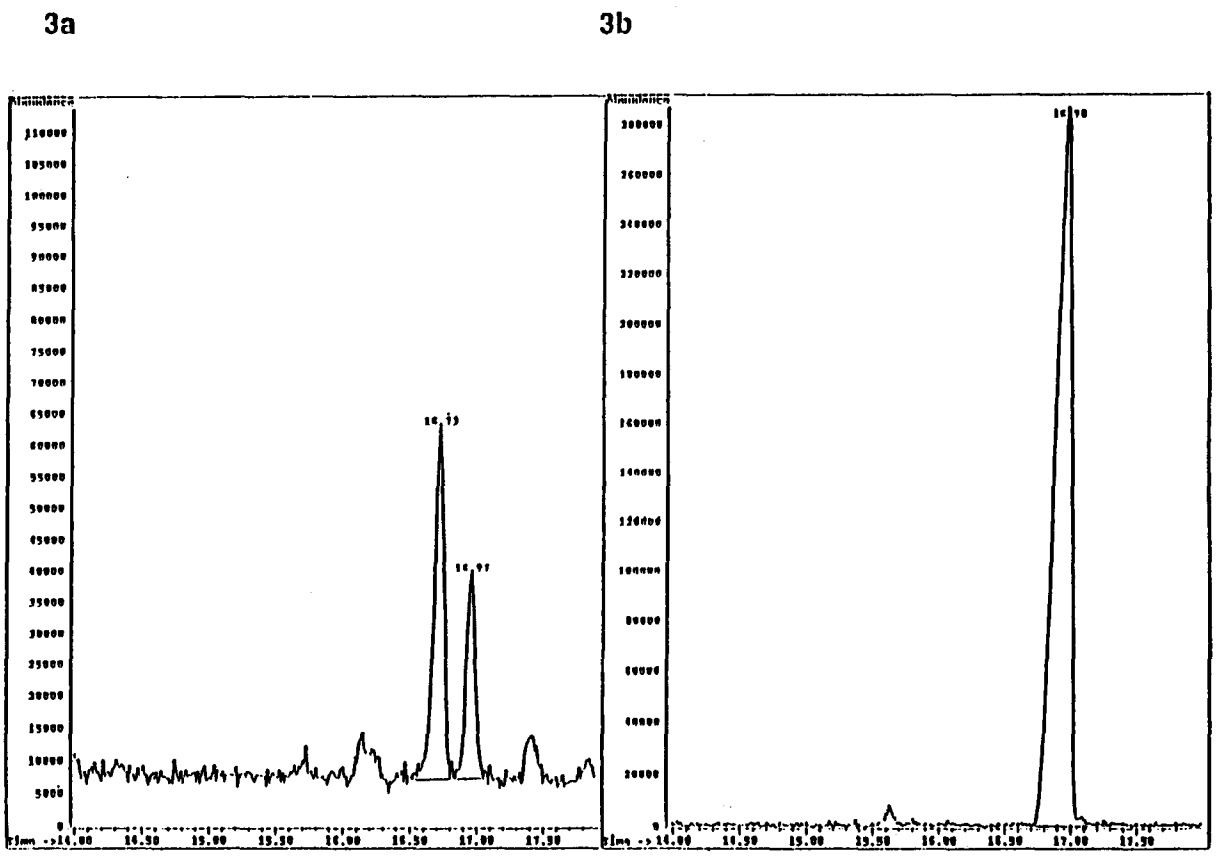

Fig. 3. Gas chromatogram of the TMS derivative of 2,2′-*O*-methylene-bis-(pivaloyl-*sn*-glycerol). Formation of the *meso* and *threo* product under non-pH-controlled conditions during the reduction of 2,2′-*O*-methylene-bis-(pivaloyl-*sn*-glyceraldehyde). Formation of the *threo* product only under pH-controlled conditions.

chromatogram only one peak, the derivatized *threo* product, is seen. The detection limit of the molar part of the *meso* product is $m > 0.002$. This means that under pH-controlled conditions no racemization of the *S,S*-enantiomer takes place and that 2,2′-*O*-methylene-bis-(1-pivaloyl-*sn*-glycerol) (**X**) is synthezised with an enantiomeric purity of at least 99.8%. After proving the enantiomeric purity of 2,2′-*O*-methylene-bis-(1-pivaloyl-*sn*-glycerol) (**X**), the central product was prepared by tritylation at *sn*-3 and alkaline methanolysis of the pivaloyl groups. The yield of the product was 65% based on the precursor 2,5-*O*-methylene-D-mannitol (**V**). The product was stable at 4°C in the dark for several months.

The synthesis of 1-*O*-alkyl-*sn*-glycerol was carried out by condensation of 1-*O*-hexadecyl methanesulfonate with the central product in the presence of potassium *t*-butylate in tetrahydrofuran. The resultant product was 2,2′-*O*-methylene-bis-(1-*O*-hexadecyl-3-*O*-trityl-*sn*-glycerol) (**XVII**). The detritylation and the cleavage of the methylene bridge by boron trifluoride/methanol resulted in 1-*O*-hexadecyl-*sn*-glycerol (**XVIII**), with a yield of 85%. This molecule can be used for the synthesis of neutral lipids and phospholipids by known methods such as acylation, tritylation and phosphorylation.

To obtain a fluorescently labeled analog, 12-BOC-aminododecyl methanesulfonate (**XVI**) was

prepared from 11-bromo-1-undecanol by the synthesis route described in Fig. 4 with a yield of 61%. The length of an alkyl chain of 12 C-atoms, linked over the amino group with the fluorescent marker, is comparable to the natural alkyl chain of 17 C-atoms. 12-BOC-amino-dodecyl methanesulfonate (**XVI**) was condensed with the central product to form 2,2′-*O*-methylene-bis-(1-*O*-(12-*N*-*t*-BOC-amino dodecyl)-3-*O*-trityl-*sn*-glycerol) (**XIX**). The amino protection group, the trityl groups and the methylene bridge were cleaved with boron trifluoride/methanol in one step. The resultant 1-*O*-(12-aminododecyl)-*sn*-glycerol (**XX**) reacted directly with NBD-Cl to the fluorescent analog 1-*O*-(12-

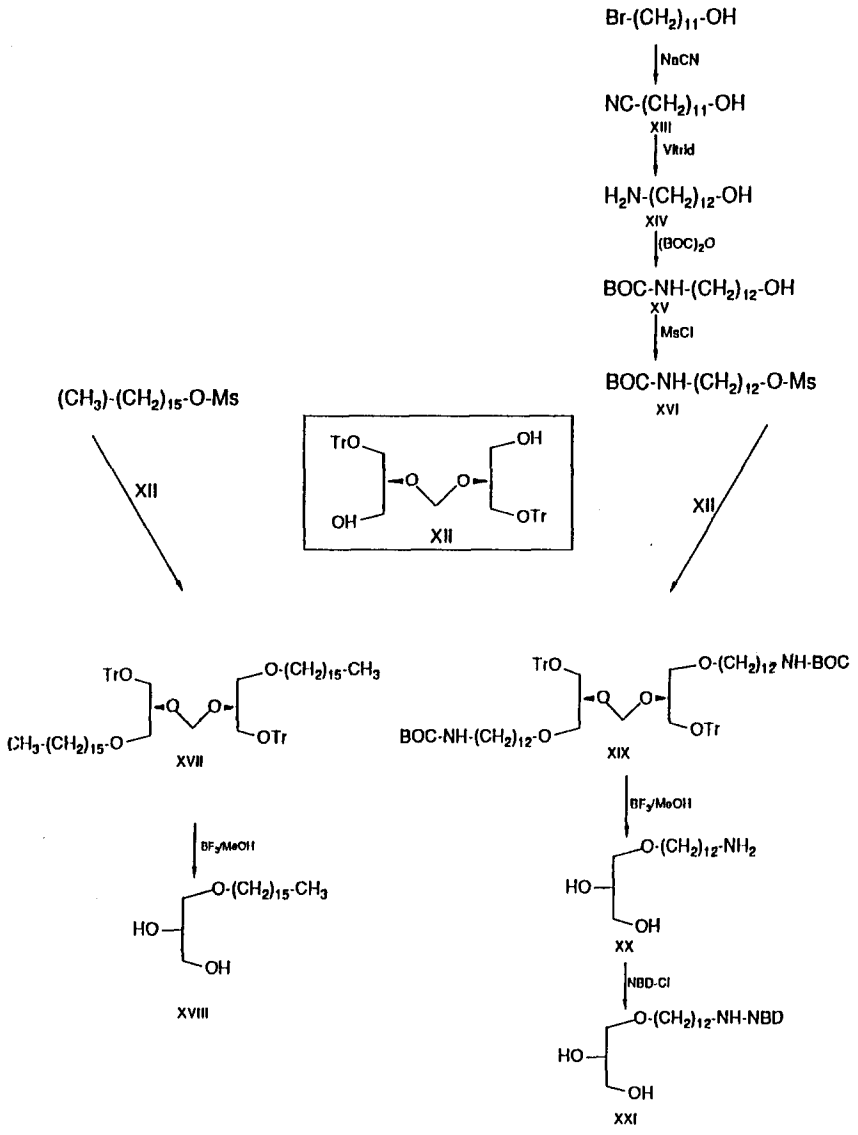

Fig. 4. Synthesis of 1-*O*-hexadecyl-*sn*-glycerol and the fluorescent analog 1-*O*-(12-NBD-aminododecyl)-*sn*-glycerol starting with the condensation of hexadecyl methanesulfonate or 12-N-t-BOC-amino-dodecyl methanesulfonate with the central product 2,2′-*O*-methylene-bis-(3-*O*-trityl-*sn*-glycerol).

NBD-aminododecyl)-*sn*-glycerol (**XXI**) with a yield of 26% based on the central product.

Fluorescent analogs of neutral lipids and phospholipids can be prepared starting from 1-*O*-(12-aminododecyl)-*sn*-glycerol (**XX**). First, the free amino group and the primary hydroxyl group have to be protected by tritylation in one step. The *N*-trityl group is more stable than the *O*-trityl group, so that the *O*-trityl group could be split off selectively with boronhydride/methanol by preservation the *N*-trityl protection group. The following steps for preparation of the different lipid classes were similar to the synthesis steps of the natural alkyl glycerols. The final steps required the cleavage of the *N*-trityl group with trifluoroacetic acid and the subsequent linking of the NBD marker with the free amino group.

4. Discussion

The synthesis route employing 2,5-*O*-methylene-D-mannitol (**VII**) as precursor is an attractive strategy for preparing alkyl glycerols or fluorescently labeled analogs with a high yield and enantiomeric purity.

After the periodate oxidation of 1,6-dipivaloyl-2,5-*O*-methylene-D-mannitol, all subsequently synthesized products up to the central molecule 2,2′-*O*-methylene-bis-(3-*O*-trityl-*sn*-glycerol) (**XII**) were symmetric molecules containing two chiral centres linked over a methylene bridge. If racemization occurred in the reaction steps between periodate oxidation and the central product, an *R,S*-diastereomer could be expected as the major impurity. Such an impurity, however, is characterized by its different physical properties, which allow for its removal by physical methods. The critical steps in the synthesis of the central product were the periodate oxidation and the reduction of the resulting dialdehyde, because of the possibility that the dialdehyde could be converted into the *R,S*-diastereomer or into the cyclic aldehyde under slightly alkaline conditions. We have shown by carrying out periodate oxidation and the subsequent reduction under pH-controlled conditions that the enantiomeric purity of 2,2′-*O*-methylene-bis-(1-pivaolyl-*sn*-glycerol) (**X**) is higher than 99%. The central product (**XII**) is well suited for the synthesis of alkyl glycerols. During the synthesis, no hydrogenation step is required. Thus, even molecules with unsaturated residues can be prepared by this method.

Fluorescent analogs of lipids are used to investigate the structure and dynamics of biological membranes, as well as the transport and the metabolism of lipids. For all these applications, fluorescent labels are an interesting alternative to radiolabels. NBD-Cl is one of the most widely used fluorescence markers for the preparation of fluorescent analogs of lipids, which are characterized by their stability and their suitable fluorescence properties. The NBD labeled ester lipids are particularly applicable to lipid visualization [23–26], photobleaching recovery measurement [27–29] and membrane fusion studies [30–32].

In this study we prepared synthetic ether lipid analogs that contain the highly fluorescent NBD marker attached to the ω-amino group of the aminododecyl chain. This position was chosen with the aim of minimizing potential perturbing effects of the NBD group on membranes and enzymes of lipid metabolism [33]. The fluorescent alkyl glycerol analog was used as biochemical precursor for lipid metabolism in cells. Isolated alveolar macrophages metabolized the precursor into neutral lipids and phospholipids (unpublished). Furthermore, the biosynthesized 1-*O*-(12-NBD-aminododecyl)-2-acyl-*sn*-glycero-3-phosphocholine was isolated and the acyl chains at C-2 were split by phospholipase A_2. The resultant product is the analog of lyso-PAF. The acetylation of lyso-PAF with acetic anhydrid in pyridine led to the fluorescent PAF analog.

5. References

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