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Synthesis of galactoglycerolipids found in the HT29 human colon carcinoma cell line

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Abstract—Synthesis of three galactoglycerolipids (3-*O*-(β-D-galactopyranosyl)-1-*O*-hexadecyl-2-*O*-palmitoyl-*sn*-glycerol, 3-*O*-(α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl)-1-*O*-hexadecyl-2-*O*-palmitoyl-*sn*-glycerol, 3-*O*-(α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl)-1-*O*-hexadecyl-*sn*-glycerol), and the corresponding glycerolipid (1-*O*-hexadecyl-2-*O*-palmitoyl-*sn*-glycerol) is described. The first two compounds were recently identified in the human colon carcinoma cell line HT29. The three-carbon synthon (*S*)-glycidol was used for construction of the glycerol moiety. Glycosylation of (*S*)-glycidol with protected galactosyl and digalactosyl donors produced galactosyl and digalactosyl glycidols. Lewis acid catalyzed opening of the epoxide produced protected galactosyl and digalactosyl glycerolipids. Deprotection, or palmitoylation followed by deprotection, yielded the target compounds. The corresponding glycerolipid was synthesized analogously and an oxidation–reduction procedure for tritiation was developed. The synthesized compounds will be used in studies of the role of galactosyl glycerolipids in differentiation and colon cancer development. © 2002 Elsevier Science Ltd. All rights reserved.

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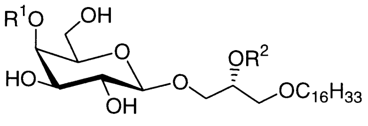
1. Introduction

Glycoglycerolipids are common constituents of plant cell membranes and bacterial cell walls.[1] In animal tissues, the expression of glycoglycerolipids is more restricted in terms of structural variability. Monogalactosyl glycerolipids and sulfated monogalactosylglycerolipids have been detected only in the testis and nervous system of mammalian species.[2]

Recently, however, monogalactosyl (3-*O*-(β-D-galactopyranosyl)-1-*O*-alkyl-2-*O*-acyl-*sn*-glycerol) and digalactosyl glycerolipids (3-*O*-(α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl)-1-*O*-alkyl-2-*O*-acyl-*sn*-glycerol) were isolated from the human colon carcinoma cell line HT29.[3] When HT29 cells were allowed to differentiate into more enterocyte-like cells, with a less tumorigenic phenotype, both galactosyl glycerolipids were lost. This suggests that they may serve as differentiation antigens in colon cancer development.[3]

The expression of galactosyl glycerolipids can potentially be involved in modulating intracellular signaling in HT29 cells. Galactosyl diacylglycerol is expressed as a minor glycolipid in cultured oligodendrocytes and was shown to enhance protein kinase C (PKC) activity in these cells.[4] In contrast, alkyl–acyl substituted glycerols were found to downregulate PKC activity.[5] The synthesis of the identified galactosyl glycerolipids would enable studies on the biological role of these glycolipids in colon cancer development.

In this paper, we describe the synthesis of two of the galactoglycerolipids that were identified in HT29 cells. In addition the corresponding glycerolipid **3**, lacking carbohydrate substitution, and the digalactosyl glycerolipid **2a**, lacking the 2-*O*-acyl substitution, were synthesized (Fig. 1). Tritiation of the galactolipids for use in metabolic studies will be performed using a galactose oxidase sodium borohydride [^{3}H] oxidation–reduction procedure.



1: R^1=H, R^2=C$_{15}$H$_{31}$CO
2a: R^1=α-Gal, R^2=H
2b: R^1=α-Gal, R^2=C$_{15}$H$_{31}$CO

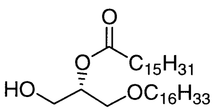


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Figure 1.

Keywords: galactoglycerolipids; oligosaccharide synthesis; glycosylation; thioglycosides; glycidol.

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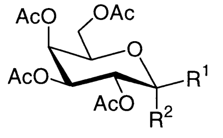


4: R^1=SEt, R^2=H
5: R^1=H, R^2=Br

Figure 2.

Usually, glycoglycerolipids are synthesized by glycosylation of an appropriate glycerol derivative. Routes to β-galactosides involve glycosylation using a neighboring participating group attached to the 2-OH of the glycosyl donor. These donors usually give significant amounts of the α-anomer, even with neighboring group participation from an acyl group atttached to the 2-OH. The potential problem of separating the anomeric mixture with long chain hydrocarbons attached to the glycerol residue, made us search for an alternative method to introduce the glycerol moiety.

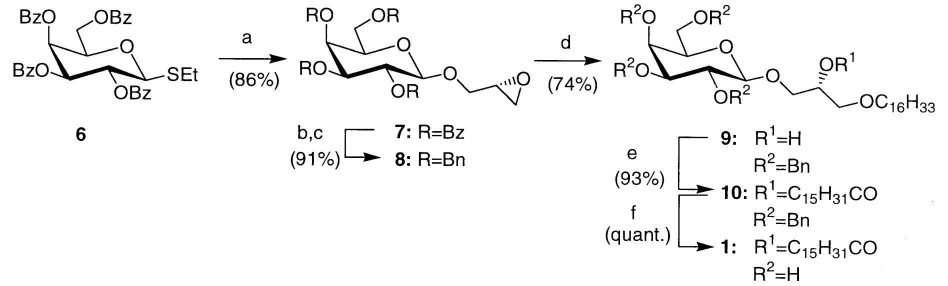
Syntheses of glycoglycerolipids from glycosylglycidols have been reported,[6–8] and we have recently demonstrated the versatility of using enantiomerically pure glycidols in the synthesis of various phospholipids.[9] The route to glycerolipids starting from commercially available (*S*)-glycidol is short, avoids protecting group manipulations and the problem with acyl migration frequently occurring when starting from glycerol derivatives.

2. Results and discussion

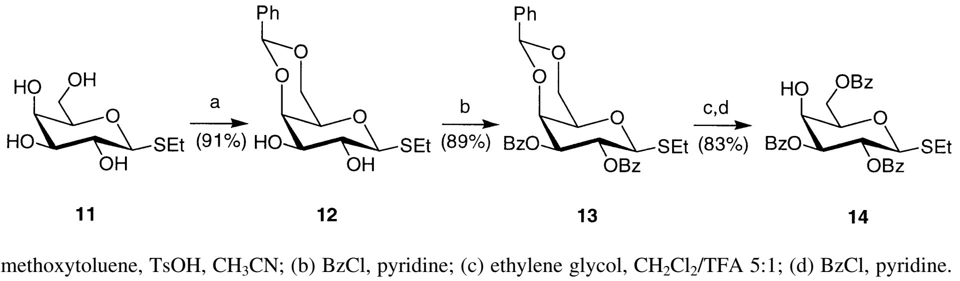
Synthesis of galactoglycerolipid **1**, in more than 20 steps, from acetobromogalactose and isopropylideneglycerol by an elaborative route has previously been published.[10] The glycerol residue was synthesized using various allyl ether protecting groups. In order to minimize the number of synthetic steps, we decided to explore the use of (*S*)-glycidol for construction of the glycerol moiety.

In the reported syntheses of glycoglycerolipids from glycosylglycidols,[6–8] the glycosylglycidols were synthesized by glycosylation of (*S*)-glycidol using mild conditions or by epoxidation of an allyl glycoside. The use of enantiomerically pure (*S*)-glycidol has the advantage over epoxidation procedures in that no diastereomers are formed. Consequently, there is no loss of valuable glycosyl donors and the purifications are simplified. Glycosylation of a deactivated acylated donor usually demands harsher conditions than the halide assisted glycosylation of glycidol, which has previously been published.[7] An obvious choice for a first attempt to glycosylate (*S*)-glycidol are acetylated donors. However, neither the thiogalactoside **4** or the corresponding bromo sugar **5** (Fig. 2) gave satisfactory results, mainly due to orthoester formation and decomposition. However, dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST)-promoted glycosylation with the benzoylated donor **6** (Scheme 1) gave the glycidyl galactoside **7** in excellent yield (86%). In order to enable the final deprotection, the benzoates were exchanged for benzyl ethers by treating **7** with methanolic sodium methoxide followed by benzylation to produce **8**.

Scandium trifluoromethanesulfonate has been shown to be an efficient Lewis acid catalyst for opening of glucosylglycidols with alcohols to produce glucoglycerolipids.[6] Opening of the epoxide in **8** with hexadecanol catalyzed by ScOTf gave **9** in 63% yield. However, when the more common catalyst boron trifluoride etherate was used, the yield was improved to 74%. Subsequent acylation with palmitic acid using standard procedures gave the protected galactoglycerolipid **10** in 93% yield. Hydrogenolytic



Scheme 1. (a) (*S*)-glycidol, DMTST, CH_2Cl_2; (b) NaOMe, MeOH; (c) BnBr, NaH, DMF; (d) $C_{16}H_{33}OH$, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (e) $C_{15}H_{31}COOH$, DCC, DMAP, CH_2Cl_2; (f) H_2, Pd/C, EtOAc/MeOH 1:1.



Scheme 2. (a) α,α-Dimethoxytoluene, TsOH, CH_3CN; (b) BzCl, pyridine; (c) ethylene glycol, CH_2Cl_2/TFA 5:1; (d) BzCl, pyridine.

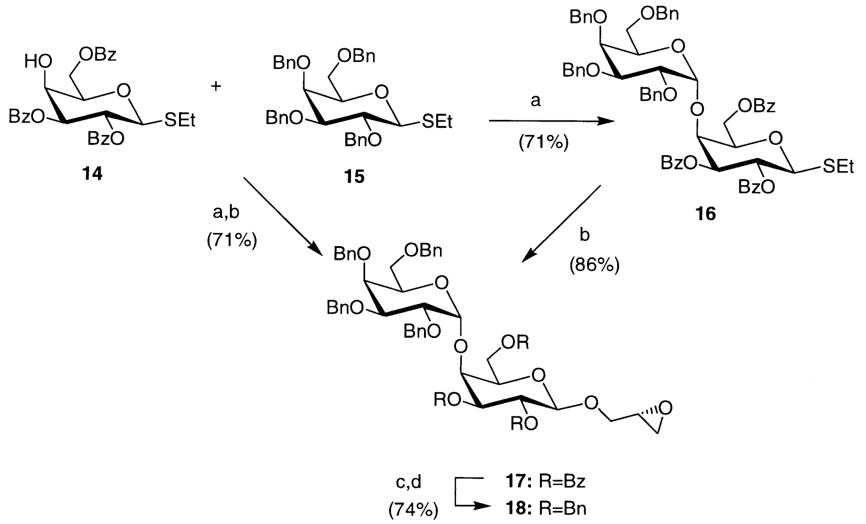
debenzylation gave the target compound **1** in quantitative yield.

The galactoglycerolipids **2a** and **2b** were synthesized analogously from the ethylthio digalactosyl donor **16**.[11] The galactoside **11** was converted to the benzylidene derivative **12** by treatment with benzaldehyde dimethyl acetal and TsOH (Scheme 2). Subsequent benzoylation produced **13**. Removal of the benzylidene acetal followed by regioselective benzoylation gave **14**.

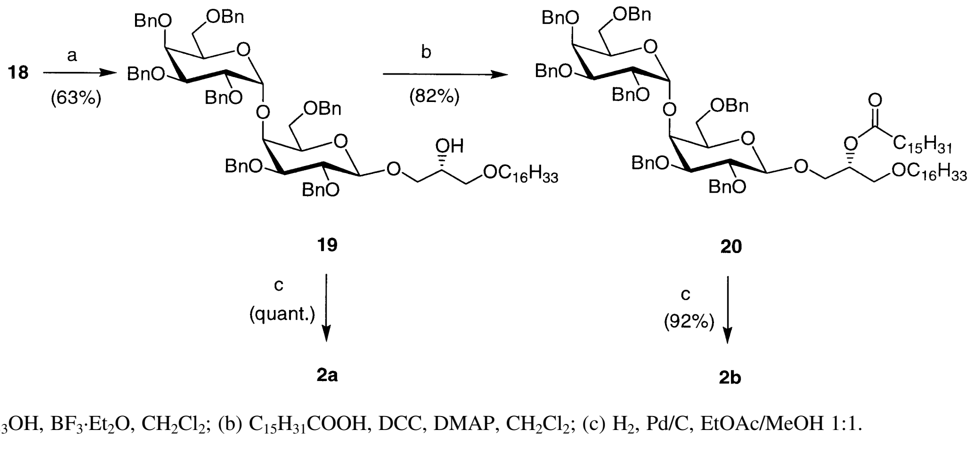
Glycosylation of acceptor **14** with tetra-*O*-benzyl-α-D-galactopyranosyl fluoride in 51% yield has been reported.[11] An alternative approach to the glycosylation of **14** is to take advantage of the 'armed–disarmed' strategy developed by Mootoo et al.[12] The original procedure, developed for 4-pentenylglycosides, has been successfully expanded to thioglycosides by others.[13,14] The more reactive 'armed' donor **15** can be selectively activated, in presence of the less reactive 'disarmed' acceptor **14**, to effect the coupling reaction without affecting the thioethyl group in **14** (Scheme 3). Thus, DMTST-promoted glycosylation in diethyl ether produced **16** in 71% yield. Subsequent glycosidation with (*S*)-glycidol gave **17** in 86% yield. In order to make the procedure even more efficient, a one-pot procedure was developed. **14** and **15** were coupled as before, then (*S*)-glycidol together with *sym*-collidine dissolved in dichloromethane were added to the reaction mixture, followed by addition of more DMTST to give **17** in 71% yield. Debenzoylation followed by benzylation produced **18** in 74% yield. Opening of the epoxide by the same procedure as for **8** using hexadecanol and boron trifluoride etherate gave **19** in 63% yield (Scheme 4). Hydrogenolytic debenzylation or palmitoylation followed by debenzylation afforded the target galactoglycerolipids **2a** and **2b**.

The glycerolipid **3** was to be tritium labeled for incorporation studies. The most convenient method for tritiation is oxidation of an alcohol followed by reduction of the resulting aldehyde using a tritiated borohydride. Synthesis of **3**, starting from isopropylideneglycerol has been reported.[15] However, a shorter route starting from (*S*)-glycidol is available (Scheme 5). Benzylation of (*S*)-glycidol,[16] followed by epoxide opening with hexadecanol produced **22**. Subsequent palmitoylation, followed by debenzylation gave **3** in 46% overall yield from (*S*)-glycidol.

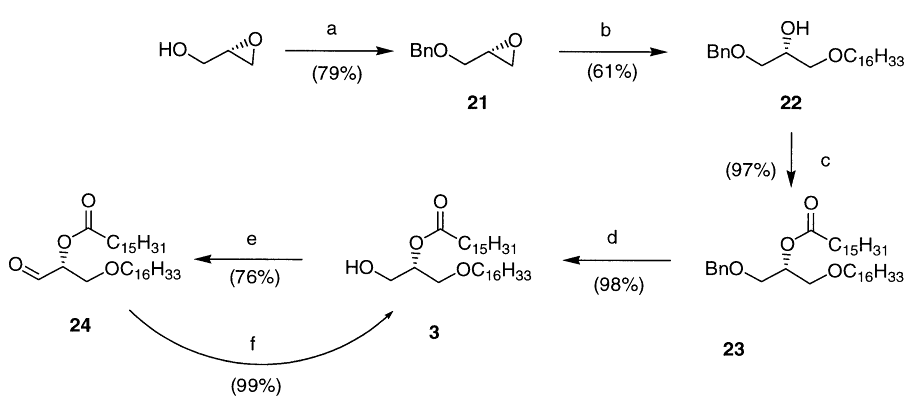
The most common procedures for oxidation of primary alcohols to aldehydes were evaluated. Attempts to oxidize **3** using Swern conditions were disappointing and the only



Scheme 3. (a) DMTST, ether; (b) (*S*)-glycidol, DMTST, CH_2Cl_2; (c) NaOMe, MeOH; (d) BnBr, NaH, DMF.



Scheme 4. (a) $C_{16}H_{33}OH$, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (b) $C_{15}H_{31}COOH$, DCC, DMAP, CH_2Cl_2; (c) H_2, Pd/C, EtOAc/MeOH 1:1.



Scheme 5. (a) BnBr, NaH, DMF; (b) $C_{16}H_{33}OH$, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (c) $C_{15}H_{31}COOH$, DCC, DMAP, CH_2Cl_2; (d) H_2, Pd/C, EtOH; (e) Dess–Martin periodinane, CH_2Cl_2; (f) $NaBH_3CN$, 2-propanol/HOAc.

product isolated was the primary halide corresponding to **3**. The Parikh–Doering procedure using DMSO activated with sulfur trioxide pyridine complex gave **24** in satisfactory 62% yield. However, the product was found to be completely racemized. Treatment of **3** with PCC in dichloromethane for 48 h produced the aldehyde **24** in 30% yield. Because of the long reaction time, messy workup and low yield in the Cr(VI) oxidation, the procedure using Dess–Martin periodinane for the oxidation was attempted. The periodinane rapidly oxidized the alcohol, to give **24** in 76% yield, without loss of enantiomeric purity.

Methods for reduction of the aldehyde using tritiated borohydride reagents were evaluated with non-tritiated reagents. Reduction, using standard conditions, with sodium borohydride in methanol gave mainly the 3-*O*-acyl derivative as a result of extensive acyl migration. Evidently, the aldehyde had to be reduced under non-basic conditions. Reduction using sodium cyanoborohydride in 2-propanol/acetic acid produced the target glycerolipid **3** containing only traces of the acyl migration product. The optical rotation of the reduction product showed that no loss of enantiomeric purity had occurred.

3. Conclusions

An efficient strategy for synthesis of glycoglycerolipids, using the the commercially available three-carbon synthon (*S*)-glycidol for construction of the glycerol moiety, has been explored. Synthesis of three galactoglycerolipids was accomplished using the strategy. (*S*)-Glycidol was glycosylated with readily available galactosyl donors to produce galactosyl and digalactosyl glycidols. Armed and disarmed galactosyl donors were successfully coupled to (*S*)-glycidol in a one-pot procedure to produce a digalactosyl glycidol derivative. Lewis acid catalyzed opening of the epoxides and subsequent deprotection or palmitoylation followed by deprotection produced the galactosyl and digalactosyl glycerolipids. Variation of the strategy provides a generally applicable route for synthesis of various glycoglycerolipids. Moreover, the ^1H, ^1H COSY of **2a** is consistent with the spectrum for the natural compound,[3] and thus provides strong support for the assignment of this galactolipid. The glycerolipid 1-*O*-hexadecyl-2-*O*-palmitoyl-*sn*-glycerol was synthesized by the same strategy, and an efficient oxidation–reduction procedure for tritiation was developed.

4. Experimental

4.1. General methods

Organic phases were dried over $MgSO_4$, filtered and concentrated in vacuo below 40°C. TLC: 0.25 mm precoated silica-gel plates (MERCK silica-gel $60F_{254}$); detection by spraying the plates with AMC-solution ($(NH_4)_2MoO_4$ 100 g and $Ce(IV)SO_4$ 2 g dissolved in 10% H_2SO_4 2 L) followed by heating at ~250°C. Optical rotations were recorded at room temperature with a Perkin–Elmer 241 polarimeter. Melting points were recorded with a Gallenkamp melting point apparatus. Flash Chromatography (FC): Silica gel MERCK 60 (0.040–0.063 mm). ^1H and ^{13}C NMR spectra were recorded on Varian Mercury 300 or Varian Inova 600 instruments at 300 MHz (^1H) and 75 MHz (^{13}C) or 600 MHz (^1H), respectively, at 25°C. Chemical shifts are given in ppm with TMS as internal standard (δ=0.00).

4.1.1. (*R*)-Glycidyl-2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside (7). To a stirred mixture of **6** (1.08 g, 1.69 mmol), (*S*)-glycidol (398 mg, 5.37 mmol), collidine (0.22 mL, 1.65 mmol) and 4 Å molecular sieves in dichloromethane (20 mL) under Ar was added DMTST (1.31 mg, 5.07 mmol). After 2 h, the reaction was quenched with NEt_3 (2.0 mL). The mixture was filtered through Celite and concentrated. FC (toluene/EtOAc 12:1→8:1) gave **7** (951 mg, 1.46 mmol, 86%) as colorless needles. R_f 0.48 (toluene/EtOAc 4:1); mp 108–109°C (from EtOAc/hexane); $[\alpha]_D$=+100 (*c* 1.1, $CHCl_3$); IR ν_{max} cm^{-1} 1069, 1094, 1258, 1450, 1600, 1721; ^1H NMR (300 MHz, $CDCl_3$), δ 8.11–7.21 (20H, m, Ph), 6.00 (1H, dd, *J*=3.6, 0.8 Hz, H-4′), 5.80 (1H, dd, *J*=10.4, 8.0 Hz, H-2′), 5.61 (1H, dd, *J*=10.4, 3.6 Hz, H-3′), 4.92 (1H, d, *J*=8.0 Hz, H-1′), 4.69 (1H, dd, *J*=11.0, 6.6 Hz, H-6a′), 4.43 (1H, dd, *J*=11.0, 6.4 Hz, H-6b′), 4.35 (1H, ddd, *J*=6.6, 6.4, 0.8 Hz, H-5′), 3.97 (1H, dd, *J*=12.4, 3.3 Hz, H-1a), 3.90 (1H, dd, *J*=12.4, 4.4 Hz, H-1b), 3.11–3.16 (1H, m, H-2), 2.62 (1H, dd, *J*=4.9, 4.1 Hz, H-3a), 2.57 (1H, dd, *J*=4.9, 2.7 Hz, H-3b); ^{13}C (75 MHz, CDCl$_3$), δ 166.0, 165.6, 165.5, 165.3, 133.6–133.3, 130.0–128.3, 101.8, 71.7, 71.5, 69.8, 69.2, 68.2, 62.1, 50.4, 44.1; Anal. calcd for $C_{37}H_{32}O_{11}$: C, 68.1; H, 4.9. Found: C, 68.0; H, 5.0.

4.1.2. (*R*)-Glycidyl-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (8). To a stirred solution of **7** (266 mg, 0.408 mmol) in MeOH (20 mL) was added NaOMe (40 mg, 0.74 mmol). After 4 h, DOWEX-NH$_4^+$ was added, and after an additional 10 min the mixture was filtered and concentrated. The residue was dissolved in DMF (5 mL). To the stirred solution was added NaH (60%, 130 mg, 3.25 mmol) and benzyl bromide (0.38 mL, 3.2 mmol). After 1 h, MeOH (0.2 mL) was added, the mixture diluted with toluene and washed with water. The organic layer was dried filtered and concentrated. FC (toluene/EtOAc 10:1, 0.1% NEt$_3$) gave **8** (222 mg, 0.372 mmol, 91%) as a white solid. R_f 0.50 (toluene/EtOAc 4:1); mp 96–97°C (from EtOAc/hexane); $[\alpha]_D$=−7.0 (c 0.9, CHCl$_3$); IR ν_{max} cm^{-1} 1047, 1117, 1452; NMR: ^{1}H (300 MHz, CDCl$_3$), δ 7.25–7.39 (20H, m, Ph), 4.93 (1H, d, J=11.5 Hz, CH_2Ph), 4.90 (1H, d, J=11.0 Hz, CH_2Ph), 4.75 (2H, d, J=11.0 Hz, CH_2Ph), 4.70 (1H, d, J=11.8 Hz, CH_2Ph), 4.61 (1H, d, J=11.8 Hz, CH_2Ph), 4.45 (1H, d, J=11.8 Hz, CH_2Ph), 4.40 (1H, d, J=11.8 Hz, CH_2Ph), 4.39 (1H, d, J=7.7 Hz, H-1′), 3.94 (1H, dd, J=11.5, 4.7 Hz, H-1a), 3.88 (1H, d, J=2.7 Hz, H-4′), 3.82 (1H, dd, J=9.9, 7.7 Hz, H-2′), 3.75 (1H, dd, J=11.5, 4.7 Hz, H-1b), 3.59–3.49 (4H, m, H-3′,5′,6a′,6b′), 3.20–3.15 (1H, m, H-2), 2.77 (1H, dd, J=5.1, 4.0 Hz, H-3a), 2.69 (1H, dd, J=5.1, 2.7 Hz, H-3b); ^{13}C (75 MHz, CDCl$_3$), δ 138.7, 138.6, 138.5, 137.9, 128.5–127.6, 104.1, 82.1, 79.5, 75.3, 74.6, 73.6, 73.5, 73.4, 73.1, 69.5, 68.8, 50.4, 44.7; Anal. calcd for $C_{37}H_{40}O_7$: C, 74.5; H, 6.8. Found: C, 74.3; H, 6.7.

4.1.3. 1-*O*-Hexadecyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-*sn*-glycerol (9). To a stirred solution of **8** (105 mg, 0.176 mmol) and hexadecanol (85 mg, 0.35 mmol) in CH$_2$Cl$_2$ (3.0 mL) was added BF$_3$·Et$_2$O (0.040 mL, 10% in CH$_2$Cl$_2$). After 15 h, the mixture was diluted with CH$_2$Cl$_2$ and washed with aqueous NaHCO$_3$ (sat.). The organic layer was dried, filtered and concentrated. FC (petroleum ether 65–75/EtOAc 5:1→3:1) gave **9** (109 mg, 0.130 mmol, 74%) as a white solid. R_f 0.37 (petroleum ether/EtOAc) 3:1; $[\alpha]_D$=−7.8 (c 1.0, CHCl$_3$); mp 43–44°C (from EtOH); IR ν_{max} cm^{-1} 1053, 1414, 1453, 2850, 2920, 3453; NMR: ^{1}H (300 MHz, CDCl$_3$), δ 7.34–7.28 (20H, m, Ph), 4.92 (1H, d, J=11.8 Hz, CH_2Ph), 4.88 (1H, d, J=11.0 Hz, CH_2Ph), 4.78 (1H, d, J=11.0 Hz, CH_2Ph), 4.74 (1H, d, J=12.1 Hz, CH_2Ph), 4.69 (1H, d, J=11.8 Hz, CH_2Ph), 4.60 (1H, d, J=11.8 Hz, CH_2Ph), 4.45 (1H, d, J=11.6 Hz, CH_2Ph), 4,39 (1H, d, J=11.5 Hz, CH_2Ph), 4.36 (1H, d, J=7.7 Hz, H-1′), 3.99–3.92 (1H, m, H-2) 3.89–3.84 (2H, m, H-4′, 1a), 3.83 (1H, dd, J=9.6, 7.7 Hz, H-2′), 3.75 (1H, dd, J=11.1, 6.7 Hz, H-1b) 3.59–3.38 (8H, m, H-3′, 5′, 6a′, 6b′, 3a, 3b, CH$_2$CH_2O), 1.56–1.52 (2H, m), 1.35–1.20 (26H, br), 0.88 (3H, t, J=7.2 Hz); ^{13}C (75 MHz, CDCl$_3$), δ 138.6, 138.5, 138.4, 137.8, 128.4–127.6, 104.8, 82.3, 79.4, 75.3, 74.6, 73.6, 73.5, 73.4, 73.3, 73.1, 71.7, 71.6, 69.7, 68.8, 31.9, 29.7–29.6, 29.5, 29.4, 26.1, 22.7, 14.1; Anal. calcd for $C_{53}H_{74}O_8$: C, 75.9; H, 8.9. Found: C, 76.0; H, 8.7.

4.1.4. 1-*O*-Hexadecyl-2-*O*-palmitoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-*sn*-glycerol (10). A mixture of **9** (55 mg, 0.066 mmol), palmitic acid (34 mg, 0.13 mmol), dicyclohexylcarbodiimide (27 mg, 0.13 mmol) and 4-(dimethylamino)pyridine (2 mg, 0.016 mmol) in dichloromethane (1 mL) was stirred for 24 h, filtered through Celite and concentrated. FC (petroleum ether 65–75/EtOAc 20:1→10:1) gave **10** (66 mg, 0.61 mmol, 93%) as a white solid. R_f 0.35 (petroleum ether/EtOAc 4:1); $[\alpha]_D$=+0.6 (c 0.9, CHCl$_3$); mp 16–17°C (from EtOH); IR ν_{max} cm^{-1} 1112, 1454, 1738, 2853, 2924; NMR: ^{1}H (300 MHz, CDCl$_3$), δ 7.38–7.22 (20H, m, Ph), 5.21–5.14 (1H, m, H-2), 4.92 (1H, d, J=11.8 Hz, CH_2Ph), 4.91 (1H, d, J=11.0 Hz, CH_2Ph), 4.75 (1H, d, J=11.8 Hz, CH_2Ph), 4.73 (1H, d, J=11.0 Hz, CH_2Ph), 4.69 (1H, d, J=11.8 Hz, CH_2Ph), 4.61 (1H, d, J=11.5 Hz, CH_2Ph), 4.45 (1H, d, J=11.8 Hz, CH_2Ph), 4.40 (1H, d, J=11.8 Hz, CH_2Ph), 4.33 (1H, d, J=7.7 Hz, H-1′), 4.00 (1H, dd, J=10.7, 4.7 Hz, H-1a), 3.88 (1H, d, J=2.7 Hz, H-4′), 3.80 (1H, dd, J=9.6, 7.7 Hz, H-2′), 3.69 (1H, dd, J=10.7, 4.8 Hz, H-1b), 3.64–3.31 (8H, m, H-3′, 5′, 6a′, 6b′, 3a, 3b, CH$_2$CH_2O), 2.28–2.23 (2H, m), 1.62–1.46 (4H, m), 1.35–1.19 (50H, br), 0.88 (6H, t, J=6.7 Hz); ^{13}C (75 MHz, CDCl$_3$), δ 173.3, 138.8, 138.6, 138.5, 137.9, 128.4–127.5, 104.3, 82.1, 79.3, 75.0, 74.6, 73.6 (2C), 73.5, 73.1, 71.6, 71.3, 69.4, 68.7, 68.3, 34.4, 31.9, 29.8–29.6, 29.5, 29.4, 29.3, 29.1, 26.1, 25.0, 22.7, 14.1; Anal. calcd for $C_{69}H_{104}O_9$: C, 76.9; H, 9.7. Found: C, 76.8; H, 9.9.

4.1.5. 3-*O*-(β-D-Galactopyranosyl)-1-*O*-hexadecyl-2-*O*-palmitoyl-*sn*-glycerol (1). A mixture of **10** (30 mg, 0.028 mmol) and 10% Pd on carbon (2 mg) in EtOAc/MeOH 1:1 (2 mL) was stirred under H$_2$ (1 atm) for 24 h, filtered through Celite and concentrated to yield **1** (20 mg, 0.028 mmol, 100%) as a white solid. R_f 0.52 (CHCl$_3$/MeOH 5:1); $[\alpha]_D$=−3.8 (c 1.2, CHCl$_3$/MeOH 1:1), lit.[10] $[\alpha]_D$=−4.2 (c 0.5, CHCl$_3$/MeOH 1:1); mp 140–142°C (from MeOH), lit.[10] 139–141°C (from MeOH); NMR: ^{1}H (600 MHz, CDCl$_3$/CD$_3$OD 5:1), δ 5.22–5.19 (1H, m, H-2), 4.23 (1H, d, J=7.3 Hz, H-1′), 4.94 (1H, dd, J=11.0, 5.9 Hz, H-1a), 3.90 (1H, d, J=3.3 Hz, H-4′), 3.84 (1H, dd, J=11.7, 6.6 Hz, H-6a′), 3.77–3.73 (2H, m, H-1b, 6b′), 3.61 (1H, dd, J=11.0, 6.6 Hz, H-3a), 3.59 (1H, dd, J=11.0, 5.5 Hz, H-3b), 3.55 (1H, dd, J=9.5, 7.3 Hz, H-2′), 3.52–3.49 (2H, m, H-3′, 5′), 3.48 (1H, ddd, J=9.5, 7.0, 7.0 Hz, CH$_2$CH_2O), 3.43 (1H, ddd, J=9.5, 7.0, 7.0 Hz, CH$_2$CH_2O) 2.34 (2H, t, J=7.5 Hz), 1.64–1.59 (2H, m), 1.57–1.53 (2H, m), 1.33–1.23 (50H, br), 0.88 (6H, t, J=7.0 Hz); ^{13}C (75 MHz, CDCl$_3$/MeOH 5:1), δ 174.3, 104.1, 75.1, 73.5, 72.0, 71.8, 71.4, 69.5, 69.0, 68.7, 61.7, 32.1, 29.9–29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 26.2, 25.1, 22.8, 14.2.

4.1.6. Ethyl 2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (14). Thiogalactoside **11** (1.00 g, 4.46 mmol), α,α-dimethoxytoluene (1.00 mL, 6.66 mmol) and TsOH·H$_2$O (25 mg) in CH$_3$CN (20 mL) were stirred at room temperature for 1.5 h. NEt$_3$ (1.0 mL) was added and the mixture concentrated. FC (EtOAc/MeOH 10:1) gave **12** (1.27 g, 4.06 mmol, 91%) as fine needles. R_f 0.19 (toluene/EtOAc 1:3); $[\alpha]_D$=−63.3 (c 1.0, CHCl$_3$), lit.[17] $[\alpha]_D$=−60.6 (c 0.7, CHCl$_3$); mp 158–159°C (from EtOAc), lit.[17] 157.6–158.6°C; ^{1}H and ^{13}C NMR spectra were in accordance with those previously published.[17]

To a stirred solution of **12** (859 mg, 2.75 mmol) in pyridine (10 mL) was added benzoyl chloride (0.8 mL, 6.88 mmol). After 1 h, the mixture was concentrated. The residue was dissolved in toluene and washed with 1 M H_2SO_4, dried filtered and concentrated. FC (toluene/EtOAc 8:1→5:1) gave **13** (1.27 g, 2.44 mmol, 89%) as fine needles. R_f 0.39 (toluene/EtOAc 4:1); $[\alpha]_D=+107.2$ (*c* 1.0, $CHCl_3$), lit.[18] $[\alpha]_D=+99.5$ (*c* 4.0, $CHCl_3$); mp 176–178°C (from EtOAc/hexane), lit.[18] 148–150°C (from EtOH); NMR: ^1H (300 MHz, $CDCl_3$), δ 7.99–7.95 (4H, m, Ph), 7.52–7.36 (11H, m, Ph), 5.96 (1H, t, *J*=10.0 Hz, H-2), 5.53 (1H, s, PhC*H*) 5.40 (1H, dd, *J*=10.0, 3.4 Hz, H-3), 4.73 (1H, d, *J*=9.6 Hz, H-1), 4.62 (1H, d, *J*=3.4 Hz, H-4), 4.41 (1H, dd, *J*=12.4, 1.4 Hz, H-6a), 4.08 (1H, dd, *J*=12.4, 1.5 Hz, H-6b), 3.71 (1H, br, H-5), 2.94 (1H, dq, *J*=12.4, 7.5 Hz, CH_3CH_2), 2.79 (1H, dq, *J*=12.4, 7.5 Hz, CH_3CH_2), 1.30 (1H, t, *J*=7.5 Hz, CH_3CH_2); ^{13}C (75 MHz, $CDCl_3$), δ 166.2, 165.4, 137.6, 133.4, 133.1, 129.9–128.2, 126.3, 101.0, 83.0, 73.9, 73.8, 70.0, 69.2, 67.3, 23.0, 14.9; The ^1H NMR spectrum in benzene was in accordance with that previously published.[18]

Compound **13** (1.06 g, 2.04 mmol) and ethylene glycol (3.0 mL) were stirred in CH_2Cl_2/TFA 5:1 (24 mL). After 1 h, the mixture was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ (sat.), dried, filtered and concentrated. The residue was dissolved in pyridine (7 mL) and benzoyl chloride (0.31 mL, 2.67 mmol) added to the stirred solution. After 30 min, the mixture was diluted with toluene and washed with 2 M H_2SO_4 and aqueous $NaHCO_3$ (sat.), dried filtered and concentrated. FC (toluene/EtOAc 20:1→6:1) gave **14** (909 mg, 1.69 mmol, 83%) as a white solid. $[\alpha]_D=+59.7$ (*c* 1.3, $CHCl_3$), lit.[11] $[\alpha]_D=+59.4$ (*c* 1.02, $CHCl_3$); mp 147–148°C (from EtOAc/hexane), lit.[11] 121–122°C; ^1H and ^{13}C NMR spectra were in accordance with those previously published.[11,19]

4.1.7. Ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (16). To a stirred mixture of **14** (102 mg, 0.190 mmol), **15** (262 mg 0.448 mmol) and 4 Å powdered molecular sieves in diethyl ether (10 mL) under Ar, was added DMTST (110 mg, 0.426 mmol). After 3 h, was added NEt_3 (0.2 mL), The mixture was filtered through Celite and concentrated. FC (petroleum ether 65–75/EtOAc 9:1) gave **16** (142 mg, 0.134 mmol, 71%) as a white solid. R_f 0.77 (toluene/EtOAc 4:1); $[\alpha]_D=+68.4$ (*c* 1.3, $CHCl_3$), lit.[11] $[\alpha]_D=+61.5$ (*c* 0.72, $CHCl_3$); ^1H and ^{13}C NMR spectra were in accordance with those previously published.[11]

4.1.8. (*R*)-Glycidyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (17). *Procedure A*. To a stirred mixture of **16** (79 mg, 0.075 mmol), (*S*)-glycidol (34 mg, 0.46 mmol) and collidine (10 μL, 0.075 mmol) in dichloromethane under Ar, was added DMTST (58 mg, 0.50 mmol). After 1.5 h, NEt_3 (0.1 mL) was added and the mixture filtered through Celite. FC (toluene/EtOAc, 0.1% NEt_3 gradient 20:1→10:1) gave **17** (69 mg, 0.064 mmol, 86%) as a colorless syrup. R_f 0.81 (toluene/EtOAc 4:1); $[\alpha]_D=+57.5$ (*c* 0.8, $CHCl_3$); IR ν_{max} cm^{-1} 1097, 1271, 1452, 1725, 2925; NMR: ^1H (600 MHz, $CDCl_3$), δ 8.02–7.91 (6H, m, Ph), 7.59–7.12 (29H, m, Ph), 5.76 (1H, dd, *J*=10.5, 5.9 Hz, H-2′), 5.22 (1H, dd, *J*=10.5, 3.0 Hz, H-3′), 4.92 (1H, d, *J*=3.4 Hz, H-1″), 4.86–4.81 (4H, m, CH_2Ph), 4.79–4.73 (3H, m, H-1′, 6a′, 6b′), 4.70 (1H, d, *J*=11.6 Hz, CH_2Ph), 4.47 (1H, d, *J*=11.6 Hz, CH_2Ph), 4.41 (1H, d, *J*=3.0 Hz, H-4′), 4.34 (1H, dd, *J*=9.7, 5.0 Hz, H-5″), 4.18 (1H, dd, *J*=10.5, 2.6 Hz, H-3″), 4.11 (1H, br, H-4″), 4.08–4.05 (2H, m, H-5′, 2″), 4.05 (1H, d, *J*=12.0 Hz, CH_2Ph), 4.02 (1H, d, *J*=12.0 Hz, CH_2Ph), 3.91 (1H, dd, *J*=12.3, 4.5 Hz, H-1a), 3.87 (1H, dd, *J*=12.3, 3.4 Hz, H-1b), 3.37 (1H, dd, *J*=9.7, 8.4 Hz, H-6a″), 3.12–3.10 (1H, m, H-2), 2.91 (1H, dd, *J*=8.4, 5.0 Hz, H-6b″), 2.61 (1H, dd, *J*=5.2, 4.1 Hz, H-3a), 2.56 (1H, dd, *J*=5.2, 2.6 Hz, H-3b); ^{13}C (75 MHz, $CDCl_3$), δ 166.4, 166.1, 165.4, 138.8–139.0, 133.2, 133.2, 133.1, 127.3–129.9, 101.5, 101.1, 79.0, 76.0, 75.6, 75.0, 74.8, 74.1, 74.0, 72.9 (2C), 72.5, 69.8, 69.6, 68.7, 67.5, 62.5, 50.4, 44.3; Anal. calcd for $C_{64}H_{62}O_{15}$: C, 71.8; H, 5.8. Found: C, 71.9; H, 6.0.

Procedure B. To a stirred mixture of **14** (100 mg, 0.186 mmol), **15** (240 mg, 0.410 mmol) and 4 Å powdered molecular sieves in diethyl ether (10 mL) under Ar, was added DMTST (105 mg, 0.41 mmol). After 2 h, (*S*)-glycidol (72 mg, 0.97 mmol) and collidine (40 μL, 0.30 mmol) in dichloromethane (10 mL) were added, followed by addition of DMTST (92 mg, 0.33 mmol). After an additional 1 h, NEt_3 (0.2 mL) was added and the mixture filtered through Celite. FC (toluene/EtOAc, 0.1% NEt_3 gradient 20:1→10:1) gave **17** (142 mg, 0.133 mmol, 71%). All data were identical to those from procedure A.

4.1.9. (*R*)-Glycidyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (18). To a stirred solution of **17** (87 mg, 0.081 mmol) in MeOH (10 mL) was added NaOMe (20 mg). After 21 h, the mixture was neutralized with DOWEX-NH_4^+, filtered and concentrated. The residue was dissolved in DMF (3 mL), NaH (60%, 20 mg, 0.50 mmol) and benzyl bromide (0.060 mL, 0.50 mmol) were added to the stirred mixture. After 1 h, MeOH (0.05 mL) was added, the mixture diluted with toluene and washed with water. The organic layer was dried filtered and concentrated. FC (toluene/EtOAc 12:1, 0.1% NEt_3) gave **18** (62 mg, 0.060 mmol, 74%) as a colorless syrup. R_f 0.18 (petroleum ether 65–75/EtOAc 3:1); $[\alpha]_D=+48.0$ (*c* 0.9, $CHCl_3$); IR ν_{max} cm^{-1} 1099, 1454, 2870; NMR: ^1H (600 MHz, $CDCl_3$), δ 7.40–7.13 (35H, m, Ph), 5.03 (1H, d, *J*=3.0 Hz, H-1″), 4.91 (1H, d, *J*=11.2 Hz, CH_2Ph), 4.89 (2H, d, *J*=11.6 Hz, CH_2Ph), 4.79 (1H, d, *J*=12.7 Hz, CH_2Ph), 4.79–4.75 (3H, m, CH_2Ph), 4.68 (1H, d, *J*=11.6 Hz, CH_2Ph), 4.55 (1H, d, *J*=11.2 Hz, CH_2Ph), 4.53 (1H, d, *J*=12.3 Hz, CH_2Ph), 4.43 (1H, dd, *J*=9.0, 4.7 Hz, H-5″), 4.37 (1H, d, *J*=7.5 Hz, H-1′), 4.25 (1H, d, *J*=12.0 Hz, CH_2Ph), 4.20 (1H, d, *J*=12.0 Hz, CH_2Ph), 4.15 (1H, d, *J*=11.6 Hz, CH_2Ph), 4.13 (1H, d, *J*=11.6 Hz, CH_2Ph), 4.12–4.07 (3H, m, H-2″, 3″, 4″), 4.02 (1H, d, *J*=3.0 Hz, H-4′), 3.96 (1H, dd, *J*=9.3, 7.5 Hz, H-6a′), 3.88 (1H, dd, *J*=11.8, 5.0 Hz, H-1a), 3.78 (1H, dd, *J*=11.8, 3.6 Hz, H-1b), 3.68 (1H, dd, *J*=9.9, 7.5 Hz, H-2′), 3.55 (1H, dd, *J*=9.0, 8.4 Hz, H-6a″), 3.52 (1H, dd, *J*=9.3, 6.0 Hz, H-6b′), 3.47 (1H, dd, *J*=7.5, 6.0 Hz, H-5′), 3.38 (1H, dd, *J*=9.9, 3.0 Hz, H-3′), 3.25 (1H, dd, *J*=8.4, 4.7 Hz, H-6b″), 3.21–3.18 (1H, m, H-2), 2.78 (1H, dd, *J*=5.2, 4.1 Hz, H-3a), 2.68 (1H, dd, *J*=5.2, 2.6 Hz, H-3b); ^{13}C (75 MHz, $CDCl_3$), δ 139.0–138.1, 128.4–127.3, 104.0, 100.4, 80.8, 79.1, 78.8, 76.6, 75.1, 74.9, 74.8, 74.6, 73.8, 73.7, 73.2, 73.0, 72.4, 72.3, 69.7, 69.3, 68.0 (2C), 50.5, 44.7; Anal. calcd for $C_{64}H_{68}O_{12}$: C, 74.7; H, 6.7. Found: C, 74.5; H, 6.5.

4.1.10. 1-*O*-Hexadecyl-3-*O*-[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl]-*sn*-glycerol (19). To a stirred solution of **18** (85 mg, 0.083 mmol) and hexadecanol (40 mg, 0.16 mmol) in dichloromethane (1.5 mL) was added $BF_3{\cdot}Et_2O$ (0.020 mL, 10% in dichloromethane). After 19 h, the mixture was diluted with CH_2Cl_2 and washed with aqueous $NaHCO_3$ (sat.). The organic layer was dried, filtered and concentrated. FC (petroleum ether 65–75/EtOAc 10:1→2:1) gave **19** (66 mg, 0.052 mmol, 63%) as a colorless syrup. R_f 0.29 (petroleum ether 650–75/EtOAc 3:1); $[\alpha]_D=+37.1$ (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 1099, 1454, 2854, 1924; NMR: ^1H (600 MHz, $CDCl_3$), δ 7.39–7.16 (35H, m), 5.00 (1H, d, *J*=3.0 Hz, H-1″), 4.91 (1H, d, *J*=11.2 Hz, C*H*$_2$Ph), 4.88 (1H, d, *J*=12.0 Hz, C*H*$_2$Ph), 4.85 (1H, d, *J*=10.8 Hz, C*H*$_2$Ph), 4.80 (1H, d, *J*=11.2 Hz, C*H*$_2$Ph), 4.79–4.75 (3H, m, C*H*$_2$Ph), 4.66 (1H, d, *J*=11.6 Hz, C*H*$_2$Ph), 4.55 (1H, d, *J*=11.2 Hz, C*H*$_2$Ph), 4.52 (1H, d, *J*=12.7 Hz, C*H*$_2$Ph), 4.41 (1H, dd, *J*=9.0, 4.7 Hz, H-5″), 4.35 (1H, d, *J*=7.5 Hz, H-1′), 4.21 (2H, s, C*H*$_2$Ph), 4.15 (1H, d, *J*=11.2 Hz, C*H*$_2$Ph), 4.13 (1H, d, *J*=11.2 Hz, C*H*$_2$Ph), 4.11–4.06 (3H, m, H-2″, 3″, 4″), 4.00 (1H, d, *J*=3.0 Hz, H-4′), 4.01–3.98 (1H, m, H-2), 3.91 (1H, dd, *J*=9.3, 7.1 Hz, H-6a′), 3.89 (1H, dd, *J*=11.2, 3.7 Hz, H-1a), 3.76 (1H, dd, *J*=11.2, 6.7 Hz, H-1b), 3.68 (1H, dd, *J*=10.1, 7.5 Hz, H-2′), 3.55 (1H, dd, *J*=9.0, 8.4 Hz, H-6a″), 3.54 (1H, dd, *J*=9.3, 6.0 Hz, H-6b′), 3.50 (1H, dd, *J*=7.1, 6.0 Hz, H-5′), 3.46 (1H, dd, *J*=9.7, 5.6 Hz, H-3a), 3.43 (1H, dd, *J*=9.7, 5.6 Hz, H-3b), 3.42–3.39 (2H, m, CH_2C*H*$_2$O), 3.38 (1H, dd, *J*=10.1, 3.0 Hz, H-3′), 3,25 (1H, dd, *J*=8.4, 4.7 Hz, H-6b″), 1.56–1.52 (2H, m), 1.31–1.21 (26H, br), 0.88 (3H, t, *J*=7.1 Hz); ^{13}C (75 MHz, $CDCl_3$), δ 139.0–138.0, 128.4–127.4, 104.6, 100.4, 80.9, 79.0, 78.9, 76.6, 75.2, 74.9, 74.8, 74.5, 73.8, 73.7, 73.4, 73.2, 73.1, 72.3 (2C), 71.7, 71.6, 69.7, 69.4, 68.0, 67.9, 31.9, 29.7–29.6, 29.5, 29.4, 26.1, 22.7, 14.1; Anal. calcd for $C_{80}H_{102}O_{13}$: C, 75.6; H, 8.1. Found: C, 75.5; H, 8.2.

4.1.11. 3-*O*-Hexadecyl-2-*O*-palmitoyl-3-*O*-[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl]-*sn*-glycerol (20). A solution of **19** (48 mg, 0.038 mmol), palmitic acid (19 mg, 0.074 mmol), dicyclohexylcarbodiimide (16 mg, 0.078 mmol) and 4-(dimethylamino)pyridine (1 mg, 0.01 mmol) in CH_2Cl_2 (1 mL) was stirred for 22 h, filtered through Celite and concentrated. FC (petroleum ether 65–75/EtOAc 10:1) gave **20** (47 mg, 0.031 mmol, 82%) as a colorless syrup. R_f 0.70 (petroleum ether 65–75/EtOAc 3:1); $[\alpha]_D=+31.8$ (*c* 0.8, $CHCl_3$); IR ν_{max} cm^{-1} 1100, 1454, 1733, 1739, 2853, 2921; NMR: ^1H (600 MHz, $CDCl_3$), δ 7.38–7.14 (35H, m), 5.21–5.18 (1H, m, H-2), 5.02 (1H, d, *J*=3.4 Hz, H-1″), 4.90 (1H, d, *J*=11.2 Hz, C*H*$_2$Ph), 4.89 (1H, d, *J*=11.2 Hz, C*H*$_2$Ph), 4.89 (1H, d, *J*=11.6 Hz, C*H*$_2$Ph), 4.78 (1H, d, *J*=12.7 Hz, C*H*$_2$Ph), 4.79–4.75 (3H, m, C*H*$_2$Ph), 4.69 (1H, d, *J*=12.0 Hz, C*H*$_2$Ph), 4.54 (1H, d, *J*=11.2 Hz, C*H*$_2$Ph), 4.53 (1H, d, *J*=12.7 Hz, C*H*$_2$Ph), 4.41 (1H, dd, *J*=9.3, 4.9 Hz, H-5″), 4.31 (1H, d, *J*=7.5 Hz, H-1′), 4.26 (1H, d, *J*=11.6 Hz, C*H*$_2$Ph), 4.21 (1H, d, *J*=12.0 Hz, C*H*$_2$Ph), 4.14 (1H, d, *J*=11.6 Hz, C*H*$_2$Ph), 4.12 (1H, d, *J*=11.6 Hz, C*H*$_2$Ph), 4.10 (1H, dd, *J*=10.1, 3.4 Hz, H-2″), 4.09 (1H, d, *J*=2.2 Hz, H-4″), 4.07 (1H, dd, *J*=10.1, 2.2 Hz, H-3″), 4.02 (1H, d, *J*=3.0 Hz, H-4′), 4.01 (1H, dd, *J*=10.8, 4.9 Hz, H-1a), 3.97 (1H, dd, *J*=9.3, 7.9 Hz, H-6a′), 3.71 (1H, dd, *J*=10.8, 5.2 Hz, H-1b), 3.65 (1H, dd, *J*=10.1, 7.5 Hz, H-2′), 3.62 (1H, dd, *J*=10.8, 6.0 Hz, H-3a), 3.60 (1H, dd, *J*=10.8, 4.5 Hz, H-3b), 3.54 (1H, dd, *J*=9.3, 8.6 Hz, H-6a″), 3.50 (1H, dd, *J*=9.3, 6.0 Hz, H-6b′), 3.45 (1H, dd, *J*=7.9, 6.0 Hz, H-5′), 3.41 (1H, ddd, *J*=9.3, 6.7, 6.7 Hz, CH_2C*H*$_2$O), 3.36 (1H, dd, *J*=10.1, 3.0 Hz, H-3′), 3.34 (1H, ddd, *J*=9.3, 6.7, 6.7 Hz, CH_2C*H*$_2$O), 3.23 (1H, dd, *J*=8.6, 4.9 Hz, H-6b″), 2.31–2.22 (2H, m), 1.60–1.55 (2H, m), 1.52–1.48 (2H, m), 1.32–1.21 (50H, br), 0.88 (6H, t, *J*=7.1 Hz); ^{13}C (75 MHz, $CDCl_3$), δ 173.3, 139.0–138.1, 128.4–127.3, 104.2, 100.4, 80.7, 79.0, 78.7, 76.5, 75.0, 74.9, 74.8, 74.5, 73.7, 73.6, 73.2, 73.0, 72.4, 72.3, 71.6, 71.3, 69.3 (2C), 68.2, 68.0, 67.8, 34.4, 31.9, 29.7–29.6, 29.5, 29.3, 29.1, 26.1, 25.0, 22.7, 14.1; Anal. calcd for $C_{96}H_{132}O_{14}$: C, 76.4; H, 8.8. Found: C, 76.4; H, 8.8.

4.1.12. 3-*O*-[α-D-Galactopyranosyl-(1→4)-β-D-galactopyranosyl]-1-*O*-hexadecyl-*sn*-glycerol (2a). A mixture of **19** (14 mg, 0.011 mmol) and 10% Pd on carbon (2 mg) in EtOAc/MeOH 1:1 (2 mL) was stirred under H_2 (1 atm) for 22 h, filtered through Celite and concentrated. FC ($CHCl_3$/MeOH 5:1) gave **2a** (7 mg, 0.011 mmol, 100%) as a white solid. R_f 0.05 ($CHCl_3$/MeOH 5:1); mp 210°C (dec.) (from MeOH/EtOAc); $[\alpha]_D=+35.3$ (*c* 0.7, $CHCl_3$/MeOH 5:1); IR ν_{max} cm^{-1} 1075, 1468, 1597, 2850, 2918, 3387; NMR: ^1H (600 MHz, $CDCl_3/CD_3OD$ 5:1), δ 5.01 (1H, d, *J*=3.7 Hz, H-1″), 4.29 (1H, d, *J*=7.0 Hz, H-1′), 4.17 (1H, dd, *J*=7.3, 4.4 Hz, H-5″), 4.01 (1H, dd, *J*=2.7, 1.3 Hz, H-4′), 3.96–3.93 (1H, m, H-2), 3.94 (1H, d, *J*=3.1 Hz, H-4″), 3.86 (1H, dd, *J*=11.0, 6.2 Hz, H-1a), 3.86–3.81 (3H, m, H-2″, 6a′, 6a″), 3.80 (1H, dd, *J*=7.1, 3.1 Hz, H-3″), 3.77 (1H, dd, *J*=11.4, 5.5 Hz, H-6b′), 3.74–3.71 (2H, m, H-1b, 6b″), 3.65–3.63 (1H, m, H-5′), 3.53 (1H, dd, *J*=10.1, 2.7 Hz, H-3′), 3.50 (1H, dd, *J*=10.1, 7.0 Hz, H-2′), 3.50–3.46 (2H, m, H-3a, 3b), 3.46 (2H, dd, *J*=6.8, 6.8 Hz, CH_2C*H*$_2$O), 1.59–1.55 (2H, m), 1.33–1.24 (26H, br), 0.88 (3H, t, *J*=7.0 Hz); ^{13}C (75 MHz, $CDCl_3/CD_3OD$ 5:1), δ 104.1, 101.8, 79.5, 74.3, 73.6, 72.1, 72.1, 72.0, 71.8 (2C), 70.1, 70.0, 69.5, 69.4, 62.2, 60.1, 32.1, 29.9–29.8, 29.7, 29.6, 29.5, 28.3, 26.2, 22.8, 14.2; Anal. calcd for $C_{31}H_{60}O_{13}$: C, 58.1; H, 9.4. Found: C, 57.9; H, 9.4.

4.1.13. 3-*O*-[α-D-Galactopyranosyl-(1→4)-β-D-galactopyranosyl]-1-*O*-hexadecyl-2-*O*-palmitoyl-*sn*-glycerol (2b). A mixture of **20** (26 mg, 0.017 mmol) and 10% Pd on carbon (4 mg) in EtOAc/MeOH 1:1 (2 mL) was stirred under H_2 (1 atm) for 24 h, filtered through Celite and concentrated. FC ($CHCl_3$/MeOH 10:1→5:1) gave **2b** (14 mg, 0.016 mmol, 92%) as a white solid. R_f 0.26 ($CHCl_3$/MeOH 5:1); mp 200–203°C (from EtOAc); $[\alpha]_D=+30.8$ (*c* 1.3, $CHCl_3$/MeOH 10:1); IR ν_{max} cm^{-1} 1075, 1470, 1733, 2849, 2916, 3344; NMR: ^1H (600 MHz, $CDCl_3/CD_3OD$ 5:1), δ 5.19–5.16 (1H, m, H-2), 5.01 (1H, d, *J*=3.7 Hz, H-1″), 4.26 (1H, d, *J*=7.7 Hz, H-1′), 4.17 (1H, dd, *J*=7.7, 4.7 Hz, H-5″), 4.02 (1H, d, *J*=3.1 Hz, H-4′), 3.96 (1H, dd, *J*=11.0, 5.9 Hz, H-1a), 3.94 (1H, d, *J*=3.1 Hz, H-4″), 3.85–3.79 (4H, m, H-2″, 3″, 6a′, 6a″), 3.77–3.70 (3H, m, H-1b, 6b′, 6b″), 3.64–3.62 (1H, m, H-5′), 3.62 (1H, dd, *J*=11.0, 5.1 Hz, H-3a), 3.60 (1H, dd, *J*=11.0, 3.3 Hz, H-3b), 3.53 (1H, dd, J=10.1, 3.1 Hz, H-3′), 3.48 (1H, ddd, J=9.5, 6.6, 6.6 Hz, $\mathrm{CH_2C}\mathit{H}_2\mathrm{O}$), 3.46 (1H, dd, J=10.1, 7.7 Hz, H-2′), 3.43 (1H, ddd, J=9.5, 6.6, 6.6 Hz, $\mathrm{CH_2C}\mathit{H}_2\mathrm{O}$), 2.36 (2H, m), 1.63–1.60 (2H, m), 1.57–1.53 (2H, m), 1.34–1.23 (50H, br), 0.88 (6H, t, J=7.1 Hz); ^{13}C (75 MHz, $\mathrm{CDCl_3/CD_3OD}$ 5:1), δ 174.2, 104.4, 101.7, 79.3, 74.4, 73.6, 72.0 (2C), 71.7 (2C), 70.1, 70.0, 69.6, 69.4, 69.0, 62.2, 60.0, 34.6, 32.1, 29.9–29.7, 29.5, 29.3, 26.2, 25.1, 22.8, 14.2; Anal. calcd for $\mathrm{C_{47}H_{90}O_{14}}$: C, 64.2; H, 10.3. Found: C, 64.0; H, 10.1.

4.1.14. 3-*O*-Hexadecyl-2-*O*-palmitoyl-D-glyceraldehyde (24). To a stirred solution of (*S*)-glycidol (365 mg, 4.93 mmol) in DMF (15 mL) was added NaH (60%, 275 mg, 6.88 mmol). After 5 min, benzyl bromide (0.82 mL, 6.9 mmol) was added. The resulting mixture was stirred for 1 h. MeOH (1 mL) was added and the mixture diluted with toluene, washed with water, dried filtered and concentrated. FC (toluene→toluene/EtOAc 4:1) gave **21** as a colorless oil (641 mg, 3.90 mmol, 79%). $[\alpha]_D$=+2.0 (c 5.9, $\mathrm{CHCl_3}$), lit.[20] $[\alpha]_D$=+1.79 (c 5.0, $\mathrm{CHCl_3}$); ^{1}H and ^{13}C NMR were in accordance with those previously published.[21] To a stirred solution of **21** (374 mg, 2.28 mmol) and hexadecanol (993 mg, 4.10 mmol) in dichloromethane (20 mL) was added $\mathrm{BF_3{\cdot}Et_2O}$ (0.20 mL, 10% in dichloromethane). After 20 h, the mixture was concentrated. FC (toluene→toluene/EtOAc 2:1) gave **22** (565 mg, 1.39 mmol, 61%) as a white solid. R_f 0.44 (toluene/EtOAc 4:1); mp 37–38°C from (MeOH), lit.[22] 36–38°C; $[\alpha]_D$=−1.1 (c 1.7, EtOH), lit.[22] $[\alpha]_D$=−0.84 (c 1.91, EtOH); ^{1}H NMR were in accordance with that previously published.[22] A solution of **22** (572 mg, 1.41 mmol), palmitic acid (738 mg, 2.88 mmol), dicyclohexylcarbodiimide (571 mg, 2.77 mmol) and 4-(dimethylamino)pyridine (36 mg, 0.29 mmol) in $\mathrm{CH_2Cl_2}$ (20 mL) was stirred for 17 h, filtered through Celite and concentrated. FC (petroleum ether 65–75/EtOAc 20:1) and recrystallization from EtOH gave **23** (879 mg, 1.36 mmol, 97%) as a white solid. R_f 0.57 (petroleum ether 65–75/EtOAc 5:1); mp 46–47°C; $[\alpha]_D$=+0.59 (c 3.0, $\mathrm{CHCl_3}$), lit.[15] $[\alpha]_D$=+0.6; ^{1}H NMR were in accordance with that previously published.[15] A stirred mixture of **23** (151 mg, 0.234 mmol) and 10% Pd on carbon (61 mg) in absolute ethanol (12 mL) was stirred under $\mathrm{H_2}$ (1 atm) for 30 min, diluted with hexane/EtOAc 1:1, filtered through Celite and concentrated. Crystallization of the residue from MeOH gave **3** (127 mg, 0.229 mmol, 98%) as a white solid. R_f 0.39 (petroleum ether 65–75/EtOAc 4:1); $[\alpha]_D$=−2.6 (c 2.1, $\mathrm{CHCl_3}$), lit.[15] $[\alpha]_D$=−1.2; mp 49–50°C (from MeOH); ^{1}H NMR were in accordance with that previously published.[15] To a stirred solution of **3** (100 mg, 0.180 mmol) in $\mathrm{CH_2Cl_2}$ (0.5 mL) was added Dess–Martin periodinane (88 mg, 0.21 mmol) dissolved in $\mathrm{CH_2Cl_2}$ (1 mL). After 45 min, the mixture was diluted with diethyl ether (10 mL) and a solution of $\mathrm{Na_2S_2O_3}$ (2.5 g) in aqueous $\mathrm{NaHCO_3}$ (sat.) was added. After 5 min, the mixture was diluted with diethyl ether (10 mL) and the phases separated. The ether layer was washed with aqueous $\mathrm{NaHCO_3}$ (sat.) and $\mathrm{H_2O}$, dried, filtered and concentrated. FC (petroleum ether 65–75/EtOAc 10:1) gave **24** (76 mg, 0.138 mmol, 76%) as a white solid. R_f 0.57 (petroleum ether 65–75/EtOAc 4:1); mp 52°C (from hexane); $[\alpha]_D$=+8.6 (c 1.5, $\mathrm{CHCl_3}$); IR ν_{max} cm^{-1} 1165, 1472, 1738, 1753, 2850, 2918; NMR: ^{1}H (300 MHz, $\mathrm{CDCl_3}$), δ 9.58 (1H, s), 5.16 (1H, dd, J=4.9, 3.6 Hz), 3.88 (1H, dd, J=11.0, 5.2 Hz), 3.77 (1H, dd, J=11.0, 3.6 Hz), 3.52–3.38 (2H, m), 2.52–2.41 (2H, m), 1.73–1.63 (2H, m), 1.57–1.50 (2H, m) 1.38–1.19 (50H, br), 0.88 (6H, t, J=6.7 Hz); ^{13}C (75 MHz, $\mathrm{CDCl_3}$), δ 197.5, 77.6, 72.1, 68.4, 33.9, 31.9, 29.8–29.6, 29.5, 29.4, 29.3, 29.1, 26.0, 24.9, 22.7, 14.1; Anal. calcd for $\mathrm{C_{35}H_{68}O_4}$: C, 76.0; H, 12.4. Found: C, 76.2; H, 12.3.

4.1.15. Reduction of the aldehyde 24. To a stirred solution of **24** (102 mg, 0.184 mmol) and sodium cyanoborohydride (7 mg, 0.11 mmol) in 2-propanol (12 mL) was added acetic acid (1.2 mL) and after 15 min, a second portion of acetic acid (0.6 mL) was added. After 17 h, sodium cyanoborohydride (30 mg, 0.48 mmol) was added. The mixture was stirred for 30 min, after which $\mathrm{H_2O}$ (40 mL) was added. The resulting precipitate was filtered off to give **3** (101 mg, 0.182 mmol, 99%). $[\alpha]_D$=−3.0 (c 1.5, $\mathrm{CHCl_3}$).

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