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Carbohydrate Research 341 (2006) 695–704
Carbohydrate RESEARCH

Syntheses of an α-D-Gal-(1→6)-β-D-Gal diglyceride, as lipase substrate

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Received 21 December 2005; received in revised form 16 January 2006; accepted 17 January 2006
Available online 3 February 2006

Abstract—Two different routes were explored to afford 3-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-1,2-di-*O*-dodecanoyl-*sn*-glycerol. In the first one, the key step was the glycosylation of the 3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol acceptor with 2-pyridyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside as the donor. In the second one, the key step was the coupling of 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-D-galactopyranosyl trichloroacetimidate donor with 1,2-*O*-isopropylidene-*sn*-glycerol. Even though the number of steps was the same in both pathways, the first one afforded a better overall yield (12.4%) than the second one (6.5%). This eight-step synthesis allowed the preparation of the expected glycolipid, which was used as substrate for recombinant GPLRP2 galactolipase using the monomolecular film technique.

Keywords: Digalactosyldiglyceride (DGDG); Glycosylation; Lipase

1. Introduction

The most abundant membrane lipids in plants are galactoglycerolipids, which constitute about 75% of the total membranes lipids in leaves.[1] Monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) are the major galactolipids found in higher plants where they represent up to 50% and 20% of the chloroplast lipids, respectively. In both families, a β-D-galactopyranosyl moiety is linked to a diacylglycerol; in DGDG, a second α-D-galactopyranosyl residue is linked at OH-6 of the first galactopyranosyl unit.

Ten years ago, Anderson et al.[2] demonstrated that the lipolysis of galactolipids was not restricted to herbivores. They reported that human pancreatic juice and duodenal contents were able to hydrolyze digalactosyl glycerolipids. Recently, we have identified pancreatic lipase-related protein 2 (PLRP2) as the enzyme from human pancreatic juice displaying the major galactolipase activity.[3] In order to investigate the biochemical properties and the substrate specificity of PLRP2, we synthesized previously two monogalactosyl diglycerides epimers at C-2 of the glycerol moiety.[3,4] Then, using the monomolecular film technique, we reported the activity of human and guinea pig PLRP2s on 1,2-di-*O*-dodecanoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol and its epimer.

The present paper is devoted to the chemical synthesis of a digalactosyl diacylglycerol, analogous with the monogalactosyl diacylglycerol described previously.[3]

2. Results and discussion

Digalactosyl glycerides with various chain lengths have been widely studied since they are easily obtained from natural sources. Among the recent papers dealing with

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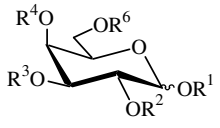
doi:10.1016/j.carres.2006.01.021

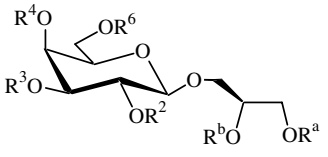
such compounds, the isolation of DGDG derivatives bearing poly-unsaturated lipid chains,[5–7] poly-unsaturated and saturated lipid chains[8,9] or saturated lipid chains only can be mentioned.[10] All these compounds have been fully characterized by ^{1}H, ^{13}C NMR and mass spectrometry. To our knowledge, there are only two examples of synthetic digalactosyl diglycerides described in the literature. The synthesis of 3-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-1,2-di-*O*-stearoyl-*sn*-glycerol by Gent and Gigg in 1975 was the first preparation of a digalactosyl diglyceride.[11,12] Very recently, Tanaka et al.[13] reported the synthesis of a series of the aforementioned compounds from a common precursor, in order to study the structure–inhibition relationship of the latter on human lanosterol synthase. The present paper deals with our own approaches to the synthesis of these biologically relevant derivatives.

Two different strategies were attempted toward the synthesis of compound **15** (Chart 1). In the first one, a monogalactosyl glycerol acceptor was synthesized and then reacted with an appropriate α-D-galactopyranosyl donor. In the second approach, the α-Gal(1→6)Gal disaccharide was prepared first, before coupling with the glycerol moiety.

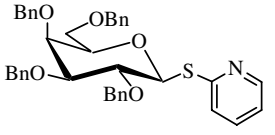
The first pathway involves the glycosylation of 1,2-*O*-isopropylidene-*sn*-glycerol with a galactopyranosyl donor able to induce the β-anomeric stereochemistry, without epimerization of the glycerol moiety. As reported previously,[3] the imidate methodology developed by Schmidt,[14] allowed to achieve this goal contrary to use galactosyl bromides, which give rise to epimerization of the glycerol moiety.[15] Therefore, a trichloroacetimidoyl glycosylation donor was prepared as follows. 1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (**1**)[16] was protected at OH-6 with a *p*-methoxyphenyl group via a Mitsunobu reaction, to afford the 6-*O*-*p*-methoxyphenyl ether **2**. After acetal cleavage in acidic conditions and acetylation, product **3** was obtained as an α,β-anomeric



1 R^1-R^2 = R^3-R^4 = $C(CH_3)_2$, R^6 = H
2 R^1-R^2 = R^3-R^4 = $C(CH_3)_2$, R^6 = $C_6H_4OCH_3$
3 R^1 = R^2 = R^3 = R^4 = Ac, R^6 = $C_6H_4OCH_3$
4 R^1= C(=NH)CCl_3, R^2 = R^3 = R^4 = Ac, R^6 = $C_6H_4OCH_3$



5 R^a-R^b = $C(CH_3)_2$, R^2 = R^3 = R^4 = Ac, R^6 = $C_6H_4OCH_3$
6 R^a-R^b = $C(CH_3)_2$, R^2 = R^3 = R^4 = Bn, R^6 = $C_6H_4OCH_3$
7 R^a-R^b = $C(CH_3)_2$, R^2 = R^3 = R^4 = Bn, R^6 = H
8 R^a-R^b = $C(CH_3)_2$, R^2 = R^3 = R^4 = R^6 = Ac
9 R^a-R^b = $C(CH_3)_2$, R^2 = R^3 = R^4 = H, R^6 = TBDMS
10 R^a-R^b = $C(CH_3)_2$, R^2 = R^3 = R^4 = Bn, R^6 = TBDMS



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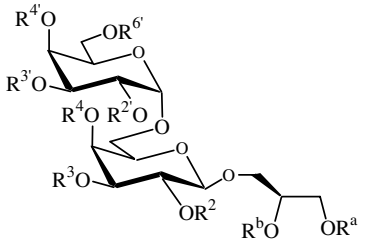
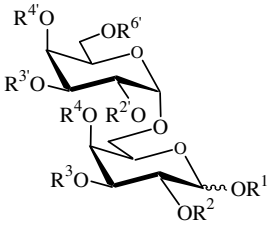


12 R^a-R^b = $C(CH_3)_2$, R^2 = R^3 = R^4 = $R^{2'}$ = $R^{3'}$ = $R^{4'}$ = $R^{6'}$= Bn
13 R^a = R^b = H, R^2 = R^3 = R^4 = $R^{2'}$ = $R^{3'}$ = $R^{4'}$ = $R^{6'}$= Bn
14 R^a = R^b = $COC_{11}H_{23}$, R^2 = R^3 = R^4 = $R^{2'}$ = $R^{3'}$ = $R^{4'}$ = $R^{6'}$= Bn
15 R^a = R^b = $COC_{11}H_{23}$, R^2 = R^3 = R^4 = $R^{2'}$ = $R^{3'}$ = $R^{4'}$ = $R^{6'}$= H
19 R^a-R^b = $C(CH_3)_2$, R^2 = R^3 = R^4 = Ac, $R^{2'}$ = $R^{3'}$ = $R^{4'}$ = $R^{6'}$= Bn



16 R^1-R^2 = R^3-R^4 = $C(CH_3)_2$, $R^{2'}$ = $R^{3'}$ = $R^{4'}$ = $R^{6'}$= Bn
17 R^1 = R^2 = R^3 = R^4 = Ac, $R^{2'}$ = $R^{3'}$ = $R^{4'}$ = $R^{6'}$= Bn
18 R^1 = C(=NH)CCl_3, R^2 = R^3 = R^4 = Ac, $R^{2'}$ = $R^{3'}$ = $R^{4'}$ = $R^{6'}$= Bn

Chart 1.

mixture. Regioselective cleavage of the anomeric acetate with hydrazine acetate, followed by reaction with trichloroacetonitrile under basic conditions afforded the imidate **4**. Glycosylation of 1,2-*O*-isopropylidene-*sn*-glycerol with imidate **4** led to the glycoside **5** in 72% yield. A change of protective groups to compound **6** was effected by O-deacetylation and benzylation (benzyl chloride and powdered potassium hydroxide in dimethylsulfoxide). Finally, the *p*-methoxyphenyl group was removed with ammonium cerium nitrate in acetonitrile to afford **7**. Another route to compound **7** involved the use of the known 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (**8**)[3] as starting material. This compound was O-deacetylated by the Zemplén method and selectively silylated at 6-*OH* using *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide. The free hydroxyl groups of **9** were benzylated to afford compound **10** in 66% yield. Finally, the silyl ether was cleaved with 1 N tetrabutylammonium fluoride in tetrahydrofurane to provide the alcohol **7** in 85% yield. It could be mentioned that the overall yield to **7** of the latter pathway (from 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl trichloroacetimidate and 1,2-*O*-isopropylidene-*sn*-glycerol)[3] is much more better than the preceding one (37.3% vs 16.3%). Glycosylation of acceptor **7** requires a donor able to induce the α-anomeric stereochemistry in smooth conditions. Trichloroacetimidoyl donors are sometimes of lower efficiency than thioglycosides in the induction of 1,2-cis stereochemistry with primary alcohol acceptors. On the other hand, the activation of thioglycosides often requires promoters that are dangerous and difficult to handle. This prompted us to use the methodology developed by Mereyala et al.[17] with 2-pyridyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**11**) as the glycosyl donor and methyl iodide as the promoter. Thus, the reaction of acceptor **7** and donor **11** afforded the α-glycoside **12**, although in a modest yield (60%) that can be explained by the formation of small amounts of the β-disaccharide by-product, difficult to separate by column chromatography. The acetal function was cleaved using 85% aqueous acetic acid and, after usual work up, the crude reaction product was treated by catalytic sodium methylate in methanol in order to remove the traces of monoacetylated derivatives formed during the acetic acid treatment. Reaction of diol **13** with dodecanoyl chloride and pyridine afforded the pure perbenzyl digalactosyl didodecanoylglyceride **14** in 92% yield. Finally, debenzylation was realized by hydrogen transfer, using cyclohexene and 20% palladium hydroxide in absolute ethanol. The deprotected product **15** was obtained in 75% yield after purification by column chromatography.

The second pathway to compound **15** involved the coupling of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) and 2-pyridyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**11**)[17] to give the α-linked disaccharide **16** in 74% yield, after purification. Treatment of the disaccharide **16** under acidic condition (80% aqueous trifluoroacetic acid) and O-reacetylation afforded the product **17** in 62% yield as a α,β-anomeric mixture. The anomeric acetate was selectively removed using benzyl amine in tetrahydrofurane, and the hemiacetal (obtained in 70% yield) was transformed into the imidate **18** (89% yield) by treatment with trichloracetonitrile and DBU in dichloromethane. Glycosylation of 1,2-*O*-isopropylidene-*sn*-glycerol with the imidate **18**, promoted by catalytic trimethylsilyl trifluoromethanesulfonate, in dichloromethane at −30 °C afforded the expected derivative **19** in 55% yield. Compound **12** was then obtained in 75% yield, after O-deacetylation and *O*-benzylation. As a summary, the first route to **15**, via compound **7** intermediate, afforded the expected derivative with a 12.4% overall yield, whereas the second one, via compound **18** intermediate, displayed a 6.5% overall yield only.

We further checked that the α-Gal(1→6)-β-Gal diglyceride (DGDG) obtained was recognized and hydrolysed by a galactolipase. Figure 1 shows the hydrolysis of the DGDG by the guinea pig pancreatic lipase-related protein 2 (GPLRP2) measured using the monomolecular film technique. The maximum enzyme activity on DGDG was measured at a surface pressure of 5 mN/m, whereas the activities of the same enzyme on monogalactosyldiglyceride (MGDG), 1,2-dicaprine (1,2-DC10) and phosphatidylglycerol (DiC12PG) were maximum at higher surface pressures. The activity on

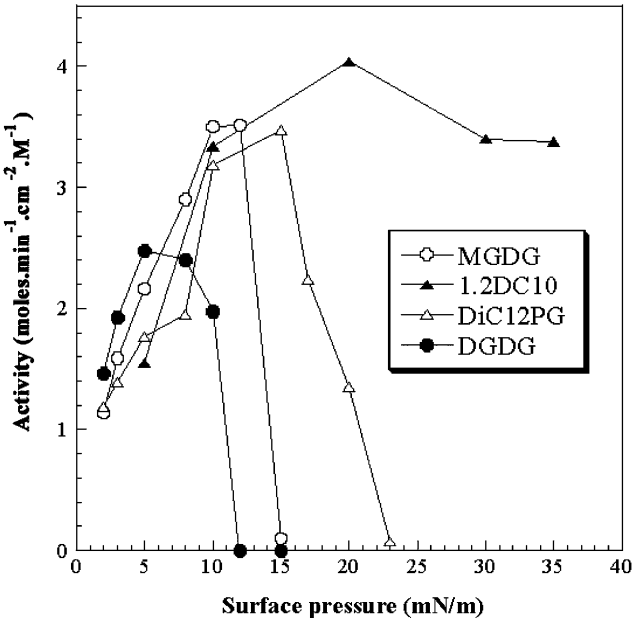


Figure 1. Variations with surface pressure of guinea pig pancreatic lipase-related protein 2 (GPLRP2) lipolytic activities on monomolecular lipid films of **15** (DGDG), 3-*O*-β-D-galactopyranosyl-1,2-di-*O*-dodecanoyl-*sn*-glycerol (MGDG),[3] 1,2-dicaprin (1.2DC10) and 1,2-didodecanoyl phosphatidylglycerol (DiC12PG).

MGDG is maximum at 12 mN/m whereas the activity on DGDG is almost abolished at this surface pressure. This observation suggests that the presence of an additional galactose residue, when compared to MGDG, decreases the enzyme interaction with the lipid–water interface when the molecular packing of the substrate molecules is increased. One hypothesis is that the increased surface density of the hydrophilic moiety of DGDG impairs the adsorption of the enzyme, which is mainly driven by hydrophobic interactions. Validation of this hypothesis will require however the simultaneous measurement of lipase adsorption and activity to complete these preliminary experiments. The detailed kinetic characterization of the galactolipase action on DGDG and MGDG studied by the monolayer technique, will be published separately.

In summary, we have tested two different routes to digalactosyl diglycerides. The best results were obtained when the glycerol residue was β-linked to the galactose, before the formation of the α-(1→6) linkage between the two D-galactose moieties. It is afore to mention that the syntheses of the titled saccharide, reported in this paper, can be compared favourably to those reported earlier in the literature. In the first route, the improvement is displayed in terms of the number of steps and yields;[11,12] in the second one, in terms of the number of steps.[13]

3. Experimental

3.1. Materials and methods

Pyridine was dried by boiling with CaH_2 prior to distillation. Dichloromethane was washed twice with water, dried with $CaCl_2$ and distilled over P_2O_5. MeOH was distilled from magnesium. Tetrahydrofuran was distilled over sodium-benzophenone. Pyridine, THF and CH_2Cl_2 were stored over 4 Å molecular sieves and MeOH over 3 Å molecular sieves. Recombinant GPLRP2 was produced in *Aspergillus orizae*.[18] Melting points were determined on a Büchi apparatus and were uncorrected. Thin layer chromatography was performed on aluminium sheets coated with Silica gel 60 F_{254} (E. Merck). Compounds were visualized by spraying the TLC plates with dilute 15% aq H_2SO_4, followed by charring at 150 °C for a few minutes. Column chromatography was performed on Silica-gel Geduran Si 60 (E. Merck). Optical rotations were recorded on a Perkin–Elmer 241 polarimeter in a 1 dm cell at 21 °C. ^1H and ^{13}C NMR spectra were recorded with a Bruker AC-200 spectrometer (working at 200 MHz and 50 MHz) or a Bruker DRX 500 (working at 500 MHz and 125 MHz), respectively, with Me_4Si as internal standard. Elemental analyses were performed by the 'Laboratoire Central d'Analyses du CNRS' (Vernaison, France). Mass spectra were recorded on a Finnigan Mat 95 XL apparatus in FAB mode.

3.2. 1,2:3,4-Di-*O*-isopropylidene-6-*O*-*p*-methoxyphenyl-α-D-galactopyranose (2)

Diisopropylazodicarboxylate (1.10 mL, 5.55 mmol) was added dropwise at 0 °C under argon to a soln of alcohol **1** (0.775 g, 4.30 mmol), triphenylphosphine (1.45 g, 5.53 mmol) and 4-methoxyphenol (1.56 g, 12.57 mmol) in THF (15 mL). The mixture was stirred for 30 min at 0 °C, then at 80 °C for 4 h. After cooling, the soln was concentrated, Et_2O (100 mL) was added and the soln was washed twice with 15% aq NaOH (2 × 20 mL), then with brine (2 × 10 mL) and dried (Na_2SO_4). Evaporation of the solvent afforded a solid residue, which was recrystallized from absolute EtOH to afford compound **2** as a white solid (1.30 g, 83%); mp 71 °C (EtOH); $[\alpha]_D$ −77.6 (*c* 1.0, $CHCl_3$); R_f 0.55 (1:3 EtOAc–petroleum ether); ^1H NMR ($CDCl_3$): δ 6.92–6.79 (m, 4H, C_6H_4), 5.58 (d, 1H, $J_{1,2}$ 5.0 Hz, H-1), 4.65 (dd, 1H, $J_{2,3}$ 7.9, $J_{3,4}$ 2.3 Hz, H-3), 4.38–4.34 (m, 2H, H-2, H-4), 4.16–4.06 (m, 3H, H-5, H-6a, H-6b), 3.76 (s, 3H, OCH_3), 1.51, 1.47, 1.36, 1.34 (4s, 12H, $2C(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 154.1, 152.8, 115.9, 114.6 (C_6H_4), 109.3, 108.6 ($2C(CH_3)_2$), 96.4 (C-1), 71.0, 70.7, 70.4 (C-2, C-3, C-5), 67.4 (C-6), 66.2 (C-4), 55.6 (OCH_3), 26.0, 25.0, 24.5 ($2C(CH_3)_2$). Anal. Calcd for $C_{19}H_{26}O_7$ (366.40): C, 62.28; H, 7.15. Found: C, 62.04; H, 7.27.

3.3. 1,2,3,4-Tetra-*O*-acetyl-6-*O*-*p*-methoxyphenyl-α-D-galactopyranose (3)

Compound **2** (1.25 g, 3.41 mmol) was dissolved in 80% aq AcOH (25 mL) and the soln was heated for 12 h at 80 °C. After cooling to rt, the soln was concentrated under diminished pressure and the residue was coevaporated twice from toluene (2 × 15 mL). The crude product was acetylated overnight in a 2:1 pyridine–Ac_2O mixture (40 mL). After concentration, the residue was purified by column chromatography (1:1 EtOAc–petroleum ether) to afford a mixture of α,β-anomers (1.40 g, 90%) from which the pure α-anomer could be recovered by recrystallization from EtOH; mp 98 °C (EtOH); $[\alpha]_D$ +70.2 (*c* 1.0, $CHCl_3$); R_f 0.80 (1:1 EtOAc–petroleum ether); ^1H NMR ($CDCl_3$): δ 6.81 (s, 4H, C_6H_4), 6.42 (d, 1H, $J_{1,2}$ 2.4, H-1), 5.68 (br s, 1H, H-4), 5.42–5.37 (m, 2H, H-3, H-2), 4.47 (bdd, 1H, $J_{5,6a}$ 5.9, $J_{5,6b}$ 7.3 Hz, H-5), 4.04 (dd, 1H, $J_{6a,6b}$ 9.5 Hz, H-6a), 3.88 (dd, 1H, H-6b), 3.76 (s, 3H, OCH_3), 2.16, 2.10, 2.04, 2.02 (4s, 12H, $4CH_3CO$); ^{13}C NMR ($CDCl_3$): δ 170.0, 169.9, 169.8, 169.0 ($4CH_3CO$), 154.4, 152.1, 115.9, 114.6 (C_6H_4), 89.8 (C-1), 69.4 (C-5), 67.8, 67.5 (C-2, C-3), 67.4 (C-6), 66.6 (C-4), 55.6 (OCH_3), 20.8, 20.6, 20.5 ($4CH_3COO$). Anal. Calcd for $C_{21}H_{26}O_{11}$ (454.42): C, 55.50; H, 5.77. Found: C, 55.46; H, 5.75.

Selected data for β-anomer (in mixture with α): ^1H NMR ($CDCl_3$): δ 5.76 (d, 1H, $J_{1,2}$ 8.2, H-1), 5.62 (br

d, 1H, $J_{3,4}$ 3.4 Hz, H-4), 5.37 (dd, 1H, $J_{2,3}$ 10.4 Hz, H-2), 5.14 (dd, 1H, H-3), 3.76 (s, 3H, OCH_3).

3.4. 2,3,4-Tri-*O*-acetyl-6-*O*-*p*-methoxyphenyl-α-D-galactopyranosyl trichloroacetimidate (4)

Hydrazine acetate (0.320 g, 3.47 mmol) was added to a soln of **3** (1.38 g, 3.03 mmol) in dry DMF and the mixture was heated at 50 °C for 1 h. The soln was cooled to rt, EtOAc (40 mL) was added and the organic extract was washed twice with brine (2 × 10 mL). After drying (Na_2SO_4) and concentration, the residue was purified by column chromatography (1:1 EtOAc–petroleum ether) to afford the expected hemiacetal as a 5:2 α,β-anomeric mixture (0.780 g, 62%); oily material; R_f 0.49–0.40 (1:2 EtOAc–petroleum ether). α-Anomer, ^{1}H NMR ($CDCl_3$): δ 6.82 (s, 4H, C_6H_4), 5.63–5.58 (m, 2H, H-4, H-1), 5.47–5.21 (m, 2H, H-2, H-3), 4.63 (br dd, 1H, $J_{4,5}$ 1.0, $J_{5,6a}$ 6.1, $J_{5,6b}$ 6.1 Hz, H-5), 4.13–3.90 (m, 2H, H-6a, H-6b), 3.76 (s, 3H, OCH_3), 2.12, 2.10, 2.01 (3s, 9H, 3CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.7, 170.5, 170.3 (3CH_3CO), 154.2, 152.3, 115.8, 114.1 (C_6H_4), 90.5 (C-1), 68.8, 68.6 (C-3, C-5), 67.6 (C-2), 67.2 (C-6), 66.8 (C-4), 55.6 (OCH_3), 20.7, 20.6, 20.5 (3CH_3COO).

Selected data for β-anomer: ^{13}C NMR ($CDCl_3$): δ 95.6 (C-1).

DBU (0.275 mL, 1.84 mmol) was added to a soln of the hemiacetal (0.780 g, 1.89 mmol) and trichloroacetonitrile (1.2 mL, 12.0 mmol) in dry CH_2Cl_2 (5.7 mL) and the mixture was stirred for 4 h at rt. After concentration under diminished pressure, the residue was purified by column chromatography (1:2 EtOAc–petroleum ether) to afford the pure imidate **4** as an oily material (0.790 g, 77%); $[\alpha]_D$ +66.3 (*c* 1.0, $CHCl_3$); R_f 0.50 (1:2 EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$): δ 8.67 (s, 1H, N*H*), 6.79 (s, 4H, C_6H_4), 6.63 (d, 1H, $J_{1,2}$ 3.2, H-1), 5.74 (dd, 1H, $J_{3,4}$ 2.9 Hz, $J_{4,5}$ 0.8, H-4), 5.49 (dd, 1H, $J_{2,3}$ 10.8 Hz, H-3), 5.39 (dd, 1H, H-2), 4.56 (ddd, 1H, $J_{5,6a}$ 6.4, $J_{5,6b}$ 7.3 Hz, H-5), 4.08 (dd, 1H, $J_{6a,6b}$ 9.7 Hz, H-6a), 3.91 (dd, 1H, H-6b), 3.76 (s, 3H, OCH_3), 2.05, 2.04, 2.03 (3 s, 9H, 3CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.9, 170.0, 169.9 (3CH_3CO), 160.8 (O*C*(NH)CCl_3), 154.4, 152.0, 115.8, 114.6 (C_6H_4), 93.6 (C-1), 90.8 (*C*Cl_3), 69.4 (C-5), 67.7, 67.6 (C-2, C-3), 67.0 (C-4), 66.1 (C-6), 55.5 (OCH_3), 20.9, 20.6, 20.5 (3CH_3COO).

Anal. Calcd for $C_{21}H_{24}Cl_3NO_{11}$ (556.762): C, 45.30; H, 4.35; N, 2.52. Found: C, 45.10; H, 4.46; N, 2.85.

3.5. 3-*O*-(2,3,4-Tri-*O*-acetyl-6-*O*-*p*-methoxyphenyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (5)

1,2-*O*-Isopropylidene-*sn*-glycerol (0.270 g, 1.89 mmol), imidate **4** (0.740 g, 1.36 mmol) and 4 Å activated molecular sieves (0.500 g) were added to alcohol-free CH_2Cl_2 (10 mL). The mixture was flushed with argon while cooling to −20 °C. A soln of TMSOTf (10 μL, 0.046 mmol) in CH_2Cl_2 (0.5 mL) was introduced through a syringe during a period of 1 h. The mixture was stirred for 4 h at −20 °C and then neutralized by the addition of Et_3N (20 μL). After filtration over Celite and dilution with CH_2Cl_2 (50 mL), the organic phase was washed with satd aq $NaHCO_3$ and dried (Na_2SO_4). Concentration of the soln gave a residue, which was purified by column chromatography (1:2 petroleum ether–EtOAc) affording compound **5** as an oil (0.520 g, 72%); $[\alpha]_D$ −14.3 (*c* 1.0, $CHCl_3$); R_f 0.60 (1:1 EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$): δ 6.82 (s, 4H, C_6H_4), 5.57 (br d, 1H, $J_{3,4}$ 3.3, $J_{4,5}$ 0.7 Hz, H-4), 5.24 (dd, 1H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.5 Hz, H-2), 5.06 (dd, 1H, H-3), 4.62 (d, 1H, H-1), 4.28 (m, 1H, H-2$_{gl}$), 4.13–3.89 (m, 5H, H-5, H-6a, H-6b, H-1a$_{gl}$, H-3a$_{gl}$), 3.82 (dd, 1H, $J_{1bgl,2gl}$ 6.0, $J_{1agl,1bgl}$ 8.2 Hz, H-1b$_{gl}$), 3.77 (s, 3H, CH_3O), 3.68 (dd, 1H, $J_{2gl,3bgl}$ 6.0, $J_{3agl,3bgl}$ 10.5 Hz, H-3b$_{gl}$), 2.11, 2.08, 2.00 (3s, 9H, 3CH_3CO), 1.43, 1.35 (2s, 6H, C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 170.2, 170.1, 169.5 (3CH_3CO), 154.4, 152.3, 115.9, 114.7 (C_6H_4), 109.4 (*C*(CH$_3$)$_2$), 101.5 (C-1), 74.3 (C-2$_{gl}$), 71.7 (C-5), 71.1 (C-3), 69.2 (C-3$_{gl}$), 68.9 (C-2), 67.5 (C-4), 66.5 (C-6), 66.2 (C-1$_{gl}$), 55.7 (OCH_3), 26.6, 25.2 (C(*C*H$_3$)$_2$), 20.7, 20.6, 20.5 (3CH_3COO). Anal. Calcd for $C_{25}H_{34}O_{12}$ (526.522): C, 57.02; H, 6.51. Found: C, 56.64; H, 6.19.

3.6. 3-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-*p*-methoxyphenyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (6)

Compound **5** (0.500 g, 0.95 mmol) was stirred overnight in MeOH (25 mL) in the presence of a chip of MeONa. After concentration, the crude product was dissolved in Me_2SO (2 mL) and added dropwise to a cold (0 °C) mixture of BnCl (0.440 mL, 3.82 mmol) and powdered KOH (0.375 g, 6.68 mmol) in Me_2SO (2.5 mL). The mixture was stirred for 16 h at rt, then poured into ice and the product was extracted with CH_2Cl_2 (2 × 30 mL). The organic phase was washed once with water (15 mL), then dried and concentrated under diminished pressure. The residue was purified by column chromatography (5:2 petroleum ether–EtOAc) affording the pure product **6** as an oil (0.450 g, 71%); $[\alpha]_D$ −6.0 (*c* 1.0, $CHCl_3$); R_f 0.58 (2:5 EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$): δ 7.43–7.25 (m, 15H, 3C_6H_5), 6.87 (s, 4H, C_6H_4), 5.02 and 4.69 (2d, 2H, *J* 11.6 Hz, CH_2Ph), 5.00 and 4.85 (2d, *J* 10.6 Hz, CH_2Ph), 4.87 and 4.80 (2d, *J* 12.1 Hz, CH_2Ph), 4.50 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 4.39 (m, 1H, H-2$_{gl}$), 4.15–3.60 (m, 13H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-1a$_{gl}$, H-1b$_{gl}$, H-3a$_{gl}$, H-3b$_{gl}$, OCH_3), 1.48, 1.42 (2s, 6H, C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 154.4, 152.3, 115.9, 114.7 (C_6H_4), 138.8, 138.6, 138.4, 128.6, 128.5, 128.4, 128.3, 128.2, 127.7, 127.6 (3C_6H_5), 109.4 (*C*(CH$_3$)$_2$), 104.4 (C-1), 82.2 (C-3), 79.5 (C-2), 75.3, 74.7, 73.3 (3CH_2Ph), 74.4 (C-2$_{gl}$), 73.2, 73.0 (C-4, C-5), 70.4 (C-3$_{gl}$), 67.2, 66.9 (C-6, C-1$_{gl}$),

55.8 (OCH_3), 27.0, 25.5 ($C(CH_3)_2$). Anal. Calcd for $C_{40}H_{46}O_9$ (670.768): C, 71.62; H, 6.91. Found: C, 71.33; H, 6.99.

3.7. 3-*O*-(2,3,4-Tri-*O*-benzyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (7)

Method A. Compound **6** (0.430 g, 0.64 mmol) was dissolved in MeCN (8.5 mL); water (2.2 mL) and $NaHCO_3$ (0.555 g, 6.60 mmol) were added and the mixture was cooled to 0 °C, before the addition of ammonium cerium nitrate (0.935 g, 1.70 mmol). The mixture was stirred for 1 h at 0 °C, then concentrated under diminished pressure and extracted with $CHCl_3$. The organic phase was washed with water, dried and concentrated again; then, the crude product was purified by column chromatography (5:2 petroleum ether–EtOAc) affording the pure product **7** as an oil (0.235 g, 69%); $[\alpha]_D$ −16.6 (*c* 1.0, $CHCl_3$); R_f 0.65 (2:5 EtOAc–petroleum ether); ^1H NMR ($CDCl_3$): δ 7.35–7.30 (m, 15H, $3C_6H_5$), 4.97 and 4.68 (2d, 2H, *J* 11.8 Hz, CH_2Ph), 4.92 and 4.74 (2d, *J* 11.0 Hz, CH_2Ph), 4.83 and 4.78 (2d, *J* 10.5 Hz, CH_2Ph), 4.40 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.34 (dddd, 1H, $J_{1agl,2gl}$ 6.3, $J_{1bgl,2gl}$ 6.2, $J_{2gl,3agl}$ 5.0, $J_{2gl,3bgl}$ 6.2 Hz, H-2_{gl}), 4.07 (dd, 1H, $J_{1agl,1bgl}$ 8.3 Hz, H-$1a_{gl}$), 3.98 (dd, 1H, $J_{3agl,3bgl}$ 10.3 Hz, H-$3a_{gl}$), 3.85 (dd, 1H, $J_{2,3}$ 9.8 Hz, H-2), 3.82 (dd, 1H, H-$1b_{gl}$), 3.80–3.79 (m, 1H, H-4), 3.77 (dd, 1H, $J_{5,6a}$ 6.3, $J_{6a,6b}$ 10.3 Hz, H-6a), 3.61 (dd, 1H, H-$3b_{gl}$), 3.54 (dd, 1H, $J_{3,4}$ 3.2 Hz, H-3), 3.50 (dd, 1H, $J_{5,6b}$ 5.4 Hz, H-6b), 3.38 (bdd, 1H, H-5), 1.64 (m, 1H, OH), 1.41, 1.36 (2s, 6H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 138.7, 138.4, 138.3, 128.7, 128.5, 128.4, 128.2, 128.0, 127.8, 127.7 ($3C_6H_5$), 109.5 ($C(CH_3)_2$), 104.5 (C-1), 82.3 (C-3), 79.6 (C-2), 75.3, 74.3, 73.4 ($3CH_2Ph$), 74.9 (C-5), 74.7 (C-2_{gl}), 73.0 (C-4), 70.8 (C-3_{gl}), 67.1 (C-1_{gl}), 61.9 (C-6), 26.9, 25.5 ($C(CH_3)_2$). Anal. Calcd for $C_{33}H_{40}O_8$ (564.65): C, 70.19; H, 7.14. Found: C, 70.05; H, 6.95.

Method B. A 1 N soln of Bu_4NF in THF (1.3 mL) was added dropwise at 0 °C under argon to a soln of silylated derivative **10** (0.679 g, 1.00 mmol) in THF (20 mL). The mixture was stirred for 1 h at 0 °C, then poured into satd aq $NaHCO_3$ (60 mL). After concentration under diminished pressure, the product was extracted with CH_2Cl_2 (4 × 20 mL). The organic phase was dried, concentrated and purified by column chromatography as described above. Pure product **7** was recovered in 85% yield.

3.8. 3-*O*-(6-*O*-*tert*-Butyldimethylsilyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (9)

3-*O*-β-D-Galactopyranosyl-1,2-*O*-isopropylidene-*sn*-glycerol (1.53 g, 5.19 mmol), obtained quantitatively by Zemplén O-deacetylation of 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (**8**),[3] was treated at 0 °C with a mixture of *tert*-butyldimethysilyl chloride (0.823 g, 5.56 mmol) and imidazole (0.707 g, 10.38 mmol) in dry DMF (10 mL). Stirring was maintained for 1 h at 0 °C, then overnight at rt. The reaction mixture was diluted with $CHCl_3$, washed successively with 10% aq HCl (10 mL), then with satd aq $NaHCO_3$ and finally with water (10 mL). The organic extract was dried (Na_2SO_4), concentrated under diminished pressure and the residue was coevaporated twice from toluene. After purification by column chromatography (10:1 $CHCl_3$–EtOH), the pure product **9** was recovered as an oil (1.88 g, 80%); $[\alpha]_D$ −12.7 (*c* 2.0, $CHCl_3$); R_f 0.80 (10:1 $CHCl_3$–EtOH); ^1H NMR ($CDCl_3$): δ 4.34 (dddd, 1H, $J_{1agl,2gl}$ 6.4, $J_{1bgl,2gl}$ 6.2, $J_{2gl,3agl}$ 5.8, $J_{2gl,3bgl}$ 5.2 Hz, H-2_{gl}), 4.30 (d, 1H, $J_{1,2}$ 7.4 Hz, H-1), 4.07 (dd, 1H, $J_{1agl,1bgl}$ 8.2 Hz, H-$1a_{gl}$), 4.05 (br d, 1H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 1.0 Hz, H-4), 3.94 (dd, 1H, $J_{5,6a}$ 5.4, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.93 (dd, 1H, $J_{3agl,3bgl}$ 10.6 Hz, H-$3a_{gl}$), 3.86 (dd, 1H, $J_{5,6b}$ 5.6 Hz, H-6b), 3.84 (dd, 1H, H-$1b_{gl}$), 3.70 (dd, 1H, $J_{2,3}$ 9.8 Hz, H-2), 3.64 (dd, 1H, H-$3b_{gl}$), 3.58 (dd, 1H, H-3), 3.50 (ddd, 1H, H-5), 1.44, 1.36 (2s, 6H, $C(CH_3)_2$), 0.90 (s, 9H, $(CH_3)_3CSi$), 0.09 (s, 6H, $(CH_3)_2Si$); ^{13}C NMR ($CDCl_3$): δ 109.5 ($C(CH_3)_2$), 103.8 (C-1), 75.1 (C-5), 74.4 (C-2_{gl}), 73.8 (C-3), 71.2 (C-2), 70.4 (C-3_{gl}), 68.7 (C-4), 66.6 (C-1_{gl}), 62.3 (C-6), 26.8, 25.3 ($C(CH_3)_2$), 25.9 ($(CH_3)_3CSi$), 18.2 ($(CH_3)_3CSi$), −5.3 ($(CH_3)_2Si$).

3.9. 3-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (10)

Sodium hydride (60% in oil, 0.700 g, 17.5 mmol) was added portionwise to a stirred soln of compound **9** (1.25 g, 3.06 mmol) in dry THF (15 mL) under argon. After 1 h, benzyl bromide (1.65 mL, 13.79 mmol) and Bu_4NI (0.185 g, 0.50 mmol) were added and the mixture was stirred for 16 h at rt. Sodium hydride (0.200 g, 5.00 mmol) and Bu_4NI (0.185 g, 0.50 mmol) were added again and the mixture was stirred at 50 °C for 16 h. After cooling to rt, the excess of benzyl bromide was destroyed by careful addition of MeOH at 0 °C (0.5 mL) and the mixture was concentrated. The residue was diluted with EtOAc (80 mL), the organic phase was washed with water (2 × 15 mL), dried (Na_2SO_4) and concentrated. After purification by column chromatography (5:1 petroleum ether–EtOAc), the pure product **10** was recovered as a solid (1.37 g, 66%); mp 63–64 °C; $[\alpha]_D$ +10.0 (*c* 1.0, $CHCl_3$); R_f 0.60 (5:1 petroleum ether–EtOAc); ^1H NMR ($CDCl_3$): δ 7.36–7.29 (m, 15H, $3C_6H_5$), 4.99–4.61 (m, 6H, $3CH_2C_6Ph$), 4.38 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.32 (dddd, 1H, $J_{1agl,2gl}$ 6.6, $J_{1bgl,2gl}$ 6.1, $J_{2gl,3agl}$ 4.7, $J_{2gl,3bgl}$ 7.0 Hz, H-2_{gl}), 4.06 (dd, 1H, $J_{1agl,1bgl}$ 8.5 Hz, H-$1a_{gl}$), 4.00 (dd, 1H, $J_{3agl,3bgl}$ 10.1 Hz, H-$3a_{gl}$), 3.87 (dd, 1H, H-$1b_{gl}$), 3.87 (br d, 1H, $J_{3,4}$ 3.2,

$J_{4,5} < 1.0$ Hz, H-4), 3.83 (dd, 1H, $J_{2,3}$ 10.2 Hz, H-2), 3.69 (br d, 2H, $J_{5,6a}$, $J_{5,6b}$ 6.6 Hz, H-6a, H-6b), 3.55 (dd, 1H, H-3b$_{gl}$), 3.52 (dd, 1H, H-3), 3.37 (br dd, 1H, H-5), 1.41, 1.36 (2s, 6H, C(CH_3)$_2$), 0.89 (s, 9H, (CH_3)$_3$CSi), 0.06 (s, 6H, (CH_3)$_2$Si); ^{13}C NMR ($CDCl_3$): δ 138.9, 138.9, 138.7, 128.5, 128.4, 128.3, 128.2, 128.2, 127.7, 127.6 (3C_6H_5), 109.3 (C(CH$_3$)$_2$), 104.4 (C-1), 82.3 (C-3), 79.6 (C-2), 75.3 (C-4), 75.3, 74.8, 73.2 (3CH_2Ph), 74.4 (C-2$_{gl}$), 73.7 (C-5), 70.3 (C-3$_{gl}$), 67.2 (C-1$_{gl}$), 61.8 (C-6), 27.0, 25.6 (C(CH$_3$)$_2$), 26.0 ((CH$_3$)$_3$CSi), 18.3 ((CH$_3$)$_3$$C$Si), −5.2, −5.3 (($CH_3$)$_2$Si). Anal. Calcd for $C_{39}H_{54}O_8Si$ (678.908): C, 68.99; H, 8.02. Found: C, 68.99; H, 8.05.

3.10. 3-*O*-[6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galacto-pyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)]-1,2-*O*-isopropylidene-*sn*-glycerol (12)

2-Pyridyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galacto-pyranoside (**11**)[17] (0.651 g, 1.027 mmol) and alcohol **7** (0.580 g, 1.027 mmol) were dissolved in CH_2Cl_2 (4 mL) containing activated crushed 4 Å molecular sieves (0.200 g). Methyl iodide (120 μL, 1.93 mmol) was added, and the mixture was stirred at 50 °C under argon for 48 h. Methyl iodide was added again (120 μL, 1.93 mmol) and stirring was maintained for 2 days. After cooling to rt and filtration, the soln was concentrated and the residue was directly purified by column chromatography (1:2 EtOAc–petroleum ether). Product **12** was recovered as a solid (0.650 g, 60% yield); mp 78 °C; $[\alpha]_D$ +21.0 (*c* 1.0, $CHCl_3$); R_f 0.65 (1:2 EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$): δ 7.37–7.27 (m, 35H, 7C$_6H_5$), 4.92 and 4.55 (2d, 2H, *J* 11.3 Hz, CH_2Ph), 4.89 and 4.75 (2d, 2H, *J* 11.4 Hz, CH_2Ph), 4.87 and 4.71 (2d, 2H, *J* 11.7 Hz, CH_2Ph), 4.80 and 4.59 (2d, 2H, *J* 11.7 Hz, CH_2Ph), 4.77 (d, 1H, $J_{1',2'}$ 3.6 Hz, H-1′), 4.75–4.74 (m, 2H, CH_2Ph), 4.72 and 4.62 (2d, 2H, *J* 11.9 Hz, CH_2Ph), 4.47 and 4.23 (2d, 2H, *J* 11.6 Hz, CH_2Ph), 4.32 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 4.25 (dddd, 1H, $J_{1agl,2gl}$ 6.3, $J_{1bgl,2gl}$ 6.3 Hz, $J_{2gl,3agl}$ 4.4, $J_{2gl,3bgl}$ 6.9 Hz, H-2$_{gl}$), 4.02 (dd, 1H, $J_{2',3'}$ 10.3 Hz, H-2′), 3.98 (dd, 1H, $J_{1agl,1bgl}$ 8.2 Hz, H-1a$_{gl}$), 3.97 (br d, 1H, $J_{3',4'}$ 2.9 Hz, H-4′), 3.93 (dd, 1H, $J_{3agl,3bgl}$ 10.5 Hz, H-3a$_{gl}$), 3.92 (dd, 1H, H-3′), 3.88 (br dd, 1H, $J_{5',6'a}$ 6.3, $J_{5',6'b}$ 6.3 Hz, H-5′), 3.82 (dd, 1H, H-1b$_{gl}$), 3.80 (m, 1H, H-4), 3.78 (d, 1H, $J_{2,3}$ 9.8 Hz, H-2), 3.74 (dd, 1H, $J_{5,6a}$, 3.8 Hz, $J_{6a,6b}$ 8.2 Hz, H-6a), 3.57–3.47 (m, 6H, H-3b$_{gl}$, H-3, H-5, H-6b, H-6′a, H-6′b), 1.37, 1.32 (2s, 6H, C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$): δ 138.9, 138.9, 138.8, 138.7, 138.1, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6 (7C_6H_5), 109.3 (C(CH$_3$)$_2$), 104.3 (C-1), 98.6 (C-1′), 82.2 (C-3), 79.5 (C-2), 79.2 (C-3′), 76.5 (C-2′), 75.3, 74.9, 74.6, 73.7, 73.7, 73.2, 73.0 (7 CH$_2$Ph), 75.0 (C-4′), 74.4 (C-2$_{gl}$), 74.1 (C-4), 73.2 (C-5), 70.2 (C-3$_{gl}$), 69.7 (C-5′), 69.1 (C-6′), 67.3 (C-6), 67.2 (C-1$_{gl}$), 61.9 (C-6), 27.1, 25.6 (C(CH$_3$)$_2$). Anal. Calcd for $C_{67}H_{74}O_{13}$ (1087.262): C, 74.01; H, 6.86. Found: C, 73.68; H, 6.95.

3.11. 3-*O*-[6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galacto-pyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)]-*sn*-glycerol (13)

Product **12** (0.326 g, 0.30 mmol) was stirred for 16 h at rt in 85% aq AcOH (6 mL). After concentration under diminished pressure and coevaporation from toluene (2 × 15 mL), the residue was treated for 2 h by a catalytic amount of MeONa in MeOH (25 mL). The soln was concentrated again, the residue was dissolved in CH_2Cl_2 (100 mL) and the organic phase was washed with water (10 mL), dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (2:1 EtOAc–petroleum ether) to provide the pure product **13** as a white crystalline material (0.251 g, 80%); mp 103–104 °C; $[\alpha]_D$ +24.0 (*c* 1.0, $CHCl_3$); R_f 0.75 (2:1 EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$): δ 7.34–7.23 (m, 35H, 7C$_6H_5$), 4.94 and 4.56 (2d, 2H, *J* 11.6 Hz, CH_2Ph), 4.93 and 4.74 (2d, 2H, *J* 11.6 Hz, CH_2Ph), 4.92 and 4.63 (2d, 2H, *J* 12.0 Hz, CH_2Ph), 4.83 (d, 1H, $J_{1',2'}$ 3.2 Hz, H-1′), 4.80 (m, 2H, CH_2Ph), 4.77 and 4.68 (2d, 2H, *J* 11.0 Hz, CH_2Ph), 4.67 and 4.60 (2d, 2H, *J* 11.0 Hz, CH_2Ph), 4.47 and 4.23 (2d, 2H, *J* 11.6 Hz, CH_2Ph), 4.33 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 4.00–3.30 (m, 17H, H-1a$_{gl}$, H-1b$_{gl}$, H-2$_{gl}$, H-3a$_{gl}$, H-3b$_{gl}$, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6′a, H-6′b); ^{13}C NMR ($CDCl_3$): δ 138.9, 138.8, 138.7, 138.5, 137.9, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6 (7C_6H_5), 104.5 (C-1), 98.6 (C-1′), 82.2 (C-3), 79.5 (C-2), 79.0 (C-3′), 76.5 (C-2′), 75.4, 74.9, 74.7, 73.8, 73.7, 73.3, 72.9 (7 CH_2Ph), 75.0 (C-4′), 74.1 (C-4), 73.6 (C-5), 72.9 (C-3$_{gl}$), 70.9 (C-2$_{gl}$), 69.6 (C-5′), 69.0 (C-6′), 67.8 (C-6), 63.6 (C-1$_{gl}$). Anal. Calcd for $C_{64}H_{70}O_{13}$ (1047.20): C, 73.40; H, 6.74. Found: C, 73.22; H, 7.07.

3.12. 3-*O*-[6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galacto-pyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)]-1,2-di-*O*-dodecanoyl-*sn*-glycerol (14)

A soln of dodecanoyl chloride (0.162 mL, 0.700 mmol) in CH_2Cl_2 (1 mL) was added dropwise for 1 h to a soln of diol **13** (0.209 g, 0.200 mmol) and pyridine (0.327 mL) in CH_2Cl_2 (2 mL). The mixture was stirred overnight at rt, then water (40 μL) was added and stirring was maintained for 3 h. The mixture was diluted with CH_2Cl_2 (50 mL) and the organic phase was washed with satd aq $NaHCO_3$ (10 mL), dried (Na_2SO_4) and concentrated. The residue was coevaporated twice from toluene (2 × 15 mL). The mixture was purified by column chromatography (1:3 EtOAc–petroleum ether). Compound **14** was obtained as a crystalline material (0.257 g, 92%); mp 47–48 °C; $[\alpha]_D$ +25.1 (*c* 1.0, $CHCl_3$); R_f 0.65

(1:3 EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$): δ 7.37–7.27 (m, 35H, 7C_6H_5), 5.24 (dddd, 1H, $J_{1agl,2gl}$ 3.5, $J_{1bgl,2gl}$ 6.9, $J_{2gl,3agl}$ 4.0, $J_{2gl,3bgl}$ 4.9 Hz, H-2$_{gl}$), 4.95 and 4.58 (2d, 2H, J 11.5 Hz, CH_2Ph), 4.93 and 4.75 (2d, 2H, J 11.0 Hz, CH_2Ph), 4.92 and 4.62 (2d, 2H, J 11.5 Hz, CH_2Ph), 4.83 and 4.65 (2d, 2H, J 12.0 Hz, CH_2Ph), 4.80–3.79 (m, 2H, CH_2Ph), 4.79 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.77 and 4.67 (2d, 2H, J 12.0 Hz, CH_2Ph), 4.51 and 4.40 (2d, 2H, J 11.7 Hz, CH_2Ph), 4.36 (dd, 1H, $J_{1agl,1bgl}$ 12.0 Hz, H-1a$_{gl}$), 4.31 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 4.21 (dd, 1H, H-1b$_{gl}$), 4.05 (dd, 1H, $J_{2',3'}$ 10.0 Hz, H-2′), 4.03–4.00 (m, 2H, H-3a$_{gl}$, H-4′), 3.94 (dd, 1H, $J_{3',4'}$ 2.8 Hz, H-3′), 3.91 (bdd, 1H, $J_{4',5'}$ 0.8, $J_{5',6'a}$ 6.5, $J_{5',6'b}$ 6.4 Hz, H-5′), 3.84 (dd, 1H, $J_{3,4}$ 2.8 Hz, H-4), 3.80 (dd, 1H, $J_{2,3}$ 9.8 Hz, H-2), 3.78–3.76 (m, 1H, H-6a), 3.63 (dd, 1H, $J_{3agl,3bgl}$ 10.2 Hz, H-3b$_{gl}$), 3.60–3.55 (m, 4H, H-5, H-6b, H-6′a, H-6′b), 3.50 (dd, 1H, H-3), 2.33–2.23 (m, 4H, 2CH_2CO), 1.64–1.50 (m, 4H, 2COCH$_2$CH_2), 1.40–1.20 (m, 36H, 18 CH_2 alkyl chains), 0.89 (t, 6H, 2CH_3 alkyl chains); ^{13}C NMR ($CDCl_3$): δ 173.4, 173.1 (2COC$_{11}$H$_{23}$), 138.8, 138.7, 138.6, 138.0, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5 (7C_6H$_5$), 104.3 (C-1), 98.6 (C-1′), 82.0 (C-3), 79.3 (C-2), 79.1 (C-3′), 76.4 (C-2′), 75.2, 74.9, 74.7, 73.7, 73.6, 73.2, 72.9 (7CH$_2$Ph), 75.0 (C-4′), 74.0 (C-4), 73.3 (C-5), 70.2 (C-2$_{gl}$), 69.7 (C-5′), 69.1 (C-6′), 68.00 (C-3$_{gl}$), 67.4 (C-6), 62.9 (C-1$_{gl}$), 34.9, 34.2, 32.0, 29.8, 29.6, 29.5, 29.4, 29.3, 29.2, 25.0, 22.8 (CH$_2$ alkyl chains), 14.3 (2CH$_3$). Anal. Calcd for $C_{88}H_{114}O_{15}$ (1411.192): C, 74.86; H, 8.14. Found: C, 74.54; H, 8.46.

3.13. 3-*O*-(6-*O*-α-D-Galactopyranosyl-β-D-galactopyranosyl)-1,2-di-*O*-dodecanoyl-*sn*-glycerol (15)

A mixture of **14** (0.200 g, 0.14 mmol), freshly distilled cyclohexene (2 mL) and $Pd(OH)_2$ (50 mg) in EtOH (4 mL) was heated for 5 h at 80 °C. After cooling and filtration on Celite, the soln was concentrated and the residue was purified by column chromatography (7:3:1 EtOAc–EtOH–water) to afford the pure product **15** as an amorphous solid (0.083 g, 75%); $[\alpha]_D$ +41.7 (*c* 1.0, $CHCl_3$); R_f 0.75 (7:3:1 EtOAc–EtOH–water); ^{1}H NMR ($CDCl_3$+CD_3OD): δ 5.24–5.18 (m, 1H, H-2$_{gl}$), 4.87 (dd, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.36 (dd, 1H, $J_{1agl,2gl}$ 3.0, $J_{1agl,1bgl}$ 12.0 Hz, H-1a$_{gl}$), 4.20 (dd, 1H, $J_{1bgl,2gl}$ 6.7 Hz, H-1b$_{gl}$), 4.17 (d, 1H, $J_{1,2}$ 7.1 Hz, H-1), 3.96–3.65 (m, 12H, H-3a$_{gl}$, H-3b$_{gl}$, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6′a, H-6′b), 3.56–3.45 (m, 2H, H-2, H-3), 2.33–2.26 (m, 4H, 2CH_2CO), 1.65–1.50 (m, 4H, 2COCH$_2$CH_2), 1.34–1.24 (m, 36H, 18 CH_2 alkyl chains), 0.84 (t, 6H, 2CH_3 alkyl chains); ^{13}C NMR ($CDCl_3$): δ 173.7, 173.4 (2COC$_{11}$H$_{23}$), 103.6 (C-1), 99.7 (C-1′), 72.8 (C-3), 72.7 (C-5), 70.9 (C-2), 70.5 (C-2$_{gl}$), 70.0 (C-3′), 69.4 (C-4′, C-5′), 68.6 (C-2′), 67.7 (C-4), 67.4 (C-3$_{gl}$), 65.8 (C-6), 62.5 (C-1$_{gl}$), 61.3 (C-6′), 33.9, 31.5, 29.2, 29.0, 28.7, 24.5, 22.3 (CH$_2$ alkyl chains), 13.5 (2CH$_3$). HRMS *m*/*z* calcd for [M+Li]: 787.5032; found 787.5039.

3.14. 6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (16)

Methyl iodide (240 μL, 3.85 mmol) was added to a mixture of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) (0.520 g, 2.00 mmol), 2-pyridyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**11**) (1.20 g, 1.89 mmol), and activated 4 Å molecular sieves in dry CH_2Cl_2 (8 mL) and the mixture was stirred for 3 days at 50 °C. After a new addition of CH_3I (120 μL, 1.92 mmol), the mixture was stirred for 1 day at 60 °C. After filtration and concentration, the residue was purified by column chromatography (1:3 EtOAc–petroleum ether) affording the pure product **16** as an oil. (1.10 g, 74%) and a 1:6 α,β-mixture (0.130 g, 9%). $[\alpha]_D$ +7.0 (*c* 1.0, $CHCl_3$) [lit.[19] $[\alpha]_D$ +10.5 (*c* 1.0, $CHCl_3$)]; R_f 0.80 (1:3 EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$): δ 7.41–7.27 (m, 20H, 4C_6H_5), 5.53 (d, 1H, $J_{1,2}$ 5.0, H-1), 5.03 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.96 and 4.59 (2d, J 11.5 Hz, CH_2Ph), 4.86 and 4.74 (2d, J 11.7 Hz, CH_2Ph), 4.77 (s, 2H, CH_2Ph), 4.57 (dd, 1H, $J_{2,3}$ 2.3, $J_{3,4}$ 7.9 Hz, H-3), 4.50 and 4.42 (2d, J 11.8 Hz, CH_2Ph), 4.33 (dd, 1H, $J_{4,5}$ 1.8 Hz, H-4), 4.31 (dd, 1H, H-2), 4.08–3.50 (m, 9H, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6′a, H-6′b), 1.54, 1.44, 1.34, 1.31 (4s, 12H, 2(CH_3)$_2$C); ^{13}C NMR ($CDCl_3$): δ 139.1, 138.9, 138.2, 128.5, 128.4, 128.3, 127.9, 127.8, 127.6, 127.5 (4C_6H$_5$), 109.3, 108.6 (2C(CH$_3$)$_2$), 97.7 (C-1′), 96.4 (C-1), 79.1 (C-3′), 76.6 (C-2′), 75.1 (C-4′), 74.9, 73.5, 73.2, 72.8 (4CH$_2$Ph), 71.0, 70.8, 70.8 (C-2, C-3, C-5), 69.3 (C-5′), 68.8 (C-6′), 66.5 (C-6), 66.0 (C-4), 26.3, 26.2, 25.1, 24.7 (2C(CH$_3$)$_2$).

Selected values for β-anomer: ^{13}C NMR ($CDCl_3$): δ 104.8 (C-1′), 96.5 (C-1).

3.15. 1,2,3,4-Tetra-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-D-galactopyranose (17)

Compound **16** (1.050 g, 1.34 mmol) was stirred for 30 min in 80% aq TFA. The mixture was concentrated under diminished pressure and coevaporated three times from toluene (3 × 15 mL). The residue was acetylated overnight in a 2:1 pyridine–Ac_2O mixture (15 mL). The soln was concentrated under diminished pressure and the crude residue was purified by column chromatography (2:3 EtOAc–petroleum ether). Compound **17** was obtained as an oily α,β-mixture (0.725 g, 62%). ^{1}H NMR ($CDCl_3$), selected values: δ 7.39–7.27 (m, 20H, 4C_6H_5), 6.38 (d, 1H, $J_{1,2}$ 2.4, H-1α), 5.63 (d, 1H, $J_{1,2}$ 8.2, H-1β), 5.58 (m, 1H, H-4α), 5.48 (br d, 1H, $J_{3,4}$ 3.2 Hz, H-4β), 5.44–5.34 (m, 2H, H-2α, H-3α), 5.32

(dd, 1H, $J_{2,3}$ 10.5 Hz, H-2β), 5.08 (dd, 1H, H-3β); ^{13}C NMR ($CDCl_3$), selected values: δ 98.8 (C-1′ β), 98.3 (C-1′ α), 92.3 (C-1 β), 89.9 (C-1 α). Anal. Calcd for $C_{48}H_{54}O_{15},H_2O$ (888.95): C, 64.85; H 6.35. Found: C, 64.70; H, 6.20.

3.16. 2,3,4-Tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-D-galactopyranosyl trichloroacetimidate (18)

Compound **17** (0.650 g, 0.746 mmol) was stirred for 16 h in THF (3 mL) in the presence of benzylamine (0.127 mL, 1.17 mmol). When TLC showed that all the starting material has been consumed (16 h), the mixture was diluted with CH_2Cl_2 (100 mL), and the organic phase was successively washed with 1 N HCl, satd aq $NaHCO_3$, then with water and dried over Na_2SO_4. After filtration and concentration, the residue was purified by column chromatography (3:4 EtOAc–petroleum ether). The hemiacetal was obtained as a 5:1 α,β-anomeric mixture (0.450 g, 70%); R_f 0.45–55 (3:4 EtOAc–petroleum ether); ^{13}C NMR (α anomer): ($CDCl_3$): δ 170.6, 170.5, 170.1 (3*C*H_3CO), 138.9, 138.6, 138.5, 137.8, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.7, 127.6 (4 *C*$_6$H$_5$), 98.8 (C-1′), 90.5 (C-1), 78.9 (C-3′), 76.7 (C-2′), 75.1 (C-4′), 74.8, 73.6, 73.5, 73.2 (4 *C*H_2Ph), 69.9 (C-5), 69.4 (C-6′), 69.3 (C-5′), 68.8 (C-3), 68.1 (C-6), 67.8, 67.5 (C-2, C-4), 20.9, 20.8, 20.7 (3*C*H_3COO).

Selected values for β-anomer: ^{13}C NMR ($CDCl_3$): δ 99.2 (C-1′), 95.6 (C-1).

DBU (0.10 mL, 3.19 mmol) was added to a soln of hemiacetal (0.430 g, 0.52 mmol) and trichloroacetonitrile (0.32 mL) in CH_2Cl_2. The mixture was stirred for 5 h, and concentrated under diminished pressure. The residue was purified by column chromatography (1:3 EtOAc–petroleum ether). A pure fraction of the α-imidate was recovered first (0.400 g, 79%) followed by an α,β-anomeric mixture (0.052 g, 10%). The pure product **18** was obtained as an oil; $[\alpha]_D$ +66.1 (*c* 1.0, $CHCl_3$); R_f 0.48 (1:3 EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$): δ 8.60 (s, 1H, N*H*), 7.38–7.27 (m, 20H, 4C$_6$*H*$_5$), 6.61 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 5.62 (br d, 1H, $J_{3,4}$ 3.0 Hz, H-4), 5.47 (dd, 1H, $J_{3,4}$ 10.8 Hz, H-3), 5.37 (dd, 1H, H-2), 4.92–4.39 (m, 10H, 4C*H*$_2$Ph, H-1′, H-5), 4.01 (dd, 1H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 10.1 Hz, H-2′), 3.99–3.92 (m, 2H, H-4′, H-5′), 3.85 (dd, 1H, $J_{3',4'}$ 2.5 Hz, H-3′), 3.72 (dd, 1H, $J_{5,6a}$ 6.2, $J_{6a,6b}$ 10.6 Hz, H-6a), 3.59 (dd, 1H, $J_{5,6b}$ 6.2 Hz, H-6b), 3.52 (d, 2H, $J_{5',6'a} = J_{5',6'b}$ 6.5 Hz, H-6′a, H-6′b), 2.09, 2.04, 2.03 (3s, 9H, 3C*H*$_3$COO); ^{13}C NMR ($CDCl_3$): δ 170.2, 170.1, 169.9 (3*C*OCH$_3$), 161.2 (O*C*(NH)CCl$_3$), 139.0, 138.8, 138.6, 138.2, 128.5, 128.4, 128.3, 128.2, 127.9, 127.7, 127.6 (4*C*$_6$H$_5$), 98.3 (C-1′), 93.8 (C-1), 91.0 (*C*Cl$_3$), 78.9 (C-3′), 76.5 (C-2′), 75.2 (C-4′), 74.9, 73.4, 73.4, 73.3 (4 *C*H_2Ph), 70.2 (C-5), 69.7 (C-5′), 68.8 (C-6′), 68.1, 67.8 (C-2, C-3), 67.3 (C-4), 66.5 (C-6), 20.8, 20.7, 20.6 (3*C*H_3CO).

3.17. 3-*O*-[2,3,4-Tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-1,2-*O*-isopropylidene-*sn*-glycerol (19)

A mixture of imidate **18** (0.360 g, 0.37 mmol), 1,2-*O*-isopropylidene-*sn*-glycerol (68 μL, 0.55 mmol) and activated 4 Å molecular sieves (0.400 g) in dry CH_2Cl_2 (4 mL) was stirred for 15 min under argon, before cooling to −30 °C. A soln of trimethylsilyl trifluoromethanesulfonate (10 μL) in CH_2Cl_2 (0.5 mL) was added dropwise and the mixture was stirred for 3 h at −30 °C. The mixture was neutralized by the addition of Et_3N (20 μL) and allowed to reach rt. After filtration on Celite, the solvent was evaporated and the product was purified by column chromatography (1:1 petroleum ether–EtOAc). A second column chromatography (1:20 MeOH–CH_2Cl_2) was necessary in order to remove the trichloroacetamide formed during the reaction. The pure product **19** was obtained as an amorphous solid (0.155 g, 55%); $[\alpha]_D$ +15.9 (*c* 1.0, $CHCl_3$); R_f 0.37 (1:2 EtOAc–petroleum ether), R_f 0.75 (1:20 MeOH–CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.38–7.27 (m, 20H, 4C$_6$*H*$_5$), 5.42 (br d, 1H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.9 Hz, H-4), 5.16 (dd, 1H, $J_{1,2}$ 7.9, $J_{2,3}$ 10.4 Hz, H-2), 4.99 (dd, 1H, H-3), 4.92 and 4.59 (2d, 2H, *J* 11.6 Hz, C*H*$_2$Ph), 4.82 and 4.72 (2d, 2H, *J* 11.6 Hz, C*H*$_2$Ph), 4.77 and 4.66 (2d, 2H, *J* 11.9 Hz, C*H*$_2$Ph), 4.77 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.48 and 4.42 (2d, 2H, *J* 11.7 Hz, C*H*$_2$Ph), 4.41 (d, 1H, H-1), 4.18 (dddd, 1H, $J_{1agl,2gl}$ 6.3, $J_{1bgl,2gl}$ 6.0 $J_{2gl,3agl}$ 4.1, $J_{2gl,3bgl}$ 6.3 Hz, H-2$_{gl}$), 4.02 (dd, 1H, $J_{2',3'}$ 10.0 Hz, H-2′), 3.97–3.92 (m, 4H, H-4′, H-5, H-5′, H-1a$_{gl}$), 3.89 (dd, 1H, $J_{3',4'}$ 2.6 Hz, H-3′), 3.82 (dd, 1H, $J_{3agl,3bgl}$ 10.4 Hz, H-3a$_{gl}$), 3.76 (dd, 1H, $J_{1agl,1bgl}$ 8.2 Hz, H-1b$_{gl}$), 3.68 (dd, 1H, $J_{5,6a}$ 6.0, $J_{6a,6b}$ 10.4 Hz, H-6a), 3.61 (dd, 1H, $J_{5,6b}$ 6.6 Hz, H-6b), 3.55 (dd, 1H, $J_{5',6'a}$ 6.6, $J_{6'a,6'b}$ 9.5 Hz, H-6′a), 3.51 (dd, 1H, H-3b$_{gl}$), 3.46 (dd, 1H, $J_{5',6'b}$ 6.0 Hz, H-6′b), 2.07, 2.07, 1.99 (3s, 9H, 3C*H*$_3$COO), 1.40, 1.33 (2s, 6H, C(C*H*$_3$)$_2$); ^{13}C NMR ($CDCl_3$): δ 170.4, 170.2, 169.7 (3*C*OCH$_3$), 138.8, 138.6, 138.4, 138.1, 128.8, 128.7, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9 (4 *C*$_6$H$_5$), 109.3 (*C*(CH$_3$)$_2$), 101.6 (C-1), 99.3 (C-1′), 79.3 (C-3′), 76.8 (C-2′), 75.5 (C-4′), 75.1, 74.0, 73.8, 73.7 (4 *C*H_2Ph), 74.6 (C-2$_{gl}$), 72.2 (C-5), 71.4 (C-3), 70.3 (C-5′), 69.6 (C-6′), 69.5 (C-3$_{gl}$), 69.4 (C-2), 68.2 (C-4), 67.4 (C-6), 66.7 (C-1$_{gl}$), 26.7, 25.3 (C(*C*H$_3$)$_2$), 20.9, 20.7, 20.7 (3*C*H_3CO). Anal. Calcd for $C_{52}H_{62}O_{16}$ (943.016): C, 66.23; H, 6.63. Found: C, 66.02; H, 6.75.

3.18. Galactolipase activity measurements

The hydrolysis of DGDG, MGDG, diglyceride and phospholipids by GPLRP2 was measured using the monomolecular film technique as previously described.[3]

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