SYNTHESIS OF A 1α,4′-DI-*O*-ALLYLATED, 2,3,2′,3′-TETRA-*O*-TETRADECYLATED LIPID A MIMIC AND ITS 4-*O*-(4-METHOXYBENZYL) PRECURSOR

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Compound **18** mimicking lipid A, containing D-glucose instead of D-glucosamine moieties in its gentiobiose skeleton, *O*-tetradecyl groups at the C-2, C-3, C-2′ and C-3′ instead of the ester- and amide-linked fatty acids, and *O*-allyl groups at the C-1α and C-4′ replacing the phosphate groups, was synthesized by the Schmidt trichloroacetamidate method in a combined 8% yield of 13 steps. Allyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside (**1**) and methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**4**) were starting materials for preparation of the respective *O*-alkylated and *O*-allylated glycosyl donor and sugar nucleophile. While boron trifluoride etherate in dichloromethane catalysed a highly preferential formation of the required β-(1→6)-glycosidic bond, α-linked lipidodisaccharide was a major product when trimethylsilyl trifluoromethanesulfonate was used as a catalyst, in both cases independently of the anomeric configuration of the starting imidate. Prolonged treatment with acid catalysts in the coupling step was exploited also for a one-pot removal of the intermediate 4-*O*-(4-methoxybenzyl) protection of the target mimic **18** of lipid A.

Keywords: Carbohydrates; Lipid A mimics; Trichloroacetimidate synthesis; Disaccharides; Glycosidations; Liposaccharides; Bacterial toxins.

Lipid A (Fig. 1a), the endotoxin responsible for toxicity of lipopolysaccharide (LPS) present in the outer surface membrane of Gram-negative bacteria[1]. The lysis of Gram-negative bacteria causes them to release LPS that can cause various health disorders. One of them is sepsis – a severe inflamatory illness which can lead to a serious organ damage and dysfunction and finally to death of the intensive care unit patients[2]. As there does not exist any effective treatment of the lethal sepsis to date, a search for an effective drug against this disease is inevitably needed.

The basic structure of almost all natural lipid A-related compounds consists of β-(1→6)-glycosidically linked D-glucosamine disaccharide 1,4′-diphosphate with the α-configuration of the anomeric phosphate. Recognized to be essential for the endotoxic activity of lipid A species are also

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several, usually six fatty acid chains per the disaccharide[3]. The asymmetric, (4+2) distribution of these fatty chains (Fig. 1a), together with the other structural features, results in an overall conical shape of the molecule that has been related to the strongly pro-inflamatory, LPS-agonistic activity of lipid A[4]. Less or no pro-inflamatory properties are characteristic for lipid A species with respective (3+2) or (2+2) distribution of the fatty chains. The interaction of pyrogenic lipid A species with their TLR4 binding site mediated by LBP, CD14 and MD-2 is believed to induce production of inflamatory mediators, including cytokines, e.g., TNFα[5–8]. High levels of the released cytokines cause severe sepsis.

Since Shiba and Kusumoto's total synthesis of the most toxic *Escherichia coli* lipid A[9,10], many synthetic lipid A mimics have been prepared with both *E. coli* LPS-agonistic and antagonistic properties. In some of them D-glucose replaces D-glucosamine at the reducing end and/or at the non-reducing end[11–13]. Especially inspiratory for the syntheses of such bis-1α,4′-*O*-phosphorylated LPS-antagonists was finding of a natural lipid A-related compound (RsDPLA, Fig. 1b) isolated from *Rhodobacter sphaeroides* and behaving as an *E. coli* LPS-antagonist[14,15]. Based on that, a compound related to RsDPLA was developed as a potent anti-septicemia drug candidate, E5564 (eritoran, Fig. 1c)[16], which passed phase II trial successfully. Eventually, the belief that the *E. coli* LPS-antagonistic lipid A mimics have to contain phosphates or other anionic groups was abandoned when *Rhizobium sin*-1 lipid A (Fig. 1d) was isolated[17]. While this unusual molecule with (3+2) distribution of the fatty chains is devoided of 4′-*O*-phosphoryl group and contains 2-amino-2-deoxy-D-glucopyranolactone instead of a usual D-glucosamine-1α-phosphate unit, it significantly inhibits *E. coli* LPS-dependent synthesis of TNFα[18]. A slightly weaker inhibition was observed with a synthetic analogue of *Rhizobium sin*-1 lipid A with (2+2) distribution of the fatty chains[18].

In our approach towards potential *E. coli* LPS-antagonistic lipid A mimics we synthesize simplified structures of *E. coli* lipid A, in which its enzymatically hydrolyzable linkages are substituted by the enzymatically non-hydrolyzable bonds, using gentiobiose as the sugar moiety of the mimics. The admittance of the substitution of the D-glucosamine units (or 2,3-diamino-2,3-dideoxy-D-glucose units, which occur in some lipid A structures as well[19]) of the lipid A disaccharide with D-glucose units is supported, e.g., by the observation that the substitution of the Vi-capsular polysaccharide in vaccines with per-*O*-acetylated pectin has not caused a change in the immunogenicity of these vaccines[20]. (Vi-capsular polysaccharide differs from pectin by *N*-acetylation on C-2 and *O*-acetylation on C-3.) Thus,

we have prepared methyl 6-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-tetradecyl-β-D-glucopyranosyl)-4-*O*-(4-methoxybenzyl)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside – the first tetra-*O*-alkylated mimic of native lipid A molecule synthesized[21], which, after its *O*-deacetylation (Fig. 1e), exhibits a remarkable *E. coli* LPS-antagonistic activity[22], though it does not contain any anionic group. Similar *E. coli* LPS-antagonistic activities have been reported for its de-*O*-4-methoxybenzylated derivative (Fig. 1f) and a glycosylamino-linked analogue (Fig. 1g)[23]. The antagonistic activity shown by the last two disaccharides specifically involved the TLR4 receptor.

In this paper we describe an expeditious synthesis of another tetra-*O*-fatty-alkylated gentiobiose **18** and its 4-*O*-(4-methoxybenzyl) precursor **17** as two other potential *E. coli* LPS-antagonists (Figs 1h, 1i), which are,

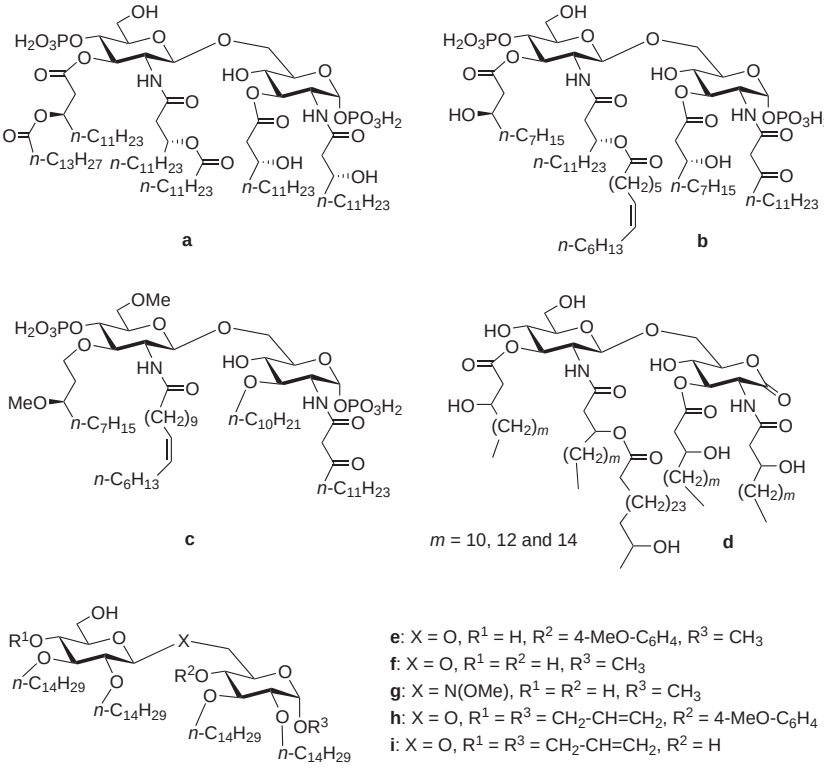
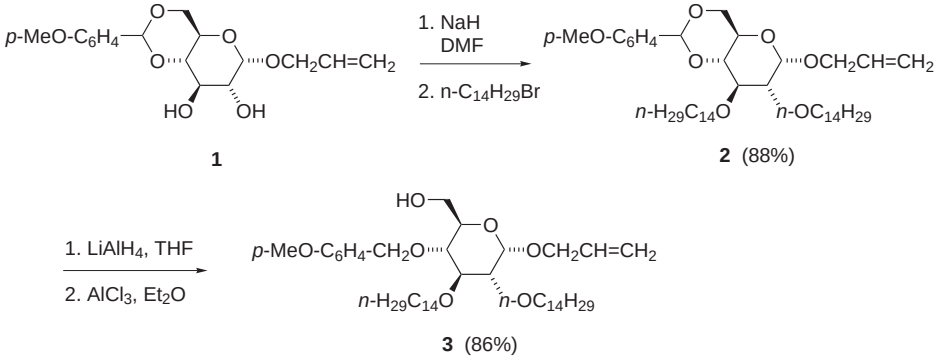


FIG. 1

Structures of some natural lipid A molecules and *E. coli* LPS-antagonists. a *Escherichia coli* lipid A; b *Rhodobacter sphaeroides* lipid A (RsDPLA); c E5564 (eritoran, synthetic *E. coli* LPS-antagonist); d *Rhizobium sin*-1 lipid A; e–g gentiobiose-based tetra-*O*-tetradecylated *E. coli* LPS-antagonists; h, i compounds **17** and **18**

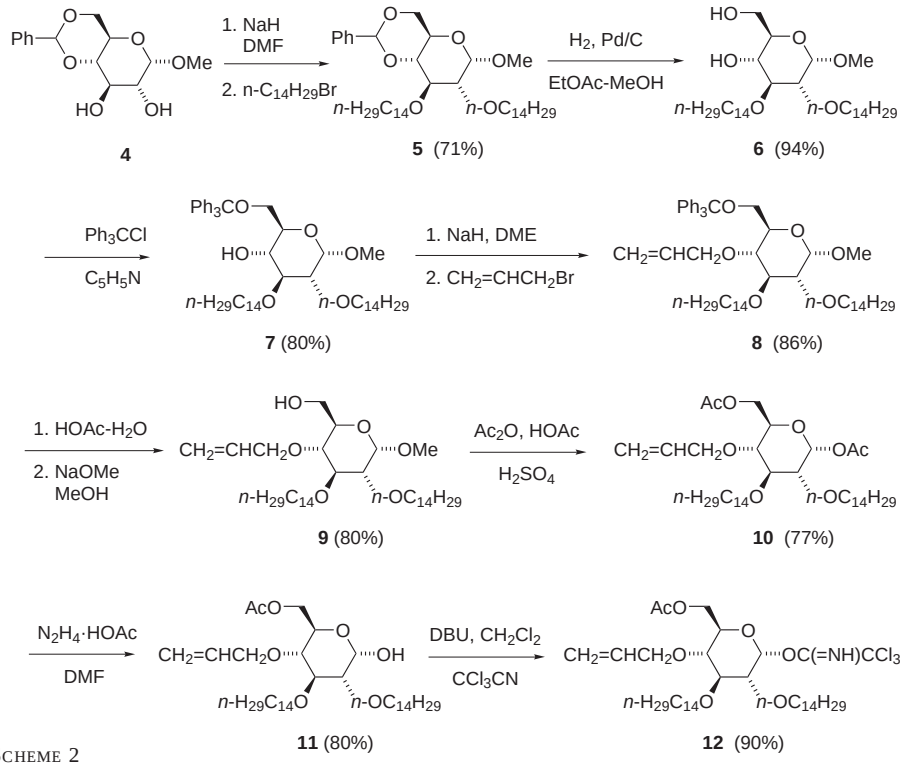
moreover, substituted at their C-1α and C-4′ hydroxy groups with allyl groups, mimicking phosphates and offering possibilities of their further transformations into other groups.

A synthetic approach based on coupling nucleophile **3** and glycosyl donor **12** was used for preparation of the target disaccharides **17** and **18**. For synthesis of **3** (Scheme 1), starting allyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside[24] (**1**), which already contains the required allyl group, was treated with NaH and tetradecyl bromide in DMF to give diether **2**. Its two fatty ether chains on C-2 and C-3 were detected in the ^{13}C NMR spectrum due to two new signals of the O-CH_2 groups at two of three chemical shifts (δ in ppm) 73.5, 71.0 and 70.8 (one of the signals belongs to C-5) as well as due to the signals of the other fatty alkyl chain carbons at δ 14.1–31.9. The *O*-alkylation appeared in the ^1H NMR spectrum of **2** as a multiplet at δ 3.59–3.85 (together with the skeletal signals of H-6a and H-6b), a broad doublet at δ 1.56, a broad singlet at δ 1.26 and a triplet at δ 0.88. Reductive opening of the 4,6-*O*-(4-methoxybenzylidene) protecting group of **2** with $LiAlH_4/AlCl_3$ afforded crystalline nucleophile **3** in an overall 76% yield based on **1**. Diagnostic for the conversion was the appearance of new signals of the (4-methoxybenzyl)methylene group at δ 77.1 and at δ 4.58 and 4.82 in its respective ^{13}C and ^1H NMR spectra, under a simultaneous disappearance of the 4-methoxybenzylidene acetal atom signals of the reaction substrate at δ 101.9 and δ 5.50. A broad singlet at δ 2.02 in the ^1H NMR spectrum confirmed also the presence of a free OH group at C-6 of nucleophile **3**.



SCHEME 1

The starting compound for the synthesis of glycosyl donor **12** (Scheme 2) was methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[25] (**4**), which was alkylated by the same procedure as compound **1**; the known fatty diether **5** was obtained. The 4,6-*O*-benzylidene group of **5** was deprotected by hydrogenolysis on Pd/C in a mixture of CH_2Cl_2 and MeOH to yield diol **6**. Both ^{13}C and ^{1}H NMR spectra of **6** documented disappearance of the signals of the benzylidene aromatic and acetal atoms under shifting the skeletal C-4 and C-6 signals to the values δ 69.4 and 62.4, respectively. A subsequent one-mol tritylation of **6** with triphenylmethyl chloride in dry pyridine resulted in protection of its C-6 hydroxy group. This was evident again in both ^{13}C and ^{1}H NMR spectra of **6** as the respective sets of the phenyl group signals at δ 127.0–143.9 and δ 7.21–7.50. Treatment of compound **7** with NaH and allyl bromide in DMF finally afforded the designed 4-*O*-allylation of the glycosyl donor precursor **8**. In the ^{13}C NMR spectrum of **8**, a set of three characteristic signals at δ 134.8, 117.0 and 78.0 appeared for the 4-*O*-allyl group, while the most characteristic in the ^{1}H NMR spectrum was a fourth-order multiplet of the allyl CH proton at δ 5.53. Standard



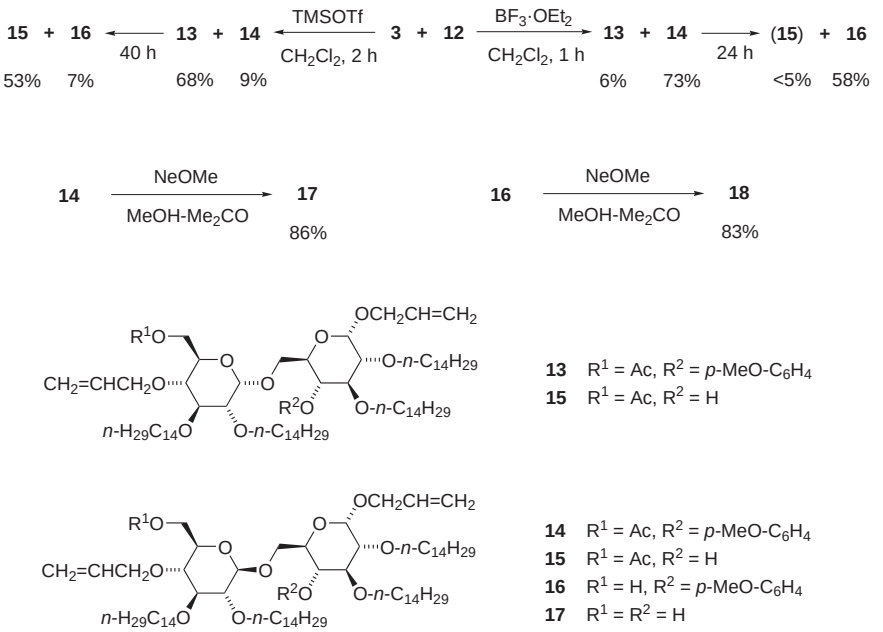
SCHEME 2

detritylation of **8** followed by acetolysis of intermediate **9** gave crystalline 1,6-diacetate **10**. In addition to the obvious skeletal carbon signals, compound **10** contained in its ^{13}C NMR spectrum only sets of substitution signals of two tetradecyl ether chains at δ 74.0, 73.8 and 14.1–32.0, two acetoxy groups at δ 170.7, 169.3, 21.0 and 20.8, and allyl ether group at δ 134.5, 117.5 and 77.2. The required structure of compound **10** was confirmed also by its ^1H NMR spectrum, which contained two characteristic, three-proton acetate singlets at δ 2.11 and 2.06, in addition to the clear signal patterns of the *O*-allyl group at δ 5.88, 5.12–2.28 and 4.02–4.35 as well as those of *O*-tetradecyl groups, which were similar to the signal patterns of all the previous di-*O*-tetradecylated precursors. The value of $J_{1,2}$ = 3.4 Hz characteristic of α-glucopyranosyl anomer completed the NMR structure assignement of compound **10**. All the six synthetic steps gave good to high yields of the corresponding products so that the overall yield of **10** from **4** was 30%.

Initially, the Koenigs–Knorr or Helferich method[26], based on using a corresponding glycosyl bromide that was supposed to be prepared from diacetate **10**, was intended to be used for coupling with nucleophile **3** as the key step of the synthesis of the target lipodisaccharide **18**. However, a standard treatment of diacetate **10** with hydrogen bromide–acetic acid solution[27] did not lead to its satisfactory, unambiguous conversion to the expected glycosyl bromide, and a mixture of several products was formed. A probable reason for the behaviour was the 4-*O*-allyl substitution of the bromide precursor since allyl ethers are known to be unstable in the presence of HBr[28]. Therefore, the initial, glycosyl bromide strategy was abandoned and a trichloroacetimidate alternative, known as the Schmidt method[29], was used for the coupling.

Hemiacetal **11**, used for the alternative approach, was obtained in 80% yield from compound **10** by regioselective *O*-deacetylation with hydrazine acetate in DMF[30]. The presence of a free hemiacetal OH group in its structure was evident from ^1H NMR spectrum. In addition to the characteristic C-6 acetate three-proton singlet at δ 2.09, it contained two well resolved doublets of α- and β-anomeric protons at δ 5.28 and 4.60 with coupling constants $J_{1,2}$ = 3.1 and 7.6 Hz, respectively, and with their total integral intensity corresponding to one proton. Following activation of **11** with trichloroacetonitrile in dichloromethane using 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) as a base afforded the isolated 90% yield of α-glycosyl imidate **12**, characterized by its coupling constant $J_{1,2}$ = 3.4 Hz (δ 6.43), which was immediately coupled with nucleophile **3**.

Two distinct catalysts for coupling of glycosyl acceptor **3** with imidate **12** were used. Thus, a commonly used strongly acid catalyst, trimethylsilyl trifluoromethanesulfonate (TMSOTf) in dichloromethane led to preferential formation of α-(1→6)-glycosidic bond between the coupling partners, disaccharide **13** being a major product of this glycosylation (Scheme 3). On the other hand, a relatively weak Lewis acid catalyst, boron trifluoride etherate ($BF_3 \cdot OEt_2$) used in the same solvent, supported the highly preferential formation of β-(1→6)-glycosidic bond. As a result, the required disaccharide **14** was isolated from the reaction mixture in a good (73%) yield. Moreover, it has to be also stressed that, in contrast to the coupling catalysts used, the anomeric configuration of glycosyl trichloroacetimidate in the activation step did not influence the final anomeric configuration of the glycosidic bond in the course of these glycosylations. Thus, practically the same ratios and yields of disaccharides **13** and **14**, depending on the aforementioned catalysts only, were obtained when nucleophile **3** was coupled either with α-imidate **12** or with 6-*O*-acetyl-4-*O*-allyl-2,3-di-*O*-tetradecyl-β-D-glucopyranosyl trichloroacetimidate, which was generated in dichloromethane from trichloroacetonitrile and K_2CO_3 as a base, i.e., under the conditions providing β-D-glucopyranosyl trichloroacetimidates[29].



SCHEME 3

Because of the acid-labile 4-methoxybenzyl group present on the C-4 carbon of nucleophile **3** and the application of the acid coupling catalysts, also the reaction time of the glycosylation step played an important role. In the 1-h glycosylation reaction of compounds **3** and **12** in dichloromethane using $BF_3 \cdot OEt_2$ as catalyst, the only products were disaccharides **13** and **14**. However, when the reaction time was prolonged to 2 h, also disaccharide **15** was detected by TLC in the reaction mixture. After 24 h, the removal of the 4-methoxybenzyl group from disaccharide **14** was complete and disaccharide **15**, obtained in a 58% isolated yield, was the only significant product present in the final reaction mixture. A similar phenomenon of de-*O*-4-methoxybenzylation in a prolonged reaction time was observed also when TMSOTf was used as a catalyst for coupling **3** and **12** in dichloromethane.

The last step of the synthesis was *O*-deacetylation of disaccharides **14** and **16**. This was accomplished by standard Zemplen deacetylation and the respective targets, β-(1→6)-linked disaccharides **17** and **18**, were obtained in the 86 and 83% yields, respectively.

The anomeric configurations of the lipidodisaccharides **13**–**18** were ascribed by ^{13}C and ^{1}H NMR spectroscopy. Thus, e.g., in the ^{13}C NMR spectra, the anomeric signals of **13** were observed at δ 98.2 and 95.2 and those of **14** at δ 104.0 and 95.2. The chemical shifts of the former signals of both the anomeric carbon atom pairs are the characteristic values for the respective α and β anomeric carbon atoms C-1′ of the glycosidic bond of 1→6 linked glucopyranobioses[31], while both the latter signals belong to the anomeric carbons C-1 bearing the α-glycosidically linked allyl group introduced into the structures of lipidodisaccharides **13** and **14** by the coupling sugar nucleophile **3**. Even more stereochemically diagnostic for the determination of the anomeric configurations were ^{1}H NMR spectra with their well resolved H-1 and H-1′ signals. Thus, the value of coupling constant $J_{1',2'}$ = 3.8 Hz ($\delta_{H\text{-}1'}$ 4.75) in the case of disaccharide **13** indicates its isomaltose α-glycosidic bond, and correspondingly, the value of coupling constant $J_{1',2'}$ = 7.6 Hz ($\delta_{H\text{-}1'}$ 4.32) in the case of disaccharide **14** is typical of a gentiobiose β-glycosidic bond. The allyl α-glycosidic configuration of both disaccharides **13** and **14** is, in both cases, characterized by the vicinal coupling constants $J_{1,2}$ = 3.5 Hz ($\delta_{H\text{-}1}$ 4.95). Anomeric NMR spectral characteristics of disaccharides **16**–**18** were very similar to those observed for disaccharide **14**. Practically the same were also the anomeric NMR spectral characteristics of disaccharides **13** and **15**.

In the NMR spectra of disaccharides **13**–**18**, the characteristic patterns of all their other structural features, skeletal and substitutional, were observed as well. Again, they were very similar to those described above in the perti-

nent sections of NMR structure proofs of the synthesis of the monosaccharide precursors of the disaccharides. In addition to the NMR analyses, the structures of all the products of all the conversions described were checked by evaluating their molecular weights by MALDI-TOF mass spectrometry as well as by elemental analysis.

EXPERIMENTAL

All reactions using air or moisture-sensitive reagents were conducted in nitrogen or argon atmosphere. Commercial solvents were dried and distilled prior to use. Melting points were determined on a Kofler hot stage. Optical rotations were measured at 20 °C in $CHCl_3$, at *c* 1.0 g ml^{-1}, on a Perkin–Elmer 141 polarimeter; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Elemental analyses were performed with a Fisons EA 1108 analyser. All reactions were monitored by TLC on glass plates precoated with silica gel (Kiesegel G, Merck) by spraying the chromatograms with a 10% sulfuric acid in ethanol and charring them on a hot plate. Preparative chromatography was performed on dry-packed silica gel (Kieselgel 60, 0.063–0.200 mm, Merck) equilibrated, prior to packing, with 40% of the mobile phase. NMR spectra (δ, ppm; *J*, Hz) were recorded at 20 °C on a Bruker Avance DPX 300 spectrometer (300.13 MHz, internal standard sodium 3-(trimethylsilyl)propanoate, δ 0.00 for ^1H and 75.47 MHz, internal standard methanol, δ 50.15 for ^{13}C). Homo and hetero nuclear correlation spectroscopy experiments were performed as well. Mass spectra were obtained with a Shimadzu-Kratos Analytical MALDI TOF IV instrument (matrix 2,5-dihydroxybenzoic acid).

Allyl 4,6-*O*-(4-Methoxybenzylidene)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (**2**)

Allyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside[24] (**1**) (1 g, 2.96 mmol) was dissolved in DMF (40 ml), the solution was cooled to 0 °C, and NaH (0.6 g, 25 mmol) was slowly added to the solution. Tetradecyl bromide (6 ml, 20 mmol) was then added dropwise over a period of 20 min. The solution was warmed slowly to 60 °C and stirred overnight. After cooling to room temperature, methanol (5 ml) was slowly added to decompose an excess of sodium hydride. Solvents were then evaporated under reduced pressure and the residue was diluted with ethyl acetate (50 ml), washed with saturated aqueous solution of citric acid (3 × 35 ml), with water (3 × 35 ml), dried (anhydrous Na_2SO_4) and evaporated. Per-*O*-substituted sugar **2** (1.9 g, 88%) was isolated by crystallization from ethyl acetate–methanol (1:3). M.p. 63–65 °C; $[\alpha]_D$ +36. For $C_{45}H_{78}O_7$ (731.1) calculated: 73.93% C, 10.75% H; found: 73.77% C, 10.63% H. ^1H NMR ($CDCl_3$): 7.41 (d, 2 H, *J* = 8.4, H_{arom}); 6.88 (d, 2 H, H_{arom}); 5.93 (m, 1 H, CH_2=C**H**CH_2-O); 5.50 (s, 1 H, CHAr); 5.19–5.38 (m, 2 H, C**H**$_2$=CHCH_2-O); 4.95 (d, 1 H, $J_{1,2}$ = 3.7, H-1); 4.22 (dt, 1 H, $J_{4,5}$ = $J_{5,6a}$ = 9.9, $J_{5,6b}$ = 4.7, H-5); 4.18 (dd, 1 H, J_1 = 5.1, CH_2=CHC**H**H-O); 4.08 (dd, 1 H, J_1 = 6.6, J_2 = 13.0, CH_2=CHCH**H**-O); 3.55–3.89 (m, 7 H, H-3, H-6a, H-6b, 2 CH_2C**H**$_2$-O); 3.80 (s, 3 H, CH_3O); 3.48 (t, 1 H, $J_{3,4}$ = 9.3, H-4); 3.33 (dd, 1 H, $J_{2,3}$ = 9.3, H-2); 1.56 (bd, 4 H, 2 C**H**$_2$$CH_2$-O); 1.26 (bs, 44 H, 2 (C**H**$_2$)$_{11}$(CH_2)$_2$-O); 0.88 (t, 6 H, *J* = 7.2, 2 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 159.6 (C_{arom}); 133.7 (CH_2=**C**HCH_2-O); 132.0 (2 C_{arom}); 129.8 (C_{arom}); 118.3 (**C**H_2=CHCH_2-O); 114.3 (2 C_{arom}); 101.9 (CHAr); 95.5 (C-1); 80.9 (C-4); 80.6 (C-2); 73.5 (C-3); 71.0, 70.8, 70.7 (C-5, 2 CH_2**C**H_2-O); 68.2 (CH_2=CH**C**H_2-O); 62.7 (C-6); 55.6 (CH_3O); 31.9, 30.4, 30.0, 29.7, 29.4, 26.1, 22.1 (2 (**C**H_2)$_{12}$$CH_2$-O); 14.1 (2 **C**$H_3$$CH_2$). MS (MALDI-TOF), *m/z*: 756.6 [M + Na^+], 772.1 [M + K^+].

Allyl 4-*O*-(4-Methoxybenzyl)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (**3**)

Solution of 1 M $LiAlH_4$ in tetrahydrofuran (11.5 ml) was added dropwise at room temperature to a stirred solution of **2** (1.5 g, 2.05 mmol) in a mixture of dichloromethane–diethyl ether (1:2, 90 ml). Then, a solution of $AlCl_3$ (1.6 g, 12 mmol) in diethyl ether (70 ml) was added dropwise and the reaction mixture was refluxed for another 4 h. The mixture was cooled to room temperature, diluted with ethyl acetate (300 ml), washed with water (3 × 400 ml), dried with anhydrous Na_2SO_4 and concentrated. The residue was purified on a silica gel column with hexane–ethyl acetate (3:1) as eluent giving pure compound **3** (1.3 g, 86.5%). M.p. 62–64 °C (ethyl acetate–methanol, 1:3); $[\alpha]_D$ +51. For $C_{45}H_{80}O_7$ (733.1) calculated: 73.72% C, 11.00% H; found: 73.80% C, 10.87% H. ^{1}H NMR ($CDCl_3$): 7.27 (d, 2 H, J = 7.9, H_{arom}); 6.87 (d, 2 H, H_{arom}); 5.90 (m, 1 H, CH_2=C**H**CH_2-O); 5.15–5.35 (m, 2 H, C**H**$_2$=CHCH_2-O); 4.92 (d, 1 H, $J_{1,2}$ = 2.5, H-1); 4.82 (d, 1 H, J = 10.6, C**H**HAr); 4.58 (d, 1 H, CH**H**Ar); 4.14 (dd, 1 H, CH_2=CHC**H**H-O); 4.04 (dd, 1 H, CH_2=CHCH**H**-O); 3.52–3.93 (m, 8 H, H-3, H-5, H-6a, H-6b, 2 CH_2C**H**$_2$-O); 3.79 (s, 3 H, CH_3O); 3.42 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.2, H-4); 3.28 (dd, 1 H, $J_{2,3}$ = 9.1, H-2); 2.02 (bs, 1 H, **H**OCH_2); 1.60 (bd, 4 H, 2 C**H**$_2$$CH_2$-O); 1.26 (bs, 44 H, 2 (C**H**$_2$)$_{11}$(CH_2)$_2$-O); 0.88 (t, 6 H, J = 7.2, 2 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 159.3 ($C_{arom}$); 133.7 ($CH_2$=CHC$H_2$-O); 130.4 ($C_{arom}$); 129.7 (2 C_{arom}); 118.0 (CH_2=CHCH_2-O); 113.8 (2 C_{arom}); 95.4 (C-1); 81.6 (C-3); 80.7 (C-2); 77.1(C-4); 74.5 (CH_2Ar); 73.6, 70.7 (2 CH_2CH_2-O); 71.4 (C-5); 68.0 (CH_2=CHCH_2-O); 61.9 (C-6); 55.2 (CH_3O); 31.9, 30.6, 30.0, 29.7, 29.4, 29.3, 26.2, 22.0, 22.6 (2 (CH_2)$_{12}$$CH_2$-O); 14.0 (2 C$H_3$$CH_2$). MS (MALDI-TOF), *m/z*: 758.0 [M + Na^+], 774.9 [M + K^+].

Methyl 2,3-Di-*O*-tetradecyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**5**)

Compound **5** was synthesized, starting from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[25] (**4**) (5 g, 17.7 mmol), in the same way as compound **2**. Purification of the obtained crude product on silica gel column with hexane–ethyl acetate (12:1) as eluent gave pure compound **5** (9 g, 71.5%). M.p. 79–80.5 °C; $[\alpha]_D$ +26 (lit.[21] m.p. 79.5–80.5 °C; $[\alpha]_D$ +26).

Methyl 2,3-Di-*O*-tetradecyl-α-D-glucopyranoside (**6**)

To a suspension of **5** (5 g, 7.4 mmol) in ethyl acetate (140 ml) and methanol (210 ml) was added 10% Pd/C (0.15 g) in methanol (25 ml) and the mixture was stirred at room temperature under hydrogen for 2 h. After filtration and evaporation of the solvents, the crude material was purified on silica gel column with hexane–ethyl acetate (3:1) as an eluent to obtain pure **6** (4.08 g, 94%). M.p. 75–76 °C (methanol); $[\alpha]_D$ +45. For $C_{35}H_{70}O_6$ (586.9) calculated: 71.62% C, 12.02% H; found: 71.55% C, 11.93% H. ^{1}H NMR ($CDCl_3$): 4.80 (d, 1 H, $J_{1,2}$ = 3.3, H-1); 3.76–3.96 (m, 3 H, H-5, H-6a, H-6b); 3.48–3.65 (m, 6 H, H-3, H-4, 2 CH_2C**H**$_2$-O); 3.42 (s, 3 H, CH_3O); 3.28 (dd, 1 H, $J_{2,3}$ = 9.0, H-2); 2.53 (bs, 1 H, OH); 2.03 (bs, 1 H, OH); 1.58 (bd, 4 H, 2 C**H**$_2$$CH_2$-O); 1.26 (bs, 44 H, 2 (C**H**$_2$)$_{11}$(CH_2)$_2$-O); 0.88 (t, 6 H, 2 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 98.1 (C-1); 80.4 (C-3); 80.2 (C-2); 73.1 (C-4); 71.7, 70.5, 67.6 (C-5, 2 CH_2CH_2-O); 63.3 (C-6); 55.2 (OCH_3); 31.7, 31.4, 30.2, 30.1, 30.0, 29.9, 29.7, 29.2, 26.1, 26.0, 22.7 (2 (CH_2)$_{12}$$CH_2$-O); 13.8 (2 C$H_3$$CH_2$). MS (MALDI-TOF), *m/z*: 611.3 [M + Na^+], 627.9 [M + K^+].

Methyl 2,3-Di-*O*-tetradecyl-6-*O*-(triphenylmethyl)-α-D-glucopyranoside (**7**)

Compound **6** (6 g, 10.2 mmol) was dissolved in dry pyridine (18 ml), trityl chloride (4.3 g, 15.4 mmol) was added and the reaction mixture was stirred at 90 °C for 3 h. The mixture was poured to an ice-cold solution of $NaHCO_3$, the yellow precipitate was collected by filtration, dissolved in chloroform (200 ml) and successively washed with an ice-cold 1 M H_2SO_4, saturated aqueous $NaHCO_3$ and water (3 × 50 ml each). The organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified on silica gel column first eluted with toluene followed by a toluene–acetone (10:1) mixture to afford compound **7** (6.8 g, 80%) as a colorless syrup. $[\alpha]_D$ +25. For $C_{54}H_{84}O_6$ (829.2) calculated: 78.21% C, 10.21% H; found: 78.08% C, 9.98% H. ^{1}H NMR ($CDCl_3$): 7.47 (d, 6 H, H_{arom}); 7.18–7.32 (m, 9 H, H_{arom}); 4.82 (d, 1 H, $J_{1,2}$ = 3.4, H-1); 3.54–3.92 (m, 8 H, H-4, H-5, H-6a, H-6b, 2 CH_2CH_2-O); 3.44 (s, 3 H, CH_3O); 3.26–3.42 (m, 2 H, H-2, H-3); 1.57 (bd, 4 H, 2 CH_2CH_2-O); 1.26 (bs, 44 H, 2 $(CH_2)_{11}(CH_2)_2$-O); 0.82 (t, 6 H, 2 CH_3CH_2). ^{13}C NMR ($CDCl_3$): 143.9, 128.7, 127.8, 127.0 (C_{arom}); 98.0 (C-1); 86.8 (CPh_3); 81.2, 80.7 (C-2, C-3); 77.2 (C-4); 73.7, 71.3, 70.0 (C-5, 2 CH_2CH_2-O); 63.9 (C-6); 55.0 (CH_3O); 31.9, 30.4, 30.1, 29.7, 29.4, 26.1, 26.0, 22.7 (2 $(CH_2)_{12}CH_2$-O); 14.1 (2 CH_3CH_2). MS (MALDI-TOF), *m/z*: 853.8 [M + Na^+], 877.1 [M + K^+].

Methyl 4-*O*-Allyl-2,3-di-*O*-tetradecyl-6-*O*-(triphenylmethyl)-α-D-glucopyranoside (**8**)

To a solution of **7** (3 g, 3.6 mmol) in dry 1,2-dimethoxyethane (21 ml) was added NaH (0.45 g, 18.7 mmol) at 0 °C. Then allyl bromide (0.85 ml, 9.8 mmol) was added dropwise and the mixture was stirred at room temperature for 2 h. Methanol (10 ml) was added and, after cooling, the reaction mixture was neutralized with dilute acetic acid (1:1) and evaporated. The residue was diluted with chloroform and water, and the water layer was washed with $CHCl_3$ (3 × 100 ml). The collected organic layers were dried with anhydrous Na_2SO_4, evaporated and the residue was purified on a silica gel column with toluene as an eluent giving pure syrup **8** (2.7 g, 86%). $[\alpha]_D$ +41. For $C_{57}H_{88}O_6$ (869.3) calculated: 78.75% C, 10.20% H; found: 78.86% C, 10.07% H. ^{1}H NMR ($CDCl_3$): 7.49 (d, 6 H, H_{arom}); 7.17–7.32 (m, 9 H, H_{arom}); 5.53 (m, 1 H, CH_2=C**H**CH_2-O); 4.90–5.01 (m, 2 H, C**H**$_2$=CHCH_2-O); 4.86 (d, 1 H, $J_{1,2}$ = 3.43, H-1); 4.09 (dd, 1 H, CH_2=CHC**H**H-O); 3.73–3.85 (m, 2 H, H-4, CH_2=CHCH**H**-O); 3.54–3.73 (m, 5 H, H-5, 2 CH_2C**H**$_2$-O); 3.44 (s, 3 H, OCH_3); 3.33–3.42 (m, 3 H, H-2, H-3, H-6a); 3.12 (dd, 1 H, $J_{5,6b}$ = 4.4, $J_{6a,6b}$ = 10.2, H-6b); 1.58 (bd, 4 H, 2 C**H**$_2$$CH_2$-O); 1.25 (bs, 44 H, 2 (C**H**$_2$)$_{11}$$(CH_2)_2$-O); 0.88 (t, 6 H, 2 C**H**$_3$$CH_2$).^{13}C NMR ($CDCl_3$): 144.0 ($C_{arom}$); 134.8 ($CH_2$=CHC**H**$_2$-O); 128.8, 127.7, 126.9 (C_{arom}); 116.7 (C**H**$_2$=CHC**H**$_2$-O); 97.9 (C-1); 86.2 (CPh_3); 81.8 (C-3); 80.8 (C-2); 78.0 (C-4); 73.8, 71.7 (2 CH_2C**H**$_2$-O); 73.7 (CH_2=CHC**H**$_2$-O); 70.2 (C-5); 62.5 (C-6); 54.8 (OCH_3); 31.9, 30.5, 30.1, 29.7, 29.3, 26.2, 26.0, 22.7 (2 $(CH_2)_{12}CH_2$-O); 14.1 (2 CH_3CH_2). MS (MALDI-TOF), *m/z*: 894.2 [M + Na^+], 911.1 [M + K^+].

Methyl 4-*O*-Allyl-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (**9**)

Compound **8** (5 g, 5.75 mmol) was dissolved in acetic acid (25 ml), water (7 ml) was added dropwise at 90 °C and the mixture was stirred at this temperature for 5 h. The reaction mixture was evaporated with a water–toluene mixture (3 × 30 ml) and the residue, free of acetic acid, was diluted with methanol (50 ml). Several drops of 1 M solution of MeONa were added and the mixture was stirred at room temperature for 30 min, neutralized with Dowex

50W (H^+), filtered and concentrated. The crude material obtained was recrystallized from acetone–diisopropyl ether to provide pure compound **9** (2.9 g, 80%). M.p. 50–51 °C; $[\alpha]_D$ +61. For $C_{38}H_{74}O_6$ (627.0) calculated: 72.88% C, 11.91% H; found: 72.80% C, 11.80% H. ^{1}H NMR ($CDCl_3$): 5.91 (m, 1 H, CH_2=C**H**CH_2-O); 5.14–5.32 (m, 2 H, C**H**$_2$=CHCH_2-O); 4.75 (d, 1 H, $J_{1,2}$ = 3.5, H-1); 4.33 (dd, 1 H, CH_2=CHC**H**H-O); 4.13 (dd, 1 H, CH_2=CHCH**H**-O); 3.56–3.84 (m, 8 H, H-3, H-5, H-6a, H-6b, 2 CH_2C**H**$_2$-O); 3.39 (s, 3 H, CH_3O); 3.31 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.4, H-4); 3.23 (dd, 1 H, $J_{2,3}$ = 9.6, H-2); 1.75 (bs, 1 H, OH); 1.58 (bd, 4 H, 2 C**H**$_2$$CH_2$-O); 1.25 (bs, 44 H, 2 (C**H**$_2$)$_{11}$(CH_2)$_2$-O); 0.88 (t, 6 H, 2 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 134.8 ($CH_2$=C**H**$CH_2$-O); 117.0 (C**H**$_2$=CH$CH_2$-O); 98.1 (C-1); 81.5 (C-3); 80.7 (C-2); 77.4 (C-4); 73.8 (CH_2=CH**C**H_2-O); 73.7, 71.8 (2 CH_2C**H**$_2$-O); 70.6 (C-5); 62.0 (C-6); 55.1 (CH_3O); 31.9, 30.5, 30.1, 29.7, 29.5, 29.3, 26.3, 26.2 (2 (**C**H_2)$_{12}$$CH_2$-O); 14.1 (2 **C**$H_3$$CH_2$). MS (MALDI-TOF), *m/z*: 651.8 [M + Na^+], 666.9 [M + K^+].

1,6-Di-*O*-acetyl-4-*O*-allyl-2,3-di-*O*-tetradecyl-α-D-glucopyranose (**10**)

A stirred suspension of **9** (3.5 g, 5.58 mmol) in acetic acid (50 ml) and acetic anhydride (75 ml) was cooled to –20 °C and concentrated H_2SO_4 (0.5 ml) was added dropwise over a period of 20 min. The mixture was warmed slowly to room temperature, stirred for 4 h and then poured into ice water (600 ml). The suspension was stirred for 3 h and compound **10** was extracted from the mixture with chloroform (3 × 100 ml). The combined extract was washed with a saturated aqueous $NaHCO_3$ solution (3×), water (2×), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel with a hexane–ethyl acetate (10:1) eluent yielding pure compound **10** (3 g, 77%), which crystallized from an diisopropyl ether–hexane (1:1) mixture. M.p. 44–45 °C; $[\alpha]_D$ +54. For $C_{41}H_{76}O_8$ (697.0) calculated: 70.64% C, 10.99% H; found: 70.85% C, 10.95% H. ^{1}H NMR ($CDCl_3$): 6.25 (d, 1 H, $J_{1,2}$ = 3.4, H-1); 5.88 (m, 1 H, CH_2=C**H**CH_2-O); 5.12–5.28 (m, 2 H, C**H**$_2$=CHCH_2-O); 4.18–4.37 (m, 3 H, CH_2=CHC**H**H-O, H-6a, H-6b); 4.07 (dd, 1 H, CH_2=CHCH**H**-O); 3.27–3.86 (m, 8 H, H-2, H-3, H-4, H-5, 2 CH_2C**H**$_2$-O); 2.11 (s, 3 H, CH_3CO); 2.06 (s, 3 H, CH_3CO); 1.40–1.64 (m, 4 H, 2 C**H**$_2$$CH_2$-O); 1.24 (bs, 44 H, 2 (C**H**$_2$)$_{11}$(CH_2)$_2$-O); 0.87 (s, 6 H, 2 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 170.7, 169.3 (2 CH_3**C**O); 134.5 (CH_2=**C**HCH_2-O); 117.5 (**C**H_2=CHCH_2-O); 89.7 (C-1); 81.3 (C-3); 79.5 (C-2); 77.2 (C-4); 74.0, 71.6 (2 CH_2**C**H_2-O); 73.8 (CH_2=CH**C**H_2-O); 71.0 (C-5); 62.9 (C-6); 32.0, 30.6, 30.0, 29.7, 29.5, 29.4, 26.2, 26.0 (2 (**C**H_2)$_{12}$$CH_2$-O); 21.0, 20.8 (2 **C**$H_3$CO); 14.1 (2 **C**$H_3$$CH_2$). MS (MALDI-TOF), *m/z*: 721.9 [M + Na^+], 738.0 [M + K^+].

6-*O*-Acetyl-4-*O*-allyl-2,3-di-*O*-tetradecyl-α,β-D-glucopyranose (**11**)

Compound **10** (1 g, 1.43 mmol) was dissolved in dry DMF (50 ml) and hydrazine acetate (0.21 g, 2.83 mmol) was added. The reaction mixture was stirred at room temperature in inert atmosphere and, after 90 min, it was diluted with ethyl acetate (50 ml). The organic layer was successively washed with a saturated aqueous $NaHCO_3$ solution, 0.1 M HCl, water (2×), then dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue crystallized from ethanol giving pure **11** (0.75 g, 80%). M.p. 67–68 °C; $[\alpha]_D$ +29. For $C_{39}H_{74}O_7$ (655.0) calculated: 71.51% C, 11.39% H; found: 71.43% C, 11.22% H. Characteristic NMR data for both anomers: ^{1}H NMR ($CDCl_3$): 5.81–5.97 (m, 1 H, (CH_2=C**H**CH_2-O)$_{\alpha,\beta}$); 5.28 (d, 0.63 H, $J_{1,2}$ = 3.1, H-1α); 5.13–5.25 (m, 2 H, C**H**$_2$=CHCH_2-O); 4.60 (d, 0.37 H, $J_{1,2}$ = 7.6, H-1β); 3.00–4.38 (m, 12 H CH_2=CHC**H**$_2$-O, H-2, H-3, H-4, H-5, H-6a, H-6b, 2 CH_2C**H**$_2$-O); 2.09 (s,

3 H, CH_3CO); 1.52–1.66 (m, 4 H, 2 C**H**$_2$CH$_2$-O); 1.24 (bs, 44 H, 2 (C**H**$_2$)$_{11}$(CH$_2$)$_2$-O); 0.87 (t, 6 H, 2 C**H**$_3$CH$_2$). ^{13}C NMR ($CDCl_3$): 170.9 (CH_3**C**O); 134.6 (CH$_2$=**C**HCH$_2$-O); 117.2 (**C**H$_2$=CHCH$_2$-O); 97.3 (C-1β); 91.1 (C-1α); 81.3 (C-3α); 80.6 (C-2α); 77.1 (C-4α); 73.8, 73.7, 73.0, 71.6 (C-5α, CH$_2$=CH**C**H$_2$-O, 2 CH$_2$**C**H$_2$-O); 63.2 (C-6α); 31.9, 30.5, 30.0, 29.7, 29.5, 29.4, 26.2, 26.0 (2 (**C**H$_2$)$_{12}$CH$_2$-O); 20.8 (**C**H$_3$CO); 14.1 (2 **C**H$_3$CH$_2$). MS (MALDI-TOF), *m/z*: 678.9 [M + Na$^+$], 696.0 [M + K$^+$].

6-*O*-Acetyl-4-*O*-allyl-2,3-di-*O*-tetradecyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranose (**12**)

A mixture of compound **11** (0.5 g, 0.76 mmol), trichloroacetonitrile (0.6 ml, 5.98 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 0.09 ml, 0.6 mmol) in dry CH_2Cl_2 (40 ml) was stirred under argon at room temperature for 90 min. The solution was evaporated and immediately chromatographed on a silica gel column with a hexane–ethyl acetate (5:1) eluent, containing also 0.3% of triethylamine, providing imidate **12** (0.55g, 90%). Characteristic signals for α anomer: ^{1}H NMR ($CDCl_3$): 8.42 (s, 1 H, NH); 6.43 (d, 1 H, $J_{1,2}$ = 3.4, H-1); 5.79 (m, 1 H, CH$_2$=C**H**CH$_2$-O); 5.08–5.50 (m, 2 H, C**H**$_2$=CHCH$_2$-O); 5.04 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.7, H-4); 3.48–4.29 (m, 11 H, H-2, H-3, H-5, H-6a, H-6b, CH$_2$=CHC**H**$_2$-O, 2 CH$_2$C**H**$_2$-O); 2.05 (s, 3 H, CH_3CO); 1.41–1.50 (m, 4 H, 2 C**H**$_2$CH$_2$-O); 1.11–1.45 (m, 44 H, 2 (C**H**$_2$)$_{11}$(CH$_2$)$_2$-O); 0.79–0.96 (m, 6 H, 2 C**H**$_3$CH$_2$). MS (MALDI-TOF), *m/z*: 824.5 [M + Na$^+$], 841.8 [M + K$^+$].

Allyl 6-*O*-(6-*O*-Acetyl-4-*O*-allyl-2,3-di-*O*-tetradecyl-β-D-glucopyranosyl)-4-*O*-(4-methoxybenzyl)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (**14**)

Procedure A. Imidate **12** (0.5 g, 0.63 mmol) and nucleophile **3** (0.4 g, 0.54 mmol) were dissolved in dry CH_2Cl_2 (15 ml) and the mixture was stirred at room temperature under argon in the presence of 4A molecular sieves for 15 min. Then the solution was cooled to –20 °C and TMSOTf (55 µl, 0.3 mmol) in CH_2Cl_2 (1 ml) was added dropwise. The mixture was left standing to warm up to room temperature, stirred for another 2 h, filtered, washed with saturated solution of $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on a silica gel column. Elution with heptane–ethyl acetate (10:1) gave first allyl 6-*O*-(6-*O*-acetyl-4-*O*-allyl-2,3-di-*O*-tetradecyl-α-D-glucopyranosyl)-4-*O*-(4-methoxybenzyl)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (**13**; 0.5 g, 68%). M.p. 58–60 °C (ethyl acetate); $[\alpha]_D$ +52. For $C_{84}H_{152}O_{13}$ (1370.1) calculated: 73.63% C, 11.18% H; found: 73.54% C, 11.26% H. ^{1}H NMR ($CDCl_3$): 7.24 (d, 2 H, J = 7.9, H_{arom}); 6.87 (d, 2 H, H_{arom}); 5.82–5.98 (m, 2 H, 2 CH$_2$=C**H**CH$_2$-O); 5.10–5.36 (m, 4 H, 2 C**H**$_2$=CHCH$_2$-O); 4.95 (d, 1 H, $J_{1,2}$ = 3.4, H-1); 4.86 (d, 1 H, $J_{1,2}$ = 3.8, H-1′); 4.82 (d, 1 H, J = 10.7, C**H**HAr); 4.56 (d, 1 H, CH**H**Ar); 3.97–4.37 (m, 9 H, H-4′, H-5, H-5′, H-6′a, H-6′b, 2 CH$_2$=CHC**H**$_2$-O); 3.78 (s, 3 H, OCH_3); 3.38–3.92 (m, 12 H, H-3′, H-4, H-6a, H-6b, 4 CH$_2$C**H**$_2$-O); 3.18–3.30 (m, 3 H, H-2, H-2′, H-3); 2.07 (s, 3 H, CH_3CO); 1.54 (bs, 8 H, 4 C**H**$_2$CH$_2$-O); 1.29 (bs, 88 H, 4 (C**H**$_2$)$_{11}$(CH$_2$)$_2$-O); 0.88 (t, 12 H, 4 C**H**$_3$CH$_2$). ^{13}C NMR ($CDCl_3$): 170.8 (CH_3**C**O); 159.3 (C_{arom}); 134.6, 133.8 (2 CH$_2$=**C**HCH$_2$-O); 129.4 (2 C_{arom}); 128.8 (C_{arom}); 118.2, 117.3 (2 **C**H$_2$=CHCH$_2$-O); 113.8 (2 C_{arom}); 98.2 (C-1′); 95.2 (C-1); 84.7, 82.0, 80.9, 80.5 (C-2, C-3, C-2′, C-3′); 77.2 (CH$_2$Ar); 74.8, 73.9, 73.8, 73.5, 73.2, 73.2, 72.8, 71.0 (C-5, C-5′, 2 CH$_2$=CH**C**H$_2$-O, 4 CH$_2$**C**H$_2$-O); 70.3, 69.0 (C-4, C-4′); 67.9 (C-6); 63.2 (C-6′); 31.9, 30.5, 30.0, 29.7, 29.5, 29.4, 26.2, 26.0 (4 (**C**H$_2$)$_{12}$CH$_2$-O); 20.8 (**C**H$_3$CO); 14.1 (2 **C**H$_3$CH$_2$); 13.9 (2 **C**H$_3$CH$_2$). MS (MALDI-TOF), *m/z*: 1394.5 [M + Na$^+$], 1411.7 [M + K$^+$].

Second fraction contained disaccharide **14** (70 mg, 9.5%). M.p. 63–65 °C; $[\alpha]_D$ +28. For $C_{84}H_{152}O_{13}$ (1370.1) calculated: 73.63% C, 11.18% H; found: 73.42% C, 10.98% H. ^{1}H NMR ($CDCl_3$): 7.24 (d, 2 H, J = 7.9, H_{arom}); 6.87 (d, 2 H, H_{arom}); 5.82–5.97 (m, 2 H, 2 CH_2=C**H**CH_2-O); 5.13–5.35 (m, 4 H, 2 C**H**$_2$=$CHCH_2$-O); 4.95 (d, 1 H, $J_{1,2}$ = 3.5, H-1); 4.82 (d, 1 H, J = 10.6, C**H**HAr); 4.58 (d, 1 H, CH**H**Ar); 4.32 (d, 1 H, $J_{1',2'}$ = 7.6, H-1′); 4.00–4.24 (m, 8 H, H-4, H-4′, H-5, H-5′, H-6a, H-6b, H-6′a, H-6′b); 3.41–3.95 (m, 12 H, 2 CH_2=CHC**H**$_2$-O, 4 CH_2C**H**$_2$-O); 3.75 (s, 3 H, OCH_3); 3.02–3.31 (m, 4 H, H-2, H-2′, H-3, H-3′); 2.07 (s, 3 H, CH_3CO); 1.41–1.50 (m, 8 H, 4 C**H**$_2$$CH_2$-O); 1.30 (bs, 88 H, 4 (C**H**$_2$)$_{11}$$(CH_2)_2$-O); 0.90 (t, 12 H, 4 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 170.8 ($CH_3$**C**O); 159.3 ($C_{arom}$); 134.6, 133.8 (2 CH_2=**C**HCH_2-O); 130.6 (C_{arom}); 129.4 (2 C_{arom}); 118.2, 117.3 (2 CH_2=$CHCH_2$-O); 113.8 (2 C_{arom}); 104.0 (C-1′); 95.2 (C-1); 84.7, 82.0, 80.9, 80.5 (C-2, C-3, C-2′, C-3′); 77.2 (**C**H_2Ar); 74.8, 73.9, 73.8, 73.5, 73.2, 73.1, 72.8, 71.0 (C-5, C-5′, 2 CH_2=CH**C**H_2-O, 4 CH_2**C**H_2-O); 70.3, 69.0 (C-4, C-4′); 67.9 (C-6); 63.2 (C-6′); 31.9, 30.5, 30.0, 29.7, 29.5, 29.4, 26.2, 26.0 (4 (**C**H_2)$_{12}$$CH_2$-O); 20.8 ($CH_3$**C**O); 14.1 (2 **C**$H_3$$CH_2$); 13.9 (2 **C**$H_3$$CH_2$). MS (MALDI-TOF), *m/z*: 1394.5 [M + Na^+], 1411.8 [M + K^+].

Procedure B. Imidate **12** (0.5 g, 0.63 mmol) and nucleophile **3** (0.4 g, 0.54 mmol) were dissolved in dry dichloromethane (15 ml) and the mixture was stirred at room temperature under argon in the presence of a 4A molecular sieve (0.4 g) for 15 min. Then the solution was cooled to 0 °C and BF_3·OEt_2 (0.13 ml) in a mixture of diethyl ether (0.5 ml) and CH_2Cl_2 (0.5 ml) was added dropwise. The mixture was left standing to warm up to room temperature and stirred for 1 h. Then $NaHCO_3$ (0.4 g) was added and, after stirring for 10 min, the mixture was filtered, washed with CH_2Cl_2 and evaporated. The residue, containing disaccharides **13** and **14** in approximate ratio 1:10 (TLC), was chromatographed on a silica gel column with heptane–ethyl acetate (10:1) as eluent giving pure **13** (45 mg, 6%) and **14** (0.55 g, 73.5%).

Allyl 6-*O*-(6-*O*-Acetyl-4-*O*-allyl-2,3-di-*O*-tetradecyl-β-D-glucopyranosyl)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (**16**)

Procedure C. Except for the 2-h additional reaction time after warming the reaction mixture to room temperature, which was in this case 40 h, procedure *A* for preparation of disaccharides **13** and **14** was followed with the same quantities of the reagents. The syrupy residue thus obtained was chromatographed on a silica gel column with a heptane–ethyl acetate (5:1) elution. First fraction contained allyl 6-*O*-(6-*O*-acetyl-4-*O*-allyl-2,3-di-*O*-tetradecyl-α-D-glucopyranosyl)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (**15**; 0.4 g, 53%). M.p. 59–62 °C (acetone); $[\alpha]_D$ +43. For $C_{76}H_{144}O_{12}$ (1249.9) calculated: 73.03% C, 11.61% H; found: 73.33% C, 11.90% H. ^{1}H NMR ($CDCl_3$): 5.80–6.00 (m, 2 H, 2 CH_2=C**H**CH_2-O); 5.11–5.40 (m, 4 H, 2 C**H**$_2$=$CHCH_2$-O); 4.91 (d, 2 H, $J_{1,2}$ = $J_{1',2'}$ = 3.5, H-1, H-1′); 3.22–4.36 (m, 24 H, H-2–H-5, H-6a, H-6b, H-2′–H-5′, H-6′a, H-6′b, 2 CH_2=CHC**H**$_2$-O, 4 CH_2C**H**$_2$-O); 2.08 (s, 3 H, CH_3CO); 1.58 (bs, 8 H, 4 C**H**$_2$$CH_2$-O); 1.26 (bs, 88 H, 4 (C**H**$_2$)$_{11}$$(CH_2)_2$-O); 0.82–0.96 (m, 12 H, 4 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 170.8 ($CH_3$**C**O); 134.7, 133.8 (2 CH_2=**C**HCH_2-O); 118.4, 117.0 (2 **C**H_2=$CHCH_2$-O); 97.3, 95.0 (C-1, C-1′); 81.6, 81.0, 80.4, 80.3, 77.2, 73.7 (3 C); 72.1, 71.5, 71.1, 69.6, 68.7, 68.3, 68.0 (C-2–C-6, C-2′–C-5′, 2 CH_2=CH**C**H_2-O, 4 CH_2**C**H_2-O); 63.2 (C-6′); 31.9, 30.6, 30.5, 30.0, 29.7, 29.5, 29.4, 26.2, 26.1 (4 (**C**H_2)$_{12}$$CH_2$-O); 20.9 ($CH_3$**C**O); 14.1 (4 **C**$H_3$$CH_2$). MS (MALDI-TOF), *m/z*: 1273.7 [M + Na^+], 1290.1 [M + K^+].

Second fraction contained disaccharide **16** (50 mg, 7%). M.p. 63–64 °C (acetone); $[\alpha]_D$ +19. For $C_{76}H_{144}O_{12}$ (1249.9) calculated: 73.03% C, 11.61% H; found: 72.88% C, 11.74% H. ^{1}H NMR ($CDCl_3$): 5.82–5.98 (m, 2 H, 2 CH_2=C**H**CH_2-O); 5.13–5.35 (m, 4 H, 2 C**H**$_2$=CHCH_2-O); 4.96 (d, 1 H, $J_{1,2}$ = 3.5, H-1); 4.32 (d, 1 H, $J_{1',2'}$ = 7.6, H-1′); 4.25–4.36 (m, 2 H, CH_2=CHC**H**H-O, H-6a); 4.14–4.25 (m, 2 H, H-6b, H-6′a); 4.02–4.14 (m, 3 H, H-4, H-6′b, CH_2=CHCH**H**-O); 3.45–3.95 (13 H, H-4′, H-5, H-5′, CH_2=CHC**H**$_2$-O, 4 CH_2C**H**$_2$-O); 3.07–3.45 (m, 4 H, H-2, H-2′, H-3, H-3′); 2.07 (s, 3 H, CH_3CO); 1.57 (bs, 8 H, 4 C**H**$_2$$CH_2$-O); 1.39–1.50 (m, 88 H, 4 (C**H**$_2$)$_{11}$(CH_2)$_2$-O); 0.88 (t, 12 H, 4 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 170.8 ($CH_3$**C**O); 134.6, 133.8 (2 CH_2=**C**HCH_2-O); 118.2, 117.3 (2 **C**H_2=CHCH_2-O); 104.0 (C-1′); 95.2 (C-1); 84.7, 82.0, 81.0, 80.5 (C-2, C-3, C-2′, C-3′); 77.4, 73.9, 73.8, 73.5, 73.2, 72.8, 71.1, 71.0 (C-5, C-5′, 2 CH_2=CH**C**H_2-O, 4 CH_2**C**H_2-O); 70.2, 69.0 (C-4, C-4′); 67.9 (C-6); 63.2 (C-6′); 31.9, 31.4, 30.5, 30.4, 30.2, 30.0, 29.7, 29.4, 26.2, 26.1, 22.7 (4 (**C**H_2)$_{12}$$CH_2$-O); 20.8 (**C**$H_3$CO); 14.1 (4 **C**$H_3$$CH_2$). MS (MALDI-TOF), *m/z*: 1274.1 [M + Na^+], 1290.2 [M + K^+].

Procedure D. A solution of imidate **12** (0.3 g, 0.38 mmol) and nucleophile **3** (0.25 g, 0.34 mmol) in CH_2Cl_2 was stirred under argon in the presence of a 4A molecular sieves (0.3 g). After cooling to 0 °C, a solution of BF_3·OEt_2 (80 µl) in diethyl ether (0.4 ml) and CH_2Cl_2 (0.4 ml) was added dropwise. The reaction mixture was then left standing to warm to room temperature and stirred for 24 h. $NaHCO_3$ (0.3 g) was added and the mixture was stirred for 15 min, filtered, washed with CH_2Cl_2 and concentrated. The residue was purified on a silica gel column with heptane–ethyl acetate (5:1) and pure disaccharide **16** (0.25 g, 58.7%) was obtained.

Allyl 6-*O*-(4-*O*-Allyl-2,3-di-*O*-tetradecyl-β-D-glucopyranosyl)-4-*O*-(4-methoxybenzyl)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (**17**)

Methanolic 1 M sodium methoxide (0.2 ml) was added to a solution of disaccharide **14** (0.3 g, 0.22 mmol) in dry acetone (2 ml) and methanol (10 ml) and the solution was stirred at room temperature for 4 h. After neutralization with Dowex 50W (H^+) resin, filtration and concentration, the residue was transferred onto a silica gel column and eluted with heptane–ethyl acetate (7:1). Compound **16** (0.25 g, 86%) was obtained as a colorless syrup. $[\alpha]_D$ +28. For $C_{82}H_{150}O_{12}$ (1328.0) calculated: 74.16% C, 11.39% H; found: 74.00% C, 11.45% H. ^{1}H NMR ($CDCl_3$): 7.24 (d, 2 H, *J* = 8.3, H_{arom}); 6.89 (d, 2 H, H_{arom}); 5.82–5.97 (m, 2 H, 2 CH_2=C**H**CH_2-O); 5.13–5.35 (m, 4 H, 2 C**H**$_2$=CHCH_2-O); 4.95 (d, 1 H, $J_{1,2}$ = 3.3, H-1); 4.82 (d, 1 H, *J* = 10.6, C**H**HAr); 4.57 (d, 1 H, CH**H**Ar); 4.28 (dd, 1 H, CH_2=CHC**H**H-O); 4.22 (d, 1 H, $J_{1',2'}$ = 7.6, H-1′); 3.96–4.21 (m, 4 H, H-6′a, CH_2=CHCH**H**-O, CH_2=CHC**H**$_2$-O); 3.42–3.94 (m, 14 H, H-3, H-5, H-5′, H-6a, H-6b, H-6′b, 4 CH_2C**H**$_2$-O); 3.80 (s, 3 H, OCH_3); 3.02–3.31 (m, 5 H, H-2, H-2′, H-3′, H-4, H-4′); 2.12 (bs, 1 H, OH); 1.41–1.50 (m, 8 H, 4 C**H**$_2$$CH_2$-O); 1.27 (bs, 88 H, 4 (C**H**$_2$)$_{11}$(CH_2)$_2$-O); 0.82–0.95 (m, 12 H, 4 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 159.2 ($C_{arom}$); 134.7, 133.8 (2 CH_2=**C**HCH_2-O); 130.6 (C_{arom}); 129.4 (2 C_{arom}); 118.1, 117.1 (2 **C**H_2=CHCH_2-O); 113.8 (2 C_{arom}); 103.8 (C-1′); 95.3 (C-1); 84.7, 82.0, 81.6, 80.6 (C-2, C-3, C-2′, C-3′); 77.4, 77.0, 75.0, 74.6, 73.8, 73.7, 73.6, 73.3, 71.4 (C-5, C-5′, **C**H_2Ar, 2 CH_2=CH**C**H_2-O, 4 CH_2**C**H_2-O); 70.0, 68.6 (C-4, C-4′); 67.9 (C-6); 63.2 (C-6′); 55.2 (CH_3O); 31.9, 30.6, 30.5, 30.4, 30.0, 29.7, 29.3, 26.2, 26.0, 22.6 (4 (**C**H_2)$_{12}$$CH_2$-O); 14.1 (4 **C**$H_3$$CH_2$). MS (MALDI-TOF), *m/z*: 1352.2 [M + Na^+], 1368.4 [M + K^+].

Allyl 6-*O*-(4-*O*-Allyl-2,3-di-*O*-tetradecyl-β-D-glucopyranosyl)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (**18**)

Compound **16** (0.15 g, 0.12 mmol) was deacetylated in a similar way as disaccharide **14** and compound **18** (0.12 g, 83%) was isolated from reaction mixture after work-up by a silica gel column chromatography with heptane–ethyl acetate (3:1) elution. M.p. 59–60 °C (ethyl acetate); $[\alpha]_D$ +24. For $C_{74}H_{142}O_{11}$ (1207.9) calculated: 73.58% C, 11.85% H; found: 73.43% C, 11.91% H. ^{1}H NMR ($CDCl_3$): 5.88–5.99 (m, 2 H, 2 CH_2=C**H**CH_2-O); 5.12–5.37 (m, 4 H, 2 C**H**$_2$=CHCH_2-O); 4.96 (d, 1 H, $J_{1,2}$ = 3.4, H-1); 4.28 (dd, 1 H, CH_2=CHC**H**H-O); 4.36 (d, 1 H, $J_{1',2'}$ = 7.7, H-1′); 4.28 (1 H, CH_2=CHCH**H**-O); 4.02–4.25 (m, 4 H, H-6′a, CH_2=CHC**H**$_2$-O, CH_2=CHCH**H**-O); 3.44–3.95 (m, 14 H, H-3, H-5, H-5′, H-6a, H-6b, H-6′b, 4 CH_2C**H**$_2$-O); 3.06–3.33 (m, 5 H, H-2, H-2′, H-3′, H-4, H-4′); 2.67 (bs, 1 H, OH); 2.33 (bs, 1 H, OH); 1.56 (bs, 8 H, 4 C**H**$_2$$CH_2$-O); 1.26 (bs, 88 H, 4 (C**H**$_2$)$_{11}$$(CH_2)_2$-O); 0.82–0.96 (m, 12 H, 4 C**H**$_3$$CH_2$). ^{13}C NMR ($CDCl_3$): 134.7, 133.7 (2 CH_2=**C**HCH_2-O); 118.1, 117.1 (2 **C**H$_2$=CHCH_2-O); 104.0 (C-1′); 95.3 (C-1); 84.6, 82.1, 81.0, 80.5 (C-2, C-3, C-2′, C-3′); 77.4, 77.0, 75.0, 73.8, 73.7, 73.5, 73.3, 71.0 (C-5, C-5′, 2 CH_2=CH**C**H$_2$-O, 4 CH_2**C**H$_2$-O); 70.4, 70.1 (C-4, C-4′); 67.9 (C-6); 62.0 (C-6′); 31.9, 30.4, 30.3, 30.0, 29.7, 29.4, 29.3, 26.2, 26.1, 22.6 (4 (**C**H$_2$)$_{12}$$CH_2$-O); 14.1 (4 **C**H$_3$$CH_2$). MS (MALDI-TOF), *m/z*: 1231.6 [M + Na^+], 1247.9 [M + K^+].

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