# Synthesis of a Precursor of a Lipid A Mimic

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The lipid A mimic precursor, methyl 6-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-tetradecyl-$\beta$-D-glucopyranosyl)-4-*O*-(4-methoxybenzyl)-2,3-di-*O*-tetradecyl-$\alpha$-D-glucopyranoside having the original *O*- and *N*-acyl substitution at the atoms C-2, C-3, C-2′, and C-3′ of the carbohydrate skeleton mimicked by ether linkages was synthesized by coupling the respective monosaccharide glycosyl bromide and 6-hydroxy nucleophile in heptane—chloroform solution promoted with silver oxide. For the 2,3-di-*O*-alkylation of both monosaccharide precursors the efficiency of potassium *tert*-butoxide/*tert*-butanol/tetradecyl bromide for the etherification was evaluated.

Lipid A has been shown to be responsible for the endotoxic activity of lipopolysaccharides (LPS), the major antigens on the surface of gram-negative bacteria [1]. These bacteria and namely their lipopolysaccharides (called also endotoxins) can cause different diseases. One of them is the sepsis when the endotoxins trigger a cascade of inflammation reactions resulting in a failure of human organs, septic shock, and death [2].

Lipid A, the most conservative part of the LPS and biologically responsible substance for the process is 1$\alpha$,4′-bisphosphorylated $\beta$-(1→6)-linked D-glucosamine disaccharide, *O*- and *N*-acylated with fatty acids (Fig. 1) [3]. The glycomimetic approach suggested for the prevention of the sepsis is based on the hypothesis that while a lipid A glycomimetic structure, possessing all the structural, conformational, functional, and electric-charge properties identical with the native lipid A molecule, could induce antibodies production enhancing the overall immunity, enzymatically nonhydrolyzable inner linkages of the mimic molecule would not be able to enter its normal metabolic degradation resulting in prevention of the inflammatory cascade occurrence.

This paper describes the synthesis of such a lipid A mimic precursor possessing tetradecyl alkylation at the hydroxyl groups at the C-2, C-3, C-2′, and C-3′ carbon atoms of the gentiobiose skeleton. The admittance of the substitution of the D-glucosamine units (or 2,3-diamino-2,3-dideoxy-D-glucose units) is supported *e.g.* by the observation that neither the substitution of the Vi-capsular polysaccharide in vaccines with per-*O*-acetylated pectin has caused a change of the immunogenicity of these vaccines [4] (Vi-capsular polysaccharide differs from pectin by *N*-acetylation

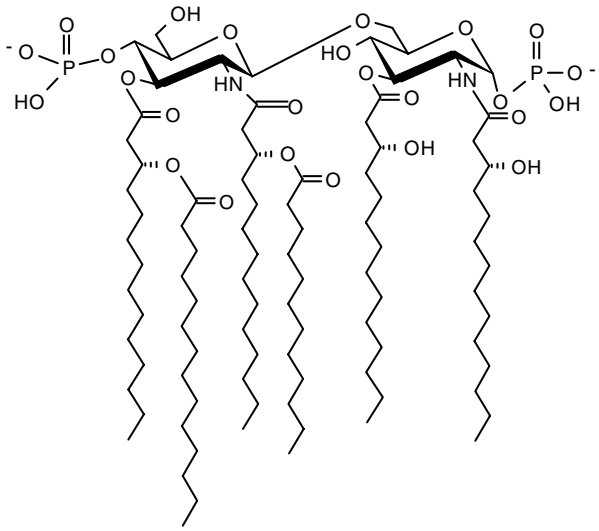
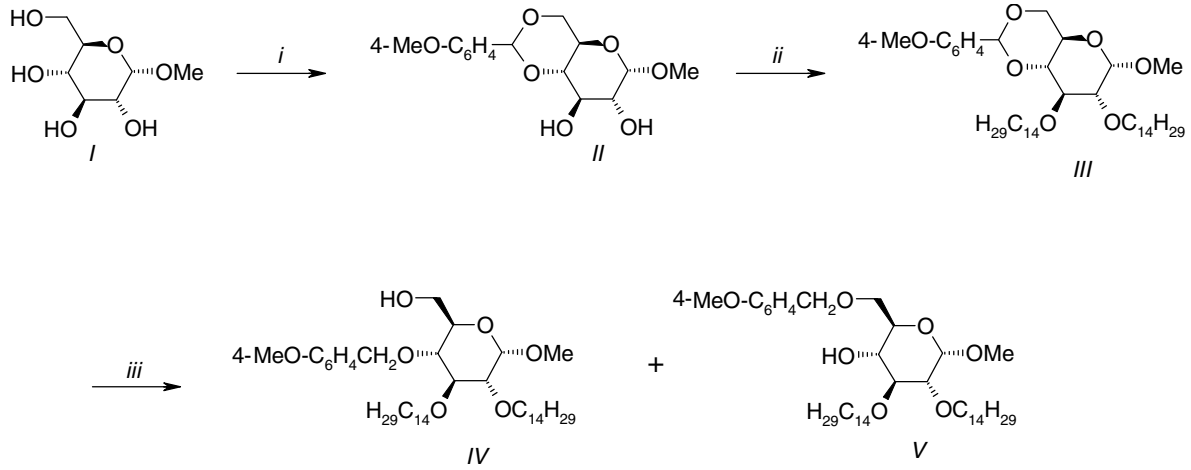


**Fig. 1.** The structure of the *E. coli* lipid A.

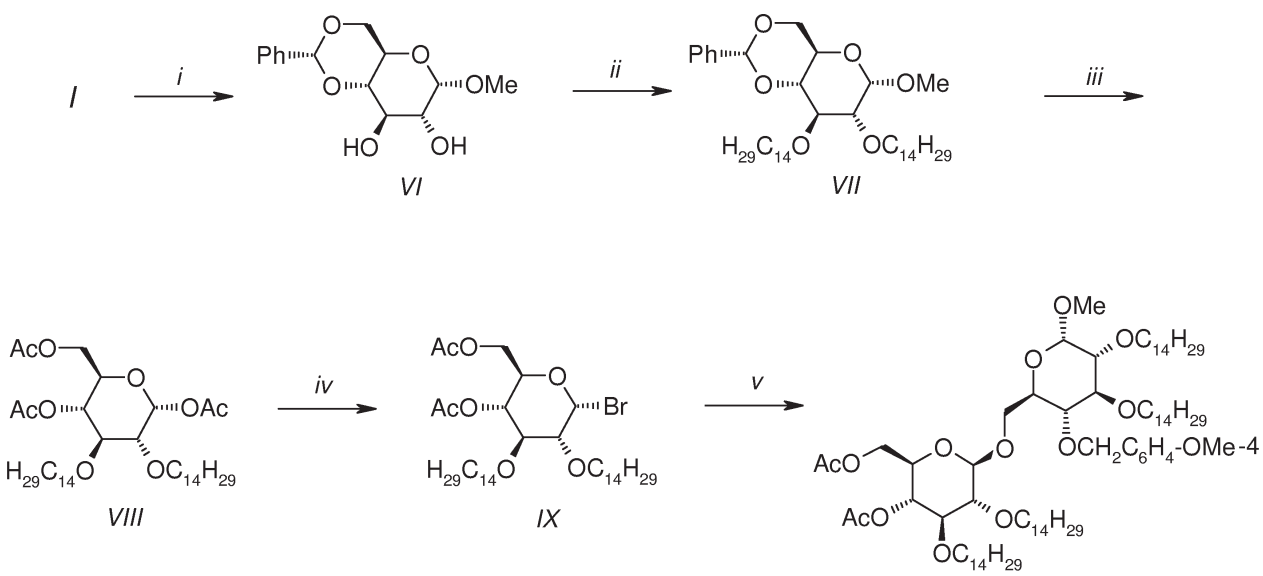
on C-2 and *O*-acetylation on C-3). The main objective of this study was to evaluate the efficiency of the *t*-BuOK/*t*-BuOH/$C_{14}H_{29}Br$ alkylation system for the etherification of suitably protected monosaccharide precursors for the synthesis of the mimics.

Both monosaccharide derivatives *II* and *VI* used for the alkylation studies with *t*-BuOK/*t*-BuOH/$C_{14}H_{29}Br$ were known acetals easily available from methyl $\alpha$-D-glucopyranoside (*I*) (Schemes 1 and 2). Anisylidene derivative *II* was prepared according to the original procedure [5] in a 93 % yield. For the synthesis of the benzylidene derivative *VI*, a modified procedure in a more convenient solvent, 1,2-dimethoxyethane (DME) was suggested allowing a




*i*: anisaldehyde dimethyl acetal, *p*-toluenesulfonic acid, DMF, 2.5 h, 50 °C, 1.6 kPa; *ii*: *t*-BuOK, *t*-BuOH, $C_{14}H_{29}Br$, 5 h, reflux; *iii*: $Me_3SiCl$, $NaBH_3CN$, MeCN, molecular sieve (0.3 nm), 5 h, r.t.

*Scheme 1*




*i*: benzaldehyde dimethyl acetal, *p*-toluenesulfonic acid, DME, Drierite, 15 h, r.t., then 2 h, reflux; *ii*: *t*-BuOK, *t*-BuOH, $C_{14}H_{29}Br$, 5 h, reflux; *iii*: $Ac_2O$, AcOH, $H_2SO_4$, 3 h, 40 °C; *iv*: HBr, AcOH, 2.5 h, r.t.; *v*: compound *IV*, $Ag_2O$, $I_2$, $CHCl_3$, heptane, Drierite

*Scheme 2*

simple, triturating isolation of the product in a 92 % yield.

Possibilities to exploit *t*-BuOK, the strong non-nucleophilic base easily soluble in *t*-BuOH for generation of the sugar alkoxides and their *O*-alkylation with tetradecyl bromide were further studied. Due to a negligible solubility of the anisylidene derivative *II* in BuOH, an additional solvent had to be applied. Being compatible with the *t*-BuOK/*t*-BuOH/$C_{14}H_{29}Br$ alkylation system suggested, DME was found to be quite convenient solvent for compound *II* (Table 1). The alkylation rate, however, was very low at room temperature. Better results were obtained at increased temperatures, however, the yields of the 2,3-di-*O*-alkylated product *III* did not exceed 45 %.

A slightly higher yield of the 2,3-di-*O*-alkylated product *VII* was obtained with the benzylidene derivative *VI* (Table 2). This was apparently due to a sufficient solubility of the starting derivative *VI* in *t*-BuOH thus enabling to avoid a cosolvent application. Analogously, at increased temperatures up to the boiling point of the solvent, the maximum yield of compound *VII* was 50 %. For both alkylations of compounds *II* and *VI*, in addition to the expected respective products *III* and *VII*, mixtures of mono-*O*-alkylated products not further analyzed were isolated.

**Table 1.** Alkylation of Methyl 4,6-*O*-(4-Methoxybenzylidene)-$\alpha$-D-glucopyranoside (*II*, 6.4 mmol) with a 1 mol dm$^{-3}$ Solution of *t*-BuOK/*t*-BuOH and $C_{14}H_{29}Br$

| $n$(*t*-BuOK) / mmol | Sugar solvent (35 cm$^3$) | Treatment with base/min | $n$($C_{14}H_{29}Br$) / mmol | Reaction time at reflux/h | Yield of *III*/% |
|---|---|---|---|---|---|
| 20 | DME | 30 | 20 | 5 | 29 |
| 40 | DME | 30 | 17 | 5 | 37 |
| 36 | DME | 45 | 20 | 5 | 37 |
| 36 | THF | 45 | 20 | 5 | 20 |
| 40 | DME | 45 | 20 | 5 | 45 |

**Table 2.** Alkylation of Methyl 4,6-*O*-Benzylidene-$\alpha$-D-glucopyranoside (*VI*, 7 mmol) Dissolved in BuOH (30 cm$^3$) with a 1 mol dm$^{-3}$ Solution of *t*-BuOK/*t*-BuOH and $C_{14}H_{29}Br$

| $n$(*t*-BuOK) / mmol | Treatment with base/min | $n$($C_{14}H_{29}Br$) / mmol | Reaction time at reflux/h | Yield of *VII*/% |
|---|---|---|---|---|
| 20 | 15 | 10 | 4[a] | 25 |
| 20 | 30 | 10 | 6 | 37 |
| 20 | 30 | 13 | 5 | 35 |
| 40 | 30 | 20 | 5 | 43 |
| 40 | 45 | 20 | 5 | 50 |

*a*) After 24 h at r.t.

A known ability of the 4,6-*O*-anisylidene derivative to be partially deprotected by a regioselective cleavage [6] was further exploited for the synthesis of nucleophile *IV*. The treatment of compound *III* with $Me_3SiCl/NaBH_3CN$ gave a $w_r = 3{:}1$ mixture of the expected derivative *IV* and its regioisomer *V*. The nucleophile *IV* was isolated by a flash chromatography in a 55 % yield. The $^{13}$C NMR spectroscopy has proved the structure of the regioisomer *V* by the presence of its substituted C-6 carbon atom at $\delta = 65.67$ with a 4-methoxybenzyloxy group. Compound *V* was not further dealt with.

A standard acetolysis of compound *VII* gave triacetate *VIII* in a 63 % yield. In a subsequent step, this compound was transformed by a standard HBr/AcOH procedure to electrophile *IX* obtained in a 90 % yield. The final coupling of *IV* and *IX* in a heptane—chloroform mixture promoted by $Ag_2O$ gave rise to the expected lipid A mimic precursor, methyl 6-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-tetradecyl-$\beta$-D-glucopyranosyl)-4-*O*-(4-methoxybenzyl)-2,3-di-*O*-tetradecyl-$\alpha$-D-glucopyranoside (*X*) as the only disaccharidic product isolated in a 38 % yield. Its $\beta$-anomeric structure is documented by the coupling constant $J_{1',2'} = 7.1$ Hz characteristic of the $\beta$-1,6-linked gentiobiose [7].

## EXPERIMENTAL

A commercial benzylidene dimethyl acetal, *t*-BuOK, and a solution of 30 % HBr in acetic acid were obtained from Aldrich. Tetradecyl bromide, sodium cyanoborohydride and trimethylsilyl chloride were obtained from Merck. Anisaldehyde dimethyl acetal was obtained from Fluka.

Specific rotations were measured on a Perkin—Elmer 141 polarimeter and the melting points were determined on a Kofler stage. Elemental analyses were done with a Fisons EA 1108 analyzer. EI mass spectra (70 eV) were recorded at 20 °C using a Finnigan MAT SSQ 710 spectrometer. MALDI mass spectra were recorded using a Shimadzu Kratos MALDI III with laser (337 nm). NMR spectra were recorded at 20 °C on a Bruker AVANCE DPX 300 spectrometer (300.13 MHz, internal standard sodium 3-(trimethylsilyl)propionate, $\delta = 0.00$ for $^1$H and 75.47 MHz, internal standard methanol, $\delta = 50.15$ for $^{13}$C). TLC was run on glass plates precoated with silica gel (0.005—0.040 mm, Aldrich), spraying the chromatograms with a 10 % ethanolic sulfuric acid and charring them on a hot plate effected detection. Column chromatography was performed using silica gel (Kieselgel 60, 0.040—0.063 mm, Aldrich) on a 50 cm × 4.5 cm column, flow rate 0.75 cm$^3$ min$^{-1}$.

### Methyl 2,3-Di-*O*-tetradecyl-4,6-*O*-(4-methoxybenzylidene)-$\alpha$-D-glucopyranoside (*III*)

To a stirred solution of methyl 4,6-*O*-(4-methoxybenzylidene)-$\alpha$-D-glucopyranoside (*II*) (2 g; 6.4 mmol) in DME (40 cm$^3$) was added *t*-BuOK (5 g; 0.04 mol) in *t*-BuOH (35 cm$^3$). The mixture was stirred for 1 h at r.t. under dry nitrogen and then tetradecyl bromide (6.2 g; 0.02 mol) was added. The mixture was further

stirred for 5 h at reflux and for 15 h at r.t. The reaction was followed by the TLC chromatography, ethyl acetate (EA)—hexane ($\varphi_r = 8:1$). The suspension was concentrated on a rotary evaporator under reduced pressure and EA (50 $cm^3$) was added to the residue. The mixture was filtered through the Celite and the solution was concentrated on a rotary evaporator *in vacuo*. The residue was purified by the column chromatography, EA—hexane ($\varphi_r = 8:1$) and crystallized. Yield = 2.15 g (48 %), m.p. = 68—70 °C, hexane—acetone ($\varphi_r = 1:1$), $[\alpha]$(D, 20 °C, chloroform, $\rho$ = 10.0 g $dm^{-3}$) = + 23°, $R_f = 0.31$. For $C_{43}H_{76}O_7$ ($M_r$ = 704.15) $w_i$(calc.): 73.29 % C, 10.80 % H; $w_i$(found): 73.49 % C, 10.48 % H. $^1$H NMR spectrum (300.13 MHz, $CDCl_3$), $\delta$: 7.40 (d, 2H, $H_{arom}$), 6.87 (d, 2H, $H_{arom}$), 5.49 (s, 1H, C*H*Ar), 4.78 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.25 (m, 1H, $J_{4,5} = J_{5,6} = 9.5$ Hz, $J_{5,6'} = 4.1$ Hz, H-5), 3.80 (s, 3H, C$H_3$—O—Ar), 3.48—3.77 (m, 8H, H-3, H-4, H-6, H-6′, C$H_2$—O—C-3, C$H_2$—O—C-2), 3.42 (s, 3H, C$H_3$—O—C-1), 3.34 (dd, 1H, $J_{2,3} = 9.3$ Hz, H-2), 1.57 (m, 4H, C$H_2$CH$_2$—O—C-2, C$H_2$CH$_2$—O—C-3), 1.24 (m, 44H, (C$H_2$)$_{11}$(CH$_2$)$_2$—O—C-2, (C$H_2$)$_{11}$(CH$_2$)$_2$—O—C-3), 0.88 (t, 6H, C$H_3$(CH$_2$)$_{13}$—O—C-2, C$H_3$(CH$_2$)$_{13}$—O—C-2). $^{13}$C NMR spectrum (75.47 MHz, $CDCl_3$), $\delta$: 159.92, 130.01, 127.30, 113.48 (6$C_{arom}$), 101.19 (*C*HAr), 99.10 (C-1), 81.95 (C-3), 80.42 (C-2), 78.25, 73.44 (*C*H$_2$—O—C-2, *C*H$_2$—O—C-3), 72.25 (C-5), 69.04 (C-4), 62.41 (C-6), 55.23 (*C*H$_3$—O—C-1, *C*H$_3$—O—Ar), 31.91, 30.30, 30.05, 29.68, 29.35, 26.11, 25.96 ((*C*H$_2$)$_{12}$CH$_2$—O—C-2, (*C*H$_2$)$_{12}$CH$_2$—O—C-3), 14.10 (2(CH$_2$)$_{13}$*C*H$_3$). Mass spectrum (EI): $m/z$ = 705 $(M)^+$.

### Methyl 2,3-Di-*O*-tetradecyl-4-*O*-(4-methoxybenzyl)-α-D-glucopyranoside (*IV*)

A solution of trimethylsilyl chloride (0.51 g; 5 mmol) in acetonitrile (4.7 $cm^3$) kept at 0 °C was dropwise added to a stirred solution of compound *III* (0.55 g; 0.78 mmol) and sodium cyanoborohydride (0.3 g; 5 mmol) in acetonitrile (16 $cm^3$) containing molecular sieve 0.3 nm (1 g). After stirring for 5 h at r.t. under a nitrogen atmosphere, the mixture was filtered through the Celite and the solution was poured into a mixture of ice and water (150 $cm^3$) saturated with $NaHCO_3$. The suspension was extracted with $CHCl_3$ (3 × 15 $cm^3$), washed with saturated aqueous $NaHCO_3$ (3 × 30 $cm^3$) and dried with $Na_2SO_4$. After filtration, the solution was concentrated on a rotary evaporator under reduced pressure. The residue was purified by the column chromatography, EA—toluene ($\varphi_r$ = 1:2) and crystallized. Yield = 0.28 g (55 %), m.p. = 67—68 °C (acetone), $[\alpha]$(D, 20 °C, chloroform, $\rho = 10.0$ g $dm^{-3}$) = + 46°, $R_f = 0.54$. For $C_{43}H_{78}O_7$ ($M_r$ = 706.38) $w_i$(calc.): 73.09 % C, 11.05 % H; $w_i$(found): 73.40 % C, 11.32 % H. $^1$H NMR spectrum (300.13 MHz, $CDCl_3$), $\delta$: 7.26 (d, 2H, $H_{arom}$), 6.87 (d, 2H, $H_{arom}$), 4.82 (d, 1H, $J$ = 10.8 Hz, C$H_2$Ar), 4.75 (d, 1H, $J_{1,2}$ = 3.5 Hz, C-1), 4.57 (d, 1H, C$H_2$Ar), 3.86 (m, 1H, $J_{4,5} = J_{5,6} = 9.1$ Hz, $J_{5,6'} = 4.2$ Hz, H-5), 3.80 (s, 3H, C$H_3$—O—Ar), 3.42—3.74 (m, 8H, H-3, H-4, H-6, H-6′, C$H_2$—O—C-2, C$H_2$—O—C-3), 3.38 (s, 3H, C$H_3$—O—C-1), 3.27 (dd, 1H, $J_{2,3} = 9.2$ Hz, H-2), 1.60 (m, 8H, (C$H_2$)$_2$CH$_2$—O—C-2, (C$H_2$)$_2$CH$_2$—O—C-3), 1.26 (m, 40H, (C$H_2$)$_{10}$(CH$_2$)$_3$—O—C-2, (C$H_2$)$_{10}$(CH$_2$)$_3$—O—C-3), 0.88 (t, 6H, C$H_3$(CH$_2$)$_{13}$—O—C-2, C$H_3$(CH$_2$)$_{13}$—O—C-3). $^{13}$C NMR spectrum (75.47 MHz, $CDCl_3$), $\delta$: 159.37, 130.44, 129.74, 113.89, (6$C_{arom}$), 98.07 (C-1), 81.66 (C-3), 80.89 (C-2), 76.60 (*C*H$_2$Ar), 74.57 (*C*H$_2$—O—C-3), 73.74 (*C*H$_2$—O—C-2), 71.78 (C-5), 70.59 (C-4), 62.01 (C-6), 55.27 (*C*H$_3$—O—Ar), 55.08 (*C*H$_3$—O—C-1), 31.92, 30.63, 30.09, 29.69, 29.36, 26.29, 26.03, 22.68 ((*C*H$_2$)$_{13}$CH$_2$—O—C-2, (*C*H$_2$)$_{13}$CH$_2$—O—C-3), 14.10 (2*C*H$_3$(CH$_2$)$_{13}$). Mass spectrum (MALDI): $m/z$ = 730.3 $(M + Na)^+$.

### Methyl 4,6-*O*-Benzylidene-α-D-glucopyranoside (*VI*)

A mixture of compounds *I* (5 g; 0.02 mol), benzylidene dimethyl acetal (13.6 $cm^3$, 0.09 mol), *p*-toluenesulfonic acid (0.45 g; 2.3 mmol), and DME (100 $cm^3$) was stirred at r.t. for 24 h under nitrogen atmosphere. Sodium hydrogen carbonate was then added and the neutral reaction mixture was triturated with petroleum ether (3 × 50 $cm^3$) and water (3 × 50 $cm^3$) and finally crystallized from ethanol. Yield = 7.3 g (93 %), m.p. = 160—161 °C, $[\alpha]$(D, 20 °C, chloroform, $\rho$ = 10.0 g $dm^{-3}$) = + 112°; Ref. [8] gives m.p. = 161—162 °C, $[\alpha]$(D, 19 °C, chloroform, $\rho = 10.0$ g $dm^{-3}$) = + 113°.

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

To a stirred solution of *VI* (2 g; 7 mmol) in *t*-BuOH (30 $cm^3$) was added *t*-BuOK (5 g; 0.04 mol) in *t*-BuOH (35 $cm^3$). The mixture was stirred for 1 h at r.t. under nitrogen atmosphere and tetradecyl bromide (6.2 g; 0.02 mol) was added. The suspension was stirred for 6 h at reflux and for 15 h at r.t. The reaction was followed by the TLC chromatography, toluene—hexane—EA ($\varphi_r$ = 3:10:1). The suspension was concentrated on a rotary evaporator under reduced pressure and EA (50 $cm^3$) was added to the residue. The mixture was filtered through the Celite and the solution was concentrated on a rotary evaporator under reduced pressure. The residue was purified by the column chromatography, toluene—hexane—EA ($\varphi_r$ = 3:10:1). Crystallization from methanol afforded compound *VII*. Yield = 2.65 g (55 %), m.p. = 79.5—80.5 °C, $[\alpha]$(D, 20 °C, chloroform, $\rho = 10.0$ g $dm^{-3}$) = + 26°, $R_f = 0.41$. For $C_{42}H_{74}O_6$ ($M_r$ = 676.13) $w_i$(calc.): 74.55 % C, 10.94 % H; $w_i$(found): 74.69 % C, 11.13 % H. $^1$H NMR spectrum (300.13 MHz, $CDCl_3$), $\delta$: 7.49 (dd, 2H, $H_{arom}$), 7.40 (m,

3H, $H_{arom}$), 5.53 (s, 1H, C*H*Ar), 4.78 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.27 (dd, 1H, $J_{4,5} = J_{5,6}$ = 9.4 Hz, $J_{5,6'}$ = 4.0 Hz, H-5), 3.49—3.80 (m, 8H, H-3, H-4, H-6, H-6′, C$H_2$—O—C-3, C$H_2$—O—C-2), 3.42 (s, 3H, $OCH_3$), 3.35 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 1.57 (m, 8H, (C$H_2$)$_2$C$H_2$—O—C-2, (C$H_2$)$_2$C$H_2$—O—C-3), 1.23 (m, 40H, (C$H_2$)$_{10}$(C$H_2$)$_3$—O—C-2, (C$H_2$)$_{10}$(C$H_2$)$_3$—O—C-3), 0.88 (t, 6H, C$H_3$(C$H_2$)$_{13}$—O—C-2, C$H_3$(C$H_2$)$_{13}$—O—C-3). $^{13}$C NMR spectrum (75.47 MHz, $CDCl_3$), $\delta$: 137.49, 128.77, 128.11, 125.97 (6$C_{arom}$), 101.19 (*C*HPh), 99.10 (C-1), 82.00 (C-3), 80.42 (C-2), 78.23 (*C*$H_2$—O—C-3), 73.45 (*C*$H_2$—O—C-2), 72.25 (C-5), 69.09 (C-4), 62.38 (C-6), 55.23 (*C*$H_3$—O), 31.90, 30.30, 30.04, 29.67, 29.34, 26.10, 25.95, 22.66 ((*C*$H_2$)$_{12}$C$H_2$—O—C-3, (*C*$H_2$)$_{12}$C$H_2$—O—C-2), 14.08 (*C*$H_3$(C$H_2$)$_{13}$—O—C-3, *C*$H_3$(C$H_2$)$_{13}$—O—C-2). Mass spectrum (MALDI): $m/z$ = 698 (M + Na)$^+$.

### 1,4,6-Tri-*O*-acetyl-2,3-di-*O*-tetradecyl-$\alpha$-D-glucopyranose (*VIII*)

To compound *VII* (0.71 g; 1 mmol) a mixture (70 cm$^3$) of acetic anhydride, acetic acid, and sulfuric acid ($\varphi_r$ = 10:10:1) was added and heated at 40 °C for 3 h. Then, the cold mixture was poured into a mixture of ice and water (300 cm$^3$) and left stirred at r.t. for 3 h. The suspension was extracted with $CHCl_3$ (3 × 20 cm$^3$), and the extract was washed with aqueous $NaHCO_3$ (3 × 20 cm$^3$) and water. The final neutral extract was dried with $Na_2SO_4$ and concentrated on a rotary evaporator under reduced pressure. The residue was purified by the column chromatography, hexane—EA ($\varphi_r$ = 3:1) and crystallized from methanol. Yield = 0.48 g (63 %), m.p. = 57—58 °C, [$\alpha$](D, 20 °C, chloroform, $\rho$ = 10.0 g dm$^{-3}$) = + 32°, $R_f$ = 0.55. For $C_{40}H_{73}O_9$ ($M_r$ = 698.76) $w_i$(calc.): 68.86 % C, 10.47 % H; $w_i$(found): 68.76 % C, 10.61 % H. $^1$H NMR spectrum (300.13 MHz, $CDCl_3$), $\delta$: 6.31 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 5.00 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.1 Hz, H-4), 4.22 (dd, 1H, $J_{5,6'}$ = 4.3 Hz, $J_{5,6}$ = 9.1 Hz, $J_{6',6}$ = 12.4 Hz, H-6′), 4.01 (dd, 1H, H-6), 3.94 (ddd, 1H, H-5), 3.78 (m, 1H, H-3), 3.44—3.62 (m, 6H, H-2, H-3, C$H_2$—O—C-2, C$H_2$—O—C-3), 2.16, 2.08, 2.07 (3s, 9H, C$H_3$CO), 1.25—1.52 (m, 48H, (C$H_2$)$_{12}$C$H_2$—O—C-2, (C$H_2$)$_{12}$C$H_2$—O—C-3), 0.88, 0.84 (2s, 6H, C$H_3$C$H_2$). $^{13}$C NMR spectrum (75.47 MHz, $CDCl_3$), $\delta$: 170.76, 169.38, 169.10 (*C*OCH$_3$), 89.70 (C-1), 79.09 (C-3), 78.74 (C-2), 73.55, 71.67 (*C*$H_2$—O—C-2, *C*$H_2$—O—C-3), 69.98 (C-5), 69.11 (C-4), 61.98 (C-6), 31.90, 30.33, 29.89, 29.65, 29.34, 26.07, 25.92, 22.66 ((*C*$H_2$)$_{12}$C$H_2$—O—C-2, (*C*$H_2$)$_{12}$C$H_2$—O—C-3), 20.99, 20.80, 20.71 (*C*$H_3$CO), 14.08 (*C*$H_3$C$H_2$). Mass spectrum (MALDI): $m/z$ = 739 (M + K)$^+$.

### 4,6-Di-*O*-acetyl-2,3-di-*O*-tetradecyl-$\alpha$-D-glucopyranosyl Bromide (*IX*)

A solution of 30 % HBr in acetic acid (2 cm$^3$) was added to a mixture containing compound *VIII* (0.2 g; 0.3 mmol) in an alcohol-free $CHCl_3$ (3 cm$^3$). The mixture was kept under a nitrogen atmosphere at r.t. for 2 h. The reaction was controlled by the TLC in toluene—acetone ($\varphi_r$ = 5:1). Anhydrous toluene (15 cm$^3$) was added to the mixture that was concentrated on a rotary evaporator under reduced pressure. This procedure was repeated 3 times. The residue was dissolved in $CHCl_3$ and washed with ice-cold water and aqueous $NaHCO_3$. The neutral solution was dried with $Na_2SO_4$. Finally the solution was concentrated on a rotary evaporator under reduced pressure. The sirupy residue crystallized from a mixture hexane—ether ($\varphi_r$ = 1:1) and compound *IX* was obtained. Yield = 0.19 g (90 %), $R_f$ = 0.69. Mass spectrum (MALDI): $m/z$ = 729.7 (M + Na)$^+$.

### Methyl 6-*O*-(4,6-Di-*O*-acetyl-2,3-di-*O*-tetradecyl-$\beta$-D-glucopyranosyl)-4-*O*-(4-methoxybenzyl)-2,3-di-*O*-tetradecyl-$\alpha$-D-glucopyranoside (*X*)

Compound *IV* (50 mg; 0.07 mmol) was dissolved in a mixture of heptane (1 cm$^3$) and alcohol-free $CHCl_3$ (1 cm$^3$). Drierite (150 mg) and freshly prepared silver oxide (35 mg) were added to the solution and the mixture was kept in dark at r.t. After 1 h a solution of iodine (0.01 g) in alcohol-free $CHCl_3$ (1 cm$^3$) was added and finally compound *IX* (60 mg; 0.09 mmol) in heptane—alcohol-free $CHCl_3$ ($\varphi_r$ = 1:1, 1 cm$^3$) was added dropwise to the mixture during 5 min. The mixture was continually stirred in dark at r.t. for 24 h. Then alcohol-free $CHCl_3$ (5 cm$^3$) was added to the reaction mixture and Drierite was removed by filtration through the Celite. The solution was concentrated on a rotary evaporator under reduced pressure. The residue was purified by column chromatography, hexane—EA ($\varphi_r$ = 2:1). The sirupy residue crystallized from dry ethanol and compound *X* was obtained. Yield = 20 mg (20 %), m.p. = 61—63 °C, [$\alpha$](D, 20 °C, chloroform, $\rho$ = 5.0 g dm$^{-3}$) = + 21°, $R_f$ = 0.78. For $C_{81}H_{148}O_{13}$ ($M_r$ = 1327.76) $w_i$(calc.): 73.19 % C, 11.15 % H; $w_i$(found): 72.98 % C, 11.61 % H. $^1$H NMR spectrum (300.13 MHz, $CDCl_3$), $\delta$: 7.25 (d, 2H, $H_{arom}$), 6.87 (d, 2H, $H_{arom}$), 4.82 (d, 1H, $J$ =10.4 Hz, C$H_2$Ar), 4.78 (d, 1H, $J_{1,2}$ = 3.33 Hz, H-1), 4.54 (d, 1H, C$H_2$Ar), 4.26 (d, 1H, $J_{1',2'}$ = 7.1 Hz, H-1′), 4.22 (m, 1H, $J_{4',5'}$ = 9.6 Hz, $J_{5',6'}$ = 3.0 Hz, $J_{5',6''}$ = 6.1 Hz, H-5′), 4.17 (m, 1H, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 3.2 Hz, $J_{5,6'}$ = 4.9 Hz, H-5), 3.67—3.92 (m, 16H, H-3, H-3′, H-4, H-4′, H-6, H-6′, H-6′, H-6″, C$H_2$—O—C-2, C$H_2$—O—C-2′, C$H_2$—O—C-3, C$H_2$—O—C-3′), 3.81 (s, 3H,

$CH_3$—O—Ar), 3.38 (s, 3H, $CH_3$—O—C-1), 3.28 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2′), 3.24 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-2), 2.05 (m, 6H, $2CH_3CO$), 1.50—1.60 (m, 8H, $CH_2CH_2$—O—C-2, $CH_2CH_2$—O—C-2′, $CH_2CH_2$—O—C-3, $CH_2CH_2$—O—C-3′), 1.20—1.30 (m, 88H, $(CH_2)_{11}(CH_2)_2$—O—C-2, $(CH_2)_{11}(CH_2)_2$—O—C-2′, $(CH_2)_{11}(CH_2)_2$—O—C-3, $(CH_2)_{11}(CH_2)_2$—O—C-3′), 0.89 (m, 12H, $CH_3(CH_2)_{13}$—O—C-2, $CH_3(CH_2)_{13}$—O—C-2′, $CH_3(CH_2)_{13}$—O—C-3, $CH_3(CH_2)_{13}$—O—C-3′). $^{13}$C NMR spectrum (75.47 MHz, $CDCl_3$), $\delta$: 170.82, 169.47 ($2COCH_3$), 159.25, 130.86, 129.44, 113.82 ($6C_{arom}$), 103.98 (C-1′), 97.95 (C-1), 82.26 (C-3′), 81.65 (C-5′), 80.78 (C-3), 77.10 ($CH_2Ar$), 74.57 (C-2′), 73.66 (C-2), 73.36, 73.30, 71.75, 71.72 ($CH_2$—O—C-3′, $CH_2$—O—C-3, $CH_2$—O—C-2′, $CH_2$—O—C-2), 69.97 (C-4′), 69.80 (C-4), 68.81 (C-5), 68.15 (C-6), 62.55 (C-6′), 55.25 ($CH_3$—O—$C_{arom}$), 55.10 ($CH_3$—O—C-1), 38.73, 31.92, 30.65, 30.35, 29.70, 29.60, 29.37, 28.92, 26.29, 26.16, 23.74, 22.97, 22.68 ($48CH_3(CH_2)_{12}CH_2$), 20.88, 20.77 ($2CH_3CO$), 14.10 ($CH_3(CH_2)_{13}$), 10.95 ($3CH_3(CH_2)_{13}$). Mass spectrum (MALDI): $m/z$ = 1351.08 $(M + Na)^+$.

## CONCLUSION

The present study of the model long-chain *O*-alkylation of the partially protected D-glucose derivatives with the *t*-BuOK/*t*-BuOH/$C_{14}H_{29}Br$ alkylation system has revealed some restrictions of the method. It can be a restricted solubility of the sugar derivatives or, what is more serious, yield limitations of the completely *O*-alkylated products. The final coupling of di-*O*-alkylated precursors has led at the conditions chosen to a single disaccharidic product, the expected derivative of gentiobiose. However, the yield of the coupling reaction was rather low. As the glycosidic linkage is being easily subjected to the enzymatic hydrolysis, this type of coupling was not further studied. Finally, taking into account a generally known low reactivity of long-chain alkylation agents, the *t*-BuOK/*t*-BuOH/$C_{14}H_{29}Br$ alkylation system was found to be quite convenient as it provides acceptable moderate yields of di-*O*-alkylated products.

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