AN EASY REGIO- AND STEREOSELECTIVE SYNTHESIS OF A VERSATILE TRISACCHARIDE PRECURSOR FOR SYNTHETIC CARBOHYDRATE BASED VACCINES

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The trisaccharide allyl 2-deoxy-2-phthalimido-6-*O*-[4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl]-β-D-glucopyranoside (**3**) was prepared directly from acceptor allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (**1**) and 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**2c**) using regio- and stereoselective synthesis in dichloromethane with trimethylsilyl triflate as a promoter.

Keywords: Trisaccharides; Oligosaccharides; Carbohydrates; Glycosidation; Regioselective synthesis; Stereoselective synthesis.

In many countries, the pneumonia caused by *Streptococcus pneumoniae* is the biggest bacteriological cause of death in children younger than 5 years and in adults over 50. Approximately 5 million children die every year of pneumonia[1,2], 27% of that induced by *S. pneumoniae* (>85 serotypes). The capsular polysaccharide is the essential factor of virulence in the *S. pneumoniae* serotype 14. Some efforts are focused on the synthesis of its structure unit, a tetrasaccharide fragment {→3)-β-D-Galp-(1→4)-β-D-Glcp-(1→6)-[β-D-Galp-(1→4)]-β-D-GlcpNAc-(1→}$_n$, for preparation of an antigen, useful in obtaining synthetic vaccines[3–5]. Bearing in mind the significance of developing new methodological approaches for preparation of this unit, we report here the synthesis of the precursor trisaccharide allyl 2-deoxy-2-phthalimido-6-*O*-[4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl]-β-D-glucopyranoside (**3**), directly from allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (**1**) (acceptor triol)

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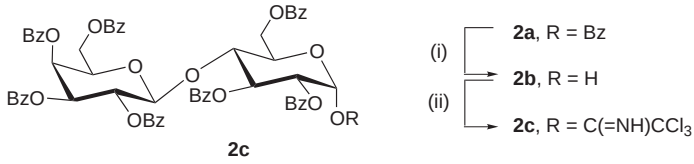
and trichloroacetimidate **2c** (Scheme 1) using regio- and stereoselective procedures, through reactions of OH at C-6 and C-1 in dichloromethane, using trimethylsilyl trifluoromethanesulfonate (TfOTMS) as a promoter.



2c

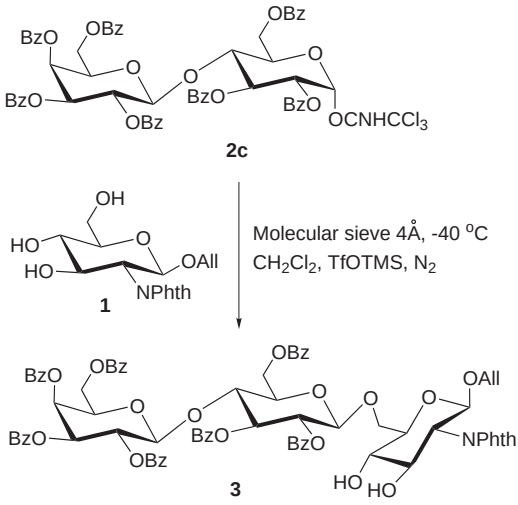
(i) ethanolamine, ethyl acetate; (ii) CCl_3CN, K_2CO_3, CH_2Cl_2

SCHEME 1

RESULTS AND DISCUSSION

The literature survey reveals that synthetic procedures for obtaining trisaccharide fragments are too long and include very tedious and time-consuming work-up operations. We avoid the use of boron trifluoride etherate as a promoter in the glycosylation[6,7], 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and polymer complexes PS–DBU as bases for generating an imidate precursor, employing TfOTMS as a sole promoter for glycosylation and K_2CO_3 (ref.[8]) as a promoter base for obtaining the imidate, which allows to reach very satisfactory yields of **3** under more accessible conditions (Scheme 2, >70%). Our strategy was focused on the previously described[5] lactosylation of the primary OH group in position C-6 in acceptor triol **1**, taking into account its highest reactivity in comparison with OH groups in positions C-3 and C-4 of **1**. The lactosylation of triol **1** with **2c** minimizes the number of reaction steps and optimizes the synthesis of **3** because it is not necessary to protect OH functionalities in positions C-3 and C-4 in the starting triol **1** (ref.[5]). The synthetic scheme is very simple: perbenzoylated lactose **2a** was selectively debenzoylated in the anomeric position C-1 using an ethanolamine–ethyl acetate mixture and **2b** (>90%) was obtained. It is noteworthy to comment in this context that the use of anhydrous hydrazine acetate in anhydrous DMF[9], a very complex and expensive system for deprotecting anomeric position (C-1 in **2b**) does not work so well. The position C-1 in **2b** was *O*-alkylated with trichloroacetonitrile–K_2CO_3 in dichloromethane to obtain **2c**. The observed signal (singlet) at 8.65 ppm corroborates the presence of the imidate group at position C-1 in **2c** with α-configuration (anomeric proton signal 6.71 ppm, $J(1,2)$ = 3.7 Hz). It is to be noted that this is the first report on the direct coupling of triol acceptor

1 with a disaccharide (lactose) imidate **2c** giving a trisaccharide with a satisfactory yield (>70%) of desired stereochemistry (100% β). To confirm the stereochemistry of the desired **3**, the coupling constants for anomeric protons were determined. The values of coupling constants for anomeric protons: H-1, $J(1,2)$ = 8.5 Hz (d, 5.14 ppm); H-1′, $J(1',2')$ = 7.8 Hz (d, 4.93 ppm) and H-1″, $J(1'',2'')$ = 9.5 Hz (d, 5.07 ppm) confirm the β-configuration of all anomeric carbons in **3**.



SCHEME 2

On the other hand, the use of protecting (participating) groups in the position C-2 (OBz) allows to obtain the β-derivative through blocking the α-face of the generated carbocation. For evaluation of the regioselectivity of the process, the variation of chemical shift (Δδ, ppm; DEPT 135) of C-6 in the acceptor triol **1** was used. A significant deshielding of the C-6 in glucose phthalimidate **1**, from 61.05 to 69.39 ppm, and deshielding of H-6 (m, Ha, Hb) from 3.90–3.97 ppm in **1** to 4.25–4.27 ppm in derivative **3** was observed[5,10–12]. These remarkable variations confirm the glycosylation route through C-6. The use of this trisaccharide as a versatile and optimal intermediate for obtaining the tetrasaccharide unit of the vaccine will be reported elsewhere soon.

In conclusion, this method provides a novel, simpler, and convenient route for the synthesis of trisaccharide allyl 2-deoxy-2-phthalimido-6-*O*-[4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl]-β-D-glucopyranoside (**3**), a versatile precursor for synthetic

carbohydrate-based vaccines, through a regio- and stereoselective glycosylation (lactosylation) without any protection of OH at C-3, C-4 in the starting acceptor triol.

EXPERIMENTAL

Optical rotations were measured with a POLAMAT A automatic polarimeter (GDR) in 1% chloroform solutions at 25 °C. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-300 instrument at 360 MHz, chemical shifts were expressed in δ, ppm, relative to the signal for internal TMS at 25 °C, using $CDCl_3$ as a solvent. Coupling constants, *J*, are given in Hz. Column chromatography was performed on Kieselgel 60 (Merck >230 mesh, 0.040–0.63 mm) and fractions were monitored by TLC on Kieselgel 60 F254 (Merck). Detection was effected by charring with 5% H_2SO_4–ethanol.

In work-up procedures, washings were carried three times with appropriate quantities of water, aqueous 10% $NaHCO_3$ or 10% NaCl solutions, unless indicated otherwise, and drying of organic solutions was perfomed with anhydrous calcium sulfate. Evaporations were done under reduced pressure at 40 °C. All solvents were distilled and dried before use.

4-*O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranose (**2b**)

To a mixture of **2a** (1.04 g, 2.40 mmol) in dried ethyl acetate (25 ml), ethanolamine (0.6 ml) was added. The reaction mixture was stirred overnight (protected from light). The reaction was monitored by TLC (silica gel plates, 5:1 v/v toluene–acetone). The product was washed twice with a NaCl solution and H_2O. The organic extract was dried (anhydrous Na_2SO_4), filtered, and concentrated. The separation by column chromatography (5:1 v/v hexane–ethyl acetate) of the residue gave 0.735 g (92%) of syrupy **2b**. ^{1}H NMR ($CDCl_3$): 5.47 d, 0.95 H, *J*(1,2) = 3.6 (H-1α); 5.40 d, 0.05 H, *J*(1,2) = 9.98 (H-1β); 5.80 t, 1 H, *J*(2,3) = 10.3 (H-3); 5.75 d, 1 H, *J*(3′,4′) = 3.5 (H-4′); 5.62 dd, 1 H, *J*(2′,3′) = 10.5 (H-2′); 5.50 dd, 1 H (H-2); 5.41 dd, 1 H, *J*(3′-4′) = 3.5 (H-3′); 5.08 d, 1 H, *J*(1′,2′) = 9.5 (H-1′β); 4.72 m, 1 H (H-5′); 4.53 m, 1 H (H-5); 4.46 m, 2 H (H-6′); 4.38 m, 2 H (H-6); 4.17 dd, 1 H (H-4). ^{13}C NMR ($CDCl_3$): 166.2–170.8 (CO); 121.4–139.8 (Ph); 100.1 (C-1′); 91.47 (C-1); 77.2 (C-4); 76.3 (C-4′); 76.1 (C-2′); 75.3 (C-2); 72.9 (C-5); 72.5 (C-5′); 72.0 (C-3′); 71.2 (C-3); 63.4 (C-6′); 63.9 (C-6).

4-*O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl Trichloroacetimidate (**2c**)

To **2b** (1.04 g, 2.33 mmol), previously dried overnight under vacuum, dry dichloromethane (in high-vacuum line) and activated K_2CO_3 (1.09 g) were added. To this mixture Cl_3CCN (8.1 ml, 8.16 mmol) was added under stirring in N_2 atmosphere. The reaction mixture was stirred overnight and monitored by TLC (silica gel plates, 5:1 v/v toluene–acetone). After completing the reaction, ether was added, the mixture was filtered through Celite, concentrated and the residue was purified by column chromatography (silica gel, 4:1 v/v toluene–acetone) to give 0.781 g (75%) of syrupy **2c**. ^{1}H NMR ($CDCl_3$): 8.65 s, 1 H (NH); 6.71 d, 1 H, *J*(1,2) = 3.7 (H-1α); 5.82 t, 1 H, *J*(2,3) = 10.1 (H-3); 5.78 d, 1 H, *J*(3′,4′) = 3.5 (H-4′); 5.60 dd, 1 H, *J*(2′,3′) = 10.5 (H-2′); 5.54 dd, 1 H (H-2); 5.40 dd, 1 H, *J*(3′,4′) = 3.5 (H-3′); 5.03 d, 1 H,

J(1′,2′) = 9.5 (H-1′β); 4.70 m, 1 H (H-5′); 4.56 m, 1 H (H-5); 4.40 m, 2 H (H-6′); 4.30 m, 2 H (H-6); 4.07 dd, 1 H (H-4). ^{13}C NMR ($CDCl_3$): 161.5 (OCNHCCl$_3$); 166.2–170.8 (CO); 121.4–139.8 (Ph); 100.4 (C-1′); 92.5 (C-1); 79.8 (CCl$_3$); 76.8 (C-4); 76.2 (C-4′); 76.0 (C-2′); 75.3 (C-2); 72.9 (C-5); 72.5 (C-5′); 72.0 (C-3′); 71.2 (C-3); 63.2 (C-6′); 62.9 (C-6).

Allyl 2-Deoxy-2-phthalimido-6-*O*-[4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl]-β-D-glucopyranoside (**3**)

A mixture of **2c** (2.161 g, 3.74 mmol), **1** (0.625 g, 1.79 mmol) and powdered molecular sieves (4Å, 2.5 g) was dried in high-vacuum line for 2 h. Dichloromethane (6 ml) was freshly distilled in the vacuum line onto reaction mixture, then TfOTMS (3 µl, 9.35 mmol) was added dropwise under stirring in N_2 atmosphere. Stirring at –40 °C was continued for 6 h. The reaction was monitored by TLC (silica gel plates, 4:3 v/v ethyl acetate–hexane, R_F 0.35). The reaction mixture was diluted with dichloromethane, filtered through Celite and washed with aqueous 10% $NaHCO_3$ solution and water, dried and concentrated. Column chromatography (silica gel, 4:3 v/v ethyl acetate–hexane) of the residue gave 0.849 g (72%) of syrupy **3**. $[\alpha]_D$ +16. For $C_{78}H_{66}O_{17}N$ (1289.4) calculated: 72.65% C, 5.15% H, 1.08% N; found: 72.33% C, 5.09% H, 1.04% N. ^{1}H NMR ($CDCl_3$): 7.71–7.67 m, 4 H (Phth); 7.05–6.97 m, 35 H (COPh); 5.80 t, 1 H, J(2′,3′) = 9.2 (H-3′); 5.77 d, 1 H, J(3″,4″) = 3.5 (H-4″); 5.70 m, 1 H (OCH$_2$C**H**=CH$_2$); 5.61 dd, 1 H, J(2″,3″) = 10.2 (H-2″); 5.55 dd, 1 H, J(2′,3′) = 9.3 (H-2′); 5.42 dd, 1 H, J(3″,4″) = 3.7 (H-3″); 5.38 dd, 1 H (H-2); 5.14 d, _ H, J(1,2) = 8.5 (H-1β); 5.10 m, 2 H (OCH$_2$CH=C**H$_2$**); 5.07 d, 1 H, J(1″,2″) = 9.5 (H-1″β); 4.93 d, 1 H, J(1′,2′) = 7.8 (H-1′β); 4.68 m, 1 H (H-5″); 4.58 m, 1 H (H-5′); 4.47 t, 1 H (H-5); 4.41 m, 2 H (H-6″); 4.28 m, 2 H (H-6′); 4.25–4.27 m, 2 H (H-6a, H-6b); 4.09 dd, 1 H (H-4′); 3.98 m, 2 H (OCH$_2$CH=C**H$_2$**); 3.94 dd, 1 H (H-4); 3.82 dd, _ H (H-3). ^{13}C NMR ($CDCl_3$): 169.63–162.92 (9 C, COPhth, COPh); 135.21, 130.37, 123.84 (Phth); 134.11, 133.55, 133.21, 131.96, 130.28, 129.68, 128.70 (C-H); 123.22, 117.18 (OCH$_2$-CH=CH$_2$); 101.1 (C-1′); 100.98 (C-1″); 97.07 (C-1); 75.71 (C-3″); 75.25 (C-2); 74.57 (C-5″); 74.43 (C-3′); 73.88 (C-2′); 73.56 (C-2″); 72.50 (C-5′); 72.05 (C-4′); 71.58 (C-5); 71.05 (C-4″); 70.70 (C-3); 69.39 (C-6, DEPT-135); 67.60 (C-4); 64.70 (O**C**H$_2$-CH=CH$_2$).

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