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Synthesis of the sialylated pentasaccharide repeating unit of the capsular polysaccharide of *Streptococcus* group B type VI

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ABSTRACT

An efficient synthetic strategy has been developed for the synthesis of the sialic acid containing pentasaccharide repeating unit of the cell wall *O*-antigen of *Streptococcus* group B type VI strain involving stereoselective α-glycosylation of sialic acid thioglycoside derivative. Stereoselective glycosylation of glycosyl trichloroacetimidate derivatives and thioglycosides were carried out using perchloric acid supported over silica ($HClO_4$–SiO_2) as a solid acid catalyst. A panel of sialic acid donors has been screened for achieving satisfactory yield and stereochemical outcome of the glycosylation reaction.

1. Introduction

Streptococcus agalactiae (Group B, GBS) is a Gram-positive organism, which is the common cause of neonatal sepsis and meningitis worldwide [1–4]. Till date, more than 10 serotypes of *Streptococcus* group B (GBS) have been isolated based on the structural variations in their capsular polysaccharides (CPSs) [5]. Most of the capsular polysaccharides of GBS serotypes contain *N*-acetylneuraminic acid (Sialic acid) residue together with other commonly available monosaccharide moieties [6]. Since CPSs are important virulent factors which play vital roles in the initial stage of invasion of the pathogenic bacteria to the host [7,8], it is quite pertinent to develop glycoconjugates based on the CPS towards the development of possible vaccine leads. Similar to other virulent strains of GBS, type VI was found responsible for significant numbers of neonatal infections [9]. Isolation of the oligosaccharide fragments from the natural sources by biofermentation culture of the bacteria suffer from several shortcomings, which include heterogeneity of the oligosaccharide fragments, presence of biological impurities, chances of self infection etc. Therefore, chemical synthetic approaches are receiving growing interest for achieving the homogeneous and pure oligosaccharides free from biological impurities. They can be used in the development of semisynthetic version of possible vaccines as well as in several immunochemical and biophysical studies [10–13]. Earlier, synthesis of the repeating units of the CPSs of GBS type II and VIII were reported by Adamo et al. [14]. and Whitfield et al. [15,16] respectively. Very recently, Guo and co-workers reported the synthesis of the sialic acid containing oligosaccharides corresponding to the CPS of type II [17] and type V [18] applying block synthetic approach using a protected sialylated galactose building block to achieve desired oligosaccharides. The structure of the repeating unit of the CPS of GBS type VI was reported by Jennings and co-workers in 1994 [19]. It is a pentasaccharide composed of D-glucose, D-galactose and sialic acid in 2:2:1 ratio and the sialic acid is linked to a D-galactose moiety at the non-reducing end through α-(2 → 3) linkage. Although the glycoconjugates derived from the pentasaccharide repeating unit corresponding to the CPS of GBS VI is a promising candidate aiming to the development of anti-GBS vaccine candidate, its chemical synthesis has not been reported so far. Therefore, it is quite pertinent to undertake the synthesis of the sialic acid containing pentasaccharide using recently reported reaction conditions for the glycosylations and functional group modifications. A straightforward sequential synthesis of the pentasaccharide corresponding to the CPS of GBS type VI is reported herein (Fig. 1).

2. Results and discussion

Starting with D-lactose derived disaccharide acceptor (**2**) [20], a series of sequential stereoselective glycosylations were carried out using suitably functionalized D-glucose, D-galactose and sialic acid thioglycoside and phosphate donors **3** [21], **4** [22], **5** [23], **5a** [24] and **5b** [25]. Compounds **2**, **3**, **4**, **5**, **5a** and **5b** were synthesized using multiple numbers of reaction steps following the reaction conditions reported earlier.

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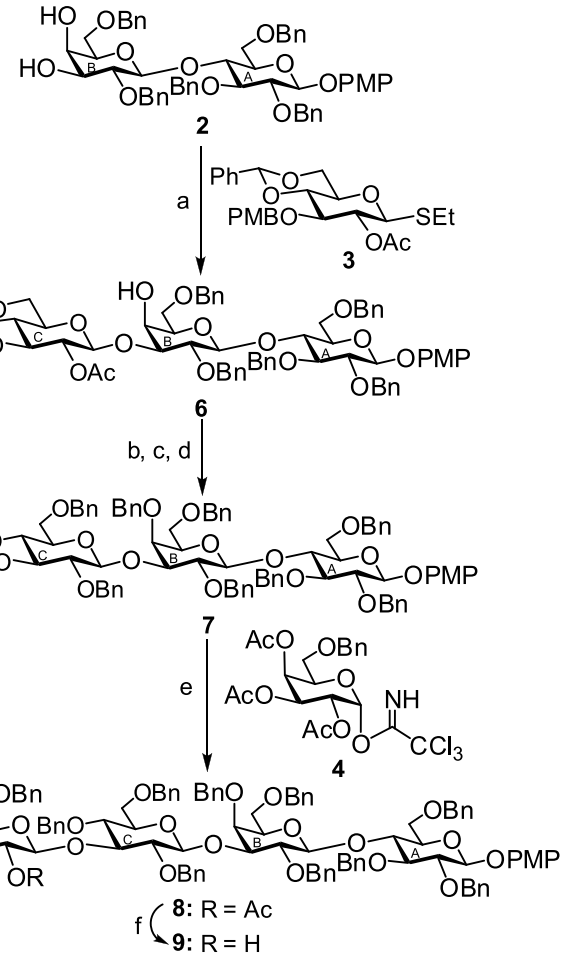
Stereoselective glycosylation of compound **2** [20] with thioglycoside donor **3** [21] using a combination [26] of *N*-iodosuccinimide (NIS) and perchloric acid supported over silica ($HClO_4$–SiO_2) [27] furnished the trisaccharide derivative **6** in 73% yield. The formation of newly formed glycosyl linkage was confirmed from the NMR spectroscopic analysis (Signals at δ 4.91 (d, $J = 9.0$ Hz, H-1_A), 4.52 (d, $J = 8.5$ Hz, H–1_C), 4.48 (d, $J = 8.0$ Hz, H–1_B) in ^{1}H NMR and δ 102.9 (C-1_A), 102.6 (C–1_B), 102.5 (C–1_C) in ^{13}C NMR spectra). Compound **6** was subjected to a series of functional group transformations involving (a) removal of benzylidene acetal using *p*-TsOH; (b) de-*O*-acetylation and benzylation using benzyl bromide and sodium hydroxide [28] and (c) oxidative removal of *p*-methoxybenzyl (PMB) group using DDQ [29] to furnish trisaccharide acceptor **7** in 62% over all yield. Stereoselective glycosylation of compound **7** with D-galactose derived trichloroacetimidate derivative (**4**) [22] in the presence of $HClO_4$–SiO_2 [30] furnished tetrasaccharide derivative **8** in 68% yield, which was de-*O*-acetylated using sodium methoxide to give the tetrasaccharide triol acceptor (**9**) suitable for the glycosylation with sialic acid glycosyl donor. The stereochemistry of the newly formed glycosidic linkages in compound **8** was confirmed from its NMR spectral analysis [signals at δ 4.94 (d, $J = 8.5$ Hz, H-1_D), 4.83 (d, $J = 8.5$ Hz, H–1_B), 4.74 (d, $J = 7.5$ Hz, H-1_A), 4.36 (d, $J = 8.0$ Hz, H–1_C) in ^{1}H NMR and δ 102.8 (2 C, C-1_A, C–1_C), 101.8 (C–1_B), 100.4 (C-1_D) in ^{13}C NMR spectra] (Scheme 1).

In order to incorporate sialic acid in compound **9**, stereoselective glycosylation reactions have been explored using conventional sialic acid thioglycoside (**5**) [23] together with a number of recently developed reaction conditions [31–38] using modified sialic acid thioglycoside (**5a**) [24] and phosphate (**5b**) [25] donors. Following earlier literature reports [31–40], the glycosylation of sialic acid thioglycosides (**5, 5a** and **5b**) was carried out using CH_3CN–CH_2Cl_2 mixed solvent allowing the nitrile effect to influence the reaction intermediate towards the formation of (2 → 3)-α-linked sialic acid glycoside. Stereoselective glycosylation of compound **9** with thioglycoside derivative **5** and **5a** in mixed solvent of CH_3CN and CH_2Cl_2 in the presence of a combination of NIS and triflic acid (TfOH) [41,42] furnished inseparable mixture of (2 → 3)-α-linked sialic acid containing pentasaccharide derivatives **10** and **10a** respectively together with inseparable corresponding sialic acid glycals by products **11** (~50%) and **11a** (~20%) respectively. Whereas, application of sialic acid phosphate donor (**5b**) using TMSOTf [25] as promoter in a mixed solvent of CH_3CN and CH_2Cl_2 led to the formation of an inseparable mixture of the desired product **10a** and corresponding glycal derivative **11a** (~25%). It was decided to carry out deprotection



Scheme 1. Reagents and conditions: (a) NIS, $HClO_4$–SiO_2, CH_2Cl_2, MS-4Å, −40 °C, 1 h, 73%; (b) *p*-TsOH, CH_2Cl_2–CH_3OH (1:1), 0–5 °C, 2 h; (c) BnBr, NaOH, THF, r t, 5 h; (d) DDQ, CH_2Cl_2–H_2O (9:1), r t, 4 h, 62% in three steps; (e) $HClO_4$–SiO_2, CH_2Cl_2, −5 °C, 45 min, 68%; (f) 0.1 M CH_3ONa, CH_3OH, r t, 3 h, 95%.

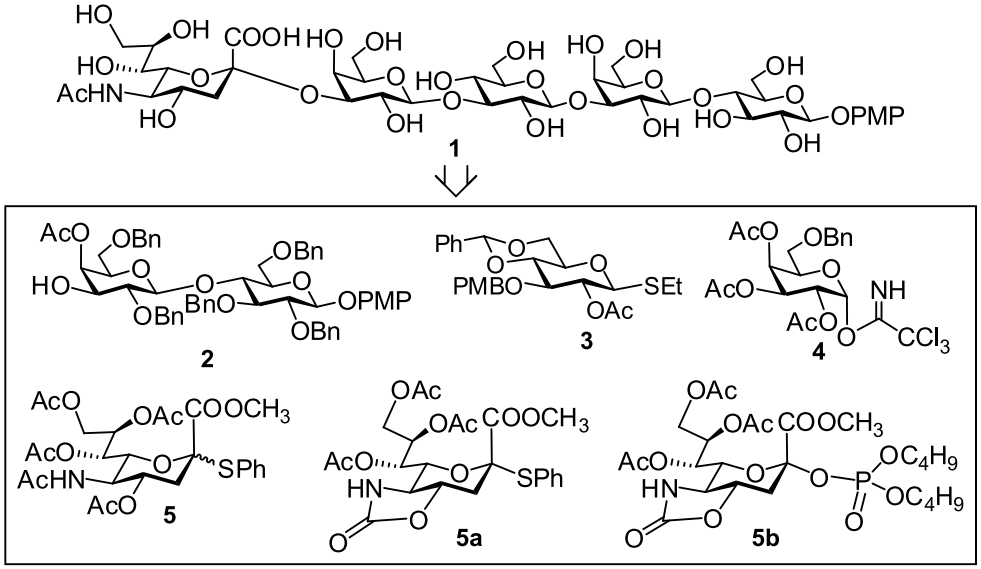
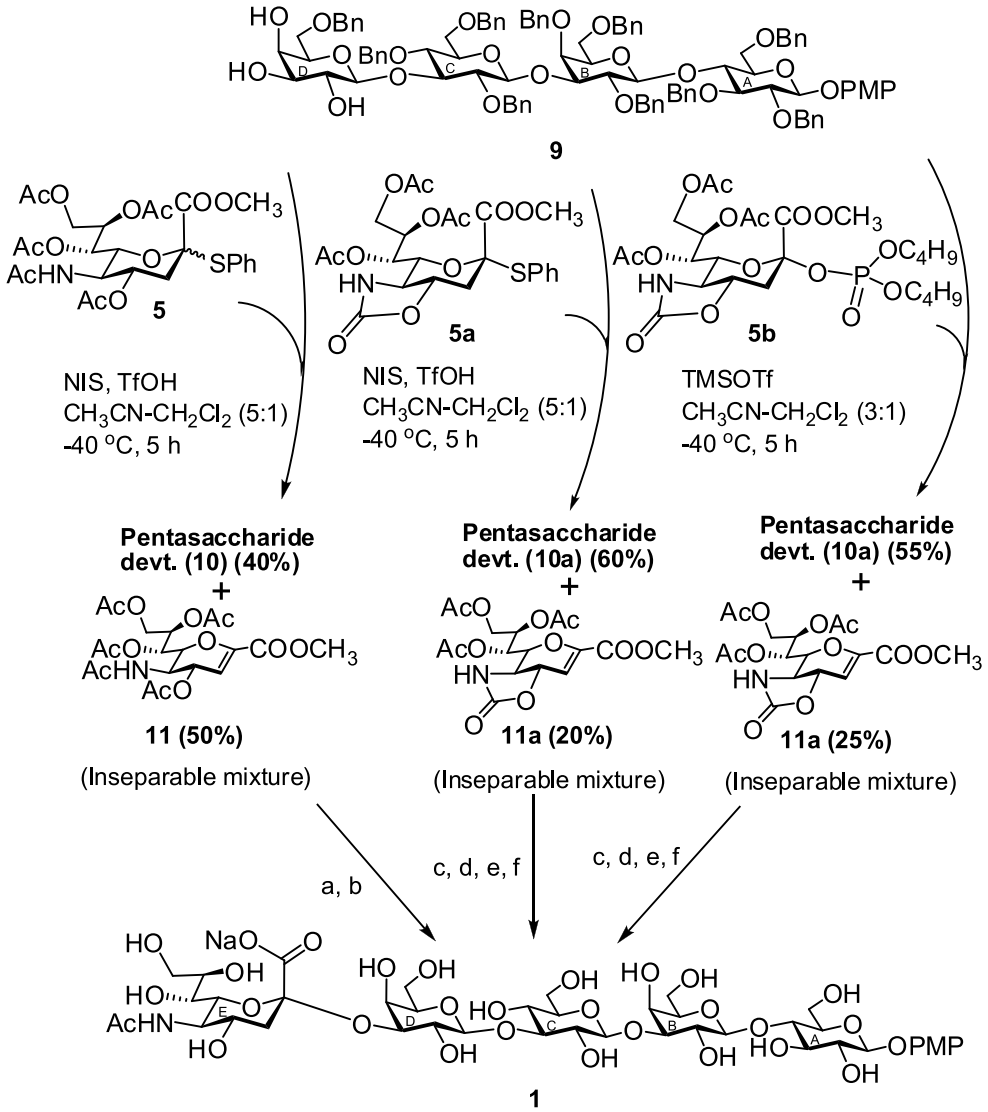


Fig. 1. Structure of the synthesized pentasaccharide repeating unit (**1**) and its synthetic intermediates.

of the inseparable mixture to get pure deprotected pentasaccharide (**1**) after removal of the functional groups. Therefore, hydrogenation of the glycoside mixture (**10** and **11**) obtained by using the sialic acid donor **5** under a positive pressure of hydrogen in the presence of 20% $Pd(OH)_2$–C [43] followed by saponification using sodium methoxide led to the deprotected pentasaccharide (**1**) in 40% over all yield. The other two product mixtures (**10a** and **11a**) obtained by using sialic acid donors **5a** and **5b** subjected to a sequence of reactions involving (a) treatment with lithium hydroxide in aqueous ethanol for the removal of 4-*O*, 5-*N*-carbonyl ring, acetyl groups and methyl ester; (b) acetylation using acetic anhydride and pyridine; (c) hydrogenation over 20% $Pd(OH)_2$–C [43] and (d) de-*O*-acetylation using sodium methoxide to furnish desired pentasaccharide in overall 60% and 55% yield respectively. The desired pentasaccharide (**1**) was purified by column chromatography over Sephadex LH-20 column and characterized using spectral analysis. Although, the stereochemical outcome in the glycosylation product could not be determined by NMR studies due to the formation of inseparable by product, the stereochemistry of the glycosidic linkages in the deprotected compound **1** was unambiguously confirmed by the NMR spectral analysis [44], which clearly showed the formation of a (2 → 3)-α-linked sialic acid glycoside (signals at δ 5.18 (d, J = 8.0 Hz, H-1_D), 4.84 (d, J = 7.5 Hz, 2 H, H-1_A, H–1_C), 4.63 (d, J = 7.5 Hz, H–1_B), 2.61 (dd, J = 13.0 Hz, 4.5 Hz, 1 H, H-3_{eE}), 1.84 (t, J = 12.0 Hz, 1 H, H-3_{aE}) in ^{1}H NMR and δ 103.2 (C-1_A), 103.1 (C–1_C), 102.9 (C-2_E), 102.8 (C–1_B), 100.9 (C-1_D) in ^{13}CNMR spectra) (Scheme 2).

3. Conclusion

In conclusion, a straightforward synthetic strategy has been developed for the synthesis of the α-linked sialic acid containing pentasaccharide repeating unit of the *Streptococcus* group B type VI strain. Perchloric acid supported over silica ($HClO_4$–SiO_2) has been used in the stereoselective activation of glycosyl trichloroacetimidate derivatives and glycosylation of thioglycosides. A panel of sialic acid donors have been evaluated for the satisfactory outcome of sialic acid glycosylation in terms of yield and stereochemical outcome. It was observed that use of 4-*O*,5-*N*-carbonylated sialic acid thioglycoside derivative furnished better yield with excellent stereochemical outcome in the glycosylation reaction in comparison to the conventional per-*O*-acetylated sialic acid thioglycoside donor.



Scheme 2. Reagents and conditions: (a) H_2, 20% $Pd(OH)_2$–C, CH_3OH, r t, 24 h; (i) 0.1 M CH_3ONa, CH_3OH, r t, 6 h, then H_2O added, r t, 10 h, 40% in two steps; (c) LiOH, EtOH–H_2O (3:1), 80 °C, 12 h; (d) Ac_2O, pyridine, r t, 5 h; (e) H_2, 20% $Pd(OH)_2$–C, H_2, CH_3OH–H_2O (1:1), r t, 24 h; (f) 0.1 M CH_3ONa, CH_3OH, r t, 6 h, then H_2O added, r t, 10 h, (60% using donor **5a** and 55% using donor **5b** in three steps).

4. Experimental

4.1. General methods

All reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on TLC were visualized by warming ceric sulphate (2% $Ce(SO_4)_2$ in 2 N H_2SO_4) sprayed plates in hot plate. Silica gel 230–400 mesh was used for column chromatography. NMR spectra were recorded on Bruker Avance 500 MHz using $CDCl_3$ as solvent and TMS as internal reference unless stated otherwise. Chemical shift value is expressed in δ ppm. The complete assignment of proton and carbon spectra was carried out by using a standard set of NMR experiments, e.g. ^{1}H NMR, ^{13}C NMR, ^{13}C DEPT 135, 2D COSY and 2D HSQC etc. ESI-MS were recorded on a Thermo Scientific Orbitrap Velos Pro TM mass spectrometer. Optical rotations were recorded in a Jasco P-2000 spectrometer. Commercially available grades of organic solvents of adequate purity are used in all reactions. $HClO_4$–SiO_2 was prepared following the reported method [27].

4.2. *p-Methoxyphenyl [2-O-acetyl-4,6-O-benzylidene-3-O-(p-methoxybenzyl)-β-D-glucopyranosyl]-(1 → 3)-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (6)*

To a solution of compound **2** (1.5 g, 1.67 mmol) and compound **3** (0.87 g, 1.83 mmol) in anhydrous CH_2Cl_2 (20 mL) was added MS-4Å (2 g) and it was cooled to −40 °C under argon. To the cooled reaction mixture were added NIS (430 mg, 1.91 mmol) and $HClO_4$–SiO_2 (25 mg) and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was filtered and washed with CH_2Cl_2 (50 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$ (50 mL), satd. $NaHCO_3$ (50 mL) and water (50 mL), dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **6** (1.6 g, 73%). Yellow oil; $[\alpha]_D$ − 7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.65–6.83 (m, 38 H, Ar–H), 5.62 (s, 1 H, PhC*H*), 5.20 (m, *J* = 10.5 Hz, 1 H, PhC*H*), 5.08 (t, *J* = 8.5 Hz, 1 H, H–2_C), 5.07 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.95 (d, *J* = 11.0 Hz, 1 H, PhC*H*), 4.91 (d, *J* = 9.0 Hz, 1 H, H-1_A), 4.88–4.82 (m, 3 H, 3 PhC*H*), 4.68 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.63 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.59 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.52 (d, *J* = 8.5 Hz, 1 H, H–1_C), 4.48 (d, *J* = 8.0 Hz, 1 H, H–1_B), 4.45 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.44 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.31 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.22–4.21 (m, 1 H, H-6_{aA}), 4.12 (d, *J* = 2.5 Hz, 1 H, H–4_B), 4.06 (t, *J* = 9.0 Hz, 1 H, H–4_C), 3.81 (br s, 6 H, 2 OCH_3), 3.79–3.74 (m, 5 H, H-3_A, H–3_B, H-6_{bA}, H-6_{abB}), 3.70 (t, *J* = 6.5 Hz, 2 H, H-4_A, H–5_C), 3.63 (dd, *J* = 7.5 Hz, 2.5 Hz, 2 H, H–3_C, H-6_{aC}), 3.57–3.49 (m, 3 H, H–2_B, H-5_A, H–5_B), 3.42–3.39 (m, 2 H, H-2_A, H-6_{bC}), 2.12 (s, 3 H, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.6 (*C*OCH$_3$), 159.2–113.6 (Ar–C), 102.9 (C-1_A), 102.6 (C–1_B), 102.5 (C–1_C), 101.3 (Ph*C*H), 82.6 (C-4_A), 81.4 (C-3_A, C–5_C), 78.2 (C–5_B), 77.8 (C–3_B), 76.8 (C–4_C), 75.6 (Ph*C*H$_2$), 75.3 (C–4_B), 75.2, 75.1 (2 Ph*C*H$_2$), 75.0 (C-2_A), 74.9 (C-5_A), 73.7 (Ph*C*H$_2$), 73.4 (C–2_C), 73.2, 73.1 (2 Ph*C*H$_2$), 72.9 (C–3_C), 68.6 (C–6_B), 68.4 (C–6_C), 68.1 (C-6_A), 65.9 (C–2_B), 55.5, 55.1 (2 O*C*H$_3$), 21.0 (CO*C*H$_3$); HRMS $[M+1]^+$: Calcd. for $C_{77}H_{82}O_{19}$: 1311.5528; found 1311.5510.

4.3. *p-Methoxyphenyl (2,4,6-tri-O-benzyl-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (7)*

To a solution of compound **6** (1.5 g, 1.14 mmol) in CH_2Cl_2–CH_3OH (20 mL; 1:1) was added *p*-TsOH (200 mg) and the reaction mixture was stirred at 0–5 °C for 2 h. The reaction mixture was quenched with Et_3N (1 mL) and the solvents were removed under reduced pressure. To a solution of the crude product in DMF (15 mL) was added benzyl bromide (1 mL, 8.42 mmol) followed by powdered NaOH (0.5 g, 12.5 mmol) and the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (100 mL). The organic layer was successively washed with satd. $NaHCO_3$ (50 mL) and H_2O (50 mL), dried (Na_2SO_4) and concentrated. To a solution of the crude product in CH_2Cl_2–H_2O (30 mL; 9:1) was added DDQ (500 mg, 2.20 mmol) and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture extracted with CH_2Cl_2 (100 mL) and the organic layer was washed with H_2O (50 mL), dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (8:1) as eluant to give pure compound **7** (1.0 g, 62%). Yellow oil; $[\alpha]_D$ − 17 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.42–6.73 (m, 49 H, Ar–H), 5.06 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.97 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.92–4.88 (m, 2 H, 2 PhC*H*), 4.85 (d, *J* = 8.5 Hz, 1 H, H–1_B), 4.80 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.78 (d, *J* = 8.5 Hz, 1 H, H-1_A), 4.69 (br s, 2 H, 2 PhC*H*), 4.63–4.39 (m, 11 H, 11 PhC*H*), 4.38 (d, *J* = 8.5 Hz, 1 H, H–1_C), 4.25 (d, *J* = 8.0 Hz, 1 H, H–4_B), 4.01 (t, *J* = 9.0 Hz, 1 H, H–4_C), 3.85 (t, *J* = 9.0 Hz, 1 H, H-4_A), 3.77 (br s, 2 H, H-6_{aA}, H-6_{aB}), 3.75 (s, 3 H, OCH_3), 3.72–3.54 (m, 8 H, H-2_A, H-3_A, H–3_B, H–3_C, H-5_A, H–5_B, H-6_{bA}, H-6_{bB}), 3.47–3.32 (m, 4 H, H–2_C, H–5_C, H-6_{abC}), 3.15 (t, *J* = 9.0 Hz, 1 H, H–2_B), 2.41 (br s, 1 H, O*H*); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.3–114.5 (Ar–C), 102.9 (C-1_A), 102.8 (C–1_B), 102.5 (C–1_C), 83.0 (C-4_A), 82.3 (C-3_A), 81.7 (C–5_C), 81.6 (C–5_B), 80.4 (C–3_B), 77.2 (C–4_C), 76.7 (C–4_B), 76.6 (C-5_A), 75.8 (Ph*C*H$_2$), 75.3 (C-2_A), 75.2, (2 Ph*C*H$_2$), 74.8 (C–3_C), 74.5, 74.0 (2 Ph*C*H$_2$), 73.9 (C–2_C), 73.4, 73.3, 73.2, 72.9 (4 Ph*C*H$_2$), 71.2 (C–2_B), 69.2 (C-6_A, C–6_B), 68.2 (C–6_C), 55.5, (O*C*H$_3$); HRMS $[M+1]^+$: Calcd. for $C_{88}H_{92}O_{17}$: 1421.6413; found 1421.6394.

4.4. *p-Methoxyphenyl (2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1 → 3)-(2,4,6-tri-O-benzyl-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (8)*

A solution of compound **7** (0.8 g, 0.56 mmol) and compound **4** (0.34 g, 0.63 mmol) in anhydrous CH_2Cl_2 (10 mL) was cooled to −5 °C under argon. To the cooled reaction mixture was added $HClO_4$–SiO_2 (50 mg) and the reaction mixture was allowed to stir at same temperature for 45 min. The reaction mixture was filtered and washed with CH_2Cl_2 (50 mL). The organic layer was successively washed with satd. $NaHCO_3$ (50 mL) and water (50 mL), dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure compound **8** (0.69 g, 68%). Yellow oil; $[\alpha]_D$ − 10 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.30–6.67 (m, 54 H, Ar–H), 5.33 (br s, 1 H, H-4_D), 5.06 (t, *J* = 9.0 Hz, 1 H, H-2_D), 4.98 (d, *J* = 12.0 Hz, 1 H, 1 PhC*H*), 4.96 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.94 (d, *J* = 8.5 Hz, H-1, H-1_D), 4.92–4.85 (m, 3 H, 3 PhC*H*), 4.83 (d, *J* = 8.5 Hz, 1 H, H–1_B), 4.80 (dd, *J* = 9.0 Hz, 2.5 Hz, 1 H, 2 H-3_D), 4.74 (d, *J* = 7.5 Hz, 1 H, H-1_A), 4.67 (d, *J* = 11.0 Hz, 1 H, PhC*H*), 4.61–4.57 (m, 2 H, 2 PhC*H*), 4.52–4.45 (m, 5 H, 5 PhC*H*), 4.40–4.37 (m, 5 H, 5 PhC*H*), 4.36 (d, *J* = 8.0 Hz, 1 H, H–1_C), 4.34 (d, *J* = 11.0 Hz, 1 H, PhC*H*), 4.26 (brs, 1 H, H–4_B), 4.15–4.12 (m, 2 H, 2 PhC*H*), 3.92 (t, *J* = 9.0 Hz, 1 H, H–4_C), 3.86 (t, *J* = 9.0 Hz, 1 H, H-4_A), 3.76–3.73 (m, 1 H, H-6_{aA}), 3.72–3.65 (m, 2 H, H-6_{abD}), 3.67 (s, 3 H, 3 OCH_3), 3.62–3.58 (m, 4 H, H–3_B, H–3_C, H-6_{aB}, H-6_{bA}), 3.52–3.48 (m, 4 H, H-3_A, H-5_A, H–5_B, H-6_{bB}), 3.41–3.37 (m, 3 H, H-2_A, H–2_C, H–5_C), 3.28–3.22 (m, 3 H, H-5_D, H-6_{abC}), 3.18 (t, *J* = 8.5 Hz, 1 H, H–2_B), 1.90 (s, 3 H, COCH_3), 1.88 (s, 3 H, COCH_3), 1.70 (s, 3 H, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8, 169.7, 169.1 (3 *C*OCH$_3$), 155.2–114.4 (Ar–C), 102.8 (C-1_A, C–1_C), 101.8 (C–1_B), 100.4 (C-1_D), 83.3 (C-5_D), 83.1 (C–5_B), 82.3 (C–2_C), 81.5 (C-2_A), 80.6 (C-4_A), 80.5 (C–3_C), 76.6 (C–4_C), 75.7 (Ph*C*H$_2$), 75.6 (C–5_C), 75.4 (C-3_A), 75.3, 75.1, 74.9, 74.5 (4 Ph*C*H$_2$), 74.4 (C-5_A), 73.7 (C–2_B), 73.5, 73.4, 73.3, 73.1, 72.6 (5 Ph*C*H$_2$), 71.6 (C-3_D), 71.2 (C–3_B), 69.8 (C-2_D, C–4_B), 69.0 (C–6_B), 68.9 (C–6_C), 68.1 (C-6_A), 67.6 (C-4_D), 66.6 (C-6_D), 55.5, (O*C*H$_3$), 20.7, 20.6 (3 CO*C*H$_3$); HRMS $[M+1]^+$: Calcd. for $C_{107}H_{114}O_{25}$: 1799.7727; found 1799.7712.

4.5. *p*-Methoxyphenyl (6-O-benzyl-β-D-galactopyranosyl)-(1 → 3)-(2,4,6-tri-O-benzyl-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (9)

A solution of compound **8** (0.6 g, 0.33 mmol) in 0.1 M CH_3ONa in CH_3OH (20 mL) was stirred at room temperature for 3 h and neutralized with Dowex 50 W X8 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure to give compound **9** (525 mg, 95%). Colorless oil; $[\alpha]_D$ − 25 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–6.73 (m, 54 H, Ar–H), 5.08 (d, J = 11.0 Hz, 1 H, PhC*H*), 5.02 (d, J = 11.0 Hz, 1 H, PhC*H*), 4.93–4.89 (m, 2 H, 2 PhC*H*), 4.90 (d, J = 8.5 Hz, 1 H, H-1_D), 4.80 (d, J = 8.0 Hz, 1 H, H–1_B), 4.77 (d, J = 8.5 Hz, 1 H, H-1_A), 4.70 (brs, 2 H, 2 PhC*H*), 4.59–4.36 (m, 14 H, 14 PhC*H*), 4.34 (d, J = 8.5 Hz, 1 H, H–1_C), 4.27 (d, J = 2.0 Hz, 1 H, H–4_B), 3.98 (t, J = 9.0 Hz, 1 H, H–4_C), 3.86 (t, J = 9.0 Hz, 1 H, H-4_A), 3.83–3.81 (m, 2 H, H-6_{abD}),3.75–3.74 (m, 2 H, H-6_{abA}), 3.72 (s, 3 H, OC*H*$_3$), 3.36–3.64 (m, 4 H, H–3_B, H–3_C, H-6_{abB}), 3.59–3.52 (m, 5 H, H-3_A, H-3_D, H-4_D, H–5_B, H–5_C), 3.43–3.40 (m, 5 H, H-2_A, H–2_C, H-5_A, H-6_{abC}), 3.38–3.31 (m, 3 H, H–2_B, H-2_D, H-5_D); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.7–114.5 (Ar–C), 104.7 (C-1_A), 102.9 (C–1_C), 102.8 (C–1_B), 102.5 (C-1_D), 83.0 (C-5_D), 82.7 (C–5_B), 82.2 (C–2_C), 81.7 (C-2_A), 81.6 (C-4_A), 80.5 (C–3_C), 77.6 (C–4_C), 75.7 (C–5_C), 75.4 (Ph*C*H$_2$), 75.3 (C-3_A), 75.2, 75.1, 74.8 (3 Ph*C*H$_2$), 74.6 (C-5_A), 74.5 (Ph*C*H$_2$), 73.8 (C–2_B), 73.7 (C–3_B), 73.5, 73.4, 73.3, (3 Ph*C*H$_2$), 73.3 (C-3_D), 73.2 (Ph*C*H$_2$), 73.0 (C-2_D), 72.9 (Ph*C*H$_2$), 71.1.(C–4_B), 69.1 (C-6_A), 69.0 (C–6_B), 68.9 (C–6_C), 68.3 (C-4_D), 68.2 (C-6_D), 55.5, (O*C*H$_3$); HRMS $[M+1]^+$: Calcd. for $C_{101}H_{108}O_{22}$: 1673.7410; found 1673.7394.

4.6. *p*-Methoxyphenyl [sodium(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate]-(2 → 3)-(β-D-galactopyranosyl)-(1 → 3)-(β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 4)-β-D-glucopyranoside (1)

4.6.1. Glycosylation of compound 9 with sialic acid donor 5

To a solution of compound **9** (500 mg, 0.298 mmol) and compound **5** (435 mg, 0.74 mmol) in anhydrous CH_3CN–CH_2Cl_2 (10 mL; 5:1 v/v) was added MS-3Å (1 g) and it was cooled to −40 °C under argon. To the cooled reaction mixture were added NIS (330 mg, 1.46 mmol) and TfOH (25 μL) and the reaction mixture was allowed to stir at same temperature for 5 h. The reaction mixture was filtered and washed with CH_2Cl_2 (50 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$ (50 mL), satd. $NaHCO_3$ (50 mL) and water (50 mL), dried (Na_2SO_4) and concentrated. The crude product was passed through a short pad of SiO_2 using hexane-EtOAc (1:1) as eluant to give glycosylated product (**10**) together with sialic acid glycal derivative (**11**). To a solution of the product mixture in CH_3OH (5 mL) was added 20% $Pd(OH)_2$–C (50 mg) and it was stirred under a positive pressure of H_2 at room temperature for 24 h. The reaction mixture was filtered through a Celite bed and concentrated under reduced pressure. A solution of the product in 0.1 M CH_3ONa in CH_3OH (10 mL) was stirred at room temperature for 6 h and then H_2O (0.2 mL) was added to the reaction mixture and allowed to stir at room temperature for 10 h and neutralized with Dowex 50 W X8 (H^+) resin. The reaction mixture was filtered and passed through a short column of Dowex 50 W X8 (Na^+) resin and concentrated under reduced pressure. The deprotected product was passed through a column of Sephadex LH-20 column using CH_3OH–H_2O (6:1) as eluant to give pure compound **1** (128 mg, 40%). White powder; $[\alpha]_D$ − 19 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 7.25–7.10 (m, 4 H, Ar–H), 5.18 (d, J = 8.0 Hz, 1 H, H-1_D), 4.84 (d, J = 7.5 Hz, 2 H, H-1_A, H–1_C), 4.63 (d, J = 7.5 Hz, 1 H, H–1_B), 4.38 (d, J = 2.5 Hz, 1 H, H-4_D), 4.31 (d, J = 2.5 Hz, 1 H, H–4_B), 4.30–4.28 (m, 1 H, H–5_C), 4.12–4.02 (m, 5 H, H-3_D, H-6_{aA}, H-6_{aC}, H-5_E, H-8_E), 3.97–3.92 (m, 6 H, H–3_B, H–3_C, H-9_{aE}, H-6_{bA}, H-6_{aB}, H-6_{bC}), 3.93 (s, 3 H, OC*H*$_3$), 3.90–3.83 (m, 8 H, H–2_B, H-2_D, H–5_B, H-5_D, H-6_{bB}, H-6_{abD}, H-9_{bE}), 3.81–3.73 (m, 4 H, H–2_C, H-4_E, H-6_E, H-7_E), 3.72–3.67 (m, 2 H, H-4_A, H–4_C), 3. 67–3.63 (m, 2 H, H-2_A, H-3_A), 3.62–3.56 (m, 1 H, H-5_A), 2.61 (dd, J = 13.0 Hz, 4.5 Hz, 1 H, H-3_{eE}), 2.17 (s, 3 H, COC*H*$_3$), 1.84 (t, J = 12.0 Hz, 1 H, H-3_{aE}); ^{13}C NMR (125 MHz, D_2O): δ 175.5 (NHCOCH$_3$), 174.7 (*C*OOH), 154.7–115.0 (Ar–C), 103.2 (C-1_A), 103.1 (C–1_C), 102.9 (C-2_E), 102.8 (C–1_B), 100.9 (C-1_D), 84.3 (C–3_C), 77.9 (C–3_B), 77.4 (C-4_A), 77.2 (C–5_B), 75.4 (C-5_D), 75.0 (C-5_A), 74.8 (C–5_C), 74.5 (C-3_A), 74.1 (C-2_A), 73.3 (C-6_E), 72.9 (C-8_E), 72.6 (C–2_C), 71.6 (C-2_D), 71.4 (C-3_D), 69.9 (C–2_B, C–4_B), 68.5 (C-7_E), 68.0 (C-4_E), 67.9 (C-4_D), 66.1 (C–4_C), 63.3 (C-9_E), 60.9.(C–6_B, C-6_D), 60.6 (C–6_C), 59.8 (C-6_A), 55.7, (O*C*H$_3$), 52.0 (C-5_E), 40.6 (C-3_E), 22.1 (CO*C*H$_3$); HRMS $[M]^+$: Calcd. for $C_{42}H_{64}NNaO_{30}$: 1085.3411; found 1085.3392. The deprotected glycal derivative (side product) could not be isolated during column chromatography.

4.6.2. Glycosylation of compound 9 with Sialic acid donor 5a

To a solution of compound **9** (100 mg, 0.06 mmol) and compound **5a** (62 mg, 0.12 mmol) in anhydrous CH_3CN–CH_2Cl_2 (5 mL; 5:1 v/v) was added MS-3Å (0.2 g) and it was cooled to −40 °C under argon. To the cooled reaction mixture were added NIS (55 mg, 0.24 mmol) and TfOH (5 μL) and the reaction mixture was allowed to stir at same temperature for 5 h. The reaction mixture was filtered and washed with CH_2Cl_2 (25 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$ (25 mL), satd. $NaHCO_3$ (25 mL) and water (25 mL), dried (Na_2SO_4) and concentrated. The crude product was passed through a short pad of SiO_2 using toluene-acetone (6:1) as eluant to give glycosylated product (**10a**) together with sialic acid glycal derivative (**11a**). To a solution of the product mixture in EtOH–H_2O (5 mL; 3:1) was added LiOH (30 mg, 1.25 mmol) and the reaction was stirred at 80 °C for 12 h. The reaction mixture was cooled to room temperature, neutralized with 10% HCl and concentrated. A solution of the crude product in pyridine (1 mL) and acetic anhydride (1 mL) was stirred at room temperature for 5 h and concentrated. The crude product mixture was extracted with EtOAc (3 × 20 mL) and the organic layer was concentrated. To a solution of the product obtained in CH_3OH (5 mL) was added 20% $Pd(OH)_2$–C (25 mg) and it was stirred under a positive pressure of H_2 at room temperature for 24 h. The reaction mixture was filtered through a Celite bed and concentrated under reduced pressure. A solution of the product in 0.1 M CH_3ONa in CH_3OH (10 mL) was stirred at room temperature for 6 h and then H_2O (0.2 mL) was added to the reaction mixture and allowed to stir at room temperature for 10 h and neutralized with Dowex 50 W X8 (H^+) resin and concentrated under reduced pressure. The deprotected product was passed through a column of Sephadex LH-20 column using CH_3OH–H_2O (6:1) as eluant to give pure compound **1** (40 mg, 60%). White powder. The analytical data was identical with the compound **1**, obtained earlier. The deprotected glycal derivative (side product) could not be isolated during column chromatography.

4.6.3. Glycosylation of compound 9 with Sialic acid donor 5b

To a solution of compound **9** (100 mg, 0.06 mmol) and compound **5b** (75 mg, 0.12 mmol) in anhydrous CH_3CN–CH_2Cl_2 (5 mL; 3:1 v/v) was added MS-3Å (0.2 g) and it was cooled to −40 °C under argon. To the cooled reaction mixture was added TMSOTf (15 μL) and the reaction mixture was allowed to stir at same temperature for 5 h. The reaction mixture was filtered and washed with CH_2Cl_2 (25 mL). The organic layer was successively washed with satd. $NaHCO_3$ (25 mL) and water (25 mL), dried (Na_2SO_4) and concentrated. The crude product was passed through a short pad of SiO_2 using toluene-acetone (6:1) as eluant to give glycosylated product (**10a**) together with sialic acid glycal derivative (**11a**). To a solution of the product mixture in EtOH–H_2O (5 mL; 3:1) was added LiOH (30 mg, 1.25 mmol) and the reaction was stirred at 80 °C for 12 h. The reaction mixture was cooled to room temperature, neutralized with 10% HCl and concentrated. A solution of the crude product in pyridine (1 mL) and acetic anhydride (1 mL) was stirred at room temperature for 5 h and concentrated. The crude product mixture was extracted with EtOAc (3 × 20 mL) and the organic layer was concentrated. To a solution of the product obtained in CH_3OH (5 mL) was added 20% $Pd(OH)_2$–C (25 mg) and it was stirred under a positive

pressure of H_2 at room temperature for 24 h. The reaction mixture was filtered through a Celite bed and concentrated under reduced pressure. A solution of the product in 0.1 M CH_3ONa in CH_3OH (10 mL) was stirred at room temperature for 6 h and then H_2O (0.2 mL) was added to the reaction mixture and allowed to stir at room temperature for 10 h and neutralized with Dowex 50 W X8 (H^+) resin and concentrated under reduced pressure. The deprotected product was passed through a column of Sephadex LH-20 column using CH_3OH–H_2O (6:1) as eluant to give pure compound **1** (35 mg, 55%). White powder. The analytical data was identical with the compound **1**, obtained earlier. The deprotected glycal derivative (side product) could not be isolated during column chromatography.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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