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ORIGINAL ARTICLE

Expedient synthesis of the pentasaccharide repeating unit of the *O*-antigen of *Escherichia coli* O86 and its conformational analysis

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Abstract Synthesis of the pentasaccharide with a 2-aminoethyl linker attached to the reducing end corresponding to the cell wall *O*-antigen of *Escherichia coli* O86 strain is reported. The synthetic strategy involves sequential glycosylation of suitably protected monosaccharide intermediates under similar glycosylation reaction conditions. Thioglycosides have been used as glycosyl donor throughout the synthetic strategy. Conformational analysis of the synthesized pentasaccharide has been carried out using 2D ROESY NMR spectral analysis and all atom explicit molecular dynamics (MD) simulation technique.

Keywords Lipopolysaccharides · Glycosylations · Antigens · *Escherichia coli* · Molecular dynamics (MD) simulation

Introduction

Development of novel therapeutics against bacterial infections is the thrust area of medicinal chemistry and drug discovery program. Emergence of bacterial strains resistant to the commonly used antibiotics poses extra challenges to this [1]. As an alternative approach for the eradication of bacterial infections several glycoconjugate based promising vaccine candidates have been developed in the recent past [2–5], in which bacterial capsular polysaccharides (CPSs) and lipopolysaccharides (LPSs) have been used as the major component. CPS and LPS are important constituents of the bacterial cell wall [6] and play important roles during the initial stage of bacterial infections to the host [7]. In Gram-negative bacteria the cell wall LPS is composed of lipid A, core oligosaccharide and *O*-antigens [8]. The *O*-antigens, which are exposed outer layer of the cell wall, are mostly responsible for the pathogenicity of the bacteria. The *O*-antigens are composed of oligosaccharide repeating units, which consist of a variety of monosaccharides. *Escherichia coli* (*E. coli*) O86 is an enteropathogenic strain, which causes enteric or diarrheal infections in human [9]. Till date, two different serotypes of this strain have been reported such as *E. coli* O86:K2:H2 [10] and *E. coli* O86:K62:B7 [11] having similar *O*-polysaccharide repeating units consisting of D-galactose, D-galactosamine and L-fucose with appropriate stereochemistry at the glycosyl linkages. The structures of the repeating units of the *O*-antigens of *E. coli* O86 are similar to the human blood group antigens and the immunochemical studies showed that both strains produce anti-B antibodies [11]. In order to get a better understanding of the antigenicity of the glycoconjugate of the *O*-antigen of *E. coli* O86:K62:B7, it is essential to have significant quantity of the pure oligosaccharide hapten for its use in a variety of immunological experiments. Isolation of the oligosaccharide from the killed bacterial cell wall is tedious and suffers from several shortcomings such as handling of live bacterial strain, difficult to remove biological impurities, batch to batch variation in the isolated oligosaccharide chain etc. Therefore, development of a concise chemical synthetic strategy would be the best option to get structurally pure oligosaccharide repeating unit corresponding to the *E. coli* O86:K62:B7 for its

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immunological studies and further use in the glycoconjugate preparation. The target pentasaccharide repeating unit contains three 1,2-*cis* glycosyl linkages, which makes the synthesis quite challenging due to the problems associated with the formation of 1,2-*cis* glycosidic bonds [12]. Very recently, Tony Mong *et al*. [13] reported the synthesis of this pentasaccharide repeating unit as its methyl glycoside applying specially designed reaction conditions for the glycosylations. However, the methoxy group present at the reducing end of the synthesized pentasaccharide cannot be easily removed keeping the cyclic structure of the sugar moiety intact at the reducing end for its further modification towards the preparation of a glycoconjugate derivative. Therefore, it was decided to synthesize the pentasaccharide repeating unit using minimum number of steps and generalized reaction conditions keeping a 2-aminoethyl group at the reducing end, which provides ready availability of an amino group for the conjugation with a suitable protein using standard reaction conditions. Understanding the structural insights of a molecule at an atomic resolution is vital to correlate its biological function. In order to get information on the conformational behaviour of the synthesized pentasaccharide in aqueous environment, NOE based NMR spectral analysis in conjugation with molecular dynamics simulation (MD) techniques have been carried out. Concise chemical synthesis of the pentasaccharide repeating unit as its 2-aminoethyl glycoside corresponding to the *O*-antigen of *E. coli* O86:K62:B7 and its conformational studies are presented herein.

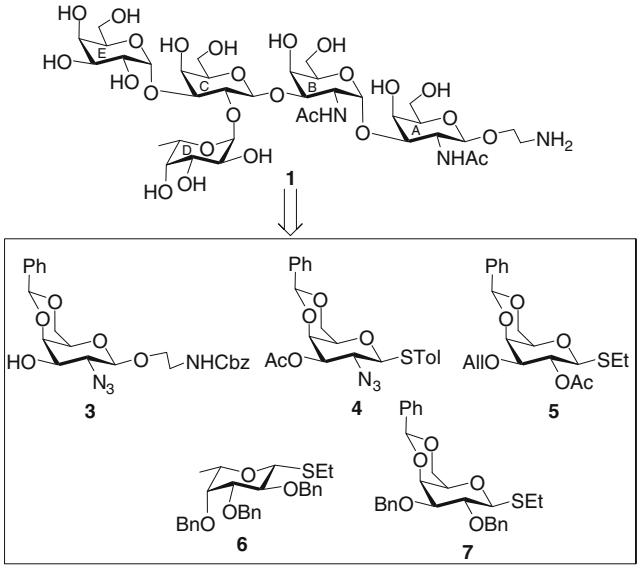


Fig. 1 Structure of the synthesized pentasaccharide as its 2-aminoethyl glycoside and its synthetic intermediates

$$\rightarrow 2\Big)-\alpha-D-Gal\text{p-}(1\rightarrow 3)-\beta-D-Gal\text{p}-(1\rightarrow 3)-\alpha-D-Gal\text{p}NAc-(1\rightarrow 3)-\alpha-D-Gal\text{p}NAc-\Big(1\rightarrow$$
$$\begin{array}{c} 2 \\ \uparrow \\ 1 \\ \alpha-L-Fuc\text{p} \end{array}$$

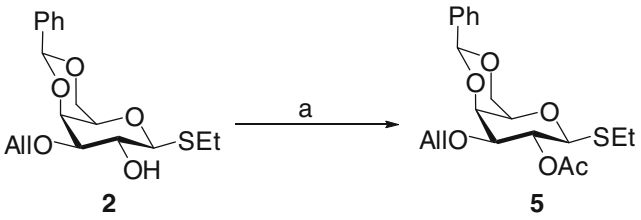
Structure of the repeating unit of the *O*-specific polysaccharide of *E. coli* O86:K62:B7 [11].

Results and discussion

The target pentasaccharide **1** as its 2-aminoethyl glycoside was synthesized using a linear glycosylation strategy. A number of suitably functionalized monosaccharide derivatives **3** [14], **4** [15], **5**, **6** [16] and **7** [17] were prepared from the commercially available reducing sugars using recently reported reaction conditions (Fig. 1). A number of recently reported reaction methodologies have been applied in the synthetic strategy. The key feathers of this report include (a) sequential linear glycosylations; (b) use of thioglycoside donor; (c) use of a combination of *N*-iodosuccinimide (NIS) and a catalytic amount of perchloric acid supported over silica gel ($HClO_4$-SiO_2) as thiophilic activator; (d) use of $HClO_4$-SiO_2 as a noncorrosive solid acid catalyst; (e) removal of benzylidene acetal and acetylation in one step; (f) use of a 2-aminoethyl linker at the reducing terminal and (g) conformational analysis using NMR spectroscopy and molecular dynamics simulation studies.

Ethyl 2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**5**) was prepared in 98 % yield by the acetylation of ethyl 3-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**2**) [18] using acetic anhydride and pyridine (Scheme 1).

Stereoselective 1,2-*cis* glycosylation of D-galactosamine derivative (**3**) with D-galactosamine thioglycoside derivative (**4**) in the presence of a combination [19] of *N*-iodosuccinimide (NIS) and $HClO_4$-SiO_2 [20] furnished disaccharide derivative **8** in 72 % yield, which was confirmed from its spectral analysis [signals at δ 5.40 (d, J = 3.0 Hz, H-1_B), 4.46 (d, J = 8.0 Hz, H-1_A) in the ^{1}H NMR and δ 100.6 (C-1_A), 94.8 (C-1_B) in the ^{13}C NMR spectra]. Presence of a non-

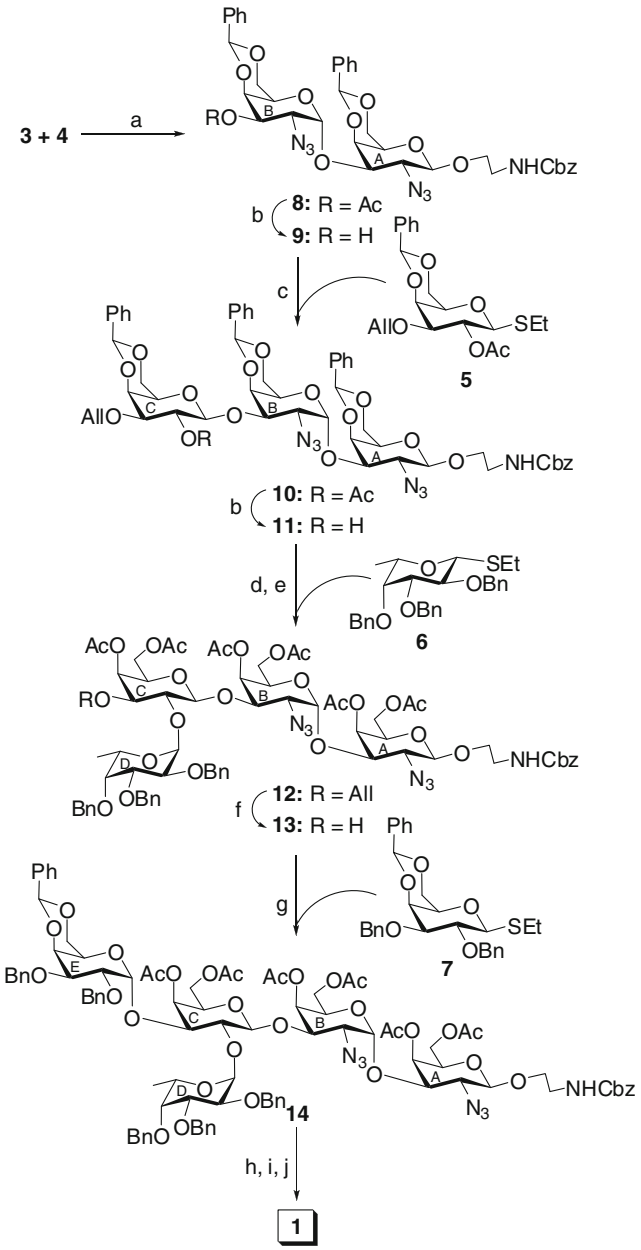


Scheme 1 Reagents: acetic anhydride, pyridine, room temperature, 2 h, 98 %

participating azido group at C-2 position of the glycosyl donor (**4**) supported the formation of 1,2-*cis* glycosylated product. De-*O*-acetylation of compound **8** using sodium methoxide [21] afforded disaccharide acceptor **9** in 93 % yield. 1,2-*Trans* selective glycosylation of compound **9** with D-galactose thioglycoside derivative **5** in the presence of a combination [19] of NIS and $HClO_4$-SiO_2 [20] at low temperature resulted in the formation of trisaccharide derivative **10** in 70 % yield. Formation of compound **10** was supported from its spectral analysis [signals at δ 5.33 (d, J = 3.0 Hz, H-1_B), 4.87 (d, J = 7.5 Hz, H-1_C), 4.33 (d, J = 8.0 Hz, H-1_A) in the ^{1}H NMR and δ 102.1 (C-1_A), 101.9 (C-1_C), 94.5 (C-1_B) in the ^{13}C NMR spectra]. Presence of *O*-acetyl group at C-2 position of the donor (**5**) having neighbouring group participation ability directed the formation of 1,2-*trans* glycoside. De-*O*-acetylation of the acetyl group in compound **10** using sodium methoxide [21] gave trisaccharide acceptor **11** in 87 % yield. Stereoselective 1,2-*cis* glycosylation of compound **11** with L-fucose thioglycoside derivative **6** in the presence of a combination [19] of NIS and $HClO_4$-SiO_2 in a mixed solvent (diethyl ether-dichloromethane) led to the formation of the required tetrasaccharide derivative together with its other isomeric product in minor quantity (~10 %). The crude product mixture was treated with acetic anhydride in the presence of $HClO_4$-SiO_2 to convert the benzylidene acetals into *O*-acetate groups in one step [22]. Purification of the acetylated products furnished tetrasaccharide **12** in 70 % overall yield. Spectral analysis of the compound **12** unambiguously confirmed its formation [signals at δ 5.45 (d, J = 3.5 Hz, H-1_D), 5.21 (d, J = 3.0 Hz, H-1_B), 4.62 (d, J = 7.5 Hz, H-1_C), 4.23 (d, J = 8.5 Hz, H-1_A) in the ^{1}HNMR and δ 102.5 (C-1_C), 102.4 (C-1_A), 97.6 (C-1_D), 94.2 (C-1_B) in the ^{13}CNMR spectra]. The formation of 1,2-*cis* glycosylated product was obtained by keeping a non-participating benzyl ether at the C-2 position of the glycosyl donor (**6**) as well as using ether in the reaction solvent [23, 24]. Removal of the allyl group from compound **12** by the treatment with palladium chloride [25] afforded tetrasaccharide acceptor **13** in 76 % yield. Further 1,2-*cis* glycosylation of compound **13** with D-galactose thioglycoside **7** in the presence of a combination [19] of NIS and $HClO_4$-SiO_2 [20] in a mixed solvent (diethyl ether-dichloromethane) led to the formation of pentasaccharide derivative **14** in 71 % yield. Spectroscopic studies of compound **14** supported its formation [signals at δ 5.36 (d, J = 3.5 Hz, H-1_B), 5.34 (d, J = 3.0 Hz, H-1_D), 5.29 (d, J = 3.5 Hz, H-1_E), 4.58 (d, J = 7.5 Hz, H-1_C), 4.25 (d, J = 7.5 Hz, H-1_A) in the ^{1}HNMR and δ 102.7 (C-1_A), 102.3 (C-1_C), 97.8 (C-1_B), 94.4 (2 C, C-1_D, C-1_E) in the ^{13}CNMR spectra]. The formation of 1,2-*cis* glycosylated product was obtained by keeping a non-participating benzyl ether at the C-2 position of the glycosyl donor (**6**) as well as tuning the reaction condition using ether in the reaction solvent [23, 24]. Finally, compound **14** was subjected to a sequence of functional group transformations, which include (a) direct transformation of azido group into acetamido group by treatment with thioacetic acid [26]; (b) de-*O*-acetylation using sodium methoxide and (c) removal of benzyl ether, benzylidene acetal and Cbz group under a catalytic transfer hydrogenation [27] using triethylsilane and 20 % palladium hydroxide on charcoal to furnish the deprotected target pentasaccharide **1** in 50 % over all yield. NMR spectral analysis of compound **1** supported its formation [signals at δ 5.26 (d, J = 3.5 Hz, H-1_D), 5.25 (d, J = 3.5 Hz, H-1_E), 5.06 (d, J = 3.5 Hz, H-1_B), 4.72 (d, J = 8.0 Hz, H-1_C), 4.60 (d, J = 8.0 Hz, H-1_A) in the ^{1}HNMR and δ 102.2 (C-1_C), 100.9 (C-1_A), 98.8 (C-1_D), 92.9 (2 C, C-1_B, C-1_E) in the ^{13}C NMR spectra] (Scheme 2).

Conformational analysis

In the present study, NMR spectroscopy in conjunction with the Molecular Dynamics (MD) simulation was used to determine the three-dimensional conformational analysis of carbohydrate structure [28]. The distance information of the molecule, which is generally used to determine the three-dimensional structure of any molecule, was collected from two-dimensional ^{1}H-^{1}H ROESY experiments with 150 ms mixing time. This information was further used in MD simulation to determine the three-dimensional structure of the molecule in solution at an atomic resolution. The computational sampling of conformation was performed for a time scale of 20 ns with inter- as well as intra-glycosidic ROE informations. A schematic presentation of the inter- and intra-glycosidic NOE in compound **1** is presented in Fig. S1 in supporting information. The proton-proton distances observed from the MD simulation (Fig. 2a, b) at the interglycosidic linkages were in good agreement to the ROEs cross peaks observed in the 2D ROESY spectrum. In particular, the inter-glycosidic linkages obtained for H-4_A/H-1_B, H-3_B/H-1_C, H-2_C/H-1_D, and H-4_C/H-1_E appeared to be within the range of 2.3 Å – 3.0 Å distance. These informations, which were conserved in the course of MD simulation, were also reflective to the fact that compound **1** attained rigid conformation to the glycosidic linkages between β-D-Gal*p*NAc (ring A) and α-D-Gal*p*NAc (ring B); as well as between α-D-Gal*p*NAc (ring B) and β-D-Gal*p* (ring C). Correspondingly strong ROEs were also



Scheme 2 Reagents: **a** NIS, $HClO_4$-SiO_2, MS 4 Å, CH_2Cl_2, − 10 °C, 1 h, 72 %; **b** 0.1 M CH_3ONa, CH_3OH, room temperature, 2 h, 93 % for compound **9** and 87 % for compound **11**; **c** NIS, $HClO_4$-SiO_2, MS 4 Å, CH_2Cl_2, − 25 °C, 1 h, 70 %; **d** NIS, $HClO_4$-SiO_2, Et_2O-CH_2Cl_2 (2:1), − 5 °C, 1 h; **e** acetic anhydride, $HClO_4$-SiO_2, room temperature, 20 min, 70 % in two steps; **f** $PdCl_2$, CH_3OH, 0–5 °C, 1.5 h, 76 %; **g** NIS, $HClO_4$-SiO_2, Et_2O-CH_2Cl_2 (3:1), − 8 °C, 1 h, 71 %; **h** CH_3COSH, pyridine, room temperature, 22 h; **i** 0.1 M CH_3ONa, CH_3OH, room temperature, 2 h; (**j**) 20 % $Pd(OH)_2$-C, Et_3SiH, CH_3OH, room temperature, 24 h, overall 50 %

observed in the NMR spectrum that reflected efficient spin-spin relaxation effect. The distances for the interglycosidic linkages of H-2_A/H-1_B, H-6_A/H-1_B, and H-6_C/H-1_E were found to be greater than 4 Å, which conferred higher degree of freedom (conformational flexibility). In addition, larger separation distance persisted between β-D-Gal*p*NAc (ring A) and α-D-Gal*p*NAc (ring B) as well as between β-D-Gal*p* (ring C) and α-D-Gal*p* (ring E). Notably, the distance between glycosidic proton H-1_A and proton of protecting group (NHAc) (Fig. 2a) is found to be within 2.7 Å – 3.5 Å that confirmed a rigid conformation around the linkage. The molecular conformational sampling was also evaluated with the consideration of torsion angle (dihedral angle of oligosaccharides) using the notion Phi angle (φ_n) as (H_n-C_n-O_n-C_{n+1}) and Psi angle (Ψ_n) as (C_n-O_n-C_{n+1}-H_{n+1}), where *n* refers to the ring number, considered for the calculation. The obtained torsion angles were within the range of −100° to +10° between β-D-Gal*p*NAc (ring A) and α-D-Gal*p*NAc (ring B) as well as between β-D-Gal*p* (ring C) and α-D-Gal*p* (ring E). Likewise, the torsion angles appeared to be within the range of +10° to +100° between B-C and C-D rings (Fig. 2c, d). Moreover, the confined density of the scatter plot obtained for the φ and Ψ correlation also indicated that these glycosidic linkages were within a rigid three-dimensional space (Fig. 2c, d). The overall root-mean-squared-deviation (RMSD) of compound **1** as indicated from the overall trajectory analysis was reflective to be in a range of 0.35 Å to 1.8 Å. It is noteworthy that the conformational deviation (RMSD) within a scale of 0–4 Å is considered to be almost identical for all the (bio) organic molecules with respect to its structural dynamicity and molecular function [29]. The superimposed snapshots of compound **1** are also displayed in Fig. 3, which dictates the stability of conformation. More importantly, the conformational rigidity is reflective for all the rings except for α-D-Gal*p* (ring E).

Conclusion

In summary, the pentasaccharide repeating unit corresponding to the cell wall *O*-antigen of *E. coli* O86:K62:B7 has been synthesized in good yield using linear glycosylation sequence. Compound **1** with a 2-aminoethyl linker at the reducing terminal has been synthesized using minimum number of reaction steps with very good yield. The conformational analysis of the pentasaccharide was carried out using MD simulation technique in conjugation with 2D ROESY NMR spectral analysis. The conformational analysis confirmed that the compound **1** is significantly rigid in solution.

Experimental section

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.

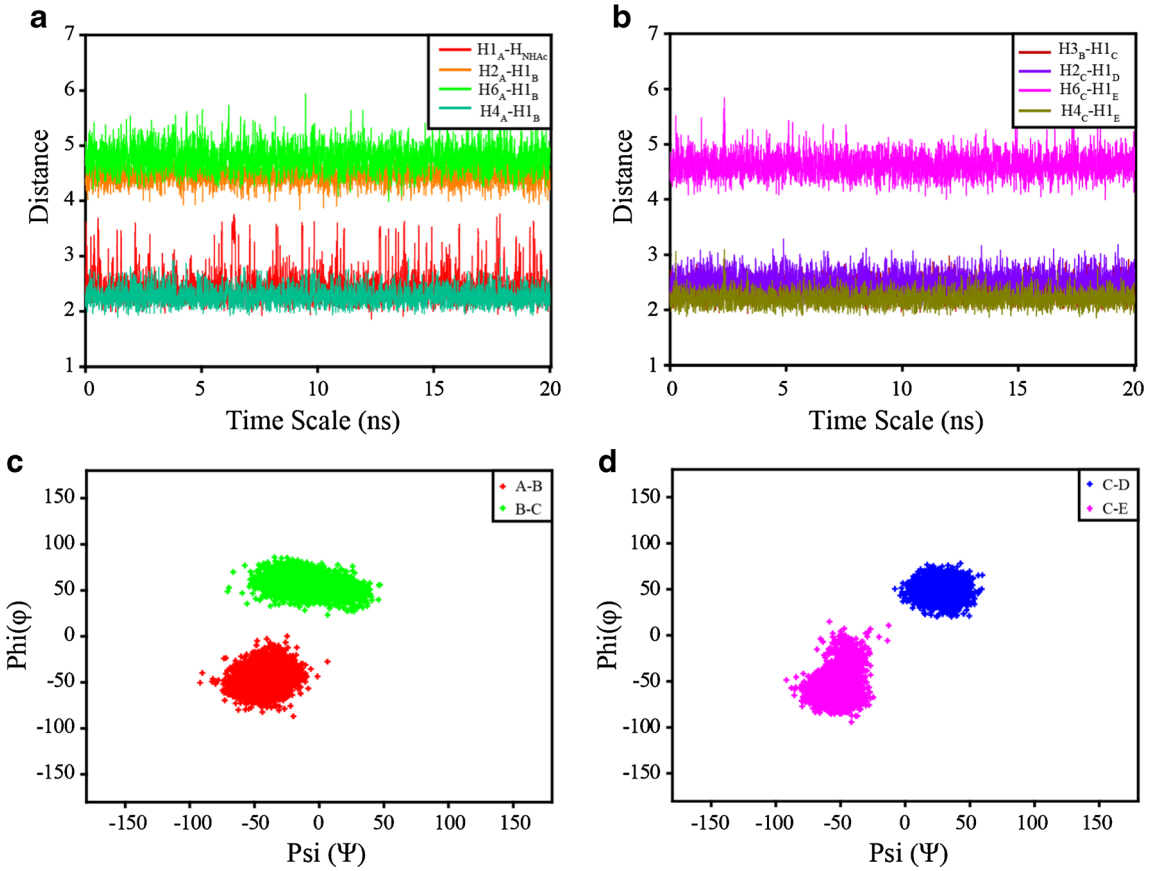


Fig. 2 Account of distance information for interglycosidic linkages **a** and **b**, and sugar ring dihedral angles (Phi and Psi) of compound **1 c** and **d**

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. In addition, 2D ROESY (300 ms mixing time) was performed to assist in the conformational analysis. The ROESY experiments were performed with 456 increments in t1 and 2 K data points in t2. The spectral width was normally 10 ppm in both dimensions. After 16 dummy scans, 80 scans were recorded per t1 increment. After zero-filling in t1, 4 K (t2) × 1 K (t1) data matrices were obtained. The two-dimensional NMR data were processed by Top Spin software suite (Bruker, Switzerland). MALDI-MS were recorded on a

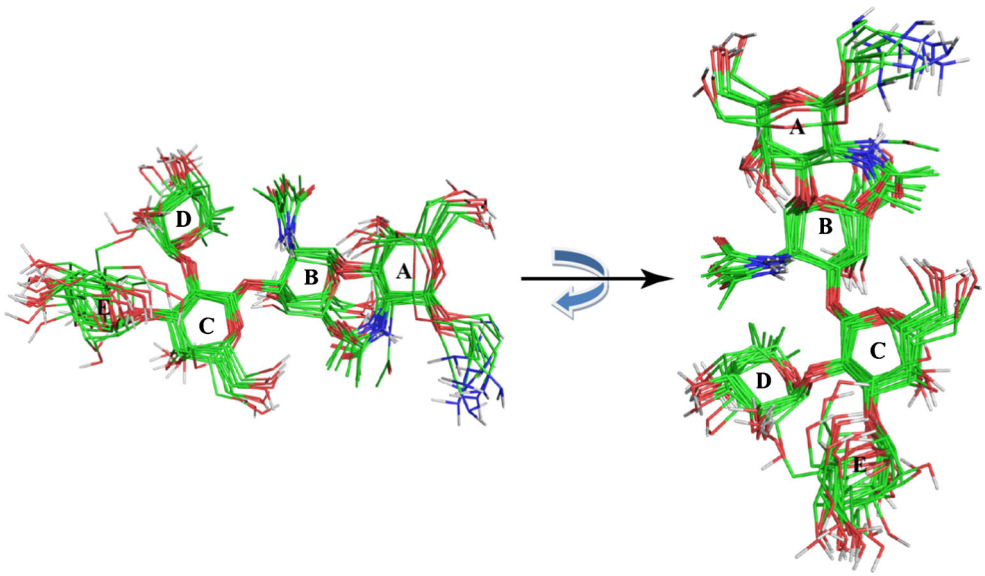


Fig. 3 Representation of conformational snapshots of compound **1** obtained using MD simulation

Bruker Daltronics 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 using the report of Chakraborti *et al*. [20].

Preparation of $HClO_4$-SiO_2 [20] $HClO_4$ (1.8 g, 12.5 mmol, as a 70 % aq solution) was added to a suspension of SiO_2 (230–400 mesh, 23.7 g) in Et_2O (70.0 mL). The mixture was concentrated and the residue was heated at 100 °C for 72 h under vacuum to furnish $HClO_4$-SiO_2 (0.5 mmol/g) as a free flowing powder. In all glycosylation reactions $HClO_4$-SiO_2 has been used in catalytic quantity (*e.g*. 3–5 mg $HClO_4$-SiO_2 per 100 mg of NIS).

Ethyl 2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (5) A solution of compound **2** (3.0 g, 8.51 mmol) in acetic anhydride (5 mL) and pyridine (5 mL) was allowed to stir at room temperature for 2 h. The solvents were removed under reduced pressure and the crude product was passed through a short pad of SiO_2 using hexane-EtOAc (2:1) as eluant to give pure compound **5** (3.3 g, 98 %). White solid; m.p. 124–125 °C [EtOH]; $[\alpha]_D^{25}$ + 0.5 (*c* 1.0, $CHCl_3$); IR (KBr): 3026, 2902, 1620, 1515, 1466, 1230, 757, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.61–7.44 (m, 5H, Ar-H), 6.08–5.92 (m, 1 H, C*H* = CH_2), 5.62 (s, 1 H, PhC*H*), 5.51 (t, *J* = 10.0 Hz, 1 H, H-2), 5.40–5.27 (m, 2 H, CH = CH_2), 4.48 (d, *J* = 9.5 Hz, 1 H, H-1), 4.59 (dd, *J* = 12.5, 1.5 Hz, 1 H, H-6_a), 4.38 (d, *J* = 3.0 Hz, 1 H, H-4), 4.30–4.18 (m, 2 H, OCH_2CH=), 4.13 (dd, *J* = 12.5, 2.0 Hz, 1 H, H-6_b), 3.67 (dd, *J* = 9.5, 3.0 Hz, 1 H, H-3), 3.56–3.54 (m, 1 H, H-5), 3.05–2.95 (m, 1 H, SC$H_2$$CH_3$), 2.86–2.79 (m, 1 H, SC$H_2$$CH_3$), 2.21 (s, 3 H, COC$H_3$), 1.40 (t, *J* = 7.5 Hz, 3 H, SCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.2 (*C*OCH_3), 137.7–117.2 (Ar-C, *C*H = CH), 101.3 (PhCH), 82.8 (C-1), 78.4 (C-4), 73.8 (C-3), 70.5 (OCH_2CH=), 70.1 (C-5), 69.3 (C-6), 68.0 (C-2), 22.4 (SC$H_2$$CH_3$), 21.0 (CO*C*$H_3$), 14.8 ($SCH_2CH_3$); ESI-MS: 417.1 $[M + Na]^+$; Anal. Calcd. for $C_{20}H_{26}O_6S$ (394.48): C, 60.89; H, 6.64 %; found: C, 60.73; H, 6.85 %.

2-(*N*-Benzyloxycarbonyl)aminoethyl *O*-(3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (8) To a solution of compound **3** (1.5 g, 3.19 mmol) and compound **4** (1.5 g, 3.39 mmol) in anhydrous CH_2Cl_2 (15 mL) was added MS 4 Å (3 g) and the reaction mixture was cooled to −10 °C. To the cooled reaction mixture were added *N*-iodosuccinimide (NIS; 800 mg, 3.55 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 through a Celite bed and washed with CH_2Cl_2 (100 mL). The combined organic layer was successively washed with 5 % $Na_2S_2O_3$ (50 mL), satd. $NaHCO_3$ (50 mL) and H_2O (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **8** (1.8 g, 72 %). White solid; m.p. 130 °C [EtOH]; $[\alpha]_D^{25}$ + 12.5 (*c* 1.0, $CHCl_3$); IR (KBr): 3014, 2932, 1741, 1506, 1457, 1216, 1056, 756, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.37–7.30 (m, 15 H, Ar-H), 5.67 (s, 1 H, PhC*H*), 5.66 (s, 1 H, PhC*H*), 5.50 (dd, *J* = 10.5, 3.0 Hz, 1 H, H-3_B), 5.48–5.45 (m, 1 H, N*H*), 5.40 (d, *J* = 3.0 Hz, 1 H, H-1_B), 5.22 (br s, 2 H, Cbz), 4.67 (d, *J* = 2.5 Hz, 1 H, H-4_B), 4.46 (d, *J* = 8.0 Hz, 1 H, H-1_A), 4.44–4.36 (m, 3 H, H-4_A, H-6_{aA}, H-6_{bA}), 4.25–4.16 (m, 3 H, H-5_A, H-6_{bA}, H-6_{bB}), 4.15–4.10 (m, 1 H, OC*H*), 4.07 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-2_B), 4.01 (t, *J* = 10.5 Hz, 1 H, H-2_A), 3.87–3.82 (m, 1 H, OC*H*), 3.76 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-3_A), 3.65–3.52 (m, 2 H, NCH_2), 3.50–3.48 (m, 1 H, H-5_B), 2.14 (s, 3 H, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3 (*C*OCH_3), 137.5–126.0 (Ar-C), 100.6 (C-1_A), 100.9 (PhCH), 100.8 (PhCH), 94.8 (C-1_B), 74.0 (C-3_A), 73.4 (C-4_B), 70.6 (C-4_A), 69.5 (OCH_2), 69.1 (2 C, C-6_A, C-6_B), 69.0 (C-3_B), 66.7 (Cbz), 66.5 (C-5_B), 63.1 (C-5_A), 61.3 (C-2_A), 56.5 (C-2_B), 40.9 (NCH_2), 20.9 (CO*C*H_3); MALDI-MS: 810.2 $[M + Na]^+$; Anal. Calcd. for $C_{38}H_{41}N_7O_{12}$ (787.77): C, 57.94; H, 5.25 %; found: 57.78; H, 5.43 %.

2-(*N*-Benzyloxycarbonyl)aminoethyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (9) A solution of compound **8** (1.7 g, 2.16 mmol) in 0.1 M CH_3ONa in CH_3OH (40 mL) was stirred at room temperature for 2 h and neutralized with Dowex 50 W X8 (H^+) resin. The reaction mixture was filtered through a Celite bed and evaporated to dryness. The crude product was passed through a short pad of SiO_2 using hexane-EtOAc (3:1) as eluant to give pure compound **9** (1.5 g, 93 %). White solid; m.p. 124–125 °C [EtOH]; $[\alpha]_D^{25}$ + 0.5 (*c* 1.0, $CHCl_3$); IR (KBr): 3018, 2918, 1509, 1457, 1218, 1049, 756, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.54–7.33 (m, 15 H, Ar-H), 5.56 (s, 2 H, 2 PhC*H*), 5.33 (m, 1 H, N*H*), 5.20 (s, 1 H, H-1_B), 5.10 (br s, 2 H, Cbz), 4.32 (d, *J* = 8.0 Hz, 1 H, H-1_A), 4.30–4.24 (m, 5 H, H-3_B, H-4_A, H-4_B, H-6_{aA}, H-6_{aB}), 4.10–3.99 (m, 3 H, H-5_A, H-6_{bA}, H-6_{bB}), 3.98–3.91 (m, 1 H, OC*H*), 3.85 (t, *J* = 10.5 Hz, 1 H, H-2_A), 3.75–3.69 (m, 1 H, OC*H*), 3.60 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-3_A), 3.59 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-2_B), 3.50–3.35 (m, 2 H, NCH_2), 3.35–3.30 (m, 1 H, H-5_B); ^{13}C NMR (125 MHz, $CDCl_3$): δ 126.3 (*C*OCbz), 129.5–124.0 (Ar-C), 102.5 (C-1_A), 101.3 (PhCH), 100.9 (PhCH), 95.1 (C-1_B), 75.5 (C-4_B), 74.2 (C-3_A), 70.6 (C-4_A), 69.5 (OCH_2), 69.1 (C-6_B), 68.9 (C-6_A), 66.7 (C-3_B), 66.6 (Cbz), 66.5 (C-5_B), 63.4 (C-5_A), 61.5 (C-2_A), 59.9 (C-2_B), 40.1 (NCH_2); MALDI-MS: 768.2 $[M + Na]^+$; Anal. Calcd. for $C_{36}H_{39}N_7O_{11}$: C, 57.98; H, 5.27 %; found: C, 57.81; H, 5.42 %.

2-(*N*-Benzyloxycarbonyl)aminoethyl *O*-(2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1 → 3)-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (10) To a solution of compound **9** (1.4 g, 1.87 mmol) and compound **5** (815 mg, 2.06 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS 4 Å (2 g) and the reaction mixture was cooled to −25 °C. To the cooled reaction mixture were added NIS (470 mg, 2.08 mmol) and $HClO_4$-SiO_2 (15 mg) and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (100 mL). The combined organic layer was successively washed with 5 % $Na_2S_2O_3$ (50 mL), satd. $NaHCO_3$ (50 mL) and H_2O (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **10** (1.4 g, 70 %). White solid; m.p. 154–155 °C [EtOH]; $[\alpha]_D^{25}$ + 11.5 (*c* 1.0, $CHCl_3$); IR (KBr): 3021, 2933, 1740, 1506, 1457, 1219, 1055, 756, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.65–7.39 (m, 20 H, Ar-H), 5.91–6.01 (m, 1 H, C*H* = CH_2), 5.69 (s, 1 H, PhC*H*), 5.65 (s, 1 H, PhC*H*), 5.63 (s, 1 H, PhC*H*), 5.50–5.40 (m, 2 H, N*H*, H-2_C), 5.41–5.36 (m, 1 H, CH = C*H*), 5.33 (d, *J* = 3.0 Hz, 1 H, H-1_B), 5.28 (dd, *J* = 10.0, 3.0 Hz, 1 H, CH = C*H*), 5.22 (br s, 2 H, Cbz), 4.87 (d, *J* = 7.5 Hz, 1 H, H-1_C), 4.65 (d, *J* = 1.5 Hz, 1 H, H-4_A), 4.45–4.34 (m, 6 H, H-3_A, H-4_B, H-4_C, H-6_{aA}, H-6_{abC}), 4.33 (d, *J* = 8.0 Hz, 1 H, H-1_A), 4.30–4.21 (m, 1 H, H-6_{aB}), 4.21–4.10 (m, 4 H, H-6_{bA}, H-6_{bB}, OCH_2CH = CH_2), 4.09–4.01 (m, 1 H, OC*H*), 4.01–3.92 (m, 3 H, H-2_A, H-2_B, H-5_A), 3.88–3.79 (m, 1 H, OC*H*), 3.75 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-3_B), 3.65 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-3_C), 3.61–3.55 (m, 2 H, NCH_2), 3.55–3.45 (m, 1 H, H-5_C), 3.41–3.37 (m, 1 H, H-5_B), 2.16 (s, 3 H, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.1 (*C*OCH_3), 156.3 (*C*OCbz), 134.7 (*C*H = CH_2), 129.1–126.1 (Ar-C), 117.1 (CH = *C*H_2), 102.1 (C-1_A), 101.9 (C-1_C), 101.1 (Ph*C*H), 100.7 (Ph*C*H), 100.4 (Ph*C*H), 94.5 (C-1_B), 77.4 (C-3_C), 75.9 (C-4_A), 73.6 (C-3_B, C-4_B), 73.4 (C-4_C), 74.4 (O*C*H_2CH = CH_2), 70.2 (C-3_A), 70.1 (C-2_C), 69.4 (C-6_C), 69.1 (C-6_A), 69.0 (C-6_B), 68.9 (O*C*H_2-), 66.5 (C-5_C, C-5_B), 63.9 (C-5_A), 61.6 (C-2_B), 58.4 (C-2_A), 40.9 (N*C*H_2), 20.9 (CO*C*H_3); MALDI-MS: 1100.4 $[M + Na]^+$; Anal. Calcd. for $C_{54}H_{59}N_7O_{17}$ (1078.08): C, 60.16; H, 5.52 %; found: C, 60.00; H, 5.70 %.

2-(*N*-Benzyloxycarbonyl)aminoethyl *O*-(3-*O*-allyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1 → 3)-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (11) A solution of compound **10** (1.2 g, 1.11 mmol) in 0.1 M CH_3ONa in CH_3OH (30 mL) was stirred at room temperature for 2 h and neutralized with Dowex 50 W X8 (H^+) resin. The reaction mixture was filtered through a Celite bed and evaporated to dryness. The crude product was passed through a SiO_2 using hexane-EtOAc (3:1) as eluant to give pure compound **11** (1.0 g, 87 %). White solid; m.p. 134–135 °C [EtOH]; $[\alpha]_D^{25}$ + 11.5 (*c* 1.0, $CHCl_3$); IR (KBr): 3016, 2917, 1508, 1455, 1216, 1048, 756, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.59–7.21 (m, 20 H, Ar-H), 5.97–5.91 (m, 1 H, C*H* = CH_2), 5.57 (s, 1 H, PhC*H*), 5.55 (s, 1 H, PhC*H*), 5.51 (s, 1 H, PhC*H*), 5.40–5.30 (m, 2 H, N*H*, CH = CH_2), 5.27 (d, *J* = 3.0 Hz, 1 H, H-1_B), 5.18 (dd, *J* = 10.5 Hz, 1 H, CH = C*H*), 5.08 (br s, 2 H, Cbz), 4.57 (d, *J* = 7.5 Hz, 1 H, H-1_C), 4.52 (d, *J* = 2.5 Hz, 1 H, H-4_A), 4.35–4.29 (m, 1 H, H-4_B), 4.28 (t, *J* = 9.5 Hz, 1 H, H-1_A), 4.26–4.15 (m, 7 H, H-4_C, H-6_{aA}, H-6_{aB}, H-6_{abC}, OCH_2CH = CH_2), 4.08–3.91 (m, 6 H, H-2_B, H-2_C, H-3_A, H-6_{bA}, H-6_{bB}, O*CH*), 3.91–3.95 (m, 1 H, H-5_A), 3.85 (t, *J* = 10.0 Hz, 1 H, H-2_A), 3.75–3.66 (m, 1 H, OC*H*), 3.66 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-3_B), 3.46–3.44 (m, 3 H, H-3_C, NCH_2), 3.39–3.35 (m, 1 H, H-5_C), 3.31–3.25 (m, 1 H, H-5_B); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.3 (*C*OCbz), 135.0 (*C*H = CH_2) 128.9–126.1 (Ar-C), 117.4 (CH = *C*H_2), 104.2 (C-1_C), 102.1 (C-1_A), 101.0 (Ph*C*H), 100.8 (2 Ph*C*H), 94.4 (C-1_B), 78.4 (C-3_C), 76.1 (C-4_A), 73.7 (C-3_B), 73.6 (C-4_B), 73.5 (C-4_C), 71.1 (*C*H_2CH = CH_2), 70.2 (2 C, C-2_C, C-3_A), 69.4 (C-6_C), 69.2 (C-6_A), 68.9 (2 C, C-6_B, O*C*H_2), 66.7 (C-5_C), 66.6 (C-Cbz), 66.5 (C-5_B), 63.9 (C-5_A), 61.5 (C-2_B), 58.4 (C-2_A), 40.9 (N*C*H_2); MALDI-MS: 1058.3 $[M + Na]^+$; Anal. Calcd. for $C_{52}H_{57}N_7O_{16}$ (1036.05): C, 60.28; H, 5.55 %; found: C, 60.11; H, 5.70 %.

2-(*N*-Benzyloxycarbonyl)aminoethyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)-*O*-(4,6-di-*O*-acetyl-3-*O*-allyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(4,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-4,6-di-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside (12) To a solution of compound **11** (900 mg, 0.87 mmol) and compound **6** (450 mg, 0.94 mmol) in anhydrous Et_2O-CH_2Cl_2 (10 mL; 2:1) was added MS 4 Å (2 g) and the reaction mixture was cooled to −5 °C. To the cooled reaction mixture were added NIS (220 mg, 0.97 mmol) and $HClO_4$-SiO_2 (10 mg) and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (50 mL). The combined organic layer was successively washed with 5 % $Na_2S_2O_3$ (50 mL), satd. $NaHCO_3$ (50 mL) and H_2O (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. To a solution of the crude product in acetic anhydride (10 mL) was added $HClO_4$-SiO_2 (250 mg) and the reaction mixture was stirred at room temperature for 20 min. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **12** (880 mg, 70 %). Colorless oil; $[\alpha]_D^{25}$ + 3.3 (*c* 1.0, $CHCl_3$); IR (KBr): 3456, 2922, 2875, 2111, 1735, 1719, 1510, 1456, 1392, 1275, 1232, 1090, 1070,

1028, 709 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.34–7.22 (m, 20 H, Ar-H), 5.79–5.69 (m, 1 H, C*H* = CH_2), 5.68 (d, *J* = 2.5 Hz, 1 H, H-4_C), 5.45 (d, *J* = 3.5 Hz, 1 H, H-1_D), 5.41 (d, *J* = 2.0 Hz, 1 H, H-4_A), 5.33 (d, *J* = 3.5 Hz, 1 H, H-4_B), 5.30–5.15 (m, 1 H, N*H*), 5.21 (d, *J* = 3.0 Hz, 1 H, H-1_B), 5.18–5.11 (m, 1 H, CH = C*H*), 5.10 (br s, 2 H, Cbz), 5.08 (dd, *J* = 10.5, 3.0 Hz, 1 H, CH = C*H*), 4.90 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.79–4.65 (4 d, *J* = 11.5 Hz, 4 H, 2 PhC*H*), 4.62 (d, *J* = 7.5 Hz, 1 H, H-1_C), 4.57 (d, *J* = 11.5 Hz, 1 H, PhC*H*) 4.39–4.30 (m, 2 H, H-3_D, H-5_D), 4.23 (d, *J* = 8.5 Hz, 1 H, H-1_A), 4.19–4.18 (m, 1 H, H-6_{aA}), 4.20–4.05 (m, 7 H, H-6_{bA}, H-6_{abC}, H-6_{abB}, CH_2CH = CH_2), 4.05–3.99 (2 dd, *J* = 10.0, 3.0 Hz, 2 H, H-2_D, H-3_B), 3.99–3.90 (m, 1 H, OC*H*), 3.85–3.70 (m, 5 H, H-2_C, H-3_A, H-3_C, H-5_A, H-5_C), 3.70–3.47 (m, 4 H, H-2_A, H-4_D, H-5_B, OC*H*), 3.46–3.42 (m, 2 H, NCH_2), 3.41 (dd, *J* = 10.5, 3.0 Hz, 1 H, H-2_B), 2.14, 2.10, 2.08, 2.03 (4 s, 18 H, 6 COCH_3), 1.19 (d, *J* = 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.0 (6 *C*OCH$_3$), 156.3 (*C*OCbz), 134.1 (*C*H = CH_2), 128.5–127.3 (Ar-C), 116.1 (CH = *C*H_2), 102.5 (C-1_C), 102.4 (C-1_A), 97.6 (C-1_D), 94.2 (C-1_B), 79.9 (C-3_C), 79.5 (C-3_B), 77.6 (C-4_D), 75.6 (C-3_D), 74.7 (Ph*C*H_2), 74.3 (Ph*C*H_2), 72.9 (C-5_D), 72.8 (C-2_D), 72.6 (2C, O*C*H_2, Ph*C*H_2), 70.8 (C-3_A), 70.7 (C-2_C), 70.5 (*C*H_2CH = CH_2), 69.9 (C-4_C), 69.7 (C-5_C), 68.1 (C-5_B), 66.8 (Cbz), 66.1 (C-4_B), 65.4 (C-4_A), 63.4 (C-5_A), 62.5 (C-6_C), 62.1 (C-6_B), 61.9 (C-6_A), 61.3 (C-2_A), 58.9 (C-2_B), 40.9 (N*C*H_2), 20.8, 20.7, 20.5 (6 CO*C*H_3), 16.7 (C*C*H_3); MALDI-MS: 1462.5 $[M + Na]^+$; Anal. Calcd. for $C_{70}H_{85}N_7O_{26}$ (1440.46): C, 58.37; H, 5.95 %; found: C, 58.20; H, 6.15 %.

2-(*N*-Benzyloxycarbonyl)aminoethyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)-*O*-(4,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(4,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-4,6-di-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside (13) To a solution of compound **12** (800 mg, 0.56 mmol) in dry CH_3OH (10 mL) was added $PdCl_2$ (50 mg, 0.28 mmol) and the reaction mixture was allowed to stir at 0–5 °C for 1.5 h. The solvents were removed under reduced pressure and the crude product was purified over SiO_2 using hexane-EtOAc (3:1) as eluant to give pure compound **13** (600 mg, 76 %). Colorless oil; $[\alpha]_D^{25}$ + 3.4 (*c* 1.0, $CHCl_3$); IR (KBr): 3434, 2112, 1741, 1275, 1247, 1132, 1069, 1029, 755, 709 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.44–7.34 (m, 20 H, Ar-H), 5.56 (d, *J* = 2.5 Hz, 1 H, H-4_C), 5.49 (s, 1 H, H-4_A), 5.41 (d, *J* = 3.0 Hz, 1 H, H-4_B), 5.39–5.31 (m, 1 H, N*H*), 5.30 (d, *J* = 3.5 Hz, 1 H, H-1_B), 5.20 (br s, 2 H, Cbz), 5.02 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.99 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.97 (d, *J* = 3.0 Hz, 1 H, H-1_D), 4.88–4.78 (m, 3 H, 3 PhC*H*), 4.71 (d, *J* = 12.0 Hz, 1 H, PhC*H*), 4.62 (d, *J* = 7.5 Hz, 1 H, H-1_C), 4.42–4.38 (m, 1 H, H-5_D), 4.38–4.32 (m, 1 H, H-1_A), 4.31–4.20 (m, 5 H, H-3_D, H-4_D, H-6_{aA}, H-6_{abC}), 4.19–4.09 (m, 6 H, H-2_D, H-3_C, H-5_A, H-6_{abB}, H-6_{bA}), 4.08–3.98 (m, 1 H, OC*H*), 3.92–3.80 (m, 4 H, H-3_A, H-3_B, H-5_C, OC*H*), 3.78–3.70 (m, 2 H, H-2_A, H-5_B), 3.68 (dd, *J* = 11.0, 3.5 Hz, H-2_B), 3.60–3.48 (m, 3 H, H-2_C, NCH_2), 2.29, 2.24, 2.22, 2.18, 2.17 (5 s, 18 H, 6 COCH_3), 1.23 (d, *J* = 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1–169.3 (6 *C*OCH$_3$), 156.1 (*C*OCbz), 128.4–127.4 (Ar-C), 102.4 (C-1_A), 101.2 (C-1_C), 101.1 (C-1_B), 93.7 (C-1_D), 81.1 (C-2_C), 79.8 (C-3_C), 77.5 (C-2_A), 76.5 (C-4_D), 74.7 (Ph*C*H_2), 74.4 (Ph*C*H_2) 72.6 (Ph*C*H_2), 72.5 (C-2_D), 72.1 (C-3_B), 71.4 (C-3_D), 71.1 (C-3_A), 70.9 (C-5_C), 69.8 (O*C*H_2), 69.5 (C-4_C), 68.5 (C-4_B), 68.1 (C-5_D), 67.1 (C-5_A), 66.6 (Cbz), 63.2 (C-4_A), 62.3 (C-6_B), 62.1 (C-5_B), 61.3 (C-6_C), 61.2 (C-6_A), 59.5 (C-2_B), 40.9 (N*C*H_2), 20.7, 20.5, 20.4, 20.2 (6 CO*C*H_3), 16.9 (C*C*H_3); MALDI-MS: 1422.5 $[M + Na]^+$; Anal. Calcd. for $C_{67}H_{81}N_7O_{26}$ (1400.39): C, 57.46; H, 5.83 %; found: C, 57.30; H, 6.05 %.

2-(*N*-Benzyloxycarbonyl)aminoethyl *O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-(1 → 3)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)-*O*]-(4,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(4,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-4,6-di-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside (14) To a solution of compound **13** (500 mg, 0.36 mmol) and compound **7** (190 mg, 0.38 mmol) in anhydrous Et_2O-CH_2Cl_2 (6 mL; 3:1) was added MS 4 Å (1 g) and the reaction mixture was cooled to −8 °C. To the cooled reaction mixture were added NIS (90 mg, 0.40 mmol) and $HClO_4$-SiO_2 (5 mg) and the reaction mixture was allowed to stir at same temperature for 1 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (25 mL). The combined organic layer was successively washed with 5 % $Na_2S_2O_3$ (25 mL), satd. $NaHCO_3$ (25 mL) and H_2O (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (3:1) as eluant to give pure compound **14** (470 mg, 71 %). Colorless oil; $[\alpha]_D^{25}$ + 4.9 (*c* 1.0, $CHCl_3$); IR (KBr): 3437, 2110, 1741, 1274, 1250, 1130, 1073, 1027, 756, 709 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.44–7.23 (m, 35 H, Ar-H), 5.61 (d, *J* = 2.5 Hz, 1 H, H-4_B), 5.42–5.19 (m, 2 H, H-4_A, H-4_C), 5.36 (d, *J* = 3.5 Hz, 1 H, H-1_B), 5.34 (d, *J* = 3.0 Hz, 1 H, H-1_D), 5.29 (d, *J* = 3.5 Hz, 1 H, H-1_E), 5.24–5.20 (m, 1 H, N*H*), 5.08 (br s, 2 H, Cbz), 5.00 (s, 1 H, PhC*H*), 4.98–4.60 (9 d, *J* = 12.0 Hz, 9 H, 9 PhC*H*), 4.58 (d, *J* = 7.5 Hz, 1 H, H-1_C), 4.58–4.56 (m, 1 H, H-4_E), 4.50–4.41 (m, 1 H, H-5_D), 4.39 (d, *J* = 11.5 Hz, 1 H, PhC*H*), 4.35–4.24 (m, 1 H, H-4_D), 4.25 (d, *J* = 7.5 Hz, 1 H, H-1_A), 4.21–3.99 (m, 10 H, H-3_C, H-3_D, H-3_E, H-6_{abA}, H-6_{abB}, H-6_{abE}, OC*H*), 3.99–3.70 (m, 10 H, H-2_A, H-2_B, H-2_D, H-3_A, H-3_B, H-5_A, H-5_B, H-6_{abC}, OC*H*), 3.70–3.63 (m, 1 H, H-5_C), 3.58–3.40 (m, 2 H, H-2_C, H-5_E), 3.39 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-2_E), 2.64, 2.14, 2.07, 2.06, 2.05 (5 s, 18 H, 6 COCH_3), 1.22 (d, *J* = 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (5 C, 5 *C*OCH$_3$), 168.7

($COCH_3$), 156.3 ($COCbz$), 128.7–126.3 (Ar-C), 102.7 (C-1_A), 102.3 (C-1_C), 100.5 (PhCH), 97.8 (C-1_B), 94.4 (2 C, C-1_D, C-1_E), 80.7 (C-3_C), 77.9 (C-2_A), 77.1 (C-4_D), 76.1 (C-3_E), 75.5 (C-2_D), 75.4 (C-3_B), 74.9 (PhCH_2), 74.5 (PhCH_2), 73.5 (PhCH_2), 72.9 (C-3_A), 72.6 (C-3_D), 72.5 (PhCH_2), 72.4 (PhCH_2), 71.8 (C-5_C), 70.9 (C-5_A), 70.8 (C-2_C), 70.5 (C-4_E), 69.9 (OCH_2), 68.7 (C-6_E), 68.2 (C-4_B), 66.8 (Cbz), 66.2 (C-5_D), 65.7 (C-4_C), 63.5 (C-4_A), 63.1 (C-5_B), 62.4 (C-6_B), 62.2 (C-5_E), 61.6 (C-6_C), 61.3 (C-6_A), 58.4 (C-2_E), 40.9 (NCH_2), 20.7 (6 $COCH_3$), 16.7 (CCH_3); MALDI-MS: 1852.7 $[M + Na]^+$; Anal. Calcd. for $C_{94}H_{107}N_7O_{31}$ (1830.88): C, 61.66; H, 5.89 %; found: C, 61.50; H, 6.10 %.

2-aminoethyl *O*-(α-D-galactopyranosyl)-(1 → 3)-*O*-[(α-L-fucopyranosyl)-(1 → 2)-*O*]-(β-D-galactopyranosyl)-(1 → 3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-2-acetamiso-2-deoxy-β-D-galactopyranoside (1)

To a solution of compound **14** (400 mg, 0.22 mmol) in pyridine (0.5 mL) was added CH_3COSH (0.2 mL, 2.78 mmol) and the reaction mixture was stirred at room temperature for 22 h. The reaction mixture was evaporated under reduced pressure and co-evaporated with toluene (3x50 mL). A solution of the crude product in 0.1 M CH_3ONa in CH_3OH (10 mL) was stirred at room temperature for 2 h, neutralized with Dowex 50 W-X8 (H^+) resin, filtered and concentrated to dryness. The crude product was passed through a short pad of SiO_2 using EtOAc as eluant to give de-*O*-acetylated product. To a solution of the product in CH_3OH (5 mL) were added 20 % $Pd(OH)_2$-C (100 mg) and Et_3SiH (2 mL, 12.52 mmol) and the reaction mixture was allowed to stir at room temperature for 24 h. The reaction mixture was filtered through a Celite bed and the filtering bed was washed with CH_3OH-H_2O (20 mL; 4:1). The combined filtrate was concentrated under reduced pressure to give compound **1**, which was passed through a Sephadex LH-20 column using CH_3OH-H_2O (4:1) as eluant to give pure compound **1** (200 mg, 50 %). White powder; $[\alpha]_D^{25}$ + 17 (*c* 0.5, H_2O); IR (KBr): 3436, 2944, 1609, 1378, 1144, 1092, 667 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 5.26 (d, *J* = 3.5 Hz, 1 H, H-1_D), 5.25 (d, *J* = 3.5 Hz, 1 H, H-1_E), 5.06 (d, *J* = 3.5 Hz, 1 H, H-1_B), 4.72 (d, *J* = 8.0 Hz, 1 H, H-1_C), 4.60 (d, *J* = 8.0 Hz, 1 H, H-1_A), 4.35–4.30 (m, 2 H, H-4_C, H-5_D), 4.29–4.24 (m, 2 H, H-5_C, H-5_E), 4.20 (dd, *J* = 10.5, 3.5 Hz, 1 H, H-2_B), 4.15–4.05 (m, 3 H, H-2_A, H-4_B, OC*H*), 4.00–3.95 (m, 4 H, H-3_B, H-3_C, H-4_A, OC*H*), 4.94–3.88 (m, 4 H, H-2_C, H-2_D, H-5_A, H-5_B), 3.85–3.73 (m, 10 H, H-2_E, H-3_A, H-4_E, H-6_{abA}, H-6_{abB}, H-6_{abC}, H-6_{aE}), 3.70–3.65 (m, 3 H, H-3_E, H-4_D, H-6_{bE}), 3.57 (dd, *J* = 10.0, 3.5 Hz, 1 H, H-3_D), 3.32–3.20 (m, 2 H, NCH_2), 2.09, 2.06 (2 s, 6 H, 2 $COCH_3$), 1.20 (d, *J* = 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, D_2O): δ 174.8, 173.8 (2 $COCH_3$), 102.2 (C-1_C), 100.9 (C-1_A), 98.8 (C-1_D), 92.9 (2 C, C-1_B, C-1_E), 76.0 (C-3_B), 75.1 (C-4_D), 74.6 (C-3_C), 74.3 (C-3_A), 74.2 (C-5_A), 72.9 (C-5_C), 71.8 (C-5_E), 71.0 (C-2_C), 70.9 (C-4_E), 69.9 (C-5_B), 69.4 (C-3_E), 69.2 (C-2_E), 68.8 (C-3_D), 68.0 (C-2_D), 67.7 (C-4_A), 66.8 (C-5_D), 65.7 (OCH_2), 63.5 (C-4_C), 63.4 (C-4_B), 61.3 (C-6_E), 61.1 (C-6_C), 60.8 (C-6_B), 60.7 (C-6_A), 50.6 (C-2_A), 48.8 (C-2_B), 39.5 (NCH_2), 22.3 ($COCH_3$), 22.0 ($COCH_3$), 15.3 (CCH_3); MALDI-MS: 960.3 $[M + Na]^+$; Anal. Calcd. for $C_{36}H_{63}N_3O_{25}$ (937.89): C, 46.10; H, 6.77 %; found: C, 61.50; H, 6.10 %.

Computational details

ROE based 2D ROESY NMR spectral analysis has been used to get detailed atomistic information of compound **1**. Further, Mestro GUI of Desmond has been used for the preparation of bond optimization, configuration for α/β forms, L/D isomeric forms, and furanose/pyranose types of sugar rings in compound **1**. Compound **1** was solvated using TIP3P water molecules [30] in a truncated octahedral box with edge distance of 10 Å. The account of non-bonded interaction was performed using a cutoff distance of 10 Å under isothermal-isobaric periodic boundary conditions. M-SHAKE algorithm was used for restraining all the hydrogen bonds with an integration time step of 2 fs [31]. Energy minimization, equilibration and production MD run for compound **1** were processed using OPLS_2005 force field in Desmond, which was already discussed in earlier reports [32, 33]. MD simulation was performed at 300 K for a time scale of 20 ns with recording an interval of 5 ps for trajectory frames.

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References

1. Andersson D.I., Hughes D.: Persistence of antibiotic resistance in bacterial populations. FEMS Microbiol. Rev. **35**, 901–911 (2011)
2. Bhatia S., Dimde M., Haag R.: Multivalent glycoconjugates as vaccines and potential drug candidates. Med. Chem. Commun. **5**, 862–878 (2014)
3. Källenius G., Pawlowski A., Hamasur B., Svenson S.B.: Mycobacterial glycoconjugates as vaccine candidates against tuberculosis. Trends Microbiol. **16**, 456–462 (2008)
4. Lucas A.H., Apicella M.A., Taylor C.E.: Carbohydrate moieties as vaccine candidates. Clin. Infect. Dis. **41**, 705–712 (2005)
5. Astronomo R.D., Burton D.R.: Carbohydrate vaccines: developing sweet solutions to sticky situations? Nat. Rev. Drug Discov. **9**, 308–324 (2010)
6. Lebeer S., Vanderleyden J., De Keersmaecker S.C.J.: Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. Nat. Rev. Microbiol. **8**, 171–184 (2010)

7. Roberts I.S., Roberts I.S.: The biochemistry and genetics of capsular polysaccharide production in bacteria. Annu. Rev. Microbiol. **50**, 285–315 (1996)
8. Raetz C.R.H., Whitfield C.: Lipopolysaccharide Endotoxins. Annu. Rev. Microbiol. **71**, 635–700 (2002)
9. Nataro J.P., Kaper J.B.: Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11**, 142–201 (1998)
10. Andersson M., Carlin N., Leontein K., Lindquist U., Slettengren K.: Structural studies of the O-antigenic polysaccharide of *Escherichia coli* O86, which possesses blood-group B activity. Carbohydr. Res. **185**, 211–223 (1989)
11. Yi W., Bystricky P., Yao Q., Guo H., Zhu L., Li H., Shen J., Li M., Ganguly S., Allen Bush C., Wang P.G.: Two different O-polysaccharides from *Escherichia coli* O86 are produced by different polymerization of the same O-repeating unit. Carbohydr. Res. **341**, 100–108 (2006)
12. Niqudkar S.S., Demchenko A.V.: Stereocontrolled 1,2-*cis* glycosylation as the driving force of progress in synthetic carbohydrate chemistry. Chemical Sc. **6**, 2687–2704 (2015)
13. Ingle A.B., Chao C.-S., Hung W.-C., Tony Mong K.-K.: Chemical synthesis of the O-antigen repeating unit of *Escherichia coli* O86 by an N-formylmorpholine-modulated one-pot glycosylation strategy. Asian J. Org. Chem. **3**, 870–876 (2014)
14. Si A., Misra A.K.: Expedient synthesis of the pentasaccharide repeating unit of the polysaccharide O-antigen of *Escherichia coli* O11. Chemistry Open. **5**, 47–50 (2016)
15. Santra A., Ghosh T., Misra A.K.: Expedient synthesis of two structurally close tetrasaccharides corresponding to the O-antigens of *Escherichia coli* O127 and *Salmonella enterica* O13. Tetrahedron-Asymmetry. **23**, 1385–1392 (2012)
16. Lönn H.: Synthesis of a tri- and a hepta-saccharide which contain α-L-fucopyranosyl groups and are part of the complex type of carbohydrate moiety of glycoproteins. Carbohydr. Res. **139**, 105–113 (1985)
17. Ghosh S., Misra A.K.: Concise synthesis of a hexasaccharide present in the cell wall lipopolysaccharide of *Azospirillum lipoferum* Sp59b. Tetrahedron-Asymmetry. **21**, 725–730 (2010)
18. Ghosh S., Misra A.K.: Concise synthesis of a hexasaccharide related to the adhesin receptor of *Streptococcus oralis* ATCC 55229. J. Carbohydr. Chem. **28**, 447–462 (2009)
19. Mukherjee, C., Misra, A. K. Glycosylation and pyranose-furanose isomerization of carbohydrates using $HClO_4$-SiO_2: synthesis of oligosaccharides containing galactofuranose. Synthesis 683–692 (2007).
20. Chakraborti A.K., Gulhane R.: Perchloric acid adsorbed on silica gel as a new, highly efficient, and versatile catalyst for acetylation of phenols, thiols, alcohols, and amines. Chem. Commun. **2003**, (1896-1897)
21. Zemplén G.: Abbau der reduzierenden Biosen, I.: Direkte konstitutions-ermittlung der cellobiose. Ber. Dtsch. Chem. Ges. **59**, 1254–1266 (1926)
22. Agnihotri G., Misra A.K.: Mild and efficient method for the cleavage of benzylidene acetals using $HClO_4$-SiO_2 and direct conversion of acetals to acetates. Tetrahedron Lett. **47**, 3653–3658 (2006)
23. Demchenko, A., Stauch, T., Boons, G.-J. Solvent and other effects on the stereoselectivity of thioglycoside glycosidations. Synlett 818–820 (1997).
24. Satoh H., Hansen H.S., Manabe S., van Gunsteren W.F., Hünenberger P.H.: Theoretical investigation of solvent effects on glycosylation reactions: stereoselectivity controlled by preferential conformations of the intermediate oxacarbenium-counterion complex. J. Chem. Theory Comput. **6**, 1783–1797 (2010)
25. Ogawa T., Yamamoto H.: Synthesis of a model linear mannohexaose for the backbone structure of fruit body polysaccharide of *Tremella fuciformis* and *Dictyophora indusiata* FISCH. Agric. Biol. Chem. **49**, 475–482 (1985)
26. Nakahara Y., Ogawa T.: Solid-phase synthesis of an *O*-linked glycopeptide based on a benzyl-protected glycan approach. Carbohydr. Res. **292**, 71–81 (1996)
27. Santra A., Ghosh T., Misra A.K.: Removal of benzylidene acetal and benzyl ether in carbohydrate derivatives using triethylsilane and Pd/C. Beilstein J. Org. Chem. **9**, 74–78 (2013)
28. Santra A., Si A., Kar R.K., Bhunia A., Misra A.K.: Linear synthesis and conformational analysis of the pentasaccharide repeating unit of the cell wall O-antigen of *Escherichia coli* O13. Carbohydr. Res. **391**, 9–15 (2014)
29. Kar R.K., Suryadevara P., Sahoo B.R., Sahoo G.C., Dikhit M.R., Das P.: Exploring novel KDR inhibitors based on pharmacoinformatics methodology. *SAR QSAR Environ. Res*. **24**, 215–234 (2013)
30. Jorgensen W.L., Chandrasekhar J., Madura J.D., Impey R.W., Klein M.L.: Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. **79**, 926–935 (1983)
31. Vincent K., van Gunsteren W.F., Hünenberger P.H.: A fast SHAKE algorithm to solve distance constraint equations for small molecules in molecular dynamics simulations. J. Comput. Chem. **22**, 501–508 (2001)
32. Dhara D., Kar R.K., Bhunia A., Misra A.K.: Convergent synthesis and conformational analysis of the hexasaccharide repeating unit of the *O*-antigen of *Shigella flexneri* serotype 1d. Eur. J. Org. Chem. **2014**(21), 4577–4584 (2014)
33. Kar R.K., Suryadevara P., Jana J., Bhunia A., Chatterjee S.: Novel G-quadruplex stabilizing agents: in-silico approach and dynamics. J. Biomol. Struct. Dyn. **31**, 1497–1518 (2013)