Carbohydrate Research 391 (2014) 9–15

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Linear synthesis and conformational analysis of the pentasaccharide repeating unit of the cell wall O-antigen of *Escherichia coli* O13

Abhishek Santra [a,†], Anshupriya Si [a,†], Rajiv Kumar Kar [b], Anirban Bhunia [b,*], Anup Kumar Misra [a,*]

[a] Bose Institute, Division of Molecular Medicine, P-1/12, C.I.T. Scheme VII-M, Kolkata 700 054, India
[b] Bose Institute, Department of Biophysics, P-1/12, C.I.T. Scheme VII-M, Kolkata 700 054, India

ARTICLE INFO

Article history:
Received 23 December 2013
Received in revised form 10 March 2014
Accepted 12 March 2014
Available online 20 March 2014

Keywords:
Pentasaccharide
O-Antigen
Escherichia coli
Conformational analysis
Molecular dynamics simulation

ABSTRACT

Synthesis of the pentasaccharide repeating unit of the O-antigen of *Escherichia coli* O13 strain has been achieved using a straightforward linear synthetic strategy. Similar reaction conditions have been used for all glycosylations as well as protective group manipulations. All intermediate steps are high yielding and the glycosylation steps are stereoselective. The synthesized pentasaccharide was subjected to conformational analysis using 2D ROESY NMR spectral analysis and molecular dynamics (MD) simulation to get detailed information on conformation of the molecule in aqueous solution.

1. Introduction

Among several bacterial infections in humans, meningitis, and sepsis,[1] diarrhoeal outbreaks[2] and urinary tract infections[3] are quite frequent. Pathogenic *Escherichia coli* (*E. coli*) strains are mostly responsible for these infections.[4] Although most of the *E. coli* strains are usually non-pathogenic members of the human colonic flora, certain strains acquire virulence factors and contribute to a variety of infections in humans and animals. *E. coli* strains are classified based on three types of antigens, which are (a) somatic (O) antigen; (b) capsular (K) antigen, and (c) flagellar (H) antigens.[5] Recently, Perepelov et al. reported the structure of the cell wall O-antigen of *E. coli* O13 comprised of D-glucose, D-glucosamine, and L-rhamnose (Fig. 1).[6] *E. coli* O13 strain is associated with diarrheal infections and shows serological cross-reactivity with *Shigella flexneri* strains.[7]

The recent thrust in the drug discovery program is to develop newer approaches to control bacterial infections due to the emergence of multi-drug resistant bacterial strains.[8] Bacterial cell wall O-antigens are unique polysaccharides and long known for their association with the bacterial virulence. As a consequence, several attempts were made in the past toward the development of novel antibacterial agents or vaccine candidates based on the cell wall glycoconjugate derivatives.[9] Bacterial cell wall O-antigens are composed of smaller oligosaccharide repeating units, which are also equally immunodominant as the whole polysaccharides. Therefore, it is quite pertinent to develop glycoconjugate derivatives corresponding to the repeating unit of the cell wall O-antigens for their evaluation in the vaccine development program.[9,10] However, isolation of oligosaccharides in adequate quantity avoiding the biological impurities is tedious and somewhat inconvenient. Development of chemical synthetic strategies for the preparation of oligosaccharides as well as their glycoconjugate derivatives is always beneficial to get significant quantity of pure compounds. In this context, synthesis of the pentasaccharide repeating unit of the O-antigen of *Escherichia coli* O13 as its *p*-methoxyphenyl (PMP) glycoside has been undertaken (Fig. 2). The PMP group has been chosen as a temporary anomeric protecting group which can be removed under an oxidative reaction condition using ceric ammonium nitrate (CAN) to give the pentasaccharide hemiacetal derivative. The hemiacetal derivative can be linked to a protein through a spacer linker to furnish glycoconjugate derivatives using methodologies reported elsewhere. Information on the conformational orientation of the oligosaccharide in aqueous environment is the prerequisite parameter for the designing of an effective glycoconjugate derivative for biological studies. For detailed understanding of the conformational properties of a molecule, 2D NOESY/ROESY NMR spectral analysis in conjunction with molecular dynamics (MD) simulation are extremely useful. A linear synthetic strategy for the synthesis of the pentasaccharide repeating unit of the O-antigen of *E. coli* O13 and its detailed conformational studies in aqueous solution using NMR spectral analysis and molecular dynamics simulation is presented herein.

* Corresponding authors. Fax: +91 33 2355 3886 (A.K.M.).
E-mail address: akmisra69@gmail.com (A.K. Misra).
† Contributed equally to this work.

http://dx.doi.org/10.1016/j.carres.2014.03.012
0008-6215/

→3)-[α-D-Glc*p*-(1→2)]-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→

Figure 1. Structure of the repeating unit of the cell wall O-antigen of *Escherichia coli* O13.

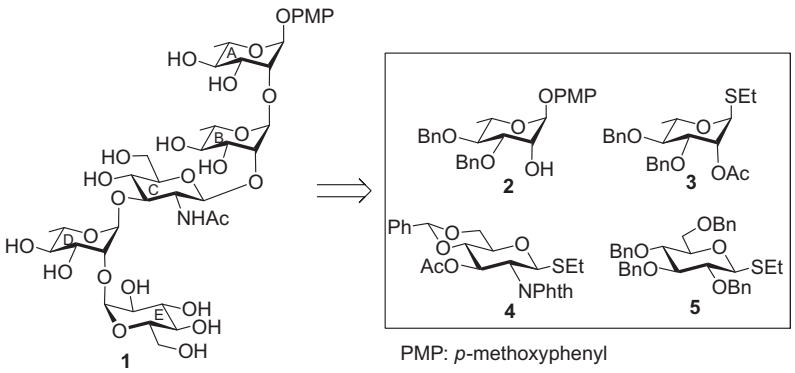


Figure 2. Structure of the synthesized pentasaccharide **1** as its *p*-methoxyphenyl glycoside and its synthetic intermediates.

2. Results and discussion

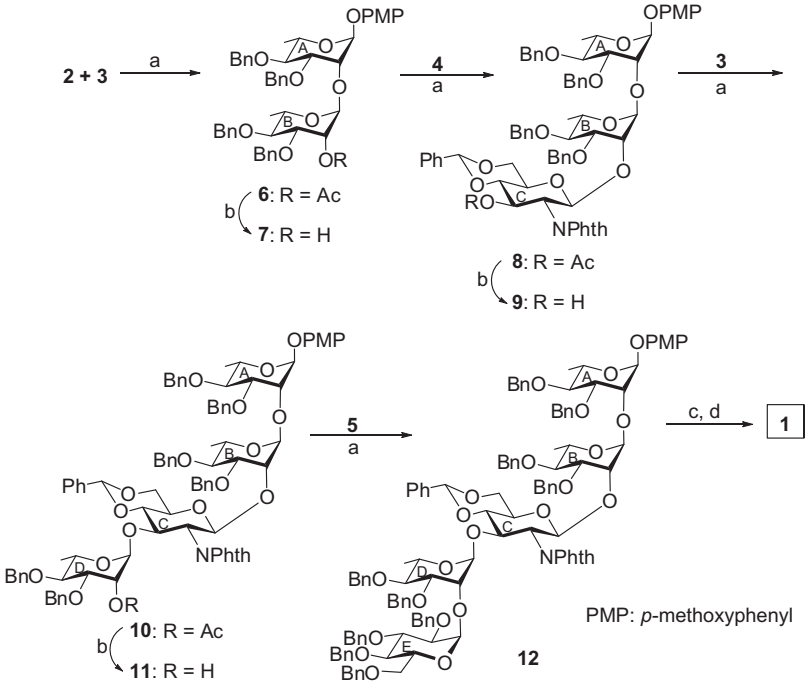
2.1. Synthesis

The target pentasaccharide **1** was synthesized from the suitably functionalized monosaccharide intermediates using a sequential glycosylation strategy using similar reaction conditions for stereoselective glycosylations and functional group manipulations. Functionalized monosaccharide derivatives **2**,[11] **3**,[12] **4**,[13] and **5**[14] were prepared from the reducing sugars using reaction methodologies reported earlier (Fig. 2). A general iodonium ion promoted stereoselective glycosylation condition was used for the synthesis of pentasaccharide derivative (**12**) as well as its synthetic intermediates **6**,[15] **8**, and **10**.

Stereoselective glycosylation of L-rhamnosyl acceptor **2** and L-rhamnosyl donor **3** following the earlier reported reaction condition[15] in the presence of a combination of *N*-iodosuccinimide (NIS) and trifluoromethane sulfonic acid (TMSOTf)[16,17] furnished disaccharide derivative **6**[15] in 88% yield. Treatment of compound **6** with sodium methoxide[18] following earlier reported reaction conditions[15] gave the disaccharide acceptor **7**[15] in 97% yield. Compound **7** was allowed to couple with D-glucosamine derived glycosyl donor **4** in the presence of a combination of NIS-TMSOTf[16,17] to furnish trisaccharide derivative **8** in 74% yield. Formation of compound **8** was confirmed from its spectral analysis [signals at δ 5.40 (d, J = 8.5 Hz, H-1_C), 5.18, (br s, H-1_A), 4.91 (br s, H-1_B) in the ^{1}H NMR and at δ 101.1 (C-1_B), 100.5 (C-1_C), 97.5 (C-1_A) in the ^{13}C NMR spectra]. Saponification of compound **8** using sodium methoxide afforded trisaccharide acceptor **9** in 97% yield. Stereoselective glycosylation of compound **8** with compound **3** in the presence of a combination of NIS-TMSOTf[16,17] resulted in the formation of tetrasaccharide derivative **10** in 76% yield, which was confirmed from its spectral analysis [signals at δ 5.24 (d, J = 8.5 Hz, H-1_C), 5.18 (br s, H-1_A), 4.92 (br s, H-1_B), 4.61 (br s, H-1_D) in the ^{1}H NMR and at δ 101.1 (C-1_C), 100.7 (C-1_B), 97.9 (C-1_D), 97.6 (C-1_A) in the ^{13}C NMR spectra]. Treatment of compound **10** with sodium methoxide produced tetrasaccharide acceptor **11** in 94% yield. Stereoselective 1,2-*cis*-glycosylation of compound **11** with D-glucosyl thioglycoside donor **5** in the presence of a combination of NIS-TMSOTf[16,17] in a mixed solvent (Et_2O–CH_2Cl_2; 4:1, v/v) furnished tetrasaccharide derivative **12** in 70% yield together with minor quantity of 1,2-*trans* glycosylated product (~10%), which was separated using column chromatography. Spectral analysis of compound **12** unambiguously supported its formation [signals at δ 5.32 (br s, H-1_A), 5.09 (br s, H-1_B), 4.92 (br s, H-1_D), 4.90 (d, J = 8.5 Hz, H-1_C), 4.75 (br s, H-1_E) in the ^{1}H NMR and at δ 102.6 (C-1_E), 101.2 (C-1_B), 97.7 (2 C, C-1_A, C-1_D), 95.1 (C-1_C) in the ^{13}C NMR spectra]. Compound **12** was subjected to a series of reactions involving (a) transformation of *N*-phthaloyl group to acetamido group by the treatment with hydrazine hydrate[19] followed by acetylation using acetic anhydride and pyridine; (b) catalytic transfer hydrogenation using triethylsilane and 10% Pd–C,[20] and (c) saponification using sodium methoxide to furnish the pentasaccharide **1** as its *p*-methoxyphenyl glycoside in 57% overall yield. Spectral analysis of compound **1** confirmed its formation [signals at δ 5.38 (br s, H-1_A), 5.10 (br s, H-1_B), 4.89 (br s, H-1_D), 4.76 (d, J = 3.5 Hz, H-1_E), 4.67 (d, J = 8.5 Hz, H-1_C) in the ^{1}H NMR and at δ 101.9 (C-1_C), 101.1 (C-1_B), 98.3 (C-1_A), 98.1 (C-1_D), 97.4 (C-1_E) in the ^{13}C NMR spectra] (Scheme 1).

2.2. Conformational analysis

In order to understand the conformation of the pentasaccharide **1**, NOE based 2D NOESY/ROESY NMR experiments in conjunction with molecular dynamics (MD) simulation were performed. The NOE cross peaks (interglycosidic linkages) of the pentasaccharide **1** were very weak at 298 K using a 500 MHz spectrometer[21] and therefore, 2D ROESY experiments were carried out to obtain the structural information of the molecule in aqueous solution. The 2D ROESY spectrum of the pentasaccharide **1** is shown in Figure 3 (and Supporting information), in which all anomeric protons of compound **1** were clearly identified. The H-1_A shows strong ROE with the aromatic ring protons of –OPMP group (see Supporting information). A strong ROE was observed between H-1_A and H-1_B and medium ROE was observed between H-1_A and H-6_B, indicating H-1_A is close in proximity to anomeric proton of L-rhamnosyl moiety B. In addition, few other interglycosidic ROEs such as H-2_A/H-1_B, H-3_A/H-1_B were also visible. The central region between L-rhamnosyl moiety B and *N*-acetyl-D-glucopyranosyl moiety C has much less conformational freedom since only three inter-glycosidic ROEs viz. H-2_B/H-1_C, H-2_B/H-2_C, and H-1_B/H-2_C were observed. The two protons, H-2_A and H-2_B display the same



Scheme 1. Reagents and conditions: (a) *N*-iodosuccinimide (NIS), TMSOTf, MS 4 Å, CH_2Cl_2 (in case of compound **12**; CH_2Cl_2–Et_2O, 1:3), −20 °C, 45 min, 88% for compound **6**, 74% for compound **8**, 76% for compound **10**, and 70% for compound **12**; (b) 0.1 M CH_3ONa, CH_3OH, room temperature, 1 h, 97% for compound **7**, 97% for compound **9**, 94% for compound **11**; (c) (i) $NH_2NH_2{\cdot}H_2O$, C_2H_5OH, 80 °C, 8 h; (ii) acetic anhydride, pyridine, room temperature, 2 h; (d) Et_3SiH, 10% Pd–C, CH_3OH–CH_2Cl_2 (5:1), room temperature, 8 h; (e) 0.1 M CH_3ONa, CH_3OH, room temperature, 3 h, 57% over all yield.

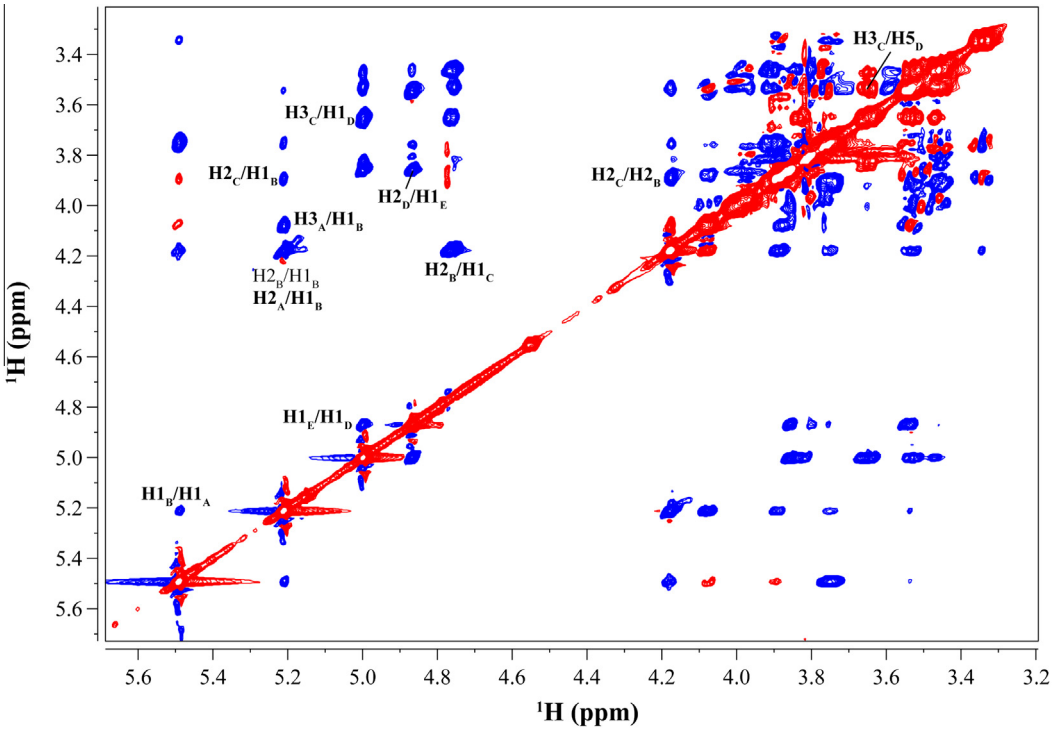


Figure 3. 2D ROESY (300 ms mixing time, 500 MHz, 298 K) spectrum of compound **1**. The key interresidual cross peaks are marked.

chemical shift, and hence it is difficult to distinguish between H-2_A/H-1_A and H-2_A/H-1_B. There were only two inter-glycosidic ROEs (H-3_C/H-1_D and H-3_C/H-5_D) observed between the *N*-acetyl-D-glucopyranosyl moiety C and L-rhamnosyl moiety D and two interglycosidic ROEs (H-2_D/H-1_E and H-1_D/H-1_E) between L-rhamnosyl moiety D and D-glucopyranosyl moiety E of the pentasaccharide **1** in aqueous solution revealing the distribution of conformation families at the glycosidic linkages.

The conformational behavior of oligosaccharides in solution cannot be restricted to a single geometry. Therefore, molecular

dynamics (MD) simulation has been used as a useful technique complementary to the 2D ROESY NMR spectral analysis for a detailed understanding on the fluctuations and conformational changes of a molecule in solution.[22] It is noteworthy to mention that the MD simulation generally performed at a nanosecond (ns) time scale whereas the NMR window can only take the snaps of conformations at micro to milli second (μs–ms) time scale.[23] Therefore, NMR experiments in combination with MD simulation can provide all types of conformations of a molecule exist in solution. The proton–proton distances at the interglycosidic linkages of compound **1** were very good in agreement with the ROE cross peaks observed in the ROESY spectrum of compound **1** in aqueous solution (Fig. 4). The terminal two interglycosidic linkages between D–E moieties and C–D moieties were rigid, as there were almost no fluctuations of inter proton distances at these linkages. However, the linkages between the B–C moieties of compound **1** are suffering from major fluctuations in the interproton distance of H-1_B/H-2_C. The inter proton distance between anomeric proton of L-rhamnopyranosyl moiety A (H-1_A) and methyl protons of B (H-6_B) also showed a huge fluctuation, presumably due to the fast correlation time of methyl protons (H-6_B). The H-1_A/H-1_B inter proton distance also fluctuates a small amount within a period of 10 ns simulation. No other significant findings were observed in the MD simulation of the compound **1**. Taken together, this result indicates that the compound **1** mostly exists in a single conformation. However, minor conformational changes may also be possible at A–B and B–C portions of compound **1**.

In summary a straightforward synthetic strategy has been developed for the preparation of the pentasaccharide repeating unit corresponding to the cell wall O-antigen of *Escherichia coli* O13. A linear glycosylation approach has been adopted for the construction of the target pentasaccharide as its *p*-methoxyphenyl glycoside. All glycosylation steps gave good yields with excellent stereoselectivity. The conformational analysis of the pentasaccharide **1** in aqueous solution was carried out using 2D ROESY NMR spectral analysis and MD simulation techniques. From the conformational analysis, it was found that the compound **1** exists as a single conformation with a possibility of minor conformational changes in A–B and B–C portions.

3. Experimental

3.1. 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 sulfate (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 Brucker 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, for example ^1H NMR, ^{13}C NMR, ^{13}C DEPT 135, 2D COSY, 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 TopSpin software suite (Bruker, Switzerland). ESI-MS were recorded on a Micromass 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.

3.1.1. *p*-Methoxyphenyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (6)

Prepared by the glycosylation of compound **2** and compound **3** in 88% yield following the similar reaction condition reported earlier.[15]

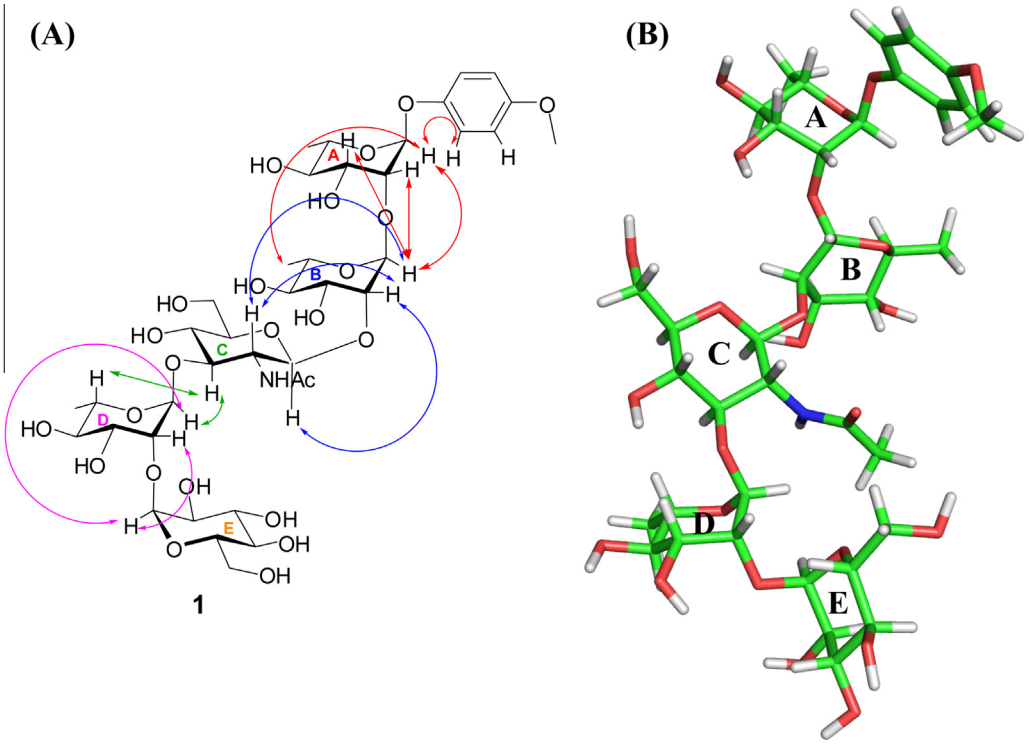


Figure 4. (A) The intermolecular ROEs of pentasaccharide **1**. (B) The global minimum conformation of pentasaccharide **1**, according to the molecular modeling technique.

3.1.2. *p*-Methoxyphenyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (7)

Prepared from compound **6** in 97% yield following the similar reaction condition reported earlier.[15]

3.1.3. *p*-Methoxyphenyl (3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (8)

A solution of compound **7** (1.5 g, 1.93 mmol), compound **4** (1 g, 2.07 mmol), and MS-4 Å (1 g) in anhydrous CH_2Cl_2 (10 mL) was stirred under argon at room temperature for 30 min. The reaction mixture was cooled to −20 °C and *N*-iodosuccinimide (NIS; 500 mg, 2.22 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf; 5 μL) were added to it and stirred at same temperature for 45 min. 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% $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane–EtOAc (6:1) as eluant to give pure compound **8** (1.7 g, 74%). White solid; mp 177–178 °C; $[\alpha]_D$ −55.7 (*c* 1.0, $CHCl_3$); IR (KBr): 3031, 2925, 2861, 1775, 1745, 1718, 1507, 1389, 1223, 1079, 1049, 722, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.88–7.07 (m, 29H, Ar-H), 6.85 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.76 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.05 (t, *J* = 10.0 Hz each, 1H, H-3_C), 5.45 (s, 1H, PhC*H*), 5.40 (d, *J* = 8.5 Hz, 1H, H-1_C), 5.18, (br s, 1H, H-1_A), 4.91 (br s, 1H, H-1_B), 4.78 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.66 (br s, 2H, PhCH_2), 4.54 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.39 (t, *J* = 10.0 Hz each, 1H, H-2_C), 4.31 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.08 (2 d, *J* = 11.0 Hz each, 2H, PhCH_2), 3.96–3.94 (m, 2H, H-2_A, PhCH_2), 3.90 (2 dd, *J* = 9.5 Hz, 3.0 Hz, 2H, H-3_A, H-3_B), 3.74 (s, 3H, OCH_3), 3.72–3.65 (m, 3H, H-2_B, H-6_{abC}), 3.64–3.59 (m, 2H, H-5_A, H-5_B), 3.56 (t, *J* = 10.0 Hz each, 1H, H-4_C), 3.54–3.50 (m, 1H, H-5_C), 3.19 (t, *J* = 9.5 Hz each, 1H, H-4_A), 3.48 (t, *J* = 9.5 Hz each, 1H, H-4_B), 1.95 (s, 3H, COCH_3), 1.18–1.15 (m, 6H, 2 CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9 (*C*OCH$_3$), 156.2–114.5 (Ar-C), 101.6 (PhCH), 101.1 (C-1_B), 100.5 (C-1_C), 97.5 (C-1_A), 80.5 (2 C, C-4_A, C-4_B), 79.2 (2 C, C-3_A, C-3_B), 78.9 (C-2_A), 77.7 (C-4_C), 75.6 (C-2_B), 75.3 (PhCH$_2$), 75.0 (PhCH$_2$), 72.7 (PhCH$_2$), 71.9 (PhCH$_2$), 69.3 (C-3_C), 68.6 (C-6_C), 68.4 (C-5_A), 68.3 (C-5_B), 65.7 (C-5_C), 55.5 (OCH$_3$), 55.3 (C-2_C), 20.7 (COCH$_3$), 18.1, 17.7 (2 CCH$_3$); ESI-MS: 1220.4 $[M+Na]^+$; Anal. Calcd for $C_{70}H_{71}NO_{17}$ (1197.47): C, 70.16; H, 5.97; found: C, 70.00; H, 6.10.

3.1.4. *p*-Methoxyphenyl (4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (9)

A solution of compound **8** (1.5 g, 1.25 mmol) in 0.1 M CH_3ONa in CH_3OH (50 mL) was allowed to stir at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50 W-X8 (H^+) resin, filtered, and concentrated. The crude product was passed through a small pad of SiO_2 using hexane–EtOAc (1:1) as eluant to give pure compound **9** (1.4 g, 97%). White solid; mp 84–85 °C; $[\alpha]_D$ −51 (*c* 1.0, $CHCl_3$); IR (KBr): 2928, 1715, 1507, 1390, 1077, 1050, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.59–7.08 (m, 29H, Ar-H), 6.85 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.76 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.49 (s, 1H, PhC*H*), 5.25 (d, *J* = 8.5 Hz, 1H, H-1_C), 5.18 (br s, 1H, H-1_A), 4.93 (br s, 1H, H-1_B), 4.81 (t, *J* = 9.5 Hz each, 1H, H-3_C), 4.76 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.66 (br s, 2H, PhCH_2), 4.53 (d, *J* = 11.0 Hz, 1 H PhCH_2), 4.36 (t, *J* = 9.5 Hz each, 1H, H-2_C), 4.28 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.12 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.06 (d, *J* = 11.0 Hz, 1H, PhCH_2), 3.97 (d, *J* = 11.0 Hz, 1H, PhCH_2), 3.95 (br s, 1H, H-2_B), 3.92–3.88 (m, 2H, H-3_B, H-6_{aC}), 3.78 (dd, *J* = 9.5 Hz, 3.0 Hz, 1H, H-3_A), 3.74 (s, 3H, OCH_3), 3.72 (br s, 1H, H-2_A), 3.71–3.69 (m, 1H, H-5_A), 3.68–3.65 (m, 1H, H-5_B), 3.64–3.53 (m, 3H, H-4_C, H-6_{bC}), 3.43–3.38 (m, 1H, H-5_C), 3.17 (t, *J* = 9.5 Hz each, 1H, H-4_A), 3.11 (t, *J* = 9.5 Hz each, 1H, H-4_B), 1.18–1.17 (m, 6H, 2 CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.8–113.7 (Ar-C), 101.9 (PhCH), 101.2 (C-1_C), 97.6 (C-1_A), 82.0 (C-4_C), 80.5 (C-4_A), 80.4 (C-4_B), 79.3 (C-2_A), 78.9 (C-3_A), 77.7 (C-3_B), 75.6 (C-2_B), 75.3 (PhCH$_2$), 75.0 (PhCH$_2$), 72.6 (PhCH$_2$), 71.9 (PhCH$_2$), 68.6 (C-3_C), 68.5 (C-6_C), 68.3 (C-5_A), 68.1 (C-5_B), 65.7 (C-5_C), 56.6 (C-2_C), 55.5 (OCH$_3$), 18.0, 17.7 (2CCH$_3$); ESI-MS: 1178.4 $[M+Na]^+$; Anal. Calcd for $C_{68}H_{69}NO_{16}$ (1155.46): C, 70.63; H, 6.01; found: C, 70.50; H, 6.15.

3.1.5. *p*-Methoxyphenyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (10)

A solution of compound **9** (1.2 g, 1.04 mmol), compound **3** (500 mg, 1.16 mmol), and MS-4 Å (1 g) in anhydrous CH_2Cl_2 (10 mL) was stirred under argon at room temperature for 30 min. The reaction mixture was cooled to −20 °C and NIS (280 mg, 1.24 mmol) and TMSOTf (3 μL) were added to it and stirred at same temperature for 45 min. 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% $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane–EtOAc (6:1) as eluant to give pure compound **10** (1.2 g, 76%). White solid; mp 175–176 °C; $[\alpha]_D$ −35 (*c* 1.0, $CHCl_3$); IR (KBr): 3030, 2932, 1774, 1744, 1715, 1507, 1454, 1388, 1232, 1103, 1075, 1051, 987, 723, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 5.46 (s, 1H, PhC*H*), 5.24 (d, *J* = 8.5 Hz, 1H, H-1_C), 5.18 (br s, 1H, H-1_A), 4.92 (br s, 1H, H-1_B), 4.89–4.88 (m, 1H, H-2_D), 4.80 (2d, *J* = 11.0 Hz each, 2H, PhCH$_2$), 4.74 (t, *J* = 9.5 Hz each, 1H, H-3_C), 4.66 (br s, 2H, PhCH_2), 4.61 (br s, 1H, H-1_D), 4.54–4.46 (m, 3H, PhCH_2), 4.41 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.36 (t, *J* = 9.5 Hz each, 1H, H-2_C), 4.31 (d, *J* = 11.0 Hz, 1H, PhCH_2), 4.09–4.06 (m, 2H, PhCH_2), 3.95–3.92 (m, 2H, H-2_A, PhCH_2), 3.90–3.86 (m, 3H, H-3_D, H-5_A, H-5_B), 3.82 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3_A), 3.74 (s, 3H, OCH_3), 3.71–3.69 (m, 2H, H-2_B, H-3_B), 3.65–3.52 (m, 4H, H-4_C, H-5_D, H-6_{abC}), 3.45–3.39 (m, 1H, H-5_C), 3.23 (t, *J* = 9.5 Hz each, 1H, H-4_A), 3.17 (t, *J* = 9.5 Hz each, 1H, H-4_B), 3.08 (t, *J* = 9.5 Hz each, 1H, H-4_D), 1.82 (s, 3H, COCH_3), 1.18–1.16 (m, 6H, 2 CCH_3), 0.80 (d, *J* = 6.0 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.6 (*C*OCH$_3$), 154.7–114.5 (Ar-C), 101.7 (PhCH), 101.1 (C-1_C), 100.7 (C-1_B), 97.9 (C-1_D), 97.6 (C-1_A), 80.5 (C-4_D), 80.4 (C-4_B), 80.2 (C-4_A), 80.0 (C-4_C), 79.1 (C-3_D), 78.9 (C-3_A), 77.7 (C-2_A), 76.9 (C-3_B), 75.5 (C-2_B), 75.3 (PhCH$_2$), 75.0 (PhCH$_2$), 74.6 (C-3_C), 72.6 (PhCH$_2$), 71.9 (PhCH$_2$), 71.4 (PhCH$_2$), 69.1 (C-2_D), 68.6 (C-6_C), 68.5 (C-5_D), 68.3 (C-5_A), 67.8 (C-5_B), 66.1 (C-5_C), 56.5 (C-2_C), 55.5 (OCH$_3$), 20.7 (*C*OCH$_3$), 18.1 (*C*CH$_3$), 17.7 (*C*CH$_3$), 17.3 (*C*CH$_3$); MALDI-MS: 1546.5 $[M+Na]^+$; Anal. Calcd for $C_{90}H_{93}NO_{21}$ (1523.62): C, 70.90; H, 6.15; found: C, 70.76; H, 6.30.

3.1.6. *p*-Methoxyphenyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (11)

A solution of compound **10** (1.1 g, 0.72 mmol) in 0.1 M CH_3ONa in CH_3OH (40 mL) was allowed to stir at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50 W-X8 (H^+) resin, filtered, and concentrated. The crude product was passed through a small pad of SiO_2 using hexane–EtOAc (1:1) as eluant to give pure compound **11** (1 g, 94%). White solid; mp 165–166 °C; $[\alpha]_D$ −49 (*c* 1.0, $CHCl_3$); IR (KBr): 3036, 2939, 1741, 1517, 1455, 1230, 1077, 1056, 977, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.61–7.09 (m, 39H, Ar-H), 6.86 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.77 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.46 (s, 1H, PhC*H*), 5.26 (d, *J* = 8.5 Hz, 1H, H-1_C), 5.19 (br s, 1H, H-1_A), 4.92 (br s, 1H, H-1_B), 4.82 (t, *J* = 9.5 Hz each, 1H, H-1_C), 4.79–4.74 (m, 2H, PhCH_2), 4.68 (br s, 1H, H-1_D),

4.67 (br s, 2H, PhCH_2), 4.57 (br s, 2H, PhCH_2), 4.55–4.48 (m, 2H, PhCH_2), 4.37 (t, J = 9.0 Hz each, 1H, H-2$_C$), 4.31 (d, J = 11.0 Hz, 1H, PhCH_2), 4.08–4.05 (m, 2H, PhCH_2), 3.95–3.88 (m, 5H, H-2$_A$, H-3$_A$, H-3$_D$, H-5$_A$, H-5$_B$), 3.76–3.75 (m, 2H, H-2$_D$, H-3$_B$), 3.74 (s, 3H, OCH_3), 3.71–3.70 (m, 1H, H-2$_B$), 3.68–3.53 (m,4H, H-4$_C$, H-5$_D$, H-6$_{abC}$), 3.48–3.43 (m, 1H, H-5$_C$), 3.23 (t, J = 10.5 Hz each, 1H, H-4$_A$), 3.22 (t, J = 10.5 Hz each, 1H, H-4$_B$), 3.07 (t, J = 10.5 Hz each, 1H, H-4$_D$), 1.20–1.17 (m, 6H, 2 CCH_3), 0.81 (d, J = 6.0 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.8–114.5 (Ar-C), 101.8 (PhCH), 101.1 (C-1$_C$), 100.6 (C-1$_B$), 99.2 (C-1$_D$), 97.6 (C-1$_A$), 80.6 (C-4$_D$), 80.5 (C-4$_B$), 80.4 (C-4$_A$), 80.0 (C-4$_C$), 79.6 (C-3$_D$), 79.0 (C-3$_A$), 78.9 (C-2$_A$), 77.7 (C-3$_B$), 75.5 (C-2$_B$), 75.3 (PhCH$_2$), 75.0 (2 C, 2 PhCH_2), 73.3 (C-3$_C$), 72.7 (PhCH$_2$), 71.9 (2 C, 2 PhCH_2), 68.6 (2 C, C-2$_D$, C-5$_D$), 68.5 (C-6$_C$), 68.3 (C-5$_A$), 67.6 (C-5$_B$), 65.9 (C-5$_C$), 56.8 (C-2$_C$), 55.5 (OCH_3), 18.1 (CCH_3), 17.3 (CCH_3); MALDI-MS: 1504.6 [M+Na]$^+$; Anal. Calcd for $C_{88}H_{91}NO_{20}$ (1481.6): C, 71.29; H, 6.19; found: C, 71.15; H, 6.36.

3.1.7. *p*-Methoxyphenyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (12)

To a solution of compound **11** (800 mg, 0.54 mmol) and compound **5** (340 mg, 0.58 mmol) in anhydrous CH_2Cl_2–Et_2O (10 mL; 1:3, v/v) was added MS-4 Å (1 g) and the reaction mixture was stirred under argon at room temperature for 30 min. The reaction mixture was cooled to −20 °C. To the cooled reaction mixture were added NIS (140 mg, 0.62 mmol) and TMSOTf (2 μL) and it was stirred at same temperature for 45 min. 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% $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane–EtOAc (3:1) as eluant to give pure compound **12** (760 mg, 70%). Colorless oil; $[\alpha]_D$ −2 (*c* 1.0, $CHCl_3$); IR (neat): 3030, 2931, 1678, 1507, 1454, 1364, 1214, 1086, 1050, 735, 696 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49–7.12 (m, 59H, Ar-H), 6.92 (d, J = 9.0 Hz, 2H, Ar-H), 6.81 (d, J = 9.0 Hz, 2H, Ar-H), 5.44 (br s, 1H, PhC*H*), 5.32 (br s, 1H, H-1$_A$), 5.09 (br s, 1H, H-1$_B$), 4.92 (br s, 1H, H-1$_D$), 4.90 (d, J = 8.5 Hz, 1H, H-1$_C$), 4.81 (t, J = 10.0 Hz each, 1H, H-3$_C$), 4.80–4.76 (m, 2H, PhCH_2), 4.75 (br s, 1H, H-1$_E$), 4.73–4.60 (m, 15H, PhCH_2), 4.50–4.45 (m, 3H, H-2$_C$, PhCH_2), 4.33 (d, J = 11.0 Hz, 1H, PhCH_2), 4.10–3.95 (m, 6H, H-2$_A$, H-2$_B$, H-2$_D$, H-3$_A$, H-5$_A$, H-5$_B$), 3.92–3.89 (m, 2H, H-3$_B$, H-4$_C$), 3.85–3.79 (m, 2H, H-3$_D$, H-5$_D$), 3.77 (s, 3H, OCH_3), 3.65–3.58 (m, 3H, H-2$_E$, H-3$_E$, H-4$_A$), 3.55–3.50 (m, 3H, H-4$_E$, H-5$_C$, H-6$_{aE}$), 3.47–3.39 (m, 5H, H-4$_B$, H-4$_D$, H-6$_{abC}$, H-6$_{bE}$), 3.36–3.30 (m, 1H, H-5$_E$), 1.70 (s, 3H, COCH_3), 1.31–1.28 (m, 6H, 2 CCH_3), 0.95 (d, J = 6.0 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.2–114.6 (Ar-C), 102.6 (C-1$_E$), 101.6 (PhCH), 101.2 (C-1$_B$), 97.7 (2 C, C-1$_A$, C-1$_D$), 95.1 (C-1$_C$), 81.6 (C-4$_B$), 80.9 (C-4$_D$), 80.3 (2 C, C-4$_A$, C-4$_C$), 79.8 (C-3$_D$), 79.2 (2 C, C-3$_A$, C-3$_B$), 78.7 (C-4$_E$), 77.7 (C-3$_E$), 77.6 (C-2$_B$), 75.6 (PhCH_2), 75.5 (PhCH_2), 75.4 (2 C, C-2$_E$, PhCH_2), 75.3 (2 C, C-2$_A$, PhCH_2), 74.9 (PhCH_2), 73.4 (2 C, 2 PhCH_2), 72.9 (2 C, C-5$_B$, PhCH_2), 72.2 (PhCH_2), 71.8 (PhCH_2), 70.2 (C-3$_C$), 68.6 (2 C, C-6$_C$, C-6$_E$), 68.5 (2 C, C-2$_D$, C-5$_D$), 68.4 (C-5$_A$), 68.3 (C-5$_C$), 66.2 (C-5$_E$), 57.5 (C-2$_C$), 55.5 (OCH_3), 18.3, 18.2, 17.9 (3 CCH_3); MALDI-MS: 2026.8 [M+Na]$^+$; Anal. Calcd for $C_{122}H_{125}NO_{25}$ (2003.85): C, 73.07; H, 6.28; found: C, 72.90; H, 6.50.

3.1.8. *p*-Methoxyphenyl (α-D-glucopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→2)-α-L-rhamnopyranoside (1)

To a solution of compound **12** (600 mg, 0.3 mmol) in C_2H_5OH (20 mL) was added hydrazine monohydrate (0.3 mL) and the reaction was allowed to stir at 80 °C for 8 h. The solvents were removed under reduced pressure and the crude product was dissolved in acetic anhydride and pyridine (5 mL, 1:1 v/v) and kept at room temperature for 2 h and concentrated under reduced pressure. To a solution of the acetylated product and 10% Pd–C (150 mg) in CH_3OH–CH_2Cl_2 (10 mL; 5:1, v/v) was added Et_3SiH (1 mL, 6.26 mmol) drop wise during 30 min and the reaction mixture was allowed to stir for 8 h. The reaction mixture was filtered through a Celite® bed, washed with CH_3OH (50 mL), and concentrated under reduced pressure. A solution of the hydrogenolyzed product in 0.1 M CH_3ONa in CH_3OH (15 mL) was allowed to stir at room temperature for 3 h. The reaction mixture was neutralized using Dowex 50W X8 (H^+) resin, filtered, and concentrated under reduced pressure to give compound **1**, which was passed through column of Sephadex LH-20 gel using CH_3OH–H_2O (3:1) as eluant to give pure compound **1** as *p*-methoxyphenyl glycoside (160 mg, 57%). White powder; $[\alpha]_D$ −0.1 (*c* 1.0, H_2O); IR (KBr): 3434, 2946, 1629, 1362, 1152, 667 cm^{-1}; ^{1}H NMR (500 MHz, D_2O): δ 6.99 (d, J = 9.0 Hz, 2H, Ar-H), 6.88 (d, J = 9.0 Hz, 2H, Ar-H), 5.38 (br s, 1H, H-1$_A$), 5.10 (br s, 1H, H-1$_B$), 4.89 (br s, 1H, H-1$_D$), 4.76 (d, J = 3.5 Hz, 1H, H-1$_E$), 4.67 (d, J = 8.5 Hz, 1H, H-1$_C$), 4.07–4.06 (m, 2H, H-2$_A$, H-2$_B$), 3.97 (dd, J = 10.0, 3.5 Hz, 1H, H-3$_A$), 3.94–3.85 (m, 2H, H-3$_C$, H-5$_D$), 3.83–3.73 (m, 5H, H-2$_D$, H-3$_B$, H-3$_D$, H-4$_C$, H-6$_{aC}$), 3.71–3.69 (m, 3H, H-2$_C$, H-6$_{abE}$), 3.70 (s, 3H, OCH_3), 3.66–3.61 (m, 3H, H-5$_A$, H-5$_E$, H-6$_{bC}$), 3.54 (t, J = 10.0 Hz each, 1H, H-4$_E$), 3.44–3.40 (m, 4H, H-2$_E$, H-3$_E$, H-5$_B$, H-5$_C$), 3.36 (t, J = 9.5 Hz each, 2H, H-4$_A$, H-4$_D$), 3.24 (t, J = 9.5 Hz each, 1H, H-4$_B$), 1.97 (s, 3H, COCH_3), 1.15–1.12 (m, 9H, 3 CCH_3); ^{13}C NMR (125 MHz, D_2O): δ 174.6 (*C*OCH$_3$), 154.9–115.2 (Ar-C), 101.9 (C-1$_C$), 101.1 (C-1$_B$), 98.3 (C-1$_A$), 98.1 (C-1$_D$), 97.4 (C-1$_E$), 81.2 (C-4$_E$), 78.8 (C-2$_A$), 78.7 (C-2$_B$), 76.2 (C-2$_D$), 75.8 (C-3$_C$), 72.7 (C-5$_C$), 72.1 (C-3$_D$), 72.0 (C-3$_E$), 71.8 (C-2$_E$), 71.7 (C-4$_B$), 71.3 (C-4$_A$), 69.7 (C-3$_A$), 69.6 (2 C, C-5$_A$, C-5$_B$), 69.4 (C-5$_E$), 69.3 (2 C, C-3$_B$, C-4$_D$), 69.2 (C-5$_D$), 68.2 (C-4$_C$), 60.6 (C-6$_C$), 60.1 (C-6$_E$), 55.8 (2 C, C-2$_C$, OCH_3), 22.3 (COCH_3), 16.6, 16.5, 16.3 (3 *C*CH_3); ESI-MS: 950.3 [M+Na]$^+$; Anal. Calcd for $C_{39}H_{61}NO_{24}$ (927.35): C, 50.48; H, 6.63; found: C, 50.31; H, 6.82.

3.2. Molecular dynamics simulation

The model structure of the pentasaccharide **1** was prepared using GLYCAM_06j force field.[24] The non-electrostatic model of *p*-methoxyphenoxy group (OPMP) was prepared and the parameters were adopted using general AMBER force field (GAFF).[25] For molecular dynamics simulation, the pentasaccharide **1** was solvated in truncated octahedral box with TIP3[26] water models with an edge distance of 8 Å. Accounts of the nonbonded interactions were done with a cutoff distance of 8 Å and simulation was performed under isothermal-isobaric periodic boundary conditions. SHAKE algorithm was applied to restrain all hydrogen bonds with an integration time step of 2 fs. Minimization of the system, was performed with 1500 steepest descent steps followed by 500 conjugate gradient steps with convergence criteria of 0.01 kcal mol^{-1}. The system annealed for a time period of 50 ps, and it was stabilized at 300 K. Density equilibrium of the system was processed for another 50 ps before the production runs with weak restraints over the solute atoms. Finally the explicit simulation was carried out for a time scale of 10 ns using sander module of AMBER11.

Acknowledgements

A.S. and A.S. thank CSIR, India for providing a Senior and Junior Research Fellowships, respectively. This work was supported by DST, India (Project No. SR/S1/OC-83/2010). Mr. Barun Mazumdar, Bose Institute NMR facility is thankfully acknowledged for carrying out the NMR experiments with great care.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2014.03.012.

References

1. Robbins, J. B.; McCracken, G. H.; Gotschlich, E. C.; Orskov, F.; Orskov, I.; Hanson, L. A. N. *Engl. J. Med.* **1974**, *290*, 1216–1220.
2. Besser, R. E.; Lett, S. M.; Weber, J. T.; Doyle, M. P.; Barrett, T. J.; Wells, J. G.; Griffin, P. M. *JAMA* **1993**, *269*, 2217–2220.
3. Johnson, J. R. *Clin. Microbial. Rev.* **1991**, *4*, 80–128.
4. Johnson, J. R.; Gajewski, A.; Lesse, A. J.; Russo, T. A. *J. Clin. Microbiol.* **2003**, *41*, 5798–5802.
5. Nataro, J. P.; Kaper, J. B. *Clin. Microbial. Rev.* **1998**, *11*, 142–201.
6. Perepelov, A. V.; Shevelev, S. D.; Liu, B.; Senchenkova, S. N.; Shashkov, A. S.; Feng, L.; Knirel, Y. A.; Wang, L. *Carbohydr. Res.* **2010**, *345*, 1594–1599.
7. DuPont, H. L.; Formal, S. B.; Hornick, R. B.; Snyder, M. J.; Libonati, J. P.; Sheahan, D. G.; LaBrec, E. H.; Kalas, J. P. *N. Engl. J. Med.* **1971**, *285*, 1–9.
8. Coates, A. R. M.; Hu, Y. *Br. J. Pharmacol.* **2007**, *152*, 1147–1154.
9. Roy, R. *Drug Discovery Today Technol.* **2004**, *1*, 327–336. and references cited therein.
10. Doores, K. J.; Gamblin, D. P.; Davis, B. G. *Chem. Eur. J.* **2006**, *12*, 656–665.
11. Guchhait, G.; Misra, A. K. *Tetrahedron: Asymmetry* **2009**, *20*, 1791–1797.
12. Sajtos, F.; Hajkó, J.; Kövér, K. E.; Lipták, A. *Carbohydr. Res.* **2001**, *334*, 253–259.
13. Kihlberg, J. O.; Leigh, D. A.; Bundle, D. R. *J. Org. Chem.* **1990**, *55*, 2860–2863.
14. Dasgupta, F.; Garegg, P. J. *Acta Chem. Scand.* **1989**, *43*, 471–475.
15. Ghosh, T.; Misra, A. K. *Carbohydr. Res.* **2012**, *362*, 8–12.
16. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.
17. Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313–4316.
18. Zemplén, G. *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 1254–1266.
19. Lee, H.-H.; Schwartz, D. A.; Harris, J. F.; Carver, J. P.; Krepinsky, J. J. *Can. J. Chem.* **1986**, *64*, 1912–1918.
20. Santra, A.; Ghosh, T.; Misra, A. K. *Beilstein J. Org. Chem.* **2013**, *9*, 74–78.
21. *NMR Spectroscopy of Glycoconjugates*; Jiménez-Barbero, J., Peters, T., Eds.; WILEY-VCH GmbH & Co. KGaA: Weinheim, 2003. pp 145–184.
22. Madsen, L. J.; Ha, S. N.; Tran, V. H.; Brady, J. W. *Comput. Model. Carbohydr. Mol.* **1990**, *430*, 69–90.
23. Fisette, O.; Lague, P.; Gagne, S.; Morin, S. *J. Biomed. Biotechnol.* **2012**. Article ID 254208.
24. Kirschener, K. N.; Yongye, A. B.; Tschampel, S. M.; Gonzalez, J. O.; Daniels, C. R.; Foley, B. L.; Woods, R. J. *J. Comput. Chem.* **2008**, *29*, 622–655.
25. Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. *J. Comput. Chem.* **2004**, *25*, 1157–1174.
26. Jorgensen, W. L.; Chandraskehar, J.; Madura, J. D.; Impey, W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926–935.