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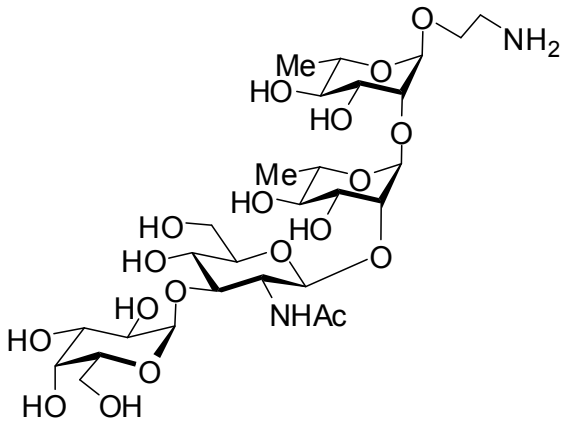
Complete List of Authors:	Jana, Manas; Bose Institute, Division of Molecular Medicine Kar, Rajiv; Bose Institute, Biophysics Bhunia, Anirban; Bose Institute, Biophysics Misra, Anup; Bose Institute, Molecular Medicine Division

Synthesis of the tetrasaccharide repeating unit of the *O*-antigen of *Escherichia coli* O69 strain and its conformational analysis

Manas Jana,[a] Rajiv Kumar Kar,[b] Anirban Bhunia[b] and Anup Kumar Misra[a*]



A tetrasaccharide corresponding to the *O*-antigen of *E. coli* O69 strain has been synthesized using two step iterative glycosylations in one pot. The conformational analysis of the synthesized tetrasaccharide was carried out using NOE based two-dimensional ROESY spectroscopy in conjugation with all atom explicit molecular dynamics simulation studies.

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ARTICLE TYPE

Synthesis of the tetrasaccharide repeating unit of the *O*-antigen of *Escherichia coli* O69 strain and its conformational analysis†

Manas Jana,[a] Rajiv Kumar Kar,[b] Anirban Bhunia[b] and Anup Kumar Misra*[a]

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A straightforward synthesis of the tetrasaccharide repeating unit of the *O*-antigen of *Escherichia coli* O69 strain as its 2-aminoethyl glycoside has been accomplished by carrying out two iterative glycosylations in one-pot. The stereochemical outcome of the glycosylations were very good. The conformational analysis of the synthesized tetrasaccharide was carried out using NOE based two-dimensional ROESY spectroscopy in conjugation with all atom explicit molecular dynamics simulation studies. The solvent-perturbation induced conformations of the tetrasaccharide **1** were found quite stable in solution.

Introduction

Urinary tract infection (UTI) in humans is a serious concern all over the world.[1] UTI is an extraintestinal bacterial infection and occurs more commonly in women than men and affects urinary tract[2] and sometimes kidney also. In some cases, UTI is termed as bladder infections or cystitis. In general, UTI are commonly treated with short term of antibiotics.[3] However, due to the emergence of the multi-drug-resistant bacterial strains, the treatment is becoming complicated and sometimes longer treatment with multiple antibiotics is required.[4] The most common bacterial strains found to cause UTI are *Escherichia coli* (*E. coli*) strains.[5] Although *E. coli* strains are found to live harmlessly in human intestine, they cause serious infections if they get into the urinary tract. Although UTI is not associated with outbreaks, certain multidrug resistant *E. coli* strains exhibited epidemic behavior causing outbreak of community acquired cystitis and pyelonephritis in Europe.[6,7] Several *E. coli* strains responsible for the UTI have been identified and characterized till date such as *E. coli* O4, O6, O14, O22, O75, O69, O83 strains.[8] Since, the virulence factors in the pathogenic *E. coli* strains are highly associated with the cell wall polysaccharide chains or *O*-antigens,[9] the structure of a number of cell wall polysaccharide repeating units have been identified in UTI causing *E. coli* strains. The structure of the O-specific side chain of the cell wall lipopolysaccharide of UTI causing *E. coli* O69 strain has been established by Erbing *et al*.[10] In order to understand the pathogenic/immunogenic role of the cell wall polysaccharide fragments, it is essential to get the access to a significant quantity of the material, which is practically inconvenient to isolate from the natural sources with adequate purity. Therefore, chemical synthesis of the oligosaccharide repeating unit of the cell wall polysaccharide would be the best option to have required quantity of oligosaccharides for their use in the glycoconjugate preparation and biological studies. For detailed understanding of the biological role of the oligosaccharide it is important to know its conformational behavior in the solution, which can be established using multi-dimensional NMR spectroscopic analysis and molecular dynamics simulation studies. In an ongoing program for the synthesis of bacterial cell wall oligosaccharides,[11,12] a straight forward synthesis of the tetrasaccharide repeating unit of the cell wall *O*-specific polysaccharide of *E. coli* O69 strain as its 2-aminoethyl glycoside using one-pot iterative glycosylations and its conformational analysis using NOE based NMR spectral analysis and molecular dynamic (MD) simulation is presented herein.

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

Structure of the tetrasaccharide repeating unit of the cell wall lipopolysaccharide of *Escherichia coli* O69.

Results and discussion

The target tetrasaccharide **1**, the 2-aminoethyl glycoside was synthesized by the stereoselective assembly of the suitably functionalized monosaccharide derivatives. The 2-aminoethyl linker present in the molecule may allow further glycoconjugate formation with a suitable aglycon moiety. The monosaccharide intermediates **2**,[13] **3**,[14] **4**[15] and **5**[16] were prepared from the commercially available reducing sugars using the reaction conditions reported in the literature (Figure 1). The tetrasaccharide **1** was synthesized by carrying out two iterative glycosylation reactions in one-pot. "Armed-disarmed" glycosylation followed by [2+2] block glycosylation were carried out in one-pot iterative glycosylations setup. To start with, known ethyl 2-*O*-acetyl-3,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**2**) was allowed to react with 2-(carbobenzyloxy)amino ethanol in the presence of a combination of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)[17,18] to give 2-(carbobenzyloxy)aminoethyl 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranoside in 81% yield, which on de-*O*-acetylation using sodium methoxide[19] furnished compound **3** in 95% yield. Stereoselective glycosylation of compound **3** with thioglycoside **2**

in the presence of a combination of NIS-TMSOTf[17,18] furnished disaccharide derivative **6** in 76% yield, which on treatment with sodium methoxide[19] resulted in the formation of disaccharide acceptor **7** in 95% yield. Spectral analysis of compound **6** confirmed its stereoselective formation [signals at δ 4.95 (br s, H-1_A), 4.70 (br s, H-1_B) in ^{1}H NMR and at δ 99.2 (C-1_A), 98.9 (C-1_B) in the ^{13}C NMR spectra] (Scheme 1). In the first step of the iterative glycosylations, D-glucosamine thioglycoside derivative **4** was treated with D-galactose thioglycoside derivative **5** in the presence of a combination of NIS and TMSOTf to generate disaccharide thioglycoside derivative **8** following "armed-disarmed glycosylation" concept.[20] The *in situ* generated compound **8** was not isolated from the reaction mixture and was allowed to react further with the disaccharide acceptor **7** as a disaccharide glycosyl donor under the similar reaction condition to furnish tetrasaccharide derivative **9** in 61% yield. Spectral analysis of compound **9** supported its formation and stereoselectivity of the glycoside bonds present in it [δ 5.53 (d, J = 3.0 Hz, H-1_D), 5.30 (d, J = 8.5 Hz, H-1_C), 5.21 (s, PhC*H*), 4.87 (br s, H-1_A), 4.56 (br s, 1 H, H-1_B) in the ^{1}H NMR and at δ 101.6 (Ph*C*H), 101.1 (C-1_C), 100.6 (C-1_A), 98.9 (C-1_B), 97.2 (C-1_D) in the ^{13}C NMR spectra]. Finally, compound **9** was subjected to a set of reactions, which include (a) treatment with hydrazine hydrate[21] followed by acetylation using acetic anhydride and pyridine to convert phthalimido group to acetamido group and (b) catalytic transfer hydrogenation using triethylsilane[22] and $Pd(OH)_2$-C to furnish target tetrasaccharide **1** as its 2-aminoethyl glycoside in 72% yield. Formation of compound **1** was unambiguously confirmed from its spectral analysis [signals at δ 5.32 (br s, H-1_D), 5.04 (br s, H-1_B), 4.82 (br s, H-1_A), 4.64 (d, J = 7.5 Hz, H-1_C) in the ^{1}H NMR and δ 102.4 (C-1_C), 101.0 (C-1_B), 99.0 (C-1_D), 98.5 (C-1_A) in the ^{13}C NMR spectra] (Scheme 2).

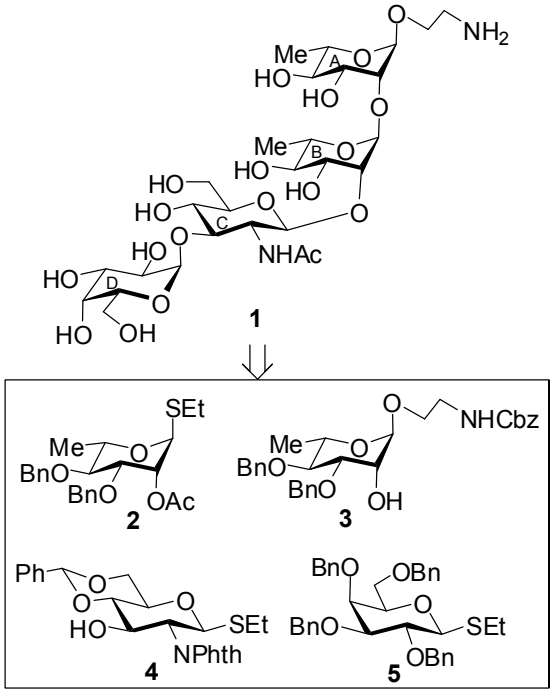
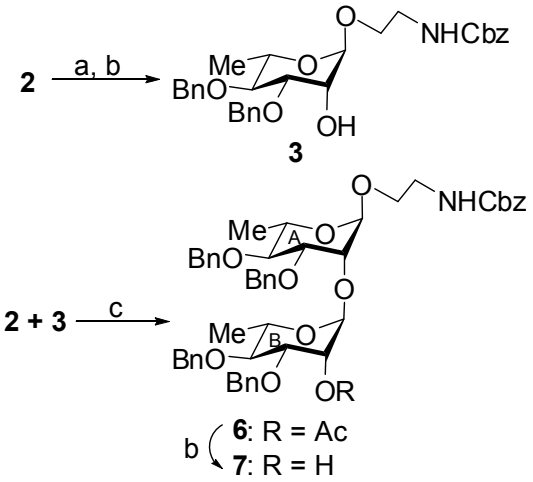
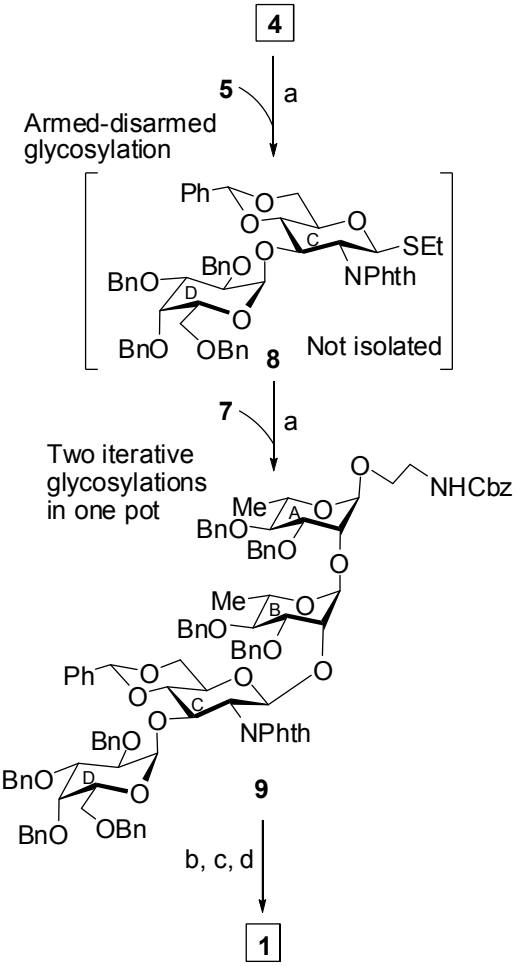


Figure 1: Structure of the synthesized tetrasaccharide **1** and its synthetic precursors.



Scheme 1: Reagents: (a) NHCbz$(CH_2)_2$OH, *N*-iodosuccinimide (NIS), TMSOTf, CH_2Cl_2, MS 4Å, – 25 °C, 1 h, 81%; (b) 0.1 M CH_3ONa, CH_3OH, room temperature, 2 h, 95% for compound **3** and 96% for compound **7**; (c) NIS, TMSOTf, CH_2Cl_2, MS 4Å, – 35 °C, 76%.



Scheme 2: Reagents: (a) NIS, TMSOTf, CH_2Cl_2, – 15 °C, 1 h, 61% for compound **9**; (b) $NH_2NH_2{\cdot}H_2O$, EtOH, 90 °C, 5 h; (c) acetic anhydride, pyridine, room temperature, 1 h; (d) Et_3SiH, 20% $Pd(OH)_2$-C, CH_3OH, 14 h, room temperature, 63%.

In order to get the information about the conformation of the tetrasaccharide **1**, molecular dynamics simulation studies in

conjugation with NMR spectroscopic analysis was carried out. The NOE-based NOESY or ROESY NMR experiments provide valuable insight on the conformation of the molecule.[23] The information as perceived from the NMR spectroscopy was used to access the conformational information of the tetrasaccharide **1** using the molecular dynamics simulation analysis. The one- and two-dimensional NMR spectra of tetrasaccharide **1** showed severe signal overlap, which can be attributed to the similar type of chemical as well as electronic environment of the sugar protons. However, certain NOEs have been identified in the 2D ROESY spectrum (supporting information), which were well correlated with the theoretical study by computing the respective proton-proton distances. It is worth mentioning that the inter-glycosidic proton-proton distances were mainly focused during the study. The trajectory analysis (Supporting information Figure 1) of the tetrasaccharide **1** revealed that the distance informations were rigid for H-1_D/H-3_C, H-1_B/H-2_A and H-1_C/H-2_B distances. Interestingly the proton-proton distance between H-1_A/-OCH_2-H also showed similar value ranging within 2.0-3.2Å. On the contrary, the H-2_A/H-2_B showed a varying range from 3.3-4.4Å. This implies that the inter-glycosidic linkage between α-L-rhamnose moiety **A** and α-L-rhamnose moiety **B** is comparatively flexible. The overview of the conformational state of the tetrasaccharide **1** was visualized based on the snapshots taken at 3.0 ns intervals and it was found that the molecular conformations were similar and rigid (Figure 2). This rigidity has also been elucidated on the basis of the ensemble structures (shown at the lower panel of Figure 2). The ensemble structures revealed that the backbone and carbohydrate rings were rigid throughout the simulation time scale. The torsional angle (oligosaccharide dihedral angle) variations, accounted for the φ_n (H_n-C_n-O_n-C_{n+1}) and ψ_n (C_n-O_n-C_{n+1}-H_{n+1}) also revealed similar information regarding the conformational rigidity (Figure 3). The torsional variation between rings (α-L-rhamnose moiety **A**)-(α-L-rhamnose moiety **B**) and rings (α-L-rhamnose moiety **B**)-(β-D-glucosamine moiety **C**) seemed to be almost similar within a range of + 100° to − 50°. Comparatively the variation between (β-D-glucosamine moiety **C**)-(α-D-galactose moiety **D**) were more rigid and majority of the conformations were within the range of + 25° to − 70°. The root-mean-squared-deviation (RMSD) plot with respect to the individual carbohydrate rings also showed constant deviations (Supporting information Figure 2) within a minimal range of 0.25Å to 1.5Å. The RMSD value calculated for the ensemble structures (Figure 2) showed a maximum deviation of 2.75Å only corresponding to the 3 ns snapshot. It should be noted that the conformational deviations within a range of 4Å can be considered similar with respect to the bio-molecular structure and functions. Hence the obtained conformation for the tetrasaccharide **1** in solvent system can be referred to as stable and rigid. Furthermore, the energetics of the tetrasaccharide **1** was also accounted in our theoretical studies which gave precise information regarding the Coulombic and van der Waals (vdW) contribution (Supporting information Figure 3). The Coulombic contribution for the tetrasaccharide **1** (solute) was found to possess a mean value of 230.94 kcal/mol and that for the contribution of water molecules (solvent) have a mean value of −144.26 kcal/mol. These values indicated that the conformation of the tetrasaccharide **1** was far more stable owing to the solvent perturbation. A similar assumption was achieved where the solute contribution showed a fluctuation in the energetics with mean value of 9.69 kcal/mol and that of the solvent contribution have a value of −26.05 kcal/mol. All these values indicated that the solvent contribution favored the energy state of the tetrasaccharide **1** and thus the conformations induced by the solvent-perturbation were found quite stable.

Conclusions

In summary, synthesis of the tetrasaccharide repeating unit of the cell wall *O*-antigen of *Escherichia coli* O69 strain has been accomplished as its 2-aminoethyl glycoside in satisfactory yield using a synthetic strategy consisting of two stereoselective iterative glycosylations in one-pot. The synthesized tetrasaccharide (**1**) was subjected to the conformational analysis using 2D NOE based NMR spectral analysis and molecular dynamics simulation study. It was found that the solvent-perturbation-induced conformations of the tetrasaccharide **1** were significantly stable in solution.

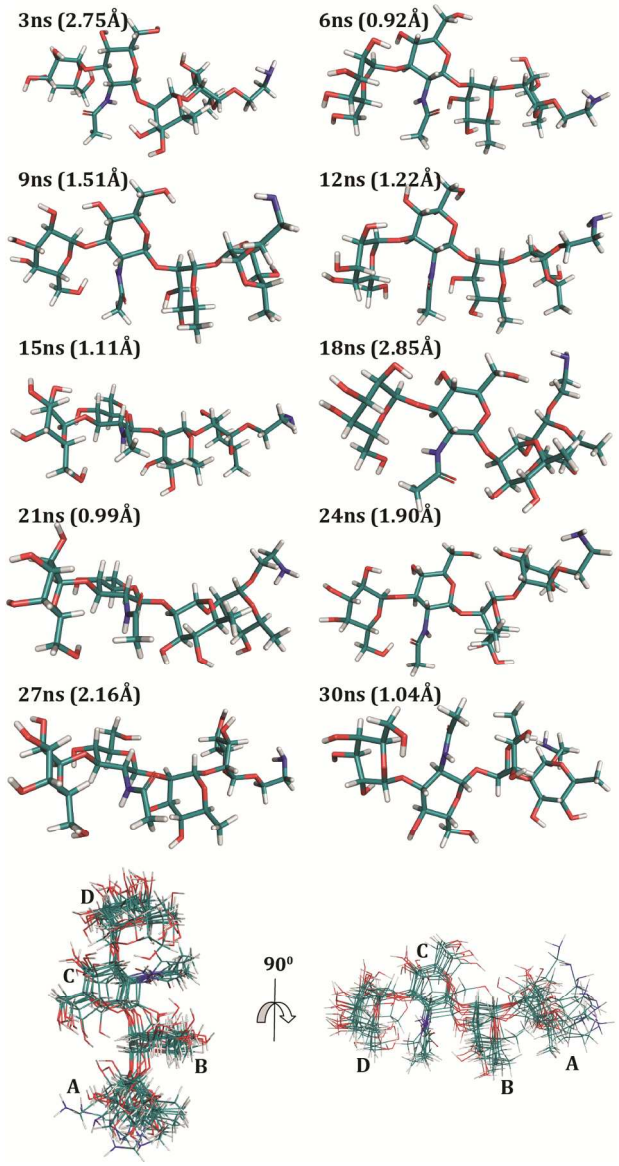


Figure 2: Conformational snapshot of tetrasaccharide **1** adopted from equal interval of the molecular dynamics simulation trajectory. The respective time point has been indicated with each conformational state. Value of RMSD (given in parenthesis) has been calculated with respect to starting structure (0 ns). Ensemble of tetrasaccharide **1** has been shown in the lower panel with 90 ° orientation.

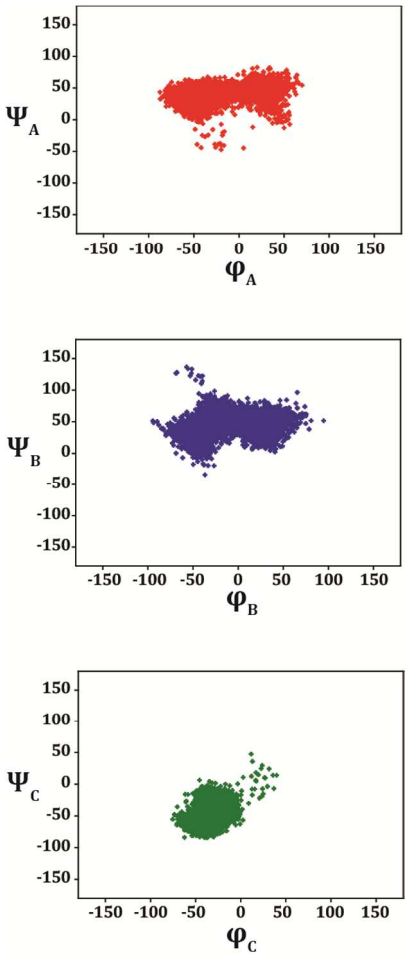


Figure 3: Account of the oligosaccharide dihedral angles between various rings (A, B, C) of tetrasaccharide **1**.

Experimental

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 2N 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, 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 2K 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, 4K (t2) × 1K (t1) data matrices were obtained. 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.

2-(CARBOBENZYLOXY)AMINOETHYL 3,4-DI-*O*-BENZYL-α-L-RHAMNOPYRANOSIDE (3): To a solution of compound **2** (1.5 g, 3.48 mmol) and 2-(carbobenzyloxy)amino ethanol (1.4 g, 7.17 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS 4Å (1 g) and the reaction mixture was cooled to – 25 °C under argon. To the cooled reaction mixture were added NIS (0.9 g, 4.0 mmol) and TMSOTf (20 µL) and the reaction was allowed to stir at same temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and successively washed with 5% aq. $Na_2S_2O_3$ and water, dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) to give pure product (1.6 g, 81%). A solution of the product in CH_3ONa (20 mL; 0.1 M solution in CH_3OH) was stirred at room temperature for 2 h. The reaction mixture was neutralized with Dowex 50W X8 (H^+) resin, filtered and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (3:1) to give pure compound **3** (1.4 g, 95%). Colorless oil; $[\alpha]_D^{25}$ – 18 (*c* 1.0, $CHCl_3$); IR (neat): 3445, 3066, 3015, 2922, 1713, 1634, 1515, 1454, 1364, 1216, 1066, 1028, 910, 755, 698, 667 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33-7.26 (m, 15 H, Ar-H), 5.10 (br s, 2 H, PhCH_2), 5.01 (br s, 1 H, N*H*), 4.87 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 4.78 (br s, 1 H, H-1$_A$), 4.68 (br s, 2 H, PhCH_2), 4.64 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 3.97 (s, 1 H, H-2$_A$), 3.79 (dd, *J* = 9.0 Hz, 3.0 Hz, 1 H, H-3$_A$), 3.75-3.70 (m, 1 H, OCH_2), 3.68-3.64 (m, 1 H, H-5$_A$), 3.56-3.48 (d, *J* = 10.0 Hz, 1 H, OCH_2), 3.45-3.40 (m, 2 H, H-4$_A$, NCH_2), 3.38-3.32 (m, 1 H, NCH_2), 1.32 (d, *J* = 6.0 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.8 (Cbz*C*O), 137.8-127.7 (Ar-C), 99.1 (C-1$_A$), 79.9 (C-3$_A$), 79.7 (C-4$_A$), 75.4 (PhCH_2), 72.1 (PhCH_2), 68.4 (C-2$_A$), 67.6 (C-5$_A$), 66.8 (2 C, OCH_2, PhCH_2), 40.8 (NCH_2), 17.9 (CCH_3); ESI-MS: 544.2 [M+Na]$^+$; Anal. Calcd. for $C_{30}H_{35}NO_7$ (521.24): C, 69.08; H, 6.76; found: C, 68.92; H, 6.90.

2-(CARBOBENZYLOXY)AMINOETHYL *O*-(2-*O*-ACETYL-3,4-DI-*O*-BENZYL-α-L-RHAMNOPYRANOSYL)-(1→2)-3,4-DI-*O*-BENZYL-α-L-RHAMNOPYRANOSIDE (6): To a solution of compound **2** (0.9 g, 2.09 mmol) and compound **3** (1 g, 1.92 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS 4Å (1 g) and the reaction mixture was cooled to – 35 °C under argon. To the cooled reaction mixture were added NIS (0.5 g, 2.22 mmol) and TMSOTf (15 µL) and the reaction was allowed to stir at same temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and successively washed with 5% aq. $Na_2S_2O_3$, water, dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) to give pure compound **6** (1.3 g, 76%). Colorless oil; $[\alpha]_D^{25}$ – 15 (*c* 1.0, $CHCl_3$); IR (neat): 3366, 3065, 3030, 2975, 2933, 1723, 1515, 1497, 1454, 1369, 1235, 1063, 1028, 983, 911, 839, 753, 689, 666 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34-7.26 (m, 25 H, Ar-H), 5.51 (br s, 1 H, H-2$_B$), 5.10 (br s, 2 H, PhCH_2), 5.02 (br s, 1 H, N*H*), 4.95 (br s, 1 H, H-1$_A$), 4.92, 4.89 (2 d, *J* = 11.0 Hz each, 2 H, PhCH_2), 4.75 (d, *J* = 11.0 Hz, PhCH_2), 4.70 (br s, 1 H, H-1$_B$), 4.67-4.59 (m, 4 H, PhCH_2), 4.56 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 3.94-3.90 (m, 2 H, H-2$_A$, H-3$_A$), 3.80-3.77 (m, 2 H, H-3$_B$, H-5$_B$), 3.74-3.66 (m, 1 H, OCH_2), 3.65-3.58 (m, 1 H, H-5$_A$), 3.50-3.45 (m, 1 H, OCH_2), 3.44-3.30 (m, 4 H, H-4$_A$, H-4$_B$, NCH_2), 2.14 (s, 3 H, COCH_3), 1.31 (d, *J* = 6.0 Hz, 3 H, CCH_3), 1.27 (d, *J* = 6.5 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ

169.8 (*C*OCH$_3$), 156.2 (Cbz*C*O), 138.4-127.5 (Ar-C), 99.2 (C-1$_A$), 98.9 (C-1$_B$), 79.9 (C- 4$_B$), 79.8 (C-4$_A$), 79.5 (C-3$_B$), 77.6 (C-3$_A$), 75.4 (Ph*C*H$_2$), 75.4 (Ph*C*H$_2$), 74.7 (C-2$_A$), 72.1 (Ph*C*H$_2$), 71.8 (Ph*C*H$_2$), 68.8 (C-2$_B$), 68.2 (2 C, C-5$_A$, C-5$_B$), 66.8 (2 C, O*C*H$_2$, Ph*C*H$_2$), 40.8 (N*C*H$_2$), 21.0 (CO*C*H$_3$), 18.0 (2 C, 2 C*C*H$_3$); ESI-MS: 912.4 [M+Na]$^+$; Anal. Calcd. for $C_{52}H_{59}NO_{12}$ (889.40): C, 70.17; H, 6.68; found: C, 70.00; H, 6.90.

2-(CARBOBENZYLOXY)AMINOETHYL *O*-(3,4-DI-*O*-BENZYL-α-L-RHAMNOPYRANOSYL)-(1→2)-3,4-DI-*O*-BENZYL-α-L-RHAMNOPYRANOSIDE (7): A solution of compound **6** (1.2 g, 1.35 mmol) in CH$_3$ONa (25 mL; 0.1 M solution in CH$_3$OH) was stirred at room temperature for 2 h. The reaction mixture was neutralized with Dowex 50W X8 (H$^+$) resin, filtered and concentrated. The crude product was purified over SiO$_2$ using hexane-EtOAc (3:1) to give pure compound **7** (1.1 g, 96%). Colorless oil; $[\alpha]_D^{25}$ – 14 (*c* 1.0, CHCl$_3$); IR (neat): 3445, 3089, 3065, 3013, 2932, 1952, 1714, 1515, 1497, 1454, 1384, 1362, 1217, 1066, 1028, 986, 911, 755, 689, 667, 628 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.39-7.26 (m, 25 H, Ar-H), 5.12 (br s, 2 H, PhC*H*$_2$), 5.07 (br s, 1 H, H-1$_A$), 5.06 (br s, 1 H, N*H*), 4.91, 4.88 (2 d, *J* = 11.0 Hz each, 2 H, PhC*H*$_2$), 4.73 (br s, 2 H, PhC*H*$_2$), 4.72 (br s, 1 H, H-1$_B$), 4.67-4.61 (m, 4 H, PhC*H*$_2$), 4.13 (br s, 1 H, H-2$_A$), 3.96 (br s, 1 H, H-2$_B$), 3.89 (dd, *J* = 10.0 Hz, 3.0 Hz, H-3$_A$), 3.83-3.79 (m, 2 H, H-3$_B$, H-5$_B$), 3.75-3.68 (m, 1 H, OC*H*$_2$), 3.67-3.62 (m, 1 H, H-5$_A$), 3.51-3.46 (m, 2 H, OC*H*$_2$, H-4$_A$), 3.45-3.35 (m, 3 H, H-4$_B$, NC*H*$_2$), 1.33, 1.32 (2 d, *J* = 6.0 Hz each, 6 H, 2 CC*H*$_3$); ^{13}C NMR (125 MHz, CDCl$_3$): δ 156.2 (Cbz*C*O), 138.4-127.1 (Ar-C), 100.7 (C-1$_A$), 99.1 (C-1$_B$), 80.2 (C-4$_B$), 80.0 (C-4$_A$), 79.5 (2 C, C-3$_B$, C-3$_A$), 75.4 (Ph*C*H$_2$), 75.3 (Ph*C*H$_2$), 74.6 (C-2$_B$), 72.3 (Ph*C*H$_2$), 72.1 (Ph*C*H$_2$), 68.7 (C-2$_A$), 68.2 (C-5$_A$), 68.0 (C-5$_B$), 66.8 (2 C, Ph*C*H$_2$, O*C*H$_2$), 40.8 (N*C*H$_2$), 18.1, 17.9 (2 C, 2 C*C*H$_3$); ESI-MS: 870.3 [M+Na]$^+$; Anal. Calcd. for $C_{50}H_{57}NO_{11}$ (847.39): C, 70.82; H, 6.78; found: C, 70.63; H, 7.00.

2-(CARBOBENZYLOXY)AMINOETHYL *O*-(2,3,4,6-TETRA-*O*-BENZYL-α-D-GALACTOPYRANOSYL)-(1→3)-*O*-(4,6-*O*-BENZYLIDENE-2-DEOXY-2-*N*-PHTHALIMIDO-β-D-GLUCOPYRANOSYL)-(1→2)-*O*-(3,4-DI-*O*-BENZYL-α-L-RHAMNOPYRANOSYL)-(1→2)-3,4-DI-*O*-BENZYL-α-L-RHAMNOPYRANOSIDE (9): To a solution of compound **4** (500 mg, 1.13 mmol) and compound **5** (670 mg, 1.14 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL) was added MS 4Å (1 g) and the reaction mixture was cooled to – 15 °C under argon. To the cooled reaction mixture were added NIS (260 mg, 1.15 mmol) and TMSOTf (5 µL) and the reaction was allowed to stir at same temperature for 1 h. The complete consumption of the starting materials and formation of a new compound was confirmed by the TLC (hexane-EtOAc 2:1) analysis. To the reaction mixture was added compound **7** (670 mg, 0.79 mmol) followed by another portion of NIS (180 mg, 0.80 mmol) and it was allowed to stir at same temperature for another 1 h. Because of the presence of TMSOTf already in the reaction mixture, further addition of it was not required. The reaction mixture was diluted with CH$_2$Cl$_2$ (100 mL) and successively washed with 5% aq. Na$_2$S$_2$O$_3$, water, dried (Na$_2$SO$_4$) and concentrated. The crude product was purified over SiO$_2$ using hexane-EtOAc (3:1) to give pure compound **9** (1.2 g, 61%). Colorless oil; $[\alpha]_D^{25}$ + 12 (*c* 1.0, CHCl$_3$); IR (neat): 3417, 3089, 3065, 3013, 2870, 1952, 1714, 1610, 1516, 1497, 1454, 1385, 1362, 1216, 1099, 1066, 1028, 986, 911, 755, 719, 689, 667, 628 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.80-6.81 (m, 54 H, Ar-H), 5.53 (d, *J* = 3.0 Hz, 1 H, H-1$_D$), 5.30 (d, *J* = 8.5 Hz, 1 H, H-1$_C$), 5.21 (s, 1 H, PhC*H*), 5.09 (br s, 2 H, PhC*H*$_2$), 4.97-4.96 (m, 1 H, H-3$_C$), 4.87 (br s, 1 H, H-1$_A$), 4.77-4.71 (m, 4 H, PhC*H*$_2$), 4.60-4.58 (m, 3 H, PhC*H*$_2$), 4.56 (br s, 1 H, H-1$_B$), 4.52-4.40 (m, 5 H, H-2$_C$, PhC*H*$_2$), 4.28-4.25 (m, 3 H, PhC*H*$_2$), 4.08-4.02 (m, 2 H, PhC*H*$_2$), 3.92-3.83 (m, 4 H, H-2$_B$, H-2$_D$, H-4$_D$), 3.81-3.73 (m, 4 H, H-3$_B$, H-4$_C$, H-5$_B$, H-5$_D$), 3.68-3.64 (m, 2 H, H-2$_A$, H-5$_C$), 3.57-3.48 (m, 4 H, H-3$_A$, H-3$_D$, OC*H*$_2$), 3.47-3.25 (m, 6 H, H-5$_A$, H-6$_{abD}$, H-6$_{aC}$, NC*H*$_2$), 3.16-3.08 (2 t, *J* = 9.0 Hz, 2 H, H-4$_A$, H-4$_B$), 2.95-2.92 (m, 1 H, H-6$_{bC}$,), 1.20-1.19 (2 d, *J* = 6.0 Hz each, 6 H, 2 CC*H*$_3$); ^{13}C NMR (125 MHz, CDCl$_3$): δ 167.8, 167.4 (Phth*C*O), 159.6 (Cbz*C*O), 138.7-113.6 (Ar-C), 101.6 (Ph*C*H), 101.1 (C-1$_C$), 100.6 (C-1$_A$), 98.9 (C-1$_B$), 97.2 (C-1$_D$), 82.8 (C-2$_D$), 80.5 (C-4$_B$), 80.3 (C-4$_A$), 78.9 (C-3$_A$), 78.8 (C-3$_B$), 77.8 (C-3$_D$), 77.7 (C-5$_C$), 75.7 (C-5$_D$), 75.5 (C-4$_D$), 75.3 (PhCH$_2$), 74.9 (Ph*C*H$_2$), 74.8 (C-2$_B$), 74.7 (Ph*C*H$_2$), 73.0 (Ph*C*H$_2$), 72.7 (Ph*C*H$_2$), 72.6 (Ph*C*H$_2$), 72.3 (C-3$_C$), 71.8 (Ph*C*H$_2$), 71.4 (Ph*C*H$_2$), 69.2 (C-4$_C$), 68.5 (2 C, C-2$_A$, C-6$_C$), 67.9 (2 C, C-5$_A$, C-6$_D$), 66.7 (2 C, Ph*C*H$_2$, O*C*H$_2$), 65.4 (C-5$_B$), 55.4 (C-2$_C$), 40.8 (N*C*H$_2$), 18.0, 17.7 (2 C, 2 C*C*H$_3$); MALDI-MS: 1771.7 [M+Na]$^+$; Anal. Calcd. for $C_{105}H_{108}N_2O_{22}$ (1748.74): C, 72.06; H, 6.22; found: C, 71.89; H, 6.40.

2-AMINOETHYL *O*-(α-D-GALACTOPYRANOSYL)-(1→3)-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→2)-*O*-(α-L-RHAMNOPYRANOSYL)-(1→2)-α-L-RHAMNOPYRANOSIDE (1): To a solution of compound **9** (1 g, 0.57 mmol) in EtOH (25 mL) was added $NH_2NH_2{\cdot}H_2O$ (0.5 mL) and reaction mixture was allowed to stir at 90 °C for 5 h. The solvents were removed under reduced pressure and a solution of the crude product in acetic anhydride and pyridine (5 mL, 1:1 v/v) was kept at room temperature for 1 h. The solvents were removed under pressure and the crude product was passed through a short pad of SiO$_2$ using hexane-EtOAc (1:1) as eluent. To a solution of the *N*-acetylated product in CH$_3$OH (10 mL) was added 20% Pd(OH)$_2$-C (150 mg) and Et$_3$SiH (4 mL, 25.04 mmol) and the reaction mixture was allowed to stir at room temperature for 14 h. The reaction mixture was filtered through a Celite bed and the filtering bed was washed with CH$_3$OH-H$_2$O (2:1). The filtrate was concentrated under reduced pressure and the product was passed through a Sephadex LH-20 column using CH$_3$OH-H$_2$O (3:1) as eluent to furnish pure compound **1** (260 mg, 63%). White powder; $[\alpha]_D^{25}$ – 10 (*c* 0.5, H$_2$O); IR (neat): ^{1}H NMR (500 MHz, D$_2$O): δ 5.32 (br s, 1 H, H-1$_D$), 5.04 (br s, 1 H, H-1$_B$), 4.82 (br s, 1 H, H-1$_A$), 4.64 (d, *J* = 7.5 Hz, 1 H, H-1$_C$), 4.05 (br s, 1 H, H-2$_B$), 3.92-3.84 (m, 3 H, H-2$_A$, H-3$_D$, OC*H*$_2$), 3.82-3.75 (m, 4 H, H-3$_A$, H-5$_C$, H-6$_{abC}$), 3.73-3.67 (m, 3 H, H-2$_C$, H-2$_D$, H-3$_B$), 3.66-3.57 (m, 8 H, H-3$_C$, H-4$_B$, H-4$_C$, H-4$_D$, H-5$_D$, H-6$_{abD}$, OC*H*$_2$), 3.40-3.36 (m, 2 H, H-5$_A$, H-5$_B$), 3.24-3.21 (m, 1 H, H-4$_A$), 3.18-3.13 (m, 2 H, NC*H*$_2$), 1.96 (s, 3 H, COC*H*$_3$), 1.20, 1.16 (2 d, *J* = 6.0 Hz each, 6 H, 2 CC*H*$_3$); ^{13}C NMR (125 MHz, D$_2$O): δ 174.6 (*C*OCH$_3$), 102.4 (C-1$_C$), 101.0 (C-1$_B$), 99.0 (C-1$_D$), 98.5 (C-1$_A$), 79.2 (C-3$_C$), 78.7 (2 C, C-2$_A$, C-3$_D$), 75.3 (C-5$_A$), 72.2 (C-4$_C$), 71.9 (C-5$_B$), 70.8 (C-3$_B$), 70.5 (C-5$_C$), 69.7 (C-4$_A$), 69.5 (C-4$_B$),

69.1 (2 C, C-2_B, C-5_D), 69.0 (C-3_A), 68.9 (C-4_D), 68.3 (C-2_D), 63.4 (OCH_2), 60.5 (C-6_D), 60.3 (C-6_C), 54.3 (C-2_C), 39.0 (NCH_2), 22.4 ($COCH_3$), 16.6 (2 C, 2 CCH_3); ESI-MS: 741.3 $[M+Na]^+$; Anal. Calcd. for $C_{28}H_{50}N_2O_{19}$ (718.30): C, 46.79; H, 7.01; found: C, 46.60; H, 7.22.

Computational Details: The three dimensional structure for the tetrasaccharide **1** was sketched in the maestro workspace of Schrodinger suite.[24] Requisite precautions were taken for preserving the relevant α/β conformational forms, isomeric forms L/D and the ring type such as furanose/pyranose type of sugar rings. Finally, the structure was cleaned for all the bond lengths, angles and dihedral parameter optimization with energy minimization according to the conjugate gradient algorithm. OPLS_2005 force field was adopted for all the conformational sampling procedure.[25] The prepared structure was then solvated in an orthorhombic box with a maximum distance of 10Å edge distance from solute atoms.[26] A cutoff distance value of 9Å was taken into consideration on the account of the non-bonded interaction. SHAKE algorithm was used to restrain appropriate bond length of the hydrogen bonds with an integration time step of 2 fs.[27] The initial minimization and equilibrium steps were preceded as per the default parameter set-up values in Desmond.[24] A production run of 30 ns time scale was carried out as per NPT micro-canonical ensemble (constant temperature-300K and constant pressure-1atm). Trajectory was saved at an interval of 4 ps and all analysis were performed using the Simulation-Even-Analysis module.[28]

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Notes and references

[a] *Bose Institute, Division of Molecular Medicine, P-1/12, C.I.T. Scheme VII-M, Kolkata-700054, India; Fax: 91-33-2355 3886; Tel: 91 33-2569 3240; E-mail: akmisra69@gmail.com*

[b] *Bose Institute, Department of Biophysics, P-1/12, C.I.T. Scheme VII-M, Kolkata-700054, India.*

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