EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201402392

Convergent Synthesis and Conformational Analysis of the Hexasaccharide Repeating Unit of the *O*-Antigen of *Shigella flexneri* Serotype 1d

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Keywords: Synthetic methods / Carbohydrates / Oligosaccharides / Glycosylation / Conformation analysis / Molecular dynamics

A hexasaccharide repeating unit of the *O*-antigen of the cell wall of *Shigella flexneri* type 1d has been synthesized using a stereoselective [3+3] block glycosylation approach. Recently developed glycosylation conditions were used in the synthesis. A thioglycoside was used as an orthogonal glycosyl donor during the synthesis. The synthesized hexasaccharide was subjected to detailed NMR spectroscopic and molecular modeling studies to determine its conformational behavior in water. The NOE-based two-dimensional NOSEY experiment and all-atom explicit molecular dynamics (MD) simulation studies suggest that the oligosaccharide is not very flexible, that it remains rigid with respect to the glycosidic linkages between the sugar moieties.

Introduction

Shigellosis is a serious health concern, and is caused by *Shigella* infections.[1] *Shigella* is a major cause of diarrheal outbreaks worldwide that cause millions of deaths annually.[2] *Shigella* is a well-studied human pathogen associated with dysentery, which is diagnosed by bloody diarrhea, abdominal cramps, fever, etc.[3] *Shigella* are mainly divided into four species, which are *Shigella dysenteriae* (group A), *Shigella flexneri* (group B), *Shigella boydii* (group C), and *Shigella sonnei* (group D).[4] These species are further classified into several serotypes on the basis of their *O*-antigen structures. In general, *Shigella O*-antigens are acidic in nature (with some exceptions), as they contain acidic sugars or functional groups.[5] Since cell-wall *O*-antigens are the main virulence factors for *Shigella* infections, several *O*-antigenic structures related to different strains of *Shigella* have been isolated and characterized to date.[5] Recently, the structure of the repeating unit of the cell-wall *O*-antigen of *Shigella flexneri* serotype 1d, isolated from diarrheal patients in China,[6] was reported by Shashkov et al.[7] The repeating unit consists of a neutral oligosaccharide with a linear backbone composed of L-rhamnose moieties, with a branch at the reducing end.

A recent thrust in drug discovery has been towards the development of drugs relying on alternative mechanisms for controlling bacterial infections, because of the emergence of multi-drug-resistant bacteria.[8] Similarly to other bacterial infections, the development of therapeutics against drug-resistant *Shigella* strains is also highly desirable. As an alternative to small-molecule therapeutics, the development of glycoconjugate vaccines based on the cell-wall *O*-antigen of *Shigella* species has been attempted in the recent past.[9–12] However, extraction from the living organism of the oligosaccharides required for the preparation of glycoconjugate derivatives is quite tedious, and does not provide sufficient quantities of material for comprehensive biological studies. Besides, the isolation of oligosaccharides without biological contamination is quite challenging. Therefore, it is often preferable or mandatory to find solutions using synthetic organic chemistry.[13,14] In this context, a convergent synthesis of the hexasaccharide repeating unit of the *O*-antigen of *Shigella flexneri* serotype 1d is reported in this paper as its 2-aminoethyl glycoside. To understand the conformational behavior of the hexasaccharide repeating unit in water, a detailed NMR spectroscopic and molecular modeling study of the synthesized hexasaccharide was undertaken, and these results are also presented.

Results and Discussion

The target 2-aminoethyl-substituted hexasaccharide **1** was synthesized using a convergent [3+3] block glycosylation strategy. For the construction of the target molecule, suitably derivatized monosaccharide intermediates **5**, **6**,[15] **7**, **8**,[16] **9**,[17] and **10**[18] were prepared from commercially available reducing sugars using earlier reported reaction

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201402392.

conditions (Figure 1). A regioselective reaction was used for the preparation of trisaccharide acceptor **12**. For this, recently developed glycosylation conditions using catalytic nitrosyl tetrafluoborate ($NOBF_4$)[19] were used to activate glycosyl trichloroacetimidate derivative **8**, and this was followed by iodonium-ion-promoted glycosylation using thioglycoside **7**, and removal of the PMB (*p*-methoxybenzyl) ether in one pot[20] to give **12** in a minimum number of reaction steps. The preparation of trisaccharide thioglycoside donor **15** was achieved using a high-yielding $NOBF_4$-catalyzed orthogonal glycosylation[21] of L-rhamnosyl thioglycoside acceptor **6** with D-glucopyranosyl trichloroacetimidate donor **9**. The key features of our synthetic strategy are the use of an in-situ-removable protecting group (PMB ether), a minimum number of steps, and convergent block glycosylations. The presence of the anomeric 2-aminoethyl linker could be useful for the preparation of glycoconjugate derivatives by providing ready access to an amino group for linking with a protein.

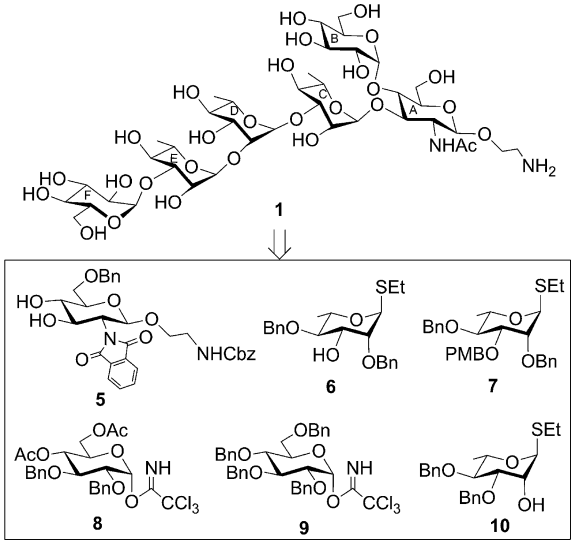
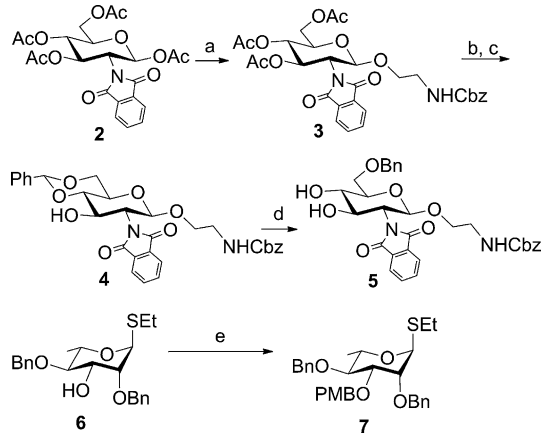


Figure 1. Structures of the hexasaccharide repeating unit and its synthetic intermediates.

Reaction of 1,2,3,4-tetra-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranose (**2**)[22] with 2-*N*-carboxybenzylamino ethanol in the presence of boron trifluoride–diethyl ether ($BF_3{\cdot}OEt_2$) at elevated temperature gave compound **3** in 76% yield. Removal of the *O*-acetyl groups using sodium methoxide,[23] followed by benzylidene acetal formation[24] using benzaldehyde dimethyl acetal in the presence of *p*TsOH gave compound **4** in 77% yield. Regioselective ring opening[25] of the benzylidene acetal in compound **4** using a combination of triethylsilane and molecular iodine gave compound **5** in 72% yield. In another experiment, ethyl 2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**6**)[15] was treated with *p*-methoxybenzyl chloride in the presence of sodium hydroxide[26] to give thioglycoside derivative **7** in 88% yield (Scheme 1).

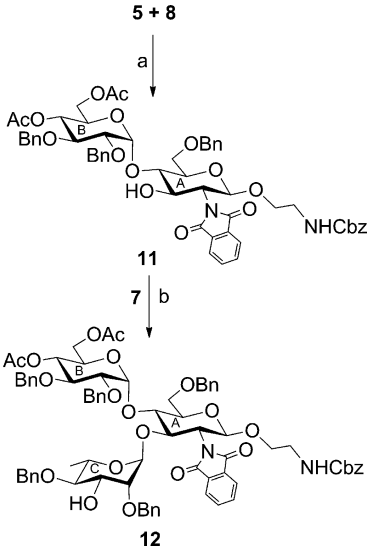
Stereo- and regioselective glycosylation of D-glucosamine diol acceptor **5** with D-glucose trichloroacetimidate derivative **8** in the presence of $NOBF_4$[19] in a CH_2Cl_2/Et_2O (1:2)



Scheme 1. Reagents: (a) $HO(CH_2)_2NHCbz$ (Cbz = benzyloxycarbonyl), $BF_3{\cdot}OEt_2$, $(CH_2Cl)_2$, 55 °C, 16 h, 76%; (b) CH_3ONa (0.05 M), CH_3OH, room temperature, 1 h; (c) $PhCH(OCH_3)_2$, *p*TsOH, DMF, room temperature, 12 h, 77% over two steps; (d) Et_3SiH, I_2, CH_3CN, 5 °C, 30 min, 72%; (e) *p*-methoxybenzyl chloride, NaOH, DMF, room temperature, 3 h, 88%.

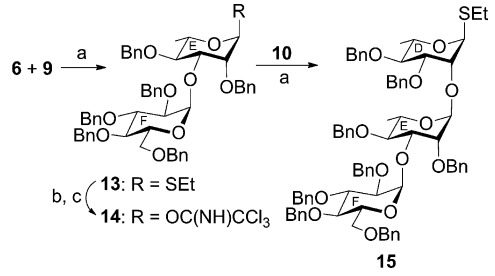
solvent mixture gave disaccharide acceptor **11** in 73% yield, together with a minor quantity (about 7%) of its regioisomer, which was removed by column chromatography. The regioselective formation of compound **11** was confirmed by analysis of its spectra [signals at δ = 5.10 (d, J = 8.0 Hz, 1_A-H), 4.62 (br. s, 1_B-H) in the ^1H NMR spectrum, and δ = 101.7 (C-1_B), 98.7 (C-1_A) in the ^{13}C NMR spectrum]. Regioselective 4-OH glycosylation of 2-deoxy-2-*N*-phthalimido-D-glucosamine 3,4-diol derivative has also been observed earlier.[27] Stereoselective glycosylation of compound **11** with L-rhamnosyl thioglycoside donor **7** in the presence of a combination of *N*-iodosuccinimide (NIS) and triflic acid (TfOH)[28,29] in CH_2Cl_2/Et_2O (2:1) followed by in situ removal[20] of the PMB group from the glycosylated product in one pot by raising the reaction temperature gave trisaccharide acceptor **12** in 71% yield. Analysis of the spectra of compound **12** confirmed its formation [signals at δ = 5.43 (d, J = 3.0 Hz, 1_B-H), 5.25 (br. s, 1_C-H), 5.20 (d, J = 8.5 Hz, 1_A-H) in the ^1H NMR spectrum, and δ = 98.1 (C-1_A), 96.2 (C-1_C), 95.4 (C-1_B) in the ^{13}C NMR spectrum]. The $J_{C\text{-}1,1\text{-}H}$ coupling constants of 154.0, 165.0, and 171.0 Hz in the ^1H-coupled ^{13}C NMR spectrum confirmed the presence of two α glycosidic linkages and one β linkage[30,31] in compound **12** (Scheme 2).

In another experiment, stereoselective 1,2-*cis* glycosylation of L-rhamnosyl thioglycoside acceptor **6** with D-glucose-derived trichloroacetimidate derivative **9** in the presence of $NOBF_4$[19] in CH_2Cl_2/Et_2O (2:1) gave disaccharide thioglycoside derivative **13** in 74% yield. This reaction relies on the orthogonal properties[21] of the anomeric thioethyl group in compound **6**. Spectral analysis of compound **13** confirmed its formation [signals at δ = 5.25 (d, J = 1.5 Hz, 1_E-H), 5.16 (d, J = 3.5 Hz, 1_F-H) in the ^1H NMR spectrum, and δ = 94.5 (C-1_F), 82.2 (C-1_E) in the ^{13}C NMR spectrum]. The anomeric thioethyl group in compound **13** was removed by treatment with *N*-bromosuccinimide (NBS)[32] to give the disaccharide hemiacetal derivative, which, on treat-



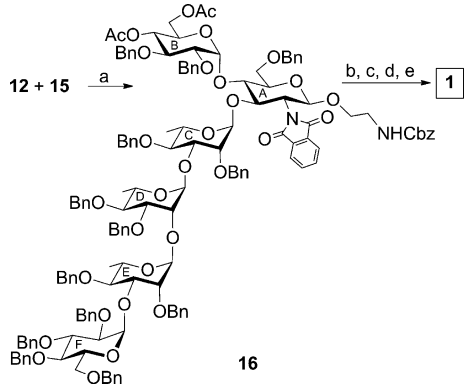
Scheme 2. Reagents: (a) $NOBF_4$, CH_2Cl_2/Et_2O (1:2), –10 °C, 30 min, 73%; (b) NIS, TfOH, CH_2Cl_2/Et_2O (2:1), MS (4 Å), –25 °C, 30 min, then 0–5 °C, 30 min, 71%.

ment with trichloroacetonitrile[33] in the presence of DBU (1,8-diazabicycloundec-7-ene), gave compound **14** in 72% yield. This compound was used immediately in the next glycosylation reaction. $NOBF_4$-catalyzed stereoselective α-glycosylation[19] of disaccharide trichloroacetimidate donor **14** with thioglycoside acceptor **10** in a CH_2Cl_2/Et_2O (1:2) solvent mixture gave trisaccharide thioglycoside derivative **15** in 70% yield. The presence of characteristic signals of compound **15** in the ^1H and ^{13}C NMR spectra, and the $J_{C\text{-}1,1\text{-}H}$ coupling constants (165.0, 171.0, and 171.5 Hz)[30,31] in the gated ^1H-coupled ^{13}C NMR spectrum confirmed its formation [δ = 5.25 (d, J = 3.0 Hz, 1_F-H), 5.21 (br. s, 1_E-H), 5.13 (br. s, 1_D-H) in the ^1H NMR spectrum, and δ = 99.8 (C-1_D), 94.6 (C-1_F), 83.7 (C-1_E) in the ^{13}C NMR spectrum] (Scheme 3).



Scheme 3. Reagents: (a) $NOBF_4$, CH_2Cl_2/Et_2O (1:2), –10 °C, 30 min, 74% for compound **13**, and 70% for compound **15**; (b) *N*-bromosuccinimide (NBS) acetone/H_2O (9:1), room temperature, 40 min; (c) CCl_3CN, DBU, CH_2Cl_2, –10 °C, 1 h, 72% over two steps.

Finally, iodonium-ion-catalyzed stereoselective convergent [3+3] α-glycosylation of trisaccharide thioglycoside donor **15** with trisaccharide acceptor **12** in the presence of a combination of NIS and TfOH[28,29] in CH_2Cl_2/Et_2O (1:1) gave branched hexasaccharide derivative **16** in 69% yield. Spectral analysis confirmed the exclusive formation of compound **16** under these reaction conditions. Signals in the ^1H and ^{13}C NMR spectra [δ = 5.72 (br. s, 1_F-H), 5.21 (d, J = 8.0 Hz, 1_A-H), 5.20–5.18 (1_B-H, 1_C-H, 1_E-H), 5.04 (br. s, 1_D-H) in the ^1H NMR spectrum, and δ = 99.9 (C-1_D), 97.9 (2 C, C-1_A, C-1_C), 97.2 (C-1_E), 94.6 (C-1_B), 94.5 (C-1_F) in the ^{13}C NMR spectrum] and the $J_{C\text{-}1,1\text{-}H}$ coupling constants[30,31] in the gated ^1H-coupled ^{13}C NMR spectrum (154.0, 165.0, 171.0, 165.0, 171.5, and 171.5 Hz) confirmed the presence of five α glycosidic linkages and one β linkage. Compound **16** was subjected to a sequence of deprotection reactions comprising (a) removal of the *N*-phthaloyl group using ethylenediamine,[34] followed by acetylation using acetic anhydride and pyridine; (b) catalytic transfer hydrogenation[35] using a combination of triethylsilane and Pd/C (10%) for the removal of the benzyl ethers and the benzyloxycarbonyl (Cbz) protecting group, and (c) removal of the *O*-acetyl groups using sodium methoxide, to give deprotected hexasaccharide **1** as its 2-aminoethyl glycoside in 54% overall yield. The product was passed through a column of LH-20 Sephadex using CH_3OH/H_2O (4:1) as eluent to give pure compound **1**. Spectral analysis of compound **1** unambiguously confirmed its formation [δ = 5.23 (d, J = 3.0 Hz, 1_B-H), 5.12 (br. s, 1_D-H), 5.03 (br. s, 1_C-H), 5.00 (d, J = 3.0 Hz, 1_F-H), 4.91 (br. s, 1_E-H), 4.49 (d, J = 7.5 Hz, 1_A-H) in the ^1H NMR spectrum, and δ = 101.9 (C-1_E), 100.7 (C-1_C), 100.6 (2 C, C-1_A, C-1_D), 97.7 (C-1_B), 95.3 (C-1_F) in the ^{13}C NMR spectrum] (Scheme 4).



Scheme 4. Reagents: (a) NIS, TfOH, CH_2Cl_2/Et_2O (1:1), MS (4 Å), –25 °C, 30 min, 69%; (b) ethylenediamine, *n*BuOH, 90 °C, 8 h; (c) acetic anhydride, pyridine, room temperature, 2 h; (d) Et_3SiH, Pd/C (10%), CH_3OH/CH_2Cl_2 (9:1), room temperature, 10 h; (e) CH_3ONa (0.1 M), CH_3OH, room temperature, 3 h, 54% over four steps.

Conformational Analysis

Molecular dynamics (MD) simulation in combination with NOE-based NOESY or ROESY NMR experiments can provide the conformations of a molecule in solution. One- and two-dimensional homonuclear NMR spectra, such as the ^1H NMR and ^1H–^1H NOESY/TOCSY spectra, of hexasaccharide **1** showed severe signal overlap, due to the similar chemical and electronic environments of the

carbohydrate protons (see Supporting Information, Figure S1). In order to explore the conformational behavior of hexasaccharide **1** in solution, an all-atoms explicit MD simulation was performed for a time period of 30 ns. The initial conformational states for hexasaccharide **1** were evaluated by examining proton–proton distances at the inter-glycosidic linkages. The proton–proton distance as analyzed from the trajectory was found to be very rigid for 3_A-H/1_C-H, 2_C-H/1_D-H, and 2_D-H/1_F-H which were all within a range of 2–4 Å (see Supporting Information, Figure S2). The fact that such a confined distance was observed throughout the time of the simulation reveals that the compound has a rigid conformation with respect to these glycosidic linkages. The distance for 2_C-H/1_D-H was found to be dynamic (see Supporting Information, Figure S2A), which suggests a greater degree of freedom with respect to the linkage between the β-D-glucosamine moiety (A) and the α-D-glucose moiety (B). On the other hand, a very small deviation for the proton–proton distance of 2_E-H/1_F-H was found, which cannot be accounted for by a conformationally flexible linkage between the α-L-rhamnose moiety (E) and the α-D-glucose moiety (F) (see Supporting Information, Figure S2B). This result is in good agreement with the NOESY spectrum of hexasaccharide **1**, where no significant NOE was observed between the α-D-glucose moiety (B) and the α-L-rhamnose moiety (C) (see Supporting Information, Figure S1). In another approach, the torsional angles were measured using the definition of the oligosaccharide dihedral angles as ϕ_n (H_n–C_n–O_n–C_{n+1}) and ψ_n (C_n–O_n–C_{n+1}–H_{n+1}). The torsional distributions for the respective moieties were found to be well conserved and within the conventional range of oligosaccharides, except for the α-D-glucose moiety (B) (see Supporting Information, Figure S3). The ϕ angle distribution (H_A–C_A–O_B–C_B) was found to be more flexible than the ψ angle (C_A–O_B–C_B–H_B), which seems to be in the range –50° to +50°. In a precise search of the conformational forms that are possible in the solution state, cluster analysis based on root-mean-square deviation (RMSD) of trajectory frames was attempted. The cluster analysis plot (Figure 2) is based on the average method of hierarchical cluster linkage, using 2 Å as the merging distance cut-off, and it is presented as a heat map with respect to hexasaccharide **1** conformations in the time of the MD simulation. The maximum numbers of populations were found to be in the range of 2–3.5 Å in comparison to the initial frame of reference. This suggests that the oligosaccharide conformation is not very flexible, that it remains rigid with respect to the glycosidic linkages between the sugar moieties. The energetics of hexasaccharide **1** in the solution state were also theoretically evaluated to get an account of the Coulombic and van der Waals (vdW) contributions, and the perturbations in these contributions over the different conformations. The Coulomb energy of hexasaccharide **1** (solute molecule) was found to be approximately 400 kcal mol^{-1}, whereas the Coulombic energy of hexasaccharide **1** owing to interaction with solvent was found to be energetically favorable (–200 kcal mol^{-1}) (see Supporting Information, Figure S4). A similar favorable energy state was also found for the vdW contribution, where the solvent energy is energetically stable in comparison to the solute itself. These results indicate that the solution-state conformations of hexasaccharide **1** are energetically favorable, and hence the solvent-perturbation-induced conformations are very stable. A simple overview of the computed conformational states of hexasaccharide **1** are shown in Figure 3 as an ensemble structure. The rigidity of the backbone for the α-L-rhamnose moiety (C), the α-L-rhamnose moiety (D), and the α-L-rhamnose moiety (E) can be observed, and the relative flexibility of the β-D-glucosamine moiety (A), the α-D-glucose moiety (B), and the α-D-glucose moiety (F) can also be observed. Interestingly the flexibility of the glycosidic linkage between the α-L-rhamnose moiety (C) and the α-L-rhamnose moiety (D) is well correlated with the weak NOE cross-peak in the NOESY spectrum (see Supporting Information, Figure S1).

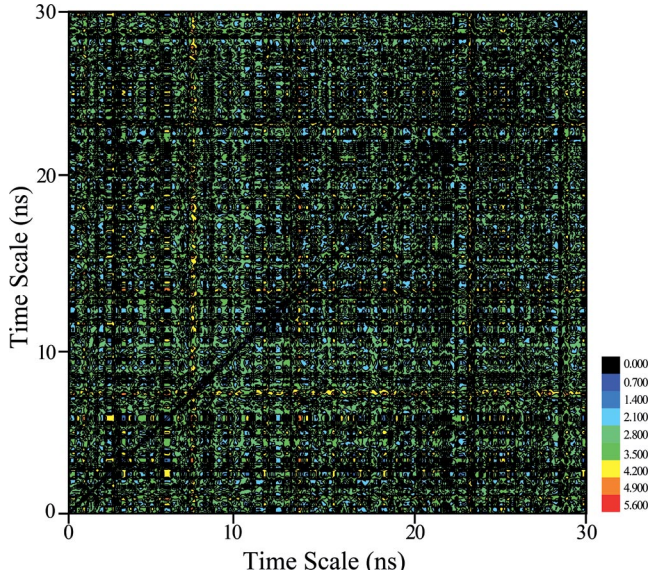


Figure 2. Hierarchical cluster analysis of conformations of hexasaccharide **1**, based on the MD simulation trajectory.

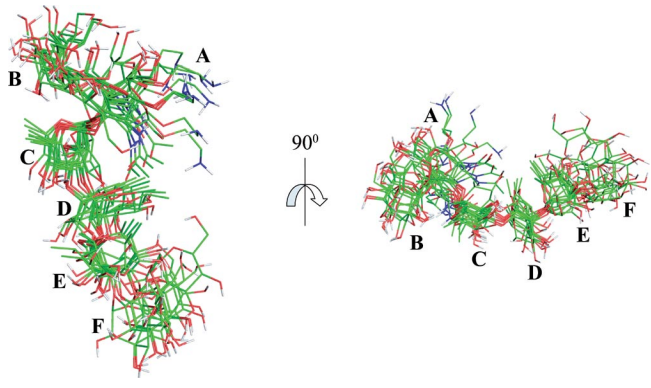


Figure 3. Ensemble conformational structures of hexasaccharide **1** sampled over a 3 ns time interval from MD simulation.

Conclusions

In summary, a concise chemical synthetic strategy has been developed for the synthesis of the hexasaccharide repeating unit of the cell-wall *O*-antigen of *Shigella flexneri* type 1d using a [3+3] block glycosylation and a minimum number of steps. The yields of both the glycosylations and the functional group manipulations were excellent. Conformational analysis of the synthesized hexasaccharide was carried out using 2D NOESY NMR spectroscopy and MD simulation. The conformational analysis showed that the molecule has a partly flexible and partly rigid structure in solution.

Experimental Section

General Methods: All reactions were monitored by thin-layer chromatography on silica-gel-coated TLC plates. The spots on TLC plates were visualized by spraying the plates with ceric sulfate [2% $Ce(SO_4)_2$ in 2 N H_2SO_4], and warming with a hot plate. Silica gel 230–400 mesh was used for column chromatography. NMR spectra were recorded with a Bruker Avance 500 MHz instrument using $CDCl_3$ as solvent and tetramethylsilane as an internal reference unless otherwise stated. Chemical shifts are expressed in ppm on the δ scale. Complete assignments of proton and carbon spectra were carried out using a standard set of NMR experiments, e.g., ^{1}H NMR, ^{13}C NMR, ^{13}C DEPT 135, 2D COSY, 2D HSQC, etc. In addition, 2D NOESY (300 ms mixing time) experiments were carried out to assist in the conformational analysis. The NOESY 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. The two-dimensional NMR spectroscopic data were processed using the TopSpin software suite (Bruker, Switzerland). ESI-MS spectra were recorded with a Micromass mass spectrometer. Optical rotations were recorded with a Jasco P-2000 spectrometer. Commercially available grades of organic solvents of adequate purity were used in all reactions.

2-(Benzyloxycarbonylamino)ethyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (3): Compound **2** (5 g, 10.47 mmol) and 2-(benzyloxycarbonylamino)ethanol (4 g, 20.5 mmol) were dissolved in $(CH_2Cl)_2$ (50 mL), and $BF_3{\cdot}OEt_2$ (1.5 mL, 12.15 mmol) was added. The reaction mixture was allowed to stir at 55 °C for 16 h. The reaction mixture was poured into satd. $NaHCO_3$ solution, and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with water, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane/EtOAc (3:1) as eluent to give pure compound **3** (4.9 g, 76%) as a white solid, m.p. 109–110 °C (EtOH). $[a]_D$ = +22 (c = 1.0, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3362, 3031, 2932, 1757, 1452, 1388, 1233, 1113, 1086, 759, 697 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.77–7.24 (m, 9 H, Ar-H), 5.69 (dd, J = 10.5, 9.0 Hz, 1 H, N*H*), 5.35 (d, J = 8.0 Hz, 1 H, 1-H), 5.14–5.07 (m, 2 H, 3-H, 4-H), 5.03–4.87 (m, 2 H, PhCH_2), 4.27–4.23 (m, 2 H, 6-H_{ab}), 4.13–4.10 (m, 1 H, 5-H), 3.81–3.74 (m, 2 H, 2-H, OCH_2), 3.69–3.62 (m, 1 H, OCH_2), 3.35–3.18 (m, 2 H, NCH_2), 2.07, 2.05, 1.82 (3 s, 9 H, 3 COCH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.5, 170.0, 169.3 (3 *C*OCH$_3$), 167.1 (2 C, Phth*C*O), 156.1 (Cbz*C*O), 136.4–123.7 (Ar-C), 98.3 (C-1), 71.9 (C-3), 70.6 (C-4), 69.5 (C-6), 68.7 (O*C*H$_2$), 66.5 (Ph*C*H$_2$), 61.8 (C-5), 54.5 (C-2), 40.8 (N*C*H$_2$), 20.7, 20.6, 20.4 (3 CO*C*H$_3$) ppm. ESI-MS: m/z = 635.1 [M + Na]$^+$. $C_{30}H_{32}N_2O_{12}$ (612.19): calcd. C 58.82, H 5.27; found C 58.65, H 5.50.

2-(Benzyloxycarbonylamino)ethyl 4,6-*O*-Benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (4): A solution of compound **3** (4 g, 6.53 mmol) in CH_3ONa (0.05 M solution in MeOH; 100 mL) was allowed to stir at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W X8 (H^+) resin, then it was filtered, and the filtrate was concentrated under reduced pressure to give the de-*O*-acetylated product.

The de-*O*-acetylated product (3.1 g) was dissolved in anhydrous DMF (5 mL), and benzaldehyde dimethyl acetal (1.5 mL, 9.99 mmol) and *p*TsOH (200 mg) were added. The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was neutralized with Et_3N (1 mL), and the solvents were removed under reduced pressure. The crude product was diluted with CH_2Cl_2 (100 mL), and the organic layer was washed with water, dried (Na_2SO_4) and concentrated. The crude product was crystallized from EtOH to give pure compound **4** (2.9 g, 77%) as a white solid, m.p. 114–115 °C (EtOH). $[a]_D$ = –32 (c = 1.0, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3448, 3019, 2883, 1773, 1715, 1388, 1233, 1113, 1086, 759, 697 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.86–7.23 (m, 14 H, Ar-H), 5.50 (s, 1 H, PhC*H*), 5.20 (d, J = 8.5 Hz, 1 H, 1-H), 5.08–5.06 (m, 1 H, N*H*), 4.95–4.85 (dd, J = 12.5 Hz, 2 H, PhCH_2), 4.55 (t, J = 9.5 Hz, 1 H, 4-H), 4.31 (dd, J = 10.0, 4.0 Hz, 1 H, 6-H_a), 4.16 (dd, J = 8.5 Hz, 1 H, 6-H_b), 3.75–3.71 (m, 2 H, OCH_2), 3.57–3.51 (m, 3 H, 2-H, 3-H, 5-H), 3.21–3.19 (m, 2 H, NCH_2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 167.1 (2 C, Phth*C*O), 156.2 (Cbz*C*O), 137.0–123.5 (Ar-C), 101.8 (Ph*C*H), 99.1 (C-1), 82.0 (C-3), 69.1 (C-6), 68.5 (O*C*H$_2$), 68.4 (C-4), 66.5 (Ph*C*H$_2$), 66.2 (C-5), 56.7 (C-2), 40.8 (N*C*H$_2$) ppm. ESI-MS: m/z = 597.1 [M + Na]$^+$. $C_{31}H_{30}N_2O_9$ (574.19): calcd. C 64.80, H 5.26; found C 64.64, H 5.44.

2-(Benzyloxycarbonylamino)ethyl 6-*O*-Benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (5): Et_3SiH (1.5 mL, 9.39 mmol) and iodine (500 mg, 1.97 mmol) were added to a solution of compound **4** (2.5 g, 4.35 mmol) in CH_3CN (15 mL) at 5 °C, and the reaction mixture was stirred at the same temperature for 30 min. The reaction mixture was diluted with CH_2Cl_2 (50 mL), and the organic layer was successively washed with $Na_2S_2O_3$ (5% aq.) and $NaHCO_3$ (satd.), then it was dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (3:1) as eluent to give pure compound **5** (1.8 g, 72%) as a yellow oil. $[a]_D$ = –14 (c = 1.0, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3390, 3031, 2924, 1713, 1520, 1391, 1069, 754, 721, 697 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.75–7.23 (m, 14 H, Ar-H), 5.36–5.34 (m, 1 H, N*H*), 5.16 (d, J = 8.0 Hz, 1 H, 1-H), 4.97–4.89 (dd, J = 12.5 Hz each, 2 H, PhCH_2), 4.54–4.46 (dd, J = 12.5 Hz, 2 H, PhCH_2), 4.25 (t, J = 9.0 Hz, 1 H, 3-H), 4.07 (t, J = 9.0 Hz, 1 H, 2-H), 3.75–3.70 (m, 3 H, 5-H, 6-H_{ab}), 3.66–3.53 (m, 3 H, 4-H, OCH_2), 3.35–3.20 (m, 2 H, NCH_2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.4 (2 C, Phth*C*O), 156.4 (Cbz*C*O), 137.6–123.5 (Ar-C), 98.6 (C-1), 74.2 (C-4), 73.6 (Ph*C*H$_2$), 73.2 (C-5), 71.7 (C-3), 69.9 (C-6), 69.5 (O*C*H$_2$), 66.5 (Ph*C*H$_2$), 56.3 (C-2), 41.1 (N*C*H$_2$) ppm. ESI-MS: m/z = 599.2 [M + Na]$^+$. $C_{31}H_{32}N_2O_9$ (576.21): calcd. C 64.57, H 5.59; found C 64.40, H 5.78.

Ethyl 2,4-Di-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-1-thio-α-L-rhamnopyranoside (7): Powdered NaOH (500 mg, 12.5 mmol) and *p*-methoxybenzyl chloride (1.4 mL, 10.32 mmol) were added to a solution of compound **6** (2 g, 5.15 mmol) in DMF (10 mL), and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was poured into water, and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with water, dried (Na_2SO_4), and con-

centrated. The crude product was purified over SiO_2 using hexane/EtOAc (10:1) as eluent to give pure compound **7** (2.3 g, 88%) as a yellow oil. $[a]_D$ = –61 (c = 1.0, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3446, 3094, 2930, 1723, 1531, 1454, 1371, 1237, 1095, 752, 698 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.40–7.23 (m, 12 H, Ar-H), 6.85 (d, J = 9.0 Hz, 2 H, Ar-H), 5.24 (br. s, 1 H, 1-H), 4.97 (d, J = 11.0 Hz, 1 H, PhCH_2), 4.74, 4.70 (2 d, J = 12.5 Hz, 2 H, PhCH_2), 4.65 (d, J = 11.0 Hz, 1 H, PhCH_2), 4.51 (br. s, 2 H, PhCH_2), 4.04–4.00 (m, 1 H, 5-H), 3.81 (s, 3 H, OCH_3), 3.79–3.76 (m, 2 H, 2-H, 3-H), 3.61 (t, J = 9.0 Hz, 1 H, 4-H), 2.62–2.52 (m, 2 H, SCH_2CH$_3$), 1.34 (d, J = 6.0 Hz, 3 H, CCH_3), 1.27 (t, J = 7.0 Hz, 3 H, SCH$_2$CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 159.2–113.7 (Ar-C), 81.9 (C-1), 80.5 (C-4), 79.9 (C-2), 76.6 (PhCH$_2$), 75.3 (PhCH$_2$), 72.2 (PhCH$_2$), 71.7 (C-3), 68.4 (C-5), 55.1 (OCH$_3$), 25.4 (SCH$_2$CH$_3$), 18.0 (CCH$_3$), 15.2 (SCH$_2$$CH_3$) ppm. ESI-MS: m/z = 531.2 $[M + Na]^+$. $C_{30}H_{36}O_5S$ (508.23): calcd. C 70.84, H 7.13; found C 70.67, H 7.28.

2-(Benzyloxycarbonylamino)ethyl (4,6-Di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (11): A solution of compound **5** (1.5 g, 2.60 mmol) and compound **8** (2.3 g, 3.90 mmol) in anhydrous CH_2Cl_2/Et_2O (1:2 v/v; 10 mL) was cooled to –10 °C under argon. $NOBF_4$ (250 mg, 2.14 mmol) was added, and the reaction mixture was stirred at the same temperature for 30 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was successively washed with satd. $NaHCO_3$ and water, then it was dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (4:1) as eluent to give pure compound **11** (1.9 g, 73%) as a colorless oil. $[a]_D$ = +16 (c = 1.0, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3410, 3019, 2927, 1710, 1518, 1389, 1210, 1047, 756 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.78–7.22 (m, 24 H, Ar-H), 5.47–5.45 (m, 1 H, NH), 5.10 (d, J = 8.0 Hz, 1 H, 1_A-H), 5.00–4.92 (dd, J = 12.5 Hz, 2 H, PhCH_2), 4.89 (t, J = 9.5 Hz, 1 H, 4_B-H), 4.79–4.72 (m, 3 H, PhCH_2), 4.67 (d, J = 11.5 Hz, 1 H, PhCH_2), 4.62 (br. s, 1 H, 1_B-H), 4.61–4.50 (m, 2 H, PhCH_2), 4.31–4.29 (m, 1 H, 3_A-H), 4.17 (dd, J = 8.5 Hz, 1 H, 2_A-H), 3.89–3.85 (m, 2 H, 3_B-H, 5_A-H), 3.76–3.66 (m, 7 H, 4_A-H, 6_A-H_{ab}, 6_B-H_{ab}, OCH_2), 3.62–3.59 (m, 1 H, 5_B-H), 3.44 (dd, J = 10.0, 3.5 Hz, 1 H, 2_B-H), 3.40–3.24 (m, 2 H, NCH_2), 1.87, 1.86 (2 s, 6 H, 2 COCH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.3, 169.3 (2 C, 2 COCH$_3$), 156.4 (Cbz$C$O), 137.9–123.4 (Ar-C), 101.7 (C-1_B), 98.7 (C-1_A), 84.6 (C-3_A), 79.2 (C-3_B), 78.9 (C-2_B), 75.5 (PhCH$_2$), 75.1 (C-4_A), 74.5 (PhCH$_2$), 73.5 (PhCH$_2$), 71.4 (C-4_B), 70.0 (OCH$_2$), 69.3 (C-6_B), 68.9 (C-5_A), 68.7 (C-5_B), 66.5 (C-6_A), 60.8 (PhCH$_2$), 55.5 (C-2_A), 41.3 (NCH$_2$), 20.7, 20.5 (2 COCH$_3$) ppm. MALDI-MS: m/z = 1025.3 $[M + Na]^+$. $C_{55}H_{58}N_2O_{16}$ (1002.38): calcd. C 65.86, H 5.83; found C 65.70, H 6.02.

2-(Benzyloxycarbonylamino)ethyl (2,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (12): Molecular seives (4 Å; 2 g) were added to a solution of compound **11** (1.5 g, 1.49 mmol) and compound **7** (900 mg, 1.77 mmol) in CH_2Cl_2/Et_2O (2:1 v/v; 10 mL), and the reaction mixture was stirred at room temperature under argon for 30 min. The reaction mixture was cooled to –25 °C, and NIS (440 mg, 1.95 mmol) and TfOH (15 μL) were added. The reaction mixture was stirred at the same temperature for 30 min, then the temperature was raised to 0 °C, and it was stirred at 0 °C for another 30 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was successively washed with $Na_2S_2O_3$ (5%), $NaHCO_3$ (satd. aq.), and water, then it was dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (8:1) as eluent to give pure compound **12** (1.4 g, 71%) as a colorless oil. $[a]_D$ = +18 (c = 1.0, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3400, 3019, 2927, 1736, 1717, 1388, 1216, 1046, 758 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.74–7.17 (m, 34 H, Ar-C), 5.43 (d, J = 3.0 Hz, 1 H, 1_B-H), 5.35–5.31 (m, 1 H, NH), 5.25 (br. s, 1 H, 1_C-H), 5.20 (d, J = 8.5 Hz, 1 H, 1_A-H), 5.05–4.94 (dd, J = 11.0 Hz, 2 H, PhCH_2), 4.85–4.60 (m, 8 H, 4_B-H, PhCH_2), 4.54–4.48 (m, 3 H, 3_A-H, PhCH_2), 4.34–4.30 (m, 2 H, 2_A-H, PhCH_2), 3.97 (t, J = 8.5 Hz, 1 H, 4_A-H), 3.85 (dd, J = 10.0, 3.0 Hz, 1 H, 3_C-H), 3.80–3.55 (m, 11 H, 2_C-H, 3_B-H, 5_A-H, 5_B-H, 5_C-H, 6_A-H_{ab}, 6_B-H_{ab}, OCH_2), 3.42 (dd, J = 10.0, 3.5 Hz, 1 H, 2_B-H), 3.38–3.25 (m, 3 H, 4_C-H, NCH_2), 1.92, 1.49 (2 s, 6 H, 2 COCH_3), 1.27 (d, J = 6.0 Hz, 3 H, CCH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.4, 169.0 (2 COCH$_3$), 167.8 (2 C, Phth$C$O), 156.2 (Cbz$C$O), 138.5–123.5 (Ar-C), 98.1 (C-1_A), 96.2 (C-1_C), 95.4 (C-1_B), 81.6 (C-4_C), 79.0 (C-2_B), 78.3 (C-3_B), 77.9 (C-2_C), 77.8 (C-3_A), 74.9 (PhCH$_2$), 74.8 (PhCH$_2$), 74.4 (C-4_A), 73.4 (PhCH$_2$), 73.3 (PhCH$_2$), 73.2 (C-3_C), 72.8 (PhCH$_2$), 71.2 (C-4_B), 69.5 (C-6_B), 68.9 (2 C, C-5_C, C-6_A), 68.8 (C-5_A), 68.7 (C-5_B), 66.6 (PhCH$_2$), 61.3 (OCH$_2$), 55.4 (C-2_A), 41.2 (NCH$_2$), 20.6, 20.4 (2 COCH$_3$), 18.0 (CCH$_3$) ppm. MALDI-MS: m/z = 1351.5 $[M + Na]^+$. $C_{75}H_{80}N_2O_{20}$ (1328.53): calcd. C 67.76, H 6.07; found C 67.57, H 6.24.

Ethyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (13): A solution of compound **6** (1.5 g, 3.86 mmol) and compound **9** (3 g, 4.38 mmol) in CH_2Cl_2/Et_2O (1:2 v/v; 15 mL) was cooled to –10 °C under argon. $NOBF_4$ (350 mg, 2.99 mmol) was added, and the reaction mixture was stirred at the same temperature for 30 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was successively washed with satd. $NaHCO_3$ and water, then it was dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (10:1) as eluent to give pure compound **13** (2.6 g, 74%) as a yellow oil. $[a]_D^{25}$ = +57 (c = 1.0, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3017, 2921, 1730, 1497, 1216, 1072, 756, 668 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.39–7.11 (m, 30 H, Ar-H), 5.25 (d, J = 1.5 Hz, 1 H, 1_E-H), 5.16 (d, J = 3.5 Hz, 1 H, 1_F-H), 4.97–4.57 (m, 10 H, PhCH_2), 4.47 (d, J = 11.5 Hz, 1 H, PhCH_2), 4.34 (d, J = 11.5 Hz, 1 H, PhCH_2), 4.13 (t, J = 9.5 Hz, 1 H, 3_F-H), 4.07–4.00 (m, 4 H, 2_E-H, 4_E-H, 5_E-H, 5_F-H), 3.77–3.65 (m, 3 H, 2_F-H, 3_E-H, 4_F-H), 3.61 (dd, J = 12.5, 3.0 Hz, 1 H, 6_F-H_a), 3.48 (dd, J = 12.5, 1.5 Hz, 1 H, 6_F-H_b), 2.70–2.50 (m, 2 H, SCH_2CH$_3$), 1.37 (d, J = 6.0 Hz, 3 H, CCH_3), 1.25 (t, J = 7.4 Hz, 3 H, SCH$_2$CH_3) ppm. ^{13}C NMR (500 MHz, $CDCl_3$): δ = 138.8–127.4 (Ar-C), 94.5 (C-1_F), 82.2 (C-1_E), 82.1 (C-3_F), 79.4 (C-2_F), 77.8 (C-3_E), 77.3 (C-4_F), 76.9 (C-2_E), 76.8 (C-4_E), 75.6 (2 C, 2 PhCH$_2$), 74.9 (PhCH$_2$), 73.4 (PhCH$_2$), 73.2 (PhCH$_2$), 72.8 (PhCH$_2$), 70.5 (C-5_F), 68.6 (C-5_E), 68.2 (C-6_F), 25.4 (SCH$_2$CH$_3$), 17.9 (CCH$_3$), 15.1 (SCH$_2$$CH_3$) ppm. MALDI-MS: m/z = 933.4 $[M + Na]^+$. $C_{56}H_{62}O_9S$ (910.41): calcd. C 73.82, H 6.86; found C 73.64, H 7.00.

(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl Trichloroacetimidate (14): NBS (470 mg, 2.64 mmol) was added to a solution of compound **13** (2 g, 2.19 mmol) in acetone/H_2O (9:1 v/v; 20 mL), and the reaction mixture was stirred at room temperature for 40 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was successively washed with $Na_2S_2O_3$ (5% aq.) and water, then it was dried (Na_2SO_4), and concentrated under reduced pressure to give the hemiacetal derivative, which was passed through a short plug of SiO_2.

The hemiacetal derivative (1.4 g) was dissolved in anhydrous CH_2Cl_2 (10 mL), and CCl_3CN (1 mL, 9.97 mmol) was added. The mixture was cooled to –10 °C, and DBU (150 μL, 1 mmol) was added. The reaction mixture was stirred at the same temperature for 1 h. The solvents were removed under reduced pressure, and the

crude product was purified over SiO_2 using hexane/EtOAc (15:1) as eluent to give pure compound **14** (1.6 g, 72%), which was used immediately in the next step without spectral characterization.

Ethyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (15): A solution of compound **10** (500 mg, 1.28 mmol) and compound **14** (1.5 g, 1.48 mmol) in CH_2Cl_2/Et_2O (1:2 v/v; 10 mL) was cooled to –10 °C under argon. $NOBF_4$ (80 mg, 0.68 mmol) was added, and the mixture was stirred at the same temperature for 30 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was successively washed with satd. $NaHCO_3$ and water, then it was dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (8:1) as eluent to give pure compound **15** (1.1 g, 70%) as a yellow oil. $[\alpha]_D$ = +1 (*c* = 1.0, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3017, 2928, 1496, 1454, 1216, 1067, 757, 668 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.39–7.13 (m, 40 H, Ar-H), 5.25 (d, *J* = 3.0 Hz, 1 H, 1_F-H), 5.21 (br. s, 1 H, 1_E-H), 5.13 (br. s, 1 H, 1_D-H), 5.00–4.34 (m, 16 H, PhCH_2), 4.20 (dd, *J* = 9.5, 3.0 Hz, 1 H, 3_E-H), 4.16 (t, *J* = 9.0 Hz, 1 H, 3_F-H), 4.12 (br. s, 1 H, 2_E-H), 4.09–4.01 (m, 3 H, 2_D-H, 5_D-H, 5_F-H), 3.87–3.84 (m, 1 H, 5_E-H), 3.80 (dd, *J* = 9.0, 3.0 Hz, 1 H, 3_D-H), 3.77 (t, *J* = 9.5 Hz, 1 H, 4_F-H), 3.72–3.64 (m, 3 H, 2_F-H, 4_D-H, 6_F-H_a), 3.54 (dd, *J* = 10.5, 1.5 Hz, 1 H, 6_F-H_b), 3.49 (t, *J* = 9.0 Hz, 1 H, 4_E-H), 2.60–2.45 (m, 2 H, SCH_2CH$_3$), 1.41, 1.32 (2 d, *J* = 6.0 Hz, 6 H, 2 CCH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 138.8–127.4 (Ar-C), 99.8 (C-1_D), 94.6 (C-1_F), 83.7 (C-1_E), 82.2 (C-3_F), 80.9 (C-4_E), 80.3 (2 C, C-4_D, C-4_F), 79.3 (C-2_F), 77.8 (C-3_D), 76.2 (C-3_E), 75.6 (2 C, 2 Ph*C*H$_2$), 75.4 (2 C, C-2_D, Ph*C*H$_2$), 75.2 (C-2_E), 74.9 (Ph*C*H$_2$), 73.5 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 72.8 (Ph*C*H$_2$), 72.4 (Ph*C*H$_2$), 70.5 (C-5_F), 68.8 (C-5_D), 68.5 (C-5_E), 68.3 (C-6_F), 25.5 (S*C*H$_2$CH$_3$), 18.2, 18.1 (2 C*C*H$_3$), 15.2 (SCH$_2$*C*H$_3$) ppm. MALDI-MS: *m*/*z* = 1259.5 $[M + Na]^+$. $C_{76}H_{84}O_{13}S$ (1236.56): calcd. C 73.76, H 6.84; found C 73.60, H 7.05.

2-(Benzyloxycarbonylamino)ethyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-]-6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (16): Molecular sieves (4 Å; 2 g) were added to a solution of compound **12** (1 g, 0.75 mmol) and compound **15** (1 g, 0.81 mmol) in CH_2Cl_2/Et_2O (1:1 v/v; 10 mL). The reaction mixture was stirred at room temperature under argon for 30 min. The reaction mixture was cooled to –25 °C, and NIS (200 mg, 0.89 mmol) and TfOH (5 μL) were added. The reaction mixture was stirred at the same temperature for 30 min, then it was diluted with CH_2Cl_2 (100 mL), and the organic layer was successively washed with $Na_2S_2O_3$ (5%), $NaHCO_3$ (satd. aq.), and water, then it was dried (Na_2SO_4) and concentrated. The crude product was purified over SiO_2 using hexane/EtOAc (8:1) as eluent to give pure compound **16** (1.3 g, 69%) as a colorless oil. $[\alpha]_D$ = +7 (*c* = 1.0, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3033, 2928, 1737, 1726, 1495, 1456, 1338, 1223, 1049, 751, 667 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.32–7.08 (m, 74 H, Ar-H), 5.72 (br. s, 1 H, 1_F-H), 5.21 (d, *J* = 8.0 Hz, 1 H, 1_A-H), 5.20–5.18 (m, 4 H, 1_B-H, 1_C-H, 1_E-H, N*H*), 5.04 (br. s, 1 H, 1_D-H), 5.00–4.40 (m, 27 H, 4_B-H, PhCH_2), 4.36 (t, *J* = 8.0 Hz, 1 H, 2_A-H), 4.25 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 4.16–4.10 (m, 3 H, 3_F-H, 5_A-H, PhCH_2), 4.08–4.02 (m, 2 H, 5_E-H, 5_F-H), 3.99 (br. s, 1 H, 2_D-H), 3.95 (br. s, 1 H, 2_E-H), 3.84–3.70 (m, 10 H, 2_C-H, 3_C-H, 3_D-H, 3_E-H, 4_A-H, 5_D-H, 6_A-H_{ab}, 6_B-H_{ab}), 3.69–3.58 (m, 5 H, 2_B-H, 2_F-H, 3_B-H, 5_C-H, 6_F-H_a), 3.56–3.40 (m, 5 H, 3_A-H, 4_C-H, 4_D-H, 4_F-H, 6_F-H_b), 3.38–3.25 (m, 3 H, 4_E-H, NCH_2), 3.22–3.19 (m, 1 H, 5_B-H), 1.86, 1.47 (2 s, 6 H, 2 COCH_3), 1.26–1.19 (3 d, *J* = 6.0 Hz, 9 H, 3 CCH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.6, 169.1 (2 *C*OCH$_3$), 167.8 (2 C, Phth*C*O), 156.5 (Cbz*C*O), 138.8–123.6 (Ar-C), 99.9 (C-1_D), 97.9 (2 C, C-1_A, C-1_C), 97.2 (C-1_E), 94.6 (C-1_B), 94.5 (C-1_F), 82.2 (2 C, C-3_C, C-3_F), 80.7 (C-4_C), 80.5 (C-4_F), 79.8 (C-4_D), 79.5 (C-2_B), 79.2 (3 C, C-2_C, C-2_E, C-3_D), 77.9 (2 C, C-2_F, C-4_A), 77.7 (2 C, C-2_D, C-4_E), 77.3 (C-3_A), 75.6 (2 C, Ph*C*H$_2$), 75.4 (C-3_E), 75.1 (Ph*C*H$_2$), 75.0 (2 C, 2 Ph*C*H$_2$), 74.9 (Ph*C*H$_2$), 73.7 (Ph*C*H$_2$), 73.4 (Ph*C*H$_2$), 73.1 (Ph*C*H$_2$), 72.7 (Ph*C*H$_2$), 72.6 (Ph*C*H$_2$), 72.5 (Ph*C*H$_2$), 72.3 (Ph*C*H$_2$), 70.4 (2 C, C-4_B, C-5_D), 69.5 (C-5_F), 69.2 (C-6_F), 68.9 (C-6_B), 68.8 (C-5_E), 68.7 (C-5_C), 68.5 (C-5_B), 68.3 (C-5_A), 68.2 (C-6_A), 66.6 (O*C*H$_2$), 61.4 (Ph*C*H$_2$), 55.5 (C-2_A), 41.0 (N*C*H$_2$), 20.6, 20.5 (2 CO*C*H$_3$), 18.3, 18.2, 18.0 (3 C*C*H$_3$) ppm. MALDI-MS: 2526.0 $[M + Na]^+$. $C_{149}H_{158}N_2O_{33}$ (2503.07): calcd. C 71.45, H 6.36; found C 71.28, H 6.50.

2-Aminoethyl (α-D-Glucopyranosyl)-(1→3)-(α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-(α-L-rhamnopyranosyl)-(1→3)-[(α-D-glucopyranosyl)-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1): Ethylene diamine (0.3 mL, 4.48 mmol) was added to a solution of compound **16** (1 g, 0.40 mmol) in *n*BuOH (20 mL), and the reaction mixture was allowed to stir at 90 °C for 8 h. The solvents were removed under reduced pressure.

The crude product was dissolved in acetic anhydride (3 mL) and pyridine (3 mL), and the solution was kept 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 to remove by-products.

The acetylated product was dissolved in CH_3OH/CH_2Cl_2 (9:1, v/v; 10 mL), and Pd/C (10%; 150 mg) and Et_3SiH (3 mL, 18.78 mmol) were added. The reaction mixture was stirred at room temperature for 10 h. The reaction mixture was filtered through a Celite® pad, and the filter pad was washed with CH_3OH (50 mL). The combined filtrate was concentrated under reduced pressure.

The hydrogenated product was dissolved in CH_3ONa (0.1 M in CH_3OH; 10 mL), and the mixture was stirred at room temperature for 3 h. The reaction mixture was neutralized with Dowex 50W X8 (H^+) resin, filtered, and concentrated to give compound **1**. This material was passed through an LH-20 Sephadex column using CH_3OH/H_2O (3:1) as eluent to give pure compound **1** (220 mg, 54%) as a white solid, $[\alpha]_D^{25}$ = +5 (*c* = 1.0, H_2O). ^1H NMR (500 MHz, D_2O): δ = 5.23 (d, *J* = 3.0 Hz, 1 H, 1_B-H), 5.12 (br. s, 1 H, 1_D-H), 5.03 (br. s, 1 H, 1_C-H), 5.00 (d, *J* = 3.0 Hz, 1 H, 1_F-H), 4.91 (br. s, 1 H, 1_E-H), 4.49 (d, *J* = 7.5 Hz, 1 H, 1_A-H), 4.18 (br. s, 1 H, 2_E-H), 4.00–3.92 (m, 4 H, 2_D-H, 4_A-H, 5_F-H, OCH_2), 3.90–3.76 (m, 8 H, 2_A-H, 3_A-H, 3_C-H, 3_D-H, 3_E-H, 4_D-H, 5_A-H, OCH_2), 3.75–3.57 (m, 11 H, 4_E-H, 4_F-H, 5_C-H, 5_D-H, 5_E-H, 6_A-H_{ab}, 6_B-H_{ab}, 6_F-H_{ab}), 3.55–3.44 (m, 4 H, 2_C-H, 3_B-H, 3_F-H, 5_B-H), 3.42–3.32 (m, 4 H, 2_B-H, 2_F-H, 4_B-H, 4_C-H), 3.18–3.05 (m, 2 H, NCH_2), 1.94 (s, 3 H, COCH_3), 1.24–1.17 (m, 9 H, 3 CCH_3) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 174.7 (*C*OCH$_3$), 101.9 (C-1_E), 100.7 (C-1_C), 100.6 (2 C, C-1_A, C-1_D), 97.7 (C-1_B), 95.3 (C-1_F), 78.3 (C-3_A), 78.1 (C-2_D), 76.9 (C-5_A), 76.6 (C-3_C), 75.2 (C-2_E), 74.3 (C-3_E), 72.9 (C-3_F), 72.8 (C-4_D), 72.6 (C-4_C), 72.1 (C-5_B), 71.7 (C-2_F), 71.6 (C-4_E), 71.5 (C-3_B), 71.4 (C-2_C), 70.2 (C-2_B), 70.1 (C-4_A), 69.8 (2 C, C-3_D, C-5_F), 69.4 (C-5_C), 69.2 (C-4_B), 69.1 (C-4_F), 69.0 (C-5_D), 66.6 (C-5_E), 65.7 (O*C*H$_2$), 60.7 (C-6_F), 60.3 (C-6_A), 60.2 (C-6_B), 54.4 (C-2_A), 39.4 (N*C*H$_2$), 22.8 (CO*C*H$_3$), 16.9, 16.8, 16.5 (3 C*C*H$_3$) ppm. ESI-MS: *m*/*z* = 1049.4 $[M + Na]^+$. $C_{40}H_{70}N_2O_{28}$ (1026.41): calcd. C 46.78, H 6.87; found C 46.56, H 7.10.

Molecular Dynamics Simulation: The structure of hexasaccharide **1** was built in the Maestro panel, and the structure was cleaned for optimal bond parameters, keeping in mind the requisite α/β config-

uration forms, L/D isomeric forms, and furanose/pyranose type of the sugar rings.[36] The OPLS_2005 force field was used to carry out the initial energy minimization and the conformational-sampling-oriented molecular dynamics simulation.[37] Hexasaccharide **1** was solvated with TIP3 water models[38] in a truncated octahedral box with an edge distance of 8 Å. The account of the nonbonded interactions was done with a cutoff distance of 8 Å, and the simulation was carried out under isothermal-isobaric periodic boundary conditions. The M-SHAKE algorithm[39] was applied to restrain all the hydrogen bonds, with an integration time step of 2 fs. The initial minimization was carried out with a convergence threshold of 1.0 kcal mol^{-1} Å^{-1} to allow initial adjustment of all the atoms with respect to the system environment. Minimization steps were then carried out similarly to previously published literature.[40] Recording intervals of 1.2 and 2 ps, respectively, were set for energy and trajectory frames. The production run was continued at a temperature of 300 K up to a time period of 30 ns.

Supporting Information (see footnote on the first page of this article): (a) copies of the ^{1}H and ^{13}C NMR spectra of all synthesized compounds: **1**, **3**, **4**, **5**, **7**, **11**, **12**, **13**, **15**, and **16**; (b) 2D NOESY spectrum of compound **1** (Figure S1); (c) Plot of the proton–proton distance information as a function of time, obtained from the MD simulation (Figure S2); (d) Scatter plot of the ϕ and ψ angles for all the hexasaccharide conformers obtained from the conformational sampling in the MD simulation (Figure S3); (e) Graphical plot indicating the energetic contributions for the hexasaccharide (Coulomb and vdW contributions). All the energy values given here are in kcal mol^{-1} (Figure S4).

Acknowledgments

D. D. thanks the Council of Scientific and Industrial Research (CSIR), New Delhi for providing a Junior Research Fellowship. The work was supported by CSIR, New Delhi [project number 02(0038)/11/EMR-II] and the Bose Institute.

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Received: April 11, 2014
Published Online: June 12, 2014

