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Synthesis of the β-1,3-glucan, laminarahexaose: NMR and conformational studies

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ABSTRACT

The synthesis of laminarahexaose is described. NMR studies of several of the intermediates leading to the β-1,3-glucan show anomalously small coupling constants for some of the C-1 hydrogens. An X-ray structure for the protected hexasaccharide shows that the small coupling constants are due to some of the glucopyranose rings adopting a twist-boat conformation. The X-ray studies also explain other unexpected NMR observations.

1. Introduction

The construction of structurally well-defined products in glycosylation reactions is one of the most important objectives in carbohydrate chemistry.[1] The production of a mixture of α and β anomers not only lowers the yield of the desired product, but also increases the workload in separation and purification processes. It is generally accepted that β-glycosidic bonds will be formed if an acyl group is present at the C-2 position of the D-glucose donor due to its neighboring group participation via the oxo-carbenium intermediate.[2] Because of the neighboring group effect, the nucleophilic acceptor attacks the upper face of the anomeric carbon of the donor to form the desired β-linkage. However, in some cases, α anomers are unexpectedly formed. For example, Du and co-workers[3] reported the synthesis of a branching hexasaccharide via 3+3 coupling yielding only the unexpected α anomer even though C-2 acetate protection was used in the trisaccharide donor. Du and co-workers concluded that the steric bulk of the C-3 hydroxyl acceptor was responsible for this unexpected result. Kong and co-workers[4] have also reported unexpected α anomer formation in the attempted synthesis of β-(1→3)-glucans. These workers proposed that the by-product of glucosylation reactions, the orthoester, rearranged in the presence of acid to give the unexpected α anomer.[5] Therefore, methodology for increasing stereoselectivity in β-glucosylations and, more importantly, reliability in β-glycosylations is still in great need.

A complicating component in the preparation of β-(1→3)-glucans is that of determining when the unexpected formation of an α anomer has occurred. In relatively simple carbohydrate systems, a small (2–6 Hz) H1–H2 coupling constant can be indicative; however, as shown below, this is not always a reliable diagnostic tool.

2. Results and discussion

2.1. Preparation of the protected hexasaccharide 16 and laminarahexaose

Recently, our group has utilized a new type of C-2 acyl protecting group, 4-acetoxy-2,2-dimethylbutanoate (ADMB),[6] as the neighboring participation group in β-glucosylations with high yield and good diastereoselectivity. We have used this C-2 acyl protecting group in the synthesis of β-(1→3)-glucans,[7] and our group is constructing a small library of linear β-(1→3)-glucans and branched glucans (having β-(1→6)-glycosidic linkages with a side chain of varying length along the linear glucan backbone) for physiological studies.[8,9] During the course of this work we prepared the linear β-(1→3)-hexaglucoside (**1**) and we noted a number of interesting spectroscopic anomalies along the way.

The synthesis started from the known 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[10] (Scheme 1). Selective silylation at the C-3 hydroxyl (to give **2**), followed by benzoylation at the C-2 hydroxyl group gave the monosaccharide donor **3**. Hydrolysis of **3** was conducted according to the method previously developed in our group.[11] The reaction resulted in not only the hydrolysis of the thioethyl group but also the migration of C-2 benzoate to the anomeric carbon to give **4**. Treatment of the crude **4** with NEt_3/CH_2Cl_2, which effected quantitative rearrangement of **4** to **5**,

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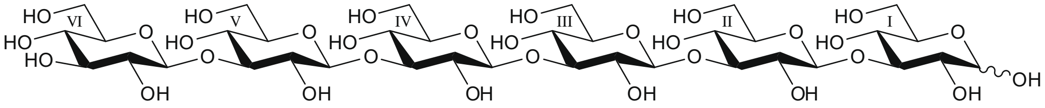
followed by standard Schmidt conditions,[12] provided the trichloroacetimidate donor **6** as the second building block.



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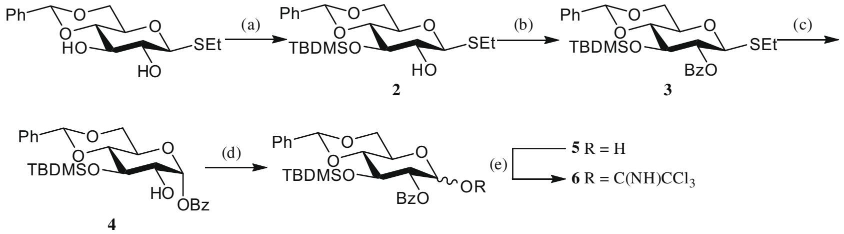
Benzoylation of compound **4**, followed by desilylation with HF/pyridine gave **8**, which would function as the reducing terminus acceptor (Scheme 2). Selective acylation of the α-thioglucoside **9**[13] at the C-2 hydroxyl by ADMB chloride in pyridine afforded **10** in 87% yield.

As shown in Scheme 3, coupling of the monosaccharide acceptor **8** and thioglucoside **3** using NIS and AgOTf as the promoter afforded the disaccharide **11** in 95% yield. The coupling constant of H-1$^{\text{II}}$ (4.96 ppm) is 7.6 Hz, and C-1$^{\text{II}}$ appears at 101.3 ppm, indicating that only the β isomer was formed. Removal of the TBDMS group using HF·Py provided the corresponding disaccharide acceptor **12**. Upon standard activation by catalytic TMSOTf, the glucosyl imidate **6** coupled with the acceptor **10** to give the disaccharide donor **13** in 92% yield. The coupling constant of H-1$^{\text{II}}$ (5.00 ppm) is 6.8 Hz, and the C-1$^{\text{II}}$ was at 99.4 ppm, again indicating that the expected β-linkage was formed.

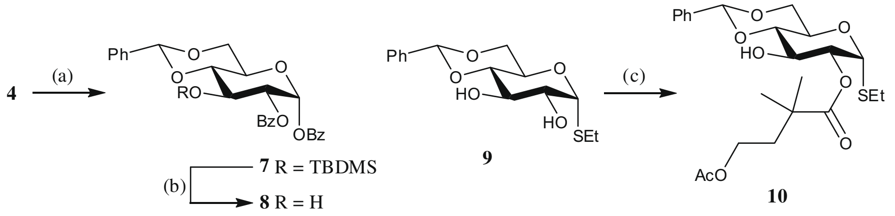
With both disaccharide donor and acceptor in hand, the stage was set for the iterative synthesis. Condensation of the β-(1→3)-disaccharide acceptor **12** and the β-(1→3)-disaccharide donor **13** (Scheme 4) with NIS and AgOTf as the promoter provided tetrasaccharide **14**. The ^{1}H NMR spectrum of the crude reaction mixture showed only one isomer formed, which was thought to be the desired tetrasaccharide with a new β-glycosidic bond. The coupling of the disaccharide donor analogous to **13** (but where the ADMB protecting group was replaced with a benzoyl group) with acceptor **12** gave a 4:3 mixture of the α and β glycosides, respectively, in 86% yield. Analysis of **14** via ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, and HMQC experiments revealed two interesting results. First, the signal of H-1$^{\text{III}}$ (4.66 ppm, see Table 1) had $J_{\text{H-1,H-2}} < 1$ Hz, which indicated that the α-glycosidic linkage had been formed. However, ^{13}C NMR showed that the chemical shift of C-1$^{\text{III}}$ was 95.9 ppm, which supported formation of the β anomer.[14] Secondly, the chemical shift of one of the benzylidene protons shifted far upfield to 3.94 ppm from its usual 5 to 6 ppm range (Table 1).

Removal of the C-3 TBDMS group of **14** yielded the corresponding tetrasaccharide acceptor **15** in 93% yield, in which the coupling constant of H-1$^{\text{III}}$ ($J_{\text{H-1,H-2}} = 2$ Hz) and the chemical shift of the benzylidene proton (4.14 ppm) remained almost the same, indicating that these two phenomena were not related to the bulky TBDMS protecting group. Coupling of the donor **13** and acceptor **15** using NIS/AgOTf gave the hexasaccharide **16** in 92% yield. The NMR spectral data for hexasaccharide **16** were similar to that presented by tetrasaccharides **14** and **15**. However, in the case of **16**, both H-1$^{\text{III}}$ and H-1$^{\text{V}}$ have $J_{\text{H-1,H-2}} < 1$ Hz. The small coupling constants for the H-1 protons suggested that the newly formed linkage had the α configuration; however, again, the ^{13}C NMR chemical shift suggested the β configuration (C-1$^{\text{III}}$ 95.8 and C-1$^{\text{V}}$ 95.9 ppm).[14] In addition, two of the six benzylidine protons were now dramatically shifted upfield in the ^{1}H NMR spectrum.

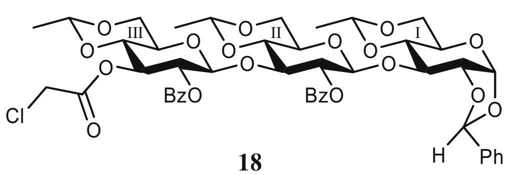
There are two examples in the literature where careful NMR analysis of oligoglucosides suggested that small coupling constants can sometimes be observed for β-glucopyranoside linkages. Collins and Ali[15] reported that the coupling constant of the third sugar residue ($J^{\text{III}}_{1,2}$) of the trisaccharide **18** is only 5.2 Hz. He attributed this anomaly to an unexplained conformational change in the trisaccharide. Also, Vetvicka and co-workers[16] noticed an exceptionally small coupling constant ($J^{\text{II}}_{1,2}$ 4.3 Hz) for the second sugar residue of trisaccharide **19**. Removal of the bulky C-3 protecting group to give **20** did not alter this value. Nuclear Overhauser effect (NOE) spectroscopy led Vetvicka and co-workers to propose that the 4,6-*O*-benzylidene acetal protection had distorted the ring conformation of the central glucose unit from the normal chair ($^{4}C_{1}$) to a boat ($^{1,4}B$ or $B_{2,5}$), thus lowering the coupling constant of H-1 in the second sugar residue.[17]



Scheme 1. Reagents and conditions: (a) TBDMSCl, Imidazole, DMF, 86%; (b) BzCl, DMAP, Py/CH$_2$Cl$_2$, 80 °C, 92%; (c) NBS, TMSOTf, CH$_2$Cl$_2$, 0 °C; (d) NEt$_3$/CH$_2$Cl$_2$, 85% for two steps; (e) Cl$_3$CCN, DBU, CH$_2$Cl$_2$, 0 °C , 92% (α/β = 8:1).



Scheme 2. Reagents and conditions: (a) BzCl, DMAP, Py, 99%; (b) HF·Py, Py/THF, 92%; (c) ADMB chloride, Py, 86%.



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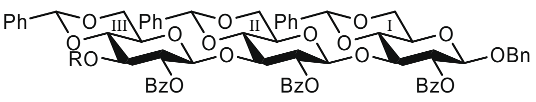


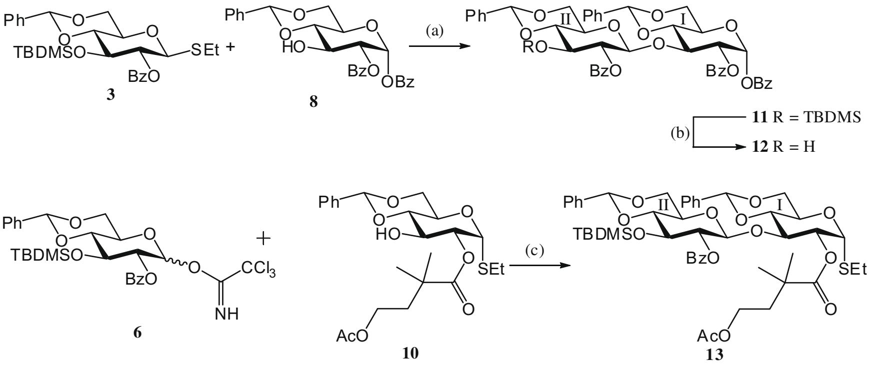
19 R=2-naphthylmethyl

20 R=H

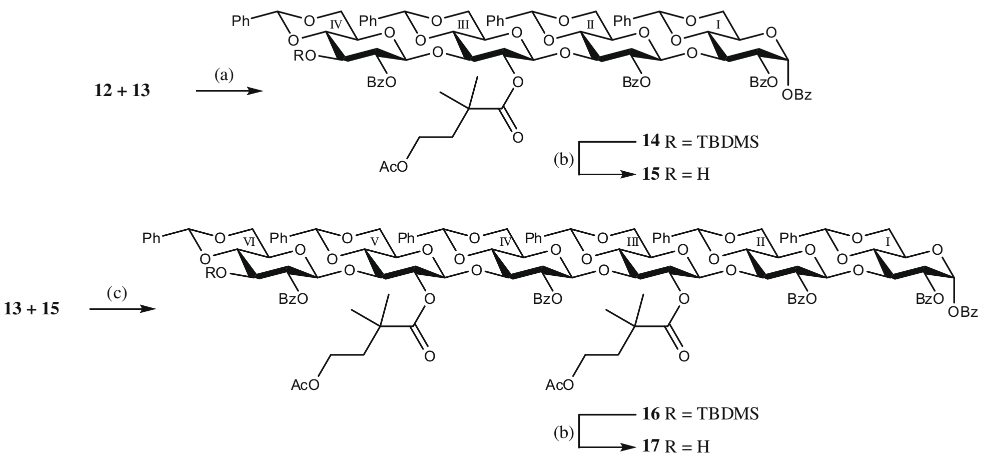
Complete deprotection of **16** was accomplished in a three-step process. The TBDMS group was removed by treatment with HF·Py in THF, and the benzylidene groups were removed by catalytic hydrogenolysis using 10% Degussa Pd/C in methanol/tetrahydrofuran/acetic acid (8:4:2.4). Removal of the acyl protecting groups (NaOMe–MeOH), followed by Sephadex LH-20 chromatography gave the hexasaccharide as a 1:1.2 mixture of α and β anomers. The laminarahexaose ^{1}H NMR, ^{13}C NMR, and HMQC spectra were identical (except for integration) to those reported by Lim and co-workers[18] for laminaraheptaose, which was prepared by partial hydrolysis of laminarin. In particular, the $J_{\text{H-1,H-2}}$ coupling constants returned to the expected 7.6–8 Hz range.

2.2. X-ray crystallography

Fortunately, after a great deal of effort, we were able to obtain crystals of protected hexasaccharide **16** (containing one cyclohexane molecule of crystallization), which were suitable for X-ray crystallography.[18] Crystal data are given in Table 2. The X-ray structure (Fig. 1) showed that all the glycosidic linkages are indeed β-linkages. Also, the third and fifth (from the reducing end) glucose rings are in a twist-boat conformation, while the remaining four glucopyranose rings are chairs. The H$_1$–H$_2$ dihedral angles of rings III and V of **16** are 63.6° and 59.3°, respectively, resulting in $J_{1,2}$ coupling constant of less than 2 Hz as predicted by the Karplus correlation.[19] Additionally, the X-ray structure shows that the benzylidene acetal proton of the third and fifth glucose units is pointed directly toward the face of the aromatic ring of the benzylidene group on the second and fourth glucose units, respectively (Figs. 2 and 3). The distance from the III-ring benzylidene proton to the C-1 carbon of the II-ring benzylidene group is 3.12 Å, and the distance from the V-ring benzylidene proton to the C-1 carbon of the IV-ring benzylidene group is 3.13 Å. The locations of these two protons near the face of the benzylidene acetal aromatic rings explain the >1 ppm upfield shift in the NMR for these two protons.[20] Although it is true that the solid-state crystal structure of **16** is not necessarily indicative of its solution conformation, the fact that the X-ray structure explains both of the unusual features of the NMR spectrum of **16** supports the fact that the conformation of **16** is the same in the solution and solid state.



Scheme 3. Reagents and conditions: (a) NIS, AgOTf/toluene, 4 Å MS, CH$_2$Cl$_2$, −20 °C, 95%; (b) HF·Py, Py/THF, 92%; (c) TMSOTf, 4 Å MS, CH$_2$Cl$_2$, −70 °C, 92%.



Scheme 4. Reagents and conditions: (a) NIS, AgOTf/toluene, 4 Å MS, CH$_2$Cl$_2$, −20 °C, 91%; (b) HF·Py, Py/THF, 93%; (c) NIS, AgOTf/toluene, 4 Å MS, CH$_2$Cl$_2$, −20 °C, 92%.

Table 1
Selected proton NMR data for compounds **1**, **14**, **15**, **16,** and **17**

Compound	δ H-1^{I} $J^{I}_{H\text{-}1,H\text{-}2}$ δ H-B^{I}	δ H-1^{II} $J^{II}_{H\text{-}1,H\text{-}2}$ δ H-B^{II}	δ H-1^{III} $J^{III}_{H\text{-}1,H\text{-}2}$ δ H-B^{III}	δ H-1^{IV} $J^{IV}_{H\text{-}1,H\text{-}2}$ δ H-B^{IV}	δ H-1^{V} $J^{V}_{H\text{-}1,H\text{-}2}$ δ H-B^{V}	δ H-1^{VI} $J^{VI}_{H\text{-}1,H\text{-}2}$ δ H-B^{VI}
14	6.56 4 5.76	4.75 7.6 5.22	4.66 <1.0 3.94	5.1 8 5.48	NA	NA
15	6.56 3.6 5.74	4.81 6.8 5.15	4.7 2 4.14	5.15 7.6 5.47	NA	NA
16	6.54 4 5.72	4.71–4.76 ND 5.23	4.62 0.8 4.09	5.19 8.4 5.33	4.71–4.76 <1.0 3.94	4.9 8 5.51
17	6.54 3.6 5.72	4.73 7.6 5.18	4.63 1.2 4.12	5.24 8 5.31	4.79 <1.0 4.1	4.90–4.97 ND 5.51
1	4.35 (β), 4.95 (α) 7.6 (β), 3.6 (α) NA	4.42–4.45 NA NA	4.51 8 NA	4.51 8 NA	4.51 8 NA	4.38 7.6 NA

δ H-1^{I} is the chemical shift of the anomeric proton on the reducing terminus pyranose; $J^{I}_{H\text{-}1,H\text{-}2}$ is the coupling constant (in Hz) of the anomeric proton on the reducing terminus pyranose; δ H-B^{I} is the chemical shift for the benzylidene proton on the reducing terminus pyranose.

Table 2
Crystal data for **16**

Empirical formula	$C_{141}H_{156}O_{42}Si$
Formula weight	2550.75
Temperature	100(2) K
Radiation (wavelength)	Mo Kα (graphite monochromated, 0.71073 Å)
Crystal size	0.26 × 0.24 × 0.24 mm
Crystal habit	Colorless block
Crystal system	Orthorhombic
Space group	$C222_1$
Unit cell dimensions	
a (Å)	17.3435(5)
b (Å)	22.9997(7)
c (Å)	73.405(2)
α (°)	90
β (°)	90
γ (°)	90
Volume	29280.9(15) Å^3
Z	8
Density (calculated)	1.157 Mg/m^3
Absorption coefficient	0.093 mm^{-1}
F(000)	10,816
θ Range for data collection	1.84–22.50°
Index ranges	$-18 \leqslant h \leqslant 5$, $-23 \leqslant k \leqslant 14$, $-78 \leqslant l \leqslant 23$
Total reflections	164,523
Unique reflections (R_{int})	15,340 (0.052)
Data ($I \geqslant 2\sigma(I)$)/restraints/parameters	14,354/1320/1579
Final *R* indices ($F_o \geqslant 4\sigma(F_o)$)	R_1 = 0.094; wR_2 = 0.2495
Final *R* indices (all data)	R_1 = 0.097; wR_2 = 0.2528
GOF (on F^2)	1.08
Largest final difference peak/hole (e Å^{-3})	0.68/−0.53

3. Experimental

3.1. General methods

^{1}H NMR spectra were recorded on a Varian Unity Inova 400 (400 MHz) spectrometer using $CDCl_3$ as solvent (except for compound **1**); multiplicities are quoted as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), doublet of triplets (dt), or multiplet (m). Carbon nuclear magnetic resonance (^{13}C) spectra were recorded on Varian Unity Inova 400 (100 MHz) spectrometers using $CDCl_3$ unless otherwise noted. Spectra were assigned using COSY, DEPT, and HMQC experiments. All chemical shifts are quoted on the δ-scale in parts per million (ppm). Residual solvent signals were used as an internal reference. Low- and high-resolution (HRMS) electrospray (ESI) mass spectra were recorded using a Bruker Microtof instrument.

Thin-layer chromatography (TLC) was carried out on Sorbent Technologies UV$_{254}$ Silica Gel plates. Flash column chromatography was carried out on silica gel (40–63 μm, Sorbent Technologies). All solvents were dried and freshly distilled using standard procedures. Organic solutions were concentrated under diminished pressure with bath temperatures <40 °C.

3.2. Ethyl 4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-1-thio-β-D-glucopyranoside (2)

To a stirred solution of ethyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[10] (10.0 g, 32.0 mmol) and imidazole (3.28 g, 48.0 mmol) in anhyd DMF (50 mL) was added *tert*-butyldimethylsilyl chloride (5.23 g, 35.2 mmol) in small portions at 0 °C. Upon being stirred at room temperature for 24 h, the reaction was quenched by addition of EtOAc (300 mL) and satd NH_4Cl (100 mL). The organic layer was washed with satd $NaHCO_3$ (2 × 250 mL) and brine (150 mL), and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded a yellow oil that was purified by flash chromatography over silica gel (1:12 EtOAc–hexanes) to give pure **2** (5.87 g, 86%) as a colorless oil: R_f 0.23 (1:10 EtOAc–hexanes); ^{1}H NMR δ 0.02 (s, 3H), 0.09 (s, 3H), 0.85 (s, 9H), 1.30 (t, *J* = 7.4 Hz, 3H), 2.65–2.80 (m, 2H), 3.41–3.51 (m, 3H, H-2, H-4 and H-5), 3.71–3.76 (m, 2H H-3 and H-6a), 4.31 (dd. *J* = 4.8 Hz and 10.4 Hz, 1H, H-6e), 4.44 (d, *J* = 9.6 Hz, 1H, H-1), 5.50 (s, 1H), 7.32–7.47 (m, 5H); ^{13}C NMR δ −4.6, −4.2, 15.5, 18.5, 24.8, 26.0, 68.8 (C-6), 70.9 (C-5), 74.3 (C-2), 76.0 (C-3), 81.3 (C-4), 86.7 (C-1), 101.8, 126.3, 128.3, 129.1, 137.3; ESI-TOF-HRMS: calcd for $C_{21}H_{34}O_5SSiNa$ $[M+Na]^+$ *m/z* 449.1794; found *m/z* 449.1790.

3.3. Ethyl 2-*O*-benzoyl-4,6-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-1-thio-β-D-glucopyranoside (3)

To a stirred solution of **2** (4.50 g, 10.6 mmol) and DMAP (645 mg, 5.28 mmol) in anhyd pyridine (20 mL) and anhyd CH_2Cl_2 (30 mL) was added benzoyl chloride (3.07 mL, 26.4 mmol) at 0 °C. The reaction mixture was heated to 80 °C for 24 h under N_2. Upon

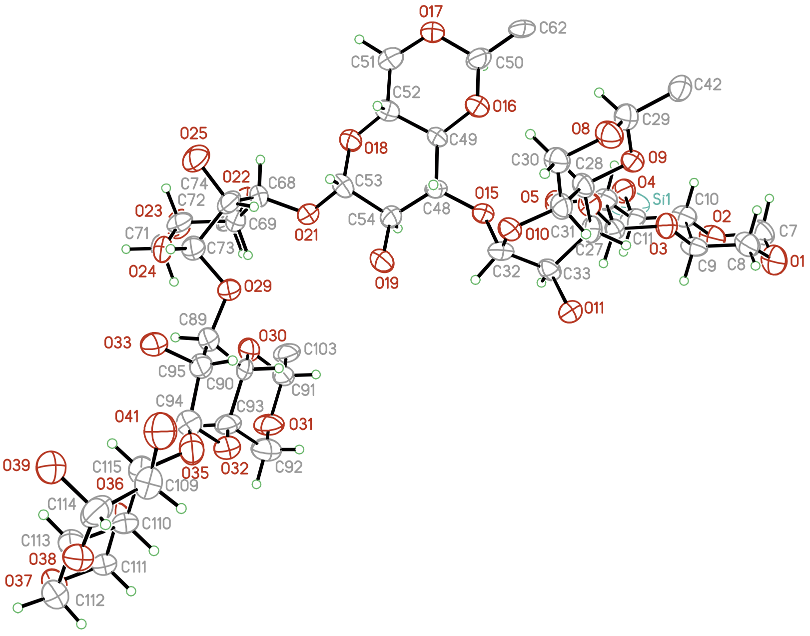


Figure 1. ORTEP of hexasaccharide **16**. Protecting groups and most hydrogens not shown for clarity. The reducing terminus is on the left.

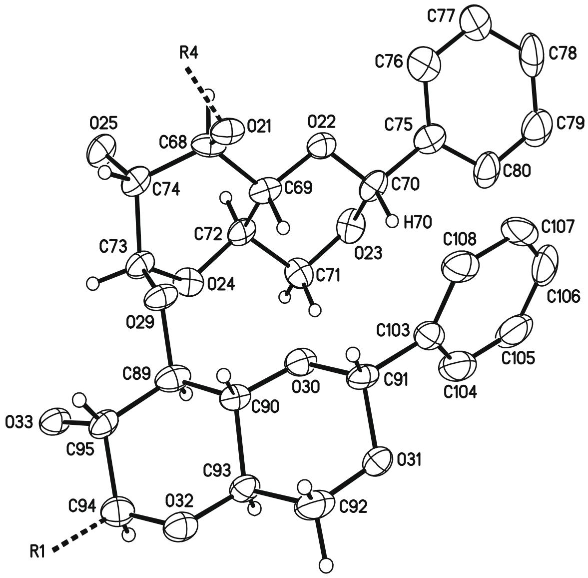


Figure 2. Partial ORTEP of hexasaccharide **16** showing the second and third glucose units from the reducing end (to the left). R1 designates the first glucose residue and R4 designates the fourth glucose residue. Notice the twist-boat conformation of the third glucose unit and the proximity of the benzylidene hydrogen (H70) on the third glucose unit protecting group and the aromatic ring of the benzylidene group on the second glucose unit.

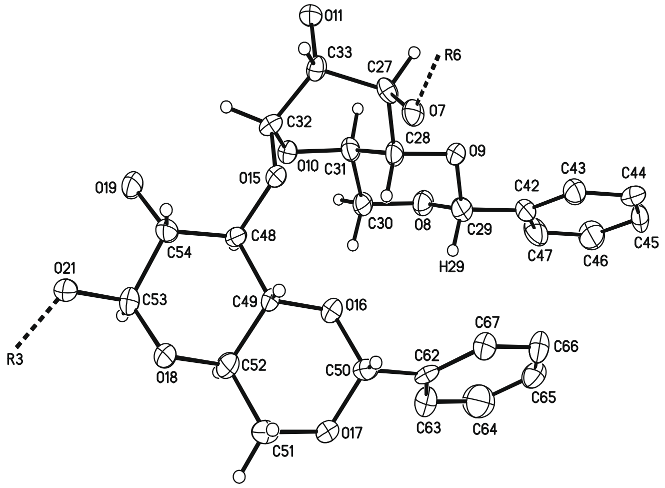


Figure 3. Partial ORTEP of hexasaccharide **16** showing the fourth and fifth glucose units from the reducing end (to the left). R3 designates the third glucose residue and R6 designates the sixth glucose residue. Notice the twist-boat conformation of the fifth glucose unit and the proximity of the benzylidene hydrogen (H29) on the fifth glucose unit protecting group and the aromatic ring of the benzylidene group on the fourth glucose unit.

removal of solvent under reduced pressure, the residue was dissolved in EtOAc (250 mL) and extracted with satd NH_4Cl (100 mL). The organic layer was washed with satd $NaHCO_3$ (2×250 mL), and brine (150 mL), and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded a yellow oil that was purified by flash chromatography over silica gel (1:15 EtOAc–hexanes) to give pure **3** (5.15 g, 92%) as a white solid: mp 38–41 °C; R_f 0.37 (1:10 EtOAc–hexanes); ^{1}H NMR δ −0.13 (s, 3H), −0.04 (s, 3H), 0.69 (s, 9H), 1.21 (t, J = 7.4 Hz, 3H), 2.67–2.77 (m, 2H), 3.55 (dt, J = 4.8 Hz and 9.6 Hz, 1H, H-5), 3.63 (t, J = 9.2 Hz, 1H, H-4), 3.79 (t, J = 10.0 Hz, 1H, H-6a), 4.05 (t, J = 8.8 Hz, 1H, H-3), 4.38 (dd. J = 4.8 Hz and 10.4 Hz, 1H, H-6e), 4.63 (d, J = 10.0 Hz, 1H, H-1), 5.29 (dd, J = 8.6 Hz and 9.8 Hz, 1H, H-2), 5.54 (s, 1H), 7.33–8.08 (m, 10H); ^{13}C NMR δ −4.9, −4.1, 14.8, 17.9, 23.9, 25.6, 68.6 (C-6), 70.8 (C-5), 73.3 (C-2), 74.2 (C-3), 81.4 (C-4), 84.1 (C-1), 101.7, 126.3, 128.1, 128.4, 129.0, 129.8, 130.0, 133.1, 137.1, 165.2; ESI-TOF-HRMS: calcd for $C_{28}H_{38}O_6SSiNa$ $[M+Na]^+$ m/z 553.2056; found m/z 553.2128.

3.4. Benzoyl 4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-α-D-glucopyranose (4)

To a stirred solution of **3** (300 mg, 0.57 mmol) in wet CH_2Cl_2 (1:200 H_2O–CH_2Cl_2) (5 mL) at 0 °C was added NBS (101 mg, 0.57 mmol). TMSOTf solution (10 μL in 1.0 mL CH_2Cl_2, 0.057 mmol) was added in one portion, and the reaction mixture was stirred at the same temperature under N_2. After the reaction was completed (~10 min, monitored by TLC), satd $NaHCO_3$ (10 mL) was added, and the mixture solution was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extract was washed with brine (2 × 50 mL) and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded a yellow oil that was purified by flash chromatography over silica gel (1:8 EtOAc–hexanes) to give pure **4** (206 mg, 75%) as a white solid: mp 61–64 °C: R_f 0.23 (1:6 EtOAc–hexanes); ^{1}H NMR δ 0.10 (s, 3H), 0.15 (s, 3H), 0.91 (s, 9H), 3.57 (t, *J* = 9.4 Hz, 1H, H-4), 3.73 (t, *J* = 10.4 Hz, 1H, H-6a), 3.86–3.90 (m, 1H, H-2), 4.00 (dt, *J* = 4.9 Hz and 9.9 Hz, 1H, H-5), 4.12 (t, *J* = 9.2 Hz, 1H, H-3), 4.29 (dd, *J* = 4.8 and 10.4 Hz, 1H, H-6e), 5.55 (s, 1H), 6.50 (d, *J* = 3.6 Hz, 1H, H-1), 7.35–8.10 (m, 10H); ^{13}C NMR ($CDCl_3$) δ -4.6, -4.0, 18.5, 26.0, 65.4 (C-5), 68.9 (C-6), 72.9 (C-2), 72.9 (C-3), 81.4 (C-4), 92.7 (C-1), 101.9, 126.3, 128.3, 128.8, 129.2, 129.5, 130.1, 133.8, 137.2, 165.3; ESI-TOF-HRMS: calcd for $C_{26}H_{34}O_7SiNa$ [M+Na]$^+$ *m/z* 509.1971; found *m/z* 509.1992.

3.5. 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-α-D-glucopyranose (5)

Crude product from the hydrolysis of **3** (300 mg, 0.566 mmol) was dissolved in a solution of 4:1 CH_2Cl_2–Et_3N (5 mL). The resulting reaction mixture was stirred at room temperature for 24 h. Removal of solvent under reduced pressure afforded a yellow oil that was purified by flash chromatography over silica gel (1:6→1:4 EtOAc–hexanes) to give an inseparable mixture of α and β **5** (234 mg, 85%, α:β = 3.2:1) as a white solid: mp 62–69 °C; (α anomer) R_f 0.25 (1:3 EtOAc–hexanes); ^{1}H NMR δ −0.05 (s, 3H), −0.02 (s, 3H), 0.68 (s, 9H), 3.59 (t, *J* = 9.4 Hz, 1H, H-4), 3.75 (t, *J* = 10.2 Hz, 1H, H-6a), 4.13 (dt, *J* = 4.8 Hz and 10.0 Hz, 1H, H-5), 4.27 (dd, *J* = 5.0 Hz and 10.2 Hz, 1H, H-6e), 4.35 (t, *J* = 9.2 Hz, 1H, H-3), 5.11 (dd, *J* = 3.8 Hz and 9.4 Hz, 1H, H-2), 5.45 (t, *J* = 3.8 Hz, 1H, H-1), 5.54 (s, 1H), 7.32–8.10 (m, 10H); ^{13}C NMR δ −4.7, −4.1, 18.1, 25.7, 62.5 (C-5), 69.0 (C-6), 69.5 (C-3), 74.8 (C-2), 82.3 (C-4), 91.3 (C-1), 102.0, 126.4, 128.2, 128.5, 129.1, 129.7, 130.1, 133.4, 137.2, 166.3; ESI-TOF-HRMS: calcd for $C_{26}H_{34}O_7SiNa$ [M+Na]$^+$ *m/z* 509.1971; found *m/z* 509.1998.

3.6. 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-α-D-glucopyranosyl trichloroacetimidate (6)

To a stirred solution of **5** (2.3 g, 4.73 mmol) in anhyd CH_2Cl_2 (20 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 50 μL). The resulting solution was cooled to 0 °C and trichloroacetonitrile (2.37 mL, 23.67 mmol) was added in one portion. After stirring at room temperature for 5 h, the reaction mixture was concentrated under reduced pressure to afford a yellow oil that was purified by flash chromatography over silica gel (1:8 EtOAc–hexanes) to give a mixture of α and β **6** (2.75 g, 92%, α/β = 8:1) as a white solid: (α anomer) mp 55–58 °C; R_f 0.37 (1:6 EtOAc–hexanes); ^{1}H NMR δ −0.07 (s, 3H), 0.02 (s, 3H), 0.70 (s, 9H), 3.72 (t, *J* = 9.4 Hz, 1H, H-4), 3.80 (t, *J* = 10.4 Hz, 1H, H-6a), 4.08 (dt, *J* = 4.9 Hz and 9.9 Hz, 1H, H-5), 4.36 (dd. *J* = 4.8 Hz and 10.4 Hz, 1H, H-6e), 4.41 (t , *J* = 9.4 Hz, 1H, H-3), 5.35 (dd, *J* = 3.6 Hz and 9.2 Hz, 1H, H-2), 5.59 (s, 1H), 6.56 (d, *J* = 4.0 Hz, 1H, H-1), 7.34–8.03 (m, 10H), 8.51 (s, 1H); ^{13}C NMR δ −4.7, −4.1, 18.2, 25.7, 65.5 (C-5), 68.8 (C-6), 69.9 (C-3), 73.4 (C-2), 81.6 (C-4), 91.1, 94.3 (C-1), 102.0, 126.4, 128.3, 128.5, 129.3, 129.4, 130.1, 133.6, 137.1, 160.9, 165.8; ESI-TOF-HRMS: calcd for $C_{28}H_{34}Cl_3NO_7SiNa$ [M+Na]$^+$ *m/z* 652.1068; found *m/z* 652.1121.

3.7. 1,2-Di-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-α-D-glucopyranose (7)

To a stirred solution of **4** (4.03 g, 8.24 mmol) and DMAP (215 mg, 1.77 mmol) in anhyd pyridine (20 mL) was added benzoyl chloride (1.6 mL, 18 mmol) in one portion. After stirring overnight at room temperature, the reaction mixture was diluted with CH_2Cl_2 (140 mL). The organic solution was washed with satd $NaHCO_3$ (2 × 100 mL), and brine (90 mL), and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded a yellow oil. The crude product was purified by flash chromatography over silica gel (1:15 EtOAc–hexanes) to give pure **7** (4.80 g, 99%) as a white solid: mp 49–52 °C; R_f 0.27 (1:10 EtOAc–hexanes); ^{1}H NMR δ −0.07 (s, 3H), 0.03 (s, 3H), 0.71, (s, 9H), 3.73–3.83 (m, 2H, H-4 and H-6a), 4.10 (dt, *J* = 4.9 Hz and 9.8 Hz, 1H, H-5), 4.34 (dd. *J* = 4.8 Hz and 10.4 Hz, 1H, H-6e), 4.42 (t, *J* = 9.2 Hz, 1H, H-3), 5.42 (dd, *J* = 3.8 Hz and 9.4 Hz, 1H, H-2), 5.60 (s, 1H), 6.60 (d, *J* = 3.6 Hz, 1H, H-1), 7.31–8.07 (m, 15H); ^{13}C NMR δ −4.7, −4.0, 18.2, 25.7, 65.5 (C-5), 68.9 (C-6), 70.3 (C-3), 73.1 (C-2), 81.8 (C-4), 91.1 (C-1), 102.1, 126.4, 128.4, 128.5, 128.9, 129.3, 129.5, 129.5, 129.9, 130.1, 133.5, 134.0, 137.1, 164.8, 165.7; ESI-TOF-HRMS: calcd for $C_{33}H_{38}O_8SiNa$ [M+Na]$^+$ *m/z* 613.2234; found *m/z* 613.2294.

3.8. 1,2-Di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranose (8)

To a stirred solution of **7** (2.5 g, 4.24 mmol) in anhyd THF (10 mL) and anhyd pyridine (1.5 mL) was added HF·Py (2.0 mL, excess) in one portion at 0 °C under N_2. After stirring at room temperature for 48 h, the reaction mixture was diluted with Et_2O (200 mL). The organic solution was washed with satd $NaHCO_3$ (2 × 150 mL), and brine (100 mL), and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded a yellow oil. The crude product was purified by flash chromatography over silica gel (1:3 EtOAc–hexanes) to give pure **8** (1.82 g, 90%) as a white solid: mp 152–154 °C; R_f 0.22 (1:3 EtOAc–hexanes); ^{1}H NMR δ 3.76–3.84 (m, 2H, H-4 and H-6a), 4.08–4.14 (m, 1H, H-5), 4.35 (dd, *J* = 5.2 Hz and 10.4 Hz, 1H, H-6e), 4.51 (t, *J* = 9.4 Hz, 1H, H-3), 5.39 (dd, *J* = 3.8 Hz and 9.8 Hz, 1H, H-2), 5.62 (s, 1H), 6.66 (d, *J* = 4.0 Hz, 1H, H-1), 7.24–8.09 (m, 15H); ^{13}C NMR δ 65.0 (C-5), 68.7 (C-6), 69.4 (C-3), 72.6 (C-2), 81.0 (C-4), 90.6 (C-1), 102.2, 126.4, 128.6, 128.9, 129.1, 129.2, 129.6, 130.0, 130.1, 133.6, 134.0, 136.9, 164.7, 166.0; ESI-TOF-HRMS: calcd for $C_{27}H_{24}O_8Na$ [M+Na]$^+$ *m/z* 499.1369; found *m/z* 499.1435.

3.9. Ethyl 2-*O*-(4-acetoxy-2,2-dimethylbutanoyl)-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside (10)

To a stirred solution of **9**[13] (1.71 g, 5.45 mmol) in anhyd pyridine (15 mL) at 0 °C was slowly added 4-acetoxy-2,2-dimethylbutyryl chloride (1.2 g, 6.2 mmol). The reaction mixture was stirred at room temperature for 24 h. Upon removal of solvent under reduced pressure, the residue was dissolved in EtOAc (150 mL) and water (30 mL). The organic layer was washed with satd $NaHCO_3$ (2 × 130 mL), and brine (100 mL), and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded a yellow oil. The crude product was purified by flash chromatography over silica gel (1:3 EtOAc–hexanes) to give pure **10** (2.20 g, 86%) as a white solid: mp 75–77 °C; R_f 0.27 (1:3 EtOAc–hexanes); ^{1}H NMR δ 1.21 (s, 3H), 1.23 (t, *J* = 7.4 Hz, 3H), 1.27 (s, 3H), 1.75–1.82 (m, 1H), 2.00 (s, 3H), 2.02–2.10 (m, 1H), 2.47–2.58 (m, 2H), 3.19 (d, *J* = 2.8 Hz, 1H, hydroxyl proton) 3.57–3.81 (m, 1H, H-4), 3.75–3.81 (m, 1H, H-6a), 3.98–4.05 (m, 1H, one of methylene protons in AcO-CH_2), 4.11 (dt, *J* = 2.7 Hz and 10.4 Hz, 1H, H-3), 4.18–4.30 (m, 3H, H-5, H-6e and one of methylene protons in AcO-CH_2), 4.90 (dd, *J* = 5.8 Hz and 9.8 Hz, 1H, H-2), 5.55 (s, 1H), 5.61 (d, *J* = 6.0 Hz, 1H, H-1), 7.33–7.37 (m, 3H), 7.48–7.50 (m, 2H); ^{13}C NMR δ 14.9, 21.0, 24.2, 24.6, 26.1, 38.2, 40.7, 61.6, 62.6 (C-5), 68.6 (C-6), 68.9 (C-3), 73.7 (C-2), 81.1 (C-4), 82.4 (C-1), 101.9, 126.4, 128.3, 129.2, 137.1, 171.3, 176.3; ESI-TOF-HRMS: calcd for $C_{23}H_{32}O_8SNa$ $[M+Na]^+$ *m/z* 491.1716; found *m/z* 491.1783.

3.10. 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranosyl]-(1→3)-1,2-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranose (11)

A mixture of the glucosyl acceptor **8** (840 mg, 1.76 mmol) and glucosyl donor **3** (1.13 g, 2.13 mmol), 4 Å molecular sieves (1.5 g) and NIS (660 mg, 2.93 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature under N_2 for 1 h. The reaction mixture was cooled to −20 °C and AgOTf solution (100 mg in 1 mL toluene, 0.389 mmol) was added. After being stirred at the same temperature under N_2 for 2 h, the reaction was quenched by addition of Et_3N (2 mL). The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to afford a yellow oil that was purified by flash chromatography over silica gel (1:4 EtOAc–hexanes) to give pure **11** (1.58 g, 95%) as a white solid: mp 124–126 °C; R_f 0.27 (1:4 EtOAc–hexanes); ^{1}H NMR δ −0.29 (s, 3H), −0.15 (s, 3H), 0.60 (s, 9H), 3.49 (dt, *J* = 4.8 Hz and 9.8 Hz, 1H, H-5$^{\rm II}$), 3.65 (t, *J* = 9.2 Hz, 1H, H-4$^{\rm II}$), 3.76 (t, *J* = 10.2 Hz, 1H, H-6a$^{\rm II}$), 3.80 (t, *J* = 10.2 Hz, 1H, H-6a$^{\rm I}$), 3.85–3.91 (m, 2H, H-3$^{\rm II}$ and H-4$^{\rm I}$), 4.11 (dt, *J* = 4.5 Hz and 9.9 Hz, 1H, H-5$^{\rm I}$), 4.30–4.36 (m, 2H, H-6e$^{\rm I}$ and H-6e$^{\rm II}$), 4.49 (t, *J* = 9.4 Hz, 1H, H-3$^{\rm I}$), 4.96 (d, *J* = 7.6 Hz, 1H, H-1$^{\rm II}$HH), 5.22 (t, *J* = 8.0 Hz, 1H, H-2$^{\rm II}$), 5.27 (dd, *J* = 3.8 Hz and 9.4 Hz, 1H, H-2$^{\rm I}$), 5.42 (s, 1H), 5.62 (s, 1H), 6.57 (d, *J* = 4.0 Hz, 1H, H-1$^{\rm I}$), 7.10–7.98 (m, 25H); ^{13}C NMR δ −5.0, −4.1, 18.0, 25.6, 65.4 (C-5$^{\rm I}$), 66.5 (C-5$^{\rm II}$), 68.7 (C-6$^{\rm I\ or\ II}$), 68.9 (C-6$^{\rm I\ or\ II}$), 72.5 (C-2$^{\rm I}$), 73.1 (C-3$^{\rm II}$), 75.4 (C-2$^{\rm II}$ and C-3$^{\rm I}$), 79.1 (C-4$^{\rm I}$), 81.4 (C-4$^{\rm II}$), 90.4 (C-1$^{\rm I}$), 101.3 (C-1$^{\rm II}$), 101.4, 101.9, 126.2, 126.4, 128.2, 128.3, 128.4, 128.5, 128.7, 129.0, 129.15, 129.19, 129.3, 129.5, 129.6, 129.0, 130.0, 132.9, 133.4, 133.9, 137.2, 137.2, 164.5, 164.9, 165.0; ESI-TOF-HRMS: calcd for $C_{53}H_{56}O_{14}SiNa$ $[M+Na]^+$ *m/z* 967.3337; found *m/z* 967.3327.

3.11. 2-*O*-Benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-1,2-di-O-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranose (12)

To a stirred solution of **11** (2.11 g, 2.22 mmol) in anhyd THF (20 mL) and anhyd pyridine (1.8 mL) was added HF·Py (1.8 mL, excess) in one portion at 0 °C under N_2. After stirring at room temperature for 48 h, the reaction mixture was diluted with diethyl ether (180 mL). The organic solution was washed with satd $NaHCO_3$ (2 × 150 mL), and brine (120 mL), and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded a yellow oil. The crude product was purified by flash chromatography over silica gel (1:2 EtOAc–hexanes) to give pure **12** (1.69 g, 92%) as a white solid: mp 134–137 °C; R_f 0.30 (1:2 EtOAc–hexanes); ^{1}H NMR δ 3.52 (dt, *J* = 4.8 Hz and 9.5 Hz, 1H, H-5$^{\rm II}$), 3.71–3.78 (m, 2H, H-4$^{\rm II}$ and H-6a$^{\rm II}$), 3.81 (t, *J* = 10.6 Hz, 1H, H-6a$^{\rm I}$), 3.86–3.93 (m, 2H, H-3$^{\rm II}$ and H-4$^{\rm I}$), 4.12 (dt, *J* = 5.2 Hz and 10.0 Hz, 1H, H-5$^{\rm I}$), 4.30–4.35 (m, 2H, H-6e$^{\rm I}$ and H-6e$^{\rm II}$), 4.53 (t, *J* = 9.4 Hz, 1H, H-3$^{\rm I}$), 5.04 (d, *J* = 7.2 Hz, 1H, H-1$^{\rm II}$HH), 5.15 (dd, *J* = 7.4 Hz and 8.2 Hz, 1H, H-2$^{\rm II}$), 5.33 (dd, *J* = 4.0 Hz and 9.6 Hz, 1H, H-2$^{\rm I}$), 5.40 (s, 1H), 5.63 (s, 1H), 6.60 (d, *J* = 4.0 Hz, 1H, H-1$^{\rm I}$), 7.15–7.99 (m, 20H); ^{13}C NMR δ 65.2 (C-5$^{\rm I}$), 66.2 (C-5$^{\rm II}$), 68.5 (C-6$^{\rm I\ or\ II}$), 68.6 (C-6$^{\rm I\ or\ II}$), 72.25 (C-2$^{\rm I}$), 72.29 (C-3$^{\rm II}$), 75.1 (C-2$^{\rm II}$), 75.5 (C-3$^{\rm I}$), 78.9 (C-4$^{\rm I}$), 80.5 (C-4$^{\rm II}$), 90.3 (C-1$^{\rm I}$), 100.9 (C-1$^{\rm II}$), 101.3, 101.7, 126.1, 126.4, 128.2, 128.29, 128.34, 128.4, 128.67, 128.7, 129.0, 129.1, 129.3, 129.6, 129.9, 133.0, 133.4, 133.9, 137.0, 137.0, 164.5, 164.9, 165.6; ESI-TOF-HRMS: calcd for $C_{47}H_{42}O_{14}Na$ $[M+Na]^+$ *m/z* 853.2472; found *m/z* 853.2445.

3.12. Ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranosyl-(1→3)-2-*O*-(4-acetoxy-2,2-dimethylbutanoyl)-4,6-*O*-benzylidene-1-thio–D-glucopyranoside (13)

A mixture of the glucosyl acceptor **10** (912 mg, 1.95 mmol) and glucosyl donor **6** (1.36 g, 2.16 mmol) and 4 Å molecular sieves (1.5 g) in CH_2Cl_2 (20 mL) was stirred at room temperature under N_2 for 1 h. Then it was cooled to −70 °C and a TMSOTf solution (39 µL in 2.16 mL CH_2Cl_2, 0.1 M, 0.216 mmol) was added. After being stirred at −70 °C under N_2 for 6 h, the reaction was quenched by addition of Et_3N (2 mL). The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to afford a yellow oil that was purified by flash chromatography over silica gel (1:5 EtOAc–hexanes) to give pure **13** (1.67 g, 92%) as a white solid: mp 68–70 °C; R_f 0.30 (1:4 EtOAc–hexanes); ^{1}H NMR δ −0.16 (s, 3H), −0.10, (s, 3H), 0.69 (s, 9H), 1.14 (s, 3H), 1.14 (s, 3H), 1.18 (t, *J* = 7.4 Hz, 3H), 1.84 (dd, *J* = 6.6 Hz and 8.2 Hz, 2H), 2.01 (s, 3H), 2.42–2.52 (m, 2H), 3.40 (dt, *J* = 4.8 Hz and 9.8 Hz, 1H, H-5$^{\rm II}$), 3.66–3.71 (m, 3H, H-4$^{\rm I}$, H-4$^{\rm II}$ and H-6a$^{\rm II}$), 3.77 (t, *J* = 10.0 Hz, 1H, H-6a$^{\rm I}$), 3.88 (dd, *J* = 7.4 Hz and 8.6 Hz, 1H, H-3$^{\rm II}$), 4.04–4.10 (m, 2H, AcO-CH_2), 4.20–4.31 (m, 4H, H-3$^{\rm I}$, H-5$^{\rm I}$, H-6e$^{\rm I}$ and H-6e$^{\rm II}$), 4.88 (dd, *J* = 6.0 Hz and 9.6 Hz, 1H, H-2$^{\rm I}$), 5.00 (d, *J* = 6.8 Hz, 1H, H-1$^{\rm II}$), 5.17 (t, *J* = 7.0 Hz, 1H, H-2$^{\rm II}$), 5.18 (s, 1H), 5.53 (s, 1H), 5.60 (d, *J* = 5.6 Hz, 1H, H-1$^{\rm I}$), 7.29–7.95 (m, 15H); ^{13}C NMR δ -4.9, -4.2, 14.9, 18.0, 21.2, 24.2, 25.08, 25.14, 25.7, 38.0, 40.8, 61.5, 62.9 (C-5$^{\rm I}$), 66.2 (C-5$^{\rm II}$), 68.8 (C-6$^{\rm I}$ and C-6$^{\rm II}$), 73.3 (C-3$^{\rm II}$), 73.7 (C-3$^{\rm I}$), 74.0 (C-2$^{\rm I}$), 75.5 (C-2$^{\rm II}$), 79.7 (C-4$^{\rm I}$), 81.1 (C-4$^{\rm II}$), 82.1 (C-1$^{\rm I}$), 99.4 (C-1$^{\rm II}$), 101.5, 101.9, 126.3, 126.4, 128.1, 128.4, 128.5, 129.0, 129.4, 129.8, 130.0, 133.2, 137.27, 137.3, 165.1, 171.0, 176.2; ESI-TOF-HRMS: calcd for $C_{49}H_{64}O_{14}SSiNa$ $[M+Na]^+$ *m/z* 959.3684; found *m/z* 959.3654.

3.13. 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranosyl-(1→3)-2-*O*-(4-acetoxy-2,2-dimethylbutanoyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-1,2 di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranose (14)

A mixture of the disaccharide acceptor **12** (370 mg, 0.446 mmol) and disaccharide donor **13** (560 mg, 0.598 mmol), 4 Å molecular sieves (1.0 g) and NIS (185 mg, 0.882 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature under N_2 for 1 h. The reaction mixture was cooled to −20 °C and a AgOTf solution (27 mg in 1.0 mL toluene, 0.105 mmol) was added. After being stirred at −20 °C under N_2 for 4 h, the reaction was quenched by addition of Et_3N (1 mL). The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to afford a yellow oil that was purified by flash chromatography over silica gel (2:5 EtOAc–hexanes) to give pure **14** (694 mg, 91%) as a white solid: mp 158–161 °C; R_f 0.37 (1:2 EtOAc–hexanes); ^{1}H NMR δ −0.17 (s, 3H), −0.07 (s, 3H), 0.67 (s, 9H), 0.83 (s, 3H), 0.87 (s, 3H), 1.55–1.61 (m, 2H), 1.90 (s, 3H), 2.58 (t, *J* = 9.6 Hz, 1H, H-4$^{\rm III}$), 3.27 (t, *J* = 10.0 Hz, 1H), 3.32–3.52 (m, 4H), 3.58–3.67 (m, 2H), 3.71–3.76 (m, 2H), 3.79–4.37 (m, 12H), 4.41 (t, *J* = 9.4 Hz, 1H, H-3$^{\rm I}$), 4.56–4.60 (m, 2H, H-2$^{\rm II}$ and H-2$^{\rm III}$), 4.66 (br s: *J* < 1.0 Hz, 1H, H-1$^{\rm III}$), 4.75 (d, *J* = 7.6 Hz, 1H, H-1$^{\rm II}$), 5.10 (d, *J* = 8.0 Hz, 1H, H-1$^{\rm IV}$), 5.22 (s, 1H), 5.29 (t, *J* = 8.0 Hz, 1H, H-2$^{\rm IV}$), 5.33 (dd, *J* = 4.0 Hz and 9.6 Hz, 1H, H-2$^{\rm I}$), 5.48 (s, 1H), 5.76 (s, 1H), 6.56 (d, *J* = 4.0 Hz, 1H, H-1$^{\rm I}$), 6.95–8.13 (m, 40H); ^{13}C NMR δ −4.9, −4.2, 18.0, 21.0, 24.77, 24.79, 25.7, 37.9, 40.5, 60.9, 64.7, 65.5, 66.3, 66.5, 68.8, 71.6, 71.8, 72.2, 73.0, 74.6, 74.95, 74.99, 75.4, 76.7, 77.9, 79.1, 81.7, 90.4 (C-1$^{\rm I}$), 95.9 (C-1$^{\rm III}$), 97.1 (C-1$^{\rm IV}$), 99.9, 100.8 (C-1$^{\rm II}$), 101.7, 101.8, 102.5, 126.1, 126.35, 126.39, 126.8, 127.8, 128.2, 128.4, 128.49, 128.51, 128.6, 128.8, 128.9, 129.1, 129.1, 129.2, 129.5, 129.59, 129.6, 129.7, 129.9, 130.0, 130.3, 133.1, 133.5, 133.9, 134.1, 137.1, 137.2, 137.3, 137.5, 164.5, 164.6, 164.8, 165.4, 170.9, 175.7; ESI-TOF-HRMS: calcd for $C_{94}H_{100}O_{28}SiNa$ $[M+Na]^+$ *m/z* 1727.6068; found *m/z* 1727.6066.

3.14. 2-*O*-Benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-(4-acetoxy-2,2-dimethylbutanoyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-1,2-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranose (15)

To a stirred solution of **14** (700 mg, 0.411 mmol) in anhyd THF (10 mL) and anhyd pyridine (1 mL) was added HF·Py (1 mL, excess) in one portion at 0 °C under N_2. After stirring at room temperature for 48 h, the reaction mixture was diluted with Et_2O (150 mL). The organic solution was washed with satd $NaHCO_3$ (2 × 90 mL), and brine (80 mL), and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded a yellow oil. The crude product was purified by flash chromatography over silica gel (4:7 EtOAc–hexanes) to give pure **15** (607 mg, 93%) as a white solid: mp 166–170 °C; R_f 0.26 (5:8 EtOAc–hexanes); ^{1}H NMR δ 0.86 (s, 3H), 0.90 (s, 3H), 1.57–1.61 (m, 2H), 1.90 (s, 3H), 2.82 (t, *J* = 9.4 Hz, 1H, H-4$^{\mathrm{III}}$), 3.31 (t, *J* = 10.2 Hz, 1H), 3.37–3.56 (m, 4H), 3.64–3.77 (m, 4H), 3.81–4.37 (m, 12H), 4.44 (t, *J* = 9.6 Hz, 1H, H-3$^{\mathrm{I}}$), 4.64 (broad s, 1H, H-2$^{\mathrm{III}}$), 4.70 (d, *J* = 2.0 Hz, 1H, H-1$^{\mathrm{III}}$), 4.73 (t, *J* = 7.6 Hz, 1H, H-2$^{\mathrm{II}}$), 4.81 (d, *J* = 6.8 Hz, 1H, H-1$^{\mathrm{II}}$), 5.15 (d, *J* = 7.6 Hz, 1H, H-1$^{\mathrm{IV}}$), 5.15 (s, 1H), 5.25 (t, *J* = 8.2 Hz, 1H, H-2$^{\mathrm{IV}}$), 5.31 (dd, *J* = 4.0 Hz and 9.6 Hz, 1H, H-2$^{\mathrm{I}}$), 5.47 (s, 1H), 5.74 (s, 1H), 6.56 (d, *J* = 3.6 Hz, 1H, H-1$^{\mathrm{I}}$), 7.04–8.14 (m, 40H); ^{13}C NMR δ 20.9, 24.6, 24.7, 37.8, 40.3, 60.8, 64.6, 65.4, 66.1, 66.2, 68.6, 71.7, 72.2, 72.4, 74.3, 74.8, 75.1, 75.2, 76.7, 77.8, 79.0, 80.9, 90.3 (C-1$^{\mathrm{I}}$), 96.1 (C-1$^{\mathrm{III}}$), 97.0 (C-1$^{\mathrm{IV}}$), 100.0, 100.5 (C-1$^{\mathrm{II}}$), 101.6, 101.8, 102.3, 126.1, 126.3, 126.4, 126.7, 127.8, 128.35, 128.38, 128.4, 128.7, 128.8, 128.97, 129.0, 129.3, 129.4, 129.6, 129.8, 130.0, 133.1, 133.4, 133.8, 134.1, 137.0, 137.1, 137.3, 164.4, 164.5, 164.8, 165.8, 170.9, 175.5; ESI-TOF-HRMS: calcd for $C_{88}H_{86}O_{28}Na$ $[M+Na]^+$ *m/z* 1613.5203; found *m/z* 1613.5166.

3.15. 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranosyl-(1→3)-2-*O*-(4-acetoxy-2,2-dimethylbutanoyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-(4-acetoxy-2,2-dimethylbutanoyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-1,2-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranose (16)

A mixture of the tetrasaccharide acceptor **15** (370 mg, 0.223 mmol) and disaccharide donor **13** (350 mg, 0.374 mmol), 4 Å molecular sieves (800 mg) and NIS (129 mg, 0.573 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature under N_2 for 1 h. The reaction mixture was cooled to −20 °C and a AgOTf solution (20 mg in 0.7 mL toluene, 0.778 mmol) was added. After being stirred at −20 °C under N_2 for 6 h, the reaction was quenched by addition of Et_3N (1 mL). The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to afford a yellow oil that was purified by flash chromatography over silica gel (4:7 EtOAc–hexanes) to give pure **16** (527 mg, 92%) as a white solid: mp 169–172 °C; R_f 0.21 (1:2 EtOAc–hexanes); ^{1}H NMR δ −0.13 (s, 3H), −0.04 (s, 3H), 0.71 (s, 9H), 0.81 (s, 3H), 0.86 (s, 3H), 0.89 (s, 3H), 0.93 (s, 3H), 1.53–1.67 (m, 4H), 1.90 (s, 3H), 1.96 (s, 3H), 2.32 (t, *J* = 9.2 Hz, 1H), 2.54 (t, *J* = 9.4 Hz, 1H) , 3.28–3.67 (m, 12H), 3.71–4.15 (m, 18H), 4.22–4.41 (m, 4H), 4.49–4.53 (m, 2H, two H-2), 4.62 (d, *J* = 0.8 Hz, 1H, H-1$^{\mathrm{III}}$), 4.71–4.76 (m, 4H, H-1$^{\mathrm{II}}$ and H-1$^{\mathrm{V}}$ and two H-2), 4.90 (d, *J* = 8.0 Hz, 1H, H-1$^{\mathrm{VI}}$), 5.19 (d, *J* = 8.4 Hz, 1H, H-1$^{\mathrm{IV}}$), 5.53 (s, 1H), 5.28–5.36 (m, 3H, H-2$^{\mathrm{I}}$, H-2$^{\mathrm{IV}}$ and benzylidene proton), 5.51 (s, 1H), 5.72, (s, 1H), 6.54 (d, *J* = 4.0 Hz, 1H, H-1$^{\mathrm{I}}$), 6.94–8.21 (m, 55H); ^{13}C NMR δ −4.9, −4.3, 17.9, 20.9, 21.0, 24.5, 24.7, 25.6, 37.8, 37.9, 40.3, 40.4, 60.7, 60.8, 64.5, 65.3, 66.0, 66.2, 66.4, 68.4, 68.6, 68.7, 71.1, 71.2, 71.6, 71.8, 71.9, 73.0, 74.2, 74.35, 74.4, 74.5, 74.8, 75.6, 76.7, 76.8, 77.9, 78.1, 79.1, 81.5, 90.3 (C-1$^{\mathrm{I}}$), 95.8 (C-1$^{\mathrm{V}}$), 95.9 (C-1$^{\mathrm{III}}$ and C-1$^{\mathrm{VI}}$), 96.5 (C-1$^{\mathrm{IV}}$), 99.9, 100.0, 100.8 (C-1$^{\mathrm{II}}$), 101.5, 101.6, 102.4, 102.7, 126.1, 126.2, 126.3, 126.7, 126.8, 127.7, 127.8, 128.1, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.1, 129.2, 129.4, 129.5, 129.6, 129.8, 130.3, 133.0, 133.3, 133.8, 134.2, 137.1, 137.16, 137.2, 137.3, 137.33, 137.5, 164.3, 164.4, 164.7, 165.0, 165.3, 170.6, 170.8, 175.6, 175.9; ESI-TOF-HRMS: calcd for $C_{135}H_{144}O_{42}SiNa$ $[M+Na]^+$ *m/z* 2487.8799; found *m/z* 2487.8750.

3.16. X-ray crystallography of 16

A colorless, crystalline mass obtained by the slow diffusion of cyclohexane into a solution of **16** in toluene was separated under a microscope to give a single crystal which was coated with Paratone oil, mounted on a thin Nylon loop and placed in the cold nitrogen stream of a Kryoflex attachment on a Bruker-AXS APEX I diffractometer. A crystal-to-detector distance of 120 mm was used because of the length of the *c*-axis, and a data collection scheme comprising 14 runs with a combination of ω and φ scans, an 0.5° width per frame, and a scan time of 30 s/frame was prepared by COSMO.[21] Data collection was controlled by the APEX2 program suite.[22] The raw data were processed with SAINT[23] to a resolution limit of 0.93 Å, which also provided a final unit cell based on a least-squares refinement of 9514 reflections chosen from diverse regions of reciprocal space, while corrections for possible crystals decay and merging of symmetry equivalent reflections were performed with SADABS.[24] Further crystallographic details are presented in Table 2. Approximately 60% of the structure was revealed by the direct methods program SHELXM,[25] and the remainder slowly developed by a combination of full-matrix, least-squares refinement, followed by computation of a difference Fourier synthesis (SHELXL[26]). Because of the limited resolution of the data set (very little significant measurable intensity beyond a 0.93 Å resolution shell and none at all beyond a 0.84 Å shell), all phenyl rings were refined as rigid groups of ideal geometry. Those carbon atoms showing extremely elongated displacement ellipsoids were restrained to approximate more isotropic behavior. Hydrogen atoms were included as riding contributions with isotropic displacement parameters tied to those of the attached carbon atoms using appropriate SHELXL HFIX instructions. Residual diffuse electron density appeared in a final difference map (following inclusion of two molecules of solvent cyclohexane, each of half occupancy). More highly disordered solvent was removed with the SQUEEZE option of the PLATON program suite.[27] Calculations other than those noted above were performed with the SHELXLS software package.[28]

3.17. 2-*O*-Benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-(4-acetoxy-2,2-dimethylbutanoyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-(4-acetoxy-2,2-dimethylbutanoyl)-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-1,2-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranose (17)

To a stirred solution of **16** (700 mg, 0.284 mmol) in anhyd THF (10 mL) and anhyd pyridine (1 mL) was added HF·Py (1 mL, excess) in one portion at 0 °C under N_2. After stirring at room temperature for 48 h, the reaction mixture was diluted with Et_2O (150 mL). The organic solution was washed with satd $NaHCO_3$ (2 × 90 mL), brine (80 mL), and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded a yellow oil. The crude product was purified by flash chromatography over silica gel (2:3 EtOAc–hexanes) to give pure **17** (621 mg, 93%) as a white solid: mp 172–175 °C; R_f 0.36 (2:3 EtOAc–hexanes); ^{1}H NMR δ 0.81 (s, 3H), 0.86 (s, 3H), 0.89 (s, 3H), 0.93 (s, 3H), 1.53–1.67 (m, 4H), 1.89 (s, 3H), 1.96 (s, 3H), 2.49 (t, J = 9.4 Hz, 1H, H-4^{V}), 2.55 (t, J = 9.4 Hz, 1H, H-4^{III}) , 2.61 (d, J = 3.6 Hz, 1H, hydroxyl proton), 3.30–3.62 (m, 10H), 3.67–4.15 (m, 20H), 4.22–4.41 (m, 4H), 4.51–4.55 (m, 2H, H-2^{II} and H-2^{III}), 4.63 (d, J = 1.2 Hz, 1H, H-1^{III}), 4.73 (d, J = 7.6 Hz, 1H, H-1^{II}) 4.79 (s, 2H, H-1^{V} and H-2^{V}), 4.90–4.97 (m, 2H, H-1^{VI} and H-2^{VI}), 5.18 (s, 1H), 5.24 (d, J = 8.0 Hz, 1H, H-1^{IV}), 5.27–5.32 (m, 3H, H-2^{I}, H-2^{IV} and benzylidene proton), 5.51 (s, 1H), 5.72, (s, 1H), 6.54 (d, J = 3.6 Hz, 1H, H-1^{I}), 7.04–8.22 (m, 55H); ^{13}C NMR δ 21.0, 21.1, 24.7, 24.9, 37.9, 37.94, 40.4, 40.6, 60.9, 61.0, 64.6, 64.7, 65.4, 66.1, 66.3, 68.5, 68.7, 68.76, 68.8, 71.3, 71.35, 72.0, 72.1, 72.5, 72.6, 74.35, 74.4, 74.9, 75.0, 75.7, 78.1, 78.4, 79.2, 81.1, 90.4 (C-1^{I}), 96.05 (C-1^{VI}), 96.1 (C-1^{V}), 96.2 (C-1^{III}), 96.6 (C-1^{IV}), 100.3, 100.9 (C-1^{II}), 101.7, 102.0, 102.5, 102.8, 126.3, 126.3, 126.3, 126.5, 126.78, 126.8, 127.9, 127.94, 128.46, 128.49, 128.6, 128.7, 128.75, 128.8, 129.0, 129.1, 129.2, 129.3, 129.4, 129.5, 129.6, 129.69, 129.7, 130.0, 130.1, 133.1, 133.5, 133.9, 134.4, 134.6, 137.1, 137.16, 137.2, 137.3, 137.4, 137.5, 164.48, 164.5, 164.8, 165.2, 166.0, 170.8, 171.0, 175.7, 175.9; ESI-TOF-HRMS: calcd for $C_{129}H_{130}O_{42}Na$ [M+Na]$^+$ *m/z* 2373.7934; found *m/z* 2373.7959.

3.18. Laminarahexaose (1)

A solution of **17** (50 mg, 0.021 mmol) in MeOH (8 mL), THF (4 mL), and HOAc (2.4 mL) was treated with Pd/C (220 mg, 10 wt %, Degussa type), and the mixture was hydrogenated (60 psi) for 4.5 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to afford a yellow oil that was co-evaporated with toluene (3 × 50 mL) to remove traces of HOAc. The crude product was purified by flash chromatography over silica gel using a gradient of 1:15→1:5 MeOH–CH_2Cl_2 to give the hydrogenolysis product (32 mg, 83%) as a white solid. To a stirred solution of the purified hydrogenolysis product (32 mg, 0.018 mmol) in MeOH (3 mL) was added NaOMe–MeOH solution (9 μL, 1.0 M, 0.009 mmol) at room temperature, and the reaction mixture was stirred for 24 h. The reaction mixture was then neutralized with Amberlite IRP®-64 ion-exchange resin (H$^+$ form, 40 mg). After removal of the resin by filtration, the filtrate was concentrated under reduced pressure, and the crude product was purified by size-exclusion chromatography over lipophilic Sephadex LH-20 (1:1 MeOH–H_2O) and lyophilized to give an inseparable mixture of α- and β-**1** (18 mg, 100% α:β = 1:1.2) as a white solid. ^{1}H NMR (DMSO/D_2O/TFAA: 95/5/trace) δ 3.00–3.69 (m, 36H), 4.35 (d, J = 7.6 Hz, β H-1^{I}), 4.38 (d, J = 7.6 Hz, 1H^{VI}), 4.42–4.45 (m, 1H, H-1^{II}), 4.51 (d, J = 8.0 Hz, 3H, H-1$^{III,\ IV,\ \mathrm{and}\ V}$), 4.95 (d, J = 3.6 Hz, α H-1); ^{13}C NMR (D_2O, acetone as standard) δ 61.2, 68.6, 70.1, 71.6, 71.8, 73.8, 73.9, 74.0, 74.4, 76.1, 76.2, 76.5, 82.8, 84.5, 84.7, 85.0, 92.6 (α C-1^{I}), 96.2 (β C-1^{I}), 103.1 (C-1$^{II,\ III,\ IV,\ \mathrm{and}\ V}$), 103.4 (C-1^{VI}); ESI-TOF-HRMS: calcd for $C_{36}H_{62}O_{31}Na$ [M+Na]$^+$ *m/z* 1013.3173; found *m/z* 1013.3160.

Supplementary data

Included in the Supplementary data are X-ray tables for compound **16** including atomic coordinates, bond lengths, and bond angles. Complete crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Center, CCDC no. 701670. Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK. (fax: +44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk or via: www.ccdc.cam.ac.uk). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.12.014.

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