Selectively Protected Disaccharide Building Blocks for Modular Synthesis of Heparin Fragments[‡]

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A modular approach for the synthesis of heparin fragments is described. Levulinoyl esters were employed to protect those hydroxy groups intended to be sulfated in the final product, while acetyl esters and benzyl ethers were used as the permanent protecting groups. A highly efficient chemoenzymatic reaction sequence was used for the deprotection of an *O*-sulfated fragment, while the final stage of the synthesis entailed a selective oxidation of a primary alcohol of a glucoside with TEMPO/NaOCl to give a glucuronic acid moiety.

Introduction

Heparan sulfates (HSs) are highly *N*- and *O*-sulfated polysaccharides that are important for a large number of biological processes, and many enzymes, growth factors, enzyme inhibitors, chemokines and extracellular matrix components, cell adhesion molecules, and microbial proteins require HSs for their functions. Among others, HS-protein complexes have been implicated in modulation of embryonic development (by growth factor modulation), inhibition of blood coagulation, organization of the extracellular matrix, angiogenesis, anchorage of cells, the presentation of enzymes and cytokines on cell surfaces, and as "co-receptors" in viral infections.[1]

The biosynthesis of HSs involves partial *N*-deacetylation of a protein-linked polysaccharide composed of β-GlcA(1−4)GlcNAc repeating units, followed by *N*-sulfation, substrate-directed epimerizations of glucuronic acid to iduronic acid moieties, and − finally − selective *O*-sulfations.[2] Although these enzymatic modifications result in a mixture of very complex polysaccharides, detailed structural studies have shown that HSs are composed of only 19 distinct disaccharide subunits, differing in their sulfation pattern and in the presence of either D-glucuronic or L-iduronic acid.

It has often been difficult to determine the oligosaccharide sequence and sulfation pattern of an HS fragment required for activation or deactivation of a given protein.[3] In order to address this important issue, we are developing a modular approach for the chemical synthesis of HSs, in which a set of presynthesized disaccharide building blocks resembling the different disaccharide motives found in HSs can be used for the assembly of a wide range of sulfated oligosaccharides. The proposed methodology would thus make it possible to synthesize any putative HS fragment in an efficient way for biological studies.

We have reported[4] a strategy for the synthesis of HS fragments previously. A sulfated oligosaccharide containing glucoside and idoside moieties was synthesized first, and this compound could then selectively be oxidized to the corresponding uronic acids. This approach avoided problems caused by the presence of uronic acids, such as epimerization of the C-5 position, poor glycosyl donating properties, and difficulties with protecting group manipulations. Another important feature of our approach is that the primary hydroxy groups of glucosamine moieties were protected as TBDPS ethers to avoid oxidation. Their removal, however, could easily be accomplished by treatment with HF in pyridine.

Here we describe a set of protecting groups that can be employed in combination with the approach described above for modular synthesis of HS fragments. In particular, it is shown that levulinoyl esters can be used to protect those hydroxy groups that should be sulfated in the end product. Furthermore, it was possible to protect those C-2 hydroxy groups that did not need sulfation as acetyl esters, and these esters could be enzymatically removed at the end of the synthetic sequence in the presence of the acid- and base-sensitive sulfate esters by use of *Pseudomonas* lipase type B.

Results and Discussion

A key issue for the development of a modular approach for heparin synthesis is the selection of a set of protecting groups that meet the following requirements:

[‡] Part 2. Part 1: Ref.[4]

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i. the C-2 hydroxy-protecting groups of the glucose or idose moieties should allow stereoselective introduction of β(1→4)-glycosidic linkages, whereas the C-2 amino group of the glucosamine derivatives need to be derivatized in such a way that α(1→4)-glycosidic linkages can be formed,

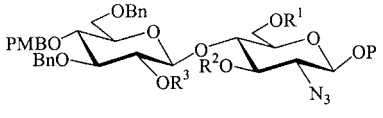
ii. a protecting group that can be selectively removed to reveal those hydroxy groups that need sulfation is required,

iii. the protecting group scheme should allow the selective oxidation of C-6 hydroxy groups to give glucuronic and iduronic acid moieties,

iv. the removal of the permanent protecting groups should be compatible with the presence of base- and acid-labile sulfate esters, and finally

v. the protecting-group scheme should be applicable to the synthesis of each of the 19 targeted disaccharides.

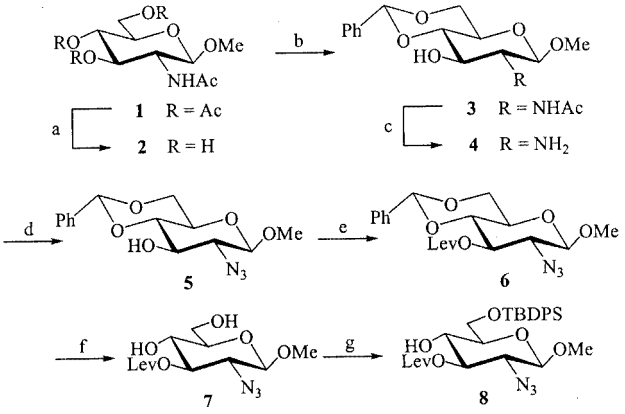
Our proposed generic protecting-group scheme is shown in Figure 1. Thus, levulinoyl esters (Lev)[5] should be employed for those hydroxy groups that need sulfation. In HSs, the C-3 and C-6 hydroxy groups of the glucosamine and the C-2 hydroxy groups of the hexuronic acid moieties may be sulfated, and so, depending on the sulfation pattern of a targeted disaccharide building block, one or more of these positions should be protected by one or more Lev groups. An important feature of the Lev ester is that, when present at the C-2′ position, it should be able to direct the formation of 1,2-*trans*-glycosides through neighboring-group participation. In cases in which the C-2′ position of a building block does not need sulfation, an acetyl group should be employed as a permanent protecting group. This ester can also enter into neighboring-group participation, but is stable under the conditions used for the removal of the Lev groups. It was anticipated that, after introduction of sulfate esters, the permanent acetyl groups should be removable without the sulfate esters being affected.[6] The latter was a concern because sulfate esters are hydrolyzed under relatively mild acidic or basic conditions. Benzyl ethers should be used as protecting groups for primary hydroxy groups intended to be oxidized to the carboxylic acids and for secondary hydroxy groups that should remain unsulfated in the final product. Finally, a *tert*-butyldiphenylsilyl (TBDPS)[7] ether should be employed for the protection of the C-6 position of the glucosamine residues. Thus, after the assembly of an oligosaccharide and the introduction of sulfate esters, the acetyl esters and benzyl ethers should be removed. Next, the free C-6 hydroxy groups of the glucosides and idosides should be selectively oxidized to the corresponding uronic acids, and finally, the remaining TBDPS protecting groups at the C-6 positions should be removed to give the desired HS fragment. This selective oxidation[4] can be performed with a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) in the presence of sodium hypochlorite as the regenerating co-oxidant[8] without affecting sulfate esters.



Sulfated	Unsulfated
R^1 = Lev	R^1 = TBDPS
R^2 = Lev	R^2 = Bn
R^3 = Lev	R^3 = Ac

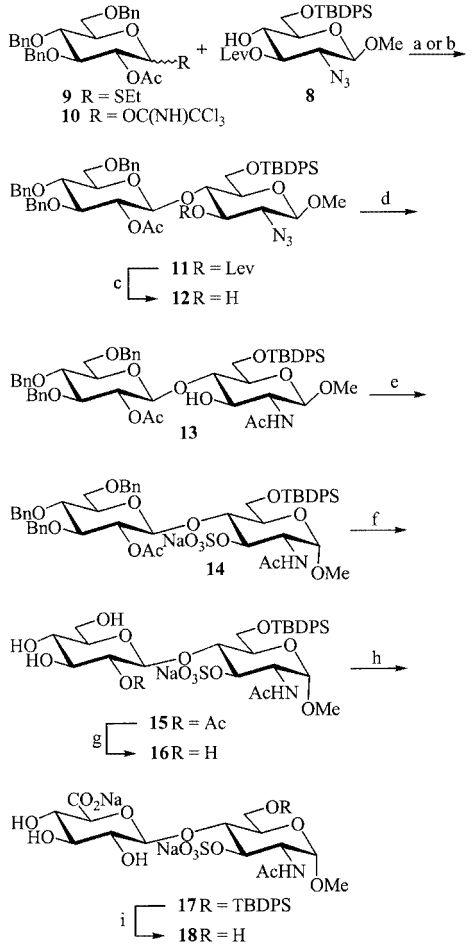
Figure 1. Proposed protecting groups for building blocks

Disaccharide **18** was prepared in order to examine whether the proposed protecting group scheme and manipulations would be suitable for a modular synthesis of HS fragments. The selectively protected disaccharide **11**, the precursor of target compound **18**, was prepared from the readily available glycosyl acceptor **8**, with a non-participating azido group at C-2, a Lev ester at C-3, and a TBDPS group at C-6 (Scheme 1), and the known glycosyl donors **9**[9] or **10**,[10] which each have an acetyl group at C-2 (Scheme 2).



Scheme 1. Reagents and conditions: (a) MeONa, MeOH, room temp., 2 h; (b) $PhCH(OMe)_2$, camphorsulfonic acid (CSA), DMF, 60 °C, 16 h; (c) $Ba(OH)_2{\cdot}8H_2O$, H_2O, MeOH, reflux, 16 h (87%); (d) TfN_3, DMAP, MeOH, room temp., 16 h (73%); (e) levulinic acid, DCC, DMAP, pyridine, room temp., 5 h; (f) 10% TFA in DCM, room temp., 3 h (81%); (g) TBDPSCl, imidazole, DMF, room temp., 16 h (73%)

Glycosyl acceptor **8** was prepared from the known methyl 2-acetamido-2-deoxy-β-D-glucopyranoside **1** (Scheme 1).[11] Thus, deacetylation of **1** under Zemplén conditions, followed by selective protection of the 4,6-diol of the resulting **2** as a benzylidene acetal by treatment with benzaldehyde dimethyl acetal[12] in the presence of catalytic camphor-10-sulfonic acid in DMF, gave partially protected **3**. *N*-Deacetylation of **3** with $Ba(OH)_2{\cdot}8H_2O$[13] afforded the free amine **4** in 87% overall yield, and this compound was treated with the diazo transfer reagent triflic azide (TfN_3) in the presence of 4-(dimethylamino)pyridine (DMAP) in methanol[14] to give the corresponding 2-azido-2-deoxy-glucopyranoside **5** in 73% yield. The free hydroxy group of **5** was protected as a levulinoyl ester by treatment with levulinic acid, 1,3-dicyclohexylcarbodiimide (DCC), and DMAP in pyridine,[5] and the benzylidene acetal of the resulting compound **6** was cleaved by treatment with trifluoroacetic acid[15] in dichloromethane (DCM) to afford diol **7** in 81% overall yield. Finally, the primary hydroxy group of **7** was selectively protected as a TBDPS ether by treatment with *tert*-butyldiphenylsilyl chloride (TBDPSCl) in the pres-



Scheme 2. Reagents and conditions: (a) **9**, **8**, NIS, TfOH, DCM, 4 Å mol. sieves, 0 °C, 15 min (56%); (b) **10**, **8**, DCM, TMSOTf, 4 Å mol. sieves, −40 °C, 30 min (72%); (c) hydrazine acetate, DCM, MeOH, room temp., 45 min (85%); (d) $CH_3C(O)SH$, pyridine, room temp., 18 h, (89%); (e) $SO_3{\cdot}NEt_3$, DMF, room temp., 2d, (87%); (f) Pd/C, H_2, EtOH, 4 bar, 10 d, (84%); (g) *Pseudomonas* sp. Lipase type B, 0.1 M phosphate buffer, 0.2 M NaCl, 3 mM $CaCl_2$, pH = 7.1, 37 °C, 3 d, (78%); (h) TEMPO, NaBr, NaOCl, H_2O, 0 °C, (76%); (i) HF·pyridine in pyridine, room temp., 8 h, (81%)

ence of imidazole in DMF[7] to yield the glycosyl acceptor **8** in 73% yield.

Coupling of glycosyl acceptor **8** with glycosyl donor **9**[9] in the presence of the promoter system *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)[16] gave the desired disaccharide **11** in 56% yield (Scheme 2). Only the β anomer was formed in this coupling, due to neighboring group participation by the acetyl group. The yield of the glycosylation was improved by use of the trichloroacetimidate donor **10**[10] in combination with trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the promoter, and in this case disaccharide **11** was obtained in 72% yield.

With the requisite disaccharide **11** in hand, attention was focussed on its conversion into the sulfated HS fragment **16**. Firstly, removal of the levulinate ester of **11** by treatment with hydrazine acetate[17] in DCM gave clean formation of compound **12**, showing that the Lev could be removed without the other protecting groups being affected. Reduction of the azido moiety with concomitant acetylation of the amino group was easily accomplished by treatment with thioacetic *S*-acid[18] to give the *N*-acetamido derivative **13** in 76% overall yield. It also proved feasible to convert the azido group into an acetamido moiety first and then to remove the levulinoyl ester under standard conditions. Next, the C-3 hydroxy group of **13** was sulfated with $SO_3{\cdot}NEt_3$ in DMF[19] to give the sulfate ester **14**. Surprisingly, the NMR spectrum of **14** showed that the GlcNAc moiety had anomerized during sulfation to the more stable α configuration, as was evident from the chemical shift of C-1 (δ = 97.8 ppm) and the $J_{C\text{-}1,1\text{-}H}$ coupling constant of 173.5 Hz. It is known[20] that glycosides can anomerize to their more stable α anomers under acidic conditions, but to the best of our knowledge no such reaction has been described for the reaction conditions used for sulfation.

The benzyl ethers of **14** were removed by catalytic hydrogenation in the presence of Pd/C, and the acetyl group was subsequently cleaved under Zemplén conditions. Unfortunately, apart from the expected compound **16**, a substantial amount of material that had lost its sulfate ester was also isolated. Several other conditions for removal of the acetyl ester were investigated, but each attempt resulted either in partial desulfation or in recovery of starting material. To address this serious problem, the use of a lipase or esterase to accomplish the selective deacetylation was considered. These enzymes have often been used for the resolution of chiral alcohols by selective hydrolysis of chiral acetyl esters,[21] but have only seldom been employed for the deprotection of acid- or base-sensitive compounds. A library of lipases was screened to select an enzyme that could perform the selective deacetylation, and these studies showed that *Pseudomonas* lipase type B in a phosphate buffer could cleave the acetyl ester of **15** efficiently, to give **16** in 78% yield. It was also found that pig liver esterase also cleaved the secondary acetyl ester, although longer reaction times were required. The transformation of **15** into **16** showed that it was possible to cleave a sterically hindered acetyl ester enzymatically without the sensitive sulfate esters being affected.

The primary hydroxy group of **16** was selectively oxidized with a catalytic amount of 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) and NaOCl as the co-oxidant[8] to give glucuronic acid **17**. In this reaction, the reduced TEMPO was regenerated by slow addition of NaOCl, and it proved important to maintain the pH between 8.5 and 9.0 by careful addition of aqueous sodium hydroxide. Because of the lipophilic TBDPS ether in **17**, purification of the product could easily be accomplished by C-18 reversed-phase column chromatography. Finally, the TBDPS ether of **17** could be removed by treatment with HF·pyridine[4] to give the target compound **18** in 81% yield.

Conclusion

A set of reaction conditions and protecting groups have been established for the modular synthesis of HS fragments.

It has been shown that levulinoyl esters can be employed as a temporary protecting group for those hydroxy groups that will ultimately need sulfation. Furthermore, C-2 hydroxy groups not requiring sulfation could be protected as acetyl esters, and these groups could be removed at the end of the synthetic sequence in the presence of the acid- and base-sensitive sulfate esters by use of a lipase. A primary hydroxy group of a sulfated disaccharide could be selectively oxidized to a carboxylic acid with a catalytic amount of TEMPO and NaOCl as the co-oxidant. It is to be expected that similar procedures and protecting groups used for the preparation of target **18** should be employable for the preparation of other disaccharides that resemble disaccharide motives found in HS. Currently, we are employing the new strategy for the assembly of a number of well defined HS fragments. For this purpose, the anomeric center of the building blocks is to be protected as a temporary allyl glycoside and the C-4′ position as a *p*-methoxybenzyl ether.

Experimental Section

General Methods: All reactions except those in which water was used as solvent were performed under argon. Reactions were monitored by TLC on 60 F_{254} Kieselgel (EM Science), and the compounds were detected by UV light and by charring with 5% sulfuric acid in methanol. Column chromatography was performed on 60 silica gel (EM Science, 70−230 mesh), size exclusion column chromatography was performed on Sephadex LH-20 (methanol/dichloromethane, 1:1, v/v, Pharmacia Biotech AB) or Sephadex G-25 (water elution) Reversed-Phase Columns. Chromatography was performed on C18 silica gel (Waters Corp.) with a methanol/water gradient as eluent. Solvents were removed under reduced pressure at < 40 °C. All organic solvents were distilled from the appropriate drying agents prior to use. Acetonitrile, dichloromethane, diethyl ether, dioxane, *N*,*N*-dimethylformamide, pyridine, and toluene were distilled from CaH_2. THF was distilled from sodium directly prior to use. Methanol was dried by refluxing with magnesium methoxide, distilled, and stored under argon. Molecular sieves were crushed and activated in vacuo at 390 °C for 3 h prior to use. NMR spectra were recorded with Varian 300 MHz, 500 MHz, and 600 MHz spectrometers equipped with Sun off-line editing workstations. Chemical shifts are reported in parts per million (ppm), with tetramethylsilane as internal standard. Matrix-assisted Laser Desorption Ionization-Time-of-Flight (MALDI-TOF) mass spectrometry was performed with an HP MALDI-TOF spectrometer with gentisic acid as matrix. Optical rotations were all measured with a JASCO P-1020 polarimeter and $[\alpha]_D$ values are given in units of deg cm^2 mg^{-1}.

Methyl 2-Amino-2-deoxy-4,6-*O*-benzylidene-β-D-glucopyranoside (4): Methyl 3,4,6-tri-*O*-acetyl-2-*N*-acetamido-2-deoxy-β-D-glucopyranoside (**1**, 4.85 g, 13.42 mmol) was dissolved in methanol (50 mL), and freshly prepared sodium methoxide was added until pH = 11 was reached. After 5 h, the reaction mixture was neutralized with Dowex H^+, the mixture was filtered, and the filtrate was concentrated in vacuo. The residue was dissolved in *N*,*N*-dimethylformamide (30 mL), and benzaldehyde dimethyl acetal (3.55 g, 23.3 mmol) was added. The pH was adjusted to 4 with 10-camphorsulfonic acid. The reaction mixture was heated (60 °C) for 18 h, neutralized with triethylamine, and then concentrated in vacuo to yield methyl 2-acetamido-2-deoxy-4,6-*O*-benzylidene-β-D-glucopyranoside, which was used without further purification. The crude product was suspended in water (60 mL) and methanol was added until a homogeneous solution was obtained. $Ba(OH)_2 \cdot 8H_2O$ (5.3 g, 17 mmol) was added, and the mixture was heated (100 °C) for 18 h. The reaction mixture was diluted with water (600 mL) and filtered, and H_2SO_4 was added (1.6 g, 17 mmol, in 100 mL H_2O). The precipitate was centrifuged, and the combined aqueous layers were concentrated in vacuo to yield compound **4** as a white solid (3.28 g, 87%). R_f = 0.12 (10% methanol in dichloromethane). $[\alpha]_D^{20}$ = −152.2 (*c* = 0.24, chloroform). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.48−7.45 (m, 2 H, ArH), 7.32−7.30 (m, 3 H, ArH), 5.56 (s, 1 H, C*H*Ph), 4.31 (d, $^3J_{1,2}$ = 8.4 Hz, 1 H, 1-*H*), 4.26 (dd, 1 H, 6-H_a), 3.77 (dd, *J* = 10.3, 6.1 Hz, 1 H, 6-H_b), 3.61−3.42 (m, 6 H, 3-*H*, 4-*H*, 5-*H*, OCH_3), 2.70 (dd, *J* = 8.1, 3.3 Hz, 1 H, 2-*H*) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 128.2−126.4 (Ar-*C*), 102.1 (*C*HPh), 104.0 (*C*-1), 68.3 (*C*-6), 72.1 (*C*-3), 81.6, 66.3 (*C*-4, *C*-5), 57.8 (*C*-2), 56.3 (O*C*H_3). MALDI-TOF MS: *m*/*z* = 282 $[M + Na]^+$.

Methyl 2-Azido-2-deoxy-4,6-*O*-benzylidene-β-D-glucopyranoside (5): A freshly prepared solution of TfN_3 (1 M, 5 × 20 mL) was added dropwise over a period of 45 min to a solution of compound **4** (3.3 g, 11.7 mmol) and 4-(dimethylamino)pyridine (1.5 g, 12.8 mmol) in methanol (140 mL). After 18 h, TLC analysis showed complete conversion of the starting material. The solvent was concentrated in vacuo to a volume of ca. 5 mL, diluted with ethyl acetate (200 mL), and washed subsequently with sat. $NaHCO_3$ (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried ($MgSO_4$) and filtered, and the solvents were evaporated in vacuo. Silica gel column chromatography (eluent: 1% methanol in dichloromethane) of the residue gave compound **5** as a white solid (2.62 g, 73%). R_f = 0.19 (1% methanol in dichloromethane). $[\alpha]_D^{20}$ = −37.8 (*c* = 0.34, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.48−7.34 (m, 5 H, ArC*H*), 5.53 (s, 1 H, PhC*H*), 4.37 (dd, *J* = 10.4, 5.1 Hz, 1 H, 6-H_a), 4.30 (d, *J* = 7.9 Hz, 1 H, 1-*H*) 3.93−3.21 (m, 8 H, 2-*H*, 3-*H*, 4-*H*, 5-*H*, 6-H_b, OCH_3), 2.78 (br. s, 1 H, 3-O*H*) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.0 (ArC_q), 129.6, 128.6, 126.5 (Ar*C*H), 103.8 (*C*H, benzylidene), 102.2 (*C*-1), 80.9, 72.4, 68.8, 66.7, 66.4 (*C*-2, *C*-3, *C*-4, *C*-5, *C*-6), 57.7 (O*C*H_3) ppm. $C_{14}H_{17}N_3O_5$: calcd. C 54.72, H 5.58, N 13.67; found C 54.55, H 5.45, N 13.65. MALDI-TOF MS: *m*/*z* = 307 $[M + Na]^+$.

Methyl 2-Azido-2-deoxy-3-*O*-levulinoyl-β-D-glucopyranoside (7): *N*,*N*′-Dicyclohexylcarbodiimide (3.81 g, 18.4 mmol) and a catalytic amount of 4-(dimethylamino)pyridine (10 mg) were added at room temperature to a stirred solution of **5** (2.57 g, 8.4 mmol) and levulinic acid (2.10 g, 18.4 mmol) in pyridine (40 mL). A few minutes later the formation of a precipitate was observed. After 18 h, TLC analysis showed complete conversion of the starting material. The precipitate was filtered off, the cake was washed with toluene (100 mL), and the combined organic phases were concentrated in vacuo to yield methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-D-glucopyranoside (**6**) as a white solid (3.4 g, 8.3 mmol, 99%), which was used without further purification. The crude product was dissolved in 10% trifluoroacetic acid in dichloromethane (100 mL), and the solution was stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo and coevaporated with toluene (5 × 10 mL), and the residue was purified by silica gel column chromatography (eluent: 1−10% methanol in dichloromethane) to give the diol **7** as a clear oil (2.13 g, 81%). R_f = 0.29 (5% methanol in dichloromethane). $[\alpha]_D^{20}$ = −31.2 (*c* = 1.29, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.87 (t, *J* = 9.6 Hz, 1 H, 3-*H*), 4.30 (d, $^3J_{1,2}$ = 7.8 Hz, 1 H, 1-*H*), 3.88 (dd, *J* = 12.0, 3.9 Hz, 1 H, 6-H_a), 3.87 (dd, *J* = 11.7, 3.6 Hz, 1 H, 6-H_b), 3.69 (t, *J* =

8.4 Hz, 1 H, 4-*H*), 3.57 (s, 3 H, OCH_3), 3.43−3.33 (m, 2 H, 2-*H*, 5-*H*), 3.19 (br. s, 2 H, 4-O*H*, 6-O*H*), 2.98−2.48 (m, 4 H, CH_2-CH_2), 2.18 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 98.8 (*C*-1), 71.6 (*C*-3), 70.5, 59.7 (*C*-2, *C*-5), 65.2 (*C*-4), 57.8 (*C*-6), 53.2 (O*C*H$_3$), 34.1, 23.9 (*C*H$_2$*C*H$_2$), 25.9 (*C*H$_3$) ppm. $C_{12}H_{19}N_3O_7$: calcd. C 45.42, H 6.04, N 13.24; found C 45.55, H 6.10, N 13.33. MALDI-TOF MS: *m*/*z* = 317.3 $[M + Na]^+$.

Methyl 2-Azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3-*O*-levulinoyl-β-D-glucopyranoside (8): *tert*-Butylchlorodiphenylsilane (2.5 mL, 9.4 mmol) was added dropwise to a solution of compound **7** (2.13 g, 6.7 mmol) and imidazole (1.15 g, 16.8 mmol) in *N*,*N*-dimethylformamide (5 mL). The mixture was stirred for 18 h at room temperature and then poured into ice-cold water (500 mL) and extracted with dichloromethane (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. Silica gel column chromatography (eluent: 2% acetone in dichloromethane) of the residue yielded **8** as a clear oil (2.70 g, 72%). R_f = 0.43 (3% acetone in dichloromethane). $[\alpha]_D^{20}$ = −18.2 (*c* = 1.0, chloroform). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.69−7.68, 7.42−7.36 (m, 10 H, ArC*H*), 4.90−4.86 (t, *J* = 9.6 Hz, 1 H, 3-*H*), 4.26 (d, $^3J_{1,2}$ = 7.8 Hz, 1 H, 1-*H*), 3.96−3.90 (2dd, *J* = 9.6, 6.3 Hz, 2 H, 6-$H_{a,b}$), 3.74−3.70 (t, *J* = 9.3 Hz, 1 H, 4-*H*), 3.55 (s, 3 H, OCH_3), 3.42−3.35 (m, 2 H, 2-*H*, 5-*H*), 2.91−2.57 (m, 4 H, CH_2CH_2), 2.17 (s, 3 H, CH_3), 1.04 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 207.7 (C=O, ketone), 173.1 (C=O, ester), 135.9−127.9 (ArC_q, Ar*C*H), 102.9 (*C*-1), 76.4, 75.8, 70.3, 64.0, 63.9 (*C*-2, *C*-3, *C*-4, *C*-5, *C*-6), 57.2 (OCH_3), 38.6, 28.4 (*C*H$_2$*C*H$_2$), 29.9 (*C*H$_3$), 27.0 [C(*C*H$_3$)$_3$], 19.5 [*C*(CH$_3$)$_3$] ppm. $C_{28}H_{37}N_3O_7Si$ (555.69): calcd. C 60.52, H 6.71, N 7.56; found C 61.09, H 6.58, N 7.46. MALDI-TOF MS: *m*/*z* = 555.6 $[M + Na]^+$.

Methyl 4-*O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3-*O*-levulinoyl-β-D-glucopyranoside (11). Method A: A suspension of donor **9** (39.8 mg, 0.07 mmol), acceptor **8** (61 mg, 0.11 mmol), and molecular sieves (4 Å, 110 mg) in dichloromethane (2 mL) was stirred at room temperature for 3 h. *N*-Iodosuccinimide (33.3 mg, 0.15 mmol) was added, and the reaction mixture was cooled (0 °C). Trifluoromethanesulfonic acid (0.6 μL, 0.007 mmol) was added; after 15 min, TLC analysis showed complete consumption of the donor. The reaction was quenched by addition of triethylamine, the resulting mixture was filtered, and the cake was washed with dichloromethane (100 mL). The combined organic phases were subsequently washed with sat. $NaHCO_3$ (1 × 10 mL) and brine (1 × 10 mL), dried ($MgSO_4$), and filtered, and the filtrate was concentrated in vacuo. Purification of the residue by silica gel flash chromatography (eluent: 2% acetone in dichloromethane), followed by Sephadex LH-20 size-exclusion column chromatography (eluent: 50% methanol in dichloromethane) yielded disaccharide **11** (42.8 mg, 56%) as a white solid. **Method B:** A suspension of trichloroacetimidate **10** (1.18 g, 1.9 mmol), acceptor **8** (788.8 mg, 1.4 mmol), and molecular sieves (4 Å, 200 mg) in dichloromethane was stirred at room temperature for 30 min. After this had been cooled to −40 °C, trimethylsilyl trifluoromethanesulfonate (30 μL, 0.17 mmol) was added. After 15 min, the reaction was quenched by addition of triethylamine, the mixture was filtered, and the cake was washed with dichloromethane (50 mL). The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (eluent: 20% ethyl acetate in hexanes), followed by size exclusion column chromatography over Sephadex LH-20 to yield disaccharide **11** (1.04 g, 72%). R_f = 0.28 (3% acetone in dichloromethane). $[\alpha]_D^{20}$ = +21.6 (*c* = 1.54, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.73−7.70, 7.40−7.23 (m, 25 H, ArC*H*), 4.97−4.88 (t, *J* = 9.6 Hz, 1 H, 3-*H*), 4.80−4.46 (m, 8 H, 1′-*H*, 2′-*H*, PhCH_2), 4.19 (d, $^3J_{1,2}$ = 8.0 Hz, 1 H, 1-*H*), 4.03−3.23 (m, 2-*H*, 4-*H*, 5-*H*, 6-$H_{a,b}$, 3′-*H*, 4′-*H*, 5′-*H*, 6′-$H_{a,b}$, OCH_3), 2.68−2.59, 2.29−2.25 (m, 4 H, CH_2CH_2), 1.97, 1.74, 1.03 (3 s, 15 H, CH_3, C(CH_3)$_3$] ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.6 (C=O, lev), 169.3 (C=O, acetyl), 137.8−128.3 (ArC_q, Ar*C*H), 102.6 (*C*-1), 100.8 (*C*-1′), 83.4, 77.7, 75.3, 75.4, 75.2, 75.1, 75.0, 74.3, 73.2, 72.9, 72.3, 68.8, 63.4, 60.6, 60.5 (*C*-3, *C*-4, *C*-5, *C*-6, *C*-2′, *C*-3′, *C*-4′, *C*-5′, *C*-6′, Ph*C*H$_2$), 29.5 (*C*H$_3$, lev), 34.2, 26.9 (*C*H$_2$*C*H$_2$), 26.4 [C(*C*H$_3$)$_3$], 20.7 (*C*H$_3$, acetyl) ppm. $C_{57}H_{67}N_3O_{13}Si$ (1030.24): calcd. C 66.45, H 6.55, N 4.08; found C 66.39, H 6.55, N 4.15. MALDI-TOF MS: *m*/*z* = 1031.9 $[M + Na]^+$.

Methyl 4-*O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-β-D-glucopyranoside (12): A solution of hydrazine acetate (81.4 mg, 0.88 mmol) in methanol (2 mL) was added to a solution of disaccharide **11** (729 mg, 0.71 mmol) in dichloromethane (18 mL), and the resulting reaction mixture was stirred until TLC analysis showed complete consumption of starting material. The solvents were evaporated in vacuo, and the residue was purified by silica gel column chromatography (1−2% acetone in dichloromethane) to yield compound **12** (561.4 mg, 84%) as a white solid. R_f = 0.15 (1% acetone in dichloromethane). $[\alpha]_D^{20}$ = +22.0 (*c* = 1.17, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.72−7.14 (m, 25 H, ArC*H*), 4.99 (t, *J* = 8.7 Hz, 1 H, 2′-*H*), 4.81−4.50 (m, 7 H, 1′-*H*, PhCH_2), 4.15 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1-*H*), 3.87−3.53 (m, 12 H, 3-*H*, 4-*H*, 6-$H_{a,b}$, 3′-*H*, 4′-*H*, 5′-*H*, 6′-$H_{a,b}$, OCH_3), 3.34−3.28 (m, 2 H, 2-*H*, 5-*H*), 1.60 (s, 3 H, CH_3, acetyl), 1.03 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.4 (*C*=O), 138.2−127.8 (ArC_q, Ar*C*H), 102.5 (*C*-1), 101.4 (*C*-1′), 83.1, 80.2, 78.0, 75.5, 75.4, 75.1, 74.8, 73.9, 73.8, 73.0, 68.6, 65.9, 62.0 (*C*-2, *C*-3, *C*-4, *C*-5, *C*-6, *C*-2′, *C*-3′, *C*-4′, *C*-5′, *C*-6′, Ph*C*H$_2$), 56.9 (O*C*H$_3$), 27.0 [C(*C*H$_3$)$_3$], 20.7 (*C*H$_3$), 19.6 [*C*(CH$_3$)$_3$] ppm. $C_{52}H_{61}N_3O_{11}Si$ (932.14): calcd. C 67.00, H 6.60, N 4.51; found C 67.19, H 6.66, N 4.61. MALDI-TOF MS: *m*/*z* = 932.8 $[M + Na]^+$.

Methyl 2-Acetamido-4-*O*-(2-O-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-6-(*O*-*tert*-butyldiphenylsilyl)-2-deoxy-β-D-glucopyranoside (13): Thioacetic *S*-acid (400 μL, 5.6 mmol) was added to a solution of disaccharide **12** (548.3 mg, 0.59 mmol) in pyridine (1 mL). After 18 h, the reaction mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (eluent: 0−5% methanol in toluene) to yield disaccharide **13** (500 mg, 89%) as a white foam. R_f = 0.14 (12% methanol in dichloromethane). $[\alpha]_D^{20}$ = +22.2 (*c* = 0.89, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.73−7.69, 7.39−7.23, 7.14−7.13 (m, 25 H, ArC*H*), 5.49 (d, ^3J = 8.0 Hz, 1 H, N*H*,), 4.99 (t, *J* = 8.75 Hz, 1 H, 2′-*H*), 4.80−4.46 (m, 7 H, 1′-*H*, PhCH_2), 3.91−3.37 (m, 14 H, 2-*H*, 3-*H*, 4-*H*, 5-*H*, 6-$H_{a,b}$, 3′-*H*, 4′-*H*, 5′-*H*, 6′-$H_{a,b}$, OCH_3), 2.00, 1.61 (2 s, 6 H, CH_3), 1.02 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 169.5 (C=O), 138.3−127.9 (ArC_q, Ar*C*H), 101.3, 101.2 ($J_{C,1\text{-}H}$ = 163.9 Hz, 164.5 Hz, *C*-1β, *C*-1′β), 83.1, 80.3, 77.9, 77.4, 75.4, 75.3, 75.1, 74.9, 73.8, 73.0, 71.8, 68.6, 62.2 (*C*-2, *C*-3, *C*-4, *C*-5, *C*-6, *C*-2′, *C*-3′, *C*-4′, *C*-5′, *C*-6′, Ph*C*H$_2$), 56.7 (O*C*H$_3$), 27.0 [C(*C*H$_3$)$_3$], 24.0 (NHAc), 20.7 (*C*H$_3$), 19.5 [*C*(CH$_3$)$_3$] ppm. $C_{54}H_{65}NO_{12}Si$ (948.18): calcd. C 68.40, H 6.91, N 1.48; found C 67.91, H 7.21, N 1.87. MALDI-TOF MS: *m*/*z* = 948.0 $[M + Na]^+$.

Methyl 2-Acetamido-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-l-β-D-glucopyranosyl)-6-(*O*-*tert*-butyldiphenylsilyl)-2-deoxy-3-*O*-sulfo-α-D-glucopyranoside Sodium Salt (14): Sulfur trioxide−triethylamine complex (250 mg, 1.38 mmol) was added to a heated solution (55 °C) of disaccharide **13** in *N*,*N*-dimethylformamide (1 mL). After 18

h, the heating was stopped, sodium acetate (200 mg) in water (20 mL) was added, and the reaction mixture was stired for 1 h. The solvents were evaporated in vacuo and coevaporated with water (5 × 10 mL) to remove remaining triethylamine. Purification on Sephadex LH-20 (eluent: 50% methanol in dichloromethane) gave compound **14** (271 mg, 78%) as a clear glass. $[\alpha]_D^{20} = -12.6$ (c = 0.1, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.63−6.97 (m, 25 H, ArC*H*), 5.33 (t, J = 8.7 Hz, 1 H, 2′-*H*), 5.12 (d, $^3J_{NH,H}$ = 3.5 Hz, 1 H, N*H*), 4.69−4.26 (m, 9 H, 1-*H*, 3-*H*, 1′-*H*, PhCH_2), 4.04−3.97 (m, 2 H, 4-*H*, 4′-*H*), 3.92−3.47 (m, 6 H, 2-*H*, 5-*H*, 6-$H_{a,b}$, 6′-$H_{a,b}$), 3.39 (t, J = 9.6 Hz, 1 H, 3′-*H*), 3.22−3.15 (m, 4 H, 5′-*H*, OCH_3), 1.84, 1.57 (2 s, 6 H, CH_3), 1.03 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.1, 171.5 (*C*=O), 139.0−126.4 (ArC_q, Ar*C*H), 102.3 ($J_{C,1\text{-}H}$ = 164.8 Hz, *C*-1′β), 97.8 ($J_{C,1\text{-}H}$ = 173.5 Hz, C-1α), 83.4, 77.4, 75.8, 75.5, 74.7, 74.3, 74.2, 73.9, 73.4, 71.1, 67.1, 61.2, 55.2, 54.7 (*C*-2, *C*-3, *C*-4, *C*-5, *C*-6, *C*-2′, *C*-3′, *C*-4′, *C*-5′, *C*-6′, Ph*C*H$_2$, O*C*H$_3$), 26.9 [C(*C*H$_3$)$_3$], 23.3 (NHAc), 20.7 (*C*H$_3$), 19.3 [*C*(CH$_3$)$_3$] ppm.

Methyl 2-Acetamido-2-deoxy-3-*O*-sulfo-4-*O*-(β-D-glucopyranosyluronic acid)-α-D-glucopyranoside Disodium Salt (18): A suspension of disaccharide **14** (15.5 mg, 14.8 μmol) and Pd/C (10 mg) in EtOH (5 mL) under H_2 was stirred for 5 d until TLC analysis showed complete conversion into one product. The reaction mixture was filtered, and the cake was washed thoroughly with 50% MeOH in chloroform. Evaporation of the solvents in vacuo yielded compound **15** (9.7 mg, 84%), which was used without further purification. Crude **15** (9.7 mg, 12.4 μmol) was dissolved in phosphate buffer (500 μL, 0.1 M, 0.2 M NaCl, 3 mM $CaCl_2$, pH = 7.1), and *Pseudomonas sp.* lipase type B (139 U/mg, 10 mg) was added. After the mixture had been shaken for 3 d at 37 °C, TLC analysis indicated complete conversion of starting material. The enzyme was filtered off and the cake was washed repeatedly with methanol/water (15 mL, 1:1, v/v). The combined extracts were concentrated in vacuo, and residual enzyme was removed by purification on a reversed-phase C-18 column (eluent: H_2O, then 30% methanol in H_2O). An aqueous NaOCl (13%) solution was adjusted to pH = 8.5 with 4 M aq. HCl and added dropwise to a cooled (0 °C) solution of **16** (9 mg, 98%, 12.2 μmol), TEMPO (0.22 mg, 1.4 μmol), and NaBr (0.6 mg, 5.5 μmol) in H_2O (350 μL). The pH of the resulting solution was carefully maintained at 8.5 ± 0.5 by addition of 1 M NaOH. Addition of the NaOCl solution and NaOH was continued until the pH remained constant for a prolonged period of time upon addition of NaOCl. The reaction mixture was directly applied to a reversed-phase chromatography column (eluent 0−30% methanol in H_2O) to give compound **17** (7.1 mg, 76%). HF·pyridine (5 μL; 70% HF in pyridine) was added to a cooled (0 °C) solution of **17** (5 mg, 6.5 μmol) in pyridine (500 μL). The mixture was allowed to warm to room temperature and was stirred for 18 h. The solution was neutralized with solid $NaHCO_3$ (9 mg), diluted with methanol (5 mL), and filtered, and the solvents were evaporated in vacuo. The residue was purified by reversed-phase column chromatography (eluent: H_2O) to give disaccharide **18** (2.8 mg, 81%) as a white solid. $[\alpha]_D^{20} = -1.47$ (c = 0.75, H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 4.67 (d, $^3J_{1,2}$ = 3.5 Hz, 1 H, 1-*H*), 4.53 (d, $^3J_{1',2'}$ = 7.5 Hz, 1 H, 1′-*H*), 4.47 (t, J = 10.0 Hz, 1 H, 3-*H*), 4.02 (dd, J = 10.0, 3.5 Hz, 1 H, 2-*H*), 3.90−3.72 (m, 5 H, 4-*H*, 5-*H*, 6-$H_{a,b}$, 5′-*H*), 3.48−3.38 (m, 2 H, 4′-*H*, 3′-*H*), 3.33−3.28 (m, 4 H, 2′-*H*, OCH_3), 1.89 (s, 3 H, CH_3) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 174.6 (*C*=O), 174.1 (*C*O$_2$Na), 101.0 (*C*-1), 97.6 (*C*-1′), 77.4 (*C*-3), 75.4, 75.0, 72.7, 72.5, 71.0, 70.8, 59.7 (*C*-4, *C*-5, *C*-6, *C*-2′, *C*-3′, *C*-4′, *C*-5′), 55.3 (O*C*H$_3$), 52.7 (*C*-2), 22.0 (NHAc) ppm.

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