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# Development of specific inhibitors for heparin-binding proteins based on the cobra cardiotoxin structure: an effective synthetic strategy for rationally modified heparin-like disaccharides and a trisaccharide

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**Abstract**—Recently, a new heparin disaccharide-binding site on the convex side of cobra cardiotoxin (CTX) was identified by NMR spectroscopy and molecular modeling. To further characterize this site two heparin-like disaccharides were synthesized for binding studies with CTX, and a trisaccharide was synthesized for testing the sequence of the disaccharide binding to CTX. Thus six differentially protected monosaccharide building blocks (three L-iduronic acids and three D-glucosamines) were prepared. These include a L-iduronic acid elongation building block namely methyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-pivaloyl-α-L-idopyranosyluronate trichloroacetimidate for which a single-crystal X-ray structure was determined to have $M_r = 576.79$, $a = 9.3098(11)$ Å $\alpha = 90°$, $b = 10.3967(12)$ Å $\beta = 90°$, $c = 28.026(3)$ Å $\gamma = 90°$, $V = 2712.7(6)$ Å$^3$, $P2_12_12_1$, $Z = 4$, $\mu = 0.71073$ Å, and $R = 0.0378$ for 7586 observed reflections. It shows that the molecular structure of the donor is in the $^1C_4$ conformation with significant 1,3-diaxial interactions between O-1 and O-3 as well as O-2 and O-4. The disaccharides and trisaccharide vary in the degree and position of O- and N-sulfation. The pivaloyl group was used as permanent protecting group of hydroxyl. The levulinoyl group was used as the temporary protecting group to protect the hydroxyl for elongation.


*Keywords:* Glycosylations; L-Iduronic acid building blocks; Heparin-like oligosaccharides; 1,3-Diaxial interactions; Cobra cardiotoxin

## 1. Introduction

Cardiotoxins (CTXs) from cobra venom are all β-sheet, slightly curved, highly basic polypeptides, which are capable of inducing general cytotoxic effects on many cell types.[1,2] They also cause severe tissue necrosis and local gangrene in humans.[3] Many workers are attracted by the abundance of positive charge on CTXs and are interested in studying its effect on various biochemical processes involving anionic molecules. CTX has been shown to inhibit protein kinase C activity in the presence of phosphatidylserine,[4] contain weak anticoagulant activity, and act as a potentiator of platelet aggregation.[5] There is also evidence to suggest a correlation between cytolytic and antiplatelet activity.[6]

Glycosaminoglycans (GAGs) represent the saccharide moieties of proteoglycans (PG) that occur abundantly in all tissues, on cell surfaces, and in the extracellular matrix (ECM).[7,8] They perform a myriad of physiological functions and are extensively sulfated, repeating copolymers of hexosamine and hexuronic acid.[9,10] Heparin and heparan sulfate are highly sulfated glycosaminoglycans (GAGs) involved in many important biological

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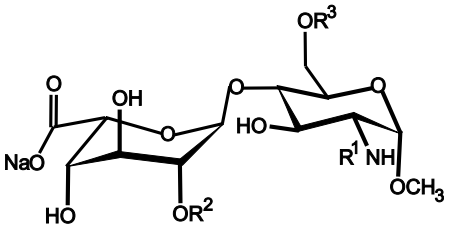
functions such as blood coagulation, cell growth and differentiation, angiogenesis, etc.[11] Recently, glycosaminoglycans (GAGs) have been suggested to be potential targets for cobra cardiotoxin (CTX) action.[12–14] The interaction of GAGs, such as high-molecular-weight heparin, with CTXs not only can induce aggregation of CTXs molecules, but also can promote its penetration into the phospholipid monolayer under physiological ionic conditions.[12] The molecular basis of the CTX–GAG interaction, however, remains elusive.

We have recently initiated a collaborative project aimed at understanding at the molecular level the binding specificity of GAGs to the CTXs in order to be able to identify specific inhibitors. For performing structure–activity relationship studies in this field, we designed and synthesized a variety of monosaccharide building blocks, which could be coupled to form oligosaccharides that would serve to determine the key structural features necessary for binding to the cobra cardiotoxin proteins (CTXs). Since heparin is heavily *O*- and *N*-sulfated, these building blocks must allow for selective sulfation. At the outset of this program it was not known, which sulfates or carboxylates were important for binding to the CTXs. During the project a heparin disaccharide-binding site on the convex side of CTX was identified by NMR spectroscopy and molecular modeling.[15] The *N*-sulfate of D-glucosamine as well as the 2-sulfate of L-iduronic acid were established as key binding points. However, the role of the 6-sulfate of D-glucosamine and the carboxylate of L-iduronic acid were not established. The former makes little or no contact with the protein in the current model, whereas the carboxylate's role cannot be determined because the uronic acid of the disaccharide used for testing has a 4,5-double bond and is not the biologically relevant L-iduronic acid. Moreover, it is not clear if the disaccharide-binding site is for an α-L-iduronic acid-(1→4)-D-glucosamine (*p*IdoA(α1,4)*p*GlcN) disaccharide or the frame-shifted *p*GlcN(α1,4)*p*IdoA disaccharide. Binding studies of a suitably sulfated trisaccharide, *p*GlcN(α1,4)*p*IdoA(α1,4)*p*GlcN, should settle this question.

It has often been difficult to determine the oligosaccharide sequence and sulfation pattern of a heparin or heparin sulfate fragment required for activation or deactivation of a given protein.[16] For this reason it is highly desirable to develop effective syntheses of this heparin-like oligosaccharide chains with defined size, sequence, and charge distribution to be used in the interaction studies. In this paper, we report a convenient synthetic strategy the effectiveness of which is illustrated by the preparation of two known[17] heparin-like disaccharides **1** and **2** (Fig. 1) and an efficient synthesis of a heparin-like trisaccharide **3** (Fig. 2). The biological properties of the disaccharides and the trisaccharide are being investigated and will be discussed in detail elsewhere.




**1** $R^1 = SO_3Na, R^2 = SO_3Na, R^3 = H$

**2** $R^1 = SO_3Na, R^2 = SO_3Na, R^3 = SO_3Na$

**Figure 1.** Disaccharides varying in the degree and position of O- and N-sulfation.

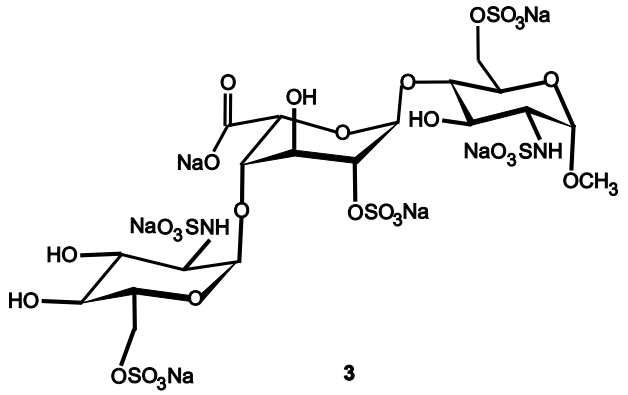


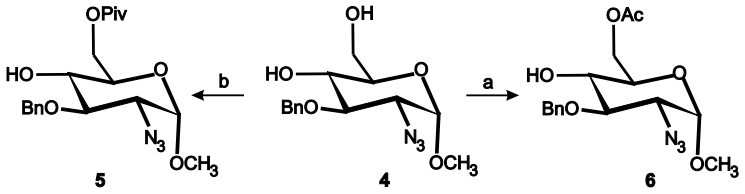

**Figure 2.** Trisaccharide used for testing the sequence of the binding disaccharide.

## 2. Results and discussion

Our synthetic approach was based on preparing and assembling uronic acid monomers and glucosamine monomers with various patterns of orthogonal protective groups. In our synthetic strategy, the pivaloyl group and the benzyl group were used as permanent protecting groups for hydroxyl functions. The levulinoyl group was used as the temporary protecting group, which was used to protect the hydroxyl group for elongation. The acetyl group was also used as a temporary protecting group, which was used to protect the hydroxyl destinated to be *O*-sulfated.

### 2.1. Preparation of glycosyl acceptors

Two monosaccharide acceptors were prepared (Scheme 1). The known acceptor methyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside **6** was obtained by selective acetylation of diol **4** using acetyl chloride in pyridine.[18] This 6-*O*-acetyl group was used as a temporary hydroxy protecting group that would be *O*-sulfated in the end product. Selective pivaloylation of diol **4** gave a new acceptor methyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-pivaloyl-α-D-glucopyranoside (**5**) in 72%

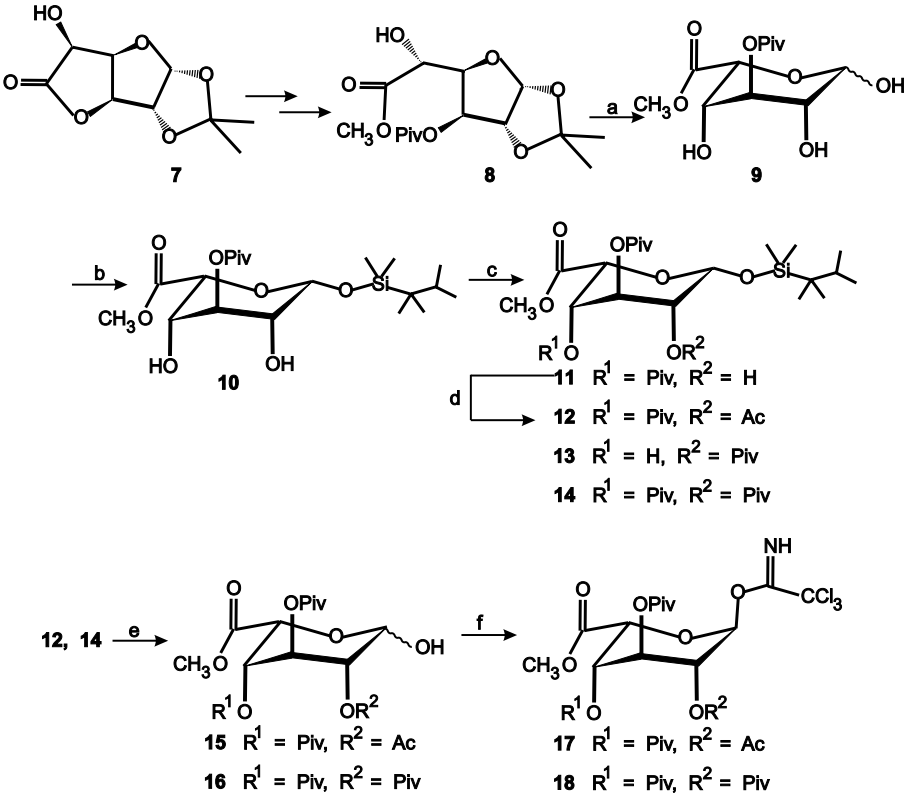



**Scheme 1.** Reagents and conditions: (a) AcCl, Py, $CH_2Cl_2$, −78 °C, 2 h; (b) PivCl, Py, $CH_2Cl_2$, −78 °C, 15 min.

yield. The reaction could be completely finished in 15 min. The pivaloyl group on the 6-position was used as a permanent hydroxy protecting group.

## 2.2. Preparation of glycosyl donors

Four differentially protected donors were prepared. The synthetic route of two L-iduronic acid derivatives, donors **17** and **18** for preparing disaccharides, are shown in Scheme 2. Literature and experimentation showed that a convenient route to L-iduronic acid building blocks was to start from the commercially available crystalline 1,2-*O*-isopropylidene-6,3-D-glucuronolactone **7**.[19–21] Taking advantage of an O-5 to O-3 migration side reaction noted in Ref. 19, we could prepare multigram quantities of methyl ester **8** under the catalysis of the organic base triethylamine at 0 °C. Treatment of the methyl ester **8** with 9:1 $CF_3COOH$–$H_2O$ afforded the L-iduronic acid derivative **9**.[22] Then by regio- and stereoselective silylation with dimethylthexylsilyl chloride (TDSCl) and imidazole in dichloromethane at −20 °C, the β-silyl glycoside **10** was obtained from **9** (Scheme 2).[23] Analysis of the $^1$H NMR (chloroform-*d*) spectrum of the key intermediate **10** indicated a preferred $^1C_4$ conformation for this compound ($J_{1,2}$ 1.0 Hz, $J_{4,5}$ 1.6 Hz ($^4C_1$: $J_{4,5} > 4.2$ Hz)[20a]). Although the OH-2 and OH-4 of the intermediate **10** have similar reactivities, the use of catalytic scandium triflate allowed for acylation to give a mixture of O-4, O-2 monoacylates, and a diacylate.[24] Pivaloylation of the key intermediate **10** with 1.1 equiv of pivaloic anhydride at 10 °C for 1.5 h gave a mixture of O-4 pivaloate **11**, O-2 pivaloate **13**, dipivaloate **14**, and traces of unreacted diol **10**. The mixture could be separated on a MPLC chromatography



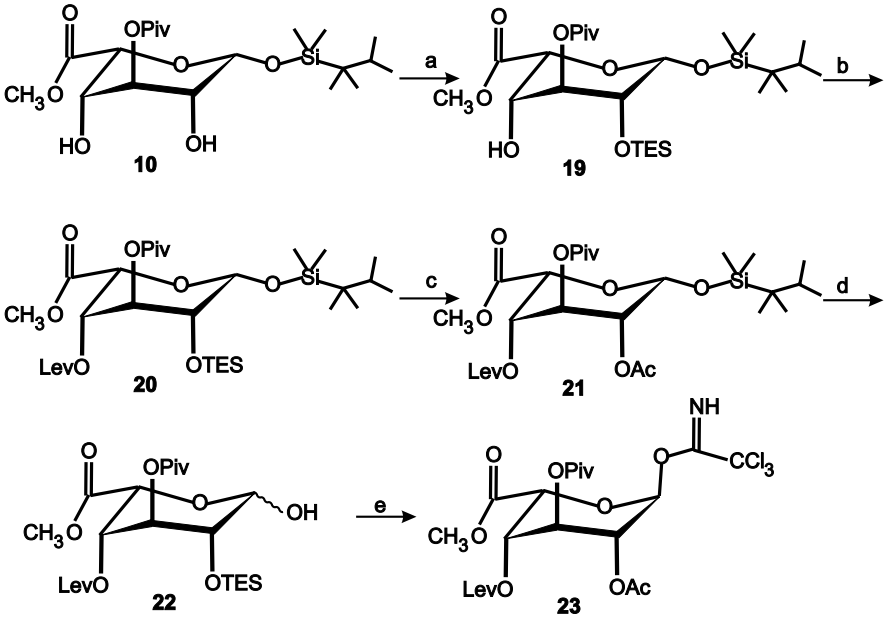

**Scheme 2.** Reagents and conditions: (a) 90% TFA, rt, 3 h; (b) imidazole, $CH_2Cl_2$, −20 °C, O/N; (c) $Piv_2O$, $Sc(OTf)_3$, $CH_2Cl_2$, 10 °C, 1 h; (d) $Ac_2O$, $Sc(OTf)_3$, $CH_2Cl_2$, rt, O/N; (e) $CH_3COOH$, $Bu_4NF$, THF, 10–15 °C, 5 h; (f) $CCl_3CN$, DBU, $CH_2Cl_2$, 0 °C, 1 h.

column eluting with 10:1 hexanes–EtOAc, and the pure pivaloates **11**, **13**, and **14** could be obtained in the ratio of 10:7:5. Then, under the catalysis of $Sc(OTf)_3$ at room temperature overnight, acetylation of **11** gave a mixture of α anomer and β anomer of the corresponding O-2 acetylated silyl glycoside **12**. Silica gel chromatography afforded pure α anomer and β anomer (5:8 α:β). Deprotection of glycoside **12** (including α anomer and β anomer) with $CH_3COOH$ and $Bu_4NF$ in THF at 10–15 °C furnished the intermediate **15**, which was then transformed into trichloroacetimidate **17** in 55% yield by standard protecting group manipulations with $CCl_3CN$ under the catalysis of DBU at 0 °C. The O-2 acetylated donor **17** allows for the preparation of O-2 sulfated L-iduronic acid-containing oligosaccharides in the end product. $^1H$ NMR analysis of building block **17** revealed a $^1C_4$ conformation as judged by the coupling constant ($J_{1,2} < 1.0$ Hz, $J_{4,5}$ 2.3 Hz). Interestingly, a strong NOE between H-1 and H-2, but not to H-5, was observed in the NOESY experiment, showing that donor **17** has an α configuration. In a similar way, another donor **18** could be obtained from the tripivaloate **14** via the intermediate **16**. $^1H$ NMR analysis of **18** also revealed a predominant $^1C_4$ conformation ($J_{1,2}$ 3.1 Hz, $J_{4,5}$ 3.5 Hz, $J_{2,3}$ 4.4 Hz, $J_{3,4}$ 4.6 Hz). This L-iduronic acid building block can be used to make only *N*-sulfated or *N*-acetylated oligosaccharides as well as for testing the glycosylation chemistry. Other variants can easily be constructed from **10**.

Another donor, L-iduronic acid building block **23**, (methyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-pivaloyl-α,β-L-idopyranosyluronate) trichloroacetimidate, is the key elongation building block for the synthesis of trisaccharide **3** (Scheme 3). Using TESOTf as reagent, a triethylsilyl group could be added on the 2-*O*-position of the diol silylate **10** selectively under the conditions of base 2,6-lutidine and $CH_2Cl_2$ at 0 °C, yielding 58% of sugar **19**.[25] The 4-*O*-position of the sugar **19** was then protected by a levulinoyl group in 64% yield. After that, compound **20** was treated with $Ac_2O$ under the catalysis of $Sc(OTf)_3$ at room temperature, resulting in more than 90% of sugar **21**. Glycoside **21** was then deprotected with $CH_3COOH$ and $Bu_4NF$ in THF at room temperature to give the intermediate **22**. Standard protecting group manipulations allowed for the formation of the chain extension donor, trichloroacetimidate **23**, in 55% yield by treatment of intermediate **22** with $CCl_3CN$ under the catalysis of DBU at 0 °C.

The single-crystal X-ray structure of the α anomer, (methyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-pivaloyl-α-L-idopyranosyluronate) trichloroacetimidate of L-iduronic acid building block **23** was obtained at room temperature by slow evaporation of a 3:1 hexanes–$CH_2Cl_2$ solvent mixture. The molecular structure and the numbering scheme for the atoms are shown in Figure 3. Crystal data and structure refinement parameters are given in Table 1; atomic coordinates and equivalent isotropic displacement parameters are shown in Table 2; and selected bond lengths, bond angles, and torsion angles are given in Table 3.

The diffraction data show that the molecular structure of the donor is in a $^1C_4$ conformation (0.841 $^1C_4$, 0.004 $B_{1,4}$, and 0.188 $^2S_O$).[26] This conformation has two strong 1,3-diaxial interactions between the substituents




**Scheme 3.** Reagents and conditions: (a) TESOTf, 2,6-lutidine, $CH_2Cl_2$, 0 °C, 1.5 h; (b) $Lev_2O$, DMAP, $CH_2Cl_2$, rt, 26 h; (c) $Ac_2O$, $Sc(OTf)_3$, $CH_2Cl_2$, rt, 1 h; (d) $CH_3COOH$, $Bu_4NF$, THF, rt, O/N; (e) $CCl_3CN$, DBU, $CH_2Cl_2$, 0 °C, 1 h.

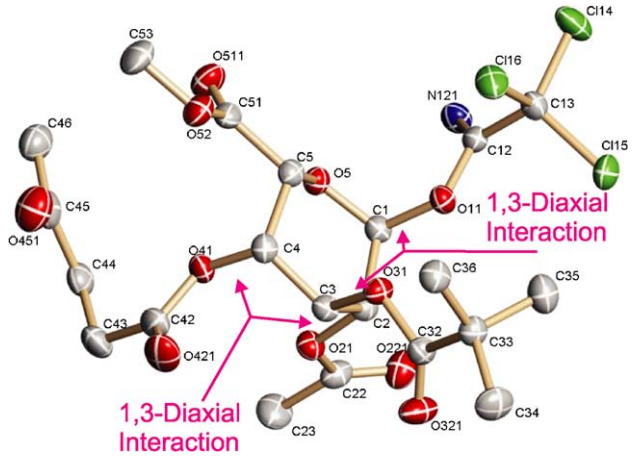



**Figure 3.** The structure and atom numbering scheme of L-iduronic acid building block **23**.

**Table 1.** Crystal data and structure refinement parameters for **23**

| | |
|---|---|
| Empirical formula | $C_{21}H_{28}Cl_3NO_{11}$ |
| Formula weight | 576.79 |
| Temperature | 125(2) K |
| Wavelength | 0.71073 |
| Crystal system | Orthorhombic |
| Space group | $P2_12_12_1$ |
| Unit cell dimensions | $a$ = 9.3098(11) Å $\alpha$ = 90° |
| | $b$ = 10.3967(12) Å $\beta$ = 90° |
| | $c$ = 28.026(3) Å $\gamma$ = 90° |
| Volume | 2712.7(6) Å$^3$ |
| $Z$ | 4 |
| $D_{calcd}$ | 1.412 Mg/m$^3$ |
| Absorption coefficient | 0.394 mm$^{-1}$ |
| $F(000)$ | 1200 |
| Crystal size | 0.35 × 0.18 × 0.05 mm$^3$ |
| $\theta$ Range for data collection | 1.45–29.60° |
| Index ranges | $-12 \leqslant h \leqslant 12$, $-14 \leqslant k \leqslant 14$, $-38 \leqslant l \leqslant 38$ |
| Reflections collected | 34,069 |
| Independent reflections | 7586 [$R$(int) = 0.0378] |
| Completeness to $\theta$ = 29.60 | 99.8% |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data/restraints/parameters | 7586/0/438 |
| Goodness-of-fit on $F^2$ | 1.009 |
| Final $R$ indices [$I > 2\sigma(I)$] | $R_1$ = 0.0332, w$R_2$ = 0.0702 |
| $R$ indices (all data) | $R_1$ = 0.0419, w$R_2$ = 0.0730 |
| Absolute structure parameter | 0.02(3) |
| Largest difference peak and hole | 0.270 and −0.173 e Å$^{-3}$ |

**Table 2.** Atomic coordinates (×10$^4$) and equivalent isotropic displacement parameters (Å$^2$ × 10$^3$) for **23**

| | $x$ | $y$ | $z$ | $U$(eq) |
|---|---|---|---|---|
| Cl(14) | 397(1) | 10700(1) | 9135(1) | 38(1) |
| Cl(15) | 1290(1) | 8947(1) | 9877(1) | 27(1) |
| Cl(16) | −652(1) | 8087(1) | 9139(1) | 31(1) |
| O(5) | 2854(1) | 6639(1) | 8280(1) | 19(1) |
| O(11) | 2405(1) | 7387(1) | 9051(1) | 19(1) |
| O(21) | 5186(1) | 5107(1) | 8716(1) | 22(1) |
| O(31) | 1688(1) | 4595(1) | 9251(1) | 20(1) |
| O(41) | 3204(1) | 4043(1) | 8073(1) | 21(1) |
| O(52) | 94(1) | 4864(1) | 7721(1) | 28(1) |
| O(221) | 6583(1) | 6544(1) | 9098(1) | 32(1) |
| O(321) | 3160(1) | 3813(1) | 9816(1) | 34(1) |
| O(421) | 3016(2) | 1979(1) | 8297(1) | 38(1) |
| O(451) | 2569(2) | 2881(2) | 7045(1) | 46(1) |
| O(511) | 1541(2) | 6431(1) | 7442(1) | 32(1) |
| N(121) | 2873(2) | 9306(1) | 8678(1) | 25(1) |
| C(1) | 3444(2) | 6820(1) | 8735(1) | 19(1) |
| C(2) | 3934(2) | 5563(1) | 8970(1) | 19(1) |
| C(3) | 2861(2) | 4458(1) | 8917(1) | 19(1) |
| C(4) | 2161(2) | 4422(1) | 8425(1) | 19(1) |
| C(5) | 1680(2) | 5757(1) | 8269(1) | 19(1) |
| C(12) | 2139(2) | 8656(1) | 8964(1) | 20(1) |
| C(13) | 847(2) | 9080(1) | 9266(1) | 22(1) |
| C(22) | 6459(2) | 5677(2) | 8817(1) | 24(1) |
| C(23) | 7645(2) | 5092(2) | 8530(1) | 36(1) |
| C(32) | 1991(2) | 4175(2) | 9705(1) | 21(1) |
| C(33) | 690(2) | 4265(2) | 10035(1) | 22(1) |
| C(34) | 920(2) | 3332(2) | 10450(1) | 34(1) |
| C(35) | 639(3) | 5663(2) | 10215(1) | 34(1) |
| C(36) | −701(2) | 3948(2) | 9771(1) | 27(1) |
| C(42) | 3563(2) | 2786(1) | 8050(1) | 27(1) |
| C(43) | 4755(2) | 2575(2) | 7695(1) | 34(1) |
| C(44) | 4866(2) | 3587(2) | 7308(1) | 28(1) |
| C(45) | 3547(2) | 3647(2) | 6995(1) | 31(1) |
| C(46) | 3497(3) | 4685(2) | 6626(1) | 37(1) |
| C(51) | 1121(2) | 5746(2) | 7757(1) | 23(1) |
| C(53) | −575(2) | 4729(2) | 7254(1) | 36(1) |

$U$(eq) is defined as one third of the trace of the orthogonalized $U^{ij}$ tensor.

at C-1 and C-3, and between C-2 and C-4. In the previously reported structure of methyl (methyl 2,3,4-tri-*O*-acetyl-α-L-idopyranosyl)uronate, the C-2 and C-4 groups twisted away from each other to avoid such 1,3 interactions.[20a] All three ester carbonyls and the C=N of the imidate are oriented *syn* to their respective sugar ring methines. The ester at C-6 is planar with respect to C-5–O-5. This solid-state structure of **23** confirms all the stereochemical and regiochemical selective reactions used in its synthesis.

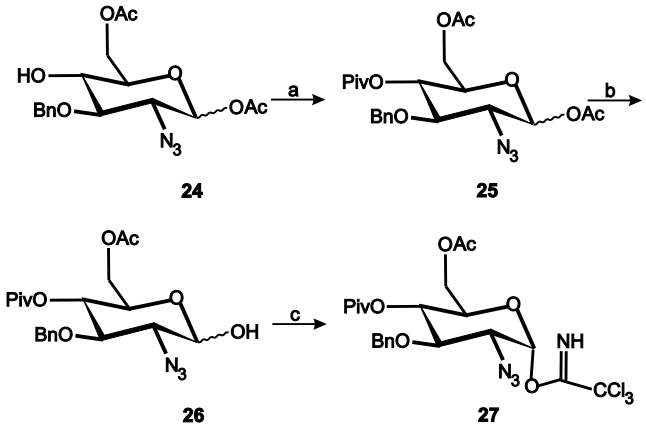
The last donor 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (**27**) is a nonreducing terminal building block used for preparing trisaccharide **3** (Scheme 4). This building block could be synthesized from the known starting material **24**.[18] Pivaloylation of **24** with pivaloyl chloride in pyridine at 45 °C for 48 h under the catalysis of small amount of DMF afforded the sugar **25**, which was deprotected with $N_2H_4$·HOAc in DMF at room temperature to give the intermediate **26**. Standard protecting group manipulation allowed for the formation of the donor **27** by treatment of intermediate **26** with $CCl_3CN$ under the catalysis of $Cs_2CO_3$ at 0 °C for 1 h.

### 2.3. Preparation of disaccharides

To test the protecting group strategy and the glycosylation chemistry, coupling of acceptor **5** with donor **17** afforded disaccharide **28** in 85% yield at −20 °C under promotion by TESOTf (Scheme 5). However, during

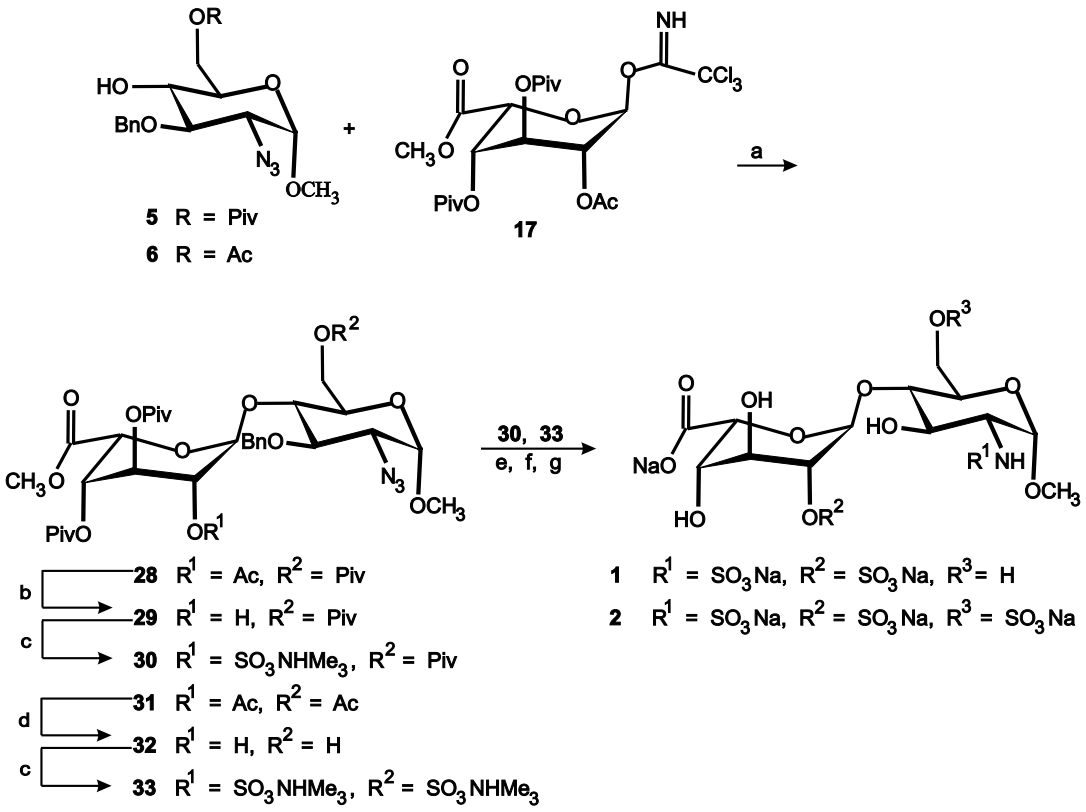
**Table 3.** Selected bond lengths, bond angles, and torsion angles of donor **23**

| | |
|---|---|
| *Bond lengths [Å]* | |
| O(5)–C(1) | 1.3995(18) |
| O(5)–C(5) | 1.4271(19) |
| C(1)–C(2) | 1.533(2) |
| C(2)–C(3) | 1.530(2) |
| C(3)–C(4) | 1.525(2) |
| C(4)–C(5) | 1.522(2) |
| C(5)–C(51) | 1.526(2) |
| *Bond angles [°]* | |
| C(1)–O(5)–C(5) | 114.03(11) |
| O(5)–C(1)–C(2) | 113.22(12) |
| C(3)–C(2)–C(1) | 113.82(12) |
| C(4)–C(3)–C(2) | 112.66(12) |
| C(5)–C(4)–C(3) | 111.33(13) |
| O(5)–C(5)–C(4) | 110.72(12) |
| *Torsion angles [°]* | |
| Ring | |
| C(1)–C(2)–C(3)–C(4) | 39.47(17) |
| C(2)–C(3)–C(4)–C(5) | −45.29(17) |
| C(3)–C(4)–C(5)–O(5) | 55.64(16) |
| C(4)–O(5)–C(5)–C(1) | −62.17(16) |
| C(5)–O(5)–C(1)–C(2) | 55.90(16) |
| O(5)–C(1)–C(2)–C(3) | −43.95(17) |
| Side chains | |
| C(5)–O(5)–C(1)–O(11) | −63.51(15) |
| C(12)–O(11)–C(1)–O(5) | −73.08(15) |
| C(1)–O(11)–C(12)–N(121) | −9.2(2) |
| O(5)–C(1)–C(2)–O(21) | 70.15(15) |
| C(22)–O(21)–C(2)–C(1) | 78.87(16) |
| C(2)–O(21)–C(22)–O(221) | −1.3(2) |
| C(1)–C(2)–C(3)–O(31) | −78.28(15) |
| C(32)–O(31)–C(3)–C(2) | −81.04(15) |
| C(3)–O(31)–C(32)–O(321) | 4.9(2) |
| C(2)–C(3)–C(4)–O(41) | 69.95(15) |
| C(42)–O(41)–C(4)–C(3) | 76.38(17) |
| C(4)–O(41)–C(42)–O(421) | 2.6(2) |
| C(3)–C(4)–C(5)–C(51) | 174.14(13) |
| O(5)–C(5)–C(51)–O(511) | −5.1(2) |
| C(53)–O(52)–C(51)–C(5) | 179.43(15) |




**Scheme 4.** Reagents and conditions: (a) PivCl, DMF, Py, 45 °C, 48 h; (b) $N_2H_4$·HOAc, DMF, rt, 2.5 h; (c) $CCl_3CN$, $Cs_2CO_3$, $CH_2Cl_2$, 0 °C, 1 h.

coupling of acceptor **6** with donor **17** at −20 °C under the same reaction conditions, disaccharide **31** could not be obtained. Only by raising the temperature to −15 to −12 °C, could the disaccharide **31** be obtained in a satisfactory yield of 64%. The key to the deprotection of disaccharides is selective removal of the *O*-acetyl groups in the presence of *O*-pivaloyl groups. When treating disaccharide **28** with 7.5% HCl in methanol[27] in order to selectively remove the *O*-acetyl group, the reaction was slow and the yield was low because of migration of the pivaloyl group from O-4 of iduronic acid to O-2. To ensure that the *O*-acetate of disaccharide **28** could be cleaved in an effective way in the presence of the *O*-pivaloate, a methanol solution of **28** was treated at −20 °C with 0.1 M DBU.[28] The expected deacetylated disaccharide **29** was then obtained in the yield of 55%. However, treatment of the other disaccharide **31** with 0.1 M DBU under the same conditions did not effectively lead to the deacetylated disaccharide **32**. Fortunately, treatment of disaccharide **31** with 7.5% HCl in methanol at room temperature overnight[26] did result in the deacetylated disaccharide **32** in a good yield of 81%. O-Sulfation of both disaccharides **29** and **32** with $SO_3$·$NMe_3$ complex in DMF at 60 °C overnight led to disaccharides **30** (yield of 88%) and **33** (yield of 83%), respectively. Next, the esters of **30** was cleaved by a cocktail of reagents (LiOH, $H_2O_2$ followed by NaOH) in aqueous tetrahydrofuran (THF). The products were purified by chromatography on Sephadex LH-20, eluting with 1:1 MeOH–$H_2O$. The *O*-benzyl group was then removed with concomitant $N_3$ to $NH_2$ reduction by 10% Pd/C and $H_2$. These amines were treated with $SO_3$·pyridine in water at pH 9.8. The products were passed through a Bio-Gel P-2 column, eluting with $H_2O$. In this way by using a series of well-known reactions (saponification, hydrogenation, and N-sulfation),[29] the *O*-sulfated disaccharides **30** was converted into the target heparin-like disaccharide **1**. The overall yield of **1** from **30** was 17%. Following the same route, we obtained **2** from **33** in an overall yield of 10%. Obviously, the yield was very low. After a long investigation of the synthesis of heparin-like disaccharide **2** from the *O*-sulfated disaccharides **33**, we found that the step of saponification and the following chromatographic process on Sephadex LH-20 greatly influenced the overall yield of disaccharide **2**. We often got complex $^1$H NMR spectra after chromatographic purification, and sometimes two or more methoxy peaks were found in the same $^1$H NMR spectrum. To solve this problem, purification on Sephadex LH-20 was avoided, and the crude product after the work-up of the saponification reaction was used to do the next hydrogenation reaction directly. In this way, we were able to obtain acceptable yields of disaccharide **2** (52% overall yield from **33**).

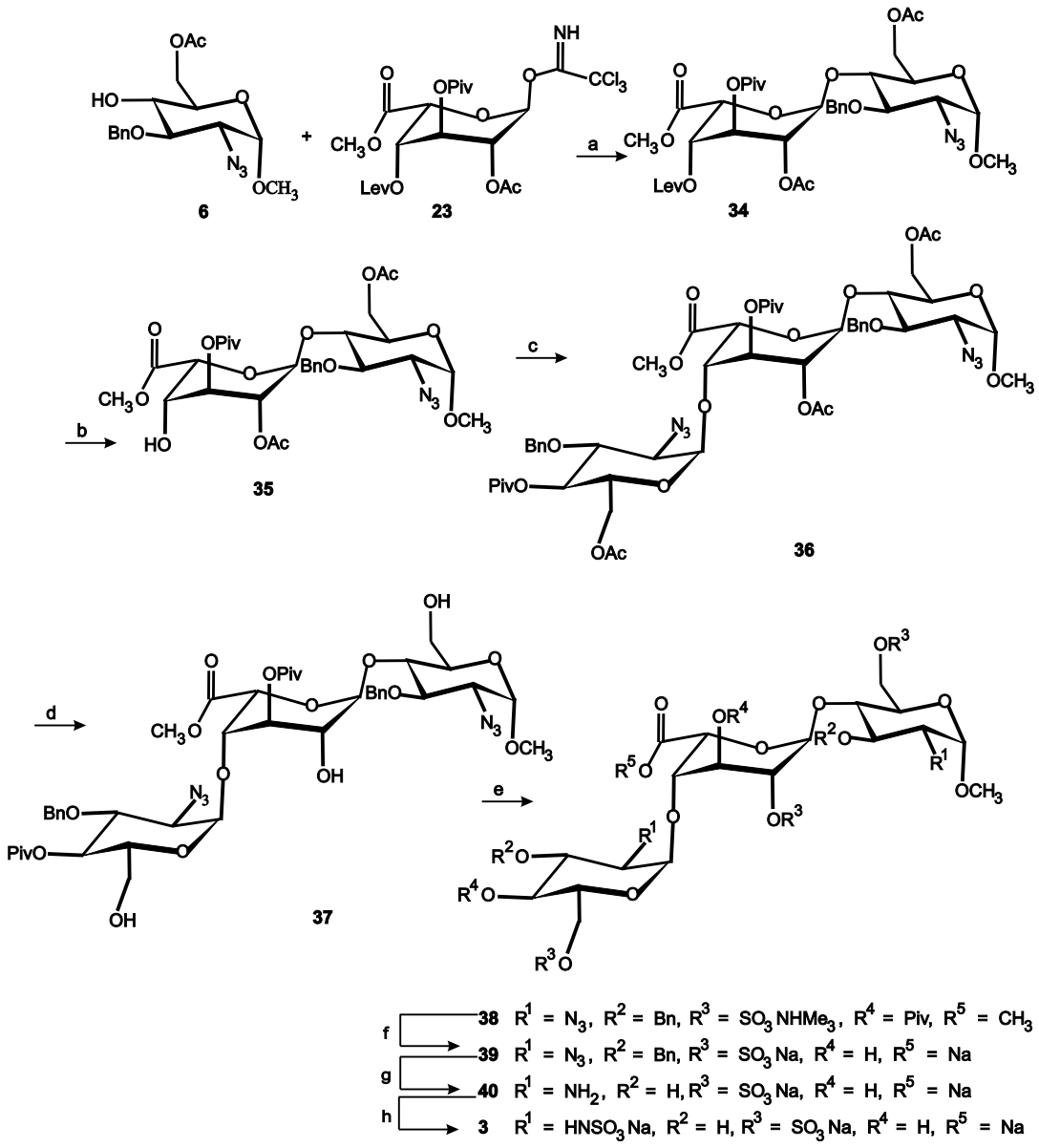



**Scheme 5.** Reagents and conditions: (a) TESOTf, 4 Å MS, $CH_2Cl_2$, −20 °C, 3 h; (b) 0.1 M DBU/MeOH, −20 °C, 3 h; (c) $SO_3{\cdot}NMe_3$, DMF, 60 °C, O/N; (d) 7.5% HCl–MeOH, 0 °C→rt, O/N; (e) $H_2O_2$, LiOH, THF, NaOH–$H_2O$, rt, 36 h; (f) 10% Pd/C, $H_2$, *t*-BuOH–$H_2O$, rt, O/N; (g) $SO_3{\cdot}$pyridine, NaOH–$H_2O$, pH 9.8, rt, 3 h.

## 2.4. Preparation of the trisaccharide

Acceptor **6** was coupled with the elongation building block **23** under the promotion of TESOTf in $CH_2Cl_2$ and 4 Å MS at −15 to −10 °C to give disaccharide **34** in 75% yield. The levulinoyl group was cleanly removed with hydrazine to give alcohol **35**, which was then coupled with nonreducing terminal building block **27** under the promotion of TESOTf in $CH_2Cl_2$ and 3 Å MS at −20 °C to give trisaccharide **36** in 40% yield. Treatment of **36** with 7.5% HCl in methanol led to a good yield (84%) of the triol **37**. The triol **37** was then reacted with $SO_3{\cdot}NMe_3$ complex in DMF at 60 °C overnight. The product **38** was purified by chromatography on Sephadex LH-20 eluting with methanol. The esters of **38** were then cleaved by a cocktail of reagents (LiOH, $H_2O_2$ followed by NaOH) in aqueous tetrahydrofuran (THF). We did this saponification reaction the same way as that of the synthesis of the heparin-like disaccharide **2**. The crude product was directly used to do the next hydrogenation reaction without purification on Sepladex LH-20. The *O*-benzyl group was removed with concomitant $N_3$ to $NH_2$ reduction to give **40**. These amines were treated with $SO_3{\cdot}$pyridine in water at pH 9.5. However, a Bio-Gel P-2 column was not effective for the purification of the residue from the N-sulfation reaction as there was always some salt mixed with the product after the column, though we got a very clean $^1$H NMR spectrum. In order to get the pure heparin-like trisaccharide **3** without salt, the mixture was passed through a Sephadex G-10 column eluting with 10% aqueous ethanol. The pure target trisaccharide **3** was then obtained in an overall yield of 47% from **38** (see Scheme 6).[28]

In conclusion, a simple and convenient synthetic route for preparing L-iduronic acid building blocks starting from readily available 1,2-*O*-isopropylidene-6,3-D-glucuronolactone was developed in less than nine steps. Based on this synthetic route, a modified and efficient synthetic method for preparing an L-iduronic acid building block from the key intermediate methyl dimethylthexylsilyl 3-*O*-pivaloyl-β-L-idopyranosiduronate was described. The single-crystal X-ray structure of the elongation building block (methyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-pivaloyl-α-L-idopyranosyluronate) trichloroacetimidate was obtained and showed that the molecular structure of the building block is in a $^1C_4$ conformation. Two heparin-like disaccharides and one heparin-like trisaccharide with different sulfation patterns have been prepared by using the L-iduronic acid building blocks developed by the above synthetic routes. Thus, an effective




**Scheme 6.** Reagents and conditions: (a) TESOTf, 4 Å MS, $CH_2Cl_2$, −15 to −10 °C, 3 h; (b) $N_2H_4$·HOAc, 2:1 EtOH–toluene, rt, 1 h; (c) TESOTf, 3 Å MS, $CH_2Cl_2$, −20 °C, 1 h; (d) 7.5% HCl–MeOH, 0 °C→rt, O/N; (e) $SO_3$·$NMe_3$, DMF, 50 °C, O/N; (f) $H_2O_2$, LiOH, THF, NaOH–$H_2O$, rt, 36 h; (g) 10% Pd/C, $H_2$, *t*-BuOH–$H_2O$, rt, O/N; (h) $SO_3$·pyridine, NaOH–$H_2O$, pH 9.5, rt, 3 h.

synthetic strategy was developed for preparing heparin-like oligosaccharide chains with defined size, sequence and charge distribution.

## 3. Experimental

### 3.1. General methods

The $^1H$ NMR spectra were obtained on Varian-500 (500 MHz) or Varian-400 (400 MHz) instruments with tetramethylsilane or the residue signal of the solvent as the internal standard. The $^{13}C$ NMR spectra were recorded on Varian-500 (125.75 MHz), Varian-400 (100.55 MHz), or Varian-200 (50.32 MHz) instruments. Optical rotations were measured at 21 ± 2 °C in a 1-dm cell on a Perkin–Elmer 341 polarimeter. Gel-permeation chromatography was performed using Sephadex LH-20, Sephadex G-10, and Bio-Gel P-2. Thin-layer chromatography (TLC) was performed on precoated plates of Silica Gel 60-$F_{254}$ (E. Merck, Darmstadt) and visualized with 1:20 $H_2SO_4$–$H_2O$, followed by heating. Unless otherwise stated, column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck). All solvents and reagents were purified and dried according to standard procedures.

### 3.2. Methyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-pivaloyl-α-D-glucopyranoside (5)

Methyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (460 mg, 1.47 mmol) was dissolved in $CH_2Cl_2$ (3.4 mL) and pyridine (3.4 mL) and cooled to −78 °C (dry ice and acetone), then pivaloyl chloride (0.67 mL, 3.6 equiv) was added at −78 °C. After 15 min, the reaction was stopped by solvent removal and purified on a flash column chromatography eluting with 2:1 hexanes–EtOAc. The final product **5** 420 mg (72%) was obtained as a colorless syrup: $[\alpha]_D$ +52.8 (*c* 2.44, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.38 (m, 5H, Ph), 4.92 (d, 1H, $J_{a,b}$ 11.1 Hz, CHaPh), 4.82 (d, 1H, $J_{b,a}$ 11.1 Hz, CHbPh), 4.79 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.43 (dd, 1H, $J_{6a,5}$ 4.7 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.25 (dd, 1H, $J_{6b,5}$ 2.3 Hz, $J_{6b,6a}$ 12.3 Hz, H-6b), 3.81 (dd, 1H, $J_{3,4}$ 1.4 Hz, $J_{3,2}$ 10.1 Hz, H-3), 3.76 (m, 1H, H-5), 3.43 (m, 4H, $OCH_3$, H-4), 3.34 (dd, 1H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 10.1 Hz, H-2), 2.69 (s, 1H, OH), 1.22 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (125.75 MHz, $CDCl_3$): $\delta$ 179.15 (C=O, Piv), 137.86, 128.66, 128.23, 128.14 (Ph), 98.68, 79.73 (C-1, C-3), 75.32 (*C*$H_2$Ph), 70.82, 70.05, 63.13, 62.94 (C-4, C-5, C-2, C-6), 55.21 ($OCH_3$), 38.91 (O=C–*C*, Piv), 27.16 ($CH_3$, Piv); Anal. Calcd for $C_{19}H_{27}N_3O_6$: C, 58.00; H, 6.92; N, 10.68. Found: C, 58.57; H, 7.23; N, 10.71. HRMS Calcd for $C_{19}H_{27}N_3O_6$ $[M-H]^+$: 392.1821. Found: 392.1796.

### 3.3. Methyl 3-*O*-pivaloyl-α,β-L-idopyranosuronate (9)

The syrup **8** (22.0 g, 66.4 mmol) was dissolved in 90% TFA (210 mL) and stirred for 3 h at room temperature under argon. The reaction was stopped by solvent removal. The product was coevaporated with toluene and then was put on the high-vacuum line. Crude product **9** (19.0 g) was obtained, which was used to do the next step directly without further purification.

### 3.4. Methyl (dimethylthexylsilyl 3-*O*-pivaloyl-β-L-idopyranosid)uronate (10)

The colorless syrup **9** (19.0 g, 65 mmol) was dissolved in dry MeCN (95 mL), followed by addition of imidazole (11.0 g, 2.5 equiv). The reaction mixture was then cooled to −20 °C, followed by addition of TDSCl (16.6 mL, 1.3 equiv). The reaction was let run overnight at −20 °C under argon. The reaction completion was verified with a TLC in 3:1 hexanes–EtOAc. Then the reaction was stopped by solvent removal. The solid was dissolved in $CH_2Cl_2$, filtered, and concentrated. The crude product was purified by flash column chromatography using 4:1 hexanes–EtOAc as eluant to give **10** 10.0 g (35%) as a colorless syrup: $[\alpha]_D$ +27.2 (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 5.22 (br t, 1H, H-3), 4.93 (d, 1H, $J_{1,2}$ 1.0 Hz, H-1), 4.32 (d, 1H, $J_{5,4}$ 1.6 Hz, H-5), 3.87 (m, 1H, H-4), 3.77 (s, 3H, $OCH_3$), 3.59 (m, 1H, H-2), 3.14 (br, 1H, OH), 1.63 (m, 1H, CH), 1.20 (s, 9H, $3CH_3$, Piv), 0.85 (m, 12H, $4CH_3$, TDS), 0.25 (s, 3H, $SiCH_3$), 0.19 (s, 3H, $SiCH_3$); $^{13}$C NMR (50.32 MHz, $CDCl_3$): $\delta$ 175.88 (C=O, Piv), 168.35 (C=O, ester), 93.49, 74.64, 69.00, 68.63, 66.99 (C-1, C-5, C-3, C-2, C-4), 52.24 ($OCH_3$), 38.75 (O=C–*C*, Piv), 33.93 (CH), 27.02 ($3CH_3$, Piv), 24.98 (Si–*C*–C), 20.16, 19.89, 18.54, 18.34 ($4\times CH_3$, TDS), −2.02, −3.69 ($2\times CH_3$, $2\times SiCH_3$). FABMS: Calcd for $C_{20}H_{38}O_8Si$ $[M+H]^+$: *m*/*z* 435.2. Found: *m*/*z* 435.2. Anal. Calcd for $C_{25}H_{46}O_9Si$: C, 57.89; H, 8.94. Found: C, 58.19; H, 9.32.

### 3.5. Methyl (dimethylthexylsilyl 3,4-di-*O*-pivaloyl-β-L-idopyranosid)uronate (11), methyl (dimethylthexylsilyl 2,3-di-*O*-pivaloyl-β-L-idopyranosid)uronate (13), and methyl (dimethylthexylsilyl 2,3,4-tri-*O*-pivaloyl-β-L-idopyranosid)uronate (14)

The colorless syrup **10** (3.2 g, 7.6 mmol) was dissolved in dry $CH_2Cl_2$ (70 mL) at 10 °C, followed by addition of pivalic anhydride (1.7 mL, 1.1 equiv). Approximately 15 min later, $Sc(OTf)_3$ (190 mg, 0.05 equiv) was added. After 1 h, a TLC in 5:1 toluene–EtOAc showed that the reaction was complete. The solvent phase was washed with satd aq $NaHCO_3$ ($2\times25$ mL) then dried over anhydrous $Na_2SO_4$. The organic phase was concentrated and dried overnight. The mixture was purified by flash column chromatography using 6:1 hexanes–EtOAc as eluant. Products **11** 1.26 g (32%), **13** 0.86 g (22%), and **14** 0.7 g (15%) were obtained (10:7:5 **11**:**13**:**14**); **11**: $[\alpha]_D$ +2.1 (*c* 0.42, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 5.18 (br t, 1H, H-3), 4.99 (m, 2H, H-1, H-4), 4.49 (d, 1H, $J_{5,4}$ 2.4 Hz, H-5), 3.73 (s, 3H, $OCH_3$), 3.51 (br m, 1H, H-2), 2.41 (br, 1H, OH), 1.65 (m, 1H, CH), 1.22 (s, 9H, $3CH_3$, Piv), 1.18 (s, 9H, $3CH_3$, Piv), 0.88 (m, 12H, $4CH_3$, TDS), 0.25 (s, 3H, $SiCH_3$), 0.20 (s, 3H, $SiCH_3$); $^{13}$C NMR (50.32 MHz, $CDCl_3$): $\delta$ 176.49, 175.64 ($2\times$C=O, $2\times$Piv), 167.23 (C=O, ester), 94.26, 73.15, 68.90, 67.82, 66.68 (C-1, C-5, C-3, C-2, C-4), 52.25 ($OCH_3$), 38.88 (O=C–*C*, Piv), 38.83 (O=C–*C*, Piv), 34.01 (CH), 27.13 ($3CH_3$, Piv), 26.93 ($3CH_3$, Piv), 25.13 (Si–*C*–C), 20.31, 19.99, 18.63, 18.41 ($4\times CH_3$, TDS), −1.98 ($CH_3$, $SiCH_3$), −3.37 ($CH_3$, $SiCH_3$); FABMS: Calcd for $C_{25}H_{46}O_9Si$ $[M+H]^+$: *m*/*z* 519.3. Found: *m*/*z* 519.3. **13**: $[\alpha]_D$ +131.8 (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 5.54 (br t, 1H, $J_{3,4}$ 7.8 Hz, $J_{3,2}$ 8.4 Hz, H-3), 5.53 (d, 1H, $J_{1,2}$ 4.3 Hz, H-1), 5.06 (dd, 1H, $J_{2,1}$ 4.3 Hz, $J_{2,3}$ 8.4 Hz, H-2), 4.66 (dd, 1H, $J_{4,5}$ 1.0 Hz, $J_{4,3}$ 7.8 Hz, H-4), 4.02 (br s, 1H, H-5), 3.80 (s, 3H, $OCH_3$), 2.93 (br, 1H, OH), 1.60 (m, 1H, CH), 1.22 (s, 9H, $3CH_3$, Piv), 1.18 (s, 9H, $3CH_3$, Piv), 0.84 (m, 12H, $4CH_3$, TDS), 0.10 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$); $^{13}$C NMR (50.32 MHz, $CDCl_3$): $\delta$ 178.35 (C=O, Piv), 177.61 (C=O, Piv), 172.75 (C=O, ester),

93.36, 76.47, 75.38, 74.11, 69.17 (C-1, C-2, C-4, C-3, C-5), 52.85 ($OCH_3$), 38.74 (O=C–*C*, Piv), 38.55 (O=C–*C*, Piv), 33.92 (CH), 27.12 ($3CH_3$, Piv), 26.92 ($3CH_3$, Piv), 24.72 (Si–*C*–C), 19.98, 19.88, 18.52, 18.47 ($4\times CH_3$, TDS), −2.62, −3.15 ($2\times CH_3$, $2\times SiCH_3$); HRMS: Calcd for $C_{25}H_{46}O_9Si$ $[M-H]^+$: *m/z* 517.2833. Found: *m/z* 517.2719. **14**: $[\alpha]_D$ −31.6 (*c* 1.18, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 5.34 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.18 (br t, 1H, $J_{3,4}$ 4.1 Hz, $J_{3,2}$ 5.3 Hz, H-3), 5.14 (br t, 1H, $J_{4,5}$ 3.9 Hz, $J_{4,3}$ 4.1 Hz, H-4), 4.84 (d, 1H, $J_{5,4}$ 3.9 Hz, H-5), 4.76 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 5.3 Hz, H-2), 3.73 (s, 3H, $OCH_3$), 1.61 (m, 1H, CH), 1.22, 1.16 ($2\times$s, 27H, $9CH_3$, $3\times$Piv), 0.84 (m, 12H, $4CH_3$, TDS), 0.16 (s, 3H, $SiCH_3$), 0.15 (s, 3H, $SiCH_3$); $^{13}C$ NMR (50.32 MHz, $CDCl_3$): $\delta$ 176.87, 176.83, 176.44 ($3\times$C=O, $3\times$Piv), 168.36 (C=O, ester), 93.46, 70.87, 68.94, 68.04, 68.02 (C-1, C-2, C-3, C-5, C-4), 52.27 ($OCH_3$), 38.80, 38.70 (overlap, $3\times$O=C–*C*, $3\times$Piv), 33.82 (CH), 27.20, 27.09, 27.08 ($3\times3CH_3$, $3\times$Piv), 24.82 (Si–*C*–C), 20.01, 19.95, 18.50, 18.47 ($4\times CH_3$, TDS), −2.42, −3.21 ($2\times CH_3$, $2\times SiCH_3$); FABMS: Calcd for $C_{30}H_{54}O_{10}Si$ $[M+H]^+$: *m/z* 603.36. Found: *m/z* 603.28.

### 3.6. Methyl (dimethylthexylsilyl 2-*O*-acetyl-3,4-di-*O*-pivaloyl-α,β-L-idopyranosid)uronate (12)

The colorless syrup **11** (430 mg, 0.8 mmol) was dissolved in dry $CH_2Cl_2$ (7.5 mL) followed by addition of $Ac_2O$ (0.8 mL, 10.0 equiv) then $Sc(OTf)_3$ (20 mg, 0.05 equiv) was added. The mixture was stirred at room temperature overnight. The solvent phase was washed with $H_2O$ ($2\times5$ mL) then dried over anhydrous $Na_2SO_4$. The organic phase was concentrated. The mixture was purified by flash column chromatography using 10:1 hexanes–EtOAc as eluant. The α anomer 100 mg (22%) and β anomer 160 mg (34%) of the product **12** was obtained (5:8 α:β); α anomer: $[\alpha]_D$ +38.5 (*c* 1.01, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 5.12 (br t, 1H, $J_{2,1}$ 1.8 Hz, $J_{2,3}$ 3.3 Hz, H-2), 5.03 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 4.98 (m, 1H, $J_{4,5}$ 2.5 Hz, H-4), 4.90 (m, 1H, H-3), 4.49 (d, 1H, $J_{5,4}$ 2.5 Hz, H-5), 3.73 (s, 3H, $OCH_3$), 2.06 (s, 3H, $CH_3$, Ac), 1.61 (m, 1H, CH), 1.23 (s, 9H, $3CH_3$, Piv), 1.19 (s, 9H, $3CH_3$, Piv), 0.85 (m, 12H, $4CH_3$, TDS), 0.22 (s, 3H, $SiCH_3$), 0.15 (s, 3H, $SiCH_3$); $^{13}C$ NMR (50.32 MHz, $CDCl_3$): $\delta$ 176.67, 175.28 ($2\times$C=O, $2\times$Piv), 169.40 (C=O, Ac), 167.17 (C=O, ester), 93.08, 72.86, 67.90, 66.72, 66.08 (C-1, C-5, C-2, C-3, C-4), 52.21 ($OCH_3$), 38.81 ($2\times$O=C–*C*, $2\times$Piv), 33.92 (CH), 27.10 ($3CH_3$, Piv), 26.95 ($3CH_3$, Piv), 25.00 (Si–*C*–C), 20.87 ($CH_3$, Ac), 20.11, 19.89, 18.55, 18.41 ($4\times CH_3$, TDS), −2.01, −3.33 ($2\times CH_3$, $2\times SiCH_3$); FABMS: Calcd for $C_{27}H_{48}O_{10}Si$ $[M-OTDS]^+$: *m/z* 401.2. Found: *m/z* 401.3. Anal. Calcd for $C_{27}H_{48}O_{10}Si$: C, 57.83; H, 8.63. Found: C, 57.74; H, 8.86; β anomer: $[\alpha]_D$ −52.9 (*c* 1.01, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 5.39 (d, 1H, $J_{1,2}$ 2.6 Hz, H-1), 5.10 (br t, 1H, H-3), 5.07 (br t, 1H, H-4), 4.87 (d, 1H, $J_{5,4}$ 3.3 Hz, H-5), 4.70 (br t, 1H, H-2), 3.75 (s, 3H, $OCH_3$), 2.03 (s, 3H, $CH_3$, Ac), 1.63 (m, 1H, CH), 1.21 (s, 9H, $3CH_3$, Piv), 1.18 (s, 9H, $3CH_3$, Piv), 0.86 (m, 12H, $4CH_3$, TDS), 0.19 (s, 3H, $SiCH_3$), 0.17 (s, 3H, $SiCH_3$); $^{13}C$ NMR (50.32 MHz, $CDCl_3$): $\delta$ 176.69, 176.45 ($2\times$C=O, $2\times$Piv), 169.33 (C=O, Ac), 168.50 (C=O, ester), 92.93, 69.43, 67.46, 67.16, 67.10 (C-1, C-2, C-3, C-4, C-5), 52.40 ($OCH_3$), 38.81, 38.78 ($2\times$O=C–*C*, $2\times$Piv), 33.82 (CH), 27.06, 26.97 ($2\times3CH_3$, $2\times$Piv), 25.04 (Si–*C*–C), 20.85 ($CH_3$, Ac), 20.04, 20.01, 18.48 ($4CH_3$, TDS), −2.46, −3.21 ($2\times CH_3$, $2\times SiCH_3$); FABMS: Calcd for $C_{27}H_{48}O_{10}Si$ $[M+H]^+$: *m/z* 561.3. Found: *m/z* 561.4. Anal. Calcd for $C_{27}H_{48}O_{10}Si$: C, 57.83; H, 8.63. Found: C, 57.94; H, 9.13.

### 3.7. Methyl 2-*O*-acetyl-3,4-di-*O*-pivaloyl-α,β-L-idopyranosuronate (15)

The colorless syrup **12** (1.0 g, 1.8 mmol) was dissolved in dry THF (13.5 mL) and cooled to 0 °C, followed by addition of HOAc (0.3 mL, 3.0 equiv) and $Bu_4NF$ (2.7 mL, 1.5 equiv, 1.0 M solution in THF). The reaction mixture was stirred with the temperature gradually increasing to 15 °C. The reaction was checked by TLC using 1:3 hexanes–EtOAc. The resulting orange solution was filtered through a bed of silica gel with 1:3 hexanes–EtOAc to give three fractions of 200 mL each, and then the three fractions were combined and concentrated. The crude product was purified by flash column chromatography using 1:2 hexanes–EtOAc as eluant to give product **15** (0.73 g, 98%), which was used directly in the next step.

### 3.8. Methyl 2,3,4-tri-*O*-pivaloyl-α,β-L-idopyranosuronate (16)

The colorless syrup **14** (1.1 g, 1.9 mmol) was dissolved in dry THF (13 mL) and cooled to 0 °C, followed by addition of HOAc (0.32 mL, 3.0 equiv) and $Bu_4NF$ (2.8 mL, 1.5 equiv, 1.0 M solution in THF). The reaction mixture was stirred at room temperature overnight. The reaction was checked by TLC using 2:1 hexanes–EtOAc. The resulting orange solution was filtered through a bed of silica gel with 1:3 hexanes–EtOAc to give three fractions of 250 mL each, and then the three fractions were combined and concentrated. The crude product was purified by flash column chromatography using 1:2 hexanes–EtOAc as eluant to give product **16** (0.81 g, 94%), which was used directly in the next step.

### 3.9. Methyl 2-*O*-acetyl-3,4-di-*O*-pivaloyl-α-L-idopyranosyluronate trichloroacetimidate (17)

Product **15** (1.25 g, 3.0 mmol) was dissolved in dry $CH_2Cl_2$ (22 mL) and cooled in an ice bath. Then,

$CCl_3CN$ (3.0 mL, 10.0 equiv) was added and allowed to cool down, followed by the addition of DBU (106.6 μL). The reaction mixture was stirred for 1 h, and TLC in 3:2 hexanes–EtOAc showed that the reaction was complete. The crude product was purified by flash column chromatography using 6:1 hexanes–EtOAc as eluant to give the final product **17** 0.92 g (55%): $[\alpha]_D$ −49.7 (*c* 1.88, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 8.86 (s, 1H, NH), 6.49 (s, 1H, 1-H), 5.13 (m, 2H, H-3, H-4), 4.97 (m, 1H, H-2), 4.89 (d, 1H, $J_{5,4}$ 2.3 Hz, 5-H), 3.74 (s, 3H, $OCH_3$), 2.07 (s, 3H, $CH_3$, Ac), 1.23 (s, 9H, $3CH_3$, Piv), 1.18 (s, 9H, $3CH_3$, Piv); $^{13}C$ NMR (50.32 MHz, $CDCl_3$): $\delta$ 176.48, 176.26 ($2\times$C=O, $2\times$Piv), 168.92 (C=O, Ac), 167.33 (C=O, ester), 159.80 (C=NH), 94.00 (C-1), 90.62 ($CCl_3$), 68.12 (C-5), 66.12 (overlap, C-2, C-3, C-4), 52.62 ($OCH_3$), 38.89, 38.85 ($2\times$O=C–*C*, $2\times$Piv), 27.08, 26.93 ($2\times3CH_3$, $2\times$Piv), 20.72 ($CH_3$, Ac); FABMS: Calcd for $C_{21}H_{30}Cl_3NO_{10}$ $[M-OTCI]^+$: *m/z* 401.2. Found: *m/z* 401.3. Anal. Calcd for $C_{21}H_{30}Cl_3NO_{10}$: C, 44.81; H, 5.37; N, 2.49. Found: C, 44.65; H, 5.58; N, 2.53.

### 3.10. Methyl 2,3,4-tri-*O*-pivaloyl-α-L-idopyranosyluronate trichloroacetimidate (18)

The product **16** (0.81 g, 1.8 mmol) was dissolved in dry $CH_2Cl_2$ (13 mL) and cooled in an ice bath. Then $CCl_3CN$ (1.8 mL, 10.0 equiv) was added and allowed to cool down, followed by the addition of DBU (62.7 μL). The reaction mixture was stirred for 1 h, and TLC in 2:1 hexanes–EtOAc showed that the reaction was complete. The crude product was purified by flash column chromatography using 6:1 hexanes–EtOAc as eluant to give the final product **18** (0.66 g, 63%); $[\alpha]_D$ −57.3 (*c* 1.0, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 8.81 (s, 1H, NH), 6.51 (d, 1H, $J_{1,2}$ 3.1 Hz, H-1), 5.26 (br t, 1H, H-3), 5.21 (br t, 1H, H-4), 5.07 (br t, 1H, H-2), 4.89 (d, 1H, $J_{5,4}$ 3.5 Hz, H-5), 3.75 (s, 3H, $OCH_3$), 1.22 (s, 9H, $3CH_3$, Piv), 1.21 (s, 9H, $3CH_3$, Piv), 1.19 (s, 9H, $3CH_3$, Piv); $^{13}C$ NMR (50.32 MHz, $CDCl_3$): $\delta$ 176.94, 176.83, 176.45 ($3\times$C=O, $3\times$Piv), 167.53 (C=O, ester), 160.12 (C=NH), 94.68 (C-1), 90.62 ($CCl_3$), 69.17, 67.83, 67.53, 67.11 (C-5, C-3, C-2, C-4), 52.53 ($OCH_3$), 38.86, 38.80, 38.78 ($3\times$O=C–*C*, $3\times$Piv), 27.06 ($9CH_3$, $3\times$Piv); FABMS: Calcd for $C_{22}H_{35}O_9$ $[M-OTI]^+$: *m/z* 443.2. Found: *m/z* 443.3. Anal. Calcd for $C_{24}H_{36}Cl_3NO_{10}$: C, 47.65; H, 6.00; N, 2.32. Found: C, 48.05; H, 6.33; N, 2.31.

The syntheses of compounds **19**, **20**, and **21** are described in Ref. 25.

### 3.11. Methyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-pivaloyl-α,β-L-idopyranosuronate (22)

The colorless syrup **21** (1.14 g, 1.98 mmol) was dissolved in dry THF (15 mL) and cooled to 0 °C, followed by addition of HOAc (0.33 mL, 3.0 equiv) and $Bu_4NF$ (3.0 mL, 1.5 equiv, 1.0 M solution in THF). Then the temperature was gradually increased to room temperature. The reaction mixture was stirred overnight. The reaction was checked by TLC using a mixture of 1:3 hexanes–EtOAc. The resulting orange solution was filtered through a bed of silica gel with 1:3 hexanes–EtOAc to give three fractions of 200 mL each, and then the three fractions were combined and concentrated. The crude product was purified by flash column using 1:2 hexanes–EtOAc as eluant to give product **22** (0.55 g, 64%), which was used directly in the next step.

### 3.12. Methyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-pivaloyl-α,β-L-idopyranosyluronate trichloroacetimidate (23)

The product **22** (390 mg, 0.91 mmol) was dissolved in dry $CH_2Cl_2$ (6.6 mL) and cooled in an ice bath. Then, $CCl_3CN$ (0.91 mL, 10.0 equiv) was added, followed by the addition of DBU (32 μL). The reaction mixture was stirred for 1.0 h at 0 °C, and TLC using 1:2 hexanes–EtOAc showed that the reaction was complete. The mixture was evaporated, and the residue was purified on a MPLC column eluting with 2:1:1 hexanes–EtOAc–$CH_2Cl_2$ to give the α anomer (290 mg, 58%) and the β anomer (79 mg, 15%) of the product **23** as colorless oils. α Anomer: $[\alpha]_D$ −68.5 (*c* 1.0, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 8.86 (s, 1H, NH), 6.50 (br s, 1H, H-1), 5.41 (m, 1H, H-3), 5.14 (br t, 1H, $J_{4,5}$ 2.3 Hz, H-4), 4.99 (m, 1H, H-2), 4.88 (d, $J_{5,4}$ 2.3 Hz, 1H, H-5), 3.79 (s, 3H, $OCH_3$), 2.76 (m, 2H, $CH_2$, Lev), 2.58 (m, 2H, $CH_2$, Lev), 2.17 (s, 3H, $CH_3$, Lev), 2.14 (s, 3H, $CH_3$, Ac), 1.24 (s, 9H, $3CH_3$, Piv); $^{13}C$ NMR (50.32 MHz, $CDCl_3$): $\delta$ 205.80 (C=O, Lev), 176.32 (C=O, Piv), 171.10 (O=C, Lev), 169.09 (C=O, Ac), 167.39 (C=O, ester), 159.93 (N=*C*–$CCl_3$), 94.16 (C-1), 90.65 ($CCl_3$), 68.04, 66.67, 66.15, 65.82 (C-5, C-4, C-2, C-3), 52.80 ($OCH_3$), 38.84 (O=C–*C*, Piv), 37.50 ($CH_2$, Lev), 29.72 ($CH_3$, Lev), 27.61 ($CH_2$, Lev), 26.99 ($3CH_3$, Piv), 20.67 ($CH_3$, Ac); FABMS: Calcd for $C_{19}H_{27}O_{10}$ $[M-OTCI]^+$: *m/z* 415.2. Found: *m/z* 415.2. β Anomer: $[\alpha]_D$ +9.5 (*c* 1.0, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 8.68 (s, 1H, NH), 6.22 (d, 1H, $J_{1,2}$ 2.3 Hz, H-1), 5.41 (br t, 1H, $J_{3,2}$ 4.7 Hz, H-3), 5.23 (dd, 1H, $J_{2,1}$ 2.3 Hz, $J_{2,3}$ 4.7 Hz, H-2), 5.14 (br t, 1H, $J_{4,5}$ 2.9 Hz, H-4), 4.71 (d, $J_{5,4}$ 2.9 Hz, 1H, H-5), 3.80 (s, 3H, $OCH_3$), 2.72 (m, 2H, $CH_2$, Lev), 2.57 (m, 2H, $CH_2$, Lev), 2.18 (s, 3H, $CH_3$, Lev), 2.12 (s, 3H, $CH_3$, Ac), 1.24 (s, 9H, $3CH_3$, Piv); $^{13}C$ NMR (50.32 MHz, $CDCl_3$): $\delta$ 205.90 (O=*C*–$CH_3$, Lev), 175.71 (C=O, Piv), 171.29 (O=C–O, Lev), 169.45 (C=O, Ac), 166.54 (O=C, ester), 160.45 (N=*C*–$CCl_3$), 94.32 (C-1), 90.33 ($CCl_3$), 73.05, 66.98, 66.82, 65.32 (C-5, C-4, C-3, C-2), 52.76 ($OCH_3$), 38.86 (O=C–*C*, Piv), 37.52 ($CH_2$, Lev), 29.74 ($CH_3$, Lev), 27.68 ($CH_2$, Lev), 27.01

($3CH_3$, Piv), 20.59 ($CH_3$, Ac); FABMS: Calcd for $C_{19}H_{27}O_{10}$ $[M-OTCI]^+$: *m/z* 415.1604. Found: *m/z* 415.1613.

Colorless and transparent crystals of the donor, methyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-pivaloyl-α-L-idopyranosyluronate) trichloroacetimidate, the α anomer of **23**, were obtained by slow evaporation of a saturated 3:1 hexanes–$CH_2Cl_2$ solution.

### 3.13. 1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-pivaloyl-α,β-D-glucopyranose (25)

Sugar **24** (0.64 g, 1.68 mmol) was dissolved in pyridine (10 mL). The temperature was raised to 45 °C. Then DMF (0.5 mL) and PivCl (1.04 mL, 5.0 equiv) were added. After 24 h another batch of PivCl (5.0 equiv) was added, and after a further 24 h of continuously stirring, the mixture was diluted with $CH_2Cl_2$ (50 mL), washed twice with $H_2O$ (2 × 20 mL). The organic phase was dried with $Na_2SO_4$ and filtered, and the solvent was evaporated. The crude product was purified on a MPLC column eluting with 8:1 hexanes–EtOAc to give the product **25** (0.32 g, 41%) as a colorless oil; $^1$H NMR (400 MHz, $CDCl_3$): δ 7.36–7.27 (m, 5Hα, 5Hβ, Phs), 6.28 (d, 1Hα, $J_{1,2}$ 3.7 Hz, H-1α), 5.52 (d, 1Hβ, $J_{1,2}$ 8.4 Hz, H-1β), 5.23 (br t, 1Hα, $J_{4,3}$ 9.6 Hz, H-4α), 4.80 (d, 1Hα, $J_{a,b}$ 10.7 Hz, CHaPhα), 4.69 (d, 1Hα, $J_{b,a}$ 10.7 Hz, CHbPhα), 4.17 (dd, 1Hα, $J_{6a,5}$ 4.6 Hz, $J_{6a,6b}$ 12.6 Hz, H-6aα), 4.01 (m, 2Hα, H-6bα, H-5α), 3.94 (br t, 1Hα, $J_{3,4}$ 9.6 Hz, $J_{3,2}$ 9.9 Hz, H-3α), 3.69 (dd, 1Hα, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 9.9 Hz, H-2α), 2.21 (s, 3Hα, $CH_3$α, Acα), 2.08 (s, 3Hα, $CH_3$α, Acα), 1.20 (s, 9Hα, Pivα); HRMS: Calcd for $C_{22}H_{29}N_3O_8M^+$: 463.1955. Found: 463.1995.

### 3.14. 6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-pivaloyl-α,β-D-glucopyranose (26)

Sugar **25** (0.28 g, 0.61 mmol) was dissolved in DMF (10 mL). Then (73 mg, 1.3 equiv) of $N_2H_4$·HOAc was added. The mixture was stirred at room temperature for 2.5 h. After that time, the mixture was diluted with $CH_2Cl_2$ (30 mL) and washed with $H_2O$ (3 × 10 mL). The organic layer was dried over $Na_2SO_4$ and filtered. The solvent was evaporated. The crude product was purified on a MPLC column eluting with 3:1 hexanes–EtOAc to give product **27** (205 mg, 80%) as a colorless oil that was directly used in the next step.

### 3.15. 6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (27)

The product **26** (205 mg, 0.49 mmol) was dissolved in dry $CH_2Cl_2$ (3 mL) and cooled in an ice bath. Then, $CCl_3CN$ (0.5 mL, 10.0 equiv) was added and allowed to cool down, followed by the addition of $Cs_2CO_3$ (1.6 g, 10.0 equiv). The reaction mixture was stirred for 1 h, and TLC in 2:1 hexanes–EtOAc showed that the reaction was complete. The mixture was filtered and evaporated. The crude product was purified on a MPLC column eluting with 8:1 hexanes–EtOAc to give the product **27** (160 mg, 58%) as a colorless oil: $[\alpha]_D$ +56.2 (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 1H, NH), 7.36–7.28 (m, 5H, Ph), 6.46 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.81 (d, 1H, $J_{a,b}$ 10.7 Hz, CHaPh), 4.72 (d, 1H, $J_{b,a}$ 10.7 Hz, CHbPh), 4.16–4.10 (m, 3H, H-6a, H-6b, H-5), 4.04 (br t, 1H, H-3), 3.79 (dd, 1H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 10.1 Hz, H-2), 2.06 (s, 3H, $CH_3$, Ac), 1.21 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (50.32 MHz, $CDCl_3$): δ 176.65 (C=O, Piv), 170.43 (C=O, Ac), 160.37 (C=NH), 136.98, 128.36, 127.87, 127.49 (Ph), 94.30 (C-1), 90.69 ($CCl_3$), 77.76 (C-3), 74.84 ($CH_2$Ph), 70.94, 69.03, 62.51, 61.52 (C-5, C-4, C-2, C-6), 38.94 (O=C–*C*, Piv), 27.12 ($3CH_3$, Piv), 20.75 ($CH_3$, Ac); HRMS: Calcd for $C_{20}H_{26}N_3O_6$ $[M-TCI]^+$: 404.1822. Found: 404.1905. Anal. Calcd for $C_{22}H_{27}Cl_3N_4O_7$: C, 46.70; H, 4.81; N, 9.90. Found: C, 47.34; H, 5.15; N, 10.03.

### 3.16. Methyl (methyl 2-*O*-acetyl-3,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-pivaloyl-α-D-glucopyranoside (28)

Glucosamine **5** (44 mg, 0.1 mmol) and L-iduronic acid **17** (94 mg, 1.5 equiv) were coevaporated with toluene and then dissolved in anhyd $CH_2Cl_2$ (4 mL). Freshly activated powdered molecular sieves (35 mg, 4 Å) were then added. The mixture was stirred for half an hour at −20 °C, then TESOTf (12.6 μL, 0.5 equiv) was added dropwise, and 3 h later *N*,*N*-ethyldiisopropylamine (25 μL, 1.0 equiv) was added. The mixture was filtered through a pad of Celite, and the solvent was removed under reduced pressure. Purification on a MPLC column eluting with (5:1 toluene–EtOAc) afforded the disaccharide **28** 75 mg (85%) as a colorless syrup: $[\alpha]_D$ +1.5 (*c* 0.66, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): δ 7.37 (m, 5H, Ph), 5.34 (d, 1H, $J_{1',2'}$ 4.7 Hz, H-1′), 5.22 (br t, 1H, H-3′), 5.08 (dd, 1H, H-4′), 4.90 (dd, 1H, $J_{2',3'}$ 1.8 Hz, $J_{2',1'}$ 4.7 Hz, H-2′), 4.84 (br t, 2H, CHaPh, CHbPh), 4.76 (br t, 2H, $J_{1,2}$ 3.7 Hz, $J_{5',4'}$ 5.5 Hz, H-5′, H-1), 4.54 (d, 1H, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.12 (dd, 1H, $J_{6a,5}$ 4.5 Hz, $J_{6a,6b}$ 11.3 Hz, H-6b), 3.87 (br t, 1H, H-3), 3.80 (m, 2H, H-4, H-5), 3.46 (s, 3H, $OCH_3$, ester), 3.42 (s, 3H, $OCH_3$), 3.39 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 9.7 Hz, H-2), 2.05 (s, 3H, $CH_3$, Ac), 1.22 (s, 9H, $3CH_3$, Piv), 1.20 (s, 9H, $3CH_3$, Piv), 1.14 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (100.55 MHz, $CDCl_3$): δ 177.76, 176.67, 176.39 (3 × C=O, 3 × Piv), 169.31 (C=O, ester), 168.17 (C=O, Ac), 137.55, 128.32, 127.62 (Ph), 98.23 (C-1), 97.99 (C-1′), 78.10, 77.33 (C-3, C-5), 74.58 ($CH_2$Ph), 69.22 (C-4), 69.05, 68.88 (C-2′, C-5′), 67.86, 67.67 (C-3′, C-4′), 63.40, 61.72 (C-2, C-6), 55.40 ($OCH_3$, ester), 52.05

($OCH_3$), 38.93, 38.80 (overlap, 3 × O=C–*C*, 3 × Piv), 27.25, 27.06, 26.97 (3 × $3CH_3$, 3 × Piv), 20.74 ($CH_3$, Ac); FABMS: Calcd for $C_{38}H_{55}N_3O_{15}$ $[M+Na]^+$: *m/z* 816.4. Found: *m/z* 816.4.

### 3.17. Methyl (methyl 3,4-di-*O*-pivaloyl-α-L-idopyranosyl-uronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-pivaloyl-α-D-glucopyranoside (29)

The disaccharide **28** (154 mg, 0.2 mmol) was dissolved in anhyd MeOH (31 mL), and DBU (460.6 μL) was added at −20 °C. About 3 h later, the solution was diluted with $CH_2Cl_2$ (350 mL), washed with 5% HCl (2 × 35 mL), and then washed with $H_2O$ (3 × 35 mL). The organic phase was dried over anhyd $Na_2SO_4$. Then the solvent was removed under reduced pressure. Purification on a MPLC column eluting with 6:1 toluene–EtOAc afforded the deacetylated disaccharide **29** (80 mg, 55%) as a colorless oil: $[\alpha]_D$ +3.5 (*c* 0.54, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.35 (m, 5H, Ph), 5.40 (d, 1H, $J_{1',2'}$ 6.0 Hz, H-1′), 5.29 (br t, 1H, H-3′), 5.12 (dd, 1H, $J_{4',5'}$ 5.9 Hz, $J_{4',3'}$ 8.2 Hz, H-4′), 4.92 (d, 1H, $J_{a,b}$ 10.3 Hz, CHaPh), 4.78 (d, 1H, $J_{b,a}$ 10.3 Hz, CHbPh), 4.75 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.68 (d, 1H, $J_{5',4'}$ 5.9 Hz, H-5′), 4.65 (dd, 1H, $J_{6a,5}$ 1.6 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.18 (dd, 1H, $J_{6b,5}$ 5.3 Hz, $J_{6b,6a}$ 12.1 Hz, H-6b), 3.88 (dd, 1H, $J_{3,2}$ 10.1 Hz, $J_{3,4}$ 10.4 Hz, H-3), 3.83 (ddd, 1H, $J_{5,6a}$ 1.6 Hz, $J_{5,6b}$ 5.3 Hz, $J_{5,4}$ 10.0 Hz, H-5), 3.73 (dd, 1H, $J_{4,5}$ 10.0 Hz, $J_{4,3}$ 10.4 Hz, H-4), 3.61 (dd, 1H, $J_{2',1'}$ 6.0 Hz, $J_{2',3'}$ 8.0 Hz, H-2′), 3.54 (s, 3H, $OCH_3$, ester), 3.41 (s, 3H, $OCH_3$), 3.39 (dd, 1H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 10.1 Hz, H-2), 1.69 (br, 1H, OH), 1.22 (s, 9H, $3CH_3$, Piv), 1.21 (s, 9H, $3CH_3$, Piv), 1.14 (s, 9H, $3CH_3$, Piv); $^{13}C$ NMR (50.32 MHz, $CDCl_3$): 178.35, 177.68, 176.69 (3 × C=O, 3 × Piv), 168.81 (C=O, ester), 137.61, 128.31, 127.95, 127.68 (Ph), 101.45 (C-1′), 98.14 (C-1), 78.59, 78.44 (C-4, C-3), 75.12 (*C*$H_2$Ph), 71.68, 70.34, 70.15 (C-2′, C-3′, C-5′), 69.88 (C-5), 68.29 (C-4′), 63.31, 62.43 (C-2, C-6), 55.27 ($OCH_3$, ester), 52.03 ($OCH_3$), 38.96, 38.89, 38.81 (3 × O=C–*C*, 3 × Piv), 27.22, 27.13, 27.02 (3 × $3CH_3$, 3 × Piv); FABMS: Calcd for $C_{36}H_{53}N_3O_{14}$ $[M+Na]^+$: *m/z* 774.3. Found: *m/z* 774.7.

### 3.18. Methyl (methyl 2-*O*-trimethylaminesulfonato-3,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-pivaloyl-α-D-glucopyranoside (30)

A solution of the deacylated disaccharide **29** (115 mg, 0.15 mmol) and $SO_3{\cdot}NMe_3$ complex (213 mg, 10.0 equiv) in dry DMF (15 mL) was stirred overnight at 60 °C. After the reaction was finished, the solution was concentrated to ~2 mL. Chromatography on Sephadex LH-20 using MeOH gave product (120 mg, 88%): $[\alpha]_D$ +13.0 (*c* 1.0, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): δ 9.72 (br, NH, $HN^+Me_3$), 7.34 (m, 5H, Ph), 5.34 (d, 1H, $J_{1',2'}$ 3.9 Hz, H-1′), 5.32 (br t, 1H, H-3′), 5.02 (br t, 1H, $J_{4',5'}$ 3.9 Hz, $J_{4',3'}$ 5.1 Hz, H-4′), 4.90 (d, 1H, $J_{a,b}$ 10.8 Hz, CHaPh), 4.83 (d, 1H, $J_{b,a}$ 10.8 Hz, CHbPh), 4.79 (d, 1H, $J_{5',4'}$ 3.9 Hz, H-5′), 4.74 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.44 (dd, 1H, $J_{6a,5}$ 1.6 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.40 (dd, 1H, $J_{2',1'}$ 3.9 Hz, $J_{2',3'}$ 4.5 Hz, H-2′), 4.34 (dd, 1H, $J_{6b,5}$ 4.1 Hz, $J_{6b,6a}$ 12.1 Hz, H-6b), 3.89 (m, 3H, H-3, H-5, H-4), 3.42 (s, 3H, $OCH_3$), 3.39 (m, 4H, H-2, $OCH_3$, ester), 2.91 (d, 9H, $J_{CH_3}$,NH 5.1 Hz, $3CH_3$, $NMe_3$), 1.23 (s, 9H, $3CH_3$, Piv), 1.20 (s, 9H, $3CH_3$, Piv), 1.13 (s, 9H, $3CH_3$, Piv); $^{13}C$ NMR (100.55 MHz, $CDCl_3$): δ 178.24, 177.05 (overlap, 3 × C=O, 3 × Piv), 168.40 (C=O, ester), 137.77, 128.27, 127.61, 127.51 (Ph), 99.02 (C-1′), 98.27, 78.05, 76.86 (C-1, C-3, C-4), 74.30 (*C*$H_2$Ph), 72.95 (C-2′), 69.08 (C-5), 68.11, 67.64, 67.33 (C-5′, C-4′, C-3′), 63.44, 62.46 (C-2, C-6), 55.29 ($OCH_3$, ester), 51.80 ($OCH_3$), 45.57 (3 × $CH_3$, $NMe_3$), 38.83, 38.82, 38.72 (3 × O=C–*C*, 3 × Piv), 27.18, 27.11, 26.88 (3 × $3CH_3$, 3 × Piv); FABMS: Calcd for $C_{39}H_{62}N_4O_{17}$ $[M+NMe_3]^+$: *m/z* 949.5. Found: *m/z* 949.8.

### 3.19. Methyl 2-*O*-sulfo-α-L-idopyranosyluronic acid-(1→4)-2-deoxy-2-sulfamido-α-D-glucopyranoside trisodium salt (1)

**3.19.1. Saponification.** The *O*-sulfated product **30** (120 mg, 135 μmol) was dissolved in THF (16 mL) and cooled to −5 °C. $H_2O_2$ (6 mL, 30%) and aq LiOH (2.8 mL, 0.7 M) were added dropwise. The reaction mixture was stirred for 1 h at −5 °C, then 2 h at 0 °C. After 20 h stirring at room temperature, MeOH (13 mL), then aq NaOH (3.6 mL, 4 N), were added dropwise at 0 °C, and the solution was left stirring for 1 h at 0 °C. After that time, the stirring was prolonged overnight at room temperature. After neutralization with 6 N HCl and extraction with $CH_2Cl_2$, the organic layer was washed with acidified aq $Na_2S_2O_3$ (pH 3.5), the water phase was combined and neutralized and then concentrated. The white solid was extracted with 1:1 $CH_2Cl_2$–MeOH, then the solvent phase was concentrated, and the residue was purified on Sephadex LH-20 using 1:1 MeOH–$H_2O$ as eluant.

**3.19.2. Hydrogenolysis.** A solution of the compound obtained above in 1:1 *t*-BuOH–$H_2O$ (3 mL) was treated with $H_2$ (50 bar) in the presence of Pd/C catalyst (120 mg, 10%) overnight. The mixture was filtered and concentrated. The residue was purified on Sephadex LH-20 using 1:1 MeOH–$H_2O$ as eluant to give product (15.0 mg).

**3.19.3. N-Sulfation.** The product obtained above (15 mg, 30 μmol) was dissolved in $H_2O$ (6 mL). $SO_3{\cdot}$pyridine (23 mg, 145 μmol) was added in five portions at $t = 0$; 0.5; 1.0; 1.5; 2.0; and 2.5 h, the pH being maintained at pH 9.8 by addition of 4 N aq NaOH. After 3 h, the reaction mixture was concentrated and layered onto a Bio-Gel P-2 column and eluted with $H_2O$ and product **1** 14.0 mg (78%) was

obtained; $^1$H NMR (500 MHz, $D_2O$): $\delta$ 5.12 (d, 1H, $J_{1',2'}$ 2.3 Hz, H-1′), 5.02 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.75 (d, 1H, $J_{5',4'}$ 2.5 Hz, H-5′), 4.23 (br t, 1H, H-2′), 4.02 (br t, 1H, H-3′), 3.97 (d, 1H, $J_{4',5'}$ 3.9 Hz, H-4′), 3.89 (br t, 1H, H-6a), 3.84 (br t, 1H, $J_{6b,5}$ 2.2 Hz, H-6b), 3.76 (m, 1H, H-5), 3.71 (br t, 1H, $J_{4,3}$ 8.6 Hz, H-4), 3.66 (br t, 1H, $J_{3,2}$ 10.1 Hz, H-3), 3.41 (s, 3H, $OCH_3$), 3.26 (br t, 1H, H-2); MALDI-TOFMS: Calcd for $C_{13}H_{20}NO_{17}Na_3S_2$ $[M+NH_4]^-$: *m/z* 613.02. Found: *m/z* 613.33.

### 3.20. Methyl (methyl 2-*O*-acetyl-3,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (31)

Glucosamine precursor **6** (145 mg, 0.41 mmol) and L-iduronic acid derivative **17** (348 mg, 1.5 equiv) were coevaporated with toluene and then dissolved in anhyd $CH_2Cl_2$ (15 mL), and freshly activated powdered molecular sieves (240 mg, 4 Å) were added. The mixture was stirred for half an hour at −20 °C, then TESOTf (46.7 μL, 0.5 equiv) was added dropwise, then stirred at −10 to −15 °C. After 3 h *N*,*N*-DIPEA (72 μL, 1.0 equiv) was added, the mixture was filtered through a pad of Celite, and the solvent was removed under reduced pressure. Purification on a MPLC column eluting with 5:1 toluene–EtOAc afforded the disaccharide **31** (199 mg, 64%) as a colorless syrup: $[\alpha]_D$ +11.5 (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.36 (m, 5H, Ph), 5.37 (d, 1H, $J_{1',2'}$ 4.6 Hz, H-1′), 5.18 (br t, 1H, H-3′), 5.08 (dd, 1H, $J_{4',5'}$ 4.6 Hz, $J_{4',3'}$ 5.9 Hz, H-4′), 4.86 (m, 3H, CHaPh, H-2′, CHbPh), 4.80 (d, 1H, $J_{5',4'}$ 4.6 Hz, H-5′), 4.77 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.50 (dd, 1H, $J_{6a,5}$ 2.0 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.18 (dd, 1H, $J_{6a,6b}$ 3.9 Hz, $J_{6b,6a}$ 12.3 Hz, H-6b), 3.89 (m, 2H, H-3, H-4), 3.79 (dd, 1H, H-5), 3.44 (s, 3H, $OCH_3$, ester), 3.44 (m, 1H, H-2), 3.42 (s, 3H, $OCH_3$), 2.12 (s, 3H, $CH_3$, Ac), 2.05 (s, 3H, $CH_3$, Ac), 1.21 (s, 9H, $3CH_3$, Piv), 1.14 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (100.55 MHz, $CDCl_3$): $\delta$ 176.56, 176.25 ($2\times$C=O, $2\times$Piv), 170.30 (C=O, ester), 169.24, 168.21 (C=O, Ac), 137.50, 128.23, 127.50, 127.39 (Ph), 98.36 (C-1), 97.62 (C-1′), 77.92, 76.60 (C-4, C-3), 74.20 (*C*$H_2$Ph), 68.84 (C-2′), 68.78 (C-5), 68.67, 67.72, 67.50 (C-5′, C-3′, C-4′), 63.16, 61.71 (C-2, C-6), 55.44 ($OCH_3$, ester), 51.99 ($OCH_3$), 38.73 (overlap, $2\times$O=C–*C*, $2\times$Piv), 26.98, 26.90 ($2\times3CH_3$, $2\times$Piv), 20.83, 20.70 ($2\times CH_3$, $2\times$Ac); FABMS: Calcd for $C_{35}H_{49}N_3O_{15}$ $[M-H]^+$: *m/z* 750.3. Found: *m/z* 750.4.

### 3.21. Methyl (methyl 3,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (32)

Disaccharide **31** (87 mg, 0.12 mmol) was dissolved in anhyd MeOH (30 mL), and AcCl (2.3 mL) was added at 0 °C. The reaction mixture was gradually warmed up to room temperature and let stand overnight. The solution was diluted with $CH_2Cl_2$ (450 mL), washed with satd aq $NaHCO_3$ (35 mL), and then washed with $H_2O$ ($3\times35$ mL). The organic phase was dried over anhydrous $Na_2SO_4$. Then the solvent was removed under reduced pressure. Purification on a MPLC column eluting with 3:1 toluene–EtOAc afforded the deacetylated disaccharide **32** (63 mg, 81%): $[\alpha]_D$ +3.4 (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.35 (m, 5H, Ph), 5.47 (d, 1H, $J_{1',2'}$ 5.5 Hz, H-1′), 5.20 (br t, 1H, H-3′), 5.14 (br t, 1H, 4′-H), 4.92 (d, 1H, $J_{a,b}$ 10.8 Hz, CHaPh), 4.79 (m, 2H, CHbPh, H-1), 4.76 (d, 1H, $J_{5',4'}$ 5.3 Hz, H-5′), 3.98 (br t, 1H, $J_{4,5}$ 9.3 Hz, 4-H), 3.85 (m, 3H, H-6a, H-3, H-6b), 3.69 (br d, 1H, H-5), 3.59 (br t, 1H, H-2′), 3.43 (m, 7H, $2OCH_3$, H-2), 1.22 (s, 9H, $3CH_3$, Piv), 1.14 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (50.32 MHz, $CDCl_3$): $\delta$ 177.95, 176.56 ($2\times$C=O, $2\times$Piv), 168.50 (C=O, ester), 137.78, 128.23, 127.46 (Ph), 101.00 (C-1′), 98.53, 78.62, 77.00 (C-1, C-3, C-4, overlap), 74.55 (*C*$H_2$Ph), 71.18 (C-2′), 71.12 (C-5), 70.58, 69.72, 68.12 (C-3′, C-5′, C-4′), 63.51, 61.12 (C-2, C-6), 55.35 ($OCH_3$, ester), 52.09 ($OCH_3$), 38.90, 38.78 ($2\times$O=C–*C*, $2\times$Piv), 27.11, 26.99 ($2\times3CH_3$, $2\times$Piv); FABMS: Calcd for $C_{31}H_{45}N_3O_{13}$ $[M+Na]^+$: *m/z* 690.29. Found: *m/z* 690.23.

### 3.22. Methyl (methyl 2-*O*-trimethylaminesulfonato-3,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-trimethylaminesulfonato-α-D-glucopyranoside (33)

A solution of the deacylated disaccharide **32** (60 mg, 90 μmol) and $SO_3{\cdot}NMe_3$ complex (250 mg, 20.0 equiv) in dry DMF (8.7 mL) was stirred overnight at 60 °C. After the reaction was finished, the solution was concentrated to ~2 mL. Chromatography on Sephadex LH-20 using MeOH as eluant gave product **33** (70 mg, 83%): $[\alpha]_D$ −0.03 (*c* 0.5, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.83 (br s, $2\times$NH, $2\times HN^+Me_3$), 7.23 (m, 5H, Ph), 5.35 (s, 1H, H-1′), 5.20 (s, 1H, H-3′), 4.93 (s, 1H, H-4′), 4.90 (s, 1H, H-5′), 4.89 (d, 1H, $J_{a,b}$ 11.5 Hz, CHaPh), 4.79 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.76 (d, 1H, $J_{b,a}$ 11.5 Hz, CHbPh), 4.34 (s, 1H, H-2′), 4.23 (s, 2H, H-6a, H-6b), 4.05 (br t, 1H, H-4), 3.82 (br t, 1H, H-3), 3.73 (br d, 1H, H-5), 3.46 (dd, 1H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 10.1 Hz, H-2), 3.38 (s, 3H, $OCH_3$), 3.16 (s, $OCH_3$, ester), 2.96 (s, 18H, $6CH_3$, $2\times NMe_3$), 1.25 (s, 9H, $3CH_3$, Piv), 1.07 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (50.32 MHz, $CDCl_3$): $\delta$ 176.60, 176.11 ($2\times$C=O, $2\times$Piv), 168.40 (C=O, ester), 137.52, 128.16, 127.22, 126.43, 126.33 (Ph), 98.31 (C-1), 97.85 (C-1′), 77.88, 73.58 (C-3, C-4), 73.02 (*C*$H_2$Ph), 69.52 (C-5), 69.38, 66.66 (C-2′, C-4′), 66.21 (overlap, C-3′, C-5′), 65.25, 63.25 (C-6, C-2), 55.44 ($OCH_3$, ester), 51.68 ($OCH_3$), 45.79 (overlap, $6CH_3$, $2\times NMe_3$), 38.76, 38.61 ($2\times$O=C–*C*, $2\times$Piv), 27.18, 26.88 ($2\times3CH_3$, $2\times$Piv); FABMS: Calcd for

$C_{37}H_{63}N_5O_{19}S_2$ $[M+NMe_3+H]^+$: *m/z* 1005.4. Found: *m/z* 1005.5.

### 3.23. Methyl (2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranoside tetrasodium salt (2)

**3.23.1. Saponification.** The *O*-sulfated product **33** (57 mg, 60 μmol) was dissolved in THF (2.7 mL) and cooled to −5 °C. $H_2O_2$ (1.3 mL, 30%) and aq LiOH (0.59 mL, 0.7 M) were added dropwise. The reaction mixture was stirred for 1 h at −5 °C, then for 2 h at 0 °C. After 20 h stirring at room temperature, MeOH (2.36 mL) then aq NaOH (0.65 mL, 4 N) were added dropwise at 0 °C, and the solution was left stirring for 1 h at 0 °C. After that time, the stirring was prolonged overnight at room temperature. After neutralization with 1 N HCl, the water phase was concentrated in vacuo under 30 °C.

**3.23.2. Hydrogenolysis.** A solution of the residue obtained above in 3:2 *t*-BuOH–$H_2O$ (5 mL) was treated with $H_2$ (50 bar) in the presence of Pd/C catalyst (80 mg, 10%) for overnight. The mixture was filtered and concentrated. The residue was purified on Sephadex LH-20 using 1:1 MeOH–$H_2O$ as eluant to give product (20 mg).

**3.23.3. N-Sulfation.** The product obtained above (20 mg, 33.6 μmol) was dissolved in water (5 mL). $SO_3$·pyridine (24 mg, 151 μmol) was added in five portions at $t$ = 0; 0.5; 1.0; 1.5; 2.0; and 2.5 h, the pH being maintained at pH 9.8 by addition of 4 N aq NaOH. After 3 h, the reaction mixture was neutralized with 1 N HCl and concentrated. The residue was layered onto a Bio-Gel P-2 column eluted with $H_2O$, and product **2** was obtained (22 mg, 94%): $^1$H NMR (400 MHz, $D_2O$): δ 5.16 (br s, 1H, H-1′), 5.04 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.74 (d, 1H, $J_{5',4'}$ 2.7 Hz, H-5′), 4.34 (br t, 2H, H-6a, H-6b), 4.26 (br t, 1H, H-2′), 4.05 (br t, 1H, H-3′), 4.00 (m, 2H, H-4′, H-5), 3.75 (br t, 1H, H-4), 3.69 (br t, 1H, H-3), 3.44 (s, 3H, $OCH_3$), 3.30 (dd, 1H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 9.9 Hz, H-2); MALDI-TOFMS: Calcd for $C_{13}H_{19}NNa_4O_{20}S_3$ $[M+K]^-$: *m/z* 735.8891. Found: *m/z* 735.0661.

### 3.24. Methyl (methyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (34)

Glucosamine **6** (121 mg, 0.34 mmol) and L-iduronic acid derivative **23** (290 mg, 1.5 equiv) were coevaporated with toluene and then dissolved in anhyd $CH_2Cl_2$ (13 mL), and freshly activated powdered molecular sieves (200 mg, 4 Å) were added. The mixture was stirred for 0.5 h at −20 °C, then TESOTf (39 μL, 0.5 equiv) was added dropwise and stirred at −15 to −10 °C for 3 h. The reaction was stopped by adding *N*,*N*-DIPEA (60 μL, 1.0 equiv). The mixture was filtered through a pad of Celite, and the solvent was removed under reduced pressure. Purification on a MPLC column eluting with 5:2 toluene–EtOAc afforded the disaccharide **34** 195 mg (75%) as a colorless syrup: $[\alpha]_D$ +8.2 (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): δ 7.34 (m, 5H, Ph), 5.35 (d, 1H, $J_{1',2'}$ 4.7 Hz, H-1′), 5.19 (br t, 1H, H-3′), 5.06 (br t, 1H, H-4′), 4.86 (m, 3H, CHaPh, H-2′, CHbPh), 4.76 (br d, 2H, H-5′, H-1), 4.48 (br d, 1H, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.18 (dd, 1H, $J_{6b,5}$ 3.8 Hz, $J_{6b,6a}$ 12.4 Hz, H-6b), 3.91 (br t, 1H, $J_{4,3}$ 9.3 Hz, H-4), 3.87 (br t, 1H, $J_{3,4}$ 9.3 Hz, H-3), 3.78 (br t, 1H, H-5), 3.51 (s, 3H, $OCH_3$, ester), 3.41 (m, 4H, $J_{2,1}$ 3.7 Hz, H-2, $OCH_3$), 2.68 (m, 2H, $CH_2$, Lev), 2.50 (m, 2H, $CH_2$, Lev), 2.16 (s, 3H, $CH_3$, Lev), 2.11 (s, 3H, $CH_3$, Ac), 2.08 (s, 3H, $CH_3$, Ac), 1.21 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (50.32 MHz, $CDCl_3$): δ 205.68 (C=O, Lev), 176.57 (C=O, Piv), 171.19 (C=O, Lev), 170.48 (C=O, ester), 169.42, 168.30 (2 × C=O, 2 × Ac), 137.64, 128.34, 127.62, 127.53 (Ph), 98.43 (C-1), 97.75 (C-1′), 78.05, 76.63 (C-3, C-4), 74.53 ($CH_2$Ph), 68.95 (C-2′), 68.85 (C-5), 68.67, 68.18, 67.69 (C-5′, C-4′, C-3′), 63.31, 61.73 (C-2, C-6), 55.46 ($OCH_3$), 52.23 ($OCH_3$, ester), 38.74 (O=C–*C*, Piv), 37.38 ($CH_2$, Lev), 29.71 ($CH_3$, Lev), 27.53 ($CH_2$, Lev), 26.92 ($3CH_3$, Piv), 20.79, 20.67 (2 × $CH_3$, 2 × Ac); HRMS: Calcd for $C_{35}H_{47}N_3O_{16}$ $[M+K]^+$: 804.2592. Found: 804.2689.

### 3.25. Methyl (methyl 2-*O*-acetyl-3-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (35)

A solution of $N_2H_4$·HOAc (230 mg, 2.5 mmol) was added to a mixture of disaccharide **34** (190 mg, 0.25 mmol) in 2:1 EtOH–toluene (48 mL), and the resulting reaction mixture was stirred at room temperature for 1 h. The solvents were then evaporated in vacuo, and the residue was diluted with $CH_2Cl_2$. The mixture was then filtered, and the solvent was removed under reduced pressure. Purification on a MPLC column eluting with 2:1 hexanes–EtOAc afforded compound **35** (113 mg, 68%) as a colorless oil: $[\alpha]_D$ +38.6 (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): δ 7.35 (m, 5H, Ph), 5.12 (d, 1H, $J_{1',2'}$ 3.3 Hz, H-1′), 4.99 (br t, 1H, H-3′), 4.86 (d, 1H, $J_{a,b}$ 11.4 Hz, CHaPh), 4.84 (d, 1H, $J_{b,a}$ 11.4 Hz, CHbPh), 4.83 (br t, 1H, H-2′), 4.79 (d,1H, $J_{1,2}$ 3.7 Hz, H-1), 4.74 (d, 1H, $J_{5',4'}$ 3.1 Hz, H-5′), 4.48 (dd, 1H, $J_{6a,5}$ 1.9 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.20 (dd, 1H, $J_{6b,5}$ 3.9 Hz, $J_{6b,6a}$ 12.5 Hz, H-6b), 3.92 (m, 1H, H-4′), 3.87 (br t, 1H, H-4), 3.86 (br t, 1H, H-3), 3.80 (m, 1H, H-5), 3.49 (s, 3H, $OCH_3$, ester), 3.44 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 9.7 Hz, H-2), 3.43 (s, 3H, $OCH_3$), 2.89 (d, 1H, $J_{OH\text{-}4',H\text{-}4'}$ 11.0 Hz, OH-4′), 2.10 (s, 6H, $2CH_3$, 2 × Ac), 1.25 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (50.32 MHz, $CDCl_3$): δ 177.40 (C=O, Piv), 170.33 (C=O, ester), 168.86 (overlap, 2 × C=O,

2 × Ac), 137.72, 128.25, 127.46, 127.05 (Ph), 98.49 (C-1), 98.07 (C-1′), 77.98, 76.79 (C-3, C-4), 73.93 ($CH_2$Ph), 70.04, 69.94 (C-3′, C-5′), 68.88 (C-5), 68.34, 68.00 (C-2′, C-4′), 63.40, 61.87 (C-2, C-6), 55.55 ($OCH_3$), 52.28 ($OCH_3$, ester), 38.89 (O=C–*C*, Piv), 27.06 ($3CH_3$, Piv), 20.90, 20.82 (2 × $CH_3$, 2 × Ac); FABMS: Calcd for $C_{30}H_{41}N_3O_{14}$ $[M+Na]^+$: *m/z* 690.2. Found: *m/z* 690.2.

### 3.26. Methyl (6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (36)

Glucosamine derivative **27** (153 mg, 0.27 mmol) and disaccharide **35** (120 mg, 0.18 mmol) were coevaporated with toluene and then dissolved in anhyd $CH_2Cl_2$ (11 mL), and freshly activated powdered molecular sieves (150 mg, 3 Å) were added. The mixture was stirred for 0.5 h at −20 °C, then TESOTf (20.4 μL, 0.5 equiv) was added dropwise. The reaction was stirred for 1 h and then stopped by adding *N*,*N*-DIPEA (30.7 μL, 1.0 equiv). The mixture was filtered through a pad of Celite, and the solvent was removed under reduced pressure. Purification on a MPLC column eluting with 7:1:1 toluene–EtOAc–$CH_2Cl_2$ afforded the trisaccharide **36** (80 mg, 40%) as a colorless syrup: $[\alpha]_D$ +47.2 (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.32 (m, 10H, 2 × Ph), 5.32 (m, 2H, H-1$^I$, H-3$^I$), 5.17 (br t, 1H, H-4$^{G'}$), 5.08 (d, 1H, $J_{1G',2G'}$ 3.7 Hz, H-1$^{G'}$), 4.89 (d, 1H, $J_{aG,bG}$ 11.3 Hz, CHaPh$^G$), 4.83 (d, 1H, $J_{bG,aG}$ 11.3 Hz, CHbPh$^G$), 4.82 (br t, 1H, H-2$^I$), 4.79 (d, 1H, H-1$^G$), 4.72 (d, 1H, $J_{aG',bG'}$ 10.4 Hz, CHaPh$^{G'}$), 4.70 (br t, 1H, H-5$^I$), 4.60 (d, 1H, $J_{bG',aG'}$ 10.4 Hz, CHbPh$^{G'}$), 4.50 (dd, 1H, $J_{6aG,5G}$ 1.7 Hz, $J_{6aG,6bG}$ 12.5 Hz, H-6a$^G$), 4.22 (dd, 1H, $J_{6bG,5G}$ 4.0 Hz, $J_{6bG,6aG}$ 12.5 Hz, H-6b$^G$), 4.09 (dd, 1H, $J_{6aG',5G'}$ 4.2 Hz, $J_{6bG',6aG'}$ 12.6 Hz, H-6a$^{G'}$), 4.04 (m, 2H, $J_{6bG',6aG'}$ 12.6 Hz, H-6b$^{G'}$, H-4$^I$), 3.84 (m, 5H, H-5$^{G'}$, H-3$^{G'}$, H-3$^G$, H-4$^G$, H-5$^G$), 3.51 (s, 3H, $OCH_3$, ester), 3.43 (m, 5H, $J_{2G',1G'}$ 3.5 Hz, H-2$^{G'}$, H-2$^G$, $OCH_3$), 2.12 (s, 3H, $CH_3$, Ac), 2.11 (s, 3H, $CH_3$, Ac), 2.05 (s, 3H, $CH_3$, Ac), 1.25 (s, 9H, $3CH_3$, Piv), 1.17 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (50.32 MHz, $CDCl_3$): $\delta$ 176.48 (overlap, 2 × C=O, 2 × Piv), 170.47, 170.37, 169.53 (3 × C=O, 3 × Ac), 168.54 (C=O, ester), 137.88, 137.02, 128.36, 128.24, 127.84, 127.48, 127.41, 127.09 (2 × Ph), 98.49 (C-1$^G$), 98.04 (overlap, C-1$^I$, C-1$^{G'}$), 78.08, 77.31 (C-3$^G$, C-4$^G$), 77.20 (C-3$^{G'}$), 74.39 ($C$H$_2$Ph$^G$), 73.95 ($C$H$_2$Ph$^{G'}$), 72.60, 69.68 (C-4$^I$, C-5$^I$), 69.14 (C-4$^{G'}$, C-5$^{G'}$), 68.89 (C-2$^I$, C-5$^G$), 67.88 (C-3$^I$), 63.31 (C-2$^G$), 62.52 (C-2$^{G'}$), 61.93 (C-6$^G$), 61.48 (C-6$^{G'}$), 55.53 ($OCH_3$), 52.12 ($OCH_3$, ester), 38.95, 38.89 (2 × O=C–*C*, 2 × Piv), 27.12 (overlap, $6CH_3$, 2 × Piv), 20.91, 20.77, 20.69 (3 × $CH_3$, 3 × Ac); FABMS: Calcd for $C_{50}H_{66}N_6O_{20}$ $[M+Na]^+$: *m/z* 1093.4. Found: *m/z* 1093.3.

### 3.27. Methyl (2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 3-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (37)

Trisaccharide **36** (80 mg, 75.7 μmol) was dissolved in anhyd MeOH (19.6 mL), and AcCl (1.51 mL) was added at 0 °C. The reaction mixture was gradually allowed to warm to room temperature and left overnight. The reaction was followed by TLC in 1:1 hexanes–EtOAc until it was completed. The solution was diluted with cold $CH_2Cl_2$ (420 mL), washed with satd aq $NaHCO_3$ (39 mL), and then washed with $H_2O$ (3 × 39 mL). The organic phase was dried over anhydrous $Na_2SO_4$. The solvent was then removed under reduced pressure. Purification on a MPLC eluting with 5:4:1 hexanes–EtOAc–$CH_2Cl_2$ afforded the deacetylated trisaccharide **37** 60 mg (84%) as a colorless oil: $[\alpha]_D$ +34.9 (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.33 (m, 10H, 2 × Ph), 5.31 (br s, 1H, H-1$^I$), 5.18 (d, 1H, $J_{1G',2G'}$ 3.7 Hz, H-1$^{G'}$), 5.16 (m, 1H, H-3$^I$), 4.98 (br t, 1H, H-4$^{G'}$), 4.84 (m, 4H, CHaPh$^G$, CHbPh$^G$, H-5$^I$, H-1$^G$), 4.75 (d, 1H, $J_{aG',bG'}$ 10.8 Hz, CHaPh$^{G'}$), 4.68 (d, 1H, $J_{bG',aG'}$ 10.8 Hz, CHbPh$^{G'}$), 4.09 (br s, 1H, H-4$^I$), 4.01 (br t, 1H, $J_{4G,5G}$ 9.5 Hz, H-4$^G$), 3.87 (m, 3H, H-3$^G$, H-3$^{G'}$, H-6a$^{G'}$), 3.81 (br d, 1H, H-6b$^{G'}$), 3.68 (d, 1H, $J_{5G,4G}$ 9.5 Hz, H-5$^G$), 3.63 (dd, 1H, $J_{2G',1G'}$ 3.7 Hz, H-2$^{G'}$), 3.61 (br d, 1H, H-2$^I$), 3.57 (d, 1H, $J_{6aG,6bG}$ 10.8 Hz, H-6a$^G$), 3.46 (m, 2H, H-2$^G$, H-5$^{G'}$), 3.43 (m, 4H, $OCH_3$, H-6b$^G$), 3.36 (s, 3H, $OCH_3$, ester), 1.28 (s, 9H, $3CH_3$, Piv), 1.17 (s, 9H, $3CH_3$, Piv); $^{13}$C NMR (50.32 MHz, $CDCl_3$): $\delta$ 178.37, 177.19 (2 × C=O, 2 × Piv), 169.06 (C=O, ester), 137.96, 137.13, 128.44, 128.31, 127.93, 127.47, 127.39, 126.75 (2 × Ph), 101.49 (C-1$^I$), 98.67 (C-1$^G$), 95.98 (C-1$^{G'}$), 78.16, 78.09 (C-3$^G$, C-3$^{G'}$), 75.94 (C-4$^G$), 75.41, 73.28 ($C$H$_2$Ph$^G$, $C$H$_2$Ph$^{G'}$), 71.64 (C-4$^I$), 71.28 (C-5$^G$), 71.11 (C-5$^{G'}$), 70.25 (C-4$^{G'}$), 68.24, 67.24 (C-5$^I$, C-2$^I$, C-3$^I$), 63.42 (C-2$^{G'}$), 63.32 (C-2$^G$), 61.16 (C-6$^{G'}$), 60.61 (C-6$^G$), 55.44 ($OCH_3$, ester), 52.08 ($OCH_3$), 38.95, 38.88 (2 × O=C–*C*, 2 × Piv), 27.10, 27.08 (2 × $3CH_3$, 2 × Piv); FABMS: Calcd for $C_{44}H_{60}N_6O_{17}$ $[M+Na]^+$: *m/z* 967.4. Found: *m/z* 967.3. Anal. Calcd for $C_{44}H_{60}N_6O_{17}$: C, 55.92; H, 6.40; N, 8.89. Found: C, 56.08; H, 6.22; N, 8.44.

### 3.28. Methyl (2-azido-3-*O*-benzyl-2-deoxy-6-*O*-trimethylaminesulfonato-α-D-glucopyranosyl)-(1→4)-(methyl 3-*O*-pivaloyl-2-*O*-trimethylaminesulfonato-α-L-idopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-trimethylaminesulfonato-α-D-glucopyranose (38)

A solution of the deacetylated trisaccharide **37** (27 mg, 30 μmol) and $SO_3$·$NMe_3$ complex (119 mg, 30.0 equiv) in dry DMF (4 mL) was stirred overnight at 60 °C. After the reaction was finished, the solution was concentrated to ~2 mL. Chromatography on Sephadex

LH-20 using MeOH as eluant gave product **38** (34 mg, 86%): $[\alpha]_D$ +36.4 (*c* 1.0, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 8.83 (br s, 2 × NH, 2 × $HN^+Me_3$), 7.39–7.23 (m, 10H, 2 × Ph), 5.58 (br t, 1H, $J_{3I,4I}$ 4.5 Hz, H-$3^I$), 5.43 (d, 1H, $J_{1I,2I}$ 2.5 Hz, H-$1^I$), 5.18 (d, 1H, $J_{1G',2G'}$ 3.3 Hz, H-$1^{G'}$), 5.05 (br t, 1H, H-$4^{G'}$), 4.91 (m, 1H, CHaPh$^G$, H-$5^I$), 4.82 (m, CHbPh$^G$, H-$1^G$), 4.79 (d, 1H, $J_{aG',bG'}$ 11.1 Hz, CHbPh$^{G'}$), 4.64 (d, 1H, $J_{bG',aG'}$ 11.1 Hz, CHbPh$^{G'}$), 4.42 (m, 2H, H-6a$^G$, H-$2^I$), 4.29 (d, 1H, $J_{6bG,6aG}$ 10.9 Hz, H-6b$^G$), 4.10 (br t, 1H, H-$4^I$), 4.02 (m, 3H, H-$3^G$, H-6a$^{G'}$, H-6b$^{G'}$), 3.91 (m, 4H, H-$4^G$, H-$5^G$, H-$5^{G'}$, H-$3^{G'}$), 3.55 (dd, 1H, $J_{2G',1G'}$ 3.5 Hz, $J_{2G',3G'}$ 10.3 Hz, H-$2^{G'}$), 3.49 (s, 3H, $OCH_3$), 3.47 (dd, 1H, $J_{2G,1G}$ 3.5 Hz, $J_{2G,3G}$ 10.0 Hz, H-$2^G$), 3.44 (s, 3H, $OCH_3$, ester), 2.93 (s, 27H, $9CH_3$, 3 × $NMe_3$), 1.30 (s, 9H, $3CH_3$, Piv), 1.18 (s, 9H, $3CH_3$, Piv); $^{13}C$ NMR (50.32 MHz, $CDCl_3$): $\delta$ 178.38, 178.21 (2 × C=O, 2 × Piv), 170.37 (C=O, ester), 139.71, 139.31, 129.43, 129.32, 128.74, 128.67, 128.51, 128.18 (2 × Ph), 100.08 (C-$1^G$), 99.90 (C-$1^{G'}$), 98.09 (C-$1^I$), 79.04 (C-$3^{G'}$, C-$3^G$), 75.81 (C-$4^G$), 75.36 ($CH_2Ph^G$), 74.01 (C-$2^I$), 73.65 ($CH_2Ph^{G'}$), 73.33 (C-$4^I$), 71.24 (C-$4^{G'}$), 70.96 (C-$5^G$, C-$5^{G'}$), 70.08, 68.45 (C-$5^I$, C-$3^I$), 67.28 (C-$6^{G'}$, C-$6^G$), 64.44 (C-$2^{G'}$), 63.93 (C-$2^G$), 55.92 ($OCH_3$, ester), 53.13 ($OCH_3$), 50.42, 50.00, 49.58, 49.15, 48.73, 48.30, 47.88, 45.91 (3 × $NMe_3$), 40.13, 40.00 (2 × O=C–*C*, 2 × Piv), 27.85, 27.74 (2 × $3CH_3$, 2 × Piv).

### 3.29. Methyl (2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranoside hexasodium salt (3)

**3.29.1. Saponification.** The *O*-sulfated product **38** (75 mg, 55 μmol) was dissolved in THF (3.0 mL) and cooled to −5 °C. $H_2O_2$ (1.43 mL, 30%) and aq LiOH (0.66 mL, 0.7 M) were added dropwise. The reaction mixture was stirred for 1 h at −5 °C, then 2 h at 0 °C. After 20 h stirring at room temperature, MeOH (2.65 mL), then aq NaOH (0.77 mL, 4 N) were added dropwise at 0 °C, and the solution was left stirring for 1 h at 0 °C. After that time, the stirring was prolonged overnight at room temperature. After neutralization with 1 N HCl, the water phase was concentrated in vacuo under 30 °C.

**3.29.2. Hydrogenolysis.** A solution of the above residue in 1:1 *t*-BuOH–$H_2O$ (5 mL) was treated with $H_2$ (50 bar) in the presence of Pd/C catalyst (80 mg, 10%) overnight. The mixture was filtered and concentrated. The residue was purified on Sephadex LH-20 using 1:1 MeOH–$H_2O$. The $^1H$ NMR spectrum showed that the reaction was not complete, and another overnight hydrogenation under the same conditions was carried out. The mixture was filtered and concentrated. The residue was purified on Sephadex LH-20 again using 1:1 MeOH–$H_2O$ to give product (22 mg).

**3.29.3. N-Sulfation.** The product obtained above (22 mg, 25.6 μmol) was dissolved in $H_2O$ (4 mL). $SO_3$·pyridine (36 mg, 226 μmol) was added in five portions at $t$ = 0; 0.5; 1.0; 1.5; 2.0; and 2.5 h, the pH being maintained at pH 9.5 by addition of 1 N aq NaOH. After 3 h, the reaction mixture was neutralized with 1 N HCl and concentrated to ~1 mL. Chromatography on a Sephadex G-10 column using 1:9 EtOH–$H_2O$ as eluant afforded product **3** (22 mg, 81%); $^1H$ NMR (400 MHz, $D_2O$): $\delta$ 5.46 (d, 1H, $J_{1G',2G'}$ 3.7 Hz, H-$1^{G'}$), 5.27 (s, 1H, H-$1^I$), 5.03 (d, 1H, $J_{1G,2G}$ 3.5 Hz, H-$1^G$), 4.90 (d, 1H, $J_{5I,4I}$ 1.8 Hz, H-$5^I$), 4.38 (m, 2H, H-$3^I$, H-6a$^{G'}$), 4.35 (br s, 2H, H-$2^I$, H-6b$^{G'}$), 4.31 (dd, 1H, $J_{6aG,5G}$ 4.9 Hz, $J_{6aG,6bG}$ 11.5 Hz, H-6a$^G$), 4.23 (m, 2H, H-6b$^G$, H-$4^I$), 4.07 (m, 1H, H-$5^G$), 3.99 (m, 2H, H-$5^{G'}$, H-$3^G$), 3.90 (br t, 1H, $J_{3G',4G'}$ 9.8 Hz, H-$3^{G'}$), 3.81 (br t, 1H, $J_{4G,3G}$ 9.4 Hz, H-$4^G$), 3.59 (br t, 1H, $J_{4G',3G'}$ 9.8 Hz, H-$4^{G'}$), 3.48 (s, 3H, $OCH_3$), 3.42 (dd, 1H, $J_{2G,3G}$ 1.2 Hz, $J_{2G,1G}$ 3.5 Hz, H-$2^G$), 3.40 (dd, 1H, $J_{2G',3G'}$ 1.1 Hz, $J_{2G',1G'}$ 3.7 Hz, H-$2^{G'}$); $^{13}C$ NMR (50.32 MHz, $D_2O$–acetone): $\delta$ 175.84 (C=O), 99.55 (C-$1^I$), 96.55 (C-$1^G$), 91.91 (C-$1^{G'}$), 77.35 (C-$4^G$), 73.63 (C-$2^I$), 71.08 (C-$4^I$, C-$3^G$), 69.90 (C-$3^{G'}$), 69.90 (C-$5^G$), 69.61, 69.40 (C-$5^{G'}$, C-$4^{G'}$), 68.01 (C-$5^I$), 66.81 (C-$6^{G'}$), 65.19 (C-$6^G$), 63.55 (C-$3^I$), 55.97 ($OCH_3$), 54.78 (C-$2^{G'}$, C-$2^G$); MALDI-TOFMS Calcd for $C_{19}H_{28}N_2O_{30}Na_6S_5$ $[M-2\times SO_3Na]^-$: *m/z* 855.98. Found: *m/z* 855.91.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