The activation of fibroblast growth factors by heparin: Synthesis and structural study of rationally modified heparin-like oligosaccharides

Rafael Ojeda, Jesús Angulo, Pedro M. Nieto, and Manuel Martín-Lomas

Abstract: Heparin-like hexasaccharide **3** and octasaccharide **4** have been synthesized using a convergent block strategy and their solution conformations have been determined by NMR spectroscopy. Both oligosaccharides contain the basic structural motif of the regular region of heparin but have been constructed as to display negatively charged sulfate groups only on one side of their solution helical structures. This charge distribution along the saccharide chain has been designed to get insight into the proposed mechanism for fibroblast growth factors (FGFs) activation that involves heparin-induced FGF dimerization.

Key words: heparin oligosaccharides, synthesis design, conformational analysis, FGF activation.

Résumé : On a réalisé la synthèse d'un hexasaccharide (**3**) et d'un octasaccharide (**4**) apparenté à l'héparine en faisant appel à une stratégie de synthèse convergente par blocs et on a déterminé leurs conformations en solution à l'aide de la spectroscopie RMN. Les deux oligosaccharides comportent le motif structurel de base de la région régulière de l'héparine, mais leurs structures ont été organisées d'une façon telle qu'elles ne comportent des groupes sulfates chargés négativement que sur un seul côté de leurs structures hélicoïdales en solution. Cette distribution des charges ainsi que la chaîne du saccharide ont été choisies afin d'avoir un aperçu du mécanisme proposé pour l'activation des facteurs de croissance du fibroblaste (FCF) qui implique une dimérisation des FCF induite par l'héparine.

Mots clés : oligosaccharides de l'héparine, modèle de synthèse, analyse conformationnelle, activation des FCF.

[Traduit par la Rédaction]

Introduction

The fibroblast growth factors (FGFs) constitute a family of signaling polypeptides that intervene in a variety of biological processes by stimulating key cellular functions after binding to specific receptor tyrosine kinases at the cell surface (FGFRs) (1). The biological activity of FGFs is tightly regulated by heparin or heparan sulfate glycosaminoglycans (GAGs) (2, 3). Binding of these GAGs to FGFs protects FGFs from degradation (4), however, the precise structural requirements for the GAG chains to regulate the biological activity of the FGFs are not yet well understood at the molecular level. A number of crystal structures of FGF complexes with heparin fragments (5–7), and of FGF-2 (basic FGF) and FGF-1 (acidic FGF) bound to variants of FGFR (8–11) have been determined and several hypotheses on the structure–activity relationship of these complexes have been expressed (12). It is widely accepted that heparin and (or) heparan sulfate would bind to FGFs to cause FGF oligomerization and that these oligomers would interact with FGFRs in the presence of divalent cations giving rise to FGFR dimerization, thus inducing the cellular response (13). According to sedimentation equilibrium experiments, the formation of FGF-2 dimers in the presence of heparin having a *trans* (with two FGF molecules on each side of the polysaccharide chain) or a *cis* (with two FGF molecules on the same side of the polysaccharide chain) configuration has been proposed (14). The existence of both types of dimeric structures has been confirmed by NMR spectroscopy and light scattering (15). The *trans* dimer has also been observed for the FGF-1-heparin complex using X-ray crystallography (7).Whether this type of mechanism, which was originally proposed for FGF-2 (14), would be generally applicable to all the members of the FGF family is still a matter of discussion as the biologically active configuration of the heparin-FGF and the heparin-FGF–FGFR complexes are still open questions (16, 17). The establishment of the molecular basis of the entire biological process and the understanding of the

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Dedicated to the memory of Ray Lemieux whose outstanding vision of chemistry and strong personality deeply influenced the early days of the Carbohydrate Group in Seville. We consider ourselves privileged to have been connected to him during the last years of his fruitful life.

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different functions of the FGF family at the molecular level remain, therefore, a difficult problem of chemical and biomedical interest.

One of the difficulties of these investigations resides in the inherent heterogeneity of the GAGs. Heparin and heparan sulfate are primarily constituted by disaccharide repeating units of L-iduronic acid and D-glucosamine linked by $\alpha 1 \rightarrow 4$ glycosidic linkages and typically containing sulfate groups at position 2 of the L-iduronic acid units and positions 2 and 6 of the D-glucosamine units. But these polysaccharides show significant heterogeneity in terms of sulfation pattern, size, and even carbohydrate sequence (18–20). For this reason it is highly desirable to develop effective syntheses of these heparin-like oligosaccharide chains with defined size, sequence, and charge distribution to be used in the interaction studies. In this context, we recently reported a synthetic strategy the effectiveness of which was illustrated by the preparation of hexasaccharide **1** and octasaccharide **2** (21). It has to be mentioned that these oligosaccharides contain L-iduronic acid residues at the reducing end, a sequence which is not available either by enzymatic or chemical degradation of heparin, and that oligosaccharides with the alternative sequence (22–24) and di- and tetrasaccharide sequences containing the above structural motif (25) have previously been synthesized. The overall structures of **1** and **2** in solution (21) resulted in stable right hand helixes with four residues per turn as those reported for natural heparin fragments (26–30). Therefore, **1** and **2** were used as chemically well-defined structural models of naturally occurring heparin-like oligosaccharides to investigate the activation of FGF-1 (21). Both compounds **1** and **2** induced the mitogenic activity of FGF-1 but, as may be expected (31–35), the activating effect of octasaccharide **2** reached a maximum at approximately the same concentration than natural low molecular weight heparin while considerably higher concentrations of **1** were needed to reach an equivalent maximal activation (21). Most interestingly, sedimentation equilibrium analysis results showed that the profile of FGF-1 solutions in the presence of activating concentrations of **2** corresponded to monomeric species thus suggesting that heparin-induced dimerization of FGF-1 in the culture medium surrounding the cell may not be an absolute requirement for FGF-1-induced mitogenesis signaling (21).

The above result prompted us to apply our synthetic strategy to the preparation of hexasaccharide **3** and octasaccharide **4** and to investigate their behavior in FGF-1 activation. We reasoned that, assuming that **3** and **4** would present a solution conformation similar to that shown by **1** and **2**, these structures (**3** and **4**) would display negatively charged sulfate groups only on one side of their right-handed helical structures (Fig. 1). Therefore, if the interaction with the polypeptide is primarily electrostatic in nature, the ability of **3** and **4** to form *trans* dimers with FGF-1would be greatly decreased and, because of their relatively reduced size, both of them would hardly be involved in the formation of *cis* dimers.

In this paper, we report the synthesis of **3** and **4** and present data that indicate that, as expected, their overall solution

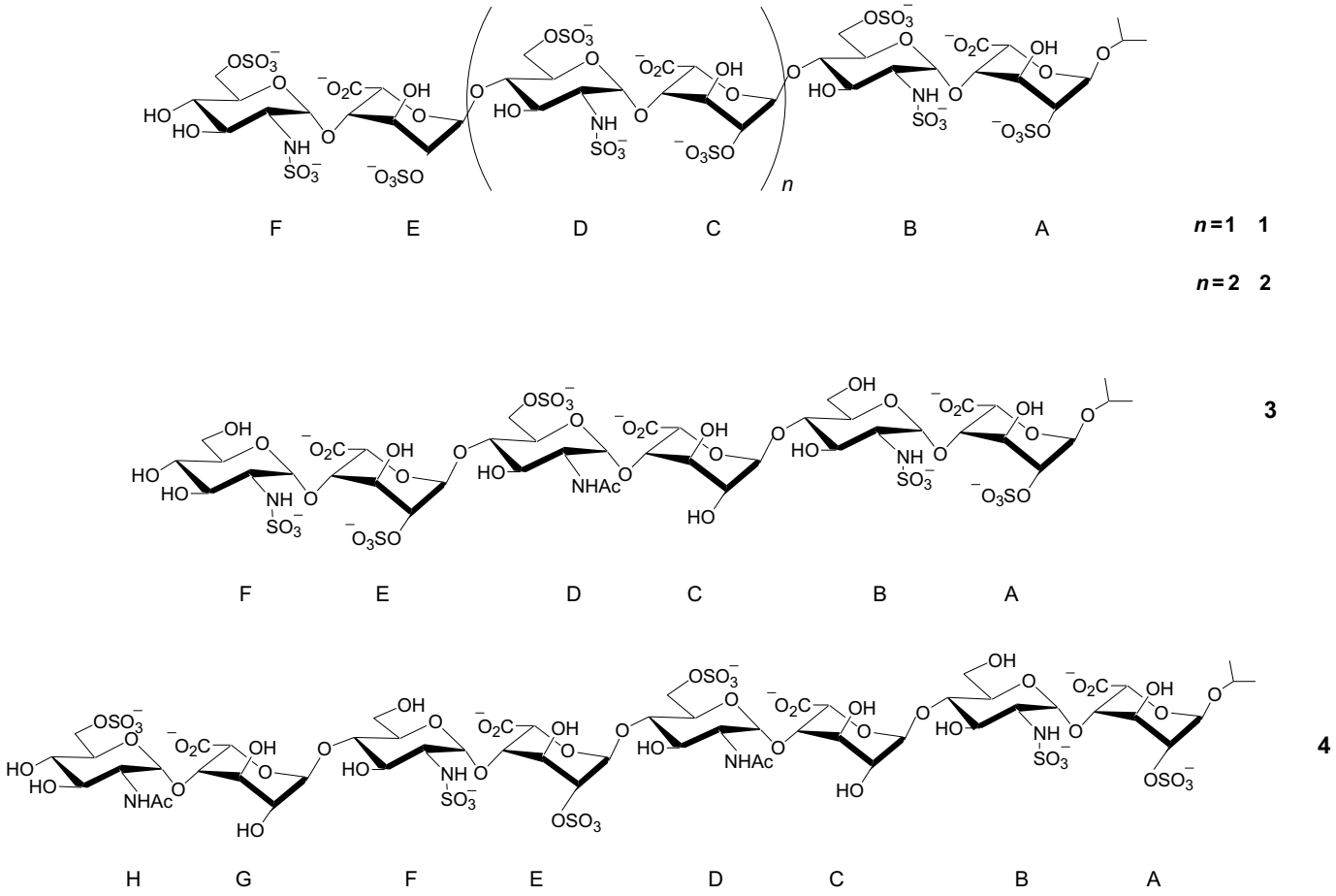
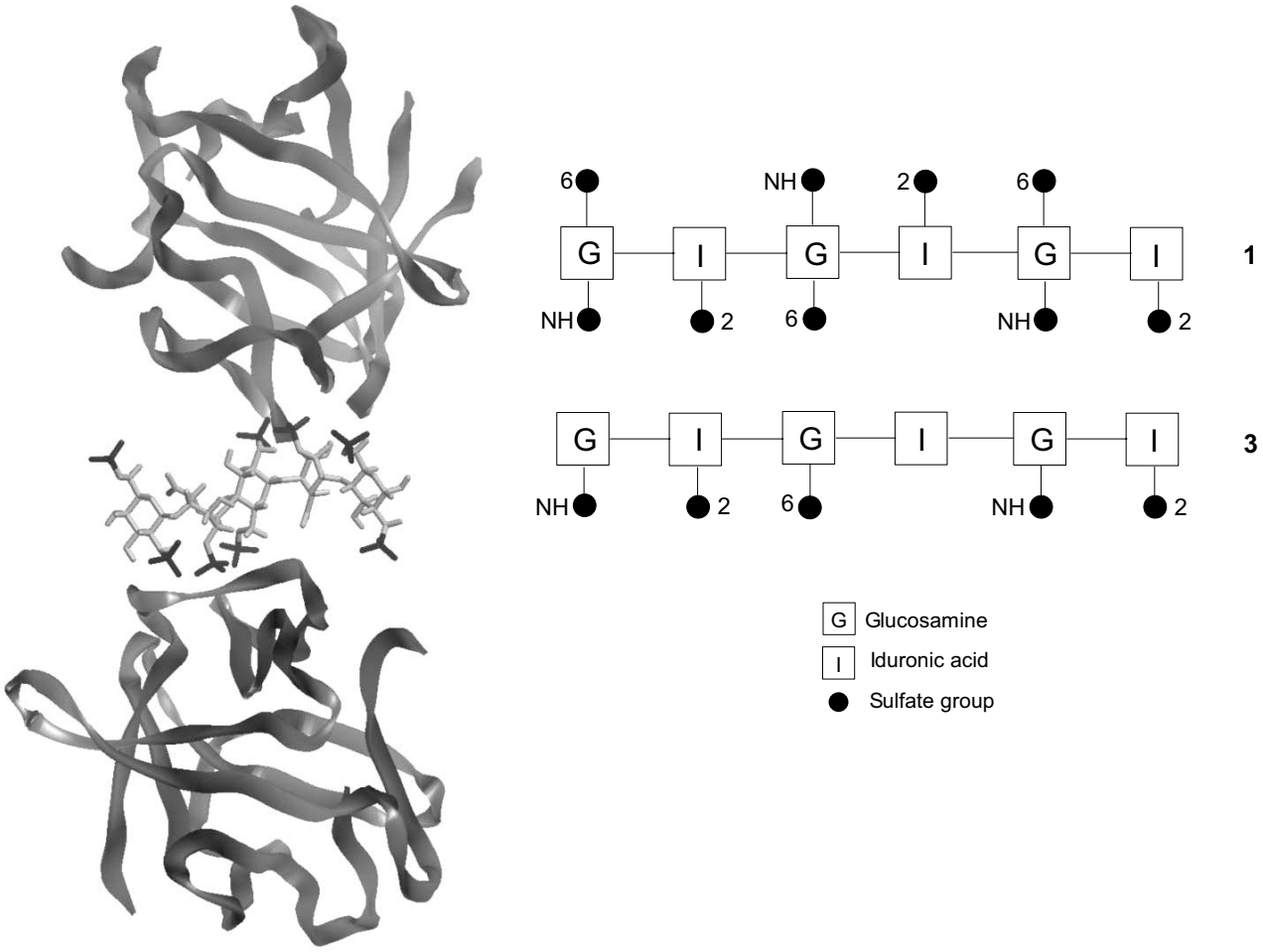


Fig. 1. Crystallographic structure of an FGF-1 *cis*-dimer (protein data bank accession code 1axm) (7) and schematic representation of hexasaccharides **1** and **3**.



structures are right-handed helixes with four residues per turn. These compounds showed an unexpected biological behavior that will be discussed in detail elsewhere.

Results and discussion

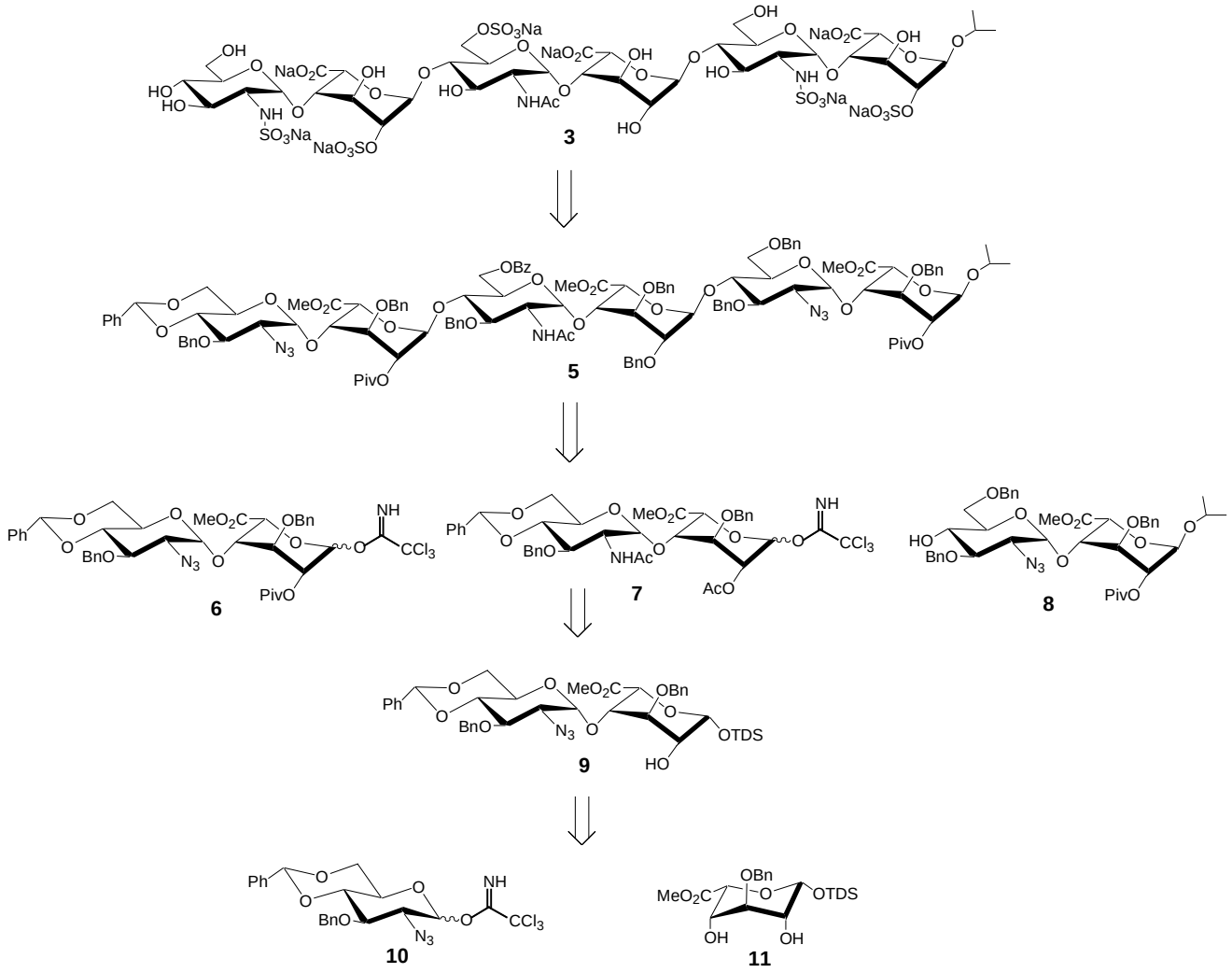
Synthesis

We have reported the synthesis of hexasaccharide **1** and octasaccharide **2** using a convergent $n + 2$ block approach from key disaccharide structures, that may operate as glycosyl donors or as glycosyl acceptors, having protective group patterns that permit formation of the required stereochemistry of the glycosidic linkages and to locate the sulfate groups at the desired positions at a later stage (21). This approach has now proven to be of wide applicability allowing the synthesis of oligosaccharide sequences with different size and sulfation patterns as the target hexasaccharides **3** and **4**. As indicated in Scheme 1 the synthesis of **3** was envisaged from the fully protected hexasaccharide **5** that could be prepared from disaccharide building blocks **6**, **7**, and **8**. These building blocks were obtained from a common disaccharide (**9**) that is readily available from the 2-azido-2-deoxy-D-glucopyranosyl trichloroacetimidate **10** and the iduronic acid glycosyl acceptor **11** (21).

Scheme 2 summarizes the synthesis of the disaccharide building blocks. The nonreducing-end building block **6** was prepared from **9** as already reported (21). This disaccharide (**6**) also provided the reducing-end building block **8** after glycosylation with isopropanol to give **12** (21) followed by regioselective reductive opening of the benzylidene acetal (36). The alternative building block **14** was also prepared from **12** that was transformed into diol **13** (21) and regioselectively acetylated (4% of **13** was recovered). The inner region building block **7** was also prepared from **9** that was transformed into **17** either by selective reduction of the azido group with thioacetic acid (37) to give **15** followed by conventional acetylation or by using the reverse sequence **9** $\rightarrow$ **16** $\rightarrow$ **17**. This latter route was more convenient as acetylation of **15** took place sluggishly most likely as a consequence of hydrogen bonding between the hydroxyl and the acetamido groups.

Glycosylation of low-reactive acceptor **8** with donor **7** afforded tetrasaccharide **19** in 50% yield and unreacted acceptor (46%) (Scheme 3). Compound **19** was deacetylated to give **20** which was benzylated under neutral conditions (38) to yield **21**. Hydrolysis of the benzylidene acetal (39) gave diol **22** that was regioselectively benzoylated (40) to afford tetrasaccharide acceptor **23**.

Scheme 1.

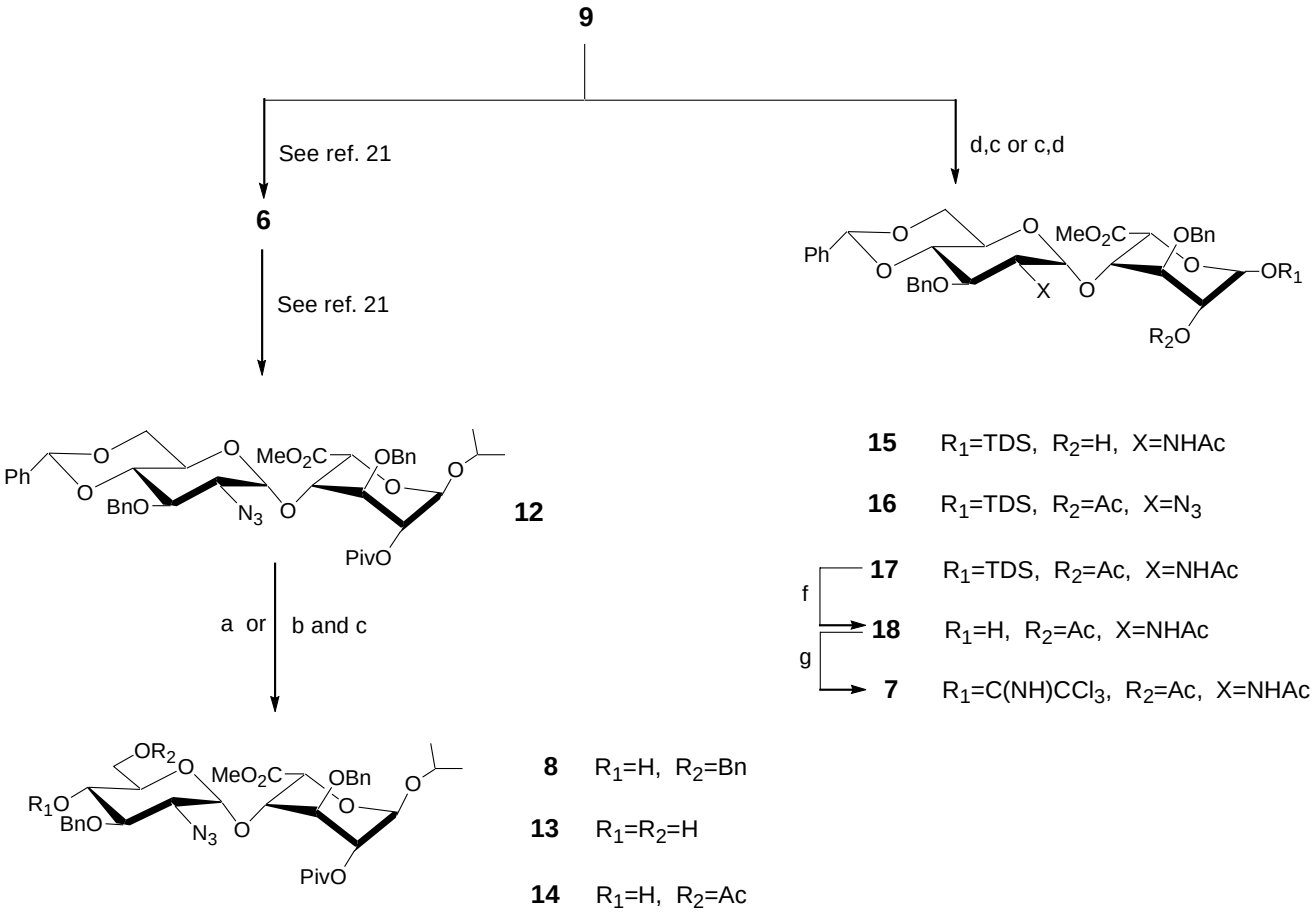


In spite of the moderate yield of the glycosylation reaction, this synthetic route to **23** was more convenient than that using acceptor building block **14** since the deactivating effect of the acetyl group in **14** further complicated the coupling with **7** to give **24** with formation of orthoester and considerable elimination (25% of 2-acetoxyglycal). Compound **24** could be conventionally deacetylated to give **25** that was benzylated to yield **21** (52%).

The coupling of **23** with donor **6** afforded hexasaccharide **5** in 65% yield. The methoxycarbonyl and acyl groups in **5** were removed by treatment with lithium hydroperoxide and then hydroalcoholic potasium hydroxide (41, 42) and the resulting partially protected hexasaccharide **26** was sulfonated and isolated as the sodium salt **27**. Hydrogenolytic cleavage of the benzylidene acetal and benzyl groups and simultaneous reduction of the azido groups in **27** followed by selective *N*-sulfonation yielded compound **3** that was purified using a protocol partially based on the reported purification of synthetic oligosaccharides of the irregular region of heparin (42).

The elongation of the oligosaccharide chain to obtain an octasaccharide (**4**) was performed by adding a new disaccharide building block (**34**) to the nonreducing end of the previously constructed hexasaccharide structure **5**. This building block (**34**) was prepared as indicated in Scheme 4 from the iduronic acid derivative **11** (21, 43) and trichloroacetimidate **29** that was obtained from **28** (21, 44). Coupling of **29** with **11** gave disaccharide **30** that was transformed into trichloroacetimidate **34** following the sequence **30** → **31** → **32** → **33** → **34** as indicated in Scheme 4. Building block acceptor **35** was prepared by regioselective reductive opening of the benzylidene acetal group in **5** (Scheme 5). As expected (21), the glycosylation reaction between **34** with **35** took place in only moderate yield (40%). However, no attempt was made to optimize this reaction since a reasonable amount of octasaccharide **36** was isolated that allowed the subsequent transformations leading to sufficient **4** for structural and biological investigation. The relevance of **4** for these further studies, which was not yet known at this stage, would dictate whether working out some changes in this synthetic strategy may be worthwhile in the near future. The introduction of a needed permanent protecting group at position 2 of the G unit was satisfactorily carried out by selective deacylation using catalytic sodium methoxide in methanol to obtain **37** (80%) followed by benzylation under neutral conditions (38) to afford **38**. The carboxymethyl and *O*-acyl groups were then removed by treatment with lithium hydroperoxide then alcoholic potasium

Scheme 2. Reagents and conditions: (*a*) $NaBH_3CN$, THF, HCl–Et_2O, 92%; (*b*) EtSH, PTSA, CH_2Cl_2, 90%; (*c*) Ac_2O, Py, CH_2Cl_2, –10°C, 85%; (*d*) AcSH, 73–77%; (*e*) Ac_2O, Py, 93–77%; (*f*) $(HF)_n$·Py, THF, –15°C, 78%; (*g*) K_2CO_3, Cl_3CCN, CH_2Cl_2, 94%.



hydroxide to give **39** that was *O*-sulfonated to yield **40**. Hydrogenolytic cleavage of the benzyl groups with concomitant reduction of the azido groups of **40** followed by *N*-sulfonation gave finally octasaccharide **4** that was purified as **3**.

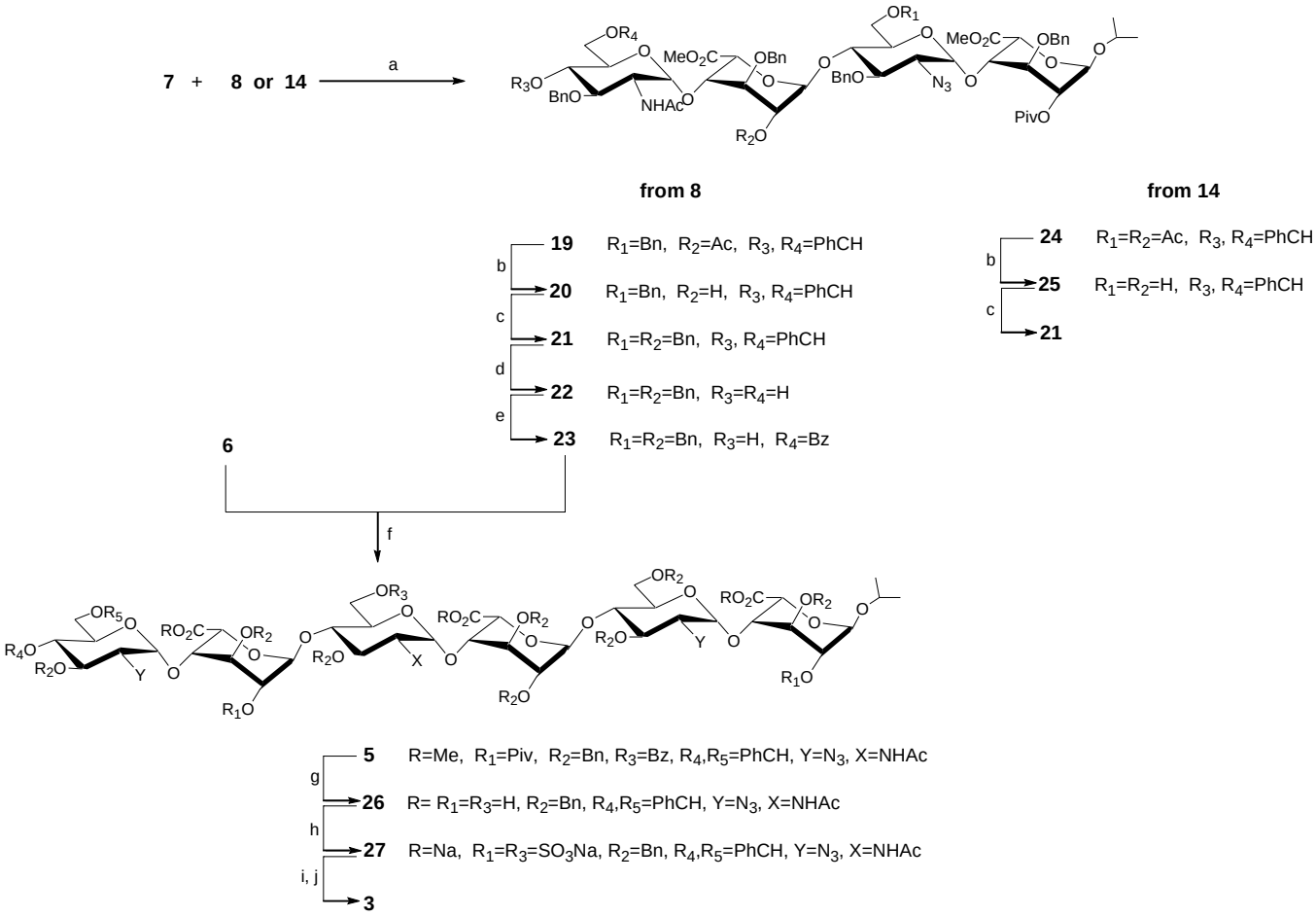
Structural study

The ^1H and ^{13}C NMR spectra of **3** and **4** were fully assigned using conventional 1D and 2D spectroscopy (Table 1). These assignments were carried out by identification of the spin systems of the residues and further connection based on inter-residue NOE. The substitution pattern in **3** and **4** led to a better signal dispersion than that observed in the spectra of hexasaccharide **1** and octasaccharide **2.** The chemical shift values were in agreement with those expected according to reported data for chemicaly modified heparin (45, 46). Three bond coupling constants were obtained from 2D DQF-COSY(47). As expected, the D-glucosaminyl residues showed a single $^4C_1$ conformation and the ω rotamer of the 6-*O*-sulfated units appeared as in **1** (21) as the *gg* conformer. The observed values for the coupling constants of the iduronate residues could be accounted for by the existence of a conformational equilibrium between $^1C_4$ and ^2S_o forms as reported for heparin (Table 2), also confirmed by the H2-H5 NOE (30). Therefore, the lack of the 2-*O*-sulfate group in the C and G units and the further modifications in the neighboring glucosamine residues, absence of 6-*O*-sulfate group in the B and F units and of *N*-sulfate group in the D and H units, do not seem to qualitatively modify the conformational behavior of these iduronate rings with respect to heparin. This result is also in agreement with previous findings that similar structural changes in natural heparin fragments do not influence the inner iduronate ring conformational equilibrium (48).

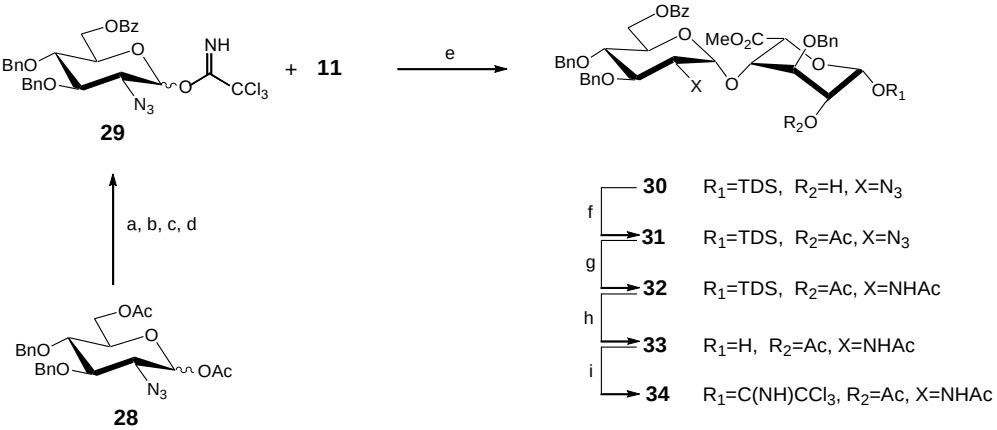
With regard to the conformation around the glycosidic linkages, the NOEs pattern observed for hexasaccharide **3** was compared to that previously obtained for the parent hexasaccharide **1** (see supplementary data).[2] There were no indications of conformational changes as no major differences were observed and only small intensity variations, which could be attributed to differences in correlation times, were detected. The possibility that the changes in charge distribution in **3** with respect to **1** may partially drive the glycosidic angles towards an *anti*-ψ arrangement could, therefore, be discarded. Furthermore, the exclusive NOEs that should be expected for such *anti*-ψ situations (H1′-H5 for the IdoA–GlcN linkages and H5′-H3 for the GlcN–IdoA ones) could not be observed in NOESY experiments with hexasaccharide **3**.

[2] Supplementary material may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada (http://www.nrc.ca/cisti/irm/unpub_e.shtml for information on ordering electronically).

Scheme 3. Reagents and conditions: (*a*) TMSOTf, CH_2Cl_2, 50%; (*b*) MeONa, MeOH, 96%; (*c*) Ag_2O, BnBr, DMF, 88%; (*d*) EtSH, PTSA, CH_2Cl_2, 83%; (*e*) BzCN, Et_3N, CH_3CN, –40°C, 88%; (*f*) TMSOTf, CH_2Cl_2, 65%; (*g*) (*i*) H_2O_2, LiOH aq; (*ii*) KOH aq, MeOH, 98%; (*h*) SO_3·Py, Py, 72%; (*i*) Pd/C, H_2, MeOH–H_2O (9:1); (*j*) SO_3·Py, H_2O, pH = 9.5, 75% from **27**.



Scheme 4. Reagents and conditions: (*a*) KOH, MeOH; (*b*) BzCl, Py, 96% from **28**; (*c*) $BnNH_2$, Et_2O, 96%; (*d*) DBU, Cl_3CCN, CH_2Cl_2, 87%; (*e*) TMSOTf, CH_2Cl_2, 0°C, 88%; (*f*) Ac_2O, Py, 43% from **29**; (*g*) AcSH, 68%; (*h*) $(FN)_n$·Py, THF, –15°C, 81%; (*i*) K_2CO_3, Cl_3CCN, CH_2Cl_2, 71%.



The above qualitative NMR study indicated that the synthetic compounds **3** and **4** present the main structural features of natural heparin oligosaccharides (46). The structural definition of **3** was further improved with a semiquantitative analysis of the NOE data. Heparin fragments larger than five monosaccharide units usually show an anisotropic hydrodynamic behavior with a shape close to a prolate ellipsoid. This feature can be determined through the analysis of σ^{NOE} and (or) the apparent correlation times for the fixed glucosamine interprotonic vectors H1-H2 and H2-H4. As the H1-H2 vector is almost parallel and the H2-H4 almost perpendicular to the main axis of inertia and both distances are

Scheme 5. Reagents and conditions: (*a*) $NaBH_3CN$, THF, HCl–Et_2O, 66%; (*b*) TMSOTf, CH_2Cl_2, 40%; (*c*) MeONa, MeOH–CH_2Cl_2, 80%; (*d*) Ag_2O, BnBr, DMF, 72%; (*e*) (*i*) H_2O_2, LiOH aq; (*ii*) KOH aq, MeOH, 94%; (*h*) SO_3·Py, Py, 64%; (*i*) Pd/C, H_2, MeOH–H_2O (9:1); (*j*) SO_3·Py, H_2O, pH = 9.5, 66% from **40**.

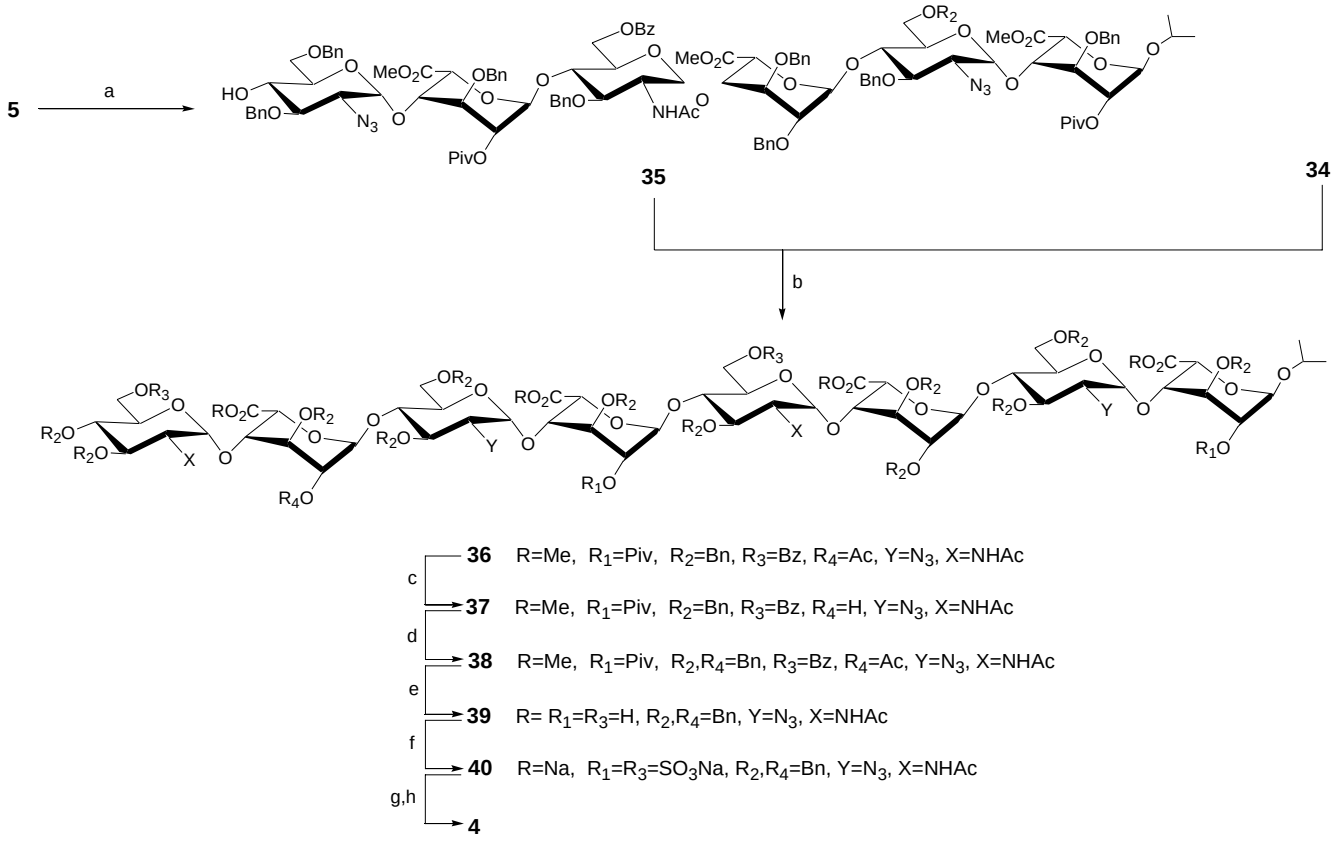


Table 1. Proton and carbon chemical shifts for hexasaccharide **3**.

	A	B	C	D	E	F
H-1	5.22	5.32	4.89	5.15	5.18	5.38
H-2	4.16	3.23	3.66	3.95	4.31	3.18
H-3	4.19	3.64	3.85	3.74	4.19	3.61
H-4	4.00	3.67	4.05	3.76	4.07	3.42
H-5	4.50	3.83	4.74	3.99	4.77	3.80
H-6/H-6′		3.84/3.78		4.37/4.23		3.85/3.76
C-1	99.8	99.7	104.2	97.1	101.9	99.4
C-2	78.6	60.8	72.5	74.1	78.5	60.7
C-3	71.0	72.1	72.2	72.4	71.7	73.8
C-4	78.6	79.7	77.5	78.4	78.4	72.6
C-5	70.8	73.6	72.6	72.0	71.9	74.4
C-6		62.5		69.0		63.0

nearly equal, the observed variation of σ^{NOE} can be attributed to a difference in parallel and perpendicular correlation times. The σ^{NOE} values for **3** were calculated from the NOE growing rate, assuming the isolated spin pairs approximation, by integration of the NOE cross-peaks at several mixing times (200–600 ms) (49). The apparent correlation times for glucosamine vectors H1-H2 and H2-H4, calculated from the σ^{NOE} ratio at 400 and 500 MHz provided a clear indication of this anisotropic tumbling (Table 3) (50). This behavior, which also was previously observed for **1** (21), prevented the accurate calculation of distances from NOE data and a complex full-motion model, which accounts both for internal motions and anisotropy, has to be considered. However, because of the above-mentioned parallel and perpendicular orientation of the H1-H2 and H2-H4 vectors with respect to the main axis of inertia, the known glucosamine H1-H2 and H2-H4 distances can be used as extreme reference values for the estimation of distances from interglycosidic NOEs. The upper and the lower distance limits may be given by considering the parallel and perpendicular correlation times derived from H1-H2 and H2-H4 σ^{NOE}, respectively. The distances thus obtained were in agreement with those expected for a *syn*-Φ disposition of the glycosidic linkages (Table 4) as that reported for heparin and related oligosaccharides.

Table 2. $^3J_{HH}$ observed for iduronate residues of **3**.

	IdoA (**A**)	IdoA (**C**)	IdoA (**E**)	$^1C_4$ (teor.)	^2S_O (teor.)	$^4C_1$ (teor.)
$^3J_{1,2}$	3.0	3.5	3.0	2.9	5.6	8.0
$^3J_{2,3}$	4.9	6.6	5.8	4.0	10.1	10.1
$^3J_{3,4}$	5.4	4.5	4.0	4.0	6.0	9.8
$^3J_{4,5}$	2.9	3.3	2.8	0.8	4.8	3.9

Table 3. σ^{NOE} (500) and σ^{NOE} (400) (s^{-1}) and apparent correlation times (ns) for internal glucosamine NOEs of **3**.

		GlcN (**B**)	GlcN (**D**)	GlcN (**F**)
H1-H2	σ^{NOE} (500)	0.16	0.20	0.13
	σ^{NOE} (400)	0.14	0.19	0.12
	τ_c	1.04	1.37	1.10
H2-H4	σ^{NOE} (500)	0.05	0.09	0.02
	σ^{NOE} (400)	0.03	0.08	0.01
	τ_c	0.60	1.22	0.55

The small differences observed for the H1′-H3:H1′-H4 ratio of the C–D linkage in comparison with those of the A–B and E–F linkages can be attributed to changes of the local flexibility as a consequence of the different substitution pattern.

Finally, a model of hexasaccharide **3** was constructed on the basis of a previous model constructed for **1**. A minimization including TIP3P water and sodium counterions was performed. The system was preequilibrated by several cycles of molecular dynamics with frozen solute coordinates. After minimization of the overall system, the relaxed structure showed the typical helical symmetry of heparin with the five sulfate groups located on one face. The backbone of this structure can be superimposed on the backbone of structure **1** (Fig. 2) This model is in agreement with the key interglycosidic distances calculated from NOE data (Table 4).

Although more detailed structural studies are presently underway, the results presented here clearly indicate that the key three-dimensional elements present in the structure of heparin, helical symmetry and iduronate ring conformational equilibrium, are adequatelly modeled by compounds **3** and **4**.

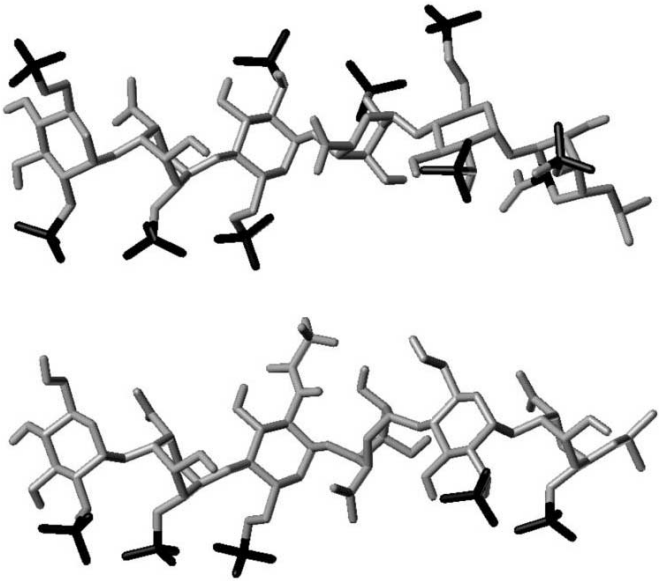
The induction of the mitogenic activity of FGF-1 by these synthetic compounds (**3** and **4**) in comparison with that promoted by compounds **1** and **2** is now under investigation. Preliminary results indicate that hexasaccharide **3** shows the maximum activating effect. On the other hand, sedimentation equilibrium analysis also shows, in this case, that the profile of FGF-1 solutions in the presence of activating concentrations of **3** and **4** correspond to monomeric species. These results and further studies on the structure–reactivity relationship will be reported in due time.

Experimental

General methods

The ^{1}H NMR spectra were measured at 500 MHz (Bruker DRX-500) with tetramethylsilane or the residual signal of the solvent as the internal standard. The ^{13}C NMR spectra were recorded at 125 MHz. Optical rotations were measured at room temperature in a 1 dm cell on a PerkinElmer 341 polarimeter. Elemental analyses were carried out in a Leco CHNS 932 analyzer. Permeation gel chromatography was performed using Sephadex LH-20 and G-25 (Pharmacia). Ionic exchange chromatography was carried out using Dowex 50WX4 resin (Fluka). Thin-layer chromatography was performed on precoated plates of silica gel (60-F254, E. Merck, Darmstadt) and visualized with H_2SO_4–EtOH (1:9, v/v) or anisaldehyde followed by heating. For column chromatography, silica gel 60 (0.2–0.5 mm, 0.2–0.063 mm, or 0.04–0.015 mm, E. Merck, Darmstadt) and distilled solvents were used. All solvents and reagents were purified and dried according to standard procedures.

Fig. 2. Relaxed structures of hexasaccharides **1** and **3**.



NMR measurements

1D and 2D experiments were recorded in D_2O at 298 K on Bruker DRX-500 and DRX-400 instruments using acetone as the external reference in a 6 mM sample. pH* was adjusted to 7.0. DQF-COSY (51), TOCSY (52), and HMQC (53) experiments were recorded using standard *z*-pulsed field gradient enhanced or selected pulse sequences when possible. Phase-sensitive experiments were performed in all cases using the TPPI (time proportional phase increment) method (54). Data were transformed into phase-sensitive mode after weighting with shifted- square sine-bells functions.

NOESY (55) experiments were carried out at 500 and 400 MHz with mixing times of 250, 300, 325, 350, 400, 500, 550, and 600 ms. The volumes of the cross and diagonal peaks were integrated using standard Bruker software. The normalized cross-peak's volume variation as a function of the mixing time indicated good linearity of the NOE build up curves for the range of mixing times considered. Cross-relaxation rates (σ) were calculated from these

Table 4. Experimental and theoretical interglycosidic distances of hexasaccharide **3**.

Residue	Distance	Minimun	Maximum	Average	Model distance
A	H2—H5	3.26	3.81	3.54	3.96
B	H1—H4(A)	2.22	2.6	2.41	2.39
	H1—H3(A)	2.42	2.83	2.62	2.33
C	H2—H5	2.62	2.97	2.79	3.78
	H1—H4(B)	2.26	2.64	2.45	2.35
	H1—H6(B)	2.42	2.83	2.62	2.31
	H1—H6′(B)	2.22	2.6	2.41	3.90
D	H1—H4(C)	2.37	2.69	2.53	2.57
	H1—H3(C)	2.1	2.38	2.24	2.22
E	H2—H5	2.72	3.08	2.90	4.02
	H1—H4(D)	2.29	2.61	2.45	2.45
	H1—H6(D)	2.16	2.45	2.3	2.42
	H1—H6′(D)	2.85	3.24	3.04	3.84
F	H1—H4(E)	1.82	2.44	2.13	2.51
	H1—H3(E)	1.92	2.57	2.24	2.21

curves assuming the isolated spin pairs approximation at short mixing times by extrapolation to zero time. The apparent correlation times were calculated using the spectral density function of an isotropic rigid molecule, from the ratio σ (500)/σ (400) (56).

Molecular modeling

Molecular modeling was performed using AMBER force field (57) (parameter set "parm91") as integrated in the AMBER 5.0 program (58), modified for carbohydrate molecules by the GLYCAM_93 parameter set (59). Specific parameters for sulfates and sulfamate groups were also included, as described by Huige and Altona (60). The initial structure of the hexasaccharide **3** was built using the ϕ/ψ values found for a minimized structure of the regular heparin-like synthetic hexasaccharide **1** (21). Minimization was carried out using periodic boundary conditions with TIP3P water molecules. Box dimensions were 53 × 50 × 50 Å, large enough to allow for the faces of the box to extend 12 Å beyond the sugar in each direction, reducing in this way the possibility of border effects that could take place if a reorientation of the solute happened during the calculation. It involved the introduction of 3933 water molecules and eight sodium counterions. To enable the cations to solvate appropriately, they were initially located randomly far enough from the solute. The initial water and counterions configuration was subjected to 500 cycles of energy minimization, with the conformation of the sugar frozen. Following this step, a 50 ps volume constant MD simulation was performed, in which only the water molecules were allowed to move. After this preequilibration of the solvent, the next step consisted of 500 cycles of energy minimization of the water molecules, keeping constant the Cartesian coordinates of the solute and counterions. To reach the equilibrium value for the density of the system, the water molecules were subjected to 50 ps pressure constant MD simulation with both the solute and the counterions frozen. Finally, the solvent equilibration procedure involved 500 cycles of steepest descent energy minimization of the water molecules. At this point, the energetic minimum of **3** was obtained using 1500 cycles of conjugated gradient minimization for the whole system.

Methyl (isopropyl 4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (13)

To a solution of **12** (800 mg, 1.02 mmol) in dry CH_2Cl_2 (9 mL), EtSH (374 μL, 5.06 mmol), and catalytic PTSA were added. After stirring for 3 h under an argon atmosphere, the reaction was neutralized with saturated $NaHCO_3$ solution, diluted with CH_2Cl_2 (200 mL), and washed with H_2O (200 mL). The organic layer was dried ($MgSO_4$) and concentrated to dryness. The mixture was purified by flash chromatography (hexane–EtOAc, 1:1) to yield **13** (639 mg, 90%). TLC: 0.14 (hexane–EtOAc, 2:1). $[\alpha]_D^{23}$ –6.6° (*c* 0.6, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.24 (m, 10H, *2 Ph*), 5.18 (d, 1H, *H-1*, $J_{1,2}$ = 4.5 Hz), 5.05 (d, 1H, *H-1′*, $J_{1,2}$ = 3.6 Hz), 4.93 (m, 1H, *H-2*), 4.89 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 11.2 Hz), 4.79–4.69 (m, 4H, *3 CH*$_2$Ph, *H-5*), 4.15 (m, 1H, *H-4*), 3.97–3.91 (m, 2H, *H-3*, *CH*$(CH_3)_2$), 3.80–3.55 (m, 5 H, *H-3′*, *H-4′*, *H-5′*, *H-6′a*, *H-6′b*), 3.75 (s, 3H, COO*CH*$_3$), 3.17 (t, 1H, *H-2′*, $J_{2,1} \approx J_{2,3}$ = 10.7 Hz), 1.22 (s, 9H, C(*CH*$_3$)$_3$), 1.19–1.15 (2d, 6H, CH(*CH*$_3$)$_2$, *J* = 6.2 Hz).

Methyl (isopropyl 4-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-pivaloyl-β-L-idopyranosyl) uronate (14)

To a solution of **13** (540 mg, 0.77 mmol) in dry CH_2Cl_2 (12 mL) at –10°C, Py (63 μL, 0.78 mmol) and acetic anhydride (74 μL, 0.78 mmol) were added. After stirring for 24 h, MeOH (1 mL) was added, and was concentrated in vacuo. The crude was purified by flash chromatography (hexane–EtOAc, 2:1) to yield **14** (488 mg, 85%) as well as nonreacting starting material **13** (22 mg, 4%). TLC: 0.33 (hexane–EtOAc, 2:1). $[\alpha]_D^{23}$ –10.1° (*c* 1.0, $CHCl_3$). MALDI-TOF (*m*/*z*): 766 (M + Na^+), 782 (M + K^+). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.23 (m, 10H, *2 Ph*), 5.18 (d, 1H, *H-1*, $J_{1,2}$ = 4.4 Hz), 5.04 (d, 1H, *H-1′*, $J_{1,2}$ = 3.4 Hz), 4.92 (m, 1H, *H-2*), 4.85 (m, 2H, *2 CH*$_2$Ph), 4.78 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 11.3 Hz), 4.74 (d, 1H, *H-5*, $J_{5,4}$ = 4.0 Hz), 4.69 (d, 1H, *1 CH*$_2$Ph *CH*$_2$Ph, J_{gem} = 11.2 Hz), 4.54 (dd, 1H, *H-6′a*, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 3.5 Hz), 4.13 (m, 2H, *H-4*, *H-6′b*), 3.93 (m, 2H, *H-3*, *CH*$(CH_3)_2$), 3.82 (m, 1H, *H-5′*), 3.76 (s, 3H, COO*CH*$_3$), 3.70 (m, 1H, *H-3′*), 3.40 (m, 1H, *H-4′*), 3.20 (dd, 1H, *H-2′*, $J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 10.1 Hz), 2.83

(d, 1H, *OH-4*, $J_{OH,4}$ = 3.4 Hz), 2.07 (s 3H, OCO*CH*$_3$), 1.21 (s, 9H, C(*CH*$_3$)$_3$), 1.19 and 1.14 (2 d, 6H, *J* = 6.0 Hz, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 172.0, 170.0 (*C=O*), 98.4 (*C-1*), 97.2 (*C-1′*), 78.8, 76.0, 75.1, 74.1, 73.1; 71.3, 70.9, 70.7, 70.6, 70.5, 70.1, 62.7, 52.2 (COO*CH*$_3$), 38.8, 27.2 (O*Piv*), 20.8 (OCO*CH*$_3$), 23.3, 21.8 (CH(*CH*$_3$)$_2$). Anal. calcd. for $C_{37}H_{49}N_3O_{13}$ (%): C 59.74, H 6.64, N 5.65; found (%): C 59.74, H 6.52, N 5.57.

Methyl (isopropyl 4-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)- 3-*O*-benzyl-2-*O*-pivaloyl-β-L-idopyranosyl) uronate (8)

To a solution of **12** (550 mg, 0.696 mmol) in dry THF (20 mL), $NaBH_3CN$ (7 mL of a solution of 1 M $NaBH_3CN$ in THF) was added, and was stirred for 10 h. Afterwards, a solution of 2.5 M HCl in Et_2O was added dropwise until the mixture became acidic. The reaction was neutralized with saturated $NaHCO_3$ solution, diluted with CH_2Cl_2 (25 mL), and washed with H_2O (15 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (hexane–EtOAc, 4:1) to yield **8** (505 mg, 92%). TLC: 0.32 (hexane–EtOAc, 4:1). $[\alpha]_D^{23}$ −4.4° (*c* 1.0, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.39–7.24 (m, 15 H, *3 Ph*), 5.21 (d, 1H, *H-1*, $J_{1,2}$ = 4.8 Hz), 5.05 (d, 1H, *H-1′*, $J_{1,2}$ =3.4 Hz), 4.93 (t, 1H, *H-2*, $J_{2,1} \approx J_{2,3}$ = 5.1 Hz), 4.84 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 11.1 Hz), 4.81 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 11.2 Hz), 4.76 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 11.2 Hz), 4.74 (d, 1H, *H-5*, $J_{5,4}$ = 5.0 Hz), 4.70 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 11.1 Hz), 4.57 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 12 Hz), 4.51 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 12 Hz), 4.14 (t, 1H, *H-4*, $J_{4,3} \approx J_{4,5}$ = 5.3 Hz), 3.97 (t, 1H, *H-3*, $J_{3,2} \approx J_{3,4}$ = 5.6 Hz), 3.94 (m, 1 H, *CH*(CH$_3$)$_2$, *J* 6.0 Hz), 3.81 (m, 1H, *H-5′*), 3.72 (s, 3H, COO*CH*$_3$), 3.71–3.67 (m, 3H, *H-3′*, *H-4′*, *H-6′a*), 3.57 (dd, 1H, *H-6′b*, $J_{6b,6a}$ = 10.2 Hz, $J_{6b,5}$ = 4.3 Hz), 3.21 (dd, 1H, *H-2′*, $J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 9.4 Hz), 2.57 (s, 1H, *OH-4*), 1.21 (s, 9H, C(*CH*$_3$)$_3$), 1.19 and 1.14 (2 d, 6H, *J* = 6.0 Hz, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.0 (*C=O*), 98.2 (*C-1′*), 97.2 (*C-1*), 79.1, 76.2, 74.8, 73.7, 73.6, 72.4, 71.3, 71.0, 70.6, 69.6, 62.6, 52.1 (COO*CH*$_3$), 38.8, 27.2 (O*Piv*), 23.3, 21.8 (CH(*CH*$_3$)$_2$). Anal. calcd. for $C_{42}H_{53}N_3O_{12}$ (%): C 63.70, H 6.75, N 5.30; found (%): C 63.62, H 6.70, N 5.26.

Methyl dimethylthexylsilyl 4-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-β-L-idopyranuronate (15)

A solution of **9** (255 mg, 0.316 mmol) in thiolacetic acid (2 mL) was stirred for 16 h at room temperature. After concentrating to dryness, the residue was purified by flash chromatography (hexane–EtOAc, 1:1) to yield **15** (639 mg, 90%). TLC: 0.35 (Hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ +59.5° (*c* 1.0, CH_2Cl_2). MALDI-TOF (*m/z*): 845 (M + Na^+), 861 (M + K^+). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.45–7.15 (m, 15H, *3 Ph*), 6.82 (d, 1H, *NH*, $J_{NH,2}$ = 9.5 Hz), 5.50 (s, 1H, Ph-*CH*-), 5.00 (s, 1H, *H-1*), 4.83 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 12.1 Hz), 4.64−4.52 (m, 5H, *3 CH*$_2$Ph, *H-1′*, *H-5*), 4.30 (m, 1H, *H-2′*), 4.21 (dd, 1H, *H-6′a*, $J_{6a,5}$ = 4.6 Hz, $J_{6a,6b}$ = 10.1 Hz), 3.94 (s, 1H, *H-4*), 3.82 (s, 3 H, COO*CH*$_3$), 3.74–3.59 (m, 6H, *H-2*, *H-3*, *H-3′*, *H-4′*, *H-5′*, *H-6′b*), 2.63 (s, 1H, *OH-2*), 1.85 (s, 3 H, NHCO*CH*$_3$), 1.64 (m, 1H, *CH*(CH$_3$)$_2$), 0.90–0.88 (m, 12H, CH(*CH*$_3$)$_2$, C(*CH*$_3$)$_2$), 0.26 and 0.19 (2 s, 6H, Si(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.4, 168.8 (*C=O*), 101.4 (Ph-*CH*-), 98.2 (*C-1*), 93.8 (*C-1′*), 82.8, 74.2, 73.7, 73.4, 72.9, 72.1, 68.7, 68.5, 63.6, 52.6 (*C-2′*), 52.1 (COO*CH*$_3$), 23.0 (NHCO*CH*$_3$), 34.0, 25.1, 20.3, 20.1, 18.6, 18.4, –1.9, –3.6 (O*TDS*). Anal. calcd. for $C_{44}H_{59}NO_{12}Si{\cdot}0.5H_2O$ (%): C 63.59, H 7.28, N 1.68; found (%): C 63.37, H 7.53, N 1.53.

Methyl dimethylthexylsilyl 2-*O*-acetyl-4-*O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-β-L-idopyranuronate (16)

Compound **9** (1 g, 1.24 mmol) was dissolved in Py (10 mL) at 0°C and acetic anhydride (5 mL) was added and was stirred for 48 h. The reaction was diluted with CH_2Cl_2 (250 mL) and washed with 1 N HCl (125 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 125 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was coevaporated with toluene twice and then purified by flash chromatography (hexane–EtOAc, 4:1), to yield **16** (1 g, 93%). TLC: 0.38 (hexane–EtOAc, 4;1). $[\alpha]_D^{23}$ +29.5° (c 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.45–7.24 (m, 15H, *3 Ph*), 5.51 (s, 1H, Ph-*CH*-), 5.07 (b.s., 1H, *H-1*), 4.97 (b. s., 1H, *H-2*), 4.76 (d, 1H, *H-1′*, $J_{1,2}$ = 3.5 Hz), 4.88−4.70 (2 d, 2H, *2 CH*$_2$Ph, J_{gem} = 11.1 Hz), 4.74−4.66 (2 d, 2H, *2 CH*$_2$Ph, J_{gem} = 11.8 Hz), 4.47 (b. s., 1H, *H-5*), 4.30 (dd, 1H, *H-6′a*, $J_{6a,5}$ = 4.9 Hz, $J_{6a,6b}$ = 10.1 Hz), 3.97–3.92 (m, 4H, *H-3′*, *H-4*, *H-5′*, *H-3*), 3.75 (s, 3H, COO*CH*$_3$), 3.64–3.59 (m, 2H, *H-4′*, *H-6′b*), 3.28 (t, 1H, *H-2′*, $J_{2,1} \approx J_{2,3}$ = 9.9 Hz), 2.03 (d, 3H, OCO*CH*$_3$), 1.61 (m, 1H, *CH*(CH$_3$)$_2$), 0.86–0.83 (m, 12H, CH(*CH*$_3$)$_2$, C(*CH*$_3$)$_2$), 0.24 and 0.14 (2s, 6H, Si(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.7, 168.8 (*C=O*), 137.8–126.1 (*Ph*), 101.6 (Ph-*CH*-), 98.4 (*C-1′*), 93.7 (*C-1*), 82.6, 76.0, 74.8 (*CH*$_2$Ph), 74.3, 73.6, 73.4 (*C-5*), 72.9 (*CH*$_2$Ph), 68.5 (*C-6′*), 67.7, 63.2, 63.1, 52.1 (COO*CH*$_3$), 20.9 (OCO*CH*$_3$), 34.0, 24.8, 20.2, 18.4, –2.0, –3.6 (O*TDS*).

Methyl (dimethylthexylsilyl 4-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-2-*O*-acetyl-3-*O*-benzyl-β-L-idopyranosid) uronate (17)

(*a*) A solution of **16** (4.3 g, 5.07 mmol) in thiolacetic acid (55 mL) was stirred for 30 h at room temperature. After concentrating to dryness, the residue was purified by flash chromatography (hexane–EtOAc, 1:1) to yield **17** (3.19 g, 73%).

(*b*) Compound **15** (790 mg, 0.98 mmol) was dissolved in Py (10 mL) at 0°C and acetic anhydride (5 mL) and catalytic DMAP were added and was stirred for 48 h. The reaction was diluted with CH_2Cl_2 (200 mL) and washed with 1 N HCl (100 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was coevaporated with toluene twice and then purified by flash chromatography (hexane–EtOAc, 1:1) to yield **17** (617 mg, 77%). TLC: 0.39 (hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ +46.3° (*c* 1.0, CH_2Cl_2). MALDI-TOF (*m/z*): 887 (M + Na^+), 903 (M + K^+). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.43–7.22 (m, 15H, *3 Ph*), 6.25 (d, 1H, *NH*, $J_{NH,2}$ = 9.6 Hz), 5.52 (s, 1H, Ph-*CH*-), 5.04 (s, 1H, *H-1*), 4.98 (s, 1H, *H-2*), 4.83 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 12.2 Hz), 4.76 (d, 1H, *H-5*, $J_{5,4}$ = 3.6 Hz), 4.67 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 11.8 Hz), 4.60−4.53 (m, 3H, *2 CH*$_2$Ph,

H-1′), 4.29 (m, 1H, *H-2′*), 4.19 (m, 1H, *H-6′a*), 3.87 (s, 1H, *H-4*), 3.85 (s, 1H, *H-3*), 3.74 (s, 3H, COOCH_3), 3.72–3.66 (m, 3H, *H-4′*, *H-5′*, *H-6′b*), 3.52 (m, 1H, *H-3′*), 1.91 (s, 3H, NHCOCH_3), 1.81 (s, 3H, OCOCH_3), 1.58 (m, 1H, *CH*$(CH_3)_2$), 0.83–0.82 (m, 12H, CH(CH_3)$_2$, C(CH_3)$_2$), 0.19 and 0.10 (2s, 6H, Si(CH_3)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.0, 169.9, 168.4 (*C=O*), 101.4 (Ph-*CH*-), 96.4 (C-1), 93.0 (*C-1′*), 82.4, 76.1, 74.0, 73.3, 73.1, 71.8, 70.3, 68.6, 67.8, 63.4, 52.2 (*C-2′*), 52.0 (COOCH_3), 23.3, 20.9 (OCOCH_3, NHCOCH_3), 33.9, 24.9, 20.1, 19.8, 18.6, 18.4, –1.9, –3.6 (O*TDS*). Anal. calcd. for $C_{46}H_{61}NO_{13}Si$ (%): C 63.94, H 7.11, N 1.62; found (%): C 63.64, H 6.82, N 1.59.

Methyl 4-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-2-*O*-acetyl-3-*O*-benzyl-α,β-L-idopyranuronate (18)

To a solution of **17** (2.75 g, 3.18 mmol) at –15°C in dry THF (90 mL) an excess of $(HF)_n$·Py (8.8 mL) was added. The reaction was warmed to 0°C and stirred for 24 h under an argon atmosphere. The mixture was diluted with CH_2Cl_2 (2 × 400 mL) and washed with H_2O (2 × 200 mL) and saturated $NaHCO_3$ solution until neutral pH. The aqueous layer was extracted with CH_2Cl_2 (2 × 200 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (hexane–EtOAc, 1:3) to yield **18** (1.8 g, 78%) as a mixture of α and β anomers. TLC: 0.16 (hexane–EtOAc, 2:3). MALDI-TOF (*m/z*): 774 (M + Na^+), 760 (M + K^+). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44–7.21 (m, 30H, *6 Ph* (α and β)), 5.87 (d, 1H, *NH* (α), $J_{NH,2}$ = 9.0 Hz), 5.71 (d, 1H, *NH* (β), $J_{NH,2}$ = 9.2 Hz), 5.54 (s, 1H, Ph-*CH*- β), 5.53 (s, 1H, Ph-*CH*-α), 5.23 (d, 1H, *H-1* (β), $J_{2,1}$ = 5.7 Hz), 5.16 (s, 1H, *H-1* (α)), 4.91–4.54 (m, 14H, *4* CH_2Ph, *H-1′*, *H-2*, *H-5* (α and β)), 4.27 (m, 2H, *H-2′* (α and β)), 4.23–4.19 (m, 2H, *H-6′a* (α and β)), 3.99–3.91 (m, 5H, *H-4*, *H-3* (α and β), *OH-1* (α)), 3.78 and 3.77 (2s, 6H, COOCH_3 (β and α)), 3.74–3.63 (m, 7H, *H-4′*, *H-5′*, *H-6′b* (α and β), *OH*-1 (β)), 3.53–3.48 (m, 2H, *H-3′* (α and β)), 1.97 (s, 3H, OCOCH_3 (α)), 1.91 (s, 3H, OCOCH_3 (β)), 1.80 (s, 3H, NHCOCH_3 (α)), 1.76 (s, 3H, NHCOCH_3 (β)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.1, 170.0, 169.8, 169.0, 168.6 (*C=O*), 138.3–127.9 (*Ph*), 101.5 (Ph-*CH*-), 97.1 (*C-1′* (α)), 96.7 (*C-1′* (β)), 93.1 (*C-1* (α and β)), 82.4, 75.9, 75.8, 73.5, 73.2, 73.1, 71.4, 71.2, 68.9, 68.6, 67.6, 67.5, 63.6, 63.5, 52.4 (*C-2′* (α and β)), 52.3 (COOCH_3 (α and β)), 23.3, 20.8 (OCOCH_3, NHCOCH_3 (α and β)). Anal. calcd. for $C_{38}H_{43}NO_{13}$·0.5H_2O (%): C 62.45, H 6.07, N 1.92; found (%): C 62.49, H 6.02, N 2.20.

***O*-(Methyl 4-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-2-*O*-acetyl-3-*O*-benzyl-α,β-L-idopyranosyluronate) trichloroacetimidate (7)**

To a solution of **18** (620 mg, 0.86 mmol) in dry CH_2Cl_2 (14 mL), Cl_3CCN (1.3 mL, 13.05 mmol) and activated K_2CO_3 (130 mg, 0.95 mmol) were added. After stirring for 1 h the residue was filtered and concentrated to dryness. The residue was purified by flash chromatography (hexane–EtOAc, 1:2) to yield **7** (701 mg, 94%) as a mixture of α and β anomers. TLC (α and β): 0.51 and 0.62 (toluene–EtOAc, 1:2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.73 (s, 1H, O(C=N*H*)CCl_3 α), 8.66 (s, 1H, O(C=N*H*)CCl_3 (β)), 7.44–7.22 (m, 30H, *6 Ph* (α and β)), 6.34 (s, 1H, *H-1* (α)), 6.22 (s, 1H, *H-1* (β)), 5.87 (d, 1H, N*H* (β), $J_{NH,2}$ = 9.3 Hz), 5.72 (d, 1H, N*H* (α), $J_{NH,2}$ = 9.4 Hz), 5.53 (s, 1H, Ph-*CH*-α), 5.52 (s, 1H, Ph-*CH*-β), 5.25 and 5.12 (m, 2 H, *H-2′* (α and β)), 4.98 (s, 1H, *H-5* (α)), 4.87–4.51 (m, 13H, *4* CH_2Ph, *H-1′* (α and β), H-2 (α and β), *H*-5), 4.30 (m, 2H, *H-2′* (α and β)), 4.24–4.17 (m, 2H, *H-6′a* (α and β)), 4.11–4.04 (m, 2H, *H-4* (α), *H-3* (β)), 3.96 (m, 1H, *H-4* (β)), 3.86 (s, 1H, *H-3* (α)), 3.77 (s, 3H, COOCH_3 (α)), 3.76 (s, 3H, COOCH_3 (β)), 3.81–3.64 (m, 6H, *H-4′*, *H-5′*, *H-6′b* (α and β)), 3.52 (m, 2H, *H-3′* (α and β)), 1.94 (s, 3H, OCOCH_3 (α)), 1.92 (s, 3H, OCOCH_3 (β)), 1.81 (s, 3H, NHCOCH_3 (α)), 1.75 (s, 3H, NHCOCH_3 (β)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.1, 170.0, 169.9, 169.7, 169.4, 168.3, 167.6 (*C=O*), 160.5, 160.1 (*C=NH*), 138.4–126.0 (*Ph*), 101.5 (Ph-*CH*-), 97.6 (*C-1′* (β)), 96.2 (*C-1′* (α)), 95.0 (*C-1* (α)), 94.5 (*C-1* (β)), 82.4, 82.3, 76.1, 75.6, 74.1, 73.9, 72.5, 72.2, 72.0, 70.4, 69.9, 69.1, 68.7, 68.6, 66.9, 65.8, 63.7, 63.5, 60.4, 52.5 (*C-2′* (α and β)), 52.3 (COOCH_3 (α and β)), 23.4, 23.2, 21.0, 20.7 (OCOCH_3, NHCOCH_3 (α and β)). Anal. calcd. for $C_{40}H_{43}N_2O_{13}Cl_3$ (%): C 55.47, H 5.00, N 3.23; found (%): C 55.78, H 5.09, N 3.52.

Methyl (isopropyl *O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (24)

To a solution of **14** (235 mg, 0.32 mmol) and **7** (362 mg, 0.42 mmol) in dry CH_2Cl_2 (0.5 mL) at room temperature, TMSOTf (20 μL of a 0.93 M solution of TMSOTf in dry CH_2Cl_2) was added under an argon atmosphere. After stirring for 7 h 30 min, saturated $NaHCO_3$ solution (2 mL) was added and diluted with CH_2Cl_2 (10 mL). The residue was washed with H_2O (7.5 mL) and then the aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (hexane–EtOAc, 1:2) to yield **24** (150 mg, 33%) and nonreacting acceptor **14** (156 mg, 43%). TLC: 0.24 (hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ +46.3° (*c* 1.0, $CHCl_3$). MALDI-TOF (*m/z*): 1470 (M + Na^+), 1486 (M + K^+). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.42–7.22 (m, 25H, *5 Ph*), 6.57 (d, 1H, N*H*, $J_{NH,2}$ = 9.2 Hz), 5.54 (s, 1H, Ph-*CH*-), 5.13 (m, 2H, *H-1a*, *H-1c*), 4.98–4.49 (m, 15H, *H-1b*, *H-1d*, *H-2a*, *H-2c*, *H-5a*, *H-5c*, *H-6b*, *8* CH_2Ph), 4.29 (m, 1H, *H-2d*), 4.17 (m, 1H, *H-6d*), 4.09 (m, 4H, *H-3c*, *H-4a*, *H-4b*, *H-6′b*), 3.93 (m, 3H, *H-3a*, *H-4c*, *CH*$(CH_3)_2$), 3.74 (s, 3H, COOCH_3), 3.60 (s, 3H, COOCH_3), 3.79–3.70 (m, 4H, *H-3b*, *H-4d*, *H-5b*, *H-5d*), 3.65 (dd, 1H, *H-6′d*, $J_{6',6}$ = 9.3 Hz, $J_{6',5}$ = 3.8 Hz), 3.54 (t, 1H, *H-3d*, $J_{3,2} \approx J_{3,4}$ = 9.6 Hz), 3.33 (dd, 1H, *H-2b*, $J_{2,1}$ = 3.2 Hz, $J_{2,3}$ = 10.2 Hz), 2.12 (s, 3H, NHCOCH_3), 2.04 (s, 3H, OCOCH_3), 1.87 (s, 3H, OCOCH_3), 1.21 (s, 9H, C*(*CH_3*)*$_3$), 1.19 and 1.16 (2d, 6H, *J* = 6.5 Hz, CH(CH_3)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.6, 171.7, 170.3, 170.1, 169.8, 169.0 (*C=O*), 137.7–127.7 (*Ph*), 101.4 (Ph-*CH*-), 98.8 (*C-1c*), 97.2 (*C-1b*), 96.9 (*C-1a*), 96.2 (*C-1d*), 82.4, 78.1, 76.3, 75.9, 75.2, 74.5, 74.0, 73.9, 73.1, 72.9, 72.1, 71.2, 70.1, 69.9, 69.8, 69.5, 68.7, 63.3, 62.9, 61.6, 52.4, 52.2, 51.9, 29.7, 27.2, 23.3, 23.1, 21.7, 21.3, 20.8 (O*Piv*, O*Ac*, NHCOCH_3, O-*i*-Pr). Anal.

calcd. for $C_{75}H_{90}N_4O_{25}\cdot 2.5H_2O$ (%): C 60.35, H 6.41, N 3.75; found: C 60.36, H 6.25, N 3.25.

Methyl (isopropyl *O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (25)

To a solution of **24** (280 mg, 0.19 mmol) in dry MeOH (3 mL), MeONa (90 μL of a 0.43 M solution of MeONa in MeOH) was added and stirred for 24 h. The reaction was diluted with CH_2Cl_2 (10 mL) and washed with saturated NH_4Cl solution (200 mL) and H_2O (5 mL). The organic layer was dried ($MgSO_4$) and concentrated to dryness. The mixture was purified by flash chromatography (hexane–EtOAc, 1:3) to yield **25** (224 mg, 85%). TLC: 0.38 (hexane–EtOAc, 1:2). $[\alpha]_D^{23}$ +20.4° (*c* 1.0, CH_2Cl_2). MALDI-TOF (*m*/*z*): 1386 (M + Na^+), 1402 (M + K^+). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.42–7.20 (m, 25H, *5 Ph*), 6.21 (d, 1H, N*H*, $J_{NH,2}$ = 7.7 Hz), 5.50 (s, 1H, Ph-*CH*-), 5.17–5.15 (m, 2 H, *H-1c*, *H-1a*), 5.04 (d, 1H, *H-1b*, $J_{2,1}$ = 3.6 Hz), 4.98 (d, 1H, *H-1d*, $J_{1,2}$ = 3.8 Hz), 4.92 (t, 1H, *H-2a*, $J_{2,1} \approx J_{2,3}$ = 5.1 Hz), 4.86 (d, 1H, *H-5c*, $J_{5,4}$ = 2.5 Hz), 4.77–4.52 (m, 8H, *8* CH_2Ph), 4.72 (d, 1H, *H-5a*, $J_{5,4}$ = 4.6 Hz), 4.18–4.09 (m, 3H, *H-2d*, *H-4a*, *H-6d*), 4.01–3.86 (m, 5H, *H-3a*, *H-3c*, *H-4b*, *H-4c*, *CH*(CH_3)$_2$), 3.69 (s, 3H, COO*CH*$_3$), 3.78–3.65 (m, 8H, *H-2c*, *H-3b*, *H-4d*, *H-5b*, *H-5d*, *H-6′d*, *H-6b*, *H-6′b*), 3.62 (t, 1H, *H-3d*, $J_{3,2} \approx J_{3,4}$ = 9.8 Hz), 3.45 (s, 3H, COO*CH*$_3$), 3.26 (dd, 1H, *H-2b*, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.2 Hz), 1.76 (s, 3H, NHCO*CH*$_3$), 1.18 (s, 9H, C(*CH*$_3$)$_3$), 1.19 and 1.17 (2d, 6H, *J* = 6.5 Hz, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.8, 170.0, 169.4 (*C=O*), 138.5–126.1 (*Ph*), 101.5 (Ph-*CH*-), 100.7 (*C-1c*), 98.3 (*C-1b*), 97.3 (*C-1a*), 96.9 (*C-1d*), 82.3, 78.2, 76.3, 75.9, 74.8, 74.3, 74.1, 74.0, 73.6, 73.2, 72.8, 72.5, 72.3, 71.3, 70.9, 68.6, 68.4, 68.1, 63.5, 63.4 (*C-2c*), 61.2, 52.8 (*C-2a*), 52.2, 52.1 (COO*CH*$_3$), 27.2, 23.3, 22.9, 22.8, 21.8, 21.3 (O*Piv*, NHCO*CH*$_3$, O-*i*-Pr). Anal. calcd. for $C_{71}H_{86}N_4O_{23}$ (%): C 62.54, H 6.36, N 4.11; found (%): C 62.45, H 6.51, N 4.12.

Methyl (isopropyl *O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (19)

To a solution of **8** (425 mg, 0.54 mmol) and **7** (600 mg, 0.693 mmol) in dry CH_2Cl_2 (0.5 mL) at room temperature, TMSOTf (8.7 μL, 48 μmol) was added under an argon atmosphere. After stirring for 4 h 30 min, saturated $NaHCO_3$ solution (2 mL) was added and diluted with CH_2Cl_2 (25 mL). The residue was washed with H_2O (20 mL) and then the aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (hexane–EtOAc, 1:1), to yield **19** (401 mg, 50%) and nonreacting acceptor **8** (195 mg, 46%). TLC: 0.33 (hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ +10.0° (*c* 1.0, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.43–7.20 (m, 30H, *6 Ph*), 5.77 (d, 1H, N*H*, $J_{NH,2}$ = 9.5 Hz), 5.52 (s, 1H, Ph-*CH*-), 5.18 (d, 1H, *H-1a*, $J_{1,2}$ = 4.7 Hz), 5.16 (d, 1H, *H-1c*, $J_{1,2}$ = 3.7 Hz), 5.03 (d, 1H, *H-1b*, $J_{1,2}$ = 3.5 Hz), 4.90 (m, 2H, *H-2a*, *H-2c*), 4.84 (d, 1H, *H-1d*, $J_{1,2}$ = 3.1 Hz), 4.81–4.49 (m, 12H, *H-5a*, *H-5c*, *10* CH_2Ph), 4.29 (m, 1H, *H-2d*), 4.15 (dd, 1H, *H-6d* $J_{6,5}$ = 4.4 Hz, $J_{6,6'}$ = 10 Hz), 4.09 (t, 1H, *H-4a*, $J_{4,3} \approx J_{4,5}$ = 5.3 Hz), 4.01 (t, 1H, *H-4b* $J_{4,3} \approx J_{4,5}$ = 9.5 Hz), 3.94–3.91 (m, 3H, *H-3a*, *H-3c*, *CH*(CH_3)$_2$), 3.86 (m, 1H, *H-4c*), 3.80 (d, 1H, *H-5b*, $J_{5,4}$ = 10 Hz), 3.72–3.59 (m, 6H, *H-3b*, *H-6b*, *H-6′b*, *H-4d*, *H-5d*, *H-6′d*), 3.67 (s, 3H, COO*CH*$_3$), 3.50 (t, 1H, *H-3d*, $J_{3,2} \approx J_{3,4}$ = 9.6 Hz), 3.41 (s, 3H, COO*CH*$_3$), 3.30 (dd, 1H, *H-2b*, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.3 Hz), 1.83 (s, 3H, OCO*CH*$_3$), 1.63 (s, 3H, NHCO*CH*$_3$), 1.19 (s, 9H, C(*CH*$_3$)$_3$), 1.18 and 1.14 (2d, 6H, *J* = 6.5 Hz, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.1, 169.8, 169.4, 168.8 (*C=O*), 138.4–126.3 (*Ph*), 101.4 (Ph-*CH*-), 98.3 (*C-1b*), 97.6 (*C-1c*), 97.1 (*C-1a*), 82.4, 78.0, 76.3, 74.9, 74.2, 74.1, 73.9, 73.7, 73.6, 73.4, 73.2, 71.6, 71.3, 70.7, 70.4, 70.1, 69.5, 68.7, 67.3, 65.6, 63.7, 62.9, 52.3 (*C-2d*), 52.1, 51.9 (COO*CH*$_3$), 38.8, 27.2, 23.2, 21.8, 20.8 (O*Piv*, NHCO*CH*$_3$, O-*i*-Pr). Anal. calcd. for $C_{80}H_{94}N_4O_{24}$ (%): C 64.24, H 6.33, N 3.75; found (%): C 63.89, H 6.36, N 3.51.

Methyl (isopropyl *O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (20)

To a solution of **19** (460 mg, 0.307 mmol) in dry MeOH (10 mL), MeONa (73 μL of 1.2 M MeONa in MeOH) was added and stirred for 24 h. The reaction was neutralized with resin (IRA-120 H^+) and filtered. The residue was purified by flash chromatography (hexane–EtOAc, 1:2) to yield **20** (429 mg, 96%). TLC: 0.28 (hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ +14.0° (*c* 1.0, CH_2Cl_2). MALDI-TOF (*m*/*z*): 1475 (M + Na^+), 1491 (M + K^+). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.42–7.21 (m, 30H, *6 Ph*), 6.11 (d, 1H, N*H*, $J_{NH,2}$ = 8.2 Hz), 5.50 (s, 1H, Ph-*CH*-), 5.17 (d, 1H, *H-1a*, $J_{1,2}$ = 4.7 Hz), 5.11 (b.s., 1H, *H-1c*), 5.06 (d, 1H, *H-1b*, $J_{1,2}$ = 3.6 Hz), 4.96 (d, 1H, *H-1d*, $J_{1,2}$ = 3.8 Hz), 4.92 (t, 1H, *H-2a*, $J_{2,1} \approx J_{2,3}$ = 5.0 Hz), 4.87 (d, 1H, *H-5c*, $J_{5,4}$ = 2.3 Hz), 4.83 (d, 1H, *1* CH_2Ph, J_{gem} = 12.2 Hz), 4.75 (2d, 2H, *2* CH_2Ph, J_{gem} = 11 Hz), 4.72 (d, 1H, *H-5a*, $J_{5,4}$ = 4.7 Hz), 4.68 (d, 1H, *1* CH_2Ph, J_{gem} = 11 Hz), 4.63 (2d, 2H, *2* CH_2Ph, J_{gem} = 11.5 Hz), 4.56–4.52 (m, 4H, *4* CH_2Ph), 4.18–4.11 (m, 3H, *H-2d*, *H-4a*, *H-6d*), 3.99–3.91 (m, 4H, *H-4b*, *H-3a*, *H-4c*, *CH*(CH_3)$_2$), 3.84 (m, 1H, *H-3c*), 3.72–3.65 (m, 6H, *H-3b*, *H-6b*, *H-6′b*, *H-4d*, *H-5d*, *H-6′d*), 3.63 (s, 3H, COO*CH*$_3$), 3.61–3.55 (m, 3H, *H-2c*, *H-3d*, *H-5b*), 3.46 (m, 1H, *H-3d*), 3.40 (s, 3H, COO*CH*$_3$), 3.30 (dd, 1H, *H-2b*, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.3 Hz), 2.60 (d, 1H, *OH-2c*, $J_{OH,2}$ = 4.7 Hz), 1.73 (s, 3H, NHCO*CH*$_3$), 1.19 (s, 9H, C(*CH*$_3$)$_3$), 1.18 and 1.13 (2d, 6H, *J* = 6.5 Hz, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.7, 170.0, 169.3 (*C=O*), 138.4–126.1 (*Ph*), 101.5 (Ph-*CH*-), 100.8 (*C-1c*), 98.3 (*C-1b*), 97.2 (*C-1a*), 96.8 (*C-1d*), 82.3, 78.2, 76.2, 75.2, 75.0, 74.1, 74.0, 73.8, 73.6, 73.2, 72.8, 72.1, 71.4, 71.3, 70.9, 70.4, 69.3, 68.6, 68.3, 68.0, 67.6, 63.4, 63.2, 52.6 (*C-2d*), 52.2, 52.0 (COO*CH*$_3$), 38.8, 29.1, 27.2, 23.3, 22.9, 21.8 (O*Piv*, NHCO*CH*$_3$, O-*i*-Pr). Anal. calcd. for $C_{78}H_{92}N_4O_{23}\cdot H_2O$ (%): C 63.66, H 6.43, N 3.81; found (%): C 63.51, H 6.19, N 4.01.

Methyl (isopropyl *O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (21)

(*a*) To a solution of **25** (170 mg, 0.124 mmol), freshly prepared Ag_2O (90 mg, 0.388 mmol) and 4 Å molecular sieves in dry DMF (0.5 mL), freshly distilled benzyl bromide (100 μL, 0.84 mmol) was added. After stirring for 40 h, the solution was filtered through Celite. The residue was purified by flash chromatography (toluene–EtOAc, 5:2) to yield **21** (101 mg, 52%).

(*b*) To a solution of **20** (440 mg, 0.303 mmol) and freshly prepared Ag_2O (161 mg, 0.7 mmol) in dry DMF (0.5 mL), freshly distilled benzyl bromide (100 μL, 0.84 mmol) was added. After stirring for 30 h, the solution was filtered through Celite. The residue was purified by flash chromatography (hexane–EtOAc, 1:1) to yield **21** (411 mg, 88%). TLC: 0.32 (hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ +47.3° (*c* 1.0, $CHCl_3$). MALDI-TOF (*m*/*z*): 1566 ($M + Na^+ + H$), 1581 ($M + K^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44–7.15 (m, 35H, *7 Ph*), 5.92 (d, 1H, N*H*, $J_{NH,2}$ = 9.7 Hz), 5.50 (s, 1H, Ph-*CH*-), 5.29 (d, 1H, *H-1c*, $J_{2,1}$ = 3.7 Hz), 5.16 (d, 1H, *H-1a*, $J_{2,1}$ = 4.6 Hz), 5.04 (d, 1H, *H-1b*, $J_{2,1}$ = 3.6 Hz), 4.91 (t, 1H, *H-2a*, $J_{2,1} \approx J_{2,3}$ = 5.0 Hz), 4.87 (d, 1H, *1* CH_2Ph, J_{gem} = 10.7 Hz), 4.81 (d, 1H, *H-1d*, $J_{2,1}$ = 3.8 Hz), 4.78–4.64 (m, 7H, *H-5c*, *H-5a*, *5* CH_2Ph), 4.57 (d, 1H, *1* CH_2Ph, J_{gem} = 11.9 Hz), 4.52–4.47 (m, 3H, *3* CH_2Ph), 4.43 (d, 1H, *1* CH_2Ph, J_{gem} = 11.9 Hz), 4.42 (d, 1H, *1* CH_2Ph, J_{gem} = 12.1 Hz), 4.31 (m, 1H, *H-2d*), 4.16 (dd, 1H, *H-6d*, $J_{6,5}$ = 4.5 Hz, $J_{6,6'}$ = 10.2 Hz), 4.12 (t, 1H, *H-4a*, $J_{4,3} \approx J_{4,5}$ = 5.2 Hz), 4.04 (t, 1H, *H-4b*, $J_{4,3} \approx J_{4,5}$ = 9.5 Hz), 3.96–3.91 (m, 3H, *H-3a*, *H-4c*, *CH*$(CH_3)_2$), 3.85–3.82 (m, 2H, *H-5b*, *H-3c*), 3.78–3.52 (m, 7H, *H-3b*, *H-3d*, *H-4d*, *H-5d*, *H-6′d*, *H-6b*, *H-6′b*), 3.62 (s, 3H, COO*CH*$_3$), 3.47 (s, 3H, COO*CH*$_3$), 3.44 (t, 1H, *H-2c*, $J_{2,1} \approx J_{2,1}$ = 4.1 Hz), 3.30 (dd, 1H, *H-2b*, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.3 Hz), 1.51 (s, 3H, NHCO*CH*$_3$), 1.20 (s, 9H, C(*CH*$_3$)$_3$), 1.19 and 1.14 (2d, 6H, *J* = 6.5 Hz, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.0, 169.1 (*C=O*), 138.6,–126.1 (*Ph*), 101.4 (Ph-*CH*-), 98.7 (*C-1c*), 98.4 (*C-1b*), 98.3 (*C-1d*), 97.2 (*C-1a*), 82.3, 78.1, 76.2, 76.0, 75.8, 74.2, 74.1, 73.7, 73.4, 73.2, 71.8, 71.3, 70.7, 70.3, 69.5, 68.7 (*C-6d*), 67.7, 65.4, 63.6, 63.0 (*C-2b*), 52.2 (*C-2d*), 52.1, 51.9 (COO*CH*$_3$), 29.7, 27.2, 23.3, 22.8, 21.8 (O*Piv*, NHCO*CH*$_3$, O*i*-Pr). Anal. calcd. for $C_{85}H_{98}N_4O_{23}$ (%): C 66.13, H 6.40, N 3.63; found (%): C 65.90, H 6.40, N 3.42.

Methyl (isopropyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (22)

To a solution of **21** (450 mg, 0.291 mmol) in dry CH_2Cl_2 (9 mL), EtSH (108 μL, 1.46 mmol), and catalytic PTSA were added. After stirring for 3 h under an argon atmosphere, the reaction was neutralized with solid $NaHCO_3$, diluted with CH_2Cl_2 (40 mL), and washed with H_2O (40 mL). The organic layer was dried ($MgSO_4$) and concentrated to dryness. The residue was purified by flash chromatography (hexane–EtOAc, 1:3) to yield **22** (351 mg, 83%). TLC: 0.23 (hexane–EtOAc, 1:3). $[\alpha]_D^{23}$ +23.2° (*c* 1.0, CH_2Cl_2). MALDI-TOF (*m*/*z*): 1477 ($M + Na^+$), 1493 ($M + K^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.34–7.16 (m, 30H, *6 Ph*), 6.19 (d, 1H, *NH*, $J_{NH,2}$ = 9.6 Hz), 5.29 (d, 1H, *H-1c*, $J_{2,1}$ = 3.1 Hz), 5.16 (d, 1H, *H-1a*, $J_{2,1}$ = 4.5 Hz), 5.04 (d, 1H, *H-1b*, $J_{2,1}$ = 3.5 Hz), 4.91 (t, 1H, *H-2a*, $J_{2,1} \approx J_{2,3}$ = 4.8 Hz), 4.86 (d, 1H, *1* CH_2Ph, J_{gem} = 10.8 Hz), 4.78–4.74 (m, 3H, *H-1d*, *H-5c*, *1* CH_2Ph), 4.73 (d, 1H, *H-5c*, $J_{5,4}$ = 4.5 Hz), 4.69–4.40 (m, 10H, *10* CH_2Ph), 4.22 (m, 1H, *H-2d*), 4.12 (t, 1H, *H-4a*, $J_{4,3} \approx J_{4,5}$ = 5.2 Hz), 4.04 (t, 1H, *H-4b*, $J_{4,3} \approx J_{4,5}$ = 9.5 Hz), 3.94 (t, 1H, *H-3a*, $J_{3,2} \approx J_{3,4}$ = 5.5 Hz), 3.92–3.90 (m, 2H, *H-4c*, *CH*$(CH_3)_2$), 3.85 (m, 1H, *H-5b*), 3.81 (t, 1H, *H-3c*, $J_{3,2} \approx J_{3,4}$ = 4.4 Hz), 3.75 (t, 1H, *H-3b*, $J_{3,2} \approx J_{3,4}$ = 9.8 Hz), 3.72–3.57 (m, 5H, *H-4d*, *H-5d*, *H-6d*, *H-6b*, *H-6′b*), 3.64 (s, 3H, COO*CH*$_3$), 3.46–3.35 (m, 3H, *H-3d*, *H-2c*, *H-6′b*), 3.42 (s, 3H, COO*CH*$_3$), 3.31 (dd, 1H, *H-2b*, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.3 Hz), 1.51 (s, 3H, NHCO*CH*$_3$), 1.20 (s, 9H, C(*CH*$_3$)$_3$), 1.18 and 1.14 (2d, 6H, *J* = 6.1 Hz, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.1, 170.0, 169.3 (*C=O*), 138.2–127.6 (*Ph*), 98.7 (*C-1c*), 98.4 (*C-1b*), 97.5 (*C-1d*), 97.3 (*C-1a*), 80.4, 78.2, 76.2, 74.9, 74.2, 74.1, 74.0, 73.9, 73.7, 73.3, 73.2, 72.5, 72.4, 72.2, 71.8, 71.3, 70.7, 70.4, 70.2, 69.2, 63.1, 62.4 (*C-2b*), 52.1, 52.0 (COO*CH*$_3$), 51.8 (*C-2d*), 38.8, 27.2, 23.3, 22.8, 21.8 (O*Piv*, NHCO*CH*$_3$, O-*i*-Pr). Anal. calcd. for $C_{78}H_{94}N_4O_{23}\cdot 0.5H_2O$ (%): C 63.96, H 6.54, N 3.82; found (%): C 63.60, H 6.48, N 3.80.

Methyl (isopropyl *O*-(2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (23)

To a solution of **22** (330 mg, 0.227 mmol) in dry CH_3CN (4 mL) at −40°C, BzCN (262 μL of a 0.9 M solution of BzCN in dry CH_3CN) and a few drops of Et_3N were added. After 7 h, MeOH was added and the mixture was allowed to reach room temperature. The solvent was evaporated and the residue was dissolved in MeOH and concentrated to dryness. The residue was purified by flash chromatography (hexane–EtOAc, 1:1) to yield **23** (312 mg, 88%). TLC: 0.29 (hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ +43.2° (*c* 1.0, CH_2Cl_2). MALDI-TOF (*m*/*z*): 1581 ($M + Na^+$), 1597 ($M + K^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.03–7.13 (m, 35H, *7 Ph*), 6.16 (d, 1H, *NH*, $J_{NH,2}$ = 9.7 Hz), 5.29 (d, 1H, *H-1c*, $J_{1,2}$ = 3.0 Hz), 5.17 (d, 1H, *H-1a*, $J_{1,2}$ = 4.6 Hz), 5.05 (d, 1H, *H-1b*, $J_{1,2}$ = 3.6 Hz), 4.92 (t, 1H, *H-2a*, $J_{2,1} \approx J_{2,3}$ = 5.0 Hz), 4.84 (d, 1H, *1* CH_2Ph, J_{gem} = 10.5 Hz), 4.83 (s, 1H, *H-1d*), 4.81 (d, 1H, *H-5c*, $J_{5,4}$ = 3.3 Hz), 4.76 (d, 1H, *1* CH_2Ph, J_{gem} = 11.2 Hz), 4.73 (d, 1H, *H-5a*, $J_{5,4}$ = 4.5 Hz), 4.70–4.47 (m, 10H, *H-6d*, *9* CH_2Ph), 4.39 (d, 1H, *1* CH_2Ph, J_{gem} = 12.0 Hz), 4.38 (dd, 1H, *H-6′d*, $J_{6',5}$ = 1.8 Hz, $J_{6',6'}$ = 12.0 Hz), 4.26 (m, 1H, *H-2d*), 4.12 (t, 1H, *H-4a*, $J_{4,3} \approx J_{4,5}$ = 5.2 Hz), 4.04 (t, 1H, *H-4b*, $J_{4,3} \approx J_{4,5}$ = 9.6 Hz), 3.99 (t, 1H, *H-4c*, $J_{4,3} \approx J_{4,5}$ = 3.9 Hz), 3.95 (t, 1H, *H-3a*, $J_{3,2} \approx J_{3,4}$ = 5.4 Hz), 3.93 (m, 1H, *CH*$(CH_3)_2$, *J* = 6.2 Hz), 3.84 (m, 1H, *H-5b*), 3.82 (t, 1H, *H-3c*, $J_{3,2} \approx J_{3,4}$ = 4.3 Hz), 3.74 (t, 1H, *H-3b*, $J_{3,2} \approx J_{3,4}$ = 10.0 Hz), 3.69–3.57 (m, 4H, *H-4d*, *H-5d*, *H-6b*, *H-6′b*), 3.63 (s, 3H, COO*CH*$_3$), 3.43 (s, 3H, COO*CH*$_3$), 3.44–3.40 (m, 2H, *H-3d*, *H-2c*), 3.31 (dd, 1H, *H-2b*, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.3 Hz), 1.52 (s, 3H,

NHCOCH_3), 1.20 (s, 9H, C(CH_3)$_3$), 1.19 and 1.14 (2d, 6H, J = 6.1 Hz, CH(CH_3)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.0, 169.2, 166.9 (*C*=*O*), 138.3–127.6 (*Ph*), 98.7 (*C-1c*), 98.4 (*C-1b*), 97.2 (*C-1a*), 97.1 (*C-1d*), 80.2, 78.1, 76.4, 74.9, 74.5, 74.2, 74.1, 73.7, 73.3, 73.2, 73.1, 73.0, 71.9, 71.8, 71.3, 71.0, 70.7, 70.3, 69.7, 69.1, 67.9, 63.1, 63.0, 52.0, 51.9 (COOCH_3, *C-2d*), 38.8, 27.2, 23.3, 22.8, 21.8 (O*Piv*, NHCOCH_3, O-*i*-Pr). Anal. calcd. for $C_{85}H_{98}N_4O_{24}\cdot 0.5H_2O$ (%): C 65.08, H 6.36, N 3.57, found (%): C 64.83, H 6.36, N 3.57.

Methyl (isopropyl *O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (5)

To a solution of **23** (90 mg, 57.7 μmol) and **6** (68 mg, 75.7 μmol) in dry CH_2Cl_2 (0.5 mL) at room temperature, TMSOTf (25 μL of a 0.1 M solution of TMSOTf in CH_2Cl_2) was added under an argon atmosphere. After stirring for 2 h 30 min, saturated $NaHCO_3$ solution (1 mL) was added and diluted with CH_2Cl_2 (20 mL). The residue was washed with H_2O (15 mL) and then the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (hexane–EtOAc, 2:3) to yield **5** (85 mg, 65%) and nonreacting acceptor **23** (27 mg, 30%). TLC: 0.33 (hexane–EtOAc, 3:2). $[\alpha]_D^{23}$ +10.4° (*c* 1.1, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.09–7.13 (m, 50H, *10 Ph*), 5.90 (d, 1H, N*H*, $J_{NH,2}$ = 9.8 Hz), 5.48 (s, 1H, Ph-*CH*-), 5.30 (d, 1H, *H-1c*, $J_{1,2}$ = 3.4 Hz), 5.25 (d, 1H, *H-1e*, $J_{1,2}$ = 3.8 Hz), 5.17 (d, 1H, *H-1a*, $J_{1,2}$ = 4.6 Hz), 5.05 (d, 1H, *H-1b*, $J_{1,2}$ = 3.7 Hz), 4.93–4.90 (m, 3H, *H-2a*, *H-2e*, *H-1f*), 4.85 (d, 1H, *1* CH_2Ph, J_{gem} = 10.9 Hz), 4.83 (d, 1H, *1* CH_2Ph, J_{gem} = 11 Hz), 4.79 (d, 1H, *H-1d*, $J_{1,2}$ = 3.7 Hz), 4.76–4.47 (m, 16H, *H-5a*, *H-5c*, *H-5e*, *H-6d*, *12* CH_2Ph), 4.42 (m, 1H, *H-6′d*), 4.39 (d, 1H, *1* CH_2Ph, J_{gem} = 12 Hz), 4.28 (m, 1H, *H-2d*), 4.27 (d, 1H, *1* CH_2Ph, J_{gem} = 10.9 Hz), 4.19 (dd, 1H, *H-6f*, $J_{6,5}$ = 4.9 Hz, $J_{6,6'}$ = 10.1 Hz), 4.12 (dd, 1H, *H-4a*, $J_{4,3}$ = 4.8 Hz, $J_{4,5}$ = 5.9 Hz), 4.05–3.99 (m, 3H, *H-4b*, *H-4d*, *H-4e*), 3.99–3.90 (m, 4H, *H-3a*, *H-3e*, *H-4c*, *CH*(CH_3)$_2$), 3.88–3.82 (m, 3H, *H-3f*, *H-5b*, *H-5f*), 3.80–3.72 (m, 3H, *H-3c*, *H-3b*, *H-5d*), 3.67 (dd, 1H, *H-6b*, $J_{6,5}$ = 3.0 Hz, $J_{6,6'}$ = 11.7 Hz), 3.63 (s, 3H, COOCH_3), 3.61–3.55 (m, 3H, *H-4f*, *H-6′b*, *H-6′f*), 3.45–3.41 (m, 2H, *H-2c*, *H-3d*), 3.39 (s, 3H, COOCH_3), 3.32 (s, 3H, COOCH_3), 3.32–3.30 (m, 1H, *H-2b*), 3.27 (dd, 1H, *H-2f*, $J_{2,1}$ = 3.8 Hz, $J_{2,3}$ = 10.0 Hz), 1.41 (s, 3H, NHCOCH_3), 1.20 (s, 9H, C(CH_3)$_3$), 1.19 and 1.13 (2d, 6H, J = 6.1 Hz, CH(CH_3)$_2$), 1.11 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.4, 177.3, 170.0, 169.8, 169.3, 169.0, 166.1 (*C*=*O*), 138.4–126.1 (*Ph*), 101.5 (Ph-*CH*-), 99.4 (*C-1f*), 98.7 (*C-1b*, *C-1c*), 98.0 (*C-1e*), 97.2 (*C-1a*, *C-1d*), 82.5, 78.1, 76.3, 76.0, 75.0, 74.9, 74.8, 74.6, 74.5, 74.1, 74.0, 73.7, 73.5, 73.3, 73.2, 71.8, 71.3, 71.1, 70.3, 70.1, 69.9, 69.6, 68.5, 67.8, 63.2, 63.0, 62.9, 62.2, 52.0, 51.9 (COOCH_3), 51.7 (*C-2d*), 38.8, 38.7, 27.2, 27.1, 23.3, 22.8, 21.8 (O*Piv*, NHCOCH_3, O-*i*-Pr). Anal. calcd. for $C_{124}H_{141}N_7O_{35}$ (%): C 65.05, H 6.21, N 4.28; found (%): C 64.85, H 6.18, N 4.23.

Isopropyl *O*-(2-azido-3-*O*-benzyl-4,6-di-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-α-L-idopyranosyluronic acid (26)

To a solution of **5** (39 mg, 17.0 μmol) in THF (2 mL) at –5°C, 30% H_2O_2 (0.7 mL) and a 1 M aqueous solution of LiOH (1.1 mL) were added. After stirring for 24 h at room temperature MeOH (1 mL) and a 3 N aqueous solution of KOH (2 mL) were added. After stirring for 24 h more the reaction was neutralized with acidic resin (IRA-120 H^+), filtered, and concentrated. The residue was purified by Sephadex LH-20 (MeOH–CH_2Cl_2, 1:1) to yield **26** (33 mg, 98%). TLC: 0.45 (CH_2Cl_2–MeOH, 8:1). ^{1}H NMR (500 MHz, MeOD) δ: 7.44–7.10 (m, 50 H, *10 Ph*), 5.56 (s, 1H, Ph-*CH*-), 5.37 (b.s., 1H, *H-1c*), 5.05–5.01 (m, 3H, *H-1a*, *H-1b*, *H-1e*), 4.89 (m, 1H, *H-1f*), 4.83 (m, 1H, *H-1d*), 1.48 (m, 3H, NHCOCH_3), 1.17–1.16 (m, 6H, CH(CH_3)$_2$).

Isopropyl *O*-(2-azido-3-*O*-benzyl-4,6-di-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyl)uronic acid hexasodium salt (27)

A mixture of **26** (33 mg, 16.7 μmol) and complex $SO_3\cdot$Py (40 mg, 0.25 mmol) in dry Py (1.5 mL) was stirred under an argon atmosphere. After 24 h, the mixture was cooled and MeOH (1 mL) and CH_2Cl_2 (1 mL) were added. The solution was eluted through a Sephadex LH-20 (MeOH–CH_2Cl_2,1:1). Fractions containing the hexasaccharide were concentrated and passed through a Dowex 50WX4-Na^+ (MeOH–H_2O, 2:1) to yield **27** (28 mg, 72%). TLC: 0.65 (EtOAc–Py–H_2O–AcOH, 8:5:3:1). ^{1}H NMR (500 MHz, MeOD) δ: 7.42–7.10 (m, 50H, 10 *Ph*), 5.56 (s, 1H, Ph-*CH*-), 5.46 (s, 1H, *H-1a* or *e*), 5.32 (s, 1H, *H-1c*), 5.28 (s, 1H, *H-1a* or *e*), 5.15 (d, 1H, *H-1b* or *f*), 5.06 (d, 1H, *H-1b* or *f*), 4.82 (m, 1H, *H-1d*), 1.26 (m, 3H, NHCOCH_3), 1.20 (d, 6H, CH(CH_3)$_2$, J = 6.0 Hz). ^{13}C NMR (125 MHz, MeOD) δ: 101.4 (Ph-*CH*-), 98.8 (*C-1c*), 98.1 (*C-1a* or *e*), 97.5 (*C-1a* or *e*), 96.1 (*C-1b* or *f*), 95.7 (*C-1b* or *f*), 95.5 (*C-1d*).

Isopropyl *O*-(2-deoxy-2-sulfamide-α-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamide-α-D-glucopyranosyl)-(1→4)-2-*O*-sulfo-α-L-idopyranosyluronic acid octasodium salt (3)

To a solution of **27** (17 mg, 7.24 μmol) in MeOH–H_2O (1.5 mL, 9:1) was hydrogenolyzed in the presence of 10% Pd/C. After 24 h, the suspension was filtered and concentrated to give the desired product which was directly used in the next step without further purification.

The hydrogenolyzed hexasaccharide was dissolved in H_2O (1mL) and the pH of the solution was adjusted to 9.5 with 1 N solution of NaOH. Complex SO_3·Py (24 mg, 10 equiv for each amine group) was added in three portions during 1 h and the pH was maintained at 9.5 by subsequent addition of a 1 N solution of NaOH. A second, third, and fourth portion of complex SO_3·Py were added after 2, 4, and 6 h stirring, respectively. After 24 h, the mixture was neutralized with 0.1 N HCl to pH 7 and then passed through a column of Sephadex G-25 with 0.9% NaCl. The appropriate fractions were pooled and passed through a column of Dowex 50WX4-Na^+ with 0.5 M NaCl and then a column of Sephadex G-25 with H_2O–EtOH (9:1). The fractions which contained the final hexasaccharide were lyophilized to give **3** (9.1 mg, 75% from **27**). Before NMR studies, it was useful to make a last elution on a column of Dowex 50WX4-Na^+ to avoid the formation of calcium salts instead of sodium salts and get a better resolution in the spectra. ^{1}H NMR (500 MHz, D_2O) δ: 5.38 (d, 1H, *H-1f*, $J_{1,2}$ = 3.7 Hz), 5.32 (d, 1H, *H-1b*, $J_{1,2}$ = 3.7 Hz), 5.22 (d, 1H, *H-1a*, $J_{1,2}$ = 3.0 Hz), 5.18 (d, 1H, *H-1e*, $J_{1,2}$ = 3.0 Hz), 5.15 (d, 1H, *H-1d*, $J_{1,2}$ = 4.0 Hz), 4.89 (d, 1H, *H-1c*, $J_{1,2}$ = 3.5 Hz), 1.99 (m, 3H, NHCO*CH*$_3$), 1.17 (d, 3H, CH(*CH*$_3$)$_2$, *J* = 6.2 Hz), 1.16 (d, 3H, CH(*CH*$_3$)$_2$, *J* = 6.2 Hz). ^{13}C NMR (125 MHz, D_2O) δ: 102.9 (*C-1c*), 99.8 (*C-1e*), 97.8 (*C-1a*), 97.6 (*C-1b*), 97.3 (*C-1f*), 94.9 (*C-1d*).

2-Azido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α,β-D-glucopyranose trichloroacetimidate (29)

To a solution of **28** (1 g, 2.12 mmol) in dry MeOH (8 mL), solid KOH (119 mg, 2.12 mmol) was added, and was stirred for 20 min. The solution was diluted with CH_2Cl_2 (200 mL) and saturated NH_4Cl solution (2 × 100 mL) and H_2O (2 × 100 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was directly used in the next step without further purification.

To this residue dissolved in Py (10 mL), BzCl (2 mL) was added and stirred at room temperature for 24 h. The reaction was diluted with CH_2Cl_2 (100 mL), washed with H_2O (75 mL) and 1 N HCl solution (75 mL), dried ($MgSO_4$), and concentrated to dryness. The residue was purified by flash chromatography and used in the next step.

To a solution of this residue (1.2 g, 2.04 mmol) in dry Et_2O (10 mL), benzylamine (1.2 mL, 10.9 mmol) was added and was stirred for 4 h. The residue was concentrated to dryness and purified by flash chromatography (hexane–EtOAc, 3:1) and then to a solution of this compound (625 mg, 1.27 mmol) in dry CH_2Cl_2 (7 mL), Cl_3CCN (1.9 mL, 19 mmol) and catalytic DBU were added. After stirring for 1 h the residue was concentrated to dryness. The residue was purified by flash chromatography (hexane–EtOAc (4:1) + 1% Et_3N) to yield **29** (703 mg, 87%) as a mixture of anomers α and β. TLC (α and β): 0.50 and 0.43 (hexane–EtOAc, 3:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.71 (s, 1H, O(C=*NH*)CCl_3 (α)), 8.69 (s, 1H, O(C=*NH*)CCl_3 (β)), 7.29–7.24 (m, 30H, *3 Ph* (α and β)), 6.42 (d, 1H, *H-1* (α), $J_{1,2}$ = 4.1 Hz), 5.65 (d, 1H, *H-1* (β), $J_{1,2}$ = 8.5 Hz), 4.99–4.84 (m, 6H, *6 CH*$_2$Ph (α and β)), 4.65–4.45 (m, 6H, *2 CH*$_2$Ph (α and β), *H-6a* (α and β), *H-6b* (α and β)), 4.17 (m, 1H, *H-5* α), 4.09 (t, 1H, *H-3* (α), $J_{3,2} \approx J_{3,4}$ = 9.5 Hz), 3.80–3.70 (m, 5H, *H-2* (α and β), *H-4* (α and β), *H-5* (β)), 3.61 (t, 1H, *H-3* (β), $J_{3,2} \approx J_{3,4}$ = 9.0 Hz).

Methyl dimethylthexylsilyl 2-*O*-acetyl-4-*O*-(2-azido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-β-L-idopyranuronate (31)

To a solution of **11** (600 g, 0.95 mmol) in dry CH_2Cl_2 (20 mL) at 0°C TMSOTf (8.6 μL, 0.048 mmol) was added under an argon atmosphere. A solution of **29** (720 mg, 1.63 mmol) in dry CH_2Cl_2 (30 mL) was added dropwise for 30 min. The reaction was neutralized with saturated $NaHCO_3$ solution and diluted with CH_2Cl_2 (100 mL). The organic layer was washed with H_2O (75 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography (hexane–EtOAc, 4:1) to yield a mixture of isomers containing **30** which was used directly in the next step.

To a solution of this mixture in Py (10 mL), acetic anhydride (5 mL) was added and was stirred for 24 h. The reaction was diluted with CH_2Cl_2 (100 mL) and washed with H_2O (80 mL) and 1 N HCl solution (80 mL). The organic layer was dried ($MgSO_4$) and concentrated to dryness. The residue was purified by flash chromatography (hexane–EtOAc, 6:1) to yield **31** (391 mg, 43% from **11**). TLC: 0.36 (hexane–EtOAc, 6:1). $[\alpha]_D^{23}$ +76.0° (*c*, 1.0, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99–7.20 (m, 20H, *4 Ph*), 5.06 (d, 1H, *H-1*, $J_{1,2}$ = 1.5 Hz), 4.99 (b.s., 1H, *H-2*), 4.90 (d, 1H, *H-1′*, $J_{1,2}$ = 3.4 Hz), 4.86 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 11.2 Hz), 4.82 (s, 2H, *2 CH*$_2$Ph), 4.74–4.58 (m, 4H, *3 CH*$_2$Ph, *H-6′a*), 4.47 (d, 1H, *H-5*, $J_{5,4}$ = 1.2 Hz), 4.41 (dd, 1H, *H-6′b*, $J_{6b,5}$ = 2.6 Hz, $J_{6b,6a}$ = 12.3 Hz), 4.22 (m, 1H, *H-5′*), 4.11 (b.s., 1H, *H-4*), 3.99–3.95 (m, 2H, *H-3′*, *H-3*), 3.73 (s, 3H, COO*Me*), 3.70 (m, 1H, *H-4′*), 3.28 (dd, 1H, *H-2′*, $J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 10.3 Hz), 2.04 (d, 3H, OCO*CH*$_3$), 1.60 (m, 1H, *CH*(CH$_3$)$_2$), 0.86–0.83 (m, 12H, CH(*CH*$_3$)$_2$, C(*CH*$_3$)$_2$), 0.24 and 0.14 (2s, 6H, Si(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.6, 168.9, 166.1 (*C=O*), 137.8–127.5 (*Ph*), 97.4 (*C-1′*), 93.6 (*C-1*), 80.0 (*C-3′*), 77.9 (*C-4′*), 75.5, 74.9, 74.3, 73.2, 72.9, 72.8, 69.9 (*C-5*), 67.2 (*C-2*), 63.5 (*C-2′*), 62.8 (*C-6*), 52.1 (COO*Me*), 34.1, 24.8, 20.9, 20.2, 19.8 18.6, –2.1, –3.5 (O*TDS*, OCO*CH*$_3$). Anal. calcd. for $C_{51}H_{63}N_3O_{13}Si·0.5H_2O$ (%): C 63.60, H 6.70, N 4.36; found (%): C 63.64, H 6.40, N 4.34.

Methyl dimethylthexylsilyl 4-*O*-(2-acetamido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-acetyl-3-*O*-benzyl-β-L-idopyranuronate (32)

A solution of **31** (375 mg, 0.39 mmol) in thiolacetic acid (6 mL) was stirred for 30 h at room temperature. After concentrating to dryness, the residue was purified by flash chromatography (hexane–EtOAc, 1:1) to yield **32** (259 mg, 68%). TLC: 0.45 (hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ +70.5° (*c*, 1.0, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ: 8.00–7.18 (m, 20H, *4 Ph*), 6.29 (d, 1H, *NH*, $J_{NH,2}$ = 9.8 Hz), 5.06 (b.s., 1H, *H-1*), 4.98 (b.s., 1H, *H-2*), 4.84–4.77 (m, 2H, *H-1′*, *2 CH*$_2$Ph), 4.84–4.77 (m, 5H, *4 CH*$_2$Ph, *H-5*), 4.48 (d, 1H, *H-6′a*, $J_{6a,6b}$ = 12.1 Hz), 4.40 (dd, 1H, *H-6′b*, $J_{6b,6a}$ = 12.1 Hz, $J_{6b,5}$ = 3.4 Hz), 4.33 (m, 1H, *H-2′*), 3.97 (b.s., 1H, *H-4*), 3.93 (b.s., 1H, *H-3*), 3.85 (m, 1H, *H-5′*), 3.78 (s, 3H, COO*Me*), 3.72 (t, 1H, *H-4′*, $J_{4,3} \approx J_{4,5}$ = 9.1 Hz), 3.58 (t, 1H, *H-3′*, $J_{3,2} \approx J_{3,4}$ = 9.6 Hz), 2.00 (d, 3H, OCO*CH*$_3$), 1.79 (d, 3H,

NHCO*CH*$_3$), 1.59 (m, 1H, *CH*(CH$_3$)$_2$), 0.86–0.82 (m, 12H, CH(*CH*$_3$)$_2$, C(*CH*$_3$)$_2$), 0.21 and 0.12 (2 s, 6H, Si(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.0, 169.8, 168.5, 166.2 (*C=O*), 138.1–127.8 (*Ph*), 95.3 (*C-1′*), 93.1 (*C-1*), 81.0 (*C-3′*), 77.6 (*C-4′*), 75.4, 74.9, 73.3, 73.0, 71.4, 70.3, 69.5, 67.9 (*C-2*), 63.0 (*C-6′*), 52.3 (COO*Me*), 52.2 (*C-2′*), 34.0, 24.9, 23.4, 20.8, 20.1, 19.8, 18.6, 13.4, –2.0, –3.5 (O*TDS*, OCO*CH*$_3$).

Methyl 4-*O*-(2-acetamido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-acetyl-3-*O*-benzyl-β-L-idopyranuronate (33)

To a solution of **32** (220 mg, 3.18 mmol) at –15°C in dry THF (7 mL) an excess of (HF)$_n$·Py (0.7 mL) was added. The reaction was warmed to 0°C and stirred for 24 h under an argon atmosphere. The mixture was diluted with CH_2Cl_2 (2 × 100 mL) and washed with H_2O (2 × 75 mL) and saturated $NaHCO_3$ solution until neutral pH. The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2–MeOH, 20:1) to yield **33** (153 mg, 81%) as a mixture of anomers α and β. TLC (α and β): 0.41 and 0.29 (hexane–EtOAc, 1:2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.00–7.18 (m, 40H, *4 Ph* (α and β)), 6.51 (d, 1H, *NH* (β), $J_{NH,2}$ = 9.7 Hz), 6.05 (d, 1H, *NH* (α), $J_{NH,2}$ = 9.7 Hz), 5.25 (b.s., 1H, *H-1* (α)), 5.18 (d, 1H, *H-1* (β), $J_{1,2}$ = 3.0 Hz), 4.96 (d, 1H, *H-2* (β), $J_{1,2}$ = 2.6 Hz), 4.93 (b.s., 1H, *H-2* (β)), 4.84–4.75 (m, 11H, *H-1′* (α and β), *H-5* (β), *4 CH*$_2$Ph (α and β)), 4.64–4.38 (m, 17H, *H-5* (α), *H-6a′* (α and β), *H-6b′* (α and β), *8 CH*$_2$Ph (α and β)), 4.37–4.30 (m, 1H, H-2′ (α and β)), 4.07 (m, 1H, *H-3* (β)), 3.99 (m, 2H, *H-3* (α), *H-4* (α)), 3.80 (s, 3H, COO*Me* (α)), 3.79 (s, 3H, COO*Me* (β)), 3.79–3.66 (m, 4H, *H-4′* (α and β), *H-5′* (α and β)), 3.54 (m, 2H, *H-3′* (α and β)), 1.99 (d, 3H, OCO*CH*$_3$ (β)), 1.95 (d, 3H, OCO*CH*$_3$ (α)), 1.81 (d, 3H, NHCO*CH*$_3$ (β)), 1.75 (d, 3H, NHCO*CH*$_3$ (α)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.4, 170.2, 170.1, 169.8, 169.4, 169.2 (*C=O*), 138.9–127.6 (*Ph*), 95.4 (*C-1′* (α)), 94.2 (*C-1′* (β)), 93.1 (*C-1* (α)), 80.7, 77.6, 77.5, 75.3, 74.9, 73.3, 73.2, 72.9, 70.5, 70.4, 69.6, 69.3, 68.3, 67.6, 63.0, 62.9, 60.4, 52.6, 52.5, 52.3, 23.3, 23.2, 20.8, 20.7 (OCO*CH*$_3$ (α and β), NH*CH*$_3$ (α and β)). Anal. calcd. for $C_{45}H_{49}NO_{14}$·0.5H_2O (%): C 64.58, H 6.02, N 1.67, found (%): C 64.63, H 5.94, N 1.67.

***O*-(Methyl 4-*O*-(2-acetamido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-acetyl-3-*O*-benzyl-α,β-L-idopyranuronate) trichloroacetimidate (34)**

To a solution of **33** (120 mg, 0.15 mmol) in dry CH_2Cl_2 (1.5 mL), Cl_3CCN (0.22 mL, 2.18 mmol) and activated K_2CO_3 (24 mg, 0.17 mmol) were added. After stirring for 3 h the residue was filtered and concentrated to dryness. The residue was purified by flash chromatography (hexane–EtOAc, 1:2) to yield **34** (100 mg, 71%) as a mixture of anomers α and β. TLC (α and β) 0.37 and 0.20 (hexane–EtOAc, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.70 (s, 1H, O(C=*NH*)CCl_3 (α)), 8.67 (s, 1H, O(C=*NH*)CCl_3 (β)), 7.99–7.19 (m, 40H, *4 Ph* (α and β)), 6.36 (d, 1H, *H-1* (α)), 6.22 (d, 1H, *H-1* (β), $J_{1,2}$ = 2.3 Hz), 6.20 (d, 1H, *NH* (β), $J_{NH,2}$ = 9.7 Hz), 5.87 (d, 1H, *NH* (α), $J_{NH,2}$ = 9.7 Hz), 5.25 (m, 1H, *H-2* (β)), 4.99 (b.s., 1H, *H-1* (α)), 5.02 (d, 1H, *H-5* (α), $J_{1,2}$ = 2.0 Hz), 4.87–4.85 (m, 2H, *H-1′* (α), *H-1′* (β)), 4.84–4.57 (m, 13H, *H-5* (β), *6 CH*$_2$Ph (α and β)), 4.54–4.36 (m, 6H, *H-2′* (α), *H-2′* (β), *H-6a′* (α), *H-6a′* (β), *H-6b′* (α), *H-6b′* (β)), 4.17–4.15 (m, 2 H, *H-3* (β), *H-4* (α)), 4.03 (m, 1H, *H-4* (β)), 3.92 (m, 1H, *H-3* (α)), 3.80–3.78 (m, 8H, *H-5′* (α), *H-5′* (β), COO*Me* (α and β)), 3.71 (t, 2H, *H-4′* (α), *H-4′* (β), $J_{4,3} \approx J_{4,5}$ = 8.5 Hz), 3.61–3.54 (m, 2H, *H-3′* (α), *H-3′* (β)), 1.98, 1.96, 1.80, 1.76 (m, 12H, OCO*CH*$_3$ (α), OCO*CH*$_3$ (β), NH*CH*$_3$ (α), NH*CH*$_3$ (β)). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.1, 169.8, 169.6, 169.3, 168.5, 167.7, 166.4, 166.1 (*C=O*), 160.5, 160.1 (*C*=NH), 138.0–127.6 (*Ph*), 96.3 (*C-1′* (α or β)), 95.0 (*C-1* (α)), 94.8 (*C-1′* (α or β)), 94.5 (*C-1* (β)), 80.9 (*C-3′* (α)), 80.8 (*C-3′* (β)), 77.6 (*C-4′* (β)), 77.5 (*C-4′* (α)), 75.5, 75.4, 75.0, 74.9, 73.9, 73.6, 72.5, 71.3, 70.5, 70.4, 69.1, 69.0, 66.9 (*C-2* (α and β)), 66.7, 62.9 (*C-6′* (α and β)), 52.6, 52.5, 52.4, 52.3 (*C-2′* (α and β), COO*Me*), 23.5, 23.3, 20.7, 20.6 (OCO*CH*$_3$ (α), OCO*CH*$_3$ (β), NH*CH*$_3$ (α), NH*CH*$_3$ (β)).

Methyl (isopropyl *O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (35)

To a solution of **5** (175 mg, 0.076 mmol) in dry THF (2 mL), $NaBH_3CN$ (1 mL of a solution of 1 M $NaBH_3CN$ in THF) was added, and was stirred for 24 h. Afterwards, a solution of 2.5 M HCl in Et_2O was added dropwise until the mixture became acidic. The reaction was neutralized with saturated $NaHCO_3$ solution (2 mL), diluted with CH_2Cl_2 (10 mL), and washed with H_2O (7 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (hexane–EtOAc, 3:2) to yield **35** (115 mg, 66%). TLC: 0.51 (hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ –9.8° (*c* 1.1, CH_2Cl_2). MALDI-TOF (*m/z*): 2311 (M + Na^+ – H), 1486 (M + K^+ – H). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.09–7.14 (m, 50H, *10 Ph*), 5.92 (d, 1H, *NH*, $J_{NH,2}$ = 9.7 Hz), 5.31 (d, 1H, *H-1e*, $J_{1,2}$ = 4.6 Hz), 5.28 (d, 1H, *H-1c*, $J_{1,2}$ = 3.4 Hz), 5.17 (d, 1H, *H-1a*, $J_{1,2}$ = 4.5 Hz), 5.05 (d, 1H, *H-1b*, $J_{1,2}$ = 3.5 Hz), 4.97 (d, 1H, *H-1f*, $J_{1,2}$ = 3.4 Hz), 4.95–4.91 (m, 2H, *H-2a*, *H-2e*), 4.85 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 10.9 Hz), 4.82 (d, 1H, *H-1b*, $J_{1,2}$ = 3.3 Hz), 4.76–4.40 (m, 21H, *16 CH*$_2$Ph, *H-5a*, *H-5c*, *H-5e*, *H-6d*, *H-6′d*), 4.33 (d, 1H, *1 CH*$_2$Ph, J_{gem} = 11.3 Hz), 4.29 (m, 1H, *H-2d*), 4.14–4.02 (m, 4H, *H-4a*, *H-4b*, *H-4d*, *H-4e*), 3.96–3.91 (m, 4H, *H-3a*, *H-3e*, *H-4c*, *CH*(CH$_3$)$_2$), 3.85–3.81 (m, 11H, *H-3b*, *H-3c*, *H-3f*, *H-4f*, *H-5b*, *H-5d*, *H-5f*, *H-6b*, *H-6′b*, *H-6f*, *H-6′f*), 3.63 (s, 3H, COO*CH*$_3$), 3.46–3.40 (m, 2H, *H-2c*, *H-3d*), 3.41 (s, 3H, COO*CH*$_3$), 3.32 (s, 3H, COO*CH*$_3$), 3.32–3.30 (m, 1H, *H-2b*), 3.19 (dd, 1H, *H-2f*, $J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 10.0 Hz), 1.43 (s, 3H, NHCO*CH*$_3$), 1.20 (s, 9H, C(*CH*$_3$)$_3$), 1.16 (s, 9H, C(*CH*$_3$)$_3$), 1.22–1.14 (m, 6H, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 177.2, 170.0, 169.8, 169.3, 169.2, 166.1 (*C=O*), 138.2–127.2 (*Ph*), 98.9 (*C-1e*), 98.4 (*C-1b*), 98.3 (*C-1f*), 97.8 (*C-1c*), 97.3 (*C-1a*), 97.0 (*C-1d*), 79.3, 78.6, 78.1, 76.3, 75.9, 75.1, 74.9, 74.8, 74.5, 74.2, 74.0, 73.7, 73.6, 73.4, 73.3, 73.2, 72.4, 72.3, 71.8, 71.3, 70.8, 70.6, 70.4, 70.1, 69.5, 69.4, 67.8,

63.0, 62.7, 62.3, 60.4, 51.9 (*C-2d*), 51.7 (COO*CH*$_3$), 51.7 (*C-2d*), 38.8, 27.2, 27.1, 23.3, 22.8, 21.8, 21.0 (O*Piv*, NHCO*CH*$_3$, O-*i*-Pr). Anal. calcd. for $C_{124}H_{143}N_7O_{35}\cdot 1.5H_2O$ (%): C 64.23, H 6.34, N 4.23; found (%): C 64.32, H 6.64, N 4.29.

Methyl (isopropyl *O*-(2-acetamido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-acetamido-6-*O*-benzoyl-3-*O*-benzyl -2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (36)

To a solution of **35** (130 mg, 56.7 μmol) and **34** (103 mg, 106 μmol) in dry CH_2Cl_2 (0.7 mL) at room temperature, TMSOTf (55 μL of a 0.22 M solution of TMSOTf in CH_2Cl_2) was added under an argon atmosphere. After stirring for 3 h, saturated $NaHCO_3$ solution (0.2 mL) was added and diluted with CH_2Cl_2 (20 mL). The residue was washed with H_2O (15 mL) and then the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL) and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (hexane–EtOAc, 1:2) to yield **36** (60 mg, 40%) and nonreacting acceptor **35** (70 mg, 54%). TLC: 0.40 (hexane–EtOAc, 1:2). $[\alpha]_D^{23}$ +33.3° (*c* 1.0, CH_2Cl_2). MALDI-TOF (*m/z*): 3119 (M + Na^+), 3135 (M + K^+). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.08–7.12 (m, 70H, *14 Ph*), 5.99 (d, 1H, *NH h*, $J_{NH,2}$ = 9.6 Hz), 5.90 (d, 1H, *NH d*, $J_{NH,2}$ = 9.4 Hz), 5.31 (d, 1H, *H-1e*, $J_{1,2}$ = 4.8 Hz), 5.29 (d, 1H, *H-1c*, $J_{1,2}$ = 3.1 Hz), 5.17 (d, 1H, *H-1a*, $J_{1,2}$ = 4.2 Hz), 5.12 (d, 1H, *H-1g*, $J_{1,2}$ = 3.2 Hz), 5.04 (d, 1H, *H-1b*, $J_{1,2}$ = 3.0 Hz), 4.96 (d, 1H, *H-1f*, $J_{1,2}$ = 3.2 Hz), 4.83 (m, 1H, *H-1h*), 4.80 (m, 1H, *H-1d*), 4.96–4.90 (m, 2H, *H-2a*, *H-2e*), 4.90–4.23 (m, 33H, *24 CH*$_2$Ph, *H-2g*, *H-5a*, *H-5c*, *H-5e*, *H-5g*, *H-6d*, *H-6′d*, *H-6h*, *H-6′h*), 4.36–4.23 (m, 2H, *H-2d*, *H-2h*), 4.12 (t, 1H, *H-4a*, $J_{4,3} \approx J_{4,5}$ = 5.2 Hz), 4.09–3.86 (m, 10H, *H-3a*, *H-3e*, *H-3g*, *H-4b*, *H-4c*, *H-4d*, *H-4e*, *H-4g*, *H-5h*, *CH*$(CH_3)_2$), 3.85–3.64 (m, 10H, *H-3b*, *H-3c*, *H-4f*, *H-4h*, *H-5b*, *H-5d*, *H-6b*, *H-6′b*, *H-6f*, *H-6′f*), 3.62 (s, 3H, COO*CH*$_3$), 3.60–3.49 (m, 3H, *H-3f*, *H-3h*, *H-5f*), 3.46–3.36 (m, 2H, *H-2c*, *H-3d*), 3.41 (s, 6H, *2* COO*CH*$_3$), 3.34–3.21 (m, 2H, *H-2b*, *H-2f*), 3.24 (s, 3H, COO*CH*$_3$), 1.83 (s, 3H, OCO*CH*$_3$), 1.61 (s, 3H, NHCO*CH*$_3$), 1.42 (s, 3H, NHCO*CH*$_3$), 1.20–1.12 (s, 24H, *2* C(*CH*$_3$)$_3$, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.1, 170.0, 169.4, 169.3, 169.2, 168.8, 166.2, 166.1, 166.0 (*C=O*), 138.0–127.2 (*Ph*), 99.2 (*C-1c*), 98.6 (*C-1b*), 98.6 (*C-1f*), 98.1 (*C-1e*), 97.8 (*C-1g*), 97.6 (*C-1a*), 97.5 (*C-1d*), 97.1 (*C-1h*), 80.8, 78.6, 78.0, 77.3, 77.0, 76.8, 75.3, 75.0, 74.8, 74.6, 74.4, 74.2, 74.0, 73.8, 73.7, 73.4, 73.2, 72.4, 71.8, 71.5, 71.3, 70.8, 70.5, 70.4, 69.7, 63.2, 63.0, 62.6, 52.4, 52.0, 51.6, 51.7 (*C-2d*), 38.8, 29.7, 27.2, 27.1, 23.3, 23.1, 22.8, 21.8, 20.7 (O*Piv*, NHCO*CH*$_3$, O-*i*-Pr). Anal. calcd. for $C_{169}H_{192}N_8O_{49}\cdot H_2O$ (%): C 65.07, H 6.20, N 3.59; found (%): C 65.08, H 6.44, N 3.57.

Methyl (isopropyl *O*-(2-acetamido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (37)

To a solution of **19** (460 mg, 0.307 mmol) in dry MeOH and CH_2Cl_2 (1:1, 4 mL), MeONa (56 μL of a 0.178 M solution of MeONa in MeOH) was added and stirred for 40 h. The reaction was neutralized with resin (IRA-120 H^+) and filtered. The residue was purified by flash chromatography (hexane–EtOAc, 1:2) to yield **37** (61 mg, 80%). TLC: 0.45 (hexane–EtOAc, 1:2). $[\alpha]_D^{23}$ +34.2° (*c* 1.0, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.07–7.12 (m, 70H, *14 Ph*), 6.19 (d, 1H, *NH h*, $J_{NH,2}$ = 8.6 Hz), 5.96 (d, 1H, *NH d*, $J_{NH,2}$ = 9.8 Hz), 5.29 (d, 1H, *H-1c*, $J_{1,2}$ = 3.1 Hz), 5.25 (d, 1H, *H-1e*, $J_{1,2}$ = 4.3 Hz), 5.17 (d, 1H, *H-1a*, $J_{1,2}$ = 4.5 Hz), 5.04 (m, 2H, *H-1g*, *H-1b*), 4.98 (d, 1H, *H-1f*, $J_{1,2}$ = 3.3 Hz), 4.94–4.90 (m, 2H, *H-2a*, *H-2e*), 4.86 (d, 1H, *H-1h*, $J_{1,2}$ = 3.1 Hz), 4.79 (m, 1H, *H-1d*), 4.88–4.38 (m, 31H, *23 CH*$_2$Ph, *H-5a*, *H-5c*, *H-5e*, *H-5g*, *H-6d*, *H-6′d*, *H-6h*, *H-6′h*), 4.31–4.26 (m, 2H, *H-2d*, *1 CH*$_2$Ph), 4.19 (m, 1H, *H-2h*), 4.11 (t, 1H, *H-4a*, $J_{4,3} \approx J_{4,5}$ = 5.6 Hz), 4.07–3.91 (m, 9H, *H-3a*, *H-3e*, *H-4b*, *H-4c*, *H-4d*, *H-4e*, *H-4g*, *H-4h*, *CH*$(CH_3)_2$), 3.84–3.64 (m, 11H, *H-3b*, *H-3c*, *H-3g*, *H-4f*, *H-5b*, *H-5d*, *H-5h*, *H-6b*, *H-6′b*, *H-6f*, *H-6′f*), 3.61 (s, 3H, COO*CH*$_3$), 3.58–3.52 (m, 4H, *H-2g*, *H-3f*, *H-3h*, *H-5f*), 3.44–3.39 (m, 2H, *H-2c*, *H-3d*), 3.38 (s, 3H, COO*CH*$_3$), 3.33 (s, 3H, COO*CH*$_3$), 3.32–3.27 (m, 2H, *H-2b*, *H-2f*), 3.18 (s, 3H, COO*CH*$_3$), 2.45 (d, 1H, *OH-2g*, $J_{OH,2}$ = 8.3 Hz), 1.65 (s, 3H, NHCO*CH*$_3$), 1.41 (s, 3H, NHCO*CH*$_3$), 1.19–1.13 (s, 24H, *2* C(*CH*$_3$)$_3$, CH(*CH*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.5, 170.1, 169.9, 169.2, 166.2 (*C=O*), 138.1–127.1 (*Ph*), 100.9 (*C-1g*), 99.1 (*C-1c*), 98.8 (*C-1b*), 98.7 (*C-1f*), 98.1 (*C-1e*), 97.6 (*C-1a*), 97.4 (*C-1d*), 97.2 (*C-1h*), 80.0, 78.7, 78.2, 78.0, 77.3, 77.1, 76.8, 75.9, 75.3, 74.9, 74.8, 74.6, 74.0, 73.7, 73.5, 73.3, 73.2, 72.8, 71.8, 71.3, 70.6, 70.5, 70.2, 70.1, 69.9, 68.2, 67.7, 63.3, 62.9, 62.6, 52.5, 52.1, 52.0, 51.9, 51.6, 38.8, 29.7, 27.2, 27.1, 23.3, 22.9, 22.8, 21.8 (O*Piv*, NHCO*CH*$_3$, O-*i*-Pr).

Methyl (isopropyl *O*-(2-acetamido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosyl) uronate (38)

To a solution of **37** (55 mg, 0.018 mmol) and freshly prepared Ag_2O (10.2 mg, 0.044 mmol) in dry DMF (0.1 mL), freshly distilled benzyl bromide (24 μL, 0.2 mmol) was added. After stirring for 24 h, the mixture was filtered

through Celite and new Ag_2O (10.2 mg, 0.044 mmol) and benzyl bromide (24 μL, 0.2 mmol) were added. After stirring for 24 h more, the mixture was filtered through Celite and concentrated to dryness. The residue was purified by flash chromatography (hexane–EtOAc, 1:1) to yield **38** (41 mg, 72%). TLC: 0.28 (hexane–EtOAc, 1:1). $[\alpha]_D^{23}$ +30.5° (*c* 1.0, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.07–7.09 (m, 75H, *15 Ph*), 6.05 (d, 1H, *NH d*, $J_{NH,2}$ = 9.8 Hz), 5.88 (d, 1H, *NH h*, $J_{NH,2}$ = 9.5 Hz), 5.30 (d, 1H, *H-1c*, $J_{1,2}$ = 2.8 Hz), 5.27 (d, 1H, *H-1e*, $J_{1,2}$ = 3.7 Hz), 5.24 (d, 1H, *H-1g*, $J_{1,2}$ = 1.1 Hz), 5.17 (m, 1H, *H-1a*, $J_{1,2}$ = 4.5 Hz), 5.05 (m, 1H, *H-1b*, $J_{1,2}$ = 3.2 Hz), 4.97 (d, 1H, *H-1f*, $J_{1,2}$ = 3.2 Hz), 4.95–4.91 (m, 2H, *H-2a*, *H-2e*), 4.86–4.38 (m, 35H, *25 CH*$_2$Ph, *H-1d*, *H-1h*, *H-5a*, *H-5c*, *H-5e*, *H-5g*, *H-6d*, *H-6′d*, *H-6h*, *H-6′h*), 4.36–4.26 (m, 3H, *H-2d*, *H-2h*, *1 CH*$_2$Ph), 4.12 (t, 1H, *H-4a*, $J_{4,3} \approx J_{4,5}$ = 4.8 Hz), 4.10–3.91 (m, 9H, *H-3a*, *H-3e*, *H-4b*, *H-4c*, *H-4e*, *H-4f*, *H-4g*, *H-4h*, *CH*$(CH_3)_2$), 3.85–3.66 (m, 12H, *H-3b*, *H-3c*, *H-3g*, *H-4d*, *H-4f*, *H-5b*, *H-5d*, *H-5f*, *H-5h*, *H-6b*, *H-6f*, *H-6′f*), 3.62 (s, 3H, COO*CH*$_3$), 3.61–3.49 (m, 3H, *H-3d*, *H-3f*, *H-6′b*), 3.45–3.41 (m, 2H, *H-2c*, *H-3h*), 3.44 (s, 3H, COO*CH*$_3$), 3.41 (s, 3H, COO*CH*$_3$), 3.32–3.27 (m, 2H, *H-2b*, *H-2f*), 3.18 (s, 3H, COO*CH*$_3$), 1.49 (s, 3H, NHCO*CH*$_3$), 1.41 (s, 3H, NHCO*CH*$_3$), 1.19–1.13 (m, 24H, *2* C$(CH_3)_3$, CH$(CH_3)_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.3, 170.1, 170.1, 169.8, 169.2, 166.2, 166.1 (*C=O*), 138.1–127.1 (*Ph*), 99.1 (*C-1c*), 98.7 (*C-1b*, *C-1f*, *C-1g*), 98.2 (*C-1e*), 97.6 (*C-1a*, *C-1d*), 97.4 (*C-1h*), 78.3, 78.0, 77.3, 77.0, 76.8, 76.3, 75.4, 74.9, 74.5, 74.0, 73.9, 73.7, 73.3, 73.2, 73.1, 73.0, 72.2, 71.8, 71.3, 70.8, 70.3, 70.1, 67.8, 63.2, 62.8, 52.5, 51.9, 51.65, 38.8, 29.7, 27.2, 27.1, 23.3, 22.9, 22.8, 21.8 (O*Piv*, NHCO*CH*$_3$, O-*i*-Pr).

Isopropyl *O*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-α-L-idopyranosyluronic acid (39)

To a solution of **38** (39 mg, 12.4 μmol) in THF (2 mL) at –5°C, H_2O_2 30% (0.7 mL) and a 1 M aqueous solution of LiOH (0.8 mL) were added. After stirring for 24 h at room temperature MeOH (0.8 mL) and a 3 N aqueous solution of KOH (2 mL) were added. After stirring for 24 h more, the reaction was neutralized with acidic resin (IRA-120 H^+), filtered, and concentrated. The residue was purified by Sephadex LH-20 (MeOH–CH_2Cl_2, 1:1) to yield **39** (32 mg, 94%). TLC: 0.47 (CH_2Cl_2–MeOH, 8:1). ^{1}H NMR (500 MHz, CD_3OD) δ: 7.37–7.06 (m, 65H, *13 Ph*), 5.30–5.19 (m, 3H, *H-1b*, *H-1f*, *H-1a* or *e*), 5.06 (s, 1H, *H-1a* or *e*), 5.00 (d, 1H, *H-1c* or *g*, $J_{1,2}$ = 3.4 Hz), 4.98 (d, 1H, *H-1c* or *g*, $J_{1,2}$ = 3.2 Hz), 4.79 (m, 2H, *H-1d*, *H-1h*), 1.61 (s, 6H, *2* NHCO*CH*$_3$), 1.30–1.15 (m, 6H, CH$(CH_3)_2$). ^{13}C NMR (125 MHz, CD_3OD) δ: 99.7 (*C-1a* or *e*), 98.2 (*C-1b* or *f*), 97.6 (*C-1a* or *e*), 97.3 (*C-1b* or *f*), 96.6, 96.4 (*C-1d*, *C-1h*), 95.1 (*C-1c*, *C-1g*).

Isopropyl *O*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyluronic acid octasodium salt (40)

A mixture of **39** (32 mg, 11.4 μmol) and complex SO_3·Py (37 mg, 0.23 mmol) in dry Py (1.5 mL) was stirred under an argon atmosphere. After 24 h, the mixture was cooled and MeOH (1 mL) and CH_2Cl_2 (1 mL) were added. The solution was eluted through a Sephadex LH-20 (MeOH–CH_2Cl_2, 1:1). Fractions containing the hexasaccharide were concentrated and passed through a Dowex 50WX4-Na^+ (MeOH–H_2O, 2:1). Finally, the product was purified by reverse phase HPLC to yield **40** (23 mg, 64%). TLC: 0.62 (EtOAc–Py–H_2O–AcOH, 8:5:3:1). ^{1}H NMR (500 MHz, CD_3OD) δ: 7.42–7.00 (m, 65H, *13 Ph*), 5.45–5.15 (5H, *H-1a*, *H-1b*, *H-1e*, *H-1f*, *H-1c* or *g*), 5.05–4.70 (3H, *H-1d*, *H-1h*, *H-1c* or *g*), 1.56 (m, 3H, NHCO*CH*$_3$), 1.46 (m, 3H, NHCO*CH*$_3$), 1.18 (d, 6H, CH*(CH*$_3$*)*$_2$, *J* = 6.0 Hz).

Isopropyl *O*-(2-acetamido-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamide-α-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamide-α-D-glucopyranosyl)-(1→4)-2-*O*-sulfo-α-L-idopyranosyluronic acid decasodium salt (4)

To a solution of **40** (23 mg, 7.15 μmol) in MeOH–H_2O (1.5 mL, 9:1) was hydrogenolyzed in the presence of 10% Pd/C. After 24 h, the suspension was filtered and concentrated to give the desired product which was directly used in the next step without further purification.

The hydrogenolyzed hexasaccharide was dissolved in H_2O (1 mL) and the pH of the solution was adjusted to 9.5 with a 1 N solution of NaOH. Complex SO_3·Py (24 mg, 10 equiv for each amine group) was added in three portions over 1 h and the pH was maintained at 9.5 by subsequent addition of a 1 N solution of NaOH. A second, third, and fourth portion of complex SO_3·Py were added after 2, 4, and 6 h stirring, respectively. After 24 h, the mixture was neutralized with a 0.1 N solution of HCl to pH 7 and then chromatographed on a column of Sephadex G-25 with a 0.9% solution of NaCl. The appropriate fractions were pooled and passed through a column of Dowex 50WX4-Na^+ with a 0.5 M solution of NaCl and then a column of Sephadex G-25 with H_2O–EtOH (9:1). The fractions which contained the final hexasaccharide were lyophilized to give **4** (10.4 mg, 66% from **40**). Before NMR studies, it was useful to make a last elution on a column of Dowex 50WX4-Na^+ to avoid the formation of calcium salts instead of sodium salts and get a better resolution in the spectra. ^{1}H NMR (500 MHz, D_2O) δ: 5.38 (d, 1H, *H-1f*, $J_{1,2}$ = 3.3 Hz), 5.30 (d, 1H, *H-1b*, $J_{1,2}$ = 3.3 Hz), 5.23 (d, 1H, *H-1a*, $J_{1,2}$ = 1.8 Hz), 5.19 (d, 1H, *H-1e*, $J_{1,2}$ = 2.7 Hz), 5.16 (d, 1H, *H-1h*, $J_{1,2}$ = 3.8 Hz), 5.14 (d, 1H, *H-1d*, $J_{1,2}$ = 3.3 Hz), 4.88–4.86 (m, 2H, *H-1c*, *H-1g*), 1.99

(m, 3H, NHCOCH_3), 1.98 (m, 3H, NHCOCH_3), 1.18–1.16 (m, 6H, CH(CH_3)$_2$). ^{13}C NMR (125 MHz, MeOD) δ: 101.1 (*C-1c*, *C-1g*), 98.2 (*C-1e*), 97.1 (*C-1b*), 97.0 (*C-1a*), 96.8 (*C-1f*), 94.4 (*C-1d*), 94.3 (*C-1h*).

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