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Synthesis and structural study of two new heparin-like hexasaccharides

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Two new heparin-like hexasaccharides, **5** and **6**, have been synthesised using a convergent block strategy and their solution conformations have been determined by NMR spectroscopy and molecular modelling. Both hexasaccharides contain the basic structural motif of the regular region of heparin but with negative charge distributions which have been designed to get insight into the mechanism of fibroblast growth factors (FGFs) activation.

Introduction

The family of fibroblast growth factors (FGFs) presently comprises more than twenty signalling polypeptides which play a significant role in important biological functions such as cell proliferation, differentiation and angiogenesis.[1] These functions are initiated after binding of FGFs to specific receptors (FGFRs) at the cell surface.[2] FGF-1 and FGF-2 are the prototypical members of the family. FGFs and FGFRs are heparin binding proteins[3,4] and, therefore, the FGF system is tightly regulated by heparan sulfate glycosaminoglycans (HS-GAGs).[5] In structural terms HS is recognised as a family of closely related linear polysaccharides (heparin is just a member) consisting of alternating units of D-glucuronic or L-iduronic acid and D-glucosamine. These units may be unsulfated and variously sulfated at specific positions (typically position 2 of the uronic acid units and *N* and 6 of the glucosamine units) and appear distributed in different domains along the polysaccharide chain.[5] The overall conformation of these biomolecules has been reported to be a well defined helical structure in which the heterogeneity of sequences and patterns of sulfation result in a diversity of charge distributions and orientations.[6] The charge orientation is further modulated by the flexibility of the internal L-iduronate units, which may adopt the ${}^{1}C_{4}$ or the ${}^{2}S_{O}$ conformation.[6,7] Since the binding of these molecules to FGFs and FGFRs is thought to be primarily electrostatic in nature, the diversity of charge distributions and orientations is believed to be related to the specificity of their interactions with the diverse and complex FGF system.[2,8] This complexity and diversity have been held responsible for apparently conflicting evidence.[2] It is generally agreed that the availability of homogeneous oligosaccharides with precisely defined structure may contribute a key step in deciphering the structural and biological consequences of this diversity.[2] These homogeneous oligosaccharides can be obtained by synthesis.[9,10] To gain insight into the biological process using these synthetic molecules, their solution conformation has to be carefully investigated in order to determine the orientation of the negative charges.[6] Having this information, binding and biological studies with these synthetic oligosaccharides may provide a rigorous insight into the molecular basis of the HS-GAGs mediated regulation of the FGF system.

In the framework of a project on the activation of FGFs we are presently using the above approach. We have previously synthesised hexasaccharides **1** and **3** and octasaccharides **2** and **4**.[11,12] Compounds **1** and **2** contain the sequence (GlcNα1 → 4IdoA) and the charge distribution of the regular region of heparin.[5] Compounds **3** and **4**, with the same disaccharide sequence, have a different charge distribution and their syntheses were designed for the final products to display the sulfate groups only on one side of the expected helical structures (Fig. 1). We have also investigated the solution conformation of these molecules using NMR spectroscopy and molecular dynamics simulations which confirmed the predicted helix-like three dimensional structures.[11,12] These compounds have shown different behaviour in inducing the mitogenic activity of FGF-1,[11,13] which clearly indicates the importance of size and charge distribution in the regulation of the FGF system and strongly suggest that a previously proposed GAG induced FGF dimerisation[14] is not an absolute requirement for biological activity.

We report in this paper the synthesis of two new hexasaccharides, **5** and **6**, and a NMR study of their solution conformation. Compounds **5** and **6** have the same sequence as **1**–**4** but their charge distribution has been designed to gain structural and biological information on the importance of sulfation at positions 2 of the L-iduronate units and 6 of the D-glucosamine residues. With the same number of negative charges, compounds **5** and **6** would present different three dimensional charge distributions which would also be different from those of **1** and **3**. The *N* and 2-*O*-sulfo groups have been reported to be essential for binding to FGF-2[15] whereas binding to FGF-1 also required the presence of 6-*O*-sulfo groups.[16] Binding to FGFRs seems to require *N*, 2 and 6 sulfation as well.[17] Fig. 1 schematically shows the different charge distribution and orientation of hexasaccharides **1** and **3** and those expected for **5** and **6**.

Results and discussion

For the synthesis of **1**–**4** we previously developed a convergent modular approach from key disaccharide structures operating as glycosyl donors and as glycosyl acceptors. These disaccharide modules were endowed with protective group patterns permitting stereochemical control during the building block assembly process and allowing subsequent sulfation at the required positions.[11,12] The synthesis of **5** and **6** has now been carried out similarly thus validating the general design of the synthetic approach and contributing further knowledge towards a much needed automation of these processes. The retrosynthetic analysis is shown in Scheme 1. A common hexasaccharide precursor (**14**) is prepared by stereoselective assembly of three modular structures that will constitute the reducing end (**11**) the inner region (**12**) and the nonreducing end (**13**) of the hexasaccharide skeleton. The sequence of

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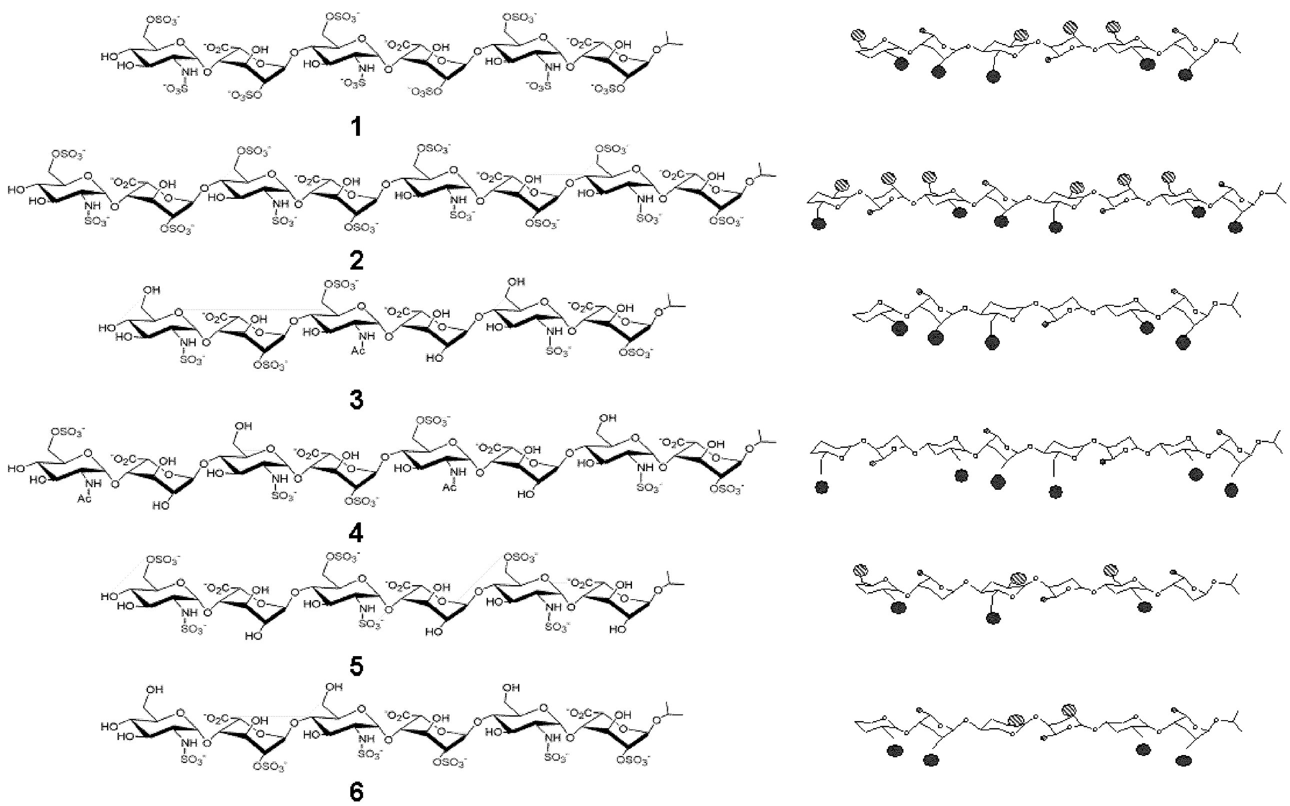


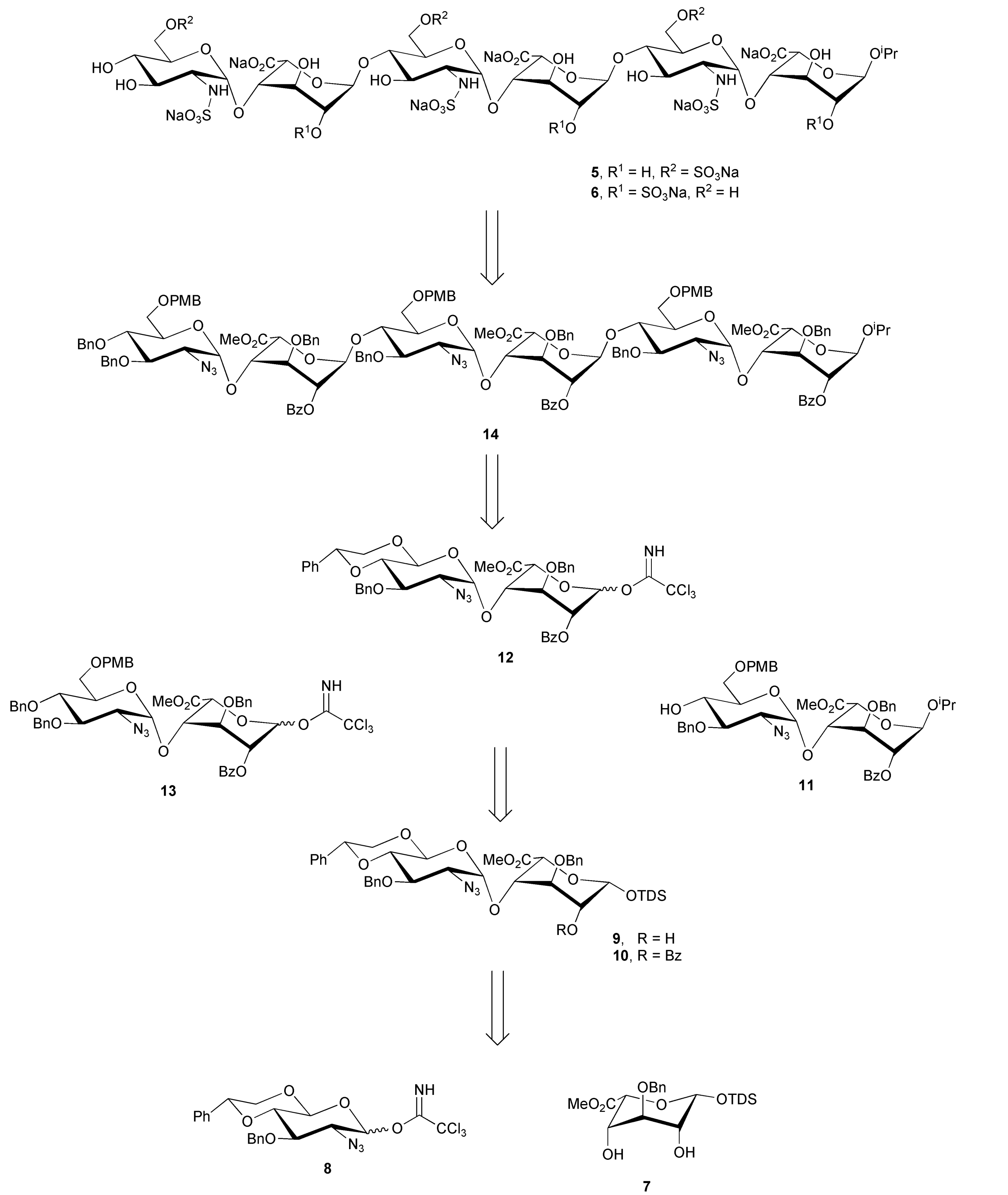
Fig. 1 Oligosaccharides **1**–**6**. Formulae and schematic representation, small and large circles indicate carboxylate and sulfate groups respectively, plain or shadowed filling indicate opposite spatial orientations.

deprotection steps preceding sulfation is a crucial aspect of the synthesis allowing the preparation of either **5** or **6** from the same common intermediate (**14**). The reducing end building block (**11**) appears as an isopropyl glycoside as, according to previous experience,[11,12,18,19] this grouping provides a convenient structural environment for the subsequent structural and binding studies of the final products. Also in agreement with extensive previous experience,[11,12] the stereoselective assembly of **11**, **12**, and **13**, which is also a crucial step for the success of the synthesis, was designed to be performed by the trichloroacetimidate glycosylation procedure.[20] These three building blocks are also prepared from a common precursor (**10**) which derives from the key disaccharide **9** which is in turn synthesised[11] from the monosaccharide derivatives **7** and **8** obtained in multigram quantities from diacetoneglucose and D-glucosamine hydrochloride respectively. For some time we have been preparing the L-iduronic acid diol (**7**) in reasonable amounts from D-glucuronolactone.[21] However, the synthesis of **7** reported by Bonnaffé *et al.*[22] has obvious advantages over ours[21] and this procedure has been used in the present work. Trichloroacetimidate **8**[23] is prepared in a straightforward manner by a sequence involving a diazo transfer reaction[24] and benzylidenation.[25] This sequence is being routinely used by us[11,12] to prepare a variety of starting materials and a number of different intermediates are currently in stock in our laboratory.

We have previously reported[11] that reaction of diol **7**[21,22] with trichloroacetimidate **8**[23] under carefully controlled experimental conditions results in the regio- and stereoselective glycosylation to give disaccharide **9**.[11] This key disaccharide has been the basic structure from which all the successful HS-GAG oligosaccharide syntheses so far performed by us[11,12] have been developed. The synthesis of the inner region and the reducing end building blocks starting from disaccharide **9** is shown in Scheme 2. Conventional benzoylation of **9** gave **10** in 95% yield. Removal of the silyl group in **10** with $(HF)_n$·Py complex[26] afforded **15** in 80% yield. Activation of the anomeric position of **15** as a trichloroacetimidate in the usual conditions[20] gave the inner region building block (**12**) in almost quantitative yield. This has been used as starting material for the preparation of the reducing end building block (**11**) as well. Reaction of **12** with isopropyl alcohol gave **16** in 70% yield. The sequence **16**⟶**17**⟶**18** leading to the reducing end building block (**11**) formally consists of a transacetalation reaction. For practical reasons, primarily based on the availability of starting benzylidene acetal derivatives and the synthetic potentiality of **17** in the modular synthesis of other GAG oligosaccharides, this route to **18**, rather than direct *p*-methoxybenzylidenation of the starting monosaccharide building block, was preferred. Treatment of **16** with EtSH and BF_3OEt_2[27] afforded diol **17** in almost quantitative yield. Compound **17** was reacted with *p*-methoxybenzaldehyde dimethyl acetal in the presence of TSOH to give **18** in 85% yield. Regioselective reductive opening of the the *p*-methoxybenzylidene ring in **18** using $NaBH_3CN$ and THF in DMF[28] yielded the reducing end building block **11** (69%).

Scheme 2 also shows the synthesis of the non reducing end building block **13**. Reductive opening of the benzylidene ring in **10** using BH_3NHMe_2in the presence of BF_3OEt_2[29] gave **19** in 80% yield. Treatment of **19** with *p*-methoxybenzyl trichloroacetimidate at −20° gave **20** in almost quantitative yield. Disaccharide **13** was obtained after removal of the silyl group[26] in **20** to give **21** (83%) and anomeric activation in the usual conditions.[20]

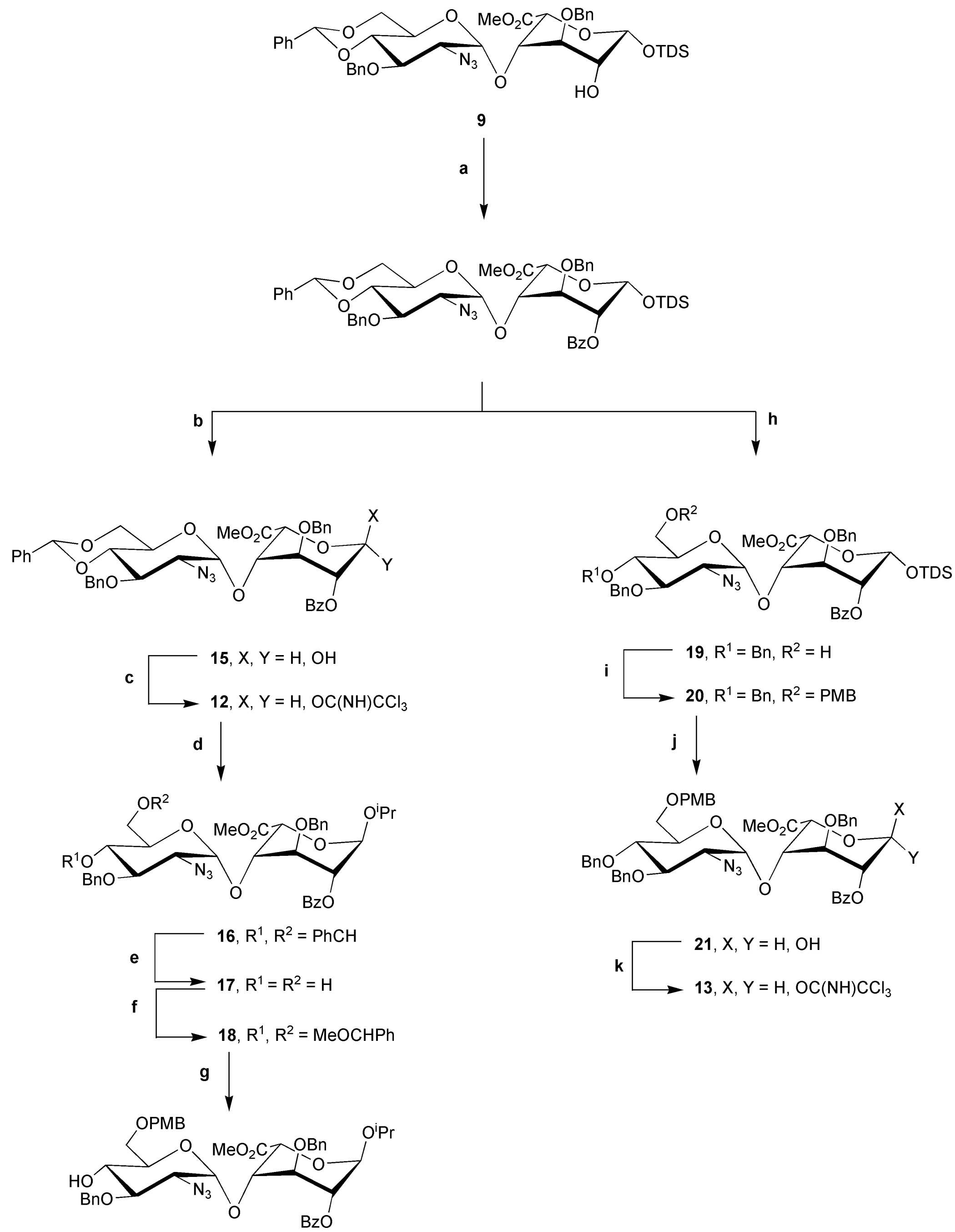
The assembly of these building blocks involved first the glycosylation of the reducing end disaccharide acceptor **11** with the inner region disaccharide donor **12** in dichloromethane at −20 °C. (Scheme 3). In these experimental conditions the donor was reactive enough and the acceptor sufficiently stable as to afford tetrasaccharide **22** in 85% yield. The reaction was highly stereoselective with the configuration of the newly formed glycosidic linkage being exclusively α as indicated by the ^{1}H NMR spectrum. This tetrasaccharide derivative was then transformed into glycosyl acceptor **25** following the sequence **22**⟶**23**⟶**24**⟶**25**. Also in this case the route involving removal of the benzylidene acetal group[27] in **22** to give diol **23** (85%), acetalation of **23** with *p*-methoxybenzaldehyde dimethyl acetal



Scheme 1 Retrosynthesis of **5** and **6**.

to give **24** in almost quantitative yield and stereoselective reductive opening[28] to **25** (52%) was preferred in spite of the moderate yield of the last step. The removal of the benzylidene group in **22** with EtSH[27] should now be carried out in the presence of CSA. The crude reaction mixture after reductive opening (**24**⟶**25**) permits the recovery of 40% unreacted **24**. Glycosylation of **25** with the non reducing end building block **13** in the conditions of the above discussed glycosylation step gave the hexasaccharide derivative **14** in 79% yield with excellent stereoselectivity. The protective group pattern in **14** permits access to the two target hexasaccharides depending on the deprotection sequence as presented in the next paragraph. As in our previous syntheses of oligosaccharides **1**–**4** using a similar strategy, this modular approach may permit also the construction of larger conveniently protected oligosaccharide skeletons allowing access to a variety of oligosaccharides of different sizes, charge distributions and orientations. The plethora of existing protecting groups,[30] the accessibility of building blocks from **7**, **8** and derivatives thereof and the effectiveness[11,12] of the trichloroacetimidate procedure[20] with regard to yield and selectivity in the building block assembly, provide a promising platform for the development of solid phase synthesis and automation. This would contribute a key step in the elucidation of the molecular basis of these GAG regulated biological processes.

For the synthesis of **5**, removal of the *p*-methoxybenzyl groups in **14** was the first step. This was achieved using 10% TFA[31] to give **26** in almost quantitative yield. Compound **26**

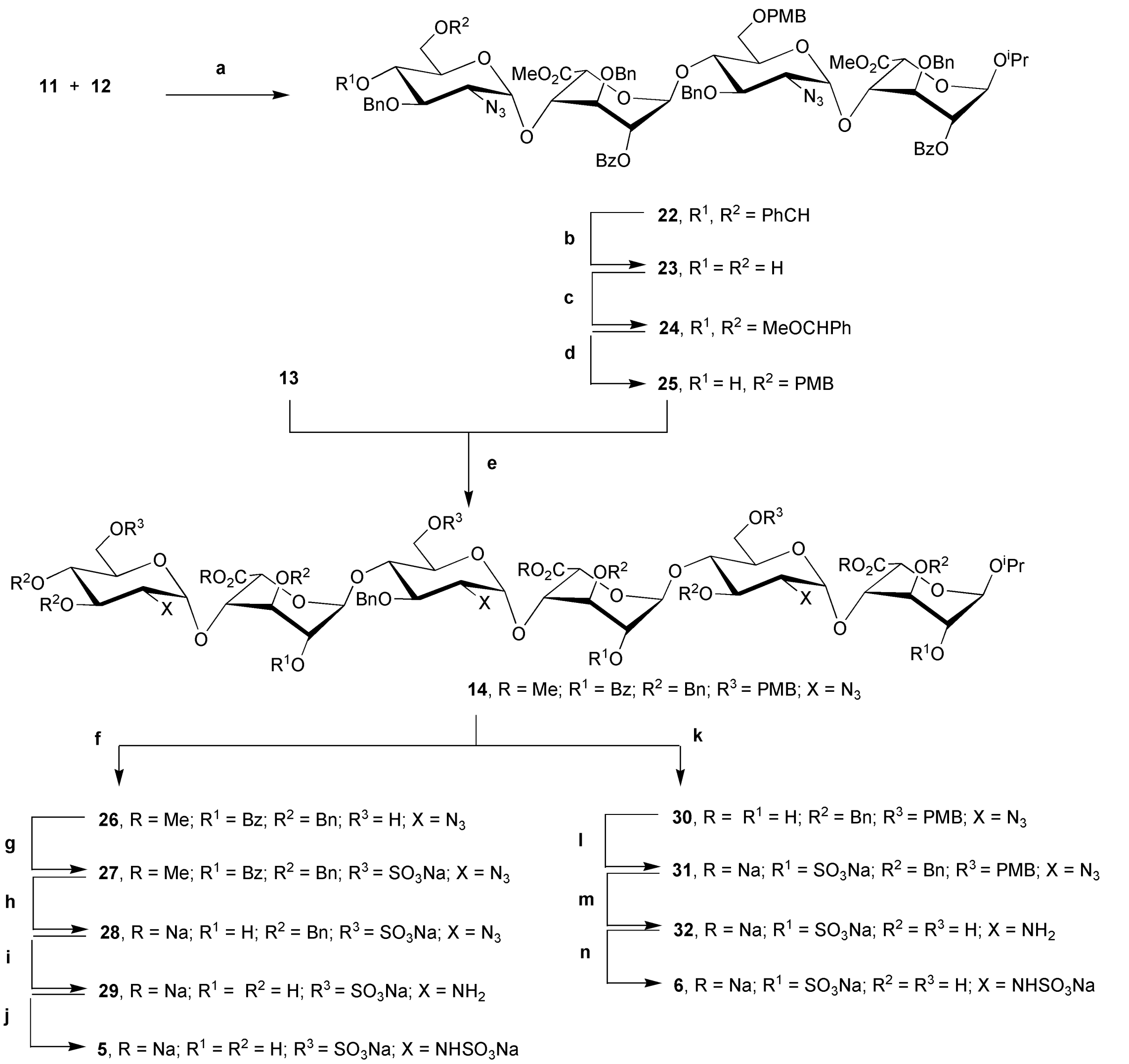


Scheme 2 Synthesis of building blocks. *Reagents and conditions:* a) BzCl, Py, 95%; b) $(HF)_n$Py, THF, −15 °C, 80%; c) K_2CO_3, Cl_3CCN, CH_2Cl_2, 70%; d) ^{i}PrOH, TMSOTf, CH_2Cl_2, 70%; e) EtSH, PTSA, CH_2Cl_2, 95%; f) *p*-MeOPhCH$(OMe)_2$, PTSA, DMF, 85%; g) $NaBH_3CN$, TFA, DMF, 69%; h) BH_3NHMe_2, BF_3OEt_2, CH_2Cl_2, −15 °C, 81%; i) $PMBOC(NH)CCl_3$, BF_3OEt_2, CH_2Cl_2, 0 °C, 96%; j) $(HF)_n$Py, THF, −4 °C, 83%; k) K_2CO_3, Cl_3CCN, CH_2Cl_2, 88%.

was treated with the SO_3·Py complex in pyridine[32] to give **27** in 98% yield. The removal of the benzoyl and methoxycarbonyl groups in **27** was then performed with LiOOH and then KOH in order to minimise elimination.[33] Compound **28**, thus obtained in quantitative yield, was then hydrogenated in the presence of 10% Pd/C to yield **29** that was immediately submitted to *N*-sulfation with the SO_3·Py complex in water at a constant pH value of 9.5. Hexasaccharide **5** was purified by gel permeation and ion exchange chromatography as reported for other synthetic heparin derived oligosaccharides.[34,35] For the preparation of **6** the ester groups in **14** were first removed as above to give **30** in almost 90% yield. *O*-Sulfation afforded **31** which was hydrogenated to **32** and this *N*-sulfated to give **6** whcih was purified as described for **5**.

The solution three-dimensional structures of **5** and **6** have been studied to determine the precise orientation of the negative charges as compared with **1**–**4** in order to correlate this charge distribution to binding and biological activity data. The key structural features, conformational equilibria of the L-iduronate units and geometry and flexibility of the glycosidic linkages, can be conveniently described by NMR spectroscopy parameters.[6,36] The ^{1}H and ^{13}C NMR spectra of **5** and **6** were assigned using standard two-dimensional techniques by identifying the spin systems and the interresidue NOEs as previously reported.[11,12] The spectra of both molecules showed considerable overlapping as a result of the identical substitution patterns of the repeating units. The values of chemical shifts (Table 1) were as expected for heparin derived oligosaccharides with those patterns of sulfation.[37]

The coupling constants (Table 2), which permitted establishment of the $^1C_4$–^2S_O conformational equilibria of the L-iduronate units[7,36,38,39] (Fig. 2), were measured from the 2D dqf-COSY cross peaks by recursive deconvolution in the frequency domain. The presence of the ^2S_O conformation was also



Scheme 3 Assembly of building blocks. *Reagents and conditions:* a) TMSOTf, CH_2Cl_2, −20 °C, 85%; b) EtSH, CSA, CH_2Cl_2, 85%; c) *p*-MeOPhCH(OMe)$_2$, CSA, CH_2Cl_2, 95%; d) $NaBH_3CN$, TFA, DMF, 52%; e) TMSOTf, CH_2Cl_2, −20 °C, 79%; f) 10% TFA, CH_2Cl_2, 96%; g) SO_3Py, Py, Dowex 50WX4 (Na+), 98%; h) H_2O_2, LiOH aq., THF, KOH aq., MeOH, 98%; i) 10% Pd/C, H_2, 9 : 1 MeOH–H_2O; j) SO_3Py, H_2O, pH 9.5, 73%; k) H_2O_2, LiOH aq., THF, 0 °C, KOH aq., MeOH, 5 °C, Dowex 50WX4 (Na+), 87%; l) SO_3Py, Py, Dowex 50WX4 (Na+), 86%; m) 10% Pd/C, H_2, 9 : 1 MeOH–H_2O; n) SO_3Py, H_2O, pH 9.5, 73%.

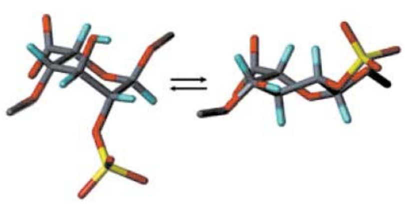


Fig. 2 Iduronate conformational equilibrium.

indicated by the H2–H5 exclusive NOE. As previously observed for compounds **1**–**4**, no significant contribution of the $^4C_1$ form was observed for the L-iduronate units at the reducing end thus confirming the suitability of this isopropyl glycoside structure for structural and binding studies.[11,12] The conformer populations in the conformational equilibria were quantified by least squares fitting of the experimental $^3J_{H,H}$ values with those calculated for the canonical forms (Table 3). The calculated 75 : 25 $^1C_4$–^2S_O conformational distribution indicated that, within the experimental error, the sulfation pattern and the L-iduronate position along the saccharide chain did not significantly influence in this case the conformational populations. This is in agreement with previous results described for heparin and heparan sulfate fragment analogues to **5** and **6**.[7,38,39]

The overall three dimensional structure of both hexasaccharides was determined from the observed interresidue NOE patterns. These, which were similar to those observed for compounds **1**–**4**[11,12] and heparin fragments,[40–42] were entirely compatible with an overall helical secondary structure. This is dictated by *syn*-ψ type glycosidic conformations characterised by H1′–H4, H1′–H3 and H5′–H4 exclusive NOEs around the GlcN–IdoA glycosidic linkage and by H1′–H4 and H1′–H6 *proR* exclusive NOEs around the IdoA–GlcN bonds[40] (Table 4). The absence of H5′–H3 and H1′–H5 NOEs for the GlcN–IdoA and the IdoA–GlcN linkages respectively permitted us to discard any contributions from arrangements of the *anti*-ψ type.

The already reported strong anisotropic hydrodynamic behaviour shown by heparin fragments longer than pentasaccharide,[43] which prevents the straightforward quantification of NOE data, was also observed for **5** and **6**. We have also described a similar behaviour for the synthetic oligosaccharides **1**–**4**[11,12] where, as for **5** and **6**, different relative intensities of the H1–H2 and H2–H4 NOEs were observed for similar H1–H2 (2.4 Å) and H2–H4 (2.5 Å) distances of the GlcN units.[12,43] The orthogonal H1–H2 and H2–H4 vectors have in this series different sensitivity to parallel and perpendicular correlation time which is a characteristic feature of prolate ellipsoid rigid molecules. This is the result of combining a linear molecular shape with a significant rigidity of the glycosidic linkages which are not usual in carbohydrate molecules other than GAGs.[36,44]

This structural study was complemented with molecular modelling data. Calculations were performed using, as in the

Table 1 Proton and carbon chemical shift in ppm for hexasaccharide **5** and **6** at 25 °C

		5		**6**	
a	1	4.94	101.4	5.23	99.5
	2	3.56	71.9	4.16	78.3
	3	4.07	70.9	4.19	70.6
	4	4.00	77.6	4.00	78.2
	5	4.50	71.1	4.51	70.5
b	1	5.34	98.1	5.31	99.4
	2	3.23	60.2	3.21	60.6
	3	3.65	72.2	3.69	71.9
	4	3.71	79.8	3.68	79.8
	5	3.98	71.4	3.85	73.5
	6	4.31[a]	68.7	3.86	62.0
	6′	4.18[b]		3.84	
c	1	5.00	104.5	5.25	101.4
	2	3.77	70.9	4.32	76.7
	3	4.10	70.0	4.22	69.9
	4	4.03	76.8	4.01	78.0
	5	4.80	70.9	4.85	70.5
d	1	5.31	98.1	5.27	99.7
	2	3.22	60.2	3.22	60.5
	3	3.64	72.2	3.69	71.9
	4	3.72	79.8	3.68	79.8
	5	3.99	71.1	3.85	73.5
	6	4.32[a]	68.7	3.86	62.0
	6′	4.18[b]		3.84	
e	1	4.99	104.5	5.24	101.5
	2	3.76	70.8	4.31	76.7
	3	4.08	70.0	4.22	69.9
	4	4.04	76.8	4.02	77.9
	5	4.79	70.9	4.84	70.5
f	1	5.33	98.1	5.28	99.7
	2	3.19	60.2	3.20	60.5
	3	3.61	73.6	3.64	73.4
	4	3.54	71.5	3.44	72.3
	5	3.91	72.2	3.80	74.1
	6	4.34[a]	68.7	3.80	62.7
	6′	4.14[b]		3.76	

[a] *pro-R*. [b] *pro-S*.

Table 2 Iduronate residues endocyclic $^3J_{HH}$ (Hz) observed for **5** and **6** and calculated for canonical $^1C_4$, ^2S_O, and $^4C_1$ structures for iduronate and 2-*O*-sulfoiduronate

Residue		$^3J_{1,2}$	$^3J_{2,3}$	$^3J_{3,4}$	$^3J_{4,5}$
IdoA-a	**5**	3.2	5.1	3.9	2.9
	6	2.5	4.8	3.6	2.8
IdoA-c	**5**	2.1	5.4[a]	4.2	2.5
	6	3.6	4.3	4.5	3.7
IdoA-e	**5**	2.1	5.4[a]	4.2	2.5
	6	3.6	4.2	3.6	3.5
IdoA·2OSO$_3$	$^1C_4$	2.1	3.0	2.9	1.0
	$^4C_1$	7.9	10.0	9.9	4.5
	^2S_O	6.9	10.4	6.8	3.0
IdoA	$^1C_4$	2.2	3.1	3.0	1.0
	$^4C_1$	7.9	10.0	9.9	4.4
	^2S_O	7.1	10.4	6.6	3.4

[a] Averaged due to overlapping.

case of **1**[11] and **3**,[12] the AMBER force field[45] with the GLYCAM_93 modification for carbohydrates[46] and specific parameters for the sulfate and sulfamate groups.[47] The energetic landscape of IdoA–GlcN and GlcN–IdoA glycosidic linkages was extensively explored resulting in a densely populated *syn*-ψ central region possibly with several accessible local subminima.[40,41] The alternative *anti*-ψ arrangement was clearly unfavourable. Models for **1**, **3**, **5** and **6** were constructed, with

Table 3 Population of $^1C_4$, and ^2S_O iduronate conformers estimated from the experimental $^3J_{HH}$ values and fitting error

	IdoA-a			IdoA-c			IdoA-e		
	$^1C_4$	^2S_O	χ^2	$^1C_4$	^2S_O	χ^2	$^1C_4$	^2S_O	χ^2
5	72	28	0.8	75	25	1.4	75	25	1.4
6	78	22	1.2	71	29	2.7	76	24	2.3

Table 4 Observed interglycosidic NOE for hexasaccharides **5** and **6**

Glycosidic linkage	**5**	**6**
f–e	H1′–H3	H1′–H3
	H1′–H4	H1′–H4
	H5′–H4[a]	H5′–H4[a]
e–d	H1′–H4	H1′–H4
	H1′–H6*proR*	H1′–H6*proR*
	H2′–H6*proR*	H2′–H6*proR*
	H1′–H6*proS*[a]	
	H5′–H3[a]	
d–c	H1′–H3	H1′–H3
	H1′–H4	H1′–H4
		H5′–H4
c–b	H1′–H4	H1′–H4
	H1′–H6*proR*	H1′–H6*proR*
	H2′–H6*proR*	H2′–H6*proR*
	H1′–H6*proS*[a]	
	H2′–H6*proS*[a]	
	H5′–H3[a]	
b–a	H1′–H3	H1′–H3
	H1′–H4	H1′–H4
		H5′–H4

[a] Weak NOE peaks.

the L-iduronate units both in the $^1C_4$ and in the ^2S_O conformation, based on the previous heparin model.[40] The resulting eight structures were subjected to several cycles of molecular dynamics runs and energy minimisation in the presence of the adequate number of counterions and including explicit water molecules in the calculations. The final structures (Fig. 3) maintained the helical structure and their backbone could be superimposed with that of heparin fragments.[40] The interprotonic distances were in all cases compatible with the interglycosidic NOEs.

Conclusions

In conclusion, compounds **5** and **6** present three dimensional structures and dynamics as compounds **1** and **3**[11,12] and all of them are structural models of GAGs which reproduce heparin basic structural features. The different negative charge distribution and orientation in these molecules result in a different biological behaviour that will be reported in due course.

Experimental

General procedures

Thin layer chromatography (TLC) analyses were performed on silica gel F_{254} precoated on aluminum plates (Merk). The compounds were detected by staining with sulfuric acid–ethanol (1 : 9) or anisaldehyde solution (25 : 25 : 450 : 1 anisaldehyde–sulfuric acid–ethanol–acetic acid) followed by heating at over 200 °C. Column chromatography was carried out on silica gel 60 (0.2–0.5 mm, 0.2–0.063 mm, or 0.040–0.015 mm; Merck) and distilled solvents were used. All solvent and reagents used in the synthesis were purified and dried according to standard procedures. Optical rotations were determined at room temperature in a 1 dm cell on a Perkin-Elmer 341 polarimeter. ^1H and ^{13}C NMR spectra were acquired on a Bruker DRX-500 spectrometer and chemical shifts are given in ppm relative to

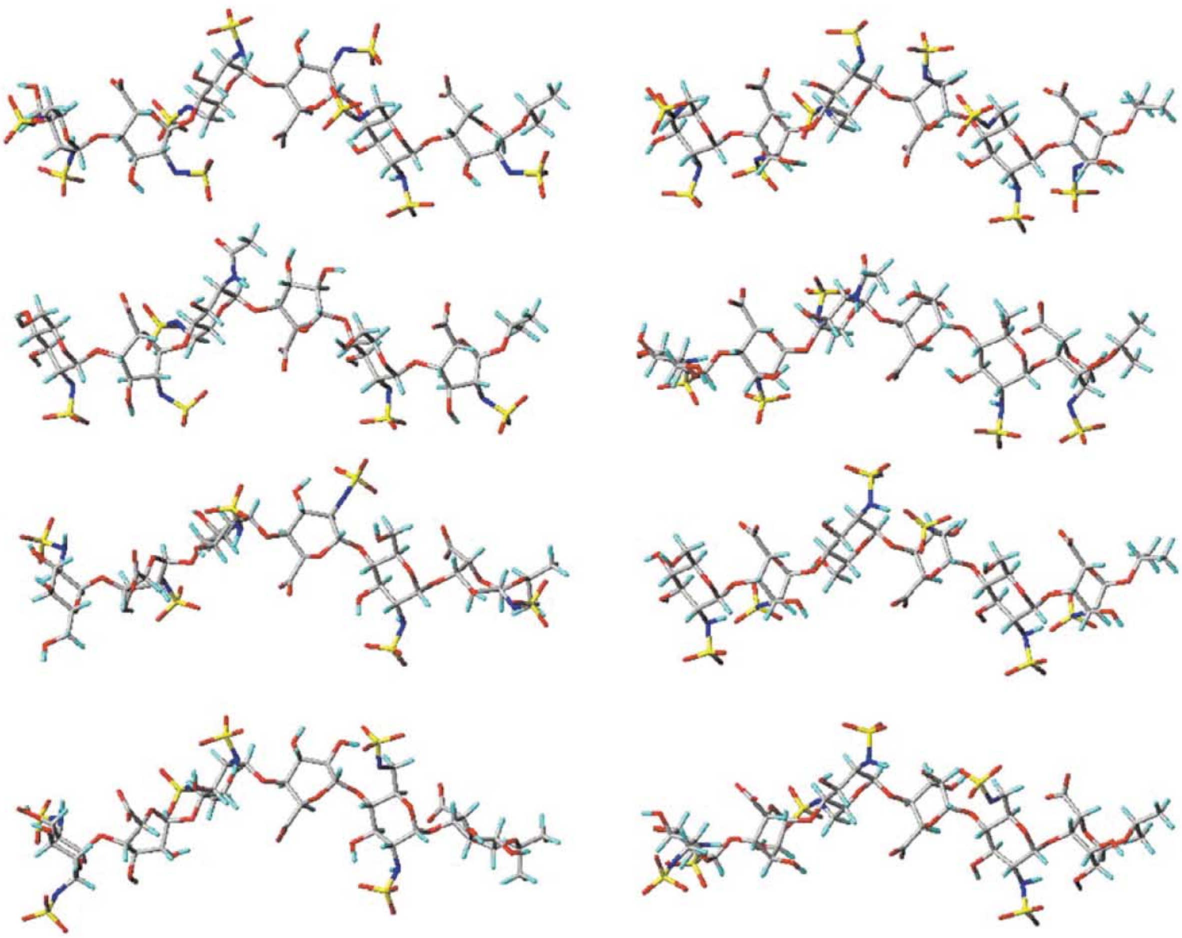


Fig. 3 Relaxed structures for hexasaccharides **1**, **3**, **5** and **6** corresponding to all iduronate residues in $^1C_4$ conformation (left) and ^2S_O one (right).

TMS. Elemental analyses were performed with a Leco CHNS-932 apparatus, after drying analytical samples over phosphorous pentoxide for 24 h. MALDI-TOF mass spectra were recorded with a MALDI-TOF GSG System spectrometer. Samples of the intermediate products were dissolved in EtOAc or MeOH at mM concentrations and 2,5-dihydroxybenzoic acid was used as the matrix. Gel filtration chromatography (Sephadex LH-20 and G-25; Pharmacia) and ion-exchange chromatography (Dowex 50WX4 Na^+; Fluka) were used in order to achieve purification of the final products.

NMR measurements

1D and 2D experiments with **5** and **6** were recorded in D_2O at 298 K on Bruker AVANCE 800 and 500 MHz instruments. The sample concentration was nearly 5mM and the pH* was adjusted to 7.0. Chemical shifts are in ppm with respect to the proton signal of the tetramethylsilane and the calibration has been made using the manufacturers software from the nucleus base frequency and the corresponding frequency ratio. DQF-COSY,[48,49] TOCSY,[50] NOESY,[51] and HSQC[52] 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. Data were transformed into phase-sensitive modes after zero filling and weighting with shifted square sine-bell functions, incrementing the number of experiments in the indirect dimension of the heteronuclear experiments by linear prediction according to the manufacturers software.

Molecular modelling

Molecular modelling was performed using AMBER force field[25] (parameter set "parm91") as integrated in the AMBER 6.0 program,[53] modified for carbohydrate molecules by the GLYCAM_93 parameter set.[46] Specific parameters for sulfate and sulfamate groups were also included, as described by Altona and Huige[47] Calculations were carried out using periodic boundary conditions with TIP3P water molecules. The initial structure of the hexasaccharides **5** and **6** were built manually using the ϕ/ψ values found for a minimised structure of the regular heparin-like synthetic hexasaccharide **1**.[11] The relaxed structures were neutralised by adding sodium atoms using "addions" as implemented in xleap from AMBER 5.0 according to the solute electrostatic potential and further radially displaced allowing appropriate solvation in the next step. Finally, water TIP3P type molecules were added. The resulting systems have the following characteristics: 3724 water molecules in a box whose dimensions were 50 Å × 50 Å × 51 Å for the **5** $^1C_4$ model; 3689 water molecules in a box whose dimensions were 50 Å × 50 Å × 51 Å for the ^2S_O model for **5**; 3759 water molecules in a box whose dimensions were 50 Å × 52 Å × 49 Å for the **6** $^1C_4$ model and 3833 water molecules in a box whose dimensions were 51 Å × 51 Å × 51 Å for the ^2S_O model for **6**. All these boxes are 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. The initial water configuration was subjected to 1000 cycles of energy minimization, with the conformation of the sugar and counterions frozen (5000 Kcal mol^{-1} $Å^{-1}$). Following this step, a 25 ps volume constant MD simulation was performed, in which only the water molecules were allowed to move. After this pre-equilibration of the solvent all the further steps were carried out using Particle Mesh Ewald (PME) electrostatic treatment with 1 Å grid and cubic B-spline interpolation. The initial velocities were assigned using a Maxwellian distribution at the corresponding temperature.

Using these conditions the protocol continues with a 25 ps molecular dynamics run with the solute atoms restrained by 500 Kcal mol^{-1} $Å^{-1}$. This restrain was weakened to 25 Kcal mol^{-1} $Å^{-1}$ during a 1000 cycles of relaxation and 3 ps of molecular dynamics. The obtained structures were subjected to five consecutive runs of 600 cycles of minimisation with the positional restrains 20, 15, 10, 5 and 0 Kcal mol^{-1} $Å^{-1}$ respectively.

Methyl (dimethyltexylsilyl-4-*O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-2-*O*-benzoyl-β-idopyranosyl) uronate (10). To a solution of **9**[11] (3.66 g, 4.54 mmol) in Py (38 mL) benzoyl chloride (2.63 mL, 22 mmol) and catalytic DMAP were added at 0 °C. The mixture was stirred at room temperature for 18 h and then diluted with CH_2Cl_2 (200 mL), and washed with water and with 1 M HCl (100 mL). The aqueous phases were washed with CH_2Cl_2 (2 × 100 mL) and the organic phases dried on Mg_2SO_4 and concentrated. The residue was purified by column chromatography (25 : 1 toluene–EtOAc) to give pure **10** (3.92 g., 95%). R_f 0.20 (25 : 1 toluene–EtOAc). $[a]_D^{20}$ −18.1 (*c* 1.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.15–7.08 (m, 20 H, *Ph*); 5.52 (s, 1 H, Ph–C*H*); 5.20 (br s, 1 H, H_1); 5.10 (br s, 1 H, H_2); 4.85 (d, 1 H, J_{gem} = 12.0 Hz, CH_2Ph); 4.76 (d, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 4.72 (d, 1 H, $J_{1',2'}$ = 3.5 Hz, $H_{1'}$); 4.49 (br s, 1 H, H_5); 4.40 (dd, 1 H, $J_{6'a,6'b}$ = 10.0 Hz, $J_{6'a,5'}$ = 5.0 Hz, $H_{6'a}$); 4.24 (m, 1 H, H_3); 4.20 (m, 2 H, CH_2Ph, $H_{5'}$); 4.07 (br s, 1 H, H_4); 3.77–3.68 (m, 4 H, CH_2Ph, COOCH_3); 3.60 (t, 1 H, $J_{3',2'}$ = $J_{3',4'}$ = 9.5 Hz, $H_{3'}$); 3.58–3.49 (m, 2 H, $H_{6'a}$, $H_{4'}$); 3.11 (dd, 1 H, $J_{2',1'}$ = 3.5 Hz, $J_{2',3'}$ = 9.5 Hz, $H_{2'}$); 1.57 (m, 1 H, C*H*$(CH_3)_2$); 0.82–0.77 (m, 12 H, CH(CH_3)$_2$, C(CH_3)$_2$); 0.31–0.15 (2 s, 6 H, Si(CH_3)$_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.5, 168.9 (*C*=O); 138.1–126.3 (*Ph*); 101.3 (Ph–*C*H–); 99.8 ($C_{1'}$); 94.2 (C_1); 82.4 ($C_{4'}$); 76.3 ($C_{3'}$); 75.3 (C_4); 75.0 (C_3); 74.7 (*C*H_2Ph); 73.4 (C_5); 73.1 (*C*H_2Ph); 68.7 (C_2); 68.4 ($C_{6'}$); 63.4 (*C*H_2Ph); 63.0 ($C_{2'}$); 52.2 (COO*C*H_3); 20.2, 20.0, 18.6, 18.5, 18.6, 14.3, −1.8, −3.4 (O*TDS*). MALDI-TOF *m*/*z* 932.1 (M + Na^+), 948.1 (M + K^+). Anal: calcd for: $C_{49}H_{59}N_3O_{12}Si\cdot 1H_2O$: C, 64.66; H, 6.53; N, 4.61; found: C, 64.70; H, 6.05; N, 3.88%.

Methyl 4-*O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-α,β-L-idopyranosyluronate (15). To a solution of **10** (1.75 g, 1.92 mmol) in dry THF (40 mL) an excess of $(HF)_n\cdot$Py (5.0 mL) was added at −10 °C. The reaction was warmed to 0 °C and stirred for 48 h under an argon atmosphere. The mixture was diluted with CH_2Cl_2 (2 × 100 mL) and washed with H_2O (2 × 100 mL) and saturated $NaHCO_3$ solution until neutral pH. The aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL) and the organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (2 : 1 hexane–EtOAc), to yield **15** (1.18 g, 80%) as a mixture of anomers α–β. TLC 0.24 (2 : 1 hexane–EtOAc). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.20–7.05 (m, 40 H, *Ph* α and β); 5.51 (m, 3 H, Ph–C*H*– α and β, H_1α); 5.24 (d, 1 H, $J_{1,OH}$ = 10.0 Hz, H_1 β); 5.08 (s, 2 H, H_2 α and β); 4.98 (d, 2 H, $J_{5,4}$ = 2.0 Hz, H_5 α); 4.92–4.75 (m, 4 H, CH_2Ph α and β); 4.66–4.58 (m, 3 H, $H_{1'}$ α CH_2Ph, $H_{1'}$ β); 4.40–4.25 (m, 6 H, H_3, $H_{6'a}$, CH_2Ph, OH) α; 4.05 (s, 2 H, H_4 α and β); 4.00–3.89 (m, 3 H, $H_{5'}$ α and β, CH_2Ph); 3.84–3.77 (m, 8 H, CH_2Ph, O*H* β, COOCH_3 α and β); 3.66–3.59 (m, 3 H, $H_{6'b}$ α and β, CH_2Ph); 3.52–3.45 (m, 4 H, $H_{3'}$, $H_{4'}$ α and β); 3.22 (m, 2 H, $H_{2'}$ α and β). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 169.4, 168.7, 165.9, 165.6 (*C*=O); 137.8–127.9 (*Ph*); 101.3 (Ph–*C*H–); 100.4 ($C_{1'}$ α); 100.3 ($C_{1'}$ β); 93.9 (C_1 α); 92.6 (C_1 β); 82.3 ($C_{4'}$ α); 82.2 ($C_{4'}$ β); 76.9, 76.7 ($C_{3'}$α and β); 76.1, 75.8 (*C*H_2Ph); 74.7 and 74.6 (C_4 β and α); 75.1 (*C*H_2Ph); 73.5 (C_3 α and β); 73.0, 72.9 (*C*H_2Ph); 68.7, 68.5, 67.4 (C_2 α and β, $C_{6'}$ α and β); 66.9 (C_5 α and β); 63.5, 63.5, 63.2 ($C_{5'}$, $C_{2'}$ α and β); 52.5–52.4 (COO*C*H_3 α and β). MALDI-TOF *m*/*z* 790 (M + Na^+); 806 (M + K^+). Anal. calcd. for $C_{41}H_{41}N_3O_{12}$: C 64.13; H, 5.38; N, 5.47; found C, 63.96; H, 5.21; N, 5.76%.

***O*-(Methyl 4-*O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α,β-L-ido-pyranosyluronate) trichloroacetimidate (12).** To a solution of **15** (600 mg, 0.78 mmol) in dry CH_2Cl_2 (6 mL), CCl_3CN (1.17 mL, 11.7 mmol) and activated K_2CO_3 (108.5 mg, 0.82 mmol) were added. After stirring for 3 h the residue was filtered and concentrated to dryness. The residue was purified by flash chromatography (3 : 1 hexane–EtOAc), to yield **12** (707 mg, 95%), as a mixture of anomers α–β. TLC α–β, 0.2–0.36 (3 : 1 hexane–EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$), β anomer; δ 8.68 (s, 1 H, *NH*); 8.20–7.08 (m, 20 H, *Ph*); 6.58 (s, 1 H, H_1); 5.52 (s, 1 H, Ph–C*H*–); 5.34 (s, 1 H, H_2); 5.03 (s, 1 H, H_5); 4.93 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.76 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.73 (d, 1 H, $J_{1',2'}$ = 3.5 Hz, $H_{1'}$); 4.37–4.34 (m, 2 H, CH_2Ph, $H_{6a'}$); 4.25 (s, 1 H, H_3); 4.18 (s, 1 H, H_4); 4.00–3.94 (m, 1 H, $H_{5'}$); 3.84 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 3.78 (s, 3 H, COOCH_3); 3.62 (t, 1 H, $J_{3',2'}$ = $J_{3',4'}$ = 9.5 Hz, $H_{3'}$); 3.58–3.45 (m, 2 H, $H_{6'b}$, $H_{4'}$); 3.22 (dd, 1 H, $J_{2',1'}$ = 3.5 Hz, $J_{2',3'}$ = 9.5 Hz, $H_{2'}$); α anomer; δ 8.66 (s, 1 H, *NH*); 8.20–7.10 (m, 20 H, *Ph*); 6.32 (s, 1 H, H_1); 5.50 (s, 1 H, Ph–C*H*–); 5.42 (s, 1 H, $J_{2,1}$ = 2.0 Hz, H_2); 4.90 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.78 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.71 (d, 1 H, $J_{5,4}$ = 1.5 Hz, H_5); 4.69 (d, 1 H, $J_{1',2'}$ = 3.5 Hz, $H_{1'}$); 4.38 (t, 1 H, $J_{3,4} \approx J_{3,2}$ = 3 Hz, H_3); 4.33 (dd, 1 H, $J_{6'a,5'}$ = 5.0 Hz, $J_{6'a,6'b}$ = 10.0 Hz, $H_{6'a}$); 4.18 (s, 1 H, H_4); 4.03 (m, 1 H, $H_{5'}$); 3.78 (d, 4 H, CH_2Ph, COOCH_3); 3.61 (t, 1 H, $J_{4',3'}$ = $J_{4',5'}$ = 9.5 Hz, $H_{4'}$); 3.54–3.49 (m, 2 H, $H_{6'b}$, $H_{3'}$); 3.20 (dd, 1 H, $J_{2',1'}$ = 3.5 Hz, $J_{2',3'}$ = 9.5 Hz, $H_{2'}$). Anal. calcd. for $C_{43}H_{41}N_4O_{12}Cl_3$: C, 56.61; H, 4.53; N, 6.14; found: C, 56.24; H, 4.51; N, 6.12%.

Methyl (isopropyl 4-*O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-α-L-idopyranosyl) uronate (16). To a cooled (0 °C) solution of **12** (2.7 g, 2.95 mmol) in dry CH_2Cl_2 (30 mL) was added isopropyl alcohol (0.56 mL, 7.39 mmol) and TMSOTf (25.7 μL, 88.7 μmol). After 15 min, the mixture was neutralised with Et_3N (1 mL) and concentrated *in vacuo*. The residue was purified by flash chromatography (6 : 1 hexane–EtOAc) to yield the α anomer **16** (1.57 g, 70%) and the β anomer (235 mg, 11%): $[a]_D^{20}$ +22.5 (*c* 1.1, $CHCl_3$); TLC 0.21 (3 : 1 hexane–EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.15–7.1 (m, 20 H, *Ph*); 5.50 (s, 1 H, Ph–C*H*–); 5.30 (s, 1 H, H_1); 5.10 (s, 1 H, H_2); 4.93 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.90 (d, 1 H, $J_{1',2'}$ = 4.0 Hz, $H_{1'}$); 4.76 (d, 1 H, $J_{5,4}$ = 4.0 Hz, H_5); 4.72 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.41 (d, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 4.33 (dd, 1 H, $J_{6'a,5'}$ = 5.0 Hz, $J_{6'a,6'b}$ = 10.0 Hz, $H_{6a'}$); 4.14 (t, 1 H, $J_{3,4}$ = $J_{3,2}$ = 9.5 Hz, H_3); 4.09 (s, 1 H, H_4); 4.03–3.95 (m, 2 H, $H_{5'}$, C*H*$(CH_3)_2$); 3.97 (d, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 3.75 (s, 3 H, COOCH_3); 3.65–3.60 (m, 2 H, $H_{3'}$, $H_{6'b}$); 3.54 (t, 1 H, $J_{4',3'}$ = $J_{4',5'}$ = 9.5 Hz, $H_{4'}$); 3.21 (dd, 1 H, $J_{2',1'}$ = 4.0 Hz, $J_{2',3'}$ = 10.0 Hz, $H_{2'}$); 1.24–1.18 (d, 6 H, CH(CH_3)$_2$).^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.1, 165.9 (*C*=O); 138.1–126.4 (*Ph*); 101.7 (Ph–*C*H–); 100.1 (C_5); 97.7 (C_1); 82.7 ($C_{4'}$); 76.8 ($C_{3'}$); 76.3 (C_4); 75.0 (*C*H_2Ph); 73.8 (C_3); 72.5 (*C*H_2Ph); 70.1 ($C_{5'}$, *C*H$(CH_3)_2$); 68.9 (C_2); 68.7 ($C_{6'}$); 67.8 ($C_{1'}$); 63.7 ($C_{2'}$); 52.5 (COO*C*H_3); 23.6–21.8 (CH(*C*H_3)$_2$). MALDI-TOF *m*/*z* 832.2 (M + Na^+), 848.2 (M + K^+). Anal. calcd. for $C_{44}H_{47}N_3O_{12}$: C, 65.24; H, 5.84; N, 5.18; found: C, 64.70; H, 5.81; N, 5.59%.

Methyl (isopropyl 4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-α-L-idopyranosyl) uronate (17). To a solution of **16** (1.0 g, 1.23 mmol) in dry CH_2Cl_2 (12 mL), EtSH (0.45 mL, 6.17 mmol) and $BF_3\cdot OEt_2$ (5 μL, 37 μmol) were added at 0 °C. After stirring for 1 h under an argon atmosphere, the reaction was neutralised with saturated $NaHCO_3$ solution (2 mL), diluted with CH_2Cl_2 (200 mL) and washed with H_2O (2 × 50 mL). The organic layer was dried with $MgSO_4$and concentrated to dryness. The residue was purified by flash chromatography (1 : 1 hexane–EtOAc) to yield **17** (840 mg, 95%). TLC 0.26 (1 : 1 hexane–EtOAc). $[a]_D^{20}$ −28.5

(*c* 1.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.15–7.16 (m, 15 H, *Ph*); 5.30 (s, 1 H, H_1); 5.09 (s, 1 H, H_2); 4.93 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.90 (d, 1 H, $J_{5,4}$ = 2.0 Hz, H_5); 4.77 (d, 1 H, $J_{1'2'}$ = 3.5 Hz, $H_{1'}$); 4.72 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.26 (d, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 4.16–4.08 (m, 3 H, H_3, H_4, CH_2Ph); 4.04–3.98 (m, H, C*H*($CH_3)_2$); 3.87–3.78 (m, 2 H, $H_{5'}$, $H_{6a'}$); 3.77 (s, 3 H, $COOCH_3$); 3.76–3.68 (m, 1 H, $H_{6'b}$); 3.49–3.40 (m, 2 H, $H_{4'}$, $H_{3'}$); 3.09 (dd, 1 H, $J_{2',1'}$ = 3.5 Hz, $J_{2',3'}$ = 10.0 Hz, $H_{2'}$); 2.35 (br s, 1 H, OH_4); 2.25 (br s, 1 H, OH_6); 1.23–1.17 (d, 6 H, CH($CH_3)_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.2, 165.6 (*C*=O); 137.8–127.7 (*Ph*); 99.0 (C_5); 97.4 (C_1); 79.9 ($C_{4'}$); 75.4 (C_4); 74.7 (CH_2Ph); 73.4 (C_3); 72.6 (CH_2Ph); 72.3 ($C_{5'}$); 71.2 ($C_{3'}$); 70.7 (*C*H($CH_3)_2$); 68.7 (C_2); 67.6 ($C_{1'}$); 63.2 ($C_{2'}$); 62.4 ($C_{6'}$); 52.3 (COO*C*H_3); 23.3–21.0 (CH($CH_3)_2$). MALDI-TOF *m*/*z* 745 (M + Na$^+$), 761 (M + K$^+$). Anal. calcd for $C_{37}H_{43}N_3O_{12}$.1H_2O: C, 60.00; H, 6.13; N, 5.67; found: C, 59.85; H, 6.30; N, 5.46%.

Methyl (isopropyl 4-*O*-(2-azido-3-*O*-benzyl-4,6-*O*-(*p*-methoxybenzylidene)-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-α-L-idopyranosyl) uronate (18). To a solution of **17** (825 mg, 1.14 mmol) in dry DMF (10 mL), 4-methoxybenzaldehyde dimethyl acetal (292 μL, 1.71 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate were added. After stirring for 3 h under argon atmosphere, the reaction was neutralised with saturated $NaHCO_3$ solution (10 mL), diluted with EtOAc (100 mL) and washed with H_2O. The organic layer was dried with $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (6 : 1 hexane–EtOAc), affording compound **18** (790 mg, 85%). TLC 0.27 (3 : 1 hexane–EtOAc). $[a]_D^{20}$ −65.4 (*c* 1.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.14 (d, 2 H, J_{ortho} = 11.0 Hz, MeO*Ph*); 7.40–7.11 (m, 15 H, *Ph*); 6.90 (d, 2 H, J_{ortho} = 11.0 Hz, MeO*Ph*); 5.43 (s, 1 H, MeOPh–C*H*–); 5.30 (s, 1 H, H_1); 5.11 (s, 1 H, H_2); 4.93 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.90 (d, 1 H, $J_{5,4}$ = 2.0 Hz, H_5); 4.75 (d, 1 H, $J_{1'2'}$ = 3.5 Hz, $H_{1'}$); 4.72 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.41 (d, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 4.30 (dd, 1 H, $J_{6'a,5'}$ = 5.0 Hz, $J_{6'a,6'b}$ = 10.0 Hz, $H_{6'a}$); 4.13 (t, 1 H, $J_{3,4} \approx J_{3,2}$ = 2.5 Hz, H_3); 4.09 (m, 1 H, H_4); 4.04–3.96 (m, 3 H, C*H*($CH_3)_2$, $H_{5'}$, CH_2Ph); 3.82 (s, 3 H, CH_3OPh); 3.75 (s, 3 H, $COOCH_3$); 3.67–3.58 (m, 2 H, $H_{3'}$, $H_{6'b}$); 3.52 (t, 1 H, $J_{4',3'} = J_{4',5'}$ = 9.5 Hz, $H_{4'}$); 3.21 (dd, 1 H, $J_{2',1'}$ = 3.5 Hz, $J_{2',3'}$ = 10.0 Hz, $H_{2'}$); 1.25–1.18 (2 d, 6 H, CH($CH_3)_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.1, 165.9 (*C*=O); 160.4 (MeO*Ph*); 137.4–127.4 (*Ph*); 113.4 (MeO*Ph*); 101.4 (MeOPh–*CH*–); 99.7 ($C_{1'}$); 97.4 (C_1); 82.4 ($C_{4'}$); 76.5 ($C_{3'}$); 76.0 (C_4); 74.7 (CH_2Ph); 73.5 (C_3); 72.2 (CH_2Ph); 70.6 (*C*H($CH_3)_2$); 68.5 ($C_{6'}$); 68.4 (C_2); 67.6 (C_5); 63.4 ($C_{5'}$); 63.2 ($C_{2'}$); 55.3 (*Me*OPh); 52.5 (COO*C*H_3); 23.3, 21.5 (CH($CH_3)_2$). MALDI-TOF *m*/*z* 863 (M + Na$^+$); 880 (M + K$^+$). Anal. calcd. for $C_{45}H_{49}N_3O_{13}$: C, 64.35; H, 5.88; N, 5.00; found C, 64.63; H, 6.02; N, 4.95%.

Methyl (isopropyl 4-*O*-(2-azido-3-*O*-benzyl-6-*O*-(4-methoxybenzyl)-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-α-L-idopyranosyl) uronate (11). A solution at 0 °C of TFA (1.17 mL, 15.22 mmol) in dry DMF (8 mL) cooled at 0 °C was added dropwise to a stirred mixture containing compound **18** (640 mg, 0.76 mmol) and $NaBH_3CN$ (1 M solution in THF 11.4 mL, 11.41 mmol). After 3 h an identical amount of $NaBH_3CN$ was added in the same conditions. Three more additions in the same conditions were made at three hour intervals. After stirring at 35 °C for 24 h, the mixture was neutralised, at 0 °C with saturated $NaHCO_3$ solution (15 mL), diluted with EtOAc (100 mL) and washed with H_2O (2 × 100 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL) and the organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash cromatography (3 : 1 hexane–EtOAc), to yield **11** (442.3 mg, 69%) as well as non-reacted starting material (160.5 mg, 25%). TLC 0.33 (2 : 1 hexane–EtOAc). $[a]_D^{20}$ −25.2°(*c* 1.0, $CHCl_3$).^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.14 (d, 2 H, J_{ortho} = 11.0 Hz, MeO*Ph*); 7.38–7.19 (m, 15 H, *Ph*); 6.90 (d, 2 H, J_{ortho} = 11.0 Hz, MeO*Ph*); 5.30 (br s, 1 H, H_1); 5.10 (br s, 1 H, H_2); 4.90 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.87 (d, 1 H, $J_{5,4}$ = 2.5 Hz, H_5); 4.79 (d, 1 H, $J_{1'2'}$ = 3.5 Hz, $H_{1'}$); 4.71 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.50 (d, 1 H, J_{gem} = 12.0 Hz, CH_2-PhOMe); 4.41 (d, 1 H, J_{gem} = 12.0 Hz, CH_2PhOMe); 4.37 (d, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 4.22 (d, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 4.13 (br s, 1 H, H_3); 4.08 (br s, 1 H, H_4); 4.04–3.98 (m, 1 H, C*H*($CH_3)_2$); 3.82 (m, 1 H, $H_{5'}$); 3.78 (s, 3 H, PhOCH_3); 3.71 (s, 4 H, $H_{6'a}$, $COOCH_3$); 3.64 (t, 1 H, $J_{4',3'} \approx J_{4',5'}$ = 9.0 Hz, $H_{4'}$); 3.55 (dd, 1 H, $J_{6'b,5'}$ = 5.0 Hz, $J_{6'b,6'a}$ = 10 Hz, $H_{6'b}$); 3.48 (t, 1 H, $J_{3',2'} \approx J_{3',4'}$ = 9.5 Hz, $H_{3'}$); 3.15 (dd, 1 H, $J_{2',1'}$ = 3.5 Hz, $J_{2',3'}$ = 9.5Hz, $H_{2'}$); 2.56 (br s, 1 H, OH_4); 1.25–1.18 (2d, 6 H, CH($CH_3)_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.0, 164.5 (*C*=O); 159.0 (MeO*Ph*); 137.4–127.4 (*Ph*); 113.6 (MeO*Ph*); 99.1 ($C_{1'}$); 97.3 (C_1); 79.5 ($C_{4'}$); 75.2 (C_4); 74.7 (CH_2Ph); 73.6 (C_3); 73.4 (CH_2PhOMe); 72.7 ($C_{3'}$); 72.4 (CH_2Ph); 70.7 (*C*H($CH_3)_2$); 70.4 ($C_{5'}$); 69.3 ($C_{6'}$); 69.0 (C_2); 68.1 (C_5); 63.0 ($C_{2'}$); 55.3 (CH_3OPh); 52.3 (COO*C*H_3); 23.3, 21.5 (CH($CH_3)_2$). MALDI-TOF *m*/*z* 866 (M + Na$^+$); 882 (M + K$^+$). Anal. calcd. for $C_{37}H_{43}N_3O_{12}$.·½ H_2O: C, 62.17; H,6.26; N, 4.83; found C, 62.20; H, 6.45; N, 4.62%.

Methyl (dimethylthexylsilyl 4-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-α,β-L-idopyranosyl) uronate (19). To a solution of **10** (100 mg, 0.10 mmol) and the complex BH_3·$NHMe_2$ (33 mg, 0.54 mmol) in dry CH_2Cl_2 (2 mL), at −15 °C, BF_3·Et_2O (68.5 μL, 0.54 mmol) was added. After stirring for 30 min at this temperature under an argon atmosphere, the mixture was neutralised with saturated $NaHCO_3$ solution (15 mL), diluted with EtOAc (75 mL) and washed with H_2O (2 × 50 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL), and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash cromatography (3 : 1 hexane–EtOAc), to yield **19** (81 mg, 81%). TLC 0.26 (2 : 1 hexane–EtOAc), $[a]_D^{20}$ −2.9 (*c* 1, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.12–7.07 (m, 20 H, 4 *Ph*); 5.16 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H_1); 5.11 (br s, 1 H, H_2); 4.84 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.76 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.72–4.65 (m, 2 H, CH_2Ph, $H_{1'}$); 4.58 (d, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 4.48 (d, 1 H, $J_{5,4}$ = 1.5 Hz, H_5); 4.22 (t, 1 H, $J_{3,2} \approx J_{3,4}$ = 2.5 Hz, H_3); 4.02 (m, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 3.98 (br s, 1 H, H_4); 3.96–3.92 (m, 1 H, $H_{5'}$); 3.90–3.82 (m, 2 H, $H_{6'a}$, CH_2Ph); 3.77 (s, 3 H, COO*Me*); 3.71–3.66 (m, 1 H, $H_{6'b}$); 3.48 (t, 1 H, $J_{3',2'} \approx J_{3',4'}$ = 9.5 Hz, $H_{3'}$); 3.37 (t, 1 H, $J_{4',3'} \approx J_{4',5'}$ = 9.5 Hz, $H_{4'}$); 3.09 (dd, 1 H, $J_{2',1'}$ = 3.5 Hz, $J_{2',3'}$ = 10.0 Hz); 1.83 (t, 1 H, O*H*); 1.55 (m, 1 H, C*H*($CH_3)_2$); 0.76–0.74 (m, 12 H, CH($CH_3)_2$, C($CH_3)_2$); 0.24, 0.12 (2 s, 6 H, Si($CH_3)_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 169.0, 166.5 (*C*=O); 138.0–127.6 (*Ph*); 99.7 ($C_{1'}$); 94.0 (C_1); 79.7 ($C_{3'}$); 77.7 ($C_{4'}$); 75.7 (C_4); 75.1 (C_3); 74.8 (CH_2Ph); 74.6 (CH_2Ph); 73.4 (C_5); 73.0 (CH_2Ph); 72.7 ($C_{5'}$); 68.6 (C_2); 63.7 ($C_{2'}$); 61.5 ($C_{6'}$); 52.2 (COO*C*H_3); 34.0, 24.7, 20.1, 19.8, 18.5, 18.3, −2.0, −3.4 (O*TDS*). MALDI-TOF *m*/*z*935 (M + Na$^+$); 951.0 (M + K$^+$). Anal. calcd. for $C_{49}H_{61}N_3O_{12}$Si: C, 64.54; H, 6.74; N, 4.60; found C, 64.33; H, 7.03; N, 4.64%.

Methyl dimethylthexylsilyl 4-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-[6-*O*-(4-methoxybenzyl)]-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-benzoyl-α,β-L-idopyranosyluronate (20). To a solution of **19** (180 mg, 0.19 mmol) and 4-methoxybenzyl trichloroacetimidate (55 mg, 0.59 mmol), in dry CH_2Cl_2 (3 mL) at −20 °C, was added BF_3·OEt_2 (6 μL, 5.91 μmol, 1 M solution in THF). After stirring for 10 min at this temperature, the mixture was neutralised with Et_3N (0.5 mL) and concentrated *in vacuo*. The residue was purified by flash chromatography (4 : 1 hexane–EtOAc), to yield **20** (186 mg, 96%). TLC 0.20 (14 : 1 toluene–EtOAc), $[a]_D^{20}$ +6.6 (*c* 0.8, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.12–7.08 (m, 20 H, *Ph*); 6.80 (d, 2 H, J_{ortho} = 8.5 Hz, MeO*Ph*); 5.19 (br s, 1 H, H_1); 5.11 (br s, 1 H, H_2); 4.85

(d, 1 H, J_{gem} = 11.0 Hz, CH_2Ph); 4.80–4.74 (m, 2 H, CH_2Ph, $H_{1'}$); 4.62–4.57 (2 d, 2 H, J_{gem} = 11.0 Hz, CH_2Ph, CH_2PhOMe); 4.50 (br s, 1 H, H_5); 4.44–4.36 (2 d, 2 H, J_{gem} = 11.0 Hz,CH_2Ph, CH_2PhOMe); 4.24 (t, 1 H, $J_{3,2} \approx J_{3,4}$ = 2.5 Hz, H_3); 4.04–3.98 (m, 3 H, CH_2Ph, H_4, $H_{5'}$); 3.85 (d, 1 H, CH_2Ph); 3.81 (dd, 1 H, $J_{6'a,6'b}$ = 11.0 Hz, $J_{6'a,5'}$ = 2 Hz, $H_{6'a}$); 3.78–3.72 (2 s, 6 H, CH_3OPh, COOCH_3); 3.68 (dd, 1 H, $J_{6'b,6'a}$ = 11.0 Hz, $J_{6'b,5'}$ = 1.5 Hz, $H_{6'b}$); 3.64 (t, 1 H, $J_{4',3'} \approx J_{4',5'}$ = 9.5 Hz, $H_{4'}$); 3.50 (t, 1 H, $J_{3',2'} \approx J_{3',4'}$ = 9.5 Hz, $H_{3'}$); 3.18 (dd, 1 H, $J_{2',1'}$ = 3.5 Hz, $J_{2',3'}$ = 9.5 Hz, $H_{2'}$); 1.57 (m, 1 H, CH(CH$_3$)$_2$); 0.78–0.75 (m, 12 H, CH(CH_3)$_2$, C(CH_3)$_2$); 0.26–0.15 (2 s, 6 H, Si(CH_3)$_2$). ^{13}C-NMR (125 MHz, CDCl$_3$): δ 168.5, 166.5 (C=O); 159.3 (MeOPh); 138.4–127.8 (Ph, MeOPh); 113.8 (MeOPh); 100.0 ($C_{1'}$); 93.9 (C_1); 79.8 ($C_{3'}$); 77.8 ($C_{4'}$); 75.6 (C_4); 75.0 (C_3); 74.8 (CH$_2$Ph); 74.5 (CH$_2$PhOMe); 73.5 (C_5); 73.2–73.0 (CH$_2$Ph); 71.7 ($C_{5'}$); 68.6 (C_2); 67.2 ($C_{6'}$); 63.7 ($C_{2'}$); 55.2 (CH$_3$OPh); 52.2 (CO-OCH$_3$); 34.0, 24.8, 20.2, 19.9, 18.6, 18.4, −1.8, −3.4 (OTDS). MALDI-TOF m/z 1054.0 (M + Na$^+$); 1070.2 (M + K$^+$). Anal. calcd. for $C_{57}H_{69}N_3O_{13}Si$: C, 66.32; H, 6.73; N, 4.07; found C, 66.21; H, 6.69; N, 3.87%.

Methyl 4-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)]-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-α,β-L-idopyranosyluronate (21). To a solution of **20** (200 mg, 0.19 mmol) at −15 °C in dry THF (6 mL) an excess of (HF)$_n$·Py (0.7 mL) was added. The reaction was warmed to 0 °C and stirred for 48 h under argon atmosphere. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (2 × 25 mL) and saturated $NaHCO_3$ solution until neutral pH. The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and the organic layers were dried over $MgSO_4$and concentrated *in vacuo*. The residue was purified by flash chromatography (2 : 1 hexane–EtOAc), to yield **21** (140 mg, 83%) as a mixture of anomers α–β. TLC 0.23 (2 : 1 hexane–EtOAc). ^{1}H-NMR (500 MHz, CDCl$_3$): δ 8.11–7.03 (m, 44 H, Ph, MeOPh α and β); 6.78–6.72 (d, 4 H, MeOPh); 5.43 (d, 1 H, $J_{1\alpha,OH}$ = 9.0 Hz, H_1α); 5.24 (d, 1 H, $J_{1\beta,OH}$ = 11.5 Hz, H_1β); 5.05 (s, 2 H, H_2 α and β); 4.88–4.84 (m, 3 H,CH_2Ph, H_5α); 4.80–4.72 (m, 2 H, CH_2Ph α and β); 4.69 (d, 1 H, $J_{1',2'\alpha}$ = 3.5 Hz, $H_{1'}$α); 4.64 (d, 1 H, $J_{1',2'\beta}$ = 3.0 Hz, $H_{1'}$ β); 4.60–4.50 (m, 5 H, H_5β, CH_2Ph α and β); 4.40–4.28 (m, 6 H, H_3, CH_2Ph α and β); 4.19 (d, 1 H, $J_{1\alpha,OH}$ = 9.0 Hz, OH α); 4.03–3.94 (m, 5 H, $H_{6'a}$, H_4,CH_2Ph, α and β); 3.85–3.75 (m, 5 H, $H_{5'}$, $H_{6'b}$, CH_2Ph, α and β); 3.73 (s, 6 H, CH_3OPh α and β); 3.71 (s, 6 H, COOCH_3 α and β); 3.65–3.55 (m, 4 H, CH_2Ph, OH β, $H_{4'}$ α and β); 3.42–3.34 (m, 2 H, $H_{3'}$ α and β); 3.24–3.18 (m, 2 H, $H_{2'}$ α and β). MALDI-TOF m/z 913 (M + Na$^+$); 928.5 (M + K$^+$). Anal. calcd. for $C_{49}H_{51}N_3O_{13}$: C, 66.13; H, 5.57; N, 4.72; found: C, 66.08; H, 6.34; N, 5.04%.

***O*-(Methyl 4-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-2-*O*-benzoyl-2-*O*-benzyl-α,β-L-idopyranosyluronate) trichloroacetimidate (13).** To a solution of **21** (140 mg, 0.15 mmol) in dry CH_2Cl_2(2 mL), CCl_3CN (236 μL, 2.35 mmol) and activated K_2CO_3 (24 mg, 0.17 mmol) were added. After stirring for 6 h the residue was filtered and concentrated to dryness. The residue was purified by flash chromatography (3 : 1 hexane–EtOAc), to yield **13** (142 mg, 88%) as a mixture of α–β anomers. TLC 0.31, 0.15 β–α (3 : 1 hexane–EtOAc). ^{1}H-NMR (500 MHz, CDCl$_3$): β anomer; δ 8.65 (s, 1 H, NHβ); 8.10–7.05 (m, 22 H, Ph, MeOPh); 6.77 (d, 2 H, J_{ortho} = 8.5 Hz, MeOPh); 6.53 (s, 1 H, H_1); 5.32 (br s, 1 H, H_2); 4.99 (d, 1 H, $J_{5,4}$ = 1.5 Hz, H_5); 4.90 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.78 (d, 1 H, $J_{1'2'}$ = 3.0 Hz, $H_{1'}$); 4.74 (d, 1 H, J_{gem} = 11.5 Hz,CH_2Ph); 4.60–4.51 (2 d, 2 H, J_{gem} = 11.0 Hz, CH_2Ph, CH_2PhOMe); 4.39–4.34 (2 d, 2 H, J_{gem} = 11.0 Hz, CH_2Ph, CH_2PhOMe); 4.23 (br s, 1 H, H_3); 4.16 (br s, 1 H, H_4); 4.06 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 3.90–3.85 (m, 2 H, $H_{5'}$, CH_2Ph); 3.78–3.70 (m, 7 H, $H_{6'a}$, CH_3OPh, COOCH_3); 3.65–3.58 (m, 2 H, $H_{4'}$, $H_{6'b}$); 3.47 (t, 1 H, $J_{3',4'} = J_{3',2'}$ = 10.0 Hz, $H_{3'}$); 3.21 (dd, 1 H, $J_{2',1'}$ = 3.0 Hz, $J_{2',3'}$ = 10.0 Hz, $H_{2'}$). ^{13}C-NMR (125 MHz, CDCl$_3$): δ 168.6, 165.4 (C=O); 160.3 (C=NH); 159.3 (MeOPh); 138.4–127.6 (Ph, MeOPh); 113.7 (MeOPh); 100.1 ($C_{1'}$); 96.1 (C_1); 80.8 ($C_{3'}$); 77.6 ($C_{4'}$); 75.7 (C_4); 74.7 (CH$_2$Ph); 74.6 (CH$_2$PhOMe); 73.1, 72.5 (CH$_2$Ph); 72.2 (C_3); 71.8 ($C_{5'}$); 69.0 (C_5); 67.2 ($C_{6'}$); 65.8 (C_2); 63.7 ($C_{2'}$); 55.2 (CH$_3$OPh); 52.45 (COOCH$_3$). Anal. calcd. for $C_{51}H_{51}N_4O_{13}Cl_3$: C, 59.22; H, 4.96; N, 5.41; found: C, 59.05; H, 5.12; N, 5.68%.

Methyl (isopropyl*O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1⟶4)-*O*-(methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-(2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyl) uronate (22). A mixture of **12** (172.5 mg, 0.18 mmol) and acceptor **11** (114.3 mg, 0.13 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and cooled at −20 °C under an argon atmosphere. A solution of TMSOTf (21.5 μL of 0.55 M in dry CH_2Cl_2) was added dropwise and the solution was stirred for 15 min at the same temperature, and then neutralised with triethylamine. The solvent was concentrated *in vacuo* and the obtained residue was purified by flash chromatography (4 : 1 hexane–EtOAc), affording compound **22** (185 mg, 85%) and a mixture of **11**–**12**, which was purified with (10 : 1 toluene–acetone) affording compound **11** (10 mg, 8.5%) and **12** (20 mg, 14%). TLC 0.29 (3 : 1 hexane–EtOAc). $[\alpha]_D^{20}$ −24.8 (c 1.0, CHCl$_3$). ^{1}H-NMR (500 MHz, CDCl$_3$): δ 8.2–6.7 (m, 39 H, Ph, PhOMe), 5.51 (s, 2 H, Ph–CH–, H_{1c}); 5.22 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H_{1a}); 5.16 (t, 1 H, $J_{2,3} \approx J_{2,1} \approx$ 4.5 Hz, H_{2c}); 5.06 (t, 1 H, $J_{2,3} \approx J_{2,1} \approx$ 2.5 Hz, H_{2a}); 4.88 (d, 1 H, J_{gem} = 11.5 Hz, CH_2-Ph); 4.84 (d, 1H, $J_{1,2}$ = 3.5 Hz, H_{1b}); 4.81–4.76 (m, 3 H,CH_2-PhOMe, H_{5c}); 4.73 (d, 1H, $J_{1,2}$ = 3.5 Hz, H_{1d}); 4.70 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.63 (d, 1 H, $J_{5,4}$ = 3.5 Hz, H_{5a}); 4.57 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.47 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.37 (s, 1 H, CH_2Ph); 4.27–4.20 (m, 2 H, H_{6d}, CH_2Ph); 4.18 (t, 1 H, $J_{3,4} = J_{3,2}$ = 5.0 Hz, H_{3c}); 4.08 (t, 1 H, $J_{3,4} \approx J_{3,2}$ = 3.5 Hz, H_{3a}); 4.00–3.92 (m, 6 H, H_{4c}, H_{4a}, CH(CH$_3$)$_2$, CH_2Ph, H_{5b}, H_{5d}); 3.71–3.67 (m, 4 H, PhOCH_3, H_{3b}); 3.67–3.59 (m, 3 H, H_{4d}, $H_{6'd}$, H_{6b}); 3.57–3.45 (m, 9 H, H_{3d}, H_{4b}, $H_{6'b}$, COOCH_3); 3.27–3.21 (m, 2 H, H_{2d}, H_{2b}); 1.25–1.15 (2 d, 6 H, CH(CH_3)$_2$). ^{13}C-NMR (125 MHz, CDCl$_3$) δ 169.9, 169.2, 165.7, 165.2 (C=O), 159.0 (MeOPh); 137.9–126.1 (Ph); 113.6 (MeOPh); 101.5 (Ph–CH–); 99.8 (C_{1b}), 99.1 (C_{1d}), 98.4 (C_{1c}), 97.2 (C_{1a}), 82.4 (C_{3d}); 78.5 (C_{4b}); 76.0 (C_{4d}); 75.6 (C_{4c}); 75.4 (C_{3c}); 75.2 (C_{4a}); 74.7 (C_{5b}); 74.2 (CH$_2$Ph); 73.9 (C_{5d}); 73.2 (C_{3a}); 73.0 (CH$_2$Ph); 72.2, 71.2 (C_{3b}); 70.5 (C_{2c}); 70.2 (CH(CH$_3$)$_2$); 69.6 (C_{5a}); 68.6 (C_{2a}); 68.5 (C_{6d}); 67.8 (C_{5c}); 67.1 (C_{6b}); 63.5 (C_{2b}); 63.3, 62.9 (C_{2d}); 55.2 (PhOCH$_3$); 52.1, 52.0 (COOCH$_3$); 23.3, 21.5 (CH(CH$_3$)$_2$). MALDI-TOF m/z 1615.5 (M + Na$^+$); 1631.5 (M + K$^+$). Anal. calcd. for $C_{86}H_{90}N_6O_{24}$: C, 64.85; H, 5.69; N, 5.27; found C, 65.09; H, 6.16; N, 5.05%.

Methyl (isopropyl*O*-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1⟶4)-*O*-(methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-(2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyl) uronate (23). To a solution of **22** (500 mg, 0.31 mmol) in dry CH_2Cl_2(8 mL), EtSH (0.34 mL, 4.70 mmol) and catalytic amount camphor sulfonic acid were added. After stirring for 24 h, the reaction was neutralised with triethylamine (2 mL). The mixture was concentrated *in vacuo* and the obtained residue was purified by flash chromatography (1 : 1 hexane–EtOAc), to yield **23** (400 mg, 85%) as well as non-reacted starting material **22** (61 mg, 12%). TLC 0.28 (1 : 1 hexane–EtOAc). $[\alpha]_D^{20}$ −6.8 (c 1.0, CHCl$_3$). ^{1}H-NMR (500 MHz, CDCl$_3$): δ 8.2–6.7 (m, 34 H, Ph), 5.51 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H_{1c}); 5.26 (s, 1 H, H_{1a}); 5.17 (t, 1 H, $J_{2,3} = J_{2,1}$ = 4.5 Hz, H_{2c}); 5.06 (m, 1 H, H_{2a}); 4.88 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.85 (d, 1H, $J_{1,2}$ = 3.5 Hz, H_{1b}); 4.79 (m, 3 H, CH_2PhOMe, H_{5c}); 4.73 (d, 1H, $J_{1,2}$ = 3.5 Hz, H_{1d}); 4.69 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.64 (d, 1 H, $J_{5,4}$ = 4.0 Hz, H_{5a}); 4.45 (m, 5 H, CH_2Ph); 4.18 (t, 1 H, $J_{3,4} \approx J_{3,2}$ = 5 Hz, H_{3c}); 4.09 (m, 1 H, H_{3a}); 4.00–3.93 (m, 5 H,

H_{4c}, H_{4a}, H_{5d}, CH_2Ph, CH(CH$_3$)$_2$); 3.71–3.67 (m, 7 H, PhO*Me*, H_{5b}, H_{6d}, H_{4b}, H_{4d}); 3.62 (m, 1 H, H_{6b}); 3.53–3.44 (m, 10 H, $H_{6'd}$, $H_{6'b}$, H_{3b}, H_{3d}, 2 COOCH_3); 3.24 (dd, 1 H, $J_{2,1}$ = 3.5 Hz, $J_{2,3}$ = 10.5 Hz, H_{2d}); 3.10 (dd, 1 H, $J_{2,1}$ = 3.5 Hz, $J_{2,3}$ = 10.0 Hz, H_{2b}); 1.20–1.14 (2 d, 6 H, CH(CH_3)$_2$). ^{13}C-NMR (125 MHz, CDCl$_3$) δ 169.8, 169.4, 165.7, 165.3 (*C*=O), 159.2 (MeO*Ph*); 138.0–127.3 (*Ph*); 113.7 (MeO*Ph*); 99.2 (C_{1b}), 98.9 (C_{1d}), 98.3 (C_{1c}), 97.2 (C_{1a}), 79.6 (C_{3d}); 78.4 (C_{3b}); 75.4 (C_{4c}, C_{4a}); 75.3 (C_{3c}); 75.0 (CH$_2$Ph); 74.7, 74.3, 73.8 (CH$_2$Ph); 72.3 (C_{3a}); 73.3, 72.2 (CH$_2$Ph); 71.7; 71.3, 71.1 (C_{4d}, C_{4b}); 71.1 (C_{6d}); 70.6 (CH(CH$_3$)$_2$); 70.4 (C_{2c}); 69.6 (C_{5a}); 69.0 (C_{2a}); 68.1 (C_{5c}); 67.3 (C_{6b}); 63.2 (C_{2d}); 62.9 (C_{2b}); 62.1, 55.2 (PhO*Me*); 52.0 (CO-OCH$_3$); 23.3, 21.5 (CH(CH$_3$)$_2$). MALDI-TOF *m*/*z* 1527 (M + Na$^+$), 1543 (M + K$^+$). Anal. calcd. for $C_{79}H_{86}N_6O_{24}$: C, 63.10; H, 5.76; N, 5.58; found: C, 63.14; H, 5.85; N, 5.46%.

Methyl (isopropyl*O*-(2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranosyl)-(1⟶4)-*O*-(methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-(2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyl) uronate (24). To a solution of **23** (799 mg, 0.53 mmol) in dry DMF (8 mL), 4-methoxybenzaldehyde dimethyl acetal (182.0 μL, 1.06 mmol) and a catalytic amount of camphor sulfonic acid were added. After stirring for 3 h under an argon atmosphere, the reaction was neutralised with saturated NaHCO$_3$ solution (10 mL), diluted with EtOAc (100 mL) and washed with H$_2$O. The organic layer was dried with MgSO$_4$ and concentrated to dryness. The residue was purified by flash chromatography (3 : 1 hexane–EtOAc), affording compound **24** (820 mg, 95%). TLC 0.26 (3 : 1 hexane–EtOAc). $[\alpha]_D^{20}$ −26.0 (*c* 1.0, CHCl$_3$). ^{1}H-NMR (500 MHz, CDCl$_3$): δ 8.2–6.7 (m, 38 H, *Ph*, *Ph*OMe), 5.52 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H_{1c}); 5.47 (s, 1 H, MePh–C*H*–); 5.27 (d, 1 H, $J_{1,2}$ = 4.5 Hz, H_{1a}); 5.18 (t, 1 H, $J_{2,3} \approx J_{2,1}$ = 4.5 Hz, H_{2c}); 5.07 (m, 1 H, H_{2a}); 4.89 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.85 (d, 1H, $J_{1,2}$ = 3.5 Hz, H_{1b}); 4.79 (m, 3 H, CH_2PhOMe, H_{5c}); 4.75 (d, 1H, $J_{1,2}$ = 3.5 Hz, H_{1d}); 4.70 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.64 (d, 1 H, $J_{5,4}$ = 4.0 Hz, H_{5a}); 4.58 (d, 1 H, J_{gem} = 11.5 Hz,CH_2Ph); 4.48 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.45 (s, 2 H, CH_2Ph); 4.27–4.19 (m, 3 H, CH_2Ph, H_{6d}, H_{3c}); 4.09 (m, 1 H, H_{3a}); 4.01–3.87 (m, 6 H, H_{4a}, H_{4c},CH_2Ph, H_{5b}, CH(CH$_3$)$_2$, H_{5d}); 3.82 (s, 3 H, CH_3OPh); 3.70–3.61 (m, 6 H, CH_3OPh, $H_{,3b}$, H_{4d} H_{6b}); 3.59–3.48 (m, 5 H, COOCH_3, H_{6d}, H_{4b}); 3.49–3.46 (m, 5 H, H_{3d}, $H_{6'b}$, COOCH_3); 3.24 (m, 2 H, H_{2d}, H_{2b}); 1.18–1.14 (2 d, 6 H, CH(CH_3)$_2$). ^{13}C-NMR (125 MHz, CDCl$_3$): δ 169.9, 169.1, 165.7, 165.2 (*C*=O), 160.1, 159.2 (MeO*Ph*); 138.0–127.3 (*Ph*); 113.7, 113.6 (MeO*Ph*); 101.4 (MeOPh–*C*H–); 99.8 (C_{1b}); 98.9 (C_{1b}); 98.4 (C_{1c}); 97.2 (C_{1a}); 82.4 (C_{4b}); 78.5 (C_{3d}); 76.1 (C_{4d}); 75.6 (C_{5b}); 75.5 (C_{3c}); 75.4 (C_{4c}); 75.3, 74.7, 74.2 (C_{4a}); 73.9 (CH$_2$Ph); 73.4 (C_{3a}); 73.2 (C_{5d}); 72.3, 71.7 (CH$_2$Ph); 71.3 (C_{3b}); 70.7 (CH(CH$_3$)$_2$); 70.3 (C_{2c}); 69.7 (C_{5a}); 69.0 (C_{2a}); 68.5 (C_{6d}); 68.1 (C_{5c}); 67.3 (C_{6b}); 63.6 (C_{2b}); 63.4 (C_{2d}); 60.0, 55.3–55.2 (CH$_3$OPh); 52.0–51.9 (CO-OCH$_3$); 23.3–21.5 (CH(CH$_3$)$_2$). MALDI-TOF *m*/*z* 1646.4 (M + Na$^+$), 1662.8 (M + K$^+$). Anal. calcd. for $C_{87}H_{93}N_6O_{25}$: C, 64.39; H, 5.77; N, 5.17; found C, 64.26; H, 5.77; N, 5.15%.

Methyl (isopropyl*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-O-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-*O*-(methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-(2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyl) uronate (25). A solution of TFA (1.17 mL, 15.22 mmol) in dry DMF (4 mL) was added dropwise at 0 °C to a stirred mixture containing compound **24** (290 mg, 0.17 mmol) and NaBH$_3$CN (1 M solution in THF 3.55 mL, 3.55 mmol). A new addition of NaBH$_3$CN and the solution of TFA in DMF at 0 °C was made after three hours. Three more identical additions were made at three hours intervals. After stirring at 35 °C for 24 h, the mixture was neutralised at 0 °C, with saturated NaHCO$_3$ solution (15 mL), diluted with EtOAc (75 mL) and washed with H$_2$O (2 × 50 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL) and the organic layers were dried (MgSO$_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (3 : 1 hexane–EtOAc), to yield **25** (156 mg, 54%) as well as non-reacted starting material **24** (122 mg, 42%). TLC 0.22 (2 : 1 hexane–EtOAc). $[\alpha]_D^{20}$ −2.8 (*c* 1.0, CHCl$_3$). ^{1}H-NMR (500 MHz, CDCl$_3$): δ 8.09–7.14 (m, 34 H, *Ph*, MeO*Ph*); 6.85–6.79 (2 d, 4 H, J_{ortho} = 8.5 Hz, MeO*Ph*); 5.54 (d, 1 H, $J_{1,2}$ = 4.5 Hz, H_{1c}); 5.25 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H_{1a}); 5.18 (t, 1 H, $J_{2,3} \approx J_{2,1}$ = 5.0 Hz, H_{2c}); 5.05 (m, 1 H, H_{2a}); 4.92 (d, 1H, $J_{1,2}$ = 3.5 Hz, H_{1b}); 4.87 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.79 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.77 (d, 1 H, $J_{5,4}$ = 4.0 Hz, H_{5c}); 4.75–4.70 (m, 2 H,CH_2Ph, H_{1d}); 4.69 (d, 1 H, J_{gem} = 11.5 Hz, CH_2Ph); 4.57 (d, 1 H, $J_{5,4}$ = 4.5 Hz, H_{5a}); 4.54 (d, 1 H, J_{gem} = 11.5 Hz,CH_2Ph); 4.50–4.40 (m, 6 H, CH_2Ph); 4.19 (t, 1 H, $J_{3,4} \approx J_{3,2} \approx$ 5.5 Hz, H_{3c}); 4.07 (t, 1 H, $J_{3,4} \approx J_{3,2} \approx$ 5.5 Hz, H_{3a}); 4.01–3.91 (m, 5 H, H_{4a}, H_{4c},CH_2Ph, CH(CH$_3$)$_2$, H_{5d}); 3.77 (s, 3 H, *Me*OPh); 3.75 (m, 1 H, H_{4b}); 3.71 (s, 3 H, CH_3OPh); 3.70–3.64 (m, 3 H, H_{4d}, H_{6d}, H_{5b}); 3.61 (m, 1 H, H_{6b}); 3.54–3.49 (m, 5 H, H_{3b}, $H_{6'd}$, COOCH_3); 3.48–3.44 (m, 5 H, H_{3d}, $H_{6'b}$, COOCH_3); 3.23 (dd, 1 H, $J_{2,1}$ = 3.5 Hz, $J_{2,3}$ = 10.0 Hz, H_{2d}); 3.17 (dd, 1 H, $J_{2,1}$ = 3.5 Hz, $J_{2,3}$ = 10.0 Hz, H_{2b}); 2.11 (br s, 1 H, OH_4); 1.20–1.13 (2 d, 6 H, CH(CH_3)$_2$). ^{13}C-NMR (125 MHz, CDCl$_3$): δ 169.8, 169.3, 165.6, 165.2 (*C*=O); 159.4, 159.2 (MeO*Ph*); 138.0, 127.2 (*Ph*); 113.9, 113.7 (MeO*Ph*); 99.2 (C_{1b}); 98.9 (C_{1d}); 98.2 (C_{1c}); 97.2 (C_{1a}); 79.2 (C_{3b}); 78.4 (C_{3d}); 75.8 (C_{3c}); 75.6 (C_{5d}); 75.4 (C_{4c}); 74.9 (C_{4a}); 74.7, 74.3, 74.0, 73.4 (CH$_2$Ph); 73.2 (C_{3a}); 72.6, 72.3 (CH$_2$Ph); 71.3, 71.0 (C_{2c}); 70.6 (C_{4b}); 70.4 (CH(CH$_3$)$_2$); 70.3 (C_{5a}); 69.3 (C_{6d}); 69.0 (C_{2a}); 68.1 (C_{5c}); 67.1 (C_{6b}); 63.4, 62.7 (C_{2b}); 64.1 (C_{2d}); 55.3, 55.2 (*Me*OPh); 51.9, 51.8 (COOCH$_3$); 23.3, 21.5 (CH(CH$_3$)$_2$). MALDI-TOF *m*/*z* 1646.5 (M + Na$^+$); 1662.7 (M + K$^+$). Anal. calcd. for $C_{87}H_{94}N_6O_{25}$: C, 64.31; H, 5.80; N, 5.17; found C, 64.26; H, 6.26; N, 5.70%.

Methyl (isopropyl*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-*O*–(2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-*O*–(3-*O*-benzyl-2-*O*-benzoyl–α-L-idopyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-ido-pyranosyl) uronate (14). A mixture of acceptor **25** (156 mg, 96 μmol) and donor **13** (170 mg, 0.16 mmol) was dissolved in dry CH$_2$Cl$_2$ (1.5 mL) and cooled at −20 °C under an argon atmosphere. A solution of TMSOTf ((25.8 μL, 4.8 μmol, 1.8 M in dry CH$_2$Cl$_2$) was added dropwise and the solution was stirred for 15 min at the same temperature, and then neutralised with Et$_3$N (0.5 mL). The solvent was evaporated and the obtained residue was purified by flash chromatography (4 : 1 hexane–EtOAc), affording compound **14** (190 mg, 79%) TLC 0.29 (2 : 1 hexane–EtOAc). $[\alpha]_D^{20}$ −1.7 (*c* 1, CHCl$_3$). ^{1}H-NMR (500 MHz, CDCl$_3$): δ 8.08–7.03 (m, 56 H, *Ph*, MeO*Ph*); 6.78 (m, 6 H, MeO*Ph*); 5.51 (d, 1 H, $J_{1,2}$ = 5.0 Hz, H_{1e}); 5.48 (d, 1 H, $J_{1,2}$ = 4.5 Hz, H_{1c}); 5.24 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H_{1a}); 5.15 (t, 2 H, H_{2e}, H_{2c}); 5.05 (t, 1 H, $J_{2,1}$ = 2.5 Hz, H_{2a}); 4.94 (d, 1 H, $J_{1,2}$ = 3.0 Hz, H_{1d}); 4.90–4.86 (m, 2 H, H_{1f}, CH_2Ph); 4.80–4.64 (m, 10 H, H_{1b}, CH_2Ph, CH_2PhOMe, H_{5a}, H_{5c}, H_{5e}); 4.53–4.26 (m, 12 H, CH_2Ph, CH_2PhOMe); 4.19 (t, 1 H, $J_{3,2} \approx J_{3,4}$ = 5 Hz, H_{3c}); 4.15 (t, 1 H, $J_{3,2} \approx J_{3,4}$ = 6.0 Hz, H_{3e}); 4.06 (t, 1 H, $J_{3,2} \approx J_{3,4}$ = 3.5 Hz, H_{3a}); 4.02 (t, 1 H, $J_{4,5} \approx J_{4,3}$ = 5.0 Hz, H_{4c}); 4.00–3.92 (m, 4 H, H_{4a}, H_{4f}, CH_2Ph, CH(CH$_3$)$_2$); 3.92–3.85 (m, 2 H, H_{4d}, H_{4e}); 3.81 (m, 1 H, H_{5d}); 3.71–3.69 (m, 10 H, 3 CH_3OPh, H_{6d}); 3.66–3.52 (m, 8 H, H_{5b}, H_{6b}, H_{3d}, H_{4d}, $H_{6'd}$, H_{3f}, H_{5f}, H_{6f}); 3.48–3.46 (2 s, 6 H, 2 COOCH_3); 3.44–3.40 (m, 2 H, H_{3b}, $H_{6'b}$); 3.33 (m, 1 H, $H_{6'f}$); 3.27 (s, 3 H, COOCH_3); 3.26–3.20 (m, 3 H, H_{2f}, H_{2d}, H_{2b}); 1.19–1.13 (2 d, 6 H, CH(CH_3)$_2$). ^{13}C-NMR (125 MHz, CDCl$_3$): δ 169.83, 169.26, 169.22, 165.62, 165.20, 165.17 (C=O); 159.3, 159.2 (MeO*Ph*); 138.2–127.5 (*Ph*, MeO*Ph*); 113.8, 113.8, 113.7 (MeO*Ph*); 99.0 (C_{1d}); 98.9 (C_{1f}); 98.8 (C_{1b}); 98.3 (C_{1e}); 98.1 (C_{1c});

97.2 (C_{1a}); 55.2, 55.2 (CH_3OPh); 51.9, 51.6 (COOCH_3); 23.2, 21.5 (CH(CH_3)$_2$). MALDI-TOF *m/z* 2516.6 (M + Na$^+$); 2532.5 (M + K$^+$). Anal. calcd. for $C_{136}H_{143}N_9O_{37}$: C, 65.46; H, 5.73; N, 5.05; found: C, 65.32; H, 5.62; N, 5.24%.

Methyl (isopropyl-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1⟶4)-*O*–(2-*O*-benzoyl-3-*O*-benzyl-α-L-ido-pyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1⟶4)-*O*-(2-*O*-benzoyl-2-*O*-benzyl-α-L-ido-pyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1⟶4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-ido-pyranosyl) uronate (26). To a stirred solution of **14** (63 mg, 25 μmol) in dry CH_2Cl_2 (6 mL), CF_3COOH (138 μL) and H_2O (3.0 μL) were added at room temperature. After stirring for 15 min at this temperature, the reaction mixture became purple. Then, the reaction was neutralised with Et_3N (150 μL) and concentrated *in vacuo*. The residue was purified by flash chromatography (1 : 1 hexane–EtOAc), to yield **26** (51.3 mg, 96%). TLC 0.23 (1 : 1 hexane–EtOAc). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.20–7.1 (m, 50 H, *Ph*); 5.44 (m, 2 H, H_{1e}, H_{1c}); 5.26 (br s, 1 H, H_{1a}); 5.13 (m, 2 H, H_{2e}, H_{2c}); 5.05 (m, 1 H, H_{2a}); 4.91–4.69 (m, 13 H, H_{1b}, H_{1d}, H_{1f}, H_{5e}, H_{5c}, CH_2Ph); 4.64–4.58 (m, 3 H, H_{5a}, CH_2Ph); 4.46–4.31 (2 d, 2 H, CH_2Ph); 4.20 (m, 2 H, CH_2Ph); 4.10 (m, 3 H, H_{3e}, H_{3a}, H_{3c}); 4.01–3.92 (m, 5 H, H_{4a}, H_{4c}, H_{4e}, CH_2Ph, *CH*(CH$_3$)$_2$); 3.62, 3.54 and 3.42 (3 s, 9 H, COOCH_3); 3.25–3.16 (3 dd, 3 H, $J_{2,1}$ = 3.5 Hz, $J_{2,3}$ = 10 Hz, H_{2f}, H_{2d}, H_{2b}); 2.04 (br s, 3 H, 3 OH_6); 1.20–1.14 (2 d, 6 H, CH(CH_3)$_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.0, 169.6, 169.4, 165.6, 165.2, 165.1 (C=O); 137.7–127.3 (*Ph*); 99.1 (C_{1d}); 98.8 (C_{1f}); 98.5 (C_{1b}); 98.1 (C_{1e}); 98.0 (C_{1c}); 97.3 (C_{1a}); 52.2, 52.0 (COOCH_3); 23.2, 21.4 (CH (CH_3)$_2$).

Methyl (isopropyl*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1⟶4)-*O*–(2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1⟶4)-*O*-(2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1⟶4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyl) uronate trisodium salt (27). A mixture of **26** (50 mg, 23.0 μmol) and the SO_3·Py complex (56 mg, 0.35 mmol) in dry Py (2.0 mL) was stirred under an argon atmosphere. After 1 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 (1 : 1 MeOH–CH_2Cl_2). Fractions containing the hexasaccharide **27** were concentrated and passed through a Dowex 50WX4-Na$^+$ (2 : 1 MeOH–H_2O), to yield pure **27** (55 mg, 98%). TLC 0.25 (18 : 5 : 3 : 1 EtOAC–Py–H_2O–AcOH). ^{1}H-NMR (500 MHz, CD_3OD): δ 8.20–7.08 (m, 50 H, *Ph*); 5.55 (d, 1 H, $J_{1,2}$ = 2.5 Hz, H_{1e}); 5.48 (br s, 1 H, H_{1c}); 5.25 (br s, 1 H, H_{1a}); 5.17 (t, 1 H, $J_{2,1} \approx J_{2,3}$ = 3.5 Hz, H_{2e}); 5.14 (br s, 1 H, H_{2c}); 4.98 (s, 1 H, H_{2a}); 4.90–4.73 (m, 13 H, H_{1b}, H_{1d}, H_{1f}, H_{5e}, H_{5c}, H_{5a}, CH_2Ph); 4.44–4.35 (2 d, 2 H, CH_2Ph); 4.32–4.27 (m, 2 H, CH_2Ph); 4.22–4.15 (m, 5 H, H_{3c}, H_{3e}, CH_2Ph); 4.12–4.08 (m, 3 H, H_{3a}, CH_2Ph); 4.02 (m, 1 H, H_{4a}); 3.99–3.90 (m, 3 H, *CH*(CH$_3$)$_2$, H_{4c}, H_{4e}); 3.72, 3.46 and 3.39 (3 s, 9 H, COOCH_3); 3.32–3.22 (m, 3 H, H_{2f}, H_{2b}, H_{2d}); 1.19–1.15 (2 d, 6 H, CH(CH_3)$_2$).

Isopropyl *O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1⟶4)-*O*–(3-*O*-benzyl-α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1⟶4)-*O*–(3-*O*-benzyl-α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-O-sulfo-α-D-glucopyranosyl)-(1⟶4)-3-*O*-benzyl-α-L-idopyranosyluronic acid hexasodium salt (28). To a solution of **27** (50 mg, 20.4 μmol) in THF (8 mL) at −5 °C, 30% H_2O_2 (0.6 mL) and a 1.25 M aqueous solution of LiOH (0.70 mL) were added. After stirring for 24 h at room temperature, MeOH (1.1 mL) and a 3 M aqueous solution of KOH (2 mL) were added. After stirring for 24 h more the reaction was neutralised with resin (IRA-120 H$^+$), filtered and concentrated. The residue was purified on Sephadex LH-20 (9 : 1 MeOH–H_2O), to yield **28** (40.1 mg, 98%). TLC 0.33 (12 : 5 : 3 : 1 EtOAc–Py–H_2O–AcOH). ^{1}H-NMR (500 MHz, MeOD): δ 7.46–7.19 (m, 35 H, *Ph*); 5.34–5.30 (m, 2 H, H_{1e}, H_{1c}); 5.10–5.00 (m, 4 H, H_{1a}, H_{1b}, H_{1d}, H_{1f}); 3.59–3.51 (m, 4 H, H_{2a}, H_{2f}, H_{2b}, H_{2d}); 1.19 (m, 6 *H*, CH(CH_3)$_2$).

Isopropyl*O*-(2-deoxy-2-sulfamide-6-*O*-sulfo-α-D-glucopyranosyl)-(1⟶4)-*O*-(α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-deoxy-2-sulfamide-6-*O*-sulfo-α-D-glucopyranosyl)-(1⟶4)-*O*-(-α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-deoxy-2-sulfamide-6-*O*-sulfo-α-D-glucopyranosyl)-(1⟶4)-α-L-idopyranosyluronic acid nonasodium salt (5). A solution of **28** (25 mg, 11.1 μmol) in MeOH–H_2O (1.5 mL, 9 : 1) was hydrogenated in the presence of 10% Pd/C. After 24 h, the suspension was filtered and concentrated to give **29** as homogenous product by TLC 0.35 (4 : 5 : 3 : 1 EtOAc–Py–H_2O–AcOH). This compound was used directly for *N*-sulfation. The crude product was dissolved in H_2O (1 mL) and the pH of the solution was adjusted to 9.5 with 1 M solution of NaOH. The pyridine–sulfur trioxide complex (24 mg, 0.145 mmol, 10 eq. for each amine group) was added in portions during 1 h and the pH was maintained at 9.5. Subsequent additions of the complex were made after stirring for 2, 4, and 6 h, respectively. After 24 h, the mixture was neutralised with 0.1 M solution of HCl and then subjected to chromatography over a Sephadex G-25 column with 0.9% solution of NaCl. The appropriate fractions were pooled and passed through a column of Dowex 50WX4-Na$^+$ (9 × 1.2 cm) with 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 **5** (11.1 mg, 73% from **28**). TLC 0.35 (4 : 5 : 3 : 1 EtOAc–Py–H_2O–AcOH). ^{1}H-NMR (500 MHz, D_2O): δ 5.33 (d, 2 H, H_{1f}, H_{1b}); 5.31 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H_{1d}); 4.99 (br s, 2 H, H_{1c}, H_{1e}); 4.94 (d, 1 H, $J_{1,2}$ = 3.0 Hz, H_{1a}); 4.79 (br s, 2 H, H_{5c}, H_{5e}); 4.49 (d, 1 H, $J_{5,4}$ = 2.5 Hz, H_{5a}); 3.24–3.18 (m, 3 H, H_{2b}, H_{2d}, H_{2f}); 1.19–1.15 (m, 6 H, CH(CH_3)$_2$).

Isopropyl*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl]-α-D-glucopyranosyl)-(1⟶4)-*O*-(3-*O*-benzyl-α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-*O*-(3-*O*-benzyl-α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-3-*O*-benzyl-α-L-idopyranosyl) uronic acid (30). To a solution of **14** (30 mg, 12.0 μmol) in THF (4 mL) at −5 °C, H_2O_2 30% (0.4 mL) and 1.25 M aqueous solution of LiOH (0.70 mL) were added. After stirring for 24 h at 5 °C, MeOH (1.1 mL) and a 3 M aqueous solution of KOH (2 mL) was added. After stirring for 24 h more at the same temperature, the reaction was neutralized with acidic resin (IRA-120 H$^+$), filtered and concentrated. The residue was purified by Sephadex LH-20 (1 : 1 CH_2Cl_2–MeOH), to yield **30** (23.1 mg, 87%). TLC 0.48 (9 : 1 CH_2Cl_2–MeOH). ^{1}H-NMR (500 MHz, MeOD): δ 7.41–7.00 (m, 41 H, *Ph*, MeO*Ph*); 6.87–6.81 (m, 6 H, MeO*Ph*); 5.20 (d, 1 H, $J_{1,2}$ = 2.5 Hz, H_{1e}); 5.18 (d, 1 H, $J_{1,2}$ = 3.0 Hz, H_{1c}); 5.12 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H_{1f}); 5.10–5.07 (m, 2 H, H_{1b}, H_{1d}); 5.04 (d, 1 H, $J_{1,2}$ = 2.5 Hz, H_{1a}); 3.70, 3.69 and 3.67 (3 s, 9 H, CH_3OPh); 1.21–1.16 (m, 6 H, CH(CH_3)$_2$). MALDI-TOF *m/z* 2161.9 (M + Na$^+$); 2178.7 (M + K$^+$).

Isopropyl*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxy-benzyl)-α-D-glucopyranosyl)-(1⟶4)-*O*-(3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl)-(1⟶4)-*O*–(3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(*p*-methoxybenzyl]-α-D-glucopyranosyl)-(1⟶4)-3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyl) uronic acid hexasodium salt (31). A mixture of **30** (18.5 mg, 8.38 μmol) and SO_3·Py complex (20 mg, 0.12 mmol) in dry Py

(1.5 mL) was stirred under an argon atmosphere. After stirring for 8 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 (1 : 1 MeOH–CH_2Cl_2). Fractions containing the hexasaccharide were concentrated and passed through a Dowex 50WX4-Na^+ (2 : 1 MeOH–H_2O), to yield **31** (18 mg, 86%). TLC 0.44 (14 : 5 : 3:1 AcOEt–Py–H_2O–AcOH). ^{1}H-NMR (500 MHz, CD_3OD): δ 7.40–7.09 (m, 41 H, *Ph*, MeO*Ph*); 6.81–6.77 (m, 6 H, MeO*Ph*); 5.42 (d, 1 H, H_{1e}); 5.38 (d, 1 H, H_{1c}); 5.12 (d, 1 H, H_{1f}); 5.17–5.14 (m, 3 H, H_{1b}, H_{1d}, H_{1a}); 3.72 (s, 6 H, CH_3OPh); 3.65 (s, 3 H, CH_3OPh); 1.21–1.17 (m, 6 H, CH(CH_3)$_2$).

Isopropyl*O*-(2-deoxy-2-sulfamido-α-D-glucopyranosyl)-(1⟶4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-deoxy-2-sulfamido-α-D-glucopyranosyl)-(1⟶4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1⟶4)-*O*-(2-deoxy-2-sulfamido-α-D-glucopyranosyl)-(1⟶4)-2-*O*-sulfo-α-L-idopyranosyl) uronic acid nonasodium salt (6). A solution of **31** (18 mg, 7.16 µmol) in MeOH–H_2O (1.5 mL, 9 : 1) was hydrogenated in the presence of 10% Pd/C. After 24 h, the suspension was filtered and concentrated to give **32** which was homogenous on TLC (6 : 5 : 3 : 1 EtOAc–Py–H_2O–AcOH. This compound was directly submitted to *N*-sulfation. The hydrogenated hexasaccharide was dissolved in H_2O (1 mL) and the pH of the solution was adjusted to 9.5 with a 1 M solution of NaOH. A pyridine–sulfur trioxide complex (24 mg, 0.145 mmol, 10 eq. for each amine group) was added in portions during 1 h and the pH was maintained at 9.5. Subsequent additions of the pyridine–sulfur trioxide complex were made after stirring for 2, 4, and 6 h, respectively. After 24 h, the mixture was neutralised with 0.1 M solution of HCl and then subjected to chromatography over a Sephadex G-25 column with 0.9% solution of NaCl. The appropriate fractions were pooled and passed through a column of Dowex 50WX4-Na^+ (9 × 1.2 cm) 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 **6** (9.1 mg, 73% from **30**). ^{1}H-NMR (500 MHz, D_2O): δ 5.18–5.05 (d, 1 H, $J_{1,2} = 3.5$ Hz, H_{1b}); 5.28 (m, 2 H, H_{1d}, H_{1f}); 5.26–5.22 (m, 3 H, H_{1e}, H_{1c}, H_{1a}); 4.85 (br s, 2 H, H_{5c}, H_{5e}); 4.51 (d, 1 H, $J_{5,4} = 2.5$ Hz, H_{5a}); 3.44 (t, 1 H, $J_{4,3} = J_{4,5} = 9.5$ Hz, H_{4f}); 2.84–2.80 (m, 3 H, H_{2b}, H_{2d}, H_{2f}); 1.20–1.16 (m, 6 H, CH(CH_3)$_2$).

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