Synthesis of Tailor-Made Glycoconjugate Mimetics of Heparan Sulfate That Bind IFN-γ in the Nanomolar Range**

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Abstract: We have recently described the preparation of three building blocks for the combinatorial synthesis of heparan sulfate (HS) fragments. Herein we show that one of these building blocks (disaccharide **4**) allows the preparation, in high yields and with total α stereoselectivity, of tetra-, hexa- and octasaccharides from the heparin (HP) regular region, by using 2+2, 2+4 and 4+4 glycosylation strategies, respectively. These oligosaccharides were processed into sulfated derivatives bearing an allyl moiety in the anomeric position. The UV-promoted conjugation of these compounds with α,ω-bis-(thio)poly(ethylene glycol) spacers of three different lengths allowed us to prepare nine benzylated glycoconjugates. After final deprotection, the glycoconjugates **1a–c**, **2a–c** and **3a–c** were obtained and their ability to inhibit the interaction between IFN-γ and HP was tested by using surface plasmon resonance detection. Compound **3b**, containing two HP octasaccharides linked by a 50-Å linker was able to inhibit the IFN-γ/HP interaction with an IC_{50} value of approximately 35 nM. In addition, the nine glycoconjugates were perfect tools in the study to ascertain the topology of the IFN-γ binding site on HS. Compounds **1a–c**, **2a–c** and **3a–c**, by mimicking the alternating sulfated and nonsulfated regions found in HS, thus comprise the first example of a library of synthetic HS mimetics giving access to the "second level of molecular diversity" found in HS.

Keywords: heparin · combinatorial chemistry · cytokines · glycosylation · oligosaccharides

Introduction

Heparan sulfate (HS) is a member of the glycosaminoglycan (GAG) family and close in structure to heparin (HP), which is clinically used for its antithrombotic activity. It is a linear sulfated polysaccharide whose basic disaccharide unit is composed of a uronic acid 1,4-linked to a 2-deoxy-2-aminoglucose. HS chains, either at the cell surface or in the extracellular matrix, interact and regulate the activity of numerous proteins, such as growth factors, cytokines, chemokines, viral proteins or coagulation factors.[1,2] HS is one of the most heterogeneous biopolymers since the uronic acid may have either the D-gluco or L-ido configuration and various sulfation patterns (sulfoforms) may occur along the chain.[3–5] There is growing evidence that the formation of different HS structures is tightly controlled during biosynthesis, with the presumed goal of generating sequences with biological specificity.[6,7] Indeed, the anti-factor-Xa property of heparin is linked to the presence of a pentasaccharide in the HP chain with a precise uronic acid content and sulfation patterns that are able to bind with high affinity and specificity to antithrombin III (AT III).[8,9] The chemical synthesis of this pentasaccharide has led to the development of Arixtra, a Food and Drug Administration (FDA) and European Medicines Agency (EMEA) approved drug against deep-vein thrombosis.[10]

Impressive improvements in the synthesis of HS fragments have been made in the last decade and have allowed HS fragments with a large structural variety to be synthesised.[10–20] In this regard, we have shown that combinatorial synthesis is an ideal tool to generate all the sulfoforms of the basic disaccharide of chondroitin sulfate (another GAG).[21] More recently we have published the synthesis of

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[**] IFN-γ = γ-interferon.

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 DOI: 10.1002/chem.200306063 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

protected disaccharide building blocks, including compound **4**,[22,23] which are the basis of a combinatorial approach toward the synthesis of HS fragment libraries.[24] However, tight and specific binding between HS and a given protein is not solely regulated by these microheterogeneities, which represent only the first level of molecular diversity in HS chains (Figure 1). In fact, the polymer, typically composed of 50–200 disaccharide units (25–100 kD), is not fully heterogeneous. Quite regular *N*-acetylated regions (NA domains), mainly composed of D-glucuronic acid and *N*-acetylated glucosamine, and thus with low global charge, separate domains rich in L-iduronic acid and *N*-sulfated glucosamine (NS domains), which are hypervariable and highly charged (Figure 1a). The latter are typically three–eight disaccharides long, while the NA domains are more regular and encompass a larger area, around 15 disaccharides in length.[25] In between, mixed NA/NS regions of variable length make the transition between the NA and NS domains. Thus, in addition to the first level of molecular diversity discussed above, HS presents a second level of diversity, since the various combinations of NS and NA–NA/NS domains generate multiple charge distribution along the polymer chain (Figure 1b).

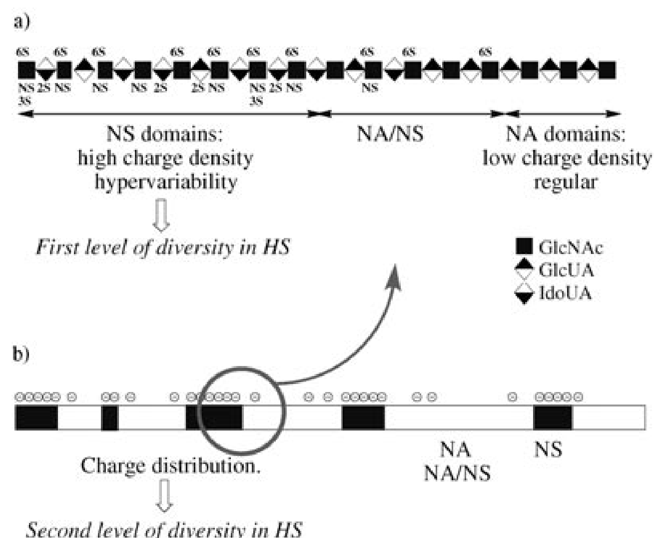

Figure 1. Dual molecular diversity in HS chains. a) Microheterogeneities resulting from the various uronic acid and sulfation motifs generate a first level of molecular diversity. b) The charge distribution due to the alternation of NS and NA–NA/NS domains of variable length leads to a second level of molecular diversity.

The primary interaction between HS and a protein is an attraction between the highly negatively charged NS domains and clusters of basic residues at the protein surface, mainly arginines and lysines. In some cases, for example, with AT-III,[8,9] fibroblast growth factors (FGFs)[1] or stromal cell-derived factor (SDF-1),[26] a single NS domain is sufficient to allow a high-affinity interaction, the specificity of which is then linked to the uronic acid and sulfation pattern of the NS domain. However, with other proteins, such as γ-interferon (IFN-γ),[27] platelet factor 4 (PF-4),[28] regulated-on-activation normally T-cell expressed and secreted proteins (RANTES) (9–68)[29] or macrophage inflamatory protein (MIP) 1α,[30] a single NS domain is not sufficient for binding and a longer fragment, including an NA domain, is needed for an efficient interaction. In fact, the proteins cited above are either multimeric in solution or multimerised upon binding to HS and the requirement for two distant NS domains reflects the fact that at least two basic domains of different subunits have to interact with the HS polymer to reach high binding constants. Recently, it has been proposed that the HS chain may adapt its conformation, and thus the distance between NS domains, in order to meet the needs of recognition of a protein (Figure 2a).[31] However, this will have an enthalpic and entropic cost and the binding of domains with the correct preformed geometry will thus be favoured. In this regard, HS glycoconjugates, in which a linker of an appropriate length separates two NS domains, should be a selective functional mimetic of the binding site of a given protein for HS (Figure 2b). Curiously, it is in the HP field that this approach has been tested, although HP is more rigid than HS and supports much less conformational accommodation.[31] HP mimetics containing a thrombin and an AT III binding site, linked by a carbohydrate[32–34] or a noncarbohydrate[35,36] spacer, have been prepared. These compounds were the first synthetic carbohydrates or mimetics displaying anti-factor-IIa and anti-factor-Xa properties. Moreover, compounds with the full anticoagulant properties of heparin but unable to bind PF-4 and thus devoid of one of the most severe side effects of HP were prepared by using this approach.[33] More recently, the "head-to-head" cross-linking of natural HP fragments, with an ethylenediamine linker, has allowed the preparation of mimetics able to bind RANTES with a greater affinity than a natural HP fragment with an equivalent length to the mimetic.[29]

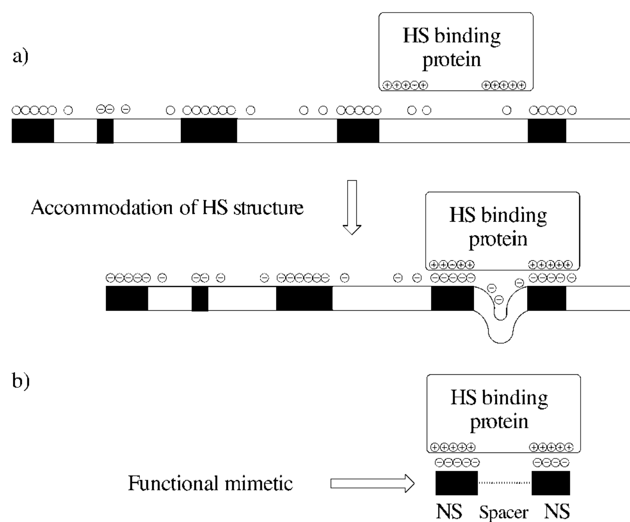

Figure 2. Model of binding on the HS polymer for a protein needing more than a single NS domain. a) Accommodation of the HS polymer conformation to meet the needs of an HS binding protein. b) Two NS domains linked by a spacer of optimum length should be a functional mimetic of the HS binding site of the protein.

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Herein we describe a strategy that opens up easy access to libraries of HS functional mimetics by combining HS synthetic fragments and α,ω-bis(thio)poly(ethylene glycol) spacers of different lengths (Figure 3b). To our knowledge, this is the first time that a methodology has been developed to address the question of the second level of molecular diversity found in HS chains. This project was started some years ago in order to prepare glycoconjugates mimicking HS and able to bind IFN-γ with high affinity and specificity.[37] IFN-γ is a Th-1 cytokine mainly secreted by NK and T cells. It was originally discovered on the basis of its antiviral activity but was later found to play a key role in the immune response and inflammatory processes. The binding of IFNγ to HS was recognised some years ago[38] and was found to control the blood clearance[39] and the subsequent tissue targeting, accumulation and localisation of the cytokine.[40] Thus, the discovery of compounds able to modulate the activity of IFN-γ could open the way to new immunotherapies,[41] especially in the field of Crohn's disease and ankylosing spondylarthropathies.[42]

IFN-γ is a C_2-symmetric homodimer in solution and binds to HS by virtue of basic residues located at the C terminus of the two subunits (Figure 3a).[43] A fragment of HS polymer, retaining the activity of the full-length polysaccharide, has been isolated by using a protection approach (an enzymatic digestion of HS polymer in the presence of IFN-γ).[27] The biochemical analysis of this fragment revealed that it was composed of two small hexa- to octasaccharide NS domains, separated by a more extended NA domain of 15–16 disaccharides (130–140 Å;[44] Figure 3a). A model of the interaction between IFN-γ and HS was thus proposed in which the KRKR domains,[45] located at the C terminus of each subunit of the IFN-γ dimer, would interact with the highly charged NS domains of the fragment.[27,39] However,

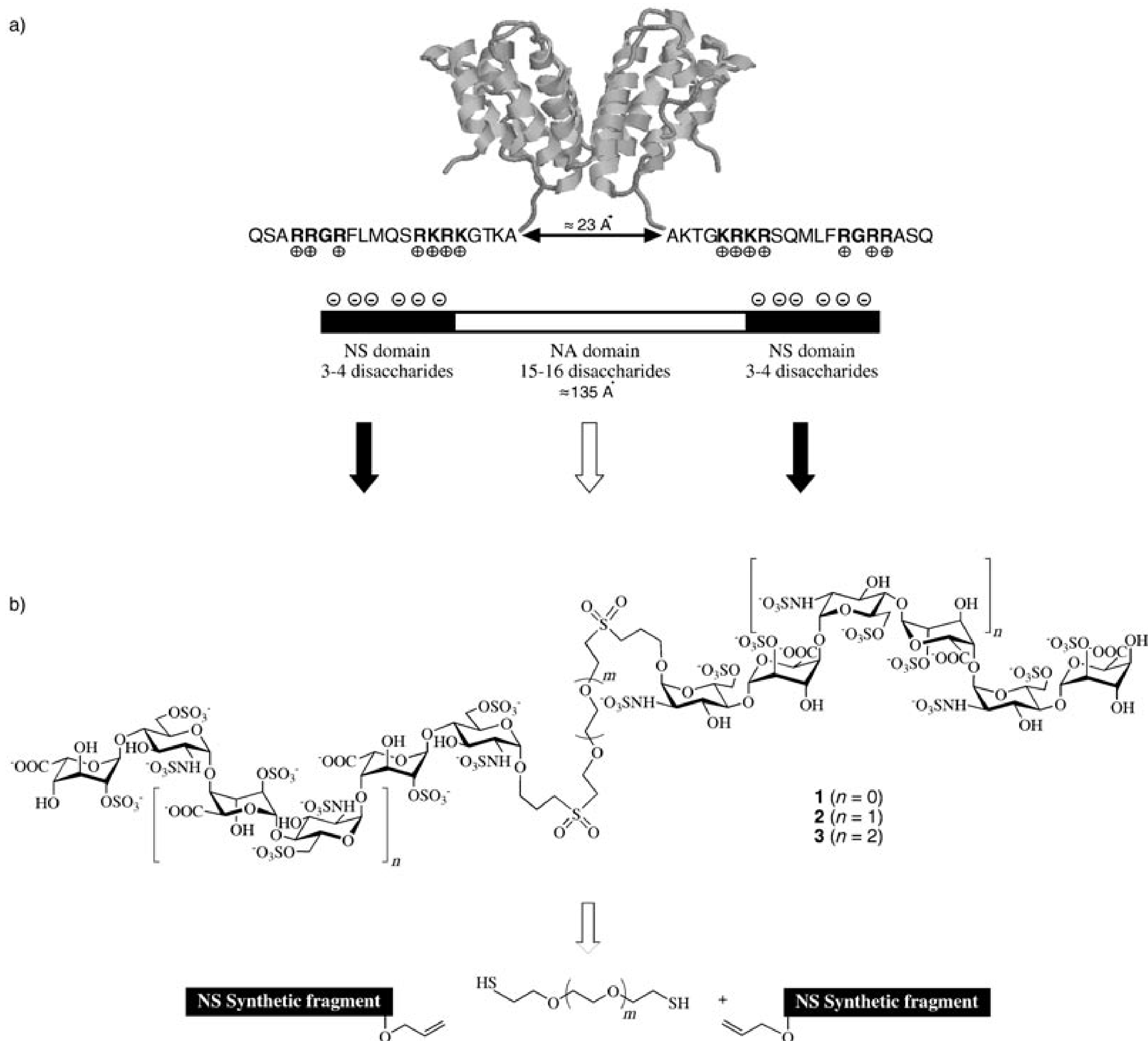


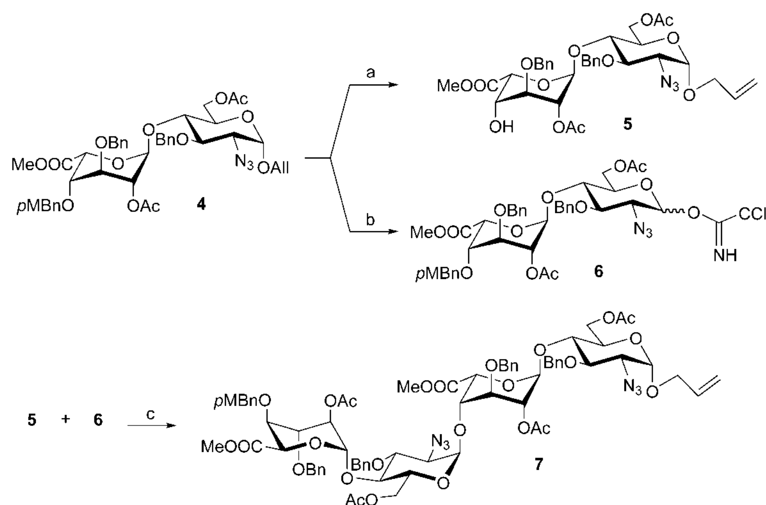
Figure 3. a) Molecular organisation of the HS binding site of IFN-γ. b) Glycoconjugates **1**–**3**, prepared by condensation of NS synthetic fragments bearing an allyl group in the anomeric position on bis(thio)PEGs, as functional mimetics of the IFN-γ binding site on HS.

X-ray diffraction crystal structures of C-termini-truncated dimers of IFN-γ,[46,47] reveal a distance of around 23 Å between the two Ala123 residues of the IFN-γ subunits; this distance is much shorter than the NA domain length (Figure 3a). This indicates that in this interaction the IFN-γ dimer has to adapt the HS chain conformation to its needs. To comfirm this hypothesis and to obtain compounds able to bind selectively and with high affinity to IFN-γ, we decided to prepare glycoconjugates in which two NS domains, ranging from tetra- to octasaccharides, would be linked by their reducing end to a spacer of the poly(ethylene glycol) (PEG) type (Figure 3b). In a model study toward this goal, we have shown that α,ω-bis(thio)PEGs add in high yields to allyl anomeric derivatives to give such C_2-symmetric glycoconjugates.[37] We chose PEG linkers for their water solubility and their availability in different lengths. In this regard, we have shown that, in the synthesis of the α,ω-bis(thio)PEG intermediate, α,ω-bis(bromo)-PEGs may be purified to near homogeneity by silica gel chromatography.[37] The use of PEGs as linkers has been criticised since their flexibility leads to lower binding constants than with more rigid spacers, due to the entropic cost needed for their organisation. However, such flexibility may be important to mimic NA domains whose conformational plasticity is thought to be important for their biological role.[31] Moreover, PEGs are essentially non-immunogenic and their incorporation into a drug is not a problem from the pharmacological point of view.

Results and Discussion

The preparation of the glycoconjugates **1–3**, relies upon the oligomerisation and functionalisation of the key disaccharide building block **4**, for which we have developed efficient, multigram syntheses.[22,23] These compounds already contain the L-iduronic moiety protected as methyl esters, in order to avoid oxidation steps that could be troublesome on elaborated material at the end of the synthesis. We opted for a protective-group pattern allowing the same building block to be transformed into a glycosyl donor or acceptor and making clear distinction between the positions to be sulfated and those remaining as free hydroxy groups. Thus, acetates protect the positions to be sulfated while benzyl ethers are used as permanent protection. A *para*-methoxybenzyl group at the 4′ position avoids the need for the preparation of a special building block for capping the nonreducing end at the end of the elongation process.[19,48] This group will be removed by hydrogenolysis with the benzyl ethers in the last step of the synthesis or, selectively, by oxidation or acidic treatment to furnish a disaccharide acceptor for chain elongation. The anomeric positions are protected as an allyl glycoside that can be removed to give, after activation of the anomeric position, a disaccharide donor.

Synthesis of tetrasaccharide 7, hexasaccharide 10 and octasaccharide 11: A 2+2, 2+4 or 4+4 strategy was planned for the preparation of tetrasaccharide **7**, hexasaccharide **10** and octasaccharide **11**; this strategy required that the *para*-methoxybenzyl group and the allyl group of disaccharide **4** and tetrasaccharide **7** could be removed orthogonally without affecting the other protecting groups. This was indeed done readily by using standard procedures (Scheme 1). The

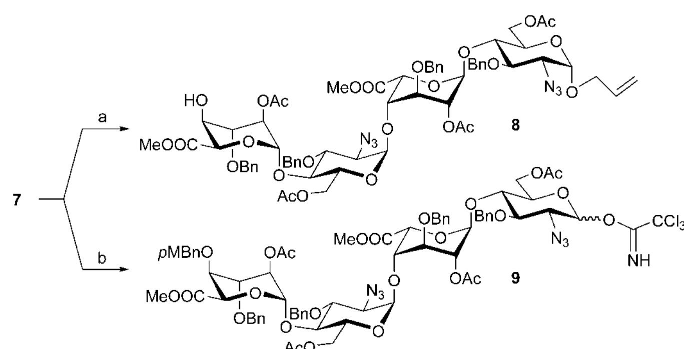


Scheme 1. a) DDQ, CH_2Cl_2, room temperature, 3 h, 83%; b) 1. H_2-activated $[Ir^I(C_8H_{14})(MePh_2P)_2]PF_6$, THF, room temperature, 2 h; 2. HgO/$HgCl_2$, acetone/H_2O (9:1), room temperature, 2 h; 3. Cl_3CCN, K_2CO_3, CH_2Cl_2, room temperature, 3 h, 88% (two steps); c) TBDMSOTf, CH_2Cl_2, $-40\rightarrow 0$°C, 90%. All = allyl, Bn = benzyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, *p*MBn = *para*-methoxybenzyl, TBDMSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate, THF = tetrahydrofuran.

para-methoxybenzyl group in the 4′ position of the known disaccharide **4**[22,23] was cleaved with DDQ, in wet CH_2Cl_2, to give the disaccharide acceptor **5**[22] in 83% yield. For the deallylation of compound **4**, $PdCl_2$/AcONa in aqueous AcOH[49] was tested first but gave only moderate isolated yields of the expected hemiacetal (60%). An isomerisation of the allyl group by using hydrogen-activated $[Ir^IC_8H_{14}$-$(MePh_2P)_2]PF_6$,[50] followed by mercuric salt promoted cleavage of the resulting anomeric 2-propenyl, gave better results.[51] The resulting hemiacetal was then treated with trichloroacetonitrile and K_2CO_3 in CH_2Cl_2 to give the imidate **6** as a 60:40 α/β mixture, in an overall yield of 88% from **4**. The β anomer of the disaccharide imidate **6** has already been prepared, by using a different protecting group strategy. However, it was only used as capping building block at the end of an elongation protocol in order to avoid sulfatation of the nonreducing-end 4-OH group of the prepared oligosaccharides.[48]

The condensation of the disaccharide acceptor **5** with the disaccharide donor **6**, in CH_2Cl_2 at −40°C and with

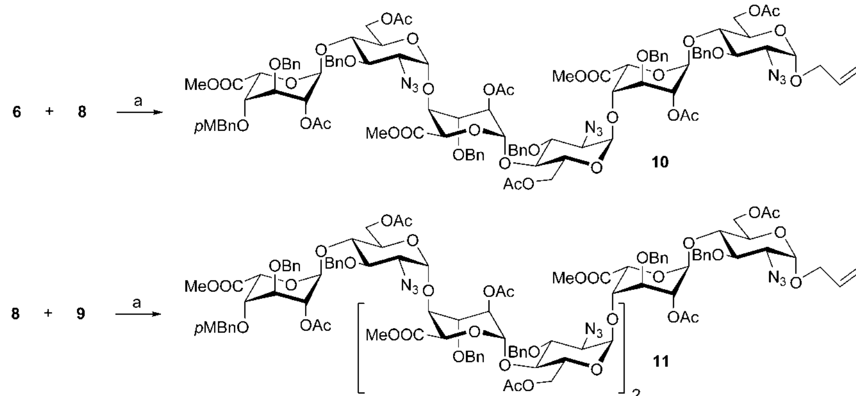
TBDMSOTf as a promotor, gave the desired tetrasaccharide **7** in excellent yield (90%) and total α stereoselectivity,[52] as expected for a donor with a nonparticipating group on the C-2 position (Scheme 1). Such a high stereoselectivity is a general tendency when 2-azidoglucose trichloroacetimidate mono- or oligosaccharide donors are condensed onto the 4-OH group of L-iduronyl acceptors.[10,16,19,48,53] This has been attributed previously to the conformations adopted by the L-iduronyl ring, which is in equilibrium between the $^1C_4$ and $^2S_0$ conformations, allowing the C-4 hydroxy group to partly occupy an axial position.[16] Indeed, in HP-fragment synthesis, lower diastereoselectivities have been observed mostly when 2-azidoglucose trichloroacetimidate donors were condensed onto D-glucuronyl acceptors that adopt the $^4C_1$ conformation.[16,24,54] Tetrasaccharide **7** was then converted into tetrasaccharide acceptor **8** and trichloroacetimidate **9** in 81% and 87% yields, respectively, by using the same protocols as described above (Scheme 2). The condensation of



Scheme 2. a) DDQ, CH_2Cl_2, room temperature, 3 h, 81%; b) 1. H_2-activated $[Ir^I(C_8H_{14})(MePh_2P)_2]PF_6$, THF, room temperature, 2 h; 2. HgO/$HgCl_2$, acetone/H_2O (9:1), room temperature, 2 h; 3. Cl_3CCN, K_2CO_3, CH_2Cl_2, room temperature, 3 h, 87% (two steps).

disaccharide and tetrasaccharide donors **6** and **9** with the tetrasaccharide acceptor **8**, under the same conditions as for the preparation of tetrasaccharide **7**, gave hexasaccharide **10** (68%) and octasaccharide **11** (93%) with total α stereoselectivity[55] (Scheme 3). This demonstrates that the single disaccharide **4** may be efficiently used as building block in a $2n+2m$ oligomerisation strategy, thereby alleviating the need for a special building block to cap the nonreducing end.

Preparation of the sulfated building blocks 21, 22 and 23: We have previously shown that the condensation of α,ω-bis(thio)PEGs onto α-allyl glucosaminyl derivatives proceeds better in water than in an organic solvent.[37] Thus, the coupling reaction has to be performed with water-soluble *O*- and *N*-sulfated oligosaccharides. Since the complete functionalisation and deprotection of the oligosaccharides **7**, **10** and **11** would result in the reduction of the allyl moiety during the last hydrogenolysis step, we chose to condense the α,ω-bis(thio)PEGs onto the benzylated, but water-soluble, oligosaccharides **21**–**23**. To this end, the oligosaccharides **7**, **10** and **11** were first deacetylated in near quantitative yields by using K_2CO_3 in MeOH (Scheme 4). The next step was to perform the reduction of the azido group without reducing the anomeric allyl moiety. The Staudinger reaction,[56] which worked well in model reactions performed on disaccharide **4**, gave a mixture of products when used on larger oligosaccharides. A catalytic reduction, with hydrogen and Lindlar catalyst in the presence of quinoline,[57] was tried next but led to concomitant reduction of the allyl group. SmI_2 has been described as an efficient and selective reagent for the reduction of azides[58] but, in our case, treatment of tetrasaccharide **7** with a solution of SmI_2 led to a mixture of products. However, 1,3-dithiopropane[59] allowed a clean reduction of the azido group in oligosaccharides **12**–**14** without any reduction or thiol addition on the allyl moiety when performed in the absence of light. Thus, the free-amino-containing tetrasaccharide **15**, hexasaccharide **16** and octasaccharide **17** were obtained in 92, 87 and 84% yields, respectively. The sulfation of both amino and hydroxy functions in compounds **15**–**17** was performed by using standard conditions. Thus, after treatment with the pyridine·SO_3 complex in pyridine, the sulfated oligosaccharides **18**–**20** were obtained in 73–96% yield. Saponification of the methyl esters was performed by using 2 M LiOH in 18% H_2O_2. The reaction on tetrasaccharide **18** proceeds smoothly at room temperature and went to completion within 24 h. With hexasaccharide **19** and octasaccharide **20**, the reaction did not go to completion within 24 h at room temperature. The temperature was thus raised to 37 °C to get reasonable reaction rates. Under these conditions, the saponification of compounds **19** and **20** was complete in 24 h without detectable epimerisation, β elimination or fragmentation. After purification by C-18 reversed-phase chromatography, the desired tetra-, hexa- and octasaccharides **21**, **22** and **23** were obtained in 73, 91 and 80% yields, respectively.



Scheme 3. a) TBDMSOTf, CH_2Cl_2, $-40\rightarrow 0$ °C, 68% (**10**), 93% (**11**).

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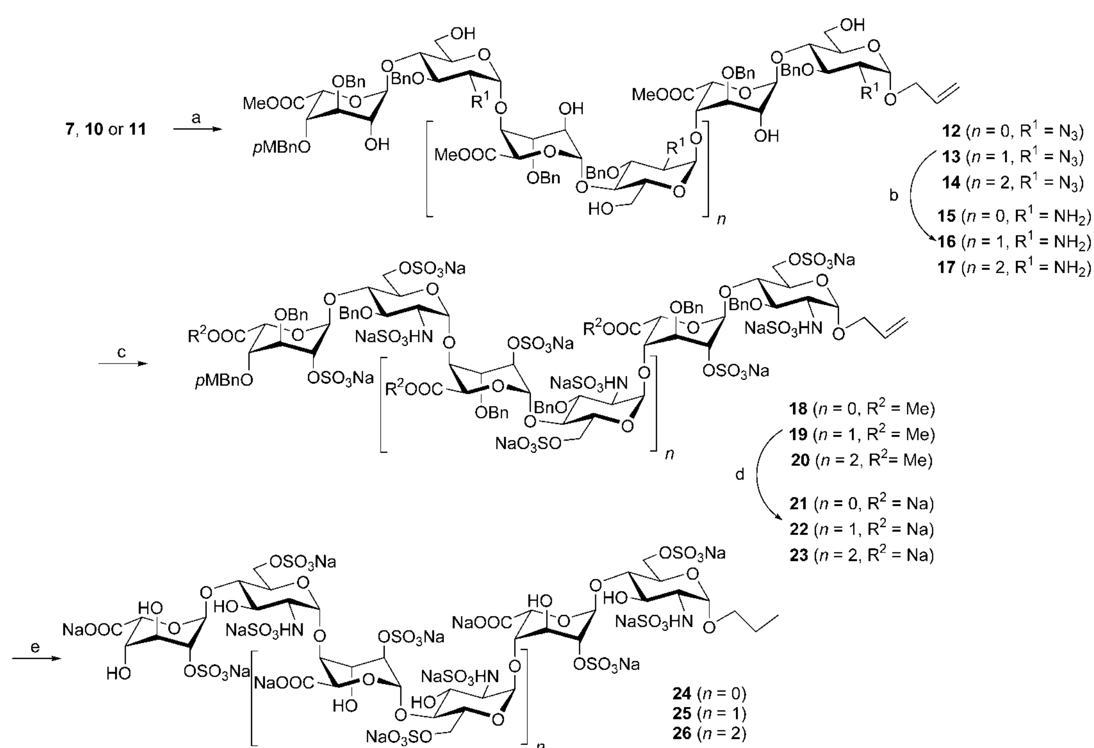

Scheme 4. a) K_2CO_3, MeOH, room temperature, 93 % (**12**), 88 % (**13**), 91 % (**14**); b) $HS(CH_2)_3SH$, NEt_3, MeOH, room temperature, 92 % (**15**), 87 % (**16**), 84 % (**17**); c) pyridine·SO_3, pyridine, 24 h, room temperature, then 24 h, 55 °C, 73 % (**18**), 95 % (**19**), 96 % (**20**); d) LiOH, H_2O_2, 73 % (**21**), 91 % (**22**), 80 % (**23**); e) $Pd(OH)_2$/C, H_2, phosphate buffer (pH 7.0), 48 h, room temperature, 94 % (**24**), 85 % (**25**), quant. (**26**).

Preparation of glycoconjugates 1a–c, 2a–c and 3a–c: For the syntheses of glycoconjugates **1a**–**c**, **2a**–**c** and **3a**–**c**, almost homogeneous α,ω-bis(thio)PEGs **27** ($m=5$) and **28** ($m=10$) were prepared from commercial PEG-300 and PEG-600, and a more polydisperse sample **29** ($m=32$) was prepared from PEG-1500.[37] These PEG lengths were chosen because, after conjugation with the allyl group, they should give linkers of different lengths (33, 50 and 114 Å), thereby allowing study of the effect of the distance between the sulfated regions on the biological activity of the glycoconjugates. The first two linkers should restrict the access of the NS domains to the first basic region of the IFN-γ C terminus (KRKR domain), while the longer linker should allow their access to the furthest basic region (RGRR domain; Figure 3a). The conditions that we had optimised in our preliminary studies[37] were first applied to the preparation of the nine targeted glycoconjugates **30a**–**c**, **31a**–**c** and **32a**–**c**. A mixture of tetrasaccharide **21** and α,ω-bis(thio)PEGs **27** was thus irradiated, in a quartz vessel, with the light of a medium-pressure Hg lamp (Scheme 5). However, the results were disappointing since HPLC analyses of the crude reaction mixture indicated that the major product was only formed in 40 %. Benzyl protecting groups have been reported recently to be reactive under short-wave UV irradiation.[60] The discrepancies between our model experiments and those performed with compounds **21** could, therefore, originate from the benzyl moieties. The coupling reactions were thus performed by using a filter with maximum transmittance at 360 nm. Under these conditions, the proportion of the desired glycoconjugates **30**–**32** rose to 70 %. Glycoconjugates **30a**, **30c**, **31a**, **31c** and **32a**–**c** were then purified by semipreparative RP-18 HPLC because we were not certain that the side products only included compounds containing changes in the number of benzyl groups. This yielded the conjugates in yields of 34–66 % and purities ranging from 92–98 %[61] (Scheme 5). These compounds were unambiguously characterised by ESI-MS mass analyses and ^{1}H NMR spectroscopy. In addition, HMQC experiments were performed on compounds **30a**, **31a** and **32b**. In our initial synthetic scheme the last step in the synthesis should have been the hydrogenolysis of the permanent benzyl protecting groups. However, due to the presence of the sulfide linkage this reaction failed. The thioether functions were thus oxidised to sulfones by using oxone,[62] to give the expected glycoconjugates **33a**, **33c**, **34a**, **34c** and **35a**–**c** (Scheme 5), unfortunately, as a mixture. Indeed products resulting from partial cleavage of the *para*-methoxybenzyl group were obtained, as shown by HPLC and ^{1}H NMR analyses. Compounds **30b** and **31b** were not purified at the thioether stage, but the coupling reaction mixtures were directly oxidised with oxone, and the resulting glycoconjugates were purified by semi-preparative RP-18 HPLC to give **33b** and **33c** in 21–31 % yield. These low yields indicate that, along with the side products arising from the UV irradiation, compounds that had the lost *para*-methoxybenzyl group were removed during the HPLC purification. To our knowledge, there is no report of oxidative removal of a *para*-methoxybenzyl group with oxone. However, the abstraction of a *para*-methoxybenzyl benzylic hydrogen atom by $KHSO_5$, with a mechanism similar to DDQ, cannot be excluded and

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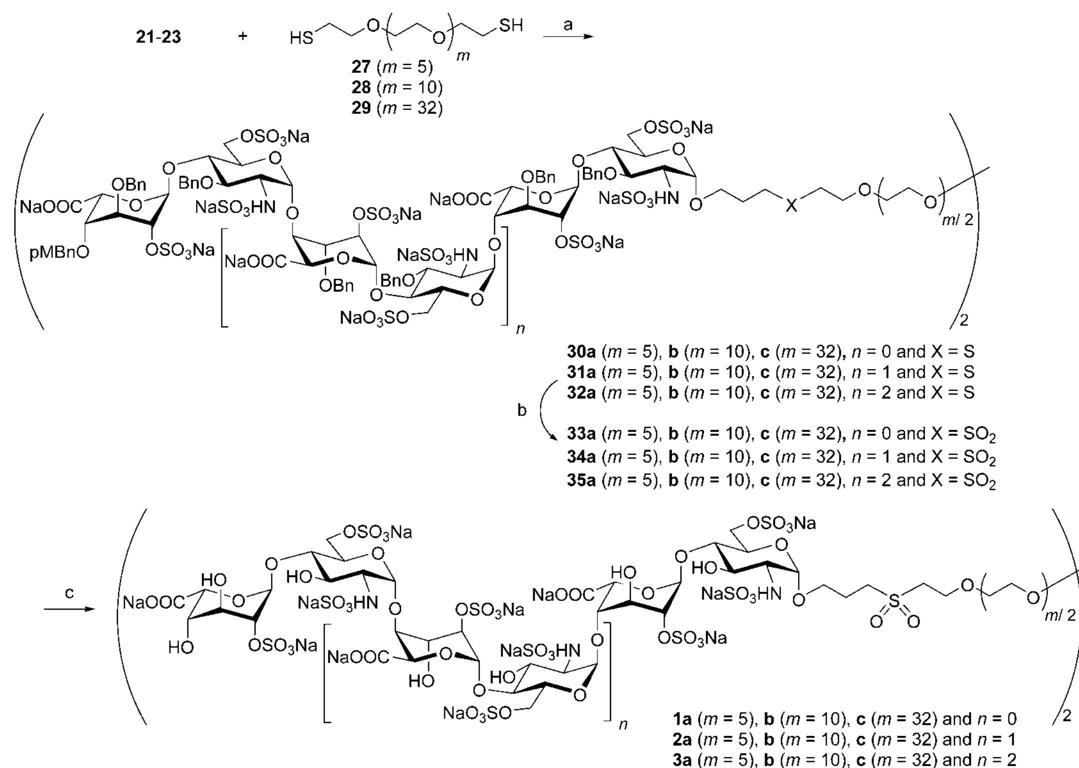

Scheme 5. a) $h\nu$ (360 nm), 34–66 %; b) $KHSO_5$ (oxone), K_2HPO_4, (pH 7), 23–31 % (combined yields for steps (a) and (b)); c) $Pd(OH)_2/C$, H_2, phosphate buffer (pH 7.0), 96 h, room temperature, 62 % to quant. (combined yields for steps (b) and (c)).

would explain the observed side reaction. Excess oxone allows efficient and quick conversion of sulfide into sulfone without detectable sulfoxide intermediates.[62] Thus, since partially deprotected side products should lead after hydrogenolysis to the same product as the fully protected ones, we decided to use this reagent in order to avoid uncompleted sulfide oxidation and formation of barely detectable sulfoxides. The final hydrogenolysis was thus performed directly after desalting (PD-10) the reaction mixture of the previous step. The oxidised glycoconjugates were thus obtained in 62% to quantitative yields[63] and were shown to be homogeneous by PAGE analysis (Figure 4), ^{1}H NMR spectroscopy and HSQC experiments. The MS analysis of large polyanionic HS- and HP-derived oligosaccharides is still a matter of intensive research.[64–67] The product signals are generally low for large oligosaccharides, even in the negative mode, since they poorly ionise. Thus, as expected, the recording of MS data on the free glycoconjugates was difficult even when using ESI techniques. A marked difference was indeed found between the signals of the debenzylated glycoconjugates and the ones obtained with the benzylated glycoconjugates, for which ESI-MS data in accordance with the proposed structures were obtained. However, correct ESI-MS data were obtained at least for compounds **1b**, **2b** and **3b**. PAGE analysis showed that synthetic tetra-, hexa- and octasaccharides **24–26** (lanes 2, 5 and 8, respectively, in Figure 4) have the same migration pattern and are more pure than the corresponding oligosaccharides obtained from natural sources (lanes 1, 4 and 7, respectively). Conjugation to PEG linkers is clearly evidenced by the shift in migration observed for **1b** (lane 3), **2b** (lane 6) and **3a–c** (lanes 9, 10 and 11, respectively). Moreover, the difference in the migration of the glycoconjugate **3a** (33 Å linker, lane 9) and the glycoconjugate **3b** (50 Å linker, lane 10) demonstrates the high-resolution power of this PAGE technique, since there is only a difference of 220 g mol^{-1} (≈4%) in molecular weight between the two compounds.

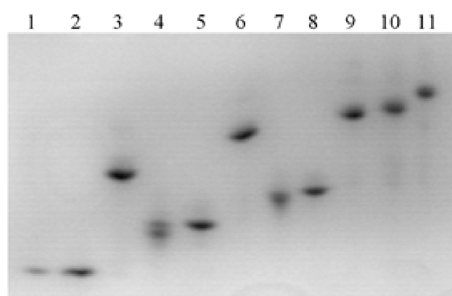

Figure 4. PAGE analysis of the glycoconjugates. Heparin-derived oligosaccharides (prepared as in ref. [25]) and glycoconjugates (1 µg each) were run through a 30% polyacrylamide gel and stained with azure A. Lane 1: heparin-derived tetrasaccharide; lane 2: **24**; lane 3: **1b**; lane 4: heparin-derived hexasaccharide; lane 5: **25**; lane 6: **2b**; lane 7: heparin-derived octasaccharide; lane 8: **26**; lane 9: **3a**; lane 10: **3b**; lane 11: **3c**.

Biological properties of the glycoconjugates 1a–c, 2a–c, 3a–c and 24–26: Surface plasmon resonance was used here as a detection system to analyse the ability of the nine glycoconjugates, **1a–c**, **2a–c** and **3a–c**, and the three nonconjugated controls, **24–26**, to inhibit the binding of IFN-γ to heparin.

Injection of IFN-γ (7.5 nM) over a Biacore sensor chip containing streptavidin and heparin produced a binding response of 175 resonance units (RU) at equilibrium. A response of 5–10 RU was observed with a similar injection over a streptavidin sensor chip, used as a blank surface (not shown). To obtain accurate glycoconjugate concentration data a colorimetric determination of the uronate content was performed.[68] The obtained values were in accordance with the weighted values. All the experiments were performed in duplicate or triplicate by using two or three batches of glycoconjugates prepared independently (standard errors were within 8–15 % of the mean). Preliminary experiments demonstrated that among the nine glycoconjugates, only **3a–c** displayed inhibition activity (not shown). The activities of the three octasaccharide glycoconjugates **3a–c** and the monomeric octasaccharide control **26** were first compared. For that purpose, IFN-γ (7.5 nM) was preincubated with 150 nM **3a–c** and **26** and injected over the heparin surface. Representative sensorgrams are shown in Figure 5A and demonstrate that the glycoconjugates **3a–c** strongly inhibit the IFN-γ/HP interaction, while the inhibition by the sole octasaccharide **26** is low. Moreover, there is a dependence of the activity on the linker length. Compound **3b** (50-Å linker length) is the most potent inhibitor while **3c** (114-Å linker length) is the least active and **3a** (33-Å linker length) has medium activity. By using the same binding assay, the concentration dependence of the inhibition by the most active glycoconjugate (**3b**) was then studied. Figure 5B shows that the activity of **3b** is dose dependant with an IC_{50} value of approximately 35 nM, that is, within the range of the dissociation constant, K_d, of IFN-γ on HS.[38]

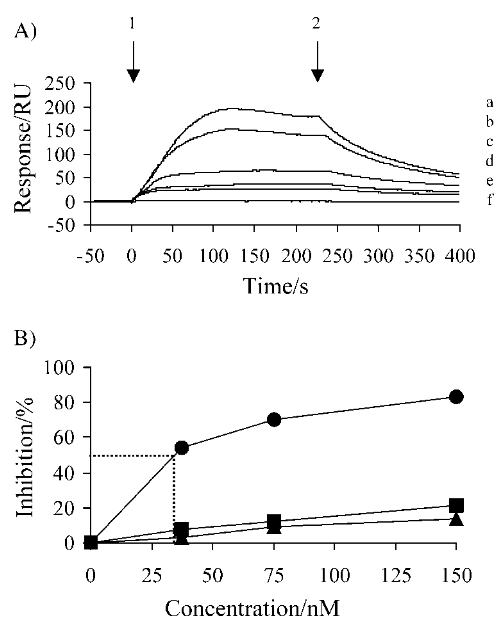

Figure 5. Inhibition of the IFNγ/heparin binding by different glycoconjugates. A) IFNγ (7.5 nM) was preincubated with 150 nM glycoconjugates and then injected (arrow 1, $t=0$) over a heparin-activated surface, as described in the experimental section. At the end of the injection phase (arrow 2, $t=240$ s), the formed complexes were washed with running buffer. The binding response (in RU) corresponding to the injection of IFNγ (a), IFNγ + **26** (b), IFNγ + **3c** (c), IFNγ + **3a** (d), IFNγ + **3b** (e), and plain buffer (f) was recorded as a function of time. B) IFNγ (7.5 nM) was preincubated with increasing concentrations (0–150 nM) of **3b** (●), **2b** (■) or **1b** (▲) and injected over a heparin-activated surface as described in the Experimental Section. The level of IFNγ bound to the heparin surface at the end of the association phase was recorded and the results were expressed as a percentage of inhibition.

These results are consistent with the view that the KRKR domains of the IFN-γ C termini are critically involved in the interaction. Moreover, it seems that the 33 Å linker is too small and needs to induce constraints in the IFN-γ dimer to allow binding, while the 114 Å linker is too long and has to adapt to the protein. Glycoconjugate **3b**, with a 50-Å linker, fits best to the IFN-γ dimer geometry and is thus the better functional mimetic of the HS binding site of IFN-γ. The optimal linker length is thus two to three times smaller than the NA domain found in the natural IFN-γ HS binding site. This confirms the hypothesis that the conformation of the natural HS binding site has to be adapted to IFN-γ geometry in order to allow an efficient binding. The C_2 symmetry of compound **3b** may also be an important factor for its activity by fitting better with the symmetry of the IFN-γ dimer than the natural HS fragment. It is thus possible that, in order to allow efficient binding, the NA domain of the natural fragment has to adopt a conformation allowing a more C_2-symmetric "head-to-head" presentation of the NS domains. Such a factor has already been found to be important in the binding of RANTES on HP.[29] The preparation of glycoconjugates with other symmetry will help to confirm this hypothesis.

Finally, as shown in Figure 5B, our results also point out the importance of the oligosaccharide size for binding. Indeed, the glycoconjugates containing tetrasaccharide (**1b**) or hexasaccharide (**2b**) NS domains, although linked with the optimal linker (50 Å), are unable to significantly interact with IFN-γ, even at the highest concentration used (150 nM).

Compound **3b** is thus a compound on which potential specific inhibitors of IFN-γ activity may be constructed.

Conclusion

We have thus demonstrated that disaccharide **4** is a potent building block for the preparation of oligosaccharides of different lengths deriving from the heparin regular region. From these oligosaccharides, we have optimised a methodology for the preparation of glycoconjugates mimicking the HS binding site of IFN-γ. We have thus found that compound **3b** was able to inhibit the IFN-γ/HP interaction with an IC_{50} value of approximately 35 nM. In addition, we have shown that the nine glycoconjugates, prepared with various oligosaccharide and PEG-linker lengths, are perfect tools to define the topology of the IFN-γ binding site on HS. However, these results give rise to new intriguing questions, since it is entirely unclear if other linker systems, linking the sugar moiety by the nonreducing end, longer oligosaccharides or modification of the sulfation and uronic acid patterns would show better properties for the exploration of this kind of interaction. We are currently developing the

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tools needed to answer these questions and the results will be reported in due course.

Experimental Section

General procedures: All moisture-sensitive reactions were performed under an argon atmosphere by using oven-dried glassware. All solvents were dried over standard drying agents[69] and freshly distilled prior to use. Evaporations were performed under reduced pressure. UV irradiation was performed using a 150 W medium-pressure Hg lamp (TQ 150, Heraeus) equipped with a midrange/longwave UV filter (45% transmittance at 360 nm, cut-off at 275 and 475 nm); during irradiation the samples were air cooled. Reactions were monitored by TLC on glass silica gel 60 F_{254} plates with detection by UV light at 254 nm and by charring with 5% ethanolic H_2SO_4 or orcinol reagent[70] for diluted solutions. Flash column chromatography was performed on Silica Gel 60 A.C.C. 6–35 μ (SDS) or on LiChroprep RP-18 (Merck). HPLC was performed by using a Waters Spherisorb ODS-2 5 μ C18 250×4.6 mm column and UV detection at 220 nm; HPLC purity is given assuming that the major compound and all the impurities have the same molar absorbtion at 220 nm. The elution was performed at a rate of 1 mL min^{-1} with a linear gradient of 10 mM AcOH-NEt_3 buffer (pH 7.0)/CH_3CN. Semipreparative HPLC was performed by using a Waters Spherisorb ODS-2 5 μ C18 250×20 mm column, eluting at 20 mL min^{-1} with a linear gradient of 5 mM AcOH-NEt_3 buffer (pH 7.0)/CH_3CN. Melting points were determined with a Büchi capillary apparatus and are uncorrected. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. NMR spectra were recorded at room temperature with Bruker AC200, AC250, AM250, AM360 or DRX400 spectrometers. Chemical shifts (δ) are given in parts per million (ppm) relative to an internal Me_4Si reference, solvent signals ($CDCl_3$: $\delta(^{13}C) = 77.0$ ppm) or acetone in D_2O ($\delta(^1H) = 2.225$ ppm and $\delta(^{13}C) = 30.5$ ppm). The allyl group carbon atoms are identified in the following way: O-C_aH_2–C_bH=C_cH_2; the two protons on C-c are identified as H-cc, for the one *cis* to H-b, and H-ct, for the one *trans* to H-b. Saccharide units in oligosaccharides are identified alphabetically from the reducing end. For compounds **10–17**, spin systems, identified with COSY experiments, were attibuted to a monosaccharide unit based on ^1H and ^{13}C chemical shifts; for compounds **18–20**, this attibution was confirmed by using HMBC experiments. Apodisations with Gaussian functions (LB = −1 to −2 Hz and GB = 50%) were used, thereby allowing measurement of coupling constants. COSY, gradient-enhanced COSY, HMBC, HMQC and HSQC experiments were performed by recording 256 FID measurements with 1024 complex data points and using standard Bruker programs. Prior to Fourier transformation, the data were zero filled in the t_1 dimension to 1024 points and multiplied with a nonshifted sinebell function in both dimensions for COSY experiments; for HMBC experiments, the data were zero filled in the t_1 dimension to 2048 points and an exponential multiplication (LB = 3 Hz) in the t_1 dimension and a π/2-shifted squared sinebell function in the t_2 dimension were used; for HSQC experiments, the data were zero filled in the t_1 dimension to 2048 points and multiplied with a π/3-shifted squared sinebell function in both dimensions. MS spectra were recorded in the positive or negative mode on a Finnigan MAT 95S spectrometer by using electrospray ionisation. IR spectra were recorded on a Fourier transformation Bruker IFS66 apparatus. Elemental analyses were performed at the CNRS (Gif sur Yvette, France).

Polyacrylamide gel electrophoresis: PAGE analysis was performed essentially as previously described.[71] Oligosaccharides (1 μg) in 20% glycerol were run initially through a stacking gel (8% acrylamide) at a constant current (15 mA), then through a resolving gel (30% acrylamide) at 20 mA, until the Phenol Red marker (applied to a separate lane as the electrophoresis marker) had reached the bottom of the gel. The discontinuous buffer system used comprised 0.125 M tris(hydroxymethyl)aminomethane (Tris)/HCl (pH 6.8) in the stacking gel, 0.375 M Tris/HCl (pH 8.8) in the resolving gel and 25 mM Tris/0.192 M glycine (pH 8.3) in the tank buffer. After electrophoresis, oligosaccharide bands were stained with 0.08% aqueous Azure A for a few minutes under constant agitation. Excess dye was removed by washing the gel in water.

IFN-γ/heparin binding analysis by using surface plasmon resonance: Binding analysis were performed on a Biacore 2000 apparatus, equilibrated with HBS-EP buffer (10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), 0.15 M NaCl, 3 mM ethylenediaminetetraacetate (EDTA), 0.05% surfactant P20, pH 7.4), at 25 °C. Size-defined heparin (9 kDa) was biotinylated at the reducing end and immobilised on a Biacore sensor chip.[26] For this purpose, two flow cells of a Biacore F1 sensor chip were activated with a mixture (50 μL) of 0.2 M 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide (EDC)/0.05 M *N*-hydroxysuccinimide (NHS) before injection of streptavidin (50 μL; 0.2 mg mL^{-1} in 10 mM acetate buffer, pH 4.2). Remaining activated groups were blocked with 1 M ethanolamine (50 μL; pH 8.5). Typically, this procedure, performed at a rate of 5 μL min^{-1}, permitted coupling of approximately 2000–2500 RU of streptavidin. Biotinylated heparin (5 μg mL^{-1}) in HBS-EP buffer was then injected at a rate of 5 μL min^{-1} over one of the two surfaces to obtain an immobilisation level of 50–60 RU. The other surface (streptavidin) was left as a blank surface. Flow cells were then conditioned with several injections of 1 M NaCl. For binding inhibition assays, IFN-γ (7.5 nM in HBS-EP buffer), either alone or preincubated with the oligosaccharides (0–150 nM; see legend of Figure 5), was injected at a rate of 50 μL min^{-1} over the two surfaces (streptavidin and streptavidin/heparin) for 4 min, after which the complexes formed were washed with buffer. The sensor-chip surface was regenerated at a rate of 50 μL mL^{-1} with a 4-min pulse of 1 M NaCl.

Allyl (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-glucopyranoside (5): DDQ (415 mg, 1.5 equiv) was added to a solution of disaccharide **4** (1.00 g, 1.2 mmol) in CH_2Cl_2 saturated with water (15 mL). After 3 h at room temperature, Et_2O (150 mL) was added and the resulting solution was successively washed with ice-cold satd aq $NaHCO_3$ solution (50 mL) and water (2×50 mL), filtered, dried ($MgSO_4$) and concentrated. Flash chromatography of the residue (silica gel, petroleum ether/AcOEt (1:1→4:6)) gave disaccharide **5** (700 mg, 83%), whose ^1H and ^{13}C NMR data were identical to those previously reported.[22]

(Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-α-L-idopyranosyluronate)-(1→4)-*O*-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-D-glucopyranoside trichloroacetimidate (6): The iridium catalyst ($(C_8H_{14}(MePh_2P)_2Ir^I$-$PF_6$; 20 mg, 0.024 mmol, 1.3 mol%) was added to a solution of disaccharide **4**[22,23] (1.49 g, 1.8 mmol) in THF (30 mL). The mixture was degassed, the Ir catalyst was activated with H_2 as previously described,[51] and, after 2 h at room temperature, the reaction was concentrated. HgO (565 mg, 2.16 mmol, 1.2 equiv) and $HgCl_2$ (538 mg, 1.98 mmol, 1.1 equiv) were then added to a solution of the residue in acetone/H_2O (9:1; 40 mL). After 2 h stirring at room temperature, the reaction mixture was filtered over a pad of celite 545 and concentrated. The residue was dissolved in Et_2O (150 mL) and the resulting solution was successively washed with 10% (w/v) aq KI solution (2×100 mL), satd aq $Na_2S_2O_6$ (50 mL) and water (2×50 mL), filtered, dried ($MgSO_4$) and concentrated. Flash chromatography of the residue (silica gel, toluene/AcOEt (8:2→6:4)) gave the anticipated hemiacetal (1.26 g, 90%) whose ^1H and ^{13}C NMR data were identical to those previously reported.[48] This compound was dissolved in CH_2Cl_2 (2.5 mL) and trichloroacetonitrile (970 μL, 9.7 mmol, 6 equiv) was added, followed by potassium carbonate (400 mg, 3.2 mmol, 2 equiv). After being stirred for 3 h at room temperature, the reaction mixture was directly applied to the top of a flash chromatography column filled with silica gel and eluted (toluene/AcOEt (9:1→8:2) with 0.1% NEt_3) to give imidate **6** (1.51 g, 98%) as an α/β (4:6) mixture. Along with the previously described β anomer[48] (60%), we also obtained the α anomer (40%): ^1H NMR (250 MHz, $CDCl_3$): $\delta = 8.73$ (s, 1H; NH), 7.40–7.22 (m, 10H; Ph), 7.11 (d, $J = 8.5$ Hz, 2H; *Ph*OMe), 6.82 (d, $J = 8.5$ Hz, 2H; *Ph*OMe), 6.37 (d, $J_{1,2} = 3.5$ Hz, 1H; H-1^A), 5.26 (d, $J_{1,2} = 4.5$ Hz, 1H; H-1^B), 4.92 (d, $J = 10.5$ Hz, 1H; C*H*$_2$Ph), 4.90 (t, $J_{2,3} = J_{2,1} = 4.5$ Hz, 1H; H-2^B), 4.75–4.64 (m, 3H), 4.63 (d, $J = 10.5$ Hz, 1H; C*H*$_2$Ph), 4.45 (d, $J = 11.5$ Hz, 1H; C*H*$_2$PhOMe), 4.39 (dd, $J_{6a,6b} = 12.5$, $J_{6a,5} = 2.0$ Hz, 1H; H-6$_a^A$), 4.37 (d, $J = 11.5$ Hz, 1H; C*H*$_2$PhOMe), 4.20 (dd, $J_{6b,6a} = 12.5$, $J_{6b,5} = 3.5$ Hz, 1H; H-6$_b^A$), 4.11 (dd, $J_{4,5} = 10.0$, $J_{4,3} = 9.0$ Hz, 1H; H-3^A or H-4^A), 3.98 (ddd, $J_{5,4} = 10.0$, $J_{5,6b} = 3.5$, $J_{5,6a} = 2.0$ Hz, 1H; H-5^A), 4.02 (dd, $J_{3,2} = 10.0$, $J_{3,4} = 9.0$ Hz, 1H; H-3^A or H-4^A), 3.95–3.90 (m with s at $\delta = 3.80$, 5H; H-4^B, H-3^B and PhO*Me*), 3.69 (dd, $J_{2,3} = 10.0$, $J_{2,1} = 3.5$ Hz, 1H; H-2^A), 3.54 (s, 3H; COOMe), 2.04 (s, 3H; CH_3 OAc), 2.03 (s, 3H; CH_3 OAc) ppm; ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta =$

170.5, 170.0, 169.8 (C=O), 160.3 (C=NH), 159.4 (*C*–OMe *p*MBn), 137.7 ($C_{quat\ arom}$), 129.6, 129.3, 128.4, 128.1, 128.0, 127.9, 127.5 (C_{arom}), 113.7 (C_m *p*MBn), 98.0 (C-1[B]), 94.4 (C-1[A]), 78.2, 75.2, 75.0, 74.7, 74.4, 73.0, 72.4, 71.8, 70.8, 70.2, 62.8 (C-2[A]), 61.6 (C-6[A]), 55.2 (CH_3 *p*MBn), 51.7 (CH_3 COOMe), 20.9, 20.6 (*C*H_3 OAc) ppm.

Allyl [(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-α-L-idopyranosyluronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside)-(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-glucopyranoside (7): Acceptor **5** (460 mg, 0.66 mmol) and imidate **6** (785 mg, 0.85 mmol, 1.3 equiv) were azeotropically dried with toluene and dissolved in CH_2Cl_2 (2 mL). Powdered 4-Å molecular sieves (1.86 g) were added and the mixture was then stirred for 30 min at room temperature. The solution was cooled to −40 °C and TBDMSOTf (0.1 M in CH_2Cl_2, 1.3 mL, 0.13 mmol, 0.2 equiv) was added. The reaction was stirred at this temperature for 30 min and then the temperature was raised to 0 °C over 30 min. The reaction was then quenched with a solution of NEt_3 (0.1 M in CH_2Cl_2, 400 μL, 0.4 mmol, 0.6 equiv), directly applied to the top of a flash chromatography column filled with silica gel and eluted (CH_2Cl_2/AcOEt/petroleum ether (85:10:5→70:25:5)) to give tetrasaccharide **7** (860 mg, 90 %): $[\alpha]_D^{30}=17$ ($c=1.05$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.24 (m, 20H; Ph), 7.13 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.82 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 5.94 (dddd, $J_{b,ct}$ = 17.0, $J_{b,cc}$ = 10.5, $J_{b,a'}$ = 6.0, $J_{b,a}$ = 5.0 Hz, 1H; H-b), 5.36 (dq, $J_{ct,b}$ = 17.0, $J_{ct,a}=J_{ct,a'}=J_{gem}$ = 1.5 Hz, 1H; H-ct), 5.28 (d, $J_{1,2}$ = 5.0 Hz, 1H; H-1[B]), 5.26 (dq, $J_{cc,b}$ = 10.5, $J_{cc,a}=J_{cc,a}=J_{gem}$ = 1.5 Hz, 1H; H-cc), 5.24 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1[D]), 4.94 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1[A]), 4.93 (dd, $J_{2,3}$ = 4.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2[D]), 4.91 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1[C]), 4.90 (d, J = 10.5 Hz, 1H; CH_2Ph), 4.86 (dd, $J_{2,3}$ = 5.5, $J_{2,1}$ = 5.0 Hz, 1H; H-2[B]), 4.82 (d, J = 10.5 Hz, 1H; CH_2Ph), 4.73 (s, 2H; CH_2Ph), 4.69 (d, J = 10.5 Hz, 1H; CH_2Ph), 4.68 (d, $J_{5,4}$ = 4.0 Hz, 1H; H-5[D]), 4.66 (s, 2H; CH_2Ph), 4.63 (d, J = 10.5 Hz, 1H; CH_2Ph), 4.59 (d, $J_{5,4}$ = 5.0 Hz, 1H; H-5[B]), 4.46 (d, J = 11.5 Hz, 1H; CH_2PhOMe), 4.42 (d, J = 11.5 Hz, 1H; CH_2PhOMe), 4.40 (br d, $J_{6a,6b}$ = 12.0 Hz, 1H; H-6_a[A]), 4.34 (dd, $J_{6a,6b}$ = 12.5, $J_{6a,5}$ = 2.0 Hz, 1H; H-6_a[C]), 4.24 (dd, $J_{6b,6a}$ = 12.0, $J_{6b,5}$ = 3.0 Hz, 1H; H-6_b[A]), 4.20 (ddt, J_{gem} = 13.0, $J_{a,b}$ = 5.0, $J_{a,cc}=J_{a,ct}$ = 1.5 Hz, 1H; H-a), 4.16 (dd, $J_{6b,6a}$ = 12.5,$J_{6b,5}$ = 3.0 Hz, 1H; H-6_b[C]), 4.05 (ddt, J_{gem} = 13.0, $J_{a',b}$ = 6.0, $J_{a',cc}=J_{a',ct}$ = 1.5 Hz, 1H; H-a′), 3.98 (dd, $J_{4,3}$ = 5.0, $J_{4,5}$ = 4.0 Hz, 1H; H-4[D]), 3.95 (dd, $J_{4,5}$ = 10.0, $J_{4,3}$ = 9.0 Hz, 1H; H-4[C]), 3.92 (dd, $J_{3,4}$ = 5.0, $J_{3,2}$ = 4.0 Hz, 1H; H-3[D]), 3.90–3.85 (m, 3H; H-3[A], H-4[A] and H-5[A]), 3.82–3.78 (m with s at δ = 3.80, 5H; H-4[B], H-5[C] and PhO*Me*), 3.76 (t, $J_{3,2}=J_{3,4}$ = 5.5 Hz, 1H; H-3[B]), 3.65 (dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 9.0 Hz, 1H; H-3[C]), 3.56 (s, 3H; COOMe), 3.49 (s, 3H; COOMe), 3.39 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2[A]), 3.29 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2[C]), 2.12 (s, 3H; CH_3 OAc), 2.06 (s, 3H; CH_3 OAc), 2.03 (s, 3H; CH_3 OAc), 2.02 (s, 3H; CH_3 OAc) ppm; ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.6, 170.5, 169.9, 169.8, 169.7, 169.3 (C=O), 159.4 (*C*–OMe *p*MBn), 137.8, 137.7, 137.6, 137.3 ($C_{quat\ arom}$), 133.0 (C-b), 129.6, 129.2, 128.4, 128.3, 128.0, 127.8, 127.6, 127.5, 127.4 (C_{arom}), 118.3 (C-c), 113.7 (C_m *p*MBn), 97.9 (C-1[D]), 97.8 (C-1[B]), 97.4 (C-1[C]), 96.5 (C-1[A]), 78.3 (C-3[A]), 77.8 (C-3[C]), 75.7 (C-4[A]), 75.5 (C-4[C]), 75.4 (C-3[B]), 75.1 (C-4[B]), 74.9 (*C*H_2Ph), 74.7 (*C*H_2Ph), 73.7 (C-3[D]), 73.5 (*C*H_2Ph), 73.3 (*C*H_2Ph), 72.8 (C-4[D]), 72.5 (CH_2 *p*MBn), 71.1 (C-5[B]), 70.9 (C-2[B]), 69.6 (C-5[C]), 69.3 (C-5[D]), 69.1 (C-5[A]), 68.8 (C-2[D]), 68.7 (C-a), 63.2 (C-2[A]), 62.8 (C-2[C]), 62.1 (C-6[A]), 61.6 (C-6[C]), 55.2 (CH_3 *p*MBn), 51.9 (CH_3 COOMe), 51.7 (CH_3 COOMe), 20.8, 20.7, 20.6 (CH_3 OAc) ppm; IR (thin film): $\tilde{\nu}$ = 3083, 3058, 3024 (C–H_{arom}), 2949, 2933, 2866, 2833 (C–H_{aliph}), 2109 (N_3), 1740 (C=O), 1613, 1514, 1455, 1438, 1372, 1295, 1245 cm^{-1}; elemental analysis: calcd (%) for $C_{73}H_{84}N_6O_{28}$ (1461.5 g mol^{-1}): C 59.99, H 5.79, N 5.75, O 28.46; found: C 60.22, H 5.76, N 5.66, O 28.16.

Allyl [(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-glucopyranoside)-(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-glucopyranoside (8): Tetrasaccharide **7** (465 mg, 0.32 mmol) was treated with DDQ (144 mg, 0.63 mmol, 2 equiv) in wet CH_2Cl_2 (10 mL) as described for the preparation of **5**. Flash chromatography (silica gel, toluene/AcOEt (8:2→6:4)) gave compound **8** (370 mg, 81 %) along with unreacted starting material (44 mg, 9 %): ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.40–7.25 (m, 20H; Ph), 5.95 (dddd, $J_{b,ct}$ = 17.0, $J_{b,cc}$ = 10.5, $J_{b,a'}$ = 6.0, $J_{b,a}$ = 5.0 Hz, 1H; H-b), 5.36 (dq, $J_{ct,b}$ = 17.0, $J_{ct,a}=J_{ct,a'}=J_{gem}$ = 1.5 Hz, 1H; H-ct), 5.27 (dq, $J_{cc,b}$ = 10.5, $J_{cc,a}=J_{cc,a}=J_{gem}$ = 1.5 Hz, 1H; H-cc), 5.20 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1[B]), 5.06 (br d, $J_{1,2}$ = 1.0 Hz, 1H; H-1[D]), 4.95 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1[A]), 4.93 (d, $J_{5,4}$ = 2.5 Hz, 1H; H-5[D]), 4.92 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1[C]), 4.93 (m, 1H; H-2[D]), 4.83 (d, $J_{5,4}$ = 2.5 Hz, 1H; H-5[B]), 4.82 (d, J = 10.5 Hz, 1H; CH_2Ph), 4.77 (d, J = 11.0 Hz, 2H; CH_2Ph), 4.75 (t, $J_{2,3}=J_{2,1}$ = 3.5 Hz, 1H; H-2[B]), 4.71 (d, J = 12.0 Hz, 1H; CH_2Ph), 4.70 (d, J = 11.0 Hz, 1H; CH_2Ph), 4.69 (d, J = 11.0 Hz, 1H; CH_2Ph), 4.62 (d, J = 12.0 Hz, 1H; CH_2Ph), 4.62 (d, J = 10.5 Hz, 1H; CH_2Ph), 4.41 (br d, $J_{6a,6b}$ = 12.0, 2H; H-6_a[A], H-6_a[C]), 4.26 (br d, $J_{6b,6a}$ = 12.0 Hz, 1H; H-6_b[A]), 4.21 (ddt, J_{gem} = 13.0, $J_{a,b}$ = 5.0, $J_{a,cc}=J_{a,ct}$ = 1.5 Hz, 1H; H-a), 4.17 (dd, $J_{6b,6a}$ = 12.0, $J_{6b,5}$ = 2.5 Hz, 1H; H-6_b[C]), 4.05 (ddt, J_{gem} = 13.0, $J_{a',b}$ = 6.0, $J_{a',cc}=J_{a',ct}$ = 1.5 Hz, 1H; H-a′), 4.03–3.96 (m, 2H), 3.96 (m, 6H), 3.78 (br d, $J_{5,4}$ = 9.5 Hz, 1H; H-5[C]), 3.73–3.58 (m, 3H), 3.48 (s, 3H; COOMe), 3.42 (s, 3H; COOMe), 3.39 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2[A]), 3.28 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2[C]), 2.59 (d, J = 10.5 Hz, 1H; OH), 2.11 (s, 3H; CH_3 OAc), 2.07 (s, 3H; CH_3 OAc), 2.06 (s, 3H; CH_3 OAc), 2.05 (s, 3H; CH_3 OAc) ppm; IR (thin film): $\tilde{\nu}$ = 3470 (O–H_{arom}), 3088, 3064, 3033 (C–H_{arom}), 2978, 2955, 2932, 2908, 2869 (C–H_{aliph}), 2111 (N_3), 1749, 1733 (C=O), 1500, 1498, 1455, 1432, 1373, 1304, 1244 cm^{-1}; ESI HRMS: m/z calcd for $C_{65}H_{76}N_6O_{28}Na$ $[M+Na]^+$: 1363.4758; found: 1363.4758.

[(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-α-L-idopyranosyluronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside)-(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-glucopyranoside trichloroacetimidate (9): Tetrasaccharide **7** (322 mg, 0.22 mmol) was treated first with $[Ir^IC_8H_{14}(MePh_2P)_2]PF_6$ (3 mg, 3.5 μmol, 1.6 mol %) in THF (3.5 mL) and then with HgO (69 mg, 0.26 mmol, 1.2 equiv) and $HgCl_2$ (67 mg, 0.24 mmol, 1.1 equiv) as described for the preparation of **6**. Flash chromatography (silica gel, toluene/AcOEt (70:30→55:45)) gave the anticipated hemiacetal (298 mg, 81 %) whose ^{1}H NMR spectrum showed the disappearance of the allyl protons. ESI HRMS: m/z calcd for $C_{70}H_{80}N_6O_{26}Na_2$ $[M+2Na]^{2+}/2$: 733.2459; found: 733.2459. This compound was then dissolved in CH_2Cl_2 (400 μL); trichloroacetonitrile (125 μL, 1.25 mmol, 6 equiv) was then added, followed by potassium carbonate (51 mg, 0.42 mmol, 2 equiv). After 1 h stirring at room temperature the reaction mixture was directly applied to the top of a flash chromatography column filled with silica gel and eluted (toluene/AcOEt (9:1→7:3) with 0.1 % NEt_3) to give imidate **9** (338 mg, 98 %) as an α/β (4:6) mixture: ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.76 (s, 0.4H; NH), 8.73 (s, 0.6H; NH), 7.40–7.24 (m, 20H; Ph), 7.13 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.82 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.39 (d, $J_{1,2}$ = 3.5 Hz, 0.4H; H-1[Aα]), 5.60 (d, $J_{1,2}$ = 8.5 Hz, 0.6H; H-1[Aβ]), 5.28 (d, $J_{1,2}$ = 5.0 Hz, 1H; H-1[D]), 5.25 (d, $J_{1,2}$ = 3.5 Hz, 0.4H; H-1[Bα]), 5.22 (d, $J_{1,2}$ = 3.5 Hz, 0.6H; H-1[Bβ]), 4.47 (d, J = 11.5 Hz, 1H; CH_2PhOMe), 4.41 (d, J = 11.5 Hz, 1H; CH_2PhOMe), 4.45 (br d, $J_{6a,6b}$ = 12.5, 0.4H; H-6_a[Aα]), 4.44 (br d, $J_{6a,6b}$ = 12.5, 0.6H; H-6_a[Aβ]), 4.36 (br d, $J_{6a,6b}$ = 12.5 Hz, 1H; H-6_a[C]), 4.23 (dd, $J_{6b,6a}$ = 12.5, $J_{6b,5}$ = 4.0 Hz, 0.6H; H-6_b[Aβ]), 4.22 (dd, $J_{6b,6a}$ = 12.5, $J_{6b,5}$ = 4.0 Hz, 0.4H; H-6_b[Aα]), 4.17 (dd, $J_{6b,6a}$ = 12.5, $J_{6b,5}$ = 2.0 Hz, 1H; H-6_b[C]), 4.04–3.88 (m, 5H), 3.88–3.74 (m with s at δ = 3.79, 5H; PhO*Me*), 3.74–3.58 (m, 3H), 3.55 (s, 3H; COOMe), 3.50 (s, 1.8H; COOMe[Bβ]), 3.49 (s, 1.2H; COOMe[Bα]), 3.43 (t, $J_{2,3}=J_{2,1}$ = 8.5 Hz, 0.6H; H-2[Aβ]), 3.30 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2[C]), 2.11 (s, 1.8H; CH_3 OAc[β]), 2.10 (s, 1.2H; CH_3 OAc[α]), 2.06 (s, 3H; CH_3 OAc), 2.04 (s, 6H; CH_3 OAc) ppm.

Allyl [(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-α-L-idopyranosyluronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside)-(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-glucopyranoside)-(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-glucopyranoside (10): Acceptor **8** (150 mg, 0.11 mmol) and imidate **6** (135 mg, 0.15 mmol, 1.3 equiv) were azeotropically dried with toluene and dissolved in CH_2Cl_2 (360 μL). Powdered 4-Å molecular sieves (330 mg) were added and the mixture was then stirred for 30 min at room temperature. The solution was cooled to −40 °C and TBDMSOTf (0.1 M in CH_2Cl_2, 220 μL, 0.022 mmol, 0.2 equiv) was added. The reaction was stirred at this temperature for 30 min and then the temperature was raised to 0 °C over 30 min. The reaction was then quenched with a solution of NEt_3 (0.1 M in CH_2Cl_2, 63 μL, 0.063 mmol, 0.6 equiv), directly applied to the top of a flash chromatography column filled with silica gel and eluted (CH_2Cl_2/AcOEt/petroleum ether (85:10:5→70:25:5)) to give hexasaccharide **10** (210 mg, 68 %): $[\alpha]_D^{30}=17$ ($c=1.50$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.23 (m, 30H; Ph), 7.13 (d, J = 8.5 Hz, 2H;

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Ph-OMe), 6.82 (d, $J=8.5$ Hz, 2H; *Ph*-OMe), 5.94 (dddd, $J_{b,ct}=17.0$, $J_{b,cc}=10.5$, $J_{b,a'}=6.0$, $J_{b,a}=5.0$ Hz, 1H; H-b), 5.36 (dq, $J_{ct,b}=17.0$, $J_{ct,a}=J_{ct,a'}=J_{gem}=1.5$ Hz, 1H; H-ct), 5.30 (d, $J_{1,2}=5.0$ Hz, 1H; H-1^{D}), 5.29 (d, $J_{1,2}=5.0$ Hz, 1H; H-1^{B}), 5.27 (dq, $J_{cc,b}=10.5$, $J_{cc,a}=J_{cc,a}=J_{gem}=1.5$ Hz, 1H; H-cc), 5.24 (d, $J_{1,2}=3.5$ Hz, 1H; H-1^{F}), 4.96 (d, $J_{1,2}=3.5$ Hz, 1H; H-1^{C}), 4.94 (d, $J_{1,2}=3.5$ Hz, 1H; H-1^{A}), 4.93 (t, $J_{2,3}=J_{2,1}=3.5$ Hz, 1H; H-2^{F}), 4.93 (d, $J_{1,2}=3.5$ Hz, 1H; H-1^{E}), 4.90 (dd, $J_{2,3}=6.0$, $J_{2,1}=5.0$ Hz, 1H; H-2^{D}), 4.90 (d, $J=10.5$ Hz, 1H; CH_2Ph), 4.87 (t, $J_{2,3}=5.0$ Hz, 1H; H-2^{B}), 4.84 (d, $J=10.5$ Hz, 1H; CH_2Ph), 4.82 (d, $J=10.5$ Hz, 1H; CH_2Ph), 4.77 (d, $J=11.5$ Hz, 1H; CH_2Ph), 4.76 (d, $J=11.0$ Hz, 1H; CH_2Ph), 4.72 (d, $J=11.0$ Hz, 1H; CH_2Ph), 4.70 (d, $J_{5,4}=4.0$ Hz, 1H; H-5^{F}), 4.69 (d, $J=10.5$ Hz, 1H; CH_2Ph), 4.69 (d, $J=11.5$ Hz, 1H; CH_2Ph), 4.69 (d, $J=12.0$ Hz, 1H; CH_2Ph), 4.65 (d, $J=10.5$ Hz, 1H; CH_2Ph), 4.65 (d, $J=12.0$ Hz, 1H; CH_2Ph), 4.64 (d, $J=10.5$ Hz, 1H; CH_2Ph), 4.59 (d, $J_{5,4}=5.0$ Hz, 1H; H-5^{B}), 4.56 (d, $J_{5,4}=4.5$ Hz, 1H; H-5^{D}), 4.47 (d, $J=11.5$ Hz, 1H; CH_2PhOMe), 4.43 (d, $J=11.5$ Hz, 1H; CH_2PhOMe), 4.40 (br d, $J_{6a,6b}=11.5$ Hz, 1H; H-6$_a^{A}$), 4.37 (dd, $J_{6a,6b}=12.0$, $J_{6a,5}=2.0$ Hz, 1H; H-6$_a^{E}$), 4.34 (dd, $J_{6a,6b}=12.5$, $J_{6a,5}=2.0$ Hz, 1H; H-6$_a^{C}$), 4.25 (dd, $J_{6b,6a}=11.5$, $J_{6b,5}=2.5$ Hz, 1H; H-6$_b^{A}$), 4.20 (ddt, $J_{gem}=13.0$, $J_{a,b}=5.0$, $J_{a,cc}=J_{a,ct}=1.5$ Hz, 1H; H-a), 4.19 (dd, $J_{6b,6a}=12.0$, $J_{6b,5}=2.5$ Hz, 1H; H-6$_b^{E}$), 4.16 (dd, $J_{6b,6a}=12.5$, $J_{6b,5}=2.5$ Hz, 1H; H-6$_b^{C}$), 4.05 (ddt, $J_{gem}=13.0$, $J_{a',b}=6.0$, $J_{a',cc}=J_{a',ct}=1.5$ Hz, 1H; H-a'), 3.99 (t, $J_{4,3}=J_{4,5}=4.0$ Hz, 1H; H-4^{F}), 3.98 (dd, $J_{4,3}=5.5$, $J_{4,5}=4.5$ Hz, 1H; H-4^{D}), 3.95 (t, $J_{4,5}=J_{4,3}=9.0$ Hz, 1H; H-4^{C}), 3.92 (dd, $J_{3,4}=5.5$, $J_{3,2}=6.0$ Hz, 1H; H-3^{D}), 3.90 (dd, $J_{3,4}=4.0$, $J_{3,2}=3.5$ Hz, 1H; H-3^{F}), 3.89–3.85 (m, 3H; H-3^{A}, H-4^{A}, H-5^{A}), 3.87 (dd, $J_{4,5}=10.0$, $J_{4,3}=9.0$ Hz, 1H; H-4^{E}), 3.83 (br t, $J_{5,4}=9.0$ Hz, 1H; H-5^{C}), 3.82–3.78 (m with s at $\delta=3.80$, 5H; H-4^{B}, H-5^{E}, PhO*Me*), 3.77 (t, $J_{3,2}=J_{3,4}=5.0$ Hz, 1H; H-3^{B}), 3.66 (dd, $J_{3,2}=10.0$, $J_{3,4}=9.0$ Hz, 1H; H-3^{E}), 3.62 (dd, $J_{3,2}=10.0$, $J_{3,4}=9.0$ Hz, 1H; H-3^{C}), 3.57 (s, 3H; COOMe), 3.53 (s, 3H; COOMe), 3.48 (s, 3H; COOMe), 3.39 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 1H; H-2^{A}), 3.29 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 1H; H-2^{C}), 3.27 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 1H; H-2^{E}), 2.12 (s, 3H; CH_3 OAc), 2.09 (s, 3H; CH_3 OAc), 2.06 (s, 3H; CH_3 OAc), 2.03 (s, 3H; CH_3 OAc), 2.02 (s, 3H; CH_3 OAc), 2.01 (s, 3H; CH_3 OAc) ppm; ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta=170.6$, 170.5 (2C), 169.9 (2C), 169.8, 169.7, 169.5, 169.3 (C=O), 159.5 (*C*−OMe *p*MBn), 137.8, 137.7, 137.6, 137.4, 137.3 ($C_{quat\ arom}$), 133.1 (C-b), 129.6, 129.3, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7 (C_{arom}), 118.3 (C-c), 113.7 (C_m *p*MBn), 98.0 (C-1^{F}), 97.8 (C-1^{B}, C-1^{C}, C-1^{D}), 97.4 (C-1^{E}), 96.5 (C-1^{A}), 78.3 (C-3^{A}), 77.8 (C-3^{C}), 77.7 (C-3^{E}), 75.7 (C-4^{A}), 75.6 (C-4^{B}), 75.5 (C-4^{C}), 75.4 (C-3^{B}), 75.3 (C-4^{D}), 75.2 (C-3^{F}), 74.9 (*C*H$_2$Ph), 74.7 (*C*H$_2$Ph), 74.0 (*C*H$_2$Ph), 73.6 (C-3^{D}, *C*H$_2$Ph), 73.4 (*C*H$_2$Ph), 73.3 (*C*H$_2$Ph), 72.6 (C-4^{F}, CH_2 *p*MBn), 71.3 (C-5^{B}), 71.1 (C-2^{B}), 69.7 (C-5^{E}), 69.6 (C-5^{C}), 69.3 (C-5^{F}), 69.2 (C-5^{A}), 68.8 (C-2^{F}), 68.7 (C-a), 63.3 (C-2^{A}), 62.9 (C-2^{E}), 62.7 (C-2^{C}), 62.1 (C-6^{A}), 61.6 (C-6^{C}, C-6^{E}), 55.2 (CH_3 *p*MBn), 51.9 (2×CH_3 COOMe), 51.7 (CH_3 COOMe), 20.8, 20.7 (CH_3 OAc) ppm; IR (thin film): $\tilde{\nu}=3100$, 3066, 3033, 2999 (C−H_{arom}), 2953, 2928, 2870 (C−H_{aliph}), 2109 (N_3), 1742 (C=O), 1613, 1515, 1497, 1455, 1438, 1371, 1304, 1235 cm^{-1}; elemental analysis: calcd (%) for $C_{104}H_{119}N_9O_{38}$ (2103.2 g mol^{-1}): C 59.39, H 5.70, N 5.99, O 28.91; found: C 59.41, H 5.88, N 5.97.

Allyl [(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-α-L-idopyranosyluronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside)-(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-glucopyranoside)-(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-glucopyranoside)-(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-glucopyranoside (11): Acceptor **8** (184 mg, 0.14 mmol) and imidate **9** (298 mg, 0.19 mmol, 1.3 equiv) were azeotropically dried with toluene and dissolved in CH_2Cl_2 (510 μL). Powdered 4-Å molecular sieves (420 mg) were added and the mixture was then stirred for 30 min at room temperature. The solution was cooled to −40 °C and TBDMSOTf (0.1 M in CH_2Cl_2, 288 μL, 0.029 mmol, 0.2 equiv) was added. The reaction was stirred at this temperature for 30 min and then the temperature was raised to 0 °C over 30 min. The reaction was then quenched with a solution of NEt_3 (0.1 M in CH_2Cl_2, 82 μL, 0.082 mmol, 0.6 equiv), directly applied to the top of a flash chromatography column filled with silica gel and eluted (CH_2Cl_2/AcOEt/petroleum ether (85:10:5→70:25:5)) to give octasaccharide **11** (343 mg, 93 %): $[\alpha]_D^{28}=19$ ($c=2.53$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.38$–7.26 (m, 40H; Ph), 7.13 (d, $J=8.5$ Hz, 2H; *Ph*-OMe), 6.83 (d, $J=8.5$ Hz, 2H; *Ph*-OMe), 5.94 (dddd, $J_{b,ct}=17.0$, $J_{b,cc}=10.5$, $J_{b,a'}=6.0$, $J_{b,a}=5.0$ Hz, 1H; H-b), 5.36 (dq, $J_{ct,b}=17.0$, $J_{ct,a}=J_{ct,a'}=J_{gem}=1.5$ Hz, 1H; H-ct), 5.31 (d, $J_{1,2}=5.0$ Hz, 1H; H-1^{D} or H-1^{F}), 5.30 (d, $J_{1,2}=6.0$ Hz, 2H; H-1^{B}, H-1^{D} or H-1^{F}), 5.26 (dq, $J_{cc,b}=10.5$, $J_{cc,a}=J_{cc,a}=J_{gem}=1.5$ Hz, 1H; H-cc), 5.24 (d, $J_{1,2}=3.5$ Hz, 1H; H-1^{H}), 4.98 (d, $J_{1,2}=3.5$ Hz, 1H; H-1$^{E(C)}$), 4.97 (d, $J_{1,2}=3.5$ Hz, 1H; H-1$^{C(E)}$), 4.94 (d, $J_{1,2}=3.5$ Hz, 1H; H-1^{A}), 4.93 (t, $J_{2,3}=J_{2,1}=3.5$ Hz, 1H; H-2^{H}), 4.93 (d, $J_{1,2}=3.5$ Hz, 1H; H-1^{E}), 4.91 (d, $J=10.0$ Hz, 1H; CH_2Ph), 4.90 (m, 2H; H-2^{D}, H-2^{F}), 4.88 (t, $J_{2,3}=6.0$ Hz, 1H; H-2^{B}), 4.86 (d, $J=10.0$ Hz, 1H; CH_2Ph), 4.85 (d, $J=10.0$ Hz, 1H; CH_2Ph), 4.83 (d, $J=11.0$ Hz, 1H; CH_2Ph), 4.78 (d, $J=11.0$ Hz, 1H; CH_2Ph), 4.76 (d, $J=11.0$ Hz, 1H; CH_2Ph), 4.74 (s, 2H; CH_2Ph), 4.73 (d, $J_{5,4}=4.0$ Hz, 1H; H-5^{H}), 4.70 (br d, $J=11.0$ Hz, 3H; 3×CH_2Ph), 4.69 (d, $J=12.0$ Hz, 1H; CH_2Ph), 4.67 (d, $J=10.0$ Hz, 1H; CH_2Ph), 4.66 (d, $J=10.0$ Hz, 1H; CH_2Ph), 4.65 (d, $J=12.0$ Hz, 1H; CH_2Ph), 4.64 (d, $J=10.0$ Hz, 1H; CH_2Ph), 4.59 (d, $J_{5,4}=5.0$ Hz, 1H; H-5^{B}), 4.58 (d, $J_{5,4}=5.0$ Hz, 1H; H-5^{D} or H-5^{F}), 4.56 (d, $J_{5,4}=5.0$ Hz, 1H; H-5^{D} or H-5^{F}), 4.47 (d, $J=11.5$ Hz, 1H; CH_2PhOMe), 4.43 (d, $J=11.5$ Hz, 1H; CH_2PhOMe), 4.40 (d, $J_{6a,6b}=12.0$ Hz, 1H; H-6$_a^{A}$), 4.37 (br d, $J_{6a,6b}=11.0$, 2H; H-6$_a^{E(C)}$, H-6$_a^{G}$), 4.34 (dd, $J_{6a,6b}=12.0$, $J_{6a,5}=1.5$ Hz, 1H; H-6$_a^{C(E)}$), 4.25 (dd, $J_{6b,6a}=12.0$, $J_{6b,5}=2.5$ Hz, 1H; H-6$_b^{A}$), 4.20 (ddt, $J_{gem}=13.0$, $J_{a,b}=5.0$, $J_{a,cc}=J_{a,ct}=1.5$ Hz, 1H; H-a), 4.22–4.16 (m, 2H; H-6$_b^{E(C)}$, H-6$_b^{E}$), 4.17 (dd, $J_{6b,6a}=12.0$, $J_{6b,5}=3.0$ Hz, 1H; H-6$_b^{C(E)}$), 4.05 (ddt, $J_{gem}=13.0$, $J_{a',b}=6.0$, $J_{a',cc}=J_{a',ct}=1.5$ Hz, 1H; H-a'), 4.20–3.97 (m, 3H; H-4^{D}, H-4^{F}, H-4^{H}), 3.96 (dd, $J_{4,5}=10.0$, $J_{4,3}=9.0$ Hz, 1H; H-4$^{C(E)}$), 3.95–3.90 (m, 3H; H-3^{D}, H-3^{F}, H-3^{H}), 3.90–3.87 (m, 3H; H-3^{A}, H-4^{A}, H-5^{A}), 3.88 (dd, $J_{4,5}=10.0$, $J_{4,3}=9.0$, 2H; H-4$^{E(C)}$, H-4^{G}), 3.87–3.79 (m with s at $\delta=3.80$, 7H; H-4^{B}, H-5$^{C(E)}$, H-5$^{E(C)}$, H-5^{G}, PhO*Me*), 3.77 (t, $J_{3,2}=J_{3,4}=6.0$ Hz, 1H; H-3^{B}), 3.67 (dd, $J_{3,2}=10.0$, $J_{3,4}=9.0$ Hz, 1H; H-3^{G}), 3.64 (dd, $J_{3,2}=10.0$, $J_{3,4}=9.0$ Hz, 1H; H-3$^{E(C)}$), 3.63 (dd, $J_{3,2}=10.0$, $J_{3,4}=9.0$ Hz, 1H; H-3$^{C(E)}$), 3.57 (s, 3H; COOMe), 3.54 (s, 3H; COOMe), 3.53 (s, 3H; COOMe), 3.48 (s, 3H; COOMe), 3.39 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 1H; H-2^{A}), 3.30 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 1H; H-2$^{C(E)}$), 3.28 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 1H; H-2$^{E(C)}$), 3.27 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 1H; H-2^{G}), 2.12 (s, 3H; CH_3 OAc), 2.10 (s, 3H; CH_3 OAc), 2.09 (s, 3H; CH_3 OAc), 2.06 (s, 3H; CH_3 OAc), 2.03 (s, 6H; CH_3 OAc), 2.02 (s, 6H; CH_3 OAc) ppm; ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=170.6$, 170.5 (3C), 169.9 (2C), 169.7, 169.6 (2C), 169.5, 169.4, 169.2 (C=O), 158.9 (*C*−OMe *p*MBn), 137.8, 137.6, 137.5, 137.4, 137.3, 137.2 ($C_{quat\ arom}$), 132.9 (C-b), 129.6, 129.2, 129.9, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4 (C_{arom}), 118.3 (C-c), 113.7 (C_m *p*MBn), 97.9 (C-1^{H}), 97.8 (C-1^{B}, C-1$^{C(E)}$, C-1^{D}, C-1^{F}), 97.4 (C-1$^{E(C)}$), 97.1 (C-1^{G}), 96.5 (C-1^{A}), 78.3 (C-3^{A}), 77.8, 77.7 (C-3^{C}, C-3^{E}, C-3^{E}), 75.6, 75.5, 75.4, 75.3, 75.2 (C-4^{A}, C-4^{B}, C-4^{C}, C-4^{D} or C-4^{F}, C-4^{E}, C-4^{G}, C-3^{B}), 74.8 (C-3^{H}, *C*H$_2$Ph), 74.7 (2*C*H$_2$Ph), 74.6 (*C*H$_2$Ph), 74.0 (*C*H$_2$Ph), 73.9 (*C*H$_2$Ph), 73.5 (C-3^{D}, C-3^{F}, *C*H$_2$Ph), 73.3 (C-4^{D} or C-4^{F}, *C*H$_2$Ph), 72.5 (C-4^{H}, CH_2 *p*MBn), 71.2 (C-5^{B}), 71.1 (C-2^{B}), 70.5 (C-5^{D} or C-5^{F}), 70.2 (C-2^{D} or C-2^{F}, C-5^{D} or C-5^{F}), 70.0 (C-2^{D} or C-2^{F}), 69.6–69.5 (3C, C-5^{C}, C-5^{E}, C-5^{G}), 69.2 (C-5^{H}), 69.1 (C-5^{A}), 68.7 (C-2^{H}), 68.6 (C-a), 63.3 (C-2^{A}), 62.8, 62.7, 62.6 (C-2^{C}, C-2^{E}, C-2^{G}), 62.0 (C-6^{A}), 61.5 (C-6^{C}, C-6^{E}, C-6^{G}), 55.2 (CH_3 *p*MBn), 51.9 (3×CH_3 COOMe), 51.7 (CH_3 COOMe), 20.8, 20.7, 20.6 (CH_3 OAc) ppm; IR (thin film): $\tilde{\nu}=3097$, 3065, 3034 (C−H_{arom}), 2955, 2932, 2877 (C−H_{aliph}), 2115 (N_3), 1749 (C=O), 1613, 1515, 1497, 1455, 1439, 1373, 1304, 1250 cm^{-1}; elemental analysis: calcd (%) for $C_{135}H_{154}N_{12}O_{50}$ (2744.8 g mol^{-1}): C 59.08, H 5.66, N 6.12, O 29.15; found: C 59.02, H 5.43, N 5.96, O 29.13.

Allyl [(methyl 3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)]-(1→4)-*O*-3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside hexasodium salt (18): Tetrasaccharide **4** (236 mg, 0.161 mmol) was stirred for 1 h with K_2CO_3 (11 mg, 0.08 mmol, 0.5 equiv) in MeOH (1 mL). The mixture was then neutralised with BioRad AG50W-X8 200 (H^+) resin, filtered and concentrated. Flash chromatography (silica gel, toluene/AcOEt (1:1→35:65)) gave the deacetylated compound **12** (192 mg, 93 %). A portion of this material (52 mg, 40 μmol) was dissolved in MeOH (400 μL), protected from light and stirred with propane-1,3-dithiol (80 μL, 0.8 mmol, 20 equiv) and triethylamine (111 μL, 0.8 mmol, 20 equiv) for 2 d. The mixture was then concentrated and purified by silica gel flash chromatography (CH_2Cl_2/MeOH (98:2→9:1) with 0.1 % NEt_3) to give **15** (46 mg, 92 %): ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta=170.1$, 169.8 (C=O), 169.2 (*C*-OMe *p*MBn), 138.6, 138.4, 137.5 ($C_{quat\ arom}$), 133.9 (C-b), 129.8, 128.8, 128.4, 128.2, 128.0, 127.8,

127.4, 127.0 (C_{arom}), 117.2 (C-c), 114.0 (C_m *p*MBn), 101.2, 100.9, 98.7, 96.7 (4×C-1), 81.9, 81.6, 75.6, 75.2, 74.8 (CH_2Ph), 74.7, 74.6, 73.7, 72.6 (CH_2Ph), 72.5, 72.0, 71.0, 69.7, 69.2, 68.5, 68.3 (CH_2Ph), 67.9, 62.1 (2×C-6), 55.9 (OCH_3), 55.1 (OCH_3), 51.8 (OCH_3) ppm; ESI HRMS: *m*/*z* calcd for $C_{65}H_{81}N_2O_{22}$ $[M+H]^+$: 1241.5281; found: 1241.5304.

Sulfur trioxide pyridine complex (200 mg, 1.25 mmol, 30 equiv) was added to a solution of **15** (52 mg, 42 μmol) in pyridine (2.5 mL). This mixture was protected from light, stirred for 24 h at room temperature and then heated for 24 h at 55 °C. MeOH (800 μL, 13.8 mmol) and NEt_3 (320 μL, 2.3 mmol) were then added to quench the reaction. The resulting mixture was stirred for 1 h at room temperature and successively purified by Sephadex LH-20 chromatography (CH_2Cl_2/MeOH (1:1)) and RP-18 flash chromatography (5 mM AcOH-NEt_3 (pH 7.0)/MeOH (100:0→60:40)), followed by ion exchange on BioRad AG50W-X8 200 (Na^+, 5 mL) resin after removal of AcOH-NEt_3 salts by lyophilisation (2×1 mL H_2O). Compound **18** (57 mg, 73 %) was thus obtained as a hexasodium salt (98 % pure by HPLC analysis): ^{1}H NMR (400 MHz, D_2O): δ = 7.59–7.41 (m, 9H; Ph), 7.41–7.33 (m, 6H; Ph), 7.30–7.21 (m, 5H; Ph), 7.11 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 7.00 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.07 (dddd, $J_{b,ct}$ = 17.0, $J_{b,cc}$ = 10.5, $J_{b,a'}$ = 6.0, $J_{b,a}$ = 5.0 Hz, 1H; H-b), 5.44 (dq, $J_{ct,b}$ = 17.0, $J_{ct,a}$ = $J_{ct,a'}$ = J_{gem} = 1.5 Hz, 1H; H-ct), 5.37 (brs, 1H; H-1^B), 5.31 (dq, $J_{cc,b}$ = 10.5, $J_{cc,a}$ = $J_{cc,a'}$ = J_{gem} = 1.5 Hz, 1H; H-cc), 5.27 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^C), 5.20 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^A), 5.13 (brs, 1H; H-1^D), 4.95 (d, $J_{5,4}$ = 1.5 Hz, 1H; H-5^B), 4.90 (d, J = 11.0 Hz, 1H; CH_2Ph), 4.88 (d, $J_{5,4}$ = 2.0 Hz, 1H; H-5^D), 4.80 (d, J = 11.5 Hz, 2H; 2×CH_2Ph), 4.79 (d, J = 11.0 Hz, 1H; CH_2Ph), 4.78 (d, J = 10.5 Hz, 1H; CH_2Ph), 4.59 (d, J = 11.5 Hz, 1H; CH_2Ph), 4.59–4.57 (m, 1H; H-2^B), 4.49 (d, J = 12.0 Hz, 1H; CH_2PhOMe), 4.48–4.45 (m, 1H; H-2^D), 4.44 (d, J = 10.5 Hz, 1H; CH_2Ph), 4.40 (brt, $J_{3,2}$ = $J_{3,4}$ = 3.0 Hz, 1H; H-3^B), 4.38 (dd, $J_{6a,6b}$ = 11.5, $J_{6a,5}$ = 2.0 Hz, 1H; H-6$_a^A$), 4.33 (d, J = 11.5 Hz, 1H; CH_2Ph), 4.30 (ddt, J_{gem} = 13.0, $J_{a,b}$ = 5.0, $J_{a,cc}$ = $J_{a,ct}$ = 1.5 Hz, 1H; H-a), 4.29 (dd, $J_{6b,6a}$ = 11.5, $J_{6b,5}$ = 5.0 Hz, 1H; H-6$_b^A$), 4.26 (d, J = 12.0 Hz, 1H; CH_2PhOMe), 4.21 (brs, 1H; H-6$_a^C$, H-6$_b^C$), 4.19–4.16 (m, 1H; H-3^D), 4.16–4.13 (m, 1H; H-4^B), 4.13 (ddt, J_{gem} = 13.0, $J_{a',b}$ = 6.0, $J_{a',cc}$ = $J_{a',ct}$ = 1.5 Hz, 1H; H-a'), 4.10 (ddd, $J_{5,4}$ = 9.5, $J_{5,6b}$ = 5.0, $J_{5,6a}$ = 2.0 Hz, 1H; H-5^A), 3.88 (s, 3H; PhO*Me*), 3.84 (t, $J_{4,5}$ = $J_{4,3}$ = 9.5 Hz, 1H; H-4^A), 3.77 (dd, $J_{4,5}$ = 10.0, $J_{4,3}$ = 9.0 Hz, 1H; H-4^C), 3.71 (brd, $J_{5,4}$ = 10.0 Hz, 1H; H-5^C), 3.67 (dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 9.5 Hz, 1H; H-3^A), 3.54 (dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 9.0 Hz, 1H; H-3^C), 3.53 (brd, $J_{4,5}$ = 2.0 Hz, 1H; H-4^D), 3.49 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^A), 3.41 (s, 3H; COOMe), 3.39 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^C), 3.13 (s, 3H; COOMe) ppm; ^{13}C NMR (100.6 MHz, D_2O): δ = 171.4, 171.1 (C=O), 159.2 (*C*-OMe *p*MBn), 137.7, 137.6, 137.5, 137.4 ($C_{quat\ arom}$), 134.0 (C-b), 131.3, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3 (C_{arom}), 118.6 (C-c), 114.3 (C_m *p*MBn), 99.3 (C-1^C), 98.3 (C-1^B), 97.8 (C-1^D), 96.8 (C-1^A), 78.1 (C-3^A), 77.7 (C-3^C), 76.0 (CH_2Ph), 75.2 (CH_2Ph), 75.0 (C-4^B), 74.4 (C-4^A), 73.9 (C-3^B), 73.2 (CH_2Ph), 72.4 (C-4^C, CH_2Ph), 72.0 (C-2^B), 70.6 (C-4^D), 70.3 (C-2^D, C-5^C, CH_2 *p*MBn), 69.8 (C-3^D), 69.4 (C-5^A), 69.3 (C-a), 67.7 (C-5^B), 67.3 (C-5^D), 67.2 (C-6^A), 66.3 (C-6^C), 58.4 (C-2^A), 57.9 (C-2^C), 55.5 (CH_3 *p*MBn), 53.0 (CH_3 COOMe), 52.6 (CH_3 COOMe) ppm; ESI HRMS: *m*/*z* calcd for $C_{65}H_{74}N_2O_{40}S_6Na_3$ $[M-3Na]^{3-}/3$: 594.3945; found: 594.3940.

Allyl [(methyl 3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-sulfonato-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)]-(1→4)-*O*-3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside nonasodium salt (19): Hexasaccharide **10** (105 mg, 50 μmol) was treated with K_2CO_3 (3.5 mg, 25 μmol, 0.5 equiv) in MeOH (1 mL) as described above. Flash chromatography (silica gel, toluene/acetone (8:2→6:4)) gave the deacetylated compound **13** (80 mg, 86 %). A portion of this material (59 mg, 32 μmol) was treated with propane-1,3-dithiol (100 μL, 1 mmol, 30 equiv) and triethylamine (140 μL, 1 mmol, 30 equiv) in MeOH (550 μL) as described above. Flash chromatography (silica gel, AcOEt/CH_2Cl_2/MeOH (50:40:10→40:40:20) with 0.1 % NEt_3) gave **16** (49 mg, 87 %): ^{13}C NMR (62.5 MHz, $CDCl_3$/[D_4]MeOH (8:2)): δ = 169.9 (C=O), 159.2 (*C*-OMe *p*MBn), 138.1, 137.2 ($C_{quat\ arom}$), 133.5 (C-b), 129.5, 128.1, 128.0, 127.9, 127.7, 127.2, 126.9, 126.4 (C_{arom}), 116.8 (C-c), 113.4 (C_m *p*MBn), 100.6, 100.5, 97.7, 96.4 (6×C-1), 81.4, 80.7, 74.8, 74.7, 74.6, 74.4 (CH_2Ph), 73.7, 73.6, 72.7 (CH_2Ph), 72.4, 72.3, 72.1 (CH_2Ph), 72.0 (CH_2Ph), 70.9, 68.7, 68.4, 67.9 (CH_2Ph), 67.8, 67.6, 60.2 (C-6), 60.0 (C-6), 59.8 (C-6), 55.0 (OCH_3), 54.8 (OCH_3), 51.5 (OCH_3) ppm; ESI HRMS: *m*/*z* calcd for $C_{92}H_{115}N_3O_{32}$ $[M+2H]^{2+}/2$: 886.8719; found: 886.8723.

Compound **16** (35 mg, 18 μmol) dissolved in pyridine (2.5 mL) was treated with sulfur trioxide pyridine complex (130 mg, 0.81 mmol, 45 equiv) as described above to yield **19** (46 mg, 95 %) as a nonasodium salt (98 % pure by HPLC analysis): ^{1}H NMR (400 MHz, D_2O): δ = 7.59–7.20 (m, 30H; Ph), 7.08 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.99 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.08 (ddt, $J_{b,ct}$ = 17.0, $J_{b,cc}$ = 10.5, $J_{b,a'}$ = $J_{b,a}$ = 6.0 Hz, 1H; H-b), 5.45 (dq, $J_{ct,b}$ = 17.0, $J_{ct,a}$ = $J_{ct,a'}$ = J_{gem} = 1.5 Hz, 1H; H-ct), 5.42 (brs, 1H; H-1^B), 5.42 (brs, 1H; H-1^D), 5.32 (dq, $J_{cc,b}$ = 10.5, $J_{cc,a}$ = $J_{cc,a'}$ = J_{gem} = 1.5 Hz, 1H; H-cc), 5.30 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^C), 5.27 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^E), 5.20 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^A), 5.19 (brs, 1H; H-1^F), 4.97 (brd, $J_{5,4}$ = 1.5 Hz, 2H; H-5^B, H-5^D), 4.92 (d, J = 11.0 Hz, 1H; CH_2Ph), 4.89 (d, $J_{5,4}$ = 2.0 Hz, 1H; H-5^F), 4.88 (d, J = 11.0 Hz, 1H; CH_2Ph), 4.83 (d, J = 11.0 Hz, 1H; CH_2Ph), 4.82 (d, J = 11.5 Hz, 1H; CH_2Ph), 4.81 (d, J = 11.0 Hz, 1H; CH_2Ph), 4.74 (d, J = 10.0 Hz, 1H; CH_2Ph), 4.71 (d, J = 10.0 Hz, 1H; CH_2Ph), 4.61–4.59 (m, 1H; H-2^B), 4.59 (d, J = 11.5 Hz, 1H; CH_2Ph), 4.56 (brd, $J_{2,3}$ = 3.0 Hz, 1H; H-2^D), 4.47 (d, J = 12.0 Hz, 1H; CH_2PhOMe), 4.48–4.46 (m, 1H; H-2^F), 4.43 (d, J = 10.0 Hz, 1H; CH_2Ph), 4.42 (brt, $J_{3,2}$ = $J_{3,4}$ = 3.0 Hz, 1H; H-3^D), 4.40–4.37 (m, 1H; H-3^B), 4.37 (d, J = 11.0 Hz, 1H; CH_2Ph), 4.36 (dd, $J_{6a,6b}$ = 10.0, $J_{6a,5}$ = 2.5 Hz, 1H; H-6$_a^A$), 4.33–4.26 (m, 3H; H-6$_a^C$, H-6$_b^A$, CH_2Ph), 4.30 (ddt, J_{gem} = 13.0, $J_{a,b}$ = 6.0, $J_{a,cc}$ = $J_{a,ct}$ = 1.5 Hz, 1H; H-a), 4.26–4.20 (m, 4H; H-6$_b^C$, H-6$_a^E$, H-6$_b^E$, CH_2PhOMe), 4.19–4.17 (m, 1H; H-3^F), 4.15–4.14 (m, 1H; H-4^B), 4.14 (ddt, J_{gem} = 13.0, $J_{a',b}$ = 6.0, $J_{a',cc}$ = $J_{a',ct}$ = 1.5 Hz, 1H; H-a'), 4.14–4.13 (m, 1H; H-4^D), 4.09 (ddd, $J_{5,4}$ = 10.0, $J_{5,6b}$ = 5.0, $J_{5,6a}$ = 2.5 Hz, 1H; H-5^A), 3.87 (s, 3H; PhO*Me*), 3.85 (dd, $J_{4,5}$ = 10.0, $J_{4,3}$ = 9.0 Hz, 1H; H-4^A), 3.83 (t, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 1H; H-4^C), 3.78 (dd, $J_{4,5}$ = 10.0, $J_{4,3}$ = 9.0 Hz, 1H; H-4^E), 3.73 (dt, $J_{5,4}$ = 10.0, $J_{5,6a}$ = $J_{5,6b}$ = 1.5 Hz, 1H; H-5^E), 3.69 (dt, $J_{5,4}$ = 10.0, $J_{5,6a}$ = $J_{5,6b}$ = 2.5 Hz, 1H; H-5^C), 3.68 (dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 9.0 Hz, 1H; H-3^A), 3.55 (dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 9.0 Hz, 1H; H-3^E), 3.53 (t, $J_{3,2}$ = $J_{3,4}$ = 10.0 Hz, 1H; H-3^C), 3.51–3.49 (m, 1H; H-4^F), 3.50 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^A), 3.44 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^C), 3.40 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^E), 3.39 (s, 3H; COOMe), 3.38 (s, 3H; COOMe), 3.12 (s, 3H; COOMe) ppm; ^{13}C NMR (100.6 MHz, D_2O): δ = 171.4, 171.2, 171.0 (C=O), 159.2 (*C*-OMe *p*MBn), 137.6, 137.4 ($C_{quat\ arom}$), 134.0 (C-b), 131.2, 129.5, 129.4, 129.1, 129.0, 128.8, 128.7, 128.5 (C_{arom}), 118.6 (C-c), 114.2 (C_m *p*MBn), 99.4 (C-1^E), 98.7 (C-1^C), 98.2 (C-1^B, C-1^D), 97.7 (C-1^F), 96.7 (C-1^A), 78.1 (C-3^A), 77.8 (C-3^C), 77.7 (C-3^E), 76.2 (CH_2Ph), 75.9 (CH_2Ph), 75.2 (CH_2Ph), 75.0 (C-4^D), 74.4 (C-4^A, C-4^B), 73.9 (C-3^B), 73.3 (C-3^D), 73.2 (CH_2Ph), 73.1 (CH_2Ph), 72.9 (C-4^C), 72.3 (CH_2Ph), 72.2 (C-4^E), 72.1 (C-2^B), 71,4 (C-2^D), 70.4 (C-4^F), 70.3 (C-5^C), 70.2 (C-2^F, C-5^E, CH_2 *p*MBn), 69.5 (C-5^A), 69.4 (C-3^F), 69.2 (C-a), 67.7/67.4 (C-5^B/C-5^D), 67.3 (C-5^F), 67.1 (C-6^A), 66.3 (C-6^C, C-6^E), 58.4 (C-2^E), 58.3 (C-2^C), 57.9 (C-2^A), 55.7 (CH_3 *p*MBn), 53.0 (CH_3 COOMe), 52.9 (CH_3 COOMe), 52.5 (CH_3 COOMe) ppm; ESI HRMS: *m*/*z* calcd for $C_{92}H_{104}N_3O_{59}S_9Na_6$ $[M-3Na]^{3-}/3$: 873.4034; found: 873.4034.

Allyl [(methyl 3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(methyl 3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)]-(1→4)-*O*-3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside dodecasodium salt (20): Octasaccharide **11** (110 mg, 40 μmol) was treated with K_2CO_3 (6 mg, 40 μmol, 1 equiv) in MeOH (2 mL) as described above. Flash chromatography (silica gel, toluene/acetone (8:2→6:4)) gave the deacetylated compound **14** (88 mg, 91 %). This compound was added to another batch of **14**, and the combined products (100 mg, 41.5 μmol) were treated with propane-1,3-dithiol (167 μL, 1.66 mmol, 40 equiv) and triethylamine (230 μL, 1.66 mmol, 40 equiv) in MeOH (550 μL) as described above. Flash chromatography (silica gel, AcOEt/CH_2Cl_2/MeOH (50:40:10→40:40:20) with 0.1 % NEt_3) gave **17** (82 mg, 84 %): ^{13}C NMR (62.5 MHz, $CDCl_3$/[D_4]MeOH (8:2)): δ = 169.9 (C=O), 159.2 (*C*-OMe *p*MBn), 138.0, 137.4, 137.2, 137.1 ($C_{quat\ arom}$), 133.4 (C-b), 129.5, 128.6, 128.2, 128.1, 128.0, 127.9, 127.6, 127.2, 127.0, 126.9, 126.7 (C_{arom}), 116.9 (C-c), 113.5 (C_m *p*MBn), 100.6, 97.7, 96.1 (8×C-1), 81.4, 80.4, 74.9, 74.8, 74.6, 74.4 (CH_2Ph), 74.3, 73.7, 73.5, 72.7 (CH_2Ph), 72.5, 72.2 (CH_2Ph), 72.1

(*C*H$_2$Ph), 68.8, 68.4, 68.3, 67.9 (*C*H$_2$Ph), 67.8, 67.5, 60.3 (C-6), 60.0 (C-6), 59.8 (C-6), 55.0 (OCH$_3$), 54.8 (OCH$_3$), 54.7 (OCH$_3$), 51.5 (OCH$_3$) ppm; ESI HRMS: *m*/*z* calcd for $C_{119}H_{148}N_4O_{42}$ $[M+2H]^{2+}/2$: 1152.4784; found: 1152.4806.

Compound **17** (30 mg, 13 µmol), dissolved in pyridine (2.5 mL), was treated with sulfur trioxide pyridine complex (125 mg, 0.78 mmol, 60 equiv) as described above to yield **20** (44 mg, 95 %) as a dodecasodium salt (97 % pure by HPLC analysis): ^{1}H NMR (400 MHz, D$_2$O): δ = 7.60–7.54 (m, 6H; Ph) 7.54–7.30 (m, 30H; Ph), 7.30–7.21 (m, 4H; Ph), 7.08 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.99 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.08 (ddt, $J_{b,ct}$ = 17.0, $J_{b,cc}$ = 10.5, $J_{b,a'}$ = $J_{b,a}$ = 6.0 Hz, 1H; H-b), 5.45 (dq, $J_{ct,b}$ = 17.0, $J_{ct,a}$ = $J_{ct,a'}$ = J_{gem} = 1.5 Hz, 1H; H-ct), 5.42 (brs, 1H; H-1^{B}), 5.40 (brs, 1H; H-1^{F}), 5.35 (brs, 1H; H-1^{D}), 5.32 (dq, $J_{cc,b}$ = 10.5, $J_{cc,a}$ = $J_{cc,a'}$ = J_{gem} = 1.5 Hz, 1H; H-cc), 5.31 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{E}), 5.30 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{C}), 5.26 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{G}), 5.21 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{A}), 5.19 (brs, 1H; H-1^{H}), 4.98–4.95 (m, 3H; H-5^{B}, H-5^{D}, H-5^{F}), 4.92 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.89 (d, $J_{5,4}$ = 2.0 Hz, 1H; H-5^{H}), 4.89 (d, J = 11.0 Hz, 2H; C*H*$_2$Ph), 4.83 (d, J = 10.0 Hz, 1H; C*H*$_2$Ph), 4.82 (d, J = 12.0 Hz, 1H; C*H*$_2$Ph), 4.81 (d, J = 11.0 Hz, 2H; 2×C*H*$_2$Ph), 4.79 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.76 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.75 (d, J = 10.0 Hz, 1H; C*H*$_2$Ph), 4.72 (d, J = 10.0 Hz, 1H; C*H*$_2$Ph), 4.70 (d, J = 10.5 Hz, 1H; C*H*$_2$Ph), 4.60 (brdd, $J_{2,3}$ = 3.5, $J_{2,1}$ = 1.5 Hz, 1H; H-2^{B}), 4.59 (d, J = 12.0 Hz, 1H; C*H*$_2$Ph), 4.56 (brd, $J_{2,3}$ = 3.0 Hz, H-2^{F}), 4.55 (brd, $J_{2,3}$ = 3.0 Hz, H-2^{D}), 4.47 (d, J = 12.5 Hz, 1H; C*H*$_2$PhOMe), 4.48 (brs, 1H; H-2^{H}), 4.43 (d, J = 10.0 Hz, 1H; C*H*$_2$Ph), 4.43–4.35 (m, 4H; H-3^{B}, H-3^{D}, H-3^{F}, H-6$_a^{A}$), 4.36 (d, J = 10.0 Hz, 1H; C*H*$_2$Ph), 4.37 (brd, $J_{6a,6b}$ = 10.0, 1H; H-6$_a^{C}$ or H-6$_a^{E}$), 4.32 (dd, $J_{6a,6b}$ = 10.0, $J_{6a,5}$ = 2.0 Hz, 1H; H-6$_a^{C}$ or H-6$_a^{E}$), 4.31 (brd, $J_{6a,6b}$ = 10.0, 1H; H-6$_b^{A}$), 4.30 (ddt, J_{gem} = 13.0, $J_{a',b}$ = 6.0, $J_{a',cc}$ = $J_{a',ct}$ = 1.5 Hz, 1H; H-a), 4.29 (d, J = 10.5 Hz, 1H; C*H*$_2$Ph), 4.29 (d, J = 10.0 Hz, 1H; C*H*$_2$Ph), 4.26–4.19 (m, 4H; H-6$_a^{G}$, H-6$_b^{C}$, H-6$_b^{E}$, H-6$_b^{G}$), 4.23 (d, J = 12.5 Hz, 1H; C*H*$_2$PhOMe), 4.18 (brt, J = 2.5 Hz, 1H; H-3^{H}), 4.17–4.11 (m, 4H; H-4^{B}, H-4^{D}, H-4^{G}, H-a'), 4.09 (ddd, $J_{5,4}$ = 10.0, $J_{5,6b}$ = 5.0, $J_{5,6a}$ = 2.5 Hz, 1H; H-5^{A}), 3.88 (s, 3H; PhO*Me*), 3.86 (dd, $J_{4,5}$ = 10.0, $J_{4,3}$ = 9.0 Hz, 1H; H-4^{A}), 3.83 (t, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 1H; H-4^{C}), 3.82 (t, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 1H; H-4^{E}), 3.78 (dd, $J_{4,5}$ = 10.0, $J_{4,3}$ = 9.0 Hz, 1H; H-4^{G}), 3.73 (brt, $J_{5,4}$ = 10.0, 1H; H-5^{G}), 3.69 (brdd, $J_{5,4}$ = 10.0, J = 2.0 Hz, 2H; H-5^{C}, H-5^{E}), 3.68 (dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{A}), 3.54 (dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{G}), 3.53 (t, $J_{3,2}$ = $J_{3,4}$ = 10.0 Hz, 2H; H-3^{C}, H-3^{E}), 3.52–3.50 (m, 1H; H-4^{H}), 3.50 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{A}), 3.44 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{E}), 3.43 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{C}), 3.40 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{G}), 3.39 (s, 3H; COOMe), 3.38 (s, 3H; COOMe), 3.35 (s, 3H; COOMe), 3.12 (s, 3H; COOMe) ppm; ^{13}C NMR (100.6 MHz, D$_2$O): δ = 171.4, 171.2, 171.1, 171.0 (C=O), 159.2 (*C*-OMe *p*MBn), 137.6, 137.4 (C$_{quat\ arom}$), 134.0 (C-b), 131.2, 129.4, 129.0, 128.8, 128.7, 128.5, 128.3 (C$_{arom}$), 118.6 (C-c), 114.2 (C$_m$ *p*MBn), 99.3 (C-1^{G}), 99.8 (C-1^{E}), 98.7 (C-1^{C}), 98.2 (C-1^{B}, C-1^{D}, C-1^{F}), 97.7 (C-1^{H}), 96.7 (C-1^{A}), 78.1 (C-3^{A}), 77.9 (C-3^{C}, C-3^{E}), 77.8 (C-3^{G}), 76.2 (*C*H$_2$Ph), 75.9 (*C*H$_2$Ph), 75.2 (*C*H$_2$Ph), 74.9 (C-4^{D}), 74.4 (C-4^{A}, C-4^{B}, C-4^{F}), 73.9 (C-3^{B} or C-3^{F}), 73.4 (C-3^{B} or C-3^{F}), 73.2 (*C*H$_2$Ph), 73.1 (*C*H$_2$Ph, C-3^{D}), 73.0 (*C*H$_2$Ph), 72.8 (C-4^{E}), 72.7 (C-4^{C}), 72.3 (*C*H$_2$Ph, C-4^{G}), 72.1 (C-2^{B}), 71.5 (C-2^{D}, C-2^{F}), 70.4 (C-2^{H}, C-4^{H}), 70.3 (C-5^{C}, C-5^{E}, C-5^{F}), 70.2 (C-5^{G}, CH$_2$ *p*MBn), 69.5 (C-5^{A}), 69.4 (C-3^{H}), 69.2 (C-a), 67.5/67.4 (C-5^{B}, C-5^{C}, C-5^{D}, C-5^{E}, C-5^{F}), 67.3 (C-5^{H}), 67.1 (C-6^{A}), 66.3 (C-6^{C}, C-6^{E}, C-6^{G}), 58.4/58.2 (C-2^{C}, C-6^{E}, C-2^{G}), 57.9 (C-2^{A}), 55.7 (CH$_3$ *p*MBn), 53.1 (CH$_3$ COOMe), 53.0 (CH$_3$ COOMe), 52.9 (CH$_3$ COOMe), 52.5 (CH$_3$ COOMe) ppm; ESI MS: $C_{119}H_{134}N_4O_{78}S_{12}Na_{12}$: *m*/*z* (%): 564.8 (53) $[M-6Na]^{6-}/6$, 682.5 (100) $[M-5Na]^{5-}/5$, 858.8 (43) $[M-4Na]^{4-}/4$, 1152.6 (29) $[M-3Na]^{3-}/3$.

Allyl [(3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)]-(1→4)-*O*-3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside octasodium salt (21): Tetrasaccharide **18** (32 mg, 17.2 µmol) was dissolved in a mixture of LiOH (42 mg, 1 mmol, 52 equiv), water (240 µL) and H$_2$O$_2$ (35 % in water, 260 µL, 3 mmol). After 24 h at room temperature, AcOH (45 µL, 0.75 mmol) was added and the mixture was purified by RP-18 flash chromatography (5 mM AcOH-NEt$_3$ (pH 7.0)/MeOH (100:0→60:40)), followed by ion exchange on BioRad AG50W-X8 200 (Na$^+$, 5 mL) resin after removal of AcOH-NEt$_3$ salts by lyophilisation (2×1 mL H$_2$O). Compound **21** (29 mg, 73 %) was thus obtained as an octasodium salt (94 % pure by HPLC analysis): ^{1}H NMR (400 MHz, D$_2$O): δ = 7.50–7.44 (m, 4H; Ph), 7.44–7.32 (m, 16H; Ph), 7.30 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.94 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.01 (dddd, $J_{b,ct}$ = 17.5, $J_{b,cc}$ = 10.5, $J_{b,a'}$ = 6.0, $J_{b,a}$ = 5.5 Hz, 1H; H-b), 5.45 (brs, 1H; H-1^{B}), 5.38 (dq, $J_{ct,b}$ = 17.5, $J_{ct,a}$ = $J_{ct,a'}$ = J_{gem} = 1.5 Hz, 1H; H-ct), 5.32 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{C}), 5.29 (brs, 1H; H-1^{D}), 5.14 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{A}), 5.14 (dq, $J_{cc,b}$ = 10.5, $J_{cc,a}$ = $J_{cc,a'}$ = J_{gem} = 1.5 Hz, 1H; H-cc), 4.85 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.76 (d, J = 11.0 Hz, 3H; C*H*$_2$Ph), 4.76 (d, $J_{5,4}$ = 2.0 Hz, 1H; H-5^{B}), 4.69 (d, $J_{5,4}$ = 1.5 Hz, 1H; H-5^{D}), 4.64 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.64 (d, J = 12.0 Hz, 1H; C*H*$_2$Ph), 4.61 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.60 (dd, $J_{2,3}$ = 3.5, $J_{2,1}$ = 1.5 Hz, 1H; H-2^{B}), 4.51 (d, J = 12.0 Hz, 1H; C*H*$_2$Ph), 4.45 (brd, $J_{2,3}$ = 2.5, 1H; H-2^{D}), 4.44 (d, J = 11.0 Hz, 1H; C*H*$_2$PhOMe), 4.40 (d, J = 11.0 Hz, 1H; C*H*$_2$PhOMe), 4.39 (dd, $J_{6a,6b}$ = 11.5, $J_{6a,5}$ = 2.5 Hz, 1H; H-6$_a^{A}$), 4.34 (dd, $J_{6b,6a}$ = 11.5, $J_{6b,5}$ = 5.0 Hz, 1H; H-6$_b^{A}$), 4.31 (brs, 2H; H-6$_a^{C}$, H-6$_b^{C}$), 4.28 (t, $J_{3,2}$ = $J_{3,4}$ = 3.5 Hz, 1H; H-3^{B}), 4.25 (dd, $J_{4,3}$ = 3.5, $J_{4,5}$ = 2.0 Hz, 1H; H-4^{B}), 4.24 (ddt, J_{gem} = 12.5, $J_{a,b}$ = 5.5, $J_{a,cc}$ = $J_{a,ct}$ = 1.5 Hz, 1H; H-a), 4.16 (brd, $J_{5,4}$ = 10.0 Hz, 1H; H-5^{C}), 4.06 (ddt, J_{gem} = 12.5, $J_{a',b}$ = 6.0, $J_{a',cc}$ = $J_{a',ct}$ = 1.5 Hz, 1H; H-a'), 4.06 (ddd, $J_{5,4}$ = 9.0, $J_{5,6b}$ = 5.0, $J_{5,6a}$ = 2.5 Hz, 1H; H-5^{A}), 3.96 (brt, $J_{3,2}$ = $J_{3,4}$ = 2.5 Hz, 1H; H-3^{D}), 3.93 (t, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 1H; H-4^{C}), 3.91 (t, $J_{4,5}$ = $J_{4,3}$ = 9.0 Hz, 1H; H-4^{A}), 3.91–3.89 (m, 1H; H-4^{D}), 3.83 (s, 3H; PhO*Me*), 3.81 (t, $J_{3,2}$ = $J_{3,4}$ = 10.0 Hz, 1H; H-3^{C}), 3.73 (dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{A}), 3.44 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{C}), 3.37 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{A}) ppm; ^{13}C NMR (100.6 MHz, D$_2$O): δ = 180.0, 179.1 (C=O, from HMBC), 163.3 (*C*-OMe *p*MBn, from HMBC), 137.9, 137.6, 137.4, 137.2 (C$_{quat\ arom}$), 134.0 (C-b), 130.8, 129.5, 129.2, 128.9, 128.8, 128.6 (C$_{arom}$), 118.4 (C-c), 114.2 (C$_m$ *p*MBn), 98.1 (C-1^{B}, C-1^{D}), 97.5 (C-1^{C}), 96.5 (C-1^{A}), 77.3 (C-3^{C}), 77.2 (C-3^{A}), 75.4 (*C*H$_2$Ph, C-3^{B}, C-4^{A}), 75.1 (C-4^{B}), 75.0 (*C*H$_2$Ph), 73.4 (C-4^{C}, C-4^{D}), 73.0 (*C*H$_2$Ph), 72.9 (C-2^{B}), 72.1 (*C*H$_2$ *p*MBn), 71.7 (*C*H$_2$Ph), 71.6 (C-2^{D}), 71.0 (C-3^{D}), 69.7 (C-5^{C}), 69.2 (C-5^{B}), 69.1 (C-5^{A}), 69.0 (C-a), 68.5 (C-5^{D}), 67.3 (C-6^{A}), 66.9 (C-6^{C}), 58.4 (C-2^{A}), 57.9 (C-2^{C}), 55.5 (CH$_3$ *p*MBn) ppm; ESI MS: $C_{63}H_{68}N_2O_{40}S_6Na_8$: *m*/*z* (%): 438.6 (91) $[M-5Na+H]^{4-}/4$, 592.4 (100) $[M-4Na+H]^{3-}/3$, 599.7 (76) $[M-3Na]^{3-}/3$, 911.0 (32) $[M-2Na]^{2-}/2$.

Allyl [(3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(-3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)]-(1→4)-*O*-3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside nonasodium salt (22): Hexasaccharide **19** (35 mg, 13 µmol) was dissolved in a mixture of LiOH (42 mg, 1 mmol, 78 equiv), water (240 µL) and H$_2$O$_2$ (35 % in water, 260 µL, 3 mmol). After 48 h at 37 °C, AcOH (45 µL, 0.75 mmol) was added and the mixture was purified as described above to give **22** (32 mg, 91 %) as a nonasodium salt (98 % pure by HPLC analysis): ^{1}H NMR (400 MHz, D$_2$O): δ = 7.55–7.45 (m, 4H; Ph), 7.45–7.28 (m, 26H; Ph), 7.30 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.94 (d, J = 8.5 Hz, 2H; *Ph*-OMe), 6.02 (dddd, $J_{b,ct}$ = 17.0, $J_{b,cc}$ = 10.5, $J_{b,a'}$ = 6.0, $J_{b,a}$ = 5.5 Hz, 1H; H-b), 5.48 (brs, 1H; H-1^{B}), 5.48 (brs, 1H; H-1^{D}), 5.38 (dq, $J_{ct,b}$ = 17.0, $J_{ct,a}$ = $J_{ct,a'}$ = J_{gem} = 1.5 Hz, 1H; H-ct), 5.31 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{C}), 5.29 (brs, 1H; H-1^{F}), 5.28 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{E}), 5.26 (dq, $J_{cc,b}$ = 10.5, $J_{cc,a}$ = $J_{cc,a'}$ = J_{gem} = 1.5 Hz, 1H; H-cc), 5.14 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{A}), 4.88 (d, J = 10.5 Hz, 1H; C*H*$_2$Ph), 4.84 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.77 (d, $J_{5,4}$ = 2.0 Hz, 1H; H-5^{D}), 4.76 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.75 (d, $J_{5,4}$ = 2.5 Hz, 1H; H-5^{B}), 4.74 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.73 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.72 (d, J = 10.5 Hz, 1H; C*H*$_2$Ph), 4.68 (d, $J_{5,4}$ = 1.5 Hz, 1H; H-5^{F}), 4.66 (d, J = 11.0 Hz, 1H; C*H*$_2$Ph), 4.64–4.59 (m, 4H; H-2^{B}, H-2^{D}, 2×C*H*$_2$Ph), 4.50 (s, 2H; 2×C*H*$_2$Ph), 4.49 (d, J = 12.0 Hz, 1H; C*H*$_2$Ph), 4.46 (brd, $J_{6a,6b}$ = 11.0 Hz, 1H; H-6$_a^{C}$ or H-6$_a^{E}$), 4.43–4.41 (m, 1H; H-2^{F}), 4.42 (d, J = 12.0 Hz, 1H; C*H*$_2$PhOMe), 4.39 (d, J = 12.0 Hz, 1H; C*H*$_2$PhOMe), 4.37 (brs, 2H; H-6$_a^{A}$, H-6$_b^{A}$), 4.34 (brd, $J_{6a,6b}$ = 11.0 Hz, 1H; H-6$_b^{C}$ or H-6$_b^{E}$), 4.33 (brd, $J_{6a,6b}$ = 10.0 Hz, 1H; H-6$_a^{C}$ or H-6$_a^{E}$), 4.29 (brt, $J_{3,2}$ = $J_{3,4}$ = 3.0 Hz, 1H; H-3^{D}), 4.28 (brd, $J_{6a,6b}$ = 10.0 Hz, 1H; H-6$_b^{C}$ or H-6$_b^{E}$), 4.26 (brt, $J_{3,2}$ = $J_{3,4}$ = 3.5 Hz, 1H; H-3^{B}), 4.26 (dd, $J_{4,5}$ = 2.0, $J_{4,3}$ = 3.0 Hz, 1H; H-4^{D}), 4.24 (ddt, J_{gem} = 13.0, $J_{a,b}$ = 5.5, $J_{a,cc}$ = $J_{a,ct}$ = 1.5 Hz, 1H; H-a), 4.23–4.21 (m, 1H; H-4^{B}), 4.12 (brd, $J_{5,4}$ = 10.0 Hz, 1H; H-5^{C}, H-5^{E}), 4.10–4.05 (m, 1H; H-5^{A}), 4.06 (ddt, J_{gem} = 13.0, $J_{a',b}$ = 6.0, $J_{a',cc}$ = $J_{a',ct}$ = 1.5 Hz, 1H; H-a'), 3.94 (dd, $J_{4,5}$ = 10.0, $J_{4,3}$ = 9.0 Hz, 1H; H-4^{C}), 3.93 (dd, $J_{4,5}$ = 10.0, $J_{4,3}$ = 9.0 Hz, 1H; H-4^{E}), 3.94–3.92 (m, 1H; H-3^{F}), 3.91 (dd, $J_{4,5}$ = 10.0, $J_{4,3}$ = 9.0 Hz, 1H; H-4^{A}), 3.90–3.87 (m, 1H; H-4^{F}), 3.83 (s, 3H; PhO*Me*), 3.82 (dd, $J_{3,2}$ = 10.5,

$J_{3,4}$ = 9.0 Hz, 1H; H-3^{E}), 3.74 (dd, $J_{3,2}$ = 10.5, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{A}), 3.70 (dd, $J_{3,2}$ = 10.5, $J_{3,4}$ = 9.0 Hz, 1H; H-3^{C}), 3.44 (dd, $J_{2,3}$ = 10.5, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{E}), 3.39 (dd, $J_{2,3}$ = 10.5, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{C}), 3.37 (dd, $J_{2,3}$ = 10.5, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{A}) ppm; ^{13}C NMR (100.6 MHz, D_2O): δ = 175.4, 174.7 (C=O), 158.9 (*C*-OMe *p*MBn), 137.9, 137.7, 137.6, 137.4, 137.2 ($C_{quat\ arom}$), 133.9 (C-b), 130.8, 130.0, 129.6, 129.5, 129.4, 129.1, 129.0, 128.9, 128.8, 128.6, 128.2 (C_{arom}), 118.3 (C-c), 114.2 (C_m *p*MBn), 98.3 (C-1^{C}, C-1^{E}), 98.0 (C-1^{D}), 97.3 (C-1^{B}), 97.2 (C-1^{F}), 96.5 (C-1^{A}), 77.8 (C-3^{C}), 77.2 (C-3^{A}), 77.1 (C-3^{E}), 75.8 (*C*H_2Ph), 75.4 (C-3^{D}), 75.3 (C-4^{A}, *C*H_2Ph), 75.1 (C-4^{D}, *C*H_2Ph), 74.9 (C-4^{B}, *C*H_2Ph), 74.6 (C-3^{B}), 73.3 (C-4^{F}, C-4^{E}), 73.0 (*C*H_2Ph), 72.9 (*C*H_2Ph), 72.7 (C-4^{C}), 72.3 (C-2^{B}), 72.1 (*C*H_2Ph), 71.8 (C-2^{D}, C-2^{F}, *C*H_2 *p*MBn), 71.0 (C-3^{F}), 69.9 (C-5^{C} or C-5^{E}), 69.4 (C-5^{C} or C-5^{E}), 69.3 (C-5^{A}), 69.0 (C-a), 68.9 (C-5^{D}), 68.5 (C-5^{F}), 68.4 (C-5^{B}), 67.3 (C-6^{A}), 67.0 (C-6^{C}), 66.9 (C-6^{E}), 58.4 (C-2^{C}), 58.3 (C-2^{E}), 57.5 (C-2^{A}), 55.6 (CH_3 *p*MBn) ppm; ESI MS: $C_{89}H_{95}N_3O_{59}S_9Na_{12}$: *m*/*z* (%): 422.0 (53) $[M-8Na+2H]^{6-}/6$, 511.0 (100) $[M-7Na+2H]^{5-}/5$, 649.8 (58) $[M-5Na+H]^{4-}/4$, 881.8 (26) $[M-3Na]^{3-}/3$.

Allyl [(3-*O*-benzyl-4-*O*-(4-methoxybenzyl)-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfonato-α-L-idopyranosyluronate)]-(1→4)-*O*-3-*O*-benzyl-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside dodecasodium salt (23): Octasaccharide **19** (37 mg, 10.5 μmol) was dissolved in a mixture of LiOH (42 mg, 1 mmol, 95 equiv), water (240 μL) and H_2O_2 (35% in water, 260 μL, 3 mmol). After 48 h at 37 °C, AcOH (45 μL, 0.75 mmol) was added and the mixture was purified as described above to give **23** (30 mg, 80%) as a nonasodium salt (96% pure by HPLC analysis): ^{1}H NMR (400 MHz, D_2O) δ = 7.55–7.45 (m, 8H; Ph), 7.45–7.31 (m, 32H; Ph), 7.29 (d, *J* = 8.5 Hz, 2H; *Ph*-OMe), 6.94 (d, *J* = 8.5 Hz, 2H; *Ph*-OMe), 6.01 (ddt, $J_{b,ct}$ = 17.0, $J_{b,cc}$ = 10.5, $J_{b,a'}$ = $J_{b,a}$ = 6.0 Hz, 1H; H-b), 5.49 (brs, 1H; H-1^{B}), 5.47 (brs, 2H; H-1^{D}, H-1^{F}), 5.39 (dq, $J_{ct,b}$ = 17.0, $J_{ct,a}$ = $J_{ct,a'}$ = J_{gem} = 1.5 Hz, 1H; H-ct), 5.33 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{G}), 5.29 (brs, 1H; H-1^{H}), 5.29 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1$^{C(E)}$), 5.28 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1$^{E(C)}$), 5.27 (dq, $J_{cc,b}$ = 10.5, $J_{cc,a}$ = $J_{cc,a'}$ = J_{gem} = 1.5 Hz, 1H; H-cc), 5.15 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{A}), 4.89 (d, *J* = 10.5 Hz, 1H; C*H*$_2$Ph), 4.88 (d, *J* = 10.5 Hz, 1H; C*H*$_2$Ph), 4.84 (d, *J* = 11.0 Hz, 1H; C*H*$_2$Ph), 4.79 (d, *J* = 11.0 Hz, 1H; C*H*$_2$Ph), 4.77 (d, *J* = 11.0 Hz, 1H; C*H*$_2$Ph), 4.78–4.76 (m, 1H; H-5^{B}), 4.76 (d, *J* = 10.0 Hz, 1H; C*H*$_2$Ph), 4.74–4.72 (m, 2H; H-5^{D}, H-5^{F}), 4.73 (d, *J* = 10.5 Hz, 1H; C*H*$_2$Ph), 4.72 (d, *J* = 10.5 Hz, 1H; C*H*$_2$Ph), 4.68 (brs, 1H; H-5^{H}), 4.67 (d, *J* = 11.0 Hz, 1H; C*H*$_2$Ph), 4.64–4.61 (m, 2H; H-2^{B}, H-2$^{D(F)}$), 4.61 (d, *J* = 12.0 Hz, 1H; C*H*$_2$Ph), 4.61 (d, *J* = 10.0 Hz, 1H; C*H*$_2$Ph), 4.53 (s, 2H; 2×C*H*$_2$Ph), 4.50 (d, *J* = 12.0 Hz, 1H; C*H*$_2$Ph), 4.49 (s, 2H; 2×C*H*$_2$Ph), 4.48 (brd, $J_{6a,6b}$ = 11.0 Hz, 1H; H-6$_a$), 4.45 (brd, $J_{6a,6b}$ = 11.0 Hz, 1H; H-6$_a$), 4.44 (d, *J* = 12.0 Hz, 1H; C*H*$_2$PhOMe), 4.44–4.42 (m, 1H; H-2^{H}), 4.40 (d, *J* = 12.0 Hz, 1H; C*H*$_2$PhOMe), 4.38 (brs, 2H; 2×H-6), 4.36 (brd, *J* = 11.0 Hz, 1H; H-6), 4.33 (brd, $J_{6a,6b}$ = 10.0 Hz, 1H; H-6), 4.32–4.20 (m, 8H; H-a, H-3^{B}, H-3^{D}, H-3^{F}, H-4^{B}, H-4^{D}, H-4^{F}, 2 H-6), 4.15–4.04 (m, 5H; H-a', H-5^{A}, H-5^{C}, H-5^{E}, H-5^{F}), 3.95 (t, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 1H; H-4$^{C(E)}$), 3.94 (dd, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 1H; H-4$^{E(C)}$), 3.95–3.93 (m, 1H; H-3^{H}), 3.93 (dd, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 1H; H-4^{G}), 3.93 (dd, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 1H; H-4^{A}), 3.90–3.88 (m, 1H; H-4^{H}), 3.83 (s, 3H; PhO*Me*), 3.82 (t, $J_{3,2}$ = $J_{3,4}$ = 10.0 Hz, 1H; H-3$^{E(C)}$), 3.74 (t, $J_{3,2}$ = $J_{3,4}$ = 10.0 Hz, 1H; H-3^{A}), 3.71 (t, $J_{3,2}$ = $J_{3,4}$ = 10.0 Hz, 1H; H-3^{G}), 3.70 (t, $J_{3,2}$ = $J_{3,4}$ = 10.0 Hz, 1H; H-3$^{C(E)}$), 3.44 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2$^{E(C)}$), 3.41 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{G}), 3.40 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2$^{C(E)}$), 3.37 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{A}) ppm; ^{13}C NMR (100.6 MHz, D_2O): δ = 175.5, 174.6, 174.5 (C=O), 158.9 (*C*-OMe *p*MBn), 137.9, 137.7, 137.6, 137.4, 137.3, 137.2 ($C_{quat\ arom}$), 133.9 (C-b), 130.8, 130.0, 129.5, 129.4, 129.3, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.2 (C_{arom}), 118.3 (C-c), 114.2 (C_m *p*MBn), 98.5 (C-1$^{C(E)}$), 98.3 (C-1^{G}), 98.1 (C-1$^{E(C)}$), 98.0 (C-1$^{F(D)}$ or C-1^{B}), 97.5 (C-1$^{D(F)}$), 97.4 (C-1$^{F(D)}$ or C-1^{B}), 97.2 (C-1^{H}), 96.5 (C-1^{A}), 77.9/77.8 (C-3$^{C(E)}$, C-3^{G}), 77.2 (C-3^{A}), 77.1 (C-3$^{E(C)}$), 76.9 (*C*H_2Ph), 76.8 (*C*H_2Ph), 75.5/75.3/75.2/75.1/74.9/74.8 (C-3^{B}, C-3^{D}, C-3^{F}, C-4^{B}, C-4^{D}, C-4^{F}, 2×*C*H_2Ph), 73.3 (C-4^{G}, C-4^{H}), 73.0 (2×*C*H_2Ph), 72.9 (C-2$^{F(D)}$, C-4$^{E(C)}$), 72.8 (*C*H_2Ph), 72.7 (C-4$^{C(E)}$), 72.1 (*C*H_2 *p*MBn), 71.8 (C-2^{B}, C-2$^{D(F)}$, C-2^{H}, *C*H_2Ph), 71.0 (C-3^{H}), 70.0/69.9/69.4/69.3 (C-5^{A}, C-5^{C}, C-5^{E}, C-5^{G}), 69.1 (C-5^{B}), 69.0 (C-a), 68.6 (C-5^{D} or C-5^{F}), 68.5 (C-5^{H}, C-5^{D} or C-5^{F}), 67.3/67.1/67.0/67.9 (4×C-6), 58.4/58.3 (C-2^{C}, C-2^{E}, C-2^{G}), 57.5 (C-2^{A}), 55.6 (CH_3 *p*MBn); ESI MS: $C_{115}H_{122}N_4O_{78}S_{12}Na_{16}$: *m*/*z* (%): 479.2 (64) $[M-9Na+2H]^{7-}/7$, 565.5 (100) $[M-7Na+H]^{6-}/6$, 683.5 (43) $[M-6Na+H]^{5-}/5$, 867.1 (29) $[M-4Na]^{4-}/4$.

1-Propyl [(2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(2-*O*-sulfonato-α-L-idopyranosyluronate)]-(1→4)-*O*-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside octasodium salt (24): $Pd(OH)_2$ (20% on charcoal, 10 mg) was added to a solution of tetrasaccharide **21** (2.5 mg, 1.3 μmol) in phosphate buffer (20 mM, pH 7.0, 200 μL). The mixture was degassed and stirred for 48 h under hydrogen (1 atm), filtered and desalted on a PD-10 column (Pharmacia) to give **24** (1.7 mg, 94%): ^{1}H NMR (400 MHz, D_2O): δ = 5.43 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{C}), 5.20 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{B}), 5.17 (brs, 1H; H-1^{D}), 5.13 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{A}), 4.86 (d, $J_{5,4}$ = 1.5 Hz, 1H; H-5^{D}), 4.74 (d, $J_{5,4}$ = 3.0 Hz, 1H; H-5^{B}), 4.35 (brs, 2H; H-6$_a^{A}$, H-6$_b^{A}$), 4.33–4.28 (m, 3H; H-2^{B}, H-2^{D}, H-6$_a^{C}$), 4.26 (brd, $J_{6b,6a}$ = 11.5 Hz, 1H; H-6$_b^{C}$), 4.17 (dd, $J_{3,2}$ = 6.5, $J_{3,4}$ = 3.5 Hz, 1H; H-3^{B}), 4.08 (brt, *J* = 3.5 Hz, 2H; H-3^{D}, H-4^{B}), 4.04–3.99 (m, 2H; H-5^{A}, H-5^{C}), 3.99–3.96 (m, 1H; H-4^{D}), 3.80–3,72 (m, 2H; H-4^{A}, H-4^{C}), 3.72–3.64 (m, 3H; H-a, H-3^{A}, H-3^{C}), 3,49 (dt, J_{gem} = 10.0, $J_{a',b}$ = 6.5 Hz, 1H; H-a'), 3.27 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{A}), 3.26 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2^{C}), 1.62 (brsextet, *J* = 7.0 Hz, 2H; H-b), 0.92 (t, *J* = 7.0 Hz, 3H; H-c) ppm; ^{13}C NMR from HMQC (100.6 MHz, D_2O): δ = 99.8 (C-1^{B}, C-1^{D}), 97.2 (C-1^{A}), 96.7 (C-1^{C}), 77.1 (C-3^{A} or C-3^{C}), 77.0 (C-2^{B}), 76.2 (C-4^{B}), 74.4 (C-2^{D}), 70.9 (C-4^{A}, C-4^{C}), 70.4 (C-a), 70.1 (C-3^{B}), 69.1 (C-3^{D}), 68.8 (C-5^{A}, C-5^{C}), 67.2 (C-6^{A}), 66.4 (C-6^{C}), 58.0 (C-2^{C}, C-2^{A}), 22.3 (C-b), 10.2 (C-c) ppm; ESI MS: $C_{27}H_{36}N_2O_{39}S_6Na_8$: *m*/*z* (%): 324.5 (62) $[M-4Na]^{4-}/4$, 440.3 (100) $[M-3Na]^{3-}/3$, 672.0 (75) $[M-2Na]^{2-}/2$.

1-Propyl [(2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(2-*O*-sulfonato-α-L-idopyranosyluronate)]-(1→4)-*O*-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside dodecasodium salt (25): Hexasaccharide **22** (2.5 mg, 0.9 μmol) was debenzylated as described above to give **25** (1.6 mg, 85%): ^{1}H NMR (400 MHz, D_2O): δ = 5.44 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1$^{C(E)}$), 5.41 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1$^{E(C)}$), 5.21 (brd, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{D}), 5.20 (brd, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{B}), 5.17 (brs, 1H; H-1^{F}), 5.13 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{A}), 4.86 (d, $J_{5,4}$ = 1.5 Hz, 1H; H-5^{F}), 4.82–4.84 (under HOD peak, H-5^{D}), 4.75 (d, $J_{5,4}$ = 3.0 Hz, 1H; H-5^{B}), 4.41 (brd, $J_{6a,5}$ = 11.5 Hz, 1H; H-6$_a^{A}$ or H-6$_a^{C}$ or H-6$_a^{E}$), 4.37–4.28 (m, 6H; H-2^{B}, H-2^{D}, H-2^{F}, 2×H-6$_a^{A}$ or H-6$_a^{C}$ or H-6$_a^{E}$, H-6$_b^{A}$ or H-6$_b^{C}$ or H-6$_b^{E}$), 4.26 (brd, $J_{6b,6a}$ = 11.5, 2H; 2× H-6$_b^{A}$ or H-6$_b^{C}$ or H-6$_b^{E}$), 4.19 (dd, $J_{3,2}$ = 6.5, $J_{3,4}$ = 3.5 Hz, 1H; H-3^{D}), 4.17 (dd, $J_{3,2}$ = 6.5, $J_{3,4}$ = 3.5 Hz, 1H; H-3^{B}), 4.13–4.08 (m, 3H; H-3^{F}, H-4^{B}, H-4^{D}), 4.08–3.99 (m, 3H; H-5^{A}, H-5^{C}, H-5^{E}), 3.99–3.96 (m, 1H; H-4^{F}), 3.81–3,71 (m, 3H; H-4^{A}, H-4^{C}, H-4^{E}), 3.71–3.61 (m, 4H; H-a, H-3^{A}, H-3^{C}, H-3^{E}), 3,49 (dt, J_{gem} = 9.5, $J_{a',b}$ = 6.5 Hz, 1H; H-a'), 3.27 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 1H; H-2$^{C(E)}$), 3.26 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 2H; H-2^{A}, H-2$^{E(C)}$), 1.63 (brsextet, *J* = 7.0 Hz, 2H; H-b), 0.92 (t, *J* = 7.0 Hz, 3H; H-c) ppm; ^{13}C NMR from HMQC (100.6 MHz, D_2O): δ = 99.6 (C-1^{B}, C-1^{D}), 98.8 (C-1^{F}), 97.2 (C-1^{A}, C-1$^{C(E)}$), 96.6 (C-1$^{E(C)}$), 77.2 (C-2^{B} or C-2^{D} or C-2^{F}), 77.0 (C-3^{A} or C-3^{C} or C-3^{E}), 76.7 (C-3^{A} or C-3^{C} or C-3^{E}), 76.1 (C-2^{B} or C-2^{D} or C-2^{F}, C-4^{B} or C-4^{D}), 74.3 (C-2^{B} or C-2^{D} or C-2^{F}), 70.4 (C-a, C-5^{A} or C-5^{C} or C-5^{E}), 70.6 (C-3$^{D(B)}$), 69.5 (C-3$^{B(D)}$, C-5^{A} or C-5^{C} or C-5^{E}), 69.1 (C-3^{F}), 69.0 (C-5^{F}), 68.8 (C-5^{A} or C-5^{C} or C-5^{E}), 67.2/66.5/66.3 (C-6^{A}, C-6^{C}, C-6^{E}), 58.3 (C-2^{A}, C-2^{C}, C-2^{E}), 22.2 (C-b), 10.4 (C-c) ppm; ESI MS: $C_{39}H_{51}N_3O_{58}S_9Na_{12}$: *m*/*z* (%): 383.5 (87) $[M-6Na+H]^{5-}/5$, 388.0 (73) $[M-5Na]^{5-}/5$, 490.7 (100) $[M-4Na]^{4-}/4$, 662.0 (78) $[M-3Na]^{3-}/3$.

1-Propyl [(2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(2-*O*-sulfonato-α-L-idopyranosyluronate)-(1→4)-*O*-(2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside)-(1→4)-*O*-(2-*O*-sulfonato-α-L-idopyranosyluronate)]-(1→4)-*O*-2-deoxy-2-sulfoamino-6-*O*-sulfonato-α-D-glucopyranoside hexadecasodium salt (26): Octasaccharide **23** (2.5 mg, 0.7 μmol) was debenzylated as described above to give **26** (1.9 mg, quant.): ^{1}H NMR (400 MHz, D_2O): δ = 5.42 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1$^{G(C,E)}$), 5.41 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1$^{E(C,G)}$), 5.40 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1$^{C(E,G)}$), 5.26 (brd, $J_{1,2}$ = 2.5 Hz, 1H; H-1$^{F(D)}$), 5.23 (brd, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{B}), 5.22 (brd, $J_{1,2}$ = 3.5 Hz, 1H; H-1$^{D(F)}$), 5.18 (brs, 1H; H-1^{H}), 5.13 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1^{A}), 4.87–4.84 (partly

under HOD peak, H-5^{D}, H-5^{F}, H-5^{H}), 4.76 (d, $J_{5,4}$ = 2.5 Hz, 1H; H-5$^{D(B)}$), 4.40 (brd, $J_{6a,5}$ = 11.5 Hz, 2H; 2×H-6$_{a}$), 4.36–4.32 (m, 5H; H-2^{B}, H-2$^{F(D)}$, 2×H-6$_{a}$, H-6$_{b}$), 4.32–4.29 (m, 2H; H-2$^{D(F)}$, H-2^{H}), 4.26 (brd, $J_{6b,6a}$ = 11.5, 3H; 3×H-6$_{b}$), 4.22–4.18 (m, 2H; H-3^{B}, H-3$^{F(D)}$), 4.18 (dd, $J_{3,2}$ = 6.5, $J_{3,4}$ = 3.5 Hz, 1H; H-3$^{D(F)}$), 4.12–4.08 (m, 4H; H-3H; H-4^{B}, H-4^{D}, H-4^{F}), 4.08–3.99 (m, 4H; 4×H-5), 3.98 (brt, J = 2.5 Hz, 1H; H-4^{F}), 3.82–3,72 (m, 4H; 4×H-4), 3.72–3.64 (m, 5H; H-a, 4×H-3), 3.49 (dt, J_{gem} = 9.5, $J_{a',b}$ = 6.5 Hz, 1H; H-a′), 3.27 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 2H; 2×H-2), 3.26 (dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 3.5 Hz, 2H; 2×H-2), 1.63 (brsextet, J = 7.0 Hz, 2H; H-b), 0.92 (t, J = 7.0 Hz, 3H; H-c) ppm; ESI MS: $C_{51}H_{66}N_4O_{77}S_{12}Na_{16}$: m/z (%): 408.6 (100) $[M-12Na+6H]^{6-}/6$, 408.6 (33) $[M-11Na+5H]^{6-}/6$, 426.8 (69) $[M-7Na+H]^{6-}/6$.

Glycoconjugate 30a: In a glass screw-capped tube *bis*-thioPEG **27** (8.5 μL of a 0.2 M solution in degassed water, 1.7 μmol) was added to lyophilised tetrasaccharide **21** (8 mg, 4.28 μmol, 2.5 equiv). The resulting viscous solution was irradiated with UV for 4 h with periodical centrifugation to allow stirring of the solution, then diluted with water (500 μL). An Ellman's test[72] performed on an aliquot of this solution confirmed completion of the reaction. The glycoconjugate was then directly purified by using reversed-phase semipreparative HPLC, followed by ion exchange on BioRad AG50W-X8 200 (Na^+, 1.5 mL) resin after removal of AcOH-NEt_3 salts by lyophilisation (2×1 mL H_2O) and desalting with a Pharmacia PD-10 prepacked column. Compound **30a** (4.6 mg, 66%) was obtained as a hexadecasodium salt (95% pure by HPLC analysis): ^{1}H NMR (400 MHz, D_2O): δ = 7.53–7.43 (m, 8H; Ph), 7.43–7.31 (m, 32H; Ph), 7.29 (d, J = 8.5 Hz, 4H; *Ph*-OMe), 6.94 (d, J = 8.5 Hz, 4H; *Ph*-OMe), 5.42 (brs, 2H; 2×H-1^{B}), 5.32 (d, $J_{1,2}$ = 3.5 Hz, 2H; 2×H-1^{C}), 5.31 (brs, 2H; 2×H-1^{D}), 5.06 (d, $J_{1,2}$ = 3.5 Hz, 2H; 2×H-1^{A}), 4.85 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.82–4.79 (under HOD signal, 2×H-5^{B}), 4.77 (d, J = 11.0 Hz, 4H; 4×CH_2Ph), 4.73 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.69 (d, $J_{5,4}$ = 2.0 Hz, 1H; 2×H-5^{D}), 4.64 (d, J = 12.5 Hz, 2H; 2×CH_2Ph), 4.62 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.62–4.60 (m, 2H; 2×H-2^{B}), 4.58 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.50 (d, J = 12.5 Hz, 2H; 2×CH_2Ph), 4.45 (brs, 2H; 2×H-2^{D}), 4.42 (s, 4H; 4×CH_2PhOMe), 4.40 (brd, $J_{6a,6b}$ = 12.5, 2H; 2×H-6$_{a}^{A}$), 4.33 (dd, $J_{6b,6a}$ = 12.5, $J_{6b,5}$ = 5.0 Hz, 2H; 2×H-6$_{b}^{A}$), 4.32–4.26 (m, 6H; 2×H-3^{B}, 2×H-6$_{a}^{C}$, 2×H-6$_{b}^{C}$), 4.25 (brs, 2H; 2×H-4^{B}), 4.16 (brd, $J_{5,4}$ = 10.0 Hz, 2H; 2×H-5^{C}), 4.03 (ddd, $J_{5,4}$ = 10.0, $J_{5,6b}$ = 5.0, $J_{5,6a}$ = 3.0 Hz, 2H; 2×H-5^{A}), 3.98–3.92 (m, 4H; 2×H-3^{D}, 2×H-4^{C}), 3.92–3.86 (m, 4H; 2×H-4^{A}, 2×H-4^{D}), 3.86–3.78 (m with s at δ = 3.83, 10H; 2×H-3^{C}, 2×H-a, 2×PhO*Me*), 3.74–3.58 (m, 26H; 2×OCH_2CH$_2$S, CH$_2$ PEG, 2×H-3^{A}), 3.52 (dt, J_{gem} = 10.0, $J_{a',b}$ = 7.0, 2H; 2×H-a′), 3.44 (dd, $J_{2,3}$ = 10.5, $J_{2,1}$ = 3.5 Hz, 2H; 2×H-2^{C}), 3.34 (dd, $J_{2,3}$ = 10.5, $J_{2,1}$ = 3.5 Hz, 2H; 2×H-2^{A}), 2.76 (t, J = 6.5 Hz, 4H; 2×OCH$_2$CH_2S), 2.70 (brt, J = 7.0 Hz, 4H; 4×H-c), 1.95 (brdq, J = 14.0, J = 7.0 Hz, 2H; 2×H-b), 1.90 (brdq, J = 14.0, J = 7.0 Hz, 2H; 2×H-b′) ppm; ^{13}C NMR from HMQC (100.6 MHz, D_2O; aromatic protons were not included in the spectral window; due to a limited signal-to-noise ratio, some cross-peaks were not detected): δ = 98.3 (C-1^{B}), 98.4 (C-1^{C}, C-1^{D}), 97.2 (C-1^{A}), 75.3 (C-4^{B}), 75.2 (2×CH$_2$Ph), 75.1 (C-3^{B}), 75.6 (C-4^{A}), 73.3 (C-4^{D}), 72.8 (CH$_2$Ph), 72.3 (C-2^{B}), 72.0 (CH$_2$ *p*MBn), 71.9 (CH$_2$Ph), 71.6 (C-2^{D}), 71.0 (C-3^{D}), 70.7–69.0 (C PEG), 68.8 (C-5^{B}), 68.6 (C-5^{D}), 67.3 (C-6^{A}), 67.0 (C-6^{C}), 58.5 (C-2^{A}), 57.7 (C-2^{C}), 55.6 (CH$_3$ *p*MBn), 31.0 (OCH$_2$CH$_2$S), 29.0 (C-c), 9.5 (C-b) ppm; ESI MS: $C_{140}H_{166}N_4O_{86}S_{14}Na_{16}$: m/z (%): 483.5 (45) $[M-10Na+2H]^{8-}/8$, 559.1 (80) $[M-8Na+H]^{7-}/7$, 656.0 (100) $[M-7Na+H]^{6-}/6$, 796.2 (32) $[M-5Na]^{5-}/5$.

Glycoconjugate 30c: Bis(thio)PEG **29** (6.5 μL of a 0.2 M solution in degassed water, 1.3 μmol) and lyophilised tetrasaccharide **21** (7 mg, 3.74 μmol, 2.5 equiv) were treated as described above to give compound **30c** (2.7 mg, 39%) as a hexadecasodium salt (97% pure by HPLC analysis): The ^{1}H NMR (400 MHz, D_2O) spectrum of compound **30c** was superimposable on that of **30a** except at δ = 3.75–3.60 (m, 134H; 2×OCH_2CH$_2$S, CH$_2$ PEG, 2×H-3^{A}) ppm; ESI MS: $C_{194}H_{274}N_4O_{113}S_{14}Na_{16}$: m/z (%): 551.8 (55) $[M-14Na+5H]^{9-}/9$, 623.9 (79) $[M-13Na+5H]^{8-}/8$, 632.1 (100) $[M-10Na+2H]^{8-}/8$, 713.0 (95) $[M-13Na+6H]^{7-}/7$, 716.1 (91) $[M-12Na+5H]^{7-}/7$, 846.5 (67) $[M-9Na+3H]^{6-}/6$, 853.7 (61) $[M-7Na+H]^{6-}/6$, 1024.7 (29) $[M-7Na+2H]^{5-}/5$.

Glycoconjugate 31a: Bis(thio)PEG **27** (6.0 μL of a 0.2 M solution in degassed water, 1.2 μmol) and lyophilised hexasaccharide **22** (9 mg, 3.30 μmol, 2.8 equiv) were treated as described above to give compound **31a** (3.7 mg, 54%) as a tetracosasodium salt (96% pure by HPLC analysis): ^{1}H NMR (400 MHz, D_2O): δ = 7.56–7.49 (m, 8H; Ph), 7.49–7.31 (m, 52H; Ph), 7.28 (d, J = 8.5 Hz, 4H; *Ph*-OMe), 6.94 (d, J = 8.5 Hz, 4H; *Ph*-OMe), 5.46 (brs, 4H; 2×H-1^{B}, 2×H-1^{D}), 5.31 (d, $J_{1,2}$ = 3.5 Hz, 2H; 2×H-1$^{C(E)}$), 5.30–5.28 (m, 2H; 2×H-1^{F}), 5.27 (d, $J_{1,2}$ = 3.5 Hz, 2H; 2×H-1$^{E(C)}$), 5.08 (d, $J_{1,2}$ = 3.5 Hz, 2H; 2×H-1^{A}), 4.89 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.86 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.84 (brs, 2H; 2×H-5$^{D(B)}$), 4.77 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.79 (brs, 2H; 2×H-5$^{B(D)}$), 4.77–4.72 (under HOD signal, 4×CH_2Ph), 4.72 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.69 (brs, 2H; 2×H-5^{F}), 4.68 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.65–4.59 (m, 10H; 2×H-2^{B}, 2×H-2^{D}, 6×CH_2Ph), 4.56 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.48 (d, J = 12.5 Hz, 2H; 2×CH_2Ph), 4.46 (m, 10H; 2×H-2^{F}, 4×H-6$_{a}$, 4×CH_2PhOMe), 4.38 (d, J = 11.0 Hz, 2H; 2×CH_2Ph), 4.33 (dd, $J_{6b,6a}$ = 11.0, $J_{6b,5}$ = 5.5 Hz, 2H; 2×H-6$_{b}$), 4.33–4.27 (m, 6H; 2×H-3^{B}, 2×H-3^{D}, 2×H-6$_{b}$), 4.27–4.21 (m, 6H; 2×H-4$^{D(B)}$, 4×H-6), 4.16 (brs, 2H; 2×H-4$^{B(D)}$), 4.08–4.00 (m, 6H; 2×H-5^{A}, 2×H-5^{C}, 2×H-5^{E}), 3.96 (brt, J = 2.0 Hz, 2×H-3^{F}), 3.95–3.92 (m, 6H; 2×H-4^{A}, 2×H-4^{C}, 2×H-4^{E}), 3.86 (brs, 2H; 2×H-4^{H}), 3.86–3.79 (m with s at δ = 3.83, 8H; 2×H-a, 2×PhO*Me*), 3.77 (brt, J = 10.5, 2H; 2×H-3$^{C(E)}$), 3.71 (t, J = 6.5 Hz, 4H; 2×OCH_2CH$_2$S), 3.74–3.60 (m, 24H; CH$_2$ PEG, 2×H-3^{A}, 2×H-3$^{E(C)}$), 3.56 (dt, J_{gem} = 10.0, $J_{a',b}$ = 7.0, 2H; 2×H-a′), 3.43 (dd, $J_{2,3}$ = 10.5, $J_{2,1}$ = 3.5 Hz, 2H; 2×H-2$^{C(E)}$), 3.41 (dd, $J_{2,3}$ = 10.5, $J_{2,1}$ = 3.5 Hz, 2H; 2×H-2$^{E(C)}$), 3.37 (dd, $J_{2,3}$ = 10.5, $J_{2,1}$ = 3.5 Hz, 2H; 2×H-2^{A}), 2.77 (t, J = 6.5 Hz, 4H; 2×OCH$_2$CH_2S), 2.72 (brt, J = 7.0 Hz, 4H; 4×H-c), 1.96 (brdq, J = 14.0, J = 7.0 Hz, 2H; 2×H-b), 1.91 (brdq, J = 14.0, J = 7.0 Hz, 2H; 2×H-b′) ppm; ^{13}C NMR from HMQC (100.6 MHz, D_2O; the spectral width did not include aromatic protons; due to a limited signal-to-noise ratio, some cross-peaks were not detected): δ = 98.7 (C-1^{C}, C-1^{E}), 98.5 (C-1^{B}, C-1^{D}), 97.3 (C-1^{A}), 75.3 (C-4$^{B(D)}$), 75.1 (C-4$^{D(B)}$), 75.2 (2×CH$_2$Ph), 75.3 (C-4$^{B(D)}$), 75.1 (C-3$^{D(B)}$, C-4$^{D(B)}$), 74.8 (C-3$^{B(D)}$), 73.1 (C-4^{F}), 72.9 (2×CH$_2$Ph), 72.0 (C-2^{F}, CH$_2$ *p*MBn), 72.1 (CH$_2$Ph, C-2^{B}, C-2^{D}), 71.0 (C-3^{F}), 70.7–69.0 (C PEG), 68.7 (C-a), 68.6 (C-5$^{F(B,D)}$, C-5^{H}), 68.6 (C-5^{F}), 66.9 (C-6), 55.6 (CH$_3$ *p*MBn), 31.1 (OCH$_2$CH$_2$S), 29.0 (C-c) ppm; ESI MS: $C_{192}H_{220}N_6O_{124}S_{20}Na_{24}$: m/z (%): 553.0 (43) $[M-11Na+H]^{10-}/10$, 617.0 (84) $[M-10Na+H]^{9-}/9$, 697.2 (100) $[M-9Na+2H]^{8-}/8$, 803.4 (68) $[M-7Na]^{7-}/7$, 940.1 (32) $[M-6Na]^{6-}/6$.

Glycoconjugate 31c: Bis(thio)PEG **29** (6.0 μL of a 0.2 M solution in degassed water, 1.2 μmol) and lyophilised hexasaccharide **22** (9 mg, 3.30 μmol, 2.8 equiv) were treated as described above to give compound **31c** (4.7 mg, 56%) as a tetracosasodium salt (96% pure by HPLC analysis): The ^{1}H NMR (400 MHz, D_2O) spectrum of compound **31c** was superimposable on that of **31a** except at δ = 3.76–3.60 (m, 136H; CH$_2$ PEG, 2×OCH_2CH$_2$S, 2×H-3^{A}, 2×H-3^{E}) ppm; ESI MS: $C_{246}H_{328}N_6O_{151}S_{20}Na_{24}$: m/z (%): 547.3 (35) $[M-18Na+6H]^{12-}/12$, 596.8 (59) $[M-18Na+7H]^{11-}/11$, 660.7 (81) $[M-16Na+6H]^{10-}/10$, 730.3 (100) $[M-18Na+9H]^{9-}/9$, 737.1 (92) $[M-15Na+6H]^{9-}/9$, 746.9 (80) $[M-11Na+2H]^{9-}/9$, 840.5 (65) $[M-11Na+3H]^{8-}/8$, 951.1 (35) $[M-14Na+7H]^{7-}/7$.

Glycoconjugate 32a: Bis(thio)PEG **27** (4.5 μL of a 0.2 M solution in degassed water, 0.9 μmol) and lyophilised octasaccharide **23** (9 mg, 2.52 μmol, 2.8 equiv) were treated as described above to give compound **32a** (2.3 mg, 34%) as a dotriacontasodium salt (92% pure by HPLC analysis): The ^{1}H NMR (400 MHz, D_2O) spectrum of compound **32a** was superimposable on that of **32b** except at δ = 3.75–3.60 (m, 26H; CH$_2$ PEG, 2×H-3^{A}, 4×H-3^{C} or H-3^{E} or H-3^{G}) ppm; ESI MS: $C_{244}H_{274}N_8O_{162}S_{26}Na_{32}$: m/z (%): 594.1 (50) $[M-15Na+3H]^{12-}/12$, 652.4 (88) $[M-13Na+2H]^{11-}/11$, 719.7 (81) $[M-12Na+2H]^{10-}/10$, 724.4 (77) $[M-10Na]^{10-}/10$, 807.4 (100) $[M-9Na]^{9-}/9$, 911.0 (76) $[M-8Na]^{8-}/8$.

Glycoconjugate 32b: Bis(thio)PEG **28** (12 μL of a 0.2 M solution in degassed water, 2.4 μmol) and lyophilised octasaccharide **23** (24 mg, 6.70 μmol, 2.8 equiv) were treated as described above to give compound **32b** (9.4 mg, 51%) as a dotriacontasodium salt (97% pure by HPLC analysis): ^{1}H NMR (400 MHz, D_2O) δ = 7.58–7.49 (m, 8H; Ph), 7.43–7.26 (m, 76H; Ph, *Ph*-OMe), 6.94 (d, J = 8.5 Hz, 4H; *Ph*-OMe), 5.49 (brs, 2H; 2×H-1$^{B(D,F)}$), 5.46 (brs, 2H; 2×H-1$^{D(B,F)}$), 5.45 (brs, 2H; 2×H-1$^{F(B,D)}$), 5.32 (d, $J_{1,2}$ = 3.5 Hz, 2H; 2×H-1$^{G(C,E)}$), 5.28 (brs, 6H; 2×H-1^{H}, 2×H-1$^{C(G)}$, 2×H-1$^{E(G)}$), 5.08 (d, $J_{1,2}$ = 3.5 Hz, 2H; 2×H-1^{A}), 4.91 (d, J = 10.0 Hz, 2H; 2×CH_2Ph), 4.88 (d, J = 10.0 Hz, 2H; 2×CH_2Ph), 4.86 (d, J = 11.5 Hz, 2H; 2×CH_2Ph), 4.85–4.79 (under HOD signal, 2×H-5^{B}, 2×H-5^{D}, 2×H-5^{F}, 8×CH_2Ph), 4.78 (d, J = 11.5 Hz, 2H; 2×CH_2Ph), 4.74 (d, J = 10.0 Hz, 2H; 2×CH_2Ph), 4.73 (d, J = 10.0 Hz, 2H; 2×CH_2Ph), 4.72 (d, J = 10.0 Hz, 2H; 2×CH_2Ph), 4.69 (brs, 2H; 2×H-5^{H}), 4.67 (d, J = 10.0 Hz, 2H; 2×CH_2Ph), 4.65–4.59 (m, 8H; H-2^{B}, H-2^{D}, H-2^{D}, 2×CH_2Ph), 4.59 (d, J = 10.0 Hz, 4H; 4×CH_2Ph), 4.55–4.49 (m, 2H; 2×H-6$_{a}$), 4.51 (d, J = 12.0 Hz, 2H; 2×CH_2Ph), 4.46–4.39 (m, 10H; 2×H-2^{H}, 4×H-6, 2×CH_2PhOMe)

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4.39–4.21 (m, 18H; 2×H-3^{B}, 2×H-3^{D}, 2×H-3^{F}, 2×H-4$^{B(D,F)}$, 10×H-6), 4.20–4.14 (brs, 4H; 2×H-4$^{D(B,F)}$, 2×H-4$^{F(B,D)}$), 4.08–4.00 (m, 8H; 2×H-5^{A}, 2×H-5^{C}, 2×H-5^{E}, 2×H-5^{G}), 3.96 (brs, 2H; 2×H-3^{H}), 3.96–3.86 (m, 8H; 2×H-4^{A}, 2×H-4^{C}, 2×H-4^{E}, 2×H-4^{G}), 3.86 (brs, 2H; 2×H-4^{H}), 3.86–3.79 (m with s at δ=3.83, 8H; 2×H-a, 2×PhO*Me*), 3.76 (brt, J=10.0, 2H; 2×H-3^{C} or H-3^{E} or H-3^{G}), 3.72 (t, J=6.5 Hz, 4H; 2×OC*H*$_2$CH$_2$S), 3.75–3.60 (m, 46H; CH$_2$ PEG, 2×H-3^{A}, 4×H-3^{C} or H-3^{E} or H-3^{G}), 3.56 (brdt, J_{gem}=10.0, $J_{a',b}$=7.0 Hz, 2H; 2×H-a′), 3.45–3.38 (m, 6H; 2×H-2^{C}, 2×H-2^{E}, 2×H-2^{G}), 3.37 (dd, $J_{2,3}$=10.0, $J_{2,1}$=3.5 Hz, 2H; 2×H-2^{A}), 2.78 (t, J=6.5 Hz, 4H; 2×OCH$_2$C*H*$_2$S), 2.73 (brt, J=7.0 Hz, 4H; 4×H-c), 1.97 (brdq, J=14.0, J=7.0 Hz, 2H; 2×H-b), 1.92 (brdq, J=14.0, J=7.0 Hz, 2H; 2×H-b′) ppm; ^{13}C NMR from HMQC (100.6 MHz, D$_2$O; due to a limited signal-to-noise ratio, some cross-peaks were not detected): δ=130.8 (C$_o$ *p*MBn), 130.5–128.0 (C$_{arom}$), 114.3 (C$_m$ *p*MBn), 98.9 (C-1$^{C(E,G)}$), 98.6 (C-1$^{E(C,G)}$, C-1$^{G(C,E)}$, C-1^{H}), 97.3 (C-1^{A}), 75.5 (*C*H$_2$Ph), 75.2 (C-4$^{F(B,D)}$), 75.1 (C-3$^{B(D,F)}$, C-4$^{B(D,F)}$, C-4$^{D(B,F)}$, *C*H$_2$Ph), 75.0 (C-3$^{F(B,D)}$), 74.9 (C-3$^{D(B,F)}$), 73.6–72.4 (*C*H$_2$Ph), 73.3 (C-4^{H}), 72.1 (C-2^{B}, C-2^{D}, C-2^{F}), 72.0 (C-2^{H}, CH$_2$ *p*MBn), 71.8 (*C*H$_2$Ph), 71.0 (C-3^{H}), 70.7–69.0 (C PEG), 68.6 (C-5$^{F(B,D)}$, C-5^{H}), 68.5 (C-5$^{B(D,F)}$, C-5$^{D(B,F)}$), 67.0 (C-6), 58.7/58.5 (C-2^{A}, C-2^{C}, C-2^{E}, C-2^{F}), 55.5 (CH$_3$ *p*MBn), 31.0 (OCH$_2$*C*H$_2$S), 29.0 (C-c) ppm; ESI MS: C$_{254}$H$_{294}$N$_8$O$_{167}$S$_{26}$Na$_{32}$: *m/z* (%): 614.9 (74) $[M-14\,\text{Na}+2\,\text{H}]^{12-}/12$, 672.1 (95) $[M-13\,\text{Na}+2\,\text{H}]^{11-}/11$, 744.4 (100) $[M-11\,\text{Na}+\text{H}]^{10-}/10$, 832.1 (96) $[M-9\,\text{Na}]^{9-}/9$, 936.2 (45) $[M-9\,\text{Na}+\text{H}]^{8-}/8$, 939.2 (47) $[M-8\,\text{Na}]^{8-}/8$.

Glycoconjugate 32c: Bis(thio)PEG **29** (4.5 µL of a 0.2 M solution in degassed water, 0.9 µmol) and lyophilised octasaccharide **23** (9 mg, 2.52 µmol, 2.8 equiv) were treated as described above to give compound **32c** (5.0 mg, 64%) as a dotriacontasodium salt (97% pure by HPLC analysis): The ^{1}H NMR (400 MHz, D$_2$O) spectrum of compound **30c** was superimposable on that of **30a** except at δ=3.77–3.60 (m, 138H; 2×OC*H*$_2$CH$_2$S, CH$_2$ PEG, 2×H-3^{A}, 4×H-3^{C} or H-3^{E} or H-3^{G}) ppm; ESI MS: C$_{298}$H$_{382}$N$_8$O$_{189}$S$_{26}$Na$_{32}$: *m/z* (%): 688.1 (91) $[M-18\,\text{Na}+6\,\text{H}]^{12-}/12$, 756.5 (100) $[M-15\,\text{Na}+4\,\text{H}]^{11-}/11$, 825.1 (100) $[M-18\,\text{Na}+8\,\text{H}]^{10-}/10$, 836.7 (75) $[M-13\,\text{Na}+3\,\text{H}]^{10-}/10$, 937.6 (59) $[M-10\,\text{Na}+\text{H}]^{9-}/9$.

Glycoconjugate 33b: Bis(thio)PEG **28** (7 µL of a 0.2 M solution in degassed water, 1.4 µmol) and lyophilised tetrasaccharide **21** (6.4 mg, 3.50 µmol, 2.5 equiv) were irradiated with UV as described above. The intermediate glycoconjugate **30b** was diluted with water (60 µL) and K$_2$HPO$_4$ (30 µL, 0.8 M in water) and oxidised with oxone (potassium monopersulfate triple salt, 56 µL, 0.2 M in water, 11.2 µmol, 8 equiv). After 4 h at room temperature, excess oxidant was reduced with Na$_2$S$_2$O$_3$ (84 µL, 0.4 M in water). The mixture was then directly purified by using reversed-phase semipreparative HPLC, followed by ion exchange on BioRad AG50W-X8 200 (Na$^+$, 1.5 mL) resin after removal of AcOH-NEt$_3$ salts by lyophilisation (2×1 mL H$_2$O) and desalting by using a Pharmacia PD-10 prepacked column. Compound **33b** (1.4 mg, 23%) was obtained as a hexadecasodium salt (96% pure by HPLC analysis): ^{1}H NMR (400 MHz, D$_2$O): δ=7.53–7.45 (m, 8H; Ph), 7.45–7.31 (m, 32H; Ph), 7.30 (d, J=8.5 Hz, 4H; *Ph*-OMe), 6.94 (d, J=8.5 Hz, 4H; *Ph*-OMe), 5.44 (brs, 2H; 2×H-1$^{B(D)}$), 5.37 (brs, 2H; 2×H-1$^{D(B)}$), 5.31 (brs, 2H; 2×H-1^{C}), 5.09 (brs, 2H; 2×H-1^{A}), 4.80 (under HOD signal, 2×H-5^{B}, 2×H-5^{D}, 2×C*H*$_2$Ph), 4.76 (d, J=11.0 Hz, 4H; 4×C*H*$_2$Ph), 4.73 (d, J=12.0 Hz, 2H; 2×C*H*$_2$Ph), 4.65 (d, J=11.0 Hz, 2H; 2×C*H*$_2$Ph), 4.63 (d, J=12.0 Hz, 2H; 2×C*H*$_2$Ph), 4.60 (brs, 2H; 2×H-2$^{B(D)}$), 4.60 (d, J=12.0 Hz, 2H; 2×C*H*$_2$Ph), 4.51 (d, J=12.0 Hz, 2H; 2×C*H*$_2$Ph), 4.45 (brs, 2H; 2×H-2$^{D(B)}$), 4.42 (m, 4H; 4×C*H*$_2$PhOMe), 4.40 (brd, $J_{6a,6b}$=11.0, 2H; 2×H-6$_a$^{A}), 4.33 (dd, $J_{6b,6a}$=11.0, $J_{6b,5}$=5.0 Hz, 2H; 2×H-6$_b$^{A}), 4.32–4.26 (m, 6H; 2×H-3$^{B(D)}$, 4×H-6), 4.24 (brs, 2H; 2×H-4$^{B(D)}$), 4.19 (brs, 2H; 2×H-5), 4.05–3.94 (m, 10H; 2×H-3$^{D(B)}$, 2×H-4^{C}, 2×H-5^{A}, 2×OC*H*$_2$CH$_2$SO$_2$), 3.94–3.84 (m, 8H; 2×H-3^{C}, 2×H-4^{A}, 2×H-4$^{D(B)}$, 2×H-a), 3.83 (s, 6H; 2×PhO*Me*), 3.73–3.63 (m, 42H; CH$_2$ PEG, 2×H-3^{A}), 3.59 (dt, J_{gem}=10.0, $J_{a',b}$=6.0, 2H; 2×H-a′), 3.51 (t, J=5.0 Hz, 4H; 2×OCH$_2$C*H*$_2$SO$_2$), 3.45 (brd, $J_{2,3}$=10.0 Hz, 2H; 2×H-2^{C}), 3.39 (brt, J=7.0 Hz, 4H; 4×H-c), 3.36 (brd, $J_{2,3}$=10.0 Hz, 2H; 2×H-2^{A}), 2.22–2.10 (m, 4H; 4×H-b) ppm; ESI MS: C$_{150}$H$_{186}$N$_4$O$_{96}$S$_{14}$Na$_{16}$: *m/z* (%): 518.2 (65) $[M-10\,\text{Na}+2\,\text{H}]^{8-}/8$, 599.4 (93) $[M-8\,\text{Na}+\text{H}]^{7-}/7$, 602.1 (100) $[M-7\,\text{Na}]^{7-}/7$, 703.4 (75) $[M-7\,\text{Na}+\text{H}]^{6-}/6$, 706.4 (87) $[M-6\,\text{Na}]^{6-}/6$, 852.7 (58) $[M-5\,\text{Na}]^{5-}/5$.

Glycoconjugate 34b: Bis(thio)PEG **28** (6.5 µL of a 0.2 M solution in degassed water, 1.3 µmol) and lyophilised hexasaccharide **22** (8.0 mg, 2.9 µmol, 2.3 equiv) were treated as described for compound **33b**. Glycoconjugate **34b** (2.4 mg, 31%) was thus obtained as a tetracosasodium salt salt (98% pure by HPLC analysis): ^{1}H NMR (400 MHz, D$_2$O): δ=7.55–7.45 (m, 12H; Ph), 7.45–7.32 (m, 48H; Ph), 7.29 (d, J=8.5 Hz, 4H; *Ph*-OMe), 6.93 (d, J=8.5 Hz, 4H; *Ph*-OMe), 5.48 (brs, 2H; 2×H-1$^{B(D)}$), 5.42 (brs, 2H; 2×H-1$^{D(B)}$), 5.32 (brs, 4H; 2×H-1$^{C(E)}$, 2×H-1^{F}), 5.28 (brs, 2H; 2×H-1$^{E(C)}$), 5.09 (brs, 2H; 2×H-1^{A}), 4.88 (d, J=12.0 Hz, 2H; 2×C*H*$_2$Ph), 4.86–4.79 (under HOD signal, 2×H-5^{B}, 2×H-5^{D}, 2×H-5^{F}, 4×C*H*$_2$Ph), 4.78 (d, J=11.0 Hz, 2H; 2×C*H*$_2$Ph), 4.75 (d, J=12.0 Hz, 2H; 2×C*H*$_2$Ph), 4.72 (d, J=12.0 Hz, 2H; 2×C*H*$_2$Ph), 4.66 (d, J=11.0 Hz, 2H; 2×C*H*$_2$Ph), 4.64–4.58 (m, 8H; 2×H-2^{B}, 2×H-2^{D}, 4×C*H*$_2$Ph), 4.57 (d, J=12.0 Hz, 2H; 2×C*H*$_2$Ph), 4.49 (d, J=11.0 Hz, 4H; 4×C*H*$_2$Ph), 4.46–4.38 (m, 10H; 2×H-2^{F}, 2×H-6$_a$^{A}, 2×H-6$_a$^{C} or H-6$_a$^{E}, 4×C*H*$_2$PhOMe), 4.33 (dd, $J_{6b,6a}$=12.0, $J_{6b,5}$=5.0 Hz, 2H; 2×H-6$_b$^{A}), 4.32–4.23 (m, 12H; 2×H-3^{B}, 2×H-3^{D}, 2×H-4$^{D(B)}$, 6×H-6), 4.22 (brs, 2H; 2×H-4$^{B(D)}$), 4.16–4.08 (m, 4H; 2×H-5^{C}, 2×H-5^{E}), 4.04–4.00 (m, 2H; 2×H-5^{A}), 3.99 (brt, J=5.0 Hz, 4H; 2×OC*H*$_2$CH$_2$SO$_2$), 3.98–3.84 (m, 14H; 2×H-3^{F}, 2×H-4^{A}, 2×H-4^{C}, 2×H-4^{E}, 2×H-a, 2×H-4^{H}, 2×H-3$^{E(C)}$), 3.82 (s, 6H; 2×PhO*Me*), 3.73–3.63 (m, 44H; CH$_2$ PEG, 2×H-3^{A}, 2×H-3$^{C(E)}$), 3.58 (dt, J_{gem}=10.0, $J_{a',b}$=6.0 Hz, 2H; 2×H-a′), 3.51 (t, J=5.0 Hz, 4H; 2×OCH$_2$C*H*$_2$SO$_2$), 3.44 (brd, $J_{2,3}$=10.0 Hz, 2H; 2×H-2$^{E(C)}$), 3.38 (brt, J=7.0 Hz, 4H; 4×H-c), 3.38 (brd, $J_{2,3}$=10.0 Hz, 2H; 2×H-2$^{C(E)}$), 3.36 (brd, $J_{2,3}$=10.0 Hz, 2H; 2×H-2^{A}), 2.21–2.10 (m, 4H; 4×H-b) ppm; ESI MS: C$_{202}$H$_{240}$N$_6$O$_{133}$S$_{20}$Na$_{24}$: *m/z* (%): 581.0 (64) $[M-11\,\text{Na}+\text{H}]^{10-}/10$, 648.8 (100) $[M-10\,\text{Na}+\text{H}]^{9-}/9$, 735.4 (85) $[M-8\,\text{Na}]^{8-}/8$, 841.3 (68) $[M-8\,\text{Na}+\text{H}]^{7-}/7$.

Glycoconjugate 1a: Compound **30a** (4.65 mg, 0.88 µmol) was oxidised as described for compound **30b** to give compound **33a**. After desalting, Pd(OH)$_2$ (20% on charcoal, 25 mg) was added to a solution of **33a** in phosphate buffer (100 mM, pH 7.0, 300 µL) and MeOH (200 µL). The mixture was degassed and stirred for 6 d under hydrogen (1 atm). The mixture was filtered over ultrafree-MC filters (Amicon) and the charcoal was rinsed with CH$_3$CN/H$_2$O (1:1, 3×200 µL) in order to desorb compound **33a** from the charcoal. The resulting solution was desalted on a PD-10 column (Pharmacia) and lyophilysed to give **33a** (2.4 mg, 85%): ^{1}H NMR (400 MHz, D$_2$O): δ=5.42 (d, $J_{1,2}$=3.5 Hz, 2H; 2×H-1^{C}), 5.19 (d, $J_{1,2}$=2.5 Hz, 2H; 2×H-1^{B}), 5.17 (brs, 2H; 2×H-1^{D}), 5.15 (d, $J_{1,2}$=3.5 Hz, 2H; 2×H-1^{A}), 4.81 (under HOD peak, 2×H-5^{D}), 4.75 (brs, 2H; 2×H-5^{B}), 4.38–4.33 (m, 4H; 2×H-6$_a$$^{A(C)}$, 2×H-6$_b$$^{A(C)}$), 4.33–4.28 (m, 6H; 2×H-2^{B}, 2×H-2^{D}, 2×H-6$_a$$^{C(A)}$), 4.26 (brd, $J_{6b,6a}$=11.5 Hz, 2H; 2×H-6$_b$$^{C(A)}$), 4.18 (dd, $J_{3,2}$=6.0, $J_{3,4}$=4.0 Hz, 2H; 2×H-3^{B}), 4.13–4.08 (m, 4H; 2×H-3^{D}, 2×H-4^{B}), 4.07–3.96 (m, 10H; 2×H-5^{C}, 2×OC*H*$_2$CH$_2$SO$_2$ (δ=4.02, t, J=5.5 Hz), 2×H-5^{A}, 2×H-4^{D}), 3.87 (dt, J_{gem}=10.0, $J_{a',b}$=5.5 Hz, 2H; 2×H-a), 3.80–3.60 (m, 30H; 2×H-4^{A}, 2×H-4^{C}, 2×CH$_2$ PEG, 2×H-3^{A}, 2×H-3^{C}, 2×H-a′), 3.55 (t, J=5.5 Hz, 4H; 2×OCH$_2$C*H*$_2$SO$_2$), 3.42 (dd, J=9.0, 7.0 Hz, 4H; 4×H-c), 3.28 (dd, $J_{2,3}$=10.0, $J_{2,1}$=3.5 Hz, 2H; 2×H-2^{A}), 3.26 (dd, $J_{2,3}$=10.0, $J_{2,1}$=3.5 Hz, 2H; 2×H-2^{C}), 2.21–2.11 (m, 4H; 4×H-b) ppm; ^{13}C NMR from HMQC (100.6 MHz, D$_2$O): δ=99.7 (C-1^{B}), 99.4 (C-1^{D}), 97.3 (C-1^{A}), 96.6 (C-1^{C}), 76.9 (C-4^{A}, C-4^{C}), 76.5 (C-2$^{B(D)}$), 76.1 (C-4^{B}), 74.1 (C-2^{D}), 69.9 (CH$_2$ PEG, C-5^{B}, C-4^{A}, C-4^{C}), 69.6 (C-3^{B}), 69.4 (C-5$^{C(A)}$), 69.3 (C-4^{D}, C-5^{C}),69.1 (C-3^{D}), 69.0 (C-5^{D}), 67.1 (C-6$^{A(C)}$), 66.6 (C-6$^{C(A)}$), 66.3 (C-a), 63.7 (O*C*H$_2$CH$_2$SO$_2$), 58.2 (C-2^{C}, C-2^{A}), 52.3 (OCH$_2$*C*H$_2$SO$_2$), 51.2 (C-c), 21.5 (C-b) ppm.

Glycoconjugate 1b: Compound **33b** (0.9 mg, 0.2 µmol) was debenzylated and purified as described for **33a** to give **1b** (0.7 mg, quant.): The ^{1}H NMR (400 MHz, D$_2$O) spectrum of compound **1b** was superimposable on that of **1a** except at δ=3.82–3.60 (m, 50H; 2×H-4^{A}, 2×H-4^{C}, CH$_2$ PEG, 2×H-3^{A}, 2×H-3^{C}, 2×H-a′) ppm; ESI MS: C$_{78}$H$_{122}$N$_4$O$_{93}$S$_{14}$Na$_{16}$: *m/z* (%): 401.8 (80) $[M-9\,\text{Na}+\text{H}]^{8-}/8$, 459.3 (100) $[M-9\,\text{Na}+2\,\text{H}]^{7-}/7$, 546.9 (59) $[M-6\,\text{Na}]^{6-}/6$.

Neoglycoconjugate 1c: Compound **30c** (2.6 mg, 0.48 µmol) was oxidised and debenzylated as described for compound **30a** to give **1c** (2.2 mg, quant.): The ^{1}H NMR (400 MHz, D$_2$O) spectrum of compound **1c** was superimposable on that of **1a** except at δ=3.82–3.60 (m, 138H; 2×H-4^{A}, 2×H-4^{C}, CH$_2$ PEG, 2×H-3^{A}, 2×H-3^{C}, 2×H-a′) ppm.

Neoglycoconjugate 2a: Compound **31a** (3.0 mg, 0.51 µmol) was oxidised and debenzylated as described for compound **30a** to give **2a** (1.7 mg, 74%): The ^{1}H NMR (400 MHz, D$_2$O) spectrum of compound **2a** was superimposable on that of **2c** except at δ=3.82–3.60 (m, 34H; 2×H-4^{A}, 2×H-4^{C}, 2×H-4^{E}, CH$_2$ PEG, 2×H-3^{A}, 2×H-3^{C}, 2×H-3^{D}, 2×H-a′) ppm.

Glycoconjugate 2b: Compound **34b** (1.6 mg, 0.27 µmol) was debenzylated and purified as described for **33a** to give **2b** (0.9 mg, 71%): The ^{1}H NMR (400 MHz, D$_2$O) spectrum of compound **2b** was superimposable on that of **2c** except at δ=3.82–3.60 (m, 54H; 2×H-4^{A}, 2×H-4^{C}, 2×

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H-4^{E}, CH_2 PEG, 2×H-3^{A}, 2×H-3^{C}, 2×H-3^{D}, 2×H-a′) ppm; ESI MS: $C_{102}H_{152}N_6O_{131}S_{20}Na_{24}$: *m*/*z* (%): 450.3 (65) $[M-11Na+H]^{10-}/10$, 501.3 (100) $[M-10Na+H]^{9-}/9$, 569.9 (83) $[M-9Na+2H]^{8-}/8$.

Neoglycoconjugate 2c: Compound **31c** (4.2 mg, 0.60 µmol) was oxidised and debenzylated as described for compound **30a** to give **2c** (2.6 mg, 76%): ^{1}H NMR (400 MHz, D_2O): $\delta=5.43$ (d, $J_{1,2}=3.5$ Hz, 2H; 2×H-1$^{C(E)}$), 5.41 (d, $J_{1,2}=3.5$ Hz, 2H; 2×H-1$^{E(C)}$), 5.21 (brd, $J_{1,2}=3.0$ Hz, 2H; 2×H-1$^{D(B)}$), 5.19 (brd, $J_{1,2}=3.0$ Hz, 2H; 2×H-1$^{B(D)}$), 5.17 (brs, 2H; 2×H-1^{F}), 5.15 (d, $J_{1,2}=3.5$ Hz, 2H; 2×H-1^{A}), 4.81 (under HOD peak, 2×H-5^{F}), 4.80 (under HOD peak, 2×H-5^{D}), 4.75 (brs, 2H; 2×H-5^{B}), 4.41 (brd, $J_{6a,5}=11.5$ Hz, 2H; 2×H-6$_a$), 4.38–4.28 (m, 12H; 2×H-2^{B}, 2×H-2^{D}, 2×H-2^{F}, 6×H-6), 4.26 (brd, $J_{6b,6a}=11.5$ Hz, 4H; 4×H-6$_b^{A}$ or H-6$_b^{C}$ or H-6$_b^{E}$), 4.19 (dd, $J_{3,2}=6.0$, $J_{3,4}=4.0$ Hz, 2H; 2×H-3^{D}), 4.18 (dd, $J_{3,2}=6.0$, $J_{3,4}=4.0$ Hz, 2H; 2×H-3^{B}), 4.13–4.08 (m, 6H; 2×H-3^{F}, 2×H-4^{B}, 2×H-4^{D}), 4.08–3.95 (m, 12H; 2×O$CH_2CH_2SO_2$ ($\delta=4.01$, t, $J=5.5$ Hz), 2×H-5^{A}, 2×H-5^{C}, 2×H-5^{E}, 2×H-4^{F}), 3.87 (dt, $J_{gem}=10.0$, $J_{a',b}=5.5$ Hz, 2H; 2×H-a), 3.83–3.60 (m, 142H; 2×H-4^{A}, 2×H-4^{C}, 2×H-4^{E}, CH_2 PEG, 2×H-3^{A}, 2×H-3^{C}, 2×H-3^{E}, 2×H-a′), 3.54 (t, $J=5.5$ Hz, 4H; 2×$OCH_2CH_2SO_2$), 3.43 (brt, $J=7.5$ Hz, 4H; 4×H-c), 3.28 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 2H; 2×H-2^{A}), 3.26 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 2H; 2×H-2$^{C(E)}$), 3.25 (dd, $J_{2,3}=10.0$, $J_{2,1}=3.5$ Hz, 2H; 2×H-2$^{E(C)}$), 2.21–2.11 (m, 4H; 4×H-b) ppm; ^{13}C NMR from HMQC (100.6 MHz, D_2O): $\delta=99.7$ (C-1^{B}, C-1^{D}), 99.4 (C-1^{F}), 97.3 (C-1^{A}), 96.9 (C-1^{E}), 96.6 (C-1$^{C(E)}$), 76.3 (C-2^{F}), 76.2 (C-2$^{B(D)}$, C-4^{A}, C-4^{C}, C-4^{E}, C-4^{B}, C-4^{D}), 74.3 (C-2$^{D(B)}$), 72.0 (C-3^{A} or C-3^{C} or C-3^{E}), 69.9 (CH_2 PEG, C-3^{A} or C-3^{B} or C-3^{D}), 69.9 (C-5$^{B(D)}$), 69.7 (C-5$^{D(B)}$), 69.6 (C-3^{B}, C-3^{D}), 69.5/69.3 (C-5^{A}, C-5^{C}, C-5^{E}, C-4^{F}), 69.1 (C-3^{F}), 67.0 (C-5^{F}), 67.1/66.6 (C-6^{A}, C-6^{C}, C-6^{E}), 66.4 (C-a), 63.8 (O$CH_2CH_2SO_2$), 58.2 (C-2^{A}, C-2^{C}, C-2^{E}), 52.4 ($OCH_2CH_2SO_2$), 51.3 (C-c), 21.5 (C-b) ppm.

Neoglycoconjugate 3a: Compound **32a** (1.5 mg, 0.19 µmol) was oxidised and debenzylated as described for compound **30a** to give **3a** (0.6 mg, 90%): The ^{1}H NMR (400 MHz, D_2O) spectrum of compound **3a** was superimposable on that of **3b** except at $\delta=3.82$–3.60 (m, 38H; 2×H-4^{A}, 2×H-4^{C}, 2×H-4^{E}, 2×H-4^{G}, CH_2 PEG, 2×H-3^{A}, 2×H-3^{C}, 2×H-3^{D}, 2×H-3^{G}, 2×H-a′).

Neoglycoconjugate 3b: Compound **32b** (9.3 mg, 1.20 µmol) was oxidised and debenzylated as compound **30a** to give **3b** (4.5 mg, 62%): ^{1}H NMR (400 MHz, D_2O): $\delta=5.46$–5.38 (m, 6H; 2×H-1^{G}, 2×H-1^{C}, 2×H-1^{E}), 5.25–5.18 (m, 6H; 2×H-1^{B}, 2×H-1^{D}, 2×H-1^{F}), 5.16 (brs, 2H; 2×H-1^{H}), 5.14 (d, $J_{1,2}=3.5$ Hz, 2H; 2×H-1^{A}), 4.81 (under HOD peak, 2×H-5^{G}), 4.80 (under HOD peak, 2×H-5$^{D(B,F)}$, 2×H-5$^{F(B,D)}$), 4.76 (d, $J_{5,4}=2.5$ Hz, 2H; 2×H-5$^{B(D,F)}$), 4.41 (brd, $J_{6a,5}=11.0$ Hz, 4H; 4×H-6$_a$), 4.37–4.28 (m, 14H; 2×H-2^{B}, 2×H-2^{D}, 2×H-2^{F}, 2×H-2^{G}, 6×H-6), 4.26 (brd, $J_{6b,6a}=11.0$ Hz, 6H; 6×H-6$_b$), 4.22–4.15 (m, 6H; H-3^{B}, H-3^{D}, H-3^{F}), 4.13–4.08 (m, 8H; 2×H-3H; 2×H-4^{B}, 2×H-4^{D}, 2×H-4^{F}), 4.08–3.96 (m, 14H; 4×O$CH_2CH_2SO_2$ ($\delta=4.01$, t, $J=5.5$ Hz), 2×H-5^{A}, 2×H-5^{C}, 2×H-5^{E}, 2×H-5^{G}, 2×H-4^{F}), 3.87 (dt, $J_{gem}=10.0$, $J_{a',b}=5.0$ Hz, 2H; 2×H-a), 3.83–3.60 (m, 58H; 2×H-4^{A}, 2×H-4^{C}, 2×H-4^{E}, 2×H-4^{G}, CH_2 PEG, 2×H-3^{A}, 2×H-3^{C}, 2×H-3^{E}, 2×H-3^{G}, 2×H-a′), 3.54 (t, $J=5.5$ Hz, 4H; 4×$OCH_2CH_2SO_2$), 3.42 (dd, $J=9.0$, 7.5 Hz, 4H; 4×H-c), 3.32–3.23 (m, 8H; 2×H-2^{A}, 2×H-2^{C}, 2×H-2^{E}, 2×H-2^{G}), 2.21–2.11 (m, 4H; 4×H-b) ppm; ^{13}C NMR from HMQC (100.6 MHz, D_2O): $\delta=99.6$ (C-1^{B}, C-1^{D}, C-1^{F}), 99.4 (C-1^{H}), 97.2 (C-1^{A}), 96.7 (C-1^{C}, C-1^{E}, C-1^{G}), 76.5 (C-2^{H}), 76.8/76.2 (C-4^{A}, C-4^{C}, C-4^{E}, C-4^{G}), 76.2 (C-2$^{B(D,F)}$, C-4^{B}, C-4^{D}, C-4^{F}), 74.1 (C-2$^{D(B,F)}$, C-2$^{F(B,D)}$), 71.9 (C-3^{A} or C-3^{C} or C-3^{E}), 69.8 (CH_2 PEG, C-3^{A} or C-3^{B} or C-3^{D}), 69.8 (C-5$^{B(D,F)}$), 69.6 (C-5$^{D(B,F)}$, C-5$^{F(B,D)}$), 69.5 (C-3^{B}, C-3^{D}, C-3^{F}), 69.5/69.3 (C-5^{A}, C-5^{C}, C-5^{E}, C-5^{G}, C-4^{H}), 69.1 (C-3^{H}), 69.1 (C-5^{F}), 67.1/66.6 (C-6^{A}, C-6^{C}, C-6^{E}, C-6^{G}), 66.4 (C-a), 63.4 (O$CH_2CH_2SO_2$), 58.1 (C-2^{A}, C-2^{C}, C-2^{E}, C-2^{G}), 52.4 ($OCH_2CH_2SO_2$), 51.3 (C-c), 21.6 (C-b) ppm; ESI MS: $C_{126}H_{182}N_8O_{169}S_{26}Na_{32}$: *m*/*z* (%): 480.2 (79) $[M-14Na+2H]^{12-}/12$, 487.7 (100) $[M-12Na]^{12-}/12$, 524.1 (84) $[M-14Na+3H]^{11-}/11$, 576.3 (86) $[M-14Na+4H]^{10-}/10$, 580.9 (63) $[M-12Na+2H]^{10-}/10$.

Neoglycoconjugate 3c: Compound **32c** (3.8 mg, 0.44 µmol) was oxidised and debenzylated as described for compound **30a** to give **3c** (2.4 mg, 77%): The ^{1}H NMR (400 MHz, D_2O) spectrum of compound **3a** was superimposable on that of **3b** except at $\delta=3.82$–3.60 (m, 146H; 2×H-4^{A}, 2×H-4^{C}, 2×H-4^{E}, 2×H-4^{G}, CH_2 PEG, 2×H-3^{A}, 2×H-3^{C}, 2×H-3^{D}, 2×H-3^{G}, 2×H-a′) ppm.

Acknowledgments

We thank the CNRS (Physique Chimie du Vivant programme), the Ministère de L'Education Nationale et de la Recherche (OG), the Paris Sud University and the Région Rhone Alpes.

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Received: December 23, 2003
Revised: April 23, 2004
Published online: July 12, 2004

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