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Synthesis of heparin-like oligosaccharides on polymer supports

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The biological functions of a variety of proteins are regulated by heparan sulfate glycosaminoglycans. In order to facilitate the elucidation of the molecular basis of glycosaminoglycan-protein interactions we have developed syntheses of heparin-like oligosaccharides on polymer supports. A completely stereoselective strategy previously developed by us for the synthesis of these oligosaccharides in solution has been extended to the solid phase using an acceptor-bound approach. Both a soluble polymer support and a polyethylene glycol-grafted polystyrene resin have been used and different strategies for the attachment of the acceptor to the support have been explored. The attachment of fully protected disaccharide building blocks to a soluble support through the carboxylic group of the uronic acid unit by a succinic ester linkage, the use of trichloroacetimidates as glycosylating agents and of a functionalized Merryfield type resin for the capping process allowed for the construction of hexasaccharide and octasaccharide fragments containing the structural motif of the regular region of heparin. This strategy may facilitate the synthesis of glycosaminoglycan oligosaccharides by using the required building blocks in the glycosylation sequence.
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Keywords: **solid-phase synthesis, carbohydrates, synthesis design, heparin, oligosaccharides**

Introduction

Heparan sulfate glycosaminoglycans regulate important biological functions by interacting with a variety of heparin-binding proteins [1]. Unfortunately, the investigation of the molecular basis of these polysaccharide-protein interactions faces serious difficulties as a consequence of the diversity and the structural heterogeneity of heparan sulfate [2]. Heparan sulfate glycosaminoglycans constitute a family of closely related linear polysaccharide species–which includes heparin– consisting of unsulfated and variously sulfated sequences of alternating $1 \rightarrow 4$ linked D-glucosamine and L-iduronic or D-glucuronic acid units [3,4]. These sequences are distributed in different domains along the linear polysaccharide chain. The size of these chains, the sequence of alternating units and the negative charge distribution and orientation within the different domains seem to play an important role in the glycosaminoglycan-protein interaction processes [3]. The recognition in the early eighties that a structurally unique pentasaccharide sequence within heparin binds antithrombin with high selectivity, strongly suggested that glycosaminoglycan-protein interactions are saccharide specific [5]. For fibroblast growth factors 1 and 2 (FGF-1 and FGF-2) which, with the exception of antithrombin, are the heparin binding proteins which have been more thoroughly studied [6], minimal carbohydrate binding motifs have been proposed [7]. However, the information available from crystallographic analysis of complexes of these proteins with heparin fragments reveals diverse patterns of stoichometry, contact sites and orientation [8–11]. It is generally agreed that the structural heterogeneity of the glycosaminoglycan is in part responsible for these apparently conflicting evidences and that the availability of homogeneous glycosaminoglycan fragments with precisely defined molecular structures would decisively contribute to decipher the molecular mechanism of these biological interactions.

As a part of a program on the molecular basis of the regulation of FGF-1 activity by heparin we have previously synthesized several heparin-like hexasaccharides and octasaccharides composed of variously sulfated $\alpha 1 \rightarrow 4$ linked D-glucosamine and L-iduronic acid units [12–15]. These synthetic molecules present a heparin-like three dimensional structure and a well defined spatial distribution of negative charge in solution [12–17], and, therefore, constitute excellent tools to investigate the molecular mechanism of FGF activation. They were synthesized using a convergent modular strategy which permitted

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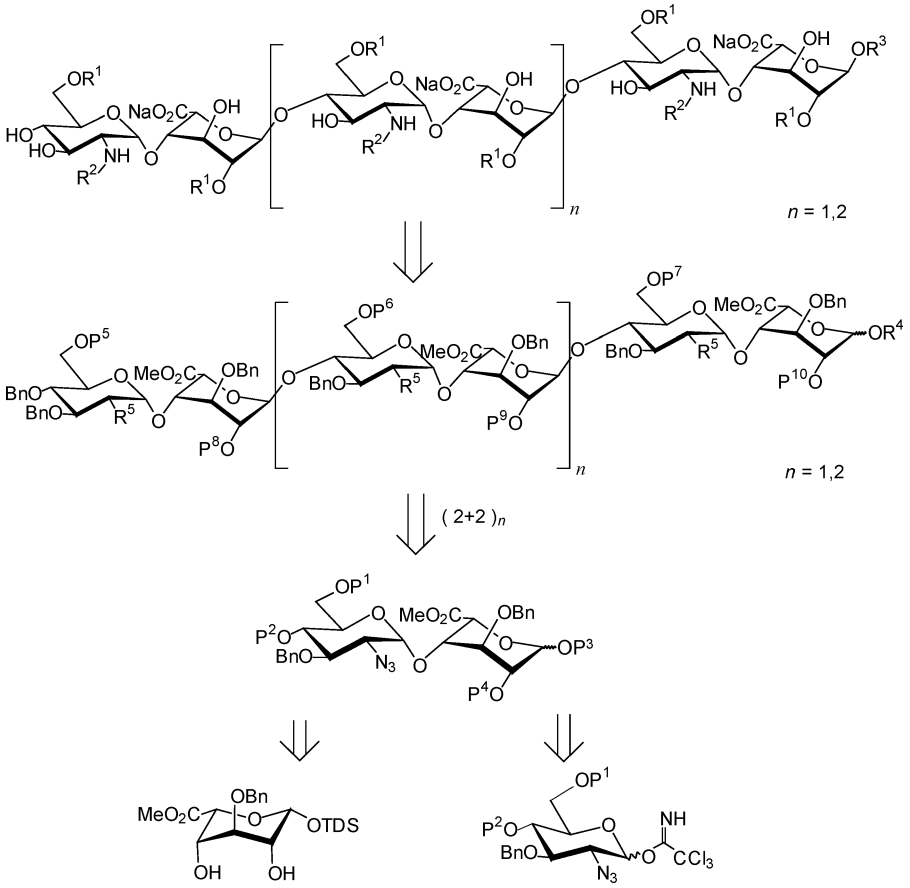


Scheme 1. General retrosynthetic analisis of heparin-like oligosaccharides.

to control the size of the final product and the pattern of sulfate groups along the oligosaccharide skeleton [15]. Effective as this approach might be, the synthesis of the diversity of oligosaccharides required for extensively investigating these biological interactions demands further simplification and automation. As in most areas in glycobiology, the development of efficient solid phase synthesis conditions to construct these oligosaccharides on automated synthesizers is much needed [18–20]. It may be expected that the solution phase modular approaches to the synthesis of glycosaminoglycan fragments recently reported by us [12–15] and by several other authors [21–24] will allow for the synthesis on solid support. Therefore we have investigated the possibility of extending our solution phase approach to the synthesis of glycosaminoglycans on polymeric supports. A preliminary communication of part of the results of this investigation has recently appeared [25]. We now give a full account of this study. In spite of the considerable effort devoted to the synthesis of glycosaminoglycan oligosaccharides [26] after the seminal contributions on the antithrombin-binding pentasaccharide [27], only a preliminary communication on the synthesis of *O*-methylated heparan sulfate—like oligomers on a soluble polymer support had appeared in the literature when we started this investigation [28].

Results and discussion

Our synthetic approach in solution was based on disaccharide building blocks with protecting group patterns specifically designed to control the stereochemistry of the glycosidic linkages and the location of the sulfate groups [12–15] (Scheme 1). The D-glucosamine unit always occupied the non reducing end. This monosaccharide sequence, which was first designed for the construction of heparan sulfate glycosaminoglycan fragments by Lay *el al*. [21], complements those obtained by chemical or enzymatic degradation of natural glycosaminoglycans. As usual in most glycosaminoglycan syntheses [26,27], *O*-sulfation positions were differentiated by temporary acyl group protection while permanent benzyl groups were used to protect the remaining hydroxyl functions. A α-isopropyl group, which resulted to be a convenient structural feature in the final oligosaccharide construct for NMR based interaction studies, was directly introduced at the reducing end building block [12–15]. The trichloroacetimidate method [29] was used in all glycosylations. Using this approach, a series of four hexasaccharides and two octasaccharides, including **I** and **II** which contain the sequence and the sulfation pattern of the regular region of heparin, were effectively prepared [12–15] (Figure 1).

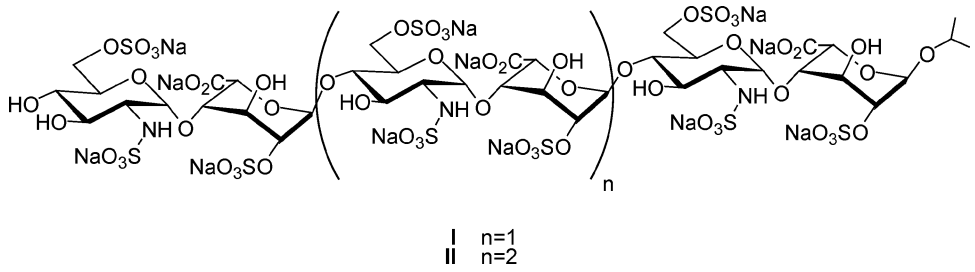


Figure 1. Hexa- and octasaccharide of the regular region of heparin.

Since these general experimental features were key for the synthesis in solution, the synthesis on polymeric support was accordingly planned on these basis using the same synthetic scheme and keeping the protecting strategy and the glycosylation method.

The only previously reported polymer supported preparation of heparan sulfate-like oligosaccharides was carried out on polyethylene glycol (PEG) also using a $n + 2$ block synthesis strategy in which, contrary to our approach, a conveniently protected D-glucopyranose unit, mimicking the D-glucosamine moiety, occupied the reducing end of the disaccharide building blocks [28]. An acceptor bound approach was used in which the conveniently protected disaccharide glycosyl acceptor was attached to PEG through the hydroxymethyl group of the D-glucopyranose unit by a succinoyl ester linker and the elongation of the oligosaccharide chain was carried out by repetitive glycosylation of the 4-OH group of the L-iduronate unit of the acceptor with a conveniently protected glycosyl donor.

We have investigated the extension of our solution phase synthesis also using the acceptor bound approach and an ester-type linker [30] which was compatible with our solution phase chemistry. Both a soluble polymer support (PEG ω-monomethyl ether, MPEG) [31] and a PEG-grafted polystyrene resin, which provide a solution-like environment, have been used and several attachment sites of the disaccharide acceptor to the polymer support have been investigated. The usual sulfation patterns of naturally occurring heparan sulfate glycosaminoglycans [3,4] involve positions R^1, R^2, R^3, and R^5. A highly convergent synthetic approach to construct a diversity of molecules with different sequence and charge distribution should, in principle, avoid these positions for attachment to the support (Figure 2). Problems associated with the established synthesis of L-iduronate monosaccharide building blocks [32–34] also precluded R^4 as attachment position. Therefore we have essentially investigated approaches in which the attachment of the acceptor include the anomeric reducing end and the carboxylate group of the L-iduronate unit although the feasibility of a scheme based on the attachment to the solid support through the hydroxymethyl group of the D-glucosamine unit, as previously reported [28], was also explored for comparison purposes.

We first studied a direct attachment of the disaccharide glycosyl acceptor to the support using a PEG-grafted polystyrene resin (ArgoGel^{TM}) functionalized with an amino group. This resin was successfully functionalized with disaccharide **3** which was prepared from **1** [12] by reaction with succinic anhydride. The functionalized resin **4** was obtained with a resin loading of 0.35 mmol/g. Following our previous solution phase chemistry,

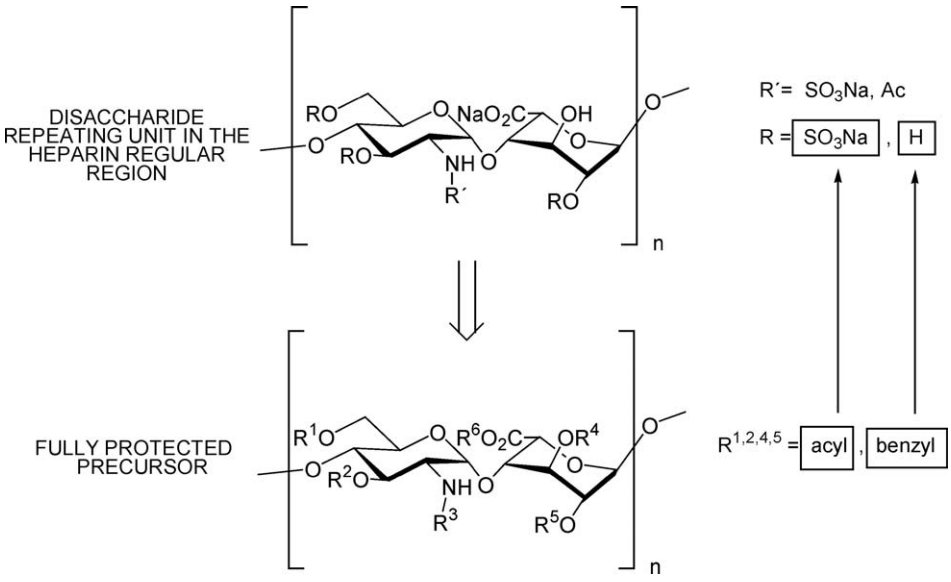
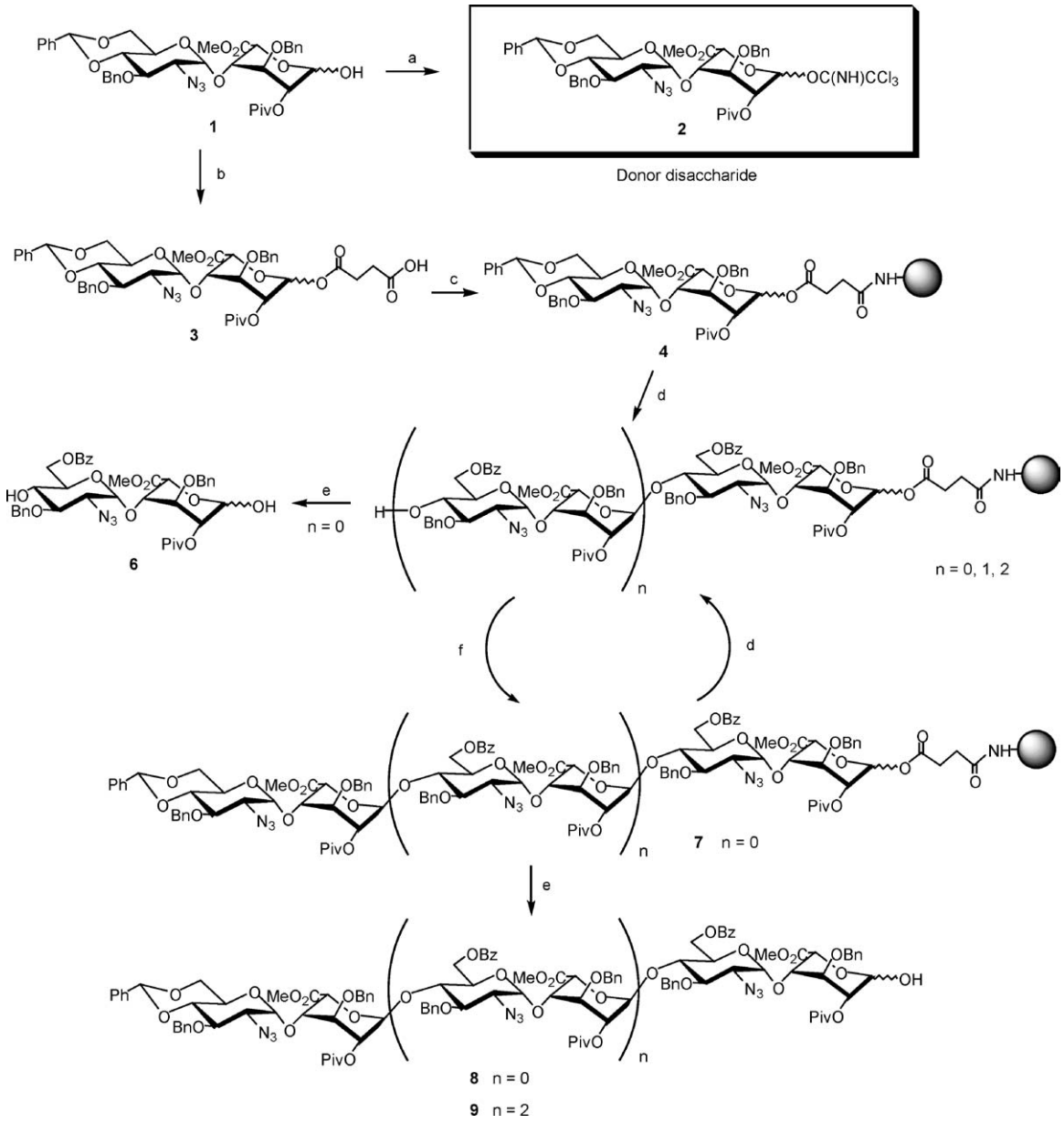


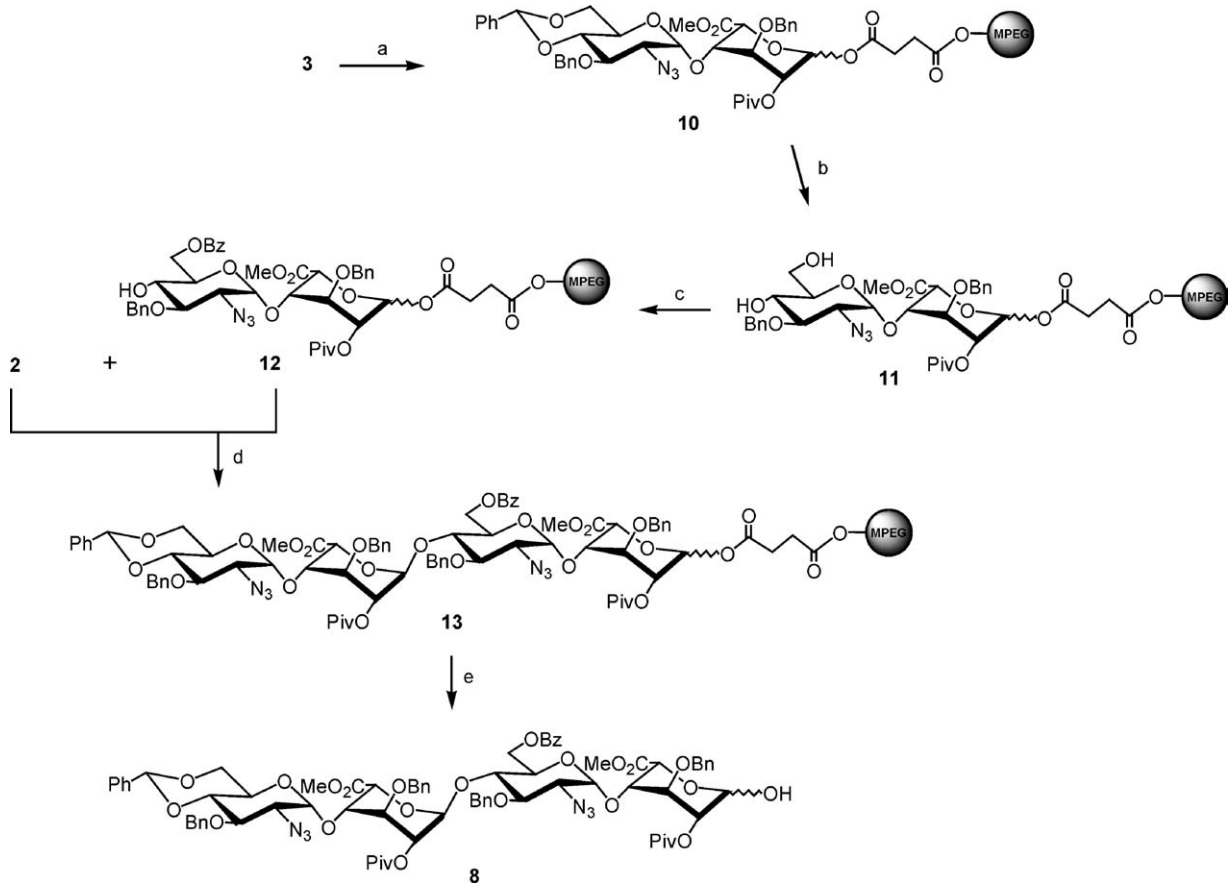
Figure 2. Retrosynthetic analisis of the disaccharide repeating unit in the heparin regular region.



Scheme 2. (a) Cl_3CCN, K_2CO_3, CH_2Cl_2; (b) Succinic anhydride, DMAP, Py; (c) i. ArgoGel-NH_2, DCC, HOBt, DMF-CH_2Cl_2; ii. Ac_2O, Py; (d) i. EtSH, *p*TsOH, CH_2Cl_2, ii. BzCN, Et_3N (cat.), CH_3CN, -20°C; (e) hydrazine acetate, THF; (f) **2**, TMSOTf, CH_2Cl_2 (three times).

the resin bound glycosyl acceptor **5** was obtained from **4** by hydrolysis of the benzylidene acetal and subsequent selective benzoylation. A preparative sample was cleaved from the resin by treatment with hydrazine acetate to yield **6** which was completely characterized. Coupling of **5** with donor **2** in the presence of TMSOTf at room temperature gave **7** in 90% yield after three glycosylation cycles. A resin aliquot was cleaved as before to give tetrasaccharide **8** that was fully characterized. The elongation of the oligosaccharide chain proceeded by successive on bead generation of the glycosyl acceptor as described above and subsequent glycosylation steps with glycosyl donor **2**. After two elongation cycles octasaccharide **9** was cleaved from the resin and characterized. The final yield was 10% from **5** after eight steps (Scheme 2).

A parallel study was carried out using the soluble polymer support MPEG. MPEG was fuctionalized by reaction with disaccharide **3** after activation with diisopropylcarbodiimide. NMR analysis showed a 90% linkage. Cleavage of the benzylidene acetal in **10** gave **11** and selective benzoylation of **11** yielded glycosyl acceptor **12**. Glycosylation of **12** with trichloroacetimidate **2** as described above afforded **13** from which tetrasaccharide **8** was released after three glycosylation cycles. The final yield was 20% from **10** after four steps (Scheme 3).

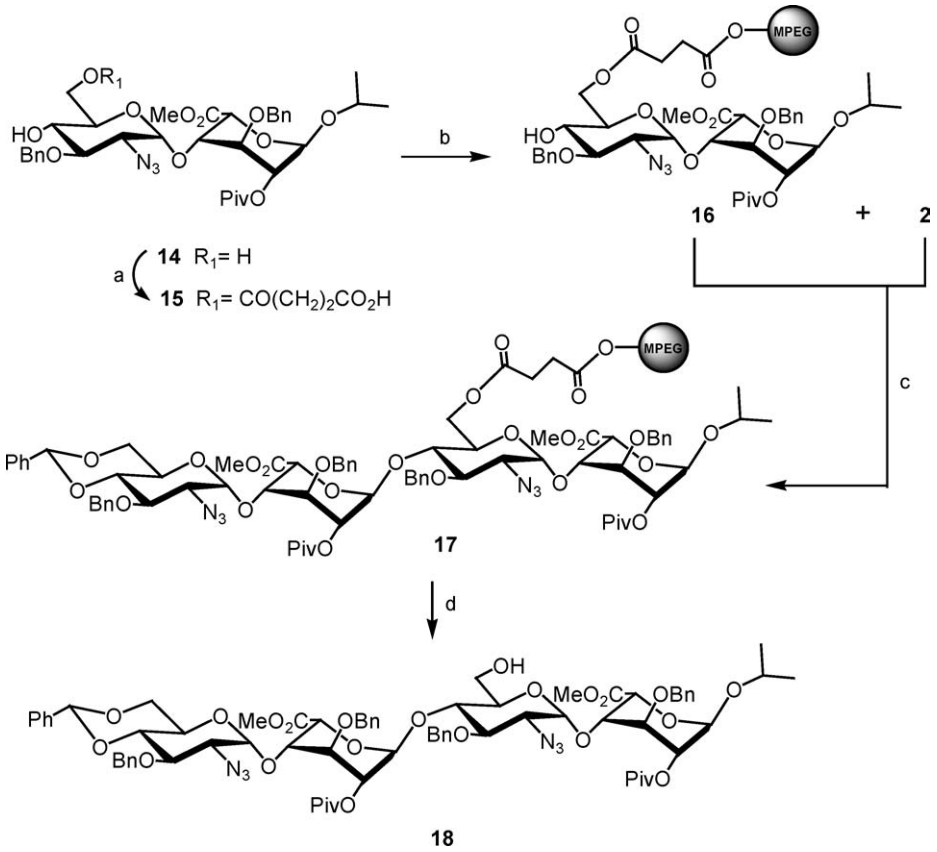


Scheme 3. (a) i. DIC, DMAP, CH_2Cl_2, MPEG; ii. Ac_2O, Py, 90%; (b) EtSH, *p*TsOH, CH_2Cl_2; (c) BzCN, Et_3N, CH_3CN, $-30^\circ C$; (d) TMSOTf, CH_2Cl_2 (three times); (e) hydrazine acetate, THF, 20% four steps.

It can be concluded that both the PEG grafted polystyrene resin and the soluble polymer support led to similar results using this synthetic scheme and that both strategies can be used to construct the oligosaccharide skeleton of these heparin-like oligosaccharides. As developed in this investigation, this approach leads to anomeric mixtures. Although a process could be envisaged allowing to stereoselectively introduce a given functionality at the anomeric position, it should be taken into account that the installation of different groupings at the reducing end of the oligosaccharide chain for structural or biological investigation, or as handles for binding studies, is more conveniently performed on the starting disaccharide module than on the final oligosaccharide construct [15].

Next the feasibility of the process using an attachment strategy through hydroxymethyl group of the D-glucosamine unit, similar to that previously reported [28] for building blocks with the reverse sequence, was explored. Diol **14** [12] was selectively transformed into the 6-*O*-succinoyl derivative **15** in 78% yield using stannilidene activation. The carboxyl group in **15** was in turn activated as above and condensed with MPEG to give the disaccharide acceptor **16**. Glycosylation of **16** with trichloroacetimidate **2** afforded **17** from which tetrasaccharide **18** was cleaved. The overall yield was 36% from **16** after two glycosylation cycles. For the reasons discussed above this strategy was not further investigated (Scheme 4).

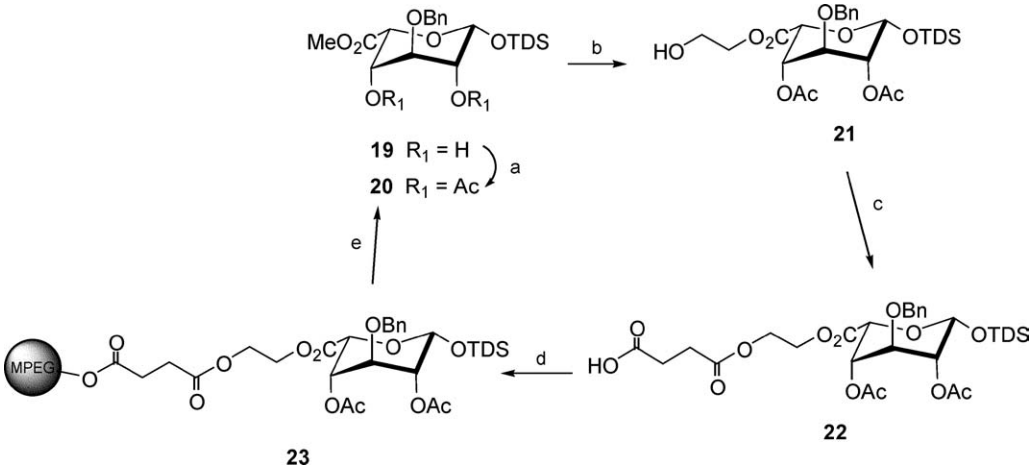
As the most convenient option it was envisaged to attach the first disaccharide module to the solid support through the carboxylate group. For this to be feasible the L-iduronate unit to be attached had to be suitably functionalized to be linked to the solid support. The synthesis of the L-iduronate building blocks is lengthy and time consuming [32–34]. These building blocks are currently prepared in our laboratory according to a procedure [34] which affords multigram amounts of L-iduronic acid methyl esters. For the sake of practicality and in order to make as much use as possible of the previously developed solution phase chemistry we decided to keep the established L-iduronate synthesis procedure and to convert the methyl L-iduronate building blocks into suitable hydroxyl ester derivatives that could be linked to the solid support. We envisioned this transformation using a transesterification reaction to obtain a 2-hydroxyethyl uronate. It has been reported that transesterification of highly funtionalized esters effectively takes place in mild conditions in the presence of catalytic dibutyltin oxide [35]. The feasibility of this approach was tested with methyl uronate **20** readily prepared from **19** [33] (Scheme 5). Compound **20** could be transformed to **21** that was reacted with succinic anhydride to



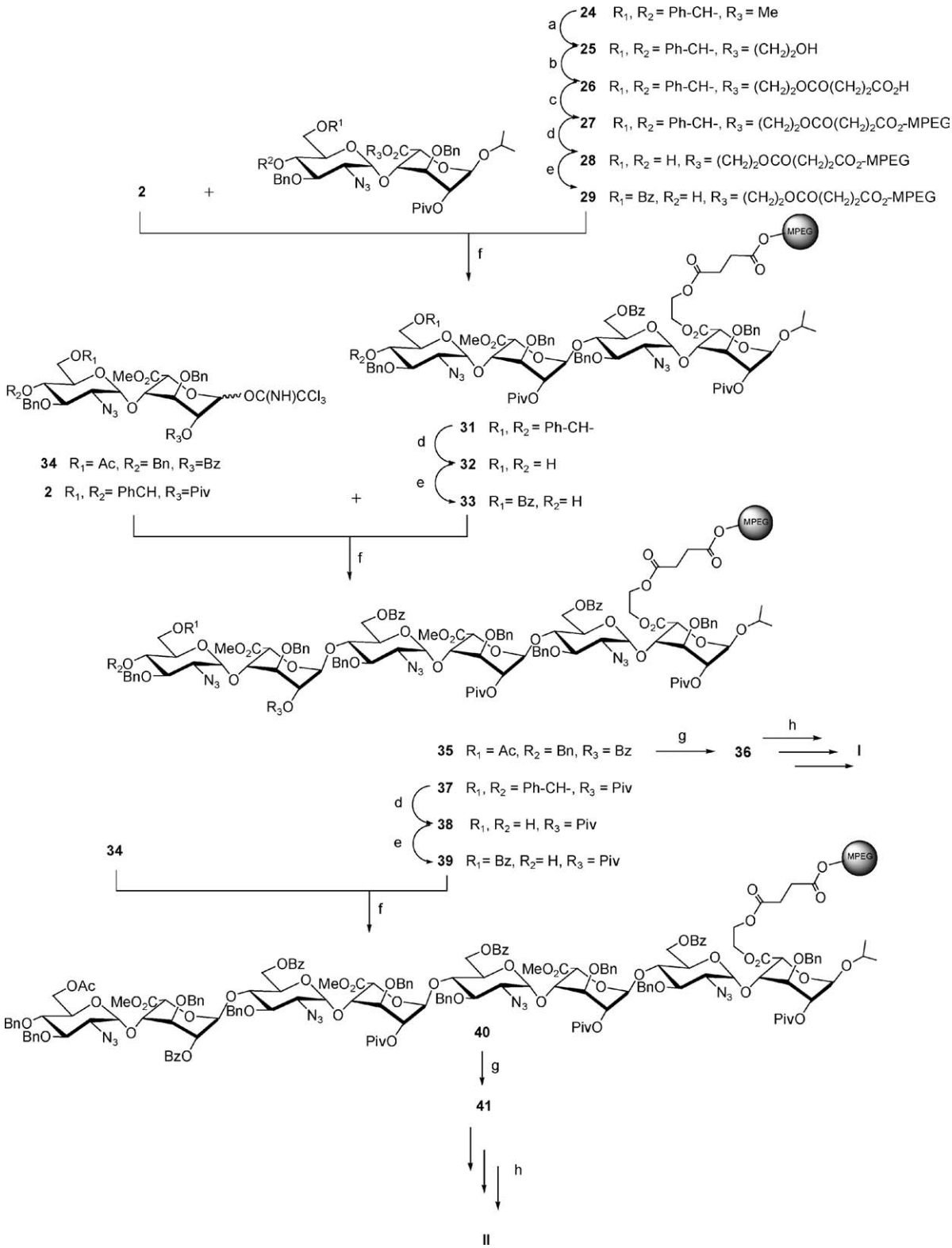
Scheme 4. (a) i. $Bu_2Sn(OMe)_2$, toluene; ii. succinic anhydride, DMAP, Py, 79%; (b) i. DIC, DMAP, CH_2Cl_2, MPEG; ii. BzCN, Et_3N, CH_3CN, $-30^\circ C$, 40%; (c) **2**, TMSOTf, CH_2Cl_2 (three times); (d) MeONa, CH_2Cl_2, 36% two steps.

afford hemisuccinate **22**. Compound **22** was then condensed with MPEG to give the support bound iduronic ester **23**. Cleavage from the support was also conveniently performed using the transesterification reaction in refluxing methanol [35].

With this promising result the solid phase synthesis was planned from disaccharide **24** [12]. Transesterification of **24** gave **25** that was reacted with succinic anhydride to give hemisuccinate **26**. Compound **26** was then used to obtain the functionalized MPEG **27**. As above, glycosyl acceptor **29** was prepared from **27** after removal of the benzylidene acetal to give **28** and regioselective benzoylation. Glycosylation with donor **2** at room temperature gave the corresponding resin bound tetrasaccharide **31** (Scheme 6). Four glycosylation cycles were performed to assure completion. The glycosylation



Scheme 5. (a) Ac_2O, DMAP, Py, 93%; (b) ethylene glycol, Bu_2SnO, toluene, $115^\circ C$, 85%; (c) Succinic anhydride, DMAP, Py, 98%; (d) MPEG, DCC, DMAP, CH_2Cl_2, 100%; (e) Bu_2SnO, MeOH, $65^\circ C$, 92%.



Scheme 6. (a) ethylene glycol, Bu_2SnO, toluene, 135°C, 81%; (b) succinic anhydride, DMAP, Py, 98%; (c) MPEG, DIC, DMAP, CH_2Cl_2; (d) EtSH, *p*TsOH, CH_2Cl_2; (e) BzCN, Et_3N (cat.), CH_3CN, −20°C. (f) i. TMSOTf, CH_2Cl_2 (four cycles); ii. PS-Suc-COOH, DMAP, DIC, CH_2Cl_2; (g) i. LiOH, H_2O_2, THF; ii. MeOH, KOH 3N; (h) see reference [12].

step was followed by capping using a procedure developed in our laboratory. This procedure is based on previously reported resin-aided capture-release strategies [36] in which the tagged unreacted PEG-bound components are captured onto the solid phase by chemoselective reaction with a functionalized resin [37]. In our approach, the free hydroxyl groups of the soluble MPEG-bound glycosyl acceptor are selectively and efficiently esterified by an insoluble Merryfield resin functionalized with an acid-ended tether and filtered off from the reaction mixture. After the capping step, glycosyl acceptor **33** was prepared after removal of the benzylidene group in **31** to give diol **32** and regioselective benzoylation. From tetrasaccharide **33** the oligosaccharide construct was further elongated either by glycosylation with trichloroacetimidate **34** [12] to yield **35** or by a further reaction with trichloroacetimidate **2** to give **37** and subsequent glycosylation of hexasaccharide acceptor **39**, prepared in the usual way via **38**, with trichloroacetimidate **2** to afford **40**. Cleavage of the fully protected oligosaccharides from **35** and **40** was attempted by transesterification in refluxing methanol [35]. However, some partial deacylation was observed. Therefore **35** and **40** were directly submitted to basic conditions. Treatment with LiOOH and then with hydroalcoholic KOH removed acyl, methoxycarbonyl and succinoyloxy carbonyl groups to afford the known partially protected oligosaccharides **36** and **41** respectively (Figure 3) [12] which showed ^{1}H NMR spectra identical in all respects to those of samples previously prepared using solution phase chemistry (Scheme 6). From **36** and **41** hexasaccharide **I** and octasaccharide **II** respectively have been previously prepared [12].

In conclusion, the solution phase modular approach previously developed by us for the synthesis of heparin-like oligosaccharides with different size and charge distribution [12–15] allows for the synthesis of these molecules on a soluble polymer support (MPEG) or on a PEG-grafted polystyrene resin (ArgoGel^{TM}). The best results have been obtained using MPEG as polymer support, a succinoyl ester linker and attaching the starting disaccharide acceptor through the carboxylate group of the L-iduronate unit taking advantage of a mild transesterification reaction in the presence of dibutyltin oxide. The diversity of disaccharide modules already prepared [12–15] for the solution phase synthesis of several oligosaccharides will permit the straightforward preparation of a diversity of heparin-like oligosaccharides for biological investigation. The reported modular synthetic procedure constitutes a convenient method for the solid phase preparation of other glycosaminoglycan fragments by combining the different building blocks as required in the glycosylation sequence.

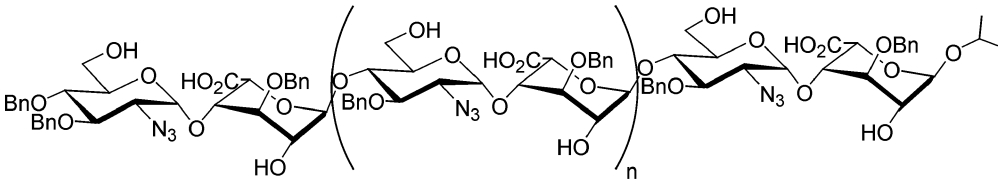
Experimental section

General procedures

Thin layer chromatography (TLC) analyses were performed on silica gel 60 F_{254} precoated on aluminium plates (Merck) and the compounds were detected by staining with sulfuric acid/ethanol (1:9) or with anisaldehyde solution [anisaldehyde (25 mL) with sulfuric acid (25 mL), ethanol (450 mL) and acetic acid (1 mL)] followed by heating at over 200°C. Column chromatography was carried out on silica gel 60 (0.2–0.5 mm, 0.2–0.063 mm or 0.040–0.015 mm; Merck). Optical rotations were determined with a Perkin-Elmer 341 polarimeter. ^{1}H- and ^{13}C-NMR spectra were acquired on Bruker DPX-300 and DRX-500 spectrometers and chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal reference or relative to D_2O. Elemental analyses were performed with a Leco CHNS-932 apparatus, after drying analytical samples over phosphorous pentoxide for 24 h. Mass spectra (fast atom bombardment, FAB MS) were carried out by the Mass Spectrometry Service, Facultad Química, Seville, with a Kratos MS-80 RFA spectrometer. MALDI-TOF MS were recorded on a GSG system. ArgoGel^{TM}-NH_2 resin was purchased from Argonaut Technologies.

Methyl (succinoyl 4-O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-D-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α,β-L-idopyranosyd)uronate (**3**)

To a solution of **1** [12] (160 mg, 0.21 mmol) in pyridine (3 mL), succinic anhydride (86 mg, 0.86 mmol) and a catalytic amount of DMAP were added. After stirring overnight, H_2O (1 mL) was added and stirring continued for a more hour. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with HCl 1M solution (2 × 40 mL) and H_2O (2 × 40 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to afford **3** (181 mg, 100%). TLC 0.42 (Toluene 6/Acetone 1). ^{1}H-NMR



36 n=1
41 n=2

Figure 3. Partially protected oligosaccharides **36** and **41**.

(500 MHz, $CDCl_3$): δ 7.44–7.24 (m, 15H; 3 Ph); 6.28 (d, J = 3.5 Hz, 0.35H; H-1 β); 6.28 (d, J = 1.7 Hz, 0.65H; H-1 α); 5.52 (s, 1H; Ph-CH-α and β); 5.08 (dd, J = 3.5 Hz, 1.7 Hz, 0.65H; H-2 α); 4.99 (t, J = 3.5 Hz, 0.35H; H-2 β); 4.96 (d, J = 3.5 Hz, 0.35H; H-1′ β); 4.91–4.88 (m, 1.65H; H-1′ α, 1 Ph-CH_2 α and β); 4.77–4.69 (m, 3.35H; H-5 β, 3 Ph-CH_2 α and β); 4.58 (d, J = 2.6 Hz, 0.65H; H-5 α); 4.29 (dd, J = 9.5 Hz, 4.5 Hz, 1H; H-6a′ α and β); 4.19 (t, J = 3.5 Hz, 0.65H; H-3 α); 4.12 (t, J = 3.5 Hz, 0.35H; H-4 β); 4.04–3.87 (m, 3H; H-3 β, H-4 α, H-3′, H-5′ α and β); 3.74 (s, 3H; COOMe α and β); 3.66–3.62 (m, 2H; H-4′, H-6′b α and β); 3.38–3.36 (m, 1H; H-2′ α and β); 2.68–2.61 (m, 4H; OCO—CH_2—CH_2—COOH); 1.24 and 1.18 (2s, 9H; $C(CH_3)_3$) α and β). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 177.9, 177.4, 176.5, 170.7, 170.0, 168.7, 168.0 (C=O); 137.8-126.1 (Ph); 101.6 (Ph-CH-); 100.1 (C-1′ α); 99.6 (C-1′ β); 91.9 (C-1 β); 90.6 (C-1 α); 82.5 (C-4′ α and β); 76.1, 75.8, 75.2, 74.9, 74.8, 74.1, 73.5, 73.4, 70.6, 68.5, 67.6, 63.4, 63.1, 63.0, 52.4, 39.1, 38.9, 28.8, 28.7, 28.4, 27.3, 27.1, 27.0.

Functionalization of ArgoGel-NH_2

*Methyl 4-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α,β-*L*-idopyranosuronate* (**6**)

To a mixture of **3** (66 mg, 77 μmol), HOBt (104 mg, 0.77 mmol) and ArgoGel-NH_2 (184 mg, 77 μmol; initial loading 0.42 mmol/g) swollen in a 1:1 mixture of DMF and CH_2Cl_2 (3 mL) under an argon atmosphere, DIC (24 μL, 0.15 mmol) was added dropwise at room temperature. After shaking for 24 h, the reaction mixture was filtered off, and the resin was washed with H_2O (2 × 10 mL), DMF (2 × 3 mL), THF (2 × 3 mL) and CH_2Cl_2 (2 × 3 mL). Finally, Ac_2O (1 mL) and pyridine (2 mL) were added and the mixture was shaken for 6 h. Then, the reaction mixture was filtered off, and the resin was washed with H_2O (2 × 3 mL), DMF (2 × 3 mL), THF (2 × 3 mL), CH_2Cl_2 (2 × 3 mL) and dried to give **4** in 84% yield according to the weight gain of resin (54 mg, 65 μmol). After drying the resin in vacuum until the weight remained constant, the new loading was determined to be 0.35 mmol/g.

To a suspension of **4** (65 μmol), swollen in dry CH_2Cl_2 (4 mL), EtSH (40 μL, 0.52 mmol) and *p*TsOH (18 mg, 66 μmol) were added. After shaking for 18 h under an argon atmosphere, the reaction was filtered off, and the resin was washed with H_2O (2 × 4 mL), DMF (2 × 4 mL), THF (2 × 4 mL) and CH_2Cl_2 (2 × 4 mL). Finally, the resin was dried at high vacuum until the weight remained constant. To resin-bound diol (59 μmol), swollen in dry CH_3CN (8 mL) at −30°C, BzCN (11 mg, 85 μmol) and one drop of Et_3N were added. After shaking for 1 h at that temperature, the reaction was filtered off, and the resin was washed with H_2O (2 × 5 mL), DMF (2 × 5 mL), THF (2 × 5 mL) and CH_2Cl_2 (2 × 5 mL). Finally, the resin was dried at high vacuum until the weight remained constant to yield **5** (304 mg, 58 μmol, 89%).

An aliquot of resin-bound acceptor **5** (52 mg, 10 μmol) was swollen in dry THF (1 mL). Then, hydrazine acetate (1 mg, 30 μmol) was added and the reaction shaken for 2 h. AcOEt (2 mL) was added and the reaction was filtered off and then the resin washed with CH_2Cl_2 (2 × 3 mL). The resin was washed with CH_2Cl_2 (5 × 3 mL), H_2O (2 × 3 mL), DMF (2 × 3 mL), THF (2 × 3 mL) and CH_2Cl_2 (2 × 3 mL). The organic layer was concentrated to 3–4 mL and then passed through a short filter of silica (eluent: AcOEt). The combined fractions containing the desired product were concentrated and purified by flash chromatography (2:1 hexane/AcOEt) to yield **6** (7 mg, 92%). TLC 0.73 (Hexane 1/AcOEt 2). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.01–7.31 (m, 15H; 3 Ph); 5.36 (br s, 0.7H; H-1 α); 5.19 (d, J = 8.0 Hz, 0.3H; H-1 β); 5.02 (d, J = 3.1 Hz, 0.7H; H-1′ α); 4.95 (d, J = 3.5 Hz, 0.3H; H-1′ β); 4.89-4.59 (m, 7H; H-2, H-5, H-6′a, 4 Ph-CH_2 α and β); 4.41–4.39 (m, 1H; H-6′b α and β); 4.12–3.50 (m, 5H; H-3, H-4, H-4′, H-5′, OH-1 α and β); 3.79 (s, 3H; COOMe α and β); 3.27–3.02 (m, 2H; H-2′, OH-4 α and β); 1.26–1.13 (2s, 9H; $C(CH_3)_3$)). MALDI-TOF MS (positive mode, DHB-matrix) m/z 786 ($M + Na^+$), 802 ($M + K^+$).

*Methyl O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-L-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α, β-L-idopyranosuronate* (**8**)

Resin-bound acceptor **5** (550 mg, 0.140 mmol) was swollen in a solution of **2** (460 mg, 0.52 mmol) in dry CH_2Cl_2 (2.5 mL), shaken for 30 min and cooled at 0°C. TMSOTf (3.1 μL, 20 μmol) was added and the reaction mixture was shaken at room temperature for 3 h. The reaction was filtered off and then the resin washed with CH_2Cl_2 (2 × 6 mL), H_2O (2 × 6 mL), DMF (2 × 6 mL), THF (2 × 6 mL) and CH_2Cl_2 (2 × 6 mL). The glycosylation and washing were repeated three times in the same conditions. Finally, the resin was dried at high vacuum until weight remained constant to yield **7** in 90% according to the weight gain of resin (92 mg, 0.125 mmol). Then, **7** (36 mg, 6.9 μmol) was treated as described above for cleavage to yield **8** (9 mg, 87%). Traces of **6** (<1 mg) were also detected. TLC 0.47 (Hexane 1/AcOEt 1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.06–7.24 (m, 30H; 6 Ph); 5.52 (s, 1H; Ph-CH-α and β); 5.42–5.34 (m, 2H; H-1a, H-1c α and β); 5.03–4.94 (m, 3H; H-1b, H-1d, H-2c α and β); 4.91–4.47 (m, 13H; H-2a, H-5a, H-5c, H-6b, H-6′b, 8 Ph-CH_2 α and β); 4.31–4.26 (m, 1H; H-6d α and β); 4.12–3.88 (m, 4H; H-3a, H-3c, H-4a, H-4c α and β); 3.88–3.19 (m, 16H; H-2b, H-2d, H-3b, H-3d, H-4d, H-4d, H-5b, H-5d, H-6′d, OH-1a, 2 COOMe α and β); 1.22–1.17 (m, 9H; $C(CH_3)_3$)). $C_{78}H_{88}O_{24}N_6$ (1493.605); FAB MS m/z 1516 ($M + Na^+ + H^+$).

Methyl isopropyl O-(6-O-acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-L-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-L-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-L-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α, β-L-idopyranosuronate (**9**).

Octasaccharide **9** was prepared according a procedure for chain elongation. This procedure consists on the generation of resin-bound acceptor (hydrolysis of benzylidene acetal followed by selective acylation, see above) and subsequent glycosylation (see above) with donor **2**. Resin-bound disaccharidic acceptor **5** (220 mg, 56 μmol) was glycosylated with donor **2** to yield the resin-bound tetrasaccharide. This compound was elongated once more to yield the corresponding resin-bound hexasaccharide which was submitted to a last cycle of elongation affording the resin bound octasaccharide. Finally, cleavage of products was performed according to the procedure described above and purified by flash chromatography (5:1 Toluene/EtOAc) to yield **9** (16 mg, 10% from **5**, 8 steps). TLC 0.53 (Hexane 1/AcOEt 1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.05–7.26 (m, 60H; 12 Ph); 5.50 (s, 1H; Ph-CH-α and β); 5.52–5.41 (m, 4H; H-1a, H-1c, H-1e, H-1g α and β); 5.03–4.38 (m, 34H; H-1b, H-1d, H-1f, H-1h, H-2a, H-2c, H-e, H-2g, H-5a, H-5c, H-5e, H-5g, H-6b, H-6′b, H-6d, H-6′d, H-6f, H-6′f, 16 Ph-CH_2 α and β); 4.16–3.28 (m, 27H; H-3a, H-3c, H-3e, H-3g, H-4a, H-4c, H-4e, H-4g, H-2b, H-2d, H-2f, H-2h, H-3b, H-3d, H-3f, H-3h, H-4b, H-4d, H-4f, H-4h, H-5b, H-5d, H-5f, H-5h, H-6′h, H-6h, OH-1a α and β); 3.77, 3.38, 3.33, 3.28 (4s, 12H; 4 COOMe); 1.20, 1.16, 1.14, 1.13 (4s, 36H; 4 $C(CH_3)_3$)). $C_{156}H_{174}O_{48}N_{12}$ (2985.192); FAB MS m/z 3007 ($M + Na^+ + 2H^+$).

Methyl (MPEG-succinoyl 4-O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-D-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α, β-L-idopyranosyd)uronate (**10**)

To a mixture of **3** (101 mg, 0.119 mmol), MPEG (358 mg, 72 μmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (2 mL), DIC (28 μL, 0.177 mmol) was added dropwise at room temperature. After stirring for 10 h, the volume was reduced to 1 mL and Et_2O (10 mL) was added with vigorous stirring until precipitation. The precipitate was filtered off and dried. Then, Ac_2O (1 mL) and pyridine (2 mL) were added and the mixture was stirred for 6 h. Et_2O (20 mL) was added with vigorous stirring until precipitation. The precipitate was filtered off and dried at high vacuum to yield **10**. NMR analysis showed 90% of linkage. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.25 (d, $J = 3.7$ Hz, 0.7H; H-1 β); 6.08 (d, $J = 2.8$ Hz, 0.3H; H-1 α); 5.48 (s, 1H; Ph-CH- α and β); 5.04 (dd, $J = 4.8$ Hz, 2.6 Hz, 0.3H; H-2 α); 4.96 (t, $J = 4.4$ Hz, 0.7H; H-2 β); 4.91 (d, $J = 3.6$ Hz, 0.7H; H-1′ β); 4.82 (d, $J = 3.6$ Hz, 0.3H; H-1′ α); 4.88–4.64 (m, 4.7H; H-5 β, 4 Ph-CH_2 α and β); 4.54 (d, $J = 3.3$ Hz, 0.3H; H-5 α); 4.27–3.88 (m, 2H; H-3, H-4 α and β); 3.35–3.29 (m, 1H; H-2′ α and β); 1.20 and 1.18 (2s, 9H; $C(CH_3)_3$) β and α).

Methyl (MPEG-succinoyl 4-O-(2-azido-3-O-benzyl-2-deoxy-α-D-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α, β-L-idopyranosyd)uronate (**11**)

To a solution of **10** (384 mg, 66 μmol), previously coevaporated with toluene, in dry CH_2Cl_2 (4 mL), EtSH (26 μL, 0.33 mmol) and catalytic pTsOH were added. After stirring for 10 h, the volume was reduced to 2 mL and Et_2O (25 mL) was added with vigorous stirring until precipitation. The precipitate was filtered off and dried at high vacuum to yield **11**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.29 (d, $J = 3.7$ Hz, 0.7H; H-1 β); 6.11 (d, $J = 2.2$ Hz, 0.3H; H-1 α); 5.05 (m, 0.3H; H-2 α); 5.00–4.89 (m, 1.7H; H-1′ α and β, H-2 β); 4.85–4.69 (m, 4.7H; H-5 β, 4 Ph-CH_2 α and β); 4.57 (d, $J = 3.1$ Hz, 0.3H; H-5 α); 4.23-3.98 (m, 2H; H-3, H-4 α and β); 3.26–3.16 (m, 1H; H-2′ α and β); 1.21 and 1.19 (2s, 9H; $C(CH_3)_3$) β and α).

Methyl (MPEG-succinoyl 4-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-D-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α, β-L-idopyranosyd)uronate (**12**)

To a cooled (−20°C) solution of **11** (365 mg, 64 μmol), previously coevaporated with toluene, in dry CH_3CN (7 mL), BzCN (16.6 mg, 0.128 mmol) and catalytic Et_3N were added. After stirring for 45 min, MeOH (1 mL) was added and the reaction was allowed to warm to room temperature. The volume was reduced to 3 mL and Et_2O (40 mL) was added with vigorous stirring until precipitation. The precipitate was filtered off and dried at high vacuum to yield **12**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.25 (d, $J = 3.5$ Hz, 0.7H; H-1 β); 6.03 (br s, 0.3H; H-1 α); 5.02 (m, 0.3H; H-2 α); 4.99–4.88 (m, 1.7H; H-1′ α and β, H-2 β); 4.85–4.54 (m, 6H; H-5, H-6′a, 4 Ph-CH_2 α and β); 4.38 (m, 1H; H-6′b α and β); 4.17–3.97 (m, 2H; H-3, H-4 α and β); 3.24–3.07 (m, 1H; H-2′ α and β); 1.19 and 1.17 (2s, 9H; $C(CH_3)_3$) β and α).

Methyl (MPEG-succinoyl O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-L-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α, β-L-idopyranosyd)uronate (**13**)

To a mixture of **12** (340 mg, 58 μmol), previously coevaporated with toluene, and **2** (104 mg, 0.117 mmol) in dry CH_2Cl_2 (1 mL), TMSOTf (4 μL, 24 μmol) was added. After stirring for 2h, one drop of Et_3N was added. Et_2O (30 mL) was added with vigorous stirring until precipitation. The white precipitate was recovered by filtration to yield **13**. This glycosylation was repeated twice more. Significant spectral data: ^{1}H-NMR

(500 MHz, $CDCl_3$): δ 6.32 (d, $J = 4.4$ Hz, 0.6H; H-1a β); 6.13 (d, $J = 2.8$ Hz, 0.4H; H-1a α); 5.46 (s, 1H; Ph-CH-α and β); 5.40–5.31 (m, 1H; H-1c α and β); 5.07–4.80 (m, 2H; H-1b, H-1d α and β); 4.92–4.40 (m, 12H; H-5a, H-5c, H-6a, H-6′a, 8Ph-CH_2 α and β); 4.24–3.90 (m, 4H; H-3a, H-3c, H-4a, H-4c α and β); 3.41–3.19 (m, 2H; H-2b, H-2d α and β).

*Methyl O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α, β-*L*-idopyranosuronate* (**8**)

To solution of **13** (321 mg, 49 μmol) in dry THF (10 mL), hydrazine acetate (5 mg, 55 μmol) was added and stirred for 8 h. AcOEt (3 mL) was added and concentrated to 2–3 mL. Then, Et_2O (30 mL) was added with vigorous stirring until precipitation. The white precipitate was removed by filtration and the soluble fraction concentrated to dryness and purified by flash chromatography (2:1 Hexane/EtOAc), to yield **8** (20 mg, 20% from **10**, four steps). Physical data were identical to those described above for this compound.

*Methyl (isopropyl 4-O-(2-azido-3-O-benzyl-2-deoxy-6-O-succinoyl-α-*D*-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**15**)

To a solution of **14** (83 mg, 0.118 mmol) in dry toluene (5 mL) at an argon atmosphere, dibutyltin dimethoxide (30 μL, 0.130 mmol) was added. Toluene (2.5 mL) was then removed by distillation and the tin acetal solution allowed to cool to room temperature. This solution was added dropwise to a solution of succinic anhydride (13 mg, 0.130 mmol), pyridine (14.3 μL, 0.177 mmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (1 mL) and stirred overnight. Then, H_2O (1 mL) was added and stirring continued one more hour. The mixture was diluted with CH_2Cl_2 (25 mL) and washed with NH_4Cl aqueous solution (15 mL) and H_2O (15 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo and the mixture was purified by flash chromatography (1:2 Hexane/EtOAc), to yield **15** (75 mg, 79%). TLC 0.44 (Hexane 1/EtOAc 3). $[\alpha]_D^{20} = +8.4^\circ$ ($c = 1$, MeOH). ^{1}H-NMR (500 MHz, MeOD): δ 7.45–7.28 (m, 15H; 3 Ph); 5.25 (d, $J = 5.7$ Hz, 1H; H-1); 5.08 (d, $J = 3.6$ Hz, 1H; H-1′); 5.02 (d, $J = 11.0$ Hz, 1H; 1 Ph-CH_2); 4.91–4.74 (m, 5H; H-2, H-5, 3 Ph-CH_2); 4.47 (dd, $J = 2.0$ Hz, 11.9 Hz, 1H; H-6′a); 4.25 (dd, $J = 5.5$ Hz, 11.9 Hz, 1H; H-6′b); 4.21 (t, $J = 5.4$ Hz, 1H; H-4); 4.04–3.96 (m, 2H; H-3, $CH(CH_3)_2$); 3.89 (m, 1H; H-5′); 3.84 (s, 3H; COOMe); 3.71 (dd, $J = 8.8$ Hz, 10.2 Hz, 1H; H-3'); 3.61 (dd, $J = 8.8$ Hz, 9.9 Hz, 1H; H-4′); 3.36–3.33 (m, 1H; H-2′); 2.66–2.59 (s, 4H; OCO—CH_2—CH_2—COOH); 1.23 (s, 9H; $C(CH_3)_3$); 1.22 and 1.18 (2d, $J = 6.2$ Hz, 6H; $CH(CH_3)_2$). ^{13}C-NMR (125 MHz, MeOD): δ 180.0–165.0 (C=O); 138.0–125.8 (Ph); 98.2 (C-1′); 97.3 (C-1); 79.6 (C-3'); 76.5 (C-3); 75.1; 74.7; 73.5; 72.0; 71.7; 71.5; 71.4; 70.9 (C-4′); 63.4; 63.3; 63.0; 51.8; 38.8; 29.0; 26.7 (OPiv); 22.6, 21.1 ($CH(CH_3)_2$). HR-FAB MS: m/z calcd. for $C_{39}H_{51}O_{15}N_3$ 824.3218; found: 824.3222.

*Methyl (isopropyl 4-O-(2-azido-3-O-benzyl-2-deoxy-6-O-MPEG-succinoyl-α-*D*-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**16**)

To a mixture of **15** (81 mg, 0.101 mmol), MPEG (360 mg, 72 μmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (3 mL), DIC (24 μL, 0.151 mmol) was added dropwise at room temperature. After stirring for 10 h, the volume was reduced to 1 mL and Et_2O (10 mL) was added with vigorous stirring until precipitation. The precipitate was filtered off and dried. Then, to a cooled (-20°C) solution of this solid in dry CH_3CN (10 mL), BzCN (8 mg, 60 μmol) and catalytic Et_3N were added. After stirring for 45 min, MeOH (1 mL) was added and the reaction was allowed to warm to room temperature. The volume was reduced to 3 mL and Et_2O (40 mL) was added with vigorous stirring until precipitation. The precipitate was filtered off and dried at high vacuum to yield **16**. NMR analysis showed 40% of linkage. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.16 (d, $J = 4.5$ Hz, 1H; H-1); 5.01 (d, $J = 3.7$ Hz, 1H; H-1′); 4.89 (t, $J = 4.9$ Hz, 1H; H-2); 4.85–4.66 (m, 5H; H-5, 4 Ph-CH_2); 4.56 (dd, $J = 3.2$ Hz, 12.5 Hz, 1H; H-6′a); 4.16–3.91 (m, 3H; H-3, H-4, H-6′b); 3.16 (dd, $J = 3.7$ Hz, 10.3 Hz, 1H; H-2′).

*Methyl (isopropyl O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-3-O-benzyl-2-deoxy-6-O-MPEG-succinoyl-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**17**)

To a mixture of **16** (406 mg, 27 μmol), previously coevaporated with toluene, and **2** (45 mg, 54 μmol) in dry CH_2Cl_2 (1 mL), TMSOTf (0.6 μL, 3.4 μmol) was added. After stirring for 2 h, one drop of Et_3N was added. Et_2O (20 mL) was added with vigorous stirring until precipitation. The white precipitate was recovered by filtration to yield **17**. This glycosylation was repeated twice more. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.46 (s, 1H; Ph—CH—); 5.22 (br s, 1H; H-1c); 5.14 (br s, 1H; H-1a); 4.99–4.52 (m, 14H; H-1b, H-1d, H-2a, H-2c, H-5a, H-5c, 8 Ph-CH_2); 3.31–3.24 (m, 2H; H-2b, H-2d).

*Methyl (isopropyl O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**18**)

To a solution of **17** (403 mg, 25 μmol) in CH_2Cl_2 (2 mL), MeONa (36 μmol) was added and stirred until complete cleavage from resin (TLC Hexane 3/EtOAc 2). The mixture was diluted with CH_2Cl_2 (30 mL) and washed with NH_4Cl aqueous

solution (20 mL) and H_2O (20 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo. The white solid was re-dissolved in CH_2Cl_2 (2 mL) and Et_2O (20 mL) was added with vigorous stirring until precipitation. The white precipitate was removed by filtration and the soluble fraction concentrated to dryness and purified by flash chromatography (4:1Hexane/EtOAc), to yield **18** (14 mg, 36% from **16**, two steps). TLC 0.44 (Hexane 3/EtOAc 2). $[\alpha]_D^{20} = -1.0^\circ$ ($c = 0.2$, CH_2Cl_2). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.45–7.22 (m, 25H; 5 Ph); 5.52 (s, 1H; Ph—CH—); 5.33 (d, $J = 5.1$ Hz, 1H; H-1c); 5.22 (d, $J = 4.9$ Hz, 1H; H-1a); 5.03 (d, $J = 3.6$ Hz, 1H; H-1b); 4.99 (t, $J = 5.3$ Hz, 1H; H-2c); 4.96 (d, $J = 3.7$ Hz, 1H; H-1d); 4.93-4.88 (m, 3H; H-2a, 2 Ph-CH_2); 4.78–4.63 (m, 8H; H-5a, H-5c, 6 Ph-CH_2); 4.21 (dd, $J = 5.0$ Hz, 10.3 Hz, 1H; H-6d); 4.11 (t, $J = 5.4$ Hz, 1H; H-4a); 4.05 (t, $J = 5.1$ Hz, 1H; H-4c); 3.97–3.61 (m, 1H; H-3a, H-3b, H-3c, H-3d, H-4b, H-4d, H-5b, H-5d, H-6b, H-6′b, H-6′d, $CH(CH_3)_2$); 3.32 (dd, $J = 3.8$ Hz, 9.9 Hz, 1H; H-2d); 3.24 (dd, $J = 3.6$ Hz, 10.2 Hz, 1H; H-2b); 1.19 (2s, 18H; $C(CH_3)_3$); 1.19 and 1.14 (2d, 6H; $J = 6.0$ Hz, $CH(CH_3)_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 180.0–165.0 (C═O); 137.3-126.1 (Ph); 101.5 (Ph—CH—); 99.1 (C-1d); 98.0 (C-1b); 97.7 (C-1c); 97.2 (C-1a); 82.4; 77.8; 77.4; 77.2; 76.9; 76.7; 76.5; 76.4; 75.9; 74.8; 74.6; 73.7; 73.6; 73.4; 72.3; 71.3; 70.8; 70.6; 68.5; 6.3; 63.0; 62.9; 60.4; 52.2; 52.1; 38.9; 38.8; 31.6; 27.2; 27.1 (OPiv); 23.3, 21.1 (CH-$(CH_3)_2$).

*Methyl (dimethylthexylsilyl 2,4-di-O-acetyl-3-O-benzyl-β-*L*-idopyranosyd)uronate* (**20**)

To a solution of **19** (300 mg, 0.68 mmol) in pyridine (4 mL), Ac_2O (2 mL) and a catalytic amount of DMAP were added. After stirring overnight, the mixture was diluted with CH_2Cl_2 (25 mL) and washed with HCl 1N solution (2 x 15 mL) and H_2O (2 × 20 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo and the mixture was purified by flash chromatography (3:1Hexane/EtOAc), to yield **20** (332 mg, 93%). $[\alpha]_D^{20} = +24.1^\circ$ ($c = 1$, CH_2Cl_2). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.40–7.28 (m, 5H; 1 Ph); 5.12 (br s, 1H; H-4); 5.10 (d, $J = 1.4$ Hz, 1H; H-1); 4.94 (br s, 1H; H-2); 4.76 (2d, $J = 11.8$ Hz, 4H; 2 Ph-CH_2); 4.62 (d, $J = 2.1$ Hz, 1H; H-5); 3.86 (d, $J = 2.7$ Hz, 1H; H-3); 3.76 (s, 3H; COOMe); 2.06 (s, 3H; OAc); 2.03 (s, 3H; OAc); 1.61 (m, 1H; $CH(CH_3)_2$); 0.87–0.82 (4s, 12H; $C(CH_3)_2$ and $CH(CH_3)_2$); 0.23–0.14 (2s, 6H; $Si(CH_3)_2$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.5, 170.4, 168.4 (C═O); 137.4-128.2 (Ph); 93.3 (C-1); 74.1 (C-3); 73.3 (Ph-CH_2); 72.9 (C-5); 68.1 (C-2); 67.5 (C-4); 52.8 ($COOCH_3$); 34.4, 25.8, 21.3, 20.6, 20.2, 19.0, 18.7, −1.6, −3.3 (OAc, OTDS). HR-FAB MS: m/z calcd. for $C_{26}H_{40}O_9Si$ 525.2520; found: 525.2494.

*2-Hydroxyethyl (dimethylthexylsilyl 2,4-di-O-acetyl-3-O-benzyl-β-*L*-idopyranosyd)uronate* (**21**)

To a solution of **20** (220 mg, 0.42 mmol) in toluene (2 mL), ethylene glycol (7 mL) and Bu_2SnO (10 mg, 42 μmol) were added. After stirring overnight at 110°C, CH_2Cl_2(10 mL) and H_2O (3 mL) were added. The hydroalcoholic layer was extracted with CH_2Cl_2 (4 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated to dryness. The mixture was purified by flash chromatography (2:1Hexane/EtOAc), to yield **21** (197 mg, 85%). TLC 0.15 (Hexane 2/EtOAc 1). $[\alpha]_D^{20} = +24.5^\circ$ ($c = 1$, CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.41–7.26 (m, 5H; 1 Ph); 5.21 (br s, 1H; H-4); 5.11 (br s, 1H; H-1); 4.94 (br s, 1H; H-2); 4.73 (s, 4H; 4 Ph-CH_2); 4.64 (d, $J = 2.6$ Hz, 1H; H-5); 4.41–4.34 (m, 1H; 1 OCO—CH_2—CH_2—OH); 4.22–4.15 (m, 1H; 1 OCO—CH_2—CH_2—OH); 3.83–3.80 (m, 3H; OCO—CH_2—CH_2—OH, H-3); 2.06 (s, 3H; OAc); 2.05 (s, 3H; OAc); 1.61 (m, 1H; $CH(CH_3)_2$); 0.88–0.79 (4s, 12H; $C(CH_3)_2$ and $CH(CH_3)_2$); 0.23–0.15 (2s, 6H; $Si(CH_3)_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.4, 170.1, 167.6 (C═O); 137.0–127.8 (Ph); 92.9 (C-1); 74.0 (C-3); 72.9 (Ph-CH_2); 72.6 (C-5); 67.6 (C-2); 67.0 (C-4); 66.9 (OCO—CH_2—CH_2—OH); 60.7 (OCO—CH_2—CH_2—OH); 34.0, 24.9, 20.8, 20.2, 19.8, 18.6, 18.3, −1.9, −3.6 (OAc, OTDS). HR-FAB MS: m/z calcd. for $C_{27}H_{43}O_{10}Si$ 555.2625; found: 555.2597 $[M + H]^+$.

*2-succinoyloxyethyl (dimethylthexylsilyl 2,4-di-O-acetyl-3-O-benzyl-β-*L*-idopyranosyd)uronate* (**22**)

To a solution of **21** (183 mg, 0.33 mmol) in pyridine (4 mL), succinic anhydride (100 mg, 1 mmol) and a catalytic amount of DMAP were added. After stirring overnight, H_2O (1 mL) was added and the solution was stirred for a more hour. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with HCl 1N solution (2 × 40 mL) and H_2O (2 × 40 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to afford **22** (212 mg, 98%). TLC 0.17 (Hexane 1/EtOAc 1). $[\alpha]_D^{20} = +20.8^\circ$ ($c = 1$, CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.41–7.26 (m, 5H; 1 Ph); 5.14 (br s, 1H; H-4); 5.11 (br s, 1H; H-1); 4.94 (br s, 1H; H-2); 4.73 (m, 4H; 4 Ph-CH_2); 4.64 (d, $J = 1.8$ Hz, 1H; H-5); 4.42–4.29 (m, 4H; OCO—CH_2—CH_2—OCO); 3.85 (br s, 1H; H-3); 2.65 (s, 4H; OCO—CH_2—CH_2—COOH); 2.05 (s, 3H; OAc); 2.04 (s, 3H; OAc); 1.60 (m, 1H; $CH(CH_3)_2$); 0.87–0.83 (4s, 12H; $C(CH_3)_2$ and $CH(CH_3)_2$); 0.22–0.14 (2s, 6H; $Si(CH_3)_2$).^{13}C-NMR (75 MHz, $CDCl_3$): δ 176.1, 172.6, 170.6, 170.5, 167.7 (C═O); 137.8-128.2 (Ph); 93.3 (C-1); 74.1 (C-3); 73.3 (Ph-CH_2); 72.8 (C-5); 68.0 (C-2); 67.4 (C-4); 63.3, 62.6 (O—CH_2—CH_2—O); 34.4, 29.3, 29.2, 25.2, 21.3, 21.2, 20.6, 20.2, 18.9, 18.7, -1.6, -3.3 (CO—CH_2—CH_2—CO, OAc, OTDS). HR-FAB MS: m/z calcd. for $C_{31}H_{46}O_{13}SiNa$ 677.2605; found: 677.2592 $[M]^+$.

*MPEG-2-succinoyloxyethyl (dimethylthexylsilyl 2,4-di-O-acetyl-3-O-benzyl-β-*L*-idopyranosyd)uronate* (**23**)

A mixture of **22** (115 mg, 0.18 mmol), MPEG (700 mg, 0.14 mmol) and a catalytic amount of DMAP, previously co-evaporated with toluene, was dissolved in CH_2Cl_2 (5 mL). Then, DCC (39 mg, 0.19 mmol) was added and the reaction was stirred overnight at room temperature. The volume was reduced to 2 mL and Et_2O (30 mL) was added with vigorous stirring until

precipitation. The white precipitate was filtered off and dried at high vacuum to yield **23**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): 7.28–7.03 (m, 5H; 1 Ph); 5.01 (br s, 1H; H-4); 4.99 (br s, 1H; H-1); 4.82 (br s, 1H; H-2); 4.73 (2d, $J = 11.8$ Hz, 2H; 2 Ph-CH_2); 4.53 (d, $J = 1.6$ Hz, 1H; H-5); 4.34–4.17 (m, 4H; OCO—CH_2—CH_2—OCO); 3.74 (br s, 1H; H-3); 1.93 (s, 3H; OAc); 1.92 (s, 3H; OAc); 1.49 (m, 1H; $CH(CH_3)_2$); 0.76–0.69 (4s, 12H; $C(CH_3)_2$ and $CH(CH_3)_2$); 0.11–0.03 (2s, 6H; $Si(CH_3)_2$).

*Methyl (dimethylthexylsilyl 2,4-di-O-acetyl-3-O-benzyl-β-*L*-idopyranosyd)uronate* (**20**)

To a solution of **23** (779 mg, 0.14 mmol) in MeOH (5 mL), Bu_2SnO (3.4 mg, 13.8 μmol) was added. After stirring overnight at room temperature, the mixture was diluted with CH_2Cl_2 (25 mL) and washed with H_2O (15 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried ($MgSO_4$) and the mixture was purified by flash chromatography (3:1Hexane/EtOAc), to yield **20** (66 mg, 92%). Physical data were identical to those described above for this compound.

*2-Hydroxyethyl (isopropyl 4-O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-*D*-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**25**)

To a solution of **24** (98 mg, 0.124 mmol) in toluene (3 mL), ethylene glycol (9 mL) and Bu_2SnO (300 mg, 1.24 mmol) were added. After stirring for 5 days at 135°C, CH_2Cl_2 (10 mL) and H_2O (3 mL) were added. The hydroalcoholic layer was extracted with CH_2Cl_2 (4 × 20 mL). The organic layer was dried ($MgSO_4$). The volume was reduced to 10 mL and then filtered through Celite. The filtrate was concentrated to dryness. The mixture was purified by flash chromatography (3:1→0:1Hexane/EtOAc), to yield **25** (83 mg, 81%) and unreacted starting material (15 mg, 15%). TLC 0.26 (Hexane 7/EtOAc 3). $[\alpha]_D^{20} = -41.1^\circ$ (*c* 0.9, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.46–7.24 (m, 15H; 3 Ph); 5.56 (s, 1H; Ph-CH-); 5.28 (d, $J = 5.5$ Hz, 1H; H-1); 5.07 (d, $J = 3.8$ Hz, 1H; H-1′); 4.95 (t, $J = 5.8$ Hz, 1H; H-2); 4.91 (d, $J = 11.0$ Hz, 1H; 1 Ph-CH_2); 4.78–4.72 (m, 4H; H-5, 3 Ph-CH_2); 4.35–4.28 (m, 2H; H-6′a, 1 OCO—CH_2—CH_2—OH); 4.20–4.17 (m, 1H; 1 OCO—CH_2—CH_2—OH); 4.11 (dd, $J = 6.6$ Hz, 5.1 Hz, 1H; H-4); 4.06–4.01 (m, 3H; H-3, H-3', H-6′b); 3.94 (m, 1H; $CH(CH_3)_2$); 3.76–3.66 (m, 4H; H-4′, H-5′, OCO-CH_2-CH_2-OH); 3.34 (dd, $J = 3.8$ Hz, 10.1 Hz, 1H; H-2′); 2.39 (br s, 1H; OCO—CH_2—CH_2—OH); 1.21 (s, 9H; $C(CH_3)_3$); 1.20 and 1.15 (2d, 6H; $J = 6.5$Hz, $CH(CH_3)_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 177.2, 169.8 (C═O); 137.8-125.9 (Ph); 101.5 (Ph—CH—); 99.4 (C-1′); 97.3 (C-1); 82.5 (C-4′); 76.1; 75.0; 74.9; 73.7; 71.7; 68.6 (OCO—CH_2—CH_2—OH); 67.1 (C-3'); 63.1; 62.9 (C-2′); 60.3 (OCO—CH_2—CH_2—OH); 38.8, 29.7, 27.2 (OPiv); 23.5, 21.9 (CH-$(CH_3)_2$). HR-FAB MS: *m*/*z* calcd. for $C_{43}H_{53}N_3O_{13}Na$ 842.3476; found: 842.3482.

*2-succinoyloxyethyl (isopropyl 4-O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-*D*-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate (26)*

To a solution of **25** (75 mg, 91 μmol) in pyridine (3 mL), succinic anhydride (55 mg, 0.55 mmol) and a catalytic amount of DMAP were added. After stirring overnight, H_2O (1 mL) was added and stirring continued for a more hour. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with HCl 1M solution (2 × 40 mL) and H_2O (2 × 40 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to afford **26** (83 mg, 98%). TLC 0.41 (Toluene 5/Acetone 1). $[\alpha]_D^{20} = -50.9^\circ$ (*c* = 1, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.44–7.24 (m, 15H; 3 Ph); 5.52 (s, 1H; Ph-CH-); 5.12 (d, $J = 3.8$ Hz, 1H; H-1); 5.00 (d, $J = 3.8$ Hz, 1H; H-1′); 4.94 (t, $J = 4.3$ Hz, 1H; H-2); 4.90 (d, $J = 11.1$ Hz, 1H; 1 Ph-CH_2); 4.81–4.69 (m, 4H; H-5, 3 Ph-CH_2); 4.37–4.21 (m, 5H; H-6′a, OCO-CH_2-CH_2-OCO); 4.14 (m, 1H; H-4); 3.99–3.93 (m, 4H; H-3, H-3', H-6′b, $CH(CH_3)_2$); 3.76–3.66–3.61 (m, 2H; H-4′, H-5′); 3.33 (dd, $J = 3.8$ Hz, 10.0 Hz, 1H; H-2′); 2.57 (s, 4H; OCO—CH_2—CH_2—COOH); 1.22 (s, 9H; $C(CH_3)_3$); 1.21 and 1.16 (2d, $J = 6.5$ Hz, 6H; $CH(CH_3)_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 177.6, 171.8, 168.8 (C═O); 137.8-126.1 (Ph); 101.5 (Ph—CH—); 99.4 (C-1′); 97.4 (C-1); 82.5 (C-4′); 76.2; 76.1; 75.4; 74.8; 72.9; 71.2; 69.7; 69.2; 68.6; 63.2; 63.0; 62.7; 61.9; 38.8, 27.2 (OPiv); 23.5, 21.9 (CH-$(CH_3)_2$). HR-FAB MS *m*/*z* 942 ($M + Na^+$). Anal. calcd. for $C_{47}H_{57}N_3O_{16}\cdot H_2O$: C 60.18%, H 6.34%, N 4.48%; found: C 60.23%, H 6.40%, N 4.50%.

*MPEG-2-succinoyloxyethyl (isopropyl 4-O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-*D*-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**27**)

A mixture of **26** (80 mg, 87 μmol), MPEG (325 mg, 65 μmol) and a catalytic amount of DMAP, previously coevaporated with toluene, was dissolved in CH_2Cl_2 (3 mL). Then, DIC (20 μL, 0.12 mmol) was added and the reaction was stirred overnight at room temperature. The volume was reduced to 2 mL and Et_2O (35 mL) was added with vigorous stirring until precipitation. The white precipitate was filtered off and dried at high vacuum to yield **27**. ^{1}H-NMR analysis confirmed the completion of the reaction. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.48 (s, 1H; Ph—CH—); 5.08 (d, $J = 3.8$Hz, 1H; H-1); 4.97 (d, $J = 3.7$ Hz, 1H; H-1′); 4.88–4.86 (m, 2H; H-2, 1 Ph-CH_2); 4.76–4.65 (m, 4H; H-5, 3 Ph-CH_2); 4.09 (m, 1H; H-4); 3.93-3.89 (m, 2H; H-3, $CH(CH_3)_2$); 3.29 (dd, $J = 3.7$ Hz, 10.0 Hz, 1H; H-2′).

*MPEG-2-succinoyloxyethyl (isopropyl 4-O-(2-azido-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**28**)

To a solution of **27** (630 mg, 0.1 mmol), previously coevaporated with toluene, in dry CH_2Cl_2 (6 mL), EtSH (120 μL,

1.5 mmol) and *p*TsOH (8 mg, 30 μmol) were added. After stirring for 10 h, the volume was reduced to 3 mL and Et_2O (40 mL) was added with vigorous stirring until precipitation. The precipitate was filtered off and dried at high vacuum to yield **28**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.16 (d, $J = 4.9$ Hz, 1H; H-1); 5.04 (d, $J = 3.3$ Hz, 1H; H-1′); 4.90 (t, $J = 4.9$ Hz, 1H; H-2); 4.80–4.70 (m, 5H; H-5, 4 Ph-CH_2); 4.11 (m, $J = 5.5$ Hz, 1H; H-4); 3.95 (m, $J = 5.5$ Hz, 1H; H-3); 3.90 (m, 1H; $CH(CH_3)_2$); 3.15 (dd, $J = 3.3$ Hz, 10.1 Hz, 1H; H-2′).

*MPEG-2-succinoyloxyethyl (isopropyl 4-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**29**)

To a cooled (−20°C) solution of **28** (605 mg, 0.1 mmol), previously coevaporated with toluene, in dry CH_3CN (12 mL), BzCN (20 mg, 0.15 mmol) and catalytic Et_3N were added. After stirring for 45 min, MeOH (1 mL) was added and the reaction was allowed to warm to room temperature. The volume was reduced to 3 mL and Et_2O (40 mL) was added with vigorous stirring until precipitation of **29**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.12 (d, $J = 4.6$ Hz, 1H; H-1); 5.01 (d, $J = 2.8$ Hz, 1H; H-1′); 4.86–4.59 (m, 7H; H-2, H-5, H-6′a, 4 Ph-CH_2); 4.40 (m, 1H; H-6′b); 3.92–3.85 (m, 3H; H-3, H-4, $CH(CH_3)_2$); 3.16 (m, 1H; H-2′).

*MPEG-2-succinoyloxyethyl (isopropyl O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**31**)

To a mixture of **29** (602 mg, 0.1 mmol), previously coevaporated with toluene, and **2** (178 mg, 0.2 mmol) in dry CH_2Cl_2 (3 mL), TMSOTf (5 μL, 30 μmol) was added. After stirring for 2 h, one drop of Et_3N was added and the volume was reduced to 2 mL. Et_2O (30 mL) was added with vigorous stirring until precipitation. The white precipitate was recovered by filtration. This glycosylation was repeated three times more. Then, a mixture of the resulting MPEG derivative, PS-Suc-COOH (400 mg, 0.49 mmol) and a catalytic amount of DMAP were swollen in dry CH_2Cl_2 (7 mL) and DIC (134 μL, 0.8 mmol) was added. After shaking overnight, the mixture was filtered and resin was washed with CH_2Cl_2 (3 × 10 mL). The combined organic filtrates were concentrated to 3-4 mL. Et_2O (40–50 mL) was added with vigorous stirring until precipitation of pure MPEG-bound disaccharide **31**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.49 (s, 1H; Ph—CH—); 5.34 (d, $J = 4.6$ Hz, 1H; H-1c); 5.05 (d, $J = 3.6$ Hz, 1H; H-1a); 4.96–4.91 (m, 2H; H-1b, H-1d); 4.93 (m, 1H; H-2c); 4.87 (m, 1H; H-2a); 4.76 (m, 1H; H-5a); 4.59 (m, 1H; H-5c); 4.10 (m, 1H; H-4a); 4.00 (m, 1H; H-4c); 3.90–3.89 (m, 2H; H-3a, H-3c); 3.32–3.25 (m, 2H; H-2b, H-2d).

*MPEG-2-succinoyloxyethyl (isopropyl O-(2-azido-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**32**)

To a solution of **31** (625 mg, 96 μmol), previously coevaporated with toluene, in dry CH_2Cl_2 (6 mL), EtSH (120 μL, 1.5 mmol) and *p*TsOH (13 mg, 50 μmol) were added. After stirring for 10h, the volume was reduced to 3 mL and Et_2O (40 mL) was added with vigorous stirring until precipitation. The precipitate was filtered off and dried at high vacuum to yield **32**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.38 (br s, 1H; H-1c); 5.07 (br s, 1H; H-1a); 5.06–4.94 (m, 2H; H-1b, H-1d); 4.98 (m, 1H; H-2c); 4.88 (m, 1H; H-2a); 4.76 (m, 1H; H-5a); 4.59 (m, 1H; H-5c); 4.12 (m, 1H; H-4a); 4.05 (m, 1H; H-4c); 3.93 (m, 1H; H-3c); 3.90 (m, 1H; H-3a); 3.20–3.14 (m, 2H; H-2b, H-2d).

*MPEG-2-succinoyloxyethyl (isopropyl O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**33**)

To a cooled (−15°C) solution of **32** (565 mg, 88 μmol), previously coevaporated with toluene, in dry CH_3CN (11 mL), BzCN (18.6 mg, 0.14 mmol) and catalytic Et_3N were added. After stirring for 45 min, MeOH (1 mL) was added and the reaction was allowed to warm to room temperature. The volume was reduced to 3 mL and Et_2O (40 mL) was added with vigorous stirring until precipitation of **33**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.33 (d, $J = 4.8$ Hz, 1H; H-1c); 5.06 (d, $J = 3.5$ Hz, 1H; H-1a); 4.98–4.95 (m, 2H; H-1b, H-1d); 4.93 (m, 1H; H-2c); 4.87 (m, 1H; H-2a); 4.77 (m, 1H; H-5a); 4.60 (m, 1H; H-5c); 4.12 (m, 1H; H-4a); 4.05 (m, 1H; H-4c); 3.89 (m, 2H; H-3a, H-3c); 3.32–3.16 (m, 2H; H-2b, H-2d).

*MPEG-2-Succinoyloxyethyl (isopropyl O-(6-O-acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate (35)*

To a mixture of **33** (270 mg, 41 μmol), previously coevaporated with toluene, and **34** (82 mg, 85 μmol) in dry CH_2Cl_2 (1.5 mL), TMSOTf (2.1 μL, 12.8 μmol) was added. After stirring for 1 h, one drop of Et_3N was added and Et_2O (25 mL) was added with vigorous stirring until precipitation. The white precipitate was recovered by filtration. This glycosylation was repeated three

times more. Then, a mixture of the resulting MPEG derivative, PS-Suc-COOH (300 mg, 0.38 mmol) and a catalytic amount of DMAP were swollen in dry CH_2Cl_2 (5 mL) and DIC (70 μL, 0.41 mmol) was added. After shaking overnight, the mixture was filtered and resin was washed with CH_2Cl_2 (3 x 10 mL). The combined organic filtrates were concentrated to 2–3 mL. Et_2O (30–40 mL) was added with vigorous stirring until precipitation of pure MPEG-bound disaccharide **35**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.53 (d, $J = 4.2$ Hz, 1H; H-1e); 5.37 (d, $J = 5.1$ Hz, 1H; H-1c); 5.13 (m, 1H; H-2e); 5.08 (d, $J = 3.8$ Hz, 1H; H-1a); 4.98–4.84 (m, 3H; H-1b, H-1d, H-1f); 4.93 (m, 1H; H-2c); 4.89 (m, 1H; H-2a); 4.76 (m, 1H; H-5a); 4.65 (m, 1H; H-5e); 4.47 (m, 1H; H-5c); 4.12 (m, 1H; H-4a); 4.11 (m, 1H; H-3e); 4.00 (m, 1H; H-4e); 3.95 (m, 1H; H-4c); 3.90 (m, 1H; H-3a); 3.86 (m, 1H; H-3c); 3.31–3.16 (m, 3H; H-2b, H-2d, H-2f); 1.92 (s, 3H; OAc).

*Isopropyl O-(2-azido-3,4-di-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-α-*L*-idopyranosyluronic acid)-(1 → 4)-O-(2-azido-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-α-*L*-idopyranosyluronic acid)-(1 → 4)-O-(2-azido-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-α-*L*-idopyranosyduronic acid* (**36**)

To a solution of **35** (100 mg, 13 μmol) in THF (2 mL) at −5°C, H_2O_2 30% (0.7 mL) and a 1 M aqueous solution of LiOH (1.1 mL) were added. After stirring for 24 h at room temperature MeOH (1 mL) and a 3 N aqueous solution of KOH (2 mL) were added. After stirring for 24 h more the reaction was neutralized with acidic resin (IRA-120H$^+$), filtered and concentrated. The residue was purified by Sephadex LH-20 (1:1 MeOH-CH_2Cl_2). Finally, fractions containing **36** and free MPEG were combined, concentrated and re-dissolved in MeOH/CH_2Cl_2 1:1 (1.5 mL). Et_2O (13 mL) was added and the mixture was cooled (0°C). The white precipitate was separated by filtration and the organic layer was concentrated in vacuo to yield pure **36** (11 mg, 37% from **26**, 8 steps). The physical data for **36** were identical in all respects with the reported values [12].

*MPEG-2-succinoyloxyethyl (isopropyl O-(2-azido-3-O-benzyl-4,6-di-O-benzylidene-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**37**)

To a mixture of **33** (280 mg, 41 μmol), previously coevaporated with toluene, and **2** (90 mg, 0.1 mmol) in dry CH_2Cl_2 (1.5 mL), TMSOTf (2.5 μL, 15 μmol) was added. After stirring for 1 h, one drop of Et_3N was added and the volume was reduced to 1 mL. Et_2O (10–15 mL) was added with vigorous stirring until precipitation. The white precipitate was recovered by filtration. This glycosylation was repeated three times more. Then, a mixture of the resulting MPEG derivative, PS-Suc-COOH (300 mg, 0.38 mmol) and a catalytic amount of DMAP were swollen in dry CH_2Cl_2 (5 mL) and DIC (80 μL, 0.48 mmol) was added. After shaking overnight, the mixture was filtered and resin was washed with CH_2Cl_2 (3 × 7 mL). The combined organic filtrates were concentrated to 2–3 mL. Et_2O (20–30 mL) was added with vigorous stirring until precipitation of pure MPEG-bound disaccharide **37**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.48 (s, 1H; Ph—CH—); 5.41 (d, 1H; H-1e, $J = 5.5$ Hz); 5.36 (d, 1H; H-1c, $J = 4.9$ Hz); 5.07 (d, 1H; H-1a, $J = 3.7$ Hz); 5.01–4.86 (m, 3H; H-1b, H-1d, H-1f); 4.96 (m, 1H; H-2e); 4.93 (m, 1H; H-2c); 4.87 (m, 1H; H-2a); 4.77–4.46 (m, 3H; H-5a, H-5c, H-5e); 4.12–3.98 (m, 3H; H-3a, H-3c, H-3e); 3.94–3.86 (m, 3H; H-4a, H-4c, H-4e); 3.37–3.22 (m, 3H; H-2b, H-2d, H-2f).

*MPEG-2-succinoyloxyethyl (isopropyl O-(2-azido-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**38**)

To a solution of **37** (260 mg, 35 μmol), previously coevaporated with toluene, in dry CH_2Cl_2 (3 mL), EtSH (40 μL, 0.4 mmol) and *p*TsOH (11 mg, 40 μmol) were added. After stirring for 10 h, the volume was reduced to 2 mL and Et_2O (20–25 mL) was added with vigorous stirring until precipitation. The precipitate was filtered off and dried at high vacuum to yield **38**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.44–5.37 (m, 2H; H-1c, H-1e); 5.04 (m, 1H; H-1a); 4.97–4.89 (m, 3H; H-1b, H-1d, H-1f); 4.96–4.91 (m, 2H; H-2c, H-2e); 4.84 (m, 1H; H-2a); 4.71 (m, 1H; H-5a); 4.50–4.42 (m, 2H; H-5c, H-5e); 4.08 (m, 1H; H-4a); 4.01–3.96 (m, 2H; H-4c, H-4e); 3.93–3.83 (m, 2H; H-3c, H-3e); 3.85 (m, 1H; H-3a); 3.31–3.08 (m, 3H; H-2b, H-2d, H-2f).

*MPEG-2-succinoyloxyethyl (isopropyl O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**39**)

To a cooled (−15°C) solution of **38** (260 mg, 35 μmol), previously coevaporated with toluene, in dry CH_3CN (5 mL), BzCN (8 mg, 60 μmol) and catalytic Et_3N were added. After stirring for 45 min, MeOH (1 mL) was added and the reaction was allowed to warm to room temperature. The volume was reduced to 2 mL and Et_2O (20 mL) was added with vigorous stirring until precipitation of **39**. Significant spectral data: ^{1}H-NMR

(500 MHz, $CDCl_3$): δ 5.43–5.31 (m, 2H; H-1c, H-1e); 5.04 (m, 1H; H-1a); 4.99–4.91 (m, 3H; H-1b, H-1d, H-1f); 4.96–4.90 (m, 2H; H-2c, H-2e); 4.85 (m, 1H; H-2a); 4.74 (m, 1H; H-5a); 4.48 (m, 2H; H-5c, H-5e); 4.10 (m, 1H; H-4a); 4.00 (m, 2H; H-4c, H-4e); 3.88 (m, 1H; H-3a); 3.87 (m, 2H; H-3c, H-3e); 3.33-3.12 (m, 3H; H-2b, H-2d, H-2f).

*MPEG-2-succinoyloxyethyl (isopropyl O-(6-O-acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyluronate)-(1 → 4)-O-(2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-2-O-pivaloyl-α-*L*-idopyranosyd)uronate* (**40**)

To a mixture of **39** (260 mg, 35 μmol), previously coevaporated with toluene, and **34** (84 mg, 88 μmol) in dry CH_2Cl_2 (1.5 mL), TMSOTf (2.2 μL, 13 μmol) was added. After stirring for 1 h, one drop of Et_3N was added and Et_2O (25 mL) was added with vigorous stirring until precipitation. The white precipitate was recovered by filtration. This glycosylation was repeated three times more. Then, a mixture of the resulting MPEG derivative, PS-Suc-COOH (320 mg, 0.39 mmol) and a catalytic amount of DMAP were swollen in dry CH_2Cl_2 (5 mL) and DIC (70 μL, 0.41 mmol) was added. After shaking overnight, the mixture was filtered and resin was washed with CH_2Cl_2 (3 × 10 mL). The combined organic filtrates were concentrated to 2–3 mL. Et_2O (25–35 mL) was added with vigorous stirring until precipitation of pure MPEG-bound disaccharide **40**. Significant spectral data: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.53 (m, 1H; H-1g); 5.45–5.35 (m, 2H; H-1c, H-1e); 5.12 (m, 1H; H-2g); 5.07 (m, 1H; H-1a); 4.99–4.82 (m, 4H; H-1b, H-1d, H-1f, H-1h); 4.97–4.92 (m, 2H; H-2c, H-2e); 4.88 (m, 1H; H-2a); 4.75–4.41 (m, 4H; H-5a, H-5c, H-5e, H-5g); 4.13-3.90 (m, 4H; H-4a, H-4c, H-4e, H-4g); 4.10 (m, 1H; H-3g); 3.89 (m, 1H; H-3a); 3.88–3.85 (m, 2H; H-3c, H-3e); 3.35–3.14 (m, 3H; H-2b, H-2d, H-2f, H-2h) 1.92 (s, 3H; OAc)

*Isopropyl O-(2-azido-3,4-di-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-α-*L*-idopyranosyluronic acid)- (1 → 4)-O-(2-azido-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-α-*L*-idopyranosyluronic acid)-(1 → 4)-O-(2-azido-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-O-(methyl 3-O-benzyl-α-*L*-idopyranosyluronic acid)-(1 → 4)-O-(2-azido-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-3-O-benzyl-α-*L*-idopyranosyduronic acid* (**41**)

To a solution of **40** (270 mg, 35 μmol) in THF (5 mL) at −5°C, H_2O_2 30% (2.1 mL) and a 1 M aqueous solution of LiOH (2.4 mL) were added. After stirring for 24 h at room temperature MeOH (2 mL) and a 3 N aqueous solution of KOH (3 mL) were added. After stirring for 24h more the reaction was neutralized with acidic resin (IRA-120 H^+), filtered and concentrated. The residue was purified by Sephadex LH-20 (1:1 MeOH-CH_2Cl_2). Finally, fractions containing **41** and free MPEG were combined, concentrated and re-dissolved in MeOH/CH_2Cl_2 1:1 (1.5 mL). Et_2O (15 mL) was added and the mixture was cooled (0°C). The white precipitate was separated by filtration and the organic layer was concentrated in vacuo to yield pure **41** (26 mg, 26% from **26**, 11 steps). The physical data for **41** were identical in all respects with the reported values [12].

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