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Exploration of the use of an acylsulfonamide safety-catch linker for the polymer-supported synthesis of hyaluronic acid oligosaccharides

José L. de Paz *, M. Mar Kayser, Giuseppe Macchione, Pedro M. Nieto *

Instituto de Investigaciones Químicas, Centro de Investigaciones Científicas Isla de La Cartuja, CSIC –US, Americo Vespucio, 49, 41092 Sevilla, Spain

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ABSTRACT

The synthesis of hyaluronic acid oligosaccharides on polyethylene glycol (PEG) using an acylsulfonamide linker has been explored. Hyaluronic acid is a challenging synthetic target that usually involves the condensation of highly disarmed glucuronic acid building blocks. Amine-ended PEG monomethyl ether was efficiently functionalized with a hydroxyl-terminated acylsulfonamide linker. Suitably protected D-glucosamine (GlcN) and D-glucuronic acid (GlcA) monosaccharide building blocks were coupled to the polymer acceptor using the trichloroacetimidate glycosylation method. The sulfonamide safety-catch linker enables simultaneous cleavage of the monosaccharide from the polymer and orthogonal functionalization for further (bio)-conjugation of the sugar sample. Subsequent glycosylation of PEG-bound glycosyl acceptor to generate hyaluronic acid oligosaccharide chain failed. Model glycosylation experiments in solution and on soluble support using the same unreactive acceptors and donors allows for the synthesis of an orthogonally protected hyaluronic acid disaccharide and suggest that the encountered difficulties could be attributed to the presence of the *N*-acylsulfonamide.

1. Introduction

The synthesis of complex oligosaccharides is still a very challenging task and there is no general procedure for the stereoselective synthesis of glycosides and complex glycoconjugates.[1] In recent years, solid phase synthesis has become a powerful tool for the effective preparation of targeted carbohydrates.[2,3] Despite the impressive advances in the solid phase synthesis of oligosaccharides, which include the formation of the challenging β-mannosidic[4] and α-galactosidic linkages,[5] several important questions still need to be addressed to make this approach usable for non-specialists.[6] Solution-phase methodologies are an attractive alternative to solid phase carbohydrate synthesis. Among others,[7–14] these methods include oligosaccharide assembly on soluble polyethylene glycol (PEG) polymers.[15–21] PEG-supported oligosaccharide synthesis has important advantages over solid phase synthesis such as higher reactivity of the soluble polymer-bound sugar and the direct monitoring of the reaction process using routine tools, for example, standard NMR spectroscopy and thin layer chromatography (TLC), while maintaining facilitated purification steps.

The linker that attaches the first monosaccharide to the polymer support is of utmost importance for the oligosaccharide synthetic process. The linker should allow for the high-yielding attachment of the first sugar unit on the support and should be stable to the reaction conditions employed for the oligosaccharide synthesis.[2] Its chemical nature also determines the cleavage conditions required to release the final compound from the polymer support. Additionally, it is highly convenient if the chosen linker enables liberation of the synthesized saccharide with an orthogonal tag for further bioconjugation.[6] For example, Seeberger and co-workers have successfully employed an octenediol linker[22] for the automated solid phase synthesis of a wide variety of complex oligosaccharides. The final product is liberated by cross metathesis using Grubbs catalyst and ethylene to afford *n*-pentenyl glycosides where the double bond can be chemically manipulated for linkage to carrier proteins and surfaces, creating carbohydrate vaccines or microarrays,[23] respectively.

Still, alternative anchors for the polymer-supported synthesis of oligosaccharides should be explored, particularly concerning the synthesis of complex glycosaminoglycans (GAGs).[24–26] Ideally, new linker designs should allow for simple and fast release from the support with simultaneous and versatile incorporation of a unique functional group that serves to attach the oligosaccharide to a chip surface, protein or other carriers without additional functionalization steps.[3,6] Importantly, in the GAG field, linker functionalization after release from the support and deprotection/sulfation steps may require more effort than the assembly of the protected oligosaccharide on the polymer. Moreover, these additional off-resin steps on valuable and elaborated compounds usually lead to a significant decrease in overall yield.

In this context, we consider an acylsulfonamide safety-catch linker[27–30] as an attractive alternative for oligosaccharide synthesis. It is completely stable under both strongly acidic and basic

* Corresponding authors. Fax: +34 954 460565 (J.L.d.P.).
E-mail address: jlpaz@iiq.csic.es (J.L. de Paz).

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reaction conditions while the sulfamyl group can be activated at the end of the synthesis to afford an *N*-alkyl-*N*-acylsulfonamide intermediate that can be then displaced by a wide range of nucleophiles to yield tailored glycoconjugates (Scheme 1). In fact, an acylsulfonamide linker has been successfully employed in the solid phase synthesis of oligosaccharides using thioglycosides as glycosyl donors.[31,32] However, to the best of our knowledge, trichloroacetimidate glycosyl donors have not been previously used with acylsulfonamide linkers.

In the context of a programme involving the development of polymer-supported synthesis of GAG oligosaccharides, we report herein an exploratory study on the use of an acylsulfonamide safety-catch linker for the synthesis of hyaluronic acid oligosaccharides[33–35] using trichloroacetimidates as glycosyl donors. We chose soluble PEG as support because it allowed us to monitor the reaction progress and identify reaction products by using standard analytical techniques. As the only unsulfated GAG, hyaluronic acid is comprised of disaccharide repeating units of β-(1→4)-D-glucuronic acid-β-(1→3)-D-*N*-acetylglucosamine. Hyaluronic acid is involved in many biological processes, including cell adhesion, cell migration and wound healing.[36–38] Due to the chemical complexity and heterogeneity of GAGs, the preparation of well-defined synthetic oligosaccharides is crucial for the establishment of structure–function relationships and the understanding of GAG–protein interactions.[39,40] GAG synthesis is a challenging task as it generally involves coupling between electron-poor building blocks. In particular, the synthesis of hyaluronic acid oligosaccharides usually requires the condensation of highly disarmed glucuronic acid building blocks due to the presence of both the electron-withdrawing carboxylic acid derivative and an acyl group at position 2 to control the 1,2-trans stereochemistry of the glycosidic bonds.[33–35]

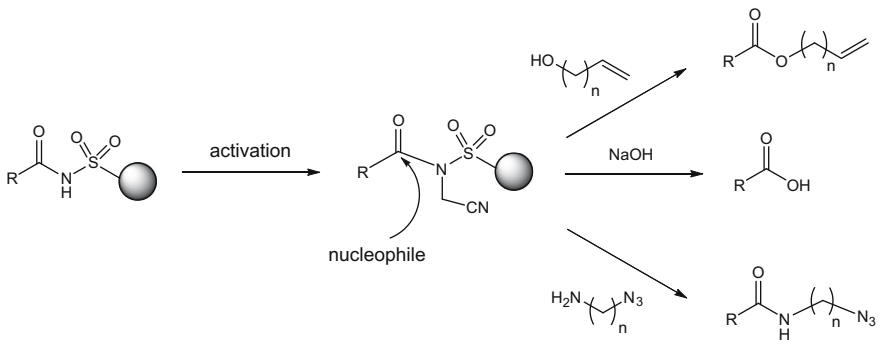
2. Results and discussion

First, we prepared PEG functionalized with an acylsulfonamide linker. Commercially available amine-ended PEG monomethyl ether was treated with 4-sulfamoyl-benzoic acid to afford sulfonamide **1** (Scheme 2). The ^{1}H NMR spectrum showed signals for amide proton at 7.51 ppm, aromatic protons at 7.98 ppm and NH_2SO_2 protons at 6.29 ppm, as well as the complete disappearance of the NH_2CH_2 methylene signal, indicating clean formation of **1**. Additionally, we employed a modified version of bromophenol blue test[41] to monitor coupling completion. Amine-terminated PEG is stained blue after the addition of 0.5% bromophenol blue solution in diethyl ether, while sulfonamide **1** is stained yellow.

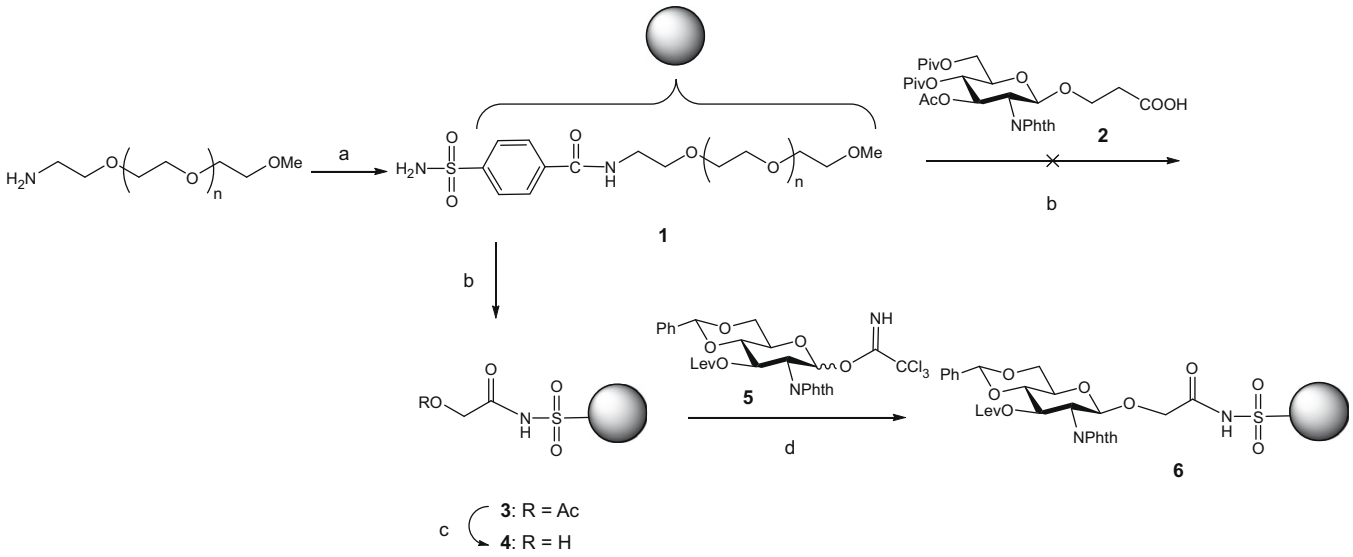
Coupling conditions were sought to load the first monosaccharide to polymer **1** through an acylsulfonamide safety-catch linker. N-Protected amino acids have been efficiently coupled to sulfonamide resins and this approach has been successfully applied to solid phase peptide synthesis.[42] Optimal loading of Boc- and Fmoc-amino acids was achieved using benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) as coupling agent.[42] In our initial studies, a carboxylic acid-terminated monosaccharide **2**, prepared by glycosylation of the corresponding trichloroacetimidate with *t*-butyl 3-hydroxypropionate followed by TFA treatment, failed to react with **1** under a host of reaction conditions, including PyBOP activation (Scheme 2). Therefore, an alternative approach was designed to introduce the first monosaccharide on PEG support involving condensation of glycosyl donors with hydroxyl-terminated polymer **4**.

Acylsulfonamide **3** was prepared by treating polymer **1** with DIPEA, catalytic DMAP, and the symmetrical anhydride of 2-*O*-acetylglycolic acid prepared in situ. Subsequent deacetylation of **3** afforded acceptor **4** quantitatively as estimated by ^{1}H NMR spectroscopy (Scheme 2). The first glycosylation attempts of **4** with trichloroacetimidate **5** in the presence of TMSOTf failed to produce the desired polymer **6** in good yield with multiple PEG-bound products formed. This complex mixture of compounds probably resulted from the coupling between oxocarbenium ion intermediate derived from **5** and deprotonated acylsulfonamide.[43] We envisaged that treatment of polymer acceptor **4** with acidic resin IR-120-H^+ prior glycosylation would ensure the protonation of the acylsulfonamide linker. Under these modified conditions, coupling of **4** with donor **5** gave PEG-bound monosaccharide **6** (Scheme 2). Interestingly, no trichloroacetimidate activation was detected in the absence of Lewis-acid catalysis, although the acylsulfonamide functional group has been recently proposed as a catalyst for glycosylation of perbenzylated trichloroacetimidate donors.[44] This fact highlights the low reactivity of donor **5** compared to perbenzylated 'armed' building blocks. Purification by selective precipitation using diethyl ether was carried out several times until TLC analysis and DOSY NMR experiments[45] showed the absence of any co-precipitated side product derived from trichloroacetimidate **5**. Integration of key ^{1}H NMR signals indicated that the coupling proceeded to completion.

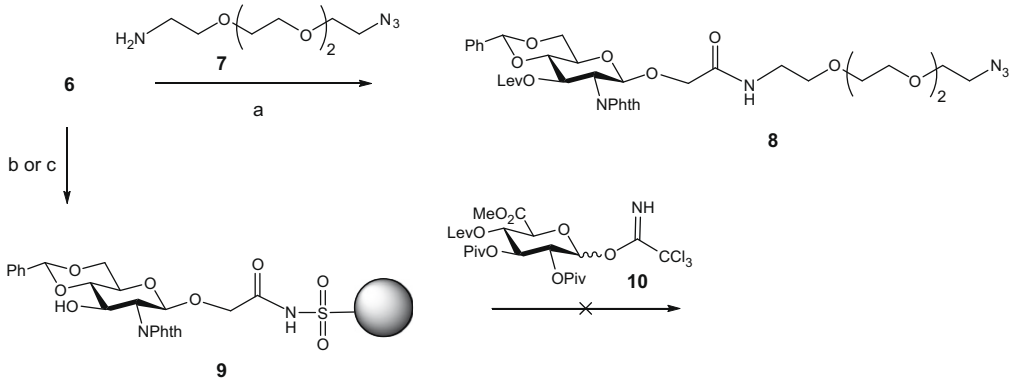
To demonstrate that the acylsulfonamide linker can be selectively activated and then displaced with an appropriate nucleophile, polymer **6** was treated with iodoacetonitrile and then with amine **7** to give the azido-functionalized glycoconjugate **8** (Scheme 3). Thereby, this linker allows for cleavage from the support and simultaneous orthogonal functionalization for further use of the sugar derivative. Next, oligosaccharide assembly on polymer **6** was explored. The levulinoyl (Lev) group of **6** was easily removed by treatment with either hydrazine acetate in CH_2Cl_2 or NaOMe in MeOH to give polymer-supported acceptor **9** (Scheme 3). Attempted glycosylation of **9**, pretreated with acidic resin as described above, with GlcA trichloroacetimidate **10** was performed under a range of temperature conditions (from room temperature to −20 °C) and catalysts (TMSOTf, $BF_3{\cdot}Et_2O$, 4 Å acid-washed molecular sieves). In all cases, efficient activation of glycosyl donor



Scheme 1. General strategy for activation and nucleophilic displacement of *N*-acylsulfonamide safety-catch linkers.



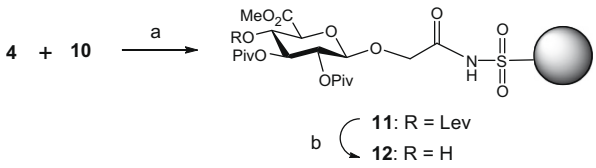
Scheme 2. Reagents: (a) 4-sulfamoyl-benzoic acid, DIC, HOBt, CH_2Cl_2; (b) 2-*O*-acetyl-glycolic anhydride, DIPEA, DMAP, CH_2Cl_2; (c) NaOMe, MeOH; (d) TMSOTf, CH_2Cl_2.



Scheme 3. Reagents: (a) (i) iodoacetonitrile, DIPEA, DMF; (ii) **7**, THF–DMF; (b) NaOMe, MeOH; (c) hydrazine acetate, CH_2Cl_2.

was monitored by TLC. NMR analysis showed a complex mixture of PEG-bound compounds and NMR signals indicative of coupling were observed. However, no pure disaccharide could be isolated after cleavage from the support. These results suggest that acidic resin treatment, prior glycosylation, does not ensure, in this case, protonation of the acylsulfonamide. It is reasonable to suppose that the presence of charged species, such as the acylsulfonamide-derived conjugate base, hinder glycosylation reactions via oxocarbenium cations. It is noteworthy that linker interference was not observed using thioglycosides as glycosylating agents and dimethylthiomethylsulfonium triflate as activator.[31,32]

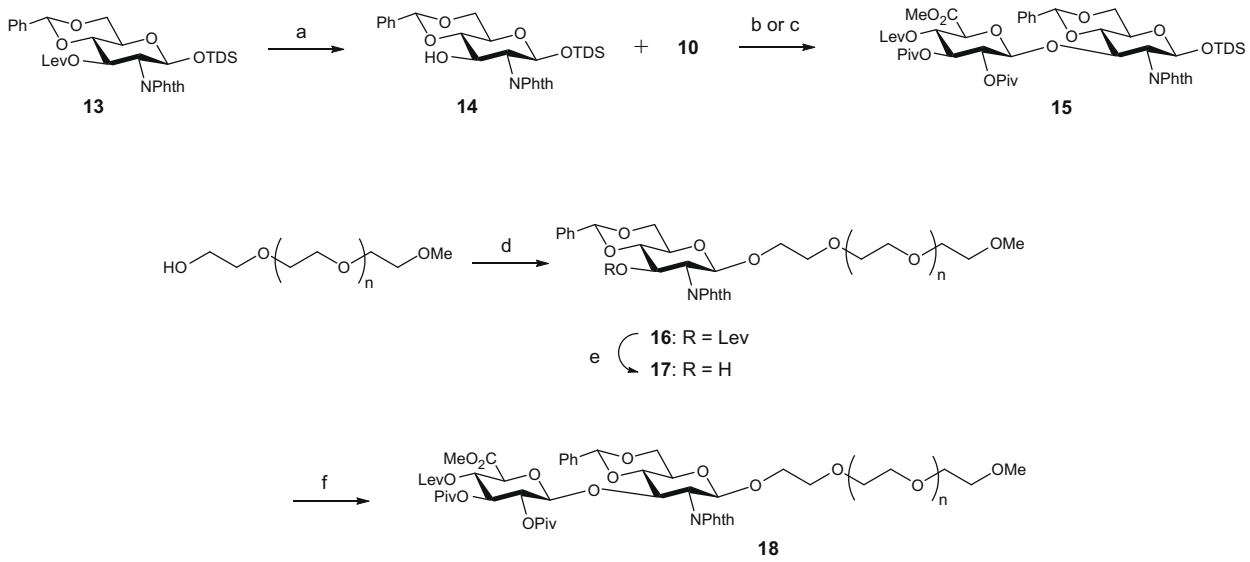
Considering the low reactivity of **10**, the alternative GlcN-GlcA glycosidic bond formation was also examined. Glycosylation of acceptor **4** with donor **10** afforded polymer-bound GlcA derivative **11** (Scheme 4). In this case, the reaction was repeated to drive it to completion as the first cycle resulted only in partial glycosylation as indicated by integration of ^{1}H NMR signals. Compound **11** was treated with hydrazine acetate to yield polymer-bound acceptor **12**. Glycosylation with **5** again did not generate the desired disaccharide.



Scheme 4. Reagents: (a) TMSOTf, CH_2Cl_2; (b) hydrazine acetate, CH_2Cl_2.

To demonstrate that encountered problems can be attributed to the acylsulfonamide linker, model glycosylations were carried out in solution and on PEG support without the *N*-acylsulfonamide linker. Monosaccharide **13** was converted into acceptor **14** that was cleanly transformed into disaccharide **15** by condensation with trichloroacetimidate **10** using either TMSOTf in CH_2Cl_2 or 4 Å acid-washed molecular sieves[46] in toluene (Scheme 5). Compound **15** is an orthogonally protected disaccharide building block for the assembly of hyaluronic acid oligosaccharides. Alternatively, glycosyl donor **5** was coupled with hydroxyl-terminated PEG monomethyl ether to give bound acceptor **17** after removal of Lev group. Polymer **17** was efficiently glycosylated with trichloroacetimidate **10** to afford bound disaccharide **18** (Scheme 5) that showed an NMR spectrum with key signals in accordance with disaccharide **15** NMR data.

In conclusion, we have explored the use of an acylsulfonamide safety-catch linker for the synthesis of hyaluronic acid oligosaccharides involving the coupling of disarmed building blocks. A synthetic procedure for the preparation of an acylsulfonamide PEG support was established. GlcN and GlcA building blocks were



Scheme 5. Reagents and conditions: (a) hydrazine acetate, CH_2Cl_2; (b) TMSOTf, CH_2Cl_2, 0 °C; (c) 4 Å acid-washed molecular sieves, toluene; (d) **5**, TMSOTf, CH_2Cl_2; (e) hydrazine acetate, CH_2Cl_2; (f) **10**, TMSOTf, CH_2Cl_2.

loaded on this PEG support by glycosylation of the corresponding trichloroacetimidates with a primary polymer-supported hydroxyl group. The safety-catch linker enables cleavage of the saccharide from the support and versatile functionalization for further conjugation of the sugar probe. However, this approach was not suitable for oligosaccharide assembly involving glycosylation of low nucleophilic acceptors with electron-poor donors. Glycosylations with the same unreactive building blocks were successfully performed both in solution and on non-modified PEG support to give an orthogonally protected disaccharide for the synthesis of hyaluronic acid sequences. These results unambiguously indicate that glycosylations of secondary hydroxyl groups failed due to the presence of the *N*-acylsulfonamide linker. It is reasonable to suppose that the chemical nature of the linker, in particular the high acidity of the NH proton, can explain the presence of charged species that hinder coupling reactions mediated by oxocarbenium ions. This investigation also highlights the advantage of using soluble polymers to optimize polymer-supported synthetic strategies because the course of the reaction can be directly monitored by solution-phase analytical methods.

3. Experimental section

3.1. General procedures

Amine-ended PEG monomethyl ether (average molecular weight 5000 Da) and PEG monomethyl ether (average molecular weight 5000 Da) were purchased from Sigma Aldrich. TLC analyses were performed on Silica Gel 60 F_{254} precoated on aluminium plates (Merck) and the compounds were detected by staining 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. Electrospray mass spectra (ES-MS) were carried out with an Esquire 6000 ESI-Ion Trap from Bruker Daltonics. High resolution FAB mass spectra (HRMS) were carried out by the Mass Spectrometry Service, Citius, University of Seville.

3.2. Polymer 1

1-Hydroxybenzotriazole (HOBt, 91 mg, 0.670 mmol) and *N*,*N*′-diisopropylcarbodiimide (DIC, 104 μL, 0.670 mmol) were added at room temperature to a solution of 4-sulfamoylbenzoic acid (135 mg, 0.670 mmol) and amine-terminated PEG monomethyl ether (3.04 g, 0.609 mmol) in CH_2Cl_2 (15 mL). After stirring for 16 h, the reaction mixture was concentrated. The residue was redissolved in pyridine (15 mL) and diethyl ether (150 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed twice with cold diethyl ether (2 × 150 mL) and dried under high vacuum to give polymer **1** (3.05 g) as a white solid. The precipitation and washings with diethyl ether were repeated, if necessary, to remove completely urea. Selected ^{1}H NMR data (300 MHz, $CDCl_3$) δ 7.98 (m, 4H, Ph), 7.51 (br s, 1H, CON*H*), 6.29 (br s, 2H, SO_2NH_2).

3.3. Polymer 3

DIC (0.54 mL, 3.48 mmol) was added at room temperature to a solution of 2-*O*-acetylglycolic acid (821 mg, 6.9 mmol) in CH_2Cl_2 (10 mL). After stirring for 12 h, the solution was cooled with an ice bath and the urea precipitate was filtered. The filtrate was added to a round-bottomed flask containing sulfonamide polymer **1** (3.6 g, 0.69 mmol), DMAP (9 mg, 70 μmol), DIPEA (0.36 mL, 2.09 mmol) and CH_2Cl_2 (20 mL). After stirring at room temperature for 24 h, the reaction mixture was concentrated. The residue was redissolved in pyridine (20 mL) and diethyl ether (120 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed twice with cold diethyl ether (2 × 120 mL) and dried under high vacuum. The residue was redissolved in methanol (20 mL) and diethyl ether (120 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed twice with cold diethyl ether (2 × 120 mL) and dried under high vacuum to give polymer **3** (3.6 g) as a white solid. Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 8.05 (d, 2H, Ph), 7.92 (d, 2H, Ph), 7.45 (br s, 1H, CON*H*), 4.56 (s, 2H, CH_2OCOCH_3), 2.09 (s, 3H, $OCOCH_3$).

3.4. Polymer 4

Compound **3** (3.6 g, 0.68 mmol) was dissolved in methanol (50 mL) and NaOMe (0.63 mL of a 2.17 M solution in MeOH) was added. After stirring at room temperature for 12 h, the reaction volume was reduced to 15 mL and 0.2 M acetic acid in diethyl ether (120 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed twice with 0.2 M acetic acid in diethyl ether (2 × 120 mL) and dried under high vacuum to give polymer **4** (3.5 g) as a white solid. Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 8.02 (d, 2H, Ph), 7.85 (d, 2H, Ph), 7.33 (br s, 1H, CON*H*), 3.93 (br s, 2H, C*H*$_2$OH).

3.5. Polymer 6

Acceptor **4** (568 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (5 mL). Amberlite IR-120-H$^+$ (2 g) was added and the mixture was stirred at room temperature for 30 min. The acidic resin was filtered off and the solvent was removed in vacuo. The residue was coevaporated with toluene, dried under high vacuum and then dissolved in CH_2Cl_2 (3 mL). A solution of trichloroacetimidate **5** (277 mg, 0.43 mmol) in CH_2Cl_2 (2 mL) and then TMSOTf (99 μL of a 0.22 M solution in CH_2Cl_2) were added at room temperature. After stirring for 30 min, diethyl ether (40 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed with cold diethyl ether (40 mL) and dried under high vacuum. Selective precipitation using diethyl ether was repeated twice to give polymer **6** (618 mg) as a white solid. Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 9.28 (br s, 1H, CON*H*SO$_2$), 8.00–7.91 (2d, 4H, Ph linker), 7.85–7.73 (m, 4H, NPhth), 7.41–7.31 (m, 5H, Ph), 5.93 (dd, $J_{2,3} = J_{3,4} = 9.8$ Hz, 1H, H-3), 5.52 (s, 1H, PhC*H*O), 5.33 (d, $J_{1,2} = 8.5$ Hz, 1H, H-1), 4.33 (m, 2H, H-2, H-6), 4.12 (br s, 2H, COC*H*$_2$Osugar), 1.85 (s, 3H, COC*H*$_3$).

3.6. Azide-functionalized glycoconjugate 8

Iodoacetonitrile (73 μL, 1.01 mmol) and DIPEA (44 μL, 0.25 mmol) were added to a solution of polymer **6** (289 mg, 51 μmol) in DMF (3 mL). After stirring in the dark overnight, diethyl ether (40 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed with cold diethyl ether (20 mL) and dried under high vacuum to give N-alkylated acylsulfonamide polymer as a brown solid. Amine **7** (101 μL, 0.51 mmol) was added to a solution of PEG-bound N-alkylated acylsulfonamide in DMF–THF (2 mL/6 mL). After stirring at room temperature overnight, THF was removed in vacuo and diethyl ether (40 mL) was added at 0 °C. The precipitated solid was filtered off and washed with cold diethyl ether (40 mL). The filtrate and the washes were combined and concentrated, and the residue was redissolved in EtOAc (40 mL), washed with 1 N HCl, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by flash chromatography (48:1 CH_2Cl_2–MeOH) afforded compound **8** (15 mg, 39%, not optimized). TLC (8:1 CH_2Cl_2–MeOH) $R_f = 0.50$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92–7.77 (m, 4H, NPhth), 7.49–7.38 (m, 5H, Ph), 6.70 (br s, 1H, CON*H*), 5.99 (dd, $J_{2,3} = J_{3,4} = 9.5$ Hz, 1H, H-3), 5.58 (s, 1H, PhC*H*O), 5.45 (d, $J_{1,2} = 8.5$ Hz, 1H, H-1), 4.45 (dd, $J_{5,6} = 4.3$ Hz, $J_{6,6'} = 10.3$ Hz, 1H, H-6), 4.39 (dd, 1H, H-2), 4.25 (d, $J_{gem} = 15.3$ Hz, 1H, OC*H*$_2$CONH), 4.07 (d, $J_{gem} = 15.3$ Hz, 1H, OC*H*$_2$CONH), 3.88–3.34 (m, 19H, H-4, H-5, H-6′, C*H*$_2$C*H*$_2$), 2.57–2.45 (m, 4H, OCO(C*H*$_2$)$_2$), 1.90 (s, 3H, COC*H*$_3$). ES-MS: *m/z*: calcd for $C_{36}H_{43}N_5O_{13}Na$: 776.3; found: 775.6 [M+Na]$^+$.

3.7. Polymer 9

3.7.1. Method A (delevulination with NaOMe)

Compound **6** (140 mg, 25 μmol) was dissolved in methanol (2 mL) and NaOMe (17 μL of a 2.17 M solution in MeOH) was added. After stirring at room temperature for 12 h, 0.2 M acetic acid in diethyl ether (15 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed twice with 0.2 M acetic acid in diethyl ether (2 × 15 mL) and dried under high vacuum to give polymer **9** (135 mg) as a white solid.

3.7.2. Method B (delevulination with hydrazine acetate)

Compound **6** (144 mg, 25 μmol) was dissolved in CH_2Cl_2 (2 mL) and hydrazine acetate (100 μL of a 0.5 M solution in MeOH) was added. After stirring at room temperature for 1 h, diethyl ether (20 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed with diethyl ether (20 mL) and dried under high vacuum to give polymer **9** (136 mg) as a white solid. Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 8.05–7.94 (2d, 4H, Ph linker), 7.89–7.77 (m, 4H, NPhth), 7.50–7.33 (m, 5H, Ph), 5.58 (s, 1H, PhC*H*O), 5.20 (d, $J_{1,2} = 8.5$ Hz, 1H, H-1), 4.70 (m, 1H, H-3).

3.8. Polymer 11

Acceptor **4** (550 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (5 mL). Amberlite IR-120-H$^+$ (2 g) was added and the mixture was stirred at room temperature for 30 min. The acidic resin was filtered off and the solvent was removed in vacuo. The residue was coevaporated with toluene, dried under high vacuum and then dissolved in CH_2Cl_2 (3 mL). A solution of trichloroacetimidate **10** (203 mg, 0.33 mmol) in CH_2Cl_2 (2 mL) and then TMSOTf (200 μL of a 0.16 M solution in CH_2Cl_2) were added at room temperature. After stirring for 1.5 h, diethyl ether (40 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed with cold diethyl ether (40 mL) and dried under high vacuum. The glycosylation and selective precipitation procedure was repeated once to give polymer **11** (545 mg) as a white solid. Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 9.33 (br s, 1H, CON*H*SO$_2$), 8.14–8.00 (2d, 4H, Ph linker), 7.43 (br s, 1H, CON*H*), 5.33–5.23 (m, 2H, H-3, H-4), 5.08 (dd, 1H, H-2), 4.62 (d, $J_{1,2} = 8$ Hz, 1H, H-1), 4.24–4.13 (2d, 2H, COC*H*$_2$Osugar), 4.09 (d, $J_{4,5} = 10$ Hz, 1H, H-5), 2.16 (s, 3H, COC*H*$_3$), 1.16–1.13 (2s, 18H, OCOC(C*H*$_3$)$_3$).

3.9. Polymer 12

Compound **11** (146 mg, 26 μmol) was dissolved in CH_2Cl_2 (2 mL) and hydrazine acetate (100 μL of a 0.51 M solution in MeOH) was added. After stirring at room temperature for 1 h, diethyl ether (20 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed with diethyl ether (20 mL) and dried under high vacuum to give polymer **12** (130 mg) as a white solid. Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 8.12–8.00 (2d, 4H, Ph linker), 5.17 (dd, 1H, H-3), 5.01 (dd, 1H, H-2), 4.60 (d, $J_{1,2} = 8$ Hz, 1H, H-1), 1.17–1.15 (2s, 18H, OCOC(C*H*$_3$)$_3$).

3.10. Dimethylthexylsilyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (14)

A solution of hydrazine acetate (407 mg, 4.52 mmol) in methanol (2 mL) was added to a solution of **13** (1.44 g, 2.26 mmol) in CH_2Cl_2 (15 mL). After stirring for 30 min, acetone (10 mL) was added and the reaction mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (3:1 hexane–EtOAc) to give **14** (1.11 g, 91%) as a white foam. TLC (2:1 hexane–EtOAc) $R_f = 0.45$; $[\alpha]_D^{20}$ −34.6 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89–7.73 (m, 4H, NPhth), 7.54–7.39 (m, 5H, Ph), 5.59 (s, 1H, PhC*H*O), 5.53 (d, $J_{1,2} = 8$ Hz, 1H, H-1), 4.68 (ddd, 1H, H-3), 4.37 (dd, $J_{5,6} = 4.5$ Hz, $J_{6,6'} = 10.3$ Hz, 1H, H-6), 4.24 (dd, $J_{2,3} = 10.5$ Hz, 1H, H-2), 3.87 (dd, 1H, H-6′), 3.68 (m, 2H, H-4, H-5), 2.46 (d, $J_{3,OH} = 3.5$ Hz, 1H, OH),

1.43 (m, 1H, C*H*(CH_3)$_2$), 0.68–0.65 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.14–0.01 (2s, 6H, Si(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.1–123.3 (Ph, NPhth), 102.0, 93.9, 82.4, 68.8, 68.5, 66.2, 58.7, 33.8, 24.5, 19.8, 19.6, 18.3, 18.2, −1.8, −3.8. HRMS: *m/z*: calcd for $C_{29}H_{37}O_7NSiNa$: 562.2237; found: 562.2261 [M+Na]$^+$.

3.11. Dimethylthexylsilyl 3-*O*-(Methyl 4-*O*-levulinoyl-2,3-di-*O*-pivaloyl-β-D-glucopyranosyluronate)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (15)

3.11.1. Method A (glycosylation with TMSOTf)

Acceptor **14** (100 mg, 0.19 mmol) and GlcA trichloroacetimidate **10** (152 mg, 0.24 mmol) were combined in a flask, coevaporated with toluene and dried under vacuum. The starting materials were dissolved in CH_2Cl_2 (2 mL) and TMSOTf (100 μL of a 0.12 M solution in CH_2Cl_2) was added at 0 °C. After 1 h, the reaction was quenched with triethylamine (0.3 mL) and the solvent was removed under reduced pressure. Purification by flash chromatography (hexane–EtOAc 3:1) yielded **15** (136 mg, 74%) and recovered acceptor (24 mg, 24%).

3.11.2. Method B (glycosylation with 4 Å acid-washed molecular sieves)

Donor **10** (116 mg, 0.19 mmol) and acceptor **14** (78 mg, 0.15 mmol) were dissolved in toluene (2 mL) in the presence of freshly activated 4 Å acid-washed molecular sieves (1.9 g, from Fluka, 1/8 in. rods). After stirring for 48 h at room temperature, triethylamine (0.1 mL) was added and the mixture was filtered and concentrated. The residue was purified by flash chromatography on silica gel (3:1 hexane–EtOAc) to give **15** (102 mg, 71%) and recovered acceptor (23 mg, 29%). TLC (2:1 hexane–EtOAc) R_f = 0.37; $[\alpha]_D^{20}$ −9.2 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85–7.72 (m, 4H, NPhth), 7.48–7.42 (m, 5H, Ph), 5.51 (s, 1H, PhC*H*O), 5.45 (d, $J_{1,2}$ = 8 Hz, 1H, H-1), 5.09 (m, 2H, H-3′, H-4′), 4.83 (dd, $J_{2,3}$ = 8.3 Hz, 1H, H-2′), 4.75 (d, $J_{1,2}$ = 7.5 Hz, 1H, H-1′), 4.69 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, 1H, H-3), 4.29 (m, 2H, H-2, H-6a), 4.03 (dd, 1H, H-4), 3.87 (dd, $J_{5,6b}$ = $J_{6a,6b}$ = 10.3 Hz, 1H, H-6b), 3.70 (s, 3H, COOC*H*$_3$), 3.66 (m, 1H, H-5), 3.55 (d, $J_{4,5}$ = 9.5 Hz, 1H, H-5′), 2.61–2.36 (m, 4H, OCO(C*H*$_2$)$_2$), 2.14 (s, 3H, COC*H*$_3$), 1.38 (m, 1H, C*H*(CH_3)$_2$), 1.01–0.73 (2s, 18H, OCOC(C*H*$_3$)$_3$), 0.63–0.60 (m, 12H, C(C*H*$_3$)$_2$ and CH(C*H*$_3$)$_2$), 0.09–(−0.06) (2s, 6H, Si(C*H*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.1, 175.7, 171.0, 167.1, 137.2–123.4 (Ph, NPhth), 101.8 (Ph*C*HO), 98.9 (C-1′), 93.4 (C-1), 81.0, 75.1, 71.8, 71.6, 71.1, 69.4, 68.9, 66.3, 57.5, 38.6, 38.2, 37.4, 33.8, 29.8, 29.7, 28.5, 27.5, 26.9, 26.6, 24.7, 24.4, 23.9, 19.8, 19.6, 18.3, 18.2, 7.9, −1.9, −3.8. HRMS: *m/z*: calcd for $C_{51}H_{69}O_{17}NSiNa$: 1018.4232; found: 1018.4234 [M+Na]$^+$.

3.12. Polymer 16

Hydroxyl-terminated PEG monomethyl ether (215 mg, 43 μmol) and glycosyl donor **5** (138 mg, 0.21 mmol), previously coevaporated with toluene and dried under high vacuum, were dissolved in CH_2Cl_2 (2 mL). TMSOTf (101 μL of a 0.11 M solution in CH_2Cl_2) was added at room temperature. After stirring for 15 min, diethyl ether (40 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed with cold diethyl ether (40 mL) and dried under high vacuum. Selective precipitation using diethyl ether was repeated twice. The solid was dissolved in pyridine (2 mL) and acetic anhydride (1 mL) was added. After stirring at room temperature for 3 h, diethyl ether (40 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed with cold diethyl ether (40 mL) and dried under high vacuum to give polymer **16** (225 mg) as a white solid. Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 7.85–7.72 (m, 4H, NPhth), 7.45–7.34 (m, 5H, Ph), 5.93 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, 1H, H-3), 5.53 (s, 1H, PhC*H*O), 5.45 (d, $J_{1,2}$ = 8.5 Hz, 1H, H-1), 4.40 (dd, $J_{5,6}$ = 4.5 Hz, $J_{6,6'}$ = 10.5 Hz, 1H, H-6), 4.30 (dd, 1H, H-2), 1.89 (s, 3H, COC*H*$_3$).

3.13. Polymer 17

Compound **16** (225 mg, 41 μmol) was dissolved in CH_2Cl_2 (2 mL) and hydrazine acetate (100 μL of a 0.82 M solution in MeOH) was added. After stirring at room temperature for 1 h, diethyl ether (30 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed with cold diethyl ether (30 mL) and dried under high vacuum to give polymer **17** (215 mg) as a white solid. Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 7.88–7.72 (m, 4H, NPhth), 7.49–7.38 (m, 5H, Ph), 5.58 (s, 1H, PhC*H*O), 5.33 (d, $J_{1,2}$ = 8.5 Hz, 1H, H-1), 4.65 (m, 1H, H-3), 4.40 (dd, $J_{5,6}$ = 4.4 Hz, $J_{6,6'}$ = 10 Hz, 1H, H-6), 4.25 (dd, 1H, H-2).

3.14. Polymer 18

Acceptor **17** (215 mg, 40 μmol) and glycosyl donor **10** (124 mg, 0.20 mmol), previously coevaporated with toluene and dried under high vacuum, were dissolved in CH_2Cl_2 (2 mL). TMSOTf (100 μL of a 0.10 M solution in CH_2Cl_2) was added at room temperature. After stirring for 30 min, diethyl ether (35 mL) was added at 0 °C. The precipitated solid was collected by filtration, washed with cold diethyl ether (35 mL) and dried under high vacuum. The glycosylation and selective precipitation procedure was repeated to drive reaction to completion, affording polymer **18** (225 mg) as a white solid. Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 7.84–7.73 (m, 4H, NPhth), 7.44–7.35 (m, 5H, Ph), 5.49 (s, 1H, PhC*H*O), 5.24 (d, 1H, H-1), 5.06 (m, 2H, H-3′, H-4′), 4.81 (dd, 1H, H-2′), 4.72 (d, 1H, H-1′), 4.68 (dd, 1H, H-3), 4.31 (m, 2H, H-2, H-6a), 2.11 (s, 3H, COC*H*$_3$), 0.99–0.69 (2s, 18H, OCOC(C*H*$_3$)$_3$).

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Supplementary data

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