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Solid-Phase Synthesis of α-Glucosamine Sulfoforms with Fragmentation Analysis by Tandem Mass Spectrometry

Runhui Liu, Chamnongsak Chanthamontri, Hongling Han, Jesús M. Hernández-Torres, Karl V. Wood, Scott A. McLuckey, and Alexander Wei*

Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907-2084

alexwei@purdue.edu

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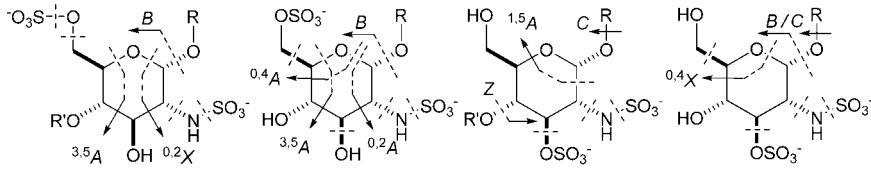

Sulfated epitopes of α-glucosamine (GlcN sulfoforms) were prepared by solid-phase synthesis as models of internal glucosamines within heparan sulfate. An orthogonally protected 2′-hydroxyethyl GlcN derivative was immobilized on a trityl resin support and subjected to regioselective deprotection and sulfonation conditions, which were optimized with the aid of on-resin infrared or Raman analysis. The sulfoforms were cleaved from the resin under mild Lewis acid conditions without affecting the *O*- or *N*-sulfate groups and purified by reversed-phase high-performance liquid chromatography (HPLC). The α-GlcN sulfoforms and their 4-*O*-benzyl ethers were examined by electrospray ionization tandem mass spectrometry (ESI-MS/MS), with product ion spectra produced by collision-induced dissociation (CID). ESI-MS/MS revealed significant differences in parent ion stabilities and fragmentation rates as a function of sulfate position. Ion fragmentation by CID resulted in characteristic mass losses with strong correlation to the positions of both free hydroxyl groups and sulfate ions. Most of these fragmentation patterns are consonant with elimination pathways, and suggest possible strategies for elucidating the structures of glucosamine-derived sulfoforms with identical *m*/*z* ratios. In particular, fragmentation analysis can easily distinguish GlcN sulfoforms bearing the relatively rare 3-*O*-sulfate from isomers with the more common 6-*O*-sulfate.

Introduction

The biological functions of cell-surface carbohydrates and related glycoconjugates are frequently regulated by postsynthetic modifications, particularly via their derivatization as sulfate esters.[1,2] In many cases, protein-carbohydrate recognition is determined by the presentation of specific sulfate epitopes, more recently referred to as sulfoforms.[2–5] A famous example of sulfoform diversity is provided by the glycosaminoglycan heparan sulfate (HS), a linear polysaccharide comprised of α-D-glucosamine (GlcN) linked in alternating 1→4 fashion to either β-D-glucuronic acid (GlcA) or α-L-iduronic acid (IdoA).[6] At least 21 disaaccharide sulfoforms have been identified within HS, assembled from six out of eight possible sulfated glucosamine derivatives (GlcNAc, GlcNAc-6*S*, GlcN*S*, GlcN*S*-3*S*, GlcN*S*-6*S*, and GlcN*S*-3*S*,6*S*) and four different uronic acid derivatives (GlcA, GlcA-2*S*, IdoA, and IdoA-2*S*);[7,8] another three sulfoforms containing a free amine ($GlcNH_3^+$, $GlcNH_3^+$-3*S*, $GlcNH_3^+$-3*S*,6*S*) have also been observed.[9] These are already sufficient to produce an enormous number of low molecular weight HS fragments: for example, HS octasaccharides generated randomly from naturally occurring disaccharide epitopes can result in well over 10^5 possible sulfoforms. Even greater structural diversity can be attained by the incorporation of unnatural (or yet to be identified) sulfoforms.

HS and other sulfated glycosaminoglycans have attracted great interest because of their important and potentially thera-

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peutic roles in vascular diseases, inflammation, viral infections, and cancer.[6,10–12] However, only a handful of high-affinity HS ligands have been characterized due to the challenges of purification and structural determination,[13,14] presenting a serious bottleneck for target-oriented synthetic efforts. The structural elucidation of HS sulfoforms with specific biological activities may be particularly challenged by their low natural abundance and their temporal or transient expression on cell-surface proteoglycans. For example, 3-*O*-sulfate esters are generated during a late stage of HS modification and considered to be relatively rare, yet are found in high-affinity ligands for extracellular proteins such as antithrombin and the herpes simplex virus glycoprotein gD.[15,16] Several recent studies have shown that the upregulation of glucosamine 3-*O*-sulfotransferases (3-OSTs) is associated with fetal and neural development[8,17] and also with several forms of human cancer.[18] The presence of 3-OSTs implies that the biosynthesis of HS ligands containing GlcN-3*S* are critical in many important biological pathways; nevertheless, the isolation and characterization of such ligands remain elusive.

Mass spectrometry (MS) has become a leading method for characterizing sulfated oligosaccharides, many of which are available only in trace quantities.[19] Three strategies have emerged in recent years. In one approach, molecular ion signals are generated by matrix-assisted laser desorption/ionization and correlated with fragments produced by enzymatic digestion.[14b,20,21] A second one is based on electrospray ionization tandem MS (ESI-MS/MS) for characterizing fragments generated in situ by collision-induced dissociation (CID)[22] with the expectation that isomers having identical *m*/*z* ratios can be distinguished by unique ion peaks or by signature patterns of mass fragments.[19,23–26] A third strategy employs MS/MS and electron detachment dissociation to produce signature mass fragment peaks, which are likely generated via radical processes and can also be used to differentiate isomeric sulfoforms.[27] These approaches are complementary and may even be performed in sequence, as the latter two are applicable toward the compositional analysis of mono- and disaccharide fragments generated from longer oligosaccharide sequences. Regardless of the method, the ionization conditions for producing mass spectra must be optimized for a given molecular structure. Organic salts such as sulfated carbohydrates are particularly sensitive to sampling conditions, as their stabilities and volatilities may be affected by counterions as well as by the parent ion structure.[28]

ESI-MS/MS with CID can be applied toward the quantitative analysis of sulfoforms within HS. Saad et al. demonstrated that the population distribution of isomeric disaccharides could be determined to within 5% by a ratiometric analysis of fragment ion peaks and validated their method by the compositional profiling of several HS oligosaccharides.[24] However, the sample set was limited to commercially available HS disaccharides and did not include rare sulfoforms containing GlcN*S*-3*S* or GlcN*S*-3*S*,6*S*. Subsequent studies on sulfated monosaccharides bearing a 3-*O*-sulfate ester indicate that these compounds may produce MS fragmentation patterns and abundance ratios distinct from those of isomeric sulfoforms.[26,29] The growing significance of 3-*O*-sulfate esters in biological signaling supports the value of including such derivatives in the compositional profiling of HS oligosaccharides.

In this paper, we present the comprehensive synthesis and ESI-MS/MS analysis of sulfoforms based on glucosamine, a primary source of structural diversity within HS (Figure 1). GlcN sulfoforms are also found in mucin-like glycoproteins and have been shown to mediate binding to the cell adhesion protein L-selectin.[30] We elected to use a solid-phase approach to generate α-GlcNAc **1** and sulfoforms **2**−**8** with up to three sulfate esters at the C2, C3, and C6 positions, in overall yields ranging from 35−88%. Solid-phase and polymeric supports have been applied toward the linear synthesis of protected oligosaccharides,[31] but there are surprisingly few reports involving the multistep deprotection of oligosaccharides[32] and no verifiable demonstrations involving sulfated carbohydrate derivatives.[33]

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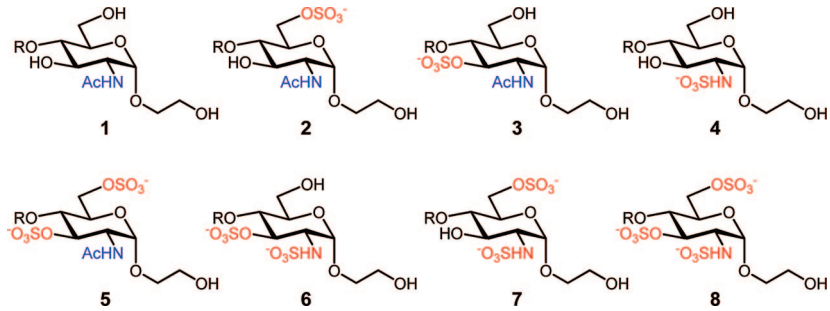


FIGURE 1. 2′-Hydroxyethyl α-*N*-acetylglucosaminoside **1** and GlcN sulfoforms **2**−**8** (**a**: R = Bn; **b**: R = H).

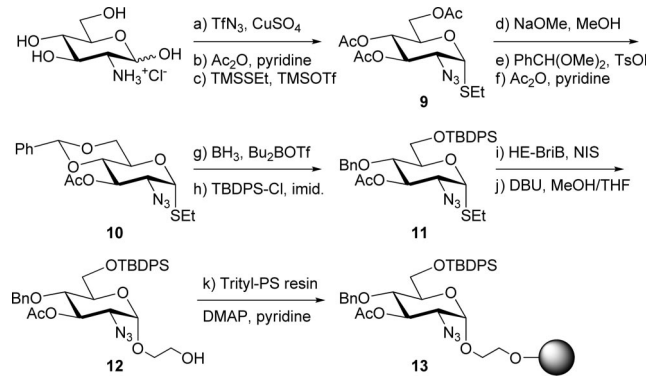
Here, we examine some practical issues enabling the solid-phase synthesis of GlcN sulfoforms from a common precursor and provide a systematic analysis of molecular ion stability and fragmentation behavior as a function of sulfonation pattern. The synthetic GlcN sulfoforms reveal important differences between positional isomers and offer new insights for structural analysis by MS/MS techniques.

Results and Discussion

Synthesis. Sulfoform diversity can be generated from combinations of carbohydrate building blocks having protecting groups at predesignated sulfate sites or by developing synthetic precursors with orthogonal protecting group systems.[3,34–36] Both approaches have their merits, but the latter offers the benefit of using a universal intermediate to generate an entire set of sulfoforms, unnatural as well as natural. The challenges of developing a unified sulfation strategy increase geometrically with the number of possible sulfate positions, as each additional protecting group reduces the chemical space for further orthogonality. We have recently addressed the issue of generating diverse sulfoforms from a common intermediate by introducing a heparan disaccharide (GlcN(α1→4)GlcA) with six orthogonal protecting groups.[35] A high density of sulfate groups also raises some concerns for the handling of highly charged intermediates in a multistep synthetic sequence. Here, we address this important practical issue by using a solid-phase approach to produce sulfated carbohydrates in deprotected forms.

α-Thioethyl 2-azidoglucoside **9** was prepared in three steps and 57% overall yield from glucosamine hydrochloride by Cu-mediated diazo transfer,[37] peracetylation, and TMSOTf-mediated glycosylation using TMSSEt,[38] added in two portions (Scheme 1). Triacetate **9** was converted into 4,6-benzylidene acetal **10** in 85% yield over three steps, followed by reductive cleavage to the 4-*O*-benzyl ether using BH_3/Bu_2BOTf and 2,6-lutidine as a proton scavenger.[39] The C6 alcohol was protected as a *tert*-butyldiphenylsilyl (TBDPS) ether to afford compound **11** in 65% overall yield from **10**. A glycol linker was installed by NIS/TfOH-promoted coupling of α-thioethyl glycoside **11** with 2′-hydroxyethyl 2-bromoisobutyrate, followed by treatment with DBU to yield a 6:1 α/β mixture of glycol-linked GlcN derivatives.[40] Careful separation by silica gel chromatography afforded 2′-hydroxyethyl α-GlcN derivative **12** in 66% isolated yield from **11**. It is worth mentioning that the coupling of β-thioglycosides with monoprotected glycol linkers generally favored the formation of β-*O*-glycosides,[40] contrary to expectations of glycosyl donors with nonparticipatory C2 groups.[41–43]

SCHEME 1. Synthesis and Loading of 2′-Hydroxyethyl α-GlcN Derivatives onto a Trityl-PS Resin[a]



[a] Select abbreviations: DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; HE-BriB = 2′-hydroxyethyl 2-bromoisobutyrate; TBDPS = *tert*-butyldiphenylsilyl.

Orthogonally protected α-GlcN **12** was tethered onto a trityl-PS resin in dry pyridine at 65 °C, assisted by 4-dimethylaminopyridine (DMAP) to produce α-GlcN-loaded resin **13**. A loading ratio of 0.8−0.9 mmol/g was attained using stoichiometric amounts of **12**; a higher ratio of 1.3 mmol/g could be obtained by using 1.5 equiv of monosaccharide relative to the maximum loading density. The loading ratios of **13** were determined by the amount of recovered **12** after cleavage from the resin.

Orthogonal cleavage conditions for the TBDPS, Ac, and N_3 groups were first optimized for homogeneous (solution) conditions, in accordance with our earlier studies of an orthogonally

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TABLE 1. Deprotection Conditions and Characteristic Vibrational Bands of Protecting Groups

group	deprotection conditions	IR, Raman bands (cm^{-1})[a]
N_3 (to -NH_2)	(i) Bu_3P (5 eq), CH_2Cl_2, 5 h; (ii) 95% aq DMF, 10 h, rt[b]	IR: 2107 (−)
N_3 (to -NHAc)	as above, then 1:1 pyridine/Ac_2O, 1 day, rt	IR: 1687 (+)
6-*O*-TBDPS[c]	I: 0.9 M TBAF in THF (pH ≥10), 1 day, rt II: 0.9 M TBAF in THF (pH 7) 1−2 days, rt	Raman: 1407, 1462, 1568 (−)
3-*O*-Ac	NaOMe or Bu_4NOH (5 eq), 1:1 MeOH/CH_2Cl_2, 5 h, rt	IR: 1753 (−)

[a] Vibrational bands used to monitor the disappearance (−) or appearance (+) of functional groups. [b] Reference 35. [c] Condition I preferred for efficient cleavage; condition II preferred for chemoselectivity.

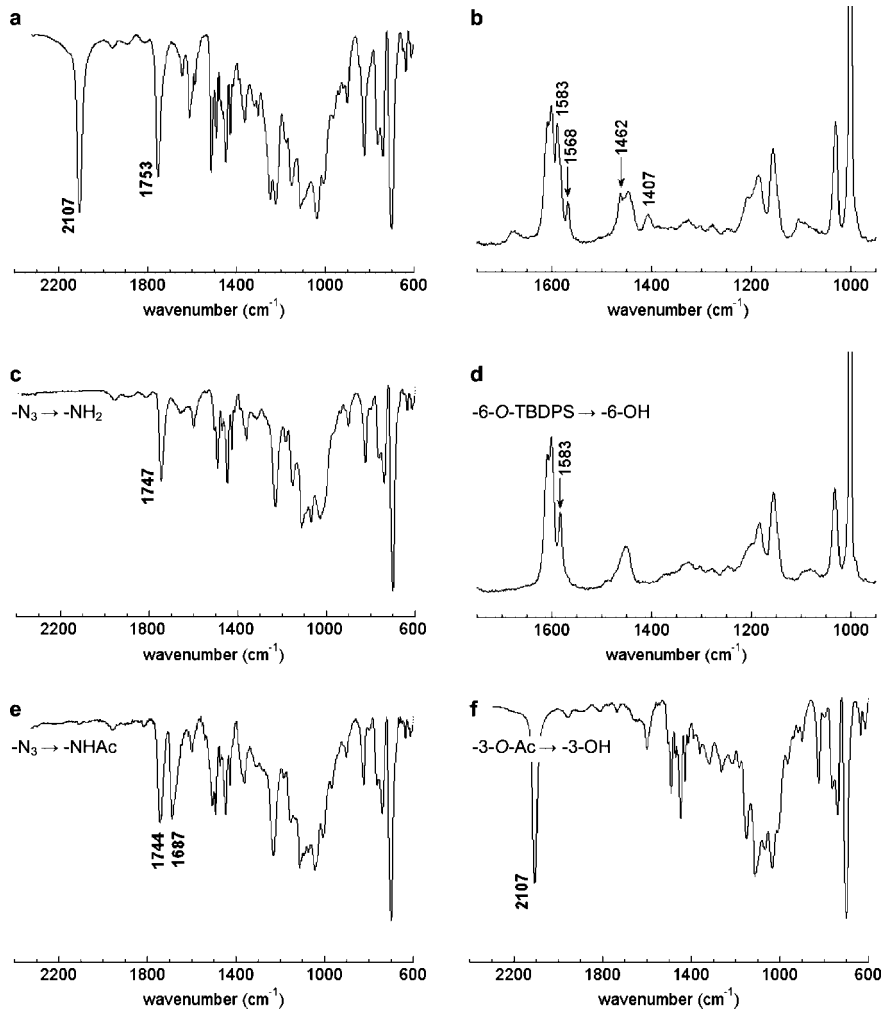


FIGURE 2. Raman and IR spectra of α-GlcN derivatives on trityl-PS resin. (a, b) IR and Raman spectra of fully protected α-GlcN (**13**).[44] (c) IR spectrum following N_3 reduction to free amine. (d) Raman spectrum following 6-*O*-TBDPS deprotection. (e) IR spectrum following conversion of N_3 to −NHAc. (f) IR spectrum following 3-*O*-Ac deprotection.

protected heparan disaccharide.[35] These deprotection conditions could also be employed in solid-phase reactions, but the order of functional group transformations and the responsivity of the supporting PS resin had some influence on reaction efficiency. In some cases, reaction speed was reduced by changes in local chemical environment; in others, a loss of chemoselectivity was observed. For example, deprotection of the TBDPS ether with unneutralized TBAF (pH ≥ 10) did not affect the C3 acetate in azidoglucose **13**, but performing the same reaction on the corresponding *N*-acetyl derivative resulted in partial C3 deacetylation. We thus employed a dual set of deprotection conditions, one optimized for speed but which could be slightly aggressive toward other functionalities, and a slower but milder condition which could be used without any concern for chemoselectivity (Table 1).

The efficacy of each deprotection step could be monitored on-resin by Fourier transform infrared (IR) or Raman spectroscopy.[31] Each protecting group exhibited one or more characteristic vibrational bands, whose presence could be correlated with the end point of the deprotection reaction (Table 1). Typical IR and Raman spectra following a deprotection reaction starting from immobilized α-GlcN derivative **13** are shown in Figure 2.[44] It is interesting to note that the IR frequency corresponding to the amide carbonyl stretch of NHAc appears at 1687 cm^{-1} while immobilized on resin, but is restored to a more typical value of 1652 cm^{-1} after cleavage from the resin support.

Partially deprotected α-GlcN derivatives were directly converted on-resin into sulfate esters using SO_3·pyridine, then

(44) The origin of the IR stretch at 1679 cm^{-1} in Figure 2a is unknown.

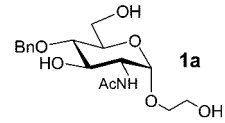
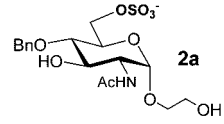
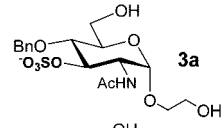
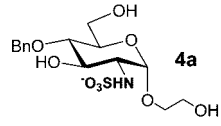
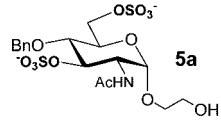
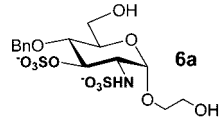
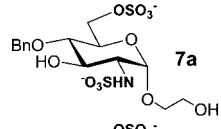
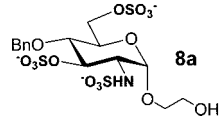
treated with a neutral Bu_4NHSO_4 solution (30−50 mM) in 1:1 MeOH/DMF to produce the Bu_4N salt. This procedure minimized any negative effects of the sulfate esters on the swelling properties of the resin, which is essential for maintaining access to the reaction medium. Mono-*O*-sulfates were readily formed at room temperature in DMF, whereas *N*-sulfate formation required basic conditions and was best achieved using 5:1 pyridine/Et_3N.[45] Multiple sulfates could be installed in a single step in some instances but more often required prolonged reaction times at 55 °C using pyridine/Et_3N or an intermediate ion-exchange step with Bu_4NHSO_4 to restore resin swelling followed by a second round of SO_3·pyridine to achieve saturation. We note that while direct sulfonation is suitably efficient, one might also consider the use of protected sulfate diesters for installing multiple sulfate groups while maintaining charge neutrality.[46] However, this approach introduces an additional deprotection step, which imposes another condition for orthogonality.

The 2′-hydroxyethyl α-GlcN derivatives were cleaved from the resin as their 4-*O*-benzyl ethers for characterization and to determine overall yield (Table 2). We first established the efficiency of the loading and deprotection steps by cleaving the unsulfated glucosamine using 3% CF_3COOH in CH_2Cl_2, and obtained α-GlcNAc **1a** in 88% yield over six operations after purification by reversed-phase HPLC. We note that this acidic condition did not cause the decomposition of α-GlcNAc-6*S* **2a**, α-GlcNAc-3*S* **3a**, or α-GlcNAc-3*S*,6*S* **5a**, which were recovered intact by adding acetic acid to the filtrate prior to concentration under reduced pressure. However, *N*-sulfated α-GlcN derivatives were more labile to acid and typically experienced partial or complete desulfation during recovery.

Prior experience suggested that Lewis acid conditions might be used to cleave the GlcN sulfoforms from the resin without sulfate ester decomposition.[35] After evaluating several possibilities we were pleased to find that both *O*- and *N*-sulfates were stable in the presence of 0.3 M BF_3·Et_2O at −20 °C. This condition was also efficient at cleaving the glycol linker from the trityl-PS resin and proved to be of general utility for obtaining 4-*O*-benzyl GlcN sulfoforms **2a**−**8a** as their Bu_4N salts (Table 2). The C4 benzyl ether facilitated HPLC purification by reducing the sulfoforms' polarity and provided a convenient UV-active chromophore for detection during elution. The benzyl ethers were then cleaved by catalytic hydrogenation, followed by treatment with an ion-exchange resin in aqueous methanol to produce the fully deprotected GlcN **1b** and sulfoforms **2b**−**8b** as Na salts (Scheme 2). The efficient sorption of Bu_4N ions is noteworthy as it can be used to purify polar organic compounds at the final stage of synthesis,[47] if care is taken to remove residual polyelectrolyte from the resin beforehand.

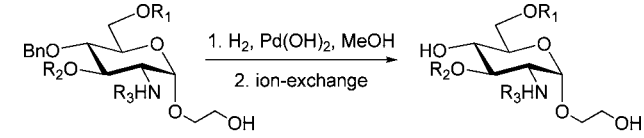
Fragmentation Analysis. ESI-MS/MS analyses were performed in negative ion mode on 2′-hydroxyethyl α-GlcN sulfoforms with 4-*O*-benzyl ethers (**2a**−**8a**) as well as on fully deprotected sulfoforms (**2b**−**8b**), with each set respectively serving as models of internal and terminal α-GlcN residues. Preliminary analyses were performed on sulfoforms **2a**−**8a** using an ion trap mass analyzer with the CID energy set to 40% of the resonance excitation rf voltage (5 V_{pp}), in order to identify differences in mass losses or fragment patterns as a function of the parent ion (Table 3). Among monosulfate derivatives **2a**−**4a**, the loss of SO_3 $[M - H - 80]^-$ was responsible for the major fragment produced from GlcNAc-3*S*, but was a minor fragmentation pathway for GlcN*S* or GlcNAc-6*S*. Among monoprotonated disulfate derivatives **5a**−**7a**, the loss of SO_3 was produced by all sulfoforms but the spectrum of GlcN*S*-6*S* also featured a significant loss of glycol $[M - H - 62; B \text{ ion}]^-$.

TABLE 2. Generation of 4-*O*-Benzyl GlcN 1a and Sulfoforms 2a−8a (Bu_4N Salts) from 13[a]

product	yield, no. operations	react cond
 **1a**	88%, 6 ops	a, b, c, d, e, f
 **2a**	58%, 8 ops	a, b, c, g, h, i, j, k
 **3a**	53%, 8 ops	a, b, c, e, h, i, d, k
 **4a**	63%, 7 ops	a, b, l, i, d, j, k
 **5a**	42%, 10 ops	a, b, c, d, e, m, i, m, i, k
 **6a**	46%, 7 ops	e, a, b, m, i, d, k
 **7a**	35%, 9 ops	d, a, b, h, i, l, i, j, k
 **8a**	38%, 9 ops	e, a, b, d, m, i, m, i, k

[a] Reagents and conditions: (a) Bu_3P, CH_2Cl_2, rt; (b) 95% aq DMF, rt; (c) Ac_2O, pyridine, rt; (d) TBAF (pH ≥10), THF, rt; (e) NaOMe, MeOH/CH_2Cl_2, rt; (f) TFA, CH_2Cl_2, rt; (g) TBAF (pH 7), THF, rt; (h) SO_3•py, DMF, rt; (i) Bu_4NHSO_4 (pH 7), DMF/MeOH, rt; (j) Bu_4NOH, MeOH/CH_2Cl_2, rt; (k) BF_3•Et_2O, CH_2Cl_2, −20 °C; (l) SO_3•py, pyridine/Et_3N, rt; (m) SO_3•py, pyridine/Et_3N, 55 °C.

SCHEME 2. Synthesis of 2′-Hydroxyethyl α-GlcN 1b and Sulfoforms 2b−8b[a]



1a-8a (Bu_4N salt) **1b-8b** (Na salt)

[a] R_1,R_2 = H or SO_3^-; R_3 = Ac or SO_3^-.

(45) Noti, C.; de Paz, J. L.; Polito, L.; Seeberger, P. H. *Chem. Eur. J.* **2006**, *12*, 8664–8686.

(46) (a) Liu, Y.; Lien, I. F. F.; Ruttgaizer, S.; Dove, P.; Taylor, S. D. *Org. Lett.* **2004**, *6*, 209–212. (b) Gunnarsson, G. T.; Riaz, M.; Adams, J.; Desai, U. R. *Bioorg. Med. Chem.* **2005**, *13*, 1783–1789. (c) Simpson, L. S.; Widlanski, T. S. *J. Am. Chem. Soc.* **2006**, *128*, 1605–1610. (d) Ingram, L. J.; Taylor, S. D. *Angew. Chem., Int. Ed.* **2006**, *45*, 3503–3506.

(47) Kaburagi, Y.; Kishi, Y. *Org. Lett.* **2007**, *9*, 723–726.

TABLE 3. ESI-MS² Fragments of 4-*O*-Benzyl GlcN Sulfoforms 2a−8a

compd[a]	*M* (amu)[b]	MS² fragment mass loss (% intensity)[c]
2a (GlcNAc-6*S*)	435	M − H (12)
		M − H − 62 (100)
		M − H − 80 (3)
3a (GlcNAc-3*S*)	435	M − H (100)
		M − H − 62 (15)
		M − H − 80 (67)
4a (GlcN*S*)	393	M − H (100)
		M − H − 62 (2)
		M − H − 80 (<1)
		M − H − 108 (5)
5a (GlcNAc-3*S*,6*S*)	515	M − H (5)
		M − H − 62 (1.4)
		M − H − 80 (100)
6a (GlcN*S*-3*S*)	473	M − H − 62 (<1)
		M − H − 80 (100)
7a (GlcN*S*-6*S*)	473	M − H − 62 (14)
		M − H − 80 (100)
8a (GlcN*S*-3*S*,6*S*)[d]	553	M − H (3)
		M − H − 80 (36)
		M − H − 160 (11)
		M − 3H + Na, Bu_4N (100)

[a] Bu_4N counterions. [b] Mass of neutral (acidic) form. [c] Negative ions. [d] ESI-MS data; no MS² was obtained from the parent ion $[M - H]^-$.

TABLE 4. Dissociation Rate Constants of 4-*O*-Benzyl GlcN Sulfoforms

compd[a]	k_P,[b] s^{-1}
2a (GlcNAc-6*S*)[c]	151 ± 12
3a (GlcNAc-3*S*)[c]	189 ± 11
4a (GlcN*S*)[d]	100 ± 9
6a (GlcN*S*-3*S*)[e]	741 ± 24
7a (GlcN*S*-6*S*)[e]	239 ± 11

[a] Bu_4N counterions. [b] 95% confidence limits. [c] CID conditions: 100 mV_{pp}, 49.016 kHz. [d] CID conditions: 100 mV_{pp}, 54.273 kHz. [e] CID conditions: 100 mV_{pp}, 90.806 kHz. Bath gas temperature = 298 K; gas pressure = 1.4×10^{-4} Torr.

The parent ion generated from diprotonated trisulfate **8a** $[M - H]^-$ was too weak for MS/MS analysis under these conditions, although standard ESI-MS revealed a substantial loss of one or two SO_3 groups. These losses are well-known to be produced from sulfonic acids and indicate the importance of performing electrospray under basic conditions.[21] Overall, the results suggest that sulfoforms bearing a 3-*O*-sulfate are significantly more prone to fragmentation, relative to their positional isomers.

Several 4-*O*-benzyl GlcN sulfoforms were also examined by ESI-MS/MS using a dueling-ion trap at lower CID voltages (100 mV_{pp}) to measure the rates of parent ion dissociation and fragment ion formation (Table 4). First-order dissociation rate constants (k_P) were obtained from the exponential decay of normalized parent ion density *P* during activated collision

$$k_P t = -\ln\left[\frac{P(t)}{\sum F + P(t)}\right] \quad (1)$$

where $\sum F$ is the sum of the normalized fragment peak intensities. A typical semilog plot of parent ion dissociation versus collision time *t* is shown in Figure 3. Differences in dissociation rates among monosulfated GlcN derivatives were modest: the k_P values of *O*-sulfated GlcNAc isomers **2a** and **3a** were greater than that of GlcN*S* **4a** by 1.5 to 2 times, respectively. On the other hand, a 3-fold rate difference was

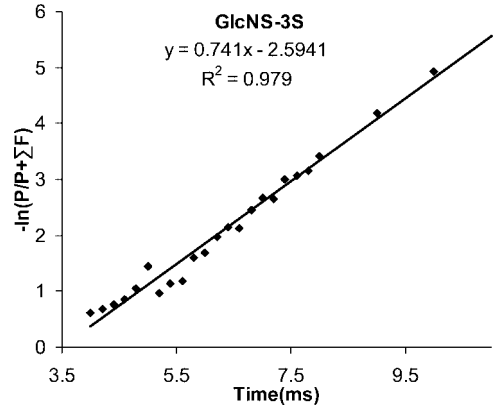


FIGURE 3. Parent ion dissociation rate data of **6a**, with each data point based on the average of 50 individual mass analysis scans. CID conditions: 100 mV_{pp}, 90.806 kHz; bath gas temperature = 298 K; total gas pressure = 1.4×10^{-4} Torr (uncorrected for ion gauge detector response to helium).

observed between isomeric disulfates **6a** and **7a** (GlcN*S*-3*S* and GlcN*S*-6*S*, respectively). The faster dissociation rate of **6a** can be attributed to the destabilizing effect of electrostatic repulsion between neighboring *N*- and *O*-sulfates at C2 and C3.

High-accuracy ion-trap CID experiments performed on a quadrupole/time-of-flight (QqTOF) tandem mass spectrometer generated characteristic daughter ions and fragmentation patterns from 4-*O*-benzyl GlcN sulfoforms under basic electrospray conditions (Figure 4 and Table 5).[48] Most fragments can be derived from their parent ions via elimination pathways and assigned according to Domon−Costello nomenclature.[49] The 3-*O*-sulfate ester again plays a defining role in fragment production: hydrogen sulfate ($HOSO_3^-$, *m*/*z* 97) is generated as the dominant fragment from GlcNAc-3*S* **3a**, whereas the mass spectra of GlcNAc-6*S* **2a** and GlcN*S* **4a** also feature ion peaks generated by ring fragmentation pathways leading to *A* and *X* ions. In these cases the C3 hydroxyl is likely to promote the formation of $^{3,5}A$ and $^{0,2}X$ fragments,[26] whereas the C2 sulfamate may contribute toward the formation of the $^{1,3}A$ fragment. Sulfamate ($H_2NSO_3^-$, *m*/*z* 96) is also produced as a major fragment ion in the spectrum of GlcN*S* **4a** but in lower relative abundance from *N,O*-disulfates **6a** and **7a**. The appearance of a small $HOSO_3^-$ peak in the GlcN*S* spectrum indicates that *N*-to-*O*-sulfate migration is possible, but minor compared with other pathways. GlcN*S*-6*S* **7a** produces fragments with *m*/*z* 374 (M − 2H − 97) and *m*/*z* 375 (M − 2H − 96), corresponding to the monovalent parent anion with loss of $HOSO_3^-$ and $H_2NSO_3^-$ respectively, whereas GlcN*S*-3*S* **6a** does not produce the latter.

The sulfation patterns have considerable influence on the generation of neutral mass losses, i.e. glycosidic and aglyconic cleavage pathways leading to *B* and *Z* ions, respectively. *B* ion formation (mass loss of 62) is especially pronounced in GlcNAc-6*S* **2a**, whereas GlcN*S* derivative **4a** has a capacity to form *Z* ions (mass loss of 108). In the case of GlcN*S*-3*S* **6a**, aglycon (C4) elimination may operate in conjunction with other fragmentation processes, not easily described by conventional nomenclature. Mechanistically speaking, "*Z* elimination" may be combined with "$^{1,5}A$ fragmentation" and various eliminations to produce conjugated

(48) It should be noted that differences in the mass analyzers and CID conditions used in this study had a significant impact on their respective ESI-MS/MS spectra. In particular, preliminary MS/MS analyses using the ion trap mass analyzer were performed with a cutoff at 30% of the parent ion *m*/*z* ratio; therefore, no ions below this threshold could be observed.

(49) Domon, B.; Costello, C. E. *Glycoconjugate J.* **1988**, *5*, 397–409.

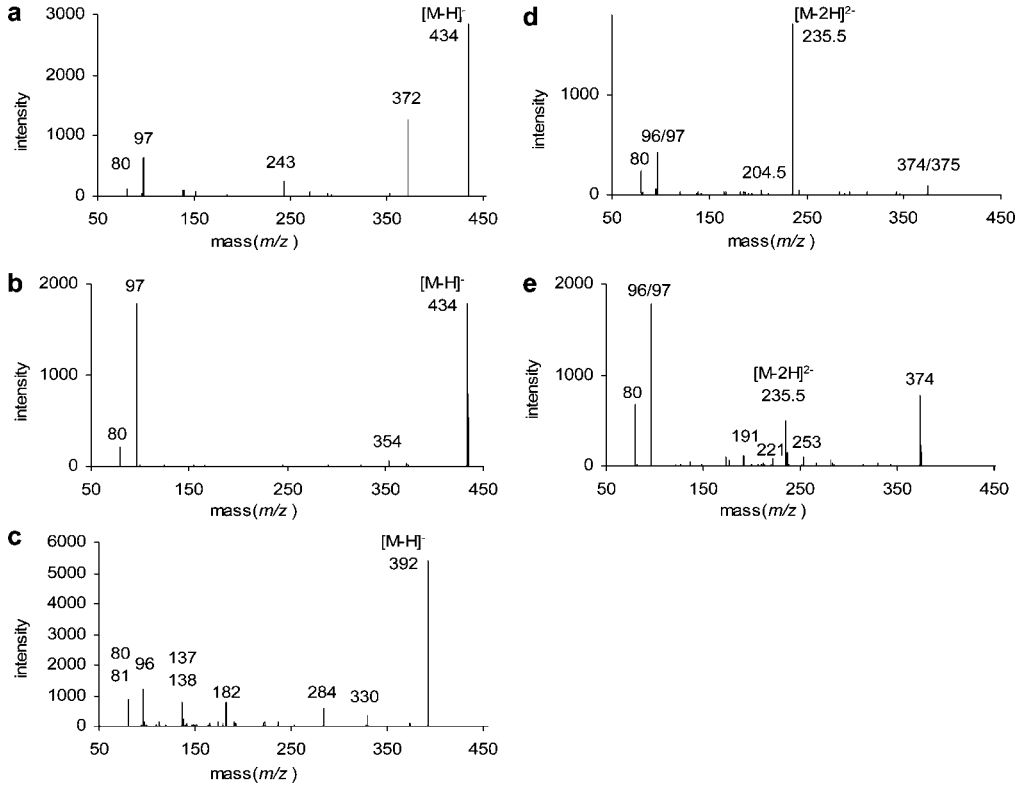


FIGURE 4. ESI-MS/MS spectra with CID of 4-*O*-benzyl GlcN sulfoforms. (a) GlcNAc-6*S* (**2a**); (b) GlcNAc-3*S* (**3a**); (c) GlcN*S* (**4a**); (d) GlcN*S*-6*S* (**7a**); (e) GlcN*S*-3*S* (**6a**). Parent ion $[M-H]^-$ for **2a**−**4a**; $[M-2H]^{2-}$ for **7a** and **6a**. See Table 5 for CID conditions.

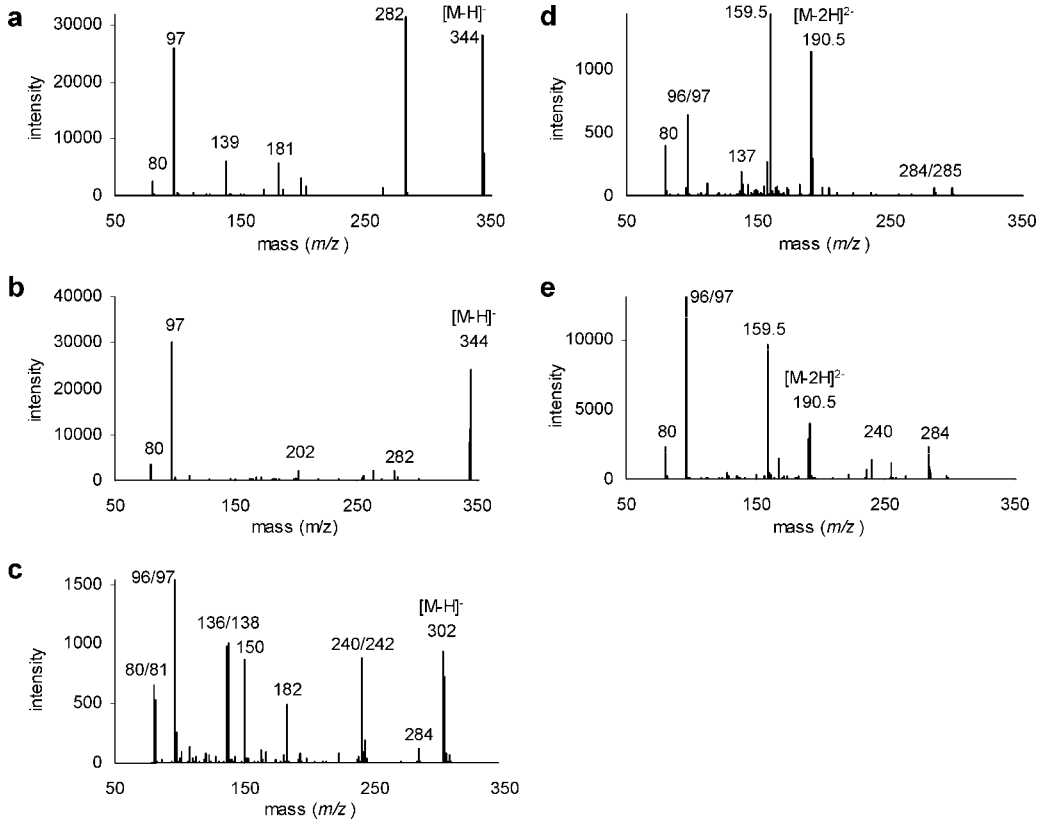
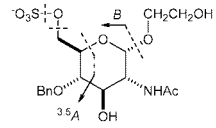
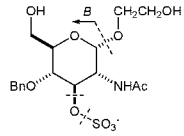
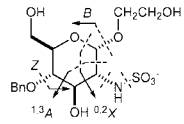
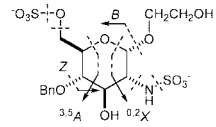
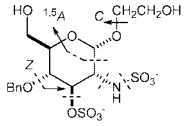


FIGURE 5. ESI-MS/MS spectra with CID of fully deprotected GlcN sulfoforms: (a) GlcNAc-6*S* (**2b**); (b) GlcNAc-3*S* (**3b**); (c) GlcN*S* (**4b**); (d) GlcN*S*-6*S* (**7b**); (e) GlcN*S*-3*S* (**6b**). Parent ion $[M - H]^-$ for **2b**−**4b**; $[M - 2H]^{2-}$ for **7b** and **6b**.[51] See Table 6 for CID conditions.

TABLE 5. Major Fragments of 4-*O*-Benzyl GlcN Sulfoforms by ESI-MS/MS with CID

compd	mass loss or ions formed (*m/z*) [a]	rel abund (%) [b]	cleavage patterns
2a [c]	SO_3^- (80)	4.3	
	$HOSO_3^-$ (97)	22.3	
	$^{3,5}A$ (243)	8.9	
	B–H_2O (354)	1.8	
	B (372)	44.1	
3a [d]	SO_3^- (80)	11.6	
	$HOSO_3^-$ (97)	100	
	B–H_2O (354)	3.7	
	B (372)	2.1	
4a [e]	SO_3^- (80)	16.0	
	HSO_3^- (81)	9.5	
	$H_2NSO_3^-$ (96)	22.3	
	$HOSO_3^-$ (97)	2.9	
	$^{1,3}A$ (137, 138)	14.5,4.5	
	$^{0,2}X$ (182)	14.7	
	Z (284)	10.8	
	B (330)	6.4	
7a [f]	SO_3^- (80)	14.1	
	$H_2NSO_3^-$ (96)	3.6	
	$HOSO_3^-$ (97)	24.8	
	$^{0,2}X$ (182)	1.6	
	B^{2-} (204.5)	2.6	
	$^{3,5}A$ (243)	3.1	
	$[M–2H–97]^-$ (374)	5.0	
	$[M–2H–96]^-$ (375)	2.3	
6a [f]	SO_3^- (80)	37.6	
	$H_2NSO_3^-$ (96)	6.2	
	$HOSO_3^-$ (97)	100.0	
	Z & $^{1,5}A$ (174, 176) [g]	5.9, 3.9	
	Z & extrusion (191) [g]	6.2	
	Z & C (221) [g]	5.0	
	Z & extrusion (253) [g]	5.2	
	$[M–2H–97]^-$ (374)	43.7	

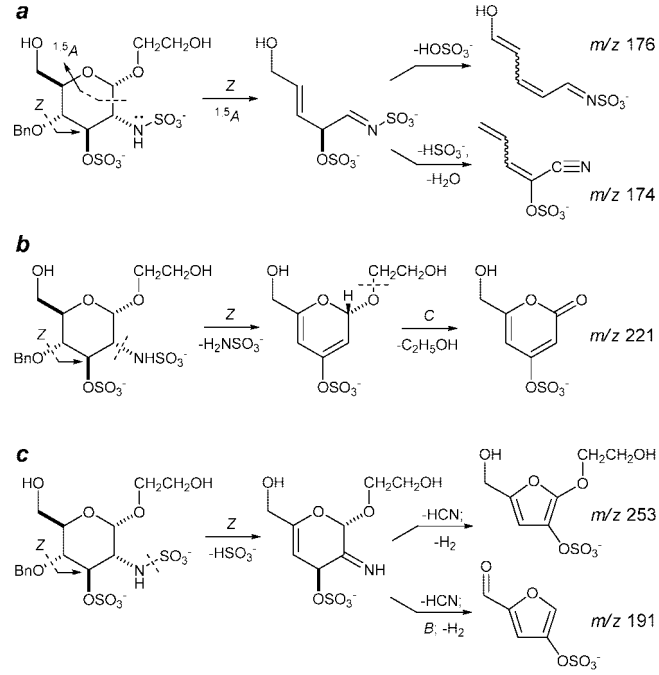
[a] Derived from parent ion $[M - H]^-$ or $[M - 2H]^{2-}$ as described in Figure 4. [b] Relative abundance based on parent ion peak, except **3a** and **6a**. [c] CID at 380 mV, 80.39 KHz (10 ms). [d] CID at 330 mV, 80.39 KHz (10 ms). [e] CID at 350 mV, 89.04 KHz (10 ms). [f] CID at 220 mV, 149.22 KHz (10 ms). [g] See Scheme 3 for mechanistic details.

fragments with *m/z* 174 and 176 (Scheme 3a), with elimination of H_2NSO_3 and "*C* elimination" (loss of ethanol) to produce an α-pyrone with *m/z* 221 (Scheme 3b), or with loss of HSO_3^- and ring contraction with extrusion of HCN and loss of H_2 to produce furans with *m/z* 191 and 253 (Scheme 3c).

ESI-MS/MS of fully deprotected sulfoforms **2b**−**4b**, **6b**, and **7b** using similar CID conditions also produces characteristic fragmentation patterns, but with somewhat greater complexity (Figure 5 and Table 6).[50] The C4 hydroxyl adds considerable diversity to the possible types of fragmentation pathways. It significantly alters the ring cleavage pathways for monosulfates **2b** and **4b** with a higher production of $^{0,n}A$ and $^{0,n}X$ fragments,

(50) Control experiments with Bu_4N salts suggest that the counterion does not have a significant effect on the negative-mode ESI-MS/MS spectra.

SCHEME 3. Proposed Fragmentation Mechanisms in the ESI-MS/MS of GlcN*S*-3*S* 6a[a]



[a] Domon−Costello nomenclature has been applied purely for descriptive purposes; fragmentation pathways may be acting in concert.

sometimes at the expense of other ring fragments such as the $^{3,5}A$ ion. The C4 hydroxyl also appears to promote the generation of SO_3^-, most likely by serving as a hydrogen bond donor to the adjacent *O*-sulfate. In the case of GlcN*S* **4b**, the relative intensity of the $HOSO_3^-$ peak (16.8%) is much higher than that produced from **4a** (2.9%), which suggests that hydrogen bonding can also promote *N*-to-*O*-sulfate migration to GlcN-4-*O*-sulfate (Scheme 4).

The 3,4-diols in **4b** and **7b** can participate in [1,2]-hydride shifts (semipinacol rearrangements), a mechanism previously reported for vicinal diols in carbohydrate rings.[52] The rearrangement of *B* ions with loss of H_2O (*B*′) can lead to fragmentation by retroaldol or retro-Diels−Alder reaction mechanisms (Scheme 5a). In addition, vicinal diols and especially enediols are easily deprotonated, due to electronic stabilization of the corresponding planar anion.[53] This can explain the observation of additional dianion fragments from GlcN*S*-6*S* **7b**, which are not observed in the spectra of the corresponding 4-*O*-benzyl sulfoform **7a** (Scheme 5b).

Conclusions

α-GlcN sulfoforms can be synthesized by orthogonal deprotection and sulfonation of a common intermediate tethered on a solid support, enabling their systematic analysis by tandem mass spectrometry. The sulfoforms were efficiently cleaved from trityl-PS resins as 4-*O*-benzyl ethers using Lewis acid conditions

(51) Isotopic parent ion peaks were observed in the MS/MS spectra of **4b**, **7b**, and **6b**. These were higher than expected after CID, possibly due to perturbations in rf resonance frequencies corresponding with the expected parent ions.

(52) Robins, M. J.; Nowak, I.; Wnuk, S. F.; Hansske, F.; Madej, D. *J. Org. Chem.* **2007**, *72*, 8216–8221.

(53) (a) Wit, G. D.; Kieboom, A. P. G.; Bekkum, H. V. *Carbohydr. Res.* **1979**, *74*, 157–175. (b) Calle, P.; Sanchez, A.; Sieiro, C. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1181–1185.

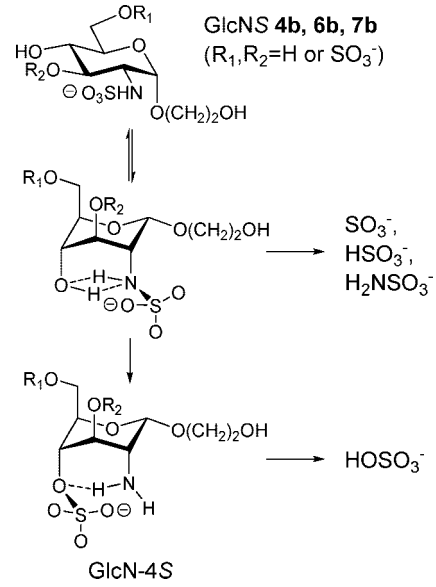
TABLE 6. Major Fragments of Fully Deprotected GlcN Sulfoforms by ESI-MS/MS with CID

compd	mass loss or ions formed (*m/z*) [a]	rel abund (%) [b]	cleavage patterns
2b [c]	SO_3^- (80)	8.1	
	$HOSO_3^-$ (97)	83.2	
	$^{0,4}A$ (139)	19.4	
	$^{0,3}A$ (169)	3.5	
	$^{0,2}A$–H_2O (181)	18.2	
	$^{0,2}A$ (199)	9.8	
	B–SO_3 (202)	5.3	
	B (282)	100	
3b [c]	SO_3^- (80)	12.3	
	$HOSO_3^-$ (97)	100	
	B–SO_3 (202)	6.6	
	B–H_2O or [M– H– SO_3]$^-$ (264)	6.5	
	B (282)	7.4	
4b [d]	SO_3^- (80)	42.4	
	HSO_3^- (81)	34.8	
	$H_2NSO_3^-$ (96)	100	
	$HOSO_3^-$ (97)	16.8	
	$H_2CNSO_3^-$ (108)	8.5	
	$^{0,2}X$–C_2H_5OH (136)	63.6	
	$^{1,3}A$ (137, 138)	25.1, 65.1	
	B' & $^{3,5}X$ (150) [e]	56.4	
	$^{0,4}X$–SO_3 (162)	6.8	
	B & $^{3,5}X$ (166) [e]	6.4	
	$^{0,2}X$ (182)	31.9	
	B'–CH_2O (192) [e]	5.3	
	B' (222) [e]	5.0	
	B (240)	57.9	
	$^{0,4}X$ (242)	12.5	
	[M–H–H_2O] (284)	8.3	
7b [f]	SO_3^- (80)	27.4	
	$H_2NSO_3^-$ (96)	4.4	
	$HOSO_3^-$ (97)	45.0	
	$[M–3H–156]^{2-}$ (112) [g]	6.8	
	B' & $^{3,5}A$ (137) [e]	13.2	
	$^{0,4}A$ (139)	6.4	
	$[M–3H–96]^{2-}$ (142) [g]	6.0	
	$^{0,4}A$ +H_2O (157)	18.9	
	B^{2-} (159.5)	100	
	$^{0,2}A$–H_2O (181)	6.1	
	$^{0,2}A$ (199)	4.6	
	$[M–2H–97]^-$ (284)	4.1	
	[M–2H–96] (285)	1.7	
6b [f]	SO_3^- (80)	17.5	
	$H_2NSO_3^-$ (96)	37.9	
	$HOSO_3^-$ (97)	100.0	
	B^{2-} (159.5)	74.0	
	C^{2-} (168.5)	11.2	
	$[M–2H–145]^-$ (236)	5.6	
	$^{0,4}X$–HSO_3 (240)	10.3	
	$[M–2H–127]^-$ (254)	8.5	
	$[M–2H–97]^-$ (284)	17.8	

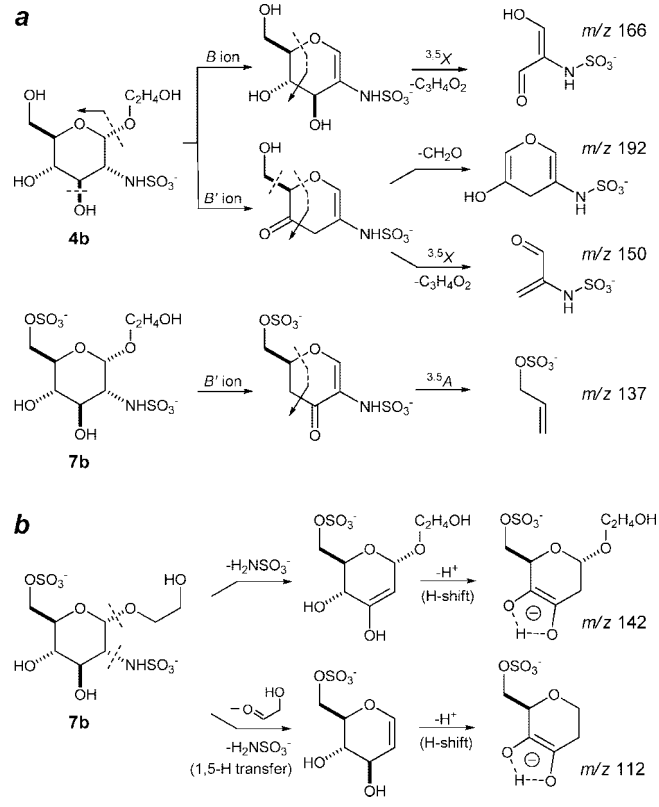
[a] Derived from parent ion $[M - H]^-$ or $[M - 2H]^{2-}$ as described in Figure 5. [b] Relative abundance based on ions with highest intensity. [c] CID at 350 mV, 101.51 KHz (10 ms). [d] CID at 400 mV, 115.60 KHz (10 ms). [e] See Scheme 5a for mechanistic details. [f] CID at 250 mV, 184.88 KHz (10 ms). [g] See Scheme 5b for mechanistic details.

at low temperature, with overall yields ranging from 88% (six operations) to 35% (nine operations) and were readily converted to the fully deprotected sulfoforms by catalytic hydrogenation. ESI-MS/MS analysis with CID revealed that GlcN derivatives

SCHEME 4. Elimination and Migration Pathways in the ESI-MS/MS of GlcN*S* Sulfoforms 4b



SCHEME 5. Proposed Fragmentation Pathways in the ESI-MS/MS of GlcN*S* 4b and GlcN*S*-6*S* 7b



with a 3-*O*-sulfate ester eliminate hydrogensulfate ion more readily than other sulfoforms, whereas derivatives with a C3 hydroxyl are more prone to ring fragmentation. GlcN*S* derivatives with a 4-*O*-benzyl ether tend to form *Z* ions, whereas those with a C4 hydroxyl produce additional $^{0,n}A$ and $^{0,n}X$ fragments. The increased number of fragmentation pathways introduced by the C4 hydroxyl suggests that GlcN sulfoforms with O4 protecting groups are better suited for modeling the MS fragmentation of internal GlcN units within HS derivatives. Lastly, the unique ESI-MS/MS spectra produced by GlcN

derivatives with identical *m/z* ratios support the use of fragmentation analysis to distinguish HS sulfoforms with 3-*O*-sulfate esters from those with 6-*O*-sulfate esters.

Experimental Section

General experimental methods. See the Supporting Information.

Solid-Phase Generation of α-GlcN Sulfoforms on Trityl-PS Resin. Trityl-PS resins were obtained from Novabiochem and used directly without activation. Standard workup conditions for solid-phase reactions involved (i) filtering and washing the resin (50 mg) three times alternatively with DMF (3 × 3 mL) and CH_2Cl_2 (3 × 3 mL) and then with additional CH_2Cl_2 (2 × 3 mL) and (ii) drying the resin under reduced pressure in the presence of KOH pellets prior to the next operation. The dry loaded resin (50 mg) was swollen in CH_2Cl_2 (3 mL) for 5 min under an inert atmosphere, cooled to −20 °C, and treated dropwise with $BF_3 \cdot Et_2O$ (114 μL, 0.9 mmol). The mixture was stirred for 1 h and then quenched with pyridine (3 mL) and slowly warmed to rt. The resin was filtered and washed with MeOH (4 × 3 mL), and the extracts were concentrated under reduced pressure at rt. Compound **1a** and sulfoforms **2a**−**8a** were purified by reversed-phase HPLC (Hydro-RP C18). The 4-*O*-benzyl group was removed using standard hydrogenation conditions in MeOH (see below); reaction mixtures were filtered then passed through an ion-exchange column (Dowex Marathon MSC, Na form) to yield fully deprotected sulfoforms **2b**−**8b** as Na salts. These were purified by reversed-phase HPLC to remove residual polystyrenesulfonate.

Mass Spectrometry. Preliminary ESI-MS/MS analyses were carried out on a mass spectrometer with an ion-trap mass analyzer. Electrospray experiments were typically performed with the needle voltage at 4.0 kV and the capillary voltage at 10 V with the capillary heated to 207 °C, the background source pressure at 1.2×10^{-2} mTorr as read by an ion gauge, and a sample flow rate of approximately 8 μL/min with N_2 as the drying gas. The sample was administered as an unbuffered aqueous solution and scanned to 1000 amu. CID experiments were conducted using helium as the bath gas, at approximately 1 mTorr for optimal trapping efficiency. The collision energy was set to 40% of the maximum resonance excitation voltage (5 V_{pp}).

High-accuracy ion-trap CID experiments were performed on parent ions using a quadrupole/time-of-flight (QqTOF) tandem mass spectrometer coupled with a nanoelectronspray ionization source.[54] Nanospray capillaries were prepared from borosilicate glass (1.5 mm o.d., 0.86 mm i.d.) using a micropipette puller. The sample was administered as a 0.5 mM solution in 98% aqueous CH_3CN containing 1% NH_4OH, with parent ions isolated by the ion-trap filter in mass-resolving mode, followed by injection into a collision chamber at 5 mTorr with nitrogen as the bath gas. The ions were cooled prior to collisional activation (10 ms) followed by QqTOF mass analysis; the final mass spectra are averages of 50 individual scans.

Parent ion dissociation rates were measured on a dueling ion-trap MS with nanoelectrospray ionization.[55] Ions were accumulated over several hundred milliseconds, followed by isolation of the parent ion and collisional activation for periods ranging from 2 to 50 ms. CID was performed by applying resonance excitation voltages with an amplitude of 100 mV_{pp} and optimized for each substrate based on the signal intensities in the product ion spectra.

Thioethyl 3,4,6-*O*-Acetyl-2-azido-2-deoxy-α-D-glucopyranoside (9). Procedure for Synthesis of TfN_3 (Caution: Potentially Explosive!). A solution of NaN_3 (37.6 g, 580 mmol) in water (48 mL) and CH_2Cl_2 (80 mL) at 0 °C was treated with Tf_2O (19.6 mL, 116 mmol). The mixture was stirred for 2 h at 0 °C, extracted with CH_2Cl_2 (2 × 35 mL), washed with saturated aqueous Na_2CO_3 solution (75 mL), dried over Na_2SO_4, and used immediately.

A solution of glucosamine hydrochloride (10 g, 46.4 mmol) in 66% aqueous MeOH (231 mL) was treated with K_2CO_3 (9.6 g, 70 mmol), $CuSO_4 \cdot 5H_2O$ (1.2 g, 4.6 mmol), and freshly prepared TfN_3 (70 mL, 1.66 M solution in CH_2Cl_2). An additional 30 mL of MeOH was added to homogenize the mixture. The mixture was stirred for 12 h at rt, concentrated, and azeotroped with toluene and then dried under reduced pressure. The crude azide was dissolved in pyridine followed by addition of Ac_2O at 0 °C. The mixture was warmed to rt, stirred for another 12 h, concentrated, azotroped with toluene, and dried under reduced pressure. The product was purified by recrystallization in 25% EtOAc in hexanes to afford the desired tetraacetate as a white solid and a mixture of anomers (14.3 g; 2.4:1 α/β). These were dissolved in CH_2Cl_2 (60 mL) and treated with TMSSEt (18.6 mL, 15 mmol, added in two portions) and TMSOTf (2.76 mL, 15.3 mmol). The mixture was heated to reflux for 20 h under Ar, quenched with saturated $NaHCO_3$ solution (50 mL), extracted with CH_2Cl_2 (3 × 35 mL), washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by recrystallization in 95% EtOH to afford α-thioethyl glycoside **9** as an amorphous white solid (9.92 g, 57% over three steps). ^1H NMR (300 MHz, $CDCl_3$): δ 5.43 (d, 1 H, J = 6 Hz), 5.27 (t, 1 H, J = 9.6 Hz), 4.99 (t, 1 H, J = 9.8 Hz), 4.44 (ddd, 1 H, J = 1.8, 4.5, 10.2 Hz), 4.29 (dd, 1 H, J = 4.8, 12.3 Hz), 4.05 (dd, 1 H, J = 2.1,12.3 Hz), 3.99 (dd, 1 H, J = 6, 10.5 Hz), 2.6 (m, 2 H), 2.06 (s, 6 H), 2.02 (s, 3 H), 1.30 (t, 3 H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 168.5, 82.8, 71.9, 68.5, 67.7, 61.7, 61.3, 24.5, 20.4, 14.4. IR (KBr): 2965, 2112, 1749, 1367, 1225, 1054, 1016 cm^{-1}. $[\alpha]^{20}_D = +180.2$ (c 1.04, CH_2Cl_2). HRESI-MS: *m/z* calcd for $C_{14}H_{21}N_3O_7SNa$ $[M + Na]^+$ 398.0998, found 398.1000.

Thioethyl 3-*O*-Acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (10). A solution of triacetate **9** (7.7 g, 20.5 mmol) in MeOH (60 mL) was treated with 1 M NaOMe solution in MeOH (8.2 mL, 8.2 mmol). The mixture was stirred at rt for 3 h, neutralized with activated Dowex 50X-W-H^+ ion-exchange resin, filtered, concentrated, and dried under reduced pressure. A solution of the crude triol in THF (48 mL) was treated with benzaldehyde dimethyl acetal (9.3 mL, 61.5 mmol) and $TsOH \cdot H_2O$ (390 mg, 2.05 mmol). The mixture was refluxed for 10 h, quenched with saturated $NaHCO_3$ solution (30 mL), extracted with CH_2Cl_2 (3 × 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude acetal was dissolved in pyridine (64 mL) followed by addition of Ac_2O (32 mL) at 0 °C. The mixture was stirred for 10 h at rt and then concentrated under reduced pressure. Purification by recrystallization in 95% EtOH yielded 4,6-*O*-benzylidene acetal **10** as an amorphous white solid (6.6 g, 85% over three steps). ^1H NMR (300 MHz, $CDCl_3$): δ 7.45 (m, 1 H), 7.35 (m, 1 H), 5.50 (s, 1 H), 5.46 (d, 2 H, J = 5.7 Hz), 5.46 (t, 1 H, J = 9.9 Hz), 4.36 (m, 1 H), 4.25 (dd, 1 H, J = 4.5, 9.9 Hz), 3.97 (dd, 1 H, J = 5.7, 9.9 Hz), 3.78 (t, 1 H, J = 9.9 Hz), 3.62 (t, 1 H, J = 10.2 Hz), 2.63 (m, 2 H), 2.12 (s, 3 H), 1.31 (t, 3 H, J = 7.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.6, 136.9, 129.2, 128.3, 126.2, 101.7, 84.1, 79.8, 70.6, 68.7, 63.3, 62.4, 24.9, 20.9, 14.9. IR (KBr): 2966, 2933, 2901, 2108, 1750, 1370, 1218, 1098, 969 cm^{-1}. $[\alpha]^{20}_D = +126.7$ (c 1.03, CH_2Cl_2); HRESI-MS: *m/z* calcd for $C_{17}H_{21}N_3O_5SNa$ $[M + Na]^+$ 402.1100, found 402.1103.

Thioethyl 3-*O*-Acetyl-2-azido-4-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α-D-glucopyranoside (11). Compound **10** (2.49 g, 6.55 mmol) was dissolved in a 1 M BH_3 solution in THF (66 mL) and then treated with a 1 M Bu_2BOTf solution in THF (6.88 mL) at 0 °C in the presence of 2,6-lutidine (2.29 mL, 19.66 mmol). The mixture was stirred for 10 h at 0 °C, cooled to −78 °C, and quenched with Et_3N (1.82 mL, 13.11 mmol) followed by the slow addition of cold MeOH (30 mL). The mixture was slowly warmed to rt, concentrated, and then purified by silica gel chromatography (20% EtOAc in hexanes) to yield the intermediate C6 alcohol (2.04

(54) Shevchenko, A.; Chernushevich, I.; Ens, W.; Standing, K. G.; Thomson, B.; Wilm, M.; Mann, M. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1015–1024.
(55) Wells, J. M.; Chrisman, P. A.; McLuckey, S. A. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 614–622.

g, 5.34 mmol). This was dissolved in DMF (7 mL) and treated with imidazole (1.1 g, 16.02 mmol) and TBDPS-Cl (2.78 mL, 10.68 mmol). The mixture was stirred for 12 h at rt, quenched with saturated $NaHCO_3$ solution (40 mL), extracted with CH_2Cl_2 (2 × 50 mL), washed with brine (40 mL), dried over Na_2SO_4, and then concentrated under reduced pressure. Purification by silica gel chromatography (6.25% EtOAc in hexanes) yielded C6 TBDPS ether **11** as an amorphous white solid (2.52 g, 62% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (m, 4 H), 7.45 (m, 6 H), 7.34 (m, 3 H), 7.25 (m, 2 H), 5.51 (d, 1 H, J = 6 Hz), 5.49 (t, 1 H, J = 9.75 Hz), 4.69 (m, 2 H), 4.26 (dd, 1 H, J = 1.2, 9.6 Hz), 4.35 (dd, 1 H, J = 3.6, 11.1 Hz), 3.94 (m, 2 H), 3.86 (t, 1 H, J = 9.3 Hz), 2.61 (m, 2 H), 2.08 (s, 3 H), 1.31 (t, 3 H, J = 7.7 Hz), 1.16 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 137.7, 135.8, 135.6, 133.4, 132.9, 129.8, 128.5, 127.9, 127.8, 127.6, 82.4, 76.5, 74.6, 73.6, 72.0, 62.5, 62.4, 26.9, 24.3, 20.9, 19.3, 14.6. IR (KBr): 3070, 2931, 2858, 2103, 1752, 1428, 1217, 1112 cm^{-1}. $[\alpha]^{20}_D$ = +77.6 (*c* 1.10, CH_2Cl_2). HRESI-MS: *m*/*z* calcd for $C_{33}H_{41}N_3O_5SSiNa$ [M + Na]$^+$ 642.2434, found 642.2435.

2′-Hydroxyethyl 3-*O*-Acetyl-2-azido-4-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α-D-glucopyranoside (12). Synthesis of 2′-Hydroxyethyl 2-Bromoisobutyrate. A solution of 2-bromopropionic acid (500 mg, 3.3 mmol) and ethylene glycol (1.8 mL, 33 mmol) in CH_2Cl_2 (16 mL) was treated at 0 °C with dicyclohexylcarbodiimide (DCC; 1.0 g, 4.95 mmol) and DMAP (37 mg, 0.3 mmol). The mixture was stirred for 5 h at 0 °C, quenched with saturated $NaHCO_3$ solution (20 mL), extracted with EtOAc (3 × 30 mL), washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and then concentrated under reduced pressure. Purification by silica gel chromatography (20% EtOAc in hexanes) afforded 2′-hydroxyethyl 2-bromoisobutyrate in 67% yield as a light yellow oil.

A mixture of thioglycoside **11** (1.24 g, 2 mmol) and 2′-hydroxyethyl 2-bromoisobutyrate (465 mg, 2.2 mmol) in 1:1 $(CH_2Cl)_2$/Et_2O (14 mL) was stirred with activated 4A molecular sieves for 1 h at rt under Ar. Then the mixture was cooled to 0 °C and treated with *N*-iodosuccinimide (495 mg, 2.2 mmol) and TfOH (53 μL, 0.6 mmol). The mixture was stirred continuously for another 5 h at 0 °C, quenched with Et_3N (167 μL, 1.2 mmol), warmed to rt, filtered through Celite, extracted with CH_2Cl_2 (3 × 40 mL), washed with brine (40 mL), dried over Na_2SO_4, and then concentrated under reduced pressure to yield the corresponding *O*-glycoside as a 6:1 mixture of anomers. These were separated by silica gel chromatography (10% EtOAc in hexane) to afford the major α-glycoside as a pure compound.

A solution of α-glycoside (1.20 g, 1.56 mmol) in 1:1 MeOH/THF (10 mL) was treated at −5 °C with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (233 μL, 1.56 mmol). The mixture was stirred for 15 h at −5 °C, quenched with saturated NH_4Cl solution (20 mL), extracted with CH_2Cl_2 (3 × 40 mL), washed with brine (40 mL), dried over Na_2SO_4, and then concentrated under reduced pressure. Purification by silica gel chromatography (20% EtOAc in hexanes) yielded α-glycol GlcN derivative **12** as a colorless oil (0.82 g, 66% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (m, 4 H), 7.46 (m, 6 H), 7.32 (m, 3 H), 7.22 (m, 2 H), 5.67 (dd, 1 H, J = 8.7, 10.8 Hz), 5.09 (d, 1 H, J = 3.6 Hz), 4.69 (s, 2 H), 3.89 (m, 7 H), 3.68 (m, 1 H), 3.26 (dd, 1 H, J = 3.3, 11.1 Hz), 2.73 (t, 1 H, J = 6 Hz), 2.09 (s, 3 H), 1.17 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8, 137.5, 135.6, 135.4, 133.2, 132.7, 129.6, 128.3, 127.7, 127.6, 127.5, 76.0, 74.6, 72.1, 71.6, 70.1, 62.4, 61.5, 61.4, 26.7, 20.7, 19.1. IR (KBr): 2931, 2858, 2109, 1752, 1428, 1225, 1113. 1037 cm^{-1}. $[\alpha]^{20}_D$ = +81.5 (*c* 0.69, CH_2Cl_2). HRESI-MS: *m*/*z* calcd for $C_{33}H_{41}N_3O_7SiNa$ [M + Na]$^+$ 642.2612, found 642.2607.

2′-Hydroxyethyl 2-Azido-3-*O*-acetyl-4-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α-D-glucopyranoside, Bound to Trityl PS Resin (13). Trityl-PS resin beads (1.07 g, 1.61 mmol) were suspended in dry pyridine for 5 min followed by addition of compound **12** (1.0 g, 1.61 mmol) and DMAP (79 mg, 0.645 mmol). The mixture was shaken for 3 days at 65 °C. The resin was filtered and washed with 5% diisopropylethylamine (DIPEA) in 8:1 CH_2Cl_2/MeOH (2 × 4 mL) and then alternatively with DMF (2 × 4 mL) and CH_2Cl_2 (2 × 4 mL), and then finally with additional CH_2Cl_2 (3 × 4 mL). The resin was dried under reduced pressure in the presence of KOH pellets to afford fully resin-bound α-GlcN derivative **13** with a loading ratio ranging from 0.83 mmol/g to 0.9 mmol/g. IR (KBr): 3026, 2929, 2107, 1753, 1600, 1493, 1448, 1224, 1040, 701 cm^{-1}. To determine the loading ratio, 50 mg of **13** was treated with a solution of 3% trifluoroacetic acid (TFA) in CH_2Cl_2 (3 mL). The mixture was stirred for 1 h at rt, and then the resin was filtered and washed with CH_2Cl_2 (3 × 3 mL). AcOH (3 mL) was added to the combined washings, which was then concentrated under reduced pressure to give compound **12**.

2′-Hydroxyethyl 2-Acetamido-4-*O*-benzyl-2-deoxy-α-D-glucopyranoside (1a). Op. 1: Resin-bound α-GlcN derivative **13** (40 mg, 0.022 mmol sugar) was swollen in CH_2Cl_2 (3 mL) for 5 min and then treated with Bu_3P (34 μL, 0.137 mmol). The mixture was shaken for 5 h at rt, filtered, and then subjected to standard workup conditions to give the intermediate *N*-ylide. Op. 2: The resin was treated with 95:5 DMF/H_2O (4 mL), shaken for 1 day at rt, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound primary amine. Op. 3: The resin was swollen in pyridine (3 mL) for 5 min, treated with Ac_2O (1.5 mL), and shaken for 1 day at rt. The resin was filtered, washed with 4:1 DMF/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the *N*-acetylated intermediate. Op. 4: The resin was swollen in THF (0.3 mL) for 5 min, treated with 1 M TBAF in THF (2.7 mL), and shaken for 1 day at rt. The resin was filtered, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the C6 alcohol. Op. 5: The resin was swollen in CH_2Cl_2 (2 mL) for 5 min, treated with 1 M NaOMe in MeOH (66 μL), and shaken for 5 h at rt. The resin was filtered, washed with 1:1 MeOH/CH_2Cl_2 (2 × 3 mL), and then subjected to standard workup conditions to give the fully deprotected resin-bound α-GlcN. Op. 6: The resin was treated with 3% TFA in CH_2Cl_2 (3 mL) for 1 h at rt, filtered, and washed with CH_2Cl_2 (3 × 2 mL). AcOH (3 mL) was added to the combined washings, which was then concentrated under reduced pressure at rt. Purification by reversed-phase HPLC yielded glycol-linked α-GlcN **1a** as an amorphous white solid (7 mg, 88% over six operations). ^{1}H NMR (300 MHz, CD_3OD): δ 7.32 (m, 5 H), 4.96 (d, 1 H, J = 11.1 Hz), 4.76 (d, 1 H, J = 3.9 Hz), 4.66 (d, 1 H, J = 11.1 Hz), 4.00 (dd, 1 H, J = 3.3, 10.8 Hz), 3.76 (m, 7 H), 3.47 (m, 2 H), 2.00 (s, 3 H). ^{13}C NMR (100 MHz, CD_3OD): δ 173.7, 140.0, 129.3, 129.0, 128.7, 98.9, 79.9, 76.0, 73.7, 73.0, 70.3, 62.3, 62.0, 55.5, 22.6. IR (neat): 3307, 2923, 1656, 1637, 1558, 1130, 1029 cm^{-1}. $[\alpha]^{20}_D$ = +73.4 (*c* 0.63, MeOH). HRESI-MS: *m*/*z* calcd for $C_{17}H_{25}NO_7Na$ [M + Na]$^+$ 378.1529, found 378.1532.

2′-Hydroxyethyl 2-Acetamido-4-*O*-benzyl-2-deoxy-6-*O*-sulfonato-α-D-glucopyranoside Tetrabutylammonium Salt (2a). Op. 1: Resin-bound α-GlcN derivative **13** (40 mg, 0.022 mmol sugar) was swollen in CH_2Cl_2 (3 mL) for 5 min and then treated with Bu_3P (34 μL, 0.137 mmol). The mixture was shaken for 5 h at rt, filtered, and then subjected to standard workup conditions to give the intermediate *N*-ylide. Op. 2: The resin was treated with 95:5 DMF/H_2O (4 mL), shaken for 1 day at rt, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound primary amine. Op. 3: The resin was swollen in pyridine (3 mL) for 5 min, treated with Ac_2O (1.5 mL), and shaken for 1 day at rt. The resin was filtered, washed with 4:1 DMF/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the *N*-acetylated intermediate. Op. 4: The resin was swollen in THF (0.3 mL) for 5 min, treated with 1 M TBAF in THF adjusted to pH 7 with AcOH (2.7 mL), and shaken for 1 day at rt. The resin was filtered, washed with 5:1 THF/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the C6 alcohol. Op. 5: The resin was swollen in dry DMF (3 mL) for 5 min, treated with SO_3·Py (22 mg, 0.137 mmol), and shaken for 10 h at rt. Op. 6: The resin was filtered, washed with DMF (2

× 3 mL), and then treated twice with Bu_4NHSO_4 (47 mg, 0.137 mmol) in 1:1 DMF/MeOH (2 × 2 mL, adjusted to pH 7 with 1 M Bu_4NOH in MeOH). The mixture was shaken for 3 h each time at rt. The resin was filtered, washed with 8:1 MeOH/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound 6-*O*-sulfate. Op. 7: The resin was swollen in CH_2Cl_2 (2 mL) for 5 min, treated with 1 M Bu_4NOH in MeOH (137 μL, 0.137 mmol), and shaken for 5 h at rt. The resin was filtered, washed with 1:1 MeOH/CH_2Cl_2 (2 × 3 mL), and then subjected to standard workup conditions to give the fully deprotected resin-bound 6-*O*-sulfate. Op. 8: The resin was treated with 0.3 M $BF_3 \cdot OEt_2$ in CH_2Cl_2 under standard cleavage conditions. Purification by reversed-phase HPLC yielded glycol-linked 6-*O*-sulfate **2a** as an amorphous white solid (10.5 mg, 58% over 8 operations). ^{1}H NMR (300 MHz, CD_3OD): δ 7.43 (dd, 2 H, J = 1.8, 8.4 Hz), 7.29 (m, 3 H), 4.92 (d, 2 H, J = 10.5 Hz), 4.76 (d, 2 H, J = 10.5 Hz), 4.75 (d, 1 H, J = 3.3 Hz), 4.25 (m, 2 H), 4.02 (dd, 1 H, J = 3, 10.5 Hz), 3.86 (m, 2 H), 3.78 (dt, 1 H, J = 3, 9.3 Hz), 3.7 (m, 2 H), 3.49 (m, 2 H), 3.24 (m, 8 H), 2.00 (s, 3 H), 1.66 (m, 8 H), 1.42 (m, 8 H), 1.03 (t, 12 H, J = 6.9 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 173.7, 139.9, 129.4, 129.2, 128.6, 98.9, 79.8, 76.1, 73.8, 70.9, 70.3, 67.8, 62.0, 59.5, 55.4, 24.8, 22.6, 20.7, 13.9. IR (neat): 3401, 2962, 2876, 1656, 1630, 1251, 1223, 1016 cm^{-1}. $[\alpha]^{20}_D$ = +34.4 (*c* 0.43, MeOH). HRESI-MS: *m*/*z* calcd for $C_{17}H_{24}NO_{10}S$ [M − NBu_4]$^-$ 434.1121, found 434.1124.

2′-Hydroxyethyl 2-Acetamido-4-*O*-benzyl-2-deoxy-3-*O*-sulfonato-α-D-glucopyranoside Tetrabutylammonium Salt (3a). Op. 1: Resin-bound α-GlcN derivative **13** (40 mg, 0.022 mmol sugar) was swollen in CH_2Cl_2 (3 mL) for 5 min and then treated with Bu_3P (34 μL, 0.137 mmol). The mixture was shaken for 5 h at rt, filtered, and then subjected to standard workup conditions to give the intermediate *N*-ylide. Op. 2: The resin was treated with 95:5 DMF/H_2O (4 mL), shaken for 1 day at rt, washed with THF (2 × 3 mL), then subjected to standard workup conditions to give the resin-bound primary amine. Op. 3: The resin was swollen in pyridine (3 mL) for 5 min, treated with Ac_2O (1.5 mL), and shaken for 1 day at rt. The resin was filtered, washed with 4:1 DMF/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the *N*-acetylated intermediate. Op. 4: The resin was swollen in CH_2Cl_2 (2 mL) for 5 min, treated with 1 M NaOMe in MeOH (82 μL), and shaken for 5 h at rt. The resin was filtered, washed with 1:1 MeOH/CH_2Cl_2 (2 × 3 mL), and then subjected to standard workup conditions to give the C3 alcohol. Op. 5: The resin was swollen in dry DMF (3 mL) for 5 min, treated with $SO_3 \cdot Py$ (22 mg, 0.137 mmol), and shaken for 10 h at rt. Op. 6: The resin was filtered, washed with DMF (2 × 3 mL), and then treated twice with Bu_4NHSO_4 (47 mg, 0.137 mmol) in 1:1 DMF/MeOH (2 × 2 mL, adjusted to pH 7 with 1 M Bu_4NOH in MeOH). The mixture was shaken for 3 h each time at rt. The resin was filtered, washed with 8:1 MeOH/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound 3-*O*-sulfate. Op. 7: The resin was swollen in THF (0.3 mL) for 5 min, treated with 1 M TBAF in THF (2.7 mL), and shaken for 1 day at rt. The resin was filtered, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the fully deprotected resin-bound 3-*O*-sulfate. Op. 8: The resin was treated with 0.3 M $BF_3 \cdot OEt_2$ in CH_2Cl_2 under standard cleavage conditions. Purification by reversed-phase HPLC yielded glycol-linked 3-*O*-sulfate **3a** as an amorphous white solid (9.5 mg, 53% over eight operations). ^{1}H NMR (300 MHz, CD_3OD): δ 7.44 (dd, 2 H, J = 1.5, 7.8 Hz), 7.29 (m, 3 H), 5.19 (d, 1 H, J = 10.2 Hz), 4.95 (d, 1 H, J = 3.3 Hz), 4.76 (dd, 1 H, J = 8.7, 11.1 Hz), 4.54 (d, 1 H, J = 10.5 Hz), 4.98 (dd, 1 H, J = 3.3, 11.1 Hz), 3.71 (m, 6 H), 3.56 (t, 1 H, J = 9 Hz), 3.47 (m, 1 H), 3.23 (m, 8 H), 1.99 (m, 8 H), 1.66 (m, 8 H), 1.42 (m, 8 H), 1.03 (t, 12 H, J = 7.2 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 173.7, 139.9, 129.9, 129.1, 128.6, 98.6, 79.5, 78.0, 76.0, 73.3, 70.6, 62.2, 62.0, 59.4, 55.1, 24.8, 22.9, 20.7, 13.9. IR (neat): 3413, 2962, 2876, 1662, 1630, 1263, 1217, 1128, 1038 cm^{-1}. $[\alpha]^{20}_D$ = +57.1 (*c* 0.62, MeOH). HRESI−MS: *m*/*z* calcd for $C_{17}H_{24}NO_{10}S$ [M − NBu_4]$^-$ 434.1121, found 434.1118.

2′-Hydroxyethyl 2-Amino-4-*O*-benzyl-2-deoxy-2-*N*-sulfonato-α-D-glucopyranoside Tetrabutylammonium Salt (4a). Op. 1: Resin-bound α-GlcN derivative **13** (40 mg, 0.022 mmol sugar) was swollen in CH_2Cl_2 (3 mL) for 5 min and then treated with Bu_3P (34 μL, 0.137 mmol). The mixture was shaken for 5 h at rt, filtered, and then subjected to standard workup conditions to give the intermediate *N*-ylide. Op. 2: The resin was treated with 95:5 DMF/H_2O (4 mL), shaken for 1 day at rt, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound primary amine. Op. 3: The resin was swollen in dry pyridine (3 mL) for 5 min, treated with Et_3N (0.6 mL), $SO_3 \cdot Py$ (87 mg, 0.548 mmol), and shaken for 10 h at rt. Op. 4: The resin was filtered, washed with 1:2 MeOH:pyridine (2 × 3 mL), and then treated twice with Bu_4NHSO_4 (47 mg, 0.137 mmol) in 1:1 DMF/MeOH (2 × 2 mL, adjusted to pH 7 with 1 M Bu_4NOH in MeOH). The mixture was shaken for 3 h each time at rt. The resin was filtered, washed with 8:1 MeOH/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound 2-*N*-sulfate. Op. 5: The resin was swollen in THF (0.3 mL) for 5 min, treated with 1 M TBAF in THF (2.7 mL), and shaken for 1 day at rt. The resin was filtered, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the C6 alcohol. Op. 6: The resin was swollen in CH_2Cl_2 (2 mL) for 5 min, treated with 1 M Bu_4NOH in MeOH (137 μL), and shaken for 5 h at rt. The resin was filtered, washed with 1:1 MeOH/CH_2Cl_2 (2 × 3 mL), and then subjected to standard workup conditions to give the fully deprotected resin-bound *N*-sulfate. Op. 7: The resin was treated with 0.3 M $BF_3 \cdot OEt_2$ in CH_2Cl_2 under standard cleavage conditions. Purification by reversed-phase HPLC yielded glycol-linked *N*-sulfate **4a** as an amorphous white solid (11 mg, 63% over seven operations). ^{1}H NMR (300 MHz, CD_3OD): δ 7.25−7.39 (m, 5 H), 5.12 (d, 1 H, J = 3.6 Hz), 4.98 (d, 1 H, J = 11.1 Hz), 4.64 (d, 1 H, J = 11.1 Hz), 3.63−3.84 (m, 8 H), 3.55 (m, 1 H), 3.42 (t, 1 H, J = 9 Hz), 3.24 (m, 8 H), 1.66 (m, 8 H), 1.42 (m, 8 H), 1.03 (t, 12 H, J = 7.5 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 140.1, 129.2, 129.1, 128.6, 99.1, 79.8, 75.6, 74.5, 72.5, 70.4, 62.5, 62.0, 60.0, 59.4, 24.8, 20.7, 13.9. IR (neat): 3383, 2962, 2876, 1630, 1455, 1382, 1151, 1029 cm^{-1}. $[\alpha]^{20}_D$ = +31.5 (*c* 0.41, MeOH). HRESI-MS: *m*/*z* calcd for $C_{15}H_{22}NO_9S$ [M − NBu_4]$^-$ 392.1015, found 392.1010.

2′-Hydroxyethyl 2-Acetamido-4-*O*-benzyl-2-deoxy-3,6-di-*O*-sulfonato-α-D-glucopyranoside Tetrabutylammonium Salt (5a). Op. 1: Resin-bound α-GlcN derivative **13** (40 mg, 0.022 mmol sugar) was swollen in CH_2Cl_2 (3 mL) for 5 min and then treated with Bu_3P (34 μL, 0.137 mmol). The mixture was shaken for 5 h at rt, filtered, and then subjected to standard workup conditions to give the intermediate *N*-ylide. Op. 2: The resin was treated with 95:5 DMF/H_2O (4 mL), shaken for 1 day at rt, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound primary amine. Op. 3: The resin was swollen in pyridine (3 mL) for 5 min, treated with Ac_2O (1.5 mL), and shaken for 1 day at rt. The resin was filtered, washed with 4:1 DMF/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the *N*-acetylated intermediate. Op. 4: This resin was swollen in THF (0.3 mL) for 5 min, treated with 1 M TBAF in THF (2.7 mL), and shaken for 1 day at rt. The resin was filtered, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the C6 alcohol. Op. 5: The resin was swollen in CH_2Cl_2 (2 mL) for 5 min, treated with 1 M NaOMe in MeOH (82 μL), and shaken for 5 h at rt. The resin was filtered, washed with 1:1 MeOH/CH_2Cl_2 (2 × 3 mL), and then subjected to standard workup conditions to give the corresponding 3,6-diol. Op. 6: The resin was swollen in dry pyridine (3 mL) for 5 min, treated with Et_3N (0.6 mL), $SO_3 \cdot Py$ (218 mg, 1.37 mmol), and shaken for 2 day at 55 °C. Op. 7: The resin was filtered, washed with 1:2 MeOH/pyridine (2 × 3 mL), and then treated twice with Bu_4NHSO_4 (94 mg, 0.274 mmol) in 1:1 DMF/MeOH (2 × 2 mL, adjusted to pH 7 with 1 M Bu_4NOH in MeOH). The mixture was shaken for

3 h each time at rt. The resin was filtered, washed with 8:1 MeOH/pyridine (2 × 3 mL), and then subjected to standard workup conditions. Op. 8,9: The sulfation and cation-exchange steps were repeated one more time to give the resin-bound 3,6-di-*O*-sulfate. Op. 10: The resin was cleaved with 0.3 M $BF_3 \cdot OEt_2$ in CH_2Cl_2 under standard conditions. Purification by reversed-phase HPLC yielded 3,6-di-*O*-sulfate **5a** as an amorphous white solid (11.5 mg, 42% over 10 operations). ^{1}H NMR (300 MHz, CD_3OD): δ 7.54 (dd, 2 H, J = 1.8, 8.1 Hz), 7.26 (m, 3 H), 5.12 (d, 1 H, J = 9.3 Hz), 4.94 (d, 1 H, J = 3.6 Hz), 4.76 (dd, 1 H, J = 9.3, 11.1 Hz), 4.64 (d, 1 H, J = 9.6 Hz), 4.26 (dd, 1 H, J = 4.2, 10.8 Hz), 4.22 (dd, 1 H, J = 2.4, 10.5 Hz), 3.98 (dd, 1 H, J = 3.6, 11.1 Hz), 3.90 (ddd, 1 H, J = 2.4, 4.4, 9.9 Hz), 3.59−3.80 (m, 4 H), 3.46 (m, 1 H), 3.24 (m, 16 H), 1.98 (s, 3 H), 1.66 (m, 16 H), 1.42 (m, 16 H), 1.03 (t, 24 H, J = 7.4 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 173.6, 139.7, 130.5, 129.0, 128.6, 98.6, 79.4, 77.7, 76.0, 71.2, 70.6, 67.5, 62.0, 59.4, 50.0, 24.8, 22.9, 20.7, 13.9. IR (neat): 3410, 2962, 2876, 1671, 1629, 1459, 1258, 1223, 1024 cm^{-1}; $[\alpha]^{20}_D$ = +20.8 (*c* 0.52, MeOH). HRESI-MS: *m*/*z* calcd for $C_{17}H_{23}NO_{13}S_2Na$ [M − 2NBu$_4$ + Na]$^-$ 536.0509, found 536.0505.

2′-Hydroxyethyl 2-Amino-4-*O*-benzyl-2-deoxy-2,3-di-*N*,*O*-sulfonato-α-D-glucopyranoside Tetrabutylammonium Salt (6a). Op. 1: Resin-bound α-GlcN derivative **13** (50 mg, 0.0274 mmol sugar) was swollen in CH_2Cl_2 (2 mL) for 5 min, treated with 1 M NaOMe in MeOH (82 μL), and shaken for 5 h at rt. The resin was filtered, washed with 1:1 MeOH/CH_2Cl_2 (2 × 3 mL), and then subjected to standard workup conditions to give the C3 alcohol. Op. 2: The resin was swollen in CH_2Cl_2 (3 mL) for 5 min and then treated with Bu_3P (34 μL, 0.137 mmol). The mixture was shaken for 5 h at rt, filtered, and then subjected to standard workup conditions to give the intermediate *N*-ylide. Op. 3: The resin was treated with 95:5 DMF/H_2O (4 mL), shaken for 1 day at rt, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound 2,3-amino alcohol. Op. 4: The resin was swollen in dry pyridine (3 mL) for 5 min, treated with Et_3N (0.6 mL) and $SO_3 \cdot Py$ (218 mg, 1.37 mmol), and shaken for 2 days at 55 °C. Op. 5: The resin was filtered, washed with 1:2 MeOH/pyridine (2 × 3 mL), and then treated twice with Bu_4NHSO_4 (94 mg, 0.274 mmol) in 1:1 DMF/MeOH (2 × 2 mL, adjusted to pH 7 with 1 M Bu_4NOH in MeOH). The mixture was shaken for 3 h each time at rt. The resin was filtered, washed with 8:1 MeOH/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound 2,3-di-*N*,*O*-sulfate. Op. 6: The resin was swollen in THF (0.3 mL) for 5 min, treated with 1 M TBAF in THF (2.7 mL), and shaken for 2 days at rt. The resin was filtered, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the deprotected, resin-bound 2,3-di-*N*,*O*-sulfate. Op. 7: The resin was treated with 0.3 M $BF_3 \cdot OEt_2$ in CH_2Cl_2 under standard cleavage conditions. Purification by reversed-phase HPLC yielded glycol-linked 2,3-di-*N*,*O*-sulfate **6a** as an amorphous white solid (12 mg, 46% over seven operations). ^{1}H NMR (300 MHz, CD_3OD): δ 7.44 (dd, 2 H, J = 1.8, 8.1 Hz), 7.28 (m, 3 H), 5.30 (d, 1 H, J = 3.3 Hz), 5.19 (d, 1 H, J = 10.8 Hz), 4.71 (dd, 1 H, J = 9, 11.1 Hz), 4.40 (d, 1 H, J = 10.2 Hz), 3.52−3.76 (m, 8 H), 3.47 (dd, 1 H, J = 3, 11.1 Hz), 3.24 (m, 16 H), 1.66 (m, 16 H), 1.42 (m, 16 H), 1.03 (t, 24 H, J = 7.5 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 139.9, 129.9, 129.1, 128.6, 98.9, 79.7, 78.3, 76.0, 72.9, 70.4, 62.3, 62.0, 59.4, 59.1, 24.8, 20.7, 13.9. IR (neat): 3402, 2961, 2875, 1630, 1460, 1382, 1225, 1035 cm^{-1}. $[\alpha]^{20}_D$ = +30.2 (*c* 0.58, MeOH); HRESI-MS: *m*/*z* calcd for $C_{15}H_{22}NO_{12}S_2$ [M − 2NBu$_4$ + H]$^-$ 472.0583, found 472.0585.

2′-Hydroxyethyl 2-Amino-4-*O*-benzyl-2-deoxy-2,6-di-*N*,*O*-sulfonato-α-D-glucopyranoside Tetrabutylammonium Salt (7a). Op. 1: Resin-bound α-GlcN derivative **13** (50 mg, 0.0274 mmol sugar) was swollen in THF (3 mL) for 5 min, treated with 1 M TBAF in THF (164 μL), and shaken for 10 h at rt. The resin was filtered, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the C6 alcohol. Op. 2: The resin was swollen in CH_2Cl_2 (3 mL) for 5 min and then treated with Bu_3P (34 μL, 0.137 mmol). The mixture was shaken for 5 h at rt, filtered, and then subjected to standard workup conditions to give the intermediate *N*-ylide. Op. 3: The resin was treated with 95:5 DMF/H_2O (4 mL), shaken for 1 day at rt, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound 2,6-amino alcohol. Op. 4: The resin was swollen in dry DMF (3 mL) for 5 min, treated with $SO_3 \cdot Py$ (21.8 mg, 0.137 mmol), and shaken for 10 h at rt. Op. 5: The resin was filtered, washed with DMF (2 × 3 mL), and then treated twice with Bu_4NHSO_4 (49 mg, 0.137 mmol) in 1:1 DMF/MeOH (2 × 2 mL, adjusted to pH 7 with 1 M Bu_4NOH in MeOH). The mixture was shaken for 3 h each time at rt. The resin was filtered, washed with 8:1 MeOH/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound 6-*O*-sulfate. Op. 6: The resin was swollen in dry pyridine (3 mL) for 5 min, treated with Et_3N (0.6 mL) and $SO_3 \cdot Py$ (88 mg, 0.548 mmol), and then shaken for 10 h at rt. Op. 7: The resin was filtered, washed with 1:2 MeOH/pyridine (2 × 3 mL), and then treated twice with Bu_4NHSO_4 (94 mg, 0.274 mmol) in 1:1 DMF/MeOH (2 × 2 mL, adjusted to pH 7 with 1 M Bu_4NOH in MeOH). The mixture was shaken for 3 h each time at rt. The resin was filtered, washed with 8:1 MeOH/pyridine (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound 2,6-di-*N*,*O*-sulfate. Op. 8: The resin was swollen in CH_2Cl_2 (2 mL) for 5 min, treated with 1 M Bu_4NOH in MeOH (137 μL), and shaken for 5 h at rt. The resin was filtered, washed with 1:1 MeOH/CH_2Cl_2 (2 × 3 mL) and then subjected to standard workup conditions to give the deprotected, resin-bound 2,6-di-*N*,*O*-sulfate. Op. 9: The resin was treated with 0.3 M $BF_3 \cdot OEt_2$ in CH_2Cl_2 under standard cleavage conditions. Purification by reversed-phase HPLC yielded glycol-linked 2,6-di-*N*,*O*-sulfate **7a** as an amorphous white solid (9.2 mg, 35% over nine operations). ^{1}H NMR (300 MHz, CD_3OD): δ 7.50 (d, 2 H, J = 8.1 Hz), 7.39 (m, 3 H), 5.10 (d, 1 H, J = 4.2 Hz), 4.94 (d, 1 H, J = 10.5 Hz), 4.74 (d, 1 H, J = 10.5 Hz), 4.27 (dd, 1 H, J = 2.7, 10.5 Hz), 4.20 (dd, 1 H, J = 4.8, 10.5 Hz), 3.69−3.88 (m, 5 H), 3.43−3.57 (m, 3 H), 3.24 (m, 16 H), 1.67 (m, 16 H), 1.42 (m, 16 H), 1.03 (t, 24 H, J = 7.5 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 140.0, 129.5, 129.2, 128.5, 99.1, 79.7, 75.8, 74.5, 70.5, 67.9, 62.0, 59.9, 59.5, 24.8, 20.7, 13.9. IR (neat): 3401, 2962, 2876, 1631, 1459, 1251, 1226, 1055, 1027 cm^{-1}. $[\alpha]^{20}_D$ = +26.9 (*c* 0.52, MeOH). HRESI-MS: *m*/*z* calcd for $C_{15}H_{21}NO_{12}S_2Na$ [M − 2NBu$_4$ + Na]$^-$ 494.0403, found 494.0406.

2′-Hydroxyethyl 2-Amino-4-*O*-benzyl-2-deoxy-2,3,6-tri-*N*,*O*-sulfonato-α-D-glucopyranoside Tetrabutylammonium Salt (8a). Op. 1: Resin-bound α-GlcN derivative **13** (50 mg, 0.0274 mmol sugar) was swollen in CH_2Cl_2 (2 mL) for 5 min, treated with 1 M NaOMe in MeOH (82 μL), and shaken for 5 h at rt. The resin was filtered, washed with 1:1 MeOH/CH_2Cl_2 (2 × 3 mL), and then subjected to standard workup conditions to give the C3 alcohol. Op. 2: The resin was swollen in CH_2Cl_2 (3 mL) for 5 min and then treated with Bu_3P (34 μL, 0.137 mmol). The mixture was shaken for 5 h at rt, filtered, and then subjected to standard workup conditions to give the intermediate *N*-ylide. Op. 3: The resin was treated with 95:5 DMF/H_2O (4 mL), shaken for 1 day at rt, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound 2,3-amino alcohol. Op. 4: The resin was swollen in THF (0.3 mL) for 5 min, treated with 1 M TBAF in THF (2.7 mL), and shaken for 10 h at rt. The resin was filtered, washed with THF (2 × 3 mL), and then subjected to standard workup conditions to give the resin-bound, fully deprotected α-GlcN. Op. 5: The resin was swollen in dry pyridine (3 mL) for 5 min, treated with Et_3N (0.6 mL) and $SO_3 \cdot Py$ (218 mg, 1.37 mmol), and shaken for 2 days at 55 °C. Op. 6: The resin was filtered, washed with 1:2 MeOH/pyridine (2 × 3 mL), and then treated twice with Bu_4NHSO_4 (140 mg, 0.411 mmol) in 1:1 DMF/MeOH (2 × 2 mL, adjusted to pH 7 with 1 M Bu_4NOH in MeOH). The mixture was shaken for 3 h each time at rt. The resin was filtered, washed with 8:1 MeOH/pyridine (2 × 3 mL), and then subjected to standard workup conditions. Op. 7,8: The sulfation

and cation-exchange steps were repeated one more time to yield the resin-bound, 2,3,6-tri-*N*,*O*-sulfate. Op. 9: The resin was treated with 0.3 M $BF_3 \cdot OEt_2$ in CH_2Cl_2 under standard conditions. Purification by reversed-phase HPLC yielded glycol-linked 2,3,6-tri-*N*,*O*-sulfate **8a** as an amorphous white solid (13.3 mg, 38% over nine operations). ^{1}H NMR (300 MHz, CD_3OD): δ 7.54 (dd, 2 H, J = 1.2, 8.1 Hz), 7.26 (m, 3 H), 5.27 (d, 1 H, J = 3.3 Hz), 5.16 (d, 1 H, J = 9.9 Hz), 4.71 (d, 1 H, J = 10.8 Hz), 4.66 (t, 1 H, J = 9.3 Hz), 4.26 (dd, 1 H, J = 3.9, 10.5 Hz), 4.20 (dd, 1 H, J = 2.1, 10.2 Hz), 3.84 (ddd, 1 H, J = 2.4, 3.9, 9.6 Hz), 3.53−3.80 (m, 5 H), 3.47 (dd, 1 H, J = 3.3, 10.8 Hz), 3.24 (m, 24 H), 1.66 (m, 24 H), 1.42 (m, 24 H), 1.03 (t, 36 H, J = 7.5 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 139.8, 130.5, 129.0, 128.5, 98.9, 79.6, 78.0, 76.0, 70.9, 70.4, 67.6, 61.9, 59.5, 59.1, 24.8, 20.7, 13.9. IR (neat): 3401, 2962, 2875, 1489, 1460, 1257, 1227, 1035 cm^{-1}. $[\alpha]^{20}_D$ = +22.0 (*c* 0.65, MeOH). HRESI-MS: *m*/*z* calcd for $C_{15}H_{20}NO_{15}S_3Na_2$ [M − $3NBu_4$ + 2Na]$^-$ 595.9791, found 595.9795.

2′-Hydroxyethyl 2-Acetamido-2-deoxy-α-D-glucopyranoside (1b). Compound **1a** (7 mg) was dissolved in MeOH (3 mL), treated with $Pd(OH)_2$ on charcoal (5 mg), and then stirred under positive H_2 pressure at rt for 20 h. The reaction mixture was filtered to remove catalyst, concentrated, and purified by reversed-phase HPLC to yield **1b** as an amorphous white solid (5.5 mg, 90%). ^{1}H NMR (500 MHz, D_2O): δ 4.85 (d, 1 H, J = 3.6 Hz), 3.92 (dd, 1 H, J = 3.6, 10.6 Hz), 3.85 (dd, 1 H, J = 2.0, 12.0 Hz), 3.69−3.80 (m, 6H), 3.53 (m, 1H), 3.46 (t, 1H, J = 9.5 Hz). ^{13}C NMR (125 MHz, D_2O): δ 174.4, 97.1, 71.8, 71.1, 69.9, 68.8, 60.5, 60.4, 53.5, 21.8. $[\alpha]^{20}_D$ = +75.7 (*c* 0.46, MeOH). HRESI-MS: *m*/*z* calcd for $C_{10}H_{19}NO_7Na$ [M + Na]$^+$ 288.1059, found 288.1061.

2′-Hydroxyethyl 2-Acetamido-2-deoxy-6-*O*-sulfonato-α-D-glucopyranoside Sodium Salt (2b). Compound **2a** (6.2 mg) was dissolved in MeOH, subjected to the hydrogenation conditions described above, and then filtered and loaded onto a column packed with a cation-exchange resin (Dowex Marathon MSC, Na form) and eluted with MeOH. The fractions were concentrated and purified by reversed-phase HPLC to yield **2b** as an amorphous white solid (2.6 mg, 82%). ^{1}H NMR (500 MHz, CD_3OD): δ 4.72 (d, 1 H, J = 3.5 Hz), 4.29 (dd, 1 H, J = 1.8, 10.8 Hz), 4.11 (dd, 1 H, J = 6.1, 10.9 Hz), 3.95 (dd, 1 H, J = 3.6, 10.6 Hz), 3.80 (m, 2H), 3.70 (m, 2H), 3.64 (dd, 1 H, J = 9.0, 10.4 Hz), 3.44 (ddd, 1 H, J = 3.7, 7.1, 10.8 Hz), 3.36 (t, 1H, J = 9.7 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 172.1, 97.4, 71.6, 70.6, 70.4, 68.7, 66.9, 60.6, 53.6, 21.1. $[\alpha]^{20}_D$ = +81.9 (*c* 0.17, MeOH). HRESI-MS: *m*/*z* calcd for $C_{10}H_{18}NO_{10}S$ [M − Na]$^-$ 344.0651, found 344.0648.

2′-Hydroxyethyl 2-Acetamido-2-deoxy-3-*O*-sulfonato-α-D-glucopyranoside Sodium Salt (3b). Compound **3a** (5.1 mg) was dissolved in MeOH, subjected to the hydrogenation conditions and ion-exchange chromatography described above, and then concentrated and purified by reversed-phase HPLC to yield **3b** as an amorphous white solid (2.1 mg, 81%). ^{1}H NMR (500 MHz, CD_3OD): δ 4.84 (d, 1 H, J = 3.5 Hz), 4.50 (dd, 1 H, J = 8.7, 10.7 Hz), 4.02 (dd, 1 H, J = 3.5, 10.7 Hz), 3.75−3.80 (m, 2H), 3.62−3.72 (m, 4H), 3.57 (t, 1H, J = 8.9 Hz), 3.47 (ddd, 1 H, J = 3.5, 6.8, 10.7 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 172.1, 97.3, 78.4, 72.4, 69.6, 69.0, 60.9, 60.5, 52.1, 21.2. $[\alpha]^{20}_D$ = +96.4 (*c* 0.14, MeOH). HRESI-MS: *m*/*z* calcd for $C_{10}H_{18}NO_{10}S$ [M − Na]$^-$ 344.0651, found 344.0652.

2′-Hydroxyethyl 2-Amino-2-deoxy-2-*N*-sulfonato-α-D-glucopyranoside Sodium Salt (4b). Compound **4a** (3.8 mg) was dissolved in MeOH, subjected to the hydrogenation conditions and ion-exchange chromatography described above, and then concentrated and purified by reversed-phase HPLC to yield **4b** as an amorphous white solid (1.4 mg, 78%). ^{1}H NMR (500 MHz, CD_3OD): δ 3.78 (dd, 1 H, J = 2.3, 11.8 Hz), 3.75 (m, 1H), 3.68−3.72 (m, 1H), 3.65 (dd, 1 H, J = 5.8, 11.8 Hz), 3.58 (dd, 1 H, J = 8.8, 10.2 Hz), 3.51−3.57 (m, 2H), 3.33 (m, 2H), 3.22 (dd, 1 H, J = 3.7, 10.3 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 97.6, 72.4, 71.9, 70.7, 68.9, 61.3, 60.6, 58.2. $[\alpha]^{20}_D$ = +131.8 (*c* 0.09, MeOH). HRESI-MS: *m*/*z* calcd for $C_8H_{16}NO_9S$ [M − Na]$^-$ 302.0546, found 302.0548.

2′-Hydroxyethyl 2-Acetamido-2-deoxy-3,6-di-*O*-sulfonato-α-D-glucopyranoside Sodium Salt (5b). A mixture of **5a** (5.7 mg) was dissolved in MeOH, subjected to the hydrogenation conditions and ion-exchange chromatography described above, and then concentrated and purified by reversed-phase HPLC to yield **5b** as an amorphous white solid (2.1 mg, 82%). ^{1}H NMR (500 MHz, CD_3OD): δ 4.83 (d, 1 H, J = 3.4 Hz), 4.50 (dd, 1 H, J = 8.8, 10.7 Hz), 4.34 (dd, 1 H, J = 1.9, 10.8 Hz), 4.10 (dd, 1 H, J = 6.5, 10.1 Hz), 4.03 (dd, 1 H, J = 3.5, 10.7 Hz), 3.91 (ddd, 1 H, J = 1.8, 6.5, 11.7 Hz), 3.81 (ddd, 1H, J = 3.1, 4.8, 10.6 Hz), 3.36−3.78 (m, 2H), 3.54 (dd, 1H, J = 9.0, 9.8 Hz), 3.47 (ddd, 1H, J = 3.4, 7.2, 8.8 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 173.6, 98.7, 79.9, 71.9, 71.2, 70.5, 68.3, 62.1, 53.7, 22.8. $[\alpha]^{20}_D$ = +77.1 (*c* 0.14, MeOH). HRESI-MS: *m*/*z* calcd for $C_{10}H_{17}NO_{13}S_2Na$ [M − Na]$^-$ 446.0039, found 446.0042.

2′-Hydroxyethyl 2-Amino-2-deoxy-2,3-di-*N*,*O*-sulfonato-α-D-glucopyranoside Sodium Salt (6b). A mixture of **6a** (3.1 mg) was dissolved in MeOH, subjected to the hydrogenation conditions and ion-exchange chromatography described above, and then concentrated and purified by reversed-phase HPLC to yield **6b** as an amorphous white solid (0.8 mg, 62%). ^{1}H NMR (500 MHz, CD_3OD): δ 5.27 (d, 1 H, J = 3.4 Hz), 4.43 (dd, 1 H, J = 8.6, 10.6 Hz), 3.78 (m, 2H), 3.65−3.72 (m, 4H), 3.58 (m, 2H), 3.39 (dd, 1 H, J = 3.4, 10.5 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 97.3, 78.5, 72.1, 69.7, 68.9, 61.0, 60.5, 56.5. $[\alpha]^{20}_D$ = +65.6 (*c* 0.05, MeOH). HRESI-MS: *m*/*z* calcd for $C_8H_{15}NO_{12}S_2Na$ [M − Na]$^-$ 403.9933, found 403.9931.

2′-Hydroxyethyl 2-Amino-2-deoxy-2,6-di-*N*,*O*-sulfonato-α-D-glucopyranoside Sodium Salt (7b). A mixture of **7a** (6.9 mg) was dissolved in MeOH, subjected to the hydrogenation conditions and ion-exchange chromatography described above, and then concentrated and purified by reversed-phase HPLC to yield **7b** as an amorphous white solid (1.9 mg, 65%). ^{1}H NMR (500 MHz, CD_3OD): δ 5.07 (d, 1 H, J = 3.6 Hz), 4.27 (dd, 1 H, J = 2.0, 10.9 Hz), 4.12 (dd, 1 H, J = 5.9, 10.9 Hz), 3.79 (m, 2H), 3.71 (m, 2H), 3.59 (dd, 1 H, J = 9.1, 10.2 Hz), 3.52 (ddd, 1 H, J = 4.1, 6.2, 10.2 Hz), 3.25 (dd, 1 H, J = 3.5, 10.2 Hz). ^{13}C NMR (125 MHz, D_2O): δ 95.8, 69.7, 68.1, 67.6, 65.5, 59.0, 56.1. $[\alpha]^{20}_D$ = +31.6 (*c* 0.13, MeOH). HRESI-MS: *m*/*z* calcd for $C_8H_{15}NO_{12}S_2Na$ [M − Na]$^-$ 403.9933, found 403.9940.

2′-Hydroxyethyl 2-Amino-2-deoxy-2,3,6-tri-*N*,*O*-sulfonato-α-D-glucopyranoside Sodium Salt (8b). A mixture of **8a** (9.8 mg) was dissolved in MeOH, subjected to the hydrogenation conditions and ion-exchange chromatography described above, and then concentrated and purified by reversed-phase HPLC to yield **8b** as an amorphous white solid (3 mg, 81%). ^{1}H NMR (500 MHz, CD_3OD): δ 5.23 (d, 1 H, J = 3.4 Hz), 4.42 (dd, 1 H, J = 8.8, 10.6 Hz), 4.33 (dd, 1 H, J = 1.9, 10.7 Hz), 4.10 (dd, 1 H, J = 6.3, 10.7 Hz), 3.88 (m, 1H), 3.80 (m, 1H), 3.72 (dd, 2 H, J = 3.8, 6.2 Hz), 3.54 (m, 1H), 3.41 (dd, 1 H, J = 3.4, 10.6 Hz. ^{13}C NMR (125 MHz, CD_3OD): δ 97.2, 78.3, 70.1, 69.6, 68.9, 66.9, 60.4, 56.6. $[\alpha]^{20}_D$ = +23.5 (*c* 0.2, MeOH). HRESI-MS: *m*/*z* calcd for $C_8H_{16}NO_{15}S_3$ [M − 3Na + 2H]$^-$ 461.9682, found 461.9670.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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