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Orthogonal Sulfation Strategy for Synthetic Heparan Sulfate Ligands

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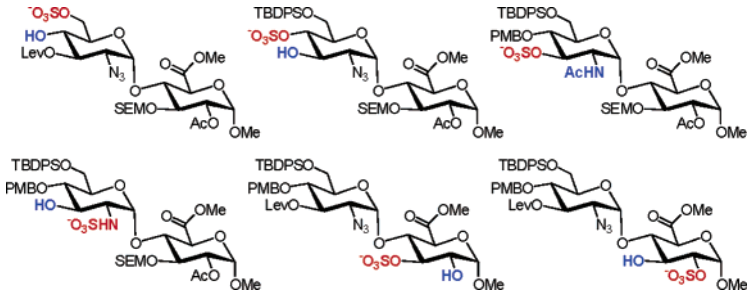
ABSTRACT



An orthogonal sulfation strategy involving six different protecting groups has been developed for generating sulfated carbohydrate libraries based on heparan. Chemoselective cleavage conditions (optimized for a heparan disaccharide) can be performed in the presence of sulfate esters as well as the remaining protecting groups.

The carbohydrate ligands referred to as heparan sulfate (HS) are a subclass of anionic linear polysaccharides known as the glycosaminoglycans.[1] HS sequences are based on repeating-unit disaccharides comprised of glucosamines linked α1→4 to pyranosyluronic acids and thus structurally related to heparin, but differ in that they are typically expressed on cell surfaces and tethered to core membrane proteins.[2] HS ligands are well-known to recruit signaling proteins such as growth factors and chemokines and are directly implicated in the activation of cell-surface receptors with key roles in vascular development and immunological response.[3] HS recognition can also be exploited pathogenically: many viral coat proteins have high affinity for heparin, and tumor cells can hijack HS-mediated signaling pathways to facilitate growth and metastatic invasion.[4,5]

The diversity of biological signaling events which rely on HS recognition is matched by the structural complexity of the sequences themselves. While the parent heparan polysaccharide is comprised of regularly repeating units of *N*-acetyl-α-D-glucosamine and *β*-D-glucuronic acid (α-D-GlcNAc and *β*-D-GlcA), several biosynthetic modifications occur in stages within localized domains, often with variable order and conversion (50−80% for any given step) to produce a prodigious number of possible stereoisomers and sulfation patterns.[6,7] These structurally complex segments are thought to be responsible for most of the biological activity in HS. Although well over 100 HS-binding proteins have been identified so far,[7] the great majority of these have yet to be matched with high-affinity ligands due to chal-

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(3) (a) Arenberg, D. A.; Polverini, P. J.; Kunkel, S. L.; Shanafelt, A.; Strieter, R. M. In *Methods in Enzymology*; Horuk, R., Ed.; Academic Press: San Diego, 1997; Vol. 288, pp 190−220. (b) Lever, R.; Page, C. P. *Nature Rev.* **2002**, *1*, 140−148.
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(5) Sasisekharan, R.; Schriver, Z.; Venkataraman, G.; Narayanasami, U. *Nature Rev.* **2002**, *2*, 521−528.
(6) Gallagher, J. T. *Biochem. Soc. Trans.* **1997**, *25*, 1206−1209.
(7) Conrad, H. E. *Heparin-Binding Proteins*; Academic Press: San Diego, 1998.

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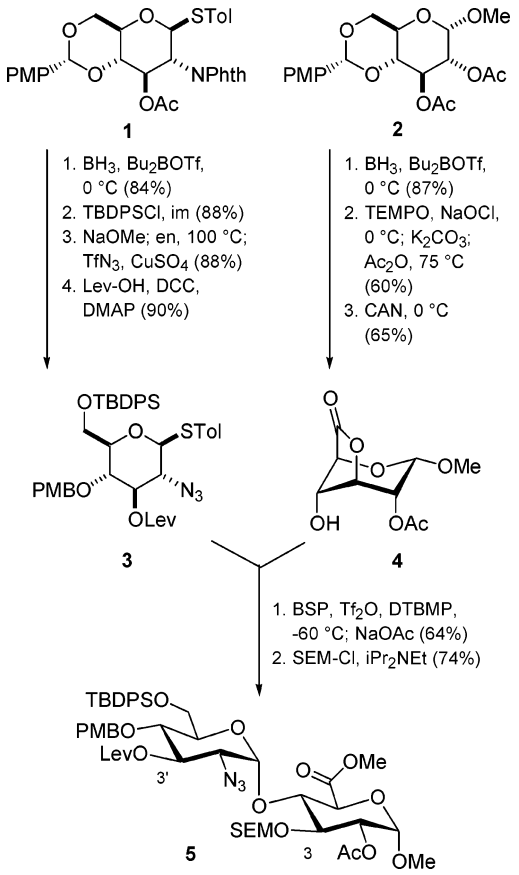
lenges in the isolation and characterization of HS sequences.[8,9]

The discovery of biologically active ligands may be accelerated by the synthesis and screening of HS-like oligosaccharides with variable sulfation profiles. Most synthetic efforts related to HS have been focused on the oligomerization of protected carbohydrate units with predesignated sulfation sites,[10,11] whereas less attention has been paid toward orthogonal protecting group systems which can be used to produce diverse sulfation patterns.[12] Both approaches have merit and are in fact quite complementary, but the challenge of the latter increases rapidly with the number of differentiable sites. To date, a focused library of eight chondroitin sulfate disaccharides has been produced,[13] and a heparan disaccharide with up to four orthogonal protecting groups has been recently reported.[12] These encouraging achievements set the stage for developing sulfation patterns of greater complexity.

Here we demonstrate an orthogonal sulfation strategy using a heparan disaccharide unit with six different protecting groups and a set of cleavage conditions that are also compatible with neighboring *O*-sulfate esters. The chemoselectivity of these conditions is demonstrated by preparing a subset of six disaccharide monosulfates, followed by deprotection of a neighboring hydroxyl group or conversion of azide to NHAc. The synthetic strategy described here is intended to enable the generation of sulfated oligosaccharide libraries derived from a common intermediate. This includes access to sulfation patterns not observed in isolated HS fragments, such as those featuring a 3-*O*-sulfate on the uronic acid moiety.[7]

Thioglycoside **1** (available in multigram quantities from D-glucosamine)[14] was converted to orthogonally protected derivative **3** by reductive cleavage of the *p*-anisylidene acetal to the 4-*O*-*p*-methoxybenzyl (PMB) ether using borane and Bu_2BOTf,[15] followed by protection of the C6 hydroxyl as a *tert*-butyldiphenylsilyl (TBDPS) ether, replacement of the phthalimide group with an azide by Cu-mediated diazo transfer onto the free amine,[16] and protection of the C3 hydroxyl as a levulinate (Lev) ester (see Scheme 1).

Scheme 1. Synthesis of Orthogonally Protected Heparan Disaccharide **5**[a]



[a] Selected abbreviations: BSP = benzenesulfinylpiperidine; DTBMP = di-*t*-Bu-4-methylpyridine; en = ethylenediamine; im = imidazole; PMP = *p*-methoxyphenyl.

Methyl D-glucoside derivative **2** was transformed into a bicyclic lactone via reductive cleavage to the 4-*O*-PMB ether, followed by tetramethyl-1-piperidineoxy (TEMPO)-mediated oxidation[17] and lactonization to the [3.2.1] isomer of 3,6-glucuronolactone.[18] Removal of the PMB group by ceric ammonium nitrate (CAN) produced glycosyl acceptor **4**, which was coupled with thioglycoside **3** using benzenesulfi-

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(9) (a) Venkataraman, G.; Shriver, Z.; Raman, R.; Sasisekharan, R. *Science* **1999**, *286*, 537−542. (b) Keiser, N.; Venkataraman, G.; Shriver, Z.; Sasisekharan, R. *Nature Med.* **2001**, *7*, 123−128. (c) Liu, J.; Shriver, Z.; Pope, R. M.; Throp, S. C.; Duncan, M. B.; Copeland, R. J.; Raska, C. S.; Yoshida, K.; Eisenberg, R. J.; Cohen, G.; Linhardt, R. J.; Sasisekharan, R. *J. Biol. Chem.* **2002**, *277*, 33456−33467.

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(12) Recent examples of orthogonally protected HS fragments: (a) Haller, M. F.; Boons, G.-J. *Eur. J. Org. Chem.* **2002**, 2033−2038. (b) Prabhu, A.; Venot, A.; Boons, G.-J. *Org. Lett.* **2003**, *5*, 4975−4978.

(13) Lubineau, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **1999**, *2523*, 3−2532.

(14) Hernández-Torres, J. M.; Liew, S.-T.; Achkar, J.; Wei, A. *Synthesis* **2002**, 487−490.

(15) (a) Jiang, L.; Chan, T.-H. *Tetrahedron Lett.* **1998**, *39*, 355−358. (b) Hernández-Torres, J. M.; Achkar, J.; Wei, A. *J. Org. Chem.* **2004**, *69*, 7206−7211.

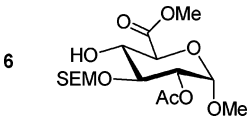
(16) (a) Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029−6033. (b) Liew, S.-T.; Wei, A. *Carbohydr. Res.* **2002**, *337*, 1319−1324.

(17) (a) Boulineau, F. P.; Wei, A. *Org. Lett.* **2002**, *4*, 2281−2283. (b) Boulineau, F. P.; Wei, A. *Org. Lett.* **2004**, *6*, 119−121.

(18) (a) Kornilov, A. V.; Sherman, A. A.; Kononov, L. O.; Shashkov, A. S.; Nifantiev, N. E. *Carbohydr. Res.* **2000**, *329*, 717−730. (b) Kornilov, A. V.; Sukhova, E. V.; Nifantiev, N. E. *Carbohydr. Res.* **2001**, *336*, 309−313.

nyl piperidine (BSP) and Tf_2O as activating agents.[11e,19] Employing a Lev group at the C3 position of the glycosyl donor **3** created some obstacles in glycosidic coupling, due to its reactivity with Tf_2O and its proximity to the activated anomeric center.[20,21] Nevertheless, the desired α-linked disaccharide could be obtained in good yield by inverting the order of reagent addition (activating BSP with Tf_2O prior to the addition of glycosyl donor) and by maintaining the reaction temperature below −55 °C. Methanolysis of the disaccharide lactone produced the corresponding methyl ester in 64% isolated yield over two steps. The free C3 hydroxyl was subsequently protected as the 2-trimethylsilylethoxymethyl (SEM) ether, resulting in orthogonally protected heparan disaccharide **5**.

The use of bicyclic lactone **4** as glycosyl acceptor is noteworthy in several respects. First, the $^1C_4$ conformation forces the C4 hydroxyl into an axial configuration with relatively low steric encumbrance, a geometry known to be favorable for α-glycosidic couplings in related systems.[22] Performing the coupling of **3** with GlcA derivative **6** under identical conditions resulted in low yields of **5** (ca. 25%) and required a tedious separation from unreacted glycosyl acceptor. Second, the lactone ring opening after glycosidic coupling provides greater flexibility in the choice of O3 protecting group at a late stage. Third, the carboxyl group can be readily converted into a variety of esters or other acyl derivatives, introducing a seventh protecting group. For example, SiO_2-mediated hydrolysis of the disaccharide lactone yielded the free acid **7** in 60% yield, followed by alkylation under biphasic conditions to produce benzyl ester **8** in 42% overall yield from **3** (See Scheme 2).[23] For the purposes of our study, we opted to constrain our investigations to the six hydroxyl and amine protecting groups featured in methyl ester **5**.



Orthogonal deprotection conditions were developed for converting disaccharide **5** to mono-*O*-sulfate esters **9**−**13** (see Tables 1 and 2).[24] In addition to the obvious need for chemoselectivity, several other factors were considered in the placement and cleavage of each protecting group. First, the deprotection conditions should also be applicable toward the generation of sulfated oligosaccharide libraries immobilized on solid-phase supports. Second, the *β*-GlcA linkage in HS suggests the placement of an acyl protecting group for the C2 hydroxyl, as their utility to assist *β*-glycosidation is well-known. Third, we wished to take advantage of orthogonal cleavage conditions which had already been developed for protecting groups with similar reactivities.[25,26] However, it must be noted that their relative stabilities are also dependent on their position, a frequent observation in the regioselective formation and cleavage of carbohydrate protecting groups.[27] For example, switching the positions of Lev (C3′) and Ac (C2) in **5** resulted in a loss of chemoselectivity during methanolysis due to the greater lability of acyl groups at the C2 position.

Scheme 2

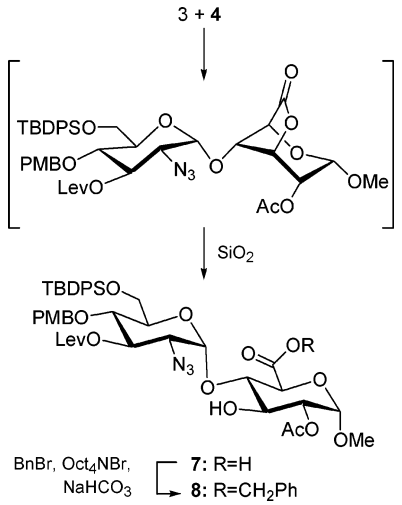
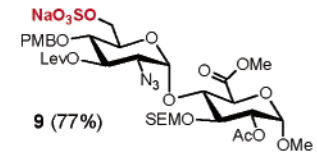
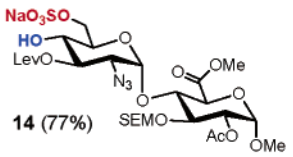
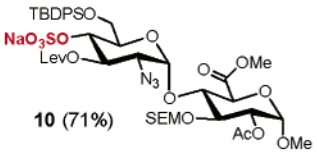
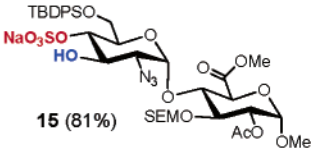
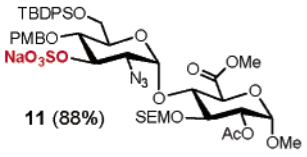
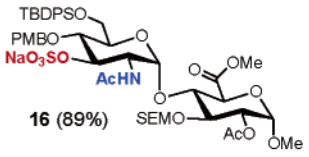
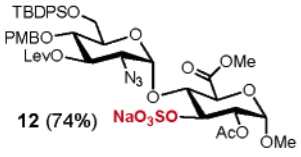
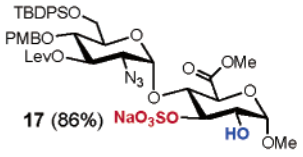
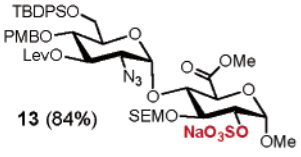
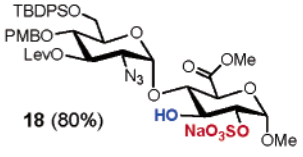
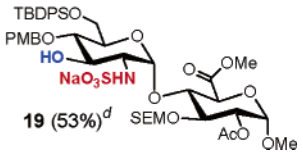


Table 1. Reagents and Conditions for Orthogonal Deprotection of **5**

group	deprotection conditions
TBDPS	TBAF (30 equiv) in THF, adjusted to pH 10, 1 day, rt
PMB	CAN in 90% aq CH_3CN (3 equiv), 12 h, 0 °C
Lev	$N_2H_4{\cdot}H_2O$ (10 equiv) in 3:2 pyridine/AcOH, 6 h, rt
SEM	$MgBr_2{\cdot}Et_2O$ (10 equiv), CH_3NO_2 (20 equiv), Et_2O, 6 h, rt
Ac	$Mg(OMe)_2$ (15 equiv) in 1:1 MeOH/CH_2Cl_2, 12 h, rt
N_3	(to −NHAc) AcSH (40 equiv), pyridine, 44 h, rt
	(to $-NH_2$) Bu_3P (1.5 equiv) in CH_2Cl_2, 5 h then 1:1 H_2O/CH_2Cl_2, 12 h, rt

(19) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015−9020.
(20) Boons and co-workers have shown that the Lev groups at the C3 position on glycosyl acceptors or at the C2 position of L-Ido donors are compatible with glycosidic coupling. See ref 12.
(21) Recent evidence by Woerpel and co-workers suggests that electronegative substituents on tetrahydropyrylium ions prefer to adopt pseudoaxial orientations: (a) Ayala, L.; Lucero, C. G.; Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2003**, *125*, 15521−15528. (b) Chamberland, S.; Ziller, J. W.; Woerpel, K. A. *J. Am. Chem. Soc.* **2005**, *127*, 5322−5323.
(22) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2128−2131.
(23) Bocchi, V.; Casnati, G.; Dossena, A.; Marchelli, R. *Synthesis* **1979**, 957−960.
(24) *O*-Sulfate esters were prepared by treating partially deprotected disaccharides with $SO_3{\cdot}Me_3N$ in pyridine for 20 h at 55 °C. See the Supporting Information for details.
(25) (a) Greene, T. W.; Wuts, P. G. M. *Protecting Groups in Organic Synthesis*, 3rd ed.; John Wiley: New York, 1999. (b) Kocienski, P. J. *Protecting Groups*, 3rd ed.; Thieme: Stuttgart, 2004.
(26) Xu, Y.-C.; Bizuneh, A.; Walker, C. *Tetrahedron Lett.* **1996**, *37*, 455−458.
(27) Selected reviews: (a) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643−663. (b) Stanek, J. *Top. Curr. Chem.* **1990**, *154*, 209−256.

Table 2. Heparan Disaccharide Monosulfates Derived from **5**[a]

1st deprotection and sulfation[b]	2nd deprotection[c]
 **9** (77%)	 **14** (77%)
 **10** (71%)	 **15** (81%)
 **11** (88%)	 **16** (89%)
 **12** (74%)	 **17** (86%)
 **13** (84%)	 **18** (80%)
	 **19** (53%)[d]

[a] Deprotection and sulfation conditons are described in Table 1 and ref 24. [b] Isolated yields over two steps. [c] Isolated yields after second deprotection. [d] Isolated yield over three steps from **5**.

To achieve full orthogonality, each deprotection condition must also be compatible with *O*-sulfate esters already present on the carbohydrate. While several cleavage reactions have been shown to be compatible with *O*-sulfates,[13] to the best of our knowledge the stability of sulfate esters under multiple deprotection conditions has not been studied methodically. We chose to address this issue by removing protecting groups adjacent to the *O*-sulfate esters in compounds **9**−**13** using the conditions listed in Table 1. These cleavage reactions proceeded smoothly to afford the disaccharide monosulfates **14**−**18** in high yields (see Table 2). In the case of 3′-*O*-sulfate **11**, the azide was converted directly to *N*-acetyl disaccharide **16** by addition of thioacetic acid.[12a,28] Last, *N*-sulfate disaccharide **19** was prepared from **5** by first removing the Lev group followed by Bu_3P reduction of the azide and hydrolysis and then selective *N*-sulfation using $PhOSO_2Cl$ in CH_2Cl_2.[29] It is worth mentioning that compound **19** could be purified by silica gel chromatography in 75% isolated yield. Chemoselective *N*-sulfation is typically used as the final step in HS oligosaccharide synthesis because of the product's sensitivity to aqueous acid, but the stability of *N*-sulfates in organic solvents may be significantly higher and warrants further study.

The orthogonal deprotection-sulfation strategy presented here demonstrates that this approach is capable of generating diverse sulfation profiles from a common synthetic heparan precursor. Further implementation will require its adaptation to oligosaccharides on solid-phase supports, so that fully deprotected HS ligands can be prepared with minimal attrition.

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Supporting Information Available: Experimental details on the synthesis and spectroscopic characterization of compounds **3**−**5** and **8**−**19** and ^1H and ^{13}C NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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