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Use of Cerny Epoxides for the Accelerated Synthesis of Glycosaminoglycans

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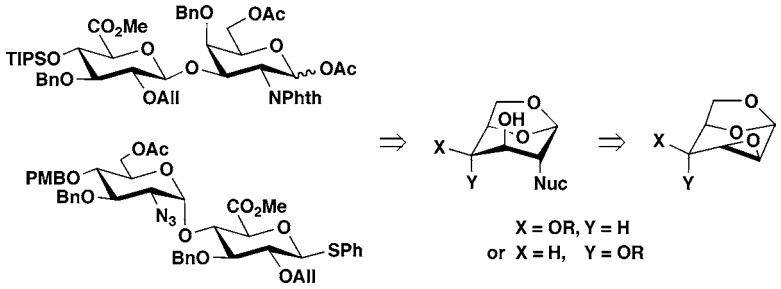
ABSTRACT



1,6:2,3-Dianhydrohexopyranoses (Cerny epoxides) are versatile intermediates for the synthesis of glycosaminoglycans. Complex heparan and chondroitin sulfate disaccharide synthons can be assembled from a single common precursor in a short sequence of steps.

Glycosaminoglycans (GAGs) are sulfated polysaccharides that modulate a wide range of physiological processes, including viral invasion, blood coagulation, and cell growth.[1] Assembled from repeating disaccharide motifs, GAGs display considerable variation in their stereochemistry and patterns of sulfation (Figure 1). Although this chemical diversity enables GAGs to interact selectively with their protein targets,[1a,2] it has also hampered efforts to isolate active sequences and to elucidate structure−activity relationships.

Rapid and efficient methods for the preparation of GAGs[3−5] are critical to advance our understanding of this important class of compounds. Synthetic approaches should ideally provide ready access to different classes (i.e., heparan, chondroitin sulfate), as well as to the variable sulfation patterns found in the native compounds. Herein we report the use of 1,6:2,3-dianhydrohexopyranoses (Cerny epoxides[6]) as versatile intermediates for the rapid assembly of heparan and chondroitin sulfate synthons. We anticipate that this approach, which is notable for its convergence and efficiency, will accelerate the generation of diverse GAG libraries.

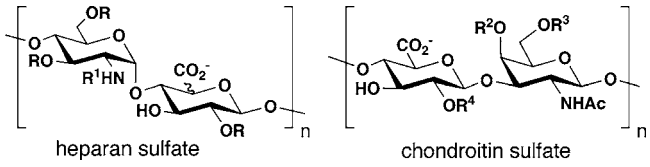


Figure 1. Structures of heparan and chondroitin sulfate, two classes of glycosaminoglycans. For heparan sulfate (HS) R = H or SO_3^-; R^1 = Ac, H, or SO_3^-; n = 20−200. For chondroitin sulfate motif C (CS−C) R^2, R^4 = H; R^3 = SO_3^-; for motif D (CS-D) R^2 = H; R^3, R^4 = SO_3^-.

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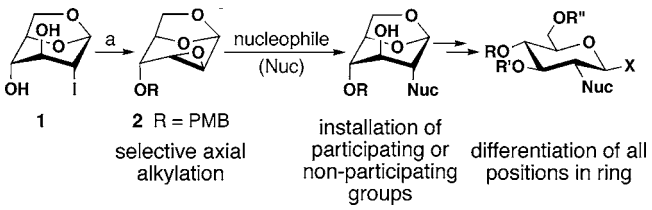
Two major challenges associated with the synthesis of GAGs are stereocontrol in the glycoside bond-forming reaction and differentiation among hydroxyl groups of comparable reactivity to access various sulfation patterns. We recognized that Cerny epoxides could be exploited to accomplish both objectives (Scheme 1). Alkylation of the

Scheme 1. Strategy for the Synthesis of Glycosaminoglycans[a]



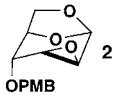
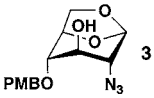
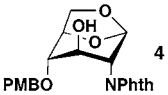
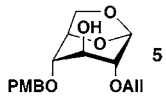
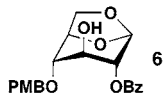
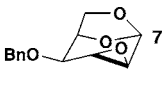
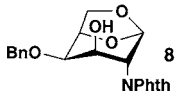
[a] Conditions: (a) NaH, PMBCl, DMF (75%).

axial C-4 hydroxyl followed by regioselective opening of the epoxide ring installs participating or non-participating groups at the C-2 position, thereby facilitating stereoselective glycosylation. Protection of the C-3 hydroxyl and opening of the 1,6-anhydro ring then delivers a diverse set of building blocks with each position of the ring differentiated. Importantly, this strategy permits rapid and efficient access to diverse GAG structures from a common precursor.

Although first reported in the 1970s, Cerny epoxides have not been widely utilized in the synthesis of GAGs. Their application has likely been limited by their tendency to undergo isomerization under alkaline conditions and by lengthy methods for their preparation.[6,7] We reasoned that the challenges associated with Cerny epoxides might be overcome through kinetic control. Accordingly, we generated iodide **1** via the one-step oxidative 1,6-iodocyclization of D-glucal according to the published procedure,[8] and much to our delight, exposure of **1** to NaH and *p*-methoxybenzyl chloride in DMF produced epoxide **2** in 75% yield with the desired regiocontrol (Scheme 1). As anticipated, the unfavorable equilibrium between the *altro* and *manno* isomers of the epoxide[9] was overcome through kinetic trapping of the desired *manno* isomer. The one-pot procedure simultaneously allowed for the introduction of a protecting group at the C-4 position. We chose the PMB group because it can withstand the conditions required for epoxide ring opening but is also orthogonal to a wide range of other protecting groups, therefore facilitating the synthesis of more complex oligosaccharides. This rapid, two-step sequence to **2** from D-glucal is a notable improvement over existing methods.

We next investigated the reactivity of trapped epoxide **2** toward a variety of nucleophiles (Table 1). In accordance

Table 1. Installation of Participating and Non-participating Groups

entry	epoxide	product	% yield
1	 **2**	 **3** C2 non-participating	76
2		 **4** C2 participating	45
3		 **5** C2 non-participating	93
4		 **6** C2 participating	73
5	 **7**	 **8**	63

with earlier reports,[6,10,11] treatment with both nitrogen- and oxygen-based nucleophiles afforded exclusively the *trans*-diaxial ring-opened forms. For instance, entry into 2-azido-2-deoxypyranoses was accomplished by reaction of **2** with NaN_3 in DMF/water[10] to afford **3**. Alternatively, a participating phthalimido functionality was regioselectively installed using potassium phthalimide to provide **4**. In this case the yield could be improved using DMSO instead of HMPA.[11] To install an oxygen functionality at C-2, reaction of **2** with allyl alcohol and NaH in DME furnished **5** in 93% yield, whereas treatment with sodium benzoate and benzoic acid in DMF afforded **6** in 73% yield. To our knowledge, these studies represent the first demonstration that allyloxy and carboxylate groups can be regioselectively introduced into

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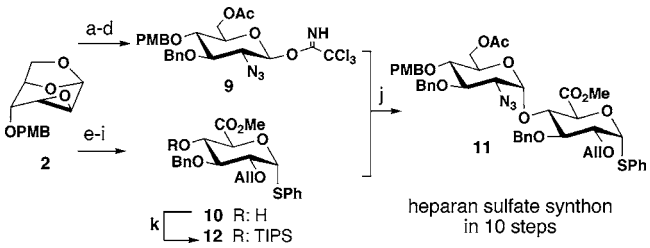
1,6:2,3 Cerny epoxides. An advantage of the approach over previously reported methods[6,7,12] is that the C-2, C-3, and C-4 positions of the ring are differentiated in a minimal number of steps. This extends earlier work and expands the versatility of Cerny epoxides toward carbohydrate synthesis.

Notably, the approach can also be applied to D-galactosamine derivatives. Oxidative 1,6-iodocyclization[13] of D-galactal followed by exposure to NaH and chloride in DMF afforded **7** (Table 1). Reaction of **7** with potassium phthalimide using a modification of Paulsen's method[14] proceeded with excellent regiocontrol to furnish **8**. Thus, galactosamine acceptors for chondroitin sulfate and other 1,3-linked amino sugars are obtained in only three steps.

To demonstrate the utility of the approach toward glycosaminoglycan synthesis, we generated a key disaccharide synthon of heparan sulfate (HS). Disaccharide **11** was chosen because related synthons have previously been shown to serve as useful building blocks for the assembly of HS oligosaccharides (Scheme 2).[4] Epoxide **2** was opened with

Scheme 2. Heparan Sulfate Synthon[a]



[a] Conditions: (a) NaN_3, DMF/H_2O (76%); (b) NaH, BnBr, DMF (81%); (c) Ac_2O, $BF_3{\cdot}Et_2O$, −65 °C (96%); (d) $BnNH_2$, Et_2O, then Cl_3CCN, K_2CO_3, CH_2Cl_2 (75%); (e) NaH, AllOH, DME (93%); (f) NaH, BnBr, DMF (83%); (g) TMSSPh, ZnI_2, CH_2Cl_2, then TBAF, THF (84%); (h) PDC, Ac_2O, *t*-BuOH (85%); (i) AcCl, MeOH (76%); (j) TMSOTf, CH_2Cl_2, (90%, all α); (k) TIPSOTf, 2,6-lutidine, CH_2Cl_2 (91%).

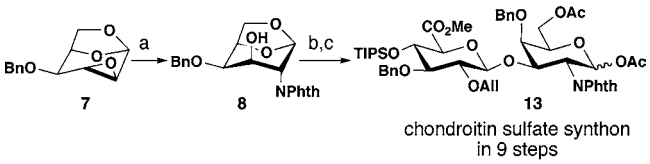
NaN_3, and the C-3 hydroxyl group was benzylated. Acetolysis using $BF_3{\cdot}OEt_2$ was successful without any loss of the PMB-protecting group at −65 °C. After selective anomeric deacetylation[15] and treatment with trichloroacetonitrile and K_2CO_3,[16] the β-trichloroacetimidate **9** was obtained. This sequence provided the key glycosyl donor, in which each hydroxyl group is differentiated, in only four steps and 44% overall yield from **2**.

Epoxide **2** also served as an entry point for the synthesis of the D-glucuronic acid acceptor of HS. Opening of **2** with NaH and allyl alcohol (the use of DME as a solvent was crucial to ensure high yield) followed by benzylation and 1,6-anhydro ring opening[17] provided a primary alcohol, which was smoothly oxidized[18] to the corresponding *tert*-butyl ester. Transesterification with concomitant deprotection of the PMB ether was accomplished by treatment with acetyl chloride and methanol to deliver HS acceptor **10**.

With **9** and **10** in hand, we assembled the core HS disaccharide. Formation of the α-glycosidic linkage is a major challenge in the synthesis of HS and is typically accomplished by masking the C-2 amino group of glucosamine as a non-participating azide.[4,5] However, anomeric selectivities have been reported to vary greatly and often depend on the electronic nature and conformational constraints of the specific donor and acceptor.[4b,5] Thus, we were delighted to find that coupling of donor **9** to acceptor **10** using the imidate methodology developed by Schmidt[19] furnished the desired α-disaccharide **11** in 90% yield, with no observed formation of the β-linked disaccharide. Traditionally, the preparation of highly differentiated HS disaccharides has required more than 20 steps. In contrast, our strategy requires approximately half the number of steps and, to our knowledge, represents the shortest and most convergent approach reported to date. Importantly, **11** can be readily converted into other HS building blocks to alter the sulfation pattern at the O-6, O-2, and N-2 positions or elaborated to assemble oligosaccharides using established methodologies.[3−5]

Finally, the versatility of the approach was further illustrated by applying it to the synthesis of chondroitin sulfate (CS). Opening of epoxide **7** with potassium phthalimide afforded acceptor **8** (Scheme 3). Coupling of **8** to donor **12**,

Scheme 3. Chondroitin Sulfate Synthon[a]



[a] Conditions: (a) phthalimide, KPhth, DMSO (63%); (b) **12**, NIS, TfOH, CH_2CN (70%, β: α 6: 1); (c) TFA, Ac_2O (83%).

which was obtained upon silylation of **10**, was accomplished using TfOH/NIS.[20] The β-selectivity of the glycosylation reaction was enhanced using CH_3CN as a solvent.[21] Upon acetolysis, the β(1-3)-linked disaccharide **13**, a key synthon for the biologically active CS-C and CS-D motifs[3c] (Figure 1), was obtained in 83% yield.

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In summary, we have demonstrated that Cerny epoxides are versatile intermediates for the accelerated synthesis of glycosaminoglycans. Our strategy allows the construction of complex HS and CS disaccharide synthons from a single common precursor in a short sequence of steps. Extension of this approach to the synthesis of focused libraries of GAGs is currently underway and will be reported in due course.

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Supporting Information Available: Experimental procedures and characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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