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New Set of Orthogonal Protecting Groups for the Modular Synthesis of Heparan Sulfate Fragments

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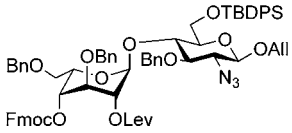
ABSTRACT



Six strategically chosen monosaccharide building blocks, which are protected by a novel set of four orthogonal protecting groups (Lev, Fmoc, TBDPS, and All), can be employed for the efficient synthesis of the 20 disaccharide moieties found in heparan sulfate. The properly protected disaccharide building blocks can be converted into glycosyl donors and acceptors, which can be used for the modular synthesis of a wide range of well-defined oligosaccharides that differ in sulfation pattern.

Heparan sulfates (HS) are highly sulfated polysaccharides that are linked to a core protein, which are found on the cell surfaces and the extracellular matrix of all eukaryotes, where they play pivotal roles in a large number of biological processes. For example, many enzymes, growth factors, enzyme inhibitors, chemokines, cell adhesion molecules, and microbial proteins require HS for their functions.[1−6] The biosynthesis of HS involves the initial formation of a simple polysaccharide composed of alternating β-D-glucuronic acid (GlcA) and α-*N*-acetyl-D-glucosamine (GlcNAc) units joined by 1−4 anomeric linkages. This structure is then modified by a series of enzymatic transformations involving *N*-deacetylation followed by *N*-sulfation, C-5 epimerization of GlcA to L-iduronic acid (IdoA), and finally *O*-sulfation. Ultimately, these modifications result in the formation of an IdoA(2-OSO_3)-GlcNSO_3(6-OSO_3) sequence. Structural studies have, however, shown that HS contains 19 other disaccharide subunits arising from incomplete or additional enzymatic modifications. Combining these different disaccharides into larger structures results potentially in enormous structural diversity.[1]

Detailed structure−activity relationship studies are beginning to unravel the biological significance of HS structural diversity. However, progress is hampered by the difficulties of identifying HS-binding motifs for specific proteins. It is to be expected that this problem will be addressed by screening a relatively large panel of well-defined HS fragments.[7−14] Organic synthesis provides the most powerful approach for obtaining well-defined HS fragments. However, no strategy for the preparation of a wide range of HS

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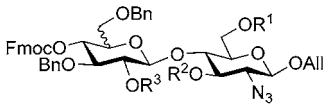

Sulfonated	Unsulfonated
R^1 = Lev	R^1 = TBDPS
R^2 = Lev	R^2 = Bn
R^3 = Lev	R^3 = Ac

Figure 1. Orthogonal protecting groups for disaccharide building blocks.

structures has been reported. To address this important issue, we are developing a modular approach for the chemical synthesis of a wide range of HS oligosaccharides whereby a set of properly protected disaccharide building blocks, resembling the different disaccharide motifs found in HS, can easily and repeatedly be used for the assembly of a library of sulfated oligosaccharides. As part of this program,[15,16] we described a strategy for HS synthesis whereby uronic acids are formed at the end of a synthetic sequence by selective oxidation of C-6 hydroxyls of idosides and glucosides using a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and sodium hypochloride as co-oxidant. This approach avoids synthetic problems associated with use of iduronic and glucuronic acids such as epimerization of C-5, poor glycosyl-donating properties, and complications associated with protecting group manipulations.

Here we report, for the first time, the synthesis of a range of properly protected disaccharides that resemble the different disaccharides found in HS and can be used for the modular synthesis of HS-oligosaccharides. A key strategic issue was the use of a levulinoyl ester (Lev), 9-fluorenylmethyl carbonate (Fmoc), a *tert*-butyldiphenylsilyl ether (TBDPS), and an allyl ether (All) as a novel set of orthogonal protecting groups. It is now shown that six strategically chosen monosaccharides can be used in a parallel combinatorial manner to prepare all structural elements found in HS.

The proposed generic protecting group scheme is shown in Figure 1. Levulinoyl esters[17] will be employed for those hydroxyls that need sulfation in the final product. In HS, the C-3 and C-6 of the glucosamine and C-2 hydroxyls of a hexuronic acid moiety can be sulfated, and therefore, depending on the sulfation pattern of a targeted disaccharide building block, one or more of these positions will need to be protected as levulinoyl groups. An important feature of the Lev ester is that when present at the C-2′ position, it directs the formation of 1,2-*trans*-glycosides by neighboring group participation.[18,19] In cases where the C-2′ position of a building block does not need sulfation, an acetyl group can be employed as a permanent protecting group. This ester can also perform neighboring group participation but is stable under the conditions needed for removing the Lev esters. An azido group could be used as an amino-masking functionality. This derivative does not perform neighboring group participation and therefore allows the introduction of α-glycosides.[20] An azido-group can easily be reduced to an amine, which can either be acetylated or sulfonated.

The C-4′ hydroxyl, which is required for extension, will be protected as 9-fluorenylmethyl carbonate. The Fmoc group can be removed with Et_3N in dichloromethane without affecting the levulinoyl ester, whereas the levulinoyl group can be cleaved with hydrazine buffered with acetic acid, conditions that do not affect the Fmoc carbonate.[21]

The anomeric center of the disaccharides will be protected as allyl glycosides as this functionality can easily be removed by isomerization to the vinyl glycoside and hydrolysis to the hemiacetal. The resulting hemiacetal can be converted into a trichloroacetimidate by employing NaH and trichloroacetonitrile in dichloromethane.[22]

Benzyl ethers could be used as protecting groups for the primary hydroxyls that will be oxidized to the carboxylic acids and for the secondary hydroxyls that will remain unsulfated in the final product.

Finally, a TBDPS ether could be employed for the protection of the C-6 position of the glucosamine residues to avoid oxidation by TEMPO.[23−25]

On the basis of the protecting group strategy outlined above, the monosaccharide building blocks **1**−**6** should allow all of the disaccharide units found in HS to be prepared and also give the capability for these disaccharides to be assembled into larger structures (Figure 2).

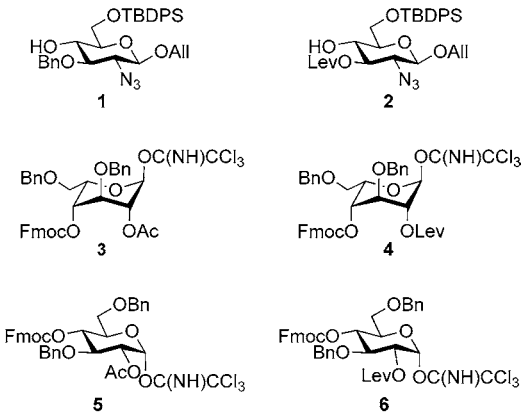

Figure 2. Building blocks for modular HS synthesis.

The preparation of the key building blocks **3** and **4** are summarized in Scheme 1. The C-2 hydroxyl of idoside **7**[7]

(14) For other attempts to develop modular synthesis, see: (a) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R. E. J. N.; Palmacci, E. R.; Seeberger, P. H. *Chem.—Eur. J.* **2003**, 9, 140−169. (b) de Paz, J. L.; Ojeda, R.; Reichardt, N.; Martin-Lomas, M. *Eur. J. Org. Chem.* **2003**, 3308−3324. These approaches however have not established a set of protecting groups that allow differential sulfation of the C-2 iduronic or glucuronic moieties. In addition, the approach by Seeberger and co-workers led to unnatural sulfation patterns.

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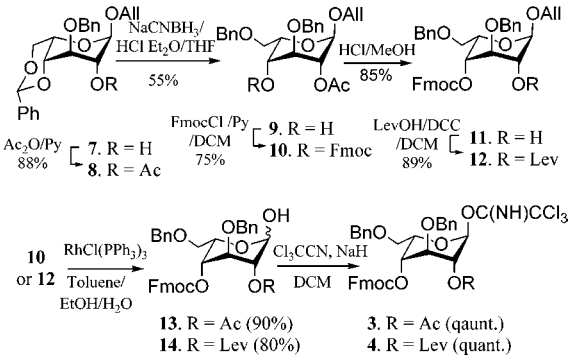
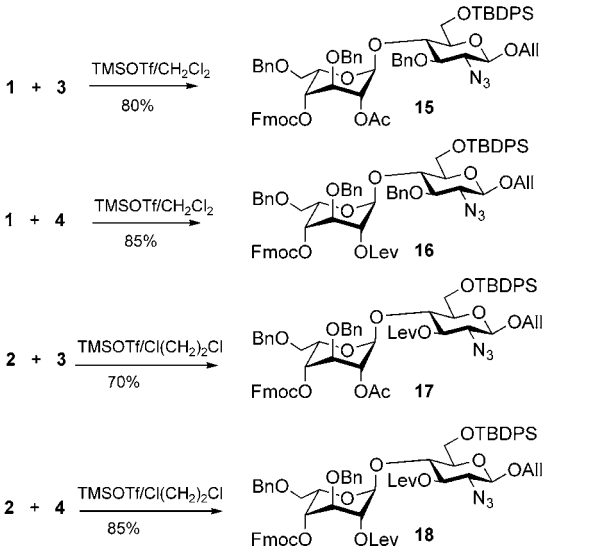
Scheme 1



Scheme 2



was protected as an acetyl ester using acetic anhydride and pyridine to give fully protected **8**. Treatment of **8** with $NaCNBH_3$ and HCl[26] opened the benzylidene acetal regioselectively to give alcohol **9**. The C-4 hydroxyl of **9** was protected as an Fmoc ester by reaction with FmocCl in dichloromethane and pyridine to give target compound **10**. It was found that **9** was susceptible to acetyl migration and therefore needed immediate protection as an Fmoc carbonate. Idosides can adopt either a $^1C_4$ or a skewed boat $^1S_0$ conformation. In the $^1C_4$ conformation, the C-2 acetyl and C-4 hydroxyl occupy axial orientations and are sufficiently close in space to allow acyl migration. Fully protected **10** was the precursor of Lev-protected **12.** It was prepared by treating **10** with dilute HCl in methanol to give **11** and protecting the C-2′ hydroxyl as a levulinoyl ester using standard conditions. Compounds **10** and **12** were converted into trichloroacetamidates **3** and **4** by a two-step procedure involving allyl ether cleavage with Wilkinson's catalyst to **13** and **14** and treatment with trichloroacetonitrile and NaH in dichloromethane. The latter condition did not affetct the base-labile Fmoc and Lev ester.

Monosaccharides **1**−**4** were employed for the preparation of properly protected disaccharides **15**−**18**. Thus, TMSOTf-mediated coupling[22] of **1**[15,16] with **3** gave disaccharide **15** in a yield of 85% as only a 1,2-*trans*-glycoside. In a similar fashion, the use of glycosyl donor **4** and acceptor **1** gave disaccharide **16**, which has a Lev ester at C-2′. TMSOTf-mediated coupling of **2**[15,16] with either **3** or **4** conveniently gave disaccharides **17** and **18**, which have a Lev ester at C-3. Surprisingly, glycosylation with acceptor **2** gave only good yields of coupling product when performed in dichloroethane. Disaccharides **15**−**18** have different combinations of Lev protecting groups at C-3 and C-2′, reflecting different sulfation patterns found in HS. Several naturally occurring HS-disaccharide moieties possess an additional sulfate at C-6 of a glucosamine moiety. It was expected that the corresponding disaccharide building blocks should be easily accessible by replacement of the TBDPS group of disaccharides **15**−**18** by a Lev ester. To investigate the compatibility of TBDPS removal with the other protecting groups, disaccharide **16** was subjected to a range of reaction conditions. It was found that treatment of **16** with HF·pyridine in acetonitrile resulted in clean formation of **19**. Other conditions such as HF·pyridine in THF or TBAF in THF with or without buffering with acetic acid resulted in removal of the Fmoc group or gave mixtures of compounds. The hydroxyl of **19** was protected as a Lev ester using standard conditions to give the modular building block **20** in an almost quantitative yield. In a similar way, compounds **15**, **17**, and **18** can be converted into derivatives that have a levulinoyl ester at C-6 to give an additional three disaccharides for modular oligosaccharide synthesis.

Next, the possibility of converting the disaccharides into glycosyl acceptors and donors was investigated. Thus, treatment of **16** with Et_3N in dichloromethane for 18 hours[21] resulted in clean removal of the Fmoc group without affecting the acetyl and Lev ester, and after purification by silica gel column chromatography, glycosyl acceptor **21** was isolated in a yield of 91%. Fortunately, compound **21** was stable for a prolonged period of time and no acyl migration was observed.

Compound **16** could also be converted into glycosyl donor **23** by first removing the allyl moiety using $PdCl_2$ and sodium acetate in aqueous acetic acid followed by treatment of the resulting hemiacetal **22** with NaH and trichoroacetonitrile.[27] It is to be expected that the disaccharides **15**, **17** and **18** can also be converted into corresponding glycosyl donors and acceptors using similar procedures. Finally, the deprotection, sulfonation, and oxidation steps were investigated. Treatment of **16** with hydrazine hydrate buffered with acetic acid

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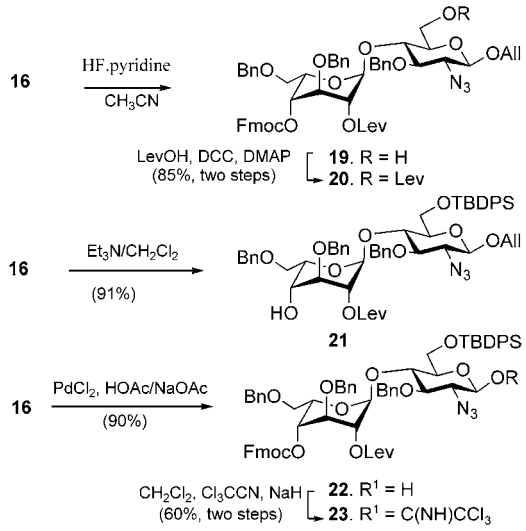
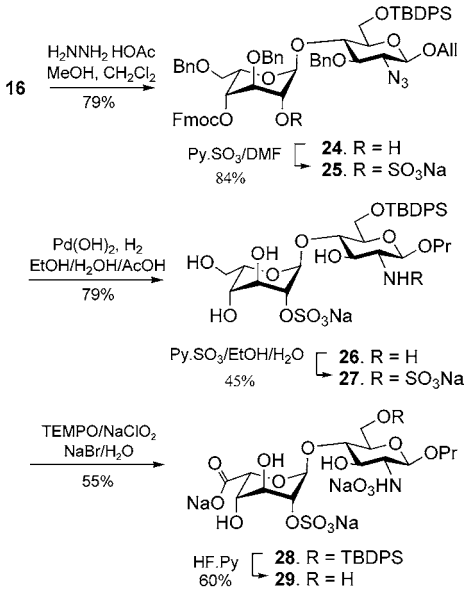
Scheme 3



Scheme 4



resulted in removal of the Lev ester without affecting the Fmoc carbonate or acetyl ester, and the resulting alcohol **24** was sulfated using SO_3·pyridine to give *O*-sulfate **25**. Catalytic hydrogenation of **25** using $Pd(OH)_2$ in a mixture of water, ethanol, and acetic acid resulted in removal of the benzyl ethers and Fmoc carbonate, and conversion of the azido moiety into an amine to give **26**. Compound **26** was immediately *N*-sulfated using SO_3·pyridine, and the primary hydroxyl of the resulting **27** was selectively oxidized using TEMPO and $NaClO_2$ as co-oxidant[23−25] to give **28**. Finally, treatment of **29** with HF·pyridine in pyridine to cleave the TBDPS ether gave, after purification by C-18 column chromatography, target compound **29**. Alternatively, *N*-acetylation of compound **26** followed by selective oxidation with TEMPO and $NaClO_2$ and removal of the TBDPS group should give an acetamido derivative. In conclusion, we have described a new set of four orthogonal protecting groups that is ideally suited for the preparation of a library of heparan sulfate fragments. The synthetic strategy was inspired by the observation that heparan sulfate is composed of 20 disaccharide units that differ in sulfation pattern and the presence of either iduronic or glucuronic acid. Properly protected disaccharide building blocks that resemble these different disaccharide motifs can now be prepared from our six strategically chosen monosaccharides. These monosaccharides are protected by four orthogonal protecting groups (Fmoc, Lev, TBDPS, and All) and could repeatedly be used for the preparation of different disaccharide building blocks. In addition, the resulting disaccharides (e.g., **15**−**17**) could easily be converted into another set of modular building blocks by replacement of the TBDPS ether at C-6 by a Lev ester. It is shown that the disaccharides can easily be converted into glycosyl donors and acceptors for the preparation of larger fragments. It is to be expected that the set of orthogonal protecting groups reported here can be applied for the synthesis of other classes of complex oligosaccharides.

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

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