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Fluorous Supported Modular Synthesis of Heparan Sulfate Oligosaccharides

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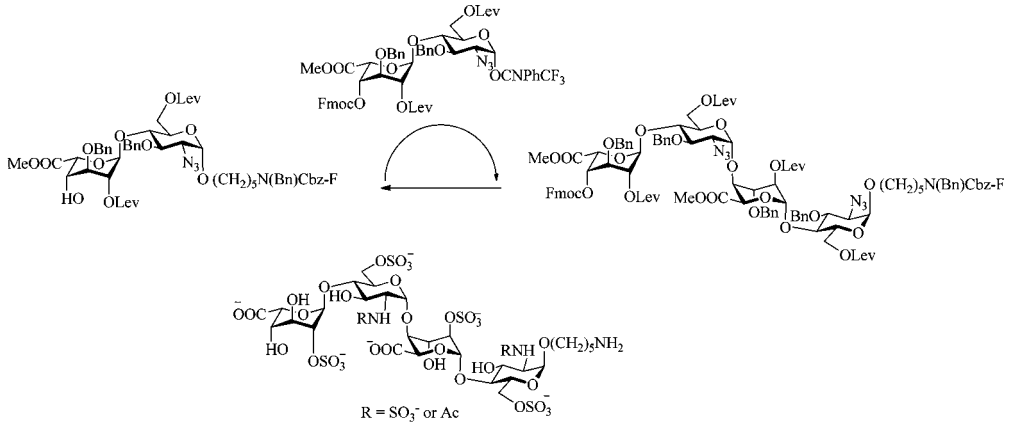
ABSTRACT



Purification: fluorous solid phase extration< 20 min

The modular synthesis of heparan sulfate fragments is greatly facilitated by employing an anomeric aminopentyl linker protected by a benzyloxycarbonyl group modified by a perfluorodecyl tag, which made it possible to purify highly polar intermediates by fluorous solid phase extraction. This tagging methodology made it also possible to perform repeated glycosylations to drive reactions to completion.

Heparan sulfates (HS) are highly *N*- and *O*-sulfated polysaccharides involved in a number of important biological processes such as embryo development, inhibition of blood coagulation, organization of the extracellular matrix, angiogenesis, the presentation of enzymes and cytokines on cell surfaces, and as coreceptors for viral infections.[1] In general, it is difficult to determine oligosaccharide sequences and sulfation patterns required for binding of HS binding proteins.[2] To address this difficulty, we have developed a modular approach for the chemical synthesis of HS oligosaccharides whereby a set of disaccharide building blocks, which resemble the different disaccharide motifs found in HS, can repeatedly be used for the assembly of a wide range of sulfated oligosaccharides.[3] In this approach, levulinoyl esters (Lev)[4] are employed for the protection of hydroxyls that need sulfation. In HS, the C-3 and C-6 of glucosamine and C-1 hydroxyls of uronic acids can be sulfated, and therefore depending on the sulfation pattern of a targeted disaccharide module, one or more of these positions are protected as Lev esters. In case the C-2′ position of a disaccharide module does not

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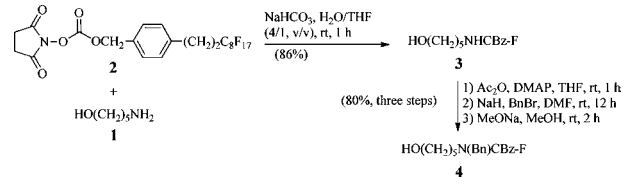
need sulfation, an acetyl ester is employed as a permanent protecting group, which is stable under the conditions used for the removal of Lev esters. An azido group is used as an amino-masking functionality because it does not perform neighboring group participation thereby allowing the introduction of α-glucosides.[5] The C-4′ hydroxyl, which is required for extension, is protected as a 9-fluorenylmethyl carbonate (Fmoc), and this protecting group can be removed with a hindered base such as Et_3N without affecting the Lev ester, whereas the Lev group can be cleaved with hydrazine buffered with acetic acid and these conditions do not affect the Fmoc carbonate.[4] The anomeric center of the modular disaccharides is protected as TDS glycosides, and this functionality can easily be removed by treatment with HF in pyridine without affecting the other protecting groups. The resulting lactol can then be converted into a leaving group for glycosylations with appropriate acceptors. Compared to conventional approaches,[3d,6] a modular synthetic strategy makes it possible to rapidly assemble libraries of HS oligosaccharides for structure−activity relationship studies.

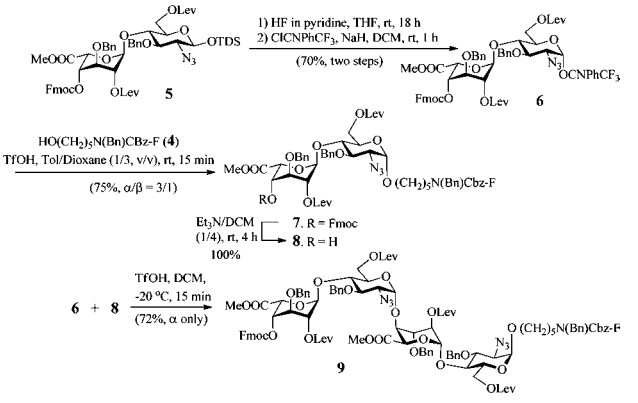
Scheme 1. Preparation of Fluorous Tagged Aminopentyl Linker



Although modular oligosaccharide assembly is very attractive,[3,7] the endgame involving selective protecting group removal, *O*- and *N*-sulfation, and global deprotection requires a relatively large number of steps providing polar compounds that are difficult to purify by conventional approaches thereby slowing down the preparation of libraries of HS oligosaccharides. Several platforms have been developed to speedup the process of oligosaccharide assembly.[8] We were attracted by light fluorous supported synthesis[9] because compounds tagged by a linear fluorous tag can easily be separated from nonfluorous material by solid phase extraction using silica gel modified by fluorocarbons.[10] This generic procedure, which resembles more filtration than chromatography, depends primarily on the presence or absence of a fluorous tag, and not on polarity or other molecular features.

We envisaged that fluorous supported synthesis would speedup modular synthesis of HS oligosaccharides and would in particular be attractive for the final modifications of the fully assembled oligosaccharides, as these procedures are high yielding but require large excesses of reagents and provide polar compounds that are difficult to purify by conventional approaches. Previously,[3d] we employed an *N*-(benzyl)benzyloxycarbonyl aminopentanol linker for the modification of the reducing end of HS oligosaccharides, and thus linker **4** was selected, which contains a benzyloxycarbonyl protecting group modified by a perfluorodecyl tag. Linker **4** could easily be prepared by treatment of aminopentanol (**1**) with **2** in aqueous sodium bicarbonate to give, after purification by fluorous solid phase extraction, benzyloxycarbonyl protected **3** in an 86% yield (Scheme 1). Selective *N*-benzylation of **3** to give **4** was accomplished by a three-step procedure involving acetylation of the hydroxyl with acetic anhydride in pyridine followed by *N*-benzylation by treatment with benzyl bromide in the presence of NaH in DMF and then saponication of the acetyl ester using NaOMe in methanol.

Scheme 2. Preparation of Fluorous Tagged Tetrasaccharide



Having at hand linker **4** modified with a perfluorodecyl tag, attention was focused on its installation into modular disaccharides by glycosylation. Thus, glycosyl donor **6** was prepared by removal of the anomeric TDS moiety of modular disaccharide **5**[4d] with HF in pyridine to give a lactol (Scheme 2), which was converted into trifluoro-*N*-phenylacetimidate **6** by reaction with *N*-phenyltrifluoroacetimidoyl chloride in the presence of NaH in DCM.[11]

Previously, we observed that glycosylations of modular disaccharides such as **6** with a regular *N*-(benzyl)-benzyloxycarbonyl aminopentanol led to mixtures of

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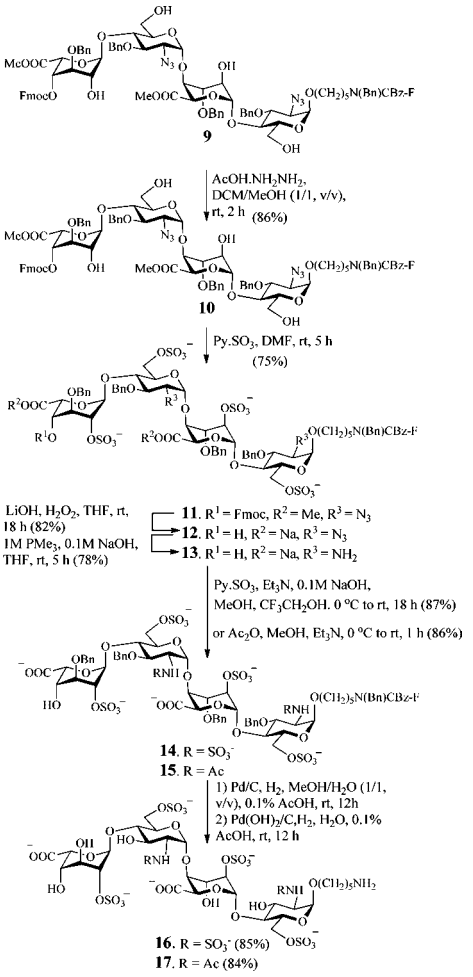
anomers that were difficult to separate by silica gel column chromatography. Therefore, an additional set of modular disaccharides, having a preinstalled linker, needed to be prepared.[3d] A number of conditions were examined to improve the anomeric selectivity of the glycosylation of linker **4** with glycosyl donor **6**. A TfOH-promoted glycosylation of **4** with **6** in DCM at −20 °C gave **7** as a mixture of anomers (α/β = 1/2, 70%), which surprisingly could readily be separated by traditional silica gel column chromatography. Increasing the temperature or the addition of thiophene[12] or DMF[13] did not have a notable effect on the anomeric ratio ($\alpha/\beta \approx 1/1$). The use of diethyl ether to improve the α anomeric selectivity[14] led to a low yield of product due to the poor solubility of linker **4**. The use of a mixture of dioxane and toluene at ambient temperature[14] and TfOH as the promoter gave compound **6** in a good yield of 75% as mainly the α anomer (α/β = 3/1) (Scheme 2). Linker **3** was also examined for tagging modular disaccharides, but the results were disappointing due to poor solubility of this compound in commonly employed solvents for glycosylation.

Next, the Fmoc protecting group of **7** was removed by treatment with Et_3N in DCM to give glycosyl acceptor **8** in a near-quantitative yield after purification by fluorous solid phase extraction. A TfOH promoted coupling of glycosyl donor **6** with acceptor **8** led to the formation of tetrasaccharide **9** as exclusively the α-anomer (Scheme 2). As expected, fluorous solid phase extraction resulted in the removal of hydrolyzed acceptor and other nonfluorous byproducts. The resulting compound **9** was, however, contaminated with glycosyl acceptor **8** due to an incomplete glycosylation. Therefore, the latter mixture was resubjected to treatment with glycosyl donor **6** (0.5 equiv) and a catalytic amount of TfOH, which led to complete consumption of the remaining acceptor to provide, after fluorous solid phase extraction, pure tetrasaccharide **9** in a yield of 72%. Solid supported synthesis often exploits repeated reaction cycles to drive reactions to completion,[8f] and the results described here highlight that such an approach is possible for fluorous supported synthesis.

The sulfate esters were installed following removal of the Lev esters from **9** with hydrazine acetate in a mixture of DCM and methanol, followed by sulfation of the hydroxyls of compound **10** using a large excess of the pyridinium sulfur trioxide−pyridine complex to provide compound **11** in high yield after purification by fluorous solid phase extraction (one reaction cycle, Scheme 3). Next, the Fmoc and methyl esters of **11** were saponified by treatment with LiOH in a mixture of hydrogen peroxide and THF to give partially deprotected **12**. Purification by fluorous solid phase extraction was troublesome probably due to the formation of micelles. However, the addition of a small amount of 2,2,2-trifluoroethanol solved this problem and pure fluorous-tagged **12** could readily be obtained.

Scheme 3. Preparation of Target Tetrasaccharides



The azido moiety of **12** was reduced with trimethylphosphine in THF in the presence of NaOH[15] to give amine **13**, which was immediately sulfated with the sulfur trioxide−pyridine complex in the presence of triethylamine in methanol to afford, after fluorous solid phase extraction with 2,2,2-trifluoroethanol (to avoid micelle formation), *N*-sulfate **14** in a yield of 86%. As expected, the modified *N*-(benzyl)benzyloxycarbonyl tag was stable under the applied basic conditions. Alternatively, acetylation of the free amine of **13** with acetic anhydride in methanol provided acetamido derivative **15**. Finally, the benzyl ethers and benzyloxycarbamate of **14** and **15** were removed by a two-step procedure[16] involving hydrogenation over Pd/C in a mixture of $MeOH/H_2O$, which led to the removal of the spacer protecting groups, followed by hydrogenation over $Pd(OH)_2$ in H_2O which resulted in the removal of the benzyl ethers to give HS oligosaccharides **16** and **17**, respectively. Addition of a small amount of acetic acid was found to be necessary to accelerate the hydrogenation.

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Prolonged hydrogenation in the absence of AcOH caused a loss of sulfate groups. The ^{1}H NMR spectra of the sulfated oligosaccharides were fully assigned by 1D and 2D NMR spectroscopy. The α-anomeric configuration of 2-azido-glucosides was confirmed by the $J_{1,2}$ coupling constants and by the ^{13}C chemical shift of C-1 (~97 ppm). Furthermore, a downfield shift of 0.5 ppm of H-6 was observed for *O*-sulfation of C-6 hydroxyls and 0.4 ppm of H-2 for *N*-sulfation.

The studies reported here highlight the appealing features of fluorous supported modular synthesis of HS oligosaccharides. During oligosaccharide assembly, the attraction of the technology is that two or more reaction cycles can easily be performed to drive the reaction to completion and thus early installation of the fluorous tag is attractive.[17] Unlike solid supported synthesis, light fluorous technology does not require large excesses of reagents to drive the reactions to completion. The fluorous-tagged compounds could easily be analyzed by standard spectroscopic methods thereby providing control over the synthesis. Furthermore, the final modifications involving selective protecting group removal, *O*- and *N*-sulfation, and global deprotection were much faster because these reactions proceed with high efficiency. The resulting intermediates are normally difficult to purify by traditional chromatographic approaches. We observed, however, that polar carbohydrates modified by a fluorous tag tend to aggregate complicating chemical transformations and solid phase extraction. This problem could easily be addressed by employing 2,2,2-trifluoroethanol as a cosolvent, which is more suitable than the use of $EtOC_4F_9$[18] or $PhCF_3$ to solve this problem.[19] Pohl and co-workers are developing a liquid handler to automate fluorous supported oligosaccharide synthesis,[20] and it is to be expected that such a system will be very attractive for modular synthesis of HS oligosaccharides.

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Supporting Information Available. ^{1}H and HSQC NMR spectra and experimental procedures for the preparation of compounds **3**–**5**, **7**–**13**, and **15**–**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.