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Modular Automated Solid Phase Synthesis of Dermatan Sulfate Oligosaccharides

Jeyakumar Kandasamy,a Frank Schuhmacher,a,b Heung-Sik Hahm,a,b James C. Kleinanda Peter H. Seebergera,b*

Dermatan sulfates are glycosaminoglycan polysaccharides that serve a multitude of biological roles as part of the extracellular matrix. Orthogonally protected D-galactosamine and L-iduronic acid building blocks and a photo-cleavable linker are instrumental for the automated synthesis of dermatan sulfate oligosaccharides. Conjugation-ready oligosaccharides were obtained in good yield.

Dermatan sulfate is a glycosaminoglycan (GAG) predominantly found in skin and present in many mammalian tissues.1 It is composed of disaccharide repeating units consisting of N-acetyl-D-galactosamine (GalNAc) and L-iduronic acid (IdoA). These alternating disaccharide units can be variably O-sulfated at the C-4 and C-6 positions in GalNAc, and C-2 position in IdoA.2 Depending on the source, 63-97% of the dermatan sulfate polymer is made up of IdoA→GalNAc4SO3 disaccharide repeating units that are considered to be characteristic for dermatan sulfate (Figure 1).3 Dermatan sulfate binds to a variety of proteoglycans and modulates various biological processes such as coagulation, angiogenesis, tumor migration and growth factor expression.4, 5 Specific biological roles of dermatan sulfate are poorly understood due to the variations and heterogeneity in its polymer structure.6, 7 In order to establish structure–activity relationships for dermatan sulfate sequences, to correlate specific sequences and sulfation patterns to protein binding and specific biological activities requires access to structurally defined dermatan sulfate oligosaccharides. Homogeneous samples of such fragments are accessible only via chemical synthesis.

To date, syntheses of dermatan sulfate oligosaccharides have been executed in solution phase relying on many manual operations.8-12 With appropriate building blocks in hand, automated solid phase synthesis enables rapid access to structurally-defined oligosaccharides.13, 14 Automated protocols for the synthesis of other glycosaminoglycans such as hyaluronan15 and chondroitin sulfate have been reported previously.16 Here, we describe the automated solid phase synthesis of conjugation ready dermatan sulfate oligosaccharides aimed at developing additional tools for biological studies of GAGs.
The remaining hydroxyl groups that were not modified during the assembly were permanently protected as benzyl ethers. Owing to the high cost of galactosamine, these building blocks are accessed from galactose via formation of galactal followed by azidoselenation, or from glucosamine via epimerization of C-4 position. Taking the latter approach, we prepared building block 1 in nine steps from glucosamine via migration of α-ester with epimerization as the key step (Scheme 1). Thioglycoside 11 was synthesized in four steps from glucosamine hydrochloride (See ESI). Levulination of the free hydroxyl group in thioglycoside 11 in 78% yield over two steps. Migration of the C-3 Lev ester to the C-4 hydroxyl with epimerization was effected by transforming the free hydroxyl group in 12 to the corresponding triflate to produce galactose thioglycoside 13 in 78% yield. Fmoc protection of the free hydroxyl group in 13 followed by the conversion of thioglycoside to glycosyl phosphate using dibutyl hydrogen phosphate and N-iodosuccinimide-triflic acid, yielded differentially protected building block 1 in 86% yield.

![Diagram](https://example.com/diagram1.png)

**Scheme 1.** Synthesis of galactosamine building block 1. Reagents and conditions: a) Lev₂O, Py, 91%. b) Et₃SiH, TFA, MS (4 Å), 77%. c) Tf₂O, Py, -10 ºC, 15 minutes; addition of H₂O, reflux at ~80 ºC for 5 h, 78%. d) FmocCl, Py, 93%. e) Dibutyl hydrogen phosphate, NIS-TfOH, MS (4 Å), 92%.

Synthesis of IdoA building block 2 was achieved in five steps from thioglycoside 14 (Scheme 2). Tetraakis(triphenylphosphine)-palladium(0) was employed for the deprotection of allyl group in thioglycoside 14 and the resulting hydroxyl group was protected as a benzyl ester to obtain thioglycoside 15 in 89% yield (over two steps). Removal of naphthyl group in 15 using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) followed by Fmoc protection of the resulting alcohol provided thioglycoside 16 in 82% overall yield. Thioglycoside 16 was then converted to the corresponding phosphate glycoside 2 in 92% yield.

![Diagram](https://example.com/diagram2.png)

**Scheme 2.** Synthesis of iduronid acid building block 2. Reagents and conditions: a) Pd(PPh₃)₄, 1,3-dimethylbarbituric acid, MeOH, 91%. b) BzCl, Py, 98%. c) DDQ, MeOH, 88%. d) FmocCl, Py, 94%. e) Dibutyl hydrogen phosphate, NIS-TIOH, MS (4 Å), 92%.

The simple non-sulfated dermatan oligosaccharides were pursued first in order to test the ability of the automated route to deliver the glycan backbone without deletion sequences. Di- and tetrasaccharides 4 and 5 were prepared using the oligosaccharide synthesizer via iterative glycosylations with building blocks 1 and 2 and linker 3 (Scheme 3). Glycosylations were carried out at temperatures between -10 ºC to 0 ºC using trimethylsilyl trifluoromethanesulfonate (TMSOTf). Each cycle involved two additions of glycosylating agent (double coupling), using five equivalents of building block. Removal of the Fmoc protecting group with triethylamine uncovered the hydroxyl group ready for elongation.

![Diagram](https://example.com/diagram3.png)

**Scheme 3.** Automated synthesis of dermatan sulfate oligosaccharide backbones. Reagents and conditions: a) TMSOTf, DCM, -10 to 0 ºC. b) 25% Et₃N in DMF, 25 ºC. c) Ac₂O, Py, 25 ºC. d) NH₂NH₂-HOAc, Py, AcOH 25 ºC or 40 ºC. e) Py-SO₃, DMF, Py, 50 ºC. f) UV irradiation using continuous flow reactor, DCM/MeOH, RT.
Synthesis of disaccharide 4 commenced with the first glycosylation/deprotection cycle using building block 1 followed by the second glycosylation/deprotection cycle using building block 2. The resulting free hydroxyl group on the terminal sugar was then acetylated on the synthesizer employing acetic anhydride in pyridine. Disaccharide 4 was released from the resin by applying UV light using a photo flow reactor. The crude product was purified by HPLC to obtain 4 in 66% overall yield for six steps based on resin loading. Tetrasaccharide 5 was assembled similarly via four glycosylation/deprotection cycles by alternating use of building blocks 1 and 2, followed by the acetylation of the free hydroxyl group on the terminal sugar. Upon cleavage from the resin, crude product was purified by HPLC to obtain 6 in 28% overall yield (10 steps). No deletion sequences were observed in the automated synthesis of 4 and 5 as judged by HPLC.

Automated synthesis of sulfated dermatan oligosaccharide required two additional steps for installation of sulfates: selective removal of the Lev protecting groups and sulfation of the resulting hydroxy groups. A solution of hydrazine acetate in pyridine-acetic acid effected removal of the Lev groups at 25 °C. Pyridine sulfur trioxide complex in dimethylformamide was used for the sulfation of free hydroxyl groups at 50 °C on the synthesizer. Synthesis of sulfated monosaccharide 6 and disaccharide 7 was achieved on a solid support in five and seven automated steps, respectively, using glycosylation/deprotection conditions established for compounds 4 and 5, followed by automated acetylation, Lev deprotection and sulfation as described above. After the cleavage of photo-sensitive linker, crude products were analysed by reverse phase (RP)-HPLC and purified by size exclusion chromatography (Sephadex LH-20) to obtain monosaccharide 6 in 43% and disaccharide 7 in 32% overall yield based on resin loading. Automated assembly of sulfated tetrasaccharide 8 using the conditions established for the synthesis of 6 and 7 yielded a mixture of compounds resulting from incomplete removal of Lev esters with hydrazine acetate at 25 °C. Raising the temperature for Lev deprotection to 40 °C in the automated run improved the purity and yields of the desired tetrasaccharide 8 that was isolated in 12% yield over 12 steps.

Scheme 4. Deprotection of sulfated disaccharide 7. Reagents and conditions: a) 1 M LiOH, H₂O₂, THF/MeOH, 79%. b) Pd/C, H₂, 80%.

After assembly of the protected oligosaccharides on solid support, deprotection of sulfated disaccharide 7 was tested in solution phase (Scheme 4). Hydrolysis of acetate, benzoate and methyl esters was achieved using lithium hydroxide/hydrogen peroxide in THF/MeOH to obtain disaccharide 9 in 79% yield. Hydrogenolysis of 9 provided fully deprotected, conjugation ready disaccharide 10 in 80% yield.

In conclusion, an efficient automated solid phase synthesis of dermatan oligosaccharides was achieved using orthogonally protected building blocks and photo-cleavable linker as well as an automated oligosaccharide synthesizer. Two-step deprotection in solution affords the conjugation ready dermatan sulfated oligosaccharides that can be directly immobilized on microarrays or conjugated to proteins.

We thank the Max-Planck Society and the European Research Council (ERC Advanced Grant AUTOHEPARIN to PHS) for generous financial support. We thank Dr. I. Vilotijevic and Dr. F. Pfrengle for their help in editing this paper.

Notes and references

References