Modular automated solid phase synthesis of dermatan sulfate oligosaccharides†

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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 the 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 of dermatan sulfate (Fig. 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 with protein binding and specific biological activities access to structurally defined dermatan sulfate oligosaccharides is required. 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 dermatan sulfate backbone may arise via assembly of orthogonally protected galactosamine (GalN) and iduronic acid building blocks (1 and 2, Fig. 1) designed for automated solid phase synthesis on solid supported photo-cleavable linker 3.16 The C-3 position of GalN and the C-4 position of IdoA are masked...
with the temporary 9-fluorenylmethoxycarbonyl (Fmoc) protecting group in anticipation of the regiospecific elongation of the oligosaccharide chain. The C-4 position of GalN carries a levulinoyl ester (Lev) that can be selectively cleaved under mild conditions to allow for sulfation of the free hydroxyl group. The GalN amino group was protected as trichloroacetamide to ensure good β-selectivities in glycosylation reactions and allow for the installation of the desired acetamide by the direct reduction at the late stage of the synthesis. The C-2 hydroxyl group of the IdoA building block was protected as a benzoate ester to ensure n-selectivity in glycosylation reactions. The remaining hydroxyl groups that were not modified during the assembly were permanently protected as benzylic ethers.

Owing to the high cost of galactosamine, these building blocks are accessed from galactose via formation of galactol followed by azido-selenol16,17 or from glucosamine via epimerization of the C-4 position.8,9 Taking the latter approach into account, we prepared building block 1 in nine steps from glucosamine via migration of an epoxy group with the Lev protecting group in 70% 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 fluoride using dibutyl hydrogen phosphate, NIS-TfOH, MS (4 Å), 93%.

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

to deliver the glycan backbone without deletion sequences. Di- and tetra-saccharides 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 °C and 0 °C using trimethylsilyl trifluoro-methanesulfonate (TMSOTf). Each cycle involved addition of a glycosylating agent (double coupling) twice, using five equivalents of building blocks. Removal of the Fmoc protecting group with triethylamine uncovered the hydroxyl group ready for elongation.

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.16 The crude product was purified by HPLC to obtain 4 in 66% overall yield for six steps based on resin loading. Tetrosaccharide 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, the crude product was purified by HPLC to obtain 6 in 28% overall yield (ten steps). No deletion sequences were observed in the automated synthesis of 4 and 5 as judged by HPLC.

Automated synthesis of sulfated dermatan oligosaccharides required two additional steps for installation of sulfates: selective removal of the Lev protecting groups and sulfation of the resulting hydroxyl groups. A solution of hydrazine acetate in pyridine–acetic acid effected removal of the Lev groups at 25 °C, as judged by HPLC. Disaccharide 4 was then converted to the corresponding triflate to produce galactose thioglycoside 13 in 86% yield.

Synthesis of IdoA building block 2 was achieved in five steps from thioglycoside 14-15 (Scheme 2). Tetrais(triphenylphosphine)-palladium(0) was employed for the deprotection of the 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 the napthyl 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.

The simple non-sulfated dermatan oligosaccharides were pursued first in order to test the ability of the automated route
Hydrogenolysis of 9 peroxide in THF–MeOH to obtain disaccharide methyl esters was achieved using lithium hydroxide/hydrogen solution phase (Scheme 4). Hydrolysis of acetate, benzoate and sulfate oligosaccharides that can be directly immobilized on microarrays or conjugated to proteins.

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Notes and references