Towards the construction of dermatan sulfate (DS) partial disaccharide library: efficient synthesis of building blocks, common intermediate, and ligand conjugate of type-B DS disaccharide

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ABSTRACT

Dermatan sulfate (DS) is composed of a repeating disaccharide unit containing iduronic acid (IdoA) and *N*-acetylgalactosamine (GalNAc). In the divergent synthesis of DS disaccharide, it is important to prepare the IdoA moiety with a diverse set of protecting groups. IdoA was efficiently obtained from glucose in 20 steps with some modifications following the method reported by van Boeckel *et al.* A disaccharide building block for constructing the DS disaccharide was synthesized by the glycosylation of the designed IdoA moiety with GalNAc. The disaccharide building block was used in the synthesis of DS-B disaccharide and its ligand conjugate.

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Dermatan sulfate (DS) is a sulfated polysaccharide, belonging to glycosaminoglycan (GAG) superfamily such as chondroitin sulfate (CS), heparan sulfate (HS), and heparin (HP). DS usually binds to a core protein and forms the corresponding glycoprotein, namely, DS-proteoglycan (DS-PG). DS-PG is a component of cell surface and extracellular matrix and interacts with many bioactive proteins such as cell surface receptors, growth factors, and cytokines to regulate their activity.^{1–7} The structure of DS consists of repeating disaccharide units containing iduronic acid (IdoA) and *N*-acetylgalactosamine (GalNAc). However, a DS polysaccharide chain is in fact heterogeneous and has various sugar and sulfation patterns because it is biologically synthesized from chondroitin (CH) or a CS chain by random enzymatic modification such as C-5 epimerization and O-sulfation.^{8,9} Recently, the specific microdomain structure in a DS polysaccharide chain has been considered to be very important for the expression of DS biofunction;¹⁰ structurally defined DS oligosaccharides are eager for elucidating the structure–function relationship of DS at the molecular level. Although many methods have been reported for the synthesis of DS and CS oligosaccharides,^{11–21} it is still challenging to design building blocks for the efficient synthesis of their oligosaccharides with a diverse set of sulfation patterns, because the previous methods are not suitable for library synthesis. In this study, we report the efficient synthesis of a DS disaccharide building block and its usage for the preparation of ligand conjugates of DS disaccharide structure towards construction of a DS disaccharide library.

For the synthesis of DS disaccharide structures based on a combinatorial approach, a disaccharide building block **1** was designed. Building block **1** has orthogonally removable protecting groups at the sulfated and glycosidic positions. Because the 3-O-sulfated structure on uronic acid (UroA) has been discovered in a CS chain obtained from lower marine organisms²² but not yet in a DS chain, the building block was designed by considering all the possible 16 types of DS disaccharide structures. DS ligand conjugates (Figure 1) were selected to fabricate “Sugar Chips”^{20,21,23,24} and investigate the binding properties of GAG-binding protein using a surface plasmon resonance (SPR) biosensor.

First, the monosaccharide moiety was synthesized as shown in Scheme 1. GalNTroc **3**²⁰ was synthesized from galactosamine hydrochloride in three steps using easily manipulated protecting groups²⁰: The amino group was protected with a triethoxy carbonyl (Troc) group, the anomeric position was protected with an allyl group, and the OH groups at the 4- and 6-positions were protected with a benzylidene group.

IdoA **15** was synthesized from D-glucose as the starting material following the method reported by van Boeckel *et al.*²⁵ with slight modifications. D-Glucose was converted to diisopropylidene glucofuranose, and the remaining OH group at the 3-position was protected with a *p*-nitrobenzyl (PNB) group.²⁶ 3-O-PNB **5** was converted into monomesylate **6** via the selective removal of the 5,6-isopropylidene group, followed by 5,6-di-O-

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Figure 1. Designed disaccharide building block **1** and target DS ligand conjugates

mesylation and 6-*O*-acetylation. Epoxide **7** was synthesized from monomesylate **6** using *t*-BuOK as the base in a mixture of CH_2Cl_2/t -BuOH (1:10 v/v). The PNB group could not be removed under this condition. Epoxide **7** was converted into tetraacetate **8** after acidic hydrolysis with TFA and acetylation. The anomeric position was then protected with an allyl group by the glycosylation with (allyloxy)trimethylsilane (AllylOTMS) in the presence of $SnCl_4$. The de-*O*-acetylation was performed with aqueous NH_3 under mild basic conditions because the PNB group is unstable under strong basic conditions such as MeONa. *p*-Methoxybenzylidenation of the resulting compound afforded *p*-methoxybenzylidene **10**. Benzoylation at the 2-position, followed by the reductive ring opening of the *p*-methoxybenzylidene group with TFA and $NaBH_3CN$ ²⁷ afforded 4-OH compound **12**. After the silylation of the 4-OH group with *tert*-butyldimethylsilyl triflate (TBDMSOTf) and triethylamine (TEA), the *p*-methoxybenzyl (MPM) group was removed with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), affording 6-OH compound

13. Oxidation of the 6-position of **13** with (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and NaOCl, followed by methyl esterification with trimethylsilyldiazomethane afforded a fully protected IdoA **14**. Deprotection of the allyl group of IdoA **14** furnished 1-OH derivative **15**, which can be used as a glycosyl donor after conversion to an imideate.

With both the glycosyl donor and acceptor moieties in hand, disaccharide building block **1** and trisaccharide intermediate **21** were synthesized (Scheme 2). After conversion of 1-OH derivative **15** to glycosyl imideate **16**, the glycosylation of GalNTroc acceptor **4** with IdoA donor **16** was performed with TMSOTf, affording the desired building block **1** in 69% yield. Stereochemistry of the anomeric position was confirmed by NMR analysis; it was determined to be the α -anomer (δ 5.28 ppm, brs). Disaccharide **1** was then converted to disaccharide donor **18** by the deprotection of the allyl group, followed by the

Scheme 1. Syntheses of GalNAc **4** and IdoA **15**. (a) TrocCl, $NaHCO_3$ in H_2O , 0 °C; (b) AllylOH, Dowex 50W-X8 (H^+), reflux; (c) $PhCH(OMe)_2$, CSA in CH_3CN , 66% yield (α only, three steps); (d) H_2SO_4 in acetone, 44% yield; (e) PNBBr, Ag_2O , MS 4A in Et_2O , 94% yield; (f) 60% AcOH aq., 40 °C; (g) MsCl in pyridine, 0 °C; (h) AcOK, 18-crown-6 in CH_3CN , reflux, 53% yield (three steps); (i) *t*-BuOK in *t*-BuOH/ CH_2Cl_2 (10:1 v/v), 40 °C; (j) TFA aq.; (k) Ac_2O in pyridine, 66% yield (three steps); (l) AllylOTMS, $SnCl_4$, MS 4A in CH_2Cl_2 , -18 °C, 86% yields; (m) NH_3 aq. in MeOH, 0 °C; (n) *p*-MeO- $PhCH(OMe)_2$, CSA in CH_3CN , 67% yield (two steps); (o) BzCl in pyridine, 95% yield; (p) TFA, $NaBH_3CN$, MS 4A in DMF, 72% yield; (q) TBDMSOTf, TEA, MS 4A in CH_2Cl_2 ; (r) DDQ, H_2O in CH_2Cl_2 , 92% yield (two steps); (s) TEMPO, $NaHCO_3$, KBr, NaOCl, H_2O in CH_3CN , 0 °C; (t) TMSCHN₂ in MeOH, 0 °C, 95% yield (two steps); (u) $Ir[(COD)(PMePh_2)_2]PF_6$, H_2 in THF; (v) H_2O , I_2 in THF, 90% yield (two steps).

Scheme 2. Synthesis of DS trisaccharide intermediate **21**. (a) CCl₃CN, Cs₂CO₃ in CH₂Cl₂; (b) **4**, TMSOTf, MS 4A in CH₂Cl₂, 69% yield (two steps); (c) Ir[(COD)(PMePh₂)₂]PF₆, H₂ in THF; (d) H₂O, I₂ in THF, 95% yield (two steps) (e) CCl₃CN, Cs₂CO₃ in CH₂Cl₂; (f) TMSOTf, MS 4A in CH₂Cl₂, -78 °C, 77% yield (two steps); (g) Zn-Cu couple in AcOH; (h) A₂O in pyridine, 94% yield (two steps).

with CCl₃CN. The glycosylation of donor **18** with glucose **19**, acting as the hydrophilic reaction site to prepare the ligand conjugate for the immobilization of disaccharide structure on the sensor chip of SPR biosensor, afforded trisaccharide **20**. Trisaccharide intermediate **21**²⁸ was synthesized by the reduction of the NO₂ group on PNB and Troc group on GalNTroc moiety, followed by *N*-acetylation.

Next, the orthogonal deprotection, selective sulfation, and conjugation of linker **28** were investigated (Scheme 3). Selective cleavage of the benzylidene group at the 4,6-position on GalNAc was achieved with a combination of TfOH/Et₃SiH, PhBCl₂/Et₃SiH, and TFA/H₂O, affording 4'-OH compound **22**, 6'-OH compound **23**, and 4',6'-OH compound **24**, respectively. For the deprotection of AAB group, DDQ was effective, affording 3''-OH compound **25** in a moderate yield. The TBDMS group was removed by treating with HF-pyridine, which was

suitable for both nonsulfated and sulfated compounds. Moreover, DS-B disaccharide ligand conjugate **29** was synthesized. Hydrolysis of the Bz group and methyl ester of compound **22**, followed by the sulfation of the free OH group with an excess amount of SO₃·pyridine afforded the corresponding disulfated compound. After the desilylation, the final deprotection of the benzyl group was achieved by hydrogenolysis with Pd/C under 7 kg/cm² H₂ pressure. Sulfate **28** was then converted to ligand conjugate **29**²⁹ in a good yield following the method reported previously.²⁰ Other sulfated dermatan disaccharides can be prepared by appropriate deprotection and sulfation.²⁴

In conclusion, a novel disaccharide building block **1** was designed for constructing a DS oligosaccharide library, possessing orthogonally removable protecting groups and capable of generating diverse sulfation patterns. DS disaccharide

Scheme 3. Selective deprotection of trisaccharide intermediate **21** and conversion to DS-B disaccharide ligand conjugates. (a) TfOH, Et₃SiH, MS 4A in CH₂Cl₂, 88% yield; (b) PhBCl₂, Et₃SiH, MS 4A in CH₂Cl₂, -78 °C, 92% yield; (c) TFA/H₂O (1:1 v/v), 86% yield; (d) DDQ, H₂O in CH₂Cl₂, 69% yield; (e) HF-pyridine in pyridine; (f) LiOH, H₂O, H₂O₂ in THF/MeOH/H₂O (5:5:1 v/v/v), then 4 M NaOH (one-pot reaction); (g) SO₃·pyridine in pyridine; (h) HF-pyridine in pyridine; (i) 10% Pd/C, H₂ (7 kg/cm²) in H₂O/MeOH/AcOH (5:5:1 v/v/v); (j) NaBH₃CN in H₂O/DMAc/AcOH (5:5:1 v/v/v), 28% yield (six steps).

building blocks **1** were efficiently synthesized using appropriate IdoA and GalNAc moieties. IdoA, which is a key monosaccharide for the disaccharide building block, was efficiently synthesized from glucose in 20 steps. DSdisaccharides were systematically and divergently synthesized from trisaccharide intermediate **21**. Although further studies are needed to construct a DS disaccharide library, the designed DS disaccharides may be useful for the synthesis of versatile DS and DS/CS hybrid oligosaccharides with minimal derivatization.

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- Trisaccharide intermediate **20**: $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ 8.13–7.10 (35H, m, aromatic, NHPh), 5.85 (1H, d, $J_{\text{NH},2'} = 6.8$ Hz, NHAc), 5.52 (1H, s, PhCH_2), 5.40 (1H, brs, H-1''), 5.25 (1H, brs, H-2''), 5.19 (1H, d, $J_{1,2'} = 8.8$ Hz, H-1'), 5.19 (1H, brs, H-5''), 4.91 and 4.69 (each 1H, d, $J_{\text{gem}} = 10.2$ Hz, PhCH_2), 4.89 and 4.69 (each 1H, d, $J_{\text{gem}} = 10.8$ Hz, PhCH_2), 4.79–4.66 (each 1H, d, $J_{\text{gem}} = 11.2$ Hz, PhCH_2), 4.82 (1H, dd, $J_{3',4'} = J_{3',2'} = 8.8$ Hz, H-3'), 4.78–4.72 (3H, m, PhCH_2 , PhCH_2 , PhCH_2), 4.69–4.66 (1H, m, PhCH_2), 4.57 (1H, d, $J_{1,2} = 8.1$ Hz, H-1), 4.37 (1H, d, $J_{6a,6b} = 12.2$ Hz, H-6a), 4.28–4.22 (2H, m, H-6a', H-4'), 4.20 (1H, s, H-4''), 4.15 (1H, d, $J_{6b,6a} = 12.2$ Hz, H-6b), 3.89–3.82 (2H, m, H-6b', H-3''), 3.78–3.54 (5H, m, H-2', H-2, H-3, H-4, H-5), 3.58–3.51 (3H, s, COOMe), 3.44 (1H, s, H-5'), 0.79 (9H, s, $\text{Si}(\text{CH}_3)_3$), -0.03 (3H, s, $\text{Si}(\text{CH}_3)_2$), -0.23 (3H, s, $\text{Si}(\text{CH}_3)_2$); HRMS (positive mode) found: m/z 1409.5799 $[\text{M}+\text{Na}]^+$, Calcd for $\text{C}_{78}\text{H}_{90}\text{Cl}_3\text{N}_2\text{NaO}_{19}\text{Si}$: 1409.5798.
- DS-B ligand conjugate: $^1\text{H-NMR}$ (600 MHz, D_2O) δ 7.25–7.18 (1H, m, aromatic), 6.97–6.88 (1H, m, aromatic), 6.87–6.77 (1H, m, aromatic), 6.73–6.63 (1H, m, aromatic), 4.90 (1H, brs, H-1''), 4.45 (1H, d, $J_{1,2} = 8.1$ Hz, H-1), 4.42–4.36 (1H, m, H-5''), 4.21–4.17 (1H, m, H-4'), 4.16 (1H, d, $J_{6a,6b} = 7.4$ Hz, H-6a), 4.04–3.75 (4H, m, H-2, H-2', H-3'', H-2''), 3.74–3.55 (7H, m, H-3, H-4, H-5, H-6b, H-3', H-5', $-\text{CH}_2\text{CH}(\text{S})\text{CH}_2-$), 3.32–3.24 (3H, m, H-1a, H-4'', H-6a'), 3.06–2.84 (3H, m, H-1b, H-6b', $-\text{SCH}_2\text{CH}_2\text{CH}=\text{}$), 2.31–2.22 (3H, m, $-\text{SCH}_2\text{CH}_2\text{CH}=\text{}$, $\text{NHCOCH}_2\text{CH}_2-$), 1.86–1.82 (4H, m, COCH_3 , $-\text{SCH}_2\text{CH}_2\text{CH}=\text{}$), 1.61–1.49 (4H, m, $-\text{CH}_2-$, $-\text{CH}_2-$), 1.34–1.29 (2H, m, $-\text{CH}_2-$ $\times 2$); HRMS (negative mode); Found: m/z 939.2057 $[\text{M}-\text{Na}]^-$, Calcd for $\text{C}_{34}\text{H}_{50}\text{N}_3\text{O}_{20}\text{S}_3$: 939.2053.

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DS ligand conjugate
 $(R^1=R^2=R^3=R^4=H \text{ or } SO_3^-)$