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Synthesis of oligomeric assemblies of a platelet-binding key disaccharide in heparin and their biological activities

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Abstract—Heparin, highly sulfated glycosaminoglycan, binds to platelets. A key disaccharide unit in heparin was previously found to be responsible for the binding, and the frequency of the disaccharide unit was important for the binding potency. A newly developed method based on the reductive amination was effectively applied to prepare structurally defined oligomeric assemblies possessing multiple units of the key disaccharide. From their platelet-binding activities measured by the competitive binding assay, the enhancement of the activity was clearly observed with increasing number of the key disaccharide.

Heparin, a glycosaminoglycan (GAG) best known as an anticoagulant drug,[1] binds to human platelets and alters their functions.[2] This event is thought to be mediated by a binding interaction between specific structures in heparin and specific protein(s) on the platelet surface. We previously found that a disaccharide unit in heparin, *O*-(2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-2-*O*-sulfo-α-L-idopyranosyluronic acid (abbreviated as GlcNS6S-IdoA2S), is a key unit responsible for binding to platelets.[3,4] Furthermore, using synthetic oligomer compounds containing two or three units of GlcNS6S-IdoA2S we identified the enhancement of the binding activity achieved by clustering the units.[5] In the synthesis of these compounds, the protected oligomeric frameworks were first prepared, and then converted through many steps of deprotection and sulfation into regiospecifically sulfated target compounds. To prepare different types of oligomer compounds based on the above strategy, the same tedious transformations must be repeated for each individual compound. When more complex structures are designed, however, it would become difficult to complete these transformations perfectly.

For the efficient preparation of complex and diverse types of oligomeric compounds, a new strategy was devised to assemble components containing the 'completed' GlcNS6S-IdoA2S unit already sulfated and deprotected. We employed a sulfated trisaccharide component **1** (Fig. 1), which is composed of the GlcNS6S-IdoA2S disaccharide unit and an additional D-glucose. Using the reducing end of the glucose moiety, the component **1** can be assembled with aromatic amino groups of linker molecules in one-step by the reductive amination method as described in the accompanying paper. The glucose unit works not only as a reducing end donor for the reductive amination, but also as the hydrophilic portion in the molecule to minimize any non-specific hydrophobic interactions between the linker and the target proteins or cells.

The synthesis of the trisaccharide **1** was carried out as shown in Schemes 1 and 2. Benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**5**) to be used as the precursor of the reducing-end glucose was prepared in four steps from benzyl β-D-glucopyranoside **2**.[6] The known L-idose derivative **6**[7] was converted to a trichloroacetimidate derivative **7**, which was then coupled with **5** in the presence of tin(II) triflate at 0°C in 1,2-dichloroethane to give the desired α-linked disaccharide **8** by the partici-

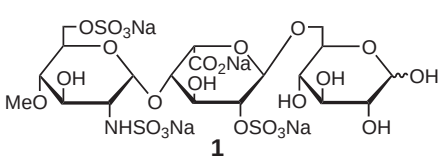

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Figure 1.

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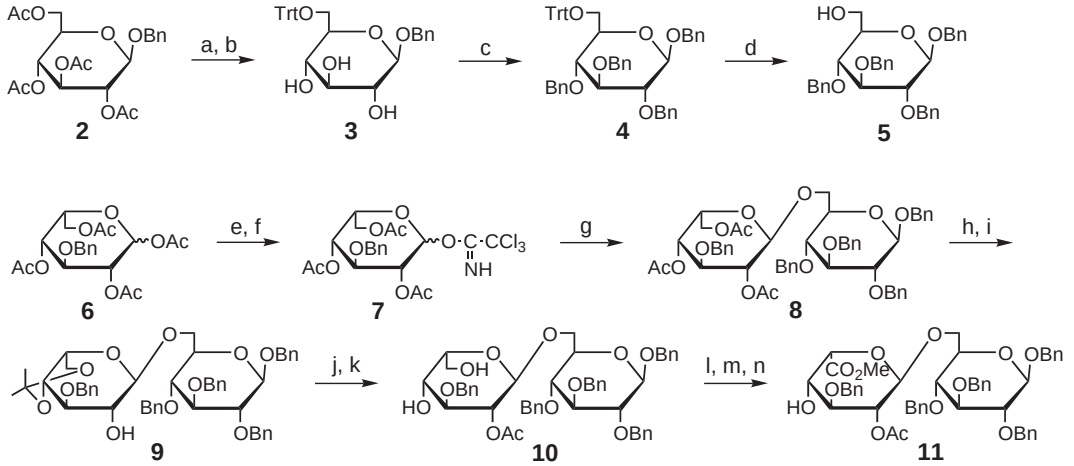
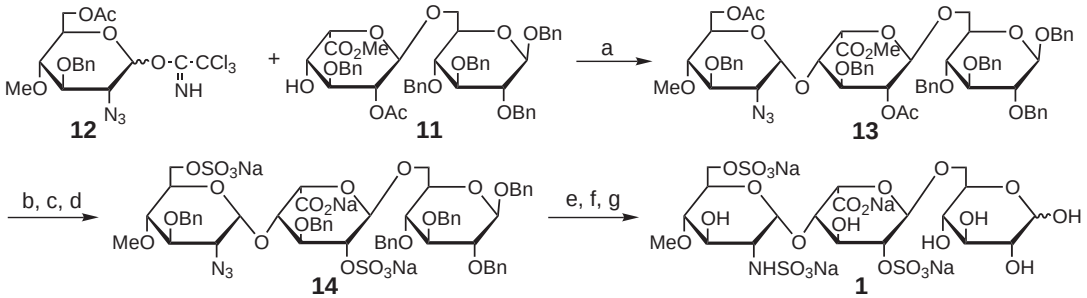


Scheme 1. (a) 0.05 M NaOMe; (b) TrtCl/pyridine, 80°C; (c) BnBr, NaH/DMF; (d) 80% AcOH, 80°C, 66% (four steps); (e) piperidine, AcOH/THF, 61%; (f) CCl_3CN, Cs_2CO_3/CH_2Cl_2, quant.; (g) **5**, $Sn(OTf)_2$, MS4A/$(CH_2Cl)_2$, 0°C, 80%; (h) 0.05 M NaOMe; (i) $(CH_3)_2C(OCH_3)_2$, CSA/acetone, 89% (two steps); (j) Ac_2O, DMAP, pyridine/CH_2Cl_2, 95%; (k) 90% AcOH, 96%; (l) TEMPO, NaClO, KBr; (m) $NaClO_2$, NaH_2PO_4/H_2O, *t*-BuOH; (n) $TMSCHN_2$, 74% (three steps).



Scheme 2. (a) TBDMSOTf, MS4A/toluene, –20°C, 82%; (b) 0.05 M NaOMe, 92%; (c) SO_3·pyridine complex/DMF; (d) NaOH/MeOH; (e) 10% Pd–C, H_2 1 kg/cm^2/THF:H_2O = 2:1; (f) SO_3·pyridine complex/H_2O, pH 9.5; (g) 10% Pd–C, H_2 7 kg/cm^2/H_2O:AcOH = 5:1; 93% (five steps).

pation of the neighboring acetyl group at the 2-position in 80% yield. After the transformations of protecting groups in four steps, the primary 6′-hydroxy group of the resultant **10** was selectively oxidized using 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) followed by $NaClO_2$ treatment. The resulting carboxyl group was then esterified with (trimethylsilyl)-diazomethane to afford the disaccharide methyl ester **11**.

The 2-azido sugar derivative **12**[5] was coupled with the disaccharide **11** using *t*-butyldimethylsilyl triflate (TBDMSOTf) at –20°C to give selectively an α-linked trisaccharide **13** in 82% yield (Scheme 2).[5,8] After all the acetyl groups of **13** were removed, the resulting 2′- and 6″-hydroxy groups were *O*-sulfated and the methyl ester was hydrolyzed to give the carboxylate **14**. The transformation of the 2″-azide into the amino group was performed by hydrogenolysis under 1 kg/cm^2 of hydrogen with Pd/C in THF:H_2O. No benzyl group was removed under this mild and neutral conditions as judged from the TLC analysis. The resulting 2″-amino group was *N*-sulfated and the product was hydrogenated (7 kg/cm^2) using Pd/C in H_2O:AcOH = 5:1 to remove all the benzyl groups. Then, purification by gel permeation chromatography using Sephadex G-25 gave the desired sulfated trisaccharide **1** in 93% yield (five steps).

The assembly of the sulfated trisaccharide **1** was performed using the method described in the accompanying paper. Coupling of **1** with the divalent linker **15** (0.4 equiv. to **1**) was performed using $NaBH_3CN$ (10 equiv.) at pH 3 (Scheme 3). After 1 day at 37°C, $NaBH_3CN$ (10 equiv.) was added again to complete the reaction. After an additional 2 days of incubation, the product was purified by gel permeation chromatography with Sephadex G-50 to give the desired dimeric assembly **18** containing two units of GlcNS6S-IdoA2S in 60% yield (based on the linker **15**). The preparation of the dimeric assembly **19**, possessing a longer methylene bridge compared to **18**, was carried out in the same manner. Using the trivalent linker **17**, the trimeric assembly **20** possessing three units of GlcNS6S-IdoA2S was prepared successfully by a similar procedure. The structure of the assemblies **18**–**20** were confirmed by 600 MHz ^{1}H NMR and ESI-MS.[9]

The platelet-binding activities of **1** and the assemblies **18**–**20** were evaluated using a competitive binding assay

according to our previous method.[5,10] In Fig. 2, the competitive binding activities of **1** and **18**–**20** were compared with those of a commercial heparin (average molecular weight 17500, from porcine intestine, Nacalai Tesque, Kyoto, Japan). The binding potency was enhanced as the number of GlcNS6S-IdoA2S moieties increased. The trisaccharide **1**, containing only one unit of GlcNS6S-IdoA2S, showed no significant activity at the concentration range tested, whereas the dimeric assemblies **18** and **19** showed distinct binding potency. The trimeric assembly **20** showed the highest activity. These results further establish the role of a clustering effect, based on the subunit structure GlcNS6S-IdoA2S.

It is often difficult to measure the biological activity of small oligosaccharide units that have low affinity by themselves. The novel methodology reported here for the assembly of structurally defined oligosaccharides, using reductive amination reaction, may be an effective way to study the biology of oligosaccharides.

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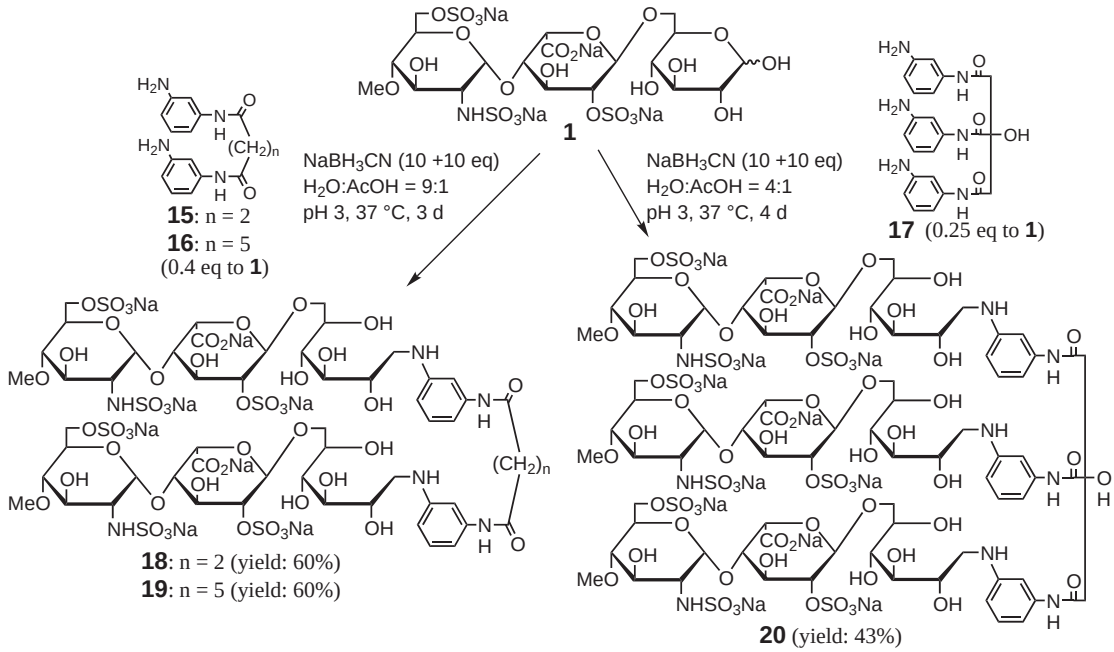

Scheme 3.

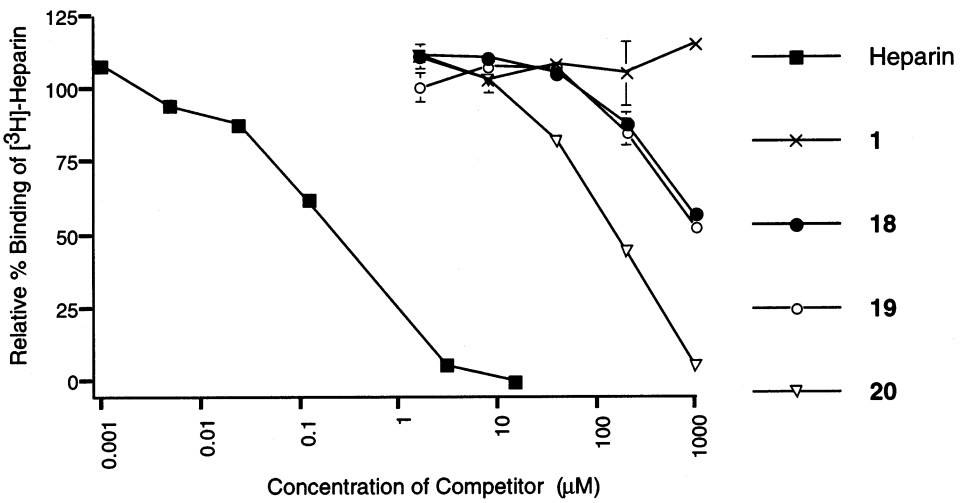

Figure 2. Binding competitive activity of synthetic assemblies and commercial heparin.

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9. Spectral data for compound **18**: ^{1}H NMR (600 MHz, D_2O), δ 7.15 (2H, t, $J=8.2$ Hz), 6.78–6.75 (4H, m), 6.58 (2H, d, $J=6.9$ Hz), 5.26 (2H, d, $J=3.6$ Hz), 5.03 (2H, s), 4.42 (2H, d, $J=2.2$ Hz), 4.20 (2H, d, $J=2.5$ Hz), 4.19 (2H, dd, $J=2.6$ Hz, $J=5.2$ Hz), 4.10 (2H, d, $J=2.8$ Hz), 4.09 (2H, d, $J=2.8$ Hz), 3.97 (2H, t, $J=3.0$ Hz), 3.89 (4H, m), 3.87 (2H, m), 3.77 (2H, m), 3.72 (2H, dd, $J=5.5$ Hz, $J=2.2$ Hz), 3.69 (2H, dd, $J=2.2$ Hz, $J=8.5$ Hz), 3.61 (2H, t, $J=9.8$ Hz), 3.58 (2H, dd, $J=5.5$ Hz, $J=11.0$ Hz), 3.47 (6H, s), 3.29 (2H, dd, $J=13.5$ Hz, $J=3.8$ Hz), 3.25 (2H, t, $J=9.8$ Hz), 3.15 (2H, dd, $J=3.5$ Hz, $J=10.6$ Hz), 3.01 (2H, dd, $J=8.7$ Hz, $J=13.6$ Hz), 2.68 (4H, s); ESI-MS (negative) m/z 903.23 [$(M-8Na+6H)^{2-}$].
   Spectral data for compound **19**: ^{1}H NMR (600 MHz, D_2O), δ 7.12 (2H, t, $J=8.0$ Hz), 6.74–6.70 (4H, m), 6.57 (2H, d, $J=6.6$ Hz), 5.26 (2H, d, $J=3.3$ Hz), 5.03 (2H, d, $J=2.8$ Hz), 4.42 (2H, d, $J=2.2$ Hz), 4.21 (2H, s), 4.19 (2H, dd, $J=4.4$ Hz), 4.11 (2H, s), 4.09 (2H, m), 3.97 (2H, t, $J=3.0$ Hz), 3.89 (4H, m), 3.87 (2H, m), 3.77 (2H, m), 3.72 (2H, dd, $J=5.8$ Hz, $J=2.2$ Hz), 3.69 (2H, dd, $J=2.1$ Hz, $J=8.4$ Hz), 3.61 (2H, t, $J=9.8$ Hz), 3.58 (2H, dd, $J=5.5$ Hz, $J=11.0$ Hz), 3.47 (6H, s), 3.28 (2H, dd, $J=12.6$ Hz, $J=3.9$ Hz), 3.25 (2H, t, $J=9.6$ Hz), 3.15 (2H, dd, $J=3.6$ Hz, $J=10.4$ Hz), 3.00 (2H, dd, $J=8.7$ Hz, $J=13.4$ Hz), 2.32 (4H, t, $J=7.3$ Hz), 1.62 (4H, dd, $J=7.6$ Hz), 1.34 (2H, t, $J=7.5$ Hz); ESI-MS (negative) m/z 924.24 [$(M-8Na+6H)^{2-}$].
   Spectral data for compound **20**: ^{1}H NMR (600 MHz, D_2O), δ 7.21 (3H, m), 6.95–6.89 (6H, m), 6.73 (3H, m), 5.25 (3H, d, $J=3.6$ Hz), 5.05 (3H, s), 4.48 (3H, s), 4.20 (3H, s), 4.18 (3H, s), 4.13 (3H, m), 4.09 (3H, d, $J=11.0$ Hz), 3.98 (3H, s), 3.87 (6H, m), 3.84 (3H, m), 3.76 (3H, m), 3.71 (3H, m), 3.66 (3H, d, $J=10.4$ Hz), 3.62 (3H, t, $J=9.6$ Hz), 3.56 (3H, m), 3.47 (9H, s), 3.36–3.21 (6H, b, overlapped with a peak at 3.25 ppm), 3.25 (t, $J=9.6$ Hz), 3.17–3.02 (10H, b, overlapped with peaks at 3.15 and 3.05 ppm), 3.15 (dd, $J=3.3$ Hz, $J=10.4$ Hz), 3.05 (d, $J=14.8$ Hz), 2.85 (4H, d, $J=14.3$ Hz); ESI-MS (negative) m/z 908.08 [$(M-12Na+9H)^{3-}$].
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