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Synthesis and biological activity of oligomer-model compounds containing units of a key platelet-binding disaccharide of heparin

Shuhei Koshida,[a] Yasuo Suda,[a,*] Yasuhiro Fukui,[a] Julie Ormsby,[b] Michael Sobel[b] and Shoichi Kusumoto[a]

[a]*Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan*
[b]*Department of Surgery, Syracuse Veterans Administration and Health Science Center, State University of New York, Syracuse, NY 13210, USA*

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Abstract

A key disaccharide unit in heparin, *O*-(2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-2-*O*-sulfo-α-L-idopyranosyluronic acid, was previously found to be responsible for the binding interaction of heparin to platelets. A clustering effect to enhance the binding was found to be dependent on the number and frequency of the disaccharide units in a heparin molecule. To systematically examine the clustering effect, three oligomer-model compounds containing two or three units of the disaccharide were synthesized. These compounds inhibited ^{3}H-labelled heparin binding to human platelets more strongly than a compound containing only one unit of the disaccharide.

Heparin, a structurally heterogeneous sulfated polysaccharide, has been used as an effective anticoagulant. Recently, it was pointed out that pharmaceutical heparin binds to platelets, directly alters platelet function and induces immuno sensitization. These responses caused by platelet-interaction are undesirable side effects in the clinical use of heparin. We identified heparin-binding proteins on the platelet surface using radio-labelled heparin,[1] and found that a key 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 NS6S-I2S), is likely to be responsible for the binding of heparin to platelets.[2,3] Furthermore, we observed that the number and frequency of NS6S-I2S in a heparin molecule influence the binding potency (a so-called clustering or polymer effect on binding).[2] To estimate the clustering effect based on NS6S-I2S in detail, we synthesized three oligomer-model compounds (**1–3**) containing two or three units of NS6S-I2S. Their structures were designed so that the influence of the number and relative orientation of the disaccharide units could be evaluated.

The synthesis of **1** and **2** was carried out as shown in Schemes 1 and 2. To predominantly form an α-D-glucosaminyl linkage to L-iduronic acid, an azide derivative was used for the precursor of

* Corresponding author. E-mail: ysuda@chem.sci.osaka-u.ac.jp

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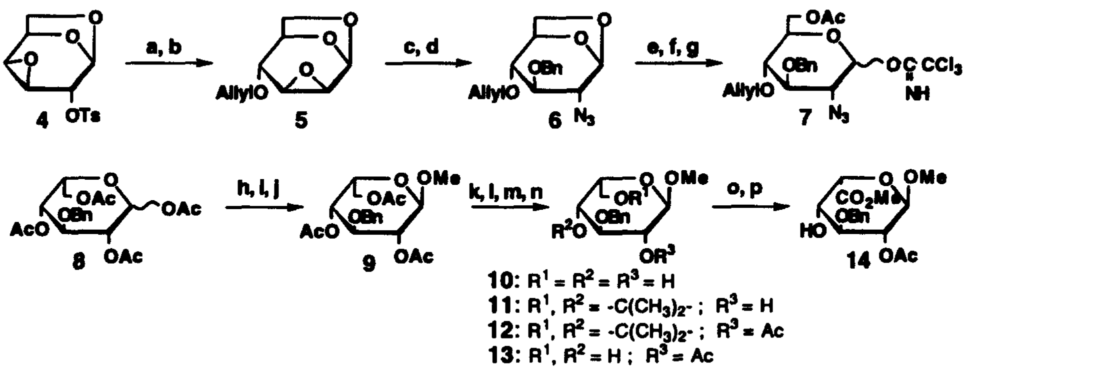
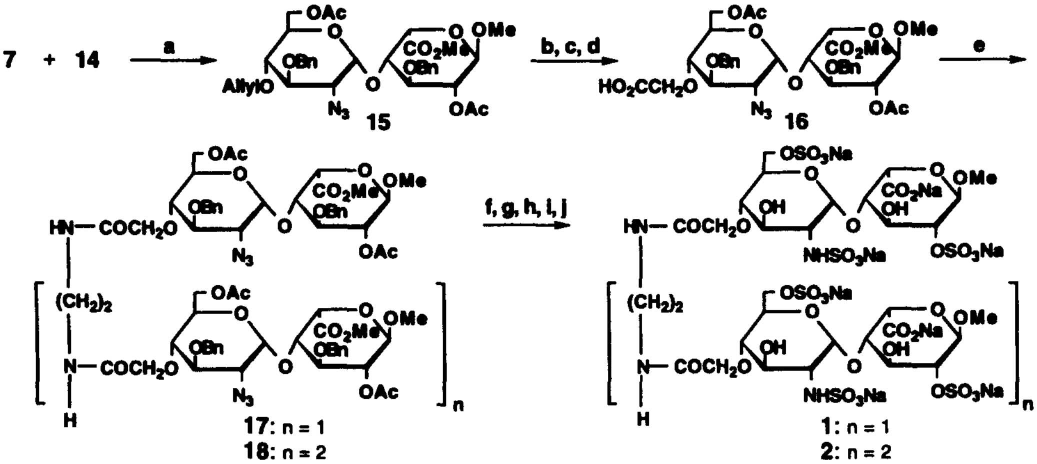
the D-glucosamine unit. An *O*-allyl group, which must be oxidized to a carboxymethyl group, as a linker moiety, was introduced at the 4-position of 1,6:3,4-dianhydro-2-*O*-tosyl-D-glucose[4] (**4**). An azide group was introduced at the 2-position of epoxide **5**, and then the 3-hydroxyl group was protected by benzylation. The 1,6-anhydro ring of **6** was opened by acetolysis using Ac_2O/AcOH/TFA. After selective removal of the anomeric acetyl group by treatment with piperidine, the resulting compound was treated with trichloroacetonitrile in the presence of cesium carbonate to give trichloroacetimidate **7** which was used as the glycosyl donor. The L-idose derivative[5] **8** was treated with piperidine to remove the anomeric acetyl group and then converted to the trichloroacetimidate derivative. To fix the α-pyranosyl structure of the L-iduronic acid unit, the imidate was coupled with methanol using BF_3·ether to form methyl glycoside **9**. After all acetyl protecting groups were removed, the 4- and 6-hydroxyl groups of the resultant **10** were protected with an isopropylidene group, and the remaining 2-hydroxyl group was again protected by acetylation. The isopropylidene of **12** was then removed with 90% acetic acid to give **13**. The primary hydroxyl group at the 6-position of **13** was selectively oxidized to carboxylic acid by 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) oxidation[6] without protection of another hydroxyl group, and the carboxyl group was esterified with (trimethylsilyl)diazomethane to afford an iduronic acid component **14**.



Scheme 1. (a) AllylOH, CSA/benzene; (b) NaOMe, 77% (2 steps); (c) LiN_3, NH_4Cl/DMF, 56%; (d) BnBr, NaH/DMF, 92%; (e) Ac_2O/AcOH/TFA, 85%; (f) piperidine/THF, 97%; (g) CCl_3CN, Cs_2CO_3/CH_2Cl_2, quant.; (h) piperidine, AcOH/THF, 61%; (i) CCl_3CN, Cs_2CO_3/CH_2Cl_2, quant.; (j) MeOH, $BF_3·OEt_2$, MS4A/CH_2Cl_2, 72%; (k) NaOMe; (l) $(CH_3)_2C(OCH_3)_2$, CSA/acetone, 67% (2 steps); (m) Ac_2O, DMAP, pyridine/CH_2Cl_2, 97%; (n) 90% AcOH, quant.; (o) TEMPO, NaClO, *n*-Bu_4NCl, KBr; (p) $TMSCHN_2$, 82% (2 steps)

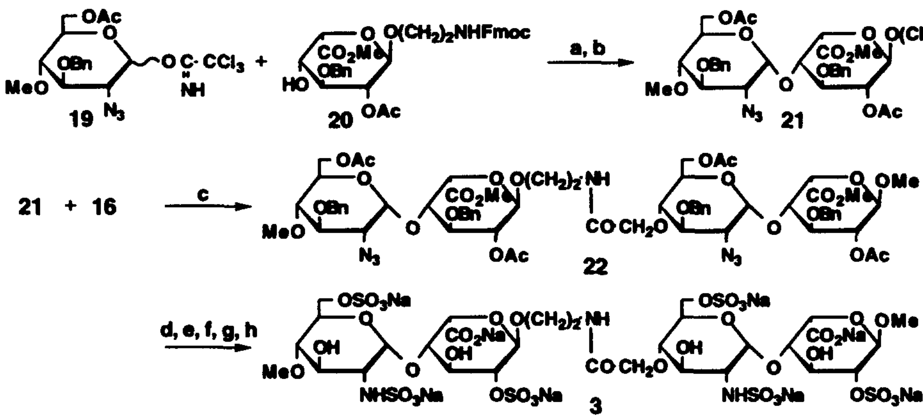
The coupling of **7** with **14** was performed in the presence of *t*-butyldimethylsilyl triflate (TBDMSOTf) at −20°C to give α-linked disaccharide **15** in 82% yield,[7] where no β-anomer was obtained. Then, the 4′-*O*-allyl group of **15** was oxidized to a carboxymethyl group in three steps: (i) osmium tetroxide/*N*-methylmorpholine-*N*-oxide; (ii) lead(IV) acetate; and (iii) sodium chlorite. Two units of **16** were coupled with ethylenediamine using pentafluorophenyl diphenylphosphinate (FDPP)[8] as a condensation reagent to give **17**. Compound **17** was treated with sodium methoxide to remove the acetyl groups, and the resulting 2- and 6′-hydroxyl groups were *O*-sulfated using sulfur trioxide–pyridine complex in dimethylformamide at room temperature, followed by neutralization with sodium hydrogencarbonate. Saponification of methyl ester, hydrogenolysis of both the benzyl and azide groups, and *N*-sulfation of the 2′-amino group were sequentially performed as reported[3] to give an oligomer-model compound **1** with two units of NS6S-I2S. By a similar procedure, three units of **16** were coupled with diethylenetriamine to give **2** via **18**.

Another type of oligomer-model **3** containing two units of the key disaccharide was prepared as shown in Scheme 3 using a similar procedure. Azide derivative **19**, which possesses a 4-*O*-methyl group instead of the *O*-allyl group in **7**, was prepared from **4**. In a similar procedure to **14**, an L-iduronic acid derivative



Scheme 2. (a) TBDMSOTf, MS4A/toluene, 82%; (b) OsO_4, NMO; (c) $Pb(OAc)_4$, 93% (2 steps); (d) $NaClO_2$, NaH_2PO_4; (e) $H_2N((CH_2)_2NH)_nH$, FDPP, DIEA/DMF, **17**: 58%, **18**: 63%; (f) NaOMe; (g) SO_3·pyridine complex/DMF; (h) NaOH/MeOH; (i) 10% Pd–C, H_2; (j) SO_3·pyridine complex/H_2O, pH=9.5, **1**: 61%, **2**: 40% (4 steps)

20 was prepared using *N*-Fmoc-ethanolamine as a linker moiety instead of methanol. After glycosidation of **19** with **20**, the *N*-Fmoc group was removed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The resulting disaccharide **21** with a free amino group was coupled using FDPP with the carboxyl component **16** described above, then sequential transformations of *O*-sulfation, saponification, hydrogenolysis, and *N*-sulfation were performed to give the oligomer-model compound **3**. Formation of compounds **1**–**3** was confirmed by ^{1}H NMR and high resolution ESI-MS.[9]



Scheme 3. (a) TBDMSOTf, MS4A/toluene, 80%; (b) DBU/CH_2Cl_2, quant.; (c) FDPP, DIEA/DMF, 45%; (d) NaOMe, 77%; (e) SO_3·pyridine complex/DMF; (f) NaOH/MeOH; (g) 10% Pd–C, H_2; (h) SO_3·pyridine complex/H_2O, pH=9.5, 59% (4 steps)

The platelet-binding activities of the synthetic compounds **1**–**3** were evaluated by the competitive binding assay according to our previous method with modifications. To find high-affinity site(s) in heparin to platelets, a higher concentration of ^{3}H-labelled heparin was employed instead of the ^{125}I-labelled one used previously.[2,3] In Fig. 1, the competitive binding activities of **1**–**3** were compared with those of a commercial heparin (average molecular weight 17 500, from porcine intestine, Nacalai Tesque, Kyoto, Japan) and **23** containing a single unit of NS6S-I2S, which was previously confirmed to be a structural element responsible for the platelet-binding of heparin.[3] At the concentration range tested, disaccharide **23** showed no significant activity due to the high concentration of the labelled heparin in the present

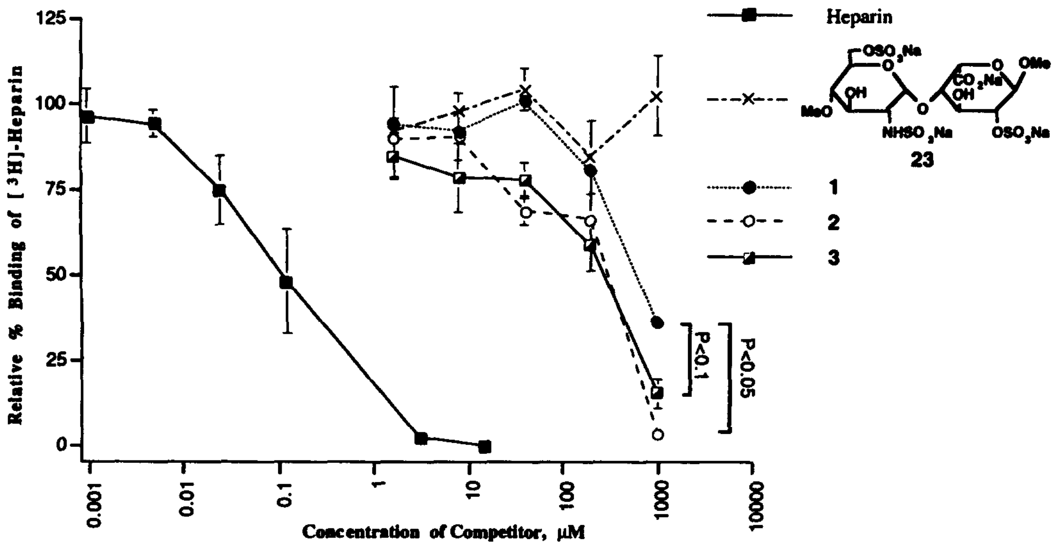


Figure 1. Binding competitive activity of oligomer-model compounds **1**, **2** and **3**, monomeric **23** and commercial heparin

assay. Even under such conditions, all oligomer-model compounds **1**–**3** exhibited distinct binding ability, although weaker than the commercial heparin of a large molecular weight. Compound **2** containing three units of NS6S-I2S showed higher binding activity than **1** containing two units. These findings clearly indicate the role of the clustering effect based on NS6S-I2S for the binding. Furthermore, the binding activity of **3** was significantly higher than that of **1**, suggesting that the relative orientation of the two units of NS6S-I2S also influence the activity.

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