Pergamon

Tetrahedron Letters, Vol. 37, No. 7, pp. 1053-1056, 1996

0040-4039/96 $15.00 + 0.00

0040-4039(95)02346-1

Synthesis and Biological Activity of a Model Disaccharide Containing a Key Unit in Heparin for Binding to Platelets

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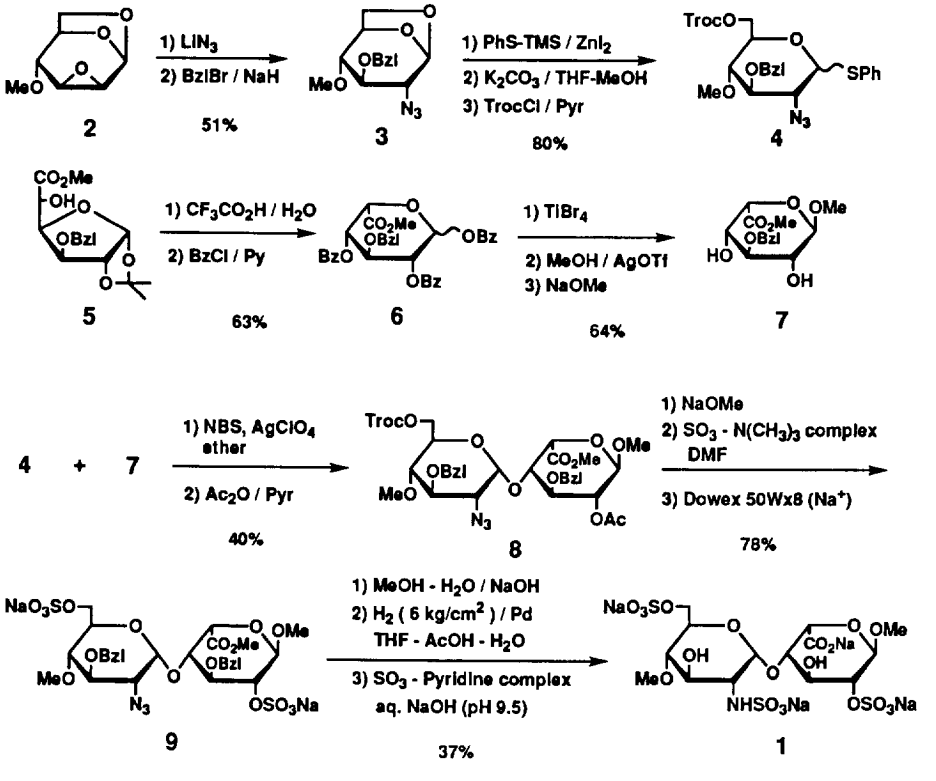
Abstract: To determine the specific site(s) in heparin necessary for binding to platelets, synthesis of a model compound containing the disaccharide sequence, O-(2-deoxy-2-sulfamido-6-O-sulfo-α-D-glucopyranosyl)-(1→4)-2-O-sulfo-α-L-idopyranuronic acid, found in heparin was performed by α-selective glycosidation using a phenyl thioglycoside as a donor. The compound inhibited ^{125}I-labelled heparin binding to human platelets to a greater extent than a heparin-derived disaccharide, obtained by the heparinase I digestion, yet contained the same number of sulfate groups per molecule.

Heparin, structurally a very heterogenous sulfated polysaccharide, has been used as an anticoagulant for over half a century.[1] Recently, however, it has been pointed out that pharmaceutical heparin binds to platelets and may directly alter platelet function and induce immuno sensitization,[2] which are undesirable side effects. Low molecular weight heparins (LMWHs), which are obtained by fractionation or depolymerization of commercial grade heparin and have lower average molecular weights (MW<10,000) than the parent heparin, possess diverse platelet reactivities,[3] but the structural basis of these differences is not clearly understood. We have shown that different methods of depolymerization yield LMWHs with disparate platelets affinities. Periodate derived LMWHs possess a higher platelet binding activity than comparable LMWHs derived by either heparinase I or nitrous acid depolymerization.[4] By considering the mechanism of depolymerization of these three methods, we predicted that a disaccharide sequence in heparin, O-(2-deoxy-2-sulfamido-6-O-sulfo-α-D-glucopyranosyl)-(1→4)-2-O-sulfo-α-L-idopyranuronic acid (abbreviated as NS6S-I2S), may be a key disaccharide in heparin responsible for heparin binding to platelets.[4] To confirm this prediction and to develop a clearer understanding of the interaction between heparin and platelets at the molecular level, we prepared a novel synthetic disaccharide, [methyl O-(2-deoxy-4-O-methyl-2-sulfamido-6-O-sulfo-α-D-glucopyranosyl)-(1→4)-2-O-sulfo-α-L-idopyranosid]uronic acid (**1**), containing NS6S-I2S.

The synthesis of the model disaccharide **1** was carried out as shown in the scheme. The key point for the synthesis was to form an α(1-4) linkage between the D-glucosamine and L-idouronic acid moieties. An azide derivative was used for the precursor of the D-glucosamine unit, instead of using D-glucosamine itself, to prevent the undesirable β-linkage.[5] According to the method reported by Carlson,[6] 1,6:2,3-dianhydro-3-O-methyl-D-glucose (**2**) was prepared. An azide group was introduced into the 2-position by reacting **2** with lithium azide.[5] The 3-hydroxyl group, which should not be O-sulfated at a later stage of synthesis, was

protected by a benzyl group to get the corresponding 1,6-anhydro-D-glucose derivative **3**. Compound **3** was reacted with phenylthiotrimethylsilane in the presence of zinc iodide,[7] followed by the hydrolysis of the 6-O-trimethylsilyl group with potassium carbonate in a mixture of tetrahydrofuran and methanol (1/1, v/v). Since the 6-O-trichloroethoxycarbonyl (Troc) group was shown to be effective for an α-predominant glycosidation in our previous work,[8] the compound was converted to a Troc-derivative to obtain the glycosyl donor **4**.

The L-iduronic acid derivative **5** was prepared from D-glucose using a modification of the procedure described by Jacquinet et al.[9] The 1,2-O-isopropylidene protective group was removed by 90% aqueous trifluoroacetic acid (r.t. for 30 min). During this acid treatment, the furanosyl derivative was converted to the pyranose form, methyl 3-O-benzyl-α- and -β-idopyranuronate, which was isolated with a yield of 68%. The L-idose derivative **6** was obtained by the treatment with benzoyl chloride (yield 63% from compound **5**). Compound **6** was converted to the corresponding bromide with titanium bromide. To fix the α-pyranosyl structure of the L-idose unit as it is in heparin, the bromide of compound **6** was reacted with methanol in the presence of silver trifluoromethansulfonate, thereby producing the methyl α-L-idopyranoside. The 2-O- and 4-O-benzoyl groups were then removed to produce the glycosyl acceptor **7**.



Scheme

Since we found that the reactivity of the 2-hydroxyl group of **7** was lower than the 4-hydroxyl group from the preliminary experiments, **7** was glycosylated without further protection. The coupling of **4** with **7** was effected using N-bromosuccinimide and silver perchlorate in ether at -20°C.[10] After the acetylation of the hydroxyl group(s) of the glycosylated products, the desired α(1-4) linked disaccharide **8** was purified by silica-gel chromatography with 40% yield. In the coupling reaction, α(1-2) linked disaccharide and α(1-2):α(1-4) linked trisaccharide were also obtained in low yields (13 and 14%, respectively). It was remarkable, however, that the linkages were all α-configuration. This high anomeric selectivity may be due to the effect of the 6-O-Troc group in the glycosyl donor, in addition to the effects of the ether as a solvent and perchlorate as a reagent.[10]

The 2-O-acetyl group and 6'-O-Troc group of **8** were removed by sodium methoxide, then the 2- and 6'-hydroxyl groups were O-sulfated using a sulfur trioxide trimethylamine complex in dimethylformamide at 50°C for 6 h.[11] The product was neutralized by passage through an ion-exchange resin (Dowex 50Wx8, Na^+ form) to obtain **9**. The sulfation of 2-O- and 6'-O-positions was confirmed by the changes in chemical shifts in the ^{1}H-NMR spectra (δ in $CDCl_3$ at 30°C): H-1 (4.91 → 4.62), H-2 (3.61 → 5.08), H-3 (3.91 → 4.28) and H-6' (3.73 → 4.19). After the treatment of **9** with a mixture of methanol and 5 M aqueous sodium hydroxide (5/1, v/v) at room temperature for 2 h, the resulting compound was hydrogenated at 6 kg/cm^2 for 4 days in the presence of palladium black in tetrahydrofuran/acetic acid/water (4/1/2, v/v/v). Finally N-sulfation was performed using a sulfur trioxide pyridine complex in aqueous sodium hydroxide (pH 9.5) at room temperature for 4 days. Formation of the desired trisulfated disaccharide **1** was confirmed by ^{1}H-NMR and negative mode FAB-MS: m/z 688.3 [a pseudo-molecular ion (M–Na)-]. Furthermore, a significant change in the chemical shift due to the N-sulfation was observed for H-2' (2.85 → 3.24).

The binding activity of **1** to platelets was evaluated by comparison with a purified disaccharide **10** (obtained by exhaustive heparinase I digestion of heparin) and a commercial heparin (from porcine intestine, Nacalai tesque, Kyoto, Japan). This structure **10** was based on the mode of action of heparinase I and determined by elemental analysis and molecular weight analysis by negative phase FAB- and ESI-MS. Homogeneity of **10** was confirmed by chromatographic profiles of high performance gel-permeation and capillary electrophoresis. The heparin-platelet competitive binding assay was performed according to a modification of a method reported previously[4] using ^{125}I-labelled heparin.[12] The results of the heparin-platelet binding assay are shown in Fig. 1.

Our previous work showed that platelet binding activity had both molecular weight and structural dependence.[4] In Fig. 1 both synthetic and heparinase-digested disaccharides (**1** and **10**) showed decreased platelet binding activity when compared to the commercial heparin (MW 17,000). The synthetic disaccharide **1**, however, demonstrated a greater inhibitory activity of ^{125}I-labelled heparin binding to platelets, thus higher platelet affinity, than the heparinase-digested disaccharide **10**, although they both contain the same number of sulfates per molecule (three). These results suggest that the unique disaccharide sequence (NS6S-I2S) contributes to specific heparin-platelet interactions. This disaccharide unit is destroyed by the specific heparinase I-digestion of heparin. The findings described here confirm our previous prediction that the NS6S-I2S sequence is a key disaccharide for heparin binding to platelets.

Acknowledgements: The authors are grateful to Mr. Masaru Miyagi at Takara Shuzo Co., Ltd (Shiga, Japan) for the analyses by ESI-MS. This work was supported in part by Organic Synthetic Society TEIJIN award (YS), by the Grant-in Aid for International Scientific Research (No. 06044138, YS) and the Grant-in Aid for Scientific Research (No. 05403035, SK) from the Ministry of Education, Science, Sports and Culture, Japan and by grant HL39903 from the National Institute of Health, USA (MS).

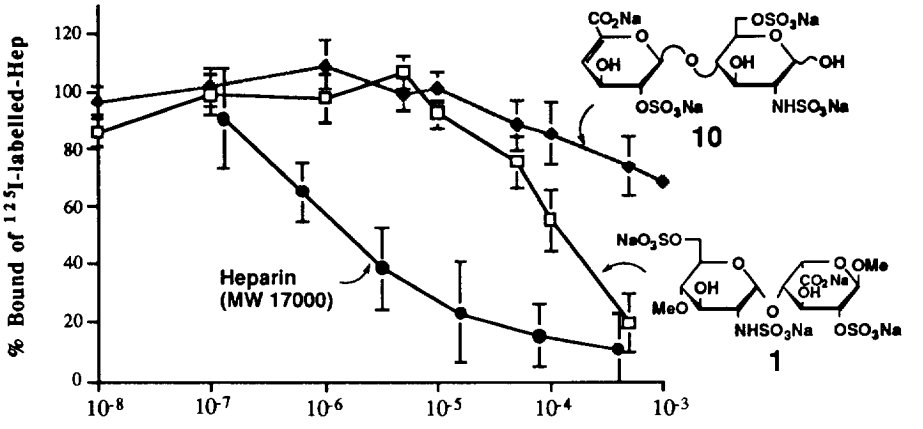


Fig. 1 Binding competition activity of disaccharides and commercial heparin.

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(*Received in Japan* 6 *November* 1995; *revised* 1 *December* 1995; *accepted* 7 *December* 1995)