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An efficient method for the assembly of sulfated oligosaccharides using reductive amination

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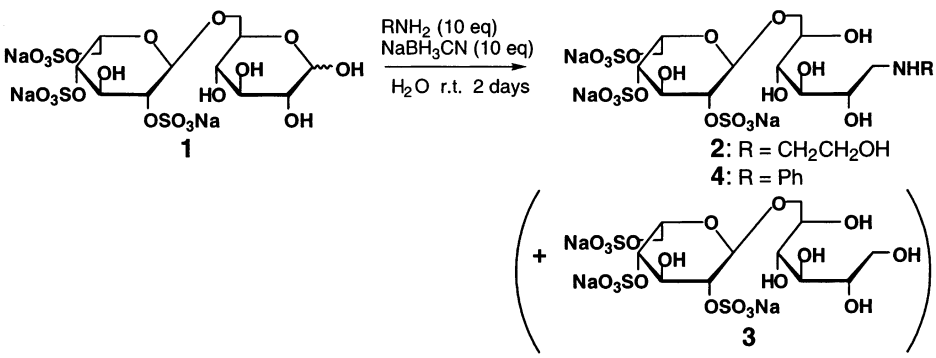
Abstract—A generally applicable efficient method was developed for assembly of structurally defined sulfated oligosaccharides. Using a reductive amination reaction at low pH with newly designed linkers possessing multiple aromatic amino groups, facile preparations of oligosaccharide assemblies were achieved without any alteration in the sulfated oligosaccharide parts. © 2001 Elsevier Science Ltd. All rights reserved.

Glycosaminoglycans (GAGs) have a number of biological functions that can be attributed to specific partial structures in the GAG chain. The weak interactions of individual subunit structures are often enhanced by their specific repetition, which is called a clustering or multi-valency effect.[1–3] For an in-depth study of the role of specific subunits, the preparation of clustered compounds is useful to obtain high-affinity ligands. Reductive amination is one efficient method for the coupling of oligosaccharides. In the past, proteins were used as carriers for oligosaccharides. But coupling occurs at unidentified amino groups in the protein, and that method gives a mixture of heterogeneous products having different numbers of carbohydrate units.[4–6] For our studies of the structure-function relations between heparin and vascular cells and proteins,[7,8] it is crucial to use structurally defined ligands possessing specific subunit structures in heparin.

We designed structurally defined carriers with aromatic amino groups as the point of linkage to carbohydrates. To establish the reaction conditions for the reductive amination, a synthetic sulfated disaccharide **1**[9] was employed as a model compound and sodium cyanoborohydride ($NaBH_3CN$) as a reducing reagent. The reagent has been widely used in the reductive amination with remarkable efficiency.[10,11] In the first attempt using a large excess (10 equiv.) of ethanolamine (Scheme 1, $R = CH_2CH_2OH$), the reaction did not proceed at neutral or acidic conditions. At higher pH around 9–10, the desired reductive amination did occur to give the coupling product **2**, but the yield was not



Scheme 1.

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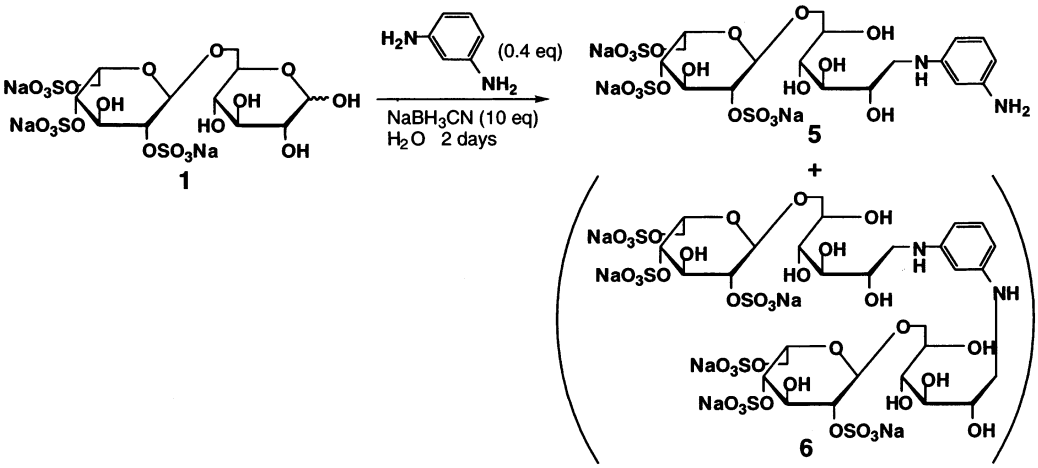
satisfactory due to the competitive reduction of the anomeric position, giving the glucitol derivative **3** as a major by-product. To favor the reductive amination, an amine derivative capable of forming a Schiff base at neutral or acidic pH range was desirable. The reaction of **1** (Scheme 1, R = Ph) with aniline ($pK_a = 4.65$) instead of ethanolamine ($pK_a = 9.52$) was performed using $NaBH_3CN$. This coupling proceeded smoothly at pH 7 to yield the desired product **4** without the formation of the by-product **3**.

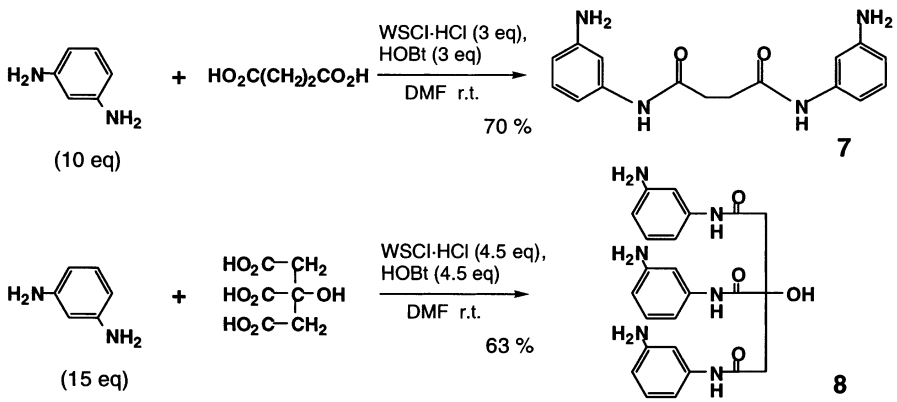
Then, the preparation of a multimeric oligosaccharides assembly by reductive amination was attempted using a divalent aromatic amine (Scheme 2). Since $NaBH_3CN$ can be used even at a pH range as low as 3,[10,11] the coupling reaction was tested at pH 3 in water–AcOH at 37°C.[12] In this case, the mono-coupled **5** was obtained in much higher yield than that at pH 7 as judged from ESI–MS, but the desired dimeric-product **6** was still not encountered at all. Reaction at a higher temperature (80°C) did not improve the results.

Although one unit of **1** was coupled with *m*-phenylenediamine almost quantitatively at pH 3, the dimeric assembly of the sulfated saccharide failed. This seems to be caused by the steric hindrance and/or the electric repulsion of the sulfated saccharide units. Therefore, a connection of *m*-phenylenediamine units using an amido spacer was next attempted. Based on this idea, linkers (**7** and **8**) having free amino groups derived from phenylenediamine moieties at the ends were prepared in one or a few steps without any protection, as illustrated in Scheme 3. A variety of more complex linkers can also be prepared using the appropriate multivalent carboxylic acids.

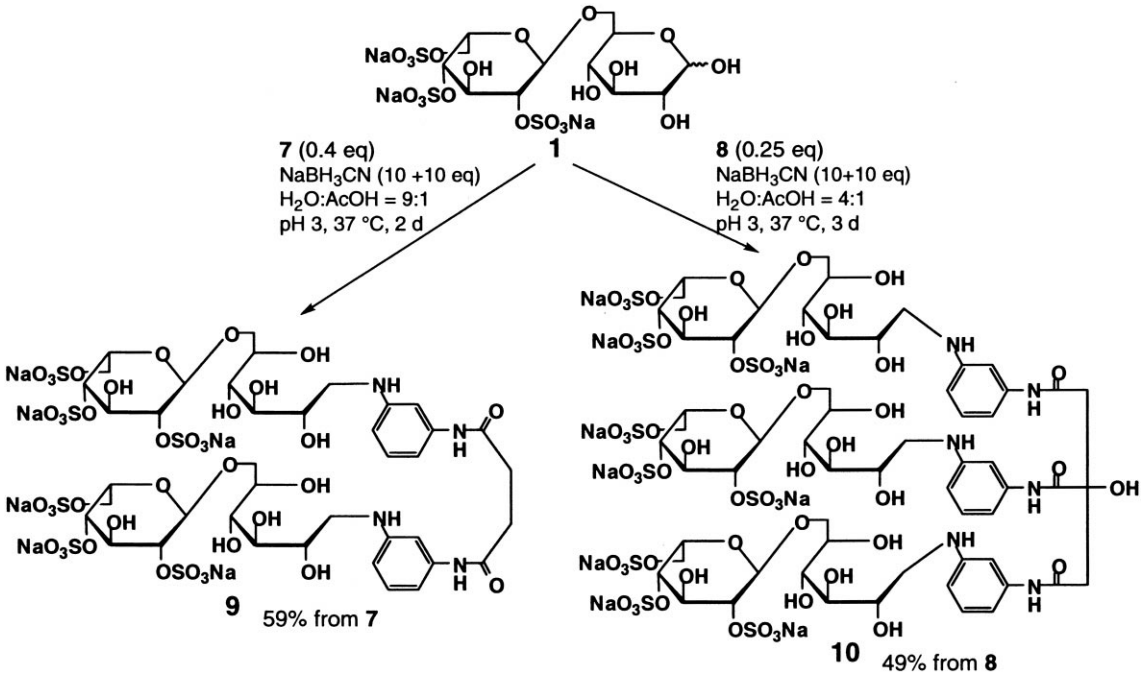
The coupling reaction of **1** with the divalent linker **7** (0.4 equiv. to **1**) was performed at pH 3 in water–AcOH at 37°C, and the reaction mixture was then purified by gel permeation chromatography (Sephadex G-50) to give the dimeric assembly **9** with a 59% yield (Scheme 4)[13] as confirmed by ^1H NMR and ESI–MS.[14] With the trivalent linker **8**, the reaction at all the three amino groups proceeded equally well to give the trimeric assembly **10**.[14] No loss of sulfate groups was observed in either case. In this manner, an easy and direct assembly of sulfated saccharides by reductive amination was made possible by using these newly designed linker compounds. This was archived under acidic conditions, without undesirable direct reduction of the reducing ends of the carbohydrate. Using the same linkers and reaction conditions, other sulfated disaccahrides, which were derived from a natural sulfated polysaccharide heparin, were also assembled.[15]



Scheme 2.



Scheme 3.



Scheme 4.

Furthermore, neutral oligosaccahrides, such as lactose or maltose, were easy to be assembled by a similar reaction conditions (data not shown). Therefore, this method may be applicable not only to sulfated saccharides but also to any oligosaccharides from natural or synthetic sources, provided that they have reducing ends.

Acknowledgements

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9. Sulfated disaccharide **1** was synthesized from the intermediate in the accompanying manuscript (see Ref. 15)
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12. No coupling reaction with *m*-phenylenediamine (0.4 equiv. to **1**) occurred, however, the most of starting material was recovered under the same conditions applied for aniline (pH 7, at room temperature). The coupling of **1** with *m*-phenylenediamine proceeded at 37°C, but only one of the amino groups of the diamine reacted to give a small amount of **5**: no desired dimeric assembly **6** was obtained and most of **1** recovered.
13. Linker **7** (0.92 mg, 3.08 μmol) and **1** (5.0 mg, 7.71 μmol) were dissolved in a mixture of water (0.9 mL) and acetic acid (0.1 mL). To the solution was added sodium cyanoborohydride (4.80 mg, 76.4 μmol), and the solution was heated at 37°C in a sealed tube. After heating for 24 h, sodium cyanoborohydride (4.80 mg, 76.4 μmol) was added, and the solution was heated again at 37°C in a sealed tube. After heating for further 24 h, the reaction solution was concentrated in vacuo. The residue was dissolved in water and $NaHCO_3$ was added, and chromatographed on Sephadex G-50 (1.7 x 90 cm, water) to give **9** as a white powder (2.84 mg, 58.9% from **7**, 47.1% from **1**).
14. Spectral data for compound **9**: ^{1}H NMR (500 MHz, D_2O), δ 7.17 (2H, t, $J=8.0$ Hz), 6.83 (2H, s), 6.78 (2H, d, $J=7.6$ Hz), 6.61 (2H, d, $J=7.6$ Hz), 4.96 (2H, s), 4.43 (2H, dd, $J=2.3$ Hz, $J=8.8$ Hz), 4.25–4.16 (8H, m), 4.12 (2H, dd, $J=8.9$ Hz, $J=11.8$ Hz), 3.90 (2H, m), 3.84 (2H, m), 3.74 (2H, m), 3.70 (2H, dd, $J=2.4$ Hz, $J=7.6$ Hz), 3.65–3.58 (2H, m), 3.57 (2H, dd, $J=6.1$ Hz, $J=10.7$ Hz), 3.31 (2H, b), 3.08 (2H, b), 2.68 (4H, s); ESI–MS (negative) m/z 714.10 [$(M-6Na+4H)^{2-}$]. Spectral data for com-

pound **10**: ^{1}H NMR (500 MHz, D_2O), δ 7.15 (3H, t, $J=8.1$ Hz), 6.76–6.71 (6H, m), 6.62–6.57 (3H, m), 4.99 (1H, s), 4.98 (2H, s), 4.47–4.43 (3H, m), 4.25–4.17 (12H, m), 4.16–4.11 (3H, m), 3.97–3.90 (3H, m), 3.90–3.80 (6H, m), 3.74 (3H, m), 3.71–3.65 (3H, m), 3.57 (3H, dd, $J=6.0$ Hz, $J=11.4$ Hz), 3.26 (2H, ddd, $J=3.4$ Hz, $J=4.4$ Hz, $J=13.4$ Hz), 3.19 (1H, dd, $J=3.4$ Hz, 13.4 Hz), 3.07–2.94 (7H, m, overlapped with the doublet peak at 3.04 ppm), 3.04 (d, 14.1 Hz), 2.84 (4H, d, $J=14.5$ Hz); ESI–MS (negative) m/z 719.14 [$(M\text{-}9Na+6H)^{3-}$].

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