Polymer-supported oligosaccharide synthesis using ultrafiltration methodology†

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Polymer-supported oligosaccharide synthesis was carried out using an ultrafiltration technique in which the synthesized polymer-bound oligosaccharides were separated from the other reagents by ultrafiltration though membranes with specifically sized pores.

The identification of functional roles of carbohydrates in biological systems has been an area of intense study in recent years,[1] resulting in a requirement for efficient access to oligosaccharides and glycoconjugates. For this reason, there have been many attempts to achieve rapid oligosaccharide synthesis over the last decade.[2] Although automated synthesis of solid-phase peptides and nucleotides has become routine, the establishment of automated oligosaccharide synthesis has begun only recently.[3]

Typically, rapid oligosaccharide synthesis has been achieved *via* solid-phase, polymer-supported, tag-supported, or fluorous-tag-supported reactions.[4] We carried out polymer-supported rapid oligosaccharide synthesis using low-molecular-weight poly(ethylene glycol)methyl ether (PEG) and demonstrated its effectiveness by synthesizing biologically active oligosaccharides with complex structures.[5] With the aim of expanding the possibilities of polymer-supported oligosaccharide synthesis, we report here an alternative polymer-supported methodology aided by ultrafiltration purification technology.

Ultrafiltration technology is currently used in polymer-immobilized recyclable catalysis systems and in the biochemistry field.[6,7] Using ultrafiltration techniques, substrates can be separated based on molecular weight by filtration though membranes (Fig. 1). High-molecular-weight species remain on the membrane, while substrates of lower molecular weights are washed out though specifically sized pores. When oligosaccharides are synthesized bound to high-molecular-weight soluble polymer, separation of polymer-bound oligosaccharides from the donor, by-products, and reagents can be achieved using this type of filtration. To demonstrate this concept, we synthesized polylactosamine oligosaccharide, widely found in nature.

Lactosamine donor **3** was synthesized from diol[8] **1** *via* selective benzoylation of the primary hydroxy group in 89% yield and subsequent chloroacetylation of the secondary hydroxy group. After removal of the *p*-methoxyphenyl group at the anomeric position under oxidative conditions, the resulting hemiacetal was

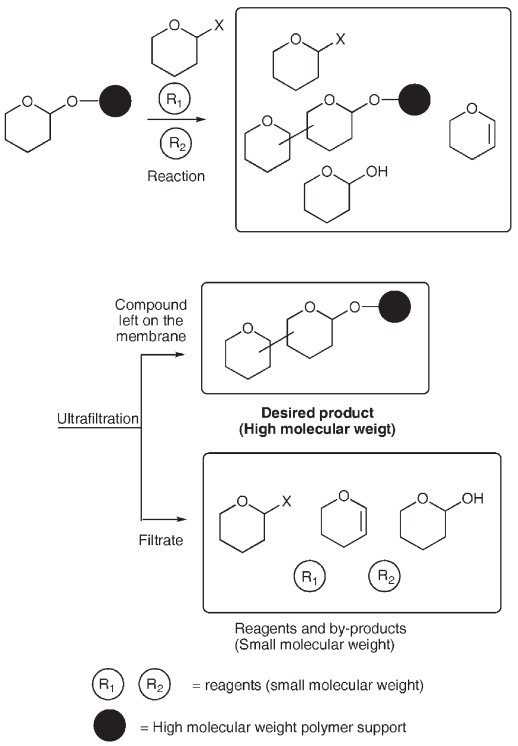
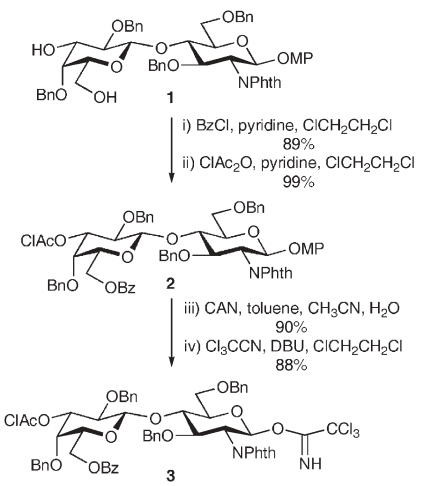


Fig. 1 Concept of ultrafiltration-dendrimer supported oligosaccharide synthesis.



Scheme 1 Synthesis of glycosyl donor.

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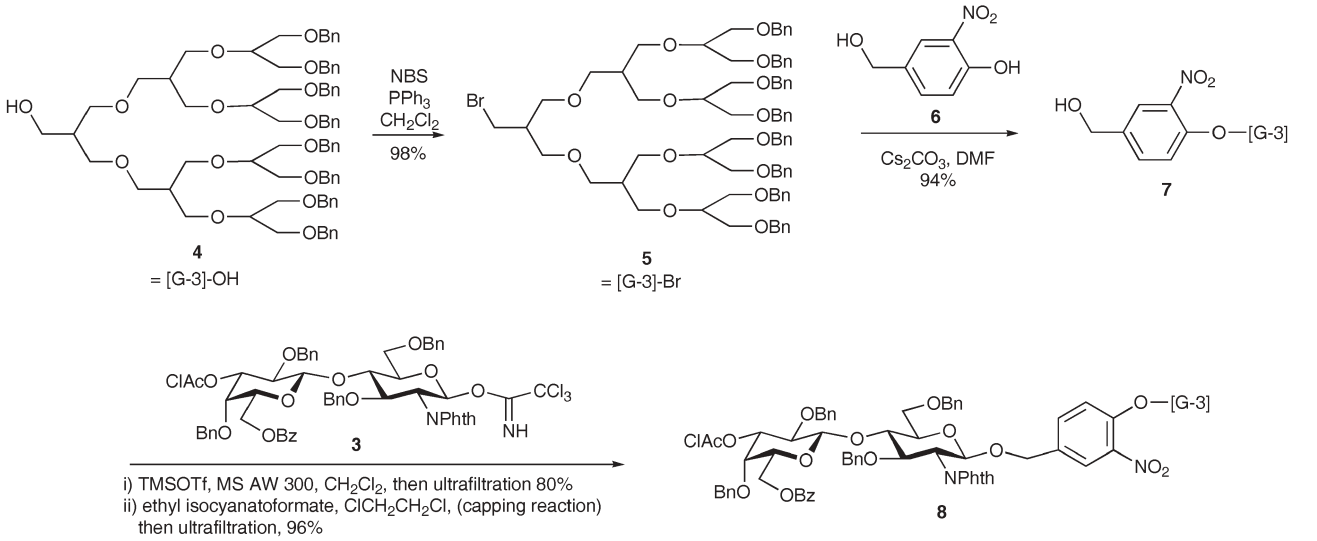


Scheme 2 Synthesis of dendrimer-bound disaccharide.

transformed to imidate **3** in good yield (Scheme 1). The polymer chosen to increase the molecular weight of the oligosaccharide was polyether type dendrimer **4**, developed by Jayaraman and Fréchet.[9] It was thought that the branched structure would be superior to that of single-strand linear polyethylene glycol ether in the ultrafiltration process, and the ether group does not interfere with the glycosylation, capping, or deprotection reactions necessary for rapid oligosaccharide synthesis. Compound **6**, a nitro-group linker which is stable under Lewis acidic conditions, as used in our previous work[10] was immobilized with dendrimer bromide **5** in 94% yield. Next, glycosylation of lactosamine imidate **3** was carried out under Lewis acidic conditions (Scheme 2). After glycosylation, the crude mixture was purified by ultrafiltration though a membrane under medium pressure.[11] Because we used a membrane with a molecular weight cutoff of 1000, the dendrimer-bound sugar was remained on the membrane (Fig. 2). Any unreacted hydroxy groups from the glycosylation reaction were then capped by reaction with ethyl isocyanatoformate[12] to avoid the formation of unnecessarily complex mixture. Next, selective deprotection of the chloroacetyl group was conducted using DABCO (Scheme 3).[13] A ultrafiltration purification process was then carried out, and the polymer-bound oligosaccharide remained on the membrane, while DABCO and side-products were washed out in the filtrate. A second glycosylation reaction was then performed in quantitative yield. All reaction steps were monitored by conventional TLC analysis.[14] The MS spectra during the ultrafiltration purification process is shown in Fig. 3. Finally, the tetrasaccharide **10** was removed from the dendrimer in 84% yield *via* a sequence of nitro group reduction,[5f] acetylation, and acid hydrolysis.

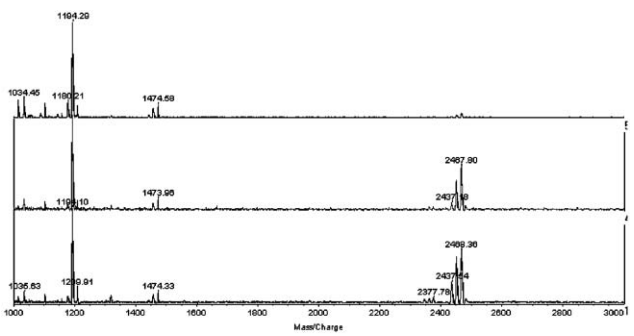
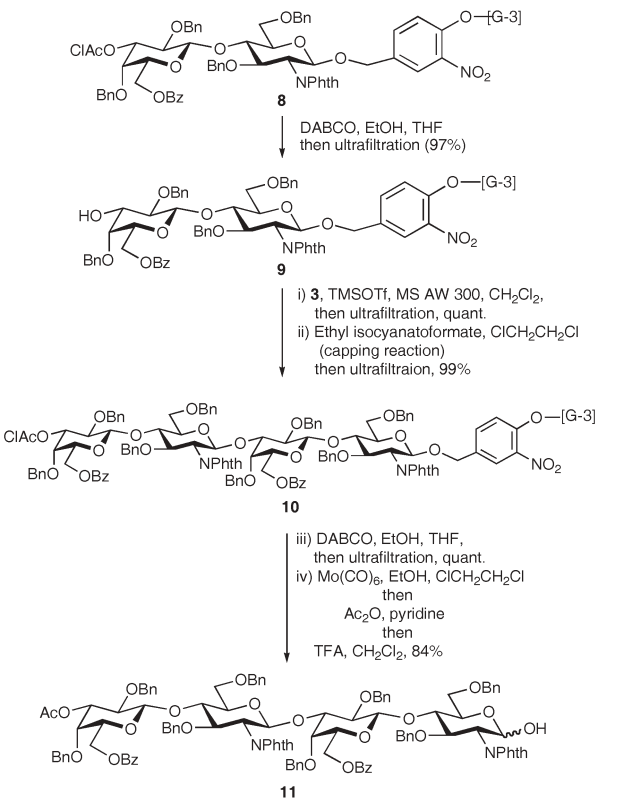


Fig. 2 The MS spectra after the glycosylation reaction between **3** and **7**. The bottom MS spectrum is for the crude mixture. The middle MS spectrum is for the portion on the membrane. The upper MS spectrum is for the filtrate. The peak of 1194 is for **3** (MW = 1157 + 39 (K)), 1473 is for **8** (MW = 1451 + 39 (K)).



Scheme 3 Tetrasaccharide synthesis.

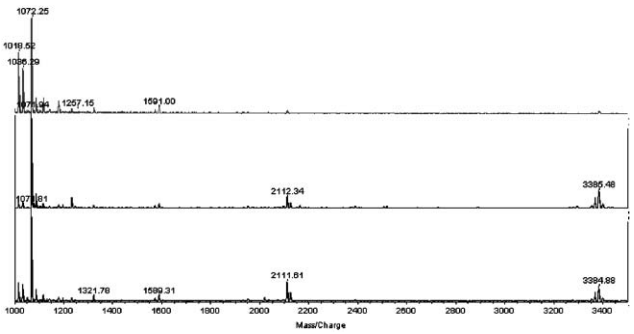


Fig. 3 The MS spectrum after glycosylation reaction from disaccharide **9** to tetrasaccharide **10**. The bottom MS spectrum is for the crude mixture, the middle MS spectrum is for the compound remaining on the membrane, and the upper MS spectrum is for the filtrate.

We have demonstrated polymer-supported oligosaccharide synthesis with ultrafiltration methodology. For a useful rapid oligosaccharide synthesis, several features are required: (I) the reactivity of the polymer-bound substrate should remain high; (ii) after the reactions, separation of the polymer-bound substrate should be simple and efficient due to the characteristic nature of polymers; (iii) it should be easy to monitor the progress of the reaction. Our new system of ultrafiltration-aided oligosaccharide synthesis fulfils the above criteria. Purification of the dendrimer-bound oligosaccharide was possible not only by ultrafiltration but also by silica gel column chromatography. Although the size selectivity and stability of the membrane towards certain organic solvents are still insufficient, we believe that with further investigation on this methodology has potential as a method of rapid oligosaccharide synthesis.

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