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Synthesis of Oligosaccharides on Soluble High-Molecular-Weight Branched Polymers in Combination with Purification by Nanofiltration

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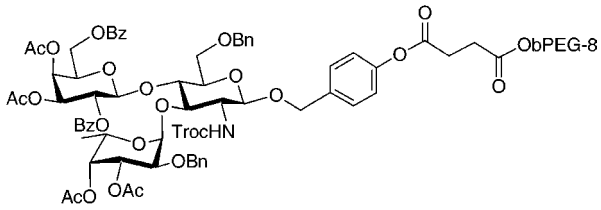
ABSTRACT



An efficient approach for polymer-supported oligosaccharide synthesis is described whereby branched and high-molecular-weight PEG derivatives are used in combination with purification by nanofiltration. This methodology was applied to the preparation of a tetraglucoside and the tumor-associated antigen Le^x.

Solid-supported synthesis provides a powerful means of preparing oligopeptides, oligonucleotides, and many small molecules.[1] These procedures eliminate time-consuming workup steps and allow the use of excess reagents to drive reactions to completion, as well as providing an opportunity for automation. Consequently, complex compounds can be synthesized within a matter of days rather than weeks or months.

The development of polymer-supported oligosaccharide synthesis has been slow, and, although the first attempts were reported in the 1970s, their success was limited and only simple di- and trisaccharides could be obtained.[2,3] These disappointing results were primarily a consequence of the absence of efficient methods for glycosidic bond formation. During the last 15 years, many powerful glycosylation approaches have been developed,[4−6] and this progress has allowed the preparation of more complex oligosaccharides by solid-supported procedures. These developments have resulted in the first example of an automated synthesis of a heptaglucoside.[7,8]

Despite these impressive advancements, polymer-supported oligosaccharide synthesis is still afflicted by many shortcomings. For example, glycosylations on a solid support often require repetitive glycosylations with large excesses of glycosyl donors or acceptors to drive reactions to completion. This represents a serious drawback since oligosaccharide chemistry requires elaborate and expensive glycosyl donors and acceptors. In addition, the rate of a reaction on a solid support is often considerably slower compared to similar solution-based reactions, making it difficult to extrapolate solution-phase conditions to solid-supported procedures.

The problems of slow reactivity and the required use of a large excess of reagents have been addressed by replacing

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insoluble cross-linked resins with *soluble* polymeric supports.[9] In this way, the reaction conditions typical of classical organic reactions are employed, while product purification can be facilitated by taking advantage of the macromolecular properties of the polymer. Poly(ethylene glycol) methyl ether (MPEG) is the most widely used polymer for liquid-phase oligosaccharide syntheses. This polymer is soluble under glycosylation and protecting group manipulation conditions but can be made insoluble during a workup procedure by the simple addition of diethyl ether or *tert*-butylmethyl ether.[10−25] Several problems are, however, associated with MPEG-supported approaches; for example, this methodology is hampered by low loading of saccharide onto MPEG and difficulties associated with selective precipitation when large saccharides are attached to the polymer, and it is not amenable to automation. These problems need to be urgently addressed if soluble-polymer-based methods are to be competitive.

Here we report a new and more efficient approach for soluble polymer-supported oligosaccharide synthesis whereby branched and high-molecular-weight PEG derivatives are used in combination with purification by nanofiltration.

Innovation in membrane technology has given rise to advanced filtration equipment containing specific pore sizes. Membrane filters are now available in a range of pore sizes, suitable for a specific application. The development of membranes compatible with organic solvents has been slower than those used for aqueous systems. However, there are now several commercially available membranes that are compatible with a range of organic solvents, and the most promising one is a Biomax membrane with a nominal molecular weight limit of 5000 Da. Thus, it was anticipated that oligosaccharides attached to a polymer of sufficiently high molecular weight can be retained by nanofiltration, and it was expected that branched poly(ethylene glycol) derivatives would be the most promising for this purpose (Figure 1).

To develop the new methodology, we set out to synthesize tetrasaccharide **10** using 4-armed PEG **4a** of MW = 10 000 Da, which is derivatized with an oxy benzoic acid function for attachment and cleavage of saccharides (Scheme 1).[15,16]

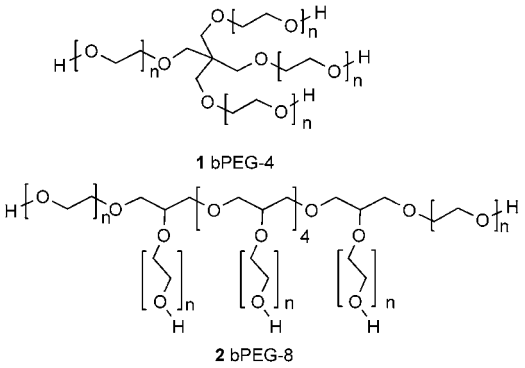
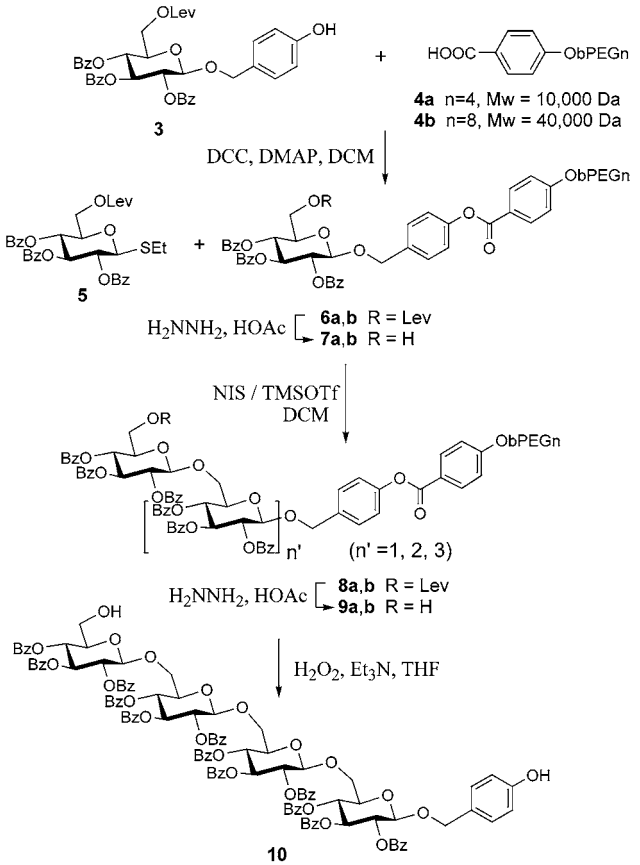


Figure 1. Structures of 4- and 8-armed PEG derivatives.

Scheme 1



Thus, glucoside **3**, which has a selectively removable levulinoyl ester (Lev) at C-6 and an anomeric *p*-hydroxybenzyl moiety, was coupled with **4a** using DCC and DMAP in dichloromethane to give immobilized **6a** (Scheme 1). Unfortunately, polymer **6a** could not be retained by nanofiltration using a Biomax filter with a nominal molecular weight limit of 5000 Da. However, polymer **6a** could be

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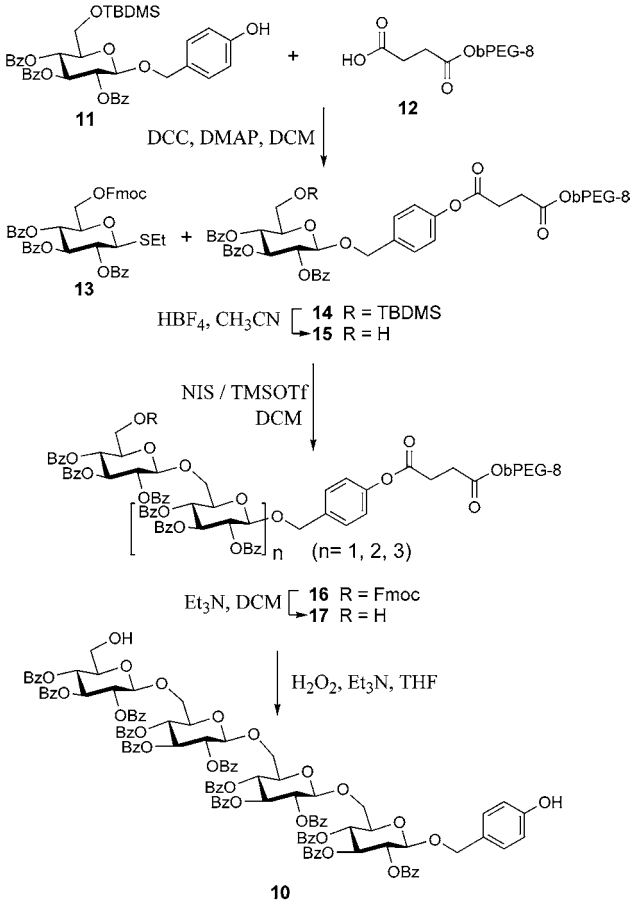
purified by either selective precipitation using diethyl ether or size exclusion column chromatography using Sephadex LH-20. ^{1}H NMR analysis of **6a** showed the absence of excess reagent and any side product, and furthermore, integration of key signals indicated that the coupling had proceeded to completion. The Lev group of **6a** could easily be removed by treatment with hydrazine acetate in dichloromethane to give polymer-bound acceptor **7a**. *N*-Iodosuccinimide (NIS)/trimethylsilyl triflate (TMSOTf)[26]-mediated coupling of **7a** with thioglycosyl donor **5** gave, after purification by precipitation or size exclusion column chromatography, polymer-bound disaccharide **8a** ($n' = 1$). It is important to note that only 1.5 equiv of glycosyl donor was required to achieve quantitative glycosylation. Furthermore, only the β-glycoside was formed due to neighboring group participation of the C-2 benzoyl ester of **5**. The process of Lev removal and glycosylation with **5** was repeated twice to give a polymer-bound tetrasaccharide, which was cleaved from the support by hydrolysis of the phenolic ester linkage using H_2O_2 and Et_3N in THF for 24 h[15,16] to give, after purification by Sephadex LH-20 size exclusion column chromatography, compound **10** in an overall yield of 24%.

Although four-armed PEG **4a** has the advantage of double the loading capacity of traditionally used MPEG (MW = 5000 Da), it did not meet our requirement of being compatible with purification by nanofiltration. To address this problem, the use of branched PEG derivative **4b** that has eight arms and a molecular weight of 40 000 Da was explored. In this case, coupling of **4b** with **3** using standard conditions gave crude **6b**, which was dissolved in a mixture of methanol/water (1/9, v/v) and applied to a Millipore high-pressure reactor that was fitted with a Biomax filter with a MW cutoff of 5000 Da. In this case, the polymer-bound saccharide could be retained and excess reagent could conveniently be removed by washing with a mixture of methanol/water. The Lev group of **6b** could be removed by treatment with hydrazine acetate in dichloromethane, and after purification by nanofiltration, the resulting polymer-bound acceptor **7b** was coupled with thioglycosyl donor **5** to give polymer-bound disaccharide **8b** ($n' = 1$). In this case, the polymer **8b** was dissolved in acetonitrile and purified by nanofiltration over a Biomax 5000 Da filter. The process of Lev removal and glycosylation was repeated twice, and after each reaction step the product was purified by nanofiltration. Finally, the resulting polymer-bound tetrasaccharide (**9b**, $n' = 3$) was released from the polymeric support by treatment with H_2O_2 and Et_3N in THF, but in this case, the reaction took more than 7 days to complete and mass spectrometric analysis of the product showed that part of the compound had lost one or more benzoyl groups. It appears that the phenolic ester linkage of **9b** is sterically more hindered than that of the four-armed counterpart **9a**, slowing the hydrolysis of the phenolic ester linkage to give **10**.

Although, the partially debenzoylated **10** could be converted into one compound by treatment with benzoyl chloride in pyridine, it was decided to explore another phenolic ester linker that can be cleaved under milder conditions. Recently, we found that a phenolic succinyl ester linker can be cleaved within a few minutes, whereas the corresponding hydroxybenzoyl oxybenzoate linker requires 18 h for cleavage.[15,16] Thus, it was anticipated that the use of succinyl-modified branched PEG **12** (Scheme 2) would allow more facile

Scheme 2



cleavage of a final compound from the polymer support. Thus, polymer **12** was coupled with **11** to give **14**, which was purified by nanofiltration using water/methanol (9/1, v/v) as solvents. The TBDMS group of **14** was cleaved by treatment with HBF_4 in acetonitrile, and after purification by nanofiltration, the polymer-bound acceptor **15** was coupled with thioglycosyl donor **13** using NIS/TMSOTf as the promoter system[26] to give **16** ($n = 1$), which was purified by nanofiltration using acetonitrile as the solvent system. The 9-fluorenylmethoxycarbonyl (Fmoc) protecting group of **16** was removed by treatment with Et_3N in CH_2Cl_2,[27] and the resulting glycosyl acceptor **17** ($n = 1$) was coupled with **13** to give a polymer-bound trisaccharide **16** ($n = 2$).

Selection of Fmoc as a temporary protecting group was important because conditions for the removal of Lev resulted

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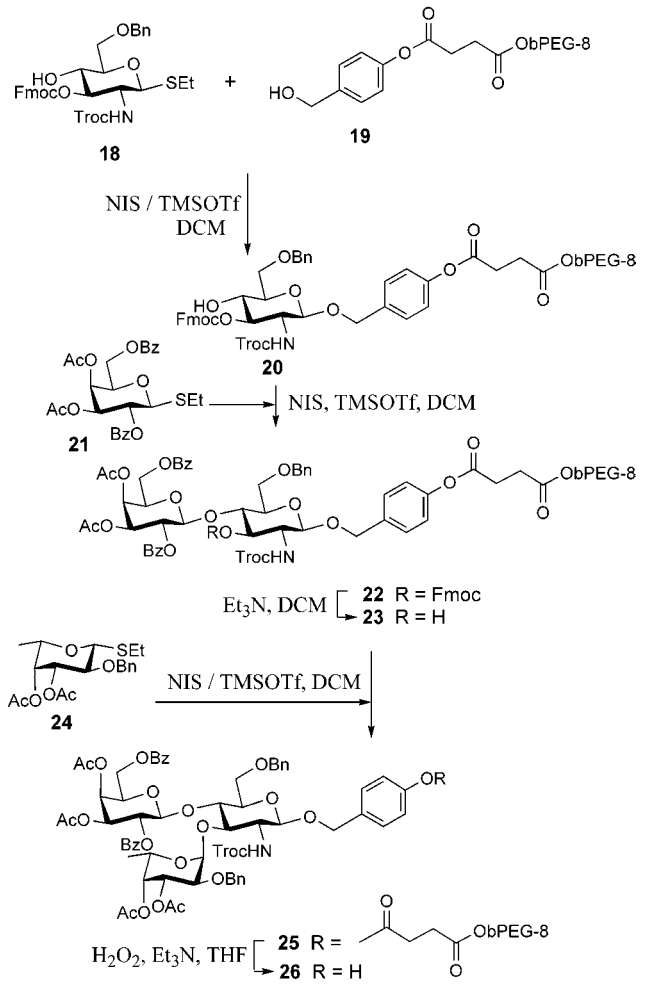
Scheme 3



in partial cleavage of the succinyl linker. The process of Fmoc removal, glycosylation, and Fmoc removal was repeated and the resulting tetrasaccharide was released from the polymeric support by treatment with $NH_2NH_2 \cdot CH_3COOH$, and in this case, **10** was obtained as a single compound in an overall yield of 40%.

Having established that an eight-armed PEG derivative in combination with a phenolic succinyl linker is convenient for oligosaccharide synthesis, attention was focused on the preparation of the tumor-associated antigen Lewis^x using a previously reported glycosylation strategy.[15] Thus, in this case, a trichloroethoxycarbonyl (Troc)-protected glucosamine derivative **18**[15] was attached to the eight-arm branched PEG polymer **19**, having a phenolic succinyl linker, by NIS/TMSOTf-mediated glycosylation[26] to give polymer-bound sugar **20**, which was immediately used in the next glycosylation without workup by addition of thiogalactosyl donor **21**[15] and another quantity of NIS/TMSOTf.[26] The glycopolymer was purified by nanofiltration, and the resulting **22** was treated with Et_3N in dichloromethane to remove the Fmoc group[27] to give glycosyl acceptor **23**, which was purified by nanofiltration and then glycosylated with fucoysl donor **24**[15,28] to give polymer bound trisaccharide **25**. The use of 1.5 equiv of glycosyl donor **24** resulted only in partial glycosylation, and the reaction had to be repeated to drive it to completion. The polymer-bound material could conveniently be cleaved from the polymer support using standard conditions to give, after purification, **26** in an overall yield of 35%. Oxidative removal of the *p*-hydroxyl benzyl moiety of **26** with DDQ gave a lactol,[15] which can be easily converted into a glycosyl donor or deprotected as described previously (Scheme 3).

In conclusion, it has been demonstrated that commercially available branched PEG derivatives are convenient for polymer-supported oligosaccharide synthesis. The use of the four-armed PEG derivative **4a** (MW = 10 000 Da) is attractive because it has a higher loading capacity than traditionally used MPEG (MW = 5000 Da), and, in this case, synthetic intermediates could be purified by precipitation or size exclusion column chromatography. Compounds synthesized on an eight-armed PEG derivative that has an average molecular weight of 40 000 Da could be purified by nanofiltration over a Biomax filter that has a nominal molecular weight limit of 5000 Da. Nanofiltration is more convenient than precipitation and will provide a future opportunity for automation. This new technology allowed the preparation of a branched trisaccharide of biological importance that has α- and β-glycosidic linkages.

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Supporting Information Available: Experimental procedures, ^{1}H NMR spectra, and HRMS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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