Carbohydrate-Based Crosslinking Agents: Potential Use in Hydrogels

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INTRODUCTION Tissue engineering requires the use of polymer scaffold biomaterials—made from either natural or synthetic polymers—that are biocompatible, porous, and biodegradable. One synthetic polymer in particular, poly(2-hydroxyethyl methacrylate) (PHEMA), has an established history of use as a biomaterial in the field of medical devices[1–5] but has also received attention as a potential scaffold material for tissue engineering purposes.[2,6–8]

The wide-spread use of PHEMA materials has been attributed to its excellent biocompatibility[2] and the fact that it can, by polymerization-induced phase separation, be easily made in macroporous forms having morphologies suitable for tissue engineering applications.[2,8–12] However, the use of PHEMA hydrogels for tissue engineering purposes is limited because PHEMA itself is not biodegradable. Fortunately, it has recently been shown that incorporation of biodegradable elements, namely peptide-based crosslinking agents, into PHEMA-based networks renders PHEMA-based materials biodegradable.[8,13] Peptide-based crosslinking agents impart biodegradability because they can be cleaved enzymatically, enabling a network polymer to fragment into soluble, linear degradation products.

Peptides have been targeted as crosslinking agents because: they are relatively easy to synthesize; there are many enzymes that can be used to cleave peptide-based crosslinking agents; the degradation properties of peptides can be tuned by selecting, for example, peptide sequences that will be targeted by particular enzymes;[8] and as peptides are naturally occurring biomacromolecules, cytotoxicity issues are unlikely for peptide-based crosslinking agents. To widen the potential for biodegradable biomaterials to be used in tissue engineering applications, there is ongoing research to develop new and more specific ways to impart biodegradability. For this reason, carbohydrates represent another class of naturally occurring biomacromolecules that has been exploited to render hydrogels biodegradable. The carbohydrates class is comprised of a broad range of compounds, ranging from monosaccharides to polysaccharides. Various polysaccharides (e.g., chitosan) have been used in the formation of hydrogels,[14,15] and have been incorporated into polymer networks to form polymer-polysaccharide hydrogel conjugates.[16,17]

Although there are many reports on using polysaccharides to form hydrogels, there are only a few reports on using monosaccharide- to oligosaccharide-based crosslinking agents to form hydrogel conjugates.[18,19] This paucity of research in this area is most likely a consequence of the difficulty of synthesizing specific carbohydrate-based compounds—the large number of hydroxyl groups on a saccharide unit dictates the use of extensive protection and deprotection chemistry when a specifically functionalized carbohydrate is required. Nevertheless, the differences between carbohydrates and peptides (e.g., glycosidic linkages vs. peptide bonds) means that hydrogels and other biomaterials involving cleavable carbohydrate-based groups may have advantages (e.g. propensity for degradation by different classes of enzymes, different rates of degradation) over similar peptide-based materials in certain applications.

Of particular interest to us are carbohydrate-based crosslinking agents, especially monosaccharide- to oligosaccharide-based crosslinking agents that could be used as components of PHEMA hydrogels. Although there have been reports on the use of 2-hydroxyethyl methacrylate (HEMA) carbohydrate conjugates as monomers, and their corresponding polymers,[20–25] to the best of our knowledge, there are no reports of monosaccharide- to oligosaccharide-based crosslinking agents. For this reason, we have developed a synthetic route to a glucopyranose-based crosslinking agent as a proof-of-concept investigation to

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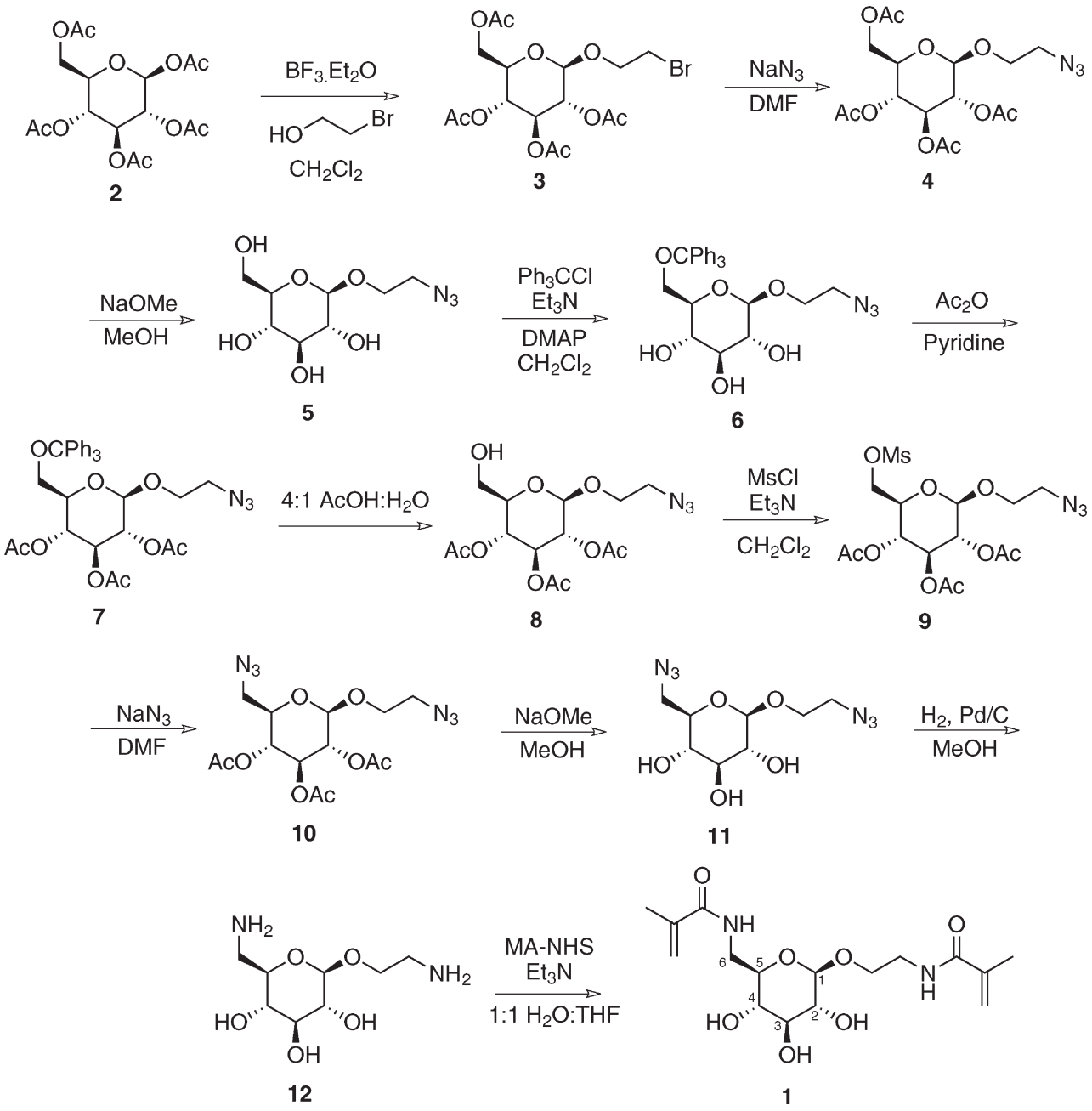

SCHEME 1 Synthesis of the carbohydrate-based crosslinking agent 1.

explore the use of monosaccharide-based crosslinking agents in PHEMA-based hydrogels. This proof-of-concept study opens the way for more elaborate carbohydrate-based crosslinkers to be used in the preparation of materials for tissue engineering. Polymers that use carbohydrate-based crosslinkers as enzyme-cleavable units will allow researchers to design new biodegradable materials that can be degraded by different classes of enzymes compared to polymers incorporating well-established peptide-based crosslinkers.

EXPERIMENTAL

The materials, details for preparation of the carbohydrate-based crosslinking agent, polymerization methods, and characterization techniques are described in Supporting Information.

RESULTS AND DISCUSSION

To explore the potential for carbohydrate-based crosslinking agents to be used in the synthesis of PHEMA-based materials, the crosslinker **1** was devised (Scheme 1). First, using a previously reported procedure,[26] the glycoside **3** was prepared using 2-bromoethanol and the pentaacetate **2**. Treatment of **3** with sodium azide gave the azide **4** in excellent yield. With one azide already installed at the C1-position, we turned our attention to the installation of the next azide at the C6-position. The azide **4** was treated with catalytic sodium methoxide to yield

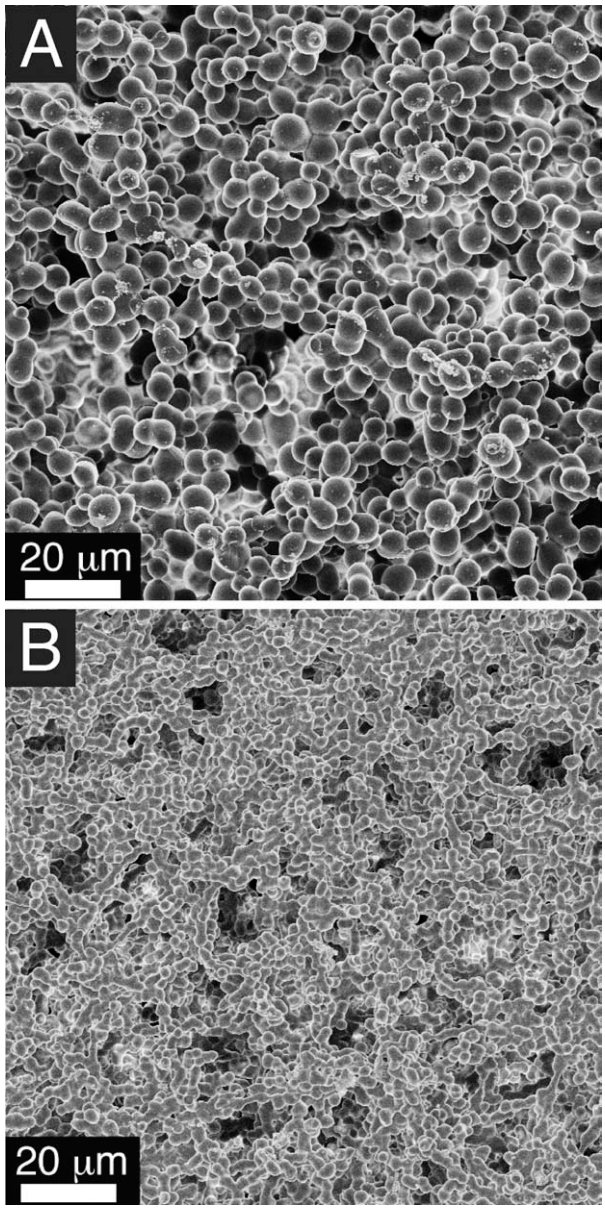

FIGURE 1 SEM images of PHEMA sponges crosslinked with (A) the carbohydrate-based crosslinking agent 1 or (B) TEGDMA.

the tetrol **5**, and selective protection of the primary alcohol in **5** using triphenylmethyl chloride gave the triol **6** in good overall yield. The triol **6** was then converted to the triacetate **7**, after which the trityl ether protecting group was removed using aqueous acid to give the alcohol **8** in good yield. Activation of the alcohol **8** using methanesulfonyl chloride gave the sulfonate **9**, and subsequent treatment of **9** with sodium azide gave the diazide **10**. Removal of the acetyl protecting groups gave the desired triol **11**. The azido groups on **11** were then reduced to amino groups using conventional hydrogenolysis conditions, and the amino groups were functionalized with methacryloyl moieties to afford the desired monosaccharide crosslinker **1**. Although the overall synthetic route involves multiple steps, each step only involved the use of relatively simple synthetic procedures, and the yield for each step was typically greater than 80%. In addition, the synthesis is robust and can be scaled easily to accommodate larger quantities. We note, however, that care needed to be taken during the functionalization of **12** with the methacryloyl groups to give **1**, and premature polymerization occurred if the reaction mixture was concentrated and subjected to even moderate heat (>33 °C) during isolation of **1**. The yield of **1** was only moderate (31%), but none of the reaction conditions used in Scheme 1 have been optimized.

An advantage of our route to **1** is that it includes the azides **5** and **11** as intermediates. In our synthesis, azido groups were used as protecting groups, but rather than simply being protecting groups in synthetic intermediates, the azido groups can also be exploited as functional groups to undergo 1,3-cycloaddition with alkynes in "click" chemistry. Click reactions of **5** could be used to functionalize alkyne-containing polymers,[27] and similar click chemistry could allow **11** to be used as a crosslinking agent for alkyne-functionalized polymers, to form polymer networks.[27–29] Furthermore, the hydroxyl groups on **1**, **5**, and **11** can be functionalized with biologically relevant groups, such as cell adhesion ligands.[30]

To test the ability of **1** to act as a crosslinking agent, PHEMA sponges (formed by polymerization-induced phase separation) that were crosslinked with **1** were compared to PHEMA sponges crosslinked with tetraethylene glycol dimethacrylate (TEGDMA). Samples crosslinked with **1** or TEGDMA appeared white, which is expected for PHEMA sponges.[8,12,31] Scanning electron microscopy (SEM) analysis of PHEMA sponges crosslinked with **1** revealed morphologies based on polymer droplets about 5 μm in diameter and pores in the order of 20 μm [Fig. 1(A)]. For PHEMA samples crosslinked with TEGDMA, SEM images showed morphologies with droplets about 2–3 μm in diameter and narrow pores with dimensions in the order of 10 μm [Fig. 1(B)]. This difference in the size of the polymer droplets and pores is not unexpected. Polymerization-induced phase separation relies on the growing polymer chains precipitating out of the polymerization solution. Any changes to the hydrophilicity of the growing polymer chains will alter the molecular weight at which the polymer chains precipitate.[31,32] If the growing polymer chains are more hydrophobic, the polymer will precipitate out of the polymerization solution at a lower molecular weight and form comparably smaller polymer droplets. Likewise, if the growing polymer chain is more hydrophilic, the polymer will stay in the polymerization solution longer to give higher molecular weight polymer chains, forming larger polymer droplets. As **1** is more hydrophilic compared to TEGDMA, mainly due to **1** being a triol, the growing polymer chains should be more hydrophilic, delaying the onset of phase separation and producing polymer droplets that are larger when compared to the more hydrophobic TEGDMA. Simple differences in hydrophilicity between TEGDMA and **1** are unlikely to fully account for the differences in polymer morphology. Other factors, such as differences between the reactivity ratios relative to HEMA of TEGDMA and **1** (which will be influenced by hydrophilicity and other factors), may also contribute to the differences in morphologies, and this possibility warrants further investigation.

CONCLUSIONS

We developed a simple, multistep synthetic route to glycosylpyranose-based crosslinking agent **1**. The route to **1**

can easily be adapted to the synthesis of other crosslinking agents suitable for click-type chemistry with alkynes. Crosslinker **1** was successfully incorporated into PHEMA sponges by copolymerization with HEMA under conditions that induce phase separation. The resulting PHEMA sponges exhibited morphologies based on polymer droplets that were larger that those seen for otherwise similar sponges crosslinked with TEGDMA. The difference in morphologies was attributed to the differences in hydrophilicity of the glucopyranose-based crosslinking agent **1** and TEGDMA. To the best of our knowledge, this is the first report of a monosaccharide-based crosslinking agent, and we believe that our synthetic route will be of benefit to researchers interested in the field of hydrogel synthesis. In addition, although the crosslinker **1** was not designed to be enzymatically degradable, we believe that further investigations into the synthesis of disaccharide- to oligosaccharide-based crosslinking agents that are enzymatically degradable could lead to the development of new and exciting biodegradable materials for tissue engineering purposes.

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