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Acidic Polysaccharide Mimics via Ring-Opening Metathesis Polymerization

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Abstract: An efficient and general synthetic strategy for the preparation of high-molecular-weight hydrophilic polymers bearing both carboxylic acid and hydroxyl pendant groups is described. Specifically, poly(5,6-dihydroxyoxanorbornane carboxylic acid) with molecular weight ranging from ~100 000 to 5 000 000 g/mol was prepared by ring-opening metathesis polymerization of methyl 5-oxanorbornene-2-carboxylate in the presence of Grubbs catalyst II and subsequently modified to tune the hydrophobic/hydrophilic properties by the introduction of either hydroxyl or carboxylic acid functionalities. These polymers mimic the natural acidic polysaccharide alginate and form hydrogels with polylysine. These polymers belong to a class of carbohydrate-like polymers, which are of interest for investigating the relationships between chemical structure and rheological properties as well as for providing new synthetic polysaccharide substitutes for applications in the biotechnology and pharmaceutical industries.

Ring-opening metathesis polymerization (ROMP) is a proven and versatile method for the synthesis of polymers with a narrow molecular weight distribution and a range of chemical functionalities.[1] Within the medical area, the use of ROMP to prepare bioactive and therapeutic polymers is actively pursued.[2] Representative examples include chemotherapeutic polymer carriers,[3] gene therapy agents,[4] selectin binders,[5] and antibacterial agents.[6] However, there have been few reports describing the use of these polymers as functional biomaterials/devices. We envisioned that ROMP could be used to synthesize polymers that mimic the properties of acidic polysaccharides, including those that form hydrogels. Polysaccharides are ubiquitous in nature and perform diverse roles from energy storage to cellular recognition.[7] In addition, these polymers have found widespread use in the biotechnology, food, and pharmaceutical industries. The molecular weight, shape, and discrete chemical functionalities (carboxylic acids, sulfonates, etc.) combined with the resulting physical and rheological properties of the polysaccharide allow for these numerous biological and industrial roles. Herein, we report the synthesis of an analogue of alginic acid, the structural dependence of its solution viscosity behavior, its cytotoxicity, and the formation of ionically cross-linked hydrogels.

Many polysaccharides possess repeating pyranose structures joined by a glycosidic linkage with the stoichiometric formula $(CH_2O)_n$. We identified the acidic polysaccharides as our first case study, and our efforts build upon the work by Mulhaupt and coworkers, who used ROMP to synthesize polyribofuranose analogues with molecular weights of 7000 to 37 000 g/mol; Clark and Lee, who prepared sulfonated polyribofuranose analogues with molecular weights of 20 000 to 70 000 g/mol; and Lienkamp et al., who synthesized diacid-functionalized norbornenes with molecular weights of 31 000 to 242 000 g/mol.[8] As shown in Figure 1, the acidic polysaccharide alginic acid is a linear block polymer of 1−4-linked β-D-mannuronic acid and α-L-glucuronic acid. Our goal was to synthesize high-molecular-weight polymers that reproduce this basic macromolecular structure of a repeating cyclic structure containing two secondary alcohols and a carboxylic acid. Thus, we chose to study poly(5,6-dihydroxyoxanorbornane carboxylic acid), which possesses these basic characteristics (Figure 1).

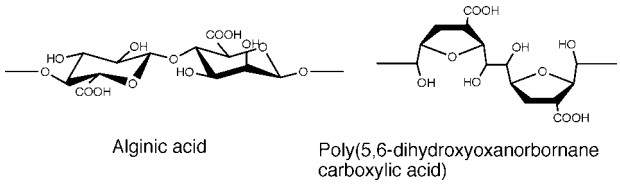
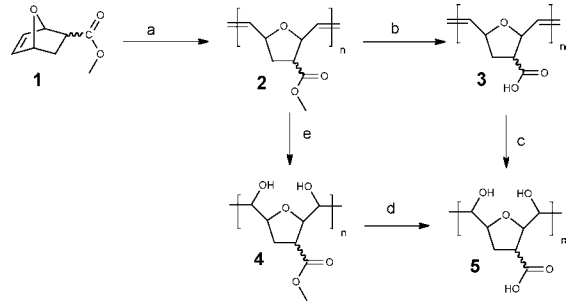


Figure 1. Structure of alginic acid and the polysaccharide mimic.

Scheme 1. Synthesis of the Polysaccharide Mimics[a]



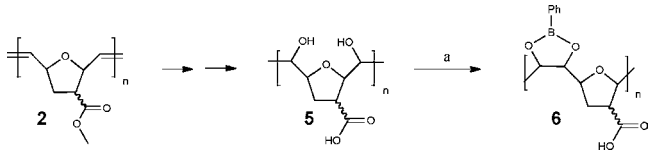
[a] Key: (a) (i) $Ru(PPh_3)_2(Cl)_2(CHPh)$, benzene, CH_2Cl_2, 25 °C, 4 h. (ii) ethyl vinyl ether (80−95%). (b) THF, NaOH, 2 days (80−90%). (c) OsO_4, NMO, H_2O, 16 h (75−85%). (d) MeOH, PTSA, reflux (99%). (e) TEA, TFA, H_2O_2, CH_2Cl_2, reflux, 16 h (80−90%).

As shown in Scheme 1, this hydrophilic polymer was synthesized via ROMP of a cyclic olefin. The monomer, methyl 5-oxanorbornene-2-carboxylate (**1**), was dissolved in 8:1 benzene/dichloromethane and polymerized using Grubbs' catalyst II at different monomer-to-catalyst ratios to afford the 10 polymers **2a**−**j** with M_n ranging from 110 000 to 5 100 000 g/mol (Table 1). The polymerization reactions were terminated by the addition of ethyl vinyl ether. To obtain the water-soluble polymer products **5**, the polyolefins were either saponified then oxidized or oxidized then saponified, as shown in Scheme 1. In the first route, the saponification of polymer **2** was performed in a mixture of 1 M NaOH and

Table 1. SEC Data for Polymers **2a**−**j** in THF Versus Polystyrene Standards

	theoretical MW	obtained *Mn*	PDI		theoretical MW	obtained *Mn*	PDI
2a	100000	91000	1.2	**2f**	3M	2.5M	1.2
2b	250000	210000	1.2	**2g**	4M	3.2M	1.3
2c	500000	370000	1.3	**2h**	5M	3.7M	1.4
2d	1 M	850000	1.2	**2i**	6M	4.4M	1.5
2e	2 M	1.6 M	1.2	**2j**	7M	5.1M	1.5

Scheme 2. Synthesis of the Boronic Ester Derivatives[a]



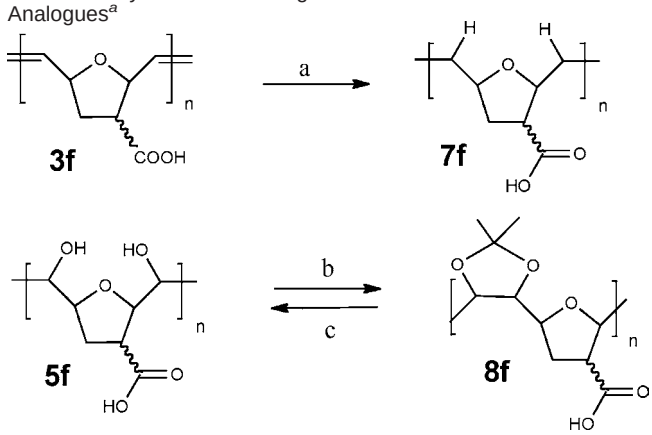
[a] Key: (a) Phenylboronic acid, MeOH, H_2O, 4 h (80−90%).

THF to afford **3**. The oxidation was then performed in water using OsO_4 and *N*-methylmorpholine *N*-oxide (NMO) to yield polymer **5**. Alternatively, **2** was oxidized in CH_2Cl_2 using a triethylamine (TEA)/trifluoroacetic acid (TFA)/H_2O_2 mixture to obtain polymer **4**. Finally, the methyl ester of **4** was cleaved to afford polymer **5**. The reactions were monitored by NMR spectroscopy, as the olefinic and methyl ester protons of the intermediates disappeared from the NMR spectrum and new proton resonances consistent with the structures of the product(s) were observed [see the Supporting Information (SI)]. The latter approach is favored since it does not require the use of OsO_4. Finally, **5** was precipitated in 5 M HCl solution, redissolved in aqueous solution using NaOH, dialyzed against a 3400 cutoff at pH 7.4, and then freeze-dried to afford a white fibrous polymer sample. Polymers **5a**−**j**, as the sodium salts, were soluble in aqueous solution. We observed no significant differences between samples of polymer **5** obtained using the **2** → **3** → **5** and **2** → **4** → **5** routes. The yields ranged between 80 and 95% for all of the reactions.

Size-exclusion chromatography (SEC) of the final polymers **5a**−**j** gave lower than expected molecular weight values, such as 100 K for an expected 3 M polymer. We suspected that the solution conformation of polymer **5** maybe different from that of polymer **2**, which possesses a rigid olefin backbone, and that this may have affected the results. Consequently, we prepared the boronic ester derivatives of polymers **5** at the 0.5, 1, 3, and 7 M molecular weights to give polymers **6c**, **6d**, **6f**, and **6j**, respectively, as shown in Scheme 2. SEC analysis of these polymers showed a good correlation between the initial molecular weight of compounds **2** and those of the boronic ester derivatives **6** ($r^2 = 0.99$; see the SI). These data also indicate that the above chemical transformations for the preparation of **6** from **2** did not degrade the polymer.

A number of interesting rheological properties became evident with these high-molecular-weight polymers. For example, polymers **3f** and **5f** with molecular weights of ∼2 500 000 g/mol were expected to give viscous solutions in water at concentrations of a few weight percent. In fact, however, only polymer **3f** gave a

Scheme 3. Synthesis of the Rigid and Flexible Backbone Analogues[a]



[a] Key: (a) H_2 (75 psi), PdOH/C (90%). (b) Benzene/acetone, H_2SO_4 cat., reflux, 46 h (70%). (c) HCl (1 M), reflux, 24 h (90%).

Figure 2. Photograph of (left) alginate:polylysine and (right) **5f**:polylysine hydrogels.

viscous solution and displayed Newtonian behavior with a viscosity of 10 Pa s [recorded at 2.5 Hz on a 2% (w/v) solution]. A polymer solution of **5f** exhibited very low viscosity (<0.1 Pa s) under the same conditions. This low viscosity with polymer **5f** may be a consequence of lost backbone rigidity. To investigate this structural dependence, we performed three additional experiments. First, polymer **3f**, which gave a viscous solution, was hydrogenated using PtOH/C under 75 psi H_2 to afford polymer **7f** (Scheme 3). An aqueous solution of polymer **7f** displayed low viscosity (<0.1 Pa s). Polymer **5f**, which did not give a viscous solution, was reacted with acetone in the presence of a catalytic amount of acid to form the acetonide analogue **8f**, in which rigidity in the polymer backbone was reintroduced. A solution of polymer **8f** possessed high viscosity [9 Pa s, recorded at 2.5 Hz on a 2% (w/v) solution]. Finally, cleavage of the acetonide group of **8f** with hydrochloric acid afforded **5f** and, again, a low-viscosity polymer solution.

Among the acidic polysaccharides, alginate is extensively used in the biomedical area for cell encapsulation, drug delivery, and tissue-engineering,[9] since it forms hydrogels[10] with cations. When polymer **5f** was mixed with polylysine at pH 7.8, a hydrogel was formed. Photographs of **5f**:polylysine and alginate:polylysine hydrogels, prepared in a similar manner, taken after the test tubes had been inverted are shown in Figure 2. The two hydrogels were viscoelastic and possessed storage modulus (G') values of 29 000 and 7500 Pa, respectively, with the **5f**:polylysine hydrogel being weaker. In addition, cytotoxicity experiments performed with polymer **5f** on mouse fibroblast cells showed no significant cytotoxicity relative to nontreated cells (see the SI).

In summary, high-molecular-weight polyanions with narrow polydispersity indexes (PDIs) were prepared in good yield via ROMP of a strained olefin. This synthetic route is attractive for preparing polymers with specific properties, given the diversity of readily available bicyclic olefins and the versatility of the olefin functionality and carboxylic acid for introduction of specific chemical groups. These polymers mimic some of the properties of acidic polysaccharides: the pendent carboxylic acid functionalities, as in alginic acid, serve as ionic cross-linking sites for cations to form hydrogels. Continued research with polysaccharide-like polymers prepared using ROMP or other synthetic strategies[5,11] will provide new structures with interesting properties as well as potential synthetic polymer substitutes for the varied industrial and biomedical applications of the acidic polysaccharides.

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Supporting Information Available: Experimental procedures and SEC and cytotoxicity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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