

## Synthesis of Cell Agglutination Inhibitors by Aqueous Ring-Opening Metathesis Polymerization

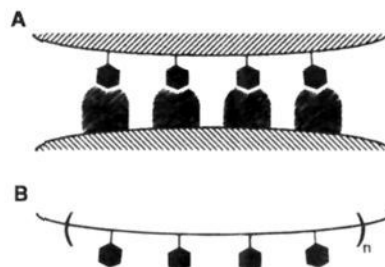
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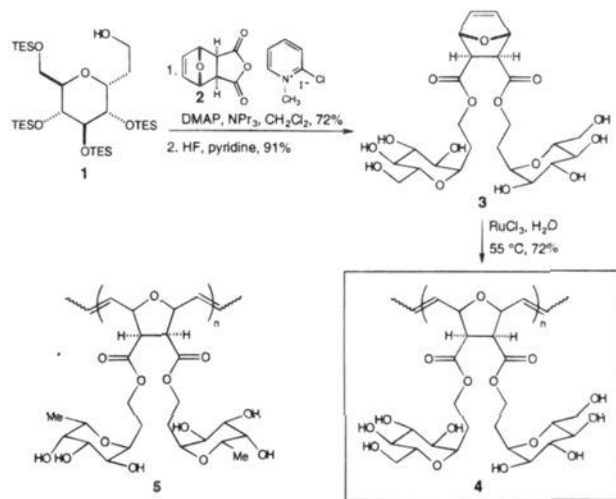
Intercellular recognition events are fundamental to many biological processes, including fertilization, development, and the mounting of an immune response. Although little is known of the molecular mechanisms underlying cell recognition and adhesion, cell surface oligosaccharides have been implicated as key participants in many of these events.<sup>1</sup> Carbohydrate receptors often bind weakly to target saccharide ligands in solution (i.e., with  $K_a = 10^3$ – $10^4$  M<sup>-1</sup>).<sup>2</sup> Thus, Nature takes advantage of multivalent carbohydrate–protein interactions to enhance the strength of cell surface binding.<sup>3</sup> Molecules that can mimic the polyvalent display of oligosaccharides presented by a cell surface should be more effective than monovalent ligands at modulating intercellular interactions<sup>4–6</sup> (Figure 1). Our desire to explore the chelate effect and to design molecules to regulate cell–cell interactions prompted us to investigate the aqueous ring-opening metathesis polymerization (ROMP)<sup>7</sup> for the synthesis of carbohydrate-substituted materials. We now describe these studies, which have resulted in the synthesis of a new class of carbohydrate-modified polymers. These polyvalent materials have unique biological properties relative to monomeric ligands: we show that a glucose-derivatized polymer acts as a potent inhibitor of concanavalin A (Con A)-induced cell agglutination.

Traditionally, carbohydrate-substituted polymers have been synthesized by polymerization of acrylamide derivatives.<sup>5a,b,8</sup> We sought an alternative polymerization method that would have the potential for greater control of polymer size, structure, and the density of carbohydrate substituents. In addition, a flexible strategy that would allow the production of copolymers could be used to synthesize materials for selective immobilization of different cell types. Finally, the chosen polymerization reaction should tolerate a highly polar monomer bearing unprotected sugars. The features of ruthenium-catalyzed ROMP suggested



**Figure 1.** (A) Notional depiction of one manner in which intercellular recognition and binding can be enhanced through multivalent carbohydrate–protein interactions. (B) Carbohydrate-substituted polymers can mimic the multivalent display of carbohydrates on the cell surface.

### Scheme 1



that this type of polymerization would offer the desired advantages.<sup>7,9</sup>

7-Oxanorbornene derivative 3, designed to produce a polymer with protein-binding activity, was synthesized to test whether carbohydrate-substituted alkenes will polymerize under aqueous ROMP conditions (Scheme 1). The carbohydrate groups were attached to the oxanorbornene skeleton via C-glycoside linkages. This mode of attachment was chosen because the C-glycoside linkage is stable toward chemical and biological assault, and C-glycosides of the  $\alpha$ -configuration, the preferred anomer for Con A binding,<sup>10</sup> are readily available.<sup>11</sup> In the synthesis of monomer 3, 2 equiv of alcohol 1<sup>12</sup> were coupled to anhydride 2<sup>13</sup> under modified Mukaiyama esterification conditions (Scheme 1).<sup>14</sup> Removal of the triethylsilyl protecting groups generated the polymer precursor 3. This route provides rapid access to monomers bearing two identical carbohydrate residues.<sup>15</sup>

The ROMP of monomer 3 was accomplished by treating it with ruthenium trichloride in water (Scheme 1).<sup>7a</sup> The reaction

(1) For recent reviews, see: (a) Hughes, R. C. *Curr. Opin. Struct. Biol.* **1992**, *2*, 687–692. (b) Drickamer, K.; Taylor, M. E. *Annu. Rev. Cell Biol.* **1993**, *9*, 237–264. (c) *Carbohydrate Recognition in Cellular Function*; Bock, G., Harnett, S., Eds.; John Wiley & Sons Ltd: Chichester, U.K., 1989; Vol. 145.

(2) Lee, Y. C. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1992**, *6*, 3193–3200.

(3) For examples in bacterial adhesion, see: (a) Ofek, I.; Sharon, N. *Infect. Immunol.* **1988**, *56*, 539–547. (b) Goldhar, J. *Methods Enzymol.* **1994**, *236*, 211–231.

(4) For an inhibitor of E-selectin-mediated adhesion, see: DeFrees, S. A.; Gaeta, F. C. A.; Lin, Y.-C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 7549–7550.

(5) For representative syntheses of sialic acid-substituted materials that inhibit influenza virus hemagglutination, see: (a) Matrosovich, M. N.; Mochalova, L. S.; Marinina, V. P.; Byramova, N. E.; Bovin, N. V. *FEBS Lett.* **1990**, *272*, 209–212. (b) Spaltenstein, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 686–687. (c) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1869–1872. (d) Kingery-Wood, J. E.; Williams, K. W.; Sigal, G. B.; Whitesides, G. M. *J. Am. Chem. Soc.* **1992**, *114*, 7303–7305. (e) Spevak, W.; Nagy, J. O.; Charych, D. H.; Schaefer, M. E.; Gilbert, J. H.; Bednarski, M. D. *J. Am. Chem. Soc.* **1993**, *115*, 1146–1147.

(6) For the synthesis of low molecular weight inhibitors of influenza virus hemagglutinin, see: (a) Glick, G. D.; Toogood, P. L.; Wiley, D. C.; Skehel, J. J.; Knowles, J. R. *J. Biol. Chem.* **1991**, *266*, 23660–23669. (b) Sabesan, S.; Duus, J. O.; Neira, S.; Domaille, P.; Kelm, S.; Paulson, J. C.; Bock, K. *J. Am. Chem. Soc.* **1992**, *114*, 8363–8375.

(7) (a) Novak, B. M.; Grubbs, R. H. *J. Am. Chem. Soc.* **1988**, *110*, 7542–7543. (b) Nguyen, S. T.; Johnson, L. K.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3974–3975. (c) Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 9858–9859.

(8) Schnarr, R. L.; Weigel, P. H.; Kuhlenschmidt, M. S.; Lee, Y. C.; Roseman, S. *J. Biol. Chem.* **1979**, *253*, 7940–7951.

(9) (a) France, M. B.; Grubbs, R. H.; McGrath, D. V.; Paciello, R. A. *Macromolecules* **1993**, *26*, 4742–4747. (b) France, M. B.; Paciello, R. A.; Grubbs, R. H. *Macromolecules* **1993**, *26*, 4739–4741. (c) Fu, G. C.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 9856–9857. (d) Feast, J. W.; Harrison, D. B. *J. Mol. Catal.* **1991**, *65*, 63–72. (e) Lu, S. Y.; Quayle, P.; Heatley, F.; Booth, C.; Yeates, S. G.; Padget, J. C. *Eur. Polym. J.* **1993**, *29*, 269–279.

(10) (a) Goldstein, I. J. In *Concanavalin A as a Tool*; Bitiger, H., Schnebli, H. P., Eds.; John Wiley & Sons, Ltd.: London, 1976; pp 55–65. (b) Mandal, D. K.; Bhattacharyya, L.; Koenig, S. H.; Brown, R. D.; Oscarson, S.; Brewer, C. F. *Biochemistry* **1994**, *33*, 1157–1162.

(11) Giannis, A.; Sandhoff, K. *Tetrahedron Lett.* **1985**, *26*, 1479–1482.

(12) For reagents and conditions for the synthesis of 1, see the supplementary material.

(13) Diels, O.; Alder, K. *Ber. Dtsch. Chem. Ges.* **1929**, *62*, 554–562.

(14) Saigo, K.; Usui, M.; Shimada, E.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1863–1866.

(15) To attach two different residues, a sequential coupling procedure can be used: Gingras, M.; Mortell, K. H., unpublished results.

afforded good yields of the carbohydrate-substituted polymer, "polyglycomer" **4**. This result demonstrates the tolerance of the ruthenium catalyst for highly functionalized monomers such as **3**. The product polymer **4** was subjected to gel filtration chromatography. By comparison of the migratory aptitude of the polymer relative to dextran standards, it is estimated that the relative molecular mass ( $M_r$ ) of the polymer is approximately  $10^6$ .<sup>16</sup> Fucose-derivatized polyglycomer **5**, synthesized analogously, exhibited the same properties upon analysis by gel filtration. These water-soluble polyglycomers possess unique amphipathic structures composed of nonpolar backbones and flexible polar side chains.<sup>17</sup>

The polymers were tested for their ability to function as multivalent ligands for the glucose-binding protein Con A. Con A is a well-studied carbohydrate binding protein<sup>18–21</sup> that exists as a homotetramer at neutral pH. Each subunit contains a carbohydrate binding site, and the tetramer can bind simultaneously to four glucose units. The propensity of Con A to agglutinate cells has long been recognized and exploited in biology.<sup>18</sup> Although the mechanism of cell agglutination is not fully understood, multivalent interactions between Con A and cell surface saccharides are required.<sup>22</sup> The requirement for multivalency in Con A activity and the available thermodynamic and structural data make this an ideal model system in which to test polyvalent inhibitors.

To assess the activity of our polymers, we compared their ability to inhibit erythrocyte agglutination by Con A with those of several controls, including the monovalent carbohydrate derivative methyl- $\alpha$ -D-glucopyranoside and the divalent glucose-substituted 7-oxanorbornene **3**. The agglutination inhibition assays were performed according to standard protocols.<sup>23</sup> Substrates were incubated with four agglutinating doses of Con A prior to the addition of rabbit erythrocytes. The concentration of glucose moieties in solution required for inhibition was determined for each substrate within a 2-fold dilution.<sup>24</sup>

The glucose polyglycomer prevented erythrocyte agglutination at a glucose residue concentration at least 2000-fold lower than that required of the monomeric methyl  $\alpha$ -D-glucopyranoside (Table 1). Inhibiting doses of 7-oxanorbornene **3** were comparable to those of methyl  $\alpha$ -D-glucopyranoside. These data suggest that Con A binds to  $\alpha$ -C-glucosides as well as it does

**Table 1.** Hemagglutination Inhibition Assays of Mono- and Multivalent Carbohydrate Derivatives

| inhibitor                          | inhibiting dose <sup>a</sup><br>[carbohydrate residues], M |
|------------------------------------|--|
| methyl $\alpha$ -D-glucopyranoside | $5.0 \times 10^{-2}$                                       |
| 7-oxanorbornene <b>3</b>           | $5.0 \times 10^{-2}$                                       |
| glucose polyglycomer <b>4</b>      | $2.5 \times 10^{-5}$                                       |
| fucose polyglycomer <b>5</b>       | $> 1.0 \times 10^{-2}$                                     |

<sup>a</sup> Inhibiting doses were obtained by averaging the results of four independent experiments. The error associated with the dose determination is a factor of 2, as dictated by the 2-fold dilutions of the assay.

to  $\alpha$ -O-glucosides, within the limits of our assay. Furthermore, Con A does not bind fucose,<sup>10</sup> and the fucose-containing polyglycomer **5** showed no inhibition at concentrations up to 10 mM. The inefficacy of the fucose polyglycomer indicates that the strong inhibition exhibited by the glucose polyglycomer is due to binding of glucose residues rather than a nonspecific effect of the polymer chain.

We have shown that ROMP can be used to create a polyvalent carbohydrate-bearing polymer that can block protein-initiated cell agglutination. The best natural inhibitors of Con A-mediated hemagglutination<sup>21</sup> and the glucose polyglycomer share a common feature: multivalency.<sup>25</sup> Progress has been slow in creating high-affinity ligands for carbohydrate binding proteins by the traditional approach of modifying the structure of the monovalent ligand;<sup>26</sup> however, imitation of the polyvalent binding environment can create better ligands for the modulation of cellular interactions. The nature of our polymers allows modifications in the flexibility of the polymer backbone and the structure of the side chains to further optimize biological activity. Additionally, the inclusion of chain transfer agents in the polymerization should afford lower molecular weight oligomers,<sup>9a,d</sup> which would retain the properties of a multivalent ligand but would be less immunogenic than the corresponding polymers. Thus, application of ROMP to the synthesis of polyglycomers offers new opportunities for design of materials for modulation of cell adhesion, immobilization of particular cell types, and study of multivalency in extracellular interactions.

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**Supplementary Material Available:** Spectral and analytical data for the precursors of polymer **4** and experimental procedures for the preparation of **3–5**; <sup>1</sup>H and <sup>13</sup>C NMR spectra for compounds **1–4** (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(25) The origin of the enhanced binding afforded by the multivalent glucose polyglycomer will be the subject of future investigations. The interaction of Con A with the polyglycomer may not be an equilibrium binding event: naturally-occurring multivalent Con A ligands can precipitate the protein. Quantitation of precipitate formed has been used to determine Con A ligand affinity. For leading references, see ref 21.

(26) For leading references to efforts in the selectin area, see: Varki, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7390–7397. For efforts directed toward inhibition of influenza virus hemagglutinin, see: Lees, W. J.; Spaltenstein, A.; Kingery-Wood, J. E.; Whitesides, G. M. *J. Med. Chem.* **1994**, *37*, 3419–3433.

(16) For a description of the determination of  $M_r$ , see the supplementary material.

(17) Analysis of <sup>1</sup>H and <sup>13</sup>C NMR data indicates that the glucose polyglycomer is composed of a 67:33 mixture of Z and E alkenes.

(18) *Concanavalin A as a Tool*; Bittiger, H., Schnebli, H. P., Eds.; John Wiley & Sons, Ltd.: London, 1976.

(19) For a crystal structure of the Con A-mannose complex, see: Derewenda, Z.; Yariv, J.; Helliwell, J. R.; Kalb, A. J.; Dodson, E. J.; Papiz, M. Z.; Wan, T.; Campbell, J. *EMBO J.* **1989**, *8*, 2189–2193.

(20) For calorimetric studies, see: (a) Ambrosino, R.; Barone, G.; Castronuovo, G.; Ceccarini, C.; Cultrera, O.; Elia, V. *Biochemistry* **1987**, *26*, 3971–3975. (b) Williams, B. A.; Chervenak, M. C.; Toone, E. J. *J. Biol. Chem.* **1992**, *267*, 22907–22911. (c) Mandal, D. K.; Kishore, N.; Brewer, C. F. *Biochemistry* **1994**, *33*, 1149–1156.

(21) For biological studies, see: (a) Mandal, D. K.; Brewer, C. F. *Biochemistry* **1993**, *32*, 5117–5120. (b) Mandal, D. K.; Brewer, C. F. *Biochemistry* **1992**, *31*, 12602–12609.

(22) (a) Walther, B. T. In *Concanavalin A as a Tool*; Bittiger, H., Schnebli, H. P., Eds.; John Wiley & Sons, Ltd.: London, 1976; pp 231–248. (b) Asahi, M.; Taniguchi, T.; Hashimoto, E.; Inazu, T.; Maeda, H.; Yamamura, H. *J. Biol. Chem.* **1993**, *268*, 23334–23338.

(23) Osawa, T.; Matsumoto, I. *Methods Enzymol.* **1972**, *28*, 323–327.

(24) The concentration of fucose residues in a fucose polyglycomer solution was determined by <sup>1</sup>H NMR integration versus a solution standard. For the agglutination assays, the carbohydrate residue concentrations of the polyglycomer solutions were determined by UV absorption at 210 nm using  $\epsilon_{210} = 4100 \text{ mol}^{-1} \text{cm}^{-1}$  for each polyglycomer. This value for  $\epsilon$  was determined for the fucose polyglycomer and assumed to be the same for all polyglycomers. For additional details, see the supplementary material.