Synthesis of end-labeled multivalent ligands for exploring cell-surface-receptor–ligand interactions

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Background: Ring-opening metathesis polymerization (ROMP) is a powerful synthetic method for generating unique materials. The functional group tolerance of ruthenium ROMP initiators allows the synthesis of a wide range of biologically active polymers. We generated multivalent ligands that inhibit cell surface L-selectin, a protein that mediates lymphocyte homing and leukocyte recruitment in inflammation. We hypothesized that these ligands function through specific, multivalent binding to L-selectin. To examine this and to develop a general method for synthesizing multivalent materials with end-labels, we investigated functionalized enol ethers as capping agents in ruthenium-initiated ROMP.

Results: We synthesized a bifunctional molecule that introduces a unique end group by terminating ruthenium-initiated ROMP reactions. This agent contains an enol ether at one end and a masked carboxylic acid at the other. We conjugated a fluorescein derivative to an end-capped neoglycopolymer that had previously been shown to inhibit L-selectin function. We used fluorescence microscopy to visualize neoglycopolymer binding to cells displaying L-selectin. Our results suggest that the neoglycopolymers bind specifically to cell surface L-selectin through multivalent interactions.

Conclusions: Ruthenium-initiated ROMP can be used to generate biologically active, multivalent ligands terminated with a latent functional group. The functionalized polymers can be labeled with a variety of molecular tags, including fluorescent molecules, biotin, lipids or antibodies. The ability to conjugate reporter groups to ROMP polymers using this strategy has broad applications in the material and biological sciences.

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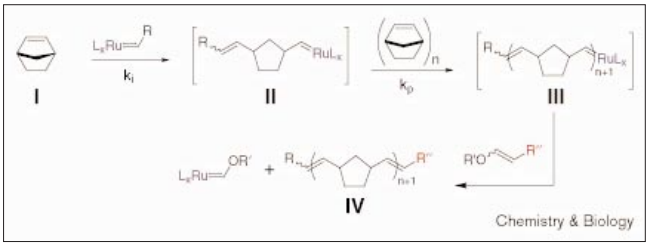
Introduction

Ring-opening metathesis polymerization (ROMP) has emerged as a powerful synthetic method for the creation of unique materials. Polymers generated by ROMP have a variety of applications in both the materials sciences, as liquid crystals, photoresists, specialty elastomers, components of superconductors and materials for solid-phase extraction [1–4], and in the biological sciences, as inhibitors of biological processes and tools for investigating multivalent interactions [5–14]. These applications have been made possible by advances in catalyst development and polymerization techniques that have provided methods to generate polymers of controlled length and defined structure [15–17].

The attachment of additional, orthogonal functional groups at polymer termini extends the applications of materials generated by ROMP [18–20]. For example, selective end-capping molybdenum–carbene-initiated ROMP reactions have facilitated the syntheses of surfaces bearing ROMP-derived polymers [19]. The end-functionalized materials generated to date have been used to create nonpolar materials with tailored physical properties [18–20]. Because ruthenium carbene initiators are highly tolerant of functional groups, including alcohols, carboxylic acids and sulfates [21,22], they can be used to synthesize highly functionalized and/or polar materials not accessible using other catalysts. This feature of ruthenium-carbene-initiated ROMP has been exploited to assemble biologically active multivalent ligands, but no methods for selectively end-labeling polymers have been described.

The importance of multivalent recognition events in biology and the utility of multivalent arrays in elucidating the features of such processes have accelerated the development of synthetic methods to generate multidentate ligands equipped with reporter groups. For example, polymers containing saccharide recognition elements and reporter groups can be generated in acrylamide copolymerization reactions. Acrylamide polymers that have reporter groups have been generated by either coupling a desired functional tag to a preformed polymer backbone or attaching a reporter group to each monomer prior to polymerization [23,24]. These strategies have been used

Figure 1

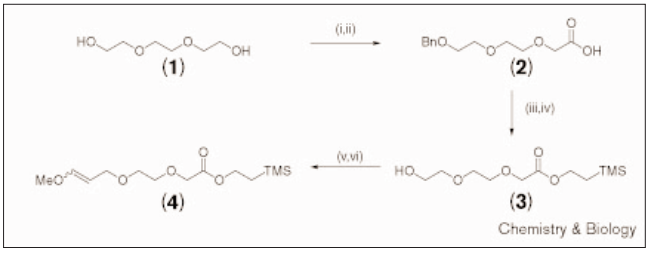


General process for ROMP using ruthenium carbene initiators [33]. For living polymerizations, nonproductive termination processes are slow relative to the rates of initiation (k_i) and propagation (k_p). When $k_i > k_p$ polymers of defined molecular mass and narrow molecular mass distribution can be generated by varying the ratio of catalyst to monomer. Reactions initiated with a ruthenium carbene are typically terminated with ethyl vinyl ether to form polymers of structure **IV** in which R″ = H. L, ligand.

to synthesize materials for assaying protein–carbohydrate interactions [25,26]. Disadvantages of the reported synthetic routes are that they do not allow control over the length of the polymer chain or the number of reporter groups incorporated. We envisioned that ROMP could be used to assemble multivalent ligands with control over these features.

The benefit of tailoring the properties of multivalent ligands is that they can provide insight into complex, cell-surface-recognition processes [27–30]. ROMP has been used to synthesize multivalent molecules that target the selectin family of cell-surface carbohydrate-binding proteins [5,6,31]. The selectins are cell-surface proteins that facilitate the recruitment of leukocytes to sites of inflammation. The selectins have been inhibited with a variety of monovalent and multivalent ligands [32] with many multivalent ligands exhibiting greater potency than their monovalent counterparts. The specificity of ligand binding in the context of the cell surface has not been addressed nor is it known whether the multivalent ligands engage their cognate selectin in multivalent interactions. Ligands equipped with reporter groups could provide insight into these issues. Here, we report the synthesis of a bifunctional capping agent that facilitates the terminal attachment of new functional groups to materials generated by ROMP. The utility of this method is illustrated by the creation of a molecular probe used to directly visualize a receptor–ligand interaction on a cell surface. Specifically, we synthesized a fluorescent multivalent carbohydrate ligand and found that it binds selectively to cell surface L-selectin. We also provide initial evidence that this ligand binds L-selectin through multivalent interactions.

Figure 2



Synthesis of capping agent **4**. Reagents and conditions: (i) BnBr, aq. NaOH, 38%; (ii) CrO_3, H_2SO_4, acetone, H_2O, 0°C→RT, 81%; (iii) $HOCH_2CH_2Si(CH_3)_3$, EDCI, DMAP, CH_2Cl_2, 0°C→RT, 97%; (iv) $Pd(OH)_2$/C, EtOH, 50 psi H_2, 77%; (v) DMSO, P_2O_5, Et_3N, CH_2Cl_2, 0°C→RT, 82%; (vi) $MeOCH_2PPh_3Cl$, *t*BuOK, CH_2Cl_2, 0°C, 59%.

Results and discussion

Synthesis of the bifunctional capping agent

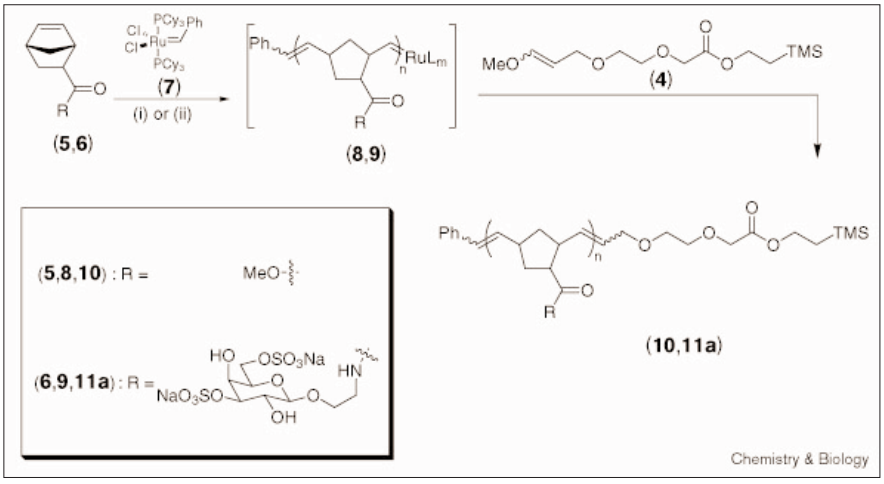
Our strategy for the synthesis and end-labeling of materials draws on the features of 'living' ROMP. Ruthenium carbenes [12,15,33] (such as **7**, see below) can facilitate living polymerization reactions in which termination and chain-transfer events are slow relative to propagation [34]. When initiation is fast relative to propagation ($k_i > k_p$), homogenous materials of controlled lengths and low polydispersities are generated (Figure 1). In living ruthenium-initiated ROMP, an active ruthenium carbene center is present at the end of each chain after the monomer is consumed (Figure 1, **III**), and this group can react with electron-rich alkenes to yield a product with a terminal alkene (**IV**) and an unreactive alkoxy-substituted ruthenium carbene. We postulated that this process could be used to introduce new functional groups at polymer termini. An advantage of this strategy is that only living chains can acquire the functionality, resulting in a more homogeneous population of end-functionalized materials.

To test the modification strategy, the bifunctional capping agent **4** (Figure 2) was designed to deliver a masked carboxylic acid to the end of living polymer chains. Our target molecule was comprised of an enol ether linked to a protected carboxylic acid via an ethylene glycol linker (Figure 2). The β-trimethylsilyl (TMS) ethyl carboxylic acid protecting group serves two purposes. First, the distinct signal of the TMS group in the ^1H nuclear magnetic resonance (NMR) spectrum provides an estimate of the capping efficiency; and second, the β-TMS ethyl group can be removed under conditions that do not affect the sulfated carbohydrate recognition epitopes employed in this study. Moreover, carboxylic acids can be activated for further functionalization easily and selectively. The target capping agent **4** was readily assembled from triethylene glycol in six steps (Figure 2).

The substrate selected for the specific end-modification strategy is a neoglycopolymer bearing 3,6-disulfogalactose epitopes. This target emerged from our interest in elucidating the features critical in L-selectin recognition. L-selectin,

Figure 3

Synthesis of end-capped polymers. Reagents and conditions: for **5**, (i) dichloroethane (DCE), 30 min; for **6**, (ii) dodecyltrimethyl ammonium bromide (DTAB) (1.6 eq), 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol (bis-tris) buffer (100 mM, pH 5.9), DCE, 40°C, 30 min.



a member of the selectin family of cell-adhesion molecules, facilitates the recruitment of white blood cells to sites of inflammation [35,36]. L-selectin ligands are mucin-like proteins that present multiple copies of anionic saccharide epitopes on an extended polypeptide backbone [37–39]. Neoglycopolymers, such as **11** (Figures 3,4), mimic mucins because they can adopt linear, extended structures that contain saccharide residues emanating from the polymer backbone. We have shown that selected sulfated, saccharide-containing neoglycopolymers related to **11** are potent inhibitors of selectin function [10,22]. By transforming **11a** into reporter ligand **16** (Figure 5), we could investigate directly ligand binding to cell surface L-selectin.

To examine the chemistry of the capping reaction, the ability of enol ether **4** to terminate ROMP reactions was evaluated in reactions of two monomers with different properties (Figures 3,4). In all reactions, a 15:1 ratio of monomer to carbene initiator was employed. To ascertain the reactivity of **4** under standard single-phase conditions, nonpolar monomer **5** was subjected to ROMP, and an excess of compound **4** was introduced to terminate the reaction. From ^{1}H NMR data, comparison of the integration of the signal from the phenyl protons with that due to the protons of the TMS group revealed that ~80% of the resulting polymer chains were capped to afford material **10**. Integration of the phenyl proton signal with that of the backbone protons indicates that the average length of these polymers was 42 monomer units. Initial attempts to end-label polymers bearing highly polar substituents revealed that the capping reaction was less effective. Specifically, when the emulsion conditions required for oligomerization of polar **6** were used, reaction termination with enol ether **4** resulted in **11a**, which was produced with a useful but more modest capping efficiency (30%) (Figure 3). The average length on these polymers was

Figure 4

Synthesis of polymer **11b**. Reagents and conditions: (i) dichloroethane (DCE), 30 min; (ii) excess compound **4** was added neat; (iii) diisopropylcarbodiimide (DIC), (2-aminoethyl)-3,6-*O*-disulfo-α-D-galactopyranoside (**15**), Et_3N, DMF, H_2O.

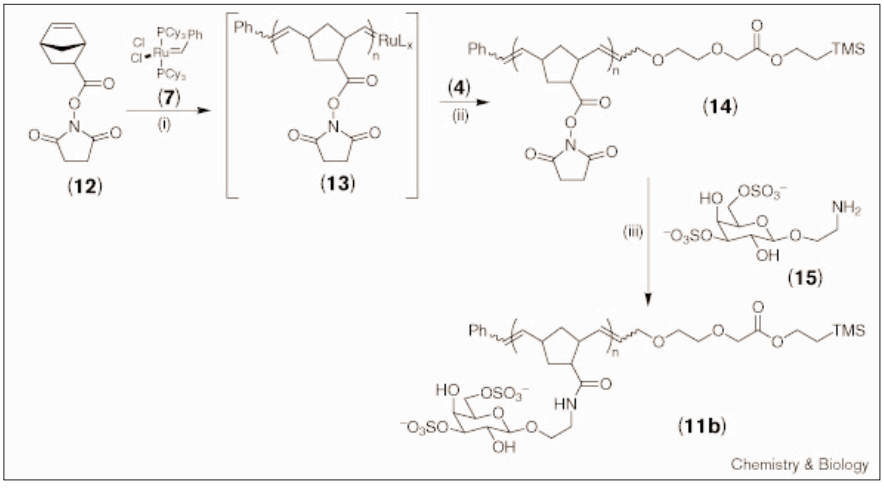
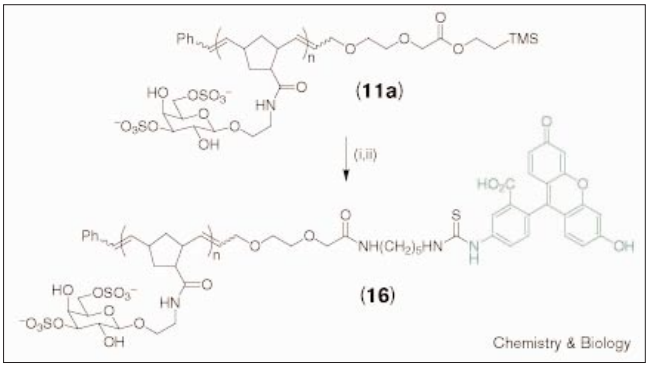


Figure 5



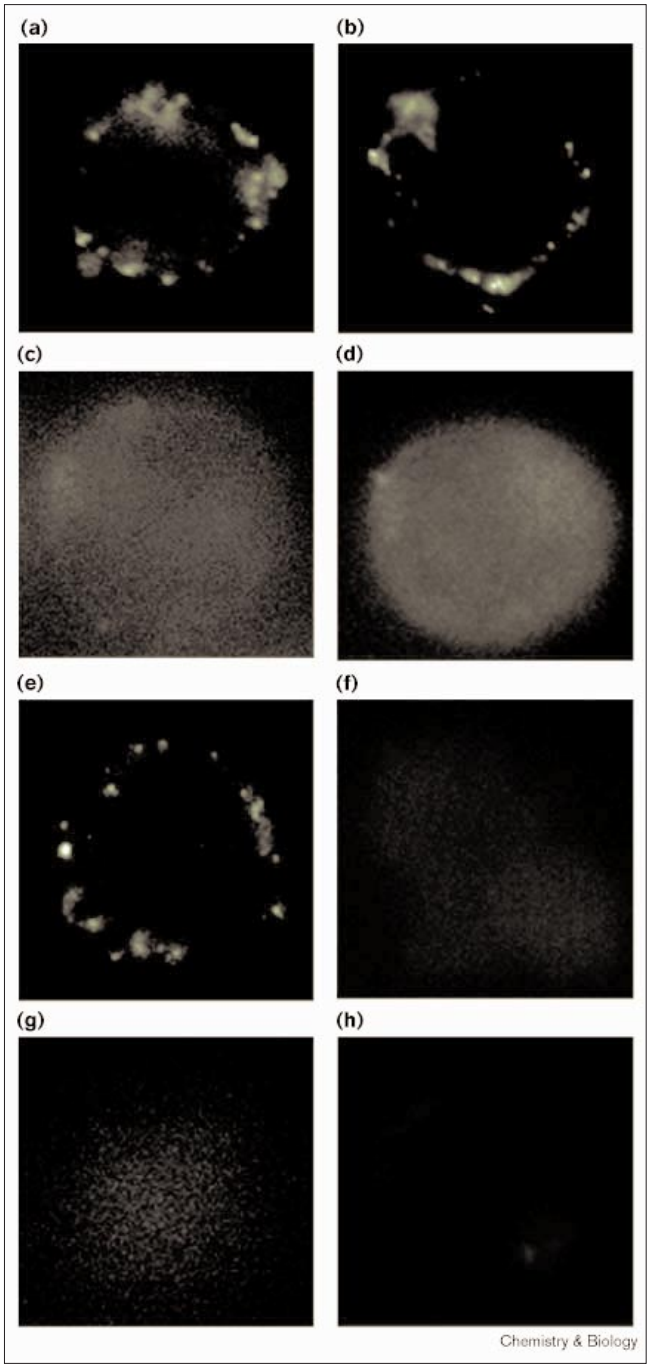
Synthesis of fluorescent neoglycopolymer **16**. Reagents and conditions: (i) 50 mM NaOH, 60°C, 2 h; (ii) 5-((5-aminopentyl)-thioureidyl) fluorescein, EDCI, N-hydroxysulfosuccinimide, H_2O, 24 h.

determined to be 15 monomer units, suggesting premature termination steps were competing with elongation and capping. We therefore explored an alternative route for the synthesis of end-labeled highly polar multivalent materials.

To minimize complications arising from phase-transfer processes, we used an alternative strategy to generate polar, functionalized polymer **11b** (Figure 4) [40]. Monomers containing N-hydroxysuccinimide esters, such as **12**, can be assembled into polymers in organic solvents using ROMP. Post-polymerization modification was accomplished by treating the resulting material (**14**) with a nucleophile to generate a new substituted polymer. As with products from reaction of methyl ester **5**, polymer **13** obtained from reaction of **12** could be terminated with capping agent **4** in efficiencies of ~80%. The polymers, which were generated using a 15:1 monomer to initiator ratio, have an average degree of polymerization (DP) of 35 monomer units. This DP is similar to that obtained with nonpolar monomer **5**, suggesting that the reaction of activated ester **12** is not subject to the side reactions that plague the assembly of the anionic monomer **6**. Polymer **14** could then be coupled to an amine-containing saccharide moiety **15** to afford the 3,6-disulfogalactose derivative **11b**. After purification of material **11b**, the NMR spectroscopic data for polymers **11a** and **11b** were virtually indistinguishable except for differences in the intensities of the signals arising from the capping agent. We postulate that the single-phase homogenous reaction conditions result in higher capping yields than do the emulsion polymerization reaction because the solubilities of the starting materials and products in the

Figure 6

Fluorescence microscopy results suggesting that neoglycopolymers bind specifically to L-selectin. **(a)** Jurkat cell labeled with a fluorescent anti-L-selectin antibody, FITC-DREG-56. **(b)** Jurkat cell labeled with fluorescent neoglycopolymer **16**. **(c)** Jurkat cell pretreated with PMA and then labeled with FITC-DREG-56. **(d)** Jurkat cell pretreated with PMA and then labeled with fluorescent neoglycopolymer **16**. **(e)** Jurkat cell treated with polyclonal chicken-derived anti-L-selectin antibody and then detected with FITC-labeled goat-anti-chicken secondary antibody. **(f)** Jurkat cell treated with polyclonal anti-L-selectin antibody and then with fluorescent neoglycopolymer **16**. Although blocking with polyclonal anti-L-selectin antibody prevented neoglycopolymer binding, blocking did not preclude binding of anti-CD44 antibody (data not shown). **(g)** HL60 cell treated with FITC-DREG-56. **(h)** HL60 cell treated with fluorescent neoglycopolymer **16**. Each image is an individual cell at 630× magnification and is representative of at least two independent experiments.



homogeneous phase conditions do not strongly influence the relative rates of polymer assembly and termination.

Conjugation of functionalized neoglycopolymer to fluorescein

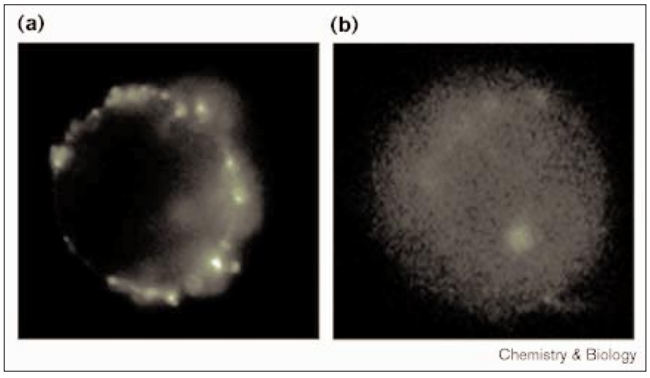
To convert a functionalized polymer into a cell-surface probe, the attachment of the fluorescein derivative 5-((5-aminopentyl)thioureidyl) fluorescein (fluorescein cadaverine) to the protected carboxylic-acid-terminated polymer **11a** was investigated (Figure 5). The fluorescein group is widely used as a reporter molecule, and we envisioned its conjugation to the glycoprotein analog would facilitate studies of receptor recognition in the cell-surface environment. The TMS-substituted ethyl ester was treated with aqueous NaOH at 60°C to afford polymer containing a terminal carboxylic acid. The coupled material was generated by condensation of the unprotected polymer with fluorescein cadaverine mediated by 1-(3-dimethylamino(propyl)-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of *N*-hydroxysulfosuccinimide (sulfo-NHS). The resulting conjugate was isolated using size-exclusion and cation-exchange chromatography to afford fluorescein-modified oligomer **16**, with a DP of 15.

Fluorescent neoglycopolymer binding to L-selectin

Our previous results led us to hypothesize that polymers such as **11** bind specifically to cell-surface L-selectin thereby inhibiting its function; consequently, we sought to gather additional evidence for such a mode of action. Accordingly, the ability of polymer **16** to bind cells displaying L-selectin was examined (Figure 6). The fluorescent polymer was added to Jurkat cells (a human acute T cell leukemia line) and the cells were viewed using fluorescence microscopy. For comparison, cells were labeled with a fluorescein-conjugated antibody to L-selectin, FITC-DREG-56 [41]. Both the antibody and the polymer bound cells at localized sites, producing similar, punctate fluorescence patterns (Figure 6a,b). The patterns are consistent with observations that L-selectin is detected as patches on the cell surface upon treatment with multivalent ligands, such as antibodies [42]. Similar patterns have been seen for E-selectin when cells are treated with multivalent ligands [43]. Binding was dependent on the presence and availability of cell surface L-selectin. Treatment of Jurkat cells with either phorbol myristate acetate (PMA), which has been shown to induce release of L-selectin from the cell surface [44], or with polyclonal anti-L-selectin antibody prevented binding of **16** (Figure 6c–f). Additionally, an L-selectin-deficient leukocytic cell line, HL60, did not bind DREG-56 or neoglycopolymer **16** (Figure 6g,h). These data suggest that this neoglycopolymer binds specifically to L-selectin on leukocyte cell surfaces.

We further investigated the mode of neoglycopolymer-binding to the cell surface by treating cells with the fluorescent neoglycopolymer in the presence of competing ligands

Figure 7



Fluorescence microscopy results support multivalent binding of neoglycopolymers. **(a)** Jurkat cell treated for 30 min at 4°C with unlabeled neoglycopolymer, washed, and then labeled with FITC-labeled anti-L-selectin antibody. **(b)** Jurkat cell treated for 30 min at 4°C with unlabeled neoglycopolymer, washed, and then treated with fluorescent neoglycopolymer **16**.

at 4°C (Figure 7). Unlabeled neoglycopolymers could be displaced from L-selectin on Jurkat cells by FITC-DREG-56. This result is expected as the antibody has higher intrinsic affinity than does the multivalent neoglycopolymer [22]. To provide preliminary insight into whether neoglycopolymers function by multivalent binding, we examined the ability of labeled polymer to displace unlabeled polymer. These neoglycopolymers inhibit L-selectin-mediated cell rolling with IC_{50} values of ~10 μM (an IC_{50} value of 152 μM based on saccharide residue concentration; [10]; E.J.G., unpublished observations). One would therefore expect a neoglycopolymer binding by single point attachments would be displaced readily by an excess of labeled neoglycopolymer, resulting in a punctate staining pattern similar to that seen in Figure 6b. If the bound neoglycopolymer is making multiple contacts, however, the labeled polymer of the same length would not rapidly displace it. Specifically, individual epitopes within the labeled ligand bind only weakly; consequently, the labeled ligand is at a disadvantage as it must form multivalent contacts to bind and effectively displace bound, unlabeled multivalent ligand. Consistent with multivalent binding, we found that an excess of labeled neoglycopolymer only weakly displaces the unlabeled counterpart over the 30 minute time period of the competition.

Our results highlight the utility of probe **16** for visualizing cell-surface recognition events. The results of these microscopy experiments, together with those obtained in our previous studies [10,31], suggest that these ROMP-derived glycoprotein mimics form specific contacts. Additionally, initial results suggest that these ligands make multivalent contacts with L-selectin on the cell surface. These and other multivalent ligands [43,45–47] have biological activities that range from their function as effective

inhibitors of the selectins to molecules that promote L-selectin downregulation from the cell surface [5,6,10,22,31]. Access to specifically end-labeled ligands, such as **16**, will facilitate a wide range of mechanistic investigations of cell surface–ligand binding events.

Significance

Ring-opening metathesis polymerization (ROMP) is a powerful synthetic method for creating biologically active polymers. The bifunctional capping agent described here is of general utility for terminating ruthenium-initiated ROMP reactions. Selective incorporation of a single end group allows the creation of specific bifunctional polymers such as those that could be appended to other oligomers, selectively immobilized, or used for quantitative binding studies. The functional group tolerance of the ruthenium carbene initiator, the versatility of the capping strategy, and the generality of the recognition element attachment protocol greatly expand the scope of useful materials that can be generated by ROMP. Our results illustrate the utility of this method in the investigation of cell surface multivalent receptor–ligand interactions.

Materials and methods

*Synthesis of capping agent **4***

2-(2-(2-benzyloxy)ethoxy)ethoxy)ethanol. Benzyl bromide (7.9 ml, 66.6 mmol) was added to a solution of triethylene glycol (**1**; 8.9 ml, 66.6 mmol) in 50% aq NaOH (5.3 ml), and the mixture was stirred at room temperature for 24 h. The reaction was diluted with H_2O (75 ml) and extracted with diethyl ether (Et_2O; 4×100 ml). The combined organic extracts were dried over Na_2SO_4, concentrated, and purified by flash column chromatography (silica, EtOAc), affording 2-(2-(2-benzyloxy)ethoxy)ethoxy)ethanol (6.1 g, 38%): Rf = 0.6 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.26 (m, 5H), 4.57 (s, 2H), 3.73-3.59 (m, 12H), 2.50 (b, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.1, 128.4, 127.8, 127.7, 73.3, 72.6, 70.7, 70.6, 70.4, 69.4, 61.7.

*10-phenyl-3,6,9-trioxadecanoic acid (**2**).* Chromium trioxide (3.33 g, 33.30 mmol) was added to 1.5 M H_2SO_4 (4.4 ml) at 0°C. A solution of 2-(2-(2-benzyloxy)ethoxy)ethoxy)ethanol (2.00 g, 8.32 mmol) in acetone (110 ml) was added, and the reaction was stirred for 5 h at room temperature. The solution was extracted with Et_2O (5×100 ml) and the combined extracts were washed with saturated aqueous NaCl (3×50 ml) and concentrated to a volume of 20 ml. Extraction with 5% $NaHCO_3$ (2×20 ml) was followed by acidification of the aqueous extracts to pH 2 with concentrated HCl and back extraction of the aqueous solution with Et_2O (3×50 ml). The combined organic extracts were washed with saturated aqueous NaCl (3×20 ml). Concentration provided 10-phenyl-3,6,9-trioxadecanoic acid (2) (1.71 g, 81%): R_f = 0.1–0.4 (10% MeOH/CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.06, (b, 1H), 7.35–7.25 (m, 5H), 4.57 (s, 2H), 4.17 (s, 2H), 3.77–3.60 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.1, 128.4, 127.8, 127.7, 73.3, 72.6, 70.7, 70.6, 70.4, 69.4, 61.7.

10-Phenyl-3,6,9-trioxadecanoic acid 2-(trimethylsilyl)ethyl ester. 10-Phenyl-3,6,9-trioxadecanoic acid (**2**) (1.71 g, 6.71 mmol) was dissolved in CH_2Cl_2 (13.4 ml) and the solution was cooled to 0°C. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (1.41 g, 7.38 mmol) and N,N-dimethylaminopyridine (DMAP) (41.0 mg, 0.34 mmol) were added, and the suspension was stirred for 10 min at 0°C. 2-(Trimethylsilyl)ethanol (0.87 g, 7.38 mmol) was added dropwise via syringe, and the solution was stirred for 20 min while warming to room temperature. The reaction was quenched with H_2O and extracted with Et_2O (3×50 ml). The combined Et_2O extracts were washed sequentially with 10% HCl (1×50 ml), 5% $NaHCO_3$ (1×50 ml), and saturated aqueous NaCl (1×50 ml), and dried over Na_2SO_4. Concentration of the solvent followed by flash chromatography (silica, 4:1 hexanes/EtOAc) afforded 10-phenyl-3,6,9-trioxadecanoic acid 2-(trimethylsilyl)ethyl ester (2.32 g, 97% yield): R_f = 0.26 (4:1 hexanes/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.23 (m, 5H), 4.54 (s, 2H), 4.24–4.18 (m, 2H), 4.10 (s, 2H), 3.72–3.59 (m, 8H), 1.01–0.95 (m, 2H), 0.01 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 138.2, 128.3, 127.6, 127.5, 73.2, 70.8, 70.6, 69.4, 68.8, 63.0, 17.3, −1.6; LRMS (LSIMS, 3-NBA) *m/z* 377.2 [M + Na$^+$, calc'd for $C_{18}H_{30}O_5NaSi$ 377.5].

*3,6-Dioxa-8-hydroxy-octanoic acid 2-(trimethylsilyl)ethyl ester (**3**).* 20% $Pd(OH)_2/C$ (100.0 mg) was added to a solution of 10-phenyl-3,6,9-trioxadecanoic acid 2-(trimethylsilyl)ethyl ester (500.0 mg, 1.4 mmol) in absolute EtOH (14 ml). The solution was shaken under 50 psi H_2 for 6 h, filtered through a pad of Celite® (EtOH eluent), and concentrated under reduced pressure to afford 3,6-dioxa-8-hydroxy-octanoic acid 2-(trimethylsilyl)ethyl ester (**3**) (284.4 mg, 77%): R_f = 0.29 (2:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.26–4.19 (m, 2H), 4.11 (s, 2H), 3.75–3.59 (m, 8H), 2.65 (b, 1H), 1.02–0.95 (m, 2H), 0.02 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 72.4, 70.8, 70.2, 68.6, 63.2, 61.5, 17.3, −1.6; LRMS (LSIMS, 3-NBA) *m/z* 287.1 [M + Na$^+$, calc'd for $C_{11}H_{24}O_5NaSi$ 287.4].

3,6-Dioxa-8-al-octanoic acid 2-(trimethylsilyl)ethyl ester. 3,6-Dioxa-8-hydroxy-octanoic acid 2-(trimethylsilyl)ethyl ester (250.0 mg, 0.95 mmol) was dissolved in CH_2Cl_2 (4.7 ml) and the solution was cooled to 0°C. Dimethyl sulfoxide (135 µl, 1.9 mmol) was added via syringe, followed by rapid addition of solid P_2O_5. After 30 min at 0°C, Et_3N (460 µl, 3.3 mmol) was added and the reaction was stirred for 30 min at 0°C. The reaction was quenched with 10% HCl (10 ml) and extracted with CH_2Cl_2 (3×20 ml). The combined organic extracts were washed with H_2O (1×25 ml) and saturated aqueous NaCl (1×25 ml), and dried over Na_2SO_4. Purification by flash chromatography (silica, 1:1 hexanes/EtOAc) afforded 3,6-dioxa-8-al-octanoic acid 2-(trimethylsilyl)ethyl ester (216.0 mg, 82%): R_f = 0.29 (1:1 hexanes/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.71 (s, 1H), 4.25–4.19 (m, 2H), 4.16 (d, J = 0.7 Hz, 2H), 4.10 (s, 2H), 3.75 (s, 4H), 1.01–0.96 (m, 2H), 0.02 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.6, 170.4, 76.8, 71.2, 71.0, 68.8, 63.2, 17.4, −1.5; LRMS (LSIMS, 3-NBA) *m/z* 285.1 [M + Na$^+$, calc'd for $C_{11}H_{22}O_5NaSi$ 285.4].

Cis, trans *3,6,10-Trioxa-8,9-ene-undecanoic acid 2-(trimethylsilyl)-ethyl ester (**4**).* Potassium tert-butoxide (36.3 mg, 0.30 mmol) was added to a suspension of (methoxymethyl)triphenylphosphonium chloride (117.6 mg, 0.34 mmol) in THF (2.0 ml) at 0°C. The dark red solution was stirred at 0°C for 5 min. 3,6-dioxa-8-al-octanoic acid 2-(trimethylsilyl)ethyl ester (42.5 mg, 0.16 mmol) was added dropwise as a 1 M solution in THF (160 µl), during which the solution turned from dark red to pale yellow. The reaction was quenched with saturated aqueous NaCl (5 ml) and extracted with Et_2O (3×15 ml). The combined Et_2O extracts were washed with H_2O (1×20 ml) and dried over Na_2SO_4. Concentration of the solvent followed by flash chromatography (silica, 9:2 hexanes/EtOAc) afforded 3,6,10-trioxa-8,9-ene-undecanoic acid 2-(trimethylsilyl)ethyl ester (**4**) (27.9 mg, 59%): R_f = 0.21 (5:1 hexanes/EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.52 (d, J = 13.1 Hz, 1H), 6.00 (dt, J = 6.5, 1.1 Hz, 1H), 4.87 (dt, J = 12.5, 7.5 Hz, 1 H), 4.53 (td, J = 7.0, 6.5 Hz, 1H), 4.24–4.17 (m, 4H), 4.10 (s, 2H), 4.09 (s, 2H), 4.07 (dd, J = 7.3, 1.2 Hz, 2H), 3.92 (dd, J = 7.4, 0.9 Hz, 2H), 3.71–3.54 (m, 8H), 3.58 (s, 3H), 3.53 (s, 3H), 1.01–0.94 (m, 4H), 0.01 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 170.5, 151.5, 149.1, 102.7, 98.7, 70.9, 70.9, 69.0, 68.8, 68.7, 68.5, 63.8, 63.0, 59.8, 55.9, 17.4, −1.6; LRMS (LSIMS, 3-NBA) *m/z* 313.2 [M + Na$^+$, calc'd for $C_{13}H_{26}O_5NaSi$ 313.4].

*Synthesis of polymer **10***

Dichloroethane (DCE) was deoxygenated by four freeze-pump-thaw (FPT) cycles. A solution of ruthenium catalyst **7** in DCE (100 µl) was

added to a solution of norbornene monomer **5** (15 mg, 0.10 mmol) in DCE (400 μl). The red mixture was stirred for 30 min at room temperature. Capping agent **4** (30 μl) was added neat, and the reaction was stirred at room temperature for 18 h. The mixture was concentrated, dissolved in a small amount of CH_2Cl_2, and filtered through a short pad of silica gel to remove excess capping agent **4**. The remaining material was eluted from the silica gel with EtOAc, and the solution was concentrated and dried. The clear, solid material was washed with hexanes (3×) and dried to afford polymer **10** (9.6 mg, 64%).

*Synthesis of polymer **11a***

DCE and, in a separate reaction vessel, a solution of the sodium salt of 3,6-disulfo galactose monomer **6** (15 mg, 0.027 mmol), dodecyltrimethyl ammonium bromide (DTAB) (13.5 mg, 0.044 mmol) and 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol (bis-tris) buffer (91 μl, 100 mM, pH 5.9) were deoxygenated by subjecting each solution to four FPT cycles. The deoxygenated dichloroethane (45 μl) was added to a vial containing ruthenium carbene **7** (1.5 mg, 0.0018 mmol) under nitrogen, and the purple solution was added to the reaction vessel containing the buffered solution of monomer and DTAB. The reaction was heated to 40–45°C for 20 min, capping agent **4** (10 μl) was added neat, and the mixture was stirred at 40–45°C for 15 min. The reaction was allowed to cool to room temperature and stirred for 6 h. The crude mixture was diluted with H_2O and MeOH until the solution was one phase and the final volume was ~1 ml. The polymer was purified by cation exchange chromatography (Sephadex®-SP C-25; 0.75 × 4.0 cm; Na^+, H_2O eluent), concentration to dryness, suspension of the residue in MeOH and centrifugation (3×). The MeOH insoluble material was dissolved in H_2O and concentration to dryness afforded capped, polymer **11a** as a light brown, flaky solid in moderate yields (60–80%).

*Synthesis of polymer **14***

DCE was deoxygenated by four FPT cycles. A solution of ruthenium catalyst **7** (3.3 mg, 0.004 mmol) in DCE (40 μl) was added to a solution of norbornene monomer **12** (15 mg, 0.064 mmol) in DCE (280 μl). The mixture was stirred for 30 min at room temperature. Capping agent **4** (13.5 μl) was added neat, and the reaction was stirred at room temperature for 18 h. The mixture was concentrated, dissolved in a small amount of CH_2Cl_2, and filtered through a short pad of silica gel to remove catalyst-derived impurities and excess capping agent. The solution was concentrated under reduced pressure and polymer **14** was used without purification in the coupling to 3,6-disulfo galactose amine **15**.

*Conversion of polymer **14** to polymer **11b***

Diisopropylcarbodiimide (DIC; 5 μl, 0.032 mmol) was added to a solution of polymer **14** (7.5 mg) in DMF (320 μl). (2-aminoethyl)-3,6-*O*-disulfo-α-D-galactopyranoside (16.7 mg, 0.048 mmol) **15** was added as a 1 M solution in H_2O (48 μl), and Et_3N (8.9 μl, 0.064 mmol) was added. The reaction was stirred at room temperature for ~40 h and then diluted with H_2O (~1 ml). The aqueous solution was extracted with $CHCl_3$ (3 × 2 ml) and concentrated under reduced pressure. The residue was washed with MeOH (3 × 2 ml), affording neoglycopolymer **11b**.

*Deprotection of polymer **11a***

Capped polymer **11a** was dissolved in H_2O (95 μl), and 1 M NaOH (5 μl) was added. The flask was fitted with a cold finger, and the solution was heated at 60°C for 2 h. After cooling to room temperature, the solution was diluted with H_2O to a final volume of 1 ml and neutralized (Amberlyst® 15 strongly acidic, macroreticular resin). The mixture was filtered through a small plug of glass wool to remove the resin and then concentrated to dryness, affording the deprotected polymer.

*Synthesis of conjugate **16***

Deprotected polymer (3.2 mg) was dissolved in H_2O (60 μl). EDCI (0.8 mg, 0.004 mmol) and sulfo NHS (0.9 mg, 0.004 mmol) were added, and the mixture was incubated at rt for 5 min. 5-((5-aminopentyl)thioureidyl) fluorescein (fluorescein cadaverine) (1.3 mg, 0.002 mmol) was added and the reaction was stirred at room temperature in the dark for 24 h. The fluorescein-coupled polymer was purified by cation exchange chromatography (Sephadex®-SP C-25; 0.75 × 4.0 cm; Na^+, H_2O eluent) and size exclusion chromatography (Sephadex® G-25, 0.75 × 22.0 cm, H_2O eluent), affording fluorescein-coupled polymer **16** (2.2 mg, 69%).

Fluorescence microscopy

Jurkat and HL60 cells were cultured at 37°C and 5% CO_2 in RPMI 1640 with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 1 mM sodium pyruvate. Cell viability was greater than 95% as determined by staining with 0.4% Trypan Blue. For each experiment, 5×10^5 live cells were used. For single labeling experiments, cells were centrifuged at 750 × *g* for 1 min and washed once with 1 ml cold PBS, then resuspended in 100 μl of cold PBS. FITC-labeled anti-L-selectin antibody (FITC-DREG-56) or fluorescein labeled polymer **16** were added. The final concentration of the polymer was 4 mM in galactose residues. Cells were incubated at 4°C for 30 min and washed twice with 2 ml cold PBS. Cells were fixed in 1 ml of fresh 2% HEPES buffered paraformaldehyde at 4°C for 30 min and washed twice with 2 ml cold PBS. Cells were centrifuged and resuspended in 50 μl of cold PBS. The cell solution was then applied to cover slips and mounted on clean glass slides with 5 μl of VectaShield (Vector Laboratories, Burlingame, CA). Competition and PMA-pretreated experiments were performed under similar conditions, except that cells were pretreated with unlabeled neoglycopolymer (n = 15) for 30 min at 4°C or 20 ng/ml PMA for 30 min at 37°C, respectively. Experiments with polyclonal anti-L-selectin antibodies (a generous gift of Dr Carrie Cook) were performed similarly to those with DREG-56 except the polyclonal antibody was used at a final concentration of 14 μg/ml and detected with goat-anti-chicken secondary antibody labeled with FITC (1:500, KPL, Gaithersburg, MD). Jurkat cells blocked with polyclonal anti-L-selectin antibody were treated with **16** at a final concentration of 4 mM. Slides were incubated overnight at 4°C and then viewed under oil-immersion (630×) on a Zeiss Axioskop (Zeiss, Germany) outfitted with a FITC-selective filter and Princeton Instruments MicroMax camera. Images were acquired using IPLab software (Signal Analytics Corporation, Vienna, VA).

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