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Precision Synthesis of Alternating Copolymers via Ring-Opening Polymerization of 1-Substituted Cyclobutenes

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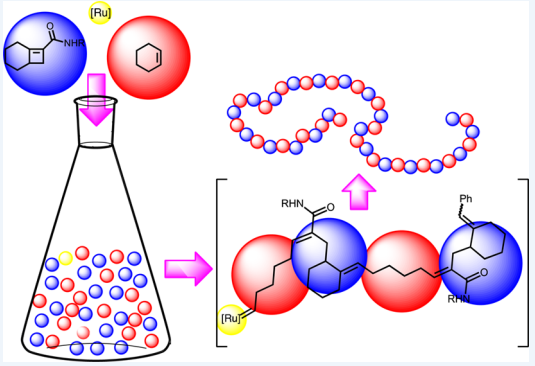
CONSPECTUS: Investigation of complex molecular systems depends on our ability to correlate physical measurements with molecular structure. Interpretation of studies that rely on synthetic polymers is generally limited by their heterogeneity; i.e., there is variation in the number and arrangement of the monomeric building blocks that have been incorporated. Superior physics and biology can be performed with materials and tools that exert precise control over the sequence and spacing of functional groups.



An interest in functional ligands combined with a desire to control the orientation and stereochemistry of monomer incorporation led to the design of new substrates for ruthenium-catalyzed ring-opening metathesis polymerization (ROMP). We discovered that ROMP of cyclobutene-1-carboxamides provides uniform and translationally invariant polymers. In contrast, cyclobutene-1-carboxylate esters ring open upon treatment with ruthenium catalyst, but they are stable to homopolymerization. However, in the presence of cyclohexene monomers, they undergo alternating ROMP (AROMP or alt-ROMP) to give copolymers with a precisely controlled sequence.

The alternating cyclobutene ester/cyclohexene pair provides access to functional group spacing larger than is possible with homopolymers. This can be desirable; for example, polymers with a regular 8−10 Å backbone spacing of cationic charge and with between four and eight cationic groups were the most effective antibacterial agents and had low cytotoxicity.

Moreover, the AROMP chemistry allows alternation of two functional moieties: one associated with the cyclohexene and one attached to the cyclobutene. In the case of antibacterial copolymers, the alternating chemistry allowed variation of hydrophobicity via the cyclohexene while maintaining a constant cation spacing through the cyclobutene. In the case of copolymers that bear donor and acceptor groups, strict alternation of the groups increased intrachain charge transfer.

Like cyclobutene-1-carboxylate esters, bicyclo[4.2.0]oct-7-ene-7-carboxylate esters ring open upon treatment with ruthenium catalyst and undergo ring opening cross-metathesis with cyclohexene to form alternating copolymers. The corresponding bicyclo[4.2.0]oct-7-ene-7-carboxyamides isomerize to the bicyclo[4.2.0]oct-1(8)-ene-8-carboxamides before they can ring open. However, the isomerized amides undergo ruthenium-catalyzed ring opening metathesis and rapidly AROMP with cyclohexene.

Our alternating copolymer systems allow functionality to be placed along a polymer chain with larger than typical spacing. We have used both homopolymers and alternating copolymers for defining the functional group density required for targeting a cell surface and for the exploration of functional group positioning within a polymer chain. These polymer systems provide access to new materials with previously inaccessible types of nanoscale structures.

■ INTRODUCTION

Iterative synthesis of polypeptides and oligonucleotides is routinely accomplished on solid supports without the use of templates. Related stepwise iterative methods have been adopted to prepare monodisperse sequence-specific polymers, for example, peptoids[17] and β-peptides,[18,19] and most recently, triazole amides and alkoxyamine amides.[20−22] Chain-growth polymerization methods based on macrocycle ROMP have been introduced to provide defined, regioregular, repeating sequences.[23−25] Herein is an account of our focus on ruthenium-catalyzed ring opening metathesis polymerization (ROMP), new ROMP monomers that we designed, and novel derived polymers, in particular, perfectly alternating copolymers.

■ ROMP IN THE STUDY OF BIOLOGY

Multivalent interactions are employed throughout receptor biology and they are commonly interrogated with multivalent probes.[1−3] These ligand-bearing probes can be synthetic polymers, self-assembled monolayers (SAMs), liposomes, biopolymers, dendrimers, and related nanoscale structures.[4−10] In the course of our work, we have employed linear synthetic polymers to investigate protein−protein and sugar−protein interactions that occur on the cell surfaces of mammalian sperm and eggs.[11−14]

Because polymerization chemistry lends itself to the rapid assembly of repeating ligand units, synthetic polymeric probes

Received: November 4, 2015
Published: February 25, 2016

DOI: 10.1021/acs.accounts.5b00490
Acc. Chem. Res. 2016, 49, 408−417

have emerged as readily accessible tools for the investigation of ligand−receptor interactions. Appropriate polymers are generated from a monomer that is connected to the desired ligand by a spacer that does not hinder the binding surface (Figure 1).

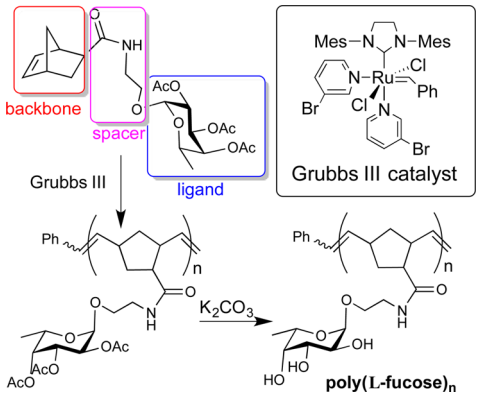


Figure 1. Schematic of functional polymer preparation with a substituted norbornene. A polymer of *n* repeating units is generated from *n* equivalents of monomer.

Successful polymer synthesis results in a multivalent scaffold that displays predictable numbers of copies of the ligand in arrays that enable engagement with the targeted receptor.

Norbornene monomers are widely used in combination with Mo or Ru catalysts for the synthesis of ROMP-based displays with defined lengths and narrow molecular mass distributions.[5,26] Norbornenes are readily obtained and affordable. Furthermore, they have the virtue of undergoing ROMP with few side reactions. The functional group-tolerant Ru catalysts[27] have the additional benefit of allowing the direct incorporation of monomers that bear a variety of ligands in the polymerization reaction. Although strongly Lewis basic groups can interfere with the Ru catalysts, standard protecting groups are generally innocuous. Therefore, these catalysts allow the use of monomers that carry diverse (although sometimes protected) functional groups. Our co-workers,[11,14] as well as those of others,[4,28−31] have prepared polynorbornenes that display either peptides or saccharides. In our laboratory, the ligand-bearing monomers are polymerized in protected form under homogeneous catalytic conditions. Subsequent deprotection provides the water-soluble chemical probe (Figure 1).

TARGETING CELL SURFACE RECEPTORS IN MAMMALIAN FERTILIZATION

Using ROMP, we developed norbornyl polymers that mimic the multivalent display of mammalian sperm protein fertilinβ.[12−14,32] Fertilinβ is a surface protein important for adhesion of the sperm to the egg plasma membrane in preparation for fusion. Monomeric peptides derived from fertilinβ are poor inhibitors of sperm-egg binding and fusion.[33] We used multivalent peptide-bearing polymers to inhibit adhesion of sperm to the egg plasma membrane.[14,32] We found that long polymers containing 100 repeating units displaying the peptide ligand were optimal inhibitors.[13] In follow-up work, we found that long polymers displaying a small number of ligands separated by nearly 100 repeating spacer units were equipotent inhibitors.[12]

Using the same norbornyl polymer backbone, we found that homopolymers displaying mannose, GlcNac, or fucose residues trigger acrosomal exocytosis in a concentration-dependent manner in mouse sperm. Each of the polymers agonizes acrosomal exocytosis independently. However, the signaling induced by these polymers converges onto the same intracellular signaling pathways.[11]

THE CYCLOBUTENE IMPERATIVE

During the evolution of our biological studies, we recognized the need for more uniform polymers, i.e., polymers with narrow dispersities, controlled regiospecificity, and specific stereochemistry. Our ability to interpret biological structure−activity relationships with our polymers at a high level of detail was limited by the stereo- and regio-irregularities of the polymerization of the racemic 5-substituted norbornenes. In principle, some (but not all) of these polymer backbone irregularities could be eliminated by the use of enantiomerically pure starting material or by relying on the achiral but synthetically less versatile norbornene-exo-2,3-dicarboximide. However, we sought approaches to new polymer backbones that would present no stereo- or regioisomeric possibilities. More well defined polymers should facilitate interpretation of structure−activity relationships in what are inherently complex systems.[15,16]

Restricting monomers to monocyclic strained olefins that are not substituted (or equivalently substituted) at the sp^3 carbon atoms removes the tacticity ambiguity in the growing chain. Thus, we considered simple cycloalkene candidates, particularly cyclobutenes in which the ring strain is similar to that of norbornene.[34] Although there are several examples of the ROMP and ROM (ring opening metathesis) of 3-substituted cyclobutenes,[35−42] polymers derived from these monomers do not meet the translational invariance criterion. On the other hand, Katz[43] had studied the ROMP of 1-methylcyclobutene[44] and 1-trimethylsilyl cyclobutene[45] with the tungsten catalyst $(CO)_5W{=}C(C_6H_5)_2$. The latter was converted to translationally invariant (all head-to-tail) polymer in which the olefinic bonds had the (*E*)-configuration. Therefore, we had some confidence that 1-substituted cyclobutenes would be useful for our purposes.

Because we were interested in studying polymers that bear peptide chains, we considered the ruthenium-catalyzed ROMP of amide-substituted monomers.[46] The development of ruthenium catalysts with improved reactivity suggested that a trisubstituted olefin might undergo metathesis.[47] Furthermore, on the basis of the reaction of ruthenium alkylidenes with acrylic acid esters,[48] we expected that the ring opening metathesis (ROM) of a 1-substituted cyclobutenecarboxylic acid amide would be regiospecific (i.e., **1** → **2** vs **1** → **3**, Figure 2). Moreover, we anticipated that metathesis would provide only one of the two geometric double bond isomers (**4**) in the ring opened product, if only because the (*E*)-isomer of **4** should be more stable than the (*Z*)-isomer.

We undertook ROM of glycine-substituted cyclobutene **1** with Grubbs III ruthenium catalyst and found that the substrate underwent ring opening to carbonyl-substituted ruthenium carbene **2**. When we included higher ratios of monomer to catalyst, polymers of repeating α,β-unsaturated amide units were obtained. One- and two-dimensional NMR characterization revealed that the ROMP of cyclobutenecarboxylic amide **1** is highly regio- and stereoselective. The ROMP reaction yields a head-to-tail ordered polymer, **4** (Figure 2), in which there are no ambiguities of tacticity, and the backbone is all *cis* (*E*-olefin). This regio- and stereoselective ROMP reaction

DOI: 10.1021/acs.accounts.5b00490
Acc. Chem. Res. 2016, 49, 408−417

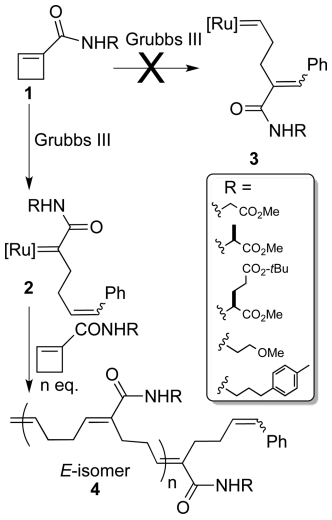


Figure 2. Cyclobutene amide ROMP.

yielded polymers with DP_n up to 50 repeating units and dispersities ranging from 1.2 to 1.6.

THE DISCOVERY OF ALTERNATING ROMP (AROMP) WITH CYCLOBUTENE MONOMERS

Although the synthesis of translationally invariant polymers with amide side chains was our initial objective, curiosity compelled us to examine the generality of the ROMP of 1-substituted cyclobutenes. We found that secondary amides, including the amides of the glycine, alanine, *t*-butyl glutamate methyl esters, and the methoxyethyl and toluylpropyl amines (Figure 2),[49] undergo facile ROMP. However, we were surprised to find that 1-cyclobutenecarboxylic acid esters **5** (Figure 3) failed to polymerize, proceeding only to the ring-opened ruthenium carbene **6** regardless of the monomer:-catalyst feed ratio.[49,50]

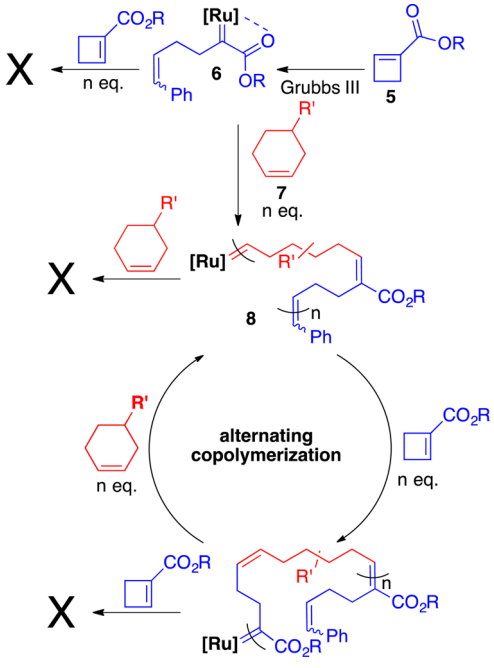


Figure 3. Alternating ROMP (AROMP).

We postulated that the stability of enoic carbene **6** to reaction with monomer resulted from the electron-withdrawing nature of the ester. However, our calculations revealed that the charge density on ester-substituted cyclobutene **5** is not significantly different from the charge density on secondary amide-substituted cyclobutene **1**, which does undergo ROMP.[49] Calculations by Fomine and Tlenkopatchev suggested that the enoic carbene is stabilized by coordination.[51] When we examined this possibility, our ab initio calculations revealed that the ester oxygen can form a chelate at the open coordination site of the 14-electron ruthenium center.[49]

We noted that the enoic carbene formed from an acrylic acid ester and Grubbs III catalyst was known to undergo ring-opening cross metathesis with cyclohexene.[48] We reasoned that our enoic carbene would also undergo this cross metathesis and that the resulting ruthenium alkylidene should be reactive with the cyclobutene ester; repetition of these events would produce an alternating copolymer (Figure 3). Indeed, mixing cyclohexene **7a** (R = H) with cyclobutene ester **5a** (R = Me) in the presence of the Grubbs III catalyst provided copolymer (Figure 3). Degrees of polymerization were as high as 150 of each unit, i.e., $(\mathbf{AB})_{150}$. ^{1}H NMR spectroscopic analysis of the polymer revealed that protons from the two different monomers integrated (approximately) at a 1:1 ratio expected for an alternating copolymer.[50] ^{1}H−^{1}H gCOSY spectra clearly showed internal connectivity between repeating units **A** and **B**, establishing that monomer incorporation alternated.

UPON FURTHER EXAMINATION: THE BACKBITING PHENOMENON

To accurately determine the extent of alternation, we performed an isotopic labeling experiment with cyclohexene-D_{10}. If the copolymer were perfectly alternating, the proton signals at δ = 5.4 and 6.8 would be reduced by 50 and 100%, respectively; in actuality, they decrease by 50 and 91% (Figure 4). Thus, the copolymer chains are predominantly alternating copolymer dyads with ∼9% **AA** dyad.

Further fractionation and spectroscopic analysis suggested the **AA** dyads are a result of intramolecular cross-metathesis, aka "backbiting", at the unhindered disubstituted alkene of the alternating copolymer. This backbiting reaction results in the formation of a cyclic copolymer; the linear copolymer does not contain **AA** dyads.[50] Moreover, the **AA** dyad is formed regardless of the monomer feed ratio. That is, it is independent of monomer concentration and cannot be suppressed by judicious selection of reaction conditions.

OPTIMIZING FOR CYCLIC ALTERNATING COPOLYMERS

We considered the possibility that backbiting would be favored by a catalyst in which the ruthenium remained coordinated to the terminus of the growing chain; this situation would enforce a cyclic conformation for the polymer backbone during the chain-lengthening steps.[52] Thus, we examined the ROMP of cyclohexene **7a** and 1-cyclobutene carboxylic acid ester **5a** or **5b** with the readily available Hoveyda−Grubbs II catalyst (Figure 5).[53,54] Integration of the ^{1}H NMR spectra of the resulting polymers and that of the polymer derived from ester **5a** and cyclohexene **7a-D10** confirmed the alternating structure and showed that the polymer contained no end groups. On the basis of NMR, mass spectroscopy, and gel phase chromatography (GPC) evidence, we estimated that the cyclic copolymers

DOI: 10.1021/acs.accounts.5b00490
Acc. Chem. Res. 2016, 49, 408−417

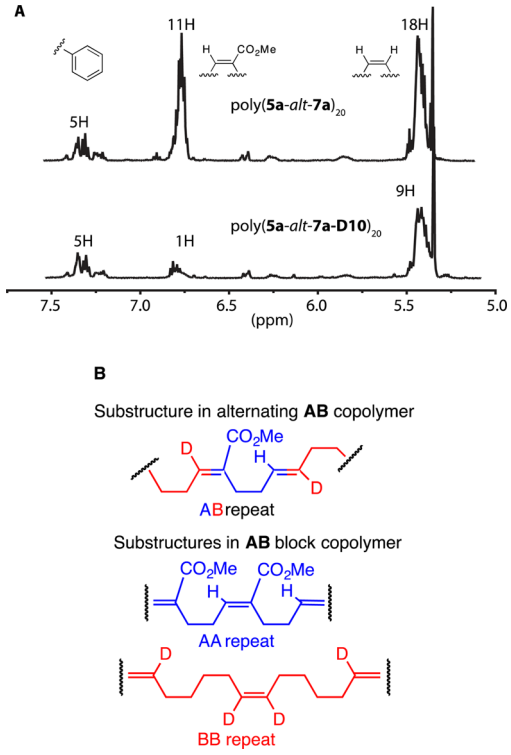


Figure 4. Isotope labeling to quantify the degree of alternation in AROMP. (A) Alkene region of ^{1}H NMR spectra (CD_2Cl_2) of poly(**5a**-*alt*-**7a**)$_{20}$ and poly(**5a**-*alt*-**7a-D10**)$_{20}$. Proton integrations and assignments are indicated above the peaks. (B) Possible substructures generated in the copolymerization of **5a** with cyclohexene-D10, **7a-D10**. Red carbons are perdeuterated. Blue carbons bear hydrogen. Adapted with permission from ref 50. Copyright (2009) American Chemical Society.

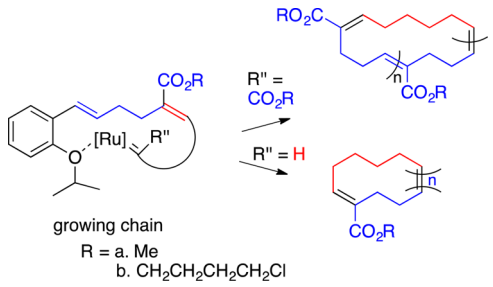


Figure 5. Cyclic alternating ruthenium-catalyzed ring-opening metathesis polymerization.[52] The Ru catalyst used is Hoveyda–Grubbs II. The monomers used are **5a** or **5b** and **7**.

contained 3–5 **AB** repeats. Thus, backbiting occurs early during the polymerization process.

BRANCHING OUT: USEFUL FUNCTIONAL GROUPS AND NEW SUBSTITUTION PATTERNS ON THE MONOMERS

We explored the variety of functionality that can be introduced into alternating copolymers under conditions that favor linear AROMP copolymers[49] and those that favor cyclic copolymers.[52] We found that a variety of alkyl esters[49,55] and a phenyl[49] ester undergo AROMP with cyclohexene with high conversion (Figure 6). The use of the electrophilic phenyl ester allows postpolymerization modification of the polymer.

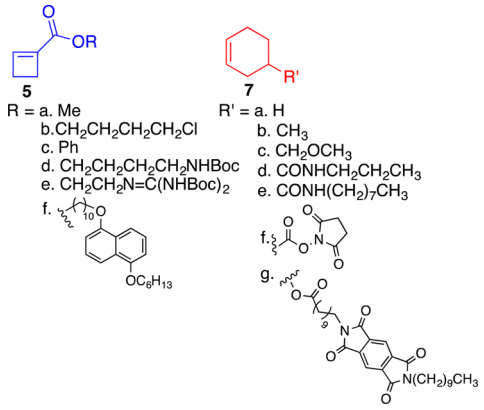


Figure 6. First generation monomers used in AROMP.

Alternatively, the alkyl esters can bear masked functionalities that yield desired functionality after the polymerization step.

When we investigated the effect of substituents on the cyclohexene, we found that a methyl group at C-1 prohibited ROMP. However, substitution at the 4-position enabled the introduction of functionality (Figure 6).[50,55,56] Thus, with a cyclobutene carboxylic acid derivative and a cyclohexene monomer that bear different functional groups, we could produce copolymers with an alternating backbone and alternating functionality in a single polymerization reaction. The alternating copolymer formation derives from the inability of the cyclobutene ester and cyclohexene monomers to self-polymerize in combination with the favorable kinetics of cross polymerization.

With the products available from ROMP and AROMP of 1-substituted cyclobutenes, we were poised to compare the binding properties of three different categories of polymers: homopolymers, random copolymers, and alternating copolymers. Experiments in two different systems show that, at least for some applications, alternating copolymers can exhibit superior properties.

ALTERNATING COPOLYMERS: EXAMINATION OF POLYMER MIMICS OF ANTIMICROBIAL PEPTIDES (AMPS)

Eukaryotes produce small "host-defense" antimicrobial peptides (AMPs, approximately 12–80 amino acid residues) as part of their innate immune response against pathogen infection.[57−59] Typically, AMPs are amphipathic with segregated hydrophobic and cationic regions of the polypeptides.[60] One approach to the development of synthetic antibiotics is to mimic the alternating cationic and lipophilic nature of these peptides with polymers that have improved chemical and biochemical stability.[61,62]

We employed AROMP chemistry with cyclobutene ester/cyclohexene pairs to prepare polymers with alternating cationic and lipophilic residues that mimic native AMPs. We varied the composition of the polymers and the distance (average or exact) between cationic groups.

Variation of the cyclobutene substituent allowed us to test a range of nitrogen functionalities as the cationic group. BOC-protected amines **5d** and **5e** could be used directly in the AROMP reactions. Alternatively, the cationic functionalities could be introduced in a postpolymerization step by reaction of the 4-chlorobutyl side chain of **5b** with trimethylamine.[55] This derivatization cleanly provided modified water-soluble polymers (Figure 7).

DOI: 10.1021/acs.accounts.5b00490
Acc. Chem. Res. 2016, 49, 408–417

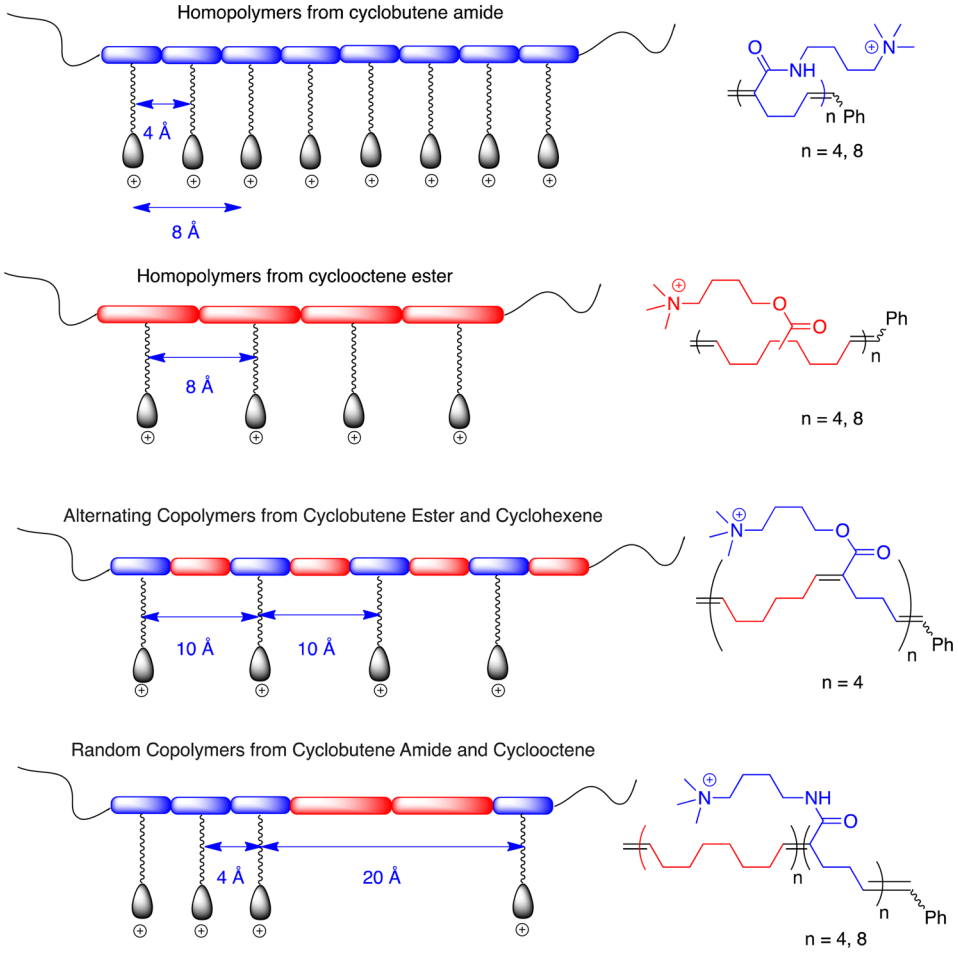


Figure 7. Structure–activity relationship for amphiphilic polymers. Polymers containing longer spacing exhibit better antibacterial activities.

Polymers bearing the trimethylammonium and guanidinium cations were the most effective antibacterials, presumably because they are charged regardless of local pH. In later experiments, we focused on the trimethylammonium-bearing polymers because they were more easily prepared than the guanidinium-bearing polymers.

Next, we undertook an investigation of the optimal spacing for antibacterial activity in combination with low host cell toxicity.[55] Using homopolymerization, alternating copolymerization, and random copolymerization, we prepared a series of polymers that display trimethylammonium ions at varying distances (Figure 7). Homopolymerization of the cyclobutene amide provided product in which the chain had a 4-carbon spacing between cationic functional groups. Homopolymerization of cyclooctene substituted at the 5-position provided an intermediate spacing of 8 carbons between cationic groups. The cyclobutene/cyclohexene alternating copolymer pair provided a 10-carbon interval along the polymer backbone between cationic side chains. Lastly, random copolymerization of substituted cyclobutene amide and cyclooctene provided copolymers in which the most frequent spacing is 4, 12, and 20 carbons. Because the alternating copolymer preparations were contaminated with cyclic polymer, we prepared cyclic alternating copolymer independently as a control.[52] The backbone, size of spacers, and functionality were maintained to the extent possible, to focus the comparison on cation–cation distances. All polymers contained between four and eight cationic groups.

The series of polymers was screened against six species of Gram-positive and Gram-negative bacteria. Linear polymers with a regular 8 to 10 Å backbone spacing and with between four and eight cationic groups were the most effective antibacterial agents against all strains. These active polymers caused bacterial membrane depolarization, lysis, and leakage of cellular contents as has been observed with other synthetic polymer mimics.[63] Erythrocyte lysis, a measure of host cell cytoxicity, was low.

The alternating cyclobutene/cyclohexene pair provided access to ligand spacing larger than is possible with homopolymers. Moreover, the alternating aspect of the polymerization enabled variation of hydrophobicity through linkages on the cyclohexene, **7b–7e**, while maintaining constant cation spacing through linkage on the cyclobutene. We found that polymers derived from the unsubstituted cyclohexene had the best antibacterial properties.[55]

POLYMERS IN WHICH ALTERNATING SUBSTITUENTS INTERACT

We presumed that any interaction between two different substituents on a polymer would be maximized if the optimum spatial relationship between them could be imposed uniformly. The donor–acceptor side-chain functionalized polymers that exhibit intrachain charge transfer seemed a likely system for the demonstration of this principle.

Weck had described polymers derived from a norbornene monomer that displayed a dialkoxynaphthalene (DAN)

DOI: 10.1021/acs.accounts.5b00490
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substructure and a cyclooctene monomer that carried a pyromellitic dianhydride (PDI) moiety.[64] The DAN/PDI functional pair is known to exhibit a charge-transfer absorbance (~460 nm) when the aromatic units are aligned in a face-to-face geometry.[65] Thus, the charge-transfer unit provides a spectroscopic reporter on the conformational arrangement of the polymer side chains in solution.[66−68] Using a Blechert−Buchmeiser unsymmetric ruthenium catalyst[69] and the recommended 1:50 ratio of the norbornene and cyclooctene monomers,[64] Weck's group prepared polymers that had a short alternating stretch and that terminated in a polycyclooctene tail. A polycyclooctene block is formed at the end of the polymerization reaction because an excess of the less reactive monomer, cyclooctene, is used to effect alternation.[64] Homopolymer tails are a common feature in alternating ROMP products.[70−73]

We employed the cyclobutene/cyclohexene system with DAN and PDI substitution (Figure 8) to test how well energy

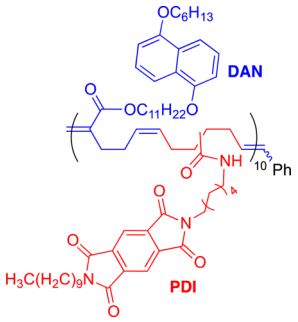

Figure 8. Alternating donor/acceptor pairs.

could flow through a copolymer that was expected to be perfectly alternating throughout. The alternating copolymer obtained had far more efficient charge transfer than the COE/NB system with nearly 50-fold higher absorbance in the charge transfer region on a per monomer pair basis.[56] The concentration dependence of the charge-transfer was consistent with intramolecular charge-transfer through π stacking along the polymer backbone. Aromatic signals in the ^{1}H NMR spectrum of the polymer shifted upfield; this effect is consistent with folding structure that aligns the donor−acceptor aromatic units for $\pi-\pi$ stacking and energy transfer. Thus, the backbone of the alternating copolymer provides a structure that is sufficient, in combination with sequence specificity, to lead to energy transfer.

EXTENDING THE MONOMER LIBRARY FOR AROMP

Fortuitously, we discovered during the course of this work that the backbiting reaction that had plagued the cyclobutene/cyclohexene alternating ROMP is inhibited when the monomers bear large substituents.[56] We surmised that the increased steric hindrance at the enoic carbene and β to the disubstituted alkene prevents secondary metathesis reactions.

The limitation of this approach to making linear polymers is that the same property that inhibits backbiting, i.e., steric bulk, also reduces the rate of propagation, thereby allowing catalyst decomposition to become a length-limiting factor in the polymerization. Therefore, we sought additional ways to control backbiting in the cyclobutene/cyclohexene alternating system. Noting the widespread utility of norbornene derivatives in ROMP for the preparation of linear polymers, we investigated the reactivity of bicyclic monomers in AROMP.

We found that methyl bicyclo[2.2.1]hept-2-ene-2-carboxylate, **9** (Figure 9), did not undergo ring-opening metathesis.

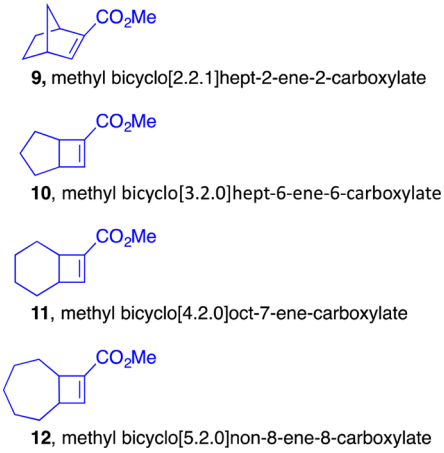

Figure 9. Second generation monomers used in AROMP.

Consequently, we postulated that the combination of steric congestion and trisubstitution on the alkene was prohibitive. Therefore, we examined the fused bicyclic esters, bicyclo[3.2.0]hept-6-ene-6-carboxylate **10**, bicyclo[4.2.0]oct-7-ene-7-carboxylate **11**, and bicyclo[5.2.0]non-8-ene-8-carboxylate **12** (Figure 9), as potential ester monomers.[74] All of the cyclobutene rings in these ester monomers rapidly ring open upon addition of Ru catalyst, but this step is not followed by homopolymerization. The rates of ring opening are proportional to the ring strain of the system; [3.2.0] ring opening was approximately four times faster than that of the [4.2.0] system and 12 times faster than that of the [5.2.0] system.

As one would predict, addition of cyclohexene to the ring-opened enoic carbene generated alternating copolymers. However, we found, somewhat to our surprise, that the most strained [3.2.0] monomer **10** was not the most effectively polymerized.[74] Inspection of spectra revealed that the growing chain, here too, undergoes backbiting reactions. In contrast, [4.2.0] monomer **11** provides a completely linear polymer as verified by deuterium labeling. Polymers with 35 repeating **AB** units on average and dispersity indices of $Đ_M = 2.0 \pm 0.1$ were obtained. We attributed the limited lengths to the slow propagation rates that ensued with introduction of the fused ring.

THE DISCOVERY OF ISOMERIZATION AROMP

Because [4.2.0] ester **11** provided polymers with unique backbone structures, we investigated the utility of the amide homologue in ROMP. To our surprise, we found that bicyclo[4.2.0]oct-7-ene-7-carboxamides **13** (Figure 10) did not readily polymerize or ring open when treated with Grubbs III catalyst. Instead, in each case tested, an alkene isomer was obtained. The isomerized amide is a bicyclo[4.2.0]oct-1(8)-ene-8-carboxamide (**14**, Figure 10); bicyclo[4.2.0]oct-1(8)-ene is the thermodynamic product in the unsubstituted [4.2.0] system.[75]

In contrast, the analogous bicyclo[3.2.0]hept-6-ene-6-carboxamide did not isomerize. This result is consistent with the thermodynamically preferred position of the olefin in the unsubstituted [3.2.0] system.[75] We verified that the

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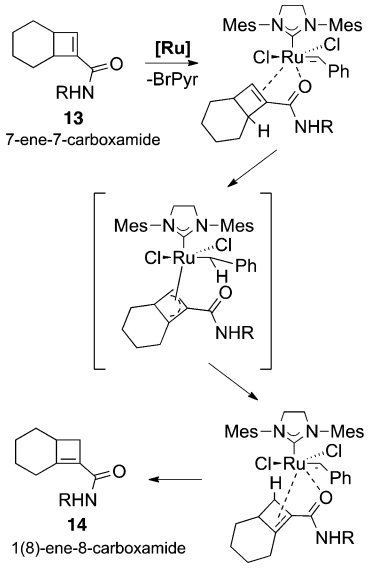

Figure 10. Ruthenium-catalyzed isomerization of bicyclo[4.2.0]oct-7-ene-7-carboxamide **13**. Adapted with permission from ref 77. Copyright (2015) American Chemical Society.

bicyclo[4.2.0]oct-7-ene-7-carboxy ester **11** does not isomerize with Grubbs III catalyst.

Further testing of the bicyclo[4.2.0]oct-7-ene-7-carboxamide isomerization reaction eliminated the possibility of the presence of a Ru–H species in the initiating solution. We determined that an open coordination site on the Ru is required for isomerization and that the substituent on the amide controls the isomerization rate. These observations suggest that formation of an amide-coordinated species (Figure 10) facilitates isomerization around the ring via a π-allyl complex[76] to form the thermodynamic product.

With facile access to tetrasubstituted olefin **14** and the precedent of 1-cyclobutene amide homopolymerization (Figure 2), we tested the homopolymerization of bicyclo[4.2.0]oct-1(8)-ene-8-carboxamides **14.** No homopolymerization was observed.[77] However, monitoring the reaction by ^{13}C NMR spectroscopy revealed that the tetrasubstituted amide does undergo ring-opening metathesis. Thus, it appeared that the combination of amide coordination in the ruthenium carbene and steric hindrance from the tetrasubstituted alkene in the monomer prevented homopolymerization by metathesis.

Prompted by the ability of cyclohexene to release enoic carbenes from a kinetic trap,[50] we added cyclohexene to the ring-opened bicyclo[4.2.0]oct-1(8)-ene-8-carboxamide **15** and found that perfectly alternating copolymer was obtained (Figure 11). The copolymer has a unique backbone in which both the alkenes formed are trisubstituted. The conjugated alkene formed is the *E*-isomer (*cis*).

The isomerization and polymerization could be performed in one reaction pot. However, the dispersities of the products from these reactions ranged from 1.6 to 1.8. We assumed catalyst was lost during isomerization; therefore, we isolated and purified the isomerized amide and subjected this intermediate to fresh catalyst. This protocol gave polymers with dispersities ranging from 1.1 to 1.2.[77]

Alternating copolymers obtained in this system were very long with DP_n up to 400 or more repeating units (800 monomers total).[77] The key advantages of the bicyclo[4.2.0]-oct-1(8)-ene-8-carboxamide **14** and cyclohexene **7** are (a) monomer economy, (b) production of long, soluble copolymers, and (c) extremely high sequence precision in the copolymer backbone. This polymer system is currently being applied to the development of polymer probes for fertilization and in the synthesis of new materials.

CONCLUSIONS

Numerous research groups have approached the assembly of functional macromolecules for various applications. This Account has focused on the inspiration of our efforts to develop easily assembled multivalent molecules for the interrogation of biological systems provided to develop new monomers for ring-opening polymerization. We found that cyclobutene-1-carboxamide monomers undergo ROMP to provide polymer backbones with high-density packing of

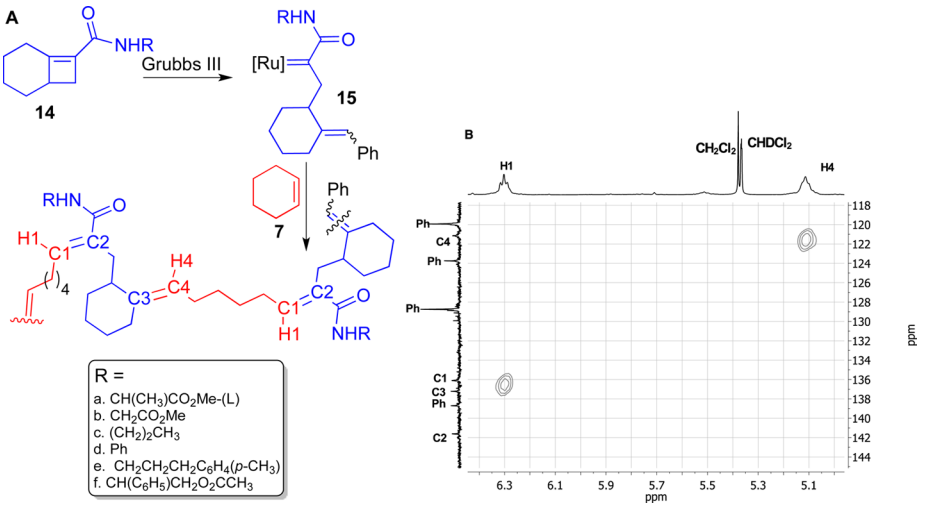

Figure 11. Third generation alternating copolymerization. (A) Alternating copolymer formed from bicyclo[4.2.0]oct-1(8)-ene-8-carboxamide **14** and cyclohexene 7. (B) Alkene region of the HSQC spectrum of poly(**14d**-*alt*-**7a**). The polymer backbone has only four alkene carbons and two alkene hydrogens corresponding to C1–C4 and H1 and H4, respectively. Panel (B) reproduced with permission from ref 77. Copyright (2015) American Chemical Society.

DOI: 10.1021/acs.accounts.5b00490
Acc. Chem. Res. 2016, 49, 408–417

functionality. The monomers are incorporated in a head-to-tail fashion to form exclusively (*E*)-alkenes.

Furthermore, we discovered cyclobutene/cyclohexene monomer pairs that ROMP to give strictly alternating copolymers and that allow functionality to be placed along a polymer chain with larger than typical spacing. Our most recent discovery of the bicyclo[4.2.0]oct-1(8)-ene-8-carboxamide/cyclohexene system allows the preparation of bulk quantities of long alternating copolymers (DP_n as high as 400 **AB** monomer units). The preparation of new materials with previously inaccessible types of nanoscale structures can now be achieved. It will be exciting to explore further the scope of functionality that can be incorporated in alternating copolymers, to investigate how regular ligand spacing affects multivalent targeting, and to expand the application of precisely alternating copolymers.

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Notes

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ACKNOWLEDGMENTS

We thank the National Institutes of Health (NIH) for Grants R01GM097971, R01HD38519 (N.S.S.), and R01GM74776 (K.A.P). We also thank our many co-workers who executed and contributed to the work described.

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