New Materials for Biological Applications Prepared by Olefin Metathesis Reactions

Thesis by

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Dedicated to my Mum,
whose strength and will have been an inspiration
Two Chinamen, behind them a third,
Are carved in lapis lazuli,
Over them flies a long-legged bird,
A symbol of longevity;
The third, doubtless a serving-man,
Carries a musical instrument.

Every discoloration of the stone,
Every accidental crack or dent,
Seems a water-course or an avalanche,
Or lofty slope where it still snows,
Though doubtless plum or cherry-branch
Sweetens the little half-way house
Those Chinamen climb towards, and I
Delight to imagine them seated there;
There, on the mountain and the sky,
On all the tragic scene they stare.
One asks for mournful melodies;
Accomplished fingers begin to play.
Their eyes mid many wrinkles, their eyes,
Their ancient, glittering eyes, are gay.

-From Lapis Lazuli
by William Butler Yeats
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Abstract

With the advent of well-defined ruthenium olefin metathesis catalysts that are highly active and stable to a variety of functional groups, the synthesis of complex organic molecules and polymers is now possible; this is reviewed in Chapter 1. The majority of the rest of this thesis describes the application of these catalysts towards the synthesis of novel polymers that may be useful in biological applications and investigations into their efficacy.

A method was developed to produce polyethers by metathesis, and this is described in Chapters 2 and 3. An unsaturated 12-crown-4 analog was made by template-directed ring-closing metathesis (RCM) and utilized as a monomer for the synthesis of unsaturated polyethers by ring-opening metathesis polymerization (ROMP). The yields were high and a range of molecular weights was accessible. In a similar manner, substituted polyethers with various backbones were synthesized; polymers with benzo groups along the backbone and various concentrations of amino acids were prepared. The results from in vitro toxicity tests of the unsubstituted polyethers are considered.

The conditions necessary to synthesize polynorbornenes with pendent bioactive peptides were explored as illustrated in Chapter 4. First, the polymerization of various norbornenyl monomers substituted with glycine, alanine or penta(ethylene glycol) is described. Then, the syntheses of polymers substituted with peptides GRGD and SRN,
components of a cell binding domain of fibronectin, using newly developed ruthenium initiators are discussed.

In Chapter 5, the syntheses of homopolymers and a copolymer containing GRGDS and PHSRN, the more active forms of the peptides, are described. The ability of the polymers to inhibit human dermal fibroblast cell adhesion to fibronectin was assayed using an *in vitro* competitive inhibition assay, and the results are discussed. It was discovered that the copolymer substituted with both GRGDS and PHSRN peptides was more active than both the GRGDS-containing homopolymer and the GRGDS free peptide.

Historically, one of the drawbacks to using metathesis is the removal of the residual ruthenium at the completion of the reaction. Chapter 6 describes a method where the water soluble tris(hydroxymethyl)phosphine is utilized to facilitate the removal of residual ruthenium from RCM reaction products.
Table of Contents

Chapter 1: General Introduction to Olefin Metathesis And Its Application to Bioactive Materials

Part 1. Olefin Metathesis
- General Aspects
- Ring-Opening Metathesis Polymerization
- Ring-Closing Metathesis
- Cross Metathesis and Acyclic Diene Metathesis Polymerization

Part 2. Manipulation of Biological Response Through the Incorporation of Poly(ethylene glycol) and Arg-Gly-Asp into Synthetic Materials
- Poly(ethylene glycol) as a Biomaterial
- Incorporating Arg-Gly-Asp to Elicit a Biological Response
- Thesis Research
- References

Chapter 2: The Synthesis of Polyethers Using a Tandem Approach: Template-Directed Ring-Closing Metathesis Followed by Ring-Opening Metathesis Polymerization

Abstract
- Introduction
- Template-Directed RCM to Synthesize Unsaturated Crown Ether Monomers
- Results and Discussion
- Polymer Synthesis and Characterization
- Depolymerization
- Toxicity of Polyether 6
- Summary
- Experimental Section
- Monomer Synthesis
- Polymer Synthesis
- Cell Culture
- Acknowledgements
- References
## Chapter 3: Synthesis of Peptide-Substituted Polyethers by Ring-Opening Metathesis Polymerization of Unsaturated Crown Ethers

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>55</td>
</tr>
<tr>
<td>Introduction</td>
<td>56</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>57</td>
</tr>
<tr>
<td>Monomer Synthesis</td>
<td>57</td>
</tr>
<tr>
<td>Polymer Synthesis and Characterization</td>
<td>62</td>
</tr>
<tr>
<td>Polymers Containing RGD</td>
<td>69</td>
</tr>
<tr>
<td>Summary</td>
<td>70</td>
</tr>
<tr>
<td>Experimental Section</td>
<td>71</td>
</tr>
<tr>
<td>Monomer Synthesis</td>
<td>72</td>
</tr>
<tr>
<td>Polymer Synthesis</td>
<td>78</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>81</td>
</tr>
<tr>
<td>References</td>
<td>81</td>
</tr>
</tbody>
</table>

## Chapter 4: Synthesis of Norbornenyl Polymers With Pendent Bioactive Oligopeptides by Ring-Opening Metathesis Polymerization

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>85</td>
</tr>
<tr>
<td>Introduction</td>
<td>86</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>88</td>
</tr>
<tr>
<td>Monomer Synthesis</td>
<td>88</td>
</tr>
<tr>
<td>Polymers With One Pendent Amino Acid: Synthesis and Characterization</td>
<td>93</td>
</tr>
<tr>
<td>Polymers With Pendent Biologically Relevant Oligopeptides: Synthesis and Characterization</td>
<td>101</td>
</tr>
<tr>
<td>Polymer Stereoisomers</td>
<td>106</td>
</tr>
<tr>
<td>Polymers With Spacer Groups and Pendent Biologically Relevant Oligopeptides</td>
<td>107</td>
</tr>
<tr>
<td>Summary</td>
<td>109</td>
</tr>
<tr>
<td>Experimental Section</td>
<td>109</td>
</tr>
<tr>
<td>Monomer Synthesis</td>
<td>111</td>
</tr>
<tr>
<td>Polymer Synthesis</td>
<td>122</td>
</tr>
<tr>
<td>Polymer Stereoisomers</td>
<td>130</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>131</td>
</tr>
<tr>
<td>References</td>
<td>131</td>
</tr>
</tbody>
</table>

## Chapter 5: Inhibition of Cell Adhesion to Fibronectin by Oligopeptide Substituted Polynorbornenes

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>135</td>
</tr>
<tr>
<td>Introduction</td>
<td>136</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>137</td>
</tr>
<tr>
<td>Chapter 6: Purification Technique for the Removal of Ruthenium from Olefin Metathesis Reaction Products</td>
<td>154</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>-----</td>
</tr>
<tr>
<td>Abstract</td>
<td>155</td>
</tr>
<tr>
<td>Introduction</td>
<td>156</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>157</td>
</tr>
<tr>
<td>Summary</td>
<td>162</td>
</tr>
<tr>
<td>Experimental</td>
<td>162</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>164</td>
</tr>
<tr>
<td>References</td>
<td>164</td>
</tr>
</tbody>
</table>
# List of Figures, Tables, and Schemes

## Chapter 1

### Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>3</td>
</tr>
<tr>
<td>Figure 2</td>
<td>6</td>
</tr>
<tr>
<td>Figure 3</td>
<td>7</td>
</tr>
<tr>
<td>Figure 4</td>
<td>10</td>
</tr>
<tr>
<td>Figure 5</td>
<td>13</td>
</tr>
<tr>
<td>Figure 6</td>
<td>15</td>
</tr>
<tr>
<td>Figure 7</td>
<td>17</td>
</tr>
<tr>
<td>Figure 8</td>
<td>18</td>
</tr>
</tbody>
</table>

### Schemes

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 1</td>
<td>2</td>
</tr>
<tr>
<td>Scheme 2</td>
<td>2</td>
</tr>
<tr>
<td>Scheme 3</td>
<td>5</td>
</tr>
<tr>
<td>Scheme 4</td>
<td>9</td>
</tr>
<tr>
<td>Scheme 5</td>
<td>9</td>
</tr>
<tr>
<td>Scheme 6</td>
<td>11</td>
</tr>
<tr>
<td>Scheme 7</td>
<td>17</td>
</tr>
</tbody>
</table>

## Chapter 2

### Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>42</td>
</tr>
<tr>
<td>Figure 2</td>
<td>43</td>
</tr>
<tr>
<td>Figure 3</td>
<td>44</td>
</tr>
</tbody>
</table>

### Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>34</td>
</tr>
<tr>
<td>Table 2</td>
<td>37</td>
</tr>
</tbody>
</table>

### Schemes

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 1</td>
<td>32</td>
</tr>
<tr>
<td>Scheme 2</td>
<td>33</td>
</tr>
<tr>
<td>Scheme 3</td>
<td>35</td>
</tr>
<tr>
<td>Scheme 4</td>
<td>36</td>
</tr>
<tr>
<td>Scheme 5</td>
<td>37</td>
</tr>
<tr>
<td>Scheme 6</td>
<td>40</td>
</tr>
</tbody>
</table>
Chapter 3

Figures
- Figure 1: 67

Tables
- Table 1: 64
- Table 2: 66

Schemes
- Scheme 1: 58
- Scheme 2: 59
- Scheme 3: 59
- Scheme 4: 61
- Scheme 5: 61
- Scheme 6: 63
- Scheme 7: 69

Chapter 4

Tables
- Table 1: 96
- Table 2: 99
- Table 3: 105

Schemes
- Scheme 1: 89
- Scheme 2: 90
- Scheme 3: 91
- Scheme 4: 91
- Scheme 5: 92
- Scheme 6: 102
- Scheme 7: 108

Chapter 5

Figures
- Figure 1: 141
- Figure 2: 142

Tables
- Table 1: 139
Chapter 6

Figures
Figure 1 ......................................................... 157
Figure 2 ......................................................... 158
Figure 3 ......................................................... 160

Tables
Table 1 ............................................................. 159

Schemes
Scheme 1 .......................................................... 158
Scheme 2 .......................................................... 161
Chapter 1

General Introduction to Olefin Metathesis

And Its Application to Bioactive Materials
Part 1. Olefin Metathesis

**General Aspects.** Olefin metathesis is a transition metal catalyzed reaction in which the groups of two substituted alkenes are transposed (Scheme 1).\(^1\) Since this method is a catalytic way to both break and form C-C double bonds, it has developed into a powerful tool for organic molecule and polymer synthesis.

![Scheme 1](image)

**Scheme 1.** General olefin metathesis reaction with unsymmetrically substituted olefins.

The mechanism of olefin metathesis, originally proposed by Chauvin et al.,\(^2\) was shown to proceed through a metallacyclobutane intermediate (Scheme 2). A \([2+2]\) cycloaddition between an olefin and a transition metal-carbene complex forms the intermediate. Cleavage of the metallacyclobutane can occur in a productive fashion to form a new metal-carbene and olefin or in a nonproductive fashion to revert back to the original starting materials. Each step in the reaction is reversible, and the reaction is under thermodynamic control.

![Scheme 2](image)

**Scheme 2.** Chauvin mechanism of olefin metathesis.
The potential of metathesis in organic synthesis was greatly increased by the advent of single component catalysts.\textsuperscript{1,3} Because these well-defined complexes react in a controlled and consistent fashion, their activities and levels of functional group tolerance can be studied. As a result, the catalyst performance has been attenuated by altering the metal or ligand sphere, leading to the development of increasingly more active and tolerant catalysts. Many of the modern catalysts (representatives shown in Figure 1) are well suited for the synthesis of complex organic molecules and polymers.

![Image of catalysts](image)

**Figure 1.** Representative single-component, metathesis-active catalysts.

The molybdenum and tungsten catalysts, particularly 1, developed by Schrock and coworkers (Figure 1), are highly active and able to affect a variety of transformations.\textsuperscript{4} However, the viability of these catalysts has been limited by their
extreme sensitivity to oxygen, water, and several common functional groups, such as aldehydes and alcohols. This limitation has restricted the application of these compounds to certain substrates and necessitated the use of rigorously purified solvents and starting materials.

Metathesis catalysts that are tolerant of both polar and protic functional groups were first discovered by Grubbs and coworkers, when the first ruthenium bisphosphine catalysts were developed (Figure 1). Since then, a number of ruthenium-based complexes have been synthesized that offer synthetic advantages over the molybdenum and tungsten alkylidenes. For example, 2 was found to be selective for olefins in the presence of a wide variety of functional groups, including alcohols and aldehydes. These ruthenium alkylidenes are also stable to many impurities including air, protic solvents, and water. In fact, catalyst 2 affects polymerizations in water under emulsion conditions using a surfactant. By altering the phosphine ligands on 2 to be water soluble, the catalyst is active in pure water or methanol. The only drawback of these bisphosphine catalysts is that they display lower activity compared to the molybdenum catalyst 1, and they are not particularly stable to primary or secondary amines.

It was discovered by Herrmann, Grubbs, and Nolan that the activity of ruthenium catalysts could be increased by substituting one or both of the phosphines with basic N-heterocyclic carbene ligands. When the substitution was made with one 2,3-dihydroimidazolyldiene ligand by Grubbs and coworkers, the resulting catalyst, 3, was found to be more active than the molybdenum-based catalyst 1. This activity was coupled with high stability and functional group tolerance demonstrated by the other ruthenium-based metathesis catalysts. This recent breakthrough has allowed for the
synthesis of materials previously inaccessible and for the reduction in catalyst loadings, making reactions more cost-effective.\textsuperscript{13,14,15}

![Scheme 3. Metathesis processes: A) ring-closing metathesis; B) cross metathesis; C) ring-opening metathesis polymerization; D) acyclic diene metathesis polymerization.](image)

Metathesis catalysts have been employed to synthesize small molecules, natural products, and polymers. These have been accomplished using the reactions shown in Scheme 3. Organic molecules have been synthesized by ring-closing metathesis (RCM) and cross metathesis (CM), while polymers have been synthesized using ring-opening metathesis polymerization (ROMP) and acyclic diene metathesis polymerization.
(ADMET). These processes are interrelated, and the particular pathway is determined by the olefin structure and reaction conditions.

**Ring-Opening Metathesis Polymerization.** Many olefin metathesis catalysts initiate the ROMP of cyclic olefins to yield poly(olefins) (Scheme 3C). ROMP is driven enthalpically by the release of ring-strain in the monomer. Highly strained monomers, such as norbornenes, cyclobutenes, and barrelenes (Figure 4, A-C), have been successfully polymerized using metal alkylidenes. Many metathesis catalysts will also affect the polymerization of less-strained olefins, such as cyclooctene, cyclopentene and cyclooctadiene (Figure 4, D-F). However, cyclohexene (Figure 4, G) will not polymerize under equilibrium conditions. Since ROMP is an equilibrium process, depending on the monomer and conditions used, depolymerization can also occur, where the growing chain “back-bites” to form cyclic olefins.

![Cyclic Olefins](image)

**Figure 2.** Cyclic Olefins: A) norbornene, B) barrelene, C) cyclobutene, D) cyclooctene, E) cyclooctadiene, F) cyclopentene, and G) cyclohexene.

Compared to other types of polymerizations methods, ROMP has several advantages. Many of the metathesis catalysts already described initiate the living polymerization of strained cyclic olefins. A living polymerization proceeds in the absence of chain termination and can be used to synthesize polymers with predictable molecular weights and narrow polydispersities. In some cases, the architecture can also
be controlled, and block copolymers have been synthesized.\textsuperscript{20} The synthesis of random copolymers is straightforward, because unlike other polymerization methods, generally the monomer incorporation is dependent only on the concentration in the feed. In addition, the synthesis of polymers with well-defined end groups (telechelic polymers) has been accomplished.\textsuperscript{21} Polymers have even been polymerized off of surfaces.\textsuperscript{22}

Many of the monomers shown in Figure 2 can be derivitized and polymerized by metal alkylidenes to form functionalized materials for a variety of purposes. Functionalized cyclooctenes have been polymerized and hydrogenated to make perfectly linear, functionalized polyethylene.\textsuperscript{23} Conducting polymers\textsuperscript{24} and polymers with photoluminescence properties\textsuperscript{25} have been made. Polymers for use in light emitting diodes (LEDs)\textsuperscript{26} and those that are liquid-crystalline\textsuperscript{27} have also been synthesized by ROMP.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{polymer Structures.png}
\caption{Polymers substituted with sugars and penicillins synthesized by ROMP.}
\end{figure}

With the advent of metathesis catalysts that react preferentially with olefins over many other functional groups and are active in water, the ROMP of monomers with
biologically relevant units has been realized. Only a few of the polymers, however, have been characterized for their biological activity. Polymers with pendant sugars have been synthesized\textsuperscript{28} and used to explore protein-saccharide interactions (Figure 3).\textsuperscript{28-c} Kiessling and coworkers found that the polymers were much more active than the monomers at inhibiting cell agglutination. This was thought to be due to multivalent interactions between the saccharide-substituted polymers and the cell surface proteins. Polymers substituted with nucleotide bases\textsuperscript{29} and amino acid esters\textsuperscript{30} have been synthesized using ruthenium and molybdenum metathesis complexes as initiators. In addition, polymers with pendant drugs such as penicillins (Figure 3)\textsuperscript{31} and vancomycins\textsuperscript{32} have been made. In the latter study, the polymers were found to have significantly increased (8 to 60 fold) antibacterial activities against resistant strains of bacteria compared to the vancomycin-derived monomer, again potentially due to multivalent interactions provided by the polymer scaffold.

**Ring-Closing Metathesis.** Ring-closing metathesis is a relatively new reaction compared to ROMP.\textsuperscript{1b,33} Unlike ROMP, RCM of an $\alpha,\omega$-diene to form a cyclic olefin is enthalpically disfavored; the reaction is entropically driven by the release of a volatile small molecule such as ethylene. The formation of the ring is limited by the relative ring strain of the product. Because of its usefulness and versatility, this reaction has been applied towards the synthesis of small to large rings, including those found in natural products.
Scheme 4. RCM of functionalized α,ω-diene to form 5-7 membered rings.

In 1992, Grubbs and coworkers demonstrated that RCM was a viable way to synthesize 5-7 membered rings with various functionality in high yields (Scheme 4).\textsuperscript{34} Several years later, it was shown that 8-membered rings could be synthesized, provided that the diene substrate contained a steric constraint to restrict the conformation to one favorable for ring-closure.\textsuperscript{35} Since then, a large number of compounds synthesized by RCM have been reported.\textsuperscript{36} Among the many examples, tri- or tetrasubstituted cyclic alkenes,\textsuperscript{37} bicyclic diaza compounds,\textsuperscript{38} chromenes,\textsuperscript{39} and bicyclic β-lactams\textsuperscript{40} have been made. RCM has been extended to include tandem reactions in which multi-cyclization can occur by attaching metathesis relays such as acetylenes or cyclic olefins.\textsuperscript{41} An example of this strategy where a one step tricyclization was accomplished using acetylene relays, is shown in Scheme 5.\textsuperscript{41c}

Scheme 5. Tandem one step tricyclization RCM reaction.
Many large-sized ring systems have been synthesized in a highly efficient manner, whether the substrates contain rigorous conformational constraints or not. Hydrogen bonding between amino acids has been exploited to synthesize conformationally-stabilized cyclic β-turn analogs (Figure 4), α-helices, and peptide-based nanotubes. RCM in the presence of a metal template accomplished the synthesis of [2]catenanes in high yields. Steric constraints built into the substrates provided a bridged calix[4]arene and the tricyclic core of roseophilin. The synthesis of large macrocycles, such as cyclic lactones and crown ethers, was accomplished in good yields in the absence of any steric constraint by slow addition techniques.

![Figure 4. Peptide β-turn covalently stabilized by RCM.](image)

The synthetic importance of RCM is demonstrated in the number of natural products that have recently been reported where ring-closing was the key step. For example, small molecules such as frontalin to complex, biologically active epothilione A and its derivatives have been synthesized where RCM was the key step.

**Cross Metathesis and Acyclic Diene Metathesis Polymerization.** The cross metathesis process resembles RCM thermodynamically in that the reaction is driven entropically by the evaporative loss of a small molecule such as ethylene.
Historically, the practical use of this reaction has been hampered due to poor selectivities for the desired products. For the CM of two terminal olefins, one desired heterodimeric product and two undesired homodimers are obtained (Scheme 6A). Despite this, cross metathesis has been used to successfully synthesize compounds in good yields, including dimers of the immunosuppressant FK 506. Recently, by using symmetric disubstituted olefins as coupling agents (Scheme 6B), the yields of the desired heterodimeric products were significantly increased.

**Scheme 6.** Cross metathesis: A) Two terminal olefins cross to form the desired heterodimer and the undesired homodimers. B) A terminal olefin crossed with a symmetric disubstituted olefin yields the desired heterodimer product.

ADMET provides a means to synthesize unsaturated polymers from linear α,ω-dienes. It is closely related to CM and has a similar thermodynamic profile. The reaction is typically carried out in neat monomer under vacuum, where the removal of a volatile compound, such as ethylene, drives the reaction. Since it is a step-growth
polymerization, where very high conversions are necessary to obtain good yields, this method typically suffers from low conversions and produces a number of undesirable side-products. It has, however, been used successfully employed to produce perfectly branched and linear poly(ethylene), ethylene/CO and ethylene/vinyl alcohol copolymers, poly(ethers), poly(acetals), and highly conjugated polymers such as poly(phenylvinylene)s, among other examples. To date, however, ADMET has not been developed as extensively as ROMP.

Part 2. Manipulation of Biological Response Through the Incorporation of Poly(ethylene glycol) and Arg-Gly-Asp into Synthetic Materials

The control of biological response through rational macromolecular design is a key issue in many biomaterial applications. For example, minimal biological response to a material may be desired in a specific application. To this end, many researchers have incorporated poly(ethylene glycol) (PEG) into materials to limit biological adhesions. Alternatively, a specific response may be desired and obtained by incorporation of a bioactive compound, such as the peptide arginine-glycine-aspartic acid (Arg-Gly-Asp, RGD), into a material. Much work in this area has focused on the modification of existing materials through the covalent attachment of bioactive moieties.

Poly(ethylene glycol) as a Biomaterial. PEG (Figure 5) is a flexible polyether that has a number of unique properties. It is soluble in most organic solvents and water and has a large exclusion volume in water. It is nontoxic, weakly immunogenic, and hospitable to biological materials. It is even FDA approved for internal consumption. As a soluble polymer, PEG has many applications. When linked to
molecules or proteins, it solubilizes them, helps move them across cell membranes, and reduces the rate of clearance through the kidney.\textsuperscript{60,61} Because of these properties, it has been widely used in the biomedical and bioengineering communities.

\begin{center}
\includegraphics[width=0.2\textwidth]{PEG.png}
\end{center}

\textbf{Figure 5.} Poly(ethylene glycol), PEG.

Because PEG resists protein absorption,\textsuperscript{62} as an insoluble material it is useful for a variety of applications. Absorbed on a surface or cross-linked as a gel, it renders the material protein rejecting and is dubbed “nonfouling.” PEG and various copolymers of PEG are utilized as protein-rejecting materials for implants and as barrier materials during the wound healing response.\textsuperscript{60,61,63} A notable example of the latter is the application of PEG-based hydrogels for use in polymeric endoluminal gel paving applications where resorbable hydrogels are extremely effective at preventing post-angioplasty adhesions in animal models.\textsuperscript{64} By covalently attaching a bound peptide to PEG-based materials, the material can be rendered selectively adhesive to certain cell types, while remaining resistant to protein absorption.\textsuperscript{65} In this manner, the wound healing response may be manipulated and controlled. This has been accomplished by attaching short bioactive peptides, such as RGD, to PEG hydrogels\textsuperscript{66} or oligo(ethylene glycol) self-assembled monolayer surfaces,\textsuperscript{67} among other examples.\textsuperscript{60,61,65} Ongoing work includes the development of functionalized PEGs or PEG-containing polymers and materials for use in various applications as a soluble or insoluble material.
Incorporating Arg-Gly-Asp to Elicit a Biological Response. Many extracellular matrix proteins bind to cell surface integrins through the short peptide sequence RGD.\textsuperscript{68,69} In fact, RGD alone has been shown to promote cell adhesion.\textsuperscript{70} Since cell attachment mediated by integrin-protein interactions influences cell survival, differentiation, and migration, this sequence has been targeted to study integrin function and provide treatments for diseases.\textsuperscript{71} For example, fibronectin and RGD containing peptides have been shown to have anti-metastatic activity and thus may have good prospects as drugs for tumor therapy.\textsuperscript{72}

Although the RGD peptide is effective in certain applications, its therapeutic potential is low. One reason for this is that the affinity of the peptide to cellular integrins is significantly lower (> 1000 times lower) than the proteins from which it is derived.\textsuperscript{68b} This is partly due to the presence of synergistic sites on the proteins that enhance RGD-integrin binding. For example, in fibronectin the sequence Pro-His-Ser-Arg-Asn (PHSRN), which is on the same side of the protein 30-40 Å from RGD (Figure 6),\textsuperscript{73} acts synergistically with RGD.\textsuperscript{74} Interestingly, PHSRN acts in synergy only when covalently attached in proximity to RGD.\textsuperscript{74} Although most biomaterials only contain RGD and not the PHSRN sequence, efforts have been made to synthesize RGD mimics that have enhanced activities. A second reason for the low therapeutic potential is because RGD, as a small peptide, has a half-life \textit{in vivo} of only a few minutes.\textsuperscript{75} As a result, efforts have been made to synthesize non-peptide RGD mimics and materials that contain this oligopeptide sequence to increase the lifetimes \textit{in vivo}. 
Efforts have been made to increase the therapeutic potential of the RGD peptide. The activities have been increased by cyclizing the peptides,\textsuperscript{75,76} altering the flanking groups,\textsuperscript{76} and synthesizing nonpeptide mimetics.\textsuperscript{76} The p-forms of the amino acids and nonpeptide mimetics also have increased lifetimes \textit{in vivo}.\textsuperscript{76,77} Since linking a peptide to a polymer can also increase lifetimes \textit{in vivo}, polymers substituted with RGD have been synthesized.\textsuperscript{77,78} The presentation of many RGDS on one molecule may lead to multivalent interactions and increased activities.\textsuperscript{79} Polypeptides with RGD repeats\textsuperscript{80} and a synthetic polymer with RGD units linked to a poly(carboxyethylmethacrylamide) backbone\textsuperscript{81} have been reported and shown to have an increased therapeutic potentials to
interfere with cancer metastasis compared to the free peptide. Ongoing research has focused on creating synthetic materials with increased lifetimes, activities, and selectivities.

**Thesis Research**

With the advent of well-defined ruthenium metathesis catalysts that are highly active and stable to a variety of functional groups, the synthesis of biologically relevant organic molecules and polymers is now possible. The majority of this thesis describes the application of 2-4 towards the synthesis of novel polymers that may be useful in biological applications. Polymers with polyether and polynorbornene backbones that are substituted with peptides have been synthesized. The efficacy of some of these polymers for biological applications is addressed.

A novel method to prepare polyethers by metathesis is described in Chapter 2. An unsaturated analog of 12-crown-4 that was made by template-directed RCM was utilized as a monomer for the synthesis of unsaturated polyethers by ROMP (Scheme 7). In contrast to previous syntheses of polyethers by metathesis,\(^{57}\) the yields were high and a range of molecular weights was accessible. The unsaturated polymer could be contacted with 2 under RCM conditions to quantitatively regenerate the unsaturated crown ether monomer. Hydrogenation of the unsaturated polymer yielded the saturated polyether. Since the polyethers were water soluble and contained PEG units, the toxicities were investigated by *in vitro* cytotoxicity tests. They were found to have similar toxicities as PEG and unlike PEG, to have a limited shelf life.
The first synthesis of substituted polyethers by olefin metathesis is described in Chapter 3. Template-directed RCM yielded unsaturated benzo-crown ethers and a benzo-crown ether with a pendent phenylalanine methyl ester. These monomers were homopolymerized and copolymerized with the 12-crown-4 analog to form polymers with different backbones and amino acid concentrations (Figure 7). Generally, peptides are attached to the ends of a polyether chain, and therefore the concentration of peptide per polymer chain is low. With this technique, the peptide is substituted along the backbone and the concentration was determined simply by the initial concentration in the feed. As a demonstration, polymers substituted with the bioactive sequence RGD were made.

Figure 7. Polyethers with different backbones and concentrations of phenylalanine.
The synthesis of polynorbornenes with pendent amino acids is described in Chapter 4. Various norbornenyl monomers substituted with glycine, alanine or penta(ethylene glycol) were polymerized using 2 as a catalyst and compared in terms of monomer and polymer yield, reaction time, and molecular weight distribution. Polymers with GRGDS and SRN, components of an integrin-cell binding domain of fibronectin, were also made. Since, catalyst 2 is unstable to amines,\textsuperscript{34} the highly active ruthenium catalysts 3 and 4 were necessary to synthesize polymers containing high concentrations of the oligopeptides. Using these catalysts, homopolymers substituted with bioactive sequences and copolymers also substituted with penta(ethylene glycol) (Figure 8) were prepared. These results demonstrate that ROMP is an excellent way to produce complex biopolymers.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure8.png}
\caption{Copolymer substituted with GRGD, SRN, and penta(ethylene glycol).}
\end{figure}

The biological activity of polymers with bioactive peptides is described in Chapter 5. Homopolymers and a copolymer containing GRGDS and PHSRN, the more active forms of the peptides, were synthesized. The ability of the polymers to compete with fibronectin for human dermal fibroblast cell binding was assayed using an \textit{in vitro} competitive inhibition assay. The homopolymer with many pendent GRGDS peptides
was significantly more active at inhibiting cell binding to fibronectin than was the free peptide, presumably due to multivalent interactions.\textsuperscript{79} The copolymer substituted with both GRGDS and PHSRN was more potent than both the GRGDS-containing homopolymer and the free peptide, demonstrating that synthetic polymers that inhibit cellular adhesion to fibronectin are now accessible through ROMP.

Historically, one of the drawbacks to using olefin metathesis is the removal of the residual ruthenium at the completion of the reaction. Residual metal can pose problems, such as olefin isomerization during distillation of the product, decomposition over time, and increased toxicity of the final material. Chapter 6 describes a method where the water soluble tris(hydroxymethyl)phosphine ligand is utilized to facilitate the removal of residual ruthenium from olefin metathesis products. Several simple procedures are described and compared for the purification of the RCM product of diethyl diallylmalonate. It was found that for an aqueous extraction, only 10 equivalents of the phosphine (based on ruthenium) were necessary to remove most of the metal. This method was also applied to the synthesis of unsaturated crown ethers to circumvent the typically time-consuming purification procedures of these RCM products.

References


(36) These have been extensively reviewed, see: 1b and 33.


(56) Valenti, D. J.; Wagener, K. B. Macromolecules 1993, 31, 2764-2773.


(73) Leahy, D. J.; Aukhil, I.; Erickson, H. P. Cell 1996, 84, 155-164.


Chapter 2

The Synthesis of Polyethers Using a Tandem Approach: Template-Directed Ring-Closing Metathesis Followed by Ring-Opening Metathesis Polymerization
Abstract

This chapter describes the synthesis of polyethers by a tandem approach of template-directed ring-closing metathesis (RCM) followed by ring-opening metathesis polymerization (ROMP) using RuCl$_2$(=CHPh)(PCy$_3$)$_2$ (1) as an initiator. Template-directed RCM is a process which utilizes non-covalent interactions between a diene and an appropriate template to enhance the RCM of the substrate devoid of other conformational constraints. Template-directed RCM was found to be an excellent synthetic protocol for the synthesis of unsaturated crown ethers in high yields with selectively for the cis olefins. ROMP of these crown ethers produces unsaturated polyethers with different molecular weights ($M_n$ from 10,900 to 206,300) depending on the initial monomer to catalyst ratios. Hydrogenation of these polymers proceeded quantitatively to yield the saturated polyethers. Template-directed depolymerization of unsaturated polyether to completely regenerate the parent crown ether is also described. Results from in vitro cytotoxicity tests are discussed which suggest that the polyethers are nontoxic to at least a concentration of 1 wt.% and have a limited shelf life.
Introduction

Polyethers are of commercial importance in areas such as polyurethane synthesis, lubricants, cosmetics, and elastomers. A notable example is poly(ethylene glycol) (PEG) which has been widely used in the bioengineering and biomedical communities due to its water solubility, low toxicity, and protein resistant properties. PEG has been attached to surfaces and to molecules such as proteins, enzymes, and small-molecule pharmaceuticals to impart stability and biocompatibility to these materials. Since PEGs are not commercially available between molecular weights of 20,000 to 200,000, a new versatile route to synthesize water soluble polyethers of various molecular weights, that can readily adapted to make functionalized polyethers, is desirable. This chapter focuses on such a route where water-soluble polyethers with PEG segments are synthesized by ring-opening metathesis polymerization (ROMP) using RuCl$_2$(=CHPh)(PCy$_3$)$_2$ (I) as an initiator.

Few examples of the synthesis of polyethers by olefin metathesis polymerization have been published. Wagener et al. reported the synthesis of polyethers having the structure [-CH$_2$=CH-(CH$_2$)$_n$O-(CH$_2$)$_n$] by acyclic diene metathesis polymerization (ADMET) using a tungsten or molybdenum catalyst. The polymerization of $\alpha,\omega$-dienes, di-4-pentenyl ether ($n=3$) and di-5-hexenyl ether ($n=4$) resulted in high yields of polymers with moderate molecular weights. However, these polyethers do not contain PEG units (which are necessary to impart water solubility to the polymers). The ADMET of ethylene glycol diallyl ether was described, but this gave only oligomers. They also reported the ROMP of 2,5-dihydrofuran to give a polymer with high molecular weight; however, the yields were low (~33%). An alternative approach described in
this chapter was undertaken to produce polyethers in high yields from unsaturated crown ether monomers.

**Template-Directed RCM to Synthesize Unsaturated Crown Ether Monomers**

Grubbs and coworkers reported the first demonstration of template-directed ring-closing metathesis (RCM), were non-covalent interactions between a diene and an appropriate template were utilized to enhance the RCM of linear dienes devoid of other conformational constraints. It was determined that the yields of crown ethers derived from linear polyethers could be significantly increased when an appropriate metal ion "template" was used to preorganize the substrate and promote the desired macrocyclization. For example, 12-crown-4 is a known ionophore for Li⁺ (Scheme 1). Analogously, it was anticipated that the preorganization of a linear α,ω-diene 2 about a complementary metal ion would provide the conformational restrictions required to enhance RCM and form the 12-crown-4 analog 3 in high yields (Scheme 2).

![Scheme 1](image)

**Scheme 1.** Complexation of a lithium ion by 12-crown-4.
Scheme 2. Synthesis of unsaturated crown ethers by template-directed RCM.

By this approach, crown ethers 3 and 5 were synthesized (Scheme 2); these are similar to known ionophores 12-crown-4 and 15-crown-5, respectively. The findings are summarized in Table 1. Noting the preferential binding of Li$^+$ by 12-crown-4, it was anticipated from the structural similarities between 3 and 12-crown-4 that the RCM of 2 would afford the highest yield of 3 when Li$^+$ is used as the template. As anticipated, a $>95\%$ yield of the cis ring-closed product was obtained when the RCM of 2 was performed with LiClO$_4$. For the 17-membered ring, 5, the RCM was enhanced by both LiClO$_4$ and NaClO$_4$ demonstrating that both Li$^+$ and Na$^+$ ions are capable of restricting the diene to a conformation favoring RCM. In general, it was evident that the ions which function best as templates to give the highest yield of ring-closed product also favored the formation of the cis isomer. This trend implies that the cis isomer allows the macrocycle to adopt a conformation which best accommodates the template ion. As predicted, yields of the ring-closed product were much lower in the absence of a complementary template.
Table 1. Template-directed Ring Closing Metathesis of 2 and 4.\textsuperscript{a}

<table>
<thead>
<tr>
<th>substrate</th>
<th>M\textsuperscript{+}</th>
<th>yield\textsuperscript{b}</th>
<th>cis : trans ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>none</td>
<td>39%</td>
<td>38 : 62</td>
</tr>
<tr>
<td></td>
<td>Li\textsuperscript{+}</td>
<td>&gt; 95 (85)%</td>
<td>100 : 0</td>
</tr>
<tr>
<td></td>
<td>Na\textsuperscript{+}</td>
<td>42%</td>
<td>62 : 38</td>
</tr>
<tr>
<td></td>
<td>K\textsuperscript{+}</td>
<td>36%</td>
<td>36 : 64</td>
</tr>
<tr>
<td>4</td>
<td>none</td>
<td>57%</td>
<td>26 : 74</td>
</tr>
<tr>
<td></td>
<td>Li\textsuperscript{+}</td>
<td>89%</td>
<td>61 : 39</td>
</tr>
<tr>
<td></td>
<td>Na\textsuperscript{+}</td>
<td>90%</td>
<td>68 : 32</td>
</tr>
<tr>
<td></td>
<td>K\textsuperscript{+}</td>
<td>64%</td>
<td>25 : 75</td>
</tr>
</tbody>
</table>

\textsuperscript{a}[Substrate]=0.02M in CH\textsubscript{2}Cl\textsubscript{2}/THF, MCIO\textsubscript{4}/substrate=5/1, 5 mol\% 1.
\textsuperscript{b}Yields determined from \textsuperscript{1}H NMR analysis of the crude reaction mixture. Bracket denotes isolated yield.

The \textit{cis} isomer of an unsaturated, 12-crown-4 analog (3) was formed in high yields utilizing this approach. After the reaction, all ionic species were removed by a single aqueous extraction. In this chapter, the ROMP of 3 to form water-soluble, unsaturated polyethers containing PEG units is described. ADMET as a route to the polyethers was also explored. Template-directed depolymerization of the unsaturated polyether to reform the crown ether monomer is discussed. Finally, results from toxicological investigations of the ethers are described.
Results and Discussion

Polymer Synthesis and Characterization. By olefin metathesis, polymers are synthesized by two pathways: ROMP and ADMET. Since the ADMET of 2 and the ROMP of 3 should both result in the desired water-soluble polyether 6 (See Scheme 3), the strengths and limitations of both routes were explored.

Scheme 3. Metathesis routes to the synthesis of polyethers with PEG segments.

Since ADMET is the most straightforward route to 6, this avenue was explored first. Subjecting 2 to standard ADMET conditions (5 mole % 1, neat, 15 mtorr) at 50 °C (Scheme 4) yielded relatively low molecular weight polymer and oligomers (total yield of 95%) with a trans to cis ratio of 6.3/1 as detected by ¹H NMR spectroscopy. The polymer had a number-averaged molecular weight ($M_n$) of 11,200 (relative to
polystyrene standards) with a broad molecular weight distribution (polydispersity index, PDI) of 3.4. These results were not surprising since ADMET is a step-growth polymerization route, which usually leads to low molecular weight polymers unless high conversions are achieved.

![Scheme 4](image)

**Scheme 4.** Synthesis of polyethers by ADMET.

ROMP, which is a chain growth polymerization route, was then explored for the synthesis of 6. In a typical polymerization, complex 1 in CH₂Cl₂ was added to a vial containing 3 (Scheme 5). The solutions were stirred for 4 hours and ethyl vinyl ether was added to terminate the reactions, as the resulting metal species is metathesis inactive. The polymers were isolated by precipitation into ether chilled to 0 °C. The polymers were all viscous oils at room temperature.

The ROMP of 3 with an initial monomer to catalyst ratio ([M]/[C]) of 100/1 gave polymer 6 in quantitative yield, as determined by ¹H NMR spectroscopy of the crude reaction mixture (78% isolated yield). The trans to cis ratio of the isolated product was 4.1/1 by ¹H NMR spectroscopy. The Mₙ determined by gel permeation chromatography (GPC) was 65,900 and the PDI was 1.96 (Table 2). The glass transition temperature (Tₕ) of this polymer at −59.0 °C was obtained by differential scanning calorimetry (DSC).
Scheme 5. Synthesis of polyethers by ROMP.

A wide range of polymer molecular weights was accessible by this methodology. The molecular weight of polymers produced by living ROMP of strained monomers can be readily controlled by the initial monomer to catalyst ratio ([M]/[C]). Although the monomers described here do not polymerize in a living fashion, since the initiator remains active throughout the reaction, the molar mass should be proportional to the [M]/[C] ratio.

<table>
<thead>
<tr>
<th>[M]/[C]</th>
<th>Yield</th>
<th>$M_n \times 10^{-4}$</th>
<th>PDI</th>
<th>trans/cis</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>&gt;95%</td>
<td>1.09</td>
<td>1.50</td>
<td>5.4</td>
</tr>
<tr>
<td>100</td>
<td>&gt;95%</td>
<td>6.59</td>
<td>1.96</td>
<td>4.1</td>
</tr>
<tr>
<td>1000</td>
<td>83%</td>
<td>9.12</td>
<td>2.29</td>
<td>3.1</td>
</tr>
<tr>
<td>3000</td>
<td>78%</td>
<td>15.5</td>
<td>1.83</td>
<td>2.6</td>
</tr>
<tr>
<td>4000</td>
<td>71%</td>
<td>20.6</td>
<td>1.73</td>
<td>2.4</td>
</tr>
</tbody>
</table>

$^a$[3]=1.2M in CH$_2$Cl$_2$, 25 °C, 4 h. $^b$Determined from $^1$H NMR spectra. $^c$Determined by GPC, polystyrene calibration.
To test this, the polymerization of 3 with monomer to catalyst ratios between 25/1 and 4000/1 was performed and the results are shown in Table 2. The molecular weights did increase as the [M]/[C] was increased to yield polymers with $M_n$'s between 10,900 and 206,300. PDI values were all between 1.7 and 2.3. As the catalyst loading was decreased, the polymer yields (determined by $^1$H NMR spectroscopy) decreased from > 95% ([M]/[C] = 25/1) to 71% ([M]/[C] = 4000/1).

At a low loading of 1, the polymerization of 3 sometimes resulted in very low yields of 6. Inspection of the crude reaction mixtures in these cases revealed the presence of a small amount (< 5%) of a vinyl ether species suggesting that isomerization of an allyl ether (from monomer and/or polymer) to a vinyl ether had occurred. It is known that the reaction of 1 with a vinyl ether forms the metathesis-inactive $\text{RuCl}_2(=\text{CHOR})(\text{PCy}_3)_2$ complex.\textsuperscript{5b} In fact, as previously indicated, ethyl vinyl ether is frequently used to terminate metathesis reactions catalyzed by 1. The isomerization and subsequent termination of 1 (Equation 1) could therefore compete with polymerization resulting in low yields of 6. Occurrence of this on a small scale would be more problematic when using low concentrations of catalyst.

\[
\begin{align*}
\text{allyl ether} & \rightarrow \text{vinyl ether} \\
\text{Cl} & \begin{array}{c} \text{PCy}_3 \end{array} & \begin{array}{c} \text{Cl} \end{array} \\
\text{Cl} & \begin{array}{c} \text{Ru} \end{array} & \begin{array}{c} \text{OR}_1 \end{array} \\
\text{H} & \begin{array}{c} \text{PCy}_3 \end{array} & \begin{array}{c} \text{OR}_1 \end{array}
\end{align*}
\]

\textbf{(1)}

To study this possibility, 3 was polymerized in CD$_2$Cl$_2$ and the propagating alkylidene of 1 was monitored by $^1$H NMR spectroscopy. In the cases where low yields
were obtained, a peak at 14.45 ppm was observed in the $^1\text{H}$ NMR spectrum in addition to the desired $\alpha$-H alkylidene resonance at 19.19 ppm. The propagating carbene resonance decreased in intensity as the peak at 14.45 ppm increased in intensity until no propagating carbene was observed. The peak at 14.45 ppm compares very closely to the $\alpha$-H peak of RuCl$_2(=\text{CHOCH}_2\text{CH}_3)(\text{PCy}_3)_2$ at 14.51 ppm obtained by the reaction of 1 with ethyl vinyl ether in CD$_2$Cl$_2$. This supports the hypothesis that isomerization to a vinyl ether and subsequent termination of 1 was occurring in these cases. The reason for this isomerization is not yet understood, although it is probably related to ruthenium hydride impurities in the catalyst. However, this detrimental reaction was not problematic unless very low concentrations of 1 (high [M]/[C]) and impure catalyst were used.

Metathesis yields polyethers with unsaturated backbones, and in order to obtain saturated backbones, the polymers must be hydrogenated. First this was attempted by subjecting 6 to hydrogen in the presence of palladium on carbon, which resulted in quantitative hydrogenation. However, it was apparent by $^1\text{H}$ NMR that a significant amount of chain scission had also occurred. Quantitative hydrogenation of 6 using Crabtree's catalyst, however, gave the desired saturated polyether (7) in 66% isolated yield. No chain scission products were apparent in the $^1\text{H}$ NMR spectrum. The $T_g$ of the resulting polymer was slightly lower than 6 (-65 °C versus -59.0 °C respectively).
**Depolymerization.** One key feature of olefin metathesis is that, unlike other carbon-carbon bond forming reactions, the reversible formation and breaking of a carbon-carbon double bonds allows the thermodynamic product distribution to be attained. This concept, with respect to template-directed RCM, implies that unproductive coupling (i.e., “mistakes” which do not result in the desired product) can be "corrected" to yield the desired product if the template effect is a significant driving force. It is also known that when dilute solutions of polymers obtained via ROMP are reacted with an olefin metathesis catalyst, an equilibrium concentration of cyclic monomers is re-established.\(^{11}\)

In an attempt to exploit these processes, the reactions between polymer 6 and catalyst 1 in the presence and absence of Li\(^+\) were undertaken. It was anticipated that the template-directed “backbiting” of 6 should yield macrocycle 3. In the absence of a template, “backbiting” should result in a complex mixture of macrocycles of various ring sizes and linear oligomers.

![Scheme 6](image.png)

**Scheme 6.** Depolymerization of polyether 6 to reform 3 (cis).

Performing the lithium ion-templated degradation of 6 under the same RCM conditions employed in the conversion of 2 to 3 resulted in a nearly quantitative conversion of 6 to the cis isomer of 3 (83% isolated yield) (Scheme 6). Polymer degradation in the absence of a Li\(^+\) template gave only \(~20\%\) combined yield of both cis
and \textit{trans} 3, the remainder consisted of low molecular weight polymer ($\text{M}_n = 9,000$) and oligomers. In addition to demonstrating the concept of template-directed backbiting, this result also illustrates the concept that if "mistakes" (i.e., ADMET dimers) are formed during the template-directed RCM of 2, they can in principle be converted to 3 via this process.

**Toxicity of Polyether 6.** PEG is a nontoxic polymer that is FDA approved for human consumption.\textsuperscript{2,3} Since the polyethers synthesized from 3 contain PEG segments, the toxicity of 6 was investigated and compared to PEG.

A quantitative \textit{in vitro} cytotoxicity test\textsuperscript{12} of 6 ($\text{M}_n \sim 69,000$) was undertaken using normal human dermal fibroblast (HDF) cells. HDF cells were harvested using trypsin/EDTA, suspended in serum-supplemented media, and seeded into 24-well tissue culture polystyrene (TCPS) plates at a density of 35,700 cells/cm\textsuperscript{2}. The cells were allowed to spread for 24 hours, at which time the media was removed. Fresh media and sterilized solutions of 6 or PEG in HBSS of various concentrations were added to the wells. The cells were incubated with the polymers for 24 hours before the polymer solutions were removed. The wells were then washed with HBSS, and the amounts of live cells were determined using a live/dead assay.

Both calcein AM and ethidium homodimer were added to the wells to allow differentiation between the live and dead cells. Under fluorescein excitation optical filters, live cells fluoresce blue/green ($\lambda = 485$ nm) due to esterase activation of calcein AM. The nuclei of dead cells fluoresce red ($\lambda = 530$ nm), as ethidium homodimer passes through the compromised membranes of the dead cells only. Because the excitation wavelengths are resolved for these two compounds, the number of live and dead cells in
each well can be counted. The average percent of live cells for 6 was compared to the average for PEG at the same concentration (Figure 1).

![Bar chart](image)

**Figure 1.** Percent live HDF cells incubated with increasing concentrations of 6 or PEG. All values are an average of 2 wells. PEG is the average of wells containing PEG molecular weight of 20K and 100K.

The results (Figure 1) indicate that polymer 6 has similar toxicity to PEG up to 0.1 wt.% (0.5 mM based on the repeat units) polymer. However, at 1 wt.% (5 mM based on the repeat units), 6 was toxic to the cells while PEG was not. The polymer in this study was synthesized using 0.1 equivalents ([M]/[C]=100) of 1, and ruthenium is known to be toxic.\(^\text{13}\) Although it was possible that the polymer itself was toxic, it was thought that residual ruthenium contained in the polymer was causing the response. To test this, 6 was synthesized using a lower concentration of catalyst (0.001 equivalents of 1) and the toxicity of the resulting polymer was studied.
Figure 2. Number of live HDF cells per well incubated with various concentrations of 6, RuCl₃, and PEG. The values are $10^3$ cells and are an average of 3 wells. The RuCl₃ concentration was 79.2 μM in PBS buffer.

Polymer 6 was synthesized using a 1000/1 initial monomer to catalyst ratio. The amount of ruthenium in a 1 wt.% solution of 6 was quantified by ICP-MS. It was found by comparing to ruthenium standards that after purification, a 1 wt.% solution of 6 contained 79.2 μM of residual ruthenium. As a control, the toxicity of a 79.2 μM solution of RuCl₃ was assessed along with $1 \times 10^{-5}$ to 1 wt.% solutions of 6 and 1 wt.% solutions of PEGs using the same method described above. Because all of the cells appeared alive when observed by phase contrast microscopy and were adhered normally with several focal contacts, the cells were enzymatically removed from the surface and counted by an automatic cell counter. The results showed (Figure 2) that the polymer
synthesized with a lower catalyst loading ([M]/[C]= 1000/1 versus 100/1) was nontoxic to the HDF cells to at least 1 wt.%. The concentration of ruthenium in the 1 wt.% solution was also nontoxic. From these results it appeared that toxicity was decreased dramatically by reducing the catalyst loading employed in the preparation of the polymer.

![Graph](image)

**Figure 3.** Percent live HDF cells incubated with increasing solutions of 6, RuCl₃, and PEG in PBS that have been aged over one month. The values are an average of 3 wells. The RuCl₃ concentration was 79.2 μM in PBS buffer.

However, when the tests were repeated for the solutions kept at room temperature for over a month, a different result was obtained. It was found that a 1 wt.% solution of 6 ([M]/[C]=1000) was now toxic to the HDF cells (Figure 3). The cells were still alive after being subjected to the RuCl₃ (79.2 μM) solution that was stored for over a month, suggesting that the ruthenium was not causing the toxic affect. The same results were
obtained if the solutions were made the day of the experiment from bulk polymer that was kept at room temperature for over a month. This indicates that 6 has a limited shelf life both in solution or in the bulk, and that over time, the polymer degrades into toxic side-products. Various autooxidation reactions can be envisioned that would result in toxic degradation products. The shelf life of the materials may be increased by hydrogenating the double bonds, as described above. Alternatively, the polymer may be investigated for applications where low concentrations or short therapeutic time periods are needed. Also, there are many nonbiological applications for these polyethers.¹³

Summary

Water-soluble polyethers were synthesized using a tandem approach involving template-directed RCM followed by ROMP. ROMP of an unsaturated analog of 12-crown-4 synthesized by templated directed RCM, yielded polymers containing PEG and butenediol units. By altering the catalyst loadings, the polymer was synthesized in a wide range of molecular weights. ADMET was not as effective and resulted in low molecular weight oligomers and polymers. Template-directed depolymerization of the unsaturated polyethers quantitatively reformed the starting crown ether monomer. Toxicity testing of the polymers showed that the materials are nontoxic up to 1 wt.% when made with a low catalyst loading, although the unsaturated polymer has a limited shelf life.

Experimental Section

Materials. Allyl bromide, sodium hydride, lithium perchlorate, ethyl vinyl ether, and ruthenium standards was purchased from Aldrich and used as received. Triethylene
glycol and ethylene glycol were purchased from Aldrich and dried over 4 Å molecular sieves (Linde). Crabtree's catalyst was purchased from Strem Chemicals and used as received. Dry tetrahydrofuran (THF) and methylene chloride (CH₂Cl₂) were rigorously degassed and passed through purification columns.¹ All other solvents were purchased from EM Science and used as received. Trypsin/EDTA was purchased from Sigma. Dulbecco's modified eagle medium (DMEM) and other cell culture reagents were purchased from GIBCO and used as received. The sterile flasks and plates were purchased from Becton Dickinson Labware. All other sterile culture materials were purchased from Falcon. The human dermal fibroblast neonatal cells isolated from foreskin tissue from a single male donor were purchased from Clonetics. The live/dead kit was obtained from Molecular Probes.

**Techniques.** Unless otherwise noted, all operations were carried out under a dry nitrogen or argon atmosphere. Dry box operations were performed in a nitrogen-filled Vacuum Atmospheres dry box. Column chromatography was performed using silica gel 60 (230-400 mesh) from EM science. ¹H NMR (300.1 MHz) and ¹³C NMR (75.49 MHz) spectra were recorded on a General Electric QE-300 spectrometer. ¹H NMR (399.65 MHz) spectra to monitor polymerizations were taken on a JEOL GX-400 spectrometer. Chemical shifts are reported downfield from tetramethylsilane (TMS). Polymer ¹H NMR spectra were obtained using a pulse delay of 60 sec. Infrared spectroscopy was performed on a Perkin Elmer Paragon 1000 FT-IR spectrometer using a thin film of sample cast on a NaCl plate. High-resolution mass spectra were provided by the Southern California Mass Spectrometry Facility (University of California, Riverside). Gel permeation chromatographs were obtained with CH₂Cl₂ as the eluent (flow rate of 1
mL/min) using an HPLC system equipped with an Altex model 110A pump, a Rheodyne model 7125 injector with a 100 µL injection loop, two American Polymer Standards 10 micron mixed bed columns, and a Knauer differential refractometer. The molecular weights and polydispersities were reported versus monodispersed polystyrene standards. Differential scanning calorimetry was measured on a Perkin-Elmer DSC-7. The results are given for the second heating using a scan rate of 20 °C/min. for all cases. All cell manipulations were performed in a vertical laminar flow hood. Phase contrast microscopy was performed on an Olympus CK 2, 100x magnification. Fluorescence microscopy was performed on a Zeiss Microscope using 485 and 530 nm optical filters. Inductive-coupled mass spectrometry data was obtained on an Elan 5000A. The samples were weighed on a microbalance and diluted in 1% nitric acid. Results for Ru isotopes 99, 101, and 102 were obtained and compared to standards.

Monomer Synthesis

**Synthesis of triethylene glycol diallyl ether (2).** To a room temperature, stirred solution of allyl bromide (4 mL, 46.2 mmol) and sodium hydride (1.35 g, 56.3 mmol) in dry THF (125 mL) was added, over a period of 1 h, a solution of triethylene glycol (3.0 mL, 22.5 mmol) in THF (25 mL). The reaction was allowed to stir at room temperature for a total of 12 h. THF was removed *in vacuo*, and the crude product was taken up in ether and extracted with water 3 times. The ether layer was separated, dried over MgSO₄, and the solvent removed *in vacuo*. The crude product was purified by column chromatography (ether eluent) to afford 3.52 g (70%) of 2 as a colorless oil. ¹H NMR (CDCl₃) δ 5.82-5.95 (m, 2H), 5.13-5.28 (m, 4H), 3.98-4.01 (m, 4H), 3.56-3.66 (m, 12 H).
Synthesis of $1,4,7,10$ tetraoxa-cyclotetradec-12-ene (3). THF (59 mL) was added to 2 (3 g, 13.0 mmol) and LiClO$_4$ (6.91 g, 65.2 mmol) and the solution stirred for 30 min or until all solids had dissolved. (Precaution should be used when handling perchlorate salts due to the explosive nature of these compounds; a blast shield should be used at all times.) Dry CH$_2$Cl$_2$ (580 mL) was added to the flask followed by a solution of 1 (537 mg, 0.652 mmol) in CH$_2$Cl$_2$ (10 mL). The mixture was heated to 40 °C for 90 min before cooling to room temperature, adding ~1 mL of ethyl vinyl ether and stirring for an additional 30 min. The solution was extracted with a minimal amount of water to remove the lithium, the organic layer was dried over MgSO$_4$, and the solvent was removed in vacuo. The residue was subjected to chromatography (ether eluent) to yield 3 as a brown liquid in 74-99 % yield. $^1$H NMR (CDCl$_3$) δ 5.75-5.78 (m, 2), 4.29-4.30 (m, 4H), 3.64-3.72 (m, 12 H). $^{13}$C NMR (75 MHz, CDCl$_3$) 129.80, 71.77, 70.21, 66.77. HRMS: calcd: 203.1284 (m+H$^+$), obsd. 203.1290 (m+H$^+$).

Polymer Synthesis

Experimental procedures and data not reported within the text are reported below.

ADMET of 2. In a round bottom, 3.6 mg of 1 (0.0044 mmol) in a minimal amount of CH$_2$Cl$_2$ was added to 20 mg (0.087 mmol) of 2. The flask was evacuated to 15 mtorr and the mixture was heated in an oil bath at 50 °C for 3 h. Ethyl vinyl ether (0.1 mL) was added and the mixture stirred for 30 min. The ethyl vinyl ether was removed
under vacuum and the $^{1}$H NMR spectrum of the crude reaction mixture taken. The polymer was isolated by precipitation into cold ether, collected by centrifugation, and dried under vacuum. Analytical data, except for the GPC results, of the isolated polymer were identical to that obtained for 6 (vide infra).

**ROMP of 3 with various [M]/[C] (6).** In a nitrogen-filled dry box, a solution of complex 1 in CH$_2$Cl$_2$ (to give a final monomer concentration of 1.2 M) was added to 3 and the reaction mixture was stirred at room temperature for 4 h. The [M]/[C] was varied between 25 and 4000. The polymerizations were terminated by ethyl vinyl ether and the solutions stirred for an additional 15-30 min. The polymers were precipitated into cold ether, stirred for 15 min, subjected to centrifugation, washed with cold ether (3x), and dried under vacuum. $^{1}$H NMR (CDCl$_3$) $\delta$ 5.78-5.81 & 5.70-5.73 (trans & cis, both m, 2H), 4.08-4.09 & 4.00-4.02 (trans & cis, both m, 4H), 3.56-3.66 (m, 12H). IR: 3570, 2864, 1457, 1353, 1296, 1249, 1114, 979, 881, 855 cm$^{-1}$.

**Hydrogenation of 6 (7).** The hydrogenation of 6 was undertaken using Crabtree's catalyst (2.5 mol %) according to literature procedure.$^{16}$ $^{1}$H NMR (CDCl$_3$) $\delta$ 3.54-3.63 (m, 12H), 3.43-3.46 (m, 4H), 1.59-1.63 (m, 4H). IR: 3926, 2866, 1479, 1449, 1351, 1297, 1247, 1115, 844 cm$^{-1}$.

**Templated-Depolymerization of 6.** The same procedure as for the synthesis of 3 was followed, with 75 mg (0.37 mmol) of polymer 6 as the substrate. $^{1}$H NMR spectroscopic analysis of the crude showed a quantitative conversion of polymer 6 to macrocycle 3. The crude was dissolved in CH$_2$Cl$_2$ and washed several times with deionized water. The organic layer was concentrated under vacuum and purified by silica
column chromatography (EtOAc) to afford 62 mg (83%) of 3. Analytical data for the product was identical to those reported for 3 above.

**Cell Culture**

**Cell Maintenance.** HDF cells were maintained in DMEM supplemented with 10% fetal bovine serum, 400 U/mL penicillin, and 400 mg/mL streptomycin in an incubator at 37 °C and 4.9% CO₂. Subculturing was accomplished by rinsing the cells with HEPES buffered saline solution (HBSS), enzymatically removing them from the surface with trypsin/EDTA, and neutralizing the trypsin with supplemented DMEM. The cells were suspended in DMEM and added to 25 cm² flasks.

**Toxicity Test.** HDF cells of passage 4-7 were harvested with trypsin/EDTA, seeded onto 24 well TCPS plates, and incubated for 24 h after which time the media was withdrawn. Fresh supplemented DMEM and polymer solutions in HBSS that had been sterile filtered through 0.2 μm filters were placed into the wells. The cells were incubated for another 24 h before the polymer solutions were removed. The wells were washed with HBSS buffer and aspirated to assure complete removal of the medium and polymer. The morphology was observed under phase contrast microscopy. The cells were then counted by one of the assays described below.

**Simple Cell Counting Assay.** If *all* the cells appeared normal and were adhered with several focal contacts (not rounded), the following simple cell counting assay was undertaken. The cells were removed using trypsin/ETDA and the number of cells per well counted by a automatic cell counter.

**Live/Dead Counting Assay.** If some of the cells were rounded and appeared dead, the following counting assay was undertaken. A solution containing 2 μM calcein
AM and 4 μM ethidium homodimer in HBSS was freshly prepared and kept in the dark until use. 100 μL of the live/dead solution was added to each well, and the plates were incubated for 10 min in the dark. The cells were viewed using fluorescence microscopy. For each view, the cells were counted first with the 485 nm filter followed by the 530 nm filter. A cell was deemed live if it was colored green/blue when viewed through the 485 nm filter and dead if the nucleus was stained red when viewed through the 530 nm filter. At least 3 views per well were counted. The percent live cells was determined by dividing the total number of live cells by the number of live and dead cells.

Acknowledgements

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References


(4) The upper molecular weight limit for poly(ethylene glycol)s is due to side reactions during the industrial scale base-catalyzed polymerization of ethylene oxide, see reference 1.


(8) Propagating alkylidene degradation could be slowed by the use of some additives such as $\alpha,\alpha$-dichlorotoluene. Maynard, H. D.; Grubbs, R. H., unpublished results.

(10) For a review of Crabtree’s catalyst, see: Crabtree, R. Acc. Chem. Res. 1979, 12, 331-339.


(14) RuCl₃ was used instead of I because the former is soluble in water, while the latter is not.


Chapter 3

Synthesis of Peptide-Substituted Polyethers by Ring-Opening Metathesis Polymerization of Unsaturated Crown Ethers
Abstract

Polyethers of various backbones and pendent peptides were synthesized by a tandem approach of ring-closing metathesis (RCM) followed by ring-opening metathesis polymerization (ROMP) using $\text{RuCl}_2(=\text{CHPh})(\text{PCy}_3)_2$ ($1$) as an initiator. An unsubstituted benzocrown ether and a benzocrown ether substituted with a phenylalanine methyl ester were synthesized in good yields using a lithium ion as a template and $1$ as a catalyst by RCM. From the phenylalanine methyl ester crown ether, crowns substituted with a phenylalanine carboxylic acid and a arginine-glycine-aspartic acid (RGD) peptide were made. ROMP of the unsaturated crown ethers formed the respective polyether homopolymers. The copolymerization of these monomers with an unsaturated analog of 12-crown-4 formed copolymers, where the compositions corresponded to the feed ratios of the monomers. In this way, polyethers of various backbones and concentrations of amino acids were synthesized.
Introduction

There are a number of applications, such as drug delivery,\(^1\) where having polyethers functionalized along the backbone is desirable; yet the number of synthetic methods to produce such polymers are few. Typically, modification is at one or both ends of the polymer, limiting the attachment sites for a linear polymer to two. Poly(oxyalkylene)s such as poly(epichlorohydrin) have been synthesized and subsequently modified with a number of functional groups.\(^2\) However, since reactions on a polymer chain can prove difficult and may not result in quantitative conversions, it is advantageous to polymerize functionalized monomers. In Chapter 2, the synthesis of unfunctionalized polyethers by a tandem approach of template-directed RCM followed by ROMP was described. This chapter discusses the synthesis of polyethers with different backbones and pendent amino acids using this approach.

As was described in Chapter 1, ruthenium alkylidene \(\text{I}^3\) is stable in the presence of many functional groups and is active in many solvents including water.\(^4\) Due to the development of this catalyst and others, the ROMP of monomers with biologically relevant units has been recently undertaken. As described in Chapter 1, polymers with pendent sugars have been synthesized\(^5\) and used to explore protein-saccharide interactions.\(^5\text{c,d}\) Additionally, polymers substituted with nucleotide bases,\(^6\) antibiotics,\(^7\) and amino acid esters\(^8\) have been prepared using ruthenium and molybdenum complexes. These examples have been based on polynorbornene and poly(7-oxanorbornene) backbones. However, polyether backbones may have advantages compared to polynorbornenes, since polyethers are flexible and water soluble. If the polyethers contain poly(ethylene glycol) (PEG) segments, they may also be biocompatible and
nontoxic. This chapter describes the synthesis of polyethers with PEG units substituted with peptides. Polymers with pendent phenylalanine (Phe, F) and the cell-binding protein fragment described in Chapter 1, arginine-glycine-aspartic acid (Arg-Gly-Asp, RGD), were synthesized.

Results and Discussion

Monomer Synthesis. The synthesis of functionalized \( \alpha, \omega \)-diene precursors for RCM from tartaric acid or serine was not successful; however, the dienes were readily prepared from catechol derivatives. The synthesis of 1,2-bis-(2-allyloxy-ethoxy)-benzene (4) and 1,2-bis-(2-allyloxy-ethoxy)-benzene substituted with a pendent phenylalanine methyl ester (5) were undertaken as shown in Scheme 1. Phenylalanine methyl ester hydrochloride salt was coupled to 3,4-dihydroxyhydrocinnamic acid using standard peptide coupling procedures employing HOBT and DCC in \( \text{CH}_2\text{Cl}_2 \) to give 2 in 75% yield (Scheme 1A). Ethylene glycol was treated with allyl bromide and base to form the ethylene glycol monoallyl ether; the alcohol was subsequently converted to a tosyl group to give 3 (38% overall yield) (Scheme 1B). Reaction of 3 with catechol or 2 in the presence of potassium carbonate in DMF gave 4 (61% yield) and 5 (72% yield), respectively (Scheme 1C). The synthesis of the unfunctionalized diene, 6, was undertaken as described in Chapter 2 from triethylene glycol, allyl bromide, and base.\(^9\)
Scheme 1. Synthesis of unsubstituted and phenylalanine substituted $\alpha,\omega$-dienes.

Template-directed RCM using a lithium ion (LiClO$_4$) as a template and complex 1 as a catalyst was undertaken to form the unsaturated crown ethers (Schemes 2 and 3). First, the unfunctionalized crown ether 7 was synthesized from 6 (Scheme 2) as described in Chapter 2.$^9$ It was demonstrated that the lithium ion preorganized the diene for RCM, giving high yields and selectivity for the cis isomer (100/1 cis/trans). In a similar manner, 4 was converted to 8 in quantitative yield as determined by $^1$H NMR.
spectroscopy (Scheme 3); however, the isolated yields were much lower (50-63%). Template-directed RCM of 5 afforded 9 in 71% isolated yield. In both cases, the cis isomer was formed preferentially (94/6 cis/trans).

Scheme 2. Synthesis of unfunctionalized crown ether by template-directed RCM.

It was described in Chapter 2 that the synthesis of 7 in the absence of a lithium ion (all other conditions the same) favored the trans isomer (38/62 cis/trans) and the yield obtained was much lower (39% yield). The templating effect of the lithium ion in this reaction was further confirmed when the synthesis of 8 or 9 was performed without a
lithium ion and monitored by $^1$H NMR spectroscopy. The overall yields of the crude mixtures obtained from the $^1$H NMR spectrum were lower than the RCM performed in the presence of a lithium ion (73\% for 8 and 60\% for 9). In both cases, the trans isomers formed predominately with a cis/trans ratio of 35/65 for 8 and 46/54 for 9.

In RCM, an equilibrium exists between the ring-closed and the ring-opened species.\textsuperscript{9,10} The template effect, coupled with running the reaction under conditions of high dilution, favored the ring-closed product over the ring-opened product in these examples.\textsuperscript{9} This was demonstrated in both the syntheses of 7 and 8 where only the ring-closed product was observed at the end of the reaction. However, in the synthesis of 9, a linear oligomer was detected in the crude reaction product by $^1$H NMR spectroscopy. Even upon rigorous purification of the substrate, this oligomer persisted as 1-5\% of the final product.

The yield of the crown product over oligomer side products was the greatest when the substituent (R, Scheme 3) of the diene substrate was CH$_2$CH$_2$-Phe-OCH$_3$ (5). For example, when R = COOH, COOCH$_3$, or CH$_2$COOCH$_3$, the desired RCM product yields were less than 32\%, and significant amounts of both linear and cyclic oligomers were detected in the crude reaction mixtures by $^1$H NMR spectroscopy. This was true whether a lithium ion was present in the reaction mixture or not. It was observed by $^1$H NMR spectroscopy that the substrates were not templated by the lithium ion.\textsuperscript{11} For the substituted products, either the spacer, bulk of the phenylalanine, or both were necessary for the substrates to be templated by the lithium ion resulting in high yields of the desired products.
Scheme 4. Generation of phenylalanine carboxylic acid monomer.

In order to obtain the crown ether with a pendent phenylalanine carboxylic acid, the saponification of 9 was carried out (Scheme 4). Reaction of 9 with hydroxide base gave 10. The linear oligomers in the starting material were removed during product isolation to afford 10 in 75% yield.

Scheme 5. Synthesis of an RGD-containing crown ether monomer.

From 10, the RGD containing crown ether was made (Scheme 5). The R(Pbf)-G-D(t-Bu)₂ peptide was synthesized by standard solution-phase FMOC chemistry,¹² and then coupled to 10 with DCC and HOBT to afford 15 in 66% yield. The protected monomer was readily soluble in organic solvents such as CH₂Cl₂.
Polymer Synthesis and Characterization. The homopolymerization of 7 to form the unfunctionalized polyether 11 (Scheme 6) was described in Chapter 2. In this chapter, the synthesis of functionalized polyethers is described. In a typical polymerization, complex 1 in CH₂Cl₂ was added to a vial containing monomer or a mixture of monomers. The solutions were stirred for 4-5 hours and ethyl vinyl ether was added to terminate the reactions. The polymers were isolated by precipitation into ether chilled to 0 °C. Many of the polymers were slightly soluble in ether, and thus the isolated yields were decreased by this purification process.¹³ The polymers generally were viscous oils or sticky solids at room temperature.

The homopolymerization of 8 and copolymerizations of 7 and 8 were undertaken (Scheme 6) and the results are given in Table 1. The homopolymerization of 8 gave a 54% isolated yield of polymer 12e with a Mₙ of 16,300 and polydispersity index (PDI) of 1.57. Thermal analysis by DSC indicated that polymer 12e possessed both a glass transition (T_g = -7.9 °C) and a melting point (T_m = 8.0 °C). Copolymers 12a-d were synthesized in good yields between 48% and 68%. The T_g of copolymers 12a-d varied between those of the two homopolymers (11 = -59.0 °C and 12e = -7.9 °C). However, the crystallization of the copolymers was inhibited by the incorporation of 7, and only copolymer 12d containing 80% of 8 exhibited a melting point (T_m = 6.6 °C). The PDI’s and trans to cis ratios were all similar and ranged from 1.57-1.84 and 2.6-3.0/1, respectively.

An important component of a copolymerization reaction is the final concentration of the monomers in the polymer (copolymer composition) relative to the initial concentrations of the monomers (feed composition).¹⁴ A desirable characteristic for the
copolymers in this research was that the incorporation into the copolymer of the two monomers be dependent on the feed composition. In this way, the copolymer composition (and pendent groups) could be changed by simply altering the initial monomer concentrations. This was the case as demonstrated by the results for 12a-d: for each copolymer the concentration of 8 incorporated was the same as that in the feed.

\[
\begin{align*}
\text{7} & \quad + \quad \text{8, 9, or 10} \\
\text{1, CH}_2\text{Cl}_2 & \quad \downarrow \\
\text{11} & \\
\text{n = 0: (11)} & \\
\text{n = 0: R = H (12a-d), CH}_2\text{CH}_2\text{CO-Phe-OCH}_3 (13a-d), \text{or CH}_2\text{CH}_2\text{CO-Phe-OH (14)}
\end{align*}
\]

Scheme 6. The synthesis of polyethers by ROMP of unsaturated crown ethers.
Table 1. Results for homopolymerization of 8 and copolymerization of 7 and 8.\(^a\)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>mol% 8 feed</th>
<th>mol% 8 polymer(^b)</th>
<th>yield(^{b,c})</th>
<th>(M_n \times 10^{-4})</th>
<th>PDI(^d)</th>
<th>trans/cis(^b)</th>
<th>(T_g (T_m), , ^\circ C)</th>
</tr>
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<tbody>
<tr>
<td>12a</td>
<td>11%</td>
<td>11%</td>
<td>&gt;95% (68%)</td>
<td>2.75</td>
<td>1.84</td>
<td>3.0</td>
<td>-56.9</td>
</tr>
<tr>
<td>12b</td>
<td>25%</td>
<td>24%</td>
<td>83% (52%)</td>
<td>2.53</td>
<td>1.63</td>
<td>2.7</td>
<td>-49.8</td>
</tr>
<tr>
<td>12c</td>
<td>50%</td>
<td>49%</td>
<td>69% (55%)</td>
<td>1.97</td>
<td>1.75</td>
<td>2.6</td>
<td>-36.5</td>
</tr>
<tr>
<td>12d</td>
<td>75%</td>
<td>80%</td>
<td>60% (48%)</td>
<td>1.76</td>
<td>1.67</td>
<td>2.6</td>
<td>-20.7 (6.6)</td>
</tr>
<tr>
<td>12e(^f)</td>
<td>100%</td>
<td>100%</td>
<td>58% (54%)</td>
<td>1.63</td>
<td>1.57</td>
<td>2.9</td>
<td>-7.9 (8.0)</td>
</tr>
</tbody>
</table>

\(^a\)[M]/[C]=100, [M]=1.2M in CH\(_2\)Cl\(_2\), 25 \, ^\circ C, 5hrs. \(^b\)Determined from \(^1\)H NMR spectra. \(^c\)Isolated yields in brackets. \(^d\)Determined by GPC, polystyrene calibration. \(^e\)Determined by DSC, 2nd heat, scan rate 20 \, ^\circ C / min. \(^f\)[M]=0.7M in CH\(_2\)Cl\(_2\).
The polymer yields decreased with increasing amounts of 8 (Table 1). The $^1$H NMR spectra of the crude reaction mixtures quenched after 5 hours indicated the presence of both polymer and unreacted 7 and 8 (residual monomers were in the same ratio as initially). When the homopolymerization of 8 was carried out for 23 hours, the $^1$H NMR spectrum of the crude reaction mixture indicated a $>95\%$ yield of 12e (compared to a 58\% yield after 5 hours). This result indicates that higher yields for these polymers may be obtained by using longer polymerization times.

The homo- and copolymerizations of 9 and 7 were conducted (Scheme 6) and the results are given in Table 2. The homopolymerization of 9 using a [M]/[C] of 50/1 gave 13e in 79\% isolated yield with a molecular weight of 3,460 and PDI of 2.00. The GPC trace contained a slight low-molecular weight shoulder. The copolymerizations with various concentrations of 9 in the feed gave polymers 13a-d in good yields. Analogous to 12a-d, the mol \% 9 incorporated into the polymers were similar to the mol \% 9 in the feed.
Table 2. Results for homopolymerization of 9 and copolymerization of 7 and 9.a

<table>
<thead>
<tr>
<th>polymer</th>
<th>mol% 9 feed</th>
<th>mol% 9 polymerb</th>
<th>yieldb,c</th>
<th>M_n x 10^{-3}d</th>
<th>PDi^d</th>
<th>M_n x 10^{-3}d,e</th>
<th>PDi^d,e</th>
<th>trans/cisb,^e</th>
<th>T_g (°C)^f</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>10%</td>
<td>13%</td>
<td>91% (76%)</td>
<td>32.0</td>
<td>2.95</td>
<td>15.8</td>
<td>1.74</td>
<td>3.8 (7.6)</td>
<td>-49.1</td>
</tr>
<tr>
<td>13b</td>
<td>25%</td>
<td>23%</td>
<td>95% (91%)</td>
<td>31.4</td>
<td>4.10</td>
<td>13.6</td>
<td>2.18</td>
<td>3.6 (10.0)</td>
<td>-34.3</td>
</tr>
<tr>
<td>13c</td>
<td>50%</td>
<td>51%</td>
<td>94% (82%)</td>
<td>9.18</td>
<td>3.73</td>
<td>4.95</td>
<td>2.25</td>
<td>3.5 (7.8)</td>
<td>-6.0</td>
</tr>
<tr>
<td>13d</td>
<td>74%</td>
<td>69%</td>
<td>84% (76%)</td>
<td>4.58</td>
<td>2.58</td>
<td>2.76</td>
<td>2.07</td>
<td>4.2 (13.9)</td>
<td>9.8</td>
</tr>
<tr>
<td>13e^g</td>
<td>100%</td>
<td>100%</td>
<td>95% (79%)</td>
<td>3.46</td>
<td>2.00</td>
<td>2.49</td>
<td>1.73</td>
<td>5.1 (16.7)</td>
<td>24.9</td>
</tr>
</tbody>
</table>

aInitial [M]/[C]=100, [M]=1.2M in CH_2Cl_2, 25 °C, 5hrs. bDetermined from ^1H NMR spectra. cIsolated yields in brackets. d Determined by GPC, polystyrene calibration. ePolymers resubjected to 1, [repeat unit]/[C]=25, [repeat unit]=2.4M in CH_2Cl_2, 25 °C, 1 week. f Determined by DSC, 2nd heat, scan rate 20 °C/min. gInitial [M]/[C]=50.
Similar to polymer 13e, the molecular weight distributions for the copolymers were all broad, and the GPC traces of 13b-d exhibited either a high or low molecular weight shoulder. Copolymer 13b had the broadest molecular weight distribution and largest shoulder as shown by the GPC trace in Figure 1a. As discussed earlier, 9 contained a small amount of a linear oligomer which could have caused chain transfer reactions during ROMP.15 After only 5 hours of reaction time the polymerizations would not have reached equilibrium, leaving some long chains and some short chains.15,16 Typically ROMP chain transfer reactions proceed for hours to days before reaching equilibrium.16,17

**Figure 1.** GPC traces for polymers 13b and 13f: a) Monomers polymerized for 5 hours with [M]/[C]=100 (13b); b) Polymer (13b) subjected to [repeat unit]/[C]=25 for 1 week; c) Monomers polymerized for 1 week with [M]/[C]=25 (13f).
With the addition of fresh catalyst to the polymers and longer reaction times, the chains could equilibrate to narrower molecular weight distributions due to back-biting along the polymer chain.\textsuperscript{15,16a} To investigate this, catalyst 1 ([repeat unit]/[C]=25)\textsuperscript{18} and isolated polymers 13a-e were reacted for 1 week. The compositions of the polymers did not change. However, the results given in Table 2 demonstrate that the molecular weights lowered and most significantly, the PDIs narrowed and molecular weight distributions were monomodal. This change was most marked for polymer 13b where the PDI went from 4.10 with a high molecular weight shoulder to 2.18 and monomodal (Figure 1a, b). Additionally, the trans to cis ratios of each of the polymers increased (Table 2). This demonstrates that the polymers were equilibrating to the thermodynamically more stable product. There was no discernible change in the trans to cis ratios for the polymers stirred in solution in the absence of catalyst.

In principle, the same narrowing effect should occur for the polymerization of 7 and 9 at long reaction times. To test this, 9 (25% in feed) and 7 were subjected to complex 1 ([M]/[C] = 25) for 1 week. The resulting polymer (13f) contained 24 mol % of 9. Also, the polymer had a monomodal GPC trace with a molecular weight of 12,300 and a PDI of 2.32. The GPC trace compared very closely to that obtained for 13b after reacting the polymer with 1 for 1 week (Figure 1b, c). These results indicate that long polymerization times are necessary for the copolymerization of 7 and 9 to obtain polymers with monomodal molecular weight distributions.

The homopolymerization of 10 was attempted, but yielded an intractable mixture due to the low solubility of the material formed. The copolymerizations of 10 and 7 were performed employing 1 with a [M]/[C] of 50/1 (Scheme 6). The polymerization of 10 (8
mol % in feed) with 7 yielded polymer 14 with ~5% of 10 incorporated into the polymer, a $M_n$ of 6,990 and PDI of 2.04 in 95% yield. Attempts at incorporating larger amounts of 10 resulted in oligomers and polymers of limited solubility. However, the copolymer made had a monomodal molecular weight distribution, which is consistent with the fact that no linear oligomers were present in this monomer.

![Scheme 7](image)

Scheme 7. Synthesis of a polyether containing the bioactive RGD sequence.

**Polymers Containing RGD.** The homopolymerization of 15 resulted in < 5% of polymer. This may be due to the rapid degradation of 1 in the presence of the monomer which contains many amines, since 1 is not stable in the presence of secondary amines. The homo- or copolymerization of an unprotected RGD containing crown ether was unsuccessful, presumably for the same reason. However, the copolymerization of the protected RGD peptide monomer 15 with 7 (Scheme 7) to form 16 was accomplished in 66% isolated yield. The resulting polymer contained 15 mol% RGD, which is slightly higher than the 10 mol% in the feed. The $M_n$ was 3,970 (with a slight low molecular weight shoulder), the PDI was 1.98, and the trans to cis ratio was 4.8/1.

It is known that very low concentrations, on the order of 5 pmol/cm$^2$, of RGD containing peptides promote cell adhesion. Although the homopolymerization of 15
was unsuccessful, copolymer 16, which contains 15 mol% RGD, was synthesized. This amount of RGD peptide is significant and could promote cell adhesion when the polymer is absorbed on a surface or cross-linked to form a gel. The preparation of 15 demonstrates the feasibility of synthesizing polyether backbone polymers by ROMP that contain bioactive peptide substituents.

**Summary**

Polyethers with different backbones and pendent peptides were prepared using a tandem approach involving template-directed RCM followed by ROMP. A benzocrown ether and a benzocrown ether with a pendent phenylalanine methyl ester were synthesized in high yields with selectivity for the cis isomer using a lithium ion as a template for RCM. Crown ethers containing phenylalanine carboxylic acid and the bioactive sequence RGD were also made. Various polyether backbones were produced by copolymerizing different ratios of the benzocrown ether and an unsaturated analog of 12-crown-4. Excellent correlation between the initial monomer concentrations and final polymer compositions was observed. In a similar manner, polymers with pendent phenylalanine methyl esters and phenylalanine carboxylic acids were made. In the case of the phenylalanine methyl ester polymers, long polymerization times were necessary to obtain polymers with monomodal molecular weight distributions. Finally, the synthesis of a protected-RGD containing polyether, with 15 mol% of RGD, was described.
Experimental Section

**Materials.** Allyl bromide, sodium hydride, 4-dimethylaminopyridine (DMAP), 
$p$-toluenesulfonyl chloride, triethylamine (Et$_3$N), catechol, lithium perchlorate (LiClO$_4$), 
ethyi vinyl ether, and 1,3-dicyclohexylcarbodiimide (DCC) were purchased from Aldrich 
and used as received. Triethylene glycol and ethylene glycol were purchased from 
Aldrich and dried over 4 Å molecular sieves (Linde). 3,4-Dihydroxyhydrocinnamic acid 
was purchased from Aldrich and dried under vacuum at 30 mtorr for 24 h. L-
Phenylalanine methyl ester hydrochloride and 1-hydroxybenzotriazole (HOBT) were 
purchased from Sigma and used as received. Anhydrous potassium carbonate (K$_2$CO$_3$) 
and potassium hydroxide were purchased from Mallinckrodt and used as received. Crabtree’s catalyst was purchased from Strzem Chemicals and used as received. The 
FMOC-protected peptides were purchased from Novabiochem and used as received. Dry 
tetrahydrofuran (THF) and methylene chloride (CH$_2$Cl$_2$) were rigorously degassed and 
passed through purification columns.$^{21}$ $N,N$-Dimethylformamide (DMF) was distilled 
from MgSO$_4$ and stored over 4 Å molecular sieves (Linde). All other solvents were 
purchased from EM Science and used as received.

**Techniques.** Unless otherwise noted, all operations were carried out under a dry 
nitrogen or argon atmosphere. Dry box operations were performed in a nitrogen-filled 
Vacuum Atmospheres dry box. Column chromatography was performed using silica gel 
60 (230-400 mesh) from EM science. $^1$H NMR (300.1 MHz) and $^{13}$C NMR (75.49 MHz) 
spectra were recorded on a General Electric QE-300 spectrometer. $^1$H NMR (399.65 
MHz) spectra to monitor polymerizations were taken on a JEOL GX-400 spectrometer. 
Chemical shifts are reported downfield from tetramethylsilane (TMS). Polymer $^1$H NMR
spectra were obtained using a pulse delay of 60 sec. Infrared spectroscopy was performed on a Perkin Elmer Paragon 1000 FT-IR spectrometer using a thin film of sample cast on a NaCl plate. High-resolution mass spectra were provided by the Southern California Mass Spectrometry Facility (University of California, Riverside). Gel permeation chromatographs were obtained with CH₂Cl₂ as the eluent (flow rate of 1 mL/min) using an HPLC system equipped with an Altex model 110A pump, a Rheodyne model 7125 injector with a 100 µL injection loop, two American Polymer Standards 10 micron mixed bed columns, and a Knauer differential refractometer. The molecular weights were reported versus monodispersed polystyrene standards. Differential scanning calorimeter (DSC) was measured on a Perkin-Elmer DSC-7. The results are given for the second heating using a scan rate of 20 °C/min for all cases.

**Monomer Synthesis**

**Synthesis of 2-[3-(3,4-dihydroxyphenyl)-propionylamino]-3-phenyl-propionic acid methyl ester (2).** To a solution of L-phenylalanine methyl ester hydrochloride (1.33 g, 6.17 mmol) in CH₂Cl₂ (30 mL) was added triethylamine (1.3 mL, 9.34 mmol) and the mixture was stirred for 15 min. Then 3,4-dihydroxyhydrocinnamic acid (1.12 g, 6.17 mmol) and HOBT (1.08 g, 8.00 mmol) were added and the solution stirred until all solids had dissolved. DCC (1.27 g, 6.17 mmol) was added and the solution stirred for 12 h. The organic layer was filtered, washed with 10% citric acid (1x), water (2x), and brine (1x), dried with MgSO₄ and the solvent removed *in vacuo*. The residue was subjected to column chromatography (ethyl acetate eluent) to yield 1.58 g (75%) of 2 as a white, extremely hydroscopic solid. ¹H NMR (CD₂Cl₂) δ 7.18-7.29 (m, 3H), 6.93-6.96 (m, 2H),
6.74 (d, J=8.1 Hz, 1H), 6.69 (d, J= 2.1 Hz, 1H), 6.55 (dd, J=2.1 Hz, J=8.1 Hz, 1H), 6.04 (bd, J=7.5 Hz, 1H), 4.77-4.83 (m, 1H), 3.66 (s, 3H), 3.00-3.03 (m, 2H), 2.76 (t, J=7.5 Hz, 2H), 2.40-2.46 (m, 2H).  

$^1$H NMR (CD$_3$Cl$_2$) $\delta$ 173.83, 172.45, 144.77, 143.38, 136.35, 133.22, 129.76, 129.08, 127.61, 120.70, 115.94, 115.84, 54.02, 52.91, 38.68, 38.23, 31.34. IR (NaCl plate): 3342, 2947, 1732, 1649, 1602, 1519, 1441, 1363, 1275, 1218, 1109, 808, 746, 699 cm$^{-1}$. HRMS (Cl) calcd for (MH)$^+$ 344.1498, found 344.1497.

**Synthesis of ethylene glycol monoallyl ether.** The compound was synthesized according to literature procedure$^{22}$ with potassium hydroxide (16.8 g, 0.3 mmol), ethylene glycol (16.7 mL, 0.3 mmol), and allyl bromide (26.1 mL, 0.3 mmol) to afford 12.9 g (42%) of the product as a colorless oil. $^1$H NMR (CDCl$_3$) $\delta$ 5.81-5.94 (m, 1H), 5.13-5.28 (m, 2H), 3.97-4.00 (m, 2H), 3.68-3.71 (m, 2H), 3.50-3.53 (m, 2H), 2.52 (bs, 1H).

**Synthesis of ethylene glycol allyl ether p-tosylate (3).** Ethylene glycol monoallyl ether (1.71 g, 16.8 mmol) and p-toluenesulfonyl chloride (3.20 g, 16.8 mmol) were dissolved in CH$_2$Cl$_2$ (50 mL), and triethylamine (3.5 mL, 25.2 mmol) and DMAP (catalytic amount) were added. The solution was stirred for 12 h before water was added and the solution acidified with 10% citric acid to a pH of 7. The organic layer was extracted with water (2x) followed by brine (1x), dried over MgSO$_4$, and concentrated in vacuo to give a colorless, viscous oil of 3 (3.90 g, 91% yield). $^1$H NMR (CDCl$_3$) $\delta$ 7.81-7.84 (m, 2H), 7.35-7.37 (m, 2H), 5.79-5.88 (m, 1H), 5.16-5.32 (m, 2H), 4.17-4.20 (m, 2H), 3.95-3.98 (m, 2H), 3.63-3.66 (m, 2H), 2.46 (s, 3H). $^{13}$C NMR (CD$_2$Cl$_2$) $\delta$ 145.65, 134.89, 133.20, 130.41, 128.39, 117.40, 72.47, 70.06, 67.94, 21.88. IR: 2868, 1598, 1452, 1357, 1190, 1177, 1097, 1019, 920, 817, 777, 665 cm$^{-1}$. HRMS (Cl) calcd for (M+NH$_4$)$^+$ 274.1113, found 274.1108.
Synthesis of 1,2-bis-(2-allyloxy-ethoxy)-benzene (4). Compound 3 (838 mg, 3.27 mmol), catechol (180 mg, 1.64 mmol), and anhydrous K$_2$CO$_3$ (905 mg, 6.55 mmol) were dissolved in dry DMF (8 mL) and the mixture was heated to 85-90 °C for 24 h. Ether was added and the solution washed with 10% NaOH (2x), water (3x), and brine (1x), dried over MgSO$_4$ and the solvent removed in vacuo. The residue was subjected to column chromatography (25% ethyl acetate, 75% hexanes eluent) to yield 280 mg (61%) of 4 as a colorless oil. $^1$H NMR (CDCl$_3$) δ 6.89–6.96 (m, 4H), 5.88-6.01 (m, 2H), 5.17-5.35 (m, 4H), 4.16-4.20 (m, 4H), 4.10-4.12 (m, 4H), 4.10-4.12 (m, 4H), 3.81-3.84 (m, 4H). $^{13}$C NMR (CDCl$_3$) δ 148.90, 134.58, 121.47, 116.90, 114.72, 72.12, 68.74, 68.46. IR: 3072, 2916, 2864, 1592, 1503, 1451, 1254, 1218, 1114, 1042, 927, 798, 798, 741 cm$^{-1}$. HRMS (EI) calcd for (M)$^+$ 278.1518, found 278.1518.

Synthesis of 2-{3-[3,4-bis-(2-allyloxy-ethoxy)-phenyl]-propionylamino}-3-phenyl-propionic acid methyl ester (5). Using the same procedure as for 4 except with 2 (1.20 g, 3.50 mmol), 3 (1.79 g, 7.00 mmol), and K$_2$CO$_3$ (1.93 g, 14.0 mmol) in DMF (17.5 mL) gave the crude product which after subjecting to column chromatography (70% ethyl acetate, 30% hexanes eluent) gave 1.29 g (72%) of 5 as a white, waxy solid. $^1$H NMR (CDCl$_3$) δ 7.21-7.26 (m, 3H), 6.92-6.97 (m, 2H), 6.68-6.84 (m, 3H), 5.84-5.96 (m, 3H), 5.15-5.32 (m, 4H), 4.85-4.89 (m, 1H), 4.06-4.14 (m, 8H), 3.75-3.80 (m, 4H), 3.69 (s, 3H), 3.05 (d, J=5.4, 2H), 2.81-2.86 (m, 2H), 2.40-2.47 (m, 2H). $^{13}$C NMR (CDCl$_3$) δ 172.48, 172.11, 149.61, 148.03, 136.26, 135.29, 134.69, 129.78, 129.10, 127.67, 121.69, 117.63, 115.65, 72.84, 69.67, 69.48, 69.20, 53.55, 52.88, 38.89, 38.41, 31.52. IR: 3294, 2931, 2868, 1741, 1651, 1510, 1451, 1433, 1261, 1216, 1139, 1116,
Synthesis of triethylene glycol diallyl ether (6). To a room temperature, stirred solution of allyl bromide (4 mL, 46.2 mmol) and sodium hydride (1.35 g, 56.3 mmol) in dry THF (125 mL) was added, over a period of 1 h, a solution of triethylene glycol (3.0 mL, 22.5 mmol) in THF (25 mL). The reaction was allowed to stir at room temperature for a total of 12 h. THF was removed in vacuo, and the crude product was taken up in ether and extracted with water 3 times. The ether layer was separated, dried over MgSO₄, and the solvent removed in vacuo. The crude product was purified by column chromatography (ether eluent) to afford 3.52 g (70%) of 6 as a colorless oil. ¹H NMR (CDCl₃) δ 5.82-5.95 (m, 2H), 5.13-5.28 (m, 4H), 3.98-4.01 (m, 4H), 3.56-3.66 (m, 12 H). ¹³C NMR (CDCl₃) δ 134.65, 116.99, 72.12, 70.52, 69.30. IR: 3072, 2864, 1462, 1420, 1348, 1291, 1249, 1109, 995, 917. HRMS (CI) calcd for (MH)⁺ 231.1596, found 231.1596.

Synthesis of 1,4,7,10 tetraoxa-cyclotetradec-12-ene (7). THF (59 mL) was added to 6 (3 g, 13.0 mmol) and LiClO₄ (6.91 g, 65.2 mmol) and the solution stirred for 30 min or until all solids had dissolved. [Precaution should be used when handling perchlorate salts due to the explosive nature of these compounds; a blast shield should be used at all times.] Dry CH₂Cl₂ (580 mL) was added to the flask followed by a solution of catalyst 1 (537 mg, 0.652 mmol) in CH₂Cl₂ (10 mL). The mixture was heated to 40 °C for 90 min before cooling to room temperature, adding ~ 1 mL of ethyl vinyl ether and stirring for 30 min. The solution was extracted with a minimal amount of water to remove the lithium, the organic layer was dried over MgSO₄, and the solvent removed in
vacuo. The residue was subjected to chromatography (ether eluent) to yield 7 as a brown liquid in 74-99% yield. \(^1\)H NMR (CDCl\(_3\)) \(\delta 5.75-5.78\) (m, 2), 4.29-4.30 (m, 4H), 3.64-3.72 (m, 12 H).

**Synthesis of 6,7,9,12,14,15-hexahydro-5,8,13,16-tetraoxa-benzocyclotetradecene (8).** Using the same procedure as for the synthesis of 7, treatment of 4 (238 mg, 0.856 mmol) and LiClO\(_4\) (454 mg, 4.28 mmol) in dry THF (3.9 mL) and CH\(_2\)Cl\(_2\) (37 mL) with catalyst 1 (35.2 mg, 0.0428 mmol) in CH\(_2\)Cl\(_2\) (1.9 mL) formed 8 in quantitative yield by \(^1\)H NMR spectroscopy. Subjecting the crude product to column chromatography (1-2 times, ether eluent) gave 50-63% (94% cis isomer) isolated yields of 8 as a white, crystalline solid. \(^1\)H NMR (CDCl\(_3\)) \(\delta 6.89-6.94\) (m, 4H), 5.75-5.78 (m, 2H), 4.41-4.42 (m, 4H), 4.15-4.18 (m, 4H), 3.86-3.88 (m, 4H). \(^13\)C NMR (CDCl\(_3\)) \(\delta 148.99, 129.81, 121.58, 114.48, 70.80, 67.82, 67.34\). IR: 2922, 2859, 1592, 1501, 1451, 1252, 1220, 1121, 1053, 971, 917, 745 cm\(^{-1}\). HRMS (EI) calcd for (M\(^+\)) 250.1205, found 250.1205.

**Synthesis of 2-(3-6,7,9,12,14,15-hexahydro-5,8,13,16-tetraoxa-benzocyclo-
tetradecen-2-yl-propionylamino)-3-phenyl-propionic acid methyl ester (9).** Using the same procedure as for 7 except with 5 (1.56 g, 3.05 mmol), LiClO\(_4\) (1.62 g, 15.3 mmol) in dry THF (13.9 mL), and dry CH\(_2\)Cl\(_2\) (137 mL) with catalyst 1 (126 mg, 0.153 mmol) in CH\(_2\)Cl\(_2\) (2 mL) gave the crude product in 77% yield by \(^1\)H NMR spectroscopy. The product was subjected to column chromatography (70% ethyl acetate, 30% hexanes eluent) and resubjected (ethyl acetate eluent) to yield 1.04 g (71% yield, 94% cis isomer, <5% linear diene) of 9. \(^1\)H NMR (CD\(_2\)Cl\(_2\)) \(\delta 7.20-7.28\) (m, 3H), 6.95-6.98 (m, 2H), 6.70-6.82 (m, 3H), 5.95 (bd, J=7.5, 1H), 5.65-5.76 (m, 2H), 4.77-4.83 (m, 1H), 4.34-4.35 (m, 4H), 4.07-4.11 (m, 4H), 3.77-3.81 (m, 4H), 3.67 (s, 3H), 3.02-3.05 (m, 2H), 2.80-2.85
(m, 2H), 2.39-2.45 (m, 2H). $^{13}$C NMR (CD$_2$Cl$_2$) δ 172.43, 171.83, 149.65, 148.03, 136.72, 134.98, 130.39, 130.25, 129.80, 128.98, 127.50, 121.56, 115.23, 115.18, 71.53, 71.26, 68.60, 68.49, 67.94, 53.64, 52.70, 38.64, 38.31, 31.43. IR (NaCl plate): 3298, 3628, 2922, 2865, 1744, 1653, 1509, 1451, 1432, 1364, 1263, 1215, 1162, 1133, 1051, 1027, 979, 695 cm$^{-1}$. HRMS (Cl) calcd for (MH)$^+$ 484.2335, found 484.2334.

**Synthesis of 2-(3,6,7,9,12,14,15-hexahydro-5,8,13,16-tetraoxa-benzocyclo tetradecen-2-yl-propionylamino)-3-phenyl-propionic acid (10).** KOH (400 mg, 7.14 mmol) was added to a solution of 9 (950 mg, 1.97 mmol) in THF (14.8 mL) and deionized water (4.9 mL) and the reaction was stirred for 13 h. Water was added and the solution extracted with CH$_2$Cl$_2$ (1x). The aqueous layer was acidified with 10% citric acid to a pH 3 and with the addition of NaCl, extracted with ether (5x). The ether layers were combined, dried over MgSO$_4$, and the solvent removed in vacuo. The residue was subjected to column chromatography (97% ethyl acetate, 3% acetic acid eluent), taken up in water, and re-extracted (adding NaCl) with ether (5x). The ether layers were consolidated, dried over MgSO$_4$, and the solvent removed in vacuo to yield 10 as a white solid in 75% yield (694 mg). $^1$H NMR (CD$_3$OD) δ 7.06-7.21 (m, 5H), 6.80-6.85 (m, 2H), 6.68-6.71 (m, 1H), 5.70-5.72 (m, 2H), 4.55-4.62 (m, 1H), 4.37-4.39 (m, 4H), 4.07-4.10 (m, 4H), 3.78-3.82 (m, 4H), 3.08-3.15 (m, 1H), 2.89-2.93 (m, 1H), 2.70-2.74 (m, 2H), 2.38-2.44 (m, 2H). $^{13}$C NMR (CD$_3$OD) δ 175.73, 174.01, 149.53, 147.88, 137.88, 135.17, 130.10, 130.00, 129.50, 128.53, 126.83, 121.60, 115.30, 115.18, 71.25, 70.97, 68.34, 68.21, 67.61, 54.90 37.98, 37.79, 31.43. IR (KBr pellet): 3310, 3030, 2926, 2864, 2584, 1732, 1649, 1514, 1451, 1426, 1265, 1228, 1161, 1135, 1047, 974, 912, 808, 741, 699 cm$^{-1}$. HRMS (FAB) calcd for (MNa)$^+$ 492.1998, found 492.2020.
Synthesis of RGD containing Monomer (15). The H₂N-Arg(Pbf)-Gly-Asp(t-Bu)₂ peptide was synthesized using standard solution FMOC chemistry,¹² and then coupled to 10. The same procedure for 2 was followed with 53 mg (0.11 mmol) 10, 90 mg (0.13 mmol) of the peptide, 0.02 mL (0.15 mmol) triethylamine, 24 mg (0.18 mmol) HOBT, 24 mg (0.11 mmol) DCC in 1.0 mL of CH₂Cl₂, resulting in 87 mg (66%) of 15 as a white solid. ¹H NMR (CD₂Cl₂) δ 7.11-7.25 (m, 5H), 6.68-6.77 (m, 3H), 6.29-6.31 (m, 1H), 5.69-5.71 (m, 2H), 4.56-4.62 (m, 2H), 4.24-4.31 (m, 5H), 4.05-4.06 (m, 4H), 3.87-4.00 (m, 2H), 3.76-3.77 (m, 4H), 3.16-3.21 (m, 2H), 3.05-3.11 (m, 2H), 2.94 (s, 2H), 2.62-2.81 (m, 6H), 2.54 (s, 3H), 2.48 (s, 3H), 2.05 (s, 3H), 1.64-1.70 (m, 4H), 1.38 (2, 18H), 1.37 (s, 6H). IR (NaCl plate): 3321, 2916, 1732, 1623, 1576, 1545, 1514, 1446, 1368, 1259, 1156, 1093, 1021, 798 cm⁻¹. HRMS (FAB, DCM/NBA/NaCl) calcd for (MNa)+ 1184.5610, found 1184.5566.

Polymer Synthesis

General procedure for the polymerization of crown ethers. In a nitrogen-filled dry box, a solution of complex 1 in CH₂Cl₂ (to give a final monomer concentration of 1.2M) was added to the monomer (homopolymers) or a mixture of monomers (copolymers) and the reaction mixture was stirred at room temperature for 5 h. The initial [M]/[C] was 100/1. The polymerizations were terminated by ethyl vinyl ether and the solutions stirred for an additional 15-30 min. The polymers were precipitated into cold ether, stirred for 15 min, isolated by centrifugation, washed with cold ether (3x), and dried under vacuum. Deviations from this literature procedure are noted in specific cases below. Data not reported within the text is also reported below.
Homopolymerization of 8 (12e). Typical procedure given above was followed, except that the initial monomer concentration was 0.7 M. $^1$H NMR (CDCl$_3$) δ 6.90-6.91 (bm, 4H), 5.82-5.84 & 5.73-5.76 (trans & cis, both bm, 2H), 4.13-4.17 (bm, 4H), 4.08-4.09 (bm, 4H), 3.77-3.81 (bm, 4H). IR: 3419, 2922, 2865, 2350, 2331, 1589, 1500, 1440, 1251, 1218, 1124, 1054, 1033, 744 cm$^{-1}$.

Copolymerizations of 7 and 8 (12a-d). Typical procedure given above was followed. For the following data, the shifts are the same for all copolymers, but peak resonances vary in intensity according to percent incorporation of the comonomers. $^1$H NMR (CD$_2$Cl$_2$) δ 6.90 (bs), 5.79-5.84 & 5.69-5.73 (trans & cis, both bm), 4.13-4.14 (bm), 4.05-4.07 (bm), 3.98-3.99 (bm), 3.76-3.79 (bm), 3.56-3.58 (bm). IR: 3474, 2921, 2864, 1591, 1501, 1452, 1354, 1253, 1217, 1115, 1030, 977, 930 cm$^{-1}$.

Homopolymerization of 9 (13e). Typical procedure given above was followed, except that a [M]/[C]=50/1 was used. $^1$H NMR (CD$_2$Cl$_2$) δ 7.20-7.22 (bm, 3H), 6.95-6.97 (bm, 2H), 6.66-6.79 (bm, 3H), 6.02 (bs, 1H), 5.77-5.83 & 5.68-5.73 (trans & cis, both bm, 2H), 4.74-4.80 (bm, 1H), 3.98-4.13 (bm, 8H), 3.70-3.73 (bm, 4H), 3.64 (bs, 3H), 2.99-3.02 (bm, 2H), 2.76-2.81 (bm, 2H), 2.36-2.42 (bm, 2H). IR: 3294, 2922, 2859, 1741, 1651, 1510, 1451, 1429, 1356, 1261, 1216, 1134, 1116, 1030, 980, 745, 700 cm$^{-1}$.

Copolymerizations of 7 and 9 (13a-d, f). Typical procedure given above was followed, except that for 13f, [M]/[C]=25/1, the initial monomer concentration was 0.8M, and the polymerization proceeded for 1 week. For the following data, shifts are the same for all copolymers, but peak resonances vary in intensity according to percent incorporation of the comonomers. $^1$H NMR (CD$_2$Cl$_2$) δ 7.23-7.25 (bm), 6.97-6.99 (bm), 6.69-6.82 (bm), 5.95 (bs), 5.77-5.84 & 5.67-5.74 (trans & cis, both bm), 4.76-4.82 (bm),

**Polymers 13a-e subjected to catalyst 1.** Typical procedure given above was followed except that 1 was reacted with polymer ([repeat unit]/[C]=25), the initial polymer concentration was 2.4M (per repeat unit), and the reaction time was 1 week. The characterization was identical to that given for polymers 13a-e above.

**Copolymerizations of 7 and 10 (14).** Typical procedure given above was followed except that a [M]/[C]=50/1 was used. The copolymerization with mol % 10 in the feed was conducted at 35 °C. $^1$H NMR (CD$_2$Cl$_2$) $\delta$ 7.20-7.25 (bm), 7.02-7.07 (bm), 6.68-6.83 (bm), 5.77-5.80 & 5.68-5.70 (trans & cis, both bm), 4.68-4.78 (bm), 4.05-4.06 (bm), 3.98-3.99 (bm), 3.75-3.77 (bm), 3.56-3.58 (bm), 3.04-3.07 (bm), 2.79-2.83 (bm), 2.39-2.44 (bm). IR: 3321, 2864, 1732, 1654, 1514, 1451, 1353, 1259, 1114, 1031, 974, 875, 850, 744, 706 cm$^{-1}$.

**Copolymerization of 7 and 15 (16).** Typical procedure given above was followed except that a [M]/[C]=50/1 and concentration of 0.9 M was used. $^1$H NMR (CD$_2$Cl$_2$) $\delta$ 7.12-7.19 (bm), 6.65-6.74 (bm), 5.77-5.78 & 5.66-6.70 (trans & cis, both bm), 4.60-4.64 (bm), 4.18-4.22 (bm), 4.03-4.05 & 3.96-4.00 (cis & trans, both bm), 3.72-3.75 (bm), 3.52-3.57 (bm), 3.06-3.10 (bm), 2.94 (s), 2.71-2.75 (bm), 2.54 (s), 2.47 (s), 2.05 (s), 1.71 (bm), 1.39 (s), 1.38 (s).
Acknowledgements

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References

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(11) The peaks corresponding to the olefinic protons of the product shift in the $^1$H NMR spectrum when the crown ether is templated by a lithium ion.


(13) Ether was more effective than hexanes for the precipitation removal of residual catalyst and monomer.


(17) Another explanation for the non-gaussian molecular weight distributions is that the monomers were forming homopolymers rather than copolymers. However, this is less likely because the $T_g$'s scale proportionally between those of the two homopolymers (-59.0 °C for 11 and 24.5 °C for 13e) as one would expect for the copolymers.

(18) In this case [M]/[C] is the ratio of repeat unit to catalyst concentration.


Chapter 4

Synthesis of Norbornenyl Polymers With Pendent Bioactive Oligopeptides by Ring-Opening Metathesis Polymerization‡
Abstract

Synthetic norbornenyl polymers substituted with cell adhesive sequences glycine-arginine-glycine-aspartic acid (GRGD) and serine-arginine-asparagine (SRN) were synthesized by ring-opening metathesis polymerization (ROMP) using newly developed ruthenium initiators. Initially, simpler polymers with pendent glycine, alanine, or penta(ethylene glycol) (EO₅) units attached directly or through ethyl and propyl spacers to various norbornenyl backbones were synthesized using Ru=CHPh(Cl)₂(PCy₃)₂ (1) as the initiator. The molecular weights, PDI's, polymerization times, yields, and glass transition temperatures were compared for these polymers. As a result of this comparison, poly(5-norbornene-2-carboxyl) was chosen as the backbone for the more complex oligopeptide containing polymers, and poly(norbornene) homopolymers and copolymers with pendent EO₅, GRGD, and SRN units were synthesized. It was found that the newly developed, more active, 2,3-dihydroimidazolylidene initiators, Ru=CHPh(Cl)₂(PCy₃)(DHIMes) (2) and Ru=CH-CH=C(CH₃)₂(Cl)₂(PCp₃)(DHIMes) (3) were necessary to synthesize polymers containing more than 10 mol% of the oligopeptides. In order to alter the presentation of the GRGD, an EO₅ containing copolymer with a propyl spacer between the GRGD and the backbone was also made.
Introduction

Considerable effort has been directed towards increasing the therapeutic potential of the cell-binding sequence Arg-Gly-Asp (RGD).\(^1\) As was discussed in Chapter 1, this sequence is part of integrin-cell binding domains of many extracellular proteins.\(^2\) One potential way to increase the binding strength of RGD-containing materials is to incorporate additional oligopeptide sequences into the materials that increase their adhesive character. The sequence Pro-His-Ser-Arg-Asn (PHSRN), that enhances integrin binding to RGD in the protein fibronectin,\(^3\) is a possible target. However, this effort has been limited by the lack of methods to synthesize RGD and PHSRN containing materials in a controllable and reliable manner. This chapter describes an excellent approach to the synthesis of copolymers substituted with RGD and the synergy peptide by ring-opening metathesis polymerization (ROMP) using newly developed ruthenium catalysts.

Previously, a synthetic polymer containing only GRGDS peptides linked to a poly(carboxylethylmethacrylamide) backbone has been reported and shown to have an increased therapeutic potential to cancer metastasis compared to the free peptide.\(^4\) However, this polymer was synthesized by nonliving, radical polymerization which provides little control over the molecular weight and resulted in PDI’s between 2 and 4.6. Also with this polymerization method, the synthesis of random or block copolymers is not straightforward, and copolymers containing the synergy site PHSRN were not synthesized.

Ring-opening metathesis polymerization (ROMP) provides a better alternative for producing well-defined copolymers with pendent bioactive oligopeptides. The copolymer composition is obtained by the feed ratio of the monomers,\(^5\) and the molecular weight is
controlled by the initial monomer to catalyst ratio. Also, ROMP of strained monomers can be living, so synthesizing block copolymers may be a possibility. In addition, the polymer architecture is readily altered; for example, a spacer group can be included between the peptide and the polymer backbone. Furthermore, ROMP of monomers substituted with amino acids has been demonstrated. In Chapter 3, polyethers with pendent amino acids were described, and the synthesis of norbornenyl polymers with one or two amino acids with alkyl side chains, but containing no functionality, has been reported.

In this chapter, the synthesis of various synthetic polymers with pendent GRGD, SRN, and/or penta(ethylene oxide) (EO₅) units by ROMP (see Copolymer Target) is described. Because deletion studies have determined that R is the important residue for PHSRN function, the synergy sequence was truncated to SRN. Since it has been shown that surfaces of oligo(ethylene oxide) are protein resistant, copolymers with EO₅ units were also made. These may be more biocompatible and water soluble than polymers with only pendent peptides. The synthesis of polymers containing these sequences, as well as various polynorbornenes with pendent glycines, alanines, and penta(ethylene glycol) using catalysts 1, 2, and 3, are discussed.
Results and Discussion

Monomer Synthesis. A series of monomers with one pendant amino acid or EO₅ was synthesized in order to study the ROMP of these monomers. First glycine and alanine monomers 9, 10, 15, and 16 were prepared (Scheme 1). Starting material 4 is commercially available and the exo methylene-bridged anhydride (5) was obtained by thermally isomerizing the commercially available endo anhydride.¹⁰ Oxa-bridged monomers 9 and 10 were made following a slightly modified literature preparation¹¹ in 9% and 25% yields, respectively. In a similar manner, methylene-bridged monomers 15 and 16 were synthesized in 59% and 51% yields, respectively. As has been found for the
synthesis of other oxa-bridged amino acid monomers, the yields for these monomers were very low. However, good yields of the methylene-bridged monomers were obtained.

Scheme 1. Synthesis of glycine and alanine substituted norbornene imide monomers.

Norbornenes substituted with glycine (19 and 20) or penta(ethylene glycol) (21) were synthesized (Scheme 2) in somewhat better yields compared to the imide-derived monomers. Glycine methyl ester hydrochloride was coupled to endo or exo 5-norbornene-2-carboxylic acid (18) with EDC, triethylamine, and HOBT in CH$_2$Cl$_2$ in 66% (19) and 73% (20) yield, respectively. The same procedure was followed to synthesize monomer 23 from 22 in 65% yield. The EO$_3$ monomer (21) was synthesized in 53% yield by reacting penta(ethylene glycol) with norbornene-exo-2-carboxylic acid chloride in the presence of base in anhydrous THF.
Scheme 2. Synthesis of norbornene monomers with a pendent glycine or penta(ethylene glycol). Reaction conditions: i. H-Gly–OCH₃·HCl, Et₃N, EDC, and HOBT in CH₂Cl₂ (19, 20, 23). ii. a) Oxalyl Cl and DMF in CH₂Cl₂; b) H(OCH₂CH₂)₅OH and K₂CO₃ in THF, reflux (21).

Next, the synthesis of monomers with ethyl and propyl spacer groups between the norbornenyl group and amino acid was undertaken. First, 4 and 5 were heated to 50 °C with 2-aminoethanol for 12 hours in a mixture of THF and MeOH to give the resulting alcohols 6 and 7 in 36% and 44% yields respectively (Scheme 3). Then monomer 12 was prepared in 49% yield by mixing 6 and methyl bromoacetate with potassium carbonate and tetrabutyl ammonium bromide in anhydrous DMF (Scheme 4). Monomers 11 and 17 with a pendent N-α-t-Boc-alanine were made in 50% and 61% yield respectively by coupling N-tert-butoxycarbonyl-l-alanine to 6 or 7 using DCC and a catalytic amount of DMAP (Scheme 4).


Monomers 13 and 14 with propyl spacers were synthesized in decent yields from 8 (Scheme 5). The acid (8) was synthesized first in 41% yield by heating 4 and aminobutyric acid in THF and MeOH at 50 °C for 12 hours. Monomer 13 was made as for 19 and 20 in 57% yield, and monomer 14 in a 36% over-all yield by using the same procedure as for 21 except the acid chloride was generated \textit{in situ} with oxalyl chloride and base.
Scheme 5. Synthesis of propyl spaced glycine and penta(ethylene glycol) containing monomers. Reaction conditions: i. H-Gly-OCH₃·HCl, Et₃N, EDC, and HOBT in CH₂Cl₂ (13). ii. a) Oxalyl Cl and DMF in CH₂Cl₂; b) H(OCH₂CH₂)₅OH and K₂CO₃ in THF, reflux (14).

Finally, the GR(Pbf)GD(O'Bu)-OH and S(O'Bu)R(Pbf)N(Trt)-OH containing monomers (24, 25, and 29) were synthesized. 24 and 25 were made by initially synthesizing the peptides on a 4-carboxyltrityl linker resin using standard Fmoc chemistry, followed by coupling 5-norbornene-exo-2-carboxylic acid to the amino terminus of the peptide. The monomer was then cleaved from the resin under mildly acidic conditions, giving the protected monomers in 76-97% and 92% yields respectively. In a similar manner, 29 was synthesized from 8 in 83% yield.
Polymers Substituted With One Amino Acid: Synthesis and Characterization. Before synthesizing polymers containing the bioactive peptides, polymers substituted with glycines, alanines, or EO₅ were studied. These were prepared by adding a solution of 1 in CH₂Cl₂ to a solution of monomer in CH₂Cl₂ to give an initial monomer concentration between 0.5 and 0.75 M. The mixtures were stirred vigorously
for 15 minutes to 3 hours before quenching with ethyl vinyl ether. The polymers were precipitated into hexanes or ether, isolated by centrifugation, and dried under vacuum before characterization. Polymers with amino acids or EO₅ units attached directly or through a spacer to the backbone were made and compared in terms of polymer yield, polymerization time, and molecular weight distribution.

First, polymers based on monomers 9, 10, 15, and 16 (Scheme 1) were synthesized using initiator 1 (Table 1). The monomers all reacted in 1 hour or less, giving excellent yields of polymer (82-95%). The polymers had glass transition temperatures between 147 and 158 °C. The number-averaged molecular weights (Mₙ) were between 17,700 and 108,000. The molecular weight distributions were narrow for poly(9) and poly(16) (PDI of 1.19 and 1.10 respectively), broader for poly(15) (1.47), and bimodal for poly(10). The broad molecular weight distribution obtained for poly(15) may be a result of catalyst decomposition which was detected by NMR spectroscopy during the course of the reaction. However, the source of the bimodal molecular weight distribution of poly(10) is unknown. This persisted regardless of the reaction solvent used (CH₂Cl₂ or benzene).

None of these monomers were suitable for further elaboration to synthesize more complex polymers. Poly(10) demonstrated a bimodal molecular weight distribution, and although poly(9) had a narrow, monomodal molecular weight distribution (1.19), the low yield of the monomer (9%) precluded its use in the synthesis of the more complex polymers. The methylene-bridged polymers both demonstrated monomodal molecular weight distributions, and the yields of both monomers were good (59% and 51%). The glycine linker was preferred to the alanine linker since GRGD is the native sequence in
fibronectin. However, attempts to saponify monomer 15 in order to obtain the carboxylic acid through which RGD could be attached failed, and resulted in the regeneration of the anhydride. The synthesis of the glycine carboxylic acid monomer directly resulted in very low yields (<25%). Therefore, monomer 15 was eliminated from the list of candidates, and the norbornene monomers were selected for further studies.

Norbornene monomers (19–21, 23) (Scheme 2) were polymerized and characterized. Since monomer 19 could be made from commercially available starting materials, it was synthesized and polymerized first. However, the reaction took 24 hours to reach 90% yield. This was not a surprising result since endo monomers often take longer to polymerize than the corresponding exo monomers.14 Exo monomer 20 was then made and reacted quickly in 45 minutes to give a quantitative yield of polymer. The penta(ethylene glycol) monomer, exo 21, also polymerized rapidly in 35 minutes. To determine if the polymer could be synthesized even faster, the highly strained monomer, 23, was subjected to ROMP. However, this monomer was too strained and polymerized quickly (<5 minutes) and uncontrollably, resulting in an extremely broad molecular weight distribution (PDI of 29.0).
Table 1. Polymerization data for norbornenyl monomers with pendent amino acids.\(^a\)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Yield</th>
<th>([M]_0/,[C]_0)</th>
<th>Time (min)</th>
<th>(M_n \times 10^3)(^b)</th>
<th>PDI(^b)</th>
<th>(T_g) (°C)(^c)</th>
<th>Trans/ Cis(^d)</th>
<th>Carbene (ppm)(^d)</th>
<th>Free PCy(_3)?(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(9)</td>
<td>82%</td>
<td>100/1</td>
<td>30</td>
<td>17.7</td>
<td>1.19</td>
<td>147.6</td>
<td>2.4</td>
<td>18.67</td>
<td>no</td>
</tr>
<tr>
<td>Poly(10)</td>
<td>83%</td>
<td>100/1</td>
<td>60</td>
<td>97.5</td>
<td>bimodal</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Poly(15)</td>
<td>90%</td>
<td>100/1</td>
<td>35</td>
<td>46.3</td>
<td>1.47</td>
<td>158.1</td>
<td>5.2</td>
<td>19.48, 18.58</td>
<td>yes</td>
</tr>
<tr>
<td>Poly(16)</td>
<td>95%</td>
<td>100/1</td>
<td>60</td>
<td>108</td>
<td>1.10</td>
<td>154.1</td>
<td>5.5</td>
<td>19.41</td>
<td>no</td>
</tr>
<tr>
<td>Poly(20)</td>
<td>&gt;99%</td>
<td>50/1</td>
<td>45</td>
<td>10.9</td>
<td>1.15</td>
<td>88.0</td>
<td>2.4</td>
<td>19.02, 18.94, 18.74</td>
<td>yes</td>
</tr>
<tr>
<td>Poly(21)</td>
<td>&gt;99%</td>
<td>36/1</td>
<td>35</td>
<td>4.61</td>
<td>1.12</td>
<td>-48.9</td>
<td>3.6</td>
<td>19.07, 19.02, 18.72</td>
<td>yes</td>
</tr>
<tr>
<td>Poly(23)</td>
<td>&gt;99%</td>
<td>100/1</td>
<td>&lt;5</td>
<td>1.00</td>
<td>29.0</td>
<td>---</td>
<td>2.9</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\) General reaction conditions: CH\(_2\)Cl\(_2\) as the solvent, rt, 1 as the initiator, \([M]_0 = 0.5\)-0.75 M.  
\(^b\) All determined by GPC, CH\(_2\)Cl\(_2\) eluent, poly(styrene) standards except poly(20) with DMF as the eluent, poly(ethylene glycol) standards.  
\(^c\) Determined by DSC, 10 °C/min, 2nd heat reported.  
\(^d\) Determined from \(^1\)H NMR spectroscopy.  
\(^e\) Determined from \(^31\)P NMR spectroscopy.
Polymers, poly(20) and poly(21) were characterized (Table 1). The $M_n$'s were 10,900 ($[M]_0/[C]_0 = 50/1$) and 4,610 ($[M]_0/[C]_0 = 35/1$) for poly(20) and poly(21) respectively and the molecular weight distributions were narrow (1.15 and 1.12). The polymers had very different glass transition temperatures depending on the substituent. The glycine substituent resulted in a polymer with a higher $T_g$ compared to unsubstituted norbornene (88.0 °C vs. 31 °C). The flexible penta(ethylene glycol) units resulted in a polymer with a lower $T_g$ (−48.9 °C). Because of high yields of both monomer and polymer, facile synthesis, monomodal molecular weight distributions, and fast polymerization times, the norbornene olefin was chosen for the synthesis of the monomers and polymers with GRGD and SRN units.

In an attempt to alter the presentation of the amino acids, polymers with ethyl (12) or propyl (13-14) spacer groups were synthesized and characterized (Table 2) concurrently with the norbornene monomers and polymers described above. Monomer 12 (Scheme 4) polymerized in 2.5 hours and 13 and 14 (Scheme 5) in 30 minutes to give poly(12), poly(13), and poly(14) in excellent yields. Again the $T_g$ of the polymers depended on the substituents. Poly(12) and poly(13) had glass transitions lower than that of poly(9) with the glycine directly attached to the backbone (86.1 and 74.5 versus 147.6 °C). Similar to poly(21), the penta(ethylene glycol) substituent resulted in poly(14) having a low glass transition at −29.9 °C. The $M_n$'s of poly(12) and poly(14) were 66,200 and 118,000. The molecular weight of poly(13) could not be determined due to the low solubility of the polymer. Poly(12) demonstrated a monomodal, molecular weight distribution of 1.31 while poly(14) had a bimodal molecular weight distribution. The
reason for this bimodal molecular weight distribution is unknown; only one propagating species was observable by $^1$H NMR spectroscopy. Attempts to saponify monomer 12 to yield the carboxylic acid functionality failed; similar to 15, saponification resulted in the regeneration of the 6.

Polymers with pendent Boc-protected alanines, poly(11) and poly(17), were synthesized in less than 2 hours in > 99% and 95% yields respectively (Table 2). The molecular weight distributions were monomodal for both poly(11) and poly(17), and the molecular weights were similar ($M_n$ of 51,900 and 55,500 respectively). The polymers also had similar glass transition temperatures ($T_g$ of 96.8 and 90.3 °C respectively).

Polymers of 11 and 17 contain an alanine linked by the carboxylic acid rather than by the amine functionality to the polymer backbone. For the purposes of this research, it was desired that the peptides be coupled to the polymer backbone through the amino terminus. However, the synthesis of poly(11) and poly(17) demonstrates the feasibility of attaching peptides through either termini, which may be useful for other types of applications. For example, since the Boc protecting group may be readily removed with acid to generate the polyamines, poly(11) and poly(17) may be useful in such applications as gene therapy.
Table 2. Polymerization data for norbornenyl monomers with amino acids attached through spacer units.\(^\text{a}\)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Yield</th>
<th>([\text{M}]_0/\text{[C]}_0)</th>
<th>Time (min)</th>
<th>(\text{M}_n \times 10^3)(^b)</th>
<th>(\text{PDI})(^b)</th>
<th>(T_g (\degree\text{C}))(^c)</th>
<th>Trans/Cis(^d)</th>
<th>Carbene (ppm)(^d)</th>
<th>Free PCy(_3)?(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(12)</td>
<td>&gt;99%</td>
<td>75/1</td>
<td>150</td>
<td>66.2</td>
<td>1.31</td>
<td>86.1</td>
<td>2.3</td>
<td>18.70</td>
<td>no</td>
</tr>
<tr>
<td>Poly(13)</td>
<td>81%</td>
<td>50/1</td>
<td>30</td>
<td>---</td>
<td>---</td>
<td>74.5</td>
<td>2.8</td>
<td>18.68</td>
<td>no</td>
</tr>
<tr>
<td>Poly(14)</td>
<td>&gt;99%</td>
<td>50/1</td>
<td>30</td>
<td>118</td>
<td>bimodal</td>
<td>-29.9</td>
<td>2.9</td>
<td>18.69</td>
<td>no</td>
</tr>
<tr>
<td>Poly(11)</td>
<td>&gt;99%</td>
<td>100/1</td>
<td>90</td>
<td>51.9</td>
<td>1.07</td>
<td>96.8</td>
<td>2.3</td>
<td>18.68</td>
<td>no</td>
</tr>
<tr>
<td>Poly(17)</td>
<td>95%</td>
<td>100/1</td>
<td>80</td>
<td>55.5</td>
<td>1.11</td>
<td>90.3</td>
<td>5.5</td>
<td>19.42, 19.20</td>
<td>yes</td>
</tr>
</tbody>
</table>

\(^{a}\)General reaction conditions: \(\text{CH}_2\text{Cl}_2\) as the solvent, rt, 1 as the initiator, \([\text{M}]_0 = 0.5\text{-}0.75 \text{ M.}\) \(^{b}\)All determined by GPC, \(\text{CH}_2\text{Cl}_2\) eluent, poly(styrene) standards except poly(14) with DMF as the eluent, poly(styrene) standards. \(^{c}\)Determined by DSC, 10 \degree\text{C/min}, 2nd heat reported. \(^{d}\)Determined from \(^1\text{H}\) NMR spectroscopy. \(^{e}\)Determined from \(^{31}\text{P}\) NMR spectroscopy.
All of the previous polymerizations were monitored by NMR. The chemical shifts of the carbene propagating species, the presence of free phosphine, and the final trans/cis ratios (vide infra) are recorded in Tables 1 and 2. All of the initiated carbenes were broad multiplets. During the polymerization of the oxa-bridged monomers 9 and 11-14, only one propagating carbene was observed at roughly the same shift (between 18.67 and 18.70 ppm). The methylene-bridged monomer 16 also demonstrated one propagating carbene at 19.41 ppm. Free phosphine was not observed in any of these reactions. However, methylene-bridged imide monomers 15 and 17 had two observable propagating species during polymerization (at 19.48 and 18.58 ppm for 15 and 19.42 and 19.20 ppm for 17) and the norbornene monomers 20 and 21 demonstrated three propagating species (at 19.02, 18.94, and 18.74 ppm for 20, and 19.07, 19.02, and 18.72 ppm for 21). For these polymers, free phosphine was observed during the reaction.

The presence of free phosphine and two propagating species during ROMP has been observed in a previous study; it was determined that the monomer was coordinating to the ruthenium resulting in the observation of a monophosphine species, in addition to the bisphosphine species.16 For the above reactions, the presence of multiple propagating species correlated to the observation of free phosphine during the reaction. Conversely, when free phosphine was not observed, only one propagating species was detected. This suggests that the two propagating species observed for the polymerization of 15 and 17 may be the monophosphine and bisphosphine species. Polymerization of the norbornene monomers resulted in three detectable propagating species. The presence of an additional propagating species may be related to the asymmetry of the monomer. Even so, the
presence of free phosphine and multiple propagating species during the reaction does not appear to have an observable detrimental effect on the final polymer characteristics, such as molecular weight distribution or reaction time.

**Polymers With Pendent Biologically Relevant Oligopeptides: Synthesis and Characterization.** Polymers with pendent GRGD, SRN, and EO₅ were first synthesized using initiator 1 for 4 hours at room temperature in CH₂Cl₂ with an initial monomer concentration of 0.7 M (Scheme 6). In this way, monomers 21 and 24 with 10, 27, and 50 mol% GRGD in the feed as well as 100 mol% 24 and 100 mol% 25 were polymerized, and the protecting groups were cleaved with TFA. However, only the polymerization with 10 mol% GRGD in the feed (26a) gave good results (Table 3), with a high over-all yield of 78% and a monomodal molecular weight distribution. Polymerizations with 27 to 100 mol% 24 or 100 mol% 25 in the feed resulted in extremely low yields of polymer (less than 26%) presumably due to catalyst decomposition. Also, the molecular weight distribution for some of these polymers was bimodal. Given these results, the more active catalysts, 2 and 3, were applied to the synthesis of these polymers.

Polymerizations were carried out using initiators 2 and 3, and the characterization results for the polymers are given in Table 3. Only a few examples of these initiators used in ROMP have been reported. However, in these examples and in ring-closing metathesis and cross-metathesis reactions, 2 and 3 were much more active than 1 both at room temperature and at higher temperatures. Also, 2 and 3 were inter-changeable with each other at higher temperatures. Based upon these initial studies it was hoped that these initiators would result in higher yields of the desired polymers.

Homopolymers and copolymers of 21, 24, and 25 were synthesized (Scheme 6) using 2 or 3 as an initiator by heating the monomers in a 1:1 mixture of CH$_2$Cl$_2$ and MeOH in an oil bath at 55 °C for 2 hours with initial monomer concentrations of 0.6 M (homopolymers) or 0.7 M (copolymers). A mixture of solvents was used to solubilize the polymers, and since the catalysts react faster at elevated temperatures, the mixtures were heated in sealed vials. The homopolymer of 24 ($26c$) was synthesized using initiator 3. The rest, including the homopolymer of 25 ($27b$), were synthesized with 2. Copolymers with GRGD and EO$_5$ units, 26b (49 mol% GRGD), and with SRN and EO$_5$ units, 27a (53
mol% SRN), were synthesized. Copolymers with both oligopeptides, **28a** (32 mol% GRGD, 21 mol% SRN, and 47 mol% EO₅) and **28b** (53 mol% GRGD and 47 mol% SRN), were also made. The amount of the peptide monomer incorporated into the polymer, determined from the ¹H NMR spectra of the purified polymers, corresponded to the amount in the feed for all of the copolymers.

The protecting groups of the polymers were cleaved to yield the unprotected amino acids. All of the polymers except the SRN homopolymer (27b) were successfully deprotected using TFA. Polymer 27b was not fully deprotected by this acid; the polymer precipitated out of the TFA solution after 10 minutes. Presumably, the more labile protecting groups (Pbf and Trt) were cleaved first, altering the solubility of 27b in TFA, resulting in the precipitation of the polymer before the t-butyl groups were removed. However, all of the protecting groups of this polymer, including the t-butyl groups, were cleaved using HF.

All of the deprotected polymers were solubilized in aqueous solution. Copolymer **26a**, with 90.8 mol% penta(ethylene glycol) units, was the only polymer soluble in water immediately after the deprotection steps. The rest of the polymers were solubilized in water by stirring in 0.1 N NaOH for 10 minutes to generate the sodium salt of the carboxylates and were isolated by precipitation into methanol. For copolymers **26b** and **27a**, this procedure cleaved off many of the penta(ethylene glycol) units (76% for 26b and 65% for 27a). However, it was later discovered that these polymers could also be made water soluble by direct treatment with milder bases such as dibasic phosphate buffer (pH 8), without saponifying the penta(ethylene glycol) units.
The over-all yields (after polymerization, cleavage, and solubilization into water) of the polymers were all between 59 and 92%, except for that of polymer 28b which was 32% (Table 3). The low yield of 28b was primarily due to polymer loss during the initial precipitation from the crude reaction solution. The $^1$H NMR spectra of all the crude reaction mixtures indicated that most of the monomers had been consumed. However, since the monomers and protected polymers had similar solubilities, polymer purification proved to be difficult. Selective precipitation of the protected polymers was achieved by precipitating into mixtures of solvents such as CH$_2$Cl$_2$/ether and MeOH/ether. The choice and relative amount of each mixture was different depending on the polymer. Polymer 26a was additionally purified by Centriprep (MWCO=3000) using ethanol as the solvent.\(^{19}\) Regardless of the purification method, except for 28b, the yields were all good to excellent.

The GPC results are given in Table 3. As desired, the number-averaged molecular weights were are fairly low between 10,700 and 18,700, and most importantly, the samples demonstrated monomodal molecular weight distributions. Copolymer 27b had the largest PDI value (1.70). This was the only polymer to be deprotected by HF; the harsh deprotection conditions could have caused the molecular weight distribution to broaden from chain scission. All the other samples had low PDI's between 1.13-1.32. Remarkably, copolymer 28a, had a low PDI of 1.21. This result indicates that synthesizing complex copolymers with three or even more monomers is possible so that drugs, for example, may also be incorporated into the polymers. The narrow molecular weight distributions also indicate that the synthesis of block copolymers may be possible.
Table 3. Polymerization data for norbornene monomers with pendent oligopeptide and EO₅ units. a

<table>
<thead>
<tr>
<th>Polymer Catalyst</th>
<th>% yield overall</th>
<th>[M]₀/[C]₀</th>
<th>mol% in feed</th>
<th>mol% in polymer</th>
<th>Mₙ (x10³)</th>
<th>PDI</th>
<th>T₉ (°C)</th>
<th>Trans/ Cis</th>
<th>GRGD</th>
<th>SRN</th>
<th>EO₅</th>
<th>GRGD</th>
<th>SRN</th>
<th>EO₅</th>
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<tbody>
<tr>
<td>26a b</td>
<td>1</td>
<td>78%</td>
<td>20/1</td>
<td>10% 0% 90%</td>
<td>9.2% 0%</td>
<td>90.8%</td>
<td>18.7</td>
<td>1.13</td>
<td>-30.7</td>
<td>4.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26b</td>
<td>2</td>
<td>81%</td>
<td>20/1</td>
<td>50% 0% 50%</td>
<td>49% 0%</td>
<td>51%</td>
<td>15.9</td>
<td>1.13</td>
<td>52.6</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26c</td>
<td>3</td>
<td>78%</td>
<td>10/1</td>
<td>100% 0% 0%</td>
<td>100% 0%</td>
<td>0%</td>
<td>13.3</td>
<td>1.32</td>
<td>98.0</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27a</td>
<td>2</td>
<td>92%</td>
<td>20/1</td>
<td>0% 52% 48%</td>
<td>0% 53%</td>
<td>47%</td>
<td>17.2</td>
<td>1.21</td>
<td>62.3</td>
<td>0.63</td>
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</tr>
<tr>
<td>27b</td>
<td>2</td>
<td>74%</td>
<td>10/1</td>
<td>0% 100% 0%</td>
<td>0% 100%</td>
<td>0%</td>
<td>10.7</td>
<td>1.70</td>
<td>131.2</td>
<td>--</td>
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<td></td>
</tr>
<tr>
<td>28a</td>
<td>2</td>
<td>59%</td>
<td>20/1</td>
<td>25% 25% 50%</td>
<td>32% 21%</td>
<td>47%</td>
<td>13.3</td>
<td>1.21</td>
<td>39.4</td>
<td>0.63</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>28b</td>
<td>2</td>
<td>32%</td>
<td>10/1</td>
<td>50% 50% 0%</td>
<td>53% 47%</td>
<td>0%</td>
<td>11.8</td>
<td>1.26</td>
<td>104.6</td>
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</table>

aGeneral reaction conditions: CH₂Cl₂:MeOH (1:1) as the solvent, 55 °C for 2 hrs. in a sealed vial, [M]₀ = 0.6-0.7 M. bReaction conditions: CH₂Cl₂ as the solvent, rt for 4 hrs., [M]₀ = 0.7 M. cCalculated from ¹H NMR spectrum. dDetermined by GPC, pH 8.0 phosphate buffer eluent, poly(ethylene oxide) standards. eDetermined by DSC, 10 °C/min, 2nd heat reported. f24% of EO₅ remaining. g35% of EO₅ remaining.
The $T_g$ values of the copolymers varied depending on the identity and concentrations of the substituents. For example, copolymers 26a and 26b had $T_g$ values of $-30.7^\circ C$ and $52.6^\circ C$ between that of homopolymers poly(21) at $-48.9^\circ C$ and 26c at 98.0 $^\circ C$. Copolymer 27a had a $T_g$ of 62.3 $^\circ C$ while homopolymer 27b had one at 131.2 $^\circ C$. The trimonomer copolymer, 28a, with 53 mol% total peptide had a lower $T_g$ (39.4 $^\circ C$) than either of the dimonomer copolymers 26b and 27a which contained 49 mol% and 53 mol% peptide, respectively. The presence of the third monomer introduces extra disorder. In addition, the GRGD and SRN containing copolymer, 28b, had a $T_g$ of 104.6 $^\circ C$, which is close to that of the GRGD containing homopolymer, 26c. The SRN and GRGD homopolymers exhibit fairly high glass transition temperatures compared to unsubstituted polynorbornene. Perhaps hydrogen bonding or other factors influenced the glass transition in these cases. Polymers containing the flexible penta(ethylene glycol) units exhibit lower $T_g$'s. Depending on the substituent, the physical state of the polymer varied widely from an oil (poly(21)) to a powdery solid (26c, 27b, and 28b).

**Polymer Stereoisomers.** The trans to cis ratios of the polymers synthesized in this research revealed an interesting observation: polymers synthesized with 1 contained more trans olefins while those synthesized by 2 had a slight excess of cis olefins. The methylene-bridged imide polymers had trans to cis ratios from 5.2/1 to 5.5/1 while the oxa-bridged imide polymers had ratios between 2.3/1 and 2.9/1. The poly(norbornene)s had ratios between 2.4/1 to 3.6/1. Similarly, the GRGD containing polymer 26a synthesized with 1 contained more trans olefins (trans/cis = 4.8/1). In contrast, the polymers synthesized with initiator 2 (26b, 27a, and 28a) exhibited slight excesses of cis
olefins \((\text{trans/cis} = 1/1.6 - 1/1.7, \text{see Table 3})\). (Polymers 26c, 27b, and 28b were not soluble in \(\text{CD}_3\text{OD}\), and the olefin peaks of the isomers were not resolved in \(\text{D}_2\text{O}\).)

To examine this further, monomers 15, 20, and 21 were polymerized under the same conditions with either 1 or 2 as the initiator. Polymerization of 15 with 1 resulted in a polymer with a \(\text{trans}\) to \(\text{cis}\) ratio of 5.2/1, while with 2 the ratio was equal (1/1). This trend was more marked with monomer 20, where 1 resulted in a polymer with a \(\text{trans}\) to \(\text{cis}\) ratio of 2.4/1 and 2 resulted in the reverse with a \(\text{trans}\) to \(\text{cis}\) ratio of 1/2.3. Similarly, for 21, initiator 1 resulted in a polymer with a \(\text{trans}\) to \(\text{cis}\) ratio of 3.6/1 and 2 with a \(\text{trans}\) to \(\text{cis}\) ratio of 1/1.7. The results obtained from polymers polymerized by 2 are unusual considering that 1 usually results, as evidenced in this research, in predominantly \(\text{trans}\) polymers.

**Polymers With Spacer Groups and Pendent Biologically Relevant Oligopeptides.** To alter the polymer architecture and the presentation of the oligopeptides, it is desirable to synthesize polymers with spacer groups between the peptide and the backbone. Since in native fibronectin, the RGD is extended from the protein as a loop,\(^{13}\) it might be especially advantageous to synthesize these polymers with a spacer between the backbone and the GRGD. As a demonstration, monomers 14 and 29 were polymerized to form 30, a polymer with a propyl spacer between the backbone and RGD and \(\text{EO}_3\) units (Scheme 7). Initially, 30 was synthesized using initiator 1, but the polymer obtained had a bimodal molecular weight distribution. As a result, the more active initiator, 2, was employed instead, and a monomodal molecular weight distribution was achieved.
Scheme 7. Synthesis of a polymer with propyl spacers and pendent RGDs.

Polymer 30 was synthesized as previously described using 2 as the initiator with a $[\text{M}]_0/\text{[C]}_0 = 10/1$ (Scheme 7). The yield was approximately 90% by $^1$H NMR spectroscopy. The polymer was deprotected with TFA and rendered soluble in water by first dissolving in pH 8 phosphate buffer (which did not saponify any of the EO$_5$ units), then precipitating into methanol. The water solubility of this polymer was dependent on the molecular weight; when higher monomer to catalyst ratios were used ($[\text{M}]_0/\text{[C]}_0 = 50/1$) the polymer did not dissolve even when subjected to strong base. The amount of 29 incorporated into the polymer (30 mol%) was slightly higher than the amount in the feed (20 mol%). The $M_n$ obtained in aqueous buffer was fairly high, at 115,000, and the PDI was narrow (1.14). The $T_g$ of the polymer was 70.2 $^\circ$C. Similar to other polymers synthesized with 2, the $trans$ to $cis$ ratio was 1/1.2. This work is readily extended to the synthesis of other homopolymers and copolymers, and demonstrates that by using the more active initiators, biopolymers with different architectures can be synthesized.
Summary

Norbornenyl polymers with pendant glycines, alanines, penta(ethylene glycol)s, or bioactive peptides GRGD and SRN attached directly or through spacers were synthesized. The ruthenium bisphosphine initiator 1 successfully polymerized monomers with glycines, alanines, and penta(ethylene glycol)s; copolymers with < 10 mol% GRGD were also prepared with this initiator. However, the synthesis of polymers with higher concentrations of the bioactive peptides required the use of the more active 2,3-dihydroimidazolylidene initiators 2 and 3. The work described in this chapter demonstrates that with the advent of catalysts 2 and 3, ROMP is a viable method to produce polymers substituted with complex peptidic structures in a controllable manner. Polymers with potential bioactivity, substituted with several different bioactive peptides, are now accessible.

Experimental

Materials. 5-Norbornene acid-endo-2-carboxylic acid (endo 18) was purchased from Aldrich. 5-Norbornene acid-exo-2-carboxylic acid (exo 18)\textsuperscript{20} and acid chloride\textsuperscript{21} as well as 5\textsuperscript{10} were synthesized according to literature procedures. 2-Aminoethanol and all other solvents were purchased from EM Science. Glycine methyl ester hydrochloride and alanine methyl ester hydrochloride were purchased from Sigma. Penta(ethylene glycol) was purchased from Aldrich and dried over 4 Å molecular sieves (Linde). The peptides were synthesized by the Beckman Institute Biopolymers Synthesis Laboratory (California Institute of Technology) using reagents purchased from Novabiochem. Centriprep flasks were purchased from Millipore. Methylene chloride used in the polymerization reactions
was dried over CaH₂, degassed, and vacuum transferred before use. All other chemicals were purchased from Aldrich and used as received.

**Techniques.** All operations were carried out under a dry nitrogen or argon atmosphere. Dry box operations were performed in a nitrogen-filled Vacuum Atmospheres dry box. Column chromatography was performed using silica gel 60 (230-400 mesh) from EM science. ¹H NMR spectra were recorded on a General Electric QE-300 (300.1 MHz) spectrometer, a JEOL GX-400 (399.65 MHz) spectrometer, or a Varian UnityPlu 600 (600.203 MHz) spectrometer as indicated. ¹³C NMR (75.49 MHz) spectra were recorded on a General Electric QE-300 spectrometer. Chemical shifts are reported downfield from tetramethylsilane (TMS). ³¹P NMR spectra were recorded on a JEOL GX-400 (161.85 MHz) spectrometer referenced to an external 85% H₃PO₄ standard. Infrared spectroscopy was performed on a Perkin Elmer Paragon 1000 FT-IR spectrometer using a thin film of sample cast on a NaCl plate or a KBr pellet as indicated. Optical rotations were recorded on a Jasco P-1010 digital polarimeter at 589 nm. High-resolution mass spectra were provided by the Southern California Mass Spectrometry Facility (University of California, Riverside). Gel permeation chromatographs with CH₂Cl₂ as the eluent (flow rate of 1 mL/min) were obtained using an HPLC system equipped with an Altex model 110A pump, a Rheodyne model 7125 injector with a 100 μL injection loop, two American Polymer Standards 10 micron mixed bed columns, a Knauer differential refractometer, and poly(styrene) as the calibration standard. Aqueous GPC (0.1 M Na₂HPO₄ dibasic buffer) or DMF GPC (both with a flow rate of 1 mL/min) were conducted using an HPLC system equipped with a Waters 515 HPLC pump, a Rheodyne model 7725 injector with a 200 μL injection loop, a Waters 2487 Dual λ.
absorbance detector, a Waters 2410 refractometer, two TSK columns (TASK 3000PW, TSK 5000PW) and poly(ethylene oxide) or poly(styrene) as the calibration standard as indicated. Differential scanning calorimetry was measured on a Perkin-Elmer DSC-7 for T_g's above 25 °C and on a Perkin-Elmer Pyris1 for T_g's below 25 °C. The results are given for the second heating using a scan rate of 10 °C/min. The HPLC results were obtained on a Beckman 126 Solvent Module HPLC equipped with a 166 UV Detector and an Altech 18-LL column using a H_2O/CH_3CN solvent system (7% CH_3CN for 6 min, 7-90% CH_3CN over 38 min, and 90% CH_3CN for 8 min).

Monomer Synthesis

**4-Hydroxymethyl-10-oxa-4-aza-tricyclo[5.2.1.0^26]dec-8-ene-exo-3,5-dione (6).**
Prepared as for the 7 (*vide infra*) with 6.10 g (36.80 mmol) 4 and 2.22 mL (36.80 mmol) of 2-aminoethanol in THF/MeOH (30 mL, 1/1) resulting in 2.78 g (36%) of 6 as a white crystalline solid. ^1H NMR (D_2O, 500 MHz) δ 6.48 (2H, s), 5.17 (2H, s), 3.56 (2H, t, J = 4.5), 3.50 (2H, t, J = 4.5), 2.99 (2H, s). ^13C NMR (D_2O, 300 MHz) δ 179.32, 136.28, 80.83, 58.12, 47.31, 40.87. IR (KBr pellet): 3475, 3001, 2969, 2931, 2894, 1766, 1688, 1438, 1407, 1386, 1335, 1316, 1268, 1219, 1169, 1155, 1099, 1054, 1014, 956, 938, 915, 878, 849, 810, 773, 723, 704, 653, 597 cm\(^{-1}\). HRMS (DCI/NH_3) calcd for (MH)^+ 210.0766 found 210.0763.

**4-Hydroxymethyl-4-aza-tricyclo[5.2.1.0^26]dec-8-ene-exo-3,5-dione (7).**
2-Aminoethanol (0.74 mL, 12.20 mmol) was added to a solution of 5 (2.00 g, 12.20 mmol) in THF/MeOH (1/1, 30 mL) and the mixture was heated to 50 °C for 12 h. After cooling to room temperature, the solvent was removed *in vacuo* and the product was
recrystallized from MeOH/hex (2/1) to afford 1.11 g (44%) of 7 as white crystals. \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 6.26-6.32 (2H, m), 3.77-3.82 (2H, m), 3.69-3.74 (2H, m), 3.30 (2H, s), 2.73 (2H, s), 2.18 (1H, bs), 1.53 (1H, d, \(J = 8.7\)), 1.35 (1H, d, \(J = 8.7\)). \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 178.65, 137.70, 60.02, 47.78, 45.14, 42.68, 41.15. IR (NaCl plate): 3506, 2984, 2954, 2885, 1758, 1689, 1423, 1403, 1341, 1329, 1166, 1152, 1063, 989, 935, 900, 881, 856, 767, 733, 649 cm\(^{-1}\). HRMS (DCI/NH\(_3\)) calcd for (MH\(^+\)) 208.0974, found 208.0969.

**4-(Exo-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0\(^2\)-6\(^\bullet\)]dec-8-en-4-yl)-butyric acid (8).** Same procedure as for the synthesis of the 7 was followed with 3.00 g (18.00 mmol) of 4 and 1.86 g (18.00 mmol) 4-aminobutyric acid in THF/MeOH (36 mL, 1:1). The crude product was subjected to column chromatography (EtOAc with 3% AcOH) to give 1.83 g (41%) of 8 as a white solid. \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 6.51 (2H, s), 5.26 (2H, s), 3.56 (2H, t, \(J = 6.9\) Hz), 2.85 (2H, s), 2.34 (2H, t, \(J = 7.5\) Hz), 1.90 (2H, t, \(J = 7.2\) Hz). \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 176.01, 168.60, 136.51, 80.95, 47.40, 37.96, 30.81, 22.59. IR (NaCl plate): 3480.6, 2977.1, 2677.2, 1770.9, 1696.0, 1562.1, 1396.1, 1165.8, 1096.1, 1015.0, 914.1, 871.2, 849.8, 807.0, 721.3, 651.6, 603.4 cm\(^{-1}\). HRMS (DCI/NH\(_3\)) calcd for (MH\(^+\)) 252.0872 found 252.0878.

**Exo-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0\(^2\)-6\(^\bullet\)]dec-8-en-4-yl)-acetic acid methyl ester (9).** The same procedure as for 15 was followed (vide infra) with 1.42 g (8.53 mmol) of 4, 1.07 g (8.53 mmol) glycine methyl ester hydrochloride, and 3.50 mL (25.16 mmol) of triethylamine in CH\(_2\)Cl\(_2\) (43 mL) to yield 0.17 g (9%) of 9 as a white solid. \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 6.54 (2H, m), 5.31 (2H, s), 4.24 (2H, s), 3.76 (3H, s), 2.97 (2H, s). \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 175.85, 167.62, 137.09, 81.45, 53.02,
IR (NaCl plate): 3100, 3029, 3011, 2992, 2958, 1747, 1707, 1423, 1373, 1326, 1267, 1221, 1180, 1154, 1102, 1081, 1014, 983, 951, 915, 874, 848, 806, 713, 656, 619, 593 cm⁻¹. HRMS (DCI/NH₃) calcd for (MH)⁺ 238.0716, found 238.0704.

*Exo*-2-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0²⁶]dec-8-en-4-yl)-propionic acid methyl ester (10). Compound 10 was synthesized according to literature procedure¹¹ in 25% yield. H NMR (CDCl₃, 500 MHz) δ 6.53 (2H, s), 5.29 (2H, s), 4.74 (1H, q, J = 7.4 Hz), 3.73 (3H, s), 2.84-2.92 (2H, m), 1.54 (3H, d, J = 7.5).

2-tert-Butoxycarbonylamino-propionic acid *exo*-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0²⁶]dec-8-en-4-ylmethyl ester (11). The same procedure as for 17 (vide infra) was followed with 0.50 g (2.39 mmol) of 6, 0.45 g (2.39 mmol) of N-tert-butoxycarbonyl-L-alanine, 0.49 g (2.39 mmol) of 1,3-dicyclohexylcarbodiimide (DCC), and 0.04 g (0.36 mmol) of 4-(dimethylamino)pyridine (DMAP) in 20 mL CH₂Cl₂. The product was purified by recrystallization from MeOH/hex (2/1) to yield 0.46 mg (50%) of 11 as white crystals. H NMR (CDCl₃, 500 MHz) δ 6.51 (2H, s), 5.27 (2H, s), 5.02 (1H, bs), 4.30 (2H, t, J = 5.3 Hz), 4.23-4.29 (1H, m), 3.73-3.81 (2H, m), 2.86-2.89 (2H, m), 1.42 (9H, s), 1.34 (3H, d, J = 7.2 Hz). C NMR (CDCl₃, 300 MHz) δ 175.91, 172.76, 154.97, 136.44, 80.79, 61.17, 49.06, 47.38, 47.36, 37.63, 28.23, 18.25. IR (NaCl plate): 3356.0, 2967.3, 2936.6, 1742.0, 1701.1, 1511.8, 1455.6, 1430.0, 1394.2, 1363.5, 1332.8, 1251.0, 1158.9, 1066.8, 1020.8, 886.0, 855.4, 713.9, 647.4. [α]D²⁵ = -10.6. HRMS (DCI/NH₃) calcd for (MH)⁺ 381.1662 found 381.1645.

*(Exo*-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0²⁶]dec-8-en-4-ylmethoxy)-acetic acid methyl ester (12). Methyl bromoacetate (1.81 mL, 19.12 mmol) was added to a solution of the 6 (1.00 g, 4.78 mmol), potassium carbonate (0.80 g, 5.76 mmol), and
tetrabutylammonium bromide (0.02 g, 0.06 mmol) in anhydrous DMF (20 mL). The solution was stirred at room temperature for 12 h. CHCl₃ (100 mL) was added, and the organic layer was successively washed with H₂O (twice) and 5% HBrₐq. The aqueous layers were combined and washed with CH₂Cl₂ (three times). The organic layers were pooled, dried over MgSO₄, and the solvent was removed in vacuo. The crude product was recrystallized from a mixture of EtOAc and ether (4:1) to give 0.66 g (49%) of 12 as a white solid. ¹H NMR (CDCl₃, 300 MHz) δ 6.51 (2H, s), 5.27 (2H, s), 4.62 (2H, s), 4.34 (2H, t, J = 5.4 Hz), 3.81 (2H, t, J = 4.5 Hz), 3.78 (3H, s), 2.89 (2H, s). ¹³C NMR (CDCl₃, 300 MHz) δ 175.87, 167.66, 154.36, 136.44, 80.81, 63.35, 52.27, 47.39, 37.46. IR (NaCl plate): 3009.3, 2966.4, 1754.9, 1701.3, 1428.2, 1396.1, 1337.1, 1288.9, 1214.0, 1149.7, 1122.9, 1021.2, 919.4, 876.6, 849.8, 785.5, 721.3, 646.3, 592.7 cm⁻¹.

[4-(Exo-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0²⁶]dec-8-en-4-yl)-butyrylamino]-acetic acid methyl ester (13). Same procedure as for 19 was followed (vide infra) with 2.21 mL (15.89 mmol) triethylamine, 0.65 g (4.77 mmol) 1-hydroxybenzotriazole (HOBT), 0.61 g (3.18 mmol) 1-[3-(dimethylamine)propyl]-3-ethylcarbodiimide hydrochloride (EDC), 0.40 g (3.19 mmol) glycine methyl ester hydrochloride, and 0.80 g (3.18 mmol) of 8 in 64 mL CH₂Cl₂. The crude product was subjected to column chromatography (EtOAc) to give 0.58 g (57%) of 13 as a white solid. ¹H NMR (CDCl₃, 300 MHz) δ 6.53 (2H, s), 5.28 (2H, s), 4.05 (2H, d, J = 5.1 Hz), 3.76 (3H, s), 3.62 (2H, t, J = 6.3 Hz), 2.86 (2H, s), 2.19 (2H, t, J = 6.9 Hz), 1.97 (2H, t, J = 6.3 Hz). ¹³C NMR (CD₂Cl₂, 300 MHz) 177.09, 172.47, 136.98, 81.56, 54.68, 52.64, 47.99, 41.66, 38.42, 33.39, 24.22. IR (NaCl plate): 3563.8, 3292.9, 3084.6, 2949.2, 1750.7, 1698.6,1547.6, 1433.0, 1401.8, 1370.5, 1282.0, 1214.3, 1162.2, 1021.6, 917.4,
4-(Exo-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0^26]dec-8-en-4-yl)-butyric acid 2-(2-(2-hydroxy-ethoxy)-ethoxy)-ethyl ester (14). The acid chloride of 8 was generated in situ using 0.70 g (2.78 mmol) of 8, 0.53 mL (6.12 mmol) oxalyl chloride, and a catalytic amount of DMF in CH2Cl2 following a literature procedure. To a solution of the crude acid chloride in anhydrous THF (45 mL), penta(ethylene glycol) (0.66 g, 2.78 mmol) and potassium carbonate (1.31 g, 9.47 mmol) were added. The mixture was heated to reflux and stirred for 12 h. After cooling to room temperature, CH2Cl2 (50 mL) was added, and the organic layer was washed with H2O (three times). The organic layer was dried over MgSO4, and the solvent was removed in vacuo. The crude product was subjected to column chromatography (EtOAc/MeOH, 4/1) to give 0.48 g (36%) of 14 as a yellow oil. 1H NMR (CDCl3, 300 MHz) δ 6.51 (2H, s), 5.24 (2H, s), 4.21 (2H, J = 4.8 Hz), 3.64-3.66 (18H, m), 3.53 (2H, t, J=6.9 Hz), 2.84 (2H, s), 2.32 (2H, t, J=7.5 Hz), 1.88 (2H, t, J=7.1 Hz). 13C NMR (CD2Cl2, 300 MHz) δ 176.80, 173.01, 136.99, 81.50, 73.04, 71.01, 70.96, 70.79, 69.53, 64.14, 62.09, 47.96, 38.34, 31.49, 23.27. IR (NaCl plate): 4015.1, 3479.7, 2878.7, 1947.7, 1767.4, 1734.6, 1696.4, 1636.3, 1439.6, 1401.4, 1352.2, 1100.9, 1019.0, 953.4, 876.9, 718.5, 647.5, 587.4 cm⁻¹. HRMS (DCI/NH₃) calcd for (MNH₄)⁺ 489.2448 found 489.2460.

Exo-(3,5-dioxo-4-aza-tricyclo[5.2.1.0^26]dec-8-en-4-yl)-acetic acid methyl ester (15). A literature procedure was followed except that CH2Cl2 was used as the solvent to give 15 in 59% yield as a white solid. 1H NMR (CDCl3, 300 MHz) δ 6.29-6.30 (2H,
m), 4.22 (2H, s), 3.73 (3H, s), 3.30-3.32 (2H, m), 2.75 (2H, d, J = 1.5 Hz), 1.70 (1H, d, J = 9.9 Hz), 1.52 (1H, d, J = 9.9 Hz).

Exo-2-(3,5-dioxo-4-aza-tricyclo[5.2.1.0²6]dec-8-en-4-yl)-propionic acid methyl ester (16). Compound 16 was synthesized according to literature procedure¹¹ in 51% yield. $^1$H NMR (CDCl₃, 300 MHz) δ 6.27-6.28 (2H, m), 4.75 (1H, q, J = 7.5 Hz), 3.70 (3H, s), 3.27-3.28 (2H, m), 2.69-2.70 (2H, m), 1.53 (3H, d, J = 7.2 Hz), 1.50-1.54 (2H, m).

2-tert-Butoxycarbonylamino-propionic acid exo-3,5-dioxo-4-aza-tricyclo[5.2.1.0²6]dec-8-en-4-ylmethyl ester (17). 7 (0.30 g, 1.45 mmol), N-(tert-butoxycarbonyl)-L-alanine (0.27 g, 1.45 mmol), DCC (0.30 g, 1.45 mmol), and DMAP (0.03 g, 0.22 mmol) were stirred in CH₂Cl₂ (18 mL) for 12 h. The solution was filtered and the solvent removed in vacuo. The residue was subjected to column chromatography twice (EtOAc, followed by EtOAc/hex, 8/2) to give 0.33 g (61% yield) of 17 as a sticky solid. $^1$H NMR (CD₃Cl, 500 MHz) δ 6.29 (2H, s), 4.99 (1H, bs), 4.11-4.32 (3H, bm), 3.75-3.79 (2H, m), 3.29 (2H, d, J = 10.1 Hz), 2.71 (2H, s), 1.52-1.54 (1H, m), 1.43 (9H, s), 1.35 (3H, d, J = 7.2 Hz), 1.27-1.29 (1H, m). $^{13}$C NMR (CDCl₃, 300 MHz) δ 177.89, 173.33, 155.33, 137.79, 79.87, 61.69, 49.33, 47.83, 45.22, 42.73, 37.49, 28.31, 18.39. IR (NaCl plate): 3366.2, 2977.5, 2936.6, 1747.1, 1696.0, 1506.7, 1450.4, 1389.1, 1363.5, 1327.7, 1251.0, 1164.0, 1061.7, 990.1, 775.3, 719.0, 642.3 cm⁻¹. [α]D²₃ = -19.0. HRMS (DCI/NH₃) calcd for (MH)+ 379.1869 found 379.1871.

[(Bicyclo[2.2.1]hept-5-ene-endo-2-carbonyl)-amino]-acetic acid methyl ester (19). Triethylamine (1.41 mL, 10.14 mmol) and glycine methyl ester hydrochloride (0.64 g, 5.06 mmol) were added to a solution of endo 18 (0.70 g, 5.06 mmol) in CH₂Cl₂ (75
mL). HOBT (1.03 g, 7.62 mmol) was added, and the solution was stirred until all solids had dissolved. Then a solution of EDC (0.97 mg, 5.06 mmol) and triethylamine (1.41 mL, 10.14 mmol) in CH$_2$Cl$_2$ (25 mL) was added and the mixture was stirred for 24 h. The organic layer was washed successively with 10% citric acid, H$_2$O, sat. NaHCO$_3$, and brine, dried over MgSO$_4$, and the solvent removed in vacuo. The crude product was subjected to column chromatography (ether) resulting in 0.70 g (66% yield, 11% exo) of 19 as a white solid. $^1$H NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 6.17-6.19 (1H, m), 5.94-5.97 (1H, m), 3.92 (2H, d, J = 5.7 Hz), 3.70 (3H, s), 3.14 (1H, bs), 2.87-2.92 (2H, m), 1.85-1.93 (1H, m), 1.28-1.43 (3H, m). $^{13}$C NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 174.62, 171.16, 138.03, 132.73, 52.58, 50.39, 46.77, 44.80, 43.26, 41.46, 29.84. IR (NaCl plate): same as for 20 (vide infra).

[(Bicyclo[2.2.1]hept-5-ene-exo-2-carbonyl)-amino]-acetic acid methyl ester (20). The same procedure as for 19 was followed with 1.40 mL (10.06 mmol) triethylamine, 0.54 g (4.30 mmol) glycine methyl ester hydrochloride, 0.57 g (4.10 mmol) exo 18, 0.83 g (6.14 mmol) HOBT, and 0.79 g (4.10 mmol) EDC in 40 mL of CH$_2$Cl$_2$ resulting in 0.63 g (73%) of 20 as an off-white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 6.10-6.13 (2H, m), 6.01 (1H, bs), 4.05 (2H, dd, J = 4.8 Hz, 17.9 Hz), 3.75 (3H, s), 2.96 (1H, s), 2.91 (1H, s), 2.06-2.08 (1H, m), 1.90-1.95 (1H, m), 1.67 (1H, d, J = 8.0 Hz), 1.34 (2H, d, J = 9.2). $^{13}$C NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 175.92, 170.97, 138.48, 136.25, 52.43, 47.48, 46.45, 44.49, 41.94, 41.42, 30.59. IR (NaCl plate): 3315.1, 3059.3, 2957.1, 2865.0, 1747.1, 1644.8, 1532.3, 1440.2, 1404.4, 1368.6, 1327.7, 1204.9, 1097.5, 1046.4, 1010.6, 898.0, 852.0, 790.6, 719.0 cm$^{-1}$. HRMS (EI) calcd for (M)$^+$ 209.1052 found 209.1048.
Bicyclo[2.2.1]hept-5-ene-exo-2-carboxylic acid 2-(2-(2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy-ethoxy)-ethoxy)-ethyl ester (21). 5-Norbornene acid-exo-2-carboxylic acid chloride (1.20 g, 7.67 mmol) was added dropwise to a stirred mixture of penta(ethylene glycol) (2.79 mL, 13.20 mmol) and potassium carbonate (3.70 g, 26.80 mmol) in THF (160 mL). The reaction was heated to reflux and stirred for 12 h, cooled, and the solvent removed in vacuo. H₂O was added and the mixture was neutralized with 10% citric acid. The aqueous layer was washed with CH₂Cl₂ (3 times), the organic layers were then consolidated, dried over MgSO₄ and the solvent was removed in vacuo. The residue was subjected to column chromatography (EtOAc/MeOH, 9/1) to give 1.4 g (53%) of 21 as a colorless oil. ¹H NMR (CDCl₃, 300 MHz) δ 6.07-6.14 (2H, m), 4.23 (2H, t, J = 4.8), 3.58-3.73 (18H, m), 3.03 (1H, s), 2.90 (1H, s), 2.60 (1H, bs), 2.22-2.27 (1H, m), 1.88-1.94 (1H, m), 1.51 (1H, d, J = 8.4 Hz), 1.32-1.38 (2H, m). ¹³C NMR (CDCl₃, 300 MHz) δ 176.01, 137.87, 135.53, 72.34, 70.38, 70.12, 69.02, 63.29, 61.45, 46.47, 46.09, 42.83, 41.43, 30.15. IR (NaCl plate): 3455.7, 2936.8, 2864.2, 1721.2, 1451.4, 1342.5, 1332.1, 1280.2, 1254.3, 1228.9, 1171.2, 1109.0, 1051.9, 942.9, 859.9, 719.8 cm⁻¹. HRMS (DCI/NH₃) calcld for (MH)⁺ 359.2070 found 359.2082.

[(Tricyclo[4.2.1.0²5]non-7-ene-exo-3-carbonyl)-amino]-acetic acid methyl ester (23). Same procedure as for 19 was followed with 0.97 mL (6.98 mmol) triethylamine, 0.44 g (3.49 mmol) glycine methyl ester hydrochloride, 0.57 g (3.49 mmol) 22, 0.71 g (5.22 mmol) HOBT, and 0.67 g (3.49 mmol) EDC in 30 mL CH₂Cl₂. The crude product was subjected to column chromatography (ether) to provide 0.53 g (65%) of 23 as a white solid. ¹H NMR (CDCl₃, 300 MHz) δ 5.90-5.98 (2H, m), 4.04 (2H, d, J = 5.1 Hz), 3.74 (3H, s), 2.71 (1H, s), 2.65 (1H, s), 2.31-2.36(2H, m), 2.14 (1H, t,
J = 7.2 Hz), 1.99 (1H, t, J = 7.8 Hz), 1.54-1.66 (2H, m), 1.32 (1H, d, J = 9.3 Hz). $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 175.68, 170.59, 135.75, 134.39, 52.22, 43.97, 43.85, 41.16, 40.35, 40.28, 38.70, 34.12, 23.68. IR (NaCl plate): 3287.8, 3062.8, 2966.4, 2355.9, 1754.9, 1647.8, 1540.7, 1433.6, 1369.3, 1203.3, 1048.0, 989.0, 699.9, 507.1 cm$^{-1}$. HRMS (DEI) calcd for (M$^+$) 235.1208 found 235.1201.

Norbornene G-R(Pbf)-G-D(O'Bu)-OH monomer (24). H$_2$N-G-R(Pbf)-G-D(O'Bu)-resin (0.50 mmol peptide, 4-carboxytrityl linker Novasyn® resin) was placed in a flask containing a frit and stopcock. The resin was swelled in 17 mL of DMF for 15 min and then rinsed with DMF (1 x 10 mL). In a vial, 0.28 g (2.00 mmol) of exo 18, 0.76 g (2.00 mmol) of 2-((1H-benzo-triazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and 0.27 g (2.00 mmol) of HOBT in 17 mL of DMF were agitated until all solids had dissolved. N,N-diisopropylethylamine (DIEA) was added (0.70 mL, 4.00 mmol) and the solution was agitated and added to the resin. Nitrogen was gently bubbled through the mixture for 2 h. The solution was removed, and the resin was then rinsed with DMF (5 x 10 mL), CH$_2$Cl$_2$ (5 x 10 mL), and MeOH (5 x 10 mL) and dried for 24 h at 30 millitorr. In a vial, 33 mL of acetic acid, CH$_2$Cl$_2$, and MeOH (5:4:1) were added to the dry resin and the vial was periodically swirled for 1.5-2 h. The solution was filtered to remove the resin, added to an excess of hex, and the solvent was removed in vacuo. The procedure was repeated to remove all of the acetic acid. The product was freeze-dried from benzene to give 24 in 76-97% yield as an off-white solid.

HPLC: single peak at 21.94 min. $^1$H NMR (CD$_2$Cl$_2$, 600 MHz) δ 7.89 (1H, bs), 7.50 (1H, bs), 7.42 (1H, bs), 7.06 (1H, bs), 6.21 (2H, bs), 6.03 (1H, s), 5.99 (1H, s), 4.65 (1H, bs), 4.38 (1H, bs), 3.96 (1H, bs), 3.87 (2H, bs), 3.75 (1H, bs), 3.13 (2H, bs), 2.88 (2H, s), 2.83
(1H, s), 2.78 (1H, s), 2.71 (2H, bs), 2.46 (3H, s), 2.40 (3H, s), 2.07 (1H, bs), 1.99 (3H, s), 1.80 (1H, bs), 1.72-1.75 (1H, m), 1.63 (1H, bs), 1.48-1.52 (3H, m), 1.35 (6H, s), 1.29 (9H, s), 1.17-1.22 (2H, m). NOESY cross peaks (CD$_2$Cl$_2$, 600 MHz) δ 7.89 (G$_{2\text{NH}}$), 4.38 (R$_{a}$); 7.50 (D$_{\text{NH}}$), 3.96 (G$_2$$_{a}$), 3.75 (G$_2$$_{a}$); 7.42 (R$_{\text{NH}}$), 3.87 (G$_1$$_{a}$); 7.06 (G$_1$$_{\text{NH}}$), 2.07 (norbornene). TOCSY cross peaks (CD$_2$Cl$_2$, 600 MHz) δ norbornene: 6.03, 5.99, 2.83, 2.78, 2.07, 1.72-1.75, 1.48-1.52, 1.17-1.22; G1: 7.06, 3.87; R: 7.42, 4.38, 3.13, 1.80, 1.63, 1.48-1.52; Pbf group: 2.88, 2.46, 2.40, 1.99; G2: 7.89, 3.96, 3.75; D: 7.50, 4.65, 2.71. $^{13}$C NMR (CD$_2$Cl$_2$, 300 MHz) δ 178.01, 173.79, 173.58, 171.27, 170.64, 170.44, 159.11, 156.99, 138.60, 138.33, 136.42, 132.84, 132.64, 128.64, 125.19, 117.82, 86.87, 81.96, 71.73, 70.68, 70.28, 69.07, 66.95, 49.77, 47.54, 46.52, 44.31, 43.43, 41.96, 37.59, 30.77, 28.66, 28.12, 25.68, 19.48, 18.15, 12.57. IR (NaCl plate): 3445.2, 3310.3, 3050.9, 2967.9, 2936.7, 1726.4, 1638.2, 1544.8, 1456.6, 1368.4, 1291.5, 1245.2, 1152.8, 1101.4, 1029.5, 956.1, 899.6, 848.2, 807.1, 786.6, 704.4, 668.4, 560.5 cm$^{-1}$. HRMS (DCM/NBA/NaCl) calcd for (MNar$^+854.3735$ found 854.3707.

**Norbornene S(O'Bu)-R(Pbf)-N(Trt)-OH Monomer (25).** The same procedure as for 24 was followed with H$_2$N-S(O'Bu)-R(Pbf)-N(Trt)-resin (0.75 mmol, 4-carboxytrityl linker Novasyn® resin), 0.47 g (3.00 mmol) of exo 18, 1.14 g (3.00 mmol) of HBTU, 0.41 g (3.00 mmol) of HOBT, and 1.00 mL (6.00 mmol) of DIEA in 19 mL of DMF to yield 0.72 g (92%) of 25 as a fluffy, white solid. HPLC: single peak at 28.86 min. $^1$H NMR (CD$_2$Cl$_2$, 600 MHz) δ 7.54 (1H, bs), 7.38 (1H, bs), 7.07-7.13 (15H, m), 6.46 (1H, bs), 6.01-6.03 (3H, m), 5.84 (1H, bs), 4.65 (1H, bs), 4.52 (1H, bs), 4.37 (1H, bs), 3.62 (1H, bs), 3.37 (1H, bm), 3.15 (1H, bs), 2.92-2.95 (2H, bm), 2.87 (2H, s), 2.75-2.79 (3H, bm), 2.43 (3H, s), 2.37 (3H, s), 2.02 (1H, bs), 1.98 (3H, s), 1.73-1.79 (2H, bm),
1.50-1.54 (2H, bm), 1.42 (2H, bm), 1.37 (6H, s), 1.18-1.26 (2H, bm), 1.07 (9H, s).

TOCSY cross peaks (CD$_2$Cl$_2$, 600 MHz) δ norbornene: 6.03, 6.01, 2.79, 2.02, 1.78, 1.52, 1.22; Ser: 7.54, 4.65, 2.96, 2.75; Arg: 7.38, 5.84, 4.52, 3.15, 2.92, 1.79, 1.51, 1.42; Pbf: 2.87, 2.43, 2.37, 1.98; Asn: 7.07, 6.46, 4.37, 3.62, 3.37. 13C NMR (CDCl$_3$, 300 MHz) δ 176.40, 176.26, 173.98, 173.16, 172.71, 170.27, 170.14, 144.14, 138.53, 138.24, 137.99, 136.14, 135.85, 132.73, 132.36, 128.68, 127.78, 126.85, 124.44, 117.46, 86.25, 73.99, 70.95, 70.53, 61.84, 53.53, 52.02, 49.89, 47.40, 46.92, 46.32, 46.21, 44.41, 43.30, 41.57, 39.40, 37.85, 30.83, 30.29, 29.71, 28.57, 27.31, 25.00, 19.28, 17.97, 12.41. IR (NaCl plate): 3434.9, 3331.1, 3061.3, 2967.9, 2926.4, 2874.5, 1726.4, 1643.0, 1550.0, 1492.9, 1446.2, 1394.3, 1368.4, 1332.1, 1254.2, 1192.0, 1155.6, 1098.6, 1031.1, 994.8, 953.3, 901.4, 854.7, 802.8, 756.1, 699.0, 667.9, 636.0, 621.1, 569.3 cm$^{-1}$. HRMS (DCM/NBA/NaCl) calcd for (MNa)$^+$ 1068.4881 found 1068.4873.

**R(Pbf)-G-D(O'Bu)-OH Monomer (29).** The same procedure as for 24 was followed with H$_2$N-R(Pbf)-G-D(O'Bu)-resin (0.25 mmol, 4-carboxytrityl linker Novasyn® resin), 0.25 g (1.00 mmol) 8, 0.38 g (1.00 mmol) HBTU, 0.14 g (1.00 mmol) HOBT, and 0.35 ml (2.00 mmol) DIEA in 8 mL DMF to yield 0.18 g (83%) of 29 as a white solid.

HPLC: single peak at 21.90 min. $^1$H NMR (CD$_2$Cl$_2$, 600 MHz) δ 7.97 (1H, bs), 7.61 (1H, bs), 7.19 (1H, bs), 6.37 (5H, bs), 5.10 (2H, s), 4.63 (1H, bs), 4.29 (1H, bs), 3.93 (1H, bs), 3.72 (1H, bs), 3.39 (2H, bs), 3.16 (2H, bs), 2.87 (2H, s), 2.75 (2H, bs), 2.68-2.72 (2H, bm), 2.46 (3H, s), 2.39 (3H, s), 2.18 (2H, bs), 1.98 (3H, s), 1.86 (2H, bs), 1.73 (1H, bs), 1.58 (1H, bs), 1.50 (2H, bs), 1.36 (6H, s), 1.33 (9H, s). TOCSY cross peaks (CD$_2$Cl$_2$, 600 MHz) δ norbornene: 6.37, 5.10, 3.39, 2.18, 1.86, 1.73; R: 7.19, 6.37, 4.29, 3.16, 1.86, 1.58, 1.50; Pbf group: 2.87, 2.46, 2.39, 1.98, 1.36; G: 7.97, 3.93, 3.72; D: 7.61, 4.63,
2.68-2.72. $^{13}$C NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 177.50, 174.09, 170.68, 170.60, 159.01, 157.04, 138.52, 136.79, 133.13, 132.55, 128.636, 125.17, 117.77, 86.85, 81.75, 81.28, 72.75, 70.47, 69.94, 61.44, 50.12, 47.90, 43.42, 38.12, 37.74, 32.33, 29.07, 28.66, 28.14, 25.76, 23.47, 19.43, 18.14, 12.55, 1.11. IR (NaCl plate): 3435.3, 3331.5, 2968.3, 2926.8, 2522.0, 1695.5, 1653.9, 1545.0, 1446.4, 1404.9, 1368.6, 1254.4, 1155.8, 1098.8, 1020.9, 917.2, 875.6, 849.7, 803.0, 730.4, 657.7, 569.5 cm$^{-1}$. HRMS (DCM/NBA/NaCl) calcd for (MH)$^+$ 888.3813 found 888.3778.

**Polymer Synthesis**

**General Synthesis for Polymers with Pendent alanine, glycine, or EO$_2$.** In a nitrogen-filled dry box, a solution of 1 in CH$_2$Cl$_2$ was added to a solution of monomer CH$_2$Cl$_2$ (or CD$_2$Cl$_2$ for NMR reactions) to give an initial monomer concentration of 0.7-0.75 M. The initial [M]$_0$/[C]$_0$ was 100/1. The reaction mixture was stirred at room temperature for 15 min to 3 h before quenching with ethyl vinyl ether and stirring for an additional 15-30 min. The polymers were precipitated into ether or hex, stirred for 15 min, and subjected to centrifugation. The solvent was removed and the solids dried under vacuum. The polymers were all white to tan powders, except for Poly(14) and Poly(21) which were viscous oils. Deviations from this literature procedure are noted in specific cases below. (Data not reported within the text is also reported below.)

**Poly(9).** $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 6.06, 5.79 (trans $&$ cis, 2H, bs), 4.91-4.97, 4.44-4.53 (cis $&$ trans, 2H, bm), 4.20 (trans $&$ cis, 2H, bs), 3.74 (trans $&$ cis, 3H, s), 3.40 (trans $&$ cis, 2H, bs). $^{13}$C NMR (CDCl$_3$, 300 MHz) $\delta$ 174.77, 167.05, 130.94, 130.71, 80.74, 53.32, 52.84, 52.34, 39.37. IR (NaCl plate): 3662.0, 3468.5, 2998.0,
Poly(10). The solvent was either CH₂Cl₂ or benzene. ¹H NMR (CD₂Cl₂, 300 MHz) δ 6.08, 5.83 (trans & cis, 2H, bs), 4.87-4.97, 4.74-4.76 (cis & trans, 1H, bm), 4.48 (trans & cis, 2H, bs), 3.71, 3.70 (trans & cis, 3H, s), 3.37 (trans & cis, 2H, bs), 1.57 (trans & cis, 3H, bd, J = 7.5 Hz).

Poly(11). ¹H NMR (CD₂Cl₂, 400 MHz) δ 6.06, 5.78 (trans & cis, 2H, bs), 5.25, 5.16 (cis & trans, 1H, bs), 4.91-5.00, 4.47 (cis & trans, 2H, bm, bs), 4.29 (trans & cis, 2H, bs), 4.15-4.19 (trans & cis, 1H, bm), 3.75 (trans & cis, 2H, bs), 3.35 (trans & cis, 2H, bs), 1.38 (trans & cis, 9H, s), 1.30 (trans & cis, 3H, bd, J = 7.3 Hz). ¹³C NMR (CDCl₃, 300 MHz) δ 175.42, 173.22, 155.08, 130.99, 130.89, 80.63, 79.83, 61.41, 53.30, 52.28, 49.12, 38.20, 28.31, 18.12. IR (NaCl plate): 3528.3, 3372.6, 2967.9, 2936.8, 2874.5, 1778.3, 1742.0, 1705.7, 1513.7, 1451.4, 1425.5, 1394.3, 1363.2, 1332.1, 1249.1, 1160.8, 1119.3, 1067.5, 1025.9, 911.8, 730.2 cm⁻¹.

Poly(12). [M]₀ was 0.6 M. ¹H NMR (CD₂Cl₂, 300 MHz) δ 6.06, 5.79 (trans & cis, 2H, bs), 4.92, 4.47 (cis & trans, 2H, bs), 4.60 (trans & cis, 2H, s), 4.32 (trans & cis, 2H, bs), 3.80 (trans & cis, 2H, bs), 3.73 (trans & cis, 3H, s), 3.38 (trans & cis, 2H, bs). ¹³C NMR (CD₂Cl₂, 300 MHz) δ 176.04, 175.91, 168.38, 155.15, 131.78, 131.49, 131.38, 81.36, 81.30, 81.24, 77.81, 65.24, 64.07, 54.76, 54.40, 52.92, 38.30. IR (NaCl plate): 4202.5, 3631.2, 3537.9, 3468.0, 3013.2, 2954.9, 2850.0, 1754.6, 1707.9, 1433.9, 1393.1, 1119.1, 1031.7, 973.4, 915.1, 850.9, 781.0, 705.2, 676.0, 629.4, 565.3 cm⁻¹.
Poly(13). \([M]_0 = 0.5\) M. \(^1\)H NMR (CD\(_2\)Cl\(_2\), 400 MHz) \(\delta\) 7.03 (trans & cis, 1H, bs), 6.03, 5.79 (trans & cis, 2H, bs), 4.96, 4.48 (trans & cis, 2H, bs), 3.93 (trans & cis, 2H, bs), 3.66 (trans & cis, 3H, s), 3.47 (trans & cis, 2H, bs), 3.37 (trans & cis, 2H, bs), 2.23 (trans & cis, 2H, bs), 1.85 (trans & cis, 2H, bs). \(^{13}\)C NMR (CD\(_2\)Cl\(_2\), 300 MHz) \(\delta\) 176.01, 173.21, 172.91, 135.87, 135.54, 135.24, 82.46, 81.56, 81.26, 55.10, 52.57, 48.23, 41.77, 41.55, 39.16, 38.49, 34.69, 34.21, 25.21. IR (NaCl plate): 3578.3, 3330.0, 3081.6, 2947.1, 2854.0, 1749.2, 1702.7, 1666.5, 1542.3, 1433.6, 1397.4, 1366.4, 1211.2, 1159.4, 1118.0, 1030.1, 973.2, 916.3, 771.4, 704.1, 564.4 cm\(^{-1}\).

Poly(14). \([M]_0 = 0.55\) M. \(^1\)H NMR (CD\(_2\)Cl\(_2\), 400 MHz) \(\delta\) 6.05, 5.79 (trans & cis, 2H, bs), 4.96, 4.45 (cis & trans, 2H, bs), 4.17 (trans & cis, 2H, bs), 3.51-3.62 (trans & cis, 20H, bm), 3.33 (trans & cis, 2H, bs), 2.33 (trans & cis, 2H, bs), 1.86 (trans & cis, 2H, bs). \(^{13}\)C NMR (CD\(_2\)Cl\(_2\), 300 MHz) \(\delta\) 176.33, 176.22, 173.03, 131.60, 81.37, 73.16, 70.95, 70.91, 70.70, 69.50, 64.20, 61.98, 54.81, 52.91, 38.67, 31.79, 31.75, 23.94. IR (NaCl plate): 4016.0, 3491.3, 2908.3, 1947.0, 1777.9, 1713.8, 1638.0, 1439.8, 833.4, 769.3, 734.3, 705.2, 670.2, 576.9, 512.8 cm\(^{-1}\).

Poly(15). \(^1\)H NMR (CD\(_2\)Cl\(_2\), 300 MHz) \(\delta\) 5.74, 5.53 (trans & cis, 2H, bs), 4.17 (trans & cis, 2H, bs), 3.72, 3.71 (trans & cis, 3H, s), 3.45, 3.09 (cis & trans, 2H, bs), 2.84, 2.76 (cis & trans, 2H, bs), 2.13-2.21 (trans & cis, 1H, bm), 1.61-1.76 (trans & cis, 1H, bm). \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 177.47, 167.32, 131.89, 131.76, 52.67, 51.04, 50.93, 45.87, 45.73, 41.91, 41.07, 39.06. IR (NaCl plate): 2994.4, 2954.0, 2853.7, 1779.4, 1751.4, 1704.5, 1413.6, 1366.7, 1324.5, 1216.6, 1169.7, 972.7, 916.4, 766.3, 733.5, 616.2 cm\(^{-1}\).
Poly(16). $^1$H NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 5.74-5.78, 5.57-5.59 (trans & cis, 2H, br m), 4.76 (trans & cis, 1H, br q, J = 7.5 Hz), 3.74, 3.71 (trans & cis, 3H, s), 3.19-3.25, 3.07-3.14 (cis & trans, 2H, br m), 2.76 (trans & cis, 2H, br s), 2.10-2.22 (trans & cis, 1H, br m), 1.82-1.90, 1.60-1.75 (cis & trans, 1H, br m), 1.55 (trans & cis, 3H, d, J = 7.2 Hz). $^{13}$C NMR (CDCl$_3$, 300 MHz) $\delta$ 177.43, 177.23, 169.70, 133.40, 132.01, 131.77, 52.75, 52.28, 50.77, 47.71, 45.78, 45.65, 41.93, 41.84, 40.87, 14.23, 14.02. IR (NaCl plate): 3003.8, 2947.5, 2863.1, 1774.8, 1742.0, 1704.5, 1451.2, 1390.2, 1357.3, 1310.4, 1230.7, 1197.9, 1118.1, 1071.2, 972.7, 911.7, 785.1, 733.5, 625.6 cm$^{-1}$.

Poly(17). $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 5.73, 5.49 (trans & cis, 2H, bs), 5.17, 5.07 (cis & trans, 1H, bs), 4.22-4.28 (trans & cis, 3H, bm), 3.70 (trans & cis, 2H, bm), 2.96-3.05 (trans & cis, 2H, bm), 3.23, 2.70 (cis & trans, 2H, bs), 2.05-2.27 (trans & cis, 1H, bm), 1.55-1.67 (trans & cis, 1H, bm), 1.40 (trans & cis, 9H, s), 1.32 (trans & cis, 3H, d, J = 7.2 Hz). $^{13}$C NMR (CDCl$_3$, 300 MHz) $\delta$ 177.91, 173.09, 155.04, 131.87, 131.75, 79.77, 61.65, 51.65, 50.78, 49.12, 47.55, 45.59, 42.19, 40.79, 37.73, 28.32, 18.27. IR (NaCl plate): 3439.0, 3364.9, 2973.2, 2930.9, 2867.4, 1766.8, 1745.6, 1703.3, 1512.7, 1449.2, 1422.7, 1391.0, 1364.5, 1332.8, 1248.1, 1163.4, 1115.7, 1068.1, 1020.5, 972.8, 914.6, 729.4 cm$^{-1}$.

Poly(19). The reaction time was 26 h. $^1$H NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 5.40-5.59 (trans & cis, 2H, bm), 3.89-4.02 (trans & cis, 2H, bm), 3.74 (trans & cis, 3H, s), 2.66 (trans & cis, 2H, bs), 2.48 (trans & cis, 1H, bs), 1.57-1.98 (trans & cis, 2H, bm), 1.10-1.45 (trans & cis, 2H, bm). $^{13}$C NMR (CD$_2$Cl$_2$, 300 MHz) and IR (NaCl plate): same as for Poly(20) (vide infra).
Poly(20). \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 6.40, 6.03 (cis \& trans, 1H, bs), 5.18-5.55 (trans \& cis, 2H, bm), 3.88-4.08 (trans \& cis, 2H, bm), 3.72 (trans \& cis, 3H, s), 3.01, 2.65 (cis \& trans, 2H, bs), 2.47, 2.38 (cis \& trans 1H, bs), 2.17 (trans \& cis, 1H, bs), 1.94, 1.84 (cis \& trans, 1H, bs), 1.57-1.61 (trans \& cis, 1H, bm), 1.15-1.20 (trans \& cis, 1H, bm). \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 176.12, 175.49, 171.97, 171.16, 170.79, 135.54, 134.96, 133.87, 133.04, 131.78, 128.77, 52.46, 51.76, 51.17, 50.86, 49.43, 48.30, 43.62, 42.99, 42.36, 41.48, 37.75, 37.55, 37.01, 36.73, 36.36, 36.15. IR (NaCl plate): 3300.0, 3082.1, 2947.2, 2843.4, 1752.4, 1648.6, 1534.4, 1437.7, 1404.7, 1363.2, 1259.4, 1202.4, 1181.6, 1031.1, 968.9, 844.3, 797.6, 750.9, 704.2 cm\(^{-1}\).

Poly(21). [M]\(_0\) was 0.5 M. \(^1\)H NMR (CD\(_2\)Cl\(_2\), 400 MHz) \(\delta\) 5.33-5.40, 5.18-5.25 (trans \& cis, 2H, bm), 4.11-4.19 (trans \& cis, 2H, bm), 3.51-3.64 (trans \& cis, 18H, bm), 2.69-3.07 (cis \& trans, 2H, bm), 2.51-2.58 (trans \& cis, 1H, bm), 1.78-2.40 (trans \& cis, 2H, bm), 1.48-1.66 (trans \& cis, 1H, bm), 1.13-1.22 (trans \& cis, 1H, bm). \(^{13}\)C NMR (CDCl\(_3\), 300 MHz) \(\delta\) 175.74, 134.36, 133.41, 132.50, 131.92, 131.06, 128.38, 125.88, 72.53, 70.42, 70.15, 69.07, 63.28, 61.52, 49.92, 49.25, 47.30, 42.83, 41.85, 40.90, 36.91, 36.82. IR (NaCl plate): 3445.3, 2936.8, 2874.5, 1726.4, 1451.4, 1347.6, 1285.4, 1249.1, 1171.2, 1114.2, 968.9, 942.9, 875.5, 854.7 cm\(^{-1}\).

Poly(23). [M]\(_0\) was 0.6 M. \(^1\)H NMR (CD\(_2\)Cl\(_2\), 300 MHz) \(\delta\) 6.43-6.81 (trans \& cis, 1H, bs), 5.08, 5.31 (trans \& cis, 2H, bs), 3.93 (trans \& cis, 2H, bs), 3.67 (trans \& cis, 3H, bs), 2.76 (trans \& cis, 2H, bs), 2.36-2.60 (trans \& cis, 2H, bm), 2.09 (trans \& cis, 1H, bs), 1.82 (trans \& cis, 1H, bs), 1.15-1.43 (trans \& cis, 3H, bm). \(^{13}\)C NMR (CD\(_2\)Cl\(_2\), 300 MHz) \(\delta\) 176.10, 175.98, 171.19, 171.10, 133.66, 133.34, 132.58, 54.50, 52.66, 42.98, 44.27, 41.70, 35.14, 32.11, 28.23, 28.27, 25.76, 23.19, 14.42. IR (NaCl plate): 3301.9,
3073.5, 2921.2, 2856.0, 1753.7, 1650.4, 1536.2, 1438.3, 1405.7, 1373.1, 1210.0, 1188.2, 1035.9, 1003.3, 965.3, 845.6, 704.3, 671.6 cm\(^{-1}\).

**General Polymerization Procedure for RGD, SRN, and EO\(_5\) Containing Polymers.** *Method 1:* In a nitrogen-filled dry box, a solution of 1 in CH\(_2\)Cl\(_2\) was added to a solution of monomer in CH\(_2\)Cl\(_2\) to give an initial monomer concentration of 0.70 M. The initial [M]\(_0\)/[C]\(_0\) was 10/1 (homopolymers) or 20/1 (copolymers). The reaction mixture was stirred at room temperature for 4 h before quenching with ethyl vinyl ether and stirring for an additional 15-30 min before isolation.

*Method 2:* In a nitrogen-filled dry box, a solution of 2 or 3 in CH\(_2\)Cl\(_2\) was added to a solution of monomer in MeOH (1:1 CH\(_2\)Cl\(_2\):MeOH) in a dram to give an initial monomer concentration of 0.6 M (polymers containing RGD/SRN only) or 0.7 M (polymers containing EO\(_5\)). The dram was sealed and removed from the box. Within 10 min, the dram was placed in an oil bath at 55 °C and the solution was stirred for 2 h. The initial [M]\(_0\)/[C]\(_0\) was 10/1 (polymers containing RGD and/or SRN only) or 20/1 (polymers containing EO\(_5\)). The polymerization mixtures were cooled to room temperature, diluted, and ethyl vinyl ether was added. The solutions were stirred for an additional 15-30 min before isolation.

The polymers were precipitated into ether (26a), ether/CH\(_2\)Cl\(_2\) (1/3) (26b, 27b, 28a), ether/CH\(_2\)Cl\(_2\) (1/1) (27a), ether/MeOH (1/3) (28b), or MeOH (26c). The polymers were subjected to centrifugation, the solvent was removed and the solids dried under vacuum. The polymers were then characterized by \(^1\)H NMR spectroscopy and deprotected.
**General Deprotection Procedure.** For all polymers, except 27b, the following procedure was undertaken. A solution of TFA, triisopropylsilane (TIS), and H₂O (95/2.5/2.5) was added to the dried polymers to make a final concentration of 20 mL/g polymer. The mixtures were stirred for 2-7 h before precipitating into cold ether. The polymers were subjected to centrifugation, the solvent was removed, and the solids washed with cold ether (2 x 5 mL) before drying under vacuum. Polymer 27b was subjected for 1 h to 9.5 mL of condensed HF and 0.5 mL of p-cresol in the proper containment apparatus. The HF was removed *in vacuo* and the solid was washed with ether before drying under vacuum.

**Solubilization in Water.** The polymers (except for 26a and 30) were subjected to a minimum amount of 0.1 N NaOH for 10 min. Polymer 26a was already soluble in water, and polymer 30 was subjected to pH 8 phosphate buffer for 10 min. The polymers were then precipitated into MeOH, subjected to centrifugation, and dried under vacuum to yield the final polymers as tan powders (26c, 27b, 28b), glassy solids (26b, 27a, 28a), and a stiff gel (26a).

**Specific Methods and Data.** Data not reported in the text is reported below. ¹H NMR copolymer spectrum is the addition of the two homopolymer spectra. All peaks are broad. Characterization, except for GPC, of 26b and 27a was performed prior to treatment with base.

**26a.** Method 1 was followed. Additional purification was achieved before deprotection by solubilizing the polymer in ethanol and subjecting the solution to centrifugation using a Centriprep flask (MWCO=3000). The removal of the monomer was monitored by HPLC. The ethanol was removed *in vacuo*, and the polymer was dried
under vacuum. $^1$H NMR (CD$_3$OD, 400 MHz): $\delta$ 5.41-5.48, 5.23-5.30, 4.74-4.79, 4.51-4.55, 4.37-4.41, 4.19-4.22, 3.91-3.94, 3.79-3.82, 3.54-3.69, 3.20-3.23, 3.01-3.17, 2.94-3.06, 2.86, 2.60-2.73, 1.95-2.05, 1.66-1.73, 1.18-1.28. IR (KBr pellet): 3420.9, 2929.7, 2881.6, 1781.2, 1728.2, 1665.6, 1631.9, 1554.9, 1545.2, 1453.7, 1381.5, 1352.6, 1251.5, 1203.4, 1174.5, 1097.4, 967.4, 948.2, 885.6, 803.7, 697.8, 582.3, 514.8 cm$^{-1}$.

**26b.** Method 2 was followed. Characterization is identical to **26a** except the peaks vary in intensity.

**26c.** Method 2 was followed except that 3 was the initiator. $^1$H NMR (D$_2$O, 400 MHz) $\delta$ 5.20-5.48 (trans & cis, 2H, bm), 4.37 (trans & cis, 1H, bs), 3.76-3.99, 3.60-3.67 (trans & cis, 4H, bm), 3.17 (trans & cis, 2H, bs), 2.84-3.02, 2.57-2.65 (cis & trans, 5H, bm), 1.63-2.01, 1.15-1.42 (trans & cis, 8H, bm). IR (KBr pellet): 3320.7, 2936.8, 1664.1, 1534.4, 1399.5, 1300.9, 1249.0, 1134.9, 1025.9, 968.8, 865.1, 750.9, 683.5, 621.2 cm$^{-1}$.

**27a.** Method 2 was followed. $^1$H NMR (CD$_3$OD, 400 MHz): $\delta$ 5.38-5.49, 5.21-5.31, 4.71-4.78, 4.52-4.54, 4.44-4.49, 4.18-4.25, 3.79-3.81, 3.63-3.69, 3.56-3.58, 2.97-3.26, 2.53-2.82, 1.19-2.09, 1.64-1.77, 1.16-1.28. IR (KBr pellet): 3468.6, 2954.9, 2872.7, 1763.4, 1732.5, 1696.6, 1450.0, 1383.2, 1301.0, 1259.9, 1218.8, 1172.6, 1105.8, 1033.9, 900.3, 746.2, 699.9, 643.4, 602.3, 561.2 cm$^{-1}$.

**27b.** Method 2 was followed. $^1$H NMR (D$_2$O, 400 MHz) $\delta$ 5.24-5.49 (trans & cis, 2H, bm), 4.34-4.50 (trans & cis, 2H, bm), 3.79 (trans & cis, 2H, bs), 3.15 (trans & cis, 2H, bs), 2.54-2.76 (trans & cis, 5H, bm), 1.18-1.96 (trans & cis, 8H, bm). IR (KBr pellet): 3351.9, 2926.4, 2864.2, 1653.8, 1524.1, 1389.2, 1306.1, 1249.1, 1197.2, 1150.5, 1083.0, 891.0, 750.9, 600.5, 553.8 cm$^{-1}$. 

28a. Method 2 was followed. $^1$H NMR (CD$_3$OD, 400 MHz): δ 5.39-5.48, 5.21-5.31, 4.75-4.80, 4.52-4.54, 4.44-4.49, 4.37-4.41, 4.21, 3.94, 3.80-3.83, 3.63-3.72, 3.57, 2.99-3.26, 2.87, 2.53-2.82, 1.90-2.09, 1.62-1.78, 1.15-1.27. IR (KBr pellet): 3460.6, 2952.0, 2870.6, 1733.4, 1586.0, 1580.8, 1453.6, 1387.5, 1362.1, 1290.8, 1189.1, 1036.5, 899.2, 746.6, 700.8, 644.9, 604.2, 558.4 cm$^{-1}$.

28b. Method 2 was followed. $^1$H NMR (CD$_3$OD, 400 MHz): δ 5.26-5.48, 4.33-4.48, 3.80-3.98, 3.17, 2.86-3.02, 2.56-2.77, 1.56-2.05, 1.12-1.29. IR (KBr pellet): 3476.4, 2947.2, 2864.2, 1767.9, 1705.7, 1643.4, 1596.7, 1575.9, 1451.4, 1378.8, 1295.8, 1254.2, 1238.7, 1129.7, 1020.8, 901.4, 839.2, 745.8, 699.1, 642.0, 600.5, 599.0 cm$^{-1}$.

30. Method 2 was followed. $^1$H NMR (D$_2$O, 400 MHz) δ 6.06, 5.79 (trans & cis, 4H, bs), 4.96, 4.46 (cis & trans, 4H, bs), 4.18 (trans & cis, 4H, bs), 3.55-3.64 (trans & cis, 20H, bm), 3.34 (trans & cis, 4H, bs), 2.89 (trans & cis, 2H, bs), 2.35 (trans & cis, 4H, bs), 1.98 (trans & cis, 2H, bs), 1.87 (trans & cis, 4H, bs). IR (KBr pellet): 3428.8, 2905.9, 2461.7, 1802.4, 1702.3, 1384.6, 1372.0, 1168.8, 1076.8, 946.7, 856.6, 548.44, 515.7 cm$^{-1}$.

Polymer Stereoisomers

Using Initiator 1. The polymerizations of monomers 15, 20, and 21 with initiator 1 are described above.

Using Initiator 2. Monomers 15, 20, and 21 were polymerized with initiator 2 under identical conditions as with 1, except that the mixtures were heated in a sealed dram in a 55 °C oil bath during polymerization. The trans to cis ratios were determined
from the $^1$H NMR spectra by integrating the peaks corresponding to the olefinic protons of the trans and cis polymers.

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**References**

‡ Portions of this chapter have been published as: Maynard, H. D.; Okada, S. Y.; Grubbs, R. H. Macromolecules 2000, in press.


Poly(15) and poly(16) have been synthesized by Baigini, et al. (ref. 6a & b) using molybdenum catalysts; since ruthenium initiators were used in our study, the syntheses and characterization of these polymers using 1 as the initiator are included here.

Leahy, D. J.; Aukhil, I.; Erickson, H. P. Cell 1996, 84, 155-164.

This is thought to be due to unfavorable steric interactions between the monomer and initiating species, see ref. 6b.


Catalyst 1 is markedly less active than 2 and 3 at higher temperatures and is less stable at higher temperatures (see reference 17). For this reason, polymerizations with 1 were conducted at room temperature, while those with 2 and 3 were conducted at 55 °C.

This method could not be used with the other protected polymers due to the insolubility of these polymers in solvents suitable for centrifugation through the membrane.


Chapter 5

Inhibition of Cell Adhesion to Fibronectin by

Oligopeptide Substituted Polynorbornenes
Abstract

Polynorbornenes substituted with peptide sequences from the RGD-containing integrin cell binding domain of fibronectin are potent inhibitors of human dermal fibroblast cell adhesion to fibronectin. Ring-opening metathesis polymerization (ROMP) using Ru=CHPh(Cl)₂(PCy₃)(DHIMes) (1) as an initiator produced polymers substituted with GRGDS and PHSRN peptide sequences. The inhibitory activity was quantified for these polymers and compared to the free peptides. A homopolymer substituted with GRGDS was significantly more active than the free GRGDS peptide (IC₅₀ of 0.18 ± 0.07 and 1.08 ± 0.5 mM respectively), and the polymer containing both GRGDS and PHSRN was the most potent inhibitor (IC₅₀ of 0.03 ± 0.007 mM). These results demonstrate the applicability of ROMP to the synthesis of highly active inhibitors of cellular-extracellular matrix protein interactions.
**Introduction**

Soluble peptides containing the arginine-glycine-aspartic acid (RGD) sequence are known to duplicate or inhibit the cell-binding of the extracellular matrix protein fibronectin. As a result, this sequence has been targeted to provide treatments for diseases such as cancer. However, as discussed in Chapter 1, because RGD peptides have low affinities and short half-lives *in vivo*, the therapeutic use of this peptide has been limited. As a result, much effort has been directed towards the synthesis of RGD-containing materials and mimics that would have increased therapeutic potentials compared to the free peptides. One feasible way is to substitute RGD-containing materials with oligopeptides that enhance the cellular adhesion strength of the RGD sequence.

In Chapter 4, an excellent method to synthesize copolymers substituted with RGD and other oligopeptides by ring-opening metathesis polymerization (ROMP) using the newly developed initiator I was described. Specifically, homopolymers with GRGD and copolymers containing GRGD and SRN, the truncated synergy domain to RGD in fibronectin, were made. This chapter describes the synthesis of homopolymers and a copolymer substituted with the more active forms of the peptides contained in the integrin cell binding domain of fibronectin, GRGDS and PHSRN. The polymer substituted with GRGES, an inactive peptide, was also made as a control. The ability of these materials to inhibit human dermal fibroblast (HDF) cell adhesion to fibronectin is discussed.
Results and Discussion

Monomer and Polymer Synthesis and Characterization. Norbornene monomers substituted with oligopeptides were synthesized by solid-phase peptide chemistry. 5-Norbornene-exo-2-carboxylic acid was coupled to the amino terminus of peptides attached to a 4-carboxyltrityl linker resin using HBTU, HOBT and DIEA in DMF. The monomers were then cleaved from the resin using mildly acidic conditions to give the protected monomers. All of the monomers were soluble in organic solvents. In this way, norbornenes substituted with the protected integrin binding sequence,
GR(Pbf)GD(O'Bu)S(O'Bu)-OH (2), synergy sequence PH(Trt)S(Trt)R(Pbf)N(Trt)-OH (3), and inactive sequence GR(Pbf)GE(O'Bu)S(O'Bu)-OH (4) were synthesized in 91%, 97%, and 47% yield respectively. While 2 and 3 were >95% pure after cleavage from the resin, 4 contained an impurity which persisted in the final compound.

Scheme 1. Synthesis of homo- and copolymers with pendent bioactive oligopeptides.

Homopolymers of 2-4 and a copolymer of 2 and 3 were synthesized by ROMP using 1° as an initiator (Scheme 1). The monomers were heated in a sealed vial in a 1:1 mixture of CH₂Cl₂ and MeOH in an oil bath at 55 °C for 2 hours. The initial monomer concentration was 0.6 M and the initial monomer to catalyst ratio was 10/1. Residual monomers were removed during isolation by precipitation into mixtures of CH₂Cl₂ and ether. The protecting groups were then cleaved using either TFA or, for polymers containing PHSRN, HF to yield the unprotected peptides. The polymers were stirred in 0.1 N NaOH for 10 minutes to generate the sodium carboxylates. After isolation, the polymers were then rigorously purified by repeated centrifugation through a membrane.
followed by lyophilization resulting in the products as spongy, tann-colored materials.

In this manner, polynorbornene homopolymers substituted with GRGDS (5), PHSRN (6), and GRGES (7) were synthesized. The copolymer containing GRGDS and PHSRN (8) was also prepared. The over-all yields (Table 1) for the GRGDS and GRGES containing polymers were excellent (91% and 84% respectively). The yields of the polymers containing PHSRN were somewhat reduced (40% for 6 and 64% for 8). This could be due to the rapid degradation of 1 in the presence of this peptide.\textsuperscript{7} The percent of GRGDS incorporated into the copolymer was 49% (determined from the \textsuperscript{1}H NMR spectrum) which corresponded to the amount in the feed (50%).

<table>
<thead>
<tr>
<th>Peptide</th>
<th>%x in feed</th>
<th>%x in polymer\textsuperscript{b}</th>
<th>% yield overall</th>
<th>Mn (x10\textsuperscript{3})\textsuperscript{c}</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRGDS</td>
<td>100%</td>
<td>100%</td>
<td>91%</td>
<td>12.0</td>
<td>1.37</td>
</tr>
<tr>
<td>PHSRN</td>
<td>100%</td>
<td>100%</td>
<td>40%</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>GRGDS/PHSRN</td>
<td>50/50%</td>
<td>49/51%</td>
<td>64%</td>
<td>9.14</td>
<td>1.30</td>
</tr>
<tr>
<td>GRGES</td>
<td>100%</td>
<td>100%</td>
<td>84%</td>
<td>11.5</td>
<td>hs</td>
</tr>
</tbody>
</table>

\textsuperscript{a}General reaction conditions: CH\textsubscript{2}Cl\textsubscript{2}:MeOH (1:1) as the solvent, 55 °C for 2 hrs. in sealed vial, M/C=10/1, [M]\textsubscript{0} = 0.6 M. \textsuperscript{b}Calculated from \textsuperscript{1}H NMR spectra. \textsuperscript{c}Determined by GPC, pH 8.0 phosphate buffer eluent, poly(ethylene oxide) standards. ns = not soluble in water. hs = high molecular weight shoulder.

Polymers 5, 7, and 8 were readily soluble in water and phosphate buffered saline (PBS). Unfortunately, the homopolymer substituted with PHSRN was not completely soluble in aqueous solutions. The number-average molecular weights (M\textsubscript{n}) determined
by GPC (compared to poly(ethylene glycol) standards) were between 9,100 and 12,000. The polydispersity indexes (PDI’s) were narrow for 5 and 8 (1.37 and 1.30 respectively). The GPC trace of 7 exhibited a slight high molecular weight shoulder. As mentioned above, the monomer 4 contained an impurity which could have caused the observed molecular weight distribution.

**Inhibitory Activity of Polynorbornenes Substituted With Oligopeptides.** It was originally hypothesized that polymers substituted with many GRGDS peptides along the backbone would exhibit stronger cell-adhesive affinities than the free peptide due to multivalent interactions provided by the polymer scaffold.\(^8\)\(^9\) Also, since PHSRN enhances cell binding to the RGD peptide in fibronectin,\(^10\) it was predicted that a copolymer substituted with both GRGDS and PHSRN may exhibit higher activities than materials containing only GRGDS. To investigate this, the ability of the polymers and peptides to inhibit HDF cell adhesion to fibronectin was determined, following a known procedure.\(^1\) Briefly, normal HDF neonatal cells were added to human plasma fibronectin coated wells containing a certain concentration of the polymer and incubated at 37 °C for 1 hour. The cells were fixed with methanol, and the number of cells adopting a normal, well-spread morphology was estimated by counting a number of randomly selected fields viewed by phase contrast microscopy. The percent cell attachment was determined by comparing the experimental wells to control wells incubated with PBS alone.
Figure 1. Inhibition of normal HDF cell attachment to fibronectin by the polymers and free peptides. The HDF cells (35,000 cells/mL) were incubated in human plasma fibronectin coated wells at 37 °C for 60 min in the presence of increasing concentrations of soluble peptides or peptide substituted polymers. Adherent cells were fixed and counted by viewing a minimum of 6 randomly selected fields per well. Each point represents the average of 3 wells.

The inhibitory effects of polymers 5, 7, and 8 and peptides GRGDS, GRGES, and PHSRN are compared in Figure 1. As expected, GRGDS and GRGDS-containing
polymers 5 and 8 have an inhibitory effect. The inactive peptide GRGES$^{1bc}$ as well as PHSRN, which is known to be inactive unless covalently bound in the vicinity of the RGD containing peptide,\(^{10}\) have no effect.

The control polymer substituted with GRGES, 7, did not inhibit HDF adhesion to fibronectin. Cells spread normally and effectively in the presence of this peptide, as observed by phase contrast microscopy. This indicates that the polymer backbone is nontoxic to the cells and the inhibitory effect observed for polymers 5 and 8 is due solely to the peptides substituted along the backbone. In addition, the percent cell attachment levels off for both 5 and 6 rather than continuing to decline, providing further indication that the polymers are nontoxic within the concentrations used for this experiment.

![Graph](image)

**Figure 2.** IC$_{50}$ values for the GRGDS containing materials. Polymer concentrations are reported as the concentration of the GRGDS repeat unit. *Within a 95% confidence level, this value is statistically different from the other two.*
The inhibitory activity was quantified for GRGDS and 5 and 8. The IC₅₀ values, or concentration of GRGDS necessary to inhibit 50% of the cells from attaching, are represented in Figure 2. For GRGDS, an IC₅₀ of 1.08 ± 0.5 mM was obtained, which is within the literature ranges obtained for this peptide.³³⁻³⁵ Polymer 5 has a lower IC₅₀ of 0.18 ± 0.07 mM, and 8 is the most active with an IC₅₀ of 0.028 ± 0.007 mM. These values are all statistically different (p < 0.04 between each value).

Both GRGDS-containing polymers inhibited HDF adhesion to fibronectin at lower concentrations than did the free peptide. This indicates that multivalent peptide-integrin interactions provided by the polymer scaffold may be important. Similar behavior has been observed for polypeptides containing many RGD repeats⁹b and a synthetic polymer substituted with RGD peptides synthesized by radical chemistry.⁹a

Polymer 8 with GRGDS and PHSRN ligands is the most potent substrate studied. The polymer backbone provides the covalent linkage necessary to obtain the PHSRN-induced enhancement of RGD adhesive activity.¹⁰ In fibronectin, PHSRN is 30-40 Å away and on the same face of the protein as RGD.¹¹ One integrin can easily span this distance. The above results indicate that the polymer backbone also provides the correct orientation and spatial separation of these ligands to effect an enhancement of GRGDS binding to the fibroblast cellular integrins.

**Summary**

These results highlight the applicability of ROMP to the synthesis of polymers for the inhibition of cell adhesion. Homopolymers and copolymers are readily synthesized containing complex oligopeptide substituents such as GRGDS and PHSRN. Multivalent
interactions provided by the polymer scaffold enhance the inhibitory activity of the GRGDS containing polymers towards HDF cell adhesion to fibronectin. A copolymer with pendent GRGDS and PHSRN peptides is a potent inhibitor of cell binding, demonstrating that ROMP provides access to synthetic materials that can inhibit the activity of proteins such as fibronectin. Such materials may be used to modulate physiologically important integrin-extracellular matrix protein interactions and may be useful as drugs for disease related applications such as tumor therapy.

Experimental

Materials. 5-Norbornene-exo-2-carboxylic acid was synthesized according to a literature procedure. All peptide coupling agents were purchased from Novabiochem. Centriprep flasks were purchased from Millipore. The peptides were synthesized and purified by the Beckman Institute Biopolymers Synthesis Laboratory (California Institute of Technology). Methylene chloride used in the polymerization reactions was dried over CaH$_2$, degassed, and vacuum transferred before use. All other chemicals were purchased from Aldrich and used as received. Dulbecco’s modified eagle medium (DMEM) and all sterile cell culture reagents were purchased from GIBCO and used as received. The sterile flasks and 24-well plates coated with human plasma fibronectin were purchased from Becton Dickinson Labware. All other sterile culture materials were purchased from Falcon. The normal human dermal fibroblast (HDF) cells isolated from neonatal foreskin tissue of a single male donor were obtained from Clonetics.

Techniques. All operations were carried out under a dry nitrogen or argon atmosphere. Dry box operations were performed in a nitrogen-filled Vacuum
Atmospheres dry box. $^1$H NMR spectra were recorded on a JEOL GX-400 (399.65 MHz) spectrometer, or a Varian UnityPlus 600 (600.203 MHz) spectrometer as indicated. $^{13}$C NMR (75.49 MHz) spectra were recorded on a General Electric QE-300 spectrometer. Chemical shifts are reported downfield from tetramethylsilane (TMS). Infrared spectroscopy was performed on a Perkin Elmer Paragon 1000 FT-IR spectrometer using a film cast on a NaCl plate or a KBr pellet as indicated. High-resolution mass spectra were provided by the Southern California Mass Spectrometry Facility (University of California, Riverside). Aqueous gel permeation chromatography (GPC) were conducted using an HPLC system equipped with a Waters 515 HPLC pump, a Rheodyne model 7725 injector with a 200 µL injection loop, a Waters 2487 Dual λ absorbance detector, a Waters 2410 refractometer, and two TSK columns (TASK 3000PW, TSK 5000PW). The eluent was 0.1 M Na$_2$HPO$_4$ dibasic buffer, the flow rate was 1 mL/min, and poly(ethylene oxide)s were used as the calibration standard. The HPLC results were obtained on a Beckman 126 Solvent Module HPLC equipped with a 166 UV Detector and an Altech 18-LL column using a H$_2$O/CH$_3$CN solvent system (7% CH$_3$CN for 6 min, 7-90% CH$_3$CN over 38 min, and 90% CH$_3$CN for 8 min). Amino acid analysis was performed by the Beckman Research Institute in the Division of Immunology (City of Hope). All cell manipulations were performed in a sterile vertical laminar flow hood. Phase contrast microscopy was performed on an inverted Nikon Eclipse TE300 microscope with 200x magnification.

**Norbornene GR(Pbf)GD(t-Bu)S(tBu)-OH Monomer (2).** H$_2$N-G-R(Pbf)-G-D(O'Bu)-S(O'Bu)-resin (0.75 mmol peptide, 4-carboxytrityl linker Novasyne® resin) was placed in a flask containing a frit and stopcock. The resin was swelled in 20 mL of DMF
for 15 min and then rinsed with DMF (1 x 10 mL). In a vial, 0.14 g (1.01 mmol) of 5-norbornene-exo-2-carboxylic acid, 0.38 g (1.00 mmol) of 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and 0.14 g (1.04 mmol) of 1-hydroxybenzotriazole (HOBT) in 6.60 mL of DMF were agitated until all solids had dissolved. *N,N*-diisopropylethylamine (DIEA) was added (0.35 mL, 2.01 mmol) and the solution was agitated and added to the resin. Nitrogen was gently bubbled through the mixture for 2 h. The solution was removed, and the resin was then rinsed with DMF (5 x 10 mL), CH₂Cl₂ (5 x 10 mL), and MeOH (5 x 10 mL) and dried for 24 h at 30 millitorr. In a vial, 33 mL of acetic acid, CH₂Cl₂, and MeOH (5:4:1) were added to the dry resin and the vial was periodically swirled for 1.5-2 h. The solution was filtered to remove the resin, added to an excess of hex, and the solvent was removed *in vacuo*. The procedure was repeated to remove all of the acetic acid. The product was freeze-dried from benzene to give 0.73 g (91%) of 2 as an off-white solid. HPLC: single peak at 21.13 min. ¹H NMR (CD₂Cl₂, 600 MHz) δ 8.09 (1H, bs, G²NH), 7.58 (1H, bs, D¹NH), 7.49 (1H, bs, R¹NH), 7.41 (1H, bs, S¹NH), 7.23 (1H, bs, G¹NH), 6.27 (1H, bs, R₂), 6.02 (1H, s, Nor₁olefin), 5.98 (1H, s, Nor₂olefin), 4.67 (1H, bm, D₂α), 4.43 (1H, bm, S₂α), 4.32 (1H, bm, R₂α), 3.88 (2H, bm, G³α + G¹α), 3.77 (1H, bm, G¹α), 3.67 (1H, bm, S₂β), 3.54 (1H, bm, Sβ), 3.12 (1H, bm, Rα), 2.87 (2H, s, R(Pbf)CH₂), 2.82 (1H, s, Nor), 2.77 (1H, s, Nor), 2.73 (1H, bm, Dβ), 2.66 (1H, bm, Dβ), 2.45 (3H, s, R(Pbf)CH₃), 2.38 (3H, s, R(Pbf)CH₂), 2.08 (1H, bs, NorCHCO), 1.98 (3H, s, R(Pbf)CH₃), 1.80 (1H, bm, R), 1.72 (1H, bm, Nor), 1.61 (1H, bm, R), 1.50 (3H, bm, R + Nor), 1.36 (6H, s, R(Pbf)CH₂furan), 1.33 (9H, s, D₁Bu), 1.19 (2H, bm, NorBridge), 1.07 (9H, s, S₁Bu). NOESY cross peaks (CD₂Cl₂, 600 MHz) δ 8.09 (G₂NH), 4.32 (Rα); 7.58 (D₁NH), 3.88 (G₂α); 3.77 (G₂α); 7.49 (R₁NH), 3.88 (G₁α); 7.41 (S₁NH), 4.67 (Dα); 7.23 (G₁NH), 2.08
(Nor_{CHCO}). TOCSY cross peaks (CD$_2$Cl$_2$, 600 MHz) $\delta$ norbornene: 6.02, 5.98, 2.82, 2.77, 2.08, 1.72, 1.50, 1.19; G1: 7.23, 3.88; R: 7.49, 4.32, 3.12, 1.80, 1.61, 1.50; G2: 8.09, 3.88, 377; D: 7.58, 4.67, 2.73, 2.66; S: 7.41, 4.43, 3.67, 3.54. $^{13}$C NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 177.74, 173.32, 172.69, 171.15, 171.01, 158.92, 156.83, 138.26, 136.29, 132.52, 128.50, 125.08, 117.70, 86.79, 82.09, 74.13, 70.78, 61.69, 53.39, 53.08, 50.37, 47.65, 46.57, 44.39, 43.76, 42.06, 40.87, 37.84, 30.85, 29.48, 28.74, 28.21, 27.93, 27.51, 25.94, 19.54, 18.25, 12.66. IR (NaCl plate): 3320.7, 3061.2, 2967.8, 2926.3, 2864.1, 1726.4, 1653.7, 1544.8, 1456.6, 1363.2, 1249.0, 1155.6, 1098.5, 953.3, 849.5, 808.0, 709.4, 667.9, 564.1 cm$^{-1}$. HRMS (DCM/NBA/PPG) calcd for (MH)$^+$ 975.4861, found 975.4876.

**Norbornene P-H(Trt)-S(Trt)-R(Pbf)-N(Trt)-OH Monomer (3).** The same procedure as for 2 was followed with H$_2$N-P-H(Trt)-S(Trt)-R(Pbf)-N(Trt)-resin (0.75 mmol, 4-carboxytrityl linker Novasyn® resin), 0.14 g (1.01 mmol) of norbornene-exo-2-carboxylic acid, 0.38 g (1.00 mmol) of HBTU, 0.14 g (1.04 mmol) of HOBT, and 0.35 mL (2.01 mmol) of DIEA in 6.25 mL DMF to yield 1.24 g (97.3%) of 3 as a fluffy white solid. HPLC: single peak at 38.85 min. $^1$H NMR (CD$_2$Cl$_2$, 600 MHz) $\delta$ 8.29 (bs), 7.59-7.38 (bm), 6.89-7.29 (bm with maxima at 6.89, 7.04, 7.13, 7.22, 7.28, 7.29), 6.63 (bm), 5.98 (s), 5.84 (bm), 5.73 (bm), 4.45 (bm), 4.30 (bm), 4.13 (bm), 3.47-3.36 (bm), 2.93-2.65 (bm, with maxima at 2.65, 2.76, 2.84, 2.93), 2.34-2.43 (bm with maxima at 2.34, 2.40), 2.10 (bm), 1.98 (s), 1.93 (bm), 1.76 (bm), 1.63 (bm), 1.54 (bm), 1.34 (s), 1.22 (bm), 0.95 (bm). $^{13}$C NMR (CD$_2$Cl$_2$, 300 MHz) $\delta$ 174.74, 172.87, 171.27, 170.01, 158.49, 156.70, 144.51, 143.85, 141.86, 138.36, 136.19, 135.91, 132.32, 129.90, 128.49, 128.06, 127.32, 127.11, 126.92, 124.62, 120.31, 117.34, 87.20, 86.49, 70.66, 63.49, 61.24, 54.93, 51.09,
47.78, 46.95, 46.24, 45.79, 43.07, 41.98, 30.81, 30.06, 28.70, 25.35, 21.26, 19.51, 18.20, 12.68. IR (NaCl plate): 3317.7, 3054.7, 2970.5, 2917.9, 1669.8, 1622.5, 1548.8, 1491.0, 1443.6, 1417.3, 1264.8, 1096.4, 738.7, 696.6, 659.6. HRMS (MALDI) calcd for (MNa)r 1708.7737, found 1708.7739.

Norbornene G-R(Pbf)-G-E(O'Bu)-S(O'Bu)-OH (4). The same procedure as for 2 was followed with G-R(Pbf)-G-E(O'Bu)-S(O'Bu)-resin (0.25 mmol, 4-carboxytrityl linker Novasyn® resin), 0.14 g (1.01 mmol) of 5-norbornene-exo-2-carboxylic acid, 0.38 g (1.00 mmol) of HBTU, 0.14 g (1.04 mmol) of HOBT, and 0.35 mL (2.01 mmol) of DIEA in 6.60 mL DMF to yield 0.11 g (46.1%) of 4 (plus an impurity detected in the 1H NMR spectrum) as a white solid. HPLC: single peak at 24.35 min. 1H NMR (CD2Cl2, 600 MHz) δ 8.07 (1H, bs), 7.62-7.53 (3H, bm), 7.23 (1H, bs), 6.28-6.23 (2H, bm), 6.02 (1H, s), 5.99 (1H, s), 4.75 (1H, bm), 4.42 (1H, bm), 4.28 (1H, bm), 3.86 (3H, bm), 3.68 (1H, bm), 3.56 (1H, bm), 3.12 (1H, bm), 2.86 (2H, s), 2.82 (1H, s), 2.77 (1H, s), 2.46 (3H, s), 2.39 (3H, s), 2.36 (1H, bm), 2.30 (1H, bm), 2.08 (1H, bs), 1.98 (3H, s), 1.93 (2H, bm), 1.80 (1H, bm), 1.73 (1H, bm), 1.61 (1H, bm), 1.50 (3H, bm), 1.36 (6H, s), 1.33 (9H, s), 1.19 (2H, bm), 1.06 (9H, s). IR (NaCl plate): 3444.0, 3286.2, 2970.6, 1727.7, 1627.8, 1543.6, 1448.9, 1364.7, 1249.0, 1154.3, 1096.4, 733.4, 665.0, 570.3. HRMS (DCM/NBA/PPG) calcd for (MH)+ 989.5018, found 989.5018.

General Polymerization Procedure. In a nitrogen-filled dry box, a solution of 1 in CH2Cl2 was added to a solution of monomer in MeOH (1:1 CH2Cl2:MeOH) in a dram to give an initial monomer concentration of 0.6 M. The dram was sealed and removed from the box. Within 10 min, the dram was placed in an oil bath at 55 °C and the solution was stirred for 2 h. The initial monomer to catalyst ratios were 10/1. The
polymerization mixtures were cooled to room temperature, diluted, and ethyl vinyl ether was added. The solutions were stirred for an additional 15-30 min before isolation by precipitation into CH$_2$Cl$_2$:ether (3:1 for 5-7, 1:1 for 8). The polymers were subjected to centrifugation, the solvent was removed, and the solids dried under vacuum.

**General Deprotection Procedure.** For 5 and 7, a solution of TFA, triisopropylsilane (TIS), and H$_2$O (95/2.5/2.5) was added to the dried polymers to make a final concentration of 20 mL/g polymer. The mixtures were stirred for 2.5 h before precipitating into cold ether. The polymers were subjected to centrifugation, the solvent was removed, and the solids washed with cold ether (2 x 5 mL) before drying under vacuum. 6 and 8 were subjected for 1 h to 10 mL of condensed HF and 0.5 mL of p-cresol in the proper containment apparatus. The HF was removed *in vacuo* and the solid was washed with ether before drying under vacuum.

**Purification.** The polymers were subjected to a minimum amount of 0.1 N NaOH for 10 min, precipitated into MeOH, isolated by centrifugation, and dried under vacuum. Deionized, doubly distilled water was added to the polymers and the polymers purified by centrifugation through a membrane using Centiprep tubes with a molecular weight cut off (MWCO) of 3,000. This procedure was repeated a minimum of 5 times for each polymer. The polymer solutions were then subjected to centrifugation, the solution decanted to remove any insoluble particulate material, and lyophilized. The resulting polymers were all spongy, tan materials and were kept at -30 °C until use.

The polymers were characterized and the data not reported in the text is reported below. The 1H NMR copolymer spectrum is the addition of the two homopolymer
spectra. All peaks are broad, and unless otherwise stated, are the composite of the trans and cis polymer proton peaks.

**GRGDS Homopolymer (5).** $^1$H NMR (D$_2$O, 600 MHz): $\delta$ 7.41 (bm, NH), 7.36 (bm, NH), 7.29 (bm, NH) 5.24-5.45 (2H, bm), 4.68 (bm, D$_o$), 4.36 (1H), 4.26 (1H, bm), 3.94-4.02 (3H, bm), 3.84 (3H, bm), 3.20 (2H), 2.94 & 2.71 (cis & trans, 2H, bm), 2.65 (2H, bm), 1.90-1.97 (2H, bm), 1.76 (1H, bm), 1.64 (4H, bm), 1.23 (2H, bm). IR (KBr pellet): 3363.1, 2933.3, 1638.7, 1528.6, 1397.6, 1240.4, 1114.6, 1036.0, 967.9, 925.9, 611.5 cm$^{-1}$. AAA expected (found): Asp 1.00 (1.03), Ser 1.00 (0.90), Gly 2.00 (1.96), Arg 1.00 (1.11) residue/mol.

**GRGES Homopolymer (7).** $^1$H NMR (D$_2$O, 600 MHz): $\delta$ 7.43 (bm, NH), 7.37 (bm, NH), 7.29 (bm, NH) 5.29-5.46 (2H, bm), 4.38 (2H, bm), 4.27 (1H), 3.92-3.98 (3H, bm), 3.85 (3H, bm), 3.20 (2H, bm), 2.98 & 2.60-2.69 (cis & trans, 2H, bm), 2.27 (2H, bm), 2.13 (1H, bm), 1.94 (4H, bm), 1.76 (1H, bm), 1.64 (3H, bm), 1.23 (2H, bm). IR (KBr pellet): 3323.8, 2936.0, 1655.7, 1540.4, 1451.3, 1398.9, 1241.7, 1115.9, 1042.5, 979.7, 848.6, 670.5, 539.5 cm$^{-1}$. AAA expected (found): Ser 1.00 (1.04), Glu 1.00 (1.09), Gly 2.00 (2.15), Arg 1.00 (0.72) residue/mol.

**GRGDS/PHSRN Copolymer (8).** $^1$H NMR (D$_2$O, 600 MHz): $\delta$ 7.70, 7.31, 6.98, 5.42, 4.48, 4.37-4.40, 4.27, 3.97, 3.86, 3.18, 2.76, 2.71, 2.66, 2.18, 1.91, 1.75, 1.64, 1.28. IR (KBr pellet): 3342.1, 2933.3, 1596.7, 1444.8, 1387.1, 1313.7, 1245.6, 1046.5, 983.6, 925.9, 616.7 cm$^{-1}$. AAA expected (found): Asp + Asn 2.00 (2.04), Ser 2.00 (1.73), Pro 1.0 (1.07), Gly 2.00 (2.24), His 1.0 (0.97), Arg 2.00 (1.94) residue/mol.

**Cell Maintenance.** HDF cells were maintained in DMEM supplemented with 10% fetal bovine serum, 400 U/mL penicillin, and 400 mg/mL streptomycin in an
incubator at 37 °C and 4.9% CO₂. Subculturing was accomplished by rinsing the cells with HEPES buffered saline solution (HBSS), enzymatically removing the cells from the surface with trypsin, and neutralizing the trypsin with supplemented DMEM. The number of viable cells was assessed using a hemacytometer with trypan blue, and the cells were dispensed into 25 or 75 cm² flasks at a density of 3500 cells/cm².

**Sample Preparation.** Samples were weighed on a microbalance and solubilized in phosphate buffered saline (PBS) to a concentration 4x the desired final concentration. The pH of the solutions were determined and adjusted to ~7.4 as necessary. Phosphate buffered saline (PBS) alone was used in the control wells. The samples were filter (0.2 μm) sterilized before use.

**Competitive Inhibition Assay.** To 24-well plates coated with human plasma, fibronectin was added 100 μl of the polymer or peptide solution. HDF cells of passage 4-7 were harvested with trypsin as described above and resuspended to 35,000 cells/mL in unmodified DMEM. Cell suspensions were allowed to recover for at least 15 min before adding 300 μl to each well. The cells were evenly dispersed by gentle rocking of the plate before incubation for 60 min at 37 °C and 4.9% CO₂. The attached cells were fixed with methanol after removal of the sample solutions and washing with deionized H₂O. The cells were observed by phase contrast microscopy and counted in a minimum of 6 randomly selected fields per well. The percent maximum cell attachment is the average number of cells per view divided by the average number obtained for the control multiplied by 100. The IC₅₀ is the concentration where 50% of the maximum possible cells are attached.
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References


(7) Typically, ruthenium catalysts are unstable in the presence of primary and secondary amines, and PHSRN contains more amines than does GRGDS or GRGES. See, for example: Fu, G. C.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 9856-9857.


(11) Leahy, D. J.; Aukhil, I.; Erickson, H. P. *Cell* **1996**, *84*, 155-164.

Chapter 6

Purification Technique for the Removal of Ruthenium from Olefin Metathesis Reaction Products"
Abstract

Ring-closing metathesis (RCM) products of reactions utilizing \( \text{RuCl}_2(=\text{CHPh})(\text{PCy}_3)_2 \) (1) as a catalyst were successfully purified of unwanted ruthenium using a water-soluble coordinating phosphine, tris(hydroxymethyl)phosphine, \( \text{P(CH}_2\text{OH)}_3 \). Several simple and efficient purification procedures were compared for the isolation of the product of the RCM of diethyl diallylmalonate. The effectiveness of this procedure was also demonstrated for the isolation of an unsaturated analog of 12-crown-4 ether.
Introduction

Metal complexes are utilized in many organic transformations, including small molecule and polymer synthesis. However, removal of the metal complex after completion of the reaction can pose a serious problem during product purification, especially on an industrial scale. This residual metal can be problematic in subsequent transformations, as well as for storage and use of the material. Specifically, as described in Chapter 1, ruthenium catalysts such as benzylidene ruthenium complex 1, are commonly utilized in olefin metathesis reactions such as RCM, cross metathesis, or ring-opening metathesis polymerization (ROMP). However, it can prove very difficult to remove the highly-colored ruthenium complexes from the products of these reactions, and the residual ruthenium can cause problems such as olefin isomerization during distillation of the product, decomposition over time, and increased toxicity of the final material. In this chapter, a method is described to alleviate these problems by using a commercially available phosphine that facilitates the removal of ruthenium during product isolation.

Tris(hydroxymethyl)phosphine (2) is a water soluble and moderately air stable phosphine. Because of these properties, there have been an increasing number of reports on the use of 2 as a ligand for water-soluble transition metal complexes used as catalysts and for applications in medicinal chemistry. Recently, two ruthenium
complexes of 2 (See Figure 1) have been described. In these cases the phosphine readily coordinates to the ruthenium resulting in complexes that are soluble in water. These studies prompted the investigations into the use of 2 for the removal of residual ruthenium from olefin metathesis reaction products as described in this chapter.

![Figure 1. Water-soluble ruthenium complexes containing tris(hydroxymethyl)-phosphine.](image)

**Results and Discussion**

The RCM of diethyl diallylmalonate by ruthenium complex 1 and purification of the product using 2 was undertaken as shown in Scheme 1. It was observed that when the crude product was added to a solution of 2 and triethylamine in methylene chloride, the solution turned from a black/brown color to pale yellow within five minutes, indicating that 2 was coordinating to the ruthenium. Upon the addition of water, the yellow color moved into the aqueous phase leaving the methylene chloride phase colorless. $^1$H NMR spectroscopic studies indicated that all of the product remained in the methylene chloride phase and all of the phosphine moved to the aqueous phase (Figure 2).
Scheme 1. Purification of diethyl diallylmalonate RCM product with phosphine 2.

Figure 2. $^1$H NMR spectra for the RCM of diethyl diallylmalonate: A) CH$_2$Cl$_2$ layer containing only the product, 3,3-diethyl ester-pentene. B) H$_2$O layer containing both the phosphine 2 and triethylamine.
To quantify this observation a series of purification experiments were undertaken and the results given in Table 1. The amount of ruthenium in a 5 mg sample of the RCM product was determined by inductive-coupled plasma mass spectrometry (ICP-MS). All purification methods achieved a more than tenfold decrease in the amount of ruthenium remaining in the sample compared to the crude sample (entry 1). The results were similar whether 86 equivalents (entry 2) or 378 equivalents (entry 3) of 2 were used followed by a single aqueous wash. The amount of ruthenium in the product could be decreased by adding 86 equivalents of 2 followed by 3 aqueous washes (entry 4) or adding 2 to the methylene chloride layer three times, followed by an aqueous wash each time (entry 5). Because 2 is polar and is known to graft onto silica gel, a purification involving stirring a solution of the product, 2, and triethylamine in methylene chloride with an excess of silica gel (entry 6) was attempted. This method gave the best result and the amount of residual ruthenium in the sample was reduced to 1 μg in 5 mg of product.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Method</th>
<th>Ruthenium (μg/5 mg product)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>crude</td>
<td>74.6 ± 0.8</td>
</tr>
<tr>
<td>2</td>
<td>86 eq. 2, 1 H₂O wash</td>
<td>5.72 ± 0.07</td>
</tr>
<tr>
<td>3</td>
<td>378 eq. 2, 1 H₂O wash</td>
<td>5.84 ± 0.07</td>
</tr>
<tr>
<td>4</td>
<td>86 eq. 2, 3 H₂O washes</td>
<td>3.35 ± 0.07</td>
</tr>
<tr>
<td>5</td>
<td>86 eq. 2, 1 H₂O wash, repeated 3 times</td>
<td>3.56 ± 0.07</td>
</tr>
<tr>
<td>6</td>
<td>86 eq. 2, stir with silica gel, filter</td>
<td>1.03 ± 0.04</td>
</tr>
</tbody>
</table>

*Table 1. Amount of ruthenium in 5 mg product by various purification methods.a*

*aNumber of eq. of 2 based on added 1. In each case, 2 eq. Et₃N to 2 was used.*

Given that in the above experiments the same results were obtained by adding 86 equivalents or greater of 2, next we studied the minimum amount of 2 that would be
necessary to draw ruthenium into the aqueous phase. A series of experiments was undertaken to determine the net ICP-MS intensity of ruthenium 101 in the aqueous phase after adding a certain number of equivalents of 2 to the methylene chloride layer followed by one aqueous wash. There was a steep rise in the net intensity and thus amount of ruthenium in the aqueous phase between 1 and 10 equivalents of 2, followed by a leveling off of intensity (Figure 3). It appears that at least 10 equivalents of 2 is necessary to efficiently extract the ruthenium into the H₂O layer from the methylene chloride phase.

![Graph showing ICP-MS signal vs. equivalents of 2](image)

**Figure 3.** Ruthenium ICP-MS signal from aqueous phase versus equivalents of 2.
Scheme 2. Distillation of 3 results in an isomerization of the allyl ether to a vinyl ether to form 4. Species 4 reacts with catalyst 1 to form a ruthenium species inert to metathesis.

With this information, the methodology was extended to another example. As discussed in Chapter 3, the RCM of bisallyl triethylene glycol with 1 yields crown ether 3 which can be polymerized by ROMP to form a polyether. However, when the purification of 3 was attempted by distillation, 2-5% of the cyclic vinyl ether 4 formed (Scheme 2). This species reacts irreversibly with 1 to form RuCl₂(=CHOR)(PCy₃)₂, which is inactive for olefin metathesis reactions. When 4 is present in solution, the ROMP of 3 is inhibited. However, when 3 was pretreated with 2 and purified with a single aqueous wash, the ruthenium concentration was reduced from 80 to 8.8 μg per 5 mg 3 as determined by ICP-MS. In this case, a tenfold decrease in the ruthenium concentration was significant enough to inhibit the detrimental isomerization during distillation, thus eliminating the need for a more time-consuming purification of 3.
Summary

This chapter describes the removal of ruthenium from RCM reaction products, facilitated by the use of water-soluble phosphine 2 during the isolation process. An aqueous extraction or silica gel purification may be used. In the case of an aqueous extraction, 10 equivalents of 2 to ruthenium is adequate to move the ruthenium to the $H_2O$ phase. This method can be extended to the purification of other RCM products, such as unsaturated crown ethers.

Experimental

Materials. Tris(hydroxymethyl)phosphine was purchased from Strem and used as received. Diethyl diallylmalonate and the ruthenium standard were purchased from Aldrich. The crown ether substrate was synthesized as described in Chapter 2. Water used to make the ICP-MS samples was purified through a nanopure column after distillation. Methylene chloride ($CH_2Cl_2$) was rigorously degassed and passed through purification columns. Triethylamine and all other solvents were purchased from EM Science and used as received.

Techniques. $^1H$ NMR (399.65 MHz) spectra were taken on a JEOL GX-400 spectrometer. $^1H$ chemical shifts are reported downfield from tetramethylsilane (TMS). Inductive-coupled mass spectrometry data was obtained on an Elan 5000A using a 180 s rinse time followed by a 120 s wash delay.

General RCM procedure. To a solution of diethyl diallylmalonate (0.10 mL, 0.41 mmol) in $CH_2Cl_2$ (19 mL) was added a solution of 1 (17 mg, 0.02 mmol) in $CH_2Cl_2$ (2 mL). The mixture was stirred for 2 h at room temperature, ethyl vinyl ether ($\sim$0.1 mL)
was added, and the mixture stirred for an additional 30 min. The solvent and ethyl vinyl ether were evaporated to give the crude 3,3-diethylester-pentene product. $^1$H NMR δ 5.59 (s, 2H), 4.14 (q, 2H, J=7.2 Hz), 2.96 (s, 2H), 1.21 (t, 3H, J=7.7 Hz).

**Typical purification procedure.** A typical purification procedure is as follows: 3,3-Diethylester-pentene (100 mg, 0.47 mmol) in methylene chloride (0.5 mL) was added to a solution of 2 (29 mg, 2.36 mmol) and triethylamine (66 μg, 4.72 mmol) in methylene chloride (1.5 mL) and stirred for 10 min. Water (~2 mL) was added and the biphasic solution vigorously stirred for 15 min. The aqueous layer was separated and the methylene chloride removed *in vacuo* to isolate the product as a yellow oil.

**General quantification by ICP-MS.** Samples of approximately 5 mg were precisely weighed on a microbalance, digested overnight with concentrated nitric acid, and diluted to 1% nitric acid. The samples were filtered through 2 μm filters to remove any particulate matter. The samples were each measured 10 times by ICP-MS, and the intensities were obtained for ruthenium isotopes 99, 101, and 102. The intensity of pure 1% nitric acid was subtracted from the sample intensities to give the net intensities. To determine the actual concentration of ruthenium in the samples, the net intensities were compared to the net intensities obtained for the ruthenium standards. The standards were obtained by diluting a ruthenium standard of 980 μg/mL Ru in 5 wt.% HCl with 1% nitric acid to get 2.04, 1.49, 0.98, 0.47, 0.1, 0.05, and 0.01 μg/mL Ru standards where each sample contained less than 0.01 wt.% HCl. The numbers given indicate the average amount of ruthenium obtained for the three isotopes measured.
Acknowledgements

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References

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(8) Phosphine 2 was purchased from Strem Chemicals, but may be synthesized from \([\text{P(CH}_2\text{OH})_3]\text{Cl}\), see reference 4.

(9) Triethylamine was necessary to catalyze a reversal in CH₂Cl₂ of the hemiacetals that are formed during the synthesis of the phosphine. (For the synthesis of 2, see reference 5a.)

