Synthesis of Norbornenyl Polymers with Bioactive Oligopeptides by Ring-Opening Metathesis Polymerization

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ABSTRACT: Synthetic norbornenyl polymers with pendent 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) (EO_5) units attached directly or through ethyl and propyl spacers to various norbornenyl backbones were synthesized using $Ru{=}CHPh(Cl)_2(PCy_3)_2$ (**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 norbornene monomers with pendent EO_5 (**21**), GRGD (**24**), and SRN (**25**) units were made. Monomers **21** and **24** were copolymerized to form a poly(norbornene) containing 9.2 mol % GRGD (**26a**) using **1** as the initiator. However, incorporating larger amounts of GRGD resulted in extremely low yields of polymers that exhibited bimodal molecular weight distributions. Homopolymers and copolymers with larger amounts of GRGD and SRN were synthesized in good yields (32−92%) with monomodal molecular weight distributions using the newly developed, more active, 2,3-dihydroimidazolylidene initiators, $Ru{=}CHPh(Cl)_2(PCy_3)$(DHIMes) (**2**) and $Ru{=}CH{-}CH{=}C(CH_3)_2(Cl)_2(PCp_3)$(DHIMes) (**3**). In this way, EO_5 containing copolymers with 49 mol % GRGD (**26b**), 53 mol % SRN (**27b**), or 32 mol % GRGD and 21 mol % SRN (**28a**) were synthesized, as well as copolymer **28b** with 53 mol % GRGD and 47 mol % SRN. To alter the presentation of the GRGD, an EO_5 containing copolymer with a propyl spacer between the GRGD and the backbone (**30**) was also synthesized.

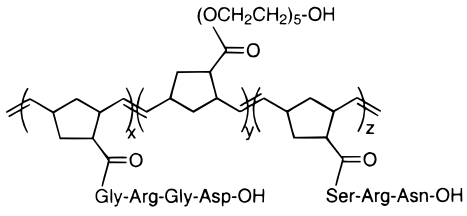
Introduction

Many extracellular matrix proteins bind to cell surface integrins through the short peptide sequence Arg-Gly-Asp (RGD).[1] 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.[2] For example, fibronectin and RGD containing peptides and mimics have been shown to have antimetastatic activity, and thus may be good prospects as drugs for tumor therapy.[3] Synthetic polymers containing pendent RGDs should be very useful in these applications, especially since multiple RGDs could lead to multivalent interactions and stronger binding.[4] In the protein fibronectin, the sequence Pro-His-Ser-Arg-Asn (PHSRN) enhances integrin binding by acting synergistically with RGD;[5] however, the majority of biomaterials containing RGD do not include this synergy site. Polymers containing both RGD and the synergy sequence, should have increased cell binding activity compared to materials with only RGD.

A synthetic polymer with RGD units linked to a poly(carboxyethylmethacrylamide) backbone has been reported and shown to have an increased therapeutic potential to cancer metastasis compared to the free peptide.[6] 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 would be difficult, 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 determined by the feed ratios of the monomers,[7] and the molecular weight is controlled by the initial monomer-to-catalyst ratio. Also, ROMP of strained systems 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 has already been proven to be a successful method to generate biologically active polymers. For example, glycopolymers have been synthesized that inhibit protein−carbohydrate recognition events.[8]

Our approach is to synthesize various synthetic polymers with pendent GRGD, SRN,[9] and/or penta(ethylene oxide) (EO_5) units by ROMP:



Polymers containing both GRGD and SRN have the advantages already discussed. Since it has been shown that surfaces of oligo(ethylene oxide) are protein resistant,[10] copolymers with EO_5 units were also made.

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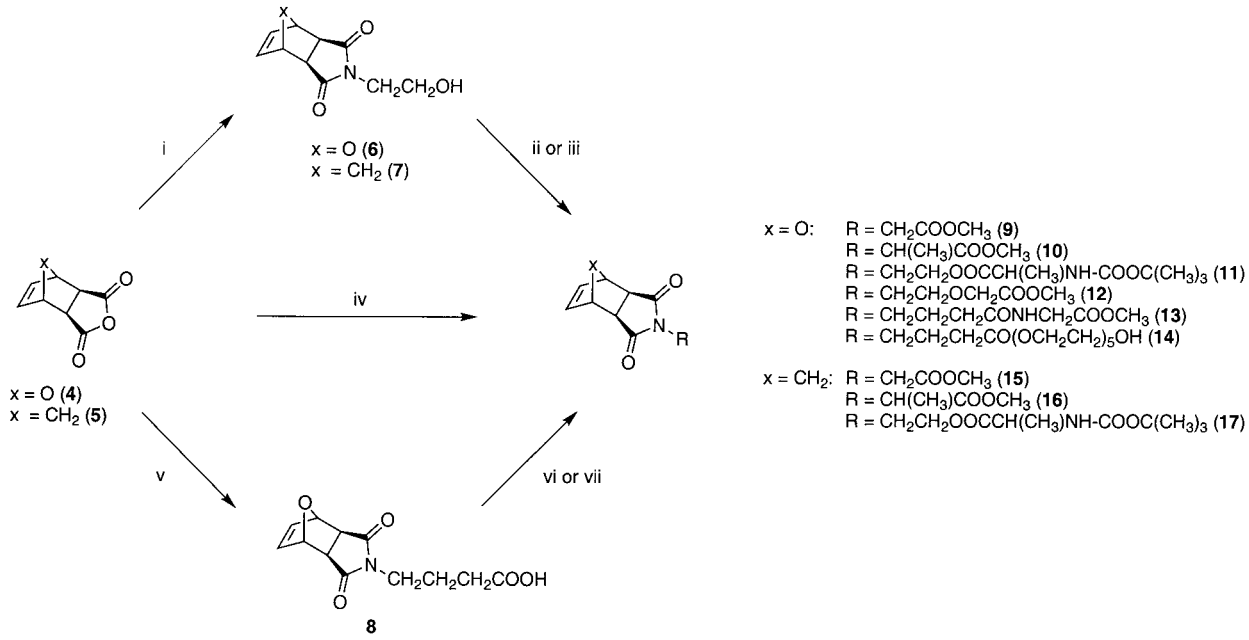
Scheme 1. Synthesis of Oxa- and Methylene-Bridged Imide Monomers 9−17[a]

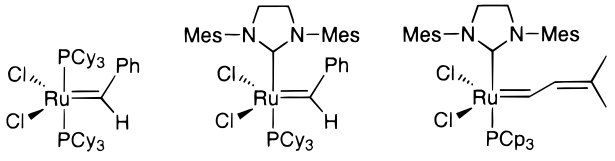


[a] Reaction conditions: i. $H_2NCH_2CH_2OH$ in THF/MeOH, 50 °C. ii. Boc-Ala-OH, DCC, and DMAP in CH_2Cl_2 (**11**, **17**). iii. $Br-CH_2COOCH_3$, K_2CO_3, and Bu_4NBr in DMF (**12**). iv. H-Gly-OCH_3·HCl or H−Ala-OCH_3·HCl and Et_3N in CH_2Cl_2, reflux (**9**, **10**, **15**, **16**). v. $H_2N(CH_2)_3COOH$ in THF/MeOH, 50 °C. vi. H-Gly-OCH_3·HCl, Et_3N, EDC, and HOBT in CH_2Cl_2 (**13**). vii. (a) Oxalyl Cl and DMF in CH_2Cl_2; (b) $H(OCH_2CH_2)_5OH$ and K_2CO_3 in THF, reflux (**14**).

These may be more biocompatible and water-soluble than polymers with only pendent peptides.

ROMP of monomers with pendent amino acids has been previously demonstrated. We have synthesized polyethers,[7] and others have synthesized norbornenyl[11] polymers with pendent amino acids. In these cases, the pendent groups were composed of one or two amino acids with alkyl side chains, containing no functionality. In addition, the norbornenyl polymers were synthesized with molybdenum catalysts which are less tolerant of functional groups than the ruthenium catalysts. To synthesize homo and copolymers with complex, biologically active groups using ruthenium based catalysts, we needed to explore the synthesis conditions, especially the choice of catalyst and monomer.

The polymers in this research were synthesized using well-defined ruthenium catalysts **1**−**3**. Catalyst **1** has



1 **2** **3**

Mes = mesityl
Cy = cyclohexyl
Cp = cyclopentyl

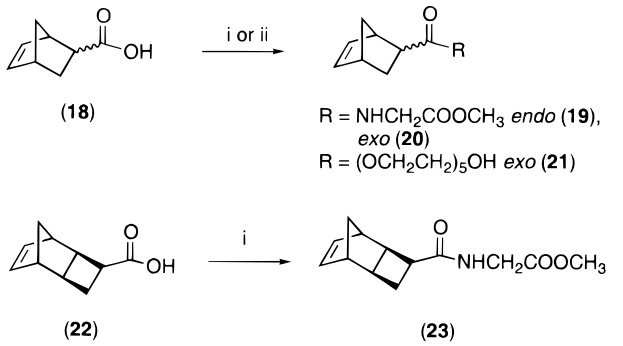
been extensively explored and has proven to be both active for ROMP and to be extraordinarily tolerant of functional groups.[12] We also recently reported catalyst **2** and **3**.[13] Although they have not yet been studied extensively in the ROMP reaction, these extremely active catalysts have been used with great success in ring-closing and cross metathesis reactions.[13] Due to the multitude of functional groups found in biologically relevant molecules such as GRGD and SRN, the utility of the dihydroimidazolylidene catalysts **2** and **3** were explored in addition to initiator **1**.

The synthesis and characterization of GRGD and SRN containing homo and copolymers is reported. Initially, polymers with different norbornenyl backbones and one pendent amino acid were synthesized. Out of the group of polymers studied, poly(5-norbornene-2-carboxyl) with a pendent glycine (poly(**20**)) demonstrated the best characteristics in terms of ease and yield of synthesis coupled with a narrow, monomodal molecular weight distribution. Accordingly, the fibronectin mimics were constructed with this backbone. GRGD (**24**), SRN (**25**), and penta(ethylene glycol) (**21**) norbornene monomers were made and polymerized, and the resulting homo and copolymers were deprotected and characterized. Initiators **1**, **2**, and **3** were compared in terms of yields, molecular weight distributions, and trans to cis ratios of the resulting polymers. Application of the more active initiators, **2** and **3**, was necessary to obtain high yields of polymers containing greater than 10 mol % pendent GRGD. Using the newly developed initiators **2** and **3**, polymers with potential biological activity containing both GRGD and SRN were synthesized.

Results and Discussion

Monomer Synthesis. A series of monomers with one pendent amino acid or EO_5 was synthesized in order to study the ROMP of these monomers. First glycine and alanine monomers **9**, **10**, **15**, and **16** were synthesized (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.[14] Oxa-bridged monomers **9** and **10** were made following a slightly modified literature preparation[15] 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 the

Scheme 2. Synthesis of Norbornene Monomers 19−21, 23[a]



[a] Reaction conditions: i. H-Gly-OCH_3·HCl, Et_3N, EDC, and HOBT in CH_2Cl_2 (**19**, **20**, **23**). ii. (a) Oxalyl Cl and DMF in CH_2Cl_2; (b) $H(OCH_2CH_2)_5OH$ and K_2CO_3 in THF, reflux (**21**).

oxa-bridged monomers were very low.[15] However, good yields of the methylene bridge monomers were obtained.

Norbornenes with a pendent glycine (**19** and **20**) or penta(ethylene glycol) (**21**) were synthesized (Scheme 2) in 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_2Cl_2 in 66% (**19**) and 73% (**20**) yields, respectively. The same procedure was followed to synthesize monomer **23** from **22** in 65% yield. The EO_5 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.

Next, the synthesis of monomers with ethyl and propyl spacer groups between the norbornenyl group and amino acid (Scheme 1) was undertaken. First, **4** and **5** were heated to 50 °C with 2-aminoethanol for 12 h in a mixture of THF and MeOH to give the resulting alcohols **6** and **7** in 36% and 44% yields, respectively. Then monomer **12** was synthesized in 49% yield by mixing **6** and methyl bromoacetate with potassium carbonate and tetrabutylammonium bromide in anhydrous DMF. Monomers **11** and **17** with pendent *N*-α-*t*-Boc-alanines were prepared 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.

Monomers **13** and **14** with propyl spacers were synthesized in decent yields from **8** (Scheme 1). The acid (**8**) was synthesized first in 41% yield by heating **4** and aminobutyric acid in THF and MeOH at 50 °C for 12 h. Monomer **13** was made as for **19** and **20** in 57% yield, and monomer **14** by using the same procedure as for **21** except the acid chloride was generated in situ using oxalyl chloride and base in a 36% overall yield.

Finally, the GR(Pbf)GD(O^tBu)−OH and S(O^tBu)R-(Pbf)N(Trt)−OH containing monomers (**24**, **25**, and **29**) were synthesized (Scheme 3). **24** and **25** were made by initially synthesizing the peptides on a 4-carboxyltrityl linker resin using standard Fmoc chemistry, followed by coupling **18** 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 with One Pendent Amino Acid: Synthesis and Characterization. Before synthesizing polymers containing the bioactive peptides, polymers with one pendent amino acid or EO_5 group were studied. These were synthesized by adding a solution of **1** in CH_2Cl_2 to a solution of monomer in CH_2Cl_2 to give an initial monomer concentration between 0.5 and 0.75 M. The mixtures were stirred vigorously for 15 min to 3 h 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 EO_5 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.

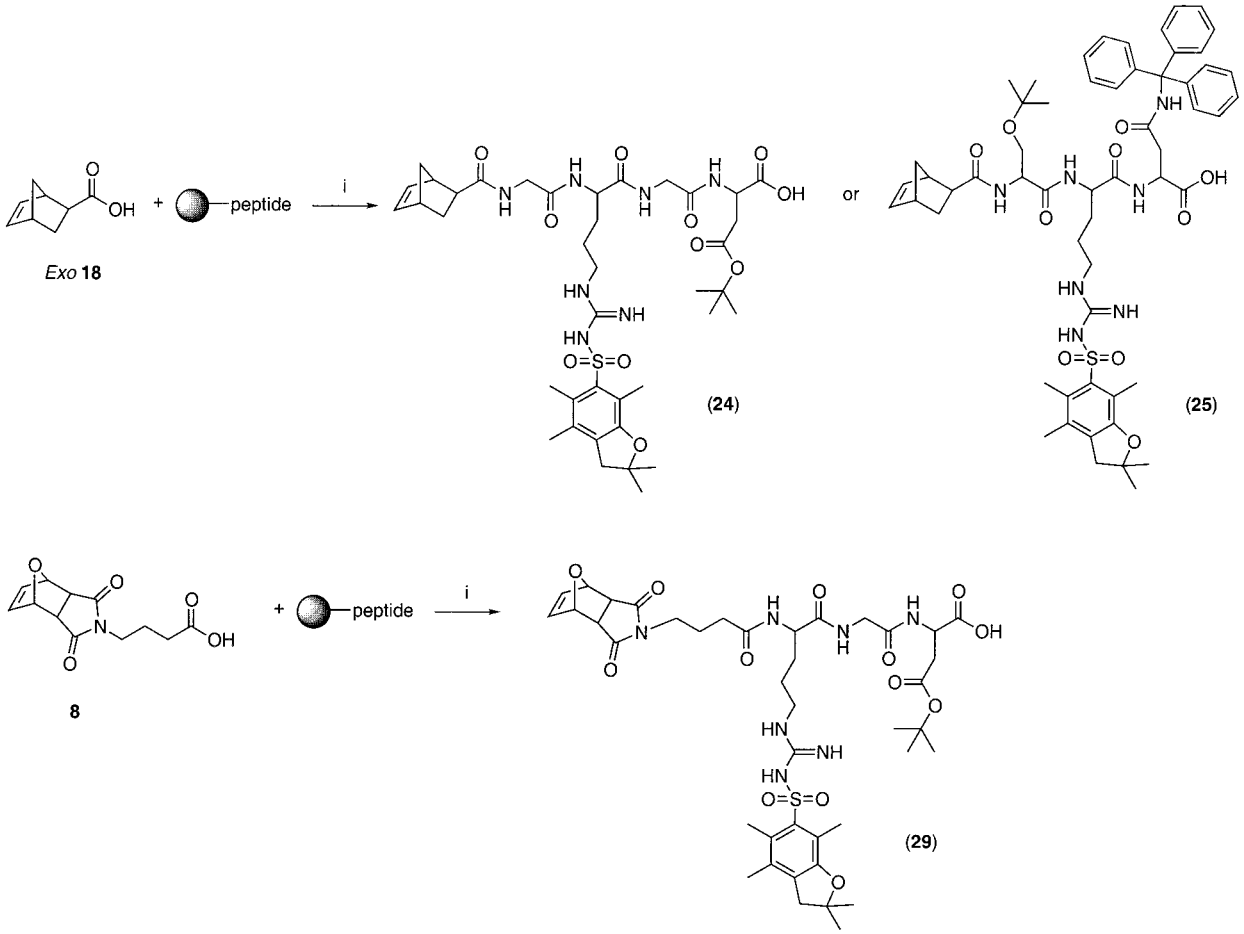
We began our study by synthesizing polymers based on monomers **9**, **10**, **15**, and **16** (Scheme 1) using initiator **1** (Table 1).[16] The monomers all reacted in 1 h or less giving excellent yields of polymer (82−95%). The polymers had glass transition temperatures between 147 and 158 °C. The number-averaged molecular weights (M_n) were between 17 700 and 108 000. The molecular weight distributions were narrow for poly(**9**) and poly(**16**) (1.19 and 1.10 respectively), broader for poly(**15**) (1.47), and bimodal for poly(**10**). The broad PDI obtained for poly(**15**) may be a result of catalyst decomposition which was detected by NMR 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 (CH_2Cl_2 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, although fairly broad for poly(**15**), 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.[17] 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 (see Table 1). Since monomer **19** could be made from commercially available starting materials, it was synthesized and polymerized first. However, the reaction took 24 h to reach 90% yield. This was not a surprising result since *endo* monomers often take longer to polymerize than the corresponding *exo* monomers.[18] *Exo* monomer **20** was then made and reacted quickly in 45 min to give a quantitative yield of polymer. The penta(ethylene glycol) monomer *exo*-**21**, also polymerized rapidly in 35 min. To determine if the polymer could be synthesized even faster, the highly strained monomer, **23**, was polymerized. However, this monomer was too strained and polymerized quickly (<5 min) and uncontrollably, resulting in an extremely broad molecular weight distribution (PDI of 29.0).

Poly(**20**) and poly(**21**) were characterized (Table 1). The M_n's were 10 900 ($[M]_0/[C]_0 = 50/1$) and 4610 ($[M]_0$/

Scheme 3. Synthesis of G−R(Pbf)-G−D(OtBu)-OH and S(OtBu)-R(Pbf)-N(Trt)-OH Containing Monomers[a]



[a] Reaction conditions: i. (a) HBTU, HOBT, and DIEA in DMF; (b) AcOH:CH_2Cl_2:MeOH (5:4:1).

Table 1. Polymerization Data for Norborneyl Monomers with Pendant Amino Acids[a]

polymer	yield (%)	$[M]_0/[C]_0$	time (min)	M_n ($\times 10^3$)[b]	PDI[b]	T_g (°C)[c]	trans/cis[d]	carbene (ppm)[d]	free PCy_3?[e]
poly(**9**)	82	100/1	30	17.7	1.19	147.6	2.4	18.67	no
poly(**10**)	83	100/1	60	97.5	bimodal				
poly(**15**)	90	100/1	35	46.3	1.47	158.1	5.2	19.48, 18.58	yes
poly(**16**)	95	100/1	60	108	1.10	154.1	5.5	19.41	no
poly(**20**)	>99	50/1	45	10.9	1.15	88.0	2.4	19.02, 18.94, 18.741	yes
poly(**21**)	>99	36/1	35	4.61	1.12	−48.9	3.6	19.07, 19.02, 18.72	yes
poly(**23**)	>99	100/1	<5	1.00	29.0		2.9		

[a] General reaction conditions: CH_2Cl_2 as the solvent, room temperature, **1** as the initiator, $[M]_0 = 0.5-0.75$. [b] All determined by GPC, CH_2Cl_2 eluent, poly(styrene) standards except poly(**20**) with DMF as the eluent, poly(ethylene glycol) standards. [c] Detemined by DSC, 10 °C/min, second heat reported. [d] Determined from ^{1}H NMR. [e] Determined from ^{31}P NMR.

$[C]_0 = 35/1$) for poly(**20**) and poly(**21**), respectively, and the PDI's 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 vs 31 °C[19]). The flexible penta(ethylene glycol) units resulted in a more disordered polymer with a low T_g of −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 acid, 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 1) polymerized in 2.5 h and **13** and **14** (Scheme 1) in 30 min 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 vs 147.6 °C). This is due to the extra disorder created by the flexible alkyl spacer group. Similar to poly(**21**), the penta(ethylene glycol) substituents 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 PDI. The reason for this bimodal molecular weight distribution is unknown; only one propagating species was observable by ^{1}H NMR. We attempted to saponify monomer **12** to yield the carboxylic acid

Table 2. Polymerization Data for Norbornenyl Monomers with Pendent Amino Acids Attached through Spacer Units[a]

polymer	yield (%)	$[M]_0/[C]_0$	time (min)	M_n ($\times 10^3$)[b]	PDI[b]	T_g (°C)[c]	trans/cis[d]	carbene (ppm)[d]	free PCy3?[e]
poly(**12**)	>99	75/1	150	66.2	1.31	86.1	2.3	18.70	no
poly(**13**)	81	50/1	30			74.5	2.8	18.68	no
poly(**14**)	>99	50/1	30	118	bimodal	−29.9	2.9	18.69	no
poly(**11**)	>99	100/1	90	51.9	1.07	96.8	2.3	18.68	no
poly(**17**)	95	100/1	80	55.5	1.11	90.3	5.5	19.42, 19.20	yes

[a] General reaction conditions: CH_2Cl_2 as the solvent, room temperature, **1** as the initiator, $[M]_0 = 0.5-0.75$. [b] All determined by GPC, CH_2Cl_2 eluent, poly(styrene) standards except poly(**20**) with DMF as the eluent, poly(ethylene glycol) standards. [c] Detemined by DSC, 10 °C/min, second heat reported. [d] Determined from ^{1}H NMR. [e] Determined from ^{31}P NMR.

Table 3. Polymerization Data for the Norbornene Monomers with Pendent Oligopeptide and EO_5 Units[a]

				mol % in feed			mol % in polymer[c]						
polymer	catalyst	% yield overall	$[M]_0/[C]_0$	GRGD	SRN	EO_5	GRGD	SRN	EO_5	M_n ($\times 10^3$)[a]	PDI[d]	T_g (°C)[e]	trans/cis[d]
23a[b]	**1**	78	20/1	10	0	90	9.2	0	90.8	18.7	1.13	−30.7	4.76
26b	**2**	81	20/1	50	0	50	49	0	51	15.9[f]	1.13	52.6	0.59
26c	**3**	78	10/1	100	0	0	100	0	0	13.3	1.32	98.0	
27a	**2**	92	20/1	0	52	48	0	53	47	17.2[g]	1.21	62.3	0.63
27b	**2**	74	10/1	0	100	0	0	100	0	10.7	1.70	131.2	
28a	**2**	59	20/1	25	25	50	32	21	47	13.3	1.21	39.4	0.63
28b	**2**	32	10/1	50	50	0	53	47	0	11.8	1.26	104.6	

[a] General reaction conditions: CH_2Cl_2:MeOH (1:1) as the solvent, 55 °C for 2 h in sealed vial, $[M]_0 = 0.6-0.7$ M. [b] Reaction conditions: CH_2Cl_2 as the solvent, room temperature for 4 h, $[M]_0 = 0.7$ M. [c] Calculated from ^{1}H NMR. [d] Detemined from GPC, pH 8.0 phosphate buffer eluent, poly(ethylene oxide) standards. [e] Determined from DSC, 10 °C/min, second heat reported. [f] 24% of EO_5 remaining. [g] 35% of EO_5 remaining.

functionality. But, similar to **15**, saponification resulted in the regeneration of the **6**.

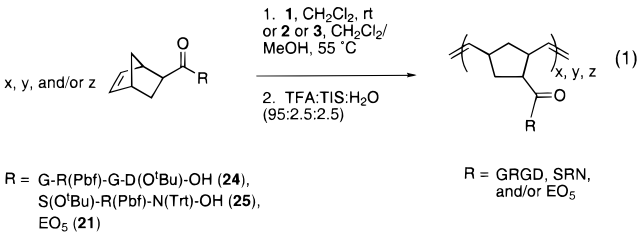
Polymers with pendent Boc-protected alanines, poly(**11**) and poly(**17**) were synthesized in less than 2 h in >99% and 95% yields respectively (Table 2). The molecular weight distributions were monomodal for both poly(**11**) and poly(**17**), and the molecular weights were similar (M_n of 51 900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

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, peptides coupled to the polymer backbone through the amino terminus were desired. 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, these polymers may be useful in such applications as gene therapy.

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** (Scheme 1) only one propagating carbene was observed at roughly the same shift (between 18.67 and 18.70 ppm). The methylene-bridged monomer **16** (Scheme 1) 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** (Scheme 1) 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** (Scheme 2) 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, and 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.[20] 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 Substituted with Biologically Relevant Oligopeptides: Synthesis and Characterization. Polymers with pendent GRGD, SRN, and EO_5 were first synthesized using initiator **1** for 4 h at room temperature in CH_2Cl_2 with an initial monomer concentration of 0.7 M (eq 1). In this way, monomers **21** (Scheme 2) and **24** (Scheme 3) 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 overall 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.



(1)

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.[21] However, in these examples and in ring-closing metathesis and cross-metathesis, **2** and **3** were more active than **1** both at room temperature and at higher temperatures.[13,21] Also, **2** and **3** were inter-changeable with each other at higher temperatures.[21] On the basis of these initial studies it was hoped that these initiators would result in higher yields of the desired polymers.

Hompolymers and copolymers of **21**, **24**, and **25** were synthesized (eq 1) using **2** or **3** as an initiator by heating the monomers in a 1:1 mixture of CH_2Cl_2 and MeOH in an oil bath at 55 °C for 2 h 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.[22] 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 % EO_5) 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 ^{1}H NMR spectrum after purification of the 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 min. 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 *tert*-butyl groups were removed. However, all of the protecting groups of this polymer, including the *tert*-butyl groups were cleaved using HF. The use of HF may be circumvented by changing the serine protecting group to the labile trityl group.

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 min to generate the sodium salt of the carboxylate 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 overall yields (after polymerization, cleavage, and solubilization into water) of the polymers were all between 59 and 92%, except for that of polymer **28b** (32%) (see Table 3). The low yield of **28b** was primarily due to polymer loss during the initial precipitation from the crude reaction solution. ^{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_2Cl_2/ether and MeOH/ether. The choice and relative amount of each mixture was different depending on the polymer. Polymer **26a** was also purified by Centriprep (MWCO 3000) using ethanol as the solvent.[23] Regardless of the purification method, except for **28b**, the yields were all good to excellent.

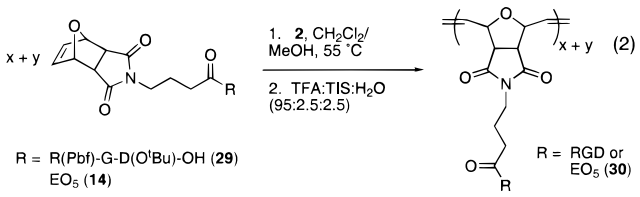
The GPC results are given in Table 3. As desired, the number-averaged molecular weights were are fairly low between 10 700 and 18 700, and most importantly, the samples demonstrated monomodal molecular weight distributions. Copolymer **27b** had the broadest PDI value (1.70). This was the only polymer 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 narrow PDI's between 1.13 and 1.32. Remarkably, the trimonomer copolymer, **28a**, had a narrow PDI of 1.21. This result indicates that synthesizing more complex copolymers with three or even more monomers is possible so that drugs, for example, may also be incorporated into the polymers. The narrow PDI's also indicate that the synthesis of block copolymers may be possible.

The T_g values of the copolymers varied depending on the identity and amounts of the substituents. For example, copolymers **26a** and **26b** had T_g values of −30.7 and +52.6 °C between that of homopolymers poly(**21**) at −48.9 °C and **26c** at 98.0 °C. Copolymer **27a** had a T_g of 62.3 °C while homopolymer **27b** had one at 131.2 °C. The trimonomer copolymer, **28a**, with 53 mol % total peptide, had a lower T_g (39.4 °C) than either of the dimonomer copolymers **26b** and **27a**, which contained 49 and 53 mol % peptide, respectively. The presence of the third monomer introduces extra disorder. In addition, the GRGDS/PHSRN copolymer, **28b**, had a T_g of 104.6 °C, which is close to that of the GRGD containing homopolymer, **26c**. The SRN and RGD 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**) had slight excesses of cis olefins (trans/cis = 1/1.6−1/1.7, see Table 3). (Polymers **26c**, **27b**, and **28b** were not soluble in CD_3OD, and the olefin peaks of the isomers were not resolved in D_2O.)

To examine this further, monomers **15** (Scheme 1), **20**, and **21** (Scheme 2) 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 trans to 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 trans to cis ratio of 2.4/1 and **2** resulted in the reverse with a trans to cis ratio of 1/2.3. Similarly, for **21**, initiator **1** resulted in a polymer with a trans to cis ratio of 3.6/1 and **2** with a trans to 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 trans polymers. The reason for this is under investigation.



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,[17] 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 copolymerized to form **30**, a polymer with a propyl spacer between the backbone and pendent RGD and EO_5 units (eq 2). 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.

Polymer **30** was synthesized as previously described using **2** as the initiator with a $[M]_0/[C]_0 = 10/1$ (eq 2). The yield was approximately 90% by ^1H NMR. 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 ($[M]_0/[C]_0 = 50/1$) 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 °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, polymer with spacer units can be synthesized.

Conclusions

Norbornenyl polymers with pendent 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% GRGD were also made with this initiator. However, the synthesis of polymers containing larger amounts of the bioactive peptides required the use of the more active 2,3-dihydroimidazolylidene initiators **2** and **3**. This is an example where the use of these more active catalysts is essential. The polymers containing GRGD and SRN have potential applications in a variety of fundamental research studies of the binding of integrins to proteins and for many disease-related applications such as in tumor therapy. Biological studies of these polymers are underway.

Experimental Section

Materials. 5-Norbornene acid-*endo*-2-carboxylic acid (*endo*-**18**) was purchased from Aldrich. 5-Norbornene acid-*exo*-2-carboxylic acid (*exo*-**18**)[24] and acid chloride[25] as well as **5**[14] were synthesized according to literature procedures. 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 CaH_2, 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. Drybox operations were performed in a nitrogen-filled Vacuum Atmospheres drybox. 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 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). ^{31}P NMR spectra were recorded on a JEOLGX-400 (161.85 mHz) spectrometer referenced to an external 85% H_3PO_4 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. High-resolution mass spectra were provided by the Southern California Mass Spectrometry Facility (University of California, Riverside). Gel permeation chromatographs with CH_2Cl_2 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 μm mixed bed columns, a Knauer differential refractometer, and poly(styrene) as the calibration standard. Aqueous GPC (0.1 M Na_2HPO_4 dibasic buffer) or DMF (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-(*exo*-3,5-Dioxo-10-oxa-4-azatricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)butyric Acid 2-(2-(2-[2-(2-Hy-

droxyethoxy)ethoxy]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 CH_2Cl_2 following a literature procedure.[25] 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, CH_2Cl_2 (50 mL) was added, and the organic layer was washed with H_2O (three times). The organic layer was dried over $MgSO_4$, 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. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.51 (2H, s), 5.24 (2H, s), 4.21 (2H, t, 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). ^{13}C NMR (CD_2Cl_2, 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/NH_3): calculated for $(MNH_4)^+$, 489.2448; found, 489.2460.

Bicyclo[2.2.1]hept-5-ene-*exo*-2-carboxylic Acid 2-(2-(2-[2-(2-Hydroxyethoxy)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, H_2O was added and the mixture was neutralized with 10% citric acid. The aqueous layer was washed with CH_2Cl_2 (3 times), the organic layers were then consolidated, dried over $MgSO_4$ 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. ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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/NH_3): calculated for $(MH)^+$, 359.2070; found, 359.2082.

Norbornene G−R(Pbf)−G−D(O^{t}Bu)−OH Monomer (24). H_2N−G−R(Pbf)−G−D(O^{t}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 × 10 mL). In a vial, 0.28 g (2.00 mmol) of *exo*-**18**, 0.76 g (2.00 mmol) of 2-(*1H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and 0.27 g (2.00 mmol) of 1-hydroxybenzotriazole (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 × 10 mL), CH_2Cl_2 (5 × 10 mL), and MeOH (5 × 10 mL) and dried for 24 h at 30 milliTorr. In a vial, 33 mL of acetic acid, CH_2Cl_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_2Cl_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_2Cl_2, 600 MHz): δ 7.89 ($G2_{NH}$), 4.38 (R_α); 7.50 (D_{NH}), 3.96 ($G2_\alpha$), 3.75 ($G2_\alpha$); 7.42 (R_{NH}), 3.87 ($G1_\alpha$); 7.06 ($G1_{NH}$), 2.07 (norbornene). TOCSY cross-peaks (CD_2Cl_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, 375; D, 7.50, 4.65, 2.71. ^{13}C NMR (CD_2Cl_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): calculated for $(MNa)^+$, 854.3735; found, 854.3707.

Norbornene S(O^{t}Bu)−R(Pbf)−N(Trt)−OH Monomer (25). The same procedure as for **24** was followed with H_2N−S(O^{t}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_2Cl_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_2Cl_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. ^{13}C 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): calculated for $(MNa)^+$, 1068.4881; found, 1068.4873.

R(Pbf)−G−D(O^{t}Bu)−OH Monomer (29). The same procedure as for **24** was followed with H_2N−R(Pbf)−G−D(O^{t}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_2Cl_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_2Cl_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_2Cl_2, 300 MHz): δ 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): calculated for $(MH)^+$, 888.3813; found, 888.3778.

Polymer Synthesis. General Synthesis for Polymers with Pendent EO_5. In a nitrogen-filled drybox, a solution of **1** in CH_2Cl_2 was added to a solution of monomer CH_2Cl_2 (or CD_2Cl_2 for NMR reactions) to give an initial monomer concentration of 0.5−0.55 M. The initial $[M]_0/[C]_0$ was 50/1 (**14**) or 36/1 (**21**). The reaction mixture was stirred at room temperature for 30−35 min before quenching with ethyl vinyl

ether and stirring for an additional 15−30 min. The polymers were precipitated into ether, stirred for 15 min, and subjected to centrifugation. The solvent was removed and the solids dried under vacuum. The polymers were viscous oils. (Data not reported within the text is reported below.)

Poly(14). ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 6.05, 5.79 (trans and cis, 2H, bs), 4.96, 4.45 (cis and trans, 2H, bs), 4.17 (trans and cis, 2H, bs), 3.51−3.62 (trans and cis, 20H, bm), 3.33 (trans and cis, 2H, bs), 2.33 (trans and cis, 2H, bs), 1.86 (trans and cis, 2H, bs). ^{13}C NMR (CD_2Cl_2, 300 MHz): δ 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, 2302.0, 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(21). ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 5.33−5.40, 5.18−5.25 (trans and cis, 2H, bm), 4.11−4.19 (trans and cis, 2H, bm), 3.51−3.64 (trans and cis, 18H, bm), 2.69−3.07 (cis and trans, 2H, bm), 2.51−2.58 (trans and cis, 1H, bm), 1.78−2.40 (trans and cis, 2H, bm), 1.48−1.66 (trans and cis, 1H, bm), 1.13−1.22 (trans and cis, 1H, bm). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 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}.

General Polymerization Procedure for RGD, SRN, and EO_5 Containing Polymers. Method 1. In a nitrogen-filled drybox, a solution of **1** in CH_2Cl_2 was added to a solution of monomer in CH_2Cl_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 drybox, a solution of **2** or **3** in CH_2Cl_2 was added to a solution of monomer in MeOH (1:1 CH_2Cl_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_2Cl_2 (1/3) (**26b**, **27b**, **28a**), ether/CH_2Cl_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 H_2O (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 × 5 mL) before drying under vacuum. Polymer **27b** was 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.

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. ^{1}H 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 (3000 MWCO). 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_3OD, 400 MHz): δ 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_2O, 400 MHz): δ 5.20−5.48 (trans and cis, 2H, bm), 4.37 (trans and cis, 1H, bs), 3.76−3.99, 3.60−3.67 (trans and cis, 4H, bm), 3.17 (trans and cis, 2H, bs), 2.84−3.02, 2.57−2.65 (cis and trans, 5H, bm), 1.63−2.01, 1.15−1.42 (trans and 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_3OD, 400 MHz): δ 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_2O, 400 MHz): δ 5.24−5.49 (trans and cis, 2H, bm), 4.34−4.50 (trans and cis, 2H, bm), 3.79 (trans and cis, 2H, bs), 3.15 (trans and cis, 2H, bs), 2.54−2.76 (trans and cis, 5H, bm), 1.18−1.96 (trans and 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_3OD, 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, 1646.9, 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_3OD, 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_2O, 400 MHz): δ 6.06, 5.79 (trans and cis, 4H, bs), 4.96, 4.46 (cis and trans, 4H, bs), 4.18 (trans and cis, 4H, bs), 3.55−3.64 (trans and cis, 20H, bm), 3.34 (trans and cis, 4H, bs), 2.89 (trans and cis, 2H, bs), 2.35 (trans and cis, 4H, bs), 1.98 (trans and cis, 2H, bs), 1.87 (trans and 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}.

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Supporting Information Available: Text giving experimental procedures and full characterization of **6**−**8**, monomers **9**−**13**, **15**−**17**, **19**, **20**, **23**−**25**, and **29**, and polymers poly(**9**), poly(**10**), poly(**11**), poly(**12**), poly(**13**), poly(**15**), poly(**16**), poly-

(**17**), poly(**19**), poly(**20**), and poly(**23**), as well as experimental procedures for the polymer stereoisomer studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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