Synthesis and Biological Applications of Oligopeptide-Substituted Polynorbornenes

Thesis by
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Chapter 1

Synthesis of Peptide-Substituted Norbornenyl Polymers as Fibronectin Mimics by Ring-Opening Metathesis Polymerization

Abstract

Synthetic fibronectin mimics have been constructed using functionalised norbornenyl polymers synthesized via ring-opening metathesis polymerization (ROMP). The ROMP reactions were catalyzed by newly developed ruthenium initiators. Prior to the synthesis of the fibronectin mimics, the polymerization reactions of a number of norbornene derivatives with pendent glycine or penta(ethylene oxide) (EO_5) units were studied in order to select the derivatives with optimal characteristics: high yields, fast polymerization times, and narrow PDI's. For these initial studies $Ru{=}CHPh(Cl_2)(PCy_3)_2$ (**1**) was used as the initiator. Based on these studies poly(5-norbornene-2-carboxyl) and poly(4-(*Exo*-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.0]dec-8-en-4-yl)-butyric acid) were chosen as the ideal backbones for a series of polymers functionalised with pendent arginine-glycine-aspartic acid (RGD) and serine-arginine-asparagine (SRN) oligopeptides. The polymers containing a propyl spacer between the pendant group and the backbone were synthesized in an attempt to demonstrate that the presentation of the peptide units could be altered to closely mimic the natural presentation of these peptides in fibronection. Homopolymers and random copolymers containing various combinations of norbornene monomers with pendant EO_5 (**21** & **14**), GRGD/RGD (**24** & **29**), and SRN (**25** & **30**) units were made. Copolymers with large RGD or SRN contents (>10 mol %) could only be synthesised in high yields and narrow PDI's by using the newly developed ruthenium 2,3-dihydroimidazolylidene initiators $Ru{=}CHPh(Cl)_2(PCy_3)$(DHIMes) (**2**) or $Ru{=}CH{-}CH{=}CH_2(CH_3)_2(Cl)_2(PCp_3)$(DIHMes) (**3**). In addition, the ROMP reactions of the norbornene monomers containing the propyl spacers also required the new initiator **2**. Attempts to synthesize these polymers using ruthenium initiator **1** resulted in either low yields, bimodal molecular weight distributions, or a combination of both.

Introduction

Integrins and extracellular matrix proteins, such as fibronectin, together provide cells with adhesion and signaling pathways.[1] Many integrins recognize proteins that contain the short peptide sequence Arg-Gly-Asp (RGD).[2] In fact, RGD alone has been shown to promote cell adhesion.[3] 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.[4] For example, fibronectin and RGD containing peptides and

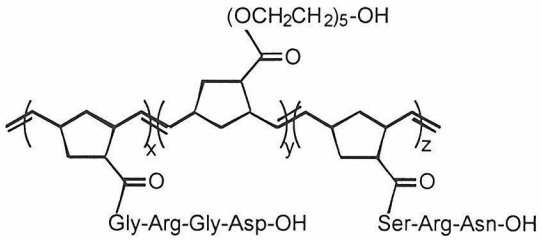
mimetics have been shown to have anti-metastatic activity, and thus may be good prospects for tumor therapy.[5]

Although RGD itself promotes cell adhesion, it is significantly less active than fibronectin, suggesting that other sites in the protein act in synergy to the tripeptide. For example, RGD demonstrates significant losses in activity and estimated binding affinity in cell adhesion assays compared to fibronectin. [6] This observation led to the discovery of a synergy site on fibronectin, Pro-His-Ser-Arg-Asn (PHSRN), that enhanced RGD binding to $\alpha_1\beta_5$.[7] Interestingly, PHSRN acted in synergy only when covalently bound by RGD.[7] Deletion studies determined that Arg was the important residue for function,[7] suggesting that the sequence could be further truncated to SRN yet still retain its synergistic activity. The crystal structure of fibronectin confirmed that PHSRN was held on the same face, 30-40 Å away from RGD, demonstrating that a single integrin molecule could access both sites.[8] Despite these findings, the majority of biomaterials containing RGD do not contain the synergy site, which could potentially enhance the cell binding ability.

Synthetic polymers containing pendent RGD and SRN[9] units would have many advantages over existing materials in terms of integrin binding strength coupled with ease and versatility of synthesis. The SRN would be covalently bound on the same molecule as RGD, which is necessary to obtain the synergistic activity.[7] Therefore, polymers containing both RGD and SRN should have increased cell binding activity compared to materials containing only RGD. The polymers would have many pendent RGD and SRN sequences per polymer chain, possibly leading to multivalent interactions and stronger binding. It has been shown that polypeptides containing RGD repeats have a greater cell binding ability than RGD itself, suggesting that having multiple integrin binding sites is advantageous.[10] The polymers would have slower clearance rates than small-molecule peptides, increasing the lifetime of the material *in vivo*.[10] Furthermore, unlike small molecule peptides and mimetics, polymers may be passive targets for such applications as tumor therapy.[11]

A synthetic polymer with RGDS units linked to a poly(carboxyethylmethacrylamide) backbone has been reported and shown to more effectively inhibit metastasis compared to the GRGDS peptide.[12] However, this polymer did not contain the synergy site and therefore did not inhibit cancer cell invasion into reconstituted basement membrane more than GRGDS. Additionally, this polymer was synthesized by nonliving, radical polymerization (resulting in

PDI's between 2 and 4.6), which would make the synthesis of copolymers or block copolymers difficult.

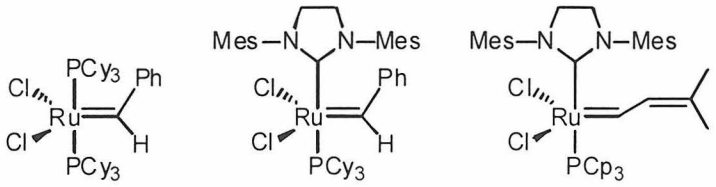


Synthetic Fibronectin Mimetic

Ring-opening metathesis polymerization (ROMP) provides a better alternative for producing well-defined copolymers as fibronectin mimetics. The copolymer composition is readily controlled by the feed ratios of the monomers,[13] and ROMP of strained systems can be living, so block copolymers may be made. In addition, the polymer architecture can be readily altered, for example to include a spacer group between the peptide and polymer backbone. Our approach is to synthesize various synthetic polymers with pendent RGD, SRN[9], and/or penta(ethylene oxide) (EO_5) units by ROMP for use in a variety of disease related applications and fundamental studies of fibronectin-$\alpha_1\beta_5$ binding (see Fibronectin Mimetic). Polymers containing RGD and SRN have the advantages already discussed. Since it has been shown that surfaces of oligo(ethylene oxide) are protein resistant[14], copolymers containing EO_5 units were made. These may be more biocompatible as well as water soluble compared to the polymers containing only the peptides.

ROMP of monomers with pendent amino acids has been previously demonstrated. We have synthesized polyethers[13] and others have synthesized polynorbornenyl[15] polymers with pendant amino acids. In these cases, the pendant groups were composed of one or two amino acids with alkyl side chains, containing no functionality. In addition, the norbornenyl polymers were synthesized with the less functional group tolerant molybdenum catalysts. In order 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 been extensively explored and has proven to be both active for ROMP and to have extraordinary functional group tolerance.[16] We also recently reported catalyst **2** and **3**.[17] 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.[17] Due to the multitude of functional groups found in biologically relevant molecules such as RGD and SRN, the utility of the dihydroimidazolylidene catalysts **2** and **3** were explored in addition to initiator **1**.



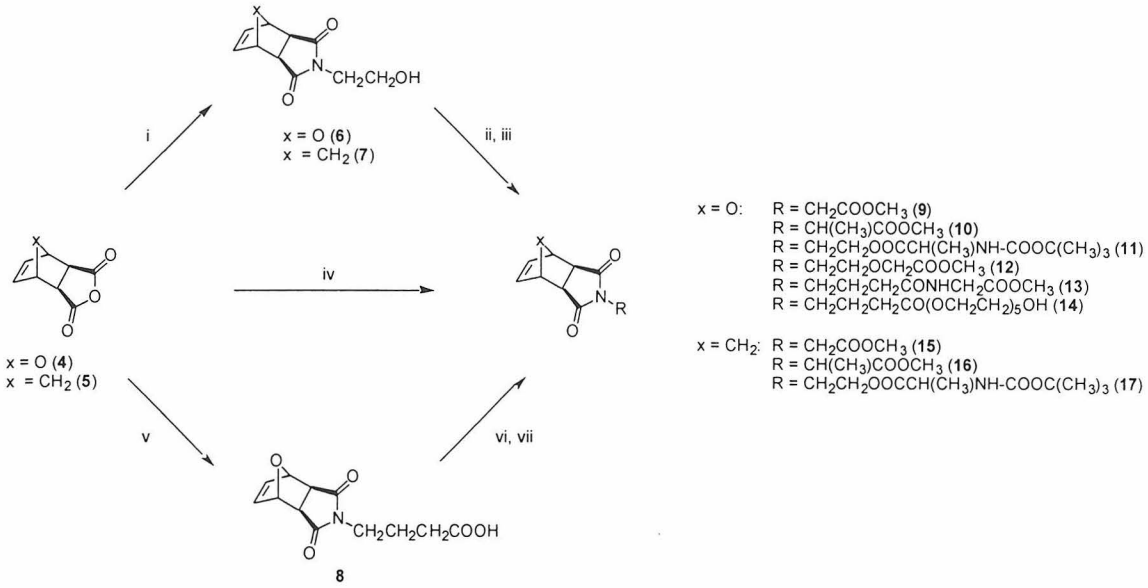
1 **2** **3**

Mes = mesityl
Cy = cyclohexyl
Cp = cyclopentyl

The synthesis of GRGD and SRN containing homo and copolymers is reported. Initially, polymers with different norborneneyl backbones and one pendent amino acid were synthesized and compared. Out of the group of polymers studied, polymers **20** and **13** with pendent glycine units 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 synthesized using these backbones. RGD (**24**, **29**), SRN (**25**, **30**), and penta(ethylene glycol) (**21**, **30**) 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 distribution, 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 larger than 10 mol% pendent RGD. Using the newly developed initiators **2** and **3**, novel fibronectin mimetics, containing both RGD and the synergy site SRN were synthesized.

Results and Discussion

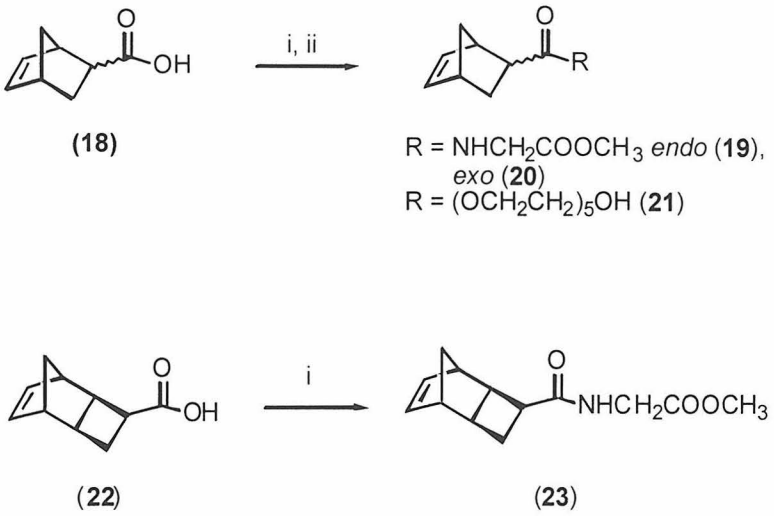
Scheme 1. Synthesis of oxa and methylene-bridged imide monomers 9-17. [a]



[a] Reaction Conditions: i. $H_2NCH_2CH_2OH$ in THF/MeOH, 50 ÞC. 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. ClH-Gly-OCH_3 or ClH-Ala-OCH3 and Et_3N in CH_2Cl_2, reflux (**9**, **10**, **15**, **16**). v. $H_2N(CH_2)_3COOH$ in THF/MeOH, 50 ÞC. vi. ClH-Gly-OCH_3, 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**).

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. In the past, simple glycine and alanine monomers **9**, **10**, **15**, and **16** (Scheme 1) were synthesized in our group, but the yields of the reactions were relatively low (10 – 50%). Therefore the screening for monomers continued with the synthesis of norbornenes with a pendent glycine (**19** and **20**) or penta(ethylene glycol) unit (**21**) (Scheme 2).

Scheme 2. Synthesis of norbornene monomers 19-21, 23. [a]



[a] Reaction conditions: i. ClH-Gly-OCH_3, 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**).

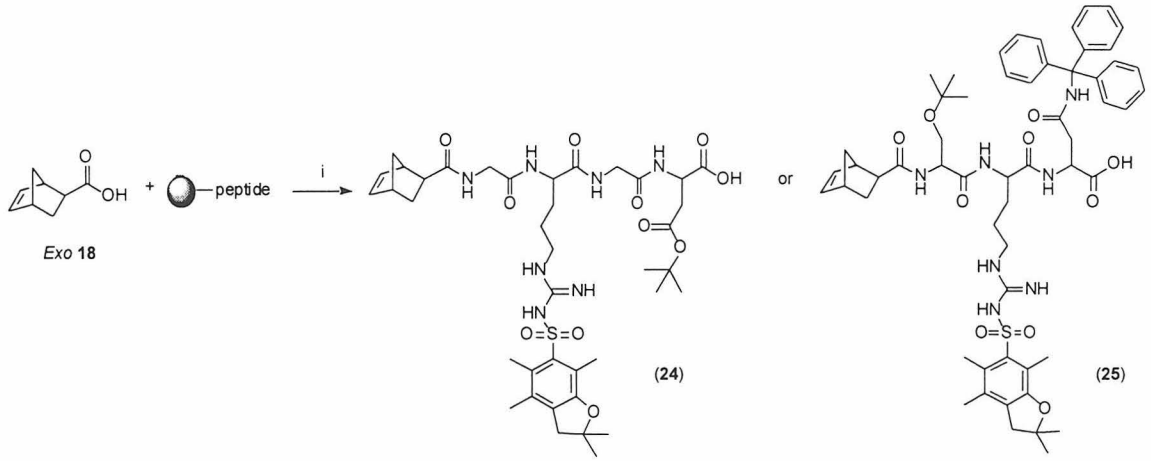
These monomers were made in somewhat better yields in comparison to the imide-derived monomers. Glycine methyl ester hydrochloride was coupled to *endo* or *exo* 5-norbornene-2-carboxylic acid (**18**) with EDC, triethylamine, and HOBT in CH_2Cl_2 in 66.0% (**19**) and 72.9% (**20**) yields, respectively. The same procedure was followed to synthesize monomer **23** from **22** in 65% yield. The EO_5 monomer (**21**) was synthesized in 52.6% yield by reacting penta(ethylene glycol) with norbornene-*cis*-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 hours in a mixture of THF and MeOH to give the resulting alcohols **6** and **7** in 36.1% and 44.4% yields respectively. Then monomer **12** was synthesized in

49% yield by mixing **6** and methyl bromoacetate with potassium carbonate and tetrabutyl ammonium bromide in anhydrous DMF. Monomers **13** and **14** with propyl spacers were synthesized in decent yields from **8**. The acid (**8**) was synthesized first in 40.5% 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 56.6% 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% over-all yield.

Finally, the GR(Pbf)GD(O^{t}Bu)-OH and S(O^{t}Bu)R(Pbf)N(Trt)-OH containing monomers (**24**, **25**, and **29**) were synthesized. **24** and **25** were created 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 92.1% yields respectively. In a similar manner, the R(Pbf)GD(O^{t}Bu)-OH and S(O^{t}Bu)R(Pbf)N(Trt)-OH monomers with propyl spacers (**29** and **30**) were synthesized in 82.9% and 85.2% yields respectively by coupling **8** to the amino terminus of the protected RGD and SRN peptides.

Scheme 3. Synthesis of norbornene G-R(Pbf)-G-D(O^{t}Bu)-OH and S(O^{t}Bu)-R(Pbf)-N(Trt)-OH monomers.[a]



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

Polymers with one pendent amino acid: synthesis and characterization. Before synthesizing polymers containing oligopeptides, polymers with one pendent amino acid or EO_5 group were studied. The polymers were synthesized by adding a solution of **1** in methylene chloride to a solution of monomer in methylene chloride 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 one pendent amino acid or EO_5 unit attached directly or through a spacer to the backbone were made and compared in terms of polymer yield, polymerization time, and polymer molecular weight distribution. Based on this comparison, norbornene and the propyl spacer imide-derivative were selected for the synthesis of the more complex, oligopeptide systems due to their optimal characteristics.

Table 1. Polymerization data for norbornenyl monomers with pendent amino acids. [a]

Polymer	Yield	$[M]_0/[C]_0$	Time (min)	M_n ($x10^3$)[b]	PDI[b]	T_g (ÞC)[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**)	100%	50/1	45	10.9	1.15	88.0	2.4	19.02, 18.94, 18.74	yes
Poly(**21**)	100%	36/1	35	4.61	1.12	-48.9	3.6	19.07, 19.02, 18.72	yes
Poly(**23**)	100%	100/1	<5	1.00	29.0	---	2.9	---	---

[a]General reaction conditions: CH_2Cl_2 as the solvent, rt, **1** as the initiator, $[M]_0$ = 0.5-0.75 M. [b]All determined from GPC, CH_2Cl_2 eluent, polystyrene standards except poly(**20**) with DMF as the eluent, poly(ethylene glycol) standards. [c]Determined from DSC, 10 ÞC/min, 2nd heat reported. [d]Determined from ^{1}H NMR. [e]Determined from ^{31}P NMR.

Polymers synthesized from monomers **9**, **10**, **15**, and **16** were deterimined to be unsuitable for use in the more complex oligopeptide systems based on the results of previous studies. These monomers and polymers suffered from unfavorable characteristics such as low monomer yields and bimodal molecular weight distributions. Therefore the norbornene monomers were selected for further studies.

Norbornene monomers (**19–21**, **23**) (Scheme 1) 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 hours to reach 90% yield. This was not a surprising result since *endo* monomers contain less strain than the corresponding *exo* monomers and often take longer to polymerize.[18] *Exo* monomer **20** was then made and reacted quickly in 45 minutes to give a quantitative yield of polymer. The penta(ethylene glycol) monomer **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 minutes) and uncontrollably, resulting in an extremely broad molecular weight distribution (PDI of 29.0).

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

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 (x10^3)[b]	PDI[b]	T_g (ÞC)[c]	Trans/ Cis[d]	Carbene (ppm)[d]	Free PCy_3?[e]
Poly(**12**)	100%	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**)	100%	50/1	30	118	bimodal	-29.9	2.9	18.69	no
Poly(**11**)	100%	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, rt, **1** as the initiator, $[M]_0$ = 0.5-0.75 M. [b]All determined from GPC, CH_2Cl_2 eluent, polystyrene standards except poly(**14**) with DMF as the eluent, polystyrene standards. [c]Determined from DSC, 10 ÞC/min, 2nd heat reported. [d]Determined from ^{1}H NMR. [e]Determined from ^{31}P NMR.

In an attempt to alter the presentation of RGD, 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** polymerized in 2.5 hours and **13** and **14** in 30 minutes to give poly(**12**), poly(**13**), and poly(**14**) quantitatively. 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). This is due to the extra disorder created by the flexible alkyl spacer groups. 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 biomodal 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 functionality. But, similar to **15**, saponification resulted in the regeneration of the **6**.

Polymers with pendant boc-protected alanines, poly(**11**) and poly(**17**) were synthesized in less than 2 hours in 100% 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 was 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.

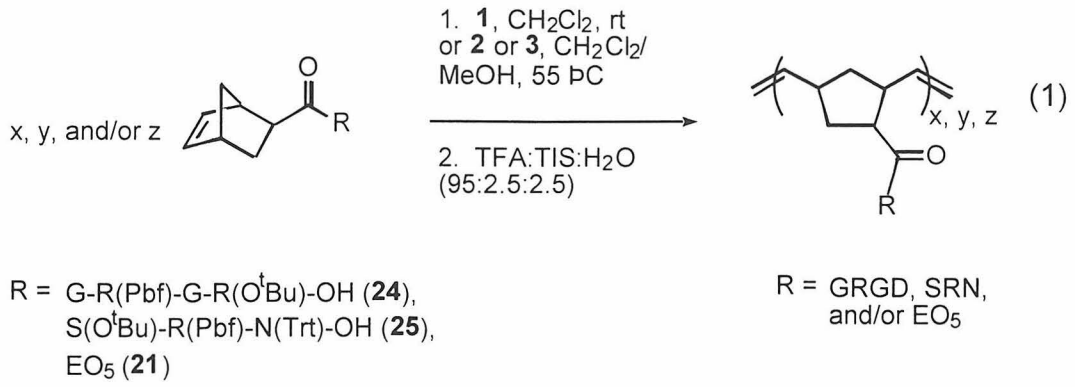
All of the previous polymerizations were monitored by NMR. The chemical shifts of the carbene propagating species, the presence of free phosphine, and the final *trans*/*cis* ratios (*vide infra*) are recorded in Tables 1 and 2. All of the initiated carbenes were broad multiplets. During the polymerization of the oxa-bridged monomers **11-14** only one propagating carbene was observed at roughly the same shift (between 18.67 and 18.70 ppm). Free phosphine was not observed in any of these reactions. However, methylene-bridged imide monomer **17** had two observable propagating species during polymerization (19.42 and 19.20 ppm) 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 had 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 multiple propagating species may be the monophosphine and bisphosphine species. Polymerization of the norbornene monomers resulted in three observable 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 pendant GRGD, SRN, and EO_5 were first synthesized using initiator **1** for 4 hours at room temperature in CH_2Cl_2 with an initial monomer concentration of 0.7 M (Equation 1). 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. 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 mol% **24**, 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. Good yields (66%) were obtained for the polymerization with 50 mol% **24** in the feed, but the GPC trace revealed a bimodal molecular weight distribution of the polymer. 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. Few examples of these initiators used in ROMP have been reported.[21] However, the catalysts have been shown to be more active than **1** for ring-closing metathesis and cross-metathesis, as well as being inter-changeable with each other at higher temperatures.[17,21] Based upon these initial studies, it was hoped and was found that these initiators would result in higher yields of the desired polymers.



Hompolymers and copolymers of **21**, **24**, and **25** were synthesized (Equation 1) using **2** or **3** as initiators by heating the monomers in a 1:1 mixture of CH_2Cl_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**, and the rest 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. Fibronectin mimetics, copolymer **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 ^{1}H NMR 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. The use of HF could be circumvented by changing the serine protecting group to the labile trityl group.

All of the deprotected polymers were solublized 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 solublized in water by stirring in 0.1 N NaOH for 10 minutes, generating the sodium salt of the peptide carboxylic acid which was 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** with a yield of 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 of all the crude reaction mixtures indicated that most of the monomers had been consumed. However, since the monomers and protected polymers had similar solubility, polymer purification proved 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 purified by Centriprep (MWCO 3000) using ethanol as the solvent.[22] Regardless of the purification method, except for **28b**, the yields were all good to excellent.

Table 3. Polymerization data for norbornene monomers with pendent oligopeptide and EO_5 units.[a]

polymer	Catalyst	% yield overall	$[M]_0/[C]_0$	mol% in feed			mol% in polymer[c]			M_n ($x10^3$)[d]	PDI[d]	T_g (ÞC)[e]	Cis/ Trans[c]
				GRGD	SRN	EO_5	GRGD	SRN	EO_5				
26a[b]	1	78%	20/1	10%	0%	90%	9.2%	0%	90.8%	18.7	1.13	-30.7	0.21
26b	2	81%	20/1	50%	0%	50%	49%	0%	51%	15.9[f]	1.13	52.6	1.7
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	1.6
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	1.6
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 ÞC for 2 hrs. in sealed vial, $[M]_0$ = 0.6-0.7 M. [b]Reaction conditions: CH_2Cl_2 as the solvent, rt for 4 hrs., $[M]_0$ = 0.7 M. [c]Calculated from ^{1}H NMR. [d]Determined from GPC, pH 8.0 phosphate buffer eluent, poly(ethylene oxide) standards. [e]Determined from DSC,10 ÞC/min, 2nd heat reported. [f]76% of EO5 repeats were saponified. [g]65% of EO5 repeats were saponified.

The GPC results are given in Table 3. The number-averaged molecular weights were fairly low between 10 700 and 18 700, and most importantly, the samples had 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. However, the other samples had narrow PDI's between 1.13-1.32. Remarkably, the trimonomer copolymer, **28a**, had a narrow PDI of 1.21. This result indicates that synthesizing more complex copolymers with four or even more monomers is possible so that drugs or reactive species may be incorporated into the polymers. The narrow PDI's also indicate that the synthesis of block copolymers may be possible.

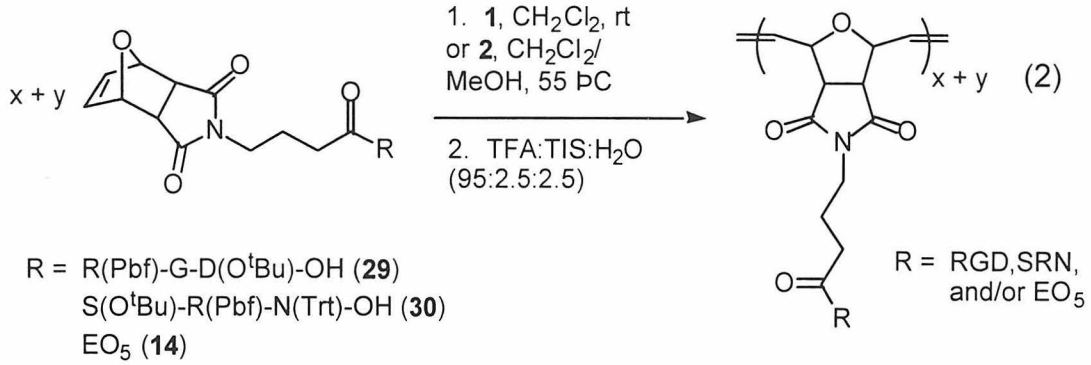
The T_g values of the copolymers varied depending on the substituents. For example, copolymers **26a** & **b** had T_g values of –30.7 °C and 52.6 °C between that of homopolymers poly(**21**) at –48.9 °C and **26c** at 98.0 °C. Copolymer **27a** had a T_g of 62.3 °C while homopolymer **27b** one of 131.2 °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 mol% and 53 mol% peptide, respectively. This indicates that the presence of the third monomer introduces extra disorder. In addition, **28b** had a T_g of 104.6 °C, which is close to that of the GRGD containing homopolymer, **26c**.

The large influence of the pendent groups on the T_g of the polymers is extremely obvious. 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 resulted in an interesting observation: polymers synthesized with **1** contained more *trans* olefins while those synthesized by **2** had slight excesses 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. 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**, contained a slight excess of *cis* olefins (*cis* /*trans* = 1.6/1 - 1.7/1, 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**, **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 *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* of 2.4/1 and **2** resulted in the reverse with a *cis* to *trans* of 2.3/1. For **21**, initiator **1** resulted in a polymer with a *trans* to *cis* ratio of 3.6/1 and **2** with a *cis* to *trans* ratio of 1.7/1. 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 change the polymer architecture and the presentation of the oligopeptides, it is useful to be able to synthesize polymers with spacer groups between the peptide and the backbone. As a demonstration, monomers **14**, **29** and **30** were polymerized to form polymers with propyl spacers between the backbone and pendent RGD, SRN and EO_5 (Equation 2). Initially, a series of homopolymers and random copolymers were synthesized using initiator **1**, but the polymers obtained had bimodal molecular weight distributions (Table 4). In addition, they were found to have large number-averaged molecular weights ranging from 118 000 to 401 000.

Polymer	% yield[a]	[M]/[C]	%RGD	%SRN	%EO_5	M_n(x10^3)[b]	PDI[b]	T_g(°C)[c]	Trans/ Cis
31	100%	50/1	0%	0%	100%	118	bimodal	-29.9	2.9
32	71%	20/1	100%	0%	0%	261	bimodal	93.7	2.3
33a	94%	20/1	0%	100%	0%	401	bimodal	53.8	1.1
33b	85%	20/1	0%	30%	70%	353	bimodal	50.2	3.7
34[d]	90%	10/1	20%	0%	80%	115	1.14	70.2	0.83

[a]Calculated from ^{1}H NMR. [b]M_n and PDI determined from GPC, DMF eluent, polystyrene standards. [c]Determined from DSC, 10°C/min, 2nd heat reported. [d]Reaction conditions: CH_2Cl_2:MeOH (1:1) as the solvent, 55°C for 2hrs. in sealed vial using initiator **2**. Note: All other reactions were performed under the following conditions: CH_2Cl_2 as the solvent, rt for 4hrs. using initiator **1**.

The EO_5 homopolymer **31** was soluble in water. The polymers **32**, **33a**, and **33b** were deprotected with TFA, but unfortunately they were not soluble in water. Treatment of the deprotected polymers with pH 8 phosphate buffer or 0.1 M NaOH did not enhance their solubility. The polymers' large molecular weights were attributed as the reason for their poor solubility. In order to address the problem of bimodal molecular weight distributions and poor solubility, initiator **2** was employed to synthesize a smaller RGD / EO_5 random copolymer.

Polymer **34** was synthesized as previously described using **2** as the initiator with a $[M]_0/[C]_0$ = 10/1 (Equation 2). The yield was approximately 90% by ^{1}H 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 demonstrates that the solubility of these types of polymers is strongly dependent upon their size. 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 and the *cis* to *trans* ratio was 1.2/1. This work is readily extended to the synthesis of other homopolymers and copolymers, and demonstrates that using the more active initiators, the polymer architecture can be altered to include spacer units.

Conclusions

A series of polymers with pendant glycine or penta(ethylene glycol) units with various norbornenyl olefins was synthesized by using initiator **1**, and the yields, molecular weights, PDI's, and T_g's were compared. From these results, it was apparent that norbornenes would be ideal for the synthesis of polymers with pendent biologically relevant peptides. In order to vary the polymer architecture, polymers with ethyl and propyl spacers between the backbone and pendent groups were also studied.

Polymers with pendent RGD, SRN, and EO_5 were synthesized. The RGD and SRN units should mimic the activity of fibronectin, and the addition of EO_5 units may reduce the toxicity of the materials. When initiator **1** was employed in the synthesis, only the polymer containing 9.2 mol% RGD was made in decent yield with a monomodal molecular weight distribution. In addition, the use of initiator **1** to synthesize the polymers containing propyl spacers between the polymer backbone and the peptide ligand resulted in bimodal molecular weight distributions. Ultimately, the synthesis of the polymers containing large amounts of peptide and the polymers with the propyl spacers required the use of the more active 2,3-dihydroimidazolylidene initiators **2** and **3**.

One of the goals of polymer chemistry has been to mimic the complexity and diversity of nature. Using the more active initiators, polymers with two and even three different types of monomers (RGD, SRN, and penta(ethylene glycol) containing) were made. With this success, the possibility of synthesizing more complex polymers with four, five or more monomer units incorporated into one polymer is possible. For example, polymers with peptides and sugars to specifically target cells, growth factors to induce cell growth, and drugs may be possible. Polymers with groups that can react with gold or silica surfaces in addition to containing antibodies or other biological moieties may also be synthesized for use in sensor technology. By contrast, the simpler RGD and SRN containing polymers described in this paper may already provide diverse uses. For example, these polymers may be used for a variety of fundamental research studies of the binding of integrins to proteins and for many disease-related applications such as in tumor therapy.

Experimental

Materials. 2-aminoethanol and solvents were purchased from EM science. Methylene chloride used in the polymerization reactions was dried over CaH_2, degassed, and vacuum transferred before use. Glycine methyl ester hydrochloride was purchased from Sigma. Penta(ethylene glycol) was purchased from Aldrich and dried over 4 Å molecular sieves (Linde). 5-Norbornene acid-*exo*-2-carboxylic acid (*exo* **18**)[23] and acid chloride[24] 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. 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. ^{1}H 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). 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 taken using an HPLC system equipped with an Altex model 110A pump, a Rheodyne model 7125 injector with a 100 □L injection loop, two American Polymer Standards 10 micron mixed bed columns, and a Knauer differential refractometer and poly(styrene) 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 □L 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 polystyrene 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 taken 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

***Exo*-(3,5-dioxo-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)-acetic acid methyl ester (15).** Literature procedure[Error! Bookmark not defined.] was followed except that CH_2Cl_2 was used as solvent to give **15** in 58.5% yield as a white solid. ^{1}H NMR ($CDCl_3$, 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).

[(Bicyclo[2.2.1]hept-5-ene-*endo*-2-carbonyl)-amino]-acetic acid methyl ester (19). Triethylamine (1.41 mL, 10.1 mmol) and glycine methyl ester hydrochloride (636 mg, 5.06 mmol) were added to a solution of *endo* **18** (700 mg, 5.06 mmol) in CH_2Cl_2 (75 mL). 1-

Hydroxybenzotriazole (HOBT, 1.03 g, 7.62 mmol) was added and the solution was stirred until all solids had dissolved. Then a solution of 1-[3-(dimethylamine)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 971 mg, 5.06 mmol) and triethylamine (1.41 mL, 10.1 mmol) in CH_2Cl_2 (25 mL) was added and the mixture was stirred for 24 h. The organic layer was washed successively with 10% citric acid, H_2O, 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 704 mg (66% yield, 11% *exo*) of **19** as a white solid. ^{1}H NMR (CD_2Cl_2, 300 MHz) δ 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_2Cl_2, 300 MHz) δ 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 1.4 mL (10.0 mmol) triethylamine, 541 mg (4.3 mmol) glycine methyl ester hydrochloride, 565 mg (4.1 mmol) *exo* **18**, 828 mg (6.1 mmol) HOBT, and 785 mg (4.1 mmol) EDC in 40 mL of CH_2Cl_2 resulting in 625 mg (72.9%) of **20** as a off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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_2Cl_2, 300 MHz) δ 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)-ethyl ester (21). 5-Norbornene acid-*exo*-2-carboxylic acid chloride (1.2 g, 7.67 mmol) was added drop wise to a stirred mixture of penta(ethylene glycol) (2.8 mL, 13.2 mmol) and potassium carbonate (3.7 g, 26.8 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 made neutral 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 (52.6%) 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 = 8.4Hz), 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) calcd for $(MH)^+$ 359.2070 found 359.2082.

[(Tricyclo[4.2.1.0^{2,5}]non-7-ene-*exo*-3-carbonyl)-amino]-acetic acid methyl ester (23). Same procedure as for **19** was followed with 973 μL (6.98 mmol) triethylamine, 438 mg (3.49 mmol) glycine methyl ester hydrochloride, 573 mg (3.49 mmol) **22**, 708 mg (5.22 mmol) HOBT, and 669 mg (3.49 mmol) EDC in 30 mL CH_2Cl_2. The crude product was subjected to column chromatography (ether) to provide 534 mg (65%) of **23** as a white solid. ^{1}H NMR ($CDCl_3$, 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

4-Hydroxymethyl-10-oxa-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-ene-*exo*-3,5-dione (6). Prepared as for the **7** (*vide infra*) with 6.1 g (36.8 mmol) **4** and 2.0 mL (36.8 mmol) of 2-aminoethanol in THF/MeOH (30 mL, 1/1) resulting in 2.78 g (36.1%) 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), 5.50 (2H, t, J = 4.5), 2.99 (2H, s). ^{13}C 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.

(*Exo*-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-ylmethoxy)-acetic acid methyl ester (12). Methyl bromoacetate (1.81 mL, 19.1 mmol) was added to a solution of the **6** (1 g, 4.78 mmol), potassium carbonate (796 mg, 5.76 mmol), and tetrabutylammonium bromide (20 mg, 0.059 mmol) in anhydrous DMF (20 mL). The solution was stirred at room temperature for 12 h. $CHCl_3$ (100 mL) was added, and the organic layer was successively washed with H_2O (twice) and 5% $HBr_{aq.}$. The aqueous layers were combined and washed with CH_2Cl_2 (three times). All of the organic layers were pooled, dried over $MgSO_4$, and the solvent was removed *in vacuo*. The crude product was recrystallized from a mixture of EtOAc and ether (4:1) to give 661 mg (49%) of **12** as a white solid. ^1H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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, 2355.9, 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}.

2-*tert*-Butoxycarbonylamino-propionic acid *exo*-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-ylmethyl ester (11). The same procedure as for **17** (*vide infra*) was followed with 500 mg (2.39 mmol) of the **6**, 453 mg (2.39 mmol) of N-tert-butoxycarbonyl-L-alanine, 494 mg (2.39 mmol) of DCC, and 44 mg (0.359 mmol) of DMAP in 20 mL CH_2Cl_2. The product was purified by recrystallization from MeOH/hex (2/1) to yield 457 mg (50.3%) of **11** as white crystals. ^1H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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. HRMS (DCI/NH_3) calcd for $(MH)^+$ 381.1662 found 381.1645.

2-*tert*-Butoxycarbonylamino-propionic acid *exo*-3,5-dioxo-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-ylmethyl ester (17). **7** (300 mg, 1.45 mmol), *N*-(*tert*-butoxycarbonyl)-L-alanine (2.74 mg, 1.45 mmol), 1,3-dicyclohexylcarbodiimide (DCC, 299 mg, 1.45 mmol), and 4-(dimethylamino)pyridine (DMAP, 27 mg, 0.22 mmol) were stirred in CH_2Cl_2 (18 mL) for 12 hours. 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 334 mg (60.9 % yield) of **17** as a sticky solid. ^1H NMR (CD_3Cl, 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 ($CDCl_3$, 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}. HRMS (DCI/NH_3) calcd for $(MH)^+$ 379.1869 found 379.1871.

4-(*Exo*-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-yl)-butyric acid (8). Same procedure as for the synthesis of the **7** was followed with 3 g (18 mmol) **4** and 1.86 g (18 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 (40.5%) of **8** as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 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) δ 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, 2495.1, 2355.9, 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.

[4-(*Exo*-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-yl)-butyrylamino]-acetic acid methyl ester (13). Same procedure as for **20** was followed with 2.21 mL (15.8 mmol) triethylamine, 645 mg (4.77 mmol) HOBT, 609 mg (3.18 mmol) EDC, 400 mg (3.19 mmol) glycine methyl ester hydrochloride, and 800 mg (3.18 mmol) of **8** in 64 mL CH_2Cl_2. The crude product was subjected to column chromatography (EtOAc) to give 580 mg (56.6%) of **13** as a white solid. ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR (CD_2Cl_2, 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, 2355.5, 1750.7, 1698.6,1547.6, 1433.0, 1401.8, 1370.5, 1282.0, 1214.3, 1162.2, 1021.6, 917.4, 875.0, 849.7, 802.9, 719.5, 651.8, 589.3, 500.8 cm^{-1}. HRMS (EI) calcd for $(MH)^+$ 323.1243 found 323.1241.

4-(*Exo*-3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.$0^{2,6}$]dec-8-en-4-yl)-butyric acid 2-(2-(2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy)-ethoxy)-ethyl ester (14). The acid chloride of **8** was generated *in situ* using 700 mg (2.78 mmol) of **8**, 530 μL (6.12 mmol) oxalyl chloride, and a catalytic amount of DMF in CH_2Cl_2 following a literature procedure.[24] To a solution of the crude acid chloride in anhydrous THF (45 mL), penta(ethylene glycol) (662 mg, 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 477 mg (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) calcd for $(MNH_4)^+$ 489.2448 found 489.2460.

Norbornene G-R(Pbf)-G-D(O^{t}Bu)-OH monomer (24). G-R(Pbf)-G-D(O^{t}Bu)-resin (0.5 mmol peptide, 4-carboxytrityl linker Novasyn® resin) was placed in a flask containing a frit and stopcock. The resin was swelled in 16.7 mL of DMF for 15 min and then rinsed with DMF (1 x 10 mL). In a vial, 276 mg (2.0 mmol) of *exo* **18**, 758 mg (2.0 mmol) of 2-(*1H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexaflurophosphate (HBTU), and 270 mg (2.0 mmol) of HOBT in 16.7 mL of DMF were agitated until all solids had dissolved. *N,N*-diisopropylethylamine (DIEA) was added (695 μL, 4.0 mmol) and the solution was agitated and pipetted onto the resin. Nitrogen was gently bubbled through the mixture for 2 h. The resin was

then rinsed with DMF (5 x 10 mL), CH_2Cl_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_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) calcd 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 S(O^{t}Bu)-R(Pbf)-N(Trt)-resin (0.75 mmol, 4-carboxytrityl linker Novasyn® resin), 471 mg (3.0 mmol) of *exo* **18**, 1.14 g (3.0 mmol) of HBTU, 405 mg (3.0 mmol) of HOBT, and 1.0 mL (6.0 mmol) of DIEA in 18.9 mL of DMF to yield 723 mg (92.1%) 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) calcd 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 R(Pbf)-G-D(O^{t}Bu)-resin (0.25 mmol, 4-carboxytrityl linker Novasyn® resin), 251 mg (1.0 mmol) **8**, 379 mg (1.0 mmol) HBTU, 135 mg (1.0 mmol) HOBT, and 348 μl (2.0 mmol) DIEA in 8.3 mL DMF to yield 184 mg (82.9%) 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, 128.63, 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.

S(O^{t}Bu)-R(Pbf)-N(Trt)-OH Monomer (30). The same procedure as for **24** was followed with S(O^{t}Bu)-R(Pbf)-N(Trt)-resin (0.25 mmol, 4-carboxytrityl linker Novasyn® resin), 251 mg (1.0 mmol) of **8**, 379 mg (1.0 mmol) of HBTU, 135 mg (1.0 mmol) of HOBT, and 0.348 mL (2.0 mmol) of DIEA in 6.25 mL of DMF to yield 247 mg (85.2%) of **30** as a fluffy, white solid. HPLC: single peak at 26.92 min. ^1H NMR (CD_2Cl_2, 600 MHz) δ 7.58 (1H, bs), 7.51 (1H, bs), 7.05-7.10 (15H, bm), 6.63 (1H, bs), 6.36 (2H, s), 5.07 (2H, s), 4.55 (1H, bs), 4.48 (1H, bs), 4.25 (1H, bs), 3.59 (1H, bs), 3.36-3.39 (2H,bm), 3.28-3.32 (4H, bm), 3.00 (2H, bs), 2.68 (2H, s), 2.80 (2H, bs), 2.42 (3H, s), 2.35 (3H, s), 1.98 (5H, s), 1.74-1.77 (2H, bm), 1.63-1.66 (1H, bm), 1.56 (1H, bs), 1.43 (2H, bs), 1.36 (6H, s), 1.07 (9H, s). TOCSY cross peaks (CD_2Cl_2, 600 MHz) δ norbornene: 6.36, 5.07, 3.36-3.39, 3.28-3.32, 1.98, 1.74-1.77, 1.63-1.66; S: 7.58, 4.55, 2.80; R: 7.51, 5.84-5.90, 4.48, 3.00, 1.74-1.77, 1.56, 1.43; Pbf group: 2.68, 2.42, 2.35, 1.98, 1.36; N: 6.63, 4.25, 3.59, 3.28-3.32. ^{13}C NMR (CD_2Cl_2, 300 MHz) δ 180.95, 177.66, 173.33, 170.58, 158.85, 156.93, 144.62, 138.56, 136.73, 133.42, 132.55, 129.04, 128.11, 127.18, 125.42, 117.63, 107.21, 86.72, 81.32, 74.22, 70.93, 61.50, 47.81, 43.46, 40.02, 38.14, 32.62, 28.65, 27.49, 25.16, 23.57, 19.45, 18.17, 12.56, 1.11. IR (NaCl plate): 3448.4, 3341.3, 2966.4, 2355.9, 1770.9, 1696.0, 1679.9, 1653.1, 1551.4, 1513.9, 1449.6, 1401.4, 1363.9, 1256.8, 1160.4, 1101.5, 1015.8, 876.6, 855.2, 812.3, 732.0, 699.8, 662.4, 571.3 cm^{-1}. HRMS (DCM/NBA/NaCl) calcd for $(MNa)^+$ 1181.4994 found 1181.4965.

Polymer Synthesis.

General Polymer Synthesis for Polymers with one pendent amino acid or EO_5. Polymers were synthesized following a typical procedure where in a nitrogen-filled dry box, a solution of catalyst in was added to a solution of monomer CH_2Cl_2 (or CD_2Cl_2 for NMR reactions) to give an initial monomer concentration of 0.7-0.75 M. The initial $[M]_0/[C]_0$ was 100. 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 minutes. 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. All polymers were white to tan powders except for poly(**21**) and poly(**14**) 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(19). The reaction time was 26 h. ^1H NMR (CD_2Cl_2, 300 MHz) δ 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_2Cl_2, 300 MHz): same as for Poly(**20**) (*vide infra*). IR (NaCl plate): same as for Poly(**20**) (*vide infra*).

Poly(20). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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 (CD_2Cl_2, 300 MHz) δ 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_2Cl_2, 400 MHz) δ 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) 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}.

Poly(23). $[M]_0$ was 0.6 M. ^{1}H NMR (CD_2Cl_2, 300 MHz) δ 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_2Cl_2, 300 MHz) δ 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}.

Poly(12). $[M]_0$ was 0.6 M. ^{1}H NMR (CD_2Cl_2, 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). ^{13}C NMR (CD_2Cl_2, 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, 2302.0, 2197.1, 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). $[M]_0$ was 0.5 M. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 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_2Cl_2, 300 MHz) δ 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). $[M]_0$ was 0.55 M. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 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_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(11). ^1H NMR (CD_2Cl_2, 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). ^{13}C NMR ($CDCl_3$, 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(17). ^1H NMR ($CDCl_3$, 300 MHz) δ 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) δ 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}.

Polymerization of RGD, SRN, and EO_5 Containing Polymers.

General Polymerization Procedure.

Method 1: Same as for simple polymers as outlined above with $[M]_0/[C]_0$ = 10/1 (homopolymers) or 20/1 (copolymers), $[M]_0$ = 0.7 M, and reaction time of 4 hr.

Method 2: Polymers were synthesized using the following procedure where in a nitrogen-filled dry box, a solution of **2** 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 hours. 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 minutes before isolation. The polymers were precipitated into ether (**26a**, **31-34**), 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 ^1H NMR and deprotected.

General Deprotection Procedure. For all polymers, except **27b**, the following procedure was observed. A solution of TFA, triisopropylsilane (TIS), and H_2O (95/2.5/2.5) solution was added to the dried polymers to make a 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 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.

Solublization in Water. After deprotection, the polymers (except for **26a**) were subjected to a minimum amount of 0.1 N NaOH 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**, **32**, **33a**), glassy solids (**26b**, **27a**, **28a**, **33b**, **34**), 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 homopolymer spectra. All peaks are broad. Except for GPC, **26b** and **27a** were characterized before treatment with base.

26c. Method 2 was followed except that **3** was the initiator. ^{1}H NMR (D_2O, 400 MHz) δ 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}.

27b. Method 2 was followed. ^{1}H NMR (D_2O, 400 MHz) δ 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}.

32. Method 1 was followed. (Note: The following data is for the protected polymer.) ^{1}H NMR (CH_2Cl_2, 400 MHz) δ 6.06, 5.79 (*trans & cis*, 2H, bs), 4.96, 4.46 (*cis & trans*, 2H, bs), 4.18 (*trans & cis*, 2H, bs), 3.51-3.60 (*trans & cis*, 4H, bm), 3.34 (*trans & cis*, 2H, 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).

33a. Method 1 was followed. (Note: The following data is for the protected polymer.) ^{1}H NMR (CH_2Cl_2, 400 MHz) δ 6.06, 5.86 (*trans & cis*, 2H, bs), 4.91, 4.50 (*cis & trans*, 2H, bs), 4.40-4.49 (*trans & cis*,, 4H, bm), 3.33-3.61 (*trans & cis*, 4H, bm), 2.80-3.01 (*trans & cis*, 4H, bm), 1.81-2.25 (*trans & cis*, 6H, bm), 1.43-1.51 (*trans & cis*, 2H, bm).

References

(1) For reviews see: (a) Hynes, R. O. *Cell* **1992**, *69*, 11-25. (b) Ruoslahti, E. *Kidney Int.* **1997**, *51*, 1413-1417. (c) Cary, L. A.; Han, D. C.; Guan, J.–L. *Histol. Histopathol.* **1999**, *14*, 1001-1009. (d) Jones, J. L.; Walker, R. A. *J. Clin. Pathol.: Mol. Pathol.* **1999**, *52*, 208-213.

(2) For reviews see: (a) Ruoslahti E. and Pierschbacher, M. D. *Science* **1987**, *238*, 491-497. (b) Ruoslahti, E. *Annu. Rev. Cell Dev. Biol.* **1996**, *12*, 697-715.

(3) For examples see: (a) Pierschbacher, M. D.; Ruoslahti, E. *Nature* **1984**, *309*, 30-33. (b) Massia, S. P.; Hubbell, J. A. *J. Cell Biol.* **1991**, *114*, 1089-1100.

(4) For reviews see: (a) Pierschbacher, M. D.; Polarek, J. W.; Craig, W. S.; Tschopp, J. F.; Sipes, N. J.; Harper, J. R. *J. Cell. Biochem.* **1994**, *56*, 150-154. (b) Ojima, I.; Chakravarty, S.; Dong, Q. *Bioorg. & Med. Chem.*, **1995**, *3*, 337-360.

(5) For reviews see: (a) Akiyama, S. K.; Olden, K.; Yamada, K. M. *Cancer & Metastasis Rev.*, **1995**, *14*, 173-189. (b) Ruoslahti, E. *Adv. Cancer Res.* **1999**, 1-20.

(6) Obara, M.; Kang, M. S.; Yamada, K. M. *Cell* **1988**, *53*, 649-657. (b) Aota, S.; Nagai, T.; Yamada, K. M. *J. Biol. Chem.* **1991**, *266*, 15938-15943, and references therein.

(7) Aota, S.; Nomizu, M.; Yamada, K. M. *J. Biol. Chem.* **1994**, *269*, 24756-24761.

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

(9) For simplicity, small peptides were chosen. However the polymers can be synthesized using the same approach with larger pendent peptides such as GRGDS and PHSRN.

(10) For a review of these materials, see Saiki, I. *Jpn. J. Pharmacol.* **1997**, *75*, 215-242.

(11) It has been shown that poly(ethylene glycol) accumulated to a significantly higher extent at tumor tissue than at normal tissue. See: Murakami, Y.; Tabata, Y.; Ikada, Y. *Drug Delivery* **1997**, *4*, 23-31.

(12) Komazawa, H.; Saiki, I.; Igarashi, Y.; Azuma, I.; Kojima, M.; Orikasa, A.; Ono, M.; Itoh, I. *J. Bioact. & Compat. Polymers* **1993**, *8*, 258-274.

(13) For an example see: Maynard, H. D.; Grubbs, R. H. *Macromolecules* **1999**, *32*, 6917-6924.

(14) For example see: Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 10714-10721 and references therein.

(15) (a) Coles, M. P.; Gibson, V. C.; Mazzariol, L.; North, M.; Teasdale, W. G.; Williams, C. M.; Zamuner, D. *J. Chem. Soc., Chem. Commun.* **1994**, 2505-2506. (b) Biagini, S. C. G.; Coles, M. P.; Gibson, V. C.; Giles, M. R.; Marshall, E. L.; North, M. *Polymer*, **1998**, *39*, 1007-1014. (c) Biagini, S. C. G.; Davies, R. G.; Gibson, V. C.; Giles, M. R.; Marshall, E. L.; North, M.; Robson, D. A. *Chem. Commun.* **1999**, 235-236.

(16) a) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2039-2041. b) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.*, **1996**, *118*, 100-110.

(17) (a) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953-956. (b) Chatterjee, A. K.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 1751-1753. (c) Chatterjee, A. K.; Morgan, J. P.; Scholl, M.; Grubbs, R. H., manuscript in preparation.

(18) For example, see ref. 15b.

(19) Andrews, R. J.; Grulke, E. A. In *Polymer Handbook*, 4th ed.; Brandrup, J.; Immergut, E. H.; Grulke, E. A., Eds.; Wiley Interscience: New York, 1999, p VI/208.

(20) Maughon, B. R.; Grubbs, R. H. *Macromolecules* **1997**, *30*, 3459-3469.

(21) Bielawski, C.; Grubbs, R. H., manuscript in prepration.

(22) 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.

(23) Ver Nooy, C. D.; Rondestvedt, Jr., C. S. *J. Am. Chem. Soc.* **1955**, *77*, 3583-3586.

(24) Stille, J. R.; Santarsiero, B. D.; Grubbs, R. H. *J. Org. Chem.* **1990**, *55*, 843-862.

(25) Peppas, N. A.; Langer, R. S. *Advances in Polymer Science, Biopolymers.* Germany: Springer, 1995.

(26) Morita, T.; Maughon, B. R.; Grubbs, R. H. *Abstr. Pap. Am. Chem.* **1998**, S214, U414.

Chapter 2

Inhibition of Cell Adhesion to Fibronectin by Oligopeptide-Substituted Polynorbornenes

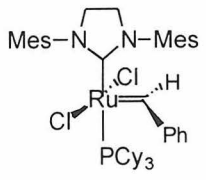
Abstract

Polynorbornenes substituted with two different peptide sequences from the RGD-containing integrin cell binding domain of fibronectin are potent inhibitors of human foreskin fibroblast cell adhesion to fibronectin-coated surfaces. Ring-opening metathesis polymerization (ROMP) using Ru=CHPh$(Cl)_2(PCy_3)$(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 and GRGES containing controls. A homopolymer substituted with GRGDS peptides was significantly more active than the free GRGDS peptide (IC_{50} of 0.18 $\pm$ 0.03 and 1.33 $\pm$ 0.20 mM respectively), and the copolymer containing both GRGDS and PHSRN was the most potent inhibitor (IC_{50} of 0.04 $\pm$ 0.01 mM). These results demonstrate that significant enhancements of observed biological activity can be obtained from polymeric materials containing more than one type of multivalent ligand and that ROMP is a useful method to synthesize such well-defined copolymers.

Introduction

In the previous chapter, the preparation of homopolymers and copolymers substituted with GRGD and SRN peptides using the 2,3-dihydroimidazolylidene catalyst **1** was described. However, these peptides represent truncated versions of the cell adhesion sequences found in fibronectin, and are much less active than expanded sequences GRGDS and PHSRN.[1,2] This chapter reports the synthesis of homopolymers and copolymers substituted with the structurally more complex, biologically active domains GRGDS and PHSRN. A homopolymer substituted with the inactive peptide GRGES[1] was synthesized and tested as a control. In addition, an

analysis of the polymers' biological potency for inhibiting cellular adhesion to fibronectin-coated surfaces was performed.



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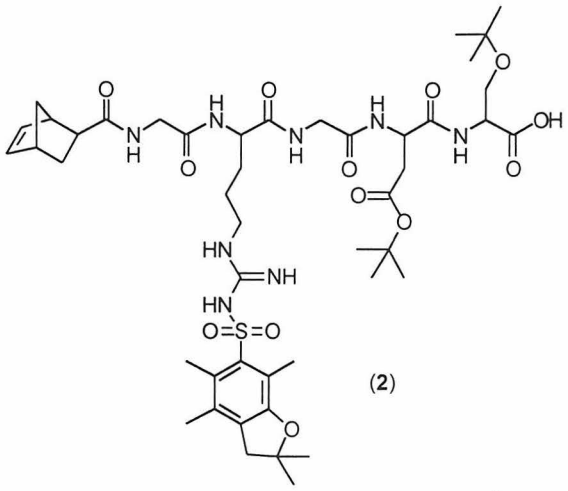

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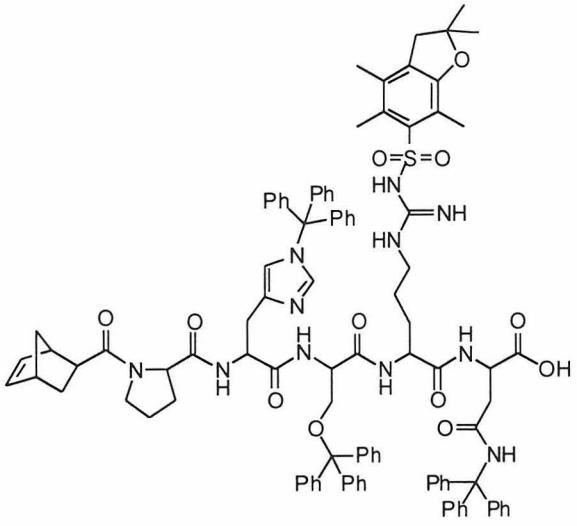

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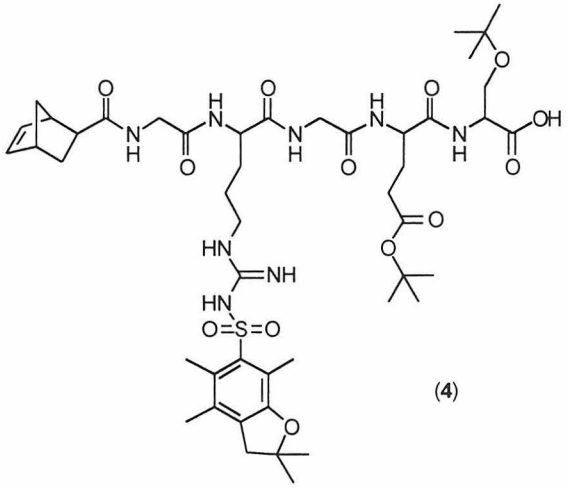

(4)

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^tBu)S(O^tBu)-OH (**2**), synergy sequence PH(Trt)S(Trt)R(Pbf)N(Trt)-OH (**3**), and inactive sequence GR(Pbf)GE(O^tBu)S(O^tBu)-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 appears to be partially deprotected monomer and which persisted in the final compound.

Hompolymers of **2-4** and a copolymer of **2** and **3** were synthesized by ROMP using **1**[3] as an initiator. The monomers were heated in a sealed vial in a 1:1 mixture of CH_2Cl_2 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 CH_2Cl_2 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 (MWCO=3000) followed by lyophilization resulting in the products as spongy, tan-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 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.[4] The percent of GRGDS incorporated into the copolymer was 49% (determined from the ^{1}H NMR spectrum) which corresponded to the amount in the feed (50%).

Table 1. Polymers substituted with oligopeptides.[a]

Peptide (#)	%x in feed	%x in polymer[b]	%yield overall	M_n(x10^3)[c]	PDI
GRGDS (**5**)	100%	100%	91%	12.0	1.37
PHSRN (**6**)	100%	100%	40%	ns	ns
GRGES (**7**)	100%	100%	84%	11.5	hs
GRGDS/PHSRN (**8**)	50/50%	49/51%	64%	9.14	1.30

[a]General reactions conditions: CH_2Cl_2:MeOH (1:1) as the solvent, 55 °C for 2 h in a sealed vial, [M]/[C]=10/1, $[M]_0$=0.6M. [b]Calculated from the ^{1}H NMR spectra. [c]Determined by GPC, pH 8.0 phosphate buffer eluent, poly(ethylene oxide) standards. ns = not soluble in aqueous solutions. 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_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, likely the partially deprotected monomer, 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 be more potent at inhibiting cell adhesion to fibronectin than the free peptide due to multivalent interactions provided by the polymer scaffold.[5,6] In addition, since PHSRN enhances cell binding to the RGD domain in fibronectin,[7] it was predicted that a copolymer substituted with both GRGDS and PHSRN would exhibit higher competitive inhibitory activity than materials containing only GRGDS. To investigate this, the ability of the polymers and peptides to inhibit HFF cell adhesion to fibronectin-coated surfaces was determined, following a known procedure.[1] Briefly, normal HFF 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 solutions were removed and the cells were fixed with methanol. 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.

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[8] as well as PHSRN, which is known to be inactive unless covalently bound in the vicinity of the RGD containing peptide,[7] demonstrate no inhibitory activity.

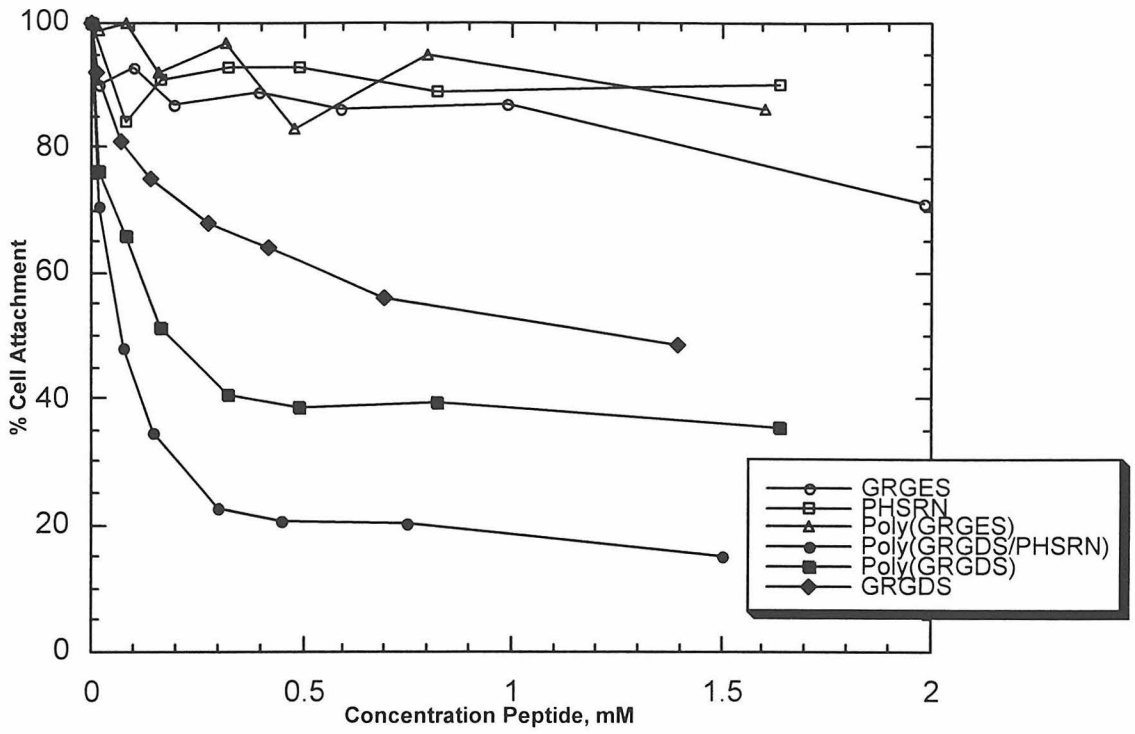

Figure 1. Inhibition of normal HDF cell attachment to fibronectin by the polymers and free peptides. The HDF cells (35,000 cells/mL) were incubated in human plasma fibronectin coated wells at 37 °C for 60 min in the presence of increasing concentrations of soluble peptides or peptide substituted polymers. 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 data for GRGDS peptide, Poly(GRGDS) and Poly(GRGDS/PHSRN) represents the averaged values from two experimental trials (as described in the text).

The control polymer substituted with GRGES (**7**), an inactive peptide,[1,2] did not inhibit HFF adhesion to fibronectin. Cells spread normally and effectively in the presence of this polymer, 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.

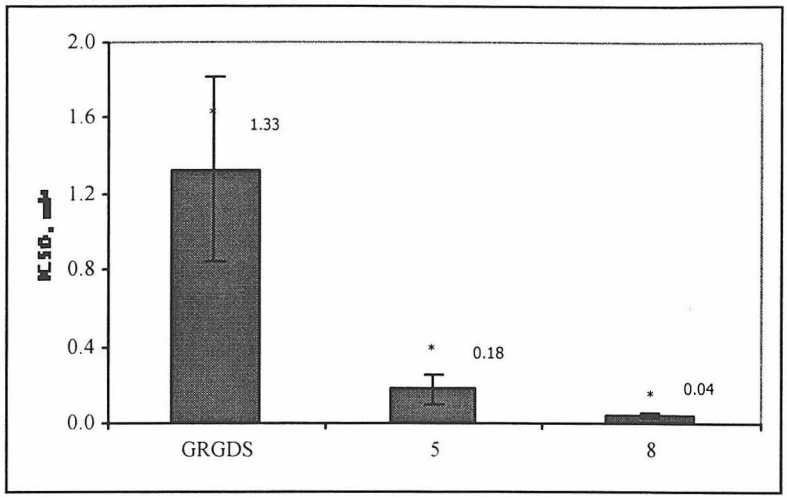

Figure 2. IC_{50} values for the GRGDS containing materials. Data is reported as the concentration of the GRGDS (peptide) or GRGDS repeat unit (polymers) and is the mean $\pm$ the standard error of the mean. *Within a >99.9% confidence level, this value is statistically different from the other two.

The inhibitory potencies were quantified for the peptide, **5**, and **8**. The IC_{50} values, or concentration of GRGDS necessary to inhibit 50% of the cells from attaching, are compared based on the concentration of GRGDS repeat unit (polymers) or peptide as shown in Figure 2.[9] For GRGDS, an IC_{50} of 1.33 $\pm$ 0.20 mM was obtained, which is within the literature ranges obtained for this peptide.[2,10] Polymer **5** has a lower IC_{50} of 0.18 $\pm$ 0.003 mM, and **8** is the most active with an IC_{50} of 0.04 $\pm$ 0.01 mM. These values are all statistically different from each other ($p < 0.004$ between each value).

The inhibition experiments involving GRGDS, **5**, and **8** were all duplicated in a second series of trial runs using peptides and polymers synthesized separately from the original batch. The results of these duplicate experiments fell with experimental error of the original results.

The data presented above for GRGDS and polymers **5** and **8** represents the averaged values from these two experimental series.

Both GRGDS-containing polymers inhibited HFF 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. In addition, polymer **8** with GRGDS and PHSRN ligands was the most potent substrate studied. The polymer backbone provides the covalent linkage necessary to obtain the PHSRN-induced enhancement of RGD adhesive activity.[7] In fibronectin, PHSRN is 30-40 Å away and on the same face of the protein as RGD.[6] 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. Overall, the results suggest that the strategy of incorporating two types of substrates into polymeric materials is a useful one to increase the biological activity of the resulting multidentate ligands.

Conclusions

These results highlight the applicability of ROMP for the synthesis of polymeric materials that are potent inhibitors of cell adhesion to the extracellular matrix protein fibronectin. Homopolymers and copolymers are readily synthesized that contain complex oligopeptide substituents of the RGD-integrin binding domain of fibronectin, GRGDS and PHSRN. The GRGDS-containing homopolymer, a multidentate ligand, was almost 750% more active in a competitive inhibition experiment than the GRGDS peptide. The presence of the synergistic peptide PHSRN, in addition to the GRGDS peptide, further enhanced the inhibitory activity; the copolymer was greater than 3300% more potent than the GRGDS peptide. Such materials may

be used to study and 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.[11] 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 tissue culture polystyrene (TCPS) 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 foreskin fibroblast (HFF) 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) 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 a 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_2HPO_4 dibasic buffer, the flow rate was 1 mL/min, and poly(ethylene oxide)s were used as the calibration standards. 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). Amino acid analysis was performed by the Beckman Research Institute in the Division of Immunology (City of Hope). All cell manipulations were conducted 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(O^{t}Bu)S(O^{t}Bu)-OH Monomer (2). H_2N-G-R(Pbf)-G-D(O^{t}Bu)-S(O^{t}Bu)-resin (0.75 mmol peptide, 4-carboxytrityl linker Novasyn® resin) was placed in a flask containing a frit and stopcock. The resin was swelled in 20 mL of DMF for 15 min and the solution was removed before rinsing 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 6.6 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 before addition 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_2Cl_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_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 until all of the acetic acid was removed. 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. ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 8.09 (1H, bm), 7.58 (1H, bm), 7.49 (1H, bm), 7.41 (1H, bm), 7.23 (1H, bm), 6.27 (1H, bm), 6.02 (1H, s), 5.98 (1H, s), 4.67 (1H, bm), 4.43 (1H, bm), 4.32 (1H, bm), 3.88 (3H, bm), 3.77 (1H, bm), 3.67 (1H, bm), 3.54 (1H, bm), 3.12 (2H, bm), 2.87 (2H, s), 2.82 (1H, s), 2.77 (1H, s), 2.73 (1H, bm), 2.66 (1H, bm), 2.45 (3H, s), 2.38 (3H, s), 2.08 (1H, bm), 1.98 (3H, s), 1.80 (1H, bm), 1.72 (1H, bm), 1.61 (1H, bm), 1.50 (3H, bm), 1.36 (6H, s), 1.33 (9H, s), 1.19 (2H, bm), 1.07 (9H, s). NOESY cross peaks (CD_2Cl_2, 600 MHz) δ 8.09 ($G2_{NH}$), 4.32 (R_α); 7.58 (D_{NH}), 3.88 ($G2_\alpha$) + 3.77 ($G2_\alpha$); 7.49 (R_{NH}), 3.88 ($G1_\alpha$); 7.41 (S_{NH}), 4.67 (D_α); 7.23 ($G1_{NH}$), 2.08 (Nor_{CHCO}). TOCSY cross peaks (CD_2Cl_2, 600 MHz) δ 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, 3.77; D: 7.58, 4.67, 2.73, 2.66; S: 7.41, 4.43, 3.67, 3.54. ^{13}C NMR (CD_2Cl_2, 300 MHz) δ 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, 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_2N-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 6.3 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_2Cl_2, 600 MHz) δ 8.29 (1H, bm), 7.59 (1H, bm), 7.47 (1H, bm), 6.89-7.29 (48H, bm with max. at 6.89, 7.04, 7.13, 7.22, 7.28, 7.29), 6.63 (1H, bm), 5.98 (2H, bm), 5.84 (1H, bm), 4.45 (1H, bm), 4.13-4.30 (4H, bm), 3.36-3.47 (4H, bm), 2.65-2.93 (10H, bm), 2.40 (3H, s), 2.34 (3H, s), 1.54-2.08 (13H, bm with max. at 1.54, 1.63, 1.76, 1.98, 2.10), 1.34 (7H, bm), 1.22 (2H, bm). ^{13}C NMR (CD_2Cl_2, 300 MHz) δ 175.65, 174.74, 174.27, 172.87, 172.10, 171.27, 170.01, 158.49, 156.70, 144.51, 143.85, 141.86, 138.37, 138.12, 136.19, 135.91, 132.32, 129.90, 128.83, 128.49, 128.42, 128.06, 127.91, 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, 43.51, 43.07, 42.76, 41.98, 41.90, 30.81, 30.33, 30.06, 29.62, 28.70, 25.35, 25.26, 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, 1264.8, 1154.3, 1096.4, 1033.3, 896.5, 738.7, 696.6 cm^{-1}. HRMS (MALDI) calcd for $(MNa)^+$ 1708.7737, found 1708.7739.

Norbornene-G-R(Pbf)-G-E(O^{t}Bu)-S(O^{t}Bu)-OH (4). The same procedure as for **2** was followed with H_2N-G-R(Pbf)-G-E(O^{t}Bu)-S(O^{t}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 6.6 mL DMF to yield 0.11 g (46.1%) of **4** (plus an impurity detected in the ^{1}H NMR spectrum) as a white solid. HPLC: single peak at 24.35 min. ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 8.07 (1H, bm), 7.53-7.62 (3H, bm), 7.23 (1H, bm), 6.23-6.28 (2H, bm), 6.02 (1H, s), 5.99 (1H, s), 4.42 (1H, bm), 4.25-4.28 (2H, bm), 3.79-3.86 (4H, bm), 3.68 (1H, bm), 3.56 (1H, bm), 3.11 (2H, 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, bm),

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): 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 cm^{-1}. 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 CH_2Cl_2 was added to a solution of monomer in MeOH (to achieve 1:1 CH_2Cl_2:MeOH) contained in a dram to give an initial monomer concentration of 0.6 M. The dram was sealed and removed from the dry 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_2Cl_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_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 2.5 h before precipitation 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. Polymers **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 cold 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 Centriprep tubes with a molecular weight cut off (MWCO) of 3000. 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 ^{1}H NMR copolymer spectrum is the addition of the two homopolymer spectra. All peaks unless otherwise stated, are the composite of the *trans* and *cis* polymer proton peaks.

GRGDS Homopolymer (5). ^{1}H NMR (D_2O, 400 MHz): δ 7.33 (NH, bm), 5.34 (2H, bm), 3.82-4.32 (7H, bm with max. at 3.82, 3.94, 4.23), 2.58-3.22 (7H, bm with max. at 2.62, 2.68, 3.16), 1.19-1.87 (8H, bm with max. at 1.61, 1.73, 1.87). 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_2O, 400 MHz): δ 7.30 (NH, bm), 5.34 (2H, bm), 3.79-4.31 (8H, bm with max. at 3.79, 3.92, 4.21, 4.31), 2.54-3.13 (5H, bm with max. at 2.54, 3.13), 1.09-2.20 (12H, bm with max. at 1.55, 1.85, 2.08, 2.20). 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_2O, 400 MHz): δ 7.62, 7.22, 6.92, 5.33, 4.14-4.43 (with max. at 4.22, 4.35, 4.43), 3.74-3.92 (with max. at 3.81, 3.92), 3.12, 2.61, 1.16-2.13 (with max. at 1.58, 1.71, 1.85). 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. HFF 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% CO_2. 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 cm^2 flasks at a density of 3500 cells/cm^2.

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, 100 μl of the polymer or peptide solutions or PBS alone (control) was added. HFF 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% CO_2. The attached cells were fixed with methanol after removal of the sample solutions and washing with deionized H_2O. 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 IC_{50} is the concentration where 50% of the maximum possible cells are attached, reported and the mean $\pm$ standard error of the mean. The p values were determined by nonparametric unpaired two group analysis using StatView®.

References

(1) Humphries, M. J.; Akiyama, S. K.; Komoriya, A.; Olden, K.; Yamada, K. M. *J. Cell Biol.* **1986**, *103*, 2637-2647.

(2) Pierschbacher, M. D.; Ruoslahti, E. *J. Biol. Chem.* **1987**, *262*, 17294-17298.

(3) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, 1, 953-956.

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

(5) Aota, S.; Nomizu, M.; Yamada, K. M. *J. Biol. Chem.* **1994**, 269, 24756-24761.

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

(7) PEG Hydrogels were grafted with RGD and PHSRN domains located in a single peptide. See: Kao, W. J.; Hubbell, J. A.; Anderson, J. M. *J. Mat. Sci. Mat. Med.* **1999**, 10, 601-605.

(8) (a) Kanai, M.; Mortell, K. H.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, 119, 9931-9932. (b) Strong, L. E.; Kiessling, L. L. *J. Am. Chem. Soc.* **1999**, 121, 6193-6196.

(9) The peptide and polymers are compared on a GRGDS peptide molar basis because PHSRN alone is known to be inactive towards cellular binding, see reference.

(10) Pierschbacher, M.D.; Ruoslahti, E. *Nature* **1984**, 309, 30-33.

(11) Ver Nooy, C. D.; Rondestvedt, C. S., Jr. *J. Am. Chem. Soc.* **1955**, *77*, 3583-3586.