Inhibition of Cell Adhesion to Fibronectin by Oligopeptide-Substituted Polynorbornenes

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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 ± 0.03 and 1.33 ± 0.20 mM respectively), and the copolymer containing both GRGDS and PHSRN is the most potent inhibitor (IC_{50} of 0.04 ± 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

Many extracellular matrix proteins bind to cell surface integrins through the short peptide sequence RGD,[1,2] and this peptide alone has been shown to mediate 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 such as cancer.[4] However, to date, the therapeutic use of this peptide has been limited. The primary disadvantage is that the affinity of the peptide to cellular integrins is significantly lower (>1000 times lower) than that of the proteins from which it is derived.[1b] Thus, the ability to synthesize RGD-containing materials with increased therapeutic potentials represents a significant undertaking.[5]

Conjugating many copies of a ligand to a single-polymer backbone has proven to be a successful way to increase ligand affinity and specificity through multivalent interactions.[6] A synthetic polymer with pendent RGD peptides synthesized by radical polymerization that inhibits lung and liver metastasis in vivo at a lower dose (1 mg) than did RGD itself (3 mg) has been reported.[7] Although this result demonstrates the usefulness of a multidentate RGD ligand, the required doses are still very high. This and most reported examples of polymeric multidentate ligands contain only one type of ligand. Our strategy is to increase the potency of RGD by the conjugation of a synergistic peptide in addition to RGD to a single polymer backbone. The important question is whether the random placement of the synergistic peptide in a copolymer and the flexibility of the polymer backbone will allow synergy between the two active peptide fragments.

Binding of cells to the extracellular matrix protein fibronectin through the RGD domain is enhanced by the oligopeptide sequence Pro-His-Ser-Arg-Asn (PHSRN).[8] Both sequences are located on the same side of the protein, approximately 30−40 Å apart.[9] Due to their accessibility and proximity, it has been proposed that one receptor binds to both sequences simultaneously.[9] This synergistic binding has been mimicked by the small peptides only, however, when the peptides were covalently attached to the same construct.[8] Therefore, the conjugation of PHSRN to RGD-containing materials should increase the cell-binding affinity of the material. Despite this, most biomaterials are substituted with RGD only,[10] partly due to the lack of synthetic methods to synthesize materials containing both sequences. For example, the synthetic RGD polymer described above was synthesized by radical chemistry, and thus the controlled synthesis of copolymers would be difficult.[7] Ring-opening metathesis polymerization (ROMP) provides a better

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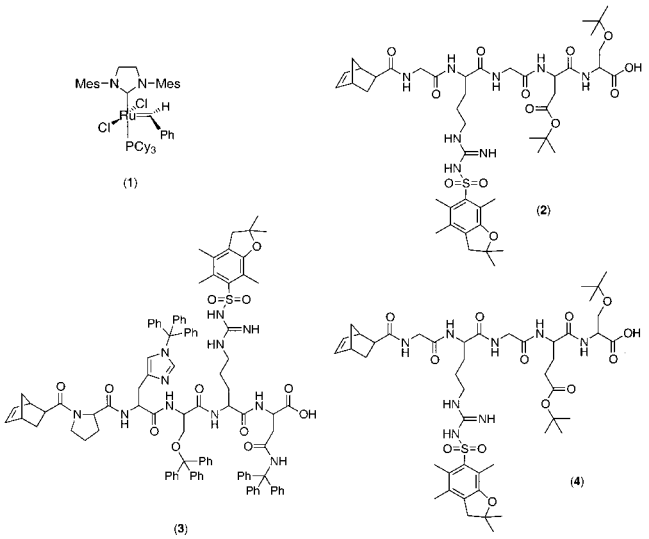
alternative for the preparation of biologically relevant copolymeric materials.

ROMP has several advantages over classical methods for the synthesis of biologically relevant copolymers. For example, the synthesis of copolymers is straightforward because the polymer composition corresponds exactly to the initial monomer feed ratios.[11] In addition, the initial monomer-to-catalyst ratio dictates the molecular weight and the polymerization can be living.[12] Also, with the advent of highly active, functional group tolerant, 2,3-dihydroimidazolylidene catalysts such as **1**,[13] the synthesis of copolymers substituted with complex, biologically relevant ligands by ROMP is now possible. We recently reported a synthetic strategy for the preparation of copolymers substituted with the truncated peptides GRGD and SRN, where the use of these catalysts was necessary to achieve high yields; however, the biological activities of these polymers were not determined.[11] Because the truncated GRGD peptide is known to be significantly less active than the GRGDS peptide,[14,15] to access the biological potency of the polymeric materials the preparation of the more structurally complex polymers substituted with GRGDS and PHSRN is necessary. Here we report the synthesis of homopolymers and copolymers substituted with the biologically active domains GRGDS and PHSRN and the inactive peptide GRGES[14,15] as a control, as well as their respective biological potency for inhibiting cellular adhesion to fibronectin-coated surfaces.



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^{t}Bu)S(O^{t}Bu)-OH (**2**), synergy sequence PH(Trt)S(Trt)R(Pbf)N(Trt)-OH (**3**), and inactive sequence GR(Pbf)GE(O^{t}Bu)S(O^{t}Bu)-OH (**4**) were synthesized in 91, 97, and 47% yield, respectively. While **2** and **3** were >95% pure after cleavage from the resin, **4** contained an impurity which appeared to be partially deprotected monomer and which persisted in the final compound.

Scheme 1. Synthesis of Homo- and Copolymers with Pendent Bioactive Oligopeptides by ROMP

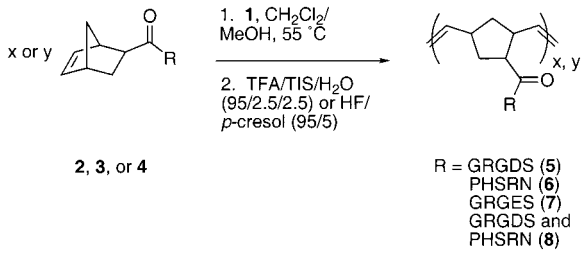


Table 1. Polymers Substituted with Oligopeptides[a]

peptide	%*x* in feed	%*x* in polymer[b]	% yield overall	M_n ($\times 10^3$)[c]	PDI[c]
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, $[M]_0$ = 0.6 M. [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.

Hompolymers of **2**−**4** and a copolymer of **2** and **3** were synthesized by ROMP using **1**[13] as an initiator (Scheme 1). 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 h. 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 min 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 (Table 1) for the GRGDS- and GRGES-containing polymers were excellent (91 and 84%, respectively). The yields of the polymers containing PHSRN were somewhat reduced (40% for **6** and 64% for **8**). This could be due to the rapid degradation of **1** in the presence of this peptide.[16] 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%).

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)

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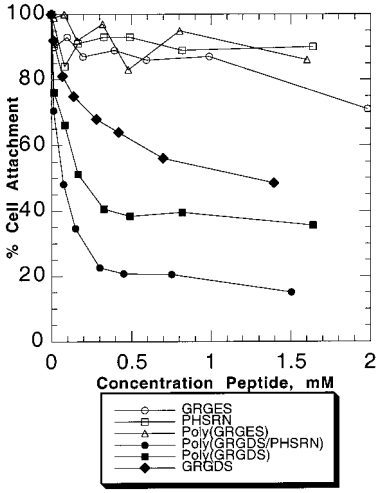

Figure 1. Inhibition of normal HFF cell attachment to fibronectin-coated surfaces by the polymers and free peptides. The HFF cells (35 000 cells/mL) were incubated in human plasma fibronectin-coated wells at 37 °C for 60 min in the presence of increasing concentrations of the soluble peptides, peptide-substituted polymers, or PBS controls. Adherent cells were fixed and counted by viewing a minimum of six randomly selected fields per well. The concentrations of the polymers are based on that of the GRGDS (**5** and **8**) or GRGES (**7**) repeat unit. The reported data represents the averaged values from three to nine experiments.

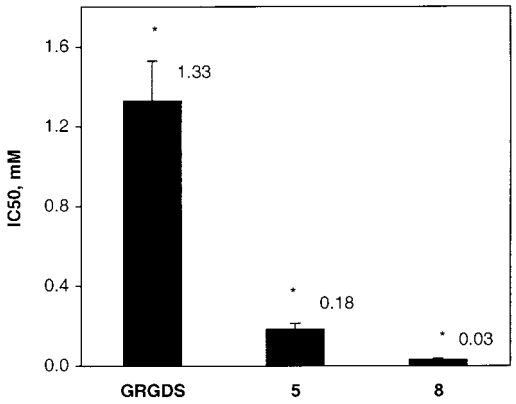

Figure 2. IC_{50} values for the GRGDS-containing materials. Data obtained from a minimum of six experiments is reported as the concentration of the GRGDS (peptide) or GRGDS repeat unit (polymers) as the mean ± the standard error of the mean. *With a >99.9% confidence level, this value is statistically different from the other two.

determined by GPC (compared to those of poly(ethylene glycol) standards) were between 9100 and 12 000. The polydispersity indexes (PDIs) were narrow for **5** and **8** (1.37 and 1.30, respectively). The GPC trace of **7** exhibited a slight high-molecular weight shoulder. As mentioned above, the monomer **4** contained an impurity which could have caused the observed molecular weight distribution.

Inhibitory Activity of Polynorbornenes Substituted With Oligopeptides. It was originally hypothesized that polymers substituted with many GRGDS peptides along the backbone would be more potent at inhibiting cell adhesion to fibronectin than the free peptide due to multivalent interactions provided by the polymer scaffold. In addition, since PHSRN enhances cell binding to the RGD domain in fibronectin,[8] 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.[14] Briefly, normal HFF neonatal cells were added to human plasma fibronectin-coated wells containing a known concentration of polymer or peptide and incubated at 37 °C for 1 h. The solutions were removed, and the cells 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** had an inhibitory effect. The inactive peptide GRGES[14,15] as well as PHSRN, which is known to be inactive unless covalently bound in the vicinity of the RGD-containing peptide,[8] demonstrated no inhibitory activity.

The control polymer substituted with GRGES (**7**), an inactive peptide,[14,15] 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** was due solely to the peptides substituted along the backbone. In addition, the percent cell attachment levels off for both **5** and **8** rather than continuing to decline (Figure 1), providing further indication that the polymers are nontoxic within the concentrations used for this experiment.

The inhibitory potencies were quantified for GRGDS, **5**, and **8**. The IC_{50} values, or concentration of GRGDS (peptide) or GRGDS repeat unit (polymers) necessary to inhibit 50% of the cells from attaching, are compared in Figure 2.[17] For GRGDS, an IC_{50} of 1.33 ± 0.20 mM was obtained, consistent with the literature.[3a,15] Polymer **5** had a lower IC_{50} of 0.18 ± 0.003 mM, and **8** was the most potent with an IC_{50} of 0.04 ± 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 within experimental error of the original results. The data presented above for GRGDS and polymers **5** and **8** represent 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, copolymer **8** with GRGDS and PHSRN ligands was the most potent substrate studied. The polymer backbone provided the covalent linkage necessary to obtain the PHSRN-induced enhancement of RGD adhesive activity. In fibronectin, PHSRN is 30−40 Å away and on the same face of the protein as RGD.[9] One integrin can easily span this distance. The above results indicate that the polymer backbone also provided 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 multifunctional substrates onto polymeric backbones is a useful way to create a diversity of biological activity of the resulting multidentate and multifunctional ligands.

(17) The peptide and polymers are compared on a GRGDS peptide molar basis because PHSRN alone is known to be inactive towards cellular binding, see ref 8.

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 a copolymer substituted with complex oligopeptide substituents of the RGD-integrin binding domain of fibronectin, GRGDS, and PHSRN were readily synthesized. 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 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 Section

Materials. 5-Norbornene-*exo*-2-carboxylic acid was synthesized according to a literature procedure.[18] 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. Drybox operations were performed in a nitrogen-filled Vacuum Atmospheres drybox. ^{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 thin 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) was 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 (TSK 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 200× 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 (0.25 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 × 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 × 10 mL), CH_2Cl_2 (5 × 10 mL), and MeOH (5 × 10 mL) and dried for 24 h at 30 mTorr. 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 0.22 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 (0.25 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 of DMF to yield 0.41 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 of 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,

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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 drybox, 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 drybox. 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 and 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 were 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 were washed with cold ether (2 × 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 solids were 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 were 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 five times for each polymer. The polymer solutions were then subjected to centrifugation, the solution was 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 are reported below. All peaks 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 4× 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 of the cell solutions 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 six 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 and is reported as the mean ± standard error of the mean. The *p* values were determined by nonparametric unpaired two group analysis using StatView.

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Supporting Information Available: Spectral data for compounds **5**, **7**, and **8** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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