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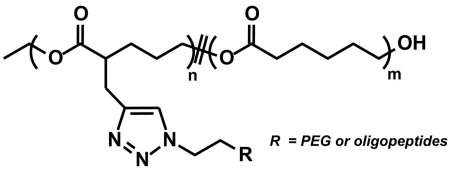

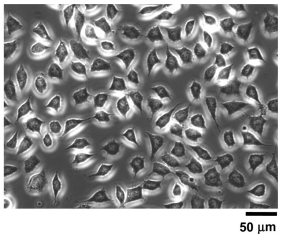

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PEG- and Peptide-Grafted Aliphatic Polyesters by Click Chemistry

Bryan Parrish, Rebecca B. Breitenkamp, and Todd Emrick*

Contribution from the Polymer Science & Engineering Department, University of Massachusetts, Conte Center for Polymer Research, Amherst, Massachusetts 01003

Received January 17, 2005; E-mail: tsemrick@mail.pse.umass.edu

Abstract: Novel aliphatic polyesters with pendent acetylene groups were prepared by controlled ring-opening polymerization and subsequently used for grafting poly(ethylene glycol) and oligopeptide moieties by the Cu(I)-catalyzed addition of azides and alkynes, a type of "click" chemistry. These aliphatic polyesters possess an acetylene graft density that can be tailored by ring-opening copolymerization of α-propargyl-δ-valerolactone (**1**) with ϵ-caprolactone. Since the mild conditions associated with the click reaction are shown to be compatible with the polyester backbone, this method is a generally useful means for grafting numerous types of functionality onto aliphatic polyesters. The amphiphilic graft polyesters prepared in this study are shown to be biocompatible by in vitro cytotoxicity evaluation, suggesting their suitability for a range of biomaterial applications.

Introduction

Aliphatic polyesters are generally considered to be well-suited for applications as polymer-based biomaterials due to their demonstrated biocompatibility and biodegradability.[1] Medical applications for aliphatic polyesters include degradable sutures, drug delivery vehicles, implant materials, and tissue engineering scaffolds.[2−6] While polymeric materials based on ϵ-caprolactone (ϵ-CL), lactide, and glycolide are currently used in numerous biomaterial applications, such polyesters are limited in scope due to their hydrophobic and semicrystalline properties and the absence of functionality on the polymer backbone, which could otherwise be used for tailoring physical properties and introducing bioactive moieties.

Numerous examples of chain-end functionalized aliphatic polyesters have been reported, prepared most commonly by the use of functional nucleophiles to initiate ring-opening lactone polymerization.[7−9] However, pendent functionalization provides a unique opportunity to alter physical and chemical properties by distributing functionality along the polymer backbone. This imparts a structural homogeneity that is distinct from other materials including block copolymers. In degradable polymers, structural homogeneity can assume considerable importance as degradation of such materials yields polymer fragments with relatively similar properties. This may be particularly relevant in biomaterial applications where the properties of the degradation products are critically important on multiple levels including their structure, function, toxicity, and clearance rate.[10,11]

Pendent functionalization of aliphatic polyesters can be achieved by polymerization of functionalized lactones, post-polymerization modification, or a combination of these two approaches. When polymerizing functionalized lactones, the functionality must be chemically compatible with the polymerization conditions and not interfere sterically with the ring-opening polymerization. In the case of post-polymerization modification, the conditions chosen must not induce degradation or undesired cross-linking, a challenging constraint, considering the labile nature of the polyester backbone. Nevertheless, several notable reports have appeared over the years including aliphatic polyesters with pendent allyl,[12] hydroxyl,[13] poly(ethylene glycol) (PEG),[14,15] and dendritic[16] functionalities. Hyperbranched[17] and dendritic[10] architectures based on aliphatic polyesters have also been prepared, which contain very high levels of chain-end functionality relative to conventional linear polyesters and thus offer multiple opportunities for covalent substitution and grafting of functional units.

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Our research in this area has focused on the ring-opening polymerization of lactones containing unsaturated substituents α to the carbonyl, such as allyl[18] and cyclopentene[14] groups, to generate polyesters with pendent olefins. In the case of allyl substitution, the pendent olefins were converted to 1,2-diols by dihydroxylation with OsO_4/*N*-methylmorpholine-*N*-oxide (NMO). These hydroxyl-substituted polyesters undergo slow degradation to low-molecular weight material at low diol grafting densities (10−15 mol %) and rapid degradation at high diol grafting densities. In contrast, dihydroxylation of polyesters with pendent cyclopentene groups affords materials with excellent shelf stability due to the steric constraints imposed by the cyclopentyl ring that prevent intramolecular cyclization. The stability of these functionalized polyesters is very useful for facilitating subsequent chemical modification as we demonstrated in the case of esterification with a carboxylic acid-terminated PEG (PEG−$C(O)CH_2CH_2CO_2H$).[14]

Here, we describe an approach to aliphatic polyesters with grafted functionality that again takes advantage of pendent unsaturation and involves fewer synthetic transformations and greater versatility than our previously reported methods. This method utilizes the stepwise analogue of the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and alkynes, a type of "click" chemistry that has been developed and utilized elegantly in recent years. For example, Sharpless, Finn, and co-workers have employed click chemistry in small-molecule organic synthesis,[19,20] Fokin, Fréchet, Hawker, and co-workers in the synthesis of dendrimers[21] and dendronized polymers,[22] and Tirrell and co-workers on biologically derived macromolecular structures.[23] This click chemistry, which results in triazole formation, is quite versatile and provides a method for coupling a wide range of molecules in a regiospecific fashion under relatively mild reaction conditions with few byproducts. Click chemistry benefits from the facile introduction of azide and alkyne groups into organic and polymer molecules, the stability of these groups to many reaction conditions, and the tolerance of the reaction to the presence of other functional groups. Thus, the application of click chemistry to aliphatic polyesters appeared promising and particularly valuable, given the sensitivity of the polyester backbone to the conditions required for many conventional organic transformations and couplings.

Our efforts to extend click chemistry to aliphatic polyesters have centered on PEG- and peptide-grafting. The known properties of PEG, including water solubility, amphiphilicity, and resistance to protein adsorption, make the polyester−*graft*−PEG structure highly desirable for biomaterial and drug delivery applications.[24] The use of click conditions for peptide grafting is of particular interest due to the incompatibility of aliphatic polyesters with conventional solid-phase peptide synthesis. It should be noted that a prior example of aliphatic polyesters with pendent peptide functionality was reported by Langer and co-workers.[25] The structure was achieved by copolymerization of L,L-lactide with an L-methyl-2,5-morpholinedione bearing a protected lysine residue. However, problems were encountered using this method including cross-linking upon reaction of deprotected lysine residues with oligopeptides. In contrast, the click method outlined here is less tedious and provides a clean route to functional aliphatic polyesters without such complications.

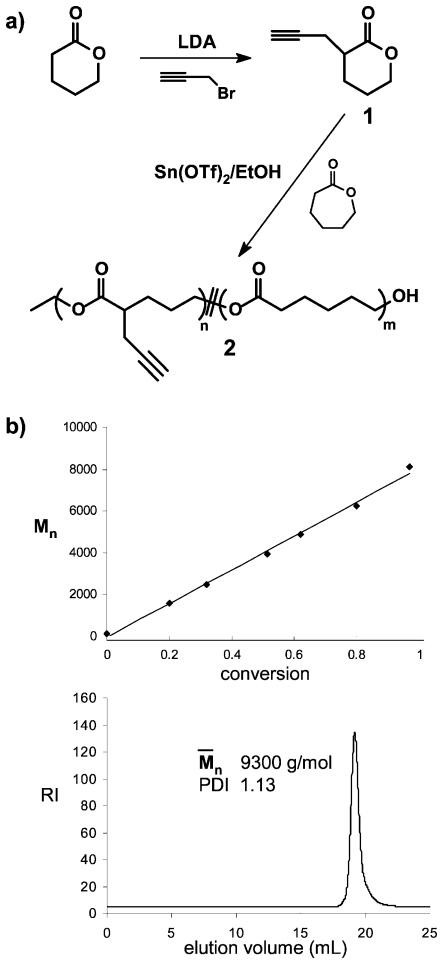

Figure 1. (a) Synthesis and homo/copolymerization of α-propargyl-δ-valerolactone; (b) plot of molecular weight vs conversion (top) and GPC trace (bottom) for homopolymerization of **1**.

We focused specifically on click reactions of aliphatic polyesters bearing pendent acetylenes with azide-terminated PEG 1100 monomethyl ethers and azide-terminated RGD-containing oligopeptides due to the well-known cell adhesion activity of the RGD sequence.[26] The alkyne functionality was introduced to polyester **2** by the ring-opening polymerization of α-propargyl-δ-valerolactone (**1**) as shown in Figure 1. The synthesis of **1** was reported previously by Schlessinger and co-workers,[27] but there is no previous account of its polymerization. Lactone **1** was incorporated into polyesters in controlled amounts based on its homopolymerization and copolymerization with ϵ-CL. Azide-terminated PEG-1100 monomethyl ether (**3**) and

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the peptide sequence GRGDS (**6**) were prepared and grafted to the polyester under Cu(I)-catalyzed click conditions. Moreover, in vitro cytotoxicity testing[28,29]of the PEG-functionalized aliphatic polyesters was conducted to confirm their suitability for biomaterial applications.

Results and Discussion

Synthesis of Aliphatic Polyesters with Pendent Acetylene Groups. Lactone (**1**) was prepared from commercially available δ-valerolactone by reaction with lithium *N*,*N*-diisopropylamide (LDA) in tetrahydrofuran (THF) at −78 °C, followed by quenching with a toluene solution of propargyl bromide. Kugelrohr distillation of the crude product at 140 °C gave **1** as a colorless, viscous liquid in 74% yield. Spectroscopic characterization of **1** confirms its structure. In the ^{1}H NMR spectrum, the terminal acetylene proton is observed at δ 1.97, and in the ^{13}C NMR spectrum, the acetylene carbons resonate at δ 81.1 and 68.6. The IR spectrum of **1** reveals an acetylene C−H stretch at 3280 cm^{-1}, and high-resolution mass spectrometry (HRMS) gives a molecular mass of 139.078 g/mol (calculated 139.076 g/mol for $[M + H]^+$).

$Sn(OTf)_2$-mediated ring-opening homopolymerization[30] of lactone **1** was performed neat at room temperature with ethanol as the initiator, yielding polymers of type **2**. The polymerizations proceeded to greater than 90% conversion in 48 h using 3 mol % catalyst relative to the initiator. The increase in polyester molecular weight as a function of lactone conversion was monitored by gel permeation chromatography (GPC) in THF (relative to polystyrene standards) as well as NMR analysis of aliquots removed from the reaction mixture. Percent conversion was calculated by integration of the ^{1}H NMR spectral signals at δ 4.28 (CH_2O in the monomer) and δ 4.03 (CH_2O in the polymer backbone). The controlled nature of the homopolymerization of lactone **1** was demonstrated by the linear increase in molecular weight with conversion and narrow polydispersity indices (PDIs) of the isolated polyesters as shown by the GPC trace in Figure 1. Molecular weights in the 6−20 K range were typically achieved, and target molecular weights were in good agreement with the monomer-to-initiator ratio. As expected, polymerizations that were allowed to proceed to full conversion displayed higher PDIs (ca. 1.3−1.4) due to transesterification that competes with propagation at low monomer concentrations.

Control over pendent acetylene density was achieved by copolymerization of **1** with ϵ-CL. While the homopolymerization rate of **1** is slower than that of ϵ-CL, the two lactones copolymerize readily over the entire range of possible incorporations. The extent of **1** incorporated into the copolymer structure was calculated by integration of the ^{1}H NMR spectral signals at δ 2.01 (C≡C-*H* from monomer **1**) against the signal at δ 4.03 (CH_2O of the polymer backbone from **1** and ϵ-CL). As shown in Table 1, the spectroscopically calculated incorporations of monomer **1** agree well with the feed ratios, and the narrow PDIs (ca. 1.11) of these aliphatic polyesters, both as homopolymers and copolymers, further demonstrate the controlled nature of ring-opening polymerization with this functional lactone.

Table 1. Number Average Molecular Weights (M_n) and Polydispersity Indices (PDI) of Homo/Co-polymers of α-Propargyl-δ-valerolactone and ϵ-Caprolactone

polymer	feed ratio (**1**:CL)	incorporation (**1**)[a]	M_n[b]	PDI[b]
$\mathbf{2_a}$	10:90	10	7.9	1.11
$\mathbf{2_b}$	25:75	23	8.0	1.10
$\mathbf{2_c}$	50:50	47	7.5	1.11
$\mathbf{2_d}$	100:0	100	6.0	1.11

[a] Determined from ^{1}H NMR spectra. [b] M_n x 10^3 g/mol determined by GPC relative to polystyrene standards.

The ^{13}C NMR spectra of these copolymers exhibit several overlapping signals in the carbonyl region (from δ 174.1 to 173.3), indicating a random distribution of functional and nonfunctional monomer units along the polyester backbone (a block copolymer composed of **1** and ϵ-CL would give only two carbonyl signals corresponding to the two homopolymer-like segments).[31] Incorporation of lactone **1** disrupts the crystallinity characteristic of conventional aliphatic polyesters.[32] Greater than 25 mol % incorporation of **1** results in polyester copolymers that are viscous liquids at room temperature.

Polyester-*graft*-PEG Copolymers of Type 4 Prepared by Click Chemistry. α,ω-PEG-1100-monomethyl ether azide **3** was synthesized by mesylation of PEG-1100 monomethyl ether, followed by nucleophilic substitution using sodium azide. The presence of the terminal azide group was characterized by a stretching frequency at 2105 cm^{-1} in the FTIR spectrum and a methylene resonance at δ 50.9 in the ^{13}C NMR spectrum ($OCH_2CH_2N_3$). Click chemistry using azides and acetylenes is most effective when performed in water or mixtures of water and polar solvents such as *tert*-butanol and in many cases proceeds readily despite low aqueous solubility. In our experiments, the click reaction was performed first by introduction of acetylene-functionalized polyester **2** as a concentrated solution in acetone to a vigorously stirring solution of **3** in water at 80 °C, followed by the addition of sodium ascorbate and copper (II) sulfate. The reaction was then stirred for 10−12 h at 80 °C. The acetone evaporates during this time period, and the polyester is solubilized in the aqueous environment as the reaction proceeds. PEG-grafted polyesters of type **4**, with 1,2,3-triazole linkages as shown in Figure 2, were isolated by extraction into CH_2Cl_2 and purified by dialysis in ethanol, followed by precipitation into hexanes. Drying under vacuum gave pure polyester-*graft*-PEG copolymers of type **4** as white semicrystalline powders. The crystallinity in these materials is due to the PEG grafts as shown by the melting transitions measured by DSC (T_m = 32 °C for polymer **4d** compared to T_m = 30 °C for PEG-1100 monomethyl ether).

The success of the click reaction to yield PEG-grafted polyester **4** was confirmed by the disappearance of the azide and alkyne signals at 2105 and 3280 cm^{-1} respectively, in the FTIR spectrum and the appearance of a singlet for the methine proton of the triazole ring at δ 7.61 in the ^{1}H NMR spectrum. Furthermore, resonances at δ 144.4 and 123.5 are found in the ^{13}C NMR spectrum of **4**, corresponding to the carbons of the triazole ring. This proved to be a clean grafting method and did not result in cross-linking or appreciable polyester degradation as demonstrated by the GPC traces in Figure 2. Table 2

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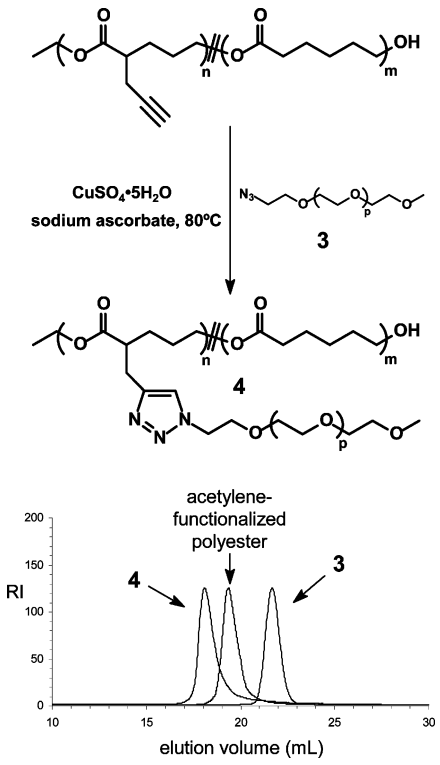

Figure 2. Synthesis of polyester-*graft*-PEG copolymers and GPC traces of PEG-azide **3**, acetylene-functionalized copolymer **2d**, and polyester-*graft*-PEG copolymer **4d**.

Table 2. Number Average Molecular Weights (M_n) and Polydispersity Indices (PDI) of Homo/Co-polyester-*graft*-PEG Copolymers Prepared by Click Chemistry

polymer	M_n[a]	PDI[a]	PEG grafts/polymer chain[b]
$\mathbf{4_a}$	10.9	1.14	5
$\mathbf{4_b}$	15.1	1.11	12
$\mathbf{4_c}$	16.3	1.13	24
$\mathbf{4_d}$	15.2	1.13	43

[a] Determined by GPC relative to polystyrene standards. [b] Determined from ^{1}H NMR spectra.

shows the molecular weight and PDI of the polyesters before and after PEG grafting as measured by GPC, as well as a calculated PEG-graft density based on ^{1}H NMR integration of the singlet at δ 3.35 (PEG OC$\underline{H}_3$) against the resonance at δ 4.03 ($CH_2C\underline{H}_2OC(O)$ polyester backbone). The product of the PEG-to-backbone ratio and the degree of polymerization, calculated from the molecular weight of the starting material, gives the average number of PEG grafts per polyester chain. It is clear that a high degree of PEG grafting density can be achieved and furthermore can be tailored by the amount of pendent acetylene in the polymer starting material.

It is interesting to note the change in GPC-derived molecular weights of the PEG-grafted polyesters as a function of PEG-grafting density. In polymer sample **4a**, with low graft density, the observed molecular weight (10.9×10^3 g/mol) is only slightly lower than the expected molecular weight calculated for the addition of 5 PEG 1100 chains to polymer **2a** (13.4×10^3 g/mol). However, in polymer sample **4d**, with high graft density, the GPC-derived molecular weight (15.2×10^3 g/mol) is much less than the molecular weight calculated for the addition of 43 PEG 1100 chains to polymer **2d** (53.3×10^3 g/mol). This underestimation in molecular weight must be due

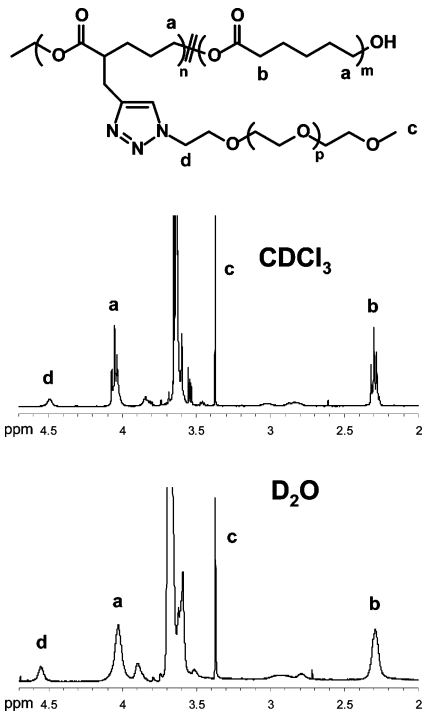

Figure 3. ^{1}H NMR spectra of polyester-*graft*-PEG copolymer **4b** prepared by click chemistry, in $CDCl_3$ (top) and D_2O (bottom).

to the impact of grafting density on hydrodynamic radius, which gives these graft copolymers more compact structures than the linear standards used to calibrate the GPC. Such an observation is not surprising, given the known influence of branching on GPC-determined molecular weights as in the case of dendrimers and hyperbranched polymers.[33]

The amphiphilic nature of PEG-grafted polyesters of type **4** distinguishes them from both the starting materials and conventional aliphatic polyesters. These copolymers are soluble in alcohols and pure water as well as most common organic solvents (acetone, THF, CH_2Cl_2, DMF, etc.). The solution structure of these materials was probed by ^{1}H NMR spectroscopy in various deuterated solvents. The spectra of **4b**, shown in Figure 3, were recorded in $CDCl_3$ and D_2O. In $CDCl_3$, the polyester backbone is solvated with clear signal splitting observed at δ 4.03 and 2.29. In contrast, dissolution of polymer **4b** in water causes a collapse of the relatively hydrophobic polyester, resulting in a broadening of the corresponding NMR signals. The solubility of PEG in both chloroform and water leads to the sharp signals at δ 3.40 in both spectra. In addition, the methylene adjacent to the triazole ring appears around δ 4.50 in both spectra.

Biocompatibility Experiments on Polyester-*graft*-PEG Copolymers. An evaluation of new synthetic polymers with regard to biocompatibility is critically important for judging the potential of new materials in biomaterial applications. Thus, the cytotoxicity of the novel polyester-*graft*-PEG copolymers was evaluated qualitatively by minimal essential medium (MEM) testing. This was accomplished by first growing a monolayer of fibroblasts (L929 mouse fibroblasts obtained from American Type Cell Culture) to near confluence in modified Eagle's medium supplemented with 10% horse serum (pH 7.0), then

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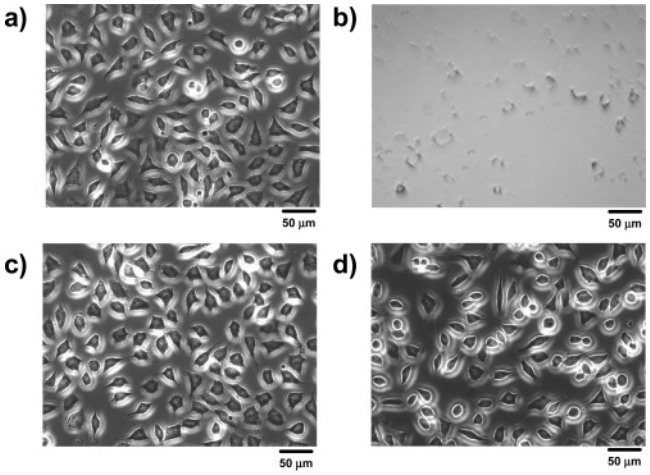

Figure 4. Phase contrast optical microscopy from minimal essential medium cell culture experiments using mouse fibroblasts in (a) medium (positive control); b) sodium dodecyl sulfate (negative control); (c) polyester-*graft*-PEG copolymer **4d**; (d) polyester-*graft*-PEG copolymer **4c**.

introducing the polyester with fresh medium to give a final concentration of 5 mg/mL. After 24 h incubation, the monolayer was observed microscopically for changes in confluence and morphology. Phase-contrast optical micrographs after 24 h exposure are shown for polymers **4c** and **4d** in Figure 4 (positive and negative controls are also shown). No qualitative change in monolayer confluence and morphology was observed in the polyester samples relative to the positive control, indicating negligible cytotoxic response of the cells to the PEG-grafted polyesters. Similar results were obtained for polymers **4b** and **4c** as described in the Supporting Information.

Hemolysis of human red blood cells was performed to provide a more quantitative cytotoxicity evaluation of the PEG-grafted polyesters. Cell lysis caused by cytotoxic material leads to release of heme into solution, which is detected by absorbance at 413 nm and compared to control experiments performed in the absence of the synthetic material. Figure 5a displays the percent hemolysis (relative to pure water = 100% hemolysis) observed for experiments performed in the presence of 5 mg/mL polymer. Results for equivalent concentrations of sodium dodecyl sulfate (SDS) and mPEG-1100 are shown for comparison. Less than 3% lysis was observed for all PEG-grafted polyesters, with the lowest value (0.5%) measured for polymer **4b**. These results are on the order of the PEG 1100 monomethyl ether starting material (3.7%) and dramatically lower than those obtained for the surfactant SDS (76%), used as a comparative control. Figure 5b shows the results of varying the concentration of polymer **4d** from 1 to 100 mg/mL. At very high polymer loading (100 mg/mL), a relatively modest hemolysis (6.7%) was observed. Thus, both MEM and hemolysis provide encouraging data regarding the biocompatibility of these graft copolymer structures and suggest further consideration of these polymers in biological applications.

Polyester-*graft*-oligopeptides by Click Chemistry. Cu(I)-catalyzed click chemistry was also applied to the grafting of oligopeptides to acetylene-functionalized aliphatic polyesters. This method enables the "biotailoring" of aliphatic polyesters that are already of interest in biomaterials research, but could be enhanced in many applications such as tissue engineering and drug delivery by the attachment of oligopeptide substituents. In this synthesis, azide-terminated oligopeptide **6** was prepared

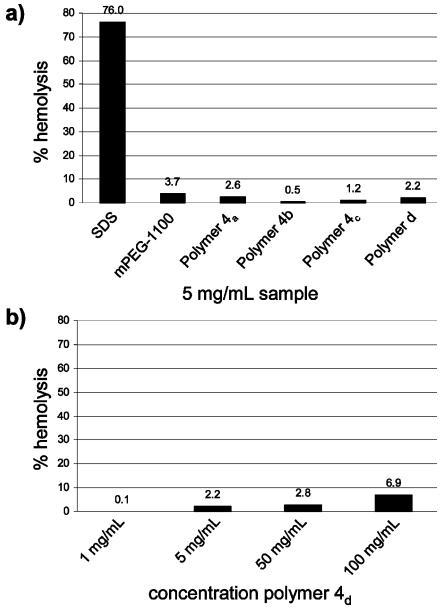

Figure 5. Hemolysis of polyester-*graft*-PEG copolymers (a) 5 mg/mL sample concentration; (b) polyester-*graft*-PEG copolymer **4d** at varying concentration.

by standard solid-phase peptide synthesis as shown in Figure 6, starting from a serine-loaded Wang resin and utilizing the peptide coupling agent HBTU.[34] The amine terminus of the resulting GRGDS oligopeptide sequence was then capped with 6-bromohexanoic acid and cleaved from the resin with 88/2/5/5 trifluoroacetic acid (TFA)/triisopropylsilane (TIPS)/H_2O/phenol, to give bromide-terminated oligopeptide **5**. Reaction of **5** with sodium azide in DMSO afforded azide-terminated pentapeptide **6**. The structure of **6** was confirmed by high-resolution mass spectrometry (HRMS FAB (*m*/*z*): $[M + H]^+$ calculated 630.296, found 630.296), as well as NMR and FTIR spectroscopy.

Our initial attempts to synthesize aliphatic polyester-*graft*-oligopeptides were carried out on the acetylene-functionalized homopolymer **4d** to give copolymer **7d**, where 5% peptide grafting was targeted. The procedure employed for this click reaction was similar to that described for PEG-grafting, except that higher temperatures (ca. 100 °C) were generally needed for successful triazole formation in the oligopeptide case. The success of this reaction was confirmed by the presence of signals in the ^{1}H NMR spectrum of polymer **7d** in the range of δ 6.70−7.80, corresponding to the peptide amide protons, and the triazole proton. The ^{1}H NMR spectrum of polymer **7d** was obtained in $CDCl_3$, a solvent in which free oligopeptide **6** is insoluble. In spectroscopic analysis performed as a control, ^{1}H NMR spectra recorded in $CDCl_3$ on mixtures of the starting materials, homopolymer **4d** and oligopeptide **6**, showed resonances only for the polyester and not the oligopeptide. To further support peptide grafting and investigate the integrity of the functionalized aliphatic polyester over time, GPC analysis was performed in DMF. As demonstrated by the GPC trace of polymer **7d** shown in Figure 7, no substantial change in polydispersity occurred following the click grafting reaction. Moreover, it appears that these polyesters have very good shelf stability (starting material **4d**: $M_n = 20.4 \times 10^3$ g/mol, PDI =

(34) Chan, W. C., White, P. D., Eds. *Fmoc Solid-Phase Peptide Synthesis: A Practical Approach,* 1st ed.; Oxford University Press: New York, 2000.

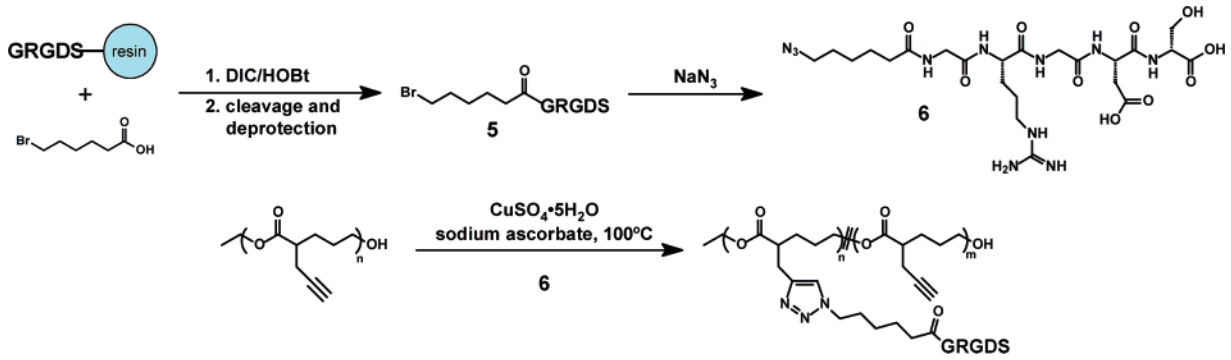

Figure 6. Synthesis of azide-terminated GRGDS oligopeptide and subsequent click chemistry to afford polyester-*graft*-GRGDS.

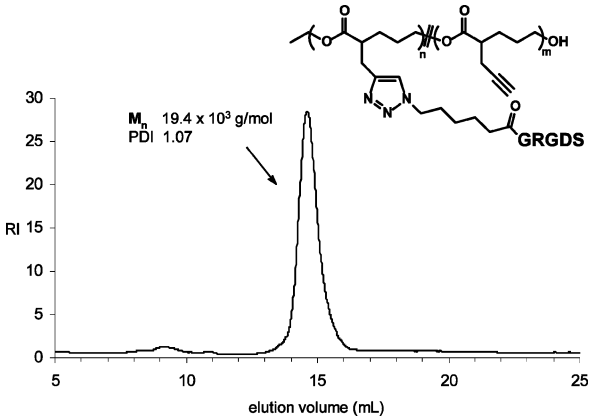

Figure 7. GPC trace of acetylene-functionalized homopolymer with 5% GRGDS-grafting prepared by click chemistry.

1.07; GRGDS-grafted polymer **7d** after 1 month storage in air: $M_n = 19.4 \times 10^3$ g/mol, PDI = 1.07). The weak signal at low retention time (high molecular weight) in the GPC trace is attributed to possible aggregation of the oligopeptide-functionalized aliphatic polyester.

Efforts are now underway to synthesize and characterize various oligopeptide-grafted aliphatic polyesters to better understand the scope and limitations of this click-induced oligopeptide grafting chemistry, as well as the properties of the peptide-functionalized aliphatic polyesters. At this stage, low grafting densities are optimal for subsequent characterization, while high grafting densities are more challenging in this regard due to solubility changes and the effect of inter- and intrapolymer interactions.

In summary, a new synthesis of aliphatic polyesters as amphiphilic and biotailored graft copolymers has been presented. The synthesis relies on polymerization of acetylene-functionalized lactones and subsequent grafting, using click chemistry in the presence of azide-terminated PEG and oligopeptides. This approach represents a convenient, rapid, and versatile synthesis of grafted aliphatic polyesters, where the mild conditions associated with click chemistry allow for grafting of functionality without appreciable polyester degradation. It is anticipated that the structural and functional variation obtained by the methods outlined here will be useful in several arenas, including the biomaterials community for polymer-based biomaterials and delivery applications.

Experimental Section

Materials. Propargyl bromide (80 wt % toluene solution), *N*,*N*-diisopropylamine (99.5+%), hexamethylphosphoramide (HMPA) (99%), δ-valerolactone (technical grade), ϵ-caprolactone (99+%), 1-hydroxybenzotriazole (HOBt) (<5% water), piperidine (99+%), *N*,*N*′-diisopropylcarbodiimide (DIC) (99%), 2,2,2-trifluoroethanol (TFE) (99.5%), 6-bromohexanoic acid (98%), triethylamine (99.5+%), sodium azide (99.5%), methanesulfonyl chloride (99.5+%), sodium dodecyl sulfate (99+%), TIPS (99%), and phenol (99+%) were purchased from Aldrich. $Sn(OTf)_2$ (97%), *n*-butyllithium (2.2 M in hexanes), and *N*,*N*-diisopropylethylamine (DIPEA) (99%) were obtained from Alfa Aesar. Copper (II) sulfate pentahydrate (ACS reagent grade) and TFA (reagent grade) were purchased from Fisher Scientific. Fmoc-Gly-OH, Fmoc-Ser(But)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Arg(Pbf)-OH, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), and Fmoc-Ser(But)-loaded Wang resin (100−200 mesh, loading density of 0.6 mmol/g) were purchased from Advanced ChemTech. Poly(ethylene glycol) 1100 monomethyl ether was obtained from Fluka, and deuterated solvents $CDCl_3$, d_6-DMSO, and D_2O, from Cambridge Isotopes. Sodium ascorbate (crystalline) was purchased from Source Naturals, and silica gel 60 (40−63 μm, 230−400 mesh) was obtained from EM Science. Dialysis tubing (Spectra/Por Membrane MWCO 6-8000), horse serum (sterile), phosphate buffered saline (1× w/o Ca or Mg), BD-Falcon tissue culture flasks (75 cm^2), and BD-Falcon six-well plates were obtained from VWR. NCTC clone 929 mouse fibroblasts and Eagle's minimum essential medium were purchased from American Type Cell Culture. *N*,*N*-diisopropylamine, triethylamine, ethyl alcohol, HMPA, δ-valerolactone, and ϵ-caprolactone were distilled over CaH_2 shortly before use. CH_2Cl_2 was washed according to standard procedures[35] and distilled over CaH_2. THF was distilled over sodium/benzophenone. Poly(ethylene glycol) 1100 monomethyl ether was purified by column chromatography on silica gel to remove PEG-diol. All other materials were used without additional purification.

Instrumentation. NMR spectra were recorded in $CDCl_3$, d_6-DMSO, or D_2O solutions using a Bruker DPX300, Bruker Avance400, or Bruker Avance600 spectrometer ($\omega_{13C} = 0.25\omega_{1H}$). Molecular weights and polydispersity indices were measured by gel permeation chromatography in THF or DMF relative to polystyrene standards (Scientific Polymer Products M_p = 503, 700, 1306, 2300, 4760, 12,400, 196,700, and 556,000 g/mol) on systems equipped with three-column sets (Polymer Laboratories 300 mm × 7.5 mm, 5 μm, 10^{-5}, 10^{-4}, and 10^{-3} Å pore sizes) and refractive-index detectors (HP 1047A) at room temperature (THF) and 50 °C (DMF) with a flow rate of 1 mL/min. High-resolution mass spectral (HRMS) data were obtained on a JEOL JMS700 MStation. UV/vis absorbance measurements were taken on a Perkin-Elmer Lambda 25 UV/vis spectrometer. IR absorbance data was obtained on a Perkin-Elmer Spectrum One FT-IR spectrometer equipped with a universal ATR sampling accessory. Melting points were measured on a Mettler DSC822 differential scanning calorimeter under $N_{2(g)}$ at a scan rate of 10 °C/min, reporting data for the second heating.

Polymerization Conditions (Synthesis of Polymer 2c). Lactone **1** and ϵ-CL were distilled over CaH_2. Glass reaction vessels were flame-

(35) Armarego, W. L. F.; Perrin, D. D. *Purification of Laboratory Chemicals*, 4th ed.; Butterworth-Heinemann: Oxford, Boston, 1996.

dried three times under a stream of $N_{2(g)}$. EtOH (110 μL, 1.7 M solution in THF) and $Sn(OTf)_2$ (150 μL, 3.7×10^{-2} M solution in THF) were introduced to the reaction vessel and allowed to stir for 15 min. **1** (1.10 g, 8.0 mmol) and ϵ-CL (0.91 g, 8.0 mmol) were then added to the vessel by syringe. The mixture was stirred at room temperature for 48 h and then dissolved in acetone and precipitated into cold hexanes. Residual **1** was removed by passage through a plug of silica gel, eluting with 50:50 EtOAc:hexanes followed by elution of the product with acetone. Solvent was removed by rotary evaporation to give pure polymer **2c** as a clear, viscous liquid (1.83 g, 91%). GPC (THF): $M_n = 7.5 \times 10^3$ g/mol, PDI = 1.11. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3 = 7.26$) (subscript PVL denotes **1**) 4.07 (m, 4H, CH_2O_{PVL+CL}), 2.58 (m, 1H, C═OCH_{PVL}), 2.45 (m, 2H, $CH_2C{\equiv}CH_{PVL}$), 2.29 (t, J = 7.3 Hz, 2H, C═OCH_{2CL}), 2.03 (m, 1H, C≡CH_{PVL}), 1.64 (br m, 8H, $CH_2CH_2CH_2O_{PVL}$ + $CH_2CH_2CH_2CH_2O_{CL}$), 1.39 (br m, 2H, CH_2CH_2-CH_2O_{CL}). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3 = 77.0$) 174.1 (*C*═O_{PVL}), 173.6 (*C*═O_{CL}), 81.1 (*C*≡CH_{PVL}), 70.2 (C≡*C*H_{PVL}), 64.6 (CH_2O_{PVL}), 64.2 (CH_2O_{CL}), 44.0 (C═O*C*H_{PVL}), 34.2 (C═O*C*H_{2CL}), 28.4 ($CH_2CH_2O_{CL}$), 27.6 (CH*C*H_{2PVL}), 26.2 ($CHCH_2CH_{2PVL}$), 25.6 (C═OCH_2-CH_2CH_{2CL}), 24.6 (C═OCH_2CH_{2CL}), 21.3 ($CH_2C{\equiv}CH_{PVL}$). IR(ATR): C≡C−H 3283, C═O 1726 cm^{-1}.

Click Reaction Conditions for Polyesters of Type 4 (4c as Representative Example). Compound **3** (2.20 g, 1.8 mmol) was dissolved in 2 mL of water in a reaction vessel. Polymer **2c** (500 mg, 1.8 mmol acetylene) was dissolved in a minimal amount of acetone and added by syringe to the rapidly stirring reaction mixture. Sodium ascorbate (71 mg, 3.6×10^{-1} mmol) and copper (II) sulfate (45 mg, 1.8×10^{-1} mmol) were added, and the resulting dispersion was heated to 80 °C overnight. The crude reaction mixture was then diluted with 50 mL of water, and the product was extracted five times with dichloromethane. The combined organics were dried over $MgSO_4$ and concentrated by rotary evaporation. Residual **3** was removed by dialysis in EtOH. The resulting product was then dissolved in a minimal amount of acetone and precipitated into cold hexanes to give pure polymer **5c** as an off-white powder (2.1 g, 78%). GPC (THF): $M_n = 16.3 \times 10^3$ g/mol, PDI = 1.13. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3 = 7.26$) 7.49 (s, 1H, R_2C═C*H*), 4.49 (m, 2H, R_2NCH_2), 4.03 (br, 4H, CH_2-OC═$O_{PVL\ +\ CL}$), 3.83 (t, J = 5.15 Hz, 2H, $R_2NCH_2CH_2$), 3.62 (br m, 96H, $CH_2CH_2O_{PEG}$), 3.45 (t. J = 5.44 Hz, 2H, CH_2OCH_{3PEG}), 3.38 (s, 3H, CH_{3PEG}), 3.00 (br, 2H, R_2CHCH_{2PVL}), 2.89 (br, 1H,C═OCH_{PVL}), 2.79 (br, 2H, C═OCH_{2CL}) 1.61 (m, 8H, $CH_2CH_2CH_2O_{PVL}$ + CH_2-$CH_2CH_2CH_2O_{CL}$), 1.38 (br m, 2H, $CH_2CH_2CH_2O_{CL}$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3 = 77.0$) 174.4 (*C*═O_{PVL}), 173.6 (*C*═O_{CL}), 144.5 (R_2C═CR), 122.5 (R_2C═*C*R), 71.6 (CH_2OCH_{3PEG}), 70.5 ($CH_2CH_2O_{PEG}$) 69.2 ($R_2NCH_2CH_2O$), 64.6 (CH_2O_{PVL}), 64.2 (CH_2O_{CL}), 58.7 (CH_{3PEG}), 53.3 (R_2NCH_2), 49.8 (R_2CHCH_{2PVL}), 44.7 (C═O*C*H_{PVL}), 34.2 (C═O*C*H_{2CL}), 28.4 ($CH_2CH_2O_{CL}$), 27.7 (CH*C*H_{2PVL}), 25.9 ($CHCH_2CH_{2PVL}$), 25.6 (C═$OCH_2CH_2CH_{2CL}$), 24.6 (C═OCH_2CH_{2CL}).

Click Reaction Conditions for Polyesters of Type 7 (7d as Representative Example). Peptide azide **6** (23 mg, 3.6×10^{-2} mmol) was dissolved in 2 mL of water and heated to 80 °C. Homopolymer **4d** (100 mg, 7.2×10^{-1} mmol acetylene) was dissolved in 1 mL of acetone and added dropwise to the rapidly stirring solution. Sodium ascorbate (122 mg, 6.1×10^{-1} mmol) and copper (II) sulfate pentahydrate (79 mg, 3.2×10^{-1} mmol) were then added, and the resulting dispersion was stirred at 80 °C until bubbling due to evaporation of acetone had ceased. The reaction vessel was then fitted with a condenser, heated to reflux, and stirred overnight. The reaction mixture was cooled to room temperature, diluted with a saturated NaCl aqueous solution, and extracted five times with CH_2Cl_2. The combined organic layers were then dried over $MgSO_4$ and concentrated by rotary evaporation. The resulting product was dried overnight under vacuum to yield polymer **7d** as a yellow, viscous liquid in 50−70% yield. GPC (DMF, after 1 month): $M_n = 19.4 \times 10^3$ g/mol, PDI = 1.07. ^{1}H NMR ($CDCl_3$, 600 MHz): δ ($CHCl_3 = 7.26$) 6.70−7.80 (m, 6 H), 4.73−4.75 (m, 1 H), 4.30−4.32 (m, 2H), 4.07−4.17 (m, 4 H), 3.64 (tr, J = 6.4 Hz, 2 H), 2.73 (m, 2 H), 2.38−2.60 (m, 6H), 2.02 (tr, J = 2.5 Hz, 1H), 1.64−1.78 (m, 8H), 1.58 (m, 6H). IR (ATR): C−H 2944.1, C−H 2864.5, C═O 1721.9 cm^{-1}.

Cell Culture. In vitro cytotoxicity was evaluated using NCTC clone L929 mouse fibroblasts purchased from American Type Cell Culture (ATCC). The fibroblasts were cultured in BD-Falcon tissue culture flasks (75 cm^2) in modified Eagle's medium supplemented with 10% horse serum. The cells were incubated at 37 °C in a 5% CO_2 atmosphere, and the medium was replaced with fresh medium every 3 days.

MEM Evaluation. L929 mouse fibroblasts were seeded at a density of 10,000 cells/cm^2 in BD-Falcon six-well polystyrene plates to reach near confluence in 24 h. After 24 h incubation, the medium was removed and replaced with fresh medium, and polymer samples dissolved in 300 μL of phosphate buffered saline (PBS) were introduced to give a final concentration of 5 mg/mL polymer. As positive and negative controls, respectively, 300 μL of PBS and 300 μL of PBS containing sodium dodecyl sulfate (SDS) to give a final concentration of 5 mg/mL were introduced (all experiments were performed in triplicate). Once the material was added, the fibroblasts were incubated for an additional 24 h and then observed microscopically for changes in confluence and morphology.

Hemolysis. Human whole blood was obtained from one of us (B.P.) and stored in EDTA stabilized vials at 4 °C until use (for a maximum of 1 week). The red blood cells (RBCs) were separated from plasma by four repeated cycles of centrifugation, removal of the supernatant, and resuspension in sterile PBS. The RBCs were diluted once more in PBS to give a 10% w/v suspension. The material to be tested was dissolved in 100 μL of PBS, introduced to 900 μL of the RBC suspension, and incubated at 37 °C for 30 min. Samples were then centrifuged, and the absorbance of the supernatant was measured at 413 nm. RBC suspension to which only 100 μL of PBS was added provided a baseline value that was subtracted from all other values. RBC suspension incubated with 100 μL of deionized water was used to determine 100% lysis. All other values are reported as a relative percentage, and each value is the average for three samples.

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Supporting Information Available: Synthetic details for preparation of compounds **1**, **3**, **5**, and **6**. Minimal essential medium evaluation of PEG-grafted polyesters **4a** and **4b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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