Synthesis of Biodegradable Amino-Acid-Based Poly(ester amide)s and Poly(ether ester amide)s with Pendant Functional Groups

Kai Guo,[1] C. C. Chu[2]

[1]*Tepha, Incorporated, Lexington, Massachusetts 02421*
[2]*Department of Fiber Science and Apparel Design and Biomedical Engineering Program, Cornell University, Ithaca, New York 14853-4401*

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ABSTRACT: A new family of biodegradable amino-acid-based poly(ester amide)s (AA–PEAs) and amino-acid-based poly(ether ester amide)s (AA–PEEAs) consisting of reactive pendant functional groups (—COOH or —NH_2) were synthesized from unsaturated AA–PEAs and AA–PEEAs via a thiol–ene reaction in the presence of a radical initiator (2,2′-azobisisobutyronitrile). The synthetic method was a one-step reaction with near 100% yields under mild reaction conditions. The resulting functional AA–PEA and AA–PEEA polymers were characterized by Fourier transform infrared spectroscopy, NMR, and differential scanning calorimetry. These new functional AA–PEA and AA–PEEA derivatives had lower glass-transition temperatures than the original unsaturated AA–PEA and AA–PEEA polymers, and their solubility in some organic solvents also improved. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 3386–3394, 2010

Key words: biodegradable; biomaterials; poly(ester amide); amino acids; graft; poly(ether ester amide)

INTRODUCTION

Tremendous efforts have been directed toward the preparation of biocompatible and biodegradable polymers with controlled chemical, physical, biodegradable, and biological properties for a wide range of potential biomedical applications, including surgical sutures,[1,2] surgical implants,[3] tissue engineering scaffolds,[4–7] and controlled drug/gene delivery.[8–14]

Poly(ester amide)s (PEAs), which comprise both ester and amide linkages in the backbones, have been proposed as a class of new biomaterials that have the potential to exhibit improved properties for a diverse range of biomedical applications.[15] There are two basic types of aliphatic PEAs: those derived from non amino acids, such as aliphatic diamine, and those derived from amino acids, such as L-phenylalanine, L-leucine, and L-lysine.[16–20] Those amino-acid-based poly(ester amide)s (AA–PEAs) are of particular interest in our laboratory because of their better biocompatibility and biodegradability compared to those from aliphatic diamines. Because of the presence of amino acids in AA–PEAs, they have amino-acid-based amide linkages and, hence, show some but not all protein behaviors. In addition, AA–PEAs also have ester linkages from non-amino-acid components. As a result, AA–PEAs combine the favorable properties, that is, enzyme-catalyzed surface erosion biodegradation and desirable mechanical, physical, and biocompatibility, of both aliphatic polyesters and polypeptides into a single entity.

Although a variety of monomers have been designed to synthesize AA–PEAs so far, there have been very few reported studies of functional AA–PEAs, that is, having reactive side chains. The incorporation of such functional side groups adds a degree of complexity to the AA–PEA synthesis because, if the side chain functional group is imparted during polymerization, it has to be protected and then deprotected to restore the intended side-chain functionality. All of these extra steps could result in a loss of yield and even in the degradation of the polymer.[21–24]

Despite this challenge, AA–PEAs containing these functionalizable amino acid side chains can be broadened for inclusion in many new and exciting applications. For example, functional handles are essential for the covalent attachment of drug molecules for drug delivery or growth factors and adhesion molecules, which are emerging as important components of medical implants and tissue engineering scaffolds.

In our recently reported studies, unsaturated amino-acid-based poly(ester amide)s (AA–UPEAs) and unsaturated amino-acid-based poly(ether ester

Correspondence to: C. C. Chu (cc62@cornell.edu).

3-mercaptopropionic acid Cysteamine

Figure 1 Chemical structures of the thiols used to synthesize functional AA–PEAs and AA–PEEAs.

amide)s (AA–UPEEAs) with built-in reactive carbon-to-carbon double bonds on the polymer backbones were designed and successfully synthesized.[20,25–27] These double bonds were incorporated into AA–UPEA with fumaryl acid or/and unsaturated diols. These >C=C< bonds not only can provide photoinduced crosslinking reactions with other photosensitive polymeric precursors (e.g., polyethylene glycol diacrylate) to form hybrid hydrogels under convenient long-wavelength ultraviolet irradiation for the controlled release of anticancer drugs[28] but also can serve as reactive sites for further modification to synthesize additional functional derivatives based on AA–PEA or amino-acid-based poly(ether ester amide) (AA–PEEA).

With the built-in >C=C< bonds in AA–UPEA and AA–UPEEA, conjugation reactions can be carried out under certain circumstances, which could be a promising alternative way to incorporate the functional side groups to AA–PEA and AA–PEEA polymers. For example, the thiol–ene reaction is a well-known organic reaction that can proceed in the presence of a radical initiator, such as 2,2′-azobisisobutyronitrile (AIBN), to conjugate a thiol moiety onto a reactive >C=C< double bond on the AA–UPEA (or AA–UPEEA) backbone to form a thioether.

In this article, we report the chemical means to provide useful functionality to AA–UPEA and AA–UPEEA by the incorporation of two pendant functional groups (—NH_2 and —COOH) onto these classes of AA–UPEAs and AA–UPEEAs for the first time. 3-Mercaptopropionic acid and cysteamine (Fig. 1) were used to graft onto AA–UPEA and AA–UPEEA to provide the pendant carboxylic acid and amine functional groups, respectively. The resulting functional AA–PEAs and AA–PEEAs could be new types of promising functional derivatives of amino-acid-based PEAs and poly(ether ester amide)s (PEEAs) with many potential exciting biomedical and tissue engineering applications

EXPERIMENTAL

Materials

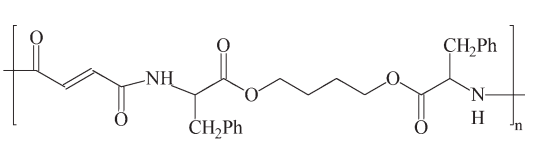
L-Phenylalanine (Alfa Aesar, Ward Hill, MA), *p*-toluenesulfonic acid monohydrate (Alfa Aesar), 3-mercaptopropionic acid (Alfa Aesar), cysteamine (Alfa Aesar), fumaryl chloride (Alfa Aesar), butane diol (Alfa Aesar), triethylene glycol (Alfa Aesar), and *p*-nitrophenol (J. T. Baker, Phillipsburg, NJ) were used without further purification. Triethylamine (Fisher Scientific, Pittsburgh, PA) was dried by refluxing with calcium hydride and then distilled. *N,N*-Dimethylacetamide (DMA; Aldrich, St. Louis, MO) was dried over calcium hydride and distilled.

Characterization

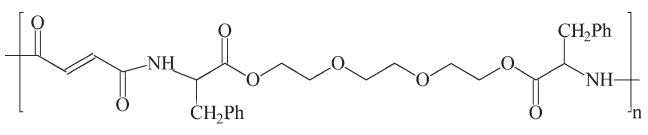
Fourier transform infrared (FTIR) spectra were recorded with a PerkinElmer Nicolet Magana 560 FTIR spectrometer (Madison, WI), and OMNIC software (Thermo Fisher Scientific, Inc., Waltham, MA) was used for data acquisition and analysis. NMR spectra were recorded by a Varian Unity INOVA-400 400-MHz spectrometer (Palo Alto, CA) operating at 400 and 100 MHz for ^{1}H- and ^{13}C-NMR, respectively. The thermal properties of the synthesized polymers were characterized by a DSC 2920 (TA Instruments, New Castle, DE). The solubility of the functionalized polymers was evaluated by the dissolution of 50 mg of the polymer in 1.0 mL of a wide range of common organic solvents at room temperature (25°C), as shown later in Table II.

Synthesis of the unsaturated PEA (FPB) and PEEA (FP3EG)

The unsaturated AA–PEA (FPB is the PEA polymer synthesized from NF (di-p-Nitrophenyl Fumarate) and PB (di-*p*-toluenesulfonic acid salts of L-phenylalanine butane-1,4-diester)) and unsaturated AA–PEEA (FP3EG is the PEA polymer synthesized from NF (di-p-Nitrophenyl Fumarate) and P3EG (L-phenylalanine triethylene glycol diester)) were synthesized according to our previously reported studies,[20,26] and those methods had the following three basic steps: (1) the synthesis of di-*p*-nitrophenyl fumarate (NF), (2) the synthesis of PB (di-p-toluenesulfonic acid salts of L-phenylalanine butane-1,4-diester) or P3EG, and (3) the solution polycondensation of NF and PB or P3EG. Figure 2 shows the chemical structures of FPB and FP3EG.

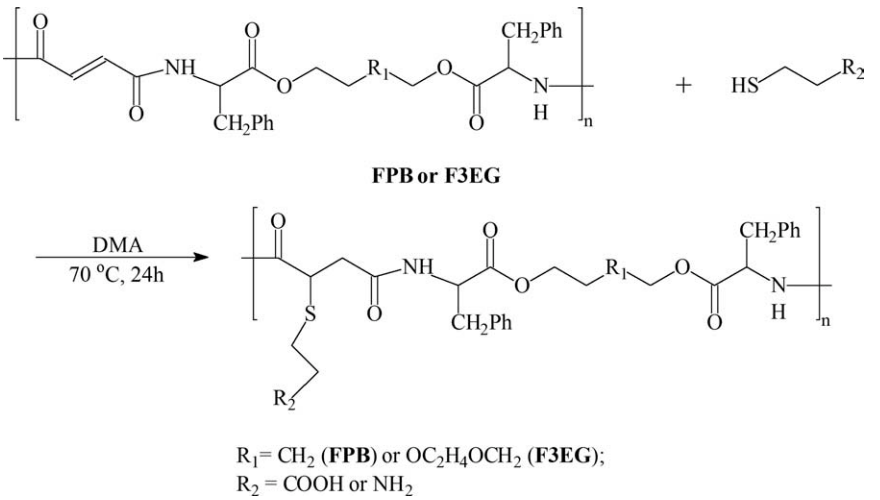


FPB



FP3EG

Figure 2 Chemical structures of FPB and FP3EG.



Scheme 1 Synthesis of functional AA–PEA and AA–PEEA with pendant functional groups.

Synthesis of functionalized FPB and FP3EG

A typical functionalization of AA–UPEA and AA–UPEEA with thiols was carried out, as shown in Scheme 1. A 10-fold excess of 3-mercaptopropionic acid (1.76 mL, 20 mmol) was added to the solution of FPB (0.93 g, 2 mmol) and AIBN (3.28 g, 20 mmol) in 20 mL of DMA. The reaction mixture was stirred at 70°C for 24 h, and then pouring the reaction solution into 400 mL of chilled diethyl ether gave the precipitated product, which was finally purified by precipitation with DMA as a solvent and diethyl ether as a nonsolvent, filtered, and dried *in vacuo* overnight.

RESULTS AND DISCUSSION

Synthesis of UPEA (FPB) and UPEEA (FP3EG)

Two different types of unsaturated polymers, FPB and FP3EG, were synthesized and used as the starting polymers to be functionalized by chemical grafting with thiol reagents. The synthesis of FPB and FP3EG were based on the method described in our previous work.[20,26]

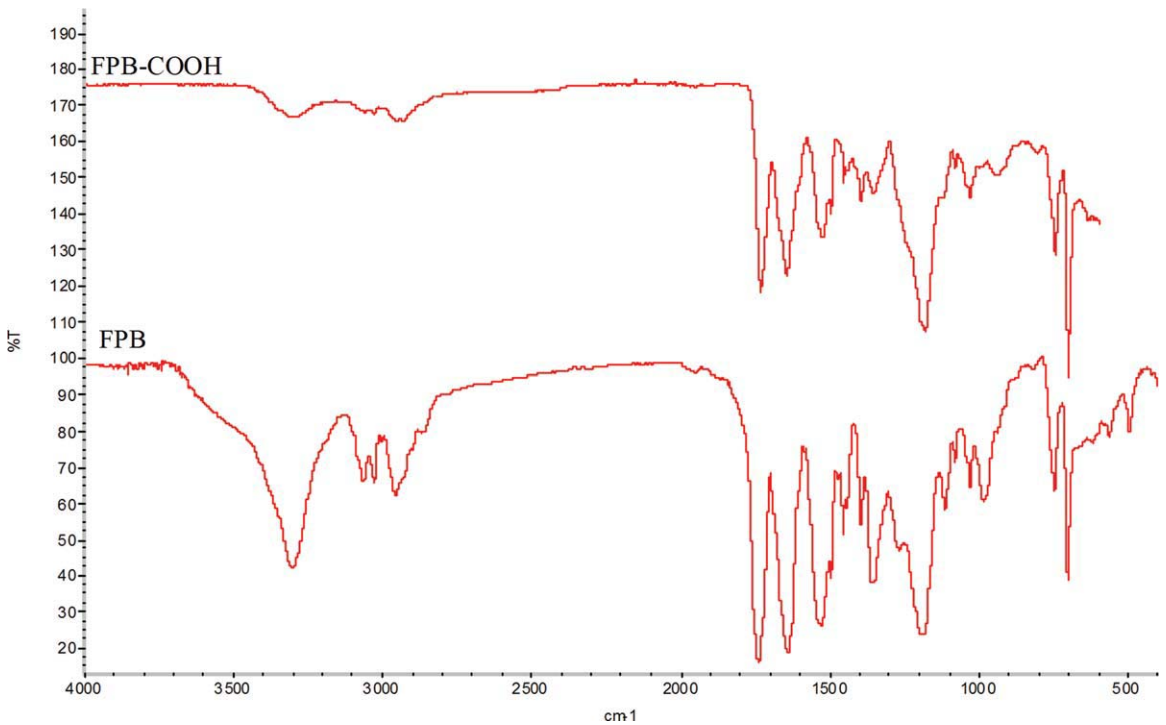


Figure 3 FTIR spectra of the FPB control and functional FPB–COOH. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

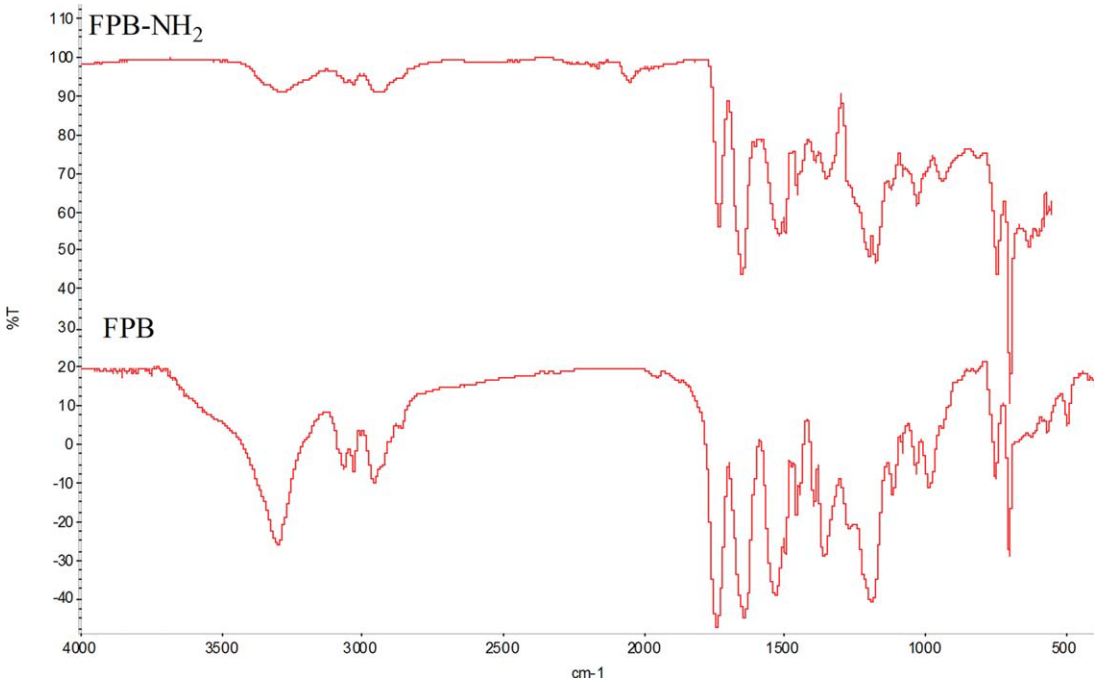


Figure 4 FTIR spectra of the FPB control and functional FPB–NH_2. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Functionalization of AA–PEAs and AA–PEEAs with thiols

The AA–UPEA (or AA–UPEEA) polymers had double bonds along the polymer backbone available for the radical addition of various thiols (Fig. 1) to provide a variety of different pendant functional groups, which could then be used as active covalent attaching sites for biologically active agents or drugs. The radical addition of thiols to the double bonds of

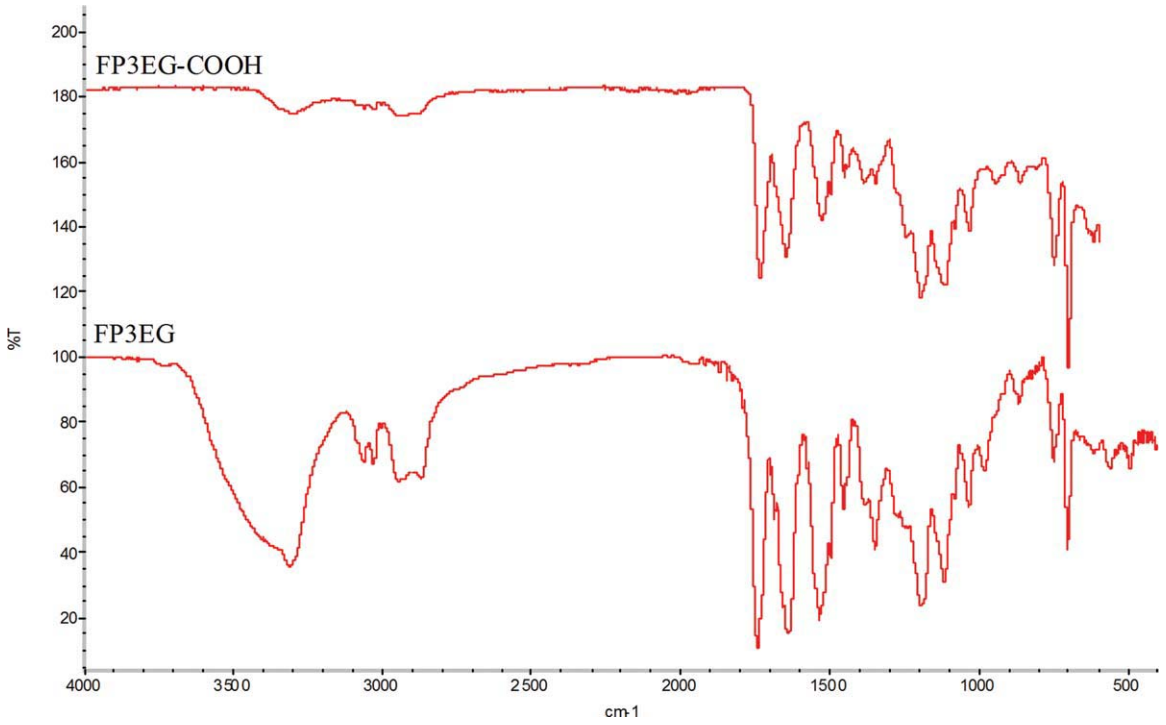


Figure 5 FTIR spectra of the FP3EG control and functional FP3EG–COOH. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

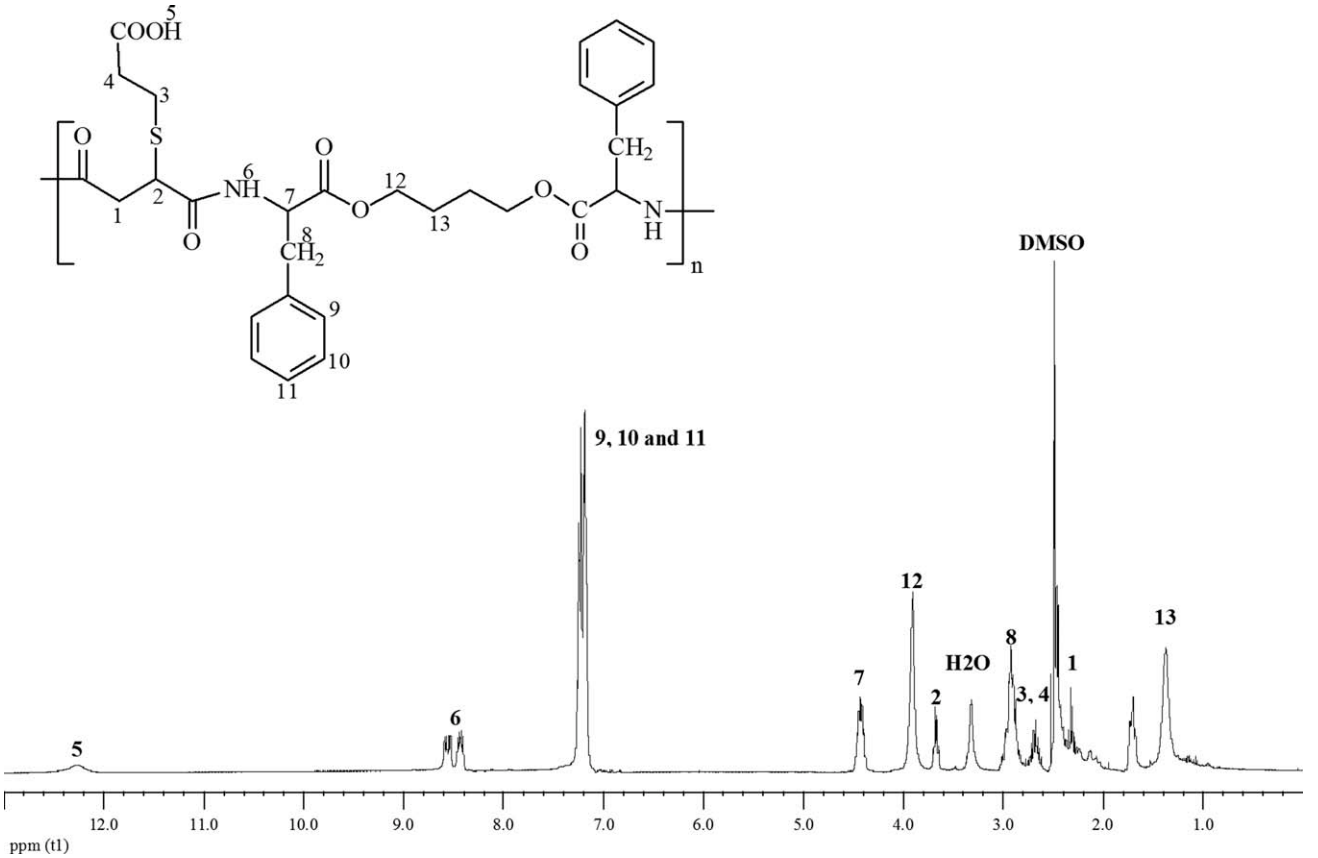


Figure 6 ^{1}H-NMR spectrum of functional FPB–COOH.

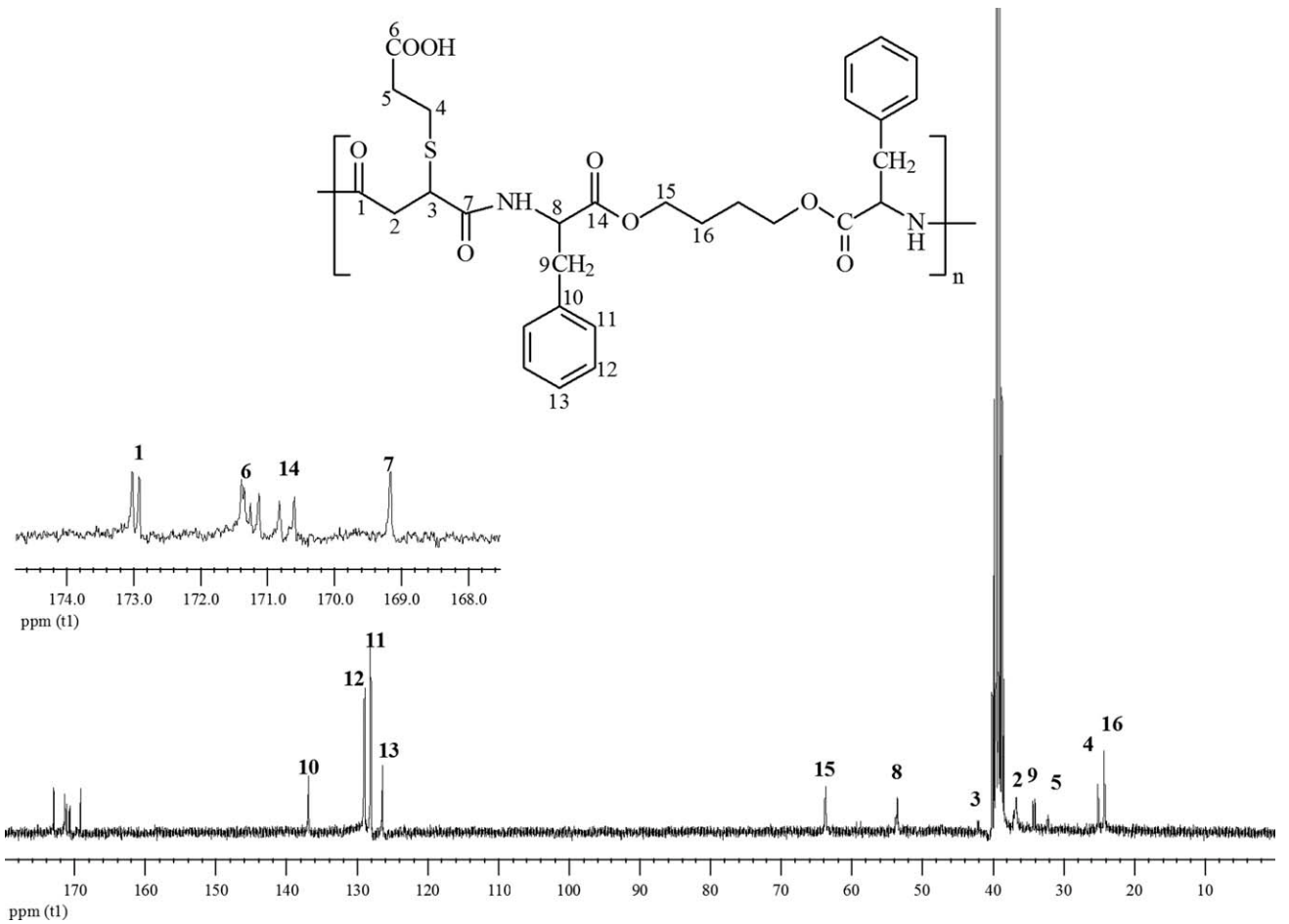


Figure 7 ^{13}C-NMR spectrum of functional FPB–COOH.

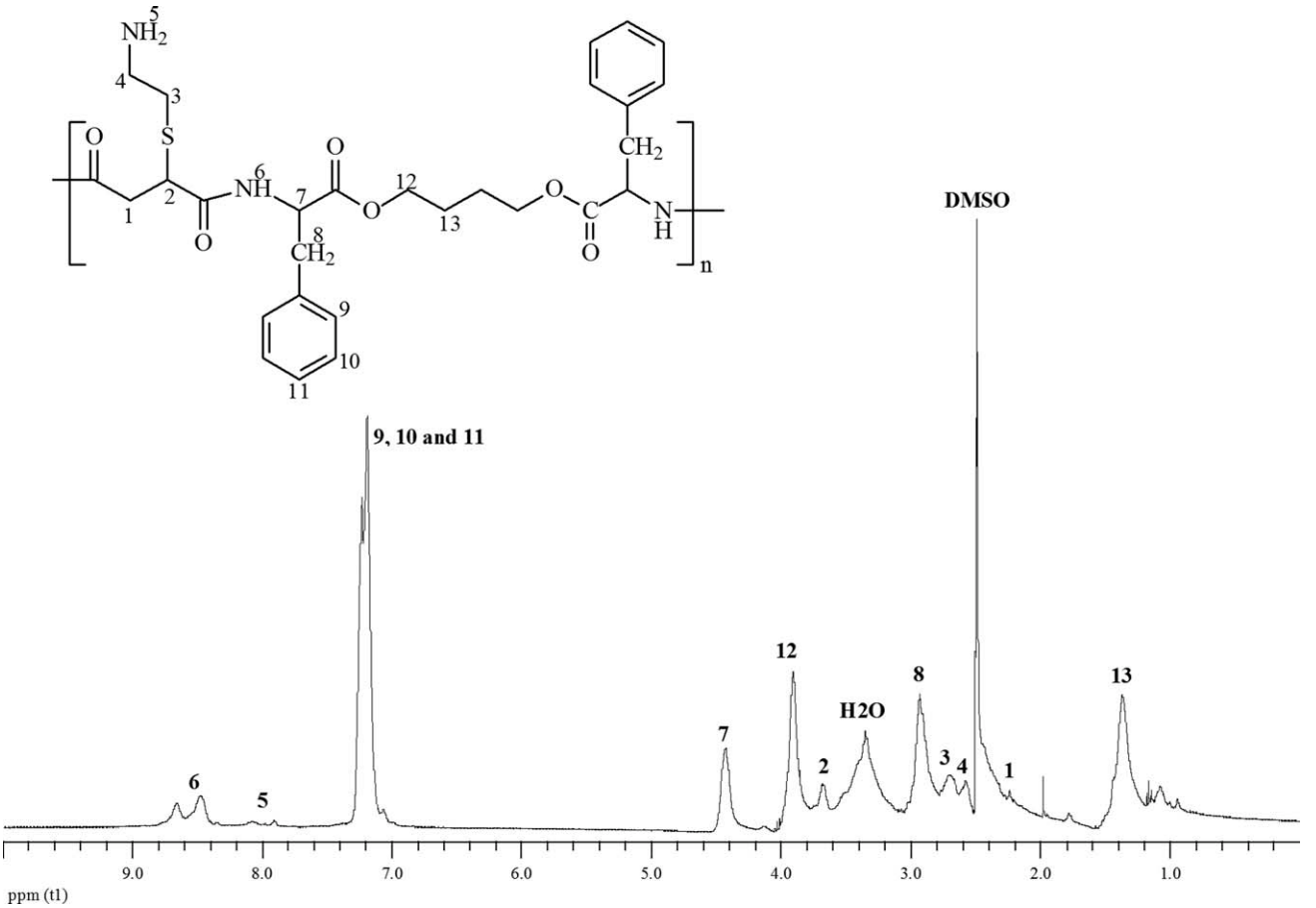


Figure 8 ^{1}H-NMR spectrum of functional FPB–NH_2.

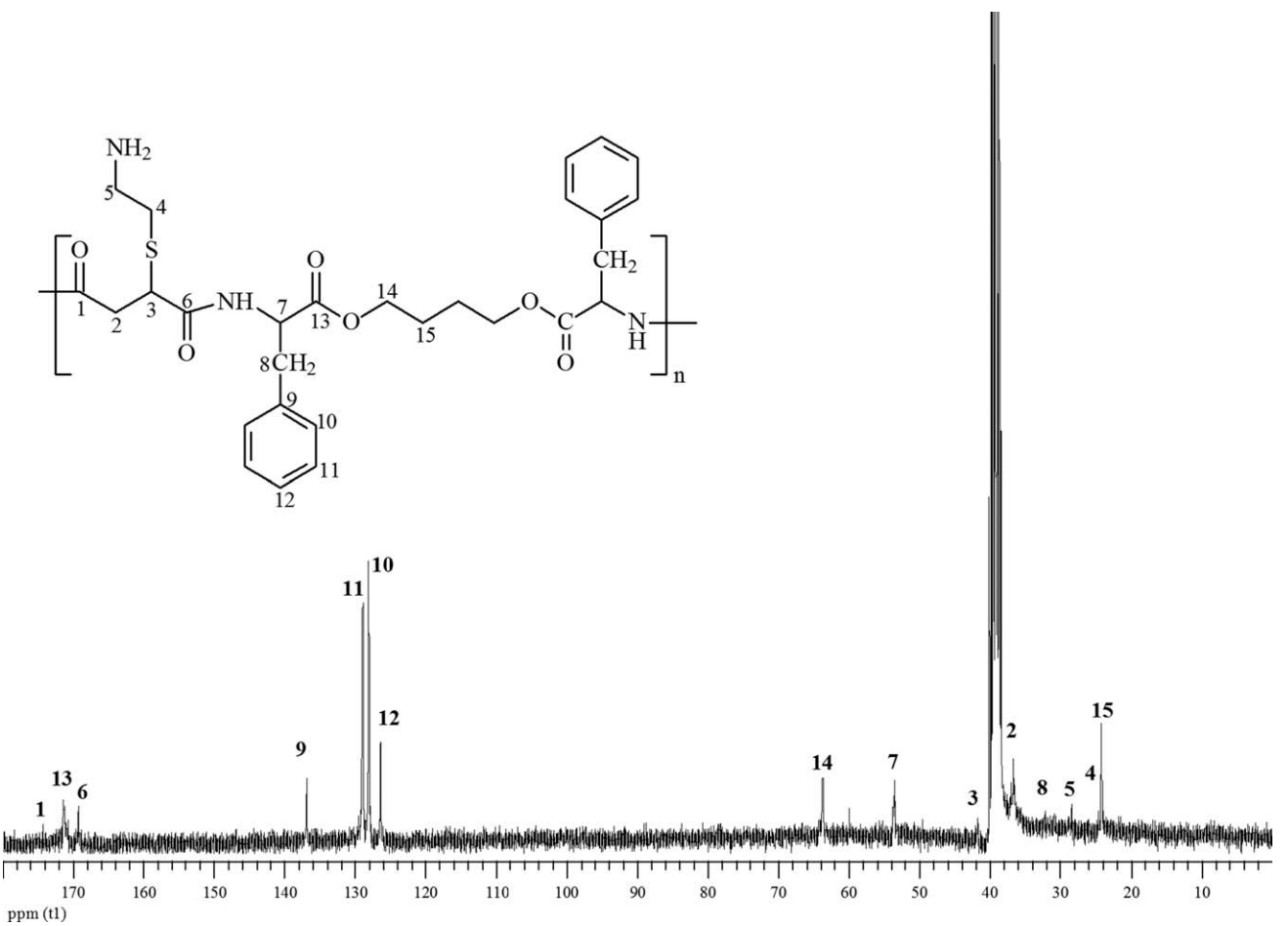


Figure 9 ^{13}C-NMR spectrum of functional FPB–NH_2.

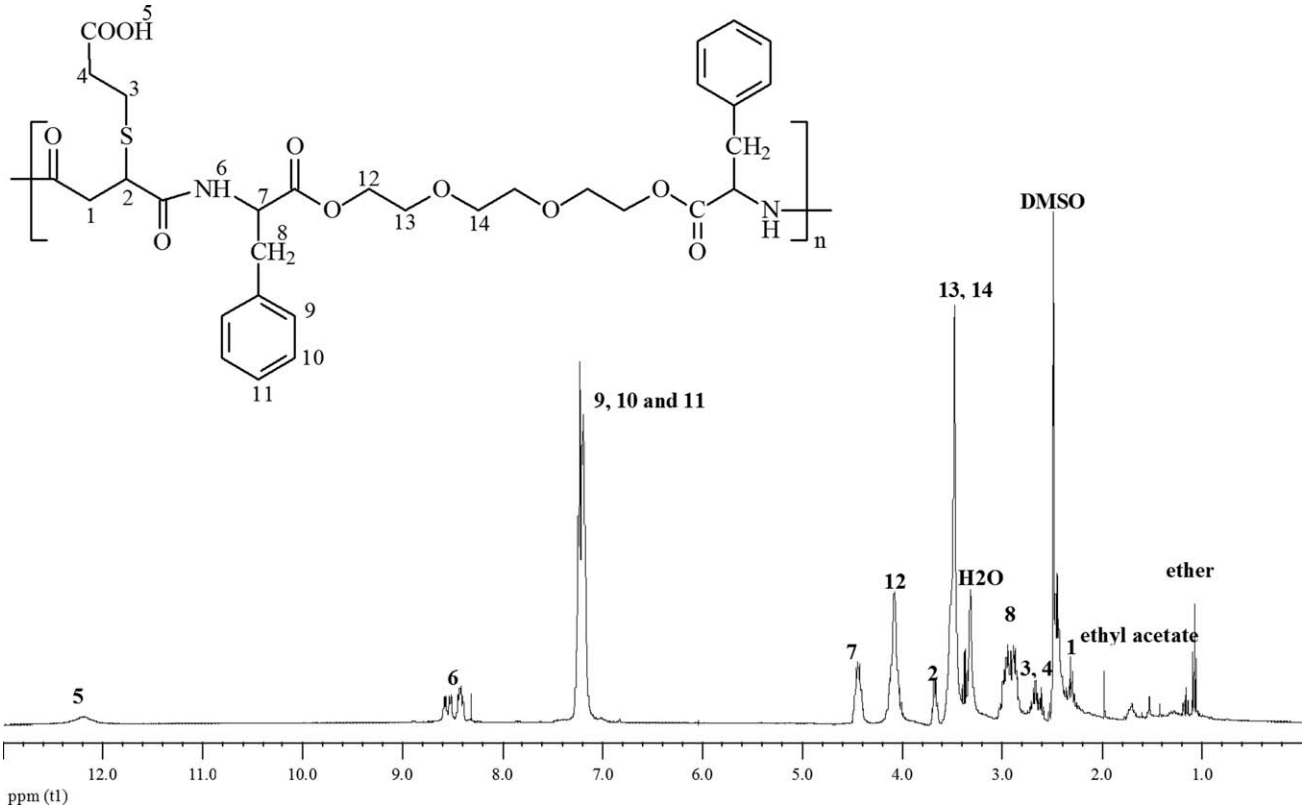


Figure 10 ^{1}H-NMR spectrum of functional FP3EG–COOH.

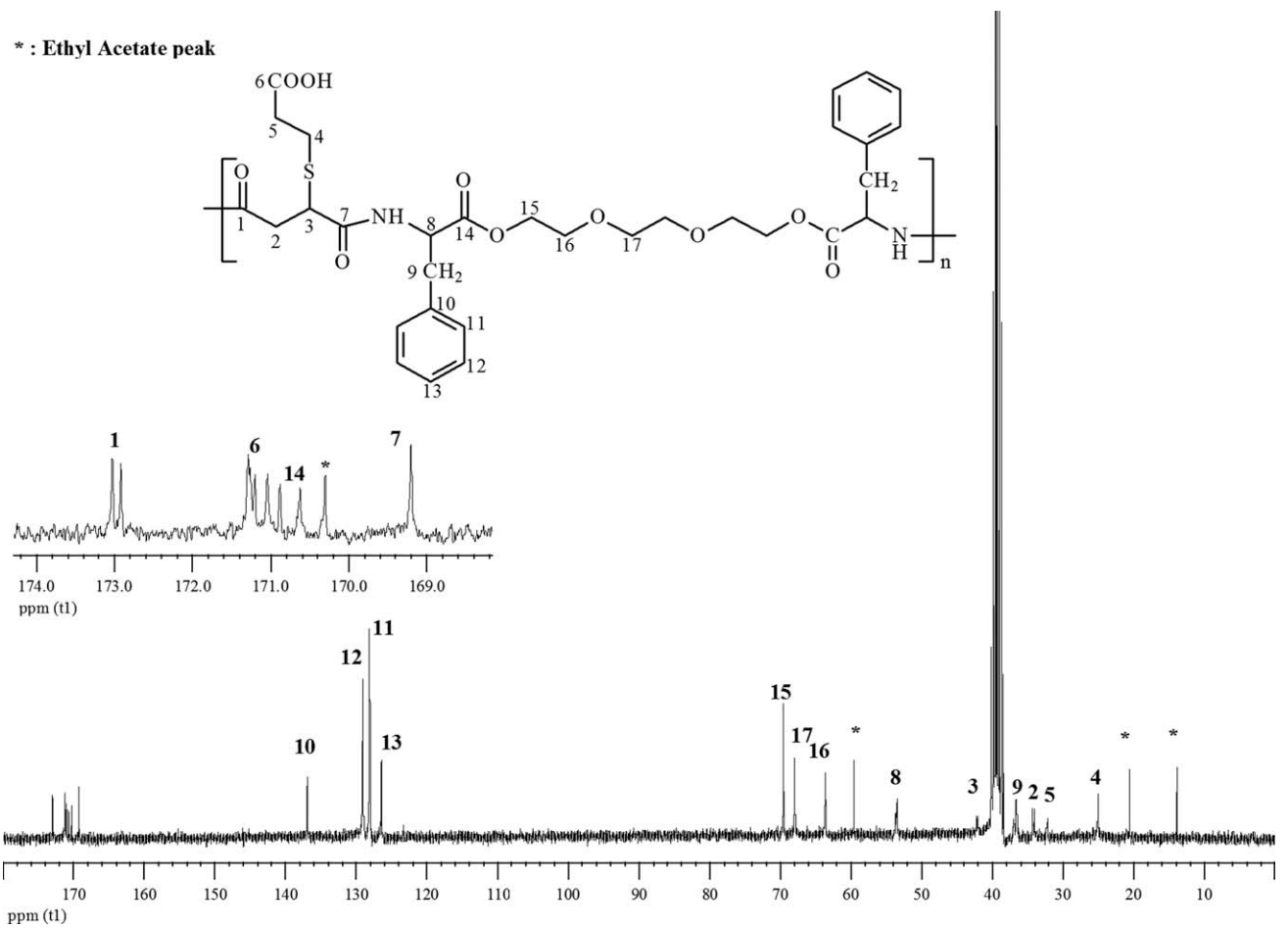


Figure 11 ^{13}C-NMR spectrum of functional FP3EG–COOH.

TABLE I
Thermal Properties of FPB, FP3EG, and Their Derivatives with Pendant Functional Groups

Polymer	T_g (°C)	T_m (°C)
FPB[20]	103	~ 250
FPB–COOH	68	N/A
FPB–NH_2	68	N/A
FP3EG[26]	67	180
FP3EG–COOH	44	N/A

N/A = not available; T_g = glass-transition temperature; T_m = melting temperature.

FPB or FP3EG was carried out at 70°C for 24 h with AIBN as a radical initiator in DMA. The addition of thiols to the FPB or FP3EG in the absence of such an initiator was not successful. After thiol attachment, the resulting functional AA–PEAs (or AA–PEEAs) polymers were purified by precipitation of the products in diethyl ether three times to remove any residual reagents. All of the resulting polymers were white to light yellow powdery solids and were characterized by NMR spectroscopy, IR spectroscopy, and differential scanning calorimetry.

The structures of the functionalized AA–PEA (or AA–PEEA) with pendant groups were confirmed by both IR and NMR spectra data. The FTIR data of these modified polymers are listed later, and their spectra are given in Figures 3–5. The FTIR data showed no residual double-bond bands compared to the FTIR spectra of their predecessors, which had characteristic unsaturated H—C= bond absorption bands (~ 3030 and ~ 984 cm^{-1}, as indicated by arrows in the figures).

The NMR spectra (^1H and ^{13}C) of FPB–COOH, FPB–NH_2, and FP3EG–COOH are shown in Figures 6–11 (see the following text for the detailed spectral band assignments of all of the polymers). The NMR spectra data were in full agreement with the anticipated chemical structure of these functional AA–PEAs and AA–PEEA polymers shown in Scheme 1.

All of the ^1H- and ^{13}C-NMR spectra of these functionalized AA–PEA and AA–PEEA showed almost no residual C=C double-bond peaks, which appeared at δ = 6.83 ppm in the ^1H-NMR spectrum and at δ = 133 ppm in the ^{13}C-NMR spectrum of the original FPB[20] and FP3EG.[26] This suggested that the yields of thiol attachment were close to 100%. These NMR data showed the presence of the corresponding introduced pendant functional groups. For example, both FPB–COOH and FP3EG–COOH showed small broad but distinctive ^1H peaks of the —COOH group (12.27 or 12.19 ppm). The ^{13}C-NMR spectra contained all of the peaks for every magnetically different carbon presented in the repeating unit of the polymer, including the ^{13}C peaks of the —COOH group (171.39 or 171.30 ppm).

Table I summarizes the thermal properties of the FPB and FP3EG and their corresponding functionalized polymers. The thermal data showed that, as the pendant functional groups were grafted to the FPB or FP3EG, the glass-transition temperatures of the resulting functional polymers were all significantly lower than those of the original polymers because of the side-group effect. For example, a 34% reduction in the glass-transition temperature was observed as the —COOH or —NH_2 functional group was grafted onto FPB. There was no difference in the level of glass-transition temperature reduction between —COOH and —NH_2 pendant functional groups or between the type of polymers (i.e., FPB vs FP3EG). The incorporation of the pendant functional groups also made the crystallization difficult, as evident in the lack of melting transition of these functionalized AA–PEA and AA–PEEA polymers.

The solubility of the functionalized FPB (or FP3EG) with the corresponding pendant functional groups in common organic solvents is shown in Table II. All of the functionalized polymers were highly soluble in trifluoroethanol, dimethyl sulfoxide, dimethylformamide, and formic acid but could not dissolve in water. However, FPB–COOH and FP3EG–COOH could dissolve in an alkaline aqueous solution (pH 10); this suggested that their pendant carboxylic acid group might have reacted with OH^- and formed a water-soluble product; however, FPB–NH_2 did not dissolve in an acidic aqueous solution (pH 4). The functional pendant groups introduced

TABLE II
Solubility of Functional PEA and PEEA at Room Temperature (25°C)

	H_2O	FA	TFE	DMF	DMSO	THF	MeOH	EA	$CHCl_3$	Acetone
FPB[20]	−	−	−	±	+	−	−	−	±	−
FPB–COOH	−	+	+	+	+	−	−	−	−	−
FPB–NH_2	−	+	+	+	+	−	−	−	−	−
FP3EG[26]	−	+	−	+	+	−	−	−	−	−
FP3EG–COOH	−	+	+	+	+			−		

FPB–COOH and FP3EG–COOH also dissolved in a basic aqueous solution (pH = 10); FPB–NH_2 did not dissolve in an acidic aqueous solution (pH = 4). + = soluble; − = insoluble; ± = partially soluble or swollen; $CHCl_3$ = chloroform; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EA = ethyl acetate; FA = formic acid; H_2O = water; MeOH = methanol; TFE = trifluoroethanol; THF = tetrahydrofuran.

also improved the solubility of the polymers in trifluoroethanol and formic acid as the original polymers (before functionalization) did not dissolve in these two solvents.

CONCLUSIONS

A new family of biodegradable and functionalized PEAs (AA–PEAs) and PEEAs (AA–PEEAs) consisting of reactive pendant functional groups (—COOH or $—NH_2$) were synthesized directly by the grafting of a thiol onto unsaturated AA–PEA and AA–PEEA via a thiol–ene reaction in the presence of the radical initiator AIBN. The one-step functionalization reaction was simple to carry out with very high yields (close to 100%) under mild conditions. The functionalized AA–PEA and AA–PEEA polymers with pendant —COOH or $—NH_2$ group were characterized and confirmed by FTIR and NMR. The thermal properties of these functionalized polymers showed that the grafting of pendant functional groups led to significantly lower glass-transition temperatures than in the original AA–UPEA and AA–UPEEA polymers because of the expansion of free volume from the grafted pendant —COOH or $—NH_2$ groups. The solubility of these new functionalized AA–PEA and AA–PEEA derivatives in organic solvents was also improved.

With the presence of these pendant —COOH or $—NH_2$ functional groups, these biodegradable and biocompatible AA–PEA and AA–PEEA polymer derivatives may have more promising biomedical applications in tissue engineering, drug/gene delivery, and wound healings. The biodegradability, mechanical properties, and feasibility of covalent conjugation of these functionalized AA–PEA and AA–PEEA with biologically active agents are currently being investigated and will be reported later.

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