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Research Article

SYNTHESIS OF AMPHIPHILIC HAIRY ROD DIBLOCK POLY(GLUTAMIC ACID)-*B*-POLY(BENZYL GLUTAMATE-*R*-OCTADECYL GLUTAMATE)

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ABSTRACT

Diblock copolypeptide poly(glutamic acid) -b-poly (benzyl glutamate-r-octadecyl glutamate) has a great potential for biological stabilization and optical orientation. This study reports on the synthesis and characterization of an amphiphilic diblock copolypeptide of poly(glutamic acid) and poly(benzyl glutamate-r-octadecyl glutamate) possessing the α-helical secondary structure. To obtain this copolypeptide, a precursor copolymer with an acid-labile protecting group for the carboxylic acid was prepared through glutamate-N-carboxy anhydride polymerization, followed by removal of the protecting group using a straightforward and highly efficient process. The precursor and the synthesized diblock copolymer were characterized by using nuclear magnetic resonance spectroscopy (NMR) and attenuated total reflection-Fourier transform infrared (ATR FT-IR). The α-helix conformation of poly (glutamic acid) -b-poly (benzyl glutamate-r-octadecyl glutamate) was identified by the characteristic α-helix amide I and amide II bands at 1651 cm^{-1} and 1547 cm^{-1} respectively, the thermal properties of this diblock copolypeptide shown on a result of TGA for observing thermally stable up to 215 °C.

Keywords: Amphiphilic diblock copolypeptide; *N*-carboxyanhydride polymerization; poly(tert-butyl glutamate)

1. Introduction

Polypeptides are composed of α-amino acid units through peptide bonds. Due to the hydrogen bonds between the carbonyl group and the amino group, polypeptides usually have different secondary structures: the α-helix, β-sheet, and the random coil. The α-helical structure has received a considerable interest because of the intriguing electro-optical properties arising from helix macrodipole (Block, 1983). Thin films of oriented α-helical polypeptides exhibit remarkable electrical and magnetic properties, with potential applications as tools in chemical biology, opto-electronics, and biosensors.

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Poly(glutamic acid) is anionic, water-soluble, non-toxic, and biodegradable. Hence, poly(glutamic acid) and its derivatives have been extensively studied for a variety of applications in industrial fields, such as medicine, cosmetics, food and water treatments (Ashiuchi et al., 2001; Ulery, Nair, & Laurencin, 2011). On the other hand, poly(benzyl glutamate) is well-known to be well-soluble in various solvents and usually exists in very stable α-helix conformation, making it like a rigid rod. A polymer block randomly consisting of benzyl glutamate and octadecyl glutamate monomer units exists in a hairy-rod structure with high molecular order and sufficient molecular mobility for good orientation, as the flexible octadecyl side chains act as an oily mantle for the rigid rods (Balavoine et al., 1999; Müller, Kessler, & Lunkwitz, 2003).

Diblock copolymers of poly(glutamic acid) and poly(benzyl glutamate-*r*-octadecyl glutamate) can combine the unique properties arising from the macrodipole along the helix axis, the amphiphilic characteristic and the liquid-like features of the side chain mantle. Such a system is very attractive as a matrix for stabilizing biomolecules as well as incorporating and orienting optical molecules.

Therefore, in this research, a new diblock copolymer, poly(glutamic acid)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) was synthesized and characterized. A precursor, poly(*tert*-butyl glutamate)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) with *tert*-butyl as an acid-labile protecting group for the carboxylic acid group was first synthesized, followed by the removal of the *tert*-butyl groups to yield the desired amphiphilic diblock copolymer.

2. Experimental section

2.1. Materials

All commercial chemicals were obtained from Sigma and used as received. Benzyl glutamate (Aldrich, 99%) was recrystallized from ethanol (70%) before use. Octadecyl glutamate was synthesized according to the procedure described by Wasserman, Garber, and Meigs (1966). Benzyl- and octadecyl-glutamate *N*-carboxyanhydrides were synthesized following the method described by Cornille, Copier, Senet, and Robin (2002). Tert-butyl glutamate *N*-carboxyanhydride was prepared by the method described by Wilder and Mobashery (1992).

2.2. Instrumentation

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in deuterated chloroform solvent ($CDCl_3$) with TMS as an internal reference, on a Bruker Avance 500 MHz spectrometer. Fourier-transform infrared spectroscopy spectra were collected as the average of 524 scans with resolution of 4 cm^{-1} on a FT-IR Tensor 27 spectrometer. Thermogravimetric analysis (TGA) measurements were performed on a Perkin-Elmer thermogravimetric analyzer at a heating rate of 10 ^{o}C/min under nitrogen atmosphere. Differential scanning calorimetry (DSC) measurements were performed on a DSC 2920 (TA instruments) at a heating rate of 10 ^{o}C/min under nitrogen atmosphere. Size exclusion chromatography (SEC) measurements were performed on a Polymer PL-GPC 50 gel permeation chromatograph system equipped with an RI detector, with tetrahydrofuran as the eluent at a flow rate of 1.0 mL/min. Molecular weight and molecular weight distribution were calculated with reference to polystyrene standards. Thermogravimetric analysis (TGA) measurements were performed on a Perkin-Elmer thermogravimetric analyzer at a heating rate of 10 ^{o}C/min under nitrogen atmosphere.

2.3. Synthesis of poly(tert-butyl glutamate)

In a round-bottom flask capped with a rubber septum and under dry nitrogen, tBuLG-NCA was dissolved in chloroform (0.08 g mL^{-1}). The reaction mixture was cooled to 5 ^{o}C and a volume of n-hexylamine was injected via a syringe. The reaction mixture was stirred at 5 ^{o}C for 5 days. Then, the reaction solution was poured into a large amount of ethanol, and $CHCl_3$ was removed by rotary evaporation. The polymer precipitate was collected by filtration and dried at 50 ^{o}C under vacuum.

2.4. Synthesis of poly(tert-butyl glutamate)-b-poly(benzyl glutamate-r-octadecyl glutamate)

Poly(*tert*-butyl glutamate)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) was synthesized by polymerization of benzyl glutamate and octadecyl glutamate (75:25, mole ratio) using poly(*tert*-butyl glutamate) as a macroinititor. The polymerization was carried out at 5 ^{o}C for a week. The product was collected by dropwise precipitation into methanol. By using the choloroform-methanol mixture, the unreacted poly(*tert*-butyl glutamate) can be eliminated upon precipitation.

2.5. Synthesis of poly(glutamic acid)-b-poly(benzyl glutamate-r-octadecyl glutamate)

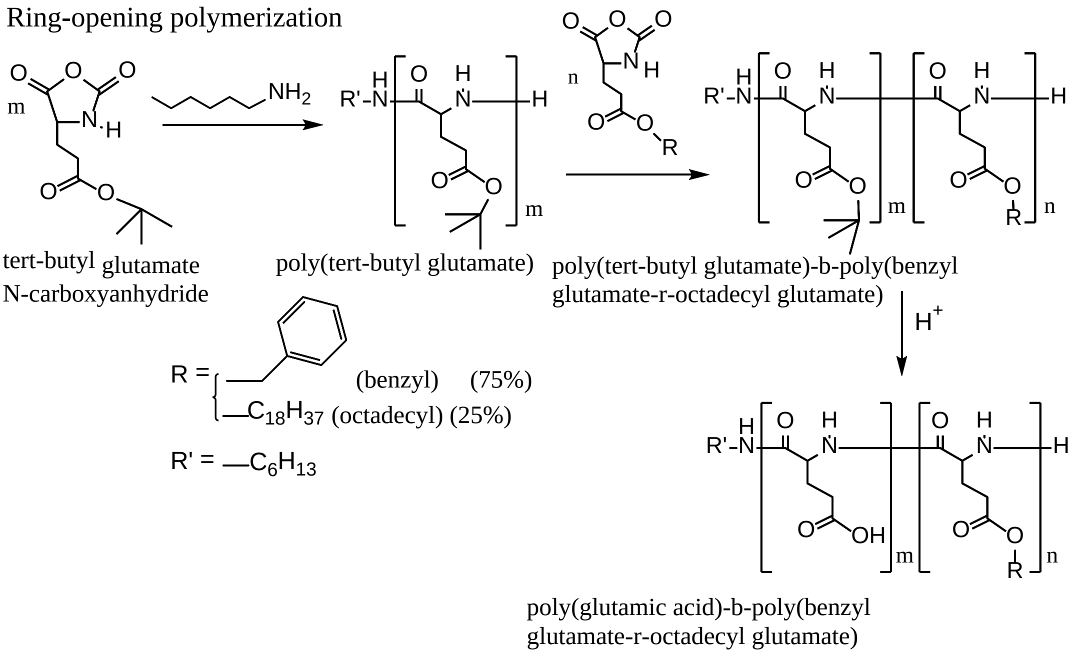
Poly(*tert*-butyl glutamate)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) was dissolved in TFA/CH_2Cl_2 (1/1, v/v) and then stirred for 2 hour. The resulting product was precipitated into ether, washed several times with ether and dried under vacuum at 60 ^{o}C.

3. Results and discussion

3.1. Synthesis of poly(tert-butyl glutamate)-b-poly(benzyl glutamate-r-octadecyl glutamate)

Poly(*tert*-butyl glutamate)-*b*-poly(benzyl glutamate-r-octadecyl glutamate) was synthesized by ring-opening polymerization of a mixture of benzyl and octadecyl glutamate *N*-carboxyanhydrides, using poly(*tert*-butyl glutamate) with a primary end group as a macroinitiator (Scheme 1).

Polymerization conditions according to a previously reported living NCA polymerization procedure (Nguyen, Vorenkamp, Daumont, Brinke, & Schouten, 2010; Vayaboury, Giani, Cottet, Deratani, & Schué, 2004) were employed. Unreacted monomers and macroinitiator were eliminated via polymer precipitation. The diblock copolymer was obtained at a relatively good yield of 70%.



Scheme 1. *Synthesis route of poly(glutamic acid)-b-poly (benzyl glutamate-r-octadecyl glutamate)*

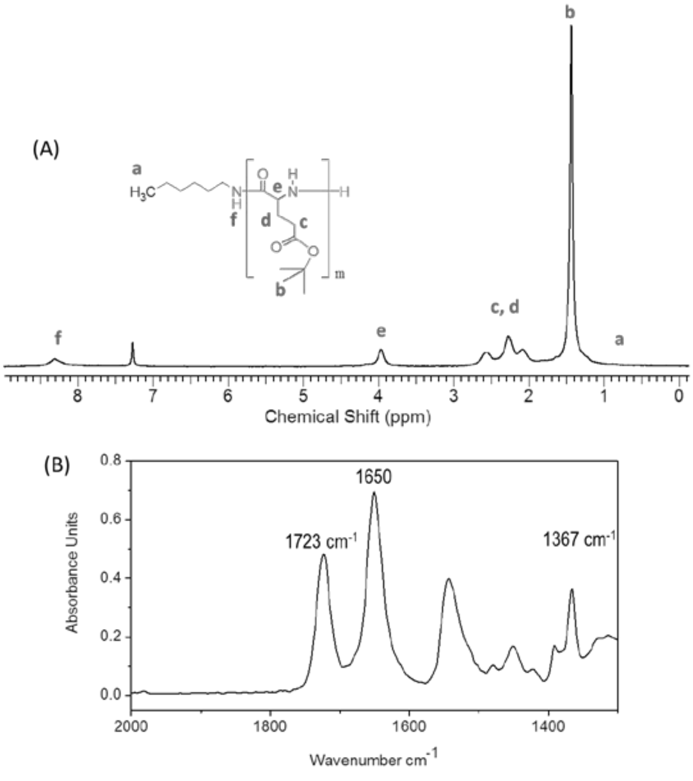


Figure 1. *^{1}H NMR (A) and FTIR (B) spectrum of poly(tert-butyl glutamate)*

The ^1H NMR spectrum of poly(*tert*-butyl glutamate) in $CDCl_3$ is presented in Figure 1A, with all characteristic peaks well assigned. The signal of the amide proton was observed at 8.2 ppm, while the signal attributed to the *tert*-butyl protons appeared at 1.45 ppm. The FT-IR spectrum of poly(*tert*-butyl glutamate) is shown in Figure 1B. It shows the characteristic absorption bands of a polyamide structure, including the bands at 1723, 1650, 1544, 1367 cm^{-1} assigned to the ester C=O stretch, amide I, amide II and *tert*-butyl C-H vibrations, respectively. The polymer conformation is completely α-helical, as identified by the amide I absorption band at 1650 cm-1 and amide II absorption band at 1544 cm^{-1}.

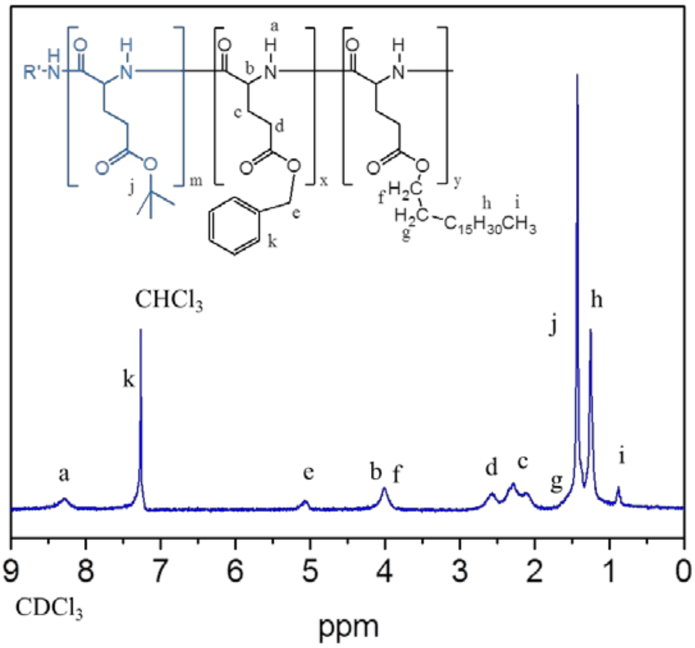


Figure 2. *^1H NMR spectrum of poly(tert-butyl glutamate)-b-poly(benzyl glutamate-r-octadecyl glutamate) in $CDCl_3$*

Figure 2 shows the ^1H NMR spectrum of the synthesized poly(*tert*-butyl glutamate)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) diblock copolypeptide with all the peaks characteristic of the chemical structures of both blocks, proving the sucessful synthesis of the diblock copolymer. All characteristic peaks could be well assigned, including amide proton peak at 8.2 ppm, aromatic peaks at 7.26 ppm, benzyl methlene peak at 5.06 ppm, peak related to the backbone methine proton and the octadecyl oxy-methylene peak overlapped with each other at 4.00 ppm, and the side group methylene protons in the range of 2.70−0.8 ppm.

The targeted number average molecular weight of poly(*tert*-butyl glutamate) was ~10000 g mol^{-1}, while the obtained polymer had a GPC-recorded number average molecular weight value of 8600 g mol^{-1} and relatively low polydispersity index (Ð) values of 1.23. The targeted number average molecular weight of poly(*tert*-butyl glutamate)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) was ~ 27000 g mol-1, while the obtained diblock copolymer had a GPC-recorded number average molecular weight value of 25400 g mol-1 and polydispersity index (Đ) values of 1.32. As shown in Figure 3, the shift to a higher molecular weight in the GPC chromatogram of poly(*tert*-butyl glutamate)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate), compared with the poly(*tert*-butyl glutamate) macroinitiator, proves the formation of the diblock copolymer. The absence of a trace of a molecular weight fraction corresponding to macroinitiator and monomers in the chromatogram of the copolymer after work-up clearly indicates the absence of homopolymer and monomer impurity.

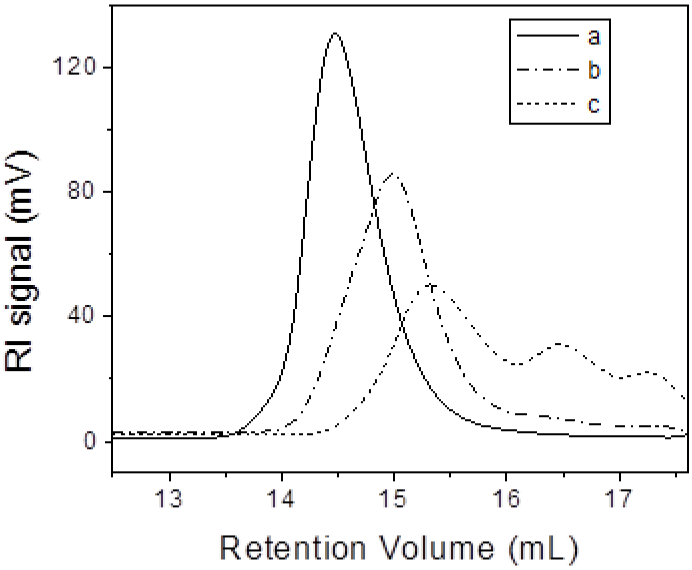


Figure 3. *GPC chromatograms (with THF as eluent) of poly(tert-butyl glutamate)-b-poly(benzyl glutamate-r-octadecyl glutamate) (a), poly(tert-butyl glutamate) (b and the extracted part containing unreacted macroinitiator and monomers (c)*

Figure 4 shows the ATR FT-IR spectrum of poly(*tert*-butyl glutamate)-b-poly(benzyl glutamate-r-octadecyl glutamate), indicating a completely α-helical conformation. The spectrum shows the characteristic absorption bands of a polyamide structure, including the bands at 1728, 1651, 1547 cm^{-1} assigned to the ester C=O stretch, amide I and amide II vibrations, respectively. The bands at 3060-3030 and 750 cm^{-1} are characteristic of the benzyl C-H stretching and deformation vibrations, respectively. The α-helix structure is identified by the characteristic α-helix amide I and amide II bands at 1651 cm^{-1} and 1547 cm^{-1}. No amide bands at lower wavenumbers corresponding to the β-sheet conformation are detected.

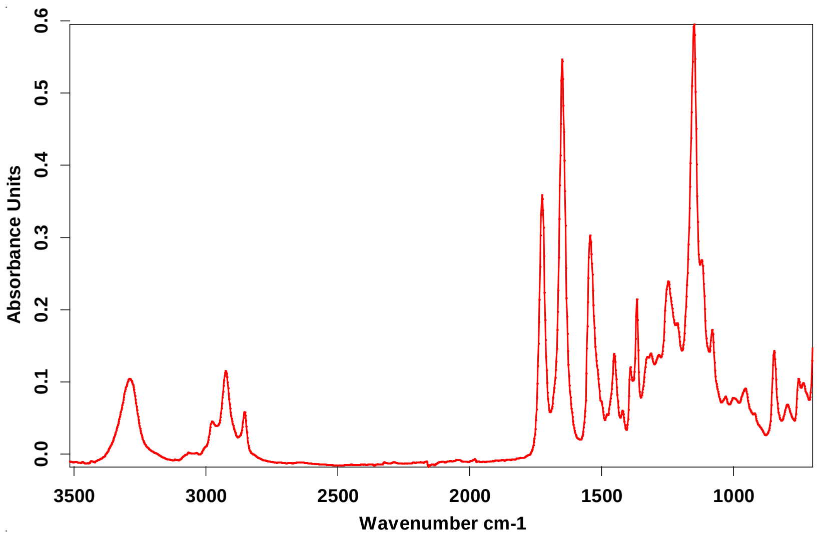


Figure 4. *FTIR spectrum of poly(tert-butyl glutamate)-b-poly(benzyl glutamate-r-octadecyl glutamate)*

3.2. Synthesis of poly(glutamic acid)-b-poly(benzyl glutamate-r-octadecyl glutamate)

The poly(glutamic acid)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) diblock copolymer was obtained by treatment of poly(*tert*-butyl glutamate)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) with trifluoroacetic acid (TFA) to remove the *tert*-butyl protecting group. Complete removal of the *tert*-butyl group was confirmed by disappearance of the ^{1}H-NMR *tert*-butyl peak at 1.43 ppm (Figure 5). As shown in Figure 6, the ATR-FTIR spectrum of the copolypeptide after hydrolysis showed disappearance of the signals of the *tert*-butyl deformation and stretching vibrations at 1365 cm^{-1}. A carboxylic absorption band appeared at 1711 cm^{-1}, along with a decrease in intensity of the carbonyl absorption band at 1728 cm^{-1}. The spectrum of the obtained copolymer shows IR amide bands at 1648 and 1545 cm^{-1}, indicating that the polymer had an α-helical conformation. The helical secondary structure is generally favorable because of the good solubility of the polymer in this conformation as well as the intriguing features that could arise from the helix macrodipole with interesting effects on charge transfer and charge transport.

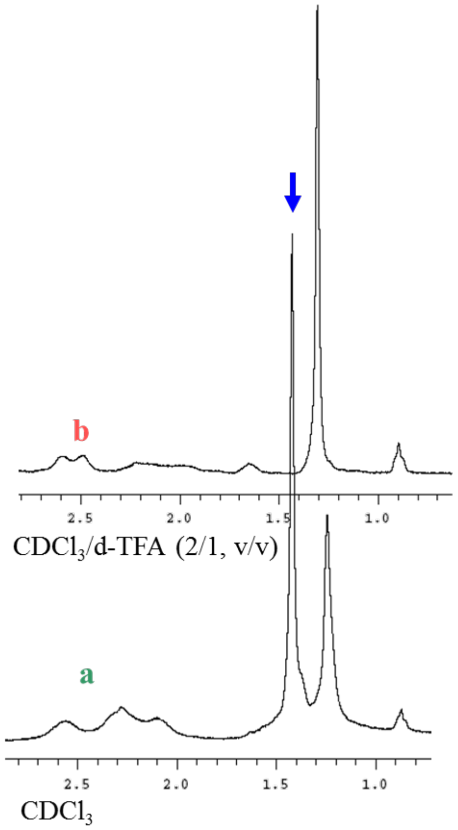


Figure 5. *^{1}H NMR spectra in the range of 3-0 ppm of poly(tert-butyl glutamate)-b-poly(benzyl glutamate-r-octadecyl glutamate) before hydrolysis (a) and after hydrolysis (b)*

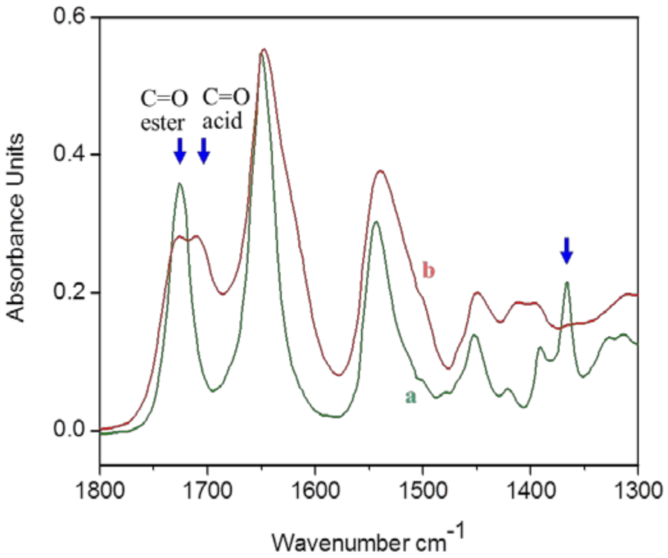


Figure 6. *FTIR spectra of poly(tert-butyl glutamate)-b-poly(benzyl glutamate-r-octadecyl glutamate) (a) and poly(glutamic acid)-b-poly(benzyl glutamate-r-octadecyl glutamate) (b)*

The thermal properties of poly(glutamic acid)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) were further studied by TGA and DSC methods. As shown by the TGA result in Figure 7, the diblock copolymer is thermally stable up to 215 °C. Above this temperature, a two-step decomposition process occurs, which is in an agreement with the appearance of two distinct endothermic peaks in the differential scanning calorimetry (DSC) curve at relevant temperature range (Figure 8). From the DSC curve of poly(glutamic acid)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate), the composition process can be assigned to the successive degradation of the poly(glutamic acid) and poly(benzyl glutamate-*r*-octadecyl glutamate) blocks, occurring at about 215 and 290°C, respectively.

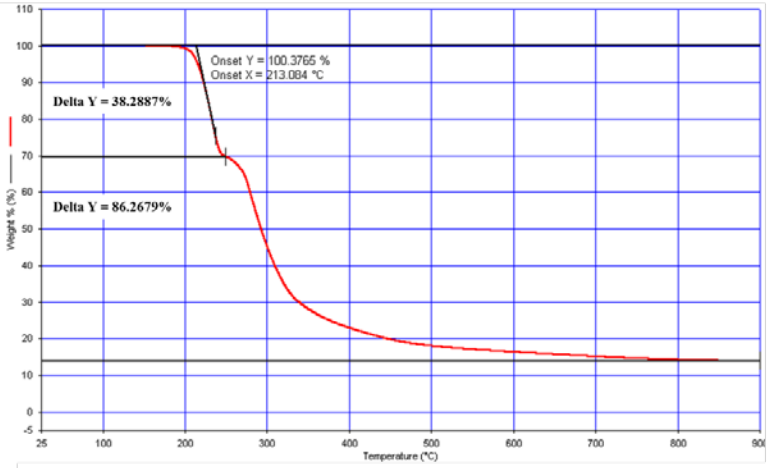


Figure 7. *TGA thermogram of poly(glutamic acid)-b-poly(benzyl glutamate-r-octadecyl glutamate recorded at a heating rate of 10 °C/min under nitrogen*

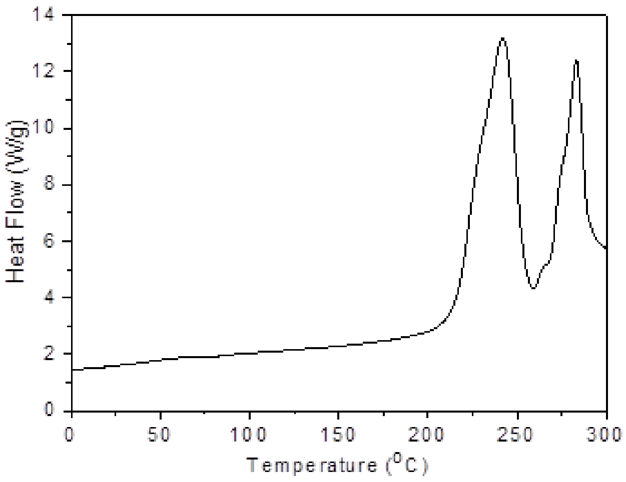


Figure 8. *DSC thermograms of poly(glutamic acid)-b-poly (benzyl glutamate-r-octadecyl glutamate*

4. Conclusions

In conclusion, the amphiphilic diblock copolypeptide was successfully synthesized by preparing poly (*tert*-butyl glutamate)-*b*-poly(benzyl glutamate-*r*-octadecyl glutamate) bearing the acid-labile protecting group for the carboxylic acid group, followed by removing this group. The structures of the copolymers were clarified through ^{1}H NMR, GPC, and FT-IR characterizations. The α-helix conformation of poly(glutamic acid)-*b*-poly (benzyl glutamate-r-octadecyl glutamate) was studied by FT-IR. Besides that, the results of DSC and TGA show that the obtained copolymers exhibited a good thermal stability of up to 215 ^{o}C.

- ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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TỔNG HỢP AMPHIPHILIC DIBLOCK POLY(GLUTAMIC ACID)-*B*-POLY(BENZYL GLUTAMATE-*R*-OCTADECYL GLUTAMATE) CẤU TRÚC HAIRY ROD

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TÓM TẮT

Chất đồng trùng hợp poly(glutamic acid)-b-poly(benzyl glutamate-r-octadecyl glutamate) có tiềm năng lớn trong việc ổn định sinh học và định hướng quang học. Bài báo giới thiệu quá trình tổng hợp và đánh giá cấu trúc và tính chất của amphiphilic diblock copolypeptide này mang các đoạn là poly(-glutamic acid) và poly(benzyl glutamate-r-octadecyl glutamate) có cấu dạng xoắn ốc. Để tổng hợp copolypeptide này, đầu tiên, một tiền chất polyme mang nhóm bảo vệ cho nhóm carboxylic acid không bền với acid được tổng hợp thông qua quá trình polyme hóa glutamate-N-carboxyanhydride. Sau đó, nhóm bảo vệ này được loại bỏ bằng quy trình đơn giản. Cấu trúc của tiền chất và diblock copolyme sau khi tổng hợp được đánh giá bằng các phương pháp phổ cộng hưởng từ hạt nhân (NMR) và phổ hồng ngoại (ATR FT-IR). Cấu trúc xoắn ốc của poly(glutamic acid)-b-poly(benzyl glutamate-r-octadecyl glutamate) được xác định bằng các dải xoắn amide bậc I và amide II được đặc trưng ở vị trí 1651 cm^{-1} và 1547 cm^{-1}, đặc tính nhiệt của copolypeptide ổn định nhiệt lên đến 215 °C thông qua kết quả TGA.

Từ khóa: Amphiphilic diblock copolypeptide; *N*-carboxyanhydride polymerization; poly(tert-butyl glutamate)