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Novel biodegradable copolymers containing pendant amine functional groups based on aspartic acid and poly(ethylene glycol)

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A new biodegradable poly(L-aspartic acid-co-poly(ethylene glycol)) having pendant amine functional groups was synthesized by the melt polycondensation reaction of prepolymer prepared from *N*-(benzyloxycarbonyl)-L-aspartic acid anhydride (*N*-Z-L-aspartic acid anhydride) and poly(ethylene glycol). The synthesized polymer was characterized by *FT*i.r., ^{1}H n.m.r., d.s.c., g.p.c. and solubility. The weight-average molecular weight of the prepolymer increased about 11 times via melt polycondensation at 160°C in a vacuum for <1 h. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: aspartic acid anhydride; poly(ethylene glycol); biodegradable)

Introduction

One of the most useful properties of synthetic polymers for biomedical applications is that the polymers should have pendant functional groups to which drugs or biologically active compounds could be covalently attached, and the polymers should be biocompatible and biodegradable for those uses involving tissue engineering and regeneration. Synthetic polymers containing amino acid residues in the main chain[1–5] or in the side chain[6–8] are some examples of functional polymers for biomedical use. Another advantage of using amino acids to prepare biologically functional biodegradable polymers is that amino acids are themselves biologically active. However, one of the major drawbacks of all available poly(amino acids) as biomaterials is the potential immunogenicity of random copolymers of more than two different amino acids[9].

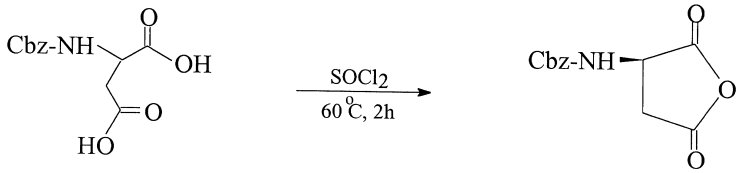
Based on the copolymer of bis(succinimidyl)carbonate derivatives of poly(ethylene glycol) (PEG) with L-lysine, Kohn and co-workers[10,11] recently prepared water-soluble poly(PEG-Lys) with multiple functional pendant groups. They also reported the attachment of antibiotics[11], doxorubicin[12] and *cis*-4-hydroxy-L-proline[13–15] onto the poly(PEG-Lys) and their biological activities.

In this paper, a series of novel biodegradable poly(ether esters) having pendant amine groups was synthesized by the melt polycondensation reaction of the prepolymer prepared from *N*-(benzyloxycarbonyl)-L-aspartic acid anhydride (*N*-Z-L-aspartic acid anhydride) and PEG using acid catalysts.

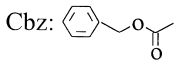
Experimental

Materials. *N*-(Benzyloxycarbonyl)-L-aspartic acid (**1**; *N*-Z-L-aspartic acid) was purchased from Sigma Chemical Co. (St Louis, MO, USA). PEGs of various molecular weights (PEG 200, 600, 2000), titanium isopropoxide (TIP) and *p*-toluenesulfonic acid as catalyst were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Palladium (10 wt%) on activated carbon and 1,4-cyclohexadiene (Aldrich) were used for catalytic transfer hydrogenation as received. Dry toluene was obtained by distillation from calcium dihydride.

*Preparation of protected N-Z-L-aspartic acid anhydride monomer (**2**).* Thionyl chloride (5.43 ml, 74.46 mmol) was slowly added to a suspension of *N*-Z-L-aspartic acid (**1**; 2 g, 7.48 mmol) in 15 ml of chloroform, and the reaction mixture was refluxed at 65°C for 2 h. The solvent and excess thionyl chloride were removed under reduced pressure. The trituration of the residue with anhydrous diethyl ether–petroleum ether produced the *N*-Z-L-aspartic acid anhydride (**2**) as fine needles. Yield of product was 96%.



1 **2**



Preparation of prepolymers. One gram (4 mmol) of *N*-Z-L-aspartic acid anhydride (**2**), PEG (**3**; 4 mmol), *p*-toluenesulfonic acid catalyst (0.008 mmol) and toluene (25 ml)

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were placed into a 50-ml round-bottomed flask equipped with a magnetic stirrer, a Deans–Stark trap with a reflux condenser and a nitrogen gas inlet. The reaction mixture was refluxed for a predetermined time under a nitrogen atmosphere. After evaporation of the solvent, a stick brown prepolymer product was obtained for subsequent melt polycondensation.

Melt polycondensation of prepolymers (postpolymerization). Ten grams of the prepolymer (**4**) and 0.25 g of titanium isopropoxide were placed into a 100-ml three-necked flask fitted with a stirrer and thermometer. The reaction mixture was stirred under vacuum at 160°C for 0.5–4 h. The polymers were dissolved in chloroform and the insoluble materials were filtered out. Finally, the

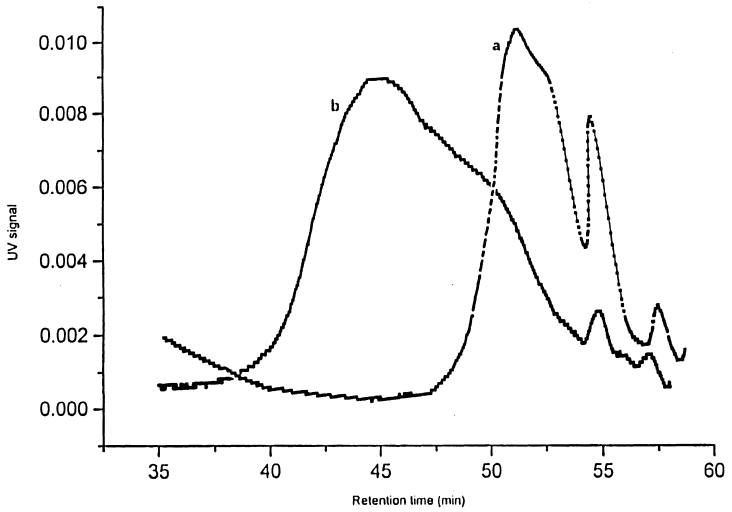


Figure 1 G.p.c. elution profiles (taken in THF at a flow rate of 1.0 ml min^{-1}) of: (a) prepolymer **4** (Run no. 5); (b) protected poly(L-aspartic acid-co-PEG) (**5**)

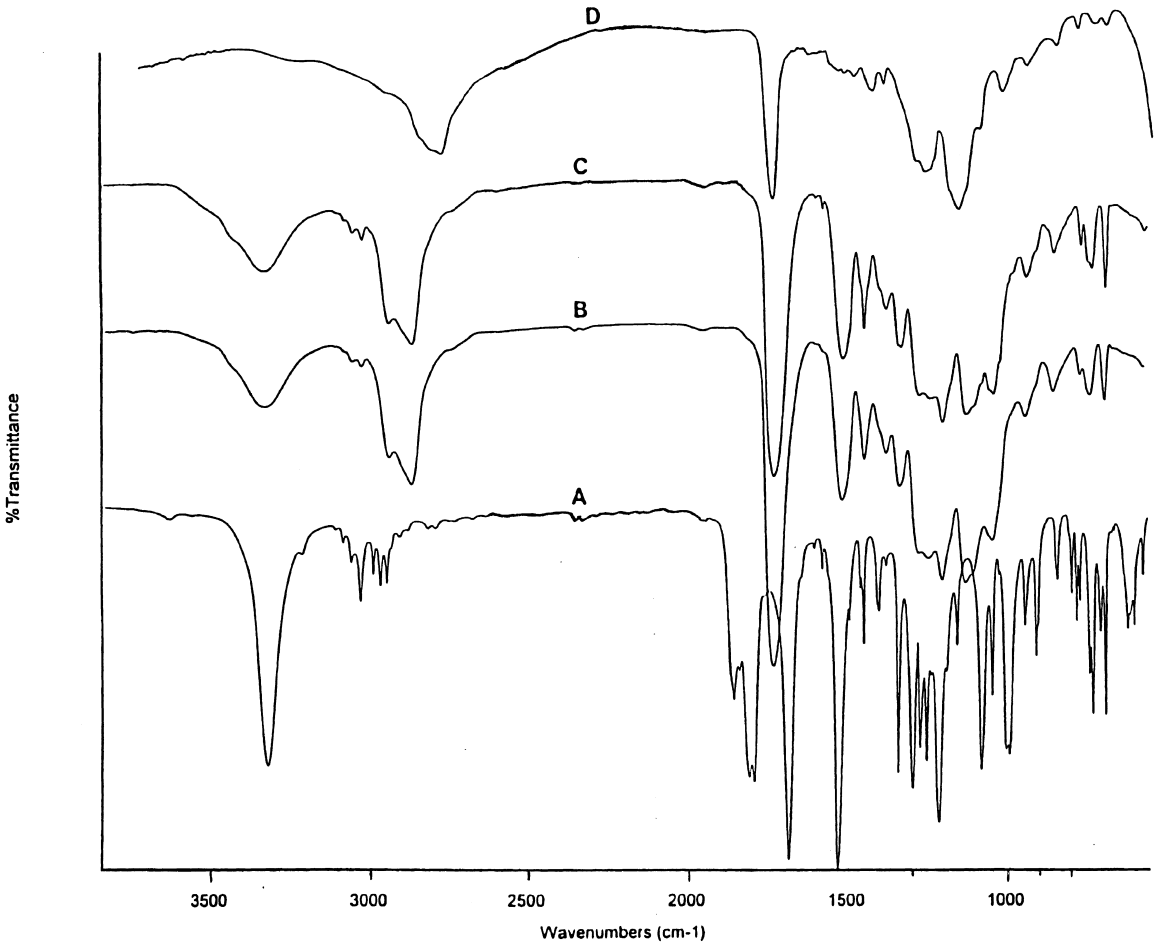
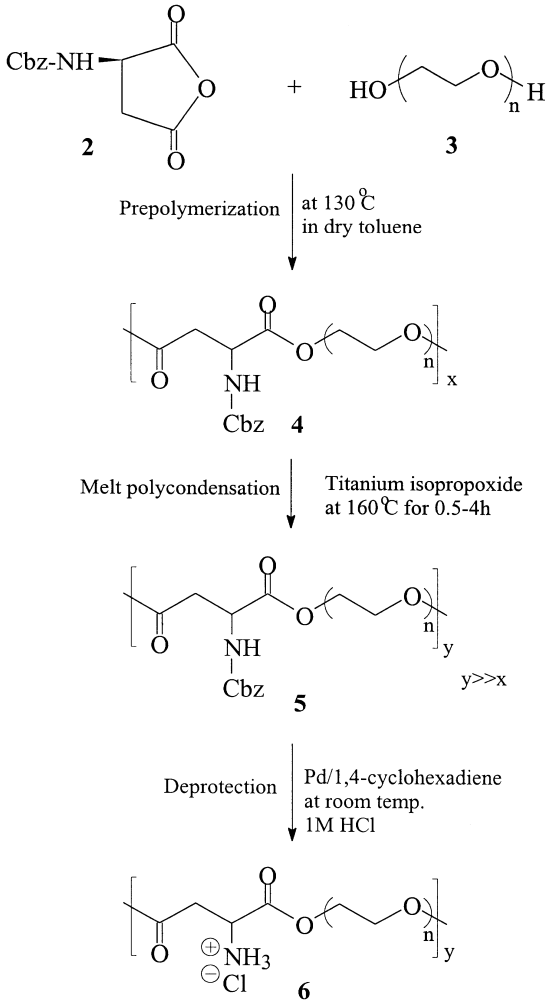


Figure 2 *FT*i.r. spectra of: (A) *N*-Z-L-aspartic acid anhydride (**2**); (B) prepolymer **4** (Run no. 5); (C) protected poly(L-aspartic acid-co-PEG) (**5**); (D) deprotected poly(L-aspartic acid-co-PEG) (**6**)

polymers were precipitated by concentrating the chloroform solution with ether and dried in a vacuum at 60°C for 24 h.

Deprotection of amino protecting group of the new polymer. A 10 wt% palladium-on-charcoal catalyst (1 g) was added to a 10 ml solution of the melt polycondensed poly(L-aspartic acid-co-PEG) whose amine group was protected by benzyloxycarbonyl (**5**; 0.33 g) in dimethylformamide (DMF). With vigorous stirring, 1,4-cyclohexadiene was slowly added to the mixture. Stirring was continued at room temperature for 12 h. After reaction, the catalyst was removed by filtration and the solution was concentrated to approximately one quarter the volume under reduced pressure. The concentrated solution was then mixed with 1 M HCl (5 ml) to form a stable formate salt (**6**). Finally, the solvent was evaporated under reduced pressure and the deprotected polymer was obtained in 83% yield.



Characterization. Average molecular weight and molecular weight distribution were determined by a Waters Model 510 gel-permeation chromatography (g.p.c.) apparatus with tetrahydrofuran (THF) as the eluent and polystyrene as the reference. Fourier transform infrared (*FT* i.r.) spectra were obtained on a Nicolet Magna 560 *FT* i.r. spectrophotometer and Nicolet data station with OMNIC 3.1 software at the resolution of 2 cm^{-1} in the region of 4000–500 cm^{-1}. ^1H n.m.r. spectra were obtained by a Bruker/Tecmag 300 using tetramethylsilane (TMS) as the internal reference. Thermal properties of the polymers were measured by a Perkin–Elmer differential scanning calorimetry (d.s.c.) model 7 under nitrogen purging at a heating rate of 10°C min^{-1}.

Results and discussion

The *N*-Z-L-aspartic acid anhydride (**2**) was prepared by cyclization reaction of *N*-Z-L-aspartic acid (**1**) in the presence of excess thionyl chloride. For the synthesis of prepolymers, *N*-Z-L-aspartic acid anhydride (**2**) and PEG (**3**) reacted to form the monoester first, which subsequently underwent polycondensation to form a low-molecular-weight prepolymer (**4**). Azeotropic removal of water by product from the reaction mixture was carried out throughout the reaction.

In order to obtain the optimal reaction conditions, several factors (reaction time, amount of catalyst, molecular weight of PEG and types of acid catalysts) were examined in this study. *Table 1* shows some results of the preparation of prepolymer **4**. The data suggest that optimal reaction conditions for achieving the highest molecular weight and yield of the prepolymers should be 24 h reaction time and the use of either *p*-toluenesulfonic acid or camphorsulfonic acid as catalyst at 0.2 mol%. The effects of the amounts of acid catalyst and the molecular weight (MW) of PEG were also examined. The molecular weight of the prepolymer reached a maximum at 0.12 mol% catalyst. Also, as the chain length of PEG increased, the percentage increase in MW of the prepolymer was not as profound as the shorter PEG chains, although PEG of MW 2000 resulted in high MW of prepolymer **4**. This result may be attributed to the availability of the terminal hydroxyl groups of PEG, as these terminal −OH groups in PEG became more hindered in the case of higher PEG MW due to long chain entanglement.

Figure 1 shows the g.p.c. data of the prepolymer (**4**) and the polymer (**5**) from the melt polymerization reaction using a low-molecular-weight prepolymer (Run no. 5, $M_w = 1589$). The M_w of the prepolymer increased from 1589 to 18 040 within 1 h at 160°C under vacuum using 0.5 wt% TIP as catalyst. A longer reaction time (>2 h) produced the polymer that was insoluble in THF but soluble in methylene chloride. This result indicates that the chain extension reaction occurred by polycondensing the end groups of the prepolymers during the melt polymerization.

The benzyloxycarbonyl (CBZ) protecting group of the polymer (**5**) could usually be removed by solvolysis with HBr/HOAc[16]. Unfortunately, the strong acid could lead to chain scissions of the polymer due to acid-catalysed ester hydrolysis. In order to avoid this problem of acid-catalysed chain fragmentation, we adopted a different deprotection method, i.e. catalytic transfer hydrogenation, which is generally used in peptide chemistry[17] to yield free amine without degrading the polymer chains. 1,4-Cyclohexadiene was used as an effective hydrogen donor under mild conditions. Palladium over activated carbon (10 wt%) was used as the catalyst for the hydrogenolysis of the aromatic CBZ protecting group.

The *FT* i.r. spectrum of *N*-Z-L-aspartic acid anhydride (**2**) (*Figure 2A*) shows characteristic absorption peaks at 3319 (NH), 1860, 1812, 1794 (C=O) and 1534 cm^{-1} (CN of urethane). The spectra of the protected prepolymer **4** (Run no. 5; *Figure 2B*), protected poly(L-aspartic acid-co-PEG) (**5**) (*Figure 2C*) and deprotected poly(L-aspartic acid-co-PEG) (**6**) (*Figure 2D*) exhibit strong bands at 1735, 1733 and 1739 cm^{-1}, assigned to the ester carbonyl. The most distinctive features of the deprotected poly(L-aspartic acid-co-PEG) (**6**) (*Figure 2D*) are the absence of N–H stretching from the CBZ protecting group at around 3320 cm^{-1}, the presence of a broad ammonium band ($-NH_3^+$) at 2600–3100 cm^{-1} (superimposed with alipatic C–H stretching),

and aromatic C–H (out-of-plane bending) absorptions at 699 and 743 cm^{-1}. This indicates the removal of the CBZ group and the formation of free pendant amine groups. The ^1H n.m.r. spectra of **2**, **5** and **6** are shown in *Figure 3*, and are consistent with the i.r. data. In particular, the disappearance of the signal at 7.3 ppm (*Figure 3B*), due to the protons of the aromatic rings in the CBZ protecting group in *Figure 3C*, is a clear indication of the deprotection reaction.

Table 1 Prepolymers (**4**) from the polycondensation of *N*-Z-L-aspartic acid anhydride (**2**) and PEG (**3**) using acid catalysts

Run no.	Catalyst[a]	MW of PEG	Mol% of catalyst	Reaction time (h)	Yield (%)	$M_n \times 10^3$	$M_w \times 10^3$	M_w/M_n
1	*p*-TsOH	200	0.02	24	86	1.11	1.42	1.28
2	*p*-TsOH	200	0.12	6	73	0.21	0.45	2.13
3	*p*-TsOH	200	0.12	24	91	1.30	2.13	1.64
4	*p*-TsOH	200	0.12	48	89	1.20	2.08	1.73
5	*p*-TsOH	200	0.12	72	84	1.19	1.58	1.33
6	*p*-TsOH	200	0.32	24	81	1.40	1.69	1.21
7	*p*-TsOH	200	0.48	24	74	0.88	1.01	1.14
8	*p*-TsOH	600	0.12	24	87	1.08	1.63	1.51
9	CSU	2000	0.2	24	88	4.29	5.16	1.20
10	*p*-TsOH	2000	0.2	24	91	4.49	5.82	1.29

[a] *p*-TsOH, *p*-toluenesulfonic acid; CSU, camphorsulfonic acid

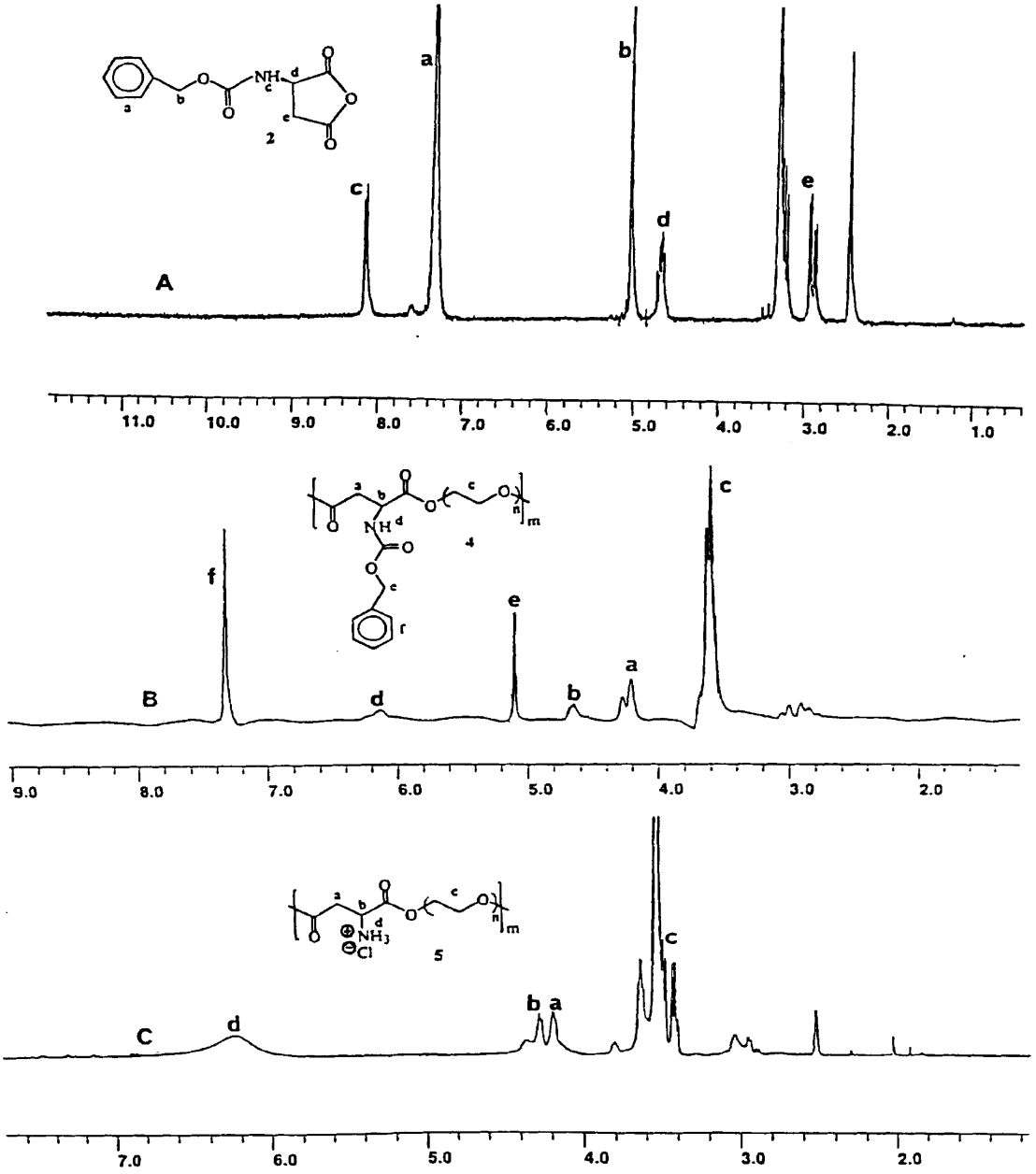


Figure 3 ^1H n.m.r. spectra of: (A) *N*-Z-L-aspartic acid anhydride (**2**); (B) protected poly(L-aspartic acid-co-PEG) (**5**); (C) deprotected poly(L-aspartic acid-co-PEG) (**6**) (300 MHz, $CDCl_3$)

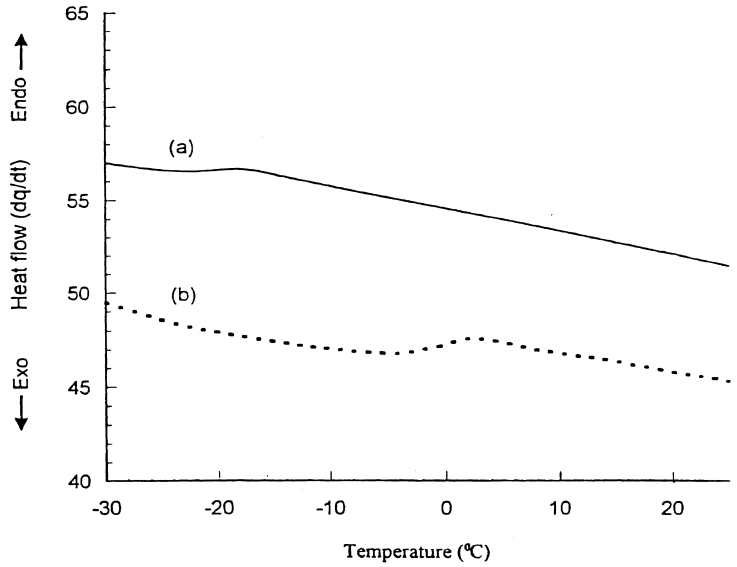


Figure 4 D.s.c. curves of: (a) prepolymer (Run no. 5); (b) protected poly(L-aspartic acid-co-PEG) (**5**)

The T_g values of the prepolymer (Run no. 5) and the protected poly(L-aspartic acid-co-PEG) (**5**) were −20.9 and 0.7°C, respectively (*Figure 4*). The T_g value of the prepolymer was significantly shifted to a higher temperature with increasing molecular weight via melt polymerization. The low T_g of the prepolymer was due to the relatively higher chain flexibility and mobility of the PEG units. Since the resulting prepolymer (**4**) and the protected polymer (**5**) are amorphous materials due to the bulky pendant amine protecting groups, the melting temperature (T_m) was not observed in the d.s.c. curves (*Figure 4*).

The protected poly(ether esters) were soluble at room temperature in polar solvents, such as THF, $CHCl_3$ and dimethylsulfoxide, whereas the deprotected polymers were partially soluble in THF and well dissolved in $CHCl_3$ and water.

In summary, a new type of biodegradable poly(ether ester) having pendant amine groups was synthesized by the melt polymerization reaction of prepolymer prepared from *N*-Z-L-aspartic acid anhydride and PEG using acid catalysts. The weight-average molecular weight of the prepolymer increased about 11 times within 1 h at 160°C *in vacuo*. *In vitro* biodegradation study and optimal reaction conditions for obtaining the highest molecular weight of copolymer are in progress and will be presented in a forthcoming full paper.

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