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λ -Benzyl-*L*-Glutamate Graft Copolymers of Cellulose and Poly[Arylene Ether Sulfone]

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λ -BENZYL-*L*-GLUTAMATE GRAFT COPOLYMERS OF CELLULOSE AND POLY(ARYLENE ETHER SULFONE)

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ABSTRACT

Macroinitiators with primary amino substituents were synthesized by one of the following techniques: a) cyanoethylation of cellulose followed by diborane reduction to produce aminopropylcellulose, *1*; b) nitration, then $SnCl_2$ reduction of poly(arylene ether sulfone), *5*, to produce poly(2-aminoarylene ether sulfone), *2*; c) phthalimidation of *5* followed by hydrazinolysis to yield poly(2-aminomethylarylene ether sulfone), *3*; and d) $LiAlH_4$ reduction of poly(cyanophenylene arylene ether) to poly(aminomethylphenylene arylene ether), *4*. Heterogeneous grafting of γ-benzyl-*L*-glutamate-*N*-carboxyanhydride, *8*, to Polymer *1* resulted in a nonrandom distribution of amino acid residues; α-helical conformations were detected at low BLG-NCA/NH_2 ratios (<5 amino acids). Using molar ratios ranging from 1 to 100 of *8*, relative to the amine concentration, grafting to Polymers *3* and *4* was effected in anhydrous THF at room temperature under homogeneous conditions. If reaction times between 24 and 48 h are utilized, high grafting efficiencies (>80%) are obtained. The conformation of the polypeptide chain was evaluated by NMR and infrared spectroscopy. Polypeptides grafted to Polymers *3* and *4* appeared to adopt the expected conformation for the chain length predicted, i.e., a progression from random coil (<8 amino acids) to β-pleated sheet (8-13 amino acids) to α-helix (>13 amino acids). The benzyl ester functions on the BLG grafts are subject to direct modification with amine nucleophiles; studies with butylamine correlate re-

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action conditions with extent of ester vs peptide cleavage. In the presence of 1-hydroxybenzotriazole, aminolysis of the ester is favored and conversions to γ-amides up to 75% without peptide cleavage are achieved.

INTRODUCTION

Graft copolymers of amino acids (AA) onto natural and synthetic polymers, such as aminopropylcellulose, *1*; poly(aminoarylene ether sulfone), *2*; poly(aminomethylarylene ether sulfone), *3*; or poly(2-aminomethyl-1,3-phenylene arylene ether), *4*, can be considered as either biodegradable spacers and carriers for pharmacons and agricultural chemicals or chiral substrates for separation of racemic mixtures. Glycoproteins and glycopeptides, proteins, and peptides containing sugar residues are widely distributed in nature, and have many bioactive properties [1]. Some of the features of these materials can be duplicated by grafting amino acids onto cellulose derivatives; unfortunately, these materials are not soluble. We have discovered that soluble graft copolymers can be produced on the synthetic polyamine matrices and plan to utilize these derivatives to produce semipermeable membranes with chiral surfaces. The synthesis and properties of the peptide graft copolymers will be the subject of this paper.

The polymerization of *N*-carboxyamino acid anhydrides (NCA's) is initiated by addition of primary amines to the anhydride ring; the initiating species becomes the end group of the protein [2]. Initiation is rapid, and polypeptides with narrow molecular weight distributions are formed [3]. Block copolymers of amine-terminated polystyrene or polybutadiene with NCA's have been prepared [4-6], and there have been a few reports on the formation of poly(amino acid) graft copolymers by using polymers containing pendent amino groups as macroinitiators. Kimura et al. carried out the graft copolymerization of the NCA's of *L*-alanine, γ-benzyl-*L*-glutamate (BLG), or β-benzyl-*L*-aspartate initiated by the copolymer of styrene and *N*-methyl-*N*-(4-vinylphenethyl)ethylene diamine [7]. Recently, cellulose-PBLG graft copolymers were prepared by polymerizing BLG-NCA by using aminoethylcellulose as a substrate [8]; these materials exhibited good blood compatibility when coated on a polyester suture. No mention of the membrane properties of these grafts was made. Ogata has just reported that poly(γ-benzyl-*L*-glutamate) has been grafted on crosslinked poly(aminomethylstyrene), aminolysis with benzyl amines was effected, and the resultant insoluble adsorbent was used to resolve (*RS*)-5-isopropylhydantoin [9].

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We are also interested in polymers with pendent reactive peptides as potential controlled-release agents. Either the free amino function at the end of the peptide chains or the benzyl ester substituents on PBLG can be coupled with an appropriate pharmacon, which can be released *in vivo* by peptidases. Our attention was focused initially on cellulose derivatives in an effort to produce a completely biodegradable polymer delivery system; initial results on this problem appear promising. Unfortunately, the cellulose grafts are insoluble in most solvents, which makes characterization of the modified derivatives very difficult. Therefore, aminated poly(arylene ether) derivatives were employed as soluble macroinitiators in an effort to produce soluble graft copolymers which could be cast into membranes. The soluble copolymers can be studied by numerous solution techniques, and some insight into the unique properties imparted by the peptide grafts can be gained.

Poly(γ-benzyl-*L*-glutamate), PBLG, was selected as the representative peptide unit, because PBLG is not only a well-characterized hydrophobic peptide known to adopt several secondary structures, but it can also be converted readily into a number of derivatives ranging in properties from hydrophobic to hydrophilic. For example, treatment of PBLG with hydroxyalkylamines converts it to poly(N^5-hydroxylalkyl-*L*-glutamines), water-soluble derivatives [10].

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EXPERIMENTAL

General Procedures and Reagents

Solution ^{1}H- and ^{13}C-NMR spectra were recorded with an IBM AF-100 or Bruker WP-200 NMR spectrometer. Chemical shifts are given in parts per million (ppm) on a σ scale downfield from the tetramethylsilane. Infrared spectra of polymer film cast from chloroform or KBr mulls were recorded with a Perkin-Elmer 283 spectrophotometer. Viscosity measurements were made with a Wells-Brookfield Viscometer (spindle, CP-40; angle, 0.8°, volume, 0.5 mL). Calibration of the viscometer with water over a shear range from 45 to 450 s^{-1} yielded a viscosity of 0.897 cP (η_{2S} = 0.8904 cP) [11]. Solvents used for general applications were of reagent grade. Dimethylsulfoxide (DMSO) and *n*-butylamine were distilled under reduced pressure in a nitrogen atmosphere. Tetrahydrofuran (THF) for the graft copolymerization and aminolysis studies was distilled from sodium benzophenone ketyl.

Macroinitiators

Aminopropylcellulose, *1*, was prepared from cellulose by cyanoethylation, D.S. = 0.3, followed by reduction of the nitrile to *1*, 1.6 meq/g NH_2, with a borane-THF complex (1.0 *M* in THF) [12]. Direct phthalimidomethylation of poly(arylene ether sulfone), *5*, was carried out at room temperature in a 50:50 v/v mixture of dichloromethane-trifluoroacetic acid with a trifluoromethane sulfonic acid catalyst; by varying the ratio of *N*-hydroxymethylphthalimide to *5*, the degree of functionalization (D.F.) was controlled to yield *6a-c*, D.F. = 0.2, 0.5, and 1.0, respectively. Subsequent homogeneous hydrazinolysis of *6* in refluxing THF-ethanol yielded poly(2-aminomethyl-1,4-phenylene ether sulfone), *3a-c*, 0.447, 1.095, and 2.123 meq/g NH_2 [13]. Poly(2-amino1,4-phenylene ether sulfone), *2*, D.F. = 0.3 (0.67 meq/g NH_2), was prepared by nitration [14] of *5*, and reduction of the nitro substituents with $SnCl_2$ and concentrated HCl [13]. Poly(2-aminomethyl-1,3-phenylene arylene ether), *4*, was prepared by reduction of poly(2-cyano-1,3-phenylene arylene ether), *7*, with lithium aluminum hydride [15]. Treatment of γ-benzyl-*L*-glutamate, BLG, with phosgene according to the procedure of Goodman [16] afforded *N*-carboxy-γ-benzyl-*L*-glutamic acid anhydride, BLG-NCA, *8*.

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Graft Copolymerizations

Aminopropylcellulose, *1*, containing 1.6 meq/g NH_2 was slurried in 20 mL anhydrous THF and stirred at room temperature while a solution of BLG-NCA in 10 mL THF was added. Graft copolymerization was allowed to proceed at room temperature for 48 h. After reducing the volume of mixture to a third under reduced pressure and pouring the slurry into tenfold excess cold MeOH, the graft copolymers, *9a-e*, were recovered by filtration and dried at 25°C under vacuum for 24 h. No peptides extractable with dioxane were detected. The results are summarized in Table 1.

The aminated poly(arylene ether sulfone) derivatives, *2* and *3*, and poly(2-aminomethyl-1,3-phenylene arylene ether), *4*, were dissolved in anhydrous THF before adding the BLG-NCA solution. The copolymerizations remained homogeneous except in the cases when the BLG-NCA/polymer ratios were greater than 15. The copolymers were isolated as described above; the results are reported in Tables 2 and 3.

Modification of Graft Copolymers

Graft copolymer *14a* was converted to the corresponding poly[(aminomethylarylene ether sulfone)-*g*-[br]-(N^5-butyl-*L*-glutamine)], *15a*, by stirring

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TABLE 1. Graft Copolymers of γ-Benzyl-*L*-glutamate on Aminopropylcellulose (*1*, D.S. = 0.3)

Structure	APC,[a] g	NCA, g	NCA/APC, mol/mol	Grafting efficiency, %	DP[b]	Conformation
9a	1.076	0.424[c]	0.94	89.0	0.8	α-Helix
9b	0.687	0.813	2.81	80.2	2.3	α-Helix
9c	0.504	0.995	4.67	76.7	3.6	α-Helix
9d	0.303	1.197	9.38	87.6	8.2	β-Sheet + α-helix
9e	0.169	1.331	18.7	70.5	13.2	α-Helix + β-sheet

[a]Aminopropylcellulose, 1.6 meq/g of NH_2.
[b]Average DP of grafts based on weight gain.
[c]Reaction conditions: total reactant concentration, 1.5 g/25 mL of THF; RT; 48 h.

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TABLE 2. Graft Copolymers of γ-Benzyl-*L*-glutamate with Poly(Aminoarylene Ether Sulfone) (*2*) and Poly(Aminomethylphenylene Arylene Ether) (*4*)

Structure	Polymer,[a] g	NCA, g	NCA/NH_2, mol/mol	Grafting efficiency, %	DP[b]	DP[c]	Conformation
10c	0.53[d]	0.47[d]	5	10.0	0.3	0.5	Random + β-sheet
10b	0.36	0.64	10	30.0	3.4	3.0	β-Sheet
10c	0.22	0.78	20	25.0	6.2	5.0	β-Sheet
10d	0.10	0.90	50	26.0	13.6	13.0	α-Helix
11a	0.20	0.12	1	90.0	0.6	0.9	Random
11b	0.15	0.27	3	96.7	2.7	2.9	Random
11c	0.10	0.42	7	91.4	6.2	6.4	β-Sheet + α-helix
11d	0.10	1.20	20	86.0	16.9	17.2	β-Sheet + α-helix

[a]Poly(aminoarylene ether sulfone), 0.67 meq/g of NH_2, substrate for Samples *10a-d*; poly(aminomethylphenylene arylene ether), 0.23 meq/g of NH_2, substrate for Samples *11a-d*.

[b]Average DP of grafts based on weight gain.

[c]Average DP of grafts based on ^1H NMR.

[d]Reaction conditions: total reactant concentration, 1.00 g/30 mL of THF; RT; 48 h for *10a-d*; 10 mL of THF, 24 h for *11a-d*.

a solution of the copolymer (0.1 meq banzyl ester/2 mL solvent) in 12.5/87.5 v/v DMSO-THF with the designated amine at 55°C; the results are summarized in Table 4. The extent of aminolysis and peptide cleavage was estimated by ^{1}H NMR(DMSO-d_6) using the resonances at 5.04 ppm (benzylic CH_2 of PBLG), 1.64 ppm (CH_3 of polysulfone backbone), and 0.79 ppm CH_3 of butyl group).

RESULTS AND DISCUSSION

Cellulose Graft Copolymers

Grafting of BLG-NCA to aminopropylcellulose, *1*, could be accomplished at room temperature in the dried THF (Scheme 1). The cellulose derivative does not appear swollen in THF, but there were enough active amino sites accessible on the surface to allow the reactions to proceed without difficulty. The graft copolymers were not soluble in most solvents, but did swell in THF, DMSO, and pyridine.

Infrared spectra were very useful in confirming the extent of grafting and for identifying the secondary structure of the peptide units grafted. Several publications document the critical chain lengths for β-pleated sheet (4-10 amino acids) or α-helix (>11 amino acids) secondary peptide structures and identify the IR bands in the amide region associate with a given conformation [17-21]. The aminopropylcellulose grafts exhibited strong peaks at 1655 and 1550 cm^{-1} for amide I and amide II bands, respectively, suggesting an α-helical conformation even though the theoretical DP of the grafts was calculated to be less than 5 (Fig. 1). Apparently the actual percentage of amino functional groups which are effective initiators is much lower than that determined by titration, and a few grafts with DP above 15 are formed. The appearance of β-pleated sheet structures at high BLG-NCA/APC ratios suggests that the rate of initiation is much slower than that of propagation due to the heterogeneous system, but incorporation of a few grafts enhances the accessibility of the amine sites. Thus long chain grafts are produced initially, followed by short chains initiated by the amine groups unveiled by the graft-induced swelling of the cellulose matrix. Both the insolubility and the obvious variation in graft structure forced us to seek a more tractable system to utilize in characterization of peptide graft copolymers.

Poly(Arylene Ether) Graft Copolymers

Soluble, well-characterized polymers with primary amine substituents, *2-4*, were used to test the accessibility theory. Poly(2-amino-1,4-phenylene ether

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TABLE 3. Graft Copolymers of γ-Benzyl-*L*-glutamate with Poly(Aminomethylphenylene Arylene Ether Sulfones) (*3a-c*)

Structure	AMPS,[a] g	NCA, g	NCA/AMPS, mol/mol	Grafting efficiency, %	DP[b]	DP[c]	Viscosity,[d] cP	Conformation
12a	0.642[e]	0.358[e]	1	90.0	0.8	0.9		Random
12b	0.375	0.625	3	93.3	2.5	2.8		Random
12c	0.204	0.796	7	100.0	6.6	7.0		Random
12d	0.152	0.848	10	90.0	8.1	9.0		β-Sheet
12e	0.082	0.918	20	82.0	16.3	16.4		β-Sheet + α-helix
13a	0.739[f]	0.261[f]	3	83.3	2.9	2.5	1.84	Random
13b	0.630	0.370	5	90.0	4.7	4.5	1.89	Random + β-sheet
13c	0.460	0.540	10	95.0	9.8	9.5	1.79	β-Sheet + α-helix
13d	0.362	0.638	15	90.7	13.4	13.6	1.79	β-Sheet + α-helix

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13e	0.254	0.746	25	96.0	23.6	24.0	1.93	α-Helix
13f	0.145	0.855	50	88.0	48.3	44.0	2.27	α-Helix
13g	0.078	0.922	100	85.0	95.5	85.0	2.26	α-Helix
14a	9.00[g]	10.38[g]	4	97.5	3.6	3.9		Random + β-sheet
14b	1.5	5.92	13.7	98.5	12.8	13.5		β-Sheet + α-helix

[a]Poly(aminomethylphenylene arylene ether sulfone), *3c*, 2.123 meq/g NH_2 for *12a-e*; *3a*, 0.447 meq/g NH_2 for *13a-g*; *3b*, 1.095 meq/g NH_2 for *14a-b*.

[b]Average DP of grafts based on weight gain.

[c]Average DP of grafts based on ^1H NMR.

[d]2.5% in pyridine at 25°C; Wells-Brookfield Viscometer (spindle, CP-40; angle, 0.8°; volume, 0.5 mL; shear rate, 450 s^{-1}).

[e]Reaction conditions: total reactant concentration, 1.00 g/25 mL of THF, RT, 24 h.

[f]Reaction conditions: total reactant concentration, 1.00 g/30 mL of THF, RT, 48 h.

[g]Reaction conditions: total reactant concentration, 7.00 g/100 mL of THF, RT, 48 h.

TABLE 4. Homogeneous Aminolysis of Poly[(Aminomethylarylene Ether Sulfone)-*g*-[br]-(γ-Benzyl-*L*-Glutamate)] (*14a*) with *n*-Butylamine

n-Butylamine,[a] g (meq)	Reaction time, h	Average branch composition			Cleavage[b]
		Benzyl[c]	Butyl[d]	Total	
0.0	0	3.9	0.0	3.9	0.0
0.293 (4.0)	24	3.2	0.6	3.8	1.6
0.586 (8.0)		1.9	1.8	3.7	5.1
1.170 (16.0)		1.7	2.2	3.9	0.0
1.755 (24.0)		1.5	2.0	3.5	10.3
2.925 (40.0)		0.4	3.2	3.6	7.7
0.586 (8.0)	5	3.6	0.3	3.9	0.0
	12	2.2	1.5	3.7	5.1
	48	1.5	1.9	3.4	12.8
	72	1.1	2.1	3.2	17.9
	96	1.0	2.1	3.1	20.5
	120	0.9	2.2	3.1	20.5
	144	0.9	2.1	3.0	23.1
	204	0.4	2.0	2.4	38.5

[a]Poly[(aminomethylarylene ether sulfone)-*g*-[br]-(γ-benzyl-*L*-glutamate)], *14a*, D.F. = 0.5, average DP = 3.9; 0.2 g (0.44 meq) in 8 mL of DMSO/THF (12.5 v/v), 55°C.

[b]% Amino acids lost.

[c]Average number of γ-benzyl-*L*-glutamate residues per graft based on ^{1}H NMR.

[d]Average number of N^5-*n*-butyl-*L*-glutamine residues per graft based on ^{1}H NMR.

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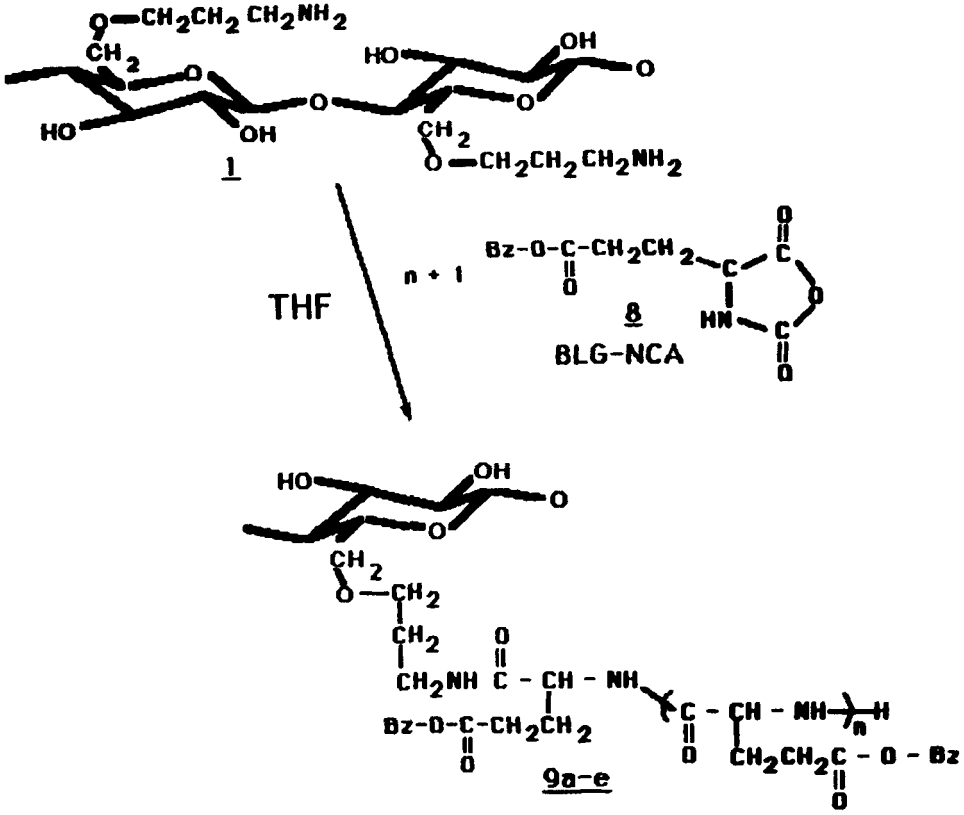

SCHEME 1. Reaction of BLG-NCA with aminopropylcellulose.

sulfone), *2*, prepared by reduction of the corresponding nitro derivative (Scheme 2), dissolved readily in THF and $CHCl_3$. Intrinsic viscosity measurements indicated that little change in molecular weight occurred during the reduction. However, we obtained very low NCA grafting efficiencies with *2* as the macroinitiator (Table 2). The nucleophilicity of these aromatic polyamines is not high enough to initiate the polymerization of NCA's effectively. Further, in contrast to the results obtained with aminopropylcellulose, a termination reaction limited the length of the peptide grafts. Even in the presence of a fifty-fold excess of BLG-NCA, the average DP of the grafts was only 13.

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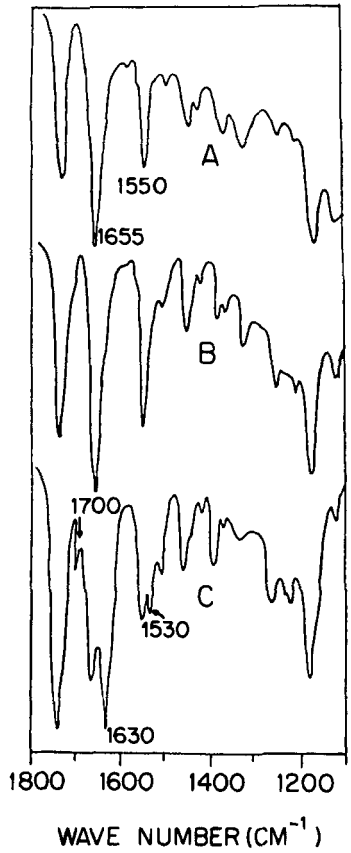

FIG. 1. Infrared spectra of aminopropylcellulose-PBLG graft copolymers. A, DP of PBLG = 0.8; B, DP = 3.6; C, DP = 13.2.

Reduction of the cyano functional group on poly(2-cyano-1,3-phenylene arylene ether), *7*, with lithium aluminum hydride produced poly(arylene ether), *4*, with benzylamine substituents (Scheme 2). Utilization of *4* as a macromolecular initiator yielded results more consistent with our expectations, i.e., the DP of the grafts could be controlled by the BLG-NCA:amine feed ratio. However, α-helical conformations were detected in the sample with an average graft length of 6.3, *11c*, indicating that an equal distribution of the monomer among all the pendent amine initiators was not obtained.

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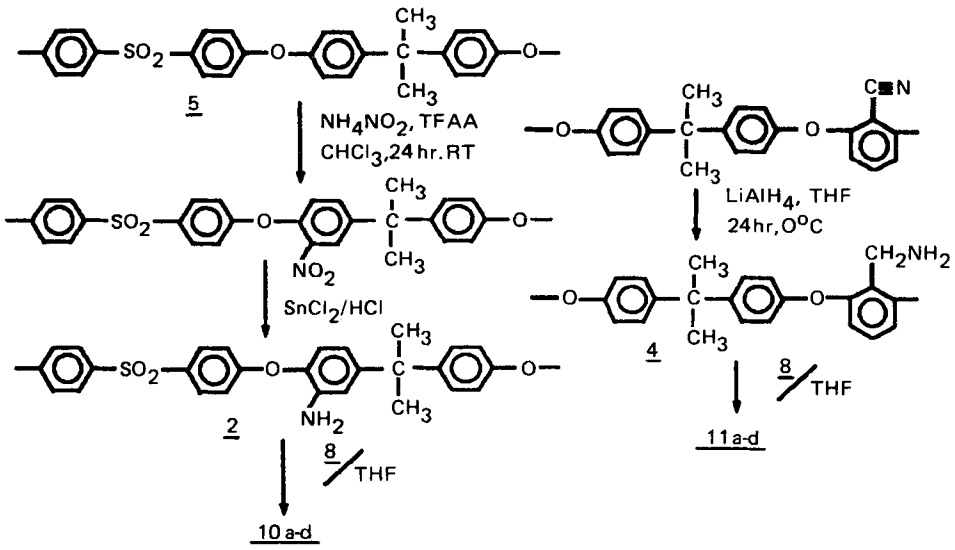

SCHEME 2. Preparation of poly(aminoarylene ethers).

Polymer *4* is rather difficult to synthesize and purify, but the potential activity of aminomethyl groups as NCA initiators was demonstrated.

The most interesting soluble macroinitiator for polymerization of BLG-NCA was *3*, which has a primary aliphatic amino function. The phthalimidomethylation of poly(arylene ether sulfone), *5*, with *N*-hydroxylmethylphthalimide in dichloromethane-trifluoroacetic acid could be controlled to any desired degree of substitution, although some reduction in the molecular weight of the highly substituted polymers was detected by viscosity measurements. The aminomethyl substituents were released by hydrazinolysis of the phthalimide substituents in a THF/ethanol solvent mixture (Scheme 3). The aminomethylated polymers with low amine contents (D.F. < 1) are soluble in $CHCl_3$, dioxane, THF, DMSO, and pyridine. Graft copolymers *12a-e* were synthesized from *3c*, D.F. = 1.0 (Table 3). The average DP of the grafts appeared to be correlated directly with the BLG-NCA:amine feed ratio.

The conformations for BLG units grafted were assigned from the IR spectra of films. The graft copolymers *12a-e* were soluble in hot pyridine and 5 vol% TFA in $CHCl_3$. However, attempts to confirm the DP's of BLG grafted to the poly(arylene ether sulfone) backbone by comparison of the integrations

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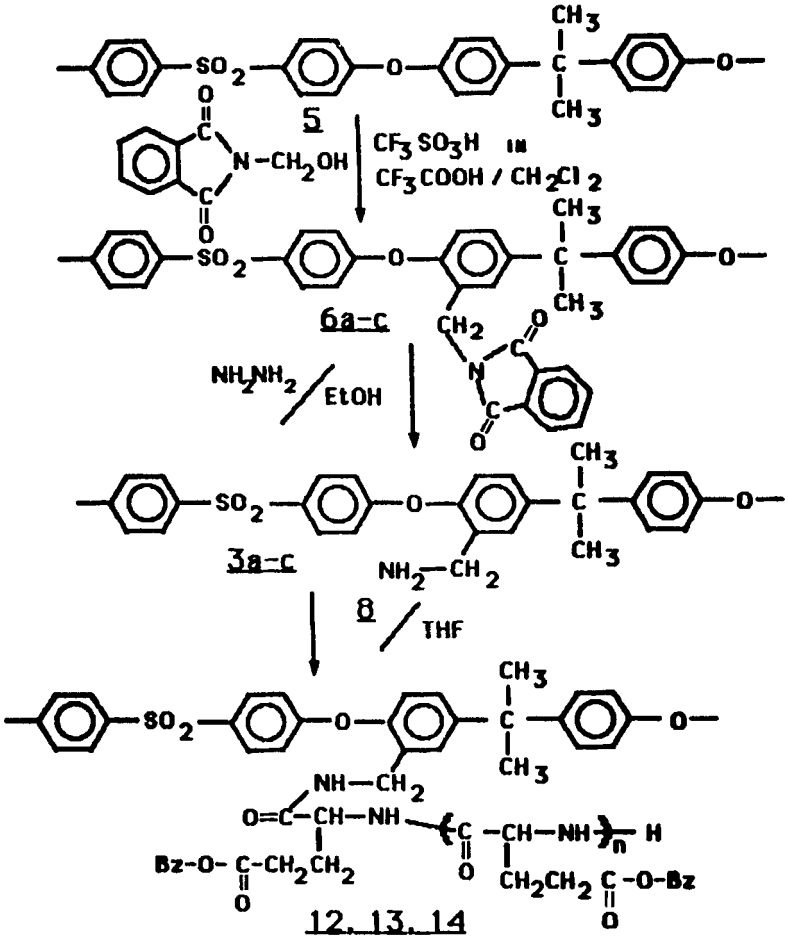

SCHEME 3. Preparation and modification of poly(aminomethylarylene ether sulfone).

of ^{1}H-NMR peaks at 5.04 ppm (benzylic H's of BLG) and 1.70 ppm (methyl H's of backbone polymer) were thwarted for these densely grafted copolymers due to the relatively high contents of PBLG units relative to the backbone protons.

Use of Polymer *3a*, D.F. = 0.2, reduced the loading on the backbone and

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produced a better balance between graft and backbone properties (Table 3). These copolymers, *13a-g*, were soluble in pyridine and DMSO; copolymers with a low DP (< 15 PBLG units) were also soluble in $CHCl_3$. The graft copolymerizations were carried out with greater than 85% efficiency, and the structures of the peptides were consistent with the average DP's calculated from the feed ratios. The secondary conformation of the PBLG units was identified by using IR and NMR spectroscopy. A disordered structure, which has the amide I band at 1660 cm^{-1} and the amide II at 1535 cm^{-1}, was predominant for copolymers with 2.5 or 4.5 BLG units. The IR spectrum of copolymer with 9.5 or 13.6 PBLG units grafted exhibited the amide I band at 1700 and 1630 cm^{-1} and the amide II band at 1530 cm^{-1}, characteristic of the antiparallel β-pleated sheet structure. Infrared spectra of copolymers with more than 15 amino acids in the PBLG units show strong bands at 1655 and 1550 cm^{-1}, characteristic of the α-helical conformation of PBLG units (Fig. 2).

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Solution Properties of the Graft Copolymers

Since the infrared studies of films revealed the presence of helical grafts in the solid state, we elected to study the structure of the copolymers in solution by NMR. Studies with oligopeptides in dilute solution reveal resolvable amide (NH) and α-methine (α-CH) resonances with differing chemical shifts for the random and α-helical conformations [22]. Since the backbone polymer would hold the grafts in close proximity, high dilution measurements would not be possible. On the other hand, some indication of the intermolecular bonding should be evident. As shown in Fig. 3, the spectra of *13d* in a 5% w/v deuterochloroform solution was essentially featureless in the peptide region. Addition of trifluoroacetic acid was required to sharpen the α-CH and, interestingly, the benzylic CH_2 resonances. Up to 3 vol% TFA could be added to the $CDCl_3$ solution without disrupting the α-helical conformation, (α-CH = 3.94 ppm). Transformation of the α-helical conformation to a random coil structure could be effected by increasing the amount of trifluoroacetic acid in the chloroform-*d* solution [23]. At 10 vol% TFA, the chemical shift of the α-methine reached 4.6 ppm, and further additions of TFA failed to produce any change, indicating that the grafts were in the random coil conformation. The conformations, however, were not changed when the concentrations of grafted copolymers in 3 vol% TFA-$CDCl_3$ were varied within the range of 0.3-11% w/v.

Determination of the average DP for the peptide grafts involves quantification of the backbone and peptide resonances. These measurements were made in 10 vol% TFA/$CDCl_3$ because the most accurate comparisons were obtained with the peptide in a random coil conformation. A typical spectrum is shown

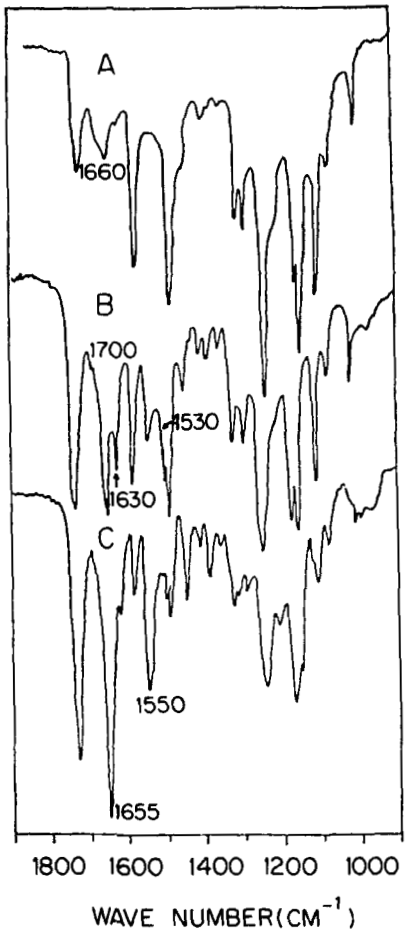

FIG. 2. Infrared spectra of poly[(aminomethylarylene ether sulfone)-*g*-[br]-(γ-benzyl-*L*-glutamate)]. Sample, BLG units: A, *13a*, 2.5; B, *13c*, 9.5; C, *13f*, 44.

in Fig. 4. The resonances associated with the poly(arylene ether sulfone) backbone are reasonably well resolved, with the exception of an overlap in the aromatic region with the aromatic protons attached to the benzyl ester and the amide protons of the peptide backbone. The average DP was calculated from

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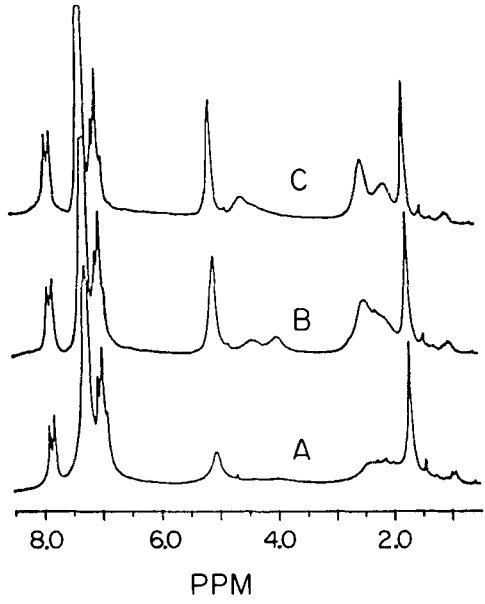

FIG. 3. Proton NMR of *13d* in $CDCl_3$ (5% w/v) with various concentrations of trifluoroacetic acid (TFA). A, 0 vol% TFA; B, 3 vol%; C, 10 vol%.

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the ratio of the benzyl CH_2 protons to the geminal CH_3 protons in the backbone polymer.

The viscosities of the graft copolymers were evaluated under various shear conditions to ascertain if the grafts promoted aggregation at low shear. As is often the case with graft copolymers, the viscosities observed at high shear rates for the copolymers did not vary much from those of the backbone (Table 3). Both the starting backbone polymer and the graft copolymers exhibited some shear thinning at low shear rates. The recovery of the low-shear viscosity was instantaneous when the shear was reduced on the backbone polymer in a stirred system. In contrast, Copolymer *13g* required 3-8 min to recover the initial low-shear viscosity. Thus, there is some indication of aggregation, but the effect is not pronounced.

Amidation of PLBG Grafts

Utilization of the graft copolymers as controlled delivery systems depends upon facile modification of the PBLG grafts. Aminolysis of the benzyl ester

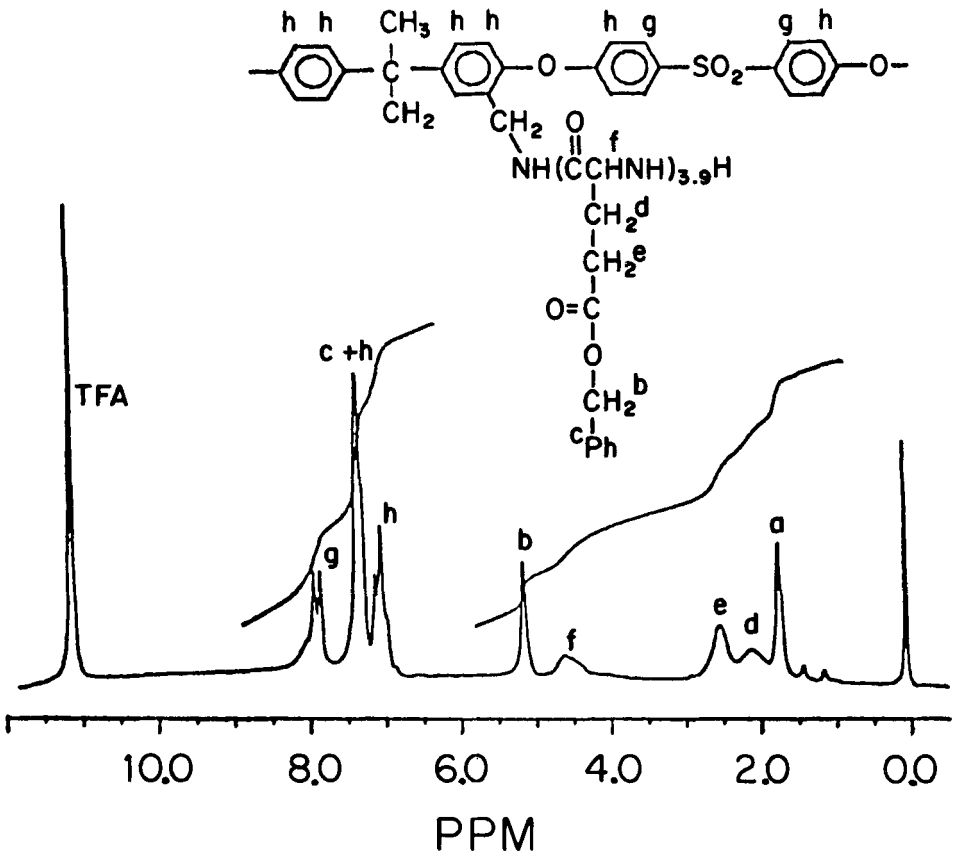

FIG. 4. A typical proton NMR spectrum of *14a* (10% w/v) in 10 vol% TFA-$CDCl_3$ at 297 K.

on PBLG homo- and copolymers with ammonia and various amines leads to the formation of γ-amides [24]. In fact, Scheraga used 3-aminopropanol and 4-aminobutanol extensively to prepare water-soluble polypeptides [10]. However, when we attempted this transformation with Graft Copolymer *14a*, the derivative precipitated rapidly from either THF or DMSO-THF solutions, indicating that crosslinking via transesterification had occurred. As the reaction proceeded, the sample redissolved in DMSO-THF but precipitated when we added water for dialysis. Anticipating that the hydroxyethylamide was not

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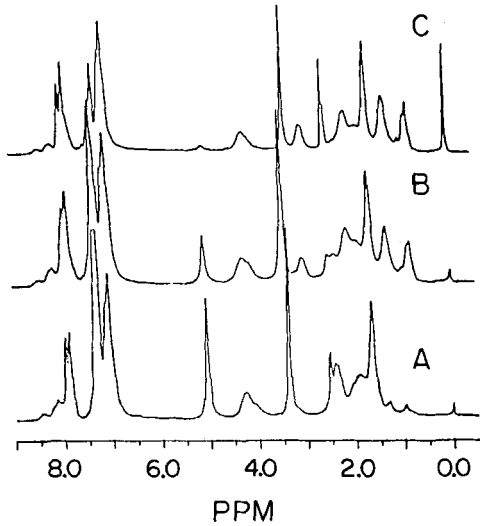

FIG. 5. Proton NMR spectra (in DMSO-d_6) of partially amidated graft copolymers from *14a*. A, 3.9 BLG units; B, 1.9 BLG + 1.8 BuLG units; C, 0.4 BLG + 2.0 BuLG units; where BuLG = N^5-*n*-butyl-*L*-glutamine.

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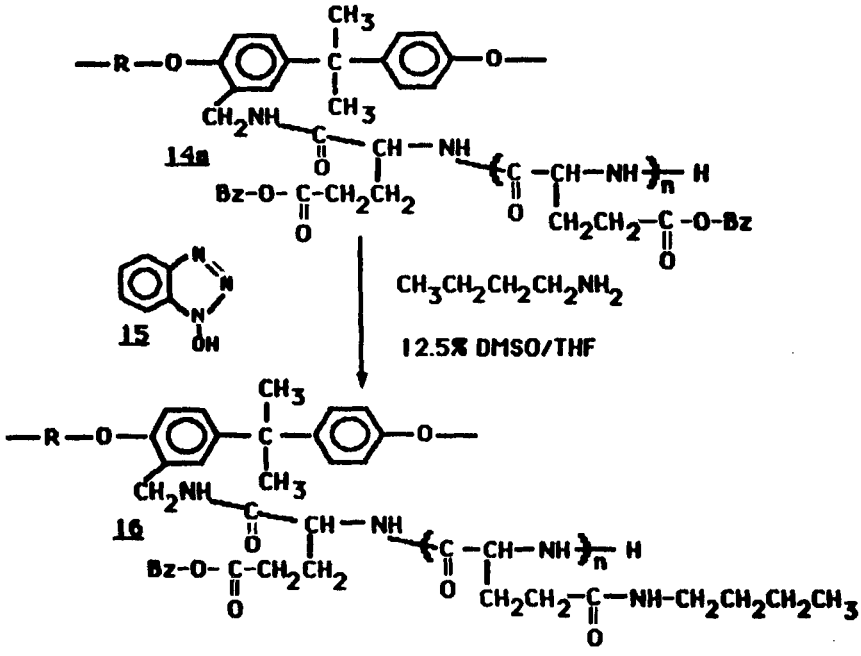
sufficiently hydrophilic to pull the backbone polymer into aqueous solution, we allowed *14a* to react with tris(hydroxymethyl)aminomethane in DMSO; conversions to the corresponding amide of greater than 50% produced an adduct which would swell in water, but homogeneous aqueous solutions could not be obtained. Clearly the backbone polymer plays an important role in the solution properties of the graft copolymers.

The rate of aminolysis of *14a* was studied under heterogeneous and homogeneous conditions with butylamine as a model nucleophile. Although the term "aminolysis" is applied primarily to cleavage of the γ-benzyl ester, a side reaction which can become significant is cleavage of the peptide chain by transamidation. By working with copolymers with short chain grafts, we were able to monitor both processes by NMR. Aminolysis of the benzyl ester led to the disappearance of the benzylic CH_2 resonances (5.04 ppm) and the appearance of butyl CH_3 at 0.79 ppm in DMSO-d_6 (Fig. 5). The ratio of glutamine peptide to backbone polymer could be monitored by comparing the peptide resonances to the peak at 1.64 ppm for the geminal CH_3 of the backbone.

When the aminolysis of *14a* was conducted in THF, the product precipitated when approximately 40% of the benzyl ester had reacted. Conversion of the



SCHEME 4. Aminolysis of PBLG grafts.

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benzyl ester to amide reached 50% within 24 h and did not change significantly thereafter. However, the extent of peptide chain cleavage continued to increase from 7.7 to 17.9% over a total reaction time of 144 h. When the product precipitated, the access to the benzyl ester was impeded, and subsequent activity was focused on the peptide bonds. Addition of 12.5% DMSO to THF produced a solvent mixture which dissolved both reactants and products in all conversion ranges. Thus, it was possible to study the aminolysis of *14a* under homogeneous conditions in this solvent mixture (Scheme 4).

The influence of reactant ratios and reaction times was studied in the 12.5/87.5 v/v DMSO-THF solvent mixture (Table 4). The composition of the copolymer isolated after 24 h of reaction with different ratios of amine to PBLG is plotted in Fig. 6. Note that the extent of peptide cleavage can be minimized by using very large excesses, i.e., >50:1, of the amine. In fact, if a hundredfold excess is used, 82% conversion of the benzyl ester to amide can be achieved in 24 h at 55°C. However, if a twentyfold excess of amine is used and the reac-

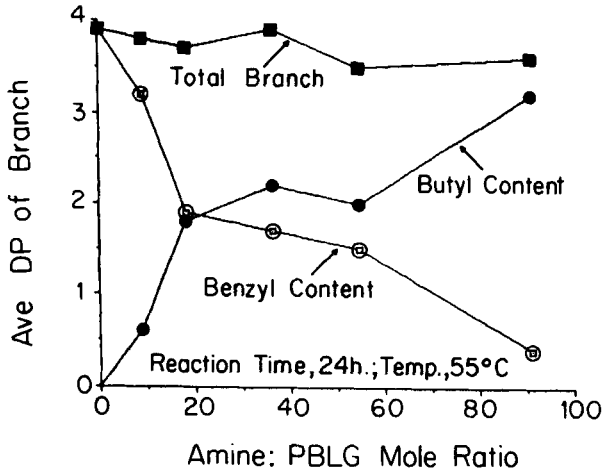

FIG. 6. Influence of reactant ratios on the aminolysis of *14a* with *n*-butylamine in 12.5/87.5 v/v DMSO-THF at 55°C.

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tion is allowed to run for several days, significant loss of peptide units is observed (Fig. 7). Only 56% of the benzyl ester initially present is converted to amide.

The aminolysis of the γ-benzyl ester can be catalyzed by 1-hydroxybenzotriazole, *15* [25]. When 1.2 equivalents of *15* was added to a solution of *14a* with a twentyfold excess of butyl amine, 64, 72, and 75% conversion of the benzyl ester to amide was obtained in 6, 11, and 24 h, respectively. Cleavage of the peptide bonds was minimized under these conditions. The average DP of the peptide branches in *14a* is 4, but only three of the amino acid residues react rapidly. It appears that the amino acid bound directly to the backbone polymer is subject to more steric hindrance and may be more difficult to transform. Further studies with single amino acid adducts are currently in progress.

CONCLUSIONS

A soluble macroinitiator, poly(aminomethylarylene ether sulfone), was effective in initiating the polymerization of the NCA of γ-benzyl-*L*-glutamate to produce soluble graft copolymers. The average DP of the peptide grafts could be predicted from the BLG-NCA/amine ratio. Infrared and NMR spec-

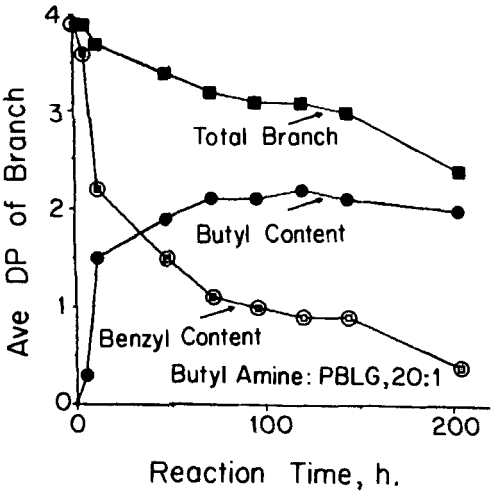

FIG. 7. Influence of reaction times on the aminolysis of *14a* with *n*-butylamine in 12.5/87.5 v/v DMSO-THF at 55°C.

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troscopy could be used to determine the conformations of the PBLG grafts. The secondary conformations depended primarily on the length of PBLG chain; little influence of the backbone polymer on the peptide orientation could be detected. The γ-benzyl ester could be modified by aminolysis, particularly in the presence of 1-hydroxybenzotriazole.

Aminopropylcellulose could also act as a macroinitiator for NCA polymerization with high grafting efficiency. However, conformational data showed that the chain length of the polypeptide grafts was not a simple function of the BLG-NCA/amine ratio.

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