

Poly(β -amino acid)s. II. Polycondensation of *p*-Nitrophenyl β -amino- β -phenylpropionate

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ABSTRACT: The polycondensation reaction of *p*-nitrophenyl β -amino- β -phenylpropionate was investigated in various solvents. In all cases, the polymers produced were precipitated during the reaction. The polymer yield was higher in chlorobenzene or toluene rather than in DMF or DMSO. The rate of the polycondensation reaction was markedly influenced by the monomer concentration in DMF. The effects of water and amine, the latter of which was used to neutralize the hydrobromide of the monomer, were also investigated. A small amount of water seemed to have little influence in chlorobenzene and in THF, but markedly depressed the polymer yield in DMF. The polymer yield was independent of the excess amount of triethylamine used. In general, the solution viscosity of the polymer obtained was not affected by the reaction conditions. The polycondensation reaction in DMF was followed by measuring spectrophotometrically the concentration of *p*-nitrophenol liberated or the *p*-nitrophenyl ester consumed. The kinetic order of the reaction was found to be second. The second order rate constant at 60°C and the apparent activation energy were 1.6×10^{-3} l/mol·sec and 3.5 kcal/mol, respectively.

KEY WORDS Polycondensation / *p*-Nitrophenyl β -Amino- β -phenylpropionate / Poly(β -amino- β -phenylpropionic acid) / Rate Constant /

Many reports have been published on the syntheses and the properties of poly(α -amino acid)s.¹ On the other hand, only a few investigations have been reported on poly(β -amino acid)s, which have only one additional methylene group per residue compared with poly(α -amino acid)s.

The high molecular weight poly(α -amino acid) can be easily obtained by the polymerization of *N*-carboxyanhydride of the α -amino acid. On the contrary, poly(β -amino acid)s were synthesized by the polymerization of *N*-carbothiophenyl derivative of β -amino acid,² the base catalyzed polymerization of acrylamide and its derivatives,³ or by the ring opening polymerization of β -lactams,⁴ but their molecular weight does not seem to be always high.

In the previous study,⁵ we reported the preparation of the polymers of phenyl-substituted β -amino acids, such as α -phenyl-, β -phenyl-, and α,β -diphenyl- β -aminopropionic acid. The thermal polycondensation of the β -amino acids

themselves and their ethyl esters resulted in certain side reactions, such as β -elimination, and did not yield high polymers. However, it was found that the polycondensation of the *p*-nitrophenyl esters of the β -amino acids gave polymers in good yields under milder reaction conditions. The *p*-nitrophenyl ester is one of the active ester reagents used in peptide syntheses. In this work, a further investigation was carried out on the polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate in detail, especially on the effects of the reaction conditions upon the polymer produced. The kinetic study on the polycondensation of *p*-nitrophenyl ester of dibasic acid and piperazine has been reported by Overberger, *et al.*,⁶ but no data are available for the polycondensation of the *p*-nitrophenyl ester of β -amino acid. The rate of the polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate was investigated by following spectrophotometrically the amount of *p*-nitrophenol originated from the aminolysis of

of the *p*-nitrophenyl ester.

EXPERIMENTAL

Reagents and Solvents

p-Nitrophenyl β -amino- β -phenylpropionate hydrobromide (monomer·HBr) was prepared from β -amino- β -phenylpropionic acid as described in the preceding paper¹ via *N*-benzyloxycarbonyl- β -amino- β -phenylpropionic acid and its *p*-nitrophenyl ester. The monomer·HBr was recrystallized from a mixture of dry acetone and ether. This was soluble in ethanol, acetone, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO). mp 173.5–175°C (decomp).

p-Nitrophenol was recrystallized from ethanol. Triethylamine (Et₃N) was fractionally distilled with a small amount of acetic anhydride and then distilled under nitrogen atmosphere from potassium hydroxide.

DMF was first dried by azeotropic distillation with dry benzene, followed by treatment with activated alumina and distillation. DMSO was stirred with activated alumina, distilled and stored on Molecular Sieves 13X. Tetrahydrofuran (THF) and dioxane were refluxed with metallic sodium and distilled from lithium aluminum hydride. Toluene and chlorobenzene were purified as usual, and the former was further dried with metallic sodium.

Polycondensation

The monomer·HBr was suspended or dissolved in a given volume of solvent in a flask, which had been flushed with dry nitrogen. Triethylamine was then added with a hypodermic syringe to the flask immersed in a thermostat. The reaction was allowed to proceed at a given temperature under an atmosphere of dry nitrogen. In the course of the reaction massive precipitates of the polymer were deposited, which were filtered after a given time of the reaction. The polymer was washed several times with ethanol by applying centrifugation to remove Et₃N·HBr. The resultant pale yellow powders were finally washed with diethyl ether, dried *in vacuo* at 60°C and weighed. The polymer was soluble in trifluoroacetic acid, dichloroacetic acid and *m*-cresol, and insoluble in usual organic solvents. It decomposed above 300°C without melting.

Kinetic Measurement

The polycondensation reaction was carried out under dry nitrogen in a 50 ml flask, which contained 1.000 g (2.72 mmol) of the monomer·HBr dissolved in 16 ml of DMF. After the flask was immersed in a constant temperature bath, 0.305 g (3.01 mmol) of Et₃N was added to the solution to start the polycondensation. From the reaction mixture, aliquots (0.05 ml) were withdrawn under a stream of dry nitrogen by a 0.05 ml micropipette at suitable time intervals and diluted to 10 ml with DMF. When the precipitation of the polymer had already occurred, a small amount of the solution was sucked up through the disc of sintered glass attached to one side of a glass tube by applying a nitrogen pressure on the surface of the reaction mixture, and a 0.05 ml aliquot was withdrawn from the filtrate. The diluted solution was analyzed spectrophotometrically. Because of the marked influence of water on the polycondensation reaction in DMF, extreme care was taken to exclude water from the reaction mixture throughout the procedure.

Measurements

The electronic spectrum was taken with a Hitachi 124 spectrophotometer. The solution viscosity of the polymer was measured in dichloroacetic acid at 30.0°C.

RESULTS AND DISCUSSIONS

Effects of Polymerization Conditions

The polycondensation was carried out in several solvents at 60°C for 24 hr. The results are shown in Table I. Since the monomer·HBr was insoluble in toluene, chlorobenzene, THF and dioxane, it was suspended in these solvents. By the addition of Et₃N the monomer was freed from HBr and dissolved, while Et₃N·HBr was separated out as a massive solid. In DMF, DMSO and ethanol, pale yellow solutions were obtained because both the monomer·HBr and Et₃N·HBr were soluble in the solvents. In every case, except that in ethanol, the polymer was precipitated during the course of the polycondensation. When the polycondensation was carried out in bulk, the reaction mixture turned to a solid mass. The polymer yield was higher in chlorobenzene or toluene than in DMF or DMSO

Table I. Polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate in various solvents^a

No.	Solvent	Yield, %	η_{sp}/c , dl/g
1	None ^b	94.6	0.08
2	Toluene	91.1	0.10
3	Chlorobenzene	87.6	0.12
4	Chlorobenzene ^c	80.0	0.09
5	Tetrahydrofuran	86.1	0.11
6	Dioxane	84.2	0.10
7	Dimethylformamide	70.6	0.09
8	Dimethyl Sulfoxide	68.6	0.09
9	Ethanol	trace	—

^a Monomer·HBr, 1.00 g; Et₃N/monomer·HBr=1.1 (mol/mol); Solvent, 7.5 ml; time, 24 hr; temp, 60°C.

^b Reaction time, 3 hr.

^c Polymerized in the absence of triethylamine hydrobromide.

which dissolved the monomer·HBr, whereas the solution viscosity of the polymer obtained was almost constant. The constant viscosity, *i.e.*, the constant molecular weight of the polymer, seems to be attributed to the precipitation of the polymer during the polycondensation reaction. The precipitation may severely depress the rate of the further growth of the polymer beyond a certain degree of polymerization, where the polymer came to be insoluble in the reaction mixture. When ethanol was used as a solvent, the solvolysis of the active ester preceded the polycondensation, resulting in the extremely low yield and low molecular weight of the product.

As described above, the polycondensation was carried out, in general, under the existence of Et₃N·HBr produced *in situ*. As a control experiment (Table I, No. 4) the polycondensation was carried out in chlorobenzene in the absence of Et₃N·HBr. This was attained by the filtration of the reaction mixture immediately after the addition of Et₃N. The operations were performed under an atmosphere of dry nitrogen using a flask equipped with a sintered glass tube, which was connected with the reaction vessel. The Et₃N·HBr was obtained quantitatively by the filtration. The result showed that the removal of Et₃N·HBr did not affect the

polycondensation. The polymer yield after 3 hr was 80%. After the polymer was collected by the filtration of the reaction mixture, dry hydrogen chloride was passed through the filtrate thus separated. By the evaporation of chlorobenzene the oligomer hydrochloride was obtained as an oily residue containing *p*-nitrophenol. An additional amount of the polymer (*ca.* 10%) was obtained from the residue by heating at 60°C for another 24 hr with Et₃N in chlorobenzene. This is an indication that the *p*-nitrophenyl ester in the solution remained active after the precipitation of the polymer had occurred.

The effects of bases on the polycondensation in DMF were investigated. The results are shown in Table II. Kovacs, *et al.*,² reported that in the polycondensation of *p*-nitrophenyl ester of γ -methyl-L-glutamyl-glycine the highest degree of polymerization was obtained in the presence of 2.5 mol of Et₃N per mole of the hydrobromide of the ester. However, in the present study, an excess of Et₃N seems to have hardly any effect on the yield and the solution viscosity of the polymer. Table II also shows the results of the polycondensations which were conducted by adding some other bases instead of Et₃N. When pyridine was used, where the base served as the solvent, the polymer yield was lowered, probably due to the low basicity of pyridine ($pK_a=5.2$) compared with that of Et₃N ($pK_a=10.9$). The water originating from the reaction of HBr with sodium hydroxide was responsible for the decreases of the yield and the solution viscosity of the polymer in the polycondensation where sodium hydroxide was applied. In the case of calcium hydride, the evolution of hydrogen gas was observed. The polymer yield was not affected, but the purification of the polymer was rather difficult on account of the occlusion of the unreacted calcium hydride in the polymer.

The effect of water on the polycondensation is shown in Table III. In chlorobenzene saturated with water, the polymer was prepared without the influence of water (Table III, No. 19). In THF, polycondensation proceeded in spite of the presence of a large excess of water against the amount of the monomer, although the yield and the solution viscosity of the polymer

Table II. Effect of base on the polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate in DMF^a

No.	Base	Base/M·HBr ^b , mol/mol	Time, hr	Yield, %	η_{sp}/c , dl/g
10	Triethylamine	1.1	3	67.5	0.10
11	"	2.5	"	54.3	0.09
12	"	3.4	"	57.7	0.08
13	"	9.4	"	60.2	0.07
14	"	28.7	"	63.7	0.08
7	"	1.1	24	70.6	0.09
15	Pyridine	1.5	"	7.6	—
16	Sodium Hydroxide	1.2	"	34.0	0.07
17	Calcium Hydride	1.2	"	73.5	0.09

^a Monomer·HBr, 0.50 g; solvent, DMF 3.7 ml; temp, 60°C.^b Monomer·HBr.**Table III.** Effect of water on the polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate^a.

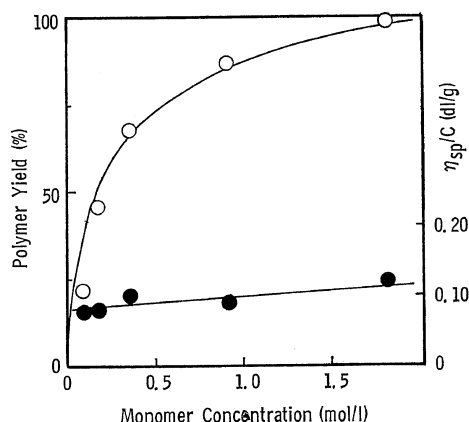
No.	Solvent	H ₂ O/Monomer, mol/mol	Time, hr	Yield, %	η_{sp}/c , dl/g
18	Chlorobenzene	0	3	76.5	0.10
19	"	0.07	"	71.7	0.09
5	Tetrahydrofuran	0	24	86.1	0.11
20	"	5.4	"	58.0	0.09
21	"	40.1	"	43.0	0.07
7	Dimethylformamide	0	"	70.6	0.09
16	Dimethylformamide ^b	1.0	"	34.0	0.07

^a Monomer·HBr 0.50 g; Et₃N/monomer·HBr=1.1 (mol/mol); solvent, 3.7 ml; temp, 60°C.^b Sodium hydrobromide was used as a base instead of triethylamine. The water was formed *in situ* from the base and hydrogen bromide attached to the *p*-nitrophenyl ester.

were lowered. On the contrary, marked depressions in the yield and the solution viscosity were found for the polymer obtained in the polycondensation in DMF with sodium hydroxide, which was used as a base to neutralize the monomer·HBr. As mentioned already, these depressions must be attributed to the water formed from sodium hydroxide and hydrogen bromide, by an equimolar amount against the monomer.

Figure 1 showed the influence of the monomer concentration on the polycondensation in DMF. The polymer yield decreased remarkably with the dilution of the monomer, but the solution viscosity of the polymer obtained was not affected.

The time—conversion curves of the polycondensations in chlorobenzene and in DMF are shown in Figure 2. The polymer yield in-

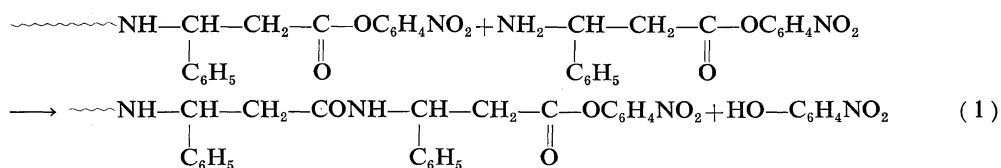
**Figure 1.** Polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate; Effect of monomer concentration: solvent, DMF; time, 3 hr; temp, 60°C; Et₃N/monomer·HBr=1.1 (mol/mol); ○, polymer yield; ●, viscosity.

creased with the reaction time until 5 hr in chlorobenzene and 10 hr in DMF, and showed almost no further changes afterwards. On the other hand, the solution viscosity of the polymer was almost constant regardless of the reaction time.

Kinetic Study of Polycondensation

The polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate proceeds as follows (eq 1).

The concentration of *p*-nitrophenyl



ester decreases accompanying the increment of that of *p*-nitrophenol with the condensation reaction. Therefore, the reaction can be followed by measuring the amount of *p*-nitrophenol liberated or the *p*-nitrophenyl ester diminished. The *p*-nitrophenyl ester of the polymer end showed a maximum absorption at 275 $m\mu$. In the determination of its molar extinction coefficient, the *N*-benzyloxycarbonyl derivative of *p*-nitrophenyl β -amino- β -phenylpropionate was used as a model compound. *p*-Nitrophenol exhibits its

absorption maximum at 315 $m\mu$ in DMF, while the absorption maximum of *p*-nitrophenolate ion shifts to 430 $m\mu$ in the same solvent. In order to obtain the molar extinction coefficients, the former was measured in DMF containing hydrogen chloride and the latter in that saturated with sodium hydroxide. The UV spectra and the molar extinction coefficients are shown in Figure 3 and Table IV, respectively. The concentrations of *p*-nitrophenyl ester and *p*-nitrophenol in the reaction mixture were calculated by the following equations.

$$A_{275} = 8.80 \times 10^3 C_1 + 2.90 \times 10^3 C_2 + 1.22 \times 10^3 C_3 \quad (2)$$

$$A_{315} = 1.95 \times 10^3 C_1 + 1.05 \times 10^4 C_2 + 1.52 \times 10^3 C_3 \quad (3)$$

$$A_{430} = 3.42 \times 10^4 C_3 \quad (4)$$

where A_{275} , A_{315} and A_{430} are the absorbances at 275, 315, and 430 $m\mu$, and C_1 , C_2 and C_3 are the concentrations of *p*-nitrophenyl ester, *p*-nitrophenol and *p*-nitrophenolate anion, respec-

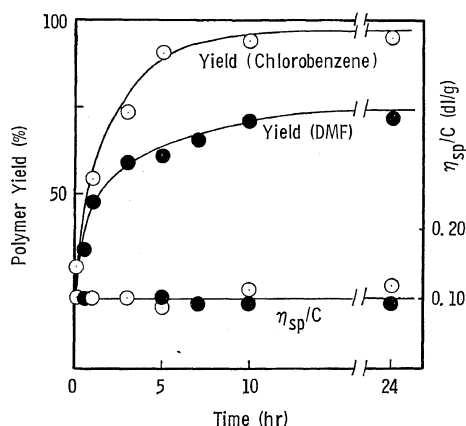


Figure 2. Polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate; time vs. polymer yield and viscosity: monomer \cdot HBr, 1.00 g; solvent, 8 ml; temp, 60°C; $\text{Et}_3\text{N}/\text{monomer} \cdot \text{HBr} = 1.1$ (mol/mol); \circ , chlorobenzene; \bullet , DMF.

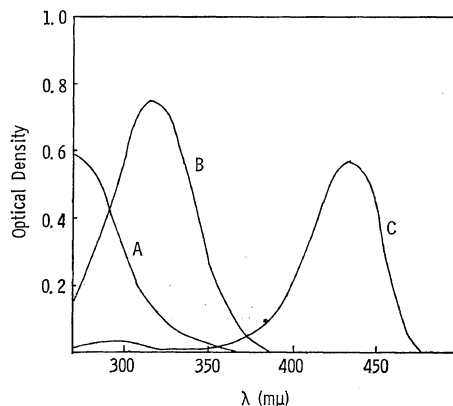


Figure 3. Electronic spectra of *p*-nitrophenol and related compounds in DMF: A, *p*-nitrophenyl *N*-benzyloxycarbonyl- β -amino- β -phenylpropionate, 0.745×10^{-4} mol/l (cell length 1 cm); B, *p*-nitrophenol, 0.685×10^{-3} mol/l, C, *p*-nitrophenolate, 1.64×10^{-4} mol/l.

Table IV. Molar extinction coefficients of *p*-nitrophenol and related compounds in DMF

	275 m μ	315 m μ	430 m μ
$\text{C}_6\text{H}_5\text{CH}_2\text{OCNHCHCH}_2\text{COC}_6\text{H}_4\text{NO}_2$ $\begin{array}{c} \text{O} \quad \quad \quad \text{O} \\ \parallel \quad \quad \quad \parallel \\ \text{C}_6\text{H}_5 \end{array}$	8800 (max)	1950	0
$\text{NO}_2\text{C}_6\text{H}_4\text{OH}$	2900	10500 (max)	0
$\text{NO}_2\text{C}_6\text{H}_4\text{O}^-$	1220	1520	34200 (max)

tively. The validity of the equations was tested by analyzing the mixtures of *p*-nitrophenyl *N*-benzyloxycarbonyl- β -amino- β -phenylpropionate and *p*-nitrophenol at various compositions of the components. The difference between the calculated and found values lay within $\pm 3\%$. In practice, however, the absorbance at 430 m μ due to *p*-nitrophenolate was not observed, *i.e.*, $C_3=0$, in the measurement of polycondensation system.*

Figure 4 shows the changes in the concentration of *p*-nitrophenyl ester and *p*-nitrophenol with the reaction time. The amount of *p*-nitrophenyl ester consumed was equal to that of *p*-nitrophenol formed. In the initial stage the aminolysis of *p*-nitrophenyl ester proceeded very rapidly but later the reaction rate dropped

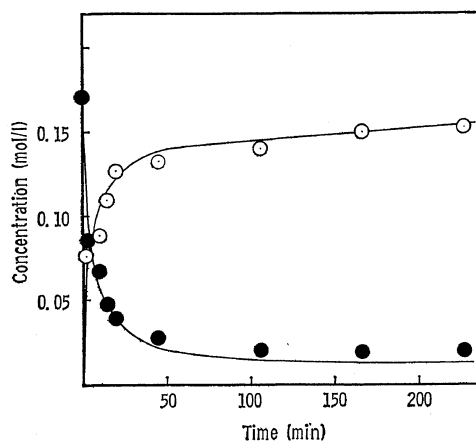


Figure 4. Polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate; concentrations of *p*-nitrophenol and *p*-nitrophenyl ester vs. reaction time: monomer·HBr, 1.00 g; solvent, DMF 16 ml; temp, 60°C; Et₃N/monomer·HBr=1.1 (mol/mol); ○, *p*-nitrophenol; ●, *p*-nitrophenyl ester.

* It was found that the phenolate anion was produced by prolonged standing after the solution of *p*-nitrophenol in DMF.

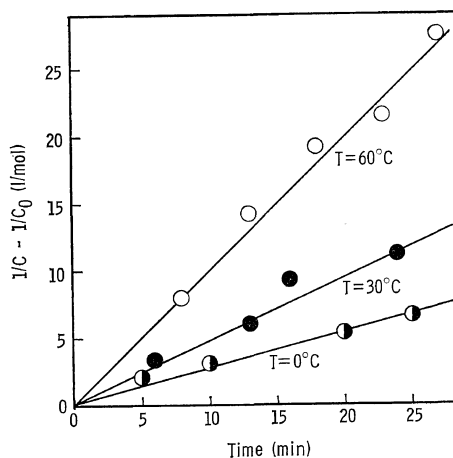


Figure 5. Second order rate plots of polycondensation reaction of *p*-nitrophenyl β -amino- β -phenylpropionate in DMF: monomer·HBr, 0.17 mol/l; Et₃N/monomer·HBr=1.1 (mol/mol).

markedly. The kinetic order with respect to the *p*-nitrophenyl ester was found to be second, at least in the first 20–30 minutes of the reaction. The second order rate plots are shown in Figure 5. From these results the rate constant k in the following equation was obtained to be 1.6×10^{-2} l/mol·sec at 60°C.

$$-dP/dt = k[P]^2 = k[\text{NH}_2\text{---}][\text{---COOC}_6\text{H}_4\text{NO}_2] \quad (5)$$

where P is the concentration of *p*-nitrophenyl ester. Overberger, *et al.*,⁶ reported that the second order reaction was fit for the formation of polyamides from various active esters of (\pm) bicyclo [2, 2, 2] octane-*trans*-2,3-dicarboxylic acid and piperazine in chloroform on the basis of the infrared spectroscopy. From the Arrhenius plot shown in Figure 6, the activation energy of the polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate was found to be 3.5 kcal/mol. This value was rather small com-

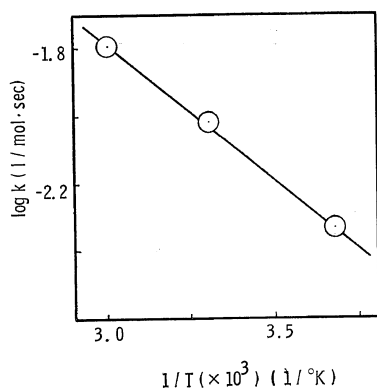


Figure 6. Polycondensation of *p*-nitrophenyl β -amino- β -phenylpropionate. Arrhenius plot of the polycondensation.

pared with 7.0 kcal/mol obtained by Nakamizo⁸ for the aminolysis of *p*-nitrophenyl *N*-benzyloxy-carbonyl-L-phenylalaninate with *t*-butyl ester of glycine in anhydrous dioxane. Nakamizo also reported that the activation energy was lowered to 3.4–3.8 kcal/mol by the addition of base or acid, such as α -pyridone, acetic acid or dichloroacetic acid, but the excess triethylamine

was not responsible for the lower activation energy. Therefore, the difference between the solvents used was responsible for the difference between the activation energies obtained by Nakamizo and in the present study, although further investigations are expected to clarify the exact mechanisms in both cases.

REFERENCES

1. "Poly- α -Amino Acids", G. D. Fasman Ed., Marcel Dekker Inc., New York, N.Y., 1967.
2. J. Noguchi and T. Hayakawa, *J. Amer. Chem. Soc.*, **76**, 2846 (1954).
3. D. S. Breslow, G. E. Hulse, and A. S. Matlack, *ibid.*, **79**, 3760 (1957).
4. H. Bestian, *Angew. Chem.*, **80**, 304 (1968).
5. H. Yuki, Y. Taketani, S. Yamashita, H. Okuno, and H. Tanaka, *Bull. Chem. Soc., Japan*, **43**, 1855 (1970).
6. C. G. Overberger and J. Sebenda, *J. Polym. Sci., Part A-1*, **7**, 2875 (1969).
7. J. Kovacs, N. Schmit, and U. R. Ghatak, *Biopolymers*, **6**, 817 (1968).
8. M. Nakamizo, *Bull. Chem. Soc., Japan*, **42**, 1071 (1969).