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Syntheses and Reactions of Functional Polymers. LXVI. Activation of Carboxylic Acid by Means of Polymers Containing Hydroxamic Acid Residue and *N*-Hydroxysuccinimide Residue

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In order to achieve solid-phase acyl activation of carboxylic acid, the syntheses and reactions of polymers containing hydroxamic acid and *N*-hydroxysuccinimide residues were investigated. A polymer containing hydroxamic acid residue was obtained by the modification of cross-linked copolymer of *p*-nitrophenyl acrylate with styrene, and one containing *N*-hydroxysuccinimide residue was prepared by copolymerization of *N*-acetoxymaleimide with styrene followed by hydrolysis. These *N*-hydroxy polymers were allowed to react with *N*-protected amino acids by means of the dicyclohexylcarbodiimide method giving the activated polymer ester. While the polymer containing hydroxamic acid residue was inferior to that containing *N*-hydroxysuccinimide residue in activation of carboxylic acid, amino acid esters of the latter were active enough to react with another amino acid to yield dipeptides almost quantitatively without racemization.

Activation of carboxylic acid under mild conditions is a useful method for selective acylation in peptide syntheses. The utilization of polymers carrying the active ester group in stepwise syntheses of peptides has been described for the polymers containing *o*-nitrophenol,[1] *p*,*p*′-dihydroxydiphenyl sulfone[2] and *N*-hydroxysuccinimide[3] moieties.

In a study on polymeric reagents, we reported on

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[1] M. Fridkin, A. Patchornik, and E. Katchalski, *J. Amer. Chem. Soc.*, **90**, 2953 (1968); G. T. Panse, D. A. Laufer, *Tetrahedron Lett.*, **1970**, 4181.

[2] Th. Wieland and Ch. Birr, *Angew. Chem.*, **78**, 303 (1966).

[3] D. A. Laufer, D. I. Marborough, T. M. Chapman, V. M. Vaidya, and E. R. Blout, *J. Amer. Chem. Soc.*, **90**, 2696 (1968).

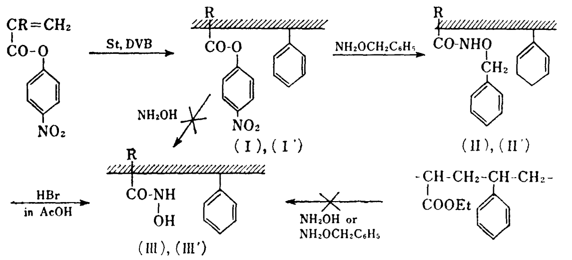
TABLE 1. IR DATA OF POLYMERS(I)—(VI) AND MODEL COMPOUNDS (KBr Disk)

Compound	Characteristic absorption (cm^{-1})			
	OH, NH	Ester	Amide	Other[assignment]
Polymer(I)		1760		1525, 1345[NO_2] 1210[-C-O-]
Polymer(II)	3200		1670—1620	
Polymer(III)	3400—3200		1620	
AcNHOH	—3200, —3100		1620	
Polymer(IV)	3400—3200	1790	1680, 1620	1180[-C-O-]
AcONHAc	3350, 3150	1790	1655	1175[-C-O-]
Polymer(VI)	3350	1790 1720—1690	1620	1520 1500
Z-GlyONHAc	3300	1790 1720—1680	1650	1520

the preparation and use of polymers having *N*-hydroxysuccinimide unit in the chain.[4] Use of such polymers would facilitate synthetic procedure, *i.e.*, separation of reaction mixture, purification of product, and recovery of the original polymer. In this article, preparation of hydroxamic acid polymer and activation of carboxylic acid by means of polymers containing hydroxamic acid and *N*-hydroxysuccinimide residues are described.

Results and Discussion

Preparation of Polymer Containing Hydroxamic Acid Residue. A series of polymer modifications was carried out as shown in scheme 1. Copolymerization of *p*-nitrophenyl acrylate with styrene (St) in the presence of divinylbenzene (DVB) was carried out to obtain cross-linked copolymer (I). Polymer (I) was reacted with benzyloxyamine and debenzylated by treatment with hydrogen bromide in acetic acid to give insoluble polymer(III). Polymer(III) was acetylated with acetic anhydride to give polymer(IV). Nitrogen analysis of polymer(I) showed that the total molar fraction of the ester was 38%. The reaction of I with benzyloxyamine proceeded almost quantitatively at 130°C for 25 hr. A characteristic feature in its IR spectrum was the presence of bands of ester at 1760 cm^{-1} and of the nitro group at 1525 cm^{-1} and 1345 cm^{-1}. In the IR spectrum of polymer(II), these absorptions disappeared and characteristic absorptions of amide appeared at 1650 cm^{-1}.



(I), (II), (III); R=H, (I′), (II′), (III′); R=CH_3

Scheme 1.

The IR absorptions of polymers(I)—(IV) and those of model compounds are shown in Table 1. The acyl content in polymer(IV) was determined from the yield of *N*-cyclohexylacetamide obtained in the reaction with excess cyclohexylamine.

Similarly, copolymerization of *p*-nitrophenyl methacrylate with styrene in the presence of DVB yielded cross-linked polymer(I′). A series of polymer modifications (scheme 1) was carried out. Polymer(I′) was less reactive than polymer(I). It was transformed into amide polymer(II′) almost quantitatively by treatment with benzyloxyamine in DMF at 120—140°C for 100 hr. Polymer(II′), however, was not debenzylated by treatment with hydrogen bromide in acetic acid at 80°C for 10 hr.

In general, amino acid *p*-nitrophenyl esters are remarkably reactive, and have been used frequently for peptide bond formation.[5] In the case of polymers(I) and (I′), however, their reactivities are so reduced as to need high temperature and long reaction time probably due to their insolubility and steric hindrance of main chain.

The direct reaction of polymer(I) with hydroxylamine was also attempted. A DMF solution containing hydroxylamine was prepared by mixing hydroxylamine hydrochloride with equimolar anhydrous triethylamine in DMF. Polymer(I), which was swelled enough in DMF, was then combined with the above solution and kept at room temperature overnight. The IR spectrum of polymer(III″) thus obtained, however, showed that it contained carboxylic acid residue in addition to hydroxamic acid residue[broad absorptions at 3400—2300 cm^{-1} and 1730—1600 cm^{-1}].

On the other hand, the reaction of ethylacrylate–styrene copolymer with hydroxylamine or benzyloxyamine was carried out under various conditions. However, the starting polymer was recovered in all cases.

In order to prepare polymers(III) and (III′), pre-

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[4] a) M. Akiyama, M. Narita, and M. Okawara, *J. Polym. Sci., Part A-I*, **7**, 1299 (1969). b) M. Akiyama, Y. Yanagisawa, and M. Okawara, *ibid.*, **7**, 1905 (1969). c) M. Narita, T. Teramoto, and M. Okawara, This Bulletin, **44**, 1084 (1971).

[5] B. Iselin, W. Rittel, and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957); W. Kern, and R. C. Schulz, *Angew. Chem.*, **69**, 153 (1957).

TABLE 2. THE CRUDE PRODUCTS OF THE REACTION OF POLYMERS(IV) AND (VI) WITH CYCLOHEXYLAMINE[a]

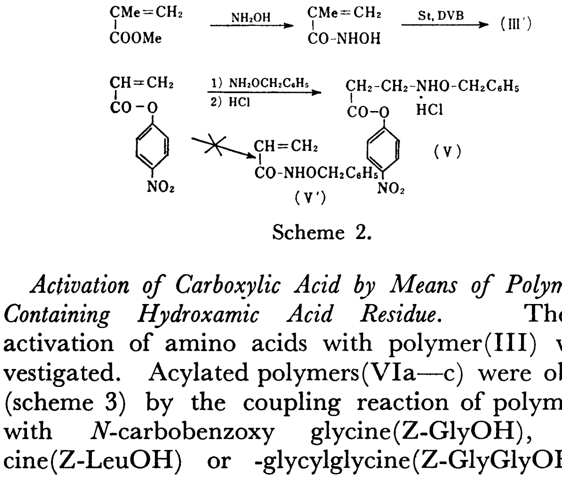
Polymer	IV	VIa	VIb	VIc
Yield of Amide (mg)	156	296	366	235
Mp (°C)	100—103	86—102	114—121	131—159
(Lit.)	(104)[7a]	(110)[8]	(129—131)[b]	(179—179.5)[c]
Acyl Content of Polymer(meq/g)[d]	1.1	1.02	1.06	0.68
Acyl Content of Polymer(meq/g)[e]		1.08	1.06	0.74

a) Polymer(1.0 g) was reacted with 1.0 g of cyclohexylamine in 30 m*l* of methylene chloride at room temperature for 20 hr. b) Recrystallized from *n*-hexane-ethyl acetate. c) Recrystallized from ethyl acetate. d) Determined from the yield of *N*-cyclohexylacylamide. e) Determined from the increase in weight of the insoluble polymer as a result of the coupling reaction.

TABLE 3. THE YIELDS OF DIPEPTIDES BY THE ACYLATION OF ETHYL ESTERS OF AMINO ACIDES WITH POLYMERS(VIa) AND (VIb)

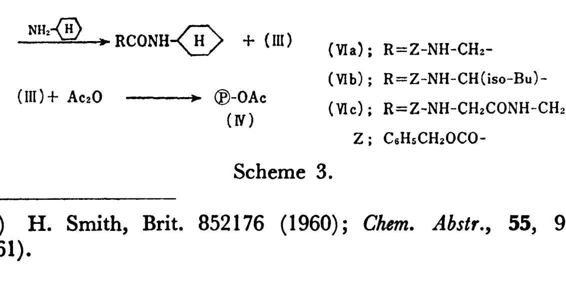
Polymer	HGlyOEt Yield of dipeptide (%)	H-L-LeuOEt Yield of dipeptide (%)
VIa	86	73
VIb	47	39

paration and polymerization of monomers having hydroxamic acid residue were attempted (scheme 2). Methacryloylhydroxamic acid was prepared by Smith's method,[6] but its copolymerization with styrene in the presence of DVB was not successful under various conditions. An attempt to prepare monomer(V′) having protected hydroxy group was also carried out. However, the reaction of *p*-nitrophenyl acrylate with benzyloxyamine produced only the Michael type addition product, *p*-nitrophenyl ester of *γ*-(*N*-benzyloxyamino)-propionic acid(V).



Scheme 2.

Activation of Carboxylic Acid by Means of Polymer(III) Containing Hydroxamic Acid Residue. The acyl activation of amino acids with polymer(III) was investigated. Acylated polymers(VIa—c) were obtained (scheme 3) by the coupling reaction of polymer(III) with *N*-carbobenzoxy glycine(Z-GlyOH), -L-leucine(Z-LeuOH) or -glycylglycine(Z-GlyGlyOH) by use of dicyclohexylcarbodiimide(DCC) as a dehydration reagent in DMF at room temperature for 40 hr. The IR spectra of polymers(VI) exhibited characteristic absorptions similar to those of a model compound (Z-GlyONHCOCH$_3$) as shown in Table 1.

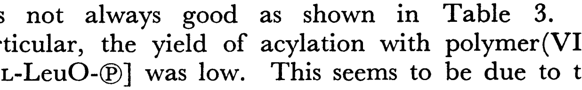


(VIa); R=Z-NH-CH$_2$-
(VIb); R=Z-NH-CH(iso-Bu)-
(VIc); R=Z-NH-CH$_2$CONH-CH$_2$-
Z; C$_6$H$_5$CH$_2$OCO-

Scheme 3.

Determination of acyl contents of polymers(VIa—c) was made: (1) base on the yields of *N*-cyclohexylacylamides obtained by the reaction with excess of cyclohexylamine and (2) from the increase in weight of the polymer obtained. The results of the two methods agreed well, as shown in Table 2.

Acylation of ethyl esters of amino acids with acylated polymer (VIa, b) was carried out as shown in scheme 4.



R; Z-NHCH$_2$-(=Z-Gly), Z-NHCH(Isobu)-(=Z-L-Leu)
R′; -CH$_2$-(=HGlyOEt), -CH(Isobu)-(=H-L-LeuOEt)

Scheme 4.

The reaction was carried out by use of a slight molar excess of polymer(VI) in DMF at room temperature for 40 hr. The yield of acylation products was not always good as shown in Table 3. In particular, the yield of acylation with polymer(VIb) [Z-L-LeuO-Ⓟ] was low. This seems to be due to the fact that the steric hindrance reduced its reactivity as well as polymer(I) carrying active ester group adjacent to the polymer chain. This shows that the polymer used for activation of carboxylic acid should have the active ester group detached from the polymer backbone.

Although activation of carboxylic acid by means of a polymer containing hydroxamic acid residue was slightly inferior to reported polymer,[1–3] it was observed

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[6] H. Smith, Brit. 852176 (1960); *Chem. Abstr.*, **55**, 9284^i (1961).

[7] J. R. A. Pollock, Ed. "Dictionary of Organic Compounds," 4th Ed., Eyre and Spottiswoode, London (1965) a) p. 790; b) p. 21.

[8] D. W. Clayton, J. A. Farrington, G. W. Kenner, and J. M. Turner, *J. Chem. Soc.*, **1957**, 1398; *ibid.*, **1956**, 375.

TABLE 4. THE COUPLING REACTION OF POLYMER(IXb) WITH Z-L-LeuOH IN VARIOUS SOLVENTS[a)]

Solvent	Methylene chloride	Chloroform	Dioxane	Ethyl acetate	DMF
Yield of polymer (g)	4.05	2.60	2.50	2.75	4.08
Acyl content of polymer (meq/g)[b)]	1.54	0.15	0	0.37	1.56
Acyl content of polymer (meq/g)[c)]	1.53				1.56

a) The coupling reaction of polymer(IXb) (2.50 g) with 3.98 g of Z-L-LeuOH by use of 4.10 g of DCC as a dehydration reagent was performed in 20 m*l* of various solvents at room temperature for 40 hr.
b) Determined from the increase in weight of the insoluble polymer as a result of the coupling reaction.
c) Determined from the yield of *N*-cyclohexylacetamide.

TABLE 5. THE CRUDE PRODUCTS OF THE REACTION OF POLYMER (X) WITH CYCLOHEXYLAMINE[a)]

Polymer	Xa	Xb	Xc
Yield of Amide (mg)	450	518	620
Mp (°C)	101—103	81—97	107—118
(Lit.)	(104)[7a)]	(110)[8)]	[129—131][b)]
Acyl content of polymer (meq/g)[c)]	3.19	1.78	1.78

a) Polymer(1.0 g) was reacted with 1.0 g of cyclohexylamine in 20 m*l* of DMF at room temperature for 24 hr.
b) Recrystallized from *n*-hexane-ethyl acetate.
c) Determined from the yield of *N*-cyclohexylacylamide.

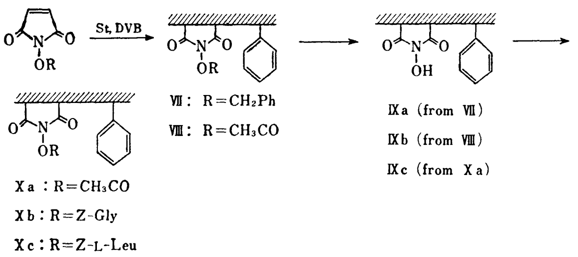
TABLE 6. IR DATA OF POLYMERS(VIII)—(XI). (KBr Disks)

Polymer	Characteristic absorption[a)] (cm^{-1})		
	OH[NH]	C=O	Other
(IXa), (IXb)	3500(m) | 2700(w)	1780(m) 1710(s), 1650(sh)	1210(s), 1050(m)
(IXc)	3500(s) | 2500(m)	1780 50(m) 1700(sh), 1650(s)	1240(s), 1070(m)
(VIII), (Xa)		1820(m), 1790(m) 1740(s)	1370(m), 1220(s) 1160(s), 1050(m)
(X), (XI)	[3400](m)	1820(m), 1785(m) 1740(s)	1510(m), 1340(m) 1250(sh), 1220(s), 1050(s)

a) Absorption intensity; s=strong, m=medium, w=weak, sh=shoulder

that insoluble polymeric reagents were useful for simplifying the procedures and making the separation of product easier.

Activation of Carboxylic Acid by Means of Polymer(IX) Containing N-*Hydroxysuccinimide Structure.* Several methods to prepare *N*-acyloxymaleimides were reported.[4c)] *N*-Benzyloxymaleimide thus obtained was copolymerized with styrene(St) in the presence of DVB to afford cross-linked copolymer(VII), which contained 47 mol% of imide moiety according to nitrogen analysis.



Scheme 5.

Polymer(VII) was debenzylated by treatment with hydrogen bromide in acetic acid to give the intended polymer(IXa) containing *N*-hydroxysuccinimide structure as shown in scheme 5. Polymer(IX) was acylated with acetic anhydride to give *N*-acetoxy polymer (Xa) whose acyl content (3.19 meq/g) was determined by the cyclohexylamine method. The content shows that the debenzylation of polymer(VII) to (IXa) about 90%. Similarly, cross-linked copolymer(VIII) of *N*-acetoxymaleimide and styrene was prepared and hydrolyzed with 6 N-hydrochloric acid at 100°C for 10 hr in dioxane to give cross-linked polymer(IXb) with 3.12 meq/g of *N*-hydroxyimide moiety.

The coupling reaction of polymer(IXb) with Z-L-LeuOH by means of DCC was examined in various solvents. The amino acid content in the acylated polymer was determined from the yield of cyclohexylamide and the weight increase. Both results agreed well as shown in Table 4. Polymer(IXb) was swelled well in DMF and methylene chloride, but not in chloroform, dioxane and ethyl acetate. Thus, the acylation reaction proceeded effectively in good swelling solvents. Similarly, the coupling reactions of polymer(IXa) with Z-GlyOH and Z-L-LeuOH were performed in DMF by means of DCC method. The acyl contents of polymer(Xb, Xc) thus obtained were

Table 7. The coupling reaction of polymer(IXb) with peptide derivative[a]

No.	Polymer (IXb) (g)	Z-Amino acid or Z-Peptide	(g)	DCC (g)	Yield of polymer (XI) (g)	Acyl content of polymer[b] (meq/g)	A fraction of reacted OH group[c] (%)
1	5.00	Z-GlyOH	6.40	8.2	7.44(XIa)	1.71(1.69)[d]	69
2	5.00	Z-LeuOH	8.0	8.2	8.15(XIb)	1.56(1.69)[d]	69
3	1.00	Z-GlyOH	1.55	0.61[e]	1.29	1.18	42
4	2.00	Z-Gly$_2$OH	2.96	3.10	3.36(XIc)	1.63(1.53)[d]	74
5	2.00	Z-Gly$_3$OH	3.56	3.10	2.79	0.93	41
6	2.00	Z-Leu$_2$OH	4.30	3.10	3.22(XId)	1.05(1.02)[d]	56
7	0.50	Z-Leu$_3$OH	1.37	0.77	0.71	0.63	24
8	1.00	Z-Leu$_4$OH	3.36	1.53	1.51	0.58	23

a) The reaction was carried out in DMF at room temperature for 40 hr. DMF was used 10 m*l*/polymer 1.0 g. In the case of Nos. 1 and 2, acyl contents of polymer were the same when the reaction time was 15 hr.
b) Determined from the increase in weight of the insoluble polymer obtained.
c) Calculated by use of acyl content of polymer determined based on b).
d) Determined from the yield of *N*-cyclohexylacylamide.
e) Ethyl chloroformate was used instead of DCC.

Table 8. The acylation of ethyl esters of amino acids with polymers(X)[a]

Ester of amino acid	Dipeptide obtained	Polymer		
		IX	Xa	Xb
HGlyOEt	Yield (%)	92	100	94
	Mp (°C)	38—42	54—67	73—91
	(Lit.)	(48)[7b]	(82)[9]	(102—103)[10]
H-L-LeuOEt	Yield (%)	100	98	86
	Mp (°C)	oil	oil	
	(Lit.)	oil	oil	

a) Polymer(1.0 g) was reacted with 1.3 mmol of ester of amino acid in 20 m*l* of DMF at room temperature for 12 hr.
b) Recrystallized from *n*-hexane.

Table 9. Optical rotation of dipeptide $[\alpha]_D^{18}$ (c=0.2, in ethanol)

Dipeptide	Prepared by using polymer(Xc)	Prepared by using the DCC method	Lit.
Z-L-LeuGlyOEt	−27.5	−25	−27.2
Z-L-Leu-L-LeuOEt	−37	−40.5	

determined by use of cyclohexylamine and the results are shown in Table 5 together with those of polymer (Xa). The regenerated *N*-hydroxyimide polymer(IXc) from X combined with cyclohexylamine (from IR data) as discussed in the preceding paper.[4c] The characteristic IR absorption data on these polymers are shown in Table 6.

For the sake of confirmation, polymer(IXc) was allowed to react with excess acetic anhydride. The acyl content in the polymer obtained was 3.19 meq/g, the same as in the above polymer(Xa).

The coupling of polymer(IXb) with various chain length acids was investigated. The acyl contents of polymer(XI) obtained are shown in Table 7. The DCC method was superior to the mixed anhydride method. The acyl content did not increase with prolonged reaction time. The fraction of reacted *N*-hydroxy group decreased with the increase of the number of amino acids in peptide. On the other hand, the fraction was higher in the case of glycine derivatives than in leucine derivatives for the same number of amino acids.

The acylation of ethyl esters of amino acids with polymer(X) was carried out. The reaction was carried out by using a slight excess of the acylated polymer in DMF at room temperature. The melting points and yields of the crude dipeptide derivatives obtained are shown in Table 8. The acylation with polymers (Xa—c) proceeded almost quantitatively. Crude products gave pure dipeptide derivatives by recrystallization. The optical rotations of Z-L-LeuGlyOEt and Z-L-Leu-L-LeuOEt obtained by the reaction of polymer (Xc) are shown in Table 9. The values agree with those in literature and those of the product obtained by the usual DCC method. Thus, acylation with polymer(Xc) seems to proceed without racemization.

In conclusion, the *N*-hydroxyimide polymer(IX) is superior to the hydroxamic acid polymer(III) in its

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9) St. Goldschmidt, and M. Wick, *Ann.*, **575**, 217 (1952).

10) G. W. Anderson, J. Bloninger, and A. D. Welcher, *J. Amer. Chem. Soc.*, **74**, 5309 (1952).

effectiveness for acyl activation, probably because the former has a reactive site detached from the polymer backbone. The bulkiness of the amino acid derivative would also affect the reactivities of polymers both in their acylation and coupling processes.

Experimental

The melting points were uncorrected. The IR spectra were obtained as potassium bromide disks with a Hitachi Infrared Photometer Model EPI-S2. Solvents used for reactions were purified by the usual method.

Preparation of Polymer(I). *p*-Nitrophenyl acrylate (17.0 g, 0.088 mol), prepared by Morawetz's method,[11] 9.15 g(0.088 mol) of styrene, 5.72 g (0.044 mol) of DVB and 0.90 g(0.0055 mol) of AIBN were dissolved in 150 m*l* of dioxane. The polymerization tube was sealed off in a vacuum, and heated in a bath thermostatted at 80°C for 20 hr. The separated polymer(I) was washed with ether, and crashed to small species, and dried in a vacuum. The total yield was 26.5 g. Polymer(I) was pale yellow. Elemental analyses; Found; C 71.21, H 5.86, N 3.68%. Nitrogen analysis showed that total molar fraction of the ester was 38%.

Preparation of Polymer(II). Polymer(I) thus obtained (25.0 g) was swelled in 200 ml of DMF and 18.5 g(0.15 mol) of benzyloxyamine was added. The mixture was heated at 120—140°C for 25 hr. Polymer(II) thus obtained was filtered off, and washed with ether, DMF and ether. It was collected and dried in a vacuum. The yield was 16.6 g.

Preparation of Polymer(III). Polymer(II)(16.0 g) was suspended in 100 m*l* of 28% HBr–AcOH solution and heated at 80—85°C for 6 hr. Polymer(III) thus obtained was separated by decantation, and washed with ether and methanol several times. Soluble parts of polymer(III) was extracted with methanol by use of a Soxhlet extractor. The yield of polymer(III) was 12.4 g. Polymer(III) was light brown.

Polymers(I′) and (II′) were prepared in similar ways. Preparation of polymer(II′) was carried out in DMF at 120—140°C for 100 hr. The reaction of polymer(II′) with hydrogen bromide in acetic acid at 80—85°C for 10 hr recovered the starting polymer(II′).

Reaction of Polymer(I) with Hydroxylamine. Ten m*l* of DMF solution containing hydroxylamine was prepared by mixing 0.7 g (0.01 mol) of hydroxylamine hydrochloride (dried at 140°C/0.1 mmHg for 3 hr) with 1.0 g (0.01 mol) of triethylamine (free from water) in 10 m*l* of DMF. Polymer(I) was swelled in 10 m*l* of DMF, and then these were combined, and kept at room temperature for 24 hr. Polymer (III″), thus obtained, was filtered off, and washed by swelling in methanol, and dried in a vacuum. The yield was 0.71 g.

Polymerization of Methacryloyl Hydroxamic Acid. Copolymerization of methacryloyl hydroxamic acid (0.51 g) prepared by Smith's method,[6] with DVB (0.65 g) in 15 m*l* of dioxane with AIBN (0.08 g) in polymerization tube sealed in a vacuum at 80°C for 12 hr gave no polymer.

Reaction of p-*Nitrophenyl Acrylate with Benzyloxyamine.* *p*-Nitrophenyl acrylate (18.5 g, 0.096 mol) and 12.0 g (0.098 mol) of benzyloxyamine were dissolved in 100 m*l* of ether, and kept at room temperature for 12 hr. The ether solution was washed with water, 1 N hydroxy ammonium solution and water by means of a separation funnel, and then washed with 1 N hydrochloric acid solution to precipitate crystalline needles. This product was filtered off. The filtrate was washed with water and dried over sodium sulfate. Although the filtrate was evaporated in a vacuum, the desired *N*-benzyloxyacrylamide was not obtained. The yield of the crystalline was 17.3 g. The product was confirmed to be *p*-nitrophenyl ester hydrochloride of γ-(*N*-benzyloxyamino)-propionic acid by elemental analysis and IR data. The yield was 17.3 g (51%), Mp 132—134°C(recrystallized from acetonitrile). Found: C, 54.28; H, 4.79; N, 7.93; Cl, 10.08%. Calcd for $C_{16}H_{17}N_2O_5Cl$: C, 54.7; H, 8.43 N, 7.97; Cl, 10.1%.

Preparation of Acetylated Polymer(IV). Polymer(III) (2.0 g) was swelled in 25 m*l* of DMF and 1.0 g of acetic anhydride was added. The mixture was kept at room temperature for 40 hr. Polymer(IV) thus obtained was filtered off and washed sith methanol. Polymer(IV) was further washed with methanol by use of a Soxhlet extractor and dried in a vacuum. The yield was 2.18 g.

Preparation of Acylated Polymer(VI). Polymer(III) (2.5 g) was swelled in 25 m*l* of DMF and 3.14 g (15 mmol) of Z-GlyOH was added. DCC (4.2 g, 20 mmol) was added to the mixture with stirring under cooling with ice-cold water, and kept at room temperature for 40 hr. The reaction mixture was diluted with methanol and precipitated *N*,*N*′-dicyclohexylurea was separated by decantation, and then polymer(VIa) was filtered off and washed with methanol. Polymer(VIa) was further washed with methanol by use of of a Soxhlet extractor and dried in a vacuum. Similar reaction were carried out with the use of 3.98 g (15 mmol) of Z-L-LeuOH and 3.74 g (15 mmol) of Z-GlyGlyOH. The yields of polymers(VIa), (VIb,) and (VIc) were 3.16 g, 3.38 g, and 3.07 g, respectively.

Preparation of N-*Acetyl*-O-(N-*carbobenzoxy*)-*glycyl Hydroxylamine*[*The Model Compound for Polymer*(*VI*)]. Acetohydroxamic acid (4.50 g, 0.051 mol) and 10.45 g (0.05 mol) of Z-GlyOH were dissolved in 100 m*l* of DMF. DCC (10.3 g) was added to the solution with stirring under cooling with ice-cold water. The reaction mixture was kept for 2 hr with stirring under cooling with ice-cold water, and then at room temperature for 12 hr. *N*,*N*′-Dicyclohexylurea precipitated was filtered off and the filtrate was concentrated in a vacuum to give *N*-acetyl-*O*-(*N*-carbobenzoxy)-glycyl hydroxylamine. It was recrystallized from the mixture of hexane and ethyl acetate (1 : 1). The yield was 5.71 g (43%). Mp 137—139°C. Found: C, 53.87; H, 5.38; N, 10.44%. Calcd for $C_{12}H_{14}N_2O_5$: C, 54.13; H, 5.30; N, 10.52%.

Acylation of Cyclohexylamine with Polymers(*IV*) *and* (*VI*). One gram of polymer(IV) or (VI) was swelled in 30 m*l* of methylene chloride and 1.0 g of cyclohexylamine was added. The reaction mixtrue was kept at room temperature for 20 hr. The polymer recovered was filtered off and washed with ethyl acetate and methanol. The filtrate and the washing were combined and concentrated in a vacuum to obtain *N*-cyclohexylacylamide. The results are shown in Table 2. The crude products gave pure products by recrystallization.

Acylation of Ethyl Ester of Amino Acid with Polymer(*VI*). Glycine ethyl ester hydrochloride (0.112 g, 0.8 mmol) or 0.156 g (0.8 mmol) of L-leucine ethyl ester hydrochloride and 0.081 g (0.8 mmol) of triethylamine were dissolved in 10 m*l* of DMF. Polymer(VIa) or (VIb) (1.0 g) was swelled in 15 m*l* of DMF. Both were mixed and kept at room temperature for 40 hr. The consumed polymer was filtered off and washed with methanol. The filtrate and washings were combined and concentrated in a vacuum. The residue was dissolved in ethyl acetate, and insoluble triethylamine

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[11] E. Gaetjens, H. Morawetz, *J. Amer. Chem. Soc.*, **83**, 1738 (1961); H. Morawetz, and P. E. Zimmering, *J. Phys. Chem.*, **58**, 753 (1954).

hydrochloride was filtered off. Ethyl acetate was evaporated in a vacuum, and the residue was worked up by the same procedure to obtain the crude products. The results are shown in Table 3.

Preparation of Polymer(VII). A mixture of 10.0 g (0.049 mol) of *N*-benzyloxymaleimide prepared by means of the method given previously,[4c] 10.0 g (0.096 mol) of styrene, 0.12 g (0.6 mol% contrary to all monomers) of DVB and 0.10 g of AIBN in 100 m*l* of benzene was heated at 40°C for 5 hr under a stream of nitrogen. The separated polymer (VII) was washed with benzene and methanol, and crashed to small species, and dried in a vacuum. The yield was 16.4 g. Found: C, 73.59; H, 5.18; N, 4.35%.

Preparation of Polymer(VIII). A mixture of 35.6 g (0.23 mol) of *N*-acetoxymaleimide prepared by means of the method given previously,[4c] 36.0 g (0.345 mol) of styrene, 1.5 g (0.0115 mol, 2 mol% toward all monomers) of DVB and 0.73 g (0.003 mol) of benzoyl peroxide in 150 m*l* of benzene was heated at 75°C for 6 hr under a stream of nitrogen. Separated polymer(VIII) was crashed to small species, and washed with methylene chloride by use of a Soxhlet extractor, and dried in a vacuum. The yield was 70.2 g.

Preparation of Polymer(IXa), A mixture of 15.0 g of polymer(VII) in 100 m*l* of 20% hydrogen bromide acetic acid solution was heated at 65—70°C for 15 hr. The reaction mixture was poured into ether. Polymer(IXa) was filtered off and washed with acetic acid, DMF, ether, and methanol. It was collected and dried in a vacuum. The yield was 10.95 g.

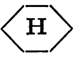
Preparation of Polymer(IXb). Polymer(VIII) was hydrolized with 6 N hydrochloric acid solution in dioxane to give polymer (IXb), and then subjected to the usual work-up.

Preparation of Polymer(Xa). Polymer(IXa) (5.0 g) was swelled in 50 m*l* of DMF and 20 m*l* of acetic anhydride was added. The mixture was kept at room temperature for 12 hr. Polymer(Xa) thus obtained was filtered off and with ether, tetrahydrofuran and ether. It was collected and under vacuum. The yield was 5.35 g.

Preparation of Peptide Derivatives. Z-GlyOH and Z-L-LeuOH were prepared according to the methods reported.[8,9] Z-GlyGlyOH and Z-GlyGlyGlyOH were prepared from Z-GlyOH and GlyOEt by the DCC method. Z-GlyGlyOH; mp 177—178°C (lit,[14] 175—176°C). Z-GlyGlyGlyOH; mp 197—198°C (lit,[15] 198°C). Z-L-Leu-L-LeuOH and Z-L-Leu-L-Leu-L-LeuOH were prepared from Z-L-LeuOH and L-LeuOEt by the DCC method. Z-L-Leu-L-LeuOEt; mp 86—88°C (recrystallized from *n*-hexane)., Found: C, 64.69; H, 8.43; N, 7.02%. Calcd for $C_{22}H_{34}N_2O_5$: C, 65.00; H, 8.43; N, 6.89%. Z-L-Leu-L-LeuOH; mp 117—119°C(recrystallized from *n*-hexane) (lit,[16] 99—99.5°C). Found: C, 63.16; H, 8.12; N, 7.38%. Calcd for $C_{20}H_{30}N_2O_5$: C, 63.47; H, 7.99; N, 7.40%. Z-L-Leu-L-Leu-L-LeuOEt; mp 137—139°C (recrystallized from carbon tetra chloride-ethyl acetate. Z-L-Leu-L-Leu-L-LeuOH; mp 150—152°C (recrystallized from carbon tetrachloride-ethyl acetate). Found: C, 63.28; H, 8.71; N, 8.49%. Calcd for $C_{26}H_{41}N_3O_6$: C, 63.52; H, 8.41; N, 8.55%.

Preparation of Polymers(X) and (XI). Polymers(Xb) and (Xc) were prepared by using polymer(IXa) as well as polymer(VI) [reaction time; 30 hr]. Polymer(X) washed with mathanol, DMF and methanol, and then dried in a vacuum. Polymer(Xb), 3.45 g, was obtained from 2.5 g polymer(IXa). Polymer(Xc), 3.84 g, was obtained from 2.4 g of polymer(IXa).

Polymer(XI) was prepared similarly in the reaction conditions shown in Table 9.

Acylation of Cyclohexylamine with Polymer(VIII), (X) and (XI). These reactions were performed as well as the reactions of cyclohexylamine with polymers(IV) and (VI). DMF was used as a solvent instead of methylene chloride. The results are shown in Table 5. In the case of polymer (XI), 1.0 g polymers(XIa—d) gave the corresponding acyl amides 490 mg, 540 mg, 530 mg, and 470 mg, respectively. These crude products offered pure products by recrystallization. Z-GlyNH-; mp 108—109°C(lit,[8] 110°C). Z-GlyGlyNH-; mp 179—179.5°C(lit,[8] 173°C). Z-L-LeuNH-; mp 129—131°C(recrystallized from *n*-hexane-ethyl acetate). Found: C, 68.96; H, 8.92; N, 7.93%. Calcd for $C_{20}H_{30}N_2O_3$: C, 69.33; H, 8.73; N, 8.09%. Z-L-Leu-L-LeuNH-; mp 194—195°C(recrystallized from ethyl acetate). Found: C, 67.36; H, 8.95; N, 8.93%. Calcd for $C_{26}H_{41}N_3O_4$: C, 67.94; H, 8.99; N, 9.14%.

Acylation of Ethyl Ester of Amino Acid with Polymer(X). Glycine ethyl ester hydrochloride(0.182 g, 1.3 mmol) or 0.245 g (1.3 mmol) of L-leucine ethyl ester hydrochloride and 0.132 g (1.3 mmol) of triethylamine were dissolved in 10 m*l* of DMF. Polymer(X)(1.0 g) was swelled in 10 ml of DMF. Both of them were mixed and kept at room temperature for 12 hr, and then treated as above. The results are shown in Table 8. The optical rotations of products were measured in ethanol(c=0.2) at 18°C using a Japan Spectroscopic Co., Model ORD/UV-5 spectropolarimeter. The results are shown in Table 9.

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