Acetohydroxamic Acid for Peptide Synthesis

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The ester derivatives of acetohydroxamic acid and N, N-diacetyl hydroxylamine, N, O-diacetyl hydroxylamine and triacetyl hydroxylamine were assured to be capable of being used as activated esters.

At first, evaluation of acyl activating ability was made by reacting the above compounds with amine, and it was shown that all of them worked to yield amides with excellent conversion and that, among them, triacetyl hydroxylamine was most powerfull, where acetylation of amines went through its imide carbonyl group. Furthermore, dipeptide synthesis was found to accomplish without any racemization and in a good yield by use of acetohydroxamic acid.

Next syntheses of polymers containing hydroxamic acid structure were carried out by the following routes. (a) methyl methacrylate was copolymerized with N-methacrylobenzyloxyamine and the copolymer obtained was debenzylated, (b) N-methacrylo-N, O-diacetyl hydroxylamine was polymerized, followed by hydrolysis and copolymers with styrene or methyl methacrylate were deacylated. The polymer obtained by route (a) was converted to the activated polymer ester of N-blocked diglycine and removal of the protecting group would provide a method for preparation of cyclic diglycine.

Introduction

At the present stage, the peptide synthesis through bifunctional activated esters has been widely utilized for its efficient asymmetric retantion and among them, more recently, the use of N-substituted hydroxylamines such as, for example, N-hydroxy succinimide[1] is recieving attention for depressing racemization. On the other hand, a variety of researches on using polymeric reagents, including polymeric acyl activating reagents[2], in peptide syntheses have been demonstrated for simplification of the process, repeating usage and preparation of cyclic peptides[3]. By choosing appropriate N-hydroxy compounds, the substances which will served the two functions mentioned above may be obtained. Thus, as N-hydroxy compounds, the simplest type of the compounds, -acetohydroxamic acid M-2 and N, N-diacetyl hydroxylamine M-1-and their polymeric derivatives were deal with for our study. This paper describes the syntheses and the possible utility of those compounds.

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Results and Discussion

N, N-diacetyl Hydroxylamine M-1

The ester derivative M-3 of N, N-diacetyl hydroxylamine M-1 which is a ring opening type of N-hydroxy succinimide appeared preparatively to be an acyl activated ester. This is coincided with the fact that the IR absorption (1810 cm^{-1}) of the carbonyl of triacetyl

hydroxylamine is shifted higher frequency than those (1720 cm^{-1}) of usual esters.

Target amides and unexpectively N, O-diacetyl derivative M-4 in spite of N, N-diacetyl derivative M-1 were obtained when M-3 was allowed to react with cyclohexylamine or aniline in ethanol for 3 hr at room temperature. The reaction path can be explained releasing O-acetyl group, N, N-diacetyl compound formed initially followed by N-O rearrangement of N-acetyl group to form N, O-diacetyl compound as shown in equation. Mechanistically this reaction path could be probable based on the reports that the preparation of N, O-diacetyl hydroxylamine by hydrolysis[4] of triacetyl hydroxylamine via N-O rearrangement. Debenzylation[5] of N, N-diacetyl-O-benzyloxyhydroxylamine which results formation of N, O-diacetyl hydroxylamine by the similar reaction machanism to the above also could support. Here the reaction of N, N-diacetyl-O-butyroylhydroxylamine with an amine was carried out to elucidate the reaction process. If the deacylation reaction proceeded via the N-O rearrangement, the products would be N, O-diacetyl hydroxylamine and N-butyroylamide. The experiment, however, showed that the products were N-acetyl-O-butyroylhydroxylamine and N-acetyl amide. Consequently, the acylation by using triacyl hydroxylamine assured to go through direct electrophile attack of N-acyl group on the imide nitrogen. These reaction schemes were shown below.

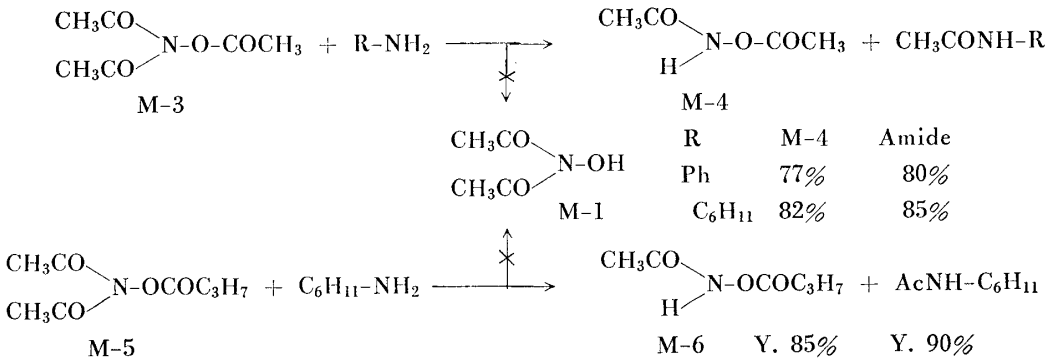
Acetohydroxamic Acid M-2

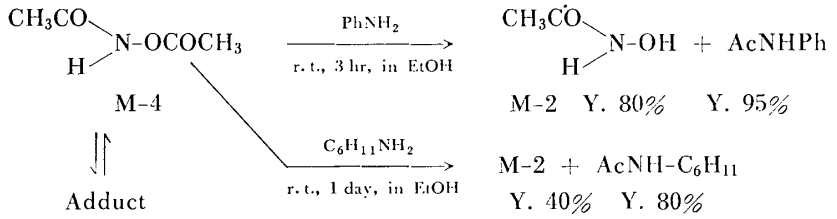
N, O-Diacetyl hydroxylamine M-4 was examined for acetylation of amines of cyclohexylamine, aniline and p-amino ethyl benzoate. In the case of cyclohexylamine, 1-2 hr after mixing both components, firstly precipitation was obseı ved and then began to dissolve slowly. The precipitate disappeared completely by standing overnight and cyclohexylacetoamide produced (Y. 80%). In the case of aniline, the reaction proceeded without precipitation to give amide and acetohydroxamic acid in good conversions of 95% and 80% correspondingly. p-Amino ethyl benzoate generated no amide. Production of acetohydroxamic acid did not indicate only the reaction on ester

Table 1 The Adducts[b] of N, O-Diacetyl hydroxylamine M-4[a] with Amines

Amine	Y. (%)	m. p. (°C)	Elemental Analysis (%)		
			C	H	N
$C_6H_{11}NH_2$	95	112～3	55.53 (55.78)	9.32 (9.35)	12.95 (12.94)
$BzNH_2$	77	79.5 ～8.0	58.91 (58.82)	7.19 (7.35)	12.64 (12.55)
$C_4H_9NH_2$	84	69～71	50.50 (50.42)	9.54 (9.77)	14.73 (14.70)
Piperizine	96	84.5 ～6.0	53.44 (52.97)	8.97 (8.93)	13.85 (13.68)
$C_3H_7NH_2$	98	67～8	47.71 (47.62)	9.15 (9.34)	15.90 (15.71)

a) M-4: m. p. 89-90°C
b) Elemental analysis and yields were calculated as the adduct (1:1).





carbonyl group with amines, because the fact that O-acetyl hydroxylamine immidiately converted into N-acetyl derivative[6] was reported. Here, N-acetyl-O-butyroyl hydroxylamine M-6 was treated with cyclohexylamine to give cyclohexyl butyroylamide. That is to say, rearrangement did not occur.

Next the precipitate was examined in the case of the reaction of N, O-diacetyl hydroxylamine M-4 with cyclohexylamine. The reaction solvent was changed ethyl acetate from ethanol in this experiment. The precipitate separated out quantitatively and did not disappeared at room temperature. Various amines except tertiary amines such as triethyl amine, pyridine, etc. and amines of weak basicity such as amino acid ethyl ester, aniline, etc. formed stable precipitates with N, O-diacetyl derivative M-4 (Table 2).

Subsequently, the precipitate was subjected for analyses and identified as follows.

1) Elemental Analysis(C, 55.53%; H, 9.23%; N, 12.95%) well agrees with the structure of AcNHOAc·$C_6H_{11}NH_2$(C_{10} $H_{20}N_2O_3$: C, 55.78%; H, 9.35%; N, 12.94%).

2) The precipitate was found to offer cyclohexylamine and M-4 when treated with benzene solution of picric acid. This reaction is assumed to proceed as discribed below.

Precipitate + HO-C_6H_2-$(NO_2)_3$ ⟶
$(O_2N)_3$-C_6H_2-OH·$H_2NC_6H_{11}$ + M-4

3) In IR spectra, there were absorptions of amine salt at a series of 3000-2500, 2200, 1685 and 1510 cm^{-1} and absorptions of ester carbonyl group and amide carbonyl groups which shifted about 40 cm^{-1} and 90 cm^{-1} to the lower frequency correspondingly. As a rule, it was recognized that the shift of carbonyl group by forming hydrogen bond could be about 40 cm^{-1} [7]. Thus hydrogen bonding through carbonyl group could contribute in this precipitate.

4) The structure of a salt type was also suggested according to data of NMR spectra (DMSO-d_6).

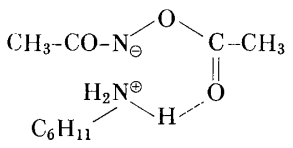


Table. 2. I. R. Spectra of the Adducts (cm^{-1})

Adduct	+NH	C=O	C=O	-C-O-
M-4·$C_6H_{11}NH_2$	3000~2500, 2200, 1585, 1510	1750	1570	1250
M-4·$BzNH_2$	3000~2500, 2200, 1595, 1500	1740	1560	1240
M-4·$C_4H_9NH_2$	3000~2500, 2200, 1595, 1520	1740	1570	1240
M-4·Piperizine	2700~2200	1720	1580	1240
M-4·$C_3H_7NH_2$	3000~2450, 2200, 1585, 1510	1710	1560	1270
M-4		1790	1660	1170

	NH	CH_3(amide)	CH_3(ester)	$+NH_3$
M-4 δ	11.4	2.02	1.81	
ppt δ		1.66	1.73	7.5

Based on the these results, the precipitate was determined to be the adduct which had hydrogen bond between ester carbonyl group and amino group and contained structure of salt. This structural analysis also suggests that no adduct formation can be observed in the case of less basic amines and tertial amines with M-4.

Peptide Synthesis by the Use of Acetohydroxamic Acid M-2

N, O-Diacetyl hydroxylamine M-4-ester derivative of acetohydroxamic acid M-2 consequently could have an ability of acyl activation because the reaction of N, O-derivative M-4 with amines gave amides aimed in excellent yield.

Peptide synthesis was then examined by the use of acetohydroxamic acid M-2.

The dioxane solution of acetohydroxamic acid M-2 and N-carbobenzyloxyamino acid was treated with the methylene dichloride solution of N, N'-dicyclohexylcarbodiimide (DCC) at 0°C for 12 hr to produce N-acetyl-O-(N'-carbobenzyloxyamino acid)-hydroxylamine M-7 in good yield (Table 3, 4).

N-Acetyl-O-(N'-carbobenzyloxyalanyl)-hydroxylamine M-7-Ala obtained was treated with cyclohexylamine or aniline at room temperature for 6 hr to provide alanylamide. The amide was obtained in high yield by means of aniline maintaining basicity such as basicity of amino acid ester.

Dipeptide synthesis was carried out between amino acid ester of acetohydroxamic acid M-7 and ethyl glycinate. The reaction schemes are shown below and the results are summarized in Table 5. Dipeptides were prepared not only

Table 3 N-Acetyl-O-(N'-carbobenzyloxyamino acid)-hydroxylamines M-7

	Y. (%)	m. p. (°C)	Elemental Analysis(%) C	H	N
Z-Gly-ONHAc (M-7-Gly)	62	137—9	53.87 (54.13)	5.38 (5.30)	10.44 (10.52)
Z-Ala-ONHAc (M-7-Ala)	85	99—102	55.69 (55.71)	5.70 (5.75)	10.01 (10.00)
Z-Phe-ONHAc (M-7-Phe)	80	104—6	64.15 (64.03)	5.58 (5.66)	7.89 (7.86)
Z-Leu-ONHAc (M-7-Leu)	76	oil	59.50 (59.61)	7.00 (6.88)	8.60 (8.69)

Table 4 I. R. Spectra of N-Acetyl-(N'-carbobenzyloxyamino acid)-hydroxylamine M-7 (cm^{-1})

	NH	C=O ester	urethane	amide	
M-7-Gly	3350	1790	1720—1700	1650	1520
M-7-Ala	3350	1800	1730—1720	1665	1530
M-7-Phe	3350	1800	1720—1710	1670	1540
M-7-Leu	3340	1800	1720—1710	1670	1530

Table 5 Peptide Synthesis Using Acetohydroxamic Acid M-2

	Y.(%)	m. p. (°C)	$[\alpha]_D$ (c=1, EtOH)
Z-Ala-Gly-OEt	92	98—9 (99—9.5)[8]	−22.9 (−21.2)[9]
Z-Phe-Gly-OEt	83	112—3 (110—1)[10]	−18.1 (−16.9)[10]
Z-Leu-Gly-OEt	71	102—3 (103—4)[11]	−29.4 (−27.2)[11]

$$\mathrm{AcNH\text{-}OH + Z\text{-}A\text{-}OH} \xrightarrow[\text{in Dioxane-}CH_2Cl_2]{\text{DCC}} \underset{\text{M-7}}{\mathrm{Z\text{-}A\text{-}ONHAc}}$$

A=Gly, L-Ala, L-Phe, L-Leu

$$\underset{\text{M-7-Ala}}{\mathrm{Z\text{-}L\text{-}Ala\text{-}ONHAc}} + \mathrm{RNH_2} \xrightarrow[\text{in } CH_3COOC_2H_5]{\text{r. t., 6 hr}} \mathrm{Z\text{-}L\text{-}Ala\text{-}NHR} + \underset{\text{M-2}}{\mathrm{(AcNHOH)}}$$

$C_6H_{11}NH_2$ Y. 68%, mp 160-1 °C

$C_6H_5NH_2$ Y. 92%, mp 158-60°C

$$\underset{\text{M-7}}{\mathrm{Z\text{-}A\text{-}ONHAc}} + \mathrm{H\text{-}Gly\text{-}OEt} \xrightarrow[\text{in THF}]{\text{0°C, 24 hr}} \mathrm{Z\text{-}A\text{-}Gly\text{-}OEt} + \underset{\text{M-2}}{\mathrm{(AcNHOH)}}$$

with high yield but also with no racemization.

It is demonstrated here that acetohydroxamic acid M-2-the simplest acyl derivative of hydroxylamine is a versatile acyl activating reagent for peptide synthesis.

Syntheses of Hydroxamic Acid Type Polymer (P-1, P-2)

Hydroxamic acid type polymer so far reported have been prepared by polymer reaction[12]. The polymer prepared by polymer reaction contain impure structure which may induce the reaction by use of the polymer to proceed unexpectedly. For the reason, syntheses and polymerization of vinyl monomer of hydroxamic acid type were examined to get the polymer having pure structure.

First of all, radical homo- and copolymerization of methacrylohydroxamic acid reported by Smith[13] was attempted but no polymer formed.

Next the treatment of benzyloxyamine with p-nitrophenyl acrylate offered not acrylobenzyloxyamide but β-N-methacrylobenzyloxyamide M-9 from methacryloyl chloride and benzyloxyamine was achieved in 72% yield. Furthermore, methacryloyl chloride was treated with N, O-diacetyl hydroxylamine M-4 to offer N-methacrylo-N, O-diacetyl hydroxylamine M-10.

Identification of the vinyl monomer M-10 was performed by means of IR spectra [1800 (ester carbonyl), 1740, 1720 (imide carbonyl), 1630 cm^{-1}(CH_2=C)], the measurement of mole weight [184.9 (calcd. 185.2)], the quantity of

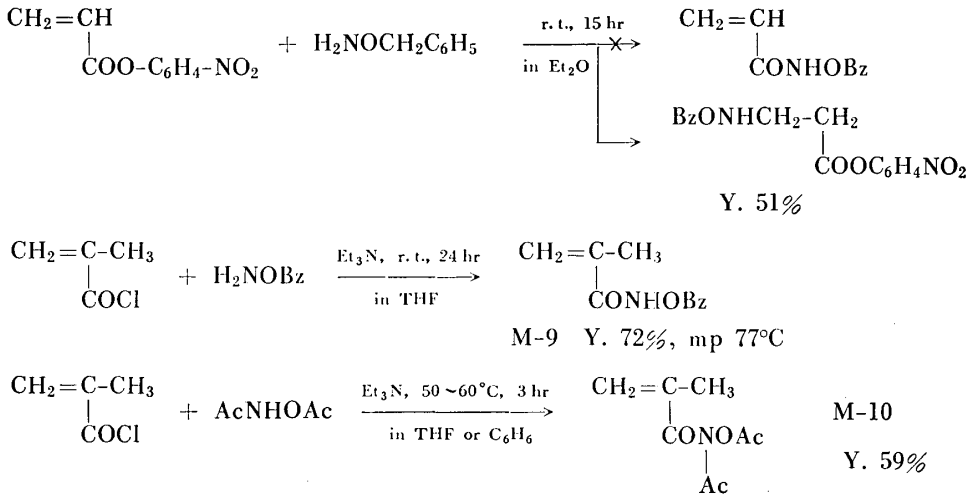
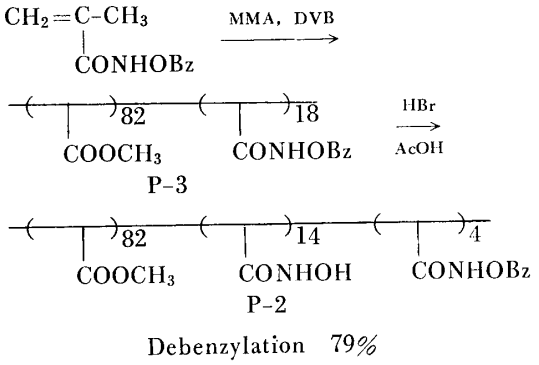


Table 6 I. R. Spectra of Polymers (cm^{-1})

	OH, NH	C=O			
		ester	imide (urethane)	amide	
P-2	3400			1620	1520
AcNHOH	3200, 3100			1620	
P-3		1800	1760, 1730		1650
Ac_2NOAc		1810	1740		1100
P-4	3400—3200	1790		1680, 1620	1180
AcNHOAc	3350, 3150	1790		1655	1175
P-5	3350	1790	(1720—1690)	1620	1520
Z-Gly-ONHAc	3350, 3250	1790	(1720—1700)	1650	1520

double bond [100.24%] and NMR spectra ($CDCl_3$) [δ 1.9(3H), 2.1(3 H), 2.2(3 H), 5.2 (1 H), 5.8(1 H)]. But the elemental analysis on carbon atom (C; 49.73%) derivated from calcd value (C; 51.88%) about 2%.

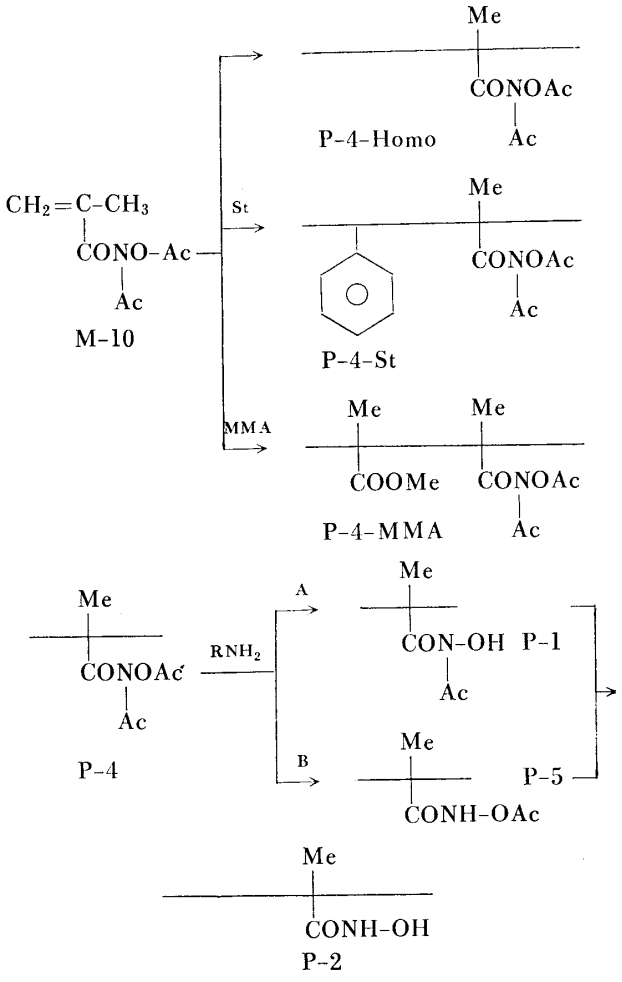
Hence, target polymer were prepared as follows. N-Methacrylobenzyloxyamine M-9 was allowed to polymerize with methyl methacrylate (MMA) and divinylbenzene (DVB) to produce insoluble cross-linked polymer P-3, followed by debenzylation in saturated HBr/AcOH to offer hydroxamic acid type polymer P-2.



Polymerization of triacyl vinyl monomer M-10 were carried out homopolymerization and copolymerization with styrene (St) or methyl methacrylate (MMA). Ester derivatives of N, N-diacyl hydroxylamine type polymer P-4 were obtained. The polymer P-4 was hydrolyzed in 6 N-HCl to produce N-hydroxyimide polymer P-1 (route A). The polymer P-4 was treated with cyclohexylamine at room temperature for 90 hr to give N, O-diacyl polymer P-5 (route B) and by adding more cyclohexylamine, the polymer P-5 introduced to hydroxamic acid type polymer P-2. The polymer P-4 did not react entirely with aniline. So that, it is impossible that the polymer P-1 is employed as an acyl activating reagent for peptide synthesis.

Table 7 Copolymerizations of N-Methacryloyl -N, O-diacetylhydroxylamine M-11 with Styrene or Methylmethacrylate

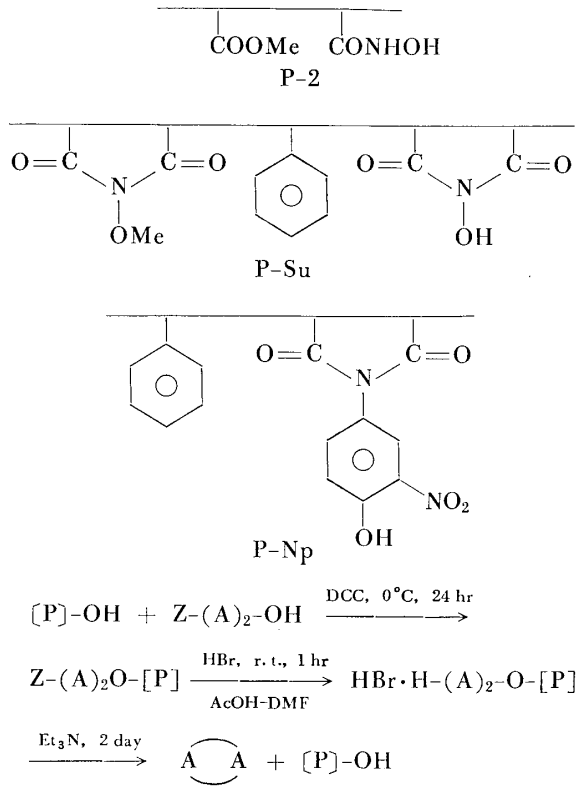
Intial Mixture (M_1/M_2)	Conversion (%)	Polymer Composition M_1 mol%
[styrene]		
3/7	91	40.3
1/1	94	40.3
7/3	79	47.5
[MMA]		
1/9	98	9.8
3/7	66	28.8
1/1	53	47.4



Cyclic Dipeptide Synthesis

Cyclic dipeptide synthesis was carried out by use of hydroxamic acid type polymer P-2 obtained via monomer M-9. Furthermore, N-hydroxysuccinimide type polymer[12] P-Su and N-(4-hydroxy-3-nitrophenyl)-succinimide type polymer[2] P-Np were also used for cyclic

dipeptide synthesis. The contents of hydroxy moiety were 1/6.9 monomer unit in the polymer P-2, 1/6.5 unit in the polymer P-Su and 1/2.3 unit in the polymer P-Np.



The polymer containing hydroxy group swelled and N-carbobenzyloxyglycylglycine were condenced at 0°C for 24 hr in DMF in the presence of DCC. After the polymer obtained was debenzylated at room temperature for 1 hr in HBr/AcOH-DMF, cyclization was carried out for 2 days by adding triethylamine. Ratio of acylation was calculated on the basis of cyclohexylamide which was obtained between acylated polymer and excess cyclohexylamine. Ratio of cyclization was calculated by based on glycylglycine introduced in the polymer. Conversion to cyclic diglycine was due to N-blocked diglycine used initially.

Cyclic diglycine was identified by means of melting point and IR spectra after recrystallization. The results are summarized in Table 8.

Table 8 Synthesis of Cyclic-diglycine

	P-Np	P-Su	P-2
Y. of Amide (mg)	242	184	164
Acyl Content (meq/1 g polymer)	0.726	0.552	0.493
Ratio of Acylation (%)	27.2	42.9	32.4
Y. of -(Gly$_2$)- (mg)	28	29	32
Ratio of Cyclization(%)	33.5	45.8	56.1
Y. of -(Gly)$_2$- (%)	9.1	19.6	18.2

As for the cyclization, hydroxamic acid type polymer P-2 was superior to others because of the interaction of amino group with N, O-diacyl hydroxylamine group. In the case of the polymer P-Np, the cyclization and the conversion were the worst values since the activating points were in the neighborhood of each other.

We document that both monomeric and polymeric hydroxamic acid have the versatility for peptide and cyclic peptide synthesis.

Experimental

Preparation of N-Acetyl-O-butyroyl Hydroxylamine M-6

Butyric anhydride(15.8 g 0.1mol) was added dropwise to a THF solution of acetohydroxamic acid (7.5 g 0.1 mol) and stirred for 24 hr. After evaporation of THF, the crude product was purified by distillation under reduced pressure to give M-6: Yield 7.5 g (51.8 %); bp 119-9.5°C (4 mm); IR 3350, 3150, 1790, 1660, 1175 cm^{-1}; Anal. Calcd for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.65. Found C, 49.51; H, 7.60; N, 9.63.

Preparation of N, N-Diacetyl-O-butyroyl Hydroxylamine M-5

N-Acetyl-O-butyroyl hydroxylamine M-6 (7.2 g 0.05 mol) dissolved in acetic anhydride (6.2 g 0.06 mol) was left for 2 days and removal of solvent under aspirator pressure. Distillation gave 9.2 g (98.9%) of a clear oil

M-5: bp 92-4°C(2.5 mm); IR 1800, 1730 cm^{-1}; Anal. Calcd for $C_8H_{13}NO_4$; C, 51.33; H, 7.00; N, 7.48. Found C, 59.81; H, 6.99; N, 7.37.

Reaction of Triacyl Hydroxylamines with Amines

Ethanol solution of triacyl hydroxylamine (0.01 mol) and amine (0.01 mol) was left for 3 hr, followed by distilling out ethanol.

[M-4-Cyclohexylamine]

The product was washed with carbon tetrachloride to give N, O-diacetyl derivative M-4 (82%) and cyclohexylacetoamide (84.5%) by evaporation of solvent.

[M-4-Aniline]

The crude product was washed with benzene -hexane to offer acetoanilide (79.4%) and M-4 (76.5%) from solution.

[M-5-Cyclohexylamine]

The residue was dissolved in ethyl acetate and cyclohexylamine (0.5 g) was added to the solution. An adduct (85%) precipitated, followed by filtering off. Distillation of solvent gave cyclohexylacetoamide (90%).

Reaction of N, O-Diacyl Hydroxylamine with Amines

[M-4, M-6-Cyclohexylamine]

Cyclohexylamine (0.01 mol) and N, O-diacyl compound (0.01 mol) were dissolved in ethanol (20 ml). A precipitate separated out after 1-2 hr and left for overnight to become clear solution. Cyclohexylamide recrystallized from hexane was extracted by hexane and the residue was washed with acetone to give acetohydroxamic acid.

[M-4-Aniline]

Aniline (2.76 g 0.03 mol) and M-4 (3.51 g 0.03 mol) were dissolved in ethanol (40 ml). The solution was left at room temperature for 3 hr and then concentrated. The crude product was washed with ether to give 1.64 g (80%) of acetohydroxamic acid recrystallized from ethyl acetate-hexane. Acetoanilide (3.36 g 95%) from ether solution was recrystallized from water.

Preparation of the Adduct between M-4 and Amines

An amine (0.01 mol) was treated dropwise with M-4 (1.17 g 0.01 mol) dissolved in ethyl acetate (10 ml). The precipitate was filtered off after 30 min.

Preparation of N-Acetyl-O-(N'-carbobenzyloxyamino acid)-hydroxylamines M-7

Acetohydroxamic acid (0.75 g 0.01 mol) and N-carbobenzyloxyamino acid (0.01 mol) were dissolved in THF (60 ml) and then treated dropwise with DCC (2.06 g 0.01 mol) dissolved in THF (30 ml) under stirring in an ice bath, followed by stirring overnight in an ice bath. After the THF solution filtered off dicyclohexylurea was evaporated, ethyl acetate (100 ml) was added. The solution was washed with 2% HCl and 5% $NaHCO_3$ and dried over anhydrous Na_2SO_4. Purification was carried out upon recrystallization from ethyl acetate-hexane after the solvent was removed.

Reaction of M-7 with Amines

An amine (5×10^{-4} mol) and M-7 (5×10^{-4} mol)were dissolved in 50 ml of THF (or ethyl acetate) and stirred at 0°C for 24 hr in the presence of triethylamine (5×10^{-4} mol). After distillation of solvent, the residue was dissolved in ethyl acetate. The solution was washed with 2% HCl and 5% $NaHCO_3$, followed by drying over Na_2SO_4. The crude product obtained by evaporation of ethyl acetate was recrystallized from ethyl acetate-hexane.

Reaction of p-Nitrophenyl Acrylate with Benzyloxyamine

Benzyloxyamine (6 g) and p-nitrophenyl acrylate (9.3 g) were left for 15 hr in ether and the solution was poured into 1N HCl to give a precipitate. Recrystallization from acetonitrile

of the precipitate filtered off yielded 51% of β-N-benzyloxyaminopropionic acid-p-nitrophenyl ester hydrochloride: Anal. Calcd for $C_{16}H_{17}N_2O_5Cl$: C, 54.70; H, 4.83; N, 7.97; Cl, 10.10. Found C, 54.28; H, 4.79; N, 7.93; Cl, 10.08.

Preparation of N-Methacrylobenzyloxyamine M-9

To methacryloyl chloride (5.3 g 0.05 mol) dissolved in THF (50 ml) was added dropwise benzyloxyamine (6.2 g 0.05 mol) and triethylamine (5.1 g 0.05 mol) dissolved in THF (20 ml) in the presence of tertial butyl catechol in an ice bath and stirred for 24 hr. After filtration of triethylamine hydrochloride precipitate, the solvent was removed off. The residue was distilled(124~6°C/1 mm) and the distillate was recrystallized from ethyl acetate-hexane to give 6.9 g (72%) of M-9: mp 77°C; IR 3200, 1650, 1620 cm^{-1}; Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.33. Found C, 68.79; H, 6.68; N, 7.37.

Preparation of N-Methacrylo-N, O-diacetylhydroxylamine M-10

Triethylamine (29.5 g) and M-4 (30 g) dissolved in benzene (or THF) were added dropwise to benzene solution of methacryloyl chloride (30 g) for 1 hr in an ice bath and then heated at 50~60°C for 3 hr in the presence of hydroquinone. After filtration of triethylamine hydrochloride and evaporation of solvent, the residue was distilled under reduced pressure in nitrogen stream to give 31.1 g of crude M-10 (98~98.5°C/3.5 mm). The distillate was redistilled from distilltaion culumn in the presence of nonflex MBP under the same condition: bp 99~100.5°C (4 mm); Anal. Calcd for $C_8H_{11}NO_4$: C, 51.88; H, 5.99; N, 7.56. Found C, 49.73; H, 5.86; N, 7.35.

Copolymerization of M-9

MMA (8 g), M-9 (3.8 g), DVB (0.65 g) and AIBN(0.01g) were dissolved in benzene(10 ml). Polymerization tube was sealed off under vacuum and heated at 70°C for 8 hr. Polymer obtained was washed in Soxlet extractor with acetone and methanol followed by vacuum drying to constant weight: Yield 10 g; IR 3300, 1720, 1650; Anal. Found C, 65.75; H, 7.78; N, 2.14.

Preparation of Polymer P-2

Polymer obtained above (2 g) was debenzylated at 80°C for 6 hr in saturated HBr/AcOH (10 ml). Polymer P-2 obtained was washed with methanol in Soxlet extractor followed by drying: Yield 1.64 g; Anal. Found C, 62.75; N, 2.41.

Polymerization of M-10 (P-4)

Homopolymerization of M-10 was carried out at 70°C for 6.5 hr in benzene by means of AIBN as initiator in a vacuum tube to give in 38.5% yield: IR 1800, 1750 cm^{-1}; Anal. Calcd for $C_8H_{11}NO_4$: C, 51.88; H, 5.99; N, 7.56. Found C, 52.04; H, 6.01; N, 7.54.

Hydrolysis of Polymer P-4-Homo

Dioxane solution of polymer P-4-Homo(1 g) was added 6 N-HCl (10 ml) and heated at 100°C for 8 hr. The solution was poured into ether to give 0.76 g of polymer P-1.

Reaction of Polymer P-4-St

Polymer P-4-St (1.0 g) was swelled in chloroform (35 ml) and cyclohexylamine (0.8 g) was added followed by standing for 90 hr. The suspension was poured into hexane in order to shrink the polymer. The polymer swelled in acetone was precipitated with hexane several times to yield 0.8 g of polymer P-4 and cyclohexylacetoamide (0.3 g) and the adduct of N, O-diacetylhydroxylamine with cyclohexylamine (0.01 g) from the solution.

The reaction of P-4-St with 1.65 g of cyclohexylamine was continued for 220 hr. The polymer was dissolved clearly. The solution

was poured into hexane in order to precipitate polymer P-2 (0.72 g) and the solution filtered off the polymer gave cyclohexylacetoamide(0.4 g): Anal. of polymer P-2: Found N, 4.41.

Synthesis of Cyclic Diglycine by the Use of P-2, P-Su, P-Np

Polymer (lg) was allowed to react with equimolar N-carbobenzyloxyglycylglycine at 0°C for 24 hr by means of DCC. Polymer was filtered and washed with methanol. Furthermore, polymer obtained was suspended in methanol and solvent was decanted in order to remove off dicyclohexylurea. After drying, the polymer was debenzylated at room temperature for 1 hr in HBr/AcOH-DMF followed by washing with DMF. Polymer debenzylated was swelled in DMF and then triethylamine was added in order to convert hydrobromide to free base followed by standing 2 days. Polymer was filtered off and washed enough with DMF. Solvent was distilled under reduced pressure. The residue was dissolved in ethyl acetate and washed with 2% HCl, 5% $NaHCO_3$ and burine. After drying over anhydrous Na_2SO_4, solvent was evaporated.

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