# STUDIES ON
# THE SYNTHESIS OF POLYPEPTIDE
# IN THE FUNCTIONAL REVERSED MICELLE

（機能性逆型ミセル中における
ポリペプチド合成に関する研究）

1981

KENJI HANABUSA

# PREFACE

The work of this thesis was done under the guidance by Professor Kiichi Takemoto and many other members of Takemoto Laboratory at the Department of Petroleum Chemistry, Faculty of Engineering, Osaka University, and at the Department of Functional Polymer Science, Faculty of Textile Science and Technology, Shinshu University, for six years since 1975.

The content of this thesis is composed of the following papers:

(1) Synthesis of poly(β-alanine) from β-alanine 4-acyl-2-nitrophenyl esters
Kenji Hanabusa, Koichi Kondo, and Kiichi Takemoto
*Makromol.Chem.*, 180, 307 (1979).

(2) Synthesis of polypeptides from dipeptide 4-acyl-2-nitrophenyl esters
Kenji Hanabusa, Koichi Kondo, and Kiichi Takemoto
*Makromol.Chem.*, 181, 635 (1980).

(3) Synthesis of poly(β-alanine) form β-alanine, β-alanyl-β-alanine, and β-alanyl-β-alanyl-β-alanine 4-dodecanoyl-2-nitrophenyl Esters
Kenji Hanabusa, Kuniko Ohno, Koichi Kondo, and Kiichi Takemoto
*Angew.Makromol.Chem.*, 84, 97 (1980).

(4) Synthesis of poly(β-alanine) in the functional reversed micelle
Kenji Hanabusa, Koichi Kondo, and Kiichi Takemoto
*Makromol.Chem.*, in press.

(5) On the intramolecular reaction of glycine 4-acyl-2-nitrophenyl esters
Kenji Hanabusa, Yoshiyuki Miwa, Koichi Kondo, and Kiichi Takemoto
*Makromol.Chem.,Rapid Commun.* 1, 433 (1980).

(6) Synthesis of poly(β-alanine) in the functional reversed micelle
Kenji Hanabusa, Hirofusa Shirai, Nobumasa Hojo, Koichi Kondo, and Kiichi Takemoto
*Makromol.Chem.*, submitted for publication.

CONTENTS

# GENERAL INTRODUCTION

Recently, the new aproach toward the reaction control in the molecular assembly has received much attention to organic chemists, and been applied to the reactions which are difficult to proceed in the conventional homogeneous systems, yielding successful results [1]. From these performance, it is clear that the reaction in the organized systems is also quite useful for the control of regioselectivity and stereoselectivity. The typical molecular assemblies include micelles, liquid crystals, monolayers and vesicles.

Extensive studies on the reaction in such molecular assembly have been performed to show the pronounced rate acceleration, rate retardation and change in the product distribution. In some cases, the remarkable rate enhancement observed is related to a enzymatic reaction [2,3], although the direct relevance to biological systems still remains in question. The specific effects realized in the organized system, however, will lead to the exciting application to new catalytic system and chemical reactions.

In the field of polymer synthesis, the association of the monomer such as micelles, liquid crystals or complexes through pendant groups gives rise to some specific circumstances in the rate acceleration of polymerization and the enhancement in the stereospecificity of the resulting polymer [4-6]. Typical micelle effect was observed for the polymerization of *p*-methacryloyloxy benzoic acid, in the presence of *p*-cetyloxy benzoic acid, accompanied by the increased rate together with high molecular weight of the polymers resulted [7]. Kämmerer et al. [8], used the matrix polymerization technique to the polycondensation of glycine bound phenol formaldehyde resin, and obtained the cyclic dimer of glycine. Kinoshita et al. [9] also reported the synthesis of 2,5-piperazine-dione derivatives from α-amino acid S-dodecyl esters on the micellar surface. In connection with polypeptide synthesis, Katchalsky et al.[10] performed the polycondensation of α-amino acid alkyl esters in which the long alkyl group serves for the monolayer formation in water, and found that the product formed was positive to a biuret reaction. However, studies on the synthesis of polypeptide in the reversed micelle appear hitherto not yet to be carried out.

The present thesis is concerned with the synthesis of polypeptide in the organized reversed micelle which would be favorable for the polycondensation.

Chapter I deals with the synthesis of active β-alanine 4-acyl-2-nitrophenyl esters as functional reversed micelle surfactant. The convenient preparation procedure of new type active esters of β-alanine which can form functional reversed micelle are described together with apparent mean aggregation numbers for model compounds.

Chapter II deals with the synthesis of poly(β-alanine) from β-alanine 4-acyl-2-nitrophenyl esters. The optimal condition for the synthesis of poly(β-alanine) are discussed in relation to the formation of reversed micelles.

Chapter III deals with the polycondensation of α-amino acid 4- acyl-2-nitrophenyl esters. This chapter also describes the intramolecular rearrangement reaction occuring in the polycondensation of the active α-amino acid esters.

Chapter IV deals with the synthesis of polypeptides from dipeptide 4-acyl-2-nitrophenyl esters, and clarifies how the hydrophilic portion of the amino acid site in the active esters can contribute to the polycondensation in the reversed micelle.

References


[1] M.D.Cohen, B.S.Green, ed., *Chemistry of Oriented Organic Molecules*, *Israel J.Chem.*, 18, 185 (1979).

[2] J.H.Fendler, E.J.Fendler, *Catalysis in Micellar and Macromolecular Systems*, Academic Press, New York, 1975.

[3] E.Cordes, ed., *Reaction Kinetics in Micelles*, Plenum Press, New York, 1973.

[4] V.A.Kabanov, O.V.Kargina, V.A.Petrovskaya, *Vysokomol.Soedin.* 13, 348 (1971).

[5] W.J.Toth, A.V.Tobolsky, *J.Polymer Sci.* B 8, 289 (1970).

[6] K.Shima, M.Kinoshita, M.Imoto, *Makromol.Chem.* 154, 247 (1972).

[7] Yu.B.Amerik, I.I.Konstantinov, B.A.Krentsel, *J.Polymer Sci.* C 23, 231 (1968)

[8] V.Böhmer, H.Kämmerer, *Makromol.Chem.* 138, 137 (1970).

[9] Y.Kawabata, M.Kinoshita, *Makromol.Chem.* 175, 105 (1974).

[10] A.Baniel, M.Frankel, I.Friedrich, A.Katchalsky, *J.Org.Chem.* 13, 791 (1948).

## Chapter I. Synthesis of β-Alanine 4-Acyl-2-nitrophenyl Esters as Functional Reversed Micelle

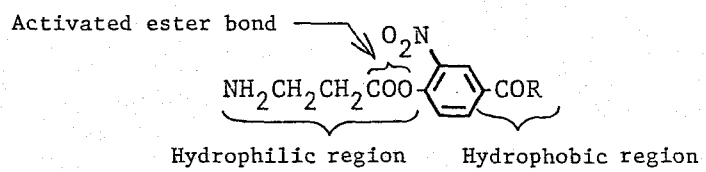
### I - 1. Introduction

The reaction in oriented organic molecular assemblies such as micelles, liquid crystals, monolayers or vesicles has attracted the interest of chemists. In such assemblies, the control of regioselectivity or stereoselectivity, the pronounced rate acceleration, rate retardation and the change in the product distribution are expected. However, a few studies on the polymer synthesis utilizing these ordered systems were carried out [1-6], particularly the investigation with respect to reversed micellar aggregation has not been performed. Therefore, the author tried to synthesize polypeptide on the reversed micellar surface. The first step to utilize the reversed micellar aggregate in the synthesis of polypeptide is to find out the favorable oriented organic molecules which form the reversed micelle and are active esters enough to cause the polycondensation.

This chapter is concerned with the preparation of β-alanine 4-acyl-2-nitrophenyl esters as functional reversed micelle, and the measurement of the apparent mean aggregation numbers of model compounds; N-acetyl-β-alanine 4-acyl-2-nitrophenyl esters.

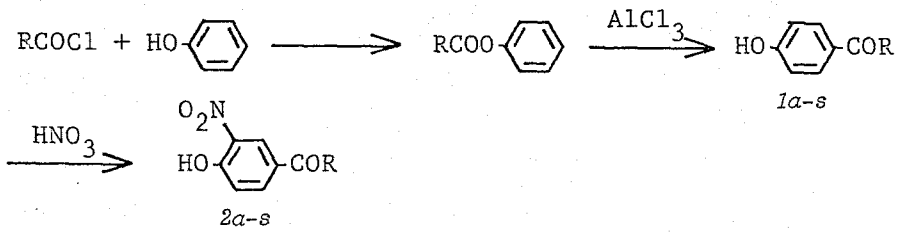
### I - 2. Results and Discussion

The author prepared active β-alanine 4-acyl-2-nitrophenyl esters to make functional reversed micelle, which can be served for the polycondensation. This ester has both a hydrophobic acyl group and a hydrophilic β-alanine region, the nitro group is introduced to the 2-position of the phenyl ester in order to activate the ester bond as shown below,

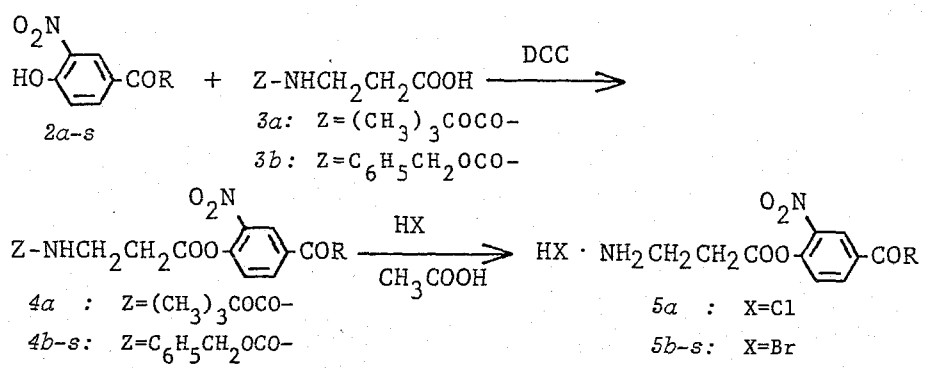


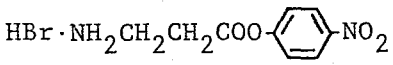

*β-Alanine 4-Acyl-2-nitrophenyl Ester*

*Synthesis of 4-acyl-2-nitrophenol (2a-s);* A variety of 4-acyl-2-nitrophenols (*1a-s*) were derived from Fries rearrangement of phenyl esters in the presence of $AlCl_3$, which *1a-i* were prepared in carbon disulfide according to the method of E.Miller et al.[7] with minor modification, and *1j-s* were obtained in nitrobenzene by the method of T.I.Briggs et al.[8]. 4-Acyl-2-nitrophenols (*2a-s*) were prepared by the nitration of 4-acylphenols (*1a-s*).

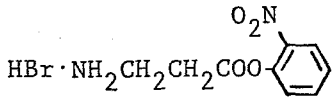



*Synthesis of β-alanine 4-acyl-2-nitrophenyl esters (5a-s);* A series of active β-alanine 4-acyl-2-nitrophenyl esters (*5b-s*) was prepared by the reaction of 4-acyl-2-nitrophenol (*2b-s*) with N-benzyloxycarbonyl-β-alanine (*3b*) in the presence of dicyclohexylcarbodiimide (DCC), and further converted into their hydrobromic acid salts (*5b-s*). N-*tert*-Butoxycarbonyl-β-alanine 4-acetyl-2-nitrophenyl ester (*4a*), was made to the hydrochloric acid salt (*5a*), since the N-benzoyloxycarbonyl ester was difficult to purify.

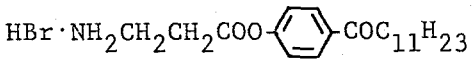




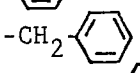
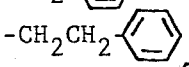
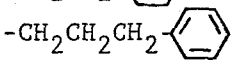
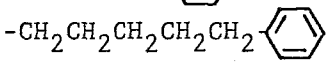
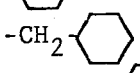
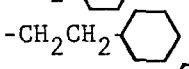
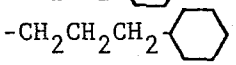
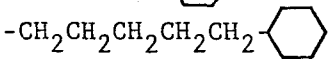
*5t*



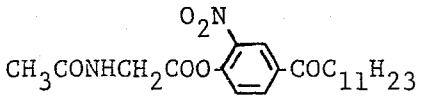
*5u*



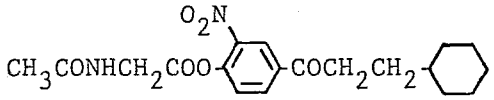
*5v*

| Symbol | -R |
|---|---|
| *a* | $-CH_3$ |
| *b* | $-C_3H_7$ |
| *c* | $-CH(CH_3)_2$ |
| *d* | $-C_5H_{11}$ |
| *e* | $-C_7H_{15}$ |
| *f* | $-C_9H_{19}$ |
| *g* | $-C_{11}H_{23}$ |
| *h* | $-C_{15}H_{31}$ |
| *i* | $-C_{17}H_{35}$ |
| *j* |  |
| *k* |  |
| *l* |  |
| *m* |  |
| *n* |  |
| *o* |  |
| *p* |  |
| *q* |  |
| *r* |  |
| *s* |  |

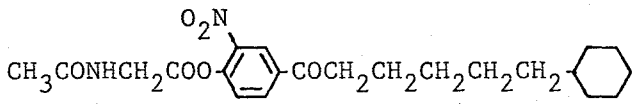
*Preparation of Model Compounds (6a-d) and Measurement of Their Apparent Mean Aggregation Numbers;* The author prepared *6a-d* as model compounds for active esters (*5*), and determined their apparent mean aggregation numbers by a vapor pressure depression method.



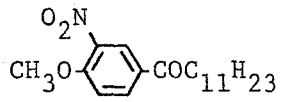
*6a*



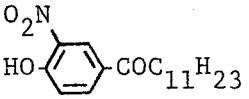
*6b*



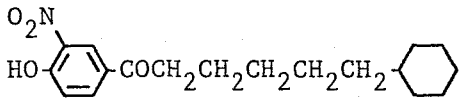
*6c*



*6d*



*2g*



*2s*

The results are summarized in Table I-1. All the values of apparent mean aggregation number of model compounds measured in methanol at 40°C were near unity over a concentration range of 0.001 to 0.01 M, which explains that these molecules are monodispersed in polar solvents such as methanol. On the other hand, the apparent mean aggregation number of *6a-c*, measured in cyclo-

Table I-1 Values of the apparent mean aggregation number for model compounds (*6a-d*,*2g*,*s*) obtained by a vapor pressure depression measurement

| Solvent | Concentration ( m mole/l ) | Apparent mean aggregation number (Z) 40°C[a)] | | | | | | 60°C[b)] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *6a* | *6b* | *6c* | *6d* | *2g* | *2s* | *6a* | *6b* | *6c* |
| Cyclohexane | 1.0 | 5.4 | 3.2 | 9.8 | 1.1 | 1.0 | 0.9 | 0.8 | 0.8 | 0.9 |
| | 2.0 | 10.1 | 4.4 | 10.6 | 0.9 | 1.0 | 1.0 | 1.0 | 0.9 | 1.1 |
| | 5.0 | 21.1 | 6.9 | 13.4 | 0.9 | 0.9 | 1.0 | 1.9 | 1.3 | 1.5 |
| | 7.5 | 26.2 | 9.8 | 15.2 | 1.0 | 1.0 | 1.0 | 2.4 | 1.5 | 1.9 |
| | 10.0 | 37.4 | 10.1 | 17.5 | 1.0 | 1.0 | 1.0 | 2.7 | 1.7 | 2.8 |
| Methanol | 1.0 | 1.2 | 1.2 | 1.0 | 1.1 | 1.4 | 1.0 | | | |
| | 2.0 | 1.0 | 1.1 | 1.0 | 1.1 | 1.1 | 1.0 | | | |
| | 5.0 | 0.8 | 0.9 | 0.9 | 1.0 | 1.0 | 1.0 | | | |
| | 7.5 | 0.8 | 0.8 | 0.8 | 1.0 | 1.0 | 1.0 | | | |
| | 10.0 | 0.8 | 0.8 | 0.8 | 1.0 | 1.0 | 1.1 | | | |

a) Exact temperature is 39.7°C in cyclohexane, 39.6°C in methanol.
b) Exact temperature is 60.2°C.

hexane at 40°C, increased with increasing in the concentration. Obviously, *6a-c* were made to aggregate, while *6d*, *2g* and *2s* were monodispersed. In other wards, it appears that *6d*, *2g* and *2s* having methoxy or hydroxy groups, cannot form the micelles due to less hydrophilicity, while *6a-c* having acetylglycinate group in the hydrophilic region can form them.

The apparent mean aggregation numbers depend also on the temperature. The value for *6a-c* in cyclohexane at 60°C was smaller than that obtained at 40°C. This indicates that the aggregation was disturbed by increasing temperature, resulting the decrease in the apparent mean aggregation numbers.

## I - 3. Experimental Part

### *Materials*

*Preparation of carboxylic acids;* Reagent grade chemicals of acetic acid, n-butyric acid, isobutyric acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, hexadecanoic acid, octadecanoic acid, benzoic acid, α-phenylacetic acid, β-phenylpropionic acid, cyclohexylcarboxylic acid, γ-cyclohexylacetic acid and ω-cyclohexylpropionic acid were purchased from Tokyo Kasei Ltd, and used without further purification. γ-Phenylbutyric acid and ω-phenylhexanoic acid were prepared by the method of D.Papa et al.[9]. γ-Cyclohexylbutyric acid and ω-cyclohexylhexanoic acid were synthesized by the method of T.L.Cairns et al.[10].

*Preparation of the esters;* The acid chloride (1.0mole) [11] was added dropwise to phenol (1.0mole) at room temperature and gently refluxed for 4h. To the cooled solution was added water and the separated ester was extracted with ether. After washing with 5 % aqueous sodium hydroxide solution and water, the etheral solution was dried over sodium salfate and evaporated. The residue was distilled under reduced pressure to give the pure ester in 80-90 % yield.

*Preparation of 4-acylphenols (1a-s);* 4-Acylphenols (*1a,d-i*) were prepared according to the method of E.Miller et al.[7], and others were obtained by the method described by T.I.Briggs et al.[8]. All the products were recrystal-

lized from chloroform-petroleum ether, except 4-isobutyroylphenol (*1c*) purified by distillation. 4-Acylphenols which were prepared include; 4-acetyl-, 4-butyryl-, 4-isobutyryl-, 4-hexanoyl-, 4-octanoyl-, 4-decanoyl-, 4-dodecanoyl- 4-hexadecanoyl-, 4-octadecanoyl-, 4-benzoyl-, 4-(α-phenylacetyl)-, 4-(β-phenylpropionyl)-, 4-(γ-phenylbutyryl)-, 4-(ω-phenylhexanoyl)-, 4-cyclohexylcarbonyl-, 4-(α-cyclohexylacetyl)-, 4-(β-cyclohexylpropionyl)-, 4-(γ-cyclohexylbutyryl)- and 4-(ω-cyclohexylhexanoyl)-phenol. The procedure used for the synthesis of 4-acylphenols (*1a-s*) are described in detail for 4-dodecanoylphenol (*1g*) and 4-(ω-phenylhexanoyl)phenol (*1n*).

*4-Dodecanoylphenol (1g);* To 50ml of carbon disulfide solution, containing 32g (0.24mole) of anhydrous aluminium chloride, 44g (0.20mole) of phenyl dodecanoate were added slowly. After refluxing for 5h, the solvent was distilled off and the residue was kept further at 80-90°C for 2h and 140-150°C for 1h. The residue was cooled, the aluminium complex was decomposed by adding 6 M HCl (100ml) slowly, made to alkaline with NaOH and extracted twice with 50ml of diethyl ether to remove phenyl dodecanoate. The alkaline solution was acidified again with hydrochloric solution, the solidified product was filtered off, and recrystallized from chloroform - petroleum ether to give 18g of 4-dodecanoylphenol in 42 % yield. After evaporation of the filtrate and recrystallization from methanol, 13g of 2-dodecanoylphenol was obtained in 33 % yield.

*4-(ω-Phenylhexanoyl)phenol (1n);* 54g (0.20mole) of phenyl ω-phenylhexanoate was added in small portions at room temperature to 50ml of nitrobenzene solution containing 35g (0.26mole) of anhydrous aluminium chloride. The reaction mixture was kept to stand at 25°C for 40h, and then the solution was poured on to ice and hydrochloric acid. When the hydrolysis was completed, the mixture was extracted with ether and the ethereal solution was evaporated in vacuo. 4-(ω-Phenylhexanoyl)phenol was obtained in 60 % yield (33g) by recrystallization from chloroform - petroleum ether.

The analytical data for *1a-s* are given in Table I-2.

*Preparation of 4-acyl-2-nitrophenols (2a-s);* The nitration of 4-acyl-

Table I-2 Analytical data of 4-acylphenol (*1a-s*)

| 4-Acyl-phenol | Yield % | Melting Point °C | Lit. °C | Formula | Analyses, % Calcd. C | Calcd. H | Calcd. N | Found C | Found H | Found N |
|---|---|---|---|---|---|---|---|---|---|---|
| *1a* | 51 | 107-108 | 106-108 [12] | $C_8H_8O_2$ | 70.57 | 5.92 | — | 70.50 | 5.94 | — |
| *1b* | 80 | 91-92 | 91 [13] | $C_{10}H_{12}O_2$ | 73.14 | 7.37 | — | 73.31 | 7.40 | — |
| *1c* | 82 | Bp 130-135(0.5mm) | Bp 133(0.5mm) [8] | | | | | | | |
| 2,4-dinitrophenyl-hydrazone of *1c* | | 167-168 | 166 [8] | $C_{16}H_{16}N_4O_5$ | 55.81 | 4.68 | 16.27 | 55.57 | 4.68 | 16.37 |
| *1d* | 55 | 62-63 | 62 [14] | $C_{12}H_{16}O_2$ | 74.97 | 8.39 | — | 75.10 | 8.41 | — |
| *1e* | 52 | 63-64 | 62.5-63.5 [15] | $C_{14}H_{20}O_2$ | 76.32 | 9.15 | — | 76.51 | 9.31 | — |
| *1f* | 44 | 64.5-65.5 | 64-65 [16] | $C_{16}H_{24}O_2$ | 77.37 | 9.74 | — | 77.45 | 9.84 | — |
| *1g* | 42 | 71-72 | 71-72 [14] | $C_{18}H_{28}O_2$ | 78.21 | 10.21 | — | 78.37 | 10.05 | — |
| *1h* | 43 | 88-89 | 84.5-85 [14] | $C_{22}H_{36}O_2$ | 79.46 | 10,92 | — | 79.53 | 10.88 | — |
| *1i* | 46 | 89-90 | 87-89 [14], 90-90.5 [17] | $C_{24}H_{40}O_2$ | 79.94 | 11.18 | — | 80.11 | 11.23 | — |
| *1j* | 54 | 134-135 | 132-133.5 [18] | $C_{13}H_{10}O_2$ | 78.77 | 5.09 | — | 78.75 | 5.10 | — |
| *1k* | 66 | 148-149 | 142 [19], 148-149 [20] | $C_{14}H_{12}O_2$ | 79.22 | 5.70 | — | 79.15 | 5.72 | — |
| *1l* | 53 | 107.5-108.5 | 74 [14], 104 [21] | $C_{15}H_{14}O_2$ | 79.62 | 6.24 | — | 79.61 | 6.26 | — |
| *1m* | 19 | 160-161 | | $C_{16}H_{16}O_2$ | 79.97 | 6.71 | — | 79.87 | 6.32 | — |
| *1n* | 60 | 103-104 | | $C_{18}H_{20}O_2$ | 80.56 | 7.51 | — | 80.55 | 7.55 | — |
| *1o* | 66 | 114.5-115.5 | | $C_{13}H_{16}O_2$ | 76.44 | 7.90 | — | 76.30 | 7.87 | — |
| *1p* | 65 | 111-112 | | $C_{14}H_{18}O_2$ | 77.03 | 8.31 | — | 77.11 | 8.33 | — |
| *1q* | 69 | 112-113 | | $C_{15}H_{20}O_2$ | 77.55 | 8.68 | — | 77.53 | 8.67 | — |
| *1r* | 54 | 150-151 | 136-137 [22] | $C_{16}H_{22}O_2$ | 78.01 | 9.00 | — | 78.23 | 8.97 | — |
| *1s* | 51 | 115-116 | | $C_{18}H_{26}O_2$ | 78.79 | 9.55 | — | 78.86 | 9.50 | — |

phenols (*1a-s*) were carried out by the two methods. One in procedure for the preparation of *2a-i,o,q-s*, using conc. sulfuric acid as solvent, the other is that for the preparation of *2j-n,p* using glacial acetic acid. The procedure used for the synthesis of 4-acyl-2-nitrophenol (*2a-s*) are described in detail for 4-dodecanoyl-2-nitrophenol (*2g*) and 4-(ω-phenylhexanoyl)-2-nitrophenol (*2n*).

*4-Dodecanoyl-2-nitrophenol (2g);* 55g of 4-dodecanoylphenol (20mmole) was dissolved in 200ml of conc. sulfuric acid at room temperature, and the solution was cooled to 0°C. To this solution an ice-cold mixture of conc. nitric acid (20mmole) and an equal volume of conc. sulfuric acid were added slowly for 1h, and stirred for 15min. After that, the solution was poured into 1000ml of ice-cold water and kept for 1h. The precipitate was filtered off and recrystallized from ethanol; yellow odorless crystals; m.p.72-73; yield:59g(91%). $^1$H NMR ($CCl_4$): δ=0.9(—$CH_3$), 1.3(—$(CH_2)_8$—), 1.7(—$CH_2$—$CH_2CO$—), 2.9(—$CH_2CH_2CO$—), 7.1, 8.1, 8.6($H_a$, $H_b$, $H_c$ in phenol), and 10.8(—OH).

*4-(ω-Phenylhexanoyl)-2-nitrophenol (2n);* 54g of 4-(ω-phenylhexanoyl)-phenol (20mmole) was dissolved in 80ml of glacial acetic acid at room temperature, and cooled to 0°C. To this solution was added fuming nitric acid (d=1.52 20mmole) slowly for 1h, and then warmed at 40°C for 0.5h. After that, the solution was evaporated in vacuo. The residue was recrystallized and further purified by column chromatographic method (solvent: benzene). By recrystallization from methanol, 4-(ω-phenylhexanoyl)-2-nitrophenol was obtained in 72% yield (45g); yellow crystals; m.p. 54.5 - 55.5°C

The analytical data for *2a-s* are given in Table I-3.

*Preparation of β-alanine active esters hydrogen bromide salts (5a-v);* The procedures used for the synthesis of active esters are described in detail for β-alanine 4-dodecanoyl-2-nitrophenyl ester (*5g*) and β-alanine 4-acetyl-2-nitrophenyl ester (*5a*).

*Hydrobromide of β-alanine 4-dodecanoyl-2-nitrophenyl ester (5g);* To 50 ml of an ethyl acetate solution containing 7.2g (22mmole) of 4-dodecanoyl-2-nitrophenol and 5.0g (22mmole) of N-benzyloxycarbonyl-β-alanie were added 4.5g (22mmole) of N,N'-dicyclohexylcarbodiimide (DCC) at 0°C. After stirring for 3h at 0°C, the mixture was kept to stand overnight at room temperature and N,N'-dicyclohexylurea was filtered off. The filtrate was evaporated in vacuo,

Table I-3 Analytical data of 4-acyl-2-nitrophenol (*2a-s*)

| 4-Acyl-2-nitrophenol | Yield % | Melting Point °C | Lit. °C | Formula | Analyses, % Calcd. C | Calcd. H | Calcd. N | Found C | Found H | Found N |
|---|---|---|---|---|---|---|---|---|---|---|
| *2a* | 88 | 132.5-133.5 | 132-132.5 [23] | $C_8H_7NO_4$ | 53.04 | 3.90 | 7.73 | 53.06 | 3.83 | 7.95 |
| *2b* | 85 | 48-49 | 47.2-48.2 [23] | $C_{10}H_{11}NO_4$ | 57.41 | 5.30 | 6.70 | 57.16 | 5.40 | 6.69 |
| *2c* | 83 | 77.5-78.5 | | $C_{10}H_{11}NO_4$ | 57.41 | 5.30 | 6.70 | 57.04 | 5.36 | 6.66 |
| *2d* | 81 | 37-38 | 37.2-37.6 [23] | $C_{12}H_{15}NO_4$ | 60.75 | 6.37 | 5.90 | 60.54 | 6.34 | 5.86 |
| *2e* | 93 | 51-52 | | $C_{14}H_{19}NO_4$ | 63.38 | 7.22 | 5.28 | 63.58 | 7.25 | 5.34 |
| *2f* | 92 | 63.5-64.5 | | $C_{16}H_{23}NO_4$ | 65.51 | 7.90 | 4.78 | 65.26 | 7.98 | 4.63 |
| *2g* | 91 | 72-73 | | $C_{18}H_{27}NO_4$ | 67.26 | 8.47 | 4.36 | 66.93 | 8.62 | 4.29 |
| *2h* | 94 | 85-86 | | $C_{22}H_{35}NO_4$ | 69.99 | 9.35 | 3.71 | 69.78 | 9.48 | 3.67 |
| *2i* | 93 | 87.5-88.5 | | $C_{24}H_{39}NO_4$ | 71.07 | 9.69 | 3.45 | 70.87 | 9.85 | 3.37 |
| *2j* | 73 | 94.5-95.5 | 94-94.5 [24] | $C_{13}H_9NO_4$ | 64.20 | 3.73 | 5.76 | 63.91 | 3.87 | 5.77 |
| *2k* | 70 | 91-92 | | $C_{14}H_{11}NO_4$ | 65.36 | 4.31 | 5.45 | 65.01 | 4.41 | 5.09 |
| *2l* | 71 | 117.5-118.5 | | $C_{15}H_{13}NO_4$ | 66.41 | 4.83 | 5.16 | 66.14 | 5.00 | 4.89 |
| *2m* | 70 | liquid | | | | | | | | |
| 2,4-dinitrophenyl-hydrazone of *2m* | | 175-176 | | $C_{22}H_{19}N_5O_7$ | 56.77 | 4.12 | 15.05 | 56.62 | 4.16 | 14.99 |
| *2n* | 72 | 54.5-55.5 | | $C_{18}H_{19}NO_4$ | 68.99 | 6.11 | 4.47 | 68.87 | 6.28 | 4.17 |
| *2o* | 77 | 95.5-96.5 | | $C_{13}H_{15}NO_4$ | 62.64 | 6.07 | 5.62 | 62.32 | 6.14 | 5.65 |
| *2p* | 72 | 56-57 | | $C_{14}H_{17}NO_4$ | 63.86 | 6.51 | 5.32 | 63.60 | 6.64 | 5.22 |
| *2q* | 71 | 60.5-61.5 | | $C_{15}H_{19}NO_4$ | 64.96 | 6.91 | 5.05 | 64.66 | 6.88 | 5.03 |
| *2r* | 70 | 69.5-70.5 | | $C_{16}H_{21}NO_4$ | 65.95 | 7.27 | 4.81 | 65.75 | 7.39 | 4.61 |
| *2s* | 75 | 76-77 | | $C_{18}H_{25}NO_4$ | 67.69 | 7.89 | 4.39 | 67.60 | 7.98 | 4.12 |

the residue was dried, and then, dry hydrogen bromide gas was passed at 80°C into the acetic acid solution containing the residue. The resulting hydrobromide was recrystallized from acetic acid, yield 9.3g (88%).

*Hydrochloride of β-alanine 4-acetyl-2-nitrophenyl ester (5a);* To 50ml of ethyl acetate solution containing 1.9g (10.6mmole) of 4-acetyl-2-nitrophenol and 2.0g(10.6mmole) of N-tert-butoxycarbonyl-β-alanine added 2.2g (10.6mmole) of DCC at 0°C. After stirring for 3h at 0°C, the mixture was kept to stand overnight at room temperature. The filtrate from N,N'-dicyclohexylurea was evaporated in vacuo, and the residue was dried. Dry hydrogen chloride gas was then passed into the ethyl acetate solution containing the residue at 0°C. The hydrochloride salt was recrystallized from ethanol, yield 2.5g (81%).

The analytical data are given in Table I-4.

*Preparation of model compounds (6a-d);* The procedure used for the synthesis of *6a-e* is described in detail for N-acetylglycine 4-dodecanoyl-2-nitrophenyl ester (*6a*).

*N-Acetylglycine 4-dodecanoyl-2-nitrophenyl ester (6a);* To 50ml of an ethyl acetate solution containing 2.3 g (20mmole) of N-acetylglycine [26] and 6.4g (20mmole) of 4-dodecanoyl-2-nitrophenol were added 4.5g (22mmole) of DCC at 0°C. After stirring for 3h at 0°C, the mixture was kept to stand overnight at room temperature and N,N'-dicyclohexylurea was filtered off. The filtrate was evaporated in vacuo. The residue was recrystallized from carbon tetrachloride. Colorless crystal; m.p.88-89°C, yield 7.2g (86%).

*4-Dodecanoyl-2-nitromethoxybenzene (6d);* 5.8g (20mmole) of 4-dodecanoylmethoxybenzene [27] was dissolved in 200ml of conc. sulfuric acid at room temperature, and the solution was cooled to 0°C. To this solution were added slowly an ice-cold mixture of conc. nitric acid (20mmole) and an equal volume of conc. sulfuric acid for 1h, and stirred for 15min. After that, the solution was poured into 1000ml of ice-cold water and kept for 1h. The precipitate was filtered off and recrystallized from methanol; Colorless crystal, m.p. 54-55°C, yield 6.2g (92%).

The analytical data for *6a-d* are given in Table I-5.

*Measurement of apparent mean aggregation number*

The apparent mean aggregation number (Z) was measured by a vapor pressure

Table I-4 Analytical data of the β-alanine active esters (*5a-v*)

| Ester | Yield % | Melting Point °C (dec.) | Formula | Analyses, % Calcd. C | H | N | Br(Cl) | Found C | H | N | Br(Cl) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *5a* | 81 | 130.5-131.5 | $C_{11}H_{13}N_2O_5Cl$ | 45.77 | 4.54 | 9.70 | 12.28 | 45.60 | 4.37 | 9.77 | 12.51 |
| *5b* | 95 | 121-122 | $C_{13}H_{17}N_2O_5Br$ | 43.23 | 4.74 | 7.76 | 22.12 | 43.01 | 4.70 | 7.64 | 22.26 |
| *5c* | 94 | 99-100 | $C_{13}H_{17}N_2O_5Br$ | 43.23 | 4.74 | 7.76 | 22.12 | 42.99 | 4.76 | 7.65 | 21.76 |
| *5d* | 85 | 127-128 | $C_{15}H_{21}N_2O_5Br$ | 46.29 | 5.44 | 7.20 | 20.53 | 46.22 | 5.32 | 7.17 | 20.26 |
| *5e* | 83 | 127-128 | $C_{17}H_{25}N_2O_5Br$ | 48.93 | 6.04 | 6.71 | 19.15 | 48.87 | 5.85 | 6.68 | 19.10 |
| *5f* | 84 | 127-128 | $C_{19}H_{29}N_2O_5Br$ | 51.25 | 6.56 | 6.29 | 17.94 | 51.14 | 6.51 | 6.30 | 17.70 |
| *5g* | 88 | 128-129 | $C_{21}H_{33}N_2O_5Br$ | 53.28 | 7.03 | 5.92 | 16.88 | 53.43 | 7.11 | 6.00 | 17.04 |
| *5h* | 89 | 133.5-134.5 | $C_{25}H_{41}N_2O_5Br$ | 56.71 | 7.81 | 5.29 | 15.09 | 56.44 | 7.97 | 5.33 | 15.22 |
| *5i* | 91 | 132.5-133.5 | $C_{27}H_{45}N_2O_5Br$ | 58.16 | 8.14 | 5.02 | 14.33 | 57.97 | 8.16 | 4.95 | 14.60 |
| *5j* | 89 | 174-175 | $C_{16}H_{15}N_2O_5Br$ | 48.63 | 3.83 | 7.09 | 20.22 | 48.50 | 3.70 | 7.10 | 19.87 |
| *5k* | 85 | 168-169 | $C_{17}H_{17}N_2O_5Br$ | 49.89 | 4.19 | 6.85 | 19.53 | 49.80 | 4.18 | 6.84 | 19.65 |
| *5l* | 85 | 164-165 | $C_{18}H_{19}N_2O_5Br$ | 51.08 | 4.52 | 6.62 | 18.88 | 51.03 | 4.49 | 6.65 | 18.65 |
| *5m* | 83 | 160-161 | $C_{19}H_{21}N_2O_5Br$ | 52.19 | 4.84 | 6.41 | 18.27 | 52.03 | 4.81 | 6.43 | 18.62 |
| *5n* | 81 | 140-141 | $C_{21}H_{25}N_2O_5Br$ | 54.20 | 5.42 | 6.02 | 17.17 | 53.91 | 5.33 | 5.98 | 17.43 |
| *5o* | 87 | 176-177 | $C_{16}H_{21}N_2O_5Br$ | 47.89 | 5.28 | 6.98 | 19.91 | 48.10 | 5.32 | 7.10 | 19.60 |
| *5p* | 85 | 176-177 | $C_{17}H_{23}N_2O_5Br$ | 49.17 | 5.58 | 6.75 | 19.24 | 48.99 | 5.45 | 6.85 | 19.64 |
| *5q* | 82 | 174.5-175.5 | $C_{18}H_{25}N_2O_5Br$ | 50.36 | 5.87 | 6.53 | 18.61 | 50.43 | 5.90 | 6.66 | 19.00 |
| *5r* | 80 | 165-166 | $C_{19}H_{27}N_2O_5Br$ | 51.48 | 6.14 | 6.32 | 18.02 | 51.50 | 6.07 | 6.35 | 18.14 |
| *5s* | 81 | 156.5-157.5 | $C_{21}H_{31}N_2O_5Br$ | 53.51 | 6.63 | 5.94 | 16.95 | 53.64 | 6.58 | 5.97 | 16.91 |
| *5t* | 82 | 196.5-197.0[a)] | $C_9H_{11}N_2O_4Br$ | 37.13 | 3.81 | 9.62 | 27.45 | 37.00 | 3.66 | 9.77 | 27.57 |
| *5u* | 94 | 178-179 | $C_9H_{11}N_2O_4Br$ | 37.13 | 3.81 | 9.62 | 27.45 | 37.13 | 3.65 | 9.54 | 27.78 |
| *5v* | 71 | 197.5-198.5 | $C_{21}H_{34}NO_3Br$ | 58.88 | 8.00 | 3.27 | 18.66 | 58.86 | 8.03 | 3.32 | 18.94 |

a) Ref.[25] 206-208°C (dec.)

Table I-5 Analytical data of model compounds (*6a-d*)

| Model Compound | Yield % | Melting Point °C | Formula | Analyses, % Calcd. C | H | N | Found C | H | N |
|---|---|---|---|---|---|---|---|---|---|
| *6a* | 86 | 88-89 | $C_{22}H_{32}N_2O_6$ | 62.84 | 7.67 | 6.66 | 62.70 | 7.68 | 6.77 |
| *6b* | 85 | 86-88 | $C_{19}H_{24}N_2O_6$ | 60.62 | 6.43 | 7.44 | 60.50 | 6.22 | 7.50 |
| *6c* | 86 | 112-113 | $C_{22}H_{30}N_2O_6$ | 63.14 | 7.23 | 6.69 | 63.44 | 6.98 | 6.95 |
| *6d* | 92 | 54-55 | $C_{19}H_{29}N_2O_6$ | 68.03 | 8.71 | 4.18 | 67.89 | 8.77 | 4.07 |

depression method (Hitachi 115 Type Molecular Weight Apparatus). Z value was calculated from the following equation

$$Z = \frac{c \cdot k}{\Delta R}$$

where c is the molar concentration of the sample, $\Delta R$ is the dial reading in ohms and k is the proportional constant obtained by using a standard ethyl stearate solution; Z has been evaluated by involving both monomer and micelle, since Z equals to $M/M_o$ ( M and $M_o$ are the apparent mean micellar weight measured and the monomeric molecular weight of sample, respectively).

## I - 4. Summary

A series of β-alanine 4-acyl-2-nitrophenyl esters having both a hydrophobic long acyl chain and a hydrophilic β-alanine region which are expected to form such aggregate as reveresed micelles in nonpolar solvents, were prepared

In order to clarify the formation of reversed micelle, the model N-acetyl-β-alanine 4-acyl-2-nitrophenyl esters corresponding to the β-alanine 4-acyl-2-nitrophenyl esters were prepared and their apparent mean aggregation numbers in methanol and cyclohexane were determined by a vapor pressure depression method.

## I - 5. References

[1] Y.Kawabata, M.Kinoshita, *Makromol.Chem.*, 176, 3243 (1975)

[2] C.M.Paleos, P.Dais, *J.Polymer Sci. Polym.Chem.Ed.*, 16, 1495 (1978)

[3] Y.B.Amerik, B.A.Krentsel, *J.Polymer Sci., Part C*, 16, 1383 (1967)

[4] E.Perplies, H.Ringsdorf, J.H.Wendorff, *Ber.Bunsenges.Phys.Chem.*, 78, 921 (1974)

[5] A.Blumstein, N.Kitagawa, R.Blumstein, *Mol.Cryst.Liq.Cryst.*, 12, 215 (1971)

[6] A.Baniel, M.Frankel, I.Friedlich, A.Katchalsky, *J.Org.Chem.*, 13, 791 (1948)

[7] E.Miller, W.H.Hartung, *Org.Syn.Coll.Vol.2*, 543 (1943)

[8] T.I.Briggs, G.G.S.Dutton, E.Merler, *Can.J.Chem.*, 34, 851 (1956)

[9] D.Papa, E.Schwenk, H.Hankin, *J.Amer.Chem.Soc.*, 69, 3018 (1947)

[10] T.L.Cairns, R.M.Joyce, R.S.Schreiber, *J.Amer.Chem.Soc.*, 70, 1689 (1948)

[11] R.E.Kent, S.M.McElvain, *Org.Syn.Coll.Vol.3*, 490

[12] A.Perkin, *J.Chem.Soc.*, 8, 87

[13] W.H.Perkin, *J.Chem.Soc.*, 55, 546 (1884)

[14] K.Nakazawa, S.Matsuura, K.Kusuda, *J.Pharm.Soc.Japan*, 74, 495 (1954)

[15] A.W.Ralston, S.T.Bauer, *J.Org.Chem.*, 5, 165 (1940)

[16] D.Woodcock, *J.Chem.Soc.*, 4391 (1955)

[17] H.E.Bell, J.E.Driver, *J.Chem.Soc.*, 835 (1940)

[18] C.R.Hauser, E.H.Man, *J.Org.Chem.*, 17, 390 (1952)

[19] L.Thoi, N.Hoang, *Israel J.Chem.*, 1, 418 (1963)

[20] A.Friedman, W.Gugig, L.Mehr, E.I.Becker, *J.Org.Chem.*, 24, 516 (1959)

[21] V.A.Zasosov, E.I.Metel'kova, S.N.Milovanova, *Zhur.Obshchei Khim.*, 26, 2499 (1956)

[22] A.Prakash, G.S.Saharia, *Def.Sci.J.*, 19, 187 (1969)

[23] F.C.Brown, *J.Amer.Chem.Soc.*, 68, 872 (1946)

[24] *Chem.Abstr.*, 44, 7172e (1950)

[25] H.Yuki, Y.Okamoto, Y.Taketani, T.Tsubota, Y.Marubayashi, *J.Polymer Sci. Polym.Chem.Ed.*, 16, 2237 (1978)

[26] R.M.Herbert, D.Shemin, *Org.Syn.Coll.Vol.*, 2, 11 (1943)

[27] S.Matsuda, M.Ogawa, T.Okazaki, *Technol.Repts.Osaka Univ.*, 6, 393 (1956)

## Chapter II. Synthesis of Poly(β-alanine) from β-Alanine 4-Acyl-2-nitrophenyl Esters

### II - 1. Introduction

The polymerization and polycondensation carried out on the surface of the ordered molecular aggregates such as micelles [1,2], liquid crystals [3-13], and monolayers [14] have attracted the interest of polymer chemists, because these surface forces are expected for the acceleration of the polymerization and polycondensation.

With respect to the synthesis of polypeptides, in 1948, Katchalsky et al. [14] reported on a modified technique for preparing polypeptides, starting from α-amino acid alkyl esters. From the results obtained at elevated temperature in bulk, it has been suggested that the alkyl groups of the esters, having definite chain length, serve to form a monolayer in which polycondensation proceeds readily, avoidiog some undesirable 2,5-piperazinedione formation.

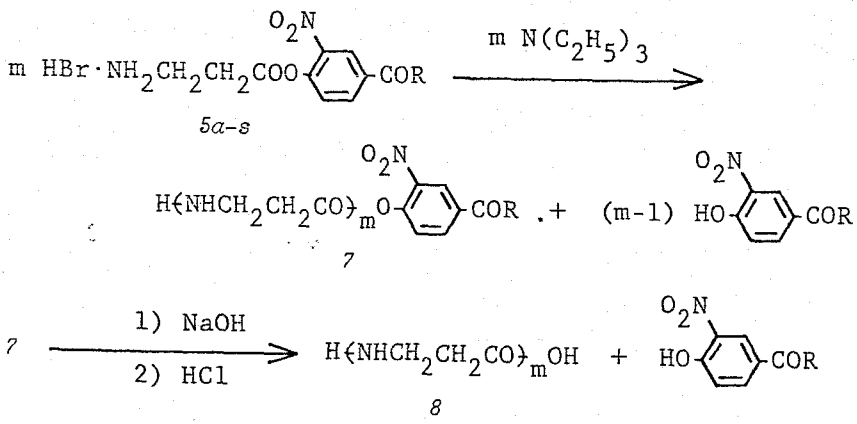
For the synthesis of poly(β-alanine), Kinoshita et al. [1] have found that the corresponding polymer could be obtained from thio-β-alanine S-alkyl esters by refluxing it in aqueous pyridine solution, and emphasized that the micellar effect due to the longer alkyl groups might be important on the polycondensation reaction, although the degree of polycondensation was still as low as about 20. However, very little is known about the effects of reversed micelle on the polymer synthesis.

This chapter is concerned with the synthesis of poly(β-alanine) from new type of active β-alanine phenyl esters as functional reversed micelle surfactant, in order to realize reactions at rather lower temperature, improving the conversion as well as the degree of polycondensation. The effect of the chain length of the acyl groups on the polycondensation was investigated, using various kinds of organic solvents for the reactions.

### II - 2. Results and Discussion

When hydrobromide salts of active esters ( *5a-v* ) were treated with triethylamine in solution at constant temperature for 24 hr, poly(β-alanine) was formed, remaining still ester residues at the chain ends. The terminal 4-acyl-2-nitrophenyl group was removed by alkali treatment as shown in the following

reaction scheme,



The conversion for the polycondensation obtained at 30°C in different solvents are summarized in Table II-1. As can be seen in Table II-1, nonpolar solvents were suitable for the polycondensation, while no polymer was formed in protic and dipolar aprotic solvents. In comparison with the esters (*5a-d*, *5t-u*) having short linear acyl groups or no acyl groups, the esters (*5f-h*) having long linear acyl group were reactive enough to give the polymer in good yield. In contrast, the esters (*5j-m*, *5o-r*) containing phenyl or cyclohexyl group were much more reactive and high in the conversion to the polymer even if the short linear acyl chain. The presence of phenyl or cyclohexyl group in the hydrophobic regin of esters resulted in the high conversion, even if the linear acyl chain is quite short as in the case of *5j*, *5k*, *5l*, *5o*, *5p* and *5q*. The phenyl and cyclohexyl group is similar in the hydrophobic character to the long linear acyl groups. The esters such as *5m* and *5s* were quite similar in the less reactivity to *5j*, suggesting that these monomers would form the undesirable rigid reversed micelles for the polycondensation owing to the presence of strong hydrophobic group.

The fact that no polymer was obtained from *5v* without nitro group suggests the presence of nitro group in the phenyl ester to be required for the polycondensation.

The effects of temperature on the polycondensation for *5a-u* in various solvents are illustrated in Figure II-1 - 21. All the esters gave no polymer in methanol, N,N-dimethylformamide and dimethylsulfoxide at any temperature. The compounds *5a-c,t* resulted in a low conversion, which was independent of the temperature (Figure II-1 - 3,20), while the conversion for *5d-j* increased until the temperature reached 30°C (Figure II-4 - 9). Among them, *5f-j* showed particularly a remarkable temperature dependence, which was the typical bell shape curves. In the case of *5j-n* containing phenyl group as hydrophobic region, the conversion from *5j* and *5k* increased with the increasing temperature (Figure II-10,11). *5l-m*, however, showed the bell shape curves, indicating the decrease in conversion with the increasing temperature through the certain optimal point (Figure II-12 - 14). For the esters (*5o-s*) which have cyclohexyl group, the bell shape curves were obtained (Figuer II-15 - 19). For the esters having certain strong hydrophobic groups, the favorable temperature for the polycondensation appears to be near 30°C, and the higher the temperature over 30°C, the less the conversion of the polycondensation. These results suggest that the reversed micellar aggregation would be thermally disturbed by the movement of hydrophobic resion and appear to be in accord with the fact that the aggregation number of reversed micelles decreases with increasing in the temperature [15-17].

Figure II-22 shows schematic representation of the ordered reversed micelles required for the polycondensation. The formation of the reversed micelles would lead to line up surfactant molecules so that the β-alaninate groups can approach more closely each other in nonpolar aprotic solvents than that in non-reversed micellar solutions. Consequently, presence of weak interactions between β-alaninate groups in the neighboring molecules results in accelerating the polycondensation favorably.

Table II-1 Results of the polycondensation in different solvents at 30°C for 24h[a)]

| Ester | Conversion in % for the water insoluble part | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cyclohexane | $CCl_4$ | Benzene | Ether | MeOH | DMF | DMSO |
| *5a* | 20 | 30 | 39 | 35 | 0 | 0 | 0 |
| *5b* | 62 | 48 | 54 | 88 | 0 | 0 | 0 |
| *5c* | 58 | 49 | 52 | 50 | 0 | 0 | 0 |
| *5d* | 75 | 62 | 60 | 76 | 0 | 0 | 0 |
| *5e* | 76 | 81 | 75 | 66 | 0 | 0 | 0 |
| *5f* | 94 | 97 | 95 | 95 | 0 | 0 | 0 |
| *5g* | 91 | 95 | 92 | 94 | 0 | 0 | 0 |
| *5h* | 51 | 93 | 86 | 71 | 0 | 0 | 0 |
| *5i* | 23 | 37 | 69 | 70 | 0 | 0 | 0 |
| *5j* | 90 | 92 | 49 | 74 | 0 | 0 | 0 |
| *5k* | 90 | 95 | 88 | 93 | 0 | 0 | 0 |
| *5l* | 91 | 86 | 85 | 92 | 0 | 0 | 0 |
| *5m* | 93 | 88 | 78 | 91 | 0 | 0 | 0 |
| *5n* | 75 | 73 | 68 | 86 | 0 | 0 | 0 |
| *5o* | 93 | 84 | 81 | 91 | 0 | 0 | 0 |
| *5p* | 91 | 88 | 80 | 91 | 0 | 0 | 0 |
| *5q* | 92 | 90 | 80 | 93 | 0 | 0 | 0 |
| *5r* | 93 | 90 | 83 | 90 | 0 | 0 | 0 |
| *5s* | 76 | 71 | 70 | 80 | 0 | 0 | 0 |
| *5t* | 6 | 2 | 20 | 8 | 0 | 0 | 0 |
| *5u* | 14 | 57 | 46 | 78 | 0 | 0 | 0 |
| *5v* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

a) [Ester]=20mmol/l; [Triethylamine]=20mmol/l

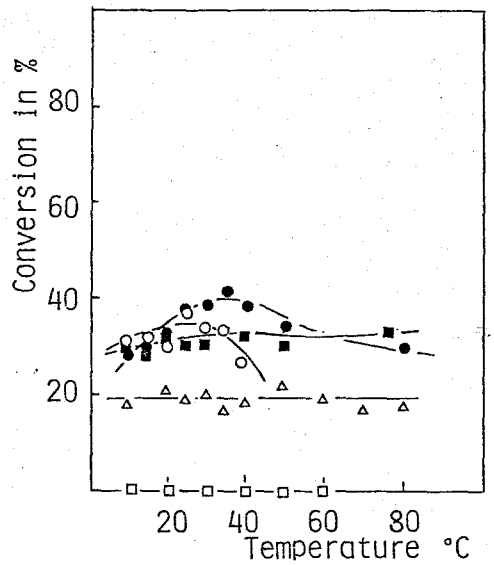



Figure II-1, Effect of temperature on the polycondensation of *5a*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5a*]=20mmole/1, [Triethylamine]=20mmole/1; reaction time 24h

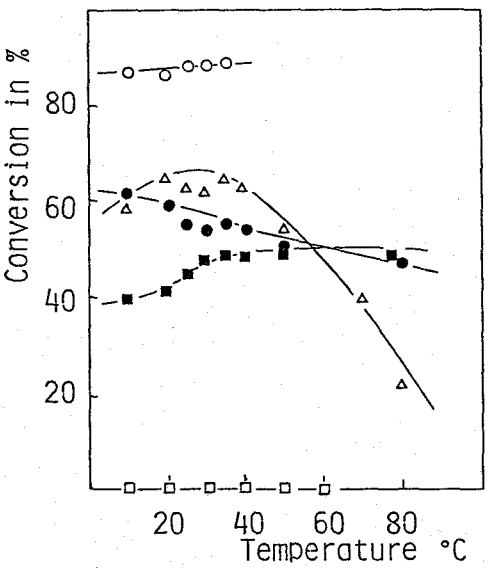



Figure II-2, Effect of temperature on the polycondensation of *5b*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5b*]=20mmole/1, [Triethylamine]=20mmole/1; reaction time 24h

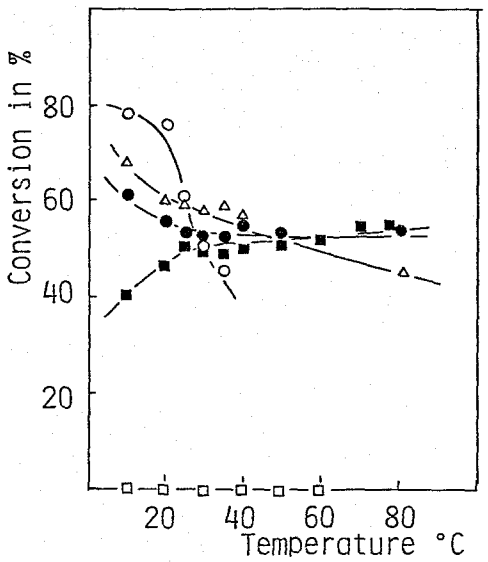



Figure II-3, Effect of temperature on the polycondensation of *5c*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5c*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

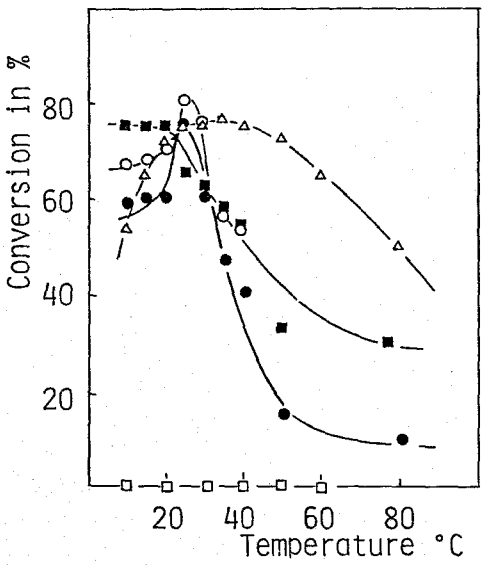



Figure II-4, Effect of temperature on the polycondensation of *5d*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5d*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

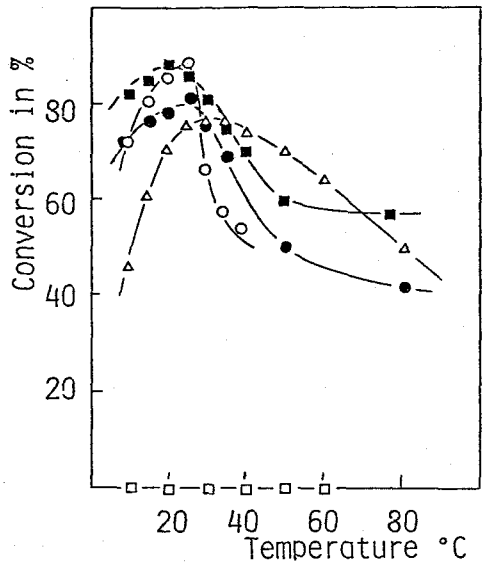



Figure II-5, Effect of temperature on the polycondensation of *5e*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5e*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

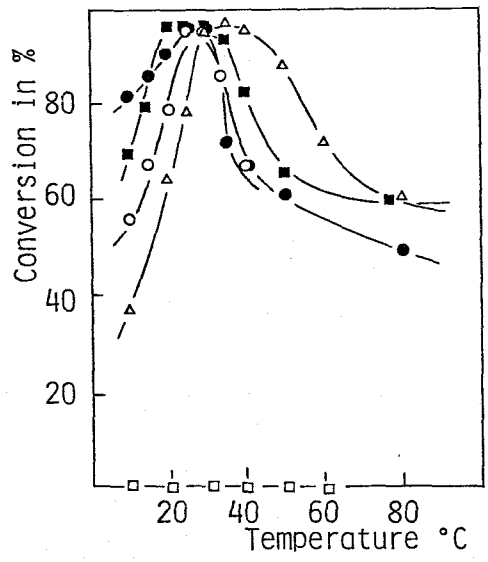



Figure II-6, Effect of temperature on the polycondensation of *5f*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5f*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

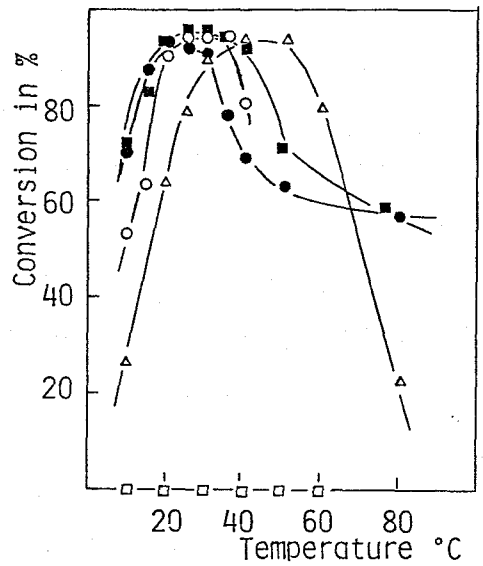



Figure II-7, Effect of temperature on the polycondensation of *5g*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5g*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

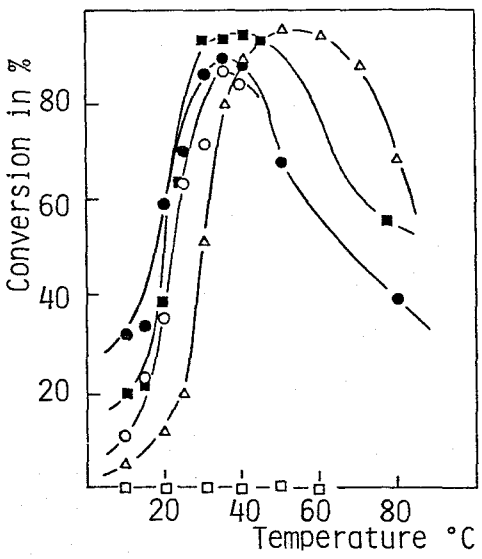



Figure II-8, Effect of temperature on the polycondensation of *5h*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5h*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

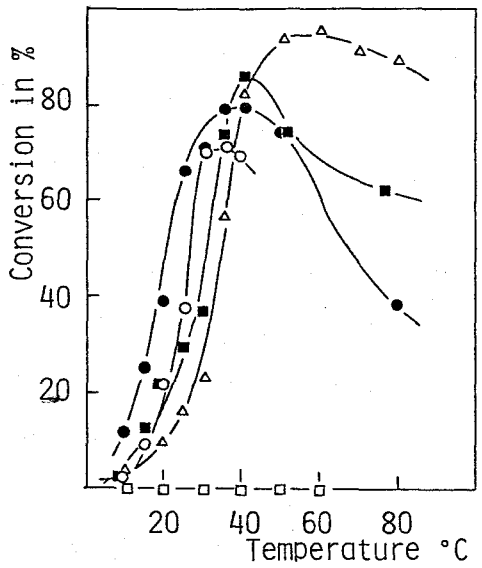



Figure II-9, Effect of temperature on the polycondensation of *5i*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5i*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

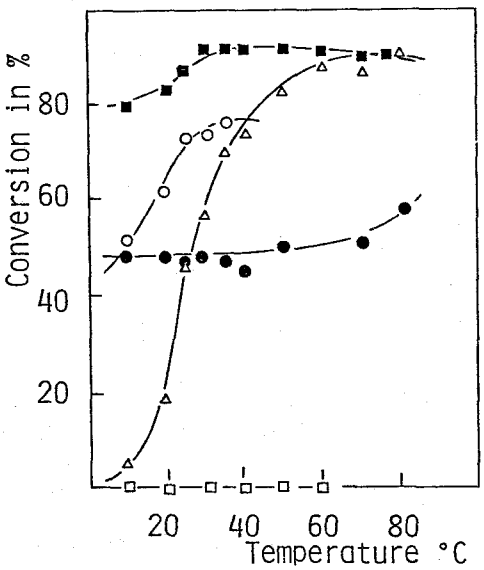



Figure II-10, Effect of temperature on the polycondensation of *5j*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5j*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

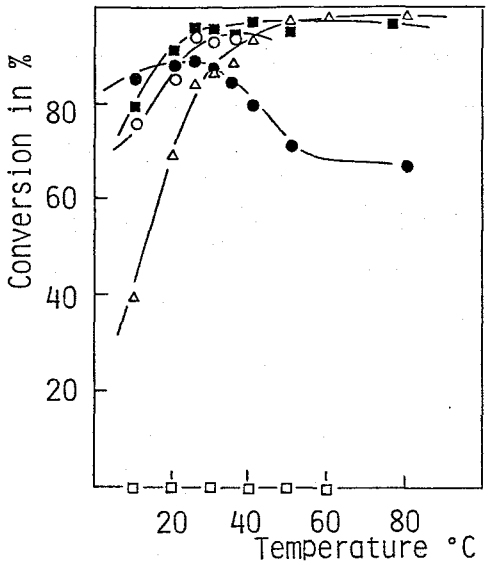



Figure II-11, Effect of temperature on the polycondensation of *5k*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5k*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

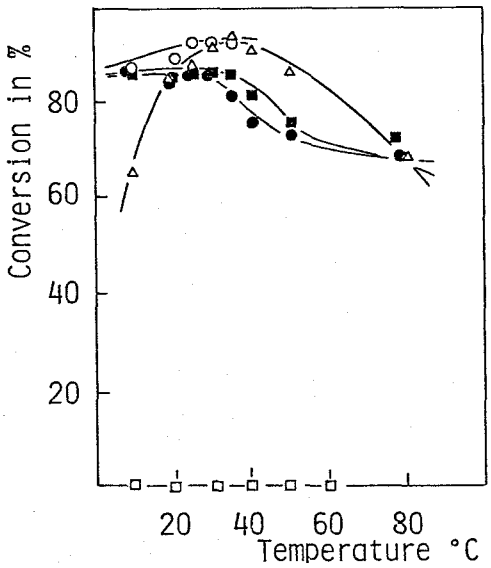



Figure II-12, Effect of temperature on the polycondensation of *5l*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5l*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

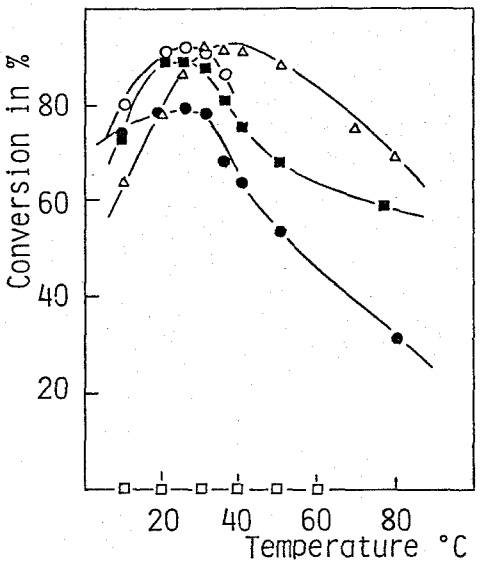



Figure II-13, Effect of temperature on the polycondensation of *5m*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5m*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

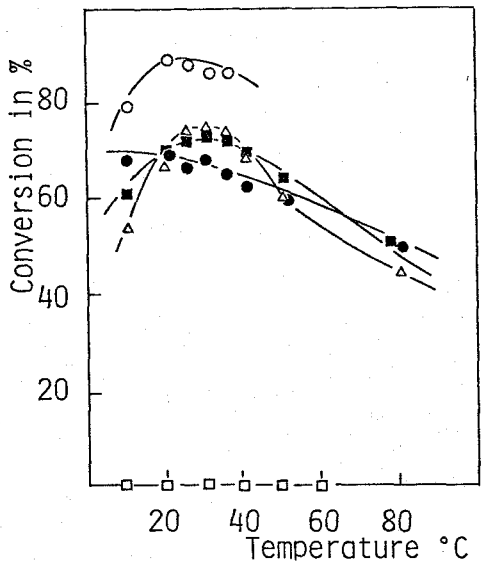



Figure II-14, Effect of temperature on the polycondensation of *5n*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●): benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5n*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

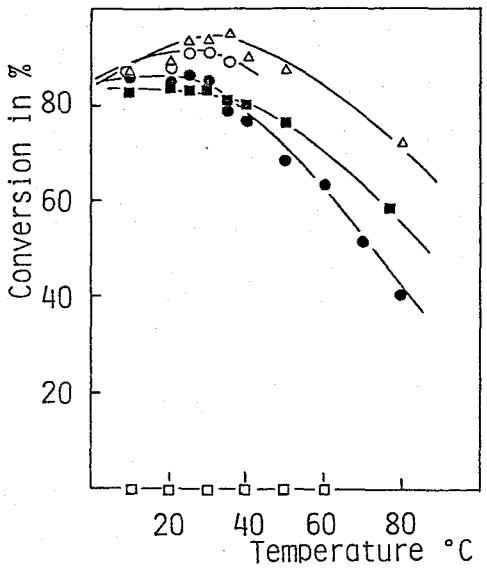



Figure II-15, Effect of temperature on the polycondensation of *5o*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5o*]=20mmole/1, [Triethylamine]=20mmole/1; reaction time 24h

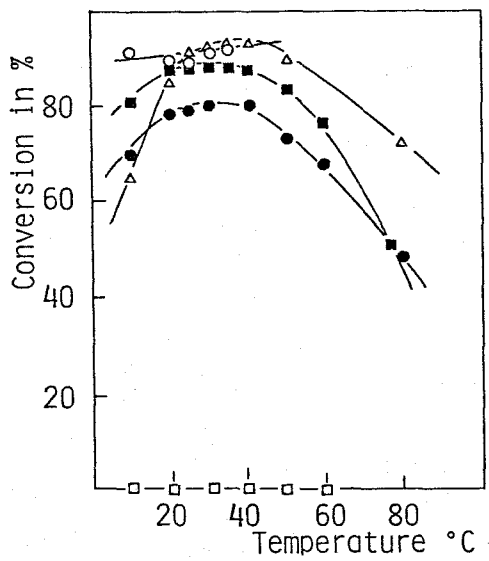



Figure II-16, Effect of temperature on the polycondensation of *5p*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5p*]=20mmole/1, [Triethylamine]=20mmole/1; reaction time 24h

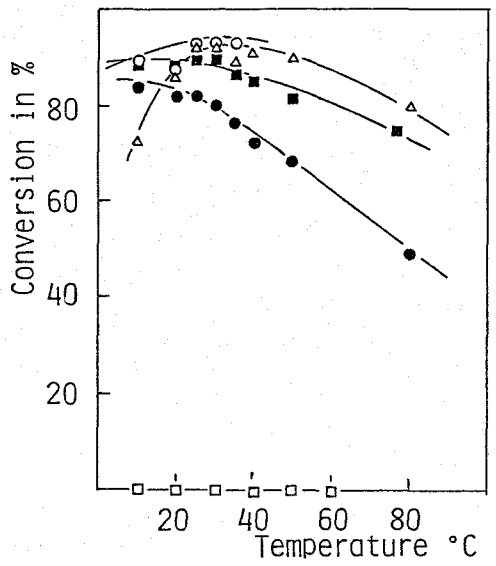



Figure II-17, Effect of temperature on the polycondensation of *5q*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5q*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

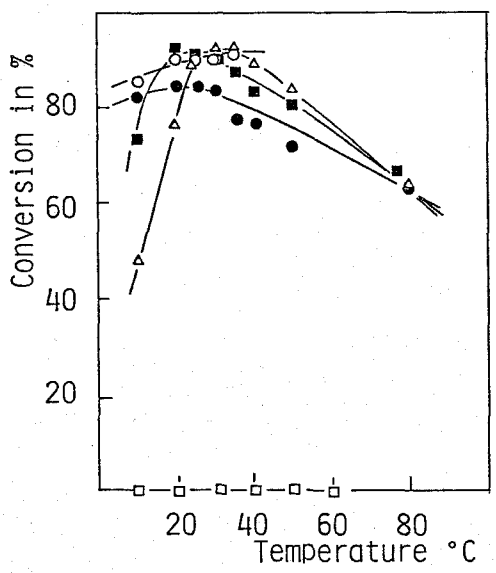



Figure II-18, Effect of temperature on the polycondensation of *5r*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5r*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

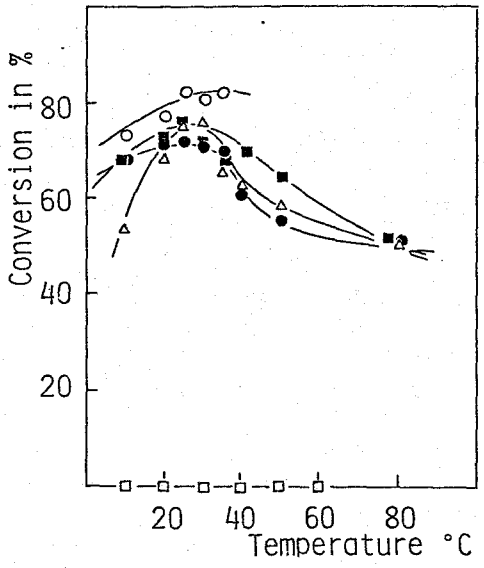



Figure II-19, Effect of temperature on the polycondensation of *5s*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5s*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

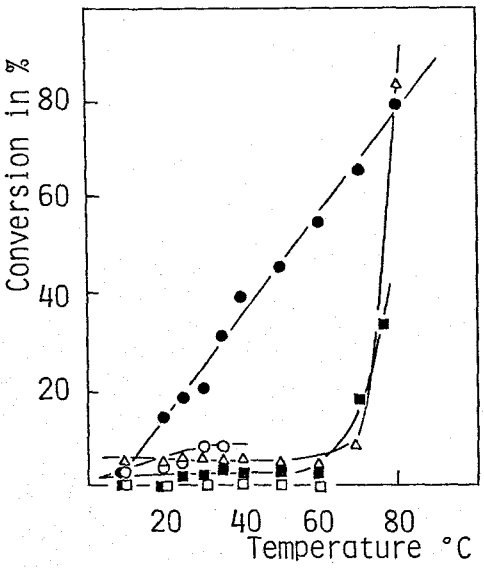



Figure II-20, Effect of temperature on the polycondensation of *5t*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5t*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

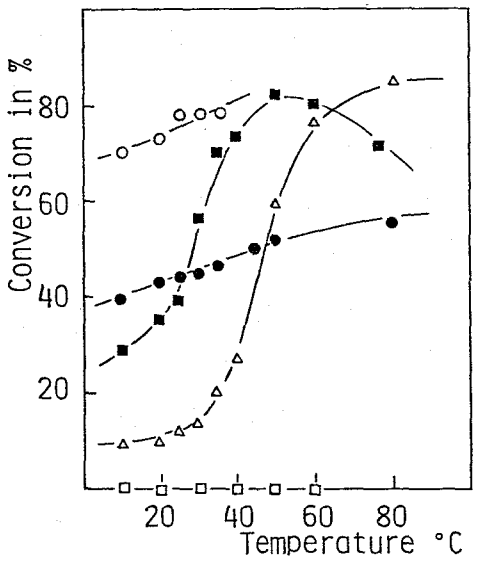



Figure II-21, Effect of temperature on the polycondensation of *5u*; relationship between the conversion and the temperature. (△):cyclohexane; (■): carbon tetrachloride; (●):benzene; (○):diethyl ether; (□):methanol, DMF, DMSO; [*5u*]=20mmole/l, [Triethylamine]=20mmole/l; reaction time 24h

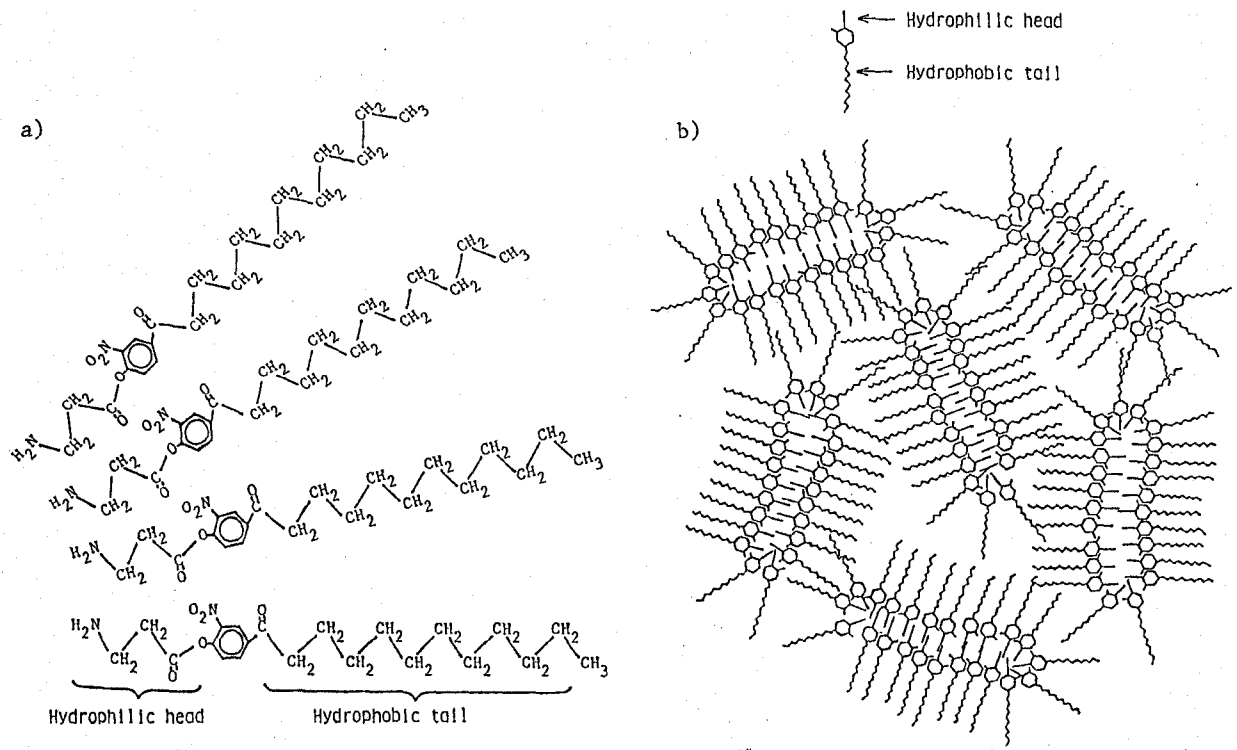



Figure II-22 Schematic illustration of polycondensation field formed by reversed micellar surface
a) Microscopic presentation
b) Macroscopic presentation

The degree (DP) and the conversion of polycondensation in diethyl ether are summarized in Table II-2. The conversion calculated for β-alanine units was in general 100% for the chloroform-insoluble polymers (*7*), which suggests the presence of the ester residue at the polymer chain end. The degree of polycondensation (DP) was calculated from the relative intensity of NMR absorption at 3.8 ppm (β-$CH_2$— in the iminoethylenecarbonyl unit) to that at 1.3 ppm (—$CH_2$— in 4 to (n-1) positions of the acyl group in the 4-acyl-2-nitrophenyl residue; in the case of *5a* 3.0 ppm of the methyl group). From Table II-2 it can be seen that the conversion as well as the DP increase with increasing acyl chain length in the esters.

Table II-2 Results of the polycondensations of the β-alanine esters *5a,d-i* in diethyl ether solution at 25°C, for 24h[a)]

| Ester | Conversion in % for the[b)] | | DP[b)c)] |
|---|---|---|---|
| | chloroform insoluble part | water insoluble part | |
| *5a* | 110 | 39 (37) | 12 |
| *5d* | 120 | 80 (77) | 16 |
| *5e* | 127 | 88 (81) | 16 |
| *5f* | 114 (117) | 97 (95) | 33 (32) |
| *5g* | 110 (113) | 94 (93) | 74 (72) |
| *5h* | 89 | 63 (64) | 96 |
| *5i* | 94 | 35 (33) | 39 |

a) [Ester]=20mmol/l;[Triethylamine]=20mmol/l.
b) Values in parentheses are those obtained from the second run.
c) Calculated from the NMR spectrum of the chloroform-insoluble part.

Figure II-23 shows typical $^1H$ NMR spectra of water and chloroform insoluble polymers obtained from *5g* ((a) and (b), respectively), together with that of authentic poly-β-alanine ((c)). The spectral analysis revealed that the 4-dodecanoyl-2-nitrophenyl group remains still at the end of the polymer chain in the case of the chloroform insoluble part ( Figure II-23 (b)).

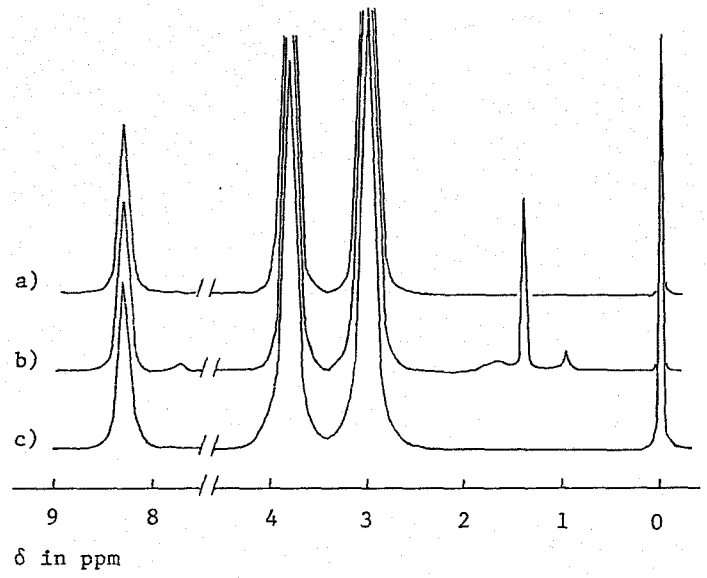



Figure II-23. $^{1}H$ NMR spectra for poly(β-alanine) from the polycondensation of *5g* in diethyl ether at 30°C (measured in trifluoroacetic acid).
a) Water in soluble part after alkali treatment,
b) Chloroform insoluble part before alkali treatment,
c) Authentic poly(β-alanine).

Solvent effects of the polycondensation for *5g* are summarized in Table II-3. In a variety of ethers used here, the reaction proceeds in good conversion on diethyl ether, while the conversion was low in 1,2-dimethoxyethane and no polymer was formed in diglyme solution. This seems to indicate that the solvents having over two ether bonds may interact with the amino group of the active ester through hydrogen bonding, which disturbs the formation of the aggregation. Other cyclic and aromatic ethers such as tetrahydrofuran, dioxane and anisole appear not to be suitable solvents for the polycondensation. Aromatic hydrocarbons such as benzene, toluene and xylene were suitable, while chlorinated benzenes were not, probably due to their large polarity. In the case of aliphatic halides, nonpolar carbon tetrachloride was found to be more effective, but the other polar solvents were not. As for nonpolar aliphatic hydrocarbons, the polycondensation took place quite readily, and particularly good yield was attained in cyclohexane or cyclooctane. In polar solvents such as alcohols, dimethyl sulfoxide, N,N-dimethylformamide and pyridine, no polymer formation was observed as well as in the case of ketone and esters.

Table II-3 Results of the polycondensation of *5g* in different solvents at 30°C for 24h[a)]

| Solvent | Conversion in %[b)] | Solvent | Conversion in % |
|---|---|---|---|
| Diethyl Ether | 94 (93) | n-Pentane | 78 (81) |
| 1,2-Dimethoxyethane | 53 (41) | n-Hexane | 83 (85) |
| Diglyme | 0 (0) | Cyclohexane | 90 (91) |
| Tetrahydrofuran | 19 (19) | n-Heptane | 79 (84) |
| 1,4-Dioxane | 8 (15) | n-Octane | 86 (88) |
| Anisole | 60 (54) | Cyclooctane | 96 (93) |
| | | Isooctane | 78 (81) |
| Benzene | 92 (91) | n-Decane | 87 (85) |
| Toluene | 92 (90) | n-Pentadecane | 85 (87) |
| *o*-Xylene | 91 (93) | | |
| *m*-Xylene | 92 (90) | Methanol | 0 (0) |
| *p*-Xylene | 93 (91) | Ethylene Glycol | 0 (0) |
| *o,m,p*-Xylene[c)] | 92 (93) | 1- Dodecanol | 0 (0) |
| Chlorobenzene | 79 (75) | Cyclohexanone | 0 (0) |
| *o*-Dichlorobenzene | 76 (75) | Acetone | 25 (21) |
| *o*-Dichlorobenzene + *p*-Dichlorobenzene[d)] | 80 (83) | Ethyl Acetate | 55 (46) |
| | | Acetonitrile | 0 (0) |
| Carbon Tetrachloride | 95 96) | Pyridine | 0 (0) |
| Chloroform | 30 (37) | Dimethyl Sulfoxide | 0 (0) |
| Dichloromethane | 36 (25) | N,N-Dimethylformamide | 0 (0) |
| 1,2-Dichloroethane | 45 (41) | | |
| Tetrachloroethane | 8 (11) | | |

a) [*5g*]=20mmol/l; [Triethylamine]=20mmol/l
b) Conversion in % for the water insoluble part
Values in parentheses are those obtained in the second run.
c) The volume ratio of *o*- : *m*- : *p*- is 1 : 1 : 1.
d) The volume ratio of *o*- : *m*- is 1 : 1.

The results of the solvent effect appear to suggest that the less the affinity of the esters to the solvent becomes with decreasing in its dielectric constant, the more the esters aggregate, which favors the polymer formation.

Based on the result of high conversions observed in the aprotic solvents such as diethyl ether or carbon tetrachloride, the mixed solvent systems provided some insight on the properties of the aprotic solvent systems. Table II-4 shows the effect of adding methanol to carbon tetrachloride on the polycondensation. Presence of 0.5 % of methanol in carbon tetrachloride reduced the conversion to 73 %, and addition of methanol over 1 % inhibited completely the polycondensation. A similar tendency was observed also for diethyl ether-methanol system (Table II-5). On the other hand, the polycondensation still proceeded smoothly in the mixed solvent system such as carbon tetrachloride-diethyl ether, regardless of their mixing ratios (Table II-6). From these results it can be well assumed that addition of a small amount of methanol seems to be enough to disturb forming the aggregation of active ester in nonpolar aprotic solvents. It can also be pointed out that the polycondensation is liable to proceed at least in nonpolar aprotic solvents which avoid the solvation towards the esters.

Table II-4 Results of the polycondensation of *5g* in the mixed solvent system containing carbon tetrachloride and methanol at 30°C for 24h[a)]

| Mixed solvent | | Conversion in % for |
|---|---|---|
| $CCl_4$ (%) | + MeOH (%) | the water insoluble part |
| 100 | 0 | 95 |
| 99.5 | 0.5 | 73 |
| 99 | 1 | trace |
| 98 | 2 | 0 |
| 95 | 5 | 0 |
| 90 | 10 | 0 |

a) [*5g*]=20mmol/l; [Triethylamine]=20mmol/l

Table II-5 Results of the polycondensation of *5g* in the mixed solvent system containing diethyl ether and methanol at 30°C for 24h[a)]

| Mixed solvent | | Conversion in % for |
|---|---|---|
| Ether (%) | + MeOH (%) | the water insoluble part |
| 100 | 0 | 94 |
| 99 | 1 | 75 |
| 98 | 2 | 38 |
| 97 | 3 | 5 |
| 96 | 4 | trace |
| 95 | 5 | 0 |
| 90 | 10 | 0 |

a) [*5g*]=20mmol/l; [Triethylamine]=20mmol/l

Table II-6 Results of the polycondensation of *5g* in the mixed solvent system containing carbon tetrachloride and diethyl ether at 30°C for 24h[a)]

| Mixed solvent | | Conversion in % for |
|---|---|---|
| $CCl_4$ (%) | + Ether (%) | the water insoluble part |
| 100 | 0 | 95 |
| 75 | 25 | 94 |
| 50 | 50 | 93 |
| 25 | 75 | 94 |
| 0 | 100 | 94 |

a) [*5g*]=20mmol/l; [Triethylamine]=20mmol/l

The effect of a variety of amines on the polycondensation was next studied for the case of *5g* in diethyl ether solution. Table II-7 shows that the addition of triethylamine leads to high conversion as compared with that of imidazole and N-methylmorpholine, whereas pyridine was found to inhibit the polycondensation completely.

Table II-7 Effect of amines in the polycondensation of *5g* in diethyl ether at 30°C for 24h[a)]

| Amine | Conversion in % for the water insoluble part |
|---|---|
| Triethylamine | 94 |
| Imidazole | 37 |
| N-Methylmorpholine | 9 |
| Pyridine | 0 |

a) [*5g*]=20mmol/l; [Amine]=20mmol/l

A mole ratio 1:1 of triethylamine/*5g* was enough to perform the polycondensation in a good yield (Figure II-24), which attains to completion almost within 24h (Figure II-25). From Figure II-26, it is clear that the highest conversion is reached at [*5g*]=1.0mmole/l.

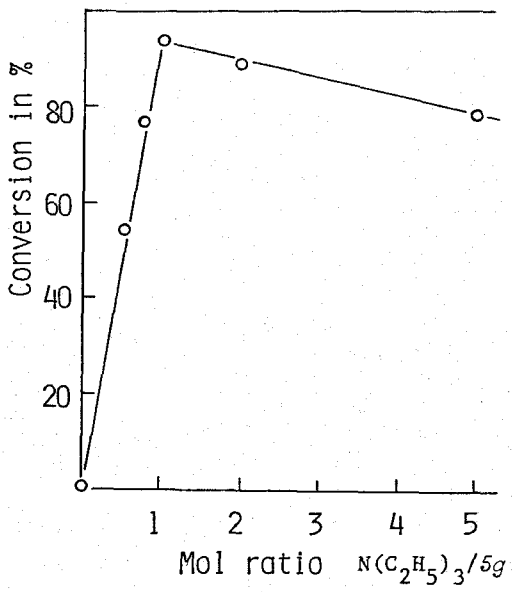



Figure II-24, Effect of triethylamine on the polycondensation of *5g*; relationship between the conversion and the amount of triethylamine; [*5g*]=20mmole/l; 30°C for 24h

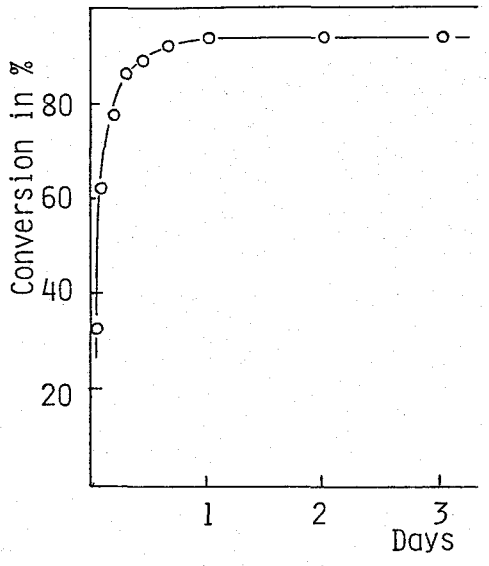



Figure II-25, Conversion of the water insoluble part as a function of the reaction time at 30°C; [*5g*]=20mmole/l; [Triethylamine]=20mmole/l

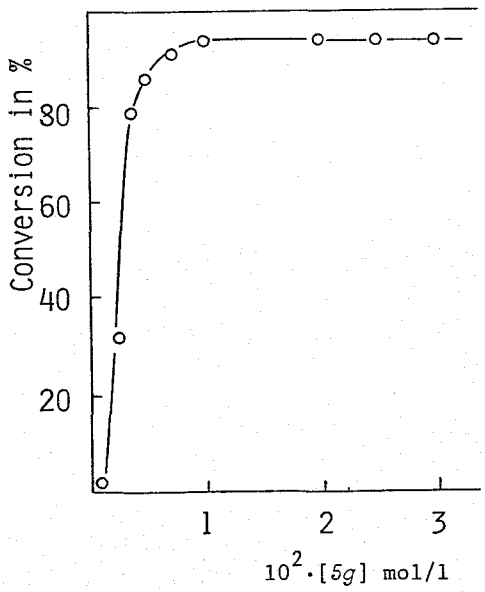



Figure II-26, Conversion of the water insoluble part as a function of the concentration of *5g*; 30°C for 24h; (mole ratio of *5g* to triethylamine is 1:1)

## II - 3. Experimental Part

The procedure used for the polycondesation of active esters is described in detail for β-alanine 4-dodecanoyl-2-nitrophenyl ester (*5g*).

*Polycondensation of 5g*: To 50ml of diethyl ether solution containing 473mg (1mmole) of *5g* were added 0.14ml (1mmole) of triethylamine. After stirring for 24h at 25°C, the solvent was evaporated. The residue was then treated and 50ml of hot chloroform were added. The chloroform-insoluble part was filtered off and washed twice with hot chloroform to remove 4-dodecanoyl-2-nitrophenol and triethylamine hydrobromide. Conversion:78mg (110%). The polymer was obtained as colorless powder of m.p. 320-325°C.

*Elimination of the 4-dodecanoyl-2-nitrophenolate group*: The polymer obtained (78mg) was mixed with aqueous 4 M NaOH (20ml) and stirred for 10 min on a water bath. After cooling, the mixture was neutralized by HCl, and chloroform (20ml) was added therein. The insoluble matter was filtered off and dried. Colorless powder; m.p. 330-335°C. Conversion; 94%.

## II - 4. Summary

The polycondensation of a series of active β-alanine 4- dodecanoyl-2-nitrophenyl esters with different structure and length of acyl groups was studied in various solvents. The polycondensation reaction of these esters by triethylamine at a given temperature show that the conversion and the degree of polycondensation of the resulting poly(β-alanine) depend on the chain length and structure of the acyl groups. No polymer was obtained in both protic and dipolar aprotic solvents such as methanol, DMF and DMSO, whereas nonpolar solvents such as diethyl ether, carbon tetrachloride, benzene or cyclohexane were found to be suitable for the polycondensation which was inhibited by the presence of a small amount of methanol. The effect of a variety of amines on the polycondesation was examined for β-alanine 4-dodecanoyl-2-nitrophenyl ester in diethyl ether solution. The temperature affected also the reaction which proceeded preferentially at near 30°C and was retarded over 30°C. The result appears to be explained by the aggregation of the active esters. It was suggested that the aggregation would play an important role in the polycondensation.

II - 5. References


[1] Y.Kawabata, M.Kinoshita, *Makromol.Chem.*, 176, 3243 (1975)

[2] C.M.Paleos, P.Dais, *J.Polymer Sci. Polym.Chem.Ed.* 16, 1495 (1978)

[3] Y.B.Amerik, B.A.Krentsel, *J.Polmer Sci.*, *(C)* 16, 1383 (1967)

[4] C.M.Paleos, T.M.Laronge, M.M.Labes, *Chem.Commun.*, 1968, 1115

[5] W.J.Toth, A.V.Tobolsky, *J. Polymer Sci.*, *(B)* 8, 289 (1970)

[6] A.C.du Visser, J.Feyen, K.de Groot, A.Bantjes, *J.Polymer Sci.*, *(B)* 8, 805 (1970); ibid. 10, 261 (1972)

[7] Y.Tanaka, S.Kabayama, Y.Shimura, A.Okada, Y.Kurihara, Y.Sakakibara *J.Polmer Sci.*, *(B)* 10, 261 (1972)

[8] E.Perplies, H.Ringsdorf, J.H.Wendorff, *Ber. Bunsenges.Phys.Chem.* 78, 921 (1974)

[9] C.M.Paleos, M.M.Labes, *Mol.Cryst.Liq.Cryst.*, 11, 385 (1970)

[10] L.Strzelecki, L.Liebert, *Bull.Soc.Chim.Fr.*, 2, 592 (1973)

[11] E.Perplies, H. Ringsdorf, J.H.Wendorff, *J.Polymer Sci.*,*(B)* 13, 243 (1975)

[12] E.Perplies, H.Ringsdorf, J.H.Wendorff, *Makromol.Chem.*, 175, 553 (1974)

[13] K.Sugiyama, T.Nakaya, *Makromol.Chem.*, 178, 271 (1977)

[14] A.Baniel, M.Frankel, I.Friedlich, A.Katchalsky, *J.Org.Chem.*, 13, 791 (1948)

[15] H.Reerink, *J.Collid Sic.*, 20, 217 (1965)

[16] J.B.Peri, *J.Am.Oil Chemists' Soc.*, 35, 110 (1958)

[17] A.Kitahara, *Bull.Chem.Soc.Japan*, 29, 15 (1956)

## Chapter III. Polycondensation of α-Amino acid 4-Acyl-2-nitrophenyl Esters

### III - 1. Introduction

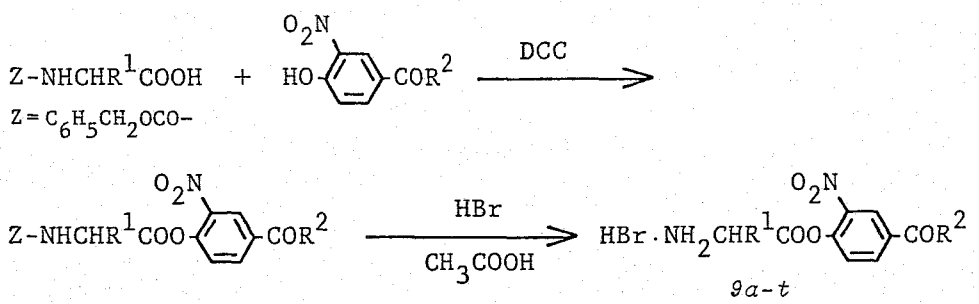
In the previous chapter, the author described the synthesis of poly(β-alanine) on the reversed micellar surface derived from β-alanine 4-acyl-2-nitrophenyl esters. The results were interpreted in terms of the aggregation of the active esters in some nonpolar solvents, which was expected particularly for the longer chain acyl groups.

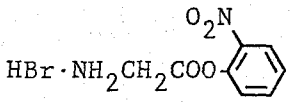
On the other hand, the synthesis of poly(α-amino acid) in oriented organic aggregate were still unsuccessful, because of unfavorable formation of the cyclic dipeptide "2,5-piperazinedione" than that of linear product "poly(α-amino acid)" [1,2].

This chapter is concerned with the preparation and polycondensation of α-amino acid 4-acyl-2-nitrophenyl esters. The reaction of these active esters brings about the intramolecular aromatic nucleophilic rearrangement as well as intermolecular nucleophilic condensation.

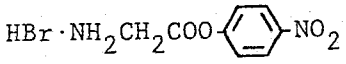
### III - 2. Results and Discussion

A series of α-amino acid esters (*9a-v*) were prepared by the reaction of 4-acyl-2-nitrophenol, 2-nitrophenol or 4-nitrophenol with N-benzyloxycarbonyl α-amino acid ( glycine, L-alanine or L-phenylalanine ) in the presence of DCC and further converted into their hydrobromide salts which were used for the polycondensation as shown below.

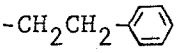
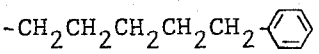
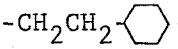
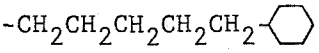
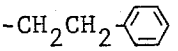
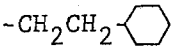
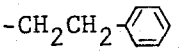
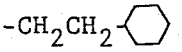




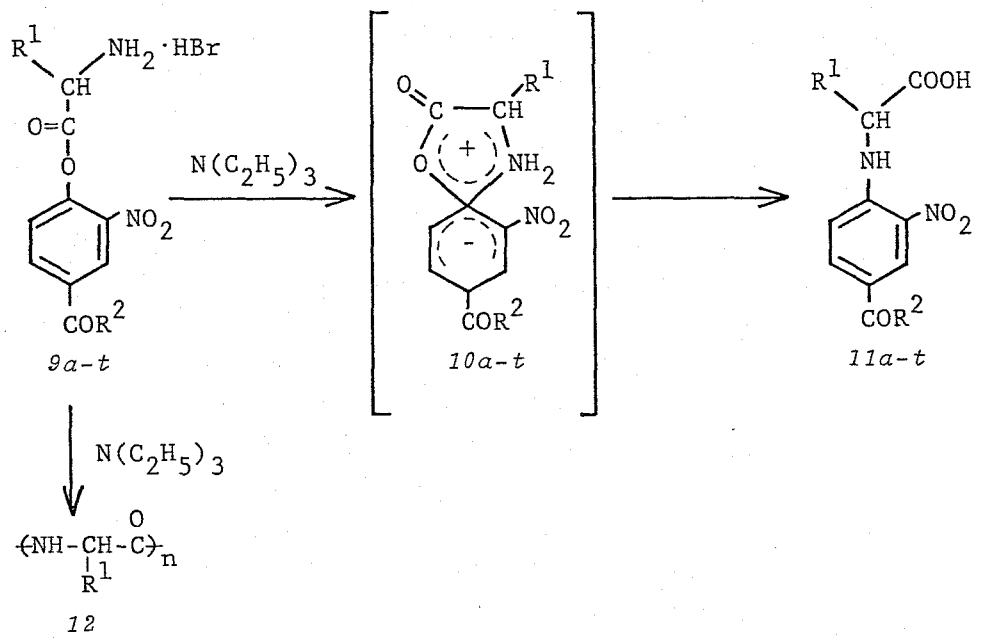
*9u*



*9v*

| Symbol | $-R^1$ | $-R^2$ |
|---|---|---|
| *a* | -H | $-CH_3$ |
| *b* | -H | $-C_5H_{11}$ |
| *c* | -H | $-C_{11}H_{23}$ |
| *d* | -H | $-C_{15}H_{31}$ |
| *e* | -H |  |
| *f* | -H |  |
| *g* | -H |  |
| *h* | -H |  |
| *i* | -H |  |
| *j* | -H |  |
| *k* | $-CH_3$ | $-C_{11}H_{23}$ |
| *l* | $-CH_3$ |  |
| *m* | $-CH_3$ |  |
| *n* | $-CH_3$ |  |
| *o* | $-CH_3$ |  |
| *p* |  | $-C_{11}H_{23}$ |
| *q* |  |  |
| *r* |  |  |
| *s* |  |  |
| *t* |  |  |

All the polycondensation were carried out in the concentration of 2.0 x $10^{-2}$M. When the active esters (*9a-v*) were treated with an equimolar amount of triethylamine in various solvents at 30°C for 24h, poly(α-amino acid) (*12*) and N-(4-acyl-2-nitrophenyl)α-amino acid (*11*) were formed.




As described in the preceding chapter, the polycondensation of β-alanine 4-acyl-2-nitrophenyl esters gave only poly(β-alanine) as the main product, whereas α-alanine 4-acyl-2-nitrophenyl esters (*9a-t*) gave under the same condition a by-product in significant amount together with poly(α-amino acid) (*12*), and this by-product was found to be N-(4-dodecanoyl-2-nitrophenyl)α-amino acid (*11a-t*).

Interestingly, the by-product was observed only in the reaction of α-amino acid 4-acyl-2-nitrophenyl esters but not in that of β-alanine active esters (cf. Chapter II). It was probably formed by an intramolecular nucleophilic rearrangement reaction of *9a-t* via its Meisenheimer complex (*10a-t*). The similar reactions have been observed in the aromatic nucleophilic substitution of nitro-activated aryl halides by amines, hydroxide ion or alkoxide ion [3-9].

The results of the polycondensation of glycine active esters (*9a-j,u,v*) are summarized in Table III-1. The conversion of by-product was high in polar solvents such as methanol or diethyl ether, particularly in methanol the intramolecular rearrangement reaction took place quantitatively. Nonpolar solvents such as cyclohexane, carbon tetrachloride and benzene retard somewhat the formation of by-product and give a certain amount of poly(glycine). The formation of poly(glycine) (*12*) from glycine active esters (*9a-j*) seems to be affected by the chain length or structure of the acyl group. In general, the conversion was found to be low in *9a*, *9e* and *9h* as compared with *9b*, *9c*, *9f*, *9g*, *9i* and *9j*, due to the decrease of the reversed micellar association. On the other hand, glycine 2-nitrophenyl ester (*9u*) gave a small amount of the rearrangement by-product and glycine 4-nitrophenyl ester (*9v*) gave no by-product. This fact indicates that the activation of esters for the aromatic nucleophilic intramolecular rearrangement reaction increases in the order of glycine 4-acyl-2-nitrophenyl ester (*9a-j*) $>$ glycine 2-nitrophenyl ester (*9u*) $\gg$ glycine 4-nitrophenyl ester (*9v*).

The results of the polycondensation of L-alanine and L-phenylalanine active esters (*9k-t*) are summarized in Table III-2. In the case of L-alanine and L-phenylalanine 4-acyl-2-nitrophenyl esters (*9k-t*), the intramolecular reaction appears to be more favorable than that of glycine 4-acyl-2-nitrophenyl ester (*9a-j*). This finding suggests that the presence of the substituents at α-carbon atom seems to stabilize the intermediate Meisenheimer complex (*10k-t*), leading to the N-(4-acyl-2-nitrophenyl)substituted amino acids (*11k-t*).

Table III-1 Results of the polycondensation of glycine active esters (*9a-j,u,v*) at 30°C for 24h.[a)]

| Ester | Conversion in % of *11* and *12* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cyclohexane | | $CCl_4$ | | Benzene | | Ether | | MeOH | |
| | *11*[b)] | *12*[c)] | *11*[b)] | *12*[c)] | *11*[b)] | *12*[c)] | *11*[b)] | *12*[c)] | *11*[b)] | *12*[c)] |
| *9a* | 84 | 13(11) | 79 | 20(18) | 84 | 11(8) | 82 | 11(10) | 99 | 0(0) |
| *9b* | 50 | 40(37) | 61 | 30(29) | 80 | 7(10) | 65 | 33(35) | 99 | 0(0) |
| *9c* | 61 | 16(19) | 53 | 39(37) | 82 | 11(11) | 82 | 13(11) | 100 | 0(0) |
| *9d* | 85 | 14(15) | 79 | 16(19) | 80 | 17(15) | 91 | 4(4) | 100 | 0(0) |
| *9e* | 89 | 2(4) | 62 | 12(15) | 83 | 5(8) | 88 | 6(5) | 100 | 0(0) |
| *9f* | 84 | 1(1) | 72 | 25(18) | 77 | 11(10) | 91 | 6(7) | 100 | 0(0) |
| *9g* | 78 | 2(4) | 49 | 39(33) | 58 | 21(19) | 78 | 4(4) | 99 | 0(0) |
| *9h* | 91 | 2(1) | 58 | 13(15) | 70 | 8(6) | 95 | 1(2) | 100 | 0(0) |
| *9i* | 53 | 34(35) | 31 | 62(67) | 51 | 7(6) | 89 | 8(11) | 100 | 0(0) |
| *9j* | 72 | 23(23) | 42 | 56(54) | 76 | 16(11) | 95 | 3(5) | 99 | 0(0) |
| *9u* | 30 | 35(33) | 30 | 60(57) | 25 | 16(15) | 23 | 55(53) | 58 | 0(0) |
| *9v* | 0 | 68(67) | 0 | 73(71) | 0 | 27(28) | 0 | 55(52) | 0 | 0(0) |

a) [Ester]=20mmole/l; [Triethylamine]=20mmole/l
b) Determined by HPLC.
c) Conversion in % for the water insoluble part.
Values in parentheses are those obtained from the second run.

Table III-2 Results of the polycondensation of L-alanine and L-phenylalanine 4-acyl-2-nitrophenyl esters (*9k-t*) at 30°C for 24h[a)]

| Ester | Conversion in % of *11*[b)] | | | | |
|---|---|---|---|---|---|
| | Cyclohexane | $CCl_4$ | Benzene | Ether | MeOH |
| *9k* | 56 | 42 | 70 | 92 | 99 |
| *9l* | 70 | 61 | 78 | 92 | 100 |
| *9m* | 97 | 84 | 96 | 99 | 99 |
| *9n* | 96 | 83 | 84 | 90 | 99 |
| *9o* | 86 | 80 | 83 | 98 | 100 |
| *9p* | 83 | 70 | 73 | 96 | 99 |
| *9q* | 85 | 53 | 81 | 95 | 100 |
| *9r* | 87 | 47 | 78 | 90 | 100 |
| *9s* | 68 | 43 | 77 | 95 | 100 |
| *9t* | 90 | 79 | 89 | 99 | 100 |

a) [Ester]=20mmole/l; [Triethylamine]=20mmole/l
b) Determined by HPLC

III - 3. Experimental Part

*Materials and Polycondensation*

The preparation and polycondensation of α-amino acid esters ( *9a-v* ) were carried out by the similar method described in the chapter I. The analytical data for α-amino acid esters (*9a-v*) are given in Table III-3. *11a-t* were purified in the form of their methyl esters because of its difficult isolation from 4-acyl-2-nitrophenol.

*N-(4-Dodecanoyl-2-nitrophenyl)glycine methyl ester;* 459mg (1mmole) of *9c* was mixed with 0.14ml (1mmole) of triethylamine in 25ml of benzene. After 24h at 30°C, the mixture was evaporated, 50ml of 4M aqueous sodium hydroxide solution was added to the residue to hydrolyze the terminal ester bond of poly(N-glycine) (*12*). After neutralyzing by 4M aqueous hydrochloric acid followed by the addition of 10ml of methanol and water, *12* was filtered off, washed with methanol and water, and dried in vacuo. The filtrate which contains 4-dodecanoyl-2-nitrophenol and *11c*, was evaporated and the residue was treated with 20ml of acetone. After the insoluble triethylamine hydrobromide salt was removed by filtration, the filtrate was evaporated in vacuo and the residue was dissolved in 20ml of methanol, further treated with hydrogen chloride gas at room temperature for 3h. The precipitate was filtered and recrystallized from methanol. M.p., $^{1}$H NMR and elemental analysis are given in Table III-4.

In a similar way, the other methyl esters (*11a*, *11b*, *11k* and *11p*) were derived. The analytical data are given in Table III-4. The conversion of *9a-v* into *11a-v* was determined indirectly by HPLC analysis of 4-acyl-2-nitrophenol liberated in the polycondensation reaction. A Varian Aerograph 4200 liquid chromatograph equipped with the column Micro PAK Si-10 (50cm in length, 2mm in diameter) was used for HPLC analysis which was carried out in benzene mobile phase (flow rate; 80ml/h) monitored by a UV detector (350 nm).

Table III-3 Anayltical data of the α-amino acid active esters (*9a-v*)

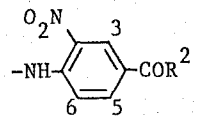
| Ester | Yield % | Melting Point °C (dec.) | Formula | Analyses,% Calcd. C | H | N | Br | Found C | H | N | Br |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *9a* | 85 | 156-157 | $C_{10}H_{11}N_2O_5Br$ | 37.64 | 3.47 | 8.78 | 25.04 | 37.51 | 3.50 | 8.81 | 25.32 |
| *9b* | 85 | 156-157 | $C_{14}H_{19}N_2O_5Br$ | 44.81 | 5.10 | 7.47 | 21.30 | 44.65 | 5.23 | 7.56 | 21.20 |
| *9c* | 86 | 152-153 | $C_{20}H_{31}N_2O_5Br$ | 52.29 | 6.80 | 6.10 | 17.39 | 52.52 | 6.75 | 6.23 | 17.67 |
| *9d* | 85 | 152-153 | $C_{24}H_{39}N_2O_5Br$ | 55.92 | 7.63 | 5.43 | 15.50 | 55.70 | 7.51 | 5.58 | 15.25 |
| *9e* | 81 | 171-172 | $C_{15}H_{13}N_2O_5Br$ | 47.26 | 3.44 | 7.35 | 20.96 | 47.03 | 3.57 | 7.32 | 20.68 |
| *9f* | 86 | 174-175 | $C_{17}H_{17}N_2O_5Br$ | 49.89 | 4.19 | 6.85 | 19.53 | 49.99 | 4.25 | 6.65 | 19.31 |
| *9g* | 87 | 161-162 | $C_{20}H_{23}N_2O_5Br$ | 53.23 | 5.14 | 6.21 | 17.70 | 53.51 | 5.11 | 6.38 | 17.95 |
| *9h* | 83 | 175.5-176.5 | $C_{15}H_{19}N_2O_5Br$ | 46.53 | 4.95 | 7.23 | 20.63 | 46.39 | 4.69 | 7.51 | 20.49 |
| *9i* | 84 | 170-171 | $C_{17}H_{23}N_2O_5Br$ | 49.17 | 5.58 | 6.75 | 19.24 | 49.40 | 5.77 | 6.48 | 19.44 |
| *9j* | 82 | 163.5-164.5 | $C_{20}H_{29}N_2O_5Br$ | 52.52 | 6.39 | 6.13 | 17.47 | 52.29 | 6.54 | 6.28 | 17.64 |
| *9k* | 76 | 155.5-156.5 | $C_{21}H_{33}N_2O_5Br$ | 53.28 | 7.03 | 5.92 | 16.88 | 53.12 | 7.12 | 5.84 | 17.01 |
| *9l* | 71 | 156.5-157.5 | $C_{16}H_{15}N_2O_5Br$ | 48.63 | 3.83 | 7.09 | 20.22 | 48.62 | 3.63 | 7.00 | 20.28 |
| *9m* | 78 | 164-165 | $C_{18}H_{19}N_2O_5Br$ | 51.08 | 4.52 | 6.62 | 18.88 | 51.37 | 4.53 | 6.79 | 18.80 |
| *9n* | 77 | 176.5-177.5 | $C_{16}H_{21}N_2O_5Br$ | 47.89 | 5.28 | 6.98 | 19.91 | 47.78 | 5.04 | 7.11 | 19.86 |
| *9o* | 77 | 173.5-174 | $C_{18}H_{25}N_2O_5Br$ | 50.36 | 5.87 | 6.53 | 18.61 | 50.44 | 5.70 | 6.51 | 18.78 |
| *9p* | 92 | 127-129 | $C_{27}H_{37}N_2O_5Br$ | 59.02 | 6.79 | 5.10 | 14.54 | 58.99 | 6.58 | 4.99 | 14.68 |
| *9q* | 93 | 167-167.5 | $C_{22}H_{19}N_2O_5Br$ | 56.07 | 4.06 | 5.94 | 16.95 | 55.89 | 4.31 | 6.09 | 17.18 |
| *9r* | 86 | 153-154 | $C_{24}H_{23}N_2O_5Br$ | 57.73 | 4.64 | 5.61 | 16.00 | 58.01 | 4.54 | 5.70 | 15.88 |
| *9s* | 90 | 161-162 | $C_{22}H_{25}N_2O_5Br$ | 55.35 | 5.28 | 5.87 | 16.74 | 55.08 | 4.98 | 6.02 | 16.87 |
| *9t* | 85 | 150.5-151.5 | $C_{24}H_{29}N_2O_5Br$ | 57.04 | 5.78 | 5.54 | 15.81 | 56.86 | 5.71 | 5.44 | 15.54 |
| *9u* | 81 | 202-203 | $C_8H_9N_2O_4Br$ | 34.68 | 3.27 | 10.11 | 28.84 | 34.87 | 2.99 | 10.11 | 29.05 |
| *9v* | 87 | 210-211 | $C_8H_9N_2O_4Br$ | 34.68 | 3.27 | 10.11 | 28.84 | 34.69 | 3.33 | 10.40 | 28.79 |

Table III-4 Analytical data of the methyl esters of *11a-c,k,p*

| Methyl ester of | M.p. °C | Formula | Analyses, % | | | $^1$H NMR absorptions δ in p.p.m.[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | Acyl-H | -COOCH$_3$ | -NHCHR$^1$ | -NH- | Phenyl-H[b] 6 | 5 | 3 |
| *11a* | 200 | $C_{11}H_{12}N_2O_5$ | Calcd. | 52.38 | 4.80 | 11.11 | 3.2 | 3.8 | 4.4 | 8.7 | 7.0 | 8.0 | 8.6 |
| | | | Found | 52.11 | 4.55 | 11.16 | | | | | | | |
| *11b* | 89 | $C_{15}H_{20}N_2O_5$ | Calcd. | 58.43 | 6.54 | 9.09 | 0.9-1.6, 3.0 | 3.8 | 4.4 | 8.7 | 7.3 | 8.0 | 8.6 |
| | | | Found | 58.26 | 6.49 | 9.05 | | | | | | | |
| *11c* | 97-98 | $C_{21}H_{32}N_2O_5$ | Calcd. | 64.26 | 8.22 | 7.14 | 0.8-1.6, 2.9 | 3.8 | 4.4 | 8.7 | 7.0 | 8.0 | 8.6 |
| | | | Found | 63.91 | 8.20 | 7.15 | | | | | | | |
| *11k* | 58-59 | $C_{22}H_{34}N_2O_5$ | Calcd. | 65.00 | 8.43 | 6.89 | 0.8-1.6, 2.9 | 3.8 | 4.8 | 8.6 | 7.1 | 8.1 | 8.7 |
| | | | Found | 64.53 | 8.28 | 6.64 | 1.5 (α-CH$_3$) | | | | | | |
| *11p* | 48-49 | $C_{28}H_{38}N_2O_5$ | Calcd. | 69.68 | 7.94 | 5.81 | 0.8-1.6, 2.9 | 3.8 | 5.1 | 8.5 | 7.1 | 8.0 | 8.6 |
| | | | Found | 69.47 | 7.88 | 5.62 | 3.3 ($C_6H_5CH_2$-) | 7.2 ($C_6H_5$-) | | | | | |

a) In $[^2H]_6$-DMSO.

b) The numbers show the position of the protons in the phenyl residue:



## III - 4. Summary

The preparation and polycondensation of α-amino acid 4-acyl-2-nitrophenyl esters was investigated. The reaction of α-amino acid 4-acyl-2-nitrophenyl esters gave a by-product derived from the intramolecular rearrangement via the Meisenheimer complex, besides poly(α-amino acid). The ratio of intermolecular reaction to intramolecular that was determined by HPLC technique. In particular, the intramolecular rearrangement reaction took place to a great extent in polr solvent such as methanol compared with nonpolar solvents such as cyclohexane, carbon tetrachloride and benzene. The by-product formed in the polycondensation was isolated and identified to be N-(4-acyl-2-nitrophenyl)α-amino acid.

## III - 5. References

[1] V.Böhmer, H.Kämmerer, *Makromol.Chem.*, 138, 137 (1970)

[2] Y.Kawabata, M.Kinoshita, *Makromol.Chem.*, 175. 105 (1974); ibid. 176, 49 (1975); ibid. 176, 2797 (1975); ibid. 176, 2807 (1975)

[3] J.F.Bunnett, R.H.Garst, *J.Amer.Chem.Soc.*, 87, 3875 (1965); ibid. 87, 3879 (1965); ibid. 90, 5965 (1968); ibid. 90, 5972 (1968)

[4] C.A.Bunton, L.Robinson, *J.Org.Chem.*, 34, 780 (1969); ibid 35, 733 (1970)

[5] C.A.Bunton, L.Robinson, *J.Amer.Chem.Soc.*, 92, 356 (1970)

[6] E.J.Fendler, J.H.Fendler, *Chem.Commn.*, 1970, 816

[7] L.M.Casilio, E.J.Fendler, J.H.Fendler, *J.Chem.Soc., (B)*, 1971, 1377

[8] J.H.Fendler, E.J.Fendler, M.V.Merritt, *J.Org.Chem.*, 36, 2172 (1971)

[9] J.H.Fendler, E.J.Fendler, S.A.Chang, *J.Amer.Chem.Soc.*, 95, 3273 (1973)

## Chapter IV. Synthesis of Polypeptide from Dipeptide 4-Acyl-2-nitrophenyl Esters

### IV - 1. Introduction

In chapter II, the author described the preparation and the polycondensation of a series of β-alanine 4-acyl-2-nitrophenyl esters having different chain length and structure of the acyl group, and found that both the conversion and the degree of polycondensation to poly(β-alanine) depend on the chain length and structure of the acyl groups and solvents used. The results were interpreted in terms of the reversed micellar aggregation of the active esters in some nonpolar solvents, which was expected particularly for the longer chain acyl groups. In chapter III, the author described the polycondensation of α-amino acid 4-acyl-2-nitrophenyl esters, and found that the formation of the significant amount of by-product through the nucleophilic rearrangement reaction besides poly(α-amino acid). Due to this rearrangement by-reaction, the synthesis of poly(α-amino acid) from α-amino acid 4-acyl-2-nitrophenyl esters gave the unsuccessful results.

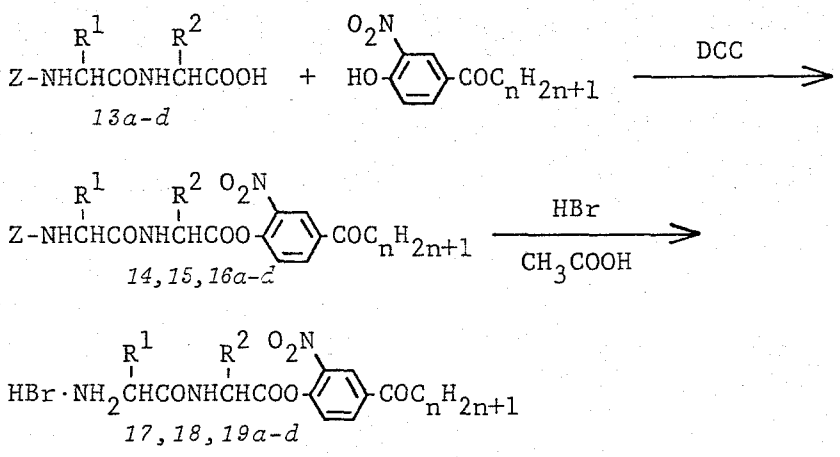
This chapter is concerned with the preparation of dipeptides (glycylglycine, D,L-alanyl-D,L-alanine, glycyl-D,L-alanine and D,L-alanylglycine) 4-acyl-2-nitrophenyl esters and the results of their polycondensation which would be correlated to the effect of sequential dipeptide ester groups. The convenient method for the synthesis of poly(α-amino acid) is described.

### IV - 2. Results and Discussion

A series of active dipeptide esters was prepared from the coupling reaction of N-benzyloxycarbonyl dipeptides (*13a-d*) with 4-acyl-2-nitrophenol in the presence of N,N'-dicyclohexylcarbodiimide (DCC), and further converted into hydrobromide salts (*17,18,19a-d*) which were used for polycondensation *(Scheme 1)*.

By treating the hydrobromide salt of the active dipeptide esters with triethylamine in certain solvents at 30°C for 24h, poly(dipeptide) with ester groups at the chain ends were formed together with 2,5-piperazinedione derivatives *(Scheme 2)*. In the case of *17*, *18* and *19a*, the C-terminal 4-acyl-2-

*Scheme 1*

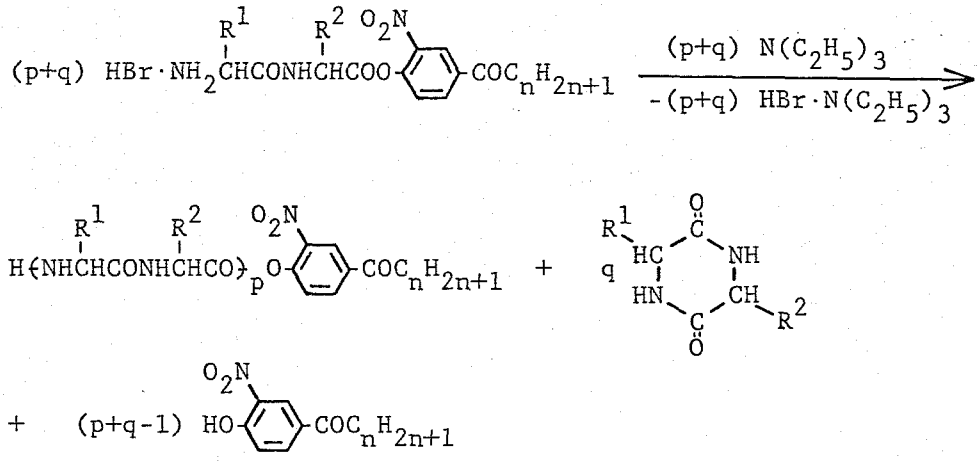



| No. | $-R^1$ | $-R^2$ |
|---|---|---|
| *13a* | -H | -H |
| *13b* | $-CH_3$ | $-CH_3$ |
| *13c* | -H | $-CH_3$ |
| *13d* | $-CH_3$ | -H |

| No. | n | $-R^1$ | $-R^2$ |
|---|---|---|---|
| *14; 17* | 1 | -H | -H |
| *15; 18* | 5 | -H | -H |
| *16a; 19a* | 11 | -H | -H |
| *16b; 19b* | 11 | $-CH_3$ | $-CH_3$ |
| *16c; 19c* | 11 | -H | $-CH_3$ |
| *16d; 19d* | 11 | $-CH_3$ | -H |

nitrophenyl group of poly(glycylglycine) was removed by alkali treatment.

*Scheme 2*



Results of polycondensation for *17*, *18* and *19a* are summarized in Table IV-1. The products obtained were first treated with chloroform and the insoluble polymer was isolated. The conversion based upon glycylglycine was generally over 100 % for the chloroform insoluble polymer, suggesting the existence of ester residues at the polymer chain end. The chloroform insoluble polymer was further treated with alkali solution to give poly(glycylglycine). The yield of water insoluble poly(glycylglycine) was very low when protic and dipolar aprotic solvents such as methanol, aqueous methanol, chloroform and dimethyl sulfoxide (DMSO) were used for the polycondensation. On the other hand, nonpolar solvents such as diethyl ether, benzene, carbon tetrachloride, hexane and cyclohexane were found to be favorable for the polycondesation, as can be seen from the high yields in water insoluble polymers. Such difference in the yield in various solvents was more prominent for *18* and *19a* than for *17*, indicating that the aggregation of the monomer molecule having the long acyl groups available for the polycondensation would be realized in nonpolar solvents, but disturbed in polar solvents showing strong enough affinity to the ester monomers.

Figure IV-1 shows typical NMR spectra of water and chloroform insoluble polymers obtained from *19a* ( a and b ), together with that of authentic 2,5-piperazinedione (c). The product ratio of poly(glycylglycine) to cyclodimer

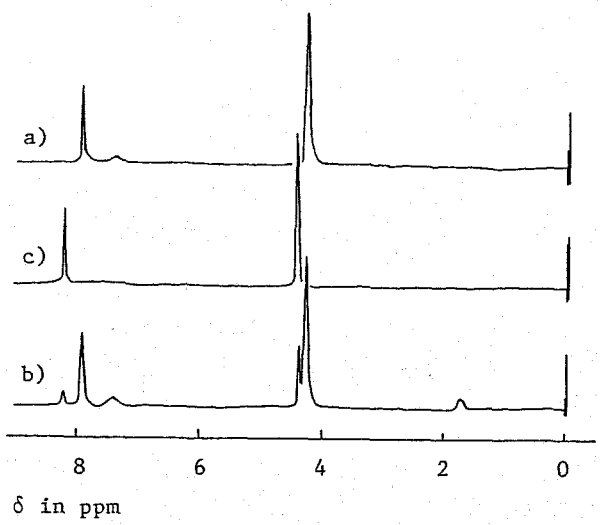



Figure IV-1 $^{1}H$ NMR spectra of poly( glycylglycine) obtained from *19a* in carbon tetrachloride at 30°C (measured in trifluoroacetic acid).
a) Water insoluble part,
b) Chloroform insoluble part,
c) Authentic 2,5-piperazinedione.

Table IV-1 Results of the polycondensation of the glycylglycine active esters *17*, *18* and *19a* in different solvents at 30°C for 24h[a)]

| Ester | Solvent | Conversion in % for the chloroform insoluble part | wt.-ratio polymer : cyclodimer | Conversion in % for the water insoluble part |
|---|---|---|---|---|
| *17* | Hexane | 139 (135)[b)] | 79:21 | 68 (65)[b)] |
| | Cyclohexane | 134 (136) | 73:27 | 45 (42) |
| | Carbon tetrachloride | 164 (160) | 81:19 | 39 (49) |
| | Benzene | 160 (159) | 73:27 | 37 (32) |
| | Diethyl ether | 130 (132) | 76:24 | 55 (59) |
| | Chloroform | 107 (105) | 44:56 | 28 (31) |
| | Methanol | 107 (108) | 48:52 | 31 (33) |
| | Methanol + Water | 108 (105) | 49:51 | 0 ( 0 ) |
| | DMSO | 111 (109) | 80:20 | 15 (17) |
| *18* | Hexane | 113 (110) | 93: 7 | 92 (88) |
| | Cyclohexane | 120 (116) | 92: 8 | 88 (87) |
| | Carbon tetrachloride | 126 (119) | 97: 3 | 93 (94) |
| | Benzene | 121 (122) | 93: 7 | 93 (93) |
| | Diethyl ether | 130 (110) | 93: 7 | 94 (92) |
| | Chloroform | 113 (112) | 49:51 | 56 (46) |
| | Methanol | 114 (112) | 69:31 | 15 (17) |
| | Methanol + Water | 120 (122) | 45:55 | 0 ( 0 ) |
| | DMSO | 117 (120) | 76:24 | 29 (24) |
| *19a* | Hexane | 119 (116) | 94: 6 | 90 (89) |
| | Cyclohexane | 119 (115) | 94: 6 | 90 (87) |
| | Carbon tetrachloride | 119 (118) | 90:10 | 96 (95) |
| | Benzene | 110 (111) | 89:11 | 84 (83) |
| | Diethyl ether | 115 (112) | 94: 6 | 94 (91) |
| | Chloroform | 100 (106) | 47:53 | 59 (45) |
| | Methanol | 104 (103) | 54:46 | 24 (22) |
| | Methanol + Water | 128 (120) | 42:58 | 0 ( 0 ) |
| | DMSO | 117 (115) | 80:20 | 20 (22) |

a) [Ester]=20mmole/l; [Triethylamine]=20mmole/l.
b) Values in parentheses are those obtained in a second run.

was estimated from the relative intensity of NMR absorption at 7.9 ppm (NH in poly(glycylglycine)) to that at 8.2 ppm (NH in 2,5-piperazinedione) (Figure IV-1). From the ratios, it is clear that the formation of poly(glycylglycine) compared to that of cyclodimer is preferred in nonpolar solvents and not in protic or dipolar aprotic solvents, and also dependent on the chain length of acyl groups of *17*, *18* and *19a*. These results suggest that the monomer aggregation in nonpolar solvents promotes the intermolecular reaction rather than intramolecular one.

In order to get information how the structure of dipeptides influences the polycondensation behavior, the other type of dipeptide esters such as *19b-c* were also studied (Table IV-2). The chloroform insoluble part, similarly obtained as in the case of *17*,*18* and *19a*, was washed thoroughly with hot

Table IV-2 Results of the polycondensation of the active esters (*19b-d*) in different solvents at 30°C for 24h[a)]

| Ester | Solvent | Conversion in % for the chloroform insoluble part | wt.-ratio polymer : cyclodimer | Conversion in % for the methanol insoluble part |
|---|---|---|---|---|
| *19b* | Hexane | 102 (100)[b)] | 68:32 | 66 (65)[b)] |
| | Cyclohexane | 104 (101) | 69:31 | 67 (64) |
| | Carbon tetrachloride | 96 ( 95) | 84:16 | 70 (72) |
| | Benzene | 84 ( 82) | 61:39 | 44 (40) |
| | Diethyl ether | 98 ( 96) | 93: 7 | 60 (61) |
| | Chloroform | 53 ( 21) | 52:48 | 17 (14) |
| | Methanol | 19 ( 20) | 43:57 | trace |
| *19c* | Hexane | 122 (120) | 90:10 | 61 (66) |
| | Cyclohexane | 128 (121) | 98: 2 | 67 (65) |
| | Carbon tetrachloride | 107 (105) | 89:11 | 54 (58) |
| | Benzene | 94 ( 95) | 59:41 | 10 ( 9 ) |
| | Diethyl ether | 106 (103) | 42:58 | 32 (34) |
| | Chloroform | 89 ( 87) | 60:40 | trace |
| | Methanol | 14 ( 12) | 47:53 | trace |
| *19d* | Hexane | 96 (101) | 95: 5 | 82 (77) |
| | Cyclohexane | 94 ( 99) | 95: 5 | 86 (83) |
| | Carbon tetrachloride | 103 (102) | 90:10 | 80 (81) |
| | Benzene | 85 ( 88) | 85:15 | 62 (58) |
| | Diethyl ether | 101 (100) | 97: 3 | 50 (43) |
| | Chloroform | 51 ( 56) | 65:35 | 22 (19) |
| | Methanol | 50 ( 45) | 55:45 | trace |

a) [Ester]=20mmole/l; [Triethylamine]=20mmole/l.
b) Values in parentheses are those obtained in a second run.

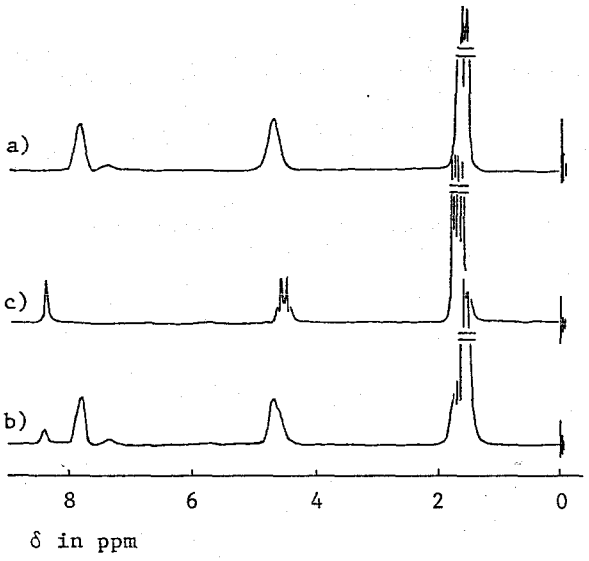



Figure IV-2 $^{1}H$ NMR spectra of poly(alanylalanine) obtained from *19b* in carbon tetrachloride at 30°C (measured in trifluoroacetic acid).
a) Methanol insoluble part,
b) Chloroform insoluble part,
c) Authentic 3,6-dimethyl-2,5-piperazinedione.

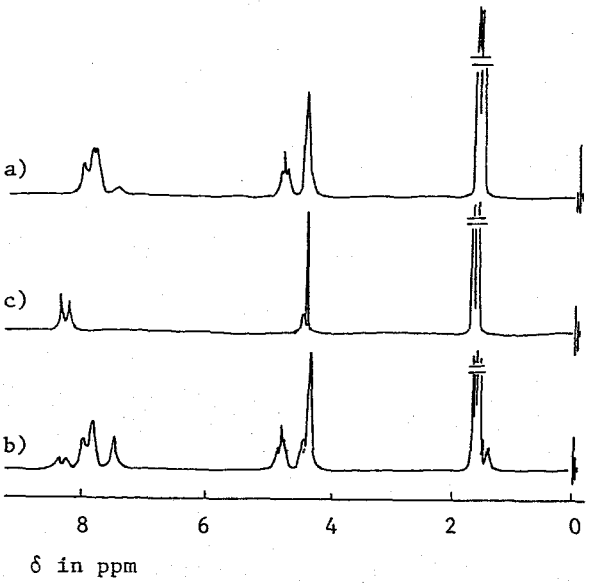


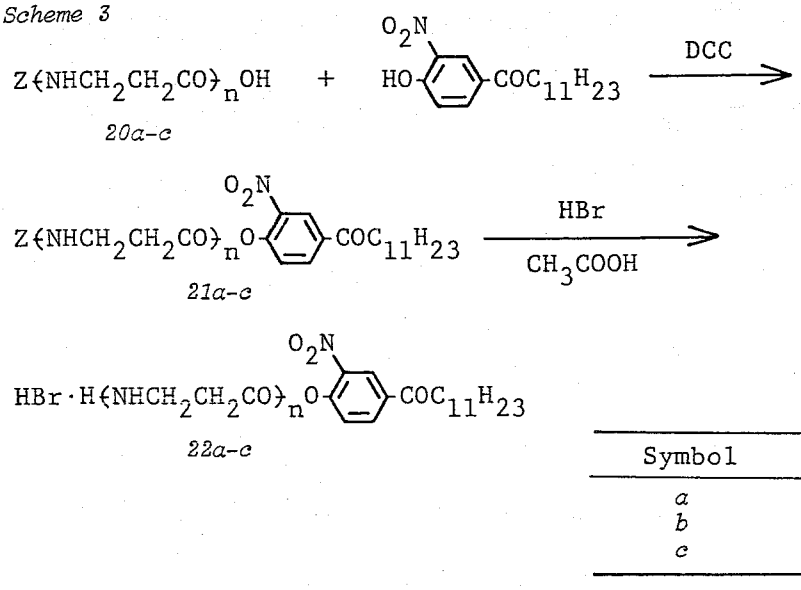

Figure IV-3 $^{1}H$ NMR spectra of poly(glycylalanine) obtained from *19c* in carbon tetrachloride at 30°C (measured in trifluoroacetic acid).
a) Methanol insoluble part,
b) Chloroform insoluble part,
c) Authentic 3-methyl-2,5-piperazinedione.

methanol to remove the cyclodimer. The NMR spectra of the products of *19b-d* are analogous to that of the product of *19a* (see Figure IV-2,3). The yield was obtained from the methanol insoluble poly(D,L-alanyl-D,L-alanine), poly( glycyl-D,L-alanine) and poly(D,L-alanylglycine), which are inherently soluble in water. The reaction in protic and dipolar aprotic solvents such as methanol and chloroform gave no polymer, or at least polymers in very low yield. Nonpolar solvents such as diethyl ether, carbon tetrachloride, hexane and cyclohexane were found to be suitable for polycondensation, while benzene was unsuitable for the purpose. Solvent effect on the product ratio of polymer to cyclodimer was also observed in the case of *19b-d*, and it was found that the cyclodimer production was particularly predominant in the case of *19b*, in all solvents used except diethyl ether. This may be interpreted in terms of solvent affinity, that is, the stronger the affinity of dipeptide esters towards a solvent, the less they are converted into polymer. In fact, the presence of the α-alanine moiety in the ester molecule tends to increase its solubility so that the polycondensation becomes more unfavorable.

Subsequently, in order to clarify how the hydrophilic parts in active esters can contribute to the polycondensation on the reversed micellar surface, the author prepared β-alanyl-β-alanine and β-alanyl-β-alanyl-β-alanine 4-dodecanoyl-2-nitrophenyl esters (*22b,c*) and investigated the polycondensation of their active esters, comparing to that of β-alanine 4-dodecanoyl-2-nitrophenyl ester (*22a*). A series of active oligopeptide esters (*22a-c*) were prepared by coupling reaction of the corresponding N-benzyloxycarbonyl peptides (*20a-c*) with 4-dodecanoyl-2-nitrophenol by DCC method, and converted into their hydrobromide salts (*Scheme 3*).

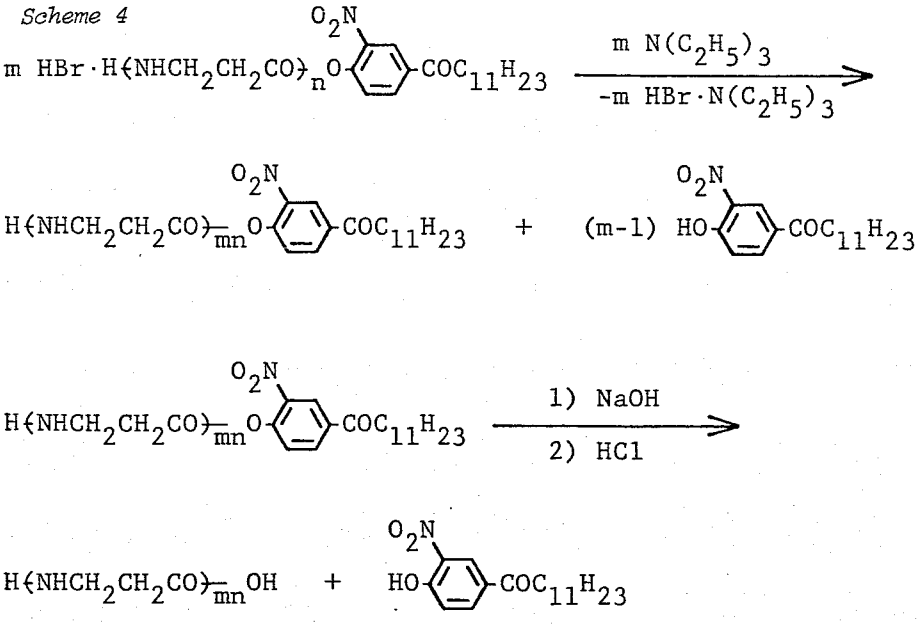
When the hydrobromide salts of active oligopeptide esters (*22a-c*) were treated with triethylamine in various solvents at given temperatures for 24h, poly(β-alanine) was formed, in which the ester residues still remained at the chain ends. The C-terminal 4-dodecanoyl-2-nitrophenyl group was removed by alkali treatmemt *(Scheme 4)*.

*Scheme 3*



| Symbol | n |
|---|---|
| *a* | 1 |
| *b* | 2 |
| *c* | 3 |

*Scheme 4*



Results of the polycondensation for *22a-c* are summarized in Table IV-3. In general, nonpolar solvents such as cyclohexane, carbon tetrachloride, benzene and diethyl ether were found to be suitable for the polycondensation. On the other hand, the yield of polymer was low in carbon tetrachloride with a little amount of methanol, and in fact, no polymer was obtained in methanol solution. These facts suggested that the aggregation would occur in nonpolar solvents, whereas it was not the case in polar solvents such as methanol. In addition, the yield decreased with increasing number of β-alanine units to avoid such aggregation.

Table IV-3 Results of the polycondensation of *22a-c* at 30°C for 24h[a)]

| Solvent | Conversion in % for the water insoluble part[b)] | | |
|---|---|---|---|
| | *22a* | *22b* | *22c* |
| Cyclohexane | 90 (91) | 56 (51) | 51 (41) |
| Carbon tetrachloride | 95 (96) | 76 (78) | 46 (44) |
| Benzene | 92 (91) | 76 (81) | 43 (45) |
| Diethyl ether | 94 (93) | 70 (70) | 42 (48) |
| Carbon tetrachloride + Methanol (95:5) | 0 ( 0 ) | 43 (39) | 21 (19) |
| Carbon tetrachloride + Methanol (90:10) | 0 ( 0 ) | 21 (22) | 10 (15) |
| Methanol | 0 ( 0 ) | 4 (trace) | 2 (trace) |

a) [Ester]=20mmole/l; [Triethylamine]=20mmole/l.
b) Values in parentheses are those obtained in a second run.

The effect of temperature on the yield of polycondensation products was studied with respect to *22a* and *22b*. Results are summarized in Table IV-4 and IV-5. As can be seen from these tables, the temperature favorable for polycondensation was near 30°C, and became less favorable over 30°C. This seemed to coincide with the fact that the aggregation number of reversed micelles in nonpolar solvents decreased with increasing temperature.

Figure IV-2 shows typical $^{1}H$ NMR spectra on the water- and chloroform-insoluble polymers obtained from *22b* in carbon tetrachloride solution at 30°C ((a) and (b), respectively), together with that of authentic cyclo-di-β-alanyl

Table IV-4 Effect of temperature on the polycondensation of *22a* for 24h[a)]

| Solvent | Conversion in % for the water insoluble part[b)] | | | |
|---|---|---|---|---|
| | 10°C | 30°C | 50°C | reflux[c)] |
| Cyclohexane | 26 (28) | 90 (91) | 93 (93) | 22 (30) |
| Carbon tetrachloride | 72 (70) | 95 (96) | 71 (73) | 59 (56) |
| Benzene | 70 (71) | 92 (91) | 63 (62) | 57 (56) |
| Diethyl ether | 53 (57) | 94 (93) | — | 80 (82) |

a) [Ester]=20mmole/l; [Triethylamine]=20mmole/l.
b) Values in parentheses are those obtained in a second run.
c) Gently refluxed.

Table IV-5 Effect of temperature on the polycondensation of *22b* for 24h[a)]

| Solvent | Conversion in % for the water insoluble part[b)] | | | |
|---|---|---|---|---|
| | 10°C | 30°C | 50°C | reflux[c)] |
| Cyclohexane | 24 (10) | 56 (51) | 65 (67) | 46 (43) |
| Carbon tetrachloride | 39 (38) | 76 (78) | 64 (63) | 50 (48) |
| Benzene | 39 (34) | 76 (81) | 54 (53) | 30 (31) |
| Diethyl ether | 19 ( 9 ) | 70 (70) | — | 66 (63) |

a) [Ester]=20mmole/l; [Triethylamine]=20mmole/l.
b) Values in parentheses are those obtained in a second run.
c) Gently refluxed.

(c). From (b) and (c), it was concluded that no cyclo-di-β-alanyl was formed under the reaction conditions.

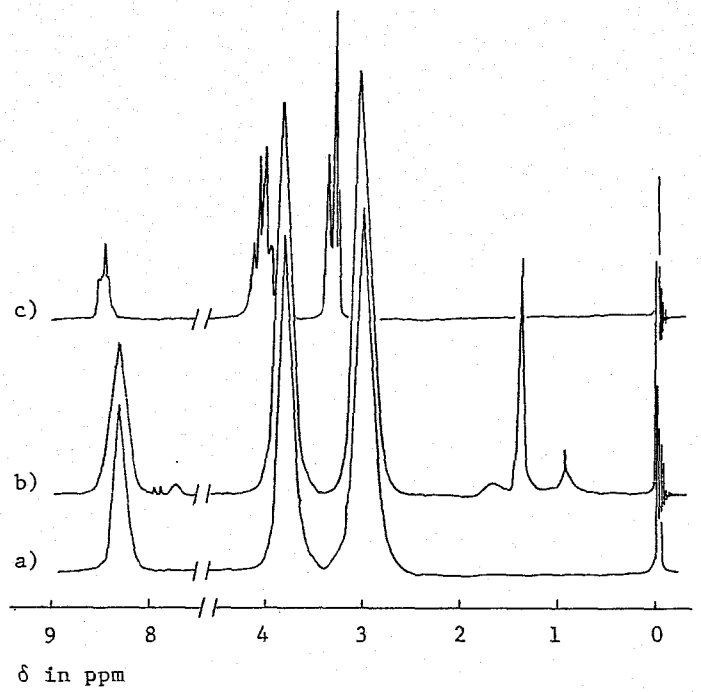



Figure IV-4. $^{1}H$ NMR spectra for poly(β-alanine) from the polycondensation of *22b* in carbon tetrachloride at 30°C (measured in trifluoroacetic acid).
a) Water insoluble part after alkali treatment,
b) Chloroform insoluble part before alkali treatment,
c) Authentic cyclo-di-β-alanyl.

## IV - 3. Experimental Part

*Materials*

*Benzyloxycarbonyl dipeptides(13a-d);* Benzyloxycarbonylglycylglycine (*13a*) was obtained by the saponification of benzyloxycarbonylglycylglycine ethyl ester which was prepared from benzyloxycarbonylglycine and glycine ethyl ester hydrochloride, using the mixed anhydride method [1]. The crude dimeric ester was dissolved in methanol and heated to 40°C stirring. Sodium hydroxide was added

to the reaction mixture, stirred further at 40°C for 2h, and then the solution was acidified to pH 2 with conc. hydrochloric acid. The solvent was removed under reduced pressure, and the residue was recrystallized from ethanol; yield 75 %, mp 177-178°C (ref.[2] 178°C)

Benzyloxycarbonyl-D,L-alanyl-D,L-alanine (*13b*), -glycyl-D,L-alanine (*13c*) and -D,L-alanylglycine (*13d*) were prepared in a similar way to *13a*. The solvent for recrystallization, yield and melting point of the products are as follows;

*13b:* Ethyl acetate-petroleum ether, 65 %, 147-149°C (ref.[2] 149°C)

*13c:* Ethanol, 78 %, 176°C (ref.[3] 176°C)

*13d:* Ethyl acetate, 71 %, 126-127°C (ref.[3] 127°C, ref.[2] 133°C)

*Glycylglycine 4-acetyl-2-nitrophenyl ester hydrobromide(17);* To 50ml of ethyl acetate solution containing 3.6g (20mmole) of 4-acetyl-2-nitrophenol and 5.3g (20mmole) of benzyloxycarbonylglycylglycine (*13a*) was added 4.1g (20mmole) of DCC at 0°C. After stirring for 3h at 0°C, the mixture was kept overnight at room temperature, and dicyclohexylurea formed was then filtered off. The filtrate was evaporated in vacuo and the residue was recrystallized from ethyl acetate-hexane to afford 7.1g (83%) of benzyloxycarbonylglycylglycine 4-acetyl-2-nitrophenyl ester (*14*). 30g of a glacial acetic acid solution saturated with dry hydrogen bromide (36%) was added to 7.1g (16.6mmole) of *14*. After stirring for 2h, the mixture was allowed to stand overnight at room temperature. The crystalline product precipitated was then filtered off and further recrystallized from formic acid-diethyl ether to give 5.7g (15.3mmole) of colorless crystals in 92 % yield; mp (dec.) 188-189°C

| | | | | | |
|---|---|---|---|---|---|
| $C_{12}H_{14}N_3O_6Br$(376.2) | Calcd. | C 38.32 | H 3.75 | N 11.17 | Br 21.24 |
| | Found | C 38.06 | H 3.65 | N 10.95 | Br 21.32 |

*Glycylglycine 4-hexanoyl-2-nitrophenyl ester hydrobromide(18);* Hydrogen bromide salt of glycylglycine 4-hexanoyl-2-nitrophenyl ester was prepared from 4.8g (20mmole) of 4-hexanoyl-2-nitrophenol and 5.3g (20mmole) of benzyloxycarbonylglycylglycine (*13a*) in a similar way to *17*. 7.9g (81%) of benzyloxycarbonylglycylglycine 4-hexanoyl-2-nitrophenyl ester (*15*) was obtained by recrystallizing from ethyl acetate-hexane. Treatment of 7.9g (16.3mmole) of *15* with hydrogen bromide in acetic acid solution gave *18*. The crude product

was recrystallized from formic acid-diethyl ether to give 6.4g (14.8mmole) of colorless crystals of *18* in 91 % yield; mp (dec.) 176-177°C.

| | | | | | |
|---|---|---|---|---|---|
| $C_{16}H_{22}N_3O_6Br$(432.3) | Calcd. | C 44.46 | H 5.13 | N 9.72 | Br 18.47 |
| | Found | C 44.13 | H 4.96 | N 9.68 | Br 18.75 |

*Glycylglycine 4-dodecanoyl-2-nitrophenyl ester hydrogenbromide(19a);* Hydrogen bromide salt of glycylglycine 4-dodecanoyl-2-nitrophenyl ester was prepared from 6.4g (20mmole) of 4-dodecanoyl-2-nitrophenol and 5.3g (20mmole) of benzyloxycarbonylglycylglycine (*13a*) in a similar way to *17* and *18*. 9.5g (83%) of benzyloxycarbonylglycylglycine 4-dodecanoyl-2-nitrophenyl ester (*16a*) was obtained after recrystallization from ethyl acetate-hexane. Treatment of 9.5g (16.7mmole) of *16a* with hydrogen bromide in acetic acid solution gave *19a*. The crude product was recrystallized from acetic acid to give 8.2g (15.9mmole) of *19a* in 95 % yield; mp (dec.) 172-173°C

| | | | | | |
|---|---|---|---|---|---|
| $C_{22}H_{34}N_3O_6Br$(516.4) | Calcd. | C 51.17 | H 6.64 | N 8.17 | Br 15.47 |
| | Found | C 50.86 | H 6.60 | N 8.24 | Br 15.86 |

*D,L-Alanyl-D,L-alanine 4-dodecanoyl-2-nitrophenyl ester hydrobromide(19b);* The compound was prepared in a similar way as described for *17*, *18* and *19a*. Reaction was run on a 20 millimolar scale. Recrystallization from ethyl acetate -hexane gave 9.4g (79%) of benzyloxycarbonyl-D,L-alanyl-D,L-alanine 4-dodecanoyl-2-nitrophenyl ester (*16b*), which was converted to 6.9g (12.7mmole) of *19b*. Recrystallization was made from formic acid-diethyl ether in 81 % yield; mp (dec.) 163-164°C, hygroscopic colorless crystals.

| | | | | | |
|---|---|---|---|---|---|
| $C_{24}H_{38}N_3O_6Br$(544.5) | Calcd. | C 52.94 | H 7.04 | N 7.72 | Br 14.68 |
| | Found | C 53.23 | H 6.92 | N 7.53 | Br 14.82 |

*Glycyl-D,L-alanine 4-dodecanoyl-2-nitrophenyl ester hydrobromide(19c);* The compound was prepared using a similar procedure to *17*. Reaction was run in a 20 millimolar scale. Recrystallization from ethyl acetate-hexane gave 9.2g (15.8mmole, 79%) of benzyloxycarbonylglycyl-D,L-alanine 4-dodecanoyl-2-nitrophenyl ester (*16c*), which was converted to 7.2g (13.6mmole) of *19c*. The product was recrystallized from acetic acid in 86 % yield;mp(dec.) 160-161°C

| | | | | | |
|---|---|---|---|---|---|
| $C_{23}H_{36}N_3O_6Br$(530.6) | Calcd. | C 52.08 | H 6.84 | N 7.92 | Br 15.06 |
| | Found | C 52.03 | H 6.88 | N 7.67 | Br 15.24 |

*D,L-Alanylglycine 4-dodecanoyl-2-nitrophenyl ester hydrobromide(19d);* The compound was prepared using a similar technique as described for the synthesis of *17*. Reaction was run also on a 20 millimolar scale. Recrystallization from ethyl acetate-hexane gave 9.5g (81%) of benzyloxycarbonyl-D,L-alanylglycine 4-dodecanoyl-2-nitrophenyl ester (*16d*), which was converted to 6.6g (12.4mmole) of *19d*. The product was recrystallized from formic acid-diethyl ether(77% yield); mp(dec.) 174-175°C. Hygroscopic colorless crystals.

| | | | | | |
|---|---|---|---|---|---|
| $C_{23}H_{36}N_3O_6Br$(530.6) | Calcd. | C 52.08 | H 6.84 | N 7.92 | Br 15.06 |
| | Found | C 51.77 | H 6.78 | N 7.83 | Br 15.05 |

*2,5-Piperazinedione derivatives;* The authentic samples, 2,5-piperazinedione (glycylglycine lactam) [4], 3,6-dimethyl-2,5-piperazinedione (D,L-alanyl-D,L-alanine lactam)[5], and 3-methyl-2,5-piperazinedione (D,L-alanylglycine lactam)[6] were prepared according to the references.

*Benzyloxycarbonyl-β-alanyl-β-alanine(20b);* The compound was obtained by the coupling reaction of benzyloxycarbonyl-β-alanine with β-alanine ethyl ester hydrochloride using the mixed anhydride method and the subsequent saponification as described by Anderson [1] and Goodman [7]. The product was recrystallized from water; mp. 144-145°C (ref.[8] 144-145°C, ref.[9] 145°C)

*Benzyloxycarbonyl-β-alanyl-β-alanyl-β-alanine(20c);* This compound was prepared from benzyloxycarbonyl-β-alanine and β-alanyl-β-alanine ethyl ester hydrochloride by the similar method as described above. The product was recrystallized from water; mp. 194-195°C (ref.[10] 194-195°C, ref.[9] 195°C).

*β-Alanine 4-dodecanoyl-2-nitrophenyl ester hydrobromide(22a);* The preparation of this compound was described in detail in the chapter I.

*β-Alanyl-β-alanine 4-dodecanoyl-2-nitrophenyl ester hydrobromide(22b);* To 50ml of acetonitrile solution containing 6.4g (20mmole) of 4-dodecanoyl-2-nitrophenol and 5.9g (20mmole) of benzyloxycarbonyl-β-alanyl-β-alanine *(21b)* 4.1g (20mmole) of DCC were added at 0°C. After stirring for 3h at 0°C, the mixture was kept to stand overnight at room temperature, and N,N'-dicyclohexyl urea formed was then filtered off. The filtrate was evaporated in vacuo, the residue was dried. 30g of glacial acetic acid solution saturated with

hydrogen bromide (36%) was added to the residue. After stirring for 2h the mixture was allowed to stand overnight at room temperature. The crystalline product precipitated was then filtered off, which was further recrystallized from acetic acid to give 7.9g (14.5mmole) of a colorless crystalline substance in a 73% yield; mp. (dec.) 127-128°C.

| | | | | | |
|---|---|---|---|---|---|
| $C_{24}H_{38}N_3O_6Br$(544.5) | Calcd. | C 52.94 | H 7.03 | N 7.72 | Br 14.68 |
| | Found | C 53.23 | H 7.09 | N 7.69 | Br 14.64 |

*β-Alanyl-β-alanyl-β-alanine 4-dodecanoyl-2-nitrophenyl ester hydrobromide (22c);* This compound was prepared in a similar way as in the case of *22b*, while 6.4g (20mmole) of 4-dodecanoyl-2-nitrophenol and 7.3g (20mmole) of benzyloxycarbonyl-β-alanyl-β-alanyl-β-alanine (*20c*) were treated in dimethyformamide instead of acetonitrile. The crude product was recrystallized from formic acid-petroleum ether to give 10.8g (17.6mmole) of hygroscopic colorless crystals of *22c* in a 88% yield; mp. (dec.) 134-136°C.

| | | | | | |
|---|---|---|---|---|---|
| $C_{27}H_{43}N_4O_7Br$(615.6) | Calcd. | C 52.68 | H 7.04 | N 9.10 | Br 12.98 |
| | Found | C 52.30 | H 7.08 | N 8.91 | Br 12.91 |

*Cyclo-di-β-alanyl (Perhydro-1,5-diazocine-2,6-dione);* This compound was prepared from cyclohexane-1,4-dione using the method described by Y.Iwakura et al.[12]. mp. 298-299°C (ref.[11] 299.5°C).

*Polycondesation;* The polycondensation technique was described in detail in the chapter II.

## IV - 4. Summary

The preparation and polycondensation of dipeptide 4-acyl-2-nitrophenyl esters was investigated, and it was found that the conversion of the polypeptide was high enough in nonpolar solvents, while the reaction in polar solvents was accompanied by the cyclodimeric products, that is, 2,5-piperazinedione derivatives. The product ratio of polypeptide to cyclodimer was estimated from the relative intensity of NMR absorption, and was correlated to the monomer aggregation in solvents promoting the intermolecular reaction rather than the intramolecular one. It was clarified how the hydrophilic region of the amino acid site in the active esters can contribute to the polycondensation in the reversed micelle.

IV - 5. References


[1] G.W.Anderson, J.E.Zimmerman, F.M.Callahan, *J.Am.Chem.Soc.*, 89, 5012 (1967)

[2] S.Goldschmidt, H.Lautenschlager, *Justus Liebigs Ann.Chem.*, 580, 68 (1953)

[3] S.Goldschmidt, M.Wick, *Justus Liebigs Ann.Chem.*, 575, 217 (1952)

[4] A.Chemizard, S.David, *Bull.Soc.Chim.Fr.*, 1, 184 (1966)

[5] Y.Shibata, T.Asahina, *Bull.Chem.Soc.Jpn.*, 1, 71 (1926)

[6] E.Brand, D.F.Erlanger, H.Sachs, *J.Am.Chem.Soc.*, 74, 1851 (1952)

[7] M.Goodman, R.Rupp, F.Naider, *Bioorg.Chem.*, 1, 294 (1971)

[8] H.T.Hanson, E.L.Smith, *J.Biol.Chem.*, 175, 833 (1948)

[9] V.W.Ried, K.Marquard, *Liebigs Ann.Chem.*, 642, 141 (1961)

[10] E.Adams, N.C.Davis, E.L.Smith, *J.Biol.Chem.*, 199, 845 (1952)

[11] Y.Iwakura, K.Uno, M.Akiyama, K.Haga, *J.Polym.Sci., (A-1)*, 7, 657 (1969)

## Conculsion

The purpose of the present study was to synthesize polypeptides under mild condition utilizing the reversed micellar surface which is realized in the oriented organic molecule association.

In Chapter I, the preparation of active β-alanine 4-acyl-2-nitrophenyl esters as functional reversed micelle surfactant was described. The apparent mean aggregation numbers of model compounds prepared for β-alanine 4-acyl-2-nitrophenyl esters were measured and refered to the polypeptide formation.

In Chapter II, the polycondensation of β-alanine 4-acyl-2-nitrophenyl esters was studied. It was found that the conversion and the degree of polycondensation of the resulting poly(β-alanine) depends on the chain length and the structure of acyl groups. In relation to solvents used, no polymer was obtained in both protic and dipolar aprotic solvents, whereas nonpolar solvents were found to be suitable for the polycondensation, suggesting that the polycondensation proceeded on the reversed micellar surface. The reaction temperature affected also the polycondensation which generally proceeded preferentially at near 30 °C and was retarded rather at higher temperature.

In Chapter III, the preparation and polycondensation of α-amino acid 4-acyl-2-nitrophenyl esters was studied. The reaction of α-amino acid 4-acyl-2-nitrophenyl esters gave a by-product derived from the intramolecular rearrangement via the Meisenheimer complex together with poly(α-amino acid). In particular the intramolecular rearrangement took place great extent in methanol as compared to nonpolar solvents such as cyclohexane, carbon tetrachloride and benzene. The by-product formed in the polycondensation was isolated and identified as N-(4-acyl-2-nitrophenyl)α-amino acid.

In Chapter IV, the preparation and polycondensation of dipeptide 4-acyl-2-nitrophenyl esters was investigated, and it was found that the conversion of the polypeptide was high enough in nonpolar solvents, while the reaction in polar solvents was accompanied by the cyclodimeric product, that is, 2,5-piperazinedione derivatives. The product ratio of polypeptide to cyclodimer was estimated from the relative intensity of NMR spectra and was correlated to the monomer aggregation promoting the intermolecular reaction rather than the intramolecular one.

ACKNOWLEDGEMENT

The work of this thesis was carried out at Osaka University and Shinshu University under the guidance of Professor Kiichi Takemoto.

I would like to express my grateful acknowledgement to Professor Kiichi Takemoto for his invaluable guidance and continuous encouragement throughout this investigation.

I am very much obliged to Dr. Koichi Kondo for numerous fruitful discussions and helpful suggestions, which facilitated the completion of this thesis, and it is a pleasure to express my thanks to Dr. Yoshiyuki Inaki and Dr. Mikiji Miyata for their encouragement and helpful advices.

I am also deeply grateful to Professor Toshio Agawa and Professor Haruo Matsuda for their helpful discussions and suggestions.

It is a pleasure to express my thanks to Professor Nobumasa Hojo and Dr. Hirofusa Shirai, Shinshu University, for help and advice with this research.

I owe it to my colleagues that this work has been accomplished, and express my thanks to Miss Kuniko Ohno and Mr. Yoshiyuki Miwa.

I further wish to thank all the members of the Takemoto Laboratory and all my friends for their friendships.

Finally, I would like to thank my father Eiichi Hanabusa and my mother Hanako Hanabusa for their heartful encouragement.

Ueda, Nagano
March, 1981

Kenji Hanabusa