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Synthesis of Poly-β-alanine from β-Alanine 4-Acyl-2-nitrophenyl Esters

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SUMMARY:

A series of active β-alanine 4-acyl-2-nitrophenyl esters with different lengths of alkyl groups was prepared, and their polycondensation was studied in various solvents. The results of the polycondensation of these esters with triethylamine at room temperature show that the conversion and the degree of polycondensation of the resulting poly(β-alanine)s depend on the chain length of the alkyl groups. No polymer was obtained in both protic and dipolar aprotic solvents such as methanol and DMSO, whereas nonpolar solvents such as diethyl ether, carbon tetrachloride, or benzene were found to be suitable for the polycondensation. The effect of a variety of amines on the polycondensation was examined for β-alanine 4-dodecanoyl-2-nitrophenyl ester (**4e**) in diethyl ether solution.

Introduction

In 1948, Baniel et al.[1] reported on a modified technique for preparing polypeptides, starting from α-amino acid alkyl esters. From the results obtained at elevated temperatures 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, avoiding some undesirable diketopiperazine formation.

For the synthesis of poly(β-alanine), Kinoshita et al.[2] 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.

In this paper, we report the synthesis of poly(β-alanine) from other types of active β-alanine phenyl esters, in order to realize reactions at rather lower temperature, with improving the conversion as well as the degree of polycondensation. The effect of the chain length of the alkyl groups on the polycondensation was investigated, using various kinds of organic solvents for the reactions.

Results and Discussion

A series of active β-alanine esters was prepared by reaction of *N*-benzyloxycarbonyl-β-alanine (**1b**) with a 4-acyl-2-nitrophenol (**2**) in the presence of dicyclohexylcarbodiimide (DCC), and

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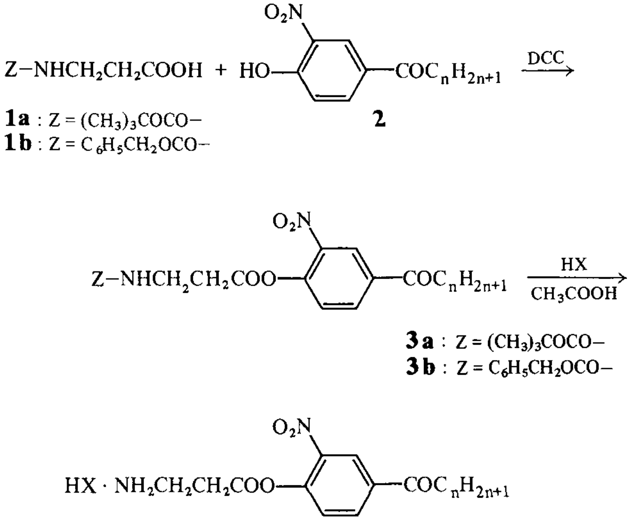
[a] Part 53 of the series: M. Sato, K. Kondo, K. Takemoto, J. Polym. Sci., Polym. Chem. Ed., in contribution.

further converted into their hydrobromic acid salts before polycondensation. Only an exception was the case of *N*-*tert*-butoxycarbonyl-*β*-alanine 4-acetyl-2-nitrophenyl ester (**3a**), from which the hydrochloric acid salt **4a** was derived, since in this case the *N*-benzoyloxycarbonyl ester was difficult to purify (Scheme 1).

Scheme 1:

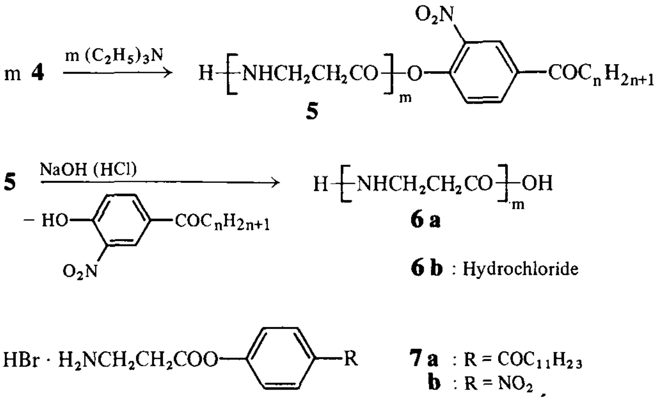


4a : X = Cl
4b-g : X = Br

	2a,3a,4a	**b**	**c**	**d**	**e**	**f**	**g**
n	1	5	7	9	11	15	17

4a–g were treated with triethylamine in ether solution at room temperature to afford poly(*β*-alanine) (**5**) with the corresponding ester residue at the chain end, which is insoluble in chloroform, but can be converted into the water-insoluble polymer **6** by alkali treatment (Scheme 2).

Scheme 2:



The results of the polycondensation are summarized in Tab. 1, which, for comparison, contains also the results of the polycondensation carried out with the hydrogen bromide of β-alanine 4-dodecanoylphenyl ester (**7a**) and β-alanine *p*-nitrophenyl ester (**7b**).

Tab. 1. Results of the polycondensations of the β-alanine esters **4a–g**, **7a** and **7b** in diethyl ether solution at 25 °C, for 24 h[a]

Ester	Conversion in % for the[b]		DP[b)c)]
	chloroform insoluble part	water insoluble part	
4a	110	39 (37)	12
4b	120	80 (77)	16
4c	127	88 (81)	16
4d	114 (117)	97 (95)	33 (32)
4e	110 (113)	94 (93)	74 (72)
4f	89	63 (64)	96
4g	94	35 (33)	39
7a	0	0	—
7b	131	12 (6)	—

[a] $[\text{Ester}] = 20\ \text{mmol} \cdot \text{l}^{-1}$; $[\text{Triethylamine}] = 20\ \text{mmol} \cdot \text{l}^{-1}$; volume of ethyl ether $= 50\ \text{cm}^3$.

[b] Values in parentheses are those obtained from the second run.

[c] Calculated from the NMR spectrum of the chloroform-insoluble part.

The conversion calculated for β-alanine units was in general 100% for the chloroform-insoluble polymers **5**, 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 **4a** 3,0 ppm of the methyl group). From Tab. 1 it can be seen that the conversion as well as the DP increase with increasing alkyl chain length in the esters. The fact that no polymer

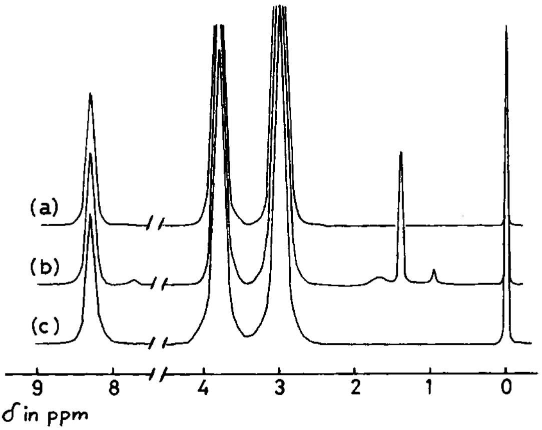


Fig. 1. ^1H NMR spectra of poly(β-alanine) from **4e** in trifluoroacetic acid. (a): Water insoluble part; (b): chloroform insoluble part; (c): poly(β-alanine)

was obtained from **7a,** which carries no nitro groups, suggests the presence of nitro groups in the ester residue to be substantial for the polycondensation.

Fig. 1 shows typical ^1H NMR spectra of water and chloroform insoluble polymers obtained from **4e** ((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 (Fig. 1(b)), which can be removed by alkali treatment (Fig. 1(a)).

Tab. 2. Results of the polycondensation of the β-alanine esters **4a–g, 7a** and **7b** in benzene solution at 25 °C, for 24 h[a)]

Ester	Conversion in % for the water insoluble part[b)]
4a	38 (46)
4b	75 (74)
4c	88 (83)
4d	96 (99)
4e	90 (92)
4f	70 (71)
4g	66 (64)
7a	0 (0)
7b	14 (23)

[a)] [Ester] = 20 mmol·l^{-1}; [Triethylamine] = 20 mmol·l^{-1}; volume of benzene = 50 cm^3.

[b)] Values in parentheses are those obtained in the second run.

Tab. 3. Results of the polycondensation of the β-alanine ester **4e** in different solvents at 25 °C for 24 h[a)]

Solvent	Conversion in % for the[b)]		DP[c)]
	chloroform insoluble part	water insoluble part	
Hexane		66 (65)	
Cyclohexane		75 (79)	
1,4-Dioxane		15 (17)	
Carbon tetrachloride	120	96 (96)	33
Benzene	123	92 (90)	24
Diethyl ether	110	94 (93)	72
Chloroform		29 (22)	
Methanol		0 (0)	
DMSO		0 (0)	

[a)] [**4e**] = 20 mmol·l^{-1}; [Triethylamine] = 20 mmol·l^{-1}; volume of solvent = 50 cm^3.

[b)] Values in parentheses are those obtained in a second run.

[c)] Values for the chloroform insoluble part.

For the polycondensation in benzene solution similar results were obtained to those run in diethyl ether solution (Tab. 2). The effect of various solvents on the polycondensation was studied with the ester **4e**. The results are summarized in Tab. 3.

It can be understood that no polymer was formed when the reaction was tried to carry out in protic and dipolar aprotic solvents such as methanol and DMSO. On the other hand, nonpolar solvents such as diethyl ether, carbon tetrachloride, or benzene were found to be suitable for the polycondensation. The use of chloroform or 1,4-dioxane as solvents is rather exceptional, which resulted in a low conversion.

The effect of a variety of amines on the polycondensation was next studied for the case of **4e** in diethyl ether solution. Tab. 4 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. A mole ratio 1:1 of triethylamine/**4e** is enough to perform the polycondensation in a good yield (Fig. 2), which attains to completion almost within 24 h (Fig. 3). From Fig. 4, it is clear that the highest conversion is reached at [**4e**] = 1,0 · 10^{-2} mol/dm^3.

Tab. 4. Effect of amines in the polycondensation of **4e** in diethyl ether at 25 °C, for 24 h[a)]

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

[a)] [**4e**] = 20 mmol · l^{-1}; [Amine] = 20 mmol · l^{-1}; volume of diethyl ether = 50 cm^3.

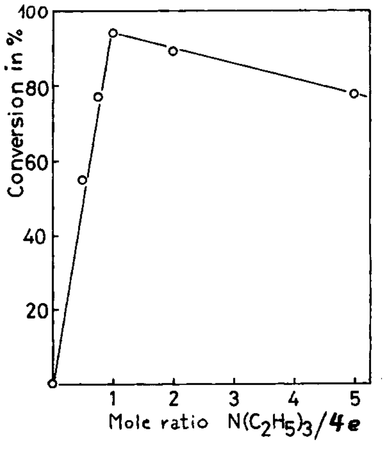


Fig. 2. Effect of triethylamine on the polycondensation of **4e**; relationship between the conversion of the water insoluble part and the amount of triethylamine. (Volume of diethyl ether = 50 cm^3; *T* = room temperature; reaction time: 24 h)

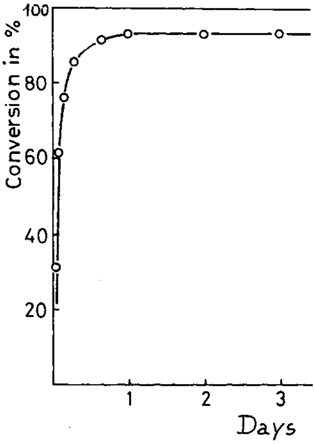


Fig. 3. Conversion of the water insoluble part as a function of the reaction time at room temperature ([**4e**] = 20 mmol · l^{-1}; [Triethylamine] = 20 mmol · l^{-1}; volume of diethyl ether = 50 cm^3)

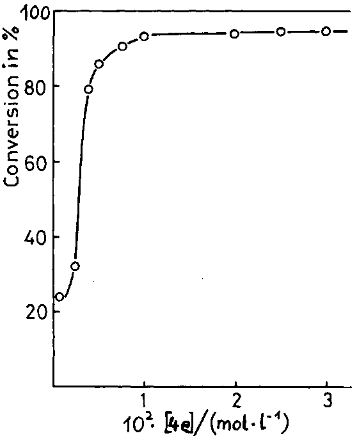


Fig. 4. Conversion of the water insoluble part as a function of the concentration of **4e** (mole ratio **4e**/triethylamine = 1 : 1; T = room temperature; reaction time: 24 h)

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 dieletric constant, the more the esters aggregate, which favors the polymer formation. The somewhat lower conversion for **4f** and **4g** may be explained by the assumption that more rigid aggregates are formed, which seem not to be suitable for the polymer formation.

Further study to support these consideration is now in progress.

Experimental Part

Materials: 4-Acylphenols as starting materials were prepared according to the method of Miller and Hartung[3] with minor modifications. The reaction of phenol with a series of acyl chlorides afforded the phenol esters, which were converted to the mixture of 2- and 4-acylphenols by the Fries rearrangement. Crude 4-acylphenols were purified by repeated recrystallization from chloroform/petroleum ether. The following 4-acylphenols were prepared: 4-acetyl-, 4-hexanoyl-, 4-octanoyl-, 4-decanoyl-, 4-dodecanoyl-, 4-hexadecanoyl-, and 4-octadecanoylphenol. The procedure used for the synthesis of active esters is described in detail for β-alanine 4-dodecanoyl-2-nitrophenyl ester (**4e**).

4-Dodecanoylphenol: To 50 cm^3 of a carbon disulfide solution, containing 32 g (0,24 mol) of anhydrous aluminium chloride, 44 g (0,20 mol) of phenyl dodecanoate were added slowly. The mixture was refluxed for 5 h with evolution of hydrogen chloride. Then, the solvent was distilled off and the content was kept further at 80–90°C for 2 h and at 140–150°C for 1 h. The residue was cooled, the aluminium complex was decomposed by adding 6 M HCl (100 ml) slowly, then alkalized with NaOH and extracted twice with 50 ml of diethyl ether to remove phenyl dodecanoate. The alkaline solution was acidified again with an HCl solution, the solidified product was filtered off, and by recrystallization from chloroform/petroleum ether 4-dodecanoylphenol was obtained in 42% yield (18 g). By evaporation of the filtrate and successive recrystallizaton from methanol, 2-dodecanoylphenol was obtained in 33% yield (13 g).

4-Dodecanoyl-2-nitrophenol (**2e**): 4-Dodecanoylphenol (55 g, 0,2 mol) was dissolved in conc. sulfuric acid (200 ml) at room temperature, and the solution was cooled to 0°C. To this solution an ice-cold mixture of conc. nitric acid (0,2 mol) and an equal volume of conc. sulfuric acid were added slowly for 1 h, and stirred for 15 min. After that, the solution was poured into 1 dm^3 of ice-cold water and kept for 1 h. The precipitate was filtered off and recrystallized from ethanol; yellow odorless crystals; m. p. 72–73°C; yield: 59 g (91%).

^{1}H NMR (CCl_4): $\delta = 0{,}9$ ($—CH_3$), 1,3 ($—(CH_2)_8—$), 1,7 ($—C\underline{H}_2—CH_2CO—$), 2,9 ($—CH_2C\underline{H}_2CO—$), 7,1, 8,1, 8,6 ($H_a$, H_b, H_c in phenol), and 10,8 (OH).

Elemental analyses and melting points for a series of 4-acyl-2-nitrophenols are given in Tab. 5.

Tab. 5. Elemental analyses and melting points of 4-acyl-2-nitrophenols

Acyl-phenol	Formula	m. p. in °C	C		H		N	
			Calc.	Found	Calc.	Found	Calc.	Found
2a	$C_8H_7NO_4$	132,5–133,5	53,04	53,06	3,90	3,83	7,73	7,95
2b	$C_{12}H_{15}NO_4$	37–38	60,75	60,54	6,37	6,34	5,90	5,86
2c	$C_{14}H_{19}NO_4$	51–52	63,38	63,58	7,22	7,25	5,28	5,34
2d	$C_{16}H_{23}NO_4$	63,5–64,5	65,51	65,26	7,90	7,98	4,78	4,63
2e	$C_{18}H_{27}NO_4$	72–73	67,26	66,93	8,47	8,62	4,36	4,29
2f	$C_{22}H_{35}NO_4$	85–86	69,99	69,78	9,35	9,48	3,71	3,67
2g	$C_{24}H_{39}NO_4$	87,5–88,5	71,07	70,87	9,69	9,85	3,45	3,37

Hydrobromide of β-alanine 4-dodecanoyl-2-nitrophenyl ester: To 50 ml of an ethyl acetate solution containing 7,2 g (22 mmol) of 4-dodecanoyl-2-nitrophenol and 5,0 g (22 mmol) of *N*-benzyloxycarbonyl-β-alanine were added 4,5 g (22 mmol) of dicyclohexylcarbodiimide (DCC) at 0°C. After stirring for 3 h at 0°C, the mixture was kept to stand overnight at room temperature and *N*,*N'*-dicyclohexylurea was filtered off. The filtrate was evaporated i. vac., 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: 9,3 g (88%).

Hydrochloride of β-alanine 4-acetyl-2-nitrophenyl ester: To 50 ml of an ethyl acetate solution containing 1,9 g (10,6 mmol) of 4-acetyl-2-nitrophenol and 2,0 g (10,6 mmol) of *N*-*tert*-butoxycarbonyl-β-alanine were added 2,2 g (10,6 mmol) of DCC at 0°C. After stirring for 3 h at 0°C, the mixture was kept to stand overnight at room temperature. The filtrate from *N*,*N'*-dicyclohexylurea was evaporated i. vac., 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 was recrystallization from ethanol. Yield: 2,5 g (81%).

Elemental analyses for **4a–g, 7a, and 7b** are summarized in Tab. 6.

Polycondensation of **4e**: To 50 ml of a diethyl ether solution containing 473 mg (1 mmol) of **4e** were added 0,14 cm^3 (1 mmol) of triethylamine. After stirring for 24 h at room temperature, the solvent was evaporated. The residue was then treated and 50 cm^3 of hot chloroform were added. The chloroform-insoluble part was filtered off and washed twice with hot chloroform to remove 4-dodecanoyl-2-nitrophenol

Tab. 6. Elemental analyses of the β-alanine esters **4a–g**, **7a**, and **7b**

Ester	Formula	C		H		N		Halogen	
		Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
4a	$C_{11}H_{13}O_5N_2Cl$	45,77	45,60	4,54	4,37	9,70	9,77	12,28	12,51
4b	$C_{15}H_{21}O_5N_2Br$	46,29	46,22	5,44	5,32	7,20	7,17	20,53	20,26
4c	$C_{17}H_{25}O_5N_2Br$	48,93	48,87	6,04	5,85	6,71	6,68	19,15	19,10
4d	$C_{19}H_{29}O_5N_2Br$	51,25	51,14	6,56	6,51	6,29	6,30	17,94	17,70
4e	$C_{21}H_{33}O_5N_2Br$	53,28	53,43	7,03	7,11	5,92	6,00	16,88	17,04
4f	$C_{25}H_{41}O_5N_2Br$	56,71	56,44	7,81	7,97	5,29	5,33	15,09	15,22
4g	$C_{27}H_{45}O_5N_2Br$	58,16	57,97	8,14	8,16	5,02	4,95	14,33	14,60
7a	$C_{21}H_{34}O_3NBr$	58,88	58,86	8,00	8,03	3,27	3,32	18,66	18,94
7b	$C_9H_{11}O_4N_2Br$	37,13	37,00	3,81	3,66	9,62	9,77	27,45	27,57

and triethylamine hydrobromide. Conversion: 78 mg (110%). The polymer was obtained as a colorless powder of m. p. 320–325 °C.

Elimination of the 4-dodecanoyl-2-nitrophenolate group: The polymer obtained (78 mg) was mixed with aqueous 4 M NaOH (20 cm^3) and stirred for 10 min on a water bath. After cooling, the mixture was neutralized by HCl, and chloroform (20 cm^3) was added therein. The insoluble matter was filtered off and dried. Colorless powder; mp. 330–335 °C. Yield: 94%.

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