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Synthesis of Poly-β-alanine from β-Alanine, β-Alanyl-β-alanine, and β-Alanyl-β-alanyl-β-alanine 4-dodecanoyl-2-nitrophenyl Esters

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SUMMARY:

Active 4-dodecanoyl-2-nitrophenyl esters of β-alanine, β-alanyl-β-alanine, and β-alanyl-β-alanyl-β-alanine were prepared, and tried to polymerize in various solvents. Non-polar solvents were found to be convenient for the polycondensation reaction. The yield of the polycondensation was high for the monopeptide ester, and less for the dipeptide and tripeptide esters. The effect of temperature on the polycondensation reaction was also studied.

ZUSAMMENFASSUNG:

Aus β-Alanin, β-Alanyl-β-alanin und dem Trimeren des β-Alanins wurden die entsprechenden 4-Dodecanoyl-2-nitrophenylester hergestellt und deren Polykondensation in verschiedenen Lösungsmitteln untersucht. Nichtpolare Lösungsmittel erwiesen sich für die Polykondensation als geeignet. Die Polykondensatausbeute war beim Monopeptidester hoch, bei den Estern der Dipeptide und Tripeptide geringer. Der Temperatureinfluß auf die Polykondensation wurde auch untersucht.

Introduction

In a previous paper[1], we reported the preparation and the polycondensation of a series of β-alanine 4-acyl-2-nitrophenyl esters with different chain lengths of the acyl groups, and found that both the yield of poly-β-alanine and the degree of polycondensation depended on the chain length of the acyl groups and the solvents used. The results were interpreted in terms of the aggregation of the active esters in certain non-polar organic solvents, which would particular-

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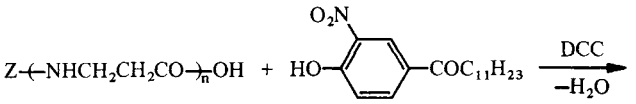
ly be expected for the longer chain groups, since the active esters with the hydrophobic long alkyl and hydrophilic amino acid group would form reversed micelles in non-polar solvents. In the successive paper[2], we further studied the effect of the amino acid structures on the polycondensation.

The present paper deals with the synthesis and polycondensation of 4-dodecanoyl-2-nitrophenyl esters containing β-alanine, β-alanyl-β-alanine, and β-alanyl-β-alanyl-β-alanine.

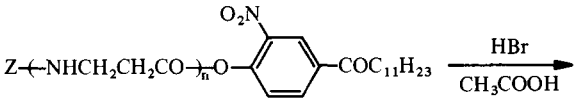
Results and Discussion

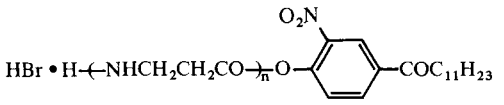
A series of active oligopeptide esters (*4-6*) were prepared by coupling reaction of the corresponding N-benzyloxycarbonyl peptides (*1-3*) with 4-dodecanoyl-2-nitrophenol by dicyclohexyl carbodiimide (DCC) method, and converted into their hydrobromide salts (Scheme 1):

Scheme 1:



1 n = 1
2 n = 2
3 n = 3



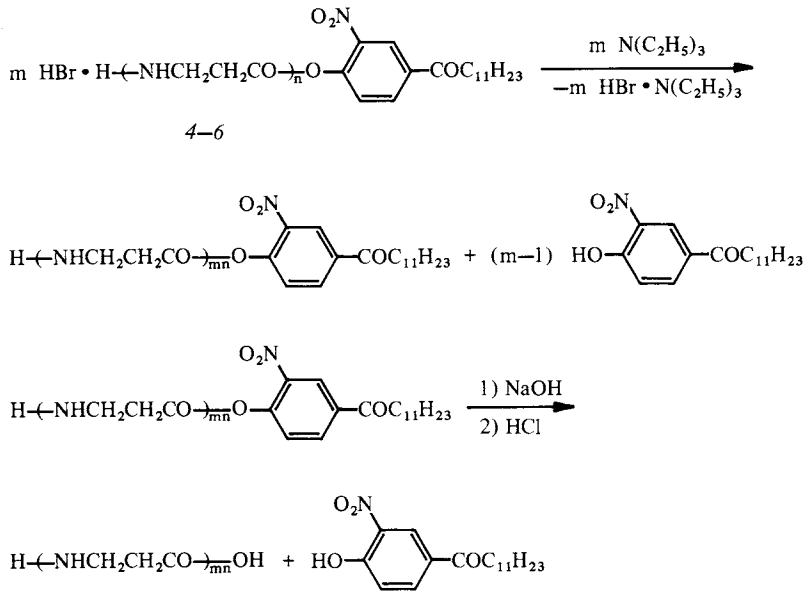


4 n = 1
5 n = 2
6 n = 3

When the hydrobromide salts of active oligopeptide esters were treated with triethylamine in various solvents at settled temperatures for 24 h, 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 treating with alkali (Scheme 2):

Scheme 2:



Results of the polycondensation for *4–6* are summarized in Tab. 1. In general, non-polar solvents such as cyclohexane, carbon tetrachloride, benzene, and diethyl ether were found to be suitable for the polycondensation. On the other hand, polymer yield was low in carbon tetrachloride if a little amount of methanol was present, and in fact, no polymer was obtained in methanol solution. These facts suggested that the aggregation would occur in non-polar solvents, while it was not the case in polar solvents such as methanol. In addition, the yield decreased with increasing number of β-alanine units. It appeared that bulkiness of the molecules resulting from the increasing number of β-alanine units disturbed such aggregation.

The effect of temperature on the yield of polycondensation products was studied with respect to *4* and *5*. Results are summarized in Tab. 2 and 3. As could be seen from these tables, the temperature was more favorable for polycondensation 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 non-aqueous solvents decreased with increasing temperature[3].

Tab. 1. Results of the polycondensation of *4* to *6* at 30 °C for 24 h[a].

Solvent	Conversion in % for the water insoluble part[b]		
	n = 1	n = 2	n = 3
Cyclohexane	90 (91)[c]	56 (51)[c]	51 (41)[c]
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] = 20 mmol · l^{-1}; [Triethylamine] = 20 mmol · l^{-1}.
[b] n = the number of β-alanine units.
[c] Values in parentheses are those obtained in a second run.

Tab. 2. Effect of temperature on the polycondensation of *4* for 24 h[a].

Solvent	Conversion in % for the water insoluble part			
	10 °C	30 °C	50 °C	reflux[b]
Cyclohexane	26 (28)[c]	90 (91)[c]	93 (93)[c]	22 (30)[c]
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] = 20 mmol · l^{-1}; [Triethylamine] = 20 mmol · l^{-1};
[b] gently refluxed;
[c] Values in parentheses are those obtained in a second run.

Fig. 1 shows typical ^{1}H NMR spectra on the water- and chloroform-insoluble polymers obtained from *5* in carbon tetrachloride solution at 30 °C ((a) and (b), respectively), together with that of authentic cyclo-di-β-alanyl (c). From (b) and (c), it was concluded that no cyclo-di-β-alanyl was formed under reaction conditions in question.

Tab. 3. Effect of temperature on the polycondensation of *5* for 24 h[a].

Solvent	Conversion in % for the water insoluble part			
	10 °C	30 °C	50 °C	reflux[b]
Cyclohexane	24 (10)[c]	56 (51)[c]	65 (67)[c]	46 (43)[c]
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] = 20 mmol · l^{-1}; [Triethylamine] = 20 mmol · l^{-1};
[b] gently refluxed;
[c] Values in parentheses are those obtained in a second run.

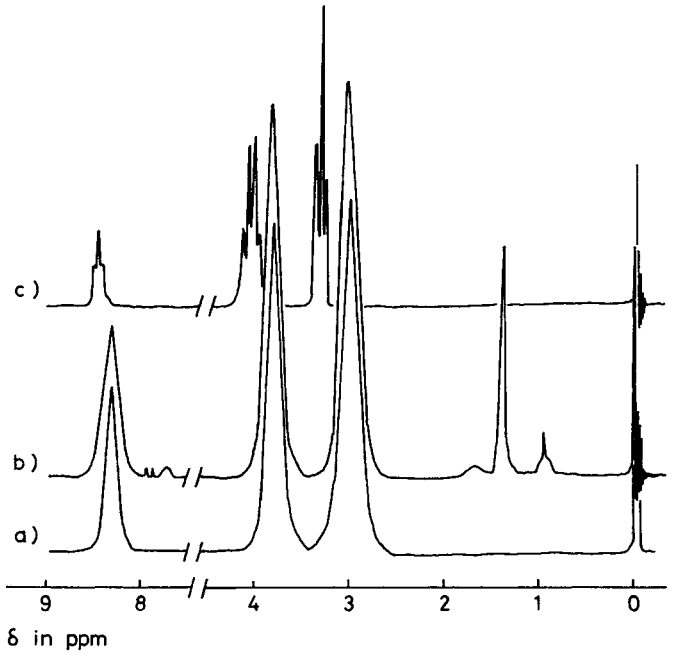


Fig. 1. ^{1}H NMR spectra for poly-β-alanine from the polycondensation of *5* in carbon tetrachloride at 30 °C (measured in trifluoroacetic acid).
a) Water insoluble part after alkali treatment,
b) Chloroform insoluble part before alkali treatment,
c) Authentic cyclo-di-β-alanyl.

Experimental Part

N-Benzyloxycarbonyl-β-alanine (1)

The compound was prepared by using the method described by Hunt and du Vigneaud[4]. The product was recrystallized from ethyl acetate/petroleum ether: mp. 105–106 °C (106 °C[5]).

N-Benzyloxycarbonyl-β-alanyl-β-alanine (2)

The compound was obtained by the coupling reaction of N-benzyloxycarbonyl-β-alanine with β-alanine ethyl ester hydrochloride using the mixed anhydride method and the successive saponification as described by Anderson[6] and Goodman[7]. The product was recrystallized from ethanol/water: mp. 144–145 °C (144–145 °C[8], 145 °C[9]).

N-Benzyloxycarbonyl-β-alanyl-β-alanyl-β-alanine (3)

This compound was prepared from N-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 (194–195 °C[10], 195 °C[9]).

β-Alanyl 4-dodecanoyl-2-nitrophenyl Ester Hydrobromide (4)

The preparation of this compound was described in detail in the previous paper[1].

β-Alanyl-β-alanine 4-dodecanoyl-2-nitrophenyl Ester Hydrobromide (5)

To 50 cm^3 of acetonitrile solution containing 6.4 g (20 mmol) of 4-dodecanoyl-2-nitrophenol[1] and 5.9 g (20 mmol) of N-benzyloxycarbonyl-β-alanyl-β-alanine *(2)* 4.1 g (20 mmol) of DCC were added 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 formed was then filtered off. The filtrate was evaporated in vac., the residue was dried. 30 g of a glacial acetic acid solution staturated with hydrogen bromide (7 N) was added to the residue. After stirring for 2 h 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 7.9 g (14.5 mmol) of a colorless crystalline substance in a 73% yield: mp. (dec.) 127–128 °C.

$C_{24}N_{38}N_3O_6Br$	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 (6)

This compound was prepared in a similar way as in the case of *(5)*, while 6.4 g (20 mmol) of 4-dodecanoyl-2-nitrophenol and 7.3 g (20 mmol) of N-benzyloxycarbonyl-β-alanyl-β-alanyl-β-alanine *(3)* were treated in dimethylformamide instead of acetonitrile. The crude product was recrystallized from formic acid/petroleum ether to give 10.8 g (17.6 mmol) of hygroscopic colorless crystals of *(6)* in a 88% yield: mp. (dec.) 134–136 °C.

$C_{27}H_{43}N_4O_7Br$	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.[11]: mp. 298–299 °C (299.5 °C[11]).

Polycondensation

50 cm^3 of carbon tetrachloride solution containing β-alanyl-β-alanine 4-dodecanoyl-2-nitrophenyl ester hydrobromide *(5)* (544.5 mg, 1 mmol) and triethylamine (0.140 cm^3, 1 mmol) were stirred for 24 h at 30 °C. After the mixture was evaporated in vac., the residue was treated with 50 cm^3 of hot chloroform, and the chloroform insoluble part was filtered and washed twice with hot chloroform to remove 4-dodecanoyl-2-nitrophenol and hydrobromide salt of triethylamine. The polymer was obtained as a colorless powder in a yield of 163.5 mg: mp. (dec.) 270–275 °C.

The NMR spectra showed that the 4-dodecanoyl-2-nitrophenyl residue had still remained in the polymer chain end.

Elimination of the 4-Dodecanoyl-2-nitrophenolate Group

The chloroform insoluble polymer (163.5 mg) was mixed with aqueous 4M NaOH (20 cm^3) and stirred for 10 min on a water bath. After cooling, the mixture was neutralized with HCl, and chloroform (20 cm^3) and water (20 cm^3) were added. The insoluble material was filtered off and dried to give colorless powder in a yield of 108 mg (76%): mp. (dec.) 330–335 °C.

From the NMR spectra, it was confirmed that the 4-dodecanoyl-2-nitrophenolate group was now removed from the polymer chain end.

[1] K. Hanabusa, K. Kondo, K. Takemoto, Makromol. Chem. **180** (1978) 307
[2] K. Hanabusa, K. Kondo, K. Takemoto, Makromol. Chem., in press
[3] H. Reerink, J. Colloid Sci. **20** (1965) 217; J. B. Peri, J. Am. Oil Chem. Soc. **35** (1958) 110; A. Kitahara, Bull. Chem. Soc. Jpn. **29** (1956) 15
[4] M. Hunt, V. du Vigneaud, J. Biol. Chem. **124** (1938) 699
[5] R. H. Siffered, V. du Vigneaud, J. Biol. Chem. **108** (1935) 753
[6] G. W. Anderson, J. E. Zimmerman, F. M. Callahan, J. Am. Chem. Soc. **89** (1967) 5012
[7] M. Goodman, R. Rupp, F. Naider, Bioorg. Chem. **1** (1971) 294
[8] H. T. Hanson, E. L. Smith, J. Biol. Chem. **175** (1948) 833
[9] V. W. Ried, K. Marquard, Liebigs Ann. Chem. **642** (1961) 141
[10] E. Adams, N. C. Davis, E. L. Smith, J. Biol. Chem. **199** (1952) 845
[11] Y. Iwakura, K. Uno, M. Akiyama, K. Haga, J. Polym. Sci., Part A-1 **7** (1969) 657