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Synthesis of a polyamide from L-aspartic acid and L-lysine

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Summary

A non-peptidic polyamide having a regular structure was synthesized by the polycondensation of N^{ε}-(β-L-aspartyl)-L-lysine via the active ester method. Amino groups of L-lysine were orthogonally protected with benzyl and *t*-butoxy carbonyl groups, and the α-carboxylic group of L-aspartic acid was masked as benzyl ester, the carboxylic group of L-lysine being activated as pentachlorophenyl ester. Coupling was achieved by the mixed anhydride method. The polycondensation of the dimeric amino acid derivative was carried out in two steps, and provided a polymer with a reduced viscosity of 0,16 dl/g (c=1%, hexafluoroisopropanol).

Introduction.

Polyamides derived from α-amino acids have been proposed as polymeric carriers of drugs because of their various properties such as low toxicity, biodegradability and the presence of functional groups for chemical coupling of the drug (1). Examples are : poly-L-lysine, poly-L-glutamic acid and poly-L-aspartic acid.

We thought an interesting approach to this subject could be the synthesis of copolyamides derived from α-amino acids with different functional side groups as it would allow us to bind different drugs and modulate the biodisponibility of that complex by introducing different biodegradable protecting groups (such as esters or amides) into the free side chains. Moreover, their processability and solubility would be improved, as compared to the polypeptides.
Here we report our first attempts to synthesize regular copolyamides derived from L-aspartic acid and L-lysine.

Results and discussion.

Among the numerous possibilities, we now present the synthesis of copolyamide **1** in which the repeating unit L-aspartyl-L-lysyl contains an isopeptidic bond :

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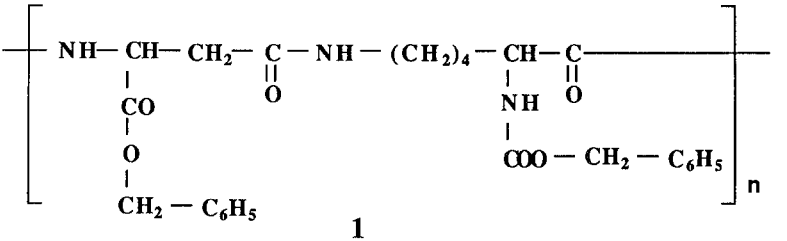
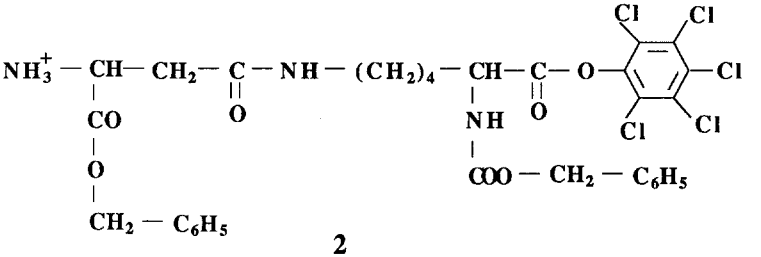


1

We have chosen to use the active ester polycondensation method ; so, we had to prepare the "heterodimer" ester 2.



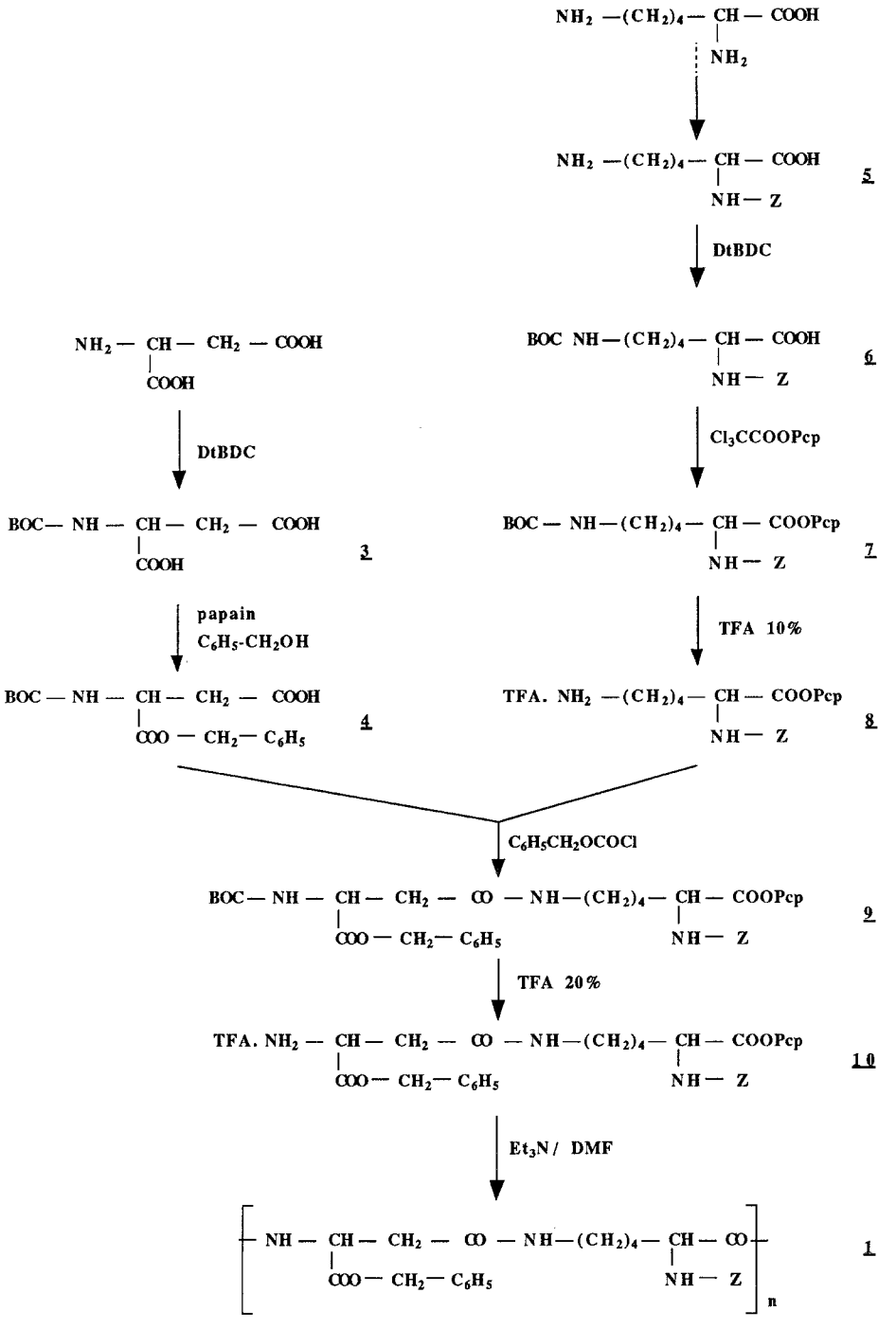
2

Several routes would lead to this heterodimer. The dipeptide ε-(β-L-aspartyl)-L-lysine was already synthesized (2). However, we have prefered to follow the series of reactions represented in Scheme 1, in which all protections and activations are achieved before coupling.

Amino groups of L-aspartic acid had to be protected with t-butyloxycarbonyl group 3 (The presence of benzyl ester group makes it impossible to use benzyloxycarbonyl group). We have used Moroder's method (3) (di-t-butyldicarbonate, DtBDC, yield 45%).

Enzymatic α-esterification of t-butyloxycarbonyl-L-aspartic acid 4 has finally been achieved by adapting Cantacuzène's method (4) to the case of benzyl alcohol. Despite the important volume of solvent needed, this procedure using free papain has given better results (yield 55% after chromatographic separation) than the immobilized papain procedure (5).

On the other hand, N^{α}-benzyloxycarbonyl-N^{ε}-t-butyloxycarbonyl-L-lysine 6 has been prepared by the classical method (condensation of ε-amino group with benzaldehyde, reaction of α-amino group with benzylchloroformate, 5, and N^{ε} protection with di-t-butyldicarbonate (6)). The activation of the carboxylic group has been carried out by transesterification with pentachlorophenyl trichloroacetate in DMF (7) to give 7, yield 79%.


NH2 —(CH2)4— CH — COOH
NH2
NH2 —(CH2)4— CH — COOH
NH — Z
5
DtBDC
NH2 — CH — CH2 — COOH
COOH
BOC NH—(CH2)4— CH — COOH
NH — Z
6
DtBDC
Cl3CCOOPcp
BOC — NH — CH — CH2 — COOH
COOH
3
BOC — NH—(CH2)4— CH — COOPcp
NH — Z
7
papain
C6H5-CH2OH
TFA 10%
BOC — NH — CH — CH2 — COOH
COO — CH2— C6H5
4
TFA. NH2 —(CH2)4— CH — COOPcp
NH — Z
8
C6H5CH2OCOCl
BOC— NH — CH — CH2 — CO — NH—(CH2)4— CH — COOPcp
COO — CH2— C6H5
NH — Z
9
TFA 20%
TFA. NH2 — CH — CH2 — CO — NH—(CH2)4— CH — COOPcp
COO— CH2— C6H5
NH — Z
10
Et3N / DMF
NH — CH — CH2 — CO — NH—(CH2)4— CH — CO
COO— CH2— C6H5
NH — Z
n
1

SCHEME 1

The study of the deprotection of 7 has shown that the BOC group is completely eliminated with 10% trifluoroacetic acid in CH_2Cl_2 at room temperature within 30 minutes, giving 8, quantitative yield.

The coupling between 4 and 8 has been achieved by the mixed carbonic-carboxylic anhydride method at -10°C, using benzyl chloroformate as a reagent and a DMF-THF mixture as a solvent. After chromatographic purification, the protected heterodimer 9 has been obtained with a yield of 33%. The infrared spectrum shows the characteristic vibration bands of : NH (3320 cm^{-1}), -CO- active ester (1783 cm^{-1}), -CO- benzyl ester (1747 cm^{-1}), -CO- carbamate (1690 cm^{-1}), -CO- amide I (1646 cm^{-1}), -NH- amide II (1532 cm^{-1})

Deprotection of 9 has been carried out with 20% trifluoroacetic acid in CH_2Cl_2 for 30 minutes. After washing, heterodimer 10 has been obtained in a nearly quantitative yield.

The polycondensation has been carried out by a two-step method in order to avoid attacks by the acidic pentachlorophenol released. The heterodimer has first been submitted to polycondensation at room temperature in DMF in the presence of an excess of triethylamine. After separation and washings, the polyamide has been obtained with a 66% yield. The reduced viscosity in hexafluoroisopropanol (HFIP, c=0,725%) at 25°C was O,O6 dl/g and its infrared spectrum showed the presence of active ester band, confirming the low molecular weight.

A second step conducted in trichlorobenzene at 60°C provided a product whose reduced viscosity at 25°C in HFIP (c=1%) reached 0,16 dl/g. Its infrared spectrum no longer showed the presence of active ester vibration band. Differential thermal analysis (DTA) curve presented a very wide peak (degradation) between 180 and 250°C.

The melting point and the reduced viscosity are in agreement with a low degree of polymerisation (DP). Elemental analysis of C (theoretical for repeating unit 64,22% ; found : 60,63%) agrees with DP=5 expressed in heterodimer repeating units (C Th. = 60,4%). We have tried to roughly estimate the DP with the help of viscosity formulas of polyhexanoamide (nylon 6) in trifluoroethanol, in spite of many differences (solvent, repeating unit, structure). We obtained a value of DP=3,5 (molecular weight 1650) using Mark-Houwink-Sakurada constants reported by Hay et al. (8) and DP=4,3 (molecular weight 2000) with those of Mattiussi et al. (9). In any case, the DP value would not be far from 5, expressed in heterodimer units, i.e. 10 in amino acid units.

Experimental.

N^{ε}-(N-t-butyloxycarbonyl-L-aspartyl-α-benzyl ester)-N^{α}-benzyloxycarbonyl-L-lysyl-pentachlorophenyl ester 9. The coupling of 4 and 8 was carried out as follows : a solution of 2 ml (9,9 mmol) of benzyl chloroformate in 20 ml of THF was stirred and cooled to -10°C and 1,35 ml (9,9 mmol) of triethylamine was added. After one minute, a

cooled solution of 3,2 g (9,9 mmol) of **4** was added. One minute later, two previously cooled (-10°C) solutions of 6,3 g (9,9 mmol) of **8** in 50 ml of DMF and of 1,35 ml (9,9 mmol) of triethylamine in 20 ml of THF were added dropwise. Stirring was continued for 10 min. at -10°C, then the bath was removed and the mixture allowed to warm to room temperature. The mixture was concentrated to one third and poured into 700 ml of cold water. The solid was filtered, dried and purified by column chromatography using $CHCl_3$ as an eluant. **9** was obtained as a white solid, 2,72 g (33%) ; m.p.=178°C.
IR spectrum (KBr) : 3320 cm^{-1} (NH), 1783 cm^{-1} (CO active ester), 1747 (CO, benzyl ester), 1690 cm^{-1} (CO carbamate), 1646 cm^{-1} (CO amide I) 1532 cm^{-1} (NH, amide II).
^{1}H-NMR ($CDCl_3$) : d=7,3 (m, phenyl), 5,1 (s, $\underline{CH_2}Ph$), 2,8(d, aspartic acid), 1,4 (s, t-butyl). Intensity ratio : 10:4:2:9

N^{ε}-(L-aspartyl α-benzyl ester trifluoroacetate)-N^{α}-benzyloxy-carbonyl-L-lysyl pentachlorophenyl ester 10. Deprotection of **9** with 20% trifluoroacetic acid in CH_2Cl_2 at room temperature. After 30 min., the solution was evaporated under vacuum and coevaporated twice with toluene and then with ether. **10** was obtained, after drying in a vacuum dessicator, as a white solid, m.p.= 88°C, with a quantitative yield.

Polycondensation of 10. The polycondensation has been carried out as follows.
First step : to a solution of 850 mg (1 mmol) of **9** in 8 ml of DMF, 139 µl (1 mmol) of triethylamine was added with stirring. After 15 min. the solution was too viscous to be stirred. After 24 h at room temperature, water was added and the suspension was triturated and centrifugated. This operation was repeated with water (x2), methanol (x2) and ether (x2). The compound **1** was obtained as a white solid, 310 mg (66%) $[\eta]$= 0,06 dl/g.
IR spectrum (KBr) : 3309 cm^{-1} (NH) ; 1774 cm^{-1} (-CO- active ester) ; 1692 cm^{-1} (-CO- carbamate) ; 1656 cm^{-1} (-CO- amide I) ; 1538 cm^{-1} (NH amide II).
^{1}H-NMR ($CDCl_3$) : d= 8,3 (broad s, $\underline{NH}$ Cbz) ; 8,1 (broad s $\underline{NH}CO$) ; 7,9 (broad s $\underline{NH}CO$ isopeptidic) ; Intensity ratio : 1:<1:1.
Second step : a mixture of 0,129 g of the preceding prepolymer, 0,3 ml of trichlorobenzene and 0,12 ml of triethylamine was maintained at 60°C for 2 hours. The polymer was then precipitated with ether. Purification by reprecipitation in ether from hexafluoroisopropanol has provided 0,076 g of polyamide. Degradation of this polymer starts at 170°C. Infrared spectrum shows no CO active ester band at 1774 cm^{-1}.

Elemental analysis : repeating unit $C_{25}H_{29}O_6N_3$
Th. % C : 64,22 H : 6,25 N : 8,98
F. % C : 60,63 H : 5,79 N : 8,63

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