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Synthesis and characterization of polyamides based on natural monomers: L-lysine and L-aspartic acid

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Summary

Nonpeptidic diamine-diacid type polyamides were prepared from natural α-amino acids, L-lysine and L-aspartic acid, under mild conditions. L-lysine carboxylic group was protected as a benzyl ester; L-aspartic acid amino group was protected as benzyloxycarbonyl (Z) or *t*-butyloxycarbonyl (BOC) derivatives. The activated ester method provided polyamides with protected amino and carboxyl side groups. The deprotection of these side groups revealed to be perfectly selective when the amino groups were protected as *t*-butyloxycarbonyl derivatives.

Introduction

Synthetic polyamides are very attractive compounds because they possess good material properties and can easily be processed. Biomedical field needs new materials for sutures and drug-delivery systems. Polyamides could be suitable if they were biocompatible and biodegradable. Until now, very few polyamides -essentially poly(α-L-amino acid)s- fulfill these requirements. For example, Miyamae (1) showed that derivatives of poly(α-L-glutamic acid) provided interesting sutures. Gonsalves (2) recently rewieved the degradable polyamides. In previous papers we described the synthesis of nonpeptidic α-amino acid containing polyamides (3-6). This article is devoted to four new nonpeptidic functionalized polyamides, containing only two natural α-amino acids: L-lysine and L-aspartic acid. Enzymatic or hydrolytic degradation should convert these polymers into metabolizable compounds. Moreover, their two different side groups could be used to bind a drug and/or a solubilizing group.

Experimental

IR characterizations were realized with a FTIR IFS 45 Brüker apparatus. Wave numbers are given in cm^{-1}. The NMR spectra were realized with an AC 200 Brüker spectrometer. Tetramethylsilane (TMS) was the reference for all chemical shifts (in ppm).
N-benzyloxycarbonyl-L-aspartic acid was synthesized according to Zervas (7) and N-*t*-butyloxycarbonyl-L-aspartic acid according to Moroder (8).

N-benzyloxycarbonyl dipentafluorophenyl-L- aspartate

In a typical way, 5,35 g (0,02 mol) of N-benzyloxycarbonyl L-aspartic acid dissolved in 100 mL of acetone was reacted with 9,5 g (0,045 mol) of dicyclohexylcarbodiimide (DCCI) and 8,5 g (0,045 mol) of pentafluorophenol. The reaction was allowed to proceed under stirring overnight. The precipitate (essentially dicyclohexylurea, DCHU) was filtered. Acetone was removed under vacuum. The residue was treated with 80 mL of acetone. The insoluble part (mostly DCHU) was discarded. This purification was repeated twice. Total removal of acetone gave 9,5 g of the desired compound (79%).

M = 599,24 g/mol. m.p. = 137°C. IR (KBr, cm^{-1}): 3327 (NH); 1783 (CO ester); 1693 (CO carbamate, amide I); 1541 (Amide II); 1524 (pentafluorophenyl moiety). ^1H NMR ($CDCl_3$): 3,5 (CH_2, 2H); 5,1 (CH and CH_2 benzyl, 3H); 5,8 (NH, 1H); 7,3 (CH aromatic, 5H). ^{13}C NMR ($CDCl_3$): 35,7 (CH_2); 50,0 (CH); 67,8 (CH_2 benzyl); 126,1 and 126,6 (C aromatic); 135,4 (C quaternary); 124,0, 136,5, 140,4 and 143,4 (CF); 155,7 (CO carbamate); 166,4 and 166,7 (2 CO ester). For BOC derivative, ^1H NMR ($CDCl_3$): 1,5 (CH_3, 9H); ^{13}C NMR ($CDCl_3$): 28,1 (CH_3); 78,3 (C quaternary).

Polycondensation

0,5 g of N,N'-L-lysinium benzylester di-*p*-toluenesulfonate (9, 10) (8,62 10^{-4} mol) and 0,52 g of N-benzyloxycarbonyl dipentafluorophenyl-L-aspartate (8,62 10^{-4} mol) were mixed during 15 minutes in the solid state. 0,66 mL of diisopropylethylamine and 0,72 mL (c = 1,2 mol/L) of THF were then added under nitrogen. A rapid dissolution was observed. The polycondensation was allowed to proceed at 60°C during 6 hours. After cooling to room temperature, the polymer was precipitated by addition of THF and then washed for several hours with water, filtered and dried under vacuum. With BOC derivative, the reaction mixture remained homogeneous even at room temperature; the polymer was precipitated by addition of water. Inherent viscosities were determined in dichloroacetic acid (DCA) at 25°C, c = 1%.

Repetitive unit M = 467,52 g/mol. IR (KBr, cm^{-1}): 3383 (NH); 3069-3039 (CH aromatic); 2937-2885 (CH_2); 1740 (CO ester); 1697 (CO carbamate); 1652 (Amide I); 1539 (Amide II). ^1H NMR (DMSO-d6): 1,2 (CH_2 lysine, 4H);1,7 (CH_2 lysine, 2 H); 2,7 (CH_2 aspartic, 2H); 3,0 (CH_2 lysine, 2H); 4,3-4,5 (CH lysine and aspartic, 2H); 5,0(CH_2 benzyl , 2H); 5,1(CH_2 Z, 2H); 7,3 (CH aromatic, 10H); 7,7, 7,9 and 8,2 (NH, 3H). ^{13}C NMR (DMSO-d6): 22,7, 28,8, 30,7 and 39,5 (CH_2 lysine); 30,4 (CH_2 aspartic); 52,1 (CH lysine and aspartic); 65,5-65,7 (CH_2 benzyl and Z); 127,8 (C aromatic); 136,0, 136,8 (C quaternary); 155,7 (CO carbamate); 166,6, 168,8, 169,3 and 169,6, (4 CO amide); 171,9 (CO ester); $[\alpha]_{25}^{578}$= 10° (dichloroacetic acid, c = 1%). For BOC derivatives: ^1H NMR (DMSO-d6): 1,5 (CH_3, 3H). ^{13}C NMR (DMSO-d6): 28,1 (CH_3); 78,1 (C quaternary).

Deprotection of N-benzyloxycarbonyl polymer I

- *HBr method*: 200 mg of totally protected polymer I was treated at room temperature by 5,0 mL of HBr/CH_3COOH (7,5%). When desired, the polymer was precipitated by addition of ether, carefully washed and filtered.
- *TFA/MSA/Anisole method*: 200 mg of totally protected polymer I were dissolved in 2,0 mL of trifluoroacetic acid (TFA) at room temperature. The mixture was brought to the chosen temperature, and reacted with 2 mL of methanesulfonic acid (MSA) and 0,5 mL of anisole. The polymer was recovered as described above.

Totally deprotected polymer (TFA/MSA/anisole method) ^1H NMR (DMSO-d6): 1,3 (CH_2 lysine, 4H); 1,7 (CH_2 lysine, 2H); 2,7 (CH_2 aspartic, 2H); 3,0 (CH_2 lysine, 2H); 4,0-4,2 (CH lysine and aspartic, 2H); 8,1 (NH_3^+, 3H); 12,5 (COOH, 1H). ^{13}C NMR (DMSO-d6): 23,0, 28,6, 30,8 and 39,5 (CH_2 lysine); 35,7 (CH_2 aspartic); 49,5 (CH aspartic); 52,3 (CH lysine); 167,7, 168,4, 168,6 and 168,9) (4 CO amide), 173,1 and 173,5 (2 CO acid).$[\alpha]_{25}^{578}$= 4° (dichloroacetic acid, c = 1%).

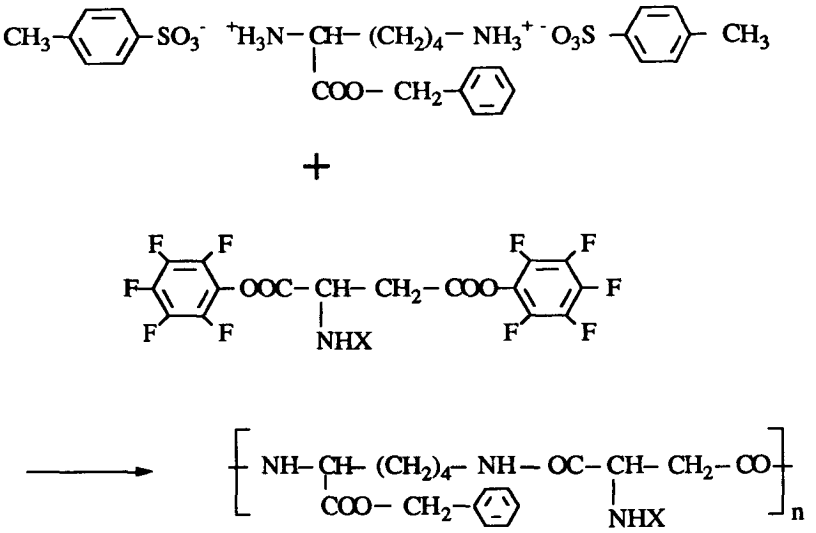
Selective deprotection of N-t-butyloxycarbonyl polymer II

1,0 g of N-tertiobutyloxycarbonyl protected polymer II (2,31 10^{-3} mol) was dissolved in 18 mL of trifluoroacetic acid (TFA). The mixture was kept under stirring for 1 hour at room temperature. The entirely N-deprotected polymer was recovered by addition of diethyl oxide. The polymer was carefully washed, filtered and dried. Repetitive unit M = 447,19 g/mol. ^1H NMR (DMSO-d6): 1,3 (CH_2 lysine, 4H); 1,7 (CH_2 lysine, 2H); 2,7(CH_2 aspartic, 2H); 3,0 (CH_2 lysine, 2H); 4,0-4,3 (CH lysine and aspartic, 2H); 5,1 (CH_2 benzyl, 2H); 7,3 (H aromatic, 5H); 8,2 (NH_3^+, 3H); 8,4, 8,7,8,9 (NH, 3H). ^{13}C NMR (DMSO-d6): 22,8, 28,5, 30,6 and 39,5 (CH_2 lysine); 35,7 (CH_2 aspartic); 49,3

(CH aspartic); 52,3 (CH lysine); 66,3 (CH_2 benzyl); 128,2 (C aromatic); 136,0 (C quaternary); 167,7, 168,3, 171,1 and 171,4 (4 CO amide); 173,1 and 173,4 (2 CO ester)

Reprotection of amino groups
500 mg of totally deprotected polymer (TFA/MSA/anisole method) was dissolved in 30 mL of water and the mixture was cooled to 2°C. pH was ajusted to 10-11 by addition of NaOH M. 0,25 mL of benzyloxycarbonyl chloride was added at once. NaOH M was progressively added to maintain pH at 9-10. After completion of the reaction (3 h), the polymer was precipitated by HCl 5M (pH 3), filtered and dried. Yield: 280 mg (45%).
Repetitive unit M = 377 g/mol. ^1H NMR (DMSO-d6): 1,3 (CH_2 lysine, 4H); 1,7 (CH_2 lysine 2H); 3,0 (CH_2 lysine, 2H); 2,5 (CH_2 aspartic); 4,1 (CH lysine and aspartic, 2H); 5,0 (CH_2 Z, 2H); 7,3 (CH aromatic, 5H); 7,7, 7,9 and 8,1 (NH, 3H); 12,6 (COOH, 1H). ^{13}C NMR (DMSO-d6): 22,8, 28,7, 30,9 and 39,5 (CH_2 lysine); 30,9 (CH_2 aspartic); 65,6 (CH_2 Z); 127,8-128,3 (CH aromatic); 137 (C quaternary); 155,7 (CO carbamate); 169,1, 169,4 and 171,0 (CO amide); 173,7 (CO acid). $[\alpha]_{25}^{578} = 17°$ (dichloroacetic acid, c = 1%).

Results and discussion
Various authors previously described the synthesis of diamine-diacid type polymers based on L-lysine. Some of them followed the interfacial route (11-13). Katsarava *et al.* (14), Muñoz-Guerra *et al.* (15) used the active ester method to prepare polyamides by reacting N^{α},N^{ε}-bis(trimethylsilyl)L-lysine alkylester with active dicarboxylic derivatives (dipentachlorophenyl esters for example). The polymers obtained can present satisfactory molecular weights. We obtained high molecular weights by simply using pentafluorophenyl esters of aliphatic diacids (adipic and glutaric acids) (6). However, contrarily to poly(L-cystyl-L-cystine), these polymers revealed to be relatively resistant to enzymatic and hydrolytic degradations (6). That is the reason why we chose to replace adipic acid by a natural α–amino diacid, L-aspartic acid. In a previous paper (3), we already described the synthesis of an alternate ω–amino acid type polyamide, containing protected β-L-aspartic acid and ε-L-lysine. The starting heterodimer was obtained by a very laborious method, involving many intermediate compounds and the two-step polycondensation led to low molecular weights. These two problems drastically limited its potentiality as a biomaterial. In the present paper, we describe the synthesis of a new family of four diamine-diacid type polyamides based on L-aspartic acid and L-lysine. Important amounts of the starting monomers can easily be obtained and purified. The presence of amino and carboxylic side groups in the neighborhood of the amide bonds should give interesting information on the conditions necessary to their cleavage.
Carboxylic group of L-lysine was protected as a benzyl ester, that can readily be removed by catalytic hydrogenolysis or by reaction with the mixture TFA/MSA/anisole (6, 16).
Activation of L-aspartic acid as a dipentafluorophenyl ester implies the protection of its amino group. Benzyloxycarbonyl group (Z) is known to be easily removable. But, we already noted that problems can arise for a purely selective deprotection in the presence of benzyl ester groups (5, 6). That is the reason why we also tested another protecting group, the *t*-butyloxycarbonyl group (BOC), orthogonal to Z and benzyl ester groups. We synthesized the dipentafluorophenylester by the DCCI method. Usually, this synthesis is carried out in THF or dichloromethane. In the present case, the elimination of the by-product (dicyclohexylurea, DCHU) revealed to be laborious in these solvents. The best results were obtained with acetone for reaction and purification, which allowed us to obtain the two N-protected L-aspartates containing less than 2% of DCHU (determined by ^1H NMR). Polycondensations were performed according to scheme I.
The polycondensations were carried out in solution for 6 hours, in the presence of a tertiary amine: diisopropylethylamine was used to neutralize the *p*-toluenesulfonic acid and the pentafluorophenol released. The salts formed were removed by washing with



with X = $C_6H_5CH_2OCO$ (P I) or X = $(CH_3)_3COCO$ (P II)

Scheme I

water. IR (KBr pellet), ^1H and ^{13}C NMR spectra (in dimethyl sulfoxide, DMSO-d6) showed the expected structure. The amide linkages could be characterized on IR spectra (1652 cm^{-1} amide I and 1539 cm^{-1} amide II), on ^1H NMR spectra (NH protons in the range 7,8-8,2 ppm), and on ^{13}C NMR traces (four peaks between 166 and 170 ppm, corresponding to the carbonyl groups of the four different amide bonds: α-α', α-ε, β-α', β-ε).

Since the active diester was not totally exempt of dicyclohexylurea, we varied the stoechiometric ratio of L-aspartate/L-lysine ester from 1 to 1,05. In tetrahydrofuran (THF, 60°C, 1,2 mol/L), the inherent viscosity (25°C, dichloroacetic acid, DCA, c = 1%) was constant for the range 1-1,02 (η = 0,19 dL/g) and significantly dropped for 1,05 (η = 0,13 dL/g).

Influence of the solvent

The influence of the solvent (60°C, 1,2 mol/L) was tested. The results obtained for **P I** are collected in table 1.

Solvent	η_{inh} (dL/g)
$CHCl_3$	0,15
THF	0,26
CH_3CN	0,14
HMPA	0,13
DMF	0,18
TCB	0,11

HMPA: hexamethylphosphorotriamide;
DMF: dimethylformamide; TCB: trichlorobenzene

Table I: Influence of the nature of the solvent on **P I** viscosity

In all cases, polymer I was soluble at 60°C, but insoluble at room temperature in THF. As we already noted for poly(adipoyl-L-lysine) (6), THF provided higher results for the polymer viscosities, that however are low. By amino end-group determination, the molecular weight of a polymer whose inherent viscosity was 0,23 was estimated to 6600 g/mol, which corresponds to 14 L-lysine and 14 L-aspartic units. Increasing the temperature to 80°C in THF (in sealed tubes) and the concentration to 1,5 mol/L did not significantly increase the viscosity. For higher concentrations, solubility problems occurred. These results seem to be higher than those obtained in ref. 3. However, they remained low and should be improved by trimethylsilylation of L-lysine benzyl ester amino groups.
Polycondensation of BOC derivative in THF was carried out for four hours at 60°C, followed by two hours at 80°C. The reaction mixture remained homogeneous even at room temperature. The polymer had to be precipitated with water. We noted a slight dependence of inherent viscosity versus concentrations of the comonomers. BOC-L-aspartic acid being much more soluble than the corresponding Z-derivative, we could test concentration up to 3,0 mol/L. (c = 1,5 mol/L, η_{inh} = 0,24; c = 1,7 mol/L, η_{inh} = 0,27).

Deprotection reactions

Selective and total deprotections of N-benzyloxycarbonyl polymer ***P I.***
We previously showed that benzyloxycarbonyl groups could be removed from entirely protected poly(L-cystyl-L-cystine) (4) and heterotrimer L-lysine-adipoyl-L-lysine (6) by HBr method with satisfactory results. We applied this method to **P I** varying both duration of the reaction and concentration of HBr solution. The results are collected in table 2.

HBr (%)	Time (min)	% Z left	% Bz left
2,5	360	10	100
7,5	15	53	100
7,5	30	20	100
7,5	45	20	100
33	4320	0	0

Table 2: Deprotection of **P I** with HBr

With low HBr concentration (2,5 and 7,5%), the partially deprotected polymer precipitated as a gum after about 20 minutes at room temperature when 80% of the benzyloxycarbonyl groups were removed. By considerably increasing the reaction time with 2,5% HBr solution (6 hours), we could obtain a polymer containing only 10% of Z groups.
In order to avoid this precipitation, the polymer (200 mg) was first dissolved in 5 mL of trifluoroacetic acid (TFA), and reacted with HBr (0,32 mL of 33% HBr, giving 2% HBr solution) for two hours. In the polymer recovered by precipitation with diethyl oxide, Z groups were totally absent and the rate of benzyl ester group was 50%.

All benzyloxycarbonyl and benzyl ester groups could be removed by action of concentrated HBr/CH_3COOH (33%) within three days. However, the polymer was slightly yellow.
So we tested a method which led to good results with poly(L-cystyl-L-cystine). According to this method, polymer **I** dissolved in TFA was reacted with methanesulfonic acid (MSA) in the presence of anisole as a scavenger. The results are collected in table 3.

T°C	Time (min)	% Z left	% Bz left
10	5	45	100
10	30	0	50
10	180	0	0
5	15	20	80
5	60	0	40
0	15	50	100
0	60	10	40
0*	30	55	100
0*	60	25	80

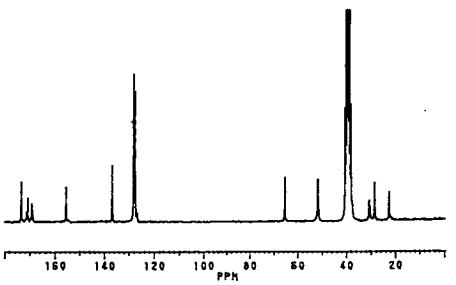
* ratio TFA/MSA/Anisole: 10/2/0,5

Table 3: Deprotection of polymer I by TFA/MSA/anisole method

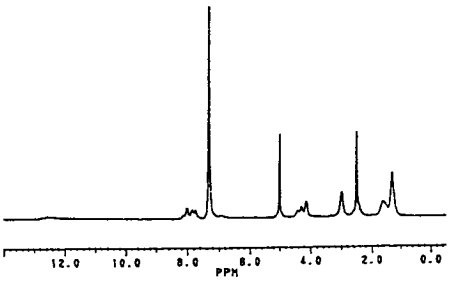
A blank realized with TFA alone showed no reaction at 18°C. The results of table 3 show that this method is not convenient for a selective regeneration of free amino groups. Total removing of Z groups while keeping all benzyl ester groups seemed impossible. But a totally deprotected polyamide bearing NH_3^+ and carboxylic free side groups could be obtained within 3 hours at 10°C.

Deprotection of N-t-butyloxycarbonyl polymer II.
The synthesis of a polymer bearing benzyl ester and free amino groups led us to the use of *t*-butyloxycarbonyl as a protecting group. The deprotection of all amino groups was realized by dissolution in trifluoroacetic acid and reaction for 1 hour. On ^{13}C NMR spectra, we noted the absence of the peak at 76 ppm relative to the quaternary carbon of the *t*-butyloxycarbonyl group. ^1H NMR spectra showed up the presence at 5,1 ppm of the peak corresponding to the CH_2 of the benzyl ester corresponding to 2 hydrogen atoms. No benzyl ester group was cleaved by TFA, but these groups could be removed by action of the mixture TFA/MSA/anisole as described above. The polycation thus obtained was easily soluble in water despite the presence of hydrophobic benzyl groups.

Reprotection of amino groups.
The entirely deprotected polymer, wich is water soluble, could be used to fix a drug directly or through a spacer. Linking a drug on amino groups could be realized on this polymer. But linking a drug on carboxylic group (by an ester or amide bond) implies the reprotection of amino group. This was realized by action of benzyloxycarbonyl chloride at 2°C, in basic medium. The degree of reprotection was 100%, determined by ^1H NMR. Finally, starting from polymer I (η_{inh}= 0,19 dL/g), we obtained totally deprotected polymer by TFA/MSA/anisole method (η_{inh}= 0,23 dL/g) and N-reprotected polymer (η_{inh}= 0,19 dL/g).



^{13}C NMR spectrum
of N-reprotected polymer



^1H NMR spectrum
of N-reprotected polymer

The solubilities of the polymers described above are collected in table 4.

Solvent	Polymer				
	I	II	III	IV	V
DMSO	+	+	+	+	+
THF	-	+/-	-	-	-
DCA	+	+	+	+	+
H_2O	-	-	+	+	-
Tris buffer 7,4 (0,2M)	-	-	+	-	+

I: completely protected polymer (Z); II: completely protected polymer (BOC); III: completely deprotected polymer by HBr or TFA/MSA; IV: N-deprotected polymer; V: N-reprotected polymer

Table 4: Solubilities of the various polymers

All polymers are soluble in DMSO. Polymer II that remained soluble in the reaction mixture (THF), could not be dissolved again after separation. The totally deprotected polymers are soluble in pure water and tris buffer 7,4, which is an important result for a biomedical application. But the N-deprotected polymers which can be dissolved in pure water, are insoluble in Tris buffer pH 7,4 (because of the neutralization of the counter-ion of amino group).

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