SYNTHESIS OF NEW BRANCHED POLYPEPTIDES WITH POLY(LYSINE)BACK BONE

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New analogues of branched polypeptides were synthesised for a further, more detailed study of the influence of the side chain terminating amino acids, particularly the hydrophobic character, configuration and the number of these amino acids, on the conformation and biological properties of the polymers. The following amino acids were coupled to poly(L-Lys-(DL-Ala_m)) in suitably protected and activated forms to study the above mentioned aspects: L-Nle, L-Ile, L-Val, L-Phe, D-Phe, D-Leu, D-Tyr, D-His, L-Glu, D-Glu, L-Lys, D-Lys and additionally the L-Glu-L-Glu, D-Glu-D-Glu, L-Lys-L-Lys and D-Lys-D-Lys dipeptides. The deprotected and purified end products were freezedried and characterized by various methods.

In the past few years we have developed a new group of high molecular weight, synthetic, branched polypeptides with poly(L-lysine) backbone[1]. Short DL-alanine oligomers were grafted to the lysine ε-amino groups by polymerisation of N^{α}-carboxy--DL-alanine anhydride. The side chain ends were covered to various degree with one particular amino acid or its oligomers. In view of the recently increased interest in biological response modifiers we were particularly interested in the immunomodulatory properties of these branched polypeptides[2]. Moreover, these polypeptides – as a versatile model system – seemed to be a realistic tool to analyse the influence of individual amino acids upon the conformation of the macromolecule and to establish the conformational requirements for the design of polypeptides with the most favourable biological properties.

The conformation of the new group of branched polypeptides has been analysed based on the CD spectra in solution[3] at various ionic strength and pH (ref.[4]). These measurements indicated a marked dependence of the conformation in solution upon the quality, charge and configuration of the side chain terminating amino acid and the number of its residues present.

While side chains consisting of DL-alanine residues only (approx. 3 residues) decrease slightly the α-helix forming capacity of the polymer as compared to poly-(L-lysine), the presence of leucine as side chain terminating amino acid increases the α-helix forming capacity and even at pH 7·4, in solution of low ionic strength, an ordered structure can be registered. A very important CD determining factor

is the absolute configuration of the side chain terminating amino acid (L- and D-glutamic acid, L- and D-lysine oligomers). Finally, the highest independent contribution of the side chains to the conformation of the molecule was observed in the case of polypeptides containing 3 glutamic acid residues at the side chain ends. These three main conformational tendencies seemed to warrant a more systematic analysis. Therefore in order to analyse these particular aspects further model compounds were selected and their synthesis is presented in this paper. The results of the conformational studies with some of these polypeptides are presented in the subsequent article.

EXPERIMENTAL

Intermediates

The following amino acid derivatives were synthesised according to procedures described in the literature (amino acids are of L-configuration unless otherwise stated): γ-benzyl-L-glutamic acid[5], γ-benzyl-D-glutamic acid[5], N-benzyloxycarbonyl-γ-benzyl-L-glutamic acid[6]. N-benzyloxycarbonyl-γ-benzyl-D-glutamic acid[6], N-benzyloxycarbonyl-γ-benzyl-L-glutamic acid pentachlorophenyl ester[7], N^{α},N^{ε}-bis(benzyloxycarbonyl)-L-lysine[8], N^{α},N^{ε}-bis(benzyloxycarbonyl)-L-lysine pentachlorophenyl ester[9], N-benzyloxycarbonyl-L-phenylalanine pentachlorophenyl ester[9], N-benzyloxycarbonyl-L-valine pentachlorophenyl ester[9], N-benzyloxycarbonyl-L-isoleucine pentachlorophenyl ester[9], N-benzyloxycarbonyl-D-phenylalanine[10], N-benzyloxycarbonyl-D-leucine[11], N--benzyloxycarbonyl-L-norleucine[12], D-tyrosine ethyl ester hydrochloride[13], D-histidine methyl ester dihydrochloride[14], N-benzyloxycarbonyl-D-histidine hydrazide[10], N-t-butoxycarbonyl--γ-benzyl-L-glutamic acid[15], N-t-butoxycarbonyl-γ-benzyl-D-glutamic acid[15], N-t-butoxycarbonyl-γ-benzyl-L-glutamic acid pentachlorophenyl ester[16], N^{ε}-benzyloxycarbonyl-L-lysine[17], N^{ε}-benzyloxycarbonyl-D-lysine[18], N^{ε}-benzyloxycarbonyl-L-lysine methyl ester[19], N^{ε}-benzyloxycarbonyl-D-lysine methyl ester hydrochloride[19], $N^{\alpha}N^{\varepsilon}$-bis(benzyloxycarbonyl)-L-lysyl-N^{ε}-(benzyloxycarbonyl)-L-lysine hydrazide[20].

N^{α},N^{ε}-Bis(benzyloxycarbonyl)-D-lysine

This compounds was synthesised by the method reported[8] in the literature for the L-lysine derivative. Yield: 53%, m.p. 80°C, R_F(b) 0·68. $[\alpha]_D^{20}$ +9·25 (*c* 2, methanol). For $C_{22}H_{26}N_2O_6$ (414·5) calculated: 63·75% C, 6·32% H, 6·76% N; found: 64·1% C, 6·78% H, 6·4% N.

Synthesis of Pentachlorophenyl Esters

The method of Kovács and coworkers[9] was adopted; characterization and analytical data are presented in Table I.

7·00 g (15·7 mmol) N-Benzyloxycarbonyl-L-norleucine dicyclohexylamine salt was suspended in 100 ml ether and treated (in two portions) with 43 ml (15·7 mmol) 5% $KHSO_4$ solution. After separation the water layer was extracted two times with ether. The combined ether extracts were dried over Na_2SO_4, the solvent evaporated and the residue dissolved in 20 ml ethyl acetate.

4·18 g (15·7 mmol) pentachlorophenol was dissolved in 63 ml ethyl acetate and cooled to 0°C. 3·24 g (15·7 mmol) dicyclohexylcarbodiimide dissolved in 8 ml ethyl acetate was added.

TABLE I

Protected pentachlorophenyl esters

Compound	M.p., °C	$[\alpha]_D^{20}$ (*c*, solvent)	R_F(b)	Formula (m.w.)	Calculated/Found			
					% C	% H	% N	% Cl
Z-D-Glu(OBzl)-OPcp	133–134	+14·4° (0·66)[a]	0·81	$C_{26}H_{20}Cl_5NO_5$ (619·7)	50·39 50·74	3·25 3·01	2·26 2·35	28·61 28·0
Z-D-Lys(Z)-OPcp	156–157	+6·1° (1, $CHCl_3$)	0·83	$C_{28}H_{26}Cl_5N_2O_6$ (66·8)	50·74 50·6	3·8 4·23	4·22 4·03	26·75 27·8
Z-D-Phe-OPcp	156–157	+50·7° (1)[b]	0·92	$C_{23}H_{16}Cl_5NO_4$ (547·7)	50·44 50·82	2·94 3·28	2·56 2·25	32·37 32·74
Z-D-Leu-OPcp	126–127	+31·9° (0·45)[c]	0·93	$C_{20}H_{18}Cl_5NO_4$ (513·7)	46·77 46·96	3·53 3·8	2·73 2·44	34·51 24·84
Z-L-Nle-OPcp	132–133	− 7·1° (1, $CHCl_3$)	0·9	$C_{20}H_{18}Cl_5NO_4$ (513·7)	46·77 46·55	3·53 3·74	2·73 2·4	34·51 34·07

[a] Ethyl acetate; [b] dimethylformamide; [c] methanol.

After 10 min stirring the N-benzyloxycarbonyl-L-norleucine containing ethyl acetate solution (20 ml) was added. Stirring of the mixture was continued for 1 h at 0°C and 1 h at room temperature. The separated N,N'-dicyclohexylurea was filtered, the solution cooled to −10°C and a further crop filtered. The solution was diluted to 150 ml with ethyl acetate, extracted 3 times with 5% $NaHCO_3$ solution, water, 0·1M-HCl solution, water and dried over $MgSO_4$. The solvent was removed by rotary evaporation below 40°C, the residue was triturated with light petroleum and kept in the refrigerator to crystallize. The solidified material was suspended in light petroleum, filtered and crystallized from ethanol.

N-Benzyloxycarbonyl-D-tyrosine Hydrazide

This compound was prepared by adopting the method of Holley and Sondheimer[21] used for the synthesis of the L-enantiomer. Yield 82%, m.p. 224—225°C. (Lit.[21] for the L-isomer: 220—221°C). R_F (b): 0·68. For $C_{17}H_{19}N_3O_4$ (329·4) calculated: 61·99% C, 5·81% H, 12·76% N; found: 62·35% C, 6·15% H, 12·45% N.

N-t-Butoxycarbonyl-γ-benzyl-D-glutamic Acid Pentachlorophenyl Ester

The synthetic route was identical with the method applied for the preparation of the L-isomer[16,22]. Yield 36%, m.p. 138—139°C, R_F(a): 0·79. $[\alpha]_D^{20}$ +16·9 (*c* 1, chloroform). For $C_{23}H_{22}Cl_5NO_6$ (585·7) calculated: 47·16% C, 3·79% H, 30·26% Cl, 2·37% N; found: 47·27% C, 4·36% H, 30·0% Cl, 2·36% N.

γ-Benzyl-glutamic Acid Pentachlorophenyl Ester Hydrochloride

0·7 g (1·3 mmol) Boc-Glu(OBzl)-OPcp was treated at room temperature with a 70 times molar excess of 2M-HCl in ethyl acetate. Proceeding of the reaction was followed by the aid of TLC and 15 min reaction time with stirring was found optimal. The deprotected derivative was immediately precipitated by the addition of excess light petroleum. The filtered precipitate was recrystallized from methanol–light petroleum, containing a few drops of ether. Yield 68%, m.p. 149—150°C, R_F(a): 0·73, $[\alpha]_D^{20}$ +29·4 (*c* 2, methanol). For $C_{18}H_{15}Cl_6NO_4$ (522·1) calculated: 41·40% C, 2·9% H, 40·75% Cl, 2·68% N; found: 41·11% C, 3·25% H, 41·37% Cl, 2·92% N.

γ-Benzyl-D-glutamic Acid Pentachlorophenyl Ester Hydrochloride

This compound was prepared as described for the L-enantiomer, M.p. 154—155°C, R_F(a): 0·73. $[\alpha]_D^{20}$ −30·1 (*c* 2, methanol). Both compounds described above were previously reported in the literature[23], but were prepared from the Nps-derivatives. Lit. data: L-isomer: m.p. 150—151°C. $[\alpha]_D^{20}$ +29·8 (*c* 2, methanol); D-isomer: m.p. 146—148°C, $[\alpha]_D^{20}$ −29·1 (*c* 2, methanol).

N^{α},N^{ε}-Bis(benzyloxycarbonyl)-L-lysyl-N^{ε}-(benzyloxycarbonyl)-L-lysine Methyl Ester

The synthesis of this dipeptide has been reported previously in the literature by the use of the mixed anhydride method[24]. 0·5 g (1·51 mmol) Lys(Z)-OMe.HCl was suspended in 5 ml dichloromethane containing 0·24 ml (1·71 mmol) triethylamine and after a few minutes stirring a solution of 1 g (1·51 mmol) Z-Lys(Z)-OPcp in 10 ml dichloromethane was added. The mixture was stirred for 24 g at room temperature. The precipitate formed was removed by filtration, the solvent evaporated under reduced pressure and the residue dissolved in ethyl acetate. The solution was washed subsequently with 1M-HCl solution, water, 5% $NaHCO_3$ solution, water, and dried over $MgSO_4$. The product was precipitated from the ethyl acetate solution by light petroleum and recrystallized from ethyl acetate–light petroleum. Yield: 58%, m.p. 121—122°C, (Lit.[24] 123°C). R_F(b): 0·71. $[\alpha]_D^{20}$ −10·7 (*c* 0·7, methanol).

N^{α},N^{ε}-Bis(benzyloxycarbonyl)-D-lysyl-N^{ε}-(benzyloxycarbonyl)-D-lysine Methyl Ester

The synthetic route applied for the L-L analogue was followed. M.p. 121–122°C, R_F(b): 0·71. $[\alpha]_D^{20}$ +10·9 (*c* 0·7, methanol). For $C_{37}H_{46}N_4O_9$ (690·8) calculated: 64·33% C, 6·71% H, 8·11% N; found: 63·95% C, 7·1% H, 8·2% N.

N^{α},N^{ε}-Bis(benzyloxycarbonyl)-D-lysyl-N^{ε}-(benzyloxycarbonyl)-D-lysine Hydrazide

This dipeptide derivative was synthesised by adopting the method of Erlanger and Brand[20] used for the synthesis of the L-L diastereomer. Yield: 84%. m.p. 185–186°C, R_F(b): 0·75. $[\alpha]_D^{20}$ +13·2 (*c* 0·3, ethanol). For $C_{36}H_{46}N_6O_8$ (690·8) calculated: 62·59% C, 6·71% H, 12·17% N; found: 62·9% C, 7·05% H, 12·31% N.

N-Benzyloxycarbonyl-γ-benzyl-L-glutamyl-γ-benzyl-L-glutamic Acid Pentachlorophenyl Ester

0·95 g (2·56 mmol) Z-Glu(OBzl)-OH was dissolved in 36 ml chloroform and 0·56 g (2·71 mmol) dicyclohexylcarbodiimide was added to the ice cooled, stirred solution. Simultaneously 1·3 g (2·43 mmol) HCl.Glu(OBzl)-OPcp was suspended in 39 ml dichloromethane and treated with 0·34 ml (2·43 mmol) triethylamine. The resulting solution was added to the above described chloroform solution, stirring continued at 0°C for 1 h and the mixture left overnight at room temperature. The precipitate was removed by filtration, the solvent evaporated and the residue dissolved in ethyl acetate. A further crop of N,N'-dicyclohexylurea was separated by filtration. The dipeptide was precipitated from the ethyl acetate solution by light petroleum, filtered and crystallized from ethanol. Yield 67·5%, m.p. 153–154°C, R_F(b): 0·88. $[\alpha]_D^{20}$ −12·7 (*c* 0·7, dimethylformamide). For $C_{38}H_{33}Cl_5N_2O_9$ (839·0) calculated: 54·4% C, 3·96% H, 21·21% Cl, 3·34% N; found: 54·2% C, 4·27% H, 21·68% Cl, 3·13% N.

N-Benzyloxycarbonyl-γ-benzyl-D-glutamyl-γ-benzyl-D-glutamic Acid Pentachlorophenyl Ester

The method described for the synthesis of the L-L analogue was applied. M.p. 153–154°C, R_F(b): 0·88, $[\alpha]_D^{20}$ +16·5 (*c* 1·0, dimethylformamide). Found: 54·05% C, 3·74% H, 21·6% Cl, 3·34% N.

Poly(Lys-(DL-Ala_m)), $m \sim 3$

The synthesis of this polymer was carried out as reported previously[1].

Poly(Lys-(X_i-DL-Ala_m)), $i < 1$, $m \sim 3$

A) The method is illustrated in the case of the coupling of Z-Glu(OBzl)-OPcp. 0·27 g (0·81 mmol) poly(Lys-(DL-Ala_m)) (hydrobromide) was suspended in 18 ml dimethylformamide and stirred for 30 min, followed by the addition of equimolar triethylamine (0·114 ml, 0·81 mmol). To the solution obtained in this way 0·9 g (1·45 mmol) Z-Glu(OBzl)-OPcp dissolved in 9 ml dimethylformamide was added. The mixture was stirred at room temperature for 24 h, the solvent removed in vacuum and the residue triturated 3 times with light petroleum and finally with ether. Yield: 0·45 g of dried polymer.

B) The method is illustrated in the case of the coupling of Z-D-Tyr-N_2H_3. 0·85 g (0·75 mmol) poly(Lys-(DL-Ala_m)) was suspended in 6 ml dimethylformamide and the mixture stirred for 30 min. Simultaneously 0·46 g (1·4 mmol) Z-D-Tyr-N_2H_3 was dissolved in 2·5 ml dimethylformamide, cooled to −15°C and under stirring 4·9 mol HCl in tetrahydrofuran added, followed

by the addition of 0·35 ml (2·6 mmol) isopentyl nitrite. The acidity of the mixture has to be controlled. After 10 min stirring 0·7 ml (4·97 mmol) triethylamine was added to the clear solution, dissolved in a mixture of 1·6 ml dimethylformamide and 1·6 ml tetrahydrofuran. The solution containing the azide derivative was united with the above described dimethylformamide solution of the polymer and stirring was continued for 12 h at 0°C and for further 12 h at room temperature. By dilution with 10 volumes of water a precipitate was formed, filtered, washed with water and dried.

Poly(Lys-((X$_2$)$_i$-DL-Ala$_m$)), $i < 1$, $m \sim 3$

Procedure *A* was applied for the coupling of L-L and D-D glutamic acid containing dipeptides, while procedure *B* was used in the case of L-L and D-D lysine containing dipeptides.

Removal of Protecting Groups from the Polypeptides

The removal of benzyl and benzyloxycarbonyl protecting groups by HBr in glacial acetic acid and the purification of end products was carried analogously to the methods reported previously[1]. The same procedure was applied for all polymers.

Methods

All compounds (except the polypeptides) were checked for purity by elemental analysis and by ascending thin layer chromatography using Merck precoated plates DC-Alufolien Kieselgel 60, solvent systems: (*a*) ethyl acetate–pyridine–acetic acid–water 240 : 20 : 6 : 11 and (*b*) ethyl acetate–pyridine–acetic acid–water 120 : 20 : 6 : 11. Melting points (uncorrected) were determined with a Boetius-type hot-stage apparatus. Optical rotations were measured with a Perkin–Elmer polarimeter (model 241).

The presumed structures were confirmed by IR spectra, recorded in KBr pellets with an IR 10 spectrometer (Carl Zeiss, Jena).

Amino acid analyses were carried out on a Chinoin Model OE 975 analyser. The samples were subjected to hydrolysis with 6M hydrochloric acid in sealed tubes at 106°C for 24h. Complete removal of benzyloxycarbonyl and benzyl blocking groups from the polypeptides was controlled by the ultraviolet and infrared absorption spectra, using Specord UV VIS and Specord IR spectrophotometers.

The identification of the terminal amino acids of the side chains and the analysis of surface topography of the branched polypeptides was realised by the aid of HPLC runs of hydrolysates of the dansylated polypeptides[25].

The relative molar mass distribution, its average and the degree of polymerisation were investigated by sedimentation analysis and gel chromatography[26]. Sedimentation analysis was carried out in a MOM 3170 ultracentrifuge as previously described[1].

RESULTS AND DISCUSSION

The interest focused on the L-leucine containing polymer by its tendency to form ordered structure at physiological pH was strengthened by the meanwhile established strong immunoadjuvant properties of this molecule, capable to protect the host against immunosuppressive action of cytostatic agents[2]. Therefore a series of polypeptides were synthesised containing similar hydrophobic chain terminating amino acids *i.e.* isoleucine, norleucine, valine.

In order to realise a systematic analysis of the dependence of conformation upon the number of chain terminating amino acid residues a stepwise elongation seemed to be required (Fig. 1, 2). Analogues with one glutamic acid or lysine, and with glutamyl-glutamic acid or lysyl-lysine dipeptides were synthesised to enable comparison with the CD spectra of the previously reported[1] polypeptides containing the oligomers (3 residues). The same synthetic route was followed with L- and D-glutamic acid, and L- and D-lysine respectively to investigate also the influence of the configuration. Additionally further D-amino acid (D-histidin, D-leucine, D-tyrosine, D-phenylalanine) containing analogues were built up, to get a better insight into this problem.

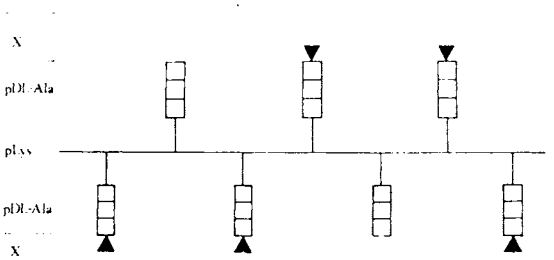

FIG. 1

Schematic presentation of branched polypeptides with a single amino acid at the side chain ends. Degree of blocking by X for the individual amino acids is indicated in Table II

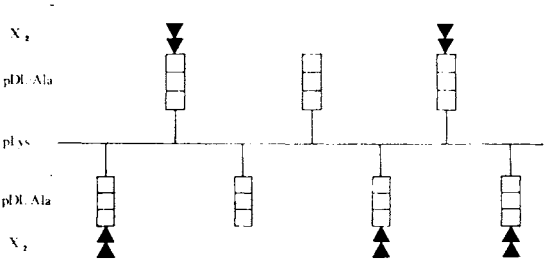

FIG. 2

Schematic presentation of branched polypeptides with a dipeptide consisting of two identical amino acids at the side chain ends. Degree of blocking by X_2 for the various dipeptides is presented in Table II

The coupling of single amino acids to the side chain ends was realised by the active ester method, using suitably protected pentachlorophenyl esters (Fig. 3), except in the case of D-histidin and D-tyrosine, when the azide method was applied (Fig. 4). Identical procedures were applied for coupling of the reactive dipeptide derivatives to the side chain ends.

The synthesis of the protected glutamic acid containing dipeptide pentachlorophenyl esters was realised by active ester coupling, as demonstrated in the scheme on Fig. 5. The route followed for the synthesis of the corresponding protected lysine dipeptide hydrazides is depicted in Fig. 6.; peptide coupling was achieved by the aid

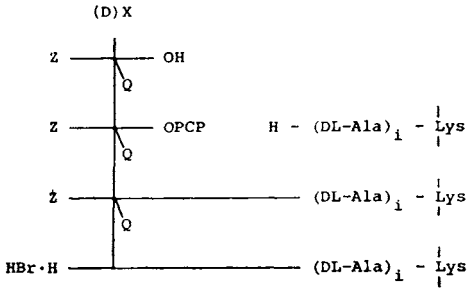

FIG. 3

Synthetic route for the coupling of protected amino acid pentachlorophenyl esters to poly-(L-Lys-(DL-Ala$_m$)). X = L-Phe, D-phe, L-Ile, L-Nle, L-Val, D-Leu, L-Lys, D-Lys, L-Glu, D-Glu; Q = H (L-Phe, D-Phe, L-Ile, L-Nle, L-Val, D-Leu); Z(L-Lys, D-Lys); OBzl (L-Glu, D-Glu)

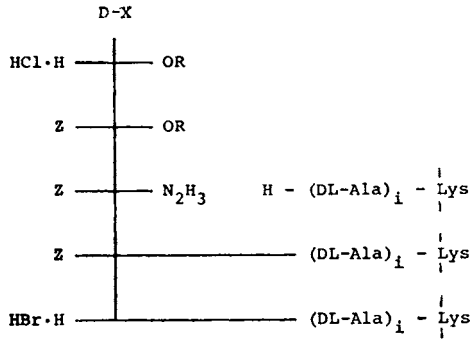

FIG. 4

Synthetic route for the coupling of protected amino acid hydrazides to poly(Lys-(DL-Ala$_m$)). X = D-Tyr, D-His; R = OEt (D-Tyr) or OMe (D-His)

of dicyclohexylcarbodiimide. Protecting groups were removed from the polymers with HBr in acetic acid, as reported previously[1]. The polypeptides were characterized by various methods; data are summarised in Table II.

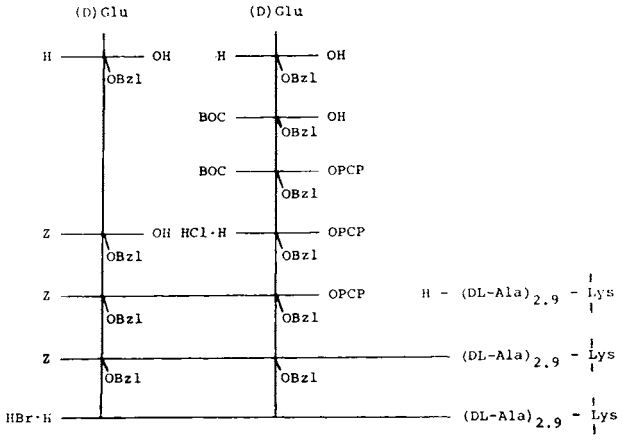

FIG. 5

Scheme of synthesis of dipeptide pentachlorophenyl esters consisting of L- or D-glutamic acids and their coupling to poly(L-Lys-(DL-Ala_m))

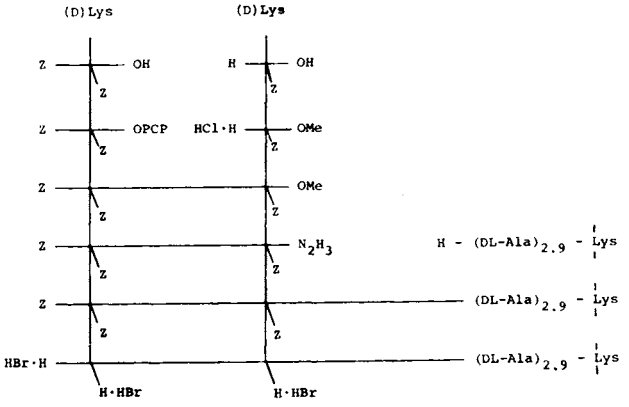

FIG. 6

Scheme of synthesis of dipeptide hydrazides consisting of L- or D-lysines and their coupling to poly(L-Lys-(DL-Ala_m))

TABLE II

Preparation and characterisation of branched polypeptides

Polypeptide	Procedure	Polymer/monomer molar ratio	Molar ratio of amino acids in the end product			M_W[a] (±5%)
			Lys	*m*	*i*	
Poly(Lys-(L-Phe$_i$-DL-Ala$_m$))[b]	*A*	1 : 1·5	1	3	0·9	54 700
Poly(Lys-(D-Phe$_i$-DL-Ala$_m$))[b]	*A*	1 : 1·5	1	3	0·92	55 200
Poly(Lys-(D-His$_i$-DL-Ala$_m$))[b]	*B*	1 : 1·5	1	2·95	0·14	75 500
Poly(Lys-(D-His$_i$-DL-Ala$_m$))[b]	*B*	1 : 1·8	1	2·95	0·53	84 800
Poly(Lys-(D-Tyr$_i$-DL-Ala$_m$))[b]	*B*	1 : 1·8	1	2·95	0·81	95 200
Poly(Lys-(Ile$_i$-DL-Ala$_m$))[b]	*A*	1 : 1·5	3	3	0·95	52 700
Poly(Lys-(Nle$_i$-DL-Ala$_m$))[b]	*A*	1 : 1·5	1	3	0·95	52 700
Poly(Lys-(D-Leu$_i$-DL-Ala$_m$))[b]	*A*	1 : 1·5	1	3	0·95	52 700
Poly(Lys-(L-Val$_i$-DL-Ala$_m$))[b]	*A*	1 : 1·5	1	3	0·75	47 700
Poly(Lys-(L-Glu$_i$-DL-Ala$_m$))	*A*	1 : 1·5	1	2·95	0·25	63 700
Poly(Lys-(L-Glu$_i$-DL-Ala$_m$))	*A*	1 : 1·8	1	2·95	0·81	76 300
Poly(Lys-(D-Glu$_i$-DL-Ala$_m$))	*A*	1 : 1·8	1	2·95	0·87	77 600
Poly(Lys-(Lys$_i$-DL-Ala$_m$))	*A*	1 : 1·5	1	2·95	0·50	83 300
Poly(Lys-(D-Lys$_i$-DL-Ala$_m$))[b]	*A*	1 : 1·5	1	2·95	0·87	91 600
Poly(Lys-(L-Glu-L-Glu)$_i$-DL-Ala$_m$))	*A*	1 : 1·5	1	2·95	0·62	87 400
Poly(Lys-((D-Glu-D-Glu)$_i$-DL-Ala$_m$))	*A*	1 : 1·5	1	2·95	0·46	78 800
Poly(Lys-((L-Lys-L-Lys)$_i$-DL-Ala$_m$))[b]	*B*	1 : 1·5	1	2·95	0·45	92 300
Poly(Lys-((D-Lys-D-Lys)$_i$-DL-Ala$_m$))[b]	*B*	1 : 1·5	1	2·95	0·2	81 200
Poly(Lys-((D-Lys-D-Lys)$_i$-DL-Ala$_m$))[b]	*B*	1 : 1·8	1	2·95	0·44	91 900

[a] Calculated from the number average degree of polymerization of poly-(L-lysine) and the side chain composition; [b] isolated as hydrobromide.

Since the purpose of the synthesis of these polymers was to enlighten the correllation between conformation and biological properties, besides the CD measurements reported in the adjoining paper, further *in vivo* animal experiments are in progress to analyse immunomodulatory potential. The results will be published elsewhere.

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