SERIES OF SEQUENTIAL POLYPEPTIDES CONTAINING LYSINE OR ARGININE: PREPARATION AND CHARACTERIZATION*

Jana PÍRKOVÁ, Svetlana CHURKINA**, Vladimír GUT, Ivo FRIČ and Karel BLÁHA

Institute of Organic Chemistry and Biochemistry Czechoslovak Academy of Sciences, 166 10 Prague 6

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The sequential polypeptides $(Lys\text{-}Ala)_n$, $(Lys\text{-}Ala\text{-}Ala)_n$, $(Lys\text{-}Ala\text{-}Ala\text{-}Ala)_n$, $(Lys\text{-}Leu\text{-}Ala)_n$, $(Lys\text{-}Leu\text{-}Ala\text{-}Ala)_n$, $(Lys\text{-}Leu\text{-}Ala\text{-}Ala\text{-}Ala)_n$, $(Lys\text{-}Ala\text{-}Leu)_n$, $(Lys\text{-}Ala\text{-}Leu\text{-}Ala)_n$, $(Orn\text{-}Leu\text{-}Ala)_n$, $(Arg\text{-}Ala\text{-}Ala)_n$, $(Arg\text{-}Leu\text{-}Ala)_n$, $(Arg\text{-}Leu\text{-}Ala\text{-}Ala)_n$, $(Arg\text{-}Ala\text{-}Leu)_n$, and $(Arg\text{-}Ala\text{-}Leu\text{-}Ala)_n$ were synthesized by polymerization of active esters (1-succinimidyl or pentafluorophenyl) of the corresponding N^{α}-deblocked monomers. The monomers were prepared using the usual methods of peptide synthesis in solution. Upon dialysis, the average molecular weight of the polymers was 6 000—9 000 as determined by sedimentation in ultracentrifuge. Polypeptides, containing leucine in addition to the basic amino acid, showed a marked tendency to aggregation. CD spectra of the products were measured for characterization.

Our studies on polycationoid polypeptides, particularly CD investigation of their conformation in solutions[1-6] and of their interaction with polyanions (DNA[7-13], polyuronic acids[14,15], and porphyrine derivatives[16]), required a set of sequential polypeptides varying in *a*) character of the basic amino acid (Lys, Orn, Arg)***, *b*) percentual representation of the basic amino acid (compare *I—III*, *IV—VI*, *XI* with *XII*), *c*) hydrophobicity of one of the other amino acid moieties (Ala vs Leu), *d*) mutual position of the basic and hydrophobic amino acid moiety (*IV* vs *VII*, *XI* vs *XIII*, *V* vs *VIII*, *XII* vs *XIV*). The preparation of such a series of polypeptides *I—XIV* and their characterization by CD spectroscopy is the subject of this communication.

The monomers were prepared using common methods of peptide synthesis in solution. In peptides containing lysine and ornithine, the α-amino group was protected with tert-butyloxycarbonyl group, the ω-amino group with benzyloxycarbonyl group. According to our previous experience, the simultaneous removal of the

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** On leave from the Department of Organic Chemistry, Leningrad State University, Leningrad, U.S.S.R.

*** The nomenclature and symbols of the amino acids and peptides obey the published IUPAC–IUB recommendations[17]. All amino acids mentioned in this paper are of the L-configuration.

benzyloxycarbonyl group in acidolytic cleavage of the tert-butyloxycarbonyl group[18] was not detectable in the final product (*I* and *IV*). The side chain in arginine-containing peptides was protected with *p*-toluenesulfonyl group, again in combination with the N^{α}-tert-butyloxycarbonyl protection. Data on synthetic intermediates in the preparation of all the monomers are given in Table I.

BocLys(Z)-Ala-OX *I*	Boc-Lys(Z)-Ala-Ala-OX *II*
Boc-Lys(Z)-Ala-Ala-Ala-OX *III*	Boc-Lys(Z)-Leu-Ala-OX *IV*
Boc-Lys(Z)-Leu-Ala-Ala-OX *V*	Boc-Lys(Z)-Leu-Ala-Ala-Ala-OX *VI*
Boc-Lys(Z)-Ala-Leu-OX *VII*	Boc-Lys(Z)-Ala-Leu-Ala-OX *VIII*
Boc-Orn(Z)-Leu-Ala-OX *IX*	Boc-Arg(Tos)-Ala-Ala-OX *X*
Boc-Arg(Tos)-Leu-Ala-OX *XI*	Boc-Arg(Tos)-Leu-Ala-Ala-OX *XII*
Boc-Arg(Tos)-Ala-Leu-OX *XIII*	Boc-Arg(Tos)-Ala-Leu-Ala-OX *XIV*

In formulae *I* — *XIV*: *a*, X = Me; *b*, X = OH; *c*, X = ONsu (1-succinimidyl); *d*, X = OPfp (pentafluorophenyl). Roman numerals without letters mean the corresponding polymers.

The polymerization was effected using activated (1-succinimidyl or pentafluorophenyl) esters. No significant difference was observed between the two activation procedures. Polymerization of the pentapeptide monomer to the polymer *VI* was not very satisfactory. It was realized using the pentafluorophenyl ester but the product was obtained only in a low yield and in low polymerization degree. The crude protected polypeptides were deblocked with hydrogen bromide in dichloroacetic acid–acetic acid (better solubility) in case of benzyloxycarbonyl protecting group, or with liquid hydrogen fluoride in case of *p*-toluenesulfonyl group. It appeared that the latter reagent degrades the guanidine grouping of arginine to ornithine to an extent detectable by amino acid analysis (3% after 30 min at 0°C), insignificant,

TABLE I

Data on Intermediates *I*—*XIV*, *a*—*d*

Compound[a]	Yield, g (%)	M.p., °C $[\alpha]_D$[b]	Formula (mol. w.)	Calculated/found		
				% C	% H	% N
Ia[c]	11·2	81—83	$C_{23}H_{35}N_3O_7$	59·34	7·58	9·03
	(80)	−14·5°	(465·6)	59·78	7·62	9·22
Ib	3·2	68—71[d]	$C_{34}H_{56}N_4O_7$[d]	64·53	8·92	8·83
	(66)	—	(623·9)	64·13	8·90	8·72
Ic	2·0	103—106	$C_{26}H_{36}N_4O_9$	56·92	6·66	10·21
	(75)	—	(548·6)	57·20	6·91	10·03
Id	1·2	132—133	$C_{34}H_{43}F_5N_4O_8$	55·89	5·93	7·67
	(84)	—	(730·7)	55·78	5·87	7·92
IIa	17·5	134—135	$C_{26}H_{40}N_4O_8$[e]	58·14	7·51	10·44
	(73)	−22·4°	(536·6)	57·65	7·52	10·48
IIb	7·4	151—152	$C_{25}H_{38}N_4O_8$	57·46	7·34	10·73
	(81)	−16·1°	(522·6)	57·13	7·32	10·70
IIc	3·8	128—131	$C_{29}H_{41}N_5O_{10}$	56·21	6·67	11·30
	(80)	—	(619·7)	56·26	6·70	11·41
IId	1·25	144—147	$C_{31}H_{37}F_5N_4O_8$	54·04	5·42	8·14
	(87)	—	(688·7)	54·08	5·70	8·48
IIIa	7·8	191—192[f]	$C_{29}H_{45}N_5O_9$	57·32	7·46	11·56
	(67)	−24·1°	(607·7)	57·08	7·24	11·71
IIIb	3·0	158—160[g]	$C_{28}H_{43}N_5O_9$	56·64	7·30	11·80
	(77)	−19·6°	(593·7)	56·38	7·18	11·84
IIIc	1·5	130—132	$C_{32}H_{46}N_6O_{11}$	55·65	6·71	12·16
	(71)	—	(690·7)	55·11	6·78	12·14
IIId	1·3	145—147	$C_{34}H_{42}F_5N_5O_9$	53·72	5·57	9·22
	(83)	—	(760·2)	53·76	5·61	9·23
IVd	1·2	132—133	$C_{34}H_{43}F_5N_4O_8$	55·89	5·93	7·67
	(84)	—	(730·7)	55·78	5·87	7·92
Va	6·2	153—154	$C_{32}H_{51}N_5O_9$	59·15	7·91	10·78
	(83)	−31·0°	(649·8)	59·41	7·96	10·76
Vb	1·3	127—130[h]	$C_{31}H_{49}N_5O_9$	57·75	7·82	10·86
	(91)	−30·0°	(644·8)	57·90	7·69	10·89
Vc	0·5	90—93	—	—	—	—
	(70)	—	—	—	—	—
VIa	1·6	199—201	$C_{35}H_{56}N_6O_{10}$	58·32	7·87	11·66
	(74)	−29·4°	(720·9)	58·63	7·80	11·88

TABLE I
(*Continued*)

Compound[a]	Yield, g (%)	M.p., °C $[\alpha]_D$[b]	Formula (mol. w.)	Calculated/found		
				% C	% H	% N
VIb	0·6	165—167	$C_{34}H_{54}N_6O_{10}$[i]	56·94	7·79	11·59
	(79)	−28·9°	(724·8)	56·71	7·55	11·67
VId	0·6	152—155	—	—	—	—
	(87)	—	—	—	—	—
VIIa	23·1	108—110	$C_{29}H_{46}N_4O_8$	60·19	8·01	9·68
	(82)	−18·7°	(578·7)	60·01	8·23	9·88
VIIb	3·4	76—78	$C_{28}H_{44}N_4O_8$	59·56	7·85	9·92
	(75)	−14·6°	(564·7)	59·30	7·69	9·75
VIIc	2·7	106—108	$C_{32}H_{47}N_5O_{10}$	58·08	7·16	10·58
	(77)	—	(661·8)	57·37	6·99	10·75
VIIIa	0·86	151—153	$C_{32}H_{51}N_5O_9$[h]	58·34	7·96	10·63
	(63)	−27·9°	(658·8)	58·21	7·68	10·54
VIIIb	0·6	154—156	$C_{31}H_{49}N_5O_9$[h]	57·75	7·82	10·91
	(90)	−24·5°	(644·8)	57·79	7·58	10·84
VIIIc	0·7	123—125	$C_{35}H_{52}N_6O_{11}$	57·36	7·15	11·47
	(93)	—	(732·8)	57·47	7·04	11·41
IXa	1·9	153—155	$C_{28}H_{44}N_4O_8$	58·94	7·85	9·92
	(47)	−25·9°	(564·6)	58·84	7·94	9·53
IXb	1·3	172—173	$C_{27}H_{42}N_4O_8$	58·89	7·69	10·18
	(83)	−35·3°[i]	(564·4)	58·42	7·51	10·04
IXd	1·2	192—194	$C_{33}H_{41}F_5N_4O_8$	55·30	5·77	7·82
	(73)	—	(716·7)	55·23	5·74	7·94
Xa	7·7	122—124	$C_{25}H_{40}N_6O_8S$	51·96	6·86	14·36
	(89)	−10·9°	(584·7)	51·35	6·90	14·38
Xb	4·4	143—146	$C_{24}H_{38}N_6O_8S$	50·07	6·64	14·54
	(91)	−6·4°	(570·7)	50·51	6·71	14·73
Xc	8·4	123—125	$C_{28}H_{41}N_7O_{10}S$	50·14	6·24	14·16
	(77)	—	(667·7)	50·36	6·19	14·68
Xd	1·8	100—102	$C_{30}H_{37}F_5N_6O_8S$	48·62	5·27	11·25
	(95)	—	(736·7)	48·91	5·06	11·40
XIa	9·6	115—118	$C_{28}H_{46}N_6O_8S$	53·65	7·40	13·41
	(77)	−17·4°	(626·8)	53·54	7·32	13·40
XIb	6·3	135—138	$C_{27}H_{44}N_6O_8S$	52·92	7·24	13·72
	(87)	−13·6°	(612·7)	52·61	7·12	13·31

TABLE I
(*Continued*)

Compound[a]	Yield, g (%)	M.p., °C $[\alpha]_D$[b]	Formula (mol. w.)	% C	% H	% N
				Calculated/found		
XIc	1·4	127—130	$C_{31}H_{47}N_7O_{10}S$	52·46	6·67	13·81
	(78)	—	(709·8)	51·78	6·47	13·69
XId	1·2	108—110	$C_{33}H_{43}F_5N_6O_8S$	50·90	5·56	10·79
	(94)	—	(778·8)	50·81	5·56	10·74
XIIa	1·0	119—120	$C_{31}H_{51}N_7O_9S$	53·35	7·34	14·05
	(47)	−23·7°	(697·8)	53·27	7·47	14·07
XIIb	0·4	127—129	$C_{30}H_{49}N_7O_9S$[j]	51·33	7·32	13·97
	(46)	—	(701·8)	51·56	6·95	13·98
XIId	0·45	95—97	$C_{36}H_{48}F_5N_7O_9S$	50·87	5·69	11·54
	(91)	—	(849·8)	51·26	5·62	10·80
XIIIa	8·5	113—115	$C_{28}H_{46}N_6O_8S$	53·65	7·40	13·41
	(56)	−9·0°	(626·8)	53·46	7·36	13·06
XIIIb	3·9	139—142	$C_{27}H_{44}N_6O_8S$	52·92	7·24	13·72
	(81)	—	(612·7)	53·18	7·29	13·29
XIIIc	1·5	128—130	$C_{31}H_{47}N_7O_{10}S$	52·46	6·67	13·81
	(65)	—	(709·8)	52·29	6·91	13·51
XIIId	1·6	103—105	$C_{33}H_{43}F_5N_6O_8S$	50·90	5·56	10·79
	(84)	—	(778·8)	50·77	5·69	10·68
XIVa	0·8	107—109	$C_{31}H_{51}N_7O_9S$	53·35	7·34	14·05
	(42)	−21·6°	(697·8)	53·34	7·23	13·85
XIVb	0·45	123—125	$C_{30}H_{49}N_7O_9S$[k]	50·96	7·37	13·79
	(58)	−1·6°[l]	(710·8)	50·56	6·81	13·27
XIVd	0·55	113—115	$C_{36}H_{48}F_5N_7O_9S$	50·87	5·69	11·54
	(98)	—	(849·8)	50·81	5·93	11·57

[a] Compounds of the series *b*—*d* were prepared as described for compounds *Ib*, *IIc*, and *IIId*, respectively. Preparation of compounds of series *a* is described individually, unless the compounds are known. [b] In dimethylformamide, *c* about 0·5 g/100 ml. [c] Reported[19] m.p. 82—84°C, $[\alpha]_D^{22}$ −12° (*c* 1, ethyl acetate). [d] Dicyclohexylammonium salt; free acid (non-crystalline) described in ref.[20]. [e] Ref.[21]. [f] Ref.[22]. [g] Sinters from 90°C. [h] Calculated for semihydrate. [i] In methanol, *c* = 0·3 g per 100 ml. [j] Calculated for a monohydrate. [k] Calculated for sesquihydrate. [l] *c* = 0·07 g/100 ml.

however, with respect to the subsequent use. The data on the obtained polypeptides are gathered in Table II. Some of the mentioned sequential polypeptides have

TABLE II

Polypeptides *I*—*XIV*: preparation by polymerization of the activated esters

Polypeptide salt	Ester, (g)[a] solvent, ml	Triethylamine, ml yield, mg[b]	M_w[c]
I	*Ic* (1·1)	0·36	6 800
HBr	3·0	350	
II[d]	*IIc* (5·8)	1·14	7 900
HBr	12·8	700	
II	*IId* (1·3)	0·18	9 200
HBr	2·0	90	
III	*IIIc* (1·7)	0·46	6 500
HBr	4·9	180	
III	*IIId* (1·1)	0·27	6 700
HBr	3·0	50	
IV	*IVd* (0·74)	0·18	9 000—
HBr	2·0	135	14 000[e]
V	*Vc* (0·35)	0·08	27 000—
HBr	0·9[f]	65	42 000[e]
VI	*VId* (0·4)	0·08	5 400
HBr	1·0[f]	10	
VII	*VIIc* (5·8)	1·2	45 000[e]
HBr	12·6	1 000	
VIII	*VIIIc* (0·55)	0·16	170 000[e]
HBr	1·8	125	
IX	*IXd* (1·2)	0·28	8 600—
HBr	3·1	120	13 200[e]
X	*Xc* (1·3)	0·30	8 000
HF	3·3	190	
X	*Xd* (1·0)	0·19	9 000
HF	2·2	140	
XI	*XIc* (1·1)	0·22	7 000—
HF	2·4	420	10 000[e]
XI	*XId* (0·9)[g]	0·17	8 700—
HF	1·9	300	12 000
XII	*XIId* (0·4)	0·17	11 000
HF	1·9	220	
XIII	*XIIIc* (1·5)	0·33	9 000—
HF	3·7	300	15 000[e]

TABLE II
(*Continued*)

Polypeptide salt	Ester, (g)[a] solvent, ml	Triethylamine, ml yield, mg[b]	M_w[c]
XIII	*XIIId* (1·6)	0·30	8 900—
HF	4·1		15 500[e]
XIV	*XIVd* (0·35)	0·19	
HF	2·1	270[h]	160 000[e]

[a] Fully protected activated ester; [b] product after deprotection, dialysis and freeze-drying, used for the CD and interaction measurements; [c] molecular weight averages measured in 0·1M-NaCl, for *IV*, *V*, *VII—IX*, *XI* also in 0·001M-HCl; [d] this compound was already described in our preceding paper; [e] sedimentation measurement indicates aggregate formation and the given values cannot represent the actual polymerization degree; [f] reaction mixture solidified within 5 min, sulfoxide (0·5 ml) was added three times in 30 min intervals and then after 2 days (one 0·5 ml portion); [g] no solidification of the polymerization mixture observed: [h] in another experiment the polymer contained 10% of ornithine after treatment with hydrogen fluoride at 0°C for 30 min and then at 17°C for 45 min.

already been described: *I* in refs[23,24], *II* in refs[3,25], *III* in refs[26,27], and *X* in refs[28,29]. Also other similar polypeptides are known: $(Arg\text{-}X\text{-}Gly)_n$ for X = Gly, Ala, Val, Leu (ref.[30]). The described preparations differ mostly in methodical details or in the character of protecting or activating groups. In some cases, however, insufficient attention has been paid to determination of the polymerization degree (cf. ref.[31]).

The molecular weights in Table II were obtained by sedimentation measurements in a centrifuge and processing the data according to Chervenka[32]. The molecular weight averages M_w for most the peptides range from 6 000 to 9 000 daltons. With polypeptides containing leucine moiety (compounds *IV*, *V*, *VII*, *VIII*, *XII—XIV*), the sedimentation measurements clearly indicate aggregation. This aggregation was studied in more detail in the polymer *IV* (see ref.[33]). From the effect of the medium on the M_w value we can conclude that the polymerization degree of non-aggregated polypeptide chains containing leucine does not differ too much from cases in which the M_w determination is not perturbed by aggregation*.

* A recent paper[34] states molecular weight of 300 000 daltons for the sequential polypeptide $(Leu\text{-}Glu(OEt)\text{-}Ala\text{-}Ala)_n$. This value was obtained using practically the same polymerization technique as in our work. Unless there is an error in the molecular weight determination (viscosimetric measurements), we assume that the formation of polymers of an order of magnitude higher molecular weight represents a phenomenon specific for the studied sequence, rather than a general situation.

Since the prepared polypeptides are further utilized for studies of interaction with polyanions mostly by chiroptical methods, they were routinely characterized by CD measurements under standard conditions – in an aqueous solvent containing 0·001M-HCl and 0·1M-NaCl (see Table III). The measured curves indicate α-helical and random-coil conformations in a variable ratio depending on the primary structure of the amino acid sequence. This ratio is characterized by the mean residual ellipticities at the n–π^* and long-wavelength π–π^* amide band maxima. The polymer *I* is typical example of a peptide in a random-coil conformation whereas the polymer *XIV* has a high content of the α-helical form. Some of the polymers formed more or less opalescent solutions (typically *VI* and *VIII*) and therefore their CD spectra are distorted by optical side effects which manifest themselves mainly by an apparent intensity decrease of the bands. For this reason, an alternative parameter suitable for assessing the polymer conformation is the band intensity ratio $[\Theta]_{\pi-\pi^*}/[\Theta]_{n-\pi^*}$, (also given in Table III) which nears 1 with increasing population of the α-helix.

TABLE III

Maxima in the CD spectra of polypeptides *I*–*XIV* in hydrochloric acid (c = 0·001 mol l^{-1}) and in sodium chloride (0·1 mol l^{-1})

Polypeptide	λ, nm ([Θ] deg cm^2 dmol^{-1})		$[\Theta]_{\pi-\pi^*}/[\Theta]_{n-\pi^*}$
	π–π^* band	n–π^* band	
I	196 (−24 100)	218 (+1 200)[a]	–
II	203 (−14 400)	221 (−8 400)	1·7
III[b]	206 (−16 600)	222 (−14 200)	1·2
III[c]	206 (−12 800)	221 (−12 200)	1·05
IV	207 (−16 100)	222 (−13 700)	1·2
V	207 (−23 300)	219 (−19 300)	1·2
VI	203 (−9 100)	221 (−5 600)	1·8
VII	204 (−16 900)	224 (−1 200)[d]	14
VIII	208 (−10 400)	220 (−9 700)	1·1
IX	199 (−15 300)	223 (−2 600)	5·9
X	207 (−14 800)	221 (−13 000)	1·1
XI	207 (−16 300)	221 (−14 500)	1·1
XII	207 (−17 400)	219 (−16 200)	1·1
XIII	206 (−16 000)	221 (−13 000)	1·2
XIV	208 (−23 700)	219 (−22 800)	1·04

[a] Another negative maximum at 233 nm (−800); [b] prepared by polymerization of 1-succinimidyl ester; [c] prepared by polymerization of pentafluorophenyl ester; [d] shoulder.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Samples for elemental analyses were dried over phosphorus pentoxide for 12 h at room temperature. The homogeneity of compounds was checked by thin-layer chromatography on silica gel coated plates (Silufol, Kavalier, Czechoslovakia) in the following systems: 2-butanol–98% formic acid–water (75 : 13·5 : : 11·5, S1), 2-butanol–25% ammonia–water (85 : 7·5 : 7·5, S2), 1-butanol–acetic acid–water–pyridine (15 : 3 : 6 : 10, S3), 1-butanol–acetic acid–water (4 : 1 : 1, S4). Paper electrophoresis was performed in a moist chamber in 1 mol l^{-1} acetic acid (pH 2·4) and in a pyridine–acetate buffer (pH 5·7) on Whatman 3MM paper at 20 V/cm for 60 min. Spots in TLC and electrophoresis were detected with ninhydrin or by the chlorination method. High performance liquid chromatography was carried out on an SP-8700 instrument and SP-8400 detector (Spectra Physics, Santa Clara, U.S.A.). All intermediates were pure according to these criteria; the purity of activated esters was checked only by TLC. A rotatory evaporator was used to concentrate solutions (bath temperature 40°C). The $[\alpha]_D$ values were estimated on a Perkin–Elmer 141 polarimeter at a concentration about 0·5 g per 100 ml. Samples for amino acid analyses were hydrolyzed with 6M-HCl at 105°C for 20 h. The analyses were performed on a Durrum D-500 analyzer.

Molecular weight averages M_w were measured by high-speed sedimentation equilibrium method according to Chervenka[32] using interference optics (ultracentrifuge Spinco, Model E) in 0·1M-NaCl; aggregating polypeptides containing leucine moiety were measured in a mixture of 0·1M-NaCl and 0·001M-HCl in which lowest M_w values were found for the individual peptides.

The CD spectra were taken on a Jobin–Yvon Dichrographe Mark V instrument equipped with data processor. The measurements were carried out at room temperature in a 0·05 cm cell in the range 190—260 nm, peptide concentration about 0·25 mg ml^{-1}. The aqueous solvent contained 0·001M-HCl and 0·1M-NaCl. The data are given in mean residue ellipticity values (deg cm^2. .$dmol^{-1}$).

Starting Material

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysine dicyclohexylammonium salt, m.p. 110—111°C, $[\alpha]_{578}$ −9·3° (*c* 1, ethyl acetate)[35]; N^{α}-tert-butyloxycarbonyl-N^{δ}-benzyloxycarbonylornithine dicyclohexylammonium salt, m.p. 128—129°C, $[\alpha]_D$ +7·8° (*c* 1, ethanol)[36]; N^{α}-tert-butyloxycarbonyl-N^{ω}-*p*-toluenesulfonylarginine[37], m.p. 96—97°C, $[\alpha]_D^{24}$ −3·6° (*c* 4, dimethylformamide). Tert-butyloxycarbonylleucyl-alanyl-alanine methyl ester[38].

2-Nitrobenzenesulfenylalanyl-alanyl-alanine Methyl Ester

A solution of alanyl-alanine methyl ester hydrochloride (liberated from 10 g of the N^{α}-2-nitrobenzenesulfenyl-protected derivative) in dichloromethane (70 ml) was added to a solution of 2-nitrobenzenesulfenylalanine (12 g) in dichloromethane (100 ml). 1-Hydroxybenzotriazole (3·8 g) was added followed, at 20°C, by 6·6 g of dicyclohexylcarbodiimide. After stirring at −20°C for 1 h, the mixture was left in refrigerator overnight and filtered. The filtrate was washed with 0·5M-H_2SO_4, 0·5M-$NaHCO_3$ and water, dried and taken down. The residue was dissolved in ethyl acetate and precipitated with diethyl ether and light petroleum; yield 6·9 g (61%), m.p. 200—202°C, $[\alpha]_D$ −45·8° (*c* 0·5, dimethylformamide). For $C_{16}H_{22}N_4O_6S$ (398·4) calculated: 48·23% C, 5·57% H, 16·06% N; found: 48·35% C, 5·60% H, 13·84% N.

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-leucine Methyl Ester (*VIIa*)

Leucine methyl ester hydrochloride (9 g) was added to a solution of 2-nitrobenzenesulfonylalanine

dicyclohexylammonium salt (21 g) in chloroform (150 ml). Dicyclohexylcarbodiimide (11·5 g) was added at −20°C and the mixture was stirred at −20°C for 1 h, set aside in a refrigerator overnight and filtered. The filtrate was washed with 0·5M-H_2SO_4, 0·5M-$NaHCO_3$ and water, dried over Na_2SO_4 and taken down. The oily residue was deblocked by standard procedure and the formed oily alanyl-leucine methyl ester hydrochloride was washed with diethyl ether and dissolved in chloroform (200 ml). N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysine dicyclohexylammonium salt (28 g) was added followed, after cooling to −20°C, with dicyclohexylcarbodiimide (12 g). After stirring at −20°C for 1 h, the mixture was allowed to stand in refrigerator overnight and filtered. The filtrate was washed with 20% (w/w) citric acid, 0·5M-$NaHCO_3$ and water, dried over Na_2SO_4 and taken down. The residue was dissolved in ethyl acetate and precipitated with diethyl ether and light petroleum; for physical and analytical data see Table I.

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanine (*Ib*)

A solution of *Ia* (4·7 g) in 2-propanol (50 ml) was mixed with 1M-NaOH (15 ml), stirred at 20°C for 2 h and diluted with water. The 2-propanol was evaporated, the aqueous solution filtered, the filtrate acidified with citric acid and extracted with ethyl acetate. The extract was washed with water, dried over sodium sulfate and taken down. The residue was dissolved in ethyl acetate and the product precipitated with diethyl ether and light petroleum. Compounds *IIb*—*XIVb* were prepared in the same manner except that *Vb* and *VIb* were crystallized from methanol–diethyl ether, and *VIIb*—*XIVb* were precipitated from 2-propanol with diethyl ether and light petroleum. The expertinent data are given in Table I.

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine 1-Succinimidyl Ester (*IIc*)

N-Hydroxysuccinimide (1·9 g) was added to a solution of *IIb* (4·0 g) in dimethylformamide (50 ml). After cooling to −20°C, dicyclohexylcarbodiimide (1·7 g) was added, the mixture was stirred at −20°C for 1 h, set aside in refrigerator overnight, filtered and taken down. The residue was dissolved in ethyl acetate and precipitated with diethyl ether and light petroleum; the obtained product was pure according to chromatography in the system S2. The same procedure was used for preparation of all the 1-succinimidyl esters employed; compound *Ic* was only triturated with hexane and could not be reprecipitated or crystallized; compounds *Xc* and *XIIIc* were crystallized from 2-propanol–diethyl ether. For data see Table I.

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanyl-alanine Pentafluorophenyl Ester (*IIId*)

Pentafluorophenol–dicyclohexylcarbodiimide complex[39] (3 : 1; 1·8 g) was added to a solution of *IIIb* in dimethylformamide–dioxane (1 : 3). Dicyclohexyl urea separated within 0·5 h. The mixture was stirred at 20°C for 1 h, set aside in refrigerator for 1 h, filtered and the filtrate was evaporated. The residue was crystallized from ethyl acetate–diethyl ether. For data see Table I.

N^{ω}-*p*-Toluenesulfonylarginyl-alanyl-alanine 1-Succinimidyl Ester Trifluoroacetate

A solution of *Xc* (1·3 g) in trifluoroacetic acid (3 ml) was allowed to stand at 20°C for 12 min, the mixture was diluted with diethyl ether, the solid was washed with diethyl ether and dried over phosphorus pentoxide; yield 1·2 g (88%), m.p. 126—128°C. The N^{α}-protecting group was analogously removed from compounds *Ic*, *IIc*, *IIIc*, *Vc*, *VIIc*, *VIIIc*, *Xc*, *XIc*, and *XIIIc*. The crude product was used in the polymerization without further purification.

N$^{\varepsilon}$-Benzyloxycarbonyllysyl-alanyl-alanine Pentafluorophenyl Ester Trifluoroacetate

Trifluoroacetic acid (1·5 ml) was added to the protected activated ester *IId* (1·3 g), the mixture was set aside for 12 min with intermittant stirring, poured into light petroleum–diethyl ether (10 : 1), the separated solid was filtered, washed with light petroleum–diethyl ether (10 : 1) and dried in vacuo. The esters *IId*, *IIId*, *Vd*, *VId*, *IXd*—*XIVd* were deblocked in the same manner and polymerized without further purification.

Poly(lysyl-alanine) Hydrobromide (*I*)

Triethylamine (0·36 ml) was added to a solution of N$^{\varepsilon}$-benzyloxycarbonyllysyl-alanine 1-succinimidyl ester trifluoroacetate (1·08 g) in dimethyl sulfoxide (3 ml). The mixture was stirred (solidified during polymerization), set aside for 7 days and diluted with water. The solid protected polypeptide was collected on filter, washed with water and dried (740 mg). The dry material was suspended in dichloroacetic acid, warmed to dissolution and the same volume of 33% hydrogen bromide solution in acetic acid was added. After standing for 1·5 h, the mixture was poured into diethyl ether, the precipitate was filtered, washed with diethyl ether, filtered and dried. The crude polyhydrobromide was dissolved in water (15 ml), dialyzed against water in a dialysis tubing (Serva, Heidelberg, F.R.G.) for 24 h and then freeze-dried. The same procedure was applied to all polypeptides containing benzyloxycarbonyl protecting group in the side chain, i.e. the lysine and ornithine derivatives *I*—*XI*. The data are summarized in Table II.

Poly(arginyl-alanyl-alanine) Hydrofluoride (*X*)

Triethylamine (0·19 ml) was added dropwise to a stirred solution of *Xd* (0·81 g) in dimethyl sulfoxide (2·2 ml). The mixture was set aside for 7 days, diluted with water and the solid was collected on filter, washed with water and dried (0·49 g). The protecting group was removed by treatment with liquid hydrogen fluoride in the presence of anisole at 0°C for 30 min. The hydrogen fluoride was evaporated, the residue was triturated with diethyl ether, filtered, washed with diethyl ether and dried. The crude polyhydrofluoride was dialyzed and freeze-dried as in the preceding experiment. The same procedure was used in the preparation of polypeptides with the *p*-toluenesulfonyl protecting group, i.e. the arginine derivatives *X*—*XIV*. For data see Table II.

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