α-AMINOACYL DERIVATIVES OF α,ω-DIAMINOPOLY(OXYETHYLENE)

Bohumil MASAŘ, Pavel SCHMIDT, Hana PIVCOVÁ and Pavel ČEFELÍN

Institute of Macromolecular Chemistry,
Czechoslovak Academy of Sciences, 162 06 Prague 6

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By reacting *p*-nitrophenyl esters of L-α-amino acids with α-(3-aminopropyl)-ω-(aminomethyl)-poly(oxyethylene), diamides having the structure $[X—NHCH(R)CONH]_2M'_x$ were prepared, X being the protective group Boc or Z, R being residues of glutamic and aspartic acid, phenylalanine and tyrosine, and M'_x being the poly(oxyethylene) chain with the $(—CH_2)_3—$ and $—CH_2—$ endgroups. The diamides were characterized by IR and ^{1}H NMR spectroscopy and thin-layer chromatography. After complete removal of tert-butyloxycarbonyl groups from diamides (X = Boc), deprotected α-aminoacyl derivatives were characterized as trifluoroacetic acid salts.

The attachment of α-aminoacids, peptides and their derivatives to poly(oxyethylene) is of importance in the preparation of block copolymers of polypeptides, the increased solubility of which makes possible an investigation of the conformation of peptides which otherwise are insoluble. Such copolymers may be used as biodegradable drug carriers. α-Aminoacids were attached to the chain of synthetic polymers by employing various procedures[1]. The method of synthesis of peptides in the liquid phase[2] makes use of joining poly(oxyethylene) with aminoacids, with an ester bond being formed by a reaction of the hydroxyl endgroup of the polyether with the carboxylic groups of the amino acid. Such ester bond is not hydrolytically stable, and already in a slightly alkaline medium (pH 8) it undergoes fast hydrolysis[3]. If poly(oxyethylene) with an attached aminoacid[4,5] is used in a living organism, one may anticipate the splitting-off of the aminoacid both selectively, due to the respective enzyme, and nonspecifically[4,6].

The attachment of phenylalanine derivatives to poly(oxyethylene) with amino or carboxyalkyl endgroups by means of the amide bond has been described by Ulbrich, Strohalm, and Kopeček[3] for the preparation of biodegradable polymers. This paper reports the preparation of poly(oxyethylene) with (1-aminoalkanecarboxamido)alkyl endgroups, *i.e.* α-[3-(1-aminoalkanecarboxamido)propyl]-ω-[(1-aminoalkanecarboxamido)methyl]poly(oxyethylene), by the reaction which employs the method of active esters[7]

$$2\ X—NHCH(R)COONp + H_2N—M'_x—NH_2 \xrightarrow{-HONp}$$

$$\longrightarrow X—NHCH(R)CONH—M'_x—NHCOCH(R)NH—X \xrightarrow{-X}$$

$$\longrightarrow H_2N—CH(R)CONH—M'_x—NHCOCH(R)—NH_2\ ;$$

here, X stands for the protective tert-butyloxycarbonyl (Boc) or benzyloxycarbonyl (Z) group, Np is the *p*-nitrophenyl group and —M'_x— is the polymer chain —$(CH_2)_3$.$(—OCH_2CH_2)_x—CH_2—$. The composition of residues R related to the α-aminoacids is defined in the text and in Table I.

The starting derivatives of α-aminoacids (names and abbreviations given according to IUPAC-IUB, J. Biol. Chem. 247, 977 (1972)) had the L-configurations. The following polymer diamides were prepared: [Boc-Asp(OBzl)NH]$_2M'_x$ (*I*), [Boc-Glu.(OBzl)NH]$_2M'_x$ (*II*), [Boc-PheNH]$_2M'_x$ (*III*), and [N-Z-TyrNH]$_2M'_x$ (*IV*). The removal of residues of *p*-nitrophenyl esters and *p*-nitrophenol from the raw product is based on the different solubility of reaction components in diethyl ether: The polymers are insoluble, while *p*-nitrophenyl esters and *p*-nitrophenol are soluble. Purity of the product, and particularly perfect removal of the last traces of the starting polymer and *p*-nitrophenol, depend on the purification procedure. Extraction of the dry residue of the reaction mixture with diethyl ether (*cf.* Experimental, procedure *A*) gives a product which according to TLC contains several tenths per cent of *p*-nitrophenol and traces of the starting polymer, while extraction of the acidified aqueous solution of dry residue with diethyl ether (*cf.* Experimental, procedure *B*) gives products which are chromatographically pure – R_F 0·30 (TLC on SiO_2, elution with the mixture DMF/H_2O 1 : 5); the only peak in gel chromatography corresponds to that of the starting polymer (identical hydrodynamic volumes of the compounds compared). The results and chemical analyses are given in Table II.

The molar ratio between aminoacid units and oxyethylene units (n_a/n_M) in polymers *I*–*IV* was determined using the signal of protons of the tert-butyloxycarbonyl group (Boc), of the phenylene group (Phen) in the tyrosine unit and of the oxyethylene unit (M). The assignment of lines in the ^{1}H NMR spectra of polymers was checked by comparing them with those of the starting compounds (including *p*-nitrophenyl esters). In the spectra of all amides containing Boc and the OCH_2CH_2 group, single

TABLE I

Absorption maxima of carbonyl groups (cm^{-1}) in the IR spectra of polymers [X—NHCH(R).CONH]$_2M'_x$ (3–5 mg of compound/g KBr)

X	R	$C_6H_5CH_2OCO$	—O—CO—NH	CH(R)CONH
$(CH_3)_3C—O—CO$	$C_6H_5CH_2OCOCH_2$	1 740	1 717	1 675
$(CH_3)_3C—O—CO$	$C_6H_5CH_2OCO.(CH_2)_2$	1 737	1 716	1 671
$(CH_3)_3C—O—CO$	$C_6H_5CH_2$	—	1 715	1 673
$C_6H_5CH_2—O—CO$	$HOC_6H_4CH_2$	—	1 724	1 672

lines were detected having the chemical shifts at δ 1·39 and δ 3·60; for amides containing the tyrosine unit, a quartett corresponding to this unit was found at δ 6·79. The value of n_a/n_M for polymers *I*–*III* was calculated from the relative band intensities using the formula $n_a/n_M = 4/9(I_{Boc}/I_M)$, for polymer *IV* it was calculated as I_{Phen}/I_M. The theoretical value of this ratio calculated on the basis of stoichiometry for the starting polymer is 0·043. According to the results in Table II, amidation in all these cases proceeded within the limits 86–95% yield.

In the infrared spectra of solid polymer diamides containing protected amino groups, an intensive band of amide I was recorded at 1 671–1 675 cm^{-1} along with those of aminoacid units (Table I); this band characterizes the linkage between the acyl group of α-aminoacid and the NH-group of the polymer chain. In polymer diamides, a maximum corresponding to the N—H stretching vibration of the CONH group appeared at 3 433 cm^{-1}, instead of the bands at 3 386 cm^{-1} (asym. stretching —NH_2) and 3 322 cm^{-1} (sym. stretching —NH_2), which characterize the starting α-(3-aminopropyl)-ω-(aminomethyl)poly(oxyethylene) (data from CH_2Cl_2 solution measurements).

Polymers *I*–*III* were deprotected by means of trifluoroacetic acid. Salts of polymers, $M'_x[NHCOCH(R)\overset{+}{N}H_3]_2\ 2\,CF_3COO^-$, were obtained in yields above

TABLE II

Yields and compositions of diamides *I*–*IV* in the extraction procedures *A* and *B*. The calculated amount of elements (mass %) is based on the analysis of the starting polymer $H_2N(CH_2)_3$—$(—OCH_2CH_2)_{46}—CH_2NH_2$. Molar content of α-aminoacid and monomer units (n_a/n_M) has been calculated according to ^1H NMR spectra, for samples isolated by the procedure *B*

Polymer diamide	Yield (*A*/*B*) mass %	Calculated			Found (*A*/*B*)			n_a/n_M
		% C	% H	% N	% C	% H	% N	
I	65	55·63	8·87	1·98	55·47	8·98	1·85	0·038
	84				55·61	8·78	1·85	
II	80	55·78	8·93	1·96	56·00	8·87	2·06	0·037
	79				56·39	9·15	1·79	
III	82	56·10	9·11	2·07	56·25	8·91	2·30	0·041
	83				55·16	9·08	1·81	
IV	65	57·20	8·56	1·93	56·96	8·40	1·87	0·037
	45[a]				56·46	8·98	1·62	

[a] Before extraction (*cf.* Experimental) acidified with 50 ml of 10% aqueous $KHSO_4$ solution instead of acetic acid.

80 mass %. The content of $NH_3CF_3COO^-$ groups, c, in these salts was determined by titration with a solution of sodium ethoxide. The calculated theoretical concentrations of ammoniumtrifluoroacetate groups in deprotected polymers were obtained by reducing the determined concentration values of amino goups in the starting polymer proportionally to the ratio between the relative molar masses of the starting polymer (2 117) and of the product of complete deprotection. The values thus obtained lie close to the calculated ones. The absence of a signal which in the ^1H NMR spectrum belongs to protons of the Boc group confirmed the completeness of deprotection.

R	$CH_2COOCH_2C_6H_5$	$(CH_2)_2COOCH_2C_6H_5$	$CH_2C_6H_5$
Salt yield, %	87	92	84
$c_{calc.}$, mol kg^{-1}	0·727	0·717	0·759
c_{found}, mol kg^{-1}	0·735	0·790	0·795

Biodegradability of peptide links on the poly(oxyethylene) chain has been proved[3]. The polymer diamides prepared by us, based on poly(oxyethylene) and having the structure $H_2NCH(R)CONH(CH_2)_3(—OCH_2CH_2)_x—CH_2NHCOCH(R)NH_2$, may serve for a further attachment of compounds able to react with amino groups, and are thus potential carriers of biologically active compounds. In contrast to polymers having the structure $H_2NCH(R)CO(—OCH_2CH_2)_y—OCOCH(R)NH_2$ and the ester attachment of α-aminoacid units, the amide attachment has an advantage in the higher chemical stability of the amide bond, especially in those cases where easy hydrolyzability with acidic and basic catalysts would compete with the selective enzyme hydrolysis. The stability of the amide bond may become an advantage in the necessary consecutive chemical processes, *e.g.* in the reduction with complex hydrides, when the amide bond does not split, unlike the ester one, or in the utilization of the second carboxylic group after hydrolysis of the ester group of the β-benzylaspartate or γ-benzylglutamate unit.

EXPERIMENTAL

Chemicals

All solvents were reagent grade, dried by employing the usual procedures and distilled before use. α-(3-Aminopropyl)-ω-(aminomethyl)poly(oxyethylene), prepared according to ref.[8] and provided by Chemlon (Humenné, Czechoslovakia), was reprecipitated from a 50% solution in benzene into a fiftyfold volume of diethyl ether and dried *in vacuo* (0·13 kPa, 25°C, 20 h); the acidometrically obtained [$—NH_2$] value (0·946 mol kg^{-1}) means that the number of oxyethylene (monomer) units (M) in the chain, x, is 46, which corresponds to the relative molar mass 2 117.

The starting derivatives of α-aminoacids had the L-configuration (Fluka, puriss.): γ-benzyl-glutamate, β-benzyl-N-(tert-butoxycarbonyl)aspartate (Boc-Asp(OBzl)OH), 4-nitrophenyl N--(tert-butoxycarbonyl)phenylalaninate, 4-nitrophenyl N-(benzyloxycarbonyl)tyrosinate.

γ-Benzyl-N-(tert-butoxycarbonyl)glutamate was prepared according to ref.[9] (yield 77%) and esterified by the DCC method to α-(4-nitrophenyl)-γ-benzyl-N-(tert-butoxycarbonyl)glutamate, yield 48% related to Boc-Glu(OBzl)OH, m.p. 119–121°C (ethyl acetate), R_F 0·47 (Silufol UV 254, ethylacetate/hexane 1 : 2). Similarly, α-(4-nitrophenyl)-β-benzyl-N-(tert-butoxycarbonyl)aspartate was prepared from Boc-Asp(OBzl)OH (yield 65%). UV spectrum (DMSO): 272 nm (ε 9 170). For $C_{22}H_{24}N_2O_8$ (444·4) calculated: 59·45% C, 5·44% H, 6·30% N; found: 59·53% C, 5·66% H, 6·26% N.

Reaction of α-Aminoacids *p*-Nitrophenyl Esters with the Polymer $H_2N—M'_x—NH_2$

To a solution of X—NHCH(R)COONp (2·2 mmol) in 20 ml dichloromethane, a solution of α-(3-aminopropyl)-ω-(aminomethyl)poly(oxyethylene) (2 mmol aminogroups) in the same volume of the same solvent was added with stirring at 25°C during 30 min. The homogeneous solution was stirred for another 3 h and then left to stand overnight. The volatile fractions were distilled off *in vacuo* (rotational evaporator, bath 40°C), and the product $[X—NHCH(R)CONH]_2M'_x$ was isolated by two alternative procedures:

A) The honey-like dry residue (1 g) was extracted ten times with 50 ml dry diethyl ether and dissolved in 150 ml dichloromethane. The solution was washed three times with 10 ml 10% $NaHCO_3$ and twice with 10 ml water. After free evaporation of the solvent the polymer compound was transferred into the aqueous solution and dried by lyophilization.

B) The aqueous solution (50 ml) of the dried residue (1 g) was acidified with 0·5–1 ml of acetic acid and extracted three times with 15 ml diethyl ether. From the aqueous solution the compound was extracted into chloroform (four times with 20 ml), the chloroform solution was washed twice with 10 ml of 10% $NaHCO_3$, then with water to neutral reaction, dried with $MgSO_4$, filtered, and evaporated. The product was dried by lyophilization.

Deprotection of polymers $[(CH_3)_3C—O—C(:O)NHCH(R)CONH]_2M'_x$: The protective groups were removed from the polymer (0·5 g) by means of 5–10 ml of a mixture of trifluoroacetic acid and dichloromethane (1 : 1 by vol.) at 25°C (30 min). The products (diammonium salts) were isolated and repurified after evaporation of the agent and solvent by repeated precipitation from the solution in ethanol into a mixture of diethyl ether and heptane (2 : 1). The salts were dried over NaOH *in vacuo* at 45°C (overnight).

Analytical Methods

The thin-layer chromatography of the starting compounds and products was carried out using Silufol UV 254 plates (Lachema, Czechoslovakia), with a $KMnO_4$ (1·6%) solution in diluted H_2SO_4 (3 ml conc. H_2SO_4 *per* liter H_2O) used for stain detection; selective detection of the presence of oxyethylene units was performed by spraying the chromatogram with aqueous soution of NH_4SCN (17%), $Co(NO_3)_2.6\,H_2O$ (3%), and finally, CH_3COOH (0·2%).

Gel chromatography was performed on a column 0·9 × 45 cm packed with Sephadex G-50, buffer 0·05M-CH_3COONH_4, flow rate 15 ml/h.

Infrared spectra were recorded with a Perkin–Elmer 580 B spectrometer (KBr discs or 5% solutions in dichloromethane). The ultraviolet spectrum was recorded with a Hewlett–Packard 8451 A spectrometer in a 0·1 mm cell. CW ^1H NMR spectra of 10% solutions of the starting compounds and of products containing a protected aminogroup in deuteriochloroform (with

hexamethyldisiloxane as the internal standard) were measured with a JEOL PS-100 spectrometer at 100 MHz.

Titration microdeterminations were performed conductometrically using a DIGI 610 apparatus (WTW, F.R.G.) with a glossy platinum electrode. The content of amino groups in the starting polymer was determined in an aqueous solution by means of 0·1M-HCl. The content of ammoniumtrifluoroacetate groups in deprotected samples was detected by titrating their solutions in isopropyl alcohol with ethanolic 0·1M-$NaOCH_2CH_3$ under nitrogen (consumption for solvent according to the blank test was subtracted).

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REFERENCES

1. Harris J. M.: J. Macromol. Sci., Rev. Macromol. Chem. Phys. *25*, 325 (1985).
2. Mutter M., Hagenmaier H., Bayer E.: Angew. Chem., Int. Ed. *10*, 811 (1971).
3. Ulbrich K., Strohalm J, Kopeček J.: Makromol. Chem. *187*, 1131 (1986).
4. Zalipsky S., Gilon C., Zilkha A.: Eur. Polym. J. *19*, 1177 (1983).
5. Ribeiro A., Saltman R., Goodman M.: Biopolymers *24*, 2431, 2449, 2469, 2495 (1985).
6. Zalipsky S., Gilon C., Zilkha A.: J. Macromol. Sci., Chem. *21*, 839 (1984).
7. Bodanszky M., Bodanszky A.: *The Practice of Peptide Synthesis*, p. 114—115. Academie Verlag, Berlin 1985.
8. Polievka M., Macho V., Balák J., Petrus T., Kunovský J.: Czech. 155578 (1974); Chem. Abstr. *82*, 170125 (1975).
9. Li Ch. H., Gorup B., Chung D., Ramachandran J.: J. Org. Chem. *28*, 178 (1963).

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