SYNTHESIS OF MODEL ALANINE-GLYCINE PEPTIDES

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Khimiya Prirodnykh Soedinenii, Vol. 4, No. 1, pp. 34-39, 1968

One of the numerous methods of synthesizing peptides is the N-acylation of amino acids and peptides or their ethyl, methyl, and benzyl esters with the thiophenyl and p-nitrophenyl esters of N-protected amino acids and peptides.

The present paper describes the synthesis of a number of DL-α-alanine-glycine peptides with various sequences of amino acids in the chain and also the production of some intermediates. The peptides were obtained both by the stepwise building up of the chain [Ala-Gly, Gly-Gly-Gly-Ala, etc. (here and below, the conventional abbreviations are used for the amino acids)], and by the condensation of fragments one of which was a thiophenyl [1] or a p-nitrophenyl ester and the second an amino acid or peptide with a free amino group. The carbobenzoxy group (cbz group) was used as the N-protective group for the first fragment [3]. The carboxy group of the second fragment was protected in the form of a benzyl ester [4] or was left unprotected.

The use of these methods made it possible to synthesize protected peptides in high yields (50-73%). The best results were achieved when using the p-nitrophenyl esters (yields 65-73%).

The initial thiophenyl and p-nitrophenyl esters of the amino acids and peptides were obtained by the usual methods of esterification in the presence of dicyclohexylcarbodiimide (DCHC) in a mixture of anhydrous tetrahydrofuran and dimethylformamide. The yields under these conditions were 60-80%.

The cbz group of the peptide was removed with hydrogen bromide in glacial acetic acid [5] with subsequent ionophoretic elimination of the bromine ions, and also by reduction with sodium in liquid ammonia [6].

The reduced peptides were freed from sodium ions by ion-exchange chromatography on IRC-50 resin (NH_4^+ form) with subsequent elimination of mineral salts by the ionophoretic method.

The synthesis of hexapeptides can be represented by the following schemes:

A. Condensation with thiophenyl esters

$$Z\text{—NH}\cdot\underset{\text{Z—I}}{\text{CHR}\cdot\text{CONHCH}_2}\cdot\text{CO}\cdot\text{NH}\cdot\text{CH}_2\text{COSC}_6\text{H}_5+\underset{\text{II}}{\text{H}_2\text{NCHR}_1\text{CONHCH}_2\text{CONHCHR}_2\text{COOH}}\rightarrow$$

$$\rightarrow Z\text{—NHCHR}\cdot\underset{\text{L—III}}{\text{CONHCH}_2\text{CONHCH}_2\text{CONH}}\cdot\text{CHR}_1\cdot\text{CONHCH}_2\text{CONHCHR}_2\text{COOH}\rightarrow$$

$$\rightarrow \xrightarrow[\text{ionophoresis}]{\text{HBr in glac. CH}_3\text{COOH}} \text{H}_2\text{NCHRCONHCH}_2\text{CONHCH}_2\underset{\text{III (a-d)}}{\text{CONHCHR}_1}\cdot\text{CONHCH}_2\times$$

$$\times\,\text{CONHCHR}_2\text{COOH}.$$

B. Condensation with p-nitrophenyl esters

$$Z\text{—NHCHR}\cdot\text{CONHCH}_2\cdot\text{CONH}\cdot\text{CH}_2\cdot\text{COOC}_6\text{H}_4\cdot\text{NO}_2\text{—p}+$$

$$+\text{p—CH}_3\cdot\text{C}_6\text{H}_4\text{SO}_3\text{H}\cdot\text{H}_2\text{N}\cdot\text{CHR}_1\cdot\text{CONHCH}_2\cdot\text{CONHCH}_2\text{COOCH}_2\text{C}_6\text{H}_5\rightarrow$$

$$\rightarrow Z\cdot\text{NH}\cdot\text{CHR}\cdot\text{CONH}\cdot\text{CH}_2\cdot\text{CONHCH}_2\text{CONHCHR}_1\cdot\text{CONHCH}_2\cdot\text{CONHCH}_2\text{COOCH}_2\text{C}_6\text{H}_5\rightarrow$$

$$\xrightarrow[\text{ionophoresis}]{\text{Na in liquid NH}_3}\text{III}$$

a. $R = CH_3$, $R_1 = R_2 = H$; c. $R = R_1 = H$, $R_2 = CH_3$;

b. $R = R_2 = H$, $R_1 = CH_3$; d. $R = R_1 = CH_3$, $R_2 = H$

Experimental

Thiophenyl esters of cbz-amino acids and peptides

A solution of 0.01 mole of amino acid or peptide and 0.011 mole of DCHC in a mixture of dry tetrahydrofuran and dimethylformamide (3:1) was cooled to -20° C and, with vigorous stirring, 0.011 mole of thiophenol was added dropwise over 30 min. After the elimination of the dicyclohexylurea, the filtrate was evaporated in vacuum in a rotary evaporator. The thiophenyl esters were isolated by treating the residue with ethanol. In individual experiments in which the product could not be precipitated in the solid state with ethanol, the residue was dissolved in ethyl acetate and the solution was washed with 0.2 N sodium bicarbonate solution and dried with sodium sulfate, and the ether was precipitated with ethanol. For purification, the product was recrystallized from ethanol.

p-Nitrophenyl esters of cbz-peptides

A solution of 0.005 mole of the cbz-peptide and 0.0063 mole of p-nitrophenol in 55 ml of a mixture of dry tetrahydrofuran and dimethylformamide (10:1) was cooled to 0° C, and 0.005 mole of DCHC was added with vigorous stirring. After stirring for 2.5 hr at room temperature, the dicyclohexylurea was filtered off and the filtrate was evaporated in a rotary evaporator. The residue, in the form of an oily liquid, was treated with 0.2 N potassium bicarbonate solution and the precipitate that deposited was filtered off and washed with 20–25 ml of 0.2 N potassium bicarbonate solution, 400 ml of water, and, finally, 10 ml of ethanol. The p-nitrophenyl esters of the cbz-peptides were crystallized from ethanol.

The results of experiments on the preparation of the thiophenyl and p-nitrophenyl esters are given in Table 1.

p-Toluenesulfonates of benzyl esters of the peptides

A mixture of 0.1 mole of a peptide and 0.11 mole of p-toluenesulfonic acid in 50 ml of anhydrous ether was heated in a Hofmann extractor (with the absorption of water by a mixture of sodium and magnesium sulfates) until the material had dissolved completely (about 30 min). 60 ml of absolute carbon tetrachloride was added and heating was continued for

Table 1

Thiophenyl and p-Nitrophenyl Esters of cbz-amino Acids and cbz-Peptides

Ester of cbz-amino acid or cbz-peptide	Yield, %	mp, °C	Formula	Nitrogen content, %	
				found	calculated
Thiophenyl esters					
Glycine	71	71—72	$C_{16}H_{15}O_3NS$	4.60	4.65
Alanine	69	73—74	$C_{17}H_{17}O_3NS$	4.37	4.44
Glycylalanine	62	97—98	$C_{19}H_{20}O_3N_2S$	7.15	7.52
Diglycylglycine (Ib)	64	129—131	$C_{20}H_{21}O_5N_3S$	10.92	10.11
Alanylglycylglycine (Ia)	65	145—147	$C_{21}H_{23}O_5N_3S$	9.39	9.80
p-Nitrophenyl esters					
Diglycylglycine (IIa)	75	178—180	$C_{20}H_{20}O_8N_4$	12.19	12.60
Alanylglycylglycine (IIb)	80	131—133	$C_{21}H_{22}O_8N_4$	12.20	12.22

Note. Here and below the tables contain the mean results of a series of experiments.

another 6.5 hr. Then 100 ml of the same solvent was added to the viscous reaction mixture and it was left in a refrigerator at 0–5° C for 12 hr. The precipitate of the sulfonate of the benzyl ester of the peptide was filtered off and recrystallized from ethanol (Table 2).

A. Preparation of cbz-peptides by the thiophenyl ester method

Over 2 hr, 0.05 mole of a free amino acid or peptide was added in 4–5 portions to a solution of 0.05 mole of a thiophenyl ester of a cbz-amino acid or cbz-peptide in 5–10 ml of glacial acetic acid heated to the boil. The solvent was distilled off in vacuum and the reaction product was precipitated with ethanol or, in individual cases, the residue was

Table 2

p-Toluenesulfonates of Benzyl Esters of Tripeptides

Compound	Yield, %	mp, °C	Formula	Nitrogen content, %	
				found	calculated
Alanylglycylglycine	79	188—190	$C_{21}H_{27}O_7N_3S$	8.67	9.01
Diglycylglycine	75	174—175	$C_{20}H_{25}C_7N_3S$	—	—

dissolved in ethyl acetate, the solution was washed with sodium bicarbonate, and the peptide was obtained from the aqueous layer by acidification with 5 N hydrochloric acid to pH 2–3. The resulting cbz-peptides were purified by crystallization from ethanol (Table 3).

Table 3

Preparation of cbz-Peptides

Peptide	Initial compounds		Yield, %	mp, °C	Formula	Analysis, %					
						found			calculated		
	Thiophenyl esters of cbz-peptides and cbz-amino acids	Amino acids or peptides				C	H	N	C	H	N
Alanylglycine	Alanine	Glycine	55	132—133	$C_{13}H_{16}O_5N_2$	—	—	9.79	—	—	9.99
Diglycylalanine (IIa)	Glycine	Glycylalanine	60	184—185	$C_{15}H_{19}O_6N_3$	—	—	12.31	—	—	12.45
Triglycylalanine	Diglycylglycine	Alanine	54	193—195	$C_{17}H_{22}O_7N_4$	—	—	14.25	—	—	14.20
Alanyltetraglycylglycine (IIIa)	Alanylglycyl-glycine	Diglycylgly-cine	51.8	232—235	$C_{21}H_{28}O_9N_6$	49.45	5.30	16.70	49.60	5.62	16.52
Triglycylalanylglycyl-glycine (IIIb)	Diglycylgly-cine	Alanylglycyl-glycine	53.8	> 195	" "	49.29	5.47	16.45	49.60	5.62	16.52
Pentaglycylalanine (IIIc)	Diglycylgly-cine	Diglycylala-nine	52	225—228	" "	49.51	5.31	16.68	49.60	5.62	16.52
Alanyldiglycylalanyl-glycylglycine	Alanylglycyl-glycine	Alanylglycyl-glycine	50.5	163—164	$C_{22}H_{30}O_9N_6$	—	—	16.17	—	—	16.08

Table 4

Benzyl Esters of cbz-Peptides

Peptide	Starting materials		Yield, %	mp, °C	Formula	Nitrogen content, %	
	cbz-Peptide p-nitrophenyl ester	Peptide benzyl ester p-toluenesulfonate				found	calculated
Glycylalanylglycine	Glycylalanine	Glycine	65	161—162	$C_{22}H_{25}O_6N_3$	10.67	9.83
Triglycylalanylglycylglycine (Ib)	Diglycylglycine	Alanylglycylglycine	68	214—215	$C_{28}H_{31}O_9N_6$	14.25	14.01
Alanyltetraglycylglycine	Alanylglycylglycine	Diglycylglycine	73.5	214—215	" "	14.02	14.01

Table 5

Ammonium Salts of the Peptides

Peptide	mp, °C	Formula	Analysis						R_f value**	Electrophoretic mobility***
			C	H	N	C	H	N		
Alanylglycine	—	$C_5H_{13}N_3O_3$	—	—	25.17	—	—	25.75	1.46	1.22
Diglycylalanine	—	$C_7H_{16}O_4N_4$	37.80	7.22	25.46	38.17	7.32	25.44	1.15	1.12
Glycylalanylglycine	—	$C_7H_{19}O_5N_5$*	40.26	6.18	20.10	41.37	6.44	20.68	—	1.12
Triglycylalanine	214—215 (decomp.)	$C_9H_{19}O_5N_5$	—	—	25.08	—	—	25.38	1.07	—
Alanyltetraglycylglycine (IIIa)	220 (decomp.)	$C_{13}H_{25}O_7N_7$	39.04	6.03	24.28	39.89	6.43	25.05	0.61	0.9
Triglycylalanylglycylglycine (IIIb)	216—218 (decomp.)	The same	40.51	5.47	24.26	39.89	6.43	25.05	—	0.94
Pentaglycylalanine	~ 240 (decomp.)	The same	40.03	5.45	24.16	39.89	6.43	25.05	0.66	0.91
(IIIc)-Alanyldiglycylalanylglycylglycine	200 (decomp.)	$C_{14}H_{27}O_7N_7$	—	—	24.01	—	—	24.18	0.77	0.91

*Glycylalanylglycine after reduction with hydrogen over Pd/C at room temperature.

**Chromatographed on Whatman 3 mm paper in the butanol—acetic acid—water (4:1:5) system, R_f relative to glycine.

***6% acetic acid, 1100 V, 4.8–7.8 mA, 1 hr. Whatman 3 mm.

B. p-Nitrophenyl ester method

A solution of 0.001 mole of the p-toluenesulfonate of the benzyl ester of an amino acid or peptide in 2–5 ml of absolute dimethylformamide cooled to 0° C was treated with 0.001 mole of triethylamine and 0.001 mole of the p-nitrophenyl ester of a cbz-amino acid or cbz-peptide. The solution was left at room temperature for 3 days. Then it was cooled to 0° C and treated with a fivefold amount of cold water. The precipitate of the benzyl ester of the cbz-peptide was filtered off and crystallized from ethanol (Table 4).

Removal of the protective groups with hydrogen bromide in glacial acetic acid

A suspension of 0.0005 mole of cbz-peptide in 1–1.5 ml of glacial acetic acid was treated with 1–1.5 ml of a 40% solution of hydrogen bromide in glacial acetic acid. After 40 min, the peptide was precipitated in the form of the hydrobromide by the addition of absolute ether. The precipitate was washed several times with ether (by decantation) and was dried over phosphorus pentoxide and sodium hydroxide in vacuum.

To obtain the ammonium salt, the peptide hydrobromide was subjected to ionophoresis in aqueous solution against 0.1 N acetic acid and 0.01 N ammonia. The ionophoresis was carried out in an instrument of the OE-101 type. The results of elementary analyses, chromatography, and electrophoresis for the ammonium salts of the peptides are given in Table 5.

Reduction with metallic sodium in liquid ammonia

Metallic sodium was added in small portions to a solution of 0.0002 mole of the benzyl ester of a cbz-peptide in 10 ml of liquid ammonia freshly distilled over sodium until a permanent blue color was produced. At the end of the reduction, the excess of sodium was removed with ammonium chloride (disappearance of the blue coloration). The ammonia was distilled off in vacuum and the residue was dissolved in 100 ml of water and treated with IRC-50 ion-exchange resin (NH_4^+ form). The solution was evaporated in a rotary evaporator and the peptide was desalted by ionophoresis against acetic acid and ammonia. The peptide was isolated in the form of the ammonium salt.

Summary

The possibility of synthesizing alanine-glycine tetra- and hexapeptides by the N-acylation of amino acids and peptides and also by that of their benzyl esters with thiophenyl and p-nitrophenyl esters of cbz-peptides has been shown.

REFERENCES

1. F. Weygand and W. Steglish, Ber., 93, 2983, 1960.
2. M. Bodansky and V. du Vigneaud, J. Am. Chem. Soc., 81, 3980, 1959.
3. M. Bergmann and L. Zervas, Ber., 65, 1192, 1932.
4. L. Zervas, M. Winitz, and G. P. Greenstein, J. org. chem., 22, 1515, 1957; H. K. Miller and H. Waelseh, J. Am. Chem. Soc., 74, 1092, 1952.
5. W. Anderson, G. Blodinger, and A. D. Welcher, J. Am. Chem. Soc., 74, 5309, 1952.
6. R. H. Srifferd and V. du Vigneaud, J. Biol. Chem., 108, 753, 1935.

1 August 1966