SYNTHESIS OF POLYPEPTIDES WITH THE SEQUENCES -Gly-Gly-Ala-, -Gly-Gly-Lys(Tos)-, AND -Gly-Gly-Glu(OCH_3)- AND THE INFLUENCE OF TRIETHYLAMINE ON THE DEGREE OF THEIR POLYCONDENSATION

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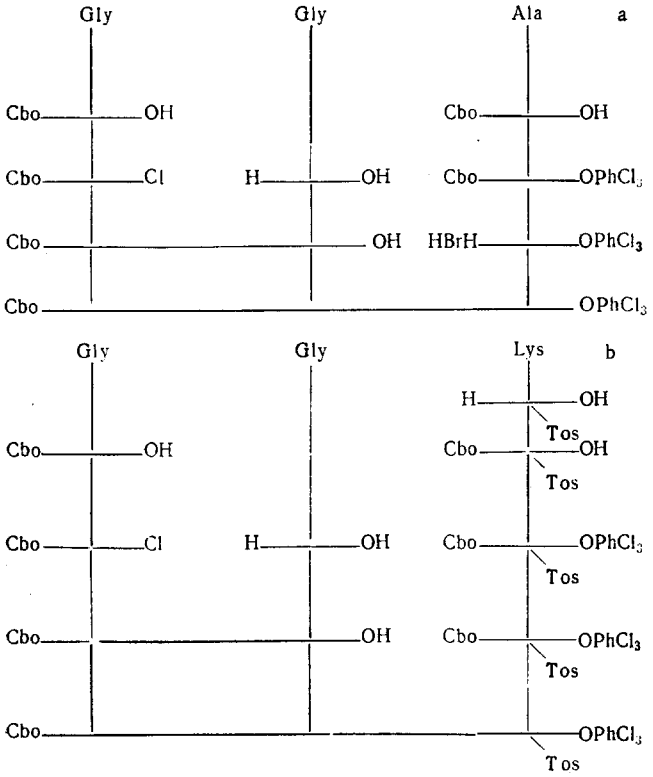
Polypeptides of regular structure are widely used as model compounds of protein structures [1]. Of all the methods for their synthesis, the most widely used is that of the polycondensation of activated esters of peptides, the 2,4,5-trichlorophenyl and pentachlorophenyl esters ensuring the highest degree of polycondensation [2]. However, there are also other factors that affect the size of the polypeptide chains of the polymers. Thus, we have established that the degree of polycondensation of peptide monomers is affected

Scheme 1



Scheme of the synthesis of Cbo-Gly-Gly-Ala-$OPhCl_3$ (a), Cbo-Gly-Gly-Lys(Tos)-$OPhCl_3$ (b).

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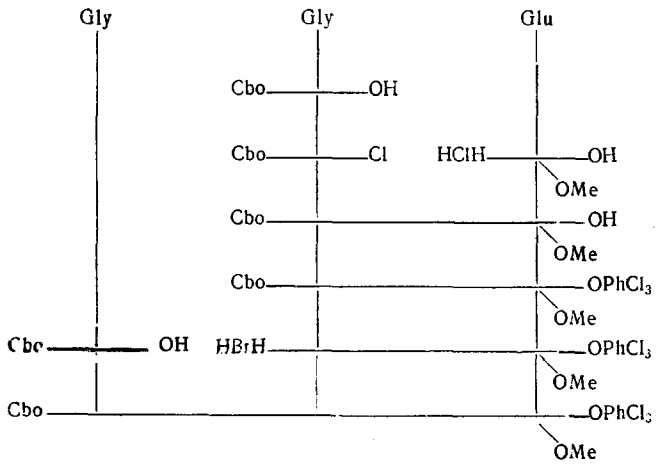
Scheme 2



Scheme of the synthesis of Cbo-Gly-Gly-Glu(OCH_3)-$OPhCl_3$

TABLE 1

Polypeptide	Amount of triethylamine, equiv.	M_{av} Van Slyke	M_{av} IR spectra
-(Gly-Gly-Ala)$_n$-	1	3460	4000
	2	11896	8800
-[Gly-Gly-Lys(Tos)]$_n$-	1	21960	24500
	2	39897	33600
-[Gly-Gly-Glu(OCH_3)]$_n$-	1	2827	3300
	2	2923	3500

by the nature of the N- and C-terminal amino acids [3]. Kovacs et al. have shown that an appreciable rise in the molecular weights of the polypeptides is achieved by increasing the amount of triethylamine to 2.5 equivalents [4]. It was desirable to consider these results for the case of monomers with different amino-acid sequences. For this purpose, we have synthesized the hydrobromides of the 2,4,5-trichlorophenyl esters of H-Gly-Gly-Ala-OH, H-Gly-Gly-Lys(Tos)-OH, and H-Gly-Gly-Glu(OCH_3)-OH. The required monomers were obtained by the hydrobrominolysis of the corresponding 2,4,5-trichlorophenyl esters of Cbo-tripeptides. The peptide bond was formed both by the acid chloride method and by the mixed anhydride method (Schemes 1 and 2). The N^{α}-amino group of lysine was protected by a Cbo group and its N^{ε} group by a tosyl radical. The use of the latter is not the optimum in the preparation of free peptides or polypeptides, since its removal takes place only under relatively severe conditions. Nevertheless, if it is not removed, as in our case, it is fairly convenient.

The mean molecular weights of the polypeptides were determined by Van Slyke's method and were also evaluated from the IR spectra. The latter method is based on measuring the relative intensities of the absorption bands of the carbonyl group of the peptide bond (amide I band in the 1650 cm^{-1} region), which is proportional to the number of these bonds in the polymer, and of the absorption band of the ester group in the 1780 cm^{-1} region:

$$n = \frac{D\ 1650\ cm^{-1}}{D\ 1780\ cm^{-1}} .$$

where n is the degree of polycondensation and D is the optical density.

The band at 1780 cm^{-1} is characteristic of an ester group of the 2,4,5-trichlorophenyl and pentachlorophenyl type. It differs from an alkyl ester group (1740 cm^{-1}) by a short-wave shift of 30-40 cm^{-1}. This difference enables us clearly to identify in the spectrum the methoxy and the 2,4,5-trichlorophenyl group simultaneously (Fig. 1a).

In agreement with this, on performing the polymerization of the hydrobromide of the 2,4,5-trichlorophenyl ester of H-Gly-Gly-Glu(OCH_3) a fall in the intensity of the absorption band in the 1780 cm^{-1} region is observed, while the intensity of the absorption band in the 1740 cm^{-1} region does not change (see Fig. 1b).

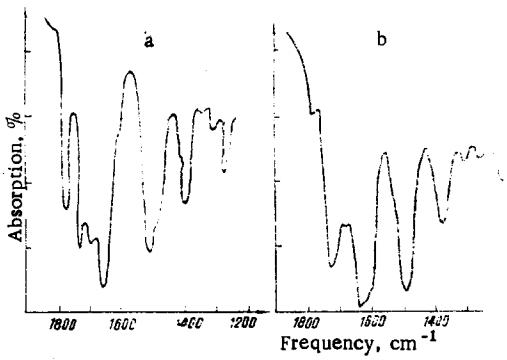


Fig. 1. IR absorption spectra of Cbo-Gly-Gly-Glu(OCH_3)-$OPhCl_3$ (a) and [-Gly-Gly-Glu(OCH_3)-]$_n$ (b).

It can be seen from Table 1 that an increase in the amount of triethylamine in the polycondensation reaction to two equivalents has an appreciable influence only in the case of the hydrobromides of the 2,4,5-trichlorophenyl esters of H-Gly-Gly-Ala-OH and H-Gly-Gly-Lys(Tos)-OH. It could not be detected in the preparation of [-Gly-Gly-Glu(OCH_3)-]$_n$. The addition of triethylamine up to an amount of three equivalents likewise did not lead to an increase in M_{av} of the polytripeptides. As the results of our observations showed, a positive effect of an excess of triethylamine is not possible in all cases. The effectiveness of the rise in M_{av} of polypeptides as a function of an increase in the amount of triethylamine is apparently connected to a considerable extent primarily with the amino-acid composition of the peptide monomer, and a positive effect can be expected in monomers containing N- or C-terminal glycine residues.

EXPERIMENTAL

Amino Acids of the L Form. Thin-layer chromatography was performed on plates with a fixed layer of silica gel (250 mesh, plate size 75 × 25 mm) in the systems butan-1-ol–acetic acid–water (4 : 1 : 1) (1) and sec-butanol–3% NH_4OH (100 : 44) (2). Chromogenic agents: a 0.5% solution of ninhydrin and iodine vapor. The IR spectra were taken on a UR-10 spectrophotometer.

Cbo-Gly-Gly-OH (I). The substance was obtained by the usual method from the acid chloride of Cbo-Gly-OH and an equimolar amount of glycine in 2 N NaOH, mp 174°C, R_f 0.72 (1), 0.43 (2).

Cbo-Gly-Glu(OCH_3)-OH (II). A solution of 8.2 g of HCl · H-Glu(OCH_3)-OH in 200 ml of water was made alkaline (pH 8) by the addition of an excess of $NaHCO_3$. The solution was cooled to −10°C and, with vigorous stirring, 9.5 g of the chloride of Cbo-Gly-OH was added in portions over 1 h. Then the solution was stirred at −5°C for another 1 h and at 20°C for 2 h, after which it was extracted with ether, and the aqueous layer was acidified with 6 N HCl. The oil that deposited was extracted with ethyl acetate. The extract was washed with water to a weakly acid pH and dried over Na_2SO_4. The solvent was evaporated off and the oily residue was crystallized by rubbing it with ether. The crystals were filtered off. The yield of (II) was 6.0 g (33.6%), $C_{10}H_{20}N_2O_7$, mp 104°C, $[\alpha]_D^{20}$ −10° (c 1.5; CH_3OH), R_f 0.89 (1) and 0.56 (2).

Cbo-Gly-Glu(OCH_3)-$OPhCl_3$(2,4,5) (III). To a solution of 2 g of Cbo-Gly-Glu(OCH_3)-OH in 10 ml of ethyl acetate was added 9.8 ml of triethylamine. The resulting solution was cooled to −10°C, and 0.72 ml of isobutyl chloroformate was added. After 40 min, a solution of 1.32 g of 2,4,5-trichlorophenol cooled to −10°C was added. The reaction mixture was stirred at −10°C for 1 h, at 0°C for 15 min, at 20°C for an hour, and at 50°C for 20 min. Then the ethyl acetate solution was washed successively with water, 1 N HCl, water, 0.5 N $NaHCO_3$, and water again, and was dried over Na_2SO_4. After evaporation of the solvent, a residue was obtained which crystallized out from ether. The yield of (III) was 2 g (66.6%), $C_{22}H_{21}Cl_3O_7N_2$, mp 98°C, R_f 0.96 (1) and 0.79 (2).

Cbo-Gly-Gly-Ala-$OPhCl_3$(2,4,5) (IV). In a similar manner to the preparation of (III), from 0.98 g of (I) dissolved in $CHCl_3$–CH_2Cl_2 (1 : 1), 0.5 ml of triethylamine, and 0.45 ml of isobutyl chloroformate the mixed anhydride was obtained, and to this was added a solution of 1.27 g of HBr · H-Ala-$OPhCl_3$(2,4,5) containing 0.5 ml of triethylamine. The subsequent reaction and purification were performed as for (III). The solvent was evaporated off and the residual oil was crystallized from hexane. The yield of (IV) was 1.21 g (60%), mp 132°C, $[\alpha]_D^{27}$ −31° (c 1.0; CH_3OH), R_f 0.47 (1) and 0.1 (2).

Cbo-Gly-Gly-Lys(Tos)-$OPhCl_3$(2,4,5) (V). This was obtained in the same way as compound (III) from 0.495 g of Cbo-Gly-Gly-OH, 0.25 ml of triethylamine, 0.225 ml of isobutyl chloroformate, and 1.05 g of HBr · H-Lys(Tos)-$OPhCl_3$(2,4,5) containing 0.25 ml of triethylamine. The yield of (V) (in amorphous form) was 1.09 g (81.3%), $C_{21}H_{33}Cl_3O_8H_4$, R_f 0.9 (2).

Cbo-Gly-Gly-Glu(OCH_3)-$OPhCl_3$(2,4,5) (VI). This was obtained similarly to compound (III) from 0.400 g of Cbo-Gly-OH, 0.26 ml of triethylamine, 0.26 ml of isobutyl chloroformate, and a solution of 0.92 g of HBr · H-Gly-Glu(OCH_3)-$OPhCl_3$(2,4,5) containing 0.26 ml of triethylamine. Yield 1.09 g (96.4%), $C_{24}H_{24}Cl_3O_8N_3$, mp 128°, $[\alpha]_D^{27}$ −20° (c 1; CH_3OH), R_f 0.89 (1) and 0.91 (2).

HBr · H-Gly-Gly-Ala-$OPhCl_3$(2,4,5) (VII). A solution of 0.95 g of Cbo-Gly-Gly-Ala-$OPhCl_3$(2,4,5) in 1.7 ml of glacial CH_3COOH was treated with 1.75 ml of 40% HBr (CH_3COOH) and the mixture was shaken for 30 min. The resulting hydrobromide (VII) was precipitated with ether, recrystallized from methanol, and dried in a vacuum desiccator over NaOH. The yield of (VII) was 0.644 g (77%), mp 163°, R_f 0.4 (1) and 0.3 (2).

HBr · H-Gly-Gly-Lys(Tos)-$OPhCl_3$(2,4,5) (VIII). This was obtained in the same way as (VII) from 1.38 g of the Cbo derivative. After recrystallization from methanol, the yield of (VIII) was 0.70 g (89%), mp 64°C, R_f 0.8 (1).

HBr · H-Gly-Gly-Glu(OCH_3)-$OPhCl_3$(2,4,5) (IX). This was obtained similarly to (VII) from 0.88 g of the Cbo derivative. The yield of (IX) was 0.65 g (81.25%), mp 98°C, R_f 0.65 (1).

Polycondensation. This was performed at a 50% concentration of the hydrobromide of the appropriate tripeptide 2,4,5-trichlorophenyl ester in dimethylformamide at 20°C with 1-2 equivalents of triethylamine in sealed tubes. After seven days, methanol was added to the reaction mixtures, and the polycondensates were precipitated as white amorphous substances.

The molecular weights of the methanol-insoluble polypeptides were determined by Van Slyke's method and, for comparison, by IR spectroscopy (see Table 1).

CONCLUSIONS

The synthesis of polytripeptides with the sequences -Gly-Gly-Ala-, -Gly-Gly-Lys(Tos)-, and -Gly-Gly-Glu(OCH_3)- having molecular weights of 11,000, 39,000, and 3000, respectively, have been effected.

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