SYNTHESIS OF A PROTECTED TETRAPEPTIDE CORRESPONDING TO THE SEQUENCE OF AMINO ACIDS 65–68 OF THE RIBONUCLEASE CHAIN

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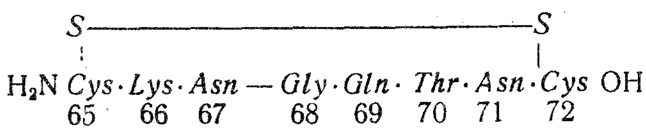
Interest in the synthesis of biologically active peptides is increasing [1] and therefore more and more attention is being given to the synthesis of peptides containing two or more cysteine residues in the peptide chain. It is known that when such peptides are oxidized disulfide bonds may arise both intramolecularly and intermolecularly in different arrangements, forming a complex mixture of cystine peptides [2]. The oxidation of reduced ribonuclease, in whose molecule there are six cysteine residues [3], takes place somewhat differently.

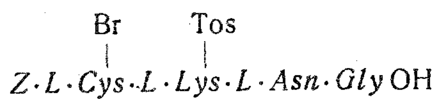
As special investigations have shown, the oxidation of reduced ribonuclease leads to an almost complete regeneration of its biological activity. This has permitted the assumption that the primary structure of ribonuclease apparently creates favorable conditions for the mutual approach of certain cysteine residues in its chain [4, 5].

In view of these results, it appeared desirable to effect the synthesis of a linear octapeptide (I) corresponding to the sequence of amino acids 65–72 in the molecule of ribonuclease [6] in order to evaluate the tendency to the formation of an intramolecular disulfide bond in this moiety, and thereby to find how necessary the remainder of the ribonuclease molecule is for the appearance of a cystine bridge in this part of the molecule.



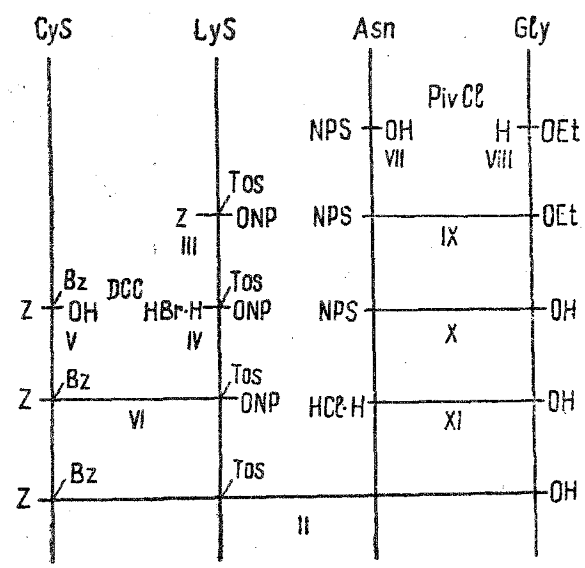
(I)

At the present time, we have synthesized the partially protected tetrapeptide*



(II)

The route for the synthesis of the peptide (II) can be seen from the following scheme:



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* The symbols used for the amino acids and their derivatives are in accordance with the decision of the 5th European Symposium, Oxford (September 1962). In addition, Asn = asparagine, Gln = glutamine, TEA = triethylamine, DMF = = dimethylformamide, DCC = dicyclohexylcarbodiimide, and PivCl = pivaloyl chloride.

The choice of the protecting groups in the synthesis of this moiety was determined by the possibility of their selective removal both in the further extension of the peptide chain and in the subsequent formation of a disulfide bond.

Experimental

The purity of all the substances was determined by thin-layer chromatography on hydrated silica containing gypsum. Acetone solutions of ninhydrin or iodine were used to reveal the spots.

The solutions, previously dried with sodium sulfate, were evaporated in vacuum. The substances for analysis were dried in vacuum (0.1–0.5 mm) at 60° C. Chromatography was carried out on Leningrad slow grade paper in the following systems: 1) butan-1-ol–acetic acid–water (4:1:5), 2) pyridine–isoamyl alcohol–water (10:10:7).

Hydrobromide of the p-nitrophenyl ester of ε-tosyl-L-lysine (IV). A solution of 9.58 g of (III) [7] in 11 ml of glacial acetic acid was treated with 5.9 ml of 5 N hydrogen bromide in glacial acetic acid and kept for 30 min at room temperature. The substance was isolated by the addition of dry ether, left in a refrigerator overnight, and filtered off. This gave 6.4 g (85%) of compound (IV), mp 182° C (from methanol), $[\alpha]_D^{20}$ +17.6° (c 2; EtOH), R_{f_1} 0.83; R_{f_2} 0.44.

Found, %: C 45.76; H 4.86; N 8.31. Calculated for $C_{19}H_{24}N_3O_6BrS$, %: C 45.42; H 4.82; N 8.37.

p-Nitrophenyl ester of N-carbobenzoxy-S-benzoyl-L-cysteinyl-ε-tosyl-L-lysine (VI). A solution of 7.22 g of (V) [8] and 10.48 g of (IV) in 50 ml of DMF was treated with 2.78 ml of $(C_2H_5)_3N$, cooled to 0° C, treated with 4.12 g of DCC, kept for 1 hr at 0° C, and left overnight at room temperature. The precipitate was filtered off and washed with 10 ml of DMF. Water was added to the filtrate until an oily substance separated, and extraction was carried out with ethyl acetate. The ethyl acetate solution was first washed with water acidified with hydrochloric acid and then with a 5% solution of sodium chloride to pH 7. It was then dried, and the solvent was distilled off to give 10.4 g (68%) of compound (VI), mp 173–174° C (from alcohol); $[\alpha]_D^{21}$ −1.7° (c 2; alcohol), R_{f_1} 0.59.

Found, %: C 58.21; H 4.99; N 7.58; S 7.98. Calculated for $C_{37}H_{38}N_4O_{10}S_2$, %: C 58.25; H 5.02; N 7.34; S 8.40.

Ethyl ester of o-nitrophenylsulfenyl-L-asparaginylglycine (IX). A solution of 5.7 g of (VII) [9] in 35 ml of chloroform was treated with 3.5 ml of TEA, and 2.7 g of PivCl was added at 10° C. The mixture was kept for 10 min without cooling and was then cooled to −10° C, and 2.2 g of (VIII) [10] dissolved in 15 ml of chloroform was added to the resulting mixed anhydride at a temperature not exceeding 0° C. The reaction solution was left for 4 hr at room temperature. The chloroform was distilled off in vacuum. The residue was triturated with a saturated solution of sodium hydrogen carbonate, filtered, and washed with water, 5% sulfuric acid, and water again. After drying, the substance was crystallized from nitromethane. The yield of compound (IX) was 4 g (54%), mp 201–203° C, $[\alpha]_D^{20}$ −117 ± 2° (c 2; DMF).

Found, %: C 45.27; H 4.89; N 15.26; S 8.51. Calculated for $C_{14}H_{18}N_4O_6S$, %: C 45.33; H 4.89; N 15.13; S 8.60.

o-Nitrophenylsulfenyl-L-asparaginylglycine (X). A solution of 3.7 g of (IX) in a mixture of 70 ml of ethanol and 30 ml of DMF was treated with 15 ml of 1 N caustic soda. After 15 min, the solution was diluted with double the volume of water and was extracted first with chloroform and then with ethyl acetate. The aqueous solution was acidified with 5% sulfuric acid and left overnight in a refrigerator. The precipitate was filtered off, washed with water, and dried. The yield of substance (X) was 3.0 g (89%), mp 130–135° C. For analysis the cyclohexylammonium salt was made: Cyclohexylamine was added to the substance dissolved in ethyl acetate–methanol (1:1) after which the solvent was distilled off in vacuum. The precipitate was filtered off, washed with ethyl acetate and petroleum ether, and recrystallized from a mixture of ethanol and ether. The melting point of the cyclohexylammonium salt of (X) was 174–175° C, $[\alpha]_D^{20}$ −63 ± 2° (c 1; methanol), R_{f_1} 0.6, R_{f_2} 0.77.

Found, %: C 48.59; H 5.92; N 15.58; S 7.13. Calculated for $C_{18}H_{27}N_5O_6S$, %: C 48.98; H 6.12; N 15.86; S 7.26.

Hydrochloride of L-asparaginylglycine (XI). A solution of 1.09 g of (X) in 12 ml of methanol was treated with 2 ml of a 3.5 N solution of hydrochloric acid in ethyl acetate. After 10-min stirring, the precipitate was filtered off, the solvent was distilled off in vacuum, and the residue was first triturated with ether–ethyl acetate (1:1) and was then dissolved in methanol, and the solution was boiled with carbon and filtered after which the solvent was distilled off to give 0.62 g of substance (XI) (86%), R_{f_1} 0.1, R_{f_2} 0.17.

Carbobenzoxy-S-benzoyl-L-cysteinyl-ε-tosyl-L-glycyl-L-asparaginylglycine (II). 1 g of (VI), 0.36 g of (XI), and 0.44 ml of TEA were dissolved in 15 ml of DMF and 12 ml of alcohol and the solution was left at room temperature for 48 hr. Then 30 ml of water acidified with hydrochloric acid was added and the mixture was placed in a refrigerator for several hours. The precipitate was filtered off, washed with water, and dried. The yield of substance (II) was 0.8 g (76%), mp 191–192° C (from 40% acetic acid), $[\alpha]_D^{20}$ −32 ± 2° (c 2; DMF), R_{f_1} 0.79, R_{f_2} 0.85.

Found, %: C 54.84; H 5.63; N 10.09; S 7.90. Calculated for $C_{37}H_{44}N_6O_{11}S_2$, %: C 54.67; H 5.46; N 10.37; S 7.89.

Summary

The synthesis of a partially protected tetrapeptide corresponding to the sequence of amino acids 65–68 of the ribonuclease chain has been effected.

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