SYNTHESIS OF A PROTECTED OCTAPEPTIDE CORRESPONDING TO SEQUENCE 65–72 OF THE RIBONUCLEASE CHAIN

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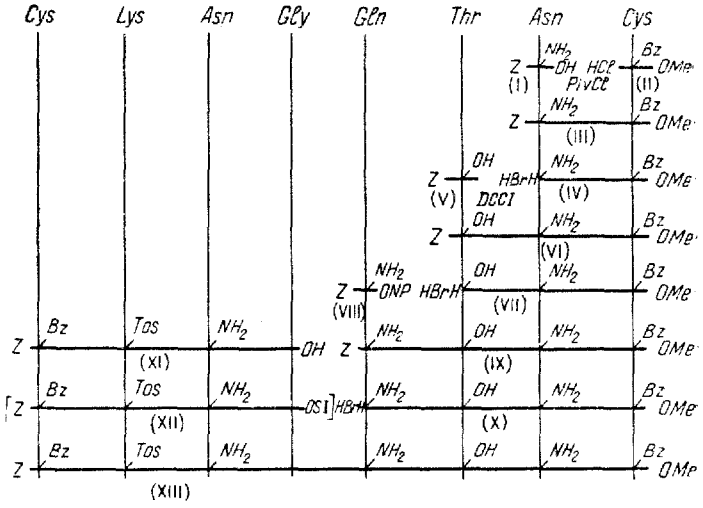
Continuing our investigations on the synthesis of a peptide fragment corresponding to the 65–72 sequence of the chain of ribonuclease [1], we have carried out the synthesis of protected peptide fragments X and XI (see scheme) corresponding to the sequences 69–72 and 65–72 of the ribonuclease molecule.

The tetrapeptide X was synthesized by the successive addition of amino acids starting with a C-terminal peptide. When carbobenzoxy-L-asparagine (I) was condensed with the methyl ester of S-benzoyl-L-cysteine (II) [2] by the mixed anhydride method, the dipeptide III was obtained. After elimination of the carbobenzoxy group by means of hydrogen bromide in glacial acetic acid, the dipeptide hydrobromide IV isolated was condensed by the dicyclohexylcarbodiimide method with the dicyclohexylammonium salt of carbobenzoxy-L-threonine (V) [3], giving the protected tripeptide VI. The tripeptide hydrobromide (VII) formed by the elimination of the carbobenzoxy group (HBr in glacial CH_3COOH) was condensed with the p-nitrophenyl ester of carbobenzoxy-L-glutamine (VIII) [4]. The tetrapeptide IX, after being treated with a solution of hydrogen bromide in glacial acetic acid, on reaction with the N-hydroxysuccinimide ester of the tetrapeptide XII obtained from the peptide XI [1], gave the peptide hydrobromide X. After recrystallization from aqueous acetic acid, the protected octapeptide XIII was obtained in the pure state.



Experimental*

For analysis, the substances were dried in vacuum for 5–6 hr. Their purity was determined chromatographically in a thin layer of alumina or silica containing 30% of gypsum. The R_f values were determined on Leningrad slow paper in the following systems: 1) pyridine–isoamyl alcohol–water (10:10:7); 2) 1-butanol–acetic acid–water (4:1:5). The melting points are uncorrected.

Methyl ester of carbobenzoxy-L-asparaginyl-S-benzoyl-L-cysteine (III)

With gentle heating, 5.3 g of I [2], 2.8 ml of TEA, and 1.6 ml of pyridine were dissolved in chloroform. The solution was cooled to −10° C and 2.7 g of PivCl was added [5]. The solution was stirred without cooling for 10 min and was then cooled to −10° C and a solution of 5.5 g of II [2] and 2.8 ml of TEA in chloroform was slowly added and the mixture was left at 20° C for 12 hr. The gel-like precipitate was evaporated to dryness, triturated with water, acidified with hydrochloric acid, washed with water, 5% sodium bicarbonate solution, and water again, dried, and crystallized from 40% acetic acid. Yield 6.7 g (67%), mp 175–177° C, $[\alpha]_D^{20}$ −7.5° (c 2; glacial CH_3COOH), R_{f_1} 0.94; R_{f_2} 0.60.

*In addition to generally accepted abbreviations for the amino acids, we have introduced the following: Asn: asparagine; Gln: glutamine; DMFA: dimethylformamide; PivCl: trimethylacetyl chloride; TEA: triethylamine; DCCI: dicyclohexylcarbodimide; and OSI: hydroxysuccinimide.

Found, %: C 57.78; H 5.12; N 8.32; S 6.48. Calculated for $C_{24}H_{25}N_3O_7S$, %: C 57.72; H 5.05; N 8.41; S 6.42.

Hydrobromide of the methyl ester of L-asparaginyl-S-benzoyl-L-cysteine (IV)

25 ml of a 35% solution of HBr in glacial CH_3COOH was added to 6.4 g of III in 6 ml of glacial acetic acid, and the mixture was stirred at 20° C for 45 min, after which dry ether was added and was decanted off; then more ether was added, and the precipitate was filtered off, carefully washed with dry ether, and dissolved on the filter in methanol. Methanol was distilled off to give 5 g (92%) of IV with R_{f_1} 0.60, R_{f_2} 0.30.

Methyl ester of carbobenzoxy-L-threonyl-L-asparaginyl-S-benzoyl-L-cysteine (VI)

A suspension of 3.31 g of V [3] and 3.3 g of IV in 20 ml of DMFA was stirred at 20° C for 30 min, and then the precipitate was filtered off and washed with DMFA, after which 1.56 g of DCCI was added in portions at 0° C and the mixture was stirred for 1.5 hr at 0° C and left overnight at 20° C. After the addition of 1.5 ml of 50% acetic acid, the precipitate of dicyclohexylurea was filtered off and was washed with DMFA. The filtrate was treated with water acidified with hydrochloric acid and the solution was kept in the refrigerator for 2 hr. The precipitate that deposited was filtered off, carefully washed with water, and crystallized from DMFA/water. This yielded 3.35 g (75%) of VI, mp 173–175° C, $[\alpha]_D^{22}$ −24°, (c 2; DMFA), R_{f_1} 0.94; R_{f_2} 0.78.

Found, %: C 55.20; H 5.39; N 9.55; S 5.57. Calculated for $C_{27}H_{32}N_4O_9S$, %: C 55.09; H 5.48; N 9.62; S 5.45.

Hydrobromide of the methyl ester of L-threonyl-L-asparaginyl-S-benzoyl-L-cysteine (VII)

A suspension of 3 g of VI in 10 ml of glacial acetic acid was treated with 7.5 ml of 40% HBr in glacial CH_3COOH and the mixture was kept for 30 min at 27° C, then 100 ml of dry ether was added and decanted off, and the residue was twice washed with dry ether, after which it was filtered off, washed with ether, and dissolved in methanol on the filter, and the solution was evaporated to dryness. This gave 2.6 g (95%) of VII, R_{f_1} 0.68.

Methyl ester of carbobenzoxy-L-glutaminyl-L-threonyl-L-asparaginyl-S-benzoyl-L-cysteine (IX)

A solution of 1.67 g of VII, 1.55 g of VIII [4], and 0.44 ml of TEA in a mixture of 15 ml of ethanol and 15 ml of DMFA was allowed to stand at 20° C for 2 hr, and then water acidified with hydrochloric acid was added and the mixture was kept in a refrigerator. The precipitate was filtered off, washed with water, dried, and crystallized from DMFA/ethyl acetate. The weight of IX was 0.88 g (40%); mp 229–231° C (decomp.), $[\alpha]_D^{23}$ −25° (c 1; DMFA).

Found %: C 53.32; H 5.63; N 11.81; S 4.33. Calculated for $C_{32}H_{40}N_6O_{11}S$, %: C 53.40; H 5.62; N 11.72; S 4.47.

Hydrobromide of the methyl ester of L-glutaminyl-L-threonyl-L-asparaginyl-S-benzoyl-L-cysteine (X)

2.8 ml of 40% HBr in glacial CH_3COOH was added to 0.72 g of IX in 2 ml of glacial acetic acid and the mixture was kept at 20° C for 45 min, treated with dry ether, and cooled in a refrigerator, after which the precipitate was filtered off, washed with dry ether, and dried in a vacuum desiccator over KOH/P_2O_5. After reprecipitation from DMFA/dry ether, 0.64 g (90%) of X was obtained with R_{f_1} 0.40; R_{f_2} 0.36.

Methyl ester of carbobenzoxy-S-benzoyl-L-cysteinyl-S-tosyl-L-lysyl-L-asparaginylglycyl-L-glutaminyl-L-threonyl-L-asparaginyl-S-benzoyl-L-cysteine (XIII)

A solution of 0.4 g of XI [1] and 0.1 g of N-hydroxysuccinimide [6] in 2 ml of DMFA was treated at 0° C with 0.12 g of DCCI in portions, the mixture was left at 0° C for an hour and at 20° C overnight, the dicyclohexylurea was filtered off and washed with 0.5 ml of DMFA, and the filtrate was treated with a solution of 0.3 g of (X) and 0.07 ml of TEA in 5 ml of methanol. It was left at 20° C for a day and was then treated with an excess of water acidified with hydrochloric acid and cooled in a refrigerator. The substance that deposited was filtered off, washed with water, and dried. The residue was dissolved in 10 ml of hot DMFA and the solution was passed through a layer of alumina (2.5 × 5 cm, activity grade II) and eluted with 5–7 ml of hot DMFA. The hot solution was treated with water acidified with hydrochloric acid until turbidity appeared and was then kept in a refrigerator, after which the precipitate was filtered off, washed with water, dried, and again crystallized from a mixture of CH_3COOH and water as described above. The yield of XIII was 0.17 g (39%), mp 180–185° C, $[\alpha]_D^{25}$ −19° (c 2; glacial CH_3COOH).

Found, %: C 54.28; H 5.95; N 12.45; S 4.91. Calculated for $C_{61}H_{76}N_{12}O_{19}S_2$, %: C 54.48; H 5.70; N 12.50; S 4.79.

Summary

The synthesis of the protected tetrapeptide and octapeptide corresponding to the sequences 69–72 and 65–72 of the

ribonuclease chain has been effected.

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