## CONCLUSION

It has been established that dictysine is an alkaloid of the denudatine type with an α, β,γ-triol system at $C_{15}$, $C_{16}$, and $C_{20}$. The structures of dictysine and dehydrodictysine acetonides have been established.

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# SYNTHESIS OF REGULAR POLYPEPTIDES INCLUDING POLYFUNCTIONAL AMINO ACIDS


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UDC 542.91 + 547.466.1 + 547.963



Polypeptides of regular structure with the sequences His-Glu, Ser-Glu, Try-Glu, His-Tyr, and Glu-His have been synthesized by the 2,4,5-trichlorophenyl ester method. Polymerization was carried out in dimethyl sulfoxide and dimethylformamide. The polymers were purified by dialysis against water. In the process of synthesizing the monomers, the α-amino groups of the acids were protected by ortho-nitrophenylsulfenyl (o-NPS-), benzyloxycarbonyl (Z-), and tert-butoxycarbonyl (t-BOC-) groups. The imidazole group of histidine was Z-protected, and the γ-hydroxy group of glutamic acid and the phenolic OH group of tyrosine were benzyl-protected (Bzl-).


Polypeptides of regular structure have shown themselves to be good catalytic models in the study of the rate of hydrolytic cleavage of the ester bond [1, 2]. The most convenient method for obtaining them is that of activated esters [3, 4]. The present investigation was devoted to the synthesis of peptide monomers — 2,4,5-trichlorophenyl (2,4,5-OTcp) esters containing serine, tryptophan, histidine, and glutamic acid residues, and their subsequent polycondensation.

In the process of synthesizing the peptide monomers, the α-amino groups of the amino acids were protected by o-nitrophenylsulfenyl (o-NPS-), benzyloxycarbonyl (Z-), and tert-butoxycarbonyl (t-BOC-) groupings, and the γ-carbonyl group of glutamic acid and the phenolic hydroxy group of tyrosine were benzyl-protected (Bzl-). The imidazole (im) group of histidine was protected by a Z group. The addition of 2,4,5-trichlorophenol to the N-protected amino acids and the synthesis of the dipeptides were carried out with the aid of the condensing agent dicyclohexylcarbodiimide (DCC). The protected peptides were purified by repeated recrystallization from organic solvents and, in some cases, by passage through a column filled with silica gel, which permitted the separation of the initial compounds that had not reacted during the process and other impurities and by-products.

The protective groups — Z, o-NPS, and t-BOC — of the activated amino acid and peptide esters were eliminated before polycondensation by the action of HBr in glacial acetic acid, 2.5 N HCl in ethyl acetate, and trifluoroacetic acid, respectively.

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TABLE 1. Conditions of the Polycondensation of the 2,4,5-Trichlorophenyl Esters of Dipeptides and Their Mean Molecular Weights

| Monomer | Amount of monomer | Amount of solvent, ml | $Et_3N$ (ml) | Time of polymerization, days | Yield, % | Mol. wt. of the protected polypeptide |
|---|---|---|---|---|---|---|
| 1. 2HBr·His (N-im-Z)-Glu (γ-Bzl)-OTcp (2,4,5) | 1.06 | 0.70 DSO | 0.4 | 10 | 0,59 | 8000—10000 |
| 2. HGl·Ser-Glu (γ-Bzl)-OTcp-(2,4,5) | 1,28 | 2,00 DSO | 0,31 | 10 | 0,30 | 10000—12000 |
| 3. 2HBr·His (N-im-Z) Tyr (O-Bzl)·OTcp (2,4,5) | 0 6618 | 0.8620 DSO | 0,23 | 10 | 0.38 | 8000—7000 |
| 4. HBr·Glu (γ-Bzl)-His× ×(N-im-Z)-OTcp(2,4,5) | 0,675 | 0.70 DMF | 0.25 | 10 | 0,22 | 8500—8000 |
| 5. $CF_3COOH$·Try-Glu (γ-Bzl)-OTcp(2,4,5) | 0,4 | 0,52 DSO | 0.14 | 10 | 0,20 | 6000—7000 |

The dipeptide 2,4,5-OTcp esters so obtained were polymerized in dimethyl sulfoxide (DSO) and dimethylformamide (DMF) solutions in the presence of triethylamine ($Et_3N$) for 10 days.

The polypeptides were precipitated from solution with methanol, and the methanol-soluble fraction with a mixture of methanol and ether (1:3), and they were dried in vacuum.

The molecular weight of the protected polypeptide of Ser-Gly(γ-Bzl)-OH was determined by the titration of the terminal amino group with perchloric acid in the presence of Crystal Violet. The molecular weights of the other polypeptides (containing histidine and tryptophan) were determined by the Van Slyke method. The values of the molecular weights of the protected polypeptides so obtained are given in Table 1.

The benzyloxycarbonyl and benzyl protective groups were eliminated from the polypeptides by hydrogenolysis over a Pd catalyst for 24 h in methanol or, for the fractions insoluble in methanol, in dioxane—acetic acid—DMF (1:1:2). The polypeptides were dialyzed in Cellophane bags against water from aqueous dioxane (1:1) solution for 6-10 h.

The molecular weights of all the dialyzed polypeptides were determined by the Van Slyke method.

To confirm the values of the molecular weights obtained, we used IR spectroscopy [5] on the basis of the ratio of the intensity of the amide I absorption band (1650 $cm^{-1}$), which is proportional to the number of peptide bonds, and of the absorption band of the terminal peptide group (1740 $cm^{-1}$), $n \leqslant D$ 1650 $cm^{-1}/D$ 1740 $cm^{-1}$, where n is the degree of polymerization, and D is the optical density. For example, for the polypeptide based on Ser-Glu, according to its spectrum (Fig. 1), $n \approx 46$ and the molecular weight ≈10,000.

## EXPERIMENTAL

L-Amino acids were used in the synthesis. The purity of the compounds obtained was checked by thin-layer chromatography on Silufol-254 plates in the following solvent systems: 1) toluene—dioxane—heptane—acetic acid (10:6:3:1); 2) toluene—dioxane—heptane—ethanol (10:6:3:1); 3) butan-1-ol—water—acetic acid (4:1:1); 4) butan-1-ol—water—pyridine—acetic acid (30:24:20:6); 5) chloroform—methanol—17% ammonia (2:2:1); and 6) methyl ethyl ketone—isobutanol—water (2:1:1). The revealing agents were iodine vapor and a 0.5% solution of ninhydrin in acetone. The results of the analysis of all the compounds corresponded to the calculated figures.

1. o-NPS-Ser-OH·Dcha. With stirring, 9.4 g of o-NPS-Cl and 30 ml of 2 N NaOH were added simultaneously to a suspension of 5.3 g of H-Ser-OH in a mixture of 60 ml of dioxane and 10 ml of water (the pH of the reaction mixture was not allowed to rise above 8), and stirring was continued for another hour. Then the serine that had not reacted was filtered off and the filtrate was treated with 150 ml of ether and was acidified with 1 N $H_2SO_4$ to pH 4. The organic layer was separated off and the aqueous layer was extracted with ether (4 × 70 ml). The combined ethereal extracts were washed with water and dried over $Na_2SO_4$. After the desiccant had been separated off, 7.5 ml of dicyclohexylamine (Dcha) was added and the mixture was left at 0-4°C for 12 h. The precipitate was filtered off, washed with ether,

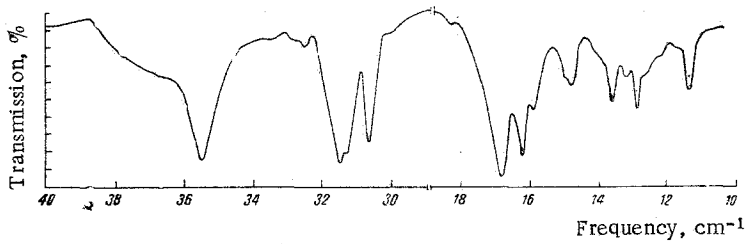


Fig. 1. IR spectra of the polypeptide $(-Ser-Glu)_n$ after dialysis.

and dried in vacuum over $P_2O_5$. The yield of (1) was 13 g (80%); $R_f$ 0.56 (system 4), mp 171-172°C; $[\alpha]_D^{22}$ −75° (c 1; DMF).

2. o-NPS-Glu-(γ-Bzl)-OH·Dcha. This was obtained in a similar manner to compound (1), starting from 4.74 g of γ-Bzl-Glu-OH and 4.17 g of o-NPS-Cl in a mixture of 24.7 ml of dioxane, 10 ml of water, 22 ml of 2 N NaOH, and 6.9 ml of Dcha. The product was recrystallized from $CHCl_3$—ether. This gave 8 g (72.2%) of the compound (2); $R_f$ 0.73 (system 1); mp 167-168°C; $[\alpha]_D^{22}$ −33° (c 3; $CHCl_3$).

3. o-NPS-Glu(γ-Bzl)-OTcp(2,4,5). With stirring at −5°C, 1.37 g of 2,4,5-OTcp was added to a solution of o-NPS-Glu(γ-Bzl)-OH (which was obtained from 2.5 g of its Dcha salt by treatment with 1 N $H_2SO_4$ in ethyl acetate) and 1.24 g of DCC in 20 ml of absolute ethyl acetate. Stirring was continued for 4-5 h and the mixture was left at 20°C for 12 h. Then 1 ml of 50% $CH_3COOH$ was added, and after 15 min the solution was filtered from the dicyclohexylurea (Dchu) that had deposited. The filtrate was poured into two volumes of ethyl acetate and the resulting mixture was washed successively with 1 N $H_2SO_4$, with water, with 0.5 N $NaHCO_3$, and with water again. Then it was dried over $Na_2SO_4$, filtered from the desiccant, and evaporated in vacuum. The residue was transferred into $CHCl_3$ and the resulting solution was passed through a column 10 × 3.5 cm) filled with silica gel (250 mesh). The main product was eluted with $CHCl_3$ and an impurity remained on the silica gel. Then the chloroform was evaporated off and the oily product was dried in vacuum. This gave 2.7 g (65.5%) of compound (3) with $R_f$ 0.90 (system 2); $[\alpha]_D^{22}$ −92° (c 2; $CHCl_3$).

4. t-BOC-Try-Glu(γ-Bzl)-OTcp(2,4,5). With stirring and cooling to −5°C, 0.453 g of HCl·H-Glu(γ-Bzl)-OTcp(2,4,5) and 0.101 ml of $Et_3N$ were added to a solution of 0.304 g of t-BOC-Try-OH and 0.206 g of DCC in 10 ml of tetrahydrofuran. The mixture was stirred at 0°C for 2 h and at 20°C for 4 h and was then left at 20°C for 12 h. The precipitate was filtered off, and the filtrate was evaporated in vacuum to dryness. The residue was dissolved in 20 ml of ethyl acetate and then 1 ml of 50% $CH_3COOH$ was added and, after 50 min, the precipitate that had deposited was filtered off, and the filtrate was washed with 1 N $H_2SO_4$, water, 0.5 N $NaHCO_3$, and water again, and was dried over $Na_2SO_4$. Then it was filtered and evaporated, to give 0.580 g of the oily compound (4) with a yield of 82%. $R_f$ 0.86 (system 3), 0.53 (system 4).

5. o-NPS-Ser-Glu(γ-Bzl)-OTcp(2,4,5). This was obtained by a similar method to that described in paragraph 4, starting from 1.09 g of NPS-Ser-OH (literated from the Dcha salt), 0.515 g of DCC, 1.1 g of HCl·H-Glu(γ-Bzl)-OTcp(2,4,5), and 0.25 ml of $Et_3N$ in 20 ml of ethyl acetate. This gave 1.3 g (80%) of the oily compound (5) with $R_f$ 0.80 (system 1).

6. o-NPS-Glu(γ-Bzl)-His($N^{im}$-Z)-OTcp(2,4,5). This was obtained similarly to the procedure of paragraph 4 starting from 2.85 g of NPS-Glu(γ-Bzl)-OH (liberated from the Dcha salt), 1.03 g of DCC, 3.43 g of 2HBr·His($N^{im}$-Z)-OTcp(2,4,5) and 0.5 ml of $Et_3N$ in 20 ml of ethyl acetate. This gave 3.0 g (75%) of compound (6), mp 123-125°C, $R_f$ 0.76 (system 1), 0.80 (system 2).

7. $N^{\alpha}$-t-BOC-His-($N^{im}$-Z)-Tyr(O-Bzl)-OTcp(2,4,5). With stirring and cooling to −5°C, 1.4 g of HCl·H-Tyr(O-Bzl)-OTcp(2,4,5) and 0.35 ml of $Et_3N$ were added to a solution of 0.9 g of $N^{\alpha}$-t-BOC-His($N^{im}$-Z)-OH and 0.54 g of DCC in 75 ml of DMF. The mixture was stirred at 0°C for 2 h and at room temperature for 4 h and was then left at room temperature for 48 h. The urea that had deposited was filtered off, and the filtrate was evaporated in vacuum to dryness. The residue was dissolved in 30 ml of chloroform and the solution was washed with 5% citric acid, with water, with 0.5 N $NaHCO_3$, and again with water and was dried over $Na_2SO_4$. This gave 1.5 g (75.3%) of the oily compound (7). $R_f$ 0.9 (system 2), 0.8 (system 1). On a chro-

matogram, an impurity corresponding to unchanged t-BOC-HIS($N^{im}$-Z)-OH with $R_f$ 0.4 (system 2) was also detected. To purify the main substance, the material was dissolved in chloroform and the solution was passed through a column (10 × 3.5 cm) filled with silica gel and was eluted with $CHCl_3$. After evaporation of the eluate, 0.9 g of pure substance was obtained with $R_f$ 0.9 (system 2), 0.8 (system 1). The residue was eluted with ethanol (0.5 g); it had $R_f$ 0.4 (system 2).

8. $N^{\alpha}$-Z-His-($N^{im}$-Z)-Glu(γ-Bzl)-OTcp(2,4,5). This was obtained in a similar manner to the procedure of paragraph 4, starting from 1.13 g of $N^{\alpha}$-Z-His-($N^{im}$-Z)-OH, 0.75 g of DCC, 1.2 g of HCl·H-Glu(γ-Bzl)-OTcp(2,4,5) and 0.45 ml of $Et_3N$ in 6 ml of DMF. The mixture was stirred at 0 to +5°C and was left at room temperature for two days, after which the precipitate that had deposited was filtered off and the filtrate was evaporated to dryness. The oil so obtained was dissolved in 30 ml of ethyl acetate. The subsequent working up was similar to that of paragraph 7. This gave 2.0 g (75%) of an oil with $R_f$ 0.80 (system 4).

9. $CF_3COOH$·Tyr-Glu(γ-Bzl)-OTcp(2,4,5). A solution of 0.774 g of compound (4) in 2 ml of $CHCl_2$ was treated with 0.11 ml (1.5 equiv.) of $CF_3COOH$, and the mixture was left for 2 h. Then absolute benzene was added and it was evaporated off in vacuum to dryness. This operation was repeated until the smell of $CF_3COOH$ had completely disappeared. Then absolute ether was added to the mixture, and it was decanted several times with absolute ether. This gave 0.68 g (90%) of an oily product with $R_f$ 0.60 (system 3), 0.74 (system 4).

10. HCl·H-Ser-Gly(γ-Bzl)-OTcp(2,4,5). To a solution of 1.0 g of (5) in 20 ml of absolute ethyl acetate was added 3 ml of 2.5 N HCl in ethyl acetate, and the mixture was stirred at 20°C for 20 min. It was worked up with ether–hexane, which gave 0.6 g (73%) of the amorphous compound (10) having $R_f$ 0.75 (system 4), 0.88 (system 6).

11. HCl·H-Glu(γ-Bzl)-His($N^{im}$-Z)-OTcp(2,4,5). This was obtained by a method similar to that of paragraph 9, starting from 1.0 g of compound (6) and 10 ml of absolute ethyl acetate, which was treated with 3 ml of 2.5 N HCl in ethyl acetate. This gave 0.6 g (68.5%) of the amorphous compound (11) with $R_f$ 0.65 (system 3), 0.46 (system 5).

12. $CF_3COOH$·$N^{\alpha}$-His-($N^{im}$-Z)-Tyr(O-Bzl)-OTcp(2,4,5). To 0.45 g of the oily $N^{\alpha}$-t-BOC-His-($N^{im}$-Z)-Tyr(O-Bzl)-OTcp(2,4,5) were added 5 ml of $CH_2Cl_2$ and 1.1 ml of $CF_3COOH$, and the mixture was left for 1 h. Then absolute benzene was added and was evaporated off to dryness several times. This operation was continued until the smell of the $CF_3COOH$ had disappeared completely. The resulting oily product was dissolved in 5 ml of $CH_3COOH$ and was precipitated with absolute ether. This gave 0.38 g (82.6%) of the amorphous compound (12) with $R_f$ 0.55 (system 3), 0.5 (system 6).

13. 2HBr·H-His($N^{im}$-Z)-Glu(γ-Bzl)-OTcp(2,4,5). A current of gaseous HBr was bubbled through a solution of 1.0 g of compound (8) in 5 ml of absolute nitromethane for 25 min. The resulting precipitate was treated with absolute ether by decantation (4 × 50 ml). This gave 0.85 g (85%) of the amorphous compound (13) with $R_f$ 0.80 (system 4).

14. Poly[Tyr-Glu(γ-Bzl)]. With heating to a temperature not exceeding 40°C, 0.4 g of $CF_3COOH$·H-Tyr-Glu(γ-Bzl)-OTcp(2,4,5) was dissolved in 0.52 g of DSO. Then the solution was cooled to 20°C and 0.14 ml of $Et_3N$ was added and the resulting mixture was kept in a sealed ampul at room temperature for 10 days. Then the ampul was opened, 3 ml of methanol was added, and the resulting precipitate was filtered off (0.09 g). The methanolic fraction was evaporated and the residue was treated with a mixture of ethanol and ether (1:3). The resulting precipitate was filtered off and washed with the same mixture. Another 0.11 g of the polymer (14) was obtained.

15. Poly[Ser(OH)-Glu(γ-Bzl)]. This was obtained by a procedure similar to that of paragraph 14 starting from 1.28 g of HCl·Ser-Glu(γ-Bzl)-OTcp(2,4,5) in 2 ml of DSO and 0.31 ml of absolute $Et_3N$. It was precipitated with a mixture of methanol and ether (1:3), which yielded 0.3 g of the polypeptide (15).

16. Poly[Glu(γ-Bzl)-His($N^{im}$-Z)]. This was obtained by a procedure similar to that of paragraph 14, starting from 0.775 g of HCl·Glu(γ-Bzl)-His($N^{im}$-Z)-OTcp(2,4,5) in 0.7 ml of DMF and 0.25 ml of $Et_3N$ and evaporated. It was precipitated with a mixture of methanol and ether (1:3) which gave 0.22 g of the polypeptide (16).

17. Poly[His($N^{im}$-Z)-Glu(γ-Bzl)]. This was obtained by a procedure similar to that of paragraph 14, starting from 1.06 g of 2HBr·His($N^{im}$-Z)-Glu(γ-Bzl)- -OTcp(2,4,5) in 0.7 ml of DSO and 0.4 ml of $Et_3N$. The yield of polymer (17) was 0.59 g.

18. Poly[His($N^{im}$-Z)-Tyr(O-Bzl)]. This was obtained by a procedure similar to that of paragraph 14, starting from 0.661 g of substance (12), 0.8% ml of DSO, and 0.23 ml of $Et_3N$. The yield of polypeptide (18) was 0.38 g.

19. Hydrogenolysis of Poly[Ser-Glu(γ-Bzl)]. In solution in 1 ml of a mixture of dioxane, acetic acid, and DMF in a ratio of 1:1:2, 0.09 g of the protected polypeptide was hydrogenated over Pd black at room temperature for 24 h. After the mixture of solvents had been evaporated off in vacuum, an oily product was left, and this was dried and was treated with a mixture of methanol and ether. The resulting amorphous product was dissolved in aqueous dioxane (1:1) and the solution was dialyzed in a cellophane bag for 6 h. After lyophilization, 0.065 g of poly(Ser-Glu) was obtained with a molecular weight of 10,000-11,000 (Van Slyke).

20. Hydrogenolysis of Poly[Tyr-Glu(γ-Bzl)]. This was performed by a procedure similar to that of paragraph 19, starting from 0.24 g of the protected polypeptide of Tyr-Glu(γ-Bzl) in 3 ml of dioxane—acetic acid—DMF (1:1:2). This gave 0.17 g of poly(Tyr-Glu) with a mean molecular weight after dialysis of 14,000-15,000 (Van Slyke).

21. Hydrogenolysis of Poly[His-($N^{im}$-Z)-Glu(γ-Bzl)]. This was performed by a procedure similar to that of paragraph 19, starting from 0.6 g of the protected polypeptide poly[His-($N^{im}$-Z)-Glu(γ-Bzl)] and 3.5 ml of methanol, and gave 0.35 g of poly(His-Glu) with a mean molecular weight after dialysis for 6 hours of 13,000 (Van Slyke).

22. Hydrogenolysis of Poly[His-($N^{im}$-Z)-Tyr(O-Bzl)]. This was performed by a procedure similar to that of paragraph 19, starting from 0.30 g of the initial compound and 18.5 ml of dioxane—acetic—DMF (1:2:2) with a mean molecular weight of 12,000-14,000 (Van Slyke).

23. Hydrogenolysis of Poly[Glu(γ-Bzl)-His-($N^{im}$-Z)]. This was performed by a procedure similar to that of paragraph 19, starting from 0.06 g of compound (16) and 3 ml of methanol, and gave 0.045 g of poly(Glu-His) with a mean molecular weight after dialysis for 10 hours of 22,000 (Van Slyke).

## CONCLUSIONS

1. Poly(His-Glu), poly(Ser-Glu), poly(His-Tyr), poly(Tyr-Glu), and poly(Glu-His) have been synthesized from the $N^{\alpha}$-hydrochlorides and trifluoroacetates of 2,4,5-trichlorophenyl esters of substituted dipeptides by polycondensation in dimethyl sulfoxide and dimethylformamide solutions in the presence of triethylamine.

2. The polypeptides have been isolated in the amorphous state by dialysis from aqueous-dioxane solutions for 6-10 h.

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