SYNTHESIS OF REGULAR POLYPEPTIDE WITH -tyr-glu- SEQUENCE

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Regular polypeptides have a number of properties that depend on the nature of the side chains of the amino acid residues. Some regular and irregular polypeptides have a catalytic effect in the hydrolysis of p-nitrophenyl acetate [1, 2]. The greatest catalytic effect in the case of the glutamic–tyrosine copolymer was observed when the ratio was 1:1. For this reason it was interesting to study this relation in more detail on the example of the regular polypeptides. In the present paper we report the preparation of a polypeptide containing the -tyr-glu- sequence. In the synthesis of the starting monomer we used the N-carbobenzoxy (Cbo) and γ-benzyl (OBzl) protective groups, in this connection leaving the free hydroxyl of the tyrosine residue. Some difficulties were encountered in the preparation of Cbo-tyr–OH, since dicarbobenzoxytyrosine was present in the product when we used the method given in [3]. We obtained the chromatographically pure Cbo-tyr–OH by a modification of the method given in [4], in contrast to which we hydrolyzed the $(Cbo)_2$tyr–OH to Cbo-tyr–OH for a longer time after isolating the $(CbO)_2$tyr–OH from the reaction mixture. From CbO-tyr–OH in the presence of dicyclohexylcarbodiimide (DCHCD) and 2,4,5-trichlorophenol we obtained the 2,4,5-trichlorophenyl ether. The Cbo group was removed by hydrobrominolysis in CH_3COOH and the obtained tyrosine ether hydrobromide was added to Cbo-glu(OBzl)–OH. It should be mentioned that a partial cleavage of the ester linkage is observed during the hydrobrominolysis of Cbo-tyr–$OPhCl_3$ (2,4,5-) in CH_3COOH. The cleavage occurs when the HBr is bubbled through a solution of the compound in nitromethane. A reduction in the hydrobrominolysis time to 3-10 min improves the quality of the tyrosine ether hydrobromide, but here the yield drops. The formation of an impurity is also observed during the hydrobrominolysis of Cbo-glu(OBzl)-tyr–$OPhCl_3$ (2,4,5-), which caused us to replace the N–Cbo group by the O-nitrosulfinyl (NPS-) protection. The NPS derivatives of γ-benzylglutamic acid and tyrosine were obtained as the dicyclohexylammonium (DCHA) salts as described in [5]. The DCHA was removed with 1.2 equiv of 1 N H_2SO_4 solution in either ethyl acetate or ether. In preparing the activated ether a better yield is obtained by using the DCHCD method. The chromatograms of NPS-tyr–OH·DCHA and NPS–tyr-glu(OBzl)–$OPhCl_3$ (2,4,5-) show slight contamination with products that contain the protected OH group of tyr. However, this contamination is less than when using Cbo protection, and, in addition, is easily separated from the NPS–tyr-glu(OBzl)–$OPhCl_3$(2,4,5-) by chromatographing on a silica gel column. The NPS group was removed with HCl in ethyl acetate. The polymerization of HCl·H–tyr-glu(OBzl)–OPh·Cl_3 (2,4,5-) was run in DMSO. The γ-benzyl group was removed by hydrobrominolysis. The averaged molecular weight (M_{av}) was determined by titration of the N-terminal groups of the polymers in absolute dioxane, containing $Hg(CH_3COO)_2$, with 0.01 $HClO_4$ solution in glacial CH_3COOH. Crystal violet was used as the indicator.

EXPERIMENTAL METHOD

The purity of the compounds was checked by TLC on a bound layer (7.5 × 2.5 cm) of silica gel (250 mesh) in the systems: H_2O–CH_3COOH–n-butanol (30:10:100) (A); 3% NH_3–sec-butanol (44:100) (B); to toluene–dioxane–heptane–CH_3COOH (10:6:3:1) (C); toluene–dioxane–cyclohexane–CH_3COOH (10:6:3:1) (D); toluene–dioxane–cyclohexane–C_2H_5OH (10:6:3:1) (E). The chromatograms were developed with iodine and ninhydrin.

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Cbo-tyr–OH (I). Starting with 15.5 g of tyrosine, as in the method of [3], we obtained 25.22 g of $(Cbo)_2$tyr–OH (R_f (D) 0.71), which contains an impurity and Cbo-tyr–OH (R_f (D) 0.65). The product was hydrolyzed with 70 ml of 2 N NaOH solution by stirring at 20°C for 4 h. The solution was extracted twice with ether, the aqueous portion was acidified with 6 N HCl solution to pH 4, and the obtained oil was extracted several times with ether. The ether extract was washed with water and dried over Na_2SO_4; the solvent was evaporated. We obtained 22.5 g of an oily product that crystallized after several weeks. The yield of (I) was 19.1 g (70.7%), mp 91-93° (from [4]: 100°); $[\alpha]_D^{25}$ 19.3 ± 0.5° (C 1.35, $CH_3COOC_2H_5$), R_f (D) 0.65.

Cbo-tyr–$OPhCl_3$ (2,4,5-) (II). To a solution of 5.0 g of 2,4,5-trichlorophenol and 7.9 g of (I) in 50 ml of ethyl acetate at −10°, 5.2 g of carbodiimide (DCHCD) was added. The mixture was stirred at −10 to 0° for 40 min, then at 20° for 1 h, and allowed to stand overnight. The reaction mass was diluted with 2-3 volumes of ethyl acetate, and 0.5 ml of 50% acetic acid solution was added. After 15 min the DCHM was filtered and the filtrate was treated in succession with 1 N HCl solution, water, three times with 0.5 N $NaHCO_3$ solution, and water, and dried over Na_2SO_4. The solvent was evaporated to give 11.33 g (91.25%) of (II), mp 138-139° (ether); $[\alpha]_D^{20.5}$ −15.8 ± 0.5° (C 1.71, $CHCl_3$), R_f (C) 0.78, R_f (D), 0.89, R_f (E) 0.89.

HBr · H–tyr–$OPhCl_3$ (2,4,5-) (III). With heating, 4.8 g of (II) was dissolved in 18 ml of glacial CH_3COOH, and 13 ml of 38% HBr solution in glacial CH_3COOH was added at 20°. After 6 min the product was precipitated with absolute ether, washed with 200 ml of absolute ether (by decantation), and dried. After reprecipitation from methanol solution with ether, the yield of (III) was 3.22 g (75.6%), mp 212-213° (decomp.); $[\alpha]_D^{18}$ +8.54 ± 0.5° (C 1.2, CH_3OH), R_f (A) 0.81 (contaminated with a small amount of HBr · H–tyr –OH with R_f (A) 0.36).

Cbo-glu(OBzl)-tyr–$OPhCl_3$ (2,4,5-) (IV). To a solution of 1.66 g of Cbo-glu(OBzl)–OH in 10 ml of ethyl acetate at −10°, 0.95 g of DCHCD was added and the mixture was stirred for 30 min. Then a suspension of 2.04 g of (III) in 20 ml of ethyl acetate, containing 0.62 ml of triethylamine and cooled to −10°, was added. The mixture was stirred at −10 to −5° for 1 h and then allowed to stand overnight at 20°. The subsequent workup was analogous to (II). The yield of (IV) was 1.9 g (59.7%), mp 124-126° ($CH_3COOC_2H_5$); $[\alpha]_D^{20}$ −25.5 ± 0.5° (C 2.0, $CH_3COOC_2H_5$); R_f (D) 0.86 (contaminated with a substance with R_f (D) 0.66).

NPS–glu(OBzl)–OH · DCHA (V). As in the method of [5], (V) was synthesized in 71.8% yield, mp 168° ($CHCl_3$–ether); $[\alpha]_D^{19.5}$ −34.4 ± 0.5° (C 1.42, $CHCl_3$), R_f (D) 0.73 and R_f (D) 0.37 (DCHA).

NPS–tyr–OH · DCHA (VI). By a method analogous to that in [5], (VI) was synthesized in 59.5% yield, mp 172° ($CHCl_3$–ether); $[\alpha]_D^{19}$ +39.8 ± 0.5° (C 1.25, CH_3OH–$CHCl_3$, 3:2), R_f (D) 0.69 and R_f (D) 0.37 for DCHA. The compound is contaminated with $(NPS)_2$tyr–OH · DCHA with R_f (D) 0.78.

NPS–glu(OBzl)–$OPhCl_3$ (2,4,5-) (VII). Analogous to (II), from 5.86 g of (V) (free of DCHA), 2.05 g of 2,4,5-trichlorophenol and 2.1 g of DCHCD in 45 ml of ethyl acetate we obtained 5.38 g (92.2%) of (VII) as an oily product; $[\alpha]_D^{21.5}$ −70.2 ± 0.5° (C 2.0, $CHCl_3$), R_f (D) 0.97 and R_f (E) 0.95.

HCl · H–glu(OBzl)–$OPhCl_3$ (2,4,5-) (VIII). To a solution of 1.12 g of (VII) in 26 ml of ethyl acetate was added 2 ml of 2.93 N HCl in ethyl acetate, and the mixture was kept for 4 min at 20°, and for 60 min in a refrigerator at −4 to −5°. The formed suspension was diluted with absolute ether, filtered, and washed with absolute ether until the filtrate was colorless. The yield of (VIII) was 0.63 g (71.0%), mp 169-169.5° (CH_3OH–ether); $[\alpha]_D^{19.5}$ +27.04 ± 0.5° (C 1.2, CH_3OH), R_f (A) 0.88 and R_f (E) 0.87.

NPS–tyr-glu(OBzl)–$OPhCl_3$ (2,4,5-) (IX). Analogous to (IV), starting with 1.14 g of (VI) (free of DCHA), 1.02 g of (VIII), 0.47 g of DCHCD, and 0.3 ml of triethylamine in 30 ml of ethyl acetate we obtained 1.6 g (97.5%) of (IX) as an amorphous product; $[\alpha]_D^{19}$ +8.02 ± 0.5° (C 1.62, $CHCl_3$), R_f (D) and R_f (E) 0.86.

HCl · H–tyr-glu(OBzl)–$OPhCl_3$ (2,4,5-) (X). To a solution of 1.88 g of (IX) in 50 ml of ethyl acetate 2.37 ml of 3.26 N HCl in ethyl acetate was added and the mixture was kept for 4 min at 20°, and for 60 min in a refrigerator at −4 to −5°. The solvent was evaporated nearly to dryness and the residue was rubbed with absolute ether; the ether was separated by decantation and, after obtaining a colorless ether layer, the product was dried. We obtained 1.31 g (82.8%) of (X) as an amorphous product; $[\alpha]_D^{18}$ −4.77 ± 0.5° (C 1.0, CH_3OH), R_f (A) 0.87.

H–$[tyr\text{-}glu(OH)]_n$–OH (XI). a) A solution of 0.5477 g of (X) in 0.6850 g of DMSO (44.5%), containing 0.304 ml of triethylamine (2.5 equiv), was allowed to stand for 15 days at 20°. Then MeOH was added and the polypeptide was precipitated with ether, filtered, and washed by rubbing with ether, and then with warm water. We obtained 0.12 g of H–$[tyr\text{-}glu(OBzl)]_n$–OH, which was dissolved in 1.2 ml of CF_3COOH, and

then 1.2 ml of 35% HBr in glacial CH_3COOH was added. After 40 min absolute ether was added until precipitation was complete and the product was repeatedly and thoroughly rubbed with ether, dissolved in MeOH, and again precipitated with ether. We obtained 0.11 g of (XI), M_{av} 2000.

b) A mixture of 0.7254 g of (X) and 0.16 ml of triethylamine (1 equiv) in 0.9675 g of DMSO (46.0%) was polymerized in a similar manner. We obtained 0.41 g of H–tyr-glu(OBzl)]$_n$–OH, which was dissolved in 4 ml of CF_3COOH, and then 4 ml of 35% HBr in glacial CH_3COOH was added. The further isolation was similar to the above. We obtained 0.3 g of (XI), M_{av} 2600-2800.

CONCLUSIONS

A polypeptide with a regular structure was synthesized, which contained the -tyr-glu(OH)- sequence and had had an average molecular weight of 2600-2800.

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