

# ENZYMATIC SYNTHESIS OF OPTICALLY ACTIVE PEPTIDES FROM GLYCOLIC ESTERS OF D,L-AMINO ACIDS

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**Abstract**

**Full Text**

**CHEMISTRY**

**M. M. BOTVINNIK and V. I. OSTOSLAVSKAYA**

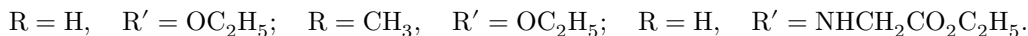
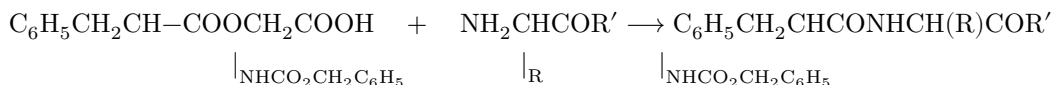
**ENZYMATIC SYNTHESIS OF OPTICALLY ACTIVE PEPTIDES FROM GLYCOLIC ESTERS OF *D, L*-AMINO ACIDS**

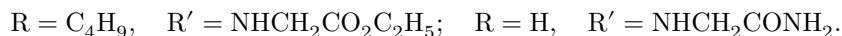
*(Presented by Academician A. N. Nesmeyanov, May 8, 1958)*

Recently one of us <sup>(1)</sup> showed that esters of benzoylphenylalanine and of  $\beta$ -hydroxyamino acids, formed through the hydroxyl group of the  $\beta$ -hydroxyamino acid—the so-called O-peptides—are capable of reacting with esters of amino acids and peptides in the presence of chymotrypsin, with the formation of new optically active N-peptides. The reaction proceeds selectively, and as a result optically active peptides are formed.

Such a reaction can be used for the synthesis of relatively inaccessible peptides. However, the use of serine O-peptides for this purpose is not very profitable, and the ethyl esters of acylated amino acids react very weakly. Therefore, in the present work experiments were undertaken to study the possibility of using accessible esters of hydroxy acids—glycolic and lactic.

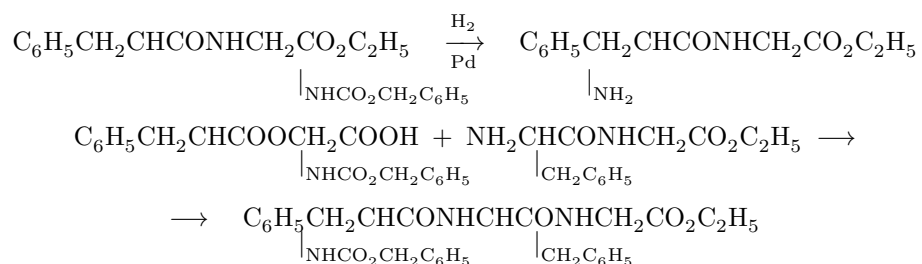
As donors of the acylamino acid, glycolic and lactic acid esters were used—hippurylglycolic, benzoyl-*D, L*-phenylalanylglycolic, benzoyl-*D, L*-phenylalanylactic, and carbobenzoxy-*D, L*-phenylalanylglycolic acids. The acceptors were: ethyl esters of glycine, *L*- and *D, L*-leucine, *L*- and *D, L*-phenylalanylglycine, *L*- and *D, L*-leucylglycine, the ester and amide of glycylglycine. In all cases, the formation of esters or amides of acylated N-peptides of *L*-phenylalanine was observed.





In those cases in which the acceptors were optically active esters of amino acids or peptides, the corresponding optically active *L, L*-peptides were obtained; when esters of racemic amino acids or peptides served as acceptors, the bulk of the isolated substance consisted of the same *L, L*-peptide, as was proved by similar values of the specific rotation and by the absence of depression of the melting point of a mixed sample.

In one of the esters of carbobenzyldipeptides obtained, the carbobenzyloxy group was removed by hydrogenation; the resulting hydrochloride of the dipeptide ester was introduced into the reaction as an acceptor. Thus the ethyl ester of carbobenzyloxy-*L*-phenylalanyl-*L*-phenylalanylglycine was obtained.



This reaction can be extended to the synthesis of peptides with a longer chain. The yields of the esters of carbobenzyloxy peptides are 30–60% calculated on the *L*-antipode. Since in most cases the substances precipitate from the reaction solution, their purification presents no particular difficulty.

Preliminary experiments have shown that, in carrying out this reaction, it is not necessary to use crystalline chymotrypsin; it may be carried out with an extract from the pancreas.

## Experimental Part

**1. Synthesis of *O*-benzoylphenylalanylglycolic acid.** 2-Phenyl-4-benzyl-5-oxazolone (from 3 g of benzoylphenylalanine) is boiled for about 4 hours with 1.2 g of glycolic acid in an ether-dioxane solution on a water bath. The solution is evaporated in vacuo, and the remaining oil is dissolved in absolute benzene. The undissolved glycolic acid is filtered off, and the solution is again evaporated to a light-yellow oil, which crystallizes on rubbing with water. Yield 3 g, 83%; m.p. 79–80° (from alcohol).

Found, %:	C 66.03; 66.14; H 5.28; 5.37
C <sub>18</sub> H <sub>17</sub> O <sub>5</sub> N. Calculated, %:	C 66.05; H 5.19

**2. Synthesis of *O*-benzoylphenylalanylactic acid.** 2-Phenyl-4-benzyl-5-oxazolone (from 3 g of benzoylphenylalanine) is boiled for 1 hour with 2 g of lactic acid in an ether solution and left for 18 hours. The ether is distilled off in vacuo, and the remaining oil is washed with water. The residue is dissolved in alcohol, and *O*-benzoylphenylalanylactic acid is precipitated with water. Yield 3 g, 80%; m.p. 160–162°.

Found, %: C 66.81; 66.71; H 5.60; 5.78; N 4.21; 4.16  
 $C_{19}H_{19}O_5N$ . Calculated, %: C 66.86; H 5.57; N 4.105

**3. Synthesis of *O*-(carbobenzoxyphenylalanyl)glycolic acid.**

a) 5 g of carbobenzoxyphenylalanine in chloroform are treated with 2.4 ml of triethylamine, and to them, on cooling to  $-10^\circ$ , 1.6 ml of chloroformic ester is added. After 10 min, a cooled chloroform solution of 2.5 g of glycolic acid and 4 ml of triethylamine is added to the solution, and the mixture is left for 24 hours at  $20^\circ$ . The solution is then washed with 2 *N* HCl and with water and evaporated; the residue is an oil which, together with *O*-carbobenzoxyphenylalanylglycolic acid, also contains impurities of an unknown neutral substance. To separate them, the oil is dissolved once more in chloroform, and the chloroform solution is treated with a 1 *N* solution of soda. For better separation of the layers, ether is added. The soda solution is rapidly acidified with 2 *N* HCl, the precipitated oil is extracted with chloroform, and the chloroform solution is evaporated in vacuo. Numerous attempts to crystallize the remaining oil did not lead to a positive result. Equivalent: found 384; 400; calculated 357.

**Saponification of the ester bond.** Samples of 0.0260 and 0.0209 g of the substance, titrated in alcoholic solution, are each treated with 10 ml of 0.01 *N* NaOH; the solutions are boiled for 1 hour on a water bath, then 10 ml of 0.01 *N*  $H_2SO_4$  is added to each and they are titrated. The amounts of 0.01 *N* NaOH consumed for binding the carbobenzoxyphenylalanine formed were, respectively, 5.4 and 4 ml. Consequently, the oil contains 80% carbobenzoxyphenylalanylglycolic acid.

b) 2.5 g of carbobenzoxyphenylalanine and 1.3 ml of triethylamine in 15 ml of tetrahydrofuran are cooled to  $-10^\circ$ , and 0.8 ml of chloroformic ester is added all at once. The mixture is left for 10 min in the cold, 2.8 ml of triethylamine is added, and immediately thereafter 1.2 g of dry glycolic acid. Evolution of  $CO_2$  is observed. The mixture is left for another 5 min in the cooling mixture, and then overnight at  $\sim 20^\circ$ . The mixture is partially evaporated and washed repeatedly with ether. The undissolved residue is acidified and again extracted with ether. The ether extracts are washed with water and evaporated. The residue is a colorless oil. Yield of carbobenzoxyphenylalanylglycolic acid, 2.1 g. Equivalent: found 372; 367; calculated 357.

**Saponification of the ester bond.** Samples of 0.0239 and 0.0256 g of the

substance, titrated in alcoholic solution, are treated with 9.472 ml of 0.01 *N* NaOH and boiled for 1 h on a water bath. Then 5 ml portions of 0.01 *N* H<sub>2</sub>SO<sub>4</sub> are added and the mixtures are titrated. For binding of the carbobenzoxyphenylalanine formed, respectively 4.68 and 5.3 ml of 0.01 *N* NaOH were consumed. Consequently, the carbobenzoxyphenylalanyl-glycolic acid content in the oil is 72–76%.

**Ammonolysis.** 0.1718 g of the substance is treated with 5 ml of 25% ammonia solution; after 1.5 h the precipitate is filtered off. Yield of the amide of carbobenzoxyphenylalanyl-glycolic acid, 0.102 g, corresponding to 75% content of *O*-carbobenzoxyphenylalanyl-glycolic acid in the oil. M.p. 181°.

Found, %: C 68.23; H 6.14; N 9.48

C<sub>17</sub>H<sub>18</sub>O<sub>3</sub>N<sub>2</sub>. Calculated, %: C 68.45; H 6.04; N 9.39

**4. Synthesis of the hydrochloride of the ethyl ester of *L*-phenylalanyl-glycine.** The hydrochloride of the ethyl ester of *L*-phenylalanyl-glycine is obtained in quantitative yield by hydrogenation of the ethyl ester of carbobenzoxy-*L*-phenylalanyl-glycine over Pd/C in an alcoholic hydrochloric-acid solution. M.p. 139° (from alcohol with ether).

Found, %: Cl 12.84; NH<sub>2</sub>/N 4.82

C<sub>13</sub>H<sub>19</sub>O<sub>3</sub>N<sub>2</sub>Cl. Calculated, %: Cl 12.56; NH<sub>2</sub>/N 5.13

The hydrochloride of the ethyl ester of *L*-leucyl-glycine and the hydrochloride of glycyl-glycine amide were obtained analogously.

**Table 1**

Interaction of glycolic and lactic esters of acylated amino acids with esters of amino acids and peptides

Exp. No.	Donor	Sample, g	Acceptor	Sample, g	D/A, mol	Time, h
1	<i>O</i> -benzoyl- <i>D, L</i> -phenylalanyl-glycolic acid	0.2	Hydrochloride of the ethyl ester of glycine	0.3	1:3.5	1
2	<i>O</i> -benzoyl- <i>D, L</i> -phenylalanyl-lactic acid	0.1	glycine	0.15	1:3.5	1

Exp. No.	Donor	Sample, g	Acceptor	Sample, g	D/A, mol	Time, h
3	<i>O</i> -carbobenzoxy- <i>D, L</i> -phenylalanylglycolic acid	2.1	glycine	3	1:3.7	24
4	Same	0.49	<i>D, L</i> -leucine	1.6	1:4	24
5	Same	0.22	<i>L</i> -leucine	0.6	1:3	2
6	Same	0.28	<i>D, L</i> -phenylalanylglycine	0.75	1:5	24
7	Same	0.22	<i>L</i> -phenylalanylglycine	from 0.55 CBZ <sup>*</sup>	1:2.5	24
8	Same	0.28	<i>D, L</i> -leucylglycine	0.7	1:3	24
9	Same	0.14	<i>L</i> -leucylglycine	0.3	1:3	1
10	Same	0.47	glycylglycine	0.7	1:3	24
11	Same	0.21	glycylglycine amide	0.4	1:4	
12	Hippurylglycolic acid	0.2	Hydrochloride of the ethyl ester of glycine	1.05	1:7	24

\* CBZ –carbobenzoxy.

**Table 2**

**Obtained esters and amides of carbobenzoxy peptides**

Experiment no.	Compound	Yield, %	Yield, mg	M.p., °C	Analysis,		
					Analysis, %, found	Analysis, %, calculated	$[\alpha]_D^{21-22}$
3	Ethyl ester of carbobenzoxy- <i>L</i> -phenylalanyl-glycine	58	650	109	—	—	$-25.78^{\circ}$ ( $C = 1.3\%$ , alcohol)
4	Ethyl ester of carbobenzoxy- <i>L</i> -phenylalanyl- <i>L</i> -leucine	32	100	114–115(1)	C 68.43H 6.50N 7.57	C 68.18H 6.36N 7.27	$-30.54^{\circ}$ ( $C = 0.7\%$ , alcohol)
5	Ethyl ester of carbobenzoxy- <i>L</i> -leucyl- <i>L</i> -leucine	34	46	114–115(1)	—	—	$-32.07^{\circ}$ ( $C = 2\%$ , alcohol)
6	Ethyl ester of carbobenzoxy- <i>L</i> -phenylalanyl-glycine	54	110	184.5–185.5(2)	C 67.89H 6.42N 7.92	C 67.70H 6.21N 7.909	$-25.62^{\circ}$ ( $C = 1.4\%$ , dioxane)
7	Ethyl ester of carbobenzoxy- <i>L</i> -phenylalanyl-glycine	30	50	185.5–186	—	—	$-26.05^{\circ}$ ( $C = 0.6\%$ , dioxane)
8	Ethyl ester of carbobenzoxy- <i>L</i> -leucyl-glycine	48	41	154–155	C 65.01H 7.16N 8.51	C 65.19H 7.04N 8.45	$-28.00^{\circ}$ ( $C = 1.4\%$ , alcohol)

Experiment no.	Compound	Yield, %	Yield, mg	M.p., °C	Analysis,		
					Analysis, %, found	Analysis, %, calculated	$[\alpha]_D^{21-22}$
9	Ethyl ester of carbobenzoxy- <i>L</i> -leucylglycine	57	55	154–155	—	—	$-26.68^\circ$ ( $C = 1.85\%$ , alcohol)
10	Ethyl ester of carbobenzoxy-glycylglycine (isolated as the amide)	30	80	196–197	C 61.14H 6.15N 13.59	C 61.16H 5.83N 13.59	$+10.31^\circ$ ( $C = 0.7\%$ , methyl alcohol)
11	Amide of carbobenzoxy- <i>L</i> -phenylalanyl-glycylglycine	21	25	196–197	Amide 3.44	Amide 3.39	—
12	Ethyl ester of hippuryl-glycylglycine	9.5	10	173	—	—	—
1	Ethyl ester of benzoyl- <i>L</i> -phenylalanyl-glycine	19	20	146–148	—	—	—
2	The same	18	10	146–147	—	—	—

\* Literature data:  $[\alpha]_D^{25} - 17.3 + 0.5^\circ$  ( $C = 2\%$ , alcohol)<sup>(3)</sup>;  $[\alpha]_D^{23-25} - 16.6^\circ$  ( $C = 2\%$ , alcohol)<sup>(4)</sup>;  $[\alpha]_D^{20.5} - 38.5^\circ$  ( $C = 0.64\%$ , alcohol);  $[\alpha]_D^{21} - 34^\circ$  ( $C = 1.25\%$ , alcohol)<sup>(5)</sup>.

5. **Synthesis of optically active peptides.** A weighed portion of the glycolic or lactic acid ester of the acylated amino acid is dissolved together

with the hydrochloride of the amino acid or peptide ester in a minimal amount of alcohol, neutralized with 1 *N* NaOH, and 2 ml of phosphate buffer, pH 8, are added. The pH of the solution is then brought to 7.8–8.2 (checked with an LP-4 potentiometer), and 1–1.5 mg of enzyme are added. The temperature of the reaction mixture is about 20°. After 5–15 min, a precipitate of peptide begins to separate. After the time indicated in Table 1 has elapsed, the precipitate is filtered off, washed with 1 *N* HCl until the ninhydrin reaction disappears and with a solution of soda until the reaction with KI and KIO<sub>3</sub>, starch disappears, and recrystallized from aqueous alcohol. If no precipitate is observed during the reaction, the entire solution is evaporated in vacuo and the residue is treated with chloroform or ethyl acetate, washed with 1 *N* HCl, water, and soda, and evaporated. The reaction conditions and the results obtained are presented in Tables 1 and 2.

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named after M. V. Lomonosov

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*Note: Figure translations are in progress. See original paper for figures.*

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