DEPSIPEPTIDE ANALOGS OF BIOLOGICALLY ACTIVE PEPTIDES

COMMUNICATION 1.* SYNTHESIS OF DEPSIPEPTIDE ANALOGS OF OPHTHALMIC ACID AND GLUTATHIONE

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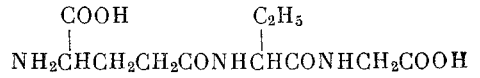
In the study of biologically active peptides an important question is the elucidation of the relation between the structure and biological activity of these compounds and the determination of which sections of the molecules are responsible for this activity. A very closely associated problem is that of the synthesis of peptide analogs having an antagonistic action. Such questions are usually resolved either by the exclusion of one or more particular amino acids for the peptide molecule, or by their replacement by other amino acids differing in spatial configuration or containing different groups. As a result of such modifications in the peptide molecule, in most cases either biologically inactive compounds are obtained, or their activity falls fairly sharply, though in individual cases highly active substances have been obtained in this way. An approach to the question of the significance of individual structural elements in the molecule of a biologically active peptide can be made somewhat differently, namely not by changing the groups in particular amino acids or their spatial configuration, but, while preserving both of these, by replacing the amide linkage by an ester linkage, i.e., by replacing in definite sections of the peptide chain one or more amino acids by the corresponding hydroxy acids.

Turning to the synthesis of compounds of this type we selected, as our first example, ophthalmic acid (I)[1-6], which, as is well known, is an antagonist of glutathione.



(I)

It was planned, in the first place, to replace the glycine residue in this acid by a glycolic acid residue. The synthesis of such a depsipeptide analog of ophthalmic acid was carried out as follows.

L-2-(Benzyloxycarbonylamino)butyric acid (II) was condensed (by the method of mixed anhydrides with benzenesulfonyl chloride) with p-nitrobenzyl glycolate (IIIa). After the usual treatment a 45% yield was obtained of the crystalline condensation product (IVa). On elimination of the benzyloxycarbonyl group with hydrogen bromide in glacial acetic acid we isolated a 95% yield of the hydrobromide (Va). Then, 1-benzyl hydrogen N-(benzyloxycarbonyl)-L-glutamate (VIa) was condensed by the method of mixed anhydrides (with isobutyl chloroformate) with the hydrobromide (Va). The yield of the protected depsipeptide (VIIa) was 72%.

Hydrogenation of the depsipeptide (VIIa) over palladium black in absolute ethanol in presence of acetic acid led to the formation of the depsipeptide analog of ophthalmic acid (VIII) in 96% yield. (VIII) was isolated as a white solid (but not crystalline) substance, and without any further purification it had good analytical and chromatographic characteristics. Since neither the chemical [1] nor the enzymic [5] methods for the synthesis of ophthalmic acid described in the literature gives a yield exceeding 20%, we considered it desirable to prepare it also in accordance with Scheme 1.

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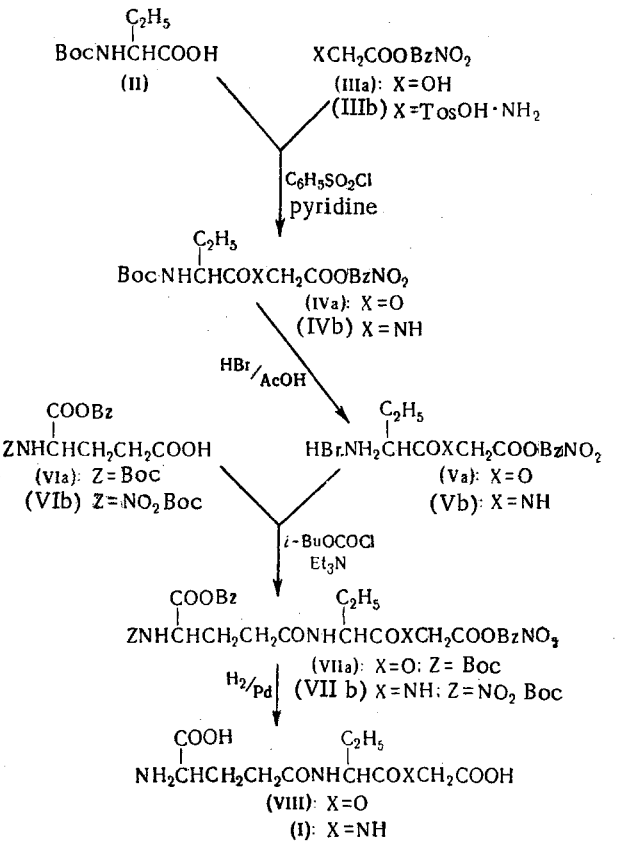


Bz = $C_6H_4CH_2$—; $BzNO_2$ = *p*-$NO_2C_6H_4CH_2$—; Boc = $C_6H_5CH_2OCO$—
NO_2Boc = *p*-NO_2—$C_6H_4CH_2OCO$—; TosOH = *p*-CH_3—$C_6H_4SO_3H$

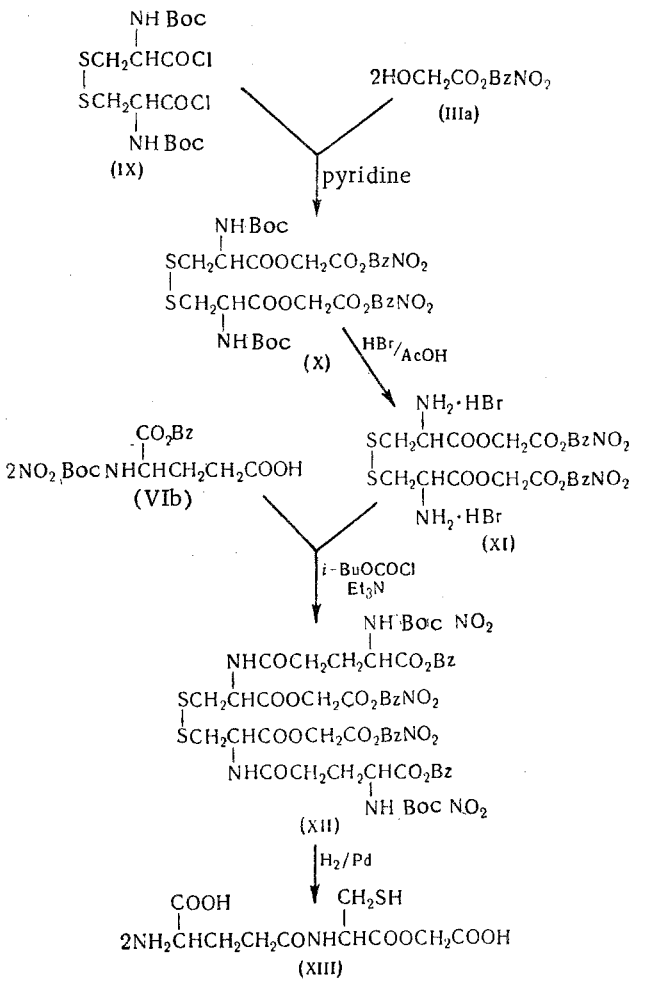
Scheme 1.

As a result of this synthesis, replacing p-nitrobenzyl glycolate (IIIa) by glycine p-nitrobenzyl ester (IIIb), and using instead of 1-benzyl hydrogen N-Boc-L-glutamate (VIa) 1-benzyl hydrogen N-(p-nitrobenzyloxycarbonyl)-L-glutamate (VIb), we obtained pure ophthalmic acid in an overall yield of 43% based on L-2-aminobutyric acid. Here we must mention that the L-2-(benzyloxycarbonylamino)butyric acid (II) prepared by the method in [1] had the described melting point of 78-79°, but its rotation was not −32°, but −12° (c 2.8, ethanol). In the hydrogenolysis of this Boc-acid (II) we isolated optically pure L-2-aminobutyric acid in 78% yield, and the use of the acid (II) in the synthesis of ophthalmic acid led to the preparation of optically pure ophthalmic acid (I). These results give us reason to suppose that there is a misprint in [1] and the true rotation of L-2-(Boc-amino)butyric acid is −12°.

It was considered to be of interest also to synthesize the depsipeptide analog of glutathione, again with the replacement of glycine by glycolic acid. The course of the synthesis of this depsipeptide analog of glutathione is presented in Scheme 2.

N,N'-Bis(benzyloxycarbonyl)cystyl chloride (IX) [7] was condensed in tetrahydrofuran solution in presence of pyridine with p-nitrobenzyl glycolate (IIIa), as a result of which the compound (X) was obtained in 40% yield. After the removal of the benzyloxycarbonyl groups with hydrogen bromide in glacial acetic acid we isolated the hydrobromide (XI) in 97% yield. The compound (XII) was synthesized in 82% yield by the condensation of 1-benzyl hydrogen N-(p-nitrobenzyloxycarbonyl)-L-glutamate (VIb) with the hydrobromide (XI). The condensation was carried out by the method of mixed anhydrides with isobutyl chloroformate in presence of triethylamine. To obtain the depsipeptide analog of glutathione (XIII) from the compound (XII) it was necessary to remove the numerous protective groups and then reduce the −S−S− linkage.

We planned to carry out this stage of the work on analogy with the work on the synthesis of glutathione [8] by hydrogenation first over 10% palladized charcoal, which should lead to the removal of all the protective groups, and then over palladium black to reduce the −S−S− linkage. Here, however, we encountered considerable difficulties associated probably with the occurrence of partial reduction of the −S−S− linkage over the palladized char-



Scheme 2.

coal with poisoning of the catalyst, so that we were unable to remove the protective groups completely by this means. The correctness of this view is confirmed by the fact that ophthalmic acid and its depsipeptide analog were readily obtained by the hydrogenation of the corresponding analogously protected compounds (VIIa) and (VIIb) over palladium.

EXPERIMENTAL

The analyses, melting points, and optical characteristics of all the compounds obtained are given in the table.

L-2-(Benzyloxycarbonylamino)butyric Acid (II). 8.3 ml of 95% benzyl chloroformate and 46 ml of 1 N NaOH were added simultaneously over a period of 30 min with vigorous stirring with maintenance of the temperature at 2-5° and the pH at 7.5-8 to a solution of 4.3 g of L-2-aminobutyric acid $[\alpha]_D^{21}$ +22° (c 2; 5 N HCl) [9] in 42 ml of 1 N NaOH. Stirring was continued further for 45 min, and the reaction mixture was then extracted with ether, acidified to Congo Red with hydrochloric acid, and extracted with ethyl acetate. The ethyl acetate solution was dried over $MgSO_4$, and the ester was vacuum-distilled off. The residue was dissolved in a little diethyl ether and poured into petroleum ether. The oily substance that separated quickly crystallized. The precipitate was filtered off, washed with petroleum ether, and vacuum-dried over P_2O_5. Weight 7 g (70%) [1].

p-Nitrobenzyl glycolate (IIIa). A mixture of 25 g of glycolic acid, 50.5 g of p-nitrobenzyl alcohol [10], 6.6 g of p-toluenesulfonic acid, and 200 ml of toluene was boiled for 30-45 min in a flask fitted with a Dean and Stark head and a condenser protected by a calcium chloride tube. 400 ml of chloroform was added to the still warm mixture, and the solution was washed with 2% $KHCO_3$ solution (about 400 ml) and then water. Solvent was vacuum-distilled off, and the residue was purified by crystallization from water with the use of activated charcoal.

Cpd. No.	Formula	Mol. wt.	M.p., °C	Found, %				Calc., %				$[\alpha]_D \pm 1°$ (concn. and solvent)
				C	H	N	Hal	C	H	N	Hal	
II	$C_{12}H_{15}O_4N$	237,25	78—79	60,54	6,37	5,79	—	60,75	6,37	5,90	—	—12° (*c*2,8; C_2H_5OH)
IIIa	$C_9H_9O_5N$	211,17	113—114	51,51	4,25	6,62	—	51,19	4,30	6,63	—	—
IVa	$C_{21}H_{22}O_8N_2$	430,40	66—67	58,55	5,08	6,44	—	58,60	5,15	6,51	—	—20° (*c*1; AcOEt)
Va	$C_{13}H_{17}O_6N_2Br$	377,20	150—152	41,44	4,69	7,30	21,13	41,39	4,54	7,43	21,19	+5,5° (*c*1; CH_3OH)
VIIa	$C_{33}H_{35}O_{11}N_3$	649,63	78—82	60,77	5,64	6,72	—	61,00	5,43	6,47	—	—25° (*c*1; DMFA
VIII	$C_{11}H_{18}O_7N_2$	290,27	—	46,02	6,45	9,78	—	45,51	6,25	9,65	—	—36° (*c*3; H_2O)
IIIb	$C_{16}H_{18}O_7N_2S$	382,38	204—207	50,26	4,65	7,82	—	50,25	4,74	7,33	—	—
IVb	$C_{21}H_{23}O_7N_3$	429,42	114—115	58,90	5,62	10,08	—	58,73	5,40	9,79	—	—10° (*c*2; AcOEt)
Vb	$C_{13}H_{18}O_5N_3Br$	376,21	133—135	41,12	5,22	11,06	21,17	41,49	4,82	11,17	21,24	+10° (*c*1; CH_3OH)
VIb	$C_{20}H_{20}O_8N_2$	416,38	99—101	—	—	6,82	—	57,69	4,84	6,73	—	—19° (*c*2; CH_3COOH)
VIIb	$C_{38}H_{35}O_{12}N_5$	693,65	171—173	56,92	5,06	10,34	—	57,14	5,09	10,10	—	—22,5° (*c*3; CH_3COOH)
X	$C_{40}H_{38}O_{16}N_4S_2$	894,86	117—119	53,71	4,54	6,36	—	53,69	4,28	6,26	—	—47,5° (*c*1; CH_3COOH)
XI	$C_{24}H_{28}O_{12}N_4S_2Br_2$	788,46	133—137 (decomp.)	36,42	3,94	6,74	20,03	36,56	3,58	7,10	20,27	—55° (*c*1; CH_3OH)
XII	$C_{64}H_{82}O_{26}N_8S_2$	1423,3	100—120	53,85	4,63	7,79	—	54,00	4,39	7,87	—	—61° (*c*1; DMFA

The p-nitrobenzyl glycolate obtained was vacuum-dried; weight 28 g (40%); m.p. 108-111°. For analysis the substance was crystallized from toluene.

p-Nitrobenyl O-[L-2-(Boc-amino)butyryl]glycolate (IVa). To a solution of 12 g of L-2-(Boc-amino)butyric acid (II) at −10°, 6.4 ml of benzenesulfonyl chloride was added with stirring, and then after ten minutes 8.44 g of p-nitrobenzyl glycolate (IIIa) was added. The mixture was stirred at −10° for 30 min, and then two hours more without cooling. The reaction solution was poured into 250 ml of water and extracted repeatedly with ethyl acetate. The ethyl acetate solution was washed successively with water, 2% $KHCO_3$ solution, and again water, and three successive portions of the washing agent were used in each case. The ethyl acetate solution was dried over anhydrous sodium sulfate, and solvent was distilled off. The residue was rubbed out with a little absolute ethanol and left overnight in a refrigerator. The crystalline precipitate was filtered off and again crystallized from absolute ethanol; weight 7.75 g (45%).

p-Nitrobenzyl O-(L-2-aminobutyryl)glycolate Hydrobromide (Va). 12 ml of a 36% solution of HBr in glacial acetic acid was added to a solution of 7.75 g of p-nitrobenzyl O-[L-2-(Boc-amino)butyryl]glycolate (IVa) in 10 ml of glacial acetic acid. After 30 min the reaction mixture was poured into 260 ml of dry ether and left in a refrigerator for two hours. The precipitate that formed was filtered off, washed several times with dry ether, and vacuum-dried over P_2O_5; weight 6.45 g (95%).

p-Nitrobenzyl O-[L-2-[(O^1-Benzyl-N-Boc-γ-L-glutamyl)-amino]butyryl]glycolate (VIIa). 5.3 g of the hydrobromide (Va) was dissolved in 30 ml of dry tetrahydrofuran, 1.95 ml of dry triethylamine was added, and the mixture was cooled to −5°. The precipitate formed was filtered off and washed with dry tetrahydrofuran. The solution obtained was then used as amine component.

A solution of 1-benzyl hydrogen N-Boc-L-glutamate (VIa) [11] and 1.95 ml of dry triethylamine in 20 ml of tetrahydrofuran was cooled to −5°, and 1.85 ml of isobutyl chloroformate [12] was added with stirring. After ten minutes the solution of the amine component, obtained as above, was added in the course of five minutes with maintenance of the temperature at −5°. The mixture was stirred at this temperature for 30 min, after which the reaction solution was left to the next day. The precipitate of triethylamine hydrochloride was filtered off and washed with dry tetrahydrofuran. The solution obtained was vacuum-evaporated to dryness. The residue was dissolved in 160 ml of ethyl acetate and washed exhaustively with water, with 0.5 N HCl, with water, with 2% sodium carbonate solution, and again with water. 100 ml of ethyl acetate was added, and the solution was dried over magnesium sulfate. Solvent was vacuum-distilled off, and the residue was rubbed out with dry ether and left in a refrigerator. The crystalline precipitate that formed was filtered off, washed with dry ether, and vacuum-dried over P_2O_5. The substance was crystallized from carbon tetrachloride; weight 6.4 g (70%).

O-[L-2-(γ-L-glutamylamino)butyryl]glycolic Acid (VIII). 5 g of (VIIa) was dissolved in 55 ml of absolute ethanol and 5 ml of glacial acetic acid and hydrogenated in presence of palladium black. When hydrogenation was complete, catalyst was filtered off, solvent was driven off in a vacuum until the volume was down to 4-6 ml, 80-100 ml of acetone was added slowly, and the mixture was left in a refrigerator. On the next day the acetone layer was poured off; the residue was washed with acetone and vacuum-dried at 60-80°. The weight of the solidified residue was 2.2 g (99%). (VIII) is very hygroscopic.

Glycine p-Nitrobenzyl Ester p-Toluenesulfonate (IIIb). A mixture of 2.5 g of glycine, 5.1 g of p-nitrobenzyl alcohol [10], 60 ml of dry toluene, and 6.6 g of p-toluenesulfonic acid was boiled in a flask fitted with a Dean and Stark head and a condenser protected by a calcium chloride tube. After two hours, when the calculated amount of water had been driven off, the precipitate formed was filtered off, washed repeatedly with benzene and ether, and crystallized from water; weight 7 g (60%).

N-[L-2-(Boc-amino)butyryl]glycine p-Nitrobenzyl Ester (IVb). 2.14 ml of benzenesulfonyl chloride was added to a solution of 2.37 g of L-2-(Boc-amino)butyric acid (II) in 10 ml of pyridine at −10°. After ten minutes 3.82 g of glycine p-nitrobenzyl ester p-toluenesulfonate (IIIb) was added, and stirring was continued for 30 min at −10° and then two hours without cooling. The reaction solution was poured into water and extracted with ethyl acetate. The ethyl acetate solution was washed successively with 5% HCl, water, 5% Na_2CO_3, and again water. The ethyl acetate solution was dried over $MgSO_4$, and solvent was driven off. The residue was rubbed out with ether and then crystallized from benzene; weight 3.82 g (88%).

N-(L-2-Aminobutyryl)glycine p-Nitrobenzyl Ester Hydrobromide (Vb). 3.3 g of N-[L-2-(Boc-amino)butyryl]-glycine p-nitrobenzyl ester (IVb) was dissolved in 4 ml of glacial acetic acid, and 4 ml of a 36% solution of HBr in glacial acetic acid was added. After 30 min the reaction mixture was poured into 120 ml of dry ether, the mixture was cooled, and the precipitate formed was filtered off. For purification the precipitate was dissolved in absolute ethanol and precipitated by the addition of dry ether; weight 2.49 g (84.5%).

1-Benzyl Hydrogen N-(p-Nitro-Boc)-L-glutamate (VIb). 11.85 g of 1-benzyl hydrogen glutamate [11] was dissolved in 25 ml of dioxane, and 50 ml of a 16.5% aqueous solution of K_2CO_3 was added with stirring. Then, at 0° over a period of 50 min 11.85 g of p-nitrobenzyl chloroformate [13] was added with maintenance of the pH at 8 by the addition of 10% aqueous K_2CO_3 solution. The reaction solution was stirred at 0° for 20 min and then without cooling for one hour. It was poured into 250 ml of water, and the precipitate formed was filtered off. The aqueous solution was extracted twice with ether, and after acidification with hydrochloric acid the solution was left in a refrigerator. On the next day the precipitate formed was filtered off and vacuum-dried over P_2O_5. The substance was crystallized from a 1:1 mixture of ethanol and water; yield 16.3 g (79%).

N-[L-2-[(O^1-Benzyl-N-p-nitro-Boc-γ-L-glutamyl)amino]butyryl]glycine p-Nitrobenzyl Ester (VIIb). 1.45 ml of dry triethylamine was added to a solution of 3.76 g of the hydrobromide (Vb) in 30 ml of dry tetrahydrofuran, and the mixture was cooled to −5°. The precipitate formed was filtered off and washed with dry tetrahydrofuran. The solution obtained was used further as an amine component. A solution of 4.2 g of 1-benzyl hydrogen N-(p-nitro-Boc)-L-glutamate in 20 ml of dry tetrahydrofuran was cooled to −5°, and 1.35 ml of isobutyl chloroformate [12] was added with stirring. After ten minutes the solution of the amine component, obtained as above, was added in the course of five minutes at −5°, after which the mixture was stirred further for 30 min and then set aside at room temperature. On the next day the precipitate of triethylamine hydrochloride was filtered off, and solvent was vacuum-distilled off. The colloidal precipitate obtained was dissolved in 300 ml of chloroform and washed successively with water, 0.5 N HCl, water, 10% K_2CO_3 solution, and again water. The chloroform solution was dried with anhydrous $CaCl_2$, and solvent was vacuum-distilled off. The residue was washed several times with hexane and vacuum-dried over P_2O_5 and paraffin wax; weight 4.3 g (62%).

Ophthalmic Acid (I). 2.42 g of (VIIb) in 45 ml of tetrahydrofuran containing 90 ml of 15% acetic acid was hydrogenated in presence of 1 g of 10% palladized charcoal. When no more hydrogen was absorbed, the palladium was filtered off and washed with alcohol. The solution was vacuum-evaporated down to 5-7 ml, after which 200 ml of acetone was added. Ophthalmic acid was precipitated as an oily mass, which, when rubbed out and cooled, crystallized. It was filtered off and vacuum-dried over P_2O_5; weight 0.98 g (98%); m.p. 178-180°, $[\alpha]_D^{20}$ −29° C 2.4; H_2O [1].

Bis-p-nitrobenzyl O,O'-(N,N'-BisBoc-L-cystyl)diglycolate (X). The bis(acid chloride) (IX), prepared from 9.17 g of N,N'-bis(benzyloxycarbonyl)cystine [7], was dissolved in 80 ml of dry tetrahydrofuran, and the resulting solution was cooled to 0° and stirred while simultaneous additions were made in the course of ten minutes of solutions of 2.9 ml

of tetrahydrofuran and 6.33 g of p-nitrobenzyl glycolate (IIIa) in 55 ml of dry tetrahydrofuran. After 30 min the cooling bath was removed, and the mixture was stirred further for one hour at room temperature and then left overnight. The precipitate of pyridine hydrochloride was filtered off, and solvent was vacuum-distilled off. The residue was dissolved in 250 ml of ethyl acetate, and the resulting solution was washed exhaustively with 5% HCl, water, 20% $KHCO_3$, and again water. The solution was dried with anhydrous $MgSO_4$, and solvent was driven off. Chloroform was added to the residue and then driven off; and this operation was repeated. The oily residue was rubbed out with hexane and left in a refrigerator. On the next day the precipitate was filtered off. After recrystallization from absolute ethanol the weight of product was 5.4 g (40%); m.p. 110-115°. A sample for analysis was crystallized, first from benzene and then from ethanol.

Bis-p-nitrobenzyl O,O'-L-Cystyldiglycolate Dihydrobromide (XI). 4 ml of a 36% solution of HBr in glacial acetic acid was added to a solution of 3.95 g of (X) in 6 ml of glacial acetic acid. After one hour the reaction mixture was poured into 80 ml of ether and left in a refrigerator until the next day. The precipitate formed was filtered off, washed several times with dry ether, and vacuum-dried over P_2O_5. For purification (XI) was dissolved in absolute ethanol and precipitated with dry ether; weight 3.37 g (97%).

Bis-p-nitrobenzyl O,O'-[N,N'-Bis-(O^1-benzyl-N-Boc-γ-L-glutamyl)-L-cystyl]diglycolate (XII). 1.12 ml of dry triethylamine was added to a solution of 3.15 g of the dihydrobromide (XI) in 15 ml of dry tetrahydrofuran, the mixture was cooled at 0-5° for 10-15 min, and the precipitate of triethylamine hydrobromide was filtered off and washed with dry tetrahydrofuran. The tetrahydrofuran solution obtained was used further as an amine component.

3.34 g of 1-benzyl hydrogen N-(p-nitro-Boc)-L-glutamate (VIb) was dissolved in 15 ml of dry tetrahydrofuran, 1.12 ml of dry ethylamine was added with stirring, the mixture was cooled to −5° and 1.05 ml of isobutyl chloroformate was added. After five minutes the solution of the amine component, prepared as above, was added in the course of five minutes at −5°. Stirring was continued at 0° for 30 min and then for two hours at room temperature, and the reaction mixture was left overnight. The precipitate of triethylamine hydrochloride was filtered off, and solvent was vacuum-distilled off at 50°. The residue was dissolved in 80 ml of ethyl acetate, and the solution was washed with water. The ethyl acetate solution was dried with anhydrous $MgSO_4$, solvent was driven off, and the residue was rubbed out with dry ether. After a few hours the crystalline precipitate was filtered off, vacuum-dried over P_2O_5 and crystallized from absolute ethanol; weight 4.67 g (82%).

SUMMARY

1. A new method for the synthesis of ophthalmic acid is proposed.

2. A method was developed for the preparation of the depsipeptide analog of ophthalmic acid and the protected depsipeptide analog of glutathione; in these glycine is replaced by glycolic acid.

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