REGIOSPECIFIC SYNTHESES OF ∝-GLUTAMYL PEPTIDES VIA 2,5-THIAZOLIDINEDIONES. PART III.

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Abstract:
Optically active N-thiocarboxyanhydride (NTA'S) of L-glutamic acid has been prepared and used for the stepwise synthesis of peptides in aqueous solution. Some ∝-glutamyl peptides were obtained by a two steps regiospecific synthesis using NTA'S.

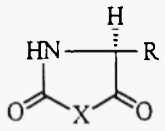
INTRODUCTION

L-Glutamyl peptides are important compounds with various applications in clinical diagnosis for detection of enzymes (1-3), or compounds with biological activity (4). The introduction of peptide bond to the ∝-carboxyl group of the L-glutamic acid is difficult, the reaction involving many steps by blocking of the ∝-carboxyl group. The literature data show that the ∝-glutamyl peptides can be prepared from phtalyl-glutamic anhydride by reaction with alcools and then coupling the resulting γ-esters with amino acid derivatives via the acid chloride, mixed anhydride or DCC method (5,6). The protecting group was then removed by a general procedure.

A new route to α-aspartyl peptides was performed by Vinick and Yung in synthesis of aspartame, a dipeptide sweetener (7). The peptide bond is achieved by the intermediate of 2,5-thiazolidinedione 1 (X=S; R=CH_2COOH), wich reacts with amino acids in aqueous solution at pH=8-9.

Some optically active N-carboxyamino acid anhydrides 1 (X=O) and N-tiocarboxyamino acid anhydrides 1 (X=S) have been prepared and used for the stepwise synthesis of peptides in aqueous solution, known as NCA or NTA methods (8,9). The use of the α-amino acid N-carboxyanhydride (NCA, X=O) in the synthesis of peptides in aqueous solution is complicated by the fact that below

pH=11 the instability of peptide carbamates leads to overreactions via decarboxylation, whereas at pH=11 overreaction via the NCA anion, formation of hydantoic acid and hydrolysis become troublesome side reactions (10).



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It was thought that analogs of the NCA'S in which the ether oxygen is replaced by sulfur might solve some of these problems because the related thiocarbamates could be expected to offer a greater stability at given pH than would the carbamates. Salts of amino acid thiocarbamates were stable to electrophoresis at pH=11, whereas the carbamate salts decomposed.

Generally N-thiocarboxyanhydrides (NTA) of good optical purity were obtained by cyclisation of thionourethans of amino acids with PBr_3, $POCL_3$, Ac_2O [8]. In addition of an NTA to an aqueous solution of an amino acid or peptide at pH=8.5-9.5 led to high yields of the peptide homolog.

The increased stability of the thiocarbamates permitted the reaction to be carried out at a lower pH (8.9-9.0), generally affording higher yields and good optical purity.

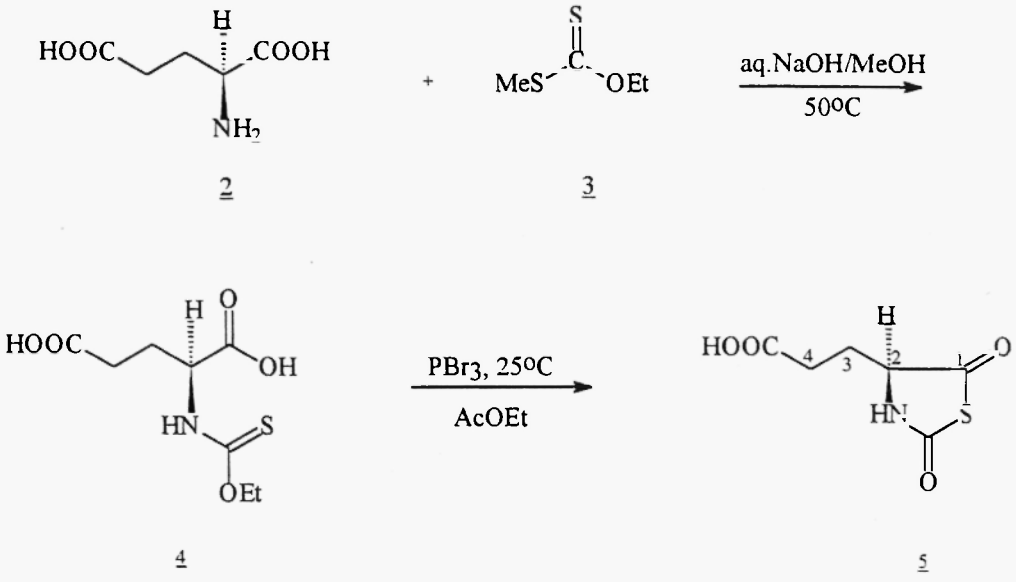
RESULTS AND DISCUSSIONS

The present paper describes the synthesis of optically active N-thiocarboxyanhydride (NTA) of L-glutamic acid 5 and its use in two steps synthesis of peptides in aqueous solution.

This compound has been alluded to in the literature but its utilization in peptide synthesis has never been reported (11).

Methyl ethyl xanthate 3 was reacted with L-glutamic acid 2 in a 1:1 mixture of aqueous sodium hydroxide and methanol to give unseparated thionourethane 4 (an oil) which cyclized in ethyl acetate with PBr_3 for 10 min. at 25°C leading to crystalline L-glutamic acid N-thiocarboxyanhydride 5 (see scheme 1).The compound 5 was characterized by IR and ^{1}H-NMR spectra.

Nevertheless, the use of PBr_3, instead of PCl_3 or Ac_2O, proved to be advantageous because the greater reactivity of PBr_3 permited NTA formation to be carried out within a shorter period of time, thus reducing exposure to acidic condition in racemization reaction.

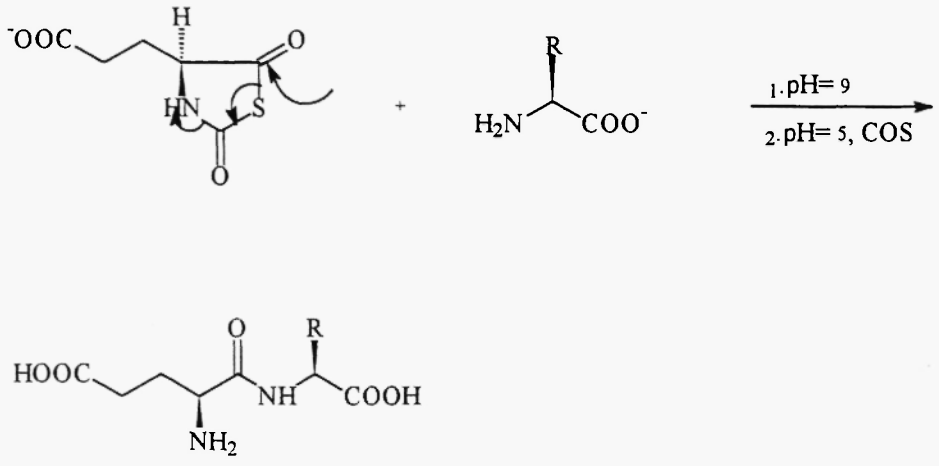


Scheme 1

The optical purity (>98%) of L-glutamic acid N-thiocarboxyanhydride $[\alpha]_D^{25} = -97°$(c=1, THF), was estimated by hydrolysis to the glutamic acid and determination of the amount of the D isomer present in the product. In this case hydrolysis of 5, gave a quantitative yield of L-glutamic acid in a high optical purity (>98%).

The reaction of N-thiocarboxyanhydride 5 with amino acids in aqueous solution was examined to determine yields and extent of racemization in peptide formation. The compound 5 was added to an aqueous solution of amino acids at pH=8-9, at 0°-5°C, then the solution was acidified to cleave the carbonyl group (see Scheme 2). The reactionswere performed in various solvents (aqueous NaOH, and 1:1 mixture of aq. NaOH 2N in methanol, or acetone).

Good yields of peptides were obtained with NTA at pH=8-9. Although the yield in NTA reaction and optical purity of the desired dipeptides decrease sharply when the reaction was carried out at a pH>10.



Scheme 2

The NTA of L-glutamic acid was reacted with L-valine, L-leucine, L-aspartic acid, L-phenylalanine t-buthylester, and 70-85% yields of the isolated dipeptides were obtained. Experimental results are given in Table 1.

Table 1. Dipeptides synthesized with L-glutamic acid NTA

Dipeptides	pH	yield, [%]	m.p. [°C]	$[\alpha]_D^{20}$ (c=1, HCl 1N)	Optical purity L-izomer [%]	FT-IR spectra (KBr, νcm^{-1})
α-L-glutamyl-L-vaiine	8.5	70	187	+23° (Lit. 13)	98.5	3340,1650,1535
α-L-glutamyl-L-leucine	9.0	85	195	+8° (Lit. 9)	99	3380,1655,1540
α-L-glutamyl-L-aspartic acid	8.5	80	140	+28° (Lit. 13)	98.7	3350,1640,1550
α-L-glutamyl-L-phenyl-alanine t-buthylester	9.0	79	127	+9° (Lit. 12)	99.2	3360,1650,1545

The IR spectra of all the dipeptides, recorded in solid state (KBr) , showed strong absorption bands at 3340-3380 cm^{-1} (νN-H stretching), 1640-1655 cm^{-1} (νC=O stretching) and 1535-1550 cm^{-1} (δN-H stretching), characteristic for the

peptide bond. If the coupling reaction is performed under conditions of pH and temperature control, a very low racemization had taken place.

In conclusion, this synthesis demonstrates that the α-glutamyl peptides can be prepared in high yield with complete regiochemical control via a short, simple series of chemical steps.

Experimental

Melting points were determined in capillaries and are uncorrected. IR spectra were recorded with a FT-IR 5300 JACSO apparatus. Mass spectrum was scanned on a Varian MAT 311 spectrometer in EI mode at 70eV. ^{1}H-NMR spectrum was measured in DMSO-d_6 on a Tesla BS487C (80Mhz) apparatus. TLC was performed with Merck silicagel 60F 254. The $[\alpha]_D^{20}$ values were estimated on a Polamat A Karl Zeis Jena photopolarimeter.

L-Glutamic acid N-thiocarboxyanhydride (L-Glu-NTA) 5

A solution of 2.94g(0.02mol) of L-glutamic acid in 10ml of 50% solution of NaOH (0.02equiv.) in methanol was stired under nitrogen at 25°C, while 2.7g(0.02mol) of O-ethyl-S-methylxanthate in 5ml methanol was dropwise added. The mixture was held at 50°C for 4 hrs. The reaction mixture was concentrated and the oil was taken up in 20ml of water and extracted with ether. The aqueous layer was acidified with 6N HCl, extracted with ethylacetate and dried with Na_2SO_4 .This solution was treated with 3ml of PBr_3 over 5min. Initially a strong exothermic reaction took place and white crystals of L-glutamic N-thiocarboxyanhydride precipitated. The filtered compound was crystallized from water, to give 1.5g (40%); m.p.=193°C; $[\alpha]_D^{20}$= -97° (c=1, THF); IR (KBr): 3200, 1740, 1653, 1399 , 1210 cm^{-1} ;^{1}H-NMR (DMSO-d_6); δ(ppm): H_2(4.70, 1H, t), H_3(2.10, 2H, m), H_4(3.17, 2H, m); M/z (EI, 70eV,%): 189(M^+,45), 87(100), 60(30).

A sample of compound was treated with silver nitrate to give silver sulfide and glutamic acid with an optical purity~98.5%.

General procedure for synthesis of dipeptides

0.01mol of L-amino acid was dissolved in 20ml of water at 0°-5°C and the pH of the solution adjusted to 9 with 2N NaOH. 1.84g(0.01mol) of L-glutamic acid-NTA was then added portionwise with vigorous stirring and the pH was maintained at 8.5-9 by addition of more base. After the completion of the NTA addition, stirring was continued at 5°C until the pH stabilized at 9 (1hr.). The reaction mixture was adjusted to pH=5-5.5 at 0°C with 12N

HCl (COS gas evolution) and then diluted with an equivalent volume of methanol. The precipitated dipeptide was collected by filtration and washed with 5ml of ice water. For purification, the precipitate was dissolved in 15ml 1M sodium carbonate, 0.1 g charcoal was added under stirring, the suspension filtered after 5min. and the product was precipitated from aqueous solution with 2N HCl at pH=4.5-5.0. The precipitate was filtered, washed with acetone, ether and dried at 60°C under low pressure. TLC, using Merck plates and i-propanol:water:acetic acid=8:3:1 as eluent, confirmed the purity of the obtained dipeptides. Some analytical data of all dipeptides prepared in this paper are given in Table 1.

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