Synthesis of a Tripeptide Containing a C-Terminal Aldehyde Moiety

by

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George Glover is an excellent teacher and research advisor. I wish to thank him for his invaluable help on this project.

Abstract: Naturally occuring peptides with C-terminal aldehyde functions have been shown to inhibit serine and thiol proteases possibly by reacting with the hydroxyl or sulfhydryl group in the active site. Tripeptides were synthesized containing a C-terminal leucine residue with the aldehyde moiety to mimic the action of these natural inhibitors.

Past investigation of proteases has shown they are involved in many biological functions such as blood clotting, maintainance of blood pressure and clot lysis. Regulation of proteases, therefore, regulates these important biological activities.

Recently, work has been directed toward finding competetive inhibitors for these proteases to function as regulators. To be effective, these inhibitors must be specific, they must be chemically stable, and be effective in low concentrations to lessen side effects. Umezawa and coworkers (1,2,3) have isolated two types of low molecular weight peptides from Actinomycetes which are extremely effective inhibitors of proteases. One type is a peptide containing one or more γ-amino-β-hydroxy acid residues derived from leucine. The second type is peptides containing C-terminal aldehydes (fig. 1) and is the subject of my research.

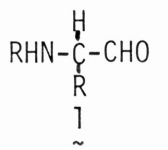
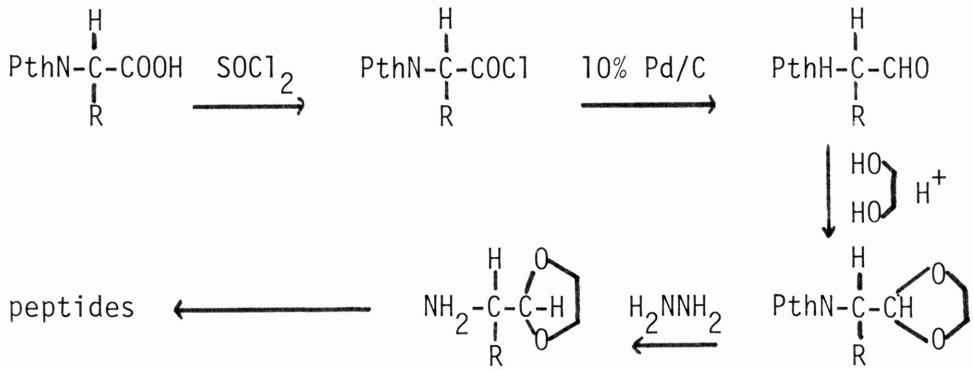


Fig. 1

As inhibitors, these C-terminal aldehydes may form stable complexes that are analogs of the transition state of the enzyme-substrate reaction. Westerik and Wolfenden (4) and Thompson (5) have shown 1 derived from glycine and alanine to be effective inhibitors of papain and elastase, respectively. These aldehydes react with the sulfur of cysteine in the active site of papain and the hydroxyl of serine in elastase to form tetrahedral intermediates similar to the actual intermediates in the enzyme-substrate complex. It has been shown by Umezawa and co-workers (1) that seemingly slight variations in the side chains of the residues result in a dramatic change in the selectivity of the inhibitor. Perhaps

by synthesizing a peptide with the correct length, side chains and N-terminal blocking group, one could effectively and selectively inhibit proteases.

With this in mind, a synthetic scheme was developed to synthesize low molecular weight peptides with C-terminal aldehyde residues derived from leucine. The following scheme was designed.



Pth=phthaloyl blocking group

R=$CH_2CH(CH_3)_2$ Fig. 2

As a control molecule the same tripeptide was synthesized with a C-terminal ester instead of the aldehyde functionality.

Experimental

Phthaloyl Leucine

Leucine (0.2 moles) and phthaleic anhydride (0.2 moles) were mixed well and heated to 140°C until the reaction was complete. The product was crystallized from ether/petroleum ether. A crystalline solid, melting point 118.5-119.5, was recovered with a 72% yield.

Phthaloyl Leucine Acid Chloride (7)

Thionyl chloride (0.21 moles) and phthaloyl leucine (0.14 moles) were mixed in dry benzene and refluxed for 2 hours. The solvent was

removed under vacuum. Dry benzene was added again and removed under vacuum to remove any thionyl chloride from the product. An oily product was recovered quantitatively.

Phthaloyl Leucine Aldehyde

Phthaloyl leucine acid chloride (33 mmoles) was placed in 130 ml of dry toluene with 2.0 g of 10% pd/C catalyst at a temperature of 90-100°C. The reaction mixture was hydrogenated and progress followed by n.m.r. Phthaloyl leucine aldehyde, an oil, was recovered with a 65% yield.

Phthaloyl Leucine Acetal (6)

Phthaloyl leucinal (59 mmoles), 14.7 ml of ethylene glycol, and 0.39 g of p-toluenesulfonic acid were mixed in benzene. The flask was fitted with a Dean-Stark trap and the solution refluxed for 3.5 hours at which time the distillation of benzene-water azeotrope ceased. After cooling, the mixture was washed with water; the benzene was dried and removed under vacuum. Phthaloyl leucine acetal was recovered as an oil with a 95% yield.

Leucine Acetal

Phthaloyl leucine (38 mmoles) was dissolved in ethanol and 57 mmoles of hydrazine were added. The solution was refluxed for 2 hours at which time phthaloyl hydrazide had formed. The reaction mixture was cooled and the phthaloyl hydrazide filtered and washed with ethanol. The solvent was removed under vacuum and the product was distilled in a micro-distillation apparatus at 54°/0.25 mm with a 45% yield.

Carbobenzoxy-valylglycyl ethyl ester (8)

Cbz-valine was prepared according to literature procedures (9) as were the hydrochloride salts of glycine and leucine ethyl esters. (10)

Dry cbz-valine (14 mmoles) and 13 mmoles of N-methyl morpholine were stirred in 70 ml of THF (distilled from lithium aluminum hydride). The solution was cooled to -15°C. Isobutyl chloroformate (18 mmoles) was added and the solution stirred for 45 seconds at which time a solution of glycine ethyl ester (14 mmoles) in dry DMF was added. [This was made by mixing glycine ethyl ester hydrochloride with one equivalent of triethylamine in dry DMF and cooling to -15°C.] After 5 min the reaction was warmed to room temperature and stirred for 15 min. The solvent was removed under vacuum and chloroform was added. The chloroform was washed with 5% bicarbonate, water, 1N HCl and finally with water. After drying the chloroform over anhydrous Na_2SO_4, the solvent was concentrated and hexane added to crystallize the product. The white crystalline product had a melting point of 166-167°C and was recovered in a 40% yield.

Cbz-valylglycine.

Cbz-valylglycine ethyl ester (8.7 mmoles) was dissolved in 5 ml of methanol. 5.22 ml of 2N NaOH in methanol (10.4 mmoles) was added and the solution stirred for 2.5 hours. When the reaction was complete the methanol was removed under vacuum and the residue was dissolved in $CHCl_3$. The $CHCl_3$ was then washed repeatedly with 5% sodium bicarbonate. The basic wash was acidified to a pH of 2 and the acid was extracted with $CHCl_3$. The $CHCl_3$ was concentrated and the acid crystallized upon addition of hexane to give a white crystalline compound with a melting point of 138.0-140.0°C in a 65% yield.

Cbz-valylglycylleucine ethyl ester.

Preparation followed method described previously (7) using leucine

ethyl ester hydrochloride and Cbz-valylglycine. The tripeptide was recrystallized in $CHCl_3$/hexane and 55% of the expected yield was recovered. Cbz-valylglycylleucine acetal.

Preparation followed previously described mixed anhydride method using cbz-valylglycine and leucine acetal (7). The tripeptide which was recovered in a 14% yield, was a white crystalline solid melting at 170-171.5°C.

Discussion.

Once prepared, the tripeptide ethyl ester and the tripeptide acetal will be used in studies to determine their inhibition properties. Several proteases will be studied including α-chymotrypsin, subtilisin Carlsberg and subtilisin BPN'. If the aldehyde moiety is responsible for binding in the active site of the serine proteases, there should be a marked difference in the K_i values for each.

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