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Papain-catalyzed selective incorporation of cystathionine derivatives into model peptides

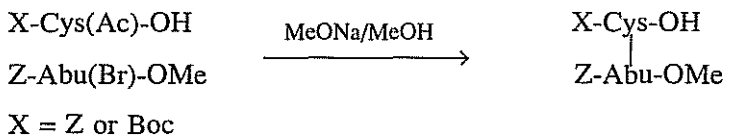
Václav Čeřovský, Pavel Šafář, Zdenko Procházka and Michal Lebl
Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 16610 Prague 6, Czechoslovakia

Introduction

Cystathionine derivatives can be used as starting compounds for the synthesis of neurohypophyseal hormone aminocarba analogues [1]. In this contribution we solve the problem of how to utilize proteolytic enzymes to selectively catalyze the formation of peptide bond at one of two carboxylic groups present in the cystathionine molecule. The method of peptide bond formation via ester aminolysis catalyzed by papain at alkaline pH ('kinetic approach') [2] has been chosen.

Results and Discussion

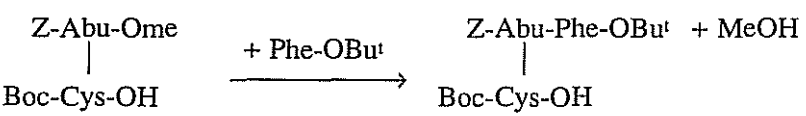
The starting Z-Abu(Boc-Cys-OH)-OMe and Z-Abu(Z-Cys-OH)-OMe cystathionine derivatives were prepared by the modified procedure [3] shown in Scheme 1.



Scheme 1.

This product was a good substrate for papain-catalyzed hydrolysis of the methyl ester at the Abu moiety at pH 9 (56% cleavage/5 min); hydrolysis of this compound at pH 4.5 was not so fast. The methyl esters Z-Abu(Z-Cys-OH)-OMe and Z-Abu(Boc-Cys-Pro-Leu-Gly-NH_2)-OMe were also cleaved by papain at pH 9 but at a slower rate. The ability of papain to recognize an ester of general structure -NH-CH(R)-CO- in the molecule of noncoded amino acids with bulky and complicated side chains (R) is surprising. Nevertheless the increase in R size had remarkable negative effects on the rate of hydrolysis.

In the reaction catalyzed by papain at pH 9 (Scheme 2) the product was isolated as a precipitate after 1 h reaction at room temperature in 56% yield.



Scheme 2.

HPLC analysis showed only a single peak of product. Amino acid analysis (cystathionine 1.00, Phe 1.03) and the presence of a molecular peak at 660 $(M + H)^+$ indicate that only the Abu carboxyl was selectively incorporated in the peptide bond. The same product was also obtained in a similar reaction under papain catalysis at pH 4.5 for 20 h at room temperature, however, the product gave a double peak on HPLC, probably due to additional incorporation of the cysteine carboxyl group in the peptide bond under the conditions of 'thermodynamic approach'. Using papain we prepared under the same conditions (pH 9, carbonate-bicarbonate buffer containing 20% DMF, 1 h) the following products: Z-Abu(Boc-Cys-OH)-Tyr-N_2H_2Ph (711 $(M + H)^+$), Z-Abu(Boc-Cys-OH)-Tyr-NH_2 (620 $(M + H)^+$) and Z-Abu(Boc-Cys-OH)-Tyr-Ile-Gln-Asn-OH (975 $(M + H)^+$). The molecular peaks of those peptides confirm that in all these instances only Abu carboxyl reacted with nucleophiles. The usefulness of some of these peptide derivatives or the application of the incorporation of cystathionine molecule in peptides by papain as a general method will be useful in the syntheses of some oxytocin and vasopressin carba analogues.

References

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