Chapter IV

Conclusion

The synthesis of a series of 13 new amino acid derivatives has been achieved by using the mixed anhydride method of Anderson and Zimmerman. One of the synthetic route started from L-valine amino acid which was converted into N-protected-L-valine with different blocking groups such as benzoyl, benzyloxycarbonyl and tertiary-butyloxycarbonyl group. In order to obtain only the L-form of the amino acid methyl ester hydrochlorides of valine, tyrosine and phenylalanine for the coupling reactions, one should be aware of many factors in each synthetic process such as pH, temperature and activation time, etc. Generally speaking, one of the most crucial problems in the peptide synthesis is the racemization. In this investigation, there was no attempt to prove the stereochemistry of the synthetic peptides, although it can be done by using a higher frequency of over 200 MHz NMR to differentiate L-form and D-form following the self-induced nonequivalent technique. Nevertheless, attempts were made to minimize the degree of racemization by controlling the essential optimum conditions especially the amount of triethylamine to be used not in excess. As a matter of fact, all the starting amino acids being used were obtained commercially in L-form and the enzyme reactions preferred

stereospecifically to L-form. Thus the final products of the enzyme inhibitors were more or less in L-form as well.

Upon the study of the enzyme inhibitions of those synthetic compounds against elastase, trypsin and chymotrypsin, various optimal conditions were carried out, such as pH, temperature, enzyme concentration and substrate concentration. It was found that the elastase was not quite stable upon storage. Although these enzymes were obtained commercially from the same company but they showed unequal activities as they lost some activities during the storage period. After all the optimal conditions for enzyme kinetic reactions were obtained, then the percentage of enzyme inhibitions were investigated. Unfortunately, the elastase gave a very low activity which was negligible. Therefore it was impossible to determine the inhibitory activities of the synthetic compounds against the elastase which might be due to its instability. However, most of the synthetic compounds showed good potential inhibitions for chymotrypsin and trypsin. Evidently, these synthetic non-specific inhibitors should not be used as antiarthritis and antiemphysema agents, in any way.

Although the attempt to find out the specific elastase inhibitors which can be used as potential antiarthritis and antiemphesema agents was not achieved but the results from this investigation can be used as a guide line for further studies in order to solve the problems occurring during the enzyme kinetic assay. In addition, the results on the

structure activity relationships in this research work can be useful for designing of the chemical structure of the synthetic enzyme inhibitors.