CHAPTER V

CONCLUSION

In the course of this research, a series of amino acid derivatives were synthesized by using the mixed anhydride method of Anderson and Zimmerman. The optimum yields were ranged between 60-90 % in high purity with a minimum degree of racemization. The results showed that the tertiary base, triethylamine, being used for mixed anhydride formation was not merely a hydrogen chloride acceptor, but it was also a good racemizer when it was used in excess. On the other hand, this base may be used without racemization if it was not in excess indicates a rapid and complete formation of the mixed anhydride in its presence.

A series of such synthetic inhibitors were tested against cathepsin G, trypsin and chymotrypsin, to determine the structural requirements necessary for specific inhibition of cathepsin G and of other serine proteases.

In the study of enzyme kinetic, each enzyme was assayed under the physiological conditions. The results showed that these chosen conditions were suitable for trypsin and chymotrypsin, but not for cathepsin G. As cathepsin G gave a very low activity which was impossible to determine the inhibitory activity. In the experiments done by both Tanaka et. al. and Nakajima et. al., regarding the determination of the specific substrates for cathepsin G, they also found the same results that cathepsin G exhibited very low reactivity toward many types of peptide

substrates and inhibitors and this particular enzyme is much less enzymatically active than many other serine proteases such as bovine pancreatic trypsin, chymotrypsin and HLE. Thus, it might be possible that the low intrinsic kinetic reactivity of cathepsin G is an inherent property of the enzyme and is related to the function of this enzyme. However, cathepsin G was found to be extremely effective at cleaving cartilage proteoglycans, fibronectin, angiotensinogen, angiotensin I and related peptide sequences in vivo with unexceptional rates, so it might be possible that this enzyme would be denatured in vitro during the time of separation.

Upon the determination of enzyme inhibition of synthetic inhibitors against trypsin and chymotrypsin, it was found that they showed a poor inhibitory activity against trypsin, but did very well against chymotrypsin. Since chymotrypsin had previously been shown to prefer aromatic amino acid residues at P_1. In addition, the results showed that the optimum length of the alkyl chain required for maximum inhibition against chymotrypsin was in the following order: 14>12>10 and that chymotrypsin preferred Phe to Tyr at P_1. In all cases, the best inhibitor against chymotrypsin in this research is Bz-Gly-Phe-NH-C_{14}.

All of these synthetic inhibitors were found to be competitive inhibitors against chymotrypsin. Considering the structure of substrate and all inhibitors as well as the preference of this enzyme with aromatic amino acid residues at P_1, it was possible that both substrate and inhibitors compete for the same binding site in the enzyme.

Although this research was not achieved to find the specific

inhibitor for cathepsin G, it gave the way for further study to find suitable conditions for studying enzyme kinetic of cathepsin G. In addition, it also gave the way to synthesize amino acid derivatives or peptides with different sequences of amino acids.