CHAPTER (I)

INTRODUCTION.
INTRODUCTION

In order to emphasize the important of amino acid derivatives, the introductory chapter highlights the plethora of amino acid derivatives that have been synthesized or isolated from natural sources. The discussion includes a review of a wide variety of uses to which these derivatives have been applied and utilized. A discussion on such derivatives which occur in nature and the role they play in biological system is included.

Unnatural and enantiomerically pure amino acids are gaining interest as components of biologically active peptides. Hruby and co-workers¹ have shown that incorporation of conformationally strained amino acids into peptides results in increased rigidity, leading to enhanced resistance towards protease enzymes. They also have different biological activity and selectivity. In addition to the 20 common amino acids, there are a number of others that have been isolated from hydrolyzates of a few specialized type of protein. All are derivatives of natural amino acids. Among these is 4-hydroxyproline, a derivative of proline, which is found in some abundance in the fibrous protein, collagen, and also in some plant proteins. Several of these unusual amino acids have been isolated and some of them are listed in scheme (I). The rare amino acids in protein are generally distinctive since there is no specific triplet code for them.
SCHEME I

Some rare amino acid derivatives that occur naturally.

In all cases, they arise by modification of their parent or precursor amino acids after these have already been incorporated into the polypeptide chain. Further, fungi and higher plants contain an extraordinary variety of amino acids, some having very curious structures. The metabolic functions of most of these specialized plant
amino acids are not yet understood, it is interesting that some plant amino acids, such as canavanine, Djenkolic acid and β-cyanoalanine are toxic to other forms of life. Some important amino acid derivatives, their biological action and synthesis are discussed as under.

(A) Haloketones derived from N-amino acids.

The specific chemical reactions carried out by enzymes occur at special locations on the protein called the active sites. The active sites are 3-dimensional structures specially adopted to bind and chemically transform their specific substrates. The active site is considered to be composed of a binding site and a catalytic site. One powerful tool for the study of enzyme active site is affinity labelling. A substrate like molecule with structural features adequate to form a complex with the enzyme under consideration similar to the enzyme-substrate complex is designed with a reactive group in its structure. Such an active site directed reagent can then covalently modify the enzymes active site upon binding.

One of the first demonstration of the usefulness of active site directed reagents for enzyme studies was the development of N-tosylphenylalaninechloromethylketone (Tos-phe-CH₂Cl) and N-tosyllysinechloromethylketone (Tos-Lys-CH₂Cl) as specific site directed reagents for chymotrypsin and trypsin respectively.
With these compounds it was possible to identify a unique histidine residue in the active site of these enzymes. Similar studies with other substrate related inhibitors have provided numerous such active site directed reagents. Haloketone derivatives of aminoacids have not only been used to characterize the functional groups present in enzyme active sites, they have also been used in X-ray crystallographic studies to map the three dimensional structure of the substrate binding site in several enzymes. Related works have proven them to be powerful tools for the study of the biological functions of the proteases. They are also expected to find use in the design of new and very selective drugs.

The α-haloketones of N-blocked amino acids are generally synthesized via the initial formation of the diazomethylketones. The initial synthesis of diazomethylketones utilized the acid chlorides of the N-blocked amino acids. However, in an attempt to synthesize the diazomethylketone starting from hippurylchloride, oxazolone formation was found to be predominant as shown in scheme (II).

\[ \text{Scheme II} \]

It was later observed that in disubstituted N-blocked amino acids, the formation of the oxazolones could be avoided, notably, phthaloyl
blocked amino acid; chlorides react with diazomethane and diazoethane to yield the diazomethylketone and diazoethylketone in good yield with no oxazolone formation$^8$-$^{10}$.

Subsequently, tosyl$^{11}$ and benzyloxycarbonyl aminoacyl chlorides have been used for the synthesis of the corresponding diazomethyl ketones. Finally, the $\alpha$-halomethylketones were prepared from the diazomethyl precursors by treatment with appropriate mineral acid (HCl, HBr etc.$^{12}$). The overall transformation is written as shown in scheme (III).

\begin{align*}
\text{Scheme III} \\
\text{Ph-N} & \quad \text{R} \quad \text{Cl} \quad \overset{\text{CH}_2\text{N}_2}{\longrightarrow} \quad \text{Ph-N} \quad \text{R} \quad \text{CH}_2\text{N}_2 \\
& \quad \overset{\text{H}X}{\longrightarrow} \quad \text{Ph-N} \quad \text{R} \quad \text{CH}_2X
\end{align*}

A close similarity of the structure of a halomethylketone inhibitor to that of a small synthetic substrate for a particular proteolytic enzyme leads to the ready understanding of the course of the inhibitor reaction.

The mechanism of inhibition of serine proteases by several chloromethylketones has been shown to involve initial formation of a non covalently bound complex (I). The histidine residue could then displace chloride from the inhibitor to yield (II) that could yield the
hemiketal (IV). Alternatively the serine of the enzyme may first add to the carbonyl group of the inhibitor to produce the tetrahedral complex (III). This mechanism was first proposed on the basis of the kinetic studies with Tos-Phe-CH₂Cl¹³.

The formation of the tetrahedral complex (III) is probably an absolute requirement for inhibition since anhydrochymotrypsin which cannot form a hemiketal of this type is not inhibited by Tos-Phe-CH₂Cl even though it binds to the substrates with an affinity comparable to the native enzyme.

Some of the reported enzyme inhibition studies with the α-haloketone inhibitors are as under.
1. Chymotrypsin is inhibited by Tos-Phe-CH₂Cl¹⁴.
2. Trypsin inhibited by Arg-CH₂Cl¹⁵.
3. Papain is inhibited by Tos-Phe-CH₂Cl, Tos-gly-CH₂Cl and Tos-Lys-CH₂Cl and a variety of Peptide Chloromethylketone¹⁶,¹⁷.

In another interesting study it has been observed that not only the α-haloketone but also the diazomethylketones are capable of inhibiting enzyme action of, especially, the thiol protease. For example, the carbobenzoxyphenylalanine analogs reacts stoichiometrically with the active centre of cysteine residue of papain. The mechanism of inhibition is shown in scheme (V).

![Scheme V](image-url)
(B) The amino acid derivatives with unsaturated side chains.

Amino acid derivatives with unsaturated side chains are a class of important compounds which occur in nature and has a variety of functions. Important among this group of compounds are the $\beta\gamma$-unsaturated amino acids and $\gamma\delta$ – unsaturated amino acids.

The $\beta\gamma$-unsaturated amino acids:

These compounds are also known as the vinylglycine. These compounds from both natural and synthetic sources have proven antimicrobial\textsuperscript{18,19,20} and enzyme inhibitory properties\textsuperscript{21}. Several $\beta\gamma$ – unsaturated amino acids have been shown to act as suicide inactivators of several vitamin-B$_6$ dependent enzymes notable among them are alanine-$\gamma$-racemase, glutamate-aspartate transaminase. The $\beta\gamma$-unsaturated amino acids are also useful synthetic intermediates\textsuperscript{22} and biochemical probes\textsuperscript{23}. The general structure of these amino acids is shown in scheme (VI).

Scheme VI

\[
\begin{align*}
&\text{Scheme VI} \\
&\begin{array}{c}
\text{I} \\
\text{II}
\end{array}
\end{align*}
\]
The closely related unstable amino acid namely ethynyl glycine (II) has been isolated from *streptomyces-sp* and has also been shown to possess antimicrobial activity as well as a suicide inhibitor of alanine rasemase.

Synthesis of vinylglycine and its analogs in the optically active form appears to be difficult primarily due to the lability of these substances towards racemization and its susceptibility towards rearrangement. The first asymmetric, stereodefined and practical method of synthesis of E-vinyl glycine derivatives is reported by Williams and Zhai and shown in scheme (VII).

Scheme (VII)
As shown in the scheme above, the bromoglycinate derivative (II) couples with trialkyltin acetylide under mild conditions to give the crystalline product (III). Treatment of these alkynes with sodium metal in liquid NH₃ / THF containing ethyl alcohol followed by a simple aqueous wash and filtration furnishes good yield of the N-t Boc- protected βγ-unsaturated amino acid (IV).

In the 1970, it was observed that βγ-unsaturated amino acids and γ-acetylenic amino acids having a leaving group at β-position are potentially good inhibitors of pyridoxal – dependent enzymes involved in amino acid metabolism.

(C) N-alkylated amino acids:

N-alkyl-L-aminoacid and more particularly N-methyl-L-amino acids are important building blocks for peptide and depsipeptide antibiotics. The antibiotic etamycin which possess interesting properties and activity against gram positive organism is reported to contain β-N-dimethylleucine as one of the amino acid residues. Further studies on the antibiotic E129 (Ostreogycrin) complex indicated the presence of further two N-alkylated amino acids namely N-methyl phenylalanine and p-dimethylamino-N-methylphenylalanine. The N-alkylated amino acid residue in peptide results in imparting rigidity to the peptide chain and makes them less susceptible to
hydrolytic degradation by the usual hydrolytic enzymes. At present hardly three methods \(^{27,28,29}\) are known for the preparation of N-methylamino acid derivatives and their peptides. A method for the preparation of N-methylaminoacid is reported by Belagalli et.al.\(^{30}\) and the different steps of which are given in scheme (VIII).

Although, preparation of optically inactive or racemic compounds present very little problem, synthesis of the enantiomerically pure N-alkylaminoacid is laborious and provides unsatisfactory yield. One possible route to enantiomerically pure N-alkylaminoacid was recently developed by Groeger et al.\(^{31}\). This method involves the synthesis of a racemic N-acetyl-N-alkylaminoacid either from the condensation of an aldehyde, an amine and hydrocyanic acid followed by acid hydrolysis of the cyanoamine or by ring opening reaction of the appropriate N-chloroacetyl derivative of azetidine-2-carboxylic acid, corresponding pipecolic acid etc. followed by resolution of the racemic mixture obtained using an aminoacylase enzymes from \textit{Aspergillus Oryzae} or procine kidney. The enzymic hydrolysis resulted in a 99.8% purity of the L-isomer of N-alkylated amino acid. The reaction sequence is shown in scheme (IX).
Scheme VIII

\[
\begin{align*}
\text{OH} & \quad \text{a} \quad \text{HCl (g), MeOH, } b = (\text{BOC})_2 \text{O, Et}_3 \text{N, CH}_2\text{Cl}_2 \\
\text{H}_2\text{N} & \quad \text{H} \quad \text{COOH} & \quad \text{OH} & \quad \text{Cl}\text{H}_3\text{N} \quad \text{COOMe} \\
\text{t Boc - N} & \quad \text{b} \quad \text{t Boc - N} \quad \text{c} \quad \text{OMe} \quad \text{OMe} \quad \text{Me} \quad \text{t Boc - N} \quad \text{COOMe} \\
\text{OMe} & \quad \text{d} \quad \text{OMe} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{t Boc - N} \quad \text{COOMe}
\end{align*}
\]

\text{a = HCl (g), MeOH, } b = (\text{BOC})_2 \text{O, Et}_3 \text{N, CH}_2\text{Cl}_2 \\
\text{c = Sod. hexamethyl disilazide (NaHMDS), MeI, THF} \\
\text{d = TFA, CHCl}_3.
**Scheme IX**

\[
\text{Ia = N-Chloro acetyl derivative of azetidine-2-carboxylic acid} \\
\text{Ib = N-Chloro acetyl derivative of proline} \\
\text{Ic = N-Chloro acetyl derivative of pipecolic acid} \\
\]
(D) Assymetric synthesis using amino acids as chiral auxiliaries

Alongside enzymatic methods, stereoselectivity in organic synthesis can be achieved by using chiral auxiliaries. These auxiliaries can either be covalently bound to the substrate (substrate control) or can be part of the reagent (reagent control). Often these chiral auxiliaries are built from amino acids. Amino acids are well suited for this purpose since most of them are cheap and commercially available. In addition, the variety of amino acids provide the opportunity of tuning additives for special purposes. Attempt is made here to give a brief introduction to the successful application of amino acids as stoichiometric "chiral inductors" in some organic reactions.

Some important amino acids which are frequently used as a building blocks for the preparation of auxiliaries are presented in scheme (X).
(i) Amino acids as chiral auxiliaries in reactions $\alpha$ to a Carbonyl group:

The condensation of secondary amines with ketones affords enamines which react with electrophiles to provide $\alpha$-alkylated iminium salts. The iminium salts can readily be hydrolyzed to the corresponding ketones. In 1969 Yomada et. al. reported the first stereoselective transformation of an amino acid derived chiral enamine $^{32}$.

In contrast to the modest chemical yields obtained, considerable selectivities were observed for the reaction of proline derivatives,
(scheme XI \( R' = \text{COOMe, COOEt-Bu} \)) with \( \alpha\beta \) unsaturated nitriles and esters. The ketones obtained after hydrolysis were isolated with 43–59% ee. The analogous reaction with alkylhalides such as methyl iodide, ethyl iodide, alkylbromide and bromomethyl acetate, proceeded with lower selectivities (< 30% ee). Different amino acid derived organotin enamines were used by De Jeso et. al. for 1,4 addition to methylacrylate and acrylonitrile. These addition reactions proceeded with moderate to excellent selectivity (up to 98% ee). The enamine derived from proline allylester and \( \alpha \) - phenylpropanal has been used in Pd-catalyzed asymmetric allylation. After hydrolysis the corresponding \( \alpha \) - alkylates aldehyde was obtained with high selectivity (90%ee).

**Scheme XI**

\[
\begin{align*}
\text{I} & \quad R' = \text{COOMe, COOEt} \\
\text{II} & \quad R' = \text{Et, CH}_2\text{Ph}
\end{align*}
\]
In 1976, Enders et al. introduced (s)-1-amino-2-methoxymethylpyrrolidine (SAMP) a chiral hydrazine derived from proline. The enantiomers of this auxiliary (RAMP) is accessible from glutamic acid after a multiple step synthesis. These chiral auxiliaries can easily be condensed with chiral aldehydes or ketones to form the corresponding hydrazones. Diastereoselective alkylation and subsequent cleavage of the auxiliary provides α-alkylated aldehydes, ketones or amines in high optical purity. Scheme (XII) shows the steps of this enantioselective alkylation and the variety of products that are accessible in high optical purity by using the methodology. The synthetic potential of these SAMP / RAMP hydrazones has been summarized in a review by Enders.

**Scheme XII**
An interesting strategy for asymmetric synthesis with the aid of amino acids was described by Seebach et al. In this method, called the "Self-regeneration of the stereogenic centre" (SRSC), an α- or β-amino acid is reacted with an aldehyde to selectively form one of the two possible diastereomeric acetals (Scheme XIII). Hydroxy and mercapto acids can also be used for the acetal formation. In the second step, the original stereogenic centre derived from the amino acid is eliminated. Electrophilic, nucleophilic or radical addition to the newly formed trigonal centre proceeds with high selectivity directed by the substituent R at the acetal centre. Hydrolysis of the modified acetals provides the corresponding acid in enantiomerically pure form. Thus, in the overall transformation, a substituent $R^2$ was introduced enantioselectively at the α-position of the starting acid. Since the amino acid is only used for the formation of the effective stereodirecting unit (acetal centre), the SCRC can be considered as an indirect way of performing asymmetric synthesis with the aid of amino acids. In scheme XIII, the general principle of the SCRC and an example of its application for the preparation of α-methylphenylalanine is presented.
Specific application in the synthesis of α-methylphenylalanine.
These are only a few examples which exemplifies the utility of amino acids and their derivatives as chiral auxiliaries. Literature is replete with such examples. Research in this field has not yet reached a saturation point and this field is still wide open for exploratory work.

**(E) Schiff Base and other derivatives of amino acid esters and their synthetic utility:***

New routes for the synthesis of amino acids both natural and unnatural, continue to present a challenge to the organic chemist. \(\alpha\) - functionalized, \(\alpha\) - amino acids can generally be obtained via the anionic and cationic amino amino acid equivalents shown below.

\[
\begin{align*}
R-C-CO\overset{\varnothing}{\equiv}O H & \quad R-C-COOH \\
NH_2 & \quad NH_2 \\
\text{anionic} & \quad \text{cationic}
\end{align*}
\]

These cationic and anionic equivalents can be obtained as an intermediate via the Schiff base derivatives of the corresponding amino acid esters.

Carbon-carbon bond formation involving the reaction of the anionic synthon of glycine or the higher amino acids with electrophiles have been utilized by numerous groups for the preparation of amino acids \(^{38-42}\). A general method is given in scheme XIV.
The same method had been applied using a phase transfer catalyst (PTC)\textsuperscript{42} for generation of the anionic equivalent. The synthesis of amino acids involving cationic amino acid equivalents is not as well developed as that using their anionic counterpart.\textsuperscript{1} The cation equivalent of glycine 1 (Scheme XV) represents a polarity reversed reagent which can be reacted with nucleophiles to yield derivatives of higher amino acids. A number of interesting amino acid derivatives which are accessible using this strategy are generally difficult to prepare via the corresponding anionic equivalent because of inaccessibility of the requisite electrophiles. The different products are obtained via the cationic equivalent is shown in scheme XV.

Scheme XIV

\[
\begin{align*}
\text{Ph}_2\text{C} = \text{N} - \text{CH}_2 - \text{COOEt} & \xrightarrow{(1) \text{basic}} \text{R} - \text{CH} - \text{COOH} \\
\text{Ph}_2\text{C} = \text{N} - \text{CH}_2 - \text{CN} & \xrightarrow{(2) \text{RX}} \text{NH}_2 \\
& \xrightarrow{(3) \text{deprotection}} 
\end{align*}
\]

with charged nucleophiles

Scheme XV

with neutral nucleophiles
The products could be prepared using either charged or neutral
nucleophiles. Thus aryl, βγ - unsaturated amino acids , as well as
β-quarternary substituted amino acids could be prepared by reacting
appropriate carbanionic species with the synthon 1 while neutral
nucleophiles, such as heteroatom nucleophiles (alcohols, thiols etc) or
activated aromatics could serve as precursors for preparation of
substituted amino acids. The discovery that a variety of
N-acylamino acetates 1 (Scheme XVI) can be brominated smoothly
to yield the α - bromo derivative(2) has made this group of glycine
equivalents easily available \(^{43,44}\). On treatment of 2 with tert. amines
or an excess of organometallic reagents, highly reactive
α - acyliminoacetate, 3, are formed in situ which reacts with a number of
C-nucleophiles to yield N-protected α - aminoacid esters.

Scheme XVI

\[
\begin{align*}
\text{R}^1 \text{H} & \quad \text{COOR}^2 \quad \text{Br}_2 \text{or} \quad \text{NBS} \quad \text{hv} \quad \text{R}^1 \text{H} \quad \text{COOR}^2 \quad \text{Br} \\
1 & \quad 2 \quad 3 \\
\text{1} \quad \text{C} - \text{Nucleophiles} & \quad \text{Nu} \quad 1} \quad \text{Nu} \quad \text{Nu} \quad \text{Nu} \\
2 \quad \text{H}_2\text{O}^+ & \quad \text{R}^1 \text{H} \quad \text{COOR}^2 \quad \text{H}_3\text{N}^+ \quad \text{COO}^- \\
\end{align*}
\]
The reaction has been carried out, so far, with Grignard reagents and enamines which are good sources of Carbon nucleophiles.

In a major modification of this method of synthesis, Bretschneider et.al. introduced the use of mixed cuprate reagents as sources of carbon nucleophiles to prepare a variety of non natural amino acids. They have observed that the reaction of two equivalents of cuprate reagent with one equivalent of 2-acylamino-2-bromoacetate in THF at -78 °C leads to the formation of the desired amino acid derivative in good yield. In this way not only alkyl but also alkenyl and aryl groups can be transferred to the N-acylimino acetates.

Scheme XVII

(F) The oxazolones and their synthetic uses.

The oxazolones or more specifically, oxazol-5-ones are important derivative of amino acids. The oxazolones are versatile heterocyclic intermediates which can be used for the synthesis of a variety of organic compounds. They have been used for the synthesis of higher amino acids from glycines. To illustrate the sequence in the preparation
of phenylalanine from glycine a temporary construction of the oxazolone ring system is necessary as shown in scheme XVIII.

Scheme XVIII

By condensing benzaldehyde with the oxazolone I the benzylidene derivative is formed which is reduced and cleaved using phosphorus and hydrogen iodide to give the desired amino acid. The oxazolones intermediates are also used for the homologation of Carboxylic acids by a -R- group containing three carbon atoms. The sequence of reaction is shown in scheme XIX.
The chemistry of the oxazolones and their synthetic utility is discussed in detail in chapters IV and V.

(G) Some other amino acid derivatives which have been synthesized or isolated from natural sources and which have very useful biological and physiological properties are briefly mentioned below.

(1) Amino acid derivatives having a four membered ring occur in sugar beets and in several marine algae. Studies have revealed that they are biosynthesized from methionine precursors $^{47}$. Some such derivatives are given in scheme XX.
(2) Some amino acid derivatives notably the coumaryl derivatives, have been synthesized and extensively used as intrinsic probe for fluorescence labelling and quantitation of peptides and proteins. Notably L-(6,7-dimethoxy-4-coumaryl)alanine have been synthesized and applied to such uses. 48

(3) Synthesis of α-hydroxy-β-amino acids is a very important field of research. These compounds have been found to show enzyme inhibitory properties. Among the different α-hydroxy-β-amino acids synthesized, the most important are Statine, Bestatine and phenylisoserine whose structures are given in scheme XXI. Statine is recognised as an inhibitor of proteolytic enzyme. Bestatine is a potent inhibitor of leucine aminopeptidase and amino peptidase-β and finally phenylisoserine which is present in the side chain of taxol contributes to the antitumor activity of that compound.
The review given in this chapter indicates some of the variety of uses to which amino acid derivatives have been applied. However, this review is by no means exhaustive and does very little justice to this important field of research. Only a few derivatives have been mentioned and only a few aspects of their reactivity is highlighted.

It will therefore be appropriate to say that this review is at best a very brief introduction. Several researchers are engaged in intensive research and many new developments finds mention in present day literature of chemical and biological sciences.
References: