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PEPTIDES ANTIMICROBIAL METAL COMPLEXES: A REVIEW

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ABSTRACT

There has always been a search for the newer antibiotics to face the various infectious diseases, which lead to the discovery of a wide range of the antibacterial drugs with a great structural diversity. Medicinal chemist and microbiologists are still seeking for new and improved antibacterial drugs. One of the main reasons is the development of resistance for the antibiotics. Structural modifications of antibiotics are the usual approach to solve the problem. Peptides, as being the part of biological systems, have always been the centre of attraction. One of the strategies could be the attachment of various peptide moieties to the antibiotics. Rare work has been done in this area to the best of our knowledge.

So a review is required for the historical background of the peptide and various methods of peptide synthesis and study so far done in this area to get new effective peptide anti-microbial.

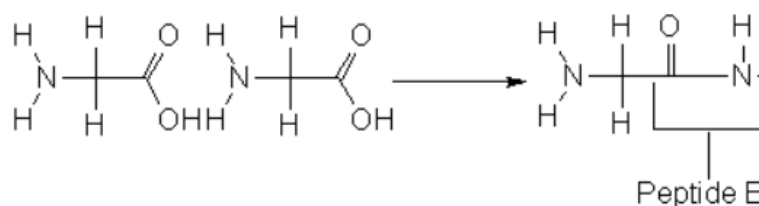
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INTRODUCTION

Peptides are chains of amino acids, in which amino acids are linked with each other with a 'Peptide Bond'. The peptide bond is an amide bond (-CONH-) which is formed by condensation of amino group of an amino acid with the carboxyl group of another amino acid, leaving a molecule of water. Thus two amino acids are joined with each other and in the same way other amino acids can be joined to the chain to form longer chain of amino acids. Peptides are one of the basic biological building blocks like proteins, and they occur naturally in all forms of life. In the human body, peptides perform a wide variety of important functions, including hormonal regulation and defence against infection etc.

A peptide bond is a covalent bond that is formed between two molecules when the carboxyl group of one molecule reacts with the

amino group of the other molecule, releasing a molecule of water. This is a condensation reaction and usually occurs between amino acids. The resulting CO-NH bond is called a peptide bond, and the resulting molecule is an amide. To form a peptide bond, two amino acids must be orientated so that the carboxylic acid group of one can react with the amino group of the other. For example, two amino acids (glycine) combining through the formation of a peptide bond to form a dipeptide.



A number of hormones, antibiotics, antitumor agents and neurotransmitters are peptides (proteins). A peptide bond can be broken down by hydrolysis. The peptide bonds

that are formed within proteins have a tendency to break spontaneously when subjected to the presence of water releasing about 10 kJ/mol of free energy. Such types of bonds are known as Metastable Bonds. This process, however, is very slow. Living organisms use enzymes either to break down or to form peptide bonds. The wavelength of absorbance for a peptide bond is 190-230 nm. X-ray diffraction studies of crystals of small peptides by Linus Pauling and R. B. Corey indicated that the peptide bond is rigid and planer. Pauling pointed out that this is largely due to the ability of the amide nitrogen to delocalize its lone pair of electrons onto the carbonyl oxygen. Because of this resonance, the C=O bond is actually longer than normal carbonyl bonds, and the N-C bond of the peptide bond is shorter than the N-C α bond. The carbonyl oxygen and amide hydrogen are in a trans configuration, rather than a cis configuration. Trans-configuration is energetically more favorable because of possible steric interactions in cis-configuration. Peptides are involved into many processes in the living organisms and it is possible to

classify them on the basis of their function. But this type of classification cannot be considered as a perfect classification, because some of the peptides can simultaneously belong to different groups. For example, Oxytocin can be considered as a hormone (transmitting signal between cells) and also oxytocin can be classified as neuropeptide because it can function as a neurotransmitter in the brain.

Hormones- Hormones are involved into carrying signals between cells. Classical examples of hormones are: bradykinin, gastrin, oxytocin etc.

1. Neuropeptides- Neuropeptides are found in neural tissues. Usually these peptides are produced in the brain and are involved into regulatory and signalling processes. Classical examples of neuropeptides are: endorphins, vasopressin, atrial-natriuretic peptide etc.

2. Alkaloids- Alkaloids are peptides, usually obtained from plants, fungi and some animals like shellfish. Alkaloids are involved to defend one organism from consuming by other organisms. Classical examples of

peptide alkaloids are: ergotamine, pandamine, dynorphinA-(1-8)-octapeptide, Nbeta-(D-Leu-D-Arg-D-Arg-D-Leu-D-Phe)-naltrexamine, etc.

3. **Antibiotics.** Antibiotics inhibit the growth of micro organisms, usually bacterial cells and locationally fungi and protozoa. Classical examples of peptide antibiotics are: tyrothricin, bacitracin, gramicidin, valinomycin etc.
4. **Toxins.** Toxin is a poison substance. Peptide toxins are the most poisonous substances. Examples of peptide toxins are: palutoxins, agatoxins, curtatoxins etc.
5. **Regulation peptides.** The group of regulation peptides is not well defined because almost any peptides can regulate some processes in organisms, but this group is used to classify peptides which do not clearly belong to other groups. Examples of regulatory peptides are: anserine, carnosine, etc.

A) According to their biological synthesis-

Peptides can be classified as

1. **Ribosomal peptides.** Ribosomal peptides (almost all known peptides) are synthesized

by translation of mRNA on ribosomes.

Usually they are subjected to further post translational modification, which can even involve the racemization of L-amino acids to D-amino acids.

2. **Non ribosomal peptides.** Non ribosomal peptides are synthesized during enzymatic catalysis. For e.g. glutathione cyclo-peptides etc.
3. **Peptones.** Peptones are peptides derived from digestion processes.

SYNTHETIC PEPTIDES

Synthetic peptides have two main uses: as peptide drugs and as peptides for diagnostic purposes.

Peptide drugs

Peptide drugs are either naturally-occurring peptides or altered natural peptides. There are many naturally occurring peptides that are biologically active. If a patient does not naturally produce a peptide that he needs, this peptide can be synthesised and given to him. In addition, the amino acids in an active peptide can be altered to make analogues of the original

peptide. If the analogue is more biochemically active than the original peptide, then it is known as an agonist and if it has the reverse effect is known as an antagonist. Contraceptives have been made by synthesising the antagonists of fertility peptides.

Diagnostic peptides

Peptides can be designed to change colour under certain conditions, and these can be used for diagnostic purposes. For example, a chromogenic peptide substrate can readily detect the presence, absence and varying blood levels of enzymes that control blood pressure and blood clotting ability. Diagnostic peptides have been used for medical research into different areas such as heart disease, leprosy and tuberculosis.

SYNTHESIS OF PEPTIDES

Peptide synthesis is a chemical process of coupling of the carboxyl group of one amino acid to the amino group of another amino acid. Usually chemical techniques are used to synthesize peptides of up to 30-40 amino acids length. The first peptide synthesis was carried out by T. Curtius in 1882 via reaction between

benzyl chloride and silver salt of glycine. During this process Curtius produced crystals of N-benzyl-glycyl-glycine. The first pure di-peptide (Gly-Gly) was synthesized by E. Fischer in 1901. In 1932, Bergmann from Fisher's lab discovered carbobenzyloxy group $C_6H_5CH_2OCO$ which is very convenient as a protective group for peptide synthesis. In 1950-1960 the first biological peptides, like oxytocin, vasopressin, insulin and others were synthesized.

([www.organic chemistry resources.com](http://www.organicchemistryresources.com))

CLASSIFICATION OF PEPTIDE SYNTHESIS

([www.peptide guide.com](http://www.peptideguide.com))

Peptide synthesis process can be classified on the basis of used techniques and type of the final product.

1) Liquid-phase peptide synthesis- Liquid-phase peptide synthesis is a classical approach to peptide synthesis. It has been replaced in most labs by solid-phase synthesis. Liquid-phase or classical peptide synthesis can be divided into two classes viz. step-by-step peptide synthesis with subsequent adding of one amino acid at ones from C-terminal to N-terminal and

'block-synthesis' with coupling of polypeptide fragments. Liquid-phase peptide synthesis is used in large-scale peptide production for industry.

2) Solid-phase peptide synthesis-Solid-phase peptide synthesis (SPPS), pioneered by Merrifield, resulted in a paradigm shift within the peptide synthesis community. It is now the accepted method for creating peptides and proteins in the lab in a synthetic manner. SPPS allows the synthesis of natural peptides which are difficult to express in bacteria, the incorporation of unnatural amino acids, peptide/protein backbone modification, and the synthesis of D-proteins, which consist of D-amino acids. Small solid beads, insoluble, yet porous, are treated with functional units ('linkers') on which peptide chains can be built. The peptide will remain covalently attached to the bead until cleaved from it by a reagent such as trifluoroacetic acid. The peptide is thus 'immobilized' on the solid-phase and can be retained during a filtration process, whereas liquid-phase reagents and by-products of synthesis are flushed away. The

general principle of SPPS is one of repeated cycles of coupling-deprotection. The free N-terminal amino group of a solid-phase attached peptide is coupled to a single N-protected amino acid unit. This unit is then deprotected, revealing a new N-terminal amine to which a further amino acid may be attached. The overwhelmingly important consideration is to generate extremely high yield in each step. For example, if each coupling step were to have 99% yield, a 26-amino acid peptide would be synthesized in 77% final yield (assuming 100% yield in each deprotection); if each step were 95%, it would be synthesized in 25% yield. Thus each amino acid is added in major excess (2~10x) and coupling amino acids together is highly optimized by a series of well-characterized agents. There are two majorly used forms of SPPS – **Fmoc Synthesis** and **Boc Synthesis**. Unlike ribosome protein synthesis, solid-phase peptide synthesis proceeds in a C-terminal to N-terminal fashion. The N-terminal of amino acid monomers is protected by these two groups and added onto a deprotected

amino acid chain. Automated synthesizers are available for both techniques, though many research groups continue to perform SPPS manually. SPPS is limited by yields, and typically peptides and proteins in the range of 70~100 amino acids are pushing the limits of synthetic accessibility. Synthetic difficulty also is sequence dependent; typically amyloid peptides and proteins are difficult to make. Longer lengths can be accessed by using native chemical ligation to couple two peptides together with quantitative yields. Solid-phase peptide synthesis is perfect for research laboratories and small quantities of production.

Boc Solid Phase Peptide Synthesis

When R. B. Merrifield invented SPPS in 1963, it was according to the tBoc method. tBoc (or Boc) stands for tert-Butyl oxy carbonyl. To remove Boc from a growing peptide chain, acidic conditions, usually neat TFA, are used. Removal of side-chain protecting groups and the peptide from the resin at the end of the synthesis is achieved by incubating in

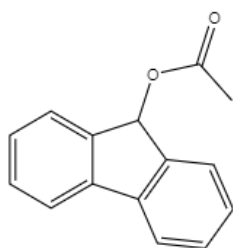
hydrofluoric acid, which can be dangerous. So for this reason Boc chemistry is generally disfavored. However for complex syntheses Boc is favourable. When synthesizing nonnatural peptide analogs which are base-sensitive, Boc is necessary.

Fmoc Solid Phase Peptide Synthesis

This method was introduced by Carpino in 1972 and further applied by Atherton in 1978. Fmoc stands for (F)luorenyl-(m)eth(o)xy-(c)arbonyl which describes the Fmoc protecting group, first described as a protecting group by Carpino in 1970. To remove an Fmoc from a growing peptide chain, basic conditions, usually 20% piperidine in DMF, are used. Removal of side-chain protecting groups and peptide from the resin is achieved by incubating in trifluoroacetic acid (TFA), deionized water, and triisopropylsilane. Fmoc deprotection is usually slow because the anionic nitrogen produced at the end is not a particularly favorable product, although the whole process is thermodynamically driven by the evolution of carbon dioxide. The main advantage of Fmoc

chemistry is that no hydrofluoric acid is needed.

It is therefore used for most routine synthesis.



9-Fluorenyl methoxy carbonyl (Fmoc)group

BOP SPPS

The use of BOP reagent was first described by Castro *et al* in 1975.

3) Homo-polymerisation. Homo-polymerisation is used to synthesize homo-polymeric chains of amino acids.

4) Enzymatic peptide synthesis. Enzymatic peptide synthesis is based on enzymes which are able to form peptide bond. Unfortunately this technique is very complicated and there is no remarkable result achieved yet.

5) Partial peptide synthesis. In partial peptide synthesis technique the natural peptides and proteins are used as a source of peptide fragments.

6) Cyclopeptide synthesis. Cyclopeptide synthesis is the cyclization of linear peptides.

7) Non-standard peptide synthesis. Non-standard peptide synthesis is used to produce peptides with non-standard peptide bonds, for example ester-bonds etc.

SOLID SUPPORTS

The physical properties of the solid support, and the applications to which it can be utilized, vary with the material from which the support is constructed, the amount of cross linking, as well as the linker and handle being used.

Polystyrene resin

This is a versatile resin, which is quite useful in multi-well, automated peptide synthesis, due to its minimal swelling in DCM.

Polyamide resin

This too is a useful and versatile resin. It seems to swell much more than polystyrene, in which case it may not be suitable for some automated synthesizers, if the wells are too small.

PROTECTING GROUPS IN PEPTIDE SYNTHESIS

Due to amino acid excesses used to ensure complete coupling during each synthesis step, polymerization of amino acids is common in reactions where each amino acid is not protected. In order to prevent this polymerization, protecting groups are used. This adds additional deprotection phases to the synthesis reaction, creating a repeating design flow as follows:

1. Protective group is removed from trailing amino acids in a deprotection reaction.
2. Deprotection reagents washed away to provide clean coupling environment.
3. Protected amino acids dissolved in a solvent such as dimethylformamide (DMF) are combined with coupling reagents are pumped through the synthesis column.
4. Coupling reagents washed away to provide clean deprotection environment.

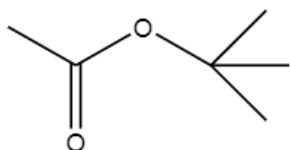
Currently, two protective groups (Fmoc, Boc) are commonly used in solid-phase peptide synthesis. Their lability is caused by the carbamate group which readily releases CO₂ for an irreversible decoupling step.

Fmoc protective group

The Fmoc (9-fluorenylmethyl carbamate) is currently a widely used protective group that is generally removed from the N terminus of a peptide in the iterative synthesis of a peptide from amino acid units. The advantage of Fmoc is that it is cleaved under very mild basic conditions (e.g. piperidine), but stable under acidic conditions. This allows mild acid labile protecting groups that are stable under basic conditions, such as Boc and benzyl groups, to be used on the side-chains of amino acid residues of the target peptide. This orthogonal protecting group strategy is common in the art of organic synthesis. FMOC is preferred over BOC due to ease of cleavage; however it is less atom-economical, as the fluorenyl group is much larger than the tert-butyl group. Accordingly, prices for FMOC amino acids were high until the large-scale piloting of one of the first synthesized peptide drugs, enfuvirtide, began in the 1990s, when market demand adjusted the relative prices of the two sets of amino acids.

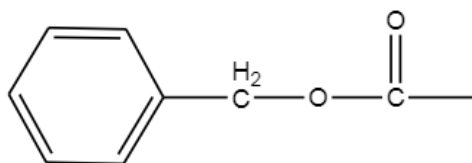
Boc protective group

Before the Fmoc group became popular, the Boc group was commonly used for protecting the terminal amine of the peptide, requiring the use of more acid stable groups for side chain protection in orthogonal strategies. It retains usefulness in reducing aggregation of peptides during synthesis. Boc groups can be added to amino acids with boc anhydride and a suitable base.^[12]



Benzyloxy-carbonyl (Z) group

Another carbamate based group is the benzyloxy-carbonyl (Z) group. It is removed in harsher conditions: HBr/acetic acid or catalytic hydrogenation. Today it is almost exclusively used for side chain protection.



Alloc protecting group

The allyloxycarbonyl (alloc) protecting group is often used to protect a carboxylic acid, hydroxyl, or amino group when an orthogonal deprotection scheme is required. It is

sometimes used when conducting on-resin cyclic peptide formation, where the peptide is linked to the resin by a side-chain functional group. The alloc group can be removed using tetrakis (triphenylphosphine) palladium(0) along with a 37:2:1 mixture of chloroform, acetic acid, and N-methylmorpholine (NMM) for 2 hours. The resin must then be carefully washed 0.5% DIPEA in DMF, 3x10 ml of 0.5% sodium diethylthiocarbamate in DMF, and then 5x10 ml of 1:1 DCM: DMF.

Lithographic protecting groups

For special applications like protein microarrays lithographic protecting groups are used. Those groups can be removed through exposure to light.

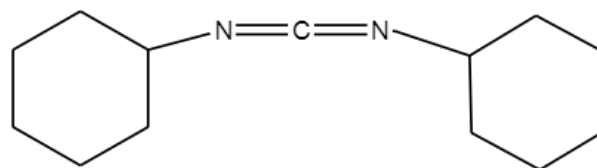
COUPLING REAGENTS IN PEPTIDE SYNTHESIS

4.5.1 Carbodiimides

These activating agents were first developed. Most common are dicyclohexylcarbodiimide (DCC) and diisopropylcarbodiimide (DIC). Reaction with a carboxylic acid yields a highly reactive O-acyl-urea. During artificial protein synthesis (such as Fmoc solid-state

synthesizers), the C-terminus is often used as the attachment site on which the amino acid monomers are added. To enhance the electrophilicity of carboxylate group, the negatively charged oxygen must first be "activated" into a better leaving group. DCC is used for this purpose. The negatively charged oxygen will act as a nucleophile, attacking the central carbon in DCC. DCC is temporarily attached to the former carboxylate group (which is now an ester group), making nucleophilic attack by an amino group (on the attaching amino acid) to the former C-terminus (carbonyl group) more efficient. The problem with carbodiimides is that they are too reactive and that they can therefore cause racemisation of the amino acid. Dicyclohexylcarbodiimide (DCC) and diisopropylcarbodiimide (DIC) are commonly used to prepare amides, esters and acid anhydrides from carboxylic acids. These reagents can also convert primary amides to nitriles, which can be useful in organic synthesis but is a troublesome side reaction of asparagine and glutamine residues in peptide synthesis. Dicyclohexylurea, the byproduct formed from DCC, is nearly insoluble in most organic solvents

and precipitates from the reaction mixture as the reaction progresses. Hence DCC is very useful in solution phase reactions, but is not appropriate for reactions on resin. DIC is used instead in solid phase synthesis since the urea byproduct is more soluble and will remain in solution. In certain applications, such as modifying proteins, ethyl-(N, N- dimethylamino) propylcarbodiimide hydrochloride (EDC) is used. This carbodiimide reagent and its urea by-product are water soluble, so the byproduct and any excess reagent are removed by aqueous extraction. Carbodiimide activation of amino acid derivatives often causes a partial racemization of the amino acid. In peptide synthesis, adding an equivalent of 1-hydroxybenzotriazole (HOBt) minimizes this problem.



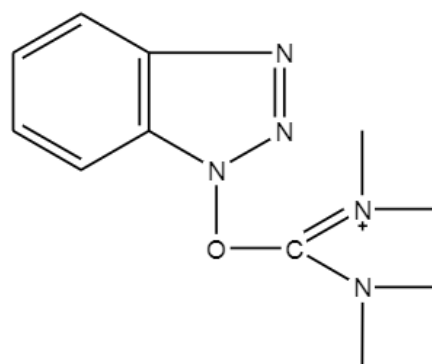
Phosponium-Based Reagents

To avoid the racemization and side reactions that can occur with carbodiimide reagents, many alternative reagents were developed to

generate OBt esters in situ. (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) is one of the first reagents developed. BOP does not generate asparagine and glutamine dehydration by products and racemization is minimal. BOP is also useful for preparing esters under mild conditions. It must be handled with caution as highly carcinogenic hexamethylphosphoramide is formed as a by product in coupling reactions. (Benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate couples amino acids as efficiently as BOP, but the by-products are less hazardous. Coupling reactions are rapid, being nearly complete within a few minutes. (Benzotriazol-1-yloxy)-tripyrrolidino-phosphonium hexafluorophosphate may be used in place of BOP in peptide synthesis without loss of coupling efficiency. Bromotripyrrolidinophosphonium hexafluorophosphate is a more reactive coupling reagent. It is especially useful in difficult coupling, such as coupling N-methylamino acids or dialkyl glycines, where other coupling reagents are inefficient. ^{[9][10][11]}

Aromatic oximes

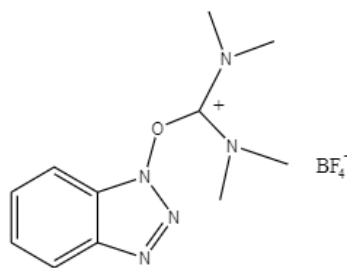
To solve the problem of Alanine attaching to DCC racemization, aromatic oximes were introduced. The most important ones are 1-hydroxybenzotriazole (HOBt) and 1-hydroxy-7-aza-benzotriazole (HOAt). Others have been developed. These substances can react with the O-acylurea to form an active ester which is less reactive and less in danger of racemization. HOAt is especially favourable because of a neighbouring group effect. Newer developments omit the carbodiimides totally. The active ester is introduced as a uronium or phosphonium salt of a non-nucleophilic anion (tetrafluoroborate or hexafluorophosphate): HBTU, HATU, PyBOP.



**O-Benzotriazole-N, N, N', N'-tetramethyl-
uronium-hexafluoro-phosphate**

Aminium-Based Reagents

Two other popular coupling reagents are O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU). As their names reflect, these reagents were believed to have a uranium structure, but crystal and solution structure studies revealed that these reagents actually have aminium structure. Both are very efficient peptide coupling reagents with little racemization. Coupling reactions are complete in as little as six minutes and when HOBt is added, racemisation can be reduced to insignificant levels. This makes these the reagents of choice in critical applications. TBTU was very effective, for instance, in key macrocyclization and coupling steps in the total synthesis of the macrocyclic peptide cyclotheonamide B. These reagents should in equal molar amounts relative to the carboxylic acid component of the coupling reaction. Excess HBTU and TBTU can react with the unprotected N-terminal of the peptide and



form a guanylidine moiety that blocks further elongation of the peptide. O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) is similar to HBTU, but reacts faster with less epimerization during coupling. HATU is preferred to HBTU in most rapid coupling protocols. HATU is utilized in the same manner as HBTU. As with HBTU, HATU should not be used in excess because it can react with the unprotected N-terminal and block further chain elongation. O-(6-Chlorobenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HCTU) remains colorless through long synthesis sequences and presumably has greater stability. It is reported to be less allergenic than other coupling reagents, but nonetheless it should be handled cautiously. DiFenza and Rovero have reported that HCTU showed reduced rates of racemisation compared to BOP.

Tetramethyluronium tetrafluoroborate

SYNTHESIZING LONG PEPTIDES

Stepwise elongation, in which the amino acids are connected step-by-step in turn, is ideal for small peptides containing between 2 and 100 amino acid residues. Another method is fragment condensation, in which peptide fragments are coupled. Although the former can elongate the peptide chain without racemization, the yield drops if only it is used in the creation of long or highly polar peptides. Fragment condensation is better than stepwise elongation for synthesizing sophisticated long peptides, but its use must be restricted in order to protect against racemisation. Fragment condensation is also undesirable since the coupled fragment must be in gross excess, which may be a limitation depending on the length of the fragment. A new development for producing longer peptide chains is chemical

ligation: Unprotected peptide chains react chemoselectively in aqueous solution. A first kinetically controlled product rearranges to form the amide bond. The most common form of native chemical ligation uses a peptide thioester that reacts with a terminal cystein residue.

PEPTIDES AS A DRUG MOIETY ^[13]

Now a day's peptides are considered to be the Active Pharmaceutical Ingredients (API) in various areas for e.g. antibiotics, antiangiogenesis, antivirals, immunomodulators, CNS disorders, neurological disorders, analgesics, antiobesity drugs etc. Over 200 new peptide based drugs are under different stages of development with 50% of them under clinical trials and prior to approval. Peptide represent 1% of the total API with a market of US\$ 300-500M per year and a growth rate of 15-25% annually with an expectation of 100% increase in the next two years. When compared with small molecule therapies, peptide therapeutics possesses certain advantages, including:

- 1) Higher activity and potency.
- 2) Higher specificity towards their target.
- 3) Lesser tendency toward adverse interactions with other drugs.
- 4) Less accumulation in tissues.
- 5) Lower toxicity.

Less favourable properties of peptides include:

- 1) Shorter persistence in the human body due to enzymatic degradation.
- 2) Ineffective in tablet form.
- 3) Potential immunogenic or allergic reactions.

PEPTIDE BASED DRUG DISCOVERY

Proteins are large molecules and are usually ineffective as drugs due to delivery and stability issues. As a large molecule, a single protein may have multiple biological functions, where each function is defined by localized interactions of a specific sequence of amino acids in the protein with another protein or a non-protein ligand. A specific sequence of the protein that represents the active site is called an epitope. An epitope can be a continuous sequence of amino acids or discontinuous sequences that are in close

proximity to each other in the three-dimensional protein structure. The first step in drug development is to “map” these active sequences of a protein in a process called epitope mapping. The purpose is to determine the minimum sequence of a peptide that constitutes the active domain of the protein, thus avoiding the inherent problems of delivering and stabilizing whole proteins as drug candidates. Once the epitope is identified the peptide sequence is then optimized and stabilized into a final drug product. Epitope mapping and sequence optimization involve the use of large numbers of peptides that constitute libraries, which are then synthesized and assayed in a parallel, high throughput manner. Successful drug discovery invariably involves protein studies, because most drugs are designed either to interact with specific target proteins, or to alter target protein-protein interactions. Conventional one protein-one experiment strategy is time consuming and expensive. Current approaches toward a successful lead development and drug discovery requires high throughput screening (HTS), that is, a fast and efficient screening of a large

number of compounds in a parallel manner. High throughput screening is made possible as a result of the merging of three distinct technologies.

1) Genomics and Proteomics – High throughput genomic analysis resulted in the identification of thousands of functionally important genes, with several of the protein products not isolated or identified. On the other hand, high throughput proteomic analysis resulted in the isolation and identification of a large number of proteins, but mostly with unknown functions. These dilemmas serve as driving forces to search for high throughput systems for identifying proteins and analyzing their structure and function relationships.

1) Combinatorial Peptide Synthesis – Technologies that allow combinatorial synthesis of large libraries of different organic compounds are now available. Although combinatorial peptide synthesis platforms have been in existence for some time, the PEPscreen platform is the first truly flexible system with the ability to

incorporate non-standard amino acids, molecular dyes, isotopic labels, varied peptide lengths, and various numbers of peptides, all in a single run. This flexibility enables synthesis of a variety of peptide libraries and addresses drug discovery research in the framework of high throughput, parallel screening.

2) Software Programming and Robotics – Advances in software programming and robotics enable automation in all aspects of drug discovery: in genomic and proteomic analysis, in the synthesis of peptide libraries and in high throughput functional assays. More importantly, software programming enables fast processing of extremely large amounts of data and customizing reports according to the experimental design.

CHALLENGES WITH PEPTIDE SYNTHESIS

- 1) Challenges during coupling reactions of same or different kind of amino acids.
- 2) Challenges during Up Scaling.

- 3) Challenges with various Coupling Reagents.
(HOBt, TBTU, EDC.HCL)
- 4) Challenges with Reactions Parameters.(
molar ratio, temperature, reaction time)
- 5) Development of various intermediates for
e.g.
- 6) Degradation of peptides during reaction.
- 7) Challenges during Work Up of reactions.
- 8) Yield and Purity of synthesized peptides.
- 9) A common problem with many peptides is
insolubility in aqueous solution. For
purification, these hydrophobic peptides
often have to be dissolved in non aqueous
solvents, or in particular buffers. These
solvents or buffers may be unsuitable for
use in biological and other assay systems,
and the customer may therefore be unable
to use the peptide for the intended
purpose.

Peptides are complex molecules and each sequence is unique with regard to its chemical and physical properties. While some peptides are difficult to synthesize, many peptides are relatively straightforward to synthesize but may still be difficult to purify after synthesis.

OPTIONS FOR PEPTIDES THAT ARE DIFFICULT TO SYNTHESIZE

1. Shorten sequence

As peptide length increases generally the purity of the crude product becomes lower. Most peptides of 15 residues or less can be obtained without major difficulty. However, as peptide length increases above 20 residues, yield of correct peptide becomes a major consideration. In many cases, decreasing the length below 20 can give more favourable results.

2. Decrease the number of hydrophobic residues

Peptides with a predominance of hydrophobic residues, especially in the region 7-12 amino acids from the C terminus, can often have assembly problems. This is thought to be due to beta-sheet formation between peptide chains during synthesis, which produces incomplete coupling. In these cases, replacing one or more residues with more polar residues, or adding a Gly or Pro

residue to help break up regular peptide structure may help.

3. **Minimise "difficult" residues**

Peptides with multiple cysteine, methionine, arginine and tryptophan can often be difficult to synthesize. Serine can often be used as a non-oxidising replacement for cysteine, and norleucine can be used as a methionine replacement. Lysine can be used in place of arginine while tyrosine or phenylalanine or other hydrophobic residues such as leucine are sometimes adequate replacements for tryptophan.

OPTIONS TO IMPROVE SOLUBILITY

1. **Change N or C terminus**

For acidic peptides (i.e. peptide has an overall negative charge at pH 7), we recommend a peptide format of Acetyl-peptide-COOH (acetyl group at amino terminus and free acid at carboxy terminus) to maximise the negative charge. For basic peptides (i.e. peptide has an overall positive charge at pH 7), we recommend a peptide

format of H-peptide-amide: (free N-terminal amino group, and amide C-terminus) to maximise the positive charge.

2. **Shorten or lengthen sequence**

Some sequences contain a large number of hydrophobic residues such as Trp, Phe, Val, Ile, Leu, Met, Tyr and Ala; generally we see solubility problems in peptides where >50% of the residues are these hydrophobic amino acids. In order to increase the polarity of the peptide, it may be useful to lengthen the sequence, provided the added amino acids increase peptide polarity. Alternatively, the sequence may be shortened to eliminate hydrophobic residues and hence increase peptide polarity. The more polar the peptide, the more likely it is to be soluble in aqueous buffers.

3. **Add solubilising residues**

For some peptides, it is possible to arbitrarily add a set of polar residues to improve solubility. We recommend for acidic peptides to add Glu-Glu to the N or C terminus and for basic peptides to add Lys-

Lys to the N or C terminus. If a charged group cannot be tolerated, we recommend the addition of Ser- Gly-Ser to the N or C terminus. Obviously there are cases where the N and C termini cannot be altered, and this approach would not be applicable.

4. Alter sequence by substituting one or more residues

Peptide solubility may be improved by changing some residues within the sequence. Often, a single replacement can dramatically improve solubility and that change may be relatively conservative; for example replacing Alanine with Glycine.

5. Alter sequence by selecting a different "frame" for a set of overlapping peptides

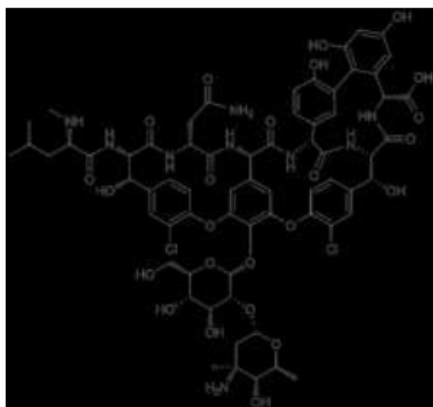
If a number of sequential or overlapping peptides of set length from a sequence are to be made, a change in the starting point of each peptide may make a difference by creating a better balance between hydrophobic and hydrophilic residues in individual peptides, or by separating "difficult" residues into different peptides

(e.g. two cysteine into separate peptides instead of together in one peptide).

PEPTIDES IN ATTACHMENT WITH ANTIBIOTICS

It is known that various peptides have been used as antibiotics. Bacitracin, Gramicidin, Polymyxin are few examples of polypeptide antibiotics. As peptides contain both acidic and basic functional groups, they can be attached to various antibiotic moieties in the same way as they are attached to other peptides as well. Charge on an antibiotic greatly influences the activity of an antimicrobial. Naturally derived cationic antimicrobial peptides typically consist of a net positive charge between +2 and +9, due to the presence of few or no acidic residues, such as glutamate or aspartate and a high number of cationic amino acids such as lysine or arginine and/or histidine [1]. Alteration of this net charge to hydrophobic ratio can vary the activity and spectrum of the peptide against a host of microorganisms. For e.g. vancomycin, represent one of the last line of effective antibiotics against Methicillin resistant *Staphylococcus aureus*. Lipoglycopeptide derivatives of vancomycin in development

include oritavancin and dalbavancin which possess increased activity against vancomycin-resistant strains [2].



Despite this, peptide antibiotics can be anionic as well. Peptides that are anionic in nature tend to be rich in glutamic and aspartic acids and include the amphibian peptide Maximin-H5 and Dermcidin, a peptide derived from human sweat. [3] [4]

Similarly to their cationic counterparts, anionic antimicrobial peptides can adopt varying amphiphilic structures such as the α -helix and the β -sheet conformations with interaction with the microbial membrane key to activity. Anionic peptides target ribosomes within the cell inhibiting ribonuclease activity, thus resulting in microbial cell death. [5] [6]

Amphibian derived peptides are excellent examples of naturally occurring, structurally diverse peptides with high antimicrobial potency. These compounds are released in skin secretions often at high concentrations and their production reflects the evolution of amphibians to their humid habitat, an environment also suitable for the growth and proliferation of opportunistic pathogenic bacteria and fungi [7]. Such peptides are also beginning to show promise against cancer, as anti-tumor compounds [8] and also possess anti-viral activity, with potential benefits against HIV [9]. They also show activity against eukaryotic cells and therefore provide a means by which amphibians may be protected from predation [10].

One of the cases where antibiotic resistance is seen is infections associated with implanted biomaterials. A study was done where the broad spectrum synthetic cationic peptide melimine has been covalently linked to a surface via two azide linkers, 4-azidobenzoic acid (ABA) or 4-fluoro-3-nitrophenyl azide (FNA), and the resulting surfaces characterized

by X-ray photoelectron spectroscopy and contact angle measurements. The antimicrobial efficacy of the two melimine-modified surfaces against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was compared by scanning electron microscopy (SEM) and fluorescence microscopy. Attachment of melimine via ABA gave an approximately 4-fold greater quantity of melimine bound to the surface than attachment via FNA.

CONCLUSION

Lots of work has been done in the field of peptide chemistry and the peptide complexes are further screened for their biological activity in many cases but till date there is no generalized rule to establish the relation between the peptide complexes and their corresponding antimicrobial activities. In due course of time certain bacteria develop naturally exhibiting more resistance to peptide complexes. This field shows a lot of promising space for further research in order to attain enhanced outcomes.

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