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EXPLORATION OF ADVANCES IN SOLID PHASE SYNTHESIS OF THERAPEUTIC PEPTIDES: HISTORICAL DEVELOPMENT, CURRENT TRENDS, AND FUTURE PERSPECTIVES

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ABSTRACT

Peptides are becoming more and more significant as drug candidates. Small peptides that are found in nature frequently have important medicinal characteristics. In recent years, peptide-based drug delivery has seen enormous advancements in therapeutic applications due to the considerable adaptability and inherent high affinity of peptides for their target sites. Although the synthesis of peptides has a long history, it still has a number of drawbacks, including high costs, significant waste production, and a need for potentially harmful reagents and solvents. Peptide medication and its therapeutic usage have progressed with time and continue to progress through medication advancements and technological changes. Peptides have been synthesized and altered utilizing chemical and biological techniques, as well as innovative design and delivery technologies, which have assisted in eliminating the inherent limitations of peptides and permitted the continuous growth of this sector. Solid-phase peptide synthesis (SPPS) is the peptide synthesis technique used for novel drug delivery systems. In order to improve the therapeutic benefits of peptide-based therapeutics, SPPS aims to produce large peptides that can be readily metabolized and modified for targeted drug delivery. This review article discusses several aspects of Advance Solid Phase Synthesis of therapeutic Peptides including their historical development, current trends, and future perspectives. Recently, 4% of overall approved drugs are peptide-based drugs or their derivatives. By utilizing various advanced synthesizers, new methods for automated peptide synthesis can meet the growing need for this process. In the future, the projected demand for polypeptides as biological products will surely promote and increase interest in these developments.

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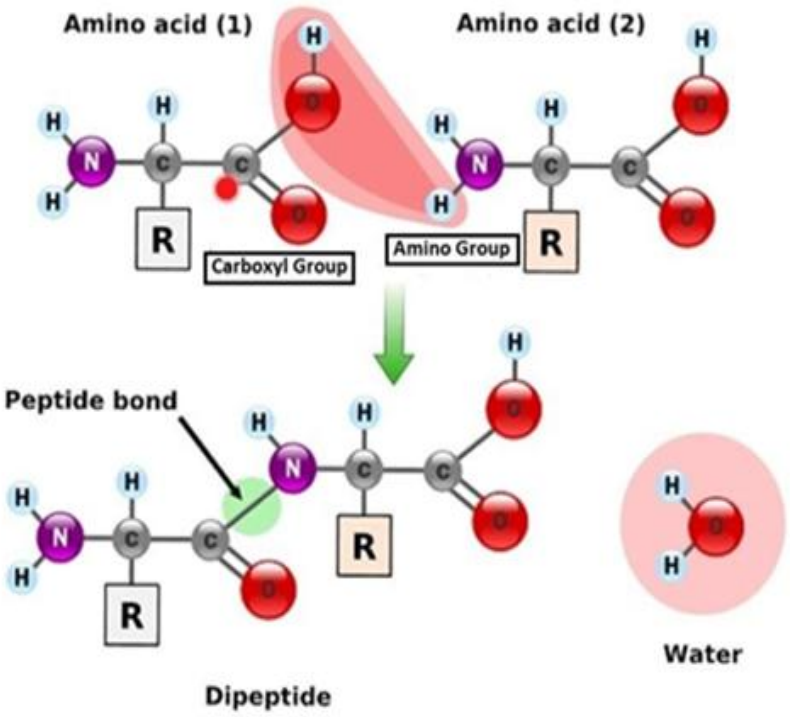
INTRODUCTION

There are twenty naturally occurring amino acids that are repeatedly found in the structure of proteins. This is because of the universal nature of the genetic code available for the incorporation of only twenty amino acids through the translation process when the proteins are synthesized in the cells. Amino acids are a group of organic compounds comprising two functional groups that are amino and carboxyl. When two or more amino acids are joined together by reacting amino (—NH_2) functional group of each amino acid and the carboxyl functional group (—COOH) of the subsequent amino acid and the bond established between those functional groups is named as peptide bond [1]. It is to be noted that a di-peptide has only two amino acids and one peptide and not two peptide bonds. These peptide bonds are functioned as the strengthening material between the individual amino acids. Peptides are a special type of pharmaceutical drug that is balanced between small molecules and proteins in terms of molecular structure but differ biochemically and therapeutically from both. Peptides offer a therapeutic intervention that closely matches natural pathways as intrinsic signaling molecules for numerous physiological activities. In living body peptides can be acts as neuro-transmitters, signaling molecules, biologically active hormones, enzymes and antibiotics [2]. Disorders for example cancer, cardiovascular diseases, inflammation, diabetes, osteoporosis and obesity can be treated by using peptide-based drug delivery. In the year 2007, about 60 peptide-based drugs have been approved by US Food and Drug Administration (FDA) and more than 150 peptides are currently in clinical trials [3]. The main objective of advanced SPPS is the rapid production of linear, intermediate, and large peptides. Producing peptides with desired pharmacokinetic properties and a high yield can be difficult with traditional peptide synthesis. In order to tackle this issue, SPPS was developed.

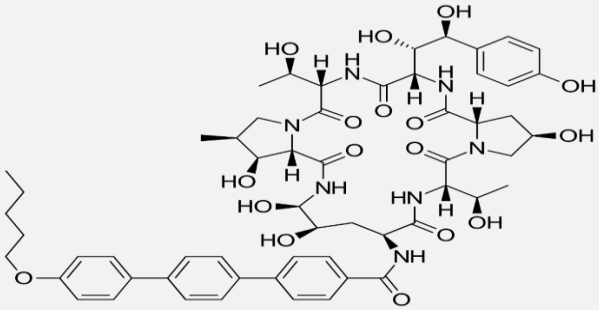
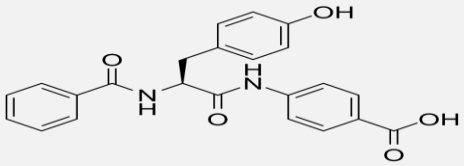
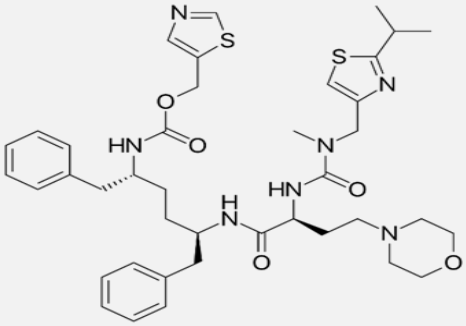
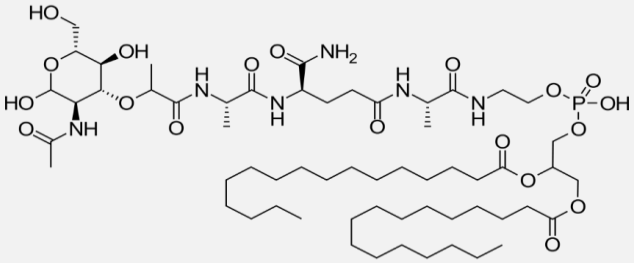
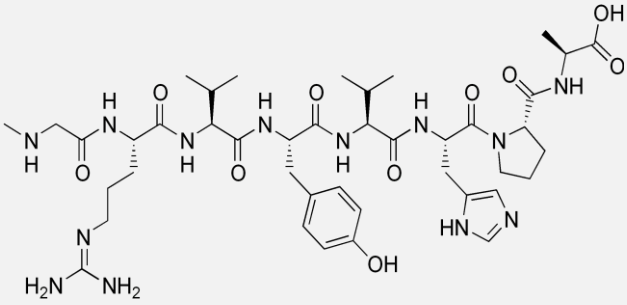


Scheme 1: Peptide bond formation by reacting two amino acids with the removal of a water molecule.

However, SPPS has some limitations like proteolytic breakdown by enzymes found in the stomach, blood, and cell plasma, which results in short half-lives of peptides, which is the primary cause of their limited bioavailability. Due to their capacity to cross membranes the drugs, an alternative route is required for administering protein and peptides such as parenteral routes that have already been stimulated in further studies [4].

Chemical reformation and alterations are of great interest in order to enhance and boost the drug ability of these commonly unstable and quickly eliminated peptide molecules from the body. Therefore, this review article aims to compile the current literature with a perspective on the advanced and effective technology for the development of peptide-based drug delivery that can be made through SPPS**.** The easy availability of synthetic peptides synthesized from SPPS has revolutionized the research and investigation in biochemistry, microbiology, combinatorial chemistry, medicinal chemistry, and new drug development technology [5].

Table 1: List of currently available marketed and clinically used peptide drugs with their structures.

Generic Name	Brand name	Structure	Disease Target
Anidulafungn	Eraxis		Antifungal action
Bentiromide	Chymex		Exocrine pancreatic
Cobicistat	Stribild		HIV infection
Mifamurtide	Mepact		Bone cancer
Saralasin	Sarenin		Hypertension

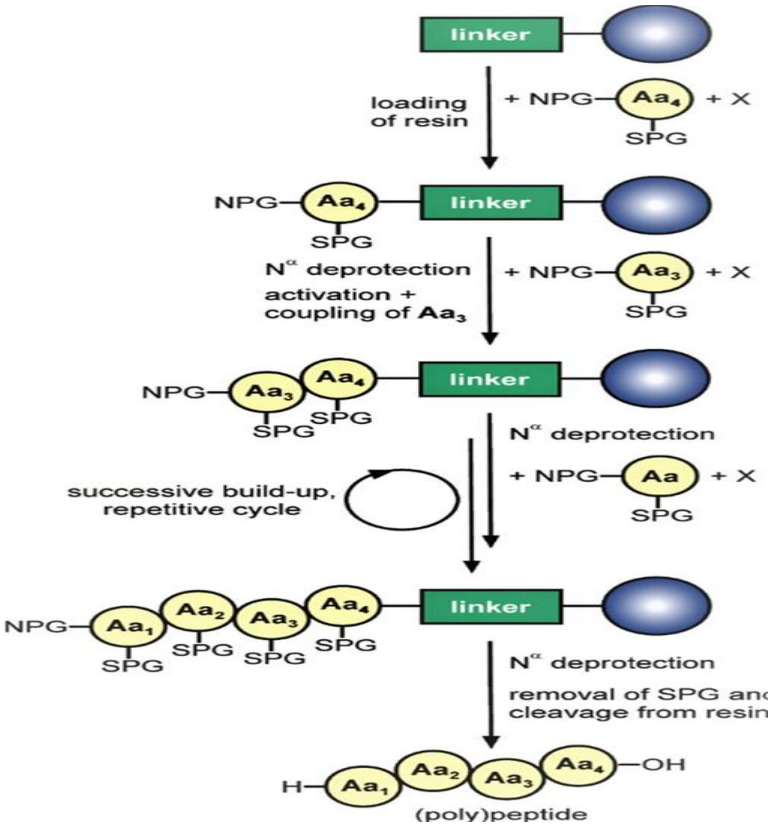
HISTORY OF SPPS

At the beginning of the 20th century, Emil Fischer was able to synthesize the first di-peptide named glycyl–glycine via peptide bond formation between the amino acids of glycine. Later in 1953, du Vigneaud made the production of poly-peptides possible by developing organic protecting groups approach to introduce trifunctional amino acids by blocking of — COOH functional group of C-terminal in amino acid and the — NH_2 functional group of N-terminal in amino acid with the intention of specific peptide-bond formation [6]. But the making of these peptides in pure form was tough to activate the free — COOH group of the N-terminal amino acid needed to get the peptide bond. Though this process guarantees good quality control and manufacture of peptides, it was actually a time-consuming and especially technical challenging method. SPPS was originally developed in the 1960s by Merrifield in order to synthesize peptide chains and it was the basis for his 1984 in Chemistry. By attaching the C-terminal amino acid of the peptide to an insoluble solid or resin support, Merrifield was able to use reagents in large excess to drive reactions for completion, then cleaved the peptide from the solid support in moderately pure form. In SPPS, an amino-protected amino acid is attached to a solid phase resin, making a covalent bond between the carbonyl group of an amino-protected amino acid. The solid phase now carries a dipeptide and this cycle is repeated to form the desired peptide chain [7].

AN OVERVIEW OF SPPS

General Procedure of Solid Phase Peptide Synthesis

The general procedure for making the peptides on a resin begins by attaching the C- terminal residue (—COOH) of the first amino acid to the resin or solid support. To avoid the polymerization of the amino acid the alpha (α) amino group (—NH2) and the reactive side chains (—R) are protected with a temporary protecting group. Once the amino acid is attached to the resin, the resin is filtered and washed to remove by-products and excess reagents [8]. Then the next new N-alpha-protected amino acid is coupled to the attached amino acid on resin. The cycle is repeated until the peptide sequence is completed [2,9].

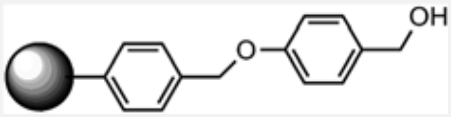
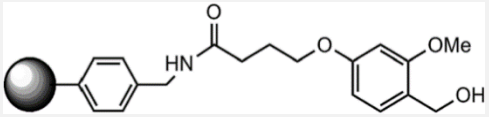


Scheme 2: Peptide assembly is shown. (1) the C- terminal (—COOH) of an amino acid is coupled to the linker and the peptide chain will be elongated by repeating a series of (1) NPG deprotection, (2) carboxy group of amino acid activation and (3) coupling. Finally, the protecting groups will be cleaved and the desired elongated peptide will be obtained. (NPG: N^{α}-protecting group, X: activator, SPG: side-chain protecting group, Aa: amino acid).

Resin Beads (Solid Phase/ Support) and linkers for SPPS

The synthesis of peptides takes place mostly in the interior of the resin, and appropriate solvation, low cross-linking for good accessibility, and good swelling properties are very essential for this process. A solid phase or resin has to be insoluble in all solvents, chemically and physically resistant, and mechanically stable to allow filtration. The small resin beads can expand up to six times their original volume in organic solvents. The swelling of the resin material in organic solvents is an important issue. The first resin or solid polymer for peptide synthesis was a copolymer consisting of polystyrene and cross-linked divinylbenzene, presented by Merrifield in 1963. The polymer needs to have a functional group for coupling the linker, which are the chemical units needed to attach an amino acid to a resin bead for making the molecules grow in the solid phase [10]. The nature of the linker governs the chemistry to be used, especially the conditions under which the products can be cleaved from the resin. The linker that is bound to the resin is called an anchor. The anchor can also be considered a protecting group for one of the functional groups of the final product, and it determines the reaction conditions under which the product can be cleaved from the support [6,10]. Different commercially available resins were used for SPPS with different linkers. They are listed in Table 2.

Table 2: Different resins with their structures used in SPPS.

Resin structure	Resin name	Cleavage conditions	Peptide product
	Merrifield resin	95% HF in CH_2Cl_2 for 2h	Acid
	Wang resin	90–95% TFA in CH_2Cl_2 for 1-2h	Acid
	HMPB resin	1% TFA in CH_2Cl_2 2–5 min	Acid

Orthogonal strategy of protecting group

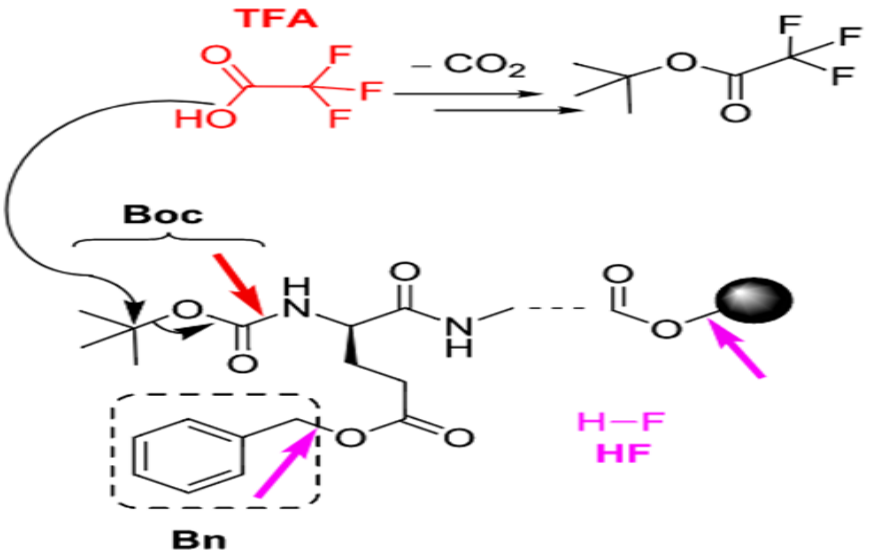
The right choice of the protecting groups for the side residues in the amino acids represents a very important step before starting the peptide synthesis on solid phase. The side chains of many amino acids are reactive and they may form side products if left unprotected. For a successful peptide synthesis on solid phase these side chains must remain protected despite of repeated exposure to N alpha deprotection conditions. Ideally the N alpha (α) protecting group and the side chain (—R) protecting groups must be removable under entirely different conditions such as basic conditions to remove the N-alpha (α) protection and acidic conditions to remove the side chain (—R) protection [11]. Such a protection approach for amino acids is called as orthogonal protection. Table 3 shows commonly applied different protecting groups for amino acids in SPPS.

Table 3: Structure of Various Protecting Groups Used In SPPS.

Name of Protecting groups	Structure
Boc (tert- butyloxy carbonyl)	
Fmoc (9-fluorenyl methoxy carbonyl)	

Boc/Bn Protecting group

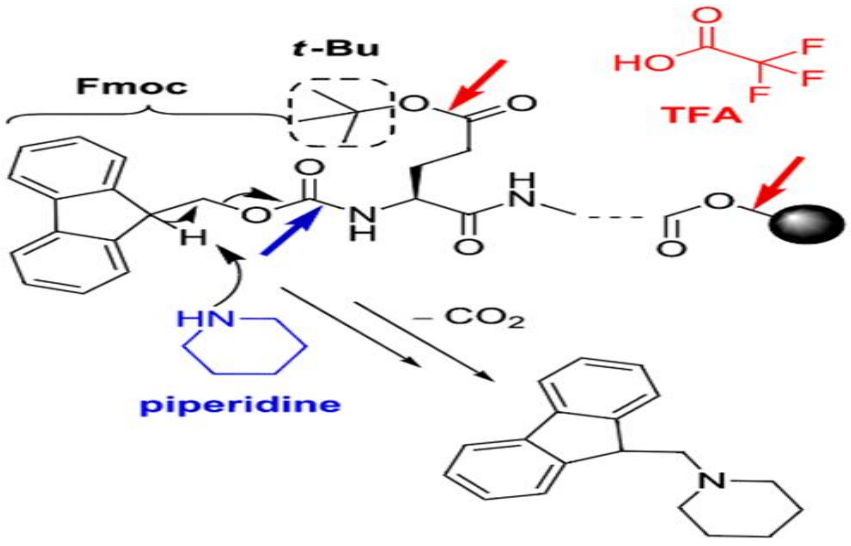
The initial process for SPPS employed by Robert Bruce Merrifield was based on the use of the Boc (tert-butyl oxy carbonyl) group as a protective group for the functional amino group (—NH_2) and Bn (benzyl) or its linked side chains' protective groups (—R) of tri-functional amino acids. Both Boc and benzyl-based protecting groups are acid labile, Therefore Boc/Bn is not an accurate orthogonal protection method. It is basically applied because the Boc group is removed under moderate conditions such as 50 % TFA in DCM while Bn (benzyl) based protection groups required very strong acids such as HF to remove them from amino acids [12].



Scheme 3: Boc /Bn protecting-group strategies applied in SPPS. Cleavage of protecting groups Boc and Bn with TFA and HF respectively occurs by acidolysis.

Fmoc/t-Bu Protection

The alpha (α) nitrogen of the amino acids is protected with the base labile Fmoc (9- fluorenyl methoxycarbonyl) group and the side chains are protected with acid labile t-Bu (tert-butyl) protecting group or the trityl (triphenylmethyl) group. This is an orthogonal protection system as the side chain protecting groups can be removed from amino acids without displacing the N-terminal protection and vice versa [13].



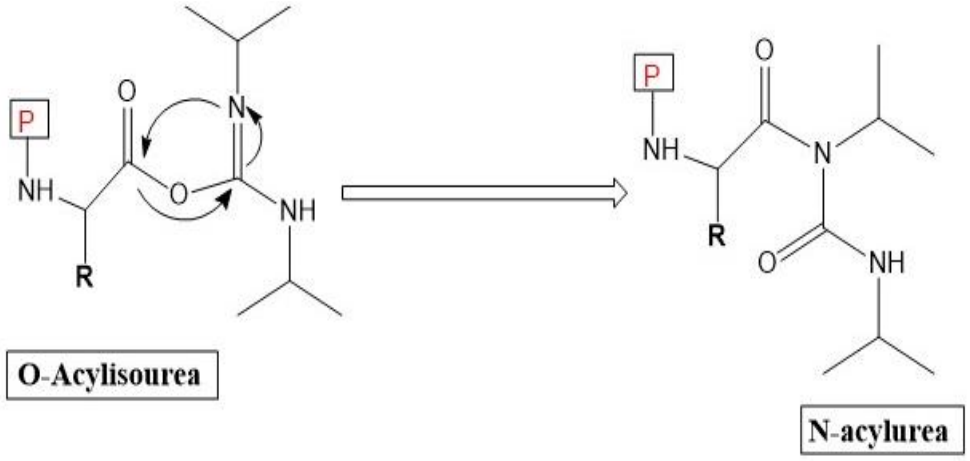
Scheme 4: Fmoc/t-Bu protecting group strategies applied in SPPS. The Fmoc group is removed by β-elimination through piperidine and t-Bu is released by acidolysis with TFA.

Comparison of Boc/Bn protecting group with Fmoc/t-Bu protecting group

The initial phase for a successful peptide synthesis using the SPPS route is to choose the protection approach. When Boc/Bn protection is used with in situ neutralization can offer excellent results for long and difficult peptide sequences. Cleaving the peptide product from the resin needs strong acids such as HF. However, HF is poisonous and reacts with ordinary laboratory glassware. It demands a special apparatus that can make scaling up difficult if a laboratory is not specifically equipped for large-scale peptide production. However, aggregation becomes a problem when Fmoc/tBu protection is applied as the peptide-resin is always in a neutral condition [11]. This is not significant with short peptide sequences but can be problematic when large peptides are manufactured. Therefore, Fmoc deprotection is rapid for small peptides and often becomes slower for longer peptides due to aggregation. Most of the time the Boc/Bn protection is suitable for long and difficult peptide sequences and base-sensitive peptides while Fmoc/tBu protection is finest for acid-sensitive peptides and peptides with side chain modifications.

Coupling Reagents

In order to make a rapid and quantitative peptide bond applying the SPPS technique, the free carboxyl terminal group of amino acid has to be induced into an active and more electrophilic species. For that reason, coupling reagents were applied and thought of as amino acid activators for many years. Carbodiimide-based coupling reagents such as DCC (Di-cyclohexyl carbo di-imide) or DIC (Di-iso propyl carbodiimide) were the first coupling reagent for carboxyl activation introduced by Merrifield and have been used for years. Dicyclohexylurea as a by-product is formed from DCC which is almost insoluble in most organic solvents. Therefore, Although DCC is not appropriate for reactions on resin or in the solid phase, it is particularly helpful for peptide synthesis in solution phase reactions [14]. Since the urea by-product is still in solution and the formed O-acylisourea is highly reactive and causes loss of chiral integrity of the amino acid due to O-N rearrangement of the O-acylisourea intermediate, DIC is used instead in solid phase synthesis, as shown in Scheme 5.



Scheme 5. Shows racemization of amino acid due to coupling reagent DIC.

Coupling reagents that are commonly used in SPPS are [14]: -

a) Phosphonium-based reagents-Benzotriazol-1-yl-N-oxy-tris (pyrrolidino) phosphonium hexafluorophosphate
b) Aminium-based reagents –O- (Benzotriazol-1-yl)- N, N, N', N'-tetramethyluronium tetrafluoroborate
c) Other Coupling Reagents –Ethyl 2-cyano-2-(hydroxy-imino) acetate (Oxyma)

SOLVENTS

CH_2Cl_2 (methylene chloride) is a solvent often used in Boc protection protocols because it easily dissolves most of the Boc-protected amino acids and causes good swelling of polystyrene (PS) based resins. CH_2Cl_2 is unreactive to TFA and used in Fmoc protocol though because it slowly reacts with piperidine forming piperidine hydrochloride which is an insoluble crystalline solid. N-methyl pyrrolidone (NMP) is the solvent well-chosen for most peptide synthesis. Most of the common peptide reagents used in SPPS are very much soluble in NMP.

On the other hand, NMP can be obtained in pure form i.e., free of reactive amines that might create problems by interfering with coupling reactions. Another solvent i.e., di-methyl formamide (DMF) is used in SPPS and can cause swelling of divinylbenzene (DVB) cross-linked polystyrene resin 4 times and have a tendency to break down over time spontaneously by releasing di-methylamine as an impurity. Di-methylamine is very reactive towards the Fmoc protecting group [15].

AGGREGATION DURING PEPTIDE SYNTHESIS

Amino acids that are susceptible to forming -sheets often accumulate during peptide strand stretching in SPPS, primarily peptides containing a high amount of Alanine (Ala), Valine (Val), Isoleucine (Ile), Asparagine (Asn), or Glutamine (Gln). Aggregation of the peptides can trigger incomplete coupling and often leads to inadequate solvation of the peptidyl-polymer, but it is not as much of noticeable when resins with a low loading are used [6]. Several strategies can be taken to suppress or reduce the hydrogen bonding causing the aggregation of peptides on resin. Some of the strategies that can be taken include:

- Apply microwave energy which is expected to reduce both inter and intra- molecular aggregation.
- Shift to in situ neutralization protocols if Boc/Bzl protection is used.
- If Fmoc deprotection is incomplete or slow during a peptide synthesis exchange piperidine with DBU (1,8-Di-aza bicyclo [5.4.0] undec-7-ene) as a deprotection reagent which can make the deprotection process fast.
- Sonication of the reaction mixture can minimize the aggregation to some extent.

MODIFICATIONS OF THERAPEUTIC PEPTIDES

In the body, peptides suffer from rapid proteolytic degradation and body clearance. Additionally, their drug ability is restricted by poor bioavailability and those complications can be overcome by chemical modification of the peptides. Therapeutic peptide demands an additional modification to improve mimic of its native peptide or protein fragment. Nearly all modifications can either be incorporated post-synthetically or during the peptide synthesis by using suitably derivatized amino acids. Some of the common modifications of peptides are listed:

Lipidation of peptides-

Acylating the N-terminal of amino acids with a fatty acid (myristic acid palmitic acid) activates the peptides to associate with plasma membranes. The processes of amidation, S or O esterification, and thioether production can all be used to create lipidated peptides. Peptide lipidation is often carried out utilizing the SPPS technique and the Fmoc/t-Bu procedures, allowing for efficient and targeted alteration [16].

Glycosylation –

An essential step in the chemical production of glycol-peptides is the integration of the sugar moiety into the peptide sequence. Afterward, amino acids can be coupled directly to resin or solid phases, or glycosylated amino acid building blocks can be produced in the solution [17].

Phosphorylation –

Peptides modified by phosphorylation are called as phosphopeptides and occur in serine, tyrosine and threonine residues being the most significant the first one. Phosphopeptides can be synthesized by either direct phosphorylation of the amino acid on resin after amino acid coupling or by applying protected phosphorylated amino acid building blocks on resin. Phosphorylated peptides are responsible for regulating most protein kinases and signal transduction [18].

PEGylation –

PEGylation is the covalent modification of peptides with polyethylene glycol (PEG) polymer units to modify the pharmacokinetic and pharmacodynamic properties of peptide-based drugs. The bulky PEG prevents degradation of the peptide by proteolytic enzymes. The hydrodynamic radius of a PEG-modified peptide is larger than the normal cross-section glomerular capillaries which extremely obstructs renal clearance. Furthermore, the PEGylated peptide is surrounded by a large cloud of water because of the ability of every single ethylene oxide unit to attach the nearby three molecules of water. This leads to enhanced solubility and great shielding of the peptide against proteolytic enzymes and antibodies [19].

INSTRUMENTS FOR SOLID-PHASE PEPTIDE SYNTHESIS

The instruments used to synthesize peptides are basically known as peptide synthesizers and these synthesizers have special capabilities to target various applications and factors determine the finest choice of synthesizer.

Manual peptide synthesizer

Solid phase peptide synthesis can be performed manually by using laboratory glassware such as round bottom flasks, sintered glass funnels, etc. that has been treated with a silylating agent to avoid the resin from sticking to the glass surfaces. To agitate the resin suspensions overhead mechanical stirrers or orbital shakers are used. Magnetic stirrers should not be used as the resin beads can be damaged if they come between the stirring bar and the inside surface of the glassware.

While performing manual solid-phase peptide synthesis with standard laboratory glassware repeated transfer is needed for filtering and washing in each amino acid coupling cycle which is very time-consuming and may result in resin loss or inadequate transfer of the resin. A number of specialized reactors are available for manual peptide synthesis on the resin which incorporates a glass frit for filtering and washing the resin without transferring it from the glassware and ports for added reaction solutions while maintaining an inert gas environment. These reactors are installed into a wrist action shaker to offer agitation. Most manual synthesizers have many reactors which permit the employer to synthesize multiple peptides simultaneously [15,20].

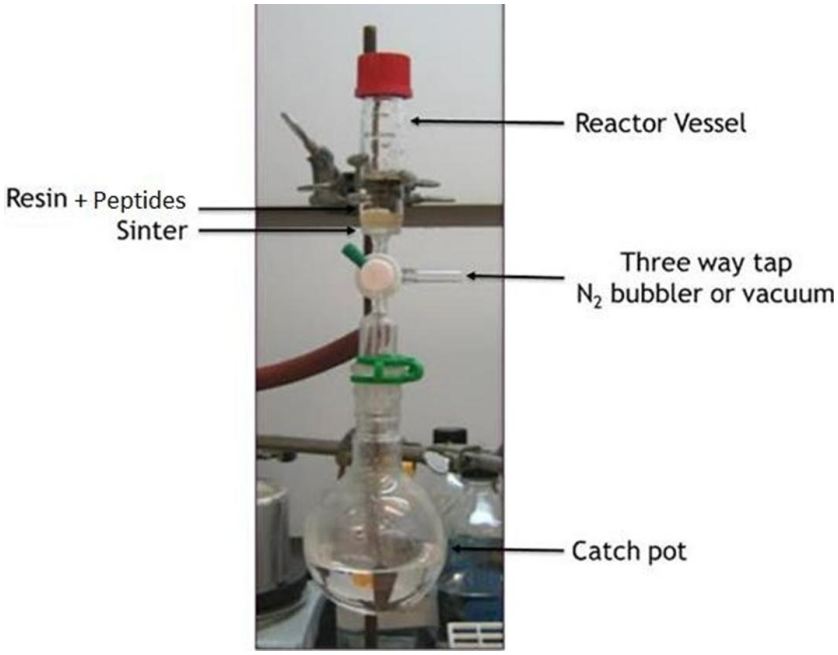


Figure 1: Schematic representation of a manual solid phase peptide synthesis.

Automated Peptide Synthesizers

Automated synthesizers can be categorized into three classes: 1. batch synthesizers, 2. continuous flow synthesizers, and 3. parallel synthesizers. Many of these synthesizers apply X-Y robotics equipped with syringe pumps to accurately supply amino acid and reagent solutions to individual reactors.

Batch synthesizers– Automated Peptide Synthesizers

This synthesizer can be capable of preparing only one or two peptides at a time although can prepare them on a larger scale. This technique of peptide synthesis is compatible with Fmoc/tBu and Boc/Bzl protocol along with a wide range of solid supports or resins for peptide synthesis. This method is mainly appropriate to produce large peptide libraries in a short time. Depending on the design of batch peptide synthesizers it employs larger scale reactors even up to 500 ml to 1000 ml able to handle up to a kilogram of resin in one reactor. These synthesizers normally use a series of valves and manifolds to supply amino acids, reagents, and wash solvents to each reactor. Many of these synthesizers depend on calibrated flow rates to supply the proper volume of amino acid or reagent solution to the reaction vessel. Batch synthesizers utilize inert gas bubbling or mechanical agitation for proper mixing. However, to separate the peptide resin from excess reagents and other by-products by filtration is an essential procedure in SPPS. In most synthesizers, this is performed either by using a vacuum beneath the reactor frit or by using inert gas pressure to the surface of the solution above the frit [20].

Batch synthesizers that are employed in solid-phase peptide synthesis are listed below:

- AAPPTec Endeavor 90
- Focus XC

- Focus Xi

Figure 2: A general representation of Batch type reactor.

Continuous flow synthesizers –

This synthesizer employs a pump to re-circulate the process solution through a column containing the resin. This machine usually needs a specific resin with low swelling properties. Typically, some automatic monitoring method is utilized to track the progress of the reactions. When the monitoring shows the reaction is complete the next process step is started. As the reaction solution continuously flows through the resin, thus continuous-flow synthesizers do not demand a mechanical means of agitation. Nearly all automated continuous-flow peptide synthesizers employ a fixed bed reactor that holds the resin in an arbitrarily set volume. This synthesizer is mostly suited to the synthesis of long and synthetically challenging peptides as it permits good swelling properties of the resin to be maintained during the process of peptide synthesis. Therefore, peptide aggregation or truncation is reduced which as a result yields higher purity peptide. During the peptide syntheses, the VBFR approach delivers a low overall system pressure, allowing the use of commercially available resins. Automated variable bed flow reactors are compatible with known peptide processes, need only four equivalents of amino acids, and have fast synthetic cycle times [21]. Continuous flow synthesizers are currently followed by in-line UV-visible monitoring of Fmoc cleavage amino acids as shown in figure 3.

Figure 3: Continuous flow synthesizer set up consisting of two R2 pump modules and one R4 reactor module.

Parallel synthesizers –

In a parallel synthesis technique individual and larger quantities of peptide can be synthesized in separate reaction vessels by proceeding parallel syntheses of the same sequence. In 1984, Geysen and his colleagues announced the creation of the first combinatorial peptide library using multipin parallel technology. In this technique, individual peptides are chemically synthesized on polyacrylic acid attached to a polyethylene pin that is arrayed in a 96-well microtiter plate [23]. The protected amino acids which are used in the making of peptides and the coupling reagents are initially dissolved and then added to the wells. Now the coated ends of the pins are immersed into a solution containing amino acids and other reagents and remained there until the coupling reactions are completed. The peptides synthesized on the pins are immersed into solutions. Furthermore, the sequence of peptides is usually dependent on the order of amino acids added to the wells. The peptides are screened by using ELISA (Enzyme-Linked Immunosorbent Assay) technique after the deprotection of amino acids but without leaving them from the polyethylene pins to measure the binding capacity of covalently attached peptides to the antibodies [22].

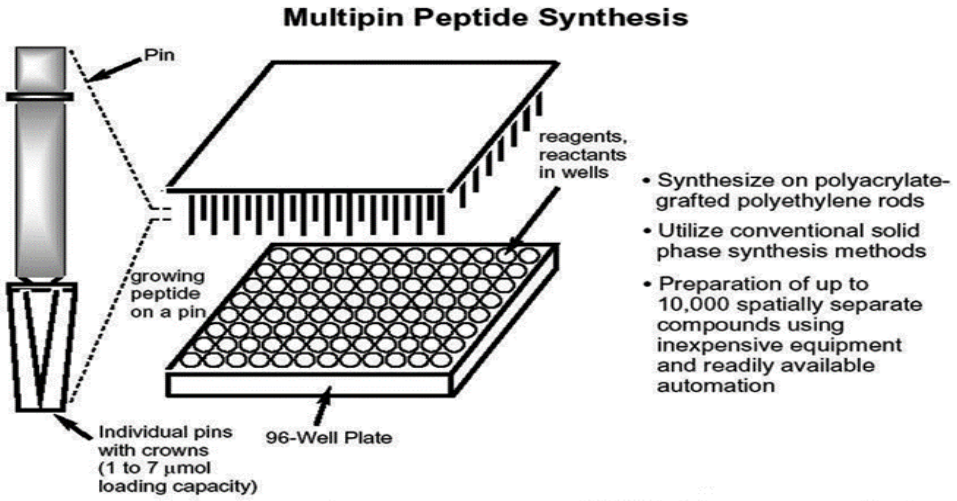


Figure 4: The Multipin apparatus for parallel peptide synthesis.

Microwave Assisted Solid Phase Peptide synthesis

The process of microwave-assisted solid phase peptide synthesis is almost the same as manual and automated peptide synthesis with the exception that the latter two processes don't use microwave irradiation or they may use conventional heating for peptide synthesis. This is a time-consuming and in- efficient process for transferring heat or energy into the reacting system. A different method for carrying out microwave-assisted organic reactions which is named enhanced microwave synthesis (EMS) has also been studied. By outwardly cooling the vessel with compressed air while at the same time administering microwave irradiation, further energy can be directly employed to the reaction mixture. EMS also guarantees an extreme and constant level of microwave energy is applied and ensuing significantly greater yields. Research published in recent years leading the peptide synthesis supports the use of EMS beneficial in synthesizing higher levels of the desired peptides from the solid phase resin when compared alone with microwave heating [23].

Microwave synthesizers

As explained above, the early studies describing microwave-assisted SPPS employed modified domestic microwave ovens which did not permit the accurate control of the microwave irradiation, resulting in homogenous heating of the reaction mixture. Two distinct microwave reactor designs are presently available for peptide synthesis one is multi-mode and another is mono-mode or single-mode reactors. In the mono-mode device, a standing wave is made when the microwave irradiation is passed by a waveguide that directs the microwave irradiation directly across the reaction vessel which is placed at a fixed distance from the microwave radiation source. The main differences between the multi-mode and mono-mode wave reactors are that parallel synthesis can be performed by using the multi-mode reactor but then again it undergoes inhomogeneous heating in the cavity. In contrast, the mono-mode reactor offers better and high yields in peptide synthesis because of a precise and homogeneous distribution of microwave radiation on the reaction mixture. Since multi-well polypropylene filter plates are heat stable so they are mostly used for microwave-assisted SPPS. Although a fiber optic internal sensor regulates the temperature, the configuration suffers from inhomogeneity across the plate. Between microwave-induced reactions, the plate is manually moved to a draining station, and the reagents and wash solutions are manually applied.

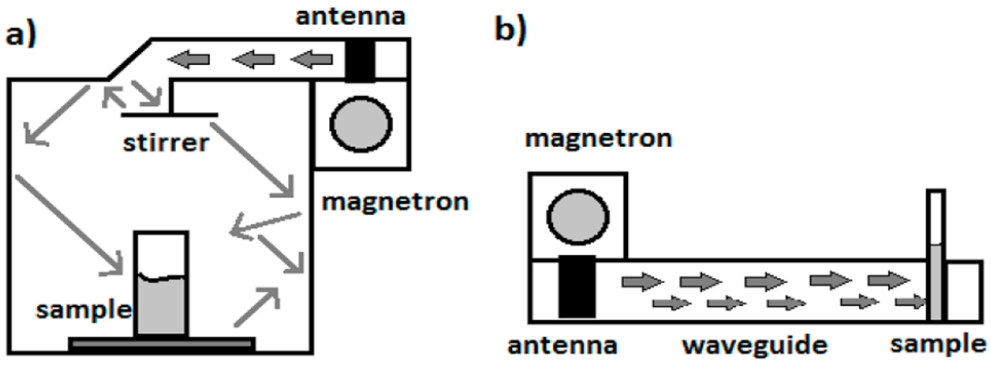


Figure 5: a) multi-mode microwave reactor, b) Mono-mode microwave reactor.

Effect of microwave radiation in SPPS

At the present time, there is hardly find any reaction that has not been performed under microwave heating conditions. It is well recorded that the majority of peptide synthesis is performed on a resin or solid phase and it has been revealed that microwave irradiation can enhance deprotection, coupling, and cleavage reactions of amino acids. The most popular solvents employed in SPPS for coupling and N-deprotection are DMF and NMP, both of which have excellent microwave heating properties. In addition to the solvent, the sample volume, vessel material, and the kind of stirring—vortexing or N2 bubbling—all have an impact on the reaction process. Additionally, it has been noted that although conventional magnetic stirring is not advised for use with SPPS due to the possibility of grinding the resin or solid support, it may be preferred to unmixed reactions. Peptoids are molecules which distinct from peptides in that the side chain is attached to the amide nitrogen instead of the α-carbon atom. Traditional procedures for peptoid synthesis need long coupling times and with microwave irradiation, each coupling time is reduced to 1 minute. Following the cleavage both homo and hetero oligomers are produced with adequate yields varying between 43% and 95%. It is then thought that this could give the explanation of why microwave heating in SPPS often does not only make the reaction faster but is also responsible for the availability of higher peptide purities compared to conventional room temperature [24].

THE FUTURE SCOPE OF PEPTIDE THERAPEUTICS

Peptides have established a special therapeutic niche since their early days as chemicals extracted from animal glands, and they will continue to play a significant role in the pharmaceutical industry. Peptide treatments have kept pace with advancements in science by diversifying into new indications and molecular targets, using cutting-edge chemical techniques to promote molecular diversity, and designing better pharmacological properties. New peptide opportunities are expected to be discovered as the study continues. Both linaclotide and afamelanotide, which are close analogs of native peptides, have received regulatory agency approval as first-in-class peptides that target guanylyl cyclase C (GC-C) and the melanocortin 1 receptor (MC1R) in the past five years. These approvals show that there is still demand for innovative peptide therapies [25]. The potential range of peptide-based medications continues to extend to new targets as research continues. In early-stage clinical trials or preclinical disease models, a huge number of peptide-addressable targets for which no medicines have yet been approved have exhibited therapeutic effects. For instance, analogs of kisspeptin that target GPR54 may be advantageous for currently utilized assisted reproductive drugs, and a melanocortin-4 receptor (MC4R) agonist may help people with inherited obesity syndromes lose weight. The pharmaceutical sector has put forward applications for patents of its own on analogs of the endogenous peptides apelin, adrenomedullin, and neuromedin U on the basis of research conducted on animals [26]. Peptide discovery for drugs will continue to benefit from advancements in computational biology and peptide screening. Bioactive peptides that may include distinctive structural properties produced by unusual post-translational modifications or non-ribosomal production can be found via metabolic, proteomic, and genomic screening of toxins and other sources of natural products. The de-opalization of poorly characterized peptide receptors might encourage research efforts for new receptor-ligand pairings, and a better understanding of the molecular basis for human genetic illnesses can produce new possible therapeutic approaches.

Ultimately, new approaches to peptide therapeutic design, transport, and half-life elongation will broaden the application potential of this unique family of compounds. Peptide therapies are under development to increase their oral availability by enhancing drug stability in the GI tract, synthesizing peptides with permeability enhancers, and enhancing their CNS availability by conjugating them to carrier molecules or administering them in nanoparticle form. In summary, peptide medicines have an even more than bright prospect for development. The visualization would continue to climb higher because hundreds of new therapeutic peptides are now in the preclinical and clinical development phases [27].

CONCLUSION

Polypeptide synthesis from automated synthesis on resin or solid substrate offers a wide range of advantages. Peptide assembly requires the concurrent execution of chemical reactions, which can be possible with several reactor tubes and enables parallel and multiple syntheses. There is no requirement for intermediary purification since coupling or deprotection processes are followed by a straightforward washing of the resin. Automated SPPS has large importance, especially for structure-activity relationship studies and backbone modification of peptide-based drugs and biologically active peptide hormones. Despite there has been significant advancement throughout the time Robert Bruce Merrifield designed the SPPS method over a decade in the past, new technology for automated peptide synthesis is still required. The overall approach of microwave-assisted SPPS shown in Fig.5 has demonstrated not only an improvement in reaction rate but also the synthesis of challenging and stiff sequences as well as sequences including unnatural amino acids. Additionally, reaction speeds and the purity of crude peptides can be improved by conductive heating. However, microwave devices offer quick, accurate heating as well as quick cooling via pressurized air, allowing for the direct application of additional energy to the reaction mixture. Although this approach is improving, it still needs work, especially in terms of utility. In the upcoming future, peptides obtained from SPPS can be further modified to generate peptide aptamers, which do not require any biological system for their production, unlike monoclonal antibodies, and have greater stability and no immunogenicity.

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Authors Contribution
All Authors have contributed equally.

Conflict of Interests
Author declares no conflict of interest.

List of Abbreviations

SPPS	-	Solid Phase Peptide Synthesis
FDA	-	Food and Drug Administration
$NPGN^{\alpha}$	-	protecting group
SPGSide	-	chain protecting group
Aa	-	Amino acid
HF	-	Fluoride hydrogen
TFA	-	Trifluoroaceticacid
Bn	-	Benzyl
Boc	-	Tert-butoxy carbonyl
CH_2Cl_2	-	Methylenechloride
DIC1,3	-	Diisopropyl carbodiimide
DMF	-	N, N-dimethylformamide
NMP	-	N-methyl pyrrolidone
EMS	-	Enhanced Microwave Synthesis
Fmo	-	Fluoren-9-ylmethyloxycarbonyl
GC-C	-	Guanylyl cyclase C
MC1R	-	Melanocortin 1 receptor

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