Research Paper

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Engineering

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Chemical Synthesis of Peptides

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ABSTRACT

The aim of a peptide or a protein synthesis is the production of pure compounds of known structure. Common elements in any chemical synthesis of both peptides and proteins are the assembly of protected amino acids or peptide chains, their deprotection, purification and characterization. The basic strategy of SPPS still persists as the initial idea, outlined by Memfield. The process requires a solid insoluble support to scaffold the first amino acid residue and subsequent stages of the peptide as it is lengthened. The carboxyl end of the peptide is scaffolded by a covalent attachment to the polymeric support. The N-terminus needs protection and deprotection at each stage of the stepwise synthesis and resulting amino group should be the free –NH2 after each deprotection. The I^-protected amino acid is activated for coupling to the growing peptide chain. For a stepwise elongation of peptides on a polymeric support, these three steps, deprotection, neutralization and coupling would be repeated until the desired sequence is assembled. Finally the covalent anchoring bond would be cleaved to liberate the free peptide.

Introduction

Peptides are the long molecular chains that make up proteins. Synthetic peptides are used either as drugs (as they are biologically active) or in the diagnosis of disease. Peptides are difficult to make as the synthetic chemist must ensure that the amino acids that make up the chain are added in the correct order and that they don't undergo any other reactions.

This involves adding one amino acid, washing away any unreacted acid then adding the next and so on. As can be imagined, this is very time consuming and only gives very low yields.

A technique that has been relatively recently developed involves attaching one end of the peptide to a solid polymer, meaning that the peptide cannot get washed away along with the excess acid. This is much quicker than classical synthesis, and leads to dramatically improved yields.

Process of Synthesis of Peptides

The process consists of five steps carried out in a cyclic fashion.

Step 1 – Attaching an amino acid to the polymer: The amino acid is reacted with a molecule known as a "linkage agent" that enables it to attach to a solid polymer, and the other end of the linkage agent is reacted with the polymer support.

Step 2 – Protection: An amino acid is an acid with a basic group at one end and an acid group at the other. To prevent an amino acid from reacting with itself, one of these groups is reacted with something else to make it unreactive.

Step 3 – Coupling: The protected amino acid is then reacted with the amino acid attached to the polymer to begin building the peptide chain.

Step 4 – Deprotection: the protection group is now removed from the acid at the end of the chain so it can react with the next acid to be added on. The new acid is then protected (Step 2) and the cycle continue until a chain of the required length has been synthesized.

Step 5 – Polymer removal: Once the desired peptide has been made the bond between the first amino acid and the linkage agent is broken to give the free peptide. Peptides synthesized at Massey University are widely used in medical research, as they can be synthesized quickly and accurately.

A peptide is a chain of special acids called amino acids linked together by bonds known as amide bonds. A protein consists of one peptide folded in a particular way, or several peptides folded together. Such peptides are synthesized very rapidly within living cells, but until recently could only be artificially synthesized in very long, slow processes that had poor yields and gave impure products. Recently a new technique known as solid phase peptide synthesis (SPPS) has been developed. SPPS results in high yields of pure products and works more quickly than classical synthesis, although still much more slowly in than living cells. This technique is discussed below.

Uses of the 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 they need, this peptide can be synthesized and given to them. In addition, the amino acids in an active peptide can be altered to make analogues of the original peptide. If the analog is more biochemically active than the original peptide it is known as an agonist and if it has the reverse effect is known as an antagonist. Contraceptives have been made by synthesizing the antagonists of fertility peptides.

Diagnostic peptides: Peptides can be designed that change color 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.

The SPPS laboratory at Massey University has supplied peptides for research purposes to universities, CRIs, research institutes and private industry since 1973. These peptides have been used for medical research into areas such as heart disease, leprosy and tuberculosis.

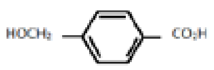
The laboratory itself is involved in research into and development of synthetic methods and peptide production.

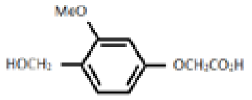
Methods of peptide synthesis – an overview

Peptide synthesis is much more complicated than simply forming amide bonds by mixing the desired amino acids together in a test tube. With twenty natural amino acids and a number of unnatural ones as well the possible combinations formed with this technique are numerous.

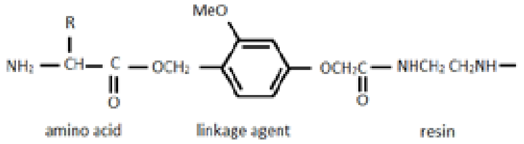
This complexity makes the synthesis of peptides both fascinating and challenging. If solutions containing two amino acids are mixed together, four different dipeptides (as well as other longer peptides) will be formed. (e.g. for a mixture of glycine and alanine the four dipeptides would be glygly, glyala, alagly, alaala. In this representation of peptides the free amino group or N-terminus is on the left hand amino acid and the free carboxylic acid group, the C-terminus is at the right hand end.). To ensure that only the desired dipeptide is formed the basic group of one amino acid and the acidic group of the other must both be made unable to react. This 'deactivation' is known as the protection of reactive groups, and the group that is unable to react is spoken of as a protected group. In classical organic synthesis the acids are protected, allowed to react and deprotected, then one end of the dipeptide is protected and reacted with a new protected acid and so on. In SPPS the amino acid that will be at one end of the peptide is attached to a water-insoluble polymer and remains protected throughout the formation of peptide, meaning both that fewer protection / deprotection steps are necessary and that the reagents can easily be rinsed away without losing any of the peptide.

Step 1 – Attaching an amino acid to the polymer: Peptide chains have two ends, known respectively as the N-terminus and the C-terminus 3, and which end is attached to the polymer depends on the polymer used. This article assumes that polyamide beads are used and hence that the C-terminus of the peptide is attached to the polymer. The attachment is done by reacting the amino acid with a linkage agent and then reacting the other end of the linkage agent with the polymer. This means that a peptide-polyamide link can be formed that will not be hydrolyzed during the subsequent peptideforming reactions. Common linkage agents are di- and tri-substituted benzenes such as those shown below:

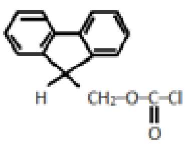




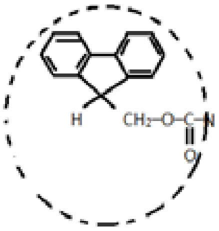
These then join the C-terminus amino acid and resin together as follows:



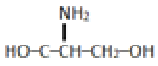
Step 2 – Protection: The next amino acid also needs to have its amino group protected to prevent the acids reacting with each other. This is done by protecting it with FMOC (9-fluorenylmethoxycarbonyl). In addition, any amino acid side chains that are aromatic, acid, basic or highly polar are likely to be reactive. These also must be protected to prevent unwanted branched chains from forming. There are four main groups used in this way: tBu (a tertiary butyl group), Trt (a triphenylmethyl group), tBoc (a tertiary butyloxycarbonyl group) and PMC (a 2, 2, 5, 7, 8-pentamethylchroman-6-sufonyl group). Examples of a carboxyl group protected with FMOC and examples of the different types of side chain protection are given below:

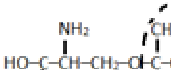


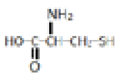
FMOC



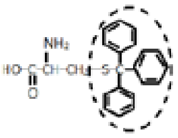
an amino acid protected with I



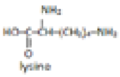




cysteine

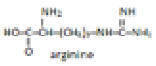


cysteine protected with Trt

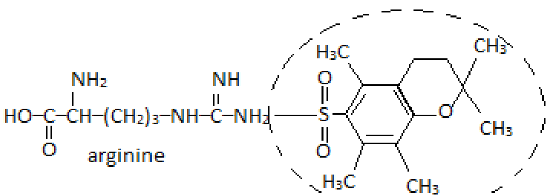


lysine

lysine protected with tBOC



arginine

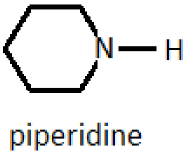


arginine

arginine protected with PMC

Step 3 – Coupling: The FMOC protected amino acid is then reacted with the last amino acid attached to the polyamide. The reaction is catalyzed by DCC (1, 3- dicyclohexylcarbodimide), which is itself reduced to DCU (1, 3- dicyclohexylurea). The reaction is shown in figure (RCOOH represents the FMOC protected acid and H_2NR represents the reactive end of the growing peptide chain).

Step 4 – Deprotection: Excess DCC is washed off the insoluble polymer with water, then the FMOC group removed with piperidine (a cyclic secondary amine). This is a trans amidification reaction.



Step 2 to 4 are repeated as each new amino acid is added onto the chain until the desired peptide has been formed.

Step 5 – Polymer removal: Once the peptide is complete it must be removed from the polyamide. This is done by cleaving the polyamide-peptide bond with a 95% solution of trifluoro acetic acid (TFA).

The side-chain protecting groups are also removed at this stage.

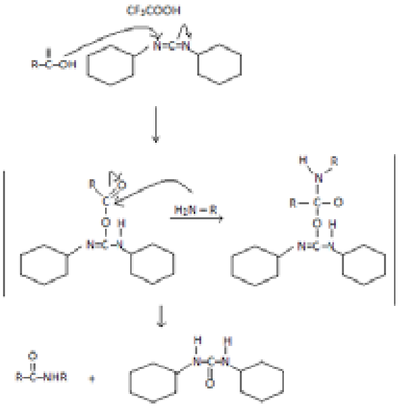


Figure – The amino acid coupling reaction

Advantages:
The potential advantages of these proposed synthetic strategies are

- Speed, simplicity and yield
- By the use of insoluble polymeric support, the scaffolded peptide also be insoluble, and it can be readily washed by simple filtration without transfer to other containers which avoids physical losses.
- All the chemical reactions during synthesis, deprotection, neutralization and coupling reactions would be driven to completion by an excess and high concentration of soluble reagents.
- It would be possible to efficiently remove excess reagents and soluble byproducts by washing using large excess of solvents, to effect a rapid partial purification at each step without the need of difficult and time consuming isolation and crystallization procedure after each synthetic cycle.

- A complete automation of the entire synthesis is possible. At the same time, some of the potential disadvantages of the stepwise SPPS involve the incomplete reactions and the gradual build up of insoluble side reaction byproducts.