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COMPARATIVE STUDY OF SYNTHESIS OF AMPICILLIN BY DIFFERENT SYNTHETIC ROUTES

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ABSTRACT

Ampicillin is one of the key antibiotics which falls under the category of β-lactam and are commonly used to manage various bacterial infections. Ampicillin is synthesised by conjugating a particular side chain to the 6-aminopenicillanic acid (6-APA) backbone which enhances its antibacterial action and oral efficacy. This review describes how ampicillin is synthesized and its mechanism of action as well as the different methods of synthesis. The paper compares some of the chemical and enzymatic synthetic pathways. Chemical techniques are mixed anhydride, acid chloride and N-carboxyanhydride techniques and enzymatic techniques mainly involve the use of penicillin acylase which selectively reacts. Each of these ways has its advantages and disadvantages regarding the yield, cost, complexity, and industry application. Enzymatic methods are more selective, easier, and environmentally friendly as opposed to chemical methods which are unfriendly, yield high and need harsh conditions and multiple steps. In general, the contemporary synthesis is moving towards greener and more effective methods based on enzymes. This study provides a clear comparison of different synthetic routes and helps in understanding the development and improvement of ampicillin production.

KEYWORDS: Ampicillin, 6-APA, chemical, enzymatic, β-lactam, bacterial infections etc.

1. INTRODUCTION

The discovery of penicillin changed the history of medicine.[1] Penicillin G was the first antibiotic used widely for the treatment of bacterial infections.[2] But it had several limitations. It was unstable in acidic conditions.[3] It showed poor oral absorption.[4] It also had limited activity against gram-negative bacteria. These problems created the need for improved penicillin derivatives.[5]

This led to the development of semisynthetic penicillin. The isolation of 6-APA made chemical modification of the penicillin nucleus possible.[6] By changing the side chain attached to 6-APA, new antibiotics with better properties could be prepared.[7] Amongst these, one of the most significant broad-spectrum penicillins was ampicillin.[8]

Ampicillin was first synthesized in the early 1960s.[9] It was obtained by attaching a D-α phenylglycine side chain to the 6-APA core.[10] The structural change enhanced the use of antibacterials in both gram-positive and gram-negative bacteria.[11]

Ampicillin quickly became an important drug in clinical practice.[12] It was used for respiratory, urinary and gastrointestinal infections. It was also effective in many systemic bacteria disease.[13] Because of its safety, low cost and broad activity, ampicillin became widely used in many countries.[14] It also became a model compound for the development of other aminopenicillins and β-lactam antibiotics.[13]

1.1. Structural basis and semisynthetic design of ampicillin

Ampicillin is a β-lactam antibiotic.[15] The structure includes a b lactam ring which is joined to thiazolidine ring.[16] This is a fundamental framework that is necessary in antibacterial activity. The alteration in this nucleus has a strong impact on biological activity.[17] The important semisynthetic penicillin intermediate is 6-APA.[18] The structural modification of penicillin was made possible with the isolation of 6-APA. It permits selective reaction of various side chains without dismantling the β-lactam ring. This finding underpinned the semisynthetic penicillin chemistry of the modern day.[19] The side chain has an a -amino group in ampicillin. This enhances the polarity of the molecule and assists in carrying it through gram negative porin channels thus broadening its scope of antibacterial action. The amino group also enhances acid stability of the β-lactam ring and promotes oral bioavailability and clinical usefulness.[21][22] The β-lactam ring is very sensitive chemically. It is not stable in acidic and basic conditions. It is also heat and nucleophilic sensitive. Due to this reason, initial synthetic techniques needed mild conditions. It could need the protection of groups.[23] Subsequently, enzymatic techniques were proposed.[24] Selective side-chain coupling in water was made possible using penicillin acylase. These reactions were done in moderate pH and temperature.[23][25] This minimized the requirements of the protection of groups and harsh reagents. Consequently, the enzymatic synthesis gained relevance in the production of ampicillin by green and industrial methods.[25] Accordingly, the production of ampicillin was subjected to more complex chemical pathways to a more selective and sustainable enzymatic synthesis. The development of synthetic chemistry and biocatalysis in the production of antibiotics is indicated by this evolution.

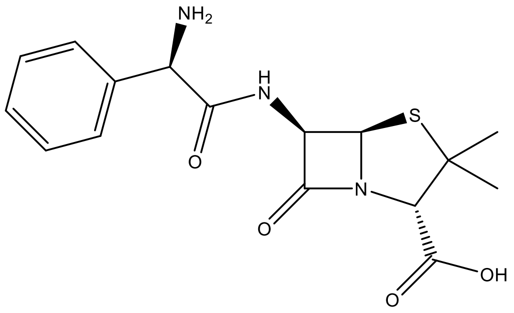

Fig. 1: Ampicilin.

1.2. Moa of ampicillin

Ampicillin is a beta-lactam antibiotic. It acts by preventing the assembly of bacterial cell wall.[26][27] Bacteria survive and shape with the help of the cell wall. Ampicillin gets attached to enzymes (penicillin-binding proteins(PBPs)[26] inside the bacterium. These enzymes are used in the synthesis of the peptidoglycan wall of the cell wall. The bacterial cell is provided with strength and structure by peptidoglycan.[28] PBPs usually assist in linking peptidoglycan chains. This is referred to as cross-linking. It renders the cell wall firm and solid.[29] Ampicillin inhibits this action by binding itself to the active site of these enzymes.[26] In the absence of inhibition of PBPs, the synthesis of peptidoglycan is defective. The cell wall is becoming thin and weak.[30] It is unable to sustain the cellular pressure. Water gets into the bacterial cell. The weakened wall breaks. The cell bursts and dies. This is referred to as bacterial lysis.[31][32]

1.2.1. Gram positive bacteria

Gram positive bacteria possess a thick layer of peptidoglycan. They lack an external envelope. Due to this, the direct exposure of the cell wall to the environment occurs.[33] The ampicillin is able to enter the PBPs on the cytoplasmic membrane with ease. The production of cells is halted once ampicillin blocks PBPs. The peptidoglycan layer is made fragile. The cell wall becomes weak. Water gets to the cell and the osmotic pressure makes the cell rupture and die.[31][32] Some of the gram-positive bacteria such as staphylococcus aureus are sensitive to ampicillin. Its effect can be enhanced when it is used with other agents.[34] Nevertheless, resistance may develop. In the case of Staphylococcus aureus(MRSA), the gene (mecA) that produces an altered PBP(PBP2a) is observed. This protein is not very sensitive to beta-lactam antibiotics and this lowers the efficacy of ampicillin.[35]

1.2.2. Gram-negative bacteria

Gram-negative bacteria are more complex in cell envelope. It comprises an outer membrane, a periplasmic space, and a thin layer of peptidoglycan. It is not exposed to the peptidoglycan layer.[36] Ampicillin has to pass through the outer membrane in order to reach PBPs.[37] It gets into the cell via small water filled channels known as points.[38] Once it enters the outer membrane, it gets into the periplasmic space and attaches to PBPs.[39] This inhibits cell wall synthesis and suppresses bacterial cell wall. The outer membrane is used as a protective shell and restricts the entry of drugs. This makes a large number of antibiotics ineffective. Other mechanisms also mediate the resistance in gram-negative bacteria.[39][40] These are beta-lactamase enzymes which destroy the antibiotic, efflux pumps which expel the drug out of the cell and alteration of the porin channels which decrease entry of the drug. Due to this mechanisms of resistance and barriers, gram-negative bacteria tend to be more difficult to treat compared to Gram-positive bacteria.[41][42] As time flowed, several synthesis pathways to ampicillin were described. These are classical chemical pathways and contemporary enzymatic pathways. Techniques like activated ester coupling, a process of mixed anhydride formation, N-carboxyanhydride reaction, Dane salt pathways and enzymatic one-pot process were invented. Both approaches vary in terms of complexity and cost, productivity and manufacturing efficiency.[43-50] This review is based on the various synthetic approaches that have been reported on ampicillin. They include both chemical and enzyme based routes. Their strengths and weaknesses are compared. This is to give a clear general view of the methods of laboratory and industrial syntheses. This will assist in the development and variation of ampicillin production.

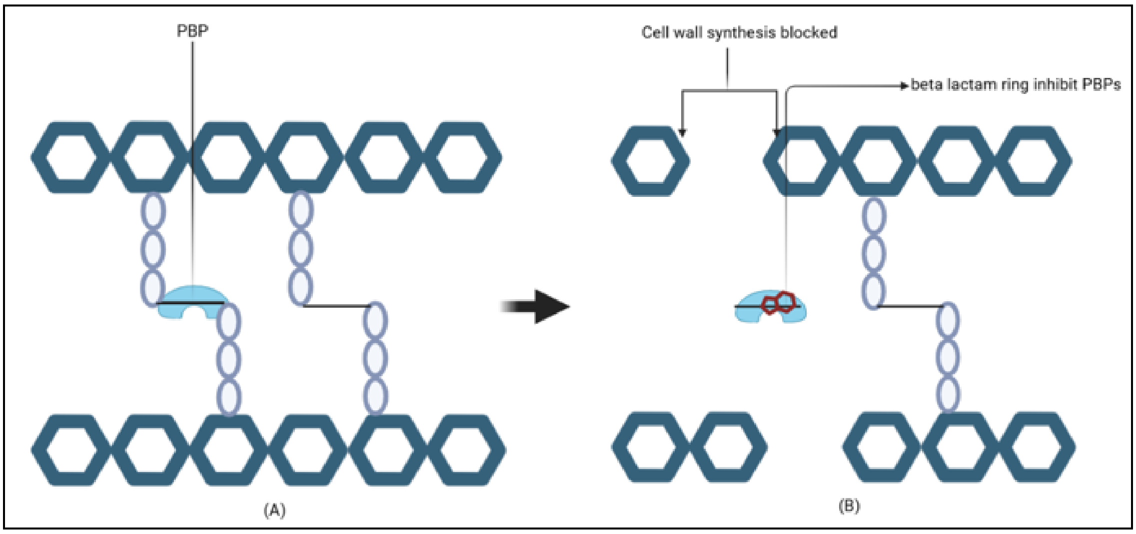

Fig. 2: (A)normal bacterial cell wall (B)cell lysis and bacterial cell death.

2. SYNTHETIC ROUTES

2.1. Chemical synthesis

2.1.1. Mixed anhydride chemical coupling route

Step 1- N-protection of D-phenylglycine (Cbz protection)

D-phenylglycine was dissolved in aqueous medium at acidic pH and cooled to 0 C, benzyl chloroformate was added in the presence of sodium hydroxide and maintained mildly basic pH and the resulting product was N-benzyloxycarbonyl-protected phenylglycine. The situation was acidified to give the shielded amino acid, which was recrystallized.

Step 2- formation of mixed anhydride of protected phenylglycine

Cbz-capped phenylglycine was dissolved in dry acetone and reacted in low temperature in the presence of triethylamine and ethyl chloroformate. Triethylamine hydrochloride rapid precipitation was taken as a sign of formation of the mixed anhydride that was not isolated.

Step 3- coupling with 6-APA

The mixed anhydride solution was placed at very low temperature and reacted with 6-APA in aqueous sodium bicarbonate. Reaction The reaction was carried out easily to obtain the Cbz-protected ampicillin intermediate in mild conditions.

Step 4- Purification and work-up of protected intermediate

Solvents extraction and pH controlled phase transfers were done to the reaction mixture to eliminate impurities. After re-acidification and solvent removal, the protected aminopenicillin intermediate was obtained.

Step 5- Catalytic hydrogenolysis(deprotection)

Hydrogenation of the protected intermediate was done in aqueous medium with palladium-barium carbonate catalyst with hydrogen pressure in the atmosphere. This was done to eliminate the benzyloxycarbonyl protecting group,which liberated free ampicillin.

Step 6- Final isolation and crystallization

Once the catalyst was removed, the solution was acidified, washed and thoroughly adjusted to the isoelectric point. The solution was then concentrated to cause the crystallization of ampicillin which was harvested as fine needle like crystals.[43]

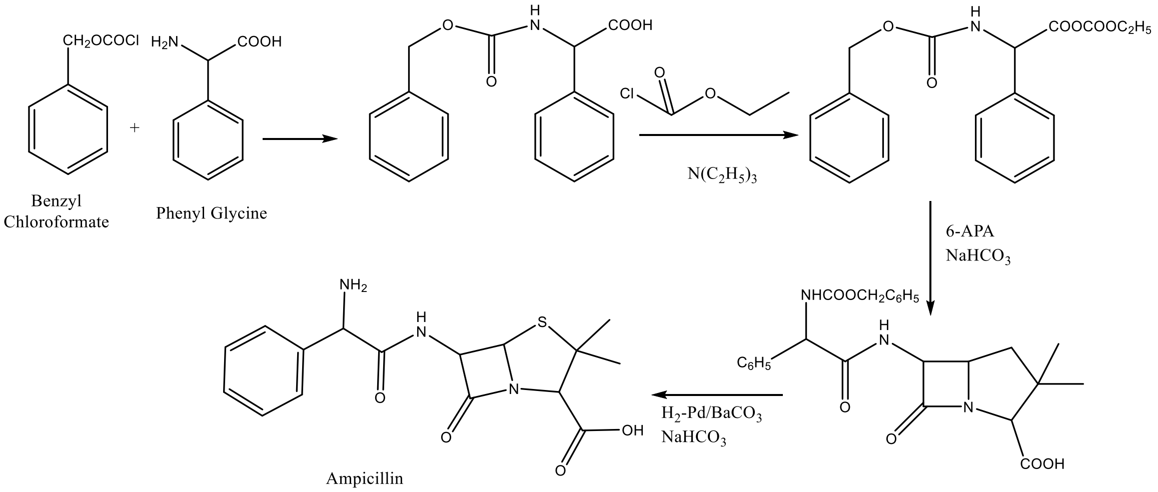

Fig. 3: Ampicillin synthesis through Mixed anhydride chemical coupling route.

2.1.2. Activated phenylglycine salt coupling route

Ampicillin was synthesized using an industrial semisynthetic method that used D-phenylglycine as a Dane salt then mixed anhydride coupled with 6-APA.

Step 1-Dane salt formation

D (-)-phenylglycine was reacted with p-nitroacetoacetanilide to yield a Dane salt form of the product. The Dane salt is a protected and activated side-chain precursor in which acylation is to take place subsequently.

Step 2- Activation of mixed anhydride

Dane salt was dissolved in methylene chloride with dimethylacetamide as co-solvent, and ethyl chloroformate was added to it at low temperature (approximately -20 to -30 C) in the presence of tertiary base (e.g., N-methylmorpholine), to produce the respective mixed anhydride.

Step 3- Silylation of 6-APA

The 6-APA was silylated with a chlorosilane reagent(e.g- trimethylchlorosilane or dimethyldichlorosilane) in the presence of triethylamine to enhance solubility and prevent nucleus degradation, β-lactam.

Step 4- Coupling to form Ampicillin intermediate

The mixed anhydride was reacted, under sub-zero temperature (c. -30 C), with the silylated 6-APA to produce the N-protected ampicillin intermediate by selective acylation.

Step 5- hydrolysis and product isolation

Free ampicillin was released by acidic hydrolysis (with a pH of about 1-1.5) which was collected in aqueous phase and crystallised close to its isoelectric point(pH approx. 5-5.5).[44]

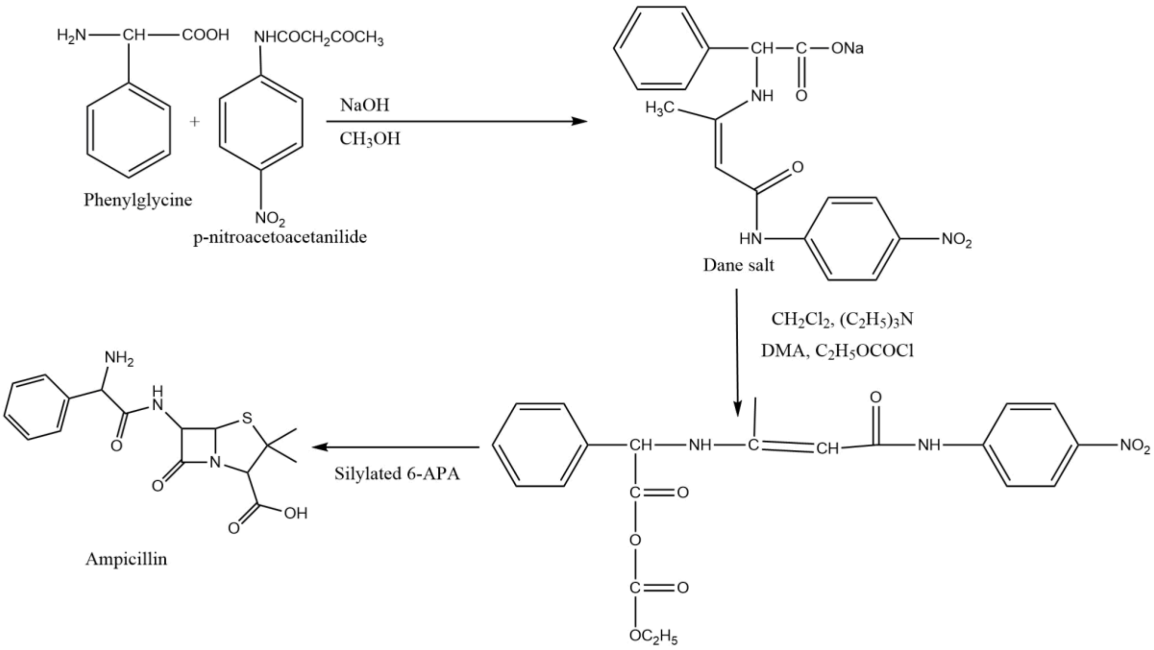

Fig. 4: Ampicillin synthesis from Activated phenylglycine salt coupling route.

2.1.3. Protected ester-trityl strategy

Ampicillin was synthesized through a protected-ester semisynthetic strategy starting from a phenacyl ester derivative of benzylpenicillin.

Step 1- Activation of benzylpenicillin Phenacyl Ester

The activation of benzylpenicillin Phenacyl Ester takes place on first step. Benzylpenicillin phenacyl ester was employed and dissolved in the anhydrous aromatic solvent then it was subjected to pyridine at the low temperature. Phosphorous pentachloride (PCl5) was used to activate the carboxyl group to give a reactive acyl intermediate in cold conditions.

Step 2- coupling

D-Phenylglycine is protected and then coupled to the product of step 1. The obtained penicillin derivatives were reacted with potassium N-trityl-D-phenylglycinate which resulted in the side-chain acylated product N-(N-trityl-D-phenylglycyl) benzylpenicillin phenacyl ester.

Step 3- Aminolysis to give a- N-tritylamino intermediate

The coupled ester continued to be reacted with butylamine to produce a derivative of a-N-tritylamino benzylpenicillin phenacyl ester. This was purified by using silica gel chromatography, significant to note was the fact that this process was not carried out in high yield.

Step 4- Thiophenolate-mediated deprotection

Sodium thiophenolate treatment of the a-N-tritylamino was then done in a polar aprotic solvent. This step produced the sodium salt of the work-up and precipitated aminopenicillin derivative after the protection.

Step 5- Final Trityl Deprotection and isolation of Ampicillin

At a room temperature, the trityl protecting group was eliminated with the help of trifluoroacetic acid (TFA). A later neutralization and aqueous treatment gave ampicillin as end-product that was freeze-dried.[45]

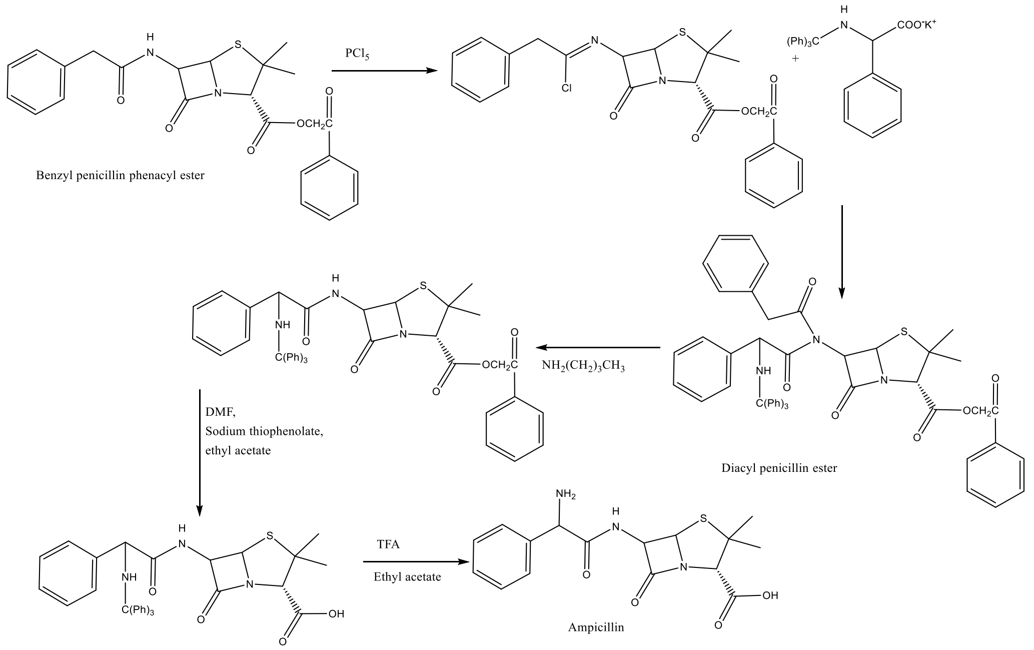

Fig. 5: Ampicillin synthesis from Protected ester-trityl strategy.

2.1.4. Trichloroethyl ester-thiocarbonyl route

Step 1- Activation of Trichloroethyl Penicillin

The trichloro ethyl ester of penicillin G was dissolved in aromatic solvent without water, and then the pyridine was added to the solution under low-temperature conditions. Phosphorus pentachloride was used to activate the b lactam nucleus and produced a reactive intermediate suitable in the introduction of the side chains.

Step 2- Coupling with thiocarbonyl-protected D-Phenylglycine

It reacted the activated penicillin derivative with potassium trichloroethoxythiocarbonyl-D-a -phenylglycinate. This reaction added the ampicillin chain of the drug in a protected form to produce a thiocarbonyl-masked aminopenicillin intermediate.

Step 3- formation and purification of the protected penicillin intermediate

Concentration and purification of the crude coupling mixture was done through silica gel chromatography. Cyclic thiocarbonyl-protected penicillin derivative was obtained as one of the major intermediates, whereby there was an assurance of eliminating any side products prior to deprotection.

Step 4- Reductive deprotection using activated zinc

The protected trichloroethyl aminopenicillin derivative was treated with activated zinc in a cold acidic solvent mixture. This reductive step removed the trichloroethyl and thiocarbonyl protected groups, releasing free ampicillin.

Step 5- Final isolation as Ampicillin hydrochloride

After filtration, the mixture was acidified and subjected to aqueous workup and organic extraction. Drying and solvent removal afforded ampicillin hydrochloride as the final isolated product.[46]

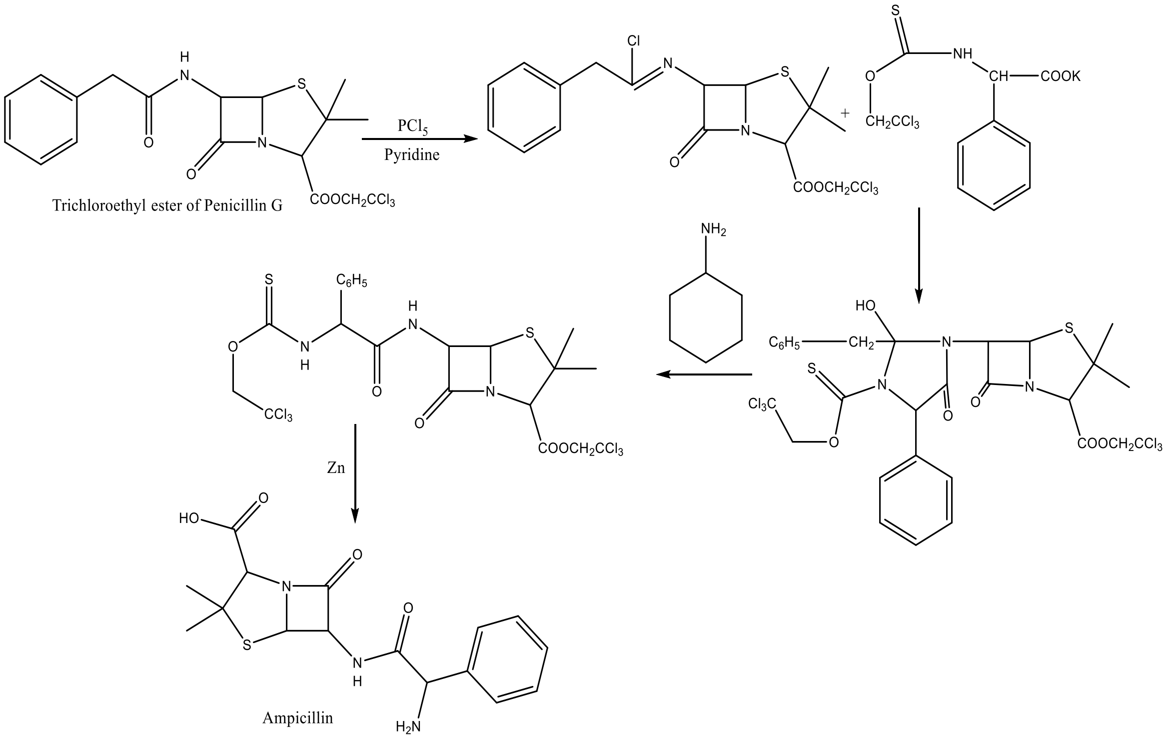

Fig. 6: Ampicillin synthesis through Trichloroethyl ester-thiocarbonyl route.

2.1.5. Direct acid chloride coupling of silylated 6-APA

Ampicillin was produced in a direct semisynthetic acylation plan of 6-APA. The first step in this approach was the silylation of 6-APA in methylene chloride with hexamethyldisilazane(HMDS) under reflux, which enhances solubility by increasing the number of silyl groups on the nucleus. The reaction mixture was allowed to cool in the presence of nitrogen then N,N-dimethylaniline was added as a base system. The substituent of the activated side chain D(-)-phenylglycyl chloride hydrochloride was added portionwise to the reaction at low temperature(0-2 C) with the result in the establishment of the ampicillin framework through acid chloride coupling.

The reaction mixture was transferred into aqueous medium, and ampicillin was isolated by precipitation as the β-napthalene sulfonate salt under strongly acidic conditions(pH approx.

1.5-1.7). Finally, treatment of the wet salt with triethylamine in aqueous isopropanol afforded anhydrous ampicillin. This patent represents an efficient industrial route based on direct acyl chloride acylation of silylated 6-APA.[47]

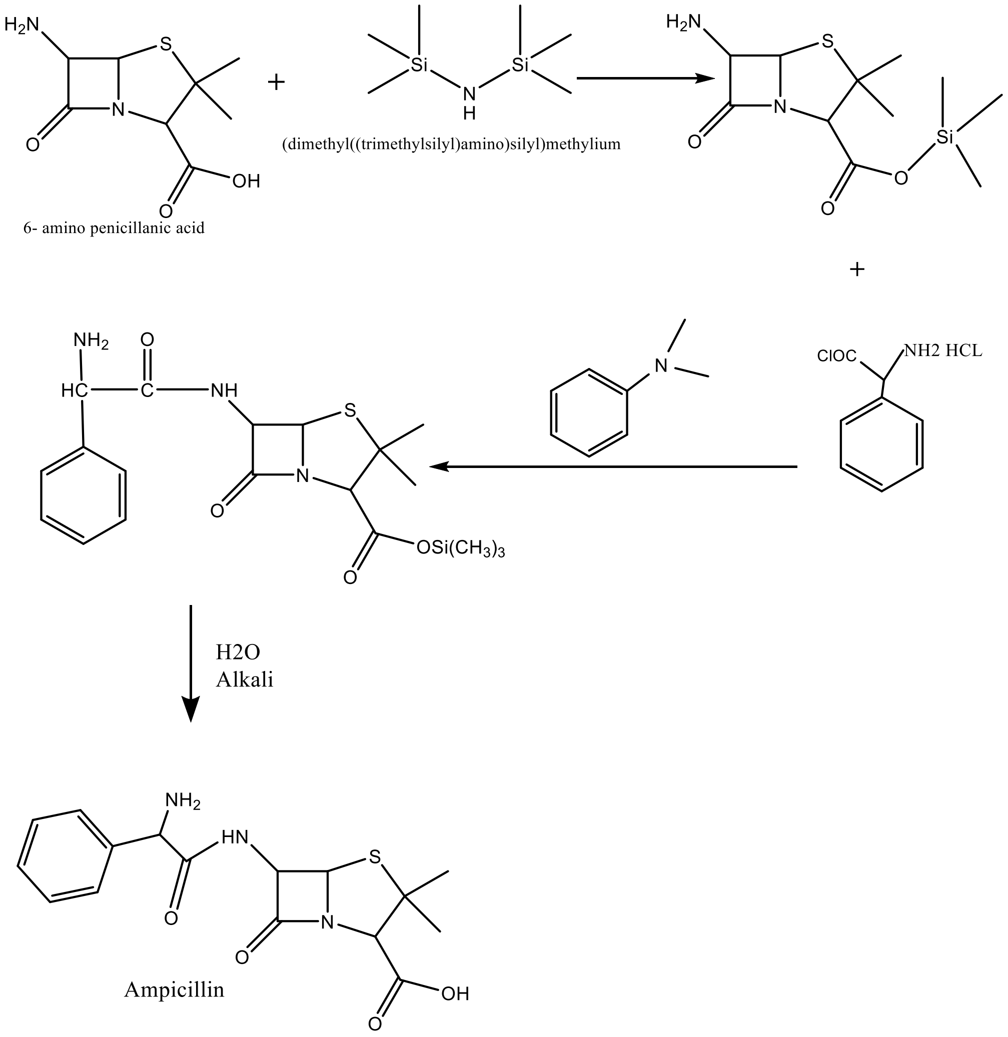

Fig. 7: Ampicillin synthesis From Direct acid chloride coupling of silylated 6-APA.

2.1.6. N-carboxyanhydride(NCA) coupling route

Ampicillin was synthesized in direct semisynthetic reaction between 6-APA and D-phenylglycine N-carboxyanhydride(NCA) in aqueous condition. Here, 6-APA was dissolved in water and the pH was adjusted to a level of about 5.0 and then the phenylglycine NCA was added as the solution was vigorously stirred by the addition of the phenylglycine NCA. This was carried out under mild conditions to produce ampicillin that was first isolated as ampicillin trihydrate after removal by taking the solution to vacuum conditions and crystallizing it. Excess 6-APA was eliminated by using an anion-exchange resin which enhances purity of the product. The subsequent trihydrate crystals were further reduced to anhydrous ampicillin by placing the product in 85% isopropanol and drying it at high temperature. This is an industrially useful process that involves NCA-based side chain acylation of controlled hydrate to anhydrate conversion.[48]

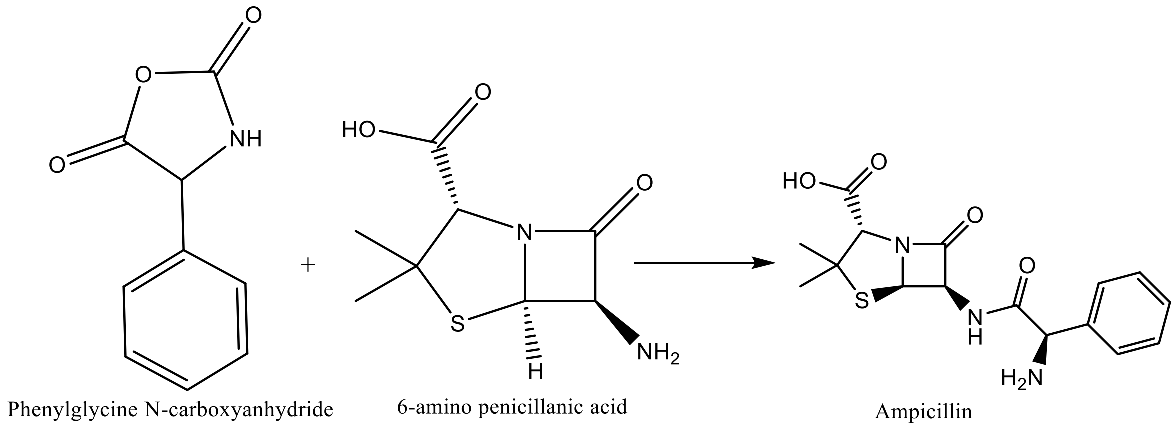

Fig. 8: From D-phenylglycine N-carboxyanhydride(NCA).

2.2. Enzymatic synthesis of Ampicillin from penicillin G

2.2.1. Two-step, one-pot enzymatic synthesis(from penicillin G Potassium salt)-

Step 1- enzymatic hydrolysis of penicillin G potassium salt

Penicillin G potassium salt was enzymatically hydrolysed in an aqueous phosphate buffer system containing ethylene glycol as a co-solvent. Immobilized penicillin acylase catalysed the selective cleavage of the phenylacetyl side chain, generating 6-APA in situ without isolation.

Step 2- Enzymatic side-chain acylation

Without isolation of the intermediate, D-phenylglycine methyl ester was introduced into the same reaction medium. The immobilized penicillin acylase catalysed the kinetically controlled acyl transfer between 6-APA and the activated side-chain donor to form ampicillin.

Step 3- One-pot cascade strategy

Both hydrolysis and coupling reactions were conducted sequentially in a single vessel using the same enzyme system. The present of ethylene glycol reduced competing hydrolysis reactions and enhanced product yield under mild conditions.

Step 5- Product recovery

After completion of the enzymatic reaction, the mixture was diluted with water to dissolve substrates and products completely. Ampicillin formation was confirmed and quantified by HPLC analysis, and the product was isolated from the aqueous phase.[49]

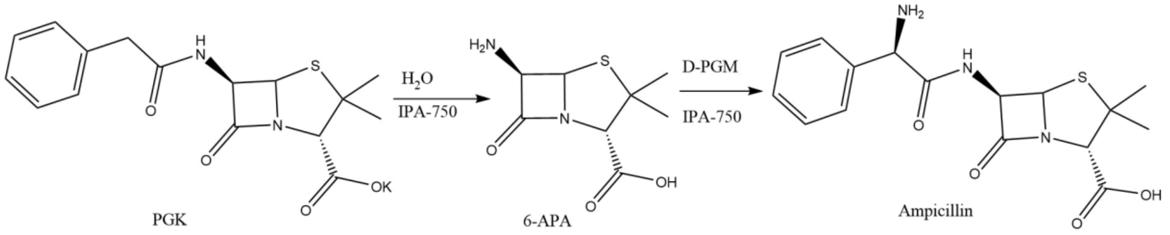

Fig. 9: Two-Step, One-Pot enzymatic Synthesis of Ampicillin.

2.2.2. One-Pot, Two-Step Synthesis(from PenG)

Blum *et al.* report two synthesis procedure of ampicillin using same substance.

One-pot, one-step enzymatic synthesis

Ampicillin was synthesized directly from penicillin G in a single reaction vessel using a dual-enzyme system. Penicillin G was dissolved in phosphate buffer (pH 7) and reacted with D-phenylglycine methyl ester as the acyl donor. The transformation was initiated by the simultaneous addition of immobilized penicillin G acylase and α -amino ester hydrolase. The reaction was carried out at ambient temperature with continuous stirring, allowing concurrent hydrolysis and side-chain acylation to proceed in one step. Under optimized substrate ratios, efficient conversion to ampicillin was achieved within a short reaction time.

One-pot, two-step enzymatic synthesis

This synthesis was conducted sequentially in the same reactor. In the first step, penicillin G was incubated in phosphate buffer with immobilized penicillin G acylase at room temperature, leading to enzymatic hydrolysis of the side chain. In the second step, D-phenylglycine methyl ester was introduced as the acylating agent, followed by the addition of a α -amino ester hydrolase. The reaction pH was carefully maintained in the neutral range during this stage to favour amide bond formation. The conversion to ampicillin was completed rapidly after the second enzyme addition.[50]

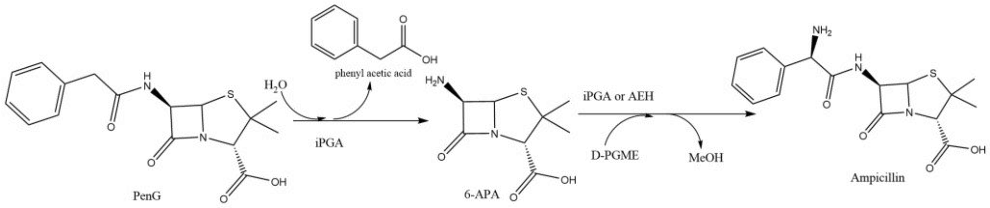

Fig. 10: One-pot, two-enzyme direct conversion of penicillin G to ampicillin.

Table 1: Comparative analysis of different synthetic routes.

	Synthetic route	**Starting material**	**Reaction mechanism**	**Yield(%)**	**Advantages**	**Disadvantages**	**Ref**
Chemical synthesis	Mixed anhydride chemical coupling route	Benzyl chloroformate	Mixed anhydride coupling+ catalytic hydrogenolysis	30-35	First reported synthesis, mild conditions	Low yield, complex protection/deprotection, expensive Pd catalyst	[43]
	activated phenylglycine salt coupling route	6-APA + Dane salt of D-phenylglycine	Dane salt activation+ mixed anhydride coupling with silylated 6-APA	75-77	High yield recyclable β-ketoamide scalable	Dane salt preparation required, low temperature (-20 to -30 º C) needed.	[44]
	protected ester-trityl strategy	Benzylpenicillin phenacyl ester	PCl_5 activation + aminolysis + thiophenolate deprotection	20-21	Direct from penicillin G derivative	Very low yield, multiple purification steps, complex	[45]
	Trichloroethyl ester-thiocarbonyl route	Trichloroethyl ester of penicillin G	PCl_5 activation + thiocarbonyl + protection + reductive deprotection	35	Avoids 6-APA isolation	Low yield, multiple protection/deprotection steps	[46]
	Direct acid chloride coupling of silylated 6-APA	6-APA + D-phenylglycyl chloride	Silylation with HMDS + acid chloride coupling	86	Highest yield, simple silylation, industrial route, scalable	Requires HMDS, acid chloride preparation	[47]
	N-carboxyanhydride(NCA) coupling route	6-APA + D-phenylglycine (NCA)	Direct NCA coupling + trihydrate crystalization	87	Very high yield, simple coupling, no protection needed	Requires NCA preparation, trihydrate-to-anhydrate conversion	[48]
Enzymatic synthesis	Two-step, one-pot enzymatic synthesis	penicillin G Potassium salt	Enzymatic hydrolysis+acylation with immobilized penicillin acylase	57.3	Moderate yield, one-pot process, ethylene glycol reduces competing hydrolysis	Requires immobilized enzyme, ethylene glycol needed	[49]
	One-pot, one-step enzymatic synthesis	Penicillin G	Dual-enzyme system(PGA+α -amino ester hydrolase)	39	Single-step conversion, ambient conditions, mild	Lower yield, dual-enzyme system required	[50]
	One-Pot, Two-Step enzymatic Synthesis	Penicillin G	Sequential enzymatic(PGA then α -amino ester hydrolase)	47	Higher yield than one-step, better control	Two-step sequential, pH control critical	[50]

3. Comparative Discussion

The production of ampicillin has been transformed by the traditional chemical technique to the more improved enzymatic technique. Chemical methods are often more yielding and necessitate harsh reaction conditions, several steps of protection and deprotection as well as organic solvents and are therefore not as environmentally friendly. Conversely, enzymatic synthesis with penicillin acylase has the benefits of high selectivity, mild reaction conditions, and low level of by-products. Nonetheless, enzymatic techniques can have drawbacks of high enzyme prices and average product. In general, the contemporary tendencies prefer enzymatic and chemoenzymatic methods, which are efficient and sustainable.

4. CONCLUSION

The review is a provision of an overview of the various synthetic routes progressed towards ampicillin. Chemical and enzymatic methods were studied in order to learn how they evolved, how effective they are, and how applicable it is in practice. The early chemical approaches contributed to the early production of ampicillin. Nevertheless, they were frequently multi-protection-deprotection reactions involving severe reagents and inappropriate reaction conditions and were rather complicated and not applicable to large-scale production in the long run. Conversely, enzymatic and chemo-enzymatic are significant progresses in the production of ampicillin. These reactions involve certain enzymes like penicillin acylase to obtain selective reactions and work under mild conditions. They also decrease the number of groups that have to be guarded, decrease side reactions and enhance the safety of the processes. Greener chemistry is also assisted by enzymatic methods, which do not require a lot of energy, and toxic solvents and reagents are also minimized. Relative study of the documented paths demonstrates apparent distinctions in yield, complexity, scalability and industrial feasibility. Silylated 6-APA coupling and N-carboxyanhydride high-yield chemical coupling routes are very efficient, and the enzymatic routes have superior selectivity and enable environmental friendliness and ease of operation. All approaches have certain strengths and weaknesses based on the purpose of their use. On the whole, the shift in classical approaches in chemical synthesis to the contemporary enzymatic strategies can be viewed as the move of the pharmaceutical production toward more efficient and sustainable methods.

The perspectives of the future are hence predicted to rely on a enhanced enzyme engineering, system streamlining, and combined chemo-enzyme systems. The innovations can also produce further improvements in yield, cost-cutting, and environmental friendly industrial production of ampicillin. The review offers a definite comparative frame of work on the heterogeneity in synthetical approaches to ampicillin. It can also become a handy source of information to the researchers and industries that worked on the synthesis of β-lactam antibiotics and the development of their processes.

5. Abbreviations

6-APA: 6-Aminopenicillanic Acid
AMP: Ampicillin
PGA: Penicillin G Acylase
DMF: Dimethylformamide
DMSO: Dimethyl sulfoxide
THF: Tetrahydrofuran
Cbz: Carbobenzyloxy

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