ß-Lactam Antibiotics: The Past, Present, and Future

Allen Chen[1], Brian Fu[2], Andrew Liang[3], Karankumar Mageswaran[1], Ayeeshi Poosarla[1]

[1] Mission San Jose High School, Fremont, CA

[2] American High School, Fremont, CA

[3] The College Preparatory School, Fremont, CA

ABSTRACT In 1928, the discovery of penicillin revolutionized the field of medicine. As the first reported β-lactam antibiotic, penicillin laid the foundation for modern antibiotics. β-lactams, 4-membered cyclic amides, can be used for the prevention and treatment of bacterial infections. Since the discovery of penicillin, many β-lactams have been discovered from natural sources, and modern methodologies in chemical synthesis have powered the design for synthetic β-lactams. This is significant in supplying the continued need for novel antibiotics in the treatment of antibiotic resistant bacteria.

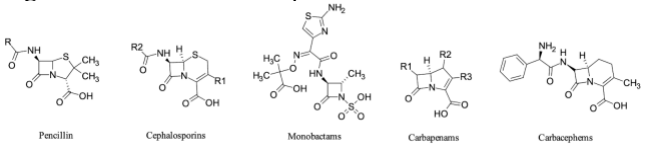
β-LACTAM-HISTORY

Penicillin, a β-lactam antibiotic, was discovered by Alexander Fleming. Fleming was growing a culture of Staphylococcus and observed that Penicillium notatum, a fungus, had contaminated the dish; a compound secreted by the fungus, later identified as penicillin, inhibited growth of the Staphylococcus culture. Years later, the β-lactam was isolated as the active component of penicillin, responsible for its antimicrobial activity. Today, as a result of the discovery of penicillin and the reactivity of β-lactams, many antibiotics contain the β-lactam moiety.[1]

Since the initial discovery of penicillin, others have sought to synthesize, purify, and characterize it. Penicillin continued to advance throughout the twentieth century. Specifically, its antiseptic capabilities, initially discovered by N.G. Heatley using mice were researched and developed.[2] In 1941 during World War II, penicillin's antibiotic capabilities proved to be crucial as it saved millions of lives. Without effective antibiotic treatment, people were dying because of common bacterial infections. At this time, Florey and Heatley enlisted the help of the U.S. government to take on the mass production of penicillin, which was made possible with deep-fermentation tanks.[3]

Following the discovery of penicillin, other β-lactam-containing natural products including penicillin derivatives, cephalosporins, monobactams, carbapenems, and carbacephems have been discovered. The structural features of the different classes of β-lactam antibiotics are shown in Figure 1.

Figure 1: Various classes of β-lactam antibiotics.



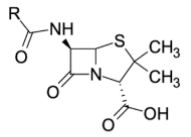
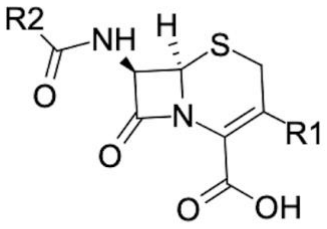
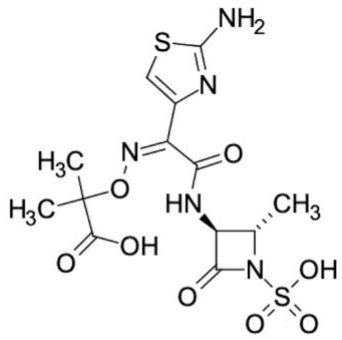
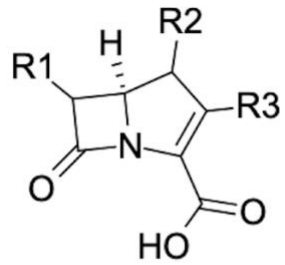
Cephalosporins are β-lactams which have historically been used for treatment against gram-positive bacteria, but recent developments have shown that they can also be effective against gram-negative bacteria. Together with cephamycins, cephalosporins form a β-lactam antibiotic subgroup called cephems.[4]

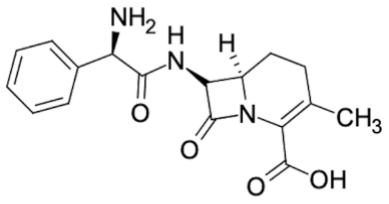
Monobactams are monocyclic, bacterially-produced β-lactam antibiotics that can act against aerobic gram-negative bacteria. In contrast to other β-lactam antibiotics, the β-lactam ring on monobactams is not fused with another ring. Monobactams did not show impressive antimicrobial activity, but the side-chain variation resulted in potent compounds.[5]

Carbapenems are a class of β-lactam antibiotics used for the treatment of severe, high-risk bacterial infections, as they are uniquely resistant to hydrolysis by many bacterial enzymes.

Carbacephems are a class of synthetic antibiotics based on the structure of cephalosporins. Carbacephems are similar to cephalosporins, but with a carbon substituted for the sulfur.[6] All of these antibiotics were results of the discovery of penicillin and contain the β-lactam ring first found in penicillin. A representative overview of the different classes of β-lactam antibiotics is illustrated in Table 1.

Table 1: Overview of the classes of β-lactam antibiotics.

Chemical Structure	Year of Discovery	Common Antibiotics	Significance	Species
 Pencillin	1928	Penicillin, Ampicillin, Azlocillin	Prevents peptidoglycan from cross-linking properly in the last stages of bacterial cell wall synthesis	Penicillium mold
 Cephalosporins	1948	Kefazol, Ancef, Ceftin, Zinacef	Effective against gram-negative bacteria	Fungus Acremonium
 Monobactams	1985	Aztreonam, Tigemonam, Nocardicin A, and Tabtoxin	Effective against aerobic gram-negative bacteria	Chromobacterium
 Carbapenams	1976	Imipenem, Panipenam, Doripenam	Largely resistant to hydrolysis by bacterial enzymes	E. coli, Klebsiella pneumoniae, Enterobacter cloacae, Citrobacter freundii, Proteus mirabilis, & Serratia marcescens

 Carbacephems	1967	Cefixime, Cefdinir, Cefotaxmine	Prevents bacterial cell division by inhibiting cell wall synthesis	E. coli, K. pneumoniae, and Enterobacter

β-LACTAM MECHANISM OF ACTION

A discovery in 1949 by Cooper and Rowley showed the irreversible binding of penicillin to the penicillin-binding proteins, or PBPs, of sensitive bacteria.[7] The crystal structure of penicillin complexed with a PBP is shown in Figure 2A, and Figure 2B shows penicillin covalently acylating the active site of a PBP.

Figure 2A: Crystal structure of PBP4 (dacB) from Escherichia coli, complexed with penicillin-G [opened] (PDB: 3PTE).

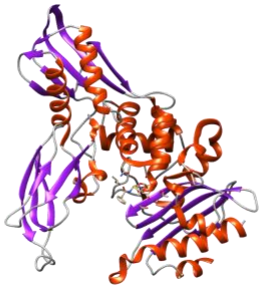
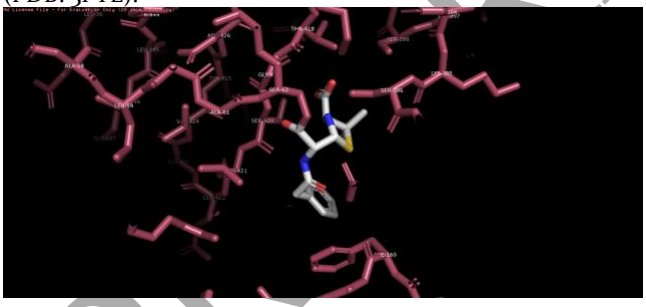
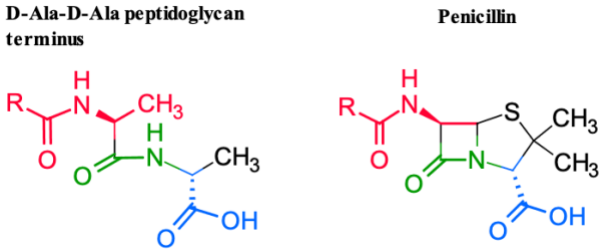


Figure 2B: Penicillin covalently acylates Serine-62 at the active site of a PBP, thus acting as a permanent inhibitor (PDB: 3PTE).



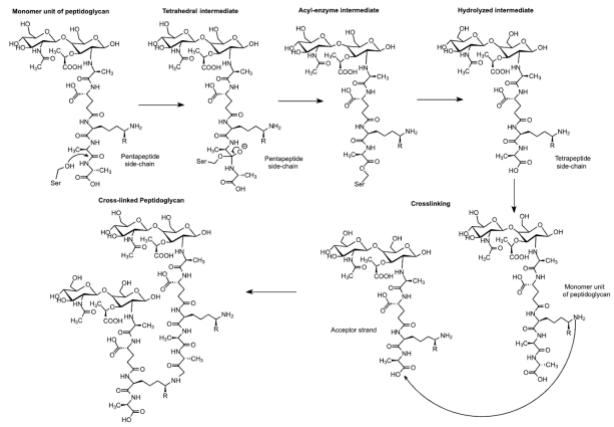
Soon after, in 1956, Lederberg discovered that penicillin converted rod-shaped E. coli bacteria into spherical protoplasts, thereby concluding that penicillin interfered with cell wall biosynthesis;[8] further work by Wise, Park, Tipper, and Trominger et al. demonstrated that penicillin targeted the cross-linking of peptidoglycan strands.[9] Ultimately, Timmer and Strominger proposed that the effectiveness of penicillin came from its structural similarity to the D-Ala-D-Ala residue of peptidoglycan, the native substrate of DD-transpeptidase, shown in Figure 3.

Figure 3: Similarities between the D-Ala-D-Ala terminus of peptidoglycan and the penicillin antibiotic.



DD-transpeptidase cross-links peptide side chains of peptidoglycan strands in the cell wall, providing structural rigidity for bacterial cells. This is followed by the breakdown of the acyl-enzyme intermediate and the formation of a new peptide bond between the carbonyl of the D-Ala moiety and the amino group of another peptidoglycan molecule, thereby crosslinking the two peptidoglycan molecules. This mechanism for this process is shown in Figure 4.

Figure 4: DD-transpeptidase catalyzes the cross-linking of peptidoglycan molecules.



β-lactam antibiotics disrupt cell wall biosynthesis via covalent inhibition of the transpeptidase enzyme. The four-membered β-lactam ring is forced into a torsed diamond geometry, as opposed to the natural 109.5° bond angles of a tetrahedral carbon. A four-membered ring has approximately 25 kcal/mol of ring strain, prompting ring opening. As such, β-lactams are often irreversible inhibitors of the enzyme because they permanently acylate the active

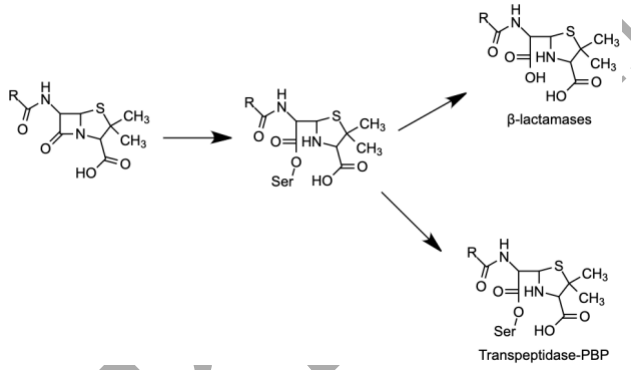
site serine of DD-transpeptidase, effectively preventing the enzyme from interacting with its native substrate, shown in Figure 5. This prevents the formation of the bacterial cell wall and promotes the osmotic lysis of the cell.

β-LACTAM RESISTANCE

Emergence of β-lactamases

From the initial discovery of penicillin as a β-lactam antibiotic, bacterial resistance has trailed closely behind in a molecular arms race between antibiotics and bacteria. Bacterial resistance to β-lactams is conferred by two major mechanisms: (1) inactivation of the β-lactam by hydrolytic enzymes called β-lactamases—shown in Figure 5—and (2) target site alterations to PBPs. Drug-resistant bacteria often express more than one of these mechanisms. The first instances of β-lactam resistance came from β-lactamases, which disrupt the amide bond of the β-lactam. Molecular modeling of various serine β-lactamases and PBP structures have demonstrated three-dimensional similarities with conserved folding patterns and preservation of topology at the active site, suggesting that β-lactamases evolved from PBPs selected for their antibiotic resistance. First found in E. coli a year prior to the clinical release of penicillin, β-lactamases have since been found in numerous gram-positive and gram-negative bacteria, often encoded as a mobile genetic element for plasmid-enabling horizontal gene transfer.[10]

Figure 5: β-lactamases hydrolyze the β-lactam ring, preventing β-lactam antibiotics from inhibiting DD-transpetidase.



β-lactamases have similar structure to DD-transpeptidase but with one key difference: β-lactams covalently bind to DD-transpeptidase while β-lactamases hydrolyze the β-lactam ring without binding to it. Thus, β-lactamases prevent β-lactams from inhibiting DD-transpeptidase.[11]

Evolution of β-lactamases

Phylogenetic analyses and nucleotide substitution rates have determined that serine β-lactamases are around 2 billion years old, while plasmid encoded OXA β-lactamases are millions of years old, both existing far before the discovery and usage of β-lactam antibiotics.[12] The expression frequencies for these early β-lactamases were low in bacterial populations and evolved in certain bacterial species as a mechanism for resistance against β-lactam containing compounds produced by fungi. However, the discovery and clinical development of β-lactams as antibiotics resulted in the evolutionary selection for relevant β-lactamases. The evolution of β-lactamases can be divided into four waves as shown in Table 2.

Table 2: The 4 Waves of β-lactamases.

Wave	Characteristics	Susceptible β-lactams	Unaffected β-lactams
1	Narrow-spectrum penicillinases TEM-1 and SHV-1 strands	Penicillin, Ampicillin	Cephalosporins, Carbapenems, Aztreonam
2	Extended spectrum cephalosporinases from point mutations to mutations to TEM and SHV	Cephalosporins	Ampicillin, Carbapenems
3	CTX-M family of β-lactamases	Cephalosporins	Carbapenems
4	Carbapenemases: KPC class β-lactamases, Metallo-β-lactamases, OXA-type enzymes	Carbapenems, Cephalosporins, Penicillin, Ampicillin	Aztreonam (Often ineffective)

Classification of β-lactamases

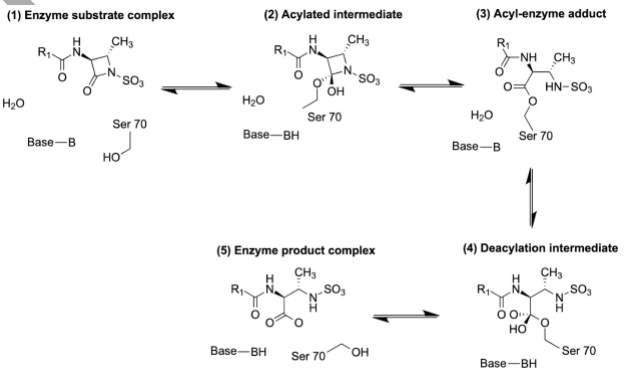
There are two classifications of β-lactamases: the first of which being the comparatively older classification that distinguished β-lactamases into classes A, B, C, and D based on amino acid sequencing.[13] In a more novel classification, Bush and Jacoby distinguished β-lactamases into Groups 1, 2, and 3, effectively combining classes A and D into a single group based on mechanism and evolutionary lineage.[14] A summary of the Bush and Jacoby classification of β-lactamases is shown in Table 3. The mechanism in which serine β-lactamases hydrolyze β-lactams is shown in Figure 6.

Table 3: Classifications of β-lactamases.

Group	Discovery	Description	Mechanism

Group 1 β-lactamases	β-lactamases were first documented in 1940 as the first bacterial enzyme capable of facilitating the breakdown of penicillin.	Group 1 β-lactamases include cephalosporinases, originally derived from Acremonium fungus.[15, 16] The most clinically relevant cephalosporinases are AmpC β-lactamases, mediating resistance to cephalothin, cefazolin, cefoxitin, and most penicillins. Overexpression of the AmpC gene confers resistance to broad-spectrum cephalosporins.[17]	The sequence of the AmpC gene was first recorded in E. coli in 1981,[18] distinct from the genetic sequence of TEM-1 and TEM-2, but still with an active site serine nucleophile.[19, 20]
Group 2 β-lactamases	TEM-1 was first documented in the early 1960s. The TEM-1 enzyme was originally found in a single strain of E. coli isolated from a blood culture from a patient named Temoniera in Greece, hence the designation TEM.	Group 2 β-lactamases include broad-spectrum, inhibitor-resistant, and extended-spectrum β-lactamases. The earliest serine carbapenemases began with the TEM-1 and SHV-1 strands which further evolved into extended-spectrum β-lactamases against aztreonam and oxycilin-hydrolyzing β-lactamases against carbapenem.	As with Group 1 β-lactamases, Group 2 β-lactamases have an active site serine, as shown in Figure 6.
Group 3 β-lactamases	Metallo-β-lactamases were initially discovered in the 1970s and attracted clinical attention in the 1990s with the spread of the IMP and VIM-type metallo-β-lactamases.	Metallo-β-lactamases have poor hydrolytic capabilities against monobactams—β-lactams without fused rings—but have recorded high hydrolytic capabilities towards penicillins, cephalosporins, and carbapenems. Due to its reliance on the zinc cation, metallo-β-lactamases are inhibited by metal ion chelators, such as EDTA.	In contrast to serine β-lactamases, metallo-β-lactamases utilize a zinc+2 cation to hydrolyze the β-lactam ring.

Figure 6: Serine β-lactamases hydrolyze β-lactams, rendering β-lactams ineffective.



An active site serine hydrolyzes the β-lactam ring, creating a transition acyl-enzyme adduct which undergoes a general base-catalyzed attack by a hydrolytic water molecule to form a second tetrahedral intermediate, which then collapses to form a product complex.[21]

Drug Inactivation by Target-site Alterations: Mutations in PBP

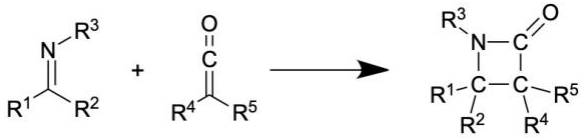
All β-lactams have the same binding target for successful inhibition: bacterial PBPs. This makes the alteration of this binding pocket extremely significant in hindering β-lactam activity. PBPs are membrane-bound DD-peptidases that evolved from serine proteases and are responsible for the crosslinking of peptidoglycan chains in bacterial cell wall formation. Due to the low expression of β-lactamases in Staphylococcus bacteria, target site alterations of PBPs are responsible for almost all β-lactam antibiotic resistance. The PBP targets in penicillin-resistant

Streptococci are modified into low-affinity targets for β-lactams, thereby reducing β-lactam inhibitory activity.[22] Meanwhile, certain bacteria such as methicillin-resistant staphylococci express novel PBPs—termed PBP2a—encoded by the mecA gene with almost no binding affinity to β-lactams. In the COL52 strain, E→K237 within the non-penicillin-binding domain, along with V→E470 and S→N643 near the SDN464 conserved DNA sequence of the penicillin-binding domain were important for resistance.[23, 24] Thus, research towards inhibitors for PBP2a is of high priority.

B-LACTAM SYNTHETIC ROUTES

The battle against bacterial resistance has necessitated the continued development of new antibiotics and methodologies, thus synthetic access to β-lactams has been of great significance.[25] The first synthetic β-lactam was prepared by Hermann Staudinger in 1907—21 years before Fleming discovered penicillin—by reaction of the Schiff base of aniline and benzaldehyde with diphenylketene in a [2+2] cycloaddition.[26, 27] This reaction, later coined the Staudinger [2+2] cycloaddition—as shown in Figure 7—still remains the most common method of β-lactam synthesis to date.[28, 29]

Figure 7: Staudinger [2+2] cycloaddition, consisting of the reaction between an imine and ketene to form a β-lactam.



There are two proposed mechanisms for the Staudinger 2+2 cycloaddition: a stepwise mechanism, wherein the nucleophilic nitrogen atom of the imine first attacks the sp-hybridized carbon atom of the ketene, followed by nucleophilic addition of the resulting enolate to the iminium; or a concerted mechanism which undergoes a pericyclic transition state to yield the β-lactam ring. Many computational studies have suggested that the mechanism for the [2+2] cycloaddition is stepwise, as reported by Cossío et al., Bachrach and Halzner, and Sordo et al.[30, 31, 32] Similar studies on the related reaction between ketene and alkenes by Burke, Houk and Wang, and Bottoni et al. have shown that the reaction has an asynchronous transition state with appreciable charge separations; however, no intermediates were isolated.[33, 34, 35] Although more than 100 years have passed since the ketene-imine cycloaddition was first reported, the reaction mechanism is still unclear.

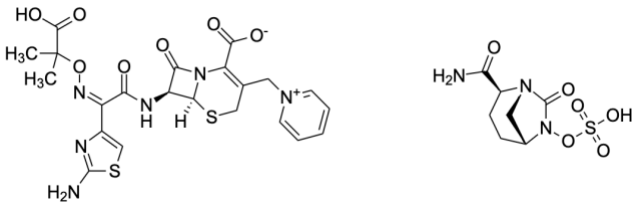
Variants of the asymmetric synthesis of β-lactams have incorporated chiral auxiliaries to control enantioselectivity and diastereoselectivity.[36, 37] Asymmetric syntheses of β-lactams yields a mix of cis and trans isomers; however, stereochemical control is necessary if a particular isomer is desired. Imines, characterized by the C=N functional group, can be prepared via condensation of an amine and aldehyde or amine and ketone.[38, 39, 40, 41] Ketenes are a highly reactive species that is characterized by the C=C=O functional group, which can be detected by infrared spectroscopy at around 2100-2200 cm-1. The most common method of ketene preparation involves activation of the carboxylic acid as a leaving group, followed by deprotonation of the α carbon and elimination of the leaving group.

MODERN ADVANCEMENTS

In an effort to produce better yields and improve reaction conditions and results, alternative synthesis methods have been produced in recent years as a substitute for the traditional Staudinger synthesis reaction. As reported by Dong et al., one such method involves a catalytic metal carbene insertion into C-H bonds. Another method consists of the activation of an unsaturated C-C bond in addition to a nucleophilic addition.[42, 43] Transition metal-assisted Staudinger reactions have also been promising alternative synthetic routes devised in recent years.[44]

Recent advancements have also been made to combat β-lactam resistance. In 2015, a novel antibacterial treatment, Ceftazidime-Avibactam (Figure 8), was approved by the U.S. Food and Drug Administration, involving a combination of the β-lactam antibacterial ceftazidime and the novel β-lactamase inhibitor, avibactam. This combination has been shown to have significant activity against β-lactamase-producing Gram-negative pathogens.[45, 46]

Figure 8: The chemical structure of ceftazidime is shown on the left, and the chemical structure of avibactam is shown on the right.



Despite recent advancements in combating resistance to β-lactam antibiotics, resistance to β-lactam antibiotics remains a concern which requires continued research development. Specifically, more needs to be done regarding plasmid-mediated β-lactamases, which transfer easily among groups of organisms and thus contribute to bacterial resistance. Induction of chromosomal β-lactamases is also a continuing problem to be resolved in terms of β-lactam antibiotic resistance.[47]

CONCLUSION

Since the discovery and initial clinical uses of penicillin in the 1900s, β-lactam antibiotics have undergone drastic improvements in order to combat the perpetual problem of bacterial resistance. The β-lactam core moiety of several natural products has attracted the attention of chemists and biologists alike for its prevalence in natural products, structural simplicity and reactivity, and effectiveness in the

mechanism of action against many strains of bacteria. The continued evolution of antibiotic resistant bacteria has made the development of new antibiotics all the more important. The development of new synthetic methodologies in accessing β-lactam-containing compounds has the potential of giving rise to improved β-lactam antibiotics that would be impactful not only in the medical field but also in the health and well-being of billions of people around the world.

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REFERENCES

1. Fleming, Alexander. "On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to Their Use in the Isolation of B. Influenzae." Reviews of Infectious Disease 2, no. 1 (1980). 129-139. doi:10.1093/clinids/2.1.129.
2. Chain, E.; Florey, H.; Gardner, A.; Heatley, N.; Jennings, M.; Orr-Ewing, J.; Sanders, A. "Penicillin As A Chemotherapeutic Agent." The Lancet 6104, no. 236 (1940). 226-228. doi:10.5555/uri:pii:S0140673601087281.
3. Gaynes, Robert. "The Discovery of Penicillin—New Insights After More Than 75 Years of Clinical Use." Emerging Infectious Diseases 23, no. 5 (2017). 849-853. doi:10.3201/eid2305.161556.
4. Abraham, E. P. "Cephalosporins 1945–1986." SpringerLink 34 (November 1987). 1-14. doi:10.2165/00003495-198700342-00003.
5. Skyes, R.B.; Bonner, D.P. Discovery and Development of Monobactams. Oxford Academia 7, no. 4 (November 1, 1985). 579-593. doi:10.1093/clinids/7.Supplement_4.S579.
6. Papp-Wallace, Kristina M.; Endimiani, Andrea; Taracila, Magdalena, A.; Bonomo, Robert A. "Carbapenems: Past, Present, and Future." Antimicrobial Agents and Chemotherapy 55, no. 11 (October 2011). 4943-4960. doi:10.1128/AAC.00296-11.
7. Abraham, E. P. "History of β-Lactam Antibiotics." Springer 67, no. 1 (1983). 1–14. doi:10.1007/978-3-642-81966-7_1.
8. Spratt, Brian. "Properties of the penicillin-binding proteins of Escherichia coli K12." European Journal of Biochemistry 72, no. 2 (1977). 341-352. doi:10.1111/j.1432-1033.1977.tb11 258.x.
9. Waxman, D. Strominger, J. "Penicillin-Binding Proteins and the Mechanism of Action of Beta-Lactam Antibiotics." Annual Review of Biochemistry 52 (1983). 825-869. doi:10.1146/annurev.bi.52.070183.004141.
10. Bush, Karen. "Past and Present Perspectives on β-Lactamases." Antimicrobial Agents and Chemotherapy 62, no. 10 (2018). doi:10.1128/aac.01076-18.
11. Rice, Louis B. "Mechanisms of Resistance and Clinical Relevance of Resistance to β-Lactams, Glycopeptides, and Fluoroquinolones." Mayo Clinic Proceedings 87, no. 2 (February 2012). 198–208. doi:10.1016/j.mayocp.2011.12.003.
12. Hall, B.; Barlow M. "Evolution of the Serine Beta-Lactamases: Past, Present and Future." Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy 7, no. 2 (2004). 111-23. doi:10.1016/j.drup.2004.02.003.
13. Ambler, R; Coulson, A; Frere, J; Ghuysen, J; Joris, B; Forsman, M; Levesque, R; Tiraby G; Waley S. "A Standard Numbering Scheme for the Class A Beta-Lactamases." The Biochemical Journal 276, pt. 1 (1991). 269–270. doi:10.1042/bj2760269.
14. Bush, K; Jacoby, A. "Updated Functional Classification of Beta-Lactamases. American Society for Microbiology." Antimicrobial Agents and Chemotherapy 54, no. 3 (March 2010). 969–976. doi:10.1128/AAC.01009-09.
15. Then, R.; Angehrn, P. "Trapping of Nonhydrolyzable Cephalosporins by Cephalosporinases in Enterobacter Cloacae and Pseudomonas Aeruginosa as a Possible Resistance Mechanism." Antimicrobial Agents and Chemotherapy 21, no. 5 (May 1982). 711-7. doi: 10.1128/aac.21.5.711.
16. Bulychev, A; Mobashery, S. "Class C Beta-Lactamases Operate at the Diffusion Limit for Turnover of Their Preferred Cephalosporin Substrates. American Society for Microbiology." Antimicrobial Agents and Chemotherapy 43, no. 7 (1999). 1743–1746. doi: www.ncbi.nlm.nih.gov/pmc/articles/PMC89354/.
17. Feller, G; et al. "Enzymes From Cold-Adapted Microorganisms. The Class C Beta-Lactamase From the Antarctic Psychrophile Psychrobacter Immobilis A5." European Journal of Biochemistry 244, no. 1 (1997). 186-91. Doi: 10.1111/j.1432-1033.1997.00186.x
18. Jaurin, B; Grundström, T. "AmpC Cephalosporinase of Escherichia Coli K-12 Has a Different Evolutionary Origin From That of Beta-Lactamases of the Penicillinase Type." Proceedings of the National Academy of Sciences of the United States of America 78, no. 8 (August 1981). 4897-901. doi:10.1073/pnas.78.8.4897.
19. Knott-Hunziker, V; Petursson, S; Jayatilake, S; Waley G; Jaurin B; Grundström T. "Active Sites of Beta-Lactamases. The Chromosomal Beta-Lactamases of Pseudomonas Aeruginosa and Escherichia Coli. National Library of Medicine." The Biochemical Journal 201, no. 3. (1982). 621-7. doi:10.1042/bj2010621.
20. Jacoby, A. "AmpC Beta-Lactamases. American Society for Microbiology." Clinical microbiology reviews 22, no. 1 (2009). 161–182. doi:10.1128/CMR.00036-08
21. Vandavasi, G; Langan, P; Weiss, K; Parks, J; Cooper, J; Ginell, C. "Active-Site Protonation States in an

Acyl-Enzyme Intermediate of a Class A β-Lactamase with a Monobactam Substrate." American Society for Microbiology Journals 61, no. 1. (2016). doi:10.1128/AAC.01636-16.
22. Tsurusaki, Y; Fukushima, K; Nishizaki, K; Takata, T; Ogawa, T; Nakashima, T; Sugata, K; Yorizane, S; Ogawara, T; Masuda, Y. "The Alteration of Penicillin-Binding Proteins (PBPs) in Drug-Resistant Streptococcus Pneumoniae Isolated From Acute Otitis Media." Acta oto-laryngologica Supplementum 540. (1999). 67-71. https://europepmc.org/article/med/10445083. (accessed January 1st). doi:
23. Park, M; Rafii, F. "Exposure to β-Lactams Results in the Alteration of Penicillin-Binding Proteins in Clostridium Perfringens." Anaerobe 45. (2017). 78-85. doi:10.1016/j.anaerobe.2017.02.004.
24. Katayama, Y.; Zhang, H.-Z.; Chambers, H. F. "PBP 2a Mutations Producing Very-High-Level Resistance to Beta-Lactams." Antimicrobial Agents and Chemotherapy 48, no. 2 (2004). 453-459. doi:10.1128/AAC.48.2.453-459.2004.
25. Hosseyni, Seyedmorteza; Jarrahpour, Aliasghar. "Recent Advances in β-Lactam Synthesis." Organic and Biomolecular Chemistry 16 (2018). 6840-6852. doi:https://doi.org/10.1039/C8OB01833B.
26. Staudinger, Hermann. "Zur Kenntniss der Ketene, Diphenylketene." European Journal of Organic Chemistry 356, no. 1-2. (1907). 51-123. doi:https://doi.org/10.1002/jlac.19073560106.
27. Tidwell, Thomas T. "Hugo (Ugo) Schiff, Schiff Bases, and a Century of β-Lactam Synthesis." Angewandte Chemie 47, no. 6 (2008). 1016-1020. doi:https://doi.org/10.1002/anie.200702965.
28. Taggi, Andrew T.; Hafez, Ahmed M.; Wack, Harald; Young, Brandon; Ferraris, Dana; Lectka, Tomas. "The Development of the First Catalyzed Reaction of Ketenes and Imines: Catalytic, Asymmetric Synthesis of β-Lactams." Journal of the American Chemical Society 124, no. 23. (2002). 6626-6635. doi:https://doi.org/10.1021/ja0258226.
29. France, Stefan; Weatherwax, Anthony; Andrew E. Taggi; Lectka, Thomas. "Advances in the Catalytic, Asymmetric Synthesis of β-Lactams." ACS Publications 37, no. 8 (2004). 592-600. doi:https://doi.org/10.1021/ar030055g.
30. Cossio, Fernando, Ana Arrieta, and Miguel Sierra."The Mechanism of the Ketene–Imine (Staudinger) Reaction in Its Centennial: Still an Unsolved Problem?" Accounts of Chemical Research 41, no. 8 (2008). 925-936. doi:https://doi.org/10.1021/ar800033j.
31. Salzner, Ulrike; Bachrach, Steven. "Cycloaddition Reactions between Cyclopentadine and Ketene. Ab Initio Examination of [2+2] and [4+2] Pathways." Journal of Organic Chemistry 61, no. 1. (1996). 237-242. doi:https://doi.org/10.1021/jo951331a.
32. Campomanes, Pablo; Menendez, Isabel M.; Sordo, Tomas L. "Mechanism of Cycloaddition reactions between Ketene and N-Silyl-, N-Germyl-, and N-Stannylimines: A Theoretical Investigation." The Journal of Physical Chemistry A 109, no. 48. (2005). 11022-11026. doi:https://doi.org/10.1021/jp054103l.
33. Burke, Luke A. "Theoretical study of (2+2) Cycloadditions." The Journal of Organic Chemistry 1985 50 (17), 3149-3155 doi:10.1021/jo00217a026.
34. Wang, Xuebao; Houk, K. N. "Carbenoid Character in Transition Structures for Reactions of Ketenes with Alkenes." Journal of the American Chemical Society 112, no. 5. (1990). 1754-1756. doi:https://doi.org/10.1021/ja00161a016.
35. Bernardi, Fernando; Bottoni, Andrea; Robb, Michael A.; Venturini, Alessandro. "MCSCF Study of the Cycloaddition Reaction Between Ketene and Ethylene. Journal of the American Chemical Society 112, no. 6. (1990). 2106-2114. doi:https://doi.org/10.1021/ja00162a010.
36. Hodous, Brian L. Fu, Gregory C. 2002. "Enantioselective Staudinger Synthesis of β-Lactams Catalyzed by a Planar-Chiral Nucleophile." Journal of the American Chemical Society 2002 124 (8), 1578-1579 doi:10.1021/ja012427r.
37. Weatherwax, A.; Abraham, C. J.; Lectka, T. "An Anionic Nucleophilic Catalyst System for the Diastereoselective Synthesis of trans-β-Lactams." 112, no. 6. (2005). 2106-2114. doi:https://doi.org/10.1021/ja00162a010.
38. Huang, J.; Zhang, J.; Dong, Y.; Gong, W. "An Effective Method To Prepare Imines from Aldehyde, Bromide/Epoxide, and Aqueous Ammonia." The Journal of Organic Chemistry 2011, no. 76. (2009), 3511-3514 doi: 10.1021/jo102455q.
39. Morales, S.; Guijarro, F. G.; Ruano, J. L. G.; Cid, M. B. "A General Aminocatalytic Method for the Synthesis of Aldimines." Journal of the American Chemical Society 136, no. 3. (2013). 1082-1089. doi:https://doi.org/10.1021/ja4111418.
40. Fontaine, P.; Chiaroni, A.; Masson, G.; Zhu, J. "One-Pot Three-Component Synthesis of α-Iminonitriles by IBX/TBAB-Mediated Oxidative Strecker Reaction." Organic Letters 10, no. 8. (2008). 1509-1512. doi:https://doi.org/10.1021/ol800199b.
41. Reeves, J. T.; Visco, M. D.; Marsini, M. A.; Grinberg, N.; Busacca, C. A.; Mattson, A. E.; Senanayake, C. H. "A General Method for Imine Formation Using B(OCH2CF3)3." Organic Letters 17, no 10. (2015). 2442-2445. doi:https://doi.org/10.1021/acs.orglett.5b00949.
42. Dong, K.; Qiu L.; Xu X. "Selective Synthesis of β-Lactams via Catalytic Metal Carbene C-H Insertion Reactions." Current Organic Chemistry. (2016). 29-40. doi:10.2174/1385272819666150626183713.
43. Hosseyni, S.; Jarrahpour, A. "Recent Advances in β-lactam Synthesis." Organic and Biomolecular Chemistry. (2018). doi:10.1039/c80b01833B.
44. Tuba, R. "Synthesis of β-Lactams by Transition Metal Promoted Staudinger Reactions: Alternative Synthetic Approaches from Transition Metal

Enhanced Organocatalysis to in situ, Highly Reactive Intermediate Synthesis and Catalytic Tandem Reactions." Organic & Biomolecular Chemistry, no. 11 (2013). doi:10.1039/c30b41048j.
45. Sharma, R.; Park, T. E.; Moy, S. "Ceftazidime-Avibactam: A Novel Cephalosporin/β-Lactamase Inhibitor Combination for the Treatment of Resistant Gram-Negative Organisms." Clinical Therapeutics, no. 38 (2016). 431-444. doi:10.1016/j.clinthera.2016.01.018.
46. Zhanel, G. G.; Lawson, C. D.; Adam, H.; ... "Ceftazidime-Avibactam: a Novel Cephalosporin/β-Lactamase Inhibitor Combination" Drugs, (2013). 159-177. doi:10.1007/s40265-013-0013-7
47. Bush, K. "Recent Developments in β-Lactamase Research and their Implications for the Future." Clinical Infectious Diseases, no. 10 (1988). 681-690. doi:10.1093/clinids/10.4.681.