arguments against the Phillips mechanism were suggestive but not completely persuasive. For example, the half-life of the proposed glycosyl cation was estimated to be 10^{-12} seconds, just longer than a molecular vibration and not long enough for the needed diffusion of other molecules. More important, lysozyme is a member of a family of enzymes called "retaining glycosidases," all of which catalyze reactions in which the product has the same anomeric configuration as the substrate (anomeric configurations of carbohydrates are examined in Chapter 7), and all of which are known to have reactive covalent intermediates like that envisioned in the alternative (S_N2) pathway. Hence, the Phillips mechanism ran counter to experimental findings for closely related enzymes.

A compelling experiment tipped the scales decidedly in favor of the S_N2 pathway, as reported by Stephen Withers and colleagues in 2001. Making use of a mutant enzyme (with residue 35 changed from Glu to Gln) and artificial substrates, which combined to slow the rate of key steps in the reaction, these workers were able to stabilize the elusive covalent intermediate. This in turn allowed them to observe the intermediate directly, using both mass spectrometry and x-ray crystallography (Fig. 6–25b).

Is the lysozyme mechanism now proven? No. A key feature of the scientific method, as Albert Einstein once summarized it, is "No amount of experimentation can ever prove me right; a single experiment can prove me wrong." In the case of the lysozyme mechanism, one might argue (and some have) that the artificial substrates, with fluorine substitutions at C-1 and C-2, that were used to stabilize the covalent intermediate might have altered the reaction pathway. The highly electronegative fluorine could destabilize an already electron-deficient oxocarbenium ion in the glycosyl cation intermediate that might occur in an S_N1 pathway. However, the S_N2 pathway is now the mechanism most in concert with available data.

An Understanding of Enzyme Mechanism Drives Important Advances in Medicine

The drugs used to treat maladies ranging from headache to HIV infection are almost always inhibitors of an enzyme. Two examples are explored here: the antibiotic penicillin (and its derivatives) and the protease inhibitors used to treat HIV infections, all of which are irreversible inhibitors.

Penicillin was discovered in 1928 by Alexander Fleming, but it took another 15 years before this relatively unstable compound was understood well enough to use it as a pharmaceutical agent to treat bacterial infections. Penicillin interferes with the synthesis of peptidoglycan (described in Chapter 20, Fig. 20–32), the major component of the rigid cell wall that protects bacteria from osmotic lysis. Peptidoglycan consists of polysaccharides and peptides cross-linked in several steps that include a transpeptidase reaction **(Fig. 6–26)**. It is

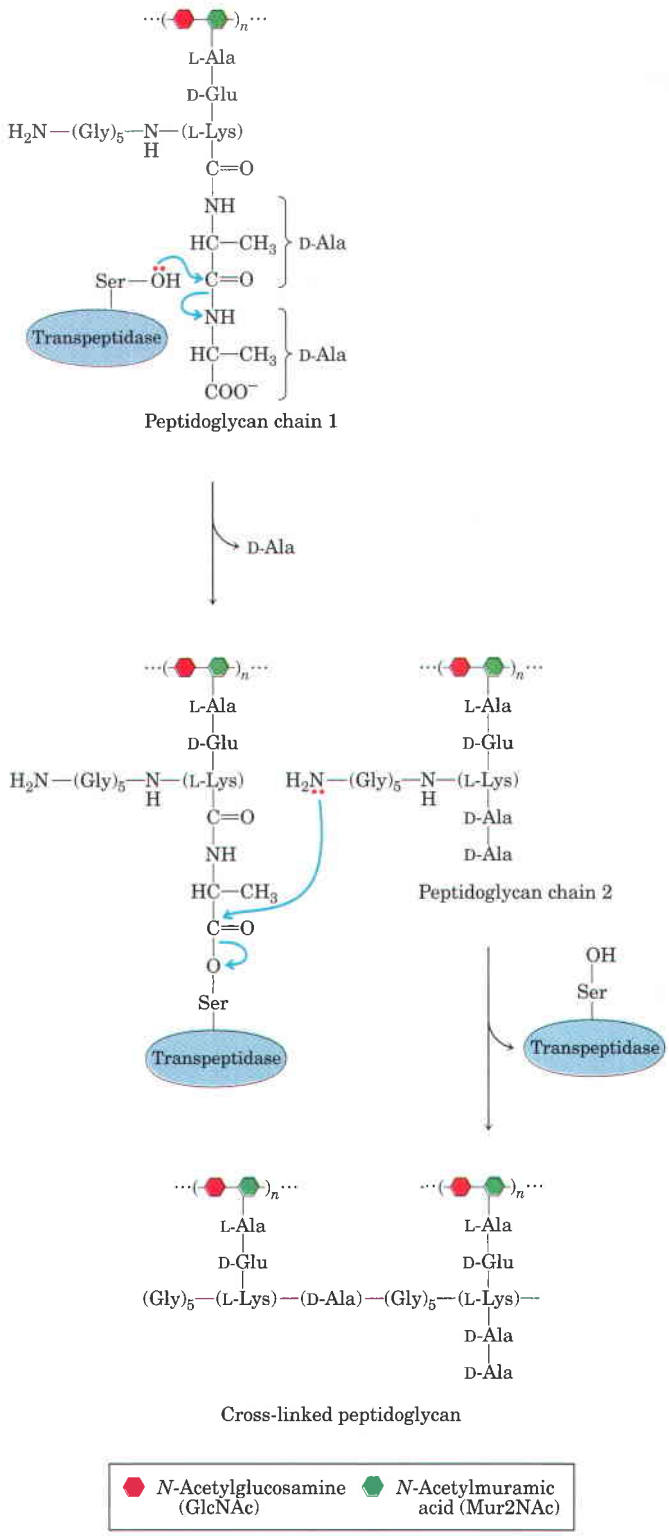
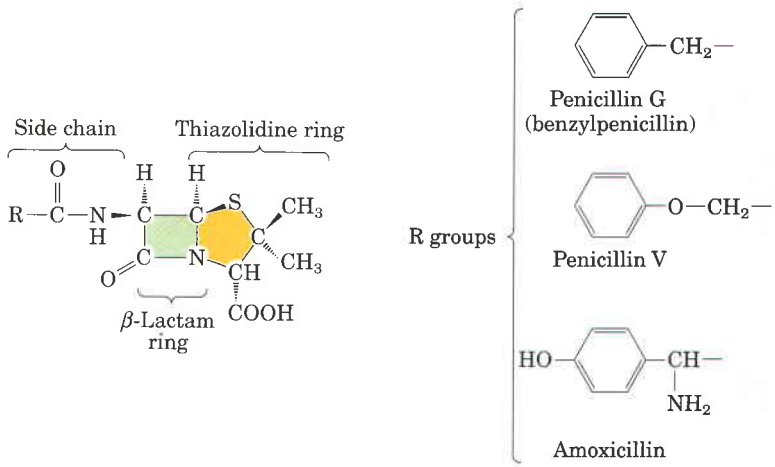
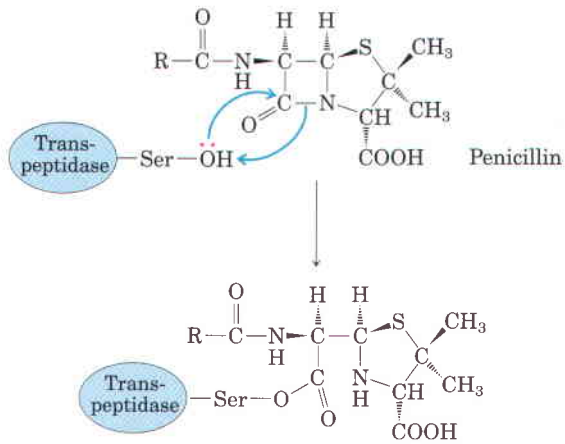


FIGURE 6–26 The transpeptidase reaction. This reaction, which links two peptidoglycan precursors into a larger polymer, is facilitated by an active-site Ser and a covalent catalysis mechanism similar to that of chymotrypsin. Note that peptidoglycan is one of the few places in nature where D-amino acid residues are found. The active-site Ser attacks the carbonyl of the peptide bond between the two D-Ala residues, creating a covalent ester linkage between the substrate and the enzyme with release of the terminal D-Ala residue. An amino group from the second peptidoglycan precursor then attacks the ester linkage, displacing the enzyme and cross-linking the two precursors.



General structure of penicillins

(a)



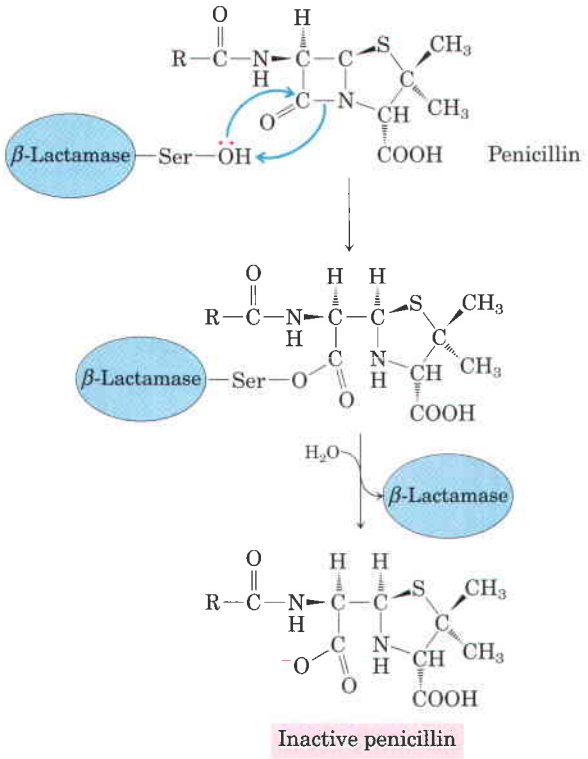
Stably derivatized, inactive transpeptidase

(b)

FIGURE 6–27 Transpeptidase inhibition by β-lactam antibiotics. (a) β-Lactam antibiotics feature a five-membered thiazolidine ring fused to a four-membered β-lactam ring. The latter ring is strained and includes an amide moiety that plays a critical role in the inactivation of peptidoglycan synthesis. The R group varies in different penicillins. Penicillin G was the first to be isolated and remains one of the most effective, but it is degraded by stomach acid and must be administered by injection. Penicillin V is nearly as effective and is acid stable, so it can be administered orally. Amoxicillin has a broad range of effectiveness, is readily administered orally, and is thus the most widely prescribed β-lactam antibiotic. **(b)** Attack on the amide moiety of the β-lactam ring by a transpeptidase active-site Ser results in a covalent acyl-enzyme product. This is hydrolyzed so slowly that adduct formation is practically irreversible, and the transpeptidase is inactivated.

this reaction that is inhibited by penicillin and related compounds **(Fig. 6–27a)**, all of which mimic one conformation of the D-Ala—D-Ala segment of the peptidoglycan precursor. The peptide bond in the precursor is replaced by a highly reactive β-lactam ring. When penicillin binds to the transpeptidase, an active-site Ser attacks the carbonyl of the β-lactam ring and generates a covalent adduct between penicillin and the enzyme. However, the leaving group remains attached because it is linked by the remnant of the β-lactam ring (Fig. 6–27b). The covalent complex irreversibly inactivates the enzyme. This, in turn, blocks synthesis of the bacterial cell wall, and most bacteria die as the fragile inner membrane bursts under osmotic pressure.

Human use of penicillin and its derivatives has led to the evolution of strains of pathogenic bacteria that express **β-lactamases (Fig. 6–28a)**, enzymes that cleave β-lactam antibiotics, rendering them inactive. The bacteria thereby become resistant to the antibiotics. The genes for these enzymes have spread rapidly through bacterial populations under the selective pressure imposed by the use (and often overuse) of β-lactam antibiotics. Human medicine responded with the development of compounds such as clavulanic acid, a



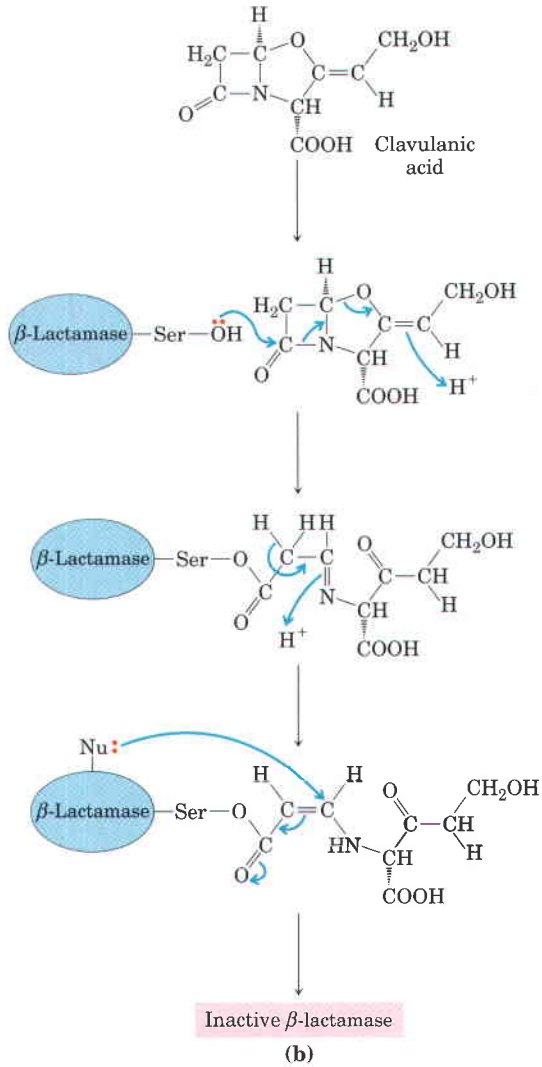


FIGURE 6–28 β-Lactamases and β-lactamase inhibition. **(a)** β-Lactamases promote cleavage of the β-lactam ring in β-lactam antibiotics, inactivating them. **(b)** Clavulanic acid is a suicide inhibitor, making use of the normal chemical mechanism of β-lactamases to create a reactive species at the active site. This reactive species is attacked by groups in the active site to irreversibly acylate the enzyme.

suicide inactivator, which irreversibly inactivates the β-lactamases (Fig. 6–28b). Clavulanic acid mimics the structure of a β-lactam antibiotic, and forms a covalent adduct with a Ser in the β-lactamase active site. This leads to a rearrangement that creates a much more reactive derivative, which is subsequently attacked by another nucleophile in the active site to irreversibly acylate the enzyme and inactivate it. Amoxicillin and clavulanic acid are combined in a widely used pharmaceutical formulation with the trade name Augmentin. The cycle of chemical warfare between humans and bacteria continues unabated. Strains of disease-causing bacteria that are resistant to both amoxicillin and clavulanic acid (reflecting mutations in β-lactamase that render it unreactive to clavulanic acid) have been discovered. The development of new antibiotics promises to be a growth industry for the foreseeable future.

Antiviral agents provide another example of modern drug development. The human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome, or AIDS. In 2005, an estimated 37 to 45 million people worldwide were living with HIV infections, with 3.9 to 6.6 million new infections that year and more than 2.4 million fatalities. AIDS first surfaced as a world epidemic in the 1980s; HIV was discovered soon after and identified as a **retrovirus**. Retroviruses possess an RNA genome and an enzyme, reverse transcriptase, capable of using RNA to direct the synthesis of a complementary DNA. Efforts to understand HIV and develop therapies for HIV infection benefited from decades of basic research on other retroviruses. A retrovirus such as HIV has a relatively simple life cycle (see Fig. 26–33). Its RNA genome is converted to duplex DNA in several steps catalyzed by a reverse transcriptase (described in Chapter 26). The duplex DNA is then inserted into a chromosome in the nucleus of the host cell by the enzyme integrase (described in Chapter 25). The integrated copy of the viral genome can remain dormant indefinitely. Alternatively, it can be transcribed back into RNA, which can then be translated into proteins to construct new virus particles. Most of the viral genes are translated into large polyproteins, which are cut by the HIV protease into the individual proteins needed to make the virus (see Fig. 26–34). There are only three key enzymes in this cycle—the reverse transcriptase, the integrase, and the protease—which thus are the potential drug targets.