

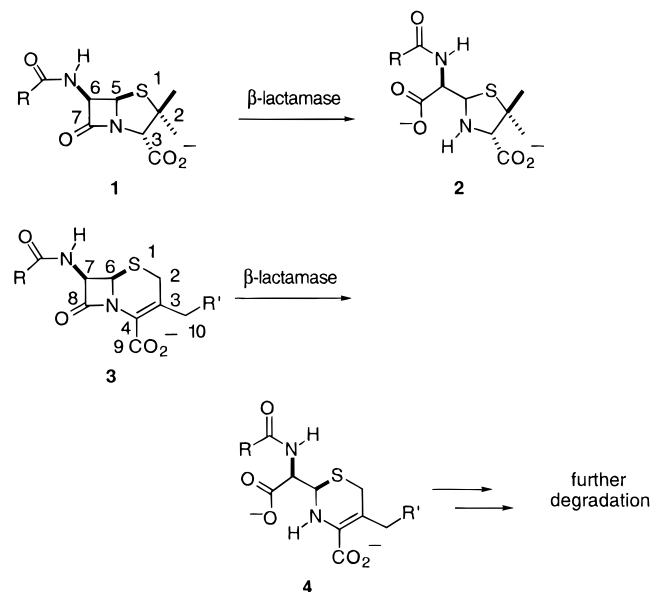
Molecular Bases for Interactions between β -Lactam Antibiotics and β -Lactamases

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The problem of bacterial resistance to multiple antibiotics has reached a crisis level such that successful chemotherapy of infections by such resistant bacteria can no longer be taken for granted.¹ Furthermore, the annual economic burden of infections due to resistant organisms in the U.S. has been estimated to have reached \$30 billion.² β -Lactam antibiotics are currently mainstays of clinical treatment of bacterial infections, a position which is secure for the foreseeable future. However, their effectiveness has been compromised by the ability of bacteria to produce β -lactamases, enzymes which hydrolyze the β -lactam moiety in these antibiotics, whereby the drug is rendered inactive. This activity is shown below for a penicillin (**1**) and a cephalosporin (**3**), two members of the β -lactam antibiotic family.



An exciting development over the past few years has been the availability of high-resolution crystal structures for several β -lactamases, as well as one for a penicillin-

binding protein (PBP), bacterial enzymes involved in cell-wall biosynthesis which are the targets of the antibacterial action of β -lactam drugs. An active-site serine in a PBP undergoes acylation by the carbonyl of the penultimate D-Ala residue in the acyl-D-Ala-D-Ala moiety of the bacterial peptidoglycan. Certain PBPs catalyze a transpeptidation step on the acyl-enzyme intermediate, whereby a second peptidoglycan strand is covalently appended to the first. This cross-linking reaction of PBPs is critical for the survival of bacteria. It has been suggested that β -lactam antibiotics mimic the structure of the acyl-D-Ala-D-Ala portion of the peptidoglycan.³ By so-doing, the lactam carbonyl acylates the active site of PBP, but the resultant acyl-enzyme species is relatively stable, whereby the bacterium is deprived of the biosynthetic activities of these enzymes, a process which results in bacterial death. An ancestral PBP is presumed to have evolved such that it acquired the ability to facilitate an effective deacylation of the acyl-enzyme species to give rise to the progenitor for β -lactamases. Such a hydrolytic activity would have had vital significance for the survival of the ancient bacteria in the face of competition for resources by the β -lactam-producing microorganisms. Selection and evolution of β -lactamases would have been favored by exposure of the resistant bacteria to these antibiotics from the environment. This process has been accelerated considerably recently by extensive use of antibiotics in both clinical settings and in animal feed.⁴ Over 190 different β -lactamases have been identified to date.⁵ Whereas amino-acid sequence homology between modern PBPs and various classes of β -lactamases⁶ is low, the fact that these enzymes are related to each other has been demonstrated by the conservation of the three-dimensional folding patterns of these functionally distinct proteins (Figure 1).⁷

Our efforts in the area of β -lactamase-mediated resistance to β -lactam antibiotics have centered on the TEM-1 β -lactamase, a prototypic member of the class A family of β -lactamases, which are the most prevalent group of such enzymes among bacterial pathogens. The class A β -lactamases are active-site-serine enzymes. That is to say, a serine in the active site undergoes acylation and deacylation in the course of substrate turnover. We have investigated the mechanisms and the nature of the

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- (5) Bush, K.; Jacoby, G. A.; Medeiros, A. A. *Antimicrob. Agents Chemother.* **1995**, *39*, 1211.
- (6) There are four functional classes of β -lactamases, classes A, B, C, and D, of which classes A, C, and D are active-site-serine enzymes, and class B constitutes a zinc-dependent family of β -lactamases. The history of classification for β -lactamases and new thoughts on this subject have been reviewed in the paper by Bush *et al.*, cited in ref 5.
- (7) Kelley, J. A.; Dideberg, O.; Charlier, P.; Werz, J. P.; Libert, M.; Moews, P. C.; Knox, J. R.; Duez, C.; Fraipoint, C.; Joris, B.; Dusart, J.; Frère, J. M.; Ghuyssen, J.-M. *Science* **1986**, *231*, 1429.

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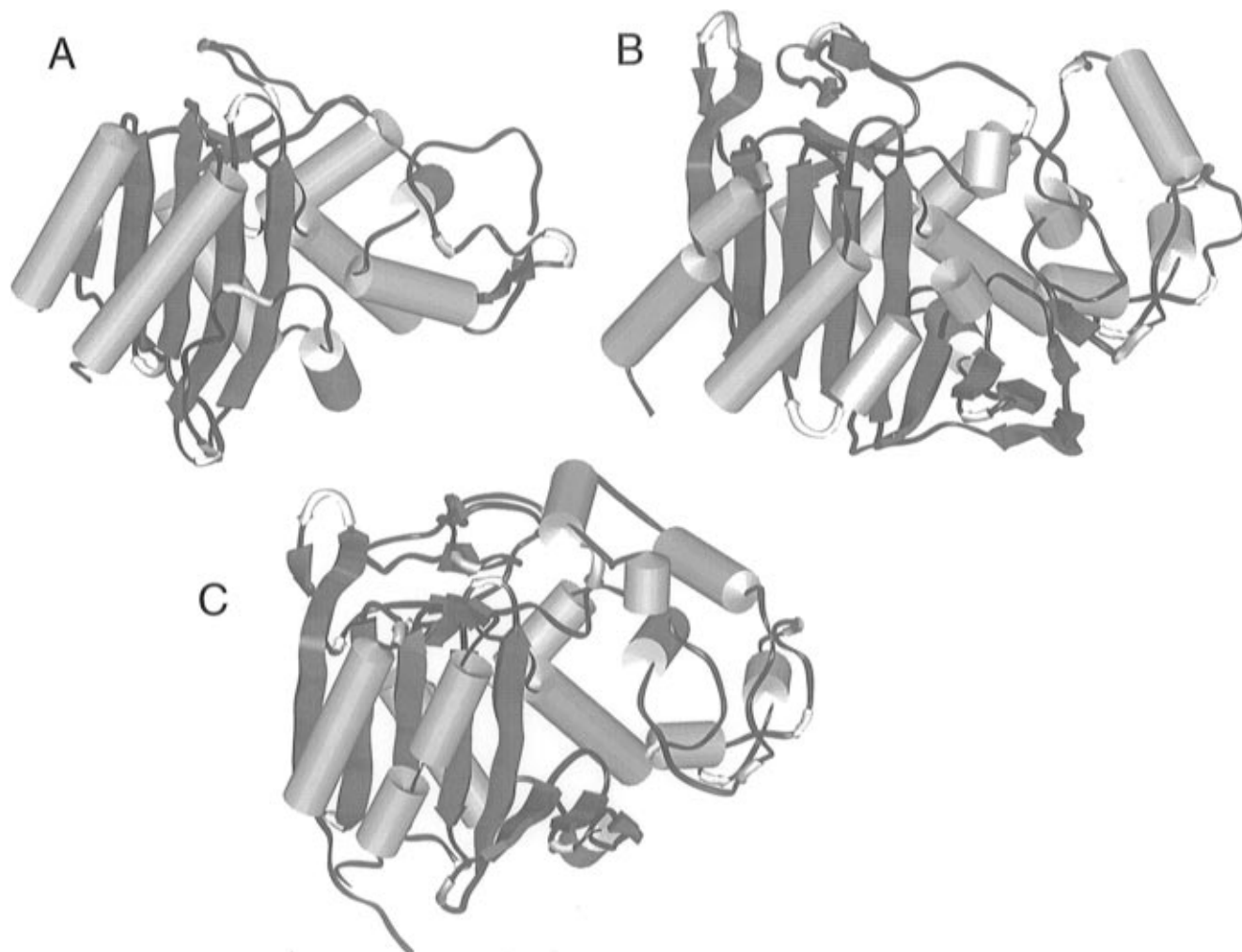
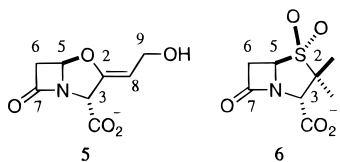


FIGURE 1. Three-dimensional fold for (A) the class A TEM-1 β -lactamase from *E. coli* (Strynadka, N. C. J.; Adachi, H.; Jensen, S. E.; Johns, K.; Sielecki, A.; Betzel, C.; Sutoh, K.; James, M. N. G. *Nature* 1992, 359, 700. Jelsch, C.; Mourey, L.; Masson, J. M.; Samama, J. P. *Proteins* 1993, 16, 364), (B) the class C β -lactamase from *Enterobacter cloacae* (Lobkovsky, E.; Moews, P. C.; Liu, H.; Zhao, H.; Frère, J. M.; Knox, J. R. *Proc. Natl. Acad. Sci. U.S.A.* 1993, 90, 11257), and (C) the bifunctional DD-peptidase/transpeptidase—a penicillin-binding protein—from *Streptomyces* R61 (Kuzin, A. P.; Liu, H.; Kelly, J. A.; Knox, J. R. *Biochemistry* 1995, 34, 9532).

interactions of a series of β -lactam agents with the TEM-1 β -lactamase, as will be outlined below.

Two strategies have been employed to counter the clinical problem posed by the β -lactamase-mediated resistance: (i) New β -lactam drugs have been developed that are less favorable substrates for many common β -lactamases, so-called extended-spectrum β -lactams, such as third-generation cephalosporins cefotaxime, ceftazidime, and ceftriaxone, the monobactam aztreonam, and the carbapenem imipenem; (ii) A mechanism-based β -lactamase inactivator, such as clavulanate (**5**), sulbactam (**6**),



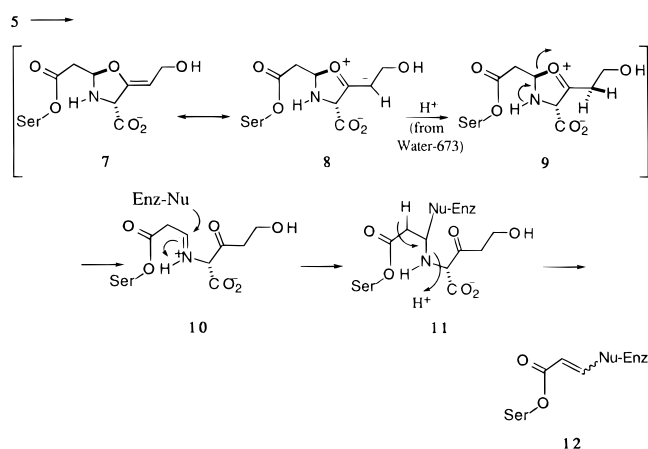
or tazobactam, is administered together with a penicillin. In the latter strategy, the inactivator inactivates the β -lactamase, thereby preserving the antibacterial activity of the penicillin and prolonging the effective life of these drugs. Whereas, at times, the phenotypic distinction between an inhibitor/inactivator and poorer substrates for

the enzyme may not be clear—as in molecules that acylate the active site but undergo the deacylation step less readily—categorization of β -lactams according to this scheme is suitable in the context of discussion in this Account.

Mechanism-Based Inactivators for β -Lactamases. The mechanisms of action of the now clinically used β -lactamase inactivators clavulanate (**5**) and sulbactam (**6**) were discussed in a review by Knowles⁸ and will not be mentioned here in depth. Both compounds acylate β -lactamases, but in competition with deacylation, other chemical events lead to inactivation of the enzyme. We have investigated these events in the context of the constraints imposed by the enzyme active site for both clavulanate (**5**)^{9,10} and sulbactam (**6**).¹¹ We generated energy-minimized models for active-site binding of the precatalytic (“Michaelis”) complexes, both for the respective acyl-enzyme intermediates and for the ultimate acylated acyclic species that lead to inactivation of class

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 (9) Imtiaz, U.; Billings, E.; Knox, J. R.; Manavathu, E. K.; Lerner, S. A.; Mobashery, S. *J. Am. Chem. Soc.* **1993**, 115, 4435.
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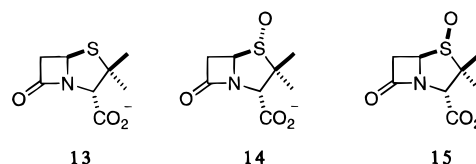
A β -lactamases by clavulanate (**5**) and sulbactam (**6**). We proposed a nonconcerted process for the formation of the inactivating species by clavulanate (**5**) for class A β -lactamases (**7–10**), as well as by sulbactam (**6**). These complexes revealed hydrogen-bonding interactions of residues Arg-244, Ser-130, Ser-235, and Water-673 with the carboxylates of the inactivators, as was described earlier for typical substrates.^{12,13} Furthermore, as it pertains to clavulanate (**5**), these models revealed that the Arg-244 side chain and the Val-216 carbonyl anchor a structurally conserved water molecule, Water-673, which serves as the most likely source of a critical proton in a stepwise sequence of events for the inactivation chemistry. Disruption of this “electrostatic anchor” for Water-673 by mutational replacement of Arg-244 with Ser in the TEM β -lactamase accounted for the resulting impairment of the efficiency of inactivation of the mutant enzyme by clavulanate (**5**) both *in vivo* and *in vitro*. These effects led to an approximately 500-fold attenuation of the first-order rate constant for enzyme inactivation (k_{inact}) by clavulanate (**5**) *in vitro*. In addition, the kinetic impact of the Arg-244-Ser mutation on the *in vivo* properties of clavulanate (**5**) was reflected by a 128-fold increase in the minimum inhibitory concentration (MIC) of ampicillin (a penicillin) in the presence of clavulanate (**5**) for a strain of *Escherichia coli* harboring the Arg-244-Ser mutant over the MIC with the wild-type enzyme; MIC is defined as the lowest concentration of the antibiotic which prevents bacterial growth. It is significant that while the Arg-244-Ser mutant enzyme was designed and studied in the laboratory, anticipating its resistance to inactivation by clavulanate (**5**) in order to study the proposed mechanism, two French groups have identified the same mutation of the TEM-1 enzyme in clinical isolates of *E. coli* which exhibit resistance to the combination of **5** and a penicillin.^{14,15} These reports of the occurrence of the plasmid-encoded Ser-244 mutant TEM-1 β -lactamase in clinical isolates herald an anticipated compromise of the effectiveness of combinations of clavulanate and penicillins for therapy against multiresistant clinical isolates.



The details of the inactivation chemistries of β -lactamases by **5** and **6** are similar.⁸ Subsequent to protonation

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 (13) Imtiaz, U.; Manavathu, E. K.; Lerner, S. A.; Mobashery, S. *Antimicrob. Agents Chemother.* **1993**, *37*, 2438.

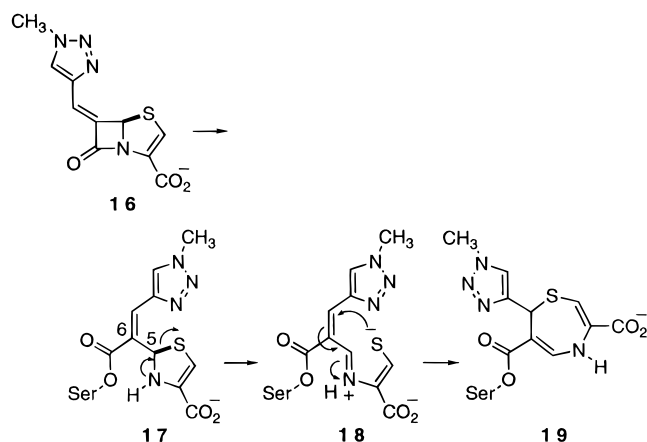
of the enol ether, departure of the leaving group from the C₅ position of **5** is a facile process. In the case of sulbactam, which would have a sulfinate as the leaving group, its departure is considerably less favorable. In order to explore the influence of the leaving group from C₅ of the inactivator on the inactivation process by sulbactam (**6**), penicillanate (**13**), penicillanate α -S-oxide (**14**), and penicillanate β -S-oxide (**15**) were prepared and



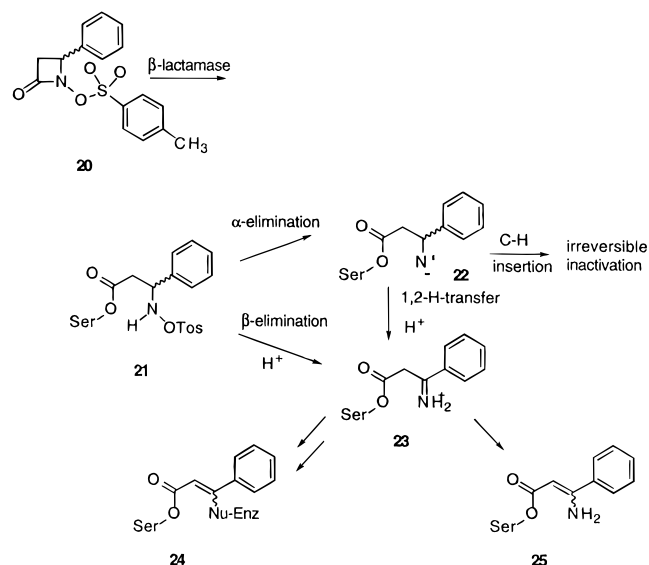
studied. Penicillanate is only a substrate, but penicillanate S-oxides are both substrates and inactivators for the TEM-1 β -lactamase. We presented an argument to rationalize these observations, based on the leaving ability of thiolate, sulfenate, and sulfinate—progressively better leaving groups—from the acyl-enzyme intermediates of penicillanate (**13**), the penicillanate S-oxides **14** and **15**, and sulbactam (**6**), respectively.¹¹ The kinetic analysis indicated that the departure of the leaving group is not rate-limiting in the inactivation process, but is an indispensable component of the irreversible inactivation of the enzyme. Furthermore, departure of the leaving group takes place unassisted (e.g., by hydrogen bonding) by the enzyme.¹¹

Woodward had predicted that penems—molecules not found to date in nature—would incorporate biological properties of penicillins and cephalosporins into one molecule.¹⁶ A few synthetic penems have been described in the literature, and it was of interest to investigate the properties of these molecules in their interactions with β -lactamases. We investigated inactivation of the TEM-1 β -lactamases by the penem BRL 42715 (**16**).¹⁷ This compound is one of only two β -lactamase inactivators that show activity against both classes A and C of β -lactamases (the other is tazobactam).¹⁸ Both class A and class C β -lactamases were inactivated efficiently with $k_{\text{inact}}/K_i = 10^6\text{--}10^7 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{\text{cat}}/k_{\text{inact}} = 1\text{--}12$. The inhibitor constants were in the nanomolar range for both enzymes.^{17,19} The inactivation chemistry was also efficient for the Arg-244-Ser mutant of the TEM-1 enzyme, which we had shown to be resistant to inactivation by clavulanate. The inactivation chemistry involved acylation of the active-site serine (**16** \rightarrow **17**), followed by a rearrangement to a dihydrothiazepine species (**19**), as suggested by Broom *et al.* in model studies.²⁰

- (14) Vedel, G.; Belaoaouaj, A.; Gilly, L.; Labia, R.; Phillippon, A.; Nevot, P.; Paul, G. *J. Antimicrob. Chemother.* **1992**, *30*, 449.
 (15) Zhou, X. Y.; Bordon, F.; Sirot, D.; Kitzis, M. D.; Gutmann, L. *Antimicrob. Agents Chemother.* **1994**, *38*, 1085.
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 (19) Two additional papers on the mechanism of inactivation of β -lactamases by BRL 42715 have appeared in the literature, the results of which are in good agreement with those reported by us: Farmer, T. H.; Page, J. W. J.; Payne, D. J.; Knowles, D. J. C. *Biochem. J.* **1994**, *303*, 825. Matagne, A.; Ledent, P.; Monnaie, D.; Felici, A.; Jamin, M.; Raquet, X.; Galleni, M.; Klein, D.; François, I.; Frère, J. M. *Antimicrob. Agents Chemother.* **1995**, *39*, 227.

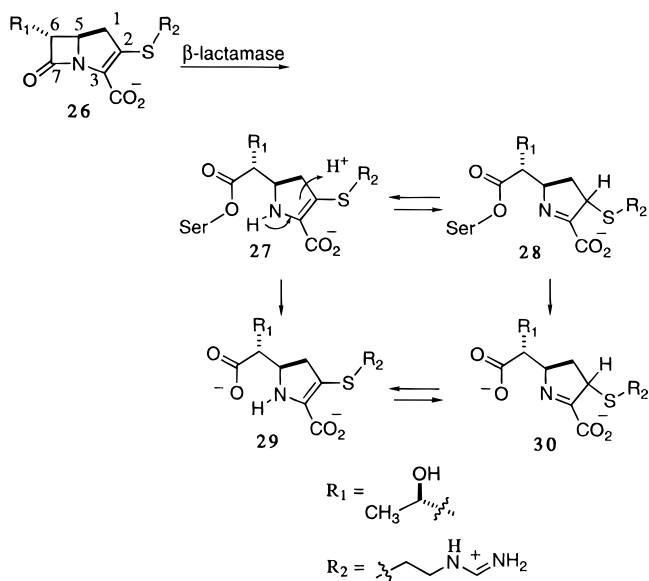


We have recently reported on a new family of β -lactamase inactivators (exemplified by **20**), which are different both in structure and in mechanism from all the known β -lactamase inactivators.²¹ The mechanism that we envisioned—as shown for **20**—could either have led to irreversible inactivation [either by nitrene (**22**) insertion or formation of **24**] or have given rise to reversible inhibition by the formation of **25**. We observed the formation of **25** in the course of enzyme inhibition. The mechanistic scheme was proposed on the basis of spectroscopic analysis of the inactivated enzyme, release and quantification of the leaving group, and kinetic evaluation of deuterated derivatives. It is significant that the sulfonate moiety in these molecules serves as a surrogate for the invariant carboxylate of the substrates and of other inactivators for β -lactamases; the exception is monobactams, which possess a sulfonate instead of a carboxylate. Two oxygens of the sulfonate moiety (those not appended to the nitrogen) are capable of forming hydrogen bonds to the side chains of residues Arg-244, Ser/Thr-235, and Ser-130 in the active site of the TEM-1 β -lactamase, as do β -lactams containing the carboxylate group.



Mechanisms of Turnover of Certain Poor Substrates for β -Lactamases. Imipenem (*N*-formimidoylthienamycin,

26) is a carbapenem in clinical use, and is considered a drug of “last resort” for treatment of nosocomial infections in the U.S. Studies by Knowles and colleagues on the mechanism of action of class A β -lactamases with carbapenems had indicated a biphasic profile for hydrolysis of carbapenems.^{22,23} It was suggested that subsequent to active-site acylation of a β -lactamase by these molecules the Δ^2 -pyrroline analog **27** may either undergo deacylation or tautomerize to the corresponding Δ^1 -derivative **28**. The ester bond of **28** was suggested to be kinetically more resistant to hydrolysis because of a less favorable substrate positioning in the active site.



Information from the crystal structure, in conjunction with kinetic findings, indicated that the imipenem carboxylate would form hydrogen bonds to the side-chain functions of Arg-244, Ser-130, and Ser-235 upon active-site anchoring of the substrate,²⁴ as was indicated previously for other β -lactamase substrates.^{12,13} This mode of active-site binding brings the Arg-244 side chain and Water-673 from the β -face of an active-site-bound imipenem to close proximity of the C₂ of the substrate. It appeared to us that Water-673, coordinated to the Arg-244 guanidinium moiety, could serve in an adventitious manner as the source of a proton for the tautomerization of **27** to **28**. To test this concept, we studied turnover of imipenem, both by the TEM-1 β -lactamase and by its Arg-244-Ser mutant variant. As stated earlier, the Arg-244-Ser mutant enzyme is believed to be incapable of retaining Water-673 in the active site. The wild-type enzyme hydrolyzed imipenem in the characteristic biphasic manner reported for other carbapenems. However, the Ser-244 mutant enzyme showed a monophasic hydrolysis of imipenem with a rate close to that of the first phase of hydrolysis by the wild-type enzyme. This observation suggests that the Ser-244 enzyme may catalyze a steady-state hydrolysis of imipenem without tautomerization of **27** to **28**. The first-order rate constants for deacylation of the two acyl-enzyme intermediates were estimated;²⁴ these determinations revealed that the rate of hydrolysis

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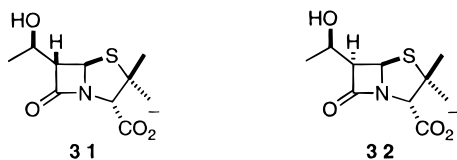
(23) Charnas, R. L.; Knowles, J. R. *Biochemistry* **1981**, *20*, 2732.

(24) Zafaralla, G.; Mobashery, S. *J. Am. Chem. Soc.* **1992**, *114*, 1505.

of **27** may be at least 12-fold faster than that of **28**. It is significant that certain other class A β -lactamases which show substitutions of serine at position 244 also hydrolyze imipenem in a monophasic manner, as observed by Rasmussen and Bush.²⁵

Analysis of the interactions of products **29** and **30** with the β -lactamase indicated that weak product inhibition existed for turnover of imipenem.²⁶ Kinetic experiments with synthetic preparations of the products of turnover showed that deacylation of the imipenem acyl-enzyme species—and not product dissociation—is the rate-limiting step; however, the enzyme–product complexes for turnover of imipenem enjoy additional stability (approximately 200-fold) over that for a typical penicillin substrate. Furthermore, β -lactamase showed an unprecedented enhancement of helicity in the course of turnover of imipenem.²⁶ This report of structure enhancement in catalysis is the first such example for turnover chemistry by β -lactamases, but its mechanistic implications are not obvious at the present.

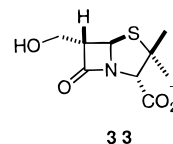
Another interesting aspect of the chemistry of turnover of imipenem is the fact that, compared to some of the more favorable penicillin substrates for the TEM-1 β -lactamase, imipenem is hydrolyzed with a k_{cat} value which is attenuated by a factor of 50 000. The differences between the K_{m} values are not significant for the two substrate types. This observation was attributed to the active-site interactions of the 6 α -1(*R*)-hydroxyethyl moiety in imipenem, which would displace the hydrolytic water molecule from its optimal position for the deacylation reaction.²⁶ In order to evaluate the effect of this moiety on the turnover chemistry, we envisioned compounds **31** and **32** as molecular probes. These molecules both



possess the 1(*R*)-hydroxyethyl moiety, but the stereochemistry at C₆ is α in **31** and β in **32**. The β -orientation was expected to have no effect on the rate of enzymic hydrolysis, as the group would point to the active-site opening. These two compounds were synthesized.²⁷ It was shown that compound **32** was indeed a reasonably good substrate for the TEM-1 β -lactamase ($k_{\text{cat}}/K_{\text{m}} = 2.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$; compared to $k_{\text{cat}}/K_{\text{m}} = 6.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ for **13**, which has two hydrogen atoms at C₆) and that **31** was an exceedingly poor substrate ($k_{\text{cat}}/K_{\text{m}} = 27 \text{ M}^{-1} \text{ s}^{-1}$). The difference in $k_{\text{cat}}/K_{\text{m}}$ values for **31** and **32** is 10 000-fold, which clearly demonstrates that the poor rate of enzymic hydrolysis of imipenem can be essentially entirely attributed to the effect of the 6 α -1(*R*)-hydroxyethyl moiety and not the carbapenem nucleus. The $K_{\text{m}} = 1.6 \mu\text{M}$ for **32**, which is in the same range as that for imipenem. However, due to the slow rate of hydrolysis of **31**, K_{m} could not be evaluated for this compound. But, the dissociation

constant for **31** was measured at $33 \pm 1 \mu\text{M}$ for this enzyme. Hence, it would appear that the differential effect seen for $k_{\text{cat}}/K_{\text{m}}$ for compounds **31** and **32** is primarily due to the kinetic effects on k_{cat} .

Concurrent with the work described above, we were investigating the mechanism of enzymic deacylation of the acyl-enzyme intermediate for the TEM-1 β -lactamase. We had earlier demonstrated that penicillanate (**13**) was a reasonably good substrate for the TEM β -lactamase.¹¹ It occurred to us that a molecule could be designed, on the basis of the minimal structure of penicillanate (**13**), which would acylate the active-site serine, but which would resist deacylation by being incorporated with a functionality that displaces the active-site hydrolytic water molecule. This would increase the longevity for the acyl-enzyme intermediate, which would be expected to provide effective inhibition of the enzyme. It was inferred from mutagenesis analysis that the active-site Water-712 is the hydrolytic water,²⁸ and we tried to design our molecule such that on active-site acylation Water-712 would be displaced from its position. Molecular modeling indicated that 6 α -(hydroxymethyl)penicillanate (**33**) should be such



a molecule. The hydroxyl group of the hydroxymethyl function of **33** was expected to displace Water-712. Modeling indicated that there are two energy minima for the acyl-enzyme intermediate with **33**; in one the active-site water is displaced, and in the other, the hydroxyl of the hydroxymethyl moiety would make a hydrogen bond to the hydrolytic water, presenting a physical barrier to its travel to the carbonyl group of the acyl-enzyme intermediate. Whereas **13** is hydrolyzed by the TEM-1 β -lactamase readily in minutes, compound **33** acylated the active site and resisted deacylation to the extent that recovery of 90% of activity required 11 h.²⁹ Furthermore, the kinetics of inhibition and the subsequent recovery of activity were both biphasic, indicative of involvement of two acyl-enzyme species.

The high-resolution crystal structure (to 1.95 Å) for one of these acyl-enzyme intermediates has recently been solved.³⁰ The complex is highly similar to the model that indicated hydrogen bonding between the hydroxymethyl moiety and the hydrolytic water. This is the first crystal structure for an acyl-enzyme intermediate with a substrate reported for a native class A β -lactamase. Prolonged incubation of the inhibitor with crystals of the TEM-1 β -lactamase resulted in cracking of the crystals, suggesting that local structural movement may be necessary to produce the second acyl-enzyme species. This assertion is consistent with the modeling results that indicated the

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(27) Miyashita, K.; Massova, I.; Mobashery, S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 319.

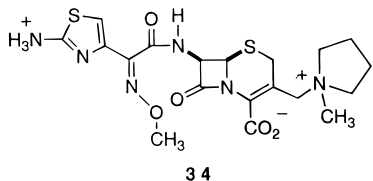
(28) Adachi, H.; Ohta, T.; Matsuzawa, H. *J. Biol. Chem.* **1991**, *266*, 3186. Escobar, W. A.; Tan, A. K.; Fink, A. L. *Biochemistry* **1991**, *30*, 10783. Delaire, M.; Lenfant, F.; Labia, R.; Masson, J. M. *Protein. Eng.* **1991**, *4*, 805.

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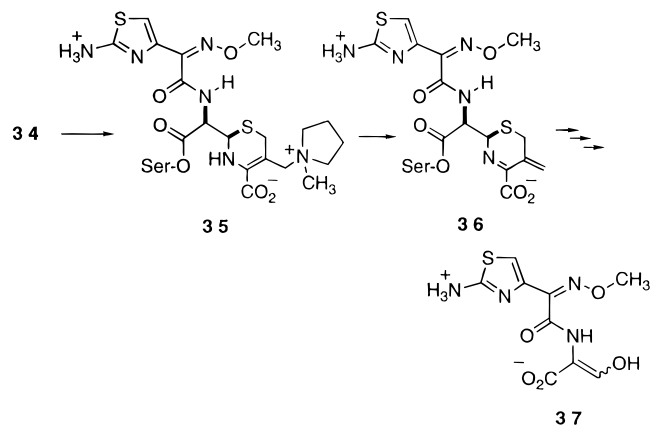
(30) Maveyraud, L.; Massova, I.; Birck, C.; Miyashita, K.; Samama, J. P.; Mobashery, S. *J. Am. Chem. Soc.* **1996**, *118*, 7435.

requirement for a change in a dihedral angle for interconversion between the two acyl-enzyme species. The design concepts outlined for **33** as an inhibitor support the mechanistic roles proposed for Glu-166 (as a general base) and Water-712 (as the hydrolytic water) in the deacylation step for turnover chemistry by class A β -lactamases.^{29,30}

The cephalosporin cefepime (**34**) has recently been approved for clinical use as an antibacterial agent. The

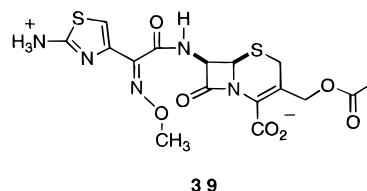
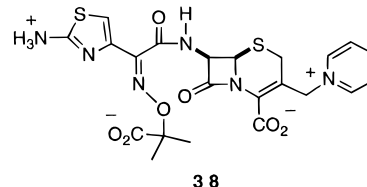


model for the active-site binding of this antibiotic indicated severe steric interactions between the active site of the TEM-1 β -lactamase and the $C_{7\beta}$ moiety of **34**. The steric interactions with amino acids Pro-167 and Asn-170, present in the Ω -loop spanning residues 164–179, are unfavorable in the interactions of the enzyme with the antibiotic.³¹ These interactions facilitated the dislocation of the hydrolytic water from its preferred position. These observations were consistent with determination of the microscopic rate constants, which showed that both acylation and deacylation were slow processes, but deacylation was rate-limiting. Indeed, the process of deacylation was sufficiently slow for the TEM-1 β -lactamase ($k_3 = 0.6 \pm 0.2 \text{ s}^{-1}$) that the *N*-methylpyrrolidine moiety of the cephalosporin was released from the acyl-enzyme intermediate prior to deacylation (**35** \rightarrow **36**), and subsequent nonenzymic fragmentation of the immediate product of β -lactam hydrolysis gave **37**.³¹ Furthermore, it was shown in circular dichroic measurements that hydrolysis of **34** by this β -lactamase is accompanied by a relaxation of the structure of the enzyme in order to accommodate the bulky $C_{7\beta}$ side chain of the antibiotic in the active site. These findings were in good agreement with dynamics simulations of the structure of the acyl-enzyme intermediate, which supported the possibility for the structural relaxation of the protein once this intermediate forms. Several mutant variants of the class A TEM-1 β -lactamase, including Asp-179-Gly and Arg-164-Asn (both residues in the Ω -loop), were prepared to explore whether an enlargement of the active site would facilitate turnover of **34**. Both mutant enzymes showed improved interactions



with cefepime, consistent with the expectations. Several mutant variants of the TEM-1 β -lactamases with substitutions of serine and histidine at position 164 have been identified in clinical isolates;⁵ hence, nature has already selected for resistance to extended-spectrum cephalosporins such as **34**.

Analysis of the details of the chemistries of enzymic hydrolysis for the clinically used extended-spectrum cephalosporins ceftazidime (**38**) and cefotaxime (**39**) were



similar to those for cefepime for the same structural reasons.³² Again, both acylation and deacylation were slow processes, and deacylation was the rate-limiting step in each case. Furthermore, the aforementioned mutations at positions 164 and 179 enhanced the rates of turnover of these two cephalosporins as well.

As a concluding thought, we point out that the enhancement or relaxation of structure for the TEM-1 β -lactamase that we have observed in hydrolyses of imipenem and cefepime, respectively, indicate the versatility of this enzyme in accommodating substrates of diverse structures. Indeed, a hallmark of catalysis by β -lactamases is that they take many β -lactams as substrates, and given time, in conjunction with the power of selection *in vivo*, mutant enzymes emerge which overcome barriers to catalysis confronted by their parental enzymes. Bacterial β -lactamase would therefore appear to be an ideal system for the study of evolution of catalytic function.

Concluding Remarks. β -Lactamases were discovered before penicillin was introduced into medical use.³³ Indeed, the issue of obsolescence of β -lactam antibiotics as a result of the β -lactamase-mediated resistance has been the subject of discussion since the late 1940s.³⁴ These antibiotics remain to the present as the mainstays in clinical utility and are expected to continue to maintain this position for the foreseeable future. Remarkably, many bacterial metabolic processes and their associated enzymes appear to be distinct from their mammalian counterparts. Furthermore, there are unique bacterial features, such as the peptidoglycan structure, that have their own distinct pathways without parallels in the mammalian systems. With such multiplicity of targets for inhibition by potential antibiotics, one would imagine that

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(32) Taibi-Tronche, P.; Massova, I.; Vakulenko, S. B.; Lerner, S. A.; Mobashery, S. Unpublished results.

(33) Abraham, E. P.; Chain, E. *Nature* **1940**, *146*, 837.

(34) Forbes, G. B. *Br. Med. J.* **1949**, *2*, 569.

a vast array of drugs should be available in the antibacterial armamentarium. In reality, this is not the case. There appear to be two main difficulties. First, the mechanistic and structural information for many of these enzymes has not been explored sufficiently to aid us in design of inhibitors as potential antibacterials. Second, many of the potent inhibitors may not gain entry into the bacterium to exert their potential antibacterial activity. This latter problem is a monumental obstacle in antibacterial drug development, which has prevented the progress of many potential antibiotics from the laboratory to the clinic. Here the advantage of β -lactam antibiotics becomes apparent. The targets of these antibiotics, the penicillin-binding proteins (PBPs), are present on the periplasmic surface of the bacterial inner membrane. Access to these enzymes in Gram-negative bacteria is made by channel-forming porin proteins, which permit the entry of most β -lactam molecules from the medium into the periplasm across the bacterial outer membrane. Since Gram-positive bacteria are devoid of the outer membrane, the access of the antibiotics to the PBPs is even more facile for these bacteria. Combined with the fact that β -lactams in general enjoy good pharmacokinetics and low toxicity, these features make β -lactam antibiotics ideal chemotherapeutic agents.

In this Account we have addressed several aspects of the catalytic activity of the bacterial TEM-1 β -lactamase,

a prototypic class A enzyme. The discussion was framed in terms of analysis of the mechanism of action of the enzyme with both clinically used agents, as well as novel molecules that have been designed, synthesized, and studied in our laboratory to further our knowledge of these enzymes. Bacteria present a continuing challenge for medicinal chemists to be on the vigil for new antibiotics with novel mechanisms of action. However, it would appear equally critical that we understand the mechanistic details at the molecular level for the resistance enzymes in order to develop new β -lactams to extend clinical usefulness of the existing types of valuable antibiotics.

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