Structure-Based Design of β-Lactamase Inhibitors. 1. Synthesis and Evaluation of Bridged Monobactams

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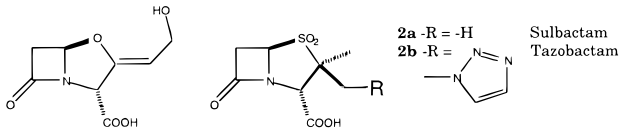
Bridged monobactams are novel, potent, mechanism-based inhibitors of class C β-lactamases, designed using X-ray crystal structures of the enzymes. They stabilize the acyl−enzyme intermediate by blocking access of water to the enzyme−inhibitor ester bond. Bridged monobactams are selective class C β-lactamase inhibitors, with half-inhibition constants as low as 10 nM, and are less effective against class A and class B enzymes (half-inhibition constants > 100 μM) because of the different hydrolysis mechanisms in these classes of β-lactamases. The stability of the acyl−enzyme complexes formed with class C β-lactamases (half-lives up to 2 days were observed) enabled determination of their crystal structures. The conformation of the inhibitor moiety was close to that predicted by molecular modeling, confirming a simple reaction mechanism, unlike those of known β-lactamase inhibitors such as clavulanic acid and penam sulfones, which involve secondary rearrangements. Synergy between the bridged monobactams and β-lactamase-labile antibiotics could be observed when such combinations were tested against strains of Enterobacteriaceae that produce large amounts of class C β-lactamases. The minimal inhibitory concentration of the antibiotic of more than 64 mg/L could be decreased to 0.25 mg/L in a 1:4 combination with the inhibitor.

Introduction

Infections caused by Gram-negative bacteria constitute about one-half of the cases of life-threatening nosocomial disease.[1,2] The past decade has seen an increase in the frequency of resistance to modern antibiotics including the third-generation cephalosporins.[3,4] In many cases, the resistance to β-lactam antibiotics is due to a high level of expression of class C β-lactamases.[2,5,6] These enzymes are mostly chromosomally encoded, but recently class C enzymes have appeared on plasmids, thus crossing interspecies boundaries.[7] Available types of β-lactamase inhibitors such as clavulanate (**1**), sulbactam (**2a**), and tazobactam (**2b**) are ineffective against these β-lactamases and are therefore not suitable for use in combination with β-lactamase-susceptible antibiotics against these emergent organisms.[2,3,8]



1 **2**

Clavulanate and the penam sulfones undergo chemical rearrangements that are triggered by attack of the enzyme on the β-lactam ring and which result in acrylic esters that are intrinsically more stable to hydrolysis.[9−14] The inefficiency of these compounds as inhibitors of the class C enzymes stems from the fact that hydrolysis of the acyl intermediate (deacylation) can occur more rapidly than the chemical rearrangement to the more stable species in the class C active site.[14] We set out to use structural and kinetic information about class C β-lactamases as a basis for the design of mechanism-based inhibitors that would specifically block the deacylation reaction of this refractory class of enzymes.

Design of Inhibitors of Class C β-Lactamases

The starting point for inhibitor design was solution of the crystal structures of *Citrobacter freundii* 1203 β-lactamase and the acyl−enzyme complex formed with aztreonam (Scheme 1) at 2.0 and 2.5 Å, respectively.[15] Comparison of the aztreonam moiety observed in the complex with the intact β-lactam molecule indicated that rotation about the C3−C4 bond by ∼70° had occurred. The counterclockwise rotation about the C3−C4 bond relaxes the eclipsed conformation of the intact β-lactam to the energetically more favorable gauche form (Scheme 1). Clockwise rotation, to adopt the alternative gauche configuration, is less favorable because it would bring the C4 methyl substituent into steric conflict with the side chains of tyrosine 150 (see Figure 2) and leucine 119. The rearrangement in the aztreonam complex leaves N1 and the sulfonate group in a position where they block one face of the ester bond formed with serine 64 (Scheme 1, upper reaction). They also displace a water molecule (WAT192 in Figure 2) that, in the native enzyme structure, is in a position where it interacts with an extensive hydrogen-bonded network of residues that is an essential part of the catalytic mechanism of class C β-lactamases. The residues involved in this network, comprising lysine 67, tyrosine 150, asparagine 152, and lysine 315, all con-

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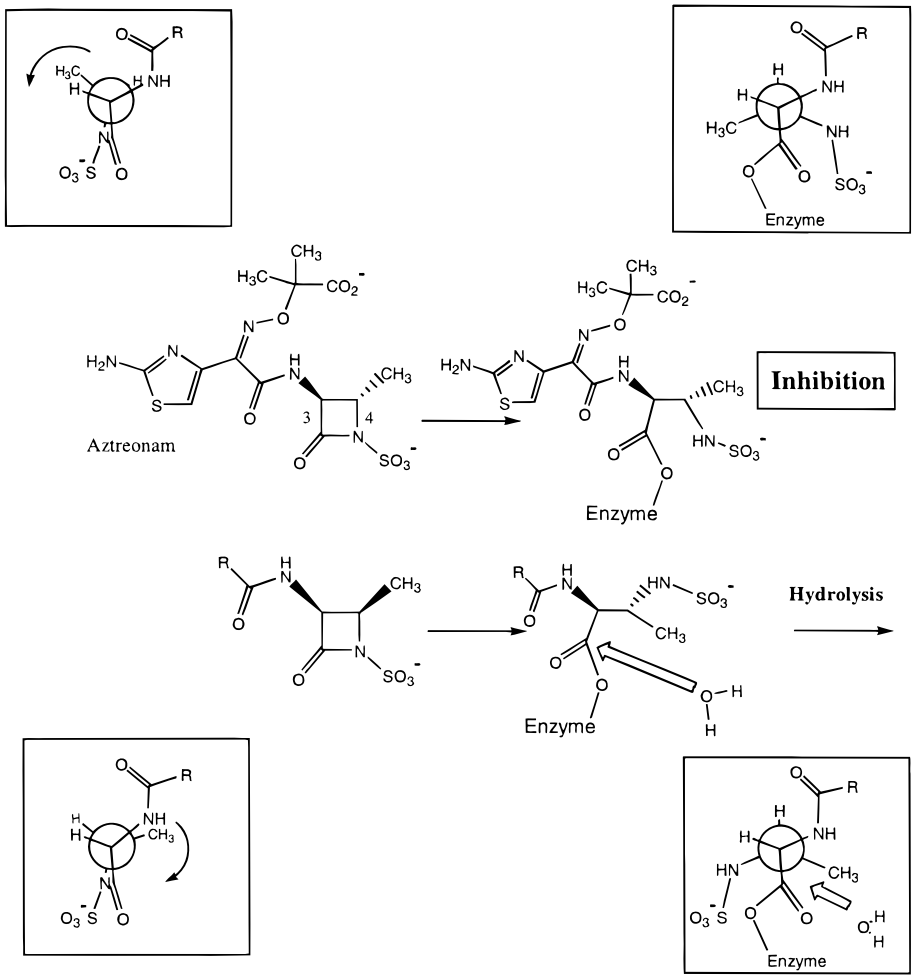
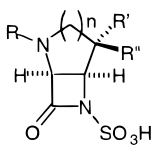
Scheme 1. Changes in the Configuration of the β-Lactam Moiety Occurring during Reaction with β-Lactamases



tribute somewhat to activation of water for hydrolysis of the acyl−enzyme complex.[15−19] It was postulated that it is this water, positioned near tyrosine 150, that is involved in deacylation.[15]

Molecular modeling, based on the *C. freundii* structure, suggested that with penicillins, cephalosporins, and monobactams where the substituents are cis to one another across the C3−C4 bond (Scheme 1, lower reaction), clockwise rotation can occur without conflict with protein side chains. The rotation leaves open the path for the water molecule to attack the ester, and therefore cis-substituted monobactams, as well as penicillins and cephalosporins, are rapidly hydrolyzed by class C enzymes.[20] Preventing this rotation in a suitable molecule should block the access of the water molecule to the ester bond and thus greatly stabilize the acyl−enzyme complex. We investigated this possibility by synthesizing monobactams (**3**) that had N3 linked to C4 by a two-carbon atom bridge.



3 (n = 1, 2)

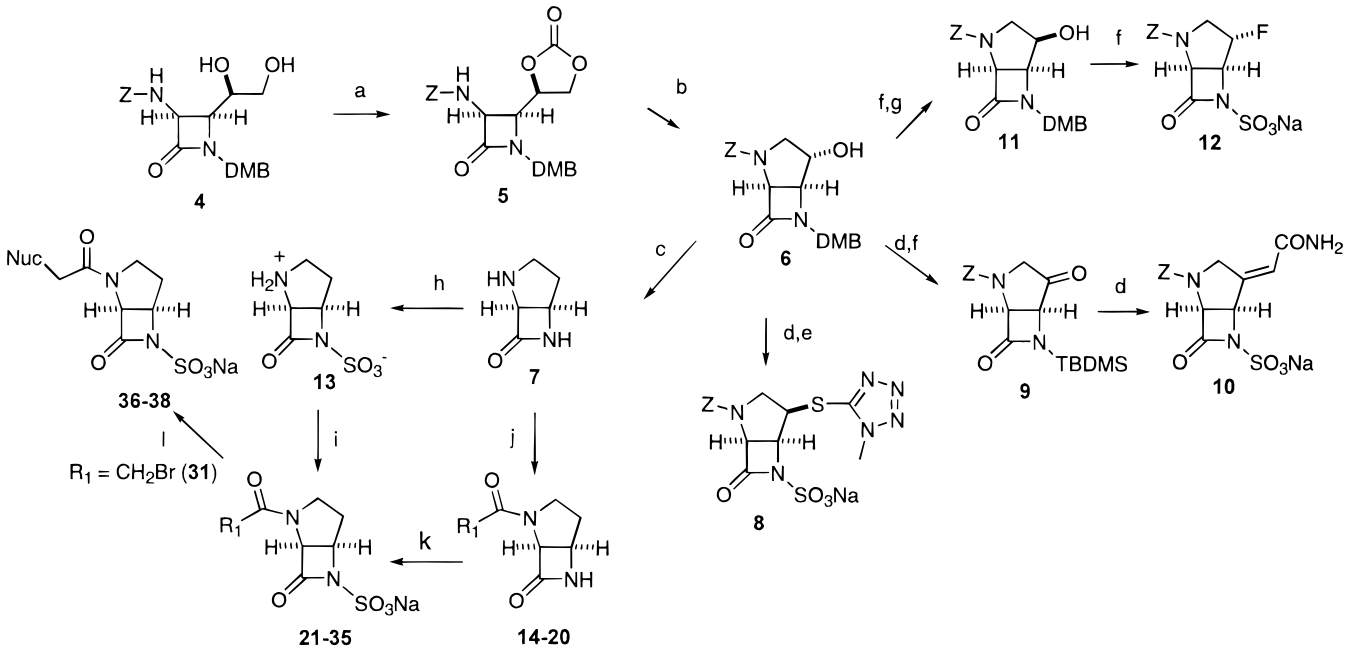
Chemistry

The key bicyclic derivatives **6** and **7** were obtained from the easily accessible[21] diol **4** by formation of the carbonate **5** and treatment with NBu_4Br[22,23] (Scheme 2). The triflate derived from the alcohol **6** was reduced with $NaBH_4$, and the protecting groups were removed by treatment with $K_2S_2O_8$ and hydrogenolysis over Pd/C to give the bridged β-lactam **7**.

The substituted monobactams **8**, **10**, and **12** were synthesized as described in Scheme 2. In a series of reactions, the 3,4-dimethoxybenzyl (DMB) protecting group on the β-lactam nitrogen of **6** was replaced by the *tert*-butyldimethylsilyl group (TBDMS) after transient THP−ether protection of the alcohol function. The resulting intermediate alcohol, activated as a triflate, was reacted with sodium 5-mercapto-1-methyltetrazole. The TBDMS group was removed, and sulfonation with DMF·SO_3 complex gave compound **8**. The intermediate alcohol was also oxidized under Swern conditions[24] to give the ketone **9** that was subjected to Wittig reaction and, after deprotection of the β-lactam nitrogen, was sulfonated to give **10**. The alcohol **6** was transformed into the isomeric alcohol **11** after Swern oxidation and $NaBH_4$ reduction. Compound **11**, activated as triflate, was reacted with NBu_4F. Removal of the dimethoxybenzyl protecting group followed by sulfonation gave compound **12**.

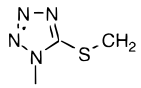
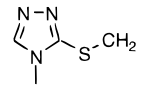
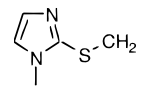
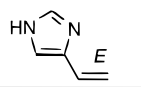
Compound **7** was treated with di-*tert*-butyl dicarbonate, sulfonated with DMF·SO_3, and hydrolyzed to give the betaine **13** (Scheme 2). Treatment of **13** with *O*-succinyl-activated esters (method A) and *O*-succinyl-activated carbamates (method B) gave the correspond-

Scheme 2[a]



[a] Reagents: (a) carbonyldiimidazole; (b) Bu_4NBr; (c) (1) Tf_2O, (2) $NaBH_4$, (3) $K_2S_2O_8$, (4) H_2, Pd/C; (d) (1) $K_2S_2O_8$, (2) dihydropyran, (3) TBDMS-Cl, imidazole, (4) pTSA; (e) (1) Tf_2O, (2) 5-mercapto-1-methyltetrazole Na salt, (3) DMF·SO_3; (f) Swern oxidation; (g) (1) $Ph_3PCHCONH_2$, (2) NBu_4F, (3) DMF·SO_3; (h) $NaBH_4$; (f) (1) Tf_2O, (2) NH_4F, (3) $K_2S_2O_8$, (4) DMF·SO_3; (h) (1) $(BOC)_2O$, (2) DMF·SO_3 then H_2O; (i) $R_1COOSucc$ (method A) or $RNHCOOSucc$ (method B); (j) ROCOX (method C) or R_1COCl (method D) or R_1COOH, DCC (method E); (k) DMF·SO_3; (l) Nuc (method F), see Table 2. DMB, 3,4-dimethoxybenzyl.

Table 1. Preparation of Bridged Monobactams **14**–**20**

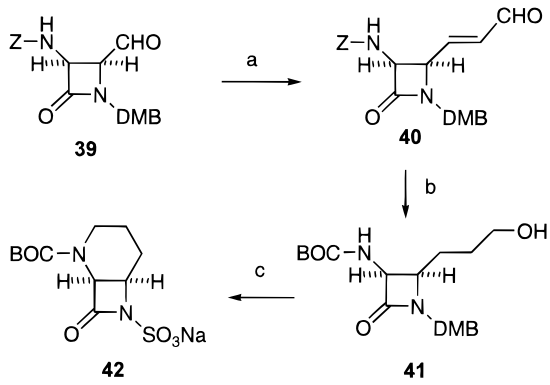
compd	R1	yield (%)	method	IR (cm^{-1})	MS (m/z)
14	$C_6H_5CH_2O$	68	C	1779, 1752, 1650	203[a]
15	$(CH_3)_3CO$	77	C	1747, 1716, 1687	169[a]
16	$BrCH_2$	75	D	1769, 1783, 1653	189[a]
17		70	E	1770, 1633	225[a]
18		51	E	1757, 1645	268[b]
19		18	E	1760, 1646	267.2[b]
20		71	E	1747, 1649, 1594	NA

[a] EI (M − CONH). [b] ISP $(M + H)^+$.

ing derivatives **21**–**28**. In another approach, compound **7** was first derivatized to give the intermediates **14**–**20** (methods C–E, Scheme 2) and then sulfonated with DMF·SO_3 to give the derivatives **29**–**35**. The bromoacetyl derivative **31**, obtained following method D, was reacted with nucleophiles to give compounds **36**–**38** (method F, Scheme 2; Tables 1 and 2).

The synthesis of the bridged β-lactam **42** is described in Scheme 3. The aldehyde **39** obtained from **4**[22] was subjected to Wittig reaction leading to the unsaturated aldehyde **40**. Reduction to the corresponding alcohol **41** was achieved by treatment with $NaBH_4$ followed by

Scheme 3[a]



[a] Reagents: (a) $Ph_3PCHCHO$; (b) (1) $NaBH_4$, (2) H_2, Pd/C, (3) $(BOC)_2O$; (c) (1) MsCl, (2) NaH, (3) $K_2S_2O_8$, (4) DMF·SO_3 then $NaHCO_3$.

hydrogenation over Pd/C and treatment with di-*tert*-butyl dicarbonate. The mesylate derived from the alcohol **41** was cyclized with NaH. Deprotection and sulfonation on the β-lactam nitrogen gave derivative **42**.

Results and Discussion

Inhibition of Class C β-Lactamases. The inhibitors, which we term bridged monobactams, were highly efficacious inhibitors of a wide variety of class C β-lactamases (Table 3). The reaction with class C β-lactamases was typically biphasic (Figure 1), reflecting two kinetically distinct conformations of the protein, as shown in Scheme 4.[18,19,25] The steady-state level of occupation of the active site, which is dependent on the ratio of deacylation rate to the acylation rate (k_4/k_2),[26] was greater than 0.999. The low deacylation rate was reflected in a very low net hydrolysis rate, with $k_{cat}/K_M < 1\ M^{-1}\ s^{-1}$ (Figure 1).

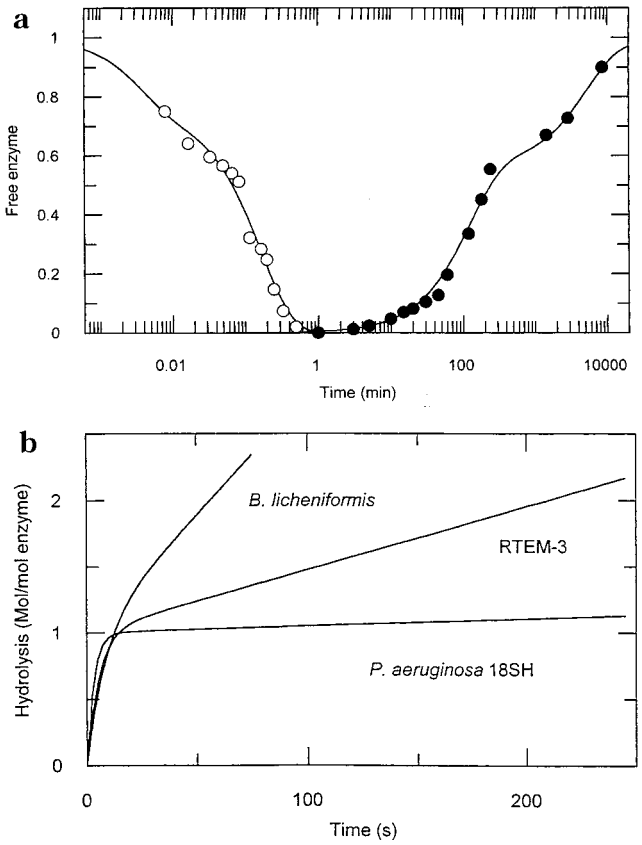
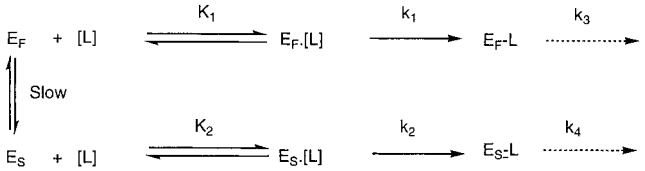


Figure 1. Time course of the reaction of β-furylacrylamido-bridged monobactam with β-lactamases. (a) Acylation of *C. freundii* 1982 class C β-lactamase. The protein concentration was 0.5 μM, and the inhibitor was present at 10 μM for acylation (○) and at 0.6 μM for deacylation (●). Free enzyme activity was determined by measuring residual nitrocefin hydrolysis.[25] The buffer was 0.1 M sodium phosphate, pH 7.0 at 37 °C. (b) Tracings of the absorption change at 310 nm during the reaction of the inhibitor with *P. aeruginosa* 18 SH class C β-lactamase (10 μM inhibitor), TEM-3 class A β-lactamase (1 mM inhibitor), and *B. licheniformis* class A β-lactamase (1 mM inhibitor). The enzymes were present at 0.1 μM; the buffer was 0.1 M sodium phosphate, pH 7.0 at 37 °C. The reactions were started by mixing equal volumes of inhibitor and enzyme solutions using an Applied Photophysics RX 1000 rapid mixing spectrophotometer accessory.

Structure of β-Lactamase−Inhibitor Complexes. The stability of the acyl−enzyme complexes formed with *C. freundii* class C β-lactamase allowed solution of their structures by X-ray crystallography (Figure 2). No significant changes in protein structure occurred, except that the side chain of aspartate 123 moved to accommodate the inhibitor bound to a neighboring protein molecule. The conformation adopted by the inhibitor moiety shows that the β-lactam ring has been opened to form an ester with the catalytic serine residue. The *N*-sulfonate moiety forms a salt bridge with lysine 315. N1 blocks the trajectory that an incoming water molecule would have to use for attack on the ester, while the sulfonate displaces the water from the position where it can be activated. The carbonyl oxygen of the ester occupies the "oxyanion hole", forming good hydrogen bonds to the main chain NH groups of serine 70 and serine 318. In this position, the reactivity of the ester toward attack by a water molecule should be comparable to that of a rapidly hydrolyzed substrate. Therefore, the stability of the acyl−enzymes formed

Scheme 4. Minimum Reaction Scheme for the Interaction of Bridged Monobactams with Class C β-Lactamases



with the bridged monobactams is attributable solely to the denial of access to the water molecule. It does not involve deactivation of the ester as a result of either chemical rearrangement or moving the carbonyl oxygen out of the oxyanion hole.

A rotation about the amide bond of the acyl side chain by approximately 35°, compared to the aztreonam complex, is observed in the inhibitor complexes. This different vector is imposed by the pyrrolidine ring. It has the consequence of placing the side chain deeper in the active site. In turn, this restricts the options for side chain variation to compounds that have small substituents in the α-position and that are relatively flexible. Indeed, bridged monobactams with rigid side chains such as that of aztreonam have very low affinities for the enzymes (Table 3).

The side chain of the inhibitor lies parallel to the strand of the β-sheet that forms one edge of the catalytic center. A hydrogen bond is possible between the carbonyl oxygen of the side chain and the amide nitrogen of asparagine 152, but its stereochemistry is far from ideal (Figure 2). There are two other interactions that appear to influence side chain specificity and affinity. First, there is the possibility to form a hydrogen bond between a donor, such as the α-amino group of **21**, and the backbone carbonyl group of serine 318 in the β-strand. This interaction appears to make a considerably smaller contribution to the overall binding energy than that involving asparagine 152 (Table 2). This may be because the side chains of tyrosine 221, valine 211, and threonine 319 provide a rather apolar protein environment at this position that counteracts the favorable hydrogen bond interaction. The second possibility is to form hydrophobic contacts with the aromatic ring of tyrosine 221, which lies at the bottom of the side chain binding pocket. The influence of this interaction was clear in a series of compounds having simple aromatic and aliphatic side chains (Table 2).

Optimization of the interactions described above through modification of the acyl side chain increased the affinity over 10000-fold, from a K_2 value > 10 mM (when none of the interactions are satisfied) to a K_2 value of 120 nM. The stability of the acyl−enzyme complex also increased (Table 4), possibly because tighter binding of the inhibitor decreases the flexibility of the acyl−enzyme complex and thus the rate of occasional access of water to the ester.

Reaction with Class A β-Lactamases. The class A enzymes generally exhibited low affinity for the bridged monobactams, although significant inhibition was observed with some side chains (Table 3). At high concentration, acylation of class A β-lactamases was also rapid, but the rate of breakdown of the acyl−enzyme was also considerably higher. Thus, lower steady-state levels of occupancy of the active site and more rapid

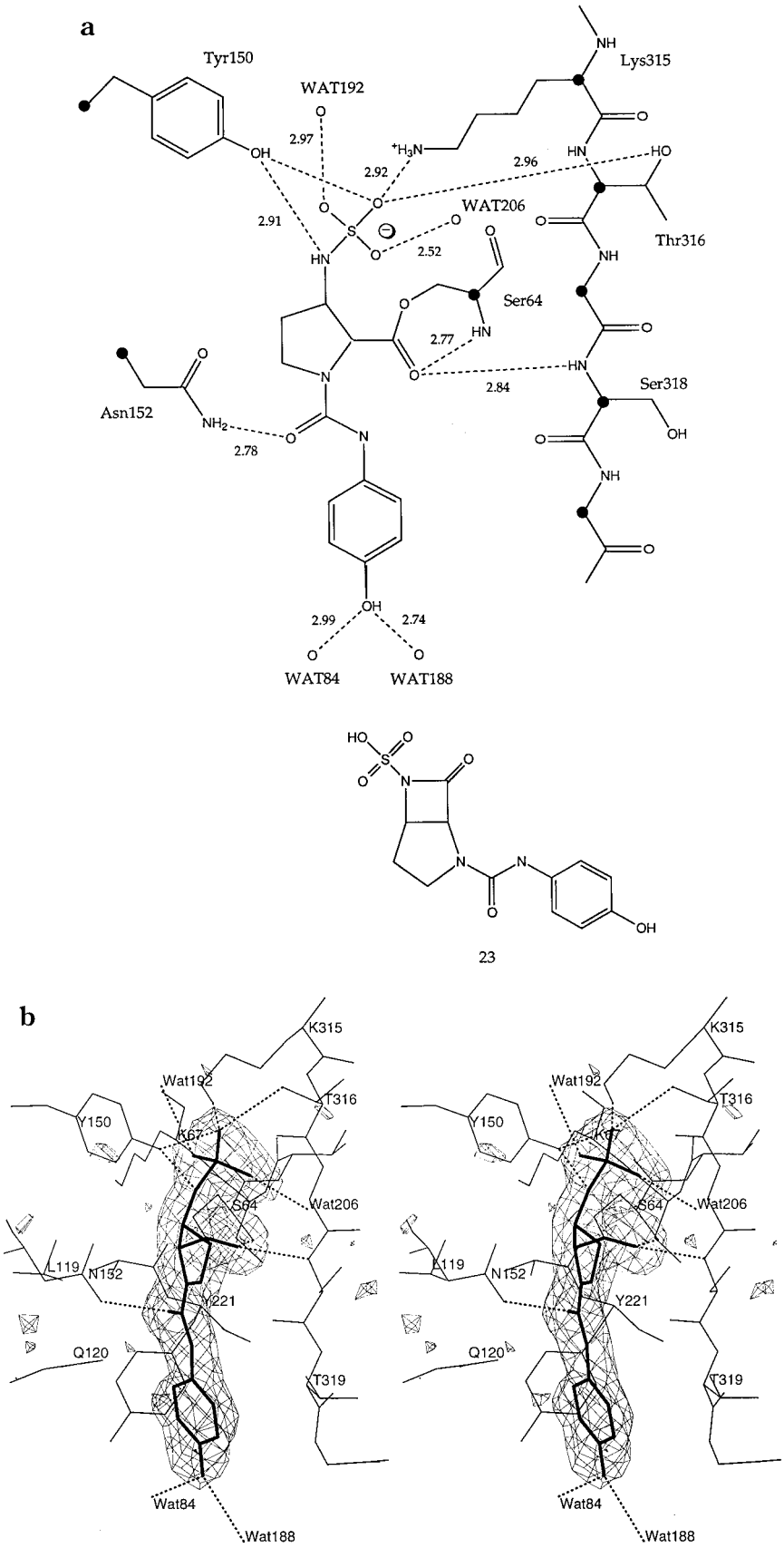
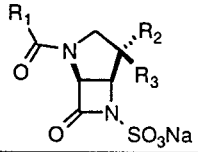
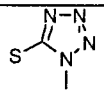
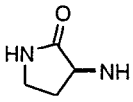
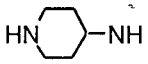
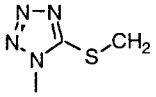
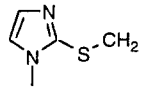
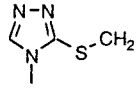
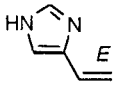
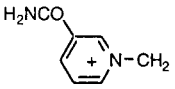
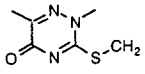


Figure 2. Binding mode of compound **23** in the active site of *C. freundii* β-lactamase. (a) Schematic representation. Hydrogen-bonding interactions of the covalently bound inhibitor moiety with the enzyme and with ordered solvent molecules are indicated by dashed lines (distances in Å). Cα positions are emphasized by filled circles. (b) Stereoview showing the inhibitor (thick lines) together with an omit electron density map (contour level 0.12 e/Å^3) calculated using the final model with the inhibitor moieties omitted. Hydrogen-bonding interactions as in panel a are indicated with broken lines.

Table 2. Preparation of Bridged Pyrrolidino Monobactams



compd	R_1	R_2	R_3	yield (%)	method[a]	IR (cm^{-1})	MS (M^-)
8	$C_6H_5CH_2O$	H		10	Exp	1771, 1710	439.0
10	$C_6H_5CH_2O$	=CHCONH$_2$ (*E*)		49	Exp	1778, 1762, 1650	380.1
12	$C_6H_5CH_2O$	F	H	65	Exp	1769, 1698	343.0
21	D-(4-HOC$_6$H$_4$)CH(NH$_2$)	H	H	20[b]	A	1766, 1660	340.0
22	C_6H_5NH	H	H	55	B	1751, 1657, 1630	309.9
23	4-HOC_6H_4NH	H	H	51	B	1755, 1647, 1610	326.0
24	3-(H_2NCO)C_6H_4NH	H	H	90	B	1756, 1661, 1607	353.1
25	4-(H_2NCO)C_6H_4NH	H	H	78	B	1756, 1661, 1608	353.1
26	4-(H_2NCONH)C_6H_4NH	H	H	47	B	1757, 1661, 1601	368.3
27		H	H	82	B	1756, 1697, 1638	316.8
28		H	H	59[b]	B	1756, 1632	317.1[c]
29	$C_6H_5CH_2O$	H	H	68	C	1759, 1707	349[c]
30	^{t}BuO	H	H	77	C	1758, 1690	291.0
31	$BrCH_2$	H	H	81	D	1760,1641	312.9
32		H	H	33	F	1758, 1646	347.0
33		H	H	35	E	1757, 1642	345.2
34		H	H	41	E	1758, 1644	346.2
35		H	H	71	E	1756, 1652, 1604	311.5
36		H	H	43	E	1763, 1689, 1665	355.2[d]
37		H	H	36	F	1759, 1647	388.0
38	4-$HOC_6H_4NHCH_2$	H	H	60	F	1759, 1648, 1517	340.1[c]

[a] Exp refers to special cases described in the Experimental Section. Methods A−F refer to general procedures described in the Experimental Section. [b] Refers to a two-step procedure; see Experimental Section. [c] $(M - Na)^-$. [d] $(M + H)^+$.

hydrolysis were observed (Figure 1), with $k_{cat}/K_M \leq 10^4$ M^{-1} s^{-1}. Inspection of class A β-lactamase crystal structures indicated that a water molecule in an equivalent position would be much less activated because there is a serine residue replacing the tyrosine of class C enzymes.[15,27] Attack in a class A enzyme occurs from the opposite side of the ester, with activation of the water molecule occurring through an extension of the hydrogen bond network that is not present in class C enzymes.[27−30] Hence, rotation about C3−C4 is not so

Table 3. Enzyme Inhibition and in Vitro Synergy with Ceftriaxone

	IC_{50}[a] (nM)			MIC[b] (μg/mL) ceftriaxone:inhibitor = 1:4		
compd	*C. freundii* 1982 (class C)	*P. aeruginosa* 18 SH (class C)	*E. coli* TEM-3 (class A)	*C. freundii* 1982	*P. aeruginosa* 18 SH	*E. coli* TEM-3
ref[c]				128	128	16
8	220	613	35	64	64	16
10	65	1035	31	>16	>16	16
12	13	380	511	>16	>16	16
21	500	90	>100000	2	8	8
22	220	420	NA	2	8	4
23	109	155	>100000	1	8	8
24	10	24	4000	1	16	16
25	54	217	>100000	0.5	4	16
26	12	48	100000	0.25	4	16
27	364	2246	>100000	1	4	16
28	2200	3900	>100000	2	8	16
29	100	200	>100000	8	128	16
30	225	1500	>100000	8	8	16
32	85	200	>100000	0.5	8	8
33	122	612	>100000	1	8	16
34	283	853	>100000	1	128	8
35	50	110	>100000	0.5	8	16
36	23	27	700	0.5	4	8
37	6	55	>100000	0.25	16	16
38	3	25	79200	8	128	8
42	5700	145000	>100000	>16	>16	16
1[d]	900	800	15	8	>32	0.25

[a] Inhibition of the isolated enzymes. [b] With the strains that produce the β-lactamases. [c] MICs of ceftriaxone in the absence of any inhibitor. [d] Values obtained with tazobactam, reference inhibitor.

Table 4. Parameters Influencing the Efficacy of Selected Bridged Monobactams

	kinetic parameters for *P. aeruginosa* 18 SH β-lactamase[a]			relative permeability (*P*)[b]	
compd	K_S (μM)	k_{on} (s^{-1})	k_{off} (10^{-5} s^{-1})	*C. freundii* 1982	*P. aeruginosa* 18 SH
8	15	3.8	4.1	10	68
21	6.9	2.7	<1	8.7	8.2
23	50	0.4	5.3	0.6	38
28	1480	1.5	120	1.7	1.0
30	127	11	3.7	NA	24
36	65	0.9	0.9	0.8	1.2
42	3500	0.5	70	NA	NA

[a] Kinetic parameters for acylation and deacylation were determined by the methods described in ref 8. [b] *P* is defined as the ratio of the observed IC_{50} for β-lactamase inhibition in intact, growing cells to the observed IC_{50} in the same cells treated with 0.7% final concentration of octyl-poly(oxyethylene). This treatment results in exposure of β-lactamase and IC_{50} values identical within experimental error to those obtained with isolated enzyme in free solution.

critical to the mechanism of deacylation in these enzymes, and restricting the rotation should have less impact on the stability of the acyl−enzyme. Thus, these observations are entirely consistent with the predictions based on the crystal structures of representative enzymes from the two classes and highlight the differences between the hydrolysis mechanisms of the two enzyme classes.

In Vitro Efficacy of the Inhibitors in Combination with β-Lactam Antibiotics. The inhibitors had no intrinsic antibacterial activity (data not shown). They were tested in combination with ceftriaxone, a third-generation cephalosporin, against strains producing class A and C β-lactamases (Table 3). As expected from the mechanism of the inhibitors, there was little synergy against strains producing class A β-lactamases. Surprisingly, despite relatively potent enzyme inhibition, the disubstituted derivatives (e.g., **8**, **10**, and **12**) showed no synergy apparently because of limited penetration through the outer membrane (Table 4). Limited permeability also seems to be a limiting factor with *P. aeruginosa*, as the magnitude of the synergy observed with the monosubstituted derivatives (**21**−**38**) was not as strong as expected. Several mechanisms might be operating in this organism,[31] but it seems that some derivatives (e.g., **23** or **30**) have difficulties in entering the periplasmic space of *P. aeruginosa* (Table 4). When this barrier could be overcome, as with Ro 48-1256 (**28**), better synergy could be obtained.[32] Penetration of the outer membrane is not such a barrier in the Enterobacteriaceae, and the monosubstituted derivatives (**21**−**38**) exhibited a strong synergy with ceftriaxone against strains producing class C β-lactamases. With the exception of compounds **29**, **30**, and **38**, the MIC values correlated rather well with the IC_{50} values.

Conclusion

The bridged monobactams are potent and selective inhibitors of class C β-lactamases. The activity arises from the formation of a stable acyl−enzyme complex that blocks the enzymes. The synergy with β-lactamase-sensitive antibiotics was less pronounced against *P. aeruginosa* than expected and could be partly attributed to a reduced penetration of the inhibitors through the outer membrane. Strong synergism with ceftriaxone and other β-lactam antibiotics was observed against Enterobacteriaceae.

Experimental Section

General Synthetic Methods. Solvents were dried by filtration through Al_2O_3 (neutral, Brockmann-number 1) when necessary and stored over a bed of molecular sieves (3 Å). All the organic solutions obtained after extraction were worked up by washing with water and brine, dried over $MgSO_4$ (unless indicated otherwise), and evaporated under reduced pressure with a water bath temperature below 35 °C. Chromatography was performed using Merck silica gel 60 (particle size 40−63

μm). Water-soluble compounds were purified by gel filtration (Mitsubishi Kasei Corp. MCI gel CHP20P 75−150 μm) using a gradient of MeOH in H_2O. The relevant fractions were pooled and lyophilized. TLC was performed on Merck TLC plates (silica gel 60 F_{254}) and were visualized with Cl_2 and a 0.5% aqueous solution of *o*-tolidine (in the presence of 2% AcOH and 0.05% KI). Proton NMR spectra were recorded on a Bruker AC250 (250 MHz) spectrophotometer. Chemical shifts (δ) are reported in ppm relative to Me_4Si as internal standard, and *J* values are in hertz (Hz). IR spectrum were recorded on a Nicolet FTIR spectrometer as KBr pellets (unless indicated otherwise). Ion spray mass spectra were recorded on a Finnigan MAT SSQ 7000 instrument, and EI mass spectra were recorded on a Perkin-Elmer Siex API III instrument. Optical rotation data were obtained on a Perkin-Elmer 241 polarimeter at 20 °C. Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. Elemental analyses are indicated by the symbols of the elements; analytical results were within 0.4% of the theoretical values.

(*3S,4S*)-[1-(3,4-Dimethoxybenzyl)-2-oxo-4-[(*R*)-2-oxo-1,3-dioxololan-3-yl]azetidin-3-yl]carbamic Acid Benzyl Ester (5). A solution of **4**[22] (215.3 g, 0.5 mol) in THF (3 L) and 1,1′-carbonyldiimidazole (121.6 g, 0.75 mol) was refluxed for 4 h. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2, washed with 1 N HCl, and worked up leaving 220.3 g (96%) of **5**, mp 135−136 °C. IR: 1836, 1850, 1765, 1721, 1687 cm^{-1}. MS (EI): 456 (M^+). NMR ($CDCl_3$): 3.68 (dd, br, 1H); 3.88 (s, 6H); 3.97−4.25 (m, br, 3H); 4.68−4.81 (m, br, 2H); 4.97−5.20 (m, br, 3H); 5.87 (NH, d, br, 1H); 6.81 (s, 1H); 6.83 (s, 1H); 7.35 (5H). Anal. ($C_{23}H_{24}N_2O_8$) C, H, N.

Benzyl (*1S,4S,5S*)-6-(3,4-Dimethoxybenzyl)-4-hydroxy-7-oxo-2,6-diazabicyclo[3.2.0]heptane-1-carboxylate (6). A solution of **5** (220 g, 0.48 mol) and Bu_4NBr (25.4 g, 0.12 mol) in DMF (3 L) was vigorously stirred under argon for 5 h at 140 °C. The solvent was evaporated, and the residue was dissolved in AcOEt, worked up, chromatographed (AcOEt/hexane, 3:1, then AcOEt), and crystallized yielding 172 g (86%) of **6**, mp 100−102 °C (AcOEt). $[\alpha]_D$ −172°($CHCl_3$, *c* 1). IR: 1731, 1707 cm^{-1}. NMR ($CDCl_3$): 2.24 (s br, 1H); 3.35 (dd, *J* = 3.5 and 13, 1H); 3.86 (s, 3H); 3.87 (s, 3H); 3.91 (br, 3H); 4.21 (d, *J* = 15, 1H); 4.40 (d, *J* = 15, 1H); 5.13 (s, 2H); 5.26 (br, 1H); 6.76 (s, 1H); 6.81 (s, 3H); 7.32 (m, 5H). Anal. ($C_{22}H_{24}N_2O_6$) C, H, N.

Benzyl (*1S,5R*)-7-Oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate (7). (a) Benzyl (*1S,4S,5S*)-6-(3,4-Dimethoxybenzyl)-7-oxo-4-[(trifluoromethyl)sulfonyloxy]-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate. A solution of **6** (206 g, 0.5 mol) and pyridine (48 mL, 0.6 mol) in CH_2Cl_2 (0.8 L) was reacted below 10 °C with $(CF_3SO_2)_2O$ (98 mL, 0.6 mol) for 2 h, then quenched with dilute $NaHCO_3$ solution, and worked up yielding 272 g (100%) of crude triflate. IR: 1771, 1712 cm^{-1}. MS (EI): 544 (M^+). NMR ($CDCl_3$): 3.50 (br, 1H); 3.87 (s, 3H); 3.88 (s, 3H); 4.20 (br, 3H); 4.52 (br, 1H); 4.71 (br, 1H); 5.17 (br, 2H); 5.33 and 5.52 (br, 1H); 6.78 (s, 1H); 6.85 (m, s, 3H); 7.34 (m, 5H).

(b) Benzyl (*1S,5R*)-6-(3,4-Dimethoxybenzyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate. A solution of the triflate **7a** (272 g, 0.5 mol) in THF/DMF (1 L; 4:1) was reacted at 0 °C overnight with $NaBH_4$ (19 g, 0.5 mol), then the mixture was adjusted to pH 4 with AcOH, and the solvents were evaporated. The oily residue was poured into 1 N HCl, extracted with AcOEt, worked up, chromatographed (AcOEt/hexane, 1:1 to 4:1), and crystallized giving 120 g (60%) of material, mp 76−78 °C (AcOEt/hexane). $[\alpha]_D$ −187°($CHCl_3$, *c* 1). IR (film): 1755, 1705 cm^{-1}. NMR ($CDCl_3$): 1.46−1.79 (m, 2H); 3.24 (m, 1H); 3.87 (s, 3H); 3.88 (s, 3H); 4.0 (m, br, 1H); 4.18 (br, 1H); 4.32 (s, 2H); 5.06−5.31 (br, 3H); 6.8 (m, 3H); 7.35 (m, 5H). Anal. ($C_{22}H_{24}N_2O_5$) C, H, N.

(c) Benzyl (*1S,5R*)-7-Oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate. The following procedure is representative for the removal of the 3,4-dimethoxybenzyl protecting group. A solution of **7b** (117 g, 0.3 mol) in MeCN/H_2O (3 L; 2:1) was warmed to 100 °C, and $K_2S_2O_8$ (164 g, 0.6 mol) was added while the pH was maintained at 4.5 with a saturated $NaHCO_3$ solution. The mixture was cooled, saturated with NaCl, and extracted with AcOEt. The aqueous layer was extracted with AcOEt, and the combined organic phases were worked up, chromatographed (AcOEt/hexane, 2:1, then AcOEt), and crystallized giving 50.4 g (68%) of **7c**, mp 59−60 °C (AcOEt/hexane). $[\alpha]_D$ −234°($CHCl_3$, *c* 1). IR: 1755, 1689 cm^{-1}. NMR ($CDCl_3$): 1.69−2.03 (m, 2H); 3.42 (m, 1H); 4.1 (br, 1H); 4.32 (br, 1H); 5.05−5.35 (br, 3H); 6.08 (br, 1H); 7.36 (m, 5H). Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

(d) (*1S,5R*)-2,6-Diazabicyclo[3.2.0]heptan-7-one (7). A solution of **7c** (13.7 g, 55.6 mmol) in MeOH (0.15 L) was hydrogenated over Pd/C. After removal of the catalyst, the filtrate was evaporated and crystallized under Ar leaving 4.5 g (72%) of **7**, mp 148−49 °C (EtOH). $[\alpha]_D$ −0.2°(EtOH, *c* 1). IR: 1735, 1713 cm^{-1}. NMR (DMSO-d_6): 1.24 (m, 1H); 1.68 (dd, *J* = 5 and 13, 1H); 2.65 (m, 1H); 3.01 (br, 1H); 3.08 (dd, *J* = 7.5 and 11.5, 1H); 3.93 (dd, *J* = 3.5 and 5, 1H); 4.35 (dd br, *J* = 3.5 and 5, 1H); 7.74 (br, 1H). Anal. ($C_5H_8N_2O$) C, H, N.

(*1S,4R,5S*)-2-(Benzyloxycarbonyl)-4-[(1-methyl-1*H*-tetrazol-5-yl)sulfanyl]-7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic Acid Sodium Salt (8). (a) Benzyl (*1S,4S,5S*)-4-hydroxy-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate was prepared in 40% yield from **6** according to the procedure described for **7c**, mp 192−193 °C (MeOH). IR: 1764, 1720, 1660 cm^{-1}. Anal. ($C_{13}H_{14}N_2O_4$) C, H, N.

(b) 1:1 Mixture of (*1S,4S,5S*)-2-(Benzyloxycarbonyl)-4-[(*R*)- and (*S*)-tetrahydropyran-2-yloxy]-2,6-diazabicyclo-[3.2.0]heptan-7-one. The previous alcohol **8a** (40.0 g, 153 mmol) was treated with *p*-toluenesulfonic acid (2.9 g, 15 mmol) and dihydropyran (28 mL, 305 mmol) in DMF (0.5 L). After 24 h the solvent was removed under reduced pressure, and the residue was purified by chromatography (EtOAc/hexane, 3:2). Yield: 40 g (76%). IR (neat): 1775, 1714 cm^{-1}. MS (CI): 364 ($M + NH_4$)$^+$.

(c) 1:1 Mixture of Benzyl (*1S,4S,5S*)-6-(*tert*-Butyldimethylsilanyl)-4-[(*R*)- and (*S*)-tetrahydropyran-2-yloxy]-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate. A solution of the lactam **8b** (39.85 g, 115 mmol) in CH_2Cl_2 (0.8 L) was treated with triethylamine (24.1 mL, 173 mmol) and *tert*-butyldimethylchlorosilane (26.0 g, 173 mmol). After 24 h the reaction mixture was worked up and chromatographed (EtOAc/hexane, 3:2). Yield: 46.8 g (88%). IR (neat): 1757, 1711 cm^{-1}. MS (CI): 478 ($M + NH_4$)$^+$.

(d) Benzyl (*1S,4S,5S*)-6-(*tert*-Butyldimethylsiloxy)-4-hydroxy-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate. The ether **8c** (2.6 g, 5.64 mmol) was stirred with pyridinium *p*-toluenesulfonate (140 mg, 0.56 mmol) in MeOH (75 mL) at 50 °C for 24 h. The solvent was removed under reduced pressure, and the residue was chromatographed (EtOAc/hexane, 2:1) yielding 1.39 g (65%) of a colorless oil which crystallized upon standing, mp 99.5−101 °C. Anal. ($C_{19}H_{28}N_2O_4Si$) C, H, N.

(e) Benzyl (*1S,4S,5S*)-6-(*tert*-Butyldimethylsilanyl)-7-oxo-4-[(trifluoromethyl)sulfonyloxy]-2,6-diazabicyclo-[3.2.0]heptane-2-carboxylate. A solution of $(CF_3SO_2)O$ (2.46 mL, 14.61 mmol) in CH_2Cl_2 (5 mL) was added dropwise at 0 °C to a solution of the alcohol **8d** (5.0 g, 13.28 mmol) and pyridine (1.18 mL, 4.61 mmol) in CH_2Cl_2 (50 mL). After 2 h the reaction mixture was worked up with diluted $NaHCO_3$ solution yielding 6.0 g (89%) of an orange oil. IR (neat): 1765, 1716 cm^{-1}. MS (ISP): 526.4 ($M + NH_4^+$).

(f) Benzyl (*1S,4S,5S*)-4-(1-Methyl-1*H*-tetrazol-5-ylsulfanyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptanecarboxylate. A solution of the triflate **8e** (1.0 g, 1.96 mmol) in DMF (20 mL) was treated under argon with sodium 5-mercapto-1-methyltetrazole (hydrate; 600 mg, 2.16 mmol) at 80 °C for 48 h. The solvent was removed under pressure and the residue taken up in EtOAc, worked up, and chromatographed (EtOAc). Yield: 150 mg (21%). IR (film): 1769, 1708 cm^{-1}. MS (ISP): 383.2 ($M + Na$)$^+$.

(*1S,4R,5S*)-2-(Benzyloxycarbonyl)-4-(1-methyl-1*H*-tetrazol-5-ylsulfanyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-

6-sulfonic Acid Sodium Salt (8). The following procedure is representative for the sulfonation. A solution of **8f** (1.7 g, 4.7 mmol) in DMF (75 mL) was cooled to 0 °C and reacted with DMF·SO_3 complex (1.0 g, 7.1 mmol). After 4 h, the solvent was evaporated under high vacuum. The residue was taken up in water (3 mL), the pH was adjusted to pH 6 with $NaHCO_3$, and the solution was chromatographed by gel filtration. Yield: 10%.

Starting Material for the Preparation of (*1S,5R*)-(*E*)-2-(Benzyloxycarbonyl)-4-(carbamoylmethylene)-7-oxo-2,6-diazabicyclo[3.2.0]heptanesulfonic Acid Sodium Salt (10). (a) Benzyl (*1S,5S*)-6-(*tert*-Butyldimethylsilanyl)-4,7-dioxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate (9). A solution of DMSO (2.08 g, 26.56 mmol) in CH_2Cl_2 (40 mL) was treated at −78 °C with $(CF_3CO)_2O$ (4.18 g, 19.92 mmol). After 15 min, a solution of **8d** (5.0 g, 13.28 mmol) in CH_2Cl_2 (45 mL) was added dropwise and the mixture was stirred for another 1.5 h. *N*-Ethyldiisopropylamine (5.12 g, 39.64 mmol) was added, and the mixture was allowed to reach room temperature and was worked up with 0.5 N HCl and saturated $NaHCO_3$ solution yielding quantitatively the product as a yellowish oil. IR (neat): 1760, 1712 cm^{-1}.

(b) 1:1 Mixture of (*E*)- and (*Z*)-Benzyl (*1S,5R*)-6-(*tert*-Butyldimethylsilanyl)-4-(carbamoylmethylene)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate. The ketone **10a** (2.43 g, 6.5 mmol) and $H_2NCOCH{=}P(Ph)_3$ (2.28 g, 7.14 mmol) were refluxed in C_6H_6 (60 mL) for 30 min. The solvent was evaporated and the residue was purified by chromatography (EtOAc) yielding quantitatively an *E*/*Z* (1:1) mixture of the product. IR (neat): 1745, 1692, 1658 cm^{-1}. MS (EI): 358 (M − tBu).

(c) Benzyl (*E*)-(*1S,5R*)-4-(Carbamoylmethylene)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate. The lactam **10b** (1.5 g, 3.6 mmol) was deprotected by reaction with NBu_4F (133 mg, 3.6 mmol) in MeOH (25 mL) for 20 min. The solvent was removed under reduced pressure, and the residue taken up in EtOAc was worked up and chromatographed (EtOAc/EtOH, 3:2). Yield: 900 mg (83%). IR: 1720, 1653 cm^{-1}. Anal. ($C_{15}H_{15}N_3O_4$) C, H, N.

Benzyl (*1S,4R,5S*)-6-(3,4-Dimethoxybenzyl)-4-hydroxy-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate (11). (a) Benzyl (*1S,5S*)-6-(3,4-Dimethoxybenzyl)-4,7-dioxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate. A solution of DMSO (3.35 mL, 47 mmol) in CH_2Cl_2 (50 mL) was treated at −78 °C with $(CF_3CO)_2O$ (4.9 mL, 35.3 mmol). After 15 min, a solution of **6** (9.7 g, 23.5 mmol) in CH_2Cl_2 (50 mL) was added dropwise, and the mixture was stirred for another 1.5 h. *N*-Ethyldiisopropylamine (12.1 mL, 70.5 mmol) was added, and the mixture was allowed to reach room temperature and was worked up with 0.5 N HCl and saturated $NaHCO_3$ solution yielding quantitatively the product as a yellowish oil. IR (neat): 1760, 1709 cm^{-1}. MS (EI): 410 (M).

(b) Benzyl (*1S,4R,5S*)-6-(3,4-Dimethoxybenzyl)-4-hydroxy-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate (11). A solution of **11a** (1.8 g, 4.4 mmol) in EtOH (50 mL) was treated with $NaBH_4$ (166 mg, 4.4 mmol) at 0 °C. The reaction mixture was stirred overnight at room temperature and then acidified with AcOH. After removal of the solvent, the residue was taken up in CH_2Cl_2, worked up, and chromatographed (AcOEt) yielding 1.2 g (66%) of **11**, mp 118−120 °C. Anal. ($C_{22}H_{24}N_2O_6$) C, H, N.

Starting Material for the Preparation of Benzyl (*1S,4S,5S*)-4-Fluoro-7-oxo-6-sulfo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate Sodium Salt (12). (a) Benzyl (*1S,4R,5S*)-6-(3,4-dimethoxybenzyl)-7-oxo-4-[(trifluoromethyl)sulfonyloxy]-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate was prepared from **11** in 58% yield according to the procedure described for the derivative **8e**. IR (KBr): 1772, 1713 cm^{-1}. MS (EI): 544 (M^+).

(b) Benzyl (*1S,4S,5S*)-4-Fluoro-6-(3,4-dimethoxybenzyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate. A solution of the triflate **12a** (1.1 g, 2.02 mmol) in THF (10 mL) was treated at room temperature with $NBu_4F{\cdot}3H_2O$ (704 mg, 2.23 mmol) for 10 min. The solvent was removed under reduced pressure and the residue taken up in EtOAc, worked up, and chromatographed (EtOAc) yielding 700 mg (92%) of a yellowish oil. IR (neat): 1764, 1708 cm^{-1}. MS (ISP): 437.2 $(M + Na)^+$.

(c) Benzyl (*1S,4S,5S*)-4-fluoro-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate was prepared in 69% yield from **12b** according to the procedure described for the preparation of **7c**. IR (neat): 1772, 1707 cm^{-1}. MS (ISN): 263.1 $(M - H)^-$.

***tert*-Butyl (*1S,5R*)-7-Oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate (15; Method C).** A solution of **7** (11.2 g, 0.1 mol), $(BOC)_2O$ (22.9 g, 0.105 mol), and DMAP (1.2 g, 10 mmol) in THF (0.25 L) was stirred at room temperature for 3 h. The solvent was evaporated, and the residue was chromatographed (AcOEt) and crystallized leaving 16.4 g (77%) of **15**, mp 125−126 °C (ether/hexane). NMR (DMSO-d_6): 1.40 (s, 9H); 1.60 (m, 1H); 1.80 (m, 1H); 3.20 (m, 1H); 3.90 (m, 1H); 4.15 (t, J = 5, 1H); 4.90 (br, 1H); 8.10 (br, 1H). Anal. ($C_{10}H_{16}N_2O_3$) C, H, N.

(*1S,5R*)-7-Oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic Acid (13). A solution of **15** (18 g, 85 mmol) in anhydrous DMF (0.2 L) was reacted below 5 °C with DMF·SO_3 (14.3 g, 93.5 mmol) for 3 h. The solvent was evaporated, and the residue was treated with water (0.1 L), stirred overnight, and then concentrated to about 15 mL. The crystals (9.4 g, 57%) were collected by filtration. IR: 1778, 1614 cm^{-1}. MS (ISN): 191 $(M - H)^-$. NMR (DMSO-d_6): 1.74 (m, 1H); 2.46 (dd, J = 6 and 14, 1H); 3.05 (m, 1H); 3.61 (dd, J = 7.5 and 14, 1H); 4.43 (t, J = 5, 1H); 5.21 (d, J = 5, 1H); 9.50 (br, 3H). Anal. ($C_5H_8N_2O_4S$) C, H, N.

(*1S,5R*)-2-[D-2-(4-Hydroxyphenyl)glycyl]-7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic Acid (21; Method A). (a) *tert*-Butyl [(*R*)-4-Hydroxy-α-[[(*1S,5R*)-7-oxo-6-sulfo-2,6-diazabicyclo[3.2.0]hept-2-yl]carbonyl]benzyl]-carbamate Triethylammonium Salt. A solution of **13** (0.8 g, 2.6 mmol) in DMF (30 mL) was reacted with TEA (0.53 g, 5.2 mmol) and (*tert*-butyloxycarbonyl)-D-(4-hydroxyphenyl)-glycine hydroxysuccinimide (1.05 g, 2.85 mmol) for 4 h. The solvent was evaporated under reduced pressure, and the residue was purified by gel filtration giving 0.41 g (30%) of material. IR: 3413, 1770, 1708, 1651 cm^{-1}. MS (FAB): 439.8 $(M)^-$.

(b) (*1S,5R*)-2-[D-2-(4-Hydroxyphenyl)glycyl]-7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic Acid (21). The ester **21a** (0.4 g, 0.74 mmol) was reacted with TFA (9 mL) at −10 °C for 1 h. The solvent was evaporated, and the residue was purified by gel filtration giving 0.15 g (60%) of **21**. IR: 1766, 1660, 1614, 1517 cm^{-1}. MS (FAB): 340 $(M - Na)^-$.

(*1S,5R*)-2-(4-Carbamoylphenylcarbamoyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic Acid Sodium Salt (23; Method B). The described methods are general for the preparation of compounds **22**−**27** as well as their required activated side chains.

(a) 4-Carbamoylphenylcarbamic Acid 2,5-Dioxopyrrolidin-1-yl Ester. A solution of 4-aminobenzamide (2.72 g, 2 mmol) and *N*,*N*′-disuccinimidylcarbonate (5.6 g, 2.2 mol) in MeCN (0.25 L) was stirred for 5 h. The crystals were collected by filtration leaving 4.83 g (87%) of colorless material. NMR (DMSO-d_6): 2.84 (s, 4H); 7.30 (b, 1H); 7.50 (d, J = 9, 1H); 7.88 (d, J = 9, 1H); 7.90 (b, 1H). Anal. ($C_{12}H_{11}N_3O_5$) C, H, N.

(b) (*1S,5R*)-2-(4-Carbamoylphenylcarbamoyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic Acid Sodium Salt (23). A solution of **13** (0.15 g, 0.78 mmol) in H_2O/MeCN (1:1; 10 mL) was reacted with $NaHCO_3$ (66 mg, 0.78 mmol) and the above-described side chain **23a** (216 mg, 0.78 mmol) for 5 h. The organic solvent was evaporated, and the crude aqueous phase was purifed by gel filtration giving 230 mg (78%) of **23**. NMR (DMSO-d_6): 1.75 (m, 1H); 3.33 (dd, J = 6 and 15, 1H); 3.12 (m, 1H); 4.00 (dd, J = 9 and 12, 1H); 4.43 (t, J = 5, 1H); 5.28 (d, J = 5, 1H); 7.10 (br, 1H); 7.56 and 7.77 (2d, J = 9, 4H); 7.8 (b, 1H); 8.80 (s, 1H). Anal. ($C_{13}H_{13}N_4O_6SNa$) C, H, N.

(*1S,5R*)-7-Oxo-2-(piperidin-4-ylcarbamoyl)-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic Acid Sodium Salt (28).

(a) (*1S,5R*)-2-(1-Benzylpiperidin-4-ylcarbamoyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic acid sodium salt was prepared in 84% yield from **13** and 1-benzylpiperidin-4-ylcarbamic acid 2,5-dioxopyrrolidin-1-yl ester. IR: 1759, 1638 cm^{-1}. MS (ISN): 407 $(M - Na)^-$.

(b) A solution of **28a** (90 mg, 0.21 mmol) in water (10 mL) was hydrogenated over Pd/C in the presence of 1 N HCl (1 mL) at 50 °C. The catalyst was removed by filtration, and the pH of the solution was adjusted to pH 6 with saturated $NaHCO_3$ solution. The resulting crude aqueous phase was purifed by gel filtration giving 50 mg (70%) of **28**.

(*1S,5R*)-2-(Bromoacetyl)-2,6-diazabicyclo[3.2.0]heptan-7-one (16; Method D). A solution of **7** (6.0 g, 53.5 mmol) and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (19.8 mL, 107 mmol) in CH_2Cl_2 (0.2 L) was cooled to 0 °C and reacted with pyridine (4.3 mL, 54 mmol) and bromoacetyl bromide (5.15 mL, 59 mmol) for 2 h. The reaction mixture was worked up and chromatographed leaving 9.3 g (75%) of **16**. NMR (DMSO-d_6): 1.73 (m, 1H); 1.89 (m, 1H); 3.21 and 3.40 (2 m, 1H); 3.95−4.40 (m, 4H); 5.21 and 5.26 (br, 1H).

Starting Material for the Preparation of (*1S,5R*)-2-[[(1-Methyl-1*H*-tetrazol-5-yl)thio]acetyl]-7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulfonic Acid Sodium Salt (32; Method E). (*1S,5R*)-2-[[(1-Methyl-1*H*-tetrazol-5-yl)-thio]acetyl]-2,6-diazabicyclo[3.2.0]heptan-7-one (17). A solution of 1-[(methyl-1*H*-tetrazol-5-yl)thio]acetic acid (940 mg, 5.35 mmol) and TEA (0.75 mL, 5.35 mmol) in DMF (30 mL) was sequentially treated with HBTU (2.0 g, 5.35 mmol) at 20 °C and **7** (500 mg, 4.46 mmol). The mixture was stirred for 5 h. The solvent was evaporated under reduced pressure, and the residue was chromatographed (EtOAc/MeOH, 10:1 to 5:1). Yield: 840 mg (70%). IR: 1770, 1633 cm^{-1}; MS (EI): 225 (M − CONH). This method is representative for the synthesis of compounds **18**−**20**. Yields and physical properties are given in Table 1.

(*1S,5R*)-2-[[(Carbamoylpyridinyl)thio]acetyl]-7-oxo-2,6-diazabicyclo[3.2.0]heptane-6-sulfonate (36; Method F). A solution of nicotinamide (122 mg, 1 mmol) and **30** (335 mg, 1 mmol) in DMF (4 mL) was stirred for 3 days. The solvent was evaporated under reduced pressure, and the residue was purified by gel filtration leaving 153 mg (43%) of a white powder.

(*3S,4S*)-[1-(3,4-Dimethoxybenzyl)-2-oxo-4-(3-oxopropenyl)azetidin-3-yl]carbamic Acid Benzyl Ester (40). A solution of the aldehyde **39**[22] (18.6 g, 46.6 mmol) in THF (0.2 L) was reacted with $P(Ph)_3CHCHO$ (15.6 g, 51.2 mmol) for 20 h. The solvent was evaporated, and the residue was taken up in AcOEt, worked up, chromatographed (AcOEt/hexane, 1:1), and crystallized (AcOEt/hexane/ether) leaving 13.8 g (70%) of **40**. IR: 1771, 1685 cm^{-1}.

(*3S,4S*)-[1-(3,4-Dimethoxybenzyl)-2-(3-hydroxypropyl)-4-oxoazetidin-3-yl]carbamic Acid *tert*-Butyl Ester (41). A solution of **40** (4.24 g, 10 mmol) in EtOH (0.12 L) was reduced with $NaBH_4$ (135 mg, 3.6 mmol) for 1 h and then hydrogenated over 10% Pd/C. At the end of the reaction the catalyst was filtered off and washed with EtOH. The filtrate was reacted with $(BOC)_2O$ (2.18 g, 10 mmol) in the presence of DMAP. The solvent was evaporated, and the residue was taken up in AcOEt, worked up, and chromatographed (AcOEt) giving 1.1 g (28%) of **41**. MS (EI): 395.1 $(M + H)^+$. NMR ($CDCl_3$): 1.43 (s, 9H); 1.46−1.80 (m, 4H); 3.57 (t br, 2H); 3.65 (m, 1H); 3.87 (s, 6H); 4.16 and 4.55 (2d, J = 16, 2H); 5.0 (m, 1H); 5.25 (d, J = 8, 1H); 6.75−6.80 (m, 3H).

***tert*-Butyl (*1S,6R*)-8-Oxo-7-sulfo-2,7-diazabicyclo[4.2.0]-octane-2-carboxylate Sodium Salt (42). (a) (*3S,4S*)-[1-(3,4-Dimethoxybenzyl)-2-[3-(methylsulfonyloxy)propyl]-4-oxoazetidin-3-yl]carbamic Acid *tert*-Butyl Ester.** A solution of **41** (4.24 g, 10 mmol) in CH_2Cl_2 (50 mL) was reacted at room temperature with $MeSO_2Cl$ (0.26 mL, 3.3 mmol) and TEA (0.5 mL, 3.6 mmol) for 2 h. The reaction mixture was worked up and chromatographed (AcOEt/hexane, 1:1 to 8:2) leaving 1.0 g (76%) of intermediate mesylate. MS (EI): 473 $(M + H)^+$. NMR ($CDCl_3$): 1.43 (s, 9H); 1.66 (m, 4H); 2.97 (s, 3H); 3.61 (m, 1H); 3.88 (s, 6H); 4.13 (m, 2H); 4.16 and 4.55 (2d, J = 16, 2H); 5.0 (m, 1H); 5.24 (d, J = 8, 1H); 6.75−6.80 (m, 3H).

(b) (*1S,6R*)-7-(3,4-Dimethoxybenzyl)-8-oxo-2,7-diazabicyclo[4.2.0]octane-2-carboxylic Acid *tert*-Butyl Ester. A solution of the mesylate **42a** (1.0 g, 2.1 mmol) in THF (20 mL) was reacted with NaH (0.1 g, 2.1 mmol; 50% dispersed in oil) for 1 h. The reaction mixture was quenched with saturated NH_4Cl solution and extracted with AcOEt. The organic solution was worked up and chromatographed (AcOEt/hexane, 1:1) leaving 0.7 g (87%) of material. MS (EI): 321 $(M - (CH{=}CMe_2))$. NMR ($CDCl_3$): 1.46 and 1.47 (2s, 9H); 1.59−1.80 (m, 4H); 3.3−3.55 (m, 2H); 3.88 (s, 6H); 3.95 (m, 1H); 4.12 and 4.45 (2d, J = 16, 2H); 5.12 and 5.38 (d, J = 8, 1H); 6.80 (s, 3H).

(c) *tert*-Butyl (*1S,6R*)-8-oxo-2,7-diazabicyclo[4.2.0]-octane-2-carboxylate was prepared in a similar way to **7c** in 55% yield from **42b**, mp 146−48 °C (ether). IR: 1755, 1663 cm^{-1}. MS (EI): 183 (M − HNCO). Anal. ($C_{11}H_{18}N_2O_3$) C, H, N.

(d) *tert*-Butyl (*1S,6R*)-8-oxo-7-sulfo-2,7-diazabicyclo-[4.2.0]octane-2-carboxylate sodium salt (42) was prepared in a similar way to **13** in 85% yield after gel filtration. IR: 1757, 1688, 1408 cm^{-1}. MS (FAB): 304.9 $(M - Na)^-$. NMR (DMSO-d_6): 1.40 (s, 9H); 1.40−1.80 (m, 4H); 2.23 (db, J = 12, 1H); 3.10−3.25 (m, 1H); 4.17 (b, 1H); 4.91 and 5.02 (2d, J = 6, 1H). Anal. ($C_{11}H_{17}N_2O_6SNa$) C, H, N.

In Vitro Antibacterial Activity. The minimal inhibitory concentrations (MICs) of the test compound were determined according to a standard method[37] by a serial 2-fold dilution method using Mueller-Hilton broth (Difco Laboratories, Detroit, MI). The inocculum size was approximately 10^5 cfu/mL. The MIC of a compound or a combination was defined as the lowest concentration that prevented visible growth of bacteria after incubation at 37 °C for 18 h.

Structure Determination. The inhibitor complex was obtained by soaking crystals of the *C. freundii* enzyme[15] for 1.5 h in 3 mM inhibitor solution in 50% saturated potassium phosphate buffer, pH 8.0. Data were collected at room temperature with a Nicolet/Siemens area detector using Cu Kα radiation from an Elliott GX21 rotating anode X-ray generator operated at 40 kV, 80 mA with a 0.3-mm focal spot and a graphite monochromator. Rotation frames of 0.15° were for two different crystal orientations. The frames were processed and scaled using the XDS software;[33] 100 963 observations in the resolution range 20−2.1 Å yielded 39 500 unique reflections (89% completeness) with a scaling *R*-factor of 3.8% on intensities. The refined cell constants (space group $P2_12_12_1$) are 97.96, 84.63, and 89.92 Å for *a*, *b*, and *c*. Analysis and refinement of the structure were performed with X-Plor version 3.1[34] and Moloc[35] on Indigo 2 workstations. Refinement started from the refined model determined for the crystal structure of the uncomplexed enzyme[15] with water molecules omitted and all *B*-values set to 25 $Å^2$. Rigid body refinement of the two molecules of the noncrystallographic dimer using data from 10−2.5 Å resolution reduced the *R*-factor from an initial 32.2% to 30.6% (free *R* from 33.0% to 31.7%). In the first round of positional refinement using data from 20.0−2.2 Å (36 289 reflections, 94% complete) the *R*-factor was decreased from an initial 31.4% to 25.5% (free *R* from 32.0% to 29.4%). A subsequent electron density difference map showed well-defined density for the inhibitor moieties in both molecules, and they were built and added to the model. Several rounds of positional and thermal parameter (restrained, isotropic) refinement and manual model rebuilding reduced the *R*-factor to 16.4% (free *R* 21.2%). A bulk solvent correction as available in X-Plor was applied throughout the refinement. During manual model building, the conformations of some side chains were changed and 252 well-defined solvent sites were gradually added to the model. The final model contained 2807 non-hydrogen atoms for each of the two complete protein chains (residues 1−361, average *B*-factors of 26.6 and 23.7 $Å^2$ for chains A and B, respectively), 22 atoms for each inhibitor moiety (average *B*-factor of 25.3 and 30.5 $Å^2$ for molecules A

and B, respectively), and 252 solvent sites (average *B*-factor 31.0 Å^2). Deviations from stereochemical ideality are 0.007 Å for bond lengths and 1.70° for bond angles using reference values.[36]

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