Synthesis and Structure−Activity Relationship of (Lactamylvinyl)cephalosporins Exhibiting Activity against Staphylococci, Pneumococci, and Enterococci

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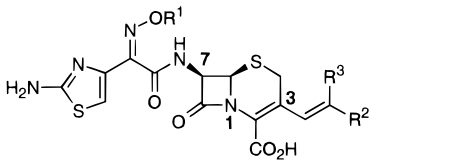
The synthesis and structure−activity relationships of a new class of vinylcephalosporins substituted with a lactamyl residue (**1**) are described. These compounds show excellent activity against enterococci and retain the broad spectrum activity of third-generation cephalosporins such as ceftriaxone.

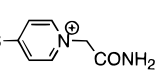
Introduction

During the last decades, the antibacterial potency of cephalosporins has been steadily increased through side-chain modifications at C-3 and C-7 (Figure 1). Most synthetic work aimed at the enhancement of activity on the Gram-negative side of the bacterial spectrum which occurred, to some extent, at the expense of the antibacterial properties on the Gram-positive side.[1] More recently, one has also attempted to improve the spectrum against Gram-positive bacteria. In this respect, the structural class of vinylcephalosporins (Figure 1) has been intensively investigated.[2−6] The representative of this group that is most advanced in clinical development is cefdinir.[7] Interest has focused in more recent investigations on vinylcephalosporins having a second substituent (R^2), *e.g.*, the development candidates TOC-39[8] and E1077[9] (Figure 1). In contrast, cephalosporins tri-substituted ($R^3 \neq H$) at the vinyl moiety, although known,[10−12] have so far received less attention.

In this paper we report the synthesis and structure−activity relationships of the new trisubstituted vinylcephalosporins **1** (Figure 2) bearing a lactamyl residue.[13] The antibacterial activity of this class has been optimized to cover methicillin-sensitive staphylococci (MSSA), enterococci, and pneumococci while maintaining good activity against Gram-negative pathogens. In addition, we attempted to obtain favorable pharmacokinetic properties.



Cefdinir: $R^1 = H$; $R^2 = H$; $R^3 = H$

TOC-39: $R^1 = H$; R^2 =  ; $R^3 = H$

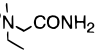
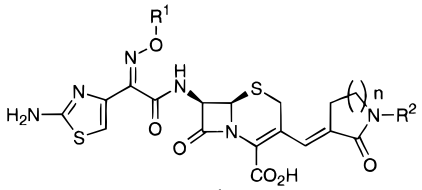
E1077: $R^1 = CH_2F$; R^2 =  ; $R^3 = H$

Figure 1.



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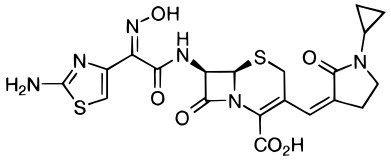
$n = 0, 1, 2$

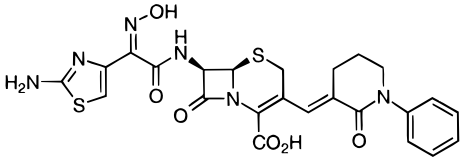
Figure 2.

Chemistry

The route used for the synthesis of γ- and δ-lactamyl derivatives ($n = 1, 2$) of **1** is described in Scheme 1. The phosphonium salts **5** were prepared from α,ω-dibromo acid chlorides[14] **2** by reaction with the appropriate amines. The best yields of **6,** by Wittig reaction of **5** with aldehyde **16**,[15] were obtained by refluxing the two components with 1,2-epoxybutane. Only minor amounts (<5%) of the *Z*-derivatives were formed under these conditions, and they were usually removed during the chromatographic purification of the reaction mixtures. However, in one case, we deliberately isolated the *Z*-isomer from a larger scale synthesis and proceeded to **19** as described below. The Δ^3 double bond in **6** was isomerized into the Δ^2 position by an oxidation−reduction sequence to yield **8**. After simultaneous removal of the BOC and benzhydryl protective groups, **9** was acylated with the activated esters of the selected aminothiazolyl oxyimino acetic acids **17**[16] and **18a**−**e**,[17] affording the vinylcephalosporins **10**−**15**, **19**, and **20**.



19



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[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.

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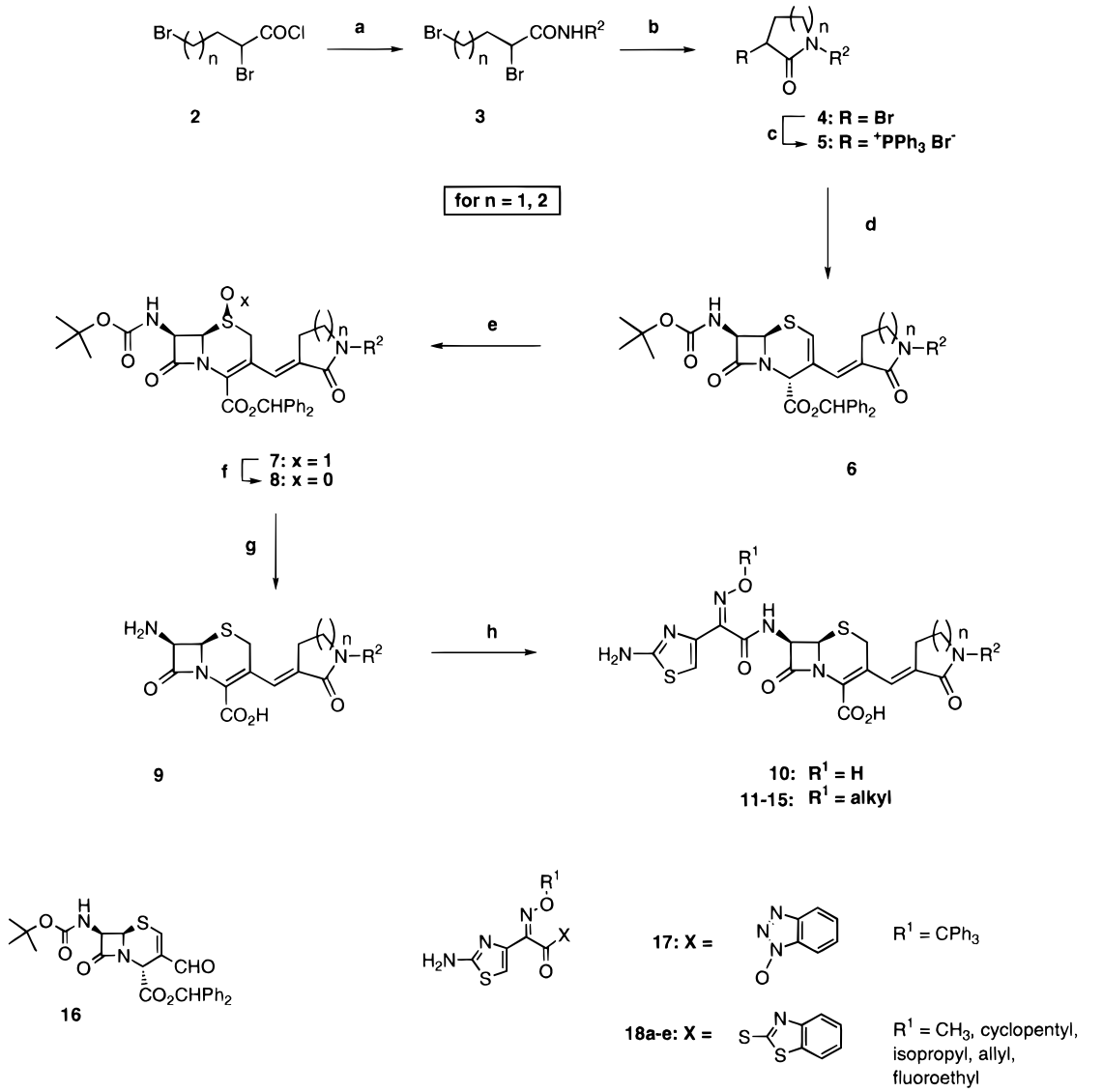
Scheme 1[a]



[a] (a) R^2-NH_2, TEA, CH_2Cl_2; (b) CH_2Cl_2, H_2O, NaOH, Dowex 2 × 10; (c) PPh_3, C_6H_6; (d) **16**, 1,2-epoxybutane, 1,2-dichloroethane; (e) mCPBA, CH_2Cl_2; (f) PBr_3, CH_2Cl_2, DMF, *N*-methylacetamide; (g) anisole, trifluoroacetic acid, CH_2Cl_2; (h) for **10**, (1) **17**, DMF, (2) trifluoroacetic acid, Et_3SiH; for **11**−**15**, **18a**−**e**, DMF.

The β-lactamyl derivatives ($n = 0$) of **1** were synthesized using an "inverse" Wittig reaction depicted in Scheme 2, since the β-lactamyl phosphonium salts **5** ($n = 0$) were not accessible by the route described above.[18] Thus, the cephalosporin phosphoranylidene **29**[19] was reacted with α-oxo-β-lactam **22**. The latter was obtained by ozonolysis of **21**.[20] The "inverse" Wittig reaction yielded a 1:1 mixture of the *Z* (**23**) and *E*- (**24**) isomers, which was separated by silica gel column chromatography. The deprotected aminocephalosporins **25** and **26** were acylated using conditions similar to those employed for the synthesis of **10** resulting in the four-membered ring analogues **27** and **28**.

Structure−Activity Relationship

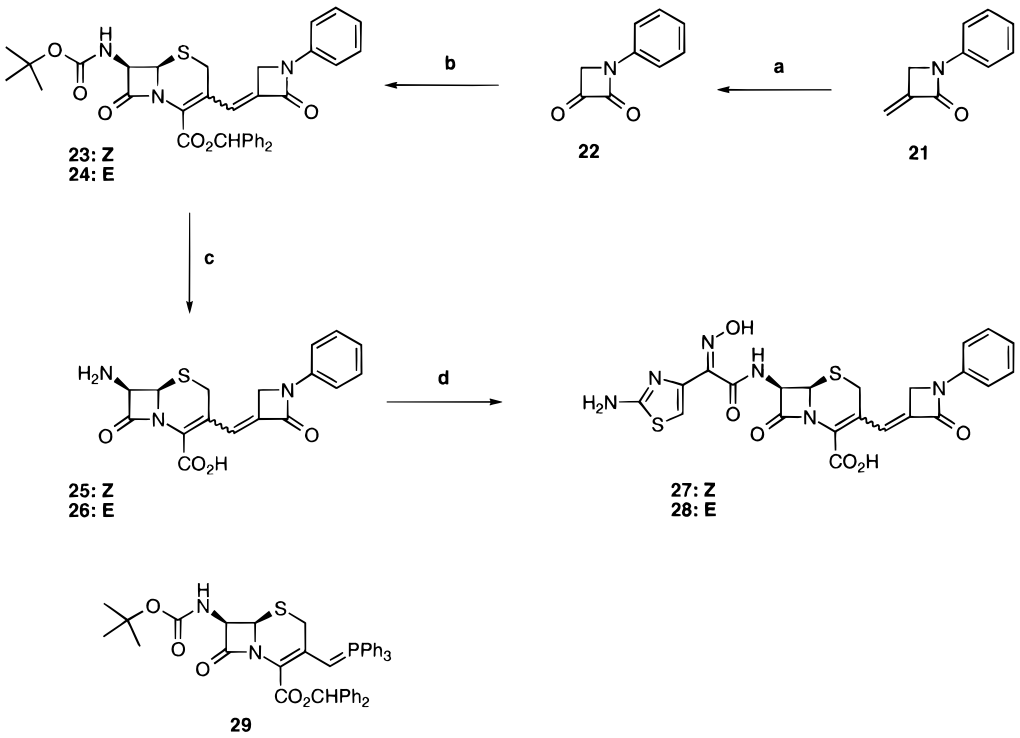
Antibacterial Activity. We investigated the influence of the substituents R^1 and R^2, the configuration at the vinyl bond, and the ring size of the C-3 lactamylvinyl substituent on the activity against various Gram-positive and Gram-negative organisms.

Thus, in a first series, different substituents R^1, hydrogen and alkyl groups, known to confer good Gram-positive activity on the oxyimino group were tested, keeping the ring size ($n = 1$) and the substituent (R^2 = trifluoroethyl) of the lactamyl residue constant (Table 1). The hydroxyimino group (R^1 = H, **10a**) provided the most balanced Gram-positive and Gram-negative spectrum, and it was also the only group to confer useful activity against *Enterococcus faecalis*.

Comparing *E*/*Z*-geometry at the C-3 vinyl bond (Table 2) in the series of γ-lactamyl derivatives revealed that the *E*-isomer **10b** was distinctly superior to the corresponding *Z*-isomer **19**, especially against *E. faecalis*.

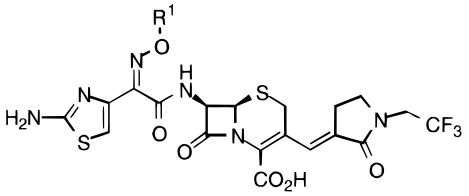
The influence of ring size of the lactamyl residue on the antibacterial spectrum was investigated with a series of derivatives with R^1 = H and R^2 = Ph (Table 3). The γ-lactamyl derivative **10c** proved to be optimal. Enlarging the ring (**20**) was detrimental to the whole spectrum, and decreasing the ring (**28**) impaired the activity against Gram-negative organisms. In contrast

Scheme 2[a]



[a] (a) O_3, EtOAc, dimethyl sulfide; (b) **29**, 1,2-epoxybutane; (c) anisole, trifluoroacetic acid; (d) (1) **17**, DMSO, (2) 90% HCOOH.

Table 1. Influence of Different Oxyimino Substituents on the *in Vitro* Antibacterial Activity[a]



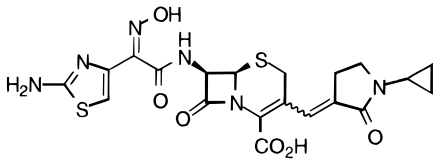
entry	R^1	*S. aureus* 887 MSSA	*S. aureus* 743 MRSA	*E. faecalis* ATCC 29212	*S. pneumoniae* Q19 pen s	*S. pneumoniae* 1/37 pen r	*E. coli* 25922	*E. cloacae* 908SSI	*C. freundii* 902	*P. aeruginosa* ATCC 27853
10a	H	1	8	1	0.06	1	≤0.06	0.5	0.25	16
11	CH_3	8	8	>32	≤0.03	0.5	0.25	1	0.25	8
12		4	8	8	≤0.06	1	2	4	4	8
13		8	4	16	≤0.06	1	2	4	4	16
14		4	4	32	≤0.06	0.5	1	4	2	8
15		4	16	32	≤0.06	1	0.5	2	1	8

[a] MICs (μg/mL).

to the five-membered ring compound **19** (Table 2), the four-membered ring *Z*-derivative **27** exhibited enhanced activity against *Staphylococcus aureus*, including a methicillin-resistant strain.

With the optimal structural attributes (R^1 = H, *E*-configuration, ring size n = 1), the influence of substituent R^2 on the antibacterial spectrum was investigated (Table 4). In general, derivatives with aryl and heteroaromatic substituents (**10j**−**p**) gave better MICs for staphylococci, pneumococci, and enterococci compared to derivatives bearing aliphatic residues (**10a,b,f**−**h**) or hydrogen (**10d**). Electron-withdrawing groups at the aromatic ring are more beneficial for the activity against Gram-positive organisms than electron-donating groups (entries **10m**,**l**). Benzylic substituents R^2 were generally less favorable to the activity than their aryl analogues (entries **10m**,**n**). Remarkably, the phenolic substituent (**10j**) also conferred good activity against *Pseudomonas aeruginosa* while keeping the good Gram-positive spectrum. Introduction of a catechol residue (**10k**) was surprisingly detrimental to anti-pseudomonas activity. The most drastic effect on the antibacterial spectrum was observed with derivatives having acidic residues R^2 (Table 4, entries **10e**,**i**,**q**),

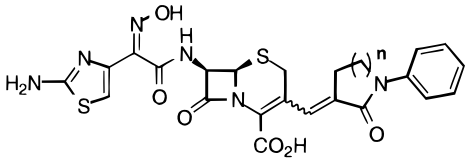
Table 2. Influence of the Double-Bond Configuration on the *in Vitro* Antibacterial Activity[a]



entry	config	*S. aureus* 887 MSSA	*S. aureus* 743 MRSA	*E. faecalis* ATCC 29212	*S. pneumoniae* Q19 pen s	*S. pneumoniae* 1/37 pen r	*E. coli* 25922	*E. cloacae* 908SSI	*C. freundii* 902	*P. aeruginosa* ATCC 27853
10b	*E*	1	8	1	0.03	2	≤0.06	0.12	0.12	8
19	*Z*	1	16	16	0.25	2	0.5	1	0.5	>32

[a] MICs (μg/mL).

Table 3. Influence of the Lactamyl Ring Size on the *in Vitro* Antibacterial Activity[a]



entry	*n*	config	*S. aureus* 887 MSSA	*S. aureus* 743 MRSA	*E. faecalis* ATCC 29212	*S. pneumoniae* Q19 pen s	*S. pneumoniae* 1/37 pen r	*E. coli* 25922	*E. cloacae* 908SSI	*C. freundii* 902	*P. aeruginosa* ATCC 27853
27	0	*Z*	0.12	2	0.5	0.12	2	2	4	4	16
28	0	*E*	0.5	8	0.25	0.5	4	1	2	1	16
10c	1	*E*	0.5	8	0.25	0.06	2	0.25	0.5	0.25	16
20	2	*E*	2	32	8	0.12	4	1	4	1	>32

[a] MICs (μg/mL).

where activity against staphylococci and enterococci was severly impaired.

Affinity for Resistant PBPs. Several PBPs that have low susceptibility to β-lactams, *i.e.*, cephalosporin-resistant mutants of PBP2x of *Streptococcus pneumoniae*,[21] PBP2′ of *Staphylococcus epidermidis*,[22] and PBP5 of *Enterococcus durans*,[23] which all confer penicillin-resistance to the parent organism, have been purified to homogeneity.[24] The IC_{50}s values measured for **10a** against these PBPs were lower than those of other β-lactams, such as ceftriaxone (Table 5). This higher affinity for penicillin-resistant PBPs correlated with improved *in vitro* activity against penicillin-resistant pneumococci, MRSA, and MRSE.

***β*-Lactamase Stability.** The cephalosporin **10a** was characterized by low affinity for class C β-lactamases, which rendered it unsusceptible to hydrolysis by these enzymes (Table 6). Among class A enzymes, only the extended broad-spectrum β-lactamases (TEM and SHV derivatives) showed appreciable activity toward **10a**. The rate of hydrolysis was less than that observed with third-generation cephalosporins such as ceftriaxone.

Conclusion

Activity against *E. faecalis* in (lactamylvinyl)cephalosporins was very sensitive to structural parameters like the lactamyl ring size, the configuration at the double bond, and, as expected, the substituent R^1 at the oxyimino group. The γ-lactamyl-*E*-vinylcephalosporins having R^1 = H and nonacidic residues R^2 (Table 4) had the best antibacterial spectrum. These compounds exhibited excellent activity against Gram-positive and Gram-negative microbes. Compared to ceftriaxone, the spectrum was extended to *E. faecalis* with improved activity against MSSA and additionally improved activity against MRSA. On the basis of pharmacokinetic studies, **10a**,**b** were selected for further investigations, which will be reported elsewere.[25]

Experimental Section

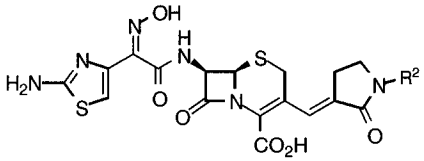
General Methods. Chromatography was carried out using Merck silica gel, 40−63 μm, 230−400 mesh. For reversed phase chromatography, Merck Lichroprep RP-18 silica gel, 25−40 μm, was used. All melting points were determined on a Buchi 510 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker FT AC-250 instrument (250 MHz) using tetramethylsilane as internal standard. IR spectra were measured on a Nicolet FT IR 170 SX spectrophotometer. Ion spray mass spectra were recorded on a Finnigan MAT SSQ 7000 instrument and EI mass spectra on a Perkin Elmer Siex API III instrument.

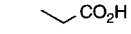
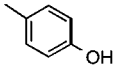
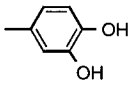
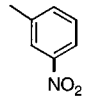
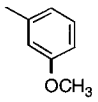
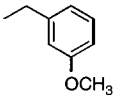
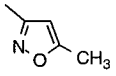
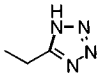
Carbonic Acid (*RS*)-4-[(2,4-Dibromobutyryl)amino]-phenyl Ester *tert*-Butyl Ester (3j). A solution of 21.15 g (80 mmol) of 2,4-dibromobutanoic acid chloride[14] in 100 mL of CH_2Cl_2 was added at −20 to −10 °C to a solution of 15.7 g (75 mmol) of carbonic acid 4-aminophenyl ester *tert*-butyl ester[26] in 200 mL of CH_2Cl_2 and 11.13 mL (80 mmol) of triethylamine. After 30 min the reaction mixture was extracted with water, and the organic phase was dried over $MgSO_4$. Evaporation of the solvent afforded a colorless oil which crystallized from Et_2O/hexane (21.24 g, 66.8%): colorless needles; mp 105−106 °C; ^{1}H-NMR ($CDCl_3$) δ 1.56 (s, 9 H), 2.50−2.85 (m, 2 H), 3.61 (m, 2 H), 4.68 (m, 1 H), 7.16 (d, 2 H, J = 8 Hz), 7.52 (d, 2 H, J = 8 Hz), 7.97 (s, 1 H); IR (KBr) 1758, 1682 cm^{-1}.

Carbonic Acid (*RS*)-4-(3-Bromo-2-oxopyrrolidin-1-yl)-phenyl Ester *tert*-Butyl Ester (4j). Dowex 2 × 10 (1.8 g) was added to a vigorously stirred mixture of 18.39 g (42 mmol) of **3j** in 200 mL of CH_2Cl_2 and 15 g of 50% NaOH solution. After 4 h at room temperature, the mixture was washed twice with water and the organic phase dried over $MgSO_4$. Evaporation of the solvent gave **4j** as colorless needles (14.6 g, 97%): mp 148−150 °C; ^{1}H-NMR ($CDCl_3$) δ 1.56 (s, 9 H), 2.46 (m, 1 H), 2.75 (m, 1 H), 3.81 (m, 1 H), 4.03 (m, 1 H), 4.57 (dd, 1 H), 7.20 (d, 2 H, J = 8 Hz), 7.66 (d, 2 H, J = 8 Hz); IR (KBr) 1751, 1697 cm^{-1}.

(*RS*)-[1-[4-[(*tert*-Butoxycarbonyl)oxy]phenyl]-2-oxopyrrolidin-3-yl]triphenylphosphonium Bromide (5j). A

Table 4. Influence of the Lactamyl Substituent on the *in Vitro* Antibacterial Activity[a]



entry	R^2	*S. aureus* 887 MSSA	*S. aureus* 743 MRSA	*E. faecalis* ATCC 29212	*S. pneumoniae* Q19 pen s	*S. pneumoniae* 1/37 pen r	*E. coli* ATCC 25922	*E. cloacae* 908SSI	*C. freundii* 902	*P. aeruginosa* ATCC 27853
10d	H	1	8	2	0.06	2	≤0.06	0.5	0.5	>16
10e	OH	1	16	4	0.06	4	≤0.06	16	0.5	8
10f		1	16	1	0.03	1	0.12	0.25	0.12	16
10g		1	16	1	≤0.06	1	≤0.06	0.25	≤0.06	16
10h		1	4	1	≤0.06	2	≤0.06	1	0.12	8
10i		16	>32	8	0.12	2	0.12	8	2	>32
10j		0.5	4	0.5	0.12	1	≤0.06	0.25	≤0.06	4
10k		2	16	0.5	≤0.06	2	0.25	0.25	0.12	32
10l		0.5	4	0.12	≤0.06	2	0.12	0.25	0.12	16
10m		1	8	0.5	≤0.06	0.5	0.5	1	0.5	>32
10n		1	8	1	≤0.06	1	2	4	2	>32
10o		1	8	0.5	0.12	2	≤0.12	≤0.12	≤0.12	16
10p		1	8	0.25	0.12	2	0.12	0.5	0.12	>32
10q		8	32	16	0.12	2	0.25	16	2	>32
Cefdinir		0.5	16	16	0.25	4	0.25	16	8	>32
Cetriaxone		4	>32	>32	≤0.06	2	≤0.06	0.5	0.25	16
Ceftazidime		8	>32	>32	0.25	16	0.25	1	0.5	1

[a] MICs (μg/mL).

Table 5. Inhibition of Purified Penicillin-Binding Proteins

	IC_{50} (μM)		
	10a	ceftriaxone	imipenem
E. coli PBP1bγ	30	0.29	0.01
S. pneumoniae PBP2x	0.3	0.5	0.5
PBP2x.505[a]	0.36	2.5	0.5
PBP2x.604[a]	0.87	5.5	5.3
S. epidermidis PBP2′ [b]	25	960	>1000
E. durans PBP5[c]	8	>500	>1000

[a] Cephalosporin-resistant mutants isolated by Laible and Hakenbeck.[21] [b] β-Lactam-resistant PBP conferring methicillin resistance. [c] β-Lactam-resistant PBP conferring broad-spectrum antibiotic resistance.

Table 6. Rates of Hydrolysis by Various β-Lactamases[a]

	hydrolysis (mol/min/mol of enzyme)	
β-lactamase	**10a**	ceftriaxone
B. licheniformis 749/C	906	1894
P. vulgaris 1028	461	897
TEM-3	14	79
TEM-4	305	643
SHV-2	1578	2314
SHV-4	1388	3339

[a] Conditions: 0.1 M sodium phosphate buffer, pH 7.0.

solution of 14.6 g (41 mmol) of **4j** and 14 g (53.4 mmol) of triphenylphosphine in 250 mL of benzene was refluxed for 48 h. The solid material was collected by filtration and washed with benzene and hexane (14.72 g of **5j**). The mother liquor was refluxed for another 72 h, yielding an additional 4.4 g of **5j**: total yield, 19.12 g (75.5%) of colorless crystals: mp 147−

150 °C; ^{1}H-NMR (DMSO-d_6) δ 1.48 (s, 9 H), 2.50 (m, 1 H), 2.76 (m, 1 H), 3.64 (m, 1 H), 4.04 (m, 1 H), 5.90 (m, 1 H), 7.18 (d, 2 H, J = 8 Hz), 7.40 (d, 2 H, J = 8 Hz), 7.7−8.0 (m, 15 H); IR (KBr) 1756, 1690 cm^{-1}. Anal. ($C_{33}H_{33}BrNO_4P$) C, H, N.

(*E*)-(2*R*,6*R*,7*R*)-7-[(*tert*-Butoxycarbonyl)amino]-3-[[1-[4-[(*tert*-butoxycarbonyl)oxy]phenyl]-2-oxopyrrolidin-3-ylidene]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic Acid Benzhydryl Ester (6j). A mixture of 12.11 g (24.49 mmol) of **16**[15] and 17.17 g (27.77 mmol) of **5j** in 250 mL of 1,2-dichloroethane:1,2-epoxybutane (1:1) was refluxed for 3.5 h. The solution was evaporated and the residue purified by silica gel column chromatography (CH_2Cl_2:EtOAc = 9:1) affording 9.81 g (53%) of **6j**: ^{1}H-NMR ($CDCl_3$) δ 1.46 (s, 9 H), 1.57 (s, 9 H), 2.83 (m, 2 H), 3.61 (t, 2 H), 5.26 (d, 1 H, J = 5 Hz), 5.28 (br s, 2 H), 5.33 (br m, 1 H), 6.61 (s, 1 H), 6.87 (s, 1 H), 6.99 (s, 1 H), 7.20 (m, 13 H), 7.73 (d, 2 H, J = 8 Hz); IR (KBr) 1781, 1750, 1691 cm^{-1}; MS (ISP) 754.5 (M + H)$^+$.

(*E*)-(5*R*,6*R*,7*R*)- and -(5*S*,6*R*,7*R*)-7-[(*tert*-Butoxycarbonyl)amino]-3-[[1-[4-[(*tert*-butoxycarbonyl)oxy]phenyl]-2-oxopyrrolidin-3-ylidene]methyl]-5,8-dioxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Benzhydryl Ester (7j). A solution of 3.27 g (15 mmol) of 80−90% *m*-chloroperbenzoic acid in 60 mL of CH_2Cl_2 was added at 4 °C to a solution of 11.3 g (15 mmol) of **6j** in 120 mL of CH_2Cl_2. After 1 h, the reaction mixture was washed successively with cold 10% aqueous $Na_2S_2O_3$, 5% aqueous $NaHCO_3$, and water. After drying the organic layer over $MgSO_4$, the solvent was removed and the residue was purified by flash silica gel column chromatography (EtOAc:hexane = 3:2) affording 10.59 g (91.7%) of **7j** as a yellow foam: ^{1}H-NMR ($CDCl_3$, 2:1 mixture of diastereomers) δ 1.47 (s, 9 H), 1.57 (s, 9 H), 2.30−3.0 (m, 2 H), 3.16 (d, major isomer, 1 H, J = 18 Hz), 3.4−3.8 (m, 3 H), 3.94 (d, major isomer, 1 H, J = 18 Hz), 4.50 (d, minor isomer, 1 H, J = 18 Hz), 4.47 (br d, major isomer, 1 H, J = 5 Hz), 4.58 (d, minor isomer, 1 H, J = 5 Hz), 5.45 (m, minor isomer, 2 H), 5.81 (m, major isomer, 2 H), 7.03 (s, minor isomer, 1 H), 7.05 (s, major isomer, 1 H), 7.1−7.5 (m, 12 H), 7.71 (m, 3 H); IR (KBr) 1799, 1757, 1723 cm^{-1}; MS (ISP) 770.5 (M + H)$^+$.

(*E*)-(6*R*,7*R*)-7-[(*tert*-Butoxycarbonyl)amino]-3-[[1-[4-[(*tert*-butoxycarbonyl)oxy]phenyl]-2-oxopyrrolidin-3-ylidene]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Benzhydryl Ester (8j). A solution of 5.1 mL of phosphorus tribromide in 15 mL of CH_2Cl_2 was added at −30 °C to a solution of 10.45 g (13.57 mmol) of **7j** in 120 mL of CH_2Cl_2, 12.1 mL of *N*-methylacetamide, and 12.8 mL of DMF. The mixture was stirred for 1 h at −30 °C and then poured into a stirred solution of 20 g of $NaHCO_3$ in 250 mL of ice−water. The organic phase was separated, washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was stirred with hexane and the solid material collected by filtration to give **8j** (9.85 g, 96.3%): ^{1}H-NMR ($CDCl_3$) δ 1.48 (s, 9 H), 1.57 (s, 9 H), 2.36 (m, 1 H), 2.69 (m, 1 H), 3.44 (m, 1 H), 3.56 (s, 2 H), 3.66 (m, 1 H), 5.02 (d, 1 H, J = 5 Hz), 5.35 (br d, 1 H), 5.69 (m, 1 H), 7.03 (s, 1 H), 7.1−7.4 (m, 12 H), 7.71 (m, 2 H); IR (KBr) 1787, 1758, 1722 cm^{-1}; MS (ISP) 754.5 (M + H$^+$).

(*E*)-(6*R*,7*R*)-7-Amino-3-[[1-(4-hydroxyphenyl)-2-oxopyrrolidin-3-ylidene]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Trifluoroacetate (9j). Trifluoroacetic acid (50 mL) was added at 0 °C to a solution of 9.78 g (12.97 mmol) of **8j** in 100 mL of CH_2Cl_2 and 10 mL of anisole. After 2 h at room temperature, the mixture was concentrated and poured into Et_2O. The resulting solid was collected by filtration and washed with Et_2O and hexane affording **9j** (5.12 g, 96.2%): ^{1}H-NMR (DMSO-d_6) δ 3.13 (m, 2 H), 3.82 (t, 2 H), 3.95 (br s, 2 H), 4.98 (d, 1 H, J = 5 Hz), 5.11 (d, 2 H, J = 5 Hz), 6.77 (d, 2 H, J = 8 Hz), 7.35 (s, 1 H), 7.55 (d, 2 H, J = 8 Hz), 9.44 (s, 1 H); IR (KBr) 1778, 1676 cm^{-1}; MS (ISP) 388.4 (M + H)$^+$.

(6*R*,7*R*)-7-[(*Z*)-[2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]amino]-3-[(*E*)-[1-(4-hydroxyphenyl)-2-oxopyrrolidin-3-ylidine]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic Acid (10j). The activated ester **17**[16] (1.4 g, 2.56 mmol) was added to a stirred suspension of 820 mg (2 mmol) of **9j** in 30 mL of DMF. After 20 h at room temperature, the mixture was concentrated and the residue dissolved in 150 mL of EtOAc and washed twice with 25 mL of water. The organic phase was concentrated to about one-third of its volume, upon which the product started to crystallize. It was collected by filtration, washed with EtOAc and Et_2O, and dried to yield 1.12 g of the trityl-protected product as yellow crystals: ^{1}H-NMR (DMSO-d_6) δ 3.16 (m, 2 H), 3.82 (t, 2 H), 3.98 (br s, 2 H), 5.30 (d, 1 H, J = 5 Hz), 6.03 (dd, 2 H, J = 5, 7 Hz), 6.62 (s, 1 H), 6.78 (d, 2 H, J = 8 Hz), 7.32 (m, 18 H), 7.56 (d, 2 H), 9.43 (s, 1 H), 9.96 (d, 1 H, J = 7 Hz), 13.88 (br s, 1 H); IR (KBr) 1784, 1679 cm^{-1}; MS (ISP) 799.4 (M + H)$^+$.

This material (1.1 g, 1.38 mmol) was added portionwise to 10 mL of ice-cold trifluoroacetic acid, the temperature being kept below 5 °C. Triethylsilane (0.4 mL) was added dropwise during 20 min resulting in a beige suspension which was poured into 100 mL of Et_2O. This mixture was stirred for 30 min, and the solid was collected by filtration and crystallized from 15 mL of 90% aqueous acetone (552.5 mg of **10j**, 72%, yellow crystals): ^{1}H-NMR (DMSO-d_6) δ 3.12 (m, 2 H), 3.82 (t, 2 H), 3.92 (br s, 2 H), 5.20 (d, 1 H, J = 5 Hz), 5.83 (dd, 2 H, J = 5, 7 Hz), 6.67 (s, 1 H), 6.78 (d, 2 H, J = 8 Hz), 7.14 (s, 2 H), 7.35 (s, 1 H, d, 2 H), 7.55 (d, 2 H, J = 8 Hz), 9.42 (s, 1 H), 9.53 (d, 1 H, J = 7 Hz), 11.50 (s, 1 H), 14.84 (br s, 1 H); IR (KBr) 1774, 1667 cm^{-1}; MS (ISP) 557.4 (M + H)$^+$. Anal. ($C_{23}H_{20}N_6O_7S_2$) C, H, N, S.

Compounds **10a**−**i**,**k**−**q** and **19** were obtained using methods similar to those used for **10j**. Compounds **11**−**15** were synthesized by reacting **9a** with the appropriate (aminothiazolyl)(alkoxyimino)acetic acid benzothiazole thioesters **18a**−**e**[17] in DMF at room temperature. The mixtures obtained were concentrated *in vacuo* and triturated with 50 mL of EtOAc:water (1:1). The solid materials were collected by filtration to yield **11**−**15**. The sodium salts **11**, **12**, and **19** were obtained by suspending the corresponding acids in water/acetone and adjusting the pH to 6.5−7 with 1 N NaOH. Purification was performed by reversed phase chromatography, using water with a gradient of acetonitrile as eluent.

1-Phenylazetidine-2,3-dione (22). Through a solution of 800 mg (5 mmol) of **21**[20] in 50 mL of ethyl acetate, cooled to −70 °C, was passed ozone for 15 min. Then 0.5 mL of dimethyl sulfide was added, and the solution was stirred for 1.5 h at −70 °C. The temperature was raised to 0 °C, and 25 mL of water was added. After 5 min the organic phase was separated, extracted with 50 mL $Na_2S_2O_3$ and $FeSO_4$ solutions, and then dried over $MgSO_4$. The solvent was evaporated and the residue purified by silica gel column chromatography (benzene) affording 114 mg (14.5%) of **22** as colorless crystals: mp 115−117°C; ^{1}H-NMR ($CDCl_3$) δ 4.34 (s, NCH_2, 2 H), 7.27 (m, 1 H), 7.50 (m, 4 H); IR (KBr) 1822, 1757 cm^{-1}; MS (EI) 161 (M). Anal. ($C_9H_7NO_2$) C, H, N.

Wittig Reaction Leading to 23 and 24. A suspension of 114 mg (0.708 mmol) of **22** in 15 mL of 1,2-epoxybutane and 695 mg (0.80 mmol) of **29**[19] was stirred at room temperature for 1.5 h. The dark-brown solution was then evaporated and the product mixture separated by silica gel column chromatography (hexane:EtOAc = 4:1, 3:1, 2:1).

(*Z*)-(6*R*,7*R*)-7-[(*tert*-Butoxycarbonyl)amino]-8-oxo-3-[(2-oxo-1-phenylazetidin-3-ylidene)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Benzhydryl Ester (23). 23 eluted first: yield, 140 mg (32%) of yellow crystals; ^{1}H-NMR ($CDCl_3$) δ 1.47 (s, 9 H), 3.78 (d, 1 H, J = 17.5 Hz), 3.99 (dd, 2 H), 4.44 (d, 1 H, J = 17.5 Hz), 5.03 (d, 1 H, J = 5 Hz), 5.26 (br d, 1 H), 5.71 (br q, 1 H), 6.75 (s, 1 H), 6.99 (s, 1 H), 7.15 (m, 1 H), 7.37 (m, 14 H); IR (KBr) 1788, 1727 cm^{-1}; MS (ISP) 624.4 (M + H)$^+$.

The second eluate (163 mg) consisted of two products that were separated by another silica gel column chromatography (CH_2Cl_2:EtOAc = 96:4) affording 82 mg (18%) of (*E*)-(6*R*,7*R*)-7-[(*tert*-butoxycarbonyl)amino]-8-oxo-3-[(2-oxo-1-phenylazetidin-3-ylidene)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid benzhydryl ester (**24**) as a yellow foam: ^{1}H-NMR ($CDCl_3$) δ 1.47 (s, 9 H), 3.57 (dd, 2 H, J = 17.5 Hz), 4.26 (d, 1 H, J = 9 Hz), 4.37 (d, 1 H, J = 9 Hz), 5.01 (d, 1 H, J = 5 Hz), 5.34 (br d, 1 H), 5.73 (br q, 1 H), 7.01 (s, 1 H), 7.13 (m, 1 H), 7.34 (m, 13 H), 7.46 (m, 2 H); IR (KBr) 1790, 1727 cm^{-1}; MS (ISP) 624.5 (M + H)$^+$.

Also, 28 mg (6%) of its Δ^3 isomer eluted as byproduct, (*E*)-(2*R*,6*R*,7*R*)-7-[(*tert*-butoxycarbonyl)amino]-8-oxo-3-[(2-oxo-1-phenylazetidin-3-ylidene)methyl]-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid benzhydryl ester: colorless foam; ^{1}H-NMR ($CDCl_3$) δ 1.47 (s, 9 H), 4.03 and 4.15 (dd, 2 H), 5.13 (s, 1 H), 5.23 (d, 1 H, J = 5 Hz), 5.29 (br d, 1 H), 5.45 (br q, 1 H), 6.70 (s, 1 H), 6.70 (s, 1 H), 6.90 (s, 1 H), 7.10 (m, 1 H), 7.26 (m, 14 H); IR (KBr) 1782, 1740 cm^{-1}; MS (ISP) 624.5 (M + H)$^+$.

(*Z*)-(6*R*,7*R*)-7-Amino-8-oxo-3-[(2-oxo-2-phenylazetidin-3-ylidene)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (25): obtained from **23** by the same procedure as described for **9j**; yield, 93%; IR (KBr) 1783, 1716 cm^{-1}. Anal. ($C_{17}H_{15}N_3O_4S$·0.1TFA) C, H, N, S, F.

(*E*)-(6*R*,7*R*)-7-Amino-8-oxo-3-[(2-oxo-2-phenylazetidin-3-ylidene)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (26): obtained from **24** by the same procedure as described for **9j**; yield, 92%; IR (KBr) 1782, 1734 cm^{-1}; MS (ISP) 358.3 (M + H)$^+$. Anal. ($C_{17}H_{15}N_3O_4S$·0.14TFA) C, H, N, S.

(6*R*,7*R*)-7-[(*Z*)-[2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]amino]-8-oxo-3-[(*Z*)-(2-oxo-1-phenylazetidin-3-ylidene)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (27). A suspension of 310 mg (0.842 mmol) of **25** and 587 mg (1.075 mmol) of the activated ester **27** in 25 mL of DMSO was stirred for 23 h at room temperature. The solvent was evaporated at 30 °C and the residue dissolved in 200 mL of EtOAc, washed twice with 100 mL of water, dried over $MgSO_4$, and concentrated to a volume of 20 mL causing the product to precipitate. It was collected by filtration and washed with EtOAc affording 387.5 mg (60%) of the trityl-protected product: ^{1}H-NMR (DMSO-d_6) δ 3.88 (d, 1 H, J = 17.5 Hz), 4.25 (br dd, 2 H), 4.62 (d, 1 H, J = 17.5 Hz), 5.34 (d, 1 H, J = 5 Hz), 6.04 (dd, 1 H, J = 5, 7 Hz), 6.62 (s, 1 H), 6.82 (s, 1 H), 7.1−7.5 (m, 22 H), 9.97 (d, 1 H, J = 7 Hz), 14.0 (br s, 1 H); IR (KBr) 1766, 1707 cm^{-1}; MS (ISP) 769.5 (M + H)$^+$.

A solution of 384 mg (0.5 mmol) of this material in 4 mL of 90% aqueous formic acid was stirred for 75 min at room temperature. The suspension was concentrated *in vacuo* and the residue triturated in 50 mL of EtOAc. The solid was collected by filtration, dried, and stirred for 1 h in 20 mL of 90% aqueous ethanol. The product was isolated by filtration and washed with hexane affording 209 mg (80%) of **27**: ^{1}H-NMR (DMSO-d_6) δ 3.80 (d, 1 H, J = 17.5 Hz), 4.22 (br dd, 2 H), 4.59 (d, 1 H, J = 17.5 Hz), 5.26 (d, 1 H, J = 5 Hz), 5.88 (dd, 1 H, J = 5 Hz, 7 Hz), 6.67 (s, 1 H), 6.78 (s, 1 H), 7.14 (m, 3 H), 7.41 (d, 4 H), 9.53 (d, 1 H, J = 7 Hz), 11.33 (s, 1 H), 14.0 (br s, 1 H); IR (KBr) 1776, 1721, 1676 cm^{-1}; MS (ISP) 527.4 (M + H)$^+$. Anal. ($C_{22}H_{18}N_6O_6S_2$) C, H, N, S.

(6*R*,7*R*)-7-[(*Z*)-[2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]amino]-8-oxo-3-[(*E*)-(2-oxo-1-phenylazetidin-3-ylidene)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (28). 28 was obtained by the same procedure as described for **27**. Trityl-protected product: yield, 74%; ^{1}H-NMR (DMSO-d_6) δ 3.82 (dd, 1 H, J = 17.5, 25 Hz), 4.54 (d, 1 H, J = 9 Hz), 4.70 (d, 1 H, J = 9 Hz), 5.34 (d, 1 H, J = 5 Hz), 6.06 (dd, 1 H, J = 5, 7 Hz), 6.62 (s, 1 H), 7.1−7.5 (m, 23 H), 9.98 (d, 1 H, J = 7 Hz), 14.2 (br s, 1 H); IR (KBr) 1788, 1742 cm^{-1}; MS (ISP) 769.5 (M + H)$^+$.

28: yield, 88%; ^{1}H-NMR (DMSO-d_6) δ 3.80 (d, 1 H, J = 17.5 Hz), 4.22 (br dd, 2 H), 4.59 (d, 1 H, J = 17.5 Hz), 5.26 (d, 1 H, J = 5 Hz), 5.88 (dd, 1 H, J = 5, 7 Hz), 6.67 (s, 1 H), 6.78 (s, 1 H), 7.14 (m, 3 H), 7.41 (d, 4 H), 9.53 (d, 1 H, J = 7 Hz), 11.33 (s, 1 H), 14.0 (br s, 1 H); IR (KBr) 1778, 1738, 1676, 1528 cm^{-1}; MS (ISP) 527.4 (M + H)$^+$.

***In Vitro* Antibacterial Activity.** Minimum inhibitory concentrations (MICs) were determined by the 2-fold agar dilution method with Mueller-Hinton agar (Difco Laboratories, Detroit, MI). The overnight broth cultures were diluted to *ca.* 10^7 colony forming units/spot with fresh broth, and an inoculum of 10^4 colony forming units/spot was applied to agar plates containing graded concentrations of each compound with an inoculating apparatus (Mast Laboratories Ltd., Liverpool, U.K.). After incubation at 37 °C for 18 h, the MIC was defined as the minimum drug concentration which completely inhibited the growth of bacteria.

Affinity for Penicillin-Binding Proteins. Several PBPs were purified to homogeneity using modifications of published procedures.[27] IC_{50} values for purified PBPs were measured using dansyl-penicillin[28] or a coumarin analogue.[29] Fluorescence bound to the protein was determined by TCA precipitation, filtration using glass fiber filter paper, and, after drying, measuring the amount of fluorescence using a Cytofluor fluorimeter.

Sensitivity to β-Lactamases. β-Lactamases were purified to homogeneity, and their hydrolytic activity was measured using standard methods.[30]

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