Dual-Action Cephalosporins Incorporating a 3'-Tertiary-Amine-Linked Quinolone

Harry A. Albrecht,* George Beskid, James G. Christenson, Kenneth H. Deitcher, Nafsika H. Georgopapadakou, Dennis D. Keith, Frederick M. Konzelmann, David L. Pruess, and Chung Chen Wei

Roche Research Center, Hoffmann-La Roche Incorporated, Nutley, New Jersey 07110

Received August 2, 1993®

We have previously reported that linking quinolones to the cephalosporin 3'-position through an ester bond, a carbamate function, or a bond through a quaternary nitrogen produced cephalosporins with a dual mode of antibacterial action. We now describe a new class of dual-action cephalosporins, with greater chemical stability than those previously reported, in which the basic nitrogen of ciprofloxacin is bonded directly to the 3'-cephalosporin position, i.e., the two moieties are linked through a tertiary amine function. These compounds have demonstrated potent activity against a broad spectrum of Gram-positive and Gram-negative bacteria, including β-lactam-resistant strains.

Introduction

When cephalosporins exert their biological activity by reacting with bacterial enzymes, opening of the β-lactam ring is accompanied by liberation of the 3'-substituent, if that substituent can function as a leaving group.[1-8] When the eliminated substance possesses antibacterial activity of its own, the cephalosporin should exhibit a dual mode of action.[9-11] As a rationale for drug design, this mechanism has been discussed in some detail, and we have reported the synthesis and the biological activity of three classes of dual-action cephalosporins.[11-23] In these previously described compounds, antibacterial quinolones were linked to the 3'-position either by an ester bond,[11-20] by a bond through a quaternary nitrogen,[21] or by a carbamate function.[22,23] These compounds demonstrated a broad spectrum of antibacterial activity derived from both cephalosporin-like and quinolone-like components.

In our continuing effort to find cephalosporin–quinolone conjugates which combine the better features of two major classes of antibacterials, we have prepared a new class of cephalosporins in which the basic nitrogen of ciprofloxacin is bonded directly to the cephalosporin 3'-position to provide a tertiary amine linkage (Schemes 1, 2, and 3; compounds **1–6**).[24,25] While the structures of the previously prepared cephalosporin–quinolones were clearly compatible with the requirement that the 3'-substituent act as a leaving group, the carbon–nitrogen bonds of amines are relatively stable. It is not obvious that under physiological conditions the substituted amine functionality should be considered as a leaving group in the usual sense, or that bifunctional cephalosporins incorporating quinolones as tertiary amines should act by the proposed dual-action mechanism. Our investigations were designed to determine the chemical and biological properties of this new class of bifunctional cephalosporins.

Chemistry

Synthetic approaches to the tertiary-amine-linked cephalosporin–quinolones are outlined in the reaction Schemes 1, 2, and 3. In Schemes 1 and 2, the 7-amino function was acylated before the quinolone was introduced into the molecule. Compound **1** was initially prepared according to Scheme 1, utilizing *tert*-butyl esters as intermediates. In work reported earlier, *tert*-butyl esters had shown less tendency than diphenylmethyl esters to undergo double-bond migration in the cephalosporin nucleus during displacement reactions.[11] However, in the present work, in displacement reactions of either the *tert*-butyl ester in Scheme 1 or the diphenylmethyl esters in Schemes 2 and 3, double-bond migration proved not to be a problem. Formation of the 3'-tertiary amine linkage through a nucleophilic displacement with the secondary amine function of ciprofloxacin is the key step in all of the reaction schemes. In Scheme 1, the reaction was carried out by stirring **7** with ciprofloxacin and sodium bicarbonate in DMF for 3 h to provide **8** in good yield (86%). This intermediate was deprotected by treatment with formic acid to remove the trityl group followed by trifluoroacetic acid in nitromethane to remove the *tert*-butyl ester. Surprisingly, use of the usual trifluoroacetic acid procedures alone on **8** did not effect complete deprotection, failing to totally remove the trityl group. The general methodology shown in Scheme 1 for the synthesis of **1** is potentially quite useful, since many 3'-iodocephalosporin esters can be conveniently prepared from 7-aminocephalosporanic acid (7-ACA) by acylation and esterification followed by reaction with iodotrimethylsilane.[26]

In practical terms, Scheme 2 often provided a more convenient route to final products of high purity. In this sequence, **9** (which is now commercially available) was acylated and the resultant chloro intermediate converted to its 3'-iodo analogue by reaction with sodium iodide in methyl ethyl ketone. The displacement reaction to establish the tertiary amine function provided an intermediate which could be chromatographically purified, if necessary, before deprotection to the final product. Compounds **4**, **5**, and **6** were prepared by this method.

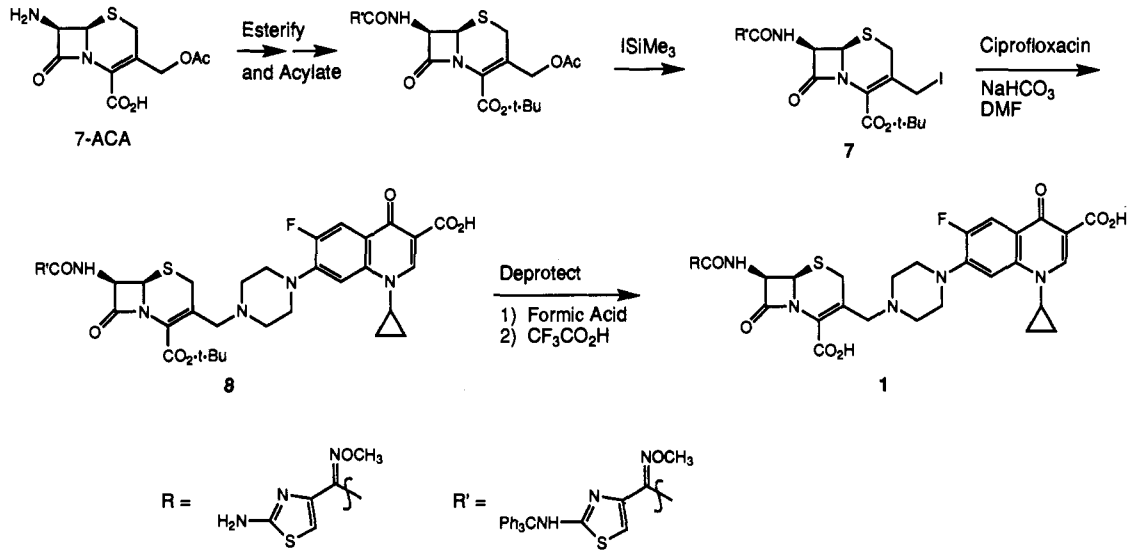
The option of introducing the quinolone component before the desired acyl group was in place was also explored (Scheme 3). Here, the 3'-alcohol **19** was converted quantitatively to the chloro derivative **20** by reaction with phosphorus pentachloride and pyridine followed by a hydrolytic work up. The displacement reaction with ciprofloxacin followed by deprotection gave **22**, a versatile common intermediate from which **2** and **3** were prepared.

The choice of which synthetic approach to use for each target compound was somewhat arbitrary and dictated largely by availability of starting materials (and a desire to experiment), rather than necessity. The decision of whether to convert 3'-chloro intermediates to the iodo analogues in a separate step, as in Scheme 2, or to use the chlorides directly for displacement reactions in the pres-

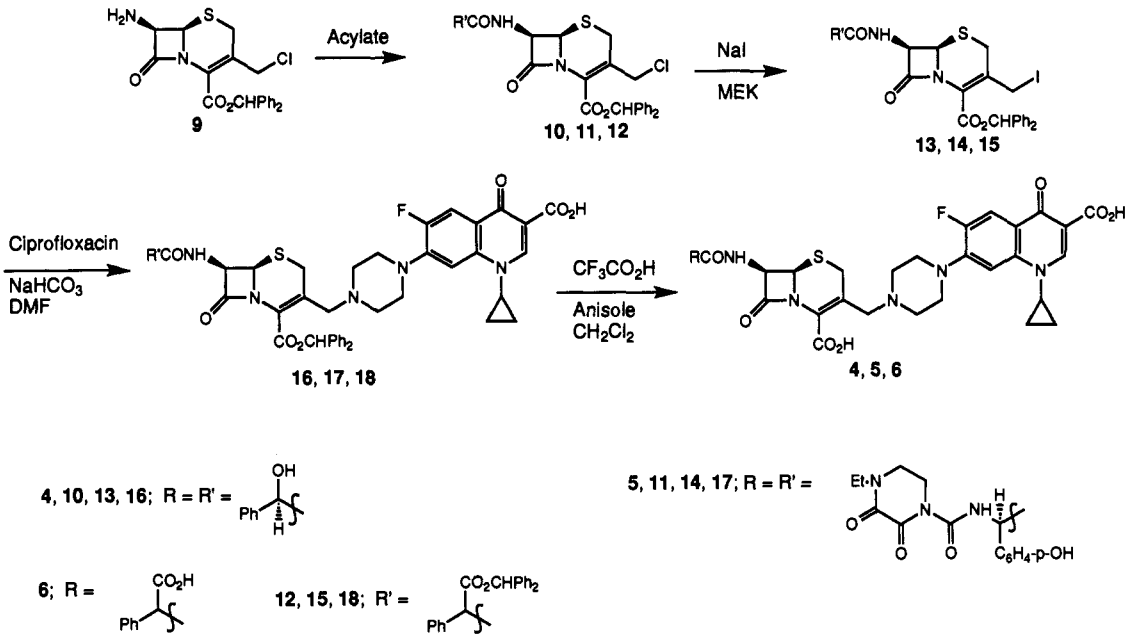
® Abstract published in *Advance ACS Abstracts*, January 1, 1994.

0022-2623/94/1837-0400$04.50/0

Scheme 1



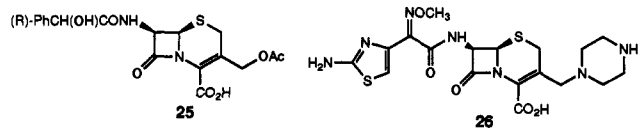
Scheme 2



ence of sodium iodide, as in Scheme 3, was also somewhat arbitrary. Although reactions of the chlorides were generally satisfactory, use of the preformed iodides permitted shorter reaction times and tended to provide intermediate products with a higher degree of purity.

Results and Conclusions

In vitro antibacterial activities of the bifunctional cephalosporins **1–6** are shown in Table 1. Activities of reference compounds are shown in Table 2. The comparison compounds include the quinolone, ciprofloxacin; a standard third-generation cephalosporin, cefotaxime; a cephalosporin with a first-generation-type spectrum, **25**; and a cephalosporin, **26**, which has a cefotaxime-type acylamino function and a 3′-piperazinyl substituent. The results for **26** confirm that these structural features are compatible with broad-spectrum activity. The fact that **26** is somewhat less potent than cefotaxime suggests that a tertiary amine substituent at the 3′-position may be less than optimal, i.e., the cephalosporin-type contribution to the activity of the bifunctional cephalosporins may be weaker than that projected on the basis of standard reference cephalosporins with a 3′-acetoxy function.



Compounds **1–6** demonstrated excellent broad-spectrum activity. Against such Gram-negative strains as *Citrobacter freundii* BS-16, *Enterobacter cloacae* P99, *Serratia marcescens* 1071, and *Proteus vulgaris* 1028 BC, which are susceptible to ciprofloxacin but are comparatively resistant to third-generation cephalosporins, the cephalosporin–quinolones showed potent activity. Against both the β-lactam-susceptible and -resistant *Staphylococcus aureus* strains, activity was generally moderate, with **4** being the most potent. Activity against methicillin-resistant *S. aureus* seems to reflect quinolone activity, since resistance of this type is mediated by overproduction of penicillin-binding protein (PBP) 2a.[27] Activity against

Scheme 3

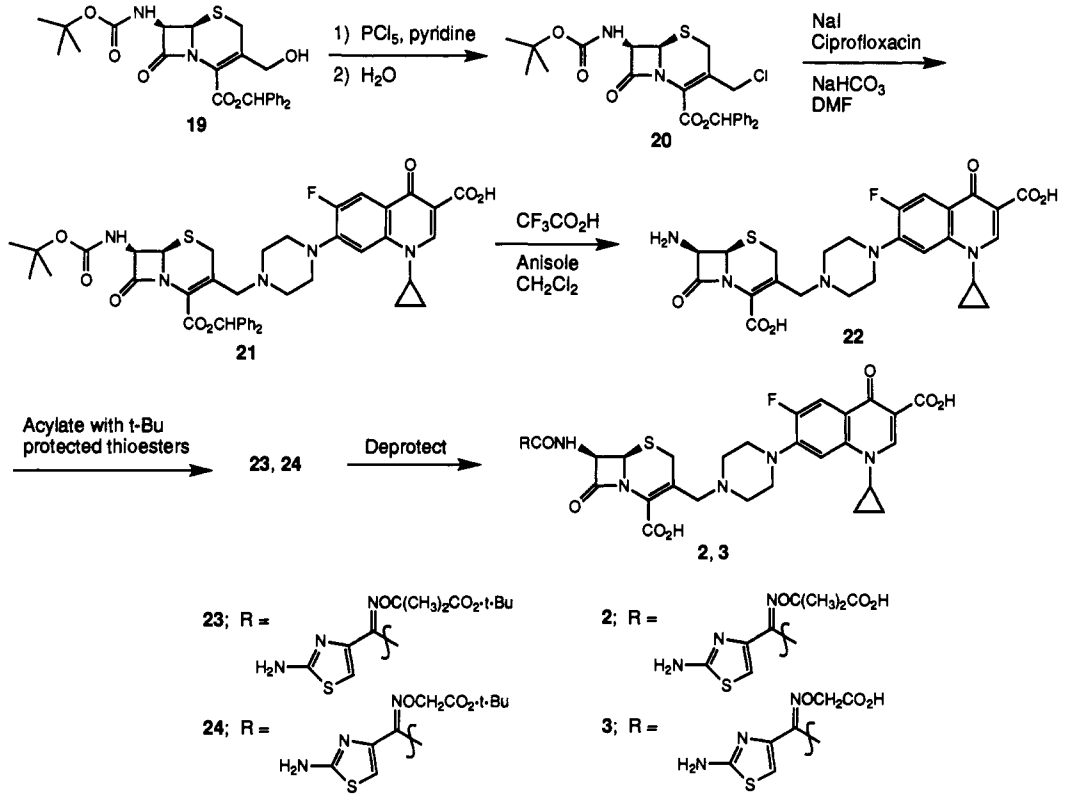


Table 1. In Vitro Activity of Tertiary-Amine-Linked Cephalosporin–Quinolones: MIC (μg/mL)

organism	1	2	3	4	5	6
Escherichia coli 257	0.125	0.25	0.25	0.125	≤0.016	0.25
E. coli ATCC 25922	0.125	0.25	0.5	0.25	≤0.016	0.25
E. coli TEM-1[a]	0.25	0.5	0.5	0.125	0.031	0.25
Citrobacter freundii BS-16[a]	0.125	0.125	1	0.125	0.063	0.063
Klebsiella pneumoniae A	1	0.5	1	0.5	0.5	1
Enterobacter cloacae 5699	0.5	1	1	0.25	0.125	0.5
E. cloacae P99[a]	0.125	0.5	0.25	0.031	≤0.016	0.063
Serratia marcescens 1071[a]	1	1	2	0.25	0.25	0.5
Proteus vulgaris 1028 BC[a]	0.25	1	1	0.063	0.063	0.25
P. mirabilis 90	2	2	2	1	1	1
Pseudomonas aeruginosa 8780	8	8	8	2	8	8
P. aeruginosa 18S/H[a]	2	2	4	2	2	4
Staphylococcus aureus Smith	4	8	8	0.5	2	2
S. aureus 67-0[b]	8	16	32	8	16	8
S. aureus 753[b]	4	8	8	1	2	2
Micrococcus luteus ATCC 9341	0.125	2	1	0.031	0.5	0.25
Streptococcus pneumoniae 6301	0.063	0.25	0.25	0.063	0.125	0.5
S. pyogenes 4	0.063	0.25	0.125	0.031	0.125	0.5
Enterococcus faecalis ATCC 29212	4	8	16	16	16	32

[a] β-Lactamase producer. [b] Methicillin-resistant.

the streptococci was rather dependent on the structure of the 7-acylamino substituent; compounds 1 and 4, the best in the series, were significantly more potent than ciprofloxacin, suggesting cephalosporin-like activity. Thus, the in vitro antibacterial spectrum of these dual-action cephalosporins seems to contain characteristics of both quinolone-like and cephalosporin-like activity. The mechanism by which the observed dual mode of action is manifested has not been unequivocally established.

In a recent publication,[28] it has been reported that compound 1 (Ro 25-8138) displays a quinolone-like ability to disrupt nucleoid segregation during replication of *Escherichia coli* cells, in an assay designed to distinguish between β-lactam and quinolone mechanisms of action. The authors attributed this quinolone response to the intact bifunctional cephalosporin rather than to the ciprofloxacin liberated in situ. The possibility that the intact bifunctional molecules may be capable of acting initially either as β-lactams or as quinolones complicates mechanistic studies, as has been discussed previously.[22] However, quinolones with large substituents on the basic nitrogen of an 8-piperazinyl substituent would not be expected to have good antibacterial activity. In view of all that is known regarding quinolone structure–activity relationships, it seems unlikely that the intact molecules could account for the potent quinolone-like antibacterial activity observed for this class of bifunctional compounds. It appears more likely that the observed quinolone activity is due to liberated ciprofloxacin.

In their behavior toward PBPs of *E. coli* (Table 3), the bifunctional compounds acted like typical cephalosporins. Affinity for PBP 3 was strongly dependent upon the nature of the 7-acylamino function.

Table 2. In Vitro Activity of Reference Compounds: MIC (μg/mL)

organism	cefotaxime	ciprofloxacin	25	26
Escherichia coli 257	0.031	0.008	2	0.25
E. coli ATCC 25922	0.063	0.008	2	0.25
E. coli TEM-1[a]	0.063	0.016	8	0.5
Citrobacter freundii BS-16[a]	32	0.031	>128	128
Klebsiella pneumoniae A	0.031	0.25	2	0.125
Enterobacter cloacae 5699	0.125	0.016	16	0.5
E. cloacae P99[a]	64	0.008	>128	128
Serratia marcescens 1071[a]	32	0.063	>128	8
Proteus vulgaris 1028 BC[a]	>128	0.016	>128	>128
P. mirabilis 90	≤0.016	0.063	2	0.5
Pseudomonas aeruginosa 8780	16	0.25	>128	64
P. aeruginosa 18S/H[a]	128	0.25	>128	>128
Staphylococcus aureus Smith	2	0.125	0.5	4
S. aureus 67-0[b]	>128	0.5	64	>128
S. aureus 753[b]	>128	0.25	128	>128
Micrococcus luteus ATCC 9341	0.031	2	0.125	0.25
Streptococcus pneumoniae 6301	≤0.016	1	0.125	0.063
S. pyogenes 4	≤0.016	0.5	0.063	0.031
Enterococcus faecalis ATCC 29212	0.25	1	16	4

[a] β-Lactamase producer. [b] Methicillin-resistant.

Table 3. Binding of Dual-Action Cephalosporins and Reference Compounds to Essential PBPs of *E. coli* UB1005 (DC0). Concentration (μg/mL) Required for 90% Inhibition of [^{14}C]Pen G Binding

compound	PBP 1a (90 kDa)	PBP 1b (90 kDa)	PBP 2 (66 kDa)	PBP 3 (60 kDa)	morphology	MIC (μg/mL)
cefotaxime	0.1	0.5	100	≤0.1	F/L[a]	0.031 (≤0.016)[b]
ceftazidime	10	10	>100	0.5	F	0.125 (0.063)
cefoperazone	10	10	2	≤0.1	F	0.031 (≤0.016)
1	>100	>100	100	0.5	F	1 (0.063)
2	100	100	100	2	F	4 (0.25)
3	>100	>100	>100	2	F	2 (4)
4	100	>100	>100	≤0.1	F	4 (2)
5	>100	>100	>100	2	F	2 (0.25)
6	>100	>100	>100	100	F	4 (2)
26	>100	100	>100	100	F	0.5 (0.25)

[a] F, filaments; L, lysis. [b] Numbers in parentheses refer to MICs for DC2, a permeability mutant.[33]

Table 4. Chemical Stability in 0.067 M pH 7.4 Phosphate Buffer at 37 °C as Determined by HPLC Analysis

compound	$t_{1/2}$ (days)	compound	$t_{1/2}$ (days)
1	6	5	2.5
2	12.5	6	9.5
4	5.5		

Chemically, the tertiary-amine-linked dual-action cephalosporins (Table 4) were much more stable than the previously reported ester-linked and carbamate-linked compounds. Degradation half-lives for compound 1 at 37 °C were determined by HPLC analysis to be 151 h in Mueller–Hinton broth and 34 h in mouse plasma. Under conditions of enzyme-mediated β-lactam hydrolysis, release of quinolone from representative tertiary-amine-linked compounds was observed. In qualitative experiments using β-lactamases derived from *S. aureus* and *E. cloacae* P99, 4 underwent rapid hydrolysis with liberation of ciprofloxacin, as determined by HPLC analysis.[24] In a quantitative study, the hydrolysis of a solution of compound 1 in the presence of the *P. vulgaris* enzyme was examined (Experimental Section). In 3 h, the concentration of 1 measured by HPLC declined from 36 to 3.6 μM, while the concentration of ciprofloxacin increased from 1.2 to 24 μM. Thus, 70% of the expected amount of free quinolone was detected.

Potent in vitro activity did not always translate into in vivo efficacy. In vitro, 4 demonstrated the best overall activity. However, in a murine pneumococcal pneumonia model (Experimental Section), 4 was inactive at the cutoff dose of 100 mg/kg. The pneumonia model is an extremely demanding test, since the infection is established by intranasal instillation of a bacterial culture and allowed to develop over a 24-h period before drug treatment. Typically, ciprofloxacin and most other quinolones are not active under these conditions. In this assay, 1 demonstrated an ED_{50} of 30 mg/kg, compared to 82 mg/kg for cefotaxime. In limited in vivo testing, the most consistently active bifunctional cephalosporin in this series was 1 (Table 5). In the mouse-protection test, 1 was active in five of six systemic infections, lacking efficacy only in the infection caused by *S. aureus*.

Table 5. Efficacy of 1, Cefotaxime, and Ciprofloxacin in Systemic Murine Infections

infecting organism	bacterial challenge (LD_{50})	ED_{50} (mg/kg)		
		1	cefotaxime	ciprofloxacin
K. pneumoniae A	>10 000	25	45	1
E. cloacae 5699	7055	14	50	0.2
S. marcescens SM	27	25	<25	<0.5
P. aeruginosa 8780[a]	>10 000	38	>250	4
S. aureus Smith	>8	>100	6	3
S. pneumoniae 6301	>10 000	54	69	>100

[a] Two treatments were administered subcutaneously, immediately after and 3 h following infection. All other infections were treated once immediately following infection.

As with the more chemically reactive bifunctional cephalosporins investigated earlier, evidence supporting the mechanism of action is suggestive rather than definitive. Properties of the tertiary-amine-linked cephalosporin–quinolones, including the nature of their broad-spectrum antibacterial activity, their behavior toward PBPs, and the rapid release of free quinolone under conditions of enzyme-mediated hydrolysis, are compatible with the proposed dual-action mechanism. Ease of

synthesis and chemical stability of the tertiary-amine linked products make this an attractive area for further molecular modification.

Experimental Section

Physical Chemistry. Infrared spectra (IR) were recorded on a Digilab FTS 15-E spectrometer. Mass spectra (MS) were obtained on a VG7070E-HF mass spectrometer in the positive-ion fast atom bombardment mode using glycerol or thioglycerol as the solvent. Proton nuclear magnetic resonance (NMR) spectra were obtained on a Varian XL-400 instrument. Chemical shifts (δ) are expressed in parts per million (ppm) downfield from tetramethylsilane, with coupling constants (J) in hertz (Hz).

HPLC Analyses. Conditions for HPLC analyses of products for purity and for monitoring stability studies were typically as follows. A Hamilton PRP-1 (250-mm × 4.1-mm) column was used, with UV detection at 280 nm and a mobile phase consisting of a 0.01 M solution of tetradecyltrimethylammonium bromide in a mixture of 70% 0.072 M pH 8.2 phosphate buffer and 30% acetonitrile. In some experiments, minor adjustments in pH and in concentration of acetonitrile were made to improve resolution. In stability studies, the decrease in integration of the product peak was followed at least until it reached 50% of the original value (i.e., through 1 half-life). In all cases, semilogarithmic plots of product-peak integrations against time were essentially linear. The major degradation product in all experiments had the same retention time and UV spectrum of ciprofloxacin.

Biological Assays. Minimum inhibitory concentrations (MICs) were determined by the agar dilution method.[29] Serial 2-fold dilutions of the compounds were prepared in water to give concentrations which, when diluted 10-fold in agar, ranged from 128 to 0.0156 μg/mL. For very active compounds, further dilutions were carried out to 0.000977 μg/mL. Three agar media were employed, brain heart infusion agar (Difco Laboratories, Detroit, MI) supplemented with 20 units/mL bovine liver catalase (Sigma Chemical Compnay, St. Louis, MO) for the streptococci; Mueller–Hinton agar (Difco) containing 3% agar for *Proteus*; and Mueller–Hinton agar for all other microorganisms. Petri dishes (10 cm) containing 20-mL final volume were inoculated with the aid of a Steers replicator (Craft Machine, Chester PA). Overnight broth cultures diluted 100-fold served as inocula. This procedure results in an inoculum of approximately 3×10^4 colony-forming units/spot. MICs were determined after overnight incubation at 36 °C.

The PBP-binding assay (Table 3) was carried out with solubilized membranes from sonicated *E. coli* UB1005, as previously described.[30] PBP binding was measured as inhibition of [^{14}C]penicillin G binding. Cell morphology was determined by microscopic examination after a 3-h incubation at 37 °C with the test compound in antibiotic medium 3 (Difco).

In vivo antibacterial activity (Table 5) was assessed in the mouse-protection test, using procedures described previously.[13] CD1 Swiss albino mice (18-20 g, Charles River Breeding Laboratories, Kingston, NY) were infected intraperitoneally with 0.5 mL of an appropriately diluted suspension of the bacterial culture. Serial dilutions of the antibacterial agents were prepared to yield concentrations ranging from 250 to 0.05 mg/kg. The infected mice were immediately treated (within 5 min) with 1.0 mL of the test solution subcutaneously at the dorsal base of the neck. Six mice were used for each treatment and for control groups. Only one treatment was given, except for experiments with *Pseudomonas aeruginosa*, where a second treatment was administered 3 h after infection. The method of Reed and Muench[31] was used to determine the 50% effective (i.e., protective) dose (ED_{50}). This method for interpolation to the 50% effective dose on a cumulative survival curve does not provide a statistical basis for calculating confidence limits. All direct comparisons of in vivo activity were made with data obtained at the same time in the same experiment with the same infection.

In the pneumococal pneumonia model,[23] pneumonia was induced in mice by intranasal instillation of an appropriately diluted culture of *Streptococcus pneumoniae* 6301. The mice were treated once subcutaneously with the test compound 24 h after infection. Mice were sacrificed 48 h after infection. The lungs were removed aseptically and cultured for the presence of the infecting organism. Untreated infected controls showed heavily positive cultures. ED_{50} values were calculated on the basis of negative lung cultures.

β-Lactamase Hydrolysis of Compound 1. In a total assay volume of 3.00 mL were combined 1.50 mL of 100 mM pH 7.40 sodium phosphate buffer, 0.30 mL of a 400 μM solution of 1, 1.15 mL of sterile deionized water, and 50 μL of a solution of *P. vulgaris* β-lactamase.[32] The amount of enzyme added was selected empirically to produce approximately 50% hydrolysis of the substrate within 1 h. The mixture was maintained at 30 °C; 50-μL samples were removed immediately after addition of the enzyme and at appropriate intervals thereafter, throughout the 3-h course of the experiment. Samples were immediately diluted with an equal volume of ice-cold acetonitrile, vortexed for 30 s, further diluted with 100 μL of solvent B (below), and kept cold until analyzed by HPLC. External standards of 1 and the quinolone, as well as substrate and enzyme blanks, were run concurrently with the study samples. The sample injection volume was 100 μL. A refrigerated autoinjector was used, with UV detection at 270 nm.

The mobile phase was prepared from solvent A (50% acetonitrile–50% solvent B) and solvent B (aqueous 50 mM monobasic potassium phosphate, 5 mM 1-octanesulfonic acid sodium salt, adjusted to pH 2.90 with HCL). The mobile phase for this experiment was 48% A and 52% B.

[6*R*-[6α,7β(*Z*)]]-3-[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-7-[[(methoxyimino)[2-[(triphenylmethyl)amino]-4-thiazolyl]-acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, 1,1-Dimethylethyl Ester (8). A mixture of 8.22 g (0.01 mol) of [6*R*-[6α,7β(*Z*)]]-3-(iodomethyl)-7-[[(methoxyimino)[2-[(triphenylmethyl)amino]-4-thiazolyl]acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 1,1-dimethylethyl ester (7), 3.31 g (0.01 mol) of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (ciprofloxacin), 1.01 g (0.012 mol) of sodium bicarbonate, and 200 mL of DMF was stirred for 3 h. The mixture was filtered to remove a small amount of undissolved solid. The solution was concentrated to dryness under reduced pressure at a bath temperature of 35 °C. The residue was shaken with 250 mL of ethyl acetate and 100 mL of water and the insoluble portion removed by filtration, and the phases were separated. The organic phase was washed with 100 mL of 5% aqueous sodium thiosulfate solution followed by 100 mL of water. After drying (Na_2SO_4), the organic solution was concentrated to dryness under reduced pressure and the residue triturated with 200 mL of ether. The solid thus obtained was filtered, washed with five 20-mL portions of ether, and dried under reduced pressure to obtain 8.86 g (86.4%) of crude 8.

This product was purified by crystallization of the hydrochloride as follows. A solution of 8.71 g (0.0085 mol) of the above product in 68 mL of acetone was acidified by addition of 68 mL (0.0204 mol) of 0.3 N HCl in acetone (prepared by diluting 1 mL of concentrated hydrochloric acid to 40 mL with acetone). The hydrochloride salt crystallized over a period of 1 h and 15 min. Acetone (100 mL) was added to thin the mixture, which was then filtered. The solid was washed with five 20-mL portions of acetone and dried to constant weight under reduced pressure to obtain 7.00 g (75%) of the hydrochloride salt.

Before deprotection, this product was converted back to the free base. The hydrochloride (5.95 g, 0.00542 mol) was stirred with 250 mL of methylene chloride and a solution of 8 g of sodium bicarbonate in 150 mL of water for 5 min. The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure to obtain a 5.24-g residue of 8: NMR (Me_2SO-d_6) δ 1.18, 1.31 (2 m, 4 H, CH_2CH_2), 1.49 (s, 9 H, *t*-Bu), 2.55 (m, 4 H, CH_2NCH_2), 3.25 (d, 1 H, J = 16 Hz, half of SCH_2), 3.3-3.5 (m, CH_2NCH_2, half of SCH_2 and water peak), 3.59 (m, 2 H, CH_2N), 3.81 (s, 3 H, MeO), 3.81 (m, 1 H, NCH), 5.17 (d, 1 H, J = 5 Hz, CH), 5.67 (dd, 1 H, J = 5 and 8 Hz, CH), 6.69 (s, 1 H, thiazole), 7.2–7.4 (m, 15 H, Ar), 7.56 (d, 1 H, J = 7 Hz, Ar), 7.91 (d, 1 H, J = 13 Hz, Ar), 8.67 (s, 1 H, =CHN), 8.84 (s, 1 H, NH), 9.56 (d, 1 H, J = 8 Hz NH); IR (KBr) 1788, 1728, 1685, 1630 cm^{-1}; MS m/z 1025.3479 $(M + H)^+$, calcd 1025.3490.

[6*R*-[6α,7β(*Z*)]]-7-[[(2-Amino-4-thiazolyl)(methoxyimino)-acetyl]amino]-3-[[4-(3-carboxy-1-cyclopropyl-6-fluoro-1,4-

dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (1), Trifluoroacetate. Deprotection of 8 was accomplished by a two-step procedure. First, the *N*-trityl group was removed with formic acid. The above 5.24-g (0.00511 mol) residue was stirred vigorously with a mixture of 75 mL of 90% formic acid and 75 mL of water for 5.5 h at room temperature. The precipitate was removed by filtration. The filtrate was concentrated to dryness under reduced pressure, using an oil pump. Four times, 100-mL portions of 4:1 CH_2Cl_2–MeOH were added, and the mixture was evaporated each time to dryness under reduced pressure. The residue was triturated with 100 mL of ether. The solid thus obtained was filtered, washed with five 20-mL portions of ether, and dried under reduced pressure to obtain 3.32 g (83%) of partially deprotected intermediate product.

Finally, the *tert*-butyl ester was converted to the carboxylic acid. A solution of 0.62 g (0.709 mmol) of the above intermediate in a mixture of 15 mL of trifluoroacetic acid and 15 mL of nitromethane was allowed to stand at room temperature for 3 h and 20 min. The mixture was concentrated to dryness under reduced pressure. Three 40-mL portions of methylene chloride were added to the residue, with evaporation being repeated each time. After trituration with 80 mL of ether, the residue solidified to provide 0.60 g (91.6%) of the title compound: NMR (Me_2SO-d_6) δ 1.19, 1.32 (2 m, 4 H, CH_2CH_2), 3.25, 3.36 (2 m, 4 H, CH_2NCH_2), 3.58 (m, 4 H, CH_2NCH_2), 3.65, 3.78 (2 d, 2 H, J = 18 Hz, CH_2S), 3.83 (m, 1 H, CH), 3.87 (s, 3 H, MeO), 4.03 (b s, 2 H, CH_2N), 5.24 (d, 1 H, J = 5 Hz), 5.85 (dd, 1 H, J = 5 and 8 Hz), 6.77 (s, 1 H, thiazole), 7.60 (d, 1 H, J = 7 Hz, Ar), 7.97 (d, 1 H, J = 14 Hz, Ar), 8.19 (s, 1 H, =CHN), 9.67 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1790, 1678, 1628 cm^{-1}; MS m/z 727 $(M + H)^+$.

(6*R*-*trans*)-3-(Chloromethyl)-7-[[(1,1-dimethylethoxy)carbonyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, Diphenylmethyl Ester (20). A solution of 9.93 g (0.02 mol) of (6*R*-*trans*)-3-(hydroxymethyl)-7-[[(1,1-dimethylethoxy)carbonyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid, diphenylmethyl ester (**19**) in 200 mL of methylene chloride was cooled to −40 °C. In one portion, 5.00 g (0.024 mol) of phosphorus pentachloride was added followed within 1–2 min by 2.00 mL (0.025 mol) of pyridine. The reaction was stirred at −30 to −40 °C for 1.5 h and then poured into a cold mixture of 250 mL of water and 100 mL of methylene chloride. The organic phase was washed with water and aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated under reduced pressure to obtain a quantitative yield of **20**: NMR ($CDCl_3$) δ 1.48 (s, 9 H, *t*-Bu), 3.49, 3.58 (2 d, 2 H, J = 14 Hz, CH_2S), 4.37, 4.47 (2 d, 2 H, J = 11 Hz, CH_2Cl), 4.99 (d, 1 H, J = 4 Hz, CH), 5.20 (d, 1 H, J = 8 Hz, NH), 5.66 (dd, 1 H, J = 4 and 8 Hz), 6.98 (s, 1 H, $CHPh_2$), 7.3–7.5 (m, 10 H, Ar); MS m/z 515 $(M + H)^+$.

(6*R*-*trans*)-3-[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-7-[[(1,1-dimethylethoxy)carbonyl]amino]-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic Acid, Diphenylmethyl Ester (21). A mixture of 2.58 g (0.005 mol) of **20**, 0.75 g (0.005 mol) of sodium iodide, and 100 mL of DMF was stirred for 30 min; 1.66 g (0.005 mol) of ciprofloxacin and 0.50 g (0.006 mol) of sodium bicarbonate were then added, and stirring continued overnight. The insoluble portion was removed by filtration, the filtrate concentrated to dryness under reduced pressure, and the residue taken up in a mixture of 200 mL of ethyl acetate, 200 mL of methylene chloride, and 100 mL of water. The aqueous phase was separated and extracted with 100 mL of 1:1 ethyl acetate–methylene chloride. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was triturated with ether to obtain 1.86 g of crude product as a solid. Chromatographic purification of 1.23 g of this material using 18 g of 70–230 mesh silica gel and 5% methanol/chloroform gave 0.99 g (37%) of **21**: NMR ($CDCl_3$) δ 1.20, 1.39 (2 m, 4 H, CH_2CH_2), 1.47 (s, 9 H, *t*-Bu), 2.53, 3.26 (2 b s, 8 H, $N(CH_2CH_2)_2N$), 3.30–3.60 (m, 4H, CH_2S and CH_2N), 3.70 (m, 1 H, CH), 5.01 (d, 1 H, J = 5 Hz, CH), 5.22 (d, 1 H, J = 8 Hz, NH), 5.64 (dd, 1 H, J = 5 and 8 Hz, CH), 6.98 (s, 1 H, $CHPh_2$), 7.25–7.45 (m, 11 H, Ar), 8.05 (d, 1 H, J = 14 Hz, Ar), 8.79 (s, 1 H, =NCH); IR (KBr) 1785, 1722, 1628 cm^{-1}; MS m/z 810 $(M + H)^+$.

(6*R*-*trans*-)-7-Amino-3-[[4-(3-carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, Trifluoroacetic Acid Salt (22). A solution of 1.32 g (0.00163 mol) of **21** in a mixture of 53 mL of trifluoroacetic acid, 53 mL of methylene chloride, and 5.3 mL of anisole was kept at 0–5 °C for 16 h. The mixture was concentrated to dryness under reduced pressure. Trituration of the residue with ether provided 1.20 g (95%) of the title compound as a solid: NMR (Me_2SO-d_6) δ 1.16, 1.32 (2 m, 4 H, CH_2CH_2), 3.02, 3.50 (2 m, 8 H, $N(CH_2CH_2)_2N$), 3.73, 3.79 (2 d, 2 H, J = 16 Hz, CH_2S), 3.84 (m, 3 H, CH_2N and CHN), 5.11, 5.20 (2 d, 2 H, J = 5 Hz, CHCH), 7.60 (d, 1 H, J = 7 Hz, Ar), 7.96 (d, 1 H, J = 13 Hz, Ar), 8.69 (s, 1 H, CHN); IR (KBr) 1789, 1712–1677, 1630 cm^{-1}; MS m/z 544 $(M + H)^+$.

[6*R*-[6α,7β(*Z*)]-7-[[[(2-Amino-4-thiazolyl)[[(1,1-dimethylethoxy)carbonyl]-1-methylethoxy]imino]acetyl]amino]-3-[[4-(3-carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (23). A mixture of 0.537 g (0.786 mmol) of **22**, 15 mL of THF, 0.317 g (3.78 mmol) of sodium bicarbonate, and 15 mL of water was stirred at 0–5 °C for 20 min. A solution of 0.768 g (1.61 mmol) of (*Z*)-2-[[[1-(2-amino-4-thiazolyl)-2-(benzothiazol-2-ylthio)-2-oxoethyl]imino]-oxy]-2-methylpropanoic acid, 1,1-dimethylethyl ester in 10 mL of THF was then added, and stirring continued for 15 min at 0–5 °C. The cooling bath was then removed and the mixture stirred overnight at room temperature. The THF was evaporated under reduced pressure, and the residual aqueous solution was washed with ethyl acetate. The pH was adjusted to 5.5 by adding 2 N HCl, to precipitate a solid. After filtering, washing with water and ethyl acetate, and drying under reduced pressure, 0.424 g (63%) of **23** was obtained, having the following data: NMR (Me_2SO-d_6) δ 1.18, 1.29 (2 m, 4 H, CH_2CH_2), 1.37, 1.41 (s, 15 H, *t*-Bu and 2 Me), 2.67 (b s, 4 H, CH_2NCH_2), 3.1–3.5 (m, includes SCH_2, CH_2NCH_2, and water peak), 3.48 (b s, 2 H, CH_2N), 3.80 (m, 1 H, CH), 5.15 (d, 1 H, J = 4 Hz, CH), 5.76 (dd, 1 H, J = 4 and 7 Hz, CH), 6.70 (s, 1 H, thiazole), 7.23 (s, 2 H, NH_2), 7.56 (d, 1 H, J = 6 Hz, Ar), 7.88 (d, 1 H, J = 11 Hz, Ar), 8.64 (s, 1 H, =NCH), 9.33 (d, 1 H, J = 7 Hz, NH); MS m/z 855 $(M + H)^+$.

[6*R*-[6α,7β(*Z*)]]-7-[[(2-Amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-3-[[4-(3-carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (2), Sodium Salt. A solution of 0.472 g (0.553 mmol) of **23** in 19 mL of trifluoroacetic acid, 1.9 mL of anisole, and 18 mL of methylene chloride was kept at 0–5 °C for 16 h. The mixture was concentrated to dryness under reduced pressure. Methylene chloride was added, and the evaporation was repeated. The residue was triturated with ether to obtain solid product in the form of a trifluoroacetic acid salt. This was dissolved in aqueous sodium bicarbonate at pH 7 and purified as the sodium salt on a column of 20 g of Waters C_{18} silica, eluting with an aqueous acetonitrile gradient of up to 30% acetonitrile. The appropriate fractions were combined, concentrated under reduced pressure, and freeze-dried to obtain 0.363 g (78%) of **2**: NMR (Me_2SO-d_6) δ 1.16, 1.33 (2 m, 4 H, CH_2CH_2), 1.37, 1.45 (2 s, 6 H, 2 Me), 2.50 (m, CH_2NCH_2 and Me_2SO), 3.2–3.4 (m, includes CH_2S, CH_2N, CH_2NCH_2, and water peak), 3.82 (m, 1 H, CH), 4.98 (d, 1 H, J = 5 Hz, CH), 5.59, 5.61 (dd, 1 H, J = 5 and 8 Hz, CH), 6.72 (s, 1 H, thiazole), 7.17 (s, 2 H, NH_2), 7.57 (d, 1 H, J = 7 Hz, Ar), 7.89 (d, 1 H, J = 14 Hz, Ar), 8.65 (s, 1 H, =CHN); IR (KBr) 1762, 1658, 1628 cm^{-1}; MS m/z 843 $(M + H)^+$.

[6*R*-[6α,7β(*Z*)]]-7-[[(2-Amino-4-thiazolyl)[(carboxymethoxy)imino]acetyl]amino]-3-[[4-(3-carboxy-1-cyclopropyl-6-fluoro-−1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (3), Sodium Salt. Via procedures similar to those described above for the preparation of **2**, intermediate **22** was acylated in 74% yield with (*Z*)-[[[1-(2-amino-4-thiazolyl)-2-(benzothiazol-2-ylthio)-2-oxoethyl]imino]oxy]acetic acid, 1,1-dimethylethyl ester to give **24**, which was then deprotected with trifluoroacetic acid–anisole in methylene chloride. After purification as the sodium salt by C_{18} reverse-phase chromatography, **3** was obtained in 71% yield: NMR (Me_2SO-*6*) δ 1.05, 1.30 (2 m, 4 H, CH_2CH_2), 2.5 (m, CH_2NCH_2 and Me_2SO), 3.2–3.4 (m, includes CH_2S, CH_2NCH_2, and CH_2N), 3.70 (m, 1 H, CHN), 4.19 (s, 2 H, NCH_2CO_2), 4.95 (d, 1 H, J = 5 Hz, CH), 5.53 (dd, 1 H, J – 5 and

8 Hz, CH), 6.83 (s, 1 H, thiazole), 7.17 (s, 2 H, NH_2), 7.50 (d, 1 H, J = 7 Hz, Ar), 7.83 (d, 1 H, J = 13 Hz, Ar), 8.59 (s, 1 H, =CHN), 12.1 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3410, 1760, 1622 cm^{-1}; MS m/z 815 $(M + H)^+$.

[6*R*-[6α,7β(*R*)]]-3-(Chloromethyl)-7-[(hydroxyphenylacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, Diphenylmethyl Ester (10). To a mixture of 1.26 g (3.05 mmol) of (6*R*-*trans*-7-amino-3-(chloromethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, diphenylmethyl ester (**9**), 0.464 g (3.05 mmol) of (*R*)-mandelic acid, and 0.412 g (3.05 mmol) of 1-hydroxybenzotriazole in 30 mL of methylene chloride was added 0.628 g (3.05 mmol) of 1,3-dicyclohexylcarbodiimide. The mixture was stirred for 16 h and the insoluble portion removed by filtration. The filtrate was washed with 0.1 M phosphoric acid followed by water. The methylene chloride solution was dried (Na_2SO_4) and concentrated to dryness under reduced pressure. The residue solidified on trituration with ether. After twice more triturating with 10-mL portions of acetonitrile, 0.881 g (53%) of **10** was obtained, having the following data: NMR ($CDCl_3$) 3.30 (d, 1 H, J = 3 Hz, OH), 3.45, 3.61 (2 d, 2 H, J = 15 Hz, CH_2S), 4.36 (s, 2 H, CH_2Cl), 5.00 (d, 1 H, J = 5 Hz), 5.16 (d, 1 H, J = 3 Hz, C*H*OH), 5.82 (dd, 1 H, J = 5 and 8 Hz, CH), 6.92 (d, 1 H, J = 8 Hz, NH), 6.98 (s, 1 H, $CHPh_2$), 7.25–7.45 (m, 15 H, Ar).

[6*R*-[6α,7β(*R*)]]-7-[(Hydroxyphenylacetyl)amino]-3-(iodomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, Diphenylmethyl Ester (13). A mixture of 0.20 g (0.364 mmol) of **10**, 0.273 g (1.82 mmol) of sodium iodide, and 6 mL of methyl ethyl ketone was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure and the residue taken up in ethyl acetate. The organic solution was washed with cold 5% aqueous sodium thiosulfate and brine, dried (Na_2SO_4), and concentrated to dryness under reduced pressure to obtain 0.217 g (93%) of **13**: NMR ($CDCl_3$) δ 3.43 (d, 1 H, J = 3 Hz, OH), 3.45, 3.76 (2 d, 2 H, J = 15 Hz, CH_2S), 4.27 (s, 2 H, CH_2I), 4.97 (d, 1 H, J = 5 Hz, CH), 5.12 (d, 1 H, J = 3 Hz, C*H*OH), 5.75 (dd, 1 H, J = 5 and 8 Hz, CH), 6.98 (s, 1 H, $CHPh_2$), 7.02 (d, 1 H, J = 8 Hz, NH), 7.2–7.4 (m, 15 H, Ar).

[6*R*-[6α,7β(*R*)]]-3-[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-7-[(hydroxyphenylacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, diphenylmethyl Ester (16). A mixture of 0.217 g (0.338 mmol) of iodide **13**, 0.112 g (0.338 mmol) of ciprofloxacin, 28.4 mg (0.338 mmol) of sodium bicarbonate, and 7 mL of DMF was stirred for 3 h. The mixture was concentrated under reduced pressure. Water and a 1:1 mixture of ethyl acetate and methylene chloride were added to the residue. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure to give 0.238 g (83%) of **16**: NMR ($CDCl_3$) δ 1.18, 1.36 (2 m, 4 H, CH_2CH_2), 2.50 (b s, 4 H, CH_2NCH_2), 3.2–3.4 (m, 6 H, CH_2NCH_2 and CH_2N), 3.50 (m, 1 H, CH), 3.42, 3.65 (2 d, 2 H, J = 14 Hz, CH_2S), 5.01 (d, 1 H, J = 5 Hz, CH), 5.17 (s, 1 H, C*H*OH), 5.80 (dd, 1 H, J = 5 and 8 Hz, CH), 6.97 (s, 1 H, $CHPh_2$), 7.04 (d, 1 H, J = 8 Hz, NH), 7.2–7.4 (m, 16 H, Ar), 8.01 (d, 1 H, J = 11 Hz, Ar) 8.77 (s, 1 H, =CHN).

[6*R*-[6α,7β(*R*)]]-3-[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-7-[(hydroxyphenylacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (4), Sodium Salt. A solution of 90.5 mg of **16** in 3.6 mL of trifluoroacetic acid, 3.6 mL of methylene chloride, and 0.36 mL of anisole was kept at 0 °C for 15 min. The mixture was concentrated to dryness under reduced pressure and the residue triturated with ether to obtain 67.0 mg of a solid trifluoroacetic acid salt. This solid was dissolved in aqueous sodium bicarbonate at pH 8 and purified by reverse-phase chromatography on 2 g of C_{18} silica, eluting with an acetonitrile–water gradient of 0–30% acetonitrile. The appropriate fractions were combined, concentrated under reduced pressure, and freeze-dried to yield 40.6 mg (54%) of **4**: NMR (Me_2SO-d_6) δ 1.00, 1.21 (2 m, 4 H, CH_2CH_2), 2.50 (m, CH_2NCH_2 and Me_2SO), 3.0–3.4 (m, 8 H, CH_2S, CH_2N, CH_2NCH_2), 3.60 (m, 1 H, CHN), 4.97 (d, 1 H, J = 5 Hz, CH), 5.12 (d, 1 H, J = 5 Hz, C*H*OH), 5.46 (dd, 1 H, J = 5 and 8 Hz, CH), 7.23–7.50 (m, 6 H, Ar), 7.82 (d, 1 H, J = 13 Hz, Ar), 8.62 (s, 1 H, =CHN); IR (KBr) 1758, 1665, 1628 cm^{-1}; MS m/z 700 $(M + H)^+$, 722 $(M + Na)^+$.

[6*R*-[6α,7β(*R*)]]-3-[[4-(Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-7-[[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino](4-hydroxyphenyl)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (5), Trifluoroacetic Acid Salt. From the starting materials (*R*)-[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino](4-hydroxyphenyl)acetic acid and **9**, using procedures similar to those used for the preparation of **4**, the chloride **11**, the iodide **14**, and the intermediate ester **17** were prepared in sequence. Deprotection then gave **5**, which was characterized as a trifluoroacetic acid salt: NMR (Me_2SO-d_6) δ 1.08 (t, 3 H, J = 7 Hz, Me), 1.20, 1.33 (2 m, 4 H, CH_2CH_2), 3.17, 3.26 (2 m, 4 H, CH_2NCH_2), 3.38 (q, 2 H, J = 7 Hz, NCH_2Me), 3.5–3.6 (m, 5 H, CH_2NCH_2 and half of CH_2S), 3.65 (d, 1 H, J = 16 Hz, half of CH_2S), 3.7–4.0 (m, includes CHN, CH_2N, NCH_2CH_2N, and water peak), 5.09 (d, 1 H, J = 5 Hz, CH), 5.51 (d, 1 H, J = 8 Hz, ArC*H*CO), 5.78 (dd, 1 H, J = 5 and 8 Hz, CH), 6.73, 7.23 (2 d, 4 H, J = 8 Hz, Ar), 7.61 (d, 1 H, J = 8 Hz, Ar), 7.98 (d, 1 H, J = 14 Hz, Ar), 8.70 (s, 1 H, =CHN); IR (KBr) 1785, 1715, 1680 cm^{-1}; MS m/z 883 $(M + H)^+$.

(6*R*-*trans*)-3-[[4-(3-Carboxyl-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]methyl]-7-[(carboxyphenylacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic Acid (6), Sodium Salt. Via the starting acid phenylmalonic acid, monodiphenylmethyl ester and via procedures similar to those used to prepare **4**, the chloride **12** (83%), the iodide **15** (95%), and the intermediate ester **18** (84%) were prepared in sequence. Deprotection of **18** and purification of the sodium salt by reverse-phase chromatography on C_{18} silica using 30% acetonitrile gave a 69% yield of **6**: NMR (Me_2SO-d_6) δ 0.99, 1.27 (2 m, 4 H, CH_2CH_2), 3.22 (b s, 4 H, CH_2NCH_2), 3.25–3.5 (m, includes CH_2S, CH_2NCH_2, CH_2N, and water peak), 3.60 (m, 1 H, CH), 3.83, 3.85 (2 s, 1 H, α-CH), 4.95, 4.96 (2 d, 1 H, J = 5 Hz, CH), 5.44, 5.54 (2 dd, 1 H, J = 5 and 8 Hz, CH), 7.10–7.45 (m, 6 H, Ar), 7.78 (d, 1 H, J = 14 Hz, Ar), 8.54 (s, 1 H, =CHN), 11.45, 11.57 (2 d, 1 H, J = 8 Hz, NH); MS m/z 750 $(M + H)^+$.

[6*R*-[6α,7β(*Z*)]]-7-[[(2-Amino-4-thiazolyl)(methoxyimino)-acetyl]amino]-8-oxo-3-[(1-piperazinyl)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (26), Trifluoroacetate. Via procedures similar to those used to prepare **1**, starting from **7**, and mono-*t*-Boc-protected piperazine, reference compound **26** was prepared, having the following data: NMR (Me_2SO-d_6) δ 2.62 (m, 4 H, CH_2NCH_2), 3.10 (b s, 4 H, CH_2NCH_2), 3.33, 3.38 (2 d, 2 H, J = 16 Hz, CH_2S), 3.58 (s, 2 H, CH_2N), 3.85 (s, 3 H, MeO), 5.14 (d, 1 H, J = 5 Hz, CH), 5.76 (dd, 1 H, J = 5 and 8 Hz, CH), 6.76 (s, 1 H, thiazole), 8.61 (b s, 2 H, NH_2), 9.12 (b s, 1 H, CO_2H), 9.61 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3050, 1785, 1665 cm^{-1}; MS m/z 482 $(M + H)^+$.

Acknowledgment. The authors gratefully acknowledge contributions of Gino Sasso for interpretation of NMR spectra; Anne Bertasso, E. John Duker, Lucy Foppiani, Eugene LaSala, Ellen R. Lipschitz, David H. McGarry, and JoAnn Siebelist for experimental work on biological assays; and BettyAnn Hedemus for assistance in preparing the manuscript.

References

(1) Hamilton-Miller, J. M. T.; Newton, G. G. F.; Abraham, E. P. Products of Aminolysis and Enzymic Hydrolysis of Cephalosporins. *Biochem. J.* **1970**, *116*, 371–384.
(2) O'Callaghan, C. H.; Kirby, S. M.; Morris, A.; Waller, R. E.; Duncombe, R. E. Correlation Between Hydrolysis of the β-Lactam of the Cephalosporin Nucleus and Expulsion of the 3-Substituent. *J. Bacteriol.* **1972**, *110*, 988–991.
(3) Faraci, W. S.; Pratt, R. F. Elimination of a Good Leaving Group from the 3'-Position of a Cephalosporin Need Not Be Concerted with β-Lactam Ring Opening. *J. Am. Chem. Soc.* **1984**, *106*, 1489–1490.
(4) Russell, A. D.; Fountain, R. H. Aspects of the Mechanism of Action of Some Cephalosporins. *J. Bacteriol.* **1971**, *106*, 65–69.
(5) Boyd, D. B.; Lunn, W. H. W. Electronic Structures of Cephalosporins and Penicillins. 9. Departure of a Leaving Group in Cephalosporins. *J. Med. Chem.* **1979**, *22*, 778–784.
(6) Boyd, D. B. Elucidating the Leaving Group Effect in the β-Lactam Ring Opening Mechanism of Cephalosporins. *J. Org. Chem.* **1985**, *50*, 886–888.

(7) Page, M. L.; Proctor, P. Mechanism of β-Lactam Opening in Cephalosporins. *J. Am. Chem. Soc.* **1984**, *106*, 3820–3825.
(8) Grabowski, E. J.; Douglas, A. W.; Smith, G. B. Ammonolysis of Cephamycins: ^{13}C NMR Characterization of the Intermediates from β-Lactam Ring Cleavage Prior to Loss of the 3'-Group. *J. Am. Chem. Soc.* **1985**, *107*, 267–268.
(9) O'Callaghan, C. H.; Sykes, R. B.; Staniforth, S. E. A New Cephalosporin with a Dual Mode of Action. *Antimicrob. Agents Chemother.* **1976**, *10*, 245–248.
(10) Greenwood, D.; O'Grady, F. Dual-Action Cephalosporin Utilizing a New Therapeutic Principle. *Antimicrob. Agents Chemother.* **1976**, *10*, 249–252.
(11) Albrecht, H. A.; Beskid, G.; Chan, K.-K.; Christenson, J. G.; Cleeland, R.; Deitcher, K. H.; Georgopapadakou, N. H.; Keith, D. D.; Pruess, D. L.; Sepinwall, J.; Specian, A. C.; Then, R. L.; Weigele, M.; West, K. F.; Yang, R. Cephalosporin 3'-Quinolone Esters with a Dual Mode of Action. *J. Med. Chem.* **1990**, *33*, 77–86.
(12) Beskid, G.; Fallat, V.; Lipschitz, E. R.; McGarry, D. H.; Cleeland, R.; Chan, K.-K.; Keith, D. D.; Unowsky, J. In Vitro Activities of a Dual-Action Antibacterial Agent, Ro 23-9424, and Comparative Agents. *Antimicrob. Agents Chemother.* **1989**, *33*, 1072–1077.
(13) Beskid, G.; Siebelist, J.; McGarry, C. M.; Cleeland, R.; Chan, K.-K.; Keith, D. D. In Vivo Evaluation of a Dual-Action Antibacterial, Ro 23-9424, Compared to Cefotaxime and Fleroxacin. *Chemotherapy* **1990**, *36*, 109–116.
(14) Georgopapadakou, N. H.; Bertasso, A.; Chan, K.-K.; Chapman, J. S.; Cleeland, R.; Cummings, L. M.; Dix, B. A.; Keith, D. D. Mode of Action of the Dual-Action Cephalosporin Ro 23-9424. *Antimicrob. Agents Chemother.* **1989**, *33*, 1067–1071.
(15) Jones, R. N.; Barry, A. L.; Thornsberry, C. Antimicrobial Activity of Ro 23-9424, a Novel Ester-Linked Codrug of Fleroxacin and Desacetylcefotaxime. *Antimicrob. Agents Chemother.* **1989**, *33*, 944–950.
(16) Georgopapadakou, N. H.; Albrecht, H. A.; Bertasso, A.; Cummings, L. M.; Keith, D. D.; Russo, D. A. Cephalosporin-Quinolone Esters; Biological Properties. 28th Intersci. Conf. Antimicrob. Agents Chemother, 1988, Abstract No. 442.
(17) Christenson, J. G.; Pruess, D. L.; Robertson, T. L. Hydrolysis of Ro 23-9424 by β-Lactamases. 28th Intersci. Conf. Antimicrob. Agents Chemother., 1988, Abstract No. 443.
(18) Christenson, J. G.; Brocks, V.; Chan, K.-K.; Keith, D. D.; Pruess, D. L.; Schaefer, F. F.; Talbot, M. K. Mode of Action of Ro 23-9424, a Dual-Action Cephalosporin. 28th Intersci. Conf. Antimicrob. Agents Chemother., 1988, Abstract No. 444.
(19) Christenson, J. G.; Chan, K.-K.; Farrish, H. H.; Patel, I. H.; Specian, A. Pharmacokinetics of Ro 23-9424, a Dual-Action Cephalosporin, in Animals. 28th Intersci. Conf. Antimicrob. Agents Chemother., 1988, Abstract No. 449.
(20) Christenson, J.; Albrecht, H.; Georgopapadakou, N.; Keith, D.; Pruess, D.; Talbot, M.; Then, R. Mode of Action of Ro 23-5068, a Dual-Action Cephalosporin. 28th Intersci. Conf. Antimicrob. Agents Chemother., 1988, Abstract No. 450.
(21) Albrecht, H. A.; Beskid, G.; Christenson, J.; Durkin, J.; Fallat, V.; Keith, D. D.; Konzelmann, F. M.; Lipschitz, E. R.; McGarry, D. H.; Siebelist, J.; Wei, C.-C.; Weigele, M.; Yang, R. Dual-Action Cephalosporins; Cephalosporin 3'-Quaternary Ammonium Quinolones. *J. Med. Chem.* **1991**, *34*, 669–675.
(22) Albrecht, H. A.; Beskid, G.; Christenson, J. G.; Georgopapadakou, N. H.; Keith, D. D.; Konzelmann, F. M.; Pruess, D. L.; Rossman, P.; Wei, C. C. Dual-Action Cephalosporins; Cephalosporin 3'-Quinolone Carbamates. *J. Med. Chem.* **1991**, *34*, 2857–2864.
(23) Beskid, G.; Albrecht, H. A.; Fallat, V.; Keith, D. D.; Lipschitz, E. R.; McGarry, C. M.; McGarry, D. H.; Rossman, P.; Siebelist. In Vitro and In Vivo Activity of Carbamate-Linked Dual-Action Antibacterial Ro 24-4383. *Chemotherapy* **1991**, *37*, 310–317.
(24) A preliminary report of this work was presented at the 31st Intersci. Conf. Antimicrob. Agents Chemother., 1991, Session 121, p 415 of Abstracts.
(25) See, also, European Patent Application EP 366 189 (Norwich-Eaton Pharmaceuticals), May 2, 1990, which discloses cephalosporin–quinolones with a variety of linkages.
(26) Bonjouklian, R.; Phillips, M. L. Reaction of TMSI with Cephalosporin Esters. *Tetrahedron Lett.* **1981**, *22*, 3915–3918.
(27) Chambers, H. F.; Hartman, B. J.; Tomasz, A. Increased Amounts of A Novel Penicillin-Binding Protein in a Strain of Methicillin-Resistant *Staphylococcus aureus* Exposed to Nafcillin. *J. Clin. Invest.* **1985**, *76*, 325–331.
(28) Georgopapadakou, N. H.; Bertasso, A. Mechanisms of Action of Cephalosporin 3'-quinolone Esters, Carbamates, and Tertiary Amines in *Escherichia coli*. *Antimicrob. Agents Chemother.* **1993**, *37*, 559–565.
(29) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard M7-A. In *National Committee for Clinical Laboratory Standards*; National Committee for Clinical Laboratory Standards: Villanova, PA, 1985.
(30) Georgopapadakou, N. H.; Liu, F. Y. Penicillin-Binding Proteins in Bacteria. *Antimicrob. Agents Chemother.* **1980**, *18*, 148–157.
(31) Reed, L. J.; Muench, H. A Simple Method of Estimating Fifty Percent End Points. *Am. J. Hyg.* **1938**, *27*, 493–497.
(32) Christenson, J.; Squires, E.; Gelger, R.; Talbot, M. Purification and Characterization of a *Proteus vulgaris* β-Lactamase That Hydrolyses Methoxyiminocephalosporins. In *Proceedings of the 12th International Congress of Chemotherapy*; Periti, P., Grassi, G. G., Eds.; American Society for Microbiology: Washington, DC, 1981; pp 748–751.
(33) Richmond, M. H.; Clark, D. C.; Wotton, S. Indirect Method for Assessing the Penetration of Beta-Lactamase-Nonsusceptible Penicillins and Cephalosporins in *Escherichia coli* Strains. *Antimicrob. Agents Chemother.* **1976**, *10*, 215–218.