portions of the membrane suspension were added to 100 µL of [^{3}H]GABA (final concentration 5 nM) and 100 µL of isoguvacine (final concentration 40 µM) followed by addition of 100 µL of the test compounds at various concentrations. Nonspecific binding, measured in the presence of 100 µM (*RS*)-baclofen, was always subtracted. The specific binding was 60 ± 4% of the total binding.

Inhibition of Synaptosomal GABA Uptake. A crude synaptosomal preparation was prepared from rat brains as described elsewhere in detail.[41] The synaptosome suspensions (500 µL) were preincubated for 10 min at 25 °C with 1.9 mL of phosphate medium containing the inhibitor. Then [^{3}H]GABA (100 µL) was added to give a final GABA concentration of 50 nM, and the incubation was continued for a further 10 min. The IC$_{50}$ values listed in Table I are the averages of at least two independent experiments in which the IC$_{50}$ values differed <10%.

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Cephalosporin 3'-Quinolone Esters with a Dual Mode of Action[1]

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According to the generally accepted mechanism by which bacterial enzymes react with cephalosporins, opening of the β-lactam ring can lead to the expulsion of a 3'-substituent. A series of dual-action cephalosporins was prepared in which antibacterial quinolones were linked to the cephalosporin 3'-position through an ester bond in the expectation that, in addition to exerting their own β-lactam activity, these cephalosporins would act as prodrugs for the second antibacterial agent. Compared to parent cephalosporins in which the 3'-substituent was acetoxy, the bifunctional cephalosporins exhibited a broadened antibacterial spectrum, suggesting that a dual mode of action may indeed be operative.

β-Lactam antibiotics exert their biological activity by acylating active-site serine residues of the transpeptidases responsible for cross-linking peptidoglycan.[2,3] Similarly, acylation of active-site serine residues by β-lactams is a key step in the mechanism by which most β-lactamases inactivate these antibiotics through hydrolysis of the β-lactam ring.[4,5] In either case, when a cephalosporin contains a potential leaving group (X) at the 3'-position, that group is eliminated (Scheme I).[6]

Published evidence suggests that opening of the β-lactam ring correlates with elimination of the nucleofugal group, although the reaction is probably not concerted.[7-13] When the leaving group possesses intrinsic antibacterial activity, the cephalosporin should exhibit a dual mode of action.[14-17] In addition to providing its own β-lactam activity, the cephalosporin should act as a targeted prodrug for the second antibacterial agent, delivering it close to its site of action. The term "dual action" has been used to describe such cephalosporins.[14] This mechanism presents an opportunity to expand the antibacterial spectrum to include oranisms which are resistant to the third-generation cephalosporins. Thus, it may be possible to design cephalosporins with significant advantages over those currently in use.

The quinolones provide a broad class of antibacterials well-suited to the role of second agent for a number of reasons.

[†] F. Hoffmann-La Roche and Company, Basle, Switzerland.

Chart I. Reference Structures
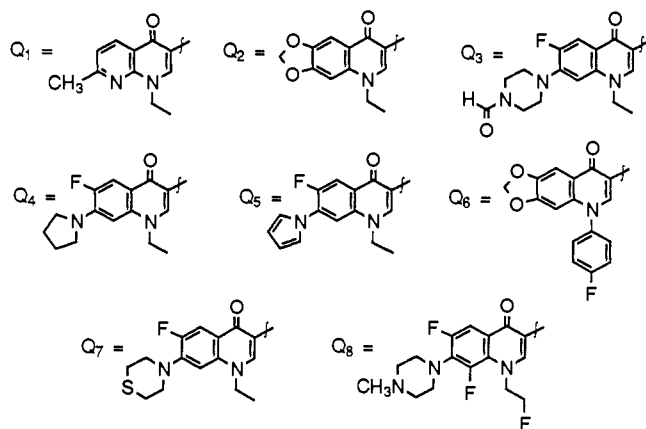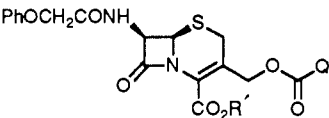
(1) The antibacterial spectra of the two classes are complementary, with quinolones being active against β-

(1) A preliminary report of this work was presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, CA, October 23–26, 1988, Abstracts 441 and 442.
(2) Frere, J. M.; Joris, B. *CRC Crit. Rev. Microbiol.* **1985**, *11*, 299.
(3) Waxman, D. J.; Strominger, J. L. *J. Biol. Chem.* **1980**, *255*, 3964.
(4) Fink, A. L. *Pharm. Res.* **1985**, *2*, 55.
(5) Knott-Hunziker, V.; Waley, S. G.; Orlek, B. S.; Sammes, P. G. *FEBS Lett.* **1979**, *99*, 59.

Table I. Intermediate Esters

no.	Q^a	R'	chromatography[b]	% yield	MS, m/z^c
20	Q_1	p-$O_2NC_6H_4CH_2$	A, EtOAc	37.0^d	714 (M + H)
21	Q_2	t-Bu	B, 4:1 EtOAc–CH_2Cl_2	59.7	664 (M + H)
22	Q_3	t-Bu	B, 20:1 CH_2Cl_2–MeOH	39.2	
23	Q_4	t-Bu	A, 4:1 EtOAc–CH_2Cl_2	49.6	706 (M + H)
24	Q_5	allyl	C, 4:1 EtOAc–CH_2Cl_2	47.0	687 (M + H)
25	Q_6	allyl	C, EtOAc	36.0	714 (M + H)
26	Q_7	allyl	A, EtOAc	45.8	723 (M + H)

^{a}See Chart I for definition of Q. ^{b}Chromatographic procedures were as follows: A, open column; B, flash; C, preparative TLC. ^{c}Mass spectrum (FAB)$^+$. ^{d}Mixture of Δ^2 and Δ^3 isomers, **20a** and **20b**, respectively.

Table II. Intermediate Esters

no.	Q^a	R''	chromatography[b]	% yield	MS, m/z^c
27	Q_1	Me	B, 9:1 EtOAc–CH_2Cl_2	70.5	925 (M + H)
28	Q_2	Me	B, 9:1 EtOAc–CH_2Cl_2	56.0	977 (M + Na)
29	Q_3	Me	B, 97:3 CH_2Cl_2–MeOH	32.8	1041 (M + H)
30	Q_4	Me	C, 4:1 EtOAc–CH_2Cl_2	46.1	988 (M + H)
31	Q_7	Me	B, 13:7 CH_2Cl_2–EtOAc	50.4	1030 (M + H)
32	Q_8	Me	B, gradient 1–8% MeOH/$CHCl_3$	41.0	1063 (M + H)
36	Q_8	CMe_2CO_2-t-Bu	B, gradient 0–10% MeOH/$CHCl_3$	58.0	
37	Q_8	CH_2CO_2-t-Bu	B, gradient 0–20% MeOH/$CHCl_3$	56.0	1163 (M + H)

$^{a-c}$See Table I for notes.

Scheme I. Mechanism of Dual Action
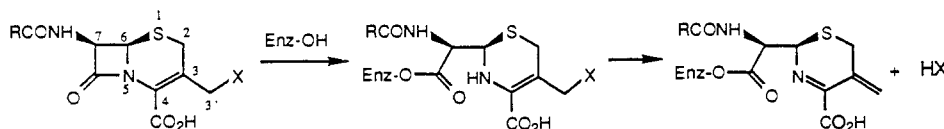
Scheme II^a
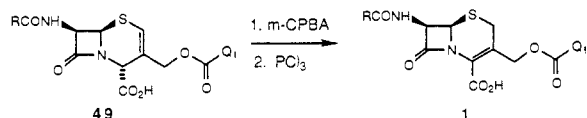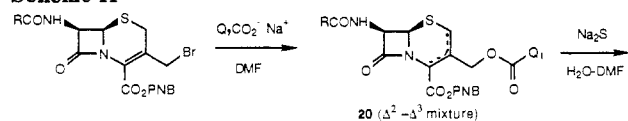
^{a}R = PhOCH$_2$, PNB = p-nitrobenzyl. For structure of Q$_1$, see Chart I.

lactam-resistant strains, while cephalosporins are more active against streptococci.

(6) For purposes of discussion, the time-honored cephem numbering system is utilized, since to do otherwise would be needlessly confusing to β-lactam chemists. Note that in the Experimental Section compounds are named according to the conventions of the Chemical Abstracts Service.

(7) Hamilton-Miller, J. M.T.; Newton, G. G. F.; Abraham, E. P. *Biochem. J.* **1970**, *116*, 371.

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(9) Faraci, W. S.; Pratt, R. F. *J. Am. Chem. Soc.* **1984**, *106*, 1489.

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Scheme III^a
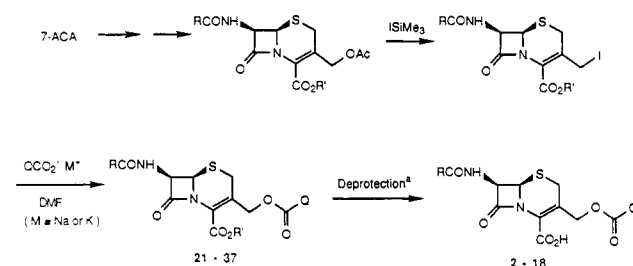
^{a}Deprotection procedures: when R' = p-nitrobenzyl, Na$_2$S in H$_2$O–DMF; when R' = t-Bu, CF$_3$CO$_2$H–anisole; when R' = allyl, Pd(0)–sodium 2-ethylhexanoate.

Scheme IV
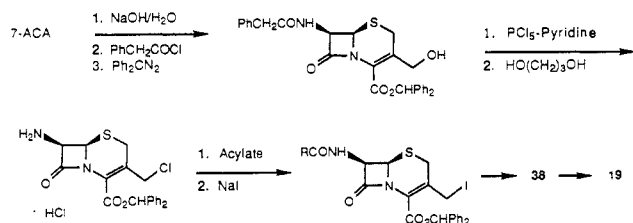
(2) The quinolones themselves are often not very soluble under physiological conditions and might benefit by in-

Table III. Intermediate Esters

no.	R	R'	Q^a	chromatography^b	% yield	MS, m/z^c
33	(thiophene)-CH$_2$-	t-Bu	Q$_2$	B, 19:1 CH$_2$Cl$_2$–MeOH	64.8	654 (M + H)
34	(thiophene)-CH$_2$-	t-Bu	Q$_3$	B, 19:1 CH$_2$Cl$_2$–MeOH	54.5	740 (M + H)
35	H–	t-Bu	Q$_8$	B, 19:1 CH$_2$Cl$_2$–MeOH	18.0	
38	EtN(CO)(CO)NCONHCH(Ph)-	CHPh$_2$	Q$_7$	A, 9:1 EtOAc–CH$_2$Cl$_2$	38.5	1173 (M + H)

$^{a-c}$ See Table I for notes.

corporation into a more soluble prodrug form which has superior pharmacokinetics and can be parenterally administered.

(3) The mechanism of action of the quinolones, which inhibit DNA gyrase, seems compatible with that of cephalosporins; i.e. there should be no antagonism.[18,19]

A series of cephalosporins has been prepared in which quinolones are linked to the 3'-position through an ester bond.

Chemistry

Cephalosporins 1–19 were prepared via the ester-protected intermediates 20–38 (Tables I–III, Chart I), according to the procedures outlined in Schemes II–IV and detailed in the Experimental Section.[20] The first bifunctional compound prepared, 1, appropriately incorporated nalidixic acid as its quinolone component and was originally prepared according to Scheme II from a 3'-bromo intermediate.[21] The displacement reaction with the sodium salt of nalidixic acid gave 20 as a Δ²/Δ³ mixture in which the desired Δ³ product was the minor component. Hydrolysis of the p-nitrobenzyl ester[22] was followed by a standard oxidation–reduction sequence, which restored the double bond to the 3-position through the intermediacy of the Δ³-sulfoxide.

Most of the new cephalosporins were prepared from 7-aminocephalosporanic acid (7-ACA) by the more efficient processes shown in Scheme III. After esterification and acylation by literature methods, the allylic acetates were cleaved by reaction with iodotrimethylsilane[23,24] to obtain

3'-iodo intermediates. The key step in this synthetic sequence was the nucleophilic displacement reaction which established the cephem–quinolone ester bond. For this reaction, the *tert*-butyl esters proved most useful, since they maintained the double bond almost exlcusively in the desired 3-position. Because salts of the quinolone acids are quite basic, base-catalyzed isomerization can occur and was observed at times (as in Scheme II) with *p*-nitrobenzyl and allyl esters.

When structural features were not compatible with the use of iodotrimethylsilane (e.g. for the synthesis of 19), the required iodo ester intermediate was obtained by an alternate procedure, (Scheme IV),[25] and utilized for the displacement reaction as in Scheme III. Deprotection of *tert*-butyl and diphenylmethyl esters was accomplished by treatment with trifluoroacetic acid–anisole, a procedure which also removed *N*-trityl groups, when present. Allyl esters were removed by Pd(0)-catalyzed transesterification.[26] After deprotection, the products were purified as sodium salts by C$_{18}$ reverse-phase chromatography.

Results and Conclusions

The bifunctional cephalosporins 1–19 (as water-soluble sodium salts), reference cephalosporins 39 and 40 (cefotaxime), and quinolones 41–48 were screened for in vitro antibacterial activity; the results are summarized in Tables IV–VIII. Compared to the reference cephalosporins 39 and 40, the cephem quinolone esters showed a broadened spectrum of in vitro antibacterial activity. As illustrated in the tables, the activity added to the spectrum of the parent cephalosporin generally paralleled the activity of the quinolone component in each case. For example, in Table IV, reference cephalosporin 39 showed significant activity only against Gram-positive organisms, while 2 displayed broad-spectrum activity, reflecting a contribution from the spectrum of 42 (oxolinic acid). This pattern of activity suggests that the quinolone carboxylic acid is being released in situ, since the quinolone ester itself would not be expected to contribute significantly in terms of quinolone activity. It is generally accepted that quinolone esters show activity only as a consequence of hydrolysis to the acid.[27] Activity of the bifunctional cephalosporins against *Staphylococcus aureus* 95 and *Enterobacter cloacae* P99

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(17) Boyd, D. B.; Lunn, W. H. W. *J. Med. Chem.* **1979**, *22*, 778.
(18) Wolfson, J. S.; Hooper, D. C. *Antimicrob. Agents Chemother.* **1985**, *28*, 581.
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(20) European Patent Application EP-251330-A, January 7, 1988.
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(22) Lammert, S. R.; Ellis, A. I.; Chauvette, R. R.; Kukolja, S. *J. Org. Chem.* **1978**, *43*, 1243.
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(25) Yamanaka, H.; Chiba, T.; Kawabata, K.; Takasugi, H.; Masugi, T.; Takaya, T. *J. Antibiot.* **1985**, *38*, 1738.
(26) Jeffrey, P. D.; McCombie, S. W. *J. Org. Chem.* **1982**, *47*, 587.
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Table IV. In Vitro Activity: MIC (μg/mL)

organisms	Q^a = CH$_3$ 39	Q = Q$_1$ 1	Q = Q$_2$ 2	Q = Q$_3$ 3	Q = Q$_4$ 4	Q = Q$_5$ 5	Q = Q$_6$ 6	Q = Q$_7$ 7
Escherichia coli ATCC 25922	128		2	8	64	8	4	1
Escherichia coli TEM-1[b]	>128		1	8	32	8	2	2
Citrobacter freundii BS-16	>128		2	16	32	32	8	8
Enterobacter cloacae P99[c]	>128	16	0.5	2	2	1	0.5	0.5
Serratia marcescens 1071	>128		1	16	32	4	1	1
Proteus vulgaris 1028 BC[d]	>128		0.25	4	32	16	64	1
Proteus mirabilis 90	64	32	4	32	32	32	32	16
Pseudomonas aeruginosa ATCC 27853	>128		>256	128	>128	>128	>128	128
Pseudomonas aeruginosa 18S/H[e]	>128		16	8	32	128	64	64
Staphylococcus aureus ATCC 29213[f]	0.5		0.25	0.5	0.5	0.25	0.5	0.125
Staphylococcus aureus 1059B[g]	0.25	0.5	0.5	1	0.25	0.125	0.5	0.25
Staphylococcus aureus 95[h]	>128		4	2	0.5	0.5	64	0.5
Streptococcus pneumoniae 6301	0.25	≤0.008			≤0.008	2	1	2
Streptococcus pyogenes 4	0.063	0.063				2	2	4
Enterococcus faecalis ATCC 29212	64		64	64	32	64	128	64

[a] See Chart I for definition of Q.   [b] TEM-1 (class IIIa) β-lactamase producer.   [c] Class Ia β-lactamase overproducer.   [d] Class Ic β-lactamase overproducer.   [e] Class Id β-lactamase overproducer.   [f] Inducible, low-level β-lactamase producer.   [g] Inducible, high-level β-lactamase producer.   [h] Methicillin-resistant, high-level β-lactamase producer.

Table V. In Vitro Activity: MIC (μg/mL)

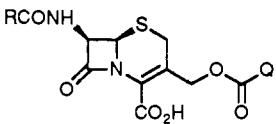| organisms	Q = CH$_3$ 40	Q = Q$_1$ 8	Q = Q$_2$ 9	Q = Q$_3$ 10	Q = Q$_4$ 11	Q = Q$_7$ 12	Q = Q$_8$ 13
E. coli ATCC 25922	0.063	1	0.5	1	2	0.5	0.125
E. coli TEM-1	0.031	0.5	0.25	0.5	0.5	0.5	0.125
C. freundii BS-16	128	32	4	32	16	8	2
E. cloacae P99	32	8	0.5	4	2	0.5	0.125
S. marcescens 1071	8	8	1	8	2	2	0.5
P. vulgaris 1028 BC	16	4	0.25	8	4	2	0.25
P. mirabilis 90	0.016	0.25	0.125	0.5	0.5	0.25	0.25
P. aeruginosa ATCC 27853	64	>128	128	>128	>128	128	8
P. aeruginosa 18S/H	128	64	16	32	32	8	4
S. aureus ATCC 29213	2	2	2	4	0.25	0.5	1
S. aureus 1059B	4	4	2	4	0.25	16	1
S. aureus 95	32	8	4	8	0.5	0.5	2
S. pneumoniae 6301	0.016				≤0.008	0.016	≤0.008
S. pyogenes 4	≤0.008				1	≤0.008	0.031
E. faecalis ATCC 29212	>128	>128	32	32	32	4	8

[a] See Chart I for definition of Q.
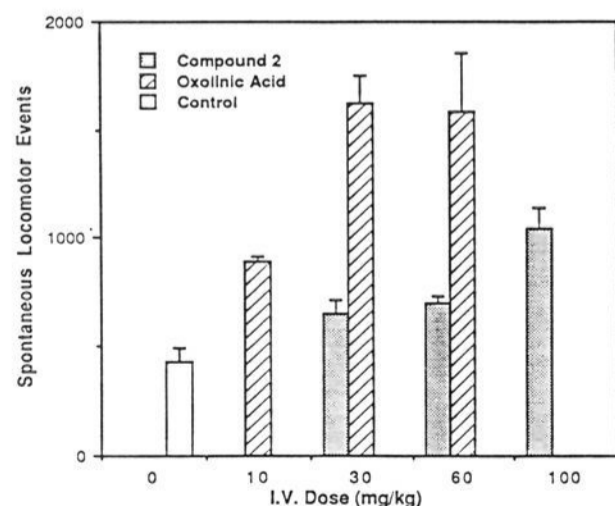
Figure 1. Effects of oxolinic acid and compound **2** on spontaneous locomotor activity in mice. Error bars represent standard error of the mean.

is noteworthy, since these strains are quite resistant to cephalosporins but susceptible to quinolones. These re-

sults lend themselves to the interpretation that a dual mode of action is operative.
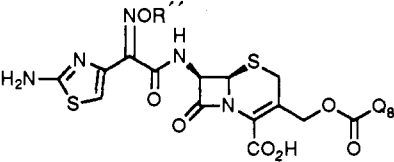
Compound **2** proved active in vivo against infections in mice with *Escherichia coli* and *Streptococcus pneumoniae* (ED$_{50}$s of 15 and 38 mg/kg sc, respectively) and showed a half-life in rats, after an iv dose of 20 mg/kg, of about 13 min, comparable to that of cefotaxime; **2** was less toxic than oxolinic acid (LD$_{50}$s of >500 and 155 mg/kg iv, respectively, in mice) and showed less potential for central nervous system (CNS) liability than oxolinic acid, which is a potent CNS stimulant. The spontaneous locomotor activity (SLA) of mice treated with oxolinic acid (30 mg/kg iv) was nearly 4 times that of the controls, while at the same dose level, SLA of mice treated with **2** increased only about 50% (Figure 1).

In their behavior toward penicillin-binding proteins (PBPs), the dual-action compounds acted like typical cephalosporins (Table IX). The major determinant of binding appeared to be the acylamino function. Com-

Table VI. In Vitro Activity: MIC (μg/mL)

organisms	R = [thiophene] $Q^a = Q_2$ 14	R = [thiophene] $Q^a = Q_3$ 15	R = H $Q^a = Q_8$ 16	R = EtN...NCONHCH $Q^a = Q_7$ 19
E. coli ATCC 25922	1	8	1	64
E. coli TEM-1	1	32	0.5	128
C. freundii BS-16	4	8	2	>128
E. cloacae P99	0.5	2	0.25	64
S. marcescens 1071	1	4	0.5	64
P. vulgaris 1028 BC	0.25	4	0.5	64
P. mirabilis 90	4	>128	32	16
P. aeruginosa ATCC 27853	>128	>128	>128	64
P. aeruginosa 18S/H	8	16	64	32
S. aureus ATCC 29213	0.5	0.5	2	1
S. aureus 1059B	1	0.5	2	0.5
S. aureus 95	4	4	32	1
S. pneumoniae 6301		0.063	0.25	0.125
S. pyogenes 4			0.25	0.016
E. faecalis ATCC 29212	32	>128	128	16

^{a} See Chart I for definition of Q.

Table VII. In Vitro Activity: MIC (μg/mL)

organisms	$R'' = CH_3$ 13	$R'' = CMe_2CO_2H$ 17	$R'' = CH_2CO_2H$ 18
E. coli ATCC 25922	0.125	0.25	0.125
E. coli TEM-1	0.125	0.5	0.25
C. freundii BS-16	2	0.5	1
E. cloacae P99	0.125	0.5	0.5
S. marcescens 1071	0.5	0.5	0.5
P. vulgaris 1028 BC	0.25	0.5	0.25
P. mirabilis 90	0.25	0.063	0.063
P. aeruginosa ATCC 27853	8	8	32
P. aeruginosa 18S/H	4	>128	4
S. aureus ATCC 29213	1	1	2
S. aureus 1059B	1	2	2
S. aureus 95	2	4	1
S. pneumoniae 6301	≤0.008	0.5	0.5
S. pyogenes 4	0.031	0.5	0.5
E. faecalis ATCC 29212	8	>128	8

pounds with the cefotaxime acyl group (**11–13** and **40**) all showed the same affinity for PBP 3 despite the variety of structures at the 3'-position. Compounds with a (phenoxyacetyl)amino substituent (**2** and **3**) more closely resembled reference cephalosporin **39** in PBP binding.

The most active bifunctional cephalosporin in the series, Ro 23-9424 (**13**), incorporates into its structure both a third-generation cephalosporin acylamino function and a 3'-ester derived from a third-generation quinolone, fleroxacin (**48**). Ro 23-9424 demonstrated excellent activity both in vitro (Table V) and in vivo (Table X) and has been selected for development as a clinical candidate.

While in vitro structure–activity relationships clearly demonstrate quinolone-like as well as cephalosporin-like activity, and thus support the dual-action hypothesis, they cannot unequivocally establish that the observed dual mode of action is due to the predicted mechanism in which the second agent is enzyme-activated. Hydrolytic instability could be a contributing factor (Tables XI and XII). Because of the labile nature of these cephalosporins, in situ hydrolysis of the allylic ester, resulting in liberation of quinolone and 3'-hydroxycephalosporin as major products, complicates mechanistic studies. Nevertheless, some data (Table XIII) suggest that the biologically active hydrolysis products alone cannot explain the observed unique properties of **13**. On a molar basis, against selected strains, **13** is more active than fleroxacin (**48**) or desacetylcefotaxime (**50**); against some organisms **13** is significantly more active than a 1:1 molar mixture of **48** and **50**. Reports on further studies with dual-action cephalosporins will be forthcoming.[28]

Experimental Section

Physical Chemistry. Infrared spectra (IR) were recorded on either a Digilab FTS 15-E or a Digilab FTS 14 spectrometer. Mass spectra (MS) were obtained on a VG7070-HF mass spectrometer in the positive-ion fast atom bombardment mode using glycerol or thioglycerol as the solvent. Proton nuclear magnetic resonance spectra (NMR) were obtained on either a Varian XL-200-FT or 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).

Chromatography. Silica gel 60 (230–400 mesh) and silica gel 60 (70–230 mesh) from Merck were used for flash[29] and open-column chromatography, respectively. Preparative TLC was performed on Merck silica gel 60 plates. Reverse-phase HPLC for purification of small (10–20 mg) samples was performed on a Waters analytical instrument using a Whatman Partisil M9 10/25 ODS-2 column. Larger scale purifications were accomplished either on a Waters Prep 500A with C_{18} silica columns, or with low-pressure (flash) columns packed with C_{18} silica from Waters.

HPLC Analyses. Stability studies (Tables XI and XII) were conducted using HPLC analysis to measure the disappearance

(28) Preliminary reports on the properties and mechanism of action of these compounds were presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, CA, October 23–26, 1988, Abstracts 442–450.

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Table VIII. In Vitro Activity of Reference Quinolones: MIC (μg/mL)

QCO₂H

organisms	$Q^a = Q_1$ 41	$Q = Q_2$ 42	$Q = Q_3$ 43	$Q = Q_4$ 44	$Q = Q_5$ 45	$Q = Q_6$ 46	$Q = Q_7$ 47	$Q = Q_8$ 48
E. coli ATCC 25922	2	0.25	0.5	0.5	0.5	0.25	0.25	0.063
E. coli TEM-1	4	0.25	0.5	1	1	0.5	0.5	0.125
C. freundii BS-16	8	0.5	>128	>128	64	32	16	1
E. cloacae P99	1	0.5	0.5	0.5	0.5	0.125	0.125	0.125
S. marcescens 1071	2	1	1	0.5	0.5	0.5	0.25	0.25
P. vulgaris 1028 BC	2	0.063	0.5	1	0.5	0.5	0.5	0.25
P. mirabilis 90	8	1	2	>128	64	32	16	0.5
P. aeruginosa ATCC 27853	128	64	16	>128				4
P. aeruginosa 18S/H	32	4	2	128	64	0.5	1	2
S. aureus ATCC 29213	32	2	1	0.125	0.125	0.5	0.063	0.5
S. aureus 1059B	32	2	1	0.063	0.125	1	0.125	0.5
S. aureus 95	8	2	1	0.063	0.125	1	0.063	0.5
S. pneumoniae 6301	128	>128	8	>128	16	64	16	4
S. pyogenes 4	128	>128	8		>128	>128	8	1
E. faecalis ATCC 29212	>128	>128	8	2	2	64	1	4

a See Chart I for definition of Q.

Table IX. Binding of Dual-Action Cephalosporin to PBP's of *E. coli* UB 1005*a*

compd	PBP 1a 90 kDa	PBP 1b 90 kDa	PBP 2 66 kDa	PBP 3 60 kDa	PBP 4 49 kDa	PBP 5/6 40 kDa	morphology*b*	MIC, μg/mL
2	0.1	30	100	10	10	>100	F	32
3	0.5	10	100	2	2	100	F	128
11	2	10	>100	0.1	10	>100	F	0.5
12	10	10	>100	0.1	100	>100	F	1
13	0.1	2	100	0.1	10	>100	F	0.12
16	0.5	100	>100	10	>100	>100	F	32
39	0.5	100	>100	10	30	>100	F	64
40	0.1	0.5	100	0.1	100	>100	F/L	0.06

a Concentration required for 90% inhibition of [¹⁴C]penicillin G binding. *b* F = filaments; L = lysis.

Table X. In Vivo Activity of Ro 23-9424, Cefotaxime, and Fleroxacin

infecting organism	mouse protection test: ED₅₀, mg/kg		
	Ro 23-9424	cefotaxime	fleroxacin
S. pyogenes 4	8.8	5.2	183
E. coli 257	1.8	1.7	0.4

Table XI. Stability of 2 in Various Media at 37 °C As Determined by HPLC Analysis

medium	half-life, h
phosphate buffer, pH 7.4	12.5
Mueller–Hinton broth	11.2
human plasma	6.0

Table XII. Stability of 13 in Various Media at 37 °C As Determined by HPLC Analysis

medium	half-life, h
phosphate buffer, pH 6.5	6.9
phosphate buffer, pH 7.4	3.0
Mueller–Hinton broth	3.4
rat serum	4.5
rat blood	5.8
dog serum	3.9
dog blood	3.4
monkey serum	5.7
human serum	6.3

Table XIII. In Vitro Activity: MIC (nmol/mL)

organism	compound			
	13*a*	48*b*	1:1 48 + 50	50*c*
Micrococcus luteus PCI	0.0746	38.2	0.597	0.597
S. pneumoniae 6301	0.0187	9.53	0.0746	0.0746
E. coli DC-O	0.299	1.19	0.299	0.597

a Ro 23-9424. *b* Fleroxacin. *c* Desacetylcefotaxime:

50
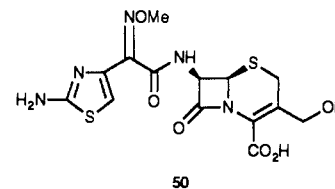
minimum inhibitory concentrations (MICs). In vivo antibacterial activity was assessed in the mouse protection test, by using procedures described in the literature.[30]

The PBP binding assay was carried out with solubilized membranes from sonicated *E. coli* UB 1005, as previously described.[31] PBP binding was measured as inhibition of [¹⁴C]-penicillin G binding (Table IX). Cell morphology was determined by microscopic examination after a 3-h incubation with the test compound.

In the spontaneous locomotor activity (SLA) test, mice were dosed iv with oxolinic acid and with 2 (15-min pretreatment time). Locomotor activity was monitored for 15 min. During the first ¹/₃ of the test session, the controls (mice treated with vehicle alone) showed their highest level of activity. These animals became gradually less active during the remainder of the test session. Thus, the final third of the test session is the most sensitive period

of starting material. Assay conditions are exemplified by those used for the analysis of 13. Solutions were incubated at 37 °C and analyzed at appropriate intervals with a Hamilton PRP-1 (250 mm × 4.1 mm) column, UV detection at 286 nm, and a mobile phase consisting of a 0.01 M solution of tetradecyltrimethyl-ammonium bromide in a mixture of 60% 0.067 M, pH 7.4, phosphate buffer and 40% acetonitrile.

Biological Assays. In vitro antibacterial screening was performed by serial 2-fold dilution in Mueller–Hinton broth according to standard methodology.[30] Results are expressed as

(30) Beskid, G.; Christenson, J. G.; Cleeland, R.; DeLorenzo, W.; Trown, P. W. *Antimicrob. Agents Chemother.* **1981**, *20*, 159.
(31) Georgopapadakou, N. H.; Liu, F. Y. *Antimicrob. Agents Chemother.* **1980**, *18*, 148.

to look for stimulant effects (Figure 1).

Mixture of (6*R-trans***)-3-[[[(1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (4-Nitrophenyl)methyl Ester (20a) and [2***R-(2α,6α,7β)***]-3-[[[(1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic Acid (4-Nitrophenyl)methyl Ester (20b).** A solution of 22.4 g (0.040 mol) of (6*R-trans*)-3-(bromomethyl)-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (4-nitrophenyl)methyl ester and 10.1 g (0.040 mol) of nalidixic acid sodium salt in 200 mL of dry DMF was stirred under argon for 5 h. The solvent was evaporated under reduced pressure. A solution of the residue in ethyl acetate was washed with brine, decolorized with charcoal, dried (Na$_2$SO$_4$), and concentrated to a low volume under reduced pressure, whereupon 3.96 g of products **20a** and **20b** crystallized. The mother liquor was purified by column chromatography on 800 g of neutral silica gel (0.063–0.200 mm, 70–230-mesh ASTM) using ethyl acetate as eluant. The appropriate fractions were combined and crystallized from ethyl acetate to obtain an additional 6.55 g of products **20a,b**; total yield 37%. The mixture of isomers was used for the subsequent reaction. Pure **20a** was obtained by fractional crystallization from ethyl acetate; pure **20b** was isolated by preparative TLC of the mother liquor on Merck PLC plates (silica gel 60F-254). Physical properties of the two isomers were as follows. **20a:** ^{1}H NMR (Me$_2$SO-d_6) δ 1.38 (t, 3 H, J = 7 Hz, Me), 2.65 (s, 3 H, Me), 3.83 (s, 2 H, CH$_2$S), 4.49 (q, 2 H, J = 7 Hz, CH$_2$), 4.63 (s, 2 H, OCH$_2$CO), 4.96, 5.19 (AB, 2 H, J_{gem} = 14 Hz, CH$_2$O), 5.21 (d, 1 H, J = 5 Hz, CH), 5.41, 5.53 (AB, 2 H, J_{gem} = 14 Hz, ArCH$_2$O), 5.84 (dd, 1 H, J = 5 and 8 Hz, CH), 6.93, 7.30 (2 m, 5 H, Ph), 7.44 (d, J = 8 Hz, 1 H, Ar), 7.72, 8.22 (2 d, 4 H, J = 8 Hz, C$_6$H$_4$NO$_2$), 8.47 (d, 1 H, J = Hz, Ar), 8.84 (s, 1 H, NCH), 9.68 (d, 1 H, J = 8Hz, NH); IR (KBr) 3405, 1785, 1735, 1697, 1637, 1520 cm^{-1}; MS m/z 736 (M + Na)$^+$, 714 (M + H)$^+$.

20b: ^{1}H NMR (Me$_2$SO-d_6) δ 1.37 (3 H, t, J = 7 Hz, Me), 2.64 (s, 3 H, ArMe), 4.46 (q, 2 H, J = Hz, CH$_2$), 4.64 (s, 2 H, OCH$_2$CO), 4.88 (s, 2 H, CH$_2$O), 5.25 (d, 1 H, J = 5 Hz, CH), 5.34 (s, 2 H, OCH$_2$Ar), 5.49 (s, 1 H, CH), 5.53 (dd, 1 H, J = 5 and 8 Hz, CH), 6.94 (s, 1 H, SCH=C), 6.95, 7.30 (2 m, 5 H, Ph), 7.43 (d, 1 H, J = 8 Hz, Ar), 7.62, 8.13 (2 d, 4 H, J = 8 Hz, C$_6$H$_4$NO$_2$), 8.46 (d, 1 H, J = 8 Hz, Ar), 8.78 (s, 1 H, NCH), 9.24 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3560, 3480, 3415, 1780, 1745, 1693, 1620, 1520 cm^{-1}; MS m/z 713 (M$^+$).

[2*R-(2α,6α,7β)***]-3-[[[(1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic Acid (49).** A solution of 2.80 g (0.0116 mol) of sodium sulfide hydrate in 20 mL of water was added dropwise to a solution of 6.11 g (0.00856 mol) of a mixture of **20a,b** in 45 mL of DMF, at –5 to –10 °C. After 35 min, the mixture was acidified to pH 3.5 by addition of 1 N HCl, to precipitate a gum. Upon addition of 50 mL of ethyl acetate and 50 mL of ether, the gum solidified. After filtration, washing with water and ether, and drying at 50 °C under reduced pressure over P$_2$O$_5$, 2.22 g of product was obtained. Addition of more ether to the filtrate produced additional precipitate, which was filtered and dissolved in aqueous NaHCO$_3$. The aqueous solution was washed with ethyl acetate and then acidified to pH 3.5. A solution of the precipitated gum in methylene chloride was filtered, washed with water, dried (Na$_2$SO$_4$), and concentrated under reduced pressure to obtain an additional 0.776 g of product; the total yield was 2.99 g (60.4%): ^{1}H NMR (Me$_2$SO-d_6) δ 1.39 (t, 3 H, J = 7 Hz, Me), 2.65 (s, 3 H, ArMe), 4.50 (q, 2 H, J = 7 Hz, CH$_2$), 4.62 (s, 2 H, OCH$_2$CO), 4.89 (s, 2 H, CH$_2$O), 5.10 (s, 1 H, CH), 5.11 (d, 1 H, J = 5 Hz, CH), 5.48 (AB, 1 H, J = 5 and 8 Hz, CH), 6.84 (s, 1 H, SCH=C), 6.94, 7.30 (2 m, 5 H, Ph), 7.44 (d, 1 H, J = 8 Hz, Ar), 8.48 (d, 1 H, J = 8 Hz, Ar), 8.84 (s, H, NCH), 9.20 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3420, 3300, 1773, 1720 cm^{-1}; MS m/z 579 (M + H)$^+$.

(6*R-trans***)-3-[[[(1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid S-Oxide.** A solution of 0.986 g (85% pure, 0.00486 mol) of *m*-chloroperbenzoic acid in 15 mL of methylene

chloride was added dropwise to a stirred suspension of 2.55 g (0.00442 mol) of **49** in 60 mL of methylene chloride at 0 °C. The mixture was stirred for 4 h at 0 °C, and filtered. The solid was washed with methylene chloride, and dried under reduced pressure to obtain 1.97 g (75%) of the sulfoxide: ^{1}H NMR (Me$_2$SO-d_6) δ 1.39 (t, 3 H, J = 7 Hz, Me), 2.65 (s, 3 H, ArMe), 3.77, 4.16 (AB, 2 H, J_{gem} = 18 Hz, CH$_2$S), 4.51 (q, 2 H, J = 7 Hz, CH$_2$), 4.69 (s, 2 H, OCH$_2$CO), 4.84, 5.43 (AB, 2 H, J_{gem} = 14 Hz, CH$_2$O), 5.00 (d, 1 H, J = 5 Hz, CH), 6.05 (dd, 1 H, J = 5 and 10 Hz, CH), 6.98, 7.33 (2 m, 5 H, Ph), 7.44 (d, 1 H, J = 8 Hz, Ar), 8.17 (d, 1 H, J = 10 Hz, NH), 8.47 (d, 1 H, J = 8 Hz, Ar), 8.85 (s, 1 H, NCH); IR (KBr) 3360, 1794, 1723, 1684, 1637 cm^{-1}; MS m/z 595 (M + H)$^+$.

(6*R-trans***)-3-[[[(1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Hydrate (1).** To a solution of 1.64 g (0.0109 mol) of sodium iodide in 40 mL of dry acetone and 20 mL of methylene chloride was added 1.30 g (0.00219 mol) of the above sulfoxide; the stirred suspension was cooled and 1.75 mL (0.0124 mol) of trifluoroacetic anhydride was added at 0 °C. After 30 min, aqueous NaHCO$_3$ was added, until the pH = 6.0. Then 1 N HCl was added until the pH = 3.5, and a small insoluble portion was removed by filtration. The organic phase was washed with aqueous sodium sulfite, dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residual solid was washed with ether, and reprecipitated from methylene chloride solution by addition of ether to obtain 0.51 g (40% yield) of product 1.

Alternatively, a mixture of 0.435 g (0.73 mmol) of sulfoxide and 7 mL of dry DMF was stirred and cooled to –12 °C, and 0.128 mL (1.4 mmol) of phosphorus trichloride was added. After 7 min, an additional 0.027 mL of phosphorus trichloride was added. The mixture was stirred for 6.5 min. A cold solution of 0.428 g (5.1 mmol) of sodium bicarbonate in 70 mL of water was then added. The precipitate was filtered, washed with water, and dried under reduced pressure over P$_2$O$_5$ to obtain 0.251 g of product 1. A second crop of solid separated from the filtrate to provide an additional 0.037 g of 1: ^{1}H NMR (Me$_2$SO-d_6) δ 1.38 (t, 3 H, J = 7 Hz, Me), 2.65 (s, 3 H, ArMe), 3.72, 3.82 (AB, 2 H, J_{gem} = 19 Hz, CH$_2$S), 4.51 (q, 2 H, J = 7 Hz, CH$_2$), 4.64 (s, 2 H, OCH$_2$CO), 4.96, 5.20 (AB, 2 H, J_{gem} = 14 Hz, CH$_2$O), 5.16 (d, 1 H, J = 5 Hz, CH), 5.76 (dd, 1 H, J = 5 and 8 Hz, CH), 6.94, 7.30 (2 m, 5 H, Ph), 7.44 (d, 1 H, J = 8 Hz, Ar), 8.48 (d, 1 H, J = 8 Hz, Ar), 8.84 (s, 1 H, NCH), 9.13 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3420, 1785, 1708, 1620 cm^{-1}; MS m/z 579 (M + H)$^+$; Anal. (C$_{28}$H$_{26}$N$_4$O$_8$S·H$_2$O) C, H, N.

General Procedures for Intermediate Esters (21–31, 33–38). A solution of 1 mmol of the sodium or potassium salt of the quinolone (41–48) in 12 mL of DMF was dried by stirring under nitrogen with 1.5 g of 4A molecular sieves for 1 h, and then combined with a solution of 1 mmol of the appropriate 3'-iodo-cephalosporin ester in 6 mL of dry DMF. After stirring for a reaction period of from 2 to 6 h, the mixture was concentrated to dryness under reduced pressure. A solution of the residue in ethyl acetate or ethyl acetate–methylene chloride was washed with aqueous NaHCO$_3$ and brine, dried (Na$_2$SO$_4$), and concentrated to dryness under reduced pressure. The residue was purified chromatographically as indicated (Tables I–III).

General Procedure for Deprotection of *tert*-Butyl and Diphenylmethyl Esters. A suspension of 0.31 mmol of the intermediate *tert*-butyl or diphenylmethyl ester in 0.66 mL of anisole under a nitrogen atmosphere was cooled in ice, and 3.3 mL of trifluoroacetic acid was added. In the event that complete solution did not immediately result, methylene chloride was added to obtain a clear solution. The reaction was kept at 0 °C and monitored by TLC to determine the optimum reaction time. After a period of from 3 to 18 h, the solution was concentrated to dryness under reduced pressure. Methylene chloride was added to the residue, and evaporation was repeated. The residue was then triturated with ethyl acetate to obtain a solid, if possible, before conversion to the sodium salt. If the precipitate did not solidify on trituration, the ethyl acetate was decanted and the residue converted directly to the sodium salt. In either case, a solution of the solid or the residual oil in 9 mL of methylene chloride was added with ice cooling to 9 mL of water containing sufficient NaHCO$_3$ to maintain a final pH of 7.2–7.4. The residue obtained,

after freeze-drying of the aqueous phase, was purified by reverse-phase HPLC using either a water–methanol or a water–acetonitrile gradient.

General Procedure for Deprotection of Allyl Esters. To a stirred solution of 0.50 mmol of the intermediate allyl ester, 0.03 mL (0.175 mmol) of triethyl phosphite, and 5.6 mg (0.025 mmol) of palladium(II) acetate in 3.9 mL of ethyl acetate and 5.6 mL of methylene chloride was added dropwise 1.68 mL of a 0.5 M solution of sodium 2-ethylhexanoate in ethyl acetate. The mixture was stirred for 30 min; 11 mL of acetone was added, and the stirring was continued for another 10 min. The product, as a sodium salt, was obtained by filtration and purified by reverse-phase HPLC.

Dual-Action Cephalosporins 2–12, 14–19. The products obtained by the above general procedures are as follows.

(**6R**-*trans*)-3-[[[(5-Ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-*g*]quinolin-7-yl)carbonyl]oxy]methyl]-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (2): ^{1}H NMR (Me$_2$SO-d_6) δ 1.34 (t, 3 H, J = 7 Hz, Me), 3.43, 3.65 (AB, 2 H, J_{gem} = 18 Hz, CH$_2$S), 4.38 (q, 2 H, J = 7 Hz, CH$_2$N), 4.63 (s, 2 H, CH$_2$O), 4.96, 5.15 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 4.99 (d, 1 H, J = 5 Hz, CH), 5.55 (dd, 1 H, J = 5 and 8 Hz, CH), 6.22 (s, 2 H, OCH$_2$O), 6.97, 7.30 (2 m, 5 H, Ph), 7.45 (s, 1 H, Ar), 7.59 (s, 1 H, Ar), 8.58 (s, 1 H, NCH), 8.97 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3410, 1765, 1692, 1635, 1528 cm^{-1}; MS m/z 630 (M + H)$^+$, 652 (M + Na)$^+$.

(**6R**-*trans*)-3-[[[[1-Ethyl-6-fluoro-1,4-dihydro-7-(4-formyl-1-piperazinyl)-4-oxoquinolin-3-yl]carbonyl]oxy]methyl]-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (3): ^{1}H (Me$_2$SO-d_6) δ 1.38 (t, 3 H, J = 7 Hz, Me) 3.2–3.6 (m, partly obscured by water peak, piperazine and CH$_2$S), 4.43 (q, 2 H, J = 7 Hz, CH$_2$), 4.64 (s, 2 H, CH$_2$O), 4.93, 5.13 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 4.99 (d, 1 H, J = 5 Hz, CH), 5.55 (dd, 1 H, J = 5 and 8 Hz, CH), 6.95, 7.29 (2 m, 5 , Ph), 7.10 (d, 1 H, J = 8 Hz, Ar), 7.85 (d, 1 H, J = 14 Hz, Ar), 8.11 (s, 1 H, NCHO), 8.66 (s, 1 H, NCH), 8.96 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3420, 1768, 1668, 1620 cm^{-1}; MS m/z 738 (M + Na)$^+$, 716 (M + H)$^+$.

(**6R**-*trans*)-3-[[[[1-Ethyl-6-fluoro-1,4-dihydro-7-(1-pyrrolidinyl)-4-oxoquinolin-3-yl]carbonyl]oxy]methyl]-7-[(phenoxyacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid (4): ^{1}H NMR (Me$_2$SO-d_6) δ 1.38 (t, 3 H, J = 7 Hz, Me), 1.95, 2.30 (2 m, 4 H, CH$_2$CH$_2$), 3.35 (m, 4 H, CH$_2$NCH$_2$), 3.41, 3.61 (AB, 2 H, J_{gem} = 17 Hz, SCH$_2$), 4.38 (q, 2 H, J = 7 Hz, CH$_2$), 4.60, 4.66 (AB, 2 H, J_{gem} = 16 Hz. CH$_2$O), 4.93, 5.09 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 5.01 (d, 1 H, J = 5 Hz, CH), 5.54 (dd, 1 H, J = 5 and 8 Hz, CH), 6.58 (d, 1 H, J = 8 Hz, Ar) 6.95, 7.30 (2 m, 5 H, Ph), 7.74 (d, 1 H, J = 15 Hz, Ar), 8.54 (s, 1 H, NCH), 8.96 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3410, 1770, 1695, 1628 cm^{-1}; MS m/z 695 (M + Na)$^+$, 673 (M + H)$^+$.

(**6R**-*trans*)-3-[[[[1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1*H*-pyrrol-1-yl)quinolin-3-yl]carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (5): NMR (Me$_2$SO-d_6) δ 1.38 (t, 3 H. J = 7 Hz, Me), 3.41, 3.64 (AB, 2 H, J_{gem} = 18 Hz, SCH$_2$), 4.50 (q, 2 H, J = 7 Hz, CH$_2$), 4.59, 4.65 (AB, 2 H, J_{gem} = 11 Hz, CH$_2$O), 4.94, 5.15 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 4.96 (d, 1 H, J = 5 Hz, CH), 5.54 (dd, 1 H, J = 5 and 8 Hz, CH), 6.38 (m, 2 H, Ar), 6.95, 7.30 (2 m, 5 H, Ph), 7.38 (m, 2 H, Ar), 7.88 (d, 1 H, J = 8 Hz, Ar), 8.10 (d, 1 H, J = 13 Hz, Ar), 8.74 (s, 1 H, NCH), 8.92 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3720, 1765, 1695, 1620 cm^{-1}; MS m/z 609 (M + H)$^+$.

(**6R**-*trans*)-3-[[[[5-(4-Fluorophenyl)-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-*g*]quinolin-7-yl]carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid sodium salt (6): ^{1}H NMR (Me$_2$SO-d_6) δ 3.35, 3.56 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$S), 4.57, 4.64 (AB, 2 H, J_{gem} = 16 Hz, CH$_2$O), 4.95, 5.15 (AB, 2 H, J_{gem} = 13 Hz, CH$_2$O), 4.97 (d, 1 H, J = 5 Hz, CH), 6.18 (s, 2 H, OCH$_2$O), 6.36 (s, 1 H, Ar), 6.95 (m, 3 H, Ph), 7.27 (m, 2 H, Ph), 7.48 (m, 2 H, Ar), 7.62 (s, 1 H, Ar), 7.70 (m, 2 H, Ar), 8.31 (s, 1 H, NCH), 8.93 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3420, 1765, 1695, 1632 cm^{-1}; MS m/z 696 (M + H)$^+$.

(**6R**-*trans*)-3-[[[(1-Ethyl-6-fluoro-1,4-dihydro-7-(4-thiomorpholinyl)-4-oxoquinolin-3-yl]carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]-

oct-2-ene-2-carboxylic acid sodium salt (7): ^{1}H NMR (Me$_2$SO-d_6) δ 1.38 (t, 3 H, J = 7 Hz, Me), 2.77 (m, 4 H, CH$_2$SCH$_2$), 3.37, 3.59 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$S), 3.48, (m, 4 H, CH$_2$NCH$_2$), 4.42 (q, 2 H, J = 7 Hz, CH$_2$), 4.57, 4.63 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 4.92, 5.12 (AB, 2 H, J_{gem} = 14 Hz, CH$_2$O), 4.96 (d, 1 H, J = 5 Hz, CH), 5.54 (dd, 1 H, J = 5 and 8 Hz, CH), 6.95, 7.30 (2 m, 5 H, Ph), 7.09 (d, 1 H, J = 8 Hz, Ar), 7.81 (d, 1 H, J = 16 Hz, Ar), 8.63 (s, 1 H, NCH), 8.93 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3420, 1768, 1695, 1622 cm^{-1}; MS m/z 727 (M + Na)$^+$, 705 (M + H)$^+$.

[**6R**-[6α,7β(**Z**)]]-7-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[[(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (8): ^{1}H NMR (Me$_2$SO-d_6) δ 1.38 (t, 3 H, J = 7 Hz, Me), 2.64 (s, 3 H, Me), 3.35, 3.63 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$S), 3.85 (s, 3 H, MeO), 4.50 (q, 2 H, J = 7 Hz, CH$_2$), 4.96, 5.15 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 5.00 (d, 1 H, J = 5 Hz, CH), 5.59 (dd, 1 H, J = 5 and 8 Hz, CH), 6.74 (s, 1 H, Ar), 7.23 (s, 2 H, NH$_2$), 7.43 (d, 1 H, J = 8 Hz, Ar), 8.48 (d, 1 H, J = 8 Hz, Ar), 8.83 (s, 1 H, NCH), 9.55 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3405, 1766, 1716, 1681, 1617 cm^{-1}.

[**6R**-[6α,7β(**Z**)]]-7-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[[(5-ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-*g*]quinolin-7-yl)carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (9): ^{1}H NMR (Me$_2$SO-d_6) δ 1.35 (t, 3 H, J = 7 Hz, Me), 3.38, 3.60 (AB, 2 H, J_{gem} = 18 Hz, CH$_2$S), 3.85 (s, 3 H, MeO), 4.37 (q, 2 H, J = 7 Hz, CH$_2$), 4.91, 5.09 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 5.00 (d, 1 H, J = 5 Hz, CH), 5.58 (dd, 1 H, J = 5 and 8 Hz, CH), 6.21 (s, 2 H, OCH$_2$O), 6.75 (s, 1 H, Ar), 7.23 (s, 2 H, NH$_2$), 7.44 (s, 1 H, Ar), 7.68 (s, 1 H, Ar), 8.58 (s, 1 H, NCH), 9.55 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3400, 1767, 1715, 1685, 1635, 1616, 1533 cm^{-1}; MS m/z 701 (M + Na)$^+$, 679 (M + H)$^+$.

[**6R**-[6α,7β(**Z**)]]-7-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[[[1-ethyl-6-fluoro-1,4-dihydro-7-(4-formyl-1-piperazinyl)-4-oxoquinolin-3-yl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (10): ^{1}H NMR (Me$_2$SO-d_6) δ 1.38 (t, 3 H, J = 7 Hz, Me), 3.25–3.45 (m, includes CH$_2$S, CH$_2$NCH$_2$, and water peak), 3.60 (m, 4 H, CH$_2$NCH$_2$), 3.86 (s, 3 H, MeO), 4.34 (q, 2 H, J = 7 Hz, CH$_2$), 4.92, 5.08 (AB, 2 H, J_{gem} = 14 Hz, CH$_2$O), 4.98 (d, 1 H, J = 5 Hz, CH), 5.56 (dd, 1 H, J = 5 and 8 Hz, CH), 6.76 (s, 1H, Ar), 7.10 (d, 1H, J = 8 Hz, Ar), 7.22 (s, 2 H, NH$_2$), 7.86 (d, 1 H, J = 13 Hz, Ar), 8.12 (s, 1 H, NCHO), 8.66 (s, 1 H, NCH), 9.54 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3420, 1765, 1712, 1622 cm^{-1}; MS m/z 764 (M + H)$^+$.

[**6R**-[6α,7β(**Z**)]]-7-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[[[1-ethyl-6-fluoro-1,4-dihydro-7-(1-pyrrolidinyl)-4-oxoquinolin-3-yl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (11): ^{1}H NMR (Me$_2$SO-d_6) δ 1.39 (t, 3 H, J = 7 Hz, Me), 1.98 (m, 4 H, CH$_2$CH$_2$), 3.36, 3.67 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$S), 3.57 (m, 4 H, CH$_2$NCH$_2$), 3.88 (s, 3 H, MeO), 4.39 (q, 2 H, J = 7 Hz, CH$_2$), 4.95, 5.06 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 4.98 (d, 1 H, J = 5 Hz, CH), 5.60 (dd, 1 H, J = 5 and 8 Hz, CH), 6.59 (d, 1 H, J = 8 Hz, Ar), 6.77 (s, 1 H, Ar), 7.20 (s, 2 H, NH$_2$), 7.72 (d, 1 H, J = 15 Hz, Ar), 8.54 (s, 1 H, NCH), 9.57 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3430, 1768, 1682, 1630 cm^{-1}; MS m/z 722 (M + H)$^+$.

[**6R**-[6α,7β(**Z**)]]-7-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[[[1-ethyl-6-fluoro-1,4-dihydro-7-(4-thiomorpholinyl)-4-oxoquinolin-3-yl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (12): ^{1}H NMR (Me$_2$SO-d_6) δ 1.38 (t, 3 H, J = 7 Hz, Me), 2.78, 3.48 (2 m, 8 H, thiomorpholine), 3.36, 3.58 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$S), 3.84 (s, 3 H, MeO), 4.41 (q, 2 H, J = 7 Hz, CH$_2$), 4.90, 5.08 (AB, 2 H, J_{gem} = 14 Hz, CH$_2$O), 4.98 (d, 1 H, J = 5 Hz, CH), 5.57 (dd, 1 H, J = 5 and 8 Hz, CH), 6.75 (s, 1 H, Ar), 7.09 (d, 1 H, J = 8 Hz, Ar); 7.22 (s, 2 H, NH$_2$), 7.81 (d, 1 H, J = 16 Hz, Ar), 8.63 (s, 1 H, NCH), 9.52 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3410, 1768, 1715, 1682, 1622, cm^{-1}; MS m/z 754 (M + H)$^+$.

(**6R**-*trans*)-3-[[[(5-Ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-*g*]quinolin-7-yl)carbonyl]oxy]methyl]-8-oxo-7-[(2-thienylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-

carboxylic acid sodium salt (14): ^{1}H NMR (Me$_2$SO-d_6) δ 1.33 (t, 3 H, J = 7 Hz, Me), 3.38, 3.61 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$S), 3.78 (s, 2 H, CH$_2$Ar), 4.38 (q, 2 H, J = 7 Hz, CH$_2$), 4.94, 5.13 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 4.96 (d, 1 H, J = 5 Hz, CH), 5.48 (dd, 1 H, J = 5 and 8 Hz, CH), 6.21 (s, 2 H, OCH$_2$O), 6.93, 7.36 (2 m, 3 H, thiophene), 7.42 (s, 1 H, Ar), 7.57 (s, 1 H, Ar), 8.58 (s, 1 H, NCH), 9.02 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3410, 1763, 1682, 1632, 1608 cm^{-1}; MS m/z 620 (M + H)$^+$.

[6R-(6α,7β)]-3-[[[[1-Ethyl-6-fluoro-1,4-dihydro-7-(4-formyl-1-piperazinyl)-4-oxoquinolin-3-yl]carbonyl]oxy]-methyl]-7-[(2-thienylacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (15): ^{1}H NMR (Me$_2$SO-d_6) δ 1.38 (t, 3 H, J = 7 Hz, Me), 3.2–3.5 (m, includes CH$_2$S, CH$_2$NCH$_2$, and water peak), 3.60 (m, 4 H, CH$_2$NCH$_2$), 3.78 (s, 2 H, CH$_2$Ar), 4.45 (q, 2 H, J = 7 Hz, CH$_2$), 4.94, 5.13 (AB, 2 H, J_{gem} = 14 Hz, CH$_2$O), 4.99 (d, 1 H, J = 5 Hz, CH), 5.43 (dd, 1 H, J = 5 and 8 Hz, CH), 6.96, 7.38 (2 m, 3 H, thiophene), 7.10 (d, 1 H, J = 8 Hz, Ar), 7.86 (d, 1 H, J = 12 Hz, Ar), 8.12 (s, 1 H, NCHO), 8.66 (s, 1 H, NCH), 9.05 (d, 1 H J = 8 Hz, NH); IR (KBr) 3430, 1765, 1715, 1662, 1623 cm^{-1}; MS m/z 706 (M + H)$^+$.

(6R-*trans*)-7-(Formylamino)-3-[[[[6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinyl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid sodium salt (16): ^{1}H NMR (Me$_2$SO-d_6) δ 2.26 (s, 3 H, MeN), 2.4–2.6 (m, CH$_2$NCH$_2$ and DMSO), 3.25–3.45 (m, CH$_2$NCH$_2$ and water peak), 3.49, 3.67 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$S), 4.74–4.88 (m, 4 H, CH$_2$CH$_2$F), 4.96, 5.19 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 5.06 (d, 1 H, J = 5 Hz, CH), 5.67 (dd, 1 H, J = 5 and 8 Hz, CH), 7.77 (d, 1 H, J = 16 Hz, Ar), 8.16 (s, 1 H, CHO), 8.53 (s, 1 H, NCH), 9.01 (d, 1 H, J = 8 Hz NH); IR (KBr) 3425, 1768, 1712, 1682, 1612 cm^{-1}; MS m/z 632 (M + H)$^+$.

[6R-[6α,7β(Z)]]-7-[[(2-Amino-4-thiazolyl)[[2-(2-carboxy-2-methyl)ethoxy]imino]acetyl]amino]-3-[[[[6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinyl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid disodium salt (17): ^{1}H NMR (Me$_2$SO-d_6) δ 1.37 (s, 3 H, Me), 1.47 (s, 3 H, Me), 2.24 (s, 3 H, MeN), 2.46, 3.29 (2 m, 8 H, piperazine), 3.27, 3.56 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$S), 4.74–4.90 (m, 4 H, CH$_2$CH$_2$F), 4.91, 5.14 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 4.99 (d, 1 H, J = 5 Hz, CH), 5.66 (dd, 1 H, J = 5 and 8 Hz, CH), 6.72 (s, 1 H, Ar), 7.17 (s, 2 H, NH$_2$), 7.77 (d, 1 H, J = 12 Hz, Ar), 8.56 (s, 1 H, NCH), 11.99 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1762, 1712, 1670, 1612 cm^{-1}; MS m/z 881 (M + H)$^+$.

[6R-[6α,7β(Z)]]-7-[[(2-Amino-4-thiazolyl)[(carboxymethoxy)imino]acetyl]amino]-3-[[[[6,8-difluoro-1-(2-fluroro-ethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinyl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid disodium salt (18): ^{1}H NMR (Me$_2$SO-d_6) δ 2.24 (s, 3 H, MeN), 2.45, 3.30 (2 m, 8 H, piperazine), 3.58 (half of AB, 2 H, J_{gem} = 17 Hz, CH$_2$S, other half of AB obscured by water peak), 4.26 (s, 2 H, OCH$_2$CO), 4.85–4.92 (m, 4 H, CH$_2$CH$_2$F), 4.94, 5.15 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 4.99 (d, 1 H, J = 5 Hz, CH), 6.63 (dd, 1 H, J = 5 and 8 Hz, CH), 6.84 (s, 1 H, Ar), 7.19 (s, 2 H, NH$_2$), 7.77 (d, 1 H, J = 12 Hz, Ar), 8.56 (s, 1 H, NCH), 11.84 (br s, 1 H, NH); IR (KBr) 3410, 1762, 1718, 1665, 1612 cm^{-1}; MS m/z 853 (M + H)$^+$.

[6R-[6α,7β(R*)]]-7-[[2-[[(4-Ethyl-2,3-dioxopiperazinyl)-carbonyl]amino]-2-phenylacetyl]amino]-3-[[[[1-ethyl-6-fluoro-1,4-dihydro-7-(4-thiomorpholinyl)-4-oxoquinolin-3-yl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid sodium salt (19): ^{1}H NMR (Me$_2$SO-d_6) δ 1.07 (t, 3 H, J = 7 Hz, Me), 1.36 (t, 3 H, J = 7 Hz, Me), 2.78 (m, 4 H, CH$_2$SCH$_2$), 3.2–3.6 (m, 10 H, CH$_2$S and 4 × CH$_2$N), 3.91 (q, 2 H, J = 7 Hz, CH$_2$), 4.33 (q, 2 H, J = 7 Hz, CH$_2$), 4.88 (d, 1 H, J = 5 Hz, CH), 4.92, 5.03 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$O), 5.49 (dd, 1 H, J = 5 and 8 Hz, CH), 5.65 (d, 1 H, J = 8 Hz, CH), 7.06 (d, 1 H, J = 8 Hz, Ar), 7.25–7.45 (m, 5 H, Ph), 7.78 (d, 1 H, J = 14 Hz, Ar), 8.60 (s, 1 H, NCH), 9.37 (d, 1 H, J = 8 Hz, NH), 9.84 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3420, 1762, 1712, 1682, 1615 cm^{-1}; MS m/z 872 (M + H)$^+$.

[6R-[6α,7β(Z)]]-7-[[(2-Amino-4-thiazolyl)(methoxy-imino)acetyl]amino]-3-[[[[6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinyl]-

carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Monosodium Salt Dihydrate (13, Ro 23-9424/001). A solution of 10.0 g (0.0123 mol) of [6R-[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 in 40 mL of DMF was added to a suspension of 5.00 g (0.0123 mol) of 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid potassium salt in 40 mL of DMF over a 5-min period. The mixture was stirred for 2 h and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and aqueous sodium bicarbonate. The organic phase was washed with aqueous sodium bicarbonate and brine, dried (MgSO$_4$), and concentrated to give 16.8 g of a brown foam. Purification by flash chromatography on 500 g of silica gel (230–400 mesh) using methanol–chloroform (gradient from 0 to 8% methanol) provided 5.42 g (41.0%) of intermediate ester **32**.

To a solution of 2.30 g (0.00216 mol) of this intermediate in 25 mL of methylene chloride at 0 °C were added 2 mL of anisole, 0.2 mL of 1,2-ethanedithiol, and 25 mL of trifluoroacetic acid. The mixture was stirred at 0 °C for 3.5 h and then concentrated under reduced pressure, at 0 °C. Methylene chloride was added to the residue, and the evaporation was repeated. To the residue was added 5 mL of cold ethyl acetate, followed by 25 mL of ether, precipitating a solid. After filtering, washing with ether, and air-drying, 3.1 g of tan solid was obtained. This solid was dissolved in 100 mL of 1:4 methanol–chloroform, and with ice cooling, 100 mL of water and 35 mL of 5% aqueous sodium bicarbonate were added, adjusting the pH to 7.8. The gum which separated during this operation was dissolved in 10 mL of DMF; 20 mL of chloroform was added, followed by water and aqueous bicarbonate until the pH = 7.8. The combined aqueous extracts containing the product were washed with chloroform and purified by reverse-phase chromatography on C$_{18}$ silica (Waters Associates), using a water–acetonitrile gradient. The appropriate fractions were concentrated under reduced pressure and lyophilized to yield 0.65 g (37.0%) of **13**: ^{1}H NMR (Me$_2$SO-d_6) δ 2.22 (s, 3 H, MeN), 2.43, 3.29 (2 m, 8 H, piperazine), 3.39, 3.58 (AB, 2 H, J_{gem} = 17 Hz, CH$_2$S), 3.84 (s, 3 H, MeO), 4.73–4.89 (m, 4 H, CH$_2$CH$_2$F), 4.94, 5.09 (AB, 2 H, J_{gem} = 12 Hz, CH$_2$O), 4.98 (d, 1 H, J = 4.5 Hz, CH), 5.58 (dd, 1 H, J = 4.5 and 8 Hz, CH), 6.74 (s, 1 H, Ar), 7.21 (s, 2 H, NH$_2$), 7.74 (d, 1 H, J = 12 Hz, Ar), 8.53 (s, 1 H, NCH), 9.53 (d, 1 H, J = 8 Hz, NH); IR (KBr) 3420, 1765, 1720, 1680, 1618 cm^{-1}; MS m/z 787 (M + H)$^+$. Anal. (C$_{31}$H$_{30}$F$_3$N$_8$NaO$_8$S$_2$·2H$_2$O) C, H, N, Na, S.

Registry No. 1, 115622-64-5; 2, 115622-46-3; 2·Na, 115699-45-1; 3, 115622-49-6; 3·Na, 115712-77-1; 4, 115622-52-1; 5, 115622-56-5; 5·Na, 122949-53-5; 6, 115622-57-6; 6·Na, 115699-51-9; 7, 115622-55-4; 7·Na, 115699-55-3; 8, 115622-47-4; 8·Na, 115699-32-6; 9, 115622-48-5; 9·Na, 115699-43-9; 10, 115622-54-3; 10·Na, 115699-39-3; 11, 115622-53-2; 11·Na, 115699-40-6; 12, 115653-74-2; 12·Na, 115699-49-5; 13, 115622-58-7; 13·Na, 115699-46-2; 14, 115622-50-9; 14·Na, 115699-37-1; 15, 122949-30-8; 15·Na, 115699-38-2; 16, 122949-31-9; 16·Na, 122967-64-0; 17, 122949-32-0; 17·2Na, 122949-54-6; 18, 122949-33-1; 18·2Na, 122949-55-7; 19, 122949-34-2; 19·Na, 122949-56-8; 20a, 122949-35-3; 20b, 122949-45-5; 21, 115622-65-6; 22, 115622-69-0; 23, 122949-36-4; 24, 122949-37-5; 25, 115622-79-2; 26, 115622-77-0; 27, 115683-76-4; 28, 122949-38-6; 29, 115622-70-3; 30, 115622-71-4; 31, 115622-80-5; 32, 122949-39-7; 33, 122967-63-9; 34, 122949-40-0; 35, 122949-41-1; 36, 122949-42-2; 37, 122949-43-3; 38, 122949-44-4; 41, 389-08-2; 41·Na, 3374-05-8; 42, 14698-29-4; 42·Na, 59587-08-5; 43, 70459-04-0; 43·Na, 122949-47-7; 44, 70459-01-7; 44·Na, 122949-48-8; 45, 91524-15-1; 45·Na, 122949-49-9; 46, 122949-46-6; 46·Na, 122949-50-2; 47, 93509-81-0; 47·Na, 115699-54-2; 48, 79660-72-3; 48·K, 115699-47-3; 49, 115622-62-3; (6R-*trans*)-3-(bromomethyl)-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (4-nitrophenyl)methyl ester, 39876-55-6; (6R-*trans*)-3-[[[(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid S-oxide, 115622-63-4;

(6R-*trans*)-3-(iodomethyl)-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid *tert*-butyl ester, 115622-66-7; (6R-*trans*)-3-(iodomethyl)-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid allyl ester, 115622-74-7; [6R-[6α,7β(Z)]]-7-[[(2-tritylamino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-(iodomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid *tert*-butyl ester, 105514-47-4; [6R-[6α,7β(Z)]]-7-[[(2-tritylamino-4-thiazolyl)[[2-(1-*tert*-butylcarboxylate-1-methyl)ethoxy]imino]acetyl]amino]-3-(iodomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid *tert*-butyl ester, 117333-74-1; [6R-(6α,7β)]-3-(io-domethyl)-7-[(2-thienylacetyl)amino]-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid *tert*-butyl ester, 37553-14-3; (6R-*trans*)-7-(formylamino)-3-(iodomethyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid *tert*-butyl ester, 122949-51-3; [6R-[6α,7β(R*)]]-7-[[2-[[(4-ethyl-2,3-dioxo-piperazinyl)carbonyl]amino]-2-phenylacetyl]amino]-3-(iodo-methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (diphenylmethyl) ester, 122949-52-4; [6R-[6α,7β(Z)]]-7-[[(2-tri-tylamino-4-thiazolyl)[[(*tert*-butylcarboxylate)methoxy]imino]-acetyl]amino]-3-(iodomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid *tert*-butyl ester, 122949-57-9.

New Leupeptin Analogues: Synthesis and Inhibition Data

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Syntheses of several tripeptide analogues of leupeptin containing C-terminal argininal, lysinal, or ornithinal units are presented. The synthetic analogues were tested as inhibitors of trypsin, plasmin, and kallikrein. (Benzyl-oxycarbonyl)-L-leucyl-L-leucyl-L-argininal (2a) was significantly less effective as an inhibitor of trypsin and plasmin activity than leupeptin. (Benzyloxycarbonyl)-L-leucyl-L-leucyl-L-lysinal (2e) and (benzyloxycarbonyl)-L-leucyl-L-leucyl-L-ornithinal (2i) display different inhibition characteristics than (benzyloxycarbonyl)-L-leucyl-L-leucyl-L-argininal (2a). While (benzyloxycarbonyl)-L-leucyl-L-leucyl-L-argininal (2a) showed moderate inhibition of all three enzymes tested, (benzyloxycarbonyl)-L-leucyl-L-leucyl-L-lysinal (2e) was less effective as an inhibitor of trypsin and plasmin activity. Of the three enzymes tested, (benzyloxycarbonyl)-L-leucyl-L-leucyl-L-ornithinal (2i) showed significant inhibition of kallikrein activity only. Modifications made in the composition and sequence of the P_2 and P_3 amino acids also resulted in variations in the inhibitory activity of the analogues. In general, plasmin showed a strong preference for inhibitors which contain an L-phenylalanyl-L-leucyl or an L-leucyl-L-valyl unit in the P_2 and P_3 positions.

Leupeptin (1), *N*-acetyl- or *N*-propionyl-L-leucyl-L-leucyl-DL-argininal, is a naturally occurring proteinase inhibitor isolated from the culture filtrates of various species of actinomyces.[1-3] Leupeptin has been shown to be a very

1: R = CH₃, CH₂CH₃
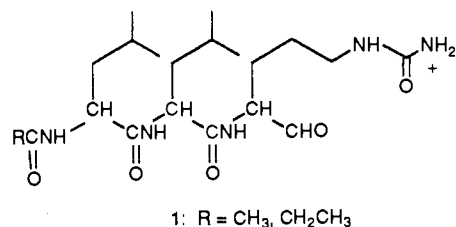
potent inhibitor of a number of proteolytic enzymes.[4-12] Leupeptin has also been shown to alter or suppress the symptoms of such disease conditions as rheumatoid arthritis,[1-13] muscular dystrophy,[14-18] allergic encephalomyelitis,[19] malaria,[20] and immunological dysregulations.[21,22] However, leupeptin is not selective among enzymes of similar substrate specificities, thus limiting its usefulness in the investigations of disease processes and as a therapeutic agent.

Several derivatives of leupeptin have been prepared that provide interesting insights into variation of biological activity with inhibitor structure.[23-25] The derivatives in which the C-terminal aldehyde of leupeptin was reduced to the alcohol, oxidized to the carboxylic acid, or protected as the dibutyl acetal[26] showed no inhibition of most enzymes that are strongly inhibited by leupeptin. Umezawa and co-workers studied the effect of minor variations in

Table I. Inhibitors

$$\underset{}{\bigcirc}\!\!-CH_2OCONHCHCONHCHCONHCHCHO$$

with substituents R_1, R_2, R_3

no.	R_1	R_2	R_3
2a	CH₂CH(CH₃)₂	CH₂CH(CH₃)₂	(CH₂)₃NHC(NH)NH₂
2b	CH₂CH(CH₃)₂	CH₂Ph	(CH₂)₃NHC(NH)NH₂
2c	CH₂Ph	CH₂CH(CH₃)₂	(CH₂)₃NHC(NH)NH₂
2d	CH₂CH(CH₃)₂	CH(CH₃)₂	(CH₂)₃NHC(NH)NH₂
2e	CH₂CH(CH₃)₂	CH₂CH(CH₃)₂	(CH₂)₄NH₂
2f	CH₂CH(CH₃)₂	CH₂Ph	(CH₂)₄NH₂
2g	CH₂Ph	CH₂CH(CH₃)₂	(CH₂)₄NH₂
2h	CH₂CH(CH₃)₂	CH(CH₃)₂	(CH₂)₄NH₂
2i	CH₂CH(CH₃)₂	CH₂CH(CH₃)₂	(CH₂)₃NH₂
2j	CH₂CH(CH₃)₂	CH₂Ph	(CH₂)₃NH₂
2k	CH₂Ph	CH₂CH(CH₃)₂	(CH₂)₃NH₂
2l	CH₂CH(CH₃)₂	CH(CH₃)₂	(CH₂)₃NH₂

the amino acid composition and sequence of leupeptin.[23,25] Using a synthetic route which he later abandoned, Ume-

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