SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW C-10 QUINOLONYL-CEPHEM ESTERS

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A series of cephalosporins derived from cephalothin containing an ester-linked quinolonyl substituent at the C-10 position (C-10 quinolonyl-cephem esters) has been prepared and evaluated for *in vitro* antibacterial activity. The C-10 quinolonyl-cephem esters exhibited a broadened spectrum of activity when compared with cephalothin and the corresponding quinolones, including activity against β-lactamase-producing bacteria.

The early work of O'CALLAGHAN *et al.*[1] and GREENWOOD and O'GRADY[2], and later MOBASHERY *et al.*[3], MOBASHERY and JOHNSTON[4,5] suggested that the incorporation of certain antimicrobial agents as cephalosporin C-10 substituents (following the numbering convention described by KUKOLJA and CHAUVETTE[6]) could result in new compounds with a spectrum of antibacterial activity that included β-lactamase-producing organisms. More recently, ALBRECHT *et al.*[7~9] described the preparation and evaluation of cephalosporins possessing quinolone substituents at C-10, such as Ro 23-9424. We now report the synthesis and antibacterial evaluation of a related series of new cephalosporins[10] derived from cephalothin which culminated in the highly potent, broad spectrum agent **1e** (Fig. 1). These compounds (**1a**~**1e**, Fig. 2, herein referred to as C-10 quinolonyl-cephem esters) possess a quinolonyl moiety attached at the C-10 position of cephalothin by an ester linkage through the quinolone-3-carboxylate. The synthetic procedures described in this report allow for the preparation of such C-10 quinolonyl-cephem esters with minimal formation of undesired Δ2-cephem side products.

Chemistry

The preparation of **1a**~**1e** proceeded as shown in Fig. 3. The coupling of the 10-iodocephem 4-nitrobenzyl ester **2**[11,12] with nalidixic acid sodium salt (**3a**) in *N*,*N*-dimethylformamide (DMF) gave a mixture of the desired Δ3-cephem adduct (**4a**) and 30~40% of the thermodynamically more stable Δ2-isomer. The quinolone carboxylate is sufficiently basic to abstract a proton from the cephalosporin

Fig. 1. Structure of **1e**.

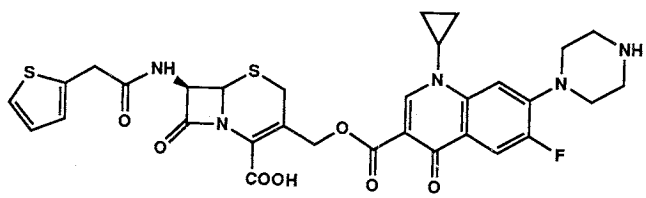
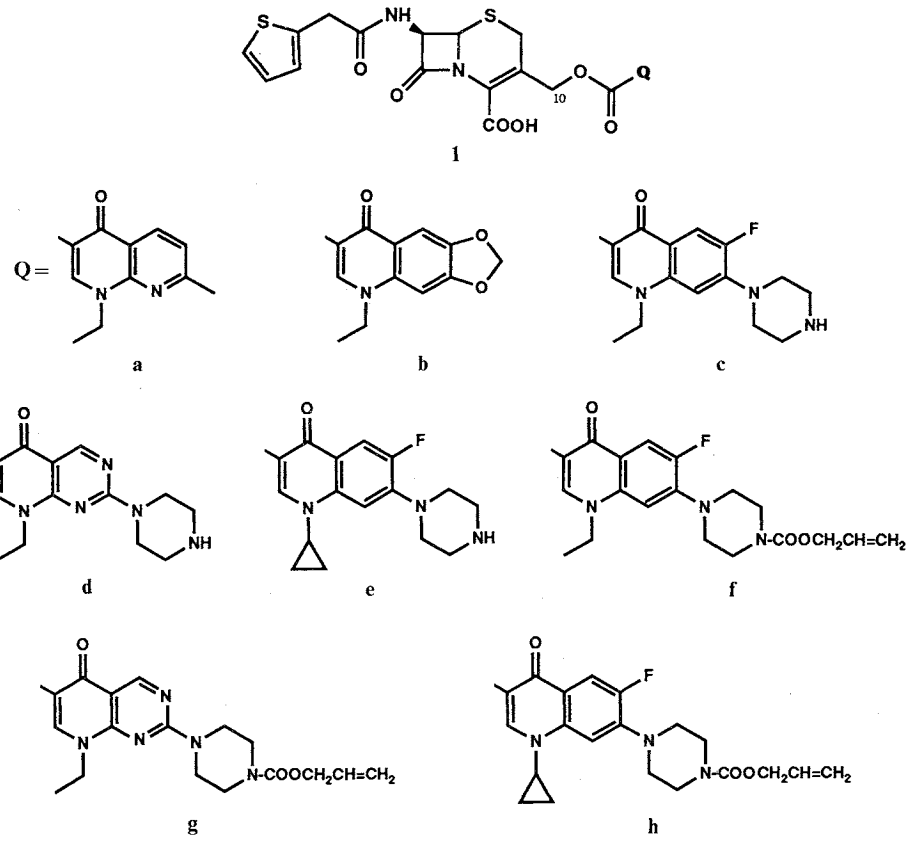


Fig. 2. Structures of C-10 quinolonyl-cephem esters.



C-2 methylene and reprotonate at C-4 to generate the Δ2-isomer[13,14]. To decrease the basicity of the quinolone carboxylate, a co-solvent (dioxane) was added to lower the polarity of the reaction medium[14]. Thus, the coupling of **2** and **3a** in 50% DMF-dioxane at 20°C for 1.5 hours afforded the isomerically pure adduct **4a** in 35% yield. Deblocking was effected by hydrogenation at 13.7 kg/cm^2 with 10% Pd-C in aqueous THF to give the target **1a** in 50% yield. The quinolonyl-cephem **1b** was similarly prepared from **2** and oxolinic acid.

The remaining C-10 quinolonyl-cephem esters (**1c**$\sim$**1e**) were prepared with allyl-protecting groups to avoid the competing, undesired hydrogenolysis of the C-10 ester linkage observed during the deblocking of the 4-nitrobenzyl groups. The quinolones norfloxacin, pipemidic acid, and ciprofloxacin were acylated with allyl chloroformate under modified Schotten-Baumann conditions and the corresponding sodium salts (**3f**$\sim$**3h**) were prepared.

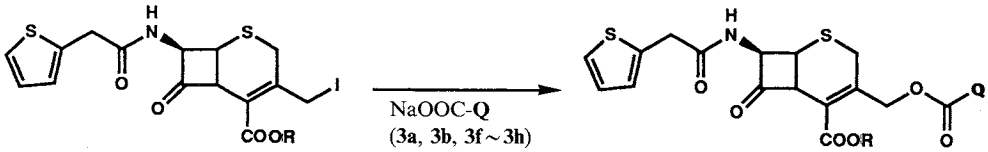
The coupling of the 10-iodocephem allyl ester (**5**, synthesized from cephalothin allyi ester and trimethylsilyl iodide) with the prepared allyloxycarbonyl-protected quinolone salts (**3f**$\sim$**3h**) in 50% DMF-dioxane afforded the quinolonyl-cephem ester adducts (**6f**$\sim$**6h**, respectively), in low to moderate yields. Degradation of the iodocephem **5** in the DMF-dioxane solution (to multiple unidentified products including cephalothin lactone) significantly competed with the desired coupling reaction. Final deblocking of the di-allyl protected **6f**$\sim$**6h** was accomplished efficiently with palladium(II) chloride, triphenylphosphine, and tributyltin hydride[15,16] to give the target cephalosporins **1c**, **1d** and **1e** in 40$\sim$75% isolated yields.

Results and Discussion

The *in vitro* antibacterial activities of the C-10 quinolonyl-cephem esters **1a**~**1e** and reference compounds are summarized in Tables 1 and 2. Compared with cephalothin (**7**), the C-10 quinolonyl-cephem esters displayed significantly enhanced activity against Gram-negative bacteria. Compared with the corresponding quinolones **3a**~**3e**, the cephems exhibited improved potency against Gram-positive organisms, of which the activity against the Streptococci was particularly noteworthy. On a molar basis, the quinolonyl-cephem **1e** was greater than ten times more potent against Streptococci than desacetylcephalothin (**8**), the quinolone **3e** or an equimolar mixture of the two agents (Table 3). A variety of β-lactamase-producing strains of bacteria were as sensitive to the C-10 quinolonyl-cephems **1a**, **1d**, and **1e** as the non-producing strains (Table 4).

The most active member of this series was the quinolonyl-cephem ester **1e**. Table 5 shows the MIC_{90} values derived for compound **1e** and competitive cephalosporin, quinolone and carbapenem agents. Against the tested Gram-negative pathogens, compound **1e** was more active than imipenem and, except for *Escherichia coli*, ceftriaxone, while against *Streptococcus pneumoniae* the quinolonyl-cephem was superior to ciprofloxacin. In addition, compound **1e** exhibited MIC_{90} values that were apparently two to eight times

Fig. 3. Preparation of C-10 quinolonyl-cephem esters.



2 R=PNB
5 R=allyl

4a, 4b R=PNB
6f~6h R=allyl
1a~1e R=H

Table 1. Antibacterial activity of C-10 quinolonyl-cephem esters.

Organism	MIC[a] (μg/ml)				
	1a	**1b**	**1c**	**1d**	**1e**
Staphylococcus aureus ATCC 29213	0.5	0.5	1	1	0.5
S. aureus MI300[b]	32	NT	8	NT	4
S. saprophyticus MI276	1	1	1	1	1
Streptococcus pneumoniae STP1	NT	≤0.12	0.12	0.12	0.06
S. pyogenes STA2	NT	≤0.12	≤0.06	≤0.06	≤0.008
viridans Streptococci STV1	NT	≤0.12	0.12	0.12	0.06
Enterococcus faecalis ATCC 29212	32	16	8	64	8
Escherichia coli ATCC 25922	32	4	1	16	0.25
Enterobacter cloacae AE63	8	2	0.25	4	0.03
Klebsiella pneumoniae KL21	4	1	0.25	4	0.06
Pseudomonas aeruginosa ATCC 27853	>128	>128	8	>64	2
Proteus mirabilis PR91	64	8	2	64	1

[a] Media: Brain-heart infusion.
[b] Methicillin-resistant strain.
NT: Not tested.

Table 2. Antibacterial activity of reference compounds.

Organism	MIC[a] (μg/ml)					
	3a	**3b**	**3c**	**3d**	**3e**	**7**
Staphylococcus aureus ATCC 29213	32	2	1	64	0.25	0.25
S. aureus MI300[b]	32	2	0.5	128	0.5	32
S. saprophyticus MI276	>128	128	2	>128	0.5	0.5
Streptococcus pneumoniae STP1	>128	128	2	128	1	0.25
S. pyogenes STA2	>128	64	2	128	0.5	0.06
viridans Streptococci STV1	>128	128	8	128	2	0.25
Enterococcus faecalis ATCC 29212	>128	32	2	>128	0.5	32
Escherichia coli ATCC 25922	4	0.25	≤0.06	2	≤0.008	16
Enterobacter cloacae AE63	1	0.5	≤0.06	1	≤0.008	64
Klebsiella pneumoniae KL21	2	0.5	0.12	1	≤0.008	0.5
Pseudomonas aeruginosa ATCC 27853	128	128	16	32	2	>128
Proteus mirabilis PR91	8	0.5	0.25	8	0.06	8

[a] Media: Brain-heart infusion.
[b] Methicillin-resistant strain.

Table 3. Comparison of **1e** with equimolar mixture of **8** and **3e**.

Organism	MIC[a] (μmol/liter)			
	1e	**3e**	**8**	1/1 **8**/**3e**
Staphylococcus aureus ATCC 29213	0.75	0.75	2.8	0.36
S. aureus MI300[b]	6.0	1.5	>90	0.73
S. saprophyticus MI276	1.5	1.5	11	1.5
Streptococcus pneumoniae STP1	0.09	3.0	2.8	1.5
S. pyogenes STA2	≤0.01	1.5	0.34	0.36
viridans Streptococci STV1	0.09	6.0	2.8	1.5
Enterococcus faecalis ATCC 29212	12	1.5	90	0.73
Escherichia coli ATCC 25922	0.37	≤0.02	180	≤0.02
Enterobacter cloacae AE63	0.04	≤0.02	90	0.04
Klebsiella pneumoniae KL21	0.09	≤0.02	45	0.02
Pseudomonas aeruginosa ATCC 27853	3.0	6.0	>180	3.0
Proteus mirabilis PR91	1.5	0.18	>180	0.17

[a] Media: Brain-heart infusion.
[b] Methicillin-resistant strain.

more potent than those previously published for Ro 23-9424 (MIC_{90} in μg/ml for *Staphylococcus aureus* 4, *S. pneumoniae* 0.25, *E. coli* 0.5, *Enterobacter cloacae* 0.5, and *Pseudomonas aeruginosa* 8[17]). This combination of high potency and broad spectrum activity positions compound **1e** as a promising new antibacterial agent.

Experimental

IR spectra were measured on a Nicolet 5MX FT-IR spectrophotometer. Mass spectra were determined on a HP5987A spectrometer in EI, CI or FAB ionization mode. ^{1}H NMR spectra were determined on a GE GN300 (300 MHz) spectrometer or an IBM WP100 (100 MHz) spectrometer. Chemical shifts are

Table 4. Antibacterial activity of C-10 quinolonyl-cephem esters against β-lactamase producing and non-producing strains of bacteria.

Organism	β-Lactamase producer	MIC (μg/ml)		
		1a[a]	**1d**[b]	**1e**[b]
Escherichia coli	−	16～32 (8)[c]	2 (2)	0.06 (5)
	+	16～32 (5)	2 (1)	0.06～0.5 (4)
Haemophilus influenzae	−	NT	1～2 (3)	0.06 (6)
	+	NT	0.5～1 (3)	≤0.03～0.06 (3)
Moraxella catarrhalis	−	NT	2 (3)	0.06～0.12 (5)
	+	NT	1～4 (3)	0.12 (5)
Enterobacter cloacae	+[d]	4～64 (5)	2 (1)	≤0.008～0.06 (4)
	+[e]	8～32 (4)	1～2 (2)	0.016 (3)

[a] Media: Brain-heart infusion.
[b] Media: Mueller-Hinton broth, except for *H. influenzae*, which was tested on GC agar with 1% Supplement B (Difco).
[c] Number of strains tested.
[d] Inducible strains (cefotaxime-sensitive) produced <0.5 U cephalosporinase/mg protein.
[e] Constitutive strains (cefotaxime-resistant) produced >10 U/mg protein.
NT: Not tested.

Table 5. Activity of **1e** and competitive agents against selected bacteria.

Organism (No. of strains)	Compound	MIC[a] (μg/ml)		
		50%	90%	Range
Staphylococcus aureus (10)	**1e**	1	2	0.5～2
	Ceftriaxone	4	4	4～>64
	Ciprofloxacin	0.25	0.25	0.12～1
	Imipenem	≤0.008	≤0.008	≤0.008～0.016
Streptococcus pneumoniae (18)	**1e**	0.008	0.06	≤0.002～0.25
	Ceftriaxone	≤0.03	≤0.03	≤0.03
	Ciprofloxacin	0.5	1	0.5～>2
	Imipenem	≤0.03	≤0.03	≤0.03
Escherichia coli (10)	**1e**	0.06	0.06	0.06～0.5
	Ceftriaxone	0.03	0.03	0.03
	Ciprofloxacin	0.008	0.016	0.008～1
	Imipenem	≤0.25	0.5	≤0.25～1
Enterobacter cloacae (10)	**1e**	0.016	0.06	0.008～0.06
	Ceftriaxone	0.25	>32	0.001～>32
	Ciprofloxacin	0.016	0.016	0.008～0.016
	Imipenem	0.25	1	0.25～4
Pseudomonas aeruginosa (21)	**1e**	0.5	2	0.25～4
	Ceftriaxone	128	>128	16～>128
	Ceftazidime	2	32	0.5～32
	Ciprofloxacin	0.12	0.5	0.06～2
	Imipenem	2	4	1～8

[a] Media: Mueller-Hinton broth. *S. pneumoniae* was tested in brain-heart infusion media.

expressed in ppm downfield from TMS. UV spectra were recorded on a Perkin-Elmer Lambda 4B UV-Vis spectrophotometer. MP's were determined on Mel-Temp apparatus and are uncorrected.

MICs were determined in brain-heart infusion or Mueller-Hinton broth by a standard 2-fold dilution method[18] with an inoculum size of 5×10^5 cfu/ml. Endpoints were determined after incubation at 37°C

for 18~20 hours. *Haemophilus influenzae* was tested on GC agar base containing 1% Supplement B (Difco) at 10^4 cfu/spot.

Cephalothin sodium salt, oxolinic acid, pipemidic acid, and nalidixic acid sodium salt were purchased from Sigma Chemical. Norfloxacin[19] and ciprofloxacin[20] were prepared by published procedures. Desacetylcephalothin (**8**) was prepared by the method of FUJIMOTO[21]. Ceftriaxone (Roche), ceftazidime (Glaxo) and imipenem (Merck) were obtained from their respective manufacturers.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-(2-propenyloxycarbonyl)-1-piperazinyl]quinoline-3-carboxylic Acid, Sodium Salt (**3f**)

A 1 N NaOH solution was added dropwise to a suspension of norfloxacin (1.50 g, 4.7 mmol) in water (60 ml) at 2°C to attain pH 12. The mixture was diluted with acetone (25 ml) and a solution of allyl chloroformate (0.50 ml, 4.7 mmol) in acetone (12 ml) was added dropwise over 15 minutes at 2°C. The mixture was stirred for 1 hour at 2°C (adding more 1 N NaOH to maintain pH 9.5~11.5) and additional allyl chloroformate (0.50 ml, 4.7 mmol) in acetone (12 ml) was added. The mixture was stirred for 1 hour at 2°C, the acetone was removed under reduced pressure, and the mixture was stirred with diethyl ether. The product was collected by filtration and recrystallized from ethanol - dichloromethane yielding 0.95 g. This material was dissolved in a minimum volume of methylene chloride and chilled to 0°C, and a solution containing 95 mg (2.4 mmol) of sodium hydroxide in 1.0 ml of methanol was added dropwise. Following the addition, stirring was continued for 60 minutes while allowing the solution to warm to room temperature. Evaporation *in vacuo* gave a white solid which was triturated in ether and collected, yielding 0.85 g (48%) of **3f**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42 (3H, t, J=6 Hz, CH_3), 3.35 (4H, br s, CH_2NCH_2), 3.61 (4H, br s, CH_2NCH_2), 4.58 (4H, m, CH_2O, CH_2N), 5.22, 5.32 (2H, 2d, J=10 and 18 Hz, $CH{=}CH_2$), 5.96 (1H, m, $CH{=}CH_2$), 7.21 (1H, d, $J_{H,F}$=8 Hz, aryl), 7.94 (1H, d, $J_{H,F}$=12 Hz, aryl), 8.97 (1H, s, aryl).

8-Ethyl-5,8-dihydro-5-oxo-2-[4-(2-propenyloxycarbonyl)-1-piperazinyl]pyrido[2,3-d]pyrimidine-6-carboxylic Acid, Sodium Salt (**3g**)

Compound **3g** was prepared from pipemidic acid (10.0 g, 33 mmol) in a procedure analogous to that described for **3f**, yielding 10.5 g (78%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (3H, t, J=6 Hz, CH_3), 3.54 (4H, br s, CH_2NCH_2), 3.94 (4H, br s, CH_2NCH_2), 4.37 (2H, q, J=6 Hz, CH_2N), 4.57 (2H, d, J=6 Hz, CH_2O), 5.23, 5.34 (2H, 2d, J=12 and 18 Hz, $CH{=}CH_2$), 5.94 (1H, m, $CH{=}CH_2$), 8.85 (1H, s, aryl), 9.18 (1H, s, aryl).

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-(2-propenyloxycarbonyl)-1-piperazinyl]quinoline-3-carboxylic Acid, Sodium Salt (**3h**)

Compound **3h** was prepared from ciprofloxacin (10.0 g, 30 mmol) in a procedure analogous to that described for **3f**, yielding 12.0 g (91%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.9 (2H, m, cyclopropyl), 1.2 (2H, m, cyclopropyl), 3.1 (4H, br s, CH_2NCH_2), 3.6 (5H, br s, CH_2NCH_2, *N*-cyclopropyl), 4.55 (2H, s, CH_2O), 5.20, 5.25 (2H, 2d, J=10 and 16 Hz, $CH{=}CH_2$), 5.9 (1H, m, $CH{=}CH_2$), 7.35 (1H, br s, aryl), 7.70 (1H, br s, aryl), 8.40 (1H, br s, aryl).

Anal Calcd for $C_{21}H_{21}FN_3NaO_5$: C 57.66, H 4.84, N 9.61, Na 5.26.
Found: C 57.30, H 5.18, N 9.55, Na 5.67.

[6*R*-(6α,7β)]-3-[[(1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)carbonyloxy]methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, 4-Nitrobenzyl Ester (**4a**)

To a solution of **2** (3.0 g, 5 mmol) in 50% DMF - dioxane (30 ml) nalidixic acid sodium salt (**3a**, 1.27 g, 5 mmol) was added at 5~10°C. The reaction was warmed to room temperature and stirred for 1.5 hours. The mixture was cooled to 5~10°C, extracted three times with chloroform and washed with 10% sodium bicarbonate. The combined organic phase was dried over sodium sulfate and filtered, and the filtrate was concentrated to dryness under reduced pressure. The residue was triturated sequentially in ethyl acetate, chloroform, and diethyl ether, and the product was collected by filtration yielding 1.23 g (35%) of **4a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (3H, t, J=7 Hz, CH_3), 2.64 (3H, s, aryl-CH_3), 3.78 (2H, s, CH_2CO), 3.81 (2H, s, CH_2S), 4.49 (2H, q, J=7 Hz, CH_2N), 4.95, 5.16 (2H, ABq, J=15 Hz, CH_2O), 5.21 (1H, d,

J=6 Hz, 6-CH), 5.39, 5.49 (2H, ABq, J=15 Hz, OCH_2-aryl), 5.80 (1H, m, 7-CH), 6.92 (2H, m, thienyl), 7.34 (1H, m, thienyl), 7.45 (1H, d, J=8 Hz, aryl), 7.72 (2H, d, J=8 Hz, aryl), 8.22 (2H, d, J=8 Hz, aryl), 8.46 (1H, d, J=8 Hz, aryl), 8.85 (1H, s, aryl), 9.22 (1H, d, NH); IR ν_{max} (KBr) cm^{-1} 3240, 1790, 1682, 1596.

Anal Calcd for $C_{33}H_{29}N_5O_9S_2$: C 56.32, H 4.15, N 9.95.
Found: C 56.27, H 4.10, N 10.08.

[6*R*-(6α,7β)]-3-[[(5-Ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-g]-7-quinolinyl)carbonyloxy]methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, 4-Nitrobenzyl Ester (**4b**)

To a suspension of oxolinic acid (3.0 g, 11 mmol) in 1,000 ml of methanol, a solution of 620 mg (11 mmol) of sodium methoxide in 2.0 ml of methanol was added dropwise at 0~5°C. Stirring was continued while allowing the reaction to warm to room temperature. After 1 hour, the reaction was heated for 4 hours at reflux. It was then filtered hot to remove the insolubles. The methanol filtrate was evaporated to dryness *in vacuo*, and the residue triturated in ether yielding 2.34 g (56%) of **3b**

Compound **4b** was prepared from **2** (9.0 g, 15 mmol) and **3b** (1.4 g, 5 mmol) in a procedure analogous to that described for **4a**. The crude **4b** obtained from the coupling procedure was sequentially triturated in ether and ethyl acetate yielding 1.70 g (21.2%) of **4b**. ^1H NMR (300 MHz, DMSO-d_6) δ 1.28 (3H, t, J=8 Hz, CH_3), 3.65~3.80 (4H, m, CH_2S, CH_2CO), 4.30 (2H, q, J=8 Hz, CH_2N), 4.90, 5.10 (2H, ABq, J=14 Hz, 3-CH_2O), 5.14 (1H, d, J=6 Hz, 6-CH), 5.41 (2H, ABq, J=16 Hz, OCH_2Ar), 5.75 (1H, m, 7-CH), 6.17 (2H, s, OCH_2O) 6.90 (2H, m, thienyl), 7.34 (1H, d, J=6 Hz, thienyl), 7.40 (1H, s, aryl), 7.52 (1H, s, aryl), 7.67 (2H, d, J=10 Hz, aryl), 8.17 (2H, d, J=10 Hz, aryl), 8.52 (1H, s, aryl) 9.17 (1H, d, NH).

Anal Calcd for $C_{34}H_{28}N_4O_{11}S_2 \cdot H_2O$: C 54.40, H 4.03, N 7.46.
Found: C 53.64, H 3.95, N 7.59.

[6*R*-(6α,7β)]-3-(Iodomethyl)-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, 2-Propenyl Ester (**5**)

Cephalothin allyl ester[14] (19.3 g, 44 mmol) was dissolved in dry dichloromethane (960 ml) under nitrogen. Trimethylsilyl iodide (16.3 ml, 71 mmol) was added at a fast drip. The solution was stirred in the dark, at room temperature until no more starting material remained (1.5 hours). The dark red solution was then cooled in ice, and 10% aqueous sodium thiosulfate solution (500 ml) was added slowly, keeping the temperature at 15°C or below. The organic phase turned from dark red to light orange. The layers were separated, and the organic phase was washed with 10% aqueous sodium thiosulfate (2×500 ml) and water (2×500 ml), dried over anhydrous magnesium sulfate and Darco, and filtered through Celite. The crude reaction product was purified by diluting with acetone, filtering through a bed of silica gel (230~400 mesh), and eluting with 5% acetone-dichloromethane. The filtrate was evaporated to near dryness. The residue was stirred, and hexane was added, precipitating a solid. The solid was collected by filtration, washed with hexane, and dried *in vacuo* yielding 12.5 g (56%) of **5**. ^1H NMR (300 MHz, $CDCl_3$) δ 3.51, 3.72 (2H, ABq, J=16 Hz, CH_2S), 3.86 (2H, s, CH_2CO), 4.31, 4.41 (2H, ABq, J=12 Hz, 3-CH_2I), 4.77 (2H, d, J=8 Hz, CH_2O), 4.97 (1H, d, J=4 Hz, 6-CH), 5.30, 5.39 (2H, 2d, J=8 and 16 Hz, $CH=CH_2$), 5.80 (1H, m, 7-CH), 5.96 (1H, m, $CH=CH_2$), 6.28 (1H, d, NH), 7.00 (2H, m, thienyl), 7.26 (1H, m, thienyl).

[6*R*-(6α,7β)]-3-[[[1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-(2-propenyloxycarbonyl)-1-piperazinyl]-3-quinolinyl]carbonyloxy]methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, 2-Propenyl Ester (**6f**)

To a solution of **5** (1.54 g, 3.1 mmol) in 50% DMF-dioxane (16 ml), **3f** (1.30 g, 3.1 mmol) was added. The mixture was stirred at room temperature for 1.5 hours, and additional **5** (240 mg, 0.5 mmol) was added. The solution was stirred for another 2.5 hours, cooled in an ice bath and diluted with chloroform. The solution was extracted with ice-cold 0.14 N NaOH (three times), water, and brine. The organic phase was dried over sodium sulfate and concentrated to dryness under reduced pressure. The residue was triturated twice in ethyl acetate and the product was collected by filtration yielding 0.46 g (19%) of **6f**. MP 114~117°C; ^1H NMR (300 MHz, DMSO-d_6) δ 1.38 (3H, t, J=8 Hz, CH_3), 3.23 (4H, br s, CH_2NCH_2), 3.61 (4H, br s, CH_2NCH_2), 3.78 (4H, br s, CH_2S, CH_2CO), 4.42 (2H, q, J=8 Hz, CH_2N), 4.59 (2H, d, J=6 Hz, CH_2O), 4.76 (2H, d, J=6 Hz, CH_2O), 4.96, 5.15 (2H, ABq, J=13 Hz, CH_2O), 5.18 (1H, d,

J=6 Hz, 6-CH), 5.2~5.4 (4H, m, 2(CH=CH_2)), 5.78 (1H, m, 7-CH), 5.9~6.0 (2H, m, 2(CH=CH$_2$)), 6.94 (2H, m, thienyl), 7.10 (1H, d, $J_{H,F}$=8 Hz, aryl), 7.58 (1H, m, thienyl), 7.85 (1H, d, $J_{H,F}$=12 Hz, aryl), 8.64 (1H, s, aryl), 9.20 (1H, d, NH).

Anal Calcd for $C_{37}H_{38}FN_5O_9S_2 \cdot \frac{1}{2}H_2O$: C 56.33, H 4.98, N 8.88.
Found: C 56.40, H 5.10, N 8.54.

[6*R*-(6α,7β)]-3-[[[8-Ethyl-5,8-dihydro-5-oxo-2-[4-(2-propenyloxycarbonyl)-1-piperazinyl]pyrido-[2,3-d]-6-pyrimidinyl]carbonyloxy]methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic Acid, 2-Propenyl Ester (**6g**)

Compound **6g** was prepared from **5** (0.95 g, 1.9 mmol) and **3g** (0.69 g, 1.7 mmol) in a procedure analogous to that described for **6f** yielding 145 mg (11%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, J=8 Hz, CH$_3$), 3.2~3.8 (m, includes CH$_2$S, CH$_2$NCH$_2$, CH$_2$CO and water), 3.91 (4H, br s, CH$_2$NCH$_2$), 4.29 (2H, q, J=8 Hz, CH$_2$N), 4.58 (2H, d, J=4 Hz, CH$_2$O), 4.75 (2H, d, J=4 Hz, CH$_2$O), 4.94, 5.13 (2H, ABq, J=13 Hz, 3-CH$_2$O), 5.2~5.4 (5H, m, 2(CH=CH_2), 6-CH), 5.78 (1H, m, 7-CH), 5.9~6.0 (2H, m, 2(CH=CH$_2$)), 6.94 (2H, m, thienyl), 7.37 (1H, m, thienyl), 8.64 (1H, s, aryl), 9.04 (1H, s, aryl), 9.19 (1H, d, NH).

Anal Calcd for $C_{35}H_{37}N_7O_9S_2$: C 55.03, H 4.88, N 12.84.
Found: C 54.84, H 4.70, N 12.68.

[6*R*-(6α,7β)]-3-[[[1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-(2-propenyloxycarbonyl)-1-piperazinyl]-3-quinolinyl]carbonyloxy]methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic Acid, 2-Propenyl Ester (**6h**)

Compound **6h** was prepared from **5** (4.03 g, 8.0 mmol) and **3h** (3.5 g, 8.0 mmol) in a procedure analogous to that described for **6f**, yielding 3.87 g (61%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.06 (2H, m, cyclopropyl), 1.12 (2H, m, cyclopropyl), 3.10 (4H, br s, CH$_2$NCH$_2$), 3.54 (5H, br s, CH$_2$NCH$_2$, *N*-cyclopropyl), 3.72, 3.73 (4H, 2s, CH$_2$CO, CH$_2$S), 4.56 (2H, d, J=6 Hz, COOCH$_2$), 4.71 (2H, br s, COOCH$_2$), 4.88, 5.10 (2H, ABq, J=12 Hz, 3-CH$_2$O), 5.1~5.4 (5H, m, 2(CH=CH_2), 6-CH), 5.70 (1H, m, 7-CH), 5.8~6.0 (2H, m, 2(CH=CH$_2$)), 6.90 (2H, m, thienyl), 7.34 (1H, d, J=4 Hz, thienyl), 7.44 (1H, d, $J_{H,F}$=8 Hz, aryl), 7.75 (1H, d, $J_{H,F}$=14 Hz, aryl), 8.40 (1H, s, aryl), 9.14 (1H, d, NH).

[6*R*-(6α,7β)]-3-[[(1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)carbonyloxy]methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**1a**)

A mixture of **4a** (0.74 g, 1.1 mmol), 20% aqueous THF (150 ml) and 10% Pd-C (0.74 g) was subjected to hydrogenation at 13.7 kg/cm^2 and room temperature for 1 hour. A solution of sodium bicarbonate (0.44 g, 5.2 mmol) in water (25 ml) was added, and the mixture was filtered through Celite. The filtrate was concentrated to about 60 ml, diluted with water (200 ml) and washed with ethyl acetate (2×100 ml). The aqueous phase was cooled and adjusted to pH 2 with cold 10% HCl. The product was extracted with ethyl acetate, the organic layer was dried over magnesium sulfate and filtered, and the filtrate was concentrated to dryness. The residue was triturated with methanol (25 ml) and the product was collected by filtration yielding 0.3 g (50%) of **1a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42 (3H, t, J=7 Hz, CH$_3$), 2.67 (3H, s, aryl-CH$_3$), 3.79 (2H, ABq, J=10 Hz, CH$_2$S), 3.84 (2H, s, CH$_2$CO), 4.51 (2H, q, J=7 Hz, CH$_2$N), 4.95, 5.10 (2H, ABq, J=15 Hz, 3-CH$_2$O), 5.18 (1H, d, J=6 Hz, 6-CH), 5.78 (1H, m, 7-CH), 6.95 (2H, m, thienyl), 7.38 (1H, m, thienyl), 7.42 (1H, d, J=8 Hz, aryl), 8.45 (1H, d, J=8 Hz, aryl), 8.83 (1H, s, aryl), 9.18 (1H, d, NH); IR ν_{max}(KBr) cm^{-1} 3240, 1786, 1728, 1696, 1640; FAB-MS m/z 591 (M+Na)$^+$; UV $\lambda_{max}^{2\%\ DMF-ethanol}$ nm (ε) 329 (12,700).

Anal Calcd for $C_{26}H_{24}N_4O_7S_2$: C 54.92, H 4.25, N 9.85.
Found: C 54.54, H 4.30, N 9.67.

[6*R*-(6α,7β)]-3-[[(5-Ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-g]-7-quinolinyl)carbonyloxy]methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**1b**)

Compound **1b** was prepared from **4b** (1.0 g, 1.4 mmol) in a procedure analogous to that described for **1a**. The crude product was purified by trituration in ether, collected by filtration, and air-dried yielding 102 mg (12%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (3H, t, J=8 Hz, CH$_3$), 3.65~3.8 (4H, m, CH$_2$S,

CH_2CO), 4.32 (2H, ABq, J=14 Hz, 3-CH_2O), 5.10 (1H, d, J=6 Hz, 6-CH), 5.68 (1H, m, 7-CH), 6.18 (2H, s, OCH_2O), 6.90 (2H, m, thienyl), 7.36 (1H, m, thienyl), 7.44 (1H, s, aryl), 7.54 (1H, s, aryl), 8.55 (1H, s, aryl), 9.12 (1H, d, NH).

Anal Calcd for $C_{27}H_{23}N_3O_9S_2 \cdot \frac{1}{2}H_2O$: C 53.46, H 3.99, N 6.93.
Found: C 53.76, H 3.99, N 6.97.

[6*R*-(6α,7β)]-3-[[[1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinyl]carbonyloxy]-methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**1c**)

To a solution of **6f** (150 mg, 0.19 mmol), dichloromethane (3.6 ml) and bis(triphenylphosphine)-palladium chloride (2.7 mg, 3.8 mmol) under nitrogen, water (19.2 ml) and tributyltin hydride (114 ml, 0.42 mmol) was added rapidly *via* syringe at 20°C. Vigorous bubbling occurred, and a precipitate formed. The mixture was stirred for 12~15 minutes, diluted with ether and stirred an additional 10 minutes, and the solid was collected by filtration. The product was triturated in acetone for 2 hours and collected by filtration, yielding 50 mg (39%) of **1c**. MP 160°C (dec); ^{1}H NMR (300 MHz, 3% TFA-d_1 in DMSO-d_6) δ 1.33 (3H, t, J=7 Hz, CH_3), 3.27 (4H, br s, CH_2NCH_2), 3.41 (4H, br s, CH_2NCH_2), 3.70 (2H, ABq, J=5 Hz, CH_2S), 3.73 (2H, s, CH_2CO), 4.42 (2H, q, J=7 Hz, CH_2N), 4.92, 5.16 (2H, ABq, J=13 Hz, 3-CH_2O), 5.09 (1H, d, J=6 Hz, 6-CH), 5.68 (1H, d, J=6 Hz, 7-CH), 6.89 (2H, m, thienyl), 7.10 (1H, d, $J_{H,F}$=8 Hz, aryl), 7.32 (1H, m, thienyl), 7.82 (1H, d, $J_{H,F}$=12 Hz, aryl), 8.61 (1H, s, aryl).

Anal Calcd for $C_{30}H_{30}FN_5O_7S_2 \cdot H_2O$: C 53.48, H 4.78, N 10.40.
Found: C 53.42, H 4.84, N 10.01.

[6*R*-(6α,7β)]-3-[[[8-Ethyl-5,8-dihydro-5-oxo-2-(piperazinyl)pyrido[2,3-d]-6-pyrimidinyl]-carbonyloxy]methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**1d**)

Compound **1d** was prepared from **6g** (100 mg, 0.13 mmol) in a procedure analogous to that described for **1c** yielding 65 mg (77%). MP 169°C (dec); ^{1}H NMR (300 MHz, 3% TFA-d_1 in DMSO-d_6) δ 1.29 (3H, t, J=10 Hz, CH_3), 3.20 (4H, br s, CH_2NCH_2), 3.70 (2H, ABq, J=20 Hz, CH_2S), 3.73 (2H, s, CH_2CO), 4.05 (4H, br s, CH_2NCH_2), 4.27 (2H, q, J=10 Hz, CH_2N), 4.88, 5.16 (2H, ABq, J=14 Hz, 3-CH_2O), 5.09 (1H, d, J=8 Hz, 6-CH), 5.68 (1H, d, J=8 Hz, 7-CH), 6.90 (2H, m, thienyl), 7.34 (1H, m, thienyl), 8.63 (1H, s, aryl), 9.05 (1H, s, aryl); IR ν_{max} (KBr) cm^{-1} 3400, 3000, 1760, 1715, 1616, 1580; MS *m/z* 640 $(M+H)^+$.

Anal Calcd for $C_{28}H_{29}N_7O_7S_2 \cdot \frac{3}{4}H_2O$: C 51.48, H 4.71, N 15.01.
Found: C 51.78, H 4.70, N 14.75.

[6*R*-(6α,7β)]-3-[[[1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinyl]-carbonyloxy]methyl]-8-oxo-7-[(2-thienyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**1e**)

Compound **1e** was prepared from **6h** (1.02 g, 1.3 mmol) in a procedure analogous to that described for **1c**. The crude product was purified by trituration in a minimum volume of acetone to give 328 mg (38%) of **1e**. ^{1}H NMR (300 MHz, 3% TFA-d_1 in DMSO-d_6) δ 1.06 (2H, m, cyclopropyl), 1.14 (2H, m, cyclopropyl), 3.28 (4H, br s, CH_2NCH_2), 3.44 (5H, br s, CH_2NCH_2, *N*-cyclopropyl), 3.70 (2H, br s, CH_2S), 3.75 (2H, s, CH_2CO), 4.85, 5.18 (2H, ABq, J=13 Hz, 3-CH_2O), 5.10 (1H, d, J=5 Hz, 6-CH), 5.68 (1H, d, J=5 Hz, 7-CH), 6.88 (2H, m, thienyl), 7.30 (1H, m, thienyl), 7.42 (1H, d, $J_{H,F}$=7 Hz, aryl), 7.80 (1H, d, $J_{H,F}$=14 Hz, aryl), 8.42 (1H, s, aryl).

Anal Calcd for $C_{31}H_{30}FN_5O_7S_2 \cdot 1\frac{1}{2}H_2O$: C 53.59, H 4.79, N 10.08.
Found: C 53.63, H 4.88, N 9.56.

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