Preparation and Antimicrobial Assessment of 2-Thioether-linked Quinolonyl-carbapenems

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This reports the synthesis and *in vitro* antimicrobial properties of a series of 2-thioether-linked quinolonyl-carbapenems. Although the title compounds exhibited broad spectrum activity, the MICs were generally higher than those observed for selected benchmark carbapenems, quinolonyl-penems, and quinolones. Enzyme assays suggested that the title compounds are potent inhibitors of penicillin binding proteins and inefficient inhibitors of bacterial DNA-gyrase. Uptake studies indicated that the new compounds are not substrates for the *norA* encoded quinolone efflux pump.

Quinolonyl-lactams are a new class of antimicrobial agents that incorporate both beta-lactam and quinolonyl substructures[1~9]. These new antimicrobial agents employed a variety of beta-lactam moieties, including cephalosporins, penems, and carbapenems, as well as several quinolonyl substituents. Linking groups between the quinolone and the lactam included ester, carbamate, dithiocarbamate, quaternary ammonium, and amines.

The quinolonyl-lactams generally exhibited high potency and a broad spectrum of activity against Gram-positive and Gram-negative bacteria[10~16]. Reports indicated that they retain activity against various antibiotic-resistant bacteria, including beta-lactamase producing Gram-negative bacteria and methicillin-resistant *Staphylococcus aureus*[14,16,17]. This activity profile reflected contribution from both the lactam and the quinolonyl pharmacophores[2,18].

We report herein on the synthesis and biological properties of thioether-linked quinolonyl-carbapenems, which are based on carbapenem substructures known to possess broad spectrum antibacterial properties. The new compounds and related reference compounds are shown in Figs. 1 and 2. Preliminary results of these studies were presented earlier.[19]

Materials and Methods

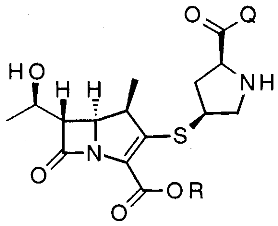
Chemistry

The key intermediates used to prepare the new quinolonyl-carbapenems are shown in Figure 3. Ciprofloxacin (**3a**) was purchased commercially. The 6,8-difluoro quinolone system was prepared by literature conditions[21], and the quinolone derivatives **3b**~**3e** were prepared using standard conditions[22,23]. The carbapenem intermediates **4a**~**4d** were prepared according to procedures reported[24,25] for the synthesis of closely related analogs. The thioacetylproline intermediate **5** was prepared according to procedures published for the synthesis of meropenem[26]. The new quinolonyl-carbapenems were prepared using a strategy whereby a quinolonyl mercaptan was added to an appropriately substituted beta-lactam such as **4a** or **4b**, or whereby a 2-hydroxyethylthio carbapenem such as **4c** or **4d** was linked to a quinolonyl moiety *via* an ester, carbamate, or related functional group[27].

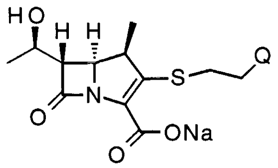
Synthesis of 1a~1c (Scheme 1)

The acid **5** was coupled with the quinolonyl compound **3b** in the presence of dicyclohexylcarbodiimide to give **6a** in good yield. This intermediate could be stored in the freezer. Conversion of **6a** to the corresponding thiol

Fig. 1. Structures of new quinolonyl-carbapenems.



1



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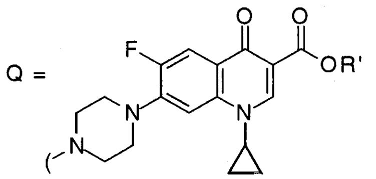
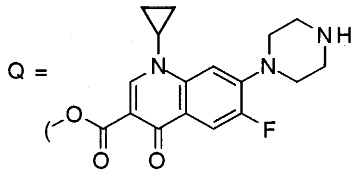
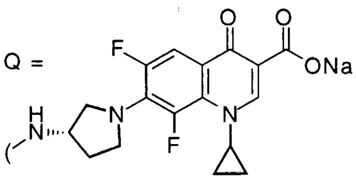


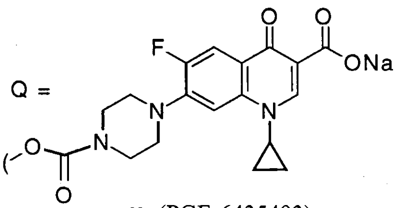
1a (PGE-5428060): R=R'=Na
1c (PGE-4136362): R=H, R'=CH_3
2c (PGE-9995108): R'=Na



2a (PGE-4372179)

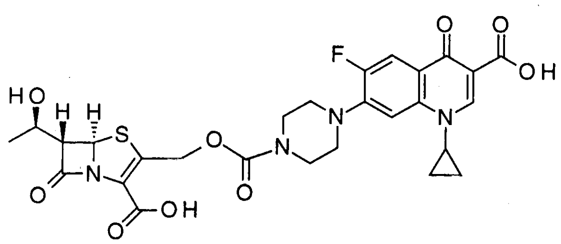


1b (PGE-2104907): R=Na

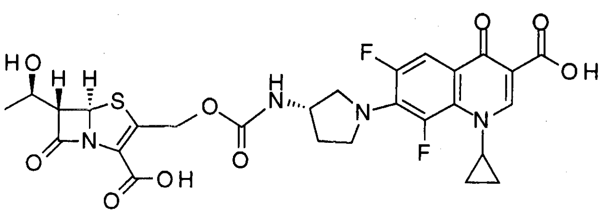


2b (PGE-6435493)

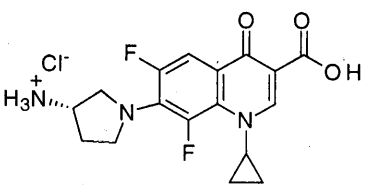
Fig. 2. Reference compounds.[20]



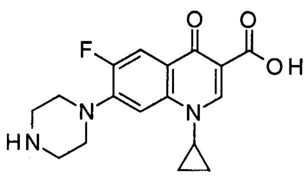
PGE-9464192



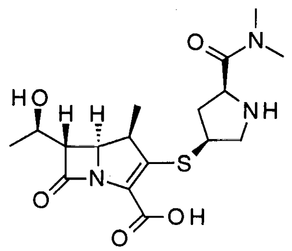
PGE-42816



PGE-1842612

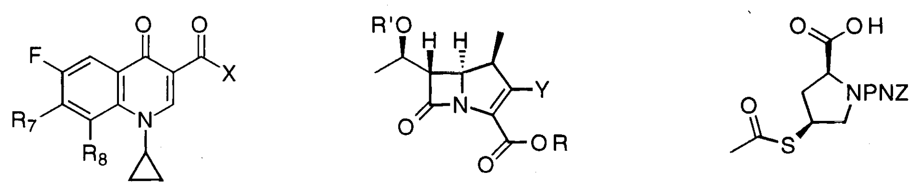


Ciprofloxacin

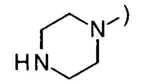
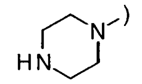
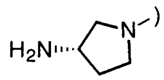
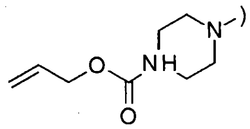
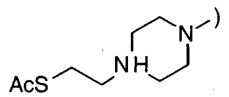


Meropenem

Fig. 3. Key intermediates.



3 4 5

Compound	X	R7	R8
3a	OH		H
3b	OPNB		H
3c	OPNB		F
3d	Cl		H
3e	O-allyl		H

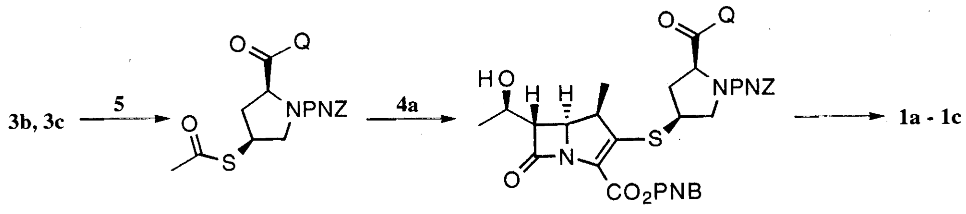
Compound	Y	R	R'
4a	$-OPO(OPh)_2$	PNB	H
4b	-OTf	allyl	H
4c	$-SCH_2CH_2OH$	allyl	H
4d	$-SCH_2CH_2OH$	allyl	alloc

was achieved *via* treatment with pyrrolidine in acetonitrile. The thiol was isolated after an acidic aqueous workup. Although disulfide formation was not observed, this intermediate was used promptly in the next step. Addition of the thiol to **4a** resulted in smooth formation of **7a**, which was purified *via* flash chromatography. Removal of the PNB and PNZ protecting groups under a hydrogen balloon required relatively long reaction times, which were associated with partial hydrolysis of the carbapenem. However, removal of these groups under 3 atmospheres of hydrogen proceeded in *ca.* 3 hours, without significant hydrolysis of the carbapenem. The diacid formed in the deprotection process was converted to the disodium salt **1a** with sodium 2-ethylhexanoate. Purification by preparative scale HPLC provided a sample for screening in bioassays.

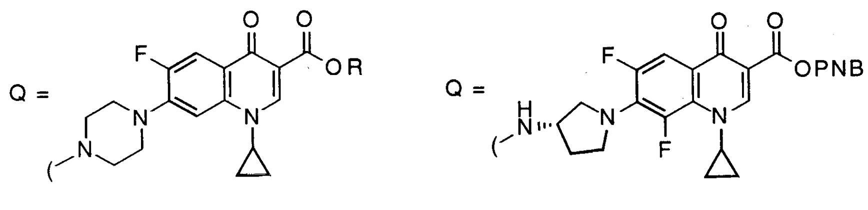
In a similar reaction sequence, **3c**, **5**, and **4a** were converted to the quinolonyl-carbapenem **1b**.

The preparation of **1c** was very similar to the preparation of **1a**. However, the conversion of **6a** to a reactive thiol nucleophile was accomplished with NaOH in aqueous methanol rather than with pyrrolidine in acetonitrile. This resulted in a facile transesterification process whereby the quinolonyl ester was converted from PNB to methyl. Treatment of this thiol with **4a** thus gave **7c**, which was purified *via* flash chromatography.

Scheme 1. Synthesis of **1a**~**1c**.



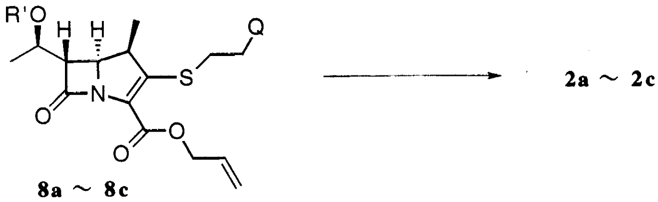
6a, 6b **7a, 7b, 7c**



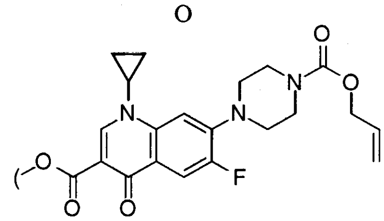
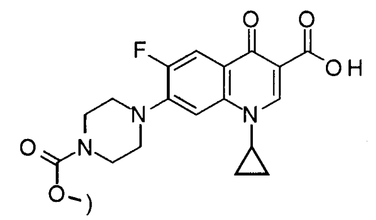
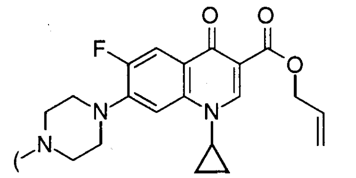
6a, 7a: R = PNB
7c: R = CH_3

6b, 7b

Scheme 2. Synthesis of **2a**~**2c**.



8a ~ **8c**

Compound (**8**)	R′	O
4d + **3d** → **8a**	alloc	
4c + **3a** → **8b**	H	
4b + **3e** → **8c**	H	

Deprotection and purification using preparative scale HPLC provided a sample of **1c** that was suitable for testing.

Synthesis of **2a**~**2c** (Scheme 2)

Quinolonyl-carbapenem **2a** was prepared by forming an ester linkage between the acid chloride **3d** (generated by treatment with oxalyl chloride) and carbapenem **4d** under basic conditions. The protected intermediate **8a** was purified *via* flash chromatography. Removal of the allyl protecting groups was achieved in one step to give **2a**.

The carbamate-linked analog **2b** was prepared by first treating **4c** with 1,2,2,2-tetrachloroethyl chloroformate and pyridine. Reaction of the unisolated intermediate with ciprofloxacin (**3a**) in the presence of aqueous base led to the formation of **8b**. The crude product was deprotected and purified *via* preparative scale HPLC to obtain a sample of **2b** for screening purposes.

The amine-linked **2c** is an example of a quinolonyl-carbapenem where the cyclic diamine substituent of the quinolonyl moiety is not acylated. To prepare this compound, the allyl ester of ciprofloxacin was alkylated with 2-bromoethanol to give the expected *N*-2-hydroxyethyl intermediate. Conversion of this primary alcohol to the corresponding mesylate followed by treatment with potassium thioacetate resulted in **3e**. Conversion to the thiol was achieved with pyrrolidine in acetonitrile. This particular thiol tended to oxidize easily to the disulfide, so **8c** was generated by treatment of unisolated thiol with a preformed solution of the the triflate **4b**. Deallylation provided **2c**.

Results and Discussion

Growth Inhibition

The new quinolonyl-carbapenems were found to possess a unique spectrum of activity (Table 1). They were comparable to, or slightly less active than, meropenem against Gram-positive organisms (Table 2). However, they were significantly less potent than meropenem against Gram-negative bacteria. Compared to the benchmark quinolones (ciprofloxacin and PGE-1842612), the quinolonyl-carbapenems were generally more active against the streptococci, but were significantly less active against *S. aureus*, *Enterobacter faecalis*, and most Gram-negative pathogens. Compared to the previously reported[20] quinolonyl-penems PGE-9464192 and PGE-42816, the new quinolonyl-carbapenems were generally less potent and more narrow in spectrum.

Variations in the quinolonyl substituents did not significantly alter the overall activity. For example, esterification of the quinolone moiety, as in **1c**, provided a compound that was comparably potent to **1a**. Likewise, the 6,8-difluoro analog **1b** retained similar potency to **1a**. Variation of the linkage appeared to alter the antimicrobial activity, with an approximate order of potency being amine $>$ ester $\sim$ amide $>$ carbamate.

Inhibition of Bacterial Enzymes

The results of bacterial enzyme inhibition assays for selected compounds are shown in Table 3. Quinolonyl-carbapenem **1a** was a potent inhibitor of *S. aureus* PBP-1, PBP-2, and PBP-3. The IC_{50} for this compound was comparable to its MIC against the organism. Compound **1a** was not found to be an efficient inhibitor of *E. coli* DNA-gyrase. The quinolonyl-penem PGE-9464192 was a more efficient inhibitor of the DNA-gyrase enzyme.

Table 1. MICs (μg/ml) of quinolonyl-carbapenems.

Organism	**1a**	**1b**	**1c**	**2a**	**2b**	**2c**
Gram positive						
S. aureus ATCC 29213 (MSCS)	0.25	0.5	0.12	0.25	2	0.06
S. aureus Mi300 (MRCS)	2	8	2	2	8	2
S. aureus Mi339 (MRCR)	64	>64	64	—	—	128
S. aureus Mi345 (MSCR)	0.25	1	0.12	—	—	0.125
S. saprophyticus Mi276	1	4	0.25	1	16	0.5
Enterobacter faecalis ATCC 29212	8	8	2	4	16	2
Streptococcus pyogenes STA2	≤0.06	≤0.06	≤0.06	0.008	0.5	0.008
Streptococcus pneumonia STP1	≤0.06	0.25	≤0.06	0.125	0.5	0.016
Streptococcus viridans STV1	≤0.06	≤0.06	≤0.06	0.06	0.5	0.016
Gram negative						
Escherichia coli ATCC 25922	8	8	8	0.25	2	0.5
Pseudomonus aeruginosa ATCC 27853	>64	>64	>64	32	32	16
P. aeruginosa PS314 (CR)	>64	>64	>64	—	—	>128
Proteus miribalis PR91	64	64	>64	8	8	4
Klebsiella pneumonia KL21	0.5	4	0.25	—	0.5	0.125
K. pneumonia KL328 (CR)	64	64	32	—	—	64
Enterobacter cloacae AE63	8	8	8	0.5	2	0.25

Table 2. MICs (μg/ml) of reference compounds.

Organism	PGE-9464192	PGE-42816	Ciprofloxacin	PGE-1842612	Meropenem
Gram positive					
S. aureus ATCC 29213 (MSCS)	0.06	≤0.06	0.25	≤0.016	≤0.06
S. aureus Mi300 (MRCS)	0.5	≤0.06	0.25	0.03	1
S. aureus Mi339 (MRCR)	128	4	32	0.5	32
S. aureus Mi345 (MSCR)	0.25	0.25	16	0.5	≤0.06
S. saprophyticus Mi276	0.5	0.25	0.25	0.06	0.25
Enterobacter faecalis ATCC 29212	2	0.5	0.25	0.06	4
Streptococcus pyogenes STA2	0.016	≤0.06	0.5	0.06	≤0.06
Streptococcus pneumonia STP1	0.03	0.12	0.5	0.06	≤0.06
Streptococcus viridans STV1	0.03	≤0.06	2	0.03	≤0.06
Gram negative					
Escherichia coli ATCC 25922	0.12	≤0.06	≤0.016	≤0.016	≤0.06
Pseudomonus aeruginosa ATCC 27853	2	1	0.25	0.06	0.25
P. aeruginosa PS314 (CR)	>128	64	32	4	2
Proteus miribalis PR91	0.5	0.25	≤0.016	0.03	≤0.06
Klebsiella pneumonia KL21	0.06	≤0.06	≤0.016	≤0.016	≤0.06
K. pneumonia KL328 (CR)	64	16	2	0.5	≤0.06
Enterobacter cloacae AE63	0.03	≤0.06	≤0.016	≤0.016	≤0.06

Table 3. Inhibition (IC_{50}, μg/ml) of bacterial enzymes.

Enzyme	**1a**		PGE-9464192[28]	
	IC_{50}	MIC (μg/ml)	IC_{50}	MIC (μg/ml)
ATCC 25922 *E. coli* DNA gyrase	>100	8	9.0	0.12
ATCC 29213 *S. aureus* PBP-1	0.2	0.25	<0.2	≤0.06
ATCC 29213 *S. aureus* PBP-2	0.2	0.25	<0.2	≤0.06
ATCC 29213 *S. aureus* PBP-3	0.2	0.25	<0.2	≤0.06

The potencies of these compounds against the organisms from which the enzymes were derived were consistent with the enzyme inhibition (Table 3).

Effect of *norA* Encoded Quinolone Efflux Pump

The uptake of **1a** (PGE-5428060) and the reference quinolone ciprofloxacin were observed (*via* fluorescence intensity) in the *S. aureus* strains SA1199 and SA1199B. The latter strain overproduces the quinolone efflux pump encoded by the *norA* gene, while the former strain does not. Observations were made before and after poisoning of the efflux pump energy source with the protonophore carbonyl cyanide *m*-chlorophenlyhydrazone (CCCP), at the time indicated by the arrows in Figure 4. The MICs of **1a** and ciprofloxacin were also measured for these organisms to help put the uptake results in perspective.

In contrast to ciprofloxacin, **1a** did not act as a substrate for the *norA* encoded quinolone efflux pump in *S. aureus*. The uptake of this compound in SA1199B was not reduced relative to the parent strain SA1199. Furthermore, the level of uptake did not increase when the energy source of the efflux pump was poisoned with CCCP (at the time indicated by the arrow in Figure 4). Thus, the cytoplasmic concentration of **1a** was independent of the efflux pump. This observation was corroborated by growth inhibition studies, which showed that the MIC for ciprofloxacin was 0.5 μg/ml against SA1199 and 16 μg/ml against SA1199B, whereas the MIC for **1a** was 0.5 μg/ml against both strains.

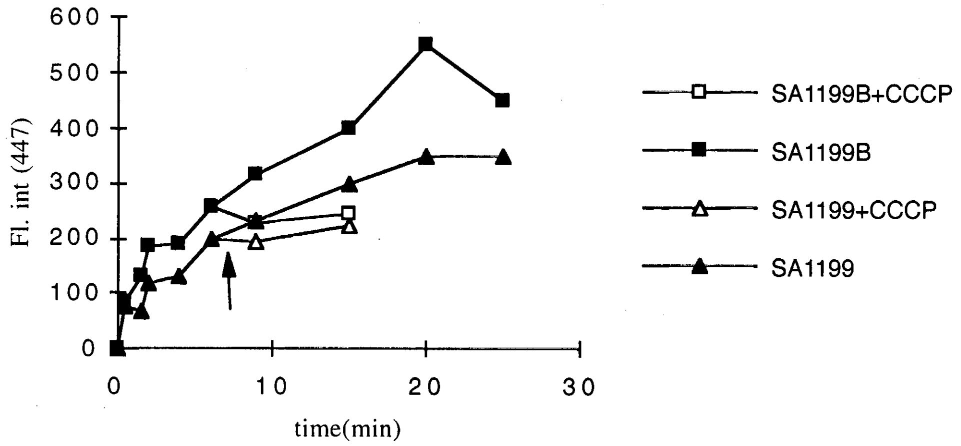
Experimental

NMR spectra were recorded using a General Electric QE-300, a General Electric GN-500, a Bruker AC-300, or a Varian 300 spectrometer. ESI-MS was performed on a Micromass Platform II mass spectrometer, using

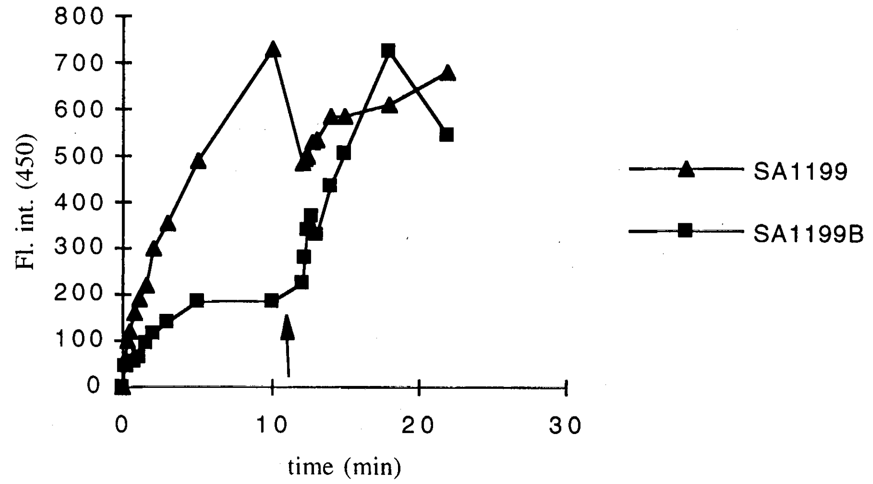
Fig. 4. Effect of *norA* encoded quinolone efflux pump.

(A) Uptake of PGE-5428060, (B) uptake of ciprofloxacin.

(A)



(B)



Uptake was monitored as fluorescence intensity (Fl. int.) at the emission wavelength indicated in Figure 4.

flow injection analysis. Fast atom bombardment MS (FAB-MS) spectra were obtained on a VG Analytical Ltd. ZAB-2F spectrometer, and plasma desorption MS (PD-MS) were obtained on an Applied Biosystem AB Bio-ION 20 252^{Cf} plasma desorption mass spectrometer. High resolution mass spectrometry (HR-MS) was performed at the Nebraska Center for Mass Spectrometry on a VG AutoSpec mass spectrometer, operated in the fast atom bombardment mode, with 3-nitrobenzyl alcohol as the matrix solvent. Flash chromatography was done using Merck silica gel 60, 230~400 mesh, 60 angstroms. Thin layer chromatography (TLC) analyses were performed on silica gel plates: E. Merck 0.20 mm pre-coated (60 F_{254}). Reversed phase TLC (RP TLC) analyses were performed on Baker Si $C_{18}F$ 200 μm octadecyl silica glass backed plates using 0.9 M aq NaCl-CH_3CN, 3:2 (v/v), as eluent. TLC plates were visualized by UV light or by the use of 15% phosphomolybdic acid in ethanol. Analytical scale reversed phase high performance liquid chromatography (RP HPLC) relating to **1a**~**1c** was performed on a Zorbax RX-C8 column (inner diameter 4.6 mm, length 25.0 cm, 5 μm particle size), eluting as follows: A-B, 3:2 (v/v), for one minute, followed by a linear ramp to A-B, 1:19 (v/v), over 19 minutes, then A-B, 1:19 (v/v), for 7 minutes, where "A" was water, "B" was MeOH, and both solvents contained 0.2% formic acid and a 5 mM concentration of NH_4OAc. Preparative RP HPLC on **1a**~**1c** was performed on a reverse phase Zorbax C8 prep scale column. **1a** was eluted with CH_3CN - water, 1 : 4 (v/v), for 15 minutes, followed by a two minute ramp to CH_3CN - water, 3 : 10 (v/v). **1b** was eluted with a gradient that ramped linearly from water - CH_3CN, 13 : 20 (v/v), to water - CH_3CN, 1 : 9 (v/v), over 30 minutes **1c** was eluted using a gradient that started at CH_3CN - water, 1 : 9 (v/v), and ramped to CH_3CN, 9 : 1 (v/v), over 30 minutes. RP HPLC relating to **2a** and **2c** was performed on a Vydac C_4 Peptide/Protein column (inner diameter 4.6 mm, length 25.0 cm, 5 μm particle size), 2.0 ml/minute flow rate, using UV detection at 300 nm: system A employed a gradient from D - C, 1 : 4 (v/v), to 100% D over 8 minutes where "C" was CH_3CN - 0.014 M aq NH_4OAc, 1 : 99 (v/v), at pH 6.88 and "D" was CH_3CN - 0.014 M aq NH_4OAc, 1 : 1 (v/v), system B employed a gradient from F - E, 1 : 4 (v/v), to 100% F over 8 minutes where "E" was water - CH_3CN - triethylamine - 0.5 M aq EDTA, 985 : 10 : 2 : 3 (in volume), and "F" was water - CH_3CN - triethylamine - 0.5 M aq EDTA, 495 : 500 : 2 : 3 (in volume), and both "E" and "F" were adjusted to pH 6.7 by the addition of concentrated HCl. Preparative RP HPLC relating to **2b** was performed using a 10 μm Waters μBondapak® C_{18}, 19 × 150 mm column, eluting with CH_3CN - water, 3 : 22 (v/v), at 9.9 ml/minute. Solvents and reagents were Aldrich sure seal grade when available. Ciprofloxacin was purchased in the form of 750 mg Cipro tablets. (3*S*,4*S*)-3-((*R*)-(*tert*-butyldimethylsilyloxy)ethyl)-4-((*R*)-carboxyethyl)-2-azetidinone was purchased from Kaneka Corp., and used as starting material to prepare key carbapenem intermediates. Rhodium (II) dioctanoate was purchased from Strem. The pH 7 buffer used in many workups was prepared from 205 g KH_2PO_4, 35 g NaOH, and one liter of water. Reactions were run under anhydrous inert atmosphere unless indicated otherwise.

Bacterial strains were obtained from clinical microbiology laboratories and the American Type Culture Collection (Rockville, MD). Organism identity was confirmed by biochemical testing, colony morphology, and gram stain. Minimal inhibitory concentration (MIC) data for aerobic organisms were determined by the standard microbroth dilution method[29] using brain heart infusion medium. The results of these assays are shown in Table 1.

PBP binding assays using *S. aureus* ATCC 29213 were performed according to SPRATT[30]. Bands were detected by autoradiography and analyzed by densitometry. IC_{50} values were determined from plots of band density *vs.* log drug concentration. The gyrase supercoiling inhibition assay was performed according to BARRETT[31]. Bands were resolved in a 1% TAE agarose gel. Overproducing gyrase clones were kindly provided by Dr. MARTIN GELLERT. Purification of gyrase was performed according to MIZUUCHI[32].

(3*S*,5*S*)-1-(4-Nitrobenzyloxycarbonyl)-3-thioacetyl-5-[4-(3-(4-nitrobenzyloxycarbonyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]-carbonylpyrrolidine (**6a**)

The acid **5**[26] (1.051 g, 2.86 mmol) was weighed into a 2-neck vessel. Dicyclohexylcarbodiimide (0.648 g, 3.14 mmol) was added as solid. Methylene chloride (28 ml) was added to produce a slurry. The *p*-nitrobenzyl ester of ciprofloxacin, **2a**, (1.435 g, 2.86 mmol) was added, followed by 10 ml of DMF. The mixture became less heterogeneous. Addition of 4-dimethylaminopyridine (0.383 g, 3.14 mmol) further reduced the amount of solid in the mixture. After 17 hours, the mixture was partitioned between 200 ml of water and 200 ml of chloroform. The organics were washed with 200 ml of water, dried over magnesium sulfate, and concentrated to provide 4.7 g of crude product. Flash chromatography on 250 ml of silica gel, eluting with acetone - $CHCl_3$, 1 : 9 (v/v), acetone - $CHCl_3$, 1 : 3 (v/v), and then with acetone - $CHCl_3$, 1 : 1 (v/v), furnished 1.8448 g of purified material that was not free of DMF and some minor impurities. A second flash chromatography was run on 200 ml of silica gel to return 1.5825 g (68%) of **6a**. ^1H NMR (300 MHz, $CDCl_3$) δ 8.57 (1H, s), 8.23 (2H, d, J=8.8), 8.21 (2H, d, J=8.7), 8.08 (1H, d, J=13.1), 7.72 (2H, d, J=8.8), 7.50 (1.6H, d, J=8.8), 7.48 (0.4H, d, J=8.8), 7.27 (1H, d), 5.48 (2H, s), 5.39 (0.2H, d, J=*ca.* 16), 5.22 (1.6H, s), 5.03 (0.2H, d, J=*ca.* 16), 4.80 (0.8H, t, J=*ca.* 7), 4.76 (0.2H, t, J=*ca.* 7), 4.2~4.0 (3H, m), 4.0~3.8 (2H, m), 3.75~3.60 (2H, m) 3.55~3.40 (2H, m), 3.40~3.2 (3H, m), 2.85~2.75 (1H, m), 2.34 (3H, s), 1.33 (2H, br d), 1.14 (2H, m). ESI-MS m/z 817 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-1-(4-Nitrobenzyloxycarbonyl)-5-[4-(3-(4-nitrobenzyloxycarbonyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]-carbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid, 4-Nitrobenzyl Ester (**7a**)

The thioacetate **6a** (0.631 g, 0.77 mmol) was dissolved in 6.6 ml of acetonitrile to give a yellow solution. Pyrrolidine (0.39 ml, 4.6 mmol) was added, and solid began to form within minutes. After 26 minutes, the mixture was partitioned between chloroform and 0.5 M HCl. The aqueous layer remained acidic to pH paper. The organics were washed with brine and then dried over magnesium sulfate. Concentration provided 0.6871 g of crude thiol as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (1H, s), 8.17 (2H, d, J=8.7), 8.15 (2H, d, J=8.7), 8.03 (1H, d, J=13), 7.66 (2H, d, J=8.8), 7.45 (1.6H, d, J=8.7), 7.40 (0.4H, d, J=*ca.* 8.7), 7.25 (1H, d), 5.42 (2H, s), 5.35 (1.6H, d, J=8.7), 7.40 (0.4H, d, J=*ca.* 8.7), 7.25 (1H, d), 5.42 (2H, s), 5.35 (0.2H, d, J=*ca.* 16), 5.17 (1.6H, br s), 4.95 (0.2H, d, J=*ca.* 16), 4.71 (0.8H, t, J=*ca.* 7), 4.65 (0.2H, t, J=*ca.* 7), 4.15~3.95 (2H, m), 3.85~3.75 (1H, m), 3.75~3.55 (3H, m), 2.8~2.65 (1H, m), 1.86 (1H, d, J=8.9), 1.27 (2H, br d), 1.07 (2H, m). Some resonances are obscured by the pyrrolidinyl acetamide by-product. The freshly prepared crude thiol (maximum 0.7 mmol) was transferred with 2.0 ml of acetonitrile to a freshly prepared ice cold solution of **4a** (maximum 1.16 mmol). The solution of **4a** already contained an excess of diisopropylethylamine. The transfer was completed with two 0.5 ml portions of acetonitrile. After stirring for 1.75 hours, during which the reaction became heterogeneous, it was partitioned between brine and chloroform. The aqueous part was extracted once more with chloroform, and the combined organics dried and concentrated to give 1.5054 g (180%) of crude **7a**. Flash chromatography was executed on 80 ml of silica gel, eluting with acetone - $CHCl_3$, 1 : 3 (v/v), followed by MeOH - CH_2Cl_2, 1 : 19 (v/v), and then with MeOH - CH_2Cl_2, 1 : 9 (v/v). Some mixed fractions were recovered and rechromatographed to maximize the yield of pure material. The total yield of pure **7a** was 0.57 g (66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (1H, s), 8.25~8.20 (6H, m), 8.07 (1H, d, J=13), 7.72 (2H, d, J=8.8), 7.64 (2H, d, J=8.8), 7.51 (1.4H, d, J=8.8), 7.45 (0.6H, d, J=*ca.* 8.8), 7.29 (1H, d), 5.49 (1H, d, J=*ca.* 14), 5.35 (0.3H, d. J=*ca.* 16), 5.22 (1H, d, J=14), 5.22 (1.4H, br s), 5.06 (0.3H, d, J=*ca.* 16), 4.82 (1.4H, t, J=*ca.* 7), 4.78 (0.6H, t, J=*ca.* 7), 4.27 (1H, dd, J=9.4, 2.6), 1.36 (3H, d, J=6.2), 1.28 (3H, d, J=7). ^{19}F NMR ($CDCl_3$) δ 43.1 (0.7F), 42.9 (0.3F). HR-MS m/z 1119.3208 $(M+H)^+$, 1119.3205 (calcd $(M+H)^+$).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]-carbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hdroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid Disodium Salt (**1a**)

The triply protected intermediate **7a** (0.249 g, 0.222 mmol) was combined in a Parr bottle with 0.237 g of 10% Pd/C (0.222 mmol), 15.5 ml of THF, 2.2 ml of EtOH, and 11.1 ml of 0.1 M ammonium acetate solution. This mixture was shaken under 45 psi of hydrogen for 3.3 hours. The catalyst was filtered off, and the bottle and filter were rinsed with THF - water, 3 : 2 (v/v), to complete the transfer. The filtrate was washed with EtOAc and the cloudy aqueous portion concentrated without warming. It was preferable to lyophilize the aqueous extracts. This provided the crude aminodiacid form of **1a** as a pale green solid. MS: MH+ = 670. The crude product was treated with 14 ml of CH_3CN - water, 1 : 1 (v/v), to produce a slurry. Addition of 340 mg of sodium 2-ethyl hexanoate made it become homogenous. After stirring for 15 minutes, 180 ml of acetone was added. A precipitate settled in the flask after 2.5 hours. The supernatent was carefully removed without disturbing the precipitate. The residue was easily dissolved in water and freeze dried to give crude **1a** as a brown solid weighing 90.8 mg. This represented a 57% yield for the triple deprotection and salt formation. HPLC was used to purify a portion of this material for biological testing. Thus, 10.6 mg of crude **1a** were purified using preparative RP HPLC. Although the material eluted within the first five minutes, fractions of pure material were obtained. The total recovery of good fractions was *ca.* 4 mg, which represented a 22% yield from **7a**. ^{1}H NMR (500 MHz, DMSO-d_6 + d-TFA) δ 8.68 (1H, s), 7.95 (1H, d, J=12.8) 7.58 (1H, d, J=7.3), 4.76 (1H, t, J=8.5), 4.0~3.9 (2H, m), 3.89 (1H, t, J=6.1), 3.85~3.7 (4H, m), 3.7 (3H, br s), 3.45~3.3 (4H, m), 3.24 (1H, dd, J=12, 7.5), 2.96 (1H, dt, J=13, 7.9), 2.85 (1H, dd, J=10.4, 6.1), 1.87 (1H, dt, J=12.8, 9), 1.3 (2H, br d), 1.18 (3H, d, J=6.1), 1.16 (3H, d, J=6.7), 1.1 (2H, m). ^{19}F NMR (300 MHz, DMSO-d_6 + d-TFA) δ 45.1. HR-MS m/z 670.2338 $(M+H)^+$, 670.2347 (calcd $(M+H)^+$ for diacid form). RP HPLC Rt = 8.97 minutes (UV detection at 283 nm, peak area = 92.4% with no detectable quinolone impurity).

(3*S*,5*S*)-1-(4-Nitrobenzyloxycarbonyl)-3-thioacetyl-5-[1-(3-(4-nitrobenzyloxycarbonyl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-7-quinolinyl)-*N*-(3*S*)-aminopyrrolidinyl]carbonylpyrrolidine (**6b**)

The acid **5** (2.36 g, 6.4 mmol) was combined with dicyclohexylcarbodiimide (1.48 g, 7.2 mmol) and 64 ml of methylene chloride. The quinolone **3c** was added, followed by 10.6 ml of DMF and finally 0.88 g (7.2 mmol) of 4-dimethylaminopyridine. The reaction was stirred overnight at room temperature, during which it became more homogenous. The mixture was diluted with chloroform and washed with three 50 ml portions of water and three 50 ml portions of brine. The organics were dried and concentrated to give 6.1 g (114%) of crude **6b**. Flash chromatography, eluting with acetone - $CHCl_3$, 1 : 19 (v/v), and then with acetone - $CHCl_3$, 1 : 3 (v/v), returned 2.2 g (41%) of pure **6b** as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ 8.6 (1H, s), 8.3~8.2 (4H, m), 7.86 (1H, br d), 7.75 (2H, d), 7.54 (2H, m), 7.15 (1H, m), 5.52 (2H, s), 5.27 (2H, br s), 4.59 (1H, m), 4.42 (1H, m), 4.18 (1H, dd), 4.1~3.95 (2H, m), 3.95~3.8 (2H, m), 3.8~3.7 (1H, m), 3.7~3.4 (2H, m), 2.7~2.4 (2H, m), 2.39 (3H, s), 2.35~2.2 (1H, m), 2.1~1.9 (1H, m), 1.3 (2H, br d), 1.15 (2H, m). ESI-MS m/z 835 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-1-(4-Nitrobenzyloxycarbonyl)-5-[1-(3-(4-nitrobenzyloxycarbonyl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-7-quinolinyl)-*N*-(3*S*)-aminopyrrolidinyl]carbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid, 4-Nitrobenzyl Ester (**7b**)

The thioacetate **6b** (0.317 g, 0.379 mmol) was dissolved in 2 ml of acetonitrile and treated with 0.19 ml (2.28 mmol) of pyrrolidine. The mixture became heterogeneous and was dissolved in chloroform and washed with 0.1 M HCl after 30 minutes. The organics were then washed with brine, dried over magnesium sulfate, and concentrated to give 0.3469 g (115%) of the corresponding crude thiol. The material was used promptly without further purification. The thiol (maximum 0.379 mmol) was transferred with 1.0 ml of acetonitrile to an ice cold solution of freshly prepared **4a** (maximum 0.564 mmol) in 3.3 ml of acetonitrile. The solution of **4a** already contained an excess of diisopropylethylamine. The transfer was completed with two 0.5 ml portions of acetonitrile. The reaction became turbid as it stirred for 2.3 hours in the ice bath. It was partitioned between chloroform and brine. The organics were dried and concentrated to give 0.7397 g (172%) of crude **7b**. Flash chromatography on 40 ml of silica gel, eluting with acetone - $CHCl_3$, 1 : 3 (v/v), followed by MeOH - $CHCl_3$, 1 : 19 and then 1 : 9 (v/v), returned 207.3 mg (48%) of **7b**. ^1H NMR (300 MHz, $CDCl_3$) δ 8.59 (1H, s), 8.3~8.1 (6H, m), 7.85 (1H, m), 7.73 (2H, d), 7.63 (2H, d), 7.44 (2H, m), 7.05 (1H, m), 5.54 (2H, s), 5.45 (1H, m), 5.35~5.2 (3H, m), 4.62 (1H, m), 4.50 (1H, m), 4.4~4.25 (2H, m), 4.1~3.9 (2H, m), 3.9~3.7 (3H, m), 3.7~3.45 (3H, m), 3.42~3.3 (2H, m), 2.95~2.8 (1H, m), 2.75~2.5 (1H, m), 2.4~2.2 (2H, m), 2.1~2.0 (2H, m), 1.42 (3H, d), 1.35 (3H, d), 1.3 (2H, m), 1.15 (2H, m). ESI-MS m/z 1137 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-[1-(3-Carboxy-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-7-quinolinyl)-*N*-(3*S*)-aminopyrrolidinyl]carbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hdroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid Disodium Salt (**1b**)

The triply protected compound **7b** (270 mg, 0.238 mmol) was combined in a Parr bottle with 14.5 ml of THF, 2.2 ml of ethanol, 11.1 ml of 0.1 M ammonium acetate solution, and 250 mg of 10% Pd/C. This mixture was shaken under 45 psi of hydrogen for 2.5 hours, after which the catalyst was removed *via* filtration. The aqueous portion was washed with ethyl acetate and then freeze dried to a yellow-green solid. The recovery of crude aminodiacid was 190 mg. This material was converted to the disodium salt without further purification. MS: MH+ = 688. The crude diacid (33.2 mg, 0.043 mmol) was dissolved in 3 ml of 50% acetonitrile/water to produce a yellow solution. Sodium 2-ethyl hexanoate (0.096 g, 0.58 mmol) was added. After 15 minutes, 40 ml of acetone was added to induce precipitation of **1b**. After the solid settled for two hours, the supernatent was carefully removed. The residue was easily dissolved in water and lyophilized to provide 26.6 mg of crude **1b**, a 72% yield based on crude aminodiacid. A portion of this sample (11.4 mg) was purified *via* preparative RP HPLC to return 4.6 mg (7%) of pure **1b**. ^1H NMR (500 MHz, D_2O) δ 8.6 (1H, br s), 7.7 (1H, br s), 4.48 (1H, m), 4.3~4.1 (2H, m), 4.1~3.8 (4H, m), 3.8~3.5 (3H, m), 3.3~3.2 (3H, m), 2.9~2.6 (1H, m), 2.3~2.2 (1H, m), 2.2~1.9 (3H, m), 1.6~1.4 (6H, m), 1.4~1.3 (2H, m). HR-MS m/z 688.2239 $(M+H)^+$, 688.2228 (calcd $(M+H)^+$ for diacid). RP HPLC Rt = 8.97 minutes (UV detection at 292 nm, peak area = 86.2% with no detectable quinolone impurity).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-1-(4-Nitrobenzyloxycarbonyl)-5-[4-(3-carboxymethyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid, 4-Nitrobenzyl Ester (**7c**)

The thioacetate **6a** (0.328 g, 0.410 mmol) was dissolved in 0.75 ml of THF and 0.75 ml of methanol. The solution was chilled in an ice bath, which caused precipitation. Another 0.25 ml of THF was added restore homogeneity. The mixture was treated with 0.11 ml of 4 N NaOH. TLC after 10 minutes indicated completion, so the reaction was quenched with pH 7 buffer and extracted with two 20 ml portions of chloroform. The organics were dried and concentrated to give the thiol methyl ester intermediate as a pale yellow solid. It was used promptly in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (1H, s), 8.25 (2H, 2d, J=8.9), 8.07 (1H, d, J=13), 7.52 (2H, 2d, J=8.8), 7.28 (1H, d), 5.39 (0.2H, d, J=*ca.* 14), 5.23 (1.6H, br s), 5.04 (0.2H, d, J=*ca.* 14), 4.84 (0.8H, t, J=*ca.* 7), 4.72 (0,2H, t, J=*ca.* 7), 3.92 (3H, s), 1.93 (1H, d, J=8.9), 1.33 (2H, br d), 1.14 (2H, m). The crude thiol methyl ester (maximum 0.410 mmol) was transferred with 0.50 ml of acetonitrile to an ice cold solution of **4a** (maximum 0.416 mmol). This solution already contained an excess of diisopropylethylamine. The transfer was completed with an additional 0.3 ml and 0.2 ml of acetonitrile. After stirring for about 2 hours, the mixture was partitioned between chloroform and brine. The organics were concentrated to give 0.6049 g (134%) of **7c** as a crude yellow solid. Flash chromatography on 60 ml of silica gel, eluting with acetone-$CHCl_3$, 1 : 1 and then 3 : 1 (v/v), furnished 0.1414 g of pure **7c**, a 35% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (1H, s), 8.21 (2H, d, J=8.8), 8.20 (2H, d, J=8.9), 8.03 (1H, d, J=13), 7.64 (2H, d, J=8.7), 7.50 (1.4H, d, J=8.7), 7.45 (0.6H, d, J=*ca.* 8.7), 7.26 (0.7H, d, J=*ca.* 8), 7.21 (0.3H, d, J=*ca.* 8), 5.48 (1H, d, J=14), 5.35 (0.3H, d, J=*ca.* 14), 5.22 (1H, d, J=14), 5.22 (1.4H, s), 5.06 (0.3H, d, J=*ca.* 14), 4.81 (0.7H, t, J=*ca.* 7), 4.79 (0.3H, t, J=*ca.* 7), 4.35~4.19 (2H, m), 4.10 (1H, m), 4.05~3.8 (2H, m), 3.90 (3H, s), 3.8~3.6 (3H, m), 3.54 (1H, m), 3.5~3.3 (3H, m), 3.3~3.1 (4H, m), 2.78 (1H, m), 2.35 (1H, m), 1.95 (1H, m), 1.36 (3H, d, J=6.2), 1.28 (3H, d, J=7.2), 1.3 (2H, m) 1.13 (2H, m). HR-MS m/z 998.3037 $(M+H)^+$, 998.3042 (calcd $(M+H)^+$).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-[4-(3-Carboxymethyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hdroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid Disodium Salt (**1c**)

The methyl ester **7c** (85 mg, 0.085 mmol) was combined in a Parr bottle with 5.9 ml of THF, 0.8 ml of ethanol, 4.2 ml of 0.1 M ammonium acetate, and 90 mg (0.085 mmol) of 10% Pd/C. The mixture was shaken under 45 psi hydrogen for 3.3 hours, after which the catalyst was removed *via* filtration. The filtrate was washed with ethyl acetate, and the aqueous portion was freeze dried to give 52.8 mg (91%) of crude **1c**. MS: MH+ =684. A 10 mg sample of this material was purified using preparative RP HPLC to recover 3.1 mg (28%) of **1c**. ^{1}H NMR (300 MHz, DMSO-d_6+d-TFA) δ 8.50 (1H, s), 7.82 (1H, d, J=13), 7.45 (1H, d, J=7), 4.76 (1H, br t), 4.08 (1H, dd), 3.70 (3H, s). ^{19}F NMR (300 MHz, DMSO-d_6+d-TFA) δ 42.1. HR-MS m/z 684.2516 $(M+H)^+$, 684.2503 (calcd $(M+H)^+$). RP HPLC Rt=15.78 minutes (UV detection at 281 nm, peak area=79.1% with no detectable quinolone impurity.

(1*R*,5*S*,6*S*)-2-[[[1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-allyloxycarbonyl)piperazinyl-3-carbonyl-quinolinyl]oxy]eth-2-ylthio]-6-[(*R*)-1-(*O*-allyloxy-carbonyl)ethyl]-1-methylcarbapen-2-em-3-carboxylic Acid, Allyl Ester (**8a**)

The acid chloride **3d** was formed using 0.035 g (0.084 mmol) of *N*-Alloc ciprofloxacin, 0.032 ml (0.047 g, 0.37 mmol) of oxalyl chloride, and 0.5 ml CH_2Cl_2. The mixture was refluxed for 15 minutes, diluted with 2 ml of carbon tetrachloride, and concentrated. The yellow solid residue of acid chloride obtained was flushed with argon, dissolved in 1 ml of CH_2Cl_2, and chilled in an ice bath. A solution of 0.036 g (0.089 mmol) of **4d** and 0.020 ml (0.015 g, 0.11 mmol) of *N*,*N*-diisopropylethylamine in 0.9 ml of $CHCl_3$ was added to the acid chloride over 1.5 minutes (the cloudy solution became clear). After 15 minutes, the ice bath was removed and the solution was stirred an additional 2.5 hours. before pouring it into 10 ml of 0.1 M HCl. The aqueous layer was extracted with two 10 ml portions of $CHCl_3$, and the combined organic layers were washed successively with H_2O and brine. The solution was dried over Na_2SO_4, filtered, concentrated, and flash chromatographed on 17 g of silica gel, eluting with EtOAc followed by $CHCl_3$-MeOH, 19 : 1 (v/v), to provide 0.030 g (0.037 mmol, 44%) of **8a**. TLC Rf 0.33 (EtOAc), 0.38 (95/5 $CHCl_3/CH_3OH$). FAB-MS m/z 809 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[[[1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-piperazinyl-3-carbonyl-quinolinyl]oxy]eth-2-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid Sodium Salt (**2a**)

The protecting groups were removed by dissolving 0.088 g (0.11 mmol) of **8a** in 5 ml of CH_2Cl_2 plus 0.015 ml of H_2O along with 0.0034 g (0.0048 mmol, 4.4 mol%) of $PdCl_2(PPh_3)_2$ under an argon atmosphere and adding 0.133 ml (0.144 g, 0.495 mmol) of tributyltin hydride to the stirred mixture. Some precipitate formed from the initially homogeneous mixture as the reaction progressed. After 1 hour the mixture was diluted with 5 ml of Et_2O and centrifuged. Only a small amount (*ca.* 7 mg) of solid was obtained. The supernatent liquid was concentrated to *ca.* 2 ml and treated with 10 ml of Et_2O. The precipitate collected after centrifuging was combined with the first precipitate. ^{1}H NMR analysis showed the presence of tributyltin residues. The precipitate was suspended in 8 ml of CH_3CN-H_2O, 1:1 (v/v), and extracted with three 5 ml portions of ether. The aq layer was partially concentrated and then freeze-dried to provide 0.032 g (47%) of solid **2a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.40 (1H, s), 7.75 (1H, d), 7.40 (1H, m), 5.0 (1H, m) 4.35~4.2 (m), 4.06 (1H, m), 3.95~3.8 (m), 3.7~2.9 (m), 1.25 (m), 1.2~1.0 (m). RP HPLC Rt=8.8 minutes (system B).

(1*R*,5*S*,6*S*)-2-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]-carbonyl]oxy]eth-2-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid, Allyl Ester (**8b**)

A mixture of 0.209 g (0.63 mmol) of ciprofloxacin **3a** and 0.261 g (3.1 mmol) of $NaHCO_3$ were suspended in 4 ml of H_2O and stirred in a 15 ml flask for 1 hour. A separate flask containing 0.178 g (0.54 mmol) of **4c** and a stir bar was fitted with a septum and flushed with argon. Dry THF (3 ml) was added, the mixture was cooled in an ice bath, and 0.102 ml (0.132 g, 0.54 mmol) of 1,2,2,2-tetrachloroethyl chloroformate was added. The dropwise addition of 0.047 ml (0.046 g, 0.57 mmol) of pyridine led to a gummy precipitate. After 35 minutes this cold solution was transferred *via* syringe into the above ciprofloxacin/$NaHCO_3$/H_2O mixture over 2 minutes. The flask was rinsed with an additional 1 ml of THF and this was also transferred. The solution was stirred for 1.5 hours at room temperature, and then diluted with 15 ml of $CHCl_3$ and 30 ml of cold 0.1 M HCl. The aqueous layer was extracted with another 15 ml of $CHCl_3$ and the combined organic layers were washed successively with 30 ml of cold 0.1 M HCl, 30 ml of H_2O, and 30 ml of brine. TLC showe a polar impurity at Rf 0.03, the intermediate monoallyl ester at Rf 0.20, and less polar impurity at Rf 0.45 (iPrOH-CH_2Cl_2, 1:9, (v/v)). After drying over Na_2SO_4, the $CHCl_3$ solution was decanted into a 150 ml Erlenmeyer flask and stirred as it was diluted to 125 ml with ether. After stirring for 30 minutes, the precipitated solid (mainly Rf=0.03 material) was removed by vacuum filtration. The filtrate was concentrated on a rotary evaporator and then dried *in vacuo* (0.2 mmHg) for 45 minutes to provide 0.152 g (0.22 mmol) of semi-pure **8b** as an off-white solid.

(1*R*,5*S*,6*S*)-2-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]-carbonyl]oxy]eth-2-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid Disodium Salt (**2b**)

Crude **8b** was dissolved in 1 ml of CH_2Cl_2 along with 0.0087 g (0.0075 mmol, 3 mol%) of $Pd(PPh_3)_4$ and 0.0035 g (0.0133 mmol, 6 mol%) of triphenylphosphine. After cooling this mixture in an ice bath, 0.037 ml (0.032 g, 0.44 mmol) of pyrrolidine was added and the mixture was stirred for 1 hour. Dilution of the reaction mixture with 10 ml of ether led to a precipitate. The solvent was removed by pipet and the solid was washed twice with 5 ml of ether and briefly dried *in vacuo*. Most of the yellow solid was dissolved in 3 ml of $CH_3CN:H_2O$, 1:1 (v/v), plus 3 ml of CH_3CN. An ion exchange column was prepared by washing 5 g of Dowex® 50×4~40 resin three times with MeOH and pouring the slurry into a column to make a 9.3×80 mm bed. The column was equilibrated with H_2O, 1 M NaOH, H_2O, and finally $CH_3CN:H_2O$, 1:1 (v/v). The above sample solution was passed through the column and eluted with $CH_3CN:H_2O$, 1:1 (v/v), while collecting 3 ml fractions. The second through fifth fractions were combined, partially concentrated, and freeze-dried to provide 0.105 g of a fluffy yellow solid. This material was taken up in 2 ml of $CH_3CN:H_2O$, 1:1 (v/v), plus 2 ml of CH_3CN, filtered through a 0.3 g Waters Sep-Pak®, C-18 cartridge (rinsed with an addition 1 ml of $CH_3CN:H_2O$, 1:1 (v/v)), concentrated, and freeze-dried. The resulting 0.097 g of yellow solid residue was purified by preparative RP HPLC. Fractions were collected at 2 minutes intervals, and product eluted in fractions #2 and #3. Partial concentration and freeze-drying provided 0.0358 g (0.052 mmol, 10%) of **2b**. ^{1}H NMR (300 MHz, CD_3CN-D_2O, 1:1 (v/v)) δ 8.87 (1H, s), 7.9 (1H, m), 7.55 (1H, m), 3.9~3.5 (m), 3.5~3.0 (m), 3.0~2.7 (m), 1.9~1.7 (m), 1.4~1.0 (m). RP TLC Rf 0.55. FAB-MS *m/z* 645 ($(M+H)^+$, acid form).

1-(2-Thioacetylethyl)-4-(3-Allyloxycarbonyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-piperazine (**3c**)

A dry 25 ml pear shaped flask containing 0.500 g (1.22 mmol) of the allyl ester of ciprofloxacin, 1.0 ml (1.38 g, 7.2 mmol) of triethylamine, 3.0 ml of anhydrous DMF, and 0.110 ml (0.194 g, 1.27 mmol) of 2-bromoethanol was fitted with a dry condenser topped with a hose connection for vacuum/argon introduction. The system was purged with argon by several vacuum/argon cycles and heated to 111°C for 15 hours. After cooling to room temperature, the precipitated salts were removed by vacuum filtration (25~50 micron frit) and rinsed with 3 ml of DMF. The DMF was removed by rotary evaporation (25°C/0.4 mmHg) and the residue partially dissolved in 20 ml of $CHCl_3$. This mixture was washed with 4 ml of 0.2 M NaOH solution saturated with NaCl, and the aqueous layer was extracted with seven 10 ml portions of $CHCl_3$. The combined $CHCl_3$ layers were washed with 10 ml of brine, stirred over Na_2SO_4, filtered, and concentrated. The 0.806 g of wet residue was flash chromatographed on 41 g of silica gel, eluting with $CHCl_3$: CH_3OH, 17 : 3 (v/v), to provide 0.333 g (0.80 mmol, 66%) of the desired hydroxyethyl compound as a white solid. TLC: Rf 0.40 ($CHCl_3$: CH_3OH, 17 : 3 (v/v). ^1H NMR (300 MHz, $CDCl_3$) δ 8.53 (1H, s), 7.95 (1H, d), 7.21 (1H, d), 6.08 (1H, m), 5.5 (1H, d), 5.32 (1H, d), 4.83 (2H, d), 3.72 (2H, t), 3.47 (1H, m), 3.38 (4H, m), 2.9 (4H, m), 2.7 (2H, m), 1.35 (2H, m), 1.15 (2H, m). ^{13}C NMR (300 MHz, $CDCl_3$) δ 8.04, 34.47, 49.97, 50.01, 52.72, 57.95, 59.38, 65.27, 104.76, 110.12, 113.01, 113.32, 118.01, 122.90, 132.44, 137.96, 144.32, 148.05, 151.67, 154.96, 165.19, 172.85. PD-MS *m/z* 416 $(M+H)^+$, 358 $(M+H-C_3H_6O)^+$. A 3.68 mmol scale repeat of the above procedure without the aqueous workup provided a 50% yield of the product after chromatography. A partially dissolved solution of 0.770 g (1.85 mmol) of this intermediate in 35 ml of CH_2Cl_2 (HPLC grade stored over sieves) was placed in a dry 50 ml flask containing 3.4 g of 4 angstrom molecular sieves and fitted with a stir bar and septum. The system was flushed with argon for a few minutes then allowed to stir at room temperature for 3.5 hours. After cooling the reaction flask in an ice bath, and adding 0.350 ml (0.254 g, 2.51 mmol) of triethylamine, 0.170 ml (0.252 g, 2.20 mmol) of methanesulfonyl chloride was added *via* syringe over 1 minutes. The suspended solids dissolved and after 35 minutes the sieves were removed by filtering through a glass wool plug. The sieves were rinsed with dry CH_2Cl_2 and the solution was concentrated in a 75 ml pear shaped flask. TLC: Rf 0.20 (iPrOH - CH_2Cl_2, 7 : 93 (v/v). A stir bar and septum was added to the flask, and the system was flushed with argon. Anhydrous DMF (10 ml) was added to the white solid residue followed by 0.251 g (2.20 mmol) of potassium thioacetate. After stirring the cloudy yellowish solution 16.5 hours at room temperature, the solvent was removed by rotary evaporation (0.4 mmHg), and the residue was taken up in 50 ml of CH_2Cl_2. This solution was washed with 50 ml of half saturated $NaHCO_3$ solution, 50 ml of water, and brine. After filtration through a cone of Na_2SO_4, the solution was concentrated, and the residue was flash chromatographed on 66 g of silica gel, eluting with iPrOH - CH_2Cl_2, 7 : 93 (v/v), to provide 0.713 g (41% overall) of **3e** as a yellowish-white solid. TLC: Rf 0.34 (iPrOH - CH_2Cl_2, 7 : 93 (v/v). ^1H NMR (300 MHz, $CDCl_3$) δ 8.43 (1H, s), 7.86 (1H, d), 7.2 (1H, m), 6.06 (1H, m), 5.49 (1H, d), 5.27 (1H, d), 4.80 (2H, m), 3.45 (1H, m), 3.29 (4H, m), 3.08 (2H, t), 2.72 (4H, m), 2.64 (2H, t), 2.38 (3H, s), 1.33 (2H, m), 1.15 (2H, m). ^{13}C NMR (300 MHz, $CDCl_3$) δ 8.01, 26.31, 30.49, 34.49, 49.74, 49.78, 52.61, 57.30, 65.17, 104.83, 109.81, 112.71, 113.02, 117.98, 122.73, 132.45, 137.66, 144.24, 148.00, 151.56, 154.86, 164.99, 172.73, 195.38. PD-MS *m/z* 474 $(M+H)^+$, 416 $(M+H-C_3H_6O)^+$.

(1*R*,5*S*,6*S*)-2-[[4-(3-Allyloxycarbonyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]-eth-2-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid, Allyl Ester (**8c**)

A 10 ml pear shaped flask containing 0.117 g (0.247 mmol) of **3e** and a stirring flea was fitted with a septum and flushed with argon. The addition of 0.1 ml of CH_2Cl_2 and 0.25 ml of CH_3CN and warming the mixture in hot tap water caused most of **3e** to dissolve. The mixture was cooled to room temperature, 0.0225 ml (0.0192 g, 0.270 mmol) of pyrrolidine was added, and the mixture was stirred at room temperature. The solution gradually clouded and became a thick slurry after about 1.5 hours. After 3.3 hours this solution of thiol was used in the subsequent step. Thus, the enol triflate **4b** was prepared in methylene chloride (TLC: Rf 0.33 (ether)). The solvent was removed under high vacuum at -25°C. Approximately 30 minutes was required to remove the CH_2Cl_2. The resulting residue was dissolved in 1.3 ml of DMF, and the solution was cooled to -50°C. The freshly prepared solution of thiol was transferred by syringe with the aid of an additional 0.8 ml of DMF. Another 0.051 ml of *N*,*N*-diisopropylethylamine was added, and the mixture was allowed to warm to -10°C over 1 hour. The cold bath was removed, and the white slurry was stirred an additional 40 minutes at room temperature before diluting with 20 ml of CH_2Cl_2. This solution was washed twice with 20 ml of half saturated $NaHCO_3$ solution and then with 20 ml of brine. After filtering through a Na_2SO_4 cone, the solution was concentrated, and the residue was purified by flash chromatography on 12 g of silica gel, eluting with iPrOH - CH_2Cl_2, 1 : 9 (v/v), to provide 0.047 g (0.069 mmol, 28%) of **8c**. TLC: Rf=0.25 (iPrOH - CH_2Cl_2, 1 : 9 (v/v). ^1H NMR (300

MHz, $CDCl_3$) δ 8.58 (1H, s), 8.04 (1H, d), 7.22 (1H, m), 6.07 (2H, m), 5.51 (2H, d), 5.32 (2H, m), 4.85 (3H, m), 4.75 (1H, m), 4.28 (2H, m), 3.49 (2H), 3.4~3.2 (6H, m), 3.07 (1H, m), 2.95 (1H, m), 2.95~2.6 (6H, m), 1.41 (3H, d), 1.37 (2H, m), 1.31 (3H, d), 1.16 (2H, m). ^{13}C NMR (300 MHz, $CDCl_3$) δ 8.12, 16.9, 21.9, 29.0, 34.7, 43.4, 49.7, 52.8, 56.5, 57.6, 59.7, 65.4, 65.6, 66.2, 105.0, 109.6, 112.7, 113.0, 118.3, 122.6, 124.3, 131.7, 132.3, 137.9, 144.4, 144.6, 148.1, 151.3, 151.6, 154.9, 160.7, 165.0, 172.8, 173.1. PD-MS m/z 681 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]eth-2-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic Acid, Sisodium Aalt (**2c**)

A 10 ml flask containing 0.102 g (0.15 mmol) of **8c**, 0.0038 g (0.0054 mmol, 3.6 mol%) of dichlorobis(triphenylphosphine)palladium (II), and a stir bar was fitted with a septum and flushed with argon. The material was dissolved in 3.0 ml of CH_2Cl_2 plus 0.020 ml of H_2O. Upon the addition of 0.135 ml (0.146 g, 0.50 mmol) of tributyltin hydride some gas evolution was seen. RP HPLC analysis (system A) of a 0.010 ml aliquot after 15 minutes (diluted with 0.50 ml of CH_3CN - H_2O, 1 : 1 (v/v), and passed through a 0.45 μm nylon filter) showed two compounds with retention times of 5.14 and 5.48 minutes. Another 0.100 ml (0.108 g, 0.37 mmol) portion of tributyltin was added (gas evolution), and after another 10 minutes only the Rt = 5.12 minutes peak was seen by HPLC. The mixture was concentrated, and the resulting brown residue was taken up in a mixture of 3 ml of ether, 3.0 ml of 0.1 M $NaHCO_3$ (0.30 mmol), and 3 ml of CH_3CN. The two-phase mixture was stirred for 10 minutes, then 5 ml of H_2O, 3 ml of CH_3CN, and 5 ml of Et_2O were added. The layers were separated and the organic layer was extracted with 2 ml of H_2O. The combined lower layers were extracted four times with 15 ml of Et_2O. Small amounts of CH_3CN were sometimes added to make the lower layer more homogeneous and to promote phase separation. The lower aqueous phase was partially concentrated on a rotary evaporator and freeze dried to give 0.0825 g (0.128 mmol, 85%) of **2c**. ^1H NMR (300 MHz, $CD_3CN : D_2O$, 4 : 3 (v/v)) δ 8.45 (1H, s), 7.8 (1H, d), 7.4 (1H, m), 3.5 (2H, br m), 3.4~3.1 (6H, br m), 3.0~2.8 (3H, br m), 2.8~2.6 (6H, br m), 1.4~1.0 (10H, br m). PD-MS m/z 646 $(M+2Na)^+$ for diacid, 623 $(M+Na)^+$, 601 $(M+H)^+$.

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References

1) ALBRECHT, H. A.; G. BESKID, K. K. CHAN, J. G. CHRISTENSON, R. CLEELAND, K. H. DEITCHER, N. F. GEORGOPAPADAKOU, D. D. KEITH, D. L. PRUESS, J. SEPINWALL, A. C. SPECIAN, Jr., R. L. THEN, M. WIEGELE, K. F. WEST & R. YANG: Cephalosporin 3′-quinolone esters with a dual mode of action. J. Med. Chem. 33: 77~86, 1990
2) DEMUTH, T. P. Jr.; R. E. WHITE, R. A. TIETJEN, R. J. STORRIN, J. R. SKUSTER, J. A. ANDERSON, C. C. MCOSKER, R. FREEDMAN & F. J. ROURKE: Synthesis and antibacterial activity of new C-10 quinolonyl-cephem esters. J. Antibiotics 44: 200~209, 1991
3) ALBRECHT, H. A.; G. BESKID, J. G. CHRISTENSON, J. W. DURKIN, V. FALLAT, N. H. GEORGOPAPADAKOU, D. D. KEITH, F. M. KONZELMANN, E. R. LIPSHUTZ, D. H. MCGARRY, J. SIEBELIST, C. C. WEI & R. YANG: Dual-action cephalosporins: Cephalosporin 3′-quaternary ammonium quinolones. J. Med. Chem. 34: 669~675, 1991
4) CORRAZ, A. J.; S. L. DAX, N. K. DUNLAP, N. H. GEORGOPAPADAKOU, D. D. KEITH, D. L. PRUESS, P. L. ROSSMAN, R. L. THEN, J. UNOWSKY & C. C. WEI: "Dual-action" penems and carbapenems. J. Med. Chem. 35: 1828~1839, 1992
5) DAX, S. L.: Dual-action beta-lactam antibacterials. Current Opinion in Therapeutic Patents 2: 1375~1384, 1992
6) ALBRECHT, H. A. & J. G. CHRISTENSON: Mechanism-Based Dual Action Cephalosporins. *In* Adv. Med. Chem., Vol. 1. *Eds.*, B. E. MAYANOFF & C. A. MARYANOFF, pp. 207~234, JAI Press, Inc., Greenwich, 1992
7) ALBRECHT, H. A.; G. BESKID, J. G. CHRISTENSON, K. H. DEITCHER, N. H. GEORGOPAPADAKOU, D. D. KEITH, F. M. KONZELMANN, D. L. PRUESS & C. C. WEI: Dual-action cephalosporins incorporating 3′-tertiary amine-linked quinolones. J. Med. Chem. 37: 400~407, 1994
8) MA, X.; B. W. DAVIS, T. L. O'HARA, D. R. ANTHONY, D. M. BILLINGS, P. A. MATSON, R. E. WHITE, H. J. KRAUSS, J. D. BERK, N. A. WOOD, P. M. KOENIGS, S. K. SHAH, D. L. SHINABARGER, T. L. TWINEM, C. C. MCOSKER, W. G. KRAFT, F. J. ROURKE & T. P. DEMUTH, Jr.: Synthesis and multifunctional antibacterial activity of new quinolonyl-cephem 3°-amines. Abstracts of Papers of 34th Intersci. Conf. on Antimicrob. Agents Chemother., No. F159, Orlando, 1994
9) GRAY, J. L.; G. P. SHRUM, C. M. GASPARSKI, A. M. WANG, N. R. RAMBERGER, B. W. DAVIS, H. D. MCKEEVER, P. M. KOENIGS, S. M. PAULE, T. L. TWINEM, W. G. KRAFT, F. J. ROURKE & T. P. DEMUTH, Jr.: Antibacterial activity of multifunctional quinolonyl-cephem 2°-amines. Abstracts of Papers of 35th Intersci. Conf. on Antimicrob. Agents Chemother., No. F16, San Francisco, 1995
10) JONES, R. N. & A. L. BARRY: *In vitro* activity of Ro 23-9424, ceftazidime, and eight other newer beta-lactams

against 100 Gram-positive blood culture isolates. Diagn. Microbiol. Infect. Dis. 12: 143~147, 1989

11) GU, J. W. & H. C. NEU: *In vitro* activity of Ro 23-9424, a dual action cephalosporin, compared with activities of other antibiotics. Antimicrob. Agents Chemother. 34: 189~195, 1990

12) JONES, R. N.: *In vitro* activity of Ro 24-6392, a novel ester-linked co-drug combining ciprofloxacin and desacetylcefotaxime, Eur. J. Clin. Microb. Infect. Dis. 9: 435~438, 1990

13) BESKID, G.; H. A. ALBRECHT, V. FALLAT, D. D. KEITH, E. R. LIPSCHUTZ, C. M. MCGARRY, P. ROSSMAN & J. SIEBELIST: *In vitro* and *in vivo* activity of carbamate-linked dual action antibacterial Ro 24-4383. Chemotherapy 37: 310~317, 1991

14) DEMUTH, T. P., Jr.; R. E. WHITE, R. A. TIETJEN, J. R. POLLACK, W. G. KRAFT, B. W. DAVIS & F. J. ROURKE: Preparation and antimicrobial activity of novel C10 quinolonyl-cephem dithiocarbamates. Abstracts of Papers of 32nd Intersci. Conf. on Antimicrob. Agents Chemother., No. 773, Anaheim, 1992

15) ROLSTON, K. V. I.; H. T. NGUYEN, D. H. HO, B. LEBLANC & G. P. BODEY: *In vitro* activity of Ro 23-9424, a dual-action antibacterial agent, against bacterial isolates from cancer patients compared with those of other agents. Antimicrob. Agents Chemother. 36: 879~882, 1992

16) ROURKE, F. J.; B. W. DAVIS, W. G. KRAFT, R. E. WHITE, C. C. MCOSKER, T. L. O'HARA & T. P. DEMUTH, Jr.: *In vitro* activity of two new quinolonyl-penem carbamates. Abstracts of Papers of 33rd Intersci. Conf. on Antimicrob. Agents Chemother., No. 1489, New Orleans, 1993

17) PACE, J.; A. BERTASSO & N. H. GEORGOPAPADAKOU: *Escherichia coli* resistant to cephalosporins and quinolones is still susceptible to the cephalosporin-quinolone ester Ro 23-9424. Antimicrob. Agents Chemother. 35: 910~915, 1991

18) GEORGOPAPADAKOU, N. H. & A. BERTASSO: Mechanisms of action of cephalosporin 3'-quinolone esters, carbamates, and tertiary amines in *Escherichia coli*. Antimicrob. Agents Chemother. 37: 559~565, 1993

19) HERSHBERGER, P. M.; A. G. SWITZER, K. E. YELM, M. C. COLEMAN, C. A. DEVRIES, F. J. ROURKE, B. W. DAVIS, W. G. KRAFT, T. L. TWINEM, P. M. KOENIGS, S. M. PAULE, R. J. SIEHNEL, P. H. ZOUTENDAM, R. IMBUS & T. P. DEMUTH: Synthesis and biological evaluation of 2-thioether-linked quinolonyl carbapenems. Abstracts of Papers of 36th Intersci. Conf. on Antimicrob. Agents Chemother., No. F170, New Orleans, 1996

20) ROURKE, F. J.; B. W. DAVIS, W. G. KRAFT, R. E. WHITE, C. C. MCOSKER, M. A. WALLING, J. A. ANDERSEN, A. M. SALVAGNO, P. M. FITZPATRICK, L. A. JAMIESON & T. P. DEMUTH, Jr.: Evaluation of new quinolonyl penem carbamates. Abstracts of Papers of 32nd Intersci. Conf. on Antimicrob. Agents Chemother., No. 772, Anaheim, 1992

21) SANCHEZ, J. P.; J. M. DOMAGALA, S. E. HAGEN, C. L. HEIFETZ, M. P. HUTT, J. B. NICHOLS & A. K. TREHAN: Quinolone antibacterial agents. Synthesis and structure-activity relationships of 8-substituted quinoline-3-carboxylic acids and 1,8-naphthyridine-3-carboxylic acids. J. Med. Chem. 31: 983~991, 1988

22) LEYSEN, D. C.; M. Q. ZHANG, A. HAEMERS & W. BOLLAERT: Synthesis of antibacterial 4-quinolone-3-carboxylic acids and their derivatives. Die Pharmazie 46: 485~501, 1991

23) LEYSEN, D. C.; M. Q. ZHANG, A. HAEMERS & W. BOLLAERT: Synthesis of antibacterial 4-quinolone-3-carboxylic acids and their derivatives. Die Pharmazie 46: 557~572, 1991

24) SHIH, D. H.; F. BAKER, L. CAMA & B. G. CHRISTIANSEN: Synthetic carbapenem antibiotics I. 1-β-Methylcarbapenem. Heterocycles 21: 29~40, 1984

25) SALZMANN, T. N.; R. W. RATCLIFFE, B. G. CHRISTIANSEN & F. A. BOFFARD: A stereocontrolled synthesis of (+)-thienamycin. J. Amer. Chem. Soc. 102: 6161~6163, 1980

26) SUNAGAWA, M.; H. MATSUMURA, T. INOUE, M. FUKASAWA & M. KATO: A novel carbappenem antibiotic, SM-7338 structure-activity relationships. J. Antibiotics 43: 519~532, 1990

27) WHITE, R. E. & T. P. DEMUTH, Jr. (Procter & Gamble Company): Antimicrobial Carbapenem Quinolones. U.S. 5,646,139, July 8, 1997

28) DEMUTH, T. P. Jr.; J. D. BERK, B. W. DAVIS, P. M. KOENIGS, W. G. KRAFT, C. C. MCOSKER, F. J. ROURKE, D. L. SHINABARGER, R. A. TIETJEN & R. E. WHITE: Preclinical evaluation of quinolonyl-lactam antibacterials: Compounds designed to combat drug-resistance in bacteria. Abstracts of Papers of Eastern Pennsylvania Branch of Amer. Soc. of Microbiol. Symposium "Antimicrobial Resistance: A Crisis in Health Care", Philadelphia, 1993

29) National Committee for Clinical Laboratory Standards: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Doc. No. M7-A2, Vol. 10, No. 8, NCCLS, Villanova, Pa., 1990

30) SPRATT, B. G.: Properties of the penicillin-binding proteins of *Escherichia coli* K12. Eur. J. Biochemistry 72: 341~352, 1977

31) BARRETT, J. F.; J. I. BERNSTEIN, H. M. KRAUSE, J. J. HILLIARD & K. A. OHEMENG: Testing potential gyrase inhibitors of bacterial DNA gyrase: A comparison of the supercoiling inhibition assay and "Cleavable Complex" assay. Analytical Biochemistry 214: 313~317, 1993

32) MIZUUCHI, K.; M. MIZUUCHI, M. H. O'DEA & M. GELLERT: Cloning and simplified purification of *Escherichia coli* DNA gyrase A and B proteins. J. Biol. Chem. 259: 9199~9201, 1984