BBr_3 (1 M in CH_2Cl_2, 2.52 mL, 2.52 mmol) over 10 min. After proceeding 1 h at −78 °C, the reaction mixture was poured into cold NH_4Cl (200 mL). The mixture was extracted with EtOAc (150 mL), and the resulting organic layer was washed with cold NH_4Cl (300 mL) and saturated NaCl. The desired **74** (50 mg, 24%) as an oil was obtained from preparative TLC (silica, 1:1 EtOAc/hexane elution). The oil crystallized on standing (mp 60–62 °C).

Method M. 4-(4-Cyanobutoxy)-2-chloro-5-propylacetophenone (70). To a 1:1 mixture of 3-chloro-2-propylphenol and 3-chloro-6-propylphenol (from Claisen rearrangement on 3-(allyloxy)chlorobenzene, followed by H_2–Pd reduction of product double bond; 17.1 g, 100 mmol) in DMF (200 mL) were added NaH (50%, 4.8 g, 100 mmol), KI (5 g), and 5-bromopentanenitrile (16.2 g, 100 mmol). The reaction was stirred at room temperature for 15 h and then treated with cold dilute HCl (200 mL). The reaction mixture was shaken with EtOAc (200 mL), and the resulting organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The desired 3-(4-cyanobutoxy)-4-propylchlorobenzene (9.6 g, 76%) was obtained as an oil by HPLC (silica, 0–30% EtOAc in hexane gradient). To CH_2Cl_2 (100 mL) were added the 4-chloropropylbenzene intermediate (1.26 g, 5 mmol) and acetyl chloride (0.4 mL, 5.5 mmol). The mixture was cooled to −10 °C, and $AlCl_3$ (1.33 g, 10 mmol) was added in portions over 30 min. The reaction was allowed to warm slowly to room temperature and was stirred for 15 h. Solvent was removed in vacuo and the residue was added to a 1:1 mixture of concentrated HCl and crushed ice with stirring. EtOAc (200 mL) was added to mixture, and stirring was continued until all organic material dissolved. The EtOAc layer was washed with saturated NaCl, dried over Na_2SO_4, filtered, and concentrated in vacuo. The desired **70** (0.6 g, 41%) was obtained by preparative TLC (silica, 40% EtOAc in hexane elution) as an oil.

Biological Methods. Binding Assay Studies. Tritiated LTB_4 preparations with a specific activity of 150–220 Ci/mmol and a radiochemical purity of ≥95% were obtained from Amersham (Arlington Heights, IL). Nonradioactive LTB_4 was purchased from Biomol Research Laboratories (Philadelphia, PA). All other chemicals were commercial reagent-grade materials. For each experiment fresh human blood from two or three individuals was obtained from the Central Indiana Regional Blood Center (Indianapolis, IN) and pooled. Neutrophils were isolated by standard techniques of Ficoll-Hypaque centrifugation, dextran 70 sedimentation, and hypotonic lysis. Preparations were ≥90% neutrophils and ≥90% viable. This procedure yielded enough cells to do concentration–response studies on seven to eight compounds. A reference antagonist, compound **18**, was included in each experiment. The effectiveness of compounds to inhibit binding of $[^3H]LTB_4$ to neutrophils was measured by using an adaptation of a radioligand-binding assay developed by Goetzl and Goldman.[12] The following were added to microcentrifugation tubes: 10 μL of DMSO containing different amounts of compound, 20 μL of radioligand (2.65 nM $[^3H]LTB_4$), and 500 μL of cells suspended at a concentration of 2×10^7 cells/mL in Hank's balanced salt solution containing 0.1% ovalbumin. The tubes were then incubated at 4 °C for 10 min. After the incubation, 300 μL of a mixture of dibutyl and dinonyl phthalate (7:2) was added, and the tubes were centrifuged for 2 min. The liquid was then decanted and the bottom tip of the tube cut off with a razor blade and placed in a counting vial. The radioactivity bound to the cell pellet was determined by scintillation spectrometry. Three incubations were carried out at each concentration of compound investigated. The individual measurements of bound label were then averaged (SEM = 1–2%) and the results expressed as a percent inhibition of specific $[^3H]$ binding after making appropriate corrections for nonspecific binding. The latter was determined by measuring the amount of label bound when cells and $[^3H]LTB_4$ were incubated with a >2000-fold excess of nonradioactive ligand. The inhibitory activity of most compounds was evaluated on only one cell preparation. The variability of the measurements from different individuals can be estimated from the inhibition observed with reference compound **18** on all 102 cell preparations studied. At 10^{-5} M, the mean percent inhibition and standard deviation for the reference compound were 93.9 and 3.9, respectively. At 10^{-6} M, the corresponding values were 56.9 and 6.9. Assuming a linear correlation between percent inhibition and standard deviation, the following estimates were calculated for the precision at different percentages of inhibition: 90 ± 4.2, 80 ± 5.0, 60 ± 6.6, 40 ± 8.2, 20 ± 9.9, and 10 ± 10.7. In cases where compounds were tested on more than one cell preparation, the precision of the measurements were equal to or better than these estimates (i.e. compound **35**, $n = 4$, 102 ± 2 at 10^{-5} M, 93 ± 1 at 10^{-6} M, 56 ± 3 at 10^{-7} M, 12 ± 3 at 10^{-8} M; compound **33**, $n = 4$, 76 ± 2 at 10^{-6} M, 28 ± 1 at 10^{-7} M, 9 ± 4 at 10^{-8} M; compound **24**, $n = 3$, 85 ± 2 at 10^{-5} M, 46 ± 2 at 10^{-6} M, 13 ± 1 at 10^{-7} M).

(12) Goldman, D. W.; Goetzl, E. J. Specific Binding of Leukotriene B_4 to Receptors on Human Polymorphonuclear Leukocytes. *J. Immunol.* **1982**, *129*, 1600–1604.

Dual-Action Penems and Carbapenems

Alfred J. Corraz, Scott L. Dax,* Norma K. Dunlap,* Nafsika H. Georgopapadakou, Dennis D. Keith, David L. Pruess, Pamela L. Rossman, Rudolph Then, Joel Unowsky, and Chung-Chen Wei

Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received November 5, 1991*

Two new series of dual-action antibacterial agents were synthesized in which penems and carbapenems were linked at the 2′-position to quinolones through either an ester or a carbamate moiety. Potent, broad-spectrum antibacterial activity was observed for both classes of compounds, indicative of a dual-mode of action.

Introduction

Dual-action agents are unique chemical entities comprised of two different types of antibacterial compounds covalently linked together in a single molecule in such a way that both components are able to exert their bactericidal properties. The antibacterial activity of quinolones occurs as a consequence of interaction with bacterial DNA gyrase,[1] while β-lactams act via inhibition of peptidoglycan transpeptidase(s).[2] By combining the two into a novel molecular hybrid, the result is inhibition of DNA replication and cell wall assembly. Furthermore, the antibacterial spectra of the two components are somewhat complementary; β-lactams possess potent Gram-positive activity, especially against *Streptococcus*, while quinolones display excellent activity against Gram-negative organisms, including *Pseudomonas aeruginosa* and β-lactam-resistant strains such as methicillin-resistant *Staphylococcus au-*

(1) Wolfson, J. S.; Hooper, D. C. The Fluoroquinolones: Structures, Mechanisms of Action and Resistance, and Spectra of Activity In Vitro. *Antimicrob. Agents Chemother.* **1985**, *28*, 581–586.

(2) Fiere, J. M.; Jovis, B. Penicillin-Sensitive Enzymes in Peptidoglycan Biosynthesis. *CRC Crit. Rev. Microbiol.* **1985**, *11*, 299–396.

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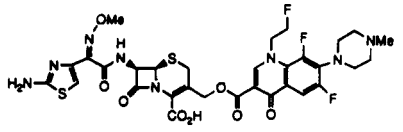
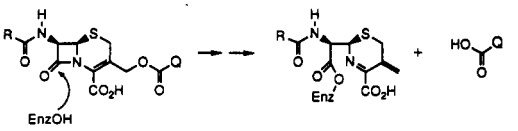


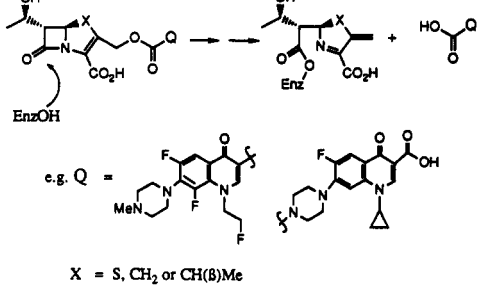
Figure 1.

reus. In addition, some of the problems associated with quinolones, such as insolubility, central nervous system effects, phototoxicity, and arthropathy, might be reduced by linking them to a β-lactam antibacterial. We have reported three classes of dual-action cephalosporins possessing a variety of linkages: ester, carbamate, and quaternary nitrogen.[3-5] All three exhibit a broad spectrum of activity, reflecting contributions from both antibacterial components. A prototype, consisting of the fluoroquinolone fleroxacin attached to the C-3′ position of desacetylcefotaxime via an ester linkage (Ro 23-9424, Figure 1), is currently in clinical trials.

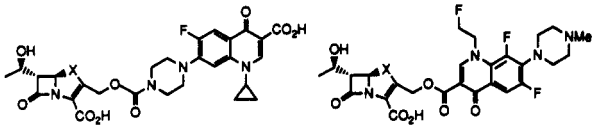
The proposed mechanism of action invokes the acylation of active-site serine residues of peptidoglycan transpeptidases via attack at the β-lactam carbonyl followed by the subsequent release of quinolone, resulting in a dual mode of antibacterial activity.[3] β-Lactamases or possibly esterases may also act to release quinolone through either β-lactam cleavage or ester hydrolysis, although the action of lactamases is particularly detrimental since β-lactam activity is lost.



The success of the cephalosporin–quinolone dual-action antibacterials has encouraged further investigation of other possibilities in the area. Penems and carbapenems are two other classes of β-lactam antibacterials which can accommodate a vinylogous-linked quinolone that would be released upon ring-opening of the β-lactam (eq 2). An attractive feature of both penems and carbapenems is their activity against anaerobic organisms which is not covered by the cephalosporin–quinolones. In addition, penems and carbapenems have other desirable properties, such as



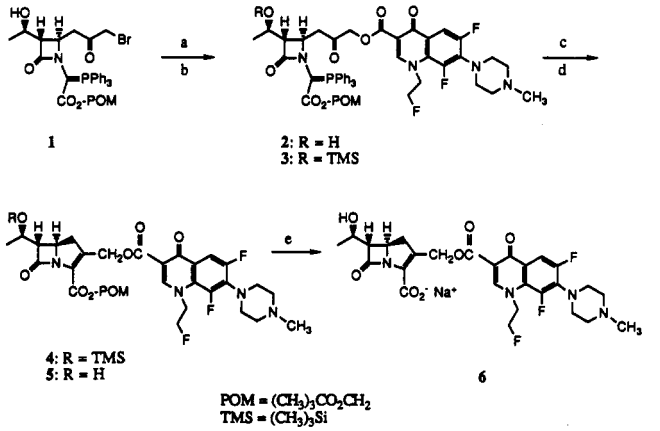
X = S, CH_2 or CH(ß)Me



X = S, CH_2 or CH(ß)Me

Figure 2.

Scheme I



a: fleroxacin (K^+ salt), NEt_3 (63%); b: TMSCl, pyridine; c: toluene, 105° C (80% for 2 steps); d: ammonium fluoride (67%); e: Porcine Liver Esterase (41%).

relatively good stability toward β-lactamases (in particular, cephalosporinases) and the potential for oral activity.[6] The renal toxicity,[7,8] associated with the susceptibility of these compounds to the mammalian enzyme dehydropeptidase, might be ameliorated by combining them with a quinolone.

We report here the preparation of both penem and carbapenem dual-action antibacterials in which the lactam component is linked at the 2′-position to quinolones through either ester or carbamate linkages. In the case of the ester-linked compounds, fleroxacin is joined via ester formation of the carboxylate group located at the C-3 position of the quinolone. With the carbamate-linked analogues, ciprofloxacin is bound by a carbamate formed with the secondary amine of the piperazine ring at C-7 of the quinolone (Figure 2). The syntheses and various biological data of these compounds are presented.

Synthesis

Carbapenems. In developing syntheses of dual-action carbapenems, two separate approaches proved successful. In the synthesis of racemic ester-linked **6** (Scheme I), the quinolone was introduced via halogen displacement upon an appropriate (3-bromo-2-oxopropyl)azetidinone **1** leading to an intermediate **3** which underwent Wittig cyclization to afford carbapenem **4**. The silyl ether protecting group used to mask the 6′-hydroxy group was then removed via

(3) Albrecht, H. A.; Beskid, G.; Chan, K.-K.; Christenson, J. G.; Cleeland, R.; Deitcher, K. H.; Georgopapadakou, N. H.; Keith, D. D.; Pruess, D. L.; Sepinwall, J.; Specian, A. C.; Then, R. L.; Weigele, M.; West, K. F.; Yang, R. Cephalosporin 3′-Quinolone Esters With a Dual Mode of Action. *J. Med. Chem.* **1990**, *33*, 77–86.

(4) Albrecht, H. A.; Beskid, G.; Christenson, J. G.; Durkin, J. W.; Fallat, V.; Georgopapadakou, N. H.; Keith, D. D.; Konzelmann, F. M.; Lipschitz, E. R.; McGarry, D. H.; Siebelist, J.; Wei, C.-C.; Weigele, M.; Yang, R. Dual-Action Cephalosporins: Cephalosporin 3′-Quaternary Ammonium Quinolones. *J. Med. Chem.* **1991**, *34*, 669–675.

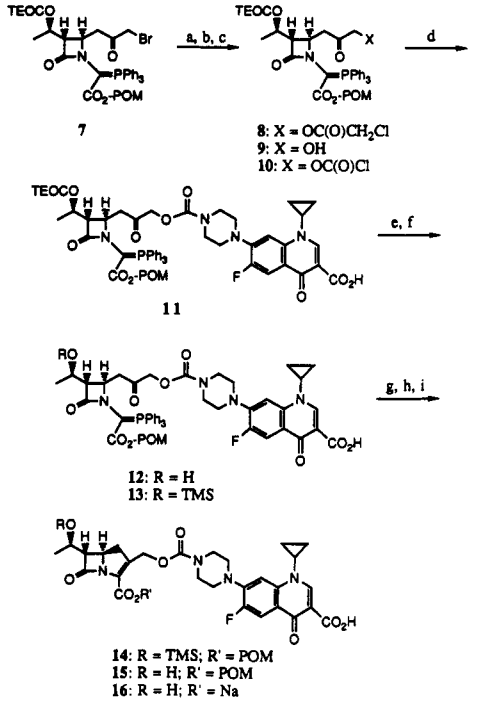
(5) Albrecht, H. A.; Beskid, G.; Georgopapadakou, N. H.; Keith, D. D.; Konzelmann, F. M.; Pruess, D. L.; Rossman, P. L.; Wei, C.-C. Dual-Action Cephalosporins: Cephalosporin 3′-Quinolone Carbamates. *J. Med. Chem.* **1991**, *34*, 2857–2864.

(6) Gural, R.; Lin, C.; Chung, M.; Oden, E.; Digiore, C. Oral Absorption and Tolerance in Man, of a New Penem Antibiotic, Sch 29482. *J. Antimicrob. Chemother.* **1982**, *9* (Suppl. C), 239–243.

(7) (a) Kahan, F. M.; Kropp, H.; Sundelof, J. G.; Birnbaum, J. Thienamycin: Development of Imipenem-Cilastatin. *J. Antimicrob. Chemother.* **1983**, *12* (Suppl. D), 1–35. (b) Kropp, H.; Sundelof, J. G.; Hajdu, R.; Kahan, F. M. Metabolism of Thienamycin and Related Carbapenem Antibiotics by the Renal Dipeptidase, Dehydropeptidase-I. *Antimicrob. Agents Chemother.* **1982**, *22*, 62–70.

(8) (a) Matsuda, K.; Sasaki, K.; Inoue, K.; Kondo, M.; Mitsuhashi, S. In Vitro Antibacterial Activity of Sch 34343 and its Stability to β-Lactamases and Renal Dehydropeptidase I. *Antimicrob. Agents Chemother.* **1985**, *28*, 684–688. (b) Appel, G. B.; Neu, H. C. *N. Engl. J. Med.* **1977**, *296*, 663–670.

Scheme II



TEOC = $Cl_3CCH_2OC(O)$ POM = $(CH_3)_3CO_2CH_2$ TMS = $(CH_3)_3Si$

a: $ClCH_2CO_2^-$ Na^+, NEt_3, DMF (67%); b: $KHCO_3$, aq. MeOH (86%); c: *i*-Pr_2NEt, Cl_2CO; d: N,O-bis(TMS)ciprofloxacin (47% for 2 steps); e: Zn, HOAc (75%); f: TMS-Cl, pyridine; g: toluene, 80°C (75% for 2 steps); h: ammonium fluoride (65%); i: Porcine Liver Esterase (<10%).

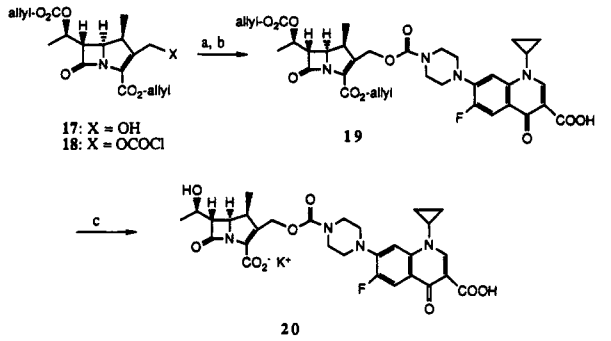
treatment with fluoride, giving **5**. Final (pivaloyloxy)-methyl (POM) ester cleavage was effected by porcine liver esterase (EC 3.1.1.1), yielding dual-action carbapenem **6**.

In the synthesis of the carbamate-linked dual-action carbapenem **16** (Scheme II), azetidinone **9** was chloroformylated and coupled to silylated ciprofloxacin. The corresponding adduct **11** was subjected to Wittig cyclization and protecting group manipulations as previously described. However, esterase-induced POM ester cleavage was inefficient, possibly due to the insolubility of **15** at pH values optimal for efficient enzyme catalysis. Other methods were tried but resulted in an unsatisfactory amount of β-lactam cleavage (and release of quinolone).

As a result of inefficient POM ester cleavage, it became necessary to modify the strategy. The synthesis of the penem analogues involved quinolone attachment after the construction of the bicyclic lactam system and was proceeding smoothly. In addition, the idea of using allyl-based protecting groups for both the 6′-hydroxy group and the 3-carboxylate ester was particularly attractive since one-step mild palladium(0)-catalyzed cleavage of both groups was effected in the case of classical carbapenems.[9] This approach could also be altered to allow for the synthesis of an optically-active carbapenem species amenable to the incorporation of quinolones. Lastly, we elected to introduce a β-methyl group at C-1 of the carbapenem since substitution of this type has been shown to increase stability of carbapenems to the mammalian enzyme dehydropeptidase (DHP).

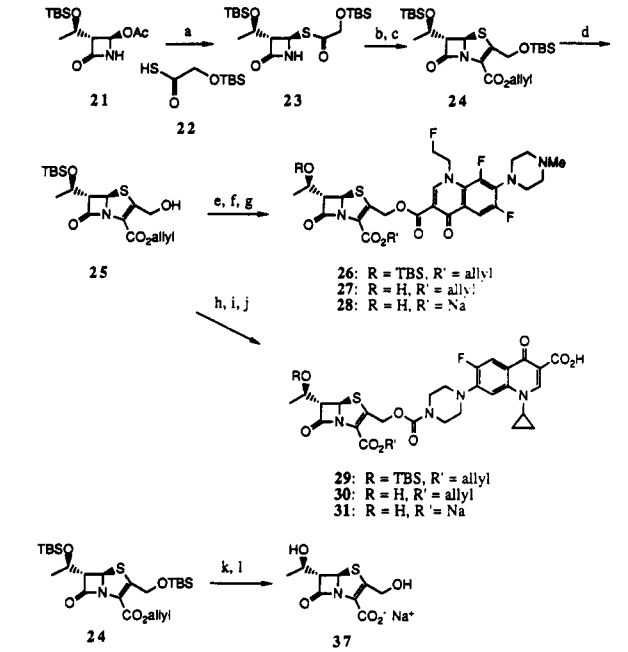
Following a known sequence,[9] carbapenem–alcohol **17** was assembled (Scheme III). This carbapenem was chloroformylated (as previously described) and coupled to silylated quinolone, which upon aqueous workup afforded

Scheme III

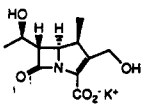


a: N,N-dimethylaniline, Cl_2CO; b: N,O-bis(TMS)ciprofloxacin (56% for 2 steps); c: $Pd(PPh_3)_4$, PPh_3, potassium 2-ethylhexanoate, 2-ethylhexanoic acid (72%).

Scheme IV



a: $NaHCO_3$ (50%); b: $CaCO_3$, $ClCOCO_2allyl$, *i*-Pr_2NEt (~100%); c: $P(OEt)_3$ (55%); d: TBAF, HOAc (85%); e: fleroxacin, *i*-BuOCOCl, NEt_3 (65%); f: TBAF, HOAc (66%); g: $Pd(PPh_3)_4$, PPh_3, sodium 2-ethylhexanoate (24%); h: 1) phosgene, *i*-Pr_2NEt, 2) N,O-bis(TMS)ciprofloxacin (89%); i: TBAF, HOAc (93%); j: $Pd(PPh_3)_4$, PPh_3, sodium 2-ethylhexanoate (24%); k: TBAF, HOAc (31%); l: $Pd(PPh_3)_4$, PPh_3, sodium 2-ethylhexanoate (64%).



17a

Figure 3.

19. Subsequent treatment with tetrakis(triphenylphosphine)palladium(0) in methylene chloride–ethyl acetate yielded the optically-active carbamate-linked dual-action carbapenem **20**. The same reaction conditions smoothly removed both allyl protecting groups of carbapenem **17** to afford **17a** (Figure 3) which, along with quinolone, allowed direct comparison of the dual-action carbapenem to its respective components.

Penems. The synthesis of four dual-action penems is outlined in Scheme IV. The approach was to prepare the 2-(hydroxymethyl)penem **25** and then couple it, either through an ester or carbamate linkage, to the desired quinolone, Several subsequent deprotection steps would afford the final products. A wide variety of 2-substituted penems have been previously synthesized[10] and, in par-

(9) Schmitt, S. A.; Saltzmann, T. N.; Shih, D. H.; Christensen, B. G. The Synthesis of 2-(Functionalized Methyl)-1-beta-Methylcarbapenems. *J. Antibiot.* **1988**, *41*, 780–787.

Table I. In Vitro Activity of Carbapenem Analogues and Reference Compounds: MIC (μg/mL)

organism	20	6	17a	fleroxacin	ciprofloxacin
Escherichia coli 257	≤0.0156	0.125	0.5	0.0313	0.0078
E. coli TEM-1[a]	0.0313	0.125	0.5	0.0625	0.0156
Citrobacter freundii BS-16[a]	0.0625	0.25	0.5	0.125	0.0313
Klebsiella pneumoniae A	0.0625	0.25	0.5	0.0625	0.25
Enterobacter cloacae P99[a]	0.0313	0.125	0.5	0.0313	0.0078
Serratia marcescens SM[a]	0.0625	0.25	1	0.0625	0.0313
Proteus vulgaris ATCC 6380[a]	0.0313	0.25	0.5	0.0625	0.0078
P. mirabilis 90	0.125	1	1	0.25	0.0625
Pseudomonas aeruginosa 8780	0.5	4	32	2	0.25
Ps. aeruginosa 18SH[a]	0.5	2	16	1	0.25
Staphylococcus aureus Smith	0.125	0.125	0.125	0.25	0.125
S. aureus 67[b]	0.5	1	32	0.5	0.5
S. aureus 753[b]	0.5	1	32	0.5	0.25
Streptococcus pneumoniae 6301	0.0313	0.0157	0.0625	4	1
S. pyogenes 4	0.0313	0.0157	0.0625	2	0.5
Bacteroides fragilis S2	1	≤0.125	nd	8	4
B. fragilis F117A	4	1	nd	64	16
B. thetaiotaomicron 62B	16	4	nd	64	>128
Clostridium histolyticum 503-86	≤0.125	nd[c]	nd	0.25	≤0.125
C. perfringens 13424	≤0.125	≤0.125	nd	0.5	0.25
C. difficile 651	4	nd	nd	16	16
C. difficile 701	1	4	nd	16	8

[a] Constitutive β-lactamase producer. [b] Methicillin-resistant. [c] No data = nd.

Table II. In Vitro Activity of Penem Analogues and Reference Compounds: MIC (μg/mL)

organism	36	28	31	37	fleroxacin	imipenem
Escherichia coli 257	0.25	0.25	0.0625	4	0.0313	0.125
E. coli TEM-1[a]	0.125	0.5	0.0625	4	0.0625	0.25
Citrobacter freundii BS-16[a]	0.25	0.5	0.125	4	0.125	2
Klebsiella pneumoniae A	0.25	0.5	0.25	4	0.0625	0.125
Enterobacter cloacae P99[a]	0.125	0.5	0.0625	8	0.0313	0.25
Serratia marcescens SM[a]	0.25	0.5	0.25	8	0.0625	0.25
Proteus vulgaris ATCC 6380[a]	0.25	0.25	0.0625	8	0.0625	1
P. mirabilis 90	1	1	1	8	0.25	2
Pseudomonas aeruginosa 8780	16	16	2	64	2	2
Ps. aeruginosa 18SH[a]	4	4	1	8	1	1
Staphylococcus aureus Smith	1	0.125	0.125	0.25	0.25	≤0.0156
S. aureus 67[b]	1	1	4	>64	0.5	32
S. aureus 753[b]	2	2	4	1	0.5	64
Streptococcus pneumoniae 6301	0.125	≤0.0156	0.0313	0.125	4	≤0.0156
S. pyogenes 4	0.125	≤0.0156	0.0313	0.125	2	≤0.0156
Bacteroides fragilis S2	nd[c]	≤0.125	16	≤0.125	8	≤0.125
B. fragilis F117A	nd	≤0.125	1	64	64	2
B. thetaiotaomicron 62B	nd	4	16	0.25	64	≥128
Clostridium histolyticum 503-86	nd	≤0.125	0.25	≤0.125	0.25	≤0.125
C. perfringens 13424	nd	≤0.125	0.25	0.5	0.5	≤0.125
C. difficile 651	nd	0.5	4	nd	16	4
C. difficile 701	nd	1	8	2	16	4

[a] Constitutive β-lactam producer. [b] Methicillin-resistant. [c] No data = nd.

ticular, 2-(hydroxymethyl)penems such as we required have been studied by the Farmitalia group.[11]

Displacement of the acetoxy group of the commercially available azetidinone **21** with the protected thioglycolic acid **22** afforded the thioester **23**. Acylation to provide the oxalamide was followed by triethylphosphite-induced cyclization to afford the penem **24**. Selective deprotection of the primary TBS ether provided the known (hydroxymethyl)penem **25**, which served as a common intermediate for the ester-linked and carbamate-linked dual-action antibacterials. In the first case, the (hydroxymethyl)penem reacted with the isobutyl mixed anhydride of fleroxacin to afford the coupled product **26**. Deprotection of the secondary TBS ether, followed by allyl ester cleavage, afforded the final product **28**. In order to prepare the carbamate-linked dual-action antibacterials, the (hydroxymethyl)penem **25** was converted to its chloroformate and then reacted with the bis-TMS derivative of ciprofloxacin. The coupled product **29** was deprotected as above to afford the final product **31**. The C-6 unsubstituted products **34** and **36** (Figure 4) were prepared in similar fashion from the corresponding (hydroxymethyl)penem **32**.[12]

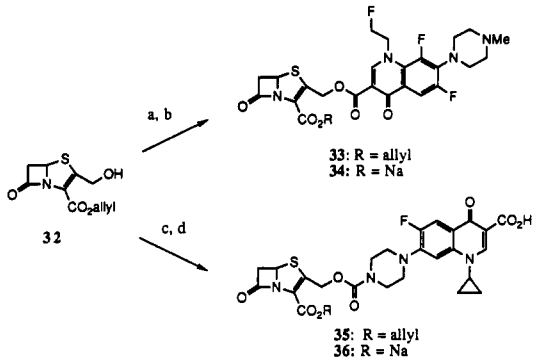
Antibacterial Activity

Carbapenems. As shown in Table I, both ester-linked and carbamate-linked dual-action carbapenems (**6** and **20**, respectively) are extremely potent broad-spectrum antibacterial agents. In fact, optically-active **20** proved to be superior to all compounds tested, exhibiting excellent activity against Gram-negative pathogens including good antipseudomonal potency and superb activity against

(10) McCombie, S. W.; Ganguly, A. K. Synthesis and In Vitro Activity of the Penem Antibiotics. *Med. Res. Rev.* **1988**, *8*, 393–440.

(11) Alpegiani, M.; Bedeschi, A.; Giudici, F.; Perrone, E.; Visentin, G.; Zarini, F.; Franceschi, G. 2-(Heteroatom-Substituted)-Methyl Penems. IV. Oxygen Derivatives. *Heterocycles* **1990**, *30*, 799–812.

(12) Perryman, B. N. J. Bicyclic β-Lactam Antibacterial Agents, Their use in Pharmaceutical Compositions, Processes for their Preparation and Intermediates for Use in Such Processes. E.P. #0,013,067, October 31, 1979.



a: fleroxacin, NEt_3, c-hexylchloroformate, DMAP (35%); b: $Pd(PPh_3)_4$, PPh_3, sodium 2-ethylhexanoate (35%); c: 1) phosgene, i-Pr_2NEt, 2) N,O-bis(TMS)ciprofloxacin (87%); d: $Pd(PPh_3)_4$, PPh_3, sodium 2-ethylhexanoate (13%).

Figure 4.

(Gram-positive) *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. In addition, this dual-action carbapenem and ester-linked **6** were quite potent against anaerobes although the penem analogues were slightly better (vide infra).

Penems. The in vitro antibacterial activity of several of the penem dual-action compounds is summarized in Table II. The ester-linked compounds **36** and **28** exhibited good activity against a broad spectrum of microorganisms, with the only weakness being low activity against *Pseudomonas aeruginosa*. This is a reflection of the spectrum of the penem portion of the molecule. An advantage gained from the penem portion is the activity against anaerobes, which is quite good. In fact, for ester-linked **28**, the anaerobe activity is better than or equal to that of imipenem against all strains tested. The carbamate-linked product **31** has weaker activity against anaerobes, but much better activity against Gram-negative and Gram-positive organisms, including surprisingly good activity against *Pseudomonas aeruginosa*.

Stability against Dehydropeptidase. In general, carbapenems and penems are susceptible to hydrolysis catalyzed by the mammalian enzyme dehydropeptidase, resulting in loss of antibacterial activity along with the formation of toxic byproducts (in the case of carbapenems). This has prompted current research to be aimed at developing DHP-resistant antibiotics, or methods to reduce the rate of DHP inactivation of particularly active but susceptible antibiotics.[13,14]

(13) (a) Graham, D. W.; Ashton, W. T.; Barash, L.; Brown, J. E.; Brown, R. D.; Canning, L. F.; Chen, A.; Springer, J. P.; Rogers, E. F. Inhibition of the Mammalian beta-Lactamse Renal Dipeptidase (Dehydropeptidase-I) by (Z)-2-(Acylamino)-3-substituted-propenoic Acids. *J. Med. Chem.* **1987**, *30*, 1074–1090. (b) Hashimoto, S.; Murai, H.; Ezaki, M.; Morikawa, N.; Hatanaka, H.; Okuhara, M.; Kohsaka, M.; Imanaka, H. Studies on New Dehydropeptidase Inhibitors I. *J. Antibiot.* **1990**, *43*, 29–42. (c) Hashimoto, S.; Murai, H.; Nitta, K.; Fujie, A.; Okuhara, M.; Kohsaka, M.; Imanaka, H. Studies on New Dehydropeptidase Inhibitors III. *J. Antibiot.* **1990**, *43*, 281–285. (d) Uchida, I.; Hashimoto, S.; Iwamoto, T.; Takase, S.; Hashimoto, M. Studies on New Dehydropeptidase Inhibitors IV. *J. Antibiot.* **1990**, *43*, 344–351.

(14) (a) Naganuma, H.; Hirouchi, Y.; Tokiwa, H.; Kawahara, Y.; Okada, R.; Kuwahara, S. Protective Effect of N-Acylamino Acids (NNAs) on Carbapenem Nephrotoxicity. Abstract 899, Thirtieth Interscience Conference on Antimicrobial Agents and Chemotherapy, October 21–24, 1990, Atlanta, GA. (b) Takahagi, H.; Hirota, T.; Matsushita, Y.; Tanaka, M.; Muramatsu, S.; Naganuma, H.; Kawahara, Y. Metabolic Studies on CS-976, a New Carbapenem Antibiotic Agent in Experimental Animals. Abstract 896, Thirtieth Interscience Conference on Antimicrobial Agents and Chemotherapy, October 21–24, 1990, Atlanta, GA.

Table III. Hydrolysis by Dehydropeptidase (Calculated for 1 μL of Enzyme Solution and 100 μM Substrate)

compound	hydrolysis (nmol/min)
glycyldehydrophenylalanine	98.6
imipenem	1.0
6	37.5
28	2.3
17a	0.88
20	0.27

With this issue in mind, our carbapenem- and penem-dual-action agents were assayed for rate of hydrolysis in the presence of hog kidney dehydropeptidase I using imipenem as a reference. Ester-linked carbapenem **6** was disappointing in that it was consumed approximately 40-fold more rapidly than imipenem. However, optically-active carbamate-linked carbapenem **20**, possessing a C-1 β-methyl group which is known to impart DHP stability, was 4-fold *more* stable than imipenem and 3-fold more stable than parent carbapenem **17a**. While this reflects the impact of a C-1 β-substituent, it may also reflect additional stabilization due to the nature of the dual-action agent. In the case of the dual-action penem **28**, DHP hydrolysis occurred twice as fast as imipenem (Table III).

Target Enzyme Activity. The target enzymes for penems and carbapenems are the penicillin-binding proteins (PBPs) whereas quinolones interact with DNA gyrase. In order to gain some insight into the mechanism of action of the dual-action compounds, some were assayed for activity against PBPs (Tables IV and V) and/or inhibition of replicative DNA biosynthesis (Table VI) which is an indication of DNA gyrase activity.[15,16] The dual-action penems bound moderately to *E. coli* and *S. aureus* PBPs. Specificity for *E. coli* PBP2 of the parent penem was preserved in the two dual-action penems **28** and **31**. Both penem and carbapenem dual-action compounds also exhibited quinolone activity (inhibition of replicative DNA biosynthesis and induction of filaments in *E. coli*), possibly due to in situ hydrolysis to the free quinolone.

Chemical Stability. Although the proposed mechanism of action for the dual-action penems and carbapenems is due to enzyme activation, an alternative possibility is simple hydrolysis of the ester or carbamate linkage to release quinolone and (hydroxymethyl)penem or carbapenem. The hydrolytic stability of these compounds was measured in pH 7.4 phosphate buffer (Table VII). The penems exhibit quite good chemical stability with **28** having a half-life of 52 h. The carbapenems have a much shorter half-life, for example, 5 h for **20**. With both classes of dual-action agents, free quinolone was liberated and easily followed by HPLC (see Experimental Section) and no intact β-lactam byproduct was observed, indicating that the lactam was the likely site of hydrolysis.

Conclusions

In summary, dual-action carbapenems and penems were assembled from azetidinone, carbapenem, or penem precursors. These agents, which contain quinolones linked to the C-2′ position of the β-lactam antibacterials either via an ester or a carbamate moiety, have been demonstrated to be potent antibacterial agents. In vitro screens indicate that a dual mode of action is operative as these compounds possess excellent anaerobe activity and good

(15) Moses, R. E.; Richardson, D. D. Replication and Repair of DNA in Cells of *Escherichia coli* Treated with Toluene. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 674–681.

(16) Pedrini, A. M.; Geroldi, A.; Siccardi, A.; Falaschi, A. Studies on the mode of Action of Nalidixic Acid. *Eur. J. Biochem.* **1972**, *25*, 359–365.

Table IV. Binding of Dual-Action Penems and Reference Compounds to *E. coli* UB1005 PBPs

	concn required (μg/mL) for 90% of inhibition of [^{14}C]Pen G binding							
compd	PBP1a 90 kDa	PBP1b 90 kDa	PBP2 66 kDa	PBP3 60 kDa	PBP4 49 kDa	PBP5/6 40 kDa	morph	MIC[b]
31	2	>100	30	100	>100	>100	F[a]	4 (1)
28	2	>100	10	30	>100	>100	F	2 (1)
37	100	100	30	>100	>100	>100	F	4 (4)

[a] F, filaments. [b] Numbers in parentheses refer to MICs for DC2, a permeability mutant of UB1005.

Table V. Binding of Dual-Action Penems and Reference Compounds to *S. aureus* ATCC 29213 PBPs

	concn required (μg/mL) for 90% of inhibn of [^{14}C]Pen G binding				
compd	PBP1 87 kDa	PBP2 80 kDa	PBP3 75 kDa	PBP4 41 kDa	MIC
31	2	2	2	2	0.25
28	0.5	0.5	0.5	>100	0.125
37	30	>100	10	>100	0.25

Table VI. Effects of Dual-Action Penems and Carbapenems and Reference Quinolones on Replication DNA Biosynthesis in *E. coli* H560 (Permeabilized Cells)

		MIC (μg/mL)	
compd	repl. DNA bios: IC_{50} (μg/mL)	*E. coli* UB1005[a]	*E. coli* 25922
6	10	1	0.12
36	2	2	0.25
31	3	4	0.06
28	4.7	2	0.25
fleroxacin	1	0.5	0.03
ciprofloxacin	0.3	0.25	0.01

[a] A nalidixic acid-resistant strain.

Table VII. Stability of Dual-Action Penems and Carbapenems in pH 7.4 Phosphate Buffer at 37 °C As Determined by HPLC Analysis

compd	half-life, h	compd	half-life, h
36	16	**20**	5
28	52	**6**	11
31	44		

potency against both Gram-negative and Gram-positive pathogens, reflective of contributions of the β-lactam as well as the quinolone.

Experimental Section

Physical Chemistry. Infrared spectra (IR) were recorded on a Digilab FTS 15-E spectrometer. Mass spectra (MS) were obtained on a VG7070E-HF mass spectrometer in positive-ion fast atom bombardment using glycerol or thioglycerol as solvent. Proton nuclear magnetic resonance spectra (NMR) were obtained on a Varian XL-400 or XL-200 instrument. Chemical shifts (δ) are expressed in parts per million (ppm) downfield from tetramethylsilane, with coupling constants (*J*) in hertz (Hz).

HPLC Analyses. Conditions for analyses of products for purity, and for monitoring stability studies were as follows: reverse-phase analytical HPLC using a Hamilton PRP-1 (250 mm × 4.1 mm) column, with UV detection typically set in the range of 275–286 nm depending upon the observed λ_{max} of the products and starting materials, and a mobile phase consisting of a 0.01 M solution of tetradecyltrimethylammonium bromide in a mixture of 0.07 M, pH 8.2 phosphate buffer and acetonitrile (70:30–64:36 v/v). In stability studies the decrease in integration of the product peak was followed at least until it reached 50% of the original value (one half-life). Semilogarithmic plots of product-peak integrations versus time were essentially linear.

Biological Assays. Minimum inhibitory concentrations (MICs) were determined by the agar dilution method. Serial 2-fold dilutions of the compounds were prepared in water to give concentrations which, when diluted 10-fold in agar, ranged from 128 to 0.0156 μg/mL. Three agar media were employed: brain heart infusion agar (Difco Laboratories, Detroit, MI) supplemented with 20 units/mL bovine liver catalase (Sigma Chemical Co., St. Louis, MO) for the streptococci; Mueller-Hinton agar (Difco) containing 3% agar for Proteus; and Mueller-Hinton agar for all other microorganisms. Petri dishes (10 cm) containing 20-mL final volume were inoculated with the aid of a Steers replicator (Craft Machine, Chester, PA). Overnight broth cultures diluted 100-fold served as inocula. Anaerobic assays were conducted in an oxygen–free hydrogen–carbon dioxide atmosphere in microtiter plates using Schaedler's broth (Difco) supplemented with coenzyme I and hemin.

Replicative DNA biosynthesis inhibition was determined by measuring the ATP-dependent incorporation of [^{3}H]thymidine into trichloroacetic acid-insoluble material by toluene-permeabilized *E. coli* H560 cells. Results are expressed as IC_{50}s, the concentrations needed for 50% inhibition.

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

Hog renal dehydropeptidase-I enzyme was partially purified from hog kidney by butanol extraction, ammonium sulfate fractionation, ion-exchange chromatography over DF-52 (Whatman Biochemicals), and gel filtration (Ultragel ACA54, LKB) as described by Kroop et al. in *Antimicrob. Agents Chemother.* **1982**, *22*, 62–70 and Campbell et al. in *Biochim. Biophys. Acta* **1966**, *118*, 386. A 42-fold purification was obtained. The specific activity of this preparation (98 mg of protein/mL), measured by the rate of hydrolysis of glycyldehydrophenylalanine, was 749 nmol hydrolyzed/min per mg of protein. Hydrolysis was measured spectrophotometrically at a concentration of 50 μM in 50 mM 3-morpholinopropanesulfonic acid (MOPS) pH 7.1 buffer and at a final volume of 0.5 or 1 mL at 37 °C. Assays of compound stability to DHP-I were measured spectrophotometrically at a fixed substrate concentration of 100 μM in pH 7.1 MOPS buffer to which enzyme (1–5 μL of the above-described preparation) was added to assure measurable hydrolysis rates. The following parameters were used for determining hydrolysis rates: glycyldehydrophenylalanine, λ = 275 nm, Δε = 15 960 M^{-1} cm^{-1}; imipenem, λ = 297 nm, Δε = 9210 M^{-1} cm^{-1}; **6**, λ = 288 nm, Δε = 11 150 M^{-1} cm^{-1}; **20**, λ = 280 nm, Δε = 7500 M^{-1} cm^{-1}; **17a**, λ = 270 nm, Δε = 7400 M^{-1} cm^{-1}; **28**, λ = 290 nm, Δε = 18 000 M^{-1} cm^{-1}.

6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylate Potassium Salt. Fleroxacin [6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid] (10 g, 27 mmol) was suspended in water (100 mL). Potassium hydroxide (27 mL of 1.0 N solution, 27 mmol) was added. Most solid material dissolved with stirring. After 5 h, the solution was filtered to remove insoluble material and the filtrate was freeze-dried to yield the desired product as a white solid (11 g, 100%).

***rac*-[2*R*,3*S*(*R*)]-2-(3-Bromo-2-oxopropyl)-3-(1-hydroxyethyl)-4-oxo-α-(triphenylphosphoranylidene)azetidine-1-acetic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (1).** A solution of rac-[2R,3S(R)]-2-(3-bromo-2,2-dimethoxypropyl)-3-(1-hydroxyethyl)-4-oxo-α-(triphenylphosphoranylidene)-1-azetidineacetic acid (2,2-dimethyl-1-oxopropyl)methyl ester[17] (6.76 g, 8.84 mmol) in acetone (230 mL) was cooled to −20 °C. With stirring, cold (−20 °C) hydrobromic acid (74 mL of 48% solution in water) was added. The temperature was observed to

(17) Gotschi, E. Canadian Patent 1,117,965, British Patent GB2,092,147, and French Patent FR2,499,081.

rise to 12 °C during this addition. The mixture was cooled to 0 °C within 2 min and stirred at this temperature for an additional 20 min. The colorless solution was then poured into a mixture of sodium carbonate (52 g), pH 7 buffer (460 mL, 1 M in phosphate), and ice (400 g). The mixture was extracted with methylene chloride (2 × 400 mL). The organic phase was washed with pH 7 buffer (300 mL, 1 M in phosphate), dried over sodium sulfate, and concentrated to dryness. Chromatography of the crude product on silica gel using ethyl acetate as the eluant, followed by crystallization of the product from ethyl acetate/hexane afforded the title compound as pale yellow crystals (3.13 g, 52%): mp 122–123 °C dec; IR ($CHCl_3$) 3515 (w), 1778, 1750, 1690 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.40–7.80 (m, 15 H, PPh_3), 5.70 (AB d, 2 H, OCH_2O), 5.32 (AB d, 2 H, CH_2Br), 4.14 (AB d, 2 H, CH_2 α to β-lactam), 2.40–3.40 (m, 4 H, β-lactam CH_2 and *CHOH*), 1.10 and 1.25 (2 s, 3 H and 9 H, $CHCH_3$ and *t*-Bu); MS *m/z* 390 (M + H).

***rac*-[2*R*,3*S*(*R*)]-6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic Acid [[3-(1-Hydroxyethyl)-1-[2-[(2,2-dimethyl-1-oxopropoxy)methoxy]-2-oxo-1-(triphenylphosphoranylidene)ethyl]-4-oxo-2-azetidinyl]acetyl]methyl Ester (2).** A solution of 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylate potassium salt (0.115 *g*, 0.282 mmol) in dry DMF (4 mL) was stirred under an argon atmosphere. Triethylamine (40 μL, 0.287 mmol) was added followed by the addition of *rac*-[2*R*,3*S*(*R*)]-2-(3-bromo-2-oxopropyl)-3-(1-hydroxyethyl)-4-oxo-α-(triphenylphosphoranylidene)-1-azetidineacetic acid (2,2-dimethyl-1-oxopropoxy)methyl ester (1) (0.200 g, 0.293 mmol). The mixture was stirred at ambient temperature for 17 h. After this time, the mixture was diluted with 25 mL of ethyl acetate and washed with water (4 × 5 mL) and then with brine (1 × 5 mL). Each aqueous washing was back-extracted using the same 20 mL of ethyl acetate. The combined organic extracts were dried (sodium sulfate) and the solvent removed via rotary evaporation. The residue was purified on a Chromatron Model 7924 preparative centrifugally accelerated radial TLC apparatus (2 mm silica gel chromatotron plate) using a methylene chloride/methanol gradient (8:1 to 4:1) as eluant to afford 0.172 g (63%) of the title compound **2** as a yellow solid: IR ($CHCl_3$) 3440, 1750 (b), 1640, 1620 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.40 (s, 1 H, NCH=), 7.99 (d, J = 11.5 Hz, 1 H, quin-Ar-H), 7.50–7.76 (m, 15 H, PPh_3), 5.64 (m, 2 H, POM-CH_2), 4.59–5.03 (m, 7 H, $C(O)CH_2O$, CH_2CH_2F, β-lactam CH), 3.48 (bs, 4 H, piperazinyl CH_2's), 2.95–3.36 (m, 5 H, CH_2 α to β-lactam, β-lactam CH, *CHC*(O*H*)CH3), 2.56 (bs, 4 H, piperazinyl CH_2's), 2.37 (s, 3 H, NMe), 1.24 (s, 9 H, *t*-Bu), 1.04 (bd, 3 H, $CHCH_3$); MS (FAB) *m/z* 971 (M + H).

***rac*-[2*R*,3*S*(*R*)]-6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic Acid [[3-[1-[(Trimethylsilyl)oxy]ethyl]-1-[2-[(2,2-dimethyl-1-oxopropoxy)methoxy]-2-oxo-1-(triphenylphosphoranylidene)ethyl]-4-oxo-2-azetidinyl]acetyl]methyl Ester (3).** Chlorotrimethylsilane (1.4 mL, 11.03 mmol) was added to a stirred solution of the lactam–quinoline **2** (0.713 g, 0.734 mmol) in dry pyridine (6.0 mL) under argon. The reaction flask was sealed and stirred at ambient temperature for 22 h. The reaction mixture was diluted with 125 mL of ethyl acetate and extracted with cold 0.25 M aqueous sodium bicarbonate solution (2 × 20 mL) and then washed with brine (1×). The organic phase was dried (sodium sulfate) and concentrated to an oil under high vacuum at 30 °C. The residue was dissolved in ethyl acetate and again concentrated under high vacuum at 30 °C. This procedure was repeated two more times to remove as much pyridine as possible, affording product **3**, which was immediately used in the following step: ^{1}H-NMR ($CDCl_3$) δ 8.45 (s, 1 H, NCH=), 7.98 (d, J = 11.5, 1 H, quin-Ar-H), 7.40–7.80 (m, 15 H, PPh_3), 5.69 (m, 2 H, POM-CH_2), 4.50–5.35 (m, 7 H, $C(O)CH_2O$, CH_2CH_2F, β-lactam CH), 3.38 (bs, 4 H, piperazinyl CH_2's), 2.80–3.15 (m, 4 H, CH_2 α to β-lactam, β-lactam CH, *CHC*(OTMS)CH_3), 2.56 (bs, 4 H, piperazinyl CH_2's), 2.35 (s, 3 H, NMe), 1.05–1.30 (s and m, 12 H, $CHCH_3$ and *t*-Bu), 0.04 (9 H, $SiMe_3$).

***rac*-[5*R*,6*S*(*R*)]-3-[[[[6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinyl]carbonyl]oxy]methyl]-7-oxo-6-[1-[(trimethylsilyl)oxy]ethyl]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (4).** The crude silylated intermediate **3** (prepared by the previous procedure) was taken up in dry toluene (35 mL) and heated at 105 °C for 75 min under an argon atmosphere. The reaction was concentrated to an oil and the residue was purified on a silica gel 2-mm chromatotron plate (methylene chloride/methanol (9:1) as eluant) to obtain 0.442 g (80%) of the desired carbapenem **4** as a white solid: IR ($CHCl_3$) 1785, 1750, 1730, 1700, 1620, 840 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.36 (s, 1 H, NCH=), 7.97 (dd, J = 12, 1, 1 H, quin-Ar-H), 5.95, 5.85 (AB dd, 2 H, OCH_2O), 5.50, 5.24 (AB dd, 2 H, $CH_2OC(O)$), 4.58–4.86 (4 m, 4 H, CH_2CH_2F), 4.16 (m, 2 H, CHOSi, β-lactam CH), 3.36 (bs, 4 H, piperazinyl CH_2's), 3.30 (dd, 1 H, carbapenem C-1 CH_2), 3.15 (m, 1 H, β-lactam CH), 3.05 (dd, 1 H, carbapenem C-1 CH_2), 2.55 (bs, 4 H, piperazinyl CH_2's), 2.36 (s, 3 H, NMe), 1.23 (m, 12 H, $CHCH_3$, *t*-Bu), 0.12 (s, 9 H, $SiMe_3$); MS (FAB) *m/z* 765 (M + H).

***rac*-[5*R*,6*S*(*R*)]-3-[[[[6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinyl]carbonyl]oxy]methyl]-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (5).** Silylated carbapenem **4** (0.335 g, 0.438 mmol) was dissolved in dry methanol (17 mL) and stirred at ambient temperature under an argon atmosphere. Ammonium fluoride (38 mg, 1.026 mmol) was added in one portion. After 75 min an additional portion of ammonium fluoride (5 mg, 0.13 mmol) was added and the solution was stirred for an additional 15 min. The mixture was diluted with 125 mL of ethyl acetate and washed with brine (2 × 25 mL). The organic phase was dried (sodium sulfate), and the solvents were removed by rotary evaporation. The residue was purified on a 2-mm silica gel chromatotron plate using methylene chloride/methanol (15:2) as eluant to afford 0.204 g (67%) of the title compound **5** as a white solid: IR (KBr) 3450, 1780, 1750, 1730, 1620 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.36 (s, 1 H, NCH=), 7.97 (dd, J = 12, 1 Hz, 1 H, quin-Ar-H), 5.96, 5.86 (AB dd, 2 H, OCH_2O), 5.50, 5.25 (AB dd, 2 H, $CH_2OC(O)$), 4.59–4.87 (4 m, 4 H, CH_2CH_2F), 4.23 (m, 2 H, β-lactam CH, *CHC*(OH)CH_3), 3.36 (bs, 4 H, piperazinyl CH_2's), 3.36 (dd, 1 H, carbapenem C-1 CH_2), 3.20 (m, 1 H, β-lactam CH), 3.05 (dd, 1 H, carbapenem C-1 CH_2), 2.55 (bs, 4 H, piperazinyl CH_2's), 2.37 (s, 3 H, NMe), 1.90 (bs, 1 H, OH), 1.32 (d, J = 6, 3 H, CH_3CH), 1.23 (s, 9 H, *t*-Bu); MS (FAB) *m/z* 693 (M + H).

***rac*-[5*R*,6*S*(*R*)]-3-[[[[6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinyl]carbonyl]oxy]methyl]-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Monosodium Salt (6).** The carbapenem **5** (0.124 g, 0.179 mmol) was dissolved in dimethyl sulfoxide (8.0 mL) with stirring at ambient temperature. To this solution was added pH 7.0 phosphate buffer (30 mL, 0.025 M in phosphate) which resulted in the precipitation of the carbapenem. Porcine liver esterase (150 μL, EC 3.1.1.1) was added followed by an additional portion of the pH 7.0 buffer (10 mL) which brought the pH of the reaction mixture to 7.8. The mixture was stirred at ambient temperature and sodium hydroxide (0.1 N) was added as needed to keep the pH of the mixture between 7.2 and 7.4. Two additional portions of the esterase were added during this period as follows: 100 μL after 1 h and 125 μL after 2 h. As the reaction progressed precipitated material dissolved into solution. After 4 h, the nearly homogeneous reaction mixture was extracted with ether (2 × 150 mL). The aqueous phase was concentrated under high vacuum to half its original volume. The remaining aqueous solution was subjected to reverse-phase column chromatography on a column (40 g of C_{18} silica gel). The column was eluted with a stepwise gradient of water to water/acetonitrile (0 to 20% acetonitrile) under pressure. Appropriate fractions were combined, concentrated, and freeze-dried to obtain 43.5 mg (41%) of product **6**: IR (KBr) 3400, 1770, 1690, 1630 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.57 (s, 1 H, NCH=), 7.75 (d, J = 12 Hz, 1 H, quin-Ar-H), 5.41 (AB d, 1 H, $CH_2OC(O)$), 5.03 (AB d, 1 H, $CH_2OC(O)$), 5.03 (m, 1 H, β-lactam CH), 4.76–4.91 (m, 4 H, CH_2CH_2F), 4.07 (m, 1 H, β-lactam CH), 3.93 (m, 1 H, *CH*(OH)), 3.29 (bs, 4 H, piperazinyl CH_2's), 2.95–3.15 (m, 2 H, carbapenem C-1 CH_2), 2.50 (bs, 4 H, piperazinyl CH_2's), 2.25 (s, 3 H, NMe), 1.13 (d, J = 6 Hz, 3 H, $CHCH_3$); MS (FAB) *m/z* 601 (M + H).

***rac*-[2*R*,3*S*(*R*)]-2-[3-[(Chloroacetyl)oxy]-2-oxopropyl]-3-[1-[[(2,2,2-trichloroethoxy)carbonyl]oxy]ethyl]-4-oxo-α-(triphenylphosphorylidene)-1-azetidineacetic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (8).** A solution of

rac-[2*R*,3*S*(*R*)]-2-(3-bromo-2-oxopropyl)-3-[1-[[(2,2,2-trichloroethoxy)carbonyl]oxy]ethyl]-4-oxo-α-(triphenylphosphoranylidene)-1-azetidineacetic acid (2,2-dimethyl-1-oxopropoxy)methyl ester (7)[17] (4.060 g, 4.73 mmol) in dry DMF (67 mL) was stirred under an argon atmosphere. Triethylamine (0.70 mL, 5.02 mmol) was added followed by the sodium salt of chloroacetic acid (0.68 g, 5.84 mmol). The mixture was stirred at ambient temperature for 1.5 h. After this time, the mixture was diluted with 400 mL of ethyl acetate and washed with water (4 × 80 mL) and then with brine (1 × 80 mL). The organic extract was dried (sodium sulfate), and volatile organics were removed via rotary evaporation. The residue was purified by flash chromatography (silica gel, ethyl acetate/methylene chloride/hexane (1:1:1) as eluant) to afford 2.725 g (66%) of the title compound 8: IR ($CHCl_3$) 1760 (b) cm^{-1}; ^1H-NMR ($CDCl_3$) (3:2 mixture of rotamers) δ 7.50–7.80 (m, 15 H, PPh_3), 5.70 and 5.42 (2 AB d, OCH_2O), 4.65–4.90 (m, 6 H, β-lactam CH and CH_2's), 3.75–3.90 (m, 1 H, $CHCH_3$), 3.08 and 2.80 (2 m, 2 H, CH_2), 1.05–1.30 (2 s and 2 d, 12 H, $CHCH_3$ and *t*-Bu); MS *m/z* 870 (M + H).

***rac*-[2*R*,3*S*(*R*)]-2-(3-Hydroxy-2-oxopropyl)-3-[1-[[(2,2,2-trichloroethoxy)carbonyl]oxy]ethyl]-4-oxo-α-(triphenylphosphorylidene)-1-azetidineacetic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (9).** The β-lactam 8 (2.587 g, 2.98 mmol) was dissolved in methanol (100 mL) and the resulting solution was cooled to 0–5 °C. To this solution was added 0.10 N sodium bicarbonate (32 mL, 3.20 mmol) and the mixture was stirred for 25 min at 0–5 °C. After this time, the solution was diluted with 500 mL of ethyl acetate and washed with water (2 × 200 mL) and then brine (1×). The organic extract was dried (sodium sulfate), and the solvents were removed via rotary evaporation. The crude residue was purified by flash chromatography (silica gel, ethyl acetate/methylene chloride/hexane (1:1:1) as eluant) to yield 2.027 g (88%) of the title compound 9: IR (KBr) 3440, 1750 (b), 1640 cm^{-1}; ^1H-NMR ($CDCl_3$) (1:1 mixture of rotamers) δ 7.50–7.80 (m, 15 H, PPh_3), 5.68 and 5.30 (2 AB d, 2 H, OCH_2O), 4.73 (apparent d, 2 H, CH_2), 4.18–4.40 (m, 2 H, CH_2), 3.87 (m, 1 H, β-lactam CH), 2.60–3.50 (m, 5 H, CH_2 and β-lactam CH), 1.05–1.30 (2 s and 2 d, 12 H, $CHCH_3$ and *t*-Bu); MS *m/z* 794 (M + H).

***rac*-[2*R*,3*S*(*R*)]-2-[3-[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)piperazinyl]carbonyl]oxy]-2-oxopropyl]-3-[1-[[(2,2,2-trichloroethoxy)carbonyl]oxy]ethyl]-4-oxo-α-(triphenylphosphorylidene)-1-azetidineacetic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (11).** Under an argon atmosphere, a solution of phosgene (0.25 mL of 20% solution in toluene, 0.483 mmol) in 1.5 mL of methylene chloride was cooled to 0 °C. A cooled and dry solution of the azetidinone 9 (0.197 g, 0.248 mmol) in 2.5 mL of methylene chloride was then added simultaneously with *N*,*N*-diisopropylethylamine (70 μL, 0.409 mmol) to the phosgene solution. The mixture was stirred at 0 °C for 30 min and then at ambient temperature for 45 min. After this time, the reaction mixture was diluted with an additional 10 mL of methylene chloride and then concentrated to the original volume at 5 °C. The resultant solution was then added to a solution of ciprofloxacin (0.070 g, 0.211 mmol) in 2.8 mL of methylene chloride which had been pretreated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (78 μL, 0.420 mmol) for 40 min at room temperature and then cooled to 0 °C. This mixture was stirred at 0 °C for 35 min and then for 75 min at ambient temperature. After this time, the mixture was concentrated under high vacuum. The residue was taken up in ethyl acetate (8 mL) and filtered to remove insoluble material. The filtrate was extracted with pH 4 phosphate buffer (2 × 3 mL) and then with brine solution (1×). The combined organic extract was dried (sodium sulfate) and the solvent removed via rotary evaporation. The crude residue was purified on a Chromatotron Model 7924 preparative centrifugally-accelerated, radial TLC apparatus (2-mm plate), using ethyl acetate followed by ethyl acetate/acetone/methanol/water (70:10:5:5) as the eluant, to obtain 112 mg (46%) of the title compound 11 which was used in the next step: ^1H-NMR ($CDCl_3$) δ 14.96 (s, 1 H, COOH), 8.73 (s, 1 H, NCH=), 8.00 (d, 1 H, quin-Ar-H), 7.35–7.75 (m, 16 H, PPh_3 and quin-Ar-H), 5.68 (AB d, 2 H, OCH_2O), 5.28 (bs, 1 H, $CHC(OTEOC)$), 4.60–4.85 (m, 4 H, CH_2CCl_3, $C(O)CH_2OC(O)$), 3.80 (b, 5 H, piperazinyl CH_2's and β-lactam CH), 2.68–3.65 (m, 8 H, piperazinyl CH_2's, β-lactam CH, CHN, and $CH_2C(O)$), 1.05–1.50 (m, 16 H, CH_2CH_2, *t*-Bu, $CHCH_3$).

***rac*-[2*R*,3*S*(*R*)]-2-[3-[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)piperazinyl]carbonyl]oxy]-2-oxopropyl]-3-(1-hydroxyethyl)-4-oxo-α-(triphenylphosphorylidene)-1-azetidineacetic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (12).** The azetidinone 11 (0.468 g, 0.406 mmol), prepared in the previous step, was taken up in 3 mL of 90% acetic acid and treated with zinc (0.95 g) for 45 min at ambient temperature. After this time, the reaction mixture was diluted with 36 mL of methylene chloride, and the mixture was filtered to remove the insoluble material. The filtrate was extracted with saturated sodium carbonate solution (2 × 15 mL) and brine (2 × 25 mL). The aqueous extracts were washed twice with methylene chloride because of the thick emulsions that formed. The organic extracts were combined and dried (sodium sulfate), and the solvents were removed by rotary evaporation. The residue was crystallized from ethyl acetate/ether to give 0.236 g (59%) of the title compound 12. An additional 0.064 g (16%) of the title compound was obtained by purifying the mother liquor on a 1-mm chromatotron plate using ethyl acetate/acetone/methanol/water (70:10:5:5) as the eluant: IR (KBr) 3460, 1750 (b), 1720, 1630 cm^{-1}; ^1H-NMR ($CDCl_3$) (rotamers) δ 14.97 (bs, 1 H, COOH), 8.79 (2 s, 1 H, NCH=), 8.07 (2 d, 1 H, quin-Ar-H), 7.40 (m, 16 H, PPh_3 and quin-Ar-H), 5.70 and 5.30 (2 AB d, 2 H, OCH_2O), 4.81 (AB d, 2 H, $C(O)CH_2OC(O)$), 3.50–3.90 (m, 5 H, piperazinyl CH_2's and β-lactam CH), 3.00–3.40 (m, 8 H, piperazinyl CH_2's and β-lactam CH and $CHC(OH)$ and $CH_2C(O)$), 2.50–2.70 (m, 2 H, CHN and OH), 1.18–1.43 (m, 4 H, CH_2CH_2), 1.28 and 1.05 (2 s, 9 H, *t*-Bu), 1.05 (m, 3 H, $CHCH_3$); MS (FAB) *m/z* 977 (M + H).

***rac*-[2*R*,3*S*(*R*)]-2-[3-[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)piperazinyl]carbonyl]oxy]-2-oxopropyl]-3-[1-[(trimethylsilyl)oxy]ethyl]-4-oxo-α-(triphenylphosphorylidene)-1-azetidineacetic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (13).** The β-lactam 12 (0.232 g, 0.237 mmol) was dissolved in 2 mL of dry pyridine and the flask was charged with argon. Trimethylchlorosilane (0.36 mL, 2.84 mmol) was then added, the flask was sealed, and the mixture was stirred at ambient temperature. After 4 h, additional trimethylchlorosilane (0.10 mL, 0.78 mmol) was added. The flask was sealed again and stirred for an additional 1.5 h. The reaction mixture was then diluted with ethyl acetate and extracted with cold 0.25 M sodium bicarbonate solution (2 × 5 mL) and then brine (1×). The organic extract was dried (sodium sulfate) and concentrated under high vacuum. The resultant oil was taken up in ethyl acetate three times and concentrated under high vacuum each time in order to remove the residual pyridine, giving product 13 as an oil which was immediately used in the following step: ^1H-NMR ($CDCl_3$) δ 8.75 (d, 1 H, NCH=), 8.05 (d, 1 H, quin-Ar-H), 7.40–7.85 (m, 16 H, PPh_3 and quin-Ar-H), 5.70 (m, 1 H, OCH_2O), 5.30 (m, 1 H, OCH_2O), 4.81 (m, 2 H, $C(O)CH_2OC(O)$), 2.50–3.90 (m, 14 H, piperazinyl CH_2's, β-lactam CH, $CHC(OTMS)$, CHN, and $CH_2C(O)$), 1.10–1.50 (m, 16 H, CH_2CH_2, $CHCH_3$, and *t*-Bu), 0.00 (s, 9 H, $SiMe_3$).

***rac*-[5*R*,6*S*(*R*)]-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)piperazinyl]carbonyl]oxy]methyl]-6-[1-[(trimethylsilyl)oxy]ethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (14).** Oil 13 was dissolved in toluene (9 mL) and heated to 105 °C for 1 h. Concentration of the reaction mixture and chromatography of the crude residue on a 1-mm chromatotron plate (ethyl acetate/acetone/methanol/water (70:10:5:5) as eluant) afforded 139 mg (75%) of the title compound 14: IR (KBr) 1790, 1750, 1730, 1710, 1630, 840 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 14.90 (s, 1 H, COOH), 8.79 (s, 1 H, NCH=), 8.07 (d, 1 H, quin-Ar-H), 7.38 (d, 1 H, quin-Ar-H), 5.90 (AB d, 2 H, OCH_2O), 5.20 (AB d, 2 H, $CH_2OC(O)$), 4.15 (m, 2 H, CHOTMS and β-lactam CH), 3.73 (bs, 4 H, piperazinyl CH_2's), 3.55 (m, 1 H, CHN), 3.32 (bs, 4 H, piperazinyl CH_2's), 3.15 (m, 1 H, β-lactam CH), 2.98 (m, 2 H, carbapenem C-1 CH_2), 1.15–1.40 (m, 4 H, CH_2CH_2), 1.24 (d, 3 H, $CHCH_3$), 1.23 (s, 9 H, *t*-Bu), 0.13 (s, 9 H, $SiMe_3$); MS (FAB) *m/z* 771 (M + H).

***rac*-[5*R*,6*S*(*R*)]-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)piperazinyl]carbonyl]oxy]methyl]-6-(1-hydroxyethyl)-7-oxo-1-azabicy-**

clo[3.2.0]hept-2-ene-2-carboxylic Acid (2,2-Dimethyl-1-oxopropoxy)methyl Ester (15). A solution of the silylated carbapenem 14 (110 mg, 0.143 mmol) in 6.5 mL of methanol was treated with ammonium fluoride (38 mg). Methylene chloride (2–3 mL) was added to obtain a clear solution. The mixture was stirred at ambient temperature for 80 min. After this time, the mixture was diluted with ethyl acetate and extracted with brine (2×). The organic extract was dried (sodium sulfate) and concentrated to an oil. The crude residue was purified by chromatography on a 1-mm chromatotron plate with ethyl acetate/acetone/methanol/water (70:10:5:5) as eluant, giving 70 mg (70%) of the title compound 15 which was used in the next step: ^{1}H-NMR (DMSO-d_6) δ 8.71 (s, 1 H, NCH=), 7.98 (d, 1 H, quin-Ar-H), 7.36 (d, 1 H, quin-Ar-H), 5.88 (AB d, 2 H, OCH_2O), 5.29 (AB d, 2 H, $CH_2OC(O)$), 4.20 (m, 2 H, β-lactam CH and OH), 3.72 (m, 4 H, piperazinyl CH_2's), 3.45–3.65 (m, 1 H, CHN), 3.36 (m, 4 H, piperazinyl CH_2's), 2.80–3.40 (m, 4 H, β-lactam CH, C*H*COH, and carbapenem C-1 CH_2), 1.15–1.50 (m, 16 H, CH_2CH_2, $CHCH_3$, and *t*-Bu).

***rac*-[5*R*,6*S*(*R*)]-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)piperazinyl]carbonyl]oxy]methyl]-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate Sodium Salt (16).** The carbapenem 15 (30 mg, 0.043 mmol) was dissolved in dimethyl sulfoxide (1 mL). To this solution was added pH 7.0 buffer (2 mL, 0.2 M in phosphate) which resulted in the precipitation of the carbapenem. Porcine liver esterase (95 μL, EC 3.1.1.1) was added which brought the pH of the mixture to 7.70. The mixture was stirred at ambient temperature and sodium hydroxide was added as needed to keep the pH of the mixture between 7.1 and 7.4. Two additional portions of the esterase were added during the early part of the reaction: 50 μL after 20 min and another 50 μL after 1 h. The reaction mixture was then stirred at ambient temperature for additional 18 h. The mixture (solid still present) was then extracted with ether (2×). The aqueous extract was chromatographed on a column containing 10 g of C_{18} silica gel. The column was eluted with a stepwise gradient of water to water/acetonitrile (0–20% acetonitrile). The appropriate fractions were combined, concentrated, and freeze-dried to obtain 2 mg of the final product 16: IR (KBr) 3440 (b), 1750 (b), 1730, 1710, 1630 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.67 (s, 1 H, NCH=), 7.94 (d, 1 H, quin-Ar-H), 7.61 (d, 1 H, quin-Ar-H), 5.32 and 4.87 (AB d, 2 H, $CH_2OC(O)$), 4.41 (bs, 1 H, OH), 3.80–3.95 (m, 3 H, β-lactam CH and C*H*COH and CHN), 3.63 (bs, 4 H, piperazinyl CH_2's), 3.33 (bs, 4 H, piperazinyl CH_2's), 3.05 (m, 1 H, β-lactam CH), 2.30–2.75 (m, 2 H, carbapenem C-1 CH_2), 1.15–1.35 (m, 4 H, CH_2CH_2), 1.13 (d, 3 H, $CHCH_3$); MS (FAB) *m*/*z* 629 (M + H).

[4*S*,5*R*,6*S*(*R*)]-1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[[[4-methyl-7-oxo-2-[(2-propenoxy)carbonyl]-6-[1-[[(2-propenoxy)carbonyl]oxy]ethyl]-1-azabicyclo[3.2.0]hept-2-en-3-yl]methoxy]carbonyl]-1-piperazinyl]-4-oxo-3-quinolinecarboxylic Acid (19). A solution of (4*S*,5*R*,6*S*(*R*))-3-(hydroxymethyl)-4-methyl-6-[1-[[(2-propenoxy)carbonyl]oxy]ethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-enecarboxylic acid 2-propenyl ester (17) (0.461 g, 1.261 mmol) in 18 mL of dry methylene chloride was cooled to 0 °C under an argon atmosphere. With stirring, *N*,*N*-dimethylaniline (0.245 mL, 1.93 mmol) was added, followed by phosgene (1.0 mL of a 1.93 M solution in toluene, 1.93 mmol). The solution was stirred at 0–5 °C for 1 h. At this time, the reaction solution was diluted with an additional 5 mL of methylene chloride and concentrated to the original volume at 0–5 °C. The resultant solution was treated with *N*,*N*-dimethylaniline (0.32 mL, 2.55 mmol) followed by the addition of a cold methylene chloride solution of *N*,*O*-bis(trimethylsilyl)ciprofloxacin (prepared from the reaction of ciprofloxacin (0.398 g, 1.201 mmol) in 7.6 mL of methylene chloride with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (0.50 mL, 2.60 mmol) for 20 min, stirred over 4A molecular sieves for 1 h and cooled to 0 °C prior to the addition to the carbapenem solution). The mixture was stirred for 1 h at 0 °C. After this time, the mixture was diluted with cold ethyl acetate and 16 mL of pH 4 buffer and the mixture was stirred vigorously for 5 min. Following separation of the layers, the aqueous layer was washed with a second portion of ethyl acetate. The two organic extracts were combined, washed with brine, dried (sodium sulfate), and concentrated via rotary evaporation. The residue was taken up in methylene chloride and a small amount of ethyl acetate and concentrated until product started precipitating out of solution. Ether was then added to complete the precipitation of the product. The solid was collected by filtration and the "crystallization" process was repeated on the solid to obtain 0.582 g (67%) of the title compound 19: IR ($CHCl_3$) 3440, 1780, 1725 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.73 (s, 1 H, NCH=), 8.00 (d, 1 H, quin-Ar-H), 7.37 (d, 1 H, quin-Ar-H), 5.86–6.02 (m, 2 H, vinylic protons), 5.25–5.50 (m, 5 H, vinylic protons and $CH_2OC(O)$), 5.13 (m, 1 H, $CHOCO_2$), 4.60–5.00 (m, 5 H, $CH_2OC(O)$ and allylic protons), 4.23 (dd, 1 H, β-lactam CH), 3.73 (bs, 4 H, piperazinyl protons), 3.57 (m, 1 H, CHN), 3.45 (dd, 1 H, β-lactam CH), 3.32 (b, 5 H, piperazinyl CH_2's and carbapenem C-1 proton), 1.47 (d, 3 H, $CHCH_3$), 1.22 and 1.44 (2 m, 4 H, cyclopropyl CH_2), 1.22 (m, 3 H, $CHCH_3$); MS (FAB) *m*/*z* 723 (M + H).

[4*S*,5*R*,6*S*(*R*)]-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)piperazinyl]carbonyl]oxy]methyl]-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Potassium Salt (20). [4*S*,5*R*,6*S*(*R*)]-1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[4-[[[4-methyl-7-oxo-2-[(2-propenoxy)carbonyl]-6-[1-[[(2-propenoxy)carbonyl]oxy]ethyl]-1-azabicyclo[3.2.0]hept-2-en-3-yl]methoxy]carbonyl]-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid (19) (0.207 g, 0.286 mmol) was combined with triphenylphosphine (30.9 mg, 0.118 mmol), tetrakis(triphenylphosphine)palladium(0) (37.1 mg, 0.032 mmol), 2-ethylhexanoic acid (46 μL, 0.306 mmol), and potassium 2-ethylhexanote (0.5 M solution in ethyl acetate; 0.62 mL, 0.310 mmol) in 12 mL of sieve-dried methylene chloride. The mixture was stirred vigorously at ambient temperature under argon, for 70 min. After this time, the solvents were removed by rotary evaporation, and the residue was triturated with ether, resulting in the formation of a solid. The solid was collected by filtration and washed with ether. This crude solid was taken up in pH 7.6 phosphate buffer and applied to a column containing 18 g of C_{18} silica gel. The column was eluted under pressure with a stepwise gradient of pH 7.6 buffer to pH 7.6 buffer/acetonitrile (0–25% acetonitrile). The fractions of desired purity were combined and concentrated to remove the acetonitrile. The aqueous solution was then applied to a new short C_{18} column and eluted with cold water to remove all of the salts. The product was then eluted from the column with cold 40% acetonitrile in water. The product-containing fractions were combined, concentrated, and freeze-dried to obtain 0.123 g (68%) of the final product 20: IR (KBr) 1780, 1720, 1700 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.74 (s, 1 H, NCH=), 7.5–7.7 (b, 2 H, quin-Ar-H), 5.4 and 4.8 (AB d partially obscured by HDO, 2 H, $CH_2OC(O)$), 4.2–4.3 (m, 2 H, β-lactam CH and OH), 3.3–3.9 (m, 14 H, β-lactam CH, piperazinyl CH_2's, CHN, carbapenem C-1 proton, C*H*CH_3), 1.05–1.50 (m, 10 H, CH_2CH_2, $CHCH_3$, and carbapenem C-1 CH_3); MS (FAB) *m*/*z* 637 (M + H).

[4*S*,5*R*,6*S*(*R*)]-6-(1-Hydroxyethyl)-3-(hydroxymethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Potassium Salt (17a). [4*S*,5*R*,6*S*(*R*)]-3-(Hydroxymethyl)-4-methyl-6-[1-[[(2-propenoxy)carbonyl]oxy]ethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-enecarboxylic acid 2-propenoxy ester (17) (84.4 mg, 0.231 mmol) was combined with triphenylphosphine (5.5 mg, 0.021 mmol), tetrakis(triphenylphosphine)palladium(0) (19.3 mg, 0.073 mmol), 2-ethylhexanoic acid (39 μL, 0.244 mmol), and potassium 2-ethylhexanoate (0.49 mL of a 0.5 M solution in ethyl acetate, 0.245 mmol) in methylene chloride/ethyl acetate (9 mL of 1:1 v/v). The mixture was stirred for 2 h at ambient temperature under an argon atmosphere. After this time, the mixture was concentrated to an oil which was triturated with ethyl ether. The solid which formed was collected by filtration. This crude product was taken up in 2% acetonitrile/water and subjected to flash reverse-phase column chromatograph (2% acetonitrile/water as eluant, C_{18} silica gel). The product-containing fraction were combined, concentrated, and freeze-dried to obtain product 17a (48 mg, 76%).

2-[(*tert*-Butyldimethylsilyl)oxy]thioacetic Acid (22). To a solution of glycolic acid (16 g, 222 mmol) in 240 mL of acetone were added benzyl bromide (26.4 mL, 244 mmol) and triethylamine (34 mL, 244 mmol). The mixture was refluxed for 22 h, during which time a heavy precipitate formed. The mixture was cooled and filtered, and the acetone was evaporated. The residue was taken up in ethyl acetate and washed with water, and the

organic phase was dried over anhydrous magnesium sulfate and evaporated to afford 29.1 g (79%) of crude benzyl ester: ^{1}H-NMR ($CDCl_3$) δ 7.36 (s, 5 H), 5.23 (s, 2 H, CH_2OPh), 4.19 (s, 2 H).

A solution of the crude benzyl ester (29.1 g, 175 mmol) and *tert*-butyldimethylchlorosilane (31.6 g, 209 mmol) in 131 mL of pyridine was stirred at 25 °C for 3 h. The mixture was filtered, taken up in ether, and washed with 1 M HCl (5×) and brine. The organic phase was dried over anhydrous magnesium sulfate and evaporated to afford crude silyl ether. Silica gel chromatography, eluting with 1:30 ethyl acetate/hexane, afforded 45.7 g (93%) of the silyl ether: ^{1}H-NMR ($CDCl_3$) δ 7.35 (s, 5 H), 5.17 (s, 2 H, CH_2OPh), 4.27 (s, 2 H), 0.90 (s, 9 H), 0.08 (s, 6 H).

A solution of the 2-[(*tert*-butyldimethylsilyl)oxy]acetic acid benzyl ester from above (42.1 g, 150 mmol) in 400 mL of ethyl acetate containing 3 g of 10% Pd/C was hydrogenated at 1 atm for 6 h. Filtration and evaporation afforded 28.5 g (99%) of the crude acid: ^{1}H-NMR ($CDCl_3$) δ 4.21 (s, 2 H), 0.92 (s, 9 H), 0.14 (s, 6 H).

The crude acid (28.5 g, 149 mmol) was dissolved in 600 mL of methylene chloride and cooled to −10 °C. Triethylamine (34 mL, 243 mmol) and then isobutyl chloroformate (17 mL, 132 mmol) were added, and the solution was stirred for 2.5 h at −10 °C to 0 °C. Hydrogen sulfide was then bubbled into the solution for 1.25 h. The mixture was poured into 250 mL of 2 N H_2SO_4 and extracted twice with methylene chloride. The organic layers were washed with brine (3×), dried over anhydrous magnesium sulfate, and evaporated to afford 32 g (>100%) of crude thioacid which was approximately 50% pure according to ^{1}H-NMR. The crude product was distilled in vacuo (3 mm) to afford 11.4 g (37%) of **22** which was 75% pure: bp 75–85 °C (3 mm); ^{1}H-NMR ($CDCl_3$) δ 4.11 (s, 2 H), 0.95 (s, 9 H), 0.13 (s, 6 H).

[4*R*,3*S*(*R*)]-3-[1(*R*)-[(*tert*-butyldimethylsilyl)oxy]ethyl]-4-[[[(*tert*-butyldimethylsilyl)oxy]acetyl]thio]-2-azetidinone (23). A solution of sodium bicarbonate (3.0 g, 35.7 mmol) in 40 mL of water was added to the thioacid **22** (4.0 g, 14.5 mmol) at 0 °C. After stirring for 20 min, a solution of (3*R*,4*R*)-4-acetoxy-3-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]-ethyl]-2-azetidinone[18] (3.0 g, 10.4 mmol) in 180 mL of dioxane was added dropwise. The mixture was stirred, warming to 25 °C, for 21 h, the dioxane was evaporated, and the residue was taken up in methylene chloride. The organic solution was washed with water, dried over anhydrous magnesium sulfate, and evaporated. The crude product was purified by silica gel chromatography, eluting with a gradient from 1:30 to 1:3 ethyl acetate/hexane to afford 2.26 g (50%) of **23**: ^{1}H-NMR ($CDCl_3$) δ 6.26 (br s, 1 H, NH), 5.22 (d, J = 2.6, 1 H, C-4H), 4.24 (bs, 3 H, CH_2O + C-3H), 3.19 (m, 1 H, CHOTBS), 1.21 (d, J = 6.2, 3 H, CH_3), 0.94 (s, 9 H), 0.87 (s, 9 H), 0.11 (s, 6 H), 0.06 (s, 6 H).

[4*S*,5*R*,6*S*(*R*)]-3-[[(*tert*-butyldimethylsilyl)oxy]methyl]-6-[1-[(*tert*-butyldimethylsilyl)oxy]ethyl]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-enecarboxylic Acid 2-Allyl Ester Penem (24). A solution of **23** (3.2 g, 7.37 mmol) in 20 mL of methylene chloride, under an argon atmosphere, was cooled to 0 °C. Calcium carbonate (2.1 g, 21 mmol) was added followed by allyloxalyl chloride[19] (1.12 mL, 7.56 mmol) and then by diisopropylethylamine (1.28 mL, 7.36 mmol). After stirring for 20 min, the mixture was filtered from excess inorganic salts, diluted with methylene chloride, and washed with cold water. The organic phase was dried over anhydrous magnesium sulfate, and the solvents were rotary evaporated to afford 4.68 g (>100%) of crude oxalamide: IR ($CHCl_3$) 1820, 1763, 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.05–5.83 (m, 1 H, vinyl H), 5.96 (d, J = 3.4, 1 H, C-4H), 5.48–6.32 (4 bs, 2 H, vinyl CH_2), 4.77 (br d, 2 H, allyl CH_2), 4.39–4.32 (m, 1 H, C-3H), 4.30 (s, 2 H, CH_2O), 3.46 (apparent t, 1 H, CHOTBS), 1.22 (d, J = 6.2, 3 H), 0.93 (s, 9 H), 0.84 (s, 9 H), 0.08 (s, 6 H), 0.03 (s, 6 H).

The crude oxalamide obtained above (4.02 g, 7.37 mmol) was dissolved in 125 mL of dry xylene, containing 20 mg of hydroquinone. The solution was heated almost to reflux, at which point triethyl phosphite (8 mL, 46.7 mmol) was added rapidly. The mixture was refluxed for 45 min and cooled to 25 °C, and the xylene was distilled off in vacuo (3 mm). The residue was dissolved in ethyl acetate and washed twice with brine, and the organic phase was dried over anhydrous magnesium sulfate and evaporated to afford crude penem. The product was chromatographed on silica gel, eluting first with hexane and then a gradient from 1:20 to 1:4 ethyl acetate/hexane to afford 2.07 g (55%) of **24**: ^{1}H-NMR ($CDCl_3$) δ 6.01–5.82 (m, 1 H), 5.52 (bs, 1 H, C-5H), 5.43–5.20 (4 bs, 2 H, vinyl CH_2), 4.84 (s, 2 H), 4.67 (m, 2 H, CH_2O), 3.66 (d, J = 4.6, 1.8, 1 H, C-6H), 1.23 (d, J = 6.8, 3 H), 0.90 (s, 9 H), 0.87 (s, 9 H), 0.08 (s, 6 H), 0.06 (s, 6 H).

[4*S*,5*R*,6*S*(*R*)]-3-(Hydroxymethyl)-6-[1-[(*tert*-butyldimethylsilyl)oxy]ethyl]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-enecarboxylic Acid 2-Allyl Ester (25). A solution of penem **24** (2.07 g, 4.04 mmol) in 340 mL of THF was cooled to 0 °C. Acetic acid (2.1 mL) and then tetrabutylammonium fluoride (16.3 mL of a 1 M solution in THF, 16.3 mmol) were added. The mixture was stirred at 0 °C for 2 h and then at 25 °C for 1.5 h. The solvent was evaporated to ca. 20 mL, and then the residue was taken up in ethyl acetate and washed successively with aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous magnesium sulfate and evaporated to afford crude hydroxymethylpenem. The product was chromatographed on silica gel, eluting first with hexane and then a gradient from 1:4 to 1:1 ethyl acetate/hexane to afford 1.37 g (85%) of **25**: $[\alpha]^{25}_D$ = +87.63° (*c* 0.21, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 5.93–5.85 (m, 1 H), 5.59 (d, 1 H, C-5H, J = 2.0), 5.44–5.22 (4 bs, 2 H, vinyl CH_2), 4.72 (m, 2 H, CH_2O), 4.55 (m, 2 H, CH_2O), 4.23 (m, 1 H, CHOSi), 3.72 (dd, J = 4.9, 1.6, 1 H, C-6H), 3.54 (t, J = 7.2, 1 H, OH), 1.23 (d, J = 6.2, 3 H), 0.86 (s, 9 H), 0.06 (s, 6 H).

[5*R*,6*S*(*R*)]-3-[[[[6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)-3-quinolinyl]carbonyl]oxy]methyl]-6-[1(*R*)-[(*tert*-butyldimethylsilyl)oxy]methyl]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Allyl Ester (26). To a stirred icewater bath cooled mixture of 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)-3-quinolinecarboxylic acid (90 mg, 0.24 mmol) in dry methylene chloride (5 mL) under dry argon were added dry triethylamine (50 μL, 36 mmol) and isobutyl chloroformate (32 μL, 0.25 mmol). After stirring at 0 °C for 1.5 h, the solution was cooled to −18 °C. A solution of **25** (65 mg, 0.16 mmol) in dry methylene chloride (4 mL) was added dropwise over 3 min at −18 °C. The stirred reaction mixture came slowly up to room temperature over 5 h and then 50 mL of methylene chloride was added. The solution was washed by extraction with water (2×) and brine. Following drying over anhydrous sodium sulfate, the desiccant was filtered off and the solvent removed under pressure, yielding 151 mg of crude product which was purified by flash chromatography (silica gel 230–400 mesh, methylene chloride/methanol (10:1)) to give 78 mg (65.0%) of **26**: $[\alpha]^{25}_D$ = +34.4° (*c* 0.81, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.057 (s, 6 H), 0.87 (s, 9 H), 1.21 (d, 3 H, J = 6.13), 2.36 (s, 3 H), 2.54 (t, 4 H, J = 4.61), 3.35 (m, 4 H), 3.73 (dd, 1 H, J = 4.11 and 1.9), 4.22 (m, 1 H), 4.69 (m, 4 H), 5.35, 571 (AB, 2 H, J_{gem} = 15.7), 5.61 (d, 1 H, J = 1.7), 5.92 (m, 1 H), 8.01 (dd, 1 H, J = 12.6 and 2.3), 8.35 (s, 1 H); MS *m/z* 751 (M + H). Anal. Calcd for $C_{35}H_{45}N_4O_7F_3SiS$: C, 55.98; H, 6.04; N, 7.46. Found: C, 55.26; H, 5.93; N, 7.23.

[5*R*,6*S*(*R*)]-3-[[[[6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)-3-quinolinyl]carbonyl]oxy]methyl]-6-(1(*R*)-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Allyl Ester (27). A stirred solution of **26** (1.87 g, 2.49 mmol) in tetrahydrofuran (185 mL) in an icewater bath was treated with glacial acetic acid (1.85 mL, 32.1 mmol) and 1 M tetrabutylammonium fluoride (18.5 mL, 18.5 mmol) in tetrahydrofuran. The reaction was stirred 3 h in an icewater bath, warmed to room temperature, and stirred for 58 h. The solvents were removed in vacuo, and the resulting residue was triturated with ether and the ether decanted off (three times). Ethyl acetate (75 mL) was added to the ether-insoluble portion and the solution was washed by extraction with aqueous sodium bicarbonate, water, and brine. After drying over anhydrous magnesium sulfate, the desiccant was removed by filtration and the solvent was evaporated under reduced pressure, yielding 1.05 g (66%) of **27**: ^{1}H-NMR ($CDCl_3$) δ 1.32 (d, 3 H, J = 6.14), 2.36 (s, 3 H), 2.56 (t, 4 H, J = 4.4), 3.35 (m, 4 H), 3.77 (dd, 1 H, J = 6.83 and 1.76), 4.23 (m, 1 H), 5.35,

(18) Commercially available from Kaneka America Corp.

(19) Frank, G. V.; Caro, W. Über Cellulose-oxalsäure-ester. *Berichte* **1930**, *63*, 1532–1543.

5.67 (AB, 2 H, J_{AB} = 16.4), 7.98 (dd, 1 H, J = 12.4 and 1.9 Hz), 8.34 (s, 1 H).

[5*R*,6*S*(*R*)]-3-[[[[6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)-3-quinolinyl]carbonyl]oxy]methyl]-6-(1(*R*)-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (28). A solution of **27** (20 mg, 0.03 mmol), methylene chloride (4 mL), ethyl acetate (4 mL), sodium 2-ethylhexanoate (7.5 mg, 0.045 mmol), triphenylphosphine (1.7 mg, 0.006 mmol), and tetrakis(triphenylphosphine)palladium(0) (1.7 mg, 0.0015 mmol) was stirred under an atmosphere of dry argon for 1 h in an icewater bath. Several volumes of ether were added, and the precipitate was centrifuged. The insoluble portion was triturated with ether and centrifuged three times. The crude product was dissolved in water and chromatographed on C_{18} silica gel (eluted with water/acetonitrile (4:1)), yielding 4.5 mg (24.3%) of **28**: IR (KBr) 1772 (β-lactam), 1730 (ester), 1618 (CO_2) cm^{-1}; ^{1}H-NMR (D_2O) δ 1.32 (d, 3 H, J = 6.32), 2.97 (s, 3 H), 3.43 (m, 4 H), 3.64 (bs, 4 H), 3.94 (d, 1 H, J = 6.0 Hz), 4.27 (t, 1 H, J = 6.2 Hz), 5.25, 5.63 (AB, 2 H, J_{AB} = 14.53), 5.69 (s, 1 H), 7.73 (d, 1 H, J = 11.96), 8.54 (s, 1 H).

[5*R*-6*S*(*R*)]-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-6-[1-[(*tert*-butyldimethylsilyl)oxy]ethyl]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Allyl Ester (29). A solution of **25** (20 mg, 0.05 mmol) in 0.5 mL of dichloromethane was cooled to 0 °C, under an argon atmosphere. Diisopropylethylamine (15 μL, 0.16 mmol) and phosgene (1.93 M in toluene; 35 μL, 0.07 mmol) were added, and the mixture was stirred at 0 °C for 30 min. Dichloromethane (1 mL) was added, and the mixture was concentrated to a volume of ca. 1 mL to afford the crude chloroformate.

While the chloroformate was being prepared, in a separate flask, ciprofloxacin (16 mg, 0.05 mmol) was suspended in 0.5 mL of dichloromethane. Bis(trimethylsilyl)acetamide (27 μL, 0.10 mmol) was added and the mixture was stirred at room temperature for 20 min, during which time the suspension becomes a solution. The solution was then cooled to 0 °C, under an argon atmosphere, and the crude chloroformate solution was added. The resulting mixture was stirred at 0 °C for 40 min and then at room temperature for 3 h. The reaction was then diluted with chloroform, washed with pH 4.0 buffer, and the aqueous phase was back-extracted with chloroform. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated to afford 31.6 mg (89%) of crude **29**, which did not require purification: IR ($CHCl_3$) 3400, 1790, 1710, 1668 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.72 (s, 1 H, enone CH), 7.98 (d, J = 13, 1 H, ArH), 7.36 (d, J = 7, 1 H, ArH), 6.02–5.81 (m, 1 H), 5.60 (d, J = 1, 1 H, C-5H), 5.52, 5.17 (AB, J = 15.4, 2 H, CH_2O(CO)), 5.43–5.26 (4 bs, 2 H, vinyl CH_2), 4.7 (m, 2 H, allyl CH_2), 4.25–4.19 (m, 1 H, CHOTBS), 3.75 (bs, 5 H, CH_2CH_2N and cyclopropyl CH), 3.55 (m, 1 H, C-6H), 3.32 (bs, 4 H, CH_2CH_2N), 1.53–1.31 (m, 4 H, cyclopropyl CH_2's), 1.21 (d, J = 6.2, 3 H, CH_3), 0.86 (s, 9 H), 0.05 (s, 6 H); MS m/z 756 (M + H).

[5*R*-6*S*(*R*)]-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-6-(1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Allyl Ester (30). A solution of **29** (20 mg, 0.03 mmol) in 2 mL of tetrahydrofuran was cooled to 0 °C. Acetic acid (20 μL, 0.35 mmol) and then tetrabutylammonium fluoride (0.2 mL of 1 M solution in THF, 0.2 mmol) was added. The mixture was stirred, warming to room temperature, for 16 h. The solvent was evaporated and the residue was then taken up in 20 mL of ethyl acetate and washed successively with pH 7.4 buffer, water, and brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to afford 15.8 mg (93%) of crude **30**: ^{1}H-NMR ($CDCl_3$) δ 8.74 (s, 1 H, enone CH), 8.00 (d, J = 13, 1 H, ArH), 7.36 (d, J = 6.8, 1 H, ArH), 6.01–5.82 (m, 1 H), 5.64 (s, 1 H, C-5H), 5.51, 5.19 (AB, J = 16, 2 H, CH_2O(CO)), 5.44–5.28 (4 bs, 2 H, vinyl CH_2), 4.76–4.67 (m, 2 H, allyl CH_2), 4.23 (m, 1 H, CHOH), 3.74 (bs, 4 H, CH_2CH_2N) and 3.55 (m, 2 H), 3.32 (bs, 4 H, CH_2CH_2N), 1.52–1.23 (m, 4 H, 2 cyclopropyl CH_2), 1.38 (d, J = 7, 3 H, CH_2).

[5*R*-6*S*(*R*)]-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-6-(1-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Monosodium Salt (31). A solution of **30** (15.8 mg, 0.025 mmol) in 0.4 mL of 1:1 dichloromethane/ethyl acetate, containing triphenylphosphine (2.8 mg, 0.01 mmol) and sodium 2-ethylhexanoate (7.5 mg, 0.045 mmol), was cooled to 0 °C under an argon atmosphere. Tetrakis(triphenylphosphine)palladium(0) (1.8 mg, 0.002 mmol) was added and the mixture was stirred at 0 °C for 40 min. The mixture was then precipitated from ether and the solid portion was purified by chromatography on C_{18} silica gel using a gradient from 1:9 to 1:1 acetonitrile/water as eluant to afford 4.4 mg (30%) of **31**: IR (KBr) 3330, 1770, 1705, 1628, 1615 cm^{-1}; ^{1}H-NMR (d_6-DMSO) δ 8.66 (s, 1 H, enone CH), 7.93 (d, J = 12.8, 1 H, ArH), 7.61 (bs, 1 H, ArH), 5.59, 5.11 (AB, J = 13.6, 2 H, CHOCON), 5.47 (s, 1 H, β-lactam CH), 5.26, 5.17 (2 bs, 2 H, allyl CH_2), 3.96 (m, 1 H, CHOH), 3.78 (m, 1 H, β-lactam CH), 3.65 (bs, 4 H, CH_2N), 1.36 (m, 2 H, cyclopropyl CH_2), 1.18 (d, J = 6, CH_3), 1.17 (m, 2 H, cyclopropyl CH_2); MS m/z 624 (M + H).

***rac*-3-[[[[6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)-3-quinolinyl]carbonyl]oxy]methyl]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Allyl Ester (33).** A solution of fleroxacin (40 mg, 0.11 mmol) in 0.7 mL of dichloromethane was cooled to 0 °C, under an argon atmosphere. Triethylamine (23 μL, 0.32 mmol) and cyclohexyl chloroformate (32 μL, 0.22 mmol) were added, and the solution was stirred at 0 °C for 1 h. The mixture was cooled to −20 °C and a solution of **32** (26 mg, 0.11 mmol) in 0.8 mL of dichloromethane was added, followed by 4-(*N*,*N*-dimethylamino)pyridine (10 mg, 0.08 mmol). The solution was stirred for 2.5 h while warming to room temperature. The mixture was then poured into aqueous sodium bicarbonate and extracted twice with chloroform. The organic layers were dried over anhydrous magnesium sulfate and filtered, and the solvents were removed via rotary evaporation. The residue was flash chromatographed on silica gel using a gradient from 1:20 to 1:10 methanol/dichloromethane as eluant to afford 23 mg (35%) of **33**: IR ($CHCl_3$) 1792, 1710, 1617 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.34 (s, 1 H, enone CH), 7.99 (dd, 1 H, J = 12.3, 1.9, ArH), 6.05–5.85 (m, 1 H, vinyl H), 5.69, 5.35 (AB, 2 H, J = 15.7, $CH_2OC(O)$), 5.66 (m, 1 H, C-5 H), 5.45–5.24 (4 bs, 2 H, vinyl CH_2), 4.91, 4.52 (b AB, 2 H, CH_2N), 4.78–4.61 (m, 4 H, allyl CH_2 and CH_2F), 3.78, 3.53 (AB of ABX, J_{AB} = 19.4, J_{AX} = 3.6, J_{BX} = 1.6, 2 H, C-6 CH_2), 3.34 (bs, 4 H), 2.54 (bt, 4 H), 2.35 (s, 3 H, NCH_3); MS m/z 593 (M + H).

***rac*-3-[[[[6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)-3-quinolinyl]carbonyl]oxy]methyl]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Monosodium Salt (34).** A solution of allyl ester **33** (84 mg, 0.14 mmol) in 1.6 mL of 1:1 dichloromethane/ethyl acetate, containing triphenylphosphine (8 mg, 0.03 mmol) and sodium-2-ethylhexanoate (41 mg, 0.25 mmol), was cooled to 0 °C under an argon atmosphere. Tetrakis(triphenylphosphine)palladium(0) (8 mg, 0.006 mmol) was added and the mixture was stirred at 0 °C for 40 min. The mixture was then precipitated from ether and the solid portion was purified by chromatography on C_{18} silica gel using a gradient from 1:9 acetonitrile/water to 1:1 acetonitrile/water as eluant to afford 28 mg (35%) of **34**: IR (KBr) 3430, 1772, 1728, 1618 cm^{-1}; ^{1}H-NMR (D_2O) δ 8.59 (s, 1 H, enone CH), 7.74 (d, J = 6, ArH), 5.75 (m, 1 H, C-5 H), 5.64, 5.28 (AB, J = 7.2, 2 H, $CH_2OC(O)$), 4.98–4.76 (m, 4 H, CH_2CH_2F), 3.82, 3.56 (AB of ABX, J_{AB} = 8.4, J_{AX} = 1.8, J_{BX} = 0, 2 H, C-6 CH_2), 3.49 (bs, 4 H), 2.98 (bs, 4 H), 2.59 (bs, 3 H, NCH_3); MS m/z 553 (acid M + H), 370.

***rac*-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Allyl Ester (35).** A solution of **32** (72 mg, 0.3 mmol) in 3.0 mL of dichloromethane was cooled to 0 °C, under an argon atmosphere. Diisopropylethylamine (55 μL, 0.6 mmol) and phosgene (1.93 M in toluene; 0.19 mL, 0.36 mmol) were added, and the mixture was stirred at 0 °C for 30 min. Dichloromethane (3 mL) was added, and the mixture was concentrated to a volume of approximately 3 mL to afford the crude chloroformate.

While the chloroformate was being prepared, in a separate flask, ciprofloxacin (95 mg, 0.3 mmol) was suspended in 3 mL of dichloromethane. Bis(trimethylsilyl)acetamide (0.16 mL, 0.96 mmol) was added and the mixture was stirred at room temperature for 25 min, during which time the suspension became a solution. The

solution was then cooled to 0 °C, under an argon atmosphere, and the crude chloroformate solution was added. The resulting mixture was stirred at 0 °C for 30 min and then at room temperature for 3 h. The reaction was then diluted with chloroform and washed with pH 4.0 buffer, and the aqueous phase was back-extracted with chloroform. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated to afford 156 mg (87%) of **35**: IR (KBr) 1795, 1712, 1620 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 8.56 (s, 1 H, C-2H), 8.09 (d, J = 12, 1 H, C-5H), 7.29 (s, 1 H, C-8H), 6.11–5.92 (m, 2 H, vinyl CH's), 5.69 (m, 1 H, β-lactam CH), 5.61–5.27 (m, 4 H, vinyl CH_2's), 5.53, 5.21 (AB q, J = 7.9, 2 H, CH_2OCON), 4.85–4.67 (m, 4 H, allyl CH_2O's), 3.81, 3.53 (ABX, J_{AB} = 16.4, J_{AX} = 3.8, J_{BX} = 1.4, 2 H, β-lactam CH_2), 3.73 (bs, 4 H, CH_2N), 3.43 (m, 1 H, cyclopropyl CH), 3.25 (bs, 4 H, CH_2N), 1.33 (m, 2 H cyclopropyl CH_2), 1.14 (m, 2 H cyclopropyl CH_2); MS m/z 639 (M + H). Anal. Calcd for $C_{31}H_{31}N_4O_8FS$: C, 58.30; H, 4.89; N, 8.77. Found: C, 58.15; H, 4.99; N, 8.77.

***rac*-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Monosodium Salt (36).** A solution of **35** (60 mg, 0.10 mmol) in 1.5 mL of 1:1 dichloromethane/ethyl acetate, containing triphenylphosphine (10 mg, 0.04 mmol) and sodium 2-ethylhexanoate (53 mg, 0.32 mmol), was cooled to 0 °C under an argon atmosphere. Tetrakis(triphenylphosphine)palladium(0) (11 mg, 0.01 mmol) was added and the mixture was stirred at 0 °C for 40 min. The mixture was then precipitated from ether and the solid portion was purified by chromatography on C_{18} silica gel using a gradient from 1:9 to 1:1 acetonitrile/water as eluant to afford 7.4 mg (13%) of **36**: IR (KBr) 3425, 1773, 1708, 1628 cm^{-1}; ^1H-NMR (D_2O + trace CD_3CN) δ 8.70 (s, 1 H, enone CH), 7.8 (m, 1 H, ArH), 7.55 (m, 1 H, ArH), 5.74 (bs, 1 H, C-5 H), 5.52, 5.17 (AB, J = 14.8, 2 H, $CH_2O(CO)$), 3.81, 3.53 (AB of ABX, J_{AB} = 17.2, J_{AX} = 3.0, J_{BX} = 0, 2 H, C-6 CH_2), 3.70 (m, 4 H), 3.35 (m, 5 H, NCH and piperazine CH_2's), 1.4 (m, 2 H, cyclopropyl CH_2), 1.1 (bs, 2 H, cyclopropyl CH_2); MS m/z 581 (M + H).

[5*R*]-[5α-6-α(*R)]-6-(1-Hydroxyethyl)-3-(hydroxymethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Monosodium Salt (37).** A solution of **24** (1.1 g, 2.14 mmol) in dry tetrahydrofuran (200 mL) was stirred under an atmosphere of argon in an icewater bath. Glacial acetic acid (1.15 mL, 10.1 mmol) was added dropwise over 1 min, followed by the dropwise addition of 1 M tetrabutylammonium fluoride (8.6 mL, 8.6 mmol) over 10 min. After the last addition, stirring was continued and the bath was allowed to slowly come to room temperature. Stirring was continued at room temperature for 24 h. The reaction mixture was allowed to stand at room temperature for an additional 20 h and concentrated to dryness under reduced pressure on a rotary evaporator (bath temperature < 25 °C). Ethyl acetate (300 mL) was added to the residue and washed by extraction with icewater, cold saturated sodium bicarbonate, and again with water. Following drying over magnesium sulfate, the desiccant was filtered off and the ethyl acetate evaporated. The residue was chromatographed on silica gel, eluting with 1:1 ethyl acetate/hexane to afford 192 mg (31%) of the diol.

A solution of the diol obtained above (190 mg, 0.67 mmol), ethyl acetate (10 mL), methylene chloride (10 mL), sodium 2-ethylhexanoate (165 mg, 1.0 mmol), triphenylphosphine (35 mg, 0.134 mmol), and tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.033 mmol) was stirred under an atmosphere of dry argon for 1 h in an icewater bath. Several volumes of ether were added, and the precipitate was centrifuged. The insoluble portion was triturated with ether and centrifuged three times. The crude product was dissolved in water and chromatographed on C_{18} silica gel, eluting with water to afford 115 mg (64%) of the known diol **37**.[11,20]

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(20) Fujimoto, K.; Iwano, Y.; Hirai, K.; Sugawara, S. From Penicillin to Penem and Carbapenem. VII. Synthesis and Antibacterial Activity of Penem Derivatives. *Chem. Pharm. Bull.* **1986**, *34*, 999–1014.

Synthetic Modification of a Novel Microbial Ionophore: Exploration of Anticoccidial Structure–Activity Relationships

E. A. Glazer,* D. A. Koss, J. A. Olson, A. P. Ricketts, T. K. Schaaf, and R. J. Wiscount, Jr.

Central Research Division, Pfizer Inc, Groton, Connecticut 06340. *Received October 23, 1991*

While fermentation-derived polyether ionophores such as salinomycin are the dominant class of anticoccidial feed additives, there is little information concerning the structural features which confer optimal potency/efficacy in this important series. The recently discovered microbial polyether **1a**, featuring potent, broad-spectrum anticoccidial activity, was employed as a template to explore structure–activity relationships. A number of single-step synthetic modifications targeted structural changes in both the lipophilic carbon backbone and the ion-binding cavity of **1a**. Although previous semisynthetic transformations among the polyether ionophores almost always resulted in a substantial loss of anticoccidial activity, we obtained several analogues, altered on the periphery of the ionophore–ion complex, which retain good potency and efficacy. Monoglycone **7** (semduramicin sodium) has the most impressive anticoccidial profile of this series, and is undergoing further biological testing under field conditions.

Avian coccidiosis, an enteric protozoal infection caused by pathogenic species of *Eimeria*, is a ubiquitous problem in the high-intensity rearing systems characteristic of the poultry industry.[1] This has resulted in a universal dependence on anticoccidial feed additives affording prophylactic disease control. Fermentation-derived polyether carboxylic acid ionophores, such as salinomycin and monensin, have been the dominant class of anticoccidial feed additives for nearly two decades. They have achieved this position because they provide excellent disease control and are relatively refractory to resistance development.[2] The anticoccidial utility of the ionophore antibiotics is well established, but there is little information concerning the structural features which confer optimal potency/efficacy in this important series.[3] While semisynthetic modifica-

(1) Fernando, M. A. Pathology and Pathogenicity. In *The Biology of the Coccidia*; Long, P. L., Ed.; University Park Press: Baltimore, Maryland, 1982; Chapter 7.

(2) (a) McDougald, L. R.; Reid, W. M. Coccidiosis. In *Diseases of Poultry*; Celnek, B. W., Ed.; Iowa State University Press: Ames, IA, 1991; pp 780–797. (b) McDougald, L. R.; Reid, W. M. Anticoccidial Drug Market: 1987. *Broiler Ind.* **1987**, *40*, 100–108. (c) McDougald, L. R. Chemotherapy of Coccidiosis. In *The Biology of the Coccidia*; Long, P. L., Ed.; University Park Press: Baltimore, Maryland, 1982; Chapter 9.

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