Discovery of MC-02,331, a New Cephalosporin Exhibiting Potent Activity Against Methicillin-resistant *Staphylococcus aureus*

SCOTT J. HECKER,* IN-SEOP CHO, TOMASZ W. GLINKA, ZHIJIA J. ZHANG, MARY E. PRICE, VING J. LEE, BURTON G. CHRISTENSEN, AMY BOGGS, SUZANNE CHAMBERLAND, FRANÇOIS MALOUIN, THOMAS R. PARR, THAMIL ANNAMALAI, JOHANNE BLAIS, EMMETT L. BOND, LAURA CASE, CHRISTINE CHAN, JAC CRASE, RIA FRITH, DAVID GRIFFITH, LAURIE HARFORD, NANCY LIU, MARIA LUDWIKOW, KRISTINA MATHIAS, DAVID REA and ROBERT WILLIAMS

Microcide Pharmaceuticals, Inc.,
850 Maude Ave., Mountain View, CA 94043, U.S.A.

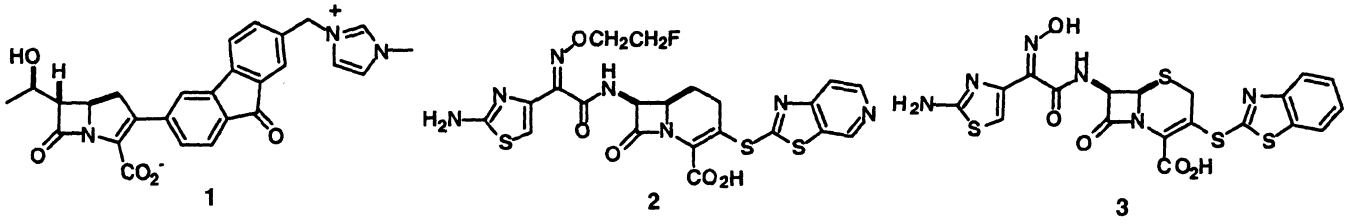
(Received for publication June 5, 1998)

A systematic approach toward building activity against methicillin-resistant staphylococci into the cephalosporin class of β-lactam antibiotics is described. Initial work focused on finding the optimal linkage between the cephem nucleus and a biphenyl pharmacophore, which established that a thio linkage afforded potent activity *in vitro*. Efforts to optimize this activity by altering substitution on the pharmacophore afforded iodophenylthio analog MC-02,002, which although highly potent against MRSA, was also highly bound to serum proteins. Further work to decrease serum protein binding showed that replacement of the iodo substituent by the positively-charged isothiouronium group afforded potent activity and reduced serum binding, but insufficient aqueous solubility. Solubility was enhanced by incorporation of a second positively-charged group into the 7-acyl substituent. Such derivatives (MC-02,171 and MC-02,306) lacked sufficient stability to staphylococcal β-lactamase enzymes. The second positive charge was incorporated into the cephem 3-substituent in order to utilize the β-lactamase-stable aminothiazolyl-(oximino)acetyl class of 7-substituents. These efforts culminated with the discovery of bis(isothiouroniummethyl)phenylthio analog MC-02,331, whose profile is acceptable with respect to potency against MRSA, serum binding, aqueous solubility, and β-lactamase stability.

The emergence of staphylococci resistant to all currently-available β-lactam antibiotics has substantially reduced the utility of these agents in the nosocomial setting. β-Lactamase mediated resistance in staphylococci was successfully addressed with the advent of β-lactamase-stable penicillins such as methicillin; however, resistance associated with production of PBP2a, a penicillin-binding protein with low affinity for currently-marketed β-lactams, has become more problematic.[1] Numerous approaches toward enhancing the PBP2a affinity of β-lactam antibiotics in order to gain coverage against methicillin-resistant staphylococci (MRS) have been reported.[2~6] Herein we describe our own efforts to discover a novel β-lactam antibiotic with utility for treatment of infections caused by MRS and other Gram-positive bacteria.

At the time we initiated this approach, we were aware of three other ongoing programs in this area (Fig. 1), at Merck[2] (2-biaryl carbapenems, **1**), Eli Lilly[3] (3-thiazolylthiocarbacephems, **2**) and Meiji Seika[4] (3-benzothiazolylthiocephems, **3**). Among the various known β-lactam nuclei, we were attracted to the cephalosporin class, because of its excellent safety record as well as the ready availability of key intermediates. Our concerns about the relative β-lactamase instability of this class, relative to the carbapenems, were alleviated by the knowledge that acceptable stability to staphylococcal β-lactamases could be gained through use of an appropriate (oximino)acetyl 7-substituent, as is found in many extended-spectrum cephalosporins. Our initial work began with attachment of a biphenyl pharmacophore to a cephem nucleus, since earlier work by the

Fig. 1.



Scheme 1.

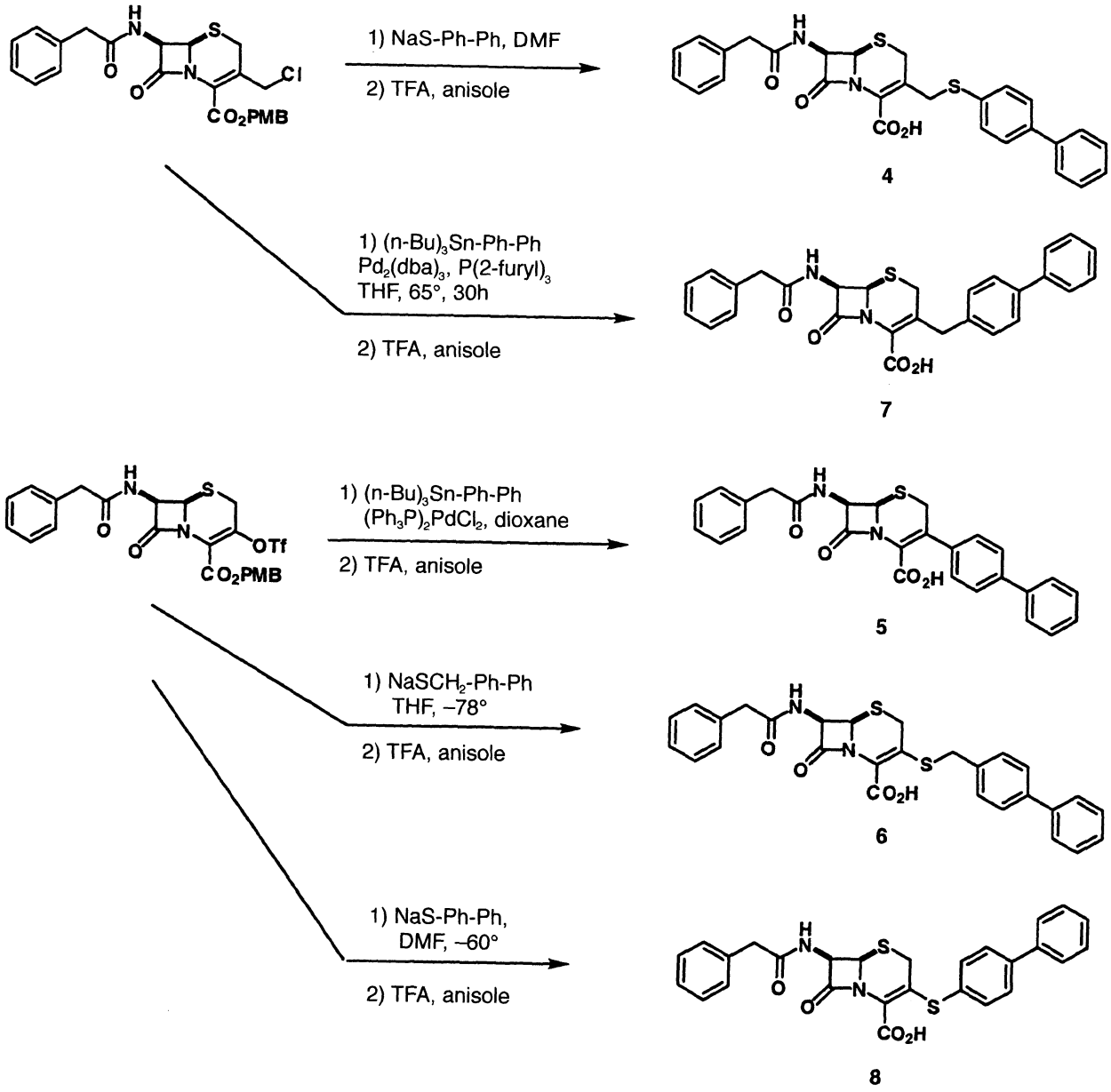
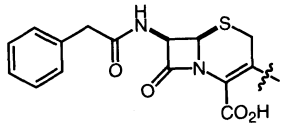
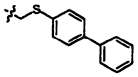
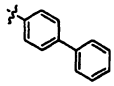
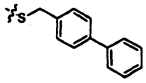
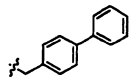
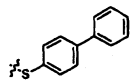


Table 1. Comparison of alternative linkages.



Compound No.	C(3)-substituent	MIC (μg/ml)				
		S. a. 29213	*S. a.* COL	*S. a.* 037	*S. a.* 76	*E. f.* 29212
—	Imipenem	≤0.02	16	32	32	1
4		≤0.06	>32	—	>32	16
5		≤0.25	—	>32	>32	16
6		≤0.13	>32	>32	>32	4
7		≤0.06	>32	>32	>32	32
8		≤0.13	4	4	>32	2

Abbreviations: *S. a.* 29213, *Staphylococcus aureus* ATCC 29213 (MSSA); *S. a.* COL, *Staphylococcus aureus* COL (MRSA, non-β-lactamase producing); *S. a.* 037, *Staphylococcus aureus* 037 (MRSA, β-lactamase-producing); *S. a.* 76, *Staphylococcus aureus* 76 (MRSA, β-lactamase-producing); *E. f.* 29212, *Enterococcus faecalis* ATCC 29212.

Merck group in the carbapenem series had demonstrated that this moiety affords potent PBP2a binding.

The C(3)-Linkage

Our first challenge was to determine the optimal linkage between the biphenyl moiety and the cephem nucleus. Five different linkages were studied; the routes by which these compounds (**4**~**8**) were prepared are shown in Scheme 1.

Table 1 shows the test results for compounds **4**~**8** against certain representative Gram-positive bacteria (MICs in μg/ml). Within this group, the biphenylthio analog **8** stands out by virtue of its superior potency against two strains of MRSA, as well as against *E. faecalis*. Based on these results, subsequent analogs retained the thio-linkage.

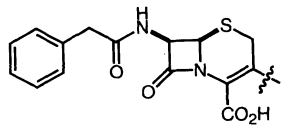
Optimizing Potency *In Vitro*

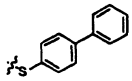
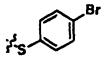
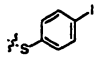
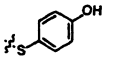
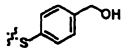
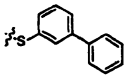
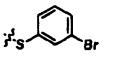
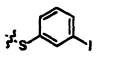
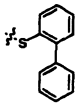
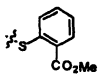
Having chosen the optimal linkage for the pharmacophore at the 3-position, we next initiated efforts to improve potency by varying substitution on the arylthio moiety. A sampling of compounds from these efforts is shown in Table 2; the general method by which these compounds were prepared is shown in Scheme 2. Two key results were identified: i) that the distal phenyl group could be replaced with another lipophilic substituent, such as halogen, but polar functionalities reduced activity, and ii) that the optimal point of substitution of the lipophilic group was the 2-position of the phenylthio moiety. The most potent compound of this series was the 2-iodophenylthio analog **21** (MC-02,002), which displayed excellent potency against both MRSA and enterococci.

Further Profiling of Early Lead MC-02,002

The compelling *in vitro* activity in the primary microbiological panel prompted extensive evaluation of MC-02,002, which included determinations of MIC_{90} values, affinity for PBP2a, solubility, efficacy in a mouse septicemia model, and binding to human serum proteins. Notably, the *in vitro* potency against both MRSA and MRCNS is impressive (Table 3), and in fact is in line

Table 2. Substituted 3-phenylthiocephems.



Compound No.	C(3)-substituent	MIC (μg/ml)				
		S. a. 29213	*S. a.* COL	*S. a.* 76	*E. f.* 29212	*E. f.* 35667
—	Imipenem	≤0.02	16	32	1	4
9		≤0.25	4	32	8	8
10		≤0.13	4	>32	2	—
11		0.06	4	16	2	4
12		0.06	16	32	2	4
13		0.13	—	32	4	4
14		≤0.25	16	>32	2	8
15		0.13	8	32	2	—
16		0.06	16	32	2	4
17		0.03	2	16	2	2
18		0.25	4	8	4	2
19		—	4	16	4	8
20		0.13	1～2	4	4	2
21		≤0.02	0.5	2	0.5	0.5
22		—	4	16	4	4
23		≤0.25	1	4	4	2
24		≤0.25	4	16	—	2

Abbreviations: *S. a.* 29213, *Staphylococcus aureus* ATCC 29213 (MSSA); *S. a.* COL, *Staphylococcus aureus* COL (MRSA, non-β-lactamase producing); *S. a.* 76, *Staphylococcus aureus* 76 (MRSA, β-lactamase-producing); *E. f.* 29212, *Enterococcus faecalis* ATCC 29212; *E. f.* 35667, *Enterococcus faecium* ATCC 35667.

Scheme 2.

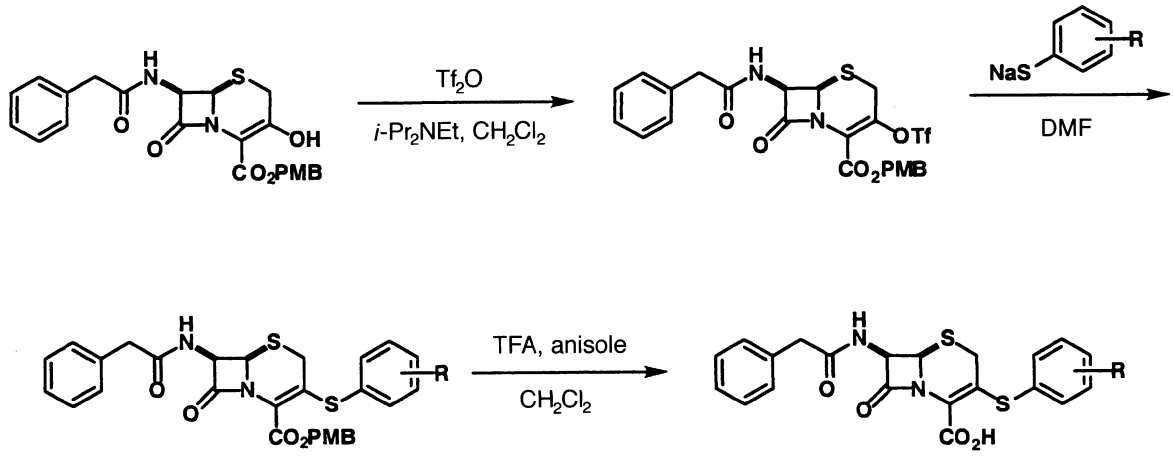
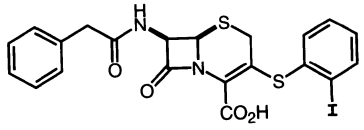


Table 3. Activity profile of early lead MC-02,002.



	MC-02,002	Imipenem
MIC_{90}(μg/ml)		
MRSA (n=37)	2	64
MRCNS (n=46)	0.5	128
E. faecalis (n=28)	4	1
PBP2a IC_{50} (μg/ml)	2.9	43
Solubility (MC-02,002 Na salt, mg/ml)	>20	10
MIC (MSSA Smith, μg/ml)	0.015	0.015
ED_{50} (SQ, MSSA Smith, mg/kg)	3.7	0.06
ED_{50}/MIC (MSSA Smith, mg/kg)	250	4
Human serum binding	>99%	20%

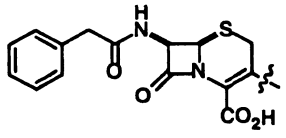
with our goals for this program. The compound did show efficacy in a mouse septicemia model when tested against a beta-lactam susceptible organism. However, the ED_{50} is higher than desired, especially given the extremely high potency of MC-02,002 against the challenge strain, MSSA Smith. This poor performance in this model is reflected by the ED_{50}/MIC ratio of 250, *vs.* a ratio of 4 for imipenem. The poor *in vivo* activity of MC-02,002 is explained by its very high protein binding, which was measured at >99%. Difficulties with high serum binding were anticipated, based on precedent in earlier programs.[3] Thus, attention turned at this point to finding ways to decrease the protein binding of MC-02,002.

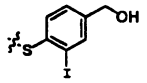
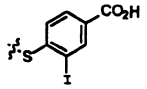
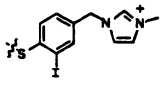
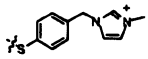
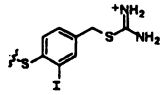
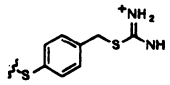
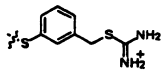
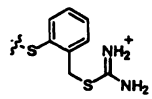
Reducing Serum Binding

To reduce the serum binding of MC-02,002, the effects of various substituents on both serum binding and antibacterial activity were studied. While we did not set a particular target for an appropriate serum binding level, we were cognizant that the term MIC/f_u (f_u is the fraction unbound) is an important determinant of efficacy against an infection, since this corresponds to the theoretical total drug concentration required to inhibit bacterial growth *in vivo*. Based on typical pharmacokinetic profiles of cephalosporins, and the knowledge that cephalosporin efficacy is generally associated with maintenance of unbound serum drug concentrations above the MIC for >50% of the dosing interval,[7] we estimated that we would need to keep the MIC_{90}/f_u ratio at about 10 or less. For a compound having the potency of MC-02,002 (MIC_{90} *vs.* MRSA = 2 μg/ml), this set a serum binding target of 80% (unbound fraction = 0.2).

The effects of various structural changes on both activity and human serum binding are shown in Table 4. Addition of a neutral polar substituent such as a hydroxyl group (**25**) modestly decreased potency, with minimal reduction in serum binding. Substitution of a carboxylate group (**26**) was equally unsuccessful, with even greater potency loss. On the other hand, incorporation of a positively-charged substituent (*e.g.* **27**) resulted in a substantial reduction in serum binding (increase in the unbound fraction by at least 3-fold), albeit with a substantial decrease in potency. While the iodo substituent contributed to increased serum binding (*e.g.*, **28**), we believed at the time that this substituent

Table 4. Effects of substituents on *in vitro* activity and serum binding.



Compound No.	C(3)-substituent	HSB	MIC (μg/ml)			
			S. a. COL	*S. a.* 76	*E. f.* 29212	*E. f.* 35667
25		>99%	1	8	2	2
26		>99%	8	32	16	8
27		97%	2	16	4	4
28		92%	16	>32	2	8
29		—	≤0.25	4	1	0.5
30		—	4	16	1	1
31		—	2	8	1	2
32		—	0.5	4	1	0.5

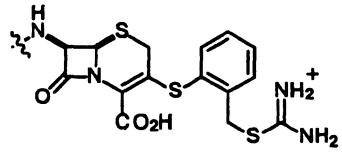
Abbreviations: HSB, human serum binding; *S. a.* COL, *Staphylococcus aureus* COL (MRSA, non-β-lactamase producing); *S. a.* 76, *Staphylococcus aureus* 76 (MRSA, β-lactamase-producing); *E. f.* 29212, *Enterococcus faecalis* ATCC 29212; *E. f.* 35667, *Enterococcus faecium* ATCC 35667.

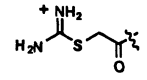
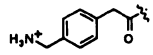
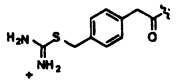
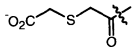
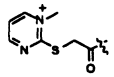
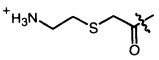
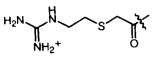
(or one like it) was required for potent anti-MRSA activity. Replacement of the imidazoliummethyl group of **27** by an isothiouroniummethyl group (**29**) gave potency comparable to that of MC-02,002 (**21**), and suggested exploring the impact on antibacterial activity of this group as the only substituent on the phenyl moiety. While the corresponding *para-* (**30**) and *meta-* (**31**) analogs were disappointing, the *ortho*-isothiouroniummethyl isomer (**32**) afforded activity comparable to that of MC-02,002. Unfortunately, the very poor aqueous solubility of this compound (and **29**~**31**) precluded determination of its serum binding. However, given the serum binding results of other compounds containing positively-charged substituents and lacking the iodo group, such as **28**, we were confident that a considerable advance had been achieved.

Improving Solubility

The insolubility of compound **32** can be attributed to the fact that it exists as a zwitterion at neutral pH. In order to improve its solubility, we sought to introduce a third charged functional group at the 7-position (Table 5, Scheme 3). While a cationic substituent alone was not sufficient for maintaining antibacterial activity (*e.g.*, **33**), it did improve solubility. Attachment of a positively-charged functional group to the phenylacetyl moiety of **32** (compounds **34** and **35**) afforded respectable potency and solubility sufficient to allow *in vivo* profiling. The solubility of these analogs did not meet the target of 10 mg/ml, and further analog work was undertaken to try to decrease the lipophilicity of compound **35** (MC-02,171). We had noted that one of the smallest and

Table 5. Effect of 7-substituent on *in vitro* activity and aqueous solubility.



Compound No.	C(3)-substituent	Sol	MIC (μg/ml)			
			S. a. COL	*S. a.* 76	*E. f.* 29212	*E. f.* 35667
33		≥10	32	>32	>32	32
34		2	4	16	1	1
35		2	2	8	0.5	1
36		1	4	8	1	1
37		≥10	>32	>32	>32	>32
38		≥10	16	16	4	8
39		≥10	8	32	4	4
40		≥10	2	8	1	0.5

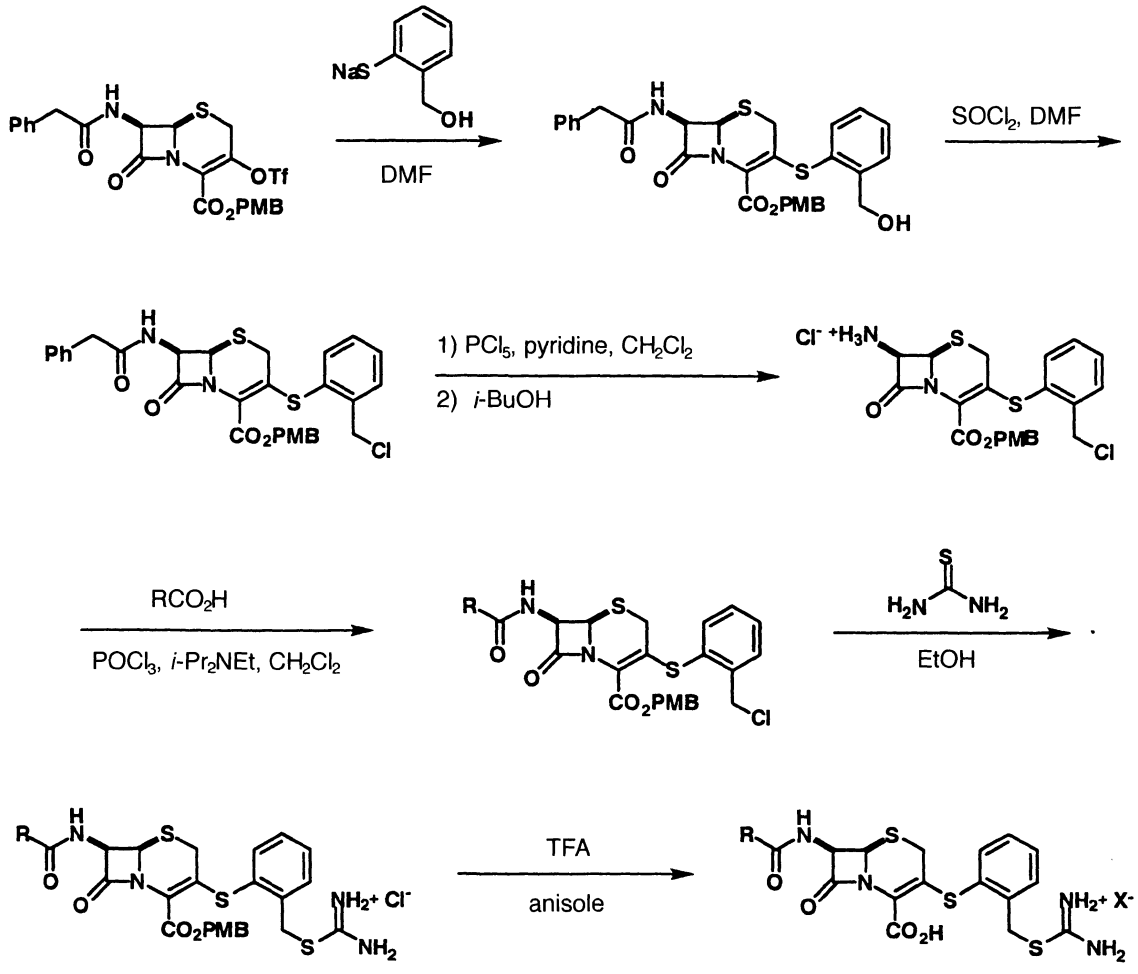
Abbreviations: Sol, aqueous solubility, mg/ml; *S. a.* COL, *Staphylococcus aureus* COL (MRSA, non-β-lactamase producing); *S. a.* 76, *Staphylococcus aureus* 76 (MRSA, β-lactamase-producing); *E. f.* 29212, *Enterococcus faecalis* ATCC 29212; *E. f.* 35667, *Enterococcus fascium* ATCC 35667.

least lipophilic 7-substituents capable of maintaining good Gram-positive potency was the methylthioacetyl group (*e.g.*, **36**). Although the solubility of **36** was insufficient (being a zwitterion), we reasoned that attachment of a charged group to this thioacetyl substituent might afford the desired combination of solubility and potency. While the desired solubility was readily attained, many of these attempts greatly reduced activity (*e.g.*, **37**~**39**). Ultimately, however, our efforts were rewarded with the discovery of *S*-(guanidinoethyl)thioacetyl derivative **40** (MC-02,306), which displayed excellent aqueous solubility as well as good potency *in vitro*.

Improving β-Lactamase Stability

The consistently better activity of our analogs against MRSA COL (β-lactamase negative) relative to MRSA 76 (β-lactamase positive) indicated that enhanced β-lactamase stability was required. Thus, we sought to utilize the aminothiazolyl(oximino)acetyl substituent found in extended-spectrum cephalosporins,[8] since these are known to afford β-lactamase stability. However, incorporation of such a substituent into a cephem containing the 3-position pharmacophore of MC-02,306 (**40**) would afford an analog with poor solubility. It appeared that a second positive charge at C(3) would be required, and therefore we pursued the synthesis of a bis(isothiouroniummethyl)phenylthio derivative. The first prototype in this series, phenylacetyl derivative **41**, displayed potent activity (Table 6). Based on these promising results, a series of analogs with modified 7-substituents was prepared; the synthetic route is exemplified with aminothiazolyl(oximino)acetyl derivative **45** (MC-02,331) in Scheme 4.

Scheme 3.



The results of evaluation of this series of analogs in which the 7-substituent is varied are shown in Table 6. Methylthioacetyl and *S*-(guanidinoethyl)thioacetyl analogs **42** and **43** displayed potent activity, but as expected, their weaker potency against MRSA 76 relative to MRSA COL is indicative of poor β-lactamase stability. The introduction of 7-aminothiazolyl(oximino)-acetyl substituents in **44** and **45** appears to remove this liability, as both analogs display equivalent activity against these two organisms. While *O*-cyclopentyl analog **44** was not active *in vivo* (presumably due to high serum binding), the unsubstitued oxime **45** (MC-02,331) showed excellent activity in the mouse septicemia model.

The activity profiles of MC-02,331, MC-02,171 and MC-02,306 are presented in Table 7. MC-02,331 displays all of the properties that we had sought in this program: potent activity *in vitro*, acceptable serum binding, good solubility, good β-lactamase stability, and good potency *in vivo*. Based on this very favorable profile, MC-02,331 was advanced to further microbiology and pharmacology studies, the results of which will be reported in due course.

Experimental

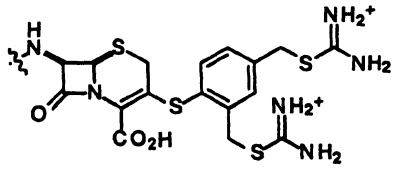
MIC Determination

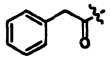
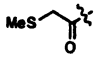
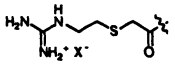
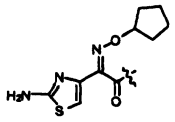
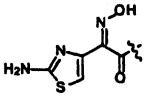
Determination of susceptibility was performed using a standard 2-fold agar dilution assay and/or broth microdilution assay according to the recommendations of the NCCLS.[9] Imipenem used as a control consisted of commercially available 1:1 imipenem/cilastatin (Primaxin).

Determination of Binding to Penicillin-binding Protein 2a (PBP2a)

The relative binding affinities for PBP2a (IC_{50}) were determined according to the method of DARGIS and MALOUIN.[10]

Table 6. Results of testing 7-acyl analogs of compound **41**.



Compound No.	C(3)-substituent	ED_{50}	MIC (μg/ml)			
			S. a. COL	*S. a.* 76	*E. f.* 29212	*E. f.* 35667
41		0.08	2	8	0.5	0.5
42		0.14	2	8	1	1
43		0.13	2	8	1	0.5
44		>5	2	2	0.25	0.5
45		0.23	4	4	0.125	0.5

Abbreviations: ED_{50}, 50% efficacious dose, *S. aureus* Smith, mg/kg; *S. a.* COL, *Staphylococcus aureus* COL (MRSA, non-β-lactamase producing); *S. a.* 76, *Staphylococcus aureus* 76 (MRSA, β-lactamase-producing); *E. f.* 29212, *Enterococcus faecalis* ATCC 29212; *E. f.* 35667, *Enterococcus faecium* ATCC 35667.

Detection of Binding to Human Serum

Percent binding was determined by ultrafiltration followed by HPLC analysis.[11,12]

Systemic Infection Model

Bacteria

Staphylococcus aureus strain Smith (ATCC 13709) was grown overnight at 37°C in brain-heart infusion broth (BHIB). The following morning, it was subcultured in fresh BHIB and incubated for 4~5 hours at 37°C. The cells were washed twice with PBS and adjusted to the desired concentration by correlation of absorbence at 600 nm with predetermined plate counts.[13] The cell suspension was mixed with an equal volume of sterile 14% hog-gastric mucin.[14] The inoculum was kept in an ice bath until used (<1 hour).

Experimental Infection

Male Swiss-Webster mice weighing 18~20 g were inoculated *via* the intraperitoneal route with 0.5 ml of bacterial suspension. Animals were observed for 72 hours. Data were analyzed by the probit method in order to determine the ED_{50} value.[15]

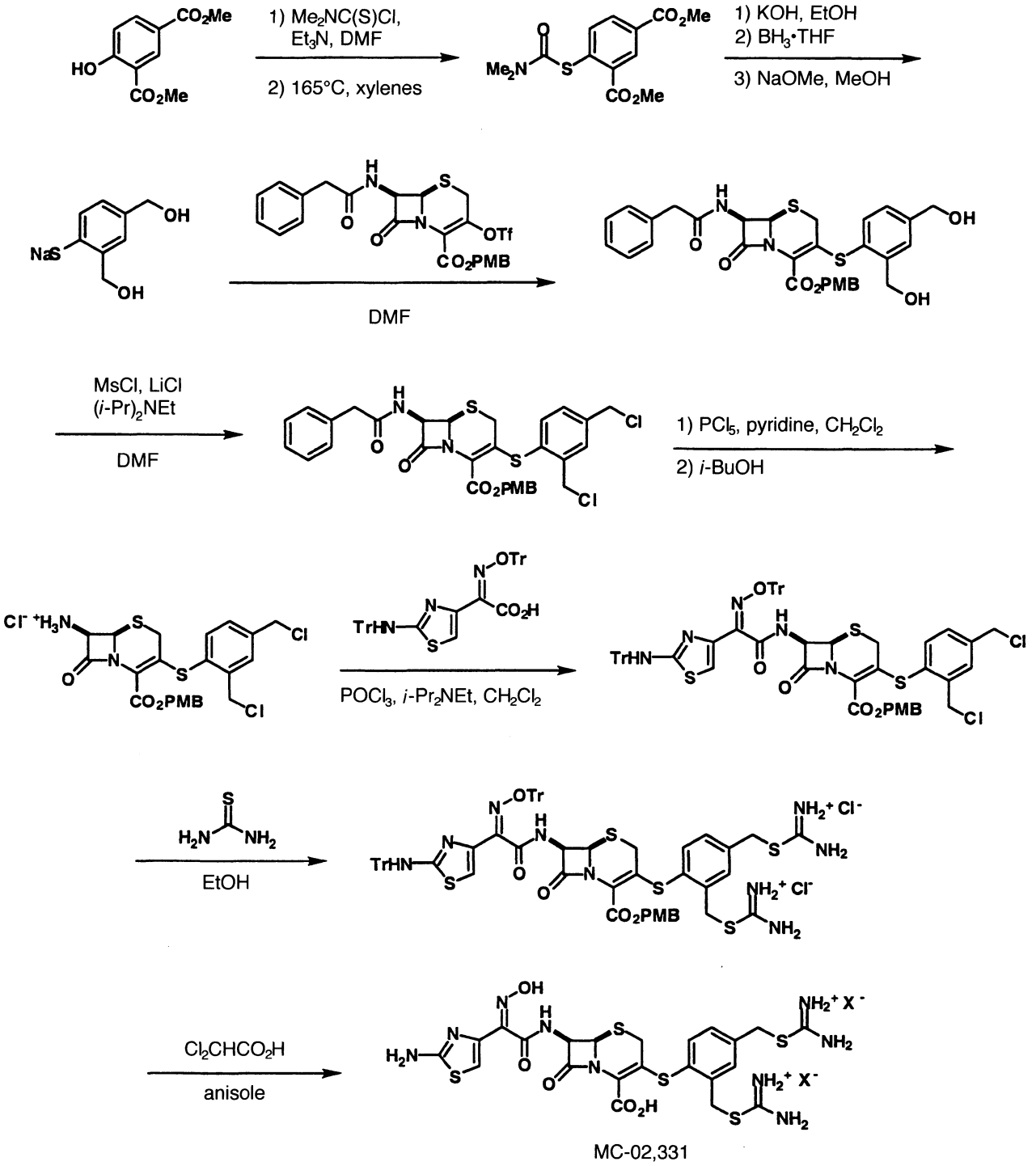
Experimental Treatment

Antibiotics were administered subcutaneously 0 and 2 hours post-challenge. ED_{50} values are reported as total dose administered.

Preparation of MC-02,331[16]

Sodium 2,4-bis(hydroxymethyl)benzenethiolate was prepared by a multi-step sequence starting with dimethyl 4-hydroxyisophthalate. Thus, a solution of 36 g (171 mmoles) of dimethyl 4-hydroxyisophthalate and 32 g (256 mmoles) of *N,N*-dimethylthiocarbamoyl chloride in dry DMF (600 ml) at 0°C was treated with 52.2 g (510 mmoles) of triethylamine. The yellow solution was stirred at room temperature for 6 hours, and was poured into 4 liters of water. The mixture was extracted with 4/1 ethyl acetate/hexane (3 × 1 liter). The organic layers were combined and washed with 1 M HCl, water and brine. The combined organic phase was dried over anhydrous sodium sulfate and concentrated. The crude product was triturated with hexanes, affording 37 g of dimethyl 4-

Scheme 4.



(*N*,*N*-dimethylthiocarbamoyloxy)isophthalate as a pale yellow solid (74%): ^{1}H NMR ($CDCl_3$) δ 3.40 (s, 3H), 3.50 (s, 3H), 3.85 (s, 3H), 3.95 (s, 3H), 7.20 (d, 1H, J=8.4), 8.20 (d, 1H, J=8.4), 8.70 (s, 1H).

Into three separate pressure tubes were each placed 12.6 g (46.3 mmoles) of 4-(*N*,*N*- dimethylthiocarbamoyloxy)isophthalate and 20 ml of *m*-xylene. The tubes were sealed and placed in an oil bath at 170°C for 5 hours. The amber reaction solutions were combined and concentrated *in vacuo*, giving 36.9 g of dimethyl 4-(*N*,*N*-dimethylcarbamoylthio)isophthalate as a cream colored solid (97%): ^{1}H NMR ($CDCl_3$) δ 3.05 (d, 6H), 3.90 (d, 6H), 7.70 (d, 1H, J=8.4), 8.10 (d, 1H, J=8.4), 8.55 (s, 1H).

A mixture of 38.0 g (680 mmoles) of potassium hydroxide, 260 ml of 95% ethanol and 36.9 g (124 mmoles)

Table 7. Activity profile of MC-02,171, MC-02,306 and MC-02,331.

	MC-02,171 (35)	MC-02,306 (40)	MC-02,331 (45)	Imipenem
MIC_{90} (μg/ml)				
MRSA	8	8	4	64
E. faecalis	1	2	0.5	1
E. faecium (Amp^R)	32	16	4	>128
Pen^R *S. pneumoniae*	0.125	0.125	0.5	—
PBP2a IC_{50} (μg/ml)	0.75	1.8	3.1	41
Solubility (mg/ml)	2	>10	>10	10
ED_{50} (mg/kg)	0.74	0.14	0.23	0.06
Human serum binding	91%	85%	87%	20%

of dimethyl 4-(*N*,*N*-dimethylcarbamoylthio)isophthalate was stirred at reflux for 4 hours and the mixture was concentrated. The residue was dissolved in 500 ml of water and was washed with ethyl acetate (2 × 500 ml). The aqueous layer was adjusted to pH 3 with concentrated hydrochloric acid, and was extracted with ethyl acetate (4 × 1 liter). The combined extracts were concentrated, and the residue was triturated with ether, giving 22.4 g of 4-mercaptoisophthalic acid as an off-white powder (91%): ^1H NMR ($CDCl_3$) δ 7.30 (d, 1H, J=8.4), 7.85 (d, 1H, J=8.4), 8.65 (s, 1H).

To a mechanically stirred solution of 22.4 (113 mmoles) of 4-mercaptoisophthalic acid in 350 ml anhydrous THF at 0°C was added dropwise 460 ml of 1 M borane in THF. The mixture was allowed to warm to room temperature and was stirred for 1.5 hours. The mixture was cooled to 0°C and was quenched by careful addition of 250 ml of methanol. After stirring for 1 hour at room temperature, the solution was concentrated *in vacuo* giving 2,4-bis(hydroxymethyl)thiophenol in quantitative yield. ^1H NMR ($CDCl_3$) δ 4.35 (s, 2H), 4.50 (s, 2H), 6.90 (d, 1H, J=8.4), 7.15 (d, 1H, J=8.4), 7.25 (s, 1H).

Freshly prepared methanolic sodium methoxide [from 2.5 g. (109 mmol) of sodium and 500 ml methanol] at 0°C was treated dropwise with 20.4 g (120 mmoles) of 2,4-bis(hydroxymethyl)thiophenol in 250 ml of methanol. The solution was stirred at room temperature for 1 hour, and was concentrated *in vacuo*. The residue was washed repeatedly with ether, and was dried *in vacuo* giving 14.1 g of sodium 2,4-bis(hydroxymethyl)benzenethiolate as a hygroscopic yellow foam (61%).

(7*R*)-7-[(Phenylacetyl)amino]-3-[2,4-bis(hydroxymethyl)phenylthio]-3-cephem-4-carboxylate, 4-Methoxybenzyl Ester

To a stirring solution of sodium 2,4-bis(hydroxymethyl)benzenethiolate (9.84 g, 51.2 mmol) in dry DMF (50 ml) at −40°C was added by cannula a −40°C solution of (7*R*)-7-[(phenylacetyl)amino]-3-trifluoromethanesulfonyloxy-3-cephem-4-carboxylate, 4-methoxybenzyl ester (25.0 g, 42.7 mmol) in dry DMF (50 ml). After 1 hour at −40°C, the mixture was allowed to warm to −30°C, and was quenched by addition of saturated ammonium chloride solution (50 ml). The mixture was partitioned between 1 liter of water and 1 liter of 3:1 ethyl acetate/hexane. The aqueous layer was furtherextracted with 1 liter of 3:1 ethyl acetate/hexane. The combined organics were washed with water (2 × 1 liter) and saturated sodium chloride (1 liter), and were dried over anhydrous sodium sulfate and concentrated to a brown solid. This material was triturated with ether, filtered and dried to afford 15.3 g (59%) of the title compound. ^1H NMR ($CDCl_3$) δ 3.01 (s, 2), 3.43 (d, 1H, J=16), 3.47 (d, 1H, J=16), 3.72 (s, 3), 4.58 (s, 2), 4.61 (d, 1H, J=14), 4.64 (d, 1H, J=14), 4.86 (d, 1H, J=4), 5.17 (s, 2), 5.54 (dd, 1H, J=4, 9), 6.80 (d, 2H, J=10), 7.1 ~ 7.4 (m, 11).

(7*R*)-7-[(Phenylacetyl)amino]-3-[2,4-bis(chloromethyl)phenylthio]-3-cephem-4-carboxylate, 4-Methoxybenzyl Ester

A solution of (7*R*)-7-[(phenylacetyl)amino]-3-[2,4-bis(hydroxymethyl)phenylthio]-3-cephem-4-carboxylate, 4-methoxybenzyl ester (15.2 g, 25.0 mmol) and lithium chloride (5.25 g, 125 mmol) in dry DMF (125 ml) at 0°C was treated sequentially with diisopropylethylamine (22 ml, 125 mmol) and methanesulfonyl chloride

(14.3 g, 125 mmol). After 45 minutes at 0°C and 45 minutes at room temperature, the mixture was added to a rapidly stirring ice/water mixture (1.5 liters). The resulting solid was filtered and washed repeatedly with water. The solid was collected and dissolved in 3 : 1 ethyl acetate/hexane (750 ml), and this solution was washed with saturated sodium chloride, dried over sodium sulfate, and concentrated with a rotary evaporator, affording 14.0 g (87%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 3.10 (d, 1H, J=18), 3.31 (d, 1H, J=18), 3.62 (d, 1H, J=16), 3.65 (d, 1H, J=16), 3.81 (s, 3), 4.60 (s, 2), 4.72 (d, 1H, J=14), 4.75 (d, 1H, J=14), 4.96 (d, 1H, J=4), 5.24 (s, 2), 5.79 (dd, 1H, J=4, 9), 6.03 (d, 1H, J=9), 6.86 (d, 2H, J=10), 7.2~7.5 (m, 10).

(7*R*)-7-Amino-3-[2,4-bis(chloromethyl)phenylthio]-3-cephem-4-carboxylate, 4-Methoxybenzyl Ester, Trifluoroacetate Salt

To a stimng solution of (7*R*)-7-[(phenylacetyl)amino]-3-[2,4-bis(chloromethyl)phenylthio]-3-cephem-4-carboxylate, 4-methoxybenzyl ester (10.0 g, 15.6 mmol) and pyridine (2.7 g, 32.2 mmol) in dichloromethane (100 ml) at 0°C was added slowly a solution of phosphorus pentachloride (5.20 g, 25.0 mmol) in dichloromethane (50 ml). After 2 hours at 0°C, isobutanol (14.5 ml, 157 mmol) was added. The mixture was stirred for 2 hours at 0°C, dichloromethane was added (350 ml), and the resulting solution was washed with saturated aqueous sodium bicarbonate (2 × 1 liter), 1 M aqueous HCl (2 × 1 liter), and brine (2 × 1 liter). The organic phase was dried (sodium sulfate), trifluoroacetic acid (1.6 ml, 20.8 mmol) was added, and the mixture was concentrated with a rotary evaporator. The resulting beige solid was triturated with ether and dried, affording the title compound (6.80 g, 68%) as a tan solid: ^{1}H NMR ($CDCl_3$/CD_3OD) δ 2.87 (d, 1H, J=18), 3.41 (d, 1H, J=18), 3.74 (s, 3), 4.51 (s, 2), 4.62 (d, 1H, J=8), 4.67 (d, 1H, J=8), 4.78 (d, 1H, J=4), 5.06 (d, 1H, J=4), 5.15 (s, 2), 6.80 (d, 2H, J=10), 7.2~7.5 (m, 5).

(7*R*)-7-[(*Z*)-2-(2-*N*-Triphenylmethylaminothiazol-4-yl)-2-(triphenylmethoxyimino)acetamido]-3-[2,4-bis(chloromethyl)phenylthio]-3-cephem-4-carboxylate, 4-Methoxybenzyl Ester

A stimng solution of (*Z*)-2-(2-*N*-triphenylmethylaminothiazol-4-yl)-2-(tliphenylmethoxyimino)acetic acid (3.70 g, 5.50 mmol) and (7*R*)-7-amino-3-[2,4-bis(chloromethyl)phenylthio]-3-cephem-4-carboxylate, 4-methoxybenzyl ester, trifluoroacetate salt (3.03 g, 4.70 mmol) and THF (8 ml) at −20°C was treated sequentially with diisopropylethylamine (2.11 ml, 12.1 mmol) and phosphorus oxychloride (0.60 ml, 6.4 mmol). After 2 hours at −20°C, 1 M phosphoric acid solution (200 ml) was added, and the mixture was extracted with ethyl acetate (2 × 200 ml). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution, water, 1 M aqueous phosphoric acid solution, and brine. After drying over sodium sulfate, the mixture was concentrated with a rotary evaporator, and the residue was subjected to chromatography on silica gel (20~30% ethyl acetate/hexane), affording 3.90 g (73%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 3.02 (d, 1H, J=18), 3.25 (d, 1H, J=18), 3.81 (s, 3), 4.58 (s, 2), 4.74 (s, 2), 5.06 (d, 1H, J=4), 5.31 (s, 2), 5.99 (dd, 1H, J=4, 9), 6.45 (s, 1), 6.90 (d, 2H, J=10), 7.1~7.6 (m, 35).

(7*R*)-7-[(*Z*)-2-(2-*N*-Triphenylmethylaminothiazol-4-yl)-2-(triphenylmethoxyimino)acetamido]-3-[2,4-bis(isothioureidomethyl)phenylthio]-3-cephem-4-carboxylate, 4-Methoxybenzyl Ester, (Bis)hydrochloride Salt

To a stirring solution of (7*R*)-7-[(*Z*)-2-(2-*N*-triphenylmethylaminothiazol-4-yl)-2-(triphenylmethoxyimino)-acetamido]-3-[2,4-bis(chloromethyl)phenylthio]-3-cephem-4-carboxylate, 4-methoxybenzyl ester (3.90 g, 3.28 mmol) in absolute ethanol (50 ml) at room temperature was added thiourea (890 mg, 11.7 mmol). After 4 days at room temperature, the solvent was removed with a rotary evaporator, and the residue was triturated with ether and dried, affording 4.40 g (100%) of the title compound: ^{1}H NMR ($CDCl_3$/CD_3OD) δ 3.01 (d, 1H, J=18), 3.09 (d, 1H, J=18), 3.71 (s, 3), 4.33 (s, 2), 4.43 (d, 1H, J=12), 4.48 (d, 1H, J=12), 5.03 (d, 1H, J=4), 5.15 (s, 2), 5.70 (d, 1H, J=4), 6.42 (s, 1), 6.89 (d, 2H, J=10), 7.1~7.6 (m, 35).

(7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[2,4-bis(isothioureidomethyl)phenylthio]-3-cephem-4-carboxylate, (Bis)acetic Acid Salt (MC-02,331)

(7*R*)-7-[(*Z*)-2-(2-*N*-triphenylmethylaminothiazol-4-yl)-2-(triphenylmethoxyimino)acetamido]-3-[2,4-bis(isothioureidomethyl)phenylthio]-3-cephem-4-carboxylate, 4-methoxybenzyl ester, (bis)hydrochloride salt (4.40 g, 3.70 mmol) was taken up in 5% anisole/dichloroacetic acid (45 ml). After 6 hours, the reaction was diluted with ether (450 ml) and was stirred overnight. The mixture was filtered, the tan solid was washed with ether and dried, and was suspended in 0.1 M ammonium acetate buffer (pH 6.0). A small amount of insoluble material was removed by filtration, and the resulting

solution was subjected to medium-pressure chromatography on a 50 ml Amberchrome column, eluting with 0~20% acetonitrile/0.1 M ammonium acetate buffer (pH 6.0). The fractions containing the desired product were concentrated on a rotary evaporator (to remove acetonitrile), and were lyophilized to afford 1.51 g (60%) of the title compound: ^{1}H NMR (D_2O/DMSO-d_6) δ 3.11 (d, 1H, J=18), 3.60 (d, 1H, J=18), 4.42 (s, 2), 4.59 (s, 2), 5.27 (d, 1H, J=4), 5.80 (d, 1H, J=4), 6.95 (s, 1), 7.4~7.6 (m, 3).

References

1) CHAMBERS, H. F.: Methicillin resistance in staphylococci: Molecular and biochemical basis and clinical implications. Clinical Microbiology Reviews 10: 781~791, 1997
2) SASOR, M. F.; L. D. CAMA, M. L. GREENLEE, M. P. DININNO & J. V. HECK: The synthesis and structure-activity relationships of a series of 2-fluorenonyl carbapenems with excellent activity against MRSA. Abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, No. F50, 1994
3) TERNANSKY, R. J.; S. E. DRAHEIM, A. J. PIKE, F. W. BELL, S. J. WEST, C. L. JORDAN, C. Y. E. WU, D. A. PRESTON, W. ALBORN, Jr., J. S. KOSHER & B. L. HAWKINS: Discovery and structure-activity relationships of a series of 1-carba-1-dethiacephems exhibiting activity against methicillin-resistant *Staphylococcus aureus*. J. Med. Chem. 36: 1971~1976, 1993
4) TSUSHIMA, M.; A. TAMURA, T. HARA, K. IWAMATSU & S. SHIBAHARA: Synthesis and biological properties of CP0467, a new cephem possessing excellent activity against MRSA. Abstracts of the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, No. 394, 1992
5) HANAKI, H.; H. AKAGI, Y. MASARU, T. OTANI, A. HYODO & K. HIRAMATSU: TOC-39, a novel parenteral broad-spectrum cephalosporin with excellent activity against methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 39: 1120~1126, 1995
6) SUNAGAWA, M.; H. YAMAGA, H. SHINAGAWA, H. HOUCHIGAI & Y. SUMITA: Synthesis and biological properties of a new series of anti-MRSA beta-lactams; 2-(thiazol-2'-ylthio)carbapenems. Bio Med. Chem. Lett. 4: 2793~2798, 1994
7) CRAIG, W. A.: Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clinical Infectious Diseases 26: 1~12, 1998
8) WEBBER, J. A. & W. J. WHEELER: Antimicrobial and pharmacokinetic properties of newer penicillins and cephalosporins. *In* Chemistry and Biology of β-Lactam Antibiotics. Vol. 1. *Eds.*, R. B. MORIN & GORMAN, pp. 372~436, Academic Press, New York, 1982
9) NCCLS (National Committee for Clinical Laboratory Standards). 1994. Methods for determining bactericidal activity of antimicrobial agents; tentative guideline. NCCLS Document M26-T, 1992. *In*: NCCLS Antimicrobial Susceptibility Testing, January 1994 edition
10) DARGIS, M. & F. MALOUIN: Use of biotinylated β-lactams and chemiluminescence for study and purification of penicillin-binding proteins in bacteria. Antimicrob. Agents Chemother. 38: 973~980, 1994
11) CRAIG, W. A. & S. BYUNGSE: Protein binding and the antimicrobial effects: Methods for the determination of protein binding. *In* Antibiotics in Laboratory Medicine, second edition, Chapter 13. *Ed.*, V. LOVIAN, pp. 477~514, Williams & Wilkins, Baltimore, 1986
12) LEGGETT, R.; S. A. WOLZ & W. A. CRAIG: Use of serum ultrafiltrate in the serum dilution test. J. Infect. Dis. 160: 616~622, 1989
13) National Committee for Clinical Laboratory Standards NCCLS Document M7-A3, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Third Edition; Approved Standard (1993)
14) TENG, N. H.; H. S. KAPLAN, J. M. HERBERT, C. MOORE, C. H. DOUGLAS, A. WUNDERLICH & A. I. BAUDS: Protection against Gram-negative bacteremia and endotoxemia with human monoclonal IgM antibodies. Proc. Natl. Acad. 82: 1790~1794, 1985
15) PASIELLO, A. P.; J. M. ESSIGMAN & G. N. WOGAN: Rapid and accurate determination of median lethal dose (LD_{50}) and its error with a small computer. J. Toxicol. Environ. Health. 3: 797~809, 1977
16) ^{1}H NMR spectra were determined at 300 MHz. Chemical shifts are expressed downfield from tetramethylsilane. Date are presented in the following order: multiplicity, number of hydrogens, coupling constant in hertz