Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 265–279

BIOORGANIC & MEDICINAL CHEMISTRY

Anti-MRSA Cephems. Part 2: C-7 Cinnamic Acid Derivatives

Dane M. Springer,* Bing-Yu Luh, Jason Goodrich and Joanne J. Bronson

Anti-infective Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, PO Box 5100, Wallingford, CT 06492, USA

Received 6 November 2001; accepted 17 June 2002

Abstract—Forty-five novel cephalosporin derivatives with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) are described. The compounds contain novel cinnamic acid moieties at C-7 that were synthesized using a key Heck reaction followed by nucleophilic aromatic substitution reactions. The most active compound (**41**) displayed an MIC_{90} against MRSA of 1.0 μg/mL, and a PD_{50} of 0.8 mg/kg. Compound **14** was found to be very safe in a mouse model of acute toxicity.

Introduction

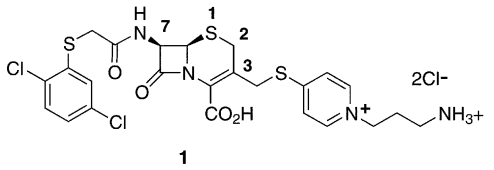
Post-surgical morbidity from bacterial infections of methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing as most strains have become multi-drug resistant.[1] The only effective treatment of MRSA currently in the clinic is vancomycin. The advent of vancomycin resistance in enterococci has raised concern that this resistance might eventually be transferred to MRSA, creating a lethal pathogen of epidemic proportions.[2] Strains of MRSA with reduced susceptibility to vancomycin have begun to appear in the clinic, heralding the beginning of the end of vancomycin's utility in the management of these infections.[3] Therefore, the search for new antibiotics with anti-MRSA activity is critical to the future maintenance of public health.[4]

We wished to develop an injectable anti-MRSA cephalosporin that would manage these life-threatening infections. Our biological and solubility criteria for a suitably active clinical candidate were as follows: MIC_{90}s against MRSA ≤ 4 μg/mL; PD_{50} in a mouse systemic MRSA infection ≤ 5 mg/kg; safety in a mouse model of acute toxicity; and, aqueous solubility ≥ 20 mg/mL.

Our program began with the observation that C-7 2,5-dichlorothiophenyl acetamides imparted excellent anti-staphylococcal activity to cephems.[5] The combination of this lipophilic C-7 group with C-3 thiopyridinium moieties provided compounds with exceptional anti-MRSA activity.[6] An early lead, aminopropyl derivative **1**, satisfied our goals for in vitro and in vivo activity. Unfortunately, compound **1** was found to be acutely toxic to mice upon iv bolus delivery to the tail vein. It was subsequently found that the C-3 amino acid derivative **2** had good activity against MRSA, and was less acutely toxic than **1** in mice.[7] However, bis-zwitterion **2** was not very soluble in a variety of aqueous media (<3 mg/mL).[8] As a result of this low solubility, a large number of derivatives were synthesized that had either a carboxylic or sulfonic acid group on the side-chain of the thiopyridinium moiety at C-3, or an acid substituted off the pyridinium ring itself.[4i] The goal was to move the acid group around the C-3 moiety in the hope of improving upon the solubility of our derivatives while maintaining sufficient anti-MRSA activity. As a result of these efforts, some general trends were observed regarding the inclusion of acid groups into our cephems. First, the extra acid functions improved the acute toxicity profiles of our compounds (vide supra regarding the toxicity of compound **1** vs **2**). In general, it was observed that positively charged molecules (such as **1**) were usually quite toxic in our acute toxicity assay. Zwitterions or bis-zwitterions (such as **2**) usually fared better in the acute toxicity screen, while compounds carrying a net negative charge were usually non-toxic in our assay. While the inclusion of an extra acid improved the solubility of our compounds (except in the case of zwitterions), and helped improve their toxicity profile, the addition of each extra acid group at the C-3 position also resulted in a concomitant loss of potency in vitro (generally 4–8 fold less active by MIC). We therefore embarked upon a program to introduce an acid at C-7

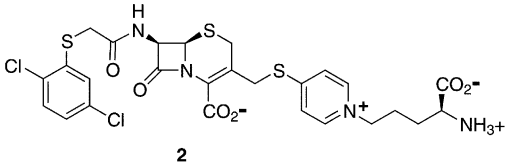
*Corresponding author at current address: Rib-X-Pharmaceuticals, 300 George Street, New Haven, CT 06511, USA; e-mail: springer@rib-x.com

0968-0896/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00336-X



1

MIC MRSA (A27223) = 0.125 μg/mL
MIC with 50% calf serum = 2 μg/mL
MIC_{90} MRSA = 2 μg/mL
PD_{50} MRSA (A27223) = 1.4 mg/kg



2

MIC MRSA (A27223) = 1 μg/mL
MIC with 50% calf serum = 4 μg/mL
MIC_{90} MRSA = 3.5 μg/mL
PD_{50} MRSA (A27223) = 4.1 mg/kg

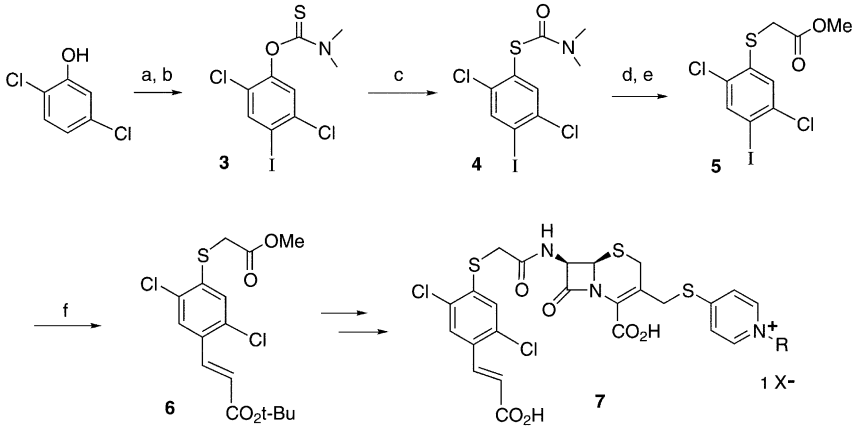
of our cephems in the hopes of improving solubility, maintaining safety in the acute toxicity assay, and retaining excellent in vitro potency against MRSA. The results of some of our efforts in this area are hereby presented.

Chemistry

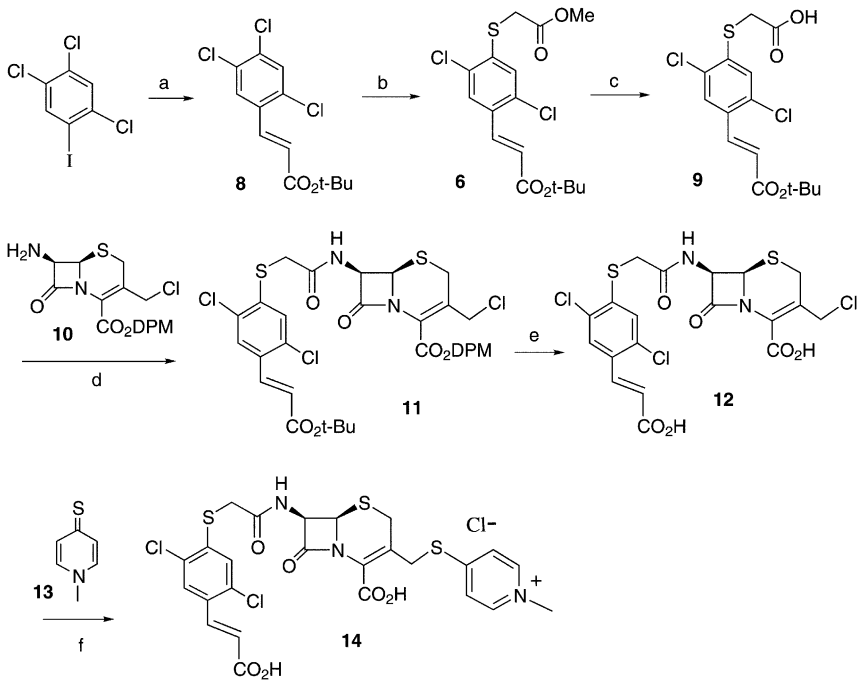
The C-7 amide-linked thiophenyl group is undoubtedly a major contributor to the lipophilicity of our derivatives. We had long maintained an interest in introducing functional groups onto the C-7 dichlorothiophenyl ring of our compounds that would improve their water solubility. One of our early routes to C-7 acid compounds is illustrated by Scheme 1. Iodination of 2,5-dichlorophenol in the *para* position, followed by conversion to the *O*-aryl dimethyl thiocarbamate, provided intermediate **3**. Newman–Kwart rearrangement of **3** gave *S*-aryl thiocarbamate **4**, which could be hydrolyzed to the thiol.[9] Alkylation of the thiol derived from **4** with methyl bromoacetate yielded iodo ester **5**. The Heck reaction of **5** with *t*-butyl acrylate afforded diester **6** in a 40% yield. Hydrolysis of the methyl ester of **6** gave the corresponding acid which could be coupled with an appropriate C-7 amino cephalosporin using DCC, and subsequently utilized to produce final cephem targets of type **7**.

While the chemistry of Scheme 1 was successful in producing the first cephalosporin targets in the C-7 acid series, it was a bit long (six steps to key diester **6**) and suffered a low yield in the Heck reaction step. We desired a shorter route to diester **6** and discovered the chemistry shown in Scheme 2. We found that 2,4,5-trichloro iodobenzene underwent clean conversion to acrylate **8** via the Heck reaction. Subsequent nucleophilic aromatic substitution of ester **8** could be achieved in good yield using the sodium salt of methyl mercaptoacetate in DMF to produce the desired diester **6** in just two steps. The efficient acquisition of **6** by this process facilitated the synthesis of our analogues as exemplified by the remaining chemistry shown in Scheme 2. Hydrolysis of **6** to acid **9** followed by DCC coupling to cephem amine **10** led to the chloromethyl diester **11**.[10] TFA deprotection of **11** gave intermediate diacid **12**, which could be treated with thiopyridones such as **13** to provide the final antibiotic targets (such as **14**).

We wished to explore the use of other substrates for the Heck reaction in an attempt to determine structure–activity relationships in this series of C-7 cinnamic acid derivatives. Unfortunately, aryl iodide **5** did not react successfully with either *t*-butyl methacrylate or *t*-butyl propiolate. Similarly disappointing was the unreactivity of aryl chlorides **15**–**19** towards nucleophilic aromatic substitution with the sodium thiolate derived from methyl mercaptoacetate. Aryl chloride **15** lacks the easily replaceable chlorine atom *para* to the acrylate group, leaving only the more hindered *ortho* chlorine available for displacement. Compounds **16** and **17** bear two *ortho* chlorine substituents that may force the acrylate



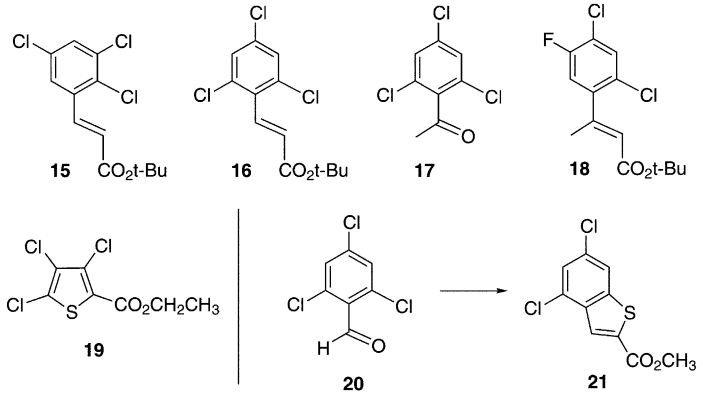
Scheme 1. Reaction conditions: (a) I_2, $AgSO_4$, CH_2Cl_2; (b) DABCO, $Me_2NC(S)Cl$, DMF; (c) heat, 220 °C, 33% from 2,5-dichlorophenol; (d) 3 N KOH, EtOH, reflux, 81%; (e) Et_3N, $BrCH_2CO_2CH_3$, CH_2Cl_2, 90%; (f) $CH_2{=}CHCO_2t$-Bu, PPh_3, Bu_3N, DMF, $Pd(OAc)_2$, 120 °C, 67%.

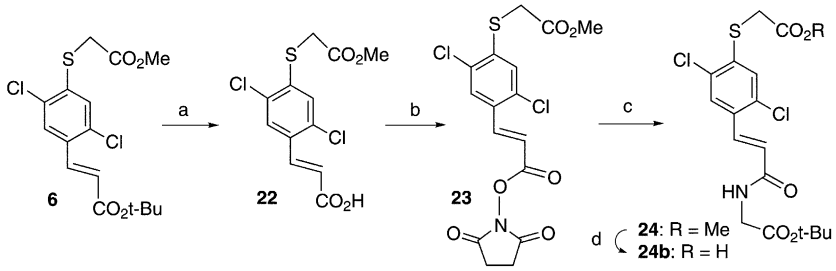


Scheme 2. Reaction conditions: (a) $CH_2{=}CHCO_2t$-Bu, PPh_3, Bu_3N, DMF, $Pd(OAc)_2$, 80 °C, 75%; (b) $NaSCH_2CO_2CH_3$, DMF, 75%; (c) NaOH, 92%; (d) CDD, THF; (e) TFA, CH_2Cl_2, 71% from **9**; (f) CH_3OH, 61%.

residue more out of plane with the aryl ring than is the case for compound **8**. This would reduce the ability of the acrylate π-system to stabilize the anion derived from addition of the thiolate to the aryl ring. Compound **18** has an additional methyl substituent at the β-carbon of the acrylate group that could similarly force the aryl ring slightly more out of conjugation with the acrylate group, reducing its reactivity relative to **8**. The failure of thiophene ester **19** to undergo the desired nucleophilic aromatic substitution of one of the chlorine atoms is not too surprising since thiophenes usually require the presence of a nitro group on the ring for nucleophilic addition to proceed efficiently. We had hoped that the aldehyde group of the bis-*ortho* substituted derivative **20** might maintain good planarity with the aryl ring, allowing the displacement of the *para* chlorine atom. Interestingly, the reaction afforded instead the benzothiophene **21** via *ortho* substitution of the thiolate, followed by intramolecular aldol condensation.

Since it appeared that we could not easily synthesize alternate chloroaryl derivatives using the aromatic substitution reaction, or easily introduce other groups on the olefinic moiety of our derivatives, we decided to make amide derivatives of the C-7 acid. Towards these ends, diester **6** was treated with TFA to afford acid **22** (Scheme 3). Acid **22** could be directly coupled with amines using DCC, however, the yields were not optimal, and purification of the products was occasionally problematic. Better results were obtained by first coupling acid **22** with *N*-hydroxy succinimide to give the activated ester **23**, and then treating **23** with amines such as *t*-butyl glycine (to provide glycinamide **24**). The corresponding diesters were selectively hydrolyzed at the methyl ester position, similarly to diester **6**, and the derived acids were then coupled to cephem amine **10** as previously described. Using the aromatic substitution chemistry described above, and a variety of different thiopyridones related to **13**, the compounds shown in Tables 1–4 were synthesized for biological evaluation.





Scheme 3. Reaction conditions: (a) TFA, CH_2Cl_2, 79%; (b) *N*-hydroxysuccinimide, DCC, THF; (c) *t*-butyl glycine, THF, 89% from **22**; (d) NaOH, 80%.

Results and Discussion

The first aspect of SAR that was investigated was the effect of chlorine substitution on activity. As Table 1 demonstrates, the C-7 dichloro aryl derivative **25** is more active than the corresponding monochloro compounds **27** and **28**. Similarly, the dichloro cephem **38** is more active than its related monochloro derivatives **39** and **40**. Amongst the monochloro compounds, the *ortho*-substituted (relative to sulfur) **28** and **40** were less active than their *meta*-substituted counterparts **27** and **39**. The bis *meta*-chlorinated cephem **29** appears to be slightly less active than compound **25**, and trichloro cephem **30** was much less potent than **25**.

Dimethyl substitution on the C-3 pyridinium ring is often well tolerated (i.e., MIC of **31** and **33**), but on occasion results in loss of potency; thus, no general trend can be predicted. The glycine acids **32**–**34**, the alanines **35**–**36**, and serine **37** were fairly active derivatives. An interesting feature of these C-7 amide-linked acids is that they often have an improved pharmacokinetic (PK) profile relative to the corresponding cinnamic acid derivative. For example, both **25** and **32** were evaluated in a mouse PK study (20 mg/kg bolus dose given iv). The following data was obtained for cinnamic acid **25**: $C_{5min} = 21$ μg/mL; AUC = 11 mg h/mL; and, $t_{1/2} = 6$ min. The data obtained from testing of **32** was: $C_{5min} = 53$ μg/mL; AUC = 38 mgh/mL; and, $t_{1/2} = 43$ min. The slightly improved in vivo activity of **32** ($PD_{50} = 1.8$, 4.1 mg/kg) relative to **25** ($PD_{50} = 4.1$, 5.4 mg/kg) might be a consequence of the compound's improved PK characteristics.

Table 2 illustrates the activity of the most potent C-7 acid derivative (in this series of cephems), the C-3 hydroxy aminopropyl thiopyridinium cephem **41**. This compound had excellent in vitro and in vivo activity, and the following mouse PK parameters: $C_{5min} = 51$ μg/mL; AUC = 22 mg h/mL; and, $t_{1/2} = 25$ min. Unfortunately, cephem **41** did not meet our solubility criteria, being rather insoluble at pH 7 (< 3 mg/mL).[8] The poor solubility of **41** is likely due to its bis-zwitterionic nature at physiological pH.

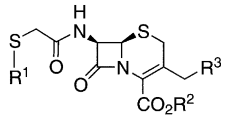
The imidazolium compounds **45**–**47** had good activity in vivo, and in vitro against our marker strain of MRSA (A27223). However, the imidazolium class of C-3 derivatives (paired with a C-7 acid group) suffered from high MIC_{90}s, as illustrated in this work by the value of 5.8 μg/mL for compound **45**. The remaining members of Table 2, the *N*-methyl thiopyridinium derivatives **14**, **48** and **49**, are quite potent in vitro. The only one of these tested in vivo was **14**, and it was found to have good activity ($PD_{50} = 5.4$, 9.5 mg/kg).[11] Compound **14** displayed an excellent MIC_{90} against MRSA (3.4 μg/mL). Cephem **14** was also found to be quite safe in our mouse acute toxicity assay. The compound was found to be safe when given iv to mice at a bolus dose of at least 850 mg/kg (the highest dose tested). By comparison, cephem **2** was safe at a dose of 240 mg/kg, but higher doses began to result in toxic effects in mice.

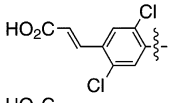
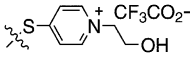
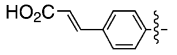
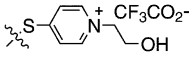
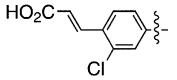
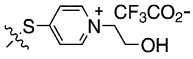
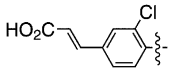
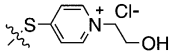
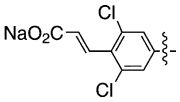
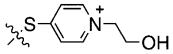
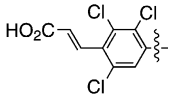
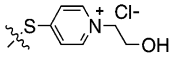
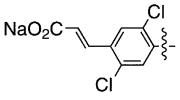
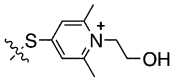
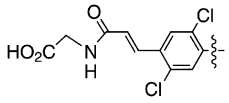
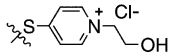
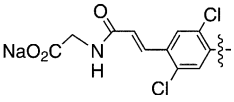
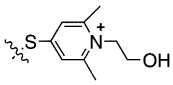
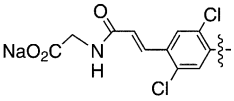
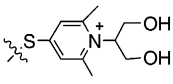
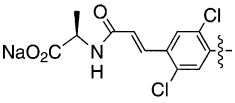
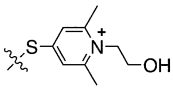
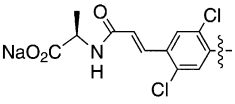
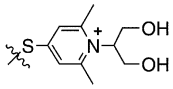
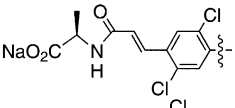
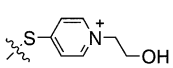
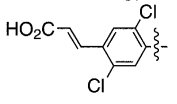
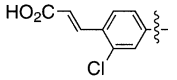
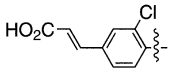
Table 3 contains the biological data for a series of C-3 salicylic acid pyridinium derivatives. Compound **50** was found to be the most interesting of the group displaying an excellent MIC_{90} (3.6 μg/mL) as well as very good activity in vivo ($PD_{50} = 3.6$, 4.1 mg/kg). Unfortunately, cephem **50** was found to have a short half-life in mice (7 min). The data for cephems **51**–**53** indicates that methyl substitution on the pyridinium ring is tolerated in vitro, but has deleterious effects on the in vivo activity of these compounds relative to cephem **50**.[12] Replacement of the salicylic hydroxyl group with a chlorine atom resulted in a loss of in vitro potency (cf. **56** with **50**). While the C-7 amide-linked acids **57** and **59** were quite active in vitro, the C-3 dimethyl pyridinium compound **58** was markedly less active than its unsubstituted counterpart cephem **57**.

Table 4 shows the biological activity for some additional C-3 derivatives in the C-7 cinnamic acid, and amide-linked acid, series of cephems. As the data in Table 4 indicate, good in vitro activity can be attained with a variety of substitution patterns. Unfortunately, good in vivo activity is lacking in most of these compounds, except for cephems **60** and **66**.[12] Cephem **66** did not meet our criteria for a clinical candidate due to its relatively high MIC_{90}. Compound **60**, while meeting all our biological goals, was found to be insoluble in a variety of aqueous media at pH 7.

Table 5 illustrates the in vitro antibacterial activity against some Gram-positive organisms for the more interesting compounds generated in the present work. These compounds are quite active against Gram-positive bacteria such as streptococci, staphylococci, and enterococci. The cephems in this class have little activity against the representative Gram-negative organisms

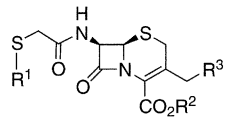
Table 1.

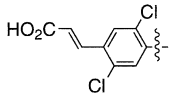
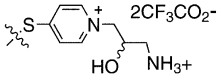
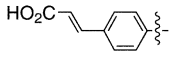
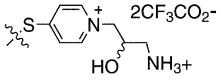
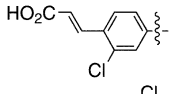
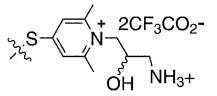
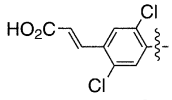
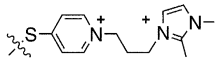
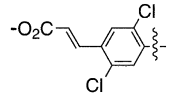
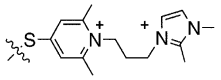
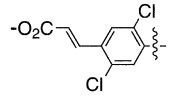
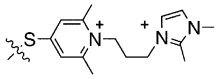
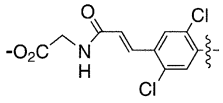
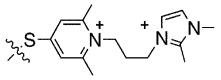
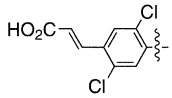
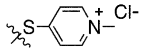
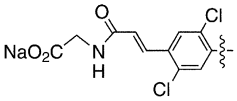
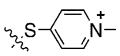
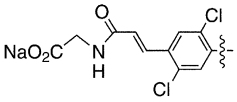
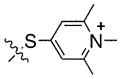


No.	R^1	R^2	R^3	MIC[a]	MIC_{90}[b]	PD_{50}[c]
25		H		1 (4)	3.3	4.1, 5.4
26		H		4 (4)		16.5, >25
27		H		2 (4)	3.6	9.5, 16.5
28		H		16 (16)		—
29		—		2 (2)		5.4, 7.2
30		H		8 (32)		—
31		—		2 (4)		>25, >25
32		H		4 (4)		1.8, 4.1
33		—		1 (2)		12.5, 12.5
34		—		2 (4)		9.5, 12.5
35		—		2 (4)		9.5, 12.5
36		—		2 (4)		21.8, >25
37		—		4 (4)		5.4
38[13]		H		2 (4)		16.5, 16.5
39[13]		H		8 (8)		12.5, >25
40[13]		H		32 (32)		—

[a]MIC (in μg/mL) versus MRSA A27223, value in parentheses is MIC in presence of 50% calf serum. The MIC of vancomycin (vs MRSA A27223) in our assay was 0.25 μg/mL, and that of imipenem was 32 μg/mL.
[b]MIC_{90} (in μg/mL) for 58 strains of methicillin-resistant *S. aureus*.
[c]PD_{50} (in mg/kg) for activity against MRSA A27223 in a mouse model of systemic infection (vancomycin PD_{50} ~0.3–0.8 mg/kg). Multiple values denote separate experiments.

Table 2.



No.	R^1	R^2	R^3	MIC[a]	MIC_{90}[b]	PD_{50}[c]
41		H		0.25 (0.5)	1.0	0.8, 0.8
42		H		4 (4)		12.5, 16.5
43		H		1 (2)	3.6	5.4, 12.5
44		H		1 (2)		9.5, 9.5
45		—		2 (4)	5.8	4.1, 9.5
46		—		2 (4)		1.4, 2.8, 4.2, 12.5
47		—		1 (2)		7.2
14		H		2 (4)	3.4	1.4, 5.4, 9.5
48		—		1 (2)		—
49		—		1 (2)		—

[a]MIC (in μg/mL) versus MRSA A27223, value in parentheses is MIC in presence of 50% calf serum. The MIC of vancomycin (vs MRSA A27223) in our assay was 0.25 μg/mL, and that of imipenem was 32 μg/mL.
[b]MIC_{90} (in μg/mL) for 58 strains of methicillin-resistant *S. aureus*.
[c]PD_{50} (in mg/kg) for activity against MRSA A27223 in a mouse model of systemic infection (vancomycin PD_{50} ~0.3–0.8 mg/kg). Multiple values denote separate experiments.

included in our screening panel (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Proteus mirabilis, Pseudomonas aeruginosa*; data not shown in Table 5).

Conclusions

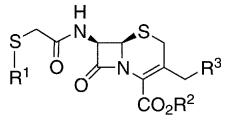
We have discovered a series C-7 cinnamic acid derivatives that often display excellent in vitro and in vivo activity against MRSA. The introduction of an extra acidic group at C-7 onto leads such as cephem **1** was found to be less deleterious to antibacterial potency than placing the acid group at C-3. Many of these compounds had good PK characteristics, and cephem **14** was shown to be a very safe compound in an acute toxicity assay in mice. Cephems such as **14**, **25**, **41**, **50** and **60** are exciting leads in the effort to bring a viable anti-MRSA cephalosporin into the clinic. The chemistry developed to synthesize these C-7 cinnamic acid derivatives will be of use to those interested in developing anti-MRSA cephems. The activity of compounds derived from further combinations of these of C-7 acid derivatives with a variety of additional C-3 substituents remains to be disclosed in future communications from the laboratories of Bristol-Myers Squibb.

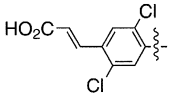
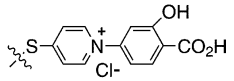
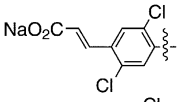
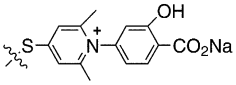
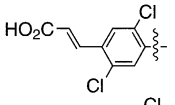
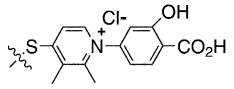
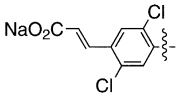
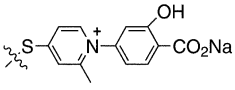
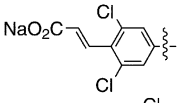
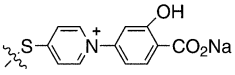
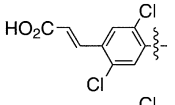
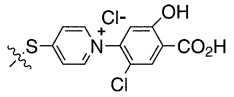
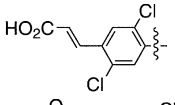
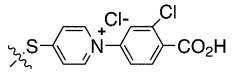
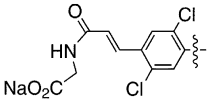
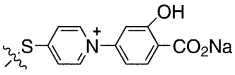
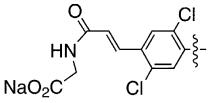
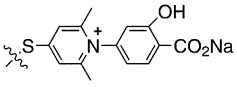
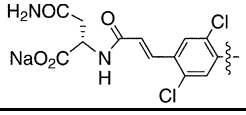
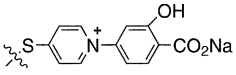
Experimental

Biology

Antibacterial MICs were determined in broth according to the standard conditions recommended by the National Committee for Clinical Laboratory Standards

Table 3.



No.	R^1	R^2	R^3	MIC[a]	MIC_{90}[b]	PD_{50}[c]
50		H		2 (8)	3.6	3.6, 4.1
51		—		4 (8)		>25, >25
52		H		4 (8)		12.5, 16.5
53		—		4 (8)		9.5, 12.5
54		—		4 (4)		9.5, 21.8
55		H		2 (8)		21.8, >25
56		H		8 (16)		—
57		—		1 (2)		7.2, 21.8
58		—		8 (8)		—
59		—		1 (1)		9.5, 21.8

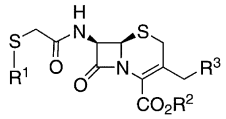
[a]MIC (in μg/mL) versus MRSA A27223, value in parentheses is MIC in presence of 50% calf serum. The MIC of vancomycin (vs MRSA A27223) in our assay was 0.25 μg/mL, and that of imipenem was 32 μg/mL.
[b]MIC_{90} (in μg/mL) for 58 strains of methicillin-resistant *S. aureus*.
[c]PD_{50} (in mg/kg) for activity against MRSA A27223 in a mouse model of systemic infection (vancomycin PD_{50} ~0.3–0.8 mg/kg). Multiple values denote separate experiments.

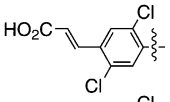
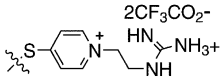
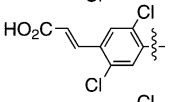
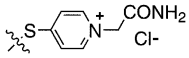
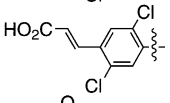
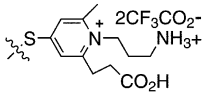
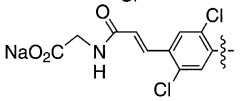
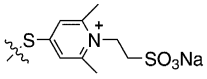
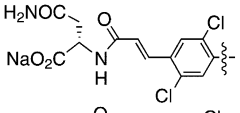
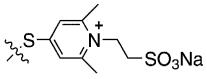
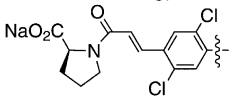
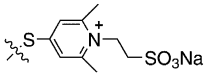
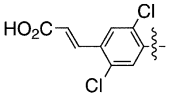
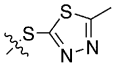
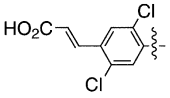
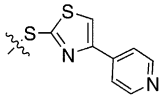
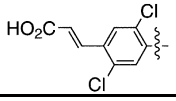
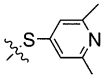
(NCCLS). MIC assays against MRSA utilized Mueller-Hinton broth + 2% NaCl, a bacterial inoculum size $\sim 5\times10^5$ CFU/mL, and were incubated at 35 °C for 24 h. MIC was defined as the lowest drug concentration inhibiting all visible growth. MIC_{90}s were determined against a panel of 58 strains of MRSA.

The PD_{50} values reported represent the concentration of the compound that protects 50% of the infected animals from death in a mouse model of systemic infection. Mice were given (ip) a lethal dose of MRSA A27233 (homogeneous strain, 2.4×10^8 CFU) on day 1, and the test compound was administered (im) twice on day 1 at 0.2 h and 2 h post infection [five animals per dose level, five animals for infected (but untreated) control animals]. Animals were monitored until the end of day five for survival.

Compounds were assayed for acute toxicity in mice as follows: the test compound was dissolved in 5% aqueous dextrose at a concentration between 5 and 25 mg/mL. The solution was filtered through a 0.2 μ filter, and between 0.1 and 1 mL of the solution was injected (in usually less than a min) into the tail vein of three mice. The usual toxic reaction observed after injection of these types of compounds was red coloration of the feet, ears, tail and muzzle of the mice, followed by respiratory distress and death. Death, when observed, usually occurred within min of injection. Mice surviving 1 h post injection usually recovered with no obvious adverse effects.

Table 4.



No.	R^1	R^2	R^3	MIC[a]	MIC_{90}[b]	PD_{50}[c]
60		H		1 (2)	1.8	>25, >25
61		H		2 (4)		21.8, 21.8
62		H		4 (8)		21.8, >25
63		—		2 (4)		21.8, >25
64		—		2 (2)		>25, >25
65		—		4 (4)		>25, >25
66[14]		H		2 (16)	4.9	5.4, 9.5
67[14]		H		1 (32)		>25, >25
68[14]		H		1 (64)		9.5, 12.5

[a]MIC (in μg/mL) versus MRSA A27223, value in parentheses is MIC in presence of 50% calf serum. The MIC of vancomycin (versus MRSA A27223) in our assay was 0.25 μg/mL, and that of imipenem was 32 μg/mL.
[b]MIC_{90} (in μg/mL) for 58 strains of methicillin-resistant *S. aureus*.
[c]PD_{50} (in mg/kg) for activity against MRSA A27223 in a mouse model of systemic infection (vancomycin PD_{50} ~0.3–0.8 mg/kg). Multiple values denote separate experiments.

Chemistry

Unless otherwise indicated all reactions were performed under a nitrogen atmosphere and all solvents were of reagent grade (anhydrous if available). Analytical thin-layer chromatography (TLC) was carried out on silica gel plates (60F-254) and visualized using UV light, iodine vapors, and/or staining by heating with ethanolic phosphomolybdic acid. 'Chromatography' or 'Chromatography on silica gel' refers to flash column chromatography using E-Merck silica gel 60 (230–400 mesh) unless otherwise noted. C-18 silica gel was purchased from Whatman.

Proton and carbon-13 nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were recorded on a Bruker AM-300 or a Varian Gemini 300 spectrometer. Chemical shifts are reported in δ (ppm) units relative to tetramethylsilane (TMS) and interproton coupling constants are reported in Hertz (Hz). Mass spectra were recorded on a Kratos MS-50 or a Finnegan 4500 instrument utilizing direct chemical ionization (DCI, isobutene) or fast atom bombardment (FAB), or on a Shimadzu/Micromass LCMS array (for ESI).

Synthesis of thiocarbamate 3. A. 2,5-Dichlorophenol (20.4 g, 0.125 mol) is placed in a 1-L round-bottom flask equipped with a large egg-shaped stir bar and is dissolved in 307 mL CH_2Cl_2. With rapid stirring, iodine (46.6 g, 0.183 mol) is added, followed by silver sulfate (42.3 g, 0.136 mol). The purple solution is stirred 1 day, at which point NMR analysis of an aliquot indicates the reaction is complete. The reaction is diluted with CH_2Cl_2 (~200 mL) and filtered through a fritted Buchner funnel to remove silver salts. The salts are washed with additional CH_2Cl_2 (~100 mL). The organic filtrate is transferred to a separatory funnel and is washed first with a solution of sodium thiosulfate

Table 5.

	Organism	A No.	MIC of compounds						
			14	**25**	**32**	**41**	**46**	**50**	**60**
1	*S. pneumoniae*/Pen. I	A27881	0.125	0.125	0.03	0.125	0.25	0.06	0.03
2	*S. pneumoniae*/Pen. Resist.	A28272	0.5	0.25	0.25	0.5	1	0.25	0.25
3	*S. pyogenes*/Todd Hewitt	A9604	0.015	0.007	0.001	0.0005	0.015	0.007	0.003
4	*E. faecalis*	A20688	1	1	0.5	0.25	2	1	0.25
5	*E. faecalis*/+ 50% calf serum	A20688	8	4	1	0.5	2	2	1
6	*E. faecium*	A24885	8	8	8	2	16	4	4
7	*E. faecium*/Amp. R, Vanco. S	A28156	128	128	>128	32	>128	64	128
8	*S. aureus*/Pen.−	A9497	0.03	0.015	0.015	0.003	0.06	0.015	0.015
9	*S. aureus*/Pen.+	A9606	0.25	0.5	0.25	0.125	1	0.5	0.125
10	*S. aureus*/+ 50% calf serum	A9606	0.5	0.5	0.25	0.25	1	0.5	0.25
11	*S. aureus*/MS/Pen. +	A20241	0.25	0.5	0.25	0.125	1	0.5	0.25
12	*S. aureus*/Hetero MR	A27218	0.5	0.5	1	0.25	2	0.5	0.5
13	*S. aureus*/+ 50% calf serum	A27218	1	2	1	0.5	2	1	0.5
14	*S. aureus*/Hetero MR	A27217	0.25	0.5	1	0.25	1	0.5	0.25
15	*S. aureus*/Homo MR	A27223	2	1	4	0.25	2	2	1
16	*S. aureus*/+ 50% calf serum	A27223	4	4	4	0.5	4	8	2
17	*S. aureus*/Homo MR	A27621	1	1	4	0.25	2	2	1
18	*S. aureus*/Homo MR	A27295	4	2	2	0.5	4	1	1
19	*S. aureus*/Homo MR	A27226	1	0.5	2	0.25	2	2	0.5
20	*S. aureus*/MR, P−	A27225	2	1	1	0.125	2	1	0.5
21	*S. epidermidis*	A24548	0.06	0.06	0.03	0.015	0.06	0.06	0.03
22	*S. epidermidis*/MR	A25783	—	0.5	0.125	0.125	1	1	0.125
23	*S. epidermidis*/Imipenem R	A27368	0.5	0.5	0.125	0.125	1	1	0.125
24	*S. haemolyticus*	A21638	0.125	0.125	0.06	0.06	0.25	0.125	0.06
25	*S. haemolyticus*/Homo MR	A27229	2	4	8	0.5	4	8	1

(~40 g in ~200 mL water; this removes excess iodine), and then brine. The organic phase is dried ($MgSO_4$), and evaporated to give 2,5-dichloro-4-iodophenol (34.59 g, 0.108 mol; 86% yield) as a pale pink/yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (s, 1H, ArH), 7.14 (s, 1H, ArH), 5.62 (br, 1H, OH).

B. 2,5-Dichloro-4-iodophenol (34.59 g, 0.108 mol) is placed into a 500-mL round-bottom flask equipped with septa, N_2 inlet, and a stir bar. The phenol is then dissolved in 130 mL DMF. DABCO (24.2 g, 0.216 mol) is added followed by dimethylthiocarbamyl chloride (21.6 g, 0.175 mol). The mixture is stirred at room temperature for ~1 h, then diluted with EtOAc (~400 mL) and poured into a separatory funnel containing ~300 mL of ice-water. The phases are separated, and the aqueous extracted twice with ~200 mL of EtOAc. The combined organic extracts are washed twice with water (~100 mL), and then brine. The organic phase is dried ($MgSO_4$), and evaporated to afford **3** as a dark oil. This material is dissolved in CH_2Cl_2 and dried again ($MgSO_4$). After evaporation a yellow solid (~35 g) is obtained. The compound was of sufficient purity to be used in the next reaction. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (s, 1H, ArH), 7.24 (s, 1H, ArH), 3.46 (s, 3H, CH_3), 3.37 (s, 3H, CH_3).

Synthesis of thiocarbamate 4. The crude material obtained above (~35 g) was heated neat under N_2 at 220 °C for 2 h. After cooling, the material was dissolved in CH_2Cl_2 and filtered through a plug of silica gel. The fractions containing the product are evaporated to afford 30.2 g of a brown solid. This material was chromatographed on silica (in portions) using a gradient elution starting with 1:1 CH_2Cl_2/hexane (material dissolved in a minimum amount of CH_2Cl_2 for column loading), and then ~70% CH_2Cl_2/hexane. Compound **4** is obtained as a yellow crystalline solid (13.0 g, 36.0 mmol; 33% yield from 2,5-dichlorophenol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (s, 1H, ArH), 7.62 (s, 1H, ArH), 3.10 (br s, 3H, CH_3), 3.00 (br s, 3H, CH_3).

Synthesis of ester 5. A. Thiocarbamate **4** (9.80 g, 0.026 mol) is dissolved in 40 mL EtOH and treated with 30 mL 3 N aqueous KOH. The mixture is heated to reflux with stirring under nitrogen for 3 h. The solution is allowed to cool and is then acidified with 3 N HCl until pH ~3. The mixture is extracted with CH_2Cl_2 (three times), and the combined organic phase washed with water and then brine. The extracts are dried ($MgSO_4$) and evaporated. The crude material is chromatographed on silica using 1:1 CH_2Cl_2/hexane. 2,5-Dichloro-4-iodothiophenol (6.43 g, 0.021 mol; 81% yield) is obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (s, 1H, ArH), 7.56 (s, 1H, ArH).

B. 2,5-Dichloro-4-iodothiophenol (6.43 g, 0.021 mol) is dissolved in 50 mL CH_2Cl_2 and triethylamine (2.52 g, 0.025 mol) is added. Methyl bromoacetate (3.82 g, 0.025 mol) is then added over 5 min. The resultant mixture is stirred at room temperature for 1.5 h, at which time ^{1}H NMR analysis indicated the reaction was complete. The mixture was diluted with CH_2Cl_2 (~200 mL) and was washed with water, 1 N HCl, water, and then brine. The organic layer was dried ($MgSO_4$) and evaporated. The crude material is chromatographed on silica using 70% CH_2Cl_2/hexane. Ester **5** is obtained as a white solid (7.20 g, 0.019 mol; 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (s, 1H, ArH), 7.42 (s, 1H, ArH), 3.75 (s, 3H, OCH_3), 3.68 (s, 2H, SCH_2).

Synthesis of diester 6 from ester 5. Ester **5** (9.00 g, 0.024 mol) is dissolved in 20 mL DMF. Triphenylphosphine (1.20 g, 0.005 mol), tributylamine (13.3 g, 0.071 mol), and *t*-butyl acrylate (17.3 g, 0.140 mol) are then added to the flask. Palladium acetate (0.95 g, 0.004 mol) is added, and the mixture heated under nitrogen to 80 °C for 4 h. The solvents are evaporated, and the crude material is partitioned between EtOAc and water. The organic phase is washed thrice with 1 N HCl, once with water, and then brine. The organic solution was dried ($MgSO_4$) and evaporated. Chromatography on silica gel using 1:1 CH_2Cl_2/hexane, followed by CH_2Cl_2 as eluant affords diester **6** (4.40 g, 0.012 mol; 50% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, 1H, J = 16 Hz, ArCH=C), 7.60 (s, 1H, ArH), 7.36 (s, 1H, ArH), 6.35 (d, 1H, J = 16 Hz, C=CHCO$_2$*t*-Bu), 3.77 (s, 3H, OCH_3), 3.72 (s, 2H, SCH_2), 1.51 (s, 9H, $C(CH_3)_3$).

Synthesis of ester 8. 2,4,5-Trichloroiodobenzene (25 g, 81.3 mmol) is dissolved in 80 mL DMF. *tert*-Butyl acrylate (48 mL, 328 mmol), tributylamine (58 mL, 243 mmol), triphenylphosphine (4.08 g, 15.5 mmol), and palladium acetate (3.23 g, 14.4 mmol) are added, and the mixture heated to 80 °C for 3 h. The solvents are evaporated and the residue is partitioned with EtOAc and water. The aqueous phase is extracted with EtOAc, and the combined organic phase is washed with brine, dried ($MgSO_4$), and evaporated. The dark red-brown oil is chromatographed on silica in a fritted Buchner funnel using vacuum filtration, with 30% CH_2Cl_2/hexane followed by 50% CH_2Cl_2/hexane as eluant. Acrylate **8** is obtained (18.7 g, 60.8 mmol; 75% yield) as a mauve solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, 1H, J = 16 Hz, ArCH=C), 7.67 (s, 1H, ArH), 7.51 (s, 1H, ArH), 6.34 (d, 1H, J = 16 Hz, C=CHCO$_2$*t*-Bu), 1.51 (s, 9H, $C(CH_3)_3$).

Synthesis of diester 6 from acrylate 8. Acrylate **8** (21.23 g, 69 mmol) is dissolved in 131 mL DMF. The sodium salt of methyl mercaptoacetate (17.7 g, crude: see note below) is added and the mixture stirred at room temperature for 1 h. The mixture is partitioned with EtOAc and water. The aqueous phase is extracted with EtOAc, and the combined organic phase is washed with brine, dried ($MgSO_4$), and evaporated. Chromatography on silica in a fritted Buchner funnel (vacuum filtration) using 50% CH_2Cl_2/hexane followed by 90% CH_2Cl_2/hexane as eluant affords diester **6** (19.6 g, 52.0 mmol; 75% yield). The spectral properties of **6** generated from **8** are identical to those determined for **6** obtained from **5**, and are reported above.

Note: The sodium salt of methyl mercaptoacetate is best made fresh before use. Approximately 30 mL of methyl mercaptoacetate is dissolved in ~250 mL THF. One equivalent of 5 N NaOH is added slowly in pipetfulls, and the mixture allowed to stir for 5 min. The solvents are removed in vacuo (including water) and the sticky solid is co-evaporated with ether (~200 mLs) and then dry THF (2×200 mLs). The solid is pumped dry for about 12 h under high vacuum until the flask is no longer cool due to evaporation. The freely mobile white solid is used as obtained. Generally it takes from 1.5 to 2 equivalents of this reagent by weight for the reaction to go to completion. Presumably the solid is a solvate/hydrate which is only about 50% pure anion by weight.

This solid is usually stored in the freezer and may remain stable anywhere from a few days to a few weeks (months?). If the color changes from white to faint beige it has usually decomposed. However, white batches that have been stored in the freezer for extended periods must also be used with caution!

The progress of this reaction is best monitored by ^{1}H NMR of aliquots. TLC is misleading at times. Use of an excess of thiolate anion results in further reaction to substitute another chlorine with a thioacetate chain.

Synthesis of acid 9. Diester **6** (4.40 g, 0.012 mol) is dissolved in 30 mL THF. To this solution is added 13 mL of 1 N NaOH, and the mixture is allowed to stir at room temperature for 1.5 h. At this time ^{1}H NMR analysis of an aliquot indicates that the reaction is complete. The THF is removed under vacuum, and the concentrate is diluted with water and extracted with EtOAc. The aqueous layer is then acidified with 1 N HCl to pH 4, and then extracted with CH_2Cl_2. The organic phase is washed with brine, dried ($MgSO_4$), and then evaporated. Acid **9** is obtained as a tan solid (3.80 g, 0.011 mol; 92% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, 1H, J = 16 Hz, ArCH=C), 7.56 (s, 1H, ArH), 7.33 (s, 1H, ArH), 6.29 (d, 1H, J = 16 Hz, C=CHCO$_2$*t*-Bu), 3.72 (s, 2H, SCH_2), 1.51 (s, 9H, $C(CH_3)_3$).

Note: This reaction was later found to be complete in less than 30 min.

Synthesis of chloromethyl cephem diester 11. Cephem amine **10** (15.04 g, 0.035 mol) is suspended under a nitrogen atmosphere in 65 mL THF. A solution of DCC in methylene chloride (1 M, 36.2 mL, 0.036 mol) is added, and the mixture allowed to stir for 5 min. Acid **9** (13.15 g, 0.035 mol) is added and the mixture is stirred for 1.5 h. Ether (~30 mL) is added, and the solids (mostly DCU) are filtered off. The red-colored filtrate is evaporated to ~25–30 mL and ether and pentane are added to precipitate the cephem product. The solid cephem is collected, washed with ether, and dried under vacuum to afford diester **11** (14.2 g, 0.019 mol; 54% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (d, 1H, J = 16 Hz, ArCH=C), 7.45–7.20 (m, 12H, ArH), 6.98 (s, 1H, Ph_2CH), 6.35 (d, 1H, J = 16 Hz, C=CHCO$_2$*t*-Bu), 5.82 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 4.97 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.37 (m, 2H, CH_2Cl), 3.76 (d, 1H, J = 16 Hz, $ArSCH_2$), 3.55 (d, 1H, J = 16 Hz, $ArSCH_2$), 3.40 (d, 1H, J = 16 Hz, $RSCH_2R$), 1.54 (s, 9H, $C(CH_3)_3$).

Synthesis of chloromethyl diacid cephem 12. Diester **11** (0.760 g, 1.00 mmol) is dissolved in 4 mL CH_2Cl_2 and 0.8 mL anisole. Trifluoroacetic acid (2 mL) is added, and the mixture is stirred for 4 h. The solvents are evaporated, and the residue triturated with CH_2Cl_2/ether. The solid is collected, washed with EtOAc and then dried under vacuum. Diacid **12** is obtained (0.420 g,

0.780 mmol; 78% yield) as a light yellow solid. ^{1}H NMR (300 MHz, DMSO): δ 9.30 (d, 1H, J = 8 Hz, RC(O)NH), 8.07 (s, 1H, ArH), 7.72 (d, 1H, J = 16 Hz, ArCH=C), 7.54 (s, 1H, ArH), 6.68 (d, 1H, J = 16 Hz, C=CHCO$_2$H), 5.71 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.13 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.55 (m, 2H, CH_2Cl), 3.97 (m, 2H, ArSCH$_2$), 3.70 (d, 1H, J = 16 Hz, RSCH$_2$R), 3.53 (d, 1H, J = 16 Hz, RSCH$_2$R).

Synthesis of thiopyridone 13. Pyran-4-thione (3.63 g, 32.4 mmol) is dissolved in a 2 M solution of methylamine in methanol (19.4 mL, 38.9 mmol), and the mixture stirred for 3 h at room temperature. The mixture is concentrated, and then chromatographed on silica using methanol/CH_2Cl_2 as eluants. ^{1}H NMR (300 MHz, DMSO): δ 7.55 (d, 2H, J = 8 Hz, C=CH), 7.16 (d, 2H, J = 8 Hz, C=CH), 3.71 (s, 3H, CH_3).

Synthesis of cephem 14. Cephem diacid **12** (5.50 g, 10.2 mmol) is dissolved in ~75 mL methanol. Thiopyridone **13** (1.28 g, 10.2 mmol) is added, and the mixture allowed to stir for ~1 h. The mixture is concentrated, and the residue is carefully treated with dilute (~0.5 N) NaOH until the pH reaches ~8.5. The solution is then chromatographed on reverse-phase C-18 silica gel, and the fractions containing the product are lyophillized. Cephem **14** (4.08 g, 6.15 mmol) is obtained as the mono sodium salt, zwitterion (a light tan lyophillate). ^{1}H NMR (300 MHz, 1:1 CD_3OD/DMSO-d_6): δ 8.44 (d, 2H, J = 7 Hz, C=CH), 8.00 (d, 2H, J = 7 Hz, C=CH), 7.73 (s, 1H, ArH), 7.43 (s, 1H, ArH), 7.20 (d, 1H, J = 16 Hz, ArCH=C), 6.39 (d, 1H, J = 16 Hz, C=CHCO$_2$), 5.39 (d, 1H, J = 5 Hz, $R_1R_2CHNR_3$), 4.83 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.40 (s, 2H, SCH$_2$), 4.10 (s, 3H, CH_3), 3.83 (m, 2H, SCH$_2$), 3.45 (d, 1H, J = 16 Hz, SCH), 3.24 (d, 1H, J = 16 Hz, SCH). Anal. ($C_{25}H_{20}Cl_2N_3Na_1O_6S_3$·2.6$H_2O$) C, H.

Synthesis of acid 22. Diester **6** (7.00 g, 0.019 mol) is suspended in 40 mL CH_2Cl_2. Anisole (1 mL) is added, followed by 15 mL of trifluoroacetic acid. The mixture is stirred for 1 h at room temperature. The solvents are concentrated to ~15 mL, and excess ether is added to precipitate a white solid. The solid is collected, washed with ether, and dried under vacuum to yield acid **22** (4.85 g, 0.015 mol; 79% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.08 (s, 1H, ArH), 7.71 (d, 1H, J = 16 Hz, ArCH=C), 7.43 (s, 1H, ArH), 6.69 (d, 1H, J = 16 Hz, C=CHCO$_2$), 4.19 (s, 2H, SCH$_2$), 3.66 (s, 3H, OCH$_3$).

Synthesis of hydroxysuccinimide ester 23. Acid **22** (8.75 g, 0.027 mol) is suspended in 55 mL THF under an atmosphere of nitrogen. Dicyclohexylcarbodiimide (1 M in CH_2Cl_2, 28.7 mL, 0.029 mol) is added, followed by *N*-hydroxysuccinimide (3.14 g, 0.027 mol). The reaction is allowed to stir for 3 h at room temperature. The mixture is diluted with ~30 mL acetone, filtered to remove dicyclohexylurea, and concentrated to ~25 mL. A solid forms which is filtered off, and the filtrate is evaporated to dryness. Crude **23** is obtained as a white solid (12.3 g) which is of sufficient purity for use in subsequent reactions. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.27 (s, 1H, ArH), 8.01 (d, 1H, J = 16 Hz, ArCH=C), 7.50 (s, 1H, ArH), 7.17 (d, 1H, J = 16 Hz, C=CHCO$_2$), 4.23 (s, 2H, SCH$_2$), 3.68 (s, 3H, OCH$_3$), 2.84 (m, 4H, CH_2CH_2).

Synthesis of diester 24. *tert*-Butylglycine hydrochloride (5.19 g, 0.031 mol) is suspended under a nitrogen atmosphere in 60 mL dry DMF. *N*-Methylmorpholine (3.90 mL, 0.036 mol) is added, and then the mixture is cooled to 0 °C. Crude hydroxysuccinimide ester **23** (12.3 g) is added, and the mixture allowed to stir for 10 min at 0 °C. The cooling bath is removed and the reaction is stirred for 1 h. The mixture is concentrated, and the residue dissolved in ethyl acetate and placed in a separatory funnel. The solution is washed with 0.4 N aqueous HCl, 0.1 N aqueous $NaHCO_3$, water and then brine. The organic phase is dried ($MgSO_4$) and evaporated to afford clean diester **24** as a light yellow solid (10.4 g, 0.024 mol; 89% yield from acid **22**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.42 (t, 1H, J = 8 Hz, NH), 7.81 (s, 1H, ArH), 7.59 (d, 1H, J = 16 Hz, ArCH=C), 7.47 (s, 1H, ArH), 6.80 (d, 1H, J = 16 Hz, C=CHCONH), 4.20 (s, 2H, SCH$_2$), 3.85 (d, 2H, J = 8 Hz, NCH_2CO_2), 3.67 (s, 3H, OCH$_3$), 1.41 (s, 9H, C(CH$_3$)$_3$).

Synthesis of acid 24b. Diester **24** (0.600 g, 1.40 mmol) is dissolved in 5 mL THF. Aqueous 1 N NaOH (1.40 mL, 1.40 mmol) is added and the mixture is allowed to stir at room temperature for 1 h. The THF is evaporated, and the residue taken up in 20 mL water. The solution is acidified to pH = 3 with 1 N HCl, and the mixture is partitioned with EtOAc and water. The organic phase is washed with brine, dried ($MgSO_4$), and evaporated. Pure acid **24b** is obtained (0.470 g, 1.12 mmol; 80% yield). ^{1}H NMR (300 MHz, DMSO): δ 8.45 (t, 1H, J = 7 Hz, NH), 7.83 (s, 1H, ArH), 7.59 (d, 1H, J = 17 Hz, C=CH), 7.43 (s, 1H, ArH), 6.80 (d, 1H, J = 17 Hz, C=CH), 4.08 (s, 2H, SCH$_2$), 3.85 (d, 2H, J = 7 Hz, NCH_2), 1.43 (s, 9H, C(CH$_3$)$_3$).

Synthesis of cephem 25. Cephem diester **11** (0.609 g, 0.802 mmol) is dissolved in 8 mL DMF. Sodium iodide (0.361 g, 2.40 mmol) is added, followed by 1-(2-hydroxy-ethyl)-1H-pyridine-4-thione (0.155 g, 1.00 mmol), and the mixture is allowed to stir for 3 h at room temperature. The DMF is evaporated, and the residue is triturated with CH_2Cl_2 and ether to afford the C-3 alkylated cephem as a solid (0.503 g, 0.557 mmol). The protected cephem is dissolved in 2 mL CH_2Cl_2 and 0.5 mL anisole, and 1.5 mL of TFA is added. The mixture is stirred 4 h at room temperature, and is then evaporated to dryness. The residue is redissolved in methanol and triturated with ether. The solid obtained is then stirred in ethyl acetate for 1 h, and then collected by filtration and dried over P_2O_5. Cephem **25** is obtained as a light tan solid (0.206 g, 0.268 mmol). ^{1}H NMR (300 MHz, DMSO; partial): δ 9.24 (d, 1H, J = 8 Hz, C(O)NH), 8.69 (d, 2H, J = 8 Hz, C=CH), 8.09 (s, 1H, ArH), 8.01 (d, 2H, J = 8 Hz, C=CH), 7.73 (d, 1H, J = 16 Hz, ArCH=C), 7.56 (s, 1H, ArH), 6.71 (d, 1H, J = 16 Hz, C=CHCO$_2$H), 5.71 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.16 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.55–4.44 (m, 2H), 4.43–4.36 (m, 2H), 3.99 (s, 2H,

$ArSCH_2$). MS (ESI) *m/e* 656 $(M+H)^+$. Anal. ($C_{26}H_{24}Cl_2N_3O_7S_3 \cdot C_2F_3O_2^-$) C, H, N.

Synthesis of cephem 32. Cephem amine **10** (6.22 g, 15.0 mmol) is suspended in 30 mL THF. DCC (1M in CH_2Cl_2; 15 mL, 15.0 mmol) is added, followed by acid **24b** (6.30 g, 15.0 mmol), and the mixture is allowed to stir for 1 h at room temperature. THF (~1 L) is added, and stirring is continued for another hour. The solids remaining are filtered off (dicyclohexylurea) and the filtrate is treated with ether to precipitate 7-(2-{4-[2-(*tert*-butoxycarbonylmethyl-carbamoyl)-vinyl]-2,5-dichlorophenylsulfanyl}-acetylamino)-3-chloromethyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid benzhydryl ester as a white solid (10.0 g, 12.2 mmol; 81% yield). ^{1}H NMR (300 MHz, DMSO): δ 9.31 (d, 1H, $J=8$ Hz, C(O)NH), 8.42 (t, 1H, $J=7$ Hz, C(O)NH), 7.81 (s, 1H, ArH), 7.60 (d, 1H, $J=16$ Hz, ArCH=C), 7.57 (s, 1H, ArH), 7.52–7.24 (m, 10H, ArH), 6.96 (s, 1H, $CHPh_2$), 6.70 (d, 1H, $J=16$ Hz, $C=CHCO_2H$), 5.80 (dd, 1H, $J=5$, 8 Hz, $R_1R_2CHNR_3$), 5.20 (d, 1H, $J=5$ Hz, CH(CNR)(SR)), 4.41 (m, 2H, CH_2Cl), 3.97 (s, 2H, $ArSCH_2$), 3.84 (d, 2H, $J=7$ Hz, $NCH_2C(O)$), 3.73 (d, 1H, $J=16$ Hz, $RSCH_2R$), 3.55 (d, 1H, $J=16$ Hz, $RSCH_2R$), 1.39 (s, 9H, $C(CH_3)_3$). MS (ESI) *m/e* 814 $(M-H)^-$. Anal. ($C_{38}H_{36}Cl_3N_3O_7S_2$) C, H.

7-(2-{4-[2-(*tert*-Butoxycarbonylmethyl-carbamoyl)-vinyl]-2,5-dichloro-phenylsulfanyl}-acetylamino)-3-chloromethyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid benzhydryl ester as obtained above (7.20 g, 8.81 mmol) is suspended in 40 mL CH_2Cl_2, and anisole (2 mL) followed by TFA (15 mL) are added, and the mixture is allowed to stir 4 h at room temperature. The solvents are evaporated, and the residue triturated with ether and CH_2Cl_2 to afford an orange solid. The solid is collected and pumped dry to afford 7-(2-{4-[2-(carboxymethyl-carbamoyl)-vinyl]-2,5-dichlorophenylsulfanyl}-acetylamino)-3-chloromethyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (4.40 g, 7.40 mmol; 84% yield). [Note: An impurity, (3-{4-[(carboxymethyl-carbamoyl)-methylsulfanyl]-2,5-dichloro-phenyl}-acryloylamino)-acetic acid, was often present in an amount of ~3–10% after this TFA deprotection. The cephem diacid material obtained is suitable for further use since the impurity is removed by trituration of the products from the subsequent C-3 alkylation reactions with thiopyridones. For a discussion on how this impurity arises in the TFA deprotection see ref. 4k.] ^{1}H NMR (300 MHz, DMSO; partial): δ 9.30 (d, 1H, $J=8$ Hz, C(O)NH), 8.40 (t, 1H, $J=7$ Hz, C(O)NH), 7.81 (s, 1H, ArH), 7.58 (d, 1H, $J=16$ Hz, ArCH=C), 7.55 (s, 1H, ArH), 6.81 (d, 1H, $J=16$ Hz, $C=CHCO_2H$), 5.73 (dd, 1H, $J=5$, 8 Hz, $R_1R_2CHNR_3$), 5.14 (d, 1H, $J=5$ Hz, CH(CNR)(SR)), 4.55 (m, 2H, CH_2Cl), 3.97 (s, 2H, $ArSCH_2$), 3.89 (d, 2H, $J=7$ Hz, $NCH_2C(O)$). MS (ESI) *m/e* 588 $(M-H)^-$.

7-(2-{4-[2-(Carboxymethyl-carbamoyl)-vinyl]-2,5-dichlorophenylsulfanyl}-acetylamino)-3-chloromethyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid as obtained above (0.800 g, 1.34 mmol) is dissolved in 4 mL methanol and 1 mL DMF. 1-(2-Hydroxy-ethyl)-1H-pyridine-4-thione (0.208 g, 1.34 mmol) is added, and the mixture is allowed to stir for 35 min. The solvents were evaporated, and the residue triturated with CH_2Cl_2 and ether. The yellow solid is collected (0.805 g), and the pH is adjusted to neutral with aqueous $NaHCO_3$. The aqueous mixture is chromatographed on C-18 silica gel using water, then 5% CH_3CN/water and finally 15% CH_3CN/water as eluants. Cephem **32** is obtained as yellow crystals of the mono sodium salt, zwitterion (0.305 g, 0.421; 31% yield). ^{1}H NMR (300 MHz, DMSO-d_6/D_2O, 2:1; partial): δ 8.52 (d, 2H, $J=8$ Hz, C=CH), 8.10 (d, 2H, $J=8$ Hz, C=CH), 7.82 (s, 1H, ArH), 7.53 (d, 1H, $J=16$ Hz, ArCH=C), 7.51 (s, 1H, ArH), 6.87 (d, 1H, $J=16$ Hz, C=CHC(O)NH), 5.42 (d, 1H, $J=6$ Hz, $R_1R_2CHNR_3$), 4.89 (d, 1H, $J=6$ Hz, CH(CNR)(SR)), 4.50–4.42 (m, 5H), 3.51 (s, 2H, SCH_2), 3.48 (d, 2H, $J=17$ Hz, $RSCH_2R$), 3.28 (d, 2H, $J=17$ Hz, $RSCH_2R$). MS (ESI) *m/e* 711 $(M-H)^-$. Anal. ($C_{28}H_{25}Cl_2N_4Na_1O_8S_3 \cdot 2.7H_2O$) C, H.

Synthesis of cephem 41. A. Synthesis of [2-hydroxy-3-(4-thioxo-4H-pyridin-1-yl)-propyl]-carbamic acid *tert*-butyl ester. A mixture of pyran-4-thione (1.10 g, 10.0 mmol) and 1-(*N*-boc)amino-3-amino-2-propanol (1.90 g, 10.0 mmol) in 25 mL absolute ethanol is stirred for 18 h at room temperature. The solution is concentrated, and the residue is triturated with a small amount of diethyl ether and filtered to provide the title thiopyridone (0.850 g, 3.01 mmol; 30%) as a tan solid (more material is present in the filtrate). ^{1}H NMR (300 MHz, DMSO): δ 7.54 (d, 2H, $J=7$ Hz, C=CH), 7.14 (d, 2H, $J=7$ Hz, C=CH), 6.88 (br t, 1H, $J=6$ Hz, NH), 5.35 (d, 1H, $J=6$ Hz, OH), 4.06 (d, 1H, $J=13$ Hz, NCH), 3.82–3.65 (m, 2H), 2.96 (br dd, 2H, $J_1=J_2=6$ Hz, NCH_2), 1.39 (s, 9H, $C(CH_3)_3$).

B. Synthesis of 1-[2-hydroxy-3-amino-prop-1-yl]-4-[(6*R*)-*trans*-2-carboxy-8-oxo-7-[(2,5-dichloro-4-(2-carboxy-ethenyl)phenylthio)acetamido]-5-thia-1-azabicyclo[4.2.0]-oct-2-en-3-yl]methylthio]pyridinium bis-trifluoroacetate. Diacid **12** (0.780 g, 1.45 mmol) is dissolved in 3 mL methanol and 8 mL of CH_2Cl_2. The above thiopyridone (0.395 g, 1.45 mmol) is added and the reaction mixture is stirred at room temperature for 4 h. The reaction mixture is then stored at 4 °C overnight. The reaction mixture is then concentrated in vacuo and the crude product is precipitated with diethyl ether. The crude product is collected by filtration and is then stirred in EtOAc for 30 min. The product is collected by filtration and dried under vacuum to provide the intermediate BOC-protected cephem as a tan solid (0.690 g, 0.850 mmol; 59% yield of a mixture of two diastereomers). ^{1}H NMR (300 MHz, DMSO, partial): δ 9.31 (d, 1H, $J=8$ Hz), 8.67 (d, 2H, $J=7$ Hz), 8.07 (s, 1H), 7.98 (d, 2H, $J=7$ Hz), 7.72 (d, 1H, $J=16$ Hz), 7.54 (s, 1H), 5.70 (dd, 1H, $J=5$, 8 Hz), 5.14 (d, 1H, $J=5$ Hz), 4.59–4.45 (m, 2H), 4.40–4.32 (m, 2H), 4.05–3.95 (m, 2H), 1.38 (s, 9H).

The above BOC-protected cephem (0.605 g, 0.747 mmol) is suspended in 3 mL of CH_2Cl_2 then 1 mL of trifluoroacetic acid is added. The reaction mixture is stirred at room temperature for 2 h. The reaction mixture is concentrated in vacuo then the residue is dissolved in

CH_2Cl_2 and precipitated with diethyl ether. The solids are collected by filtration, stirred in EtOAc for 30 min then again collected by filtration and dried under vacuum in the presence of P_2O_5. Cephem **41** is obtained (0.410 g, 0.609 mol; 82% yield) as the bis-trifluoroacetate salt. ^{1}H NMR (300 MHz, DMSO, partial): δ 9.40–9.35 (m, 1H, RC(O)NH), 8.77–8.72 (m, 2H, SpyrH), 8.23–8.18 (m, 2H, SpyrH), 8.12 (s, 1H, ArH), 7.77 (d, 1H, $J=16$ Hz, ArCH=C), 7.62 (s, 1H, ArH), 6.75 (d, 1H, $J=16$ Hz, C=CHCO$_2$H), 5.68–5.61 (m, 1H, $R_1R_2CHNR_3$), 5.11 (d, 1H, $J=5$ Hz, CH(CNR)(SR)), 4.07–3.97 (m, 2H, ArSCH$_2$). MS (ESI) *m/e* 685 (M^+).

Synthesis of 1-[3-(2,6-dimethyl-4-thioxo-4H-pyridin-1-yl)-propyl]-2,3-dimethyl-3H-imidazol-1-ium; chloride salt. A. 3-Bromopropylamine hydrobromide (20.5 g, 93.6 mmol) and triphenylmethyl chloride (25.0 g, 103 mmol) are dissolved in 200 mL CH_2Cl_2. A solution of 25 g of triethylamine in 10 mL of CH_2Cl_2 is added dropwise, and the mixture is stirred for 2 h. The mixture is washed with water, 10% phosphoric acid, water, and then brine. The organic phase is dried ($MgSO_4$), and concentrated in vacuo. Upon concentration, a solid forms that is collected by filtration and washed with diethyl ether and hexane to provide 1-triphenylmethyl-3-bromopropylamine (30.0 g, 87.2 mmol; 93% yield).

B. 1-Triphenylmethyl-3-bromopropylamine as obtained above (6.87 g, 20.0 mmol) and 1,2-dimethylimidazole (2.2 g, 22.9 mmol) are dissolved in 80 mL DMF and heated at 80 °C for 3 h. The reaction mixture is concentrated in vacuo, and the residue is diluted with diethyl ether. A precipitate forms that is collected by filtration and washed with diethyl ether to provide 4.5 g of product. The filtrate is concentrated in vacuo, then treated with a small amount of diethyl ether to afford an additional 2.2 grams of product. The solids are combined to yield 1-(3-triphenylmethylamino)propyl-2,3-dimethylimidazolium bromide (6.70 g, 15.3 mmol; 77%).

C. 1-(3-Triphenylmethylamino)propyl-2,3-dimethylimidazolium bromide as obtained above (6.50 g, 14.8 mmol) is dissolved in 70 mL methanol and cooled to 0 °C. 3 N HCl (15 mL) is added, and the cooling bath was removed. The reaction mixture is stirred 30 min at ambient temperature, and then the organic solvents are evaporated. The aqueous phase is washed with diethyl ether (two times), and with CH_2Cl_2 (three times), and then concentrated. The 1-(3-amino)propyl-2,3-dimethylimidazolium dichloride obtained (2.30 g, 10.2 mmol; 69%) is used in subsequent reactions without further purification.

D. 1-(3-Amino)propyl-2,3-dimethylimidazolium dichloride as obtained above (2.30 g, 10.2 mmol) and 2,6-dimethylpyran-4-thione (1.6 g, 11.4 mmol) are dissolved in 30 mL absolute ethanol. 2 N NaOH (6.3 mL, 12.6 mmol) is added, and the reaction mixture is stirred at room temperature for 18 h. The reaction mixture is heated at 60 °C for 6 h. The mixture is concentrated, and acidified with 1 N HCl to pH ~2. The mixture is extracted with CH_2Cl_2 (three times), and the combined organic extracts are washed with water and then brine. The organic layer is dried ($MgSO_4$), and concentrated in vacuo. The residue is filtered through a short plug of silica gel by eluting with 5% methanol/CH_2Cl_2 as the solvent. The title thiopyridone was obtained as the chloride salt (1.70 g, 5.46 mmol; 54%). ^{1}H NMR (300 MHz, DMSO): δ 7.72 (s, 1H, imidazolium), 7.61 (s, 1H, imidazolium), 7.03 (s, 2H, 2×C=CH), 4.23 (t, 2H, $J=8$ Hz, NCH$_2$), 4.00–3.92 (m, 2H, NCH$_2$), 3.75 (s, 3H, NCH$_3$), 2.69 (s, 3H, CH$_3$), 2.37 (s, 6H, 2×CH$_3$), 2.18–2.02 (m, 2H, CH$_2$).

Synthesis of cephem 46. Cephem diacid **12** (0.550 g, 1.02 mmol) is dissolved in 3 mL DMF, and 1-[3-(2,6-dimethyl-4-thioxo-4H-pyridin-1-yl)-propyl]-2,3-dimethyl-3H-imidazol-1-ium; chloride salt (0.270 g, 0.877 mmol) is added. The mixture is allowed to stir for 20 min at room temperature, and then the solvents are evaporated. The residue is triturated with ether, and the solids that are collected are washed with ether, ethyl acetate and acetone. The material is then treated with 0.4 N aqueous NaOH until the pH is ~7.5, and the solution is chromatographed on reverse-phase C-18 silica. A gradient elution beginning with water, and ending with 20% CH$_3$CN/water, delivers bis-zwitterion cephem **46** (0.310 g, 0.399 mmol) as a light yellow lyophillate. ^{1}H NMR (300 MHz, DMSO; partial): δ 9.12 (d, 1H, $J=8$ Hz, RC(O)NH), 8.13 (s, 2H, SpyrH), 7.79 (s, 1H, imidazolium), 7.71 (s, 1H, ArH), 7.64 (s, 1H, imidazolium), 7.45 (s, 1H, ArH), 7.16 (d, 1H, $J=16$ Hz, ArCH=C), 6.39 (d, 1H, $J=16$ Hz, C=CHCO$_2$H), 5.36 (dd, $J=5$, 8 Hz, $R_1R_2CHNR_3$), 4.86 (d, 1H, $J=5$ Hz, CH(CNR)(SR)), 4.42–4.21 (m, 6H), 3.99–3.80 (m, 2H), 3.75 (s, 3H, CH$_3$), 2..70 (s, 6H, 2×CH$_3$), 2.63 (s, 3H, CH$_3$). MS (ESI) *m/e* 777 (M^+).

Synthesis of cephem 50. A. Synthesis of 2-hydroxy-4-(4-thioxo-4H-pyridin-1-yl)-benzoic acid, sodium salt. 3-Aminosalicylic acid (1.50 g, 9.87 mmol), pyran-4-thione (0.640 g, 5.71 mmol) and sodium bicarbonate (0.840 g, 10.0 mmol) are suspended in 20 mL absolute ethanol, and the mixture is refluxed for 6 h. The mixture is concentrated, and placed on a reverse-phase C-18 silica gel column. Elution with water and then acetonitrile/water gives the title thiopyridone as a red sodium salt. ^{1}H NMR (300 MHz, DMSO): δ 7.87 (d, 2H, $J=8$ Hz, C=CH), 7.80 (d, 1H, $J=8$ Hz, ArH), 7.21 (d, 2H, $J=8$ Hz, C=CH), 6.81 (d, 1H, $J=2$ Hz, ArH), 6.72 (dd, 1H, $J=2$, 8 Hz, ArH).

B. Cephem diacid **12** (0.430 g, 0.799 mmol) is dissolved in 2 mL DMF, and 2-hydroxy-4-(4-thioxo-4H-pyridin-1-yl)-benzoic acid, sodium salt (0.210 g, 0.781 mmol) is added and the mixture stirred at room temperature for 20 min. The crude product is precipitated from the solution with ether. The material obtained is treated with 0.5 N NaOH to ~pH 8, and chromatographed on a reverse-phase C-18 silica gel column. Elution with water and then 10% acetonitrile/water affords cephem **50** (0.131 g, 0.170 mmol) as a light yellow lyophillate. ^{1}H NMR (300 MHz, DMSO; partial): δ 9.05 (d, 1H, $J=8$ Hz, RC(O)NH), 8.87 (d, 2H, $J=7$ Hz, SpyrH), 8.34 (d, 2H, $J=7$ Hz, SpyrH), 7.82 (d, 1H, $J=7$

Hz, ArH), 7.46 (s, 1H, ArH), 7.09 (d, 1H, $J=16$ Hz, ArCH=C), 7.01 (d, 1H, $J=2$ Hz, ArH), 6.89 (dd, 1H, $J=2$, 7 Hz, ArH), 6.30 (d, 1H, $J=16$ Hz, C=$CHCO_2H$), 5.40 (dd, $J=5$, 8 Hz, $R_1R_2CHNR_3$), 4.92 (d, 1H, $J=5$ Hz, CH(CNR)(SR)), 4.66 (d, 1H, $J=13$ Hz, SCH_2), 4.45 (d, 1H, $J=13$ Hz, SCH_2), 3.96–3.75 (m, 2H), 3.50 (d, 1H, $J=17$ Hz, SCH_2), 3.36 (d, 1H, $J=17$ Hz, SCH_2). MS (ESI) m/e 748 $(M+H)^+$. Anal. ($C_{31}H_{21}Cl_2N_3Na_2O_9S_3\cdot 4.7H_2O$) C, H, N.

Synthesis of cephem 60. A. Synthesis of *N*,*N*′-di(*tert*-butoxycarbamyl)-*N*″-[2-(4-thioxo-4H-pyridin-1-yl)-ethyl]-guanidine. Pyran-4-thione (0.110 g, 0.982 mmol) and *N*,*N*′-di(*tert*-butoxycarbamyl)-*N*″-[2-aminoethyl]-guanidine (0.385 g, 1.28 mmol) are dissolved in 10 mL absolute ethanol and allowed to stir 18 h at room temperature. ^{1}H NMR analysis indicates the reaction is only ~50% complete. The mixture is heated to 80 °C for 4 h, at which time ^{1}H NMR analysis indicates no more product is forming, but the presence of decomposition products is increasing. The reaction is concentrated, and then chromatographed on silica using CH_3OH/CH_2Cl_2 as eluants to afford the title thiopyridone (0.110 g, 0.285 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.48 (t, 1H, $J=7$ Hz, CNH), 7.41 (d, 2H, $J=8$ Hz, C=CH),), 7.15 (d, 2H, $J=8$ Hz, C=CH), 4.06 (t, 2H, $J=7$ Hz, CH_2), 3.63 (dt, $J=7$, 7 Hz, CH_2), 1.47 (s, 9H, $C(CH_3)_3$), 1.46 (s, 9H, $C(CH_3)_3$).

B. Cephem diacid **12** (0.153 g, 0.285 mmol) is dissolved in 5 mL of 1:1 CH_3OH/CH_2Cl_2, and *N*,*N*′-di(*tert*-butoxycarbamyl)-*N*″-[2-(4-thioxo-4H-pyridin-1-yl)-ethyl]-guanidine (0.110 g, 0.285 mmol) is added. The mixture is stirred at room temperature for 1 h, and then concentrated. The residue is triturated with ether and ethyl acetate, and the solids collected are then stirred in ethyl acetate for ~30 min and then filtered. The material is suspended in 3 mL CH_2Cl_2, and 1 mL TFA is added. The mixture is stirred at rt for 3 h and then evaporated. The residue is suspended in some CCl_4, which is then evaporated. Ether is added to triturate the cephem, and this material is collected by filtration, redissolved in methanol, and then triturated again using ether. The solids are collected and washed with ethyl acetate to afford cephem **60** bis-trifluoroacetate (0.063 g, 0.068 mmol) as a yellow solid. ^{1}H NMR (300 MHz, DMSO): δ 8.69 (d, 2H, $J=8$ Hz, C=CH), 8.07 (s, 1H, ArH), 8.02 (d, 2H, $J=8$ Hz, C=CH), 7.72 (d, 1H, $J=16$ Hz, ArCH=C), 7.55 (s, 1H, ArH), 6.69 (d, 1H, $J=16$ Hz, C=$CHCO_2H$), 5.69 (dd, $J=5$, 8 Hz, $R_1R_2CHNR_3$), 5.14 (d, 1H, $J=5$ Hz, CH(CNR)(SR)), 4.56–4.46 (m, 2H), 4.40–4.31 (m, 2H), 3.97 (s, 2H, SCH_2). MS (ESI) m/e 697 (M^+).

Acknowledgements

We thank our colleagues in the Department of Microbiology in Wallingford, CT for the biological data reported in this work. We also thank the Department of Analytical Research in Wallingford, CT, for analytical data, and Mr. Robert Perrone of the Department of Pharmaceutics Research and Development in New Brunswick, NJ for solubility determinations in support of this work.

References and Notes

1. (a) Ayliffe, G. A. J. *Clinical Infectious Diseases* **1997**, *24*, S74. (b) Ehlert, K. *Curr. Pharm. Des.* **1999**, *5*, 45.
2. Rotschafer, J. C.; Petersen, M.; Hoang, A. T. D.; Wright, D. *J. Infect. Dis. Pharm.* **1998**, *3*, 29.
3. (a) Hiramatsu, K.; Hanaki, H.; Ino, T.; Yabuta, K.; Oguri, T.; Tenover, F. C. *J. Antimicrob. Chemother.* **1997**, *40*, 135. (b) Smith, T. L.; Pearson, M. L.; Wilcox, K. R.; Cruz, C.; Lancaster, M. V.; Robinson-Dunn, B.; Tenover, F. C.; Zervos, M. J.; Band, J. D.; White, E.; Jarvis, W. R. *New Eng. J. Med.* **1999**, *340*, 493.
4. (a) For some recent publications concerning the development of anti-MRSA cephems see: Hecker, S. J.; Glinka, T. W.; Cho, A.; Zhang, Z. J.; Price, M. E.; Chamberland, S.; Griffith, D.; Lee, V. J. *J. Antibiot.* **2000**, *53*, 1272. (b) Glinka, T. W.; Cho, A.; Zhang, Z. J.; Ludwikow, M.; Griffith, D.; Huie, K.; Hecker, S. J.; Dudley, M. N.; Lee, V. J.; Chamberland, S. *J. Antibiot.* **2000**, *53*, 1045. (c) Ishikawa, T.; Iizawa, Y.; Okonogi, K.; Miyake, A. *J. Antibiot.* **2000**, *53*, 1053. (d) Ishikawa, T.; Kamiyama, K.; Matsunaga, N.; Tawada, H.; Iizawa, Y.; Okonogi, K.; Miyake, A. *J. Antibiot.* **2000**, *53*, 1071. (e) Tsushima, M.; Iwamatsu, K.; Umemura, E.; Kudo, T.; Sato, Y.; Shiokawa, S.; Takizawa, H.; Kano, Y.; Kobayashi, K.; Ida, T.; Tamura, A.; Atsumi, K. *Bioorg. Med. Chem.* **2000**, *8*, 2781. (f) Pae, A. N.; Lee, J. E.; Kim, B. H.; Cha, J. H.; Kim, H. Y.; Cho, Y. S.; Choi, K. I.; Koh, H. Y.; Lee, E.; Kim, J. H. *Tetrahedron* **2000**, *56*, 5657. (g) Yamazaki, H.; Tsuchida, Y.; Satoh, H.; Kawashima, S.; Hanaki, H.; Hiramatsu, K. *J. Antibiot.* **2000**, *53*, 546. (h) Yamazaki, H.; Tsuchida, Y.; Satoh, H.; Kawashima, S.; Hanaki, H.; Hiramatsu, K. *J. Antibiot.* **2000**, *53*, 551. (i) For prior publications from the laboratories of Bristol-Myers Squibb see: Springer, D. M.; Luh, B.-Y.; Bronson, J. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 797. (j) D'Andrea, S. V.; Bonner, D.; Bronson, J. J.; Clark, J.; Denbleyker, K.; Fung-Tomc, J.; Hoeft, S. E.; Hudyma, T. W.; Matiskella, J. D.; Miller, R. F.; Misco, P.F.; Pucci, M.; Sterzycki, R.; Tsai, Y.; Ueda, Y.; Wichtowski, J. A.; Singh, J.; Kissick, T. P.; North, J. T.; Pullockaran, A.; Humora, M.; Boyhan, B.; Vu, T.; Fritz, A.; Heikes, J.; Fox, R.; Godfrey, J. D; Perrone, R.; Kaplan, M.; Kronenthal, D.; Mueller, R. H. *Tetrahedron* **2000**, *56*, 5687. (k) Singh, J.; Kim, O. K.; Kissick, T. P.; Natalie, K. J.; Zhang, B.; Crispino, G. A.; Springer, D. M.; Wichtowski, J. A.; Zhang, Y.; Goodrich, J.; Ueda, Y.; Luh, B. Y.; Burke, B. D.; Brown, M.; Dutka, A. P.; Zheng, B.; Hsieh, D.-M.; Humora, M. J.; North, J. T.; Pullockaran, A. J.; Livshits, J.; Swaminathan, S.; Gao, Z.; Schierling, P.; Ermann, P.; Perrone, R. K.; Lai, M. C.; Gougoutas, J. Z.; DiMarco, J. D.; Bronson, J. J.; Heikes, J. E.; Grosso, J. A.; Kronenthal, D. R.; Denzel, T. W.; Mueller, R. H. *Org. Process Res. Dev.* **2000**, *4*, 488. (l) Kim, O. K.; Hudyma, T. W.; Matiskella, J. D.; Ueda, Y.; Bronson, J. J.; Mansuri, M. M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2753. (m) Kim, O. K.; Ueda, Y.; Mansuri, M. M.; Russell, J. W.; Bidwell, V. W. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1945.
5. A group from Eli Lilly found that in a series of C-3 acetate cephems, a derivative bearing a 2,5-dichlorothiophenyl acetamide group at C-7 was extremely potent against staphylococcus species. The optimal halogen substitution to maximize anti-staphylococcus activity on the thiophenyl ring was the 2,5-dichloro pattern. In largely unpublished observations, our group at Bristol-Myers Squibb has found this SAR trend at C-7 to be quite general for anti-MRSA activity irrespective of the nature of the C-3 group. For the original work by Lilly, see: Huffman, G. W. US Patent 3,907,784, 1975. *Chem. Abstr.* **1975**, *84*, 17395.

6. Kim, C. U.; Misco, P. F.; Wichtowski, J. A.; Ueda, Y.; Hudyma, T. W.; Matiskella, J. D.; D'Andrea, S. V.; Hoeft, S. E.; Miller, R. F. US Patent 5,567,698, 1996. *Chem. Abstr.* **1996**, *126*, 7903.
7. Compound **1**, given at a dose of ~200 mg/kg, resulted in death to all three mice tested. Compound **2**, given at a dose of ~300 mg/kg, was safe to all three mice tested. See the Experimental details of the acute toxicity assay.
8. For solubility determinations, a known quantity of test compound is dissolved in water, 0.9% saline, or any other buffer and the pH adjusted to the desired point by the addition of min amounts of 1 N NaOH. The solutions are stirred for 2 h, and then filtered through a 0.2-μ filter. The filtrate is then assayed by HPLC and the concentration of dissolved compound determined by comparison to a standard curve.
9. (a) Kwart, H.; Evans, E. R. *J. Org. Chem.* **1966**, *31*, 410. (b) Newman, M. S.; Karnes, H. A. *J. Org. Chem.* **1966**, *31*, 3980. (c) Sebok, P.; Timar, T.; Eszenyi, T.; Patonay, T. *J. Org. Chem.* **1994**, *59*, 6318. (d) Robertson, D. W.; Beedle, E. E.; Krushinski, J. H.; Pollock, G. D.; Wilson, H.; Wyss, V. L.; Hayes, J. S. *J. Med. Chem.* **1985**, *28*, 717.
10. Chloromethyl cephem amine **10** was purchased from Otsuka Chemical Co., Ltd.
11. Unfortunately, compounds **48** and **49**, synthesized towards the end of our MRSA cephem program, remained unevaluated in vivo at the close of our work.
12. Compounds that were found to be inactive in vivo were not generally evaluated in mouse pharmacokinetic or pharmacodynamic studies. Thus, there remain many possible (but unevaluated) explanations for the lack of in vivo activity of many of our cephems. Depending on the nature of the pendant groups on the C-3 thiopyridinium ring, the compounds may have differential metabolic and distribution profiles. One possible common explanation could be that inactive compounds remain localized at the site of intramuscular injection limiting the exposure of the animal to the test drug. This could either be a consequence of low solubility, or poor absorption into the vascular system from the muscle tissue, or both.
13. (a) These compounds were synthesized from the known *t*-butyl ester of 7-aminocephalosporanic acid by acylation and then TFA deprotection, as described for other derivatives in the experimental section. For syntheses of *t*-butyl 7-aminocephalosporanate see: Blacklock, T. J.; Butcher, J. W.; Sohar, P.; Lamanec, T. R.; Grabowski, E. J. J. *J. Org. Chem.* **1989**, *54*, 3907. (b) Mangia, A.; Scandroglio, A. *Org. Prep. Proced. Int.* **1986**, *18*, 13. For reference purposes, the C-3 acetate derivative with a 2,5-dichlorothiophenyl group at C-7 (no acrylic acid substituent) had an MIC of 2 μg/mL versus MRSA A27223. Thus, with this C-3 group, the inclusion of the acid substituent at C-7 (compare with compound **38**) caused no loss of in vitro potency. Usually, a 2-fold loss of in vitro potency is observed by incuding an acid at C-7, while in general an acid placed at C-3 results in a 4-fold loss of activity.
14. These compounds were generally prepared in methanol using the requisite chloromethyl cephem diacid, and the sodium thiolate of the C-3 side-chain group.