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Anti-MRSA Cephems. Part 3: Additional C-7 Acid Derivatives

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

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

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Abstract—Twenty-seven novel cephalosporin derivatives with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) are described. The compounds contain novel acid moieties at C-7 that were synthesized using nucleophilic aromatic substitution reactions and Stille couplings. The most interesting compound (**6**) displayed an MIC_{90} against MRSA of 3.7 μg/mL, and an average PD_{50} of 3.9 mg/kg.

Introduction

One facet of our program to develop an injectable cephalosporin with activity against methicillin-resistant *Staphylococcus aureus* that met with considerable success was the C-7 dichlorothiophenyl cinnamic acid series of cephems.[1,2] The C-7 acid group in these molecules often imparted to them an increased safety profile, while maintaining sufficient potency against the target organism. We wished to expand the C-7 acid series of cephems to include derivatives with alternate linkers between the pendant acid group and the dichlorothiophenyl moiety, with the goal of further improving upon the biological activity and safety profile of these types of compounds.

The overall synthetic strategy is depicted by the successful example shown in Scheme 1. The coupling of a suitably protected acid derivative such as **1** with cephem amine **2** lead to the diprotected chloromethyl cephem **3**. Cleavage of the protecting groups with TFA provided chloromethyl cephem diacid **4**, which was exposed to alkylation with thiopyridones such as **5**, to afford the final cephalosporin products as exemplified by **6**.

The strategy of Scheme 1 required the ready synthesis of a variety of substituted dichlorothiophenyl acetic acid derivatives, a challenge that was initially met with the tactics depicted by Scheme 2. Commercially available 2,4,5-trichlorothiophenol was alkylated with *t*-butyl bromoacetate to afford intermediate ester **7**. Oxidation of **7** with one equivalent of *m*-CPBA gave sulfoxide **8**. The sulfoxide was found to be sufficiently activated towards nucleophilic aromatic substitution with the sodium salt of methyl mercaptoacetate in DMF, providing differentially protected diester **9**. Selective hydrolysis of **9** lead to the sulfoxide-linked acid **10**, subsequently utilized for couplings to cephem amine **2**. Alternatively, sulfoxide **9** could be reduced using NaI and trifluoroacetic anhydride to yield the orthogonally protected sulfide-linked diester **11**. Again, selective hydrolysis of **11** set the stage for the coupling of acid **1** with amine **2**. The same sequence (cephem coupling after aromatic substitution and selective hydrolysis) was performed on sulfone-linked ester **13**, formed from **7** by oxidation with excess m-CPBA. The above chemistry lead to the series of C-7 thio-, sulfoxide-, and sulfone-linked cephems whose biological data are depicted in Table 1.

Further experimentation revealed that we could synthesize sulfonamide-linked C-7 acid derivatives using the chemistry shown in Scheme 3. Available 2,4,5-trichlorobenzene sulfonyl chloride (**15**) was coupled with *t*-butyl glycine to afford sulfonamide **16**. This sulfonamide was also found to be suitable for the aromatic substitution and hydrolysis protocol described above ultimately delivering acid **18** for coupling to cephem amine **2**.

*Corresponding author at current address: Rib-X Pharmaceuticals, 300 George Street, New Haven, CT 06511, USA. Fax: +1-203-624-5627; e-mail: springer@rib-x.com

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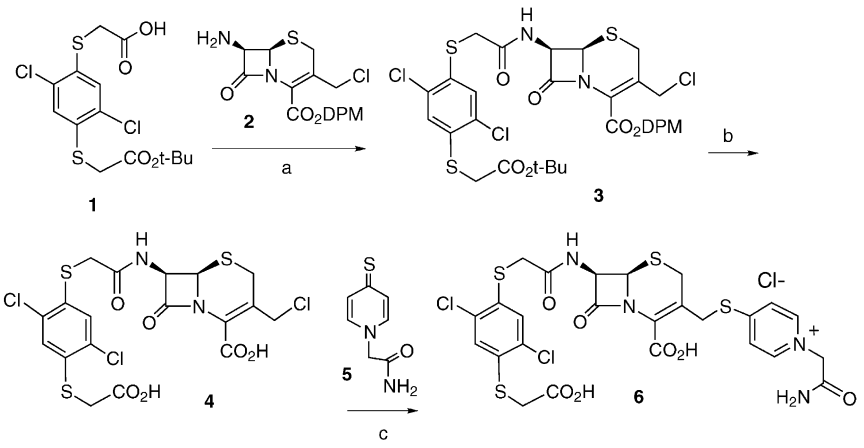
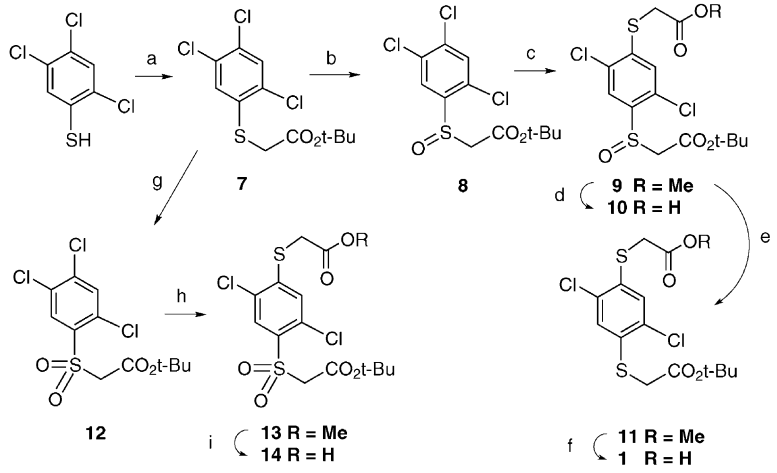
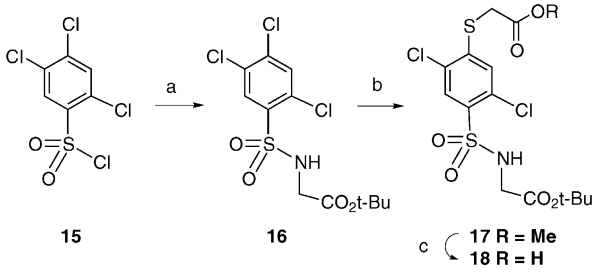


Scheme 1. Reaction conditions: (a) DCC, THF; (b) TFA, CH_2Cl_2, anisole, 61% from **1**; (c) DMF, 39%.



Scheme 2. Reaction conditions: (a) *t*-butyl bromoacetate, Et_3N, CH_2Cl_2, 94%; (b) *m*-CPBA, $CHCl_3$, 67%; (c) $NaSCH_2CO_2CH_3$, DMF, 75%; (d) 1 N NaOH, THF, 75%; (e) NaI, $(CF_3CO)_2O$, acetone, 87%; (f) 1 N NaOH, THF, 95%; (g) *m*-CPBA, $CHCl_3$, 93%; (h) $NaSCH_2CO_2CH_3$, DMF, 94%; (i) 1 N NaOH, THF, 92%.



Scheme 3. Reaction conditions: (a) *t*-butyl glycine, Et_3N, $CHCl_3$, 94%; (b) $NaSCH_2CO_2CH_3$, DMF, 74%; (c) 1 N NaOH, THF, 79%.

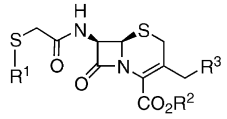
We decided to produce a few cephems that had the carboxylic acid directly attached to the C-7 dichlorothiophenyl ring. For this purpose, we found an earlier intermediate (**19**) to be an excellent starting material.[1] As Scheme 4 illustrates, trichloro cinnamate **19** was ozonized to afford 2,4,5-trichlorobenzaldehyde, which was easily oxidized under Jones conditions to 2,4,5-trichlorobenzoic acid. Protection of the acid with isobutylene, followed by aromatic substitution with the sodium salt of methyl mercaptoacetate and then hydrolysis, provided acid **23**. Selective cleavage of the *t*-butyl ester of diester **22** with TFA gave acid **24**, which was converted to acid **25** by DCC coupling with *t*-butyl glycine, followed by hydrolysis of the methyl ester.

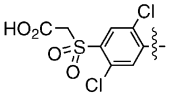
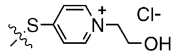
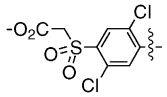
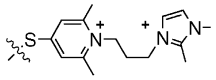
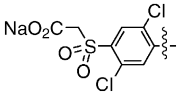
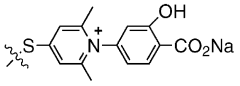
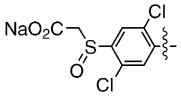
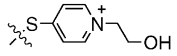
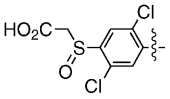
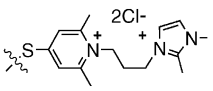
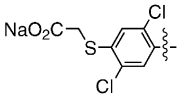
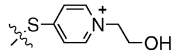
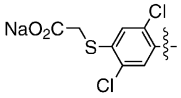
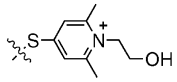
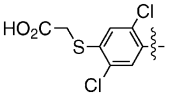
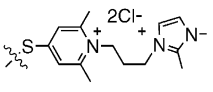
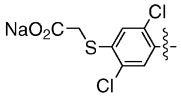
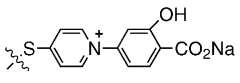
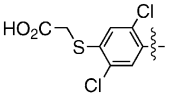
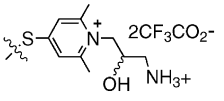
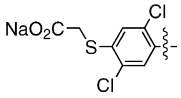
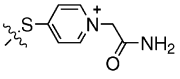
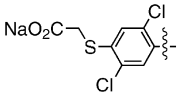
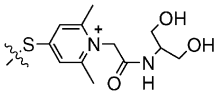
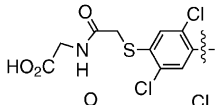
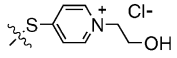
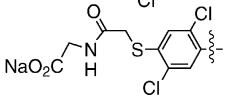
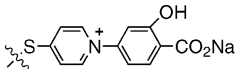
A final series of C-7 acid cephems was produced via the Stille coupling protocol shown in Scheme 5. Known aryl iodide **26** was coupled to stannanes **27** and **28** to afford diesters **29** and **30**.[1] Hydrolysis of the methyl esters of **29** and **30** gave acids **31** and **32**, again suitable for coupling to cephem amine **2**. The biological data for the cephem compounds synthesized using the chemistry of Schemes 3–5 is presented in Table 2.

Results and Discussion

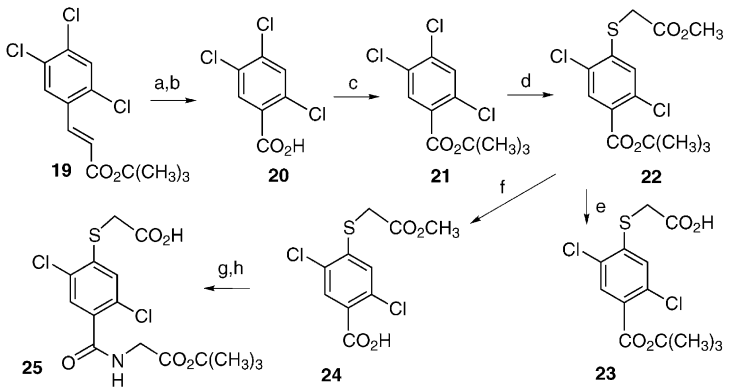
The compounds presented in Tables 1 and 2 all contain the 2,5-dichloro substitution pattern on the C-7 aryl ring since earlier work had repeatedly shown that this array produced compounds with the most potent antistaphylococcal activity.[1,3] A comparison of the data in Table 1 for the C-7 sulfone, sulfoxide and sulfide linked derivatives, containing identical C-3 groups, seems to indicate a slight (but general) preference for the sulfide-

Table 1.

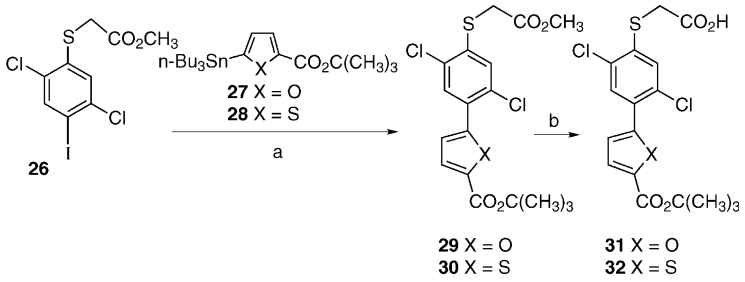


No.	R^1	R^2	R^3	MIC[a]	MIC_{90}[b]	PD_{50}[c]
33		H		2 (2)		9.5, 9.5
34		—		4 (4)		2.4, 5.4
35		—		4 (4)		>25, >25
36		—		4 (8)		—
37		H		4 (4)		5.4, 7.2
38		—		2 (2)	3.3	7.2, 9.5
39		—		2 (2)		21.8, >25
40		H		2 (4)	7.3	5.4, 5.4
41		—		2 (2)		12.5, >25
42		H		1 (4)		9.5, 12.5
6		—		2 (2)	3.7	2.4, 5.4
43		—		4 (8)		2.4, 5.4
44		H		4 (4)		21.8, 21.8
45		—		8 (8)		12.5, >25

[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.



Scheme 4. Reaction conditions: (a) O_3, $(CH_3)_2S$, CH_2Cl_2, 79%; (b) Jones reagent, 96%; (c) isobutylene, dioxane, H_2SO_4, 70%; (d) $NaSCH_2CO_2CH_3$, DMF, 80%; (e) 1 N NaOH, THF, 72%; (f) TFA, CH_2Cl_2, 92%; (g) $NH_2CH_2CO_2C(CH_3)_3$, DCC, THF, 49%; (h) 1 N NaOH, THF, 94%.



Scheme 5. Reaction conditions: (a) $PdCl_2(PPh_3)_2$, THF, reflux, X = O 78%, X = S 53%; (b) 1 N NaOH, MeOH, THF, X = O quant, X = S 98%.

linked compounds relative to the similarly active sulfones and sulfoxides. For example, in the C-3 hydroxyethyl pyridinium series of compounds, C-7 sulfide **38** seems to be a bit more potent than sulfone **33** and sulfoxide **36**. This effect is again evident when comparing sulfide **40** with sulfone **34** and sulfoxide **37**. The dimethyl substitution at C-3 of **39** does not negatively impact in vitro activity when compared to the unsubstituted derivative **38**, but renders **39** less potent in vivo than **38**. The most potent of the sulfur-linked derivatives shown in Table 1 is the C-3 acetamido pyridinium derivative **6**, a compound that has suitable in vitro and in vivo activity for further development. Unfortunately, the solubility and acute toxicity of **6**, synthesized towards the close of our program, remain undetermined since resources were deployed in support of an alternate cephem clinical candidate, BMS-247243.[4]

As Table 2 indicates, the sulfonamide-linked acids **46**–**48**, the directly attached acids **49**–**51**, and the amide-linked acid **52** were generally less active than the sulfur-linked derivatives of Table 1. The furan- and thiophene-linked derivatives **53**–**58** were potent in vitro (except for their generally high serum effect), but suffered from weak in vivo activity.

Conclusions

A variety of C-7 acid containing cephems have been produced that have different linkers between the dichloroaryl ring and the carboxylate. Of these compounds, the sulfide-linked C-7 acids were the most potent series of cephems presented in this report. The most interesting compound in vitro and in vivo is the C-7 sulfide-linked acid **6**. This derivative has the potency required of a candidate for further pre-clinical development.

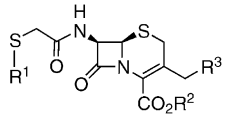
While these compounds are generally less active than our C-7 dichlorothiophenyl cinnamic acid series of cephems, they represent interesting alternate leads for development of a viable anti-MRSA cephalosporin. The chemistry presented here should prove useful to chemists developing new β-lactam derivatives for treatment of MRSA infections. The production of cephems comprised of these novel C-7 moieties and other C-3 groups may yield a successful clinical candidate. Further efforts in this regard by other researchers at Bristol-Myers Squibb await future disclosure.

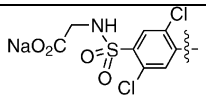
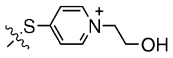
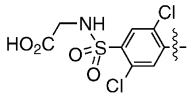
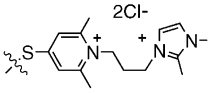
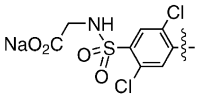
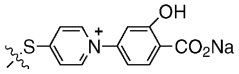
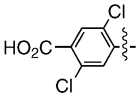
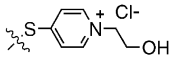
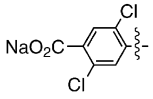
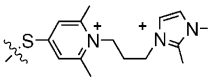
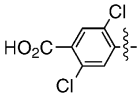
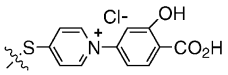
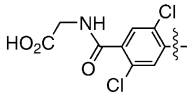
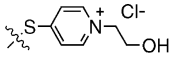
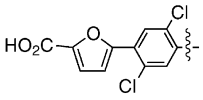
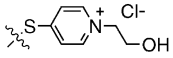
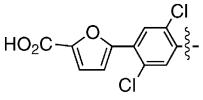
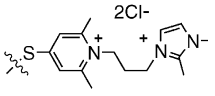
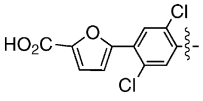
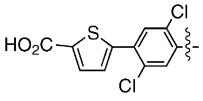
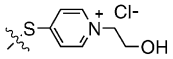
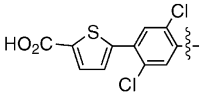
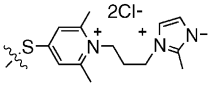
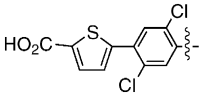
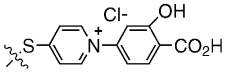
Experimental Procedures

Biology

Antibacterial MICs were determined in broth according to the standard conditions recommended by the National Committee for Clinical Laboratory Standards (NCCLS). 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 by MRSA A27233. The details of these assays are provided in ref 1.

Table 2.



No.	R^1	R^2	R^3	MIC[a]	MIC_{90}[b]	PD_{50}[c]
46		—		4 (4)		9.5, 9.5, >25
47		H		4 (8)		5.4, 5.4, 16.5
48		—		8 (8)		>25. >25
49		H		4 (4)		>25, >25
50		—		2 (2)	13.2	5.4, 7.2
51		H		4 (4)		16.5, >25
52		H		4 (4)		7.2, 7.2
53		H		2 (2)		12.5, 12.5
54		H		4 (8)		5.4, 9.5
55[5]		H		2 (16)		9.5, 9.5
56		H		2 (8)		16.5, >25
57		H		2 (32)		12.5, >25
58		H		2 (32)		21.8, >25

[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.

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.

Proton and carbon-13 nuclear magnetic resonance (^{1}H 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 ester 7. 2,4,5-Trichlorothiophenol (35.0 g, 0.166 mol) was dissolved in 500 mL CH_2Cl_2 and cooled to 0 °C. Triethylamine (22.0 g, 0.217 mol) was added to the solution, followed by addition of a solution of tertiary-butyl bromoacetate (35.1 g, 0.180 mol) in 100 mL CH_2Cl_2 over a period of 5 min. After stirring for 20 min at 0 °C, the ice-bath was removed, and stirring continued for another hour. The mixture was placed in a separatory funnel and washed with water (2×), 10% aqueous H_3PO_4, and then brine. The organic phase was dried ($MgSO_4$), and evaporated to afford a white solid, which is washed with hexane. Ester **7** (51 g, 0.156 mol; 94%) as obtained was of suitable purity for subsequent reactions. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.47 (s, 1H, ArH), 7.45 (s, 1H, ArH), 3.66 (s, 2H, SCH_2), 1.43 (s, 9H, $C(CH_3)_3$).

Synthesis of sulfoxide 8. Ester **7** (50.0 g, 0.153 mol) is dissolved in 500 mL chloroform and cooled to 0 °C. *m*-Chloroperoxybenzoic acid (50–60% from Aldrich, 48.0 g, 0.140–0.168 mol) is added in small portions over 30 min. The ice-bath is removed and stirring continued for 2.5 h at room temperature. The solids were removed by filtration, and the filtrate washed with dilute aqueous $NaHSO_3$, 5% aqueous Na_2CO_3, saturated aqueous $NaHCO_3$, and then brine. The organic phase is dried ($MgSO_4$), and evaporated. The crude material is chromatographed twice on silica gel using CH_2Cl_2 and then 3% methanol/CH_2Cl_2 to afford the sulfoxide **8** (35.0 g, 0.102 mol; 67% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (s, 1H, ArH), 7.53 (s, 1H, ArH), 4.05 (d, 1H, J = 14 Hz, $CH_2S(O)$), 3.70 (d, 1H, J = 14 Hz, $CH_2S(O)$), 1.43 (s, 9H, $C(CH_3)_3$).

Synthesis of diester 9. Sulfoxide **8** (35.0 g, 105 mmol) is dissolved in 300 mL DMF, and the sodium salt of methyl mercaptoacetate (17.92 g, 140 mmol) is added, and the mixture is stirred 20 min at room temperature.[6] Thin-layer chromatographic analysis indicates significant starting material **8** remains, and more of the sodium salt is added (8.27 g, 64.6 mmol). After 30 min, ^{1}H NMR analysis indicates ~10% **8** is present, and more of the salt is added (3.20 g, 25.0 mmol). After stirring another 30 min, the reaction mixture is filtered, and the bulk of the DMF evaporated. The residue is taken up in 350 mL ethyl acetate and washed with water. The organic layer is washed with brine, dried ($MgSO_4$) and evaporated. The crude material is chromatographed on silica via vacuum filtration using CH_2Cl_2 then 3% methanol/CH_2Cl_2 as eluants. Diester **9** (32.5 g, 78.7 mmol; 75% yield) is obtained as an ivory solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (s, 1H, ArH), 7.28 (s, 1H, ArH), 3.92 (d, 1H, J = 14 Hz, $CH_2S(O)$), 3.77 (s, 3H, OCH_3), 3.71 (s, 2H, SCH_2), 3.57 (d, 1H, J = 14 Hz, $CH_2S(O)$).

Synthesis of acid 10. Diester **9** (2.00 g, 4.95 mmol) is dissolved in 30 mL THF. Aqueous 1 N NaOH (5.7 mL, 5.70 mmol) is added and the mixture is allowed to stir at room temperature for 45 min. The THF is evaporated, and the residue is treated with 10% aqueous H_3PO_4 until the solution reaches a pH of 4. The solution is extracted with CH_2Cl_2, and the organic layer is washed with water and brine. The organic phase is dried ($MgSO_4$) and evaporated. Acid **10** is obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (s, 1H, ArH), 7.33 (s, 1H, ArH), 3.94 (d, 1H, J = 15 Hz, SOCH), 3.77 (s, 2H, SCH_2), 3.59 (d, 1H, J = 15 Hz, SOCH), 1.43 (s, 9H, $C(CH_3)_3$).

Synthesis of diester 11. Diester **9** (28.0 g, 67.8 mmol) was dissolved in 500 mL acetone. Sodium iodide (48.7 g, 325 mmol) was added, followed by trifluoroacetic anhydride (40.0 g, 191 mmol) over 5 min. After stirring at room temperature for 1 h, the reaction mixture was concentrated in vacuo. Methylene chloride is added to and evaporated from the residue twice. The residue is taken up in methylene chloride and washed with saturated aqueous $NaHSO_3$ solution (3×), water, and then brine. The organic phase is dried ($MgSO_4$), and evaporated. Chromatography of the residue on silica using CH_2Cl_2 affords the diester **11** (23.5 g, 59.2 mmol; 87% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, partial): δ 7.38 (s, 1H, ArH), 7.35 (s, 1H, ArH), 3.74 (s, 3H, OCH_3), 3.66 (s, 2H, SCH_2), 3.60 (s, 2H, SCH_2).

Synthesis of acid 1. Diester **11** (21.0 g, 52.9 mmol) is dissolved in 350 mL THF. Aqueous 1 N NaOH (60 mL, 60 mmol) is added and the mixture is allowed to stir at room temperature for 40 min. The THF is evaporated, and the residue is treated with 10% aqueous H_3PO_4 until the solution reaches pH of 4. The solution is extracted with CH_2Cl_2, and the organic layer is washed with water and brine. The organic phase is dried ($MgSO_4$) and evaporated. Acid **1** is obtained as a white solid (19.3 g, 50.4 mmol; 95% yield) of suitable purity for coupling to cephem amines. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (s, 1H, ArH), 7.37 (s, 1H, ArH), 3.68 (s, 2H, SCH_2), 3.58 (s, 2H, SCH_2), 1.42 (s, 9H, $C(CH_3)_3$).

Synthesis of sulfone 12. Ester **7** (7.00 g, 21.4 mmol) was dissolved in 40 mL chloroform and treated with *m*-chloroperoxybenzoic acid (~60% from Aldrich, 12.0 g, ~42 mmol). After stirring for 1 h at room tempera-

ture, the solids are removed by filtration, and the filtrate was washed with dilute aqueous $NaHSO_3$, 5% aqueous Na_2CO_3, saturated aqueous $NaHCO_3$, and then brine. The organic phase was dried ($MgSO_4$), and evaporated. Chromatography of the residue on silica using CH_2Cl_2 then 3% methanol/CH_2Cl_2 as eluant affords the sulfone **12** (7.00 g, 19.5 mmol; 93% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H, ArH), 7.67 (s, 1H, ArH), 4.32 (s, 2H, SCH_2), 1.33 (s, 9H, $C(CH_3)_3$).

Synthesis of diester 13. Using the procedure described above for the synthesis of diester **9**, sulfone **12** (7.20 g, 20.0 mmol) was converted to diester **13** (8.05 g, 18.8 mmol; 94% yield). The compound was isolated as a white solid after chromatography on silica using CH_2Cl_2 then 3% methanol/CH_2Cl_2 as eluant. ^{1}H NMR (300 MHz, $CDCl_3$, partial): δ 8.05 (s, 1H, ArH), 7.40 (s, 1H, ArH), 4.28 (s, 2H, SO_2CH_2), 3.78 (s, 3H, OCH_3), 3.77 (s, 2H, SCH_2).

Synthesis of acid 14. Diester **13** (2.00 g, 4.95 mmol) is dissolved in 30 mL THF. Aqueous 1 N NaOH (5.7 mL, 5.70 mmol) is added and the mixture is allowed to stir at room temperature for 45 min. The THF is evaporated, and the residue is treated with 10% aqueous H_3PO_4 until the solution reaches pH of 4. The solution is extracted with CH_2Cl_2, and the organic layer is washed with water and brine. The organic phase is dried ($MgSO_4$) and evaporated. Acid **14** is obtained as a white solid (1.80 g, 4.56 mmol; 92% yield) of suitable purity for coupling to cephem amines. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (s, 1H, ArH), 7.40 (s, 1H, ArH), 4.30 (s, 2H, SO_2CH_2), 3.80 (s, 2H, SCH_2), 1.31 (s, 9H, $C(CH_3)_3$).

Synthesis of cephem diester 3. Acid **1** (14.0 g, 35.4 mmol) and cephem amine **2** (16.9 g, 40.8 mmol; purchased from Otsuka Chemical Co. Ltd.) are dissolved in 600 mL dry THF. A solution of DCC (1 M in CH_2Cl_2, 50 mL, 50 mmol) is added, and the mixture is stirred for 4 h. The solids are filtered off, and the filtrate is concentrated. The residue is taken up in methylene chloride and washed with water, then brine. The organic layer is dried ($MgSO_4$) and evaporated. Diester **3** is obtained (30 g; contains a small amount of dicyclohexylurea) of suitable purity for subsequent reactions. ^{1}H NMR (300 MHz, DMSO): δ 9.28 (d, 1H, J = 8 Hz, NH), 7.56–7.23 (m, 12H, ArH), 6.96 (s, 1H, $OCHAr_2$), 5.77 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.19 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.48–4.33 (m, 2H), 3.95 (s, 2H, SCH_2), 3.89 (s, 2H, SCH_2), 3.71 (d, 1H, J = 17 Hz, SCH), 3.55 (d, 1H, J = 17 Hz, SCH), 1.37 (s, 9H, $(CH_3)_3$).

Synthesis of cephem diacid 4. Cephem diester **3** as obtained above (30-g) is dissolved in 140 mL methylene chloride and 10 mL anisole. Trifluoroacetic acid (25 mL) is added, and the mixture allowed to stir for 30 min. Ether is added to triturate the cephem, and the solids are collected by filtration. ^{1}H NMR analysis indicates the *t*-butyl ester is intact. The material is redissolved in 70 mL methylene chloride and treated with 24 mL TFA. After 2 h, 24 more mL of TFA is added. The mixture is stirred an additional h, and then evaporated to dryness. The residue is triturated with ether, and the solid is collected and washed with ether. Diacid **4** is obtained (12.0 g, 21.6 mmol; 61% from acid **1**) as a light brown solid, of suitable purity for coupling with thiopyridones. ^{1}H NMR (300 MHz, DMSO): δ 9.24 (d, 1H, J = 8 Hz, NH), 7.55 (s, 1H, ArH), 7.40 (s, 1H, ArH), 5.70 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.15 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.60–4.50 (m, 2H, CH_2Cl), 3.96 (s, 2H, SCH_2), 3.86 (s, 2H, SCH_2), 3.70 (d, 1H, J = 16 Hz, SCH), 3.51 (d, 1H, J = 16 Hz, SCH).

Synthesis of thiopyridone 5. Pyran-4-thione (0.336 g, 3.00 mmol) and glycineamide hydrochloride (0.300 g, 3.19 mmol) are dissolved in 10 mL ethanol. 1 N NaOH (3 mL, 3.00 mmol) is added, and the mixture is stirred for 18 h at room temperature. The ethanol is evaporated, and the residue is taken up in methylene chloride. The solution is washed with water, and then brine. The organic phase is dried ($MgSO_4$) and evaporated. The material is then triturated with ether and a little hexane to afford **5**. ^{1}H NMR (300 MHz, DMSO): δ 7.72 (br s, 1H, NH), 7.47 (d, 2H, J = 7 Hz, pyr), 7.41 (br s, 1H, NH), 7.12 (d, 2H, J = 7 Hz, pyr), 4.67 (s, 2H, CH_2).

Synthesis of cephem 6. Cephem diacid **4** as obtained above (0.350 g, 0.629 mmol) is dissolved in 2 mL DMF. Thiopyridone **5** (0.061 g, 0.363 mmol) is added, and after 10 min ~15% thiopyridone **5** is detected by ^{1}H NMR. More diacid **4** (0.070 g, 0.123 mmol) is added, and the mixture is stirred for another 10 min. The solution is concentrated, then added dropwise to ether. The precipitate is collected, and treated with 0.5 N NaOH until the pH is ~7.7. The solution is chromatographed on C-18 silica, with water and then 15% CH_3CN/water as eluants. Mono-sodium salt, zwitterion cephem **6** is obtained (0.100 g, 0.140 mmol; 39%) as a light yellow lyophillate. MS (ESI) *m/e* 687 $(M)^-$. Anal. ($C_{25}H_{21}Cl_2N_4Na_1O_7S_4 \cdot 3.8H_2O$) C, H, N. ^{1}H NMR (300 MHz, DMSO, partial): δ 9.07 (d, 1H, J = 8 Hz, NH), 8.55 (d, 2H, J = 7 Hz, pyr), 8.45 (d, 2H, J = 7 Hz, pyr), 8.03 (br s, 1H, NH), 7.65 (br s, 1H, NH), 7.45 (s, 1H, ArH), 7.34 (s, 1H, ArH), 5.39 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.18 (s, 2H), 4.92 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.76 (d, 1H, J = 14 Hz), 4.26 (d, 1H, J = 14 Hz), 3.84–3.70 (m, 2H).

Synthesis of sulfonamide 16. Tertiary-butyl glycine ester (2.62 g, 20.0 mmol) and triethylamine (2.50 g, 25.0 mmol) are dissolved in 20 mL chloroform. The solution is cooled with an ice-bath, and 2,4,5-trichlorobenzenesulfonyl chloride (5.60 g, 20.0 mmol) dissolved in 30 mL chloroform is added over 5 min. The cooling bath is removed and the mixture allowed to stir at room temperature for 1 h. The solution is washed with 10% aqueous H_3PO_4, water, and then brine. The organic phase is dried ($MgSO_4$), and evaporated to afford clean sulfonamide **16** (7.00 g, 18.8 mmol; 94% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10 (s, 1H, ArH), 7.63 (s, 1H, ArH), 3.72 (s, 2H, NCH_2CO_2), 1.33 (s, 9H, $C(CH_3)_3$).

Synthesis of diester 17. Using the procedure described previously for the synthesis of diester **9**, sulfonamide **16**

(3.70 g, 10.0 mmol) is converted to diester **17** (2.37 g, 7.40 mmol; 74% yield). The compound is isolated as a white solid after chromatography on silica using CH_2Cl_2 then 5% methanol/CH_2Cl_2. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (s, 1H, ArH), 7.36 (s, 1H, ArH), 5.65 (t, 1H, $J=5$ Hz, NH), 3.85 (s, 3H, OCH_3), 3.78 (s, 2H, SCH_2), 3.71 (d, 2H, $J=5$ Hz, NCH_2CO_2), 1.34 (s, 9H, $C(CH_3)_3$).

Synthesis of acid 18. Diester **17** (1.83 g, 4.24 mmol) is dissolved in 20 mL THF. Aqueous 1 N NaOH (15 mL, 15.0 mmol) is added and the mixture is allowed to stir at room temperature for 1 h. The reaction is concentrated and the aqueous solution is washed with diethyl ether. The aqueous layer is acidified to a pH of 4 with 1 N HCl, and the solution is extracted with CH_2Cl_2. The organic layer is washed with water and brine. The organic phase is dried ($MgSO_4$) and evaporated. Acid **18** is obtained as a white solid (1.40 g, 3.35 mmol; 79% yield) of suitable purity for coupling to cephem amines. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (s, 1H, ArH), 7.37 (s, 1H, ArH), 5.65 (t, 1H, $J=6$ Hz, NH), 3.80 (s, 2H, SCH_2), 3.71 (d, 2H, $J=6$ Hz, NCH_2), 1.33 (s, 9H, $C(CH_3)_3$).

Synthesis of acid 20. Ester **19**[1] (1.03 g, 3.35 mmol) is dissolved in 200 mL CH_2Cl_2 and 100 mL methanol. The mixture is cooled to −78 °C, and ozone is bubbled through the solution until it turns blue. The mixture is purged with oxygen, and then 1.3 mL of methyl sulfide is added. The mixture is allowed to warm to room temperature, and the solvents are evaporated. The crude residue is partitioned between diethyl ether and water. The diethyl ether layer is washed with water and brine, and then dried ($MgSO_4$). Concentration of the diethyl ether layer, followed by chromatography of the resulting residue on silica using 25% CH_2Cl_2/hexane as eluant, affords 2,4,5-trichlorobenzaldehyde (0.55 g, 2.63 mmol; 79% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.34 (s, 1H, CHO), 7.98 (s, 1H, ArH), 7.58 (s, 1H, ArH).

2,4,5-Trichlorobenzaldehyde (0.275 g, 1.31 mmol) obtained above is dissolved in 8 mL acetone. A slight excess of Jones Reagent is added, and the mixture stirred at room temperature for 1 h. Methanol (~6 mL) is added, and after 5 min the mixture is partitioned between methylene chloride and water. The aqueous phase is extracted with chloroform (2×), and the combined organic extracts are washed with water, then brine. The organic phase is dried ($MgSO_4$), and concentrated to afford pure 2,4,5-trichlorobenzoic acid **20** (0.285 g, 1.26 mmol; 96% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (s, 1H, ArH), 7.61 (s, 1H, ArH).

Synthesis of ester 21. Acid **20** (2.93 g, 13.0 mmol) placed in a Parr hydrogenation bottle (under an atmosphere of nitrogen) is dissolved in 55 mL of dioxane. The bottle is cooled to −78 °C, and 5 mL concd sulfuric acid is added cautiously, followed by ~50 mL liquid isobutylene (cooled to −78 °C). The bottle is sealed and agitated on a Parr shaker for approximately 19 h. The sealed bottled is vented, and the solution slowly added to a separatory funnel containing half-saturated aqueous $NaHCO_3$ and diethyl ether. The aqueous layer is extracted with diethyl ether, and the combined organic phase was dried ($MgSO_4$) and concentrated. Chromatography of the residue on silica using hexane, followed by 25% CH_2Cl_2/hexane, affords ester **10** as a pale yellow oil (2.56 g, 9.10 mmol; 70% yield) which solidifies overnight in the refrigerator to an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81 (s, 1H, ArH), 7.53 (s, 1H, ArH), 1.58 (s, 9H, $C(CH_3)_3$).

Synthesis of diester 22. Using the method described previously for the synthesis of diester **9**, ester **21** (2.5 g, 11.3 mmol) is converted to diester **22** (3.20 g, 9.12 mmol; 81% yield). Diester **22** was obtained as a white solid by chromatography on silica gel using 80% CH_2Cl_2/hexane. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (s, 1H, ArH), 7.30 (s, 1H, ArH), 3.75 (s, 3H, OCH_3), 3.72 (s, 2H, SCH_2), 1.58 (s, 9H, $C(CH_3)_3$).

Synthesis of acid 23. Diester **22** (1.60 g, 4.60 mmol) is dissolved in 12 mL THF. Aqueous 1 N NaOH (4.6 mL, 4.60 mmol) is added and the mixture is allowed to stir at room temperature for 1.5 h. The THF is evaporated, and the residue taken up in ~40 mL water. The aqueous layer is extracted with EtOAc (compound is in organic layer at this point!), and evaporated to yield a white solid. The material is re-dissolved in EtOAc, and acidified by shaking in a separatory funnel with ~0.5 N HCl. The organic layer is washed with brine, dried ($MgSO_4$), and evaporated. Pure acid **23** is obtained (1.12 g, 3.32 mmol; 72% yield). ^{1}H NMR (300 MHz, DMSO): δ 7.80 (s, 1H, ArH), 7.42 (s, 1H, ArH), 4.10 (s, 2H, CH_2), 1.52 (s, 9H, $C(CH_3)_3$).

Synthesis of acid 24. Diester **22** (1.60 g, 4.56 mmol) is dissolved in 5 mL CH_2Cl_2. Trifluoroacetic acid (2 mL) is added to this solution, and the mixture stirred for 4 h at room temperature. The reaction mixture is concentrated to provide **24** (1.24 g, 4.20 mmol; 92% yield) as a tan solid of sufficient purity for use in subsequent reactions. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.87 (s, 1H, ArH), 7.46 (s, 1H, ArH), 4.23 (s, 2H, SCH_2), 3.68 (s, 3H, OCH_3).

Synthesis of acid 25. Acid **24** (1.24 g, 4.20 mmol) is dissolved in 14 mL dry THF. Dicyclohexylcarbodiimide (0.866 g, 4.20 mmol) is added, followed by tertiary-butyl glycine ester (0.550 g, 4.20 mmol), and the mixture stirred at room temperature for 2 h. Diethyl ether is added to the flask, and the solids are removed by filtration. The filtrate is evaporated to yield 1.96 g of crude material. Chromatography of the crude material on silica using 80% CH_2Cl_2/hexane, followed by a second chromatography on silica using 20% EtOAc/hexane affords the *t*-butyl glycine amide of acid **24** (0.844 g, 2.07 mmol; 49% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (s, 1H, ArH), 7.31 (s, 1H, ArH), 4.12 (d, 2H, $J=6$ Hz, NCH_2CO_2), 3.77 (s, 3H, OCH_3), 3.72 (s, 2H, SCH_2), 1.48 (s, 9H, $C(CH_3)_3$).

The amide obtained above (0.844 g, 2.07 mmol) is dissolved in 7 mL THF. Aqueous 1 N NaOH (2.2 mL, 2.20 mmol) is added and the mixture is allowed to stir at

room temperature for 20 min. The THF is evaporated, and the residue taken up in 20 mL water. The solution is acidified to a pH of 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 **25** is obtained (0.770 g, 1.95 mmol; 94% yield). ^{1}H NMR (300 MHz, DMSO): δ 8.87 (t, 1H, $J=7$ Hz, NH), 7.50 (s, 1H, ArH), 7.40 (s, 1H, ArH), 4.07 (s, 2H, SCH_2), 3.86 (d, 2H, $J=7$ Hz, NCH_2), 1.43 (s, 9H, $C(CH_3)_3$).

Synthesis of stannane 27. Bis(tributyltin) (29.5 g, 50.9 mmol) is dissolved under a nitrogen atmosphere in 70 mL dry THF. The solution is cooled to −20 °C, and butyllithium (1.6 M in hexane, 31.2 mL, 49.9 mmol) is added dropwise over 20 min, maintaining the temperature of the bath at −20 °C. The solution is cooled to −50 °C, and then copper(I) bromide methylsulfide complex (5.10 g, 24.8 mmol) is added. The mixture is allowed to stir at −40 °C for 15 min, and is then cooled to −78 °C. 5-Bromofuroic acid *tert*-butyl ester (4.10 g, 16.6 mmol) dissolved in 15 mL THF is added, and the mixture allowed to stir for 3 h at −78 °C. The reaction mixture is poured into 1 L of diethyl ether and ∼300 mL half-saturated aqueous ammonium chloride solution. After stirring for 5 min the diethyl ether layer is decanted onto another ∼300 mL of half-saturated aqueous ammonium chloride solution. After 5 min the biphasic mixture is separated, and the organic phase is washed with brine, dried ($MgSO_4$) and evaporated. Chromatography on silica using hexane, then 25% CH_2Cl_2/hexane affords stannane **27** (5.05 g, 11.1 mmol; 67% yield) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.04 (d, 1H, $J=4$ Hz, HetArH), 6.56 (d, 1H, $J=4$ Hz, HetArH), 1.59–1.47 (m, 3H, $SnBu_3$), 1.37–1.24 (m, 9H, $SnBu_3$), 1.13–1.05 (m, 6H, $SnBu_3$), 0.89 (t, 9H, $J=6$ Hz, $SnBu_3$).

Synthesis of diester 29. Stannane **27** (1.50 g, 3.28 mmol) is dissolved in 8 mL dry THF. Aryl iodide **26**[1] (0.928 g, 2.46 mmol) is added, followed by bis(triphenylphosphine)-palladium(II) chloride (0.160 g, 0.228 mmol). The solution is heated to reflux for 6 h. The mixture is diluted with ∼15 mL THF, 4 mL saturated aqueous KF is added, and the mixture is stirred for 20 min. Diethyl ether is added, and the mixture is then filtered to remove insoluble tin solids. The biphasic filtrate is separated, and the aqueous layer is extracted with diethyl ether. The combined organic phases are washed with brine, dried ($MgSO_4$) and evaporated. During evaporation crystals began to form, and when only ∼5 mL of liquid remains it is decanted. The solids are washed with hexane and then pumped dry. Diester **29** (0.793 g, 1.91 mmol; 78% yield) is obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (br s, 1H, ArH), 7.40 (br s, 1H, ArH), 7.19 (d, 1H, $J=2$ Hz, HetArH), 7.13 (d, 1H, $J=2$ Hz, HetArH), 3.75 (s, 3H, OCH_3), 3.71 (s, 2H, SCH_2), 1.60 (s, 9H, $C(CH_3)_3$).

Synthesis of acid 31. Diester **29** (0.793 g, 1.91 mmol) is dissolved in 8.65 mL methanol and 10 mL THF. Aqueous 1 N NaOH (2.01 mL, 2.01 mmol) is added and the mixture is allowed to stir at room temperature for 20 min. The reaction mixture is partitioned with $CHCl_3$ and aqueous 0.5 N HCl. The aqueous phase is extracted with $CHCl_3$, and the combined organic phase is washed with brine, dried ($MgSO_4$), and evaporated. Acid **31** is obtained (0.771 g, 1.91 mmol; quantitative yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (s, 1H, ArH), 7.40 (s, 1H, ArH), 7.17 (d, 1H, $J=4$ Hz, furyl H), 7.12 (d, 1H, $J=4$ Hz, furyl H), 3.76 (s, 2H, CH_2), 1.56 (s, 9H, $C(CH_3)_3$).

Synthesis of stannane 28. Using the method described above for the synthesis of stannane **27**, 5-bromo-2-thiophenecarboxylic acid, tertiary-butyl ester (4.52 g, 17.2 mmol) is converted to stannane **28** (4.33 g, 9.16 mmol; 53% yield). The compound is isolated as a light yellow oil after chromatography on silica using 25% CH_2Cl_2/hexane. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, 1H, $J=3$ Hz, HetArH), 7.12 (d, 1H, $J=3$ Hz, HetArH), 1.60–0.80 (m, 27H, $SnBu_3$).

Synthesis of diester 30. Using the method described above for the synthesis of diester **29**, stannane **28** (1.70 g, 3.60 mmol) and aryl iodide **26** (1.03 g, 2.73 mmol) are converted to diester **30** (0.920 g, 2.13 mmol; 78% yield as a light yellow solid). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66 (d, 1H, $J=3$ Hz, HetArH), 7.55 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.27 (d, 1H, $J=3$ Hz, HetArH), 3.78 (s, 3H, OCH_3), 3.73 (s, 2H, SCH_2), 1.58 (s, 9H, $C(CH_3)_3$).

Synthesis of acid 32. Diester **30** (1.05 g, 2.43 mmol) was dissolved in 5 mL methanol and 5 mL THF. Aqueous 1 N NaOH (2.55 mL, 2.55 mmol) was added. To the nonhomogeneous mixture was added 8 mL of CH_2Cl_2 and the mixture was allowed to stir at room temperature for 20 min. The reaction mixture was partitioned with $CHCl_3$ and aqueous 0.5 N HCl. The aqueous phase was extracted with $CHCl_3$, and the combined organic phase was washed with brine, dried ($MgSO_4$), and evaporated. Acid **32** was obtained (1.05 g, 2.51 mmol; 98% yield) of suitable purity for coupling to cephem amines. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (d, 1H, $J=4$ Hz, thiophene H), 7.55 (s, 1H, ArH), 7.45 (s, 1H, ArH), 7.29 (d, 1H, $J=4$ Hz, thiophene H), 3.77 (s, 2H, CH_2), 1.59 (s, 9H, $C(CH_3)_3$).

Synthesis of cephem 34. Cephem **2** (2.08 g, 5.01 mmol) and acid **14** (1.80 g, 4.56 mmol) are dissolved in 60 mL THF, and DCC (1.10 g, 5.33 mmol) is added. The mixture is stirred for 4 h at room temperature, and then the solids are filtered off. The filtrate is treated with 10 mL of ether, and the solids filtered off again. The filtrate is evaporated to afford the expected cephem amide (3.40 g, 4.29 mmol; 94%), of suitable purity for subsequent reactions. ^{1}H NMR (300 MHz, DMSO): δ 9.39 (d, 1H, $J=8$ Hz, NH), 7.88 (s, 1H, ArH), 7.75 (s, 1H, ArH), 7.51–7.28 (m, 10H, ArH), 6.97 (s, 1H, $OCH(Ar)_2$), 5.80 (dd, 1H, $J=5$, 8 Hz, $R_1R_2CHNR_3$), 5.21 (d, 1H, $J=5$ Hz, CH(CNR)(SR)), 4.63 (s, 2H, SO_2CH_2), 4.47–4.39 (m, 2H, CH_2Cl), 4.09 (s, 2H, $ArSCH_2$), 3.73 (d, 1H, $J=17$ Hz, $RSCH_2R$), 3.57 (d, 1H, $J=17$ Hz, $RSCH_2R$), 1.22 (s, 9H, $C(CH_3)_3$).

The amide obtained above (3.39 g, 4.28 mmol) is dissolved in 60 mL methylene chloride and 3 mL anisole.

TFA (6 mL) is added and the mixture is stirred for 2 h. The mixture is concentrated, and the reaction is found to be incomplete. The material is redissolved in 60 mL methylene chloride and treated with TFA (8 mL) for another 1.5 h. The mixture is concentrated, and triturated with ether. The solids are collected and dissolved in DMF, then re-precipitated with ether. The solids are collected to afford the expected cephem diacid (1.60 g, 2.80 mmol; 65%) as a tan solid. ^{1}H NMR (300 MHz, DMSO): δ 9.38 (d, 1H, J = 8 Hz, NH), 7.90 (s, 1H, ArH), 7.74 (s, 1H, ArH), 5.71 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.16 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.63 (s, 2H, SO_2CH_2), 4.60–4.50 (m, 2H, CH_2Cl), 4.09 (s, 2H, $ArSCH_2$), 3.70 (d, 1H, J = 17 Hz, $RSCH_2R$), 3.52 (d, 1H, J = 17 Hz, $RSCH_2R$).

The above acid (0.350 g, 0.613 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[1] (0.180 g, 0.577 mmol) is added and the mixture allowed to stir for 30 min at room temperature. Ether is added to precipitate the crude product. The material is re-dissolved in DMF (~2 mL) and triturated with ether. The solids are collected and washed with ethyl acetate and acetone, and then pumped dry to afford crude cephem **34** as the dichloride salt. ^{1}H NMR (300 MHz, DMSO, partial): δ 9.35 (d, 1H, J = 8 Hz, NH), 7.88 (s, 1H, ArH), 7.81 (s, 2H, pyrH), 7.76 (d, 1H, J = 2 Hz, imidazolium), 7.68 (s, 1H, ArH), 7.66 (d, 1H, J = 2 Hz, imidazolium), 5.66 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.13 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.59 (s, 2H, SO_2CH_2), 3.76 (s, 3H, CH_3), 2.73 (s, 6H, 2 X CH_3), 2.64 (s, 3H, CH_3).

The above dichloride salt of **34** is treated with 0.5 N NaOH until the pH is ~7.5, and the solution is then chromatographed on C-18 silica gel, using water then acetonitrile/water as eluants, to afford bis-zwitterion **34** (0.130 g, 0.157 mmol; 27%) as a light orange lyophillate. Anal. ($C_{33}H_{35}Cl_2N_5O_8S_4 \cdot 3.8H_2O$) C, H, N.

Synthesis of cephem 38. Cephem diacid **4** (0.390 g, 0.701 mmol) is dissolved in 4 mL methanol and 2 mL methylene chloride, and 1-(2-hydroxy-ethyl)-1H-pyridine-4-thione (0.101 g, 0.650 mmol) is added. After stirring at room temperature for 1 h, the product is precipitated with ether and collected. The material is dissolved in a little DMF and triturated again with ether. The solid is collected and washed with ether, ethyl acetate, and acetone to afford crude cephem **38** (0.260 g) as the chloride salt. This material is then dissolved in 0.5 N NaOH until the pH is ~7.5, and chromatographed on C-18 silica gel, using water then acetonitrile/water as eluants, to afford mono-sodium salt, mono-zwitterion **38** (0.170 g, 0.243 mmol; 37%) as a light yellow lyophillate. MS (ESI) m/e 675 $(M+H)^+$. Anal. ($C_{25}H_{22}Cl_2N_3Na_1O_7S_4 \cdot 3.0H_2O$) C, H, N. ^{1}H NMR (300 MHz, DMSO, partial): δ 9.07 (d, 1H, J = 8 Hz, NH), 8.63 (d, 2H, J = 7 Hz, pyr), 8.35 (d, 2H, J = 7 Hz, pyr), 7.45 (s, 1H, ArH), 7.35 (s, 1H, ArH), 5.38 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 4.90 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.68 (d, 1H, J = 14 Hz), 4.54–4.42 (m, 2H), 4.30 (d, 1H, J = 14 Hz), 3.78 (s, 2H), 3.82–3.70 (m, 4H).

Synthesis of cephem 50. Cephem amine **2** (3.17 g, 7.64 mmol) is dissolved in 13 mL THF. Dicyclohexylcarbodiimide (1.58 g, 7.66 mmol) is added, followed by acid **23** (2.58 g, 7.64 mmol). The mixture is stirred for 1.5 h at room temperature, and then filtered to remove dicyclohexylurea. The filtrate is evaporated to afford the expected chloromethyl cephem diester (5.52 g, 7.52 mmol; 98%) as an orange foam. ^{1}H NMR (300 MHz, DMSO): δ 9.36 (d, 1H, J = 8 Hz, NH), 7.79 (d, 1H, J = 1 Hz, ArH), 7.54 (d, 1H, J = 1 Hz, ArH), 7.53–7.26 (m, 10H, ArH), 5.80 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.21 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.47–4.36 (m, 2H, CH_2Cl), 4.00 (s, 2H, SCH_2), 3.74 (d, 1H, J = 17 Hz, SCH), 3.56 (d, 1H, J = 17 Hz, SCH), 1.54 (s, 9H, $(CH_3)_3$).

The above cephem diester is dissolved in 25 mL methylene chloride, and 3 mL anisole. Trifluoroacetic acid (8 mL) is added, and a precipitate begins to form. The suspension is stirred for 4.5 h and then filtered to afford some of the desired diacid product. The filtrate is triturated with methylene chloride and ether, and filtered to afford more product. The combined solids are stirred in ethyl acetate overnight (~16 h), and then filtered and pumped dry to afford the expected chloromethyl cephem diacid (1.99 g, 3.89 mmol; 52%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO): δ 9.31 (d, 1H, J = 8 Hz, NH), 7.84 (s, 1H, ArH), 7.54 (s, 1H, ArH), 5.71 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.13 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.59–4.49 (m, 2H, CH_2Cl), 3.99 (s, 2H, SCH_2), 3.71 (d, 1H, J = 17 Hz, SCH), 3.52 (d, 1H, J = 17 Hz, SCH).

The above chloromethyl cephem diacid (0.300 g, 0.586 mmol) is dissolved in 4 mL methanol. 1-[3-(2,6-Dimethyl-4-thioxo-4H-pyridin-1-yl)-propyl]-2,3-dimethyl-3H-imidazol-1-ium; chloride salt[1] (0.171 g, 0.549 mmol) is added and the mixture allowed to stir for 1 h at room temperature. The solvents are evaporated, and the material is triturated with methylene chloride and ether. The solid is collected and pumped dry to afford crude cephem **50** (0.295 g) as the dichloride salt. ^{1}H NMR (300 MHz, DMSO, partial): δ 9.34 (d, 1H, J = 8 Hz, NH), 7.86 (s, 1H, ArH), 7.83 (s, 2H, pyrH), 7.80 (d, 1H, J = 2 Hz, imidazolium), 7.68 (d, 1H, J = 2 Hz, imidazolium), 7.56 (s, 1H, ArH), 5.70 (dd, 1H, J = 5, 8 Hz, $R_1R_2CHNR_3$), 5.15 (d, 1H, J = 5 Hz, CH(CNR)(SR)), 4.36–4.31 (m, 2H, CH_2Cl), 4.05–3.95 (m, 2H, SCH_2), 3.77 (s, 3H, CH_3), 2.75 (s, 6H, 2×CH_3), 2.66 (s, 3H, CH_3), 2.31–2.20 (m, 2H, CH_2). MS (ESI) m/e 750 $(M-H)^+$.

The above material is dissolved in 1 N NaOH until the pH is ~8, and chromatographed on C-18 silica gel using water and acetonitrile/water as eluants to afford pure bis-zwitterion **50** (0.081 g, 0.108 mmol; 20%) as a tan lyophillate. Anal. ($C_{32}H_{33}Cl_2N_3O_6S_3 \cdot 3.9H_2O$) C, H, N.

Synthesis of cephem 53. Cephem amine **2** (0.510 g, 1.24 mmol) is dissolved in 4 mL THF. DCC (0.269 g, 1.30 mmol) is added, followed by acid **31** (0.500 g, 1.24 mmol). The mixture is stirred for 1.5 h, and then filtered to remove dicyclohexyl urea. The filtrate is evaporated,

and the crude cephem diester product is dissolved in 2 mL methylene chloride and then treated with 2 mL TFA. After stirring about 5 h at room temperature ether is added to precipitate the expected cephem diacid product. Anal. ($C_{21}H_{15}Cl_3N_2O_7S_2 \cdot 0.9H_2O$) C, H, N. ^{1}H NMR (300 MHz, DMSO): δ 9.31 (d, 1H, $J = 8$ Hz, NH), 7.88 (s, 1H, ArH), 7.63 (s, 1H, ArH), 7.36 (d, 1H, $J = 3$ Hz, furyl), 7.27 (d, 1H, $J = 3$ Hz, furyl), 5.73 (dd, 1H, $J = 5$, 8 Hz, $R_1R_2CHNR_3$), 5.15 (d, 1H, $J = 5$ Hz, CH(CNR)(SR)), 4.60–4.50 (m, 2H, CH_2Cl), 4.00 (s, 2H, SCH_2), 3.70 (d, 1H, $J = 17$ Hz, SCH), 3.51 (d, 1H, $J = 17$ Hz, SCH).

The above cephem diacid (0.150 g, 0.260 mmol) is dissolved in 2 mL methanol, 0.3 mL DMF and 0.5 mL methylene chloride. 1-(2-Hydroxy-ethyl)-1H-pyridine-4-thione (0.040 g, 0.258 mmol) is added, and the mixture is stirred at room temperature for 1.5 h. The precipitate that forms is filtered and washed with methanol. The filtrate is then triturated with ether and the solids collected and washed with ether. Cephem **53** is obtained (0.099 g, 0.135 mmol; 52%) as a tan solid. MS (ESI) m/e 696 $(M)^+$. Anal. ($C_{28}H_{24}Cl_3N_3O_8S_3 \cdot 0.9H_2O$) C, H, N. ^{1}H NMR (300 MHz, DMSO): δ 9.31 (d, 1H, $J = 8$ Hz, NH), 8.66 (d, 2H, $J = 7$ Hz, pyr), 8.02 (d, 2H, $J = 7$ Hz, pyr), 7.86 (s, 1H, ArH), 7.61 (s, 1H, ArH), 7.35 (d, 1H, $J = 3$ Hz, furyl), 7.24 (d, 1H, $J = 3$ Hz, furyl), 5.69 (dd, 1H, $J = 5$, 8 Hz, $R_1R_2CHNR_3$), 5.15 (d, 1H, $J = 5$ Hz, CH(CNR)(SR)), 4.53 (m, 2H, CH_2), 4.42–4.36 (m, 2H, CH_2Cl), 4.03–3.93 (m, 2H, SCH_2), 3.83–3.78 (m, 2H, CH_2), 3.76 (d, 1H, $J = 17$ Hz, SCH), 3.51 (d, 1H, $J = 17$ Hz, SCH).

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5. For a description of the synthesis of this type of compound, see ref 1, footnote 13.
6. The sodium salt of methyl mercaptoacetate is made fresh prior to use. For a discussion of the synthesis and handling of this reagent, see ref 1.