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Synthesis and *anti*-MRSA Activity of Novel Cephalosporin Derivatives

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Abstract—Cephalosporin derivatives containing a unique combination of lipophilic *C*-7 sidechains and polar *C*-3 thiopyridinium groups were synthesized and found to exhibit potent *anti*-MRSA activity in vitro and in vivo. The optimum *C*-7 sidechains utilized were 2,5-dichlorophenylthioacetamido and 2,6-dichloropyrid-4-ylthioacetamido. The *C*-3 thiopyridinium rings were substituted at nitrogen with amino acid and pyruvic acid groups that were designed to confer aqueous solubility as required for IV formulation. This paper describes the characteristics of these novel cephalosporins and highlights synthetic methods developed to allow their practical, large-scale syntheses.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA)[1] is resistant to β-lactam antibiotics due to the presence of an additional penicillin-binding protein (PBP2a) for which β-lactams have poor affinity.[2] Vancomycin[3] and Synercid[4] are the only marketed drugs that are effective against MRSA infections. In addition to the complications caused by limited treatment options, vancomycin intermediate-resistant MRSA[5] has recently been detected in hospitals in Japan[6] and the USA.[7] Furthermore, the hospital setting is no longer the exclusive domain of MRSA infections. Recently, there have been alarming reports of its spread in nursing homes, extended-care facilities, and a day care center.[8] The need for new anti-MRSA agents is clear.

Many companies are looking for novel classes of anti-bacterial agents. Another effective strategy to identify compounds with activity against resistant organisms is to improve on a known class of antibacterial agents.[9] Since cephalosporins have an excellent safety profile and are synthetically accessible from commercial sources, we initiated a program to develop a cephalosporin with anti-MRSA activity. The goals of the program were to identify a cephalosporin with (1) in vitro and in vivo activity against MRSA, (2) suitable pharmacokinetics in animals to predict convenient dosing in humans, (3) sufficient water solubility to allow I.V. dosing, and (4) an excellent safety profile. The 2,5-dichlorophenylthioacetamido *C*-7 group was disclosed in the late 1970s by researchers at Lilly as a lipophilic sidechain that conferred excellent gram-positive activity to the cephem class.[10] For instance, their *C*-3 acetate

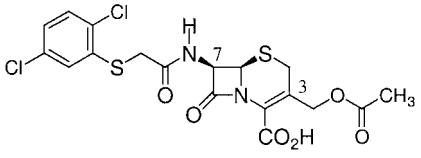

Figure 1.

Keywords: anti-MRSA activity; 2,5-dichlorophenylthioacetamido; 2,6-dichloropyrid-4-ylthioacetamido; novel cephalosporins.
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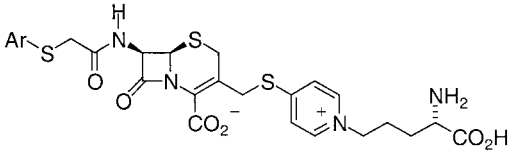
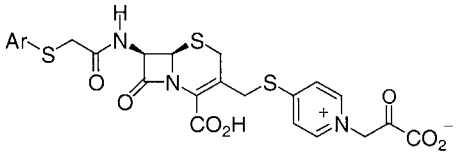


1: Ar = 2,5-dichlorophenyl (diClPh)
2: Ar = 2,6-dichloropyrid-4-yl (diClPyr)



3: Ar = 2,5-dichlorophenyl (diClPh)
4: Ar = 2,6-dichloropyrid-4-yl (diClPyr)

Figure 2.

derivative (Fig. 1) had MICs of ~1 μg/mL against Methicillin-susceptible *Staphylococcus aureus* (MSSA). In our own hands, this Lilly compound had MICs of 2 μg/mL against MRSA (strain A 27223[11]). Unfortunately, this acetate derivative suffered from poor pharmacokinetics. Our efforts at Bristol–Myers Squibb were aimed at finding an improved cephalosporin containing a lipophilic *C*-7 sidechain. Some of our earlier work in this area has been published.[12]

Results and Discussion

Through a series of optimizations at the *C*-7 and *C*-3 positions, we discovered cephems **1**, **2**, **3**, and **4** as having excellent properties for use as anti-MRSA agents (Fig. 2). This paper describes the activity and synthesis of these four compounds.

All four cephems contain a lipophilic *C*-7 group (2,5-dichlorophenylthioacetamido or 2,6-dichloropyrid-4-ylthioacetamido) and a thiopyridinium group at *C*-3. Compounds **1** and **2** are substituted with an amino acid sidechain while **3** and **4** contain a pyruvic acid moiety. Key parameters that were optimized included MICs, IC_{50}s vs. PBP2a, PD_{50}s, and solubility. The MICs[13] and PBP2a IC_{50}s are shown in Table 1. Cephems **1**–**4** have excellent activity against homogeneous and heterogeneous methicillin-resistant strains of MRSA.[14] In addition, these cephems have excellent in vitro activity against a variety of gram-positive bacteria including resistant strains (e.g., penicillin-resistant *S. pneumoniae*, methicillin-resistant *S. epidermitis*, and methicillin-resistant *S. haemolyticus*). In terms of PBP2a inhibition, these new cephalosporins have a much greater affinity for this critical PBP than traditional cephalosporins (methicillin PBP2a IC_{50} ~100 μg/mL). Cephems **1**–**4** also retained good binding affinities (<0.6 μg/mL) to the other important staphylococcal PBPs.

Table 1. Minimum Inhibitory Concentrations (MICs) in μg/mL (MR=methicillin-resistant; P-=penicillinase negative; M=Methicillin; IM=imipenem; NT=not tested; IC_{50}=the concentration at which 50% inhibition has occurred)

Organism	A No.	1	2	3	4	M	IM
S. aureus/Hetero MR	A27218	0.5	1	0.5	1	32	1
S. aureus/+50% calf serum	A27218	0.5	1	1	1	8	NT
S. aureus/Hetero MR	A27217	0.5	0.5	0.5	1	64	1
S. aureus/Hetero MR	A25795	1	2	1	1	128	8
S. aureus/Homo MR	A27223	2	2	2	2	128	32
S. aureus/+50 calf serum	A27223	4	4	16	2	64	NT
S. aureus/Homo MR	A27621	1	2	2	2	64	16
S. aureus/Homo MR	A27295	2	4	4	2	128	64
S. aureus/Homo MR	A27226	1	2	1	2	64	4
S. aureus/MR, P-	A27225	1	2	2	2	128	NT
PBP2a IC_{50} (μg/mL)		28	NT	10	4.5	100	NT

Table 2. PD_{50}s and solubility (NT=not tested)

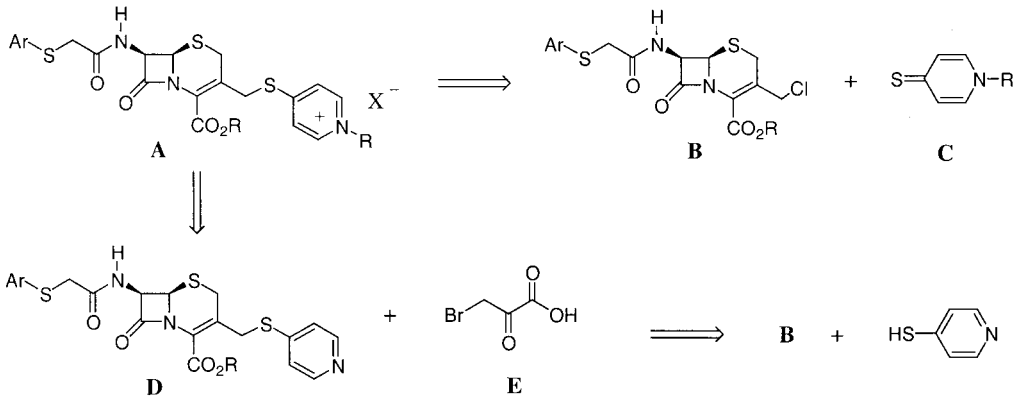
Cmpd	PD_{50} (mg/Kg/total dose) vs. MRSA (A27223)	Aqueous solubility (mg/mL at pH 7, rt)
1	8.2	2–3
2	4.8	NT
3	8.0	23
4	9.6	40

Table 2 summarizes the efficacy and solubility properties of **1**, **2**, **3**, and **4**. All were efficacious in a systemic murine model of infection (MRSA, homogeneous) with PD_{50}s ranging from 8–9.6 mg/Kg.[15] The aqueous solubility of the pyruvate derivatives **3** and **4** is greatly improved over that of cephems **1** and **2** especially at neutral pH.

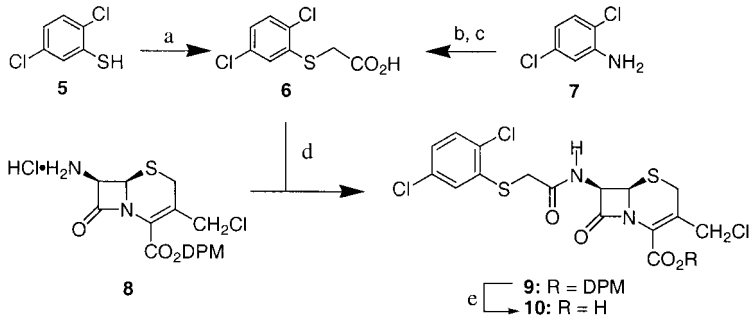
The synthetic approaches to cephems **1**–**4** are shown retrosynthetically in Scheme 1. The thiopyridinium sidechain at C-3 can be introduced by reacting allylic chloride **B** with thiopyridone **C**. Alternatively, the thiopyridine nitrogen of **D** can be quaternized with an electrophile such as bromopyruvic acid **E**. Cephem **D** is synthesized by reacting allylic chloride **B** with 4-thiopyridine.

The preparation of the *C*-7 sidechains is shown in Schemes 2 and 3. The earlier synthesis of **6**[16] relied on the expensive thiophenol **5**. Alternatively, careful diazotization of aniline **7** followed by reaction with the copper salt of thioglycolic acid (mercaptoacetic acid) produced **6** in 70% overall yield.[17] The initial route from **6** to **10** utilized a DCC coupling of the acid **6** with 7-aminocephalosporonic acid diphenylmethane ester **8** to provide DPM ester **9**. While this proved satisfactory for small scale reactions, the large scale preparations of **10** were best accomplished by converting **6** to its acid chloride followed by coupling with amine **8** to give ester **9**. Deprotection of **9** with trifluoroacetic acid gave the chloroacid **10** (Scheme 2).

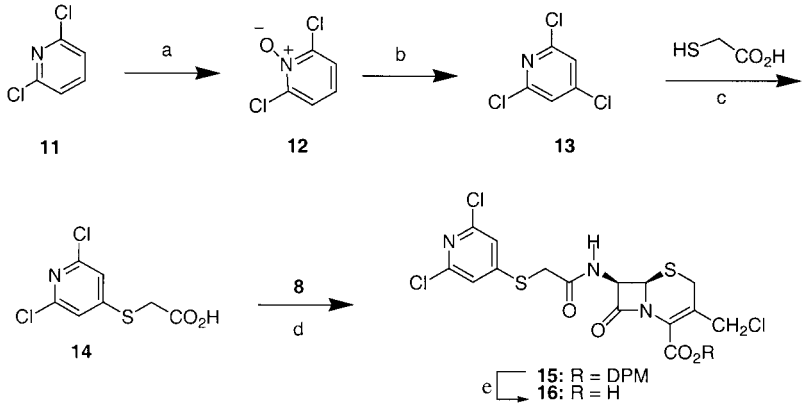
For the C-7 dichloropyrid-4-yl derivatives, the key acid **14** was prepared from 2,6-dichloropyridine as shown in Scheme 3. While the general route from **11** to **14** was previously reported,[18] modifications were made to facilitate work on a larger scale. Commercially available 2,6-dichloropyridine (**11**) was oxidized with hydrogen peroxide to give the *N*-oxide **12**. A major improvement over the literature conditions for the large scale conversion of **12** to 2,4,6-trichloropyridine (**13**) was realized when LiCl was added to the reaction mixture. This moderated an otherwise



Scheme 1.



Scheme 2. Synthesis of chloroester **9** and chloroacid **10**. Reaction conditions and yields: (a) $BrCH_2CO_2H$, aq NaOH, 100°C; ~100%; (b) HCl, $NaNO_2$; (c) $HSCH_2CO_2H$, $CuSO_4$; 55–70% from **7**; (d) $(COCl)_2$, NMM, DMF; 88%; (e) TFA, anisole, CH_2Cl_2, 0°C, 94%.

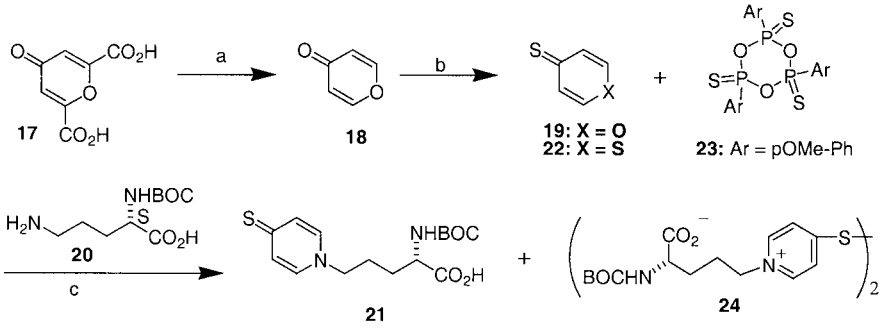


Scheme 3. Synthesis of chloroester **15** and chloroacid **16**. Reaction conditions and yields: (a) 30% H_2O_2, TFA, 100°C, 3 h, 84%; (b) LiCl, $POCl_3$, 115°C, 6 h, 71%; (c) aq. NaOH, MeOH, 25°C, 17 h, 70%; (d) EDC, NMM, CH_2Cl_2, 0°C, 1 h, 93%; (e) TFA, anisole, 0°C, 1 h, 98%.

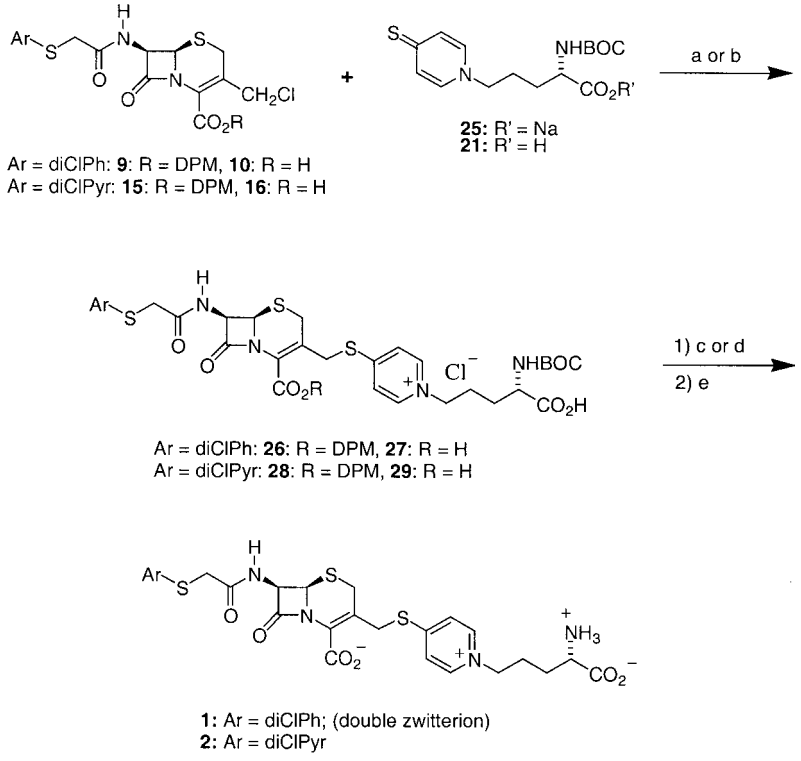
overly vigorous reaction. Acid **14** was coupled efficiently on a large scale to the cephem nucleus using EDC. The TFA deprotection of DPM ester **15** gave an excellent yield of the acid **16**.

Scheme 4 illustrates the synthesis of thiopyridone **21**. Improvements were made to the literature preparations of **18**[19] and **19**.[20] The expensive γ-pyrone (**18**) was prepared by decarboxylating chelidonic acid (**17**) in di-*n*-butylphthalate (bp 340°C), a solvent with a considerably higher boiling point than **18** (bp ~210°C). Isolation by distillation rather than extraction led to an 81% yield of **18**. Treatment of **18** with Lawesson's reagent under anhydrous conditions afforded **19** with minimal amounts (<3%) of the side product **22**. Purification of **19** by bulb-to-bulb distillation rather than chromatography effectively removed the impurities **22** and **23**. Reaction of *N*-Boc-ornithine **20** with **19** gave the expected thiopyridone **21** plus small amounts of the dimer **24**. Method development work revealed that replacing NaOEt with pyridine minimized the formation of colored impurities during the formation of **21**.

With the requisite cephem and thiopyridone units in hand, several approaches to cephems **1** and **2** were studied. The



Scheme 4. Synthesis of 4-mercaptopyridyl-*N*-Boc-L-ornithine **21**. Reaction conditions and yields: (a) Cu-bronze, 2,2′-dipyridyl, *n*-butylphthalate, ~210°C; 80–90% distilled; (b) Lawesson's reagent, toluene, 80°C; 98% distilled; (c) *N*-Boc-L-ornithine **20**, pyridine, aq EtOH, reflux; 91%.

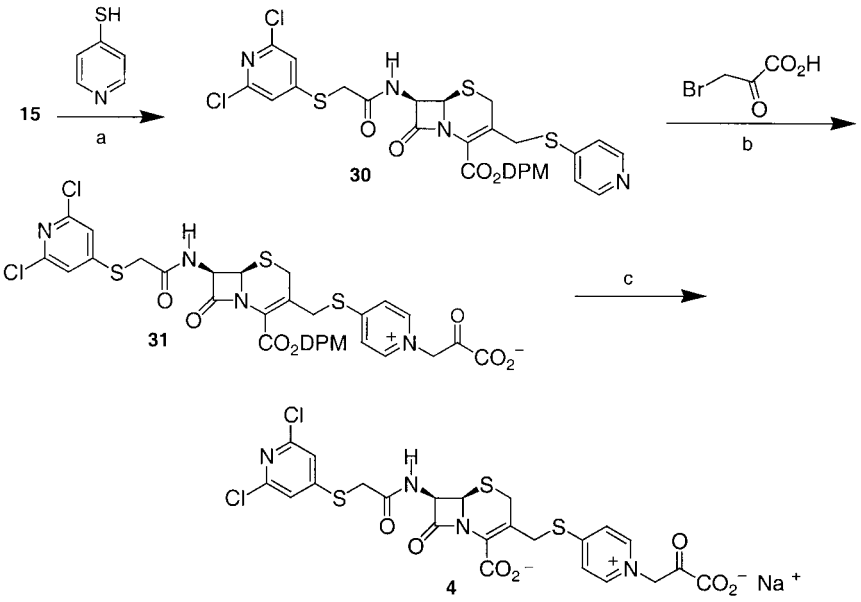


Scheme 5. Synthesis of cephems **1** and **2**. Reaction conditions: (a) **16**+**25**→**29**: DMF, 5 h, rt; (b) **10**+**21**→**27**: THF, 3 h, rt; (c) TFA, anisole, CH_2Cl_2; (d) HCO_2H, HCl; (e) NaOAc, MeOH.

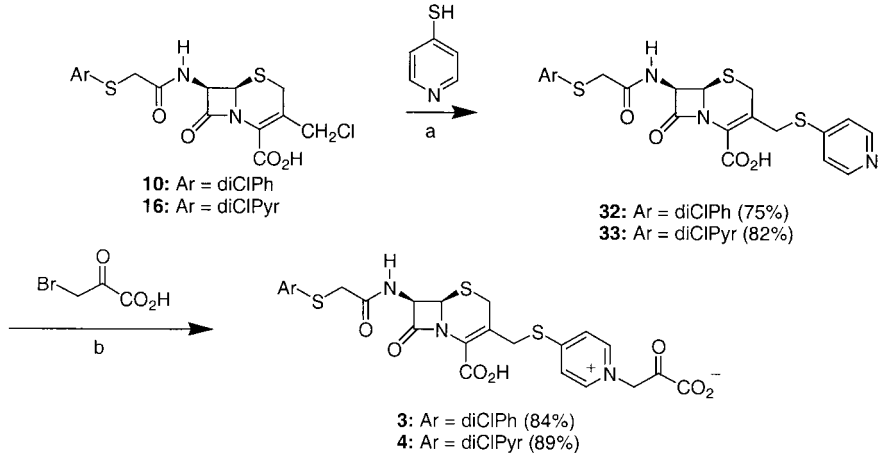
coupling of thiopyridone **21** (acid form) with DPM ester **9** gave cephem **26** in a crude yield of 80%; however, the presence of the corresponding delta-2 isomer (10%) made purification difficult. Based on this result, the analogous approach to cephem **28** was not attempted. An improvement in purity was realized when thiopyridone **25** (Na salt) was reacted with the chloroacids **10** and **16** to give the thiopyridinium compounds **27** and **29** (97 and 71% yield, respectively) (Scheme 5). Work to optimize the large scale synthesis of **1** led to the coupling of thiopyridone **21** with chloroacid **10**. This procedure minimized the formation of the delta-2 isomer and other impurities and allowed the isolation of cephem **27** in 97% yield. Cephems **27** and **29** typically were deprotected using TFA/anisole/CH_2Cl_2 conditions (70–90% yield). However, a mixture of formic acid and hydrochloric acid was also effective for the large scale deprotection of **27** (99.7% yield). The crude deprotected salts (TFA or HCl) of cephems **1** and **2** were purified further by conversion to the bis-zwitterionic cephems **1** and **2**, respectively.

The pyruvate derivatives **3** and **4** were prepared by the alternative quaternization route (Scheme 1: D+E→A). Our initial route to compound **4** (and **3**) involved the use of the DPM esters, which underwent quaternization at the *C*-3 thiopyridine group when treated with 5 mol equiv. of bromopyruvic acid (Scheme 6). This procedure was complicated by (1) incomplete quaternization, (2) secondary reaction of the product with bromopyruvic acid,[21] and (3) the necessity of C-18 column chromatography to purify the crude product.

It was subsequently found that the DPM esters **9** and **15** were not the preferred starting materials. In an improved



Scheme 6. Synthesis of **4** via thionation of chloroester **15**. Reaction conditions and yields: (a) 2,6-lutidine, NaI, DMF, 25°C, 1.5 h, 80%; (b) DMF, 25°C, 18 h, 80%; (c) TFA, anisole, 0–25°C, 1.5 h, 90%; aq NaOH, C-18 chromatography; 40%.



Scheme 7. Synthesis of **3** and **4** via chloro acids **10** and **16**. Reaction conditions: (a) 2,6-lutidine, NaI, DMF, 25°C, 40 min; (b) BSA, THF, 25°C, 2 h.

sequence, chloroacids **10** and **16** were treated with 4-mercaptopyridine to give cephems **32** and **33**, respectively. Quaternization of **32** and **33** with bromopyruvic acid was best accomplished using a novel in situ silylation procedure (Scheme 7).[22] This involved pretreatment of **32** and **33** with BSA in THF to afford a homogeneous solution. The addition of 2.5 equiv. of bromopyruvic acid led to the efficient quaternization of the pyridyl groups in just 2 h at rt. The final products **3** and **4** were isolated in excellent yield and high purity while avoiding the pitfalls of the DPM ester route (Scheme 6).

In summary, cephalosporins **1**–**4** with potent anti-MRSA activity and promising physical characteristics have been identified. In **1**–**4**, the cephalosporin core is appended with a novel combination of a lipophilic *C*-7 substituent and a polar thiopyridinium group at *C*-3. The original routes to cephems **1**–**4** were modified substantially to allow the practical, large scale synthesis of these derivatives. This chemistry also proved critical in the synthesis of a variety of related analogs. Our continuing studies on the optimization of anti-MRSA cephalosporins will be reported in future publications.

Experimental

Abbreviations: rt=room temperature, EDC=*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, DCU=dicyclohexylurea, DCC=dicyclohexylcarbodiimide, BSA=bistrimethylsilylacetamide, TFA=trifluoroacetic acid, NMM=*N*-Methyl Morpholine.

[6*R*-[3(S*),6α,7β]]-1-(4-Amino-4-carboxybutyl)-4-[[[2-carboxy-7-[[[(2,5-dichlorophenyl)thio]-acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]thio]-pyridinium, hydroxide, inner salt (1). Powdered chloride salt **27** (122.44 g, 141.9 mmol) was added in portions to

98% formic acid (236 mL) with rapid stirring so that the solid dissolved. The solution was stirred for 4 h. Conc. HCl (14.07 mL, 170.3 mmol) was added and the solution was stirred for 20 min. The mixture was added rapidly over 2 min to EtOAc (2600 mL) in a Morton flask with mechanical stirring. The residue from the reaction flask was washed into the slurry with HCO_2H (2×5 mL) and EtOAc (2×10 mL). The mixture was stirred for 15 min, filtered, and the filter cake was transferred to a Morton flask with 1000 mL EtOAc and stirred for 1 h to remove most of the entrained HCO_2H. The slurry was filtered and the filter cake was washed thoroughly with EtOAc (2×100 mL). The solid was dried under vacuum overnight to give 117.11 g (99.7%) of the chloride salt of **1** as a golden yellow powder, mp ~100°C (eff.). $[\alpha]_D$=+28.0 (*c*=1, CH_3CN/H_2O 2:1, pH=1.8). Anal. Calcd for $C_{26}H_{26}N_4O_6S_3Cl_2 \cdot 2\ HCl \cdot 1.0\ H_2O \cdot 0.5\ EtOAc \cdot 0.24\ HCO_2H$: C, 42.20; H, 4.32; N, 6.97; S, 11.97; Cl, 17.65; H_2O, 2.24. Found: C, 41.99; H, 4.41; N, 6.73; S, 11.53; Cl, 17.46; H_2O, 2.26 (KF).

The chloride salt of **1** (115.12 g, 143.25 mmol) was powdered with a Teflon spatula and added slowly over 20 min to mechanically stirred 50% aqueous EtOH (6 L) at 25°C (pH=3.0). After 15 min a mixture of a solution of 1N NaOH (23.6 mL) and NaOAc (40.21 g, 295.7 mmol) in 50% aqueous EtOH (492 mL) was added via an addition funnel as a rapid stream of droplets over 20 min. The mixture (pH 5.2) was stirred for 1.5 h and the solid was filtered through a medium porosity sintered glass funnel. The product was washed by stirring it in the funnel with 50% aqueous EtOH (2 L). The material was transferred to a Buchner funnel containing a Whatman 1 (18.5 cm) filter paper for a faster filtration. The washing was repeated two more times with EtOH/H_2O (1:1, 1 L), each time transferring the product to a beaker for slurrying and then filtering through a fresh filter paper in the Buchner funnel. After further washing with EtOH (3×800 mL) and EtOAc (3×800 mL) the product was dried for 17 h under vacuum to give 78.4 g (77.4%) of the double zwitterion **1**, mp ~165°C (dec.). ^{1}H NMR (DMSO-d_6-TFA, 270 MHz) δ 1.8–2.1 (m, 4H), 3.7 (ABq, *J*=18.2 Hz, 2H), 4.0 (s, 2H), 4.4–4.7 (m, 4H), 5.2 (d, *J*=4.7 Hz, 1H), 5.8 (dd, *J*=4.7 and 8.0 Hz, 1H), 7.3 (dd, *J*=2.3 and 8.2 Hz, 1H), 7.5 (d, *J*=9.4 Hz, 1H), 8.1 (d, *J*=7.0 Hz, 2H), 8.4 (br s, 3H), 8.8 (d, *J*=7.0 Hz, 2H), 9.4 (d, *J*=8.2 Hz, 1H); ^{13}C NMR (DMSO-d_6-TFA, 68 MHz) δ, 26.9, 27.7, 34.1, 38.3, 51.9, 58.0, 60.0, 123.4, 124.1, 126.8, 127.5, 129.5, 131.1, 133.1, 138.2, 143.1, 158.5, 159.0, 159.6, 160.1, 162.6, 163.4, 164.7, 168.9, 171.1; IR (KBr) 3500, 1763, 1630 cm^{-1}; MS (ESI) M+H=657, M−H=655; $[\alpha]_D$=+35.7 (*c*=1, 0.1N HCl/CH_3CN 2:3, pH=3.2). Anal. Calcd for $C_{26}H_{26}N_4O_6S_3Cl_2 \cdot 2.73\ H_2O$: C, 44.18; H, 4.49; N, 7.93; S, 13.61; Cl, 10.03; H_2O, 6.96. Found: C, 44.04; H, 4.33; N, 7.69; S, 13.50; Cl, 10.00; H_2O, 6.93 (KF).

[6*R*-[3(S*),6α,7β]]-1-(4-Amino-4-carboxybutyl)-4-[[[2-carboxy-7-[[[(2,6-dichloro-4-pyridinyl)thio]-acetyl]-amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]-methyl]thio]pyridinium, hydroxide, inner salt (2). The sodium salt of **29** (576 mg, 0.704 mmol) was stirred with CH_2Cl_2 (24 mL) at 0°C while anisole (2.4 mL) and TFA (8.7 mL) were added. This solution was stirred for 1 h at 0°C followed by concentration in vacuo to remove CH_2Cl_2 and TFA. The residue was stirred with diethyl ether (100 mL) for 15 min, and the resulting solid was collected by filtration. This solid was stirred with acetone (25 mL) for 20 min, and then the solid was collected by filtration to give 386 mg (68%) of **2** as the monochloride, mono TFA salt. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.72 (br s, 2H), 1.98 (br s, 2H), 3.42 (d, *J*=17 Hz, 1H), 3.58–3.74 (m, 3H), 3.61 (d, *J*=17 Hz, 1H), 3.99 (d, *J*=15 Hz, 1H), 4.07 (d, *J*=15 Hz, 1H), 4.37–4.58 (m, 4H), 5.02 (d, *J*=5 Hz, 1H), 5.57 (dd, *J*=5.8 Hz, 1H), 7.51 (s, 2H), 8.10 (d, *J*=6 Hz, 2H), 8.73 (d, *J*=6 Hz, 2H), 9.42 (d, *J*=8 Hz, 1H); MS (ESI) M+H=658.

(6*R-trans*)-4-[[[2-Carboxy-7-[[[(2,5-dichlorophenyl)thio]-acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]thio]-1-(2-carboxy-2-oxoethyl)-pyridinium, hydroxide, inner salt (3). (*Method A*) The sodium salt form of **3** was prepared from (6*R*)-*trans*-3-[(4-pyridylthio-methyl]-7-[(2,5-dichlorophenyl)-thioacetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, diphenyl-methyl ester[23] using the same procedure described for compound **4** (method A): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.35 (d, *J*=17 Hz, 1H), 3.51 (d, *J*=17 Hz, 1H), 3.91 (s, 2H), 4.30 (d, *J*=14 Hz, 1H), 4.74 (d, *J*=14 Hz, 1H), 4.97 (d, *J*=5 Hz, 1H), 5.46 (dd, *J*=5.8 Hz, 1H), 5.75 (s, 2H), 7.24 (dd, *J*=9.2 Hz, 1H), 7.45–7.50 (m, 2H), 8.38 (d, *J*=7 Hz, 2H), 8.48 (d, *J*=7 Hz, 2H), 9.20 (d, *J*=8 Hz, 1H); MS (ESI) M^+=627. Anal. Calcd for $C_{24}H_{18}N_3O_7S_3Cl_2Na \cdot 3\ H_2O$: C, 40.91; H 3.43; N, 5.96; S, 13.65; Na, 3.26. Found: C, 40.62; H, 3.26; N, 5.82; S, 13.78; Na, 3.30.

(*Method B*) Bistrimethysilylacetamide (BSA)(3.4 g, 14.9 mmol) was added to a stirred suspension of mercaptopyridyl acid **32** (2.03 g, 3.5 mmol) in anhydrous CH_3CN (11.0 mL) at 25°C under an argon atmosphere. A clear orange solution was obtained after 5 min. Anhydrous bromopyruvic acid (1.46 g, 8.8 mmol) was added and the reaction mixture was stirred for 3 h. The solution was diluted with CH_3CN (11.0 mL) and H_2O (30 mL) was added dropwise with good stirring to obtain a precipitate. After 1.5 h the solid was filtered, washed with water/CH_3CN (2:1, 30 mL), and CH_3CN and dried with suction under nitrogen overnight to give 2.23 g of **3**. A suspension of 2.1 g of this solid in CH_3CN (20.0 mL) was sonicated for 10 min, filtered and dried under vacuum to give 1.85 g (yield ~84%) of zwitterion **3**.

(6*R-trans*)-4-[[[2-Carboxy-7-[[[(2,6-dichloro-4-pyridinyl)-thio]acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]thio]-1-(2-carboxy-2-oxoethyl)-pyridinium, hydroxide, inner salt (4). (*Method A*) A stirred solution of DPM ester **15** (10 g, 15.75 mmol) in 100 mL of DMF was cooled to 0°C (ice bath) under a N_2 atmosphere. To this solution was added NaI (2.36 g, 15.75 mmol) followed by 4-mercaptopyridine (1.92 g, 17.3 mmol) and 2,6-lutidine (2.02 g, 18.9 mmol). The ice bath was removed and the reaction was allowed to stir at rt for 1.5 h. Water was added dropwise to the reaction mixture to precipitate a yellow solid which was collected by filtration, washed with water, and dried on the filter while under suction. This gave 12 g of solid which was stirred with 200 mL of ether for 1 h. The solid was again collected by filtration and dried on the filter to give 10 g of **30** as a yellow solid. Bromopyruvic acid (12.5 g, 74.9 mmol) was added all at

once to a DMF solution (120 mL) of cephem **30** (9.0 g, 12.8 mmol). This mixture was stirred at rt for 18 h at which time the DMF was removed in vacuo (~1 torr, ≤40°C) to give a dark brown residue. The residue was stirred with 200 mL of water for 30 min and the resulting tan solid was collected by filtration and dried (with P_2O_5 in a vacuum dessicator). The dry solid was slurried in EtOAc and stirred for 1 h. The solid was collected by filtration to give 9 g (81%) of **31** as a tan solid. DPM ester **31** (10 g, 11.4 mmol) was slurried with CH_2Cl_2 (50 mL) and anisole (15 mL), and then cooled to 0°C. TFA (50 mL) was added and the mixture was stirred at rt for 1.5 h. The reaction was concentrated in vacuo to remove most of the CH_2Cl_2 and TFA. The residue was stirred with 100 mL of ether and the resulting solid was collected by filtration. This solid was slurried in 100 mL of ethyl acetate for 0.5 h, collected by filtration, and dried in vacuo to give 7.4 g (89%) of **4** as the TFA salt. The TFA salt of **4** was dissolved in ~200 mL of water adjusted to pH 8 with NaOH. Purification over a C18 column (~125 g) was accomplished by eluting with water followed by increasing amounts of acetonitrile up to 30% to give 3.25 g of **4** as the monosodium salt. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.39 (d, J=17 Hz, 1H), 3.53 (d, J=17 Hz, 1H), 3.96 (d, J=16 Hz, 1H), 4.03 (d, J=16 Hz, 1H), 4.31 (d, J=14 Hz, 1H), 4.76 (d, J=14 Hz, 1H), 4.99 (d, J=5 Hz, 1H), 5.46 (dd, J=5.8 Hz, 1H), 5.76 (s, 2H), 7.54 (s, 2H), 8.39 (d, J=7 Hz, 2H), 8.49 (d, J=7 Hz, 2H), 9.25 (d, J=8 Hz, 1H); MS (ESI) M^+=628.

(*Method B*) Bistrimethysilylacetamide (BSA)(7.99 mL, 32.4 mmol) was added to a suspension of the mercaptopyridyl acid **33** (5.0 g, 8.59 mmol) stirred in THF (28.6 mL) at 25°C under an argon atmosphere. A clear solution was obtained after 30 min. Anhydrous bromopyruvic acid (3.59 g, 21.5 mmol) was added and the reaction mixture was stirred for 2 h. The solution was chilled in an ice-bath and H_2O (120 mL) was added dropwise with good stirring to obtain a precipitate. After 15 min the solid was filtered, washed twice with water, and dried under vacuum overnight to give 5.04 g (89%) of the zwitterion **4**. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.39 (d, J=17 Hz, 1H), 3.53 (d, J=17 Hz, 1H), 3.96 (d, J=16 Hz, 1H), 4.03 (d, J=16 Hz, 1H), 4.31 (d, J=14 Hz, 1H), 4.76 (d, J=14 Hz, 1H), 4.99 (d, J=5 Hz, 1H), 5.46 (dd, J=5.8 Hz, 1H), 5.76 (s, 2H), 7.54 (s, 2H), 8.39 (d, J=7 Hz, 2H), 8.49 (d, J=7 Hz, 2H), 9.25 (d, J=8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 68 MHz) δ 27.1, 33.2, 33.7, 57.4, 59.3, 66.2, 119.2, 122.2, 122.4, 127.7, 143.6, 144.3, 149.1, 155.3, 161.9, 162.4, 163.1, 163.9, 167.8, 194.3; IR (KBr) 3265, 1770, 1629 cm^{-1}; MS (ESI) M^+=628; Anal. Calcd for $C_{23}H_{18}N_4O_7S_3Cl_2 \cdot 0.72\ H_2O$: C, 41.82; H 3.27; N, 8.48; S, 14.56; Cl, 10.73; H_2O 4.69. Found: C, 41.55; H, 3.09; N, 8.43; S, 14.47; Cl, 11.11; H_2O, 4.71 (KF). Cephem **4** could be further purified as the meglumine (MGA) salt as described below. Powdered zwitterion **4** (3.0 g, 4.54 mmol) was added in portions to a solution of methyl-D-glucamine (0.887 g, 4.54 mmol) in 20 mL H_2O. The solid dissolved after stirring for 15 min (pH=6.8). Acetone (80 mL) was added dropwise over 15 min to form a precipitate, which was stirred for 15 min. The solid was filtered, washed twice with 10% H_2O/acetone, and dried under vacuum to give 2.93 g (73%) of the meglumine salt of **4**. The salt was powdered and added to water (15 mL) with stirring at 25°C. Acetone (75 mL) was added dropwise to the slurry. The salt initially dissolved, then a gummy precipitate formed which solidified upon stirring after 15 min. The solid was filtered and the filter cake was washed twice by slurrying with 5% H_2O/acetone in the filtration funnel. The product was dried under vacuum to give 2.21 g (57% overall yield from zwitterion) of the meglumine salt of **4**, mp 130°C (dec.). ^{1}H NMR (THF-d_8-TFA, 270 MHz) δ 2.7 (m, 1H), 2.8 (t, J=5.3 Hz, 3H), 2.9 (m, 1H), 3.3–3.4 (m, 2H), 3.6–4.0 (m, 8H), 4.1–4.2 (m, 4H), 4.6 (m, 2H), 4.8 (s, 1H), 5.3 (d, J=4.7 Hz, 1H), 5.8 (dd, J=4.7 and 8.2 Hz, 1H), 6.2 (s, 1H), 7.5 (s, 2H), 8.0 (s, 2H), 8.1 and 8.2 (pair of doublets due to TFA, J=6.5 Hz each, 2H), 8.6 (br s, 2H), 8.8 (m, 2H), 9.3 (d, J=8.2 Hz, 1H); $[\alpha]_D$=1.5 (c=1.5, H_2O, pH=6.1). IR (KBr) 3368, 1750, 1661, 1630 cm^{-1}. MS (ESI) M+H_2O+H=647, M+H_2O−H=645. Anal. Calcd for $C_{23}H_{18}N_4O_7S_3Cl_2 \cdot C_7H_{17}NO_5 \cdot 1.0\ H_2O$: C, 42.75; H 4.43; N, 8.31; S, 11.41; Cl, 8.41; H_2O 2.14. Found: C, 42.60; H, 4.26; N, 8.11; S, 11.50; Cl, 8.40; H_2O, 1.83 (KF).

[(2,5-Dichlorophenyl)thio]acetic acid (6). (*Method A*) Conc. HCl (76.5 mL, 925.9 mmol) was added to 2,5-dichloroaniline **7** [50.0 g, 308.6 mmol, (CAUTION, lachrymator)] in a 2 L beaker. The large lumps that developed were broken up with a stirring rod. The mixture of white crystals was stirred for 30 min to ensure that all of the aniline was converted to the HCl salt. Water (400 mL) was added to form a suspension, which was stirred magnetically. Ice (~450 mL) was added to bring the temperature to 10°C. A solution of $NaNO_2$ (22.36 g, 324.0 mmol) in H_2O (80 mL) was added dropwise with rapid stirring over 5 min. Small portions of ice were added to keep the temperature at 10°C throughout the diazotization reaction. The resulting solution contained a small amount of insoluble material. After stirring at 10°C for 1 h the excess nitrite was destroyed by addition of a solution of sulfamic acid (2.10 g, 21.6 mmol) in H_2O (10 mL) dropwise (carefully so that the resulting foam didn't spill out of the beaker). The mixture was filtered and the filter cake was washed with a small amount of H_2O. Mercaptoacetic acid (26.5 mL, 432.0 mmol) was added to a solution of $CuSO_4 \cdot 5H_2O$ (77.05 g, 308.6 mmol) in water (300 mL) stirred mechanically under N_2 in a 2 L round-bottomed flask. After stirring for 30 min a dark precipitate formed. The solution of the diazonium salt was added in small portions through a dropping funnel to the insoluble copper complex with rapid stirring. The temperature of the mixture was kept at 20°C with a water bath. The mixture was stirred for 30 min, conc. HCl (80 mL) was added, and the mixture was stirred at 40–50°C for 1 h. The warm reaction mixture was filtered to collect the solid, which was washed twice with 1% HCl and water. The filter cake was washed into a 4 L beaker with 1.5 L H_2O and dissolved by the addition of 38 g Na_2CO_3. After heating to boiling, 30 g Darco was added and the mixture was boiled for 15 min. The mixture was filtered hot through Celite and washed with water. Conc. HCl (30 mL) was added to the warm filtrate dropwise until the pH reached 1 (foaming!). The precipitated product was collected by filtration and thoroughly washed with water three times. Each time the filter cake was sucked dry on the filter funnel. The lightly colored solid was dried under vacuum over P_2O_5 overnight to give **6**: 43.81 g (60%). Hexane (300 mL) was added to a solution of crude material in hot EtOAc

(100 mL); crystals formed immediately. The mixture was cooled to room temperature and let stand for 2 h. The crystals were filtered, washed with hexane, and dried under vacuum over P_2O_5 at 50°C overnight to give 32.48 g (first crop corrected yield 44%) of **6**, mp 128–129°C. The mother liquor was evaporated to ~10% volume. After standing for 4 h, the crystals were filtered, washed with hexane, and dried under vacuum overnight to give a second crop of **6**: 7.19 g (~10%), total yield for two crops ~54%. ^{1}H NMR (DMSO-d_6, 270 MHz) δ 4.0 (s, 2H), 7.24–7.27 (d, J=8.8 Hz, 1H), 7.37 (s, 1H), 7.47–7.50 (d, J=8.8 Hz, 1H), 13.0 (br s, 1H). ^{13}C NMR (DMSO-d_6, 68 MHz) δ 33.6, 126.0, 128.9, 130.7, 132.4, 137.8, 169.9. IR (KBr) 3500, 1707 cm^{-1}. MS (ESI) M−H=235. Anal. Calcd for $C_8H_6Cl_2SO_2$·0.081 H_2O: C, 40.25; H, 2.60; Cl, 29.72; S, 13.44; H_2O, 0.61. Found: C, 40.53; H, 2.49; Cl, 29.51; S, 13.18; H_2O, 0.61 (KF).

(*Method B*) A mixture of 2,5-dichlorothiophenol (10.3 g, 57.5 mmol) and bromoacetic acid (8.03 g, 57.8 mmol) in water (225 mL) was treated with 10N NaOH (13 mL, 130 mmol) and the mixture was heated at 100°C for 1 h. The reaction mixture was then cooled to 0°C and acidified to pH 1 with 6N HCl. The product precipitated and was collected by filtration to give 13.0 g (95% yield) of 2,5-dichlorophenylthioacetic acid as white crystals, mp 118°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (s, 2H), 7.15 (dd, J=2.9 Hz, 1H), 7.32 (d, J=9 Hz, 1H), 7.36 (d, J=2 Hz, 1H). Anal. Calcd for $C_8H_6O_2SCl_2$: C, 40.53; H, 2.55. Found: C, 40.46; H, 2.64.

(6-*R*-*trans*)-3-(chloromethyl)-7-[[[(2,5-dichlorophenyl)thio]-acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, diphenylmethyl ester (9). (*Method A*) Oxalyl chloride (7.06 mL, 80.9 mmol) and DMF (0.10 mL. 1.35 mmol) were added to a stirred suspension of acid **6** (16.08 g, 64.7 mmol) in 50 mL CH_2Cl_2 at −25°C. Vigorous gas evolution occurred. The solution was stirred for 2 h, evaporated to dryness, and the residue was re-evaporated from toluene (10 mL×3). The product acid chloride was dissolved in 50 mL CH_2Cl_2 and the solution was chilled to −30°C. In a separate flask, *N*-methylmorpholine (13.7 mL, 124.7 mmol) was added to a suspension of **8** (25.8 g, 57.3 mmol) in 300 mL CH_2Cl_2. The resulting solution was added dropwise over 20 min to the above acid chloride solution, keeping the temperature <−20°C. The bath temperature was kept at −35 to −40°C to allow a fast rate of addition. After stirring at −25°C for 20 min, the bath was removed, and the reaction was quenched with water and stirred for 5 min. The mixture was washed with 400 mL water (pH 1), 200 mL water, 400 mL 5% $NaHCO_3$, and 200 mL water. All aqueous layers were backwashed with CH_2Cl_2 (20 mL×2). The combined organic layers were dried ($MgSO_4$), stirred with Darco (30 g) for 15 min, and filtered through a pad of Celite. The filtrate was evaporated to ~100 mL, and *t*-butylmethyl ether (300 mL) was added. The mixture was stirred at 25°C for 1 h and overnight at 5°C. The crystals were filtered and the filter cake was stirred with *t*-butylmethyl ether on the funnel and filtered. This washing process was repeated one more time and then the product was dried overnight under vacuum to give 31.96 g (88%) of **9**, mp 157–158°C. Anal. Calcd for $C_{29}H_{23}N_2O_4S_2Cl_3$·0.03 H_2O: C, 54.89; H, 3.66; N, 4.42; S, 10.11; Cl, 16.76; H_2O, 0.09. Found: C, 54.53; H, 3.69; N, 4.20; S, 9.87; Cl, 16.75; H_2O, 0.10 (KF).

(6*R*)-*trans*-3-Chloromethyl-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, diphenylmethyl ester, HCl salt (8). (*Method B*) The mentioned compound (248 g, 0.55 mol) was treated with $NaHCO_3$ (56 g, 0.66 mol) in water (1.6 L) at 0°C. The mixture was stirred at 0°C for 0.5 h and then CH_2Cl_2 (1.5 L) was added. The biphasic mixture was filtered through Celite and the Celite pad was washed with CH_2Cl_2 (2 L total). The layers were separated and the organic solution was dried over anhydrous $MgSO_4$, filtered, and concentrated to a volume of ca. 2 L. The free amine solution was then added to a mixture of 2,5-dichlorothiophenylacetic acid (130 g, 0.55 mol) and dicyclohexylcarbodiimide (144 g, 0.70 mol) in THF (1 L) at rt. The reaction mixture was stirred for 2.5 h and then was filtered through Celite, washing the Celite pad with several portions of acetone. The filtrate was concentrated in vacuo to give a solid mass. The solid was slurried in Et_2O and then collected by filtration, washing the solid with several portions of Et_2O. The solid was dried under high vacuum over P_2O_5 to give 268 g (77%) of **9**, mp 120°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.43 (d, J=18 Hz, 1H), 3.59 (d, J=18 Hz, 1H), 3.69 (d, J=17 Hz, 1H), 3.79 (d, J=17 Hz, 1H), 4.36 (d, J=12 Hz, 1H), 4.41 (d, J=12 Hz, 1H), 4.98 (d, J=5 Hz, 1H), 5.81 (dd, J=5.9 Hz, 1H), 6.98 (s, 1H), 7.14–7.44 (m, 14H). Anal. Calcd for $C_{29}H_{23}N_2O_4S_2Cl_3$: C, 54.94; H, 3.66; N, 4.42. Found: C, 55.18; H, 3.84; N, 4.62.

(6*R*,7*R*)-7-[(2,5-Dichlorophenyl)thiomethylcarbonyl-amino]-3-chloromethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid (10). To a slurry of benzhydryl ester **9** (93.0 g, 146.7 mmol) in CH_2Cl_2 (465 mL) chilled in an ice-bath was added anisole (18.6 mL) and then TFA (139.5 mL, added over 5 min). After 40 min the ice-bath was removed and the solution was diluted with toluene (930 mL). The mixture was evaporated on a rotary evaporator at a bath temp. of 40°C to ~500 mL volume. During the evaporation a solid formed. CH_2Cl_2 (300 mL) was added and the resulting solution was evaporated to ~500 mL volume. This last process was repeated two more times to remove any residual TFA. The mixture was stirred at rt for 1 h. The solid was filtered and the filter cake was washed with toluene (4×270 mL); each time the solid was slurried in the funnel and sucked dry by compacting. Finally, the product was dried under vacuum overnight to give 75.28 g of microscopic glassy beads. The material was dissolved in THF (120 mL) and hexane (10 mL) was added. A massive precipitate formed immediately. The mixture was stirred as more hexane (400 mL) was added slowly. After stirring for 1 h the solid was filtered, washed with hexane, and dried under vacuum overnight to give 70.08 g (93.8%) of **10**, mp ~136–140°C dec. ^{1}H NMR (THF-d_8, 270 MHz) δ 3.4–3.7 (ABq, J=18.1 Hz, 2H), 3.7 (s, 2H), 4.5–4.6 (AB q, J=11.7 Hz, 2H), 5.0 (d, J=4.7 Hz, 1H), 5.7–5.8 (dd, J=4.7 and 8.5 Hz, 1H), 7.0–7.1 (dd, J=2.4 and 8.5 Hz, 1H), 7.3 (d, J=8.2 Hz, 1H), 7.5 (d, J=2.3 Hz, 1H), 8.4 (d, J=8.5 Hz, 1H); ^{13}C NMR (THF-d_8, 68 MHz) δ, 27.4, 35.9, 44.4, 58.9, 60.7, 125.7, 127.2, 128.3, 131.2, 134.0, 140.0, 163.1, 165.2, 168.6; IR (KBr) 3500, 3270, 1775, 1653 cm^{-1}, $[\alpha]_D$=61.3 (c=1, THF); Anal. Calcd for $C_{16}H_{13}N_2O_4S_2Cl_3$·0.13 H_2O·0.85 THF: C, 44.05; H, 3.73;

N, 5.14; S, 11.80; Cl, 19.62; H_2O 0.41. Found: C, 43.85; H, 3.81; N, 5.27; S, 12.07; Cl, 20.01; H_2O 0.44 (KF).[24]

2,6-Dichloropyridine, 1-oxide (12). An aqueous solution of H_2O_2 (30%, 62.3 mL, 550 mmol) was added dropwise over 5 min to a solution of 2,6-dichloropyridine (**11**) (44.4 g, 300 mmol) in TFA (350 mL). The solution was heated to reflux with a heating mantle behind a safety shield; vigorous evolution of gas was observed. After 1 h, HPLC showed starting material and product in a ratio of 8.5:91.5. This ratio was unchanged after 2 h. After 3 h, the reaction mixture was cooled to 25°C and evaporated to 2/3 volume behind a safety shield. The residue was added with stirring to a mixture of 700 mL H_2O and 100 mL ice. After stirring for 10 min, the precipitated solid was filtered off and washed with 50 mL of H_2O. The filter flask was changed and the solid on the filter was washed further with H_2O (4×50 mL). The solid was dried under vacuum overnight to give 6.48 g of 2,6-dichloropyridine (15% recovered, contained ~10% product **12**). The pH of the aqueous filtrate was carefully adjusted to pH 9 (pH meter) with solid K_2CO_3 (foaming!) and then extracted with CH_2Cl_2 (4×200 mL). The organic extract was dried ($MgSO_4$) and evaporated to ~50 mL. Crystals started forming in the flask. Heptane (200 mL) was added slowly with rapid stirring. The mixture was evaporated to remove ~50 mL of the solvent. The precipitated solid was filtered, washed with heptane, and dried under vacuum to give 41.63 g (84% yield, 99% based on the recovered starting material) of **12**, mp 137–138°C. Anal. Calcd for $C_5H_3NOCl_2 \cdot 0.04\ H_2O \cdot 0.02\ CH_2Cl_2$: C, 36.23; H, 1.89; N, 8.42; Cl, 43.46; H_2O, 0.43. Found: C, 36.60; H, 1.81; N, 8.33; Cl, 43.82; H_2O, 0.49 (KF).

2,4,6-Trichloropyridine (13). A 5 L four-necked Morton flask was equipped with a mechanical stirrer, a liquid addition funnel, a thermocouple, and a nitrogen inlet. The flask was flushed with N_2 gas and charged with finely powdered LiCl (87 g, 2.05 mol) and then $POCl_3$ (520 mL) via the addition funnel. The mixture was heated to 75°C and the liquid addition funnel was replaced with a solid addition funnel. *N*-Oxide **12** (350 g, 2.13 mol) was introduced portionwise via the solid addition funnel. An exotherm was observed in a few min. The temperature was maintained at 85–90°C by controlling the rate of addition of **12** and by external cooling when necessary; the addition was complete in 2 h. After an additional 3 h at 85°C the reaction was complete by HPLC. The mixture was allowed to slowly cool to 60°C and then poured in portions into a mixture of 1 kg ice and 500 mL H_2O in a beaker cooled in an ice-bath. The temperature of the mixture was ~30°C during the later part of the quenching process. After stirring for 0.5 h 1 L of hexane was added. Aqueous NaOH (1N, 900 mL) was added with cooling at a rate such that the temperature remained 30–40°C. The organic layer was separated and the aqueous layer was extracted with *n*-heptane (2×1 L). The combined organic extract was dried over finely powdered anhydrous Na_2SO_4 and filtered. The product partly crystallized on concentration of the filtrate. After cooling at −5°C for 4 h the liquid portion was decanted and then the solid was filtered with suction and dried under vacuum for a few minutes to give 231.8 g (60%) of product **13.**

[(2,6-Dichloropyrid-4-yl)thio]acetic acid (14). MeOH (100 mL) was added to a solution of NaOH (16.06 g, 401.56 mmol) in H_2O (30 mL) at 25°C and the solution was sparged with argon for 15 min. After cooling to 0°C, the reaction vessel was charged with mercaptoacetic acid (17.99 g, 195.34 mmol) and the colorless solution was sparged with argon for an additional 15 min. In a separate flask a solution of 2,4,6-trichloropyridine (**13**) (35.64 g, 195.3 mmol) in MeOH (65 mL) was sparged with argon for 15 min at 25°C and then added in one portion to the above solution. The transfer was completed with MeOH (25 mL), the resulting heterogeneous mixture was further diluted with MeOH (250 mL) to obtain a clear solution. The solution was stirred at 0°C for 1.5 h and then warmed to rt and stirred overnight. After a total reaction time of 16.5 h the milky mixture was diluted with MeOH and H_2O to solubilize the insoluble material. The solution was concentrated to remove most of the MeOH and then partitioned between H_2O (600 mL) and CH_2Cl_2 (250 mL). The aqueous layer was separated and washed with CH_2Cl_2 (100 mL). The volatile organics were removed from the milky aqueous phase in vacuum and the resulting clear solution was stirred and heated to 80°C. The pH of the hot solution was lowered from 9.90 to 3.45 with conc. H_3PO_4 and then the mixture was seeded with crystals of acid **14** and stirred for 1 h. Since there was no evidence of crystallization, the pH of the solution was lowered further to 3.15 with H_3PO_4. After 1 h a significant amount of a white crystalline material had formed. The pH was lowered further stepwise in a fashion for slow crystallization at 80°C; pHs: 3.00 (15 min), 2.85 (15 min) and 2.35 (2.5 h). The heating was stopped and the mixture was stirred overnight at 25°C. The solid was filtered, washed with H_2O (2×250 mL), air-dried (45 min), and then placed under vacuum overnight to give **14** as a white crystalline material (39.07 g, 84%). Recrystallization of 25.12 g of this material from EtOH (50 mL)/heptane (450 mL) furnished 20.46 g (68% overall yield) of **14** as fluffy white crystals, mp 149°C. Anal. Calcd for $C_7H_5NO_2SCl_2$: C, 35.31; H, 2.12; N, 5.88; S, 13.47; Cl, 29.78. Found: C, 35.19; H, 1.86; N, 5.79; S, 13.35; Cl, 30.09.

(6*R-trans*)-3-(Chloromethyl)-7-[[[(2,6-dichloro-4-pyridinyl)thio]acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid, diphenylmethyl ester (15). NMM (1.93 mL, 17.54 mmol) was added to a suspension of acid **14** (4.16 g, 17.5 mmol) and **8** (7.91 g, 17.54 mmol) in CH_2Cl_2 (88 mL) stirred at ice-bath temperature to obtain a clear solution.[25] EDC[26] (4.03 g, 21.0 mmol) was added as a solid and the resulting solution was stirred in the ice-bath. After 1 h, TLC analysis[27] showed no **8** remaining. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed successively with very dilute aqueous HCl (0.04N, 50 mL), brine, 5% $NaHCO_3$, and brine. The solution was dried ($MgSO_4$) and evaporated to ~50 mL. Crystals formed during evaporation of the solvent. *t*-Butylmethyl ether (140 mL) was added slowly, and after stirring for 30 min, the crystals were filtered, washed with *t*-butylmethyl ether, and dried under vacuum to give 10.37 g (93%) of chloroester **15** as microscopic needles, mp 178–180°C (dec.). ^{1}H NMR ($CDCl_3$, 270 MHz) δ 3.4–3.8 (m, 4H), 4.4 (d, J=3 Hz, 1H), 4.9 (d, J=4.7 Hz, 1H), 5.8 (m, 1H), 6.9 (s, 1H), 7.3–7.4 (m, 11H); ^{13}C NMR ($CDCl_3$, 68 MHz) δ, 27.3, 34.4, 43.0, 57.5, 59.3, 80.0, 119.1, 125.6, 127.0, 127.1,

127.8, 128.2, 128.4, 128.5, 128.6, 138.8, 138.9, 150.9, 151.8, 160.3, 163.8, 166.5; IR (KBr) 3500, 3271, 1777, 1723, 1653 cm^{-1}; MS (ESI) M+H=635; $[\alpha]_D$=24.1 (c=0.5, THF). Anal. Calcd for $C_{28}H_{22}N_3O_4S_2Cl_3$: C, 52.96; H, 3.49; N, 6.62; S, 10.10; Cl, 16.75. Found: C, 52.95; H, 3.26; N, 6.60; S, 10.11; Cl, 16.45.

(6*R*-*trans*)-3-(Chloromethyl)-7-[[[(2,6-dichloro-4-pyridinyl)thio]acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid (16). TFA (25.0 mL) was added dropwise to a stirred suspension of ester **15** (16.70 g, 26.3 mmol) and anisole (8.56 mL, 78.9 mmol) in CH_2Cl_2 (83.0 mL) chilled in an ice-bath. After 40 min, TLC (silica gel, EtOAc/hexane 3:7) showed no ester **15**. After a total of 1 h, toluene (217 mL) was added to the reaction mixture and the mixture was evaporated to ~1/2 volume (a precipitate formed during the evaporation). Toluene (100 mL) was added and the mixture was evaporated to ~200 mL and then stirred in an ice-bath for 30 min. The product was filtered, washed twice with cold toluene (40 mL), and dried under vacuum over NaOH pellets overnight to give 12.41 g (98%) of chloroacid **16**,[28] mp 173–175°C (dec). ^{1}H NMR ($CDCl_3$, DMSO-d_6, 270 MHz) δ 3.5–3.7 (ABq, J=17.8 Hz, 2H), 3.8–4.0 (ABq, J=15.6 Hz, 2H), 4.4–4.6 (ABq, J=11.1 Hz, 2H), 5.0 (d, J=4.7 Hz, 1H), 5.2 (br s, 2H), 5.70–5.75 (dd, J=4.7 Hz, 1H), 7.3 (s, 2H), 9.3 (d, J=8.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, DMSO-d_6, 68 MHz) δ 26.8, 34.0, 43.5, 57.8, 59.8, 119.1, 124.9, 126.7, 150.0, 154.3, 162.8, 164.2, 168.1; IR (KBr) 3263, 1776, 1714, 1651 cm^{-1}; MS (ESI) M+H=464, M−H=462; $[\alpha]_D$=80.5 (c=0.8, THF). Anal. Calcd for $C_{15}H_{12}N_4O_4S_2Cl_3$·0.065 H_2O·0.085 TFA: C 37.99; H, 2.57; N, 8.76; S, 13.37; Cl, 22.17; F, 1.01; H_2O, 0.24. Found: C, 38.31; H, 2.33; N, 8.61; S, 13.18; 22.23; F, 1.28; H_2O, 0.25 (KF).[29]

4-Pyranone (18). A literature procedure[19] was modified as follows. A 1 litre, three-necked flask was equipped with a mechanical stirrer, thermometer, and a Claison adapter connected to a condenser on top. The flask was charged with anhydrous chelidonic acid (**17**)(100 g, 530 mmol), dibutylphthalate (300 mL), Cu-bronze (10.85 g), and 2,2′-dipyridyl (3.8 g, 24.4 mmol). The mixture was stirred slowly under argon and heated with a heating mantle to ~210°C under an argon atmosphere. The flow of argon gas was stopped and the progress of the reaction was monitored by the evolution of CO_2. After ~1/2 h the rate of evolution of CO_2 decreased considerably. The mixture was stirred vigorously for about 30 s, cooled, and then filtered through a glass wool plug for a rapid filtration. Insoluble material was washed separately with EtOAc and filtered, and the filtrate was evaporated to remove EtOAc. This mixture of product **18** and dibutyl phthalate was further dried under pump vacuum for 1 h and combined with the first bulk filtrate. The product, a solid, was distilled under vacuum without the use of a condenser, leaving behind the high boiling solvent dibutyl phthalate. The receiver flask was cooled in an ice-bath. During distillation a heat-gun was used to melt any solid in the receiver adapter. The product **18** distilled at 60–65°C/0.6 mm, bath temp. ~110–130°C. The solid was melted and stirred with 50 mL heptane. After stirring in an ice-bath for 0.5 h the solid product was filtered, washed with heptane, and dried under vacuum for 0.5 h to give 45.46 g (89%) of 4-pyranone **18**.

4-Thiopyranone (19). A literature procedure[20] was modified as follows. Molecular sieves (2.0 g, 4 Å, spherical) were added to a solution of pyranone **18** (24.25 g, 250 mmol) in dry toluene (400 mL) and the mixture was deoxygenated twice by evacuation and release of vacuum with argon. After the mixture was stirred overnight, Lawesson's reagent (57.26 g, 142 mmol) was added and the mixture was heated to 80°C in an oil-bath. The mixture stayed heterogeneous due to the crystallization of the trimer byproduct **23**. After 1 h the mixture was cooled and filtered through 200 mL of silica gel in a sintered glass funnel. The cake was washed with 200 mL toluene and 300 mL 20% EtOAc in toluene until the red-orange color was removed. The filtrate was evaporated to give 62 g of a solid. The product was distilled in a Kugelrohr assembly with the receiver bulb cooled in a dry ice-acetone bath. Distillation at 45–80°C (oven temperature) under vacuum (1 mm) gave 28 g (quantitative, uncorrected yield) of 4-thiopyranone **19**. The product was redistilled as above to furnish 26.57 g (95%) of 4-thiopyranone **19**.

(*S*)-α-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-thioxo-1(4*H*)-pyridinepentanoic acid (21). A mixture of 4-thiopyrone **19** (7.43 g, 66.27 mmol)], L-α-*N*-Boc-ornithine **20** (15.39 g, 66.27 mmol), absolute ethanol (70 mL), distilled water (70 mL), and pyridine (5.4 mL, 66.27 mmol) was refluxed under an argon atmosphere. Within 5 min. all the material was in solution. After 6.3 h the heating bath was removed and stirring was continued at 25°C for 30 min. Methanesulfonic acid (4.4 mL, 66.27 mmol) was added and the mixture was stirred for 10 min. The volatiles were removed under vacuum and the resulting orange solution was treated with EtOAc (200 mL) and H_2O (200 mL). The aqueous phase was saturated with NaCl, stirred vigorously for 10 min, and then the phases were separated. The aqueous fraction was re-saturated with NaCl and extracted with EtOAc (4×200 mL). The combined organic fractions were dried ($MgSO_4$) and stirred with activated charcoal (0.226 g) for 20 min. The mixture was filtered through a pad of Celite and the filtrate was evaporated. The residue was dried under high vacuum to a constant weight to give **21** as a yellow amorphous solid (16.84 g, 78%). mp 90°C (eff., contraction at 60°C). ^{1}H NMR (DMSO-d_6, 270 MHz) δ 1.4 (s, 9H), 1.5–1.8 (m, 4H), 3.9 (m, 3H), 7.1 (m, 1H), 7.2 (d, J=7.0 Hz, 2H), 7.6 (d, J=7.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, 68 MHz) δ 27.1, 27.4, 28.2, 53.0, 56.0, 78.1, 130.2, 136.2, 155.6, 173.8, 188.9; IR (KBr) 3490, 1703, 1624 cm^{-1}; MS (ESI) M+H=327; $[\alpha]_D$=+1.4 (c=1.0, CH_3CN). Anal. Calcd for $C_{15}H_{22}N_2O_4S$·0.16 H_2O·0.31 EtOAc: C, 54.69; H, 7.01; N, 7.86; S, 8.99; H_2O, 0.8. Found: C, 54.73; H, 7.02; N, 7.78; S, 8.86; H_2O, 0.8 (KF).

[6*R*-[3(S*),6α,7β]]-(4-[[[2-Carboxy-7[[[(2,5-dichlorophenyl)thio]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-3-yl]methyl]thio]-1[[[4-carboxy-4(1,1-dimethylethoxy)-carbonyl]amino]butyl]pyridinium, chloride (27). To a solution of **10** (80.93 g, 153 mmol) in THF (400 mL) under an argon atmosphere was added **21** (54.89 g, 153 mmol), and the mixture was stirred at 25°C. The reaction was monitored by HPLC; after 2.8 h the solution

was added over 2 min to *t*-butylmethyl ether (1700 mL) stirred mechanically in a Morton flask. The mixture was stirred for 1 h, filtered, and then the filter cake was washed thoroughly by slurrying with *t*-butylmethyl ether (3×200 mL) in the filtration funnel. The product was dried under vacuum overnight to give 123.68 g (96.8%) of chloride salt **27** as a yellow powder, mp. 155–160°C (eff.). ^{1}H NMR (DMSO-d_6, 270 MHz) δ 1.4 (s, 9H), 1.7–1.9 (m, 5H), 3.3–3.9 (m, 4H), 4.0 (s, 2H), 4.5 (m, 4H), 5.2 (d, J=5.3 Hz, 1H), 5.6–5.7 (dd, J=4.7 and 8.2 Hz, 1H), 7.1 (d, J=8.2 Hz, 1H), 7.2 (d, J=2.3 Hz, 1H), 7.4 (d, J=2.3 Hz, 1H), 7.5 (s, 1H), 8.0 (d, J=7 Hz, 2H), 8.8 (d, J=6.5 Hz, 2H), 9.4 (d, J=8.0, 1H); IR (KBr) 3500, 1778, 1695, 1630 cm^{-1}; MS (ESI) M−Cl=757, M−HCl−H=755; $[\alpha]_D$=−5.9 [c=1, THF]. Anal. Calcd for $C_{32}H_{35}N_4O_8S_3Cl_3$·0.65 H_2O·0.2 THF·0.2 $(CH_3)_3COCH_3$: C, 47.10; H 4.85; N, 6.69; S, 11.48; Cl, 12.69; H_2O, 1.40. Found: C, 46.70; H 4.91; N, 6.64; S, 11.03; Cl, 12.81; H_2O, 1.36 (KF).

[6*R*-[3(S*),6α,7β]]-(4-[[[2-Carboxy-7[[[(2,6-dichloro-4-pyridinyl)thio]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-3-yl]methyl]thio]-1[[[4-carboxy-4(1,1-dimethyl-ethoxy)-carbonyl]amino]butyl]pyridinium, chloride (29). Chloroacid **16** (517 mg, 1.1 mmol) was dissolved in DMF (4 mL) and then thiopyridone **25** (348 mg, 1.0 mmol) was added. This mixture was stirred for 5 h at rt. The DMF solution was added dropwise to 150 mL of vigorously stirred diethyl ether. The precipitated solid was collected by filtration. This solid was stirred with 25 mL of acetone for 15 min and then collected by filtration to give 584 mg (71%) of **29** as its sodium salt. This material was used without further purification for the preparation of cephem **2**.

(6*R-trans*)-7-[[[2,5-Dichlorophenyl)thio]acetyl]amino]-3-[(pyridinylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid (32). NaI (4.62 g, 30.8 mmol) was added portionwise over 2 min to an orange solution of 4-mercaptopyridine (3.78 g, 34 mmol) in 100 mL of anhydrous DMF under an argon atmosphere. The mixture was stirred vigorously with an overhead stirrer for 8 min to dissolve most of the salt. Chloroacid **10** (14.4 g, 30.1 mmol) was added and, after stirring for 5 min (exotherm 22° to 29.4°C), 2,6-lutidine (3.82 g, 35.6 mmol) was added. After 40 min the dark red-brown solution was diluted with 60 mL CH_3CN and 40 mL of water and stirred for 5 min. The solution was further diluted with 140 mL of CH_3CN and 60 mL water to precipitate the product. The suspension was stirred for 35 min at 25°C. The mixture was filtered and the solid was washed with 100 mL of CH_3CN, 100 mL of 1:1 CH_3CN/H_2O, and CH_3CN (2×50 mL). The isolated solid was dried under vacuum over P_2O_5 for 60 h to give 14.3 g (83.0%) of **32** as a yellow solid. ^{1}H NMR (DMSO-d_6, 270 MHz) δ 3.5–3.6 (ABq, J=17.8 Hz, 2H), 3.9 (s, 3H), 4.2–4.3 (ABq, J=12.9 Hz, 2H), 5.1 (d, J=4.7 Hz, 1H), 5.7 (dd, J=4.7 and 7.7 Hz, 1H), 7.2 (d, J=8.8 Hz, 1H), 7.3 (d, J=4.7 Hz, 2H), 7.5 (m, 2H), 8.4 (d, J=4.7 Hz, 2H), 9.3 (d, J=8.2 Hz, 1H); ^{13}C NMR (DMSO-d_6, 68 MHz) δ 27.2, 32.8, 34.1, 57.6, 59.2, 121.3, 125.8, 126.15, 126.2, 128.8, 130.7, 132.5, 137.8, 147.8, 149.2, 163.0, 164.2, 168.1; IR (KBr) 3270, 1767, 1649 cm^{-1}; Anal. Calcd for $C_{21}H_{17}N_3O_4S_3Cl_2$·0.67 CH_3CN·0.37 H_2O: C, 46.53; H, 3.45; N, 8.92; S, 16.68; Cl, 12.3; H_2O, 1.16. Found: C, 46.79; H, 3.19; N, 9.13; S, 16.49; Cl, 12.16; H_2O, 1.18 (KF). This material was used in the next reaction without further purification. A 3 g sample of crude product was crystallized from DMF–CH_3CN to give 2.71 g (90.3%) of **32**, mp >145°C (dec.).

(6*R-trans*)-7-[[[(2,6-Dichloro-4-pyridinyl)thio]acetyl]-amino]-3-[(4-pyridinyl-thio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (33). NaI (0.937 g, 6.26 mmol) was added portionwise to a rapidly stirred solution of 4-mercaptopyridine (0.768 g, 6.56 mmol) in DMF (15.6 mL) at 25°C. Chloroacid **16** (3.0 g, 6.26 mmol) was added to the reaction mixture and after 2 min 2,6-lutidine (0.87 mL, 7.51 mmol) was added. The product **33** precipitated from the reaction mixture. After 15 min, isopropyl alcohol (31 mL) was added dropwise and the mixture was stirred for 15 min. The solid was filtered, washed with isopropyl alcohol (25 mL), water (25×2 mL),[30] acetone (25 mL), and dried under vacuum to give 3.15 g of crude **33**. This material was powdered with a stirring rod and added to DMF (19 mL) with rapid stirring. The solid initially dissolved and then precipitated. After stirring the suspension for 10 min, THF (19 mL) was added and the mixture was stirred further for 15 min. The solid was filtered, washed with THF (15 mL×2), and dried under vacuum overnight to give 2.99 g (82%) of the mercaptopyridyl acid **33** mp 160–161°C (effer.). ^{1}H NMR (DMSO-d_6, 270 MHz) δ 3.5–3.8 (ABq, J=18.2 Hz, 2H), 4.0 (s, 1H), 4.2–4.3 (ABq, J=12.9 Hz, 2H), 5.1 (d, J=4.7 Hz, 1H), 5.6–5.7 (dd, J=4.7 Hz, 1H), 7.3 (m, 2H), 7.5 (s, 2H), 8.4 (d, J=5.8 Hz, 2H), 9.3 (d, J=8.3 Hz, 1H); ^{13}C NMR (DMSO-d_6, 68 MHz) δ 27.2, 32.8, 33.2, 57.5, 59.3, 119.2, 121.3, 125.8, 126.2, 147.7, 149.1, 149.2, 155.3, 163.0, 164.1, 167.7. IR (KBr) 3272, 1779, 1672 cm^{-1}; $[\alpha]_D$=34.3 (c=0.5, pyridine); MS (ESI) M+H=543, M−H=541. Anal. Calcd for $C_{20}H_{16}N_4O_4S_3Cl_2$·0.09 H_20·0.5 DMF: C, 44.40; H, 3.41; N, 10.84; Cl, 12.19; H_2O, 0.28. Found: C, 44.06, H, 3.59, N, 11.12; Cl, 12.22; H_2O, 0.27 (KF).

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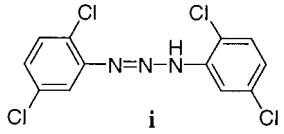
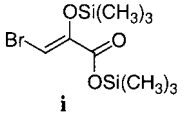


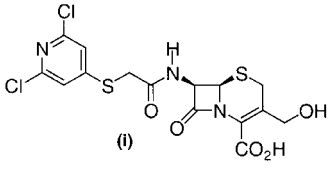
i

18. Kaneko, C.; Uchiyama, K.; Sato, M.; Katagiri, N. *Chem. Pharm. Bull.* **1986**, *34*, 3658.
19. DeSouza, C.; Hajikarimian, Y.; Sheldrake, P. W. *Synth. Comm.* **1992**, *22*, 755.
20. Inoue, S.; Iwamatsu, K.; Kai, F.; Katano, K.; Kondo, S.; Ogino, H.; Okamoto, R.; Sezaki, M.; Tsuruoka, T.; Yoshida, T. Patent, EP020971, Jan. 28, 1987 (**19** isolated by chromatography).
21. Quaternization of **33** in the absence of BSA was incomplete even after the use of 5 mol equiv. of bromopyruvic acid; also, a low quality product (HPLC AP 40–50%) was obtained. The product **4** reacted further with bromopyruvic acid according to LC/MS data (Ms. B. Warrack, AR&D, Princeton).
22. In a separate experiment, reaction of bromopyruvic acid with 2 mol of BSA formed the silylenolester (i). The structure of this compound was consistent with its ^{1}H NMR. Silyl enolester (i) was unreactive towards presilylated **33**.

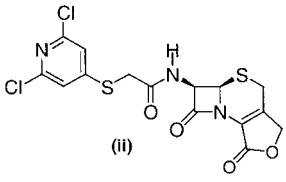


i

23. Bronson, J.; D'Andrea, S. V.; Hoeft, S. E.; Matiskella, J. D.; Misco Jr., P. F.; Luh, B. Y.; Springer, D. M.; Ueda, Y.; Wichtowski, J. A. U.S. patent 5,668,290, Sep. 16, 1997.
24. For ^{1}H NMR in DMSO-d_6, a fresh sample of **10** was used, since it is unstable in DMSO-d_6. An impurity observed in a freshly made solution increased from 2–5% to 25% over 8 h at rt. Over 1.5 days it decomposed to a mixture of products. $CDCl_3$ with a few drops of DMSO-d_6 is a better solvent system for NMR spectra. We have found that **10** has much better stability in THF; the ^{1}H NMR was almost unchanged after 24 h at rt. The NMR spectra were taken in deuterated THF.
25. This in situ procedure was developed to prevent scale up problems associated with extraction of the free base for use in the acylation reaction. The free base also is less stable.
26. The coupling reagent DCC was replaced by water soluble carbodiimide EDC to avoid purification problems caused by the presence of the DCU byproduct.
27. TLC: Silica gel, EtOAc, aliquot was spotted after neutralization with Et_3N, visualized by UV, R_f **8** as free base=0.53.
28. **16** decomposed to the hydroxy acid (i), lactone (ii) and other byproducts during HPLC analysis.



(i)



(ii)

29. Due to the instability of **16** in DMSO-d_6, a freshly prepared solution in $CDCl_3$/DMSO-d_6 was used for the NMR analyses.
30. Inorganic salts were removed during slurrying of the product with water in the funnel. DMF was also removed during this wash.