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Synthesis and Structure–Activity Relationships of Cephalosporins, 2-Isocephems, and 2-Oxaisocephems with C-3′ or C-7 Catechol or Related Aromatics

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Abstract—A series of cephalosporins, 2-isocephems, and 2-oxaisocephems with C-3′ catechol-containing (pyridinium-4-thio)methyl groups and 2-isocephems with C-7 catechol related aromatics have been prepared and evaluated for antimicrobial activity. It turns out that these compounds have highly potent activity against Gram-negative bacteria, especially resistant pathogens such as *Pseudomonas aeruginosa*. The most active compound of the series was (6*S*,7*S*)-7-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-[(1,5-dihydroxy-4-pyridon-2-yl)methoxy]imino]acetamido]-3-[[[(4-methyl-5-carboxymethyl)thiazol-2-yl] thio] methyl] -8-oxo-1-aza-4-thiabicyclo [4.2.0] oct-2-ene-2-carboxylic acid which exhibited potent in vitro activity against clinically isolated *P. aeruginosa* and *Acinetobacter baumanii* which is also resistant to many anti-infectives, and good in vivo efficacy against clinically isolated *P. aeruginosa*.

Introduction

Recently, compromised hosts from the use of antitumour drugs, immunosuppressants, and radiotherapy have increased, while the increasing aging society has brought about an augmentation of patient with chronic disease, so that the opportunistic infections attributed to miscellaneous Gram-negative bacteria such as *Serratia marcescens*, *Proteus vulgalis*, *A. baumanii*, and *P. aeruginosa* have come to be a serious problem in chemotherapy. In particular, *P. aeruginosa* has resistance to most anti-infectives, and causes bacterial pneumonia, meningitis, and sepsis. In addition, some strains of this bacteria are highly resistant to clinically used anti-pseudomonal agents, such as gentamicin,[1] cefsulodin,[2] ceftazidime,[3] cefpiramide,[4] aztreonam,[5] and imipenem.[6]

Recently, it has been reported that the introduction of a catechol moiety or its bioisostere at C-7[7–11] or C-3′[12–18] of cephalosporins give rise to improved activity against Gram-negative bacteria including *P. aeruginosa*. As a part of our efforts to find more effective antimicrobials against resistant pathogen like *P. aeruginosa*, we investigated 2-isocephems[19–23] bearing a catechol moiety or its bioisostere at C-7 to find that 1,3-dihydroxy-4-pyridone moiety showed potent activity against *P. aeruginosa*.[24] Further investigation on the antipseudomonal activity of new cephalosporins with these substituents revealed that cephalosporins having C-3 catechol-containing (pyridinium-4-thio)methyl groups possessed good in vitro and in vivo activity against *P. aeruginosa*.[25] Encouraged by this result, we examined the activity of the corresponding 2-isocephems and 2-oxaisocephems.

In this paper, we describe the synthesis and structure–activity relationships of cephalosporins, 2-isocephems, and 2-oxaisocephems with C-3′ or C-7 catechol or its bioisostere.

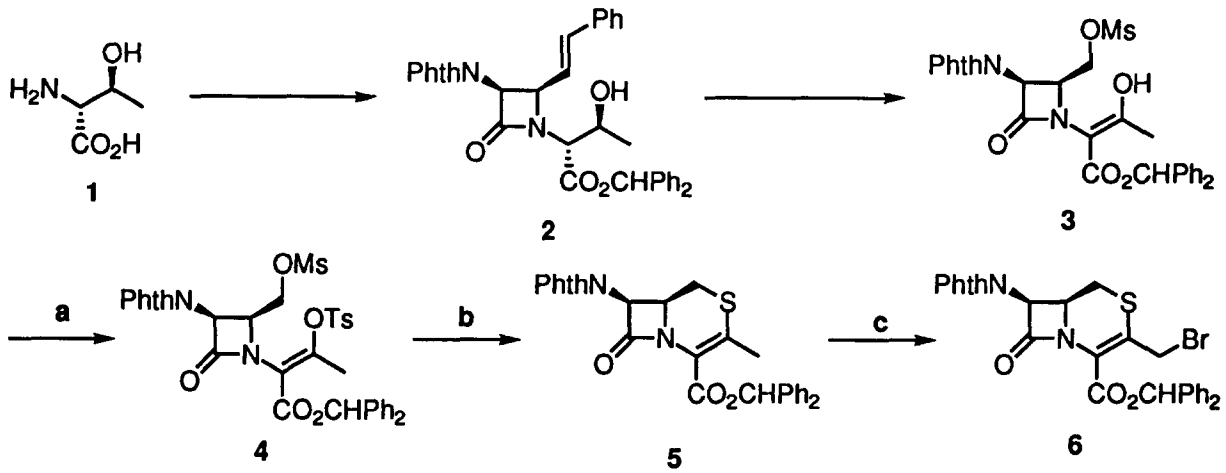
Key words: *Pseudomonas aeruginosa*; cephalosporin; 2-isocephem; catechol; 1,3-dihydroxy-4-pyridone.

Chemistry

2-Isocephems bearing a catechol or 1,3-dihydroxy-4-pyridone moiety at C-7

We selected the 3-bromomethyl derivative **6**[24] as the key intermediate in the preparation of new optically active 2-isocephems. Compound **6** was easily obtained from the lactam enol (**3**) derived in five steps from D-threonine (**1**) via **2**.[26] Tosylation of **3** with *p*-toluenesulfonyl chloride in the presence of *N*-methylpyrrolidine gave tosylate **4**. (6*S*,7*S*)-7-Phthalimido-3-methyl-2-isocephem (**5**) was obtained by treatment of **4** with hydrogen sulfide. The resulting **5** was converted to **6** by free radical bromination with *N*-bromosuccinimide (NBS) in the presence of 2,2′-azobis(isobutyronitrile) (AIBN) (Scheme 1).

Next, C-3′ substituents were introduced by treatment of **6** with thiol derivative[27–32] (Scheme 2) to afford **7a–g** after esterification with diphenyldiazomethane in the case of thiol derivative bearing carboxyl group. After deprotection of the phthalimido group by methylhydra-



Scheme 1. (a) *p*-TsCl/*N*-methylpyrrolidine; (b) H_2S/Et_3N; (c) NBS/AIBN.

zine, generated amines **8a–g** were reacted with 1-hydroxybenzotriazole (HOBT) ester of protected 2-aminothiazole derivatives with 1,3-dihydroxypyridone **9**,[7] or catechol **12** to give compounds **10a–g**, **13b,e**. Finally, removal of all of the protective groups of **10a–f**, **13b,e** was achieved using trifluoroacetic acid (TFA) in the presence of anisole or 95% TFA to afford 2-isocephems **11a–f**, **14b,e**. [(4′-Pyridyl)thio]methyl derivative **10g** was quaternarized by *t*-butyl bromoacetate before removal of the protective groups.

The synthetic route to the aminothiazole derivative with catechol (**12**) is shown in Scheme 3. Substituted benzyl bromide **15**[33] was converted to *N*-hydroxyphthalimide derivative **16**, which was subjected to hydrazinolysis followed by condensation with aminothiazole glyoxylic acid **17**[34] to afford **12**.

Cephalosporins, 2-isocephems, and 2-oxaisocephems bearing a catechol or 3-hydroxy-4-pyridone moiety at C-3′

Cephalosporin derivatives were prepared from *p*-methoxybenzyl (6*R*,7*R*)-7-amino-3-chloromethyl-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate hydrochloride **18**[35] (Scheme 4). The [(4-pyridyl)thio]methyl group was introduced to C-3′ by treatment of **18** with 4-mercaptopyridine. The obtained **19** was coupled with active ester derived from 2-aminothiazole derivative **20**[36] and HOBT to afford **21**. After quaternarization of **21** by alkyl halides **22a**,[37]**b**, deprotection was achieved using TFA in the presence of anisole to give catechol derivatives **23a,b**. In case of the pyridone derivative **22c**,[38] **21** led first to the amino acid **24**. Compound **24** was quaternarized by iodide derived from **22c** after treatment with *N,O*-(bistrimethylsilyl)acetamide (BSA), then the benzhydryl group was removed by TFA in the presence of anisole to furnish **23c**.

2-Isocephem derivatives were synthesized from 3-[(4-pyridyl)thio]methyl derivative **7g** (Scheme 5), while 2-oxaisocephem derivatives were synthesized from 3-bromomethyl derivative **29**[39] (Scheme 6). After introduction of the (4-pyridyl)thio group at C-3′ and the aminothiazole derivative **20** at C-7, **7g** and **29** were converted to amino acids **26** and **32**, respectively. The obtained amino acids were quaternarized by alkyl halides **22a–c** after treatment with BSA to afford **27a–c** and **33a–c** after removal of the benzhydryl group when **22c** was employed. All compounds with a C-3′ catechol or 3-hydroxy-4-pyridone moiety, **23a–c**, **27a–c**, and **33a–c** synthesized in this investigation are listed in Table 1.

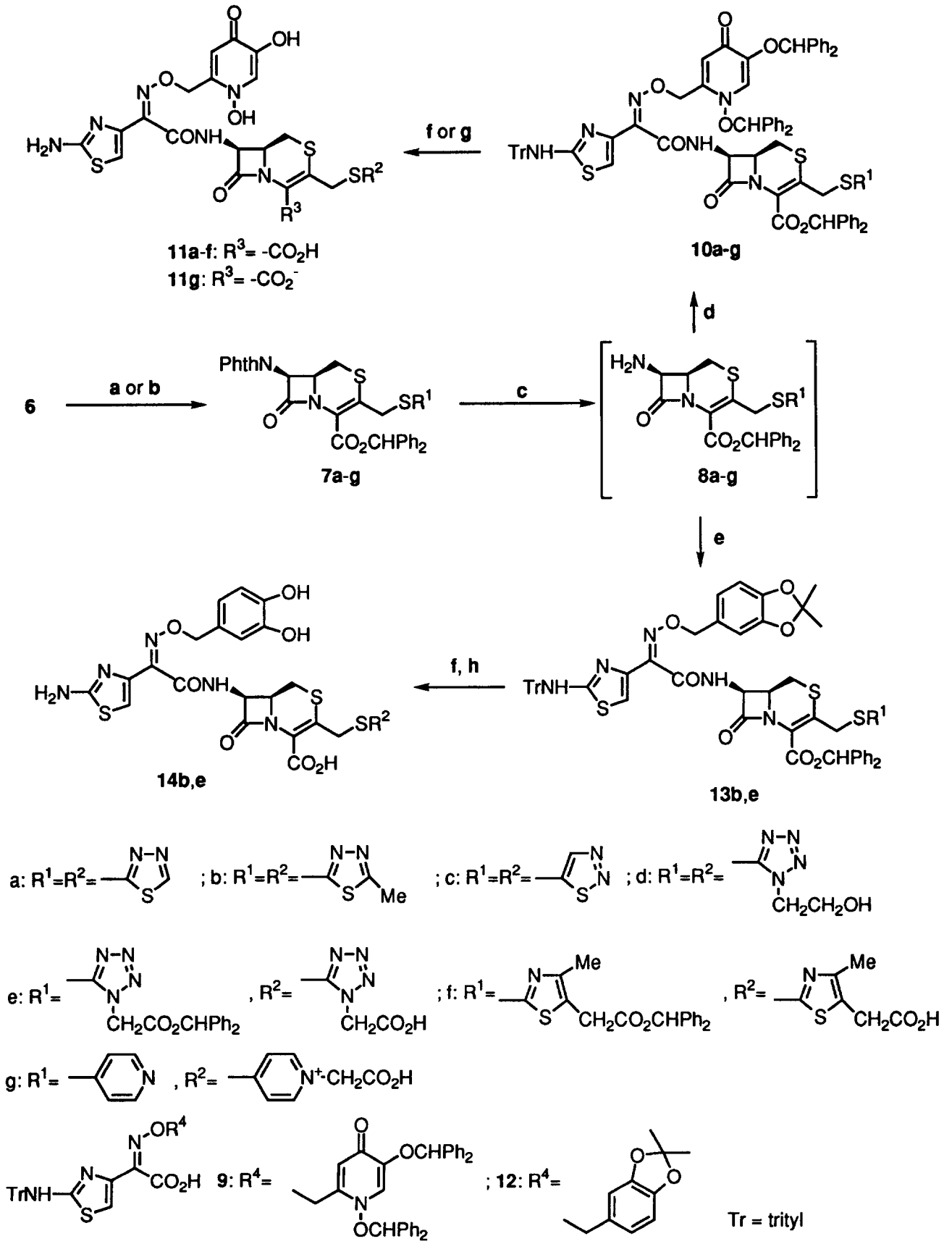
A new catechol containing compound, **22b**, was prepared as depicted in Scheme 7. Ethyl 3,4-dihydroxybenzoate was converted to hydrazide **34** by hydrazine monohydrate. Compound **34** was treated with bromoacetyl bromide to give the desired acetamide **22b**.

Biological Results and Discussion

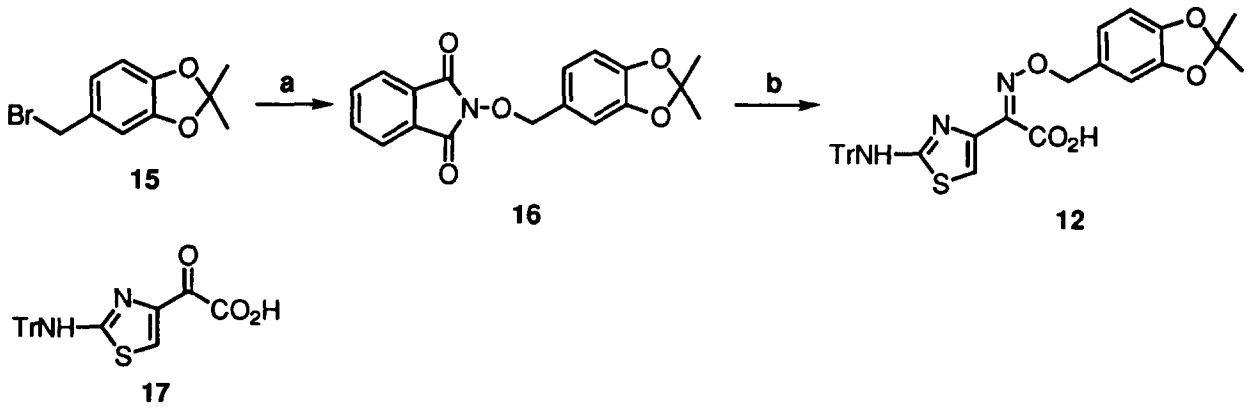
In vitro antibacterial activity

Compounds **11a–g**, **14b,e**, **23a–c**, **27a–c**, and **33a–c** were evaluated for in vitro antibacterial activity against Gram-positive (*Staphylococcus aureus* FDA 209P and *Staphylococcus epidermidis* ATCC 12228) and Gram-negative (*Escherichia coli* NIHJ JC-2, *Klebsiela pneumoniae* NCTC 9632, *S. marcescens* IFO 12648, *P. vulgalis* OX 19, *P. aeruginosa* ATCC 10145, *P. aeruginosa* NCTC 10490, *P. aeruginosa* E-2, and *A. baumanii* Ac 54) bacteria by using a two-fold agar dilution method.[40] The minimum inhibitory concentrations (MICs) of the tested compounds compared with ceftazidime and cefsulodine as reference compounds are displayed in Table 2.

In the C-7 substituted isocephem series, 1,3-dihydroxypyridone derivatives **11a–g** were more active against *P.*



Scheme 2. (a) $R^1SH/NaHCO_3$; (b) (1) R^2SH/Et_3N. (2) Ph_2CN_2; (c) $MeNHNH_2$; (d) **9**/DCC/HOBT; (e) **12**/DCC/HOBT; (f) TFA/anisole; (g) (1) $BrCH_2CO_2{}^tBu$, (2) TFA/anisole; (h) 95% TFA.



Scheme 3. (a) *N*-Hydroxyphthalimide, K_2CO_3; (b) (1) $NH_2NH_2 \cdot H_2O$, (2) **17**.

aeruginosa than others **14b,e**. Among them, **11e,f** with the carboxyl groups at C-3′ exhibited outstanding activity not only against *P. aeruginosa* but also *A. baumanii*, which is usually resistant to β-lactam antibiotics, although they were virtually devoid of activity against Gram-positive bacteria. The replacement of the 1,3-dihydroxypyridone moiety **11b,e** by the catechol moiety, **14b,e**, increased the activity against *S. aureus*, but decreased the activity against *P. aeruginosa* as mentioned above.

In the series of compounds with the C-3′ catechol-containing (pyridinium-4-thio)methyl groups, 2-oxaisocephem derivatives **33a–c** were less active against *S. marcescens* and *P. aeruginosa* than others. Among the cephalosporin derivatives **23a–c**, the *N*-(3,4-dihydroxybenzamido)carbamoylmethyl group improved the potency against *E. coli* and *S. marcescens*, while the (5-hydroxy-4-pyridon-2-yl)methyl group reduced activity against *E. coli*, *P. aeruginosa*, and *A. baumanii*. In the 2-isocephem derivatives **27a–c**, only the *N*-(3,4-dihydroxybenzamido)carbamoylmethyl group gave moderate activity against *A. baumanii*. Against other bacteria, the level of antimicrobial activity was independent of the nature of the C-3 substituent. Among the 2-oxaisocephem derivatives **33a–c**, the 3,4-dihydroxybenzoylmethyl group showed the best activity against all the organisms tested, while the *N*-(3,4-dihydroxybenzamido)carbamoylmethyl group provided least potency overall.

In comparison with cephalosporin derivatives **23a–c**, 2-isocephem derivatives **27a–c** exhibited more potent activity against *P. aeruginosa* E-2, with similar activity against other organisims except *A. baumanii*. Isocephem derivatives with the 1,3-dihydroxypyridone moiety at C-7, **11a–g**, displayed the same activity against *P. aeruginosa* E-2 as 2-isocephem derivatives substituted at C-3′, **27a–c** did. Almost all of the new compounds were more potent against Gram-negative bacteria, especially *P. aeruginosa*, than the reference compounds were.

As mentioned above, *A. baumanii* is one of the representative opportunistic pathogens[41] that are usually resistant to β-lactam antibiotics. So we selected **11e,f** which exhibited potent activity against both *P. aeruginosa* and *A. baumanii* as candidates for further evaluation.

In vitro antibacterial activity of these compounds against clinically isolated *P. aeruginosa* and *A. baumanii* is shown in Table 3. Data for ceftazidime and cefsulodin are also given. 2-Isocephem **11e,f** possessed similar potency against clinically isolated *P. aeruginosa*, and their activity were superior to reference compounds, while against clinically isolated *A. baumanii*, **11f** displayed better activity than **11e** did. Furthermore, **11f** displayed potent activity even against gentamicin, ceftazidime, cefpiramide, aztreonam, or imipenem resistant *P. aeruginosa*. The results are shown in Table 4..

In vivo antibacterial activity

The in vivo efficacy against systemic infections with *P. aeruginosa* E-2, clinically isolated *P. aeruginosa* Nos 58, 61, and 110, in mice of **11e,f**, ceftazidime and aztreonam is summarized in Table 6. One hour after intraperitoneal infection of bacteria, a single dose of each compound was subcutaneously administered to mice. The efficacy of each compound was expressed as 50% effective dose values (ED_{50}) which were calculated by the probit method from the number of mice surviving seven days after infection.

As shown in Table 5, the in vivo antibacterial activity of **11f** against *P. aeruginosa* E-2 was most potent. Furthermore, **11f** exhibited better in vivo efficacy against all clinically isolated *P. aeruginosa* tested than ceftazidime or aztreonam by approximately 5–40-fold.

In summary, 2-isocephems with a 1,3-dihydroxy-4-pyridone moiety at C-7, cephalosporins or 2-isocephems with catechol-containing (pyridinium-4-thio) methyl group at C-3 showed strong in vitro antibacterial activity against Gram-negative bacteria including *P. aeruginosa*. In addition, compound **11f** displayed in vitro potency against clinically isolated *A. baumanii*, are better protective effects on systemic infection in mice owing to clinically isolated *P. aeruginosa* than reference compounds.

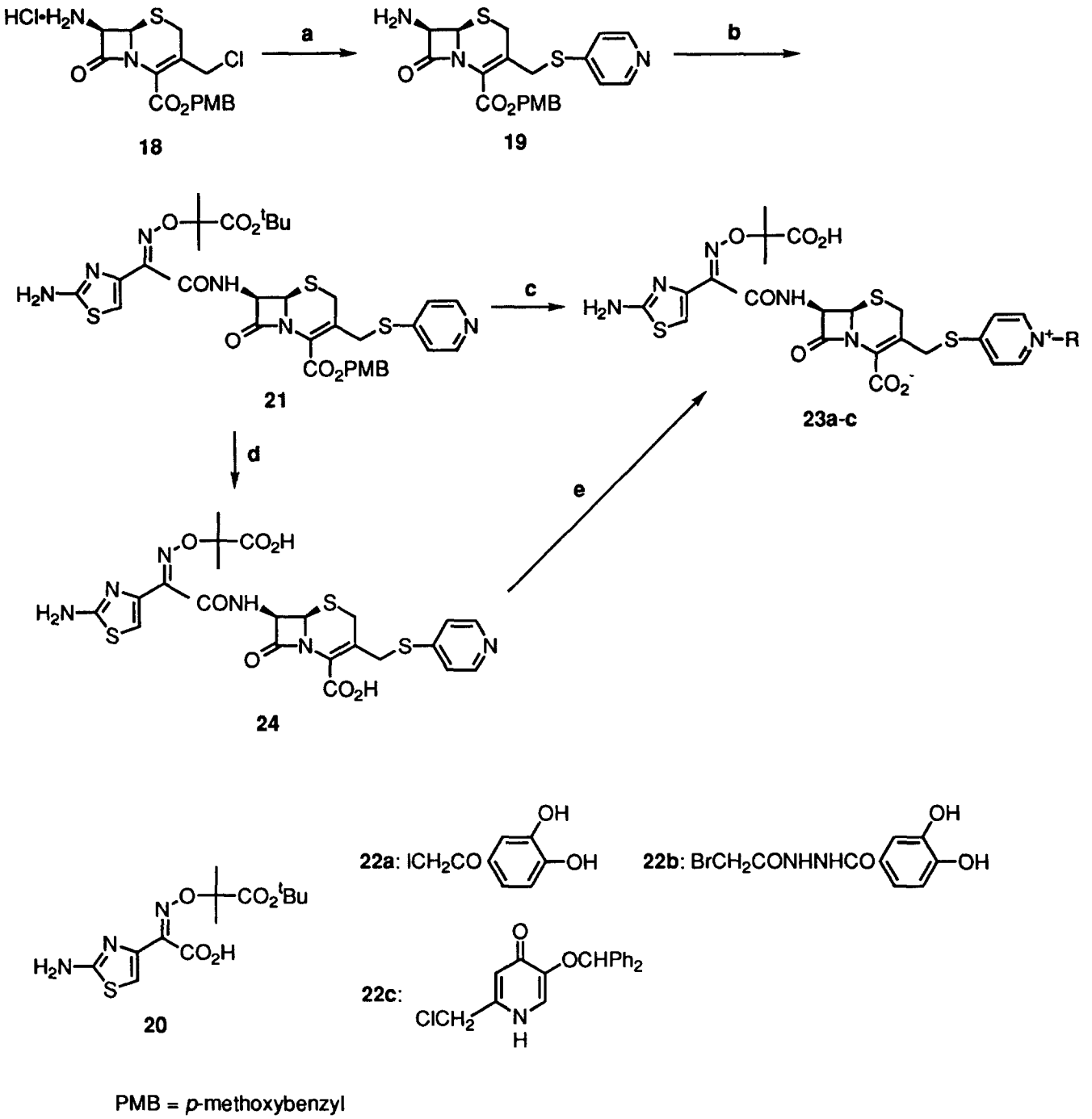
Experimental

General

Reagents were used as supplied unless otherwise noted. All the melting points were taken on a Yanaco MP-500D apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker AC250 spectrometer operating at 250 MHz. The chemical shifts are reported in parts per million (ppm) on the δ scale downfield relative to tetramethylsilane as an internal standard and coupling constants in Hz. IR spectra were measured for KBr pellets with a Jasco IR-810 infrared spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter.

(3*S*,4*R*)-1-[(*R*)-1-[(Benzhydryloxy)carbonyl]-(*S*)-2-hydroxypropyl]-3-phthalimido-4-styrylazetidin-2-one (2). This compound was prepared as described in our preceding paper.[26]

(3*S*,4*S*)-1-[1-[(Benzhydryloxy)carbonyl]-2-hydroxypropenyl]-4-[(mesyloxy)methyl]-3-phthalimidoazetidin-2-one (3). This compound was prepared in the same



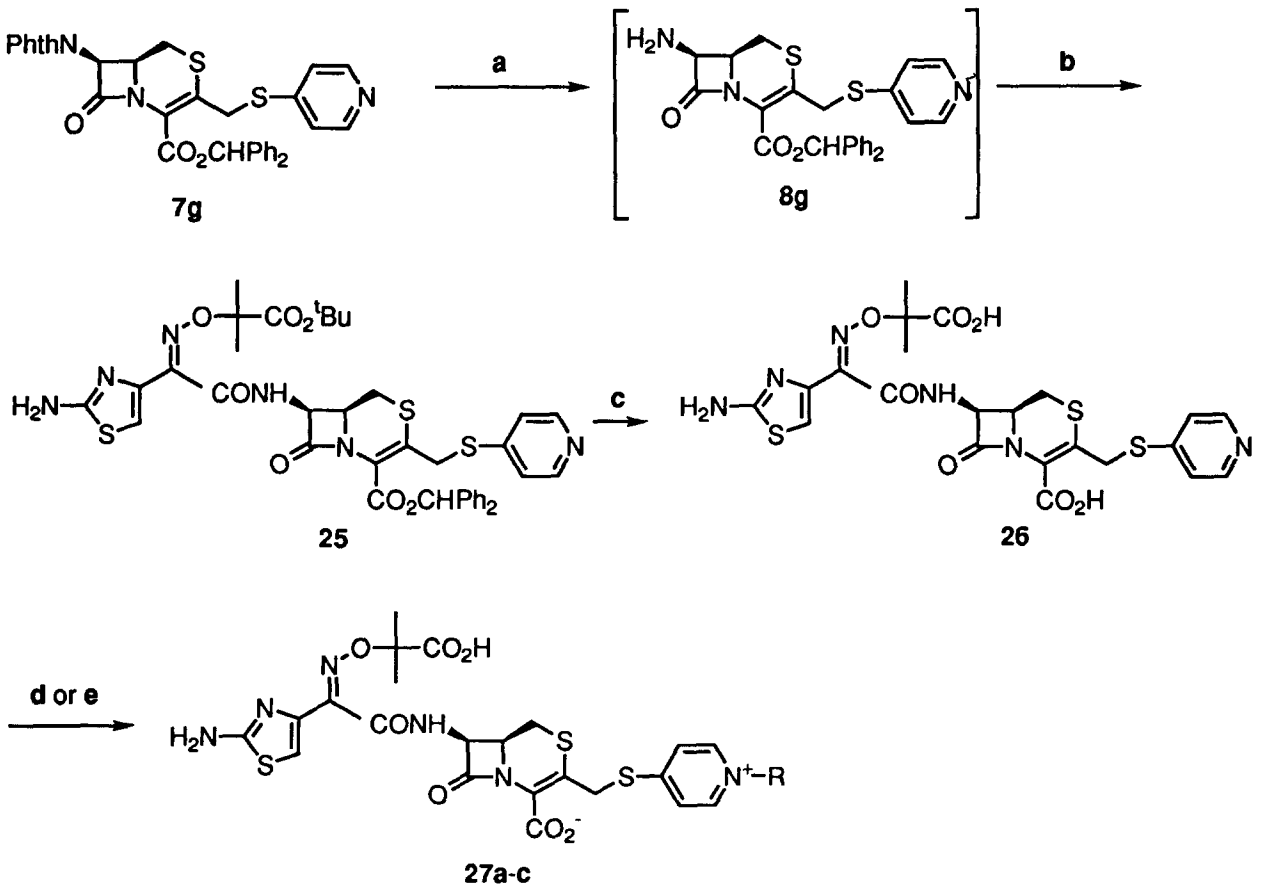
Scheme 4. (a) (1) NaOH, (2) 4-mercaptopyridine; (b) **20**/DCC/HOBT; (c) (1) **22a,b**, (2) TFA/anisole; (d) (1) TFA/anisole, (2) Et_3N; (e) (1) BSA, (2) **22c**/NaI, (3) *i*-PrOH, (4) TFA/anisole.

manner as described for the preparation of the 3-(4-nitrophthalimido)azetidinone congener:[26] white powder. ^{1}H NMR ($CDCl_3$): δ 2.37 (3H, s), 2.81 (3H, s), 4.33–4.63 (3H, m), 5.52 (1H, d. J = 5.2 Hz), 6.96 (1H, s), 7.22–7.53 (10 H, m), 7.75–7.96 (4H, m). IR (cm^{-1}): 3500, 1790, 1780, 1720, 1420, 1380, 1180.

Benzhydryl-(6*S*,7*S*)-3-methyl-8-oxo-7-phthalimido-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (5). *N*-Methylpyrrolidine (6.9 mL, 0.066 mol) was added dropwise to a soln of **3** (39.0 g, 0.066 mol) and *p*-toluenesulfonyl chloride (12.5 g, 0.066 mol) in CH_2Cl_2 (280 mL) at 0 °C. After stirring for 1 h, the mixture was washed with dil. HCl (250 mL) and water (250 mL × 2). Evaporation of dried ($MgSO_4$) organic phase furnished the crude **4**. A stream of H_2S was bubbled through a soln of triethylamine (18.4 mL, 0.13 mol) in CH_2Cl_2 (340 mL) at 5 °C. To this soln, a soln of the crude **4** in CH_2Cl_2 (100 mL) was added dropwise, and the mixture was stirred for 1 h. After washing with 10% HCl (450 mL) and water (450 mL × 2), the resulting mixture was dried over $MgSO_4$ and evapd. AcOEt (80 mL) was added to the residue and the mixture was heated to reflux. After cooling, the resulting precipitates were filtered, washed with AcOEt and dried under reduced pressure to give **5** (26.6 g, 79%) as white prisms, mp 183–183.5 °C. $[\alpha]_D^{28}$ −9.9 (*c* 0.78, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.29 (3H, s), 2.84 (1H, dd, J = 3.3, 11.9 Hz), 3.54 (1H, dd, J = 10.4, 11.9 Hz), 4.02–4.14 (1H, m), 5.80 (1H, d, J = 5.1 Hz), 6.90 (1H, s), 7.15–7.61 (10H, m), 7.70–7.95 (4H, m). IR (cm^{-1}): 1780, 1720. Anal. calcd for $C_{29}H_{22}N_2O_5S$: C, 68.22; H, 4.34; N, 5.49. Found: C, 68.43; H, 4.20; N, 5.51.

Benzhydryl-(6*S*,7*S*)-3-bromomethyl-8-oxo-7-phthalimido-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (6). A suspension of **5** (25.5 g, 0.05 mol), NBS (11.6 g, 0.065 mol), and AIBN (0.82 g, 0.005 mol) in CCl_4 (500 mL) was refluxed for 1 h. After cooling to room temperature, the mixture was evapd. To the residue were added AcOEt (75 mL), water (250 mL), and hexane (25 mL), and the mixture was stirred for 1.5 h. The resulting precipitates were filtered, washed with water (50 mL) and hexane:AcOEt (1:2) (60 mL), and dried under reduced pressure to afford **6** (17.9 g, 61%) as white needles, mp <300 °C. $[\alpha]_D^{28}$ −30.6 (*c* 1.24, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.95 (1H, dd, J = 3.2, 12.0 Hz), 3.56 (1H, dd, J = 10.3, 12.0 Hz), 4.10–4.21 (1H, m), 4.30 (1H, d, J = 10.9 Hz), 4.74 (1H, d, J = 10.9 Hz), 5.82 (1H, d, J = 5.3 Hz), 6.98 (1H, s), 7.17–7.60 (10H, m), 7.74–7.97 (4H, m). IR (cm^{-1}): 1770, 1720. Anal. calcd for $C_{29}H_{21}BrN_2O_5S$: C, 59.09; H, 3.59; N, 4.75. Found: C, 59.19; H, 3.58; N, 4.71.

Benzhydryl-(6*S*,7*S*)-8-oxo-7-phthalimido-3-[[(1,3,4-thiadiazol-2-yl)thio]methyl]-1-aza-4-thiabicyclo-[4.2.0]oct-2-ene-2-carboxylate (7a). To a soln of 2-mercapto-1,3,4-thiadiazole[27] (2.36 g, 0.020 mol) and

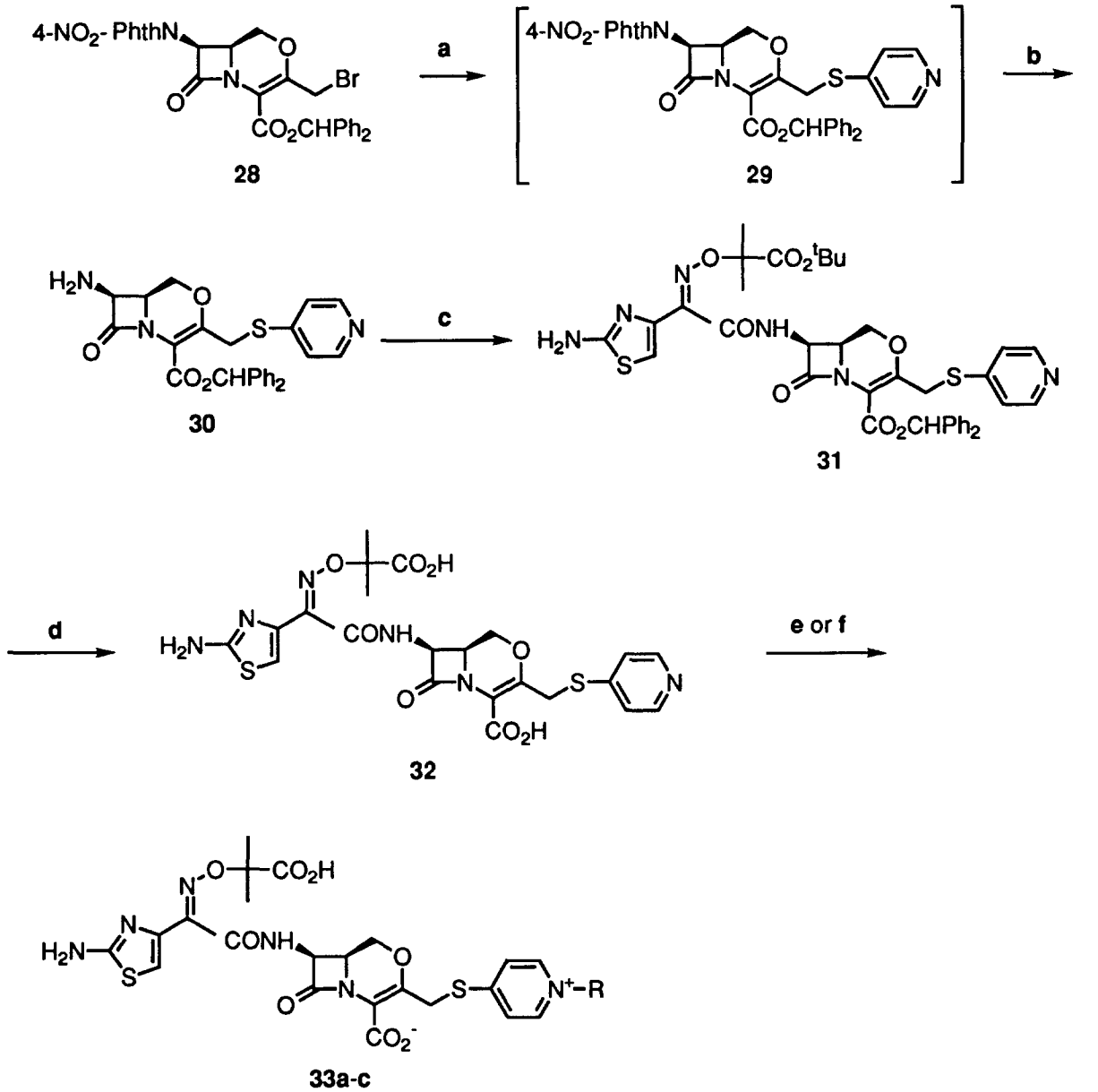


Scheme 5. (a) $MeNHNH_2$; (b) **20**/DCC/HOBT; (c) (1) TFA/anisole, (2) Et_3N; (d) (1) BSA, (2) **22a,b**, (3) *i*-PrOH; (e) (1) BSA, (2) **22c**, (3) *i*-PrOH, (4) TFA/anisole.

$NaHCO_3$ (3.37 g, 0.040 mol) in acetone (50 mL) and water (5 mL) was added **6** (11.8 g, 0.020 mol). After stirring for 2 h at room temperature, the mixture was evapd. The residue was dissolved in CH_2Cl_2 (50 mL), washed with water (50 mL × 3), dried over $MgSO_4$, and evapd to give **7a** (12.5 g, 100%) as a yellow powder. ^1H NMR ($CDCl_3$): δ 2.90 (1H, dd, J = 3.0, 12.0 Hz), 3.51 (1H, dd, J = 10.4, 12.0 Hz), 4.02–4.17(1H, m), 4.44 (1H, d, J = 13.9 Hz), 4.81 (1H, d, J = 13.9 Hz), 5.83 (1H, d, J = 5.2 Hz), 6.96 (1H, s), 7.18–7.60 (10H, m), 7.71–7.96 (4H, m), 8.97 (1H, s). IR (cm^{-1}): 1790, 1720, 1380.

Compounds **7b–d,g** were obtained by the same procedure as described for **7a**.

Benzhydryl-(6*S*,7*S*)-3-[[[5-[(benzhydryloxycarbonyl)-methyl]-4-methylthiazol-2-yl]thio]methyl]-8-oxo-7-phthalimido-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (7f). To a soln of **6** (5.89 g, 0.010 mol) and triethylamine (2.79 mL, 0.020 mol) in CH_2Cl_2 (25 mL) was added 5-carboxymethyl-2-mercapto-4-methylthiazole[32] (1.88 g, 0.010 mol), and the resulting mixture was stirred for 40 min at room temperature. After addition of water (25 mL), the mixture was acidified with 10% HCl. The organic phase was sepd and the aq phase extracted with CH_2Cl_2 (25 mL). The combined organic extracts were washed with water (25 mL × 2), dried over $MgSO_4$, and filterd. Diphenyldiazomethane (2.14 g, 11 mmol) was added to the filtrate and the mixture

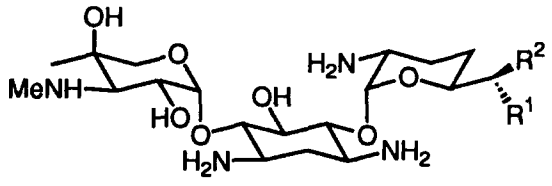


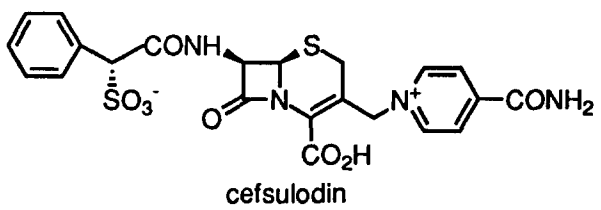
Scheme 6. (a) 4-Mercaptopyridine/Et_3N; (b) (1) $MeNHNH_2$, (2) AcOH; (c) **20**/DCC/HOBT; (d) $AlCl_3$/anisole/$MeNO_2$; (e) (1) BSA, (2) **22a,b**, (3) *i*-PrOH; (f) (1) BSA, (2) **22c**, (3) *i*-PrOH, (4) TFA/anisole.

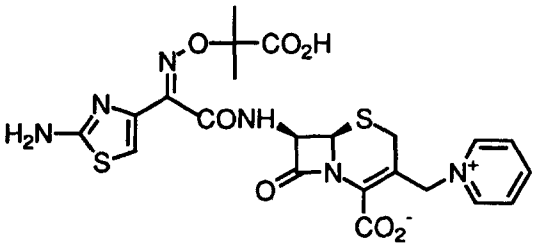
was stirred for 3 h at room temperature. Removal of the solvent under reduced pressure gave **7f** (9.10 g, 100%) as a brown powder. ^{1}H NMR ($CDCl_3$): δ 2.24 (3H, s), 2.78 (1H, dd, J=3.2, 11.9 Hz), 3.45 (1H, dd, J = 10.3, 11.9 Hz), 3.75 (2H, s), 3.95–4.08 (1H, m), 4.13 (1H, d, J=14.0 Hz), 4.69 (1H, d, J=14.0 Hz), 5.77 (1H, d, J=5.2 Hz), 6.87 (1H, s), 6.92 (1H, s), 7.08–7.67 (20H, m), 7.72–8.00 (4H, m). IR (cm^{-1}) 1790, 1720.

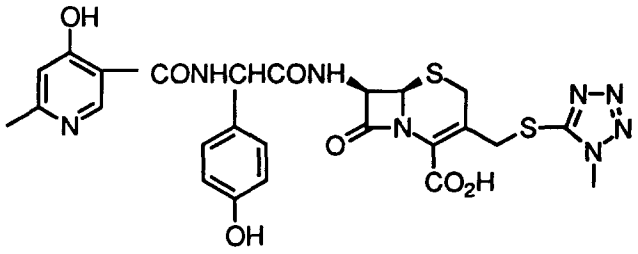
Compound **7e** was obtained by the same procedure as described for **7f**.

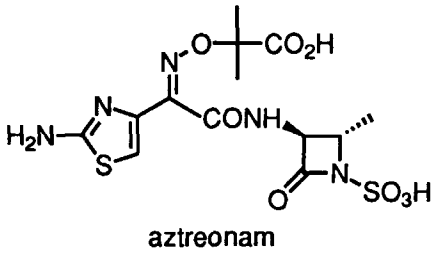
Benzhydryl-(6*S*,7*S*)-3-[[[5-[(Benzhydryloxycarbonyl) methyl]-4-methylthiazol-2-yl]thio]methyl]-7-[2-[(*Z*)-[[1,5-bis(benzhydryloxy)-4-pyridon-2-yl]methoxy]imino]-2-(2-tritylaminothiazol-4-yl)acetamido]-8-oxo-1-aza-4-











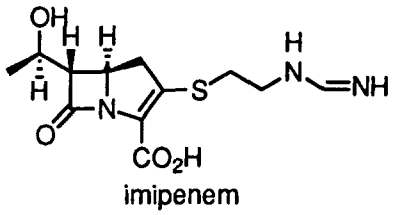
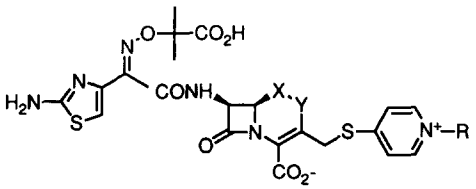
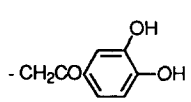
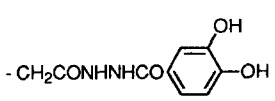
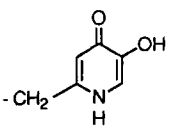
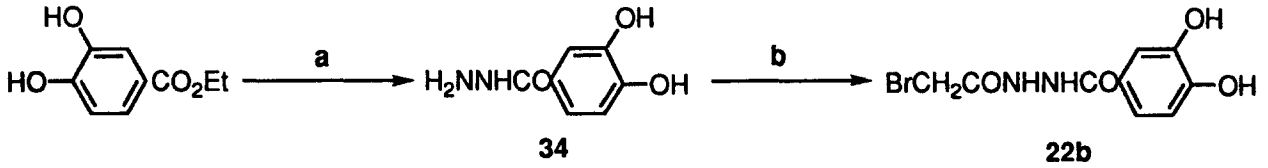


Chart 1.

Table 1. Cephalosporins **23a–c**, 2-isocephems **27a–c**, and 2-oxaisocephems **33a–c** bearing a catechol or 3-hydroxy-4-pyridone moeity at C-3′



	23a	**27a**	**33a**	**23b**	**27b**	**33b**	**23c**	**27c**	**33c**
X	S	CH_2	CH_2	S	CH_2	CH_2	S	CH_2	CH_2
Y	CH_2	S	O	CH_2	S	O	CH_2	S	O
R									



Scheme 7. (a) $NH_2NH_2 \cdot H_2O$; (b) $BrCH_2COBr$.

Table 2. Antibacterial activity of cephalosporins, 2-isocephems, and 2-oxaisocephems with a catechol or its bioisostere at C-7 or C-3′ (MICs,[a] μg/mL)

Compound	Gram-positive organisms[b]		Gram-negative organisms[b]							
	S. au. FDA 209P	*S. ep.* ATCC 12228	*E. c.* NIHJ JC-2	*K. pn.* NCTC 9632	*S. m.* IFO 12648	*P. v.* OX 19	*P. ae.* ATCC 10145	*P. ae.* NCTC 10490	*P. ae.* E-2	*A. b.* Ac 54
11a	25	12.5	0.2	<0.025	0.05	<0.025	0.1	<0.025	0.1	0.78
11b	50	12.5	0.2	<0.025	0.05	<0.025	0.2	<0.025	0.2	1.56
11c	25	25	0.2	0.1	0.05	<0.025	0.39	<0.025	0.2	6.25
11d	50	25	0.1	<0.025	<0.025	<0.025	0.05	<0.025	0.05	1.56
11e	>100	100	0.1	<0.025	0.05	<0.025	0.1	<0.025	0.2	0.2
11f	>100	>100	0.1	0.05	0.05	<0.025	0.05	<0.025	0.2	0.39
11g	12.5	6.25	0.1	<0.025	0.05	0.05	0.1	<0.025	0.2	0.78
14b	1.56	0.78	0.2	<0.025	0.1	<0.025	0.78	0.2	1.56	0.78
14e	25	12.5	0.2	0.05	0.2	0.05	0.39	0.05	1.56	>100
23a	3.13	3.13	0.1	<0.025	0.05	<0.025	0.05	<0.025	0.78	0.39
23b	3.13	1.56	<0.025	<0.025	<0.025	<0.025	0.1	<0.025	1.56	0.78
23c	12.5	12.5	0.78	<0.025	0.05	<0.025	0.39	<0.025	3.13	50
27a	6.25	3.13	0.39	<0.025	0.05	<0.025	0.05	<0.025	0.1	>100
27b	6.25	3.13	0.39	<0.025	0.05	<0.025	0.1	<0.025	0.2	1.56
27c	12.5	6.25	0.39	<0.025	<0.025	<0.025	0.1	<0.025	0.2	>100
33a	3.13	3.13	0.1	<0.025	0.1	<0.025	0.1	0.05	0.78	0.78
33b	6.25	6.25	0.78	<0.05	0.78	<0.025	1.56	0.78	12.5	12.5
33c	3.13	3.13	0.39	<0.025	0.1	<0.025	0.78	0.1	12.5	1.56
Ceftazidime	6.25	3.13	0.39	0.05	0.1	<0.025	1.56	1.56	1.56	12.5
Cefsulodin	6.25	1.56	50	50	50	100	3.13	0.78	3.13	50

[a]Minimum inhibitory concentrations (10^6 cells/mL).
[b]Definitions of organism abbreviations: *S. au.* = *S. aureus*, *S. ep.* = *S. epidermidis*, *E. c.* = *E. coli*, *K. pn.* = *K. pneumoniae*, *S. m.* = *S. marcescens*, *P. v.* = *P. vulgaris*, *P. ae.* = *P. aeruginosa*, *A. b.* = *A. baumanii*.

Table 3. In vitro antibacterial activity (MICs, μg/mL) of 2-isocephems **11e,f** against clinically isolated *P. aeruginosa* and *A. baumanii*

Strains	Compound	MIC_{50}[a]	MIC_{80}[b]	MIC_{90}[c]	MIC range[d]
P. aeruginosa[e]	**11e**	0.1	0.78	3.13	<0.025–>100
	11f	0.1	0.78	3.13	<0.025–>100
	ceftazidime	3.13	12.5	>100	1.56–>100
	cefsulodin	6.25	25	100	0.78–>100
A. baumanii[f]	**11e**	0.78	3.13	6.25	0.2–>100
	11f	0.78	1.56	3.13	0.025–25
	ceftazidime	6.25	12.5	12.5	0.78–25
	cefsulodin	50	50	100	12.5–>100

[a]The MIC value for 50% of isolates.
[b]The MIC value for 80% of isolates.
[c]The MIC value for 90% of isolates.
[d]The range of MIC value for isolates.
[e]155 clinical isolates.
[f]64 clinical isolates.

Table 4. In vitro antibacterial activity (MICs μg/mL) of 2-isocephem **11f** against gentamicin, ceftazidime, cefpiramide, aztreonam, and imipenem resistant *P. aeruginosa*

Strain	**11f**	Gentamicin	Ceftazidime	Cefpiramide	Aztreonam	Imipenem
P. aeruginosa No. 33	0.39	>100	6.25	25	25	3.13
P. aeruginosa No. 59	1.56	6.25	50	>100	25	50
P. aeruginosa No. 47	1.56	6.25	50	100	25	25
P. aeruginosa No. 56	1.56	12.5	12.5	25	50	25
P. aeruginosa No. 69	3.13	3.13	50	100	25	50

thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (10f). A 2 M soln of methylhydrazine in THF (23.4 mL, 0.0468 mol) was added to a soln of **7f** (40.4 g, 0.0468 mol) in DMF (80 mL) at −10 °C. After stirring for 30 min, AcOEt (100 mL) and water (1 L) were added to the reaction mixture. The organic phase was sepd, and the aq phase extracted with AcOEt (200 mL × 2). The combined organic layers were washed with water (600 mL × 2) and brine (600 mL), and dried over Na_2SO_4. After removal of the solvent, the residue was dissolved in CH_2Cl_2 (100 mL) and the soln stirred for 4 h. The resulting precipitates were filtered off and washed with CH_2Cl_2 (130 mL). The washings were mixed with the filtrate. To the resulting solution were added aminothiazole derivative **9**[7] (42.2 g, 0.0468 mol), HOBT (6.46 g, 0.0468 mol), and 1,3-dicyclohexylcarbodiimide (DCC) (9.66 g, 0.0468 mol) without isolation of **8f**. After stirring for 16 h at room temperature, the precipitates were filtered off and the filtrate was washed with 5% aq $NaHCO_3$ soln (250 mL) and water (250 mL × 2), and dried over $MgSO_4$. The solvent was evapd and the residue purified by silica gel column chromatography to afford **10f** (49.8 g, 66%) as a yellow powder. ^{1}H NMR ($CDCl_3$): δ 2.22 (3H, s), 2.68 (1H, dd, J = 2.1, 12.3 Hz), 3.03 (1H, dd, J = 10.2, 12.3 Hz), 3.71 (2H, s), 3.85–4.00 (1H, m), 4.12 (1H, d, J = 14.0 Hz), 4.60 (1H, d, J = 14.0 Hz), 4.61 (1H, d, J = 15.0 Hz), 4.90 (1H, d, J = 15.0 Hz), 5.44 (1H, dd, J = 4.9, 6.4 Hz), 5.86 (1H, s), 6.06 (1H, s), 6.30 (1H, s), 6.65 (1H, s), 6.83 (1H, s), 6.86 (1H, s), 6.88 (1H, s) 6.95 (1H, br s), 7.04–7.61 (55H, m), 8.18 (1H, d, J = 4.9 Hz). IR (cm^{-1}): 1780, 1580, 1500, 710.

Compounds **10a–e,g**, **13b,e** were obtained by the same procedure as described for **10f**.

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-[(1,5-dihydroxy-4-pyridon-2-yl)methoxy]imino]acetamido]-3-[[[(5-carboxymethyl-4-methyl)thiazol-2-yl]thio]methyl]-8-oxo-1-aza-4-thiabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (11f). TFA (120 mL) and anisole (24 mL) were added to **10f** (46.9 g, 0.03 mol) and the resulting soln was stirred for 2 h at room temperature. *i*-Pr_2O (400 mL) was added to the mixture and the generating precipitates filtered and washed with *i*-Pr_2O. Thus obtained crude **11f** was dissolved in 5% aq $NaHCO_3$ soln (200 mL) and the insoluble substance filtered off. The filtrate was subjected to chromatography on Sephadex G-25 using water as solvent. The fractions containing the product were combined and adjusted to pH 4 with 10% HCl. The resultant precipitates were collected by filtration, washed with water, and dried in vacuo to give **11f** (14.1 g, 66%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6): δ 2.22 (3H, s), 2.92–3.18 (2H, m), 3.75 (2H, s), 3.90–4.03 (1H, m), 4.18 (1H, d, J = 13.9 Hz), 4.52 (1H, d, J = 13.9 Hz), 5.13 (2H, s), 5.63 (1H, dd, J = 5.0, 8.7 Hz), 6.70 (1H, br s), 6.85 (1H, s), 7.26 (2H, br s), 7.70 (1H, s), 9.41 (1H, d, J = 8.7 Hz). IR (cm^{-1}): 3330, 1760, 1670.

Table 5. In vivo efficacy of isocephems **11e,f** against systemic infections with *P. aeruginosa* in mice[a] in comparison with ceftazidime and aztreonam

Test organisms	Compound	MIC (μg/mL)	Challenge dose (cells/mouse)	ED_{50} (mg/kg)[b]	95% confidence limits (mg/kg)
P. aeruginosa E-2	**11e**	0.2	3.83×10^5	14.0	9.82–27.87
	11f	0.2		10.37	3.16–<23.08
	ceftazidime	1.56		18.70	6.93–34.84
	aztreonam	6.25		47.19	26.84–139
P. aeruginosa No. 58	**11f**	0.05	3.40×10^3	3.2	1.58–4.96
	ceftazidime	3.13		31.17	12.26–128.29
	aztreonam	12.5		122.35	66.34–129.05
P. aeruginosa No. 61	**11f**	0.025	2.50×10^3	6.82	2.67–10.17
	ceftazidime	3.13		93.84	46.20–223.10
P. aeruginosa No. 110	**11f**	0.10	2.50×10^5	32.5	17.10–79.67
	ceftazidime	12.5		166.8	115.29–254.08
	aztreonam	100		>400	

[a]Experimental infections were produced by intraperitoneal injection with the challenge organisms suspended in 5% gastric mucin. The infections were lethal to all untreated mice.
[b]Dose required to prevent death in 50% of animals (subcutaneous administration).

Compounds **11a–e** were obtained by the same procedure as described for **11f**; yields and NMR data are given in Table 6.

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1,5-dihydroxy-4-pyridon-2-yl)methoxyimino]acetamido]-3-[[(1-carboxymethylpyridinium-4-yl)thio]methyl]-8-oxo-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (11g). To a soln of **10g** (1.77 g, 1.3 mmol) in DMF (5 mL) was added *t*-butyl bromoacetate (0.46 mL, 2.8 mmol). After stirring at room temperature for 16 h and at 50 °C for 2 h, the mixture was concentrated. The residue, anisole (1 mL), and TFA (4 mL) were stirred at ambient temperature for 2 h, after which time, *i*-Pr_2O (50 mL) was added. The resulting precipitates were filtered and washed with *i*-PrOH, and dissolved in 5% aq $NaHCO_3$ soln. This soln was purified by Diaion HP-20 column chromatography using *i*-PrOH:H_2O as eluent. After removal of *i*-PrOH, the fractions containing product were freeze-dryed to furnish **11g** (0.37 g, 41%) as a light orange powder. 1H NMR (DMSO-d_6): δ 2.65–2.83 (2H, m), 3.75–3.89 (1H, m), 4.32 (1H, d, J = 15.7 Hz), 4.38 (1H, d, J = 15.7 Hz), 4.76 (2H, s), 5.09 (2H, s), 5.23 (1H, dd, J = 3.4, 5.2 Hz), 6.86 (1H, s), 6.92 (1H, s), 7.21 (2H, br s), 7.60 (1H, s), 7.94 (2H, d, J = 6.5 Hz), 8.52 (2H, d, J = 6.5 Hz), 10.31 (1H, d, J = 5.2 Hz). IR (cm^{-1}): 3400, 1760, 1640, 1530, 1380.

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-[(3,4-dihydroxy)benzyloxy]imino]acetamido]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (14b). TFA (12 mL) and anisole (6 mL) were added to **13b** (3.25 g, 3 mmol) at 0 °C. After stirring for 30 min, *i*-Pr_2O (150 mL) was added to the mixture and the resultant precipitates were filtered and washed with *i*-Pr_2O. The obtained powder was dissolved in 95% TFA (20 mL) at 0 °C and the resulting soln stirred for 30 min. *i*-Pr_2O (150 mL) was added and the generating precipitates were collected by filtration and washed with *i*-Pr_2O, then dissolved in 5% aq $NaHCO_3$ soln. This soln was chromatographed on Diaion HP-20 eluted with *i*-PrOH:H_2O mixtures. The appropriate fractions were collected, evapd to remove *i*-PrOH, and freeze-dryed to afford **14b** (0.68 g, 36%) as a pale yellow powder. 1H NMR (DMSO-d_6): δ 2.67 (3H, s), 2.85–3.05 (2H, m), 3.72–3.86 (1H, m), 4.37 (1H, d, J = 13.2 Hz), 4.61 (1H, d, J = 13.2, Hz), 4.91 (2H, s), 5.45 (1H, dd, J = 4.9, 8.9 Hz), 6.61 (1H, d, J = 7.9 Hz), 6.68 (1H, d, J = 7.9 Hz), 6.74 (1H, s), 6.76 (1H, s), 7.22 (2H, br s), 9.06 (2H, br s), 9.17 (1H, d, J = 8.9 Hz). IR (cm^{-1}): 3320, 3200, 1760, 1610, 1530, 1380.

Compound **14e** was obtained by the same procedure as described for **14b**; yield and NMR data are given in Table 6.

Table 6. 2-Isocephems with 1,3-dihydroxy-4-pyridone or catechol moiety at C-7 **11a–e**, **14e**, cephalosporin, 2-isocephem, or 2-oxaisocephem with catechol moiety at C-3′ **23a**, **27a**, **33a**

Compound	Yield (%)[a]	1H NMR δ (250 MHz, DMSO-d_6)
11a	57	3.01–3.24 (2H, m), 3.92–4.03 (1H, m), 4.42 (1H, d, J = 14.1 Hz), 4.63 (1H, d, J = 14.1 Hz), 5.13 (2H, s), 5.66 (1H, dd, J = 5.1, 9.0 Hz), 6.69 (1H, s), 6.85 (1H, s), 7.25 (2H, br s), 7.70 (1H, s), 9.38 (1H, d, J = 9.0 Hz), 9.56 (1H, s).
11b	72	2.69 (3H, s), 3.01–3.25 (2H, m), 3.91–4.04 (1H, m), 4.36 (1H, d, J = 14.2 Hz), 4.56 (1H, d, J = 14.2 Hz), 5.15 (2H, s), 5.66 (1H, dd, J = 4.9, 9.0 Hz), 6.71 (1H, s), 6.85 (1H, s), 7.26 (2H, br s), 7.72 (1H, s), 9.37 (1H, d, J = 9.0 Hz).
11c	78	3.01–3.26 (2H, m), 3.95–4.07 (1H, m), 4.31 (1H, d, J = 14.5 Hz), 4.37 (1H, d, J = 14.5 Hz), 5.15 (2H, s), 5.67 (1H, dd, J = 5.0, 8.9 Hz), 6.72 (1H, s), 6.86 (1H, s), 7.26 (2H, br s), 7.72 (1H, s), 8.92 (1H, s), 9.43 (1H, d, J = 8.9 Hz).
11d	47	3.01–3.24 (2H, m), 3.71–3.87 (2H, m), 3.91–4.03 (1H, m), 4.28–4.54 (5H, m), 5.14 (2H, s), 5.65 (1H, dd, J = 4.9, 8.6 Hz), 6.70 (1H, s), 6.85 (1H, s), 7.26 (2H, br s), 7.71 (1H, s), 9.38 (1H, d, J = 8.6 Hz).
11e	68	3.00–3.21 (2H, m), 3.91–4.05 (1H, m), 4.38 (1H, d, J = 14.0 Hz), 4.45 (1H, d, J = 14.0 Hz), 5.15 (2H, s), 5.22 (1H, d, J = 16.7 Hz), 5.29 (1H, d, J = 16.7 Hz), 5.62 (1H, dd, J = 5.0, 8.5 Hz), 6.75 (1H, s), 6.86 (1H, s), 7.26 (2H, br s), 7.81 (1H, s), 9.36 (1H, d, J = 8.5 Hz).
14e	64	2.82 (1H, dd, J = 2.3, 12.6 Hz), 3.02 (1H, dd, J = 9.9, 12.6 Hz), 3.84–3.97 (1H, m), 4.36 (1H, d, J = 13.8 Hz), 4.49 (1H, d, J = 13.8 Hz), 4.91 (2H, s), 5.24 (1H, d, J = 18.0 Hz), 5.36 (1H, d, J = 18.0 Hz), 5.60 (1H, dd, J = 5.1, 8.7 Hz), 6.62 (1H, d, J = 8.0 Hz), 6.68 (1H, d, J = 8.0 Hz), 6.74 (1H, s), 6.78 (1H, s), 7.22 (2H, br s), 8.89 (2H, br s), 9.18 (1H, d, J = 8.7 Hz).
23a	26	1.43 (3H, s), 1.44 (3H, s), 3.41 (1H, d, J = 17.5 Hz), 3.59 (1H, d, J = 17.5 Hz), 4.47 (1H, d, J = 12.5 Hz), 4.55 (1H, d, J = 12.5 Hz), 5.08 (1H, d, J = 4.5 Hz), 5.68 (1H, dd, J = 4.5, 9.0 Hz), 6.11 (2H, s), 6.72 (1H, s), 6.94 (1H, d, J = 7.7 Hz), 7.28 (2H, br s), 7.39 (1H, s), 7.44 (1H, d, J = 7.7 Hz), 8.25 (2H, d, J = 5.0 Hz), 8.56 (2H, d, J = 5.0 Hz), 9.55 (1H, d, J = 9.0 Hz).
27a	29	1.39 (3H, s), 1.41 (3H, s), 3.01–3.23 (2H, m), 3.88–3.98 (1H, m), 4.53 (1H, d, J = 14.3 Hz), 4.75 (1H, d, J = 14.3 Hz), 5.48 (1H, dd, J = 5.2, 8.6 Hz), 6.10 (2H, s), 6.74 (1H, s), 6.93 (1H, d, J = 8.4 Hz), 7.27 (2H, br s), 7.42 (1H, d, J = 8.4 Hz), 7.44 (1H, s), 8.24 (2H, d, J = 6.6 Hz), 8.58 (2H, d, J = 6.6 Hz), 9.36 (1H, d, J = 8.6 Hz).
33a	15	1.38 (3H, s), 1.39 (3H, s), 3.80–4.06 (2H, m), 4.53 (1H, d, J = 14.8 Hz), 4.63–4.71 (1H, m), 4.81 (1H, d, J = 14.8 Hz), 5.64 (1H, dd, J = 6.5, 9.3 Hz), 6.12 (2H, s), 6.74 (1H, s), 6.94 (1H, d, J = 9.0 Hz), 7.27 (2H, br s), 7.41 (1H, d, J = 9.0 Hz), 7.43 (1H, s), 8.20 (2H, d, J = 6.3 Hz), 8.60 (2H, d, J = 6.3 Hz), 9.49 (1H, d, J = 9.3 Hz).

[a] **11a–e**, **14e**, **23a**, **27a**, **33a**: Yields from **10a–e**, **13e**, **21**, **25**, **32**, respectively.

***N*-[(2,2-Dimethyl-1,3-benzodioxol-5-yl)methoxy]phthalimide (16).** A mixture of *N*-hydroxyphthalimide (16.3 g, 0.10 mol), substituted benzyl bromide **15**[33] (27.0 g, 0.11 mol), and K_2CO_3 (15.3 g, 0.11 mol) in DMF (300 mL) was stirred at room temperature for 3 h. After removal of the solvent, the residue was dissolved in CH_2Cl_2 (200 mL) and water (200 mL). The organic phase was sepd, and the aq phase extracted with CH_2Cl_2 (50 mL × 2). The combined organic extracts were washed with water (200 mL × 3), dried over $MgSO_4$, and evapd. The residue was purified by silica gel chromatography to afford **16** (22.5 g, 69%) as white prisms, mp 136–137 °C. ^{1}H NMR ($CDCl_3$): δ 1.67 (6H, s), 5.09 (2H, s), 6.69 (1H, d, J = 7.8 Hz), 6.93 (1H, dd, J = 1.5, 7.8 Hz), 6.97 (1H, d, J = 1.5 Hz), 7.69–7.88 (4H, m); IR (cm^{-1}): 2990, 1730, 1500, 1240, 970, 710. Anal. calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.49; H, 4.72; N, 4.03.

2-[(*Z*)-[(2,2-Dimethyl-1,3-dibenzodioxol-5-yl)methoxy]imino]-2-(2-tritylaminothiazol-4-yl)acetic acid (12). To a suspension of **16** (11.39 g, 0.035 mol) in EtOH (170 mL) was added hydrazine hydrate (1.70 mL, 0.035 mol). The resulting mixture was refluxed for 1 h and cooled. The precipitates were filtered off and the filtrate was concd. The residue was dissolved in CH_2Cl_2 (100 mL), and insoluble material was filtered off. To the filtrate were added EtOH (100 mL) and aminothiazole glyoxylic acid **17**[34] (14.51 g, 0.035 mol). After stirring at room temperature for 17 h, the solvent was removed under reduced pressure and the residue triturated with Et_2O. The resultant precipitates were filtered, washed with Et_2O, and dried in vacuo to give **12** (15.91 g, 77%) as yellow prisms, mp 133–136 °C. ^{1}H NMR (DMSO-d_6): δ 1.63 (6H, s), 4.95 (2H, s), 6.76 (1H, s), 6.77 (1H, d, J = 4.6 Hz), 7.14–7.44 (18H, m), 8.75 (1H, s). IR (cm^{-1}): 3060, 2990, 1500, 1450, 1260, 1240. Anal. calcd for $C_{34}H_{29}N_3O_5S$: C, 69.02; H, 4.94; N, 7.10. Found: C, 68.84; H, 4.82; N, 7.23.

***p*-Methoxybenzyl-(6*R*,7*R*)-7-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-[1-(*t*-butoxycarbonyl)-1-methylethoxy]imino]acetamido]-8-oxo-3-[[(4-pyridyl)thio]methyl]-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (21).** To a suspension of **18**[35] (20.3 g, 0.050 mol) in CH_2Cl_2 (200 mL) was added aq 1 N NaOH soln (100 mL, 0.10 mol) at 0 °C. After stirring for 40 min, the organic layer was sepd, washed with brine (200 mL), dried over $MgSO_4$, and filtered. 4-Mercaptopyridine (6.1 g, 0.055 mol) and MeOH (25 mL) were added to the filtrate, which was stirred for 15 h. After this, the mixture was washed with aq 5% $NaHCO_3$ soln (200 mL) and brine (200 mL), dried over $MgSO_4$, and evapd to give crude **19**. DCC (11.3 g, 0.055 mol) was added to a soln of aminothiazole **20**[36] (18.1 g, 0.055 mol) and HOBT (7.4 g, 0.055 mol) in THF (250 mL) at 0 °C. After stirring for 1 h at room temperature, insoluble dicyclohexylurea was filtered off, and crude **19** was added to the filtrate. The resulting mixture was stirred for 16 h at room temperature and evapd. The residue was dissolved in CH_2Cl_2 (400 mL), washed with 2% HCl (300 mL), aq 5% $NaHCO_3$ soln (300 mL), and brine (300 mL), dried over $MgSO_4$, and evapd. The residue was purified by silica gel chromatography to afford **21** (23.9 g, 63%) as a brown powder. ^{1}H NMR ($CDCl_3$): δ 1.42 (9H, s), 1.57 (3H, s), 1.59 (3H, s), 3.45 (1H, d, J = 17.9 Hz), 3.61 (1H, d, J = 17.9 Hz), 3.80 (3H, s), 4.03 (1H, d, J = 13.3 Hz), 4.25 (1H, d, J = 13.3 Hz), 5.00 (1H, d, J = 4.9 Hz), 5.18 (1H, d, J = 11.9 Hz), 5.26 (1H, d, J = 11.9 Hz), 5.95 (1H, dd, J = 4.9, 8.7 Hz), 6.32 (2H, br s), 6.87 (1H, s), 6.88 (2H, d, J = 8.5 Hz), 7.08 (2H, d, J = 6.1 Hz), 7.34 (2H, d, J = 8.5 Hz), 7.84 (1H, d, J = 8.7 Hz), 8.35 (2H, d, J = 6.1 Hz); IR (cm^{-1}): 3300, 3130, 2980, 1790, 1730, 1520, 1140.

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-[*N*-(3,4-dihydroxybenzamido)carbamoylmethyl]pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (23b). To a soln of **21** (1.0 g, 1.3 mmol) in DMF (10 mL) was added bromide **22b** (1.15 g, 4 mmol). After stirring for 40 h at room temperature, the reaction mixture was triturated successively with hexane: Et_2O (1:1) (200 mL) and Et_2O (200 mL × 2). The resultant precipitates were filtered, washed with Et_2O, then added to the mixture of anisole (0.3 mL) and TFA (5 mL) at 0 °C. The resulting soln was stirred for 1.5 h, then Et_2O (80 mL) was added. The resultant precipitates were filtered, washed with Et_2O, and dissolved in aq 5% $NaHCO_3$ soln (100 mL), and insoluble material was filtered off. The pH of the filtrate was adjusted to 4 with 10% HCl. The aq soln was subjected to chromatography on Diaion HP-20 using *i*-PrOH:H_2O mixtures as solvent. The appropriate fractions were combined and concd. The resulting precipitates were filtered and dried in vacuo to furnish **23b** (0.61 g, 60%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6): δ 1.42 (3H, s), 1.44 (3H, s), 3.42 (1H, d, J = 17.5 Hz), 3.60 (1H, d, J = 17.5 Hz), 4.42 (1H, d, J = 15.4 Hz), 4.52 (1H, d, J = 15.4 Hz), 5.09 (1H, d, J = 4.7 Hz), 5.42 (2H, br s), 5.69 (1H, dd, J = 4.7, 9.1 Hz), 6.71 (1H, s), 6.78 (1H, d, J = 8.1 Hz), 7.11–7.45 (5H, m), 7.52 (1H, d, J = 7.0 Hz), 8.23 (2H, d, J = 6.7 Hz), 8.64 (2H, d, J = 6.7 Hz), 9.45 (1H d, J = 9.1, Hz), 10.24 (1H, br s), 10.38 (1H, br s). IR (cm^{-1}): 3300, 1770, 1640, 1540, 1300.

Compound **23a** was obtained by the same method as described for **23b**; yield and NMR data are given in Table 6.

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-(5-hydroxy-4-pyridon-2-yl)methylpyridinium-4-yl]thio]methyl]-8-oxo-1-aza-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (23c). TFA (10 mL) and anisole (2 mL) were added to **21** (2.0 g, 2.65 mmol) at 0 °C. After stirring for 1 h at 0 °C and for 1.5 h at room temperature, Et_2O (50 mL) was added to the mixture. The resulting precipitates were filtered, washed with Et_2O, and suspended in CH_2Cl_2 (50 mL). After neutralization by triethylamine (0.74 mL, 5.3 mmol), Et_2O (100 mL) was again added to the mixture. The resultant precipitates were filtered and washed with Et_2O to give crude **24**, which was dissolved in DMF (8 mL) and BSA (1.96 mL, 7.95 mmol) was added to this soln. The mixture was stirred for 1 h at

room temperature. Meanwhile, NaI (0.79 g, 5.30 mmol) was added to a soln of chloride **22c**[38] (1.73 g, 5.30 mmol) in DMF (8 mL). After stirring for 1 h at room temperature, this soln was added to the soln of silylated **24** and the resulting soln was stirred for 20 h at room temperature. At this time, *i*-PrOH (100 mL) was added to the mixture and the resulting precipitates were filtered, washed with *i*-PrOH and CH_2Cl_2, and dried in vacuo. To this precipitates were added anisole (1.5 mL) and TFA (5 mL) at 0 °C. After stirring for 30 min at 0 °C, Et_2O (100 mL) was added to the soln. The resulting precipitates were filtered, washed with Et_2O, and dissolved in 2% HCl (300 mL). Insoluble substance were filtered off and the pH of the filtrate was adjusted to 4 with aq 5% $NaHCO_3$ soln. The aq soln was chromatographed on Diaion HP-20 eluted with *i*-PrOH:H_2O mixtures, the appropriate fractions were collected and concd. The resulting precipitates were filtered, washed with water, and dried in vacuo to afford **23c** (0.05 g, 3%) as a yellow powder. ^{1}H NMR (DMSO-d_6): δ 1.42 (6H, s), 3.44 (1H, d, J=17.3 Hz), 3.63 (1H, d, J=17.3 Hz), 4.34 (1H, d, J=14.2 Hz), 4.45 (1H, d, J=14.2 Hz), 5.07 (1H, d, J=3.9 Hz), 5.54 (2H, br s), 5.70 (1H, dd, J=3.9, 7.0 Hz), 6.71 (1H, s), 6.86 (1H, s), 7.27 (2H, br s), 7.81 (1H, s), 8.21 (2H, d, J=7.8 Hz), 8.72 (2H, d, J=7.8 Hz), 9.53 (1H, d, J=7.0 Hz). IR (cm^{-1}): 3320, 1770, 1630, 1570, 1110.

Benzhydryl-(6*S*,7*S*)-7-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-[1-(t-butoxycarbonyl)-1-methylethoxy]imino]acetamido]-8-oxo-3-[[(4-pyridyl)thio]methyl]-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (25). Compound **7g** (9.92 g, 0.016 mol) was treated with methylhydrazine by the same method as described for **8f** to give crude amine **8g**, which was condensed with active ester derived from aminothiazole **20**[36] and HOBT by the similar method as described for **21** to furnish **25** (7.53 g, 60%) as a yellow powder. ^{1}H NMR ($CDCl_3$): δ 1.43 (9H, s), 1.54 (3H, s), 1.58 (3H, s), 3.02–3.25 (2H, m), 3.99–4.10 (1H, m), 4.10 (1H, d, J=14.3 Hz), 4.40 (1H, d, J=14.3 Hz), 5.64 (1H, dd, J=5.3, 7.0 Hz), 6.00 (2H, br s), 6.88 (1H, s), 6.94 (1H, s), 7.04 (2H, d, J=5.9 Hz), 7.21–7.55 (10H, m), 8.04 (1H, d, J = 7.0 Hz), 8.33 (2H, d, J = 5.9 Hz). IR (cm^{-1}): 3290, 2980, 1780, 1710, 1370, 1140.

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-[N-(3,4-dihydroxybenzamido)carbamoylmethyl]pyridinium-4-yl]thio]methyl]-8-oxo-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (27b). Compound **25** (0.79 g, 1.0 mmol) was deprotected by TFA as the same procedure as described for **24** to afford crude **26**, which was mixed with BSA (0.74 mL, 3.0 mmol) in DMF (3 mL), and the resulting mixture was stirred for 1 h at 40 °C. To this soln was added a soln of bromide **22b** (0.87 g, 3.0 mmol) and BSA (0.74 mL, 3.0 mmol) in DMF (2 mL). After stirring for 40 h at room temperature, *i*-PrOH (30 mL) was added and the mixture was evaporated. The residue was triturated with Et_2O and the resultant precipitates were filtered, washed with Et_2O, dissolved in aq 5% $NaHCO_3$ solution (80 mL), and insoluble material was filtered off. The pH of the filtrate was adjusted to 4 with 10% HCl. The aq soln was subjected to chromatography on Diaion HP-20 using *i*-PrOH:H_2O mixtures as solvent. The appropriate fractions were combined and concd. The resulting precipitates were filtered, washed with water and MeOH, and dried in vacuo to give **27b** (0.49 g, 57%) as a yellow powder. ^{1}H NMR (DMSO-d_6): δ 1.39 (3H, s), 1.40 (3H, s), 2.93–3.23 (2H, m), 3.88–4.00 (1H, m), 4.51 (1H, d, J=14.3 Hz), 4.73 (1H, d, J=14.3 Hz), 5.43 (3H, br s), 6.74 (1H, s), 6.78 (1H, d, J=8.1 Hz), 7.04–7.36 (5H, m), 7.52 (1H, d, J=6.5 Hz), 8.22 (2H, d, J=5.7 Hz), 8.66 (2H, d, J=5.7 Hz), 9.37 (1H, br s), 10.26 (1H, br s), 10.86 (1H, br s); IR (cm^{-1}): 3200, 1760, 1640, 1290.

Compound **27a** was obtained by the same method as described for **27b**; yield and NMR data are given in Table 6.

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-(5-hydroxy-4-pyridon-2-yl)methylpyridinium-4-yl]thio]methyl]-8-oxo-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carboxylate (27c). Crude **26** (0.78 g, 1.0 mmol) was quaternarized and deprotected by the same manner as described for **23c** to give **27c** (0.05 g, 7%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6): δ 1.42 (6H, s), 2.98–3.25 (2H, m), 3.91–4.07 (1H, m), 4.51 (1H, d, J = 15.0 Hz), 4.78 (1H, d, J = 15.0 Hz), 5.58 (3H, br s), 6.78 (1H, s), 6.92 (1H, s), 7.26 (2H, br s), 7.83 (1H, s), 8.17 (2H, d, J = 5.0 Hz), 8.78 (2H, d, J = 5.0 Hz), 9.53 (1H, d, J = 7.5 Hz). IR (cm^{-1}): 3310, 1760, 1630, 1580.

Benzhydryl-(6*S*,7*S*)-7-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-[1-(t-butoxycarbonyl)-1-methylethoxy]imino]acetamido]-8-oxo-3-[[(4-pyridyl)thio]methyl]-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (31). To a soln of **28**[39] (6.18 g, 10 mmol) and 4-mercaptopyridine (1.33 g, 12 mmol) in DMF (50 mL) was added triethylamine (1.67 mL, 12 mmol) at 0 °C dropwise. After stirring for 30 min, methylhydrazine (0.60 mL, 11 mmol) was added at −50 °C to the reaction mixture without isolation of **29**, which was stirred for 30 min. Then AcOH (2.5 mL) was added to the soln, which was allowed to warm to room temperature and stirred for 2 h. The precipitates were filtered off and the filtrate diluted with AcOEt (120 mL) and washed with 5% aq $NaHCO_3$ soln (300 mL). The aq layer was extracted with additional AcOEt (120 mL). The combined organic extracts were washed with 5% aq $NaHCO_3$ soln (300 mL × 3), brine (300 mL), dried over Na_2SO_4, and evapd to afford crude **30**, which was treated with active ester derived from aminothiazole **20**[36] and HOBT by the similar procedure as described for **21** to give **31** (5.80 g, 74%) as a orange powder. ^{1}H NMR ($CDCl_3$): δ 1.41 (9H, s), 1.51 (3H, s), 1.56 (3H, s), 3.85–4.03 (2H, m), 4.24 (1H, d, J = 14.0 Hz), 4.33 (1H, d, J = 14.0 Hz), 4.65 (1H, d, J = 7.4 Hz), 5.69 (1H, dd, J = 4.5, 6.8 Hz), 6.29 (2H, br s), 6.84 (1H, s), 6.91 (1H, s), 7.14 (2H, d, J = 6.3 Hz), 7.19–7.61 (10H, m), 8.15 (1H, d, J = 6.8 Hz), 8.29 (2H, d, J = 6.3 Hz). IR (cm^{-1}): 3300, 2980, 1790, 1720, 1380, 1140.

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-8-oxo-3-[[(4-pyridyl)-thio]methyl]-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (32). To a mixture of **31** (5.80 g, 7.4 mmol) and anisole (0.6 mL) in CH_2Cl_2 (60 mL) was added a soln of $AlCl_3$ (7.89 g, 59.2 mmol) in nitromethane (25 mL) at 0 °C. After stirring for 40 min at 0 °C, the mixture was poured into ice-water (300 mL) and the aq layer separated. The aq layer was washed with CH_2Cl_2 (10 mL). The combined organic layer was extracted with 1N HCl (30 mL). The pH of the combined aq layer was adjusted to 3.5 with 5% aq $NaHCO_3$ soln, and the aq soln subjected to chromatography on Diaion HP-20 using *i*-PrOH:H_2O mixtures as solvent. The appropriate fractions were combined, concd, and freeze-dried to furnish **32** (2.58 g, 62%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6): δ 1.39 (6H, s), 3.84–4.09 (2H, m), 4.27 (1H, d, J = 13.5 Hz), 4.43 (1H, d, J = 13.5 Hz), 4.58 (1H, d, J = 8.0 Hz), 5.66 (1H, dd, J = 3.5, 7.5 Hz), 6.76 (1H, s), 7.26 (2H, br s), 7.35 (2H, d, J = 6.0 Hz), 8.37 (2H, d, J = 6.0 Hz), 9.12 (1H, d, J = 7.5 Hz). IR (cm^{-1}): 3300, 1770, 1370, 1120.

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-[*N*-(3,4-dihydroxybenzamido)carbamoylmethyl]pyridinium-4-yl]-thio]methyl]-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (33b). Compound **32** (0.70 g, 1.24 mmol) was treated with bromide **22b** by the same method as described for **27b** to give **33b** (0.48 g, 50%) as a yellow powder. ^{1}H NMR (DMSO-d_6): δ 1.32 (6H, s), 3.78–4.00 (1H, m), 4.17–4.36 (1H, m), 4.52 (1H, d, J = 14.0 Hz), 4.58 (1H, d, J = 14.0 Hz), 4.79 (1H, d, J = 9.5 Hz), 5.42 (2H, s), 5.63 (1H, dd, J = 6.7, 9.5 Hz), 6.74 (1H, s), 6.78 (1H, d, J = 8.3 Hz), 7.21 (1H, d, J = 8.3 Hz), 7.26 (2H, br s), 7.29 (1H, s), 7.52 (1H, d, J = 6.8 Hz), 8.20 (2H, d, J = 6.5 Hz), 8.67 (2H, d, J = 6.5 Hz), 9.58 (1H, d, J = 9.5 Hz), 10.20 (1H, br s), 10.27 (1H, br s). IR (cm^{-1}): 3200, 1760, 1630, 1530, 1290.

Compound **33a** was obtained by the same procedure as described for **33b**; yield and NMR data are given in Table 6.

(6*S*,7*S*)-7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[1-(5-hydroxy-4-pyridon-2-yl)methylpyridinium-4-yl]thio]methyl]-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (33c). Compound **32** (1.00 g, 1.78 mmol) was quaternarized and deprotected by the same manner as described for **23c** to give **33c** (0.16 g, 13%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6): δ 1.41 (6H, s), 3.75–3.98 (1H, m), 4.12–4.30 (1H, m), 4.54 (1H, d, J = 14.3 Hz), 4.70 (1H, d, J = 14.3 Hz), 4.85 (1H, d, J = 9.2 Hz), 5.78 (3H, br s), 6.81 (1H, s), 7.18 (1H, s), 7.27 (2H, br s), 8.02 (1H, s), 8.10 (2H, d, J = 6.0 Hz), 8.92 (2H, d, J = 6.0 Hz), 9.21 (1H, d, J = 8.0 Hz). IR (cm^{-1}): 3320, 1760, 1620, 1540.

2-Bromo-[*N*-(3,4-dihydroxybenzamidoyl)]acetamide (22b). To ethyl 3,4-dihydroxy-benzoate (2.5 g, 13.7 mmol) was added hydrazine monohydrate (10 mL). After stirring for 1 h at 40 °C, the mixture was evaporated and the residue was triturated with *i*-PrOH to afford crude **34**. Bromoacetylbromide (1 mL, mmol) was added to a suspension of crude **34** in dioxane (20 mL). After stirring for 1 h at room temperature, the mixture was evapd, and the residue dissolved in AcOEt (50 mL). The soln was washed with brine (40 mL), dried over $MgSO_4$, and evapd. The residue was triturated with Et_2O and the resulting precipitates were filtered, washed with Et_2O, and dried in vacuo to give **22b** (1.8 g, 45%) as white prisms, mp 183 °C (decomp.). ^{1}H NMR (DMSO-d_6): δ 3.96 (2H, s), 6.78 (1H, d, J = 8.3 Hz), 7.24 (1H, dd, J = 1.8, 8.3 Hz), 7.30 (1H, d, J = 1.8 Hz), 9.24 (1H, br s), 9.57 (1H, br s), 10.17 (1H, s), 10.26 (1H, s). IR (cm^{-1}): 3480, 3270, 1690, 1630, 1600, 1500, 1290. Anal. calcd for $C_9H_9BrN_2O_4$: C, 37.39; H, 3.14; N, 9.69. Found: C, 37.27; H, 2.89; N, 9.78.

In vitro antibacterial activity

Minimum inhibitory concentrations (MICs) were determined by the twofold agar dilution method[40] with Müller–Hinton agar (Difco Laboratories, Detroit, MI, U.S.A.). The overnight broth cultures were diluted to approximately 10^6 CFU/mL with fresh broth, and an inoculum of 10^4 CFU per spot was applied to agar plates containing graded concentrations of each compound with an inoculating apparatus (Microplanter: Sakuma Seisakusyo, Tokyo, Japan). After incubation at 37 °C for 18 h, the MICs were defined as the minimum drug concentration which completely inhibited the growth of bacteria.

In vivo antibacterial activity

In vivo activities were determined against systemic infections caused by several strains of *P. aeruginosa*. Male ICR strain mice weighting approximately 20±1 g, in groups of 10, were used for each dosage group. Mice were challenged intraperitoneally with 0.5 mL of approximately 10–100 times the 50% lethal doses (LD_{50}) of the respective strains. The bacterial suspensions, which were prepared by overnight cultures at 37 °C on Müller–Hinton broth for several strains of *P. aeruginosa*, were suspended with the same fresh broth of overnight culture containing 5% gastric mucin. One hour after infection, various doses of each compound were subcutaneously administered to mice. The number of mice surviving at each dose was counted on the seventh day after infection, and the 50% effective dose values (ED_{50}) were calculated by the probit method.

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