STUDIES ON β-LACTAM ANTIBIOTICS

IV. AN IMPROVED SYNTHESIS OF 3-(ISOTHIAZOLYLTHIOMETHYL)CEPHALOSPORINS AND ITS APPLICATION TO NEW DERIVATIVES

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An improved synthesis and *in vitro* activity of cephalosporins with a (4-carboxy-3-hydroxy-5-isothiazolyl)thiomethyl group at the 3-position and its application to the preparation of new derivatives are described. These compounds showed excellent activity against Gram-negative bacteria including β-lactamase producing strains. Among them, **2f** was the most interesting because of its broad spectrum of antibacterial activity, including Gram-positive bacteria, and its outstanding inhibitory potency against *Pseudomonas aeruginosa*.

The opportunistic infectious diseases caused by various Gram-negative bacteria such as *Pseudomonas aeruginosa, Escherichia coli* and *Serratia marcescens* have become a serious problem in chemotherapy[1,2]. In the course of our synthetic studies of anti-pseudomonal agents[3], we described the synthesis and structure-activity relationships of cephalosporins bearing an isothiazolylthiomethyl group at the 3-position. Among them, YM-13115, **1**, showed the strongest activity against Gram-negative bacteria, including *P. aeruginosa*. However, it was poorly active against Gram-positive bacteria, and its synthesis was rather impractical due to the poor yield (10%) of acylation at the 7-position.

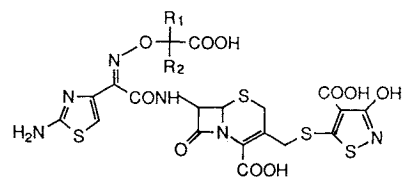
In this paper, we wish to describe an improved synthesis of 3-[(4-carboxy-3-hydroxy-5-isothiazolyl) thiomethyl]cephem compounds and its application to new derivatives, **2**, in which lipophilic alkyl- or arylthiomethoxyimino groups are introduced in order to enhance the activity against Gram-positive bacteria.

Chemistry

A general synthetic method for the preparation of (*Z*)-[[2-tritylamino-4-thiazolyl]-2-*tert*-butoxycarbonyl(alkyl or arylthio)methoxyimino]carboxylic acids **9a**～**9d**, **9e′**, **9f′** is outlined in Scheme 1.

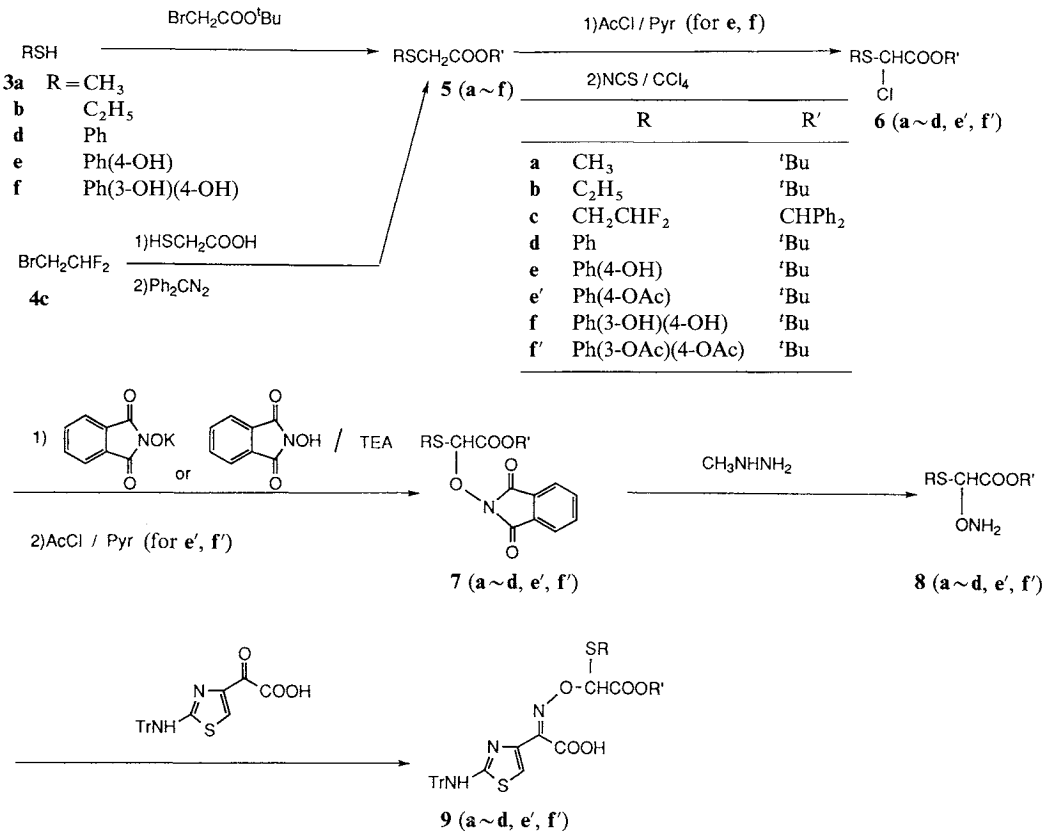
Alkyl- or arylmercaptoacetates **5** were synthesized from readily accessible starting materials: *tert*-Butyl bromoacetate was allowed to react with mercaptans **3a**, **3b**, **3d**～**3f** to afford **5a**, **5b**, **5d**～**5f**. The synthesis of **3f** was performed according to the literature procedure from catechol and thiourea[4].

Fig. 1. Structure of YM-13115 and its related derivatives.



1 $R_1=R_2=CH_3$ (YM-13115)
2 $R_1=H$, $R_2=$ –S-alkyl or –S-aryl
17a $R_1=H$, $R_2=CH_3$
17d $R_1=H$, $R_2=Ph$
17f $R_1=H$, $R_2=Ph(3\text{-}OH)(4\text{-}OH)$

Scheme 1.

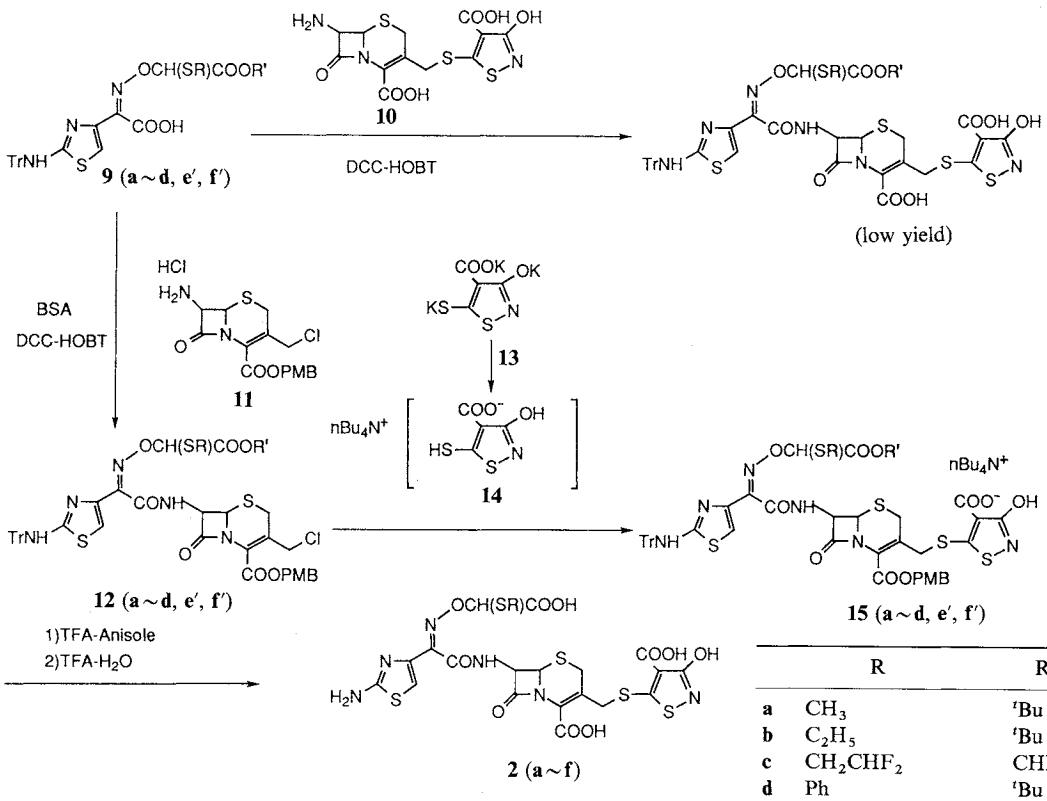


The difluoroethyl derivative **5c** was synthesized *via* the reaction of the corresponding bromide **4c** with mercaptoacetic acid, followed by esterification using diphenyldiazomethane.

Oxidation of **5a**~**5d** with *N*-chlorosuccinimide (NCS)[5] gave α-chloro derivatives **6a**~**6d**, which were converted to **9a**~**9d** *via* oxyamino compounds **8a**~**8d** in three steps. As for **5e** and **5f**, protection of the aromatic hydroxy groups was needed prior to the NCS oxidation. Acetylation followed by NCS oxidation gave **6e'** and **6f'**. The substitution reaction of **6e'** and **6f'** using *N*-hydroxyphthalimide yielded **7e'** and **7f'**. Since the acetyl groups were partially cleaved by nucleophilic attack during the reaction, crude **7e'** and **7f'** were thoroughly acetylated before they were converted to **9e'** and **9f'**, respectively.

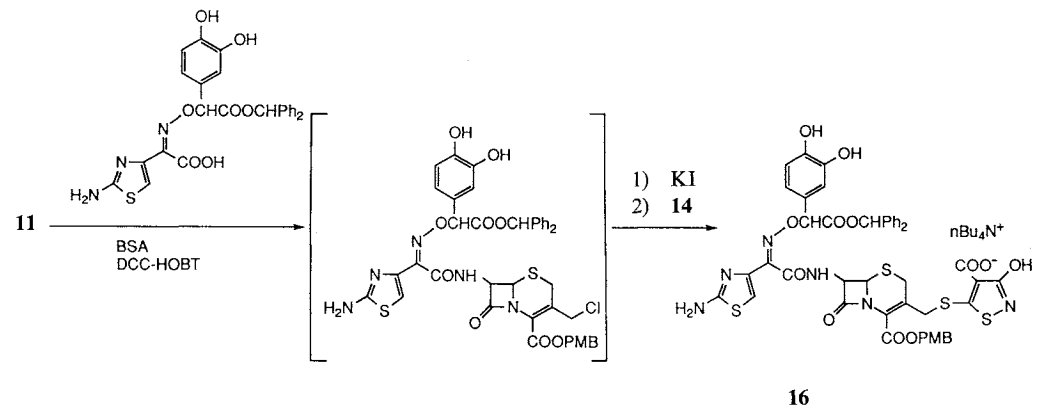
Since the yield of the coupling reaction of imino acids, **9**, with 7-amino-3-(4-carboxy-3-hydroxy-5-isothiazoly)thiomethylcephalosporanic acid, **10**[3], was poor, we chose *p*-methoxybenzyl 7-amino-3-chloromethylcephalosporanate hydrochloride, **11**, as one of the starting materials (Scheme 2). Thus acylation of **11** with **9a**~**9d**, **9e'**, **9f'** by the DCC-HOBT method gave **12a**~**12d**, **12e'**, **12f'** in good yield. To our disappointment, the subsequent conversion of the chloro group into the isothiazolylthio group using 4-carboxy-3-hydroxy-5-mercaptoisothiazole tripotassium salt, **13**, failed because of its strong basicity in aqueous solution and extremely poor solubility in organic solvents. Conversion using commonly employed acidic conditions[6] also failed due to the rapid decomposition of **13**. We found, however, that **13** formed stable salt **14** when treated with tetrabutylammonium hydrogen sulfate (TBAHS) and reacted with chloride **12** in the H_2O-CH_2Cl_2 system to give the desired product **15** (Method A). More conveniently,

Scheme 2.



	R	R'
a	CH_3	^{t}Bu
b	C_2H_5	^{t}Bu
c	CH_2CHF_2	$CHPh_2$
d	Ph	^{t}Bu
e	Ph(4-OH)	—
f	Ph(3-OH)(4-OH)	—
e'	Ph(4-OAc)	^{t}Bu
f'	Ph(3-OAc)(4-OAc)	^{t}Bu

Scheme 3.



this conversion was performed in a one-pot procedure from **11** (Method B). Thus, dethia compound, **16**, was obtained from **11** with the DMF-CH_2Cl_2 system without isolating the corresponding 7-acyl-3-chloromethylcephem (Scheme 3). Results and NMR data are shown in Tables 1a and 1b.

Table 1a. Yield and IR data for 3-isothiazolylthiomethyl derivatives.

Compound	Method of preparation	Yield (%)	IR (KBr) β-lactam (cm^{-1})	Compound	Method of preparation	Yield (%)	IR (KBr) β-lactam (cm^{-1})
15a	A	62[a]	1792	**15e'**	A	60[a]	1792
15b	A	74[a]	1792	**15f'**	A	78[a]	1780
15c	A	74[a]	1792	**16**	B	83[b,c]	1778
15d	A	72[a]	1790				

[a] Mixture of diastereomers.
[b] Absolute configuration is (*S*).
[c] Overall yield from **11**.

Table 1b. NMR data for 3-isothiazolylthiomethyl derivatives.

Compound	^{1}H NMR (90 MHz, δ in DMSO-d_6)					
	C(2)–H (2H, ABq)	C(3)–CH_2 (2H, q)	C(6)–H (1H, d)	C(7)–H (1H, dd)	Thiazole (1H, s)	Others[b]
15a	(3.68, 3.71)[a]	(4.06, 4.27) (4.07, 4.28)	5.15 5.19	5.52 5.53	6.75 6.76	1.41 (9H, s, ^{t}Bu), 2.11, 2.13 (3H, each s, SCH_3), 3.74 (3H, s, $ArOCH_3$), 5.1~5.3 (1H, m, C*H*SCH_3), 5.27 (2H, s, CH_2Ar), 6.9~7.5 (19H, m, Ar), 8.82 (1H, s, TrNH), 9.60, 9.64 (1H, each d, CONH)
15b	(3.49, 3.56)[a]	(4.11, 4.17)[a]	5.14 5.17	5.67 5.68	6.73 6.75	1.15, 1.16 (3H, each t, SCH_2CH_3), 1.41 (9H, s, ^{t}Bu), 2.67, 2.70 (2H, each q, SCH_2CH_3), 3.73 (3H, s, $ArOCH_3$), 5.4~5.6 (3H, m, C*H*SCH_2, CH_2Ar), 6.8~7.5 (19H, m, Ar), 8.82 (1H, s, TrNH), 9.59, 9.62 (1H, each d, CONH)
15c	(3.52, 3.57)[a]	(4.05, 4.17)[a]	5.18 5.21	5.64 5.65	6.82 6.88	3.56 (2H, dt, CH_2CHF_2), 3.73 (3H, s, $ArOCH_3$), 5.1~5.3 (1H, m, C*H*SCH_2), 5.76 (1H, tt, CHF_2), 6.8~7.5 (30H, m, Ar, C*H*Ph_2), 8.96 (1H, s, TrNH), 9.7~9.9 (1H, m, CONH)
15d	(3.71, 3.73)[a]	(3.98, 4.04)[a]	5.16 5.18	5.73 5.74	6.77 6.80	1.25 (9H, s, ^{t}Bu), 3.72 (3H, s, $ArOCH_3$), 5.27 (2H, s, CH_2Ar), 5.73, 5.76 (1H, each s, CHSAr), 6.86, 7.21 (4H, ABq, *Ar*OCH_3), 7.1~7.6 (20H, m, S*Ar*, Tr), 8.87 (1H, s, TrNH), 9.66, 9.70 (1H, each d, CONH)
15e'	(3.51, 3.75)[a]	(3.97, 4.14) (4.00, 4.14)	5.16 5.18	5.74[a]	6.78 6.78	1.31 (9H, s, ^{t}Bu), 2.35 (3H, s, CH_3COO-Ar), 3.71 (3H, s, $ArOCH_3$), 5.75, 5.78 (3H, m, CHSAr, CH_2Ar), 6.8~7.6 (23H, m, Ar), 8.86 (1H, brs, OH), 9.65, 9.71 (1H, each d, CONH)
15f'	(3.45, 3.52)[a]	(3.92, 3.97)[a]	5.13 5.21	5.65 5.66	6.79 6.88	1.25 (9H, s, ^{t}Bu), 2.26 (6H, s, CH_3COO-Ar), 3.72 (3H, s, $ArOCH_3$), 5.14, 5.21 (2H, ABq, CH_2Ar), 5.81, 5.83 (1H, each s, CHSAr), 6.86, 7.21 (4H, ABq, *Ar*OCH_3), 7.1~7.5 (18H, m, S*Ar*, Tr), 8.86 (1H, s, TrNH), 9.72 (1H, d, CONH)
16	(3.37, 3.65)	(3.97, 4.17)	5.11	5.77	6.75	3.74 (3H, s, $ArOCH_3$), 5.20 (2H, ABq, CH_2Ar), 5.60 (1H, s, CHAr), 6.85 (1H, s, C*H*Ph_2), 6.6~7.5 (17H, m, Ar)

[a] Diastereomers were indistinguishable.
[b] Signals for tetrabutylammonium ion were observed as follows: δ=0.9 (12H, t), 1.1~1.7 (16H, m), 3.1~3.3 (8H, m).

Finally **15a**~**15d**, **15e'**, **3f'** were deprotected by a stepwise procedure (TFA - anisole then TFA - H_2O, for removing the *p*-methoxybenzyl and trityl groups, aq $NaHCO_3$ for the acetyl group). Crude **2a**~**2f** were purified using a HP-20 column. Diastereomers were further separated in the case of **2f** (more polar isomer: **2f-I**, less polar: **2f-II**) by preparative HPLC.

Biological Results

The MICs of the new cephalosporins (**2a**~**2f**) against selected Gram-positive and Gram-negative bacteria are summarized in Table 2. For comparison, the MIC values for **1** (YM-13115), **17a**, **17d**, **17f** (the dethia congeners of **2a**, **2d**, **2f**)[3,7], ceftazidime (CAZ) and ceftriaxone (CTRX) are also listed.

The introduction of the alkyl- or arylthio group tends to enhance the activity against Gram-positive bacteria as shown. The activity of **2a**, **2d** against *Staphylococci* and *Streptococci* was mostly higher than

Table 2. Comparative activity (MIC, μg/ml)[a] of 3-[(4-carboxy-3-hydroxy-5-isothiazolyl)thiomethyl]cephem compounds.

Compound	*S.a.1*	*S.a.2*	*S.e.*	*S.p.*	*E.c.*[b]	*K.p.*[b]
1 (YM-13115)	100	100	25	6.25	0.05	0.025
2a	12.5	12.5	6.25	0.39	0.05	0.05
2b	25	25	12.5	6.25	0.025	0.1
2c	25	12.5	6.25	0.78	0.1	0.1
2d	50	25	12.5	12.5	0.05	0.2
2e	50	25	12.5	0.78	0.1	0.2
2f	6.25	6.25	1.56	1.56	0.05	0.2
2f-I	3.13	6.25	3.13	3.13	0.05	0.2
2f-II	6.25	12.5	3.13	3.13	0.1	0.2
17a	—[d]	50	25	—	<0.2	<0.2
17d	—	25	25	—	<0.2	<0.2
17f[c]	6.25	6.25	3.13	3.13	0.05	0.1
CAZ	6.25	6.25	3.13	0.2	0.1	0.1
CTRX	1.56	3.13	1.56	0.05	0.05	0.025

Compound	*E.cl.*	*S.m.*[b]	*P.r.*[b]	*P.a.1*	*P.a.2*[b]	*P.a.3*
1 (YM-13115)	0.2	0.05	0.05	0.2	50	0.025
2a	0.2	0.025	0.2	0.05	25	0.025
2b	0.2	0.013	0.05	0.05	12.5	0.025
2c	0.39	0.05	0.2	0.39	50	0.05
2d	0.39	0.025	0.2	0.1	12.5	0.1
2e	0.78	0.05	0.2	0.1	25	0.1
2f	0.78	0.05	0.2	<0.006	3.13	<0.006
2f-I	1.56	0.05	0.2	0.013	6.25	0.013
2f-II	0.39	0.025	0.1	<0.006	1.56	<0.006
17a	0.78	1.56	0.39	<0.2	0.39	0.039
17d	1.56	1.56	<0.2	1.56	0.78	0.78
17f[c]	0.2	0.1	0.2	0.013	1.56	<0.006
CAZ	0.2	<0.006	0.78	0.78	50	0.78
CTRX	0.05	<0.006	0.05	1.56	>100	6.25

Abbreviations: *S.a.1*, *Staphylococcus aureus* FDA 209P JC-1; *S.a.2*, *S. aureus* Smith; *S.e.*, *Streptococcus epidermidis* IID866; *S.p.*, *S. pyogenes* Cook; *E.c.*, *Eschelichia coli* 0-1; *K.p.*, *Klebsiella pneumoniae* ATCC 10031; *E.cl.*, *Enterobacter cloacae* 963; *S.m.*, *Serratia marcescens* IID620; *P.r.*, *Providencia rettgeri* Y-1; *P.a.1*, *Pseudomonas aeruginosa* NCTC 10490; *P.a.2*, *P. aeruginosa* ATCC 8689; *P.a.3*, *P. aeruginosa* IID5142.

[a] Agar dilution method: Mueller-Hinton agar; 10^6 cfu/ml.

[b] β-Lactamase producing strains.

[c] Known in ref 7), but no biological data was given therein. Absolute configuration of the C-7 substituent is (*S*).

[d] —: Not done.

that of the corresponding dethia congeners (**17a**, **17d**).

Among all the compounds tested, the activity of **2f-I** against *Staphylococci* was most improved. Since the dethia congener **17f** was equally potent to either **2f-I** or **2f-II**, this exceptionally improved activity was putatively due to its catechol moiety.

There was no significant loss in activity against Gram-negative bacteria compared with the original compound **1**. Excellent activity was maintained not only against *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *S. marcescens* and *Providencia rettgeri* but also against *P. aeruginosa*. It is worth noting that non-catechol type cephems with comparatively high molecular weight (*e.g.* **2d**; MW: 724) did show anti-pseudomonal activity, since recent theoretical studies suggest that they have only poor outer membrane permeability[8,9].

Table 3. Protein binding and half lives of **2f**, CTRX and CAZ[a].

Compound	Protein binding (%)			$T_{1/2}$ in mice (minutes)
	Human	Rat	Mouse	
2f	92.7	97.8	98.2	24
CTRX	92.7	85.2	68.3	36
CAZ	21.9	10.4	4.7	17

[a] Determined by bioassay.
CTRX, ceftriaxone; CAZ, ceftazidime.

Both of the catechol type cephems (**2f**, **17f**) showed excellent anti-pseudomonal activity as anticipated[10]. Particularly, the MIC of **2f-II**, the less polar isomer of **2f**, was 32 times better than that of CAZ against *P. aeruginosa* ATCC 8689, the CAZ resistant strain.

The plasma half-lives in mice were 24 minutes for **2f**, while that of CAZ and CTRX were 17 minutes and 36 minutes, respectively. Protein binding data for **2f**, CAZ and CTRX are also given in Table 3.

Experimental

NMR spectra were recorded at 90 MHz using a JEOL FX-90Q spectrometer and at 500 MHz using a JEOL GX-500 spectrometer with tetramethylsilane as an internal standard. IR spectra were taken using a Hitachi 270-30 spectrometer. Mass spectra were obtained using a JEOL DX-300 spectrometer. For column chromatography, silica gel (Wakogel C-200) was used. Preparative HPLC was performed with KHD-W-1000 (KYOWA SEIMITU CO., LTD) apparatus with a UV detector (KLC-200A) set using ODS column (YMC RI-355-15 I-15 120A ODS) at 280 nm.

Biological Evaluation

MICs were determined by the 2-fold serial agar dilution method using Mueller-Hinton agar (pH 7.2) after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml. Protein binding for the compounds was determined by an ultrafiltration method using Centrifree micropartition system (Amicon, U.S.A.). Blood samples were collected from the abdominal cava under ether anaesthesia and centrifuged. Biological samples were assayed by an agar-well method using *E. coli* NIHJ as a test organism. The plasma elimination half-life was calculated by log-linear regression.

Tetrabutylammonium 3-Hydroxy-5-mercaptoisothiazole-4-carboxylate (**14**)

To an aqueous solution of **13** (14.6 g, 50.1 mmol, in H_2O, 250 ml) was added tetrabutylammonium hydrogen sulfate (37.3 g, 109.9 mmol) in H_2O (100 ml) and the reaction mixture was stirred for 40 minutes at room temperature. Precipitated crystals were filtered, washed twice with H_2O (100 ml) and dried over P_2O_5 *in vacuo* to yield **14** (20.65 g, 98.5%): MP 119~120°C;

Anal Calcd for $C_{20}H_{38}N_2O_3S_2$: C 57.38, H 9.15, N 6.69, S 15.32.
Found: C 57.23, H 9.15, N 6.65, S 15.35.

Diphenylmethyl (2,2-Difluoroethylthio)acetate (**5c**)

Diphenyldiazomethane was slowly added to a CH_2Cl_2 solution of (2,2-difluoroethylthio)acetic acid (1.95 g, 12.5 mmol) with stirring at room temperature until the evolution of N_2 ceased. After concentration,

the product was fractionated by silica gel column chromatography (*n*-hexane - EtOAc, 20 : 1) to give the product (3.08 g, 76%): IR neat (cm^{-1}) 3048, 1744, 1276; ^1H NMR ($CDCl_3$) δ 2.91 (2H, dt, CF_2HCH_2), 3.41 (2H, s, CH_2CO), 5.82 (1H, tt, CF_2H), 6.90 (1H, s, $CHAr_2$), 7.33 (1OH, m, Ar); EI-MS *m/z* 322 (M).

tert-Butyl (3,4-Dihydroxyphenylthio)acetate (**5f**)

Carbamimidothioic acid 3,4-dihydroxyphenyl ester monoacetate[4] (23.4 g, 95.9 mmol) was dissolved in 1 N NaOH (383 ml) at room temperature. To the solution was added *tert*-butyl bromoacetate (15.5 ml, 96 mmol) and allowed to react for 3 hours. The reaction mixture was neutralized and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was then chromatographed on a silica gel column ($CHCl_3$ - EtOAc, 4 : 1) to give the product (17.7 g, 72%): IR (KBr) cm^{-1} 3248, 1706, 1602; ^1H NMR (DMSO-d_6) δ 1.33 (9H, s, ^{t}Bu), 3.46 (2H, s, SCH_2), 6.6~6.9 (3H, m, Ar), 9.08 (2H, br s, OH); EI-MS *m/z* 256 (M).

tert-Butyl (3,4-Diacetoxyphenylthio)acetate (**5f'**)

To a solution of **5f** (17.7 g, 69 mmol) in CH_2Cl_2 (70 ml) was added pyridine (11.4 ml, 141 mmol) and acetyl chloride (10.0 ml, 141 mmol) at 4°C. Aqueous workup and purification on a silica gel column (*n*-hexane - EtOAc, 7 : 3) gave **5f'** (19.7 g, 84%): IR (KBr) cm^{-1} 2992, 1780, 1734; ^1H NMR ($CDCl_3$) δ 1.37 (9H, s, ^{t}Bu), 2.35 (3H, s, Ac), 2.26 (3H, s, Ac), 3.61 (2H, s, SCH_2), 7.0~7.4 (3H, m, Ar).

tert-Butyl 2-Chloro-2-(3,4-diacetoxyphenylthio)acetate (**6f'**)

NCS (9.3 g, 70 mmol) was gradually added to a solution of **5f'** (19.7 g, 58 mmol) in CCl_4 (100 ml) and stirred overnight at room temperature. The precipitate was filtered off and the solvent was removed *in vacuo*. The residual crude oil (17.6 g) was used for the next reaction without purification: ^1H NMR ($CDCl_3$) δ 1.47 (9H, s, ^{t}Bu), 2.29 (6H, s, Ac), 5.41 (1H, s, SCHCl), 7.1~7.6 (3H, m, Ar); EI-MS *m/z* 374 (M).

tert-Butyl 2-(3,4-Diacetoxyphenylthio)-2-phthalimidoyloxy)acetate (**7f'**)

Crude **6f'** 17.6 g, (obtained above) was added dropwise to a solution of *N*-hydroxyphthalimide (11.1 g, 68 mmol) and triethylamine (9.5 ml, 68 mmol) DMF (250 ml) at −40°C. Then the temperature was gradually raised to 25°C. The reaction mixture was diluted with EtOAc, washed with H_2O and brine successively and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and precipitated *N*-acetoxyphthalimide was removed by filtration with the aid of a small amount of Et_2O. The filtrate was concentrated to give a crude oil, which contained partially deacetylated **7f'**. This crude mixture was acetylated once again as described for **5f'** and chromatographed on a silica gel column (*n*-hexane - AcOEt, 6 : 4) to give pure **7f'** (3.27 g, 11.3% from **5f'**): MP 154°C; IR (KBr) cm^{-1} 3000, 1780, 1500, 1380; ^1H NMR ($CDCl_3$) δ 1.35 (9H, s, ^{t}Bu), 2.28 (3H, s, Ac), 2.29 (3H, s, Ac), 5.94 (1H, s, OCHS), 7.1~7.3 (3H, m, Ar), 7.7~7.9 (4H, m, Ar).

tert-Butyl 2-Aminoxy-2-(3,4-diacetoxyphenylthio)acetate (**8f'**)

To a solution of **7f'** (3.10 g, 6.19 mmol) in CH_2Cl_2 (50 ml) was added methylhydrazine (0.33 ml, 6.20 mmol) at −60°C. The temperature was raised to 0°C in 1.5 hours. Insoluble materials were removed by suction and solvent was evaporated *in vacuo* to give **8f'** (2.50 g), which could not be purified by column chromatography because of decomposition: IR (KBr) cm^{-1} 2992, 1778, 1740, 1224; ^1H NMR ($CDCl_3$) δ 1.42 (9H, s, ^{t}Bu), 2.28 (6H, s, Ac), 5.31 (1H, s, OCHS), 5.7 (2H, br s, NH_2), 6.9~7.6 (3H, m, Ar); FAB-MS (positive) *m/z* 372 (M+H).

(*Z*)-2-[[(*tert*-Butoxycarbonyl)-(3,4-diacetoxyphenylthio)methoxy]imino]-2-(2-tritylamino-4-thiazolyl)acetic Acid (**9f'**)

To a solution of **8f'** (2.50 g, 6.73 mmol) in methanol was added 2-oxy-2-(2-tritylamino-4-thiazolyl) acetic acid (2.38 g, 5.75 mmol), and the reaction mixture was stirred for 1 hour at room temperature. After removing methanol by evaporation, the residue was purified by column chromatography on silica gel to afford **9f'** as a colorless powder (2.28 g, 48% from **7f'**): MP 90~93°C; IR (KBr) cm^{-1} 2992, 1778, 1740, 1494; ^1H NMR (DMSO-d_6) δ 1.26 (9H, s, ^{t}Bu), 2.26 (6H, s, Ac), 5.96 (1H, OCHS), 6.90 (1H, s,

Table 4. NMR data for **9a**~**9d**, **9e'**.

Compound	^{1}H NMR (90 MHz, δ)	(Solvent)
9a	1.26 (9H, s, ^{t}Bu), 2.10 (3H, s, CH_3), 5.54 (1H, s, OCHS), 6.88 (1H, s, thiazole), 7.0~7.6 (15H, m, Ar)	(DMSO-d_6)
9b	1.15 (3H, t, CH_3), 1.38 (9H, s, ^{t}Bu), 2.58 (2H, q, CH_2), 5.41 (1H, s, OCHS), 6.68 (1H, s, thiazole), 7.0~7.5 (15H, m, Ar)	(CD_3OD)
9c	2.08 (2H, dt, CH_2), 5.44 (1H, s, OCHS), 6.06 (1H, tt, CHF_2), 6.92 (1H, s, thiazole), 6.92 (1H, s, $CHPh_2$), 7.2~7.5 (20H, m, Ar)	(DMSO-d_6)
9d	1.24 (9H, s, ^{t}Bu), 5.80 (1H, s, OCHS), 6.81 (1H, s, thiazole), 7.0~7.5 (20H, m, Ar)	(DMSO-d_6)
9e'	1.22 (9H, s, ^{t}Bu), 5.80 (1H, s, OCHS), 6.80 (1H, s, thiazole), 7.03, 7.48 (4H, ABq, S*Ar*OAc), 7.0~7.4 (15H, m, Ar)	(DMSO-d_6)

For NMR data of **9f'**, see experimental section.

thiazole), 7.1~7.5 (18H, m, Ar), 8.86 (1H, s, COOH); FAB-MS (positive) *m/z* 768 (M+H).

The other acids (**9a**~**9d**, **9e'**) were similarly prepared. ^{1}H NMR data are summarized in Table 4.

p-Methoxybenzyl 7*β*-[(*Z*)-2-[[(*RS*)-(*tert*-Butoxycarbonyl)-(3,4-diacetoxyphenylthio)methoxy]imino]-2-(2-tritylamino-4-thiazolyl)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (**12f'**)

To a solution of **9f'** (783 mg, 1.02 mmol) and HOBT (138 mg, 1.02 mmol) in a mixed solvent of DMF (1.5 ml) and CH_2Cl_2 (4 ml) was added DCC (210 mg, 1.02 mmol). The solution was stirred for 1 hour under ice cooling. The active ester thus obtained was added to a solution of **11** (413 mg; 1.01 mmol) and bistrimethylsilylacetamide (BSA) (0.4 ml) in CH_2Cl_2 (10 ml) and reacted for 1.5 hours at room temperature. The reaction mixture was diluted with 2-butanone and washed successively with H_2O, aq. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel ($CHCl_3$ - AcOEt, 95 : 5) to afford **12f'** as an amorphous solid (896 mg, 78.6%): IR (KBr) cm^{-1} 1780, 1740, 1372, 1248, 1208, 1170; ^{1}H NMR ($CDCl_3$) δ 1.26 (9H, s, ^{t}Bu), 2.23~2.25 (6H, each s, Ac), 3.46, 3.72 (2H, ABq, 2-CH_2), 3.73 (3H, s, OCH_3), (4.43, 4.52) and (4.43, 4.53) (2H, two ABqs of diastereomers, 3-CH_2), 5.2~5.4 (3H, m, CH_2Ar and 6-CH), 5.70~5.73 (1H, m, 7-CH), 5.83 and 5.85 (1H, each s, OCHS), 6.80 (1H, s, thiazole), 6.9~7.5 (22H, m, Ar), 9.77 (1H, d, CONH); FAB-MS (positive) *m/z* 1,118 (M+H).

Tetrabutylammonium Salt of *p*-Methoxybenzyl 7*β*-[(*Z*)-2-[[(*RS*)-(*tert*-Butoxycarbonyl)-(3,4-diacetoxyphenylthio)methoxy]imino]-2-(2-tritylamino-4-thiazolyl)acetamido]-3-[(4-carboxy-3-hydroxy-5-isothiazolyl)thio]methyl-3-cephem-4-carboxylate (**15f'**) (Method A)

To a mixture of H_2O (4 ml) and CH_2Cl_2 (4 ml) was added 4-carboxy-3-hydroxy-5-mercaptoisothiazole tripotassium salt (263 mg, 0.90 mmol), TBAHS (613 mg, 1.80 mmol) and **12f'** (857 mg, 0.77 mmol), and the reaction mixture was stirred for 12 hours at room temperature. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$ - MeOH - HCOOH, 95 : 2 : 1) to afford **15f'** (912 mg, 78%): IR (KBr) cm^{-1} 1780, 1730, 1678, 1248, 1210, 1170; FAB-MS (negative) *m/z* 1,257 (M−Bu_4N). The ^{1}H NMR data are shown in Table 1b.

Tetrabutylammonium Salt of *p*-Methoxybenzyl 7*β*-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-[[(*S*)-(3,4-dihydroxyphenyl)-(diphenylmethoxycarbonyl)methoxy]imino]acetamido]-3-[(4-carboxy-3-hydroxy-5-isothiazolyl)thio]methyl-3-cephem-4-carboxylate (**16**) (Method B)

Compound **11** (2.03 g, 5.01 mmol) was allowed to react with (*Z*)-2-(2-amino-4-thiazolyl)-2-[[(*S*)-(3,4-dihydroxyphenyl)-(diphenylmethoxycarbonyl)methoxy]imino]acetic acid (3.12 g, 6.00 mmol) for 4 hours using the method described for **12f'**. Instead of quenching the reaction by adding H_2O, KI (1.66 g, 10 mmol) in DMF (10 ml) and **14** (2.50 g, 6.00 mmol) were added to the mixture. The cooling bath was removed, and stirring was continued for 2 hours at ambient temperature. After removal of CH_2Cl_2, ice-water was added and the mixture was extracted with 2-butanone. The organic layer was washed with H_2O, brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was thoroughly triturated with ethanol and dried *in vacuo* to afford the product (5.19 g, 83%): IR (KBr) cm^{-1} 3380, 1792, 1730, 1678, 1618, 1250;

FAB-MS (positive) *m/z* 1,011 ($M-Bu_4N+2H$). The ^1H NMR data are shown in Table 1b.

Trisodium Salt of 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-[[(*RS*)-carboxy-(3,4-dihydroxyphenylthio)-methoxy]imino]acetamido]-3-[(4-carboxy-3-hydroxy-5-isothiazolyl)thio]methyl-3-cephem-4-carboxylate (2f)

To a mixture of **15f'** (6.72 g, 4.48 mmol) and anisole (7 ml) was added TFA (35 ml), and the mixture was stirred for 1 hour at room temperature. After removing TFA *in vacuo*, the residue was triturated with Et_2O. The collected precipitate was treated with TFA (30 ml) and H_2O (15 ml) for 3 hours at room temperature, and the mixture was concentrated and triturated with Et_2O. The powder obtained was dissolved in aq-$NaHCO_3$ (10 ml), and stirring was continued for 3 hours at room temperature. The reaction mixture was purified using a HP-20 (500 ml) column. The fractions containing the desired compound were collected, concentrated and lyophilized to give **2f** (718 mg, 20%): FAB-MS (negative) *m/z* 799 ($M-Na$).

Both diastereomers were separated by preparative HPLC (mobile phase; 10% CH_3CN-0.02 M KH_2PO_4).

2f-I: IR (KBr) cm^{-1} 3388, 1722, 1624, 1386; ^1H NMR (D_2O) δ 3.35, 3.72 (2H, ABq, 2-CH_2), 3.93, 4.48 (2H, ABq, CH_2S), 5.13 (1H, d, 6-CH), 5.71 (1H, d, 7-CH), 5.83 (1H, s, CHSAr), 7.09 (thiazole) 6.8~7.1 (3H, m, Ar).

2f-II: IR (KBr) cm^{-1} 3452, 1772, 1620, 1388; ^1H NMR (D_2O) δ 3.35, 3.72 (2H, ABq, 2-CH_2), 3.92, 4.43 (2H, ABq, CH_2S), 5.14 (1H, d, 6-CH), 5.74 (1H, d, 7-CH), 5.82 (1H, s, CHSAr), 7.09 (thiazole) 6.8~7.1 (3H, m, Ar).

2a~**2e** were prepared in a similar way.

2a: yield 14%; IR (KBr) cm^{-1} 3392, 1778, 1638, 1388, 1014; ^1H NMR (DMSO-d_6) δ 2.17 and 2.19 (3H, each s, SCH_3), 3.56, 3.76 (2H, ABq, 2-CH_2), 3.74 (3H, s, OCH_3), 4.12, 4.28 (2H, ABq, 3-CH_2), 5.2 (1H, s, C*H*SCH_3), 5.56 (1H, d, 6-CH), 5.73~5.93 (1H, m, 7-CH), 6.77 and 6.79 (1H, each s, thiazole), 9.59 and 9.65 (1H, each d, CONH).

2b: yield 20%; IR (KBr) cm^{-1} 3458, 1776, 1614, 1390, 1064; ^1H NMR (D_2O) δ 1.24 (3H, t, SCH_2CH_3), 2.70 and 2.73 (2H, each q, SCH_2CH_3), 3.47, 3.79 (2H, ABq, 2-CH_2), 5.20 and 5.22 (1H, each, s, *CH*SCH_2), 5.64 (1H, d, 6-CH_2), 5.79 and 5.82 (1H, each d, 7-CH), 7.07 and 7.08 (1H, each s, thiazole).

2c: yield 20%; IR (KBr) cm^{-1} 3444, 1766, 1624, 1390, 1010; ^1H NMR (D_2O) δ 3.0~3.3 (2H, m, SCH_2CHF_2), 3.48 and 3.82 (2H, ABq, 2-CH_2), 3.88 and 4.42 (2H, ABq, 3-CH_2), 5.23 (1H, d, 6-CH), 5.77 (1H, d, 7-CH), 5.82 and 5.84 (1H, each s, C*H*SCH_2), 6.09 (1H, m, CHF_2), 7.12 (1H, s, thiazole).

2d: yield 10%; IR (KBr) cm^{-1} 3396, 1778, 1638, 1384, 1014; ^1H NMR (DMSO-d_6) 3.52, 3.82 (2H, ABq, 2-CH_2), 4.07, 4.24 (2H, ABq, 3-CH_2), 5.18 (1H, d, 6-CH), 5.7~5.9 (1H, m, 7-CH), 5.81 and 5.84 (1H, each s, CHSPh), 6.78 and 6.80 (1H, each s, thiazole), 7.2~7.6 (5H, m, SPh), 9.67 and 9.71 (1H, each d, CONH).

2e: yield 12%; IR (KBr) cm^{-1} 3464, 1770, 1628, 1540, 1388; ^1H NMR (DMSO-d_6) 3.28, 3.57 (2H, ABq, 2-CH_2), 5.00 (1H, d, 6-CH), 5.40 and 5.46 (1H, each s, CHSAr), 5.51~5.60 (1H, m, 7-CH), 6.62, 7.26 (4H, ABq, Ar), 6.82 and 6.86 (1H, each, s, thiazole).

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