STUDIES ON β-LACTAM ANTIBIOTICS

I. SYNTHESIS AND *IN VITRO* ANTI-PSEUDOMONAL ACTIVITY OF 3-ISOTHIAZOLE-CEPHALOSPORIN DERIVATIVES

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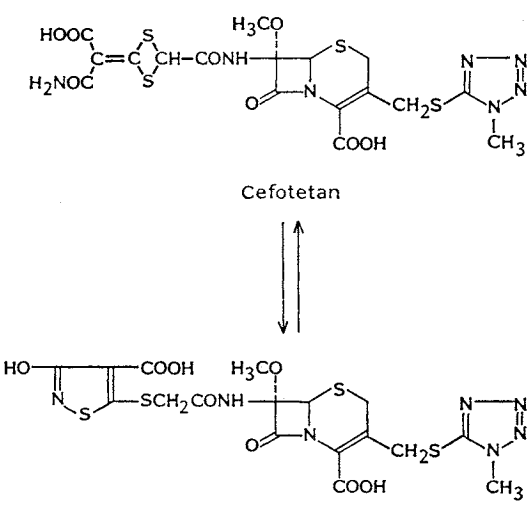
The synthesis and *in vitro* activity of 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(2-carboxy-2-alkoxyimino)acetamido]cephalosporins with a (4-carboxy-3-hydroxy-5-isothiazolyl)thiomethyl group at the 3-position are described. These cephalosporins (**9a**~**9i**) showed excellent activity against Gram-negative bacteria including β-lactamase producing strains. The most interesting compound of the series was 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(2-carboxy-2-propoxyimino)acetamido]-3-cephem-4-carboxylic acid (**9g**, YM-13115) because of its outstanding inhibitory potency against *Pseudomonas aeruginosa* and highly prolonged plasma half-life in rats.

At present, the opportunistic infectious diseases have progressively increased and become a serious problems in chemotherapy. These diseases have been mainly caused by various Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* sp. and *Serratia marcescens*, *etc.* Ceftazidime has been reported to be a very broad spectrum antibacterial agent with high degree of resistance to most β-lactamases and potentially active against *P. aeruginosa*[1]. It is a parenteral aminothiazolyl cephalosporin bearing a pyridinium methyl group at the 3-position. In a previous paper[2], we reported the synthesis and antimicrobial activity of a semi-synthetic cephamycin, cefotetan (YM-09330). As shown in Fig. 1, the tautomer of cefotetan possessing an isothiazole ring on 7β-substituent also had potent broad-spectrum antibacterial activity. This paper describes the synthesis and *in vitro* activity of several kinds of cephems (**9a**~**9i**) bearing an isothiazole ring at the 3-position. These compounds had high activities against Gram-negative bacteria, especially against strains of *P. aeruginosa*, and showed long plasma half-lives in rats.

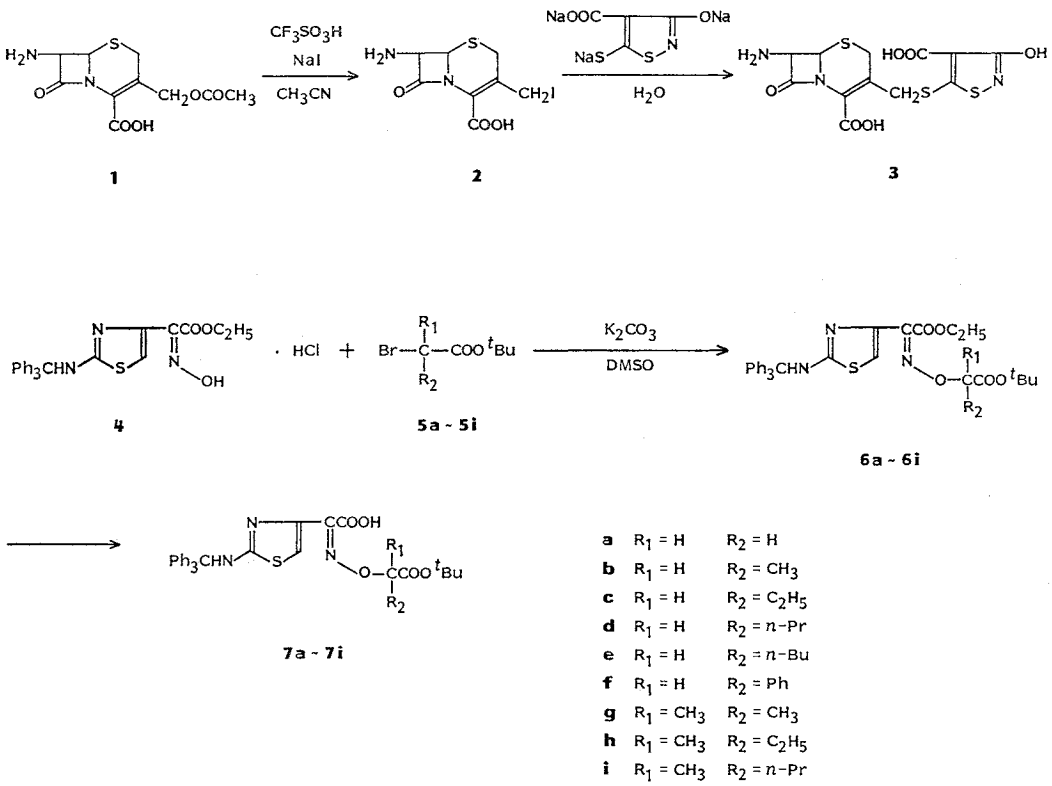
Fig. 1.



Chemistry

As one of the starting materials, 7β-amino-3-(4-carboxy-3-hydroxy-5-isothiazolyl)-3-cephem-4-carboxylic acid (**3**) was prepared from trisodium 4-carboxy-3-hydroxy-5-mercaptoisothiazole and a 3-iodomethylcephem compound (**2**) which was prepared in one-pot process from iodination of 7-ACA (**1**). This iodination method[3], which was developed in our laboratory, was carried

Scheme 1.

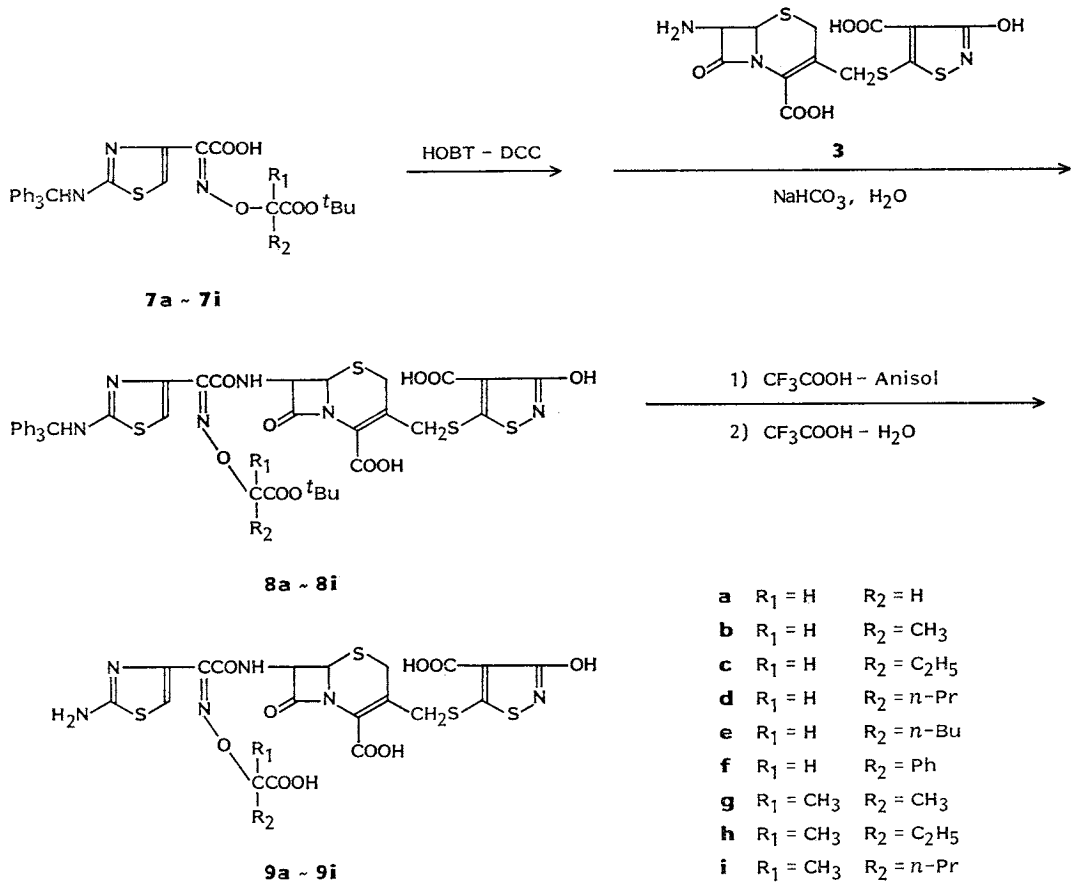


out with sodium iodide and trifluoromethanesulfonic acid. The other starting materials, (*Z*)-2-(2-trityl-amino-4-thiazolyl)-2-(*tert*-butoxycarbonylalkoxyimino)acetic acids (**7b, 7c, 7d, 7e, 7i**) were prepared from **4** by alkylation with α-bromo-*tert*-butyl esters (**5b, 5c, 5d, 5e, 5i**), followed by subsequent alkaline hydrolysis as shown in Scheme 1, and the other acids (**7a, 7f, 7g, 7h**) were obtained in a similar manner as reported by the Glaxo group[4] and the Roussel-Uclaf group[5]. The coupling of the acids (**7a~7i**) to the 7β-aminocephalosporin nucleus (**3**) was accomplished *via* their active esters, formed by well-known method such as DCC-HOBT. Stepwise removal of two protective groups of **8a~8i** with trifluoro-acetic acid-anisol and trifluoroacetic acid - water gave the products (**9a~9i**) as shown in Scheme 2.

Biological Results

The *in vitro* antibacterial activities of the new 3-[(4-carboxy-3-hydroxy-5-isothiazolyl)thiomethyl]-cephem compounds (**9a~9i**) against selected Gram-positive and Gram-negative organisms were shown in Table 1. The antibacterial activities of **9a~9i** against *Staphylococcus aureus* were clearly inferior to those of ceftriaxone and ceftazidime. Against most of the Gram-negative bacteria, the activities were similar. However, against *P. aeruginosa*, **9a~9i** were clearly more active than ceftazidime. Especially, **9g** (YM-13115)[6,7], **9h** and **9i** showed very strong activities against *P. aeruginosa* and their activities were 4 to 16 times as strong as that of ceftazidime. Furthermore, the relationship between MIC of **9a~9i** against *P. aeruginosa* and substituent groups (R_1 and R_2) of **9a~9i** was investigated. In Fig. 2, the mean MIC of **9a~9i** against *P. aeruginosa* was plotted against the values of substituent lipophilicity (π)[8] of R_1 and R_2 of **9a~9i**. When R_1 is hydrogen, a significant parabolic relationship is observed

Scheme 2.



between the anti-pseudomonal activity and the values of π. The compounds which showed the strongest *in vitro* activity against *P. aeruginosa*, had π values in the range of 1.4 to 1.8. When R_1 is methyl, the increasing lipophilicity of R_2 (Me, Et and *n*-Pr) has little influence on the anti-pseudomonal activity, and all of the compounds have strong activities against *P. aeruginosa*. The value for substituent lipophilicity of **9g** was 1.78. The plasma half-lives in rats[9] were 48 minutes for **9g**, 34 minutes for ceftriaxone, 33 minutes for **9h** and 14 minutes for ceftazidime. The stated above higher inhibitory potency of **9g** against *P. aeruginosa* and its markedly longer plasma half-life in rats in comparison with those of ceftazidime seem to be attributed to the difference in 3-position moiety of cephem ring.

Experimental

NMR spectra were recorded at 90 MHz on a Jeol 90-Q spectrometer and at 100 MHz on a Jeol MH100NMR spectrometer using tetramethylsilane as an internal standard. IR spectra were taken on a Hitachi 260-10 spectrophotometer. For column chromatography, silica gel (Wakogel C-200) was used. Melting points of the cephalosporins are not accurately reproducible because of extensive decomposition.

General Preparation of (*Z*)-2-(2-Tritylamino-4-thiazolyl)-2-(*tert*-butoxycarbonylalkoxyimino)-acetic Acid (7)

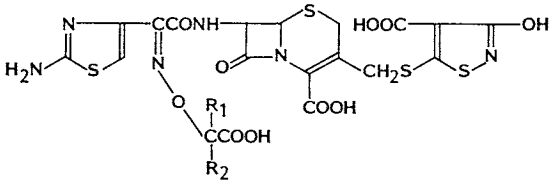
To a solution of ethyl (*Z*)-2-hydroxyimino-2-(2-tritylamino-4-thiazolyl)acetate hydrochloride (**4**,

Table 1. Antibacterial activities of 3-[(4-carboxy-3-hydroxy-5-isothiazolyl)thiomethyl]cephem compounds (**9a**~**9i**).

Strain	MIC (μg/ml)										
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	CAZ	CTRX
R_1/R_2:	H/H	H/Me	H/Et	H/*n*-Pr	H/*n*-Bu	H/Ph	Me/Me	Me/Et	Me/*n*-Pr		
Staphylococcus aureus Smith	50	50	50	25	25	25	50	50	50	12.5	3.13
Escherichia coli O-1	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2
E. coli Ebara	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	0.78	≦0.2
Klebsiella pneumoniae ATCC 10031	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2	≦0.2
K. pneumoniae V-17	3.13	≦0.2	≦0.2	≦0.2	≦0.2	1.56	≦0.2	≦0.2	≦0.2	0.39	0.78
Serratia marcescens IID-620	≦0.2	1.56	≦0.2	0.39	0.78	1.56	≦0.2	≦0.2	≦0.2	0.39	0.78
Proteus rettgeri Y-1	≦0.2	0.39	0.39	0.39	0.78	≦0.2	≦0.2	≦0.2	≦0.2	3.13	≦0.2
Enterobacter cloacae 963 (MS-1)	—	0.78	0.78	0.78	1.56	1.56	0.39	0.78	≦0.2	0.39	≦0.2
E. aerogenes NY-2	3.13	1.56	0.78	0.78	1.56	3.13	0.78	0.78	≦0.2	6.25	0.78
Pseudomonas aeruginosa NCTC 10490	0.78	≦0.2	≦0.2	≦0.2	≦0.2	1.56	≦0.2	≦0.2	0.39	0.78	0.78
P. aeruginosa IID 5142	1.56	0.39	≦0.2	0.39	0.39	0.78	≦0.2	≦0.2	≦0.2	3.13	6.25
P. aeruginosa NC-5	3.13	0.78	0.39	1.56	3.13	0.39	0.39	≦0.2	≦0.2	3.13	50
P. aeruginosa 99	1.56	0.78	0.39	1.56	3.13	3.13	0.78	≦0.2	0.39	3.13	100

CAZ: Ceftazidime, CTRX: ceftriaxone.

Fig. 2. Relationship between the anti-pseudomonal activity and π of R_1 and R_2 of **9a**~**9i**.



9a R_1 = H R_2 = H
9b R_1 = H R_2 = CH_3
9c R_1 = H R_2 = C_2H_5
9d R_1 = H R_2 = *n*-Pr
9e R_1 = H R_2 = *n*-Bu
9f R_1 = H R_2 = Ph
9g R_1 = CH_3 R_2 = CH_3
9h R_1 = CH_3 R_2 = C_2H_5
9i R_1 = CH_3 R_2 = *n*-Pr

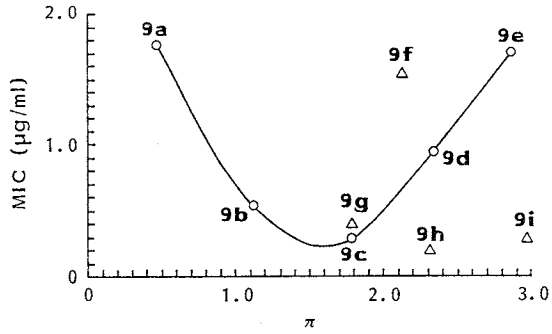


Table 2. Yields, mp, analytical and IR data of **7b**, **7c**, **7d**, **7e** and **7i**.

Compounds No.	R_1	R_2	Yield (from **4**) mp (°C)	*Anal* Calcd for Calcd: Found:	IR (KBr) cm^{-1} COO^tBu
				$C_{31}H_{31}N_3O_5S$ (557.63)	
			20%	C 66.77, H 5.54, N 7.54, S 5.75	
7b	H	Me	166~167	C 66.68, H 5.85, N 7.29, S 5.74	1710
				$C_{32}H_{33}N_3O_5S \cdot \frac{1}{4}H_2O$ (576.20)	
			44%	C 66.71, H 5.85, N 7.29, S 5.56	
7c	H	Et	172~173	C 66.84, H 5.81, N 7.31, S 5.63	1735
				$C_{33}H_{35}N_3O_5S$ (587.72)	
			43%	C 67.67, H 6.02, N 7.17, S 5.47	
7d	H	*n*-Pr	168~169	C 67.63, H 6.06, N 7.22, S 5.51	1735
				$C_{34}H_{37}N_3O_5S$ (599.75)	
			31%	C 68.09, H 6.22, N 7.01, S 5.35	
7e	H	*n*-Bu	102~105	C 68.25, H 6.00, N 6.98, S 5.39	1735
				$C_{34}H_{37}N_3O_5S$ (599.75)	
			30%	C 68.09, H 6.22, N 7.01, S 5.35	
7i	Me	*n*-Pr	175~176	C 67.83, H 6.43, N 6.72, S 5.37	1735

Table 3. ^1H NMR spectral data of **7b**, **7c**, **7d** and **7e**.

Compounds No.	R_2	^1H NMR δ value (DMSO-d_6) ppm NH	Thiazole 5-H	—CH(H)—R_2	*tert*-Bu	R_2
7b	Me	8.76	6.80	4.52	1.38	1.33 (3H, d)
7c	Et	8.82	6.84	4.37	1.40	0.88 (3H, t), 1.72 (2H, m)
7d	*n*-Pr	8.81	6.84	4.43	1.40	0.90 (3H, t), 1.22~1.51 (2H, m), 1.54~1.80 (2H, m)
7e	*n*-Bu	8.80	6.83	4.21	1.35	0.88 (3H, t), 1.12~1.55 (4H, m), 1.55~1.83 (2H, m)

0.005 mol) in DMSO (20 ml) was added powdered potassium carbonate (1.0 g) and *tert*-butyl α-bromoalkylacetate (**5**, 0.006 mol). After being stirred at room temp for one night, the mixture was partitioned between EtOAc and ice-water. The organic layer was washed with brine, dried ($MgSO_4$)

Table 4. ^{1}H NMR spectral data of **8**.

Compounds			^{1}H NMR δ value (DMSO-d_6) ppm						
No.	R_1	R_2	CONH (1H, d)	Thiazole 5-H (1H, s)	C(7)-H (1H, dd)	C(6)-H (1H, d)	C(3)-CH_2 (2H, q)	*tert*-Bu (9H, s)	R_1, R_2
8a	H	H	9.38	6.72	5.69	5.13	4.16	1.42	4.50 (2H, s)
8b	H	Me	9.32, 9.41	6.71, 6.72	5.72	5.15	4.18	1.39	4.49 (1H, q), 1.27 (3H, d)
8c	H	Et	9.40	6.74, 6.78	5.74	5.21	4.22	1.44	4.38 (1H, t), 1.70, 0.88
8d	H	*n*-Pr	9.44	6.72, 6.74	5.74	5.18	4.22	1.40	4.39 (1H, t), 1.2~1.9, 0.88
8e	H	*n*-Bu	9.43	6.73, 6.71	5.74	5.18	4.20	1.40	4.39 (1H, t), 1.2~1.9, 0.88
8f	H	Ph	9.54	6.76, 6.73	5.69	5.11	4.15	1.36	5.44 (1H, s), 7.38 (5H)
8g	Me	Me	9.26	6.60	5.68	5.15	4.16	1.36	1.38 (6H, s)
8h	Me	Et	9.27	6.66, 6.64	5.71	5.13	4.16	1.39	1.22 (3H, s), 1.71, 0.84
8i	Me	*n*-Pr	9.34, 9.40	6.66, 6.68	5.72	5.20	4.20	1.40	1.40 (3H, s), 1.0~1.9, 0.84

Table 5. ^{1}H NMR spectral data of **9**.

Compounds			^{1}H NMR δ value (DMSO-d_6) ppm						
No.	R_1	R_2	CONH (1H, d)	Thiazole 5-H (1H, s)	C(7)-H (1H, dd)	C(6)-H (1H, d)	C(3)-CH_2 (2H, q)	C(2)-2H (2H, q)	R_1, R_2
9a	H	H	9.44	6.76	5.78	5.17	4.16	3.64	4.57 (2H, s)
9b	H	Me	9.48, 9.44	6.80, 6.79	5.93, 5.79	5.22	4.20	3.69	1.40 (3H, d)
9c	H	Et	9.48, 9.46	6.78, 6.76	5.96, 5.72	5.21	4.22	3.68	4.49 (1H), 1.81 (2H), 0.96 (3H, t)
9d	H	*n*-Pr	9.48	6.78, 6.76	5.98, 5.72	5.22	4.22	3.70	4.54 (1H), 1.98~1.2, 0.91 (3H, t)
9e	H	*n*-Bu	9.48	6.78, 6.77	5.98, 5.72	5.22	4.20	3.68	4.50 (1H), 1.98~1.2, 0.86 (3H, t)
9f	H	Ph	9.60, 9.50	6.82, 6.78	5.95, 5.68	5.18, 5.15	4.18	3.55	5.57 (1H), 7.64~7.10
9g	Me	Me	9.38	6.70	5.82	5.17	4.15	3.66	1.41 (6H, s)
9h	Me	Et	9.38	6.71	5.90, 5.70	5.18	4.18	3.68	1.31, 1.41, 1.82, 0.86 (3H, t)
9i	Me	*n*-Pr	9.36	6.72	5.96, 5.72	5.20	4.16	3.66	1.04~1.92, 0.84 (3H, s)

and evaporated under reduced pressure. The residue was triturated with *n*-hexane to afford ethyl (*Z*)-2-(2-tritylaminothiazolyl)-2-(*tert*-butoxycarbonylalkoxyimino)acetate (**6**). A solution of above diester (**6**) and powdered potassium carbonate (500 mg) in methanol (55 ml) and water (6 ml) was refluxed for 4~5 hours. The mixture was concentrated to about 6 ml and extracted with EtOAc after acidification (pH 1.0) with 1 N HCl. The separated organic layer was washed with brine, dried ($MgSO_4$), and then evaporated under reduced pressure. The residue was triturated with Et_2O to afford acid **7**.

Preparation of 7-β-Amino-3-[(4-carboxy-3-hydroxy-5-isothiazolyl)thiomethyl]-3-cephem-4-carboxylic Acid (**4**)

To a solution of the 7-amino-3-iodomethylcephem compound (34 g, 0.1 mol) and $NaHCO_3$ (8.4 g, 0.1 mol) in water (600 ml) was added trisodium 4-carboxy-3-hydroxy-5-mercaptoisothiazole (26.7 g, 1.11 mol) and the mixture was stirred for 3 hours at room temp. The reaction solution was adjusted to pH 1.6 with 2 N HCl (114 ml) under ice-cooling, the separated solid was collected by suction and washed with cold water and dried (P_2O_5) to give **3** as pale brown powder (29 g, 74.6%): MP 170~200°C (dec); IR (KBr) 1770 cm^{-1}; NMR ($D_2O+NaHCO_3$) δ 3.58 (2H, q, 2-CH_2), 3.98 (2H, q, 3-CH_2), 5.04 (1H, d, 6-H), 5.41 (1H, d, 7-H).

General Preparation of 3-[(4-Carboxy-3-hydroxy-5-isothiazolyl)thiomethyl]-7β-[(*Z*)-2-(2-tritylamino-4-thiazolyl)-2-(*tert*-butoxycarbonylalkoxyimino)acetamido]-3-cephem-4-carboxylic Acid (**8**)

A mixture of **7** (3.3 mmol), 1-hydroxybenzotriazole (3.6 mmol) and DCC (3.6 mmol) in dioxane (16.6 ml) was stirred for 1 hour at room temp. The mixture was filtered to remove a small amount of insoluble material and the filtrate was added to a solution of **3** (3 mmol) and $NaHCO_3$ (6.2 mmol) in water (10 ml). After stirring at room temp for one night, the mixture was concentrated under reduced pressure. The residue was treated with 5% aqueous $NaHCO_3$ (10 ml) and washed with EtOAc. The aqueous layer was then extracted with methyl ethyl ketone (MEK) after acidification (pH 1.5) with 2 N HCl. The separated organic layer was washed with brine, dried ($MgSO_4$) and then evaporated under reduced pressure without heating. The residue was fractionated by silica gel chromatography ($CHCl_3$ - 2-PrOH - HCOOH, 90: 10: 2) to give, after trituration with mixture of Et_2O and *n*-hexane, **8** as a powder.

General Preparation of 3-[(4-Carboxy-3-hydroxy-5-isothiazolyl)thiomethyl]-7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(hydroxycarbonylalkoxyimino)acetamido]-3-cephem-4-carboxylic Acid (**9**)

Trifluoroacetic acid (6 ml) was added to a mixture of **8** (0.589 mmol) in anisol (0.5 ml) under ice-cooling, and then mixture was stirred for 1 hour at 15~20°C. After removing of the trifluoroacetic acid under reduced pressure without heating, the residue was triturated with Et_2O. The collected precipitate was added in a mixture of trifluoroacetic acid (6 ml) and water (2 ml) under ice-cooling. After being stirred at 15~20°C for 1 hour, the trifluoroacetic acid and water were evaporated under reduced pressure. The residue was triturated with Et_2O to give **9** as a powder.

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