SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 7β-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-3-(SUBSTITUTED)-2-PROPENOYL-AMINO]-3-CEPHEMS WITH C-3 SUBSTITUTIONS

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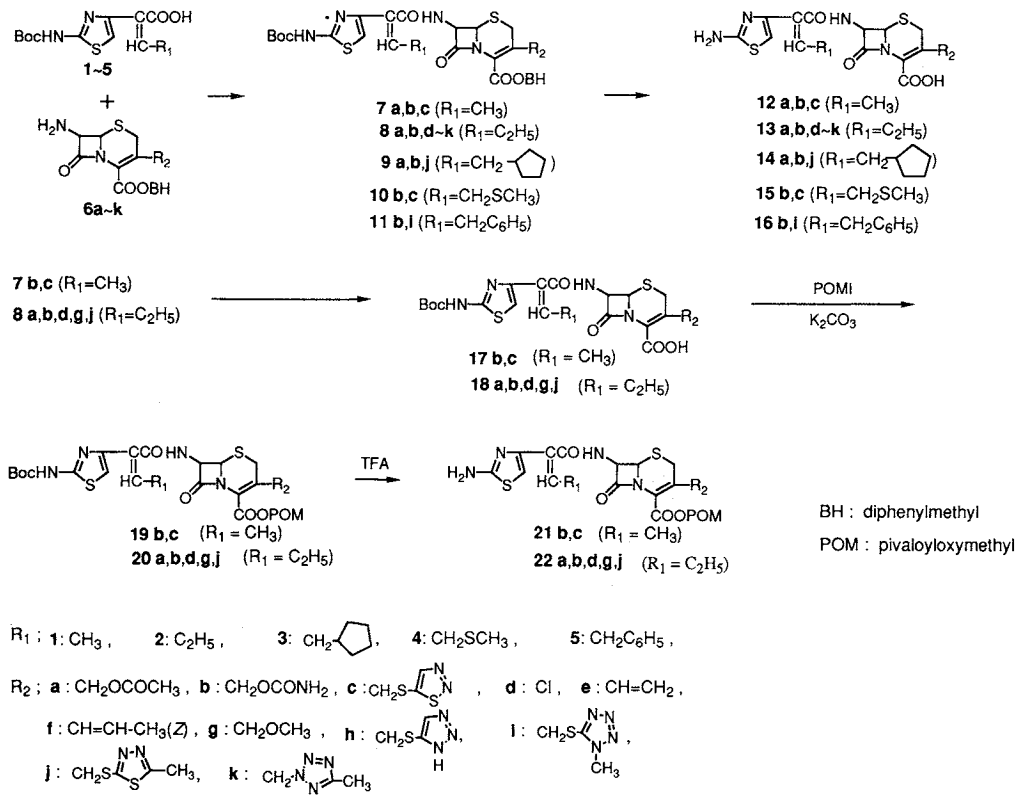
Synthesis and biological activity of a series of 7β-[(Z)-2-(2-aminothiazol-4-yl)-3-(substituted)-2-propenoylamino]-3-cephem-4-carboxylic acids with C-3 substitutions and their pivaloyloxymethyl esters are described. These acid compounds exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria. Pivaloyloxymethyl esters of selected compounds in this series were found to be well absorbed from small intestine in mice. Pivaloyloxymethyl 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-pentenoylamino]-3-carbamoyloxymethyl-3-cephem-4-carboxylate hydrochloride hydrate (S-1108) was finally selected as the candidate for clinical evaluation.

The need still exists for the development of new orally active, semi-synthetic cephalosporins which exhibit potent, broad-spectrum, antibacterial activity. In a previous paper[1] relating to the antibacterial activity and oral absorption of 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-alkenoylamino]-3-desacetoxymethyl-3-cephalosporins, we reported that the pivaloyloxymethyl (POM) ester of 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-pentenoylamino]-3-cephem-4-carboxylic acid showed high plasma level after oral administration to mice. The free acid, the active form of this cephalosporin ester, showed excellent activity against both of Gram-positive and Gram-negative bacteria, but did not show satisfactory activity against *Staphylococcus aureus* strain. We then focused our attention to modification of the substituent at C-3 position to improve the antibacterial activity against *S. aureus* while retaining the high antibacterial activity against other Gram-positive and Gram-negative bacteria. As a result, we found that pivaloyloxymethyl 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-pentenoylamino]-3-carbamoyloxymethyl-3-cephem-4-carboxylate (**22b**, S-1108) showed excellent oral activity and S-1006 (**13b**), the active form of S-1108, showed a potent and balanced antibacterial activity against Gram-positive and Gram-negative bacteria including *S. aureus*. Currently, S-1108 is under clinical evaluation as a new orally active cephalosporin. Herein reported are the synthesis and biological activity of 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-alkenoylamino]-3-(substituted)-3-cephem-4-carboxylic acids and some of their POM esters.

Chemistry

Based on the result of antibacterial activity and oral absorbability of 3-unsubstituted cephem derivatives in the previous report, 2-(2-aminothiazol-4-yl)-3-(substituted)-2-propenoylamino side chain moieties with methyl, ethyl, cyclopentylmethyl, methylthiomethyl and benzyl were selected as the 7β-side chains to be attached to cephems having various substituents at C-3 position. Coupling reaction of these 7β-side chain acids **1**~**5** and 7β-aminocephem esters having C-3 substituents **6a**~**6k** was carried out in the presence of an activating reagent such as methanesulfonyl chloride (MsCl) or phenylphosphoryl dichloride under

Scheme 1.



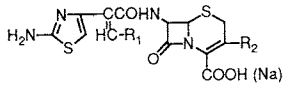
mild reaction conditions. Subsequent deblocking of these esters **7**~**11** was effected by treatment with trifluoroacetic acid (TFA) or aluminium chloride at ambient temperature to give **12**~**16**, respectively. POM esters of cephem acids **12b**, **12c**, **13a**, **13b**, **13d**, **13g**, **13j** and **14b** beside **15b** selected from their antibacterial activity were prepared to test for oral absorbability in mice. Selective deprotection of **7b**, **7c**, **8a**, **8b**, **8d**, **8g** and **8j** was performed by treating with TFA in dichloromethane at 0°C to give acids **17b**, **17c**, **18a**, **18b**, **18d**, **18g** and **18j** with remaining the *tert*-butoxycarbonyl group, which were reacted with POM iodide (POMI) in the presence of potassium carbonate in dimethylformamide (DMF) giving the corresponding esters **19b**, **19c**, **20a**, **20b**, **20d**, **20g** and **20j**, respectively. These esters obtained were deblocked with TFA at room temperature to afford **21b**, **21c**, **22a**, **22b**, **22d**, **22g** and **22j**, which were purified by column chromatography. The POM ester of **15b** was prepared similarly. Synthetic details for representative derivatives are described in the experimental section.

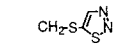
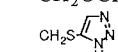
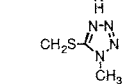
Biological Evaluation

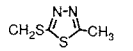
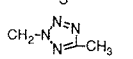
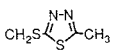
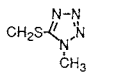
The *in vitro* antibacterial activity of the new cephalosporins **12**~**16** against Gram-positive and Gram-negative bacteria and peak plasma levels of selected cephalosporins after oral administration (40 mg/kg) of the corresponding POM esters **21** and **22** in mice are summarized in Table 1. For comparison, the MIC values of cefteram (CFTM) are also listed.

As shown in Table 1, almost all compounds synthesized showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria. Antibacterial activity against *S. aureus* including methicillin-resistant strain (SR3131) was enhanced by introducing cyclopentylmethyl or methylthiomethyl

Table 1. Antibacterial effects of C-3 substituents of cephalosporins and plasma levels of POM esters.



Compound	R_1	R_2	MIC (μg/ml)										PL (μg/ml)[b]
			S.a.	*S.a.* (R)	*S.py.*	*E.c.*	*K.p.*	*E.cl.*	*P.m.*	*P.v.*	*H.i.*	*P.a.*1	*P.a.*2[a]	
12a	CH_3	CH_2OCOCH_3	0.78	50	≤0.012	0.78	0.2	1.56	0.1	0.1	N.D.	1.56	100	
12b	CH_3	CH_2OCONH_2	0.78	50	≤0.012	0.78	0.2	0.78	0.05	0.1	0.1	3.13	100	14.4
12c	CH_3		0.1	1.56	≤0.012	0.78	0.2	0.78	0.39	0.2	0.05	12.5	100	2.0
13a	C_2H_5	CH_2OCOCH_3	0.39	50	≤0.012	1.56	0.39	1.56	0.39	0.2	0.025	0.39	25	1.4
13b	C_2H_5	CH_2OCONH_2	0.39	25	≤0.012	0.78	0.2	0.78	0.1	0.1	0.025	0.39	25	13.3
13d	C_2H_5	Cl	1.56	N.D.[c]	≤0.012	6.25	1.56	6.25	0.2	0.2	N.D.	6.25	100	4.7
13e	C_2H_5	$CH=CH_2$	0.78	N.D.	≤0.012	12.5	3.13	6.25	0.39	0.78	N.D.	12.5	>100	
13f	C_2H_5	$CH=CH-CH_3(Z)$	1.56	>100	≤0.012	12.5	6.25	12.5	1.56	1.56	0.1	12.5	>100	
13g	C_2H_5	CH_2OCH_3	0.78	>100	≤0.012	25	6.25	12.5	1.56	1.56	0.2	>100	>100	21.4
13h	C_2H_5		0.39	N.D.	≤0.012	3.13	0.78	1.56	0.78	0.78	N.D.	1.56	50	
13i	C_2H_5		0.39	6.25	≤0.012	1.56	0.39	1.56	0.78	0.78	≤0.012	0.78	25	

13j	C_2H_5		0.2	N.D.	≤0.012	1.56	0.78	1.56	1.56	1.56	N.D.	0.78	50	3.0
13k	C_2H_5		0.78	50	≤0.012	12.5	3.13	12.5	1.56	1.56	0.05	12.5	>100	
14a		CH_2OCOCH_3	0.2	6.25	≤0.012	6.25	1.56	3.13	3.13	3.13	0.05	3.13	25	
14b		CH_2OCONH_2	0.2	6.25	≤0.012	3.13	0.78	1.56	1.56	1.56	0.025	3.13	12.5	0.7
14j			0.1	1.56	≤0.012	3.13	1.56	1.56	1.56	3.13	0.1	3.13	12.5	
15b	CH_2SCH_3	CH_2OCONH_2	0.39	12.5	0.025	0.78	0.2	0.78	0.2	0.1	0.05	0.78	25	1.5
15c	CH_2SCH_3		0.1	3.13	≤0.012	1.56	0.39	1.56	1.56	0.78	0.025	3.13	25	
16b	$CH_2C_6H_5$	CH_2OCONH_2	0.2	12.5	≤0.012	3.13	0.78	3.13	1.56	1.56	0.025	3.13	25	
16i	$CH_2C_6H_5$		0.1	12.5	≤0.012	6.25	1.56	3.13	3.13	6.25	0.05	3.13	25	
Cefteram[d]			1.56	100	≤0.012	0.78	0.1	0.78	0.05	0.05	0.025	100	>100	

[a] *S.a.*, *Staphylococcus aureus* FDA 209P JC-1; *S.a.* (R), *Staphylococcus aureus* SR3131; *S.py.*, *Streptococcus pyogenes* C-203; *E.c.*, *Escherichia coli* NIHJ JC-2; *K.p.*, *Klebsiella pneumoniae* SR1; *E.cl.*, *Enterobacter cloacae* SR233; *P.m.*, *Proteus mirabilis* PR-4; *P.v.*, *Proteus vulgaris* CN-329; *H.i.*, *Haemophilus influenzae* SR3508; *P. a.*1, *Pseudomonas aeruginosa* ATCC 25619; *P.a.*2, *Pseudomonas aeruginosa* SR24.

[b] Plasma levels were measured at 15 minutes after oral administration of corresponding POM esters (**21** and **22**) to mice in 40 mg/kg dose.

[c] N.D.: Not determined.

[d] 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(5-methyl-2*H*-tetrazol-2-yl)methyl-3-cephem-4-carboxylic acid.

Table 2. Pharmacokinetic parameters in mice and *in vivo* activity (ED_{50}) against infection in mice.

Compound No.	Pharmacokinetic parameters[a]			ED_{50} (mg/kg/dose)[b]		
	C_{max} (μg/ml)	AUC (μg·hours/ml)	UR (%)	*S.a.*[c]	*E.c.*[c]	*H.i.*[c]
21b	14.4	12.2	15.3	2.25 (1.56)[d]	1.00 (0.1)	0.28 (0.05)
22b	13.3	10.4	14.0	2.88 (0.78)	2.18 (0.2)	0.085 (0.006)
	(4.7	3.4	7.1)			
CFTM-PI	11.5	15.9	10.3	10.8 (1.56)	0.91 (0.2)	0.085 (0.006)

[a] Dose; 40 mg/kg (po) for **21b** and **22b**, and 20 mg/kg (po) for cefteram pivoxil (CFTM-PI). Numbers in parentheses are data of 20 mg/kg (po) of **22b** for comparison with CFTM-PI.
[b] Challenge dose: 4×10^6 CFU/mouse for *S.a.*; 2×10^5 CFU/mouse for *E.c.*; 1×10^6 CFU/mouse for *H.i.*
[c] *S.a.*, *Staphylococcus aureus* Smith; *E.c.*, *Escherichia coli* EC-14; *H.i.*, *Haemophilus influenzae* 88562.
[d] Numbers in parentheses are MICs of 10^6 CFU/ml inoculum size.

moiety as the 7β-side chain olefinic substituent (R_1). Compound **15b** showed potent and well balanced antibacterial activity, though the oral absorbability of its POM ester was poor. Introduction of heteroarylthiomethyl or 5-methyl-2*H*-tetrazol-2-ylmethyl group at C-3 position such as **12c**, **13h**～**13k**, **14j**, **15c** and **16i** resulted in reduction of antibacterial activity against Gram-negative bacteria. Compounds **13d**～**13g** with chloro, vinyl, propenyl(*Z*) and methoxymethyl groups at C-3 position had reduced antibacterial activity against both Gram-positive and Gram-negative bacteria. Reduced antibacterial activity against Gram-negative bacteria was observed in compounds **14a**, **14b**, **14j**, **16b** and **16i** having cyclopentylmethyl and benzyl substituents as the 7β-side chain olefinic moiety (R_1). It is revealed that **12b** and **13b** having carbamoyloxymethyl group at C-3 position showed well balanced antibacterial activity against both Gram-positive and Gram-negative bacteria. Those acid compounds were found not to be absorbed from small intestine in mice (data are not shown). Thus, POM esters **21b**, **21c** **22a**, **22b**, **22d**, **22g** and **22j** of selected compounds **12b**, **12c**, **13a**, **13b**, **13d**, **13g** and **13j** were prepared and tested for oral absorbability. As shown at the right column in Table 1, **22g** showed the highest plasma level in mice, though its acid **13g** had less antibacterial activity. Compounds **21b** and **22b** with the carbamoyloxymethyl substituent at C-3 position showed high plasma level in mice. Thus, numbers of orally absorbable esters of the corresponding acids **12b** and **13b** were prepared and tested for oral absorbability (no data are shown here)[2], only to find that the POM ester showed the highest plasma level in mice.

As listed in Table 2, **21b** and **22b** showed parallel pharmacokinetic parameters in C_{max}, AUC and urinary recovery. Therapeutic efficacy of **21b** and **22b** was also excellent and better than that of cefteram pivoxil (CFTM-PI) against staphylococci infection[3], however, the C_{max}, AUC and urinary recovery of **21b** and **22b** were lower than those of CFTM-PI. Antibacterial activity of **13b**, the active form of **22b**, against *S. aureus* and *Haemophilus influenzae* was better than that of **12b**. Thus, **13b** was selected for further evaluation. Compound **13b** is stable against β-lactamases[4] and the POM ester **22b** showed very low nephrotoxicity[5] and oral single-dose toxicity potentials[6] in rabbits and rats, respectively. These data indicated that compound **22b** hydrchloride hydrate designated as S-1108, had the most preferable profile as an oral cephem in the compounds prepared and was selected as the candidate for clinical evaluation.

Experimental

Chemistry

All reactions involving air-sensitive reactions or compounds were carried out under nitrogen in dry

solvents. IR spectra were taken on a Hitachi 260-10 or Jasco IR-700 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian EM-390 (90 MHz) or a VXR 200 (200 MHz) spectrophotometer using TMS or sodium 3-(trimethylsilyl)-1-propane-sulfonate (in D_2O) as an internal standard.

Determination of Antibacterial Activity

The *in vitro* antibacterial activity was given as minimum inhibitory concentration (MIC) in μg/ml as determined by the serial agar dilution method (sensitivity test agar) after incubation at 37°C for 18~20 hours with an inoculum size of one loopful of 10^6 CFU/ml. Sensitivity test agar containing 3% horse serum for *Streptococcus pyogenes* C-203 and sensitivity test agar containing 5% Fildes Enrichment for *Haemophilus influenzae* SR3508 were used.

In Vivo Antibacterial Activity Test

ICR female mice (age: 5 weeks, weight: 19~23 g) were infected intraperitoneally with bacterial suspension in 5% mucin and compounds were administered orally 1 and 5 hours after infection. ED_{50} values were calculated from survival ratio of mice on 7th day after infection by the probit method.

Oral Absorption Study

Male ICR-strain mice aged 6 weeks weighing 24~30 g were used in groups of 5. The antibiotics were given to mice orally in a single dose of 40 mg (potency)/kg or 20 mg/kg for CFTM-PI. Plasma samples were collected at 0.25 and 2 hours after dosing and urine specimens were collected over a period of 2 hours after dosing. The concentrations of the active compounds were determined by the Band Culture method[7] using *Escherichia coli* 7437 as the test organism and trypto-soy agar as the test medium.

Diphenylmethyl 7β-[(*Z*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-butenoylamino]-3-acetoxymethyl-3-cephem-4-carboxylate (**7a**)

To a solution of 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-2(*Z*)-butenoic acid (**1**) (142 mg, 0.5 mmol) and triethylamine (TEA) (76 μl, 0.55 mmol) in CH_2Cl_2 (4 ml) was added MsCl (40 μl, 0.52 mmol), and the reaction mixture was stirred for 4 hours at −50°C, to which was added dropwise a solution of diphenylemethyl 7β-amino-3-acetoxymethyl-3-cephem-4-carboxylate (**6a**, 263 mg, 0.6 mmol) and TEA (180 μl, 1.3 mmol) in CH_2Cl_2 (4 ml) and the resulting mixture was stirred for 3 hours at the same temperature. The reaction mixture was acidified with dil HCl and extracted with EtOAc. The extract was washed with brine and dil $NaHCO_3$, dried and concentrated. The residue was subjected to silica gel column chromatography (eluent: EtOAc-CH_2Cl_2, 1:2) to give **7a** (240 mg, 68%) as a pale yellow powder.

^1H NMR ($CDCl_3$) δ 1.52 (9H, s, $C(CH_3)_3$), 2.0 (3H, d, J=7.5 Hz, CH_3), 2.07 (3H, s, CH_3), 3.36, 3.62 (2H, ABq, J=16.5 Hz, 2-H), 4.89, 5.17 (2H, ABq, J=14.0 Hz, CH_2O), 5.06 (1H, d, J=4.5 Hz, 6-H), 5.87 (1H, dd, J=4.5, 8.0 Hz, 7-H), 6.45 (1H, q, J=7.5 Hz, $=CHCH_3$), 6.81 (1H, s, thiazole H), 6.83 (1H, s, Ph_2CH), 7.18~7.40 (10H, m, Ph_2), 7.82 (1H, d, J=8.0 Hz, NH); IR ($CHCl_3$) cm^{-1} 3410, 1783, 1723, 1670, 1160.

Diphenylmethyl 7β-[(*Z*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-butenoylamino]-3-carbamoyloxymethyl-3-cephem-4-carboxylate (**7b**)

7b (colorless powder by the reaction of **1** and **6b** in 70.8% yield).

^1H NMR ($CDCl_3$) δ 1.51 (9H, s, $C(CH_3)_3$), 2.10 (3H, d, J=7.5 Hz, $CHCH_3$), 3.25 (2H, br s, 2-H), 4.83 (2H, br s, NH_2), 4.78, 5.03 (2H, ABq, J=15.0 Hz, CH_2O), 4.98 (1H, d, J=5.0 Hz, 6-H), 5.75 (1H, dd, J=5.0, 9.0 Hz, 7-H), 6.51 (1H, q, J=7.5 Hz, $=CHCH_3$), 6.66 (1H, s, thiazole H), 6.83 (1H, s, Ph_2CH), 6.83 (1H, t, J=7.5 Hz, $=CHCH_2$), 7.21~7.40 (10H, m, Ph_2), 7.96 (1H, d, J=9.0 Hz, NH), 10.02 (1H, br, NH); IR ($CHCl_3$) cm^{-1} 3530, 3440, 1782, 1730, 1675, 1157.

Diphenylmethyl 7β-[(*Z*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-butenoylamino]-3-(1,2,3-thiadiazol-5-ylthio)methyl-3-cephem-4-carboxylate (**7c**)

To a mixture of **1** (156 mg, 0.55 mmol), diphenylmethyl 7β-amino-3-(1,2,3-thiadiazol-5-ylthio)methyl-3-cephem-4-carboxylate (**6c**, 225 mg, 0.5 mmol), *N*-methylmorpholine (NMM) (200 μl, 1.8 mmol) and CH_2Cl_2 (8 ml) was added phenylphosphoryl dichloride (90 μl, 0.6 mmol) at −30°C. After being stirred at

$-30 \sim -20°C$ for 2.5 hours, the reaction mixture was treated with dil HCl and extracted with EtOAc. The extract was washed with brine and dil $NaHCO_3$, dried and concentrated. The residue was purified by silica gel column chromatography (eluent: EtOAc-CH_2Cl_2, 1 : 5) to give **7c** (248 mg, 69%).

^1H NMR ($CDCl_3$) δ 1.50 (9H, s, $C(CH_3)_3$), 2.05 (3H, d, $J=7.0$ Hz, CH_3), 3.36, 3.53 (2H, ABq, $J=18.0$ Hz, 2-H), 4.03, 4.13 (2H, ABq, $J=12.0$ Hz, CH_2O), 4.98 (1H, d, $J=5.0$ Hz, 6-H), 5.80 (1H, dd, $J=5.0$, 9.0 Hz, 7-H), 6.52 (1H, q, $J=7.0$ Hz, $=CHCH_3$), 6.70 (1H, s, thiazole H), 6.85 (1H, s, Ph_2CH), 7.18~7.40 (10H, m, Ph_2), 8.12 (1H, d, $J=9.0$ Hz, NH), 8.46 (1H, s, thiadiazole H), 9.41 (1H, br s, NH).

Diphenylmethyl 7β-[(*Z*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-pentenoylamino]-3-carbamoyloxymethyl-3-cephem-4-carboxylate (**8b**)

Compound **8b** was prepared in 86% yield by coupling of 2-(2-*tert*-butoxycarbonylaminothiazol-4-yl)-2(*Z*)-pentenoic acid (**2**) with **6b**·TsOH in a manner similar to that used for the synthesis of **7a**.

^1H NMR ($CDCl_3$) δ 1.10 (3H, t, $J=7.5$ Hz, CH_3), 1.51 (9H, s, $C(CH_3)_3$), 2.55 (2H, quint, $J=7.5$ Hz, $=CHCH_2$), 3.16 (2H, br s, 2-H), 4.80, 4.97 (2H, ABq, $J=14.4$ Hz, CH_2O), 4.82 (2H, br s, NH_2), 4.93 (1H, d, $J=4.5$ Hz, 6-H), 5.67 (1H, dd, $J=4.5$, 8.0 Hz, 7-H), 6.37 (1H, t, $J=7.5$ Hz, $=CHCH_2$), 6.68 (1H, s, thiazole H), 6.80 (1H, s, Ph_2CH), 7.22~7.40 (10H, m, Ph_2), 7.82 (1H, d, $J=8.0$ Hz, NH), 10.0 (1H, br, NH); IR ($CHCl_3$) cm^{-1} 3430, 1785, 1726, 1672, 1158.

Synthesis of Diphenylmethyl 7β-[(*Z*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-pentenoylamino]-3-(substituted)-3-cephem-4-carboxylates (**8a**, **8d**~**8k**)

These compounds were synthesized similarly by reaction of **2** with the corresponding 3-substituted methyl cephem esters **6**. Chemical yields, ^1H NMR and IR spectra are listed in Table 3.

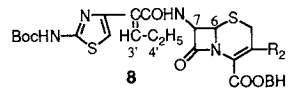
Syntheis of Diphenylmethyl 7β-[(*Z*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-4-(substituted)-2-butenoylamino]-3-(substituted)-3-cephem-4-carboxylates (**9a**, **9b**, **9j**, **10b**, **10c**, **11b** and **11i**)

These compounds were synthesized similarly by reaction of **3**, **4** or **5** with the corresponding 3-substituted methyl cephem esters **6**. Chemical yields, ^1H NMR and IR spectra are listed in Table 4.

Sodium 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-butenoylamino]-3-acetoxymethyl-3-cephem-4-carboxylate (**12a**)

A mixture of **7a** (261 mg, 0.37 mmol), TFA (1.0 ml), anisole (0.5 ml) and CH_2Cl_2 (1.5 ml) was stirred

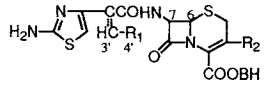
Table 3. Yields, ^1H NMR and IR spectral data of cephalosporin esters (**8**).



8

Compound No.	R_2	Yield (%)	^1H NMR δ in $CDCl_3$ (J=Hz)					IR ($CHCl_3$) cm^{-1}
			6-H (d)	7-H (dd)	3'-H (t)	4'-H (quint)	Thiazole H	(C=O)
8a	CH_2OCOCH_3	78	5.03 (5)	5.82 (5, 8)	6.33 (8)	2.41 (8)	6.80	1790
8d	Cl	88	4.95 (5)	5.78 (5, 9)	6.40 (7.5)	2.63 (8)	6.68	1785
8e	$CH=CH_2$	66	4.99 (5)	5.68 (5, 9)	6.42 (7.5)	2.63 (7.5)	6.72	1782
8f	$CH=CH-CH_3$(*Z*)	55	5.05 (4.5)	5.70 (4.5, 8)	6.42 (7.5)	2.53 (7.5)	6.72	1783
8g	CH_2OCH_3	65	5.02 (5)	5.85 (5, 8)	6.42 (7)	2.47 (7)	6.68	1781
8h		52	4.97 (5)	5.77 (5, 8)	6.39 (7)	2.52 (7)	6.72	1785
8i		75	5.02 (5)	5.88 (5, 8)	6.42 (7)	2.50 (7)	6.72	1782
8j		82	5.03 (5)	5.87 (5, 8)	6.45 (8)	2.51 (8)	6.72	1786
8k		83.5	4.99 (5)	5.81 (5, 8)	6.34 (8)	2.53 (8)	6.78	1783

Table 4. Yields, ^{1}H NMR and IR spectral data of cephalosporin esters (**9**, **10** and **11**).



Compound No.	R_1	R_2	Yield (%)	^{1}H NMR δ in $CDCl_3$ (J=Hz)					IR ($CHCl_3$) cm^{-1}
				6-H (d)	7-H (d)	3'-H (t)	4'-H	Thiazole H	(C=O)
9a		CH_2OCOCH_3	76	5.02 (5)	5.83 (5, 8)	6.34 (7.5)	2.50 (t, 7.5)	6.78	1788
9b		CH_2OCONH_2	78	4.95 (5)	5.76 (5, 8)	6.43 (8)	2.55 (t, 8)	6.70	1780
9j			62.5	5.02 (5)	5.90 (5, 8)	6.49 (8)	2.50 (t, 8)	6.78	1783
10b	CH_2SCH_3	CH_2OCONH_2	38	4.99 (5)	5.75 (5, 8)	6.50 (8)	3.51 (d, 8)	6.78	1780
10c	CH_2SCH_3		96	5.01 (5)	5.77 (5, 8)	6.50 (8)	3.53 (d, 8)	6.79	1780
11b	$CH_2C_6H_5$	CH_2OCONH_2	17.7	4.99 (5)	5.77 (5, 8)	6.56 (8)	3.87 (d, 8)	6.78	1785
11i	$CH_2C_6H_5$		23.9	5.01 (5)	5.87 (5, 8)	6.59 (8)	3.83 (d, 8)	6.66	1785

at room temperature for 1 hour. After concentration, the residue was triturated with Et_2O to give a light brown powder. The crude product dissolved in dil $NaHCO_3$ was subjected to chromatography on a Diaion HP-20 (eluent: 10% MeOH). The product eluates were lyophilized to give **12a** Na salt (105 mg, 60.4%).

Anal Calcd for $C_{17}H_{17}N_4O_6S_2Na \cdot 2H_2O$: C 41.43, H 4.26, N 11.28, S 12.92, H_2O 7.25.
Found: C 41.28, H 4.38, N 11.00, S 12.58, H_2O 7.53.

^{1}H NMR (D_2O) δ 2.36 (3H, d, J=8.0 Hz, CH_3), 2.60 (3H, s, CH_3), 3.83, 4.14 (2H, ABq, J=18.0 Hz, 2-H), 5.03, 5.34 (2H, ABq, J=11.0 Hz, CH_2O), 5.67 (1H, d, J=5.0 Hz, 6-H), 6.28 (1H, d, J=5.0 Hz, 7-H), 6.90 (1H, q, J=8.0 Hz, =C*H*CH_3), 6.96 (1H, s, thiazole H).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-butenoylamino]-3-carbamoyloxymethyl-3-cephem-4-carboxylic Acid (**12b**)

12b (colorless crystalline powder in 82.3% yield from **7b**).

Anal Calcd for $C_{16}H_{17}N_5O_6S_2 \cdot 1.5H_2O$: C 41.20, H 4.32, N 15.02, S 13.75, H_2O 5.79.
Found: C 41.23, H 4.42, N 15.22, S 13.41, H_2O 6.07.

^{1}H NMR (DMSO-d_6-CD_3OD) δ 2.25 (3H, d, J=8.0 Hz, CH_3), 3.86 (2H, br s, 2-H), 4.96, 5.32 (2H, ABq, J=12.0 Hz, CH_2O), 5.50 (1H, d, J=5.0 Hz, 6-H), 6.11 (1H, d, J=5.0 Hz, 7-H), 6.67 (1H, s, thiazole H), 6.67 (1H, t, J=8.0 Hz, =C*H*CH_2).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-butenoylamino]-3-(1,2,3-thiadiazol-5-ylthio)methyl-3-cephem-4-carboxylic Acid (**12c**)

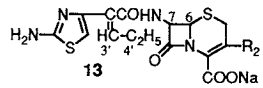
12c (light brown powder in 73% yield as a TFA salt from **7c**).

^{1}H NMR (D_2O) δ 2.35 (3H, d, J=8.0 Hz, =CHC*H*$_3$), 5.64 (1H, d, J=5.0 Hz, 6-H), 6.22 (1H, d, J=5.0 Hz, 7-H), 6.92 (1H, q, J=8.0 Hz, =C*H*CH_3), 6.95 (1H, s, thiazole H).

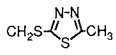
7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-pentenoylamino]-3-carbamoyloxymethyl-3-cephem-4-carboxylic Acid (**13b**)

To a solution of $AlCl_3$ (40.2 g, 0.3 mol) in anisole (400 ml) was added a solution of **8b** (43.4 g, 0.06 mol) dissolved in CH_2Cl_2 (210 ml) at -30°C, and the mixture was stirred at the same temperature for 30 minutes. To the reaction mixture was added 1 N HCl (430 ml) and triturated with EtOAc (430 ml). The aqueous layer was mixed with dil $NaHCO_3$ to adjust pH to 3.5 precipitating a light brown solid, which was filtered off and added into a mixture of $NaHCO_3$ (24 g), water (530 ml), acetylacetone (7.4 ml) and CH_2Cl_2 (350 ml). 1 N HCl was added to the mixture to adjust pH to 7.5. The aqueous solution after separation was adjusted to pH 3.5 by addition of conc HCl to precipitate a colorless solid. After filtration, the solid was washed

Table 5. Yields, ^{1}H NMR and IR spectral data of cephalosporins (**13**).



13

Compound No.	R_2	Yield (%)	^{1}H NMR δ (J=Hz)						IR cm^{-1}[b] (C=O)
			6-H (d)	7-H (d)	3'-H (t)	4'-H (quint)	Thiazole H	Solvent[a]	
13a	CH_2OCOCH_3	80	5.16 (5)	5.82 (5)	6.32 (8)	2.36 (8)	6.69	a	1780 (N)[b]
13d	Cl	60	5.71 (5)	6.23 (5)	6.82 (8)	2.69 (8)	6.93	c	1763 (N)
13e	$CH=CH_2$	42	5.68 (5)	6.22 (5)	6.81 (8)	2.71 (8)	6.95	b	1770 (N)[b]
13f	$CH=CH-CH_3(Z)$	83	5.21 (5)	5.81 (5)	6.35 (8)	2.30 (8)	6.44	a	1750 (N)
13g	CH_2OCH_3	76	5.66 (5)	6.23 (5)	6.80 (7)	2.70 (7)	6.94	c	1760 (K)
13i		63	5.61 (5)	6.20 (5)	6.80 (7)	2.70 (7)	6.95	c	1760 (K)
13j		75	5.60 (5)	6.19 (5)	6.79 (8)	2.68 (8)	6.81	c	1758 (N)
13k		78	5.11 (5)	5.81 (5)	6.33 (8)	2.17 (8)	6.47	b	1780 (N)[b]

[a] a, CD_3OD-D_2O-$NaHCO_3$; b, D_2O-$NaHCO_3$; c, D_2O.
[b] IR spectral data of free acids are listed. N, Nujol; K, KBr.

with water and dried to give **13b** (28.5 g, 87.2%).

^{1}H NMR (DMSO-d_6) δ 1.00 (3H, t, J=8.0 Hz, CH_3), 2.18 (2H, quint, J=8.0 Hz, =CHCH_2), 3.48, 3.59 (2H, ABq, J=18.0 Hz, 2-H), 4.62, 4.85 (2H, ABq, J=13.0 Hz, CH_2O), 5.18 (1H, d, J=4.5 Hz, 6-H), 5.98 (1H, dd, J=4.5, 8.0 Hz, 7-H), 6.20 (1H, s, thiazole H), 6.22 (1H, t, J=8.0 Hz, =CHCH$_2$); IR (Nujol) cm^{-1} 1780.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-pentenoylamino]-3-(1,2,3-triazol-4-ylthio)methyl-3-cephem-4-carboxylic Acid (**13h**)

13h (colorless crystalline powder in 65% yield from **8h**).

^{1}H NMR ($CDCl_3$-CD_3OD) δ 1.09 (3H, t, J=7.0 Hz, CH_3), 2.35 (2H, quint, J=7.0 Hz, =CHCH_2), 3.56, 3.84 (2H, ABq, J=16.0 Hz, 2-H), 4.03 (2H, br s, CH_2O), 5.10 (1H, d, J=5.0 Hz, 6-H), 5.76 (1H, d, J=5.0 Hz, 7-H), 6.38 (1H, t, J=8.0 Hz, =CHCH$_2$), 6.43 (1H, s, thiazole H); IR (Nujol) cm^{-1} 1762.

Synthesis of Sodium 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-pentenoylamino]-3-(substituted)-3-cephem-4-carboxylates (**13a**, **13d**~**13g** and **13i**~**13k**)

These compounds were synthesized by applying the method used for the synthesis of **12a**. Chemical yields, ^{1}H NMR and IR spectral data are listed in Table 5.

Sodium 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-3-(substituted)-2-propenoylamino]-3-(substituted)-3-cephem-4-carboxylates (**14a**, **14b**, **14j**, **15b**, **15c**, **16b** and **16i**)

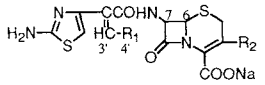
These compounds were synthesized by applying the method used for the synthesis of **12a**. Chemical yileds, ^{1}H NMR and IR spectral data of these compounds are listed in Table 6.

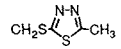
Pivaloyloxymethyl 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-pentenoylamino]-3-carbamoyloxymethyl-3-cephem-4-carboxylate (**22b**)

To an ice-cooled solution of **8b** (885 mg, 1.23 mmol) dissolved in anisole (3.3 ml) and CH_2Cl_2 (8.3 ml) was added TFA (1.9 ml), and the mixture was stirred at 0°C for 2 hours. After concentration, the residue was triturated with Et_2O and petroleum ether to give crude **18b** (675 mg) as a light brown powder.

A suspension of **18b** (3.45 g, 6 mmol), K_2CO_3 (1.65 g, 7.2 mmol) and DMF (35 ml) was treated with POMI (1.15 ml, 6.8 mmol) at −40°C for 1.5 hours. The reaction mixture was mixed with 10% H_3PO_4 and extracted with EtOAc. The extract was washed with brine and water, dried and concentrated. The residue

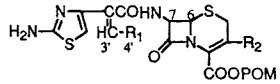
Table 6. Yields, ^{1}H NMR and IR spectral data of cephalosporins (**14**, **15** and **16**).

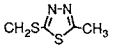


Compound No.	R_1	R_2	Yield (%)	^{1}H NMR δ (J=Hz)						IR cm^{-1}[b] (C=O)
				6-H (d)	7-H (d)	3′-H (t)	4′-H	Thiazole H	Solvent[a]	
14a		CH_2OCOCH_3	65	5.66 (5)	6.25 (5)	6.86 (8)	2.73 (t, 8)	6.94	c	1760 (N)
14b		CH_2OCONH_2	73.6	5.49 (5)	6.09 (5)	6.62 (8)	2.4～2.6 (m)	6.53	a	1750 (N)
14j			87	5.60 (5)	6.19 (5)	6.79 (8)	2.68 (m)	6.92	c	1758 (N)
15b	CH_2SCH_3	CH_2OCONH_2	82	5.12 (5)	5.74 (5)	6.67 (8)	3.35 (d, 8)	6.36	a	1769 (K)
15c	CH_2SCH_3		79	5.61 (5)	6.16 (5)	6.78 (8)	3.81 (d, 8)	6.79	c	1759 (K)
16b	$CH_2C_6H_5$	CH_2OCONH_2	30.8	5.23 (5)	6.84 (5)	6.39 (8)	3.53 (d, 8)	6.39	c	1750 (K)
16i	$CH_2C_6H_5$		73.3	5.16 (5)	5.85 (5)	6.53 (8)	3.68 (d, 8)	6.54	b	1775 (K)[b]

[a] a: CD_3OD-D_2O-$NaHCO_3$; b, D_2O-$NaHCO_3$; c, D_2O.
[b] IR spectral data of free acids are listed. N, Nujol; K, KBr.

Table 7. Yields, ^{1}H NMR and IR spectral data of 7β-acylaminocephalosporin POM esters (**21** and **22**).



Compound No.	R_1	R_2	Yield (%)	^{1}H NMR δ in $CDCl_3$ (J=Hz)					IR ($CHCl_3$) cm^{-1} (C=O)
				6-H (d)	7-H (dd)	3′-H	4′-H (R_1)	Thiazole H	
21b	CH_3	CH_2OCONH_2	29.8	5.02 (5)	5.7～5.9[a]	6.45 (q, 7)	1.90 (d, 7)	6.22	1786
21c	CH_3		20.3	5.08 (5)	5.92 (5, 8)	6.52 (q, 8)	1.95 (d, 8)	6.28	1785
22a	C_2H_5	CH_2OCOCH_3	27.2	5.09 (4.5)	5.95 (4.5, 8)	6.44 (t, 8)	2.38 (quint, 8)	6.31	1785
22d	C_2H_5	Cl	32.1	5.12 (5)	5.8～6.0[a]	6.41 (t, 7.5)	2.40 (quint, 7.5)	6.32	1783
22g	C_2H_5	CH_2OCH_3	40.1	5.06 (5)	5.95 (5, 9)	6.45 (t, 8)	2.40 (quint, 8)	6.35	1785
22j	C_2H_5		47.6	5.02 (5)	5.90 (5, 9)	6.39 (t, 8)	2.36 (quint, 8)	6.31	1785

[a] Overlapped with other proton signals.

was subjected to silica gel column chromatography (eluent: EtOAc-C_6H_6, 2:1) to obtain **20b** (2.05 g, 51.1%) as colorless crystals.

Anal Calcd for $C_{28}H_{37}N_5O_{10}S_2$: C 50.37, H 5.59, N 10.49, S 9.60.
Found: C 50.29, H 5.53, N 10.28, S 9.32.

^{1}H NMR ($CDCl_3$) δ 1.07 (3H, t, J=7.5 Hz, CH_3), 1.22 (9H, s, $C(CH_3)_3$), 1.52 (9H, s, $C(CH_3)_3$), 2.46 (2H, quint, J=7.5 Hz, $=CHCH_2$), 3.23~3.70 (2H, m, 2-H), 4.77, 5.04 (2H, ABq, J=14.0 Hz, CH_2O), 4.83 (2H, br s, NH_2), 5.01 (1H, d, J=4.5 Hz, 6-H), 5.86 (2H, s, OCH_2O), 5.88 (1H, dd, J=4.5, 8.0 Hz, 7-H), 6.40 (1H, t, J=7.5 Hz, $=CHCH_2$), 6.74 (1H, s, thiazole H), 7.65 (1H, d, J=8.0 Hz, NH), 8.70 (1H, br s, NH).

A solution of **20b** (2.7 g, 4.04 mmol) in CH_2Cl_2 (10 ml) was treated with TFA (30 ml) at room temperature for 1.5 hours. After concentration, the residue was partitioned between EtOAc and dil $NaHCO_3$. The organic solution was washed with brine, dried and concentrated. The residue was chromatographed on a silica gel column (eluent: EtOAc-CH_2Cl_2, 2:1) obtaining **22b** (1.83 g, 73%) as a pale yellow powder.

^{1}H NMR ($CDCl_3$) δ 1.05 (3H, t, J=7.5 Hz, CH_3), 1.22 (9H, s, $C(CH_3)_3$), 2.40 (2H, quint, J=7.5 Hz, $=CHCH_2$), 3.51, 3.82 (2H, ABq, J=18.0 Hz, 2-H), 4.80, 5.13 (2H, ABq, J=13.0 Hz, CH_2O), 5.12 (1H, d, J=5.0 Hz, 6-H), 5.27 (2H, br s, NH_2), 5.83~6.01 (3H, m, 7-H, OCH_2O), 6.32 (1H, s, thiazole H), 6.41 (1H, t, J=7.5 Hz, $=CHCH_2$), 8.27 (1H, d, J=8.0 Hz, NH); IR ($CHCl_3$) cm^{-1} 3470, 3385, 1783, 1756, 1670, 1602, 1523, 1330, 1122, 1103, 985.

The above solid was dissolved in EtOAc and mixed with HCl in EtOAc precipitating crystalline powder. The precipitate was filtered off and washed with EtOAc and MeOH to give pure **22b**·HCl (S-1108, 1.4 g, 53.7% from **20b**).

Anal Calcd for $C_{23}H_{29}N_5O_8S_2 \cdot HCl \cdot 1.2H_2O$: C 44.15, H 5.22, N 11.19, S 10.25, Cl 5.67, H_2O 3.45.
Found: C 44.19, H 5.19, N 11.16, S 10.51, Cl 5.57, H_2O 3.63.

^{1}H NMR (CD_3OD) δ 1.13 (3H, t, J=7.6 Hz, CH_3), 1.21 (9H, s, $(CH_3)_3$), 2.37 (2H, quint, J=7.6 Hz, $=CHCH_2$), 3.58, 3.71 (2H, ABq, J=18.4 Hz, 2-H), 4.76, 5.08 (2H, ABq, J=13.6 Hz, CH_2O), 5.02 (1H, d, J=4.8 Hz, 6-H), 5.84, 5.93 (2H, ABq, J=5.7 Hz, OCH_2O), 5.88 (1H, d, J=4.8 Hz, 7-H), 6.34 (1H, t, J=7.6 Hz, $=CHCH_2$), 6.69 (1H, s, thiazole H); ^{13}C NMR (CD_3OD) δ 13.81, 24.58, 27.28, 39.81, 58.95, 60.86, 64.08, 81.10, 105.13, 125.42, 127.56, 131.09, 139.18, 159.15, 161.76, 165.47, 168.41, 172.50, 178.34; IR (Nujol) cm^{-1} 3347, 1796, 1745, 1691, 1651, 1509.

Synthesis of Pivaloyloxymethyl 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-3-(substituted)-2-propenoylamino]-3-(substituted)-3-cephem-4-carboxylates (**21b**, **21c**, **22a**, **22d**, **22g** and **22j**)

These compounds were prepared by the similar procedures to those used for preparation of **22b** as described above. ^{1}H NMR and IR spectral data and chemical yields are listed in Table 7.

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