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

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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 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.

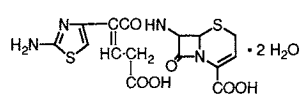
Intensive efforts in our laboratories to expand the antibacterial spectra of existing oral β-lactams[1] have led to a new type of orally absorbable cephalosporin, ceftibuten (Fig. 1), which shows a broad and potent antibacterial activity against most Gram-negative bacteria with limited activity against Gram-positive ones[2,3].

Next attention has been focused to find new orally absorbable cephalosporins having broader and more potent antibacterial activity, especially against Gram-positive bacteria. We thought that the elimination of the 7β-side chain carboxyl group of ceftibuten would improve the activity against Gram-positive bacteria, with retaining high antibacterial activity against Gram-negative bacteria. Thus, 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-butenoylamino]-3-cephem-4-carboxylic acid (**16a**) was synthesized, which was found to have potent and broad antibacterial activity against both Gram-positive and Gram-negative bacteria, although with less oral absorbability. Pivaloyloxymethyl (POM) ester **21a** of this compound was found to be well absorbed from the intestine in mice. In an attempt to improve the biological activity further, a number of 7β-[(Z)-2-(2-aminothiazol-4-yl)-3-(substituted)-2-propenoylamino]-3-cephem derivatives **16b**~**16o** were synthesized to examine antibacterial activity and, in addition, corresponding POM esters, **21b**~**21f** and **21h**, of some of these derivatives were prepared to test oral absorbability in mice.

Chemistry

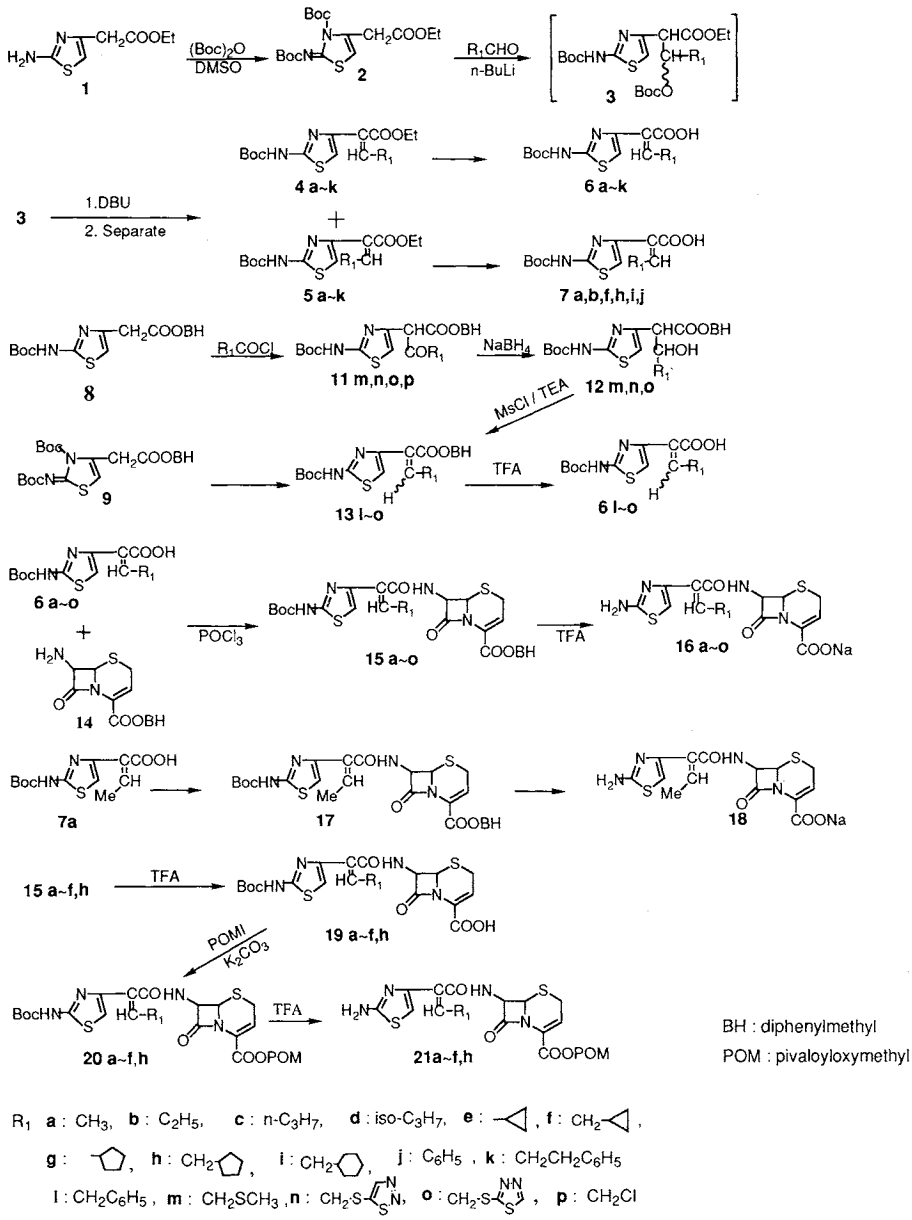
Most of 7β-side chain acid fragments were synthesized according to G. KINAST's method[4] as shown in Scheme 1. In all cases, olefinic esters were obtained as mixtures of *Z* and *E* isomers **4** and **5**, which were separated by recrystallization or column chromatography. The geometry of these isomers was determined from the chemical shift of observed characteristic olefin proton signals in their ^{1}H NMR spectra. The olefinic proton at C-3 in **4** located *trans* to the carbonyl group should resonate at a higher field than that in corresponding isomers **5** because of the

Fig. 1.



Ceftibuten

Scheme 1.



less deshielding effect by the ester carbonyl group[5]. The ratio of the *Z*/*E* isomers (**4**/**5**) in the elimination reaction of **3** depends on the R_1 substituent. The data as described in experimental section show that bulky R_1 substituents tend to increase formation of the *Z* olefins. These olefinic ethyl esters **4** and **5** underwent alkaline hydrolysis to give olefinic acids **6** and **7**, respectively, where alkaline hydrolysis of *Z* esters required rather drastic reaction conditions than that of corresponding *E* ester **5**. *Z* esters with benzyl, methylthiomethyl or heteroarylthiomethyl substituents at C-2 did not give isolable hydrolysis products. To prepare these olefinic acids, we used diphenylmethyl 2-[(*Z*)-2-*tert*-butoxycarbonylaminothiazol-4-yl]acetate (**8**) as the starting material, and compounds **12m**~**12o** were prepared by successive reaction of

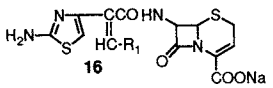
8 with acyl chlorides (**10m**, **10p**) followed by reduction with sodium borohydride, while **11n** and **11o** were prepared by the reaction of **11p** with 5-mercapto-1,2,3-thiadiazole and 2-mercapto-1,3,4-thiadiazole, respectively. Elimination of mesylates of **12m**~**12o** gave a mixture of *E*, *Z* olefinic esters **13m**~**13o**. Ester **13l** was prepared by reaction of **9** with phenylacetaldehyde. *Z* forms of compounds **13l** and **13m** were separated by column chromatography of the corresponding *E*, *Z* mixtures and successively deblocked with trifluoroacetic acid (TFA) to give **6l** and **6m**, respectively. In cases of **13n** and **13o**, no effective separation was successful and thus the partial deblocking was carried out in the state of mixture to obtain **6n** and **6o** as the *E*, *Z* mixture. Olefinic protons (3-H) of *Z* acids **6** also resonate at a higher field than those of the corresponding *E* acids **7** (described in experimental section). Acylation of diphenylmethyl 7β-amino-3-cephem-4-carboxylate (**14**) with various 7β-acyl side chain acids **6a**~**6m** was efficiently achieved with appropriate activating agents such as phosphoryl chloride, methanesulfonyl chloride (MsCl), phenylphosphoryl dichloride or dicyclohexylcarbodiimide (DCC) under mild reaction conditions. Subsequent deprotection of the acylated products **15a**~**15m** was carried out by treatment with TFA to afford 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-3-(substituted)-2-propenoylamino]-3-cephem-4-carboxylic acids (Na salts) (**16a**~**16m**). In the reactions of **6n** and **6o** with **14**, corresponding acylated cephem esters were obtained as mixtures of *E* and *Z* isomers, which were separated by column chromatography. Deprotection of the separated *Z* isomers **15n** and **15o** were carried out by treating with TFA to the corresponding acids (Na salts) **16n** and **16o**, respectively. 7β-[(*E*)-2-(2-Aminothiazol-4-yl)-2-butenoylamino]-3-cephem-4-carboxylic acid Na salt (**18**) was also synthesized *via* its ester **17** for comparison of the spectral data and antibacterial activity with that of *Z* isomer **16a**. Olefinic geometry at 7β-side chain moiety in these esters and acids was retained during the reactions (data are not shown). As the acid derivatives **16** synthesized in this series were not absorbed from small intestine in mice, POM esters of selected cephem acids were prepared and tested for oral absorbability. Selective deprotection of **15** was performed by treating with TFA in dichloromethane at 0°C to give acids **19** 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 POM esters **20**. The *tert*-butoxycarbonyl group in these esters was deblocked with TFA at room temperature to afford the objective compounds **21**, which were purified by column chromatography. Synthetic details for representative compounds are described in the experimental section.

Biological Evaluation

The *in vitro* antibacterial activity of the new cephalosporins **16a**~**16o** against Gram-positive and Gram-negative bacteria in Table 1 and peak plasma levels of selected cephalosporins **16a**, **16b**, **16h** and POM esters **21a**~**21f** and **21h**, after oral administration (40 mg/kg) to mice in Table 2, are summarized. For comparison, the MIC values and the peak plasma levels for cefaclor and ceftibuten are also listed.

As shown in Table 1, antibacterial activity against Gram-positive bacteria was potentiated by increasing the alkyl moiety in the 7β-side chain with decreasing the activity against Gram-negative ones. Compounds **16h** and **16i** having cyclopentylmethyl and cyclohexylmethyl moieties in the 7β-side chain exhibited the most potent activity against *Staphylococcus aureus* including methicillin-resistant strain (SR3131). Compounds **16d**, **16e** and **16g** having functional groups such as isopropyl, cyclopropyl and cyclopentyl substituted directly to the vinyl carbon showed antibacterial activity similar to those of **16c**, **16f** and **16h** substituted *via* the methylene group such as propyl, cyclopropylmethyl and cyclopentylmethyl. The former compounds were slightly less active against Gram-positive bacteria and more active against

Table 1. Antibacterial effects (MIC, μg/ml) of R_1 substituents in cephalosporins (**16a**~**16o** and **18**).



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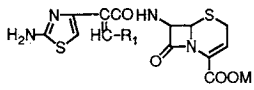
Compound No.	R_1	MIC (μg/ml)										
		S.a.	*S.a.* (R)	*S.py.*	*E.c.*	*K.p.*	*E.cl.*	*P.m.*	*P.v.*	*H.i.*	*S.m.*	*P.a.*1	P.a.2[a]
16a	CH_3	3.13	>100	0.025	0.78	0.1	0.78	0.05	0.05	N.D.	1.56	12.5	>100
18[b]	CH_3	25	>100	0.39	6.25	1.56	12.5	0.39	0.78	N.D.	25	>100	>100
16b	C_2H_5	1.56	>100	≤0.012	0.78	0.2	0.78	0.05	0.1	N.D.	1.56	1.56	50
16c	n-C_3H_7	0.78	25	≤0.012	1.56	0.78	1.56	0.39	0.39	0.025	3.13	0.78	25
16d	i-C_3H_7	1.56	N.D.	≤0.012	1.56	0.39	0.78	0.2	0.2	N.D.	3.13	1.56	25
16e		6.25	>100	≤0.012	1.56	0.39	1.56	0.2	0.2	0.025	3.13	3.13	100
16f		0.39	100	≤0.012	3.13	0.78	1.56	0.39	0.78	0.012	1.56	0.78	25
16g		0.78	25	≤0.012	1.56	0.78	1.56	0.78	0.78	0.012	3.13	0.78	12.5
16h		0.1	12.5	≤0.012	1.56	0.78	1.56	0.78	1.56	0.025	3.13	1.56	12.5
16i		0.1	6.25	≤0.012	6.25	3.13	6.25	3.13	6.25	0.2	12.5	12.5	50
16j	C_6H_5	1.56	N.D.	0.025	1.56	0.39	1.56	0.39	0.39	N.D.	1.56	0.78	25
16k	$CH_2CH_2C_6H_5$	0.39	50	≤0.012	6.25	3.13	6.25	3.13	3.13	0.1	6.25	12.5	50
16l	$CH_2C_6H_5$	0.1	50	≤0.012	1.56	0.78	1.56	0.78	1.56	0.025	3.13	1.56	12.5
16m	CH_2SCH_3	0.78	50	≤0.012	0.78	0.2	0.78	0.2	0.1	0.025	0.78	0.78	25
16n		0.39	100	≤0.012	1.56	0.39	1.56	0.39	0.39	0.012	1.56	6.25	25
16o		0.78	50	≤0.012	1.56	0.2	1.56	0.2	0.2	0.012	0.78	1.56	25
Ceftibuten		100	>100	0.39	0.1	0.012	0.39	0.025	0.025	0.1	0.1	6.25	100
Cefaclor		0.39	50	0.1	3.13	0.78	100	0.78	25	1.56	>100	>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; *S.m.*, *Serratia marcescens* ATCC 13880; *P.a.*1, *Pseudomonas aeruginosa* ATCC 25619; *P.a.*2, *Pseudomonas aeruginosa* SR24.

[b] *E* isomer of **16a**.

N.D.: Not determined.

Table 2. Plasma levels of selected cephalosporins **16** and POM esters **21**.



Compound No.	R_1	M	Plasma level (μg/ml)
16a	CH_3	Na	0.4
16b	C_2H_5	Na	<0.4
16h	CH_2⬠	Na	<0.4
21a	CH_3	POM	8.6
21b	C_2H_5	POM	14.1
21c	n-C_3H_7	POM	3.5
21d	i-C_3H_7	POM	4.6
21e	◁	POM	3.1
21f	CH_2◁	POM	4.8
21h	CH_2⬠	POM	<0.3
Cefaclor		H	29.6
Ceftibuten		H	16.7

Gram-negative ones than the latters. Compounds **16k** and **16l** possessing phenethyl and benzyl groups exhibited potent antibacterial activity against Gram-positive bacteria, although they were far less active against Gram-negative bacteria. Compound **16m** having methylthiomethyl group exhibited higher antibacterial activity against both Gram-positive and Gram-negative bacteria than **16b** having ethyl group. Antibacterial activity of **16n** and **16o** having heteroarylthiomethyl substituents was well balanced against both Gram-positive and Gram-negative bacteria though they were slightly less active than the methylthiomethyl analog **16m**. As can be seen from data on **16a** (*Z* isomer) and **18** (*E* isomer), the effect of the olefin stereochemistry on the antibacterial activity is significant, indicating that the antibacterial activity of other *E* isomers in this series are considered to be far less than that of corresponding *Z* isomers. Not unexpectedly, representative acids **16a**, **16b** and **16h** were found not to be absorbed from intestine in mice. To improve their oral absorbability, POM esters **21a**~**21f** and **21h** were prepared and examined for oral absorbability in mice. Peak plasma level data on these compounds are listed in Table 2. Derivatives **21a** and **21b** having 2-(2-aminothiazol-4-yl)-2-butenoyl and 2-(2-aminothiazol-4-yl)-2-pentenoylamino substituents at C-7 showed high plasma levels and derivatives **21d** and **21f** with 2-(2-aminothiazol-4-yl)-4-methyl-2-pentenoyl and 2-(2-aminothiazol-4-yl)-4-cyclopropyl-2-butenoylamino substituents at C-7 showed moderate plasma levels in mice. In addition, **16a**, **16b**, **16d** and **16f** have potent and well balanced antibacterial activity against both Gram-positive and Gram-negative bacteria. These data indicate that these four cephem POM esters mentioned above have possibility to be applicable as orally absorbable pro-drugs.

Experimental

Chemistry

All reactions involving air-sensitive reactions or compounds were carried out under nitrogen in dry solvents. Melting points were recorded on a Yanagimoto melting point apparatus and uncorrected. IR spectra were taken on a Hitachi 260-10 or Jasco IR-700 spectrophotometer. ^{1}H 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-propanesulfonate (in D_2O) as an internal standard.

Determination of Antibacterial Activity

The *in vitro* antibacterial activity is given as minimum inhibitory concentration (MIC) in μg/ml as determined by the 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.

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 for **16**, **21** and cefaclor or 20 mg/kg for ceftibuten. Plasma samples were collected at 0.25 and 2 hours after dosing. Plasma levels were determined by the Band Culture method[6] using *Escherichia coli* 7437 as a test organism and Trypto-soy agar as the test medium.

Ethyl 2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-butenoate (**4a** and **5a**)

Compound **4a** and **5a** were prepared as follows by modifying the procedure of G. KINAST[4]. To a solution of **2** (19.3 g, 0.05 mol) in THF (100 ml) were successively added *n*-butyllithium (15% *n*-hexane solution, 34.5 ml) and acetaldehyde (3.27 ml) at −55°C. After stirring at the same temperature for 2.5 hours, the reaction mixture was treated with 10% citric acid (50 ml). The organic layer was separated and the aqueous layer was extracted with EtOAc. The organic layer and the extract were combined and washed with brine, dried and concentrated to leave **3** as an oily residue, which was dissolved in benzene (130 ml) and treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 4.5 g) at room temperature for 2 hours. The mixture was washed with 1 N HCl and brine, dried and concentrated to give a mixture of **4a** and **5a**, which was chromatographed on a silica gel column to obtain *Z* isomer from earlier eluates and *E* isomer from later ones.

Z isomer (**4a**, 2.7 g, 17.3% yield as an oil).

TLC Rf 0.55 (cyclohexane-Et_2O, 1:1); ^1H NMR ($CDCl_3$) δ 1.34 (3H, t, J=8 Hz, CH_3), 1.52 (9H, s, $C(CH_3)_3$), 2.01 (3H, d, J=7.5 Hz, CH_3), 4.33 (2H, q, J=8 Hz, CH_2CH_3), 6.84 (1H, q, J=7.5 Hz, $=CHCH_3$), 6.91 (1H, s, thiazole H), 8.89 (1H, br s, NH).

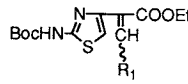
E isomer (**5a**, 3.45 g, 22.1% yield as an oil).

TLC Rf 0.35 (cyclohexane-Et_2O, 1:1); ^1H NMR ($CDCl_3$) δ 1.21 (3H, t, J=8.0 Hz, CH_3), 1.52 (9H, s, $C(CH_3)_3$), 1.89 (3H, d, J=7.5 Hz, CH_3), 4.16 (2H, q, J=8.0 Hz, CH_2CH_3), 6.88 (1H, s, thiazole H), 7.16 (1H, q, J=7.5 Hz, $=CHCH_3$), 8.91 (1H, br s, NH).

Preparation of **4b**~**4k** and **5b**~**5k**

These compounds were prepared by the similar procedure to that used for preparation of **4a** and **5a**

Table 3. Yields, ^1H NMR and IR spectral data of 7-side chain acid ethyl esters (**4** and **5**).



Compound No.	R_1	*Z*/*E*	Yield (%)	^1H NMR δ in $CDCl_3$ (*J*=Hz)			IR ($CHCl_3$) cm^{-1}
				3-H	4-H (R_1)	Thiazole H	(C=O)
4b	C_2H_5	*Z*	41	6.74 (t, 8)	2.41 (quint, 8)	6.88	1730
5b	C_2H_5	*E*	51	7.02 (t, 8)	2.31 (quint, 8)	6.84	1725
4c	*n*-C_3H_7	*Z*	35	6.78 (t, 7)	2.40 (m)	6.92	1726
5c	*n*-C_3H_7	*E*	40	7.06 (t, 7)	2.28 (m)	7.06	1725
4d	*i*-C_3H_7	*Z*	38	6.57 (d, 11)	2.7~3.1 (m)	6.87	1726
5d	*i*-C_3H_7	*E*	35	6.84 (d, 10)	2.6~3.1 (m)	6.84	1726
4e		*Z*	5	6.13 (d, 10)	2.1~2.5 (m)	6.94	1725
5e		*E*	80	6.39 (d, 11)	1.8~2.2 (m)	7.04	1720
4f		*Z*	29	6.87 (t, 9)	2.32 (t, 9)	6.90	1726
5f		*E*	35	7.14 (t, 9)	2.21 (t, 9)	6.86	1723
4g		*Z*	35	6.66 (d, 10)	2.7~3.2 (m)	6.88	1720
5g		*E*	51	6.98 (d, 10)	2.4~2.9 (m)	6.76	1717
4h		*Z*	49	6.78 (t, 8)	2.41 (t, 8)	6.89	1725
5h		*E*	38	7.12 (t, 8)	2.31 (t, 8)	6.87	1720
4i		*Z*	41	6.82 (t, 8)	2.32 (t, 8)	6.91	1727
5i		*E*	37	7.11 (t, 8)	2.18 (t, 8)	6.82	1725
4j	C_6H_5	*Z*	12	6.91 (s)	—	7.56	1723
5j	C_6H_5	*E*	56	6.66 (s)	—	7.86	1720
4k	$CH_2CH_2C_6H_5$	*Z*	32	6.7~6.9 (m)	2.6~3.0 (m, 4H)	6.89	1720
5k	$CH_2CH_2C_6H_5$	*E*	30	6.9~7.3 (m)	2.4~2.8 (m, 4H)	6.73	1717

as described above. The yields and IR and NMR spectral data of these compounds are listed in Table 3.

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2(*Z*)-butenoic Acid (**6a**)

A mixture of **4a** (3.12 g), EtOH (20 ml) and 2 N NaOH (20 ml) was stirred at 60°C for 2 hours. The reaction mixture was concentrated to remove EtOH and the residue was mixed with water and acidified with 1N HCl. The precipitate was extracted with EtOAc, and the extract was washed with water, dried and concentrated. The crystalline residue was recrystallized from CH_3CN to give **6a** as colorless needles, mp 178°C (lit. mp 183° C)[4]. Yield, 2.48 g (94%).

^{1}H NMR ($CDCl_3$-CD_3OD) δ 1.53 (9H, s, $C(CH_3)_3$), 2.11 (3H, d, J=7.0 Hz, CH_3), 6.84 (1H, q, J=7.0 Hz, =C*H*CH_3), 6.96 (1H, s, thiazole H).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2(*E*)-butenoic Acid (**7a**)

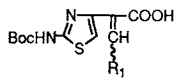
To an ice-cooled solution of **5a** (3.12 g) in MeOH (15 ml) was added 2 N NaOH (10 ml). After 2 hours, the mixture was concentrated to remove MeOH and the residue was acidified with 2 N HCl (12 ml) to precipitate brown solid, which was filtered off, washed with water and dried. The solid was recrystallized from EtOH affording pure **7a**. Yield, 2.35 g (89%), mp 193°C (lit. mp 195°C)[4].

^{1}H NMR ($CDCl_3$-CD_3OD) δ 1.54 (9H, s, $C(CH_3)_3$), 1.94 (3H, d, J=8.0 Hz, CH_3), 6.84 (1H, s, thiazole H), 7.32 (1H, q, J=8.0 Hz, =C*H*CH_3).

Synthesis of **6b**~**6k**, **7b**, **7f**, **7h**, **7i** and **7j**

These compounds were prepared by the similar procedure to that used for preparation of **6a** and **7a** as described above. The yields, IR and ^{1}H NMR spectral data of these compounds are listed in Table 4.

Table 4. Yields, ^{1}H NMR and IR spectral data of 7-side chain acids (**6** and **7**).



Compound No.	R_1	Z/E	Yield (%)	^{1}H NMR δ in $CDCl_3$ (J=Hz)			IR ($CHCl_3$) cm^{-1}
				3-H	4-H (R_1)	Thiazole H	(C=O)
6b	C_2H_5	*Z*	80	6.70 (t, 8)	2.60 (quint, 8)	6.98	1728
7b	C_2H_5	*E*	92	7.19 (t, 7.5)	2.33 (quint, 7.5)	6.81	1726
6c	*n*-C_3H_7	*Z*	78	6.67 (t, 7)	2.55 (m)	6.95	1725
6d	*i*-C_3H_7	*Z*	82	6.46 (t, 11)	3.25 (m)	6.97	1725
6e		*Z*[a]	63	6.06 (t, 11)	2.3~2.8 (m)	6.94	1724
6f		*Z*	73	6.78 (t, 9)	2.51 (t, 9)	6.93	1723
7f		*E*[a]	80	7.26 (t, 8)	2.21 (t, 8)	6.78	1724
6g		*Z*	63	6.58 (d, 10)	3.0~3.4 (m)	6.92	1730
6h		*Z*	85	6.67 (t, 8)	2.10 (t, 8)	6.92	1725
7h		*E*[a]	92	7.21 (t, 7.5)	2.28 (t, 7.5)	6.72	1728
6i		*Z*	73	6.73 (t, 8)	2.51 (t, 8)	6.96	1722
7i		*E*	82.7	7.22 (t, 8)	2.19 (t, 8)	6.78	1730
6j	C_6H_5	*Z*[a]	63	7.41 (s)	—	6.93	1718
7j	C_6H_5	*E*	75	7.95 (s)	—	6.72	1715
6k	$CH_2CH_2C_6H_5$	*Z*	62	6.65 (m)	2.56~3.0 (m)	6.84	1723
6l	$CH_2C_6H_5$	*Z*[a]	58.2	6.84 (t, 8)	3.86 (d, 8)	7.00	1722
6m	CH_2SCH_3	*Z*[a]	76	6.78 (t, 8)	3.54 (d, 8)	7.02	1728
6n		M[c]	78	6.73 (t, 8), 7.24 (t, 8)	4.25 (d, 8), 3.86 (d, 8)	7.10 6.88	1720 (KBr) 1718 (KBr)
6o		M[c]	59.8	N.D.[b]	N.D.[b]	6.82 6.96	1720 (KBr)

[a] NMR spectra were measured in $CDCl_3$-CD_3OD.

[b] Not analyzable.

[c] Mixture of Z/E isomer.

Diphenylmethyl 2-(2-*tert*-Butoxycarbonylimino-3-*tert*-butoxycarbonyl-1,3-thiazolin-4-yl)acetate (**9**)

A mixture of diphenylmethyl 2-(2-aminothiazol-4-yl)acetate (32.4 g, 0.1 mol), di-*tert*-butyl dicarbonate (50 g, 0.23 mol) and DMSO (30 ml) was allowed to stand at room temperature for 5 days. To the reaction mixture was added crushed ice and the precipitate was filtered off, washed with water and dissolved in CH_2Cl_2. The solution was washed with water, dried, concentrated and then mixed with petroleum ether precipitating **9** as colorless crystals, 30.2 g (57.5%), mp 125~126°C.

Anal Calcd for $C_{28}H_{32}N_2O_6S$: C 64.11, H 6.15, N 5.34, S 6.11.
Found: C 64.00, H 6.17, N 5.32, S 6.12.

^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s, $C(CH_3)_3$), 1.54 (9H, s, $C(CH_3)_3$), 3.84 (2H, s, CH_2), 6.23 (1H, s, thiazoline H), 6.89 (1H, s, Ph_2CH), 7.31 (10H, br s, Ph_2).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-4-phenyl-2(*Z*)-butenoic Acid (**6l**)

To a cooled solution of **9** (9.0 g, 17.3 mmol) in THF (10 ml) was added dropwise *n*-butyllithium (10.8 ml of 1.6 M *n*-hexane solution) at −70°C. After stirring at the same temperature for 20 minutes, a solution of phenylacetaldehyde (2.28 g, 19 mmol) in THF (10 ml) was added. After further stirring for 2 hours, the reaction mixture was treated with 10% citric acid (40 ml) and extracted with EtOAc. The extract was evaporated to dryness and the residue (8.1 g, 12.6 mmol) dissolved in toluene (80 ml) was treated with DBU (2.26 ml, 15 mmol) at 0°C. After being stirred at the same temperature for 1 hour, the mixture was acidified with 10% HCl and extracted with EtOAc, and the extract was washed with brine, dried and concentrated. The residue was subjected to silica gel column chromatography to give **13l** (5.7 g, 85.9%) as a colorless powder. To an ice-cooled solution of **13l** (1.0 g) in CH_2Cl_2 (2 ml) and anisole (1 ml) was added TFA (2 ml). After being stirred for 1.5 hours at 0°C, the reaction mixture was concentrated and the residue was mixed with Et_2O to give precipitate, which was recrystallized from Et_2O to give **6l** (390 mg, 58.2%) as colorless needles.

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-4-methylthio-2(*Z*)-butenoic Acid (**6m**)

To a solution of **8** (5.24 g, 10 mmol) in THF (50 ml) was added dropwise 1 M THF solution of lithium hexamethyldisilazane (33 ml) at −65°C. After 20 minutes, methylthioacetyl chloride (**10m**, 1.87 g, 15 mmol) in THF (4 ml) was added and the mixture was stirred for further 30 minutes at the same temperature. The cooling bath was removed and 10% HCl (20 ml) was added to the reaction mixture, which was extracted with EtOAc and the extract was washed with brine, dried and concentrated. The residue was purified by silica gel column chromatography to give **11m** (2.51 g, 49%) as a mixture of keto-enol isomers.

^{1}H NMR ($CDCl_3$) δ 1.53 (9H, s, $C(CH_3)_3$), 1.96 (6/3H, s, CH_3), 2.14 (3/3H, s, CH_3), 3.20 (2/3H, s, SCH_2), 3.33 (4/3H, s, SCH_2), 5.48 (2/3H, s, CHCO), 6.79 (1/3H, s, thiazole H), 6.87 (1H, s, Ph_2CH), 7.05~7.30 (10·2/3H, m, Ph_2 and thiazole H), 9.75 (1H, br s, NH), 13.3 (1/3H, br s, OH); IR ($CHCl_3$) cm^{-1} 3420, 1720, 1540, 1370, 1155.

To an ice-cooled solution of **11m** (2.51 g, 4.93 mmol) in CH_2Cl_2 (9 ml) and THF (1 ml) was added $NaBH_4$ (86 mg) and the mixture was stirred at 0°C for 10 minutes. The reaction mixture was acidified with aq HCl and extracted with EtOAc, and the extract was washed with brine and water, dried and concentrated. The oily residue was subjected to silica gel column chromatography to give **12m** (2.45 g, 81%) as an epimeric mixture.

^{1}H NMR ($CDCl_3$) δ 1.54 (9H, s, $C(CH_3)_3$), 2.07 (3/2H, s, CH_3), 2.11 (3/2H, s, CH_3), 2.2~2.8 (2H, m, SCH_2), 3.9~4.8 (3H, m, COCHCH and OH), 6.78 (1H, s, Ph_2CH), 6.83 (1H, s, thiazole H), 7.0~7.25 (10H, m, Ph_2), 9.2 (1H, br s, NH); IR ($CHCl_3$) cm^{-1} 3410, 1725, 1540, 1370, 1150.

To an ice-cooled solution of **12m** (2.0 g, 3.9 mmol) in CH_2Cl_2 (40 ml) were successively added TEA (1.6 ml) and MsCl (0.38 ml, 49 mmol), and the mixture was stirred at 0°C for 20 minutes and then at room temperature for 20 minutes. Thereto was added 10% HCl (2 ml) and the resulting mixture was extracted with EtOAc. The extract was washed with brine and dil $NaHCO_3$, dried and concentrated. To the residue were added CH_2Cl_2 (30 ml) and TEA (6 ml) and the mixture was kept at room temperature for 7 hours. The residue obtained after concentration was dissolved in EtOAc and the solution was washed with brine and dil $NaHCO_3$, dried and concentrated. The residue was chromatographed on a silica gel column to give **13m** (*Z* isomer, 496 mg, 25%) as a colorless powder.

^{1}H NMR ($CDCl_3$) δ 1.52 (9H, s, $C(CH_3)_3$), 2.15 (3H, s, CH_3), 3.34 (2H, d, J=8.0 Hz, =CHCH_2S),

6.76 (1H, s, thiazole H), 6.78 (1H, t, J=8.0 Hz, =C*H*CH_2), 6.81 (1H, s, Ph_2CH), 7.12~7.30 (10H, m, Ph_2); IR ($CHCl_3$) cm^{-1} 1720.

A mixture of **13m** (208 mg, 0.42 mmol), anisole (4 ml) and TFA (0.6 ml) was stirred at room temperature for 25 minutes. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography to give **6m** as a colorless powder (105 mg, 76%).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-4-(1,2,3-thiadiazol-5-ylthio)-2-butenoic Acid (**6n**)

To a solution of **8** (4.25 g, 10 mmol) in THF (50 ml) was added dropwise 1 M THF solution (30 ml) of lithium hexamethylsilazane at −78°C. After 30 minutes, chloroacetyl chloride (**10p**, 1.0 ml, 12.6 mmol) was added. After being stirred for further 1 hour, the reaction mixture was treated with 10% citric acid and extracted with EtOAc. The usual work-up of the extract followed by silica gel column chromatography gave **11p** (2.68 g, 54%). To an ice-cooled solution of sodium salt of 5-mercapto-1,2,3-thiadiazole (1.12 g, 8.0 mmol) in EtOH (50 ml) was added **11p** (2.5 g, 5 mmol) in EtOH (20 ml). After being stirred at 0°C for 2 hours, the reaction mixture was evaporated and the residue was extracted with EtOAc. The extract was washed with brine, dried and concentrated. The residue was subjected to silica gel column chromatography obtaining **11n** (2.11 g, 65%) as a powder. To a solution of **11n** (2.1 g, 3.6 mmol) in CH_2Cl_2 (20 ml) and MeOH (40 ml) was added $NaBH_4$ (164 mg, 4.32 mmol) at −20°C and the mixture was stirred at the same temperature for 20 minutes, treated with 10% HCl and extracted with EtOAc. The extract was washed with brine, dried and concentrated to give **12n** (2.06 g) as a crude mixture, which was dissolved in CH_2Cl_2 (30 ml) and treated with TEA (1.28 ml, 9.2 mmol) and MsCl (0.36 ml, 4.6 mmol) under ice-water cooling for 30 minutes. DBU (1.05 ml, 7.0 mmol) was added to the above mixture. After being stirred for 20 minutes at the same temperature, the reaction mixture was acidified with 10% HCl and extracted with CH_2Cl_2. The extract was washed with brine, dried and concentrated. The residue was chromatographed on a silica gel column to give **13n** (1.65 g) as an isomeric mixture, which was dissolved in CH_2Cl_2 (30 ml) and treated with anisole (3 ml) and TFA (9 ml) at 0~5°C for 2 hours. After concentration, the residue dissolved in EtOAc was washed with brine and extracted with satd $NaHCO_3$ solution 4 times. The aqueous layers were washed with Et_2O and acidified to pH 2~3 with conc HCl under cooling. Extraction with EtOAc and subsequent usual work-up gave **6n** (0.89 g, 78%) as an isomeric mixture (1 : 2).

2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-4-(1,3,4-thiadiazol-2-ylthio)-2-butenoic Acid (**6o**)

According to the procedures similar to those used for **6n**, **6o** was prepared by reaction of **11p** with sodium salt of 2-mercapto-1,3,4-thiadiazole giving **11o** (59.6%) followed by $NaBH_4$ reduction to **13o** (72%, 1 : 1 isomeric mixture) and TFA deprotection to **6o** (59.8%). As they were difficult to separate each other the mixture was used for the next reaction as it is.

Diphenylmethyl 7β-[(*Z*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-butenoylamino]-3-cephem-4-carboxylate (**15a**)

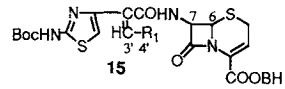
To a mixture of **6a** (284 mg, 1 mmol), TEA (166 μl, 1.2 mmol) and CH_2Cl_2 (10 ml) was added MsCl (80 μl, 1.02 mmol) at −60°C and the stirring was continued at the same temperature for 3 hours. Thereto was added dropwise a pre-cooled mixture of **14** (366 mg, 1 mmol), TEA (166 μl, 1.2 mmol) and CH_2Cl_2 (10 ml) and the mixture was further stirred for 2 hours at the same temperature. The reaction mixture was poured into 1 N HCl and the organic layer was separated, washed with brine and dil $NaHCO_3$, dried and concentrated to leave a resinous residue, which was purified by silica gel column chromatography to give **15a** as a colorless oil (574 mg, 90%).

^1H NMR ($CDCl_3$) δ 1.53 (9H, s, $C(CH_3)_3$), 2.15 (3H, d, J=8.0 Hz, CH_3), 3.27 (2H, m, 2-H), 4.87 (1H, d, J=5.0 Hz, 6-H), 5.89 (1H, dd, J=5.0, 9.0 Hz, 7-H), 6.47 (1H, m, 3-H), 6.51 (1H, q, J=8.0 Hz, =C*H*CH_3), 6.61 (1H, s, thiazole H), 6.74 (1H, s, Ph_2CH), 7.20~7.48 (10H, m, Ph_2), 7.96 (1H, d, J=9.0 Hz, NH); IR ($CHCl_3$) cm^{-1} 3410, 1778, 1723, 1670, 1280, 1160.

Synthesis of Diphenylmethyl 7β-[(*Z*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-3-(substituted)-2-propenoylamino]-3-cephem-4-carboxylates (**15b**~**15m**)

These compounds were synthesized by applying the method as described above for the synthesis of **15a**. NMR and IR spectra and chemical yields are listed in Table 5.

Table 5. Yields, ^{1}H NMR and IR spectral data of 7β-acylaminocephalosporin esters (**15b**~**15o**).



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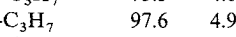
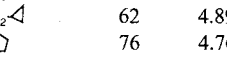
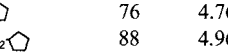
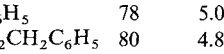
Compound No.	R_1	Yield (%)	^{1}H NMR δ in $CDCl_3$ (J=Hz)					IR ($CHCl_3$) cm^{-1} (C=O)
			6-H (d)	7-H (dd)	3'-H	4'-H (R_1)	Thiazole H	
15b	C_2H_5	83	4.88 (5)	5.91 (5, 9)	6.42 (t, 8)	2.62 (quint, 8)	6.68	1781
15c	n-C_3H_7	75.3	4.82 (5)	5.91 (5, 8)	6.45 (t, 8)	2.62 (quint, 8)	6.65	1775
15d	i-C_3H_7	97.6	4.92 (5)	5.94 (5, 8)	6.27 (d, 11)	3.0~3.6 (m)[a]	6.70	1785
15e		82	4.69 (5)	5.82 (5, 8)	6.32 (t, 4)	2.8~3.1 (m)[a]	6.58	1775
15f		62	4.89 (5)	5.89 (5, 9)	6.54 (t, 8)	2.48 (t, 8)	6.73	1782
15g		76	4.76 (5)	5.87 (5, 8)	6.37 (d, 11)	3.2~3.7 (m)	6.67	1778
15h		88	4.96 (5.5)	5.96 (5.5, 8)	6.48 (t, 8)	2.07 (t, 8)	6.72	1782
15i		74	4.95 (5)	5.94 (5, 9)	6.48 (t, 8)	2.41 (t, 8)	6.69	1782
15j	C_6H_5	78	5.00 (5)	6.02 (5, 8)	7.3~7.4[a]	—	6.94	1775
15k	$CH_2CH_2C_6H_5$	80	4.86 (5)	5.88 (5, 8)	6.4~6.6[a]	3.1~3.4 (m)	6.63	1775
15l	$CH_2C_6H_5$	58	4.93 (5)	5.98 (5, 8)	6.6~6.8[a]	3.8~4.0 (m)	6.65	1778
15m	CH_2SCH_3	39	4.98 (5)	5.93 (5, 8)	6.53 (t, 8)	3.52 (d, 8)	6.71	1780
15n		11	4.95 (5)	5.86 (5, 8)	6.45 (t, 8)	3.9~4.5 (m)	6.74	1778
15o		18.7	5.00 (5)	5.96 (5, 8)	6.73 (t, 8)	4.2~4.6 (m)	6.85	1780

[a] Overlapped with other proton signals.

Diphenylmethyl 7β-[(*Z*)-2-(*tert*-Butoxycarbonylaminothiazol-4-yl)-4-(1,2,3-thiadiazol-5-ylthio)-2-butenoylamino]-3-cephem-4-carboxylate (**15n**) and Its *E* Isomer

To a mixture of **6n** (400 mg, 1.0 mmol), **14** (366 mg, 1.0 mmol) and CH_2Cl_2 (18 ml) were added successively *N*-methylmorpholine (NMM) (330 μl, 3.0 mmol) and phenylphosphoryl dichloride (165 μl, 1.1 mmol) at $-30°C$. After being stirred at -30~$-20°C$ for 2 hours, the reaction mixture was treated with 10% citric acid and extracted with EtOAc. The extract was washed with brine and dil $NaHCO_3$, dried and concentrated, and the residue was subjected to silica gel column chromatography to give **15n** (77 mg, 11%) as a pale yellow powder and the corresponding *E* isomer (230 mg, 34.3%) from the later eluates.

Compound **15n**.

^{1}H NMR ($CDCl_3$) δ 1.52 (9H, s, $C(CH_3)_3$), 3.35~3.46 (2H, m, 2-H), 3.96~4.50 (2H, m, $=CHCH_2$), 4.95 (1H, d, J=5.0 Hz, 6-H), 5.86 (1H, dd, J=5.0, 8.0 Hz, 7-H), 6.45 (1H, t, J=8.0 Hz, $=CHCH_2$), 6.53~6.63 (1H, m, 3-H), 6.74 (1H, s, thiazole H), 6.90 (1H, s, Ph_2CH), 7.30~7.40 (10H, m, Ph_2), 8.35 (1H, d, J=8.0 Hz, NH), 8.51 (1H, s, thiadiazole H), 9.33 (1H, br, NH).

E isomer.

^{1}H NMR ($CDCl_3$) δ 1.53 (9H, s, $C(CH_3)_3$), 3.2~3.3 (2H, m, 2-H), 3.90 (2H, d, J=8.0 Hz, $=CHCH_2$), 4.80 (1H, d, J=5.0 Hz, 6-H), 5.75 (1H, dd, J=5.0, 8.0 Hz, 7-H), 6.4~6.5 (1H, m, 3-H), 6.75 (1H, s, thiazole H), 7.13 (1H, t, J=8.0 Hz, $=CHCH_2$), 7.3~7.4 (11H, m, Ph_2 and Ph_2CH), 7.90 (1H, s, thiadiazole H), 10.3 (1H, br, NH).

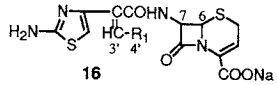
Diphenylmethyl 7β-[(*Z*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-4-(1,3,4-thiadiazol-2-ylthio)-2-butenoylamino]-3-cephem-4-carboxylate (**15o**)

Compound **15o** (pale yellow powder, 18.7%) was prepared by the similar method to that used for the preparation of **15n** as described above.

Diphenylmethyl 7β-[(*E*)-2-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-butenoylamino]-3-cephem-4-carboxylate (**17**)

To a solution of **7a** (170 mg, 0.6 mmol) and **14** (183 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) was added DCC (104 mg, 0.5 mmol) at room temperature. After being stirred for 15 hours, the precipitate was filtered off and the filtrate was concentrated. The residue dissolved in EtOAc was washed with brine, dried and

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



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Compound No.	R_1	Yield (%)	^{1}H NMR δ (J=Hz)						IR cm^{-1} (C=O)
			6-H (d)	7-H (d)	3'-H	4'-H (R_1)	Thiazole H	Solvent[b]	
16b	C_2H_5	59.5	5.63 (5)	6.29 (5)	6.81 (t, 8)	2.71 (quint, 8)	6.96	a	1765 (N)[a]
16c	n-C_3H_7	59.5	5.63 (5)	6.28 (5)	6.82 (t, 8)	2.68 (q, 8)	6.94	b	1775 (N)[a]
16d	i-C_3H_7	63.2	5.63 (5)	6.29 (5)	6.54 (d, 11)	2.8~3.3 (m)	6.94	b	1775 (N)[a]
16e		65.1	5.63 (5)	6.31 (5)	6.24 (d, 10)	2.0~2.4 (m)	6.90	a	1760 (K)
16f		84	5.33 (5)	6.00 (5)	6.70 (t, 8)	2.45 (t, 8)	6.57	a	1775 (N)[a]
16g		73	5.60 (5)	6.27 (5)	6.71 (d, 10)	2.8~3.3 (m)	6.91	a	1762 (K)
16h		82	5.11 (5)	5.82 (5)	6.82 (t, 8)	2.22 (t, 8)	6.74	b	1770 (N)[a]
16i		66.5	5.58 (5)	6.22 (5)	6.80 (t, 8)	2.59 (t, 8)	6.87	a	1755 (N)
16j	C_6H_5	63	5.24 (5)	5.84 (5)	7.01 (s)	—	7.16	c	1770 (N)
16k	$CH_2CH_2C_6H_5$	68	5.20 (5)	5.85 (5)	6.37 (t, 8)	2.5~2.9[c]	6.54	b	1775 (K)[a]
16l	$CH_2C_6H_5$	42	5.15 (5)	5.87 (5)	6.50 (m)	3.4~3.7 (m)	7.24	c	1770 (N)
16m	CH_2SCH_3	72	5.32 (5)	6.30 (5)	6.78 (t, 8)	3.46 (q, 8)	6.88	b	1775 (K)[a]
16n		80	5.13 (5)	5.82 (5)	6.34 (t, 8)	4.03 (d, 8)	6.43	c	1775 (K)[a]
16o		70	5.58 (5)	6.26 (5)	6.92 (t, 7)	4.60 (d, 7)	7.06	c	1765 (K)

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

concentrated to leave a viscous oil. Purification by silica gel column chromatography gave **17** (140 mg, 67%) as a colorless powder.

^{1}H NMR ($CDCl_3$) δ 1.53 (9H, s, $C(CH_3)_3$), 1.93 (3H, d, J=7.5 Hz, CH_3), 3.13 (2H, d, J=5.0 Hz, 2-H), 4.69 (1H, d, J=4.5 Hz, 6-H), 5.70 (1H, dd, J=4.5, 8.0 Hz, 7-H), 6.32 (1H, d, J=5.0 Hz, 3-H), 6.55 (1H, s, thiazole H), 6.61 (1H, s, Ph_2CH), 7.11~7.47 (11H, m, Ph_2 and $=CHCH_2$), 7.73 (1H, d, J=8.0 Hz, NH); IR ($CHCl_3$) cm^{-1} 3340, 1777, 1721, 1623, 1158.

Sodium 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-butenoylamino]-3-cephem-4-carboxylate (**16a**)

Compound **15a** (135 mg, 0.21 mmol) was treated with TFA (2 ml) at room temperature for 1.5 hours. After concentration, the residue was partitioned between EtOAc and dil $NaHCO_3$. The aqueous layer was washed with Et_2O and chromatographed on a Diaion HP-20 column. The eluates containing the product were lyophylized to give **16a** (56 mg, 68.5%) as a pale yellow powder.

Anal Calcd for $C_{14}H_{13}N_4O_4S_2Na \cdot 3H_2O$: C 38.02, H 4.56, N 12.67, H_2O 12.21.
Found: C 37.87, H 4.24, N 12.67, H_2O 12.03.

^{1}H NMR (D_2O) δ 2.32 (3H, d, J=8.0 Hz, CH_3), 3.95~4.32 (2H, m, 2-H), 5.62 (1H, d, J=5.0 Hz, 6-H), 6.30 (1H, d, J=5.0 Hz, 7-H), 6.77 (1H, m, 3-H), 6.85 (1H, q, J=8.0 Hz, $=CHCH_3$), 6.95 (1H, s, thiazole H); IR (Nujol) cm^{-1} 1752.

Synthesis of Sodium 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-3-(substituted)-2-propenoylamino]-3-cephem-4-carboxylates (**16b**~**16o**)

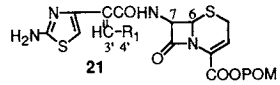
These compounds were synthesized by applying the method for the synthesis of **16a** as described above. NMR and IR spectra and chemical yields are listed in Table 6.

Sodium 7β-[(*E*)-2-(2-Aminothiazol-4-yl)-2-butenoylamino]-3-cephem-4-carboxylate (**18**)

Compound **18** was obtained as a pale yellow powder in 68% yield from **17**.

^{1}H NMR (D_2O) δ 2.32 (3H, d, J=7.5 Hz, CH_3), 3.92~4.09 (2H, m, 2-H), 5.58 (1H, d, J=5.0 Hz, 6-H), 6.21 (1H, d, J=5.0 Hz, 7-H), 6.74 (1H, m, 3-H), 7.11 (1H, s, thiazole H), 7.39 (1H, q, J=7.5 Hz,

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



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Compound No.	R_1	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	C_2H_5	48.5	5.01 (5)	5.95 (5, 8)	6.41 (t, 8)	2.38 (quint, 8)	6.34	1785
21c	n-C_3H_7	41.6	5.05 (5)	5.95 (5, 8)	6.41 (t, 8)	2.30 (m)	6.37	1790
21d	i-C_3H_7	43.8	5.00 (5)	5.96 (5, 8)	6.22 (d, 11)	2.7~3.1 (m)[a]	6.79	1780
21e		27.7	5.03 (5)	6.03 (5, 9)	5.80 (d, 11)	2.0~2.5 (m)[a]	6.33	1785
21f		27.5	5.06 (5)	6.00 (5, 8.5)	6.55 (t, 7.5)	2.30 (t, 7.5)	6.38	1784
21h		33.3	5.02 (5.5)	5.98 (5.5, 8.5)	6.45 (t, 7.5)	2.39 (t, 7.5)	6.33	1797

[a] Overlapped with other proton signals.

=C*H*CH_3).

Pivaloyloxymethyl 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-butenoylamino]-3-cephem-4-carboxylate (**21a**)

To an ice cooled solution of **15a** (300 mg, 0.47 mmol) and anisole (1.5 ml) in CH_2Cl_2 (4 ml) was added TFA (2 ml) and the mixture was stirred at 0°C for 2 hours. The residue after concentration to remove TFA was mixed with Et_2O to precipitate the acid (**19a**) as a pale yellow crystalline powder (200 mg), which was used for the next reaction without further purification.

^{1}H NMR ($CDCl_3$-CD_3OD) δ 1.54 (9H, s, $C(CH_3)_3$), 2.03 (3H, d, J=8.0 Hz, CH_3), 3.55 (2H, m, 2-H), 5.05 (1H, d, J=5.0 Hz, 6-H), 5.91 (1H, d, J=5.0 Hz, 7-H), 6.40~6.65 (2H, m, =C*H*CH_3 and 3-H), 6.77 (1H, s, thiazole H).

A mixture of **19a** (200 mg, 0.43 mmol), K_2CO_3 (90 mg, 0.65 mmol), POMI (80 μl, 0.47 mmol) and DMF (3 ml) was stirred at −30°C for 30 minutes. The reaction mixture was treated with 10% citric acid and extracted with EtOAc, and the extract was washed with brine and water, dried and concentrated. The oily residue was subjected to silica gel column chromatography affording pure **20a** (140 mg, 56.2%).

^{1}H NMR ($CDCl_3$) δ 1.23 (9H, s, $C(CH_3)_3$), 1.52 (9H, s, $C(CH_3)_3$), 2.04 (3H, d, J=8.0 Hz, CH_3), 3.37 (1H, dd, J=6.0, 19.0 Hz, 2-H_a), 3.63 (1H, dd, J=3.0, 19.0 Hz, 2-H_b), 5.01 (1H, d, J=5.0 Hz, 6-H), 5.83 (2H, s, OCH_2O), 5.88 (1H, dd, J=5.0, 8.0 Hz, 7-H), 6.52 (1H, dd, J=3.0, 6.0 Hz, 3-H), 6.55 (1H, q, J=8.0 Hz, =C*H*CH_3), 6.71 (1H, s, thiazole H), 7.81 (1H, d, J=8.0 Hz, NH); IR ($CHCl_3$) cm^{-1} 3415, 3100, 1786, 1746, 1725, 1678, 1545, 1155.

Compound **20a** (100 mg, 0.17 mmol) was treated with TFA (1 ml) at room temperature for 1 hour. To the residue after concentration were added dil $NaHCO_3$ and EtOAc. The organic extract was washed with brine, dried and concentrated. The residue was subjected to silica gel column chromatography to give **21a** as a pale yellow powder (70 mg, 85%).

^{1}H NMR ($CDCl_3$) δ 1.21 (9H, s, $C(CH_3)_3$), 1.93 (3H, d, J=7.0 Hz, CH_3), 3.40 (1H, dd, J=5.0, 18.9 Hz, 2-H_a), 3.60 (1H, dd, J=3.0, 18.9 Hz, 2-H_b), 5.03 (1H, d, J=5.0 Hz, 6-H), 5.29 (2H, br s, NH_2), 5.80, 5.91 (2H, AB q, J=6.0 Hz, OCH_2O), 5.96 (1H, dd, J=5.0, 8.0 Hz, 7-H), 6.25 (1H, s, thiazole H), 6.49 (1H, q, J=7.0 Hz, =C*H*CH_3), 6.59 (1H, dd, J=3.0, 5.0 Hz, 3-H), 8.40 (1H, d, J=8.0 Hz, NH); IR ($CHCl_3$) cm^{-1} 1783.

Synthesis of Pivaloyloxymethyl 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-3-(substituted)-2-propenoylamino]-3-cephem-4-carboxylates (**21b**~**21f** and **21h**)

These compounds were prepared by the similar procedures to those used for preparation of **21a** as described above. NMR and IR spectral data and chemical yields are listed in Table 7.

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