STUDIES ON CEPHALOSPORIN ANTIBIOTICS

V. SYNTHESIS, ANTIBACTERIAL ACTIVITY AND ORAL ABSORPTION OF NEW 3-[(*Z*)-2-METHOXYCARBONYLVINYLTHIO]-7β-[(*Z*)-2-(2-AMINOTHIAZOL-4-YL)-2-(OXYIMINO)ACETAMIDO]CEPHALOSPORINS

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A series of new 3-[(*Z*)-2-methoxycarbonylvinylthio]-7β-[(2-aminothiazol-4-yl)acetamido]-cephalosporins (**1**) having various oxyimino groups (*Z*-form) at the α position of the C-7 side chain was synthesized and evaluated for antibacterial activity and oral absorption in rats. Of these, the cephalosporin (**1a**) with a hydroxyimino group in the C-7 side chain showed a potent antibacterial activity against Gram-negative bacteria and Gram-positive *Staphylococcus aureus* as well as good oral absorption in rats. The structure-activity relationships of **1** are also presented.

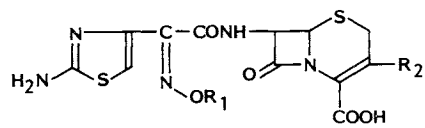
Recently, we have studied the synthesis and biological properties of cephalosporins with a hetero-atom attached directly to the C-3 position of the cephem nucleus[1~4]. In a previous paper[4], we reported that 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]cephalosporins with a lower alkoxycarbonylvinylthio group (*Z*-form) at the C-3 position, represented by **2** as shown in Fig. 1, display good oral absorption in rats, potent antibacterial activity against Gram-negative bacteria and improved activity against *Staphylococcus aureus* as compared with cefixime[5]. Subsequently, we studied the chemical modification of the C-7 side chain of **2** in order to find cephalosporins showing both higher activity, especially against *S. aureus* and better oral absorption than **2**.

Herein we describe the synthesis, antibacterial activity and oral absorption in rats of new 3-[(*Z*)-2-methoxycarbonylvinylthio]cephalosporins (**1**) having various oxyimino groups (*Z*-form) at the α position of the 7β-(2-aminothiazol-4-yl)acetamido side chain.

Chemistry

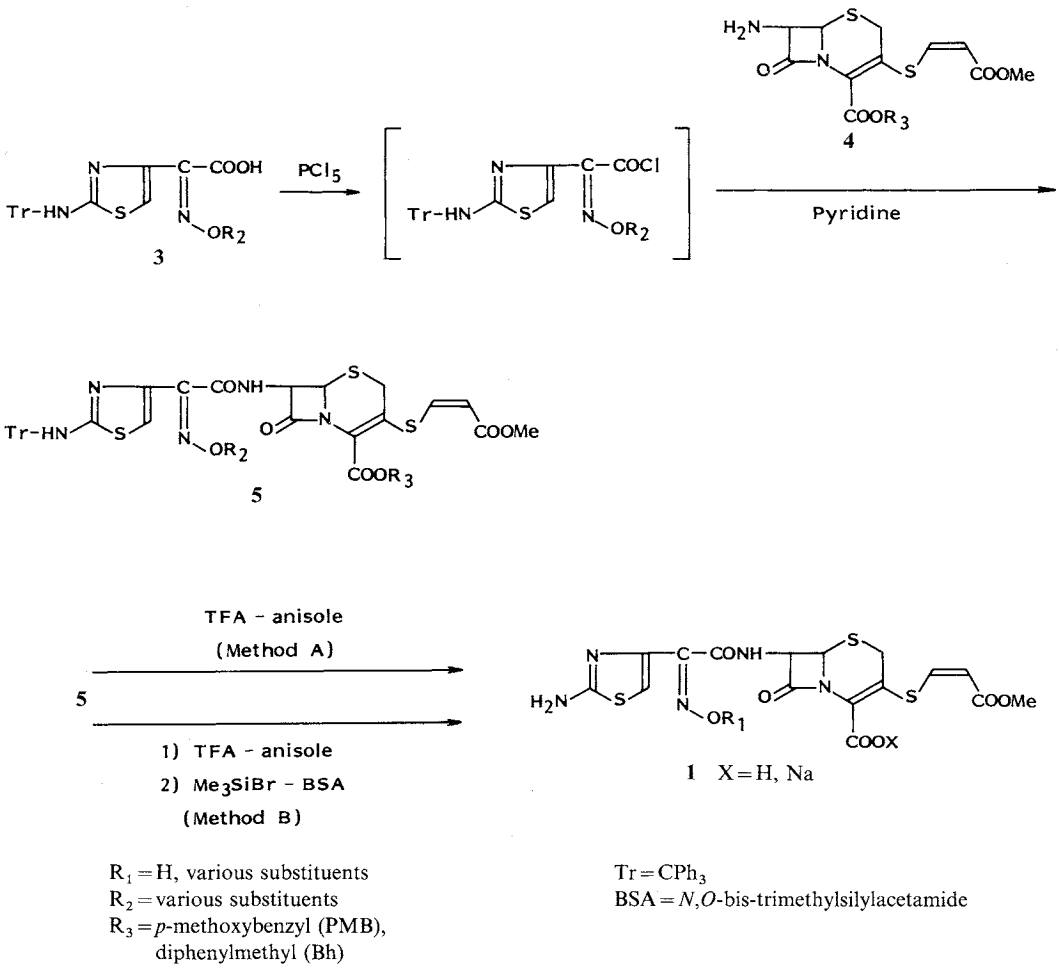
The new cephalosporins (**1a**~**1l**) listed in Table 1 were prepared by the synthetic route as shown in Scheme 1. 4-(2-Aminothiazole)acetic acid derivatives (**3**) having various oxyimino groups at the α position were converted into the acid chlorides with phosphorus pentachloride in dichloromethane at low

Fig. 1. Structure of **1**, **2** and cefixime.



1	R_1=H, various substituents	R_2=SCH=CHCO$_2$Me(*Z*)
2	R_1=CH_2CO_2H	R_2=SCH=CHCO$_2$Me(*Z*)
Cefixime	R_1=CH_2CO_2H	R_2=CH=CH_2

Scheme 1.



R_1 = H, various substituents
R_2 = various substituents
R_3 = *p*-methoxybenzyl (PMB), diphenylmethyl (Bh)

Tr = CPh_3
BSA = *N*,*O*-bis-trimethylsilylacetamide

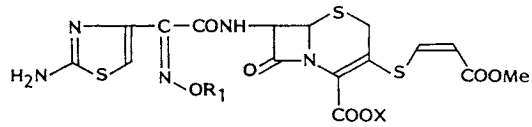
temperature. Then, the acid chlorides (not isolated) were reacted with 7β-amino-3-[(*Z*)-2-methoxycarbonylvinylthio]cephalosporanic acid ester derivatives (**4**)[6] in the presence of pyridine to afford the 7β-acylamino derivatives (**5**). Subsequently, the protecting groups in **5** (except **5i**) were removed by a conventional manner (Method A) with trifluoroacetic acid (TFA) and anisole to yield the desired cephalosporins (**1**). In the case of **5i** bearing *O*,*O*-diethylphosphonomethoxyimino group in the C-7 side chain, trimethylsilyl halide was used to hydrolyze the phosphoric acid ester group after treating with TFA-anisole[7] (Method B).

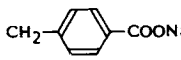
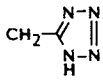
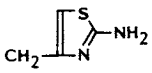
Antibacterial Activity and Oral Absorption

The *in vitro* antibacterial activities of the new cephalosporins (**1a**～**1l**) against selected Gram-positive and Gram-negative bacteria and their peak serum levels after oral administration (50 mg/kg) to rats are summarized in Table 1. For comparison, the MIC values and peak serum levels of **2**[4] and cefixime[5] are listed at the bottom of Table 1.

Against *S. aureus* 209P JC-1, most of these new cephalosporins showed improved activity compared with **2**, though the analogues **1g** and **1i** having carboxylic and phosphoric acid as an acidic group,

Table 1. *In vitro* antibacterial activity and peak serum level of **1a**~**1l**.



Compound No.	R_1	X	MIC (μg/ml, 10^6 cfu/ml)[a] *S.a.*	*E.c.*	*K.p.*	*M.m.*	*S.m.*	*P.m.*	Peak serum level (μg/ml)[b] po, 50 mg/kg rats ($n=3$)
1a	H	Na	0.39	≦0.1	≦0.1	≦0.1	1.56	≦0.1	18.5
1b	CH_3	Na	1.56	≦0.1	≦0.1	≦0.1	1.56	≦0.1	7.4
1c	CH_2CH_2F	Na	0.78	≦0.1	0.2	≦0.1	0.39	≦0.1	4.1
1d	$CH_2CH{=}CH_2$	Na	0.39	0.2	0.78	0.2	0.78	0.2	6.5
1e	CH_2CO_2Et	Na	0.78	1.56	≦0.1	0.2	1.56	≦0.1	<3.4
1f	CH_2CONH_2	Na	1.56	≦0.1	≦0.1	≦0.1	0.39	≦0.1	4.9
1g	$C(CH_3)_2CO_2Na$	Na	25	1.56	0.39	0.2	1.56	0.2	<1.3
1h		Na	3.13	1.56	0.39	0.78	3.13	≦0.1	<2.8
1i	$CH_2PO_3Na_2$	Na	50	0.78	≦0.1	≦0.1	0.78	≦0.1	<3.1
1j		Na	6.25	0.78	≦0.1	≦0.1	1.56	≦0.1	<2.3
1k		Na	0.39	≦0.1	0.2	0.39	0.39	≦0.1	<2.6
1l	$CH_2CH_2NH_2$ (TFA salt)	H	6.25	0.2	≦0.1	≦0.1	0.39	0.39	3.7
2[c]	CH_2CO_2Na	Na	12.5	0.2	≦0.1	≦0.1	1.56	≦0.1	38.9
Cefixime[c]			25	0.78	≦0.1	0.2	0.78	≦0.1	28.6

[a] The MICs were determined by a standard agar dilution method using Sensitive Test agar (Eiken, Japan).

[b] The peak serum levels were measured by a disc-plate method using *Escherichia coli* SC 507 or *Micrococcus luteus* NIHJ as the test organism.

[c] For compounds **2** and cefixime see refs 4 and 5, respectively.

Abbreviations: *S.a.*; *Staphylococcus aureus* 209P JC-1, *E.c.*; *Escherichia coli* NIHJ JC-2, *K.p.*; *Klebsiella pneumoniae* IFO 3317, *M.m.*; *Morganella morganii* IID 602, *S.m.*; *Serratia marcescens* IID 618, *P.m.*; *Proteus mirabilis* IFO 3849.

respectively, in the oxime moiety of C-7 side chain exhibited no significant activity. In particular, compounds **1a**, **1d** and **1k** with hydroxyimino, allyloxyimino and 2-aminothiazol-4-ylmethoxyimino group, respectively, in the C-7 side chain showed fairly potent activity.

On the other hand, against the Gram-negative bacteria, these new cephalosporins exhibited a potent antibacterial activity comparable to cefixime and **2**, though **1g** and **1h** were somewhat less active than the others.

According to these results, we found that introduction of the acidic groups (except hydroxyimino group) into the oxime moiety was liable to reduce the activity against *S. aureus*, due to the high hydrophilicity.

In the oral absorption study in rats, only compound **1a** exhibited prominent concentrations in serum. However, the peak serum level of **1a** was inferior to those of **2** and cefixime. Contrary to our expectation, the oral absorption of **1i**, a phosphoric acid analogue of **2**, was much lower than that of **2**.

The most favorable compound **1a** in this series was then advanced to a preliminary *in vivo* efficacy trial by oral administration. As shown in Table 2, compound **1a** exhibited good efficacy against systemic infections in mice induced by *Klebsiella pneumoniae* 6 and *Escherichia coli* TM-36, but its efficacy was

Table 2. *In vivo* antibacterial activity of **1a** against systemic infections in mice.

Organisms	Challenge dose[a] (cfu/mouse)	Compound	ED_{50}[b] (mg/kg)	MIC[c] (μg/ml)
Klebsiella pneumoniae 6	1.0×10^7	**1a**	3.64 (2.07~ 6.59)	0.025
		2	0.49 (0.17~ 1.48)	0.05
Escherichia coli TM-36	1.3×10^7	**1a**	6.25 (3.48~11.36)	0.1
		2	1.73 (1.00~ 3.30)	0.2

Drugs were administered orally 1 hour after infection. Mouse: Male ICR strain, 4 weeks, 10 mice/group.
[a] ip, (5% mucin).
[b] Probit method (95% confidence limits).
[c] Inoculum size: 10^6 cfu/ml.

inferior to that of **2**, probably due to the lower bioavailability of **1a**.

Further biological evaluation of the promising compound **1a**, as well as its prodrugs designed for improving the bioavailability are now under study.

Experimental

IR spectra were taken on a Jasco DS-701G IR spectrometer. ^{1}H NMR spectra were recorded on a Varian XL-200 NMR spectrometer using TMS or sodium trimethylsilyl propionate-d_4 (in D_2O) as an internal standard. Mass spectra (MS) were measured on a Jeol JMX-DX303 or JMS-SX102 mass spectrometer. Chromatographic separations were done by using Wako Silica gel C-200 (100~200 mesh, Wako, Japan) or Sephadex LH-20 (Pharmacia, Sweden). Analytical HPLC was performed on a TSK gel LS-410 column (5 μm, 150 × 4.6 mm, i.d., Tosoh, Japan) eluted with 35% aq acetonitrile containing tetra-*n*-amylammonium bromide (10 mmol) and ammonium acetate (10 mmol), flow rate 1.0 ml/minute at ambient temperature monitoring UV absorbance at 290 nm.

In Vitro and *In Vivo* Antibacterial Activities

MICs were determined by the 2-fold agar dilution method using Sensitive Test agar (Eiken, Japan) after incubation at 37°C for 18 hours with an inoculum size 10^6 cfu/ml. Mouse protecting experiments were conducted by use of male ICR mice ($n=10$) infected intraperitoneally with 0.5 ml of a bacterial suspension containing 100% or more minimal lethal doses. Hog gastric mucin (5%, w/v) was added to the suspension before injection. The test drugs in 5% gum arabic were administered orally 1 hour after the infection. Mortality of the animals was recorded daily over a period of 7 days and the ED_{50} values were calculated by the method of probit[8].

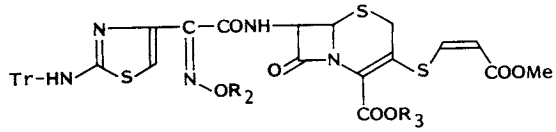
Oral Absorption Study

Male SLC/Wister rats ($n=3$) weighing 180~220 g were fasted overnight and orally dosed with 50 mg/kg of the test compounds in 5% gum arabic. Serum samples were collected at 0.5, 1, 2, and 4 hours, respectively, after dosing. Serum levels of the test compounds were measured by the disc-plate method using *Escherichia coli* SC 507 or *Micrococcus luteus* NIHJ as a test organism and Sensitive Test agar as the test medium.

Diphenylmethyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-methoxyimino]acetamido]-3-[(*Z*)-2-methoxycarbonylvinylthio]-3-cephem-4-carboxylate (**5b**)

To a solution of 2-(2-tritylaminothiazol-4-yl)-2-[(*Z*)-methoxyimino]acetic acid (**3**, R_2=Me, 591 mg, 1.33 mmol) in dry CH_2Cl_2 (16 ml) were successively added pyridine (532 mg, 5.0 equiv) and phosphorus pentachloride (277 mg, 1.0 equiv) at 0°C with stirring, and the reaction mixture was stirred for 20 minutes. Then, a solution of diphenylmethyl 7β-amino-3-[(*Z*)-2-methoxycarbonylvinylthio]cephalosporanate (**4**[6], R_3=$CHPh_2$, 560 mg, 0.87 equiv) in dry CH_2Cl_2 (5 ml) was added to the reaction mixture at −10°C and stirred for 30 minutes at 0°C. After the reaction, 0.5% HCl (50 ml) was added to the reaction mixture and extracted with EtOAc (100 ml). The extract was washed with brine (50 ml), dried ($MgSO_4$) and evaporated.

Table 3. ^{1}H NMR, MS and IR spectral data of **5**.



5

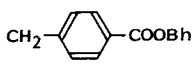
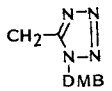
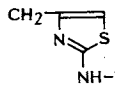
Compound			^{1}H NMR δ ($CDCl_3$)	MS (m/z)	IR (KBr) cm^{-1}
No.	R_2	R_3			
5a	DMB	PMB	3.12 (1H, d, J=17 Hz), 3.60 (1H, d, J=17 Hz), 3.70 (3H, s), 3.75 (3H, s), 3.77 (3H, s), 3.80 (3H, s), 4.94 (1H, d, J=5 Hz), 5.22 (2H, s), 5.23 (1H, d, J=10 Hz), 5.34 (1H, d, J=10 Hz), 5.81 (1H, dd, J=5, 9 Hz), 5.94 (1H, d, J=10 Hz), 6.35~6.47 (2H, m), 6.83 (1H, s), 6.86 (2H, d, J=8 Hz), 6.93 (1H, d, J=10 Hz), 6.98 (1H, br s), 7.20~7.40 (19H, m)	998[b]	1790, 1700
5c	CH_2CH_2F	PMB	3.49 (1H, d, J=18 Hz), 3.79 (3H, s), 3.80 (1H, d, J=18 Hz), 3.81 (3H, s), 4.52 (2H, dt, J=30, 4 Hz), 4.70 (2H, dt, J=48, 4 Hz), 5.09 (1H, d, J=5 Hz), 5.23 (2H, s), 5.92 (1H, d, J=10 Hz), 5.95 (1H, dd, J=5, 9 Hz), 6.76 (1H, s), 6.82 (1H, d, J=9 Hz), 6.87 (2H, d, J=9 Hz), 6.96 (1H, d, J=10 Hz), 7.00 (1H, s), 7.26~7.40 (17H, m)	893[a]	1780, 1680
5d	$CH_2CH{=}CH_2$	PMB	3.47 (1H, d, J=18 Hz), 3.77 (3H, s), 3.79 (1H, d, J=18 Hz), 3.80 (3H, s), 4.79 (2H, d, J=5 Hz), 5.09 (1H, d, J=5 Hz), 5.22 (2H, s), 5.25 (1H, d, J=12 Hz), 5.32 (1H, d, J=20 Hz), 5.92 (1H, d, J=10 Hz), 5.92~6.12 (2H, m), 6.72 (1H, s), 6.87 (2H, d, J=9 Hz), 6.96 (1H, d, J=10 Hz), 7.00 (1H, s), 7.24~7.42 (17H, m)	887[a]	1780, 1680
5e	CH_2CO_2Et	PMB	1.26 (3H, t, J=7 Hz), 3.49 (1H, d, J=18 Hz), 3.88 (3H, s), 3.89 (1H, d, J=18 Hz), 3.92 (3H, s), 4.22 (2H, q, J=7 Hz), 4.88 (2H, s), 5.09 (1H, d, J=5 Hz), 5.24 (2H, s), 5.91 (1H, dd, J=5, 9 Hz), 5.92 (1H, d, J=10 Hz), 6.82 (1H, s), 6.88 (3H, d, J=9 Hz), 6.97 (1H, d, J=10 Hz), 7.00 (1H, s), 7.26~7.40 (17H, m)	933[a]	1780, 1725, 1690
5f	CH_2CONH_2	PMB	3.49 (1H, d, J=18 Hz), 3.76 (1H, d, J=18 Hz), 3.77 (3H, s), 3.80 (3H, s), 4.70 (2H, br s), 5.09 (1H, d, J=5 Hz), 5.21 (2H, s), 5.89 (1H, dd, J=5, 9 Hz), 5.93 (1H, d, J=10 Hz), 6.65 (1H, s), 6.89 (2H, d, J=9 Hz), 6.92 (1H, d, J=10 Hz), 7.00 (1H, br s), 7.20~7.46 (17H, m), 8.20 (1H, br s)	905[b]	1780, 1670
5g	$C(CH_3)_2CO_2Bh$	Bh	1.69 (6H, s), 3.27 (1H, d, J=17 Hz), 3.66 (1H, d, J=17 Hz), 3.77 (3H, s), 5.03 (1H, d, J=5 Hz), 5.82 (1H, d, J=10 Hz), 6.00 (1H, dd, J=5, 8 Hz), 6.65 (1H, s), 6.83 (1H, d, J=10 Hz), 6.88 (1H, s), 6.92 (1H, br s), 7.03 (1H, s), 7.14~7.48 (36H, m)	1,146[b]	1790, 1740, 1700
5h		PMB	3.33 (1H, d, J=18 Hz), 3.68 (1H, d, J=18 Hz), 3.78 (3H, s), 3.80 (3H, s), 5.04 (1H, d, J=5 Hz), 5.23 (2H, s), 5.39 (2H, s), 5.90 (1H, d, J=10 Hz), 5.93 (1H, dd, J=5, 9 Hz), 6.72 (1H, d, J=9 Hz), 6.73 (1H, s), 6.87 (2H, d, J=9 Hz), 6.91 (1H, d, J=10 Hz), 7.01 (1H, s), 7.12 (1H, s), 7.26~7.52 (29H, m), 8.13 (2H, d, J=8 Hz)	1,147[a]	1780, 1710
5i	$CH_2PO(OEt)_2$	PMB	1.29 (6H, t, J=7 Hz), 3.48 (1H, d, J=18 Hz), 3.75 (1H, d, J=18 Hz), 3.76 (3H, s), 3.80 (3H, s), 4.05~4.20 (4H, m), 4.59 (1H, dd, J=5, 18 Hz), 4.68 (1H, dd, J=5, 18 Hz), 5.07 (1H, d, J=5 Hz), 5.21 (2H, s), 5.82 (1H, dd, J=5, 9 Hz), 5.90 (1H, d, J=10 Hz), 6.76 (1H, s), 6.85 (2H, d, J=9 Hz), 6.94 (1H, s), 6.95 (1H, d, J=10 Hz), 7.20~7.45 (17H, m), 8.62 (1H, d, J=9 Hz)	998[b]	1780, 1680

Table 3. (Continued)

Compound No.	R_2	R_3	^{1}H NMR δ ($CDCl_3$)	MS (*m/z*)	IR (KBr) cm^{-1}
5j		PMB	3.40 (1H, d, J=17 Hz), 3.67 (1H, d, J=17 Hz), 3.76 (6H, s), 3.79 (3H, s), 3.80 (3H, s), 5.02 (1H, d, J=5 Hz), 5.16 (1H, d, J=12 Hz), 5.22 (1H, d, J=12 Hz), 5.43 (2H, s), 5.45 (1H, d, J=14 Hz), 5.52 (1H, d, J=14 Hz), 5.85 (1H, dd, J=5, 8 Hz), 5.91 (1H, d, J=10 Hz), 6.40~6.50 (2H, m), 6.77 (1H, s), 6.85 (2H, d, J=8 Hz), 6.96 (1H, br s), 6.97 (1H, d, J=10 Hz), 7.20~7.37 (18H, m), 8.56 (1H, d, J=8 Hz)	1,080[b]	1790, 1700
5k		PMB	3.20 (1H, d, J=18 Hz), 3.68 (1H, d, J=18 Hz), 3.78 (3H, s), 3.79 (3H, s), 5.01 and 5.14 (2H, ABq, J=12 Hz), 5.08 (1H, d, J=5 Hz), 5.12 and 5.22 (2H, ABq, J=14 Hz), 5.80 (1H, dd, J=5, 7 Hz), 5.91 (1H, d, J=10 Hz), 6.16 (1H, s), 6.82 (1H, s), 6.83 (2H, d, J=9 Hz), 6.94 (1H, d, J=10 Hz), 7.02 (1H, s), 7.12~7.40 (32H, m), 9.98 (1H, d, J=7 Hz)	1,202[b]	1780, 1690
5l	CH_2CH_2NHBoc	PMB	1.30 (9H, s), 3.35~3.50 (2H, m), 3.51 (1H, d, J=18 Hz), 3.77 (3H, s), 3.78 (1H, d, J=18 Hz), 3.80 (3H, s), 4.36 (2H, t, J=5 Hz), 4.98 (1H, t, J=7 Hz), 5.08 (1H, d, J=5 Hz), 5.22 (2H, s), 5.90 (1H, d, J=10 Hz), 5.96 (1H, dd, J=5, 9 Hz), 6.64 (1H, s), 6.87 (2H, d, J=9 Hz), 6.98 (1H, d, J=10 Hz), 7.01 (1H, s), 7.14~7.40 (17H, m), 8.09 (1H, d, J=9 Hz)	991[b]	1780, 1690

[a] FD, M^+.

[b] FAB or SI-MS, $(M+H)^+$.

Abbreviations: DMB; 2,4-dimethoxybenzyl, PMB; *p*-methoxybenzyl, Bh; diphenylmethyl, Tr; trityl, Boc; *tert*-butoxycarbonyl.

The residue was purified by column chromatography on silica gel (eluent; EtOAc-*n*-hexane, 2:3) to yield 243 mg (23%) of **5b** as a pale yellow powder: IR (KBr) cm^{-1} 1780 (β-lactam), 1720, 1680; ^{1}H NMR ($CDCl_3$) δ 3.48 (1H, d, J=18 Hz, 2-Hα), 3.76 (3H, s, CO_2CH_3), 3.81 (1H, d, J=18 Hz, 2-Hβ), 4.09 (3H, s, =$NOCH_3$), 5.12 (1H, d, J=5 Hz, 6-H), 5.83 (1H, d, J=10 Hz, =$CHCO_2CH_3$), 5.96 (1H, dd, J=5 and 9 Hz, 7-H), 6.76 (1H, s, thiazole 5-H), 6.87 (1H, d, J=9 Hz, CONH), 6.89 (1H, d, J=10 Hz, SCH=), 7.02 (2H, s, TrN*H* and $CHPh_2$), 7.10~7.44 (25H, m, aromatic H); FD-MS *m/z* 908 (M^+).

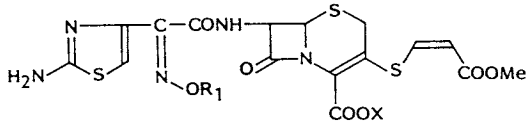
Similarly, compounds **5a** and **5c**~**5l** were prepared from **4** with various 4-(2-aminothiazole)acetic acid derivatives **3** according to the procedure described for **5b**. Their spectral data are summarized in Table 3.

Sodium 7β-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-methoxyimino]acetamido]-3-[(*Z*)-2-methoxycarbonylvinylthio]-3-cephem-4-carboxylate (**1b**) (Method A)

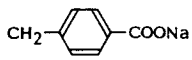
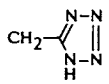
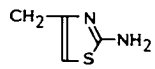
To a mixture of TFA (3.5 ml) and anisole (0.7 ml) was added compound **5b** (237 mg, 0.26 mmol) under ice-cooling, and the reaction mixture was stirred for 40 minutes at the same temperature. Then, the reaction mixture was added dropwise into a mixture of Et_2O and *n*-hexane (1:2, 40 ml). The precipitated TFA salt of the desired product was collected by filtration. Subsequently, the TFA salt (150 mg) was dissolved in water (5 ml) with $NaHCO_3$ (44 mg, 2.0 equiv) and chromatographed on Sephadex LH-20 column (eluent; H_2O), and then lyophilized to afford 110 mg (81%) of **1b** as a white amorphous solid: IR (KBr) cm^{-1} 1760 (β-lactam), 1660; ^{1}H NMR (D_2O) δ 3.57 (1H, d, J=18 Hz, 2-Hα), 3.76 (3H, s, CO_2CH_3), 3.97 (1H, d, J=18 Hz, 2-Hβ), 4.00 (3H, s, =$NOCH_3$), 5.30 (1H, d, J=5 Hz, 6-H), 5.85 (1H, d, J=5 Hz, 7-H), 6.04 (1H, d, J=10 Hz, =$CHCO_2CH_3$), 7.02 (1H, s, thiazole 5-H), 7.31 (1H, d, J=10 Hz, SCH=); HPLC analysis 96% purity.

According to Method A, compounds **1a**, **1c**~**1h** and **1j**~**1l** were prepared from the corresponding cephalosporin derivatives **5**. In the case of **1l**, the crude TFA salt obtained was purified by Sephadex

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



1

Compound No.	R_1	X	^{1}H NMR δ (D_2O)	IR (KBr)[a] cm^{-1}
1a	H	Na	3.57 (1H, d, J=17 Hz), 3.77 (3H, s), 3.97 (1H, d, J=17 Hz), 5.32 (1H, d, J=5 Hz), 5.89 (1H, d, J=5 Hz), 6.04 (1H, d, J=10 Hz), 7.00 (1H, s), 7.32 (1H, d, J=10 Hz)	1770
1c	CH_2CH_2F	Na	3.58 (1H, d, J=17 Hz), 3.77 (3H, s), 3.98 (1H, d, J=17 Hz), 4.50 (2H, dt, J=32, 4 Hz), 4.80 (2H, dt, J=48, 4 Hz), 5.32 (1H, d, J=5 Hz), 5.88 (1H, d, J=5 Hz), 6.06 (1H, d, J=10 Hz), 7.07 (1H, s), 7.34 (1H, d, J=10 Hz)	1760
1d	$CH_2CH{=}CH_2$	Na	3.59 (1H, d, J=18 Hz), 3.79 (3H, s), 4.00 (1H, d, J=18 Hz), 4.77 (2H, d, J=5 Hz), 5.33 (1H, d, J=5 Hz), 5.35 (1H, d, J=11 Hz), 5.41 (1H, d, J=20 Hz), 5.89 (1H, d, J=5 Hz), 6.07 (1H, d, J=11 Hz), 6.10 (1H, m), 7.06 (1H, s), 7.35 (1H, d, J=11 Hz)	1760
1e	CH_2CO_2Et	Na	1.28 (3H, t, J=7 Hz), 3.56 (1H, d, J=17 Hz), 3.77 (3H, s), 3.97 (1H, d, J=17 Hz), 4.29 (2H, q, J=7 Hz), 4.86 (2H, s), 5.31 (1H, d, J=5 Hz), 5.89 (1H, d, J=5 Hz), 6.05 (1H, d, J=10 Hz), 7.10 (1H, s), 7.34 (1H, d, J=10 Hz)	1760
1f	CH_2CONH_2	Na	3.58 (1H, d, J=17 Hz), 3.77 (3H, s), 3.98 (1H, d, J=17 Hz), 4.76 (2H, s), 5.33 (1H, d, J=5 Hz), 5.89 (1H, d, J=5 Hz), 6.06 (1H, d, J=10 Hz), 7.13 (1H, s), 7.34 (1H, d, J=10 Hz)	1760
1g	$C(CH_3)_2CO_2Na$	Na	1.50 (3H, s), 1.52 (3H, s), 3.60 (1H, d, J=17 Hz), 3.78 (3H, s), 3.98 (1H, d, J=17 Hz), 5.33 (1H, d, J=5 Hz), 5.89 (1H, d, J=5 Hz), 6.07 (1H, d, J=10 Hz), 7.02 (1H, s), 7.35 (1H, d, J=10 Hz)	1760
1h		Na	3.42 (1H, d, J=17 Hz), 3.77 (3H, s), 3.86 (1H, d, J=17 Hz), 5.24 (1H, d, J=5 Hz), 5.33 (2H, s), 5.83 (1H, d, J=5 Hz), 6.07 (1H, d, J=10 Hz), 7.04 (1H, s), 7.29 (1H, d, J=10 Hz), 7.54 (2H, d, J=8 Hz), 7.90 (2H, d, J=8 Hz)	1770
1j		Na	(DMSO-d_6) 3.38 (1H, d, J=17 Hz), 3.62 (3H, s), 3.90 (1H, d, J=17 Hz), 5.08 (1H, d, J=5 Hz), 5.23 (2H, s), 5.63 (1H, dd, J=5, 8 Hz), 5.86 (1H, d, J=10 Hz), 6.72 (1H, s), 7.29 (2H, br s), 7.46 (1H, d, J=10 Hz), 10.17 (1H, d, J=8 Hz)	1770
1k		Na	3.50 (1H, d, J=17 Hz), 3.78 (3H, s), 3.94 (1H, d, J=17 Hz), 5.09 (2H, s), 5.25 (1H, d, J=5 Hz), 5.84 (1H, d, J=5 Hz), 6.07 (1H, d, J=10 Hz), 6.78 (1H, s), 7.05 (1H, s), 7.33 (1H, d, J=10 Hz)	1760
1l	$CH_2CH_2NH_2$ (TFA salt)	H	3.42 (2H, t, J=5 Hz), 3.58 (1H, d, J=17 Hz), 3.75 (3H, s), 3.98 (1H, d, J=17 Hz), 4.53 (2H, t, J=5 Hz), 5.32 (1H, d, J=5 Hz), 5.87 (1H, d, J=5 Hz), 6.03 (1H, d, J=10 Hz), 7.18 (1H, s), 7.30 (1H, d, J=10 Hz)	1770

[a] β-Lactam.

LH-20 column (eluent; H_2O). The purities of these compounds were 94~97% by HPLC analysis, and their spectral data are summarized in Table 4.

Sodium 7β-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-phosphonomethoxyimino]acetamido-3-[(*Z*)-2-methoxycarbonylvinylthio]-3-cephem-4-carboxylate (**1i**) (Method B)

Compound **5i** (569 mg, 0.57 mmol) was added to a mixture of TFA (4.0 ml) and anisole (0.8 ml) under ice-cooling, and stirred for 1 hour at the same temperature. Then, the reaction mixture was added dropwise to a mixture of Et_2O and *n*-hexane (1 : 2, 40 ml), and the precipitate TFA salt of 7β-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-*O*,*O*-diethylphosphonomethoxyimino]acetamido]-3-[(*Z*)-2-methoxycarbonylvinylthio]-3-

cephem-4-carboxylic acid (370 mg, 87%) was collected by filtration: IR (KBr) cm^{-1} 1780 (β-lactam), 1670; ^1H NMR (DMSO-d_6) δ 1.25 (6H, t, $J=7$ Hz, $PO(OCH_2CH_3)_2$), 3.65 (3H, s, CO_2CH_3), 3.68 (1H, d, $J=18$ Hz, 2-Hα), 4.00~4.17 (5H, m, $PO(OCH_2CH_3)_2$ and 2-Hβ), 4.50 (2H, d, $J=7$ Hz, $=NOCH_2$), 5.23 (1H, d, $J=5$ Hz, 6-H), 5.81 (1H, dd, $J=5$ and 8 Hz, 7-H), 6.03 (1H, d, $J=10$ Hz, $=CHCO_2CH_3$), 6.80 (1H, s, thiazole 5-H), 7.51 (1H, d, $J=10$ Hz, SCH=), 9.76 (1H, d, $J=8$ Hz, CONH).

Subsequently, the above TFA salt (150 mg, 0.2 mmol) was dissolved in dry dichloromethane (10 ml), and *N,O*-bis-trimethylsilylacetamide (0.25 ml, 5.0 equiv) was added to the solution at room temperature. Then the mixture was stirred for 20 minutes. To the mixture was added trimethylsilylbromide (0.13 ml, 5.0 equiv), and stirred for 6 hours. After the reaction, the solvent was evaporated *in vacuo*, and the resulting residue was dissolved in water (5 ml), and then the aqueous solution was adjusted to pH 8 with $NaHCO_3$. After filtration over Celite, the filtrate was chromatographed on Sephadex LH-20 column (eluent; H_2O), and lyophilized to afford 70 mg (54%) of **1i** as an amorphous solid: IR (KBr) cm^{-1} 1760 (β-lactam), 1600; ^1H NMR (D_2O) δ 3.58 (1H, d, $J=18$ Hz, 2-Hα), 3.76 (3H, s, CO_2CH_3), 3.98 (1H, d, $J=18$ Hz, 2-Hβ), 4.27 (2H, d, $J=7$ Hz, $=NOCH_2$), 5.31 (1H, d, $J=5$ Hz, 6-H), 5.85 (1H, d, $J=5$ Hz, 7-H), 6.05 (1H, d, $J=10$ Hz, $=CHCO_2CH_3$), 7.02 (1H, s, thiazole 5-H), 7.33 (1H, d, $J=10$ Hz, SCH=); HPLC analysis: 95% purity.

References

1) YOKOO, C.; M. GOI, A. ONODERA, M. MURATA, T. NAGATE, Y. WATANABE & K. SOTA: Studies on cephalosporin antibiotics. I. Synthesis, antibacterial activity and oral absorption of new 3-(*O*-substituted)-7β-[D-α-amino-α-(4-hydroxyphenyl)acetamido]cephalosporins. J. Antibiotics 41: 170~180, 1988
2) YOKOO, C.; M. GOI, A, ONODERA, M. MURATA, T. NAGATE, Y. WATANABE & K. SOTA: Studies on cephalosporin antibiotics. II. Synthesis, antibacterial activity and oral absorption of 3-alkoxycarbonylmethoxy-7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(*O*-substituted oxyimino)acetamido]cephalosporins. J. Antibiotics 41: 181~192, 1988
3) YOKOO, C.; M. GOI, A. ONODERA, M. MURATA, T. NAGATE & Y. WATANABE: Studies on cephalosporin antibiotics. III. Synthesis, antibacterial activity and oral absorption of new 3-(substituted-alkylthio)-7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]cephalosporins. J. Antibiotics 44: 498~506, 1991
4) YOKOO, C.; M. GOI, A. ONODERA, H. FUKUSHIMA & T. NAGATE: Studies on cephalosporin antibiotics. IV. Synthesis, antibacterial activity and oral absorption of new 3-(2-substituted-vinylthio)-7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]cephalosporins. J. Antibiotics 44: 1422~1431, 1991
5) YAMANAKA, H.; T. CHIBA, K. KAWABATA, H. TAKASUGI, T. MASUGI & T. TAKAYA: Studies on β-lactam antibiotics. IX. Synthesis and biological activity of a new orally active cephalosporin, cefixime (FK027). J. Antibiotics 38: 1738~1751, 1985
6) WATANABE, Y.; C. YOKOO, M. GOI, A. ONODERA, M. MURATA, H. FUKUSHIMA & K. SOTA (Taisho): Cephalosporin derivatives. U.S. 4,699,981, Oct. 13, 1987
7) INAMOTO, Y.; T. CHIBA, K. SAKANE, T. KAMIMURA & T. TAKAYA: Studies on FK-482. II. Synthesis and structure-activity relationships of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-substituted acetamido]-3-vinyl-3-cephem-4-carboxylic acid derivatives. Yakugaku Zasshi (Japanese) 110: 246~257, 1990
8) FINNEY, D. J.: The maximum likelihood solution. *In* Probit Analysis. 2nd Ed. *Ed.*, D. J. FINNEY, pp. 48~64, Cambridge University Press, 1952