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

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The synthesis, antibacterial activity and oral absorption in rats of new 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]cephalosporins (**1**) having various substituted-alkylthio groups at the C-3 position of the cephem nucleus are described. Of these, the cephalosporins with a cyanomethylthio group (**1d**) and fluoroethylthio group (**1p**) at the C-3 position showed a potent *in vitro* antibacterial activity against Gram-positive and Gram-negative bacteria as well as good oral absorption in rats. When administered orally to mice infected with *Klebsiella pneumoniae*, **1d** had stronger protective effect than **1p**. The structure-activity relationships of **1** are also presented.

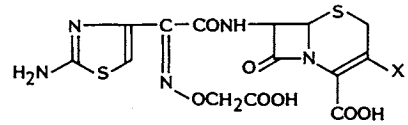
In recent years, research on cephalosporin antibiotics having an aminothiazole-oxime moiety at the C-7 position of the cephem nucleus, so-called third generation cephalosporins, has been undertaken extensively due to their potent antibacterial activity and remarkable stability toward bacterial β-lactamases. However, there have been few reports[1~3] on the analogues possessing a hetero-atom attached directly to the C-3 position.

In the course of our research program directed toward orally administered third generation cephalosporins such as cefixime[4] and cefteram pivoxil[5], we have already found that 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]cephalosporins with an alkoxycarbonylmethoxy group at the C-3 position of the cephem nucleus, represented by **2** as shown in Fig. 1, display good oral absorption in rats as well as potent antibacterial activities against Gram-negative bacteria[6].

In connection with that work, we then designed the new analogues (**1**) (Fig. 1) having various substituted-alkylthio groups at the C-3 position, including the 3-thio congener of **2**, in order to improve the activity of **2** against Staphylococci.

This paper describes the synthesis of **1**, and the effects of the new *S*-substituents at the C-3 position on antibacterial activity and oral absorption in rats.

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

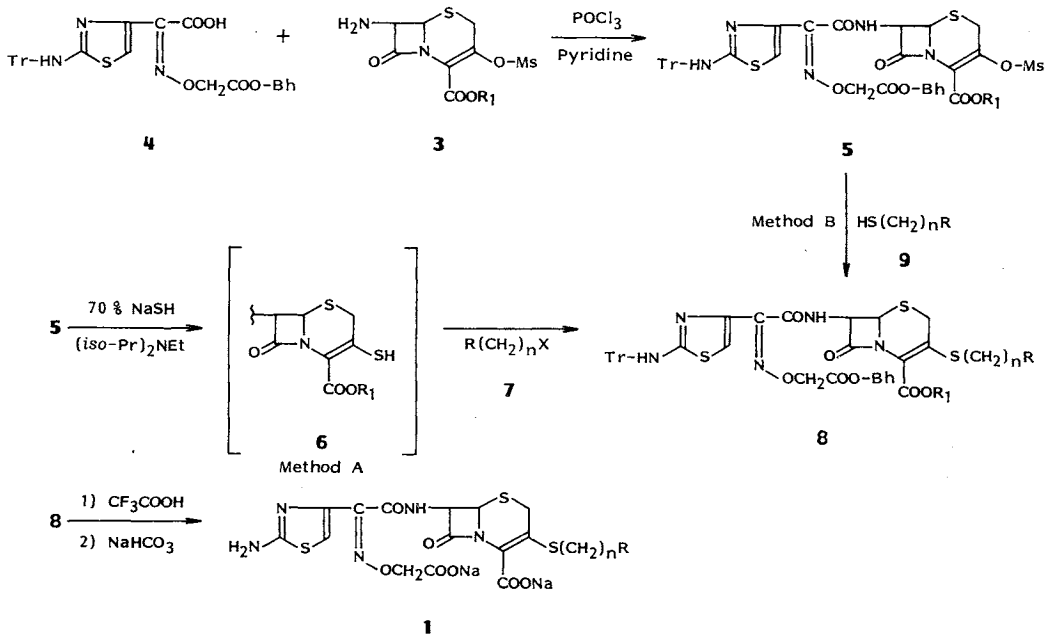


1	$X=-S(CH_2)_nR$
2	$X=-OCH_2COOCH_3$
Cefixime	$X=-CH=CH_2$

Chemistry

The new cephalosporins (**1a**~**1q**) were synthesized by the route as shown in Scheme 1. Diphenylmethyl (Bh) or *p*-methoxybenzyl (PMB) 7β-amino-3-methanesulfonyloxycephalosporinate (**3**)[7] was coupled with the protected 2-aminothiazole-4-acetic acid derivative (**4**) in the presence of

Scheme 1.



Tr=$-CPh_3$, Ms=$-SO_2CH_3$, Bh=$-CHPh_2$, X=Halogen, R_1=Bh or PMB, n=1 or 2.

phosphorus oxychloride and pyridine in THF to afford the 7β-acylaminocephalosporin derivative (**5**). Subsequently, compound **5** was reacted with sodium hydrosulfide in DMF to give the 3-mercaptocephalosporin (**6**). After isolation or without isolation of **6**, the 3-mercapto group in **6** was alkylated with various alkyl halides (**7**) in the presence of *N,N*-diisopropylethylamine as a base to yield the protected 3-substituted-alkylthio cephalosporins (**8**) (Method A). In this way, there was no formation of the Δ^2-isomer of **8**.

Compound **8** was also prepared from **5** by the alternative route using alkyl mercaptans (**9**) (Method B). However, considerable amounts of the Δ^2-isomer were formed during the reaction[8].

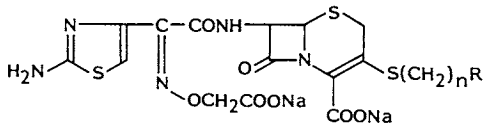
Finally, the protecting groups in **8** were removed by the conventional method using TFA and anisole to afford the desired cephalosporin derivatives (**1**).

Antibacterial Activity and Oral Absorption

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

Against *Staphylococcus aureus* 209P JC-1, the cephalosporins having the cyanomethylthio group (**1d**), benzylthio group (**1h**), or fluoroethyl group (**1p**) at the C-3 position of the cephem nucleus showed more potent activity than the others. And their activities were 4 to 8 times higher than those of cefixime and **1r**[1,2] having an unsubstituted-alkylthio group ($-SCH_3$) at the C-3 position. Judging from the comparison of the activity between **2** having a methoxycarbonylmethoxy group at the C-3 position and the corresponding 3-thio congener (**1a**), it is clear that the sulfur atom directly attached to the C-3 position of the cephem nucleus plays an important role to enhance the antibacterial activity against *S. aureus*, probably due to

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



1

Compound No.	R	n	MIC (μg/ml, 10^6 cfu/ml)[a] *S.a.*	*E.c.*	*K.p.*	*M.m.*	*S.m.*	Peak serum level (μg/ml)[b] po, 50 mg/kg rats ($n=3$)
1a	$COOCH_3$	1	25	0.78	≦0.1	≦0.1	1.56	8.7
1b	$CONH_2$	1	25	1.56	≦0.1	≦0.1	0.78	11.9
1c	$CH(NH_2)COOH$ (D)	1	>400	50	1.56	100	50	NT
1d	CN	1	6.25	1.56	≦0.1	≦0.1	0.78	28.7
1e	$CH=CH_2$	1	25	1.56	≦0.1	≦0.1	0.78	15.6
1f	CF_3	1	12.5	1.56	≦0.1	0.2	1.56	16.0
1g	SCH_3	1	12.5	1.56	≦0.1	≦0.1	1.56	10.8
1h	Ph	1	6.25	1.56	≦0.1	0.2	1.56	10.3
1i	Pyridin-2-yl	1	12.5	0.39	≦0.1	≦0.1	0.78	10.6
1j	2-Aminothiazol-4-yl	1	12.5	0.39	≦0.1	≦0.1	0.78	3.0
1k	$COOCH_3$	2	50	0.78	≦0.1	≦0.1	1.56	2.4
1l	CN	2	12.5	0.78	≦0.1	≦0.1	0.78	14.7
1m	OH	2	50	1.56	≦0.1	0.2	3.13	6.7
1n	OCH_3	2	25	0.78	≦0.1	≦0.1	1.56	20.8
1o	$OCONH_2$	2	12.5	0.39	≦0.1	≦0.1	0.78	6.1
1p	F	2	6.25	1.56	≦0.1	≦0.1	1.56	25.6
1q	NHAc	2	25	0.78	≦0.1	0.2	0.78	0.7
1r	H[c]	1	50	1.56	≦0.1	0.39	1.56	30.4
2	$(3\text{-}OCH_2COOCH_3)$[c]		>400	1.56	≦0.1	0.2	1.56	24.4
Cefixime[c]			25	0.78	≦0.1	0.2	0.78	30.2

[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 **1r**, **2** and cefixime see ref 1 or 2, 6 and 4, respectively.

NT: Not tested.

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.

the higher lipophilicity of the sulfur atom than that of the oxygen atom.

On the other hand, against all the Gram-negative bacteria, these new compounds except **1c** with an amino acid group at the C-3 position exhibited potent antibacterial activity comparable to cefixime and compound **2**.

Most of these new compounds displayed good oral absorption in rats. In particular, compounds **1d** and **1p** showed high peak serum levels after oral administration, and the peak serum level of **1d** was almost equal to that of cefixime. Contrary to our expectation, the oral absorption of **1a** was much lower than that of the 3-oxy congener **2** in rats.

The representative compounds in this series (**1d** and **1p**) possessing the potent *in vitro* antibacterial activity and good oral absorption in rats were then advanced to a preliminary *in vivo* efficacy trial by oral administration. As shown in Table 2, the two compounds, particularly **1d**, showed good *in vivo* efficacy against a systemic infection in mice induced by *Klebsiella pneumoniae* 6, though less potent than that of

cefixime.

In this study, we found some new orally administered cefixime type cephalosporins with improved activity against *S. aureus* by introduction of a substituted-alkylthio group into the C-3 position. Further chemical modifications of the C-3 position of the cephalosporins are now under study to enhance still more the activity against *Staphylococci.*

Table 2. *In vivo* antibacterial activity of **1d** and **1p** against systemic infections in mice induced by *Klebsiella pneumoniae* 6.

Compound	ED_{50} (mg/kg)[a]	MIC (μg/ml)[b]
1d	1.09 (0.49~1.95)	0.05
1p	3.20 (1.62~6.03)	≦0.025
Cefixime	0.59 (0.22~1.23)	≦0.025

Drugs were administered orally 1 hour after infection.
Infective challenge dose: 2.9×10^7 cfu/mouse, ip, (5% mucin).
Mouse: Male ICR strain, 4 weeks, 10 mice/group.
[a] Probit method (95% confidence limits).
[b] Inoculum size: 10^6 cfu/ml.

Experimental

MP's were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. 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. Most chromatographic separations were done using Wako Silica gel C-200 (100~200 mesh, Wako, Japan) or Sephadex LH-20 (Pharmacia, Sweden).

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 of 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[9].

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. 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*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-methanesulfonyloxy-3-cephem-4-carboxylate (**5a**)

To a solution of 7β-amino-3-methanesulfonyloxycephalosporanic acid Bh ester **3a**[7] (2.65 g, 5.76 mM) in THF (80 ml) was added 2-(2-tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetic acid (**4**) (4.14 g, 1.1 equiv) and pyridine (1.37 g, 3.0 equiv) under ice-cooling. After being stirred at -10°C, phosphorus oxychloride (1.76 g, 2.0 equiv) was added dropwise to the solution, and the mixture was stirred at $-10 \sim -5$°C for 20 minutes. Then, brine (100 ml) was added to the mixture and extracted with EtOAc (100 ml). The extract was washed successively with 0.5% aq $NaHCO_3$ (50 ml) and brine (100 ml), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; EtOAc-*n*-hexane, 1 : 1) to afford 4.56 g (72%) of **5a** as an amorphous solid: IR (KBr) cm^{-1} 1795 (β-lactam), 1736, 1692; ^{1}H NMR (DMSO-d_6) δ 3.18 (3H, s, SO_2CH_3), 3.65 (1H, d, $J=17$ Hz, 2-H$_\alpha$), 3.97 (1H, d, $J=17$ Hz, 2-H$_\beta$), 4.82 (2H, br s, $=NOCH_2$), 5.31 (1H, d, $J=5$ Hz, 6-H), 5.86 (1H, dd, $J=5$ and 8 Hz, 7-H), 6.75 (1H, s, thiazole 5-H), 6.89 (1H, s, C*H*Ph$_2$), 6.91 (1H, s, C*H*Ph$_2$), 7.17~7.53 (35H, m, aromatic H), 8.90 (1H, br s, TrN*H*), 9.70 (1H, d, $J=8$ Hz, CONH); FAB-MS m/z 1,096 $(M+H)^+$.

p-Methoxybenzyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-methanesulfonyloxy-3-cephem-4-carboxylate (**5b**)

This compound was prepared from **3b** (PMB ester) in 75% yield as described for **5a** from **3a**. The

spectral data of **5b** are as follows: IR (KBr) cm^{-1} 1794 (β-lactam), 1735, 1688; ^1H NMR (DMSO-d_6) δ 3.36 (3H, s, SO_2CH_3), 3.60 (1H, d, J=18 Hz, 2-H_α), 3.74 (3H, s, OCH_3), 3.96 (1H, d, J=18 Hz, 2-H_β), 4.82 (2H, br s, $=NOCH_2$), 5.11 and 5.23 (2H, ABq, J=12 Hz, OCH_2Ph), 5.26 (1H, d, J=5 Hz, 6-H), 5.80 (1H, dd, J=5 and 8 Hz, 7-H), 6.74 (1H, s, thiazole 5-H), 6.88 (1H, s, $CHPh_2$), 6.92 (2H, d, J=9 Hz, aromatic H), 7.10~7.60 (27H, m, aromatic H), 8.89 (1H, br s, TrN*H*), 9.65 (1H, d, J=8 Hz, CONH); FAB-MS m/z 1,050 $(M+H)^+$.

Diphenylmethyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-cyanomethylthio-3-cephem-4-carboxylate (**8d**) (Method A)

To a solution of **5a** (500 mg, 0.46 mM) in DMF (5 ml) were added 70% sodium hydrosulfide (41 mg, 1.1 equiv) and *N*,*N*-diisopropylethylamine (89 mg, 1.5 equiv) dissolved in DMF (2 ml) at −5°C. After being stirred for 20 minutes at the same temperature, chloroacetonitrile (69 mg, 2.0 equiv) was added to the solution at 0°C, and then stirred for 20 minutes at the same temperature. Subsequently, 0.5% HCl (10 ml) was added to the reaction mixture and extracted with EtOAc (20 ml). The extract was washed with 0.5% aq $NaHCO_3$ (10 ml) and brine (20 ml), successively, and then dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; EtOAc-*n*-hexane, 1 : 1) and crystallized from MeOH to give 385 mg (78%) of **8d**: MP 129~131°C; IR (KBr) cm^{-1} 2315 (nitrile), 1780 (β-lactam), 1730, 1680; ^1H NMR ($CDCl_3$) δ 3.21 and 3.40 (2H, ABq, J=17 Hz, SCH_2CN), 3.42 (1H, d, J=17 Hz, 2-H_α), 3.66 (1H, d, J=17 Hz, 2-H_β), 4.93 and 5.05 (2H, ABq, J=16 Hz, $=NOCH_2$), 5.06 (1H, d, J=5 Hz, 6-H), 5.95 (1H, dd, J=5 and 9 Hz, 7-H), 6.81 (1H, s, thiazole 5-H), 6.98 (1H, s, $CHPh_2$), 6.99 (1H, s, $CHPh_2$), 7.02 (1H, br s, TrN*H*), 7.20~7.55 (35H, m, aromatic H), 8.17 (1H, d, J=9 Hz, CONH); FAB-MS m/z 1,073 $(M+H)^+$.

Anal Calcd for $C_{61}H_{48}N_6O_7S_3$: C 68.26, H 4.51, N 7.83.
Found: C 67.96, H 4.46, N 7.66.

Compounds **8a**, **8b**, **8e~8n**, **8p** and **8q** were similarly prepared from **5a** or **5b** with a corresponding substituted-alkyl halide **7** according to the procedure described for **8d**. Their spectral data are summarized in Table 3.

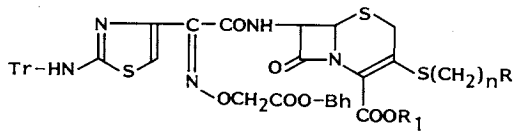
Diphenylmethyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-mercapto-3-cephem-4-carboxylate (**6**)

After the reaction of **5a** with sodium hydrosulfide according to the procedure for **8d**, water was added to the reaction mixture and washed with Et_2O. The aqueous layer was adjusted to pH 2 with 0.5% HCl and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$) and evaporated. The residue was dissolved in a small amount of CH_2Cl_2, and added dropwise to isopropyl ether with stirring. The precipitate formed was collected by filtration to give **6** (85%) as a pale yellow powder: IR (KBr) cm^{-1} 1785 (β-lactam), 1733, 1687; ^1H NMR (DMSO-d_6) δ 3.73 (2H, br s, 2-$H_{\alpha,\beta}$), 4.85 (2H, br s, $=NOCH_2$), 5.26 (1H, d, J=5 Hz, 6-H), 5.68 (1H, dd, J=5 and 8 Hz, 7-H), 6.85 (thiazole 5-H), 6.86 (1H, s, $CHPh_2$), 6.90 (1H, s, $CHPh_2$), 7.16~7.61 (35H, m, aromatic H), 8.93 (1H, br s, TrN*H*), 9.58 (1H, d, J=8 Hz, CONH); FAB-MS m/z 1,034 $(M+H)^+$.

Diphenylmethyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-[2*R*-(*N*-*tert*-butoxycarbonylamino)-2-diphenylmethoxycarbonylethylthio]-3-cephem-4-carboxylate (**8c**) (Method B)

To a solution of **5a** (300 mg, 0.27 mM) in CH_3CN (5 ml) were added D-*N*-(*tert*-Boc)cystein diphenylmethyl ester **9c** (138 mg, 1.3 equiv) and *N*,*N*-diisopropylethylamine (40 mg, 1.1 equiv) under ice-cooling. After stirring for 2 hours at the same temperature, 0.5% HCl (5 ml) was added to the reaction mixture and extracted with EtOAc (10 ml). The extract was washed with brine (10 ml), and then dried ($MgSO_4$) and evaporated. The residue was chromatographed on a Lobar column packed with LiChroprep Si 60 (size B, 40~63 μm, Merck), (eluent; acetone-*n*-hexane, 2:3) to give 112 mg (30%) of the title compound **8c** and the corresponding Δ^2-isomer (38 mg, 10%). **8c**: IR (KBr) cm^{-1} 1780 (β-lactam), 1710, 1490; ^1H NMR ($CDCl_3$) δ 1.40 (9H, s, $C(CH_3)_3$), 3.09 (1H, d, J=17 Hz, 2-H_α), 3.15 (2H, d, J=5 Hz, SCH_2), 3.36 (1H, d, J=17 Hz, 2-H_β), 4.52~4.62 (1H, m, CHNHBoc), 4.76 (1H, d, J=5 Hz, 6-H), 4.91

Table 3. ^{1}H NMR, MS and IR spectral data of **8**.



8

Compound No.	R/R_1	n	^{1}H NMR, δ ($CDCl_3$)	MS (m/z)[a]	IR (KBr) cm^{-1}
8a	$COOCH_3$/Bh	1	3.32 (1H, d, J=17 Hz), 3.34 (1H, d, J=15 Hz), 3.45 (1H, d, J=15 Hz), 3.60 (1H, d, J=17 Hz), 3.68 (3H, s), 4.94 (1H, d, J=17 Hz), 5.03 (1H, J=5 Hz), 5.06 (1H, d, J=17 Hz), 5.87 (1H, dd, J=5, 9 Hz), 6.83 (1H, s), 6.95 (1H, s), 6.97 (1H, s), 7.01 (1H, br s), 7.14~7.54 (35H, m), 8.14 (1H, d, J=9 Hz)	1,106	1,780, 1,730, 1,685
8b	$CONH_2$/Bh	1	3.30 (1H, d, J=17 Hz), 3.35 (2H, br s), 3.51 (1H, d, J=17 Hz), 4.92 (1H, d, J=17 Hz), 5.02 (1H, d, J=5 Hz), 5.03 (1H, d, J=17 Hz), 5.33 (1H, br s), 5.87 (1H, dd, J=5, 9 Hz), 6.38 (1H, br s), 6.80 (1H, s), 6.96 (2H, s), 7.02 (1H, br s), 7.24~7.50 (35H, m), 8.12 (1H, d, J=9 Hz)	1,091	1,785, 1,730, 1,685
8e	$CH=CH_2$/Bh	1	3.25 (1H, d, J=17 Hz), 3.26~3.40 (2H, m), 3.40 (1H, d, J=17 Hz), 4.94 (1H, d, J=17 Hz), 5.01 (1H, d, J=5 Hz), 5.06 (1H, d, J=17 Hz), 5.10~5.22 (2H, m), 5.64~5.77 (1H, m), 5.81 (1H, dd, J=5, 9 Hz), 6.84 (1H, s), 6.96 (2H, s), 7.01 (1H, br s), 7.23~7.55 (35H, m), 8.15 (1H, d, J=9 Hz)	1,074	1,780, 1,735, 1,685
8f	CF_3/Bh	1	3.00~3.30 (2H, m), 3.26 (1H, d, J=17 Hz), 3.53 (1H, d, J=17 Hz), 4.91 (1H, d, J=17 Hz), 5.03 (1H, d, J=5 Hz), 5.05 (1H, d, J=17 Hz), 5.91 (1H, dd, J=5, 9 Hz), 6.81 (1H, s), 6.96 (1H, s), 7.00 (2H, s), 7.14~7.50 (35H, m), 8.12 (1H, d, J=9 Hz)	1,116	1,790, 1,735, 1,690
8g	SCH_3/Bh	1	2.09 (3H, s), 3.39 (1H, d, J=17 Hz), 3.60 (1H, d, J=17 Hz), 3.65 (1H, d, J=13 Hz), 3.77 (1H, d, J=13 Hz), 4.94 (1H, d, J=17 Hz), 5.05 (1H, d, J=5 Hz), 5.06 (1H, d, J=17 Hz), 5.87 (1H, dd, J=5, 9 Hz), 6.84 (1H, s), 6.97 (2H, s), 7.01 (1H, br s), 7.16~7.53 (35H, m), 8.14 (1H, d, J=9 Hz)	1,094	1,780, 1,725, 1,680
8h	Ph/Bh	1	3.16 (1H, d, J=17 Hz), 3.30 (1H, d, J=17 Hz), 3.82 (1H, d, J=13 Hz), 3.89 (1H, d, J=13 Hz), 4.91 (1H, d, J=17 Hz), 4.91 (1H, d, J=5 Hz), 5.04 (1H, d, J=17 Hz), 5.81 (1H, dd, J=5, 9 Hz), 6.81 (1H, s), 6.96 (2H, s), 7.00 (1H, br s), 7.19~7.52 (40H, m), 8.16 (1H, d, J=9 Hz)	1,124	1,775, 1,730, 1,675
8i	Pyridin-2-yl/Bh	1	3.29 (1H, d, J=17 Hz), 3.64 (1H, d, J=17 Hz), 3.96 (1H, d, J=14 Hz), 4.08 (1H, d, J=14 Hz), 4.92 (1H, d, J=5 Hz), 4.93 (1H, d, J=17 Hz), 5.05 (1H, d, J=17 Hz), 5.79 (1H, dd, J=5, 8 Hz), 5.82 (1H, s), 6.93 (1H, s), 6.95 (1H, s), 7.00 (1H, br s), 7.10~7.66 (38H, m), 8.11 (1H, d, J=8 Hz), 8.47 (1H, d, J=5 Hz)	1,125	1,775, 1,730, 1,675
8j	2-Tritylaminothiazol-4-yl/Bh	1	3.09 (1H, d, J=17 Hz), 3.38 (1H, d, J=17 Hz), 3.60 (1H, d, J=14 Hz), 3.73 (1H, d, J=14 Hz), 4.70 (1H, d, J=5 Hz), 4.90 (1H, d, J=16 Hz), 5.04 (1H, d, J=16 Hz), 5.77 (1H, dd, J=5, 9 Hz), 6.00 (1H, s), 6.53 (1H, br s), 6.84 (1H, s), 6.94 (2H, s), 7.00 (1H, br s), 7.18~7.54 (50H, m), 8.13 (1H, d, J=9 Hz)	1,388	1,780, 1,735, 1,680

Table 3. (Continued)

Compound No.	R/R_1	n	^{1}H NMR, δ ($CDCl_3$)	MS (m/z)[a]	IR (KBr) cm^{-1}
8k	$COOCH_3$/Bh	2	2.53 (2H, t, J=7 Hz), 2.93 (2H, t, J=7 Hz), 3.27 (1H, d, J=17 Hz), 3.44 (1H, d, J=17 Hz), 3.68 (3H, s), 4.94 (1H, d, J=17 Hz), 5.03 (1H, d, J=5 Hz), 5.06 (1H, d, J=17 Hz), 5.83 (1H, dd, J=5, 8 Hz), 6.83 (1H, s), 6.96 (2H, s), 7.01 (1H, br s), 7.25~7.62 (35H, m), 8.19 (1H, d, J=8 Hz)	1,120	1,780, 1,730, 1,680
8l	CN/Bh	2	2.40 (2H, t, J=7 Hz), 2.78 (2H, t, J=7 Hz), 3.20 (1H, d, J=17 Hz), 3.45 (1H, d, J=17 Hz), 4.92 (1H, d, J=17 Hz), 5.03 (1H, d, J=5 Hz), 5.05 (1H, d, J=17 Hz), 5.85 (1H, dd, J=5, 9 Hz), 6.80 (1H, s), 6.96 (1H, s), 6.98 (1H, s), 7.00 (1H, br s), 7.22~7.48 (35H, m), 8.12 (1H, d, J=9 Hz)	1,087	1,780, 1,730, 1,680
8m	OH/PMB	2	2.70~2.96 (2H, m), 3.30 (1H, d, J=18 Hz), 3.50 (1H, d, J=18 Hz), 3.60~3.78 (2H, m), 3.80 (3H, s), 4.93 (1H, d, J=16 Hz), 5.01 (1H, d, J=5 Hz), 5.04 (1H, d, J=16 Hz), 5.22 (1H, d, J=12 Hz), 5.29 (1H, d, J=12 Hz), 5.85 (1H, dd, J=5, 9 Hz), 6.79 (1H, s), 6.90 (2H, d, J=9 Hz), 6.98 (1H, s), 7.02 (1H, br s), 7.16~7.48 (27H, m), 8.10 (1H, d, J=9 Hz)	1,032	1,775, 1,725, 1,675
8n	OCH_3/Bh	2	2.84 (2H, t, J=6 Hz), 3.26 (1H, d, J=17 Hz), 3.29 (3H, s), 3.46 (1H, d, J=17 Hz), 3.48 (2H, t, J=6 Hz), 4.94 (1H, d, J=17 Hz), 5.03 (1H, d, J=5 Hz), 5.06 (1H, d, J=17 Hz), 5.80 (1H, dd, J=5, 8 Hz), 6.84 (1H, s), 6.96 (2H, s), 7.00 (1H, br s), 7.12~7.53 (35H, m), 8.16 (1H, d, J=8 Hz)	1,092	1,780, 1,730, 1,680
8p	F/Bh	2	2.80~3.03 (2H, m), 3.25 (1H, d, J=17 Hz), 3.44 (1H, d, J=17 Hz), 4.28~3.40 (1H, m), 4.50~4.62 (1H, m), 4.94 (1H, d, J=17 Hz), 5.04 (1H, d, J=5 Hz), 5.06 (1H, d, J=17 Hz), 5.84 (1H, dd, J=5, 9 Hz), 6.84 (1H, s), 6.96 (2H, s), 7.00 (1H, br s), 7.23~7.52 (35H, m), 8.16 (1H, d, J=9 Hz)	1,080	1,785, 1,735, 1,685
8q	NHAc/Bh	2	1.83 (3H, s), 2.62~2.93 (2H, m), 3.07~3.48 (3H, m), 3.50 (1H, d, J=17 Hz), 4.94 (1H, d, J=17 Hz), 5.04 (1H, d, J=17 Hz), 5.06 (1H, d, J=5 Hz), 5.87 (1H, dd, J=5, 9 Hz), 6.37 (1H, t, J=6 Hz), 6.81 (1H, s), 6.96 (1H, s), 6.98 (1H, s), 7.03 (1H, br s), 7.10~7.48 (35H, m), 8.12 (1H, d, J=9 Hz)	1,119	1,775, 1,725, 1,660

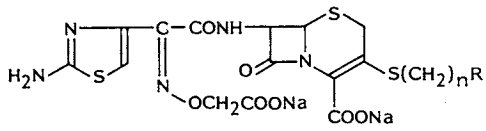
[a] $(M+H)^+$.

and 5.04 (2H, ABq, J=17 Hz, =$NOCH_2$), 5.38 (1H, d, J=8 Hz, BocN*H*), 5.79 (1H, dd, J=5 and 9 Hz, 7-H), 6.82 (1H, s, thiazole 5-H), 6.83 (1H, s, C*H*Ph_2), 6.94 (2H, s, C*H*Ph_2×2), 7.00 (1H, br s, TrN*H*), 7.16~7.52 (45H, m, aromatic H), 8.12 (1H, d, J=9 Hz, CONH); FAB-MS m/z 1,387 $(M+H)^+$.

p-Methoxybenzyl 7*β*-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-(2-carbamoyloxyethylthio)-3-cephem-4-carboxylate (**8o**) (*via* **8m**)

To a solution of the 3-(2-hydroxyethylthio) derivative **8m** (400 mg, 0.39 mM) in CH_2Cl_2 (10 ml) was added chlorosulfonyl isocyanate (0.044 ml, 1.2 equiv) at −30°C, and the reaction mixture was stirred for 15 minutes at −10°C. Then, 20% aq Na_2SO_3 (5 ml) was added to the mixture and stirred for 1 hour at room temperature. The separated organic layer was washed with brine (10 ml×3), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; benzene-acetone, 7:1) to yield 240 mg (58%) of **8o** as an amorphous solid: IR (KBr) cm^{-1} 3240, 1775 (*β*-lactam), 1700, 1510; ^{1}H NMR ($CDCl_3$) δ 2.92 (2H, t, J=6 Hz, 3-SCH_2), 3.30 (1H, d, J=17 Hz, 2-H_α), 3.52 (1H, d, J=17 Hz, 2-H_β), 3.80 (3H, s, OCH_3), 4.16 (2H, t, J=6 Hz, SCH_2CH_2O), 4.72 (2H, br s, $CONH_2$), 4.95

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



1

Compound No.	R	n	^{1}H NMR, δ (D_2O)	IR (KBr)[a] cm^{-1}
1a	$COOCH_3$	1	3.56 (1H, d, J=17 Hz), 3.57 (1H, d, J=15 Hz), 3.68 (1H, d, J=15 Hz), 3.76 (3H, s), 3.86 (1H, d, J=17 Hz), 4.60 (2H, s), 5.27 (1H, d, J=5 Hz), 5.84 (1H, d, J=5 Hz), 7.08 (1H, s)	1,755
1b	$CONH_2$	1	3.52 (2H, s), 3.56 (1H, d, J=17 Hz), 3.82 (1H, d, J=17 Hz), 4.60 (2H, s), 5.28 (1H, d, J=5 Hz), 5.85 (1H, d, J=5 Hz), 7.07 (1H, s)	1,760
1c	$CH(NH_2)COONa$ (D)	1	2.91 (1H, dd, J=11, 15 Hz), 3.45 (1H, dd, J=4, 15 Hz), 3.56~3.62 (2H, m), 3.80 (1H, d, J=17 Hz), 4.59 (2H, s), 5.31 (1H, d, J=5 Hz), 5.85 (1H, d, J=5 Hz), 7.08 (1H, s)	1,755
1e	$CH=CH_2$	1	3.34~3.54 (2H, m), 3.54 (1H, d, J=17 Hz), 3.79 (1H, d, J=17 Hz), 4.59 (2H, s), 5.10~5.28 (2H, m), 5.25 (1H, d, J=5 Hz), 5.82 (1H, d, J=5 Hz), 5.78~6.00 (1H, m), 7.08 (1H, s)	1,755
1f	CF_3	1	3.30~3.68 (2H, m), 3.58 (1H, d, J=17 Hz), 3.91 (1H, d, J=17 Hz), 4.60 (2H, s), 5.28 (1H, d, J=5 Hz), 5.85 (1H, d, J=5 Hz), 7.08 (1H, s)	1,760
1g	SCH_3	1	2.20 (3H, s), 3.61 (1H, d, J=17 Hz), 3.88 (1H, d, J=14 Hz), 3.89 (1H, d, J=17 Hz), 4.00 (1H, d, J=14 Hz), 4.60 (2H, s), 5.29 (1H, d, J=5 Hz), 5.84 (1H, d, J=5 Hz), 7.08 (1H, s)	1,760
1h	Ph	1	3.27 (1H, d, J=17 Hz), 3.52 (1H, d, J=17 Hz), 3.97 (1H, d, J=12 Hz), 4.07 (1H, d, J=12 Hz), 4.58 (2H, s), 5.11 (1H, d, J=5 Hz), 5.78 (1H, d, J=5 Hz), 7.06 (1H, s), 7.30~7.47 (5H, m)	1,755
1i	Pyridin-2-yl	1	3.16 (1H, d, J=17 Hz), 3.54 (1H, d, J=17 Hz), 4.03 (1H, d, J=13 Hz), 4.14 (1H, d, J=13 Hz), 4.58 (2H, s), 5.13 (1H, d, J=5 Hz), 5.79 (1H, d, J=5 Hz), 7.06 (1H, s), 7.32~7.42 (1H, m), 7.50 (1H, d, J=8 Hz), 7.86 (1H, t, J=8 Hz), 8.47 (1H, d, J=5 Hz)	1,750
1j	2-Aminothiazol-4-yl	1	3.14 (1H, d, J=17 Hz), 3.57 (1H, d, J=17 Hz), 3.75 (1H, d, J=14 Hz), 3.90 (1H, d, J=14 Hz), 4.59 (2H, s), 5.18 (1H, d, J=5 Hz), 5.80 (1H, d, J=5 Hz), 6.54 (1H, s), 7.07 (1H, s)	1,755
1k	$COOCH_3$	2	2.72 (2H, t, J=7 Hz), 3.02 (2H, t, J=7 Hz), 3.54 (1H, d, J=17 Hz), 3.72 (3H, s), 3.84 (1H, d, J=17 Hz), 4.59 (2H, s), 5.27 (1H, d, J=5 Hz), 5.83 (1H, d, J=5 Hz), 7.08 (1H, s)	1,755
1l	CN	2	2.80 (2H, t, J=7 Hz), 3.04 (2H, m), 3.56 (1H, d, J=17 Hz), 3.88 (1H, d, J=17 Hz), 4.59 (2H, s), 5.29 (1H, d, J=5 Hz), 5.85 (1H, d, J=5 Hz), 7.08 (1H, s)	1,755
1m	OH	2	2.77~3.08 (2H, m), 3.58 (1H, d, J=17 Hz), 3.75 (1H, t, J=6 Hz), 3.82 (1H, d, J=17 Hz), 4.60 (2H, s), 5.30 (1H, d, J=5 Hz), 5.85 (1H, d, J=5 Hz), 7.10 (1H, s)	1,750
1n	OCH_3	2	2.98 (2H, t, J=7 Hz), 3.39 (3H, s), 3.56 (1H, d, J=17 Hz), 3.66 (2H, t, J=7 Hz), 3.86 (2H, t, J=17 Hz), 4.60 (2H, s), 5.28 (1H, d, J=5 Hz), 5.84 (1H, d, J=5 Hz), 7.09 (1H, s)	1,755
1o	$OCONH_2$	2	2.98~3.10 (2H, m), 3.57 (1H, d, J=17 Hz), 3.86 (1H, d, J=17 Hz), 4.10 (2H, t, J=6 Hz), 4.59 (2H, s), 5.28 (1H, d, J=5 Hz), 5.83 (1H, d, J=5 Hz), 7.08 (1H, s)	1,750
1p	F	2	3.00~3.09 (1H, m), 3.11~3.20 (1H, m), 3.57 (1H, d, J=17 Hz), 3.87 (1H, d, J=17 Hz), 4.52 (1H, t, J=7 Hz), 4.60 (2H, s), 4.77 (1H, t, J=7 Hz), 5.28 (1H, d, J=5 Hz), 5.84 (1H, d, J=5 Hz), 7.09 (1H, s)	1,755

Table 4. (Continued)

Compound No.	R	n	^{1}H NMR, δ (D_2O)	IR (KBr)[a] cm^{-1}
1q	NHAc	2	1.99 (3H, s), 2.70~3.06 (2H, m), 3.26~3.48 (2H, m), 3.54 (1H, d, J=17 Hz), 3.80 (1H, d, J=17 Hz), 4.60 (2H, s), 5.29 (1H, d, J=5 Hz), 5.82 (1H, J=5 Hz), 7.08 (1H, s)	1,755

[a] β-Lactam.

and 5.05 (2H, ABq, J=17 Hz, =NOCH_2), 5.01 (1H, d, J=5 Hz, 6-H), 5.24 (2H, s, OCH_2Ph), 5.78 (1H, dd, J=5 and 9 Hz, 7-H), 6.81 (1H, s, thiazole 5-H), 6.90 (2H, d, J=9 Hz, aromatic H), 6.97 (1H, s, CHPh$_2$), 7.03 (1H, br s, TrNH), 7.24~7.46 (27H, m, aromatic H), 8.11 (1H, d, J=9 Hz, CONH); FAB-MS m/z 1,075 $(M+H)^+$.

Sodium 7β-[2-(2-Aminothiazol-4-yl)-2-[(Z)-carboxymethoxyimino]acetamido]-3-cyanomethylthio-3-cephem-4-carboxylate (**1d**)

To a mixture of TFA (5 ml) and anisole (1 ml) was added **8d** (400 mg, 0.37 mM) under ice-cooling, and stirred for 45 minutes at the same temperature. Then, the reaction mixture was added dropwise to a mixture of Et_2O and n-hexane (1 : 2, 50 ml). The precipitated TFA salt of the desired product was collected by filtration, and washed with a small amount of a mixture of Et_2O and n-hexane. Subsequently, the TFA salt was dissolved in H_2O with $NaHCO_3$ (93 mg, 3.0 equiv), and chromatographed on Sephadex LH-20 column (eluent; H_2O), then lyophilized to afford 140 mg (70%) of **1d** as a white solid: IR (KBr) cm^{-1} 2320 (nitrile), 1760 (β-lactam), 1600; ^{1}H NMR (D_2O) δ 3.65 (1H, d, J=17 Hz, 2-H$_\alpha$), 3.96 (1H, d, J=17 Hz, 2-H$_\beta$), 4.60 (2H, s, =NOCH_2), 5.32 (1H, d, J=5 Hz, 6-H), 5.88 (1H, d, J=5 Hz, 7-H), 7.08 (1H, s, thiazole 5-H).

The others were similarly prepared from compound **8** according to the procedure for **1d** and their spectral data are listed in Table 4.

References

1) YAMANAKA, H.; K. KAWABATA, K. MIYAI, H. TAKASUGI, T. KAMIMURA, Y. MINE & T. TAKAYA: Studies on β-lactam antibiotics. X. Synthesis and structure-activity relationships of 7β-[(Z)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]cephalosporin derivatives. J. Antibiotics 39: 101~110, 1986
2) SAKAGAMI, K.; T. WATANABE, S. FUKATSU, H. NITTA, M. HATANAKA & T. ISHIMARU: Synthetic cephalosporins V. Synthesis and antibacterial activity of 3-alkylthio-7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(O-substituted oxyimino)acetamido]cephalosporins and related compounds. Yakugaku Zasshi (Japanese) 109: 913~925, 1989
3) NISHIMURA, S.; N. YASUDA, H. SASAKI, K. KAWABATA, K. SAKANE & T. TAKAYA: Synthesis and biological activity of 3-vinylthio- and 3-vinylthiomethylcephem derivatives. J. Antibiotics 43: 1160~1168, 1990
4) 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
5) SADAKI, H.; H. IMAIZUMI, T. INABA, T. HIRAKAWA, Y. MUROTANI, Y. WATANABE, S. MINAMI & I. SAIKAWA: Studies on β-lactam antibiotics for medicinal purpose. XVIII. Synthesis and structure-activity relationships of 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-substituted methyl-3-cephem-4-carboxylic acid derivatives. Yakugaku Zasshi (Japanese) 106: 129~146, 1986
6) 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
7) SCARTAZZINI, R.; P. SCHNEIDER & H. BICKEL: 263. Neue β-Lactam-Antibiotica. Über die Funktionalisierung der Cephem-3-Stellung mittels Schwefel oder Stickstoff. Helv. Chim. Acta 58: 2437~2450, 1975
8) SAKAGAMI, K.; T. WATANABE, S. FUKATSU, H. NITTA, M. HATANAKA & T. ISHIMARU: Synthetic Cephalosporins. II. The synthesis and oral activity of 7-[R-2-amino-2-(3-chloro-4-hydroxyphenyl)acetamido-3-methylthio-3-cephem-4-carboxylic acid and related compounds. J. Antibiotics 40: 1325~1330, 1987
9) FINNEY, D. J.: The maximum likelihood solution. *In* Probit Analysis. 2nd. Ed. *Ed.*, D. J. FINNEY, pp. 48~64, Cambridge University Press, 1952