STUDIES ON ORALLY ACTIVE CEPHALOSPORIN ESTERS

VI†. SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 3-(3-ISOXAZOLYL)OXYMETHYLCEPHALOSPORIN DERIVATIVES

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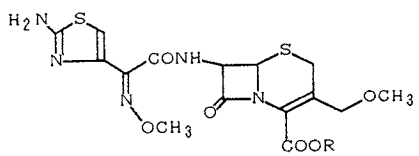
The synthesis and biological activities of a series of 3-(isoxazol-3-yl)oxymethyl cephalosporins are described. 7-[2-(2-Aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(isoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylic acid (**7a**) showed potent activity against both Gram-positive and Gram-negative bacteria including some β-lactamase producing species. Its pivaloyloxymethyl ester provided a good urinary recovery after oral administration to mice.

In recent years, extensive studies have been performed on cephalosporin antibiotics having an aminothiazole-oxime moiety at the C-7 position of the cephem nucleus, so-called that third generation cephalosporins, which show a broad spectrum of activity against a variety of microorganisms. Most of them are for parenteral use. Quite recently, however, some third generation cephalosporins have been developed for oral use[2,3].

In a previous paper[4], we reported on the synthesis and biological activities of 3-methoxymethyl cephalosporin derivatives, one of which, cefpodoxime proxetil (CS-807) (Fig. 1), has been successfully developed as a prodrug for oral use. It showed good antibacterial activity against both Gram-positive and Gram-negative bacteria including β-lactamase producing species and significantly good absorption from the intestine.

It has been well recognized that electron-withdrawing substituents of the C-3 position of the cephem nucleus generally enhance antibacterial activity[5]. 3-Heterocyclic thiomethyl cephalosporins generally show higher antibacterial activity than 3-methylthiomethyl ones[6]. Therefore, cephalosporins bearing a heterocyclic oxymethyl moiety on the C-3 position may be expected to be more potent in the activity than the corresponding 3-methoxymethyl derivatives. There have been found, however, few reports[7,8] on the 3-heterocyclic oxymethyl derivatives, probably because of synthetic difficulties. Our further elaboration for

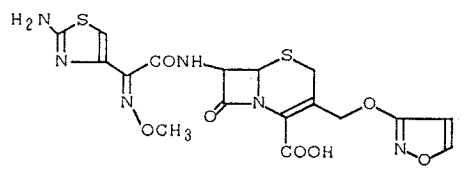
Fig. 1. Structure of cefpodoxime and cefpodoxime proxetil.



Cefpodoxime R=H

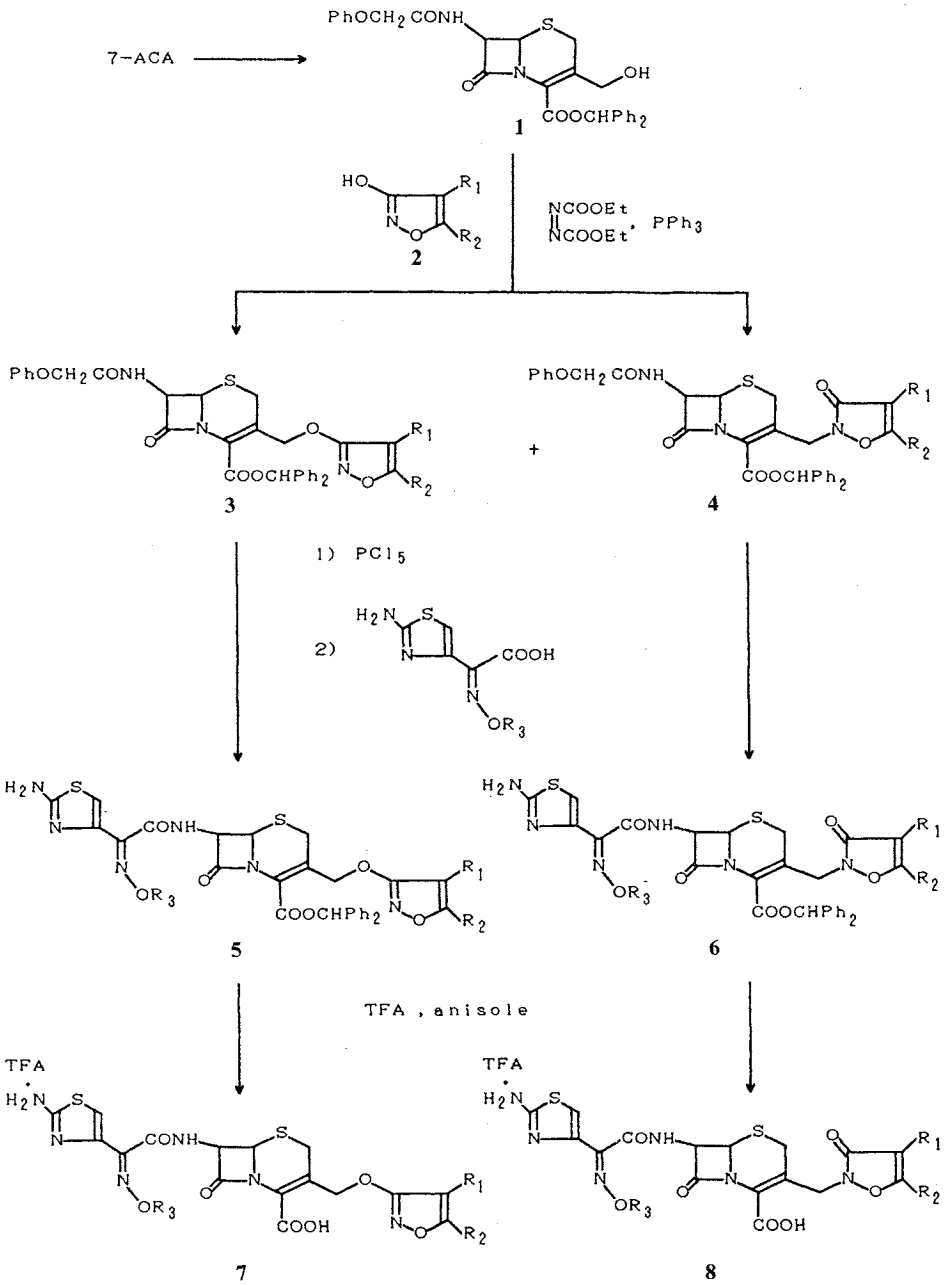
Cefpodoxime proxetil (CS-807) R=$CHOCOOCHCH_3$ (CH_3, CH_3)

Fig. 2. Structure of a 3-isoxazolyl oxymethyl cephalosporin, **7a**.



† Paper V[1].

Scheme 1.



a $R_1=R_2=H$ $R_3=CH_3$
b $R_1=H$ $R_2=R_3=CH_3$
c $R_1=F$ $R_2=R_3=CH_3$
d $R_1=H$ $R_2=CH_2COOCH_3$ $R_3=CH_3$
e $R_1=H$ $R_2=CH_2COOCHPh_2$ $R_3=CH_3$
f $R_1=H$ $R_2=COOCH_3$ $R_3=CH_3$
g $R_1=H$ $R_2=COOCHPh_2$ $R_3=CH_3$
h $R_1=H$ $R_2=CH_3$ $R_3=CH_2CH_2F$

In the case of **7** and **8**, for (**e**, **g**) R_2 means CH_2COOH and COOH, respectively.

developing orally usable cephalosporin has been made in the direction of chemical modification of the C-3 substituent.

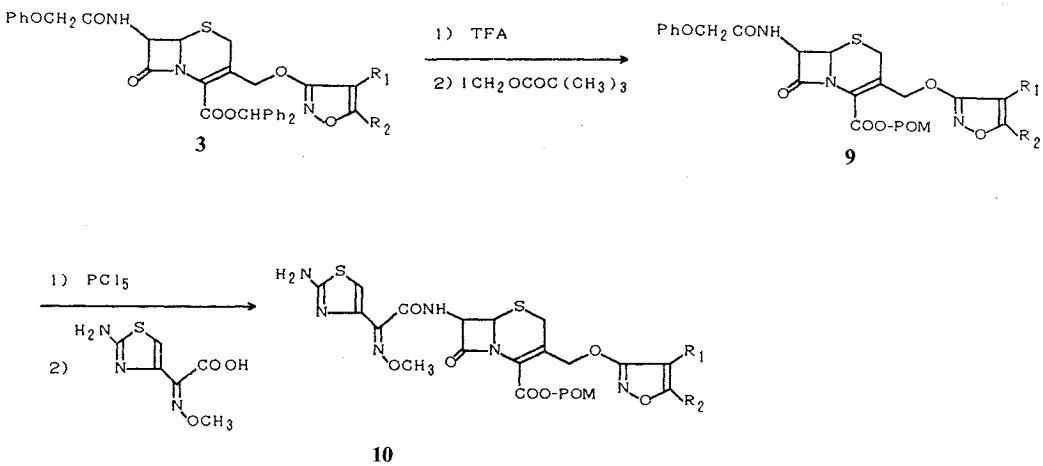
The introduction of an isoxazolyl moiety to the C-3 position instead of the methyl group in cefpodoxime provided 7-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(isoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylic acid (**7a**) (Fig. 2), which showed potent antibacterial activity, and its pivaloyloxymethyl (POM) ester **10a** exhibited good urinary recovery after po administration to mice.

In this paper, we describe the synthesis and biological activities of the cephalosporin derivatives bearing isoxazole moiety at the C-3 position, especially 3-isoxazolyl oxymethyl groups, and their POM esters.

Chemistry

In the synthesis of 3-heterocyclic oxymethyl cephalosporins, the reaction of a hydroxyheterocyclic compound including 3-hydroxyisoxazole with 3-acetoxymethyl cephems did not proceed in contrast to preparing 3-heterocyclic thiomethyl derivatives from the corresponding heterocyclic thiols. Therefore, the MITSUNOBU reaction, which is one of excellent methods for preparation of aryl ethers[9], was employed to synthesize 3-isoxazolyl oxymethyl cephalosporin derivatives using 3-hydroxyisoxazoles as an acidic hydroxyl component. The desired compound **3**, together with *N*-substituted isoxazolone isomer **4**, was successfully prepared from the 3-hydroxymethyl cephem **1**, readily available[4] from 7-ACA, and 3-hydroxyisoxazoles **2** according to the method under mild conditions without Δ^2 isomerization. Both isomers **3** and **4** were conventionally progressed to the corresponding acids **7** and **8** as follows; the phenoxyacetyl groups of **3** and **4** were removed according to the CHAUVETTE's modification[10] followed by acylation with 2-(2-

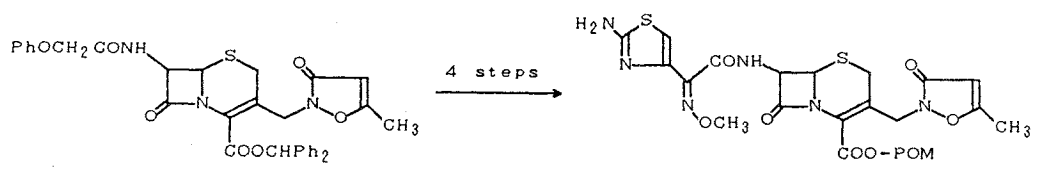
Scheme 2.



3 **9** **10**

a R_1=H R_2=H
b R_1=H R_2=CH_3
POM: $CH_2OCOC(CH_3)_3$

Scheme 3.



4b **11**

aminothiazol-4-yl)-(Z)-2-alkoxyiminoacetic acid to give **5** and **6**, which were treated with trifluoroacetic acid (TFA) to afford the acids **7** and **8**, respectively (Scheme 1).

POM ester **10** was prepared in an alternative procedure (Scheme 2). The POM ester **9** was obtained from **3** by treatment of the diphenylmethyl ester **3** with TFA followed by esterification with iodomethyl pivalate. Exchange of the acyl group in **9** was carried out by the same method as that mentioned above to give the desired POM ester **10**. Similarly, the POM ester of isoxazolone isomer **11** was also prepared from **4b** (Scheme 3).

Biological Results and Discussion

The *in vitro* antibacterial activities of the compounds in this series are shown in Tables 1 and 2.

The compound **7a** having the isoxazolyloxymethyl moiety at the C-3 position of the cephem nucleus exhibited potent activity against both Gram-positive and Gram-negative bacteria. Lipophilic substituents on the isoxazole ring such as methyl (**7b**), methoxycarbonylmethyl (**7d**) and methoxycarbonyl (**7f**) group showed only moderate activity against Gram-positive bacteria. The acidic substituents such as carboxymethyl (**7e**) and carboxy (**7g**) group showed no improvement in activity against Gram-negative species compared with the unsubstituted compound **7a**.

Further elaboration of the isoxazole ring was not fruitful. The compound **7c** possessing an electronegative and hydrophobic fluorine atom on the isoxazole ring showed unexpectedly diminished activity against both Gram-positive and Gram-negative bacteria.

Introduction of a 2-fluoroethyl group (**7h**) in place of the methyl group on the oxime moiety at the C-7 side chain of the cephem nucleus, which gave good antibacterial activity, especially against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a previous paper[11], showed no significant improvement in the

Table 1. Antibacterial activity (MIC, μg/ml) of **7** (**a**~**h**).

Compound	*S.a.*	*S.a.* (R)	*E.c.*	*E.c.* (R)	*K.p.*	*K.p.* (R)	*E.cl.*	*S.m.*	*P.v.*	*M.m.*
7a	0.4	0.4	0.1	0.4	0.1	0.2	0.4	0.05	≦0.01	1.5
7b	0.2	0.8	0.2	1.5	0.2	0.4	0.8	0.1	≦0.01	1.5
7c	3.1	3.1	0.4	1.5	0.2	1.5	1.5	0.8	0.02	25
7d	0.4	1.5	0.2	0.8	0.2	1.5	0.8	0.1	≦0.01	3.1
7e	3.1	3.1	0.1	0.4	0.05	0.8	0.4	0.05	≦0.01	6.2
7f	0.8	3.1	0.4	0.8	0.2	3.1	1.5	0.2	0.02	25
7g	1.5	6.2	0.4	0.4	0.1	0.8	0.4	0.1	≦0.01	12.5
7h	0.2	0.8	0.4	0.8	0.4	1.5	0.8	0.2	≦0.01	1.5
Cefpodoxime	0.8	0.8	0.4	0.4	0.2	0.8	1.5	0.2	0.02	50

Abbreviations: *S.a.*, *Staphylococcus aureus* 209P JC-1; *S.a.* (R), *S. aureus* 56; *E.c.*, *Escherichia coli* NIHJ JC-2; *E.c.* (R), *E. coli* 609; *K.p.*, *Klebsiella pneumoniae* 806; *K.p.* (R), *K. pneumoniae* 846; *E.cl.*, *Enterobacter cloacae* 963; *S.m.*, *Serratia marcescens* 1184; *P.v.*, *Proteus vulgaris* 1420; *M.m.*, *Morganella morganii* 1510. (R): Means β-lactamase producing strains.

Table 2. Antibacterial activity (MIC, μg/ml) of **8** (**a**, **b**, **d**~**g**).

Compound	*S.a.*	*S.a.* (R)	*E.c.*	*E.c.* (R)	*K.p.*	*K.p.* (R)	*E.cl.*	*S.m.*	*P.v.*	*M.m.*
8a	0.8	1.5	0.1	0.4	0.05	0.8	0.4	0.05	≦0.01	12.5
8b	0.4	1.5	0.1	0.4	0.05	0.2	0.2	0.05	≦0.01	3.1
8d	1.5	3.1	0.4	1.5	0.4	1.5	0.8	0.2	≦0.01	12.5
8e	6.2	6.2	0.4	1.5	0.2	1.5	0.8	0.05	≦0.01	25
8f	3.1	6.2	0.4	0.4	0.2	0.8	0.4	0.1	≦0.01	1.5
8g	6.2	25	0.8	0.8	0.2	0.8	0.8	0.1	≦0.01	50

Abbreviations mean the same strains as in Table 1.

activity.

The isomeric isoxazoles (**8a**, **8b**, **8d**~**8g**) also showed favorable activities comparable to that of 3-isoxazolyl oxymethyl derivatives (**7a**~**7g**).

Although the acid derivatives **7a**, **7b** and **8a** exhibited potent antibacterial activity, they showed poor urinary recovery after po administration to mice, 8.6, 1.2, and 2.1%, respectively. Consequently, the acids were converted to POM esters, which are generally useful as prodrugs for the po administration of β-lactam antibiotics such as cefteram pivoxil[3]. The results of urinary recovery of the parent acids after po administration of POM esters **10a**, **10b** and **11** are shown in Table 3. The compounds **10a** and **10b** gave good recovery comparable to that of cefteram pivoxil. The isoxazolone derivative **11**, however, gave poor results.

Table 3. Urinary recovery (% of dose)[a] of POM esters.

Compound	**10a**	**10b**	**11**	CFTM-PI[b]
Urinary recovery (%)	34.9	41.5	12.9	39

[a] Urinary recovery was determined by disk method on nutrient agar using *Bacillus subtilis* ATCC 6633 as a test strain after po administration of POM esters (50 mg/kg as a parent cephalosporin) in slc *dd*Y(SPF) mice ($n=5$, 0~24 hours).
[b] Cefteram pivoxil.

According to these results, it was found that introduction of an electron-withdrawing isoxazolyl moiety on the C-3 position of the cephem nucleus gave an improvement in antibacterial activity compared with cefpodoxime and, especially, 3-isoxazolyl oxymethyl isomers (**7**) were favorable parent compounds for use as prodrugs.

Experimental

IR spectra were recorded on a Nicolet NIC-60SX spectrometer. NMR spectra were determined on a Varian EM-360 (60 MHz) or Jeol GX-270 (270 MHz) spectrometer using TMS as an internal standard. The mp's were determined using a Yanagimoto micro-melting point apparatus and are uncorrected.

MICs were determined in nutrient agar medium (Eiken) by the 2-fold dilution method with 10^7 cfu/ml inoculum size after incubation at 37°C for 18 hours.

3-Hydroxyisoxazoles (**2a**~**2g**)

2a~**2g** were prepared according to the reported procedures[12~15] except **2c**. It was prepared as described below.

4-Fluoro-3-hydroxy-5-methylisoxazole (**2c**)

To a stirred mixture of ethyl 2-fluoroacetoacetate[16] (13.06 g) and ethylene glycol (27.36 g) in benzene (270 ml) was added boron trifluoride etherate (1.25 g). After being refluxed for 4 hours, the resulting mixture was washed with brine, aq $NaHCO_3$ and brine successively. The organic layer was dried ($MgSO_4$) and concd *in vacuo* to give a colorless oil. The oil was chromatographed on a silica gel column (EtOAc-cyclohexane, 1:4) to give ethyl 3,3-ethylenedioxy-2-fluorobutanoate (13.62 g, 80.4%). ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.32 (3H, t, $J=7$ Hz, CH_2CH_3), 1.48 (3H, d, $J=3$ Hz, CH_3), 4.07 (4H, s, OCH_2CH_2O), 4.29 (2H, q, $J=7$ Hz, CH_2CH_3), 4.76 (1H, d, $J=50$ Hz, FCH).

To a stirred solution of ethyl 3,3-ethylenedioxy-2-fluorobutanoate (2.08 g) and hydroxylamine hydrochloride (0.92 g) in pyridine (11 ml) was added dropwise a 28-% solution of sodium methoxide in methanol (4.52 ml) during 5 minutes at room temperature. After stirring for 25 minutes, the insoluble solid was filtered off. To the filtrate was added AcOH (1.36 ml) and was concd *in vacuo*. To the residue was added THF and the insoluble solid was removed by filtration. The filtrate was evaporated *in vacuo* and the resulting residue was chromatographed on a silica gel column ($CHCl_3$-MeOH, 10:1) to give 3,3-ethylenedioxy-2-fluoro-*N*-hydroxybutanamide (1.77 g, 91.3%). ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.44 (3H, d, $J=2.5$ Hz, CH_3), 3.95 (4H, s, OCH_2CH_2O), 4.83 (1H, d, $J=47$ Hz, FCH), 7.81 (2H, br, NHOH).

A suspension of 3,3-ethylenedioxy-2-fluoro-*N*-hydroxybutanamide (11.1 g) in conc H_2SO_4 (27 ml) was heated at 70°C for 1 hour. The resulting solution was poured into ice water, the mixture was extracted with

EtOAc (twice) and the combined extract was dried ($MgSO_4$). The solvent was removed under reduced pressure, followed by recrystallization from hexane to give colorless needles of **2c** (6.44 g, 88.7%). MP 116~117°C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.33 (3H, d, J=2.5 Hz CH_3), 10.46 (1H, s, OH).

Anal Calcd for $C_4H_4NO_2F$: C 41.03, H 3.44, N 11.96, F 16.23.
Found: C 41.38, H 3.43, N 11.84, F 16.05.

Diphenylmethyl 7-Phenoxyacetamido-3-[(isoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylate (**3a**) and Diphenylmethyl 7-Phenoxyacetamido-3-[(2,3-dihydro-3-oxoisoxazol-2-yl)methyl]-3-cephem-4-carboxylate (**4a**)

Diethyl azodicarboxylate (418 mg) was added to a stirred solution of **1** (1.06 g), 3-hydroxyisoxazole (204 mg) and triphenylphosphine (630 mg) in dry THF (20 ml) within 7 minutes at 5°C. After stirring for 1 hour at the same temperature, the solvent was evaporated *in vacuo*. The residue was chromatographed on a silica gel column (EtOAc-cyclohexane, 1:1~5:1) to give **3a** (209 mg, 17.5%) and **4a** (137 mg, 11.5%). ^{1}H NMR (60 MHz, $CDCl_3$; **3a**) δ 3.51 (2H, s, 2-CH_2), 4.54 (2H, s, PhOCH_2), 4.95 and 5.22 (2H, ABq, J =13 Hz, 3′-CH_2), 4.96 (1H, d, J=5 Hz, 6-CH), 5.83 (1H, d, J=2 Hz, isoxazole 4-H), 5.87 (1H, dd, J=5 and 9 Hz, 7-CH), 6.7~7.7 (17 H, m, Ph_2CH, PhO and NHCO), 8.05 (1H, d, J=2 Hz, isoxazole 5-H). ^{1}H NMR (60 MHz, $CDCl_3$; **4a**) δ 3.33 (2H, s, 2-CH_2), 4.53 (2H, s, PhOCH_2), 4.90 (2H, s, 3′-CH_2), 4.96 (1H, d, J=5 Hz, 6-CH), 5.73 (1H, d, J=2.5 Hz, isoxazole 4-H), 5.91 (1H, dd, J=5 and 9 Hz, 7-CH), 6.7~7.7 (17 H, m, Ph_2CH, PhO and NHCO), 7.86 (1H, d, J=2.5 Hz, isoxazole 5-H).

Diphenylmethyl 7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(isoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylate (**5a**)

To a stirred solution of **3a** (662 mg) and pyridine (162 mg) in dry CH_2Cl_2 (6 ml) was added PCl_5 (288 mg) in one portion at −35°C. The mixture was gradually warmed to room temperature, and then cooled to −35°C again. To the resulting solution was added PrOH (1 ml) slowly. After stirring for 30 minutes at room temperature, the solution was washed with brine and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was washed with petroleum ether by decantation. The residue was triturated with ether and the resulting powder was collected by filtration to give diphenylmethyl 7-amino-3-[(isoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylate hydrochloride (448 mg, 80.7%). IR (KBr) cm^{-1} 1789 (β-lactam C=O). ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.79 (2H, s, 2-CH_2), 4.97 and 5.08 (2H, ABq, J =12.7 Hz, 3′-CH_2), 5.27 (1H, d, J=5.1 Hz, 6-CH), 5.31 (1H, d, J=5.1 Hz, 7-CH), 6.26 (1H, d, J=1.7 Hz, isoxazole 4-H), 6.95 (1H, s, C*H*Ph_2), 7.2~7.5 (10 H, m, CH*Ph*$_2$), 8.68 (1H, d, J=1.7 Hz, isoxazole 5-H).

To a stirred solution of 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (233 mg) and 1-hydroxybenzotriazole (177 mg) in DMF (4 ml) was added dicyclohexylcarbodiimide (DCC, 239 mg) at room temperature. After stirring for 1 hour, the precipitate was removed by filtration. The filtrate was then added to a solution of the 7-aminocephalosporin ester (526 mg) and *N*,*N*-diethylaniline (157 mg) in CH_2Cl_2 (4 ml) at 5°C. The solution was stirred for 1 hour and diluted with EtOAc. The resulting solution was washed with brine and water successively, and then dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column (EtOAc-cyclohexane, 4:1) to give **5a** (405 mg, 59.5%). ^{1}H NMR (60 MHz, $CDCl_3$) δ 3.55 (2H, s, 2-CH_2), 3.99 (3H, s, OCH_3), 5.06 (1H, d, J=5 Hz, 6-CH), 4.95 and 5.23 (2H, ABq, J=14 Hz, 3′-CH_2), 5.84 (2H, br, NH_2), 5.86 (1H, d, J=2 Hz, isoxazole 4-H), 6.07 (1H, dd, J=5 and 9 Hz, 7-CH), 6.70 (1H, s, thiazole ring-H), 6.97 (1H, s, C*H*Ph_2), 7.1~7.6 (10 H, m, CH*Ph*$_2$), 8.09 (1H, d, J=2 Hz, isoxazole 5-H), 8.19 (1H, d, J=9 Hz, NHCO).

7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(isoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylic Acid TFA Salt (**7a**)

To a stirred solution of **5a** (405 mg) in anisole (1.5 ml) and 1,2-dichloroethane (2 ml), TFA (2 ml) was added at 5°C. After stirring for 40 minutes, the solvent was removed *in vacuo* and the residue was triturated with ether. The resulting powder was chromatographed on a silica gel column (EtOAc-EtOH-H_2O, 5:2:1) to give **7a** (199 mg, 53.4%). IR (KBr) cm^{-1} 1781 (β-lactam C=O). ^{1}H NMR (Table 4).

7(**b**~**h**) and **8** (**a**, **b**, **d**~**g**) were prepared by the similar manner as mentioned above and the ^{1}H NMR

Table 4. ^{1}H NMR (270 MHz) data of **7** (**a**~**h**).

	DMSO-d_6, δ ppm, J in Hz
7a	3.60, 3.69 (2H, ABq, J=18.3, 2-CH_2), 3.86 (3H, s, OCH_3), 4.94, 5.13 (2H, ABq, J=12.0, 3′-CH_2), 5.18 (1H, d, J=4.9, 6-CH), 5.81 (1H, dd, J=4.9, 7.8, 7-CH), 6.32 (1H, d, J=1.7, isoxazole 4-H), 6.77 (1H, s, thiazole ring-H), 8.67 (1H, d, J=1.7, isoxazole 5-H), 9.64 (1H, d, J=7.8, NHCO)
7b[a]	2.30 (3H, s, CH_3), 3.55 (2H, s, 2-CH_2), 3.98 (3H, s, OCH_3), 5.15 (1H, d, J=5, 6-CH), 5.07, 5.33 (2H, ABq, J=15, 3′-CH_2), 5.80 (1H, s, isoxazole ring-H), 5.82 (1H, d, J=5, 7-CH), 6.86 (1H, s, thiazole ring-H)
7c	2.35 (3H, d, J=2.0, CH_3), 3.61, 3.70 (2H, ABq, J=17.8, 2-CH_2), 3.84 (3H, s, OCH_3), 4.97, 5.21 (2H, ABq, J=11.7, 3′-CH_2), 5.18 (1H, d, J=4.9, 6-CH), 5.81 (1H, dd, J=4.9, 8.3, 7-CH), 6.73 (1H, s, thiazole ring-H), 9.60 (1H, d, J=8.3, NHCO)
7d	3.60, 3.70 (2H, ABq, J=18.1, 2-CH_2), 3.66 (2H, s, isoxazole-CH_2), 3.86 (3H, s, OCH_3), 3.93 (3H, s, $COOCH_3$), 4.92, 5.10 (2H, ABq, J=12.2, 3′-CH_2), 5.19 (1H, d, J=4.9, 6-CH), 5.81 (1H, dd, J=4.9, 8.3, 7-CH), 6.17 (1H, s, isoxazole ring-H), 6.77 (1H, s, thiazole ring-H), 9.65 (1H, d, J=8.3, NHCO)
7e	3.59, 3.69 (2H, ABq, J=18.0, 2-CH_2), 3.79 (2H, s, isoxazole-CH_2), 3.86 (3H, s, OCH_3), 4.91, 5.10 (2H, ABq, J=12.2, 3′-CH_2), 5.19 (1H, d, J=4.9, 6-CH), 5.81 (1H, dd, J=4.9, 8.3, 7-CH), 6.13 (1H, s, isoxazole ring-H), 6.77 (1H, s, thiazole ring-H), 9.65 (1H, d, J=8.3, NHCO)
7f	3.60, 3.70 (2H, ABq, J=18.1, 2-CH_2), 3.86 (3H, s, OCH_3), 3.88 (3H, s, $COOCH_3$), 5.01, 5.18 (2H, ABq, J=12.0, 3′-CH_2), 5.19 (1H, d, J=4.9, 6-CH), 5.82 (1H, dd, J=4.9, 8.3, 7-CH), 6.77 (1H, s, thiazole ring-H), 7.08 (1H, s, isoxazole ring-H), 9.64 (1H, d, J=8.3, NHCO)
7g	3.60, 3.70 (2H, ABq, J=18.1, 2-CH_2), 3.86 (3H, s, OCH_3), 5.00, 5.18 (2H, ABq, J=12.2, 3′-CH_2), 5.19 (1H, d, J=4.9, 6-CH), 5.81 (1H, dd, J=4.9, 8.3, 7-CH), 6.77 (1H, s, thiazole ring-H), 6.94 (1H, s, isoxazole ring-H), 9.63 (1H, d, J=8.3, NHCO)
7h	2.31 (3H, s, CH_3), 3.57, 3.74 (2H, ABq, J=18.0, 2-CH_2), 4.30 (2H, dt, J=3.9, 29.8, OCH_2CH_2F), 4.66 (2H, dt, J=3.9, 47.9, OCH_2CH_2F), 4.89, 5.09 (2H, ABq, J=12.2, 3′-CH_2), 5.19 (1H, d, J=4.9, 6-CH), 5.82 (1H, dd, J=4.9, 8.3, 7-CH), 5.97 (1H, s, isoxazole ring-H), 6.79 (1H, s, thiazole ring-H), 9.67 (1H, d, J=8.3, NHCO)

[a] Measured at 60 MHz (CD_3OD).

Table 5. ^{1}H NMR (270 MHz) data of **8** (**a**, **b**, **d**~**g**).

	DMSO-d_6, δ ppm, J in Hz
8a	3.32, 3.50 (2H, ABq, J=18.1, 2-CH_2), 3.85 (3H, s, OCH_3), 4.76, 4.94 (2H, ABq, J=15.9, 3′-CH_2), 5.16 (1H, d, J=4.9, 6-CH), 5.78 (1H, dd, J=4.9, 8.3, 7-CH), 5.98 (1H, d, J=2.7, isoxazole 4-H), 6.76 (1H, s, thiazole ring-H), 8.51 (1H, d, J=2.7, isoxazole 5-H), 9.63 (1H, d, J=8.3, NHCO)
8b[a]	2.21 (3H, s, CH_3), 3.43 (2H, s, 2-CH_2), 3.88 (3H, s, OCH_3), 4.76, 4.86 (2H, ABq, J=16, 3′-CH_2), 5.16 (1H, d, J=5, 6-CH), 5.63 (1H, s, isoxazole ring-H), 5.80 (1H, dd, J=5, 8, 7-CH), 6.82 (1H, s, thiazole ring-H), 9.70 (1H, d, J=8, NHCO)
8d	3.23, 3.48 (2H, ABq, J=18.1, 2-CH_2), 3.72 (2H, s, isoxazole-CH_2), 3.85 (3H, s, $COOCH_3$), 3.86 (3H, s, OCH_3), 4.75, 4.97 (2H, ABq, J=16.1, 3′-CH_2), 5.13 (1H, d, J=4.9, 6-CH), 5.78 (1H, dd, J=4.9, 8.3, 7-CH), 5.88 (1H, s, isoxazole ring-H), 6.78 (1H, s, thiazole ring-H), 9.64 (1H, d, J=8.3, NHCO)
8e	3.32, 3.45 (2H, ABq, J=18.1, 2-CH_2), 3.72 (2H, s, isoxazole-CH_2), 3.85 (3H, s, OCH_3), 4.74, 4.97 (2H, ABq, J=16.1, 3′-CH_2), 5.12 (1H, d, J=4.4, 6-CH), 5.77 (1H, dd, J=4.4, 7.8, 7-CH), 5.83 (1H, s, isoxazole ring-H), 6.76 (1H, s, thiazole ring-H), 9.62 (1H, d, J=7.8, NHCO)
8f	3.34, 3.54 (2H, ABq, J=18.1, 2-CH_2), 3.84 (3H, s, $COOCH_3$), 3.87 (3H, s, OCH_3), 4.89, 5.10 (2H, ABq, J=16.4, 3′-CH_2), 5.15 (1H, d, J=4.9, 6-CH), 5.79 (1H, dd, J=4.9, 7.8, 7-CH), 6.71 (1H, s, isoxazole ring-H), 6.75 (1H, s, thiazole ring-H), 9.63 (1H, d, J=7.8, NHCO)
8g	3.35, 3.54 (2H, ABq, J=18.1, 2-CH_2), 3.86 (3H, s, OCH_3), 4.87, 5.08 (2H, ABq, J=16.4, 3′-CH_2), 5.16 (1H, d, J=4.9, 6-CH), 5.79 (1H, dd, J=4.9, 8.3, 7-CH), 6.56 (1H, s, isoxazole ring-H), 6.76 (1H, s, thiazole ring-H), 9.63 (1H, d, J=8.3, NHCO)

[a] Measured at 60 MHz.

data are listed in Tables 4 and 5.

Pivaloyloxymethyl 7-Phenoxyacetamido-3-[(isoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylate (**9a**)

To a stirred solution of **3a** (1.50 g) in anisole (3 ml) and 1,2-dichloroethane (3 ml) was added TFA (3 ml) with ice-cooling. After stirring for 35 minutes, the solvent was removed *in vacuo*. The residue was

dissolved in EtOAc and the solution was extracted with aq $NaHCO_3$. The aqueous extract was washed with EtOAc and then acidified with 10% HCl. The resulting mixture was extracted with EtOAc twice and the combined extract was washed with brine. The solution was dried ($MgSO_4$) and evaporated *in vacuo* to afford the corresponding acid (1.10 g).

To a solution of the acid (582 mg) in *N,N*-dimethylacetamide (2.5 ml) were added dicyclohexylamine (245 mg) and iodomethyl pivalate (392 mg) successively under cooling. After stirring for 30 minutes, EtOAc (15 ml) was added to the mixture. The resulting precipitate was filtered off and the filtrate was washed with dil HCl, aq $NaHCO_3$ and brine successively. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo* to give a yellow oil. The oily residue was chromatographed on a silica gel column (EtOAc - cyclohexane, 1 : 1) to give **9a** (356 mg, 48.3%). ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.20 (9H, s, *tert*-Bu), 3.57 (2H, s, 2-CH_2), 4.52 (2H, s, PhOCH_2), 4.99 (1H, d, J=5 Hz, 6-CH), 4.98 and 5.29 (2H, ABq, J=14 Hz, 3′-CH_2), 5.7~6.0 (4H, m, 7-CH, $COOCH_2$ and isoxazole 4-H), 6.7~7.5 (6H, m, Ph and NHCO), 8.11 (1H, d, J=2 Hz, isoxazole 5-H).

Pivaloyloxymethyl 7-Phenoxyacetamido-3-[(5-methylisoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylate (**9b**)

9b was prepared from **3b** as described for **9a**. ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.20 (9H, s, *tert*-Bu), 2.28 (3H, s, CH_3), 3.56 (2H, s, 2-CH_2) 4.55 (2H, s, PhOCH_2), 5.01 (1H, d, J=5 Hz, 6-CH), 4.98 and 5.28 (2H, ABq, J=13 Hz, 3′-CH_2), 5.62 (1H, s, isoxazole ring-H), 5.8~6.1 (3H, m, 7-CH and $COOCH_2$), 6.8~7.6 (6H, m, Ph and NHCO).

Pivaloyloxymethyl 7-[2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(isoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylate (**10a**)

To a solution of **9a** (541 mg) in dry CH_2Cl_2 (5.5 ml) was added pyridine (145 mg) and PCl_5 (258 mg) at −35℃ with stirring. The mixture was stirred for 15 minutes after removal of the cooling bath. After cooling the solution to −35℃ again, PrOH (1 ml) was added. The solution was then stirred for 20 minutes at room temperature and concd *in vacuo*. The oily residue was crystallized in a mixture of CH_2Cl_2 and ether. The resulting crystals were collected by filtration to give pivaloyloxymethyl 7-amino-3-[(isoxazol-3-yl)oxymethyl]-3-cephem-4-carboxylate hydrochloride (228 mg, 51.3%). IR (KBr)cm^{-1} 1781 (β-lactam C=O). ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.14 (9H, s, *tert*-Bu), 3.80 (2H, s, 2-CH_2), 4.99 and 5.12 (2H, ABq, J=12.7 Hz, 3′-CH_2), 5.25 and 5.28 (2H, ABq, J=5.4 Hz, 6-CH and 7-CH), 5.81 and 5.91 (2H, ABq, J=6.1 Hz, $COOCH_2$), 6.33 (1H, d, J=1.7 Hz, isoxazole 4-H), 8.69 (1H, d, J=1.7 Hz, isoxazole 5-H).

To a stirred solution of 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (109 mg) and 1-hydroxybenzotriazole (82.8 mg) in DMF (2 ml) was added DCC (112 mg) and the mixture was stirred for 50 minutes at room temperature. The resulting precipitate was removed by filtration and the filtrate was added to a solution of the 7-aminocephalosporin ester (220 mg) and *N,N*-diethylaniline (74 mg) in CH_2Cl_2

Table 6. ^{1}H NMR (270 MHz) data of the POM esters (**10a**, **10b** and **11**).

	$CDCl_3$, δ ppm, J in Hz
10a[a]	1.18 (9H, s, *tert*-Bu), 3.58 (2H, s, 2-CH_2), 3.97 (3H, s, OCH_3), 5.05 (1H, d, J=5, 6-CH), 4.97, 5.28 (2H, ABq, J=13, 3′-CH_2), 5.79 (2H, br, NH_2), 5.87 (2H, s, $COOCH_2$), 5.93 (1H, d, J=2, isoxazole 4-H), 6.01 (1H, dd, J=5, 9, 7-CH), 6.66 (1H, s, thiazole ring-H), 8.09 (1H, d, J=2, isoxazole 5-H), 8.10 (1H, d, J=9, NHCO)
10b	1.22 (9H, s, *tert*-Bu), 2.33 (3H, s, CH_3), 3.56, 3.67 (2H, ABq, J=18.5, 2-CH_2), 4.07 (3H, s, OCH_3), 5.09 (1H, d, J=4.9, 6-CH), 4.99, 5.29 (2H, ABq, J=13.4, 3′-CH_2), 5.43 (2H, br, NH_2), 5.62 (1H, d, J=1.0, isoxazole ring-H), 5.87, 5.94 (2H, ABq, J=5.6, $COOCH_2$), 6.03 (1H, dd, J=4.9, 8.8, 7-CH), 6.89 (1H, s, thiazole ring-H), 7.47 (1H, d, J=8.8, NHCO)
11	1.23 (9H, s, *tert*-Bu), 2.25 (3H, s, CH_3), 3.47 (2H, s, 2-CH_2), 4.05 (3H, s, OCH_3), 4.88, 4.95 (2H, ABq, J=16.7, 3′-CH_2), 5.09 (1H, d, J=4.9, 6-CH), 5.51 (1H, s, isoxazole ring-H), 5.52 (2H, br, NH_2), 5.44, 5.47 (2H, ABq, J=5.5, $COOCH_2$), 6.01 (1H, dd, J=4.9, 8.8, 7-CH), 6.88 (1H, s, thiazole ring-H), 7.53 (1H, d, J=8.8, NHCO)

[a] Measured at 60 MHz.

(2 ml) under cooling. The solution was stirred for 30 minutes on an ice bath and for an additional 30 minutes after removal of the cooling bath. Then the solution was diluted with EtOAc and washed with water (twice) and brine successively. The organic layer was dried ($MgSO_4$) and concd *in vacuo*. The residue was chromatographed on a silica gel column (EtOAc-cyclohexane, 3:1) to give **10a** (187 mg, 64.1%). IR (KBr) cm^{-1} 1787 (β-lactam C=O). ^1H NMR (Table 6).

10b was prepared from **9b** as described for **10a**. IR(KBr) cm^{-1} 1788 (β-lactam C=O). ^1H NMR (Table 6).

11 was also prepared by the similar manner as mentioned above using **4b** as a starting material. IR (KBr) cm^{-1} 1789 (β-lactam C=O). ^1H NMR (Table 6).

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