SEMISYNTHETIC β-LACTAM ANTIBIOTICS

IV. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 7β-[2-(HETERO AROMATIC METHOXYIMINO)-2-(2-AMINOTHIAZOL-4-YL)ACETAMIDO]CEPHALOSPORINS

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A series of 7β-[2-(hetero aromatic methoxyimino)-2-(2-aminothiazol-4-yl)acetamido]-cephalosporins have been synthesized and bacteriologically evaluated. Several substances in this series showed exceptional *in vitro* activity, especially those with a five-membered hetero aromatic substituent moiety at the 7-position and a quaternary ammonium group as the 3-function of the cephem nucleus. The most active derivative, 7β-[2-(imidazol-4-ylmethoxyimino)-2-(2-aminothiazol-4-yl)acetamido]-3-(pyridiniomethyl)ceph-3-em-4-carboxylate (**13a**) was the most evenly balanced with respect to activity against Gram-positive and Gram-negative bacteria. Furthermore, **13** was stable to various types of β-lactamases and had high affinities for penicillin binding protein-3 and -1Bs of both *Escherichia coli* and *Pseudomonas aeruginosa*.

The search for newer cephalosporin antibiotics for the treatment of infectious disease has recently made significant progress, and during these advances a large number of semisynthetic cephalosporin derivatives were prepared and tested. Extensive improvements of antibacterial activity against Gram-negative strains, especially against *Pseudomonas aeruginosa*, and of stability to β-lactamase gave rise to the so-called third-generation cephalosporin antibiotics. However, these drugs are less potent than the older cephalosporins against Gram-positive bacteria. For example, cefoperazone[1] and cefotaxime[2] have high intrinsic activities against Gram-negative strains, but further improvement in activity against *Pseudomonas* and *Serratia* sp. is desirable. Ceftazidime[3] is highly active against these organisms but has poor activity against Staphylococci.

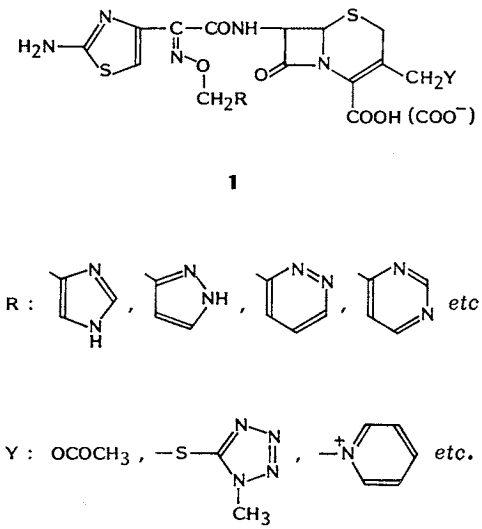
In our previous paper,[4] we reported that the introduction of basic and polar carbamoyl groups into the oxyimino moiety at the 7-position of the cephem nucleus yields a broad antibacterial spectrum and high activity against Gram-negative bacteria including *P. aeruginosa* and Staphylococci.

The third-generation cephalosporin antibiotics, except cefoperazone and latamoxef,[5] have a common methoxyimino group at the α-position of the 2-aminothiazol-4-ylacetyl group, and ceftazidime has 2,2-dimethylacetic acid in place of a methyl group at the 7-position of the cephem nucleus. None of them have basic groups.

On the basis of these facts and in view of the bacteria membrane penetration and pharmacokinetic properties, we planned the synthesis and microbiological evaluation of aromatic methoxyimino derivatives, introducing a polar basic hetero aromatic ring in order to increase the activities against *P. aeruginosa* and Gram-positive strains, which are weak points of the third-generation cephalosporin antibiotics. As shown in general structure **1**, an acyl group bearing a five- or six-membered hetero

aromatic ring, such as imidazole, pyrazole, pyridazine, and pyrimidine, as R at the 7-position, and an acetoxymethyl, 1-methyl-5-tetrazol-5-yl-thiomethyl or pyridium methyl group as the substituent at the 3-position were introduced into the cephem nucleus, and the effects of these substitutions on antibacterial activity were examined. In the present paper, we describe the synthesis and *in vitro* antibacterial activity of newly synthesized compounds **1**.

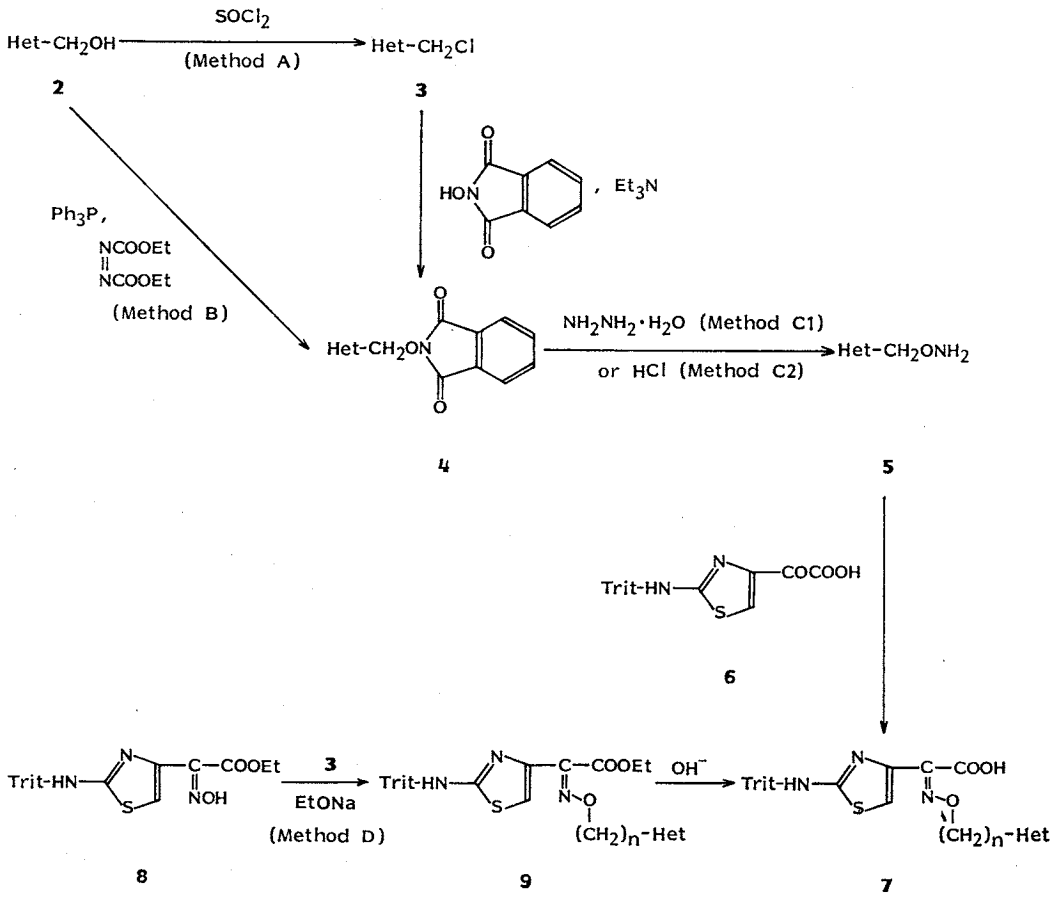
Fig. 1. Structure of new synthesized cephalosporin.



Chemistry

Scheme 1 summarizes two general methods for the synthesis of [(*Z*)-2-(aryl substituted methoxyimino)-2-(2-tritylaminothiazol-4-yl)]acetic acid (**7**). As the starting materials, we used

Scheme 1.

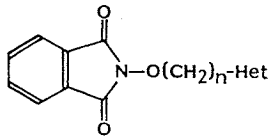


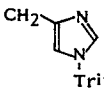
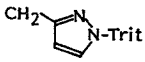
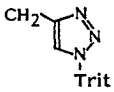
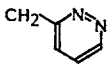
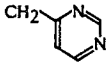
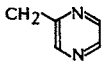
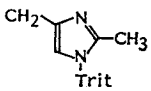
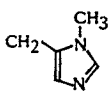
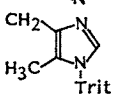
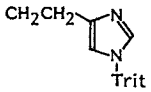
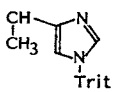
Trit=$C(C_6H_5)_3$

various hetero aromatic methanols (**2**). From each alcohol compound (**2**), the phthalimidoyloxy derivative (**4**) was prepared by chlorination with thionyl chloride to the chloromethyl derivative (**3**), which was treated with *N*-hydroxyphthalimide in the presence of triethylamine. Depending on the specific compound **4**, at this step, protection by tritylation was sometimes performed and the resulting substance was used in the next step (Method A). Also, **2**, with the nitrogen on its hetero aromatic ring if necessary protected by tritylation, was treated with diethyl azodicarboxylate, triphenyl phosphine and *N*-hydroxyphthalimide for conversion to the phthalimidoyloxy derivatives (**4**) (Method B).

Thiazolylacetic acids (**7**) were obtained by condensation of glyoxylic acid (**6**) with aromatic alkoxy-

Table 1. Physical properties of hetero aromatic methoxyiminophthalimide (**4a**~**4k**).



Compound No.	$(CH_2)_n$-Het	MP (°C)	Recrystallized solvent	Method	Formula	IR (KBr), cm^{-1}
4a		155~157	Petroleum ether - $CHCl_3$	A	$C_{31}H_{23}N_3O_3$	1780, 1725
4b		177~179	Et_2O - $CHCl_3$	A	$C_{31}N_{23}N_3O_3$	1790, 1735
4c		195~200	Et_2O - $CHCl_3$	A	$C_{30}H_{22}N_4O_3$	1790, 1735
4d		139~140	Diisopropyl ether - $CHCl_3$	A, B	$C_{13}H_9N_3O_3$	1785, 1725
4e		150~155	Diisopropyl ether	B	$C_{13}H_9N_3O_3$	1780, 1730
4f		145~147	Et_2O - $CHCl_3$	B	$C_{13}H_9N_3O_3$	1780, 1725
4g		Amorphous		A	$C_{32}H_{25}N_3O_3 \cdot \frac{1}{3}H_2O$	1780, 1720
4h		170~171	Et_2O - $CHCl_3$	B	$C_{13}H_{11}N_3O_3 \cdot H_2O$	1780, 1725
4i		220~223	Et_2O - $CHCl_3$	A	$C_{32}H_{25}N_3O_3$	1780, 1730
4j		177~178	Petroleum ether - $CHCl_3$	A	$C_{32}H_{25}N_3O_3$	1780, 1725
4k		179~181	Diisopropyl ether - $CHCl_3$	A	$C_{32}H_{25}N_3O_3$	1780, 1725

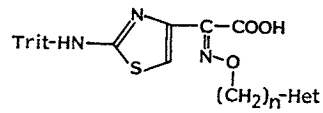
Trit: $C(C_6H_5)_3$. Het: Hetero aromatic ring.

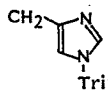
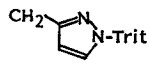
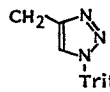
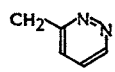
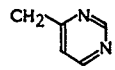
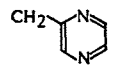
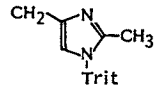
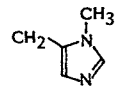
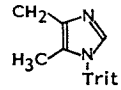
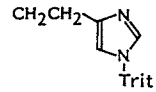
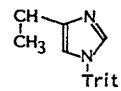
amines (**5**) which were prepared by treatment of phthalimide derivatives (**4**) with hydrazine hydrate (Method C1). In the case of unprotected aromatic ring compound such as pyridazine, the phthalimide derivatives (**4**), prepared similarly from **2**, were converted to the oxyamine compounds (**5**) by hydrolysis with hydrochloric acid (Method C2).

Furthermore, compounds **7** were prepared from ethyl hydroxyiminoacetic acid (**8**) by treatment with chloromethyl compounds (**3**) in the presence of a base, followed by subsequent alkaline hydrolysis of the ester derivatives (Method D). In both Methods C and D, only the *Z*-isomer of **7** was obtained, perhaps because of the bulky substituted methoxyimino group.

Various phthalimidoyloxy derivatives (**4**) and thiazolylacetic acids (**7**) are listed in Tables 1 and 2.

Table 2. Physical properties of 2-(2-tritylaminothiazol-4-yl)-2-(hetero aromatic methoxyimino)acetic acid (**7a**~**7k**).

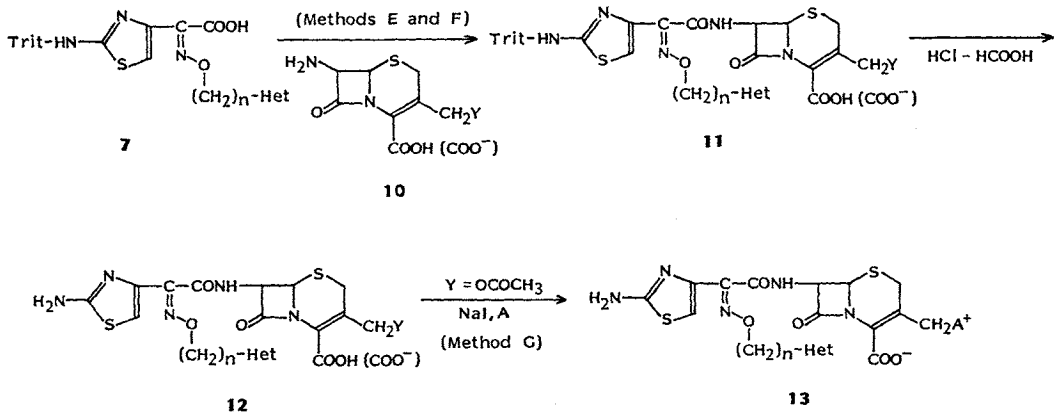


Compound No.	$(CH_2)_n$-Het	MP (dec) (°C)	Recrystallized solvent	Method	Formula	IR (KBr), cm^{-1}
7a		186~188	MeOH	C1, D	$C_{47}H_{37}N_5O_3S$	1720, 1615
7b		143~146	MeOH	C1	$C_{47}H_{37}N_5O_3S$	1720, 1520
7c		133~145	*n*-Hexane - $CHCl_3$	C1	$C_{46}H_{36}N_6O_3S$	1720, 1590
7d		160~166	Et_2O - $CHCl_3$	C2, D	$C_{29}H_{23}N_5O_3S$	1715, 1620
7e		171~174	Et_2O - $CHCl_3$	C2, D	$C_{29}H_{23}N_5O_3S$	1725, 1625
7f		131~134	Et_2O - $CHCl_3$	C2	$C_{29}H_{23}N_5O_3S$	1720, 1520
7g		192~193	Diisopropyl ether - $CHCl_3$	C1, D	$C_{48}H_{39}N_5O_3S$	1740, 1600
7h		231~232	Et_2O - $CHCl_3$	C2, D	$C_{29}H_{25}N_5O_3S$	1725, 1615
7i		175~176	MeOH	C1	$C_{48}H_{39}N_5O_3S$	1740, 1600
7j		200~202	Diisopropyl ether - $CHCl_3$	C1	$C_{48}H_{39}N_5O_3S$	1710, 1585
7k		163~164	Et_2O - $CHCl_3$	C1	$C_{48}H_{39}N_5O_3S$	1710, 1510

The 7-acyl cephalosporins listed in Table 3 were prepared by one of the methods outlined in Scheme 2 (Methods E~G) and Scheme 3 (Method H).

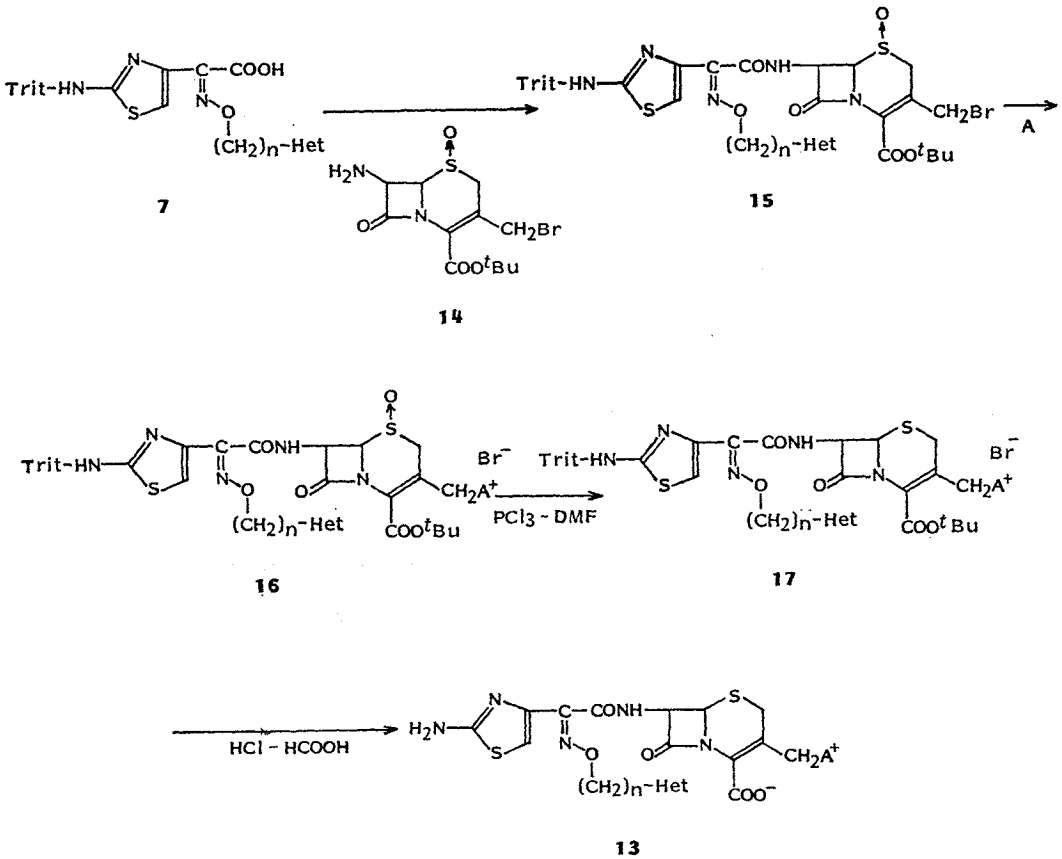
The procedure of Method E was found to be more applicable for the synthesis of various cephem derivatives. The protected cephalosporins (**11**) were prepared by acylation of 7-amino-ceph-3-em-4-carboxylic acid (**10**) (variously substituted at the C-3 position) with aminothiazolylacetic acid (**7**).

Scheme 2.



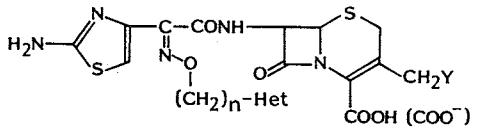
7 **10** **11** **12** **13**

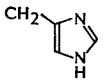
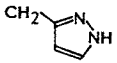
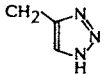
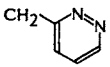
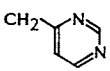
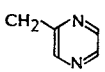
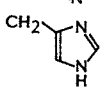
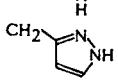
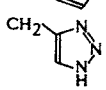
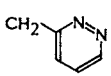
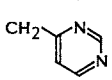
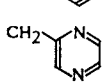
Scheme 3. Method H.

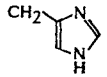
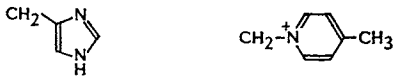
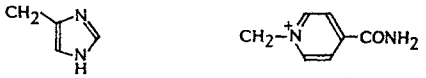
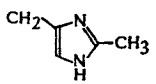
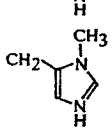
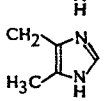
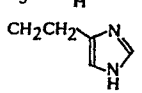
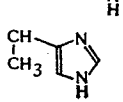


7 **14** **15** **16** **17** **13**

Table 3. 7β-[2-(2-Aminothiazol-4-yl)-2-(hetero aromatic methoxyimino)acetamido]-3-(substituted methyl)ceph-3-em-4-carboxylic acid.



Compound No.	$(CH_2)_n$-Het	CH_2Y	MP (dec) (°C)	Method	Formula	IR (KBr), cm^{-1}
12a		CH_2OAc	155~180	E	$C_{19}H_{19}N_7O_7S_2 \cdot 2HCl \cdot 3H_2O$	1770
12b		CH_2OAc	170~180	E	$C_{19}H_{19}N_7O_7S_2 \cdot 2HCl \cdot H_2O$	1770
12c		CH_2OAc	160	E	$C_{18}H_{18}N_8O_7S_2 \cdot 2HCl \cdot 2H_2O$	1760, 1660
12d		CH_2OAc	145~158	E	$C_{20}H_{19}N_7O_7S_2 \cdot 2HCl \cdot 2H_2O$	1780
12e		CH_2OAc	150~165	E	$C_{20}H_{19}N_7O_7S_2 \cdot 2HCl \cdot \frac{1}{2}H_2O$	1770, 1610
12f		CH_2OAc	170~180	E	$C_{20}H_{19}N_7O_7S_2 \cdot 2HCl \cdot 2\frac{1}{2}H_2O$	1770
13a		CH_2Py^+	150~180	F, G, H	$C_{22}H_{20}N_8O_5S_2 \cdot 3HCl \cdot H_2O$	1780, 1630
13b		CH_2Py^+	170~195	F, G	$C_{22}H_{20}N_8O_5S_2 \cdot 3HCl \cdot 3H_2O$	1770, 1660
13c		CH_2Py^+	175	F, G	$C_{21}H_{19}N_9O_5S_2 \cdot 3HCl \cdot 3H_2O$	1770, 1610
13d		CH_2Py^+	145~155	F, G	$C_{23}H_{20}N_8O_5S_2 \cdot 2\frac{1}{2}H_2O$	1770
13e		CH_2Py^+	160~175	F, G	$C_{23}H_{20}N_8O_5S_2 \cdot H_2O$	1770, 1610
13f		CH_2Py^+	180~195	F, G	$C_{23}H_{20}N_8O_5S_2 \cdot 2\frac{1}{2}H_2O$	1780

12g		H	160~190	F	$C_{16}H_{15}N_7O_5S_2\cdot\frac{1}{2}H_2O$	1770
12h			170~185	F	$C_{19}H_{19}N_{11}O_5S_3\cdot H_2O$	1775
13g			155~170	G	$C_{23}H_{22}N_8O_5S_3\cdot 3HCl\cdot 2H_2O$	1770
13h			165	G	$C_{22}H_{26}N_8O_5S_2\cdot 3HCl\cdot 2H_2O$	1770, 1615
13i			155~170	H	$C_{23}H_{22}N_8O_5S_2\cdot 3HCl\cdot 3H_2O$	1770, 1600
13j			165~190	H	$C_{23}H_{21}N_9O_6S_2\cdot 3HCl\cdot 3H_2O$	1775, 1680
13k			160~180	H	$C_{22}H_{19}ClN_8O_5S_2\cdot 3HCl\cdot \frac{1}{2}H_2O$	1770
13l			160~185	G	$C_{21}H_{19}N_9O_5S_2\cdot 3HCl\cdot 4H_2O$	1780, 1630
13m		CH_2Py^+	150~168	G	$C_{23}H_{22}N_8O_5S_2\cdot 3HCl\cdot 2H_2O$	1780, 1630
13n		CH_2Py^+	155~180	G, H	$C_{23}H_{22}N_8O_5S_2\cdot 3HCl\cdot 1\frac{1}{2}H_2O$	1775
13o		CH_2Py^+	155~185	G	$C_{23}H_{22}N_8O_5S_2\cdot 3HCl\cdot 3H_2O$	1780, 1630
13p		CH_2Py^+	165~190	H	$C_{23}H_{22}N_8O_5S_2\cdot 3HCl\cdot 2\frac{1}{2}H_2O$	1780
13q		CH_2Py^+	145~165	H	$C_{23}H_{22}N_8O_5S_2\cdot 3HCl\cdot 3H_2O$	1785, 1675

Py: Pyridine.

7 was activated with *N,N*-dicyclohexylcarbodiimide (Method E) or with phosphorus pentachloride (Method F), and converted to **11**. The desired cephalosporin **12** was derived from **11** by subsequent removal of the trityl group by the treatment of formic acid containing hydrochloric acid at room temperature.

Compounds **12**, which have an acetoxymethyl group at the 3-position of cephem, were led to derivatives **13** by nucleophilic displacement with a substituted tertiary amine, such as a pyridine derivative, methylpyrrolidine, or a heterocyclic thiol, in the presence of sodium iodide (Method G).

Method H uses 3-bromomethyl cephem *tert*-butyl ester, 1-oxide (**14**) as the cephem compound. Activation of **7** by the acid chloride method followed by coupling with **14** afforded **15** bearing a bromomethyl group at the 3-position. Then **16** was prepared by treating **15** with a pyridine derivative or methylpyrrolidine. Reduction of the *S*-oxide of **16** was performed by treatment with phosphorus trichloride in dimethylformamide, and subsequent removal of the protecting groups afforded **13** in the same way as in Method E.

The structures of **12** and **13** were confirmed from IR and NMR spectral data shown in Table 10 and by Elemental Analysis.

Antibacterial Activity and Discussion

The MIC values of this series of cephalosporins against selected strains of Gram-positive and Gram-negative bacteria were determined by the standard serial 2-fold dilution method.[6]

The effects of various substituents at oxyimino group in the acyl side chain moiety of the 7-position and the commonly used C-3 substituents of the cephem nucleus were examined. Table 4 shows the antibacterial activity of a series of 3-acetoxymethylcephalosporins with various hetero aromatic substituents at position R in structure **1**. Cefotaxime serves as a standard.

Of these, compound **12a** showed the greatest effectiveness against all kinds of Gram-positive and Gram-negative strains shown in Table 4. In activity against only Gram-negative strains, including activity against *P. aeruginosa*, the five-membered hetero aromatic ring (**12a**~**12c**) tended to be more potent than the six-membered (**12d**~**12f**) ring.

Table 5 demonstrates the effect of various substituents at the position R of the cephem (**1**) nucleus with a quaternary pyridinium methyl group in place of the acetoxymethyl of **12** (Y=OAc). As in the case of the substances in Table 4, not all compounds showed significant activity against Staphylococci, but the activities of the five-membered ring derivatives (**13a**~**13c**) against Gram-negative strains were somewhat superior to those of the six-membered ring (**13d**~**13f**) compounds. There was a particularly distinct tendency of this sort in the case of *P. aeruginosa*. Of these five-membered

Table 4. Effect on antibacterial activity (MIC, μg/ml) of the oxime ether group of 3-acetoxymethylcephalosporins.

Compound No.	*S.a.* 209P	*S.a.* Smith	*E.c.* NIHJ	*K.p.* Type 2	*E.cl.* 3402	*S.m.* 10104	*P.a.* 32234
12a	0.78	0.78	0.05	0.10	0.20	0.78	6.25
12b	1.56	0.78	0.05	0.39	0.20	1.56	12.5
12c	3.13	3.13	0.05	0.20	0.20	0.78	12.5
12d	3.13	1.56	0.05	0.20	0.78	1.56	12.5
12e	1.56	1.56	0.10	1.56	1.56	12.5	25
12f	1.56	0.78	0.20	1.56	3.13	6.25	25

Abbreviations: *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *E.cl.*, *Enterobacter cloacae*; *S.m.*, *Serratia marcescens*; *P.a.*, *Pseudomonas aeruginosa*.

Table 5. Effect on antibacterial activity (MIC, μg/ml) of the oxime ether group of 3-pyridiniomethylcephalosporins.

Compound No.	*S.a.* 209P	*S.a.* Smith	*E.c.* NIHJ	*K.p.* Type 2	*E.cl.* 3402	*S.m.* 10104	*P.a.* 32234
13a	0.78	0.78	0.05	0.20	0.20	0.10	0.78
13b	0.39	0.39	0.05	1.56	0.20	0.20	1.56
13c	0.78	0.39	0.05	0.39	0.78	0.10	1.56
13d	0.78	0.78	0.05	0.78	1.56	0.10	3.13
13e	0.78	0.39	0.10	6.25	6.25	1.56	12.5
13f	0.39	0.78	0.05	6.25	3.13	1.56	12.5

Abbreviations: See footnote in Table 4.

Table 6. Effect on antibacterial activity (MIC, μg/ml) of the 3-substituent of imidazolylmethoxyiminocephalosporins.

Compound No.	*S.a.* 209P	*S.a.* Smith	*E.c.* NIHJ	*K.p.* Type 2	*E.cl.* 3402	*S.m.* 10104	*P.a.* 32234
12g	6.25	1.56	0.05	0.10	0.20	0.78	12.5
12a	0.78	0.78	0.05	0.10	0.20	0.78	6.25
12h	0.78	0.78	0.05	0.10	0.10	0.39	12.5
13g	0.39	0.78	0.05	0.20	0.20	0.20	6.25
13h	1.56	1.56	0.05	0.20	0.05	0.20	1.56
13a	0.78	0.78	0.05	0.20	0.20	0.10	0.78
13i	1.56	1.56	0.05	0.20	0.10	0.10	1.56
13j	1.56	1.56	0.05	0.20	0.10	0.20	0.78
13k	0.78	0.78	0.10	0.39	0.10	0.20	1.56
13l	0.78	0.78	0.10	0.78	0.78	0.20	1.56

Abbreviations: See footnote in Table 4.

derivatives, the imidazole compound (**13a**) showed the best activity, again as in the case of the 3-acetoxymethylcephalosporins.

On the basis of the results shown in Tables 4 and 5, the effects of various C-3 substituents of cephem bearing the 2-(imidazol-4-ylmethoxyimino)-2-(2-aminothiazol-4-yl)acetamido group on the antibacterial activity were examined (Table 6). Among compounds that generally have C-3 substituents as Y (compounds **12g, 12a, 12h, 13g, 13h,** and **13a**), only **12g** did not show good activity against Gram-positive strain, *Staphylococcus aureus*. On the other hand, against Gram-negative, ionized compound (**13g, 13h,** and **13a**), especially the pyridium derivative (**13a**), showed high inhibitory potency, particularly to *P. aeruginosa*. A few quaternary aromatic pyridinium and pyridazinium derivatives (**13i**~**13l**) were therefore prepared and tested, but no more active compound was found than the parent compound **13a.**

Table 7 lists the activity of a series of pyridinium methyl cephem compounds with the imidazolylmethoxyimino group bearing a methyl group on the imidazole ring (**13m**~**13o**) and with the methylene moiety modified to ethylene (**13p** and **13q**). These compounds, which may depend on increased lipophilicity or a steric effect contributed by the bulky substituent, had a negative effect in comparison with the parent compound **13a.**

In this study, compound **13a** exhibited the best antibacterial activity. Table 8 presents the MICs of compound **13a** and several reference antibiotics. The activities of **13a** against Gram-negative organisms other than *P. aeruginosa* were almost equivalent to those of the reference drugs; but against *P. aeruginosa*, **13a** was more active than ceftazidime, and against *S. aureus*, as active as, or more active

Table 7. Effect on antibacterial activity (MIC, μg/ml) of the alkyl group on imidazolylmethyl.

Compound No.	*S.a.* 209P	*S.a.* Smith	*E.c.* NIHJ	*K.p.* Type 2	*E.cl.* 3402	*S.m.* 10104	*P.a.* 32234
13m	0.78	1.56	0.20	0.78	0.20	0.20	1.56
13n	0.78	0.78	0.05	0.78	0.78	0.39	6.25
13o	0.78	1.56	0.05	0.39	0.39	0.20	1.56
13p	1.56	1.56	0.20	0.78	0.20	0.39	3.13
13q	3.13	3.13	0.20	3.13	0.78	1.56	25

Abbreviations: See footnote in Table 4.

Table 8. Comparative activities (MIC, μg/ml) of the third-generation cephalosporins.

Compound No.	*S.a.* 209P	*S.a.* Smith	*E.c.* NIHJ	*K.p.* Type 2	*E.cl.* 3402	*S.m.* 10104	*P.a.* 32234
13a	0.78	0.78	0.05	0.20	0.20	0.10	0.78
Cefotaxime	3.13	0.78	0.05	0.05	0.10	0.39	12.5
Cefmenoxime	3.13	0.78	0.05	0.10	0.10	0.10	25
Ceftizoxime	6.25	0.78	0.05	0.05	0.05	0.10	25
Ceftazidime	12.5	3.13	0.10	0.39	0.20	0.20	1.56

Abbreviations: See footnote in Table 4.

Table 9. Stability of compound **13a** to hydrolysis by β-lactamase.

β-Lactamase type	Source	Relative rate of hydrolysis (Vmax)[a]				
		CER	CPZ	CAZ	CTX	**13a**
I_a	*Enterobacter cloacae* GN7471	100	2.8	1	1	1
I_b	*Escherichia coli* 1154	100	4.1	1	1	1
I_c	*Proteus vulgaris* GN76	100	8.1	1	21	3.9
I_d	*Pseudomonas aeruginosa* 2006	100	12	1	1	1

[a] Hydrolysis of each substance is expressed as the relative rate of hydrolysis, taking the absolute rate of cephaloridine as 100. Vmax: Maximum rate of hydrolysis.

CER, Cephaloridine; CPZ, cefoperazone; CAZ, ceftazidime; CTX, cefotaxime.

than cefotaxime and other cephalosporins.

The stabilities of compound **13a** to a few β-lactamases were tested. Table 9 shows the hydrolysis rates of the antibiotics, relative to that of cephaloridine.

It was found that **13a** has good stability to hydrolysis by all of the lactamases employed, although it is less stable than ceftazidime. This stability probably contributes to its broad spectrum of activity.

The comparative affinities of **13a** for penicillin binding proteins (PBPs) were examined, and it became clear that **13a** has a remarkably high affinity for PBP-3 and PBP-1Bs of both *Escherichia coli* and *P. aeruginosa*. PBP-1Bs was known to participate in cell elongation, and PBP-3 participates in septum formation. The excellent activity of **13a** may be attributed in part to its high affinities for these target proteins.

Further, it was learnt that, after intravenous administration in rats and monkeys, **13a** was well distributed to the kidneys, trachea, lungs, liver and muscles and was excreted efficiently in an unchanged form in the urine.[7]

Experimental

MP's were determined on a Yanagimoto MP-1 micro melting point apparatus and are uncor-

rected. IR spectra were taken on a Hitachi 285 spectrophotometer. NMR spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-20B and at 200 MHz on a Varian XL-200 spectrometer using TMS or 3-(trimethylsilyl)propanesulfonic acid, sodium salt (DSS) as the internal standard. HPLC separations were performed with a Waters Model 6000A pump, a Series 440 refractometer detector, and a μ-Porasil (30 cm, 4 mm) column. Mass spectra were measured with a Hitachi RMU-6M mass spectrometer. The results of the elemental analyses were within 0.4% of the theoretical values, except where noted. Organic solvents were dried over anhydrous Na_2SO_4 and all concentrations by evaporation were carried out *in vacuo*.

Methyl *N*-Trityl-1,2,3-triazol-4-ylcarboxylate

A solution of methyl 1,2,3-triazol-4-ylcarboxylate[8] (15.3 g, 0.12 mol) and trityl chloride (36.2 g, 0.13 mol) in $CHCl_3$ (300 ml) was cooled to 5°C, and triethylamine (15.2 g, 0.15 mol) was added thereto. After stirring for 1 hour at room temperature, the resulting mixture was washed successively with diluted HCl and brine, dried over Na_2SO_4, and evaporated to give an oil. Chromatography on a silica gel column, eluting with benzene - $CHCl_3$ (gradient elution), give 14.9 g (34%) of 1 (or 2)-trityl and 21.0 g (47%) of 2 (or 1)-trityl compound. For 1 (or 2)-trityl compound: MP 128~135°C; IR (KBr) cm^{-1} 1745, 1225; ^1H NMR ($CDCl_3$) δ 3.84 (3H, s), 7.0~7.4 (15H, m), 8.14 (1H, s). For 2 (or 1)-trityl compound: MP 191~192°C; IR (KBr) cm^{-1} 1740, 1720; ^1H NMR ($CDCl_3$) δ 3.92 (3H, s), 7.0~7.5 (15H, m), 8.04 (1H, s).

N-Trityl-1,2,3-triazol-4-ylmethanol

To an ice-cooled suspension of $LiAlH_4$ (2.2 g, 0.058 mol) in THF (200 ml) was added dropwise a solution of methyl 1 (or 2)-trityltriazol-4-ylcarboxylate (21.5 g, 0.058 mol) in THF (300 ml). After stirring for 40 minutes at room temperature, successive additions of H_2O (2.2 ml), 15% aqueous NaOH (2.2 ml), and H_2O (4.4 ml) were made to the resulting mixture. The precipitate was removed by filtration and the filtrate was evaporated to give 19.0 g (95%) of 1 (or 2)-trityltriazol-4-ylmethanol as a white solid: MP 168~169°C; IR (KBr) cm^{-1} 3600~3100, 1600, 1490, 1450; ^1H NMR ($CDCl_3$) δ 2.40 (1H, t, J=6.5 Hz), 4.68 (2H, d, J=6.5 Hz), 7.0~7.4 (15H, m), 7.66 (1H, s).

In the same manner, 2 (or 1)-trityltriazol-4-ylmethanol was obtained from 2 (or 1)-trityltriazol-4-ylcarboxylate methyl ester (yield 92%): MP 205~207°C; IR (KBr) cm^{-1} 3600~3100, 1445; ^1H NMR ($CDCl_3$) δ 4.78 (2H, s), 7.0~7.5 (16H, m).

4-Chloromethylimidazole Hydrochloride (**3a**)

4-Hydroxymethylimidazole hydrochloride[9] (673 mg, 5 mmol) was suspended in benzene (3 ml) and to this stirred mixture was added dropwise a solution of thionyl chloride (0.5 ml, 7 mmol) in benzene (5 ml) with stirring. The resulting mixture was then refluxed for 2 hours, and was chilled to room temperature. The precipitates formed were collected by filtration, and washed with benzene to give 750 mg (98%) of the desired compound as a crystalline solid: MP 135~140°C.

The following examples are representative of each procedure in Methods A~H.

Method A: *N*-Tritylimidazol-4-ylmethoxyphthalimide (**4a**)

i) Imidazol-4-ylmethoxyphthalimide: *N*-Hydroxyphthalimide (3.26 g, 20 mmol) was added to an ice-cooled solution of triethylamine (4.0 g, 0.04 mol) in $CHCl_3$ (50 ml). To this solution, 4-chloromethylimidazole hydrochloride (**3a**: 1.53 g, 0.01 mol) was added portionwise at the same temperature and stirred for 14 hours. After evaporation of the solvent, the residue was dissolved in 5% aqueous Na_2CO_3, and was extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4 and evaporated. The residue was crystallized from a mixture of diisopropyl ether and Et_2O to give 1.61 g (66%) of the title compound as a crystalline solid: MP 163~165°C; IR (KBr) cm^{-1} 1780, 1730, 1390; ^1H NMR (DMSO-d_6) δ 5.12 (2H, s), 7.15 (1H, s), 7.53 (1H, s), 7.78 (4H, s).

Anal Calcd for $C_{12}H_9N_3O_3$: C 59.26, H 3.73, N 17.28.
Found: C 59.45, H 3.70, N 17.33.

ii) *N*-Tritylation of Imidazol-4-ylmethoxyphthalimide: To a solution of imidazol-4-ylmethoxyphthalimide (1.216 g, 5 mmol) in $CHCl_3$ (50 ml) was added tritylchloride (1.39 g, 5 mmol), and thereto triethylamine (505 mg, 5 mmol) was added while cooling the mixture in an ice-bath. The resulting

fluid was washed successively with 5% aqueous $NaHCO_3$ and brine, then dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from a mixture of Et_2O and petroleum ether to give 1.62 g (67%) of *N*-tritylimidazol-4-ylmethoxyphthalimide (**4a**): MP 155~157°C; IR (KBr) cm^{-1} 1780, 1725; ^1H NMR ($CDCl_3$) δ 5.18 (2H, s), 7.0~7.4 (17H, m), 7.25 (4H, s).

Anal Calcd for $C_{31}H_{23}N_3O_3$: C 76.68, H 4.77, N 8.65.
Found: C 76.69, H 4.76, N 8.87.

Method B: Pyridazin-3-ylmethoxyphthalimide (**4d**)

Diethyl azodicarboxylate (6.55 g, 0.025 mol) was added to a mixture of 3-hydroxymethylpyridazine[10] (2.20 g, 0.02 mol), *N*-hydroxyphthalimide (3.26 g, 0.02 mol) and triphenylphosphine (5.24 g, 0.02 mol) in THF (30 ml). After stirring for 2 hours at room temperature, the resulting mixture was evaporated and the residue was dissolved in EtOAc, washed successively with 5% aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4 and evaporated. Trituration of the residue with diisopropyl ether gave 3.32 g (65%) of **4d** as a crystalline solid: MP 139~140°C; IR (KBr) cm^{-1} 1785, 1725; ^1H NMR ($CDCl_3$) δ 5.52 (2H, s), 7.5~7.9 (1H, m), 7.7~7.9 (1H, m), 9.20 (1H, m).

Anal Calcd for $C_{13}H_9N_3O_3$: C 61.18, H 3.55, N 16.46.
Found: C 61.23, H 3.48, N 16.51.

Method C1: 2-(2-Tritylaminothiazol-4-yl)-2-(*N*-tritylimidazol-4-ylmethoxyimino)acetic Acid (**7a**)

To a suspension of **4a** (9.71 g, 0.02 mol) in EtOH (100 ml) was added a solution of 100% hydrazine hydrate (0.02 mol) in MeOH (7 ml) ar room temperature. The resulting mixture was stirred for 4 hours. The insoluble material precipitated was removed by filtration and the filtrate was adjusted to pH 7.0 with diluted HCl. To this neutral solution was added 2-tritylaminothiazolylglyoxylic acid (**6**) (0.02 mol), and the mixture was stirred at room temperature for 8 hours. The precipitate formed was collected by filtration and washed with EtOH and Et_2O to afford 11.5 g (76.5%) of the title compound **7a**: MP 186~188°C (dec); IR (KBr) cm^{-1} 1720, 1615; ^1H NMR (DMSO-d_6) δ 4.96 (2H, s), 6.77 (1H, s), 6.9~7.5 (32H, m), 8.77 (1H, s).

Anal Calcd for $C_{47}H_{37}N_5O_3S$: C 75.08, H 4.96, N 9.31.
Found: C 74.99, H 4.95, N 9.38.

Method C2: 2-(2-Tritylaminothiazol-4-yl)-2-(pyridazin-3-yl)acetic Acid (**7d**)

A suspensions of pyridazin-3-ylmethoxyphthalimide (**4d**) (1.28 g, 5 mmol) in 6 N HCl (20 ml) was refluxed for 1 hour. The resulting mixture was evaporated to dryness. The resulting crystalline residue was dissolved in H_2O (4 ml), and was adjusted to pH 7 with 5% $NaHCO_3$. To this solution was added a suspension of 2-tritylaminothiazolylglyoxylic acid (**6**) (2.07 g, 5 mmol) in EtOH (40 ml), and the mixture was stirred overnight at room temperature. The precipitate formed was collected by filtration and recrystallized from Et_2O - $CHCl_3$ to give 1.08 g (69%) of the title compound **7d**: MP 160~166°C (dec); IR (KBr) cm^{-1} 1715, 1620; ^1H NMR ($CDCl_3$) δ 5.50 (2H, s), 6.68 (1H, s), 7.26 (15H, s), 7.5~7.8 (2H, m), 9.04 (1H, m).

Anal Calcd for $C_{29}H_{23}N_5O_3S$: C 66.78, H 4.44, N 13.47.
Found: C 66.83, H 4.52, N 13.57.

Method D: 2-(2-Tritylaminothiazol-4-yl)-2-(1-methylimidazol-4-ylmethoxyimino)acetic Acid (**7h**)

To an ice-cooled solution of **8** (8.51 g, 0.02 mol) and EtONa (3.04 g, 0.044 mol) in DMF (50 ml) was added 3-methyl-4-chloromethylimidazole hydrochloride (3.67 g, 0.022 mol; prepared as described for 4-chloromethylimidazole hydrochloride (**3a**) from the hydroxymethyl compound[11]) and the mixture was stirred for 1 hour at the same temperature, and for another 2 hours at room temperature. The resulting mixture was poured into EtOAc (100 ml), washed with H_2O and dried over Na_2SO_4. The EtOAc was evaporated *in vacuo* to afford oily **9** (Het=1-methylimidazol-5-yl), which was used in the following reaction without further purification.

This oil (**9**) was dissolved in MeOH (100 ml), and refluxed with 2 N NaOH (25 ml) for 1.5 hours. After removing the solvent, the residue was dissolved in H_2O. The aqueous solution was adjusted to pH 5~6 with diluted HCl under ice-cooling, and the resulting mixture was stirred at this temperature for 30 minutes. The precipitate was collected by filtration, washed with H_2O, and crystallized from

Et_2O - $CHCl_3$ to afford 6.73 g (64.3%) of **7h**: MP 231~232°C (dec); IR (KBr) cm^{-1} 1725, 1615; ^1H NMR ($CDCl_3$) δ 3.63 (3H, s), 5.22 (2H, s), 6.83 (1H, s), 6.9~7.5 (17H, m).

Anal Calcd for $C_{29}H_{25}N_5O_3S$: C 66.52, H 4.81, N 13.38.
Found: C 66.48, H 4.92, N 13.67.

Method E: 7β-[2-(2-Aminothiazol-4-yl)-2-(imidazol-4-ylmethoxyimino)acetamido]-3-acetoxymethyl-ceph-3-em-4-carboxylic Acid (**12a**)

i) Acylation of Cephalosporanic Acid: *N,N*-Dicyclohexylcarbodiimide (412 mg, 2 mmol) was added to a mixture of **7a** (1.5 g, 2 mmol) and 1-hydroxybenztriazole (306 mg, 2.26 mmol) in CH_2Cl_2 (40 ml). After 5 hours of stirring at room temperature, the mixture was filtered and the filtrate was added to a stirred solution of 7β-aminocephalosporanic acid (**10**; Y=OAc) (545 mg, 2 mmol) and triethylamine (1.4 ml) in CH_2Cl_2 (10 ml). The mixture was stirred for 12 hours at room temperature. After evaporation of the solvent, the residue was diluted with H_2O, made acidic with 10% citric acid, and extracted with EtOAc. The organic layer was separated, washed with H_2O, dried over Na_2SO_4 and evaporated, Trituration of the residue with Et_2O afforded a powdery product containing protected compound **11**.

ii) Removal of Protecting Groups: The product **11** was dissolved in a mixture of formic acid (50 ml) and concentrated HCl (4 ml), and stirred for 2 hours at room temperature. The reaction mixture was evaporated to dryness and acetone (100 ml) was added the residue. The precipitate formed was collected by filtration and dissolved in 5% $NaHCO_3$ to adjust the pH to 7, and subjected to column chromatography on a non-ionic adsorption resin (Diaion HP-20). The column was washed with H_2O and eluted with 15% aqueous MeOH, followed by lyophilization of fractions containing the desired product to afford the desired compound. With addition of HCl, this product was converted to HCl salt **12a**: MP 155~180°C (dec); IR (KBr) 1770 cm^{-1}; ^1H NMR (D_2O) δ 2.12 (3H, s), 3.46 (1H, d, J=18 Hz), 3.75 (1H, d, J=18 Hz), 5.25 (1H, d, J=5 Hz), 5.44 (2H, s), 5.86 (1H, d, J=5 Hz), 7.25 (1H, s), 7.66 (1H, s), 8.80 (1H, s).

Anal Calcd for $C_{19}H_{19}N_7O_7S_2 \cdot 2HCl \cdot 3H_2O$: C 35.19, H 4.20, N 15.12.
Found: C 35.01, H 4.38, N 15.33.

Method F: 7β-[2-(2-Aminothiazol-4-yl)-2-(pyrazol-3-ylmethoxyimino)acetamido]-3-pyridiniomethyl-ceph-3-em-4-carboxylic Acid (**13b**)

A solution of phosphorus pentachloride (417 mg, 2 mmol) in CH_2Cl_2 (12 ml) was cooled to −15°C, and **7b** (1.5 g, 2 mmol) was added thereto. After 15 minutes of stirring, triethylamine (202 mg, 2 mmol) was added to the mixture, and after 5 minutes, a solution of 7-amino-3-pyridiniomethyl-ceph-3-em-4-carboxylate dihydrochloride[12] (729 mg, 2 mmol) and bis(trimethylsilyl)acetamide (2.4 ml) in CH_2Cl_2 (20 ml) was added. The resulting mixture was stirred for 30 minutes at the same temperature. The solution was washed with 5% $NaHCO_3$ solution. The organic layer was washed with 5% citric acid and then washed with H_2O, dried and evaporated to dryness to give a powder (2.1 g) of protected compound **11**.

Removal of the protecting group was performed as described in Method E to afford the title compound **13b**: MP 170~195°C (dec); IR (KBr) cm^{-1} 1770, 1660; ^1H NMR (D_2O) δ 3.12 (1H, d, J=18 Hz), 3.60 (1H, d, J=18 Hz), 5.23 (1H, d, J=5 Hz), 5.26 (2H, s), 5.36 (1H, d, J=13 Hz), 5.57 (1H, d, J=13 Hz), 5.83 (1H, d, J=5 Hz), 6.47 (1H, d, J=2 Hz), 6.98 (1H, s), 7.63 (1H, d, J=2 Hz), 8.10 (2H, t, J=7 Hz), 8.59 (1H, t, J=7 Hz), 8.96 (2H, d, J=7 Hz).

Anal Calcd for $C_{22}H_{20}N_8O_5S_2 \cdot 3HCl \cdot 3H_2O$: C 37.53, H 4.15, N 15.92.
Found: C 37.72, H 4.44, N 16.22.

Method G: 7β-[2-(2-Aminothiazol-4-yl)-2-(imidazol-4-ylmethoxyimino)acetamido]-3-acetoxymethyl-ceph-3-em-4-carboxylic Acid (**13i**)

12a (2.72 g, 5 mmol) was added to a mixture of 4-methylpyridine (95 mg, 10 mmol), NaI (4.5 g) and H_2O (0.5 ml) at 80°C with stirring, which was continued for 1 hour at the same temperature. The mixture was cooled to room temperature, diluted with H_2O and adjusted to pH 2.5 with 1 N HCl. An insoluble material was filtered off and the filtrate was washed with EtOAc, evaporated to remove solvent and subjected to column chromatography (Diaion HP-20). After the column was washed

Table 10. Spectral data of compounds **12** and **13**.

Compound No.	^{1}H NMR δ value (D_2O+DCl)								IR (KBr) (cm^{-1})
	2-CH_2 (2H, ABq, J=18 Hz)	3-CH_2 (2H, ABq, J=13 Hz)	6-H (1H, d, J=5 Hz)	7-H (2H, s, J=5 Hz)	OCH_2 (2H, s)	Thiazole (1H, s)	Other protons		
							Het	Y	
12a	3.61	4.37	5.25	5.86	5.44	7.25	7.66 (1H, s), 8.80 (1H, s)	2.12 (3H, s)	1770
12b	3.50	[a]	5.16	5.87	5.40	7.02	6.50 (1H, s), 7.65 (1H, d)	2.10 (3H, s)	1770
12c	3.48	[a]	5.17	5.80	5.41	7.06	8.04 (1H, s)	2.10 (3H, s)	1760, 1660
12d	3.47	4.83	5.17	5.84	5.57	7.08	7.8~8.0 (2H, m), 9.1~9.2 (2H, m)	2.08 (3H, s)	1780
12e	3.53	4.83	5.25	5.91	5.42	7.11	7.67 (1H, d,) 8.83 (1H, d), 9.13 (1H, s)	2.10 (3H, s)	1770, 1610
12f	3.45	[a]	5.16	5.81	5.43	7.05	8.5~8.8 (3H, br s)	2.10 (3H, s)	1770
13a	3.51	[a]	5.32	5.90	5.42	7.23	7.64 (1H, s), 8.78 (1H, s),	8.16 (2H, t), 8.65 (1H, t), 9.00 (2H, d)	1780, 1630
13b	3.36	5.47	5.23	5.83	5.26	6.98	6.47 (1H, d), 7.63 (1H, d)	8.10 (2H, t), 8.59 (1H, t), 8.96 (2H, d)	1770, 1660
13c	3.37	5.47	5.23	5.84	5.39	7.04	8.02 (1H, s)	8.13 (2H, t), 8.63 (1H, t), 8.96 (2H, d)	1770, 1610
13d	3.35	5.51	5.26	5.89	5.55	7.06	7.70 (2H, m), 9.15 (1H, m)	8.15 (2H, t), 8.63 (1H, t), 9.00 (2H, d)	1770
13e	3.40	5.46	5.30	5.94	5.37	7.07	7.62 (1H, d), 8.78 (1H, d)	8.10 (2H, t), 8.56 (1H, t), 9.02 (2H, d)	1770, 1610
13f	3.40	5.45	5.26	5.88	5.42	7.04	8.5~8.8 (3H, m)	8.05 (2H, t), 8.65 (1H, t), 9.01 (2H, d)	1780

12g	3.52	[a]	5.16	5.85	5.40	7.25	7.70 (1H, s), 8.80 (1H, s)	6.8 (1H, m)	1770
12h	3.74	[a]	5.23	5.80	5.43	7.24	7.66 (1H, s), 8.80 (1H, s)	4.10 (3H, s)	1775
13g	3.67	[a]	5.23	5.81	5.43	7.25	7.65 (1H, s), 8.78 (1H, s)	4.23 (3H, s), 7.83 (2H, d), 8.46 (2H, d)	1770
13h	[a]	[a]	5.36	5.86	5.40	7.20	7.64 (1H, s), 8.76 (1H, s)	2.1~2.4 (4H, br), 3.00 (3H, s), 3.4~3.8 (4H, br s)	1770, 1615
13i	3.47	5.44	5.30	5.85	5.40	7.22	7.64 (1H, s), 8.76 (1H, br s)	2.66 (3H, s), 7.94 (2H, d), 8.76 (2H, br)	1770, 1600
13j	3.51	[a]	5.31	5.89	5.39	7.20	7.62 (1H, s), 8.75 (1H, s)	8.42 (2H, d), 9.17 (2H, d)	1775, 1680
13k	3.53	5.61	5.34	5.91	5.41	7.24	7.64 (1H, s), 8.77 (1H, s)	8.15 (1H, br), 8.71 (1H, d), 9.01 (1H, d)	1770
13l	3.52	5.80	5.30	5.88	5.42	7.22	7.65 (1H, s), 8.78 (1H, s)	8.62 (2H, m)	1780, 1630
13m	3.51	5.54	5.30	5.90	5.32	7.21	2.60 (3H, s), 7.44 (1H, s)	8.16 (2H, t), 8.64 (1H, t), 9.00 (2H, d)	1780, 1630
13n	3.45	5.53	[a]	5.87	5.44	7.20	3.90 (3H, s), 7.65 (1H, s), 8.76 (1H, d)	8.14 (2H, t), 8.63 (1H, t), 8.97 (2H, d)	1775
13o	3.48	5.54	5.30	5.88	5.36	7.21	2.36 (3H, s), 8.61 (1H, s)	8.16 (2H, t), 8.64 (1H, t), 9.00 (2H, d)	1780, 1630
13p	3.47	5.54	5.28	5.85	[a]	7.15	7.30 (1H, s), 8.64 (1H, s)	8.14 (2H, t), 8.64 (1H, t), 8.99 (2H, d)	1780
13q	3.51	[a]	5.15	6.15	[a]	7.26	7.61 (1H, s), 8.75 (1H, s)	8.16 (1H, t), 8.66 (1H, t), 9.01 (2H, d)	1785, 1675

[a] It was difficult to read the values because the signals overlapped with those of H_2O or other protons.

with H_2O, the elution was carried out with 20% aqueous MeOH. The fractions containing the product were lyophilized to afford a powder. With the addition of diluted HCl, this compound was converted to 922 mg of the HCl salt of **13i**: MP 155~170°C (dec); IR (KBr) cm^{-1} 1770, 1600; ^1H NMR (D_2O) δ 2.66 (3H, s), 3.26 (1H, d, J=18 Hz), 3.68 (1H, d, J=18 Hz), 5.30 (1H, d, J=5 Hz), 5.40 (2H, s), 5.58 (1H, d, J=13 Hz), 5.85 (1H, d, J=5 Hz), 7.22 (1H, s), 7.64 (1H, s), 7.94 (2H, d, J=7 Hz), 8.76 (3H, br).

Anal Calcd for $C_{23}H_{22}N_8O_5S_2 \cdot 3HCl \cdot 3H_2O$: C 38.47, H 4.35, N 15.61.
Found: C 38.68, H 4.20, N 15.89.

Method H: 7β-[2-(2-Aminothiazol-4-yl)-2-(imidazol-4-ylmethoxyimino)acetamido]-3-(3-chloropyridiniomethyl)ceph-3-em-4-carboxylate (**13k**)

i) *tert*-Butyl 7β-[2-(2-Aminothiazol-4-yl)-2-(2-tritylimidazol-4-ylmethoxyimino)acetamido]-3-bromomethyl-ceph-3-em-4-carboxylate, 1-Oxide (**15**): A solution of phosphorus pentachloride (420 mg, 2 mmol) in CH_2Cl_2 (10 ml) was added to **7a** (1.5 g, 2 mmol) with stirring and cooling at −20°C. The mixture was stirred for 30 minutes at −15°C to −12°C. To this mixture containing acid chloride was added triethylamine (0.5 ml) at −15°C. After stirring for 5 minutes, a solution of *tert*-butyl 7β-amino-3-bromomethyl-ceph-3-em-4-carboxylate, 1-oxide, hydrochloride[13] (803 mg, 2 mmol) and triethylamine (0.28 ml, 2 mmol) in CH_2Cl_2 (10 ml) was added. The resulting mixture was stirred for 40 minutes at the same temperature, cooled CH_2Cl_2 (50 ml) was added, and this was followed by washing successively with H_2O, 5% $NaHCO_3$ solution and saturated NaCl solution. The product was dried and evaporated to dryness to give 1.92 g of the desired compound **15** as foam, which was used for the next step without further purification: IR (KBr) cm^{-1} 1795, 1715, 1675; ^1H NMR ($CDCl_3$) δ 1.55 (9H, s), 4.65 (1H, d, J=5 Hz), 5.32 (2H, s), 6.70 (1H, s), 6.74~7.60 (17H, m).

ii) *tert*-Butyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-(*N*-tritylimidazol-4-ylmethoxyimino)acetamido]-3-(3-chloropyridiniomethyl)ceph-3-em-4-carboxylate, 1-Oxide, Bromide (**16**): The above product (**15**: 1.90 g) was dissolved in acetone (50 ml) and 3-chloropyridine (2 g) was added during cooling in an ice-bath. The resulting mixture was stirred for 14 hours at room temperature. The solvent was distilled off and the residue was dissolved in acetone (3 ml). After the addition of Et_2O to the mixture with stirring, the precipitate was collected by filtration to give 1.53 g of **16**.

iii) Removal of *S*-Oxide and Deprotection of Compound **16**: Compound **15** was dissolved in DMF (15 ml) and the solution was cooled to −50°C to −60°C. Phosphorus trichloride (0.24 ml, 2.7 mmol) was added while stirring. The resulting mixture was stirred for 30 minutes at −40°C and then poured into $CHCl_3$ (50 ml). This mixture was washed twice with saturated NaCl solution and with H_2O. The organic layer was dried and evaporated. The residue was deprotected as described in Method E to afford the HCl salt of desired compound **13k**: MP 160~180°C (dec); IR (KBr) 1770 cm^{-1}; ^1H NMR (D_2O) δ 3.30 (1H, d, J=18 Hz), 3.76 (1H, d, J=18 Hz), 5.34 (1H, d, J=5 Hz), 5.41 (2H, s), 5.42 (1H, d, J=13 Hz), 5.79 (1H, d, J=13 Hz), 5.91 (1H, d, J=5 Hz), 7.24 (1H, s), 7.64 (1H, s), 8.15 (1H, br), 8.71 (1H, d, J=7 Hz), 8.77 (1H, s), 9.01 (1H, d, J=7 Hz), 9.21 (1H, s).

Anal Calcd for $C_{22}H_{19}ClN_8O_5S_2 \cdot 3HCl \cdot \frac{1}{2}H_2O$: C 38.11, H 3.34, N 16.16.
Found: C 37.92, H 3.35, N 16.28.

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