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# Studies on Anti-MRSA Parenteral Cephalosporins

## I. Synthesis and Antibacterial Activity of 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido]-3-(substituted imidazo[1,2-*b*]-pyridazinium-1-yl)methyl-3-cephem-4-carboxylates and Related Compounds

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In order to improve the antibacterial activity of cefozopran (CZOP) against methicillin-resistant *Staphylococcus aureus* (MRSA), we initiated chemical modification to introduce a 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyimino acetyl group at the C-7 position and a 3- or 6-substituted imidazo[1,2-*b*]pyridazinium or 5-substituted imidazo[1,2-*a*]pyridinium group at the C-3′ position. Although this approach successfully enhanced the anti-MRSA activity of CZOP two to eight times, a slight decrease in the activity against Gram-negative bacteria including *Pseudomonas aeruginosa* was involved. Among the novel derivatives, 3-(6-aminoimidazo[1,2-*b*]pyridazinium-1-yl)methyl-7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido]-3-cephem-4-carboxylate (**44a**) showed an excellent balance of activity against MRSA and Gram-negative bacteria.

Nosocomial infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is still a big problem in many countries[1,2], although MRSA was first identified nearly four decades ago in England[3]. In Japan, the selection pressure resulting from the 1980's incorrect use of third-generation cephalosporins having insufficient antibacterial activity against *S. aureus* has promoted the wide prevalence of MRSA, which continues to be as high as 60 percent of all clinical isolates of *S. aureus* strains[4]. Although cephalosporins such as cefepime[5], cefpirome[6], cefozopran[7] (CZOP), cefluprenam[8] and cefoselis[9], so-called fourth-generation cephalosporins, have better antibacterial activity against *S. aureus* than their third-generation counterparts, their activity against MRSA proved to be insufficient. Additionally, there is no β-lactam antibiotic which can be used for treatment of MRSA infection. Other antibiotics available for treatment of MRSA infection are the glycopeptide antibiotics vancomycin (VCM) and teicoplanin, and the aminoglycoside antibiotic arbekacin. Certain populations of MRSA, however, have been found to be resistant to teicoplanin and arbekacin. Although VCM-resistant *S. aureus* was not isolated until recently[10], the undesirable side effects that can occur with the use of VCM[11] have been a problem. The development of second- or third-line alternatives for VCM in the treatment of MRSA infection has, therefore, been accelerated.

MRSA is characterized by production of penicillin-binding protein 2′ (PBP-2′), of which the affinity for most β-lactam antibiotics is quite low. Since PBP-2′ of MRSA is a multi-functional PBP capable of playing the role of both PBP-1 and PBP-2, MRSA can synthesize peptidoglycan even when only PBP-2′ is functioning[12]. Thus, the activity of a β-lactam antibiotic against MRSA is correlated with its affinity for PBP-2′[13]. According to a recent report, certain chemical modification of β-lactam antibiotics can augment

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their affinity for PBP-2′ and enhances the activity against MRSA. In cephalosporin derivatives, replacement of the C-7 acyl group with a lypophilic phenyl acetyl group generally improves the antibacterial activity against Gram-positive bacteria including MRSA but decreases the activity against Gram-negative bacteria[14]. In the series of zwitter ionic cephalosporin derivatives with a C-7 aminothiazole substituent, combination of a C-7 hydroxyiminoaminothiazolyl group and a lipophilic but polar C-3 substituent can afford a compound with potent antibacterial activity against both Gram-negative and Gram-positive bacteria including MRSA[13]. In these studies, TOC-39[15], TOC-50[16] and MC-02,331[17] were reported as potential candidates for development as the next generation of cephalosporin derivatives with anti-MRSA activity.

Considering the safety of cephalosporin derivatives compared with other classes of antibiotics, we have started chemical modification of CZOP in hopes of discovering a novel anti-MRSA antibiotic. In this paper, synthesis and structure-activity relationships of the CZOP derivatives bearing a 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyimino acetyl group at the C-7 position and a 3- or 6-substituted imidazo[1,2-*b*]pyridazinium or 5-substituted imidazo[1,2-*a*]pyridinium group at the C-3′ position are described.

## Chemistry

### Synthesis of 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido] CZOP Derivative **11**

In order to obtain the novel oxyiminoacetic acid derivative **1a**, bearing an acid-removable methoxymethyl (MOM) group at the oxyimino moiety, we successfully applied the practical method[18] for preparation of 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-methoxyiminoacetic acid (**1b**), which is the C-7 acyl moiety of CZOP (Scheme 1).

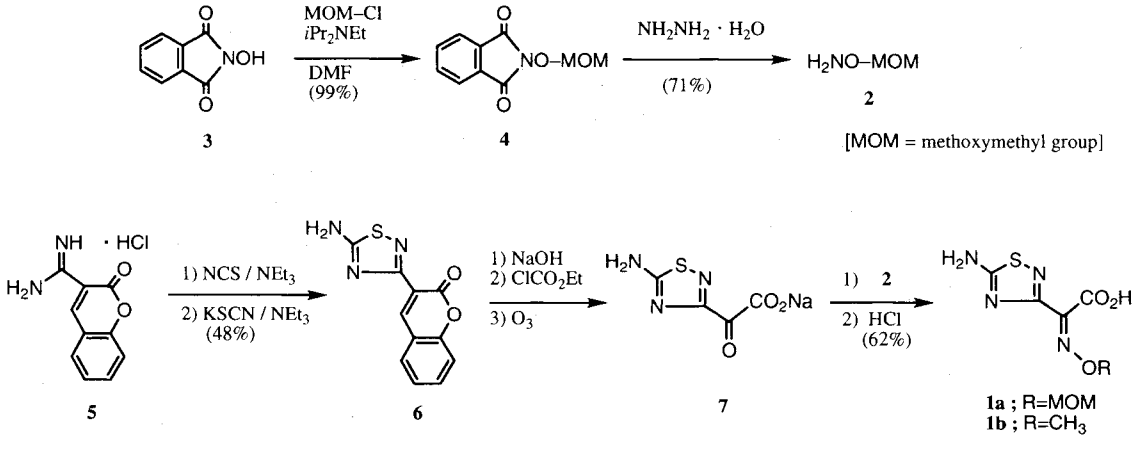
*O*-Methoxymethylhydroxylamine (**2**) was prepared from *N*-hydroxyphthalimide (**3**) in 70% overall yield. Condensation of **2** with the sodium salt of α-ketoacid **7** prepared from 3-amidinocoumarin[18] (**5**) followed by acidification gave the desired *Z*-isomer **1a** as a single product in 62% yield.

Scheme 2 illustrates the method for preparation of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**11**) from **1a**. Treatment of the acid **1a** with phosphorus pentachloride gave the acid chloride hydrochloride **8** in 76% yield. Condensation of **8** with 7β-amino-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate dihydrochloride[19,20] (**9**) in the presence of tri-*n*-butylamine under aqueous conditions gave 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-methoxymethoxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**10**) in 72% yield. Removal of the MOM group in **10** was achieved by treatment with TFA/anisole to afford the desired compound **11** in 50% yield.

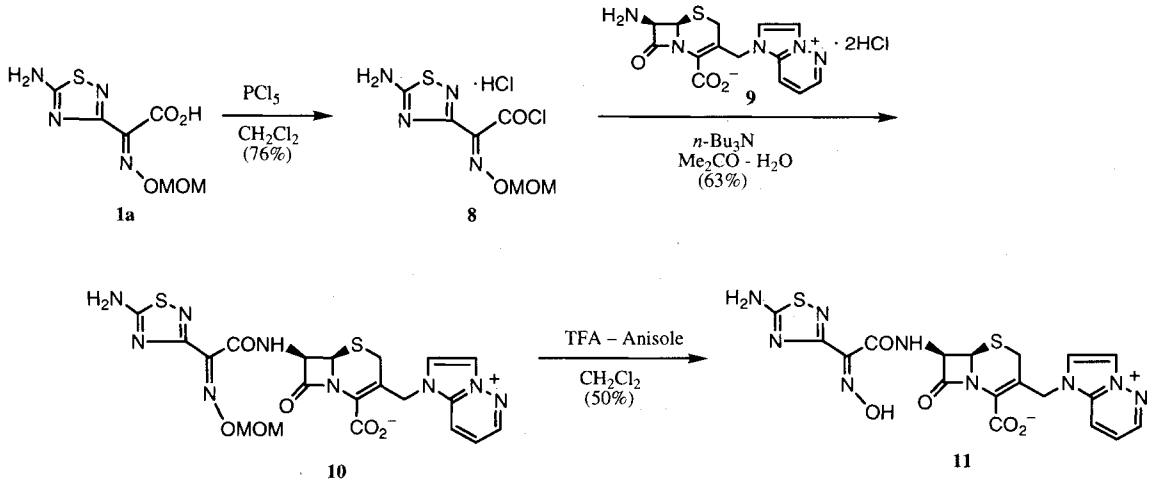
### Synthesis of the Substituted Imidazo[1,2-*b*]pyridazine and Imidazo[1,2-*a*]pyridine Derivatives

6-Chloroimidazo[1,2-*b*]pyridazine[21] (**12**) is a suitable precursor for modification of the imidazo[1,2-*b*]pyridazine skeleton at the 3- and 6-positions as shown in Scheme 3 and Scheme 4. Nucleophilic substitution reactions of the 6-chloro group with ammonia, methylamine and sodium methoxide afforded the 6-amino **13**, 6-methylamino **14** and

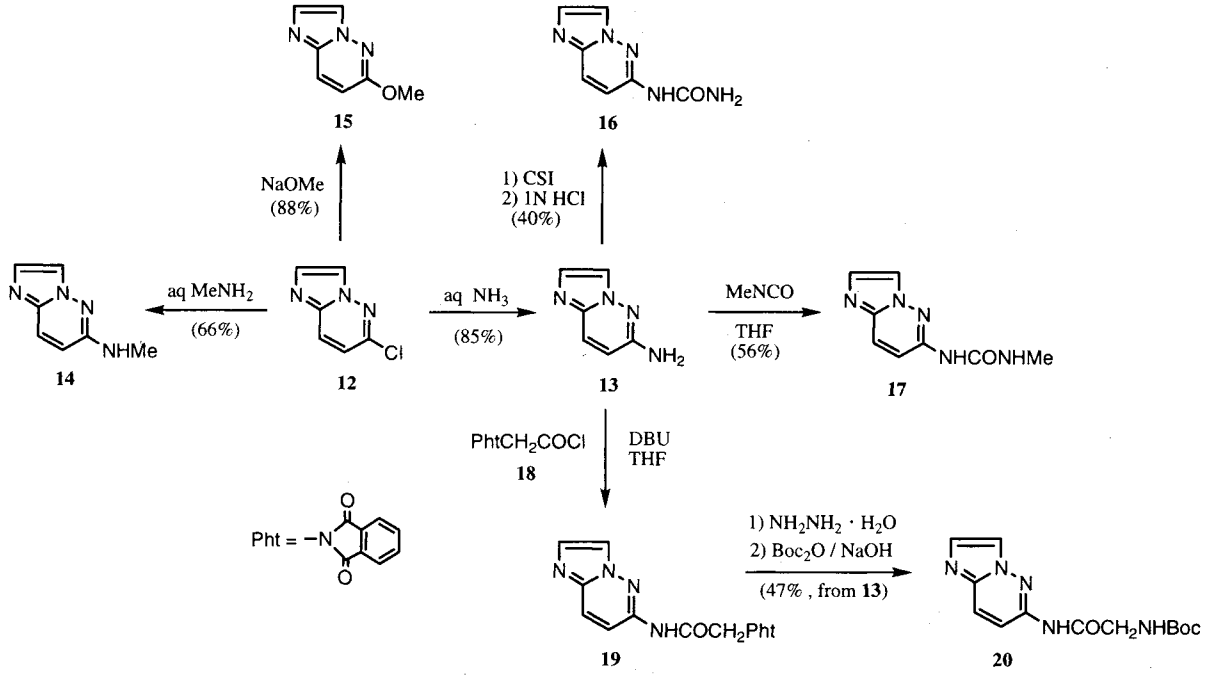
Scheme 1.



Scheme 2.



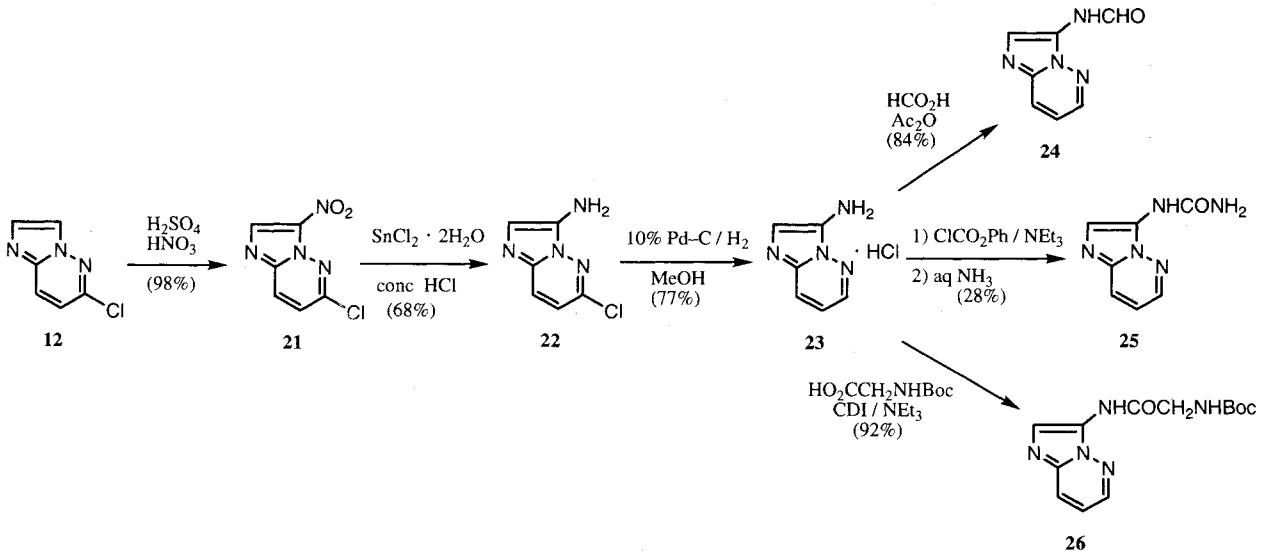
Scheme 3.



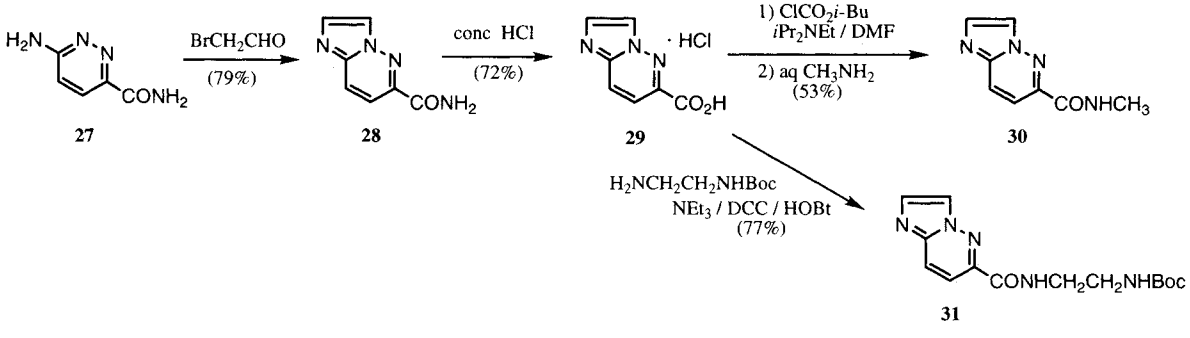
6-methoxy **15** derivatives, respectively, in good yields. Further transformation of the 6-amino derivative **13** gave a variety of the imidazo[1,2-*b*]pyridazines. Reaction of **13** with chlorosulfonyl isocyanate (CSI) followed by 1 N HCl treatment gave the ureido derivative **16** in 40% yield. Similarly, treatment of **13** with methyl isocyanate in THF afforded the *N*-methylureido derivative **17** in 56% yield. Acylation of **13** with phthalimidoacetyl chloride[22] (**18**) proceeded under reflux conditions in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to provide the intermediate **19**, which was converted into the Boc protected derivative **20** by deprotection followed by Boc protection in 47% overall yield from **13**.

Nitration of **12** under the usual conditions afforded the

Scheme 4.




Scheme 5.

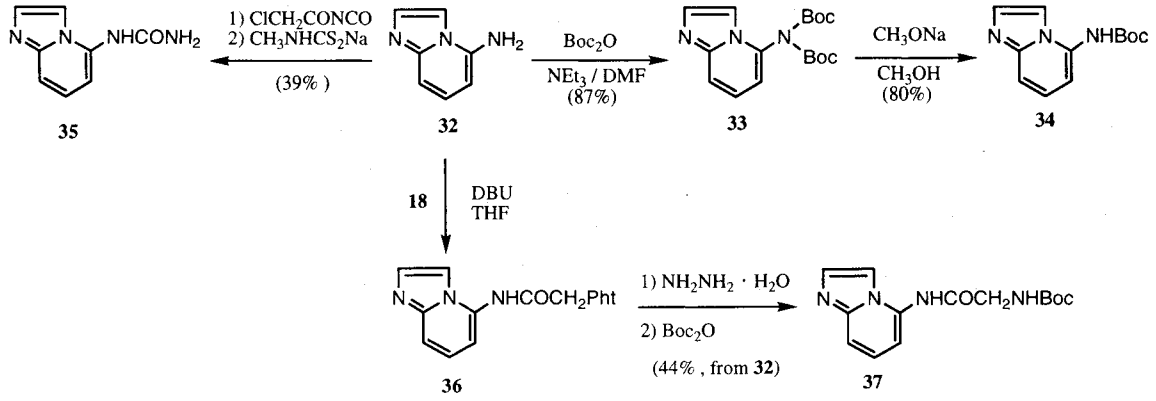



6-chloro-3-nitroimidazo[1,2-*b*]pyridazine[23] (**21**) in 98% yield. The stepwise reduction procedure, reduction of the nitro group with stannous chloride in conc HCl and hydrogenolysis of the chloro group in the presence of 10% palladium on charcoal under a hydrogen atmosphere, converted **21** into the novel intermediate 3-aminoimidazo[1,2-*b*]pyridazine hydrochloride (**23**) *via* the intermediate **22**. Formylation of **23** gave **24** in 84% yield. Treatment of **23** with phenyl chloroformate followed by ammonia treatment afforded the ureido derivative **25** in 28% yield. Introduction of the Boc protected glycine unit was achieved by using 1,1′-carbonyldiimidazole (CDI) as a condensing agent in 92% yield.

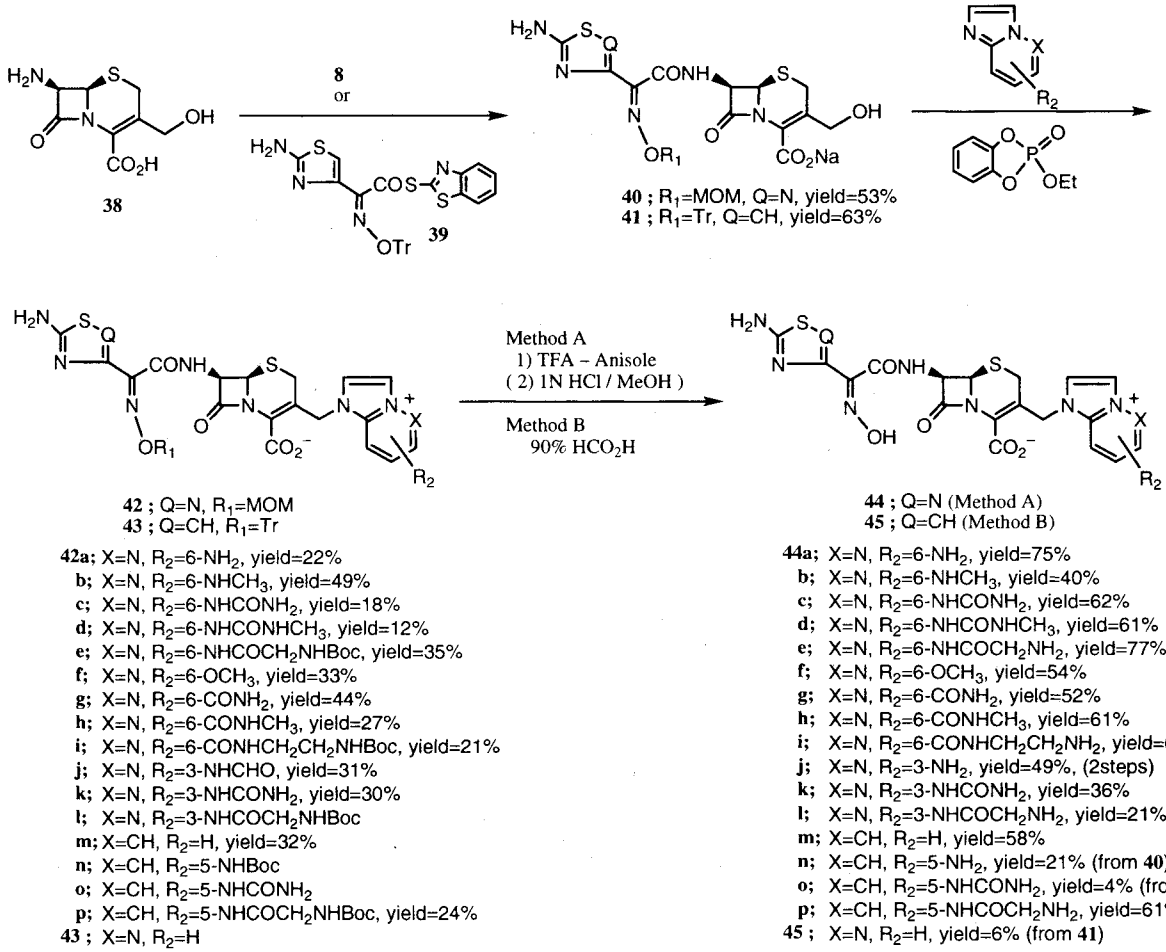
Scheme 5 shows the methods for preparation of the 6-carbamoyl derivatives of imidazo[1,2-*b*]pyridazine from the reported 3-aminopyridazine-6-carboxamide[24] (**27**). The key compound 6-carboxyimidazo[1,2-*b*]pyridazine hydrochloride (**29**) was obtained by hydrolysis of 6-carbamoylimidazo[1,2-*b*]pyridazine (**28**) prepared by condensation of **27** with bromoacetaldehyde in 57% overall yield. In the presence of base, condensation of **29** with methylamine and 2-*tert*-butoxycarbonylaminoethylamine[25] afforded the carbamoyl derivatives **30** and **31** in 53% and 77% yield, respectively.

Methods for preparation of the 5-substituted imidazo[1,2-*a*]pyridine derivaties **34**, **35** and **37** is shown in Scheme 6. The known starting material, 5-aminoimidazo[1,2-*a*]pyridine[26] (**32**), was converted into

Scheme 6.




Scheme 7.




**42** ; Q=N, $R_1$=MOM
**43** ; Q=CH, $R_1$=Tr

**42a**; X=N, $R_2$=6-$NH_2$, yield=22%
**b**; X=N, $R_2$=6-$NHCH_3$, yield=49%
**c**; X=N, $R_2$=6-$NHCONH_2$, yield=18%
**d**; X=N, $R_2$=6-$NHCONHCH_3$, yield=12%
**e**; X=N, $R_2$=6-$NHCOCH_2NHBoc$, yield=35%
**f**; X=N, $R_2$=6-$OCH_3$, yield=33%
**g**; X=N, $R_2$=6-$CONH_2$, yield=44%
**h**; X=N, $R_2$=6-$CONHCH_3$, yield=27%
**i**; X=N, $R_2$=6-$CONHCH_2CH_2NHBoc$, yield=21%
**j**; X=N, $R_2$=3-NHCHO, yield=31%
**k**; X=N, $R_2$=3-$NHCONH_2$, yield=30%
**l**; X=N, $R_2$=3-$NHCOCH_2NHBoc$
**m**; X=CH, $R_2$=H, yield=32%
**n**; X=CH, $R_2$=5-NHBoc
**o**; X=CH, $R_2$=5-$NHCONH_2$
**p**; X=CH, $R_2$=5-$NHCOCH_2NHBoc$, yield=24%
**43** ; X=N, $R_2$=H

**44** ; Q=N (Method A)
**45** ; Q=CH (Method B)

**44a**; X=N, $R_2$=6-$NH_2$, yield=75%
**b**; X=N, $R_2$=6-$NHCH_3$, yield=40%
**c**; X=N, $R_2$=6-$NHCONH_2$, yield=62%
**d**; X=N, $R_2$=6-$NHCONHCH_3$, yield=61%
**e**; X=N, $R_2$=6-$NHCOCH_2NH_2$, yield=77%
**f**; X=N, $R_2$=6-$OCH_3$, yield=54%
**g**; X=N, $R_2$=6-$CONH_2$, yield=52%
**h**; X=N, $R_2$=6-$CONHCH_3$, yield=61%
**i**; X=N, $R_2$=6-$CONHCH_2CH_2NH_2$, yield=66%
**j**; X=N, $R_2$=3-$NH_2$, yield=49%, (2steps)
**k**; X=N, $R_2$=3-$NHCONH_2$, yield=36%
**l**; X=N, $R_2$=3-$NHCOCH_2NH_2$, yield=21% (from **40**)
**m**; X=CH, $R_2$=H, yield=58%
**n**; X=CH, $R_2$=5-$NH_2$, yield=21% (from **40**)
**o**; X=CH, $R_2$=5-$NHCONH_2$, yield=4% (from **40**)
**p**; X=CH, $R_2$=5-$NHCOCH_2NH_2$, yield=61%
**45** ; X=N, $R_2$=H, yield=6% (from **41**)

the Boc protected derivative **34** in two steps *via* the bis-Boc derivative **33**. The ureido derivative **35** was prepared by reaction with chloroacetyl isocyanate followed by treatment with sodium *N*-methyldithiocarbonate in 39% overall yield. Introduction of the Boc protected glycine unit was achieved by the same method as that used for the preparation of the

imidazo[1,2-*b*]pyridazine derivative **20**.

Synthesis of C-3′ Modified Cephalosporin Derivatives

The general procedure for the preparation of C-3′ modified cephalosporin derivatives is shown in Scheme 7. The aminothiadiazolyl intermediate **40** was prepared by acylation of 7$\beta$-amino-3-hydroxymethyl-3-cephem-4-carboxylic acid (**38**) with the acid chloride **8** in 53% yield. The corresponding aminothiazolyl intermediate **41** was prepared by acylation of **38** with *S*-(2-benzothiazolyl) 2-(2-aminothiazol-4-yl)-2(*Z*)-trityloxyiminothioacetate (**39**), obtained from 2-(2-aminothiazol-4-yl)-2(*Z*)-trityloxyiminoacetic acid by the modified procedure[27], in 63% yield. Introduction of the substituted imidazo[1,2-*b*]pyridazine or imidazo[1,2-*a*]pyridine at the C-3′ position was accomplished by treatment with ethyl *o*-phenylenephosphate[28] to give the protected derivatives **42a**~**42p**, **43** in 12~49% yield. Following TFA-anisole treatment gave the desired hydroxyimino derivatives **44a**~**44i**, **44k**~**44p**. (Method A) In the case of **44j**, additional HCl-methanol treatment was needed for removal of the formyl group. Treatment of **43** with 90% formic acid afforded the desired aminothiazolyl derivative **45** in 6% overall yield from **41**. (Method B)

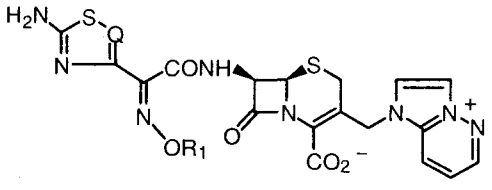
## Biological Results and Discussion

Our first challenge was to determine the optimal acyl moiety for enhancing the anti-MRSA activity of CZOP. Imidazo[1,2-*b*]pyridazinium derivatives having three different acyl moieties were examined. *In vitro* antibacterial activity of these derivatives including CZOP is shown in Table 1.

Among these derivatives, the 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyimino acetyl derivative **11** showed the most potent anti-MRSA activity, although its activity against Gram-negative bacteria was decreased. The corresponding 2-aminothiazol-4-yl derivative **45** exhibited inferior activity against most strains including MRSA. It was supposed that both the 5-amino-1,2,4-thiadiazol-3-yl ring and hydroxyimino group contributed to anti-MRSA activity. Replacement of methyl group with a methoxymethyl (MOM) group in acyl group of CZOP reduced the activity against most bacteria, particularly MRSA. Thus, the 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyimino acetyl moiety was chosen as the optimal acyl group for anti-MRSA activity.

We next tried to improve the potency by varying the azole moiety. Antibacterial activity of the azole variants is summarized in Table 2. Introduction of a heteroatom-based substituent at the 6-position of the imidazo[1,2-*b*]pyridazinium group in **11** did not improve the antibacterial activity practically (**44a**~**44f**). On the other hand, the 6-carboxamide derivatives **44g**~**44i** showed slightly improved anti-MRSA activity but inferior potency against *Pseudomonas aeruginosa*. Among the derivatives bearing an amine-based substituent at the 3-position of the imidazo[1,2-*b*]pyridazinium group in **11**, the 3-amino derivative **44j** showed antibacterial activity with a broader spectrum than the corresponding 6-amino derivative **44a** but slightly less potent anti-MRSA activity. Although the 5-aminoimidazo[1,2-*a*]pyridinium derivative **44n** showed activity and a spectrum similar to those of **11**, except for the activity against *P. aeruginosa*, introduction of an amine-based substituent at the 5-position in the azole decreased

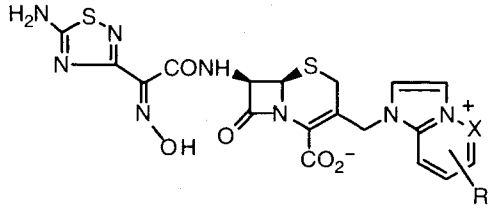
Table 1. Antibacterial activity (MIC, $\mu$g/ml) of C-7 acyl modified CZOP derivatives (**10**, **11**, **45**) and CZOP.




| Compd. | $R_1$ | Q | *S. a.* | MRSA1 | MRSA2 | MRSA3 | *E.c.* | *E.cl.* | *S.m.* | *P.v.* | *P.a.*1 | *P.a.*2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10** | MOM | N | 0.78 | 50 | 100 | >100 | 0.1 | 0.2 | 0.2 | 0.39 | 1.56 | 25 |
| **11** | H | N | 0.39 | 3.13 | 12.5 | 50 | 0.78 | 0.78 | 1.56 | 3.13 | 1.56 | 25 |
| **45** | H | CH | 0.2 | 12.5 | 50 | 100 | 0.2 | 0.78 | 0.39 | 0.78 | 50 | >100 |
| CZOP | Me | N | 0.78 | 25 | 25 | 100 | 0.05 | 0.1 | 0.1 | 0.2 | 1.56 | 6.25 |

*S. a.*, *Staphylococcus aureus* 308A-1 ; MRSA1, *S. aureus* J-108 ; MRSA2, *S. aureus* N133 ; MRSA3, *S. aureus* OFU4 ; *E.c.*, *Escherichia coli* NIHJ JC-2 ; *E. cl.*, *Enterobacter cloacae* CS4495 ; *S. m.*, *Serratia marcescens* IFO 12648 ; *P.v.*, *Proteus vulgaris* IFO 3988 ; *P.a.*1, *Pseudomonas aeruginosa* P9 ; *P.a.*2, *P. aeruginosa* U31.

Table 2. Antibacterial activity (MIC, $\mu$g/ml) of 7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyimino-acetamido]-3-(substituted imidazo[1,2-*b*]pyridazinium or imidazo[1,2-*a*]pyridinium-1-yl)methyl-3-cephem-4-carboxylates (**11**, **44a**~**44p**) and CZOP.



| Compd. | R | X | *S. a.* | MRSA1 | MRSA2 | MRSA3 | *E.c.* | *E.cl.* | *S.m.* | *P.v.* | *P.a.*1 | *P.a.*2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **11** | H | N | 0.39 | 3.13 | 12.5 | 50 | 0.78 | 0.78 | 1.56 | 3.13 | 1.56 | 25 |
| **44a** | 6-$NH_2$ | N | 0.2 | 6.25 | 12.5 | 25 | 0.39 | 0.39 | 0.78 | 1.56 | 3.13 | 25 |
| **44b** | 6-NHMe | N | 0.2 | 12.5 | 50 | 50 | 0.39 | 0.78 | 0.78 | 1.56 | 25 | 100 |
| **44c** | 6-$NHCONH_2$ | N | 0.39 | 6.25 | 6.25 | 25 | 0.39 | 0.78 | 0.78 | 1.56 | 3.13 | 50 |
| **44d** | 6-NHCONHMe | N | 0.39 | 6.25 | 12.5 | 25 | 0.78 | 0.78 | 1.56 | 1.56 | 6.25 | 50 |
| **44e** | 6-$NHCOCH_2NH_2$ | N | 0.39 | 6.25 | 12.5 | 25 | 1.56 | 1.56 | 1.56 | 3.13 | 6.25 | 100 |
| **44f** | 6-OMe | N | 0.39 | 6.25 | 12.5 | 50 | 0.39 | 0.78 | 0.78 | 1.56 | 12.5 | 50 |
| **44g** | 6-$CONH_2$ | N | 0.78 | 3.13 | 6.25 | 25 | 1.56 | 1.56 | 1.56 | 3.13 | 6.25 | 50 |
| **44h** | 6-CONHMe | N | 0.78 | 6.25 | 12.5 | 50 | 1.56 | 1.56 | 1.56 | 6.25 | 25 | >100 |
| **44i** | 6-$CONH(CH_2)_2NH_2$ | N | 0.78 | 3.13 | 6.25 | 12.5 | 3.13 | 3.13 | 3.13 | 6.25 | 25 | 100 |
| **44j** | 3-$NH_2$ | N | 0.39 | 12.5 | 12.5 | 25 | 0.39 | 0.39 | 0.78 | 1.56 | 1.56 | 12.5 |
| **44k** | 3-$NHCONH_2$ | N | 1.56 | 12.5 | 25 | 100 | 0.78 | 1.56 | 1.56 | 6.25 | 6.25 | 50 |
| **44l** | 3-$NHCOCH_2NH_2$ | N | 1.56 | 12.5 | 25 | 50 | 1.56 | 3.13 | 3.13 | 12.5 | 3.13 | 12.5 |
| **44m** | H | CH | 0.1 | 12.5 | 25 | 50 | 0.78 | 0.78 | 1.56 | 3.13 | 12.5 | >100 |
| **44n** | 5-$NH_2$ | CH | 0.1 | 6.25 | 12.5 | 25 | 0.39 | 0.78 | 0.39 | 1.56 | 6.25 | 100 |
| **44o** | 5-$NHCONH_2$ | CH | 0.2 | 12.5 | 12.5 | 50 | 0.78 | 1.56 | 0.78 | 1.56 | 12.5 | >100 |
| **44p** | 5-$NHCOCH_2NH_2$ | CH | 0.78 | 50 | 50 | 100 | 1.56 | 6.25 | 3.13 | 6.25 | 25 | >100 |
| CZOP | | | 0.78 | 25 | 25 | 100 | 0.05 | 0.1 | 0.1 | 0.2 | 1.56 | 6.25 |

*S. a.*, *Staphylococcus aureus* 308A-1 ; MRSA1, *S. aureus* J-108 ; MRSA2, *S. aureus* N133 ; MRSA3, *S. aureus* OFU4 ; *E.c.*, *Escherichia coli* NIHJ JC-2 ; *E. cl.*, *Enterobacter cloacae* CS4495 ; *S. m.*, *Serratia marcescens* IFO 12648 ; *P.v.*, *Proteus vulgaris* IFO 3988 ; *P.a.*1, *Pseudomonas aeruginosa* P9 ; *P.a.*2, *P. aeruginosa* U31.

the activity against most strains (**44o** and **44p**).

Extensive *in vivo* evaluation using three strains, *S. aureus* 308A-1, *S. aureus* N133(MRSA) and *P. aeruginosa* P9, was conducted for the six derivatives, **11**, **44a**, **44c**, **44g**, **44j** and **44n**, which showed potent *in vitro* antibacterial activity (Table 3). The selected compounds showed also potent effects against experimental systemic infections caused by *S. aureus* including MRSA in comparison with those of CZOP, while all the compounds except **11** and **44a** showed inferior activity against infection caused by *P. aeruginosa*. The *in vivo* activity of the six compounds and CZOP reflected their *in vitro* antibacterial activity. Since *in vitro* anti-MRSA activity of the selected compounds was 8 to 16 times less potent than that of VCM (MIC value against *S. aureus* N133, 0.78 $\mu$g/ml), it was not surprising that the *in vivo* efficiency of none of the compounds against MRSA infection exceeded that of VCM. However, **44a** showed potency comparable to that of VCM against MRSA-infection. Considering the balanced and good antibacterial activity against both MRSA and *P. aeruginosa*, **44a** was considered to be the most promising CZOP derivative for further evaluation.

In conclusion, among the novel CZOP derivatives, 3-(6-aminoimidazo[1,2-*b*]pyridazinium-1-yl)methyl-7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyimino-acetamido]-3-cephem-4-carboxylate (**44a**) showed an excellent balance of activity against MRSA and Gram-negative bacteria. Practically, **44a** showed a protective effect comparable to that of VCM against experimental systemic infection in mice caused by MRSA, *S. aureus* N133 strain. Further evaluation as well as modification of **44a** is now on progress.

Table 3. Protective effects of **11**, **44a**, **44c**, **44g**, **44j**, **44n**, CZOP and VCM against experimental systemic infection in mice. ($ED_{50}$, mg/kg)

| Compd. | *S. aureus* 308A-1 | *S. aureus* N133 (MRSA) | *P. aeruginosa* P9 |
|---|---|---|---|
| **11** | 0.55 | 7.02 | 1.25 |
| CZOP | 1.10 | 17.7 | 2.41 |
| VCM | NT | 4.42 | NT |
| **44a** | 0.44 | 5.02 | 2.48 |
| CZOP | 1.25 | 17.7 | 2.41 |
| VCM | NT | 4.42 | NT |
| **44c** | 0.57 | 4.82 | 3.51 |
| CZOP | 1.31 | NT | 2.15 |
| VCM | NT | 2.21 | NT |
| **44g** | 1.25 | 7.29 | 14.0 |
| CZOP | 2.62 | NT | 2.41 |
| VCM | NT | 5.02 | NT |
| **44j** | 0.67 | 17.7 | 3.87 |
| CZOP | 1.74 | >25.0 | 2.41 |
| VCM | NT | 4.43 | NT |
| **44n** | 0.14 | 20.2 | >12.5 |
| CZOP | 1.10 | >25.0 | 4.21 |
| VCM | NT | 4.06 | NT |

NT; not tested

## Experimental

MPs were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 215 spectrophotometer. $^1$H NMR spectra were recorded on a Varian gemini 200 (200 MHz) spectrometer using TMS as the internal standard. Column chromatography was carried out on Merck Kieselgel 60 (Art No. 7734) and Mitsubishi Chemical MCI gel CHP-20P. Ion exchange chromatography was carried out using Amberlite IRA-401 (Cl form).

### Determination of *In Vitro* Antibacterial Activity

The MICs against selected strains of Gram-positive and Gram-negative bacteria were determined by the standard serial 2-fold agar dilution method with Mueller-Hinton agar as the test medium. The agar plates were inoculated with about $10^4$ CFU of microorganisms per spot and were incubated overnight at 37°C.

### Determination of *In Vivo* Antibacterial Activity

Bacterial strains were cultured overnight at 37°C in brain heart infusion broth, suspended in 5% mucin and inoculated intraperitoneally into ICR male mice. Compounds were administered subcutaneously immediately after the bacterial challenge. The 50% effective dose ($ED_{50}$) was calculated from the survival rate recorded on day 5 after infection.

### *N*-Methoxymethoxyphthalimide (**4**)

Under ice-cooling, chloromethylmethyl ether (22.4 ml, 294 mmol) was added to a solution of *N*-hydroxyphthalimide (40 g, 245 mmol) in DMF (380 ml) containing *N*-ethyldiisopropylamine (46.9 ml, 269 mmol), and the mixture was stirred at room temperature for 4 hours. The volatile was removed under reduced pressure, and the residue was poured into ice-water (1.5 liter). The resulting crystals were collected by filtration, washed with water (100 ml) and MeOH (20 ml) successively and dried under a vacuum to give **4** (50.2 g, 99%): MP 127~128°C; *Anal* Calcd for $C_{10}H_9NO_4$: C 57.97, H 4.38, N 6.76. Found: C 57.75, H 4.26, N 6.61.; IR (KBr) $cm^{-1}$ 3500, 1790, 1720, 1610; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.59 (3H, s, $OCH_3$), 5.10 (2H, s, $OCH_2$), 7.89 (4H, s, Ph).

### *O*-Methoxymethylhydroxylamine (**2**)

Hydrazine monohydrate (19.4 ml, 400 mmol) was added dropwise to a solution of **4** (41.4 g, 200 mmol) in a mixture of $CH_2Cl_2$ (200 ml) and MeOH (26 ml), and the mixture

was stirred at room temperature for 5 hours. The resulting precipitate was filtered off, and the filtrate was washed with 5 N aqueous ammonia (150 ml). The aqueous layer was extracted three times with $CH_2Cl_2$ (200 ml), and the combined extract was washed with brine (150 ml). The organic layer was dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to give **2** as a pale yellow oil (10.9 g, 71%): IR (KBr) $cm^{-1}$ 3320, 2930, 1590; $^1H$ NMR ($CDCl_3$) $\delta$ 3.42 (3H, s, $OCH_3$), 4.71 (2H, s, $OCH_2$), 5.52 (2H, br s, $NH_2$).

3-(5-Amino-1,2,4-thiadiazol-3-yl)coumarin (**6**)

Triethylamine (140 ml, 1.0 mol) was added dropwise to a suspension of 3-amidinocoumarin[18] (**5**, 224 g, 1.0 mol) and *N*-chlorosuccinimide (130 g, 0.97 mol) in MeOH (2.5 liter) with maintaining temperature at 10~16°C, and the mixture was stirred at 5°C for 30 minutes. The resulting crystals were collected by filtration. The obtained crystals and potassium thiocyanate (76 g, 0.78 mol) were suspended in a mixture of MeOH (1.26 liter) and $Me_2CO$ (630 ml), and the mixture was stirred at room temperature for 16 hours. The resulting crystals were collected by filtration, washed with $H_2O$ (300 ml) and dried under vacuum to give **6** (109 g, 48%): MP 220~228°C; *Anal* Calcd for $C_{11}H_7N_3O_2S$: C 53.87, H 2.88, N 17.13. Found: C 53.99, H 2.77, N 17.13.; IR (KBr) $cm^{-1}$ 3320, 3125, 1700, 1630, 1605, 1570, 1530; $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.23~7.57 (4H, m, Ph), 7.97 (2H, br s, $NH_2$), 8.58 (1H, s, CH).

2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-methoxymethoxyiminoacetic Acid (**1a**)

To a suspension of **6** (24.5 g, 100 mmol) in EtOH (200 ml), 1 N NaOH (200 ml) was added, and the mixture was stirred at 40°C for 1 hour to afford a clear solution. Under ice-cooling, ethyl chloroformate (10 ml, 104.6 mmol) was added to the reaction mixture, and the mixture was stirred at 5°C for 5 minutes. $CH_2Cl_2$ (300 ml) and 1 N HCl (100 ml) were successively added to the mixture, and the $CH_2Cl_2$ layer was separated. The aqueous layer was extracted with $CH_2Cl_2$ (100 ml), and the combined extract was cooled at −78°C. An excess amount of $O_3$ gas was bubbled into the solution under cooling at −78°C for 5 hours. After the excess $O_3$ gas was removed by nitrogen bubbling, sodium acetate (8.2 g, 100 mmol) and dimethylsulfide (50 ml, 680 mmol) were added to the mixture, and the mixture was stirred below 0°C for 15 minutes. The resulting sodium salt was then extracted twice with water (200 ml, 100 ml), and the combined aqueous layer was washed with EtOAc (100 ml). The separated aqueous layer was used for further reaction without purification. To the aqueous solution was added **2** (8.08 g, 105 mmol). After the pH of the aqueous solution was adjusted to 5.0, the mixture was stirred overnight at room temperature. 1 N HCl (110 ml) was added to the mixture which was then extracted with a mixture of EtOAc (300 ml) and THF (100 ml). The aqueous layer was saturated with NaCl and extracted three times with THF (200 ml). The combined organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (500 g: eluents=$Me_2CO$~$Me_2CO$ - MeOH=4 : 1) to give a solid residue which was crystallized from EtOAc to give **1a** as white crystals (14.3 g, 62%): MP 176~177°C; *Anal* Calcd for $C_6H_8N_4O_4S$: C 31.03, H 3.47, N 24.13. Found: C 31.16, H 3.55, N 23.93.; IR (KBr) $cm^{-1}$ 3450, 3120, 1740, 1650, 1620, 1540; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.31 (3H, s, $OCH_3$), 5.00 (2H, s, $OCH_2$), 7.92 (2H, br s, $NH_2$).

2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-methoxymethoxyiminoacetyl Chloride Hydrochloride (**8**)

Under cooling at −20°C, phosphorus pentachloride (6.87 g, 33 mmol) was added portionwise to a suspension of **1a** (6.96 g, 30 mmol) in $CH_2Cl_2$ (90 ml), and the mixture was stirred at −20~−5°C for 2 hours. To the reaction mixture was added diisopropyl ether (90 ml), and the mixture was stirred at −5~0°C for 30 minutes. The resulting precipitate was collected by filtration, washed with diisopropyl ether (10 ml) and dried under a vacuum to give **8** (5.72 g, 76%): IR (KBr) $cm^{-1}$ 1780, 1660, 1460; The NMR spectra was measured after conversion into the corresponding methyl ester by treatment with MeOH. $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.35 (3H, s, $OCH_3$), 3.86 (3H, s, $CO_2CH_3$), 5.19 (2H, s, $OCH_2$), 6.60 (2H, br s, $NH_2$).

7$\beta$-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-methoxymethoxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**10**)

Under cooling at −5°C, **8** (502 mg, 2.0 mmol) was added all at once to a solution of 7$\beta$-amino-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate dihydrochloride[19,20] (**9**, 2.21 g, 2.4 mmol) in a mixture of $Me_2CO$ (12 ml) and $H_2O$ (12 ml) containing tri-*n*-butylamine (2.38 ml, 10 mmol), and the mixture was stirred at −5°C~10°C for 2 hours. The reaction mixture was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (150 g: eluents=$Me_2CO$~70% aq $Me_2CO$). The eluted fractions were concentrated under reduced pressure. The concentrate was further purified by MCI gel CHP-20P column chromatography (300 ml: eluents=$H_2O$~7% aq EtOH).

Table 4. $^1$H NMR spectral data for 7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-methoxymethoxyimino-acetamido]-3-(substituted imidazo[1,2-*b*]pyridazinium or imidazo[1,2-*a*]pyridinium-1-yl)methyl-3-cephem-4-carboxylates (**10**, **42a**~**42p**).

| Compound No. | Chemical shift (*J*=Hz) (DMSO-$d_6$, $\delta$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cephem nuclei | | | | | Thiadiazole | | Imidazo[1,2-*b*]pyridazinium or Imidazo[1,2-*a*]pyridinium |
| | $C_2$-H ABq (17) | $C_3$-$CH_2$ ABq (14) | $C_6$-H d (5) | $C_7$-H dd (5&8) | $C_7$-NH d (8) | $NH_2$ br s | MOM s, s | |
| **10** | 3.03 | 5.28 | 5.00 | 5.66 | 9.56 | 8.13 | 3.31 | 7.95 (1H,dd,*J*=9&5Hz), 8.75 (2H,s), |
| | 3.45 | 5.49 | | | | | 5.07 | 9.04 (1H,dd,*J*=1&5Hz),9.33 (1H,dd,*J*=1&9Hz). |
| **42a** | 2.98 | 5.19 | 4.99 | 5.65 | 9.56 | 8.14 | 3.32 | 7.21 (2H,br s), 7.21 , 8.79 (each1H,d,*J*=10Hz), |
| | 3.43 | 5.46 | | | | | 5.08 | 8.17, 8.33 (each1H,d,*J*=2Hz). |
| **42b** | 3.00 | 5.13 | 4.99 | 5.65 | 9.58 | 8.15 | 3.32 | 2.83 (3H,s), 7.26, 8.75 (each1H,d,*J*=10Hz), 7.95 (1H,m), |
| | 3.44 | 5.35 | | | | | 5.08 | 8.25, 8.32 (each1H,d,*J*=2Hz). |
| **42c** | 3.02 | 5.17 | 5.02 | 5.67 | 9.58 | 8.14 | 3.31 | 7.07 (2H,br s), 8.17 , 9.07 (each1H,d,*J*=10Hz), |
| | 3.48 | 5.41 | | | | | 5.08 | 8.48, 8.58 (each1H,d,*J*=2Hz). |
| **42d** | 3.05 | 5.17 | 5.02 | 5.68 | 9.58 | 8.14 | 3.31 | 2.74 (3H,d,*J*=4Hz), 7.72 (1H,m), 8.16 , 9.03 |
| | 3.48 | 5.41 | | | | | 5.08 | (each1H,d,*J*=10Hz), 8.43, 8.58 (each1H,d,*J*=2Hz). |
| **42e** | 3.03 | 5.21 | 5.01 | 5.67 | 9.56 | 8.13 | 3.31 | 1.40 (9H,s), 3.88 (2H,d,*J*=4Hz), 7.17 (1H,m), 8.42 , 9.18 (each |
| | 3.48 | 5.44 | | | | | 5.07 | 1H,d,*J*=10Hz), 8.56, 8.68 (each1H,d,*J*=2Hz), 11.48 (1H,br s). |
| **42f** | 2.98 | 5.19 | 5.00 | 5.65 | 9.56 | 8.13 | 3.31 | 4.04 (3H,s), 7.64 , 9.23 (each1H,d,*J*=10Hz), |
| | 3.44 | 5.46 | | | | | 5.07 | 8.54, 8.60 (each1H,d,*J*=2Hz). |
| **42g** | 3.02 | 5.28 | 5.02 | 5.68 | 9.59 | 8.15 | 3.31 | 8.22, 8.56 (each1H,br s), 8.36 , 9.39 (each1H,d,*J*=10Hz), |
| | 3.50 | 5.51 | | | | | 5.07 | 8.69, 8.94 (each1H,d,*J*=2Hz). |
| **42h** | 3.02 | 5.28 | 5.01 | 5.67 | 9.55 | 8.11 | 3.30 | 2.88 (3H,d,*J*=5Hz), 8.34 , 9.40 (each1H,d,*J*=10Hz), |
| | 3.43 | 5.51 | | | | | 5.06 | 8.67, 8.93 (each1H,d,*J*=2Hz), 9.15 (1H,m). |
| **42i** | 3.01 | 5.27 | 5.01 | 5.67 | 9.55 | 8.12 | 3.30 | 1.38 (9H,s), 3.0~3.8 (4H,m), 6.92 (1H,m), 8.35 , 9.39 (each |
| | 3.51 | 5.50 | | | | | 5.06 | 1H,d,*J*=10Hz), 8.67, 8.94 (each1H,d,*J*=2Hz), 9.19 (1H,m).. |
| **42j** | 3.05 | 5.30 | 5.00 | 5.67 | 9.59 | 8.13 | 3.31 | 7.91 (1H,dd,*J*=5&9Hz), 8.52 (1H,s), 8.83 (1H,s), |
| | 3.50 | 5.59 | | | | | 5.06 | 9.09 (1H,d,*J*=5Hz), 9.35 (1H,d,*J*=9Hz). |
| **42k** | 3.02 | 5.26 | 5.00 | 5.66 | 9.58 | 8.13 | 3.31 | 6.72 (2H,br s), 7.83 (1H,dd,*J*=5&10Hz), 8.45 (1H,s), |
| | 3.39 | 5.54 | | | | | 5.06 | 9.03 (1H,d,*J*=5Hz), 9.28 (1H,d,*J*=10Hz), 9.89 (1H,br s). |
| **42m** | 2.97 | 5.29 | 5.00 | 5.66 | 9.58 | 8.16 | 3.31 | 7.52, 8.00 (each1H,m), 8.39, 8.53 (each1H,d,*J*=2Hz), |
| | 3.46 | 5.45 | | | | | 5.08 | 8.70 (1H,d,*J*=9Hz), 8.94 (1H,d,*J*=6Hz). |
| **42p** | 3.03 | 5.31 | 5.02 | 5.68 | 9.57 | 8.12 | 3.31 | 3.94 (2H,m), 7.09 (1H,m), 7.70 (1H,d,*J*=8Hz), 7.94 |
| | 3.44 | (m) | | | | | 5.07 | (1H,t,J=8Hz), 8.10 (1H,m), 8.36 (1H,s), 8.39 (1H,s). |

The fractions eluted with 7% aq EtOH were concentrated under reduced pressure, and the concentrate was lyophilized to give **10** (690 mg, 72%). The analytical results are shown in Table 4.

7$\beta$-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**11**)

Under ice-cooling, TFA (6.0 ml) was added to a suspension of **10** (500 mg, 0.92 mmol) in $CH_2Cl_2$ (18 ml) containing anisole (1.2 ml), and the mixture was stirred at room temperature for 4 hours. The reaction mixture was concentrated under reduced pressure. The concentrate was diluted with $H_2O$ (20 ml), and the pH of the mixture was adjusted to 4.0 with aq $NaHCO_3$. The mixture was washed with diethyl ether (20 ml) and concentrated under reduced pressure. The concentrate was purified by MCI gel CHP-20P column chromatography (200 ml: eluents=$H_2O$~5% aq EtOH). The fractions eluted with 5% aq EtOH were concentrated under reduced pressure, and the concentrate was lyophilized to give **11** (230 mg, 50%). The analytical results are shown in Table 5 and Table 6.

6-Aminoimidazo[1,2-*b*]pyridazine (**13**)

A mixture of 6-chloroimidazo[1,2-*b*]pyridazine[21] (**12**) (9.21 g, 60 mmol) and 25% aq ammonia (150 ml) was heated at 180°C in a stainless sealed tube for 8 hours. After cooling, the mixture was concentrated under reduced pressure. The resulting crystals were collected by filtration, washed with $H_2O$ (20 ml) and dried under a vacuum to give **13** (6.85 g, 85%): MP 200~203°C; *Anal* Calcd for $C_6H_6N_4$: C 53.72, H 4.51, N 41.77. Found: C 53.60, H 4.37, N

Table 5. Yield, IR and analytical data for 7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido]-3-(substituted imidazo[1,2-*b*]pyridazinium or imidazo[1,2-*a*]pyridinium-1-yl)methyl-3-cephem-4-carboxylates (**11**, **44a**~**44p**) and **45**.

| Compound No. | Yield(%) | Formula | *Anal* Calcd (%) C | H | N | Found (%) C | H | N | IR (KBr, $cm^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **11** | 50 | $C_{18}H_{15}N_9O_5S_2 \cdot 3.3H_2O$ | 38.51 | 3.85 | 22.46 | 38.77 | 3.65 | 22.16 | 1770 | 1670 | 1610 |
| **44a** | 75 | $C_{18}H_{16}N_{10}O_5S_2 \cdot 3.0H_2O$ | 37.89 | 3.89 | 24.55 | 38.18 | 3.50 | 24.20 | 1770 | 1670 | 1620 |
| **44b** | 40 | $C_{19}H_{18}N_{10}O_5S_2 \cdot CF_3CO_2H \cdot 3.5H_2O$ | 35.64 | 3.68 | 19.80 | 35.59 | 4.00 | 20.11 | 1770 | | 1605 |
| **44c** | 62 | $C_{19}H_{17}N_{11}O_6S_2 \cdot 3.8H_2O$ | 36.31 | 3.92 | 24.52 | 36.66 | 3.65 | 24.30 | 1770 | 1700 | 1605 |
| **44d** | 61 | $C_{20}H_{19}N_{11}O_6S_2 \cdot 4.0H_2O$ | 37.21 | 4.22 | 23.86 | 37.39 | 3.88 | 23.52 | 1770 | 1680 | 1600 |
| **44e** | 77 | $C_{20}H_{19}N_{11}O_6S_2 \cdot 2HCl \cdot 6.5H_2O$ | 31.46 | 4.49 | 20.18 | 31.29 | 4.34 | 19.98 | 1780 | 1720 | 1630 |
| **44f** | 54 | $C_{19}H_{17}N_9O_6S_2 \cdot 4.0H_2O$ | 37.81 | 4.17 | 20.89 | 38.02 | 3.98 | 20.53 | 1770 | 1670 | 1610 |
| **44g** | 52 | $C_{19}H_{16}N_{10}O_6S_2 \cdot 3.2H_2O$ | 37.87 | 3.72 | 23.25 | 38.28 | 3.97 | 22.74 | 1770 | 1690 | 1610 |
| **44h** | 61 | $C_{20}H_{18}N_{10}O_6S_2 \cdot 4.0H_2O$ | 38.09 | 4.16 | 22.21 | 38.02 | 3.88 | 22.08 | 1770 | 1680 | 1610 |
| **44i** | 66 | $C_{21}H_{21}N_{11}O_6S_2 \cdot 2CF_3CO_2H \cdot 3.5H_2O$ | 34.17 | 3.44 | 17.53 | 34.29 | 3.35 | 17.82 | 1780 | 1680 | 1630 |
| **44j** | 49 | $C_{18}H_{16}N_{10}O_5S_2 \cdot 2.5H_2O$ | 38.50 | 3.77 | 24.94 | 38.75 | 3.85 | 24.77 | 1770 | 1660 | 1610 |
| **44k** | 36 | $C_{19}H_{17}N_{11}O_6S_2 \cdot 5.0H_2O$ | 35.13 | 4.19 | 23.72 | 35.02 | 3.83 | 23.38 | 1760 | | 1605 |
| **44l** | 21* | $C_{20}H_{19}N_{11}O_6S_2 \cdot 2HCl \cdot 3.5H_2O$ | 33.86 | 3.98 | 21.71 | 33.67 | 4.22 | 21.68 | 1780 | 1720 | 1640 |
| **44m** | 58 | $C_{19}H_{16}N_8O_5S_2 \cdot 2.5H_2O$ | 41.83 | 3.88 | 20.54 | 41.69 | 3.77 | 20.25 | 1770 | 1650 | 1610 |
| **44n** | 21* | $C_{19}H_{17}N_9O_5S_2 \cdot 2.5H_2O$ | 40.71 | 3.96 | 22.49 | 40.75 | 3.75 | 22.71 | 1770 | 1660 | 1610 |
| **44o** | 4* | $C_{20}H_{18}N_{10}O_6S_2 \cdot 3.0H_2O$ | 39.21 | 3.95 | 22.86 | 38.99 | 3.95 | 22.43 | 1770 | 1705 | 1610 |
| **44p** | 61 | $C_{21}H_{20}N_{10}O_6S_2 \cdot 2HCl \cdot 8.0H_2O$ | 31.94 | 4.85 | 17.74 | 32.01 | 4.68 | 17.93 | 1780 | 1720 | 1650 |
| **45** | 6* | $C_{19}H_{16}N_8O_5S_2 \cdot 4.0H_2O$ | 39.86 | 4.22 | 19.57 | 39.64 | 4.12 | 19.26 | 1770 | 1670 | 1610 |

* overall yield from **40** or **41**

41.61.; IR (KBr) $cm^{-1}$ 3400, 3320, 3200, 1630, 1620, 1560, 1500; $^1H$ NMR (DMSO-$d_6$) $\delta$ 6.26 (2H, br s, $NH_2$), 6.61, 7.68 (each 1H, d, $J$=9.2 Hz, $C_7$-H and $C_8$-H), 7.37, 7.70 (each 1H, s, $C_2$-H and $C_3$-H).

6-Methylaminoimidazo[1,2-*b*]pyridazine (**14**)

A mixture of **12** (9.21 g, 60 mmol) and 40% aq methylamine (100 ml) was heated at 180°C in a stainless sealed tube for 8 hours. After cooling, the mixture was concentrated under reduced pressure. The concentrate was extracted with a mixture of THF (100 ml) and EtOAc (200 ml). The separated organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. To the residue was added a mixture of *n*-hexane-diethyl ether (50 ml, 1:2), and the resulting precipitate was collected by filtration and dried under a vacuum to give **14** (5.83 g, 66%): IR (KBr) $cm^{-1}$ 3240, 1630, 1580, 1500, 1400; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.78 (3H, d, $J$=4.8 Hz, $CH_3$), 6.91 (1H, m, NH), 6.61, 7.64 (each 1H, d, $J$=9.4 Hz, $C_7$-H and $C_8$-H), 7.35, 7.78 (each 1H, d, $J$=1 Hz, $C_2$-H and $C_3$-H).

6-Methoxyimidazo[1,2-*b*]pyridazine (**15**)

To a suspension of **12** (1.53 g, 10 mmol) in MeOH (4 ml), 28% sodium methoxide MeOH solution (2.45 ml) was added, and the mixture was stirred at room temperature. After 6 hours, additional 28% sodium methoxide MeOH solution (1.6 ml) was added to the mixture, and the reaction mixture was stirred at room temperature for an additional 16 hours. The reaction mixture was portioned with $H_2O$ (50 ml) and EtOAc (50 ml). The separated organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. To the residue was added *n*-hexane (20 ml), and the resulting crystals were collected by filtration, washed with *n*-hexane (10 ml) and dried under a vacuum to give **15** as white crystals (1.3 g, 88%): MP 109~110°C; *Anal* Calcd for $C_7H_7N_3O$: C 56.37, H 4.73, N 28.17. Found: C 56.50, H 4.67, N 28.15.; IR (KBr) $cm^{-1}$ 3130, 1620, 1550, 1500; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.98 (3H, s, $OCH_3$), 6.67, 7.77 (each 1H, d, $J$=9.6 Hz, $C_7$-H and $C_8$-H), 7.60, 7.74 (each 1H, s, $C_2$-H and $C_3$-H).

6-Ureidoimidazo[1,2-*b*]pyridazine (**16**)

Under ice-cooling, chlorosulfonyl isocyanate (CSI) (3.0 ml, 34.5 mmol) was added to a suspension of **13**

Table 6. $^1$H NMR spectral data for 7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido]-3-(substituted imidazo[1,2-*b*]pyridazinium or imidazo[1,2-*a*]pyridinium-1-yl)methyl-3-cephem-4-carboxylates (**11**, **44a**~**44p**) and **45**.

| Compd. No. | Chemical shift (*J*=Hz) (DMSO-$d_6$, $\delta$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cephem nuclei | | | | Thia(dia)zole | | | Imidazo[1,2-*b*]pyridazinium or Imidazo[1,2-*a*]pyridinium |
| | $C_2$-H ABq (17) | $C_3$-$CH_2$ ABq (14) | $C_6$-H d (5) | $C_7$-H dd (5&8) | $C_7$-NH d (8) | $NH_2$ br s | N-OH br s | |
| **11** | 3.01<br>3.49 | 5.26<br>5.47 | 4.98 | 5.64 | 9.34 | 8.00 | 11.86 | 7.95 (1H,dd,*J*=9&5Hz), 8.75 (2H,s),<br>9.04 (1H,dd,*J*=1&5Hz),9.32 (1H,dd,*J*=1&9Hz). |
| **44a** | 2.98<br>3.44 | 5.14<br>5.33 | 4.97 | 5.64 | 9.36 | 8.02 | 11.85 | 7.20 (2H,br s), 7.20 , 8.77 (each1H,d,*J*=10Hz),<br>8.16, 8.32 (each1H,d,*J*=2Hz). |
| **44b** | 3.08<br>3.46 | 5.11<br>5.45 | 4.98 | 5.73 | 9.70 | 7.80 | 11.87 | 2.83 (3H,d,*J*=4Hz), 7.35 , 8.85 (each1H,d,*J*=10Hz),<br>8.26, 8.31 (each1H,d,*J*=2Hz). |
| **44c** | 3.04<br>3.47 | 5.19<br>5.40 | 5.00 | 5.66 | 9.38 | 8.02 | 11.85 | 7.08 (2H,br s), 8.17 , 9.05 (each1H,d,*J*=10Hz),<br>8.47, 8.56 (each1H,d,*J*=2Hz), 10.32 (1H,br s). |
| **44d** | 3.04<br>3.46 | 5.18<br>5.40 | 5.01 | 5.67 | 9.36 | 8.00 | 11.86 | 2.74 (3H,d,*J*=4Hz), 7.73 (1H,m), 8.17 , 9.02<br>(each1H,d,*J*=10Hz), 8.43, 8.56 (each1H,d,*J*=2Hz). |
| **44e** | 3.27<br>3.64 | 5.48<br>(m) | 5.18 | 5.89 | 9.45 | 8.07 | 11.91 | 3.98 (2H,m), 8.33 , 8.71 (each1H,d,*J*=2Hz),<br>8.49, 8.90 (each1H,d,*J*=10Hz), 12.18 (1H,br s). |
| **44f** | 2.97<br>3.43 | 5.20<br>5.45 | 4.98 | 5.64 | 9.34 | 8.01 | 11.86 | 4.05 (3H,s), 7.64 , 9.22 (each1H,d,*J*=10Hz),<br>8.53, 8.59 (each1H,d,*J*=2Hz). |
| **44g** | 3.01<br>3.51 | 5.28<br>5.49 | 4.99 | 5.66 | 9.36 | 8.02 | 11.85 | 8.21, 8.56 (each1H,br s), 8.35 , 9.36 (each1H,d,*J*=10Hz),<br>8.68, 8.90 (each1H,d,*J*=2Hz). |
| **44h** | 3.02<br>3.50 | 5.29<br>5.50 | 5.00 | 5.66 | 9.36 | 8.02 | 11.86 | 2.89 (3H,d,*J*=4Hz), 8.36 , 9.40 (each1H,d,*J*=10Hz),<br>8.70, 8.94 (each1H,d,*J*=2Hz), 9.19 (1H,m). |
| **44i** | 3.0~<br>3.6(m) | 5.49<br>(m) | 5.13 | 5.84 | 9.42 | 8.03 | 11.88 | 3.0~3.8 (4H,m), 8.45 , 9.14 (each1H,d,*J*=10Hz),<br>8.61, 8.76 (each1H,d,*J*=2Hz). |
| **44j** | 3.00<br>3.44 | 5.19<br>5.37 | 4.99 | 5.66 | 9.36 | 8.03 | 11.89 | 6.43 (2H,br s), 7.66 (1H,dd,*J*=5&9Hz), 7.84 (1H,s),<br>8.91 (1H,d,*J*=5Hz), 8.97 (1H,d,*J*=9Hz). |
| **44k** | 2.99<br>3.37 | 5.23<br>5.59 | 4.97 | 5.68 | 9.21 | 7.85 | 11.84 | 6.60 (2H,br s), 7.79 (1H,dd,*J*=5&10Hz), 8.39 (1H,s),<br>8.94 (1H,d,*J*=5Hz), 9.35 (1H,d,*J*=10Hz). |
| **44l** | 3.27<br>3.53 | 5.38<br>5.55 | 5.15 | 5.82 | 9.48 | 8.07 | 11.85 | 4.25 (2H,m), 8.03 (1H,dd,*J*=5&10Hz), 8.57 (1H,s),<br>9.06 (1H,d,*J*=10Hz), 9.17 (1H,d,*J*=5Hz). |
| **44m** | 2.96<br>3.43 | 5.29<br>5.42 | 4.98 | 5.65 | 9.36 | 8.03 | 11.88 | 7.51, 8.00 (each1H,m), 8.38, 8.50 (each1H,d,*J*=2Hz),<br>8.67 (1H,d,*J*=9Hz), 8.93 (1H,d,*J*=7Hz). |
| **44n** | 2.96<br>3.40 | 5.24<br>(m) | 4.97 | 5.65 | 9.35 | 8.01 | 11.87 | 6.51 , 7.56 (each1H,d,*J*=7Hz), 7.75 (1H,t,*J*=7Hz),<br>7.83 (2H,br s), 8.22, 8.34 (each1H,d,*J*=2Hz). |
| **44o** | 3.10<br>3.47 | 5.07<br>(m) | 5.10 | 5.73 | 9.39 | 8.00 | 11.84 | 6.80 (1H,br s), 7.7~8.0 (3H,m),<br>8.44, 8.56 (each1H,d,*J*=2Hz). |
| **44p** | 3.24<br>3.61 | 5.44<br>(m) | 5.17 | 5.88 | 9.47 | 8.11 | 11.90 | 4.17 (2H,m), 7.76 (1H,m), 8.0~9.0 (6H,m),<br>12.43 (1H,br s). |
| **45** | 3.03<br>3.43 | 5.27<br>5.49 | 5.00 | 5.62 | 9.38 | 7.08 | 11.28 | 6.60 (1H,s,Thiazole-H), 7.95 (1H,dd,*J*=10&5Hz), 8.76 (2H,s),<br>9.04 (1H,d,*J*=5Hz),9.34 (1H,d,*J*=10Hz). |

(4.02 g, 30 mmol) in THF (60 ml), and the mixture was stirred at 5°C for 1 hour and then allowed to stand at room temperature for 1 hour. To the reaction mixture was added 1 N HCl (60 ml), and the mixture was heated at 80°C for 1 hour. After cooling, the pH of the mixture was adjusted to 10.0 with 1 N NaOH. The resulting crystals were collected by filtration, washed with $H_2O$ (10 ml) and $Me_2CO$ (10 ml) successively and dried under a vacuum to give **16** (2.14 g, 40%): MP 244~246°C; *Anal* Calcd for $C_7H_7N_5O \cdot 0.2H_2O$: C 46.47, H 4.09, N 38.72. Found: C 46.33, H 4.11, N 38.62.; IR (KBr) cm$^{-1}$ 3400, 3100, 1705, 1635, 1610, 1560, 1540, 1440, 1410; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.98 (2H, br s, $NH_2$), 7.53, 7.98 (each 1H, d, *J*=9.8 Hz, $C_7$-H and $C_8$-H), 7.59, 8.06 (each 1H, s, $C_2$-H and $C_3$-H), 9.61 (1H, br s, NH).

6-*N'*-Methylureidoimidazo[1,2-*b*]pyridazine (**17**)

Methyl isocyanate (1.77 ml, 30 mmol) was added to a suspension of **13** (1.34 g, 10 mmol) in THF (50 ml), and the mixture was refluxed at 80°C under a nitrogen atmosphere.

After 7 hours, additional methyl isocyanate (5.0 ml, 84.7 mmol) was added to the reaction mixture, and the mixture was refluxed at 80°C for 5 days under a nitrogen atmosphere. After cooling, the mixture was evaporated under reduced pressure. Treatment of the solid residue with EtOH (20 ml) gave **17** (1.07 g, 56%) as crystals: MP 223~225°C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.76 (3H, d, $J$=4 Hz, $CH_3$), 7.44 (1H, m, NH), 7.85, 8.19 (each 1H, d, $J$=10 Hz, $C_7$-H and $C_8$-H), 7.96, 8.29 (each 1H, s, $C_2$-H and $C_3$-H), 9.98 (1H, br s, NH).

6-(*tert*-Butoxycarbonylaminoacetyl)aminoimidazo[1,2-*b*]pyridazine (**20**)

Phthalimidoacetyl chloride[22] (**18**) (4.5 g, 20 mmol) was added to a suspension of **13** (1.8 g, 13.4 mmol) in THF (200 ml) containing 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (3.3 ml, 22 mmol), and the mixture was refluxed at 90°C for 5 hours. After cooling, the reaction mixture was evaporated under reduced pressure. To the residue was added a mixture of $H_2O$ (50 ml) and EtOAc (100 ml), and the resulting crystals, 6-(phthalimidoacetyl)-aminoimidazo[1,2-*b*]pyridazine (**19**), were collected by filtration. From the organic layer, **19** was recovered by concentration. The crystals and the recovered **19** were dissolved in EtOH (200 ml). To the solution was added hydrazine monohydrate (2.19 ml, 45 mmol), and the mixture was refluxed at 90°C for 3 hours. The reaction mixture was evaporated under reduced pressure. $H_2O$ (100 ml) and 1 N HCl (50 ml) were successively added to the residue. The resulting precipitate was filtered off, and the filtrate was washed with EtOAc (100 ml). To the separated aqueous layer were added 1 N NaOH (80 ml) and di-*tert*-butyl dicarbonate (11.5 ml, 50 mmol), and the mixture was stirred at room temperature for 16 hours. The reaction mixture was extracted with EtOAc (300 ml). The organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. Treatment of the residue with diethyl ether (30 ml) gave **20** (1.85 g, 47%) as crystals: MP 186~187°C; *Anal* Calcd for $C_{13}H_{17}N_5O_3 \cdot 0.5H_2O$: C 51.99, H 6.04, N 23.32. Found: C 51.88, H 6.24, N 23.25; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.40 (9H, s, Boc), 3.82 (2H, d, $J$=5.8 Hz, $CH_2$), 7.08 (1H, t, $J$=5.8 Hz, NH), 7.88, 8.10 (each 1H, d, $J$=10 Hz, $C_7$-H and $C_8$-H), 7.70, 8.11 (each 1H, d, $J$=1.0 Hz, $C_2$-H and $C_3$-H), 10.82 (1H, br s, NH).

3-Amino-6-chloroimidazo[1,2-*b*]pyridazine (**22**)

6-Chloro-3-nitroimidazo[1,2-*b*]pyridazine[23] (**21**) (0.50 g, 2.5 mmol) was added to an ice-cooled suspension of stannous chloride dihydrate (3.39 g, 15 mmol) in conc HCl (5 ml), and the mixture was heated gradually and stirred at 100°C for 15 minutes. After cooling, the reaction mixture was diluted with cooled water (10 ml), and the pH of the mixture was adjusted to 5.0 with 2 N NaOH. The mixture was extracted with a mixture of EtOAc (100 ml) and THF (50 ml), and the organic layer was washed with brine (100 ml). The extract was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. Treatment of the residue with diethyl ether (10 ml) gave **22** (0.29 g, 68%): MP 160~164°C; $^1$H NMR ($CDCl_3$) $\delta$ 4.00 (2H, br s, $C_3$-$NH_2$), 6.83, 7.78 (each 1H, d, $J$=9.0 Hz, $C_7$-H and $C_8$-H), 7.26 (1H, s, $C_2$-H).

3-Aminoimidazo[1,2-*b*]pyridazine Hydrochloride (**23**)

A solution of **22** (2.0 g, 11.9 mmol) in EtOH (80 ml) was treated with 10% palladium-charcoal (1.5 g) under a hydrogen atmosphere for 2 hours. After the catalyst was filtered off, the filtrate was concentrated under reduced pressure. Filtration followed by drying of the resulting crystals under a vacuum gave **23** (1.55 g, 77%): MP 250°C (dec.); $^1$H NMR ($CDCl_3$) $\delta$ 7.39 (1H, s, $C_2$-H), 7.5~9.0 (3H, m, $C_6$-H, $C_7$-H and $C_8$-H).

3-Formylaminoimidazo[1,2-*b*]pyridazine (**24**)

Under ice-cooling, acetic anhydride (5 ml) was added to formic acid (15 ml), and the mixture was stirred at 5°C for 30 minutes. To the mixture was added **23** (0.75 g, 4.4 mmol), and the reaction mixture was stirred at room temperature for 1 hour. After concentration, the residual solid was dissolved in cooled water (10 ml). With aq $NaHCO_3$, the pH of the solution was adjusted to 7.0. Filtration followed by drying of the resulting crystals under a vacuum gave **24** (0.6 g, 84%): MP 242~244°C; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.1~8.7 (3H, m, $C_6$-H, $C_7$-H and $C_8$-H), 7.91, 8.42 (each 1H, s, $C_2$-H and CHO).

3-Ureidoimidazo[1,2-*b*]pyridazine (**25**)

Under ice-cooling, phenyl chloroformate (17.6 ml, 140 mmol) was added dropwise to a mixture of **23** (10 g, 56.4 mmol) and triethylamine (19.7 ml, 141 mmol) in $CH_2Cl_2$ (150 ml), and the mixture was stirred at 5°C for 1 hour. The reaction mixture was washed with $H_2O$ (100 ml), and the organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. The residual solid was dissolved in 1,4-dioxane (25 ml). To the solution was added 25% aq ammonia (25 ml). After being stirred for 20 minutes, the reaction mixture was concentrated under reduced pressure. Filtration followed by drying of the residual crystals under a vacuum gave **25** (2.95 g, 28%): MP >300°C; *Anal* Calcd for $C_7H_7N_5O$: C

47.46, H 3.98, N 39.53. Found: C 47.25, H 4.14, N 39.61; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.35 (2H, br s, $NH_2$), 7.08 (1H, dd, $J$=9.2 and 4.5 Hz, $C_7$-H), 7.72 (1H, s, $C_2$-H), 8.03, 8.51 (each 1H, each dd, $J$=9.2, 1.6 Hz and $J$=4.5, 1.6 Hz, $C_6$-H and $C_8$-H), 8.99 (1H, br s, NH).

3-(*tert*-Butoxycarbonylamino)acetylaminoimidazo[1,2-*b*]pyridazine (**26**)

1,1′-Carbonyldiimidazole (6.4 g, 39.5 mmol) was added to an ice-cooled solution of Boc-glycine (6.3 g, 36 mmol) in $CH_2Cl_2$ (60 ml), and the mixture was stirred at 5°C for 15 minutes. To the reaction mixture were added **23** (5.1 g, 30 mmol) and triethylamine (4.62 ml, 33 mmol), and the mixture was stirred at 5°C for 15 minutes and then at room temperature for additional 20 hours. The reaction mixture was washed with brine (50 ml), dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure. Filtration followed by drying of the residual crystals under a vacuum gave **26** (8.0 g, 92%): MP 155~156°C; *Anal* Calcd for $C_{13}H_{17}N_5O_3$: C 53.60, H 5.88, N 24.04. Found: C 53.43, H 5.89, N 23.94.; $^1$H NMR ($CDCl_3$) $\delta$ 1.51 (9H, s, Boc), 4.09 (2H, d, $J$=6.6 Hz, $CH_2$), 5.36 (1H, br s, NH), 6.98 (1H, dd, $J$=9.2 and 4.4 Hz, $C_7$-H), 7.93, 8.30 (each 1H, each d, $J$=9.2, 4.4 Hz, $C_6$-H and $C_8$-H), 8.16 (1H, s, $C_2$-H), 9.08 (1H, br s, NH).

6-Carbamoylimidazo[1,2-*b*]pyridazine (**28**)

A mixture of bromoacetaldehyde diethylacetal (12.64 ml, 84 mmol) and 47% aq HBr (3.52 ml) was refluxed under heating at 120°C for 30 minutes. After cooling, EtOH (100 ml), $NaHCO_3$ (3.2 g, 38 mmol) and 3-amino-pyridazine-6-carboxamide[24] (**27**, 1.66 g, 12 mmol) were successively added to the mixture. The reaction mixture was stirred at 80°C for 4 hours. Then the mixture was cooled and evaporated under reduced pressure. The residue was diluted with cooled water (200 ml), and the pH of the mixture was adjusted to 7.0 with aq $NaHCO_3$. The mixture was extracted three times with a mixture of EtOAc (200 ml) and THF (100 ml). The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (60 g: eluent $=CH_2Cl_2$~$CH_2Cl_2$-MeOH=20:1) to give **28** (1.54 g, 79%): MP 228~230°C; IR (KBr) cm$^{-1}$ 3420, 3280, 1720, 1660, 1605, 1460, 1440; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.71, 8.24 (each 1H, d, $J$=9.8 Hz, $C_7$-H and $C_8$-H), 7.93, 8.31 (each 1H, d, $J$=1.0 Hz, $C_2$-H and $C_3$-H), 8.21, 7.89 (each 1H, br s, $CONH_2$).

6-Carboxyimidazo[1,2-*b*]pyridazine Hydrochloride (**29**)

A solution of **28** (20.45 g, 126 mmol) in conc HCl (200 ml) was refluxed at 100°C for 1 hour. After the mixture was cooled at 5°C, the resulting precipitate was collected by filtration. The precipitate was washed with cooled water (20 ml) and diethyl ether (30 ml) successively and dried under a vacuum to give **29** (18.0 g, 72%): *Anal* Calcd for $C_7H_6N_3O_2Cl$: C 42.12, H 3.03, N 21.05. Found: C 41.93, H 3.32, N 21.32.; IR (KBr) cm$^{-1}$ 2800, 1740, 1640, 1530, 1490, 1450, 1400; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.05, 8.50 (each 1H, d, $J$=9.6 Hz, $C_7$-H and $C_8$-H), 8.31, 8.73 (each 1H, d, $J$=1.7 Hz, $C_2$-H and $C_3$-H).

6-*N*-Methylcarbamoylimidazo[1,2-*b*]pyridazine (**30**)

Under cooling at −10°C, isobutyl chloroformate (1.56 ml, 12 mmol) was added to a mixture of **29** (2.0 g, 10 mmol) and *N*-ethyldiisopropylamine (3.83 ml, 22 mmol) in DMF (20 ml), and the mixture was stirred at −10°C for 15 minutes. Under ice-cooling, the reaction mixture was added dropwise to a mixture of 40% aq methylamine (10 ml) and EtOAc (20 ml) over 15 minutes. The reaction mixture was stirred at room temperature for 10 minutes and then concentrated under reduced pressure. The concentrate was diluted with $H_2O$ (10 ml), and the mixture was extracted with $CH_2Cl_2$ (50 ml). The organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. Treatment of the residue with diisopropyl ether (20 ml) gave **30** (928 mg, 53%): IR (KBr) cm$^{-1}$ 1670, 1520, 1260; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.85 (3H, d, $J$=4 Hz, $CH_3$), 7.70, 8.25 (each 1H, d, $J$=9.4 Hz, $C_7$-H and $C_8$-H), 7.93, 8.30 (each 1H, d, $J$=1.4 Hz, $C_2$-H and $C_3$-H), 8.85 (1H, m, NH).

6-*N*-(2-*tert*-Butoxycarbonylaminoethyl)carbamoyl-imidazo[1,2-*b*]pyridazine (**31**)

A mixture of triethylamine (2.54 ml, 18.2 mmol) and *N*-(*tert*-butoxycarbonyl)ethylenediamine[25] (3.50 g, 21.8 mmol) in DMF (4 ml) was added to a suspension of **29** (3.63 g, 18.2 mmol) in DMF (36 ml). Under ice-cooling, to the mixture were added 1-hydroxybenzotriazole (2.46 g, 18.2 mmol) and 1,3-dicyclohexylcarbodiimide (4.50 g, 21.8 mmol). The reaction mixture was stirred at 5°C for 1 hour and then at room temperature for additional 23 hours. The resulting precipitate was filtered off and washed with EtOAc (30 ml). The combined filtrate and washing were evaporated under reduced pressure. The residue was dissolved in EtOAc (300 ml). The solution was washed with aq $NaHCO_3$ (200 ml) and $H_2O$ (200 ml) successively, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. Treatment of the residue with *n*-hexane

(50 ml) gave **31** (4.27 g, 77%): IR (KBr) $cm^{-1}$ 3400, 3300, 1680, 1520, 1280; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.37 (9H, s, Boc), 3.0~3.5 (4H, m, $CH_2CH_2$), 6.93 (1H, m, N*H*Boc), 7.71, 8.25 (each 1H, d, $J$=9.5 Hz, $C_7$-H and $C_8$-H), 7.94, 8.31 (each 1H, s, $C_2$-H and $C_3$-H), 8.84 (1H, m, NH).

5-Di(*tert*-butoxycarbonyl)aminoimidazo[1,2-*a*]pyridine (**33**)

Triethylamine (3.0 ml, 22 mmol) and di-*tert*-butyl dicarbonate (4.8 ml, 21 mmol) were added to a solution of 5-aminoimidazo[1,2-*a*]pyridine[26] (**32**) (1.33 g, 10 mmol) in DMF (20 ml), and the mixture was stirred at room temperature for 3 hours. After evaporation of the solvent, the residue was portioned with a mixture of EtOAc (200 ml) and $H_2O$ (100 ml). The separated aqueous layer was extracted with EtOAc (200 ml), and the combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. Treatment of the residue with diethyl ether (50 ml) gave **33** (2.88 g, 87%) as crystals: MP 133~135°C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.58 (9H, s, Boc), 1.68 (9H, s, Boc), 7.23, 7.95 (each 1H, d, $J$=8 Hz, $C_6$-H and $C_8$-H), 7.48, 8.18 (each 1H, d, $J$=2.8 Hz, $C_2$-H and $C_3$-H), 7.65 (1H, t, $J$=8 Hz, $C_7$-H).

5-*tert*-Butoxycarbonylaminoimidazo[1,2-*a*]pyridine (**34**)

A solution of **33** (2.4 g, 7.21 mmol) in 1 N sodium methoxide MeOH solution (10 ml) was stirred at room temperature for 30 minutes. After the mixture was evaporated under reduced pressure, the residue was portioned with a mixture of EtOAc (200 ml) and $H_2O$ (100 ml). The separated aqueous layer was extracted twice with EtOAc (200 ml), and the combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. Treatment of the residue with diethyl ether (50 ml) gave **34** (1.35 g, 80%) as crystals: MP 158~159°C; *Anal* Calcd for $C_{12}H_{15}N_3O_2$: C 61.79, H 6.48, N 18.01. Found: C 61.80, H 6.48, N 17.94.; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.51 (9H, s, Boc), 6.95, 7.38 (each 1H, d, $J$=9 Hz, $C_6$-H and $C_8$-H), 7.25 (1H, t, $J$=9 Hz, $C_7$-H), 7.57, 7.88 (each 1H, s, $C_2$-H and $C_3$-H), 9.86 (1H, br s, NH).

5-Ureidoimidazo[1,2-*a*]pyridine (**35**)

Under ice-cooling, chloroacetyl isocyanate (4.0 ml, 50 mmol) was added to a suspension of **32** (2.66 g, 20 mmol) in THF (200 ml), and the mixture was stirred at room temperature for 16 hours. To the reaction mixture was added sodium *N*-methyldithiocarbonate (16.5 g, 100 mmol) in $H_2O$ (100 ml), and the reaction mixture was stirred at room temperature for 4 hours. After the mixture was saturated with NaCl, the aqueous layer was extracted three times with THF (200 ml). The combined extract was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (60 g: eluent=$CH_2Cl_2$~$CH_2Cl_2$-MeOH=10:1) to give crude **35**, which was purified by crystallization from diethyl ether (1.38 g, 39%): MP 185~188°C; IR (KBr) $cm^{-1}$ 3360, 3150, 1675, 1640, 1530, 1510; $^1H$ NMR (DMSO-$d_6$) $\delta$ 6.39 (2H, br s, $NH_2$), 7.17~7.27 (3H, m, $C_6$-H, $C_7$-H and $C_8$-H), 7.60, 7.83 (each 1H, d, $J$=1.0 Hz, $C_2$-H and $C_3$-H), 8.91 (1H, br s, NH).

5-(*tert*-Butoxycarbonylaminoacetyl)aminoimidazo[1,2-*a*]pyridine (**37**)

1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (3.68 ml, 24.6 mmol) and phthalimidoacetyl chloride (**18**) (5.0 g, 22.4 mmol) were added to a solution of **32** (2.0 g, 15 mmol) in THF (150 ml), and the mixture was refluxed at 90°C for 16 hours. After cooling, the reaction mixture was evaporated under reduced pressure. A mixture of $H_2O$ (100 ml) and EtOAc (200 ml) was added to the residue, and the resulting crystals, 5-(phthalimidoacetyl)aminoimidazo-[1,2-*a*]pyridine (**36**), were collected by filtration. From the organic layer, **36** was recovered by concentration. The crystals and the recovered **36** were dissolved in EtOH (200 ml). To the solution was added hydrazine monohydrate (2.92 ml, 60 mmol), and the mixture was refluxed at 90°C for 3 hours. After the reaction mixture was evaporated under reduced pressure, $H_2O$ (100 ml) and 1 N HCl (30 ml) were added to the residue. The resulting precipitate was filtered off, and the filtrate was washed with EtOAc (100 ml). To the separated aqueous layer were added 1 N NaOH (50 ml) and di-*tert*-butyl dicarbonate (11.5 ml, 50 mmol), and the mixture was stirred at room temperature for 16 hours. The reaction mixture was extracted with EtOAc (300 ml), and the extract was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (60 g: eluent=$CH_2Cl_2$~$CH_2Cl_2$-MeOH=20:1) to give **37** (1.92 g, 44%): IR (KBr) $cm^{-1}$ 3300, 2900, 1700, 1640, 1510, 1410, 1360; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.42 (9H, s, Boc), 3.89 (2H, d, $J$=6 Hz, $CH_2$), 7.00, 7.45 (each 1H, d, $J$=6.2 Hz, $C_6$-H and $C_8$-H), 7.20 (1H, t, $J$=6 Hz, NH), 7.60, 7.88 (each 1H, s, $C_2$-H and $C_3$-H), 10.41 (1H, br s, NH).

Sodium 7$\beta$-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-methoxymethoxyiminoacetamido]-3-hydroxymethyl-3-cephem-4-carboxylate (**40**)

Under ice-cooling, 7$\beta$-amino-3-hydroxymethyl-3-cephem-4-carboxylic acid (**38**) (1.93 g, 8.4 mmol) and $NaHCO_3$

(1.88 g, 22.4 mmol) were dissolved in a mixture of $H_2O$ (20 ml) and THF (20 ml). To the mixture was added **8** (2.0 g, 7.0 mmol). The reaction mixture was stirred at 5°C for 2 hours and then concentrated under reduced pressure. The concentrate was purified by MCI gel CHP-20P column chromatography (300 ml: eluents=$H_2O$~5% aq EtOH). The fractions eluted with 5% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **40** (1.73 g, 53%): *Anal* Calcd for $C_{14}H_{15}N_6O_7S_2Na \cdot 3.5H_2O$: C 31.76, H 4.19, N 15.87. Found: C 31.88, H 4.22, N 15.71.; IR (KBr) $cm^{-1}$ 3430, 1760, 1670, 1600, 1400; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.37 (3H, s, $OCH_3$), 3.26, 3.48 (2H, ABq, $J$=17 Hz, $C_2$-H), 3.81, 4.19 (2H, m, $C_3$-$CH_2$), 4.95 (1H, d, $J$=4.6 Hz, $C_8$-H), 5.14 (2H, s, $OCH_2$), 5.62 (1H, dd, $J$=4.6 and 8.4 Hz, $C_7$-H), 6.00 (1H, br s, OH), 8.19 (2H, br s, $NH_2$), 9.54 (1H, d, $J$=8.4 Hz, $C_7$-NH).

*S*-(2-Benzothiazolyl) 2-(2-Aminothiazol-4-yl)-2(*Z*)-trityloxyiminothioacetate (**39**)

Under cooling at −20°C, triethylphosphite (4.23 ml, 24.7 mmol) was added to a mixture of 2-(2-aminothiazol-4-yl)-2(*Z*)-trityloxyiminoacetic acid[27] (7.29 g, 17 mmol), 2,2′-dibenzothiazolyl disulfide (6.78 g, 20.4 mmol) and tri-*n*-butylamine (8.1 ml, 34 mmol) in acetonitrile (85 ml). The reaction mixture was stirred at 5°C for 15 hours. The resulting precipitate was collected by filtration, washed with acetonitrile (20 ml) and dried under a vacuum to give **39** (6.39 g, 65%): IR (KBr) $cm^{-1}$ 3450, 3050, 1700, 1620, 1540, 1440; $^1H$ NMR (DMSO-$d_6$) $\delta$ 6.84 (1H, s, $C_5$-H), 7.25~7.36 (15H, m, Trityl), 7.40 (2H, br s, $NH_2$), 7.58~7.65 (2H, m, Ph), 8.12 (1H, m, Ph), 8.26 (1H, m, Ph).

Sodium 7$\beta$-[2-(2-Aminothiazol-4-yl)-2(*Z*)-trityloxyiminoacetamido]-3-hydroxymethyl-3-cephem-4-carboxylate (**41**)

Under ice-cooling, **38** (2.53 g, 11 mmol) was suspended in $H_2O$ (100 ml), and the pH of the mixture was adjusted to 7.0 with 1 N NaOH. To the mixture was added a solution of **39** (6.3 g, 11 mmol) in THF (300 ml), and the reaction mixture was stirred at room temperature for 29 hours. After the mixture was concentrated under reduced pressure, the resulting precipitate was filtered off. The filtrate was purified by MCI gel CHP-20P column chromatography (200 ml: eluents=$H_2O$~40% aq EtOH). The fractions eluted with 40% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **41** (4.6 g, 63%): *Anal* Calcd for $C_{32}H_{26}N_5O_6S_2Na \cdot 2.0H_2O$: C 54.93, H 4.32, N 10.01. Found: C 54.86, H 4.24, N 10.28.; IR (KBr) $cm^{-1}$ 3420, 1752, 1664, 1593, 1528, 1392, 1355; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.27, 3.46 (2H, ABq, $J$=17 Hz, $C_2$-H), 3.78, 4.16 (2H, m, $C_3$-$CH_2$), 5.01 (1H, d, $J$=4.5 Hz, $C_8$-H), 5.69 (1H, dd, $J$=4.5 and 8 Hz, $C_7$-H), 6.40 (1H, br s, OH), 6.62 (1H, s, thiazolyl), 7.23 (2H, br s, $NH_2$), 7.26~7.38 (15H, m, trityl), 9.80 (1H, d, $J$=8 Hz, $C_7$-NH).

3-(6-Aminoimidazo[1,2-*b*]pyridazinium-1-yl)methyl-7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-methoxymethoxyiminoacetamido]-3-cephem-4-carboxylate (**42a**)

Under ice-cooling, a solution of ethyl *o*-phenylenephosphate[28] (1.5 g, 7.5 mmol) in DMF (2 ml) was added to a mixture of **13** (402 mg, 3.0 mmol) and **40** (699 mg, 1.5 mmol) in DMF (12 ml). The mixture was stirred at room temperature for 4 hours. The reaction mixture was diluted with diethyl ether (120 ml). After being stirred at room temperature for 30 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation. The residual oil was purified by silica gel column chromatography (40 g: eluents=$Me_2CO$~70% aq $Me_2CO$). The eluted fractions were concentrated under reduced pressure. The concentrate was further purified by MCI gel CHP-20P column chromatography (100 ml: eluents=$H_2O$~20% aq EtOH). The fractions eluted with 20% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **42a** (182 mg, 22%). The analytical results are shown in Table 4.

The cephalosporin derivatives (**42b**~**42p**) were prepared by a method similar to that used for the preparation of **42a**. The yields were as follow: **42b** (49%); **42c** (18%); **42d** (12%); **42e** (35%); **42f** (33%); **42g** (44%); **42h** (27%); **42i** (21%); **42j** (31%); **42k** (30%); **42m** (32%); **42p** (24%). The analytical results are shown in Table 4. The crude products, **42l**, **42n** and **42o**, purified by silica gel column chromatography were used for the following deprotection step without further purification.

3-(6-Aminoimidazo[1,2-*b*]pyridazinium-1-yl)methyl-7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido]-3-cephem-4-carboxylate (**44a**)

TFA (10 ml) was added to a mixture of **42a** (200 mg, 0.36 mmol) and anisole (2.0 ml), and the mixture was stirred at room temperature for 6 hours. The reaction mixture was concentrated under reduced pressure. The residue was diluted with $H_2O$ (20 ml), and the pH of the residue was adjusted to 4.0 with aq $NaHCO_3$. The mixture was washed with diethyl ether (20 ml) and concentrated under reduced pressure. The concentrate was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=$H_2O$~15% aq EtOH). The fractions eluted with 15% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **44a** (139 mg,

75%). The analytical results are shown in Table 5 and Table 6.

The cephalosporin derivatives **44b**, **44c**, **44d**, **44f**, **44g**, **44h**, **44i**, **44k**, **44m**, **44n** and **44o** were prepared by a method similar to that used for the preparation of **44a**. The analytical results and yields are shown in Table 5 and Table 6.

3-(6-Aminomethylcarbonylaminoimidazo[1,2-*b*]pyridazinium-1-yl)methyl-7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(Z)-hydroxyiminoacetamido]-3-cephem-4-carboxylate Dihydrochloride (**44e**)

TFA (40 ml) was added to a mixture of **42e** (490 mg, 0.68 mmol) and anisole (8.0 ml), and the mixture was stirred at room temperature for 6 hours. After concentration under reduced pressure, the concentrate was diluted with $H_2O$ (30 ml). The resulting precipitate was filtered off. The filtrate was purified by MCI gel CHP-20P column chromatography (200 ml: eluents=$H_2O$~0.01 N HCl). The fractions eluted with 0.01 N HCl were concentrated under reduced pressure. The concentrate was passed through Amberlite IRA-401 (50 ml, Cl form), and the eluent was lyophilized to give **44e** (340 mg, 77%). The analytical results are shown in Table 5 and Table 6.

**44l** and **44p** were prepared by a method similar to that used for the preparation of **44e**. The analytical results and yields are shown in Table 5 and Table 6.

3-(3-Aminoimidazo[1,2-*b*]pyridazinium-1-yl)methyl-7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido]-3-cephem-4-carboxylate (**44j**)

TFA (28 ml) was added to a mixture of **42j** (330 mg, 0.56 mmol) and anisole (5.6 ml), and the mixture was stirred at room temperature for 6 hours. The reaction mixture was concentrated under reduced pressure. To the residue were added MeOH (4 ml) and 1 N HCl (2 ml). The reaction mixture was stirred at room temperature for 7 hours. After concentration under reduced pressure, the concentrate was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=$H_2O$~5% aq EtOH). The fractions eluted with 5% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **44j** (143 mg, 49%). The analytical results are shown in Table 5 and Table 6.

7$\beta$-[2-(2-Aminothiazol-4-yl)-2(*Z*)-hydroxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**45**)

Ethyl *o*-phenylenephosphate[28] (1.0 g, 5.0 mmol) was added to a solution of **41** (640 mg, 1.0 mmol) and imidazo[1,2-*b*]pyridazine (143 mg, 1.2 mmol) in DMF (8 ml), and the mixture was stirred at room temperature for 4 hours. The reaction mixture was diluted with diethyl ether (120 ml). After being stirred at room temperature for 30 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation. The residual oil was purified by silica gel column chromatography (60 g: eluents=$Me_2CO$~75% aq $Me_2CO$). The eluent was concentrated under reduced pressure, and the concentrate was lyophilized. The lyophilized product was dissolved in 90% aq formic acid (5 ml), and the mixture was stirred at room temperature for 1 hour. After evaporation under reduced pressure, the residue was suspended in $H_2O$ (10 ml). With aq $NaHCO_3$, the pH of the mixture was adjusted to 4.0. The mixture was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=$H_2O$~10% aq EtOH). The fractions eluted with 10% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **45** (28 mg, 6%). The analytical results are shown in Table 5 and Table 6.

## References


1) CHAMBERS, H. F.: Methicillin resistance in *Staphylococci*: Molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev. 10: 781~791, 1997
2) YOKOTA, T.: Methicillin-resistant *Staphylococcus aureus* (MRSA) infection in Japan: Its prophylaxis and treatment based on the mechanisms. J. Infect. Chemother. 2: 1~7, 1996
3) JEVONS, M. P.: "Celbenin"-resistant *Staphylococci*. Br. Med. J. 1: 124~126, 1961
4) KONNO, M.: Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* in Japan. J. Infect. Chemother. 1: 30~39, 1995
5) NAITO, T.; S. ABURAKI, H. KAMACHI, Y. NARITA, J. OKUMURA & H. KAWAGUCHI: Synthesis and structure-activity relationships of a new series of cephalosporins, BMY-28142 and related compounds. J. Antibiotics 39: 1092~1107, 1986
6) LATTRELL, R.; J. BLUMBACH, W. DUERCKHEIMER, H.-W. FEHLHABER, K. FLEISCHMANN, R. KIRRSTETTER, B. MENCKE, K.-H. SCHEUNEMANN, E. SCHRINNER, W. SCHWAB, K. SEEGER, G. SEIBERT & M. WIEDUWILT: Synthesis and structure-activity relationships in the cefpirome series. I. 7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-oxyiminoacetamido]-3-[(substituted-1-pyridinio)methyl]-ceph-3-em-4-carboxylates. J. Antibiotics 41: 1374~1394, 1988
7) MIYAKE, A.; Y. YOSHIMURA, M. YAMAOKA, T. NISHIMURA, N. HASHIMOTO & A. IMADA: Studies on condensed-heterocyclic azolium cephalosporins IV. Synthesis and antibacterial activity of 7$\beta$-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-(condensed-heterocyclic azolium)methyl cephalosporins including SCE-2787. J. Antibiotics 45: 709~720, 1992

8) WATANABE, N.; R. HIRUMA & K. KATSU: *In vitro* evaluation of E1077, a new cephalosporin with a broad anti-bacterial spectrum. Antimicrob. Agents Chemother. 36: 589~597, 1992
9) MINE, Y.; Y. WATANABE, H. SAKAMOTO, K. HATANO, K. KUNO, Y. HIGASHI, T. KAMIMURA, Y. MATSUMOTO, S. TAWARA, F. MATSUMOTO & S. KUWAHARA: *In vitro* antibacterial activity of FK037, a novel parenteral broad-spectrum cephalosporin. J. Antibiotics 46: 71~87, 1993
10) HIRAMATSU, K.; H. HANAKI, T. INO, K. YABUTA, T. OGURI & F. C. TENOVER: Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J. Antimicrob. Chemother. 40: 135~136, 1997
11) FARBER, B. F. & R. C. MOELLERING, Jr.: Retrospective study of the toxicity of preparations of vancomycin from 1974 to 1981. Antimicrob. Agents Chemother. 23: 138~141, 1983
12) OKONOGI, K.; Y. NOJI, M. NAKAO & A. IMADA: The possible physiological roles of penicillin-binding proteins of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. J. Infect. Chemother. 1: 50~58, 1995
13) HANAKI, H.; H. AKAGI, T. OTANI & K. HIRAMATSU: Structure-activity relationships between linkage style at the C-3 position and nuclear analogues of C(7)-hydroxyiminoaminothiazol cephalosporins against methicillin-resistant *Staphylococcus aureus*. J. Infect. Chemother. 2: 143~147, 1996
14) CHO, I.-S.; T. GLINKA, M. PRICE, Z. J. ZHANG, L. CASE, J. CRASE, R. FRITH, N. LIU, M. LUDWIKOW, D. REA, V. J. LEE & S. J. HECKER: Discovery of MC-02,331 and analogs, new cephalosporins active against resistant Gram-positive organisms. Abstracts of Papers of 36th Intersci. Conf. on Antimicrob. Agents Chemother., No. 138, Louisiana, 1996
15) HANAKI, H.; H. AKAGI, Y. MASARU, T. OTANI, A. HYODO & K. HIRAMATSU: TOC-39, a novel parenteral broad-spectrum cephalosporin with excellent activity against methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 39: 1120~1126, 1995
16) NOMURA, S.; H. HANAKI & N. UNEMI: *In vitro* antibacterial activity of TOC-50, a new parenteral cephalosporin against methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Chemotherapy 42: 253~258, 1996
17) HECKER, S. J.; I.-S. CHO, T. W. GLINKA, Z. J. ZHANG, M. E. PRICE, V. J. LEE, B. G. CHRISTENSEN, A. BOGGS, S. CHAMBERLAND, F. MALOUIN, T. R. PARR, T. ANNAMALAI, J. BLAIS, E. L. BOND, L. CASE, C. CHAN, J. CRASE, R. FRITH, D. GRIFFITH, L. HARFORD, N. LIU, M. LUDWIKOW, K. MATHIAS, D. REA & R. WILLIAMS: Discovery of MC-02,331, a new cephalosporin exhibiting potent activity against methicillin-resistant *Staphylococcus aureus*. J. Antibiotics 51: 722~734, 1998
18) SAKURAI, A.; H. MIDORIKAWA & Y. HASHIMOTO: The cyclization of ethyl cyanoacetate and salicylaldehyde or 3-methoxysalicylaldehyde with ketones by means of ammonium acetate. Bull. Chem. Soc. Jpn. 43: 2925~2933, 1970
19) NISHIMURA, S.; Y. FUJIWARA & H. HIDAKA (Takeda Chemical Industries, Ltd.): Production of 7-aminocephalosporanic acid derivative. WO 9739002, Oct 23, 1997 [Chem. Abstr. 127: 331324, 1998]
20) YOSHIDA, K.; S. NISHIMURA & S. MAEDA (Takeda Chemical Industries, Ltd.): Preparation of crystalline cephalosporin derivatives. JP 10,291,993, Nov 4, 1998 [Chem. Abstr. 130: 24909, 1999]
21) STANOVNIK, B. & M. TISLER: Synthesis of pyridazine derivatives-VIII. Imidazo[1,2-*b*]pyridazines and some tricyclic aza analogs. Tetrahedron 23: 387~395, 1967
22) SHEEHAN, J. C. & V. S. FRANK: A new synthetic route to peptides. J. Am. Chem. Soc. 71: 1856~1861, 1949
23) FABIO, P. F.; A. E. LANZILOTTI & S. A. LANG, Jr.: Synthesis of carbon-14 and deuterium labelled 3-nitro-6-propoxyimidazo[1,2-*b*]pyridazine–An antiparasitic agent. J. Labelled Comp. Radiopharm. 15: 407~412, 1978
24) MOURAD, A. E.; D. S. WISE & L. B. TOWNSEND: Methyl imidazo[1,2-*b*]pyridazine-2-carbamates and related compounds as potential antifilarial agents. J. Heterocyclic Chem. 29: 1583~1592, 1992
25) RAVIKUMAR, V. T.: A convenient large synthesis of *N*-Boc-ethylenediamine. Synth. Commun. 24: 1767~1772, 1994
26) PAOLINI, J. P. & R. K. ROBINS: Aromaticity in heterocyclic systems III. The structure and proton magnetic resonance spectra of certain imidazo[1,2-*a*]pyridines (1). J. Heterocyclic Chem. 2: 53~62, 1965
27) KOBORI, T.; R. YAMAMOTO, M. FUJITA, T. HIYAMA & T. NAGATE (Taisho Pharmaceutical Co., Ltd.): Preparation of benzothiazolethiol esters as intermediates for cephalosporin. WO 92 07,840, May 14, 1992 [Chem. Abstr. 117: 150798, 1992]
28) NAITO, K.; M. KATO & K. TSUKAMURA (Takeda Chemical Industries, Ltd.): Preparation of 1,3,2-dioxaphospholes as catalysts and use in preparation of cephem carboxylates. CA 1,260,004, Sep 26, 1989 [Chem. Abstr. 113: 132506, 1990]