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Studies on Anti-MRSA Parenteral Cephalosporins

III. Synthesis and Antibacterial Activity of 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-[(*E*)-2-(1-alkylimidazo[1,2-*b*]pyridazinium-6-yl)thiovinyl]-3-cephem-4-carboxylates and Related Compounds

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In the course of our exploration for a novel cephalosporin derivative having excellent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), we modified the C-3 linked spacers of cephem derivatives bearing a 1-methylimidazo[1,2-*b*]pyridazinium-6-yl group at the C-3′ position and 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetyl group at the C-7 position. The optimal spacers were the (*E*)-2-vinyl and (*E*)-2-thiovinyl groups seen in **19a** and **29aa**, respectively. Their anti-MRSA activity was 16 to 32 times as potent as that of cefozopran (CZOP). Focusing on the (*E*)-2-vinyl and (*E*)-2-thiovinyl spacers, we further modified the alkoxyimino groups in the C-7 acyl moiety and the 1-alkylimidazo[1,2-*b*]pyridazinium moieties at the C-3′ position and investigated the structure-activity relationships (SAR) of the derivatives. Consequently, we selected 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-fluoromethoxyiminoacetamido]-3-[(*E*)-2-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)thiovinyl]-3-cephem-4-carboxylate (**29ca**) as a new anti-MRSA parenteral cephalosporin candidate for further biological evaluation. The selected **29ca** showed anti-MRSA activity comparable to that of vancomycin (VCM) both *in vitro* and *in vivo*, high affinity (IC_{50}=2.7 μg/ml) for penicillin binding protein 2′ (PBP2′) of MRSA and potent activity against Gram-negative bacteria as well.

In our previous papers[1,2], we reported that a new series of cefozopran (CZOP) derivatives, bearing a lipophilic alkoxyimino group in the C-7 acyl moiety, showed potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Specifically, some cyclopentyloxyimino derivatives with amino-based substituent(s) in the C-3′ azole moiety had anti-MRSA activity comparable to that of vancomycin (VCM), reflecting their high affinity for the target enzyme, penicillin binding protein 2′ (PBP2′) of MRSA. In this study, we envisioned further improvement of the anti-MRSA activity of the derivatives by extensive chemical modification.

In an attempt to improve the biological properties of cephalosporins dramatically, chemical modification of the cephem nucleus has been an effective strategy, as represented by development of flomoxef[3], cefdinir[4] and cefditoren pivoxil[5]. Recently, the availability of inexpensive synthetic intermediates, which give easy access to the cephem nucleus having a C-3 linked spacer different from that of the naturally occurring cephems, has enabled extensive modification of the C-3 function. Structural alteration of the C-3 moiety is known to influence the reactivity of the β-lactam ring as well as the affinity for the target PBPs, both physically and electronically[6~8]. Recent reports have indicated that introduction of a thiovinyl

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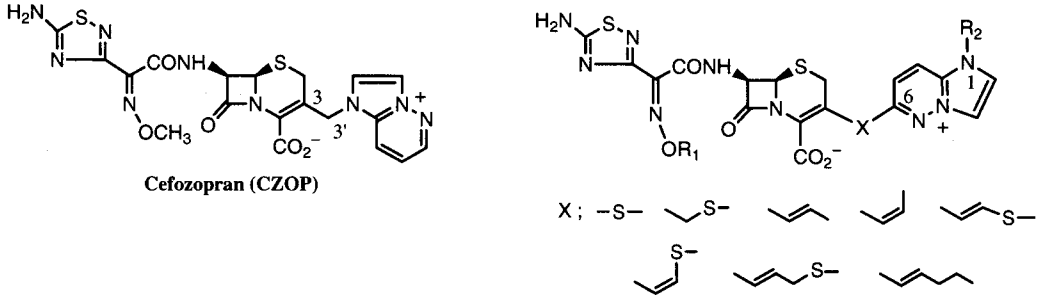
group[9] or thio group[10, 11] as the C-3 spacer between the C-3 position and the C-3′ pharmacophore results in improvement of anti-MRSA activity.

Based on the structure-activity relationships (SAR) described above, we next focused on determining the optimal C-3 spacer (-X-) between the cephem nucleus and the C-3′ pharmacophore. We selected a 1-methylimidazo-[1,2-*b*]pyridazinium-6-yl group as the C-3′ pharmacophore and prepared spacer-modified cephem derivatives bearing lipophilic oxyimino groups in the C-7 acyl moiety (Fig. 1). Among the prepared compounds, we found that the (*E*)-2-thiovinyl and (*E*)-2-vinyl spacers afford potent activity against various species of bacteria including MRSA. Further refinement by chemical modification of the lipophilic oxyimino group in the C-7 acyl moiety and the alkyl substituent of the imidazo[1,2-*b*]pyridazinium ring at the C-3′ position was conducted. In this report, we describe the synthesis and biological evaluation of the spacer-modified cephem derivatives.

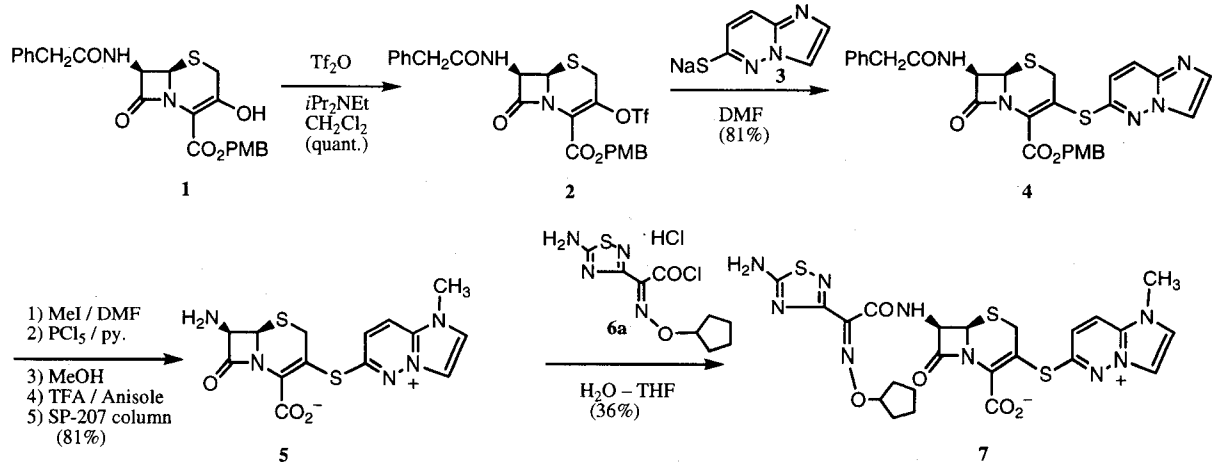
Chemistry

Synthesis of the thio spacer-bearing derivative **7** is illustrated in Scheme 1. Reaction of sodium imidazo[1,2-*b*]pyridazine-6-thiolate (**3**) with the 3-triflate derivative **2**, which was easily prepared from *p*-methoxybenzyl (PMB) 3-hydroxy-7β-phenylacetamido-3-cephem-4-carboxylate (**1**), gave the 3-(imidazo[1,2-*b*]pyridazine-6-yl)thio derivative **4** in 81% yield. The novel 6-substituted imidazo[1,2-*b*]pyridazines represented by **3**, requisite for synthesis of the C-3′ moiety, were prepared in a standard fashion (*vide infra*). After methylation of **4** with iodomethane, successive deprotection of the phenylacetyl group (by phosphorus pentachloride/pyridine followed by MeOH treatment) and PMB group (by TFA/anisole) was achieved to give the 7β-amino derivative **5** in 81% yield. Condensation of **5** with 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetyl chloride hydrochloride[12] (**6a**) under aqueous conditions gave the desired 7β-[2-(5-amino-1,2,4-thiadiazol-

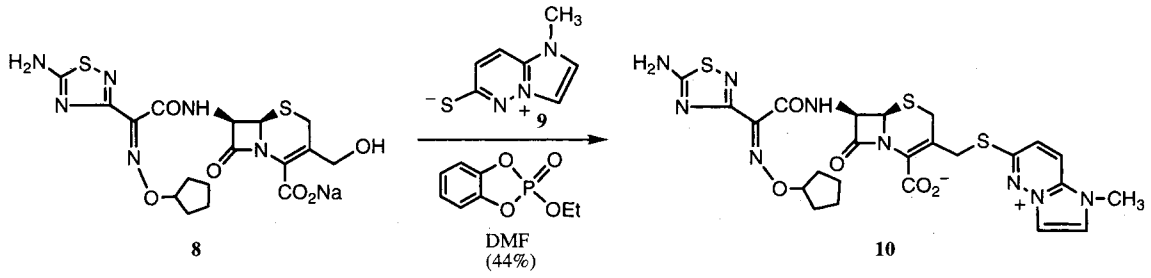
Fig. 1.



Scheme 1.



Scheme 2.



3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)thio-3-cephem-4-carboxylate (**7**) in 36% yield.

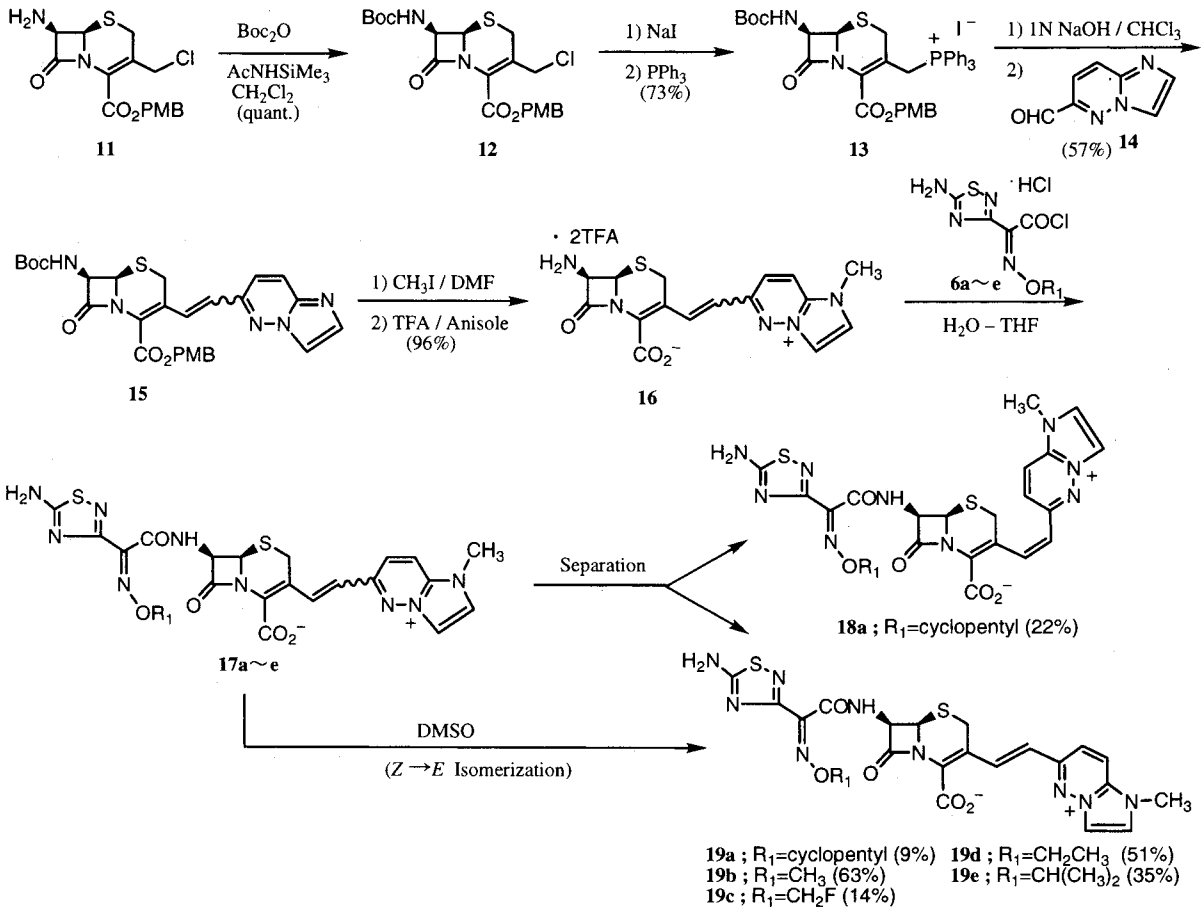
The prototype thiomethyl derivative **10** was obtained by reaction of sodium 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-hydroxymethyl-3-cephem-4-carboxylate[2] (**8**) with 1-methylimidazo[1,2-*b*]-pyridazinium-6-thiolate (**9**) in the presence of ethyl-*o*-phenylenephosphate[13] in DMF as shown in Scheme 2.

The preparation method for the (*Z*)- and (*E*)-2-vinyl derivatives is shown in Scheme 3. WITTIG reaction of 6-formylimidazo[1,2-*b*]pyridazine (**14**) with the alkaline treated 3-triphenylphosphoniummethyl derivative **13**, which was easily prepared from the 7β-amino-3-chloromethyl derivative **11** in 3 steps (73% yield), afforded 7β-*t*-butoxycarbonylamino-3-[2-(imidazo[1,2-*b*]pyridazine-6-yl)vinyl]-3-cephem-4-carboxylic acid PMB ester (**15**) in 57% yield as a mixture of (*Z*)- and (*E*)-geometrical isomers (2 : 1), which was used in the following reaction without separation of the isomers. After methylation of the azole moiety, the *t*-butoxycarbonyl (Boc) and PMB groups were removed with TFA/anisole to give the 7β-amino derivative **16** in 96% yield as a mixture of (*Z*)- and (*E*)-geometrical isomers (2 : 1). Without separation of the isomers, **16** was condensed with **6a** under aqueous conditions. At this stage, column chromatographic separation and subsequent precipitation of the obtained (*Z*)- and (*E*)-geometrical mixture **17a** afforded 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-[(*Z*)-2-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)vinyl]-3-cephem-4-carboxylate (**18a**) and its (*E*)-isomer **19a** in 22% and 9% yield, respectively. Interestingly, we found that dissolution of **18a** in DMSO-d_6 for ^{1}H-NMR measurement promoted complete geometrical isomerization to give **19a**. The isomerization reaction in DMSO proved to be a very simple method to obtain the other derivatives with (*E*)-geometry, **19b**~**19e**, from the corresponding geometrical mixtures **17b**~**17e**.

The (*Z*)- and (*E*)-2-thiovinyl derivatives were synthesized from (*Z*)- and (*E*)-vinyltosylates (**20** and **25**) prepared by the reported method[14] as shown in Scheme 4. Addition and elimination type replacement reaction of **20** and **25** with **3** in DMF proceeded smoothly to give the corresponding 7β-(Boc)amino-3-[2-(imidazo[1,2-*b*]pyridazine-6-yl)thiovinyl]-1-oxide-3-cephem-4-carboxylates (**21** and **26**) with complete retention of (*Z*)- or (*E*)-configuration in good yields, respectively. After reduction of the sulfoxide group in **21** and **26** by treatment with phosphorus trichloride in a mixture of dichloromethane and dimethylacetamide (DMA), methylation of the obtained sulfide derivatives **22** and **27** with iodomethane followed by deprotection of the Boc and benzhydryl (BH) groups gave the 7β-amino derivatives **23** and **28a**. Replacement of iodomethane with other alkylhalides afforded the novel (*E*)-derivatives **28b**~**28g**. Condensation of **23** with **6a** under aqueous conditions gave the desired 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-[(*Z*)-2-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)thiovinyl]-3-cephem-4-carboxylate (**24**) in 59% yield. As well as the (*Z*)-isomer, the desired (*E*)-isomers **29aa**~**29ia** were obtained by reaction of **28a**~**28g** with a variety of acid chlorides **6a**~**6i** in 7~60% yields. In the case of **29fa**, additional treatment with aqueous 90% formic acid was needed for removal of the trityl group.

Introduction of the longer spacer, a 1-butenyl group, at the C-3 position was accomplished by WITTIG reaction of 3-(imidazo[1,2-*b*]pyridazine-6-yl)propanal (**30**) with the alkaline treated **13**, although the generated olefin moiety was a (*Z*)- and (*E*)-geometrical mixture (4 : 1) (Scheme 5). The following isomerization reaction accomplished by treat-

Scheme 3.

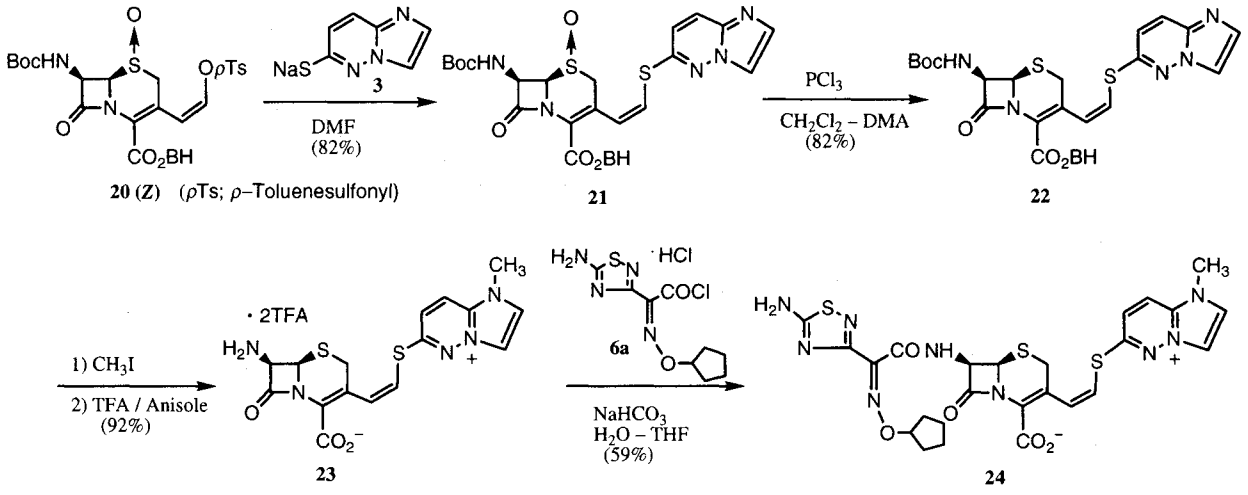


ment with iodine in toluene gave the (*E*)-isomer **31** as a single product. Methylation of **31** with iodomethane followed by deprotection of the Boc and PMB groups afforded the 7β-amino derivative **32**. Condensation of **32** with **6a** under aqueous conditions gave 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-[(*E*)-4-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)-1-butenyl]-3-cephem-4-carboxylate (**33**) in 56% yield.

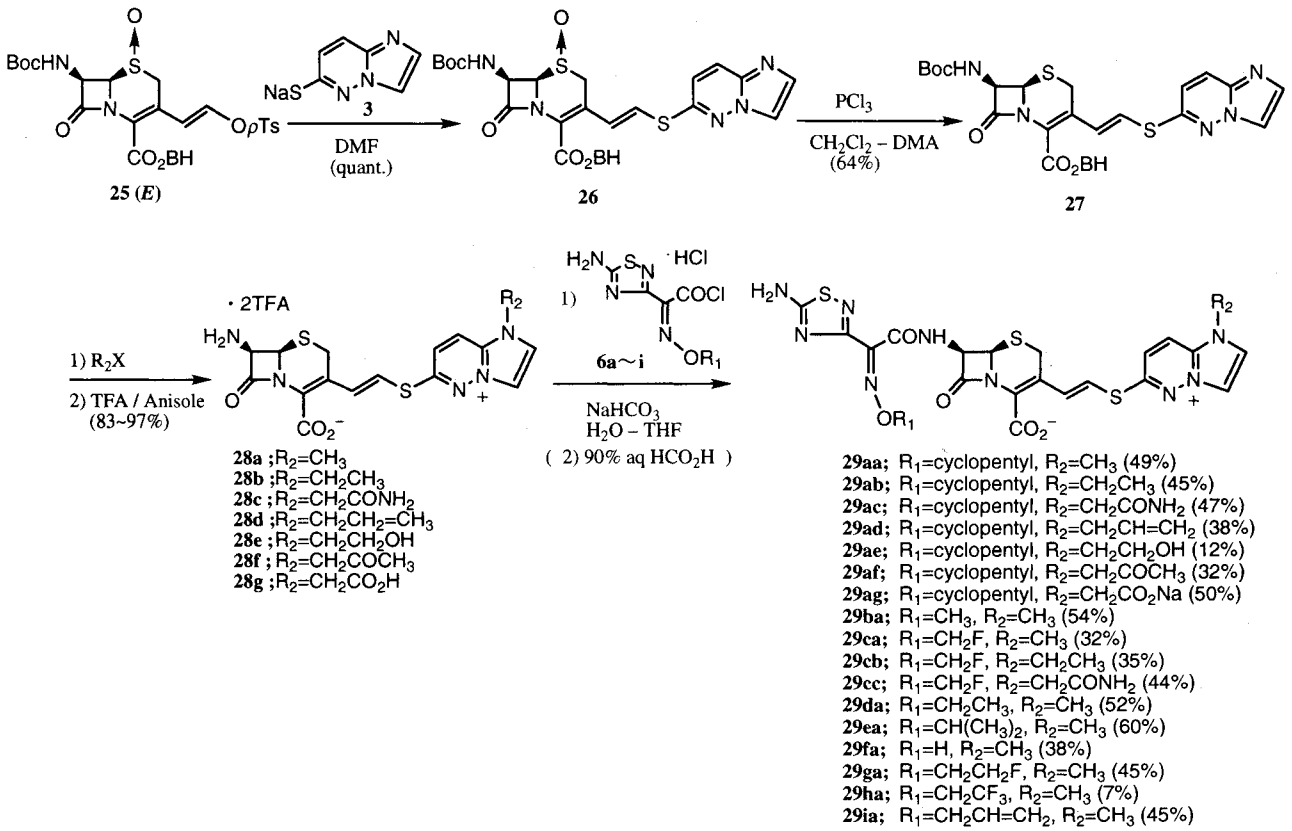
The derivative bearing a 3-thio-(*E*)-1-propenyl spacer was synthesized as illustrated in Scheme 6. WITTIG reaction of chloroacetaldehyde with **13** in the presence of potassium carbonate followed by treatment with sodium iodide afforded the 3-iodo-1-propenyl derivative **34**, which was reacted with **3** to give 7β-(Boc)amino-3-[3-(imidazo[1,2-*b*]pyridazine-6-yl)thio-1-propenyl]-3-cephem-4-carboxylate PMB ester (**35**) in 30% yield as a (*Z*)- and (*E*)-geometrical mixture (1 : 2), which was used in the following reaction without separation of the isomers. After methylation of **35** with iodomethane, the Boc and PMB groups were deprotected by treatment with TFA/anisole to give the 7β-amino derivative **36**. Without isolation, **36** was condensed with **6a** under aqueous conditions. Although separation of the (*Z*)- and (*E*)-isomers by column chromatography could not be achieved, preferential precipitation from the mixture afforded the (*E*)-isomer (**37**) as a pure state in 20% yield.

The preparation method for the 6-substituted imidazo[1,2-*b*]pyridazine derivatives is shown in Scheme 7. The reported 6-chloroimidazo[1,2-*b*]pyridazine[15] (**38**) was selected as a starting material. Sodium imidazo[1,2-*b*]pyridazine-6-thiolate (**3**) was obtained by reaction of **38** with methyl 3-mercaptopropionic acid in the presence of methanolic sodium methoxide in 90% yield, with reference to the reported method[16]. Synthesis of 1-methylimidazo-[1,2-*b*]pyridazinium-6-thiolate (**9**) was achieved by methylation of **38** with iodomethane followed by reaction with potassium hydrogen sulfide. For preparation of 3-(imidazo[1,2-*b*]pyridazine-6-yl)propanal (**30**), carbon-carbon bond formation at the C-6 position was carried out by the

Scheme 4–1.



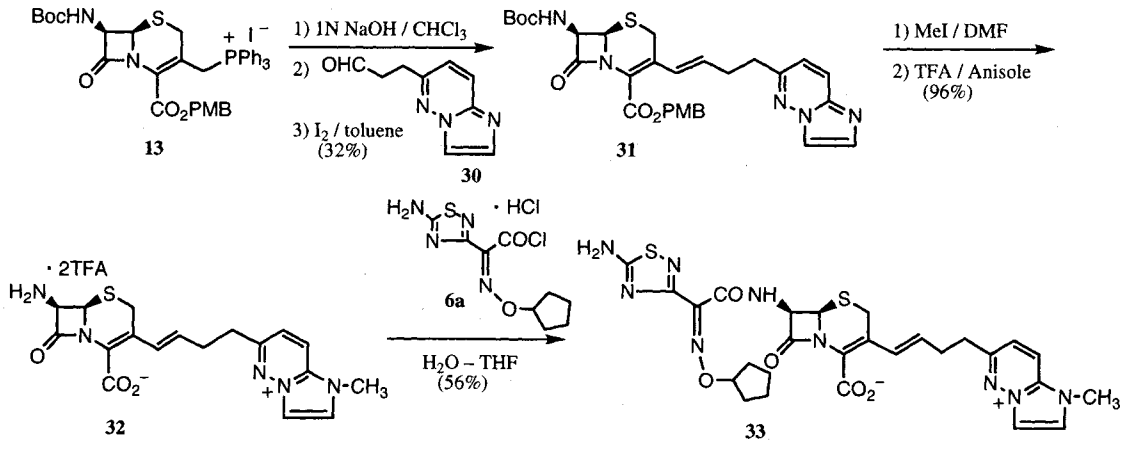
Scheme 4–2.



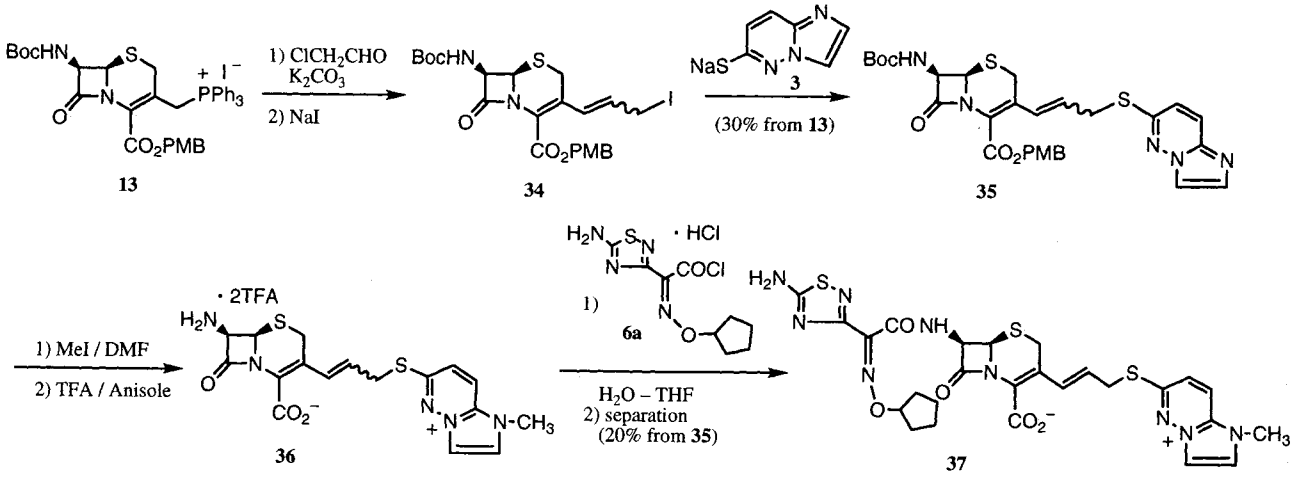
28a ;R_2=CH_3
28b ;R_2=CH_2CH_3
28c ;R_2=CH_2CONH_2
28d ;R_2=CH_2CH_2=CH_3
28e ;R_2=CH_2CH_2OH
28f ;R_2=CH_2COCH_3
28g ;R_2=CH_2CO_2H

29aa; R_1=cyclopentyl, R_2=CH_3 (49%)
29ab; R_1=cyclopentyl, R_2=CH_2CH_3 (45%)
29ac; R_1=cyclopentyl, R_2=CH_2CONH_2 (47%)
29ad; R_1=cyclopentyl, R_2=CH_2CH=CH_2 (38%)
29ae; R_1=cyclopentyl, R_2=CH_2CH_2OH (12%)
29af; R_1=cyclopentyl, R_2=CH_2COCH_3 (32%)
29ag; R_1=cyclopentyl, R_2=CH_2CO_2Na (50%)
29ba; R_1=CH_3, R_2=CH_3 (54%)
29ca; R_1=CH_2F, R_2=CH_3 (32%)
29cb; R_1=CH_2F, R_2=CH_2CH_3 (35%)
29cc; R_1=CH_2F, R_2=CH_2CONH_2 (44%)
29da; R_1=CH_2CH_3, R_2=CH_3 (52%)
29ea; R_1=$CH(CH_3)_2$, R_2=CH_3 (60%)
29fa; R_1=H, R_2=CH_3 (38%)
29ga; R_1=CH_2CH_2F, R_2=CH_3 (45%)
29ha; R_1=CH_2CF_3, R_2=CH_3 (7%)
29ia; R_1=CH_2CH=CH_2, R_2=CH_3 (45%)

Scheme 5.



Scheme 6.



reported procedure[17]. The palladium catalyzed reaction of **38** with the zinc reagent, which was prepared *in situ* by treatment of ethyl 3-iodopropionate with copper activated zinc[18], gave ethyl 3-(imidazo[1,2-*b*]pyridazine-6-yl)-propionate (**40**) in 62% yield. Reduction of **40** with diisobutylaluminum hydride (DIBAL) gave the desired **30** in 56% yield. 6-Formylimidazo[1,2-*b*]pyridazine (**14**) was prepared from 6-carbamoylimidazo[1,2-*b*]pyridazine[1] (**41**). Dehydration of **41** with phosphorus oxychloride gave 6-cyanoimidazo[1,2-*b*]pyridazine (**42**) in 85% yield. Reduction of **42** was accomplished with commercially available Raney-Nickel in 75% aqueous formic acid to give **14** in 48% yield.

Biological Results and Discussion

The SAR of the spacers (-X-) of the cyclopentyloxyimino derivatives bearing a 1-methylimidazo[1,2-*b*]pyridazinium-6-yl group as the C-3′ pharmacophore were examined (Table 1). Compounds with a thio spacer (**7**), 2-vinyl spacer (**18a** and **19a**) and 2-thiovinyl spacer (**24** and **29aa**) showed anti-MRSA activity superior to that of CZOP. In contrast, compounds with a thiomethyl (**10**), 1-butenyl (**33**) and 3-thio-1-propenyl (**37**) spacer had decreased activity against all the tested strains, although the compounds with such spacers showed anti-MRSA activity similar to that of CZOP. In the case of 2-vinyl and 2-

Scheme 7.

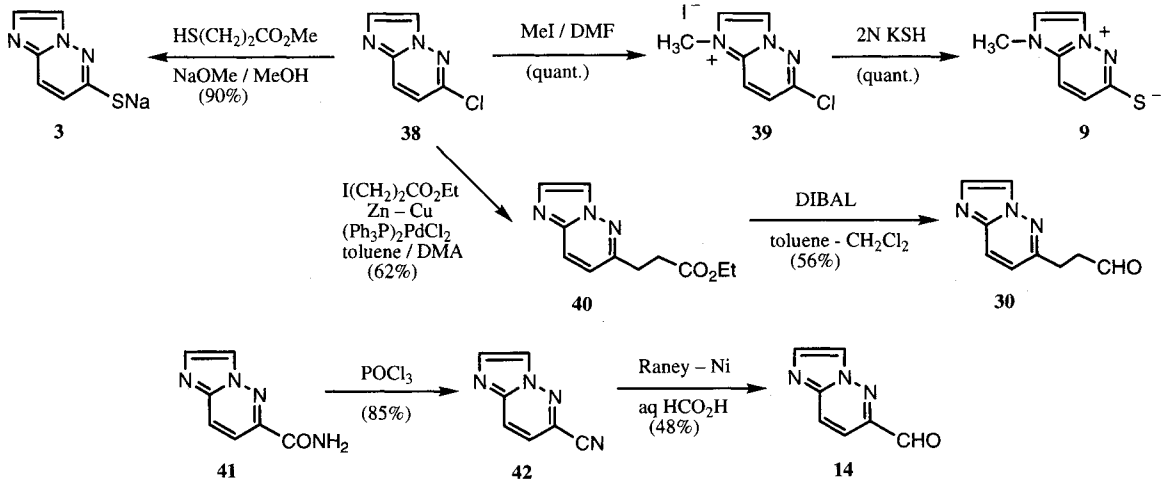
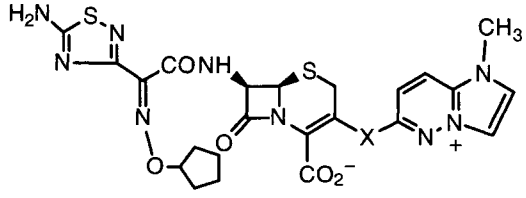
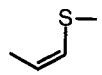
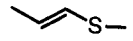
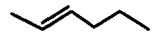
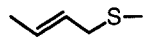


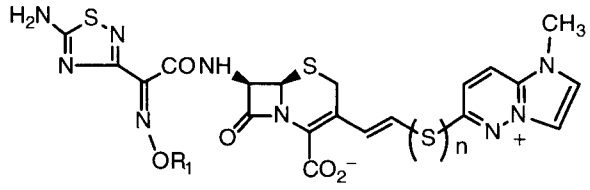
Table 1. Antibacterial activity (MIC, μg/ml) of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-linked spacer (1-methylimidazo[1,2-*b*]pyridazinium-6-yl)-3-cephem-4-carboxylates (**7**, **10**, **18a**, **19a**, **24**, **29aa**, **33**, **37**) and CZOP.



Compd.	X	*S. a.*	MRSA1	MRSA2	MRSA3	*E.c.*	*E.cl.*	*S.m.*	*P.v.*	*P.a.*1	*P.a.*2
7	S	0.78	3.13	3.13	6.25	0.78	1.56	3.13	1.56	12.5	50
10		0.39	25	25	50	1.56	1.56	6.25	12.5	25	12.5
18a		0.78	6.25	12.5	12.5	1.56	1.56	6.25	3.13	50	50
19a		0.39	1.56	1.56	3.13	0.39	1.56	0.78	0.39	12.5	12.5
24		0.2	3.13	3.13	6.25	0.78	1.56	3.13	3.13	6.25	50
29aa		0.39	1.56	1.56	3.13	0.78	6.25	1.56	1.56	6.25	25
33		0.78	25	50	50	12.5	25	25	50	100	50
37		3.13	50	50	100	12.5	12.5	25	25	100	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* GN5788 ; *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, μg/ml) of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyimino-acetamido]-3-[2(*E*)-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)vinyl and thiovinyl]-3-cephem-4-carboxylates (**19a**~**19e**, **29aa**~**29ia**).



Compd.	n	R_1	*S. a.*	MRSA1	MRSA2	MRSA3	*E.c.*	*E.cl.*	*S.m.*	*P.v.*	*P.a.*1	*P.a.*2
19a	0		0.39	1.56	1.56	3.13	0.39	1.56	0.78	0.39	12.5	12.5
19b	0	CH_3	0.78	6.25	12.5	25	0.006	0.025	0.013	0.05	25	12.5
19c	0	CH_2F	0.39	3.13	6.25	12.5	0.006	0.025	0.013	0.025	12.5	12.5
19d	0	CH_2CH_3	0.78	3.13	6.25	12.5	0.025	0.1	0.05	0.05	12.5	12.5
19e	0		0.78	3.13	6.25	6.25	0.1	0.39	0.39	0.2	12.5	12.5
29aa	1		0.39	1.56	1.56	3.13	0.78	6.25	1.56	1.56	6.25	25
29ba	1	CH_3	0.39	3.13	3.13	6.25	0.025	0.39	0.2	0.1	6.25	100
29ca	1	CH_2F	0.2	0.78	1.56	3.13	0.013	0.1	0.2	0.05	6.25	100
29da	1	CH_2CH_3	0.39	3.13	3.13	6.25	0.05	0.39	0.2	0.2	3.13	25
29ea	1		0.39	3.13	3.13	3.13	0.2	3.13	0.78	0.39	3.13	25
29fa	1	H	0.2	1.56	1.56	6.25	0.1	1.56	1.56	0.39	100	>100
29ga	1	CH_2CH_2F	0.39	1.56	3.13	3.13	0.05	0.78	0.2	0.2	3.13	50
29ha	1	CH_2CF_3	0.39	3.13	3.13	6.25	0.2	0.78	0.78	0.78	6.25	50
29ia	1	$CH_2CH{=}CH_2$	0.39	1.56	3.13	6.25	0.2	0.78	0.39	0.39	6.25	50

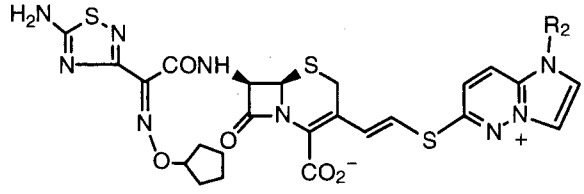
Details of each strain are shown in Table 1.

thiovinyl spacers, the (*E*)-isomer (**19a** and **29aa**) exhibited more potent activity than the (*Z*)-isomer (**18a** and **24**) against most of the bacterial species including MRSA. From the point of view of the electronic effect, the electron deficient pharmacophore at the C-3′ position might play an important role in the potent antibacterial activity of **19a** and **29aa** by enhancing the β-lactam ring reactivity through the conjugated spacer.

Having chosen (*E*)-2-vinyl and (*E*)-2-thiovinyl groups as the optimal spacers for the 1-methylimidazo[1,2-*b*]-pyridazinium-6-yl moiety, we next tried to further improve the activity by varying the oxyimino groups in the C-7 acyl moiety. Table 2 shows the antibacterial activity of the derivatives **19a**~**19e** and **29aa**~**29ia** against Gram-positive and Gram-negative bacteria. In the case of the (*E*)-2-vinyl spacer, replacement of the cyclopentyloxyimino group with a less bulky and lipophilic alkoxyimino group decreased the activity against *S. aureus*, especially MRSA, but increased the activity against Gram-negative bacteria other than *Pseudomonas aeruginosa*. A similar trend was observed among (*E*)-2-thiovinyl derivatives **29aa**~**29ia**, though the anti-MRSA activity was not influenced by replacement of the cyclopentyloxyimino group with other alkoxyimino groups. Consequently, the modification in the alkoxyimino group brought about well-balanced activity against Gram-positive and Gram-negative bacteria other than highly resistant *P. aeruginosa* (*P.a.*2). Among these derivatives, the fluoromethoxyimino derivative **29ca**, isopropoxyimino derivative **29ea** and 2-fluoroethoxyimino derivative **29ga** showed the most potent anti-MRSA activity. In addition to the excellent activity against MRSA, the fluoromethoxyimino derivative **29ca** showed antibacterial activity superior to that of CZOP against Gram-negative bacteria other than *P. aeruginosa*.

We next examined the effect of modification of the alkyl group at the N-1 position of the imidazo[1,2-*b*]-pyridazinium ring on the antibacterial activity of the (*E*)-2-thiovinyl derivatives. Among the cyclopentyloxyimino

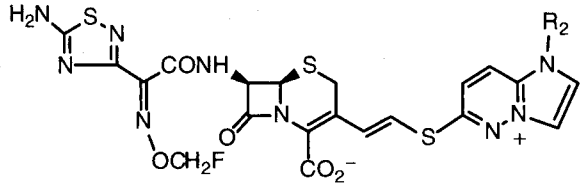
Table 3. Antibacterial activity (MIC, μg/ml) of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyimino-acetamido]-3-[2(*E*)-(1-alkylimidazo[1,2-*b*]pyridazinium-6-yl)thiovinyl]-3-cephem-4-carboxylates (**29aa**~**29ag**).



Compd.	R_2	*S. a.*	MRSA1	MRSA2	MRSA3	*E.c.*	*E.cl.*	*S.m.*	*P.v.*	*P.a.*1	*P.a.*2
29aa	CH_3	0.39	1.56	1.56	3.13	0.78	6.25	1.56	1.56	6.25	25
29ab	CH_2CH_3	0.39	1.56	1.56	3.13	0.78	3.13	1.56	0.78	6.25	25
29ac	CH_2CONH_2	0.39	1.56	1.56	3.13	0.39	1.56	1.56	0.78	6.25	25
29ad	$CH_2CH{=}CH_2$	0.39	1.56	1.56	3.13	1.56	3.13	1.56	1.56	6.25	25
29ae	CH_2CH_2OH	0.39	3.13	3.13	6.25	0.78	3.13	1.56	1.56	12.5	25
29af	CH_2COCH_3	0.39	1.56	3.13	3.13	1.56	3.13	3.13	1.56	6.25	50
29ag	CH_2CO_2Na	1.56	6.25	6.25	12.5	0.78	1.56	1.56	0.39	12.5	>100

Details of each strain are shown in Table 1.

Table 4. Antibacterial activity (MIC, μg/ml) of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-fluoromethoxy-iminoacetamido]-3-[2(*E*)-(1-alkylimidazo[1,2-*b*]pyridazinium-6-yl)thiovinyl]-3-cephem-4-carboxylates (**29ca**~**29cc**) and CZOP.



Compd	R_2	*S. a.*	MRSA1	MRSA2	MRSA3	*E.c.*	*E.cl.*	*S.m.*	*P.v.*	*P.a.*1	*P.a.*2
29ca	CH_3	0.2	0.78	1.56	3.13	0.013	0.1	0.2	0.05	6.25	100
29cb	CH_2CH_3	0.39	1.56	3.13	6.25	0.025	0.39	0.2	0.1	6.25	100
29cc	CH_2CONH_2	0.39	0.78	1.56	3.13	0.025	0.1	0.1	0.05	3.13	25
CZOP		0.78	25	25	100	0.05	0.1	0.1	0.2	1.56	6.25

Details of each strain are shown in Table 1.

derivatives, the ethyl derivative **29ab**, carbamoylmethyl derivative **29ac**, allyl derivative **29ad** and acetylmethyl derivative **29af** showed anti-MRSA activity similar to that of the methyl derivative **29aa**, but replacement of the methyl group with a hydroxyethyl (**29ae**) or carboxymethyl (**29ag**, sodium salt) group decreased the anti-MRSA activity (Table 3). Similarly, the effect of the introduction of ethyl and carbamoylmethyl groups at the N-1 position on the antibacterial activity of the fluoromethoxyimino derivatives was examined (Table 4). Although the anti-MRSA activity of the ethyl derivative **29cb** was lower than that of the methyl derivative **29ca**, the carbamoylmethyl derivative **29cc** was as active as **29ca** against Gram-positive and Gram-negative bacteria including MRSA. The consistent evaluation of these series of derivatives revealed that **29ca** and **29cc** had acceptable spectrum and potency of

Table 5. Protective effect against MRSA systemic infection, MIC_{90} against MRSA clinical isolates and PBP2′ affinity of the selected compounds, CZOP and VCM.

Compd.	ED_{50}* (mg/kg)	MIC_{90}# (μg/ml)	$IC_{50}$$ (μg/ml)
19a	6.25	NT	NT
29aa	4.42	3.13	NT
29ac	3.50	NT	NT
29ca	4.82	3.13	2.7
29cc	3.90	3.13	NT
CZOP	>15	100	78.0
VCM	2.21 – 5.05	1.56	NT

NT; not tested
* Effect against experimental systemic infection caused by *S. aureus* N133 in mice. Compounds were administered subcutaneously immediately after the bacterial challenge.
# Value against 54 clinically isolated MRSA.
$ Affinity for PBP2' of *S. aureus* N200P.

the antibacterial activity.

Based on the antibacterial activity profiles, **19a**, **29aa**, **29ac**, **29ca** and **29cc** were selected for further evaluation. We examined their *in vivo* effect using mice with experimental systemic *S. aureus* N133 (MRSA) infection (Table 5). Although the (*E*)-2-vinyl derivative **19a** showed *in vivo* activity slightly inferior to that of VCM, the (*E*)-2-thiovinyl derivatives **29aa**, **29ac**, **29ca** and **29cc** had protective effects comparable to that of VCM. In addition, Table 5 shows *in vitro* MIC_{90} values of the selected derivatives **29aa**, **29ca** and **29cc** against 54 clinically isolated MRSA strains, which is an index of the expected clinical effectiveness. Their MIC_{90} values were proved to be 3.13 μg/ml, which is comparable to that of VCM. Since their MIC_{90} values are identical to their MICs (1.56 μg/ml) against *S. aureus* N133, the *in vivo* infecting organism, **29aa**, **29ca** and **29cc** would be effective against infections caused by most of clinical isolates. As expected from the potent anti-MRSA activity, the typical compound in the new series, **29ca**, showed high affinity for PBP2′ of *S. aureus* N200P (IC_{50}=2.7 μg/ml), which is nearly thirty times as high as that of CZOP.

In conclusion, members of a new class of cephem derivatives having an (*E*)-2-thiovinyl spacer at the C-3 position has been found to exhibit strong anti-MRSA activity which is comparable to that of VCM both *in vitro* and *in vivo*. After considering the preliminary pharmacological and toxicological profile (data not shown), we finally chose 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-fluoromethoxyiminoacetamido]-3-[(*E*)-2-(1-methylimidazo-[1,2-*b*]pyridazinium-6-yl)thiovinyl]-3-cephem-4-carboxylate (**29ca**) as a candidate of new anti-MRSA cephalosporin for further biological evaluation. Because of its potent anti-MRSA activity and well-balanced antibacterial spectrum, **29ca** would be a promising candidate as an agent of first choice for MRSA infection.

Experimental

MPs were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 215 or Horiba FT-200 spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian gemini 200 (200 MHz) spectrometer using TMS as the internal standard. IP is used as an abbreviation for imidazo[1,2-*b*]pyridazine. Column chromatography was carried out on Merck Kieselgel 60 (Art No. 7734), Pharmacia Fine Chemicals Sephadex LH-20 and Mitsubishi Chemical MCI gel CHP-20P, HP-20 and SP-207.

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.

In some experiments, clinical isolates of MRSA, which were kindly supplied by three clinical laboratories in Japan in 1993, were used for the determination of anti-MRSA activity of test compounds. MIC_{90} was defined as the concentration of a compound required to inhibit the growth of 90% of the strains.

Determination of *In Vivo* Antibacterial Activity

S. aureus N133 strain was 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.

Determination of Affinity for Penicillin Binding Protein 2′

Membrane was prepared from *S. aureus* N200P cells grown to the late exponential phase in trypticase soy broth and incubated with [^{14}C]benzylpenicillin. PBPs were separated by SDS-polyacrylamide gel electrophoresis and detected by fluorography. Binding affinity of antibiotic for PBP2′ was assessed by a competition assay in which the membrane was incubated with dilutions of antibiotic at 30°C for 10 minutes before being labeled with [^{14}C]-benzylpenicillin for 10 minutes. Binding affinity was expressed in terms of the concentration required to prevent [^{14}C]benzylpenicillin binding by 50% (IC_{50}).

ρ-Methoxybenzyl 7*β*-Phenylacetylamino-3-trifluoromethylsulfonyloxy-3-cephem-4-carboxylate (**2**)

Under an argon atmosphere with cooling at −78°C, diisopropylethylamine (1.91 ml, 11 mmol) and trifluoromethanesulfonic anhydride (1.85 ml, 11 mmol) were successively added dropwise to a solution of *ρ*-methoxybenzyl 7*β*-phenylacetylamino-3-hydroxy-3-cephem-4-carboxylate (**1**) in dichloromethane (50 ml), and the mixture was stirred at −78°C for 30 minutes. The reaction mixture was diluted with dichloromethane (400 ml), and the resulting solution was washed twice with H_2O (300 ml). The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure. Treatment of the residual solid with diisopropyl ether (30 ml) gave **2** (5.95 g, quant.): ^{1}H NMR ($CDCl_3$) δ 3.32 (1H, d, J=17 Hz, C_2-H), 3.4~3.8 (3H, m, C_2-H and PhCH_2), 3.80 (3H, s, OCH_3), 4.99 (1H, d, J=4.8 Hz, C_6-H), 5.15, 5.34 (2H, ABq, J=11.6Hz, CO_2CH_2), 5.85 (1H, dd, J=4.8 and 8.8 Hz, C_7-H), 6.08 (1H, d, J=8.8 Hz, C_7-NH), 6.88 (2H, d, J=8.8 Hz, Ph), 7.2~7.4 (7H, m, Ph).

ρ-Methoxybenzyl 3-(Imidazo[1,2-*b*]pyridazine-6-yl)thio-7*β*-phenylacetylamino-3-cephem-4-carboxylate (**4**)

Under ice-cooling, sodium imidazo[1,2-*b*]pyridazine-6-thiolate (**3**, 380 mg, 2.2 mmol) was added to a solution of **2** (1.17 g, 2.0 mmol) in DMF (4 ml), and the mixture was stirred at 5°C for 30 minutes. The reaction mixture was diluted with EtOAc (100 ml), and the resulting solution was washed twice with brine (100 ml). The organic layer 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: eluents=*n*-hexane~EtOAc). The fractions eluted with EtOAc were concentrated under reduced pressure to give **4** (956 mg, 81%): ^{1}H NMR ($CDCl_3$) δ 3.40, 3.81 (2H, ABq, J=18 Hz, C_2-H), 3.66 (2H, d, J=2.6 Hz, PhCH_2), 3.77 (3H, s, OCH_3), 5.06 (1H, d, J=5 Hz, C_6-H), 5.17, 5.24 (2H, ABq, J=11 Hz, CO_2CH_2), 5.88 (1H, dd, J=5 and 9.4 Hz, C_7-H), 6.17 (1H, d, J=9.4 Hz, C_7-NH), 6.77 (2H, d, J=8.6 Hz, Ph), 6.88 (1H, d, J=9.4 Hz, IP-H), 7.2~7.4 (7H, m, Ph), 7.73 (1H, d, J=1 Hz, IP-H), 7.79 (1H, d, J=9.4 Hz, IP-H), 7.85 (1H, d, J=1 Hz, IP-H).

7*β*-Amino-3-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)thio-3-cephem-4-carboxylate (**5**)

Iodomethane (5 ml, 80 mmol) was added to a solution of **4** (500 mg, 0.85 mmol) in DMF (5 ml), and the mixture was stirred at room temperature for 16 hours and concentrated under reduced pressure. The concentrate was diluted with a mixture of *n*-hexane (75 ml) and diethyl ether (75 ml). After being stirred at room temperature for 10 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation. The residual oil was dissolved in dichloromethane (10 ml). Under ice-cooling, the solution was added to a reagent suspension, prepared from pyridine (0.34 ml, 4.26 mmol) and phosphorus pentachloride (886 mg, 4.25 mmol) in dichloromethane (4 ml), and stirred at 5°C for 30 minutes. The mixture was stirred at 5°C for 1 hour. Under cooling at −40°C, MeOH (5 ml) was added to the reaction mixture, and the mixture was stirred at −40~−20°C for 1 hour. The reaction mixture was diluted with diethyl ether (100 ml). After being stirred at room temperature for 10 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation, and the residual oil was dissolved in dichloromethane (5 ml). To the solution were added anisole (2 ml) and TFA (2.5 ml) at room temperature successively, and stirring was continued for 30 minutes. The reaction mixture was diluted with diethyl ether (100 ml). After being stirred at room temperature for 10 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation, and the residue was purified by MCI gel SP-207 column chromatography (100 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 **5** (228 mg, 81%): ^{1}H NMR (DMSO-d_6) δ 3.22, 3.88 (2H, ABq, J=17 Hz, C_2-H), 4.07 (3H, s, CH_3), 4.67 (1H, d, J=5 Hz, C_6-H), 5.02 (1H, d, J=5

Hz, C_7-H), 7.91 (1H, d, J=10 Hz, IP-H), 8.37 (1H, d, J=2 Hz, IP-H), 8.64 (1H, d, J=2 Hz, IP-H), 8.63 (1H, d, J=10 Hz, IP-H).

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)thio-3-cephem-4-carboxylate (**7**)

Under ice-cooling, 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetyl chloride hydrochloride[12] (**6a**, 0.4 g, 1.3 mmol) was added portionwise to a solution of **5** (210 mg, 0.63 mmol) in a mixture of THF (1 ml) and H_2O (1 ml) containing 0.6 M aq $NaHCO_3$ (5.1 ml). The reaction mixture was stirred at 5°C for 30 minutes and concentrated under reduced pressure. The concentrate was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=H_2O~25% aq EtOH). The fractions eluted with 25% aq EtOH were concentrated under reduced pressure, and the concentrate was lyophilized to give **7** (136 mg, 36%). The analytical results are shown in Table 6 and Table 7.

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)thiomethyl-3-cephem-4-carboxylate (**10**)

A solution of ethyl *o*-phenylenephosphate[13] (1.0 g, 5.0 mmol) in DMF (2 ml) was added to a mixture of sodium 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-hydroxymethyl-3-cephem-4-carboxylate[2] (**8**, 490 mg, 1.0 mmol) and 1-methylimidazo-[1,2-*b*]pyridazinium-6-thiolate (**9**, 330 mg, 2.0 mmol) in DMF (8 ml). The reaction mixture was stirred at room temperature for 4 hours and diluted with diethyl ether (100 ml). After being stirred at room temperature for 15 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 (50 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~30% aq EtOH). The fractions eluted with 30% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **10** (268 mg, 44%). The analytical results are shown in Table 6 and Table 7.

p-Methoxybenzyl 7β-*t*-Butoxycarbonylamino-3-chloromethyl-3-cephem-4-carboxylate (**12**)

N-Trimethylsilylacetamide (7.88 g, 60 mmol) was added to a suspension of *p*-methoxybenzyl 7β-amino-3-chloromethyl-3-cephem-4-carboxylate (**11**, 4.05 g, 10 mmol) in dichloromethane (50 ml). To the resulting clear solution, di-*t*-butyldicarbonate (4.37 g, 20 mmol) was added. The reaction mixture was stirred at room temperature for 15 hours and concentrated under reduced pressure. The residue was dissolved in EtOAc (200 ml), and the solution was washed with H_2O (200 ml) and brine (100 ml) successively. The organic layer 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: eluents=*n*-hexane~*n*-hexane-EtOAc=2:1). The fractions eluted with *n*-hexane-EtOAc=2:1 were concentrated under reduced pressure to give **12** (5.1 g, quant.): ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s, Boc), 3.46, 3.66 (2H, ABq, J=18 Hz, C_2-H), 3.81 (3H, s, OCH_3), 4.43, 4.54 (2H, ABq, J=12 Hz, C_3-CH_2), 5.03 (1H, d, J=5 Hz, C_6-H), 5.22 (2H, s, CO_2CH_2), 5.45 (1H, d, J=9 Hz, C_7-NH), 5.64 (1H, dd, J=5 and 9 Hz, C_7-H), 6.90, 7.32 (each 1H, d, J=8.8 Hz, Ph).

p-Methoxybenzyl 7β-*t*-Butoxycarbonylamino-3-(triphenylphosphonium)methyl-3-cephem-4-carboxylate Iodide (**13**)

Sodium iodide (7.50 g, 50 mmol) was added to a solution of **12** (5.1 g, 10 mmol) in methyl ethyl ketone (100 ml), and the mixture was stirred at room temperature for 1 hour under a nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (200 ml) and was washed with H_2O (150 ml), aq sodium thiosulfate (150 ml) and brine (150 ml) successively. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. The residue was dissolved in toluene (100 ml). To the solution was added triphenylphosphine (4.72 g, 18 mmol), and the mixture was stirred at room temperature for 1 hour under a nitrogen atmosphere. The reaction mixture was diluted with *n*-hexane (50 ml). The resulting precipitate was collected by filtration, washed with diethyl ether (10 ml) and dried under a vacuum to give **13** (6.08 g, 73%): ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s, Boc), 3.24, 4.27 (2H, ABq, J=18 Hz, C_2-H), 3.85 (3H, s, OCH_3), 4.87 (2H, s, CO_2CH_2), 4.95 (1H, d, J=4.2 Hz, C_6-H), 5.0~5.3 (2H, m, C_3-CH_2 and C_7-NH), 5.5~5.9 (2H, m, C_3-CH_2 and C_7-H), 6.87, 7.17 (each 1H, d, J=8.8 Hz, Ph), 7.6~7.9 (15H, m, Ph).

p-Methoxybenzyl 7β-*t*-Butoxycarbonylamino-3-[(*E*,*Z*)-2-(imidazo[1,2-*b*]pyridazine-6-yl)vinyl]-3-cephem-4-carboxylates Iodide (**15**)

Saturated aq NaCl (9.25 ml) and 1 N NaOH (9.25 ml) were successively added to a solution of **13** (3.73 g, 4.53 mmol) in chloroform (18.5 ml), and the mixture was stirred

at room temperature for 1 hour. To the separated organic layer was added a solution of 6-formylimidazo[1,2-*b*]-pyridazine (**14**, 680 mg, 4.62 mmol) in chloroform (20 ml), and the mixture was stirred at room temperature for 5.5 hours. The reaction mixture was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (100 g: eluents=*n*-hexane~*n*-hexane - EtOAc=1 : 3). The fractions eluted with *n*-hexane - EtOAc were concentrated under reduced pressure. Treatment of the residual solid with diethyl ether - EtOAc (10 : 1, 30 ml) gave **15** (1.45 g, 57%) as a geometrical mixture (*Z* : *E*=2 : 1): ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s, Boc), 3.35, 3.54 (2H×2/3, ABq, *J*=18 Hz, C_2-H), 3.65, 3.82 (2H×1/3, ABq, *J*=18 Hz, C_2-H), 3.80 (3H×2/3, s, OCH_3), 3.83 (3H×1/3, s, OCH_3), 5.01~5.45 (4H, m, CO_2CH_2, C_6-H and C_7-NH), 5.66 (1H, m, C_7-H), 6.52 (1H×2/3, d, *J*=12 Hz, CH=CH), 6.7~7.9 (9H+1H×1/3, m, Ph, CH=CH and IP-H).

7β-Amino-3-[(*E*,*Z*)-2-(imidazo[1,2-*b*]pyridazine-6-yl)-vinyl]-3-cephem-4-carboxylates Bistrifluoroacetic Acid (**16**)

Iodomethane (1.87 ml, 30 mmol) was added to a solution of **15** (564 mg, 1.0 mmol) in DMF (5.0 ml). The reaction mixture was stirred at room temperature for 21 hours and concentrated under reduced pressure. The concentrate was diluted with diethyl ether (60 ml). After being stirred at room temperature for 10 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation. To the residual oil were added anisole (4.0 ml) and TFA (5.0 ml) successively, and the mixture was stirred at room temperature for 2.5 hours. The reaction mixture was diluted with diethyl ether (60 ml). The resulting precipitate was collected by filtration, washed with diethyl ether (20 ml) and dried under a vacuum to give **16** (455 mg, 96%) as a geometrical mixture (*Z* : *E*=2 : 1): ^{1}H NMR (DMSO-d_6) δ 3.3~3.7 (2H, m, C_2-H), 4.11 (3H, s, CH_3), 5.1~5.4 (2H, m, C_6-H and C_7-H), 6.81, 7.07 (2H×2/3, d, *J*=12 Hz, vinyl), 6.65, 7.64 (2H×1/3, d, *J*=16 Hz, vinyl), 7.9~8.8 (4H, m, IP-H).

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyl-oxyiminoacetamido]-3-[(*E*,*Z*)-2-(1-methylimidazo[1,2-*b*]-pyridazinium-6-yl)vinyl]-3-cephem-4-carboxylates (**18a** and **19a**)

Under ice-cooling, the pH of a solution of **16** (120 mg, 0.26 mmol) in a mixture of THF (10 ml) and H_2O (10 ml) was adjusted to 7.5 with saturated aq $NaHCO_3$. After **6a** (90 mg, 0.29 mmol) was added portionwise to the solution at 5°C, the pH of the mixture was adjusted to 8.0 with saturated aq $NaHCO_3$. The reaction mixture was stirred at 5°C for 30 minutes and concentrated under reduced pressure. The concentrate was purified by MCI gel HP-20 column chromatography (100 ml: eluents=H_2O~30% aq EtOH). The fractions eluted with 30% aq EtOH were concentrated under reduced pressure. The resulting precipitate was collected by filtration, washed with H_2O (2 ml) and dried under a vacuum to give **19a** (14 mg, 9%). The filtrate was further purified by sephadex LH-20 column chromatography (300 ml: eluent=H_2O). The fractions eluted with H_2O were concentrated under reduced pressure. The concentrate was lyophilized to give **18a** (34 mg, 22%). The analytical results are shown in Table 6 and Table 7.

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-fluoromethoxy-iminoacetamido]-3-[(*E*)-2-(1-methylimidazo[1,2-*b*]pyri-dazinium-6-yl)vinyl]-3-cephem-4-carboxylate (**19c**)

Under ice-cooling, the pH of a solution of **16** (455 mg, 0.97 mmol) in a mixture of THF (20 ml) and H_2O (20 ml) was adjusted to 7.5 with saturated aq $NaHCO_3$. After 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-fluoromethoxyimino-acetyl chloride hydrochloride[19] (**6c**, 240 mg, 0.87 mmol) was added portionwise to the solution at 5°C, the pH of the mixture was adjusted to 8.0 with saturated aq $NaHCO_3$. The reaction mixture was stirred at 5°C for 30 minutes and concentrated under reduced pressure. The concentrate was purified by MCI gel HP-20 column chromatography (100 ml: eluents=H_2O~15% aq EtOH). The fractions eluted with 15% aq EtOH were concentrated under reduced pressure and lyophilized. The obtained powder was dissolved in DMSO (18 ml) and allowed to stand at room temperature for 1.5 hours. After evaporation of the mixture under reduced pressure, the residue was purified by MCI gel HP-20 column chromatography (100 ml: eluents=H_2O~15% aq EtOH). The fractions eluted with 15% aq EtOH were concentrated under reduced pressure. The resulting precipitate was collected by filtration, washed with H_2O (2 ml) and dried under a vacuum to give **19c** (75 mg, 14%).

The derivatives **19b**, **19d** and **19e** were prepared by a similar method. The yields are shown in Scheme 3. The analytical results are shown in Table 6 and Table 7.

Benzhydryl 7β-*t*-Butoxycarbonylamino-3-[(*Z*)-2-(imidazo-[1,2-*b*]pyridazine-6-yl)thiovinyl]-1-oxide-3-cephem-4-carboxylate (**21**)

To a solution of benzhydryl 7β-*t*-butoxycarbonylamino-3-[(*Z*)-2-tosyloxyvinyl]-1-oxide-3-cephem-4-carboxylate[14] (**20**, 0.3 g, 0.44 mmol) in DMF (3 ml) was added **3** (150 mg, 0.88 mmol), and the mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with EtOAc (100 ml), and the solution was washed twice

with H_2O (100 ml). The organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give **21** (240 mg, 82%): ^1H NMR ($CDCl_3$) δ 1.47 (9H, s, Boc), 3.45, 4.16 (2H, ABq, J=18 Hz, C_2-H), 4.57 (1H, d, J=4 Hz, C_6-H), 5.86 (2H, m, C_7-H and C_7-NH), 6.78 (1H, d, J=9.4 Hz, IP-H), 6.97 (1H, s, CH), 7.2~7.6 (12H, m, Ph and vinyl), 7.71 (1H, s, IP-H), 7.79 (1H, d, J=9.4 Hz, IP-H), 7.89 (1H, s, IP-H).

Benzhydryl 7β-*t*-Butoxycarbonylamino-3-[(*Z*)-2-(imidazo-[1,2-*b*]pyridazine-6-yl)thiovinyl]-3-cephem-4-carboxylate (**22**)

Under cooling at −10°C, phosphorus trichloride (0.061 ml, 0.70 mmol) was added to a solution of **21** (0.23 g, 0.35 mmol) in a mixture of dichloromethane (3.8 ml) and *N*,*N*-dimethylacetamide (0.15 ml), and the mixture was stirred at −10°C for 1 hour. The reaction mixture was evaporated under reduced pressure. The residue was portioned in a mixture of EtOAc (100 ml) and aq $NaHCO_3$ (100 ml). The separated organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give **22** (0.18 g, 82%): ^1H NMR ($CDCl_3$) δ 1.47 (9H, s, Boc), 3.73 (2H, m, C_2-H), 5.04 (1H, d, J=4 Hz, C_6-H), 5.66 (2H, m, C_7-H and C_7-NH), 6.80 (1H, d, J=9.4 Hz, IP-H), 6.96 (1H, s, CH), 7.2~7.6 (12H, m, Ph and vinyl), 7.72 (1H, s, IP-H), 7.81 (1H, d, J=9.4 Hz, IP-H), 7.88 (1H, s, IP-H).

7β-Amino-3-[(*Z*)-2-(imidazo[1,2-*b*]pyridazine-6-yl)-thiovinyl]-3-cephem-4-carboxylate Bistrifluoroacetic Acid (**23**)

Iodomethane (0.12 ml, 1.93 mmol) was added to a solution of **22** (120 mg, 0.19 mmol) in DMF (1 ml), and the mixture was stirred at room temperature for 16 hours and concentrated under reduced pressure. The concentrate was diluted with a mixture of diethyl ether (60 ml) and *n*-hexane (60 ml). After being stirred at room temperature for 10 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation. The residual oil was dissolved in dichloromethane (5 ml). To the solution were added anisole (2 ml) and TFA (2.5 ml) successively, and the mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with diethyl ether (100 ml). The resulting precipitate was collected by filtration, washed with diethyl ether (20 ml) and dried under a vacuum to give **23** (106 mg, 92%): ^1H NMR (DMSO-d_6) δ 3.92 (2H, m, C_2-H), 4.11 (3H, s, CH_3), 5.24 (1H, d, J=5 Hz, C_6-H), 5.31 (1H, d, J=5 Hz, C_7-H), 7.08, 7.17 (each 1H, d, J=11 Hz, vinyl), 8.03 (1H, d, J=9.8 Hz, IP-H), 8.45 (1H, d, J=2 Hz, IP-H), 8.72 (1H, d, J=2 Hz, IP-H), 8.74 (1H, d, J=9.8 Hz, IP-H).

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyl-oxyiminoacetamido]-3-[(*Z*)-2-(1-methylimidazo[1,2-*b*]-pyridazinium-6-yl)thiovinyl]-3-cephem-4-carboxylate (**24**)

Under ice-cooling, the pH of a solution of **23** (100 mg, 0.16 mmol) in a mixture of THF (20 ml) and H_2O (20 ml) was adjusted to 7.5 with aq $NaHCO_3$. To the solution was added portionwise **6a** (60 mg, 0.19 mmol), and the mixture was stirred at 5°C for 30 minutes. The pH of the reaction mixture was adjusted to 8.0. The reaction mixture was concentrated under reduced pressure. The concentrate was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=H_2O~30% aq EtOH). The fractions eluted with 30% aq EtOH were concentrated under reduced pressure, and the concentrate was lyophilized to give **24** (64 mg, 59%). The analytical results are shown in Table 6 and Table 7.

Benzhydryl 7β-*t*-Butoxycarbonylamino-3-[(*E*)-2-(imidazo-[1,2-*b*]pyridazine-6-yl)thiovinyl]-1-oxide-3-cephem-4-carboxylate (**26**)

Under ice-cooling, **3** (8.0 g, 46.2 mmol) was added to a solution of benzhydryl 7β-*t*-butoxycarbonylamino-3-[(*E*)-2-tosyloxyvinyl]-1-oxide-3-cephem-4-carboxylate[14] (**25**, 21 g, 30.9 mmol) in DMF (200 ml), and the mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with EtOAc (600 ml), and the solution was washed twice with brine (400 ml). The organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give **26** (20.5 g, quant.): ^1H NMR ($CDCl_3$) δ 1.47 (9H, s, Boc), 3.27, 4.15 (2H, ABq, J=18 Hz, C_2-H), 4.53 (1H, d, J=4 Hz, C_6-H), 5.85 (2H, m, C_7-H and C_7-NH), 6.77 (1H, d, J=9.4 Hz, IP-H), 6.97 (1H, s, CH), 7.1~7.6 (12H, m, Ph and vinyl), 7.68 (1H, s, IP-H), 7.75 (1H, d, J=9.4 Hz, IP-H), 7.89 (1H, s, IP-H).

Benzhydryl 7β-*t*-Butoxycarbonylamino-3-[(*E*)-2-(imidazo-[1,2-*b*]pyridazine-6-yl)thiovinyl]-3-cephem-4-carboxylate (**27**)

Under cooling at −10°C, phosphorus trichloride (4.8 ml, 55.0 mmol) was added to a solution of **26** (20.3 g, 30.9 mmol) in a mixture of dichloromethane (330 ml) and *N*,*N*-dimethylacetamide (12 ml), and the mixture was stirred at −10°C for 45 minutes. The reaction mixture was evaporated under reduced pressure. The residue was portioned in a mixture of EtOAc (600 ml) and aq $NaHCO_3$ (500 ml). The separated organic layer was washed with brine (300 ml), dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (300 g: eluents=*n*-hexane~EtOAc). The fractions eluted with

EtOAc were concentrated under reduced pressure to give **27** (12.2 g, 61%): ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s, Boc), 3.56, 3.70 (2H, ABq, J=18 Hz, C_2-H), 4.98 (1H, d, J=5 Hz, C_6-H), 5.66 (1H, dd, J=5 and 9.2 Hz, C_7-H), 6.30 (1H, d, J=9.2 Hz, C_7-NH), 6.73 (1H, d, J=9.2 Hz, IP-H), 6.95 (1H, s, CH), 7.1~7.6 (12H, m, Ph and vinyl), 7.69 (1H, s, IP-H), 7.78 (1H, d, J=9.2 Hz, IP-H), 7.87 (1H, s, IP-H).

7β-Amino-3-[(*E*)-2-(imidazo[1,2-*b*]pyridazine-6-yl)-thiovinyl]-3-cephem-4-carboxylate Bistrifluoroacetic Acid (**28a**)

Iodomethane (100 ml, 1.6 mol) was added to a solution of **27** (29 g, 45 mmol) in DMF (90 ml), and the mixture was stirred at room temperature for 22 hours and concentrated under reduced pressure. The concentrate was diluted with a mixture of diethyl ether (400 ml) and *n*-hexane (400 ml). After being stirred at room temperature for 10 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation. The residual oil was dissolved in dichloromethane (225 ml). To the solution were added anisole (100 ml) and TFA (125 ml) successively, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with diethyl ether (1 liter). The resulting precipitate was collected by filtration, washed with diethyl ether (50 ml) and dried under vacuum to give **28a** (26 g, 93%): ^{1}H NMR (DMSO-d_6) δ 3.83, 4.09 (2H, ABq, J=17 Hz, C_2-H), 4.10 (3H, s, CH_3), 5.15 (1H, d, J=5Hz, C_6-H), 5.26 (1H, d, J=5 Hz, C_7-H), 7.38 (2H, s, vinyl), 8.05 (1H, d, J=9.8 Hz, IP-H), 8.42 (1H, d, J=2 Hz, IP-H), 8.69 (1H, d, J=2 Hz, IP-H), 8.72 (1H, d, J=9.8 Hz, IP-H).; TFA content: 33.0% (measured by ion chromatography)

The derivatives **28b**~**28g** were prepared by a method similar to that used to prepare **28a**. The yields, analytical data and the reaction conditions were as follow.

28b (170 mg, 86%) was obtained from the reaction of iodoethane (2.5 ml) with **27** (0.2 g, 0.312 mmol) in DMF (2 ml) at 40°C for 48 hours followed by TFA (2.5 ml)/anisole (2 ml) treatment: ^{1}H NMR (DMSO-d_6) δ 1.49 (3H, t, J=7.2 Hz, CH_3), 3.87, 4.11 (2H, ABq, J=17 Hz, C_2-H), 4.54 (2H, q, J=7.2 Hz, CH_2), 5.24 (1H, d, J=5 Hz, C_6-H), 5.30 (1H, d, J=5 Hz, C_7-H), 7.41 (2H, s, vinyl), 8.06 (1H, d, J=9.6 Hz, IP-H), 8.54 (1H, d, J=2 Hz, IP-H), 8.71 (1H, d, J=2 Hz, IP-H), 8.80 (1H, d, J=9.6 Hz, IP-H).

28c (182 mg, 88%) was obtained from the reaction of iodoacetamide (1.73 g) with **27** (0.2 g, 0.312 mmol) in DMF (2 ml) at room temperature for 22 hours followed by TFA (2.5 ml)/anisole (2 ml) treatment: ^{1}H NMR (DMSO-d_6) δ 3.85, 4.10 (2H, ABq, J=17 Hz, C_2-H), 5.20 (1H, d, J=5 Hz, C_6-H), 5.29 (1H, d, J=5 Hz, C_7-H), 5.31 (2H, s, CH_2), 7.41 (2H, s, vinyl), 7.64, 7.95 (each 1H, br s, $CONH_2$), 8.08 (1H, d, J=9.4 Hz, IP-H), 8.45 (1H, d, J=2 Hz, IP-H), 8.70 (1H, d, J=9.4 Hz, IP-H), 8.73 (1H, d, J=2 Hz, IP-H).

28d (195 mg, 97%) was obtained from the reaction of allyl bromide (2.7 ml) with **27** (0.2 g, 0.312 mmol) in DMF (2 ml) at 40°C for 16 hours followed by TFA (2.5 ml)/anisole (2 ml) treatment: ^{1}H NMR (DMSO-d_6) δ 3.88, 4.15 (2H, ABq, J=17 Hz, C_2-H), 5.1~5.4 (6H, m, C_6-H, C_7-H and allyl), 5.9~6.2 (1H, m, allyl), 7.42 (2H, s, vinyl), 8.10 (1H, d, J=9.8 Hz, IP-H), 8.46 (1H, d, J=2 Hz, IP-H), 8.73 (1H, d, J=9.8 Hz, IP-H), 8.76 (1H, d, J=2 Hz, IP-H).

28e (170 mg, 84%) was obtained from the reaction of 2-iodoethanol (2.43 ml) with **27** (0.2 g, 0.312 mmol) in DMF (2 ml) at 40°C for 48 hours followed by TFA (2.5 ml)/anisole (2 ml) treatment: ^{1}H NMR (DMSO-d_6) δ 3.79 (2H, m, CH_2), 3.86, 4.10 (2H, ABq, J=17 Hz, C_2-H), 4.58 (2H, m, CH_2), 5.22 (1H, d, J=5 Hz, C_6-H), 5.28 (1H, d, J=5 Hz, C_7-H), 7.40 (2H, s, vinyl), 7.96 (1H, d, J=9.6 Hz, IP-H), 8.41 (1H, d, J=2 Hz, IP-H), 8.66 (1H, d, J=9.6 Hz, IP-H), 8.71 (1H, d, J=2 Hz, IP-H).

28f (185 mg, 90%) was obtained from the reaction of chloroacetone (0.14 ml) with **27** (0.2 g, 0.312 mmol) in a mixture of dichloromethane (1.2 ml) and acetonitrile (0.46 ml) in the presence of sodium iodide (0.26 g, 1.73 mmol) at room temperature for 18 hours followed by TFA (2.5 ml)/anisole (2 ml) treatment: ^{1}H NMR (DMSO-d_6) δ 2.30 (2H, s, CH_3), 3.85, 4.11 (2H, ABq, J=17 Hz, C_2-H), 5.16 (1H, d, J=5 Hz, C_6-H), 5.28 (1H, d, J=5 Hz, C_7-H), 5.65 (2H, s, CH_2), 7.40 (2H, s, vinyl), 8.01 (1H, d, J=10 Hz, IP-H), 8.24 (1H, d, J=2 Hz, IP-H), 8.59 (1H, d, J=10 Hz, IP-H), 8.75 (1H, d, J=2 Hz, IP-H).

28g (85 mg, 83%) was obtained from the reaction of *t*-butyl bromoacetate (2.5 ml) with **27** (0.1 g, 0.156 mmol) in DMF (1 ml) at room temperature for 16 hours followed by TFA (1.25 ml)/anisole (1 ml) treatment: ^{1}H NMR (DMSO-d_6) δ 3.84, 4.10 (2H, ABq, J=17 Hz, C_2-H), 5.18 (1H, d, J=5 Hz, C_6-H), 5.28 (1H, d, J=5 Hz, C_7-H), 5.48 (2H, s, CH_2), 7.40 (2H, s, vinyl), 8.12 (1H, d, J=9.6 Hz, IP-H), 8.45 (1H, d, J=2 Hz, IP-H), 8.75 (1H, d, J=2 Hz, IP-H), 8.80 (1H, d, J=9.6 Hz, IP-H).

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-[(*E*)-2-(1-methylimidazo[1,2-*b*]-pyridazinium-6-yl)thiovinyl]-3-cephem-4-carboxylate (**29aa**)

Under ice-cooling, the pH of a solution of **28a** (1.88 g, 3.04 mmol) in a mixture of THF (150 ml) and H_2O (150 ml) was adjusted to 7.5 with aq $NaHCO_3$. To the solution was added portionwise **6a** (1.22 g, 3.92 mmol), and the mixture was stirred at 5°C for 30 minutes. The pH of the reaction

mixture was adjusted to 8.0. The reaction mixture was concentrated under reduced pressure, and the concentrate was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=H_2O~30% aq EtOH). The fractions eluted with 30% aq EtOH were concentrated under reduced pressure, and the concentrate was lyophilized to give **29aa** (0.93 g, 49%). The analytical results are shown in Table 7 and Table 8.

The derivatives **29ab**~**29ia** except for **29fa** were prepared by a similar method. The yields are shown in Scheme 4-2. The analytical results are shown in Table 6 and Table 7.

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-hydroxyiminoacetamido]-3-[(*E*)-2-(1-methylimidazo[1,2-*b*]-pyridazinium-6-yl)thiovinyl]-3-cephem-4-carboxylate (**29fa**)

Under ice-cooling, 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-trityloxyiminoacetyl chloride hydrochloride[12] (1.22 g, 3.92 mmol) was added to a solution of **28a** (193 mg, 0.312 mmol) in a mixture of THF (20 ml) and H_2O (20 ml) containing tri-*n*-butylamine (0.52 ml, 2.18 mmol), and the mixture was stirred at 5°C for 30 minutes. The reaction mixture was concentrated under reduced pressure. To the concentrate was added 90% aq formic acid (6 ml), and the mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure. The concentrate was diluted with aq $NaHCO_3$ (100 ml), and the solution was washed with EtOAc (60 ml). The separated aqueous layer was 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, and the concentrate was lyophilized to give **29fa** (67 mg, 38%). The analytical results are shown in Table 6 and Table 7.

p-Methoxybenzyl 7β-*t*-Butoxycarbonylamino-3-[(*E*)-4-(imidazo[1,2-*b*]pyridazine-6-yl)-1-butenyl]-3-cephem-4-carboxylate (**31**)

Saturated aq NaCl (5.88 ml) and 1 N NaOH (5.88 ml) were successively added to a solution of **13** (2.42 g, 2.94 mmol) in chloroform (10 ml), and the mixture was stirred at room temperature for 1 hour. To the separated organic layer was added a solution of 3-(imidazo[1,2-*b*]pyridazine-6-yl)propanal (**30**, 468 mg, 2.67 mmol) in chloroform (10 ml), and the mixture was stirred at room temperature for 24 hours. The reaction mixture was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (100 g: eluents=*n*-hexane~*n*-hexane - EtOAc=1 : 3). The fractions eluted with *n*-hexane - EtOAc were concentrated under reduced pressure to give geometrical mixture of **31** (870 mg, *Z*:*E*=4 : 1). The obtained geometrical mixture was dissolved in toluene (60 ml). To the solution was added iodine (37.4 mg), and the mixture was stirred at 100°C for 40 hours. The reaction mixture was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (20 g: eluents=*n*-hexane~*n*-hexane - EtOAc=1 : 2). The fractions eluted with *n*-hexane - EtOAc were concentrated under reduced pressure to give **31** (570 mg, 32%) as the (*E*)-isomer: ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s, Boc), 2.64 (2H, m, CH_2), 2.92 (2H, t, *J*=8 Hz, IP-CH_2), 3.48, 3.64 (2H, ABq, *J*=18 Hz, C_2-H), 3.78 (3H, s, OCH_3), 4.95 (1H, d, *J*=5 Hz, C_6-H), 5.18, 5.26 (2H, ABq, *J*=12 Hz, CO_2CH_2), 5.24 (1H, d, *J*=9.8 Hz, C_7-NH), 5.58 (1H, dd, *J*=5 and 9.8 Hz, C_7-H), 6.03 (1H, dt, *J*=16 and 7 Hz, CH=C*H*CH_2), 6.8~7.0 (4H, m, Ph, C_3-CH and IP-H), 7.35 (2H, d, *J*=9 Hz, Ph), 7.74 (1H, d, *J*=1 Hz, IP-H), 7.88 (1H, d, *J*=10 Hz, IP-H), 7.91 (1H, d, *J*=1 Hz, IP-H).

7β-Amino-3-[(*E*)-4-(1-methylimidazo[1,2-*b*]-pyridazinium-6-yl)-1-butenyl]-3-cephem-4-carboxylate Bistrifluoroacetic Acid (**32**)

Iodomethane (2.9 ml, 46.6 mmol) was added to a solution of **31** (550 mg, 0.93 mmol) in DMF (5.0 ml), and the mixture was stirred at room temperature for 3 hours and concentrated under reduced pressure. The concentrate was diluted with diethyl ether (60 ml). After being stirred at room temperature for 10 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation. To the residual oil were added anisole (3.6 ml) and TFA (4.5 ml) successively, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with diethyl ether (60 ml). The resulting precipitate was collected by filtration, washed with diethyl ether (10 ml) and dried under a vacuum to give **32** (550 mg, 96%): ^{1}H NMR (DMSO-d_6) δ 2.69 (2H, m, CH_2), 3.13 (2H, t, *J*=7 Hz, IP-CH_2), 3.66, 3.82 (2H, ABq, *J*=18 Hz, C_2-H), 4.09 (3H, s, CH_3), 5.11 (1H, d, *J*=5 Hz, C_6-H), 5.20 (1H, d, *J*=5 Hz, C_7-H), 6.27 (1H, dt, *J*=16 and 7 Hz, CH=C*H*CH_2), 6.86 (1H, d, *J*=16 Hz, C_3-CH), 8.00 (1H, d, *J*=10 Hz, IP-H), 8.44 (1H, d, *J*=2 Hz, IP-H), 8.71 (1H, d, *J*=2 Hz, IP-H), 8.74 (1H, d, *J*=10 Hz, IP-H).

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-[(*E*)-4-(1-methylimidazo[1,2-*b*]-pyridazinium-6-yl)-1-butenyl]-3-cephem-4-carboxylate (**33**)

Under ice-cooling, the pH of a solution of **32** (150 mg, 0.24 mmol) in a mixture of THF (9 ml) and H_2O (9 ml) was

adjusted to 7.5 with aq $NaHCO_3$. After **6a** (84 mg, 0.27 mmol) was added portionwise to the solution at 5°C, the pH of the mixture was maintained at 8.0 by addition of aq $NaHCO_3$. The reaction mixture was stirred at 5°C for 30 minutes and concentrated under reduced pressure. The concentrate was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=H_2O~35% aq EtOH). The fractions eluted with 35% aq EtOH were concentrated under reduced pressure. The resulting precipitate was collected by filtration, washed with H_2O (1 ml) and dried under a vacuum to give **33** (90 mg, 56%). The analytical results are shown in Table 6 and Table 7.

p-Methoxybenzyl 7*β*-*t*-Butoxycarbonylamino-3-[(*E*,*Z*)-3-(imidazo[1,2-*b*]pyridazine-6-yl)thio-1-propenyl]-3-cephem-4-carboxylates (**35**)

40% aq chloroacetaldehyde (8.3 ml) and H_2O (11 ml) were successively added to a solution of **13** (2.48 g, 3.0 mmol) in dichloromethane (40 ml). Under ice-cooling, to the mixture was added a solution of potassium carbonate (465 mg, 3.36 mmol) in H_2O (10 ml), and the mixture was stirred at 5°C for 4 hours. The organic layer was separated, and the aqueous layer was extracted twice with dichloromethane (100 ml). The combined organic layer was washed with brine (100 ml), 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: eluents=*n*-hexane~*n*-hexane - EtOAc=5 : 2). The fractions eluted with *n*-hexane - EtOAc were concentrated under reduced pressure, and the residue was dissolved in Me_2CO (40 ml). To the solution was added sodium iodide (1.75 g, 11.7 mmol), and the mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure. The concentrate was portioned into ice-water (50 ml) and EtOAc (100 ml), and the organic layer was separated. The aqueous layer was extracted twice with EtOAc (100 ml). The combined organic layer was washed with sodium thiosulfate (100 ml) and brine (100 ml) successively, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure, and the residue was dissolved in DMF (1 ml). To the solution was added **3** (425 mg, 2.46 mmol), and the mixture was stirred at room temperature for 4 hours. The reaction mixture was diluted with EtOAc (100 ml), and the solution was washed twice with H_2O (100 ml). The separated 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 (90 g: eluents=*n*-hexane~EtOAc). The fractions eluted with EtOAc were concentrated under reduced pressure to give **35** (550 mg, 30%) as a geometrical mixture (*Z* : *E*=1 : 2): ^{1}H NMR ($CDCl_3$) δ 1.45, 1.47 (9H, eachs, Boc), 3.4~4.0 (7H, m, C_2-H, SCH_2 and OCH_3), 4.94 (1H, d, *J*=5 Hz, C_6-H), 5.1~5.4 (4H, m, CO_2CH_2, C=C*H* and C_7-NH), 5.57 (1H, m, C_7-H), 6.35 (1H×1/3, d, *J*=11 Hz, C_3-CH), 6.6~7.0 (3H, m, Ph and IP-H), 7.22 (1H×2/3, d, *J*=15 Hz, C_3-CH), 7.3~7.5 (2H, m, Ph), 7.6~8.1 (3H, m, IP-H).

7*β*-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-[(*E*)-3-(1-methylimidazo[1,2-*b*]pyridazinium-6-yl)thio-1-propenyl]-3-cephem-4-carboxylate (**37**)

Iodomethane (2.5 ml, 40 mmol) was added to a solution of **35** (236 mg, 0.39 mmol) in DMF (2.0 ml), and the mixture was stirred at room temperature for 3 hours and concentrated under reduced pressure. The concentrate was diluted with a mixture of diethyl ether (75 ml) and *n*-hexane (75 ml). After being stirred at room temperature for 15 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation. The residual oil was dissolved in dichloromethane (5 ml). To the solution were added anisole (2 ml) and TFA (2.5 ml) successively, and the mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with diethyl ether (100 ml). The resulting precipitate was collected by filtration, washed with diethyl ether (10 ml) and dissolved in a mixture of THF (20 ml) and H_2O (20 ml). Under ice-cooling, the pH of the solution was adjusted to 7.5 with aq $NaHCO_3$. After **6a** (182 mg, 0.58 mmol) was added portionwise to the solution at 5°C, the pH of the mixture was maintained at 8.0 by addition of aq $NaHCO_3$. The reaction mixture was stirred at 5°C for 30 minutes and concentrated under reduced pressure. The concentrate was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=H_2O~35% aq EtOH). The fractions eluted with 35% aq EtOH were concentrated under reduced pressure. The resulting precipitate was collected by filtration, washed with H_2O (1 ml) and dried under a vacuum to give **37** (50 mg, 20%) as the (*E*)-isomer. The analytical results are shown in Table 6 and Table 7.

Sodium Imidazo[1,2-*b*]pyridazine-6-thiolate (**3**)

Methyl 3-mercaptopropionate (88.6 ml, 0.8 mol) and 28% sodium methoxide in MeOH (154.4 ml, 0.8 mol) were added successively to a solution of 6-chloroimidazo[1,2-*b*]pyridazine[15] (**38**, 61.4 g, 0.4 mol) in MeOH (1.2 liter), and the mixture was stirred at 80°C for 3 hours. After cooling, the reaction mixture was evaporated under reduced pressure. The residue was treated with a mixture of THF (400 ml) and MeOH (400 ml), and the resulting precipitate

was filtered off. The filtrate was evaporated under reduced pressure. The residual solid was treated with EtOAc (400 ml). The resulting precipitate was collected by filtration, washed four times with EtOAc (200 ml) and dried under a vacuum to give **3** (62.5 g, 90%): MP>300°C; ^{1}H NMR (DMSO-d_6) δ 6.82, 7.11 (each 1H, d, J=9.6 Hz, C_7-H and C_8-H), 7.21, 7.54 (each1H, s, C_2-H and C_3-H).

6-Chloro-1-methylimidazo[1,2-*b*]pyridazinium Iodide (**39**)

Iodomethane (10 ml, 160 mmol) was added to a solution of **38** (2.0 g, 13 mmol) in DMF (8 ml), and the mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with Me_2CO (100 ml). The resulting crystals were collected by filtration, washed with Me_2CO (20 ml) and dried under a vacuum to give **39** (85 g, 100%): MP 270~275°C (dec.); ^{1}H NMR (DMSO-d_6) δ 4.14 (3H, s, CH_3), 8.10, 8.82 (each 1H, d, J=10 Hz, C_7-H and C_8-H), 8.48, 8.75 (each 1H, d, J=2 Hz, C_2-H and C_3-H).

1-Methylimidazo[1,2-*b*]pyridazinium-6-thiolate (**9**)

A solution of **39** (3.54 g, 12 mmol) in 2 N aq potassium hydrogen sulfide (25 ml) was stirred at room temperature for 1 hour and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (50 g: eluent=MeOH). The fractions eluted were concentrated under reduced pressure. Treatment of the residue with diethyl ether (50 ml) gave **9** (2.43 g, quant.) as crystals: MP 240~245°C (dec.); ^{1}H NMR (DMSO-d_6) δ 3.88 (3H, s, CH_3), 7.31, 7.65 (each 1H, d, J=10 Hz, C_7-H and C_8-H), 7.91, 7.99 (each 1H, d, J=2 Hz, C_2-H and C_3-H).

Ethyl 3-(Imidazo[1,2-*b*]pyridazine-6-yl)propionate (**40**)

Copper activated zinc[18] (4.91 g, 75 mmol) was added to a solution of ethyl 3-iodopropionate (11.4 g, 50 mmol) in a mixture of toluene (60 ml) and hexamethylphosphoramide (6 ml), and the mixture was stirred at 80°C for 3 hours under a nitrogen atmosphere. To the reaction mixture were

Table 6. IR and analytical data for 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-linked spacer (1-alkylimidazo[1,2-*b*]pyridazinium-6-yl)-3-cephem-4-carboxylates.

Compound No.	Formula	*Anal* Calcd (%) C	H	N	Found (%) C	H	N	IR (KBr, cm^{-1})		
7	$C_{23}H_{23}N_9O_5S_3\cdot4.0H_2O$	41.00	4.64	18.71	40.68	4.51	19.08	1760	1670	1615
10	$C_{24}H_{25}N_9O_5S_3\cdot4.0H_2O$	41.91	4.84	18.33	41.69	4.63	18.03	1760	1670	1600
18a	$C_{25}H_{25}N_9O_5S_2\cdot4.0H_2O$	44.97	4.98	18.88	45.17	4.86	18.48	1768	1668	1606
19a	$C_{25}H_{25}N_9O_5S_2\cdot3.0H_2O$	46.22	4.81	19.40	46.03	4.64	19.15	1772	1655	1610
19b	$C_{21}H_{19}N_9O_5S_2\cdot3.5H_2O$	41.72	4.33	20.85	41.98	4.15	20.96	1767	1670	1614
19c	$C_{21}H_{18}FN_9O_5S_2\cdot2.5H_2O$	41.72	3.83	20.85	41.99	4.05	20.63	1770	1672	1616
19d	$C_{22}H_{21}N_9O_5S_2\cdot3.5H_2O$	42.71	4.56	20.38	42.95	4.61	20.32	1767	1672	1616
19e	$C_{23}H_{23}N_9O_5S_2\cdot4.0H_2O$	43.05	4.87	19.65	42.94	4.63	19.36	1768	1670	1618
24	$C_{25}H_{25}N_9O_5S_3\cdot6.0H_2O$	40.81	5.07	17.13	40.94	4.57	17.49	1760	1650	1600
29aa	$C_{25}H_{25}N_9O_5S_3\cdot5.5H_2O$	41.31	4.99	17.34	41.57	4.77	17.38	1760	1660	1600
29ab	$C_{26}H_{27}N_9O_5S_3\cdot4.5H_2O$	43.20	5.02	17.44	43.12	4.79	17.24	1760	1660	1600
29ac	$C_{26}H_{26}N_{10}O_6S_3\cdot7.0H_2O$	39.19	5.06	17.58	39.12	4.59	17.09	1760	1680	1600
29ad	$C_{27}H_{27}N_9O_5S_3\cdot5.0H_2O$	43.60	5.01	16.95	43.88	4.74	17.46	1760	1670	1600
29ae	$C_{26}H_{27}N_9O_6S_3\cdot5.5H_2O$	41.26	5.06	16.66	41.50	4.67	16.47	1760	1660	1600
29af	$C_{27}H_{27}N_9O_6S_3\cdot7.0H_2O$	40.75	5.19	15.84	40.98	4.96	16.28	1760	1675	1600
29ag	$C_{26}H_{24}N_9O_7S_3Na\cdot6.0H_2O$	38.95	4.53	15.72	38.87	4.23	15.52	1760		1610
29ba	$C_{21}H_{19}N_9O_5S_3\cdot5.0H_2O$	38.00	4.40	18.99	38.03	4.09	19.04	1760	1660	1600
29ca	$C_{21}H_{18}FN_9O_5S_3\cdot3.0H_2O$	39.06	3.75	19.52	39.15	3.69	19.27	1760	1670	1600
29cb	$C_{22}H_{20}FN_9O_5S_3\cdot5.0H_2O$	37.98	4.35	18.12	38.07	3.90	18.46	1760	1680	1610
29cc	$C_{22}H_{19}FN_{10}O_6S_3\cdot3.5H_2O$	37.87	3.76	20.08	37.98	3.63	20.18	1760	1680	1610
29da	$C_{22}H_{21}N_9O_5S_3\cdot4.5H_2O$	39.51	4.52	18.85	39.73	4.58	19.19	1760	1670	1600
29ea	$C_{23}H_{23}N_9O_5S_3\cdot5.5H_2O$	39.42	4.89	17.99	39.41	4.54	18.10	1760	1670	1600
29fa	$C_{20}H_{17}N_9O_5S_3\cdot4.0H_2O$	38.03	3.99	19.96	38.15	4.25	19.69	1760	1670	1600
29ga	$C_{22}H_{20}FN_9O_5S_3\cdot3.5H_2O$	39.52	4.07	18.85	39.84	3.80	18.74	1760	1670	1600
29ha	$C_{22}H_{18}F_3N_9O_5S_3\cdot5.0H_2O$	36.11	3.86	17.23	36.03	3.86	17.02	1770	1680	1605
29ia	$C_{23}H_{21}N_9O_5S_3\cdot4.0H_2O$	41.13	4.35	18.77	40.96	4.06	18.56	1760		1600
33	$C_{27}H_{29}N_9O_5S_2\cdot4.0H_2O$	46.61	5.36	18.12	46.54	5.21	18.18	1774	1653	
37	$C_{26}H_{27}N_9O_5S_3\cdot4.0H_2O$	43.75	4.94	17.66	43.58	4.89	17.87	1760	1670	1600

Table 7. ^{1}H NMR spectral data for 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-linked spacer (1-alkylimidazo[1,2-*b*]pyridazinium-6-yl) -3-cephem-4-carboxylates.

Compd. No.	Chemical shift (*J*=Hz) (DMSO-d_6, δ)									
	Cephem nuclei					Thiadiazole		Imidazo[1,2-*b*]pyridazinium		
	C_2-H Abq (17)	C_6-H d (5)	C_7-H dd (5&8)	C_7-NH d (8)	NH_2 br s	N-OR_1	X	R_2	C_2&C_3-H d(2)	C_7&C_8-H d(10)
7	3.24	5.20	5.74	9.57	8.14	1.4~1.9(8H,m)		4.07(3H,s)	8.37	7.91
	3.95					4.74(1H,m)			8.61	8.62
10	3.27	4.94	5.76	9.41	8.13	1.4~2.0(8H,m)	3.99,4.99	4.06(3H,s)	8.31	7.89
	3.60					4.72(1H,m)	(2H,ABq,*J*=14Hz)		8.81	8.55
18a*	3.42	5.34	5.81	–	–	1.4~2.0(8H,m)	6.70,6.85	4.12(3H,s)	8.08	7.83
	3.75		(d)			4.92(1H,m)	(each1H,d,*J*=12Hz)		8.32	8.37
19a	3.48	5.14	5.67	9.55	8.16	1.4~2.0(8H,m)	6.65,8.08	4.07(3H,s)	8.37	7.90
	3.70					4.75(1H,m)	(each1H,d,*J*=16Hz)		8.72	8.65
19b*	3.80	5.36	5.90	–	–	4.10(3H,s)	6.92,7.77	4.11(3H,s)	8.04	8.08
	3.92		(d)				(each1H,d,*J*=16Hz)		8.29	8.40
19c	3.56	5.15	5.71	9.80	8.25	5.81(2H,d,*J*=54Hz)	6.64,8.08	4.07(3H,s)	8.37	7.90
	3.71						(each1H,d,*J*=16Hz)		8.72	8.65
19d*	3.80	5.37	5.90	–	–	1.34(3H,d,*J*=7Hz)	6.91,7.78	4.12(3H,s)	8.04	8.08
	3.92		(d)			4.38(2H,q,*J*=7Hz)	(each1H,d,*J*=16Hz)		8.29	8.40
19e*	3.79	5.37	5.90	–	–	1.37(6H,d,*J*=6Hz)	6.91,7.77	4.11(3H,s)	8.04	8.08
	3.93		(d)			4.61(1H,m)	(each1H,d,*J*=16Hz)		8.29	8.40
24	3.66	5.08	5.64	9.49	8.15	1.4~1.9(8H,m)	6.56,7.36	4.08(3H,s)	8.38	7.96
	3.85					4.74(1H,m)	(each1H,d,*J*=11Hz)		8.74	8.65
29aa	3.54	5.09	5.64	9.51	8.15	1.3~2.0(8H,m)	6.62,7.48	4.08(3H,s)	8.37	7.95
	3.65					4.74(1H,m)	(each1H,d,*J*=16Hz)		8.69	8.64
29ab	3.60	5.09	5.64	9.52	8.16	1.5~2.0(8H,m)	6.62,7.48	1.47(3H,t,*J*=7Hz)	8.47	7.96
	(m)					4.75(1H,m)	(each1H,d,*J*=16Hz)	4.51(2H,q,*J*=7Hz)	8.72	8.70
29ac	3.61	5.10	5.64	9.52	8.15	1.4~2.0(8H,m)	6.64,7.48	5.27(2H,s), 7.61,	8.39	7.98
	(m)					4.75(1H,m)	(each1H,d,*J*=16Hz)	7.93(each1H,br s)	8.74	8.62
29ad	3.61	5.09	5.64	9.51	8.16	1.4~2.0(8H,m)	6.63,7.47	5.16,5.31(each2H,	8.38	7.98
	(m)					4.75(1H,m)	(each1H,d,*J*=16Hz)	m), 6.06(1H,m)	8.75	8.62
29ae	3.61	5.10	5.65	9.52	8.16	1.4~1.9(8H,m)	6.65,7.45	3.79,4.58(each2H,	8.40	7.93
	(m)					4.74(1H,m)	(each1H,d,*J*=16Hz)	m), 5.42(1H,m)	8.70	8.65
29af	3.60	5.10	5.64	9.51	8.14	1.4~2.0(8H,m)	6.64,7.48	2.30(3H,s)	8.24	8.00
	(m)					4.74(1H,m)	(each1H,d,*J*=16Hz)	5.65(2H,s)	8.75	8.58
29ag	3.56	5.11	5.64	9.52	8.17	1.4~2.0(8H,m)	6.68,7.49	4.79(2H,s)	8.31	7.86
	3.66					4.75(1H,m)	(each1H,d,*J*=16Hz)		8.63	8.48
29ba	3.60	5.08	5.65	9.57	8.17	3.94(3H,s)	6.61,7.48	4.08(3H,s)	8.37	7.95
	(m)						(each1H,d,*J*=16Hz)		8.69	8.65
29ca	3.54	5.11	5.67	9.78	8.26	5.80(2H,d,*J*=55Hz)	6.61,7.47	4.08(3H,s)	8.37	7.95
	3.65						(each1H,d,*J*=16Hz)		8.69	8.66
29cb	3.55	5.11	5.65	9.78	8.25	5.80(2H,d,*J*=56Hz)	6.61,7.49	1.47(3H,t,*J*=7Hz)	8.47	7.96
	3.65						(each1H,d,*J*=16Hz)	4.51(2H,q,*J*=7Hz)	8.73	8.71
29cc	3.56	5.12	5.67	9.79	8.26	5.81(2H,d,*J*=56Hz)	6.64,7.49	5.30(2H,s),7.62,	8.40	7.98
	3.67						(each1H,d,*J*=16Hz)	8.00(each1H,br s)	8.74	8.63
29da	3.60	5.09	5.66	9.56	8.17	1.28(3H,t,*J*=7Hz)	6.61,7.46	4.08(3H,s)	8.37	7.95
	(m)					4.20(2H,q,*J*=7Hz)	(each1H,d,*J*=15Hz)		8.68	8.64
29ea	3.55	5.10	5.66	9.52	8.16	1.28(6H,m)	6.61,7.48	4.08(3H,s)	8.37	7.94
	3.66					4.42(1H,m)	(each1H,d,*J*=15Hz)		8.69	8.65
29fa	3.60	5.09	5.70	9.45	8.05	11.99(1H,br s)	6.62,7.47	4.06(3H,s)	8.37	7.88
	(m)						(each1H,d,*J*=16Hz)		8.72	8.65
29ga	3.53	5.10	5.66	9.63	8.21	4.40(2H,dt,*J*=28&3Hz)	6.61,7.46	4.08(3H,s)	8.37	7.95
	3.65					4.69(2H,dt,*J*=48&3Hz)	(each1H,d,*J*=15Hz)		8.70	8.64
29ha	3.59	5.10	5.67	9.73	8.25	4.80(2H,m)	6.62,7.47	4.08(3H,s)	8.38	7.95
	(m)						(each1H,d,*J*=16Hz)		8.69	8.66
29ia	3.53	5.09	5.67	9.61	8.20	4.68(2H,m),5.30(2H,	6.61,7.46	4.08(3H,s)	8.38	7.95
	3.65					m),5.99(1H,m)	(each1H,d,*J*=15Hz)		8.69	8.66
33	3.31	4.95	5.53	9.43	8.13	1.4~2.0(8H,m)	2.58(2H,m),3.07(2H,	4.09(3H,s)	8.39	7.94
	(m)					4.74(1H,m)	t,*J*=7Hz),5.60(1H,m),		8.73	8.70
							6.80(1H,d,*J*=16Hz)			
37	3.51	5.01	5.60	9.45	8.19	1.4~2.0(8H,m)	3.93(2H,m), 5.55(1H,	4.06(3H,s)	8.36	7.83
	(m)		(m)			4.75(1H,m)	m),7.33(1H,d,*J*=15Hz)		9.10	8.55

* measured in D_2O.

added **38** (6.14 g, 40 mmol) and bis(triphenylphosphine)-palladium chloride (561 mg, 0.8 mmol) successively. The mixture was stirred at 80°C for 1.5 hours under a nitrogen atmosphere. Under ice-cooling, a mixture of H_2O (100 ml), 25% aq ammonia (30 ml) and EtOAc (100 ml) was added to the reaction mixture. The mixture was stirred at room temperature for 30 minutes and filtered with Celite. The organic layer of the filtrate was separated. The aqueous layer was extracted twice with EtOAc (300 ml). The combined organic layer was washed with 25% aq ammonia (200 ml) and brine (200 ml) successively, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (180 g: eluent=dichloromethane~dichloromethane - EtOAc - MeOH=20 : 20 : 1). The fractions eluted with dichloromethane - EtOAc - MeOH were concentrated under reduced pressure to give **40** (5.47 g, 62%) as crystals: MP 52~54°C; ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J=7 Hz, CH_3), 2.84, 3.17 (each 2H, t, J=7.4 Hz, CH_2CH_2), 4.16 (2H, q, J=7 Hz, OCH_2), 6.95, 7.84 (each 1H, d, J=9 Hz, C_7-H and C_8-H), 7.70, 7.88 (each 1H, d, J=1 Hz, C_2-H and C_3-H).

3-(Imidazo[1,2-*b*]pyridazine-6-yl)propanal (**30**)

Under cooling at −78°C, a 1.0 M diisobutylaluminum hydride solution in toluene (30 ml) was added dropwise to a solution of **40** (3.29 g, 15 mmol) in a mixture of dichloromethane (45 ml) and toluene (15 ml). The mixture was stirred at −78°C for 2 hours, and additional 1.0 M diisobutylaluminum hydride solution in toluene (11 ml) was added dropwise to the mixture. After being stirred at −78°C for an additional 45 minutes, MeOH (12 ml) was gradually added to the reaction mixture. The mixture was stirred at −78°C for 25 minutes and at room temperature for 30 minutes. H_2O (50 ml) and EtOAc (150 ml) were successively added to the reaction mixture. After filtration with Celite, the separated 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 (50 g: eluent=*n*-hexane~EtOAc). The fractions eluted with EtOAc were concentrated under reduced pressure to give **30** (1.47 g, 56%) as crystals: MP 89~91°C; ^{1}H NMR ($CDCl_3$) δ 3.04, 3.20 (each 2H, t, J=6.6 Hz, CH_2CH_2), 6.96, 7.87 (each 1H, d, J=9.6 Hz, C_7-H and C_8-H), 7.72, 7.88 (each 1H, d, J=1 Hz, C_2-H and C_3-H), 9.92 (1H, s, CHO).

6-Cyanoimidazo[1,2-*b*]pyridazine (**42**)

A suspension of 6-carbamoylimidazo[1,2-*b*]pyridazine[1] (**41**, 21.0 g, 0.13 mol) in phosphorus oxychloride (150 ml, 1.6 mol) was stirred at 115°C for 4.5 hours. The mixture was concentrated under reduced pressure. The concentrate was poured into ice-water (200 ml). The pH of the mixture was adjusted to 7.0 with aq potassium carbonate. The separated crude **42** was collected by filtration. The remaining **42** was recovered from the filtrate. The obtained crude **42** was combined and dissolved in dichloromethane (100 ml). The mixture was purified by silica gel column chromatography (225 g: eluent=*n*-hexane~EtOAc). The fractions eluted with EtOAc were concentrated under reduced pressure to give **42** (15.9 g, 85%): ^{1}H NMR (DMSO-d_6) δ 7.72, 8.41 (each 1H, d, J=9.4 Hz, C_7-H and C_8-H), 8.07, 8.55 (each 1H, d, J=1 Hz, C_2-H and C_3-H).

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

Commercially available Raney-Nickel (2.66 g) was added to a solution of **42** (2.61 g, 18 mmol) in 75% aq formic acid (92 ml), and the mixture was stirred at 100°C for 1 hour. After cooling at room temperature, the reaction mixture was filtered. The filtrate was diluted with H_2O (90 ml) and concentrated to about 50 ml under reduced pressure. The pH of the concentrate was adjusted to 7.0 with aq potassium carbonate. The aqueous mixture was extracted twice with dichloromethane (200 ml), and the separated 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 (40 g: eluent=*n*-hexane~EtOAc). The fractions eluted with EtOAc were concentrated under reduced pressure to give **14** (1.29 g, 48%) as crystals: MP 107~109°C; ^{1}H NMR (DMSO-d_6) δ 7.65, 8.10 (each 1H, d, J=9.4 Hz, C_7-H and C_8-H), 7.97, 8.12 (each 1H, d, J=1 Hz, C_2-H and C_3-H), 10.05 (1H, s, CHO).

References

1) Ishikawa, T.; Y. Iizawa, K. Okonogi & A. Miyake: 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. J. Antibiotics 53: 1053~1070, 2000

2) Ishikawa, T.; K. Kamiyama, T. Matsumoto, N. Matsunaga, H. Tawada, Y. Kawano, Y. Iizawa, K. Okonogi & A. Miyake: Studies on anti-MRSA parenteral cephalosporins. II. Synthesis and antibacterial activity of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-(substituted imidazo[1,2-*b*]-pyridazinium-1-yl)methyl-3-cephem-4-carboxylates and related compounds. J. Antibiotics 53: 1071~1085, 2000

3) Tsuji, T.; H. Satoh, M. Narisada, Y. Hamashima & T. Yoshida: Synthesis and antibacterial activity of 6315-S,

a new member of the oxacephem antibiotic. J. Antibiotics 38: 466~476, 1985

4) INAMOTO, Y.; T. CHIBA, T. KAMIMURA & T. TAKAYA: FK 482, a new orally active cephalosporin. Synthesis and biological properties. J. Antibiotics 41: 828~830, 1988
5) SAKAGAMI, K.; K. ATSUMI, Y. YAMAMOTO, A. TAMURA, T. YOSHIDA, K. NISHIHATA & S. FUKATSU: Synthesis and oral activity of pivaloyloxymethyl 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3(*Z*)-(4-methylthiazol-5-yl)vinyl-3-cephem-4-carboxylate (ME1207) and its related compound. Chem. Pharm. Bull. 39: 2433~2436, 1991
6) COENE, B.; A. SCHANCK, J. -M. DEREPPE & M. V. MEERSSCHE: Substituent effects on reactivity and spectral parameters of cephalosporins. J. Med. Chem. 27: 694~700, 1984
7) NISHIKAWA, J. & K. TORI: 3-Substituent effect and 3-methylene substituent effect on the structure-reactivity relationship of 7β-(acylamino)-3-cephem-4-carboxylic acid derivatives studied by carbon-13 and IR spectroscopies. J. Med. Chem. 27: 1657~1663, 1984
8) BOYD, D. B.: Substituent effects in cephalosporins as assessed by molecular orbital calculations, nuclear magnetic resonance, and kinetics. J. Med. Chem. 26: 1010~1013, 1983
9) 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
10) 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
11) TSUSHIMA, M.; K. IWAMATSU, A. TAMURA & S. SHIBAHARA: Novel cephalosporin derivatives possessing a bicyclic heterocycle at the 3-position. Part I: Synthesis and biological activities of 3-(benzothiazol-2-yl)thiocephalosporin derivatives, CP0467 and related compounds. Bioorg. Med. Chem. 6: 1009~1017, 1998
12) GOTO, J.; K. SKANE & T. TERAJI: Studies of 7β-[2-(aminoaryl)acetamido]-cephalosporin derivatives. III. Synthesis and structure-activity relationships in the aminothiadiazole series. J. Antibiotics 37: 557~571, 1984
13) 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]
14) FARGE, D.; P. L. ROY, C. MOUTONNIER & J. F. PEYRONEL (Rhone-Poulenc Industries S. A.): 3-Vinylcephalosporins. DE 3,019,445, Dec 04, 1980 [Chem. Abstr. 95: 7307, 1981]
15) STANOVNIK, B. & M. TISLER: Synthesis of pyridazine derivatives-VIII. Imidazo[1,2-*b*]pyridazines and some tricyclic aza analogs. Tetrahedron 23: 387~395, 1967
16) TASAKA, A.; N. TAMURA, Y. MATSUSHITA, K. TERANISHI, R. HAYASHI, K. OKONOGI & K. ITOH: Optically active antifungal azoles. I. Synthesis and antifungal activity of (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-mercapto-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol and its stereoisomers. Chem. Pharm. Bull. 41: 1035~1042, 1993
17) SAKAMOTO, T.; S. NISHIMURA, Y. KONDO & H. YAMANAKA: Palladium-catalyzed coupling reaction of *N*-heteroaryl halides with functionalized alkylzinc iodides. Synthesis: 485~486, 1988
18) LEGOFF, E.: Cyclopropanes from an easily prepared, highly active Zinc-Copper couple, dibromomethane, and olefins. J. Org. Chem. 29: 2048~2050, 1964
19) KANAI, T.; Y. MORITA, H. SHINOHARA, Y. KAI & K. OGURA: Reaction of insoluble 2-(5-amino-1,2,4-thiadiazol-3-yl)-2-[(fluoromethoxy)imino]acetic acid with insoluble PCl_5 in CH_2Cl_2. Chem. Express 7: 805~808, 1992