VOL. 55 NO. 11, NOV. 2002 THE JOURNAL OF ANTIBIOTICS pp. 975 — 992

S-3578, A New Broad Spectrum Parenteral Cephalosporin Exhibiting Potent Activity Against both Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* Synthesis and Structure-activity Relationships

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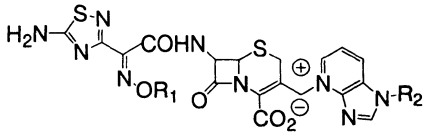
(Received for publication June 10, 2002)

A series of 7-aminothiadiazolylcephalosporins having a 1-(substituted)-1*H*-imidazo[4,5-*b*]pyridinium group at the C-3′ position of the cephem nucleus were synthesized and evaluated for *in vitro* antibacterial activities. Among the cephalosporins prepared in this study, 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetamido]-3-[1-(3-methylaminopropyl)-1*H*-imidazo[4,5-*b*]pyridinium-4-yl]methyl-3-cephem-4-carboxylate sulfate (**S-3578**) showed extremely potent broad spectrum activity against both Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacteria including *Pseudomonas aeruginosa*, and good water solubility.

The so-called fourth-generation cephalosporins bearing the quaternary ammonium group at the C-3′ position, such as cefpirome (CPR)[1], cefepime (CFPM)[2], cefozopran (CZOP)[3], and cefoselis (CFSL)[4], have potent activity against Gram-positive bacteria and Gram-negative bacteria including *Pseudomonas aeruginosa*, and are widely used for the treatment of bacterial infections. However, their activity against methicillin-resistant *Staphylococcus aureus* (MRSA) is not sufficient for clinical use. MRSA as well as *Pseudomonas aeruginosa* is a nosocomial pathogen associated with serious infections and considerable mortality. Also, the incidence of mixed infection by MRSA and *Pseudomonas aeruginosa* has been increasing[5], and antibacterial agents having high activity against these two pathogens are needed. We attempted to enhance the anti-MRSA activity of C-3′ quaternary ammonium cephalosporins while retaining potent activity against Gram-negative bacteria including *Pseudomonas aeruginosa*. It was a challenge to find such a parenteral C-3′ quaternary ammonium cephalosporin with potent activity against MRSA and sufficient water solubility for administration because quaternary ammonium cephems possessing anti-MRSA activity may not be sufficiently soluble in water due to their zwitterionic structure.

In the course of searching for novel C3′-quaternary cephems, we found 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetamido]-3-[1*H*-imidazo[4,5-*b*]-pyridinium-4-yl]methyl-3-cephem-4-carboxylate (**1**) (Fig. 1), which showed potent activity against MRSA and *Pseudomonas aeruginosa*. We further investigated the influence of various substitutions (R_1, R_2) on both antibacterial activity and water solubility. Eventually, we discovered a promising compound for further evaluation,

Fig. 1.



1: R_1 = —Et, R_2 = —H
S-3578: R_1 = —Et, R_2 = —(CH2)3—NMe · H · H2SO4

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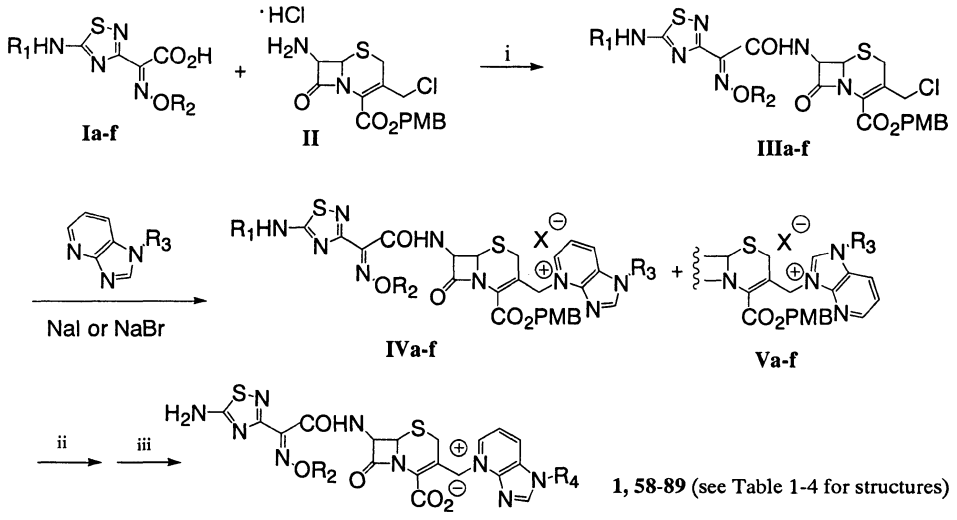
Scheme 1.



i) $POCl_3$ or $Cl_2P(O)OPh$, N-methylmorpholine, ii) $AlCl_3$-anisole, $TiCl_4$-anisole or H_2SO_4-HCO_2H, iii) Purification by HP-20 chromatography

	R_1	R_2
a	Boc	$-Me$
b	Boc	$-Et$
c	H	$-Et$
d	Boc	$-CH(CH_3)_2$
e	Boc	$-CH_2F$
f	Boc	$-CH_2CH_2F$

3-5, 7, 8, 10-17, 28-34, 43, 44, 46, 48, 49, 51, 52, 55 or 57 (see Scheme 2-9 for structures)

X : Br or I
PMB: *p*-methoxybenzyl

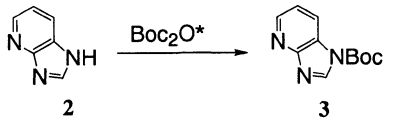
7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyimino-acetamido]-3-[1-(3-methylaminopropyl)-1*H*-imidazo[4,5-*b*]pyridinium-4-yl]methyl-3-cephem-4-carboxylate sulfate (**S-3578**), which displayed excellent activities against MRSA as well as *Pseudomonas aeruginosa*, and good water solubility (>100 mg/ml, at pH 2~7). Herein, we describe the synthesis and structure-activity relationships of a series of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-[1-(substituted)-1*H*-imidazo[4,5-*b*]pyridinium-4-yl]methyl-3-cephem-4-carboxylates.

Synthesis

Cephalosporin derivatives bearing the 1-(substituted)-1*H*-imidazo[4,5-*b*]pyridine at the C-3′ position were synthesized as shown in Scheme 1. The cephem nucleus[6] (**II**) was acylated with α-alkoxyiminoacetic acid[7~9] (**Ia~f**) using phosphorus oxychloride or phenyl phosphorodichloridate in the presence of *N*-methylmorpholine. The C-3 chloromethyl cephalosporin intermediate (**IIIa~f**) was treated with sodium bromide or sodium iodide to give the corresponding bromide or iodide, which was displaced by the corresponding 1-(substituted)-1*H*-imidazo[4,5-*b*]pyridine (**3~5**, **7**, **8**, **10~17**, **28~34**, **43**, **44**, **46**, **48**, **49**, **51**, **52**, **55** or **57**) to afford a mixture of **IVa~f** and a regioisomer (**Va~f**), which was treated with an $AlCl_3$-anisole, $TiCl_4$-anisole or H_2SO_4-HCO_2H system. Purification on reversed phase (HP-20) column chromatography yielded cephalosporin derivatives (**1**, **58~89**).

The methods of synthesizing 1-(substituted)-1*H*-imidazo[4,5-*b*]pyridine are shown in Schemes 2~9. 1-*tert*-Butoxycarbonyl-1*H*-imidazo[4,5-*b*]pyridine (**3**) was prepared by treatment of commercially available 1*H*-imidazo[4,5-*b*]pyridine (**2**) with di-*tert*-butyl dicarbonate (Boc_2O) (Scheme 2). To introduce an alkyl group onto the 1-position of 1*H*-imidazo[4,5-*b*]pyridine, three methods

Scheme 2.



2 3

*Boc_2O : di-*tert*-butyl dicarbonate

(Method A~C in Scheme 3) were employed with reference to KHANNA's procedure[10]. In Method A, compounds **4**~**17** were prepared by a reaction of 1*H*-imidazo[4,5-*b*]pyridine (**2**) with the corresponding alkyl halide or methanesulfonate (**4'**~**17'**) in the presence of a base (NaH or Cs_2CO_3). In this method, an undesired regioisomer, 3-(substituted)-3*H*-imidazo[4,5-*b*]pyridine was also produced. In Method B, compounds **11**, **12**, **28**~**34** were obtained regioselectively by reaction of 3-amino-2-formamidopyridine (**18**) and the corresponding aldehyde (**19**~**27**) in the presence of borane-pyridine complex. In Method C, regioselectively reductive alkylation of 2,3-diaminopyridine (**35**) with aldehyde **20**, **36**~**38** was accomplished by palladium-catalyzed hydrogenation in a mixture of MeOH and AcOH to give the corresponding diaminopyridine derivatives **39**~**42**. These compounds **39**~**42** were then treated with triethyl orthoformate or trimethyl orthoformate in the presence of *p*-toluenesulfonic acid (TsOH) catalyst to afford imidazopyridine derivatives **12** or **43**~**45**, respectively. Compound **46** was prepared by treatment of ester **9** with 28% NH_4OH (Scheme 4). As shown in Scheme 5, the Boc group of compound **12** was removed with HCl-MeOH to give amine **47**, which was treated with formaldehyde and formic acid to give compound **48**. As shown in Scheme 6, the chloride **6** was substituted by cyclopropylamine and the resulting amine was protected with a Boc group by treatment of Boc_2O in the presence of 4-dimethylaminopyridine (DMAP) catalyst to give **49**. As shown in Scheme 7, compound **50** was prepared by the reaction of **11** with (2-bromoethoxy)-triethylsilane in the presence of NaH.

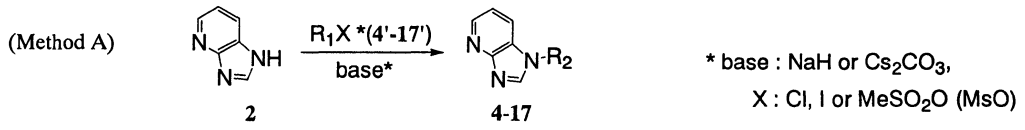
The triethylsilyl group of **50** was removed with a system of AcOH-THF-H_2O to afford the alcohol **51** followed by substitution with di-*tert*-butyl iminodicarboxylate (Boc_2NH) by the MITSUNOBU reaction using the 1,1'-(azodicarbonyl) dipiperidine (ADDP)-tributylphosphine system[11] to give **52**. Compound **55** was prepared as shown in Scheme 8. Compound **11** was treated with HCl-MeOH to give the amine **53**, which was then reacted with 1*H*-pyrazole-1-[*N*,*N'*-bis(*tert*-butoxycarbonyl)]-carboxamidine[12] (**54**) to afford the guanidine derivative **55**. Compound **57** was prepared by the method shown in Scheme 9. Esterification of **45** was achieved by using diazomethane followed by treatment with 28% NH_4OH to afford compound **57**.

Results and Discussion

Table 1 shows the antibacterial activities of cephems bearing the 1*H*-imidazo[4,5-*b*]pyridine derivative, and the reference compounds CZOP, CFSL and vancomycin (VCM). MICs were determined by the standard serial twofold dilution method using Mueller-Hinton medium.

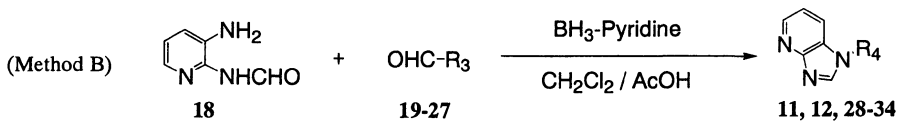
Compound **1** showed potent antibacterial activities against Gram-positive bacteria including MRSA and Gram-negative bacteria including *Pseudomonas aeruginosa*. In particular, the anti-MRSA activity of **1** was superior to that of the reference compounds CZOP and CFSL but inferior to that of VCM. The introduction of a methyl group at the 1-position of imidazopyridine (**58**) enhanced the antibacterial activity against MRSA and *Pseudomonas aeruginosa*. Lengthening of the methylene chain (**59**~**61**) did not further improve the anti-MRSA activity, but decreased potency against *Pseudomonas aeruginosa* compared with **58**. Compounds **62**~**64** containing functional groups, such as a difluoromethyl (**62**), a hydroxy ethyl (**63**) or a carbamoylmethyl (**64**) group, were less active than **58** against both MRSA and *Pseudomonas aeruginosa*. However, compound **65** having an aminoethyl group showed the same activity against MRSA and *Pseudomonas aeruginosa* as that of **58**. Among these compounds (**1**, **58**~**65**), **58** and **65** were the most active against MRSA and *Pseudomonas aeruginosa*. Although **58** had low water solubility (solubility of **58**; <10 mg/ml), compound **65** had good water solubility due to salt formation (solubility of **65** hydrochloride; >100 mg/ml). These findings led us to explore a variety of carbon lengths

Scheme 3.

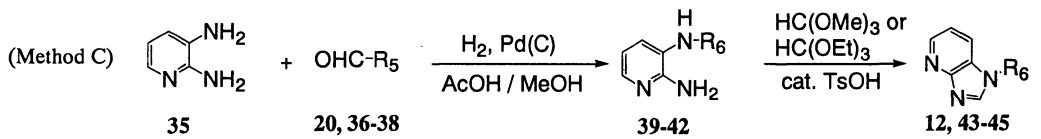


R_1X	R_1X	R_1X	R_1X
4' I–Me	8' I–(CH2)3–OSiEt3	12' MsO–(CH2)3–N(Me)–Boc	15' MsO–(3-pyrrolidine)–NBoc
5' I–Et	9' I–(CH2)2–CO2Et	13' MsO–(CH2)4–NHBoc	16' MsO–(2-pyrrolidine)–NBoc
6' I–(CH2)3–Cl	10' MsO–(CH2)2–NHBoc	14' MsO–(3-azetidine)–NBoc	17' MsO–(4-piperidine)–NBoc
7' Cl–CHF_2	11' MsO–(CH2)3–NHBoc		

R_2	R_2	R_2	R_2
4 –Me	8 –(CH2)3–OSiEt3	12 –(CH2)3–N(Me)–Boc	15 (3-pyrrolidine)–NBoc
5 –Et	9 –(CH2)2–CO2Et	13 –(CH2)4–NHBoc	16 (2-pyrrolidine)–NBoc
6 –(CH2)3–Cl	10 –(CH2)2–NHBoc	14 (3-azetidine)–NBoc	17 (4-piperidine)–NBoc
7 –CHF_2	11 –(CH2)3–NHBoc		

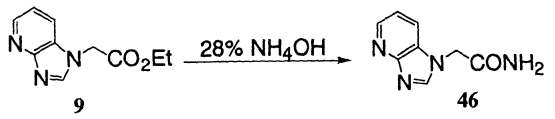


OHC-R_3	OHC-R_3	R_4	R_4
19 OHC–(CH2)3–NHBoc	24 OHC–CH(Me)–(CH2)2–NHBoc	11 –(CH2)3–NHBoc	31 –CH(Me)–(CH2)2–NHBoc
20 OHC–(CH2)2–N(Me)–Boc	25 OHC–CH(Me)–(CH2)2–NHBoc	12 –(CH2)3–N(Me)–Boc	32 –CH(Me)–(CH2)2–NHBoc
21 OHC–(CH2)2–Me	26 OHC–(1-cyclopropyl)–NHBoc	28 –(CH2)2–Me	33 –(1-cyclopropyl)–NHBoc
22 OHC–(cyclopropyl)	27 OHC–CH(CF3)–(CH2)2–NHBoc	29 –(cyclopropyl)	34 –CH(CF3)–(CH2)2–NHBoc
23 OHC–(CH2)2–N(Et)–Boc		30 –(CH2)3–N(Et)–Boc	



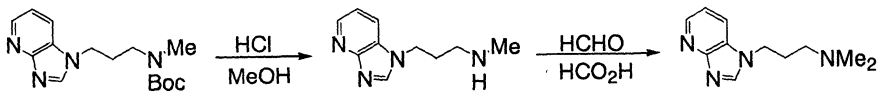
OHC-R_5	OHC-R_5	R_6	R_6
20 OHC–(CH2)2–N(Me)–Boc	37 OHC–CH(Et)–NHBoc	39, 12 –(CH2)3–N(Me)–Boc	41, 44 –CH(Et)–NHBoc
36 OHC–CH(Me)–NHBoc	38 OHC–CH(CO2CHPh2)–NHBoc	40, 43 –CH(Me)–NHBoc	42, 45 –CH(CO2H)–(CH2)2–NHBoc

Scheme 4.



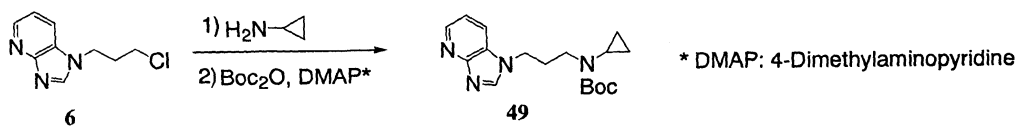
9 46

Scheme 5.



12 47 48

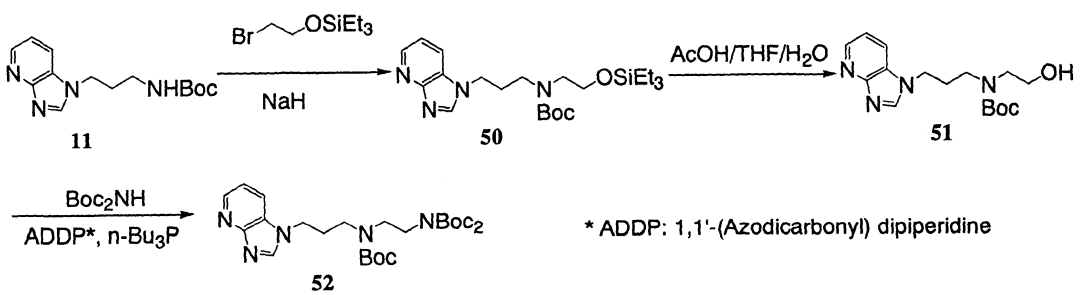
Scheme 6.



6 49

* DMAP: 4-Dimethylaminopyridine

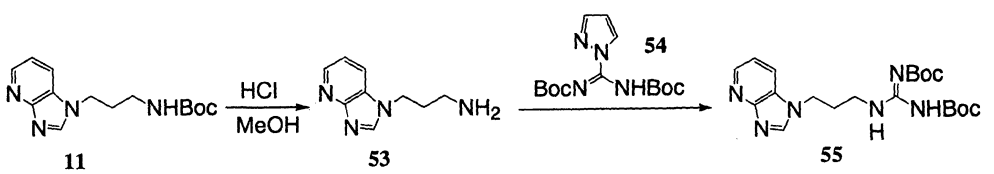
Scheme 7.



11 50 51 52

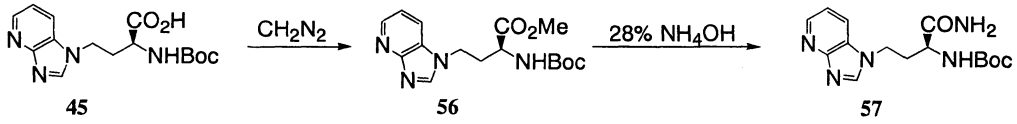
* ADDP: 1,1'-(Azodicarbonyl) dipiperidine

Scheme 8.



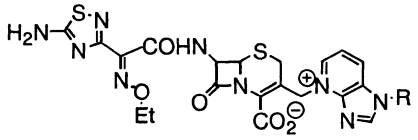
11 53 55

Scheme 9.



45 56 57

Table 1. Antibacterial activity (MIC, μg/ml) of **1**, **58**~**65**, CZOP, CFSL and VCM.



R: −H −Me −Et −Me  −CHF_2 −OH $CONH_2$ NH_2

1 **58** **59** **60** **61** **62** **63** **64** **65**

Compound	*S. a.*	MRSA 1	MRSA 2	*E. c.*	*P. a.* 1	*P. a.* 2
1	1.56	12.5	12.5	0.2	1.56	12.5
58	0.78	6.25	6.25	0.2	0.78	3.13
59	1.56	6.25	6.25	0.1	1.56	3.13
60	0.78	6.25	6.25	0.2	1.56	6.25
61	0.78	6.25	6.25	0.2	1.56	6.25
62	1.56	12.5	12.5	0.2	3.13	12.5
63	1.56	12.5	12.5	0.2	1.56	3.13
64	1.56	12.5	12.5	0.39	1.56	6.25
65	1.56	6.25	6.25	0.39	0.78	3.13
CZOP	0.78	50	50	0.05	0.39	1.56
CFSL	0.78	25	25	0.05	3.13	6.25
VCM	1.56	0.78	1.56	>100	>100	>100

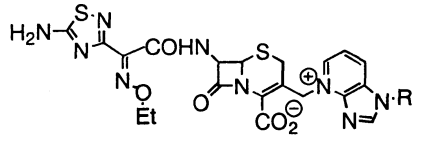
S. a., *Staphylococcus aureus* SMITH; MRSA 1, *S. aureus* SR3626; MRSA 2, *S. aureus* SR3637; *E.c.*, *Escherichia coli* NIHJ JC-2; *P. a.* 1, *Pseudomonas aeruginosa* SR24; *P. a.* 2, *P. aeruginosa* SR5393
CZOP, cefozopran; CFSL, cefoselis; VCM, vancomycin

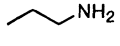
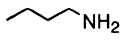
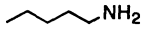
for the 2-aminoethyl group of **65**.

Table 2 shows the antibacterial activity of cephems bearing the 1-(aminoalkyl)-1*H*-imidazo[4,5-*b*]pyridine derivative. The data show that anti-MRSA activity was further enhanced by elongation of the spacer carbon chain between the amino group and the imdazopyridine moiety of compound **65**. Although the 2-aminopropyl derivative **66** and the 2-aminobutyl derivative **67** did not enhance anti-MRSA activity compared with 2-aminoethyl derivative **65**, the 3-aminopropyl derivative **68** and 4-aminobutyl derivative **69** displayed more potent antibacterial activity than **65** against MRSA. Regarding the activity against *Pseudomonas aeruginosa*, **65** and **68** were more active than **69**. Among compounds **65**~**69**, the 3-aminopropyl derivative **68** showed the most potent activity against MRSA and *Pseudomonas aeruginosa*. Therefore, our attention was next focused on the preparation of cephalosporin derivatives bearing a variety of 1-(3-aminopropyl)-1*H*-imidazo[4,5-*b*]pyridinium analogs. We explored the effects of the substituent of the 3-aminopropyl moiety of compound **68**.

Table 3 shows the activities of cephalosporin derivatives bearing a variety of 1-(3-aminopropyl)-1*H*-imidazo[4,5-*b*]pyridinium analogs. Further improvement of antibacterial

Table 2. Antibacterial activity (MIC, μg/ml) of **65**~**69**.



R:  **65**  **66** Me  **67** Et  **68**  **69**

Compound	*S. a.*	MRSA 1	MRSA 2	*E. c.*	*P. a.* 1	*P. a.* 2
65	1.56	6.25	6.25	0.39	0.78	3.13
66	1.56	6.25	6.25	0.39	1.56	6.25
67	1.56	6.25	12.5	0.39	3.13	6.25
68	0.78	3.13	3.13	0.39	0.78	3.13
69	0.78	3.13	3.13	0.39	1.56	6.25

abbreviations: see footnote in Table 1.

activity against MRSA and *Pseudomonas aeruginosa* was not observed by modification of compound **68**. The introduction of a methyl group (**70**) or a hydroxyethyl group (**74**) on the primary amine and a methyl group (**77**, **78**) or a cyclopropyl group (**79**) on a spacer carbon chain of 3-aminopropyl derivative **68** had no effect on anti-MRSA activity compared to that of compound **68**. Compounds **82**~**85** having a cyclic amino group or compound **76** having a guanidyl group instead of an amino group also showed the same activity as compound **68** against MRSA. However, introduction of dimethyl groups (**71**) or a larger substituent than the methyl group, such as an ethyl (**72**) or a cyclopropyl (**73**) group on the primary amine, and an electron-withdrawing group such as a trifluoromethyl (**80**) or a carbamoylmethyl group (**81**) on the spacer carbon chain diminished activities against MRSA. Regarding the antibacterial activity against *Pseudomonas aeruginosa*, the activities of all compounds were inferior to that of compound **68**.

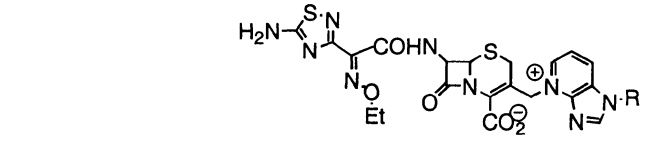
While optimizing the C-3 substituent, we investigated the substituent effect of the C-7 oxime moiety. Some examples are shown in Table 4. In analogs of compound **70**, the ethoxyimino moiety was modified. The isopropyl analog **87** had the same activity as **70** against MRSA and *Pseudomonas aeruginosa*, but was much less active against *Escherichia coli*. Other compounds, methyl analog **86**, fluoromethyl analog **88** and fluoroethyl analog **89**, were less active than **70** against MRSA and *Pseudomonas aeruginosa*. The ethyl analog **70** showed well-balanced activity against Gram-positive bacteria including MRSA and Gram-negative bacteria including *Pseudomonas aeruginosa*.

Among the novel cephalosporin derivatives having a 1-(substituted)-1*H*-imidazo[4,5-*b*]pyridinium group at C-3′, **68** had the highest activity against MRSA and *Pseudomonas aeruginosa*. However, subsequent evaluation of **68** revealed that its mouse acute toxicity (i.v.) was relatively strong. The other analogs were also evaluated for mouse acute toxicity. And compounds **70** and **74** were selected based primarily on a favorable combination of antibacterial activity and mouse acute toxicity. Ultimately, on the basis of physicochemical property, we selected crystalline **70** sulfate, not amorphous **74**, as a promising candidate for further evaluation and designated as **S-3578**.

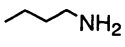
Experimental

IR spectra were taken on a JASCO IR-700 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Gemini-300 (300 MHz) or Varian Gemini-200 (200 MHz) spectrometer. Chemical shifts are reported in ppm from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS in D_2O) or TMS (in $CDCl_3$ and DMSO-d_6) as internal standard. The following abbreviations are used: s singlet, d doublet, dd double doublet, t triplet, q quartet, m multiplet, ABq AB quartet,

Table 3. Antibacterial activity (MIC, μg/ml) of **68**, **70**~**85**.



R=



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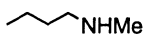

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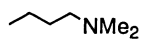

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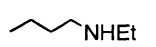

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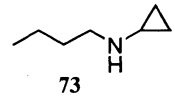

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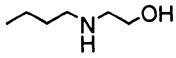

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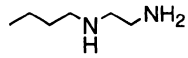

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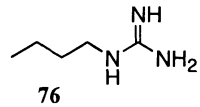

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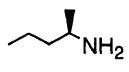

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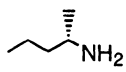

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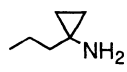

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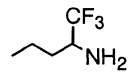

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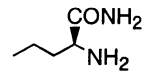

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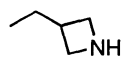

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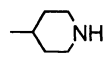
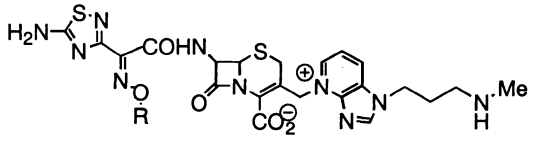


85

Compound	*S. a.*	MRSA 1	MRSA 2	*E. c.*	*P. a.* 1	*P. a.* 2
68	0.78	3.13	3.13	0.39	0.78	3.13
70	0.78	3.13	3.13	0.39	1.56	6.25
71	0.78	6.25	6.25	0.39	3.13	6.25
72	1.56	3.13	6.25	0.39	1.56	6.25
73	0.78	6.25	6.25	0.2	3.13	12.5
74	1.56	3.13	3.13	0.39	1.56	6.25
75	1.56	6.25	6.25	0.39	1.56	6.25
76	0.78	3.13	3.13	0.2	1.56	6.25
77	0.78	3.13	3.13	0.39	1.56	6.25
78	0.78	3.13	3.13	0.39	1.56	6.25
79	0.78	3.13	3.13	0.2	1.56	6.25
80	1.56	12.5	12.5	0.39	6.25	12.5
81	1.56	12.5	12.5	0.2	3.13	6.25
82	1.56	3.13	3.13	0.39	1.56	6.25
83	0.78	3.13	3.13	0.2	1.56	6.25
84	0.78	3.13	3.13	0.2	1.56	6.25
85	0.78	3.13	3.13	0.2	1.56	6.25

abbreviations: see footnote in Table 1.

Table 4. Antibacterial activity (MIC, μg/ml) of **70**, **86**~**89**.



R= —Et —Me —CH(CH$_3$)$_2$ —CH$_2$F —CH$_2$CH$_2$F

70 **86** **87** **88** **89**

Compound	*S. a.*	MRSA 1	MRSA 2	*E. c.*	*P. a.* 1	*P. a.* 2
70	0.78	3.13	3.13	0.39	1.56	6.25
86	1.56	6.25	6.25	0.39	3.13	12.5
87	0.78	3.13	3.13	1.56	1.56	6.25
88	0.78	6.25	6.25	0.2	3.13	6.25
89	1.56	3.13	6.25	0.2	3.13	12.5

abbreviations: see footnote in Table 1.

bs broad singlet. Column chromatography was carried out on Merck Kieselgel and Mitsubushi Chemical HP-20.

Measurement of *In Vitro* Antibacterial Activity

MICs were determined by a serial twofold dilution method in Sensitivity Disk Agar-N (Nissui Pharmaceutical, Tokyo, Japan). The overnight cultures of bacterial strains in Mueller Hinton broth (Becton Dickinson) were diluted to about 10^6 CFU/ml. Bacterial suspensions of 1 μl were spotted onto agar plates containing various concentrations of an antibiotic and incubated for 20 hours at 37°C before the MICs were scored.

General Preparation 1-(Substituted)-1*H*-imidazo[4,5-*b*]-pyridine

1-*tert*-Butoxycarbonyl-1*H*-imidazo[4,5-*b*]pyridine (**3**)

To a solution of 1*H*-imidazo[4,5-*b*]pyridine (**2**) (775 mg, 6.5 mmol) in DMF (8 ml) was added di-*tert*-butyl dicarbonate (1.65 ml, 7.15 mmol) under cooling on an ice-water bath, and the mixture was allowed to stand overnight at room temperature. After evaporation of the solvent, the residue was purified by silica gel column chromatography to give the title compound **3** (1.16 g, 81% yield); ^{1}H-NMR ($CDCl_3$) δ 1.71 (9H, s), 7.32 (1H, dd, J=4.8 , 8.1 Hz), 8.28 (1H, dd, J=1.8, 8.1 Hz), 8.62 (1H, dd, J=1.8, 4.8 Hz), 8.66 (1H, s).

1-[3-(*tert*-Butoxycarbonyl-methylamino)-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**12**)

(Method A) To a solution of 1*H*-imidazo[4,5-*b*]pyridine (**2**) (5.15 g, 34.8 mmol) in DMF (35 ml) was added 60% NaH (1.53 g, 38.2 mmol) under cooling on an ice-water bath, and the mixture was stirred at room temperature for 15 minutes. To the mixture was added a solution of 3-*tert*-butoxycarbonyl-methylaminopropyl methanesulfonate (**12'**) (10.2 g, 38.2 mmol) in DMF (20 ml). The reaction mixture was stirred at 50°C for 1.5 hours. After evaporation of the solvent, the residue was chromatographed on silica gel column. The fraction eluted with EtOAc was concentrated to give a regioisomer, 3-[3-(*tert*-butoxycarbonyl-methyl-amino)-propyl]-3*H*-imidazo[4,5-*b*]pyridine (5.94 g, 59% yield); ^{1}H-NMR ($CDCl_3$) δ 1.44 (9H, s), 2.18 (2H, m), 2.85 (3H, s), 3.30 (2H, t, J=6.8 Hz), 4.32 (2H, t, J=7.2 Hz), 7.25 (1H, dd, J=8.1, 4.8 Hz), 8.08 (1H, dd, J=8.1, 1.4 Hz), 8.15 (1H, s), 8.40 (1H, dd, J=4.8, 1.4 Hz).

The fraction eluted with MeOH : EtOAc=6 : 94 was concentrated to give the objective compound **12** (2.90 g, 29% yield); ^{1}H-NMR ($CDCl_3$) δ 1.45 (9H, s), 2.12 (2H, m), 2.85 (3H, s), 3.32 (2H, t, J=6.8 Hz), 4.22 (2H, t, J=7.0 Hz), 7.25 (1H, dd, J=8.2, 4.8 Hz), 7.74 (1H, dd, J=8.2, 1.4 Hz), 8.20 (1H, s), 8.60 (1H, dd, J=4.8, 1.4 Hz).

(Method B) To a suspension of *N*-(3-amino-2-pyridinyl)-formamide (**18**) (5.76 g, 42 mmol) and 3-*tert*-

butoxycarbonyl-methylaminopropionaldehyde (**20**) (8.22 g, 42 mmol) in CH_2Cl_2 (40 ml) was added an ice-cooled mixture of CH_2Cl_2 (20 ml) and AcOH (60 ml) at −10°C. To this borane-pyridine complex (4.44 ml, 46.2 mmol) was added immediately. After stirring for 1 hour at room temperature, the reaction mixture was neutralized to pH 7 using aqueous ammoium hydroxide and extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$, and filtered. After concentration, the crude residue was chromatographed on silica gel column (MeOH : EtOAc=1 : 9) to give compound **12** (1.25 g, 92% yield) which had properties identical to the product reported from Method A.

(Method C) To a solution of 2,3-diaminopyridine (**35**) (220 mg, 2 mmol) and 3-*tert*-butoxycarbonyl-methylaminopropionaldehyde (**20**) (450 mg, 2.4 mmol) in MeOH (2.0 ml) and AcOH (2.0 ml) was added 10% Pd-C (128 mg). The mixture was stirred under hydrogen atmosphere for 20 minutes under ambient pressure and at room temperature. The catalyst was filtered off and to the solution was added EtOAc (20 ml) and 1 N NaOH (5 ml). The aqueous layer was extracted with EtOAc (15 ml). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$: MeOH=80 : 1~10 : 1) to give **39** as a solid (560 mg, 84%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.44 (9H, s), 1.83 (2H, m), 2.85 (3H, s), 3.1 (2H, t, J=6.3 Hz), 3.36 (2H, t, J=6.3 Hz), 4.0 (1H, bs), 4.5 (2H, bs), 6.65 (1H, bs), 6.76 (1H, d, J=7.5 Hz), 7.56 (1H, bs).

To a mixture of compound **39** (7.89 g, 28 mmol) and triethyl orthoformate (46.6 ml) was added *p*-toluenesulfonic acid monohydrate (53 mg, 0.28 mmol) and stirred at 90°C for 40 minutes. The reaction mixture was concentrated *in vacuo* and to the residue was added EtOAc and saturated $NaHCO_3$. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography to give compound **12** (7.17 g, 88% from **38**) with properties identical to the product reported above.

The other 1-(substituted)-imidazo[4,5-*b*]pyridines (**4**~**11**, **13**~**17**, **28**~**34**, **43**~**45**) were prepared by procedures (Method A~C) similar to those described for the preparation of **12**.

1-Methyl-1*H*-imidao[4,5-*b*]pyridine (**4**)

(Method A) Compound **4** was obtained in 70% yield from **2** using iodide **4′** and NaH as a base; ^{1}H-NMR ($CDCl_3$) δ 3.87 (3H, s), 7.23 (1H, dd, J=5.1, 8.1 Hz), 7.68 (1H, dd, J=1.5, 8.1 Hz), 8.11 (1H, s), 8.50 (1H, dd, J=1.5, 5.1 Hz).

1-Ethyl-1*H*-imidao[4,5-*b*]pyridine (**5**)

(Method A) Compound **5** was obtained in 72% yield from **2** using iodide **5′** and NaH as a base; ^{1}H-NMR ($CDCl_3$) δ 1.57 (3H, t, J=7.5 Hz), 4.27 (2H, q, J=7.5 Hz), 7.25 (1H, dd, J=5.1, 8.1 Hz), 7.76 (1H, dd, J=1.5, 8.1 Hz), 8.16 (1H, s), 8.58 (1H, dd, J=1.5, 5.1 Hz).

1-(3-Chloropropyl)-1*H*-imidazo[4,5-*b*]pyridine (**6**)

(Method A) Compound **6** was obtained in 21% yield from **2** using iodide **6′** and Cs_2CO_3 as a base (reaction temperature; 5°C): ^{1}H-NMR ($CDCl_3$) δ 2.33 (2H, m), 3.49 (2H, t, J=5.7 Hz), 4.44 (2H, t, J=6.9 Hz), 7.26 (1H, dd, J=4.8, 8.1 Hz), 7.80 (1H, dd, J=1.5, 7.8 Hz), 8.17 (1H, s), 8.60 (1H, dd, J=1.5, 4.5 Hz).

1-Difluoromethyl-1*H*-imidazo[4,5-*b*]pyridine (**7**)

(Method A) Compound **7** was obtained in 48% yield from **2** using chloride **7′** and Cs_2CO_3 as a base; ^{1}H-NMR ($CDCl_3$+CD_3OD) δ 7.39 (1H, dd, J=4.8, 8.1 Hz), 7.41 (1H, d, J=60.3 Hz), 8.04 (1H, dd, J=0.9, 8.1 Hz), 8.46 (1H, s), 8.64 (1H, dd, J=1.5, 4.5 Hz).

1-(2-Triethylsilanyloxy-ethyl)-1*H*-imidazo[4,5-*b*]-pyridine (**8**)

(Method A) Compound **8** was obtained in 30% yield from **2** using iodide **8′** and NaH as a base; ^{1}H-NMR ($CDCl_3$) δ 0.46 (6H, q, J=8.2 Hz), 0.81 (9H, t, J=8.2 Hz), 3.94 (2H, t, J=5.0 Hz), 4.30 (2H, t, J=5.0 Hz), 7.22 (1H, dd, J=7.8, 1.6 Hz), 7.77 (1H, dd, J=1.2, 7.8 Hz), 8.17 (1H, s), 8.56 (1H, dd, J=1.6, 4.6 Hz).

1-Ethoxycarbonylmethy-1*H*-imidazo[4,5-*b*]pyridine (**9**)

(Method A) Compound **9** was obtained in 53% yield from **2** using iodide **9′** and NaH as a base; ^{1}H-NMR ($CDCl_3$) δ 1.29 (3H, t, J=7.2 Hz), 4.27 (2H, q, J=7 Hz), 5.10 (2H, s), 7.28 (1H, dd, J=8.0, 4.8 Hz), 7.76 (1H, dd, J=8.0, 1.4 Hz), 8.49 (1H, dd, J=4.8, 1.4 Hz), 8.51 (1H, s).

1-(2-*tert*-Butoxycarbonylamino-ethyl)-1*H*-imidazo[4,5-*b*]pyridine (**10**)

(Method A) Compound **10** was obtained in 36% yield from **2** using methanesulfonate **10′** and NaH as a base; ^{1}H-NMR ($CDCl_3$) δ 1.45 (9H, s), 3.57 (2H, q, J=6.0 Hz), 4.36 (2H, t, J=6.0 Hz), 5.51 (1H, s), 7.17 (1H, dd, J=8.2, 4.8 Hz), 7.75 (1H, dd, J=8.2, 1.6 Hz), 8.01 (1H, s), 8.47 (1H, dd, J=4.8, 1.6 Hz).

1-(3-*tert*-Butoxycarbonylamino-propyl)-1*H*-imidazo[4,5-*b*]pyridine (**11**)

(Method A) Compound **11** was obtained in 27% yield from **2** using methanesulfonate **11'** and NaH as a base; ^{1}H-NMR ($CDCl_3$) δ 1.45 (9H, s), 2.11 (2H, m), 3.20 (2H, m), 4.27 (2H, t, J=10.5 Hz), 4.79 (1H, br s), 7.25 (1H, dd, J=11.7, 7.2 Hz), 7.75 (1H, dd, J=11.7, 1.5 Hz), 8.21 (1H, s), 8.59 (1H, dd, J=7.2, 1.5 Hz).

(Method B) Compound **11**, which had properties identical to the product reported from Method A, was obtained in 80% yield from **18** using aldehyde **19**.

1-(4-*tert*-Butoxycarbonylamino-butyl)-1*H*-imidazo[4,5-*b*]pyridine (**13**)

(Method A) Compound **13** was obtained in 22% yield from **2** using methanesulfonate **13'** and NaH as a base; ^{1}H-NMR ($CDCl_3$) δ 1.44 (9H, s), 1.52 (2H, m), 1.93 (2H, m), 3.18 (2H, m), 4.25 (2H, t, J=6.9 Hz), 4.65 (1H, s), 7.23 (1H, dd, J=8.1, 4.8 Hz), 7.77 (1H, dd, J=8.1, 1.2 Hz), 8.12 (1H, s), 8.58 (1H, dd, J=4.8, 1.2 Hz).

1-(1-*tert*-Butoxycarbonyl-azetidin-3-ylmethyl)-1*H*-imidazo[4,5-*b*]pyridine (**14**)

(Method A) Compound **14** was obtained in 22% yield from **2** using methanesulfonate **14'** and NaH as a base; ^{1}H-NMR ($CDCl_3$) δ 1.44 (9H, s), 3.08 (1H, m), 3.70 (2H, m), 4.06 (2H, m), 4.43 (2H, d, J=7.8 Hz), 7.27 (1H, dd, J=4.6, 7.8 Hz), 7.76 (1H, dd, J=1.6, 7.8 Hz), 8.14 (1H, s), 8.61 (1H, dd, J=1.6, 4.6 Hz).

1-[(3*R*)-1-*tert*-Butoxycarbonyl-pyrrolidin-3-yl]-1*H*-imidazo[4,5-*b*]pyridine (**15**)

(Method A) Compound **15** was obtained in 13% yield from **2** using methanesulfonate **15'** and Cs_2CO_3 as a base; ^{1}H-NMR (DMSO-d_6) δ 1.39~1.43 (9H, m), 2.43~2.47 (2H, m), 3.52~3.60 (2H, m), 3.83~3.89 (1H, m), 5.20 (1H, br s), 7.30 (1H, dd, J=4.8, 8.1 Hz), 8.14 (1H, dd, J=1.5, 8.1 Hz), 8.44 (1H, dd, J=1.5, 4.8 Hz), 8.55 (1H, br s).

1-[(3*S*)-1-*tert*-Butoxycarbonyl-pyrrolidin-3-yl]-1*H*-imidazo[4,5-*b*]pyridine (**16**)

(Method A) Compound **16** was obtained in 16% yield from **2** using methanesulfonate **16'** and Cs_2CO_3 as a base.

1-(1-*tert*-Butoxycarbonyl-piperidin-4-yl)-1*H*-imidazo-[4,5-*b*]pyridine (**17**)

(Method A) Compound **17** was obtained in 20% yield from **2** using methanesulfonate **17'** and sodiumhydride as a base; ^{1}H-NMR ($CDCl_3$) δ 1.51 (9H, s), 2.14 (4H, m), 2.94 (1H, m), 4.37 (4H, m), 7.25 (1H, dd, J=8.2, 4.6 Hz), 7.79 (1H, dd, J=8.2, 1.4 Hz), 8.21 (1H, s), 8.60 (1H, dd, J=4.6, 1.4 Hz).

1-Propyl-1*H*-imidazo[4,5-*b*]pyridine (**28**)

(Method B) Compound **28** was obtained from **18** in 41% yield using aldehyde **21**; ^{1}H-NMR ($CDCl_3$) δ 0.97 (3H, t, J=7 Hz), 1.93 (2H, m), 4.16 (2H, q, J=7 Hz), 7.25 (1H, dd, J=8.2, 4.2 Hz), 7.74 (1H, d, J=8.2 Hz), 8.12 (1H, s), 8.59 (1H, d, J=4.2 Hz).

1-Cyclopropylmethyl-1*H*-imidazo[4,5-*b*]pyridine (**29**)

(Method B) Compound **29** was obtained from **18** in 71% yield using aldehyde **22**; ^{1}H-NMR ($CDCl_3$) δ 0.43~0.56 (4H, m), 1.3 (1H, m), 2.12 (2H, m), 4.15 (2H, d, J=7 Hz), 7.20 (1H, dd, J=8.2, 4.8 Hz), 8.14 (1H, d, J=8.2 Hz), 8.4 (1H, d, J=4.8 Hz), 8.54 (1H, s).

1-[3-(*tert*-Butoxycarbonyl-ethylamino)-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**30**)

(Method B) Compound **30** was obtained from **18** in 81% yield using aldehyde **23**; ^{1}H-NMR ($CDCl_3$) δ 1.08 (3H, t, J=6.9 Hz), 1.44 (9H, s), 2.12 (2H, m), 3.25 (4H, m), 4.22 (2H, t, J=7.5 Hz), 7.20 (1H, dd, J=8.4, 4.5 Hz), 7.74 (1H, dd, J=8.4, 1.5 Hz), 8.20 (1H, s), 8.59 (1H, dd, J=4.5, 1.5 Hz).

1-[(3*R*)-3-*tert*-Butoxycarbonylamino-butyl]-1*H*-imidazo-[4,5-*b*]pyridine (**31**)

(Method B) Compound **31** was obtained from **18** in 85% yield using aldehyde **24**; ^{1}H-NMR ($CDCl_3$) δ 1.19 (3H, d, J=6.9 Hz), 1.46 (9H, s), 2.01 (2H, m), 3.80 (1H, bs), 4.27 (2H, m), 4.48 (1H, d, J=7.5 Hz), 7.23 (1H, dd, J=4.5, 8.4 Hz), 7.74 (1H, dd, J=1.5, 8.1 Hz), 8.23 (1H, bs), 8.58 (1H, dd, J=1.5, 4.8 Hz).

1-[(3*S*)-3-*tert*-Butoxycarbonylamino-butyl]-1*H*-imidazo-[4,5-*b*]pyridine (**32**)

(Method B) Compound **32** was obtained from **18** in 79% yield using aldehyde **25**.

1-[2-(1-*tert*-Butoxycarbonylamino-cyclopropyl)-ethyl]-1*H*-imidazo[4,5-*b*]pyridine (**33**)

(Method B) Compound **33** was obtained from **18** in 15% yield using aldehyde **26**; ^{1}H-NMR ($CDCl_3$) δ 0.48 (2H, t, J=5.7 Hz), 0.76 (2H, t, J=5.7 Hz), 1.43 (9H, s), 2.11 (2H, t, J=7.5 Hz), 4.40 (2H, t, J=6.9 Hz), 5.09 (1H, bs), 7.23 (1H, dd, J=4.8, 8.1 Hz), 7.73 (1H, dd, J=1.5, 7.8 Hz), 8.27 (1H, s), 8.57 (1H, dd, J=1.5, 4.8 Hz).

1-(3-*tert*-Butoxycarbonylamino-4,4,4-trifluoro-butyl)-1*H*-imidazo[4,5-*b*]pyridine (**34**)

(Method B) Compound **34** was obtained from **18** in 58% yield using aldehyde **27**; ^{1}H-NMR ($CDCl_3$) δ 1.48 (9H, s), 2.07~2.47 (2H, m), 4.37 (3H, bs), 5.34 (1H, d, J=9.6 Hz), 7.27 (1H, dd, J=8.2, 4.8 Hz), 7.76 (1H, dd, J=8.2, 1.6 Hz), 8.26 (1H, s), 8.60 (1H, dd, J=4.8, 1.4 Hz).

1-[(2*S*)-2-*tert*-Butoxycarbonylamino-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**43**)

(Method C) Compound **43** was obtained from 2,3-diaminopyridine (**35**) in 51% yield using aldehyde **36**; ^{1}H-NMR ($CDCl_3$) δ 1.18 (3H, d, J=6.9 Hz), 1.43 (9H, s), 4.05 (1H, m), 4.29 (2H, m), 4.64 (1H, d, J=6.6 Hz), 7.24 (1H, dd, J=4.8, 8.1 Hz), 7.87 (1H, d, J=8.4 Hz), 8.07 (1H, s), 8.56 (1H, dd, J=1.8, 5.1 Hz).

1-[(2*S*)-2-*tert*-Butoxycarbonylamino-butyl]-1*H*-imidazo-[4,5-*b*]pyridine (**44**)

(Method C) Compound **44** was obtained from diaminopyridine **35** in 46% yield using aldehyde **37**; ^{1}H-NMR ($CDCl_3$) δ 1.00 (3H, t, J=7.2 Hz), 1.42 (9H, s), 1.4~1.7 (2H, m), 3.83 (1H, m), 4.32 (2H, m), 4.71 (1H, d, J=8.1 Hz), 7.23 (1H, dd, J=4.5, 8.1 Hz), 7.86 (1H, d, J=7.8 Hz), 8.07 (1H, s), 8.55 (1H, dd, J=1.5, 5.1 Hz).

1-[(3*S*)-3-*tert*-Butoxycarbonylamino-3-carboxy-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**45**)

(Method C) Compound **45** was obtained from diaminopyridine **35** using aldehyde **38** in 68% yield; ^{1}H-NMR (DMSO-d_6) δ 1.41 (9H, s), 2.0~2.40 (2H, m), 3.80 (1H, m), 4.34 (2H, t, J=6.6 Hz), 7.28 (1H, dd, J=4.5, 7.8 Hz), 7.37 (1H, d, J=7.8 Hz), 8.08 (1H, d, J=6.9 Hz), 8.41 (1H, s), 8.42 (1H. d, J=6.9 Hz).

1-Carbamoylmethyl-1*H*-imidazo[4,5-*b*]pyridine (**46**)

To a solution of compound **9** in EtOH (20 ml) was added 28% NH_4OH and the reaction mixture was stirred at room temperature for 30 minutes. After evaporation of the solvent, the residue was purified by HP-20 resin chromatography to afford compound **46** (1.41 g, 48%); ^{1}H-NMR (DMSO-d_6) δ 4.96 (2H, s), 7.27 (1H, dd, J=8.2, 4.8 Hz), 7.37 (1H, s), 7.76 (1H, s), 7.92 (1H, dd, J=8.2, 1.6 Hz), 8.40 (1H, dd, J=4.8, 1.6 Hz), 8.41 (1H, s).

1-(3-Methylamino-propyl)-1*H*-imidazo[4,5-*b*]pyridine (**47**)

Compound **12** (2.90 g, 10 mmol) was dissolved in 3.3 N HCl/MeOH, and stirred at room temperature overnight. After evaporation of the solvent, the residue was dissolved in H_2O then K_2CO_3 was added to make the solution alkaline. The mixture was concentrated under reduced pressure and the residue was rinsed with $CHCl_3$, and precipitate was filtered off. The filtrate was condensed under reduced pressure to afford compound **47** (1.43 g, 100%); ^{1}H-NMR ($CDCl_3$) δ 2.02 (2H, m), 2.4 (3H, s), 2.56 (2H, t, J=6.6 Hz), 4.33 (2H, t, J=6.9 Hz), 7.23 (1H, dd, J=4.8, 8.1 Hz), 7.79 (1H, dd, J=1.5, 7.8 Hz), 8.15 (1H, s), 8.57 (1H, dd, J=1.5, 4.5 Hz).

1-(3-Dimethylamino-propyl)-1*H*-imidazo[4,5-*b*]pyridine (**48**)

To compound **47** was added HCO_2H (1.43 ml) and 35% HCHO (0.97 ml) and this was heated at 100°C for 30 minutes. After evaporation of solvent, the residue was dissolved in H_2O (10 ml) then K_2CO_3 was added until the solution became alkaline. The mixture was concentrated under reduced pressure, the residue was rinsed with $CHCl_3$, and then the precipitate was filtered off. The filtrate was condensed under reduced pressure to afford compound **48** (1.57 g, 100%); ^{1}H-NMR δ 2.03 (2H, m), 2.25 (6H, s), 2.27 (2H, t, J=6.9 Hz), 4.30 (2H, t, J=6.9 Hz), 7.23 (1H, dd, J=4.8, 8.1 Hz), 7.79 (1H, dd, J=1.2, 8.4 Hz), 8.15 (1H, s), 8.57 (1H, dd, J=1.5, 4.8 Hz).

1-[3-(*tert*-Butoxycarbonyl-cyclopropyl-amino)-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**49**)

To compound **6** (660 mg, 3.25 mmol) was added cyclopropyl amine (2.4 ml) and this was stirred at room temperature for 2 hours. To the reaction mixture was added cyclopropylamine (1.2 ml) and the reaction mixture was stirred overnight at room temperature then refluxed for 3 hours. After evaporation, the residue was dissolved in DMF (6 ml) then Boc_2O (0.85 ml) was added to the solution. After stirring the reaction at room temperature for 2 hours, 4-dimetylaminopyridine (82 mg) was added and stirring was continued overnight. After evaporation, the residue was chromatographed on a silica gel column to give compound **49** (566 mg, 49% from **6**); ^{1}H-NMR ($CDCl_3$) δ 0.55 (2H, m), 0.74 (2H, m), 1.44 (9H, s), 2.15 (2H, m), 2.47 (1H, m), 3.30 (2H, t, J=6.6 Hz), 4.22 (2H, t, J=7.2 Hz), 7.25 (1H, dd, J=5.01, 8.1 Hz), 7.75 (1H, dd, J=1.8, 7.8 Hz), 8.21 (1H, s), 8.59 (1H, dd, J=1.8, 4.5 Hz).

1-[3-[*tert*-Butoxycarbonyl-(2-triethylsilanyloxy-ethyl)-amino]-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**50**)

To a solution of compound **11** (1.14 g, 4.13 mmol) in DMF (6 ml) was added 60% NaH (250 mg, 6.2 mmol) and the mixture was stirred at room temperature for 10 minutes. To the reaction mixture was added a solution of (2-bromo-

ethoxy)-triethylsilane (1.48 g, 6.2 mmol) in DMF and stirred at the same temperature for 1 hour. To the reaction mixture was added 60% NaH (170 mg, 4.13 mmol) and (2-bromo-ethoxy)-triethylsilane (990 mg, 4.13 mmol). This was stirred at room temperature for 2 hours, then poured into a mixture of cold water and EtOAc. The organic layer was washed successively with water and brine, dried over $MgSO_4$ and filtered off. After evaporation, the residue was chromatographed on a silica gel column to give compound **50** (1.26 g, 70%); ^{1}H-NMR ($CDCl_3$) δ 0.57 (9H, q, J=7.8 Hz), 0.93 (6H, t, J=7.8 Hz), 1.46 (9H, s), 2.15 (2H, m), 3.25 (2H, bs), 3.38 (2H, bs), 3.68 (2H, m), 4.21 (2H, t, J=7.2 Hz), 7.25 (1H, dd, J=7.8, 4.5 Hz), 7.75 (1H, dd, J=7.8, 1.2 Hz), 8.30 (1H, s), 8.59 (1H, dd, J=4.5, 1.2 Hz).

1-[3-[*tert*-Butoxycarbonyl-(2-hydroxy-ethyl)-amino]-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**51**)

To a solution of compound **50** (1.26 g, 2.9 mmol) of THF (6 ml) was added AcOH (3 ml) and water (6 ml) then this was stirred at room temperature for 30 minutes. The reaction mixture was poured into a mixture of ice water and EtOAc. The aqueous layer was adjusted to pH 8 with Na_2CO_3 and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give compound **51** (0.93 g, 100%); ^{1}H-NMR ($CDCl_3$) δ 1.42 (9H, s), 2.18 (2H, m), 3.38 (4H, m), 3.78 (2H, t, J=5.1 Hz), 4.23 (2H, t, J=7.5 Hz), 7.21 (1H, dd, J=8.1, 3.9 Hz), 7.74 (1H, dd, J=8.1, 1.2 Hz), 8.17 (1H, s), 8.55 (1H, dd, J=3.9, 1.2 Hz).

1-[3-[*tert*-Butoxycarbonyl-(2-di-*tert*-butoxycarbonyl-amino-ethyl)-amino]-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**52**)

To a solution of compound **51** (0.97 g, 3.02 mmol) of THF (15 ml) was added tri-*n*-butylphosphine (1.13 ml, 4.53 mmol), di-*tert*-butyl iminodicarboxylate (0.995 g, 4.53 mmol) and 1,1′-(azodicarbonyl)dipiperidine (1.15 g, 4.53 mmol) under cooling on an ice-water bath, stirred for 15 minutes, and stirred at room temperature for 3 hours. Next, tri-*n*-butylphosphine (0.37 ml, 1.5 mmol), di-*tert*-butyl iminodicarboxylate (0.33 g, 1.5 mmol) and 1,1′-(azodicarbonyl)dipiperidine (0.38 g, 1.5 mmol) were added to the reaction mixture and this was stirred for 2 hours. After filtration of insoluble material, EtOAc was added to the filtrate followed by washing with H_2O, dried over $MgSO_4$, filtration and concentration under reduced pressure. The residue was chromatographed on a silica gel column to give compound **52** (1.14 g, 72%); ^{1}H-NMR ($CDCl_3$) δ 1.48 (27H, s), 2.13 (2H, m), 3.37 (4H, m), 3.73 (2H, d, J=6.2 Hz), 4.21 (2H, d, J=7.4 Hz), 7.21 (1H, dd, J=7.8, 4.8 Hz), 7.75 (1H, dd, J=7.8, 1.2 Hz), 8.24 (1H, s), 8.58 (1H, dd, J=4.8, 1.2 Hz).

1-(3-Amino-propyl)-1*H*-imidazo[4,5-*b*]pyridine dihydrochloride (**53**)

To compound **11** (2.76 g, 10 mmol) was added 3.3 N HCl/MeOH (30 ml) and this was stirred at room temperature for 22 hours. After evaporation of the solvent, crystallization was performed with MeOH and 2-propanol to give compound **53** (2.34 g, 94%); ^{1}H-NMR (DMSO-d_6) δ 2.22 (2H, m), 2.84 (2H, m), 4.63 (2H, t, J=7.2 Hz), 7.68 (1H, dd, J=5.1, 8.1 Hz), 8.32 (2H, bs), 8.69 (1H, d, J=5.7 Hz), 8.71 (1H, d, J=9.9 Hz), 9.45 (1H, s).

1-[3-*N*,*N*′-Bis(*tert*-butoxycarbonyl)guanidino-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**55**)

To a solution of compound **53** (747 mg, 13 mmol) in DMF (10 ml) was added 1*H*-pyrazole-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)carboxamidine (**54**) (978 mg, 14.3 mmol) and this was stirred at room temperature overnight. After addition of EtOAc and water, the organic phase was washed with water and brine, dried over $MgSO_4$, and filtered off. After evaporation of solvent, the residue was rinsed with Et_2O and the precipitate was collected by filtration to give compound **55** (1.06 g, 84%); ^{1}H-NMR (DMSO-d_6) δ 1.39 (9H, s), 1.47 (9H, s), 2.08 (2H, m), 3.31 (2H, m), 4.31 (2H, t, J=6.6 Hz), 7.26 (1H, dd, J=4.8, 8.1 Hz), 8.07 (1H, dd, J=1.2, 8.4 Hz), 8.35 (1H, t, J=6.0 Hz), 8.41 (1H, dd, J=1.5 Hz, 4.5 Hz), 8.58 (1H, s).

1-[(3*S*)-3-*tert*-Butoxycarbonylamino-3-methoxy-carbonyl-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**56**)

To a solution of compound **45** in THF was added diazomethane-Et_2O under cooling ice bath. After evaporation of the solvent, the residue was chromatographed on a silica gel column to give compound **56** (4.5 g, 66%); ^{1}H-NMR ($CDCl_3$) δ 1.46 (9H, s), 2.20~2.52 (2H, m), 3.64 (3H, s), 4.34 (2H, t, J=6.3 Hz), 4.2~4.39 (1H, m), 5.45 (1H, d, J=7.5 Hz), 7.25 (1H, dd, J=4.8, 8.1 Hz), 7.75 (1H, dd, J=1.5, 8.1 Hz), 8.22 (1H, s), 8.59 (1H, dd, J=1.5, 4.5 Hz).

1-[(3*S*)-3-*tert*-Butoxycarbonylamino-3-carbamoyl-propyl]-1*H*-imidazo[4,5-*b*]pyridine (**57**)

To a solution of compound **56** (4.5 g, 13.5 mmol) was added 28% NH_4OH (10 ml) with stirring at room temperature for 4 hours then the mixture was left standing at 4°C overnight. After evapotration of the solvent, crystallization was performed from the residue with EtOH to give compound **57** (2.39 g, 55%): ^{1}H-NMR (DMSO-d_6) δ

1.40 (9H, s), 1.96~2.20 (2H, m), 3.80 (1H, m), 4.29 (2H, t, J=7.2 Hz), 7.01 (1H, s), 7.12 (1H, d, J=8.1 Hz), 7.27 (1H, dd, J=4.5, 8.1 Hz), 7.29 (1H, s), 8.04 (1H, dd, J=1.5, 8.4 Hz), 8.38 (1H, s), 8.42 (1H, dd, J=1.5, 4.8 Hz).

General Preparation of Cephalosporins

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(Z)-ethoxyiminoacetamido]-3-[1-(3-methylaminopropyl)-1H-imidazo[4,5-b]pyridinium-4-yl]methyl-3-cephem-4-carboxylate Sulfate (**70** Sulfate, **S-3578**)

To a suspension of compound **II** (102 g, 252 mmol) in EtOAc (1 liter) was added N-methylmolpholine (28 ml, 254 mmol) at 0°C and the reaction mixture was stirred at −5°C for 30 minutes. To the mixture was added acid **Ib** (80.67 g, 252 mmol) and phosphorus oxychloride (24 ml, 264 mmol) at the same temperature, followed by dropwise addition of N-methylmolpholine (86.7 ml, 792 mmol) at −27°C. The reaction mixture was stirred at from −15°C to 0°C for 3 hours. After addition of ice-cold brine (1 liter), the organic phase was washed successively with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* to give crude **IIIb** (188 g, 92% purity, 82% yield).

To a solution of imidazopyridine **12** (28.9 g, 99.8 mmol) in DMF (92 ml) was added the chloromethyl derivative **IIIb** (81.2 g, 99.8 mmol) and NaBr (20.5 g, 199 mmol). The reaction mixture was stirred at 5°C for 3.5 days. After addition of MeCN (50 ml), the mixture was poured into 5% NaCl. The precipitate was filtered and dried *in vacuo* to give a mixture of quaternary ammonium **IVb** and **Vb** in the ratio of 61:13 determined by HPLC (total 173 g). To 98% HCO_2H (170 ml), the mixture of **IVb** and **Vb** (173 g) and 62% H_2SO_4 (511 ml) was successively at 0°C and stirred at 3°C for 1 hour. The reaction mixture was poured into a mixture of 2-propanol (8.5 liters) and acetone (1 liter). The precipitate was collected by filtration and dissolved in H_2O (300 ml). The solution was chromatographed on HP-20 resin. The target product was eluted with 2% MeCN/0.001 N H_2SO_4. The solution containing the target product was adjusted to pH 4.5 by addition of poly(4-vinylpyridine) and filtered. The filtrate was concentrated and lyophilized to give **70** sulfate (crude **S-3578**) (31 g, 42% yield from **IIIb**); IR (KBr) cm^{-1} 1779, 1671, 1634, 1527, 1488, 1464; *Anal* Calcd for $C_{24}H_{28}N_{10}O_5S_2 \cdot 0.54H_2SO_4 \cdot 4.6H_2O$: C 39.14, H 5.24, N 19.02, S 11.06. Found: C 39.22, H 5.18, N 19.22, S 10.88.

To a solution of the described crude S-3578 (**70** sulfate) 4.0 g in H_2O (12 ml) was added 10 N H_2SO_4 (0.65 ml) and THF (13 ml) at 10°C. After standing at the same temperature for a week, the crystallized solid was filtered and dried under reduced pressure (15 mmHg at room temperature for 1.5 hour) to afford pure **S-3578** (1.67 g, 37.3%); MP >200°C; *Anal* Calcd for $C_{24}H_{28}N_{10}O_5S_2 \cdot H_2SO_4 \cdot 7H_2O$: C 34.95, H 5.38, N 16.98, S 11.66. Found: C 34.67, H 5.30, N 17.16, S 11.72; ^{1}H-NMR (D_2O) δ 1.30 (3H, t, J=7.5 Hz), 2.42 (2H, m), 2.74 (3H, s), 3.16 (2H, t like, J=8.1 Hz), 3.34 and 3.64 (2H, ABq, J=18.3 Hz), 4.33 (2H, q, J=7.5 Hz), 4.65 (2H, t like, J=7.5 Hz), 5.25 (1H, d, J=4.8 Hz), 5.71 and 5.94 (2H, ABq, J=15 Hz), 5.87 (1H, d, J=4.8 Hz), 7.89 (1H, dd, J=6.6, 8.1 Hz), 8.82 (1H, d, J=8.1 Hz), 8.86 (1H, d, J=6.6 Hz), 8.89 (1H, s); *Anal* Calcd for $C_{24}H_{28}N_{10}O_5S_2 \cdot H_2SO_4 \cdot 7H_2O$: C 34.95, H 5.38, N 16.98, S 11.66. Found: C 34.67, H 5.30, N 17.16, S 11.72.

The other cephalosporins (**1**, **58**~**69**, **71**~**89**) were prepared by a procedure similar to that described for the preparation of **S-3578** (**70** sulfate).

Compound **1**

Compound **1** was obtained in 28% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6) δ 1.18 (3H, t, J=7.2 Hz), 3.10 and 3.50 (2H, ABq, J=18 Hz), 4.10 (2H, q, J=7.2 Hz), 5.12 (1H, d, J=5.1 Hz), 5.62 (2H, ABq, J=14.4 Hz), 5.79 (1H, dd, J=5.0, 8.5 Hz), 7.54 (1H, dd, J=6.4, 8.0 Hz), 8.10 (2H, br s), 8.55 (1H, d, J=5.1 Hz), 8.56 (1H, s), 8.72 (1H, d, J=6 Hz), 9.53 (1H, d, J=9 Hz); IR (KBr) cm^{-1} 1773, 1665, 1609, 1527, 1388.

Compound **58**

Compound **58** was obtained in 58% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6) δ 1.18 (3H, t, J=7.2 Hz), 2.95 and 3.52 (2H, ABq, J=17.4 Hz), 4.06 (3H, s), 4.04~4.19 (2H, m), 5.02 (1H, d, J=5.1 Hz), 5.64~5.69 (3H, m), 7.95 (1H, dd, J=6.3, 8.1 Hz), 8.13 (2H, br s), 8.88 (1H, dd, J=0.9, 8.1 Hz), 9.04 (1H, s), 9.44 (1H, d, J=8.7 Hz), 9.71 (1H, d, J=5.7 Hz); IR (KBr) cm^{-1} 1774, 1671, 1614, 1528.

Compound **59**

Compound **59** was obtained in 62% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6) δ 1.18 (3H, t, J=7.0 Hz), 1.51 (3H, t, J=7.2 Hz), 2.99 and 3.53 (2H, ABq, J=17.4 Hz), 4.10 (2H, q, J=6.9 Hz), 4.51 (2H, q, J=7.2 Hz), 5.01 (1H, d, J=4.5 Hz), 5.66~5.69 (3H, m), 7.94 (1H, t, J=6.6 Hz), 8.12 (2H, br s), 8.94 (1H, d, J=8.1 Hz), 9.12 (1H, s), 9.44 (1H, d, J=8.7 Hz), 9.67 (1H, d, J=6.3 Hz); IR (KBr) cm^{-1} 1775, 1669, 1634, 1613, 1526.

Compound **60**

Compound **60** was obtained in 36% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6) δ 0.91 (3H, t, J=6.9 Hz), 1.2 (3H, t, J=6.9 Hz), 1.9 (2H, m), 3.0~3.6 (2H, m), 4.1 (2H, q, J=6.9 Hz), 4.4 (2H, t, J=6.9 Hz), 5.0 (1H, d, J=5.1 Hz), 5.6 and 6.0 (2H, ABq, J=14 Hz), 5.8 (1H, dd, J=5.1, 8.4 Hz), 8.0 (1H, dd, J=6.9, 8.4 Hz), 8.1 (2H, bs), 9.0 (1H, d, J=8.4 Hz), 9.1 (1H, d, J=6.9 Hz), 9.13 (1H, s), 9.5 (1H, d, J=8.4 Hz); IR (KBr) cm^{-1} 1781, 1676, 1632, 1523.

Compound **61**

Compound **61** was obtained in 22% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6) δ 0.5~0.6 (4H, m), 1.21 (3H, t, J=6.9 Hz), 1.21 (3H, t, J=7.2 Hz), 1.4 (1H, m), 3.3 and 3.5 (2H, ABq, J=17 Hz), 4.15 (2H, q, J=7.2 Hz), 4.4 (2H, d, J=6.9 Hz), 5.1 (1H, d, J=5.1 Hz), 5.6 and 6.1 (2H, ABq, J=14.4 Hz), 5.8 (1H, dd, J=5.1, 8.8 Hz), 8.0 (1H, dd, J=6, 7.8 Hz), 8.1 (2H, bs), 8.9 (1H, d, J=6 Hz), 9.0 (1H, d, J=7.8 Hz), 9.1 (1H, s), 9.5 (1H, d, J=8.7 Hz); IR (KBr) cm^{-1} 1781, 1675, 1633, 1524.

Compound **62**

Compound **62** was obtained in 38% yield from **IIIb** using $TiCl_4$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6+D_2O) δ 1.16 (3H, t, J=7.2 Hz), 3.12 and 3.50 (2H, ABq, J=17.1 Hz), 4.10 (2H, q, J=7.2 Hz), 5.0 (1H, d, J=4.8 Hz), 5.64 and 5.76 (2H, ABq, J=14.4 Hz), 5.69 (1H, d, J=4.8 Hz), 8.02 (1H, m), 8.10 (1H, d, J=59.1 Hz), 8.92 (1H, d, J=7.8 Hz), 9.34 (1H, s), 9.50 (1H, d, J=6.3 Hz); IR (KBr) cm^{-1} 1774, 1669, 1611, 1528.

Compound **63**

Compound **63** was obtained in 35 % yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.16 (3H, t, J=7.2 Hz), 3.01 and 3.56 (2H, ABq, J=17.7 Hz), 3.80 (2H, t, J=4.5 Hz), 4.08 (2H, q, J=7.2 Hz), 4.55 (2H, m), 5.02 (1H, d, J=5.1 Hz), 5.61 and 5.70 (2H, ABq, J=13.5 Hz), 5.69 (1H, dd, J=5.1, 8.4 Hz), 7.91 (1H, dd, J=6.0, 8.1 Hz), 8.15 (2H, br s), 8.91 (1H, d, J=8.1 Hz), 9.05 (1H, s), 9.45 (1H, d, J=8.4 Hz), 9.51 (1H, d, J=6.0 Hz): IR (KBr) cm^{-1} 1774, 1670, br 1613, 1527.

Compound **64**

Compound **64** was obtained in 43% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.29 (3H, t, J=7 Hz), 3.28 and 3.61 (2H, ABq, J=18 Hz), 4.31 (2H, q, J=7 Hz), 5.22 (1H, d, J=4.6 Hz), 5.40 (2H, s), 5.66 and 5.91 (2H, ABq, J=14.6 Hz), 5.86 (1H, d, J=4.6 Hz), 7.89 (1H, dd, J=6.2, 8.4 Hz), 8.69 (1H, d, J=8.4 Hz), 8.83 (1H, s), 8.90 (1H, d, J=6.2 Hz); IR (KBr) cm^{-1} 1770, 1684, 1613, 1525.

Compound **65** Hydrochloride

Compound **65** hydrochloride was obtained in 35% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.30 (3H, t, J=7.0 Hz), 3.31 and 3.63 (2H, ABq, J=18 Hz), 3.67 (2H, t, J=6.0 Hz), 4.33 (2H, q, J=7.0 Hz), 4.90 (2H, t, J=6.0 Hz), 5.22 (1H, d, J=5.0 Hz), 5.63 and 5.94 (2H, ABq, J=14.8 Hz), 5.85 (1H, d, J=5.0 Hz), 7.92 (1H, dd, J=6.4, 8.2 Hz), 8.82~8.89 (2H, m), 8.91 (1H, s); IR (KBr) cm^{-1} 1772, 1669, 1634, 1524, 1488, 1464.

Compound **66** Sulfate

Compound **66** sulfate was obtained in 50% yield from **IIIb** using H_2SO_4-HCO_2H for deprotection; ^{1}H-NMR (D_2O) δ 1.31 (3H, d, J=7.2 Hz), 1.45 (3H, d, J=6.6 Hz), 3.32 and 3.63 (2H, ABq, J=18 Hz), 4.07 (1H, q like, J=6.6 Hz), 4.36 (2H, q, J=7.2 Hz), 4.8 (2H, m), 5.22 (1H, d, J=4.5 Hz), 5.63 and 5.93 (2H, ABq, J=14.7 Hz), 5.86 (1H, d, J=4.5 Hz), 7.93 (1H, dd, J=6.6, 7.5 Hz), 8.84 (1H, d, J=7.5 Hz), 8.88 (1H, d, J=6.6 Hz), 8.92 (1H, s); IR (KBr) cm^{-1} 3406, 2979, 1772, 1614, 1527.

Compound **67** Sulfate

Compound **67** sulfate was obtained in 70% yield from **IIIc** using H_2SO_4-HCO_2H for deprotection; ^{1}H-NMR (D_2O) δ 1.11 (3H, t, J=7.2 Hz), 1.30 (3H, J=7.2 Hz), 1.84 (2H, m), 3.32 and 3.63 (2H, ABq, J=18 Hz), 3.92 (1H, m), 4.32 (2H, q, J=7.2 Hz), 5.22 (1H, d, J=4.8 Hz), 5.66 and 5.96 (2H, ABq, J=14.7 Hz), 5.86 (1H, d, J=4.8 Hz), 7.93 (1H, dd, J=6.3, 8.1 Hz), 8.83 (1H, d, J=8.1 Hz), 8.88 (1H, d, J=6.3 Hz), 8.92 (1H, s); IR (KBr) cm^{-1} 1776, 1671, 1634, 1528, 1488, 1463.

Compound **68** Hydrochloride

Compound **68** hydrochloride was obtained in 31% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.28 (3H, t, J=7.0 Hz), 2.36 (2H, m), 3.11 (2H, t, J=8.6 Hz), 3.28 and 3.61 (2H, ABq, J=18.0 Hz), 4.61 (2H, t, J=7.0 Hz), 4.30 (2H, q, J=7.0 Hz), 5.21 (1H, d, J=4.6 Hz), 5.60 and 5.87 (2H, ABq, J=14.7 Hz), 7.86 (1H, dd, J=8.2, 6.2 Hz), 8.78 (1H, d, J=8.2 Hz), 8.81 (1H, d, J=6.2 Hz), 8.85 (1H, s); IR (KBr) cm^{-1} 1772, 1615, 1524, 1387.

Compound **69** Hydrochloride

Compound **69** hydrochloride was obtained in 27% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.30 (3H, t, J=7 Hz), 1.75 (2H, m), 2.05 (2H, m),

3.04 (2H, t, J=6.8 Hz), 3.31 and 3.64 (2H, ABq, J=18.1 Hz), 4.33 (2H, q, J=7 Hz), 4.57 (2H, t, J=7 Hz), 5.23 (1H, d, J=5 Hz), 5.64 and 5.89 (2H, ABq, J=14.8 Hz), 5.85 (1H, d, J=5 Hz), 7.86 (1H, dd, J=8.2, 6.6 Hz), 8.78 (1H, d, J=8.2 Hz), 8.81 (1H, d, J=6.6 Hz), 8.85 (1H, s); IR (KBr) cm^{-1} 1774, 1671, 1617, 1523, 1489, 1462.

Compound **71** Sulfate

Compound **71** sulfate was obtained in 37% yield from **IIIc** using H_2SO_4-HCO_2H for deprotection; ^{1}H-NMR (D_2O) δ 1.30 (3H, t, J=6.9 Hz), 2.46 (2H, m), 2.91 (6H, s), 3.27 (2H, bs), 3.31 and 3.63 (2H, ABq, J=17.7 Hz), 4.32 (2H, q, J=7.2 Hz), 4.63 (2H, t, J=7.8 Hz), 5.23 (1H, d, J=4.8 Hz), 5.64 and 5.89 (2H, ABq, J=15 Hz), 5.85 (1H, d, J=4.8 Hz), 7.89 (1H, dd, J=6.6, 8.1 Hz), 8.80 (1H, d, J=8.1 Hz), 8.86 (1H, d, J=6.6 Hz), 8.88 (1H, s); IR (KBr) cm^{-1} 1774, 1670, 1610, 1527, 1488, 1463.

Compound **72** Hydrochloride

Compound **72** hydrochloride was obtained in 33% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.29 (6H, m), 2.40 (2H, m), 3.12 (4H, m), 3.34 and 3.65 (2H, ABq, J=18.4 Hz), 4.33 (2H, q, J=7.2 Hz), 4.64 (2H, t, J=7 Hz), 5.25 (1H, d, J=4.6 Hz), 5.70 and 5.94 (2H, ABq, J=14.8 Hz), 5.88 (1H, d, J=4.6 Hz), 7.89 (1H, dd, J=8.2 Hz, 6.4 Hz), 8.81 (1H, d, J=8.2 Hz), 8.85 (1H, d, J=6.4 Hz), 8.89 (1H, s); IR (KBr) cm^{-1} 1779, 1671, 1633, 1526, 1488, 1463.

Compound **73** Hydrochloride

Compound **73** hydrochloride was obtained in 26% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6) δ 0.68 (2H, d, J=6 Hz), 0.88 (2H, bs), 1.18 (3H, t, J=7.2 H), 2.31 (center, 2H, m), 2.61 (1H, m), 3.03 (2H, t, J=7.2 Hz), 3.08 and 3.47 (2H, ABq, J=16 Hz), 4.10 (2H, q, J=7.2 Hz), 4.64 (2H, t, J=7.2 Hz), 5.04 (1H, d, J=4.8 Hz), 5.56 and 5.74 (2H, ABq, J=14.4 Hz), 5.75 (1H, d, J=4.8 Hz), 7.87 (1H, dd, J=6.2, 8.1 Hz), 8.12 (2H, bs), 9.00 (1H, d, J=8.4 Hz), 9.16 (1H, s), 9.26 (1H, d, J=6.2 Hz), 9.46 (1H, d, J=8.1 Hz); IR (KBr) cm^{-1} 1774, 1670, 1635, 1612, 1527.

Compound **74** Hydrochloride

Compound **74** hydrochloride was obtained in 12% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.30 (3H, t, J=6.9 Hz), 2.42 (2H, m), 3.22 (4H, m), 3.31 and 3.64 (2H, ABq, J=18.2 Hz), 3.83 (2H, t, J=5.4 Hz), 4.33 (2H, q, J=6.9 Hz), 4.65 (2H, t, J=6.6 Hz), 5.23 (1H, d, J=4.8 Hz), 5.62 and 5.90 (2H, ABq, J=14.6 Hz), 5.86 (1H, d, J=4.8 Hz), 7.89 (1H, dd, J=8.1, 6.6 Hz), 8.80 (1H, d, J=8.1 Hz), 8.84 (1H, d, J=6.6 Hz), 8.88 (1H, s); IR (KBr) cm^{-1} 1773, 1669, 1611, 1527, 1388.

Compound **75** Hydrochloride

Compound **75** hydrochloride was obtained in 14% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.31 (3H, t, J=7 Hz), 2.45 (2H, m), 3.42 (6H, m), 4.33 (2H, q, J=7 Hz), 4.66 (2H, d, J=7.4 Hz), 5.23 (1H, d, J=5 Hz), 5.61 and 5.91 (2H, ABq, J=14.8 Hz), 5.85 (1H, d, J=5 Hz), 7.89 (1H, dd, J=8 Hz, 6.4 Hz), 8.80 (1H, d, J=8 Hz), 8.84 (1H, d, J=6.4 Hz), 8.88 (1H, s); IR (KBr) cm^{-1} 1772, 1668, 1610, 1524, 1488, 1462.

Compound **76** Sulfate

Compound **76** sulfate was obtained in 51% yield from **IIIc** using H_2SO_4-HCO_2H for deprotection; ^{1}H-NMR (D_2O) δ 1.29 (3H, t, J=7.2 Hz), 2.30 (2H, m), 3.29 (2H, t, J=6.3 Hz), 3.30 and 3.63 (2H, ABq, J=17.7 Hz), 4.32 (2H, q, J=6.9 Hz), 4.61 (2H, t, J=7.2 Hz), 5.23 (1H, d, J=4.8 Hz), 5.64 and 5.90 (2H, ABq, J=15.0 Hz), 5.84 (1H, d, J=4.8 Hz), 7.88 (1H, dd, J=6.3, 7.8 Hz), 8.78 (1H, d, J=9.0 Hz), 8.84 (1H, d, J=6.3 Hz), 8.87 (1H, s); IR (KBr) cm^{-1} 1774, 1670, 1633, 1527, 1488, 1461.

Compound **77** Hydrochloride

Compound **77** hydrochloride was obtained in 45% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6+D_2O) δ 1.20 (3H, t, J=6.9 Hz), 1.31 (3H, d, J=6.6 Hz), 2.10~2.40 (2H, m), 3.17 and 3.51 (2H, ABq, J=18 Hz), 3.25 (1H, m), 4.13 (2H, q, J=6.9 Hz), 4.61 (2H, t, J=7.8 Hz), 5.05 (1H, d, J=4.8 Hz), 5.61 and 5.82 (2H, ABq, J=13.8 Hz), 5.75 (1H, d, J=4.8 Hz), 7.90 (1H, dd, J=6.3, 8.1 Hz), 8.94 (1H, d, J=7.8 Hz), 9.10 (1H, s), 9.25 (1H, d, J=6.0 Hz); IR (KBr) cm^{-1} 1774, 1669, 1633, 1525, 1489, 1462.

Compound **78** Hydrochloride

Compound **78** hydrochloride was obtained in 15% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6+D_2O) δ 1.30 (3H, t, J=7.2 Hz), 1.44 (3H, d, J=6.6 Hz), 2.2~2.6 (2H, m), 3.32 and 3.64 (2H, ABq, J=18 Hz), 3.53 (1H, m), 4.32 (2H, q, J=7.2 Hz), 4.65 (2H, t, J=7.5 Hz), 5.25 (1H, d, J=4.5 Hz), 5.7 and 5.94 (2H, ABq, J=14.7 Hz), 5.87 (1H, d, J=4.5 Hz), 7.89 (1H, dd, J=6.0, 8.4 Hz), 8.81 (1H, d, J=8.1 Hz), 8.85 (1H, d, J=6.3 Hz), 8.90 (1H, s); IR (KBr) cm^{-1} 1772, 1608, 1525, 1488, 1462.

Compound **79** Hydrochloride

Compound **79** hydrochloride was obtained in 33% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 0.85 (2H, bs), 1.09 (2H, bs), 1.29 (3H, t, J=7.2 Hz), 2.40 (2H, m), 3.30 and 3.63 (2H, ABq, J=18 Hz), 4.33 (2H, q, J=7.2 Hz), 4.76 (2H, m), 5.24 (1H, d, J=5 Hz), 5.66 and 5.91 (2H, ABq, J=13.8 Hz), 7.87 (1H, m), 8.79 (1H, d, J=7.8 Hz), 8.85 (1H, d, J=6.0 Hz), 8.90 (1H, s); IR (KBr) cm^{-1} 1779, 1671, 1633, 1523, 1488, 1463.

Compound **80** Hydrochloride

Compound **80** hydrochloride was obtained in 28% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6+D_2O) δ 1.20 (3H, t, J=7.2 Hz), 1.90~2.28 (2H, m), 3.24 (1H, m), 3.34 and 3.43 (2H, ABq, J=19.2 Hz), 4.14 (2H, q, J=7.2 Hz), 4.66 (2H, t, J=8.7 Hz), 5.06 (1H, d, J=5.1 Hz), 5.59 and 6.01 (2H, ABq, J=14.1 Hz), 5.83 (1H, d, J=5.1 Hz), 7.95 (1H, dd, J=7.5, 10.8 Hz), 8.99 (1H, d, J=7.5 Hz), 9.05 (1H, d, J=10.8 Hz), 9.07 (1H, s); IR (KBr) cm^{-1} 1773, 1670, 1633, 1526, 1489, 1462.

Compound **81** Sulfate

Compound **81** sulfate was obtained in 43% yield from **IIIc** using H_2SO_4-HCO_2H for deprotection; ^{1}H-NMR (D_2O) δ 1.29 (3H, t, J=7.2 Hz), 2.64 (2H, m), 3.30 and 3.63 (2H, ABq, J=18 Hz), 4.25 (2H, t, J=6.6 Hz), 4.32 (2H, q, J=7.2 Hz), 4.6~4.8 (1H, m). 5.24 (1H, d, J=5.1 Hz), 5.65 and 5.91 (2H, ABq, J=14.7 Hz), 5.86 (1H, d, J=5.1 Hz), 7.89 (1H, dd, J=6.6, 8.1 Hz), 8.81 (1H, d, J=8.4 Hz), 8.85 (1H, d, J=6.3 Hz), 8.89 (1H, s); IR (KBr) cm^{-1} 1774, 1689, 1632, 1526, 1490, 1462.

Compound **82** Hydrochloride

Compound **82** hydrochloride was obtained in 28% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.30 (3H, t, J=7 Hz), 3.31 and 3.64 (2H, ABq, J=17.6 Hz), 5.23 (1H, d, J=4.6 Hz), 5.64 and 5.91 (2H, ABq, J=15.4 Hz), 5.86 (1H, d, J=4.6 Hz), 7.90 (1H, dd, J=7.8, 6.4 Hz), 8.80 (1H, d, J=7.8 Hz), 8.86 (1H, d, J=6.4 Hz), 8.89 (1H, s); IR (KBr) cm^{-1} 1773, 1670, 1616, 1524, 1487, 1463, 1450.

Compound **83** Hydrochloride

Compound **83** hydrochloride was obtained in 24% yield from **IIIb** using $AlCl_3$-anisole for deprotection, ^{1}H-NMR (D_2O) δ 1.30 (3H, t, J=7.2 Hz), 2.68~2.80 (1H, m), 2.85~2.97 (1H, m), 3.31 and 3.63 (2H, ABq, J=18 Hz), 3.63~3.88 (3H, m), 4.08~4.19 (1H, m), 4.33 (2H, q, J=6.9 Hz), 5.22 (1H, d, J=4.5 Hz), 5.62 and 5.94 (2H, ABq, J=14.4 Hz), 5.62~5.72 (1H, m), 5.85 (1H, d, J=4.5 Hz), 7.92 (1H, dd, J=6.3, 8.4 Hz), 8.85 (1H, d, J=8.4 Hz), 8.89 (1H, d, J=5.7 Hz), 9.03 (1H, s); IR (KBr) cm^{-1} 3398, 2982, 1771, 1668, 1611, 1461, 1391.

Compound **84** Hydrochloride

Compound **84** hydrochloride was obtained in 38% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (DMSO-d_6) δ 1.17 (3H, t, J=6.6 Hz), 2.69 (center, 2H, m), 3.15 and 3.47 (2H, ABq, J=18 Hz), 3.47~3.81 (4H, m), 4.10 (2H, q, J=6.6 Hz), 5.04 (1H, d, J=5.1 Hz), 5.31 and 5.88 (2H, ABq, J=13.5 Hz), 5.60 (1H, m), 5.80 (1H, dd, J=5.1, 8.4 Hz), 7.94 (1H, dd, J=7.2, 7.5 Hz), 8.12 (2H, s), 9.03~9.05 (2H, m), 9.05 (1H, d, J=8.4 Hz); IR (KBr) cm^{-1} 1777, 1677, 1636, 1528, 1463, 1406.

Compound **85** Hydrochloride

Compound **85** hydrochloride was obtained in 25% yield from **IIIb** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 1.30 (3H, t, J=7.5 Hz), 2.50 (4H, m), 3.33 (3H, m), 3.70 (3H, m), 4.33 (2H, q, J=7.5 Hz), 5.07 (1H, m), 5.23 (1H, d, J=4.8 Hz), 5.64 and 5.91 (2H, ABq, J=14.7 Hz), 5.85 (1H, d, J=4.8 Hz), 7.89 (1H, m), 8.85 (2H, m), 8.98 (1H, s); IR(KBr) cm^{-1} 1773, 1670, 1616, 1524, 1460.

Compound **86** Hydrochloride

Compound **86** hydrochloride was obtained in 39% yield from **IIIa** using $AlCl_3$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 2.40 (2H, m), 2.73 (3H, s), 317 (2H, t, J=8.2 Hz), 3.30 and 3.64 (2H, ABq, J=17.9 Hz), 4.05 (3H, s), 4.64 (2H, t, J=7.0 Hz), 5.22 (1H, d, J=4.8 Hz), 5.62 and 5.89 (2H, ABq, J=14.6 Hz), 5.85 (1H, d, J=4.8 Hz), 7.88 (1H, dd, J=8.6, 6.6 Hz), 8.80 (1H, d, J=8.6 Hz), 8.84 (1H, d, J=6.6 Hz), 8.88 (1H, s); IR (KBr) cm^{-1} 1773, 1669, 1611, 1525, 1389.

Compound **87** Sulfate

Compound **87** sulfate was obtained in 41% yield from **IIId** using H_2SO_4-HCO_2H for deprotection; ^{1}H-NMR (DMSO-d_6+D_2O) δ 1.19 (6H, d, J=6.3 Hz), 2.29 (2H, m), 2.25 (3H, s), 2.99 (2H, t, J=6.9 Hz), 3.10, 3.53 (2H, ABq, J=18 Hz), 4.35 (1H, m), 4.61 (2H, bs), 5.05 (1H, d, J=4.8 Hz), 5.59, 5.75 (2H, ABq, J=13.5 Hz), 5.75 (1H, d, J=4.8 Hz), 7.86 (1H, dd, J=6.0, 8.1 Hz), 8.94 (1H, d, J=8.1 Hz), 9.11 (1H, s), 9.31 (1H, d, J=6.0 Hz); IR (KBr) cm^{-1} 1776, 1671, 1633, 1525, 1488, 1463.

Compound **88** Hydrochloride

Compound **88** hydrochloride was obtained in 31% yield from **IIIe** using $TiCl_4$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 2.40 (2H, m), 2.73 (3H, s), 317 (2H, t, J=8.2 Hz), 3.29 and 3.64 (2H, ABq, J=18.0 Hz), 4.63 (2H, t, J=7.2 Hz), 5.24 (1H, d, J=4.6 Hz), 5.63 and 5.90 (2H, ABq, J=14.2 Hz), 5.82 (2H, d, J=54.2 Hz), 5.86 (1H, d, J=4.6 Hz), 7.87 (1H, dd, J=8.2, 6.2 Hz), 8.79 (1H, d, J=8.2 Hz), 8.85 (1H, d, J=6.2 Hz), 8.87 (1H, s); IR (KBr) cm^{-1} 1774, 1671, 1617, 1525, 1393.

Compound **89** Hydrochloride

Compound **89** hydrochloride was obtained in 26% yield from **IIIf** using $TiCl_4$-anisole for deprotection; ^{1}H-NMR (D_2O) δ 2.43 (2H, m), 2.75 (3H, s), 319 (2H, t, J=8.4 Hz), 3.32 and 3.65 (2H, ABq, J=18.0 Hz), 4.66 (6H, m), 5.26 (1H, d, J=5.1 Hz), 5.65 and 5.92 (2H, ABq, J=14.6 Hz), 5.87 (1H, d, J=5.1 Hz), 7.90 (1H, dd, J=8.4 Hz, 6.3 Hz), 8.81 (1H, d, J=8.4 Hz), 8.87 (1H, d, J=6.3 Hz), 8.89 (1H, s); IR (KBr) cm^{-1} 1774, 1671, 1615, 1526, 1387.

Acknowledgements

The authors are grateful to Prof. J. SHIMADA, St. Marianna Univ. Sch. of Med., and Prof. S. KUWAHARA, Toho Univ., for their informative discussions.

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