STUDIES OF 7β-[2-(AMINOARYL)ACETAMIDO]-CEPHALOSPORIN DERIVATIVES

I. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS IN THE AMINOPYRIDINE SERIES

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(Received for publication December 8, 1983)

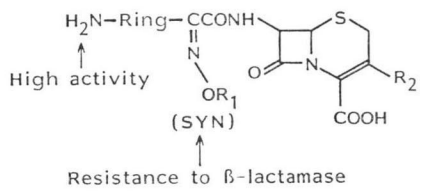
The synthesis and *in vitro* activity of 7β-(2-aminopyridyl-2-alkoxyiminoacetamido)cephalosporins with various substituents at the 3-position are described. The effects of substitution pattern on the pyridine ring, oxime substituent and 3-substituent were studied as a function of the MIC values. Of these various kinds of derivatives, 7β-[2-(2-aminopyridin-6-yl)-2-alkoxyiminoacetamido]cephalosporins exhibited significantly higher activity against most of microorganisms.

In the field of cephalosporins, the antibacterial activity strongly depends on the acyl moiety at the 7-position. Among a number of potent cephalosporins produced during the past twenty years, the aminothiazolyl oxyimino series such as ceftizoxime[1], cefotaxime[2,3], cefmenoxime[4] and ceftriaxone[5] in particular have not only excellent antibacterial properties but also marked resistance to β-lactamases. One of the common structural characteristics of these compounds is the amino function on the thiazole ring, and it would seem that this amino function is important for potent antibacterial activities.

Our effort was concentrated on elucidating the effect of the amino function on the aromatic ring and finding a new acyl moiety superior to the aminothiazole derivatives. The alternation of the heteroaromatic ring possessing an amino function has not been extensively studied in the past.

In a first approach to a new acyl moiety, we assumed that the structure shown in Scheme 1 may be necessary for high antibacterial activity and resistance to β-lactamase. In order to evaluate this concept, we chose pyridine as the ring and designed the synthesis of pyridyl side chain acids bearing the amino function and the oxyiminoacetic acid substituent at the various positions on the ring.

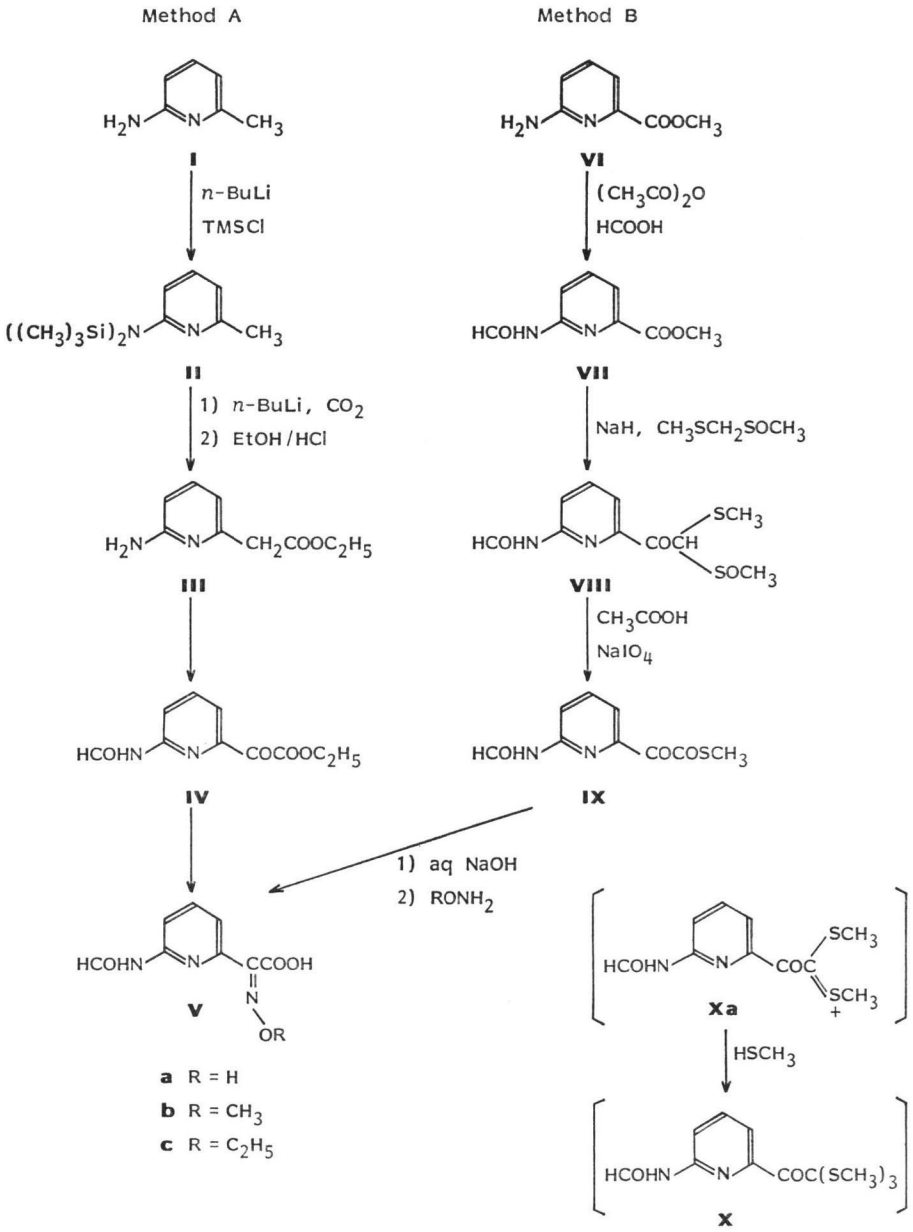
Scheme 1. Prerequisite to new acyl moieties.



We succeeded in synthesizing 2-amino-6-pyridyl-, 2-amino-5-pyridyl-, 2-amino-4-pyridyl- and 4-amino-2-pyridylacetic acids possessing an oxime group in the α-position, all of which were new compounds. The antibacterial activities of their cephalosporin derivatives were examined as a function of the MIC values.

In this paper, we report the preparation of aminopyridyl side chain acids, the new antibiotics and the result of the structure-activity studies[6].

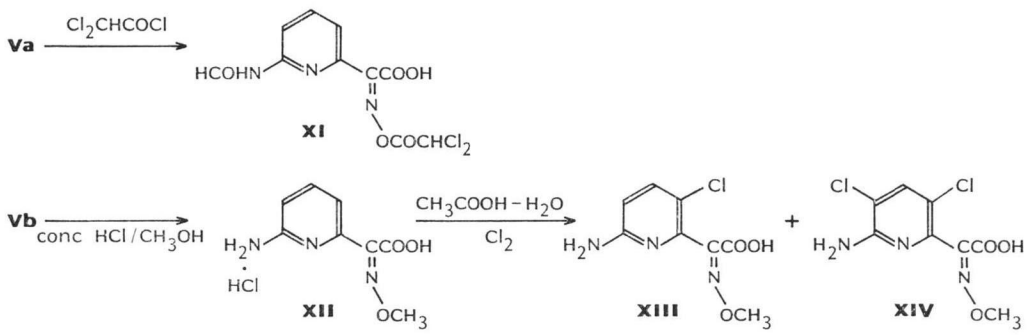
Scheme 2.



Chemistry

The side chain 2-(2-formamidopyridin-6-yl)-2-alkoxyiminoacetic acids were prepared by the two methods outlined in Scheme 2. According to Method A, aminopicoline (**I**) was converted to **III** *via* the disilylated compound (**II**), which was obtained by reaction with *n*-butyllithium and trimethylsilyl chloride (TMSCl). Attempts to carboxylate the *N*-acyl, phthaloyl or monosilylated derivatives of **I** were not successful. **III** was protected by formylation with a mixture of formic acid and acetic anhydride and oxidized with SeO_2 to afford the keto ester (**IV**), which was hydrolyzed with sodium hydroxide in 80% aqueous ethanol and then converted to the desired compound (**V**) by reaction with an appro-

Scheme 3.



priate oxyamine $RONH_2$.

In addition, we developed an improved method which avoids the use of large amounts of *n*-butyllithium for a large scale preparations. According to Method B, methyl 2-formamidopyridyl-6-carboxylate (**VII**), obtained by *N*-protection of **VI**, was transformed to **VIII** by reaction with sodium hydride and methyl methylthiomethyl sulfoxide according to the OGURA method[7]. **VIII** was converted to the corresponding ketothioester (**IX**) by heating with sodium periodate in glacial acetic acid. In this rearrangement, without sodium periodate, a significant amount of **X** was obtained. This product (**X**) might have been generated from the intermediate (**Xa**) through addition of the methanethiol arising from the reaction. To transform the orthothioformate (**X**) into the ketothioester (**IX**), the reagent ($NaIO_4$) was throughly effective, without any unfavored side reaction. However other known oxidizing reagents such as hydrogen peroxide, periodic acid and *m*-chloroperbenzoic acid were not effective. Compound **IX** thus obtained was converted to the desired compound (**V**) by hydrolysis and subsequent reaction with an appropriate oxyamine $RONH_2$.

2-(2-Formamidopyridin-5-yl)-2-methoxyiminoacetic acid, 2-(2-formamidopyridin-4-yl)-2-methoxyiminoacetic acid and 2-(4-formamidopyridin-2-yl)-2-methoxyiminoacetic acid were obtained in a manner similar to that of Method B. However in the case of these compounds, a mixture of acetic anhydride and formic acid (volume ratio: approximately 1: 10) was used to convert **VIII** to the ketothioester (**IX**), because these formyl groups were very easily lost by heating in acetic acid.

The hydroxyiminoacetic acid (**Va**) was converted to the corresponding dichloroacetoxyimino compound (**XI**) by treating with dichloroacetyl chloride in order to protect the hydroxyl group. The dichloroacetoxy group was smoothly removed by treating with aqueous $NaHCO_3$.

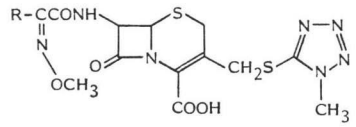
Monochloro and dichloro aminopyridine derivatives were prepared by chlorination of the *N*-deprotected compound (**XII**) of **V** (Scheme 3).

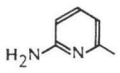
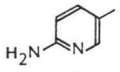
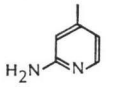
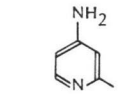
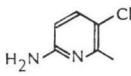
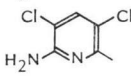
The cephalosporins were prepared either by coupling the 2-formamidopyridyl-2-alkoxyiminoacetic acid with 7β-aminoceph-3-em-4-carboxylic acids possessing various substituents at the 3-position or by displacing the 3-acetoxy group with thiol compounds after acylation of 7β-aminocephalosporanic acid. The acylated cephalosporins were deformylated by treatment with concentrated hydrochloric acid in methanol.

Antibacterial Activity

Table 1 shows the MICs of aminopyridylcephalosporins possessing a methoxyimino moiety at the α-position of the side chain acid. The 3-position of the cephalosporin nucleus is methyltetrazolylthio-

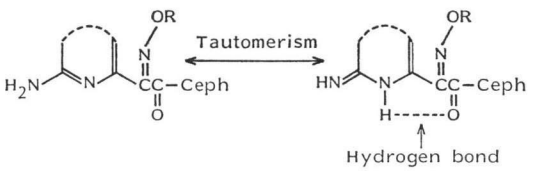
Table 1. Antibacterial activity (MIC μg/ml) of aminopyridyl cephalosporins.



Compound No.	R	*S. aureus* 209p JC-1	*E. coli* NIHJ JC-2	*P. vulgaris* IAM-1025	*P. aeruginosa* NCTC-10490	*P. aeruginosa* IAM-1095	*S. marcescens* 35	*E. cloacae* 60
1		1.56	0.39	0.025	1.56	25	3.13	3.13
2		1.56	3.13	0.39	12.5	400	>100	>100
3		1.56	1.56	3.13	50	>800	>100	50
4		12.5	0.78	0.39	100	800	50	3.13
5		6.25	25	0.78	50	800	>100	100
6		3.13	>100	50	400	>800	>100	>100
	Ceftizoxime	6.25	0.025	0.025	0.39	25	1.56	6.25

methyl group which is typical in this field[4,5]. In the 2-aminopyridine series (compounds **1**, **2** and **3**), the activity of compound **1** is significantly higher than the other two (**2** and **3**) against all organisms except *Staphylococcus aureus*. On the other hand, a shift of the amino function in the 4-aminopyridin-2-yl derivative (**4**) showed considerable activity, but which was still lower than that of **1**, especially against *Pseudomonas aeruginosa*.

Scheme 4. Improved structure of new acyl moieties.



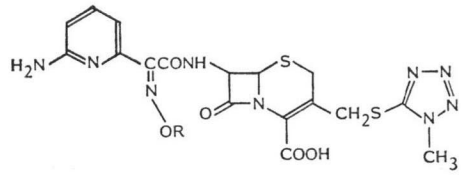
These results suggest that the positions of the amino function and oxyiminoacetic moiety were very important for good antibacterial activities. Therefore we assumed that the partial structure shown in Scheme 4 might be necessary for good activities. Interestingly the partial structure is consist with the aminothiazolyloxyiminocephalosporins.

The successful synthesis of 2-aminopyridin-6-ylcephalosporin derivatives which exhibited potency comparable to that of ceftizoxime prompted us to make further chemical modifications.

The monochlorinated derivative (**5**) had poor activity against Gram-negative bacteria except *Proteus vulgaris* and the dichlorinated compound (**6**) was generally inactive against Gram-negative bacteria but exhibited higher activity than compound **5** against *S. aureus*.

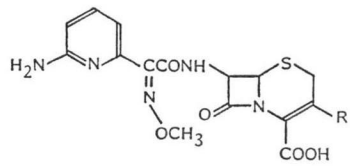
Exchanging the alkoxyimino part led to no significant change of activity except that the hydroxyimino derivative (**7**) improved the activity against *S. aureus* and *E. coli*, but poorer activity against *P.*

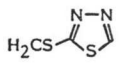
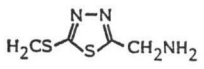
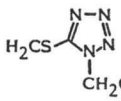
Table 2. Antibacterial activity (MIC μg/ml) of 2-aminopyridin-6-ylcephalosporins.



Compound No.	R	*S. aureus* 209p JC-1	*E. coli* NIHJ JC-2	*P. vulgaris* IAM-1025	*P. aeruginosa* NCTC-10490	*P. aeruginosa* IAM-1095	*S. marcescens* 35	*E. cloacae* 60
7	H	0.39	0.2	0.2	6.25	100	25	6.25
8	C_2H_5	3.13	1.56	0.1	1.56	12.5	12.5	6.25
9	*n*-Pr	1.56	6.25	0.1	1.56	25	12.5	3.13
10	*iso*-Pr	3.13	6.25	0.2	1.56	25	6.25	3.13
11	*n*-Bu	0.78	6.25	0.78	1.56	12.5	12.5	3.13
12	*iso*-Bu	1.56	12.5	0.39	1.56	25	25	12.5
13	$CH_2CH=CH_2$	3.13	3.13	0.05	1.56	12.5	6.25	3.13
14	$CH_2C\equiv CH$	1.56	3.13	0.05	1.56	12.5	12.5	3.13
15	CH_2CF_3	3.13	6.25	0.1	1.56	25	25	6.25

Table 3. Antibacterial activity (MIC μg/ml) of 2-aminopyridin-6-ylcephalosporins.



Compound No.	R	*S. aureus* 209p JC-1	*E. coli* NIHJ JC-2	*P. vulgaris* IAM-1025	*P. aeruginosa* NCTC-10490	*P. aeruginosa* IAM-1095	*S. marcescens* 35	*E. cloacae* 60
16	CH_2OCOCH_3	3.13	0.39	0.025	1.56	25	25	12.5
17	CH_2OCONH_2	3.13	0.78	0.05	3.13	100	50	50
18		1.56	0.78	0.025	1.56	12.5	12.5	3.13
19		1.56	0.78	0.10	6.25	50	12.5	3.13
20		50	0.78	0.025	1.56	25	6.25	12.5
21	H	12.5	0.20	0.025	6.25	400	12.5	12.5
22	CH_3	100	25	1.56	100	>800	>100	>100
23	Cl	3.13	0.78	0.10	25	400	>100	>100

aeruginosa (Table 2).

Table 3 shows the activities of 2-aminopyridin-6-ylcephalosporins with various substituents at the 3-position. Heteroaromatic thiomethyl derivatives exhibited good activities, but none exceeded *N*-

methyltetrazolylthiomethyl compound except a slight improvement against *P. aeruginosa* in the thiadiazolylthiomethyl derivative (**18**).

As a result of our investigations, we have succeeded in finding a new acyl moiety which confers potent antibacterial activities, and have demonstrated experimentally that the partial structure shown in Scheme 4 is associated with potent antibacterial activities.

Experimental

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 260-10 spectrophotometer or Shimadzu IR-420 spectrophotometer. NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spectrometer and at 100 MHz on a Jeol-MH 100 NMR spectrometer using TMS as an internal standard. The following abbreviations are used: s singlet, d doublet, dd double doublet, t triplet, q quartet, m multiplet, ABq AB quartet, bs broad singlet. Organic solvents were dried over anhydrous $MgSO_4$ and all concentrations by evaporation were carried out *in vacuo*.

Determination of *In Vitro* Antibacterial Activity

All the *in vitro* antibacterial activities are given as MIC in μg/ml required to prevent growth of the bacterial culture. MIC's were determined by the agar dilution method using heart infusion agar (Difco) after incubation at 37°C for 20 hours with an inoculum size of about 10^6 cfu/ml.

General Preparation of V

Method A:

1) 2-[*N*,*N*-Bis(trimethylsilyl)amino]-6-methylpyridine (**II**): A 15% *n*-hexane solution (636 g) of *n*-butyllithium was added to a solution of **I** (64.8 g) in tetrahydrofuran (500 ml) at −20 to −30°C over 1 hour, and stirred at −8 to −10°C for 30 minutes. To the solution was added trimethylsilylchloride (161.7 g) at −15 to −5°C over 40 minutes, and the resultant solution was stirred at room temperature overnight. The solution was filtered through by a column packed with silica gel (180 g), washed with tetrahydrofuran and then the filtrate was concentrated. The residue was purified by fractional distillation to give **II** (127.6 g, 84.4%); bp 95~97°C/5~6 mmHg.

2) Ethyl 2-(2-Aminopyridin-6-yl)acetate (**III**): A 15% *n*-hexane solution (338.6 g) of *n*-butyllithium was added dropwise to a solution of **II** (100 g) in anhydrous THF (300 ml) at −20 to −30°C over 1 hour, then the solution was stirred at 20 to 23°C for 1 hour. The resultant solution was added in small portions to crushed dry ice (1 kg) with stirring, and stirring was continued until room temperature was reached. After removing tetrahydrofuran from the solution *in vacuo*, absolute ethanol (1 liter) was added to the residue. An anhydrous 30% ethanol solution (660 ml) of hydrochloric acid was added dropwise to the solution at −5 to −10°C and further hydrogen chloride gas was bubbled at 0 to 5°C for 30 minutes, then the solution was stirred at 10°C overnight. After removing the ethanol, the residue was dissolved in water, and washed with ethyl acetate 3 times. The solution was adjusted to pH 7 to 8 with sodium bicarbonate and extracted with ethyl acetate. The extract was dried and concentrated to give the crude product (54 g, 75.0%). The product was purified by column chromatography on silica gel (1 kg) (eluant; EtOAc - C_6H_6, 3: 1) to give **III** (30.2 g, 41.9%); mp 66~68°C.

Anal Calcd for $C_9H_{12}N_2O_2$: C 59.98, H 6.71, N 15.55.
Found: C 59.77, H 6.50, N 15.21.

3) Ethyl 2-(2-Formamidopyridin-6-yl)glyoxylate (**IV**): Acetic anhydride (16.6 ml) and 98% formic acid (7.32 ml) were mixed at room temperature and stirred at 50 to 60°C for 30 minutes. The solution was added dropwise to a solution of **III** (26.5 g) in ethyl acetate (250 ml) at 20 to 23°C over 30 minutes, and stirred at the same temperature for 1 hour. Cold water was added to the resultant solution and the mixture was shaken thoroughly. The ethyl acetate layer was separated, washed with water, aqueous sodium bicarbonate and water in turn, dried and concentrated to give ethyl 2-(2-formamidopyridin-6-yl)acetate (28 g, 91.5%); mp 35~38°C: IR (Nujol) 3250, 3100, 1738, 1690, 1580, 1460, 1305, 1277 cm^{-1}. To a solution of the above compound (26 g) in dioxane (260 ml) was added selenium

dioxide (16.65 g) in small portions at 85 to 90°C over 1 hour and stirred at the same temperature for 1 hour. After cooling the resultant mixture, the dioxane layer was separated, concentrated and residue was dissolved in ethyl acetate. The solution was washed with water, and treated with activated charcoal and then concentrated to give **IV** (14.3 g, 51.5%); mp 124~126°C; IR (Nujol) 3220, 3100, 1737, 1720, 1690, 1273, 1233 cm^{-1}.

4) 2-(2-Formamidopyridin-6-yl)-2-methoxyiminoacetic Acid (**Vb**, *Z* isomer): 2 N Sodium hydroxide solution in 80% aqueous ethanol (14.9 ml) was added to a solution of **IV** (6.0 g) in ethanol (180 ml) at room temperature and stirred at that temperature for 20 minutes. Methoxyamine hydrochloride (2.7 g) was added to the resultant solution, stirred at room temperature for 1.5 hours and then concentrated to a small volume. The precipitates were collected by filtration, washed with ethyl acetate and water, dissolved in methanol and then treated with activated charcoal. The solution was concentrated and then the precipitates were collected by filtration to give **Vb** (*Z* isomer) (3.6 g, 60.2%), which was recrystallized from EtOAc - Et_2O; mp 170~171°C (dec).

Anal Calcd for $C_8H_9N_3O_4$: C 48.43, H 4.06, N 18.83.
Found: C 48.79, H 4.28, N 18.84.

The configuration of the oxyimino group of **Vb** and the following side chain acids must be *Z* form since in these cephalosporin derivatives, the NMR chemical shift of the amide proton at the C-7 position was observed at very low field as same as that of aminothiazolyl cephems[1,9].

Method B:

1) Methyl 2-Formamido-6-pyridinecarboxylate (**VII**): A mixture of formic acid (559.3 g) and acetic anhydride (1,033.4 g) was stirred for 30 minutes at 40 to 50°C and thereto was added **VI**[10] (616 g) at 40°C, and then the mixture was stirred for 1 hour at 80°C. After the removal of the solvent from the reaction mixture, to the residue was added a mixture of benzene (2 liters) and *n*-hexane (6 liters). The precipitates were collected by filtration and then recrystallized from benzene (2 liters) to give **VII** (647.8 g, 88.8%); mp 134~136°C.

Anal Calcd for $C_8H_8N_2O_3$: C 53.33, H 4.48, N 15.55.
Found: C 53.37, H 4.40, N 15.58.

2) 2-Formamido-6-(2-methanesulfinyl-2-methylthioacetyl)pyridine (**VIII**): To a mixture of **VII** (435.7 g), methyl methylthiomethyl sulfoxide (300 g) and *N*,*N*-dimethylformamide (2.2 liters) was added portionwise 50% oil suspension sodium hydride (348 g) with stirring and ice cooling, and the mixture was stirred for 30 minutes at room temperature. To the reaction mixture was added methylene chloride (4.4 liters) with ice cooling, the precipitates were collected by filtration and then added to a mixture of methylene chloride (3 liters), ice (2 kg) and concentrated hydrochloric acid (730 ml). The mixture was adjusted to pH 7 with sodium bicarbonate and extracted with methylene chloride. The extract was dried, concentrated and crystallized from diethyl ether to give **VIII** (430 g, 65.3%); mp 125~128°C.

Anal Calcd for $C_{10}H_{12}N_2O_3S_2$: C 44.10, H 4.44, N 10.29, S 23.54.
Found: C 44.26, H 4.36, N 10.42, S 23.55.

3) *S*-Methyl 2-(2-Formamidopyridin-6-yl)thioglyoxylate (**IX**): A mixture of **VIII** (424 g) and sodium periodate (100 g) in acetic acid (2.1 liters) was stirred for 30 minutes at 70°C. After the removal of acetic acid, to the residue were added water (5 liters) and sodium thiosulfate (116 g), and then the mixture was adjusted to pH 7 with sodium bicarbonate. The precipitates were collected by filtration, washed with water and then dried to give **IX** (246.4 g, 70.6%), which was recrystallized from EtOAc; mp 170~173°C.

Anal Calcd for $C_9H_8N_2O_3S$: C 48.21, H 3.59, N 12.49, S 14.30.
Found: C 48.28, H 3.62, N 12.59, S 14.54.

In this reaction, the treatment of **VIII** in acetic acid without sodium periodate afforded **IX** in a low yield of 30.3% and 2-formamido-6-(2,2,2-trimethylthioacetyl)pyridine (**X**) in 48.7% yield which was separated by silica gel column chromatography, eluting with C_6H_6 - EtOAc, 4: 1. In addition, **X** was converted into **IX** in 74.8% yield by heating with sodium periodate in acetic acid. Compound **X** was crystallized from diisopropyl ether; mp 93~95°C; IR (Nujol) 1690, 1680, 1595, 1580, 1450, 1380, 1370, 1320, 1290, 1265 cm^{-1}; NMR (DMSO-d_6) δ 2.04 (9H, s), 6.8~7.1 (1H, m, ring proton), 7.4~7.9 (2H, m, ring protons), 10.4~10.7 (1H, m, NH).

Table 4. Yield, mp and IR data of regio-isomers of aminopyridines obtained by Method B.

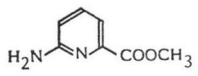
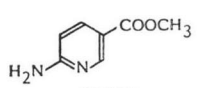
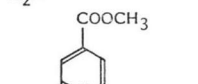
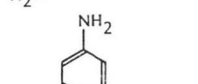
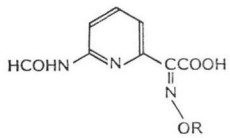
Substrate		VII	VIII	IX	Vb
	Yield (%)	88.1	65.3	70.6	57.4
	mp (°C)	134~136	130~132	163~165	170~171
	IR $\nu_{C=O}^{Nujol}$ (cm^{-1})	1740, 1700	1710, 1690	1700, 1670	1745, 1680
	Yield (%)	90.6	65.0	69.5	34.8
	mp (°C)	218~220	125~127	152~154	159~161
	IR $\nu_{C=O}^{Nujol}$ (cm^{-1})	1720, 1710	1710, 1660	1730, 1680	1735, 1665
	Yield (%)	94.2	69.3	49.0	56.6
	mp (°C)	190~197	123~125	165~167	170~172
	IR $\nu_{C=O}^{Nujol}$ (cm^{-1})	1740, 1710	1700, 1690	1710, 1680	1710, 1640
	Yield (%)	68.7	20.0	65.0	90.0
	mp (°C)	185~186	132~133	145~148	168~170
	IR $\nu_{C=O}^{Nujol}$ (cm^{-1})	1690, 1675	1700, 1680	1690, 1670	1725, 1650

Table 5. Yield, mp and IR data of 2-(2-formamidopyridin-6-yl)-2-alkoxyiminoacetic acids.



R	Yield (%)	mp (°C dec)	IR (Nujol cm^{-1})		
			NH	COOH	CHO
H	48.2	190~192	3120	1700	1665
C_2H_5	48.0	183~184	3220	1760	1680
n-Pr	45.6	155~156	3250	1740	1650
iso-Pr	34.7	140~142	3250	1755	1670
n-Bu	40.5	140~145	3300	1750	1670
iso-Bu	38.5	129~131	3150	1755	1670
$CH_2CH=CH_2$	50.6	153~155	3250	1750	1680
$CH_2C\equiv CH$	55.2	140	3250	1760	1670
CH_2CF_3	38.0	145~150	3250	1755	1685

4) 2-(2-Formamidopyridin-6-yl)-2-ethoxyiminoacetic Acid (**Vc**, *Z* isomer): A mixture of **IX** (4.5 g), methanol (20 ml) and 1 N aqueous sodium hydroxide (20 ml) was stirred for 50 minutes at room temperature to give a solution containing 2-(6-formamidopyridin-2-yl)glyoxylic acid. To the solution was added *O*-ethylhydroxylamine hydrochloride (2.2 g), and the mixture was stirred for 35 minutes at that temperature. The reaction mixture was adjusted to pH 7 with hydrochloric acid and the methanol was distilled off. The remaining aqueous mixture was washed with ethyl acetate, then layered with ethyl acetate and adjusted to pH 1 with 10% hydrochloric acid. The ethyl acetate layer was separated, washed with water, treated with activated charcoal and then concentrated to give **Vc** (*Z* isomer) (2.3 g, 48.0%), which was recrystallized from EtOAc - Et_2O; mp 183~184°C (dec).

Anal Calcd for $C_{10}H_{11}N_3O_4$: C 50.63, H 4.67, N 17.72.
Found: C 50.21, H 4.89, N 17.35.

2-Aminopyridin-5-yl, 2-aminopyridin-4-yl, 4-aminopyridin-2-yl derivatives were obtained respectively from 2-amino-5-pyridinecarboxylate[11], 2-amino-4-pyridinecarboxylate[12], 4-amino-2-pyridinecarboxylate[13] according to Method B. The properties of these compounds are listed in Table 4. 2-(2-Formamidopyridin-6-yl)acetic acids derivatives bearing various oxime substituents which were obtained by the reaction with the corresponding alkoxyamine are listed in Table 5.

2-(2-Formamidopyridin-6-yl)-2-dichloroacetoxyiminoacetic Acid (**XI**, *Z* isomer)

A mixture of **Va** (3.6 g), dichloroacetyl chloride (7.6 g) and methylene chloride (100 ml) was stirred at room temperature for 5 hours. The precipitates were collected by filtration, washed with diethyl ether and dried to give **XI** (4.6 g, 83.6%); mp 88~90°C; IR (Nujol) 1800, 1720, 1620 cm^{-1}.

Preparation of the Monochloro and Dichloro Derivatives (**XIII**, **XIV**, *Z* isomer)

A mixture of **Vb** (*Z* isomer) (5.0 g) and concentrated hydrochloric acid (2.3 ml) in methanol (50 ml) was stirred for 40 minutes at room temperature. After the removal of methanol from the reaction mixture, the residue was pulverized in diethyl ether, collected by filtration to give 2-(2-aminopyridin-6-yl)-2-methoxyiminoacetic acid hydrochloride (**XII**) (*Z* isomer) (5.2 g, ~100%) as a pale brown powder; NMR (DMSO-d_6+D_2O) δ 4.10 (3H, s), 6.84 (1H, d, J=7 Hz), 7.23 (1H, d, J=10 Hz), 7.99 (1H, dd, J=7 Hz, J=10 Hz).

To a mixture of **XII** (8.0 g), acetic acid (350 ml) and water (10 ml) was introduced chlorine gas for 1 hour at 8°C. After the removal of excess chlorine gas by bubbling air into the reaction mixture, the solvent was distilled off. The residue was pulverized in diethyl ether and collected by filtration. After the addition of water and ethyl acetate to the resultant powder (9.8 g), the aqueous layer was separated and washed with ethyl acetate twice and then the water was distilled off. The remaining water in the residue was azeotropically removed with benzene twice to yield a brownish powder, which was dried in a desiccator to give 2-(2-amino-5-chloropyridin-6-yl)-2-methoxyiminoacetic acid (**XIII**) (*Z* isomer) (3.3 g, 41.3%); mp 142~145°C; NMR (DMSO-d_6+D_2O) δ 3.81 (3H, s), 6.50 (1H, d, J=9 Hz), 7.48 (1H, d, J=9 Hz).

The remaining ethyl acetate layer was dried and concentrated and the residue was washed with diethyl ether to give 2-(2-amino-3,5-dichloropyridin-6-yl)-2-methoxyiminoacetic acid (**XIV**, *Z* isomer) (2.4 g, 26.3%); mp 139~144°C; NMR (DMSO-d_6+D_2O) δ 3.96 (3H, s), 7.83 (1H, s).

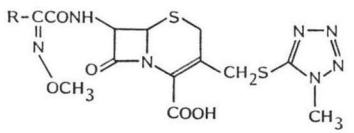
General Procedure for the Acylation of 7β-Aminoceph-3-em-4-carboxylic Acids

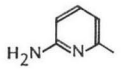
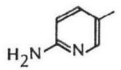
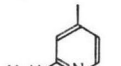
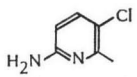
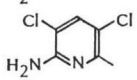
A) Synthesis of Cephalosporins **1**~**18**, **22** (*Z* isomer): (1) Phosphoryl chloride (6.5 mmol) was added to *N*,*N*-dimethylformamide (5 ml) and stirred at 40°C for 30 minutes. To the solution was added a solution of 2-(2-formamidopyridin-6-yl)-2-alkoxyiminoacetic acid (**V**) (5 mmol) in *N*,*N*-dimethylformamide (5 ml) at −15°C, and stirred at −10 to −8°C for 50 minutes [solution A]. Separately, the 7-aminocephem derivative (5 mmol) and trimethylsilylacetamide (7.2 g) were dissolved in methylene chloride (20 ml) at 40°C and cooled. To the cool solution was added the above solution A at −20 to −15°C and stirred at that temperature for 40 minutes. The resultant solution was poured into a solution of saturated aqueous sodium bicarbonate (30 ml) and water (40 ml) with ice cooling. The aqueous layer was separated, washed with ethyl acetate, and then ethyl acetate (50 ml) was added to the aqueous layer and adjusted to pH 3 with 10% hydrochloric acid. The organic layer was separated and extracted with ethyl acetate twice. The extracts were combined, washed with water and concentrated to a small volume. The resulting precipitates were collected by filtration, washed with ethyl acetate and dried, to give the acylated compounds in 40~70% yield.

(2) Conc hydrochloric acid (0.4 ml) was added to a solution of the above product (*Z* isomer, 2 mmol) in methanol (15 ml) and stirred at room temperature for 40 minutes. After the methanol removed, water (100 ml) was added to the residue, which was then dissolved by adding 10% hydrochloric acid. After some insoluble material was filtered off, the filtrate was adjusted to pH 3 with aqueous sodium bicarbonate and then submitted to column chromatography on macroporous, non-ionic adsorption resin (Diaion HP-20), with an eluant of aqueous methanol. The eluate was lyophilized to give the desired cephalosporins (40~65%).

B) Synthesis of Cephalosporins **19**, **20** (*Z* isomer): A solution of 7-[2-(2-formamidopyridin-6-yl)-2-methoxyiminoacetamido]cephalosporanic acid (*Z* isomer) (5 mmol) and disodium 2-(5-mercapto-1*H*-tetrazol-1-yl)acetate (6 mmol) in water (40 ml) was adjusted to pH 7 with sodium bicarbonate, and stirred at 65°C for 6 hours at pH 7 to 7.4. The resultant solution was washed with ethyl acetate, adjusted to pH 2.5 with 10% hydrochloric acid and stirred. The precipitates were collected by filtration, washed with water and diethyl ether to give 7-[2-(2-formamidopyridin-6-yl)-2-methoxyiminoacetamido]-3-[(1-carboxymethyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid (*Z* isomer) (1.3 g, 44.0%);

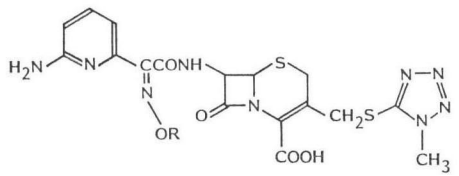
Table 6. IR and ^{1}H NMR data of aminopyridyl cephalosporins.



Compound No.	R	IR (Nujol cm^{-1}) β-Lactam	NMR δ value (DMSO-d_6)							
			N-CH_3 3H, s	O-CH_3 3H, s	CONH 1H, d J=8 Hz	C_7-H 1H, dd J=5, 8 Hz	C_6-H 1H, d J=5 Hz	C_3-H_2 2H, ABq J=13 Hz	C_2-H_2 2H, bs	Ring proton
1		1780	3.75	3.78	9.52	5.85	5.17	4.30 4.37	3.73	6.53 (1H, d, J=8 Hz) 6.93 (1H, d, J=8 Hz) 7.48 (1H, t, J=8 Hz)
2		1780	3.90	3.97	9.73	5.82	5.17	4.35[a]	3.75	6.57 (1H, d, J=9 Hz) 7.67 (1H, dd, J=2, 9 Hz), 8.03 (1H, d, J=2 Hz)
3		1775	3.98	3.97	9.79	5.83	5.18	4.35[a]	3.73	6.67~6.80 (2H, m) 8.00 (1H, d, J=6 Hz)
4		1778	3.77	3.97	9.52	5.80	5.12	4.32[a]	3.64	6.60 (1H, dd, J=2, 7 Hz) 6.97 (1H, d, J=2 Hz) 8.00 (1H, d, J=7 Hz)
5		1790	3.94	4.09	9.70	5.80	5.14	4.21 4.37	3.70	6.97 (1H, d, J=10 Hz) 7.80 (1H, d, J=10 Hz)
6		1785	3.98	3.98	9.43	5.81	5.17	4.35[a]	3.77	7.87 (1H, s)

[a] bs.

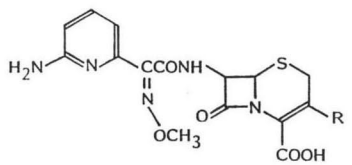
Table 7. IR and ^1H NMR data of cephalosporins possessing various oxime parts.

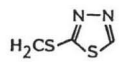
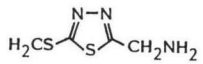
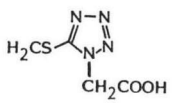


Compound No.	R	IR (Nujol cm^{-1}) β-Lactam	NMR δ value (DMSO-d_6) N-CH_3 3H, s	CONH 1H, d J=8 Hz	C_7-H 1H, dd J=5, 8 Hz	C_6-H 1H, d J=5 Hz	C_3-H_2 2H, ABq J=13 Hz	C_2-H_2 2H, bs	R
7	H	1770	3.95	9.46	5.84	5.13	4.30[a]	3.68	
8	C_2H_5	1780	3.95	9.45	5.85	5.15	4.24 4.38	3.64[b] 3.76	1.28 (3H, t, J=7 Hz) 4.18 (2H, q, J=7 Hz)
9	*n*-Pr	1780	3.93	9.30	5.83	5.13	4.30[a]	3.7	0.9 (3H, t, J=8 Hz) 1.67 (2H, m) 4.07 (2H, t, J=8 Hz)
10	*iso*-Pr	1780	3.97	9.43	5.87	5.17	4.33[a]	3.70	1.27 (6H, d, J=6 Hz) 4.35 (1H, m)
11	*n*-Bu	1775	3.92	9.50	5.78	5.14	4.30[a]	3.70	0.90 (3H, t, J=7 Hz) 1.80~1.16 (4H, m) 4.16 (2H, t, J=7 Hz)
12	*iso*-Bu	1780	3.92	9.44	5.84	5.12	4.24 4.36	3.92	0.88 (6H, d, J=7 Hz) 1.96 (1H, m) 3.88 (2H, d, J=7 Hz)
13	$CH_2CH=CH_2$	1780	3.93	9.47	5.80	5.10	4.33[a]	3.70	4.67 (2H, d, J=5 Hz) 5.17~5.57 (2H, m) 5.83~6.27 (1H, m)
14	$CH_2C\equiv CH$	1765	3.90	9.54	5.80	5.12	4.26 4.34	3.62[b] 3.76	3.48 (1H, t, J=2 Hz) 4.76 (2H, d, J=2 Hz)
15	CH_2CF_3	1780	3.95	9.68	5.83	5.17	4.32[a]	3.72	4.66, 4.92 (2H, ABq, J=9 Hz)

[a] bs, [b] ABq, J=18 Hz.

Table 8. IR and ^1H NMR data of cephalosporins with various substituents at the 3-position.



Compound No.	R	IR (Nujol cm^{-1}) β-Lactam	NMR δ value (DMSO-d_6)						
			O-CH_3 3H, s	CONH 1H, d J=8 Hz	C_7-H 1H, dd J=5, 8 Hz	C_6-H 1H, d J=5 Hz	C_2-H_2 2H, bs	C_3-H_2 2H, ABq J=13 Hz	R
16	CH_2OCOCH_3	1780	3.88	9.4	5.83	5.15	3.5	4.67 5.04	2.00 (3H, s)
17	CH_2OCONH_2	1780	3.88	10.0	5.92	5.15	3.44[a] 3.60	4.62 4.88	
18		1780	3.83	9.57	5.82	5.13	3.67[a] 3.73	4.30 4.55	
19		1770	3.88	9.5	5.75	5.05	3.53	4.35[b]	4.35 (2H, br)
20		1780	3.98	9.50	5.88	5.18	3.67[a] 3.80	4.27 4.50	5.33 (2H, s)
21	H	1785	4.06	10.0	5.85	5.18	3.64		6.52 (1H, t)
22	CH_3	1780	4.11	9.55	5.77	5.18	3.35[a] 3.70		2.07 (3H, s)
23	Cl	1780	4.13	10.07	5.88	5.37	3.57[a] 4.13		

[a] ABq, J=18 Hz, [b] bs.

mp 166~168°C (dec), which was deprotected with methanolic hydrochloric acid in the same manner as General Procedure A (2) to give compound **20**.

Compound **19** was obtained in the same manner as the preparation of **20**.

C) Synthesis of Cephalosporins **21**, **23** (*Z* isomer): A mixture of *N*,*N*-dimethylformamide (3 ml) and phosphoryl chloride (3 mmol) was stirred at 37 to 40°C for 30 minutes. To the solution were added methylene chloride (3 ml) and 2-(2-formamidopyridin-6-yl)-2-methoxyiminoacetic acid (3 mmol) at −20 to −25°C and stirred at −10 to −15°C for 1 hour. A solution of *p*-nitrobenzyl 7-amino-3-cephem-4-carboxylate (3 mmol) and trimethylsilylacetamide (2 g) in methylene chloride (200 ml) was added to the above solution at −10 to −15°C, and then stirred at that temperature for 30 minutes. After the solution was concentrated, ethyl acetate and water were added to the residue. The ethyl acetate layer was separated, washed with water and then concentrated. The residue was triturated with diethyl ether to give *p*-nitrobenzyl 7-[2-(2-formamidopyridin-4-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylate (730 mg, 45.1%); mp 195~200°C (dec); IR (Nujol) 3350, 3200, 1790, 1725, 1690, 1660 cm^{-1}.

10% Palladium on carbon (216 mg) was added to a solution of the above product (1 mmol) in tetrahydrofuran (10 ml), methanol (5 ml), acetic acid (0.075 ml) and water (0.75 ml). The mixture was subjected to catalytic hydrogenation at room temperature under ordinary pressure for 5 hours. After filtering off the catalyst, the filtrate was concentrated. Ethyl acetate and aqueous sodium bicarbonate were added to the residue, and the aqueous layer was separated. The aqueous solution was adjusted to pH 2 with 10% hydrochloric acid. The precipitates were collected by filtration and washed with water to give 7-[2-(2-formamidopyridin-6-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylic acid (300 mg, 71.9%); mp 202~204°C (dec); IR (Nujol) 3250, 3200, 1780, 1720, 1660 cm^{-1}.

A solution of the above compound (5 mmol) and conc hydrochloric acid (1 ml) in methanol (12 ml) was stirred at room temperature for 1 hour. To the resultant solution was added diethyl ether (100 ml), and the precipitates were collected by filtration and dissolved in a mixture of methanol (50 ml) and water (10 ml). The solution was adjusted to pH 3 with aqueous sodium bicarbonate, treated with activated charcoal (1 g) and concentrated to a volume of about 20 ml. The precipitating crystals were collected by filtration, washed with water and dried to give 7-[2-(2-aminopyridin-6-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylic acid (**21**) (1.48 g, 76.5%); mp 215~220°C (dec).

Compound **23** was prepared from *p*-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate in substantially the same manner.

Acknowledgments

The authors express their gratitude to Dr. Y. MORIMOTO and his coworkers for microanalyses and spectra measurements and Dr. M. NISHIDA and his coworkers for the antimicrobial tests.

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