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

II. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS IN THE AMINOPYRIMIDINE SERIES

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The synthesis and the antibacterial activity of 7β-[2-(aminopyrimidinyl)-2-oxyiminoacetamido]cephalosporins with various substituents at the 3-position in the cephem nucleus are described. The 7β-[2-(4-aminopyrimidin-2-yl)-2-methoxyiminoacetamido]cephalosporin derivative (**1**) showed significantly higher activity than the corresponding 2-aminopyrimidin-4-yl derivative (**2**) against Gram-negative bacteria. It was also higher in potency against *Escherichia coli* and *Serratia marcescens* than the aminopyridyl compound (**4**).

During the course of our extensive research on modified cephalosporins, our efforts have been concentrated on synthesizing new cephalosporins with an aminoaromatic ring and an oxyimino group in the acyl moiety in order to elucidate the effect of the amino function on the antibacterial properties. The remarkable properties of 7β-[2-(2-aminopyridin-6-yl)-2-alkoxyiminoacetamido]cephalosporins described in the preceding paper[1] prompted us to synthesize two new types of aminopyrimidyl cephalosporins.

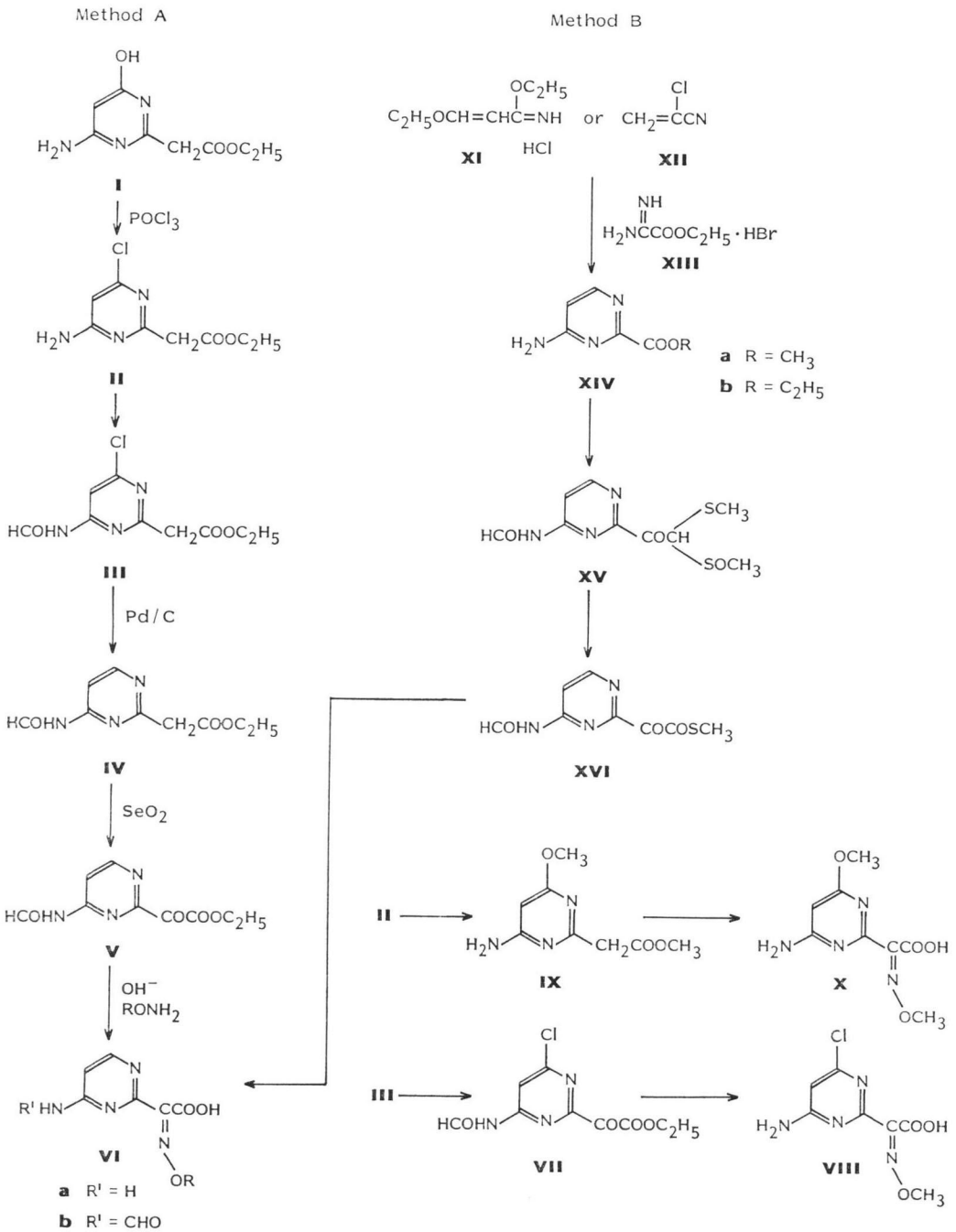
The 2-aminopyrimidin-4-yl derivative (**2**) first synthesized showed relatively weak activity against *Escherichia coli, Proteus vulgaris, Serratia marcescens* and *Enterobacter cloacae*, whereas the 4-aminopyrimidin-2-yl derivative (**1**) exhibited strong activity against these same species. The marked activity against Gram-negative bacteria encouraged us to modify the C-3 substituent and the oxyimino moiety and to introduce certain substituents at the 6-position on the pyrimidine ring.

In this paper we report the preparation of the aminopyrimidyl side chain acids, their conversion to new cephalosporin antibiotics and the result of the structure-activity investigation as a function of the MIC values.

Chemistry

The new compounds **VI**, which are the side chain acids at the C-7 position of the cephem nucleus, were prepared by the two methods outlined in Scheme 1. According to Method A, ethyl 2-(4-amino-6-hydroxypyrimidin-2-yl)acetate (**I**) was converted to *N*-protected compound (**III**) *via* **II**, which was obtained by reaction with phosphoryl chloride. **III** was hydrogenated with 10% palladium on carbon to afford **IV**. The amino acids (**VIa**) possessing an oxyimino group were prepared from the acetate (**IV**) by oxidizing with SeO_2, followed by hydrolysis and then condensation with appropriate oxyamines $RONH_2$. **VIII** was obtained from **III** in a manner similar to that of the preparation of **VIa**. The formyl groups of **V** and **VII** were also hydrolyzed under the conditions of hydrolysis of the ester. Similarly, **X** was prepared from **IX**, which had been obtained by treating **II** with sodium methoxide in methanol.

Scheme 1.



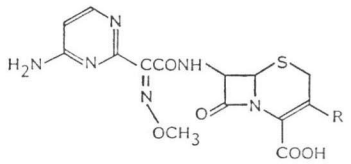
According to Method B, **XIV** was prepared by cyclization of either ethyl 3-ethoxyacrylimidate hydrochloride (**XI**) or 2-chloroacrylonitrile (**XII**) with 1-ethoxycarbonylformamidine hydrobromide (**XIII**). The reaction of **XII** with **XIII** to afford ethyl 4-aminopyrimidin-2-carboxylate (**XIVb**) was carried out in ethanol since, in methanol, a significant amount of by-products was detected by TLC. The resultant ethyl ester (**XIVb**) was converted to the methyl ester (**XIVa**) with a catalytic amount of triethyl-

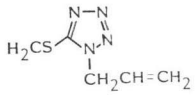
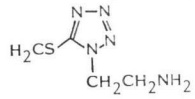
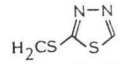
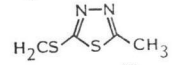
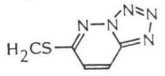
Table 1. Antibacterial activity (MIC μg/ml) of aminoaromatic cephalosporins.

Compound No.	*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	12.5	0.10	0.025	1.56	50	0.78	0.78
2	6.25	1.56	0.20	6.25	200	100	25
3a*	3.13	50	6.25	50	800	>100	>100
3b*	6.25	100	12.5	200	800	>100	>100
4	1.56	0.39	0.025	1.56	25	3.13	3.13

* The C-3 substituent is 1,3,4-thiadiazol-2-yl thiomethyl group.

Table 2. Antibacterial activity (MIC μg/ml) of 4-amino-2-pyrimidylcephalosporins.



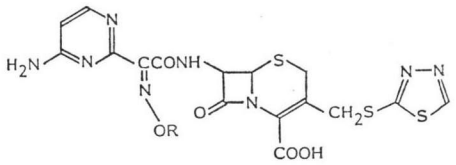
Compound No.	R	*S. aureus* 209p JC-1	*E. coli* NIHJ JC-2	*P. vulgaris* IAM-1025	*P. aeruginosa* NCTC-10490	*P. aeruginosa* IAM-1095	*S. marcescens* 35	*E. cloacae* 60
5	H	>100	0.10	0.10	6.25	200	3.13	3.13
6	CH_2OCOCH_3	25	0.20	0.20	1.56	100	50	12.5
7	CH_2OCONH_2	25	0.10	0.20	1.56	100	>100	25
8		12.5	0.05	0.025	6.25	50	3.13	1.56
9		12.5	0.025	0.025	1.56	25	0.78	0.10
10		12.5	0.05	0.025	1.56	25	3.13	3.13
11		6.25	0.10	0.025	6.25	100	12.5	6.25
12		6.25	0.39	0.025	6.25	50	12.5	0.78

amine in methanol in quantitative yield in order to facilitate the reaction with methyl methylthiomethyl sulfoxide. **XIVa** was converted to **VIb** in a similar manner to the preparation of the 2-(aminopyridyl)-2-alkoxyiminoacetic acids[1].

The condensation of the keto acid obtained from **XVI** with an appropriate oxyamine $RONH_2$ gave the *Z* isomer of **VIb** by conducting the reaction in an aqueous solution at pH 3 ~ 4 and room temperature, but it was very difficult to determine the configuration of the oxyimino group of **VIb** by comparison of NMR chemical shifts with a minor product which was the geometric isomer. However after acylation of the 7β-aminocephems, it was possible to show by the NMR chemical shift of the amide proton at the C-7 position that **VIb** was free from contamination of its *E* isomer[8].

All carboxylic acids (**VIb**) were activated by the Vilsmeier reagent prepared from *N*,*N*-dimethyl-

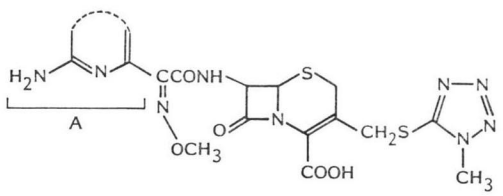
Table 3. Antibacterial activity (MIC μg/ml) of 4-amino-2-pyrimidylcephalosporins.

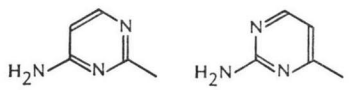


Compound No.	R	*S. aureus* 209p JC-1	*E. coli* NIHJ JC-2	*P. vulgaris* IAM-1025	*P. aeruginosa* NCTC-10490	*P. aeruginosa* IAM-1095	*S. marcescens* 35	*E. cloacae* 60
13	C_2H_5	6.25	0.10	0.025	1.56	12.5	12.5	3.13
14	*n*-Pr	3.13	0.78	0.025	1.56	12.5	6.25	1.56
15	$CH_2CH=CH_2$	6.25	0.20	0.05	1.56	25	6.25	1.56
16	CH_2Ph	0.78	1.56	0.05	1.56	50	25	3.13
17		0.78	0.78	0.20	1.56	6.25	12.5	1.56

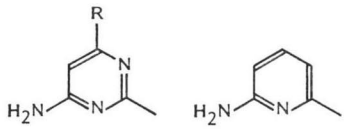
formamide and phosphoryl chloride. Amino acids **VIa** and **VIII** were stirred with phosphoryl chloride at 0 to 5°C in methylene chloride in order to protect the amino group, and then *N*,*N*-dimethylformamide was added to the resultant mixture. These activated acids, without an isolation, were coupled with 7β-aminoceph-3-em-4-carboxylic acids possessing various substituents at the C-3 position. The removal of the *N*-protecting group was carried out by treating with a solution of conc hydrochloric acid and methanol to afford the new antibiotics.

Scheme 2.





A:



a R = Cl

b R = OCH_3

Antibacterial Activity

The following structure-activity relationships are derived from Tables 1, 2 and 3. The 4-aminopyrimidin-2-yl compound (**1**) showed stronger activity than compounds **2**, **3a**, **3b** and **4** against Gram-negative bacteria. The differences between compounds **1** and **2** in their antibacterial activities might be caused by the basicity of the amino function. Thus in the partial structure A (Scheme 2), the second ring nitrogen should be introduced on the oxyimino side rather than on the amino side for maximum activity.

The introduction of a 6-chloro or 6-methoxy substituent into the pyrimidine ring, (**3a**, **3b**), resulted in a significant decrease in activity against *S. marcescens* and *E. cloacae*. A similar effect was noted with the introduction of a chloro function into the aminopyridine ring[1].

The modification of the 3-position, especially in the cases of the heteroaromatic thiomethyl compounds (**8**, **9**, **10**, **11** and **12**) showed generally parallel activities with the parent compound (**1**) except for

a slight improvement with the aminoethyltetrazolyl compound (**9**) and the thiadiazolyl compound (**10**) against *Pseudomonas aeruginosa* strain IAM-1095. The aminoethyltetrazolyl thiomethyl group may be a very interested 3-substituent but for disadvantages arising from metabolic products since compound **9** showed the best overall antibacterial spectrum. All derivatives exhibited weak activities against *Staphylococcus aureus*.

The activity against *S. aureus* was improved by modification of the oxyimino moiety, with the benzyloxyimino and phenoxyimino compounds (**16, 17**) exhibited relatively strong activities (5 times as active as the compound **1**). At the same time, the phenoxyimino derivative (**17**) had increased activity against *P. aeruginosa* IAM-1095, but its activity against *S. marcescens* decreased.

Our further studies will be presented in subsequent papers.

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 10^8 cfu/ml.

General Preparation of VI

Method A:

1) Ethyl 2-(4-Amino-6-chloropyrimidin-2-yl)acetate (**II**): A mixture of ethyl 2-(4-amino-6-hydroxypyrimidin-2-yl)acetate (**I**)[4] (15.8 g) and phosphoryl chloride (75 ml) was stirred for 4 hours with heating at 80 to 90°C. After the phosphoryl chloride was distilled off, the remaining oily substance was poured into a mixture of ice-water (200 ml) and ethyl acetate (200 ml). The resultant mixture was neutralized with aqueous ammonia and extracted with ethyl acetate. The extract was washed with water and concentrated. The resultant residue was washed with diisopropyl ether to give **II** as pale brown crystals (8.1 g, 47.0%); mp 127~128°C; IR (Nujol) 3400~3250, 1700, 1650, 1580~1520, 1320, 1210~1160, 860, 840 cm^{-1}.

2) Ethyl 2-(6-Chloro-4-formamidopyrimidin-2-yl)acetate (**III**): To the suspension of **II** (135 g) in ethyl acetate (3 liters) was added a mixture of formic acid (130 g) and acetic anhydride (288 g) which had been stirred for 0.5 hour at room temperature. The mixture was heated for 8 hours at 80°C and then evaporated to dryness. The residue was dissolved in ethyl acetate, washed with water and evaporated. The oily product (123.5 g) was purified by column chromatography on silica gel (650 g) using a mixture of benzene and ethyl acetate (2: 1) as the eluant to give oily **III** (88 g, 57.7%); IR (film) 3600~2800, 1730~1680, 1560, 1190~1140, 1020 cm^{-1}; NMR ($CDCl_3$) δ 1.30 (3H, t, J=8 Hz), 3.92 (2H, s), 4.23 (2H, q, J=8 Hz), 8.3~9.3 (1H, br), 9.4~10.4 (2H, br).

3) Ethyl 2-(4-Formamidopyrimidin-2-yl)acetate (**IV**): To a solution of **III** (2.3 g) and sodium acetate (0.9 g) in 80% aqueous ethanol (50 ml) was added 10% palladium on carbon (0.2 g), and the mixture was stirred under a hydrogen atmosphere for 8 hours at room temperature. The reaction mixture was filtered and the filtrate was concentrated. The residue (2.2 g) was purified by column chromatography on silica gel (40 g) using a mixture of benzene and ethyl acetate (1: 1) as the eluant to give a pale brown solid (1.3 g, 66.1%); IR (Nujol) 1710, 1670, 1530, 1310, 1170, 840 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (3H, t, J=8 Hz), 3.78 (2H, s), 4.33 (2H, q, J=8 Hz), 6.5~8.3 (1H, br), 8.37 (1H, d, J=5 Hz),

9.15 (1H, bs), 9.45 (1H, bs).

4) Ethyl 2-(4-Formamidopyrimidin-2-yl)glyoxylate (**V**): A mixture of **IV** (2.9 g), selenium dioxide (1.7 g) in dimethyl sulfoxide (30 ml) was stirred with heating at 50 to 52°C for 1 hour and at 70 to 72°C for 0.5 hour. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated to a volume of about 5 ml and the residue was poured into water (50 ml) and an insoluble material was filtered off. The filtrate was adjusted to pH 7 with aqueous sodium bicarbonate and saturated with sodium chloride and then extracted with a mixture of ethyl acetate and ethanol (2: 1). The extract was concentrated to give **V** as a deep yellow oil (2.4 g, 76.8%). The product was used in the subsequent step without further purification.

5) 2-(4-Aminopyrimidin-2-yl)-2-methoxyiminoacetic Acid (**VIa**, R=CH_3, *Z* isomer): To a solution of **V** (2.4 g) in ethanol (30 ml) was added dropwise a 1 N ethanol solution of potassium hydroxide (11 ml) with ice cooling and stirring, and then stirring was continued for 2 hours at room temperature. The reaction mixture was concentrated to a volume of about 15 ml and to the residue was added diethyl ether (20 ml). The precipitates of potassium 2-(4-aminopyrimidin-2-yl)glyoxylate (1.2 g) were collected by filtration and added to a methanolic solution of *O*-methylhydroxylamine hydrochloride (0.50 g) with stirring at room temperature. The reaction was allowed to stand overnight, then filtered. The filtrate was concentrated and the residue was pulverized in acetone (15 ml) to give **VIa** (R=CH_3) as a pale brown powder, which was recrystallized from water. (*Z* isomer, 290 mg, 13.8%); mp 138~143°C; IR (Nujol) 3400~3100, 2900~2500, 1660~1540, 1250, 1040~990 cm^{-1}; NMR (D_2O+$NaHCO_3$) δ 4.05 (3H, s), 6.63 (1H, d, J=6 Hz), 8.13 (1H, d, J=6 Hz).

Anal Calcd for $C_7H_8N_4O_3 \cdot 2H_2O$: C 36.21, H 3.47, N 24.13.
Found: C 36.41, H 3.45, N 24.25.

As in a preceding paper[1], the configuration of the oxyimino group of **VIa** and the following side chain acids must be *Z* form, since in these cephem derivatives, the resonance of the amide protons at very low field (9.40~9.60 ppm in DMSO-d_6).

Ethyl (6-Chloro-4-formamidopyrimidin-2-yl)glyoxylate (**VII**) from **III**

A mixture of **III** (24.3 g) and selenium dioxide (16.6 g) in *N*,*N*-dimethylformamide (243 ml) was stirred for 1 hour at 70 to 75°C. The precipitated solid was filtered off, and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (500 ml), washed with water and saturated aqueous sodium chloride and evaporated to dryness. The residue was triturated with diisopropyl ether to give **VII** as a powder (17.7 g, 68.9%). This product (1 g) was recrystallized from ethyl acetate (10 ml) to afford the purified product (570 mg); mp 114~117°C; IR (Nujol) 3400, 3230~3100, 1760, 1720~1680, 1580, 1550, 1250, 1200, 850, 730 cm^{-1}.

2-(6-Chloro-4-aminopyrimidin-2-yl)-2-methoxyiminoacetic acid (**VIII**, *Z* isomer) was prepared from **VII** according to Method A-5); mp 165~167°C; IR (Nujol) 3350, 3200, 1740~1695, 1660, 1365, 1030 cm^{-1}; NMR (DMSO-d_6+D_2O) δ 3.80 (3H, s), 6.77 (1H, s).

Methyl 2-(4-Amino-6-methoxypyrimidin-2-yl)acetate (**IX**)

To a solution of **II** (21.5 g) in methanol (200 ml) was added a solution of sodium metal (7.3 g) in methanol (130 ml) and the mixture was refluxed for 3.5 hours. The reaction mixture was cooled in an ice-salt bath and saturated with dry hydrogen chloride gas and then allowed to stand overnight at room temperature. The mixture was evaporated to dryness and the residue was dissolved in a mixture of ethyl acetate and cold aqueous sodium bicarbonate. The organic layer was evaporated to give **IX** (14.2 g, 56.0%); mp 91~94°C; IR (Nujol) 3480, 3390, 3210, 1738, 1660, 1600 cm^{-1}; NMR (DMSO-d_6) δ 3.66 (5H, s), 3.82 (3H, s), 5.68 (1H, s), 6.66 (2H, bs).

The following compounds were obtained in a similar way to that described above.

2-(4-Amino-6-methoxypyrimidin-2-yl)-2-methoxyiminoacetic acid (*Z* isomer, **X**) from **IX**; mp 127~129°C; IR (Nujol) 3420, 3380, 1650, 1615, 1590, 1250, 1050, 1025 cm^{-1}; NMR (DMSO-d_6+D_2O) δ 3.78 (3H, s), 3.95 (3H, s), 5.78 (1H, s).

2-(2-Formamidopyrimidin-4-yl)-2-methoxyiminoacetic acid from methyl 2-(2-aminopyrimidin-4-yl)-acetate[5]; mp 180~182°C (dec); IR (Nujol) 3300, 1750, 1670, 1590, 1573, 1408 cm^{-1}; NMR (DMSO-d_6) δ 4.00 (3H, s), 7.47 (1H, d, J=5 Hz), 8.60 (1H, d, J=5 Hz).

Method B:

1) Methyl 4-Aminopyrimidin-2-carboxylate (**XIVa**): a) To a solution of ethyl 3-ethoxyacrylimidate hydrochloride (**XI**)[6] (4.0 g) and 1-ethoxycarbonylformamidine hydrobromide (**XIII**)[7] (4.4 g) in methanol (110 ml) was added dropwise a solution of sodium metal (1.0 g) in methanol (110 ml) at 0°C. The reaction mixture was stirred for 1 hour at 0 to 5°C and for an additional 4 hours at room temperature. The solution was evaporated to dryness and the residue was dissolved in a mixture of ethyl acetate and saturated aqueous sodium chloride. The organic layer was separated and the aqueous layer was extracted with ethyl acetate five times. All organic layers were combined and evaporated. The residue was triturated with diethyl ether to give **XIVa** (1.3 g, 38.9%), which was recrystallized from ethyl acetate; mp 140~142.5°C; IR (Nujol) 3450, 3300, 3180, 1730, 1630, 1585, 1540 cm^{-1}; NMR (DMSO-d_6) δ 3.81 (3H, s), 6.54 (1H, d, J=6 Hz), 7.23 (2H, s), 8.16 (1H, d, J=6 Hz).

Anal Calcd for $C_6H_7N_3O_2$: C 47.06, H 4.61, N 27.44.
Found: C 47.41, H 4.83, N 27.08.

b) To a solution of 2-chloroacrylonitrile (**XII**) (437 mg) and **XIII** (985 mg) in ethanol (5 ml) was added dropwise triethylamine (1.01 g) at 0°C. The reaction mixture was stirred for 4 hours at room temperature and evaporated to dryness. The residue was dissolved in a mixture of ethyl acetate and water, and extracted with ethyl acetate three times. The combined extracts were dried and evaporated to dryness. The residue was triturated with diethyl ether to give ethyl 4-aminopyrimidin-2-carboxylate (**XIVb**) (470 mg, 56.3%), which was recrystallized from a mixture of ethyl acetate and benzene (1: 2); mp 101~104°C; IR (Nujol) 3450, 3300, 3180, 1730, 1630, 1580, 1540 cm^{-1}; NMR (DMSO-d_6) δ 1.30 (3H, t, J=7 Hz), 4.30 (2H, q, J=7 Hz), 6.60 (1H, d, J=6 Hz), 7.31 (2H, s), 8.20 (1H, d, J=6 Hz). **XIVb** was refluxed with a catalytic amount of triethylamine in methanol to give methyl ester (**XIVa**), which was identified by comparison of the spectra data with **XIVa** prepared by Method B-1a).

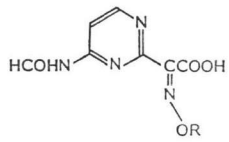
2) 4-Formamido-2-(2-methanesulfinyl-2-methylthioacetyl)pyrimidine (**XV**): A mixture of formic acid (100 g) and acetic anhydride (204 g) was stirred for 0.5 hour at room temperature. To the solution was added **XIVa** (30 g) and the mixture was stirred for 1.5 hours at 70 to 75°C and then evaporated to dryness. The residue was triturated with ethanol, collected by filtration and washed with ethanol to give methyl 4-formamidopyrimidin-2-carboxylate (20.0 g, 56.3%); mp 234~236°C.

To a solution of the above compound (1.3 g) and methyl methylthiomethyl sulfoxide (0.9 g) in *N*, *N*-dimethylformamide (10 ml) was added 50% oil suspension sodium hydride (1.0 g) at 10°C with stirring and the stirring was continued for 1.5 hours at room temperature. The mixture was cooled in an ice bath and thereto was added methylene chloride (30 ml). The precipitates which were collected by filtration were added portionwise to a mixture of methylene chloride (50 ml), ice water (50 ml) and concentrated hydrochloric acid (2.1 ml) with stirring. The methylene chloride layer was separated and the aqueous layer was extracted with methylene chloride. The combined extracts were dried and evaporated to dryness. The residue was triturated with diethyl ether to give **XV** (1.2 g, 61.0%); IR (Nujol) 1690, 1560, 1450, 1370 cm^{-1}.

3) *S*-Methyl 2-(4-Formamidopyrimidin-2-yl)thioglyoxylate (**XVI**): A mixture of formic acid (4.8 g) and acetic anhydride (9.7 g) was stirred for 0.5 hour at room temperature. To the solution was added **XV** (2.6 g) and the mixture was stirred for 1.5 hours at 50°C and then for 1 hour with an addition of sodium periodate (610 mg) at the same temperature. The mixture was evaporated to dryness and the residue was dissolved in a mixture of ethyl acetate (50 ml) and saturated aqueous sodium chloride (20 ml). The organic layer was separated and the aqueous layer was extracted with ethyl acetate three times. The combined organic layers were evaporated to dryness. The residue (2.0 g) was subjected to column chromatography over silica gel (13 g) using a mixture of ethyl acetate and benzene (1: 1 by volume) as an eluant. The fractions containing the desired compound were collected, evaporated to dryness and crystallized from a small amount of ethyl acetate to give pure **XVI** (840 mg, 39.2%); mp 112~114°C; IR (Nujol) 3480, 3380, 1715, 1680, 1585 cm^{-1}; NMR (DMSO-d_6) δ 2.17 (3H, s), 7.20 (1H, bs), 8.12 (1H, d, J=6 Hz), 9.17 (1H, bs), 11.08 (1H, d, J=7 Hz).

4) 2-Ethoxyimino-2-(4-formamidopyrimidin-2-yl)acetic Acid (**VIb**, R=C_2H_5): To a suspension of **XVI** (3.0 g) in water (26 ml) was added dropwise 1 N aqueous sodium hydroxide (12 ml) at room temperature and the mixture was stirred for 0.5 hour at the same temperature. To the solution was added

Table 4. Yield, mp, IR and ^{1}H NMR data of **VIb**.



R	Yield[a] (%)	mp (°C dec)	IR (Nujol cm^{-1})			NMR δ value (DMSO-d_6)	
			NH	COOH	CHO	R	Ring proton[b] 2H
CH_3	62.5	165~166	3250	1740	1700	4.00 (3H, s)	7.4~7.8 (1H, m) 8.72 (1H, d, J=6 Hz)
C_2H_5	70.0	130~135	3250	1720	1710	1.28 (3H, t, J=7 Hz) 4.32 (2H, q, J=7 Hz)	7.4~7.7 (1H, m) 8.72 (1H, d, J=6 Hz)
n-Pr	71.6	145~148	3150	1750	1690	0.97 (3H, t, J=7 Hz) 1.4~2.0 (2H, m) 4.23 (2H, t, J=7 Hz)	7.4~7.7 (1H, m) 8.70 (1H, d, J=6 Hz)
$CH_2CH=CH_2$	64.0	120~122	3250	1710	1710	4.8 (2H, d, J=5 Hz) 5.1~5.6 (2H, m) 5.7~6.4 (1H, m)	7.3~7.8 (1H, m) 8.73 (1H, d, J=6 Hz)
CH_2Ph	72.5	75~ 77	3250	1720	1700	5.33 (2H, s) 7.40 (5H, s)	7.3~7.6 (1H, m) 8.73 (1H, d, J=6 Hz)
Ph	25.8	131~133	3150	1740	1680	7.1~7.9 (5H, m)	7.5~7.8 (1H, m) 8.87 (1H, d, J=6 Hz)

[a] Yield from **XVI** according to Method B.

[b] The chemical shifts of C_5-H on the pyrimidine ring were not apparent because of coupling with the proton of the formamido group.

an aqueous solution of ethoxyamine prepared by ethoxyamine hydrochloride (1.3 g), water (10 ml) and sodium bicarbonate (1.1 g). The reaction mixture was stirred for 0.5 hour at room temperature and adjusted to pH 4 with 1 N hydrochloric acid (1.5 ml). The solution was stirred for 10 minutes at room temperature and adjusted to pH 3 with 1 N hydrochloric acid and then washed with ethyl acetate. The aqueous layer was saturated with sodium chloride, adjusted to pH 1 with 10% hydrochloric acid and extracted with ethyl acetate. The extract was evaporated to dryness. The crystalline residue was washed with *n*-hexane to give the title compound (*Z* isomer, 2.2 g, 70.0%); mp 130~135°C (dec).

Anal Calcd for $C_9H_{10}N_4O_4$: C 45.38, H 4.23, N 23.52.
Found: C 45.67, H 4.05, N 23.24.

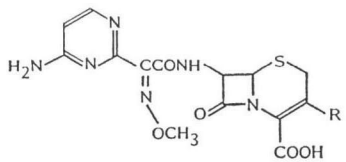
Various **VIb** derivatives, synthesized according to Method B and bearing different oxyimino groups, are listed in Table 4.

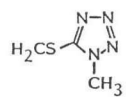
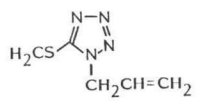
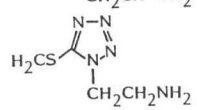
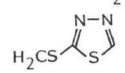
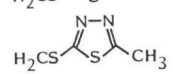
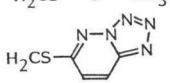
General Procedure for the Acylation of 7β-Aminoceph-3-em-4-carboxylic Acids with a Carboxylic Acid; Compounds **1** and **5~17**

A mixture of *N*,*N*-dimethylformamide (12 ml) and phosphoryl chloride (12 mmol) was stirred for 30 minutes at room temperature. To the mixture were added methylene chloride (12 ml) and 2-(4-formamidopyrimidin-2-yl)-2-methoxyiminoacetic acid (*Z* isomer) (10 mmol) at −5 to 0°C, and then the reaction mixture was stirred for 1 hour at that temperature. Meanwhile, a mixture of the 7-aminoceph-3-em-4-carboxylic acid (10 mmol) and trimethylsilylacetamide (12 g) in methylene chloride (120 ml) was warmed to make a clear solution. The solution was cooled to −10°C and added to the activated acid solution obtained above. The reaction mixture was stirred for 40 minutes at 0°C, and then poured into cold aqueous sodium bicarbonate. The aqueous layer was separated, adjusted to pH 2 with 10% hydrochloric acid and extracted with ethyl acetate. The extract was dried and evaporated to dryness. The residue was triturated with diethyl ether to give the cephalosporin derivative in 50~70% yield.

A solution of the formamidopyrimidyl cephem (8 mmol) obtained above and concentrated hydrochloric acid (0.73 ml) in methanol (80 ml) was stirred for 1.5 hours at room temperature. The solvent was evaporated to dryness and the residue was dissolved in water (100 ml). The aqueous solution was

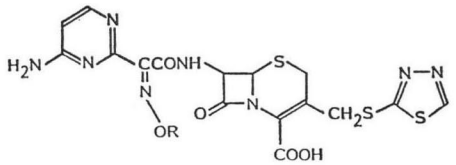
Table 5. IR and ^{1}H NMR data of cephalosporins with various substituents at the 3-position.



Compound No.	R	IR (Nujol cm^{-1}) β-Lactam	NMR δ value (DMSO-d_6)							
			R			N-OCH_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	Pyrimidine ring proton, each of 2H, d, J=7 Hz
			C_3-H_2, 2H, ABq, J=13 Hz	C_2-H_2 2H, bs	Other proton					
1		1780	4.32[a]	3.6	3.94 (3H, s)[b]	3.94[b]	9.43	5.80	5.11	6.44 8.10
5	H	1780		3.6	6.6 (1H, m)	4.00	9.47	5.91	5.13	6.50 8.17
6	CH_2OCOCH_3	1780	4.7 5.0	3.36[c] 3.62	2.03 (3H, s)	3.93	9.40	5.77	5.10	6.43 8.10
7	CH_2OCONH_2	1775	4.62 4.90	3.38[c] 3.61		3.94	9.41	5.80	5.11	6.44 8.10
8		1780	4.23 4.48	3.7	4.8~5.4 (4H, m) 4.7~6.2 (1H, m)	3.95	9.45	5.80	5.10	6.45 8.10
9		1770	4.3~ 4.7[b]	3.6	3.1~3.8 (2H, m) 4.3~4.7 (2H, m)[b]	3.99	9.45	5.82	5.12	6.61 8.25
10		1780	4.28 4.65	3.73	9.63 (1H, s)	3.95	9.47	5.87	5.18	6.48 8.15
11		1780	4.21 4.58	3.7	2.70 (3H, s)	3.97	9.47	5.81	5.22	6.47 8.12
12		1770	4.23 4.60	3.63[c] 3.77	7.75 (1H, d, J=10 Hz) 8.60 (1H, d, J=10 Hz)	3.93	9.43	5.85	5.12	6.45 8.12

[a] bs, [b] the signals overlapped each other, [c] ABq, J=18 Hz.

Table 6. IR and ^{1}H NMR data of cephalosporins possessing various oxyimino groups.



Compound No.	R	IR (Nujol cm^{-1}) β-Lactam	NMR δ value (DMSO-d_6)							
			R	C_3-ring proton 1H, 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	Pyrimidine ring proton, each of 2H, d, J=7 Hz
13	C_2H_5	1780	1.27 (3H, t, J=7 Hz) 4.22 (2H, q, J=7 Hz)	9.57	9.37	5.87	5.17	4.33 4.58	3.72	6.45 8.12
14	*n*-Pr	1780	0.90 (3H, t, J=7 Hz) 1.6 (2H, m) 4.08 (2H, t, J=7 Hz)	9.52	9.36	5.80	5.12	4.26 4.54	3.85[a] 3.74	6.40 8.06
15	$CH_2CH=CH_2$	1780	4.68 (2H, d, J=5 Hz) 5.3 (2H, m) 6.0 (1H, m)	9.57	9.43	5.85	5.13	4.30 4.57	3.70	6.42 8.10
16	CH_2Ph	1780	5.28 (2H, s) 7.40 (5H, s)	9.60	9.55	5.87	5.15	4.33 4.57	3.6	6.47 8.13
17	Ph	1780	7.1~7.9 (5H, m)	9.56	9.48	5.86	5.18	4.30 4.54	3.7	6.45 8.10

[a] ABq, J=18 Hz.

washed with ethyl acetate and adjusted to pH 3 with aqueous sodium bicarbonate and then subjected to column chromatography over a nonionic adsorption resin (Diaion HP-20). The column was washed with water and eluted with 50% aqueous methanol. The eluate containing the desired compound was evaporated to remove methanol and then lyophilized to give the new cephalosporins (presented in Tables 5 and 6).

Compound **9**, which was prepared from 7β-amino-3-[1-(2-*t*-butoxycarbonylaminoethyl)-1*H*-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid, was prepared by deprotection of the *t*-butoxycarbonyl group with formic acid prior to the removal of *N*-formyl group.

7-[2-(2-Aminopyrimidin-4-yl)-2-methoxyiminoacetamido]-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (**2**, *Z* isomer); Mp 181~182.5°C (dec); IR (Nujol) 3440, 3320, 1790, 1693, 1660, 1630, 1525, 1043 cm^{-1}; NMR (DMSO-d_6) δ 3.68 (2H, bs), 3.90 (3H, s), 3.93 (3H, s), 4.25, 4.33 (2H, ABq, J=14 Hz), 5.12 (1H, d, J=5 Hz), 5.82 (1H, dd, J=5, 8 Hz), 6.85 (1H, d, J=5 Hz), 8.27 (1H, d, J=5 Hz), 9.50 (1H, d, J=8 Hz).

The following compounds were obtained by coupled with the corresponding amino acids (**VIII**, **X**), which were activated by stirred with 3 equivalent phosphoryl chloride in methylene chloride for 30 minutes at 0 to 5°C and followed by addition of 3.5 equivalent *N*,*N*-dimethylformamide.

7-[2-(4-Amino-6-chloropyrimidin-2-yl)-2-methoxyiminoacetamido]-3-(1,3,4-thiadiazol-2-yl)thiomethyl-3-cephem-4-carboxylic acid (**3a**, *Z* isomer); mp 173~178°C (dec); IR (Nujol) 3400, 3280, 1780, 1680, 1630, 1575, 1530, 1380, 1040, 900, 800 cm^{-1}; NMR (DMSO-d_6) δ 3.72 (2H, bs), 4.00 (3H, s), 4.28, 4.63 (2H, ABq, J=13 Hz), 5.17 (1H, d, J=4.5 Hz), 5.85 (1H, dd, J=4.5, 8.0 Hz), 6.50 (1H, s), 7.4 (2H, bs), 9.50 (1H, d, J=8.0 Hz), 9.58 (1H, s).

7-[2-(4-Amino-6-methoxypyrimidin-2-yl)-2-methoxyiminoacetamido]-3-(1,3,4-thiadiazol-2-yl)-thiomethyl-3-cephem-4-carboxylic acid (**3b**, *Z* isomer); mp 161~163°C; IR (Nujol) 3400, 3250, 1780, 1675, 1620, 1580, 1380, 1040 cm^{-1}; NMR (DMSO-d_6) δ 3.73 (2H, bs), 3.83 (3H, s), 3.97 (3H, s), 4.27, 4.63 (2H, ABq, J=13 Hz), 5.17 (1H, d, J=4.5 Hz), 5.73 (1H, s), 5.87 (1H, dd, J=4.5, 8 Hz), 6.77 (2H, bs), 9.45 (1H, m), 9.57 (1H, s).

Acknowledgment

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