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

III. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS IN THE AMINOTHIADIAZOLE SERIES

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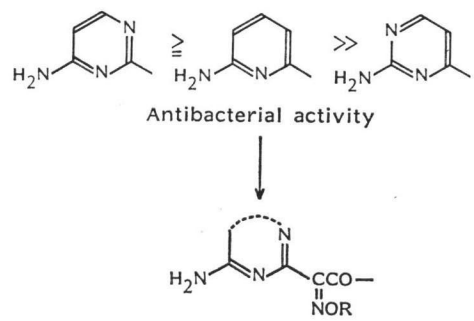
The synthesis and *in vitro* antibacterial activity of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]cephalosporins with various substituents at the 3-position in the cephem nucleus are described. Aminothiadiazolyl cephalosporins having pyridiniomethyl groups at the 3-position exhibited excellent activity against all organisms, particularly against *Pseudomonas aeruginosa*.

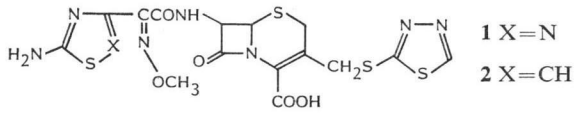
In preceding papers[1,2], we reported the studies of the antibacterial activity of aminopyridyl and aminopyrimidyl cephalosporin derivatives which suggested an attractive partial structure for high activities (Scheme 1). We have now applied that partial structure to a five-membered ring, that is, 5-amino-1,2,4-thiadiazolyl derivatives[3,4]. The aminothiadiazolyl cephalosporin (**1**) first synthesized was compared with the corresponding aminothiazolyl cephalosporin (**2**) (Table 1). The higher antibacterial activity of **1** against *Pseudomonas aeruginosa* and *Enterobacter cloacae* encouraged further extensive research on aminothiadiazolyl cephalosporins.

Scheme 1. A partial structure for high activity.



In this paper, we report the preparation of 2-(5-amino-1, 2, 4-thiadiazol-3-yl)-2-oxyiminoacetic acids and the result of our structure-activity studies of their cephalosporin derivatives as a function of the minimum inhibitory concentration (MIC) values.

Table 1. Comparative activity (MIC μg/ml) of aminothiadiazolyl and aminothiazolyl 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	3.13	0.05	0.025	1.56	12.5	1.56	3.13
2	0.78	0.05	0.025	1.56	25	1.56	6.25

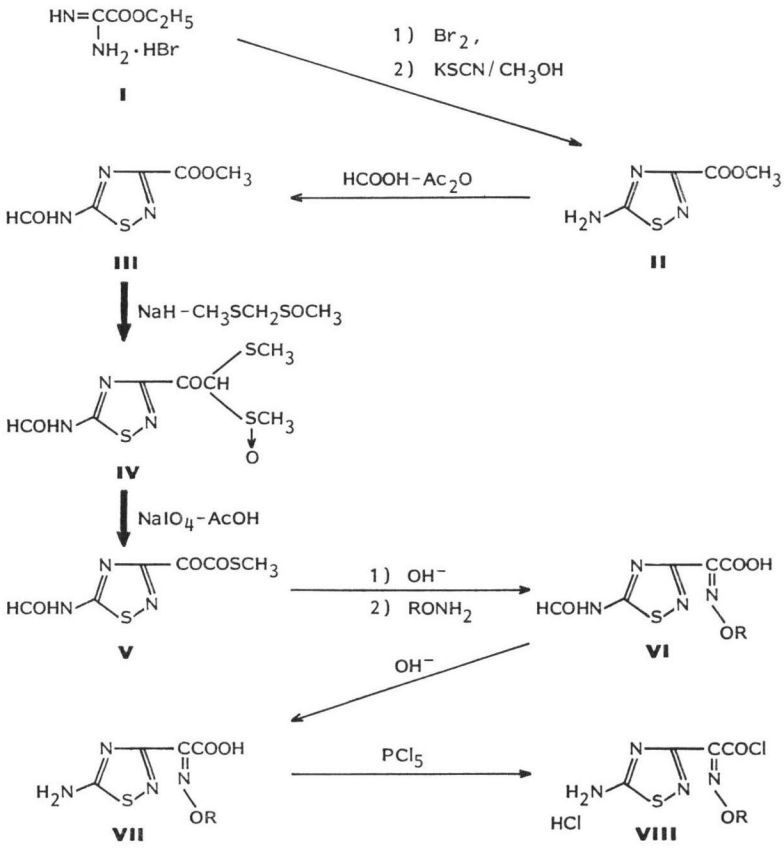
Chemistry

Most of the 2-aminothiadiazolyl-2-oxyiminoacetic acids were prepared by the route shown in Scheme 2, which was similar to that described in the previous papers[1,2]. Methyl 5-formamido-1,2,4-thiadiazole-3-carboxylate, which was prepared by cyclization of the amidino ester (**I**) by a modification of GOERDELER's general procedure[5] and then formylation, was condensed with formaldehyde dimethyl dithioacetal *S*-oxide, and the acyl adduct (**IV**) was rearranged to the ketothioester (**V**) by heating in a mixture of sodium periodate and acetic acid. The thioester (**V**) was hydrolyzed, then reacted with an alkoxyamine to give the corresponding alkoxyiminoacetic acid (**VI**) (*Z* isomer).

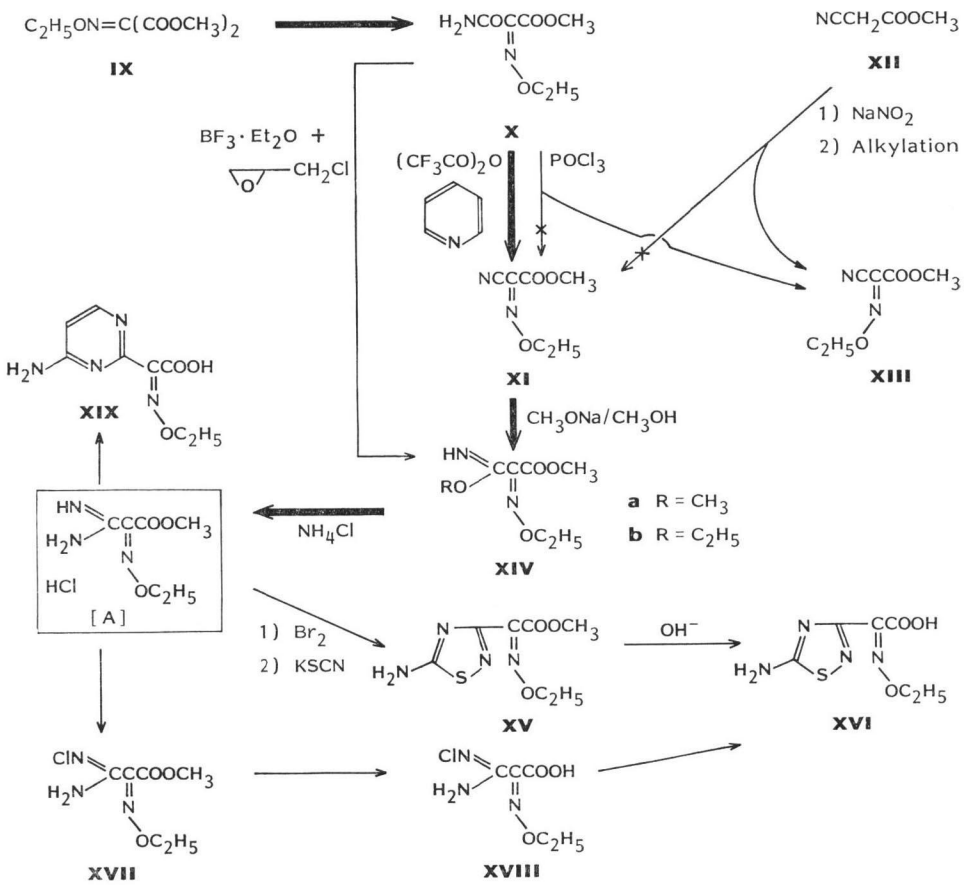
In the case of pyridine and pyrimidine derivatives, the formyl group, which was the protecting group of the amino function, was easily deprotected with dilute acid after being coupled with 7β-aminoceph-3-em-4-carboxylic acids. In contrast, the formyl group on the aminothiadiazole ring was resistant to mild acidic and basic hydrolysis. Therefore the removal of the formyl group was carried out under alkaline conditions prior to coupling with 7β-aminoceph-3-em-4-carboxylic acids. Aminothiadiazolylacetic acids (**VII**) were successfully activated with phosphorous pentachloride to give the acid chlorides (**VIII**).

In addition, we developed another synthetic route, shown as Method B in Scheme 3. The amidation of dimethyl ethoxyiminomalonate (**IX**) with aqueous ammonia gave only the *Z* isomer of the amide

Scheme 2. A useful procedure for 2-substituted oxyimino-2-(5-amino-1,2,4-thiadiazol-3-yl)acetic acid (*Z* isomer); Method A.



Scheme 3. Key intermediate [A]; Method B.



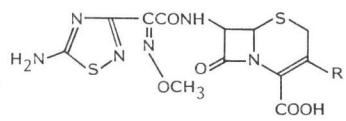
(**X**) in 58.7% yield. **X** was dehydrated with trifluoroacetic anhydride and pyridine in methylene chloride with cooling to give the *Z* isomer of methylethoxyiminocyanoacetate (**XI**) in 88.9% yield. Conversely the dehydration of **X** with phosphoryl chloride gave only the *E* isomer (**XIII**), which could also be obtained by reaction of ethyl cyanoacetate (**XII**) with sodium nitrite and subsequent alkylation. The *Z* isomer (**XI**) was then converted to the imidate (**XIV**) with a catalytic amount of sodium methoxide in methanol. Similar treatment of the *E* isomer (**XIII**) did not give the corresponding imidate. **XIV** was then converted to the key intermediate [A], a valuable intermediate in the preparation of hetero-aromatic *Z*-oxyiminoacetic acids. Additionally, a one step conversion of amide (**X**) to imidate (**XIV**) was successful by using Meerwein reagent. The cyclization of the amidine [A] with bromine followed by potassium thiocyanate gave the desired ester (**XV**) in an nearly quantitative yield. The ester was hydrolyzed to the acid (**XVI**) in 1 N aqueous sodium hydroxide. The *N*-brominated compound of [A] was too unstable to be isolated and purified, but both the *N*-chlorinated ester (**XVII**) and the carboxylic acid (**XVIII**) were stable enough to be recrystallized from water. **XVIII** afforded the desired acid (**XVI**) by reaction with potassium thiocyanate in the presence of excess triethylamine in methanol in 77.2% yield.

Furthermore the amidine [A] could be converted to 2-ethoxyimino-2-(4-aminopyrimidin-2-yl)-acetic acid (**XIX**) by ring closure with 2-chloroacrylonitrile and subsequent hydrolysis of the ester.

Antibacterial Activity

During the course of our evaluation of the structure-activity relationships of the 3-substituents on the cephem nucleus, we found that pyridiniomethyl derivatives have excellent activity against all organisms, especially against *Pseudomonas aeruginosa* (Table 2). Therefore, the effects of variation of the oxime moiety in the pyridiniomethyl derivatives were studied. The results are shown in the Table 3. Most of these derivatives showed remarkable activity against all microorganisms. Especially compound **12** with an ethoxyimino substituent showed the best balanced spectrum of activity. Both the hydroxyimino cephem **11** and the cyclopentyloxyimino cephem **18** displayed higher activity than the other against *Staphylococcus aureus*. The former compound showed a decrease of activity against Gram-negative bacteria. However the latter showed high inhibitory potency against these same species. It has been reported[8] that the introduction of acidic substituents at the C-3 or C-7 position of the cephem nucleus resulted in a significant decrease the anti-staphylococcal activity. Nevertheless the compound **20** with a carboxymethyloxyimino substituent preserved good activity against *S. aureus*. In addition, we studied the effects of the replacement of the pyridinium group with other heteroaromatic rings. Most of the resulting onium derivatives exhibited good activity against all organisms (Table 4). In particular, the pyridazine and pyrazole derivatives (compounds **21** and **24**) exhibited activity comparable to the

Table 2. Antibacterial activity (MIC μg/ml) of aminothiadiazolyl cephalosporins.



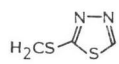
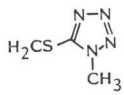
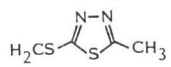
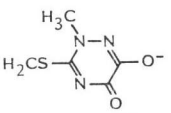
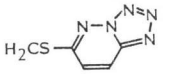
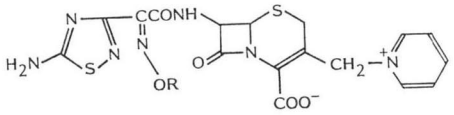
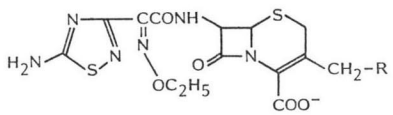
Compound No.	R	*S. aureus* 209p JC-1	*E. coli* NIHJ JC-2	*P. vulgaris* IAM-1025	*P. aeruginosa* NCTC-10490	*P. aeruginosa* IAM-1095	*S. marcescens* 35	*E. cloacae* 60
1		3.13	0.05	0.025	1.56	12.5	1.56	3.13
3	H	25	0.05	0.20	1.56	12.5	3.13	6.25
4	CH_2OCOCH_3	12.5	0.05	0.10	1.56	12.5	6.25	25
5	CH_2OCONH_2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

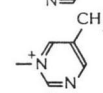
Table 3. Antibacterial activity (MIC μg/ml) of aminothiadiazolyl cephalosporins.



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
10	CH_3	1.56	0.20	0.39	0.78	0.78	1.56	0.10
11	H	0.39	1.56	3.13	0.78	0.78	25	1.56
12	C_2H_5	1.56	0.05	0.10	0.39	1.56	0.78	0.10
13	$CH(CH_3)_2$	1.56	0.10	0.20	0.78	3.13	1.56	0.39
14	$CH_2CH=CH_2$	0.78	0.10	0.20	0.39	1.56	3.13	0.20
15	$CH_2C\equiv CH$	1.56	0.10	0.39	0.78	0.78	1.56	0.39
16	CH_2CF_3	0.78	0.10	0.10	0.78	3.13	1.56	0.39
17	CH_2SCH_3	0.78	0.10	0.20	0.39	1.56	6.25	0.39
18		0.39	0.39	0.10	1.56	1.56	6.25	0.78
19		0.78	0.20	0.10	1.56	1.56	3.13	1.56
20	CH_2COOH	3.13	0.39	0.39	1.56	1.56	0.39	0.39

Table 4. Antibacterial activity (MIC μg/ml) of aminothiadiazolyl 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
21		0.78	0.10	0.10	0.78	1.56	0.78	0.39
22		3.13	1.56	3.13	12.5	200	25	3.13
23		3.13	0.20	0.39	1.56	6.25	6.25	0.78
24		0.78	0.20	0.20	0.78	1.56	1.56	0.39
25		0.78	0.10	0.20	3.13	3.13	3.13	0.78
26		1.56	0.05	0.10	0.78	1.56	0.78	0.39
27		1.56	0.20	0.20	1.56	3.13	6.25	0.39
28		3.13	0.20	0.39	1.56	3.13	6.25	0.39
29		1.56	0.20	0.39	0.78	3.13	0.78	0.78

pyridinium derivative, with improved activity against *S. aureus*. The pyrimidine derivative (compound **22**) showed significantly poor activity against Gram-negative organisms.

In summary, we have reported the antibacterial activity against a variety of organisms of 7β-[2-(aminoaryl)acetamido]cephalosporin derivatives containing the aminopyridyl, aminopyrimidyl and aminothiadiazolyl ring systems. Of these three classes, the aminothiadiazolyl cephalosporins showed not only marked antibacterial activities which were comparable to that of the aminothiazolyl cephalosporins, but also specially high inhibitory potency against *P. aeruginosa*.

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 spectrophotometer 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*. Column chromatography was carried out on Merck silica gel 60 (70~230 mesh ASTM), Rf values on TLC were measured on Merck silica gel F_{254} (Type 60).

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 VII

Method A:

1) Methyl 5-Amino-1,2,4-thiadiazole-3-carboxylate (**II**): To a solution of 1-ethoxycarbonylformamidine hydrobromide (**I**)[7] (16.6 g) in absolute methanol (340 ml) was added dropwise bromine (12.8 g) at −5°C. To the mixture were added triethylamine (21.2 g) and then potassium thiocyanate (8.1 g) in absolute methanol (100 ml) at 0°C. The reaction mixture was stirred for 1 hour at 0°C and for an additional 3 hours at room temperature. The resulting precipitates were collected by filtration and washed with methanol to give the title compound (10.0 g, 70.6%); mp 202~205°C; IR (Nujol) 3400, 3250, 3100, 1710, 1610, 1540 cm^{-1}; NMR (DMSO-d_6) δ 3.85 (3H, s), 8.25 (2H, s).

Anal Calcd for $C_4H_5N_3O_2S$: C 30.19, H 3.17, N 26.40, S 20.14.
Found: C 30.15, H 3.05, N 26.41, S 20.14.

2) Methyl 5-Formamido-1,2,4-thiadiazole-3-carboxylate (**III**): To a mixture of formic acid (33 g) and acetic anhydride (22 g) was added **II** (6.2 g), and then the mixture was stirred for 2 days at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was triturated with a mixture of diethyl ether (50 ml) and *n*-hexane (50 ml) to give the title compound (7.2 g, 98.7%); mp 210~215°C; IR (Nujol) 3100, 1720, 1680 cm^{-1}; NMR (DMSO-d_6) δ 3.90 (3H, s), 8.85 (1H, s).

Anal Calcd for $C_5H_5N_3O_3S$: C 32.09, H 2.67, N 22.46, S 25.67.
Found: C 32.08, H 2.67, N 22.38, S 25.60.

3) 5-Formamido-3-(2-methanesulfiny-2-methylthioacetyl)-1,2,4-thiadiazole (**IV**): To a suspension of **III** (9.2 g) and 50% oil suspension sodium hydride (7.1 g) in *N*,*N*-dimethylformamide (100 ml) was added dropwise formaldehyde dimethyl dithioacetal *S*-oxide (6.1 g) at 40 to 50°C. The mixture was stirred for 1 hour at room temperature and methylene chloride (300 ml) was added to the reaction mixture. The resulting precipitates were collected by filtration and washed with methylene chloride and then added to a stirred mixture of conc hydrochloric acid (14.7 ml) and ice-water (200 ml). An insoluble product was collected by filtration and washed with water to give the title compound (10.7 g, 78.2%), which was recrystallized from a mixture of dioxane and ethyl acetate (1: 1); mp 158~160°C; IR (Nujol)

3100, 1700, 1680 cm^{-1}; NMR (DMSO-d_6) δ 2.22, 2.28 (3H, 2s), 2.68, 2.85 (2H, 2s), 5.70, 5.80 (1H, 2s), 8.86 (1H, s).

Anal Calcd for $C_7H_9N_3O_3S_3$: C 30.21, H 2.90, N 15.10, S 34.56.
Found: C 30.20, H 2.88, N 15.04, S 34.27.

4) *S*-Methyl (5-Formamido-1,2,4-thiadiazol-3-yl)thioglyoxylate (**V**): A mixture of **IV** (0.85 g) and sodium periodate (0.2 g) in glacial acetic acid (10 ml) was stirred for 45 minutes at 70°C. The reaction mixture was evaporated and the residue was dissolved in a mixture of ethyl acetate and water. The mixture was adjusted to pH 7 with an aqueous solution of sodium bicarbonate and treated with 10% aqueous solution of sodium thiosulfate (10 ml). The organic layer was separated, dried and evaporated to dryness. The residue was triturated with *n*-hexane to give the title compound (280 mg, 39.6%), which was recrystallized from ethyl acetate to give the analytically pure material as pale yellow prisms; mp 195~197°C; IR (Nujol) 3100, 1715, 1695, 1650 cm^{-1}; NMR (DMSO-d_6) δ 2.55 (3H, s), 8.95 (1H, s).

Anal Calcd for $C_6H_5N_3O_3S_2$: C 31.17, H 2.19, N 18.17, S 27.73.
Found: C 31.15, H 2.13, N 18.06, S 27.68.

5) 2-Ethoxyimino-2-(5-formamido-1,2,4-thiadiazol-3-yl)acetic Acid (**VI**, R=C_2H_5, *Z* isomer) from **IV**: A mixture of **IV** (10.0 g) and sodium periodate (2.0 g) in glacial acetic acid (50 ml) was stirred for 50 minutes at 70°C. The solvent was evaporated and the residue was washed with *n*-hexane. To the residue was added 1 N aqueous sodium hydroxide (160 ml) and stirred for 1 hour at room temperature. To the reaction mixture was added ethoxyamine hydrochloride (3.5 g) and adjusted to pH 3 to 4 with 10% hydrochloric acid and then stirred for 1 hour at room temperature. After an insoluble material was filtered off, the filtrate was washed with ethyl acetate, adjusted to pH 1 with 10% hydrochloric acid and extracted with ethyl acetate. The extract was dried and evaporated to dryness. The residue was triturated with diisopropyl ether to give 2-ethoxyimino-2-(5-formamido-1,2,4-thiadiazol-3-yl)acetic acid (*Z* isomer) (4.5 g, 51.2%); mp 165~168°C (dec); IR (Nujol) 3350, 1730, 1690, 1595, 1565 cm^{-1}; NMR (DMSO-d_6) δ 1.30 (3H, t, J=7 Hz), 4.30 (2H, q, J=7 Hz), 8.87 (1H, s).

6) 2-Ethoxyimino-2-(5-amino-1,2,4-thiadiazol-3-yl)acetic Acid (**VII**, R=C_2H_5, *Z* isomer): A mixture of **VI** (4.4 g) and 1 N aqueous sodium hydroxide (54 ml) was stirred for 2 hours at 50 to 55°C. The mixture was cooled in an ice bath, adjusted to pH 1 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 title compound (2.9 g, 75.1%), which was recrystallized from *N*,*N*-dimethylacetamide and washed with methylene chloride; mp 168~170°C (dec).

Anal Calcd for $C_6H_6N_4O_3S$: C 33.33, H 3.73, N 25.91, S 14.83.
Found: C 33.31, H 3.72, N 25.72, S 14.69.

The *E* isomer of a higher Rf value of **VII** was isolated as pale brown crystals from the mother liquid on standing for two weeks at room temperature; mp 145~148°C; IR (Nujol) 3450, 3370, 1725 cm^{-1}; NMR (DMSO-d_6) δ 7.98 (2H, bs, NH_2), 4.18 (2H, q, J=7 Hz, $-CH_2-$), 1.22 (3H, t, J=7 Hz, $-CH_3$).

The configuration of these isomers was finally established by coupled with 7β-aminocephem-4-carboxylic acids, that is, in the *E* isomer of the cephalosporin derivative (**12**), the NMR chemical shift of the amide proton at C-7 position was obserbed at 9.10 ppm in DMSO-d_6 higher than the corresponding *Z* isomer (9.55 ppm). In addition its antibacterial activity was significantly decreased.

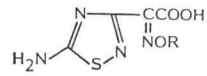
The properties of various alkoxyimino derivatives are summarized in Table 5.

Method B:

1) Dimethyl Ethoxyiminomalonate (**IX**): To a mixture of dimethyl isonitrosomalonate[8] (12.3 g) and diethyl sulfate (14.3 g) in *N*,*N*-dimethylformamide (12 ml) was added dropwise with stirring triethylamine (9.4 g) at 30 to 40°C. Stirring was continued for 1.5 hours at the same temperature. The mixture was diluted with methylene chloride (45 ml) and water (30 ml), and then the organic layer was separated, washed with 5% aqueous potassium carbonate and water, dried and evaporated to give an oily residue (11.5 g, 68.4%). The residue was distilled under reduced pressure (5 mmHg) to give **IX** (5.5 g); bp 95~105°C (5 mmHg); IR (film) 3000, 2970, 1755, 1730, 1610 cm^{-1}; NMR ($CDCl_3$) δ 1.30 (3H, t, J=7 Hz), 3.83 (6H, s), 4.32 (2H, q, J=7 Hz).

2) Methyl 2-Carbamoyl-2-ethoxyiminoacetate (*Z* isomer, **X**): A mixture of **IX** (57.4 g) and conc

Table 5. Mp, IR and ^{1}H NMR data of **VII**.



R	mp (°C dec)	IR (Nujol cm^{-1})		NMR δ value (DMSO-d_6)	
		NH_2	COOH	R	NH_2 2H, bs
CH_3	180~182	3450, 3150	1715	3.90 (3H, s)	8.10
C_2H_5	168~170	3450, 3370	1665	1.22 (3H, t, J=7 Hz), 4.17 (2H, q, J=7 Hz)	8.17
$CH(CH_3)_2$	152~155	3450, 3300	1730	1.22 (6H, d, J=6 Hz), 4.1~4.6 (1H, m)	8.20
$CH_2CH=CH_2$	93~ 95	3430, 3100	1710	4.22 (2H, d, J=6 Hz), 5.1~5.5 (2H, m), 5.7~6.3 (1H, m)	8.17
$CH_2C\equiv CH$	155~157	3500, 3310	1745	3.53 (1H, t, J=2 Hz), 4.87 (2H, d, J=2 Hz)	8.23
CH_2CF_3	140~143	3450, 3350	1745	4.72, 4.95 (2H, ABq, J=9 Hz)	8.25
CH_2SCH_3	140~143	3500, 3300, 3150	1740	2.22 (3H, s), 5.33 (2H, s)	8.20
	160~165	3470	1715	1.17~2.10 (8H, m), 4.60~4.97 (1H, m)	8.22
	150	3300	1710	1.80~2.50 (4H, m), 5.30~5.50 (1H, m), 5.83~6.30 (2H, m)	8.20
CPh_3	173~174	3450	1735	7.35 (15H, s)	8.22
CH_2COO*t*-Bu	150~155	3420, 3230, 3100	1725	1.45 (9H, s), 4.70 (2H, s)	8.12

ammonium hydroxide (50 ml) in methanol (150 ml) was stirred for 2.5 hours at room temperature. The mixture was adjusted to pH 4 with 10% hydrochloric acid with cooling and concentrated to 70 ml under reduced pressure. The aqueous solution was stored in a refrigerator for 5 hours and the resulting precipitates were collected by filtration, washed with cold water and dried to give **X** (Z isomer) (31.0 g, 58.7%), which was recrystallized from diethyl ether to afford colorless prisms; mp 73~75°C; IR (Nujol) 3450, 3300, 3200, 1740, 1680, 1660, 1600 cm^{-1}; NMR (DMSO-d_6) δ 1.28 (3H, t, J=7 Hz), 3.83 (3H, s), 4.28 (2H, q, J=7 Hz), 7.70 (2H, bs).

Anal Calcd for $C_6H_{10}N_2O_4$: C 41.38, H 5.75, N 16.09.
Found: C 41.55, H 5.53, N 16.06.

The configuration of the ethoxyimino moiety was finally determined by its conversion as follows into **VII** (R=C_2H_5), which had been obtained by Method A.

3) Methyl 2-Cyano-2-ethoxyiminoacetate (Z isomer, **XI**): To a solution of **X** (Z isomer) (5.2 g) in pyridine (50 ml) was added dropwise with stirring trifluoroacetic anhydride (15.7 g), keeping the temperature below 25°C with ice cooling, stirring was continued for 30 minutes, then the reaction mixture was poured into water (200 ml), followed by addition of diisopropyl ether (200 ml). The mixture was adjusted to pH 2 with 6 N hydrochloric acid (80 ml) at 15 to 20°C and the organic layer was separated, washed with water, dried and evaporated to give **XI** (Z isomer) (4.2 g, 88.9%) as an oil, which was purified by fractional distillation; bp 97~98°C(12 mmHg); IR (film) 2250, 1760, 1557, 1440, 1270, 1050 cm^{-1}.

Anal Calcd for $C_6H_8N_2O_3$: C 46.15, H 5.13, N 17.95.
Found: C 46.25, H 4.97, N 18.05.

4a) Methyl 3-Imino-3-methoxy-2-ethoxyiminopropionate (*Z* isomer, **XIVa**): To a solution of **XI** (*Z* isomer) (4.7 g) in methanol (50 ml) was added a 1 N methanolic solution (5 ml) of sodium methoxide with cooling in an ice-bath. The mixture was kept for 15 minutes at room temperature, then was neutralized to the end point of phenolphthalein with a 1 N methanolic solution of acetic acid, then evaporated. The residue was dissolved in methylene chloride (80 ml), washed with water, dried and evaporated to give **XIVa** (*Z* isomer) (4.8 g. 83.3%) as an oil; IR (film) 3340, 1755, 1665, 1645, 1610 cm^{-1}; NMR ($CDCl_3$) δ 1.32 (3H, t, J=7 Hz), 3.84 (3H, s), 3.87 (3H, s), 4.28 (2H, q, J=7 Hz), 8.25 (1H, bs).

4b) Methyl 3-Imino-3-ethoxy-2-ethoxyiminopropionate (*Z* isomer, **XIVb**) from **X**: To a solution of Meerwein reagent (triethyloxonium tetrafluoroborate) [prepared from boron fluoride etherate (2.8 g) by a method of Org. Syn., 46: 113, 1966] in methylene chloride (30 ml) was added **X** (*Z* isomer, 2.6 g) and the mixture was stirred for 18 hours at room temperature. The reaction mixture was cooled in an ice-bath and triethylamine (3.0 g) was added thereto, followed by addition of water (20 ml). The organic layer was separated, dried and evaporated to give crude **XIVb** (*Z* isomer) (5.0 g) as an oil, which was used in subsequent steps without further purification.

5) Methyl 2-Amidino-2-ethoxyiminoacetate Hydrochloride (*Z* isomer, [A]): A solution of crude **XIVa** (*Z* isomer) (5.0 g) and ammonium chloride (802 mg) in methanol (25 ml) was stirred for 6 hours at room temperature and evaporated to dryness to give a residue. The residue was crystallized from isopropanol to give [A] (*Z* isomer) (1.75 g, 31.4%), which was purified by recrystallization from methanol to give colorless prisms; mp 174~175°C (dec); IR (Nujol) 2600, 2490, 1740, 1680, 1595 cm^{-1}; NMR (DMSO-d_6) δ 1.33 (3H, t, J=7 Hz), 3.90 (3H, s), 4.45 (2H, q, J=7 Hz).

Anal Calcd for $C_6H_{11}N_3O_3 \cdot HCl$: C 32.80, H 5.47, N 23.69, Cl 16.91.
Found: C 32.75, H 5.46, N 23.61, Cl 16.90.

XIVb also gave the amidino ester [A] in a manner similar to that described above in 45% yield from **X**.

6) The Treatment of **X** with Phosphoryl Chloride: A mixture of **X** (376 mg) and phosphoryl chloride (620 mg) was heated at 45°C for 6 hours, then poured into ice-water and then extracted with ethyl acetate. The extract was washed with saturated aqueous sodium bicarbonate and evaporated to give an oily product (300 mg), which was identified as **XIII** (*E* isomer) obtained by reacting methyl cyanoacetate (**XII**) with sodium nitrite and following alkylation[9] by comparison with Rf values and IR spectra. That is, the Rf values of the *Z* isomer (**XI**) and the *E* isomer (**XIII**) were 0.38 and 0.34 respectively using benzene as a solvent. In addition, especially the C≡N absorption of **XIII** in IR spectrum was extremely weak.

7) Methyl 2-Ethoxyimino-2-(5-amino-1,2,4-thiadiazol-3-yl)acetate (**XV**): To a solution of [A] (1.05 g) and triethylamine (1.26 g) in methanol (10 ml) was added dropwise bromine (0.8 g) at −5°C with stirring. After several minutes, a solution of potassium thiocyanate (485 mg) in methanol (5 ml) was added dropwise to the reaction mixture at −5°C. After storage for 2 hours at 0 to 5°C, the mixture was evaporated, and the residue was dissolved in ethyl acetate, washed with water and saturated aqueous sodium chloride, and then evaporated to give **XV** as a crystalline solid (1.12 g, 97.4%); mp 179~181°C (dec); IR (Nujol) 3400, 3250, 3100, 1745, 1620, 1600, 1530 cm^{-1}.

Anal Calcd for $C_7H_{10}N_4O_3S$: C 36.52, H 4.69, N 24.33, S 13.93.
Found: C 36.40, H 4.55, N 24.30, S 13.84.

8) 2-Ethoxyimino-2-(5-amino-1,2,4-thiadiazol-3-yl)acetic Acid (**XVI**, *Z* isomer) from **XV**: **XV** (460 mg) was stirred in 1 N aqueous sodium hydroxide (4 ml) at room temperature for 2 hours. To the reaction mixture was added 1 N aqueous hydrochloric acid (4 ml), then it was extracted with ethyl acetate and the extracts were evaporated to give a colorless solid (230 mg, 53.2%) which gave an identical NMR spectrum, IR spectrum and Rf values on TLC with **VII** (R=C_2H_5) obtained by Method A.

Preparation of **XVI** from Methyl 2-(*N*-Chloro)amidino-2-ethoxyiminoacetate (**XVII**)

To a solution of [A] (12.6 g) in methanol (300 ml) was added dropwise an ethereal solution (90 ml) of hypochlorous acid (obtained from mixing a 10% aqueous solution (100 ml) of sodium hypochlorite and 3 N hydrochloric acid (60 ml) with diethyl ether (100 ml)) at 0 to 5°C with cooling in an ice-bath and stirring. Stirring was continued for 30 minutes at the same temperature, then triethylamine (14.0 g) was added dropwise to the reaction mixture, which was then evaporated to dryness. The residue was

triturated with cold water (150 ml) and stirred for 30 minutes in an ice-bath. The resulting precipitates were collected by filtration washed with cold water and dried to give **XVII** (*Z* isomer) (7.2 g, 57.8%); mp 38~40°C; IR (Nujol) 3470, 3350, 1750, 1635, 1600, 1560, 1030, 840 cm^{-1}; NMR (CD_3OD) δ 1.23 (3H, t, J=7 Hz), 3.76 (3H, s), 4.20 (2H, q, J=7 Hz).

Anal Calcd for $C_8H_{10}N_8O_3Cl$: C 34.71, H 4.86, N 20.24, Cl 17.08.
Found: C 34.59, H 4.80, N 20.22, Cl 17.04.

A suspended solution of **XVII** (6.0 g) in 1 N aqueous sodium hydroxide (30 ml) was stirred for 1 hour at room temperature. The solution was cooled in an ice-bath, acidified with 6 N hydrochloric acid (5.5 ml) and extracted with ethyl acetate (30 ml) twice. The extracts were dried, evaporated and the residue was triturated in a mixed solvent of diisopropyl ether and petroleum ether (1: 1) to give 2-(*N*-chloro)-amidino-2-ethoxyiminoacetic acid (**XVIII**, *Z* isomer) (5.0 g, 89.4%), which was recrystallized from water; mp 125~126°C (dec); IR (Nujol) 3480, 3370, 2800~2200, 1740, 1620, 1600, 1380, 1040, 970, 820 cm^{-1}; NMR (CD_3OD) δ 1.30 (3H, t, J=7 Hz), 4.25 (2H, q, J=7 Hz).

Anal Calcd for $C_5H_8N_3O_3Cl$: C 31.02, H 4.17, N 21.71, Cl 18.31.
Found: C 31.37, H 4.22, N 21.47, Cl 18.51.

To a solution of potassium thiocyanate (970 mg) and triethylamine (2.5 g) in methanol (40 ml) was added **XVIII** (1.94 g) at −5 to 0°C with cooling in an ice-salt bath and stirring. The mixture was stirred for 30 minutes at the same temperature and allowed to store overnight in a refrigerator. The mixture was evaporated to dryness, the residue was dissolved in water (10 ml), adjusted to pH 1.5 with 3 N hydrochloric acid and extracted with ethyl acetate. The extract was dried, evaporated to dryness and triturated with diisopropyl ether to give **XVI** (1.67 g, 77.2%).

Preparation of 2-Ethoxyimino-2-(4-aminopyrimidin-2-yl)acetic Acid (**XIX**) from [A]

A mixture of [A] (*Z* isomer) (2.1 g), 2-chloroacrylonitrile (875 mg) and triethylamine (2.0 g) in ethanol (21 ml) was stirred for 8 hours at room temperature and allowed to store at the same temperature overnight. The mixture was evaporated to dryness and the residue was dissolved in a mixture of ethyl acetate (20 ml) and water (10 ml). The organic layer was separated, treated with activated charcoal and evaporated to dryness. The residue was purified by column chromatography on silica gel (30 g) using ethyl acetate - benzene (1: 1) as an eluant to give a mixture (1.0 g) of methyl 2-ethoxyimino-2-(4-amino-pyrimidin-2-yl)acetate (*Z* isomer) and ethyl 2-ethoxyimino-2-(4-aminopyrimidin-2-yl)acetate (*Z* isomer) (1: 1).

In this reaction, with a similar way in methanol, only the corresponding methyl ester was obtained in a low yield of 24.8% by purified on silica gel chromatography, eluting with ethyl acetate; mp 120~122°C; IR (Nujol) 3500, 3320, 3200, 1740, 1645, 1600, 1590, 1540, 1495, 1250, 1040, 990 cm^{-1}; NMR (DMSO-d_6) δ 1.31 (3H, t, J=7 Hz), 3.87 (3H, s), 4.30 (2H, q, J=7 Hz), 6.50 (2H, d, J=6 Hz), 7.20 (2H, bs), 8.17 (2H, d, J=6 Hz).

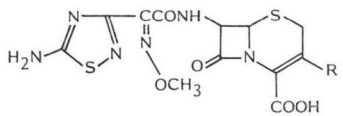
Anal Calcd for $C_9H_{12}N_4O_3$: C 48.21, H 5.36, N 25.00.
Found: C 48.20, H 5.32, N 24.96.

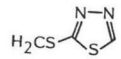
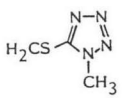
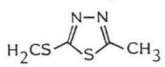
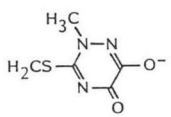
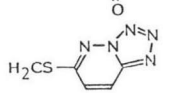
The mixture (930 mg) of methyl and ethyl ester obtained above in 1 N aqueous sodium hydroxide (4.95 ml) was stirred for 3.5 hours at room temperature. The solution was passed through an ion exchange resin (16 ml), Amberlite IRC-50 and evaporated to dryness. The residue was triturated with a mixture of acetone (20 ml) and water (2 ml) to give **XIX** (*Z* isomer) (0.8 g, 41.0% from [A]); mp 180~182°C (dec); IR (Nujol) 3560, 3400, 3250, 1640, 1605, 1530 cm^{-1}; NMR (D_2O) δ 1.33 (3H, t, J=7 Hz), 4.33 (2H, q, J=7 Hz), 6.67 (1H, d, J=7 Hz), 8.05 (1H, d, J=7 Hz).

General Procedure for the Activation of Carboxylic Acids (**VII**)

2-Ethoxyimino-2-(5-amino-1,2,4-thiadiazol-3-yl)acetyl Chloride Hydrochloride (**VIII**, R=C_2H_5, *Z* isomer): To a solution of phosphorus pentachloride (54.6 g) in methylene chloride (500 ml) was added **VII** (R=C_2H_5) (54.0 g) with stirring and cooling at −20°C. The mixture was stirred for 30 minutes at −15 to −12°C and for 2 hours at −5°C. To the mixture containing partially precipitated product was added diisopropyl ether (500 ml) at −5°C and the mixture was stirred for 30 minutes at −5 to 10°C. The resulting precipitates were collected by filtration, washed with diisopropyl ether and dried to give the title compound (60.2 g, 89.1%); mp 125~127°C (dec); IR (Nujol) 1785, 1625, 1055 cm^{-1}.

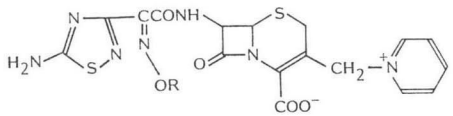
Table 6. IR and ^1H NMR data of aminothiadiazolyl cephalosporins.



Compound No.	R	IR (Nujol cm^{-1}) β-Lactam	NMR δ value (DMSO-d_6)						
			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=14 Hz	C_2-H_2 2H, bs	=N-OCH_3 s, 3H	Other protons
1		1775	9.67	5.90	5.22	4.38, 4.67	3.80	4.00	9.63 (1H, s)
3	H	1775	9.55	5.92	5.17		3.63 (d, J=3 Hz)	3.93	6.67 (1H, t, J=3 Hz)
4	CH_2OCOCH_3	1780	9.50	5.77	5.08	4.67, 4.93	3.50	3.87	1.97 (3H, s)
5	CH_2OCONH_2	1780	9.54	5.80	5.14	4.62, 4.88	3.52	3.92	
6		1770	9.57	5.83	5.13	4.33 (bs)	3.73	4.00	3.97 (3H, s)
7		1780	9.58	5.83	5.16	4.25, 4.53	3.70	4.00	2.72 (3H, s)
8		1760		5.85 (d, J=5 Hz)	5.20	4.0~4.5	3.48, 3.78 (2H, ABq, J=18 Hz)	4.08	3.64 (3H, s)
9		1785	9.56	5.80	5.14	4.20, 4.62	3.82, 3.62 (2H, ABq, J=18 Hz)	3.92	7.72 (1H, d, J=8 Hz), 8.56 (1H, d, J=8 Hz)

Compound **8** is a disodium salt and its NMR spectrum was recorded in D_2O.

Table 7. IR and ^{1}H NMR data of aminothiadiazolyl cephalosporins.

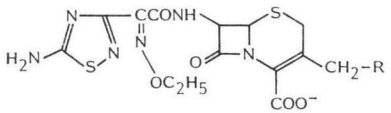


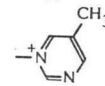
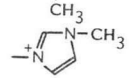
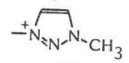
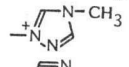
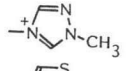
Compound No.	R	IR (Nujol cm^{-1}) β-Lactam	NMR δ value (DMSO-d_6+D_2O)[a]				
			=N-OR	C_7-H 1H, d, J=5 Hz	C_6-H 1H, d, J=5 Hz	C_3-H_2 2H, ABq, J=14 Hz	C_2-H_2 2H, ABq, J=18 Hz
10	CH_3	1770	3.86 (3H, s)	5.73	5.06	5.19, 5.69	3.07, 3.57
11	H	1780		5.86	5.08	5.28, 5.62	3.14, 3.54
12	C_2H_5	1770	1.21 (3H, t, J=7 Hz), 4.12 (2H, q, J=7 Hz)	5.7	5.05	5.19, 5.68	3.10, 3.52
13	$CH(CH_3)_2$	1770	1.22 (6H, d, J=6 Hz), 4.1~4.6 (1H, m)	5.78	5.12	5.33, 5.70	3.15, 3.57
14	$CH_2CH=CH_2$	1770	4.44~4.76 (2H, m), 5.0~6.1 (6H, m)[b]		5.10		3.12, 3.50
15	$CH_2C\equiv CH$	1770	3.47 (1H, t, J=2 Hz), 4.73 (2H, d, J=2 Hz)	5.82	5.08	5.25, 5.65	3.10, 3.53
16	CH_2CF_3	1780	4.63, 4.93 (2H, ABq, J=9 Hz)	5.83	5.17	5.37, 5.67	3.23, 3.50
17	CH_2SCH_3	1770	2.17 (3H, s), 5.22 (2H, s)	5.73	5.10	5.00, 5.83	3.00, 3.62
18		1780	1.4~2.0 (8H, m), 4.60~4.83 (1H, m)	5.87	5.10	5.30, 5.83	3.17, 3.53
19		1780	1.6~2.6 (4H, m), 5.8~6.2 (2H, m)	5.81	5.19	4.9 ~5.6 (2H)	3.39, 3.61
20	CH_2COOH	1780	4.70 (2H, s)	5.93	5.30	5.40, 5.60	3.27, 3.63

a) Pyridinium ring proton: 8.0~8.3 (2H, m) 8.4~8.7 (1H, m), 9.3~9.6 (2H, m).

b) Containing C_7-H and C_3-H_2.

Table 8. IR and ^{1}H NMR data of aminothiadiazolyl cephalosporins.



Compound No.	R	IR (Nujol cm^{-1}) β-Lactam	NMR δ value (DMSO-d_6+D_2O)					
			=N-OC_2H_5 3H, t, J=7 Hz 2H, q, J=7 Hz	C_7-H 1H, d, J=5 Hz	C_6-H 1H, d, J=5 Hz	C_3-H_2 2H, ABq, J=14 Hz	C_2-H_2 2H, ABq, J=18 Hz	Onium ring proton
21		1770	1.23, 4.20	5.77	5.10	5.4~5.9#	3.03~3.92#	7.67 (1H, m), 8.42 (1H, m), 9.20 (1H, m), 9.52 (1H, m)
22		1770	1.32, 4.33	5.90	5.30	5.37, 5.60	3.28, 3.72	2.58 (3H, s), 9.25 (2H, s), 9.60 (1H, s)
23		1770	1.30, 4.33	5.90	5.30	5.47, 5.75	3.27, 3.72	9.13 (2H, bs), 9.45 (2H, bs)
24		1770	1.34, 4.37	5.88	5.27	5.23, 5.57	3.19, 3.57	4.11 (3H, s), 6.78 (1H, t, J=3 Hz), 8.21 (2H, d, J=3 Hz)
25		1770	1.23, 4.15	5.66	5.02	5.02, 5.18	3.12, 3.54	3.86 (3H, s), 7.65 (1H, s), 7.97 (1H, s), 9.32 (1H, s)
26		1770	1.23, 4.13	5.67	5.00	5.30, 5.59	3.15, 3.55	4.30 (3H, s), 8.82 (1H, s), 9.06 (1H, s)
27		1770	1.23, 4.12	5.63	4.98	5.0~5.5#	3.1~3.8#	3.91 (3H, s), 9.03 (1H, s), 10.25 (1H, s)
28		1770	1.22, 4.13	5.63	4.98	4.7~5.3#	3.3~3.7#	4.05 (3H, s), 9.30 (1H, s), 10.25 (1H, s)
29		1770	1.30, 4.32	5.83	5.25	5.29	3.20	8.19 (1H, d, J=4 Hz), 8.42 (1H, d, J=4 Hz)

It was difficult to read the δ value because the signals overlapped with those of water or other protons.

Anal Calcd for $C_6H_3O_2N_4SCl_2$: C 26.57, H 2.95, N 20.66, S 11.81, Cl 26.20.
Found: C 26.13, H 2.99, N 20.49, S 11.81, Cl 26.41.

The other carboxylic acids were activated in a similar way.

General Procedure for the Acylation of 7β-Aminoceph-3-em-4-carboxylic Acids with an Acid Chloride (**VIII**)

A) Synthesis of Cephalosporins **1**, **3**~**9**: A mixture of the 7β-aminocephem (5 mmol) and trimethylsilylacetamide (8 g) in methylene chloride (40 ml) was warmed to make a solution. The solution was cooled to −25°C and an acid chloride (5 mmol) was added. The reaction mixture was stirred for 30 minutes at −8 to −10°C, then poured into cold aqueous sodium bicarbonate. The mixture was stirred for 30 minutes at room temperature and the aqueous layer was separated. The aqueous solution was adjusted to pH 1.5 with 10% hydrochloric acid and extracted with ethyl acetate. The extract was dried, evaporated and the residue was triturated with diethyl ether to give a crude product, which was dissolved in aqueous sodium bicarbonate and reprecipitated by addition of 10% hydrochloric acid to give the pure compound in 40~70% yield.

IR and ^{1}H NMR data of compounds **1** and **3**~**9** were listed in Table 6.

B) Synthesis of Cephalosporins **10**~**20**: To a solution of 1-[(7β-amino-4-carboxy-3-cephem-3-yl)methyl]pyridinium chloride hydrochloride dihydrate (10 mmol) and trimethylsilylacetamide (16 g) in methylene chloride (50 ml) were added an acid chloride (11 mmol) prepared above at −20°C and the mixture was stirred for 25 minutes at −18 to −12°C and for an additional 20 minutes at −12 to −3°C. A solution of sodium bicarbonate (4 g) in water (30 ml) was added to the reaction mixture and the aqueous layer was separated, adjusted to pH 1.5 with 6 N hydrochloric acid, washed with ethyl acetate and then readjusted to pH 4 with aqueous sodium bicarbonate. The aqueous solution was passed through a column packed with alumina (16 g) and then subjected to column chromatography on a non-ionic adsorption resin Diaion HP-20 (100 ml). After the column was washed with water, the elution was carried out with 20% aqueous methanol. The eluates containing the product were combined, evaporated to remove methanol *in vacuo* and lyophilized to give the desired cephalosporin in 50 to 80% yield.

Compounds **11** and **20** were prepared by the respective deprotection of trityl or *t*-butyl groups with trifluoroacetic acid after the acylation.

IR and ^{1}H NMR data of compounds **10**~**20** were listed in Table 7.

General Procedure for Displacement at the 3-Position with Heteroaromatic Nucleophile (**21**~**29**)

To a mixture of the heteroaromatic compound (11 mmol), sodium iodide (18 g) and water (3.0 ml) was added sodium 7-[2-ethoxyimino-2-(5-amino-1,2,4-thiadiazol-3-yl)acetamido]cephalosporinate (*Z* isomer) (10 mmol) at 80°C with stirring, which was continued for 1 hour at that temperature. The mixture was cooled to room temperature, diluted with water (25 ml) and adjusted to pH 2.8 with 1 N hydrochloric acid. An insoluble material was filtered off and the filtrate was washed with ethyl acetate, evaporated to remove ethyl acetate and subjected to column chromatography on a non ionic adsorption resin Diaion HP-20 (160 ml). After the column was washed with water, the elution was carried out with 20% aqueous methanol. The eluates containing the desired compound were combined, evaporated to remove methanol *in vacuo* and lyophilized to give compounds **21**~**29** in 30 to 50% yield.

IR and ^{1}H NMR data of compounds **21**~**29** were listed in Table 8.

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References

1) GOTO, J.; K. SAKANE, Y. NAKAI, T. TERAJI & T. KAMIYA: Studies of 7β-[2-(aminoaryl)acetamido]cephalosporin derivatives. I. Synthesis and structure-activity relationships in the aminopyridine series. J. Antibiotics 37: 532~545, 1984

2) GOTO, J.; K. SAKANE, Y. NAKAI, T. TERAJI & T. KAMIYA: Studies of 7β-[2-(aminoaryl)acetamido]cephalosporin derivatives. II. Synthesis and structure-activity relationships in the aminopyrimidine series. J. Antibiotics 37: 546~556, 1984
3) TERAJI, T.; K. SAKANE & J. GOTO: Cephem compounds, processes for their preparation, pharmaceutical compositions containing them and their starting compounds. Eur. Pat. Appl. 7,470, July 2, 1979
4) CSENDES, I.; B. W. MÜLLER & W. TOSCH: Cephalosporin antibiotics. Synthesis and antimicrobial activity of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-oxyiminoacetamido]cephalosporin derivatives. J. Antibiotics 36: 1020~1033, 1983
5) GOERDELER, J.: Über 1,2,4-Thiodiazole. I. Darstellung und Eigenschaften der 5-Amino-1,2,4-thiodiazole. Chem. Ber. 87: 57~67, 1954
6) IWAMATSU, K.; S. INOUYE, T. TSURUOKA, K. MIZUTANI, S. OMOTO, H. OGINO, K. MIYAUCHI, T. WATANABE & T. NIIDA: Synthesis and biological activity of 7β-(2-amino-2-carboxy)ethylthioacetamido-7α-methoxycephalosporin derivatives. J. Antibiotics 36: 229~241, 1983
7) SCHAEFER, C. F.: Synthesis of the *s*-triazine system. VI. Preparation of unsymmetrically substituted *s*-triazines by reaction of amidine salts with imidates. J. Org. Chem. 27: 3608~3613, 1962
8) ZAUGG, H. E.; M. FREIFELDER, H. J. GLENN, B. W. HORROM, G. R. STONE & M. R. VERNSTEN: 3-Carboxy-2,5-piperazinedione and derivatives. J. Am. Chem. Soc. 78: 2626~2631, 1956
9) MÜLLER, P. TH.: Bulletin de la Societe Chimique de France. (3), 27: 1015. Beilsteins Handbuch Der Organischen Chemie 3: 775