SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS IN THE CEFPIROME SERIES

I. 7-[2-(2-AMINOTHIAZOL-4-YL)-2-(*Z*)-OXYIMINOACETAMIDO]-3-[(SUBSTITUTED-1-PYRIDINIO)METHYL]-CEPH-3-EM-4-CARBOXYLATES†

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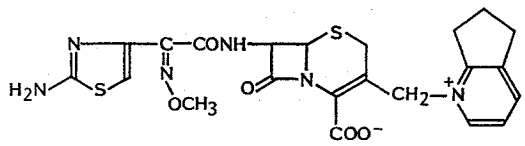
7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-oxyiminoacetamido]-3-[(substituted-1-pyridinio)methyl]-ceph-3-em-4-carboxylates **II** are a group of β-lactam antibiotics with extraordinary high antibacterial activity. The promising member of this group, cefpirome (HR 810, **II-1**) is a candidate for clinical use. Synthetic pathways to **II** starting from cefotaxime derivatives **I** or 7-aminocephalosporanic acid (7-ACA) are described. A preferred method for the conversion of **I** to **II** or 7-ACA to precursors **III** respectively employs iodotrimethylsilane and an excess of the pyridine base. Structure-activity studies reveal an optimum overall activity in the series of pyridines with fused saturated and unsaturated rings or cyclopropyl- and alkoxy substituents. Favorable oxyimino substituents are methyl, ethyl, difluoromethyl and carbamoylmethyl groups. Acidic substituents lead to decreased activity against *Staphylococcus aureus* SG 511. Introduction of halogen in the thiazole nucleus causes improvement of activity against the K1 β-lactamase producing *Klebsiella aerogenes* 1082 E strain.

In the last 10 years, aminothiazolyl cephalosporin antibiotics such as cefotaxime brought considerable progress in the treatment of bacterial infections[1]. As a result of extended modifications in the aminothiazolyloxyiminoacetamido moiety and of the C-3′ substituent in cefotaxime, 3′-pyridinium derivatives have been found to be very potent antibacterial compounds. Among them cefpirome (HR 810)[2~5] (Fig. 1) was selected as a candidate for clinical use.

Fig. 1. Structure of cefpirome (HR 810, **II-1**).



In this paper syntheses and structure-activity relationships in the series of pyridinium cephalosporins related to cefpirome will be described.

Chemistry

The title compounds **II** of Tables 1~5 have been prepared according to methods A~D (Scheme 1). Typical working examples are given for each method. In spite of extensive variations of the traditional method A in aqueous medium[6], the yields exceeded 40% only in a few cases (see examples

† Dedicated to Professor WILHELM BARTMANN on the occasion of his 60th birthday.

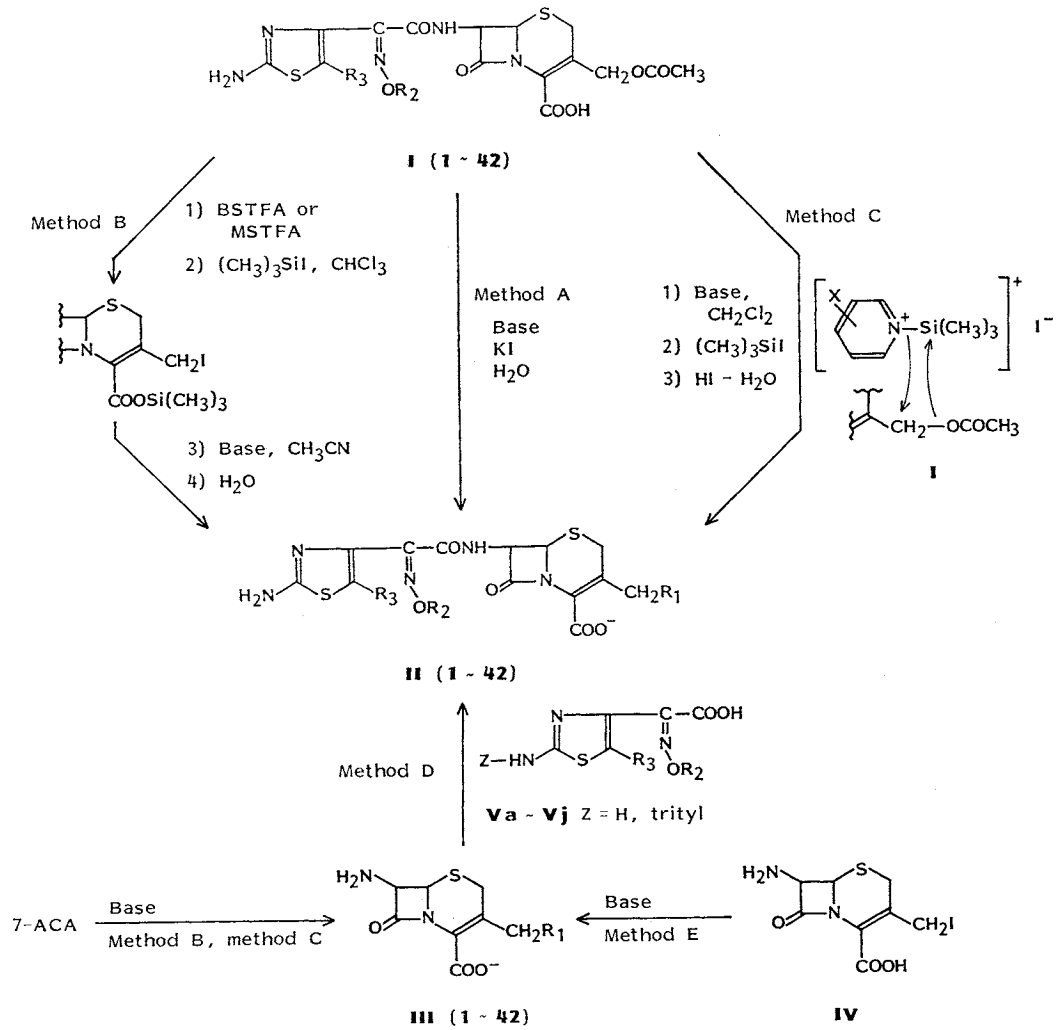
II-8, **II-17** and **II-21**). Pure amorphous betaines were obtained after chromatography of the crude products over silica gel with acetone - water mixtures as eluents. For preparative purposes this synthesis was not practical. Therefore considerable efforts were undertaken to explore alternative methods, preferably displacement reactions under anhydrous conditions in order to avoid competitive hydrolysis of the β-lactam ring. Method B using 3-(iodomethyl)cephems brought decisive progress. The 3-iodomethyl precursors usually have been prepared indirectly from the chlorides or bromides. The preparation of the 3-(iodomethyl)cephems directly from the 3-(acetoxymethyl)cephems brought an easier access to these reactive intermediates. This type of reaction was first described by the Eli Lilly group in 1981[7,8]. They applied the general procedure from JUNG and LYSTER[9], and HO and OLAH[10] for the cleavage of esters with iodotrimethylsilane. The reaction involves a silylation of the starting 3-acetoxycephem followed by an iodination with iodotrimethylsilane in methylenchloride or chloroform to give the iodomethyl intermediate. Change of the solvent to acetonitrile and reaction with base yields the final product. In most cases, the yields were higher than 50%. After addition of water to the reaction mixtures, the products are isolated as monohydroiodide salts (see example **II-6**). A disadvantage of this method was the use of acetonitrile and the need for the expensive *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) or bis(trimethylsilyl)trifluoroacetamide (BSTFA) as silylating agent.

We therefore developed a simplified method which avoids the silylation procedure and acetonitrile[11]. The displacement reaction occured smoothly in boiling methylene chloride if the base was used in excess compared to iodotrimethylsilane (Method C). With 2,3-cyclopentenopyridine as base, pure crystalline cefpirome (**II-1**) dihydroiodide was obtained from cefotaxime (**I-1**) in >65% yield. It was converted into the sulfate by means of the liquid anion exchange resin Amberlite LA-2 followed by the addition of sulfuric acid.

The ease of the displacement in general depends on the substitution pattern of the pyridine component. 2,3-Cyclopenteno- or 2,3-cyclohexenopyridine gives high yield, the corresponding 3,4-cyclopenteno- or 3,4-cyclohexenopyridine gives lower yield because a higher reaction temperature in boiling chloroform is needed, and the 2-cephem is formed predominantly. In Method C the quaternary 1-trimethylsilylpyridinium iodide formed from iodotrimethylsilane and the pyridine base, probably is the reactive intermediate. The displacement reaction may be rationalized as shown in Scheme 1. Crystalline 1 : 1 adducts from bromo- or iodotrimethylsilane and pyridine and their X-ray structure are known[12,13]. The role of tertiary amines-iodotrimethylsilane adducts in the preparation of 7-amino-3-(ammoniomethyl)cephems from bistrimethylsilylated 7-aminocephalosporanic acid (7-ACA) has been discussed recently in detail[14]. 7-Amino-3-[(*N*-methylpyrrolidinio)methyl]ceph-3-em-4-carboxylate (**III**, $R_1 = -\overset{+}{N}\square_{CH_3}$) has been prepared from *N*-methylpyrrolidine and an excess of iodotrimethylsilane in yields up to 63%. In this case the intermediacy of a 3-(iodomethyl)cephem is assumed. Using an excess of *N*-methylpyrrolidin according to Method C, the authors[14] found no 3′-exchange product, thus indicating the different reactivity of tertiary aliphatic amines and pyridines.

Alternatively, compounds **II** have been prepared by acylation of 7-ACA derivatives **III** with activated 2-(2-aminothiazol-4-yl)-2-oxyiminoacetic acids **V**, *e.g.* acid chlorides or active esters, such as hydroxybenzotriazole (HOBT) ester[15] (Method D). Compounds **III** were prepared from 7-ACA and pyridines in aprotic solvents preferably according to Method C[16] or from 7-amino-3-(iodomethyl)-ceph-3-em-4-carboxylic acid **IV**[17], bistrimethylsilylacetamide and substituted pyridines in acetonitrile

Scheme 1. Synthetic routes to pyridinium cephalosporins II.



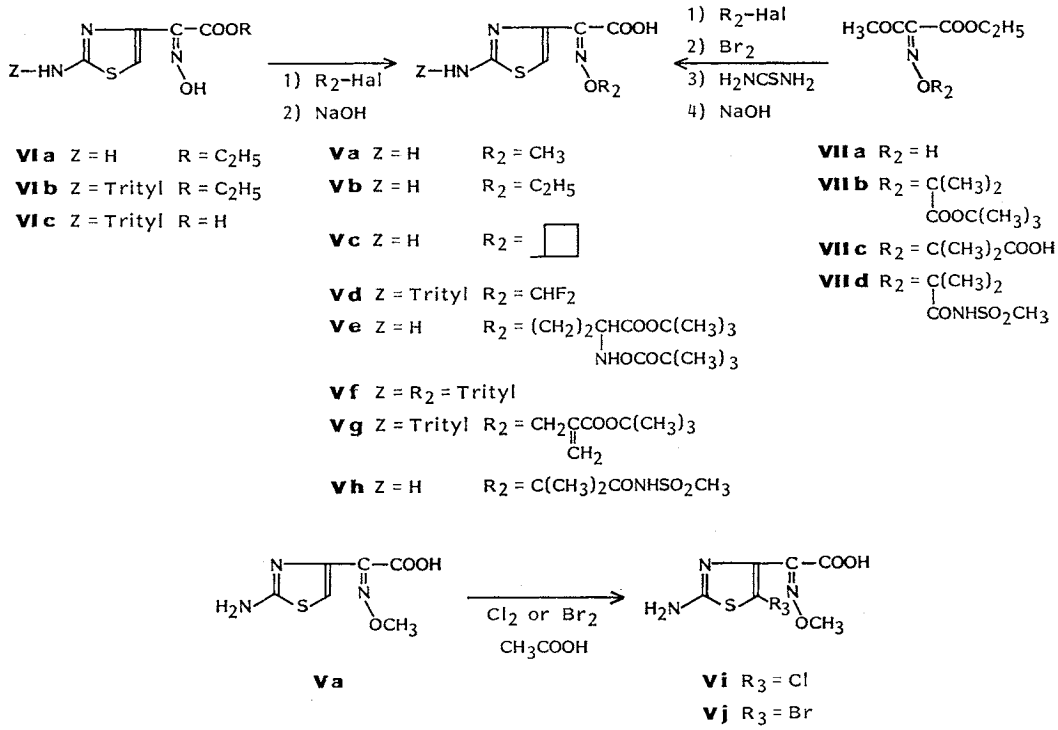
For definition of R_1, R_2 and R_3 see Tables 1~5; X=pyridine substituent.

or DMF as solvents (Method E). **III-1**, the precursor of cefpirome (**II-1**), has also been prepared from bistrimethylsilylated 7-ACA derivatives and 2,3-cyclopentenopyridine in yields of 76~94%[14].

Starting material for compounds **II** with R_2=CH_3, R_3=H was cefotaxime (**I-1**). Compounds **I** with neutral oxime substitution other than CH_3 as well as 5-halogen derivatives (R_3=Cl, Br) have been prepared by acylation of 7-ACA or its *tert*-butyl ester with the appropriately substituted acids **V** preferably *via* HOBT active esters.

The acids **V**, some already described, were prepared by alkylation of ethyl 2-(2-aminothiazol-4-yl)-2-(*Z*)-hydroxyiminoacetate (**VIa**) or its *N*-trityl derivative **VIb** with appropriate halides (see preparation of **Vc**, **Ve** and **Vf**) or by alkylation of the 2-(*Z*)-hydroxyimino-2-(2-tritylaminothiazol-4-yl)-acetic acid (**VIc**) dianion with alkyl halides (preparation of **Vg**). Another pathway involved alkylation of ethyl 2-(*Z*)-hydroxyiminoacetoacetate (**VIIa**) with alkyl halides, followed by bromination, cyclization with thiourea and alkaline hydrolysis of the ester group (preparation of **Vh**). Halogena-

Scheme 2. Synthesis of 2-(2-aminothiazol-4-yl)-2-(*Z*)-oxyiminoacetic acids (V).



tion of **Va** with chlorine or bromine gave the acids **Vi** and **Vj**, respectively (Scheme 2).

Antibacterial Properties and Structure-activity Relationships

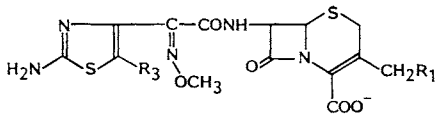
The *in vitro* activity of compounds **1**~**42** (Tables 1~5) against Gram-positive and Gram-negative aerobic bacteria were determined by an agar and serial dilution technique respectively. Cefpirome and analogues with saturated and unsaturated rings fused to the pyridine moiety exhibit excellent activity and β-lactamase stability (Table 1: **1**, **2**, **5**~**10**). With increasing ring size (**1**→**2**→**3**→**4**) the activity is diminished, mainly against *Pseudomonas*. This could be due to decreasing cell wall penetration as indicated by comparison of the MICs of **1**, **2**, **3** and **4** against *Pseudomonas aeruginosa* 1771 and its cell wall deficient mutant 1771 M. The halogenated analogues **11** and **12** of cefpirome are highly active against the K1 β-lactamase producing *Klebsiella aerogenes* 1082 E whereas the activity against *P. aeruginosa* 1771 decreases from H to Cl to Br (Table 1).

Table 2 gives the MICs of a series of pyridinium compounds monosubstituted by an alkyl, cycloalkyl and aromatic substituent. The antibacterial activity against *P. aeruginosa* decreases with increasing size of the substituent, except the cyclopropyl derivative **18**, which is still comparable to cefpirome (**1**).

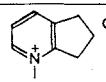
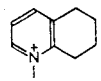
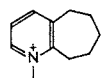
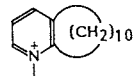
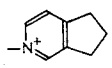
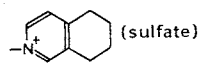
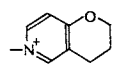
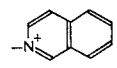
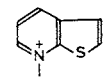
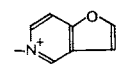
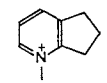
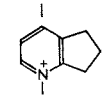
Table 3 shows compounds containing electron donating and electron withdrawing substituents. The most active are alkoxy and methylthio derivatives (**21**~**24**). Compounds **25**~**29** carrying electron withdrawing substituents (Hammet σ values from 0.3~0.7) exhibit reduced antibacterial potency, especially against *Pseudomonas*.

In cefpirome analogues with neutral oxime substitution (Table 4) the ethoxyimino compound **31**,

Table 1. Effect of annelated saturated and unsaturated rings and of 5-halogen substitution on the antibacterial activity[a].



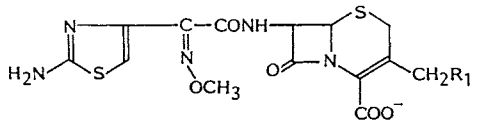
II

Compound[b]	R_1	*S.a.* SG 511	*S.p.* 77 A	*S.f.* D	*P.a.* 1771	*P.a.* 1771 M	*E.c.* TEM	*K.a.* 1082 E	*K.a.* 1522 E	*E.cl.* P 99
1	 Cefpirome sulfate	0.31	0.008	3.13	1.56	0.62	0.078	12.5	0.062	3.13
2	 (sulfate)	0.39	0.015	6.25	6.25	0.39	0.125	50	0.062	6.25
3[c]		07.8	0.007	12.5	12.5	3.13	0.39	6.25	0.19	3.13
4[c]		07.8	0.002	6.25	100	25	0.78	12.5	3.13	50
5		0.78	0.004	>100	0.78	0.62	0.019	6.25	0.031	25
6	 (sulfate)	0.31	0.002	100	1.56	1.25	0.062	25	0.031	50
7		0.62	0.004	100	0.39	0.62	0.12	6.25	0.062	25
8		0.31	0.002	1.56	1.56	0.78	0.015	25	0.031	6.25
9		0.62	0.008	25	1.56	0.39	0.062	6.25	0.031	6.25
10		0.78	0.015	6.25	1.56	1.56	0.031	100	0.062	100
11		0.62	0.008	6.25	3.13	0.39	1.56	1.56	1.56	12.5
12		0.19	0.002	50	12.5	1.56	0.098	1.56	0.78	12.5

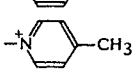
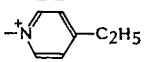
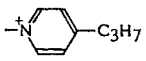
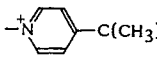
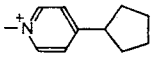
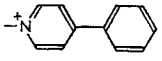
[a] MIC (μg/ml): Serial dilution test, Mueller-Hinton medium (Difco); inoculum 5×10^4 cfu/ml. [b] Compounds **1**~**10** R_3=H, compound **11** R_3=Cl, compound **12** R_3=Br. [c] Agar dilution values.

S.a.: *Staphylococcus aureus*, *S.p.*: *Streptococcus pyogenes*, *S.f.*; *Streptococcus faecium*, *P.a.*: *Pseudomonas aeruginosa*, *E.c.*: *Escherichia coli*, *K.a.*: *Klebsiella aerogenes*, *E.cl.*: *Enterobacter cloacae*.

Table 2. Effect of alkyl-, cycloalkyl- and aryl-substitution on the antibacterial activity[a].

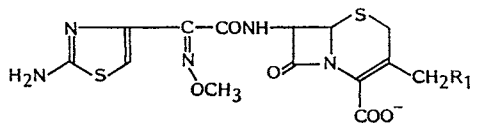


II

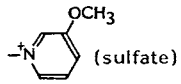
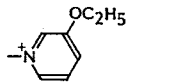
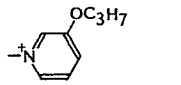
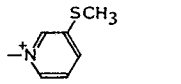
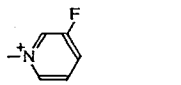
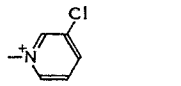
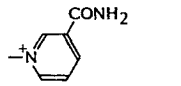
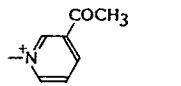
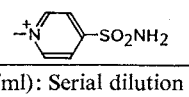
Compound	R_1	*S.a.* SG 511	*S.p.* 77 A	*S.f.* D	*P.a.* 1771	*P.a.* 1771 M	*E.c.* TEM	*K.a.* 1082 E	*K.a.* 1522 E	*E.cl.* P 99
13		1.56	0.015	50	7.81	1.25	0.31	31.2	0.15	125
14		1.56	0.015	100	12.5	1.56	0.15	62.5	0.078	62.5
15		0.78	0.015	100	15.6	3.13	0.078	62.5	0.039	62.5
16		1.56	0.008	>100	12.5	1.56	0.078	62.5	0.078	50
17		3.13	0.031	>100	15.6	1.95	0.31	>100	0.15	125
18		0.31	0.002	50	0.78	0.15	0.031	6.25	0.031	25
19		0.78	0.008	6.25	25	6.25	0.31	50	0.62	50
20		0.78	0.004	100	12.5	3.13	0.15	6.25	0.31	50

[a] MIC (μg/ml): Serial dilution test, Mueller-Hinton medium (Difco); inoculum 5×10^4 cfu/ml.
Abbreviations: See footnote in Table 1.

Table 3. Effect of electron donating and electron withdrawing substituents on the antibacterial activity[a].

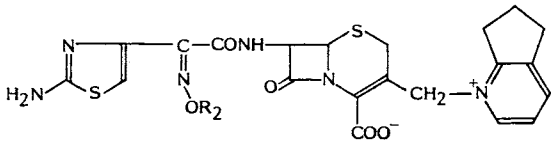


II

Compound	R_1	*S.a.* SG 511	*S.p.* 77 A	*S.f.* D	*P.a.* 1771	*P.a.* 1771 M	*E.c.* TEM	*K.a.* 1082 E	*K.a.* 1522 E	*E.cl.* P 99
21	 (sulfate)	0.31	0.002	6.25	0.78	0.15	0.078	6.25	0.019	25
22		0.39	0.002	12.5	6.25	0.39	0.078	25	0.078	12.5
23		0.39	0.002	6.25	3.13	0.78	0.062	25	0.25	12.5
24		0.39	0.004	25	6.25	3.13	0.15	100	0.078	100
25		0.39	0.008	100	25	0.62	0.62	125	0.039	31.2
26		0.78	0.015	50	15.6	2.5	0.62	125	0.15	62.5
27		0.39	0.008	50	15.6	0.31	0.15	31.2	0.039	15.6
28		0.78	0.008	12.5	31.2	3.13	0.31	125	0.15	31.2
29		3.13	0.008	>100	100	0.625	0.625	>100	0.078	>100

[a] MIC (μg/ml): Serial dilution test, Mueller-Hinton medium (Difco); inoculum 5×10 cfu/ml.
Abbreviations: See footnote in Table 1.

Table 4. Antibacterial activity of 3′-cyclopentenopyridinium cephalosporins II with different neutral oxime substitution[a].



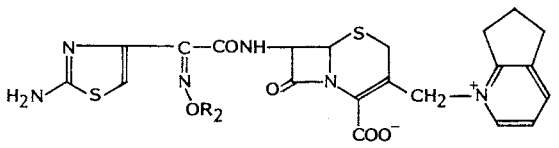
II

Compound	R_2	*S.a.* SG 511	*S.p.* 77 A	*S.f.* D	*P.a.* 1771	*P.a.* 1771 M	*E.c.* TEM	*K.a.* 1082 E	*K.a.* 1522 E	*E.cl.* P 99
1	CH_3 (cefpirome sulfate)	0.19	<0.002	1.56	1.56	0.39	0.013	1.56	0.002	0.78
30	H	0.098	<0.002	12.5	100	0.78	0.19	50	0.19	25
31	C_2H_5	0.19	<0.002	12.5	1.56	0.78	0.049	1.56	0.049	1.56
32	C_3H_7	0.19	<0.002	25	6.25	3.13	0.078	6.25	0.39	6.25
33		0.19	0.004	100	12.5	3.13	0.098	12.5	0.78	3.13
34	$(CH_2)_{11}CH_3$	0.098	<0.002	50	>100	12.5	12.5	>100	25	>100
35	CHF_2	0.098	<0.002	25	1.56	0.098	0.004	0.78	0.004	0.098
36	CH_2CONH_2	0.098	<0.002	50	3.13	0.19	0.098	3.13	0.025	3.13

[a] MIC (μg/ml): Agar dilution test, Mueller-Hinton agar (Difco); inoculum 5×10^4 cfu/spot.

Abbreviations: See footnote in Table 1.

Table 5. Antibacterial activity of 3′-cyclopentenopyridinium cephalosporins II with different acidic oxime substitution[a].



II

Compound	R_2	*S.a.* SG 511	*S.p.* 77 A	*S.f.* D	*P.a.* 1771	*P.a.* 1771 M	*E.c.* TEM	*K.a.* 1082 E	*K.a.* 1522 E	*E.cl.* P 99
37	CH_2COOH	3.13	0.049	>100	1.56	0.19	0.049	1.56	0.025	6.25
38	$C(CH_3)_2COOH$	6.25	0.098	>100	1.56	0.39	0.39	0.78	0.19	12.5
39	$CH_2C(=CH_2)COOH$	0.78	0.025	>100	1.56	0.39	0.098	6.25	0.013	25
40		6.25	0.098	>100	1.56	0.39	0.19	0.78	0.098	3.13
41	$C(CH_3)_2CONHSO_2CH_3$	25	0.19	>100	12.5	0.78	1.56	6.25	1.56	25
42	$CH_2CH_2CH(NH_2)COOH$	6.25	0.098	>100	50	3.13	0.78	25	0.39	50
Ceftazidime		3.13	0.025	>100	0.78	0.025	0.049	0.39	0.007	100

[a] MIC (μg/ml): Agar dilution test, Mueller-Hinton agar (Difco); inoculum 5×10^4 cfu/spot.

Abbreviations: See footnote in Table 1.

the difluoromethyl **35** and the carbamoylmethyl compound **36** have activities comparable to cefpirome except *Streptococcus faecium* D. From all examples of Table 4 the hydroxyimino compound **30** has the best activity against *Staphylococcus aureus* but a considerably less activity against Gram-negatives, especially *P. aeruginosa* and β-lactamase producing organisms, *e.g. K. aerogenes* 1082 E. As a rule, the activity against Gram-negative bacteria decreases with increasing lipophilicity of the oxime substituent.

Compounds with acidic oxime substitution (Table 5) except **41** and **42** have excellent activities against Gram-negative bacteria, similar to ceftazidime. The activity against Gram-positive bacteria is significantly reduced (*S. aureus* 3.13~6.25 μg/ml) except that of **39**. None of the prepared compounds is active against Streptococci of the serogroup D.

In Vivo Activity

The *in vitro* effectiveness of compounds with general structure **II** is reflected by high *in vivo* activity. In the mouse infection model with *Streptococcus pyogenes* 77 A or *Escherichia coli* 078 as infective organisms, ED_{100} values after sc application are in the range of about 0.5 to 4 μg/20 g mouse, *e.g.* cefpirome (**1**); 1.95 μg and 0.98 μg for *S. pyogenes* 77 A and *E. coli* 078 respectively[5]. No derivative is absorbed by the oral route to an appreciable extent (ED_{100}>30 μg/20 g mouse.)

Pharmacokinetics

The substitution pattern of the pyridinium moiety has no marked influence on the pharmacokinetics in mouse, dog and monkey. Mean half lives are 20~30 minutes in mice and 40~60 minutes in dogs with somewhat variable urinary recoveries of 60 to 80%. The half-life in monkey is between 40 (compound **21**) and 90 minutes (cefpirome, **1**) for a 20-mg/kg dose. In human, cefpirome has an elimination half-life of 2 hours[18].

Experimental

IR spectra (Perkin-Elmer 113) were measured as KBr-pellets. ^1H NMR spectra were recorded on Bruker WP 60 and AM 270 spectrometers using TMS as an internal standard. UV spectra were measured in aqueous solution using a Perkin-Elmer 554 spectrometer. Fast atom bombardment mass spectra (FAB-MS) were recorded using a Kratos MS 902 mass spectrometer with glycerol as liquid matrix. Analytical HPLC was carried out with Nucleosil 7C18, using H_2O - MeOH (3:1) +0.7% phosphate buffer, pH 6.5 as eluent. All mp's are uncorrected. Pyridine bases were either commercially available or prepared by literature procedures. The 2-aminothiazolylacetic acid derivatives **VIa** and **VIb** are manufactured by Lonza Ltd., Basel, Switzerland. Compounds **Vd**[19] and **Vi**[20] were prepared as described.

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-3-[(3-methoxy-1-pyridinio)methyl]ceph-3-em-4-carboxylate (**II-21**)

Method A: A mixture of 7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]cephalosporanic acid (**I-1**) (13.7 g, 0.03 mol), potassium iodide (149 g, 0.9 mol), ascorbic acid (0.9 g) and 3-methoxypyridine (16.4 g, 0.15 mol) in H_2O - Me_2CO (3:1, 300 ml) was heated at 66~68°C for 4 hours while stirring. After cooling, the solution was extracted with CH_2Cl_2 (3×100 ml; from the CH_2Cl_2 - solution 11 g of 3-methoxypyridine could be recovered by distillation) and the aqueous phase was diluted with Me_2CO (1.7 liters). The resulting mixture was chromatographed over a 5×70 cm column of silica gel, eluting with Me_2CO - H_2O (8:1) to remove the potassium iodide. The solvent was changed to Me_2CO - H_2O (4:1) for elution of the product. Lyophilization gave 7.3 g (48.3%) of an amorphous pale yellow solid. 2 g of this product was dissolved in water (8 ml) and acidified with 6 N

H_2SO_4 (0.7 ml) to pH 1.3. After the addition of cold EtOH (10 ml) a crystalline precipitate formed. The slurry was cooled for 2 hours at 5°C, the crystals were filtered, washed with cold EtOH - H_2O (1 : 1, 4 ml) and EtOH (4 ml), and dried to give 2.1 g (87%) of **II-21** sulfate, mp 195~200°C (dec).

Anal Calcd for $C_{20}H_{20}N_6O_6S_2 \cdot H_2SO_4$(602.6): C 39.9, H 3.7, N 13.9, S 15.9.
Found: C 39.5, H 3.7, N 13.9, S 15.8.

Yields and NMR spectra of the compounds prepared according to method A are listed in Tables 6~9.

II-21 monohydroiodide was prepared from **I-1** and 3-methoxypyridine as described below for **II-6** (method B, yield 77%). **II-21** dihydroiodide was obtained in 61% yield according to method C as described below for **II-1**.

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-3-[(3,4-cyclohexeno-1-pyridinio)methyl]-ceph-3-em-4-carboxylate (**II-6**)

Method B: A mixture of **I-1** (11.8 g, 0.026 mol), BSTFA (21.8 g, 0.085 mol) and CH_2Cl_2 (60 ml) was heated under reflux for 1 hour while stirring. The resulting dark colored solution was cooled to 15°C, iodotrimethylsilane (14 g, 0.07 mol) was added and stirring was continued for 20 minutes at room temperature. The solution was evaporated to remove most of the CH_2Cl_2 and the oily residue was dissolved in CH_3CN (50 ml). THF (2.5 ml) was added, followed by a solution of 3,4-cyclohexenopyridine (4.26 g, 0.032 mol) and BSTFA (12.85 g, 0.05 mol) in CH_3CN (25 ml). After standing for 3 hours at room temperature the solution was cooled to 5°C and water (3.5 ml) was added while stirring. A precipitate formed which was filtered, washed with CH_2Cl_2 (20 ml), Me_2CO (20 ml) and Et_2O (50 ml) and dried to give 13.2 g (77.4%) of **II-6** monohydroiodide. Analytical HPLC: 91 area % purity.

Anal Calcd for $C_{23}H_{24}N_6O_5S_2 \cdot HI$(656.5): C 41.9, H 3.9, I 19.3, N 12.8, S 14.6.
Found: C 41.4, H 4.2, I 18.5, N 12.5, S 14.2.

Preparation of **II-6** Sulfate

A mixture of the above **II-6** monohydroiodide (13.1 g), Amberlite LA-2 anion exchange resin (20 ml, Rohm & Haas), toluene (60 ml) and water (40 ml) was stirred at room temperature until the salt was dissolved. The phases were separated, the organic phase was washed with water (5 ml) and the combined aqueous phases were washed with toluene (40 ml), treated with activated charcoal (1 g) for 15 minutes and filtered. The aqueous solution was cooled to 5°C and acidified to pH 1.3 with 6 N H_2SO_4. Cold EtOH (80 ml) was added whereupon a crystalline precipitate begun to separate. The suspension was stirred for 2 hours at 5°C, filtered and the solid was washed with EtOH (30 ml) and dried *in vacuo* to constant weight to give 5.6 g (43%) of colorless microneedles, mp >200°C (dec). ^1H NMR Table 6.

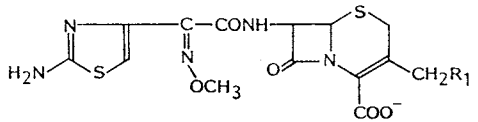
Anal Calcd for $C_{23}H_{24}N_6O_5S_2 \cdot H_2SO_4 \cdot H_2O$(644.7): C 42.8, H 4.4, N 13.0, S 14.9, H_2O 2.8.
Found: C 42.1, H 4.2, N 13.1, S 14.8, H_2O 2.3.

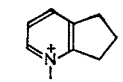
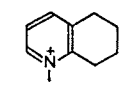
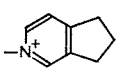
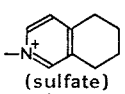
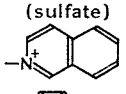
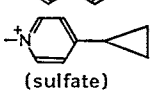
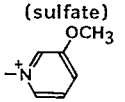
II-8, **II-9**, **II-18** and **II-21** were prepared in an analogous way from **I-1** and the corresponding pyridines. The crude hydroiodide salts were dissolved in aq $NaHCO_3$ and chromatographed over a column of silica gel, eluting with Me_2CO - H_2O (5 : 1). The products were obtained as amorphous solids after lyophilization. Yields and NMR spectra are listed in Tables 6~9.

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-3-[(2,3-cyclopenteno-1-pyridinio)methyl]-ceph-3-em-4-carboxylate (**II-1**)

Method C: To a solution of iodotrimethylsilane (140 g, 100 ml, 0.7 mol) in dry CH_2Cl_2 (900 ml) cooled to 5°C, 2,3-cyclopentenopyridine (102 g, 0.85 mol) was added, maintaining the temperature below 20°C. **I-1** (45.5 g, 0.1 mol) was added and the mixture was heated under reflux for 2 hours. The solution was cooled to 5°C and a solution of potassium iodide (60 g) in 2 N HCl (200 ml) was added over a period of 5 minutes while cooling and stirring. A precipitate formed which was collected by filtration after standing in a refrigerator overnight. It was stirred with three 100 ml portions of ice-water in a beaker, being filtered off with suction each time, then washed with Me_2CO (3×200 ml), and dried to yield 54.5 g (69%) of **II-1** dihydroiodide as yellow crystals, mp 179~181°C (dec). Analytical HPLC: 96 area % purity.

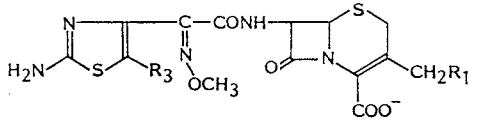
Table 6. Method of preparation, yield and ^{1}H NMR data of pyridinium cephalosporins **II**.

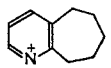
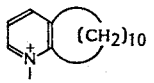
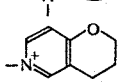
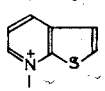
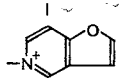
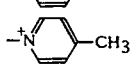
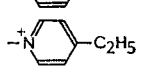
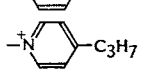
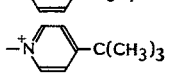


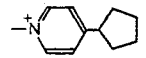
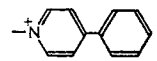
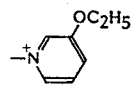
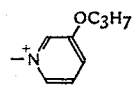
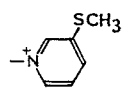
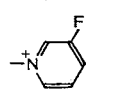
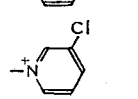
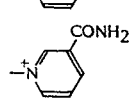
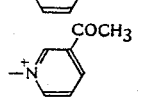
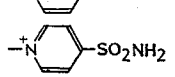
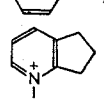
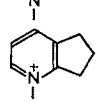
Compound	R_1	Method of preparation (yield, %)	^{1}H NMR δ (DMSO-d_6) (J in Hz)								
			Thiazole-H (1H, s)	OCH_3 (3H, s)	CONH (1H, d, J=8)	6-H (1H, d, J=5)	7-H (1H, dd, J=5, 8)	2-CH_2 (2H, AB, J=18)	3'-CH_2 (2H, AB, J=15)	Pyridine	Pyridine substituent
1	 (sulfate)	A (23) B (55) C (56)	6.71	3.80	9.66	5.18	5.85	3.41[a]	5.44, 5.55	7.92 (1H, dd, J=7), 8.42 (1H, d, J=7), 8.68 (1H, d, J=7)	2.12~2.30 (2H, m), 3.06~3.17 (2H, m), 3.20~3.31 (2H, m)
2	 (sulfate)	C (58)	6.72	3.82	9.62	5.18	5.86	3.37[a]	5.47, 5.84	7.93 (1H, dd, J=7), 8.36 (1H, d, J=7), 8.72 (1H, d, J=7)	1.7~2.0 (4H, m), 2.9~3.1 (4H, m)
5		A (29)	6.70	3.80	9.52	5.06	5.62	3.03, 3.50	4.98, 5.10	8.01 (1H, dd, J=7), 9.22~9.30 (3H, m)	2.1~2.22 (2H, m), 3.05~3.15 (4H, m)
6	 (sulfate)	B (43)	6.72	3.82	9.63	5.19	5.87	3.38, 3.50	5.39, 5.48	7.89 (1H, d, J=7), 8.64 (1H, d, J=7), 8.80 (1H, s)	1.82 (4H, br s), 2.86 (2H, br s), 2.99 (2H, br s)
8		A (48) B (60) C (36)	6.68	3.78	9.42	5.08	5.68	3.42[a]	5.25 5.62	7.6~8.7 (5H, m), 9.35 (1H, d, J=7), 10.23 (1H, s)	
18	 (sulfate)	B (55) D (49)	6.73	3.80	9.61	5.18	5.86	3.36, 3.51	5.36, 5.46	7.87 (4H, AA'BB', J=7), 8.76	1.08~1.17 (2H, m), 1.37~1.47 (2H, m), 2.24~2.38 (1H, m)
21	 (sulfate)	A (48) B (77) C (61)	6.72	3.80	9.63	5.19	5.88	3.42, 3.54	5.45, 5.56	8.13 (1H, dd, J=7), 8.29 (1H, d, J=7), 8.66 (1H, d, J=7), 8.89 (1H, s)	4.03 (3H, s)

[a] 2H, br s.

Table 7. Method of preparation, yield and ^1H NMR data of pyridinium cephalosporins II.



Compound	R_1	Method of preparation (yield, %)	^1H NMR δ (CF_3COOD) (J in Hz)							
			Thiazole-H (1H, s)	OCH_3 (3H, s)	6-H (1H, d, J=5)	7-H (1H, d, J=5)	2-CH_2 (2H, AB, J=18)	3′-CH_2 (2H, AB, J=15)	Pyridine	Pyridine substituent
3		A (15)	7.42	4.25	5.42	6.10	3.40, 3.65	5.80, 6.05	7.6~8.65 (3H, m)	1.7~2.2 (6H, br s), 3.0~3.6 (4H, m)
4		A (8)	7.43	4.35	5.41	6.02	3.52, 3.71	5.75, 6.02	7.6~8.72 (3H, m)	1.4~2.1 (16H, br s), 2.9~3.5 (4H, m)
7		A (19)	7.43	4.24	5.38	6.12	3.46, 3.63	5.12, 5.86	7.65 (1H, d, J=7), 8.3~8.6 (2H, m)	2.0~2.4 (2H, m), 2.7~3.3 (2H, m), 4.4~4.8 (2H, m)
9		B (56)	7.44	4.28	5.44	6.16	3.54, 3.80	5.82, 6.28	7.83 (1H, d, J=7), 8.0~8.12 (2H, m), 8.5~9.1 (2H, d)	
10		A (16) C (39)	7.40	4.20	5.40	6.08	3.33, 3.83	5.50, 6.25	8.0~8.25 (2H, m), 8.88 (1H, d, J=7), 9.42 (1H, s)	7.30 (1H, d, J=2)
13		A (23) C (61)	7.41	4.22	5.40	6.08	3.50, 3.83	5.53, 6.22	7.9~9.3 (5H, m)	
14		A (28) C (42)	7.42	4.19	5.40	6.11	3.49, 3.77	5.23, 5.95	7.86 (4H, AA′BB′, J=7), 8.76	2.75 (3H, s)
15		A (35)	7.37	4.23	5.38	6.12	3.50, 3.77	5.35, 6.18	7.91 (4H, AA′BB′, J=7), 8.80	1.46 (3H, t), 3.07 (2H, q)
16		A (38)	7.35	4.22	5.40	6.10	2.87~4.06[a] (4H, m)	5.25, 6.05	7.86 (4H, AA′BB′, J=7), 8.78	1.09 (3H, t), 1.85 (2H, br s), 2.87~4.06 (4H, m)[b]
17		A (45)	7.36	4.23	5.38	6.09	3.48, 3.79	5.45, 6.15	8.07 (4H, AA′BB′, J=7), 8.82	1.51 (9H, s)

19		A (19)	7.39	4.23	5.41	6.10	1.38~4.23 (11H, m)[c]	5.38, 6.03	7.95 (4H, AA'BB', J=7), 8.82	1.38~4.23 (11H, m)[b]
20		A (12) C (55)	7.35	4.20	5.42	6.12	3.55, 3.82	5.42 6.15	8.26 (4H, AA'BB', J=7), 8.91	7.51~7.89 (5H, m)
22		A (24)	7.42	4.23	5.41	6.12	3.50, 3.82	5.40, 6.08	7.9~8.15 (2H, m), 8.4~8.7 (2H, m)	1.56 (3H, t), 4.30 (2H, q)
23		A (17)	7.40	4.22	5.38	6.11	3.45, 3.82	5.38, 6.10	7.9~8.2 (2H, m), 8.45~8.7 (2H, m)	1.10 (3H, t), 1.98 (2H, m), 4.20 (2H, m)
24		B (27)	7.42	4.23	5.40	6.10	3.50, 3.81	5.40, 6.10	7.8~8.85 (4H, m)	2.67 (3H, s)
25		A (27)	7.41	4.22	5.40	6.11	3.50, 3.82	5.41, 6.10	7.85~9.2 (4H, m)	
26		A (18)	7.38	4.22	5.38	6.08	3.52, 3.83	5.38, 6.15	7.9~9.1 (4H, m)	
27		A (14) C (45)	7.37	4.22	5.37	6.08	3.55, 3.86	5.42, 6.18	8.1~8.4 (1H, m), 8.9~9.36 (2H, m), 9.58 (1H, s)	
28		A (36)	7.36	4.24	5.38	6.12	3.61, 3.86	5.41, 6.17	8.15~9.6 (4H, m)	2.91 (3H, s)
29		A (13)	7.40	4.22	5.38	6.12	3.52, 3.82	5.38, 6.10	8.62 (4H, AA'BB', J=7), 9.13	
11		A (20)	—[f]	4.21	5.39	6.10	3.1~4.2 (6H, m)[d]	5.40, 6.01	7.6~8.6 (3H, m)	2.2~2.8 (2H, m), 3.1~3.8 (6H, m)[e]
12		A (14)	—[f]	4.20	5.38	6.08	3.0~4.2 (6H, m)[d]	5.39, 6.08	7.5~8.4 (3H, m)	2.2~2.8 (2H, m), 3.0~3.8 (6H, m)[e]

[a] With 2H propyl. [b] With 2H, 2-CH_2. [c] With 9H cyclopentyl. [d] With 4H cyclopenteno. [e] With 2H, 2-CH_2. [f] Cl, Br instead of H. Compound **11** R_3=Cl, compound **12** R_3=Br.

Anal Calcd for $C_{22}H_{22}N_6O_5S_2 \cdot 2HI \cdot H_2O$(788.4): C 33.51, H 3.32, I 32.19, N 10.66, S 8.13, H_2O 2.3.
Found: C 33.6, H 3.6, I 31.3, N 10.7, S 7.1, H_2O 2.5.

The sulfate, colorless microneedles, mp >200°C (dec), was prepared in 78% yield exactly as described for **II-6** sulfate, except that 12 g of **II-1** dihydroiodide was used.

Anal Calcd for $C_{22}H_{22}N_6O_5S_2 \cdot H_2SO_4$(612.7): C 43.13, H 3.95, N 13.72, S 15.70, SO_4^{2-} 15.69.
Found: C 43.2, H 3.9, N 13.3, S 15.3, SO_4^{2-} 15.8.

^{1}H NMR Table 6; UV $\lambda_{max}^{H_2O}$ nm (ε) 265 (21,100); $[\alpha]_D^{25}$ −4.7° (*c* 5, H_2O); IR (KBr) 1793 cm^{-1} (s, β-lactam C=O); FAB-MS *m/z* 515 and 613 ($M+H^+$ and $M \cdot H_2SO_4+H^+$).

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-3-([2,3-cyclohexeno-1-pyridinio)methyl]-ceph-3-em-4-carboxylate (**II-2**)

II-2 dihydroiodide was prepared from **I-1** and 2,3-cyclohexenopyridine as described for **II-1**·2HI. Yield 72%.

Anal Calcd for $C_{23}H_{24}N_6O_5S_2 \cdot 2HI \cdot 2H_2O$(820.5): C 33.7, H 3.7, N 10.2, S 7.8, I 30.9, H_2O 4.4.
Found: C 32.5, H 3.6, N 9.8, S 7.3, I 30.5, H_2O 3.8.

The sulfate, mp >190°C (dec), was obtained from the dihydroiodide as described for **II-6** sulfate in 80% yield. ^{1}H NMR Table 6.

Anal Calcd for $C_{23}H_{24}N_6O_5S_2 \cdot H_2SO_4 \cdot H_2O$(644.7): C 42.8, H 4.4, N 13.0, S 14.9, H_2O 2.8.
Found: C 42.6, H 4.2, N 13.0, S 14.7, H_2O 3.1.

II-10, **II-13**, **II-14**, **II-20**, **II-21** were analogously prepared from **I-1** and the corresponding pyridines, **II-31** from **I-31** and 2,3-cyclopentenopyridine. The betaines were obtained as amorphous products after chromatography of the dihydroiodide salts (yields and NMR spectra are listed in Tables 6~9).

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-3-[4-cyclopropyl-1-pyridinio)methyl]-ceph-3-em-4-carboxylate Sulfate (**II-18**)

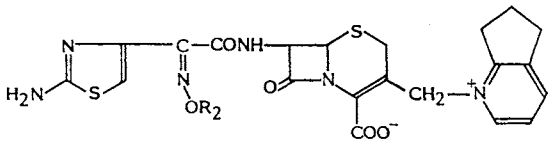
Method D: A solution of 7-amino-3-[(4-cyclopropyl-1-pyridinio)methyl]ceph-3-em-4-carboxylate (**III-18**) hydroiodide (4.6 g, 10 mmol) in DMF (75 ml) was cooled to 5°C and 2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetic acid (**Va**) 1-hydroxybenzotriazole active ester[15] (3.8 g, 12 mmol) was added while stirring. After standing overnight in the refrigerator, the solution was diluted with diisopropyl ether. A dark colored solid precipitated, which was filtered, suspended in a mixture of water (15 ml), EtOAc (25 ml) and Amberlite LA-2 anion exchange resin (10 ml) and stirred at room temperature until all material was dissolved (approx 15 minutes). The aqueous phase was separated, washed with EtOAc (2×20 ml) and acidified with 2 N H_2SO_4 to pH 1.5 EtOH (40 ml) was added whereupon a crystalline precipitate formed. After standing for 3 hours at 0°C, the solid was filtered, washed with EtOH and dried *in vacuo*. Yield 3.0 g (49%) of pale yellow microneedles. Analytical HPLC: 95 area % purity.

Anal Calcd for $C_{22}H_{22}N_6O_5S_2 \cdot H_2SO_4$(612.7): C 43.1, H 3.9, N 13.7, S 15.7.
Found: C 42.9, H 3.7, N 13.2, S 15.2.

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-hydroxyiminoacetamido]-3-[(2,3-cyclopenteno-1-pyridinio)methyl]-ceph-3-em-4-carboxylate Formate (**II-30**)

Method D: A mixture of 2-(2-tritylaminothiazol-4-yl)-2-(*Z*)-trityloxyiminoacetic acid (**Vf**) (6.05 g, 9 mmol), 1-hydroxybenzotriazole hydrate (1.4 g, 9.1 mmol), *N,N'*-dicyclohexylcarbodiimide (1.88 g, 9.1 mmol) and DMF (50 ml) was stirred at room temperature for 2 hours. A solution of 7-amino-3-[(2,3-cyclopenteno-1-pyridinio)methyl]ceph-3-em-4-carboxylate hydroiodide (**III-1**) (4.6 g, 10 mmol) in DMF (50 ml), water (3 ml) and pyridine (1 ml) was then added and stirring was continued for 6 hours at room temperature. Dicyclohexyl urea was filtered and the solvent was removed *in vacuo*. The oily residue was chromatographed over a column of silica gel, eluting with EtOAc - 2-propanol - water (10 : 4 : 1). The product fractions were concentrated to 20 ml. A colorless precipitate formed which was filtered and dried to give 3.6 g. It was dissolved in 80% aq HCOOH. After 4 hours at 25~30°C, precipitated triphenylcarbinol was filtered and the solution evaporated to dryness. The residue was triturated with toluene, Me_2CO and Et_2O and dried to give 1.18 g (24.5%) of a colorless solid.

Table 8. Method of preparation, yield and ^{1}H NMR data of cyclopentenopyridinium cephalosporins II.



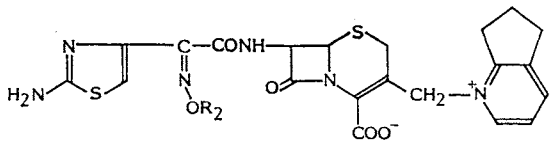
Compound	R_2	Method of preparation (yield, %)	^{1}H NMR[a] δ (DMSO-d_6) (J in Hz)						
			R_2	Thiazole-H (1H, s)	CONH (1H, d, J=8)	6-H (1H, d, J=5)	7-H (1H, dd, J=5, 8)	2-CH_2 (2H, AB, J=18)	3′-CH_2 (2H, AB, J=15)
30	H	D (24)	—	6.62	9.38	5.05	5.65	3.23~3.5 (4H, m)[b]	5.23, 5.44
31	C_2H_5	C (72) D (56)	1.18 (3H, t), 4.06 (2H, q)	6.68	9.55	5.16	5.85	3.38[c]	5.42, 5.53
33		D (42)	1.3~2.5 (8H, m),[b] 4.62 (1H, m)	6.69	9.47	5.04	5.67	3.30 3.80	5.18, 5.45
34	$(CH_2)_{11}CH_3$	D (40)	1.0~1.4 (23H, m), 4.01 (2H, q)	6.61	9.45	5.08	5.71	3.18 3.62	5.16, 5.52

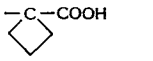
[a] Cyclopentenopyridine: 2.1~2.3 (2H, m), 3.0~3.2 (2H, m), 3.2~3.3 (2H, m), 7.88 (1H, dd), 8.3~8.4 (1H, dd), 9.1~9.2 (1H, d).

[b] With 2 cyclopentene-H.

[c] 2H, br s.

Table 9. Method of preparation, yield and ^{1}H NMR data of cyclopentenopyridinium cephalosporins II.



Compound	R_2	Method of preparation (yield, %)	^{1}H NMR[a] δ (CF_3COOD) (J in Hz)					
			R_2	Thiazol-H (1H, s)	6-H (1H, d, J=5)	7-H (1H, d, J=5)	2-CH_2 (2H, AB, J=18)	3′-CH_2 (2H, AB, J=15)
32	C_3H_7	A (9)	1.02 (3H, t), 1.86 (2H, m), 4.45 (2H, t)	7.42	5.40	6.15	3.1~3.75 (6H, m)[b]	5.50, 5.95
35	CHF_2	D (16)	6.70 (1H, t, J=72)	7.45	5.38	6.12	3.1~3.8 (6H, m)[b]	5.48, 6.05
36	CH_2CONH_2	A (31)	5.08 (2H, s)	7.43	5.42	6.10	3.1~3.8 (6H, m)[b]	5.45, 5.93
37	CH_2COOH	A (21)	5.09 (2H, s)	7.43	5.41	6.12	3.1~3.7 (6H, m)[b]	5.50, 5.90
38	$C(CH_3)_2COOH$	A (24)	1.79 (6H, s)	7.40	5.40	6.11	3.1~3.8 (6H, m)[b]	5.48, 5.95
39	$CH_2=C(CH_3)COOH$	D (12)	5.18 (2H, s), 6.28 (1H, br s), 6.75 (1H, br s)	7.42	5.40	6.12	3.2~3.8 (6H, m)[b]	5.45, 5.90
40		A (32)	2.0~3.0 (8H, m)[c]	7.42	5.42	6.15	3.1~3.8 (6H, m)[b]	5.42, 5.93
41	$C(CH_3)_2CONHSO_2CH_3$	D (48)	1.75 (6H, s), 3.25 (3H, s)	7.42	5.41	6.10	3.1~3.7 (6H, m)[b]	5.43, 5.93
42	$(CH_2)_2CH(NH_2)COOH$	D (15)	2.65~2.8 (2H, m), 4.55~4.75 (3H, m)	7.28	5.39	6.06	3.45, 3.72	5.46, 5.92

[a] Cyclopentenopyridine: 2.2~2.7 (2H, m), 3.1~3.8 (4H, m), 7.6~8.6 (3H, m).
[b] With 4 cyclopentene-H.
[c] With 2 cyclopentene-H.

Compounds **II-11**, **II-12**, **II-31**, **II-33** and **II-41** were prepared in an analogous way from **III-1**·HI and the HOBT active ester from **Vi**, **Vj**, **Vb**, **Vc** and **Vh**. **III-1**·HI and the HOBT active esters of **Vd**, **Vg** and **Ve** gave the protected **II-35**, **II-39** and **II-42**. Chromatography of the crude products followed by removal of the protective groups with 90% aq TFA afforded the trifluoroacetates. These were dissolved in aq $NaHCO_3$ and chromatographed over silica gel with Me_2CO - H_2O (3 : 1), to give the betaines as amorphous solids. Yields and NMR spectra are listed in Tables 8 and 9.

7-Amino-3-[(2,3-cyclopenteno-1-pyridinio)methyl]ceph-3-em-4-carboxylate Hydroiodide (**III-1**)

Method C: 2,3-Cyclopentenopyridine (35.7 g, 0.3 mol) was added to a solution of iodotrimethylsilane (36 ml, 0.25 mol) in CH_2Cl_2 (500 ml) at 10~15°C while stirring, then 7-ACA (13.6 g, 0.05 mol) was added in one portion and the mixture heated under reflux for 2 hours. The dark colored solution was cooled to 5°C and hydrolyzed by the addition of EtOH - H_2O (7 : 1, 400 ml). A dark yellow precipitate formed which was filtered after standing overnight at 5°C, washed with 2-propanol (2× 80 ml) and Me_2CO (2×80 ml) and dried *in vacuo* to give 19.5 g (82%) of the monohydroiodide salt.

Anal Calcd for $C_{16}H_{17}N_3O_3S \cdot HI \cdot H_2O$(477.3): C 40.26, H 4.22, I 26.59, N 8.80, S 6.72.
Found: C 40.2, H 4.2, I 25.7, N 8.7, S 6.5.

IR (KBr) 1786 cm^{-1} (s, β-lactam C=O); ^1H NMR (60 MHz, CF_3COOD) δ 2.3~2.8 (2H, m, cyclopentene-H), 3.1~3.9 (6H, m, 4 cyclopentene-H, SCH_2), 5.7 and 6.0 (2H, AB, J=15 Hz, CH_2N), 5.62 (2H, br s, β-lactam-H), 7.6~8.8 (3H, m, pyridine).

7-Amino-3-[(4-cyclopropyl-1-pyridinio)methyl]ceph-3-em-4-carboxylate Hydroiodide (**III-18**)

Method E: A mixture of 7-amino-3-(iodomethyl)ceph-3-em-4-carboxylic acid (**IV**) (11 g, 32 mmol), bis(trimethylsilyl)acetamide (22 ml, 89 mmol) and CH_3CN (250 ml) was stirred for 10 minutes at room temperature to obtain a clear solution. 4-Cyclopropylpyridine (11.4 g, 96 mmol) was added and stirring was continued for 5 hours at room temperature. Water (2 ml) was then added whereupon a precipitate formed. After 1 hour at 0°C, the solid was filtered, washed with Me_2CO and dried to give 10.1 g (69%) of **III-18** hydroiodide.

Anal Calcd for $C_{16}H_{17}N_3O_3S \cdot HI$(459.3): I 27.6.
Found: I 25.3.

^1H NMR (60 MHz, CF_3COOD) δ 1.0~2.5 (5H, m, cyclopropyl), 3.72 (2H, s, SCH_2), 5.31 and 6.03 (2H, AB, J=15 Hz, CH_2N), 5.57 (2H, s, lactam-H), 7.68 and 8.71 (4H, AA'XX', J=7 Hz, pyridine).

7-Amino-3-(iodomethyl)ceph-3-em-4-carboxylic Acid (**IV**)

To a suspension of 7-ACA (30 g, 0.11 mol) and NaI (20.7 g, 0.14 mol) in CH_3CN (150 ml) was added trifluoromethanesulfonic acid (50 g, 29.5 ml, 0.33 mol) dropwise during 10 minutes while stirring and cooling, maintaining the temperature between 10 and 15°C. The resulting dark colored solution was then stirred at 20~25°C for 30 minutes and kept overnight at 5°C. To the cold solution EtOH - H_2O (1 : 1, 120 ml) was added during 15 minutes. The precipitate formed was filtered, washed with Me_2CO (2×100 ml) and dried *in vacuo*. Yield 22 g (59%) of dark yellow crystals: MP 176°C (dec); ^1H NMR (60 MHz, CF_3COOD) δ 3.65 and 3.85 (2H, AB, J=16 Hz, SCH_2), 4.6 and 4.7 (2H, AB, J= 7 Hz, CH_2N), 5.3 and 5.4 (2H, AB, J=4 Hz, lactam-H).

2-(2-Aminothiazol-4-yl)-2-(*Z*)-(3-*tert*-butoxycarbonylamino-3-*tert*-butoxycarbonyl-prop-1-yl-oxyimino)acetic Acid (**Ve**)

A mixture of ethyl 2-(2-amino-thiazol-4-yl)-2-(*Z*)-hydroxyiminoacetate (**VIa**) (17.2 g, 0.08 mol), *tert*-butyl 4-bromo-2-*tert*-butoxycarbonylaminobutyrate (prepared from *tert*-butyl 4-bromo-2-aminobutyrate and di-*tert*-butyl dicarbonate in dioxane - 1 N NaOH) (27. 1g, 0.08 mol), K_2CO_3 (48 g, 0.35 mol) and Me_2CO (400 ml) was stirred for 42 hours at room temperature. After filtration, the solvent was removed *in vacuo* and the residue chromatographed over a column of silica gel, eluting with toluene - EtOAc (2 : 1). The product fractions gave 4.1 g of the ethyl ester after evaporation of the solvent and trituration with Et_2O. This product was dissolved in EtOH (60 ml) and 1 N NaOH (10 ml). After 20 hours at room temperature, 1 N HCl (10 ml) was added, the solvent was removed by evaporation and

the residue chromatographed over a Lobar-C column of silica gel, eluting with EtOAc - 2-propanol - water (4 : 3 : 2). Lyophilization of the product fractions gave 0.55 g of an amorphous solid: ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.40 (18H, br s, *tert*-butyl), 1.8~2.3 (2H, m, CH_2CH_2CH), 3.6~4.5 (3H, m, CH_2CH_2CH), 6.72 (1H, s, thiazole).

2-(2-Aminothiazol-4-yl)-2-(*Z*)-cyclobutyloxyiminoacetic Acid (**Vc**)

2-(2-Aminothiazol-4-yl)-2-(*Z*)-cyclobutyloxyiminoacetic acid (**Vc**) was similarly prepared from **VIa** and bromocyclobutane followed by saponification with 1 N NaOH - EtOH: MP 180°C (dec); ^{1}H NMR (60 MHz, DMSO-d_6) δ 1.2~2.3 (6H, m, cyclobutane), 4.6 (1H, quintet, J=7 Hz, cyclobutane), 6.82 (1H, s, thiazole), 7.2 (2H, br s, NH_2).

2-(2-Tritylaminothiazol-4-yl)-2-(*Z*)-trityloxyiminoacetic Acid (**Vf**)

A solution of tritylchloride (37.6 g, 0.135 mol) in $CHCl_3$ (60 ml) was added dropwise to a stirred suspension of ethyl 2-(2-aminothiazol-4-yl)-2-(*Z*)-hydroxyiminoacetate (**VIa**) (13.7 g, 0.064 mol) and triethylamine (18.9 ml, 0.136 mol) in $CHCl_3$ (225 ml), cooled to +5°C. Stirring was continued overnight at room temperature. The mixture was then washed with water (100 ml), 1 N HCl (100 ml) and water (2×100 ml). $CHCl_3$ was evaporated, the oily residue was suspended in EtOH (500 ml) and 2 N NaOH (50 ml), and heated at 70°C for 24 hours while stirring. After cooling, a precipitate of the sodium salt formed which was filtered, washed with EtOH and dried. This salt (34 g, 49 mmol) was suspended in EtOAc (500 ml), 1 N HCl (50 ml) was added and the mixture was shaken vigorously for 3 minutes. A small amount of the insoluble salt was removed by filtration, the organic layer was separated, washed with cold water (200 ml) and concentrated to a volume of 150 ml. The precipitated product was filtered, washed with diisopropyl ether and dried *in vacuo*. Yield 20 g (61%) of colorless crystals: MP 157°C (dec); ^{1}H NMR (60 MHz, DMSO-d_6) δ 6.70 (1H, s, thiazole), 7.62 (30H, s, trityl).

2-(*Z*)-(2-*tert*-Butoxycarbonyl-2-propen-1-yl-oxyimino)-2-(2-tritylaminothiazol-4-yl)acetic Acid (**Vg**)

2-(*Z*)-Hydroxyimino-2-(2-tritylaminothiazol-4-yl)acetic acid (**VIc**) (17.2 g, 40 mmol, prepared by treatment of the ethyl ester **VIb** with NaOH in dioxane) was added to a solution of potassium *tert*-butoxide (9.6 g, 85.6 mmol) in dry THF (80 ml). A mixture of water (1 ml) and THF (20 ml) was added dropwise to the suspension at 25°C while stirring. The acid dissolved slowly. After 15 minutes a deep blue solution was formed. *tert*-Butyl-2-(bromomethyl)acrylate (10.5 g, 47 mmol) was then added dropwise whereupon the color changed to red and KBr precipitated. Stirring was continued for 2 hours at room temperature. The suspension was filtered and THF removed by evaporation. The residue was dissolved in EtOAc (300 ml), the solution washed with cold 1 N HCl (100 ml), water (2× 200 ml) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue triturated with Et_2O. After drying 17.2 g (76%) of a brown solid were obtained: ^{1}H NMR (60 MHz, DMSO-d_6) δ 1.45 (9H, s, *tert*-butyl), 4.57 (2H, br s, CH_2), 5.85 and 6.03 (2H, br s, $=CH_2$), 6.67 (1H, s, thiazole), 7.27 (15H, br s, trityl).

2-(2-Aminothiazol-4-yl)-2-(*Z*)-(2-methylsulfonylaminocarbonylprop-2-yl-oxyimino)acetic Acid (**Vh**)

Ethyl-2-(*Z*)-(2-methylsulfonylaminocarbonylprop-2-yl-oxyimino)acetoacetate (**VIId**): A mixture of ethyl 2-(*Z*)-hydroxyiminoacetoacetate (**VIIa**) (15.9 g, 0.1 mol), K_2CO_3 (15.2 g, 0.11 mol), *tert*-butyl-2-bromoisobutyrate (22.3 g, 0.1 mol) and DMF (50 ml) was stirred for 6 hours at room temperature. The salts were filtered, DMF was removed *in vacuo*, the residue dissolved in Et_2O and the etheral solution was washed with 1 N HCl, 1 N NaOH and water. After drying ($MgSO_4$), Et_2O was removed to give **VIIb** as an oil (16.3 g, 54%). This was dissolved in TFA (60 ml). After stirring for 1 hour at 20°C, TFA was evaporated, the residue dissolved in Et_2O, the solution washed with water and dried with $MgSO_4$. Et_2O was removed *in vacuo* to obtain the acid **VIIc** as an oil (11 g, 45 mmol, 84%). This crude product was dissolved in CH_2Cl_2 (50 ml) and a solution of methylsulfonyl isocyanate (6.06 g, 50 mmol) in CH_2Cl_2 (25 ml) was added dropwise at room temperature while stirring. CO_2 evolved and after 2 hours the solvent was removed by evaporation. The residue was dissolved in 10% aq $NaHCO_3$, impurities extracted with Et_2O and the aqueous phase acidified to pH 1 with 2 N HCl. An

oilseparated which was extracted with CH_2Cl_2. After evaporation of the solvent, the residue was triturated with Et_2O to give a crystalline solid (5.6 g, 39%): MP 86~88°C; ^1H NMR (60 MHz, $CDCl_3$) δ 1.32 (3H, t, CH_2CH_3), 1.55 (6H, s, $2\times CH_3$), 2.35 (3H, s, CH_3CO), 3.25 (3H, s, CH_3SO_2), 4.31 (2H, q, CH_2CH_3).

Preparation of **Vh**: A solution of bromine (1 ml) in CH_2Cl_2 (10 ml) was added dropwise to a solution of **VIId** (6.45 g, 20 mmol) and benzoyl peroxide (0.1 g) in CH_2Cl_2 (200 ml). After standing overnight the color had disappeared and the solution was washed with water and evaporated to obtain the ethyl 4-bromoacetoacetate derivative as an amorphous solid (7.8 g, 98%). This product (19.5 mmol) was dissolved in THF (10 ml) and added dropwise to a solution of thiourea (1.52 g, 20 mmol) in EtOH - H_2O (2:1, 15 ml). The pH was adjusted to 5.0 by dropwise addition of concd NH_3. After standing overnight the mixture was concentrated *in vacuo*, the residue dissolved in concd aq $NaHCO_3$ (10 ml) and the aqeous solution washed with CH_2Cl_2. The aqueous phase was acidified with 2 N HCl to pH 5. A gummy product separated which was recrystallized from EtOH to give the **Vh** ethyl ester as pale yellow crystals (2.9 g, 40%, mp 152~155°C). The ester was dissolved in 1 N NaOH (20 ml). After standing 3 hours at room temperature, 1 N HCl (20 ml) was added. A crystalline precipitate separated which was filtered, washed with cold water and dried to give 1.3 g. Concentration of the mother liquor afforded a further crop of 0.8 g (total yield 78%): MP 197°C (dec); ^1H NMR (60 MHz, DMSO-d_6) δ 1.43 (6H, $2\times CH_3$), 3.25 (3H, s, CH_3SO_2), 6.90 (1H, s, thiazole).

2-(2-Amino-5-bromothiazol-4-yl)-2-(*Z*)-methoxyiminoacetic Acid (**Vj**)

A solution of bromine (32 g) in AcOH (100 ml) was added dropwise to a cooled suspension (15~20°C) of **Va** (40.2 g, 0.2 mol) in AcOH (300 ml) while stirring. Bromination took place immediately. The addition of the bromine occupied about 1 hour and the color of the suspension changed from colorless to yellow. After 2 hours, the precipitate was filtered, washed with AcOH and water. The crude product was recrystallized from EtOH. Yield 48.2 g (86%) of yellow crystals: MP 135~137°C (dec); ^1H NMR (60 MHz, DMSO-d_6) δ 3.86 (3H, s, OCH_3).

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