NEW BROAD-SPECTRUM CEPHALOSPORINS WITH ANTI-PSEUDOMONAL ACTIVITY

III. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 7β-[D-2-(4-HYDROXY-6-METHYLPYRIDINE-3-CARBONYLAMINO)-2-(4-HYDROXYPHENYL)ACETAMIDO]-3-(METHYL OR SUBSTITUTED METHYL)-CEPH-3-EM-4-CARBOXYLIC ACIDS

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The influence of various 3-substituents on the antibacterial activity of 7β-[D-2-(4-hydroxy-6-methylpyridine-3-carbonylamino)-2-(4-hydroxyphenyl)acetamido]ceph-3-em-4-carboxylic acids (**III**) was investigated. Introduction of an acidic substituent, such as a sulfo or a carboxyl group, to a 3-(1-methyl-1*H*-tetrazolyl)thiomethyl substituent (**IIIf~i**) resulted in a marked loss of activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*, in contrast to an in crease of activity against *Proteus mirabilis*. Displacement of the acetoxy group of **IIIb** with pyridines (**IIIm~p**) enhanced the activity against *P. aeruginosa* and *E. aerogenes*: their activity against those strains were superior to that of the cephalosporin **IIId** having a 3-(1-methyl-1*H*-tetrazolyl)thiomethyl substituent. As a result of extensive studies in addition to the study of *in vitro* activity in this series, 7β-[D-2-(4-hydroxy-6-methylpyridine-3-carbonylamino)-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid, code No. SM-1652, cefpiramide (generic name), was selected as a candidate for further biological and clinical investigations.

In a preceding paper[1], we reported the study of the influence of various *N*-acyl moieties on the antibacterial activity of 7β-[2-acylamino-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)-thiomethyl]ceph-3-em-4-carboxylic acids. This property as well as the pharmacokinetic characteristics of the compounds may, however, also be influenced by the nature of the 3-substituents of the cephem nucleus.

We report here a study on the influence of various 3-substituents on the antibacterial activity of this type of cephalosporin. The 4-hydroxy-6-methylpyridine-3-carbonyl group, which has been found favorably affect the activities of the cephalosporins[1], was chosen as the *N*-acyl moiety.

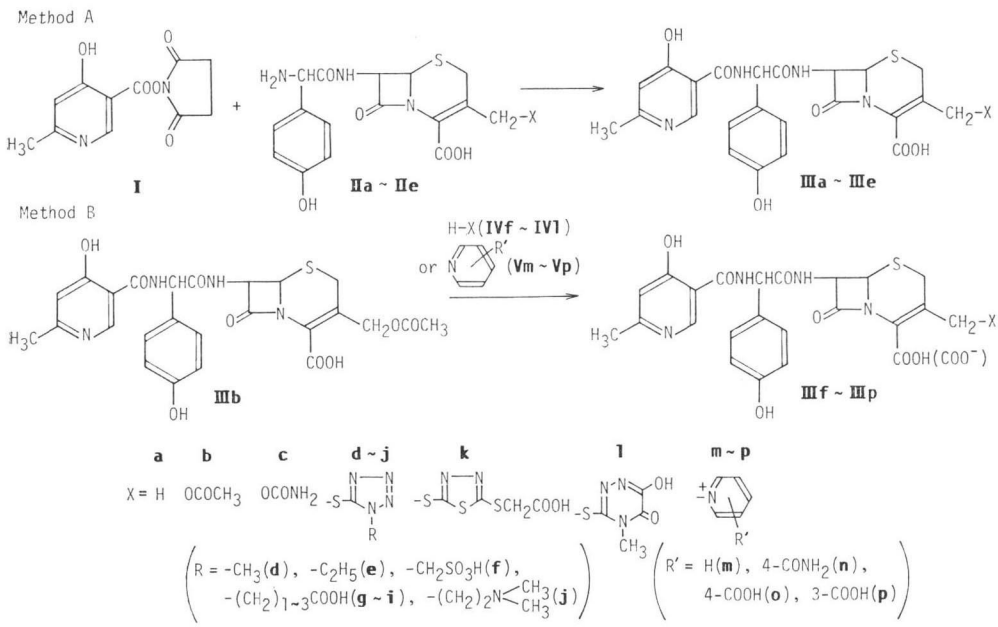
Chemistry

The cephalosporins listed in Table 1 were prepared by the two general methods outlined in Scheme 1.

Only cephalosporins having the *R*-configuration of the chiral center of the 7β-side chain were prepared because a preceding investigation[1] had demonstrated that in at least one case the corresponding *S*-epimer has inferior biological activity.

The general synthetic procedures (Methods A and B) were the same as those described in a previous report[2] except for the preparation of **IIIm~p**, which were obtained by displacing the acetoxy group of

Scheme 1.



IIIb with appropriate pyridines according to a well-known procedure[3,4].

Biological Results and Discussion

The minimal inhibitory concentration (MIC) values of the cephalosporins thus synthesized against three species of Gram-positive bacteria and seven species of Gram-negative bacteria were determined by the serial two-fold agar dilution method[13]. The results are listed in Table 1. The following structure-activity relationships are derived from Table 1.

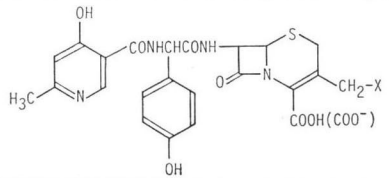
Compound **IIIa** having a methyl group in the 3-position of the cephem nucleus was less active than its other compounds against the bacteria tested except *Staphylococcus aureus* and *Klebsiella pneumoniae*.

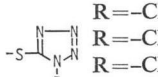
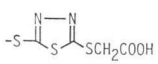
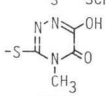
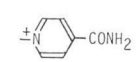
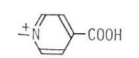
Compounds **IIIb** and **IIIc**, having a 3-acetoxymethyl group and a 3-carbamoyloxymethyl group respectively, showed good *in vitro* activity against most of the strains tested, but they were less active against *Escherichia coli*, *K. pneumoniae*, and *Serratia marcescens* than **IIId**, having a 3-(1-methyl-1*H*-tetrazolyl)thiomethyl group.

Replacement of the methyl group of the 1-methyl-1*H*-tetrazolylthio residue of **IIId** by an ethyl group gave a compound, **IIIe**, almost as active as **IIId**.

Replacement with an acidic substituent, such as a sulfomethyl or a carboxymethyl group, gave compounds (**IIIf, g**) with significantly decreased activity against *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *E. coli*, *K. pneumoniae*, and *Enterobacter aerogenes*, but with increased activity against *Proteus mirabilis*. The same pattern of this activity was found when the number of methylenes in the 1-carboxyalkylene-1*H*-tetrazolylthio group was increased (**IIIh, i**). Compound **IIIk**, carrying a carboxyl substituent on a 1,3,4-thiadiazolylthio group, also showed activity similar to that of **IIIf~i** against Gram-negative bacteria, but unlike **IIIf~i**, it retained activity similar to **IIId** against *S. epidermidis* and *S. faecalis*.

Table 1. *In vitro* antibacterial activity of the cephalosporins.



Compound No.	X	MIC (μg/ml)[a]									
		S. a.	*S. e.*	*S. f.*	*E. c.*	*K. p.*	*P. m.*	*P. v.*	*E. a.*	*P. a.*	*S. m.*
IIIa	–H	1.56	12.5	100	100	0.39	100	25	>100	50	>100
b	$-OCOCH_3$	0.39	1.56	6.25	3.13	0.39	3.13	0.10	3.13	0.78	>100
c	$-OCONH_2$	0.78	1.56	6.25	6.25	0.20	3.13	0.20	6.25	0.78	100
d	$R=-CH_3$	0.39	3.13	6.25	0.39	0.05	3.13	0.05	1.56	0.78	6.25
e	$R=-CH_2CH_3$	0.39	1.56	3.13	0.39	≤0.025	3.13	≤0.025	1.56	1.56	6.25
f	 $R=-CH_2SO_3H$	6.25	25	100	3.13	0.20	0.20	≤0.025	6.25	0.78	12.5
g	$R=-CH_2CO_2H$	6.25	50	100	3.13	0.39	0.20	≤0.025	12.5	1.56	25
h	$R=-(CH_2)_2CO_2H$	3.13	25	50	1.56	0.20	0.39	≤0.025	12.5	1.56	12.5
i	$R=-(CH_2)_3CO_2H$	3.13	25	50	1.56	0.20	0.39	≤0.025	12.5	1.56	6.25
j	$R=-(CH_2)_2N(CH_3)_2$	1.56	25	50	0.39	0.05	25	0.05	12.5	12.5	12.5
k		1.56	3.13	6.25	1.56	0.10	0.78	0.05	6.25	0.78	12.5
l		0.20	6.25	6.25	0.39	≤0.025	6.25	≤0.025	6.25	3.13	12.5
m		0.20	0.39	12.5	3.13	0.20	12.5	0.20	0.39	0.20	25
n		0.39	1.56	50	1.56	0.10	12.5	0.10	0.20	0.10	25
o		1.56	1.56	50	3.13	0.78	1.56	≤0.025	0.78	0.20	>100
p		1.56	1.56	>100	6.25	0.78	6.25	0.05	1.56	0.39	>100

[a] The MIC's were determined by the serial two-fold dilution method[13].
Test organisms and abbreviations: *S. a.*, *Staphylococcus aureus* 209 P; *S. e.*, *Staphylococcus epidermidis* IAM 1296; *S. f.*, *Streptococcus faecalis* NCTC 8213; *E. c.*, *Escherichia coli* NIHJ JC-2; *K. p.*, *Klebsiella pneumoniae* ATCC 10031; *P. m.*, *Proteus mirabilis* GN 2425; *P. v.*, *Proteus vulgaris* OX-19; *E. a.*, *Enterobacter aerogenes* ATCC 13048; *P. a.*, *Pseudomonas aeruginosa* IFO 3451; *S. m.*, *Serratia marcescens* X100.

Introduction of a dimethylaminoethyl group onto the 1*H*-tetrazolylthio group produced a partly different effect on activity compared with that of acidic groups: **IIIj** showed a significant decrease of activity against *P. mirabilis* and *P. aeruginosa* in addition to a decrease of activity against *S. aureus, S. epidermidis, S. faecalis* and *E. aerogenes*, but retained activity against *E. coli, K. pneumoniae, P. vulgaris* and *S. marcescens* similar to that of **IIId**. It has been reported that the 1-dimethylaminoethyl-1*H*-tetrazol-5-ylthiomethyl substituent increased the activity of 7-[2-(2-aminothiazol-4-yl)acetamido]-cephalosporins[5]. However, in the present cephalosporin structure, this substituent was not as effective as the 1-methyl-1*H*-tetrazol-5-ylthiomethyl substituent.

Replacement of the 1-methyl-1*H*-tetrazolylthio group with a 4-methyl-5-oxo-6-hydroxy-4,5-dihydro-1,2,4-triazin-3-ylthio group (**IIIl**) resulted in a decrease of the *in vitro* activity against *P. aeruginosa* and *E. aerogenes*, but did not significantly change the activity against the other species.

Replacement of the 3-acetoxy group of **IIIb** with pyridine leads to **IIIm**, which was less active than **IIId** against *E. coli, K. pneumoniae, P. mirabilis, P. vulgaris*, and *S. marcescens*, but more active against *S. epidermidis, E. aerogenes* and *P. aeruginosa*. In particular, the antipseudomonal activity of **IIIm** was noteworthy. Introduction of a 4-carbamoyl substituent into the pyridine ring (**IIIn**) resulted in a slight increase of the *in vitro* activity against *E. coli, K. pneumoniae, P. vulgaris, E. aerogenes*, and *P. aeruginosa* and a decrease of *in vitro* activity against *S. aureus, S. epidermidis* and *S. faecalis*. A 4-carboxyl substituent on the pyridine ring (**IIIo**) remarkably increased the *in vitro* activity against *P. mirabilis* and *P. vulgaris* but decreased it against *S. aureus, S. epidermidis, S. faecalis, K. pneumoniae* and *S. marcescens*. Compound **IIIp**, having a 3-carboxyl substituent on the pyridine ring, was less active than compound **IIIo**.

Among the various 3-substituents tested, the 1-methyl- and 1-ethyl-1*H*-tetrazol-5-ylthiomethyl substituents gave the cephalosporins with the highest *in vitro* activity and the broadest antibacterial spectrum. In addition to the study of structure-activity relationships in *in vitro* activity in this series[1,2], extensive studies on pharmacokinetics, effectiveness in protecting small animals against bacterial infection, physicochemical properties (solubility and stability), *etc.* were carried out. As a result sodium 7-[D-2-(4-hydroxy-6-methylpyridine-3-carbonylamino)-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylate [sodium salt of **IIId**, code No. SM-1652, cefpiramide (generic name)] was selected as a candidate for further biological and clinical investigations. Laboratory evaluation[6,14] and the preliminary study in humans[7] of cefpiramide have been reported.

Experimental

Infrared spectra were recorded on a Hitachi model EPI-G3 spectrophotometer. NMR spectra were recorded on a JEOL FX-90Q (90 MHz) spectrometer, a Varian EM-390 (90 MHz) spectrometer or a Varian T-60 (60 MHz) spectrometer using TMS as an internal standard; all chemical shifts are reported in δ values. Synthetic methods, empirical formulas and IR data, and NMR data of the cephalosporins synthesized are shown in Tables 2 and 3, respectively.

Materials

IVe[8], **IVf**[9], **IVg**[9,10], **IVh**[9], **IVi**[9], **IVj**[5], **IVk**[11], and **IVl**[12] were prepared as reported in the reference.

IIId was reported in the preceding paper[1].

7β-[D-2-(4-Hydroxy-6-methylpyridine-3-carbonylamino)-2-(4-hydroxyphenyl)acetamido]cephalosporanic Acid Triethylamine Salt (Triethylamine Salt of **IIIb**)

To a stirred suspension of triethylamine (3.03 g, 30 mmole) and *N*-(4-hydroxy-6-methylpyridine-3-

Table 2. Synthetic methods, empirical formulas, and IR data of the cephalosporins.

Compound No.	Method	Formula[a]	IR(KBr) β-lactam (cm^{-1})	Compound No.	Method	Formula[a]	IR(KBr) β-lactam (cm^{-1})
IIIa	A	$C_{23}H_{22}N_4O_7S \cdot 1.5H_2O$	1755	**IIIi**	B	$C_{23}H_{28}N_8O_9S_2 \cdot 3.5H_2O$	1780
b	A	$C_{25}H_{24}N_4O_9S \cdot 1.5H_2O$	1765	**j**	B	$C_{28}H_{30}N_9NaO_7S_2 \cdot 8H_2O$[d]	1763
c	A	$C_{24}H_{23}N_5O_9S \cdot H_2O$	1770	**k**	B	$C_{27}H_{24}N_6O_9S_4 \cdot 7.5H_2O$	1775
d	A	$C_{25}H_{24}N_8O_7S_2 \cdot 2.5H_2O$	1770	**l**	B	$C_{27}H_{25}N_7O_9S_2 \cdot 8H_2O$[e]	1775
e	A	$C_{26}H_{26}N_8O_7S_2 \cdot 2.5H_2O$	1775	**m**[1]	B	$C_{28}H_{25}N_5O_7S \cdot 5.5H_2O$	1770
f	B	$C_{25}H_{22}N_8Na_2O_{10}S_3 \cdot 7.5H_2O$[b]	1760	**n**[1]	B	$C_{29}H_{26}N_6O_8S \cdot 5H_2O$[f]	1770
g	B	$C_{26}H_{23}N_8NaO_9S_2 \cdot 3H_2O$[c]	1768	**o**[1]	B	$C_{29}H_{25}N_5O_9S \cdot 3H_2O$[g]	1775
h	B	$C_{27}H_{26}N_8O_9S_2 \cdot 2H_2O$	1773	**p**[1]	B	$C_{29}H_{25}N_5O_9S \cdot 4.5H_2O$[h]	1775

[a] Compounds were analyzed for C, H, and N. Unless otherwise indicated, analyses are within ±0.4% of the theoretical values.
[b] Disodium salt of **IIIf**, N: calcd. 12.85; found 12.20.
[c] Monosodium salt of **IIIg**.
[d] Sodium salt of **IIIj**, N: calcd. 15.08; found 14.22.
[e] H: calcd. 5.17; found 4.47.
[f] H: calcd. 5.12; found 4.32, N: calcd. 11.86; found 11.24.
[g] H: calcd. 4.64; found 4.11.
[h] H: calcd. 4.89; found 4.07.
[1] It is presumed that 3-pyridinium forms an inner salt with a 4-carboxyl group of the cephem nucleus.

carbonyloxy)succinimide[1] (2.50 g, 10 mmole) in 27 ml of dimethylsulfoxide was added **IIb**[2] (trifluoroacetic acid salt; 5.35 g, 10 mmole). The reaction mixture was stirred at room temperature for 30 minutes and then filtered to remove a small amount of insoluble material. The clear filtrate was added to stirred acetone (600 ml). The precipitate formed was collected, washed on the filter with acetone and dried *in vacuo*; yield 5.98 g. This triethylamine salt of **IIIb** was used for the following displacement reaction without further purification.

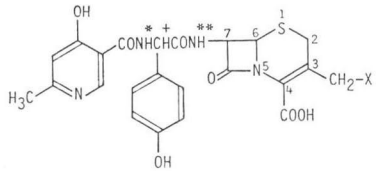
NMR (DMSO-d_6) δ 1.16 (t, 9H, J=7.5 Hz, $-CH_2CH_3$), 1.91 (s, 3H, $OCOCH_3$), 2.30 (s, 3H, pyridine-CH_3), 3.05 (q, 6H, J=7.5 Hz, $-CH_2CH_3$), 3.33 (broad s, 2H, C_2-H_2), 4.72 (broad s, 2H, C_3-CH_2), 5.00 (d, 1H, J=5 Hz, C_6-H), 5.77 (dd, 1H, J=5, 8 Hz, C_7-H), 6.20 [broad, 1H, $-CH(NH)$], 6.29 (s, 1H, pyridine 5-H), 6.57, 7.13 (each d, 2H×2, J=8 Hz, phenyl protons), 8.17 (s, 1H, pyridine 2-H), 9.17 (d, 1H, –CONH–), 11.65 (d, 1H, J=8 Hz, –CONH–).

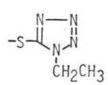
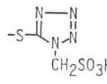
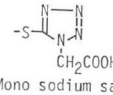
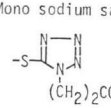
General Procedure for Displacing the 3-Acetoxy Group of the Cephalosporin **IIIb**, with 1-Carboxy (or Sulfo)-alkyl-1*H*-tetrazole-5-thiols or 2-Carboxymethylthio-1,3,4-thiadiazole-5-thiol

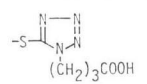
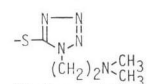
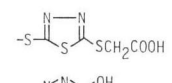
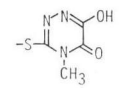
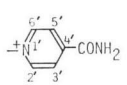
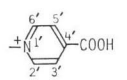
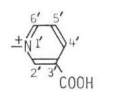
Synthesis of Cephalosporins **IIIf**~**i, k**: A solution containing the triethylamine salt of the cephalosporin **IIIb** (10 mmole), 1-carboxy (or sulfo)-alkyl-1*H*-tetrazole-5-thiol (14 mmole) or 2-carboxymethylthio-1,3,4-thiadiazole-5-thiol (14 mmole), and $NaHCO_3$ (21 mmole) in 90 ml of phosphate buffer (pH 6.4) was heated at 55°C for 18~24 hours. When the reaction was complete (monitored by HPLC), the solution was cooled to room temperature and adjusted to pH 2~2.5 by the addition of 2 N HCl. The precipitate was collected, washed on a filter with water and dried *in vacuo* to give a crude product, which was purified by preparative liquid chromatography on a reverse phase column, LiChroprep RP-8 or μBondapak C-18, with a mobile phase consisting of 0.01 M phosphate buffer (pH 6.8) and methanol or tetrahydrofuran. Each mobile phase used was as follows: **IIIf** and **IIIg**, 94% Buffer+6% THF; **IIIh**, 85% Buffer+15% MeOH; **IIIi**, 83% Buffer+17% MeOH; **IIIk**, 84% Buffer+16% MeOH. The fractions containing the product were combined and concentrated *in vacuo* to a small volume and adjusted to pH 2 by the addition of 2 N HCl at 0~5°C. The precipitate formed was collected, washed on a filter with water and dried *in vacuo* over phosphorus pentoxide.

Sodium salts of **IIIf** and **IIIg** were prepared by lyophilization of solutions consisting of equimolecular amounts of the cephalosporin (acid form) and $NaHCO_3$.

Table 3. ^{1}H NMR data of the cephalosporins **III**.



Compound No.	X	NMR δ value (DMSO-d_6)[a)]								
		-CONH*- 1H, d J=7~8 Hz	-CONH**- 1H, d J=8~9 Hz	C_7-H, -CH$^+$- 2H	C_6-H 1H, d J=5 Hz	C_3-CH_2 2H	C_2-H_2 2H	Phenyl protons J=8~9 Hz	Pyridine ring protons	X
IIIa	-H	11.08	9.23	5.54~5.74 (m)	4.95	—	3.23 3.48 (ABq, J=18 Hz)	6.69 (d, 2H) 7.22 (d, 2H)	2.25 (s, 3H, CH_3) 6.25 (s, 1H, 5-H) 8.26 (m, 1H, 2-H)	1.98 (s, 3H)
IIIb	-OCOCH$_3$	11.03	9.23	5.60~5.76 (m)	4.97	4.63 4.94 (ABq, J=13 Hz)	3.46 (br)	6.66 7.19	2.23 (s, 3H, CH_3) 6.23 (s, 1H, 5-H) 8.23 (m, 1H, 2-H)	2.01 (s, 3H, CH_3)
IIIc	-OCONH$_2$	11.09	9.26	5.62~5.77 (m)	5.01	4.57 4.83 (ABq, J=13 Hz)	3.43 (br)	6.70 7.23	2.26 (s, 3H, CH_3) 6.26 (s, 1H, 5-H) 8.26 (m, 1H, 2-H)	6.57 (s, 2H, NH_2)
IIIe		11.05	9.26	5.60~5.77 (m)	4.97	4.28[ov)]	3.59 (br)	6.69 7.20	2.25 (s, 3H, CH_3) 6.24 (s, 1H, 5-H) 8.26 (m, 1H, 2-H)	1.40 (t, 3H, CH_3) 4.28 (q, CH_2)[ov)]
IIIf		10.50	9.30	5.57~5.80 (m)	4.97	4.23 (br)	3.62 (br)	6.57 7.23	2.37 (s, 3H, CH_3) 6.57 (s, 1H, 5-H) 8.46 (s, 1H, 2-H)	5.03 (s, 2H, CH_2)
IIIg	 (Mono sodium salt)	11.29	9.17	5.63 (dd, C_7-H) 5.85 (d, -CH$^+$-)	4.93	4.20 (br)	3.45 (br)	6.59 7.17	2.27 (s, 3H, CH_3) 6.23 (s, 1H, 5-H) 8.18 (s, 1H, 2-H)	4.80 (s, CH_2)
IIIh		11.03	9.27	5.58~5.80 (m)	4.97	4.27~4.57[ov)] (m)	3.62 (br)	6.83 7.27	2.27 (s, 3H, CH_3) 6.27 (s, 1H, 5-H) 8.27 (s, 1H, 2-H)	2.93 (t, 2H, N-CH_2) 4.27~4.57[ov)] (-CH_2COOH)

IIIi		11.08	9.30	5.58~5.82 (m)	4.98	4.20~ 4.45[ov]	3.63 (br)	6.73 7.27	2.27 (s, CH_3) 6.28 (s, 1H, 5-H) 8.28 (s, 1H, 2-H)	2.00~2.33 (m, N–CH_2CH_2–) 4.20~4.45[ov] (broad s, –CH_2COOH)
IIIj	 (Sodium salt)	11.52	9.13	5.68 (dd, C_7-H) 6.20 (d, –CH+)	4.93	4.0~ 4.4[ov] (m)	3.0~ 3.8[ov] (m)	6.50 7.10	2.32 (s, 3H, CH_3) 6.25 (s, 1H, 5-H) 8.12 (s, 1H, 2-H)	2.03 (s, 6H, $N(CH_3)_2$) 3.0~3.8[ov] ($-CH_2N<^{Me}_{Me}$) 4.0~4.4[ov] (tetrozol-CH_2)
IIIk		10.39	9.27	5.65~5.78 (m)	4.98	4.20 4.47 (ABq, J=13Hz)	3.58 (br)	6.72 7.25	2.27 (s, 3H, CH_3) 6.28 (s, 1H, 5-H) 8.30 (s, 1H, 2-H)	4.12 (s, 2H, SCH_2COOH)
IIIl		11.05	9.26	5.60~5.77 (m)	4.97	[b]	3.56	6.69 7.21	2.26 (s, 3H, CH_3) 6.24 (s, 1H, 5-H) 8.24 (m, 1H, 2-H)	3.28 (s, 3H, CH_3)
IIIm		11.02	9.28	5.4~6.0[ov] (m)	5.07	5.4~ 6.0[ov]	3.43 (br)	6.70 7.23	2.26 (s, 3H, CH_3) 6.25 (s, 1H, 5-H) 8.27 (s, 1H, 2-H)	8.15 (t, 2H, 3′-H, 5′-H) 8.58 (d, 1H, 4′-H) 9.02 (d, 2H, 2′-H, 6′-H)
IIIn		11.12	9.24	5.60~5.77[ov] (m)	4.97	5.20 (d,1H) 5.60~ 5.77[ov]	3.11 3.44 (ABq, J=18Hz)	6.65 7.18	2.27 (s, 3H, CH_3) 6.25 (s, 1H, 5-H) 8.29 (s, 1H, 2-H)	8.45 (d, 2H, 3′-H, 5′-H) 9.49 (d, 2H, 2′-H, 6′-H)
IIIo		11.08	9.1~ 9.3[ov]	5.2~6.0[ov]	5.01	5.2~ 6.0[ov]	3.38 (br)	6.67 7.20	2.29 (s, 3H, CH_3) 6.25 (s, 1H, 5-H) 8.30 (s, 1H, 2-H)	8.43 (d, 2H, 3′-H, 5′-H) 9.25[ov] (d, 2H, 2′-H, 6′-H)
IIIp		11.06	9.2~ 9.4[ov]	5.3~6.0[ov]	5.01	5.3~ 6.0[ov]	3.23 3.46 (ABq, J=18Hz)	6.65 7.18	2.27 (s, 3H, CH_3) 6.24 (s, 1H, 5-H) 8.28 (s, 1H, 2-H)	8.19 (t, 1H, 5′-H) 8.91 (d, 1H, 4′-H) 9.2~9.4[ov] (6′-H) 9.57 (s, 1H, 2′-H)

[a] In NMR descriptions, s=singlet, d=doublet, dd=double doublet, m=multiplet, q=quartet, t=triplet, br=broad singlet, ABq=AB quartet, [ov] signals overlapped each other.

[b] It was difficult to read the δ value because the signals overlapped with those of water.

Synthesis of **IIIj**: Displacement was carried out by the procedure described above except that the amount of $NaHCO_3$ was changed from 21 mmole to 17 mmole.

7β-[D(−)-2-(4-Hydroxy-6-methylpyridine-3-carbonylamino)-2-(4-hydroxyphenyl)acetamido]-3-[(4-methyl-5-oxo-6-hydroxy-4,5-dihydro-1,2,4-triazin-3-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**IIIl**)

A solution containing the triethylamine salt of the cephalosporin **IIIb** (5.0 g, 7.6 mmole), 3-mercapto-4-methyl-5-oxo-6-hydroxy-4,5-dihydro-1,2,4-triazine (1.45 g, 9.1 mmole), and $NaHCO_3$ (0.703 g, 8.4 mmole) in 66 ml of phosphate buffer (pH 6.0) was heated at 55°C for 20 hours. The solution was cooled to room temperature and adjusted to pH 2.0 by the addition of 2 N HCl. The precipitate was collected, washed on a filter with water and dried *in vacuo* over phosphorus pentoxide; yield 4.83 g. It was purified by preparative liquid chromatography on a reverse phase column (μBondapak C-18) with a mobile phase consisting of 0.01 M phosphate buffer (pH 6.8) and MeCN (9: 1, by volume).

General Procedure for Displacing the 3-Acetoxy Group of the Cephalosporin **IIIb** with Pyridines

Synthesis of Cephalosporins **IIIm**~**p**: A solution containing the triethylamine salt of the cephalosporin **IIIb** (10 mmole), potassium thiocyanate (25 g), pyridine or its derivative (15 mmole) and $NaHCO_3$ (15 mmole: it was added only for the preparation of **IIIo, p**) in 25 ml of water was heated at 55°C for 18~24 hours. When the reaction was complete (monitored by HPLC), the solution was cooled to room temperature. Water (60 ml) was added to the reaction mixture and the precipitate was removed by filtration. The filtrate was adjusted to pH 2 by the addition of 3 N HCl and cooled in an ice-bath with stirring. The precipitate was collected, washed on a filter with water and dried *in vacuo*.

This was purified by preparative liquid chromatography on a reverse phase column (LiChroprep RP-8) with a mobile phase consisting of 0.01 M phosphate buffer (pH 6.8) and MeOH. Each mobile phase used was as follows: **IIIm**, 80% Buffer+20% MeOH; **IIIn** and **IIIp**, 82% Buffer+18% MeOH; **IIIo**, 87% Buffer+13% MeOH (by volume).

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