SEMISYNTHETIC CEPHAMYCINS. II

STRUCTURE-ACTIVITY STUDIES RELATED TO CEFMETAZOLE (CS-1170)

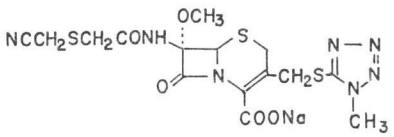
HIDEO NAKAO, HIROAKI YANAGISAWA, SADAO ISHIHARA, EIJI NAKAYAMA, AKIKO ANDO, JUN-ICHI NAKAZAWA, BUNJI SHIMIZU, MASAKATSU KANEKO, MITSUO NAGANO and SHINICHI SUGAWARA

Central Research Laboratories, Sankyo Co., Ltd., Shinagawa-ku, Tokyo, 140, Japan

(Received for publication November 21, 1978)

Synthesis and *in vitro* antimicrobial activity of a number of cephalosporins related to cefmetazole (CS-1170) are described.

In a previous paper[1], we reported the synthesis and antimicrobial activity of a new semisynthetic cephamycin, cefmetazole (CS-1170), which showed not only potent broad-spectrum antibacterial activity but also remarkable stability against β-lactamase. Recently a semisynthetic cephamycin (SKF-73678)[2~4] similar to cefmetazole was reported to have similar activities. As an extension of our studies, to synthesize new cephamycins which are highly resistant to β-lactamase as well as highly potent against Gram-positive and Gram-negative bacteria, we attempted further modification of cefmetazole. This article presents some results of our structure-activity studies.



Cefmetazole (CS-1170)

1. Modification of the 7β-Acyl Group

The (cyanomethylthio)acetyl group in cefmetazole was replaced by other (substituted-thio)acetyl groups. The cephamycins were prepared by either of the two general methods outline in Scheme 1. One method employed acylation of diphenylmethyl 7β-amino-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate(**II**) with an appropriate acyl chloride(**I**) followed by hydrolysis of the esters(**III**). The other method was to react an appropriate mercaptan with 7β-chloroacetamido-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acid(**IV**). The *in vitro* antibacterial activities of these compounds are shown in Table 1.

The data in Table 1 show that when the cyanomethyl group is replaced by other groups, the activity against Gram-negative bacteria is generally decreased except for the 2-carboxyethyl congener(**13**). The cyanobenzyl derivative (**5**) is only weakly active against Gram-negative organisms, however, its activity against Gram-positive bacteria is slightly better than that of cefmetazole. The α-methyl analog (**6**) is also significantly less active than other congeners. The cyanomethyloxyacetyl analog (**16**) is about four times less active than cefmetazole against both Gram-positive and Gram-negative bacteria.

Scheme 1.

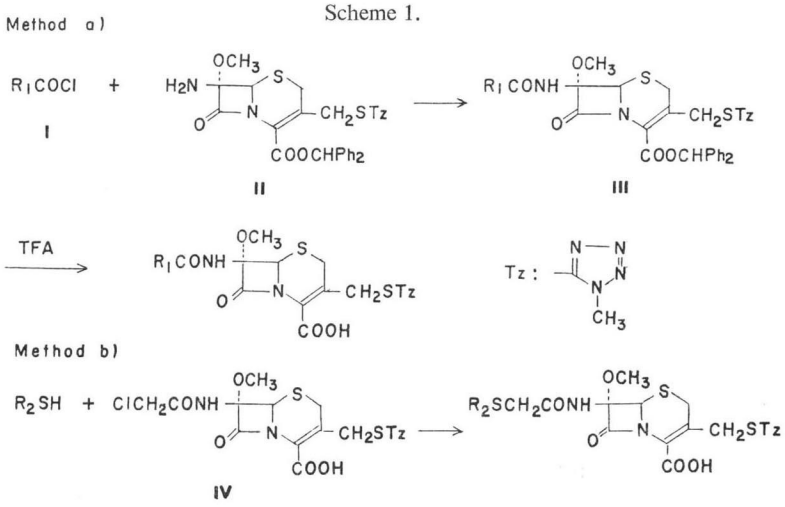
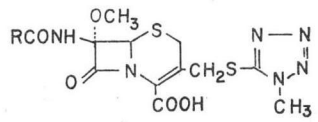


Table 1. Antibacterial activities of 3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl cephamycins.



Compound	R	MIC (μg/ml)[a]							
		S. aureus		*E. coli*		*Kleb. pneum.*	*Prot. vulg.*	*Salm. ent.*	*Shig. flex.*
		209P	R[b]	NIHJ	R[c]				
CMZ[d]	$NCCH_2SCH_2$	0.2	0.8	0.8	0.8	0.8	1.5	0.4	0.8
1	CH_3SCH_2	0.8	1.5	3.1	3.1	1.5	3.1	0.8	1.5
2	$CH_3CH_2SCH_2$	0.2	0.8	6.2	6.2	3.1	1.5	0.8	1.5
3	$NCCH(CH_3)SCH_2$	0.4	0.8	1.5	3.1	1.5	0.8	0.4	1.5
4	$NCC(CH_3)_2SCH_2$	0.4	0.8	6.2	12.5	6.2	1.5	3.1	3.1
5	$NCCH(C_6H_5)SCH_2$	0.1	0.4	200	200	200	1.5	50	200
6	$NCCH_2SCH(CH_3)$	0.8	1.5	25	25	25	6.2	6.2	12.5
7	$NCCH_2CH_2SCH_2$	0.1	0.8	1.5	6.2	1.5	3.1	0.8	3.1
8	$NCCH(C_2H_5)SCH_2$	0.2	0.4	6.2	6.2	6.2	0.8	1.5	6.2
9	$N_3CH_2SCH_2$	0.4	0.4	3.1	3.1	3.1	1.5	0.8	1.5
10	$HC\equiv CCH_2SCH_2$	0.2	0.8	3.1	3.1	3.1	3.1	0.8	3.1
11	$H_2NCOCH_2SCH_2$	0.8	3.1	3.1	3.1	3.1	12.5	1.5	3.1
12	$HOOCCH_2SCH_2$	25	25	3.1	3.1	3.1	6.2	1.5	3.1
13	$HOOCCH_2CH_2SCH_2$	6.2	6.2	0.8	0.8	0.8	1.5	0.2	0.8
14	$HOOCCH_2CH_2CH_2SCH_2$	12.5	12.5	12.5	6.2	0.8	1.5	0.4	3.1
15	$HOCH_2CH_2SCH_2$	0.8	1.5	1.5	1.5	1.5	3.1	0.8	1.5
16	$NCCH_2OCH_2$	0.8	1.5	3.1	3.1	3.1	12.5	1.5	3.1
17[e]	CF_3SCH_2	0.4	0.8	1.5	1.5	0.8	0.4	0.2	0.8
CFX	Cefoxitin	0.4	1.5	3.1	3.1	3.1	3.1	1.5	3.1

[a] The MIC's were determined by the two-fold serial dilution method on Nutrient agar. (Incubation: 37°C, 18 hours, Inoculum size: one loopful (10^7 cells/ml)).

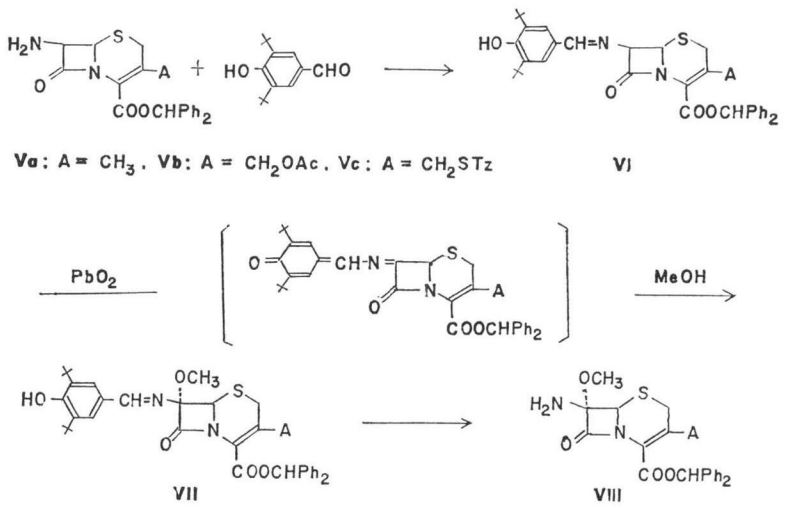
[b] Penicillinase producer. [c] Cephalosporinase producer.

[d] Cefmetazole. [e] SKF-73678.

2. Modification at the 3-Position

It is known[5,6] that *in vitro* activity can be increased against a number of bacteria by substituting an appropriate heterocyclicthiomethyl group for the methyl or acetoxymethyl group at the 3-position of the cephalosporin nucleus. In the case of cefmetazole, the corresponding 3-methyl (**21**) and 3-acetoxymethyl (**22**) analogs were prepared analogously to cefmetazole (Scheme 2). 3-Heterocyclicthiomethyl analogs (**18**, **19** and **20**) were prepared by the reaction of **22** with an appropriate heterocyclicthiol. As shown in Table 2, 3-methyl analog (**21**) was quite inactive and the 3-acetoxymethyl analog (**22**) was about two times less active than cefmetazole. Other congeners (**18**, **19** and **20**) were also less active, especially the N-phenyltetrazolyl analog (**19**) which was almost inactive against Gram-negative bacteria. The 3-carbamoyloxymethyl analog (**23**) prepared from cephamycin C by the usual method[7] was as active as **22**. As anticipated, 3-hydroxymethyl analog (**24**), which was prepared by enzymatic hydrolysis of **22** with *Bacillus subtilis*, and its lactone (**25**) were almost inactive.

Scheme 2.



3. Modification of the 7α-Methoxy Group

As analogs of cefmetazole, the 7α-ethoxy (**27**), propyloxy (**28**), methylthio (**29**) and cyano (**30**) derivatives were synthesized by a procedure similar to the methoxylation[8] (Scheme 4). As shown in Table 3, all analogs were almost inactive except for **27** which showed weak activity. On the other hand, the corresponding 7α-hydrogen analog (**26**)[9] showed remarkable activity against tested organisms except for β-lactamase-producing *Escherichia coli*. Thus, in the series of 7β-(substituted-thio) acetyl cephamycins, cefmetazole was the most active compound.

Scheme 3.

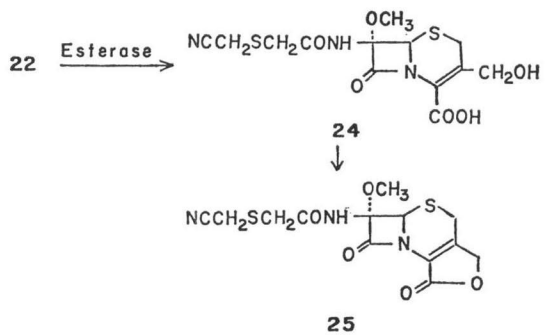
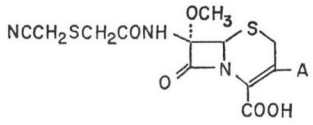
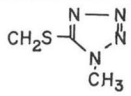
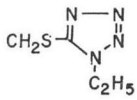
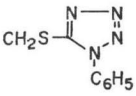
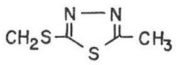


Table 2. Antibacterial activities of 7β-(cyanomethylthio)acetamidocephamycins.



Compound	A	MIC (μg/ml)*							
		S. aureus		*E. coli*		*Kleb. pneum.*	*Prot. vulg.*	*Salm. ent.*	*Shig. flex.*
		209P	R	NIHJ	R				
CMZ		0.2	0.8	0.8	0.8	0.8	1.5	0.4	0.8
18		0.2	0.8	1.5	1.5	1.5	1.5	0.8	1.5
19		0.2	0.4	50	100	100	0.8	50	50
20		0.8	1.5	12.5	12.5	12.5	3.1	1.5	12.5
21	CH_3	>200	>200	>200	>200	>200	>200	>200	>200
22	CH_2OAc	0.2	0.8	1.5	1.5	1.5	6.2	0.8	3.1
23	CH_2OCONH_2	0.8	3.1	1.5	1.5	3.1	6.2	1.5	3.1
24	CH_2OH	50	200	200	200	200	200	200	>200
25	Lactone	50	>200	>200	>200	>200	>200	>200	>200

* See footnote a) in Table 1.

Scheme 4.

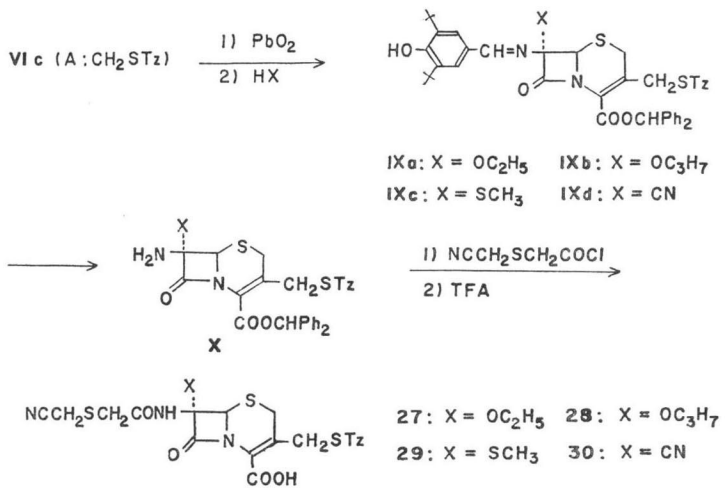
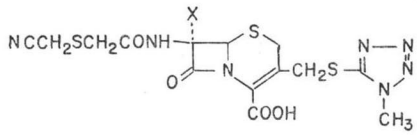


Table 3. Antibacterial activities of 7α-substituted cephalosporins.



Compound	X	MIC (μg/ml)*							
		S. aureus		*E. coli*		*Kleb. pneum.*	*Prot. vulg.*	*Salm. ent.*	*Shig. flex.*
		209P	R	NIHJ	R				
CMZ	OCH_3	0.2	0.8	0.8	0.8	0.8	1.5	0.4	0.8
26	H	0.1	0.4	0.8	12.5	0.8	3.1	0.4	0.8
27	OC_2H_5	6.2	12.5	50	50	50	50	25	25
28	OC_3H_7	200	200	>200	>200	>200	>200	>200	>200
29	SCH_3	25	100	>200	>200	200	>200	200	>200
30	CN	25	50	100	100	100	100	25	100

* See footnote a) in Table 1.

Experimental Section

In most cases the final cephalosporins were purified by preparative TLC over silica gel (layer thickness: 0.5 mm, solvent: *n*-BuOH - AcOH - H_2O (4: 1: 1)). NMR spectra were determined on a Varian T-60 spectrometer.

Antibacterial activity

The MIC's (μg/ml) of the cephalosporins were determined in two-fold agar dilution method (Medium: Nutrient agar, Incubation: 37°C, 18 hours, Inoculum size: one loopful (10^7 cells/ml)).

(Substituted-thio)acetyl chloride

General procedures: Method a) To a cold solution of 0.02 mol of (substituted-thio)acetic acid in 20 ml of anhydrous ether was added 4.2 g of PCl_5 and the mixture was stirred for 2 hours at 5~15°C. The reaction mixture was concentrated at room temperature and diluted with 10 ml of CCl_4 then concen-

Table 4. Physical properties of substituted acetic acid derivatives.

Compound	Bp, °C (mm) Mp, °C or Appearance	Compound	Bp, °C (mm) Mp, °C or Appearance
CH_3SCH_2COCl[10]	69(35)	$NCCH_2CH_2SCH_2COOH$[12]	127(14)
$C_2H_5SCH_2COCl$[10]	90(40)	$NCCH(CH_3)SCH_2COOH$	syrup
$NCCH_2CH_2SCH_2COCl$	130(2)	$NCC(CH_3)_2SCH_2COOH$	syrup
$NCCH(CH_3)SCH_2COCl$	90(2)	$NCCH(C_2H_5)SCH_2COOH$	syrup
$NCC(CH_3)_2SCH_2COCl$	75(2)	$NCCH_2SCH(CH_3)COOH$	139(2)
$NCCH(C_2H_5)SCH_2COCl$	100(2)	$NCCH(C_6H_5)SCH_2COOH$	mp 70°C
$NCCH_2SCH(CH_3)COCl$	not dist.	$HC\equiv CCH_2SCH_2COOH$[13]	mp 57°C
$NCCH(C_6H_5)SCH_2COCl$	163(3)	$H_2NCOCH_2SCH_2COOH$[14]	mp 128°C
$HC\equiv CCH_2SCH_2COCl$	73(80)	$N_3CH_2SCH_2COOH$	oil
$H_2NCOCH_2SCH_2COCl$	84(1.5)	$NCCH_2OCH_2COOH$	130(0.5)
$N_3CH_2SCH_2COCl$	78(2)	$NCCH(CH_3)SCH_2COOC_2H_5$	76(1)
$NCCH_2OCH_2COCl$	75(2)	$NCC(CH_3)_2SCH_2COOC_2H_5$	80(1)
CH_3SCH_2COOH	lit.[11]	$NCCH(C_2H_5)SCH_2COOC_2H_5$	102(1)
$C_2H_5SCH_2COOH$	122(20)	$NCCH(C_6H_5)SCH_2COOC_2H_5$	150(3)

trated at 35~40°C. This procedure was repeated once more to remove $POCl_3$. The resulting residue was distilled to give the corresponding acyl chloride (Table 4).

Method b) To a solution of 0.02 mol of (substituted-thio)acetic acid in 10 ml of benzene was added 3 ml of $SOCl_2$. The mixture was heated at 65~70°C for 3 hours with stirring and then concentrated *in vacuo* to remove benzene and excess $SOCl_2$. The residue was distilled to give the corresponding acyl chloride.

(Substituted-thio)acetic acid

General procedure: A mixture of 0.1 mol of ethyl (substituted-thio)acetate and 30 ml of 20% aqueous KOH was stirred at 5~10°C for 1.5~2.5 hours until the ester was completely dissolved. The resulting clear solution was washed with ether, then adjusted to pH 1.5~2 with HCl at 5~15°C, saturated with NaCl and extracted with ether. The extract was dried ($MgSO_4$) and evaporated to give crude acid, which was employed in the next step with or without purification (Table 4).

Ethyl (substituted-thio)acetate

General procedure: To a solution of 2.3 g of sodium in 100 ml of EtOH was added dropwise 12 g of ethyl thioglycolate followed by 0.1 mol of an appropriate halocyanoalkane at 10~20°C and the mixture was stirred for 3 hours at room temperature. After removal of EtOH *in vacuo*, 100 ml of water was added and the mixture was extracted with ether. The extract was dried ($MgSO_4$) and concentrated, and the residue was distilled to give ethyl (substituted-thio)acetate (Table 4).

Ethyl (azidomethyl)thio acetate

A mixture of 6.7 g of ethyl [(chloromethyl)thio]acetate, 5 g of sodium azide and 15 ml of DMF was stirred at 45°C for 17 hours. After addition of 150 ml of ether, the mixture was washed with water. The organic layer was evaporated and the residual oil was distilled to give 6.1 g of ethyl [(azidomethyl)-thio]acetate, bp 80°C (3 mmHg).

Ethyl [(cyanomethyl)oxy]acetate

To an ice-cooled solution of 6 g of glycolonitrile in 150 ml of anhydrous THF was added 2.3 g of a 52% dispersion of NaH in paraffin oil. The mixture was stirred at room temperature for 6 hours and then diluted with 200 ml of DMF under ice cooling. To the mixture was added 10 g of ethyl bromoacetate. The mixture was stirred at room temperature overnight and then poured into 500 ml of ice-water and extracted with ether. The extract was washed with saturated NaCl solution, dried (Na_2SO_4) and evaporated. The residual oil was distilled to give 3.7 g of ethyl [(cyanomethyl)oxy]acetate as colorless oil, bp 93°C (3 mmHg).

Anal. Calcd. for $C_6H_9NO_3$: C, 50.34; H, 6.34; N, 7.79.
Found : C, 50.35; H, 6.59; N, 7.55.

[(Cyanomethyl)oxy]acetic acid

By a similar procedure to preparation of (substituted-thio)acetic acid described above, hydrolysis of 2 g of ethyl [(cyanomethyl)oxy]acetate gave the desired acetic acid (1 g) as colorless oil, bp 132°C (0.5 mmHg).

Anal. Calcd. for $C_4H_5NO_3$: C, 41.74; H, 4.38; N, 12.17.
Found : C, 41.88; H, 4.28; N, 11.93.

[(Cyanomethyl)oxy]acetyl chloride

By a similar procedure to preparation of (substituted-thio)acetyl chloride described above, the reaction of 0.9 g of the above acid and 1.65 g of PCl_5 gave the desired acyl chloride (0.7 g) as colorless oil, which was used immediately for the next step without further purification.

7α-Methoxy-7β-(substituted-thio)acetamido-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acid

General procedure: Method a). Acylation of diphenylmethyl 7β-amino-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate (**II**).

To a cold solution of 1.05 g (2 mmol) of **II** in 15 ml of 1,2-dichloroethane was added dropwise 330 mg (2.2 mmol) of N,N-diethylaniline followed by a solution of 2.2 mmol of (substituted-thio)acetyl chlo-

ride in 1 ml of 1,2-dichloroethane. The mixture was cooled and stirred for 30~40 minutes, then washed with water, 3% aqueous $KHSO_4$ and water successively. The dried ($MgSO_4$) organic layer was concentrated to dryness *in vacuo* to give the corresponding 7β-(substituted-thio)acetamido derivative as a pale yellow amorphous powder, which was employed in the next step without purification.

To a chilled stirred solution of the above ester (1 g) in 6 ml of 1,2-dichloroethane and 1 ml of anisole was added dropwise 2 ml of TFA. After 30 minutes, the mixture was evaporated *in vacuo* below 35°C to remove excess TFA. The resulting residue was dissolved in 15 ml of EtOAc, washed with water and then extracted with 10% aqueous K_2HPO_4. The extract was washed twice with EtOAc, covered with 30 ml of EtOAc and adjusted to pH 2.0 with 10% HCl. After separation of the organic layer, the aqueous layer was extracted twice with EtOAc. The combined extracts were washed with water, dried ($MgSO_4$) and evaporated *in vacuo* to give about 500 mg of the desired acid as yellowish powder.

Method b). Reaction of (substituted-alkyl)mercaptan and 7β-chloroacetamido-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acid (**IV**).

a) To a cold solution of 435 mg (1 mmol) of **IV** in 3 ml of DMF was added 300 mg (3 mmol) of triethylamine followed by a solution of 1 mmol of carboxyalkylmercaptan in 0.5 ml of DMF. The mixture was stirred at room temperature for 3 hours. To the reaction mixture was added water (30 ml) and EtOAc (30 ml) followed by 10% HCl to adjust to pH 2. The organic layer was separated and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with saturated NaCl solution and then extracted with 10% aqueous K_2HPO_4 solution. The aqueous extract was washed with EtOAc. To the ice-chilled aqueous extract was added EtOAc (30 ml) followed by 10% HCl to adjust to pH 2 with stirring. The organic layer was separated and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with saturated NaCl solution, dried ($MgSO_4$) and evaporated *in vacuo* to give the desired compound.

b) A mixture of 520 mg (5.2 mmol) of sodium 2-hydroxyethylmercaptide, 1.74 g (4 mmol) of **IV** and 420 mg (5 mmol) of $NaHCO_3$ in 20 ml of water was stirred for 1 hour under ice-cooling. After addition of NaCl (7 g), the reaction mixture was layered with 20 ml of EtOAc and adjusted to pH 2 with 10% HCl. The organic layer was removed and the insoluble oily material and aqueous layer were extracted with *n*-BuOH (30 ml × 2). The extract was concentrated *in vacuo* and the residue was dissolved in 5 ml of acetone. Insoluble material was removed by filtration and the filtrate was added dropwise to 200 ml of *n*-hexane. The precipitated oily substance was separated and then dissolved in acetone. The acetone solution was evaporated *in vacuo* and the residue was dissolved in 10 ml of dioxane. The dioxane solution was lyophilized to give 990 mg of **15** as a pale yellow powder.

3-Carbamoyloxymethyl-7β-[(cyanomethyl)thio]acetamido-7α-methoxy-3-cephem-4-carboxylic acid (**23**)

A mixture of 1.5 g of di(diphenylmethyl) 7β-(D-5-*tert*-butoxycarbonylamino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7-methoxy-3-cephem-4-carboxylate[15], 1.5 g of N-(trimethylsilyl)trichloroacetamide and 5 ml of 1,2-dichloroethane was heated at 45°C for 30 minutes. To the mixture was added 1.4 g of freshly distilled [(cyanomethyl)thio]acetyl chloride and the mixture was stirred for 20 hours at 45°C. After removal of the solvent *in vacuo*, the resulting residue was triturated with *n*-hexane and then chromatographed on a silica gel dry column (5 × 50 cm) with EtOAc-benzene (1 : 1) mixture. Extraction of the part of Rf value about 0.2 with EtOAc and evaporation of the solvent gave 420 mg of diphenylmethyl 3-carbamoyloxymethyl-7β-[(cyanomethyl)thio]acetamido-7α-methoxy-3-cephem-4-carboxylate as a pale yellow amorphous powder. This powder was dissolved into 4.2 ml of anisole. To the solution was added 2.1 ml of trifluoroacetic acid under ice-cooling. After stirring for 30 minutes at 5°C, the excess trifluoroacetic acid was removed *in vacuo* at room temperature. The residual oil was dissolved in 15 ml of EtOAc and extracted with 10% K_2HPO_4 solution. The extract was covered with 30 ml of EtOAc and adjusted with 10% HCl to pH 2. The organic layer was separated and the aqueous layer was extracted three times with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo* to give 150 mg of **23**.

3-Acetoxymethyl-7β-[(cyanomethyl)thio]acetamido-7α-methoxy-3-cephem-4-carboxylic acid (**22**)

(a) Diphenylmethyl 3-acetoxymethyl-7β-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)-7α-methoxy-3-cephem-4-carboxylate (**VIIb**)

A mixture of 10 g of diphenylmethyl 7-aminocephalosporanate (**Vb**) and 5 g of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde in 200 ml of benzene was heated under reflux for 40 minutes using a water separater. To the reaction mixture was added 10 ml of 1,2-dichloroethane. After cooling with ice-water, 20 g of freshly prepared PbO_2 was added and the mixture was stirred for 1 hour and then filtered by suction. The red-brown filtrate was concentrated to about half volume *in vacuo* and 200 ml of methanol was added. The mixture was allowed to stand overnight at room temperature, then concentrated *in vacuo*. The resulting residue was dissolved in 100 ml of benzene, dried and concentrated to about 40 ml *in vacuo* and the solution was chromatographed on about 200 g of silica gel which was dried at 130°C for 3 hours *in vacuo* (solvent: benzene - EtOAc (10: 1)). Concentration of the fraction which showed Rf value 0.5 on silica gel TLC (solvent: cyclohexane - EtOAc (3: 1)) afforded 6.6 g of **VIIb** as pale yellow amorphous powder. NMR($CDCl_3$) 1.45 (18H, s, *t*-butyl), 1.98 (3H, s, CH_3CO), 3.35 (2H, dd, C_2-H), 3.55 (3H, s, CH_3O), 4.80 (2H, dd, C_3-CH_2O-), 5.03 (1H, s, C_6-H), 5.50 (1H, s, OH), 6.97 (1H, s, $COOCHPh_2$), 7.30 (10H, s, $COOCH(C_6H_5)_2$), 7.63 (2H, s, benzene ring-H), 8.45 (1H, s, CH=N).

(b) Diphenylmethyl 7β-amino-7α-methoxycephalosporanate (**VIIIb**)

To a solution of 5 g of **VIIb** in 50 ml of EtOAc was added a solution of 3 g of GIRARD T reagent in 60 ml of methanol. The mixture was stirred for 3 hours at room temperature, then concentrated *in vacuo*. To the residue was added 50 ml of EtOAc and 30 ml of water. The separated aqueous layer was extracted with EtOAc and the combined organic layers were washed twice with water, dried and evaporated *in vacuo* to give 3 g of **VIIIb** as yellowish powder. TLC Rf 0.5 (silica gel, benzene - EtOAc (2: 1)).

(c) Acylation followed by hydrolysis of **VIIIb**

According to the general procedure described above, acylation with [(cyanomethyl)thio]acetyl chloride followed by hydrolysis with trifluoroacetic acid gave the desired compound (**22**) as a pale yellow powder.

7β-[(Cyanomethyl)thio]acetamido-7α-methoxy-3-heterocyclicthiomethyl-3-cephem-4-carboxylic acid (**18**, **19**, **20**).

General procedure: A mixture of 400 mg of **22**, 100 mg (1.19 mmol) of $NaHCO_3$ and 1.93 mmol of heterocyclic thiol in 14 ml of pH 7 phosphate buffer solution was heated at 65°C for 4 hours. After cooling, the reaction mixture was adjusted to pH 2 with 10% HCl and extracted with EtOAc three times. The combined extracts were washed with NaCl solution. To the extract was added diphenyldiazomethane until the red color of the solution no longer disappeared and the mixture was allowed to stand for 3 hours and then concentrated *in vacuo*. The resulting residue was purified by silica gel preparative layer (thickness: 0.5 mm) chromatography (solvent: benzene - EtOAc (3: 1)). Extraction of the main band with EtOAc and evaporation of the extract *in vacuo* gave the diphenylmethyl esters of the desired compounds (yield, 25~35%).

Hydrolysis of the esters with trifluoroacetic acid in the usual manner gave the desired compounds (**18**, **19**, **20**).

7β-[(Cyanomethyl)thio]acetamido-3-hydroxymethyl-7α-methoxy-3-cephem-4-carboxylic acid (**24**).

To a solution of 1.7 g of **22** and 0.4 g of $NaHCO_3$ in 30 ml of water was added 1 g of lyophilized *Bacillus subtilis* ATCC 6633. The mixture was stirred at 36°C for 3.5 hours maintaining pH 7.2~7.5. After cooling in an ice-bath, the reaction mixture was layered with EtOAc and adjusted carefully to pH 2.5 with 10% HCl and filtered by suction using Celite. The organic layer was separated and the aqueous layer was extracted with EtOAc twice. The combined organic layers were dried and evaporated to yield 540 mg (35%) of crude acid (**24**) as pale yellow powder. The crude acid was dissolved in 10 ml of EtOAc and to this was added excess of 2 M sodium 2-ethylhexanoate in *n*-BuOH solution. The precipitate was collected, washed with EtOAc and dried under vacuum to give 200 mg of sodium salt of **24**.

Lactone (**25**)

To an ice-cold solution of 200 mg of **24** in 5 ml of THF was added 0.2 ml of trifluoroacetic anhydride. After stirring for 10 minutes, evaporation of the solvent and addition of EtOAc-ether to the residue gave a precipitate which was collected and dried to yield 65 mg (34%) of **25**.

7β-[(Cyanomethyl)thio]acetamido-7α-substituted-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acids (**27**, **28**, **29**, **30**).

(a) 7α-Alkoxy derivatives (**27**, **28**)

By a similar procedure to the preparation of **22**, oxidation of SCHIFF base (**VIc**) followed by addition of EtOH gave the diphenylmethyl 7β-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)-7α-ethoxy-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate (**IXa**). Treatment of **IXa** with GIRARD T reagent yielded 7β-amino-7α-ethoxy compound (**Xa**), which was acylated with (cyanomethylthio)-acetyl chloride. Removal of the diphenylmethyl group in the usual manner afforded 7β-[(cyanomethyl)-thio]acetamido-7α-ethoxy-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acid (**27**).

By the same procedure, 7α-propoxy analogue (**28**) was prepared.

(b) 7α-Methylthio derivative (**29**)

A mixture of 2 g of diphenylmethyl 7-amino-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate and 1 g of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde in 50 ml of benzene was refluxed for 45 minutes using a water separater. To the cooled mixture was added 15 ml of 1,2-dichloroethane followed by 3 g of PbO_2. After stirring for 1 hour, the reaction mixture was filtered by suction and 20 ml of a 48% solution of methylmercaptan in ether was added. After stirring for 30 minutes at room temperature, the reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in 50 ml of methanol and 3.5 g of GIRARD T reagent was added. After stirring for 1.5 hour at room temperature, the mixture was evaporated *in vacuo*. To the residue was added 50 ml of EtOAc - ether (1 : 1) and insoluble material was removed by filtration. The filtrate was washed with water three times, dried and evaporated *in vacuo* to give 2.1 g of crude diphenylmethyl 7β-amino-7α-methylthio-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate (**Xc**) as a brownish viscous oil.

The reaction of 2.1 g of **Xc** and 550 mg of [(cyanomethyl)thio]acetyl chloride in the usual manner gave the 2.1 g of diphenylmethyl 7β-(cyanomethylthio)acetamido-7α-methylthio-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate which was purified by column chromatography on silica gel. NMR ($CDCl_3$) 2.30 (3H, s, CH_3S), 3.46 (6H, m, C_2-H, $NCCH_2SCH_2$-), 3.72 (3H, s, N-CH_3), 4.32 (2H, dd, C_3-CH_2S-), 4.89 (1H, s, C_6-H), 6.82 (1H, s, $COOCHPh_2$), 7.30 (10H, s, $COOCH(C_6H_5)_2$), 7.87 (1H, brs, NH).

Removal of the diphenylmethyl group with trifluoroacetic acid in the usual manner yielded **29**.

(c) 7α-Cyano derivative (**30**)

To a solution of 3 g of diphenylmethyl 7-(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylen)methylimino-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate[1] in 40 ml of benzene and 20 ml of 1,2-dichloroethane was added liquid HCN which was prepared from 4.9 g of NaCN. The mixture was allowed to stand for 1.5 hour at room temperature and then concentrated *in vacuo* and purified by silica gel column chromatography (benzene - EtOAc (10 : 1)) to give 1.5 g of diphenylmethyl 7α-cyano-7β-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate (**IXd**). NMR ($CDCl_3$) 1.37 (18H, s, *t*-butyl), 3.70 (5H, s, C_2-H, N-CH_3), 4.33 (2H, dd, C_3-CH_2S), 5.35 (1H, s, C_6-H), 5.73 (1H, s, OH), 6.92 (1H, s, $COOCHPh_2$), 7.35 (10H, m, $COOCH(C_6H_5)_2$), 7.72 (2H, s, benzene ring-H), 8.68 (1H, s, CH=N).

A mixture of 1.5 g of **IXd**, 3.5 g of GIRARD T reagent, 20 ml of THF and 100 ml of methanol was stirred overnight at room temperature and then concentrated *in vacuo*. To the residue was added 50 ml of EtOAc - ether (1 : 1) and insoluble material was removed by filtration. The filtrate was washed with water, dried and evaporated *in vacuo* to give 540 mg of the 7β-amino-7α-cyano compound (**Xd**) as a viscous oil, which was treated without further purification with [(cyanomethyl)thio]acetyl chloride in the usual manner. Treatment of the [(cyanomethyl)thio]acetyl derivative with trifluoroacetic acid yielded the desired compound (**30**).

Acknowledgement

We are very grateful to Dr. K. ARIMA, the former director of our research laboratories, and Dr. Y. KISHIDA, the director of chemical research for their encouragement throughout this work.

References

1) NAKAO, H.; H. YANAGISAWA, B. SHIMIZU, M. KANEKO, M. NAGANO & S. SUGAWARA: A new semisynthetic 7α-methoxycephalosporin, CS-1170. J. Antibiotics 29: 554~558, 1976
2) DEMARINIS, R. M.; J. V. URI & J. A. WEISBACH: Synthesis and *in vitro* antibacterial activity of 7-trifluoromethylthioacetamidocephamycins related to SK&F 59962 (cefazaflur). J. Antibiotics 29: 973~975, 1976
3) MIYADERA, T.; T. HASHIMOTO, H. NAKAO, B. SHIMIZU & S. SUGAWARA: Synthesis and *in vitro* antibacterial activity of 7α-methoxy-7β-trifluoromethylthioacetamido-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid. Ann. Rep. Sankyo Res. Lab. 29: 143~146, 1977
4) URI, J. V.; P. ACTOR, J. R. GUARINI, L. PHILLIPS, D. PITKIN, R. M. DEMARINIS & J. A. WEISBACH: Biological and chemotherapeutic studies on three semisynthetic cephamycins. J. Antibiotics 31: 82~91, 1978
5) DEMARINIS, R. M.; J. R. E. HOOVER, G. L. DUNN, P. ACTOR, J. V. URI & J. A. WEISBACH: A new parenteral cephalosporin, SK&F 59962: Chemistry and structure activity relationships. J. Antibiotics 28: 463~470, 1975
6) WICK, W. E. & D. A. PRESTON: Biological properties of three 3-heterocyclicthiomethylcephalosporin antibiotics. Antimicr. Agents & Chemoth. 1: 221~234, 1972
7) (a) KARADY, S.; S. H. PINES, L. M. WEINSTOCK, F. E. ROBERTS, G. S. BRENNER, A. M. HOINOWSKI, T. Y. CHENG & M. SLETZINGER: Semisynthetic cephalosporins *via* a novel acyl exchange reaction. J. Am. Chem. Soc. 94: 1410~1411, 1972
(b) CHRISTENSEN, B. G.; M. SLETZINGER, S. KARADY & L. D. CAMA: Japan Patent Provisional Publication, 47-931, 1972
8) YANAGISAWA, H.; M. FUKUSHIMA, A. ANDO & H. NAKAO: A novel simple synthesis of 7α-substituted cephalosporins. Tetrahed. Lett. 1976: 259~262, 1976
9) NANNINI, G.; G. MOLGORA, G. BIASOLI, P. N. GIRALDI, G. MEINARDI & I. CARNERI: New broad-spectrum alkylthio cephalosporins. Arzneim. Forsch. 25: 1665, 1975
10) MOORADIAN, A.; C. J. CAVALLITO, A. J. BERGMAN, E. J. LAWSON & C. M. SUTER: A new series of testosterone esters. J. Am. Chem. Soc. 71: 3372~3374, 1949
11) MELLANDER, A.: Alkylmercapto and alkylsulfonyl carboxylic acid. Chem. Abstr. 28: 5408, 1934
12) SIEGFRIED, A. G.; R. GRIOT & C. E. BARRELET: Cyanoethylthioglycolic acid. Chem. Abstr. 52: 18220b, 1958. Swiss Pat. 325,456
13) POURCELOT, G. & P. CADIOT: Preparation of propargylic, allenic and acetylenic derivatives of the elements of Group VIB. Bull. Soc. Chim. Fr. 1966: 3016~3024, 1966
14) SOLLADIE-CAVALLO, A. & P. VIELES: Some compounds related to oxy and thiodiacetic acid series compounds. Bull. Soc. Chim. Fr. 1967: 517~523, 1967
15) SHIMIZU, B.; M. KANEKO, M. KIMURA & S. SUGAWARA: Synthesis of 7α-methoxy-7-[2-(substituted-thio)acetamido]cephalosporin derivatives and their antibacterial activities. Chem. Pharm. Bull. (Tokyo) 24: 2629~2636, 1976