SYNTHESIS AND BIOLOGICAL ACTIVITY OF 7β-(2-AMINO-2-CARBOXY)-ETHYLTHIOACETAMIDO-7α-METHOXYCEPHALOSPORIN DERIVATIVES

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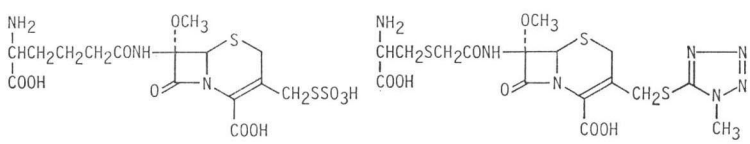
C-7 and C-3 substituents of a new antibiotic SF-1623 were chemically modified to improve the bioactivity, and substituent effect at C-7 and C-3 was examined on the basis of MIC and ED_{50} values against selected bacteria. Among a large number of derivatives, MT-141 possessing 2-D-2-amino-2-carboxyethylthioacetamido residue at C-7 and *N*-methyltetrazolylthiomethyl residue at C-3 showed a high order of antibacterial activity *in vitro* and especially *in vivo*.

In the preceding paper[1], we reported the isolation and structural elucidation of a new β-lactam antibiotic SF-1623 (**1**) belonging to the cephamycin group. Although it was active against some Gram-negative bacteria, the activity of the antibiotic was not high enough for practical application. Successful synthesis of analogs of cephamycin C such as cefoxitin[2,3] and cefmetazole[4,5] with marked resistance to β-lactamases and broad spectrum activity prompted us to make extensive effort to improve the bioactivity of SF-1623 (**1**) by chemical modification. Our effort was concentrated on modifying the D-aminoadipoyl group of SF-1623 (**1**), because alteration of this group has not been extensively studied in the past. The synthetic derivatives were screened not only by *in vitro* evaluation but also *in vivo*, because these properties may not always be in parallel, in particular in β-lactam antibiotics.[6]

In this paper, we report the synthesis of thio analogs of SF-1623 (**1**) where the central methylene of the α-aminoadipoyl group is replaced by a sulfur atom. The compounds were prepared either by direct modification of **1** or by synthesis from 7β-amino-7α-methoxycephalosporanic acid. The structure-activity study resulted in the synthesis of MT-141 (**8**) having potent antibacterial properties.*

Chart 1.



1 SF-1623 **8** MT-141

Synthesis of Analogs of SF-1623

Semisynthetic cephamycins in this study were prepared by the three general methods (A~C)

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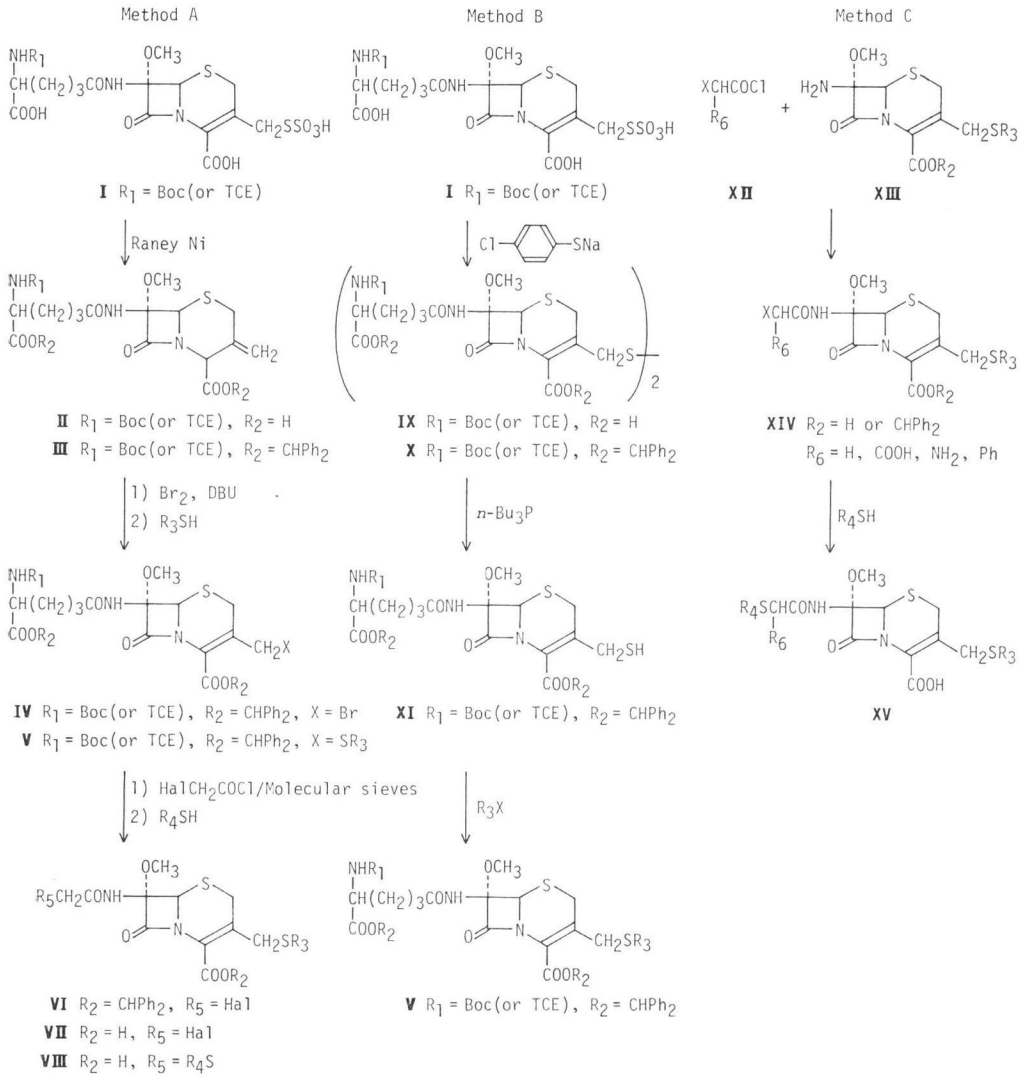
outlined in Chart 2. Antibiotic SF-1623 (**1**) was used as a starting material in methods A and B, and 7β-amino-7α-methoxy-3-cephem compound (**XIII**) in method C. According to method A, *N*-protected **I** was converted to the desulfurized exomethylene compound **II**[7], which was transformed to **V** *via* 3-bromomethyl compound **IV**[8]. Application of the acyl exchange method[9] to **V** followed by deprotection gave 7β-halogenoacetamido derivative **VII**, which was easily converted to the desired compound **VIII** by reaction with an appropriate mercaptan R_4SH.

According to method B, disulfide **IX** obtained by reaction of *N*-protected **I** with sodium *p*-chlorothiophenolate was reduced with tributylphosphine to afford mercaptan **XI**, which was converted to **V** by substitution with a diazoalkane or heterocyclic bromide. Compound **V** thus obtained could be transformed into **VIII** by the successive reactions shown in method A.

For the preparation of a variety of semisynthetic cephamycins containing various heterocyclic thio-

Chart 2.



methyl groups, method C was most suitable. The starting material **XIII** in this route could be prepared by the known process[4] or a new process developed in these laboratories[10]. Direct acylation or *S*-alkylation of halogenoacetyl intermediates (**XIV**) yielded the final cephamycins (**XV**). Other synthetic routes that are not comprised in methods A~C are described in the experimental section.

Effect of the C-3 Substituents on the Bioactivity

We examined first the effect of C-3 substituents on the antibacterial activity. Six pathogenic bacteria, one Gram-positive and five Gram-negative, were used as test organisms, and the results are listed in Table 1. 7β-Side chain employed in this study was either the 2-D-amino-2-carboxyethylthioacetamido group or 2-aminoethylthioacetamido group. The first C-3 substituent examined was the sulfothio group, which is a characteristic moiety of antibiotic SF-1623 (**1**) and has not been extensively studied. It was found, however, that compounds **3** and **4** having this group showed poor activity except compound **4** against *Proteus vulgaris*.

Replacement of the sulfothio group by an acetoxy group (**5** and **6**) showed remarkable improvement of the antibacterial activity and spectrum. Replacement of the acetoxy group with azide (**10**) resulted in further improvement of the activity. However, compound **10** was less active than **8** having a *N*-methyltetrazolylthiomethyl group, especially against *Staphylococcus aureus* and *P. vulgaris*. Compounds **13** and **14** possessing neutral heterocycles were also less active than compound **8**. Compounds **11** and **12** possessing acidic heterocycles were almost inactive against *S. aureus* but rather active against Gram-negative bacteria. The *N*-dimethylaminoethyltetrazolylthiomethyl group in **15** enhanced the bioactivity of the compound similar to the *N*-methyltetrazolylthiomethyl group in **8** except for *Serratia marcescens*. Compounds **16** and **17** containing the pyridinium moieties were less active against Gram-negative bacteria than **8**. Essentially, the same effect of the C-3 substituents was observed when the 7β-side chain was an aminoethylthioacetamido instead of a 2-D-amino-2-carboxyethylthioacetamido group.

Effect of C-7 Substituents on the Bioactivity

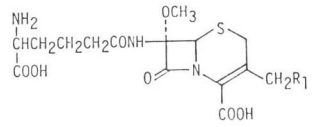
Effects of different 7β-side chains on the antibacterial activity *in vitro* are listed in Table 2. All compound had the *N*-methyltetrazolylthiomethyl group at the C-3 substituent. Compound **2** having a D-α-aminoadipoyl side chain was a parent cephamycin to compare with.

Compound **7**, in which the 7β-aminoadipoyl group was replaced with a 2-aminoethylthioacetamido group, exhibited improved activity, especially against *S. aureus*, *P. vulgaris* and *S. marcescens* as compared to compound **2**. Introduction of a phenyl group in the α-position of the side chain reduced the activity (compound **18**).

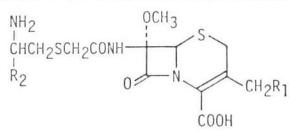
Notably, replacement of the central methylene of the α-aminoadipoyl group with a sulfur atom as in **8** contributed to greater activity against Gram-negative bacteria, and MICs of **8** were the lowest among the cephamycins studied. The activity against *S. aureus* was also improved. Addition of one more methylene carbon to the C-7 side chain of **8** resulted in a small change in the bioactivity (compound **19**). Cyclization of the amino acid function *via* a carbamoyl group caused moderate reduction of the bioactivity (**21**), but other substitutions such as methoxyimination at α-position (**23**), carboxylation at α-position (**24**) and dimethylation at γ-position (**22**) brought about considerable decrease in bioactivity. An amino group in the α-position reduced the activity considerably (**25**).

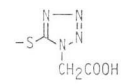
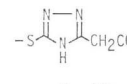
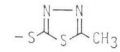
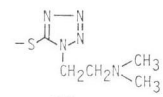
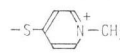
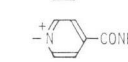
We have attempted further modification of the amino acid moiety of compound **8**. Introduction of an acyl group, such as *N*-acetyl (**26**), *N*-carbamoyl (**27**), *N*-glycyl (**28**) and *N*-alanyl group (**29**) caused

Table 1. Effect of C-3 substituents on MIC to six pathogens.



Compound	R_1	MIC (μg/ml)					
		S. aureus[a]	*E. coli*[b]	*K. pneumoniae*[c]	*S. typhi*[d]	*P. vulgaris*[e]	*S. marcescens*[f]
1	$-SSO_3H$	200	50	50	12.5	3.13	200
2		50	6.25	6.25	3.13	1.56	12.5



Compound	R_2	R_1	MIC (μg/ml)					
			S. aureus[a]	*E. coli*[b]	*K. pneumoniae*[c]	*S. typhi*[d]	*P. vulgaris*[e]	*S. marcescens*[f]
3	–H	$-SSO_3H$	200	50	100	50	25	100
4	–COOH	$-SSO_3H$	200	50	50	25	3.13	200
5	–H	–OAc	12.5	12.5	25	6.25	12.5	25
6	–COOH	–OAc	100	6.25	6.25	3.13	0.39	50
7	–H		1.56	3.13	6.25	1.56	0.39	3.13
8	–COOH		6.25	0.78	1.56	0.39	0.05	1.56
9	〃	$-SCH_3$	100	50	100	25	25	100
10	〃	$-N_3$	25	1.56	1.56	0.78	0.20	1.56
11	〃		100	25	6.25	3.13	0.39	12.5
12	〃		100	6.25	3.13	3.13	0.78	50
13	〃		50	3.13	3.13	1.56	0.39	6.25
14	〃		25	3.13	1.56	0.78	0.20	12.5
15	〃		12.5	1.56	3.13	1.56	0.78	25
16	〃		6.25	6.25	12.5	3.13	0.78	3.13
17	〃		3.13	12.5	12.5	6.25	3.13	6.25

a) *Staphylococcus aureus* Smith, b) *Escherichia coli* NIHJ JC-2, c) *Klebsiella pneumoniae* PCI-602, d) *Salmonella typhi* O-901-W, e) *Proteus vulgaris* OX-19, f) *Serratia marcescens* No. 1.

decrease in the antibacterial activity against all the test organisms. *N*-2-Hydroxybenzyl analog **30**, which is a *N*-aralkyl derivative of **8**, was 4 times more active than **8** against *S. aureus*, but markedly less active against Gram-negative bacteria. Ester and amido derivatives (**31** and **32**) of carboxyl function were also 2~4 times more active than **8** against *S. aureus*, but they were inferior to **8** against Gram-negative bacteria.

These results indicated that the intact amino acid function of **8** contributed to its high antibacterial activity, especially against Gram-negative bacteria. Compound **8** was coded MT-141.

Effect of 7α-Methoxy Group on the Antibacterial Activity

In a series of compounds having the 7β-2-D-amino-2-carboxyethylthioacetamido group in common, the *in vitro* activities of 7α-hydrogen and 7α-methoxy compounds were compared.

For this purpose, 7α-hydrogen analogs **33**, **34** and **35** corresponding to the respective 7α-methoxy congeners **8**, **13** and **17** were prepared and their activity examined against 7 pathogens including β-lactamase-producing strains (Table 3). It was found that the 7α-hydrogen compounds had enhanced activity against *S. aureus*, but that they were as active as the 7α-methoxy compound against sensitive Gram-negative bacteria. However, against *P. vulgaris*, *Proteus morganii* and *S. marcescens*, all of the 7α-methoxy compounds tested were definitely more active than the 7α-hydrogen congeners. An exception was *Pseudomonas aeruginosa*, against which 7α-hydrogen compounds, especially compound **35** possessing pyridinium cation at C-3 were more active than the 7α-methoxy congeners.

Configurational Effect of α-Amino Acid Moiety at C-7

As a result of screening of C-3 and C-7 substituents described above, MT-141 (**8**) comprising 7β-2-D-2-amino-2-carboxyethylthioacetamido and 3-*N*-methyltetrazolylthiomethyl groups was selected as a promising candidate for further evaluation. However, the 2-amino-2-carboxyethylthioacetamido group contained an asymmetric carbon, and MT-141 (**8**) existed as the D-isomer. Therefore, it was necessary to compare the activity of the D- and L-isomers, before final selection was made. For this purpose, L-isomers **36**, **37** and **38** corresponding to **8**, **31** and **27** respectively were prepared, and MIC values against six pathogens determined (Table 4).

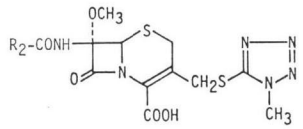
Among all the compounds tested, MT-141 (**8**) with D-configuration was the most active. Moreover, it was found that in the three paired compounds listed in Table 4, definite superiority of D-isomer over L-isomer was only observed in the pair **8** and **36**, both possessing the intact amino acid moiety. In the other couples, *i.e.* the ethyl ester analogs (**31** and **37**), and the carbamoyl congeners (**27** and **38**) D- and L-isomers showed similar activities. Contrary to expectation, L-isomer **37** was even more active than D-isomer **31** against some organisms such as *S. aureus* and *S. marcescens*. Thus, acyl and ester derivatization of the α-amino acid moiety caused a loss of the configurational effect that was clearly observed in the original pair, **8** and **36**.

As described in the preceding section, substitution of the α-amino acid function of D-congener **8** caused a marked decrease in antibacterial activity. However, this was not observed among the L-isomers. The ester **37** and *N*-acyl derivative **38** showed almost the same activity as that of the parent **36**.

In Vivo Evaluation

In vivo activities of MT-141 (**8**) and other compounds synthesized in this study were compared on the basis of ED_{50} values which were obtained by using a mouse model intraperitoneally infected with *Escherichia coli* No. 29. The results are summarized in Table 5, together with MIC values. At an

Table 2. Effect of C-7 substituents on MIC to six pathogens.



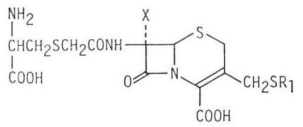
Compound	R_2	MIC (μg/ml)					
		S. aureus	*E. coli*	*K. pneumoniae*	*S. typhi*	*P. vulgaris*	*S. marcescens*
7	NH_2 $CH_2CH_2SCH_2-$	1.56	3.13	6.25	1.56	0.39	3.13
18	NH_2 Ph CH_2CH_2SCH-	3.13	6.25	12.5	1.56	12.5	6.25
8	D NH_2 $CHCH_2SCH_2-$ COOH	6.25	0.78	1.56	0.39	0.05	1.56
19	DL NH_2 $CHCH_2CH_2SCH_2-$ COOH	6.25	0.78	0.78	0.78	0.20	1.56
20	D NH_2 $CHCH_2SCH_2CH_2-$ COOH	3.13	3.13	1.56	1.56	1.56	3.13
21	DL CONH NHCO $CHCH_2SCH_2-$	3.13	3.13	3.13	0.78	1.56	6.25
22	D NH_2 CH_3 $CH-C-S-CH_2-$ COOH CH_3	12.5	6.25	6.25	3.13	3.13	6.25
23	D NH_2 $CHCH_2SCH_2C-$ COOH $N-OCH_3$	50	6.25	12.5	6.25	50	12.5
24	D NH_2 $CHCH_2SCH-$ COOH COOH	12.5	6.25	—*	3.13	—*	50
25	D NH_2 $CHCH_2SCH-$ COOH NH_2	100	50	50	50	25	100
26	D $NHCOCH_3$ $CHCH_2SCH_2-$ COOH	12.5	3.13	—*	0.78	0.39	12.5
27	D $NHCONH_2$ $CHCH_2SCH_2-$ COOH	25	3.13	3.13	1.56	1.56	6.25
28	D $NHCOCH_2NH_2$ $CHCH_2SCH_2-$ COOH	25	3.13	6.25	1.56	6.25	6.25
29	D CH_3 $NHCOCHNH_2$ $CHCH_2SCH_2-$ COOH	12.5	6.25	12.5	3.13	1.56	12.5

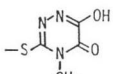
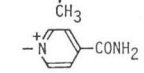
Table 2. (continued)

30	D	$NHCH_2$–C_6H_4-*o*-OH | $CHCH_2SCH_2$– | COOH	1.56	12.5	6.25	3.13	6.25	25
31	D	NH_2 | $CHCH_2SCH_2$– | $COOCH_2CH_3$	3.13	3.13	3.13	1.56	0.78	12.5
32	D	NH_2 | $CHCH_2SCH_2$– | $CONH_2$	1.56	3.13	3.13	1.56	3.13	6.25

* Not determined.

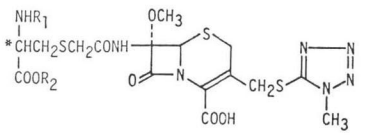
Table 3. Effect of 7α-methoxy group on MIC to eight pathogens.



Compound	R_1	X	MIC (μg/ml)							
			S. aureus	*E. coli*	*K. pneumoniae*	*S. typhi*	*P. vulgaris*	*P. morganii*[a]	*S. marcescens*	*P. aeruginosa*[b]
8		$-OCH_3$	6.25	0.78	1.56	0.39	0.05	0.78	1.56	25
33		H	3.13	0.78	0.78	0.78	0.39	50	25	25
13		$-OCH_3$	50	3.13	3.13	1.56	0.39	1.56	6.25	25
34		H	1.56	3.13	1.56	1.56	3.13	50	100	25
17		$-OCH_3$	3.13	12.5	12.5	6.25	3.13	3.13	6.25	25
35		H	1.56	3.13	6.25	6.25	6.25	12.5	12.5	3.13

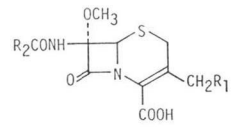
a) *Proteus morganii* 1510 (β-Lactamase-producing strain).
b) *Pseudomonas aeruginosa* MB-3829.

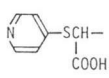
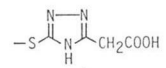
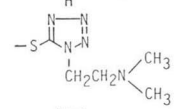
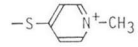
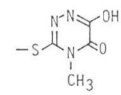
Table 4. Configurational effect of α-amino acid moiety on MIC to six pathogens.



Compound	R_1	R_2	Configuration (*)	MIC (μg/ml)					
				S. aureus	*E. coli*	*K. pneumoniae*	*S. typhi*	*P. vulgaris*	*S. marcescens*
8	H	H	D	6.25	0.78	1.56	0.39	0.05	1.56
36	H	H	L	12.5	3.13	6.25	3.13	3.13	6.25
31	H	Et	D	3.13	3.13	3.13	1.56	0.78	12.5
37	H	Et	L	1.56	3.13	6.25	1.56	3.13	6.25
27	$CONH_2$	H	D	25	3.13	3.13	1.56	1.56	6.25
38	$CONH_2$	H	L	50	3.13	6.25	3.13	3.13	6.25

Table 5. Comparison of *in vitro* and *in vivo* activities to *E. coli* No. 29.



Compound		R_2	R_1	MIC (μg/ml)	ED_{50} (mg/mouse)	Geometric standard deviation
39[*1]				0.20	1.0 (0.57 ~1.76)[*2]	2.15
7		$H_2NCH_2CH_2SCH_2-$	〃	1.56	0.50 (0.35 ~0.72)	1.81
8	D	NH_2 $CHCH_2SCH_2-$ $COOH$	〃	0.78	0.026 (0.021~0.032)	2.66
36	L	NH_2 $CHCH_2SCH_2-$ $COOH$	〃	3.13	0.41 (0.234~0.718)	3.57
31	D	NH_2 $CHCH_2SCH_2-$ $COOEt$	〃	1.56	0.045 (0.021~0.096)	1.10
32	D	NH_2 $CHCH_2SCH_2-$ $CONH_2$	〃	3.13	0.90 (0.60 ~1.35)	1.91
26	D	$NHCOCH_3$ $CHCH_2SCH_2-$ $COOH$	〃	3.13	0.22 (0.124~0.380)	2.46
6	D	NH_2 $CHCH_2SCH_2-$ $COOH$	$-OAc$	1.56	0.25 (0.143~0.438)	4.66
10		〃	$-N_3$	3.13	0.066 (0.062~0.070)	1.22
12		〃		6.25	0.15 (0.091~0.248)	3.15
15		〃		1.56	0.052 (0.034~0.08)	1.36
16		〃		6.25	0.50 (0.33 ~0.75)	3.17
13		〃		3.13	0.09 (0.041~0.198)	6.51
40		$H_2NCH_2CH_2SCH_2-$	〃	6.25	>2	—

[*1] 7α-Hydrogen compound.

[*2] Figures in parenthesis indicate 95% confidence limits.

initial stage of this investigation, we have found that compound **39** which was synthesized in the exploratory work, showed a very low MIC value against *E. coli* No. 29, but appeared not as active *in vivo* as expected. This aroused our attention towards the importance of ED_{50} values in evaluating the antibacterial activity of β-lactam antibiotics.

Among the test compounds, MT-141 (**8**) showed the lowest ED_{50} value, which was far less than that expected from the *in vitro* MIC value. Indeed, **8** was 4 times less active than **39** in MIC value, but 38 times more active in ED_{50} value. Low ED_{50} values were also shown by compounds **31**, **15**, **10** and **13**. All of them contained the same 7β-substituent as **8** or the ethyl ester of it, but the C-3 substituents were varied. Compounds **12** and **16** also exhibited relatively low ED_{50} values in spite of high MIC values.

Configurational and substitutional effect of α-amino acid moiety of the 7β-side chain was also observed *in vivo*. Compound **36** that is a corresponding L-isomer of **8**, was 16 times less active than D-congener **8** *in vivo*, though the MIC was only 4 times inferior. It was of interest to note that ED_{50} values of the amido congener **32** and the decarboxyl congener **7** were 20 and 35 times less active than **8** *in vivo*, but 2 and 4 times less active in MIC values. The same was true for compound **40** that was almost inactive *in vivo*, in spite of a MIC of 6.25 μg/ml.

These results revealed that the 2-D-amino-2-carboxyethylthioacetyl function, which is a sulfur isoster of D-aminoadipoyl moiety in natural SF-1623, played an important role not only for the *in vitro* but also for the *in vivo* activity.

Experimental

Methods

IR spectra were measured in KBr disk using a Hitachi Model 260-10 IR spectrometer. NMR spectra were recorded on a Varian XL-100 spectrometer using TMS as external reference in D_2O unless otherwise stated. The MICs (μg/ml) of the synthetic cephamycins were determined in two-fold agar dilution method using heart-infusion agar and incubating at 37°C for 20 hours. Inoculum size was 10^6 colony forming unit (CFU)/ml.

For determination of *in vivo* activity, Slc: *dd*Y female mice of 4 weeks old were used, ten in a group. The animals were inoculated intraperitoneally with 10 LD_{50} CFU of *E. coli* No. 29 suspended in 2.5% mucin, and were given a single subcutaneous dose of test compound dissolved in distilled water immediately after challenge. The ED_{50} values (mg/mouse) were determined by the probit method from survival rate of mice after a week.

7β-Chloroacetamido-7α-methoxy-3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-3-cephem-4-carboxylic Acid (**VIIa**) from SF-1623 (**1**)

Method A: Two grams of sodium 7β-(5-D-*t*-butoxycarbonylamino-5-carboxyvaleramido)-7α-methoxy-3-sulfothiomethyl-3-cephem-4-carboxylate (**I**) in 50% aqueous EtOH (50 ml) were hydrogenated at room temperature for 5 hours in the presence of 10 g of RANEY nickel. The catalyst was filtered off and washed with water (50 ml). The filtrate and washings were combined, adjusted to pH 2 with 2 N HCl and extracted with AcOEt (200 ml). The AcOEt extract was washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography with elution by AcOEt - MeOH (25: 3) to give 1.1 g of 7β-(5-D-*t*-butoxycarbonylamino-5-carboxyvaleramido)-7α-methoxy-3-methylene-3-cephem-4-carboxylic acid (**II**); IR 1760, 1680 cm^{-1}; NMR (acetone-d_6) δ 1.41 (9H, s, *t*-But), 1.82 (4H, m, CH_2), 2.40 (2H, m, CH_2CO), 3.44 (3H, s, OCH_3), 3.46 (2H, ABq, J=16 Hz, C-2), 4.16 (1H, m, CH), 5.13 (1H, s, C-4), 5.26 (2H, ABq, J=12 Hz, C-3), 5.36 (1H, s, C-6), 6.03 (1H, d, NH), 8.15 (1H, bs, NH).

To a cooled (−78°C) stirred solution of 1,8-diazabicyclo[5.4.0]nndec-7-ene (340 mg) and Br_2 (0.13 ml) in 20 ml of THF were added 615 mg of diphenylmethyl 7β-(5-D-*t*-butoxycarbonylamino-5-diphenyl-

methoxycarbonylvaleramido)-7α-methoxy-3-methylene-3-cephem-4-carboxylate (**III**) prepared from **II** in 15 ml THF, and a mixture was kept at $-78°C$ for 20 minutes, and then at 0°C for 20 minutes. After addition of $P(OMe)_3$ (0.2 ml), the reaction mixture was evaporated and chromatographed over Sephadex LH-20 to give 390 mg of diphenylmethyl 7β-(5-D-*t*-butoxycarbonylamino-5-diphenylmethoxycarbonylvaleramido)-7α-methoxy-3-bromomethyl-3-cephem-4-carboxylate (**IV**); IR 1780, 1720, 1670 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (9H, s, *t*-But), 1.76 (4H, m, CH_2), 2.28 (2H, m, CH_2CO), 3.50 (3H, s, OCH_3), 3.52 (2H, ABq, J=18 Hz, C-2), 4.08 (2H, ABq, J=13 Hz, C-3), 4.40 (1H, m, CH), 5.05 (1H, s, C-6), 6.90 (2H, bs, CH), 7.32 (20H, m, Ph).

To a solution of 350 mg of **IV** in DMF (5 ml) was added 5-mercapto-2-methyl-1,3,4-thiadiazole (45 mg), and the mixture was stirred at room temperature for 15 hours. The reaction mixture was diluted with AcOEt (70 ml), and washed with water (30 ml×2). The solvent layer was dried over $MgSO_4$, evaporated, and chromatographed over Sephadex LH-20 to afford diphenylmethyl 7β-(5-D-*t*-butoxycarbonylamino-5-diphenylmethoxycarbonylvaleramido)-7α-methoxy-3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-3-cephem-4-carboxylate (**V**); IR 1780, 1710, 1680 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (9H, s, *t*-But), 1.75 (4H, m, CH_2), 2.20 (2H, m, CH_2CO), 3.49 (3H, s, OCH_3), 2.73 (3H, s, CH_3), 3.56 (2H, bs, C-2), 4.15 (2H, ABq, J=13 Hz, C-3), 4.43 (1H, m, CH), 5.02 (1H, s, C-6), 6.88 (1H, s, CH), 6.90 (1H, s, CH), 7.32 (20H, m, Ph).

Compound **V** (190 mg) was dissolved in 6 ml of dichloromethane, and to this solution were added Molecular Sieves 4A (1.9 g) and chloroacetyl chloride (100 mg), and a mixture was stirred at room temperature for 48 hours. The reaction mixture was filtered off and the filtrate was washed with 10% aqueous $NaHCO_3$ and water. The solvent layer was dried over $MgSO_4$, and evaporated to give 210 mg of diacyl compound, which was subsequently treated with a mixture of CF_3COOH (1.5 ml) and anisole (3 ml) at 0°C for 50 minutes to afford 7β-chloroacetamido-7α-methoxy-3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-3-cephem-4-carboxylic acid (**VIIa**); IR 1775, 1680, 1610 cm^{-1}; NMR (acetone-d_6) δ 2.76 (3H, s, CH_3), 3.54 (3H, s, OCH_3), 3.70 (2H, ABq, J=18 Hz, C-2), 4.26 (2H, s, CH_2CO), 4.45 (2H, ABq, J=14 Hz, C-3), 5.12 (1H, s, C-6).

7β-Bromoacetamido-7α-methoxy-3-methylthiomethyl-3-cephem-4-carboxylic Acid (**VIIb**) from SF-1623 (**1**)

Method B: A mixture of 3.0 g of 7β-(5-D-*t*-butoxycarbonylamino-5-carboxyvaleramido)-7α-methoxy-3-sulfothiomethyl-3-cephem-4-carboxylic acid (**I**) and sodium *p*-chlorothiophenolate (0.8 g) in water (50 ml) was stirred at room temperature for 2.5 hours.

When the reaction was over, the mixture was washed with AcOEt (30ml), and the aqueous layer was adjusted to pH 2 with 1 N HCl, and extracted with AcOEt (50 ml×2). The solvent layer was washed with water, dried over $MgSO_4$ and evaporated to give 2.25 g of 1,1′-bis [7β-(5-D-*t*-butoxycarbonylamino-5-carboxyvaleramido)-7α-methoxy-4-carboxy-3-cephem-3-yl]dimethyl disulfide (**IX**); IR 1760, 1705, 1620 cm^{-1}; NMR (acetone-d_6) δ 1.40 (9H, s, *t*-But), 1.90 (4H, m, CH_2), 2.45 (2H, m, CH_2CO), 3.51 (3H, s, OCH_3), 3.56 (2H, ABq, J=18 Hz, C-2), 3.97 (2H, ABq, J=13 Hz, C-3), 4.25 (1H, m, CH), 5.11 (1H, s, C-6), 7.05 (1H, d, NH), 8.17 (1H, bs, CONH).

To a solution of 850 mg of 1,1′-bis[7β-(5-D-*t*-butoxycarbonylamino-5-diphenylmethoxycarbonylvaleramido)-7α-methoxy-4-diphenylmethoxycarbonyl-3-cephem-3-yl]dimethyl disulfide (**X**) prepared from **IX** in 40% aqueous dioxane (25 ml) was added *n*-tributyl phosphine (0.25 ml), and the mixture was stirred at 40°C for 45 minutes. The reaction mixture was evaporated, diluted with water (40 ml) and extracted with AcOEt (100 ml). The solvent layer was dried over $MgSO_4$, evaporated and chromatographed over Sephadex LH-20 with elution by AcOEt to give 720 mg of diphenylmethyl 7β-(5-D-*t*-butoxycarbonylamino-5-diphenylmethoxycarbonylvaleramido)-7α-methoxy-3-thiomethyl-3-cephem-4-carboxylate (**XI**); IR 2550, 1770, 1720, 1685 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (9H, s, *t*-But), 1.73 (4H, m, CH_2), 1.88 (1H, dd, SH), 2.20 (2H, m, CH_2CO), 3.47 (2H, bs, C-2), 2.92 & 3.72 (2H, each dd, C-3), 3.52 (3H, s, OCH_3), 4.42 (1H, m, CH), 5.04 (1H, s, C-6), 6.90 (1H, s, CH), 6.92 (1H, s, CH), 7.32 (20H, m, Ph).

To a solution of **XI** (640 mg) in AcOEt (30 ml) was added an ether solution of diazomethane at 0°C. When the reaction was completed, the mixture was evaporated and chromatographed over silica gel with benzene - AcOEt (5: 1) as a developing solvent to give 530 mg of diphenylmethyl 7β-(5-D-*t*-butoxycarbonylamino-5-diphenylmethoxycarbonylvaleramido)-7α-methoxy-3-methylthiomethyl-3-cephem-4-

carboxylate (**V**); IR 1780, 1720, 1680 cm^{-1}; NMR ($CDCl_3$) δ 1.42 (9H, s, *t*-But), 1.83 (4H, m, CH_2), 1.96 (3H, s, CH_3), 2.28 (2H, m, CH_2CO), 3.40 (2H, bs, C-2), 3.52 (3H, s, OMe), 3.58 (2H, bs, C-3), 4.51 (1H, m, CH), 5.08 (1H, s, C-6), 6.92 (2H, s, CH), 7.34 (20H, m, Ph).

Application of the known diacyl exchange reaction to **V** (440 mg) as described above using bromoacetyl chloride afforded 125 mg of 7β-bromoacetamido-7α-methoxy-3-methylthiomethyl-3-cephem-4-carboxylic acid (**VIIb**); IR 1760, 1670, 1600 cm^{-1}; NMR (acetone-d_6) δ 2.0 (3H, s, CH_3), 3.44 (2H, ABq, J=18 Hz, C-2), 3.52 (3H, s, OCH_3), 3.61 (2H, ABq, J=13 Hz, C-3), 4.09 (2H, s, CH_2CO), 5.16 (1H, s, C-6).

7β-(2-D-Amino-2-carboxyethylthioacetamido)-7α-methoxy-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (**8**)

Method C: A mixture of 7β-bromoacetamido-7α-methoxy-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (**XIV**) (920 mg) and D-cysteine hydrochloride (470 mg) in water (15 ml) was adjusted to pH 6.8 with aqueous $NaHCO_3$, and stirred under ice-cooling for 2 hours. The reaction mixture was chromatographed on a column of Diaion HP-20 with elution by water to give 1.1 g of sodium salt of **8** which was crystallized from H_2O - EtOH; mp 90~91°C; *Anal.* Calcd. for $C_{16}H_{20}N_7O_7S_3Na \cdot 7H_2O$: C 28.78, H 5.13, N 14.68, Found: C 28.84, H 4.96, N 14.21; IR 1755, 1670, 1610 cm^{-1}; NMR (D_2O) δ 3.22 (2H, m, CH_2S), 3.50 (2H, s, SCH_2CO), 3.55 (3H, s, OCH_3), 3.59 (2H, ABq, J=18 Hz, C-2), 3.97 (1H, m, CH), 4.04 (3H, s, N-CH_3), 4.16 (2H, ABq, J=13 Hz, C-3), 5.15 (1H, s, C-6).

Similar reaction as described above, using DL-homocysteine, DL-cysteinehydantoin, D-penicillamine, D-cysteine ethyl ester, D-cysteine amide and L-cysteine in place of D-cysteine afforded the respective products **19**, **21**, **22**, **31**, **32**, and **36**.

7β-(2-D-Amino-2-carboxyethylthioacetamido)-7α-methoxy-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**6**)

Treatment of 7β-bromoacetamido-7α-methoxycephalosporin (425 mg) with D-cysteine (200 mg) by a similar procedure as described for the preparation of **8** gave 320 mg of sodium salt of **6**; IR 1760, 1670, 1605 cm^{-1}; NMR (D_2O) δ 2.10 (3H, s, OAc), 3.20 (2H, m, CH_2S), 3.50 (2H, ABq, J=18 Hz, C-2), 3.51 (2H, s, SCH_2CO), 3.57 (3H, s, OCH_3), 3.98 (1H, m, CH), 4.78 (2H, ABq, J=13 Hz, C-3), 5.19 (1H, s, C-6).

7β-(2-D-Amino-2-carboxyethylthioacetamido)-7α-methoxy-3-(heterocyclicthiomethyl or pyridiniummethyl)-3-cephem-4-carboxylic Acid (**11**, **12**, **15**, **16** and **17**) by Substitution Reaction of **6**

General Procedure: A mixture of 485 mg (1 mmole) of sodium salt of **6** and heterocyclic thiol or substituted pyridine (each 2 mmole) was dissolved in 15 ml of 0.1 M phosphate buffer (pH 6.8), and stirred at 60~65°C for 6 hours under nitrogen atmosphere. When the reaction was over, the mixture was chromatographed on Diaion HP-20 column with elution by water to give desired compounds. Spectral data of some typical compounds were as follows.

7β-(2-D-Amino-2-carboxyethylthioacetamido)-7α-methoxy-3-(1-carboxymethyl-1*H*-tetrazol-5-yl)-thiomethyl-3-cephem-4-carboxylic Acid (**11**)

IR 1755, 1610 cm^{-1}; NMR (D_2O) δ 3.16 (2H, m, CH_2S), 3.44 (2H, s, SCH_2CO), 3.55 (3H, s, OMe), 3.59 (2H, ABq, J=18 Hz, C-2), 3.92 (1H q, CH), 4.21 (2H, ABq, J=13 Hz, C-3), 5.00 (2H, s, N-CH_2), 5.15 (1H, s, C-6).

7β-(2-D-Amino-2-carboxyethylthioacetamido)-7α-methoxy-3-(4-carbamoylpyridinium)methyl-3-cephem-4-carboxylic Acid (**17**)

IR 1760, 1670, 1620 cm^{-1}; NMR (D_2O) δ 3.20 (2H, m, CH_2S), 3.47 (2H, s, SCH_2CO), 3.55 (3H, s, OCH_3), 3.57 (2H, ABq, J=18 Hz, C-2), 3.95 (1H, m, CH), 5.20 (1H, s, C-6), 5.48 (2H, ABq, J=13 Hz, C-3), 8.37 & 9.11 (4H, each d, J=6 Hz, pyridinium)

7β-[3-(2-D-Amino-2-carboxyethylthio)-2-methoxyimino]propionamido-7α-methoxy-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (**23**)

To an ice-cooled solution of 525 mg of diphenylmethyl 7β-amino-7α-methoxy-3-(1-methyl-1*H*-

tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylate (**XIII**) and *N*,*N*-dimethylaniline (0.15 ml) in CH_2Cl_2 (25 ml) was added dropwise under stirring 3-bromo-2-methoxyiminopropionyl chloride (215 mg) in CH_2Cl_2 (5 ml).

After reaction at 10°C for 3 hours, the mixture was washed sequentially with 0.2 N HCl, aqueous 5% $NaHCO_3$ and water. The solvent layer was dried over $MgSO_4$ and evaporated to give 630 mg of diphenylmethyl 7β-(3-bromo-2-methoxyimino)propionamido-7α-methoxy-3-(1-methyl-1*H*-tetrazol-5-yl)-thiomethyl-3-cephem-4-carboxylate; IR 1760, 1720, 1670 cm^{-1}; NMR ($CDCl_3$) δ 3.54 (3H, s, OMe), 3.60 (2H, ABq, J=18 Hz, C-2), 4.01 (3H, s, N-CH_3), 4.04 (3H, s, OCH_3), 4.22 (2H, s, $BrCH_2C$), 4.25 (2H, ABq, J=13 Hz, C-3), 5.09 (1H, s, C-6), 6.90 (1H, s, CH), 7.30 (10H, m, Ph).

Removal of diphenylmethyl group from this ester (350 mg) by treatment with CF_3COOH and anisole, followed by reaction with D-cysteine gave 210 mg of **23**; IR 1760, 1660, 1620 cm^{-1}; NMR (D_2O) δ 3.10 (2H, m, CH_2S), 3.55 (3H, s, OCH_3), 3.60 (2H, ABq, J=18 Hz, C-2), 3.62 (2H, bs, SCH_2), 3.95 (1H, m, CH), 4.01 (3H, s, N-CH_3), 4.04 (3H, s, OCH_3), 4.18 (2H, ABq, J=13 Hz, C-3), 5.14 (1H, s, C-6).

Compounds **18** and **24** were prepared analogously using α-bromophenylacetyl chloride and half acid chloride of bromomalonic acid, respectively.

7β-(2-D-Carbamoylamino-2-carboxyethylthioacetamido)-7α-methoxy-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (**27**)

A mixture of sodium salt of **8** (300 mg) and 150 mg of potassium cyanate in water (20 ml) was stirred at room temperature for 24 hours. The reaction mixture was subjected to a column chromatography of Diaion HP-20 developing with water to give sodium salt of **27** (180 mg); IR 1770, 1660, 1610 cm^{-1}; NMR (D_2O) δ 3.07 (2H, m, CH_2S), 3.47 (2H, s, SCH_2CO), 3.56 (3H, s, OCH_3), 3.60 (2H, ABq, J=18 Hz, C-2), 4.05 (3H, s, N-CH_3), 4.17 (2H, ABq, 14 Hz, J=13 Hz, C-3), 4.22 (1H, m, CH), 5.14 (1H, s, C-6).

7β-[2-D-(Aminomethylcarbonyl)amino-2-carboxyethylthioacetamido]-7α-methoxy-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (**28**)

To an ice-salt cooled solution of *N*-*t*-butoxycarbonylglycine (175 mg) in THF (7 ml) were added triethylamine (0.14 ml) and ethyl chloroformate (0.1 ml), and the mixture was stirred at −20°C for 40 minutes. After addition of sodium salt of **8** (405 mg) dissolved in 50% aqueous THF (8 ml), the mixture was stirred at −20°C for 30 minutes, and then at 0~5°C for 2 hours. When the reaction was over, the solution was diluted with water (20 ml), layered over with AcOEt (70 ml), brought to pH 2 with 1 N HCl and shaken under cooling. The AcOEt extract was washed with water, and evaporated to give 496 mg of *N*-*t*-butoxycarbonylglycyl derivative of **8** (**28a**); IR 1760, 1670, 1605 cm^{-1}; NMR (acetone-d_6) δ 1.45 (9H, s, *t*-But), 3.19 (2H, m, CH_2S), 3.48 (2H, s, SCH_2CO), 3.55 (3H, s, OCH_3), 3.70 (2H, ABq, J=18 Hz, C-2), 3.82 (2H, bs, CH_2NH), 4.03 (3H, s, N-CH_3), 4.45 (2H, ABq, J=13 Hz, C-3), 4.80 (1H, m, CH), 5.11 (1H, s, C-6).

A cooled solution of **28a** (450 mg) in $CHCl_3$ (2 ml) was treated with CF_3COOH (4 ml) at 0°C for 30 minutes, and evaporated. The residue was washed with *n*-hexane, dissolved in small amount of water and adjusted to pH 6 with 1 N NaOH. Chromatographic purification over Diaion HP-20 afforded 150 mg of sodium salt of **28**; IR 1760, 1670, 1610 cm^{-1}; NMR (D_2O) δ 3.10 (2H, m, CH_2S), 3.43 (2H, s, SCH_2), 3.53 (3H, s, OCH_3), 3.57 (2H, ABq, J=18 Hz, C-2), 3.87 (2H, bs, CH_2NH), 4.03 (3H, s, N-CH_3), 4.16 (2H, ABq, J=13 Hz, C-2), 4.46 (1H, m, CH), 5.12 (1H, s, C-6).

7β-[2-D-(2-Hydroxybenzylamino)-2-carboxyethylthioacetamido]-7α-methoxy-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (**30**)

To a mixture of **8** (295 mg) and salicylaldehyde (70 mg) in 50% aqueous MeOH was added $NaBH_4$ (50 mg), and a solution was stirred at 5~10°C for 3 hours. The reaction mixture was evaporated and chromatographed over Diaion HP-20 with elution by 20% aqueous EtOH to give 180 mg of **30**; IR 1755, 1610 cm^{-1}; NMR (D_2O-acetone-d_6) δ 3.20 (2H, m, CH_2S), 3.45 (2H, bs, SCH_2), 3.55 (3H, s, OCH_3), 3.61 (2H, ABq, J=18 Hz, C-2), 3.81 (2H, bs, $NHCH_2$), 4.03 (3H, s, N-CH_3), 4.20 (1H, m, CH), 4.26 (2H, ABq, J=13 Hz, C-3), 5.12 (1H, s, C-6), 6.91 & 7.28 (4H, each m, Ph).

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