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Synthesis of 7α-Methoxy-7-[2-(substituted thio)acetamido]cephalosporin Derivatives and Their Antibacterial Activities

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The synthesis of new 7α-methoxy-7-(2-substituted thio)acetamido derivatives of cephalosporin by nucleophilic displacement of the halogen atom of 7β-(2-halogenoacetamido)-7-methoxy cephalosporanic acid derivatives was described. The *in vitro* antimicrobial activities were also described.

The recent isolation of a new family of β-lactam antibiotics "cephamycins" which have an α-methoxy group at the 7-position of the cephalosporin nucleus from *Streptomyces*[2–5] has aroused considerable interest. The enhanced activities against gram-negative bacteria of these new antibiotics prompted a search for more active cephalosporin derivatives.

Karady[6] reported a method for converting the aminoadipoyl residue of cephamycin C to other acyl groups by an acyl exchange reaction *via* a diacyl derivative of cephamycin C. Although, the application of this method makes possible the synthesis of a number of derivatives of cephamycin C,[7] it seems very difficult to obtain such cephamycin C derivatives having an acyl group at 7 position wherein the synthesis of the acid chloride is impossible.

The purpose of this report is to describe a convenient and versatile synthesis of 7α-methoxy-7-[2-(substituted thio)acetamido]-3-substituted methyl-3-cephem-4-carboxylic acids by nucleophilic displacement of the halogen atom of 7β-(2-halogenoacetamido)derivatives with various mercapto compounds.

7β-(D-5-*tert*-Butoxycarbonylamino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7-methoxy-3-cephem-4-carboxylic acid (**2a**) was obtained by tert-butoxycarbonylation of cephamycin C (**1**) with *tert*-butoxycarbonyl azide in the presence of one equivalent of 4-dimethylaminopyridine[8] according to the slightly modified method reported by Christensen, *et al.*[7]

The acid (**2a**) was esterified by diphenyldiazomethane in ethyl acetate to give diphenylmethyl 7β-(D-5-*tert*-butoxycarbonylamino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7-methoxy-3-cephem-4-carboxylate (**3a**).

The diphenylmethyl ester (**3a**) was heated with chloroacetyl chloride in the presence of bis(trimethylsilyl)trifluoroacetamide in dry dichloromethane at 40° for 90 hr under anhydrous

1) Location: 2-58, 1-*chome*, *Hiromachi*, *Shinagawa-ku*, *Tokyo*.
2) R. Nagarajan, L.D. Boeck, M. Gorman, R.L. Hamill, C.E. Higgens, M.M. Hoehn, W.M. Stark, and J.G. Whitney, *J. Am. Chem. Soc.*, **93**, 2308 (1971).
3) E.O. Stapley, M. Jackson, S. Hernandez, S.B. Zimmerman, S.A. Currie, S. Mochales, J.M. Mata, H.B. Woodruff, and D. Hendlin, *Antimicrob. Ag. Chemother.*, **2**, 122 (1972).
4) M. Arai, Y. Ito, M. Nakahara, H. Kayamori, and S. Sugawara, Japan Patent Provisional Publication, 49-42893 (1974).
5) H. Imanaka, J. Hosoda, K. Jomon, I. Ueda, D. Morino, and H. Sakai, Japan Patent Provisional Publication, 49-30593(1974).
6) S. Karady, S.H. Pines, L.M. Weinstock, F.E. Roberts, A.M. Hoinowsky, T.Y. Cheng, and M. Sletzinger, *J. Am. Chem. Soc.*, **94**, 1410 (1972).
7) B.G. Christensen, M. Sletzinger, S. Karady, and L.D. Cama, Japan Patent Provisional Publication, 47 931 (1972).
8) E. Guibe-Jampel and M. Wakselman, *Chem. Commun.*, **1971**, 267.

condition. The reaction mixture was worked up as usual and crude 7β-[N-(D-5'-*tert*-butoxycarbonylamino-5'-carboxyvaleryl)-2-chloroacetylamino]-3-carbamoyloxymethyl-7-methoxy-3-cephem-4-carboxylic acid dibenzhydryl ester (**4a**) was obtained as a chromatographically homogeneous foam. This was treated with trifluoroacetic acid at 0° for 5 min in the presence of small amount of anisole and after evaporation of the solvent, the residual product was worked up as usual, and 7β-(2-chloroacetamido)-7-methoxy-3-carbamoyloxymethyl-3-cephem-4-carboxylic acid (**5a**) was extracted with ethyl acetate. The crude acid (**5a**) was purified by column chromatography on silica gel; **5a** was eluted by 10% methanol in chloroform and was obtained as a chromatographically pure amorphous powder.

The reaction period required for the completion of the diacylation of **3a** (3→4) was surprisingly shortened by reaction with bromoacetyl bromide instead of chloroacetyl chloride. In this case, **3a** was completely consumed after only 2 hr from the addition of bistrimethylsilyl trifluoroacetamide and bromoacetyl bromide at room temperature. After usual work up and successive treatment with trifluoroacetic acid, 7β-(2-bromoacetamido)-7-methoxy-3-carbamo-

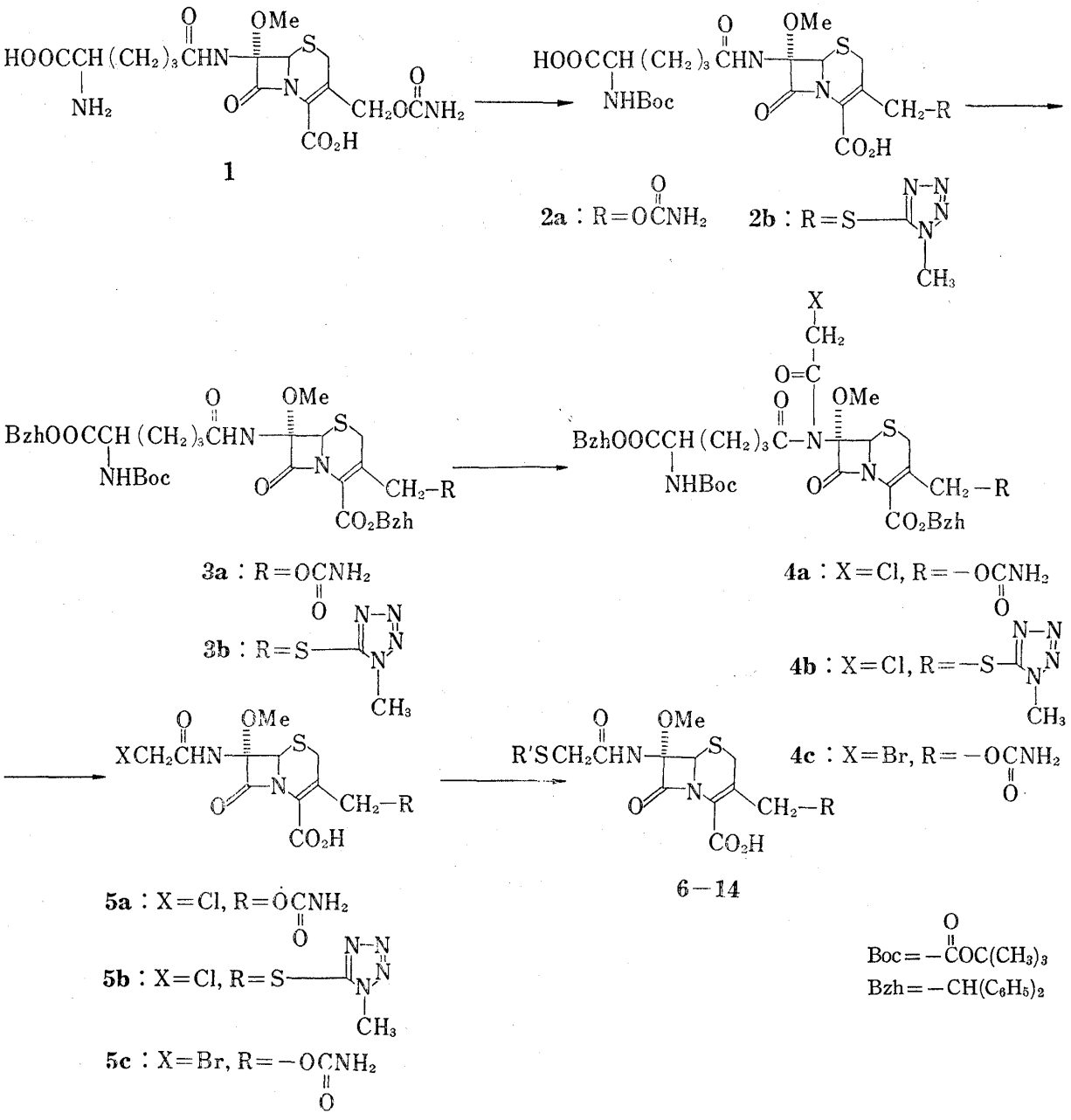


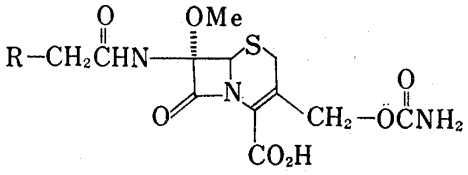
Chart 1

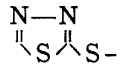
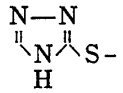
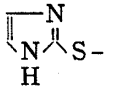
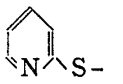
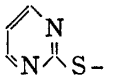
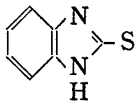
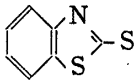
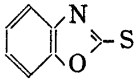
yloxymethyl-3-cephem-4-carboxylic acid (**5c**) was obtained as an amorphous powder. This derivative (**5c**) is relatively unstable compared with **5a**.[9] Considerable decomposition was observed during the post treatment and prolonged storage. From these reasons the chloroacetamido derivative (**5a**) is a better intermediate than bromoacetamido derivative (**5c**).

7β-(2-Chloroacetamido)-7-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thio] methyl-3-cephem-4-carboxylic acid (**5b**) was also obtained by a similar procedure to that for the preparation of **5a** from 7β-(D-5-*tert*-butoxycarbonylamino-5-carboxyvaleramido) π-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acid dibenzhydryl ester (**3b**) which was prepared by heating **2a** at 95° for 30 min in phosphate buffer (pH 7.0) in the presence of 1-methyl-5-mercaptotetrazole and successive esterification with diphenyldiazomethane after the usual work up.

The structures of compounds (**5a**) and (**5b**) were confirmed by the nuclear magnetic resonance (NMR), ultraviolet (UV), and infrared (IR) spectra. The NMR spectrum of com-

Table I. Physical Properties of Substituted Thio Acetamido Derivatives of Cephamycin C



Compound	R	NMR (δ)[a] 7-OCH_3	NMR (δ)[a] $-SCH_2C(=O)-$	NMR (δ)[a] 6-H	NMR (δ)[a] Others[g]	UV λ_{max}[b]	IR[c]	TLC Rf[d] Sol. A[e]	TLC Rf[d] Sol. B[f]
6		3.46(s)	4.16(s)	5.03(s)	9.30(s)	263	1760	0.49	0.29
7		3.37(s)	3.90(s)	5.00(s)	8.32(s)	263	1770	0.37	0.28
8		3.45(s)	3.82(s)	5.08(s)	7.16(s)	257	1765	0.21	0.31
9		3.39(s)	3.89(s)	5.02(s)	7.0—8.5	266 239	1770	0.57	0.43
10		3.46(s)	3.96(s)	5.04(s)	8.57(d) 7.22(t)	265 242	1760 1700	0.43	0.43
11		3.41(s)	4.16(s)	4.95(s)	7.2—7.8	287 281	1780 1680	0.48	0.51
12		3.45(s)	4.02(s) (in CD_3OD)	5.04(s)	7.2—7.9	297 271	1770	0.58	0.48
13		3.50(s)	4.14(s)	5.05(s)	7.2—7.8	283 276 245	1780 1720	0.61	0.48

a) measured in CD_3CN+D_2O
b) measured in pH 6.86 phosphate buffer (nm)
c) KBr pellet (cm^{-1})
d) thin layer chromatographic *Rf* value on silica gel plate
e) *n*-butanol: acetic acid: water=5: 4:1
f) methanol: chloroform=1: 1
g) heterocyclic-H

9) About 35% of **5c** decomposed after 5 hr in 0.025 M borate buffer (pH 10.0) at room temperature, while 80% of **5a** remained under the same conditions.

pound (**5a**) showed proton signals (ppm from TMS) for a 7-OCH_3 (δ 3.48, 3H, s), Cl-CH_2- (δ 4.11, 2H, s), 6-H (δ 5.10, 1H, s) and the UV spectrum in pH 6.86 phosphate buffer showed absorption maximum at 267 nm. The IR spectrum band at 1775 cm^{-1} (KBr) showed the presence of a β-lactam carbonyl group. The NMR spectrum of compound (**5b**) showed the proton signals (ppm from TMS) for 7-OCH_3 (δ 3.43, 3H, s), Cl-CH_2- (δ 4.12, 2H, s), 6-H (δ 5.00, 1H, s) and tetrazole N-CH_3 (δ 3.87, 3H, s). The UV spectrum in pH 6.86 phosphate buffer showed the absorption maximum at 271 nm in this case and the IR spectrum band at 1775 cm^{-1} also showed the presence of a β-lactam carbonyl group. The structures of compounds (**5a**) and (**5b**) were also supported by the relative intense antibacterial activities.[10]

The nucleophilic substitution reaction of **5** by various heterocyclic mercapto compounds in the presence of 2 equivalents of sodium hydroxide gave 7α-methoxy-7-substituted thioacetamido derivatives (**6**—**14**) as an amorphous powder.

The NMR, UV, IR spectra and the thin layer chromatographic behaviors of these compounds (**6**—**13**) are given in Table I.

Antibacterial Activity

The *in vitro* antibacterial activities of the compounds (**6**—**13**) were tested by the serial agar dilution method. The minimal inhibitory concentrations (MIC) against a variety of gram-positive and gram-negative bacteria of the above compounds (**6**—**13**) are shown in Table II.

Table II. Antibacterial Activity of 7-Substituted Thio Acetamido Derivatives of Cephamycin C (MIC μg/m)

Organism	Compound								Cefoxitin	Cephalothin
	6	**7**	**8**	**9**	**10**	**11**	**12**	**13**		
Staph. aureus 209P	0.4	1.5	0.8	0.4	0.8	0.4	≦0.1	0.4	0.4	0.05
Staph. aureus (R)[a]	1.5	6.2	3.1	1.5	6.2	1.5	0.2	1.5	1.5	0.2
E. coli NIHJ	3.1	6.2	6.2	50	6.2	25	400	200	3.1	6.2
E. coli 609 (R)[b]	3.1	12.5	6.2	50	12.5	25	≧400	200	3.1	50
Sh. flexneri 2a	3.1	12.5	12.5	50	6.2	25	400	200	3.1	12.5
Klebsiella 806	3.1	6.2	6.2	50	6.2	25	≧400	200	3.1	3.1
Proteus vulgaris	6.2	25	12.5	6.2	12.5	6.2	1.5	6.2	3.1	6.2
Salm. enteritidis	1.5	6.2	6.2	25	6.2	12.5	400	200	1.5	3.1

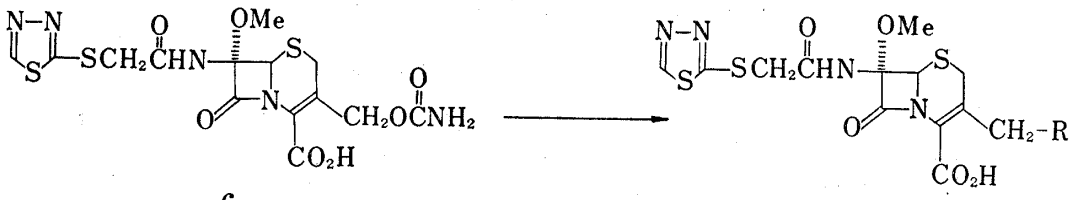
a) penicillinase producer
b) cephalosporinase producer
nutrient agar: inocula were diluted 100-fold after overnight culture.

From the data, it is apparent that the compounds which have a five membered heterocyclic ring in the acylamido group have relative more potent antibacterial activities than the compounds having six membered or fused five membered heterocyclic ring in the acylamide moiety. In particular, compound (**6**) has nearly the same activities as that of new semi-synthetic antibiotic "cefoxitin"[2,11,12] and is superior to cephalothin in their activities against gram-negative bacteria. Compound (**8**) is relatively unstable under the conditions used for the synthesis and gradually lost its potency on the storage even in the refrigerator. This

10) The *in vitro* MIC of **5a** and **5b** against *Staphylococcus aureus* 209P and *Escherichia coli* NIHJ are 0.8, 6.2 μg/ml for **5a** and 0.4, 3.1 μg/ml for **5b**, respectively.
11) H. Wallick and D. Hendlin, *Antimicrob. Ag. Chemother.*, **5**, 25 (1974).
12) A.K. Miller, E. Celozzi, Y. Kong, B.A. Pelak, D. Hendlin, and E.D. Staplay, *Antimicrob. Ag. Chemother.*, **5**, 33 (1974).

instability may be explained by the intramolecular catalytic effect of imidazole ring reported by Bundgaard[13] in the case of penicillin derivatives.

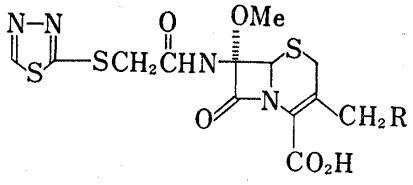
As can be seen from the above reasons, it became clear that [(1,3,4-thiadiazol-2-yl)thio]-acetamido group is one of the best groups as a 7β-substituent of cephamycin C. Subsequently exchanging the substituent at position-3 of compound (**6**) was undertaken for the purpose of finding a more effective substituent. Compound (**6**) was heated in the presence of an excess of 5-mercapto-1-methyltetrazole, sodium acetate, sodium azide or 2-mercaptopyridine N-oxide respectively in 0.5M-phosphate buffer at pH 7.5 according to the method reported by Mata.[14]

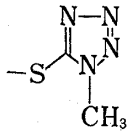
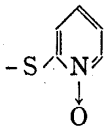


6 **14—17**

Chart 2

TABLE III. Physical Properties of 7α-Methoxy-7-thiadiazolylthioacetamido cephalosporin Derivatives



Compound	R	NMR (δ)[a] 7-OCH$_3$	NMR (δ)[a] $-SCH_2C(=O)-$	NMR (δ)[a] 6-H	NMR (δ)[a] Others	UV λ_{max}[b]	IR[c]	TLC Rf[d] Sol. A	TLC Rf[d] Sol. B
14		3.46(s)		5.02(s)	3.94(s)[e] 9.28(s)[h]	266	1760	0.43[f]	0.41[g]
15	$-OCCH_3$	3.42(s)	4.11(s)	5.00(s)	1.98(s)[i] 9.23(s)[h]	263	1760	0.46[f]	0.45[g]
16	$-N_3$	3.41(s)		5.01(s)	9.23(s)[h]	263	1760 2100	0.43[j]	0.39[g]
17		3.37(s)		4.95(s)	9.03(s)[h]	264	1755	0.18[j]	0.23[k]

a) measured in CD_3CN+D_2O
b) measured in pH 6.86 phosphate buffer (nm)
c) KBr pellet (cm^{-1})
d) thin-layer chromatographic *Rf* value on silica gel plate
e) tetrazole-N-CH_3
f) *n*-buthanol: acetic acid: water=5: 4: 1
g) methanol: chloroform=1: 1
h) thiadiazole-H
i) acetyl-CH_3
j) *n*-buthanol: acetic acid: water=4: 1: 1
k) chloroform: methanol: water=6: 4: 1

13) H. Bundgaard, *J. Pharm. Pharmacol.*, **24**, 985 (1972).
14) J.M. Mata, *Japan. Patent Provisional Publication*, **46**-3286 (1971).

After completion of the reaction and usual work up, the desired 3-substituted methyl derivatives of compound (**6**) were obtained as an amorphous powder (compound **14**—**17**) in relatively low yields. The NMR, UV, IR spectra and chromatographic behaviors are presented in Table III.

The *in vitro* antibacterial activities of these compounds (**14**—**17**) are given in Table IV. From the data in Table IV, it can be seen that the [(1-methyl-1*H*-tetrazol-5-yl)thio]methyl group is the most effective substituent in the 3-position of compound (**6**).

Table IV. Antibacterial Activity of 7-α-Methoxy-7-thiadiazolylthioacetamido cephalosporin Derivatives (MIC μg/ml)

Organism	Compound			
	14	**15**	**16**	**17**
Staph. aureus 209P	0.4	0.8	≦0.1	≦0.1
Staph. aureus (R)[a]	0.8	1.5	0.4	0.4
E. coli NIHJ	1.5	3.1	3.1	6.2
E. coli 609 (R)[b]	1.5	6.2	3.1	25
Sh. flexneri 2a	1.5	6.2	1.5	6.2
Klebsiella 806	1.5	3.1	3.1	6.2
Proteus vulgaris	1.5	6.2	3.1	12.5
Salm. enteritidis	0.8	3.1	3.1	3.1

a) penicillinase producer
b) cephalosporinase producer
nutrient agar: inocula were diluted 100-fold after overnight culture.

7α-Methoxy-7-[2-(1,3,4-thiadiazol-2-yl) thio] acetamido-3-[(1-methyl-1-*H*-tetrazol-5-yl)-thio]methyl-3-cephem-4-carboxylic acid (**14**) has good antimicrobial activity and is 1—2 times more active than cefoxitin and far more active than cephalothin against the β-lactamase producing *Escherichia coli*.

The nucleophilic displacement reaction of halogenoacetamido derivatives (**5**) by various mercapto compounds is a convenient and versatile synthetic method for preparing heterocyclic thioacetamido derivatives of 7α-methoxy cephalosporin derivatives. Furthermore, this nucleophilic displacement reaction of **5b** is also applicable for the synthesis of a noncyclic thioacetamido derivative of 7α-methoxy cephalosporin, CS-1170[15]; 7-cyanomethylthioacetamido-7α-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acid, which will be reported as one of the most active β-lactam antibiotics.[15]

Experimental

Melting points were determined using a Yanagimoto melting points apparatus and are uncorrected. IR spectra were obtained in KBr pellets using JASCO-A_2. NMR spectra were obtained (unless indicated otherwise) in $CD_3CN+D_2O=1:1$ on a Varian T-60 spectrometer using TMS as an external standard. UV spectra were obtained using Hitachi 124 spectrophotometer.

7β-(D-5-*tert*-Butoxycarbonylamino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7-methoxy-3-cephem-4-carboxylic Acid (2a)——To a solution of 34.5 g of cephamycin C (**1**) (80% purity 56.3 mmol) in 1090 ml of H_2O containing 5% K_2HPO_4 were added 715 ml of acetone and 8.1 g (70.5 mmol) of 4-dimethylaminopyridine at room temperature. After adjusting the pH of the solution to 9.5 with 2.5 N NaOH, 34.5 ml of *tert*-butoxycarbonyl azide was added dropwise and the solution was stirred for 3.5 hr at room temperature. The reaction solution was allowed to stand at 4° overnight and the insoluble material was removed by filtration and then the filtrate was washed with 1.5 liters of EtOAc. The H_2O layer was added 700 ml of EtOAc and the pH of the solution was adjusted to 2.5 with conc. HCl at 0—2°. The H_2O layer was extracted with two portions of 1.5 liters of EtOAc. The combined organic layer was washed with saturated NaCl solution until the pH of the washings was 3—4. The EtOAc solution was dried over anhydrous Na_2SO_4 and the solvent was remov-

15) H. Nakao, H. Yanagisawa, B. Shimizu, M. Kaneko, M. Nagano, and S. Sugawara, *J. Antibiotics*, **29**, 554 (1976).

ed by evaporation under reduced pressure to give 22.1 g of crude **2a** as a pale yellowish amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1790 (β-lactam), UV λ_{max} nm (ε) in THF: 263 (6820) NMR δ (ppm from TMS) in DMSO-d_6; 3.30 (3H, s, 7-OMe), 5.06 (1H, s, 6-H), 1.38 (9H. s, *tert*-butyl).

7β-(D-5-*tert*-Butoxycarbonylamino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7-methoxy-3-cephem-4-carboxylic Acid Dibenzhydryl Ester (3a)——The crude **2a** (23.7 g, 34.5 mmol) obtained above was dissolved in 800 ml of EtOAc and the solution was added to an ether solution of diphenyldiazomethane which was freshly prepared from 26.7 g of benzophenone hydrazone, 30.8 g of anhydrous Na_2SO_4, 72.5 g yellow HgO and 9.7 ml of EtOH saturated with KOH in 450 ml of ether. The red solution was stirred for 2 hr at room temperature in the dark. To the solution was added 50 ml of AcOH and the solution was stirred for 1 hr at room temperature. The reaction solution was washed with two portions of 500 ml of 20% NaCl solution and two portions of 500 ml of 5% $NaHCO_3$ solution successively. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation *in vacuo* to give 44.3 g of crude (**3a**) as an amorphous solid. This crude material was purified by silica gel column chromatography. The column was eluted with $CHCl_3$ containing 1% MeOH. The fractions containing **3a** were collected and the solvent was removed by evaporation *in vacuo* to give 26.9 g of **3a** as a colorless amorphous solid. *Anal.* Calcd. for $C_{47}H_{50}O_{11}N_4S$: C, 64.22; H, 5.73; N, 6.37; S, 3.65. Found: C, 64.34; H, 5.42; N, 6.15; S, 3.45. NMR δ (ppm from TMS) in $CDCl_3$; 3.60 (3H, s, 7-OMe), 5.10 (1H, s, 6-H).

7β-(2-Chloroacetamido)-7-methoxy-3-carbamoyloxymethyl-3-cephem-4-carboxylic Acid (5a)——Chloroacetyl chloride (3.18 ml, 40 mmol) was added to the dry CH_2Cl_2 solution (100 ml) containing 10.28 g of bis(trimethylsilyl)trifluoroacetamide (40 mmol) and stirred for 25 min at room temperature. To this solution was added 8.8 g (10 mmol) of **3a** and the solution was stirred for 74 hr at 40° under anhydrous condition. After addition of the reagents (one equivalent) the reaction solution was stirred for 16 hr at 40°. The reaction solution was added dropwise to the 250 ml of chilled 10% $NaHCO_3$ solution and was stirred for 30 min in an ice bath. The organic layer was separated and washed with 2 portions of 100 ml of saturated NaCl solution and dried over anhydrous $MgSO_4$. The solvent was removed by evaporation *in vacuo* to give pale yellow, amorphous solid. The solid dissolved in 10 ml of anisole was added 20 ml of trifluoroacetic acid and stirred for 5 min at 0°. After evaporation of the solvent, the residue was dissolved in a mixture of 60 ml of 1 M-phosphate buffer (pH 7.5), 45 ml of 5% $NaHCO_3$ and 60 ml of EtOAc. The organic layer separated was extracted with additional 10 ml of 1 M-phosphate buffer (pH 7.5). The pH of the combined inorganic layers was adjusted to 2.5 with 3 N HCl and the solution was saturated with NaCl. The solution was extracted with 7 portions of 70 ml of EtOAc and the combined extracts were washed with a small amount of saturated NaCl solution. The solution was dried over anhydrous Na_2SO_4 and the solvent was evaporated to dryness *in vacuo* to give 3.3 g of crude **5a**, and which was purified by silica gel column chromatography eluting with $CHCl_3$–MeOH (9: 1) to give 1.0 g of pure **5a**[9] as an amorphous solid. *Anal.* Calcd. for $C_{12}H_{14}O_7N_3S$: C, 41.86; H, 4.10; N, 12.20; S, 9.31. Found: C, 41.58; H, 4.03; N, 12.33; S, 9.03. UV λ_{max} nm (ε) in phosphate buffer (pH 6.86); 267 (6800).

7β-(D-5-*tert*-Butoxycarbonylamino-5-carboxyvaleramido)-7-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)-thio]methyl-3-cephem-4-carboxylic Acid Dibenzhydryl Ester (3b)——To a solution of the crude **2a** (17.0 g) in 90 ml of 1 M phosphate buffer (pH 7.0) containing 3.0 g of $NaHCO_3$ was added 5.4 g of 5-mercapto-1-methyltetrazole. The pH of the solution was adjusted to 6—7 with $NaHCO_3$ solution, and was kept at 95° for 75 min. After cooling to 0°, the pH of the solution was adjusted to 3.0 and which was extracted with 3 portion of 300 ml of EtOAc. To the washed and dried solution was added diphenyldiazomethane in ether which was freshly prepared from 28.6 g of benzophenone hydrazone and 73.6 g of yellow HgO. After stirring the solution for 2 hr at room temperature in the dark, 40 ml of AcOH was added to decompose the excess diphenyldiazomethane and the mixture was stirred until the color of the solution turned yellow. The reaction solution was treated as above described, 27.0 g of an amorphous solid was obtained, and which was chromatographed on a silica gel column (4×48 cm) with $CHCl_3$. The eluate with $CHCl_3$ containing 1% of MeOH gave 8.0 g of chromatographically homogeneous **3b** as a colorless amorphous solid. *Anal.* Calcd. for $C_{48}H_{51}O_9N_7S_2$: C, 61.72; H, 5.50; N, 10.50; S, 6.87. Found: C, 61.50; H, 5.25; N, 10.30; S, 6.58. UV λ_{max} nm (ε) in THF: 259 (7950).

7β-(2-Chloroacetamido)-7-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl) thio] methyl-3-cephem-4-carboxylic Acid (5b)——To a solution of chloroacetyl chloride (1.81 g) and bis(trimethylsilyl)trifluoroacetamide in 40 ml of CH_2Cl_2, 3.74 g of dibenzhydryl ester (**3b**) was added. After the reaction mixture was stirred for 67 hr at 40°, the solution was poured into 100 ml of 10% $NaHCO_3$ at 0—5°. The separated aqueous layer was extracted with two portions of 50 ml $CHCl_3$. The combined $CHCl_3$ layer was dried over anhydrous $MgSO_4$ and the solvent was evaporated to dryness *in vacuo* to give 5.1 g of amorphous solid. This solid was dissolved into 4 ml of anisole and was added 10 ml of trifluoroacetic acid under cooling with water. After 5 min, the solvent was removed by evaporation *in vacuo*. The residual product was dissolved into a solution containing 25 ml of 1 M phosphate buffer (pH 7.5), 30 ml of 5% $NaHCO_3$ and washed with three portions of 30 ml of EtOAc. The pH of the H_2O layer was adjusted to 2.5 with 3 N H_3PO_4. The solution was saturated with NaCl and was extracted with four portions of 50 ml of EtOAc. The combined extract was dried over $MgSO_4$ and concentrated to dryness *in vacuo* to give 1.65 g of amorphous solid which was purified by silica gel column chromatography (3×30 cm) eluting with $CHCl_3$ containing 15% MeOH. Pure **5b** (999 mg) was obtained as

a colorless amorphous solid. *Anal.* Calcd. for $C_{13}H_{15}O_5N_6S_2$: C, 39.09; H, 3.79; N, 21.04; S, 16.06. Found: C, 39.03; H, 3.58; N, 21.13; S, 15.95. UV λ_{max} nm (ε) in pH 6.86 phosphate buffer; 271 (9050), NMR and IR data was shown in the text.

General Procedure of 7β-(Substituted thio) acetamido Derivatives (6—14)

a) 7α-Methoxy-7-[2-(1,3,4-thiadiazol-2-yl) thio] acetamido-3-carbamoyloxymethyl-3-cephem-4-carboxylic Acid (6)——To a solution of 2-mercapto-1,3,4-thiadiazole (177 mg) in 1.5 ml of 1 N NaOH was added 380 mg of **5a** dissolved in 10 ml of 0.1 N NaOH. After stirring the solution for 2 hr, the pH of the solution was adjusted to 2.5 by addition of 3 N H_3PO_4 which was saturated with NaCl and extracted with five portions of 30 ml of EtOAc. The combined extract was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* giving 280 mg of crude **6** which was purified by preparative TLC plates using $CHCl_3$–MeOH (1: 1) as a developing solvent giving 145 mg of pure **6** as a colorless amorphous solid. *Anal.* Calcd. for $C_{14}H_{15}O_7N_5S_3$: C, 36.44; H, 3.28; N, 15.18; S, 20.84. Found: C, 36.22; H, 3.15; N, 15.24; S, 20.58.

b) 7α-Methoxy-7-[2-(1,3,4-thiadiazol-2-yl) thio] acetamido-3-[(1-methyl-1*H*-tetrazol-5-yl) thio] methyl-3-cephem-4-carboxylic Acid (14)——To a solution of the compound (**5b**) 434 mg in 10 ml of 0.1 N NaOH was added 123 mg of 2-mercaptothiadiazole dissolved in 10.5 ml of 0.1 N NaOH and the mixture was stirred for 2 hr at room temperature which was added 20 ml of EtOAc and the pH of the solution was adjusted to 2.0 with 6 N HCl at 0—5°. After saturation of the solution with NaCl, the aqueous layer was extracted with three portions of EtOAc. The extracts were combined and dried over anhydrous $MgSO_4$. Evaporation of the solvent left 555 mg of slight yellow residue which was purified by preparative TLC plates using a solution (*n*-BuOH: AcOH: H_2O=8: 1: 1) as a developing solvent. The band corresponding to **14** was cut out and extracted with MeOH. The residue was dissolved in 10 ml of H_2O and 20 ml of EtOAc was added. The pH of the solution was adjusted to 2.0 with 6 N HCl and the solution was extracted with two portions of 20 ml of EtOAc. The extracts were dried over anhydrous $MgSO_4$ and concentrated to dryness *in vacuo* to give 412 mg of **14** as a chromatographically homogeneous, colorless amorphous powder. *Anal.* Calcd. for $C_{15}H_{16}O_5N_8S_4$: C, 34.87; H, 3.12; N, 21.69; S, 24.83. Found: C, 34.57; H, 3.00; N, 21.47; S, 24.55. UV λ_{max} nm (ε) in pH 6.86 phosphate buffer, 266 (11400).

Dicyclohexylamine Salt of (14)——To a solution of 5 ml of acetone containing 400 mg of amorphous powder (**14**) was added 270 mg of dicyclohexylamine and stirred for 5 min. The solution was poured into 50 ml of diisopropylether under stirring and the precipitated powder was collected by filtration, then washed with ether. The white powder was dissolved in 5 ml of MeOH to which was added 20 ml of EtOH. The solution was concentrated to about half of its original volume *in vacuo* at 10—20° and allowed to stand overnight in refrigerator. The crystals were collected by filtration to give 420 mg of dicyclohexylamine salt as fine needles. mp 132—133° (decomp.). *Anal.* Calcd. for $C_{27}H_{36}O_5N_9S_4 \cdot 1H_2O$: C, 45.49; H, 5.37; N, 17.68; S, 17.99. Found: C, 45.52; H, 5.45; N, 17.82; S, 18.21. UV λ_{max} nm (ε) in pH 6.86 phosphate buffer: 267 (14100). IR ν_{max}^{KBr} cm^{-1}; 1770, 1695. NMR δ (ppm from TMS) in D_2O: 9.38 (1H, s, thiadiazole C–H), 5.09 (1H, s, 6-H), 4.23 (2H, s, SCH_2CO–), 4.02 (3H, s, tetrazole N–CH_3), 3.51 (3H, s, 7-OCH_3), 1.0—2.2 (dicyclohexyl–CH_2).

General Procedure of Substitution Reaction of Carbamoyloxy Group (14—17)

7α-Methoxy-7-[2-(1,3,4-thiadiazol-2-yl) thio] acetamido-3-[(1-methyl-1*H*-tetrazol-5-yl) thio] methyl-3-cephem-4-carboxylic Acid (14)——To a solution of **6** (660 mg) in 10 ml of 0.5 M phosphate buffer (pH 7.5) was added 166 mg of 5-mercapto-1-methyltetrazole. The solution was kept at 90—94° for 42 min.[16] The pH of the solution was adjusted to 2.5 with 3 N H_3PO_4 and the solution was saturated with NaCl which was extracted with 7 portions of 10 ml of EtOAc. The extracts were combined and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation *in vacuo* giving 293 mg of amorphous powder which was purified by preparative TLC with $CHCl_3$–MeOH (6: 4) as a developing solvent. This purification by TLC was repeated two times and 77 mg of pure (**14**) was obtained as an amorphous powder. UV λ_{max} nm (ε) in pH 6.86 phosphate buffer: 266 (11200). Other physical properties were completely consistent with that of described above.

Antibacterial Activity of Compounds (6—17)——The 7α-methoxy cephalosporin derivatives (**6**—**17**) were dissolved in 3% $NaHCO_3$ and their MIC against a variety of bacteria were determined by the serial agar dilution method using cefoxitin Na salt and cephalotin Na salt as reference compounds.

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16) At this time, although a considerable amount of starting material remained in the reaction solution, prolonged heating caused decomposition of the starting material and the product.