SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 7β-[2-(2-AMINOTHIAZOL-4-YL)ACETAMIDO]CEPHALOSPORIN DERIVATIVES

V. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 7β-[2-(2-AMINOTHIAZOL-4-YL)-2-METHOXYIMINOACETAMIDO]-CEPHALOSPORIN DERIVATIVES AND RELATED COMPOUNDS

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In order to improve the antibacterial activity of 7β-[2-(2-aminothiazol-4-yl)acetamido]-cephalosporins new derivatives having a methoxyimino moiety in the 7-acyl side chain and related compounds were synthesized. Of these, 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]cephalosporins were found to possess excellent activity against a variety of Gram-positive and Gram-negative bacteria including β-lactamase-producing strains.

An extensive study of structure-activity relationships led to the selection of 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-ceph-3-em-4-carboxylic acid, SCE-1365*, for further biological and clinical evaluation.

In previous papers[1] we described various chemical modifications of 7β-[2-(2-aminothiazol-4-yl)-acetamido]cephalosporins[2] in an effort to improve the antibacterial activity especially against β-lactamase-producing strains. In continuing these investigations for further improvement of antibacterial activity, we reported in the previous paper[3] the synthesis and configuration of 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid derivatives and related acids (Table 1) which are used for the acylation of 7-aminocephalosporins**.

In this paper the synthesis, the configuration and the antibacterial activity of 7β-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]cephalosporin derivatives will be described.

Chemistry

1. Synthesis of 7β-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]cephalosporins

Acylation of various 7-aminocephalosporins was conducted with the oxyiminoacetic acids listed in Table 1. 2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (**1b**) was readily converted into an acid chloride (**4b**) hydrochloride by the reaction with phosphorus pentachloride. After acylation of 7-aminocephalosporanic acid (7-ACA, **5a**) with **4b** hydrochloride in N,N-dimethyl-

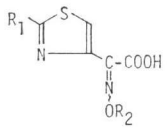
* Generic name: cefmenoxime.

** Part of the results was reported as a brief communication[4], and presented at the 18th Interscience Conference on Antimicrobial Agents and Chemotherapy[5]. Another group working independently has reported partially similar results[6].

acetamide (DMA), the chloroacetyl group was removed by the action of thiourea in the presence of sodium acetate to give 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]cephalosporanic acid isolated as its sodium salt (**7a**). From the acylation products a small amount of (*E*)-isomer (**13d**) was also isolated presumably due to geometrical isomerization of the methoxyimino moiety under acidic conditions[7].

In order to avoid the isomerization under acidic conditions, **1b** was reacted with phosphorus pentachloride in the presence of triethylamine to give an acid chloride (**4b**). Acylation of 7-ACA (**5a**) in aqueous THF, containing 3 equivalents of triethylamine, with **4b** readily afforded an acylated compound (**6a**). Removal of the protecting group

Table 1. 2-(2-Chloroacetamidothiazol-4-yl)-2-methoxyiminoacetic acid derivatives and related compounds.



Compound	Configuration	R_1	R_2
1a	*E*	$ClCH_2CONH$	CH_3
1b	*Z*	〃	〃
2a	*Z*	$ClCH_2CONH$	C_2H_5
2b	*Z*	〃	i-C_3H_7
2c	*Z*	〃	$CH_2COOC_4H_9(t)$
3a	*E*	CH_3	CH_3
3b	*Z*	〃	〃

Chart 1.

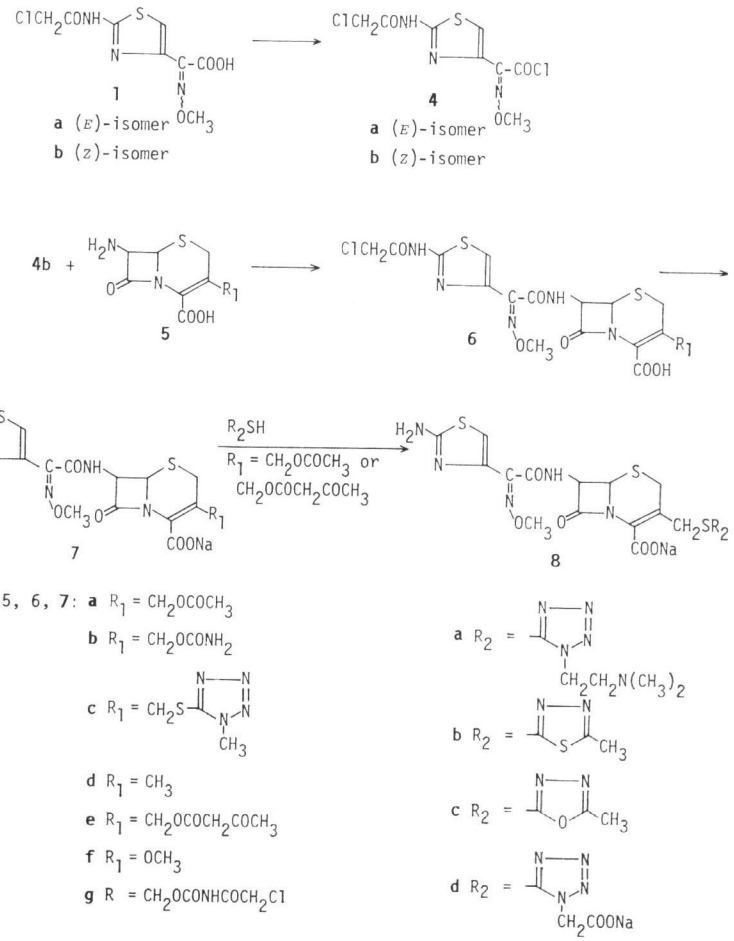
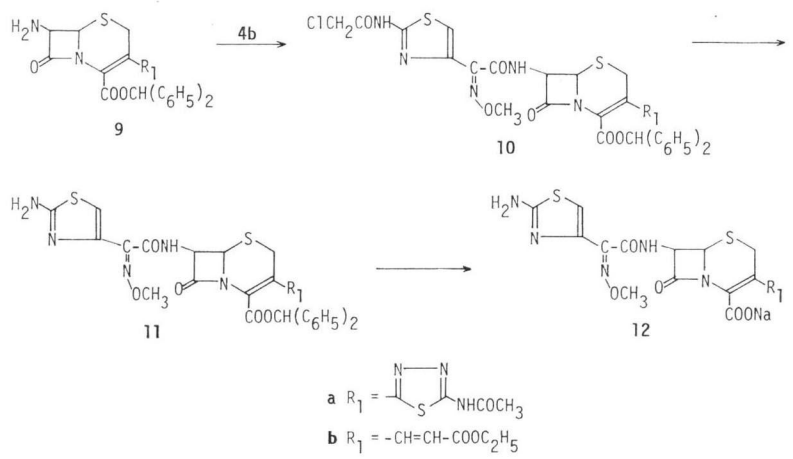


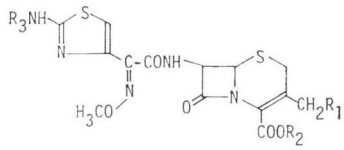
Chart 2.



from **6a** with thiourea gave **7a** without substantial contamination with the (*E*)-isomer (**13d**).

Similar treatment of other 7-aminocephalosporins bearing various substituents at the 3-position with **4b** followed by deprotection afforded several 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]cephalosporins (**7b**~**7g**). Esters (**9**) of 7-aminocephalosporins were also acylated with **4b** to give **10**. Removal of the chloroacetyl group followed by treatment with trifluoroacetic acid-anisole gave the anticipated 7-acylated compounds (**12**).

Chart 3.



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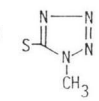
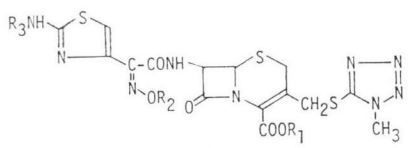
	R_1	R_2	R_3
a	$OCOCH_3$	t-C_4H_9	$ClCH_2CO$
b	$OCOCH_3$	t-C_4H_9	H
c	$OCOCH_3$	H	H
d	$OCOCH_3$	Na	H
e	$OCONHCOCH_2Cl$	H	$ClCH_2CO$
f	$OCONH_2$	Na	H
g		Na	H

t-Butyl 7-aminocephalosporanate was acylated with 2-(2-chloroacetamidothiazol-4-yl)-(*E*)-2-methoxyiminoacetyl chloride (**4a**) hydrochloride generated from the corresponding acid (**1a**) and phosphorus pentachloride to give **13a**. The 2-aminothiazol-4-yl compound (**13b**), obtained by removal of the chloroacetyl group from **13a**, was treated with trifluoroacetic acid-anisole to afford trifluoroacetic acid salt of 7β-[2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetamido]cephalosporanic acid (**13c**) which was converted into its sodium salt (**13d**). Acid chloride (**4a**) hydrochloride was also used for the acylation of 7-amino-3-(N-chloroacetylcarbamoyloxymethyl)ceph-3-em-4-carboxylic acid (**5g**)[8] to give **13e**. Both of the protecting groups in **13e** were removed simultaneously with thiourea to afford **13f**.

New compounds (**8**, **13g**) having heterocyclicthiomethyl group at the 3-position were prepared by reacting various thiol compounds with 3-acetoxymethyl (**7a**, **13c**) or 3-acetoacetoxymethyl (**7e**) derivatives.

Chart 4.



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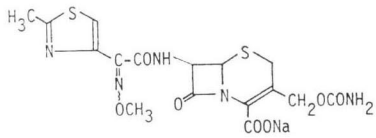
	R_1	R_2	R_3
a	H	C_2H_5	$ClCH_2CO$
b	Na	C_2H_5	H
c	H	i-C_3H_7	$ClCH_2CO$
d	Na	i-C_3H_7	H
e	H	$CH_2COOC_4H_9(t)$	$ClCH_2CO$
f	H	CH_2COOH	$ClCH_2CO$
g	Na	CH_2COONa	H



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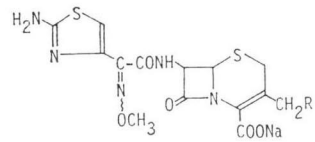
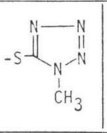
a (E)-isomer

b (Z)-isomer

Table 2. NMR Spectra of 7β-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]cephalosporins.



Compound	Configuration	R	Chemical shift (90 MHz, d_6-DMSO, ppm)	
			CONH	Thiazole 5-H
7c	Z		9.50	6.70
13g	E		9.25	7.40
7a	Z	$-OCOCH_3$	9.46	6.69
13d	E		9.28	7.46

2. Removal of Chloroacetyl Group with Sodium N-Methyldithiocarbamate

In the deprotection of the amino group with thiourea described above, formation[9] of 2-imino-4-thiazolidinone as a by-product is inevitable which causes, especially in a large scale, a purification problem due to its poor solubility in organic solvents. After an extensive screening of the reagents which might effect the removal of the chloroacetyl group sodium N-methyldithiocarbamate was found[10] to be an efficient reagent for this purpose.

Deprotection of **6a** with sodium N-methyldithiocarbamate proceeded readily at room temperature in a short period and the by-product, 3-methyl-4-thiazolidinone-2-thione[11], was easily removed by extraction with an organic solvent. This reagent was also effectively used for the conversion of **6g** into **7b**. Sodium N-methyldithiocarbamate also proved to be practical in a large scale preparation of **7c**.

3. Synthesis of Related Compounds

For an extensive study of structure-activity relationships between 7β-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]cephalosporins and related compounds, several new compounds with closely related structures were also synthesized. Thus, (Z)-2-ethoxyimino- (**14b**), (Z)-2-isopropyloxyimino- (**14d**) and (Z)-2-carboxymethoxyimino (**14g**) compounds were prepared from their corresponding acid derivatives (**2a**$\sim$**2c**) by reactions similar to those employed for the synthesis of **7c**.

In the case of **14g**, the t-butyloxycarbonyl group was removed before the removal of the chloroacetyl group from 7β-[2-(2-chloroacetamidothiazol-4-yl)-(Z)-2-(t-butyloxycarbonyl)methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**14e**). 7β-[2-(2-Methylthiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-carbamoyloxymethylceph-3-em-4-carboxylic acid salt (**15b**) and its (E)-isomer (**15a**) were obtained by acylating 7-amino-3-(N-chloroacetylcarbamoyloxy-

methyl)ceph-3-em-4-carboxylic acid (**5g**) with the corresponding methoxyiminoacetic acids (**3a**, **3b**) *via* an acid chloride or a mixed anhydride followed by removal of the chloroacetyl group.

4. Configuration of the Methoxyimino Moiety

Based on the facts that 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]cephalosporins and the related (*Z*)-isomers were prepared from the corresponding (*Z*)-acids under conditions designed to avoid acid catalyzed isomerization and that the chemical shifts of a pair of isomers (Table 2) were consistent with those observed for the parent acids[3], the assigned configuration of the oxyimino moiety of each compound was assumed to be reasonable*.

For further confirmation of the assignments, the chemical shift of the carboxamide moiety of each pair of isomers was examined and is listed in Table 2. As was observed for the configuration assignment of other antibiotics[12] or semi-synthetic cephalosporins[13] with related oxyimino structures, the chemical shift of CONH of **7c** appeared at a lower field than that of **13g** by 0.25 ppm. Similar differences in chemical shift were also observed with another pair of isomers.

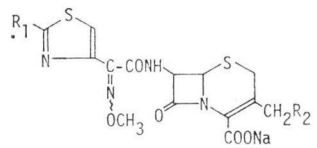
These imply that an intramolecular hydrogen-bonding[12] between the oxygen atom of the oxyimino moiety and the proton of the CONH group can exist in **7c** and other (*Z*)-compound (**7a**) which is ascribable to the (*Z*)-configuration.

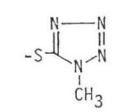
Antibacterial Activity

The *in vitro* antibacterial activity of newly synthesized compounds against several bacteria, especial-

Table 3. *In vitro* antibacterial activity of methoxyiminoacetyl derivatives.

(MIC: μg/ml)



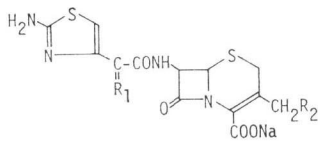
No.		7a	13d	7b	13f	7c	13g	15b	15a	Cefotiam	Cefazolin	Cefuroxime
Compound	R_1	$-NH_2$		$-NH_2$		$-NH_2$		$-CH_3$				
	R_2	$-OCOCH_3$		$-OCONH_2$				$-OCONH_2$				
	Config.	*Z*	*E*	*Z*	*E*	*Z*	*E*	*Z*	*E*			
S. aureus 1840		3.13	25	3.13	100	3.13	50	6.25	12.5	1.56	0.78	1.56
E. coli T-7		0.78	12.5	0.39	25	0.78	50	25	50	3.13	100	25
S. marcescens TN 24		0.78	3.13	0.20	6.25	0.20	3.13	12.5	25	100	>100	>100
P. vulgaris GN 4413		0.78	25	1.56	>100	0.39	>100	100	100	>100	>100	>100
E. cloacae TN 1282		6.25	25	6.25	25	1.56	50	50	>100	100	>100	>100

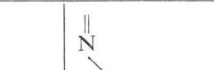
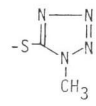
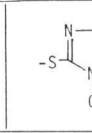
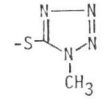
The MICs were determined by a standard agar dilution method in Trypticase soy agar (BBL).

* This assignment of configuration was confirmed by X-ray analysis of cefmenoxime which will be reported elsewhere.

Table 4. Effect of 7-acyl group on antibacterial activity.

(MIC: μg/ml)



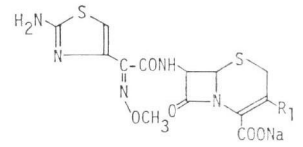
No.		7c	14b	14d	14g	*
Compound	R_1					
	R_2					$-OCOCH_3$
S. aureus 1840		3.13	3.13	3.13	50	12.5
E. coli T-7		0.78	1.56	6.25	0.39	0.78
S. marcescens TN 24		0.20	3.13	3.13	0.78	3.13
P. vulgaris GN 4413		0.39	1.56	12.5	0.025	0.78
E. cloacae TN 1282		1.56	3.13	6.25	0.78	1.56

* Potassium salt was used.
Preparation of this nitrone compound is reported in the forthcoming paper.[16]

ly against β-lactamase-producing strains, is shown in Tables 3, 4 and 5.

From Table 3 it is apparent that all the (*Z*)-isomers (**7a, 7b, 7c**) exhibit excellent activity [*ca.* 10~100 times as active as the corresponding (*E*)-isomers (**13d, 13f, 13g**)]. Substitution of the annular amino group by a methyl group caused marked decrease in activity thus indicating the importance of the amino group for excellent antibacterial activity.

Table 5. Effect of 3-substituent



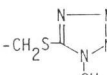
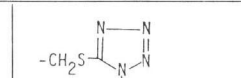
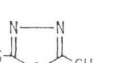
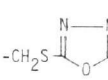
No.		7c	8a	8b	8c
Compound	R_1				
S. aureus 1840		3.13	3.13	1.56	3.13
E. coli T-7		0.78	1.56	1.56	0.78
S. marcescens TN 24		0.20	0.78	0.78	0.39
P. vulgaris GN 4413		0.39	1.56	1.56	3.13
E. cloacae TN 1282		1.56	6.25	3.13	12.5

Table 3 also shows that the (*Z*)-isomers all possess higher activity than cefazolin, cefuroxime and cefotiam, especially against strains resistant to these cephalosporins.

Table 4 indicates the effect of the 7-acyl group on antibacterial activity. Simple homologation of the methoxyimino group (**14b, 14d**) caused a decrease in activity especially against Gram-negative bacteria. Contrary to this, compound **14g** which has a carboxymethyl function exhibited a remarkable improvement in activity against all the Gram-negative bacteria but less activity against *Staphylococcus aureus*. A similar tendency was observed with a nitrone compound*.

From these structure-activity relationships it appears that the combination of the annular amino and (*Z*)-methoxyimino groups is one of the most promising structural features.

The effects of substituent variation at the 3-position on the cephalosporin having this 7-acyl group are shown in Table 5.

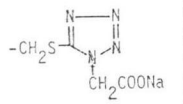
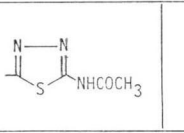
It is apparent that all the compounds with a heterocyclicthio group either *via* the methylene group or directly attached to the cephem ring surpass those with aliphatic substituents, and **7c** is the most potent compound.

The extensive study of structure-activity relationships thus far described led to the selection of 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-ceph-3-em-4-carboxylic acid, cefmenoxime, for further biological evaluation.

Cefmenoxime also possesses high resistance to various β-lactamases[14] and excellent *in vivo* activity as well as good pharmacokinetic properties[15].

For the large scale production of 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid which is required for further biological and clinical evaluations, a stable and unique hemihydrochloride** was prepared[17] and has proved to be of great practical use.

on antibacterial activity.
(MIC: μg/ml)

8d	**12a**	**7d**	**7e**	**7f**	**12b**
		$-CH_3$	$-CH_2OCOCH_2COCH_3$	$-OCH_3$	$-CH=CHCOOC_2H_5$
25	6.25	100	12.5	100	25
1.56	0.78	3.13	0.78	6.25	25
0.78	0.78	0.78	0.78	1.56	6.25
0.025	1.56	1.56	1.56	12.5	50
>100	0.78	50	6.25	100	3.13

* Preparation of this new compound is reported in the forthcoming paper[16].
** X-Ray analysis of the crystallographic structure will be reported elsewhere.

Experimental

Infrared spectra were measured on a Hitachi Type 215 spectrophotometer. NMR spectra were measured on a Varian EM-390 (90 MHz) or T-60 (60 MHz) spectrometer using tetramethylsilane as a standard. UV spectra were done on a Shimadzu UV-210 spectrophotometer. $[\alpha]_D$ was measured on a Perkin Elmer Model 141 polarimeter. All melting points are uncorrected.

2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl chloride (**4b**) hydrochloride

To an ice-cooled suspension of 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (**1b**) (278 mg) in CH_2Cl_2 (5 ml) was added PCl_5 (208 mg) with stirring. After stirring for 30 minutes at room temperature, *n*-hexane (2 ml) was added to the mixture. The separated solids were collected by suction to give **4b** as its hydrochloride, a colorless crystal, 276 mg. *Anal.* Calcd. for $C_8H_7Cl_2N_3O_3S \cdot HCl$: C, 28.89; H, 2.42; N, 12.63. Found: C, 28.47; H, 2.73; N, 12.12.

2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl chloride (**4b**)

To a solution of **1b** (55.6 g) and triethylamine (24.3 g) in CH_2Cl_2 (600 ml) was added PCl_5 (41.8 g) in two portions under ice-cooling. The ice-bath was removed 5 minutes after addition. After stirring for 20 minutes at room temperature, the mixture was concentrated under reduced pressure. To the residue was added *n*-hexane (1 liter) and the mixture was stirred vigorously. The precipitated oil was separated by decantation of the *n*-hexane and dissolved in THF (600 ml). Triethylamine hydrochloride separated from the THF solution and was removed by filtration to give a THF solution of **4b**.

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]cephalosporanate (**7a**)

(a) To an ice-cooled suspension of 7-ACA (**5a**) (762 mg) in DMA (15 ml) was added **4b** hydrochloride (931 mg) with stirring. After stirring for 15 minutes under ice-cooling and then 2 hours at room temperature, the mixture was poured into water (10 ml) and extracted twice with AcOEt. The combined extract was, after washing and drying over $MgSO_4$, concentrated under reduced pressure to give **6a** as an oil, 1.40 g. To a solution of **6a** (1.40 g) in THF (30 ml) were added thiourea (500 mg) and $AcONa \cdot 3H_2O$ (895 mg). After stirring for 4 hours at room temperature, the separated solids were collected by suction and washed with Et_2O. This was dissolved in water (6 ml) and the solution was adjusted to pH 7.0 with $NaHCO_3$ which was chromatographed on an Amberlite XAD-2 column. Elution with water and lyophilization gave **7a**, a colorless powder, 78 mg. *Anal.* Calcd. for $C_{16}H_{16}N_5O_7S_2Na \cdot 2.5H_2O$: C, 36.78; H, 4.05; N, 13.40. Found: C, 36.93; H, 3.80; N, 12.68. NMR (60 MHz, D_2O) δ: 2.07 (3H, s, $COCH_3$), 3.53 (2H, q, 2-CH_2), 3.98 (3H, s, OCH_3), 4.75 (2H, q, 3-CH_2), 5.21 (1H, d, 6-H), 5.81 (1H, d, 7-H), 7.01 (1H, s, thiazole 5-H).

Further elution with 5% aqueous EtOH gave rise to the (*E*)-isomer (**13d**), 62 mg. The identity was confirmed by comparison of the NMR spectrum of the sample prepared unambiguously (see below).

(b) To an ice-cooled solution of 7-ACA (**5a**) (10.0 g) and triethylamine (11.0 g) in 50% aqueous THF (120 ml) was added dropwise a THF solution of **4b** [prepared from **1b** (10.0 g)]. After addition, the mixture was stirred for 1 hour at room temperature. Water and AcOEt were added to the mixture and the aqueous layer was adjusted to pH 2.0 with H_3PO_4. The organic layer was separated after shaking and the aqueous layer was extracted with AcOEt. Conventional work-up of the combined organic layer afforded **6a**, as a powder, 10.7 g. NMR (60 MHz, d_6-DMSO) δ: 2.00 (3H, s, $COCH_3$), 3.55 (2H, bs, 2-CH_2), 3.86 (3H, s, OCH_3), 4.33 (2H, s, $ClCH_2$), 4.84 (2H, q, 3-CH_2), 5.15 (1H, d, 6-H), 5.83 (1H, dd, 7-H), 7.40 (1H, s, thiazole 5-H).

6a was dissolved in DMA (40 ml) and thiourea (3.40 g) was added. After stirring for 15 hours at room temperature, the mixture was poured into ice-water and adjusted to pH 3.5 with $NaHCO_3$. The separated solids were collected by suction and dissolved in 5% aqueous $NaHCO_3$ and chromatographed on Amberlite XAD-2 column. Elution with water followed by 2% aqueous EtOH and lyophilization gave **7a**, as a colorless powder, 4.20 g. This was identical with the specimen obtained in section (a).

Several (*Z*)-methoxyiminoacyl derivatives (**7b**~**7f**) were obtained by treatment similar to that described above (section (b)) for 7-aminocephalosporins (**5b**~**5d**, **5e**[18], **5f**[19]). Analytical and spectral data of these compounds are given below.

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-carbamoyloxymethylceph-3-em-4-carboxylate (**7b**)

Anal. Calcd. for $C_{15}H_{15}N_6O_7S_2Na \cdot 3H_2O$: C, 33.84; H, 3.98; N, 15.78. Found: C, 33.94; H, 3.82; N, 15.42. NMR (60 MHz, D_2O) δ: 3.47 (2H, q, 2-CH_2), 3.92 (3H, s, OCH_3), 4.68 (2H, q, 3-CH_2), 5.27 (1H, d, 6-H), 5.72 (1H, d, 7-H), 6.95 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)-thiomethyl]ceph-3-em-4-carboxylate (**7c**)

Anal. Calcd. for $C_{16}H_{16}N_9O_5S_3Na \cdot 2H_2O$: C, 33.74; H, 3.54; N, 22.13. Found: C, 34.18; H, 3.57; N, 21.79. NMR (60 MHz, D_2O) δ: 3.59 (2H, q, 2-CH_2), 3.93 and 3.98 (3H×2, s×2, OCH_3 and N-CH_3), 4.08 (2H, q, 3-CH_2), 5.12 (1H, d, 6-H), 5.72 (1H, d, 7-H), 6.93 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methylceph-3-em-4-carboxylate (**7d**)

Anal. Calcd. for $C_{14}H_{14}N_5O_5S_2Na \cdot 1.5H_2O$: C, 37.67; H, 3.84; N, 15.68; Found: C, 37.37; H, 3.98; N, 15.38. NMR (60 MHz, D_2O) δ: 1.94 (3H, s, 3-CH_3), 3.46 (2H, q, 2-CH_2), 4.00 (3H, s, OCH_3), 5.17 (1H, d, 6-H), 5.76 (1H, d, 7-H), 6.99 (1H, s, thiazole 5-H).

Sodium 3-acetoacetoxymethyl-7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]ceph-3-em-4-carboxylate (**7e**)

Anal. Calcd. for $C_{18}H_{18}N_5O_8S_2Na \cdot 3H_2O$: C, 37.69; H, 4.21; N, 12.21. Found: C, 37.97; H, 4.01; N, 12.49. NMR (60 MHz, D_2O) δ: 2.31 (3H, s, $COCH_3$), 3.35 (2H, q, 2-CH_2), 4.01 (5H, s, OCH_3 and $COCH_2CO$), 4.92 (2H, q, 3-CH_2), 5.24 (1H, d, 6-H), 5.83 (1H, d, 7-H), 7.02 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methoxyceph-3-em-4-carboxylate (**7f**)

Anal. Calcd. for $C_{14}H_{14}N_5O_6S_2Na \cdot 2.5H_2O$: C, 34.99; H, 3.99; N, 14.58. Found: C, 35.18; H, 3.66; N, 14.28. NMR (60 MHz, D_2O) δ: 3.60 (2H, q, 2-CH_2), 3.76 (3H, s, 3-OCH_3), 4.01 (3H, s, N-OCH_3), 5.24 (1H, d, 6-H), 5.66 (1H, d, 7-H), 7.06 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-(5-acetamido-1,3,4-thiadiazol-2-yl)ceph-3-em-4-carboxylate (**12a**)

To a solution of diphenylmethyl 7-amino-3-(5-acetamido-1,3,4-thiadiazol-2-yl)ceph-3-em-4-carboxylate (**9a**)[20] (202 mg) in DMA (3 ml) was added a THF solution of **4b** [prepared from **1b** (133 mg)]. After stirring for 2 hours at room temperature, the mixture was poured into water and extracted with AcOEt. Conventional work-up of the extract afforded **10a**, as a powder, 145 mg. To a solution of **10a** (100 mg) in DMA (1.5 ml) was added thiourea (25 mg). After stirring for 18 hours at room temperature, the mixture was poured into water and extracted with AcOEt. Conventional work-up of the extract gave rise to **11a** which was dissolved in a mixture of CF_3COOH (4 ml) and anisole (1 ml). After stirring for 30 minutes at room temperature, the mixture was poured into Et_2O and the separated solids were collected by suction. This was dissolved in water (0.5 ml) and adjusted to pH 7.0 with $NaHCO_3$. The solution was chromatographed on an Amberlite XAD-2 column. Elution with water and lyophilization gave **12a**, as a powder, 29 mg. *Anal.* Calcd. for $C_{17}H_{15}N_8O_6S_3Na \cdot 4.5H_2O$: C, 32.58; H, 3.85; N, 17.85. Found: C, 32.19; H, 3.29; N, 17.14. NMR (60 MHz, D_2O) δ: 2.26 (3H, s, $COCH_3$), 4.02 (5H, bs, 2-CH_2 and N-OCH_3), 5.37 (1H,d, 6-H), 5.92 (1H, d, 7-H), 7.01 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-ethoxycarbonylvinylceph-3-em-4-carboxylate (**12b**)

To a solution of diphenylmethyl 7-amino-3-ethoxyvinylceph-3-em-4-carboxylate (**9b**)[21] (900 mg) in DMA (50 ml) was added a CH_2Cl_2 solution of **4b** [prepared from **1b** (613 mg)]. After stirring for 2.5 hours at room temperature, the mixture was extracted with AcOEt. Conventional work-up of the extract gave an oil which was chromatographed on a silica gel column. Elution with $CHCl_3$-AcOEt (7: 3) afforded **10b**, as a powder, 0.70 g. This was dissolved in AcOEt (50 ml) and to this

solution was added a solution of sodium N-methyldithiocarbamate (0.13 g) in MeOH (10 ml). After stirring for 1 hour at room temperature, the mixture was poured into water and extracted with AcOEt. Conventional work-up of the extract followed by chromatography on silica gel column ($CHCl_3$-AcOEt=1: 1) afforded **11b**, as a powder, 0.39 g.

This was dissolved in a mixture of CF_3COOH (7 ml) and anisole (1.5 ml). After stirring for 30 minutes at room temperature the mixture was poured into Et_2O and the separated solids were collected by suction which were dissolved in water (1 ml) by adding $NaHCO_3$. After neutralization (pH 7.0) with $NaHCO_3$, the solution was chromatographed on an Amberlite XAD-2 column. Elution with water followed by lyophilization gave rise to **12b**, as a colorless powder, 66 mg. NMR (60 MHz, D_2O) δ: 1.28 (3H, t, $CH_2C\underline{H}_3$), 3.65 (2H, bs, 2-CH_2), 4.00 (3H, s, N-OCH_3), 4.23 (2H, q, $C\underline{H}_2CH_3$), 5.31 (1H, d, 6-H), 5.86 (1H, d, 7-H), 6.04 (1H, d, CH=C<u>H</u>-CO), 6.96 (1H, s, thiazole 5-H), 7.70 (1H, d, C<u>H</u>=CHCO).

2-(2-Chloroacetamidothiazol-4-yl)-(*E*)-2-methoxyiminoacetyl chloride (**4a**) hydrochloride

To an ice-cooled suspension of **1a** (555 mg) in CH_2Cl_2 (5 ml) was added PCl_5 (416 mg) in one portion with stirring. While stirring for 30 minutes, **1a** went into solution followed by separation of solid. The solids were, after addition of *n*-hexane (5 ml), collected by suction to afford **4a** as its hydrochloride, as a colorless powder, 620 mg. *Anal.* Calcd. for $C_8H_7Cl_2N_3O_3S \cdot HCl$: C, 28.89; H, 2.42; N, 12.63. Found: C, 28.35; H, 2.81; N, 12.60.

t-Butyl 7β-[2-(2-chloroacetamidothiazol-4-yl)-(*E*)-2-methoxyiminoacetamido]cephalosporanate (**13a**)

To an ice-cooled solution of *t*-butyl 7-aminocephalosporanate (4.0 g)[22] and pyridine (2.66 g) in CH_2Cl_2 (60 ml) was added **4a** hydrochloride (5.26 g). After stirring for 1 hour at room temperature, $CHCl_3$ (60 ml) was added to the reaction mixture. The solution was washed with 0.5 N HCl and then with water and dried over $MgSO_4$. Evaporation of organic solvents gave **13a**, as a colorless powder, 5.0 g. *Anal.* Calcd. for $C_{22}H_{26}ClN_5O_8S_2$: C, 44.93; H, 4.46; N, 11.91. Found: C, 44.74; H, 4.64; N, 11.61. NMR (60 MHz, $CDCl_3$) δ: 1.50 (9H, s, *t*-C_4H_9), 2.10 (3H, s, $OCOCH_3$), 3.45 (2H, q, 2-CH_2), 4.10 (3H, s, OCH_3), 4.28 (2H, s, $ClCH_2$), 4.90 (2H, q, 3-CH_2), 4.96 (1H, d, 6-H), 5.80 (1H, dd, 7-H), 7.84 (1H, s, thiazole 5-H), 8.05 (1H, d, NH).

t-Butyl 7β-[2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetamido]cephalosporanate (**13b**)

A solution of **13a** (5.0 g), thiourea (970 mg) and benzyltriethylammonium bromide (250 mg) in a mixture of EtOH (25 ml) and THF (50 ml) was stirred overnight at room temperature. The mixture was poured into 10% aqueous $NaHCO_3$ and extracted with AcOEt. Conventional work-up of the extract followed by chromatography on a silica gel column afforded **13b**, as a powder, 2.23 g. NMR (60 MHz, $CDCl_3$) δ: 1.54 (9H, s, *t*-C_4H_9), 2.08 (3H, s, $OCOCH_3$), 3.50 (2H, q, 2-CH_2), 4.12 (3H, s, OCH_3), 4.96 (2H, q, 3-CH_2), 5.06 (1H, d, 6-H), 5.52 (2H, bs, NH_2), 6.01 (1H, dd, 7-H), 7.45 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetamido]cephalosporanate (**13d**)

To a mixture of CF_3COOH (16 ml) and anisole (1.6 ml) was dissolved **13b** (1.74 g) and the solution was stirred for 2 hours at room temperature. Addition of Et_2O (200 ml) to the mixture caused separation of a solid which was collected by suction and washed with Et_2O to give the trifluoroacetic acid salt of **13c**, as a powder, 1.45 g.

A solution of the trifluoroacetic acid salt of **13c** (450 mg) and $NaHCO_3$ (170 mg) in water (5 ml) was chromatographed on an Amberlite XAD-2 column. Elution with water and lyophilization afforded **13d**, as a colorless powder, 141 mg. *Anal.* Calcd. for $C_{16}H_{16}N_5O_7S_2Na \cdot 2H_2O$: C, 37.43; H, 3.93; N, 13.64. Found: C, 37.10; H, 4.13; N, 13.34. NMR (90 MHz, D_2O) δ: 2.11 (3H, s, $OCOCH_3$), 3.49 (2H, q, 2-CH_2), 4.06 (3H, s, OCH_3), 4.84 (2H, q, 3-CH_2), 5.17 (1H, d, 6-H), 5.81 (1H, d, 7-H), 7.50 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetamido]-3-carbamoyloxymethylceph-3-em-4-carboxylate (**13f**)

To an ice-cooled solution of **5g** (1.05 g) in DMA (20 ml) was added **4a** hydrochloride (869 mg).

After stirring for 2 hours at room temperature, the mixture was poured into water (50 ml) and extracted with AcOEt. Conventional work-up of the extract gave **13e**, 2.2 g. This was dissolved in THF (50 ml) and thiourea (913 mg) and $AcONa \cdot 3H_2O$ (1.63 g) were added to this solution. After stirring for 17 hours at room temperature, the separated solids were collected by suction and dissolved in water (10 ml). The solution, after neutralization (pH 7.0) with $NaHCO_3$, was chromatographed on an Amberlite XAD-2 column. Elution with water and lyophilization afforded **13f**, as a colorless powder, 360 mg. *Anal.* Calcd. for $C_{15}H_{15}N_6O_7S_2Na \cdot 2.5H_2O$: C, 34.42; H, 3.85; N, 16.05. Found: C, 34.43; H, 3.70; N, 15.68. NMR (60 MHz, D_2O) δ: 3.55 (2H, q, 2-CH_2), 4.11 (3H, s, OCH_3), 4.81 (2H, q, 3-CH_2), 5.21 (1H, d, 6-H), 5.82 (1H, d, 7-H), 7.55 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*E*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)-thiomethyl]ceph-3-em-4-carboxylate (**13g**)

To a solution of 1-methyl-1*H*-tetrazole-5-thiol (272 mg), $NaHCO_3$ (555 mg) and benzyltriethylammonium bromide (68 mg) in water (10 ml) was added trifluoroacetic acid salt of **13c** (1.0 g) and the mixture was stirred for 6 hours at 60°C under an atmosphere of nitrogen. After cooling, the mixture was chromatographed on an Amberlite XAD-2 column. Elution with water, and then with 2.5% aqueous EtOH followed by lyophilization gave **13g**, as a powder, 205 mg. *Anal.* Calcd. for $C_{16}H_{16}N_9O_5S_3Na \cdot 2H_2O$: C, 33.74; H, 3.54; N, 22.13. Found: C, 34.25; H, 3.81; N, 21.69. NMR (90 MHz, D_2O) δ: 3.57 (2H, q, 2-CH_2), 3.98, 4.05 (3H×2, s×2, N-CH_3 and OCH_3), 4.18 (2H, q, 3-CH_2), 5.12 (1H, d, 6-H), 5.75 (1H, d, 7-H), 7.48 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[[1-(2-N,N-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thiomethyl]ceph-3-em-4-carboxylate (**8a**)

To a solution of **7e** (993 mg) in water (10 ml) were added 1-(2-N,N-dimethylaminoethyl)-1*H*-tetrazole-5-thiol[2,18] (346 mg) and $NaHCO_3$ (168 mg) and the mixture was heated at 55°C for 1 hour with stirring. After cooling, the mixture was chromatographed on an Amberlite XAD-2 column. Elution with water followed by lyophilization gave **8a**, as a powder, 170 mg. *Anal.* Calcd. for $C_{19}H_{23}N_{10}O_5S_3Na \cdot 3H_2O$: C, 35.40; H, 4.53; N, 21.72. Found: C, 35.75; H, 4.43; N, 21.00 NMR (60 MHz, D_2O) δ: 2.20 (6H, s, $N(CH_3)_2$), 2.85 ($\left(\text{2H, t, } CH_2N\langle^{CH_3}_{CH_3}\right)$), 3.48 (2H, q, 2-$CH_2$), 3.88 (3H, s, N-$OCH_3$), 4.16 (2H, q, 3-$CH_2$), 4.44 (2H, t, CH_2), 5.10 (1H, 2, 6-H), 5.66 (1H, d, 7-H), 6.90 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl]ceph-3-em-4-carboxylate (**8b**)

A solution of **7e** (993 mg) and soidum salt of 2-methyl-1,3,4-thiadiazole-5-thiol (308 mg) in water (10 ml) was heated at 55°C for 2 hours with stirring. Similar work-up as described for the preparation of **8a** afforded **8b**, as a powder, 122 mg. *Anal.* Calcd. for $C_{17}H_{16}N_7O_5S_4Na \cdot 3H_2O$: C, 32.48; H, 3.74; N, 16.57. Found: C, 32.01; H, 4.01; N, 15.98.

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(2-methyl-1,3,4-oxadiazol-5-yl)thiomethyl]ceph-3-em-4-carboxylate (**8c**)

A solution of **7a** (113 mg) and the sodium salt of 2-methyl-1,3,4-oxadiazole-5-thiol (46 mg) in water (1 ml) was heated at 60°C for 7 hours. The mixture was, after cooling, chromatographed on an Amberlite XAD-2 column. Elution with 2% aqueous EtOH followed by lyophilization gave **8c**, as a powder, 41 mg. *Anal.* Calcd. for $C_{17}H_{16}N_7O_6S_3Na \cdot 2H_2O$: C, 35.85; H, 3.54; N, 17.21. Found: C, 35.73; H, 3.72; N, 17.01.

Disodium salt of 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-carboxymethyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**8d**)

To a solution of **7a** (9.25 g) were added $NaHCO_3$ (3.01 g) and 1-carboxymethyl-1*H*-tetrazole-5-thiol (3.0 g)[23] in water (90 ml) and the mixture was heated at 55°C for 9 hours with stirring. After cooling, the mixture was chromatographed on an Amberlite XAD-2 column. Elution with water followed by lyophilization afforded **8d**, as a powder, 4.20 g. *Anal.* Calcd. for $C_{17}H_{15}N_9O_7S_3Na_2 \cdot 4.5H_2O$: C, 30.00; H, 3.26; N, 18.56. Found: C, 30.33; H, 3.52; N, 18.34.

These new 3-heterocyclicthiomethyl derivatives (**8b**~**8d**) gave reasonable NMR spectra.

Removal of the chloroacetyl group with sodium N-methyldithiocarbamate

(a) Preparation of **7a**

To a suspension of **6a** (2.4 g) in water (7 ml) were added $NaHCO_3$ (384 mg) and sodium N-methyldithiocarbamate (650 mg). After stirring for 1 hour at room temperature, the mixture was extracted with AcOEt. Conventional work-up of the AcOEt extract gave 3-methyl-4-thiazolidinone-2-thione as crystals (from Et_2O). Mp 71~72°C (lit[11]. 72°C). *Anal.* Calcd. for $C_3H_5NOS_2$: C, 32.63; H, 3.42; N, 9.52. Found: C, 32.43; H, 3.40; N, 9.49. NMR (60 MHz, $CDCl_3$) δ: 3.36 (3H, s, CH_3), 4.01 (2H, s, CH_2).

The aqueous layer was chromatographed on an Amberlite XAD-2 column. Elution with water followed by lyophilization gave rise to **7a**, as a powder, 1.11 g.

(b) Preparation of **7b**

To an ice-cooled suspension of **5g** (10.5 g) in 50% aqueous THF (150 ml) was added triethylamine (9.09 g). To the resulting solution was added a THF solution of **4b** [prepared from **1b** (8.75 g)]. After stirring for 2 hours under ice-cooling, the mixture was adjusted to pH 2.0 with 1 N HCl and extracted with AcOEt. Conventional work-up gave **6g**, as crystals, 14.8 g. NMR (60 MHz, $CDCl_3$) δ: 3.50 (2H, q, 2-CH_2), 3.99 (3H, s, N-OCH_3), 4.04 and 4.30 (2H×2, s×2, $ClCH_2CO$×2), 4.95 (2H, q, 3-CH_2), 5.10 (1H, d, 6-H), 5.73 (1H, d, 7-H), 7.32 (1H, s, thiazole 5-H).

A suspension of **6g** (1.6 g) in water (10 ml) was adjusted to pH 7.0 with $NaHCO_3$ to dissolve **6g**. To the solution was added sodium N-methyldithiocarbamate (0.80 g). After stirring for 1 hour at room temperature, the mixture was washed with AcOEt and the aqueous layer was chromatographed on an Amberlite XAD-2 column. Elution with water followed by lyophilization afforded **7b**, as a powder, 650 mg.

(c) Preparation of **7c**

A suspension of 7β-[2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**6c**) (160 g) [prepared by acylating **5c** with **4b** in the presence of triethylamine; NMR (90 MHz, d_6-DMSO) δ: 3.66 (2H, q, 2-CH_2), 3.87, 3.92 (3H×2, N-CH_3 and OCH_3), 4.30 (2H, q, 3-CH_2), 4.32 (2H, s, $ClCH_2$), 5.10 (1H, d, 6-H), 5.76 (1H, dd, 7-H), 7.40 (1H, s, thiazol 5-H), 9.63 (1H, d, NH)] in a mixture of water (650 ml) and AcOEt (250 ml) was adjusted to pH 6.5 with $NaHCO_3$ under ice-cooling to dissolve **6c**. To the solution was added sodium N-methyldithiocarbamate (41.5 g) under ice-cooling. After stirring for 3 hours at room temperature, the mixture was washed with AcOEt (300 ml×3). The aqueous layer was concentrated under reduced pressure to about 0.4 liter and was chromatographed on an Amberlite XAD-2 column using water and subsequently 4% aqueous EtOH as eluant. From the fraction eluted with 4% aqueous EtOH, after lyophilization, was obtained crude **7c**, as a powder, 71.4 g. This was dissolved in water (300 ml) and chromatographed on a Sephadex LH-20 column. Elution with water followed by lyophilization afforded **7c**, as a colorless powder, 58.8 g.

The compounds prepared in these sections (a), (b), (c) were all identical with the compounds described earlier in the Experimental section.

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-ethoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)-thiomethyl]ceph-3-em-4-carboxylate (**14b**)

To an ice-cooled suspension of **2a** (1.17 g) in CH_2Cl_2 (12 ml) were added triethylamine (506 mg) and PCl_5 (833 mg). After stirring for 30 minutes with ice-cooling, the mixture was concentrated under reduced pressure. To the residue was added *n*-hexane and, after shaking, *n*-hexane was removed by decantation. The residual oil was dissolved in THF (5 ml) and the separated solids (triethylamine hydrochloride) were removed by filtration to obtain a THF solution of the acid chloride of **2a**.

To an ice-cooled solution of **5c** (1.31 g) and triethylamine (1.24 g) in 50% aqueous THF (16 ml) was added the THF solution of the acid chloride of **2a**. After stirring for 30 minutes under ice-cooling and then for 1 hour at room temperature, the mixture was adjusted to pH 2.0 with H_3PO_4 and extracted with AcOEt. The extract was washed with water and dried over $MgSO_4$. Evaporation of the solvent

afforded **14a**, as a powder, 2.40 g. NMR (90 MHz, d_6-DMSO) δ: 1.24 (3H, t, $C\underline{H}_3CH_2$), 3.61 (2H, q, 2-CH_2), 3.91 (3H, s, N-CH_3), 4.34 (2H, s, $ClCH_2$), 5.12 (1H, d, 6-H), 5.78 (1H, dd, 7-H), 7.38 (1H, s, thiazole 5-H), 9.61 (1H, d, NH).

To a solution of **14a** (1.81 g) and $NaHCO_3$ (252 mg) in water (20 ml) was added sodium N-methyldithiocarbamate (517 mg). After stirring for 3 hours at room temperature, the mixture was washed with AcOEt and the aqueous layer was chromatographed on an Amberlite XAD-2 column. Elution with 5% aqueous EtOH followed by lyophilization gave rise to **14b**, as a colorless powder, 832 mg. *Anal.* Calcd. for $C_{17}H_{18}N_9O_5S_3Na\cdot 2H_2O$: C, 34.99; H, 3.80; N, 21.60. Found: C, 34.67; H, 3.70; N, 21.76. NMR (90 MHz, D_2O) δ: 1.31 (3H, t, $C\underline{H}_3CH_2$), 3.64 (2H, q, 2-CH_2), 4.03 (3H, s, N-CH_3), 4.18 (2H, q, 3-CH_2), 4.26 (3H, q, $CH_3C\underline{H}_2$), 5.18 (1H, d, 6-H), 5.77 (1H, d, 7-H), 6.98 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-isopropyloxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylate (**14d**)

Similar reactions as above using **2b** (1.22 g) and **5c** (1.31 g) gave **14d** (551 mg) *via* **14c** (2.30 g).

14c, colorless powder. NMR (90 MHz, d_6-DMSO) δ: 1.25 (6H, d, $CH_3\times 2$), 3.67 (2H, q, 2-CH_2), 3.91 (3H, s, N-CH_3), 4.32 (2H, s, $ClCH_2$), 5.12 (1H, d, 6-H), 5.79 (1H, dd, 7-H), 7.35 (1H, s, thiazole 5-H), 9.54 (1H, d, NH), 12.85 (1H, bs, COOH).

14d, colorless powder. *Anal.* Calcd. for $C_{18}H_{20}N_9O_5S_3Na\cdot 3H_2O$: C, 35.12; H, 4.26; N, 20.48. Found: C, 35.03; H, 4.00; N, 20.47. NMR (90 MHz, D_2O) δ: 1.31 (6H, d, $CH_3\times 2$), 3.64 (2H, q, 2-CH_2), 4.04 (3H, s, N-CH_3), 4.18 (2H, q, 3-CH_2), 4.50 (1H, septet, $C\underline{H}Me_2$), 5.18 (1H, d, 6-H), 5.77 (1H, d, 7-H), 6.97 (1H, s, thiazole 5-H).

Disodium salt of 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-carboxymethoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**14g**)

Similar reaction as described for **14b** using **2c** (1.13 g) and **5c** (985 mg) afforded **14e**, as a powder, 1.95 g. NMR (60 MHz, d_6-DMSO) δ: 1.30 (9H, s, *t*-Bu), 3.60 (2H, q, 2-CH_2), 3.81 (3H, s, N-CH_3), 4.26 (2H, s, $ClCH_2$), 4.49 (2H, s, OCH_2), 5.04 (1H, d, 6-H), 5.71 (1H, dd, 7-H), 7.32 (1H, s, thiazole 5-H).

14e (1.72 g) was dissolved in a mixture of CF_3COOH (10 ml) and anisole (2 ml) and the mixture was stirred for 30 minutes at room temperature. After concentration under reduced pressure, water and AcOEt were added to the condensate. After shaking, the organic layer was separated and washed with water and dried over $MgSO_4$. Evaporation of the solvent afforded **14f**, as a powder, 1.20 g.

To a solution of **14f** (1.01 g) and $NaHCO_3$ (268 mg) in water (10 ml) was added sodium N-methyldithiocarbamate (258 mg). After stirring for 2 hours at room temperature, the mixture was washed with AcOEt and chromatographed on an Amberlite XAD-2 column. Elution with water followed by lyophilization gave rise to **14g**, as a powder, 106 mg. *Anal.* Calcd. for $C_{17}H_{15}N_9O_3S_3Na_2\cdot 3H_2O$: C, 31.24; H, 3.24; N, 19.29. Found: C, 31.05; H, 3.38; N, 18.94. NMR (90 MHz, D_2O) δ: 3.64 (2H, q, 2-CH_2), 4.04 (3H, s, N-CH_3), 4.20 (2H, q, 3-CH_2), 4.60 (2H, s, OCH_2), 5.19 (1H, d, 6-H), 5.78 (1H, d, 7-H), 7.04 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-methylthiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-carbamoyloxymethylceph-3-em-4-carboxylate (**15b**)

To a solution of **3b** (601 mg) in a mixture of CH_2Cl_2 (2 ml) and DMA (2 ml) was added PCl_5 (625 mg) with cooling (−10°C). Stirring was continued for 1 hour to obtain a solution of the acid chloride of **3b**. This was added to a solution of **5g** (1.05 g) in DMA and the mixture was stirred for 2 hours under cooling (−5°C). After addition of water, the mixture was extracted with AcOEt. Conventional work-up of the extract gave 3-(N-chloroacetylcarbamoyloxymethyl)-7β-[2-(2-methylthiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]ceph-3-em-4-carboxylic acid, as a powder, 1.20 g.

The above was dissolved in 3% aqueous $NaHCO_3$ (300 ml) and the solution was lyophilized after stirring for 4 hours at room temperature. The powdery residue was dissolved in a small amount of water and chromatographed on an Amberlite XAD-2 column. Elution with water followed by lyophilization afforded **15b**, as a colorless powder, 211 mg. *Anal.* Calcd. for $C_{16}H_{16}N_5O_7S_2Na\cdot 1.5H_2O$: C, 38.09; H, 3.80; N, 13.88. Found: C, 38.88; H, 3.82; N, 12.47. NMR (60 MHz, D_2O) δ: 2.65

(3H, s, thiazole 2-CH_3), 3.49 (2H, q, 2-CH_2), 4.00 (3H, s, N-OCH_3), 4.64 (2H, q, 3-CH_2), 5.14 (1H, d, 6-H), 5.74 (1H, d, 7-H), 7.57 (1H, s, thiazole 5-H).

Sodium 3-carbamoyloxymethyl-7β-[2-(2-methylthiazol-4-yl)-(*E*)-2-methoxyiminoacetamido]ceph-3-em-4-carboxylate (**15a**)

To a cooled (−10°C) solution of **3a** (601 mg) and triethylamine (303 mg) in THF (35 ml) was added isobutyloxycarbonylchloride (408 mg) dropwise with stirring. After addition the mixture was stirred for 70 minutes at −10°C. To this was added dropwise a solution of **5g** (1.05 g) and triethylamine (303 mg) in 50% aqueous THF (28 ml). After stirring for 1 hour under ice-cooling and then for 2 hours at room temperature, the mixture was poured into water and adjusted to pH 2.0 with 1 N HCl followed by extraction with AcOEt. Conventional work-up of the extract gave crude 3-(N-chloroacetylcarbamoyloxymethyl)-7β-[2-(2-methylthiazol-4-yl)-(*E*)-2-methoxyiminoacetamido]ceph-3-em-4-carboxylic acid as an oil, 1.3 g.

The above oil was dissolved in THF (35 ml) and to this solution were added thiourea (372 mg) and $AcONa \cdot 3H_2O$ (831 mg). After stirring for 15 hours at room temperature, the separated solids were collected by suction and dissolved in water (10 ml). The aqueous solution was adjusted to pH 7.0 with $NaHCO_3$ and chromatographed on an Amberlite XAD-2 column. Elution with water followed by lyophilization afforded **15a**, as a colorless powder, 395 mg. *Anal.* Calcd. for $C_{16}H_{16}N_5O_7S_2Na \cdot 1.5H_2O$: C, 38.09; H, 3.80; N, 13.88. Found: C, 38.45; H, 3.89; H, 13.03. NMR (60 MHz, D_2O) δ: 2.60 (3H, s, CH_3), 3.40 (2H, q, 2-CH_2), 3.98 (3H, s, OCH_3), 4.63 (2H, q, 3-CH_2), 5.08 (1H, d, 6-H), 5.70 (1H, d, 7-H), 7.95 (1H, s, thiazole 5-H).

7β-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid hemihydrochloride

To a solution of **7c** (165.0 g) in water (3.3 liters) was added 10% HCl (0.3 liter) at 25.5°C dropwise with stirring. During addition a colorless solid (free acid of **7c**) separated and subsequently went into solution. The solution was stirred for 1 hour at this temperature and newly separated colorless crystals were collected by suction and washed with water followed by drying over anhydrous silica gel under reduced pressure to give the hemihydrochloride, as colorless crystals, 154.5 g. *Anal.* Calcd. for $C_{16}H_{17}N_9O_5S_3 \cdot \frac{1}{2}HCl \cdot \frac{1}{2}H_2O$: C, 35.67; H, 3.46; N, 23.40; S, 17.80; Cl, 3.29. Found: C, 35.78; H, 3.41; N, 23.46; S, 17.88; Cl, 3.46. Moisture content (determined by KARL FISHER method): 2.1%. IR λ_{max}^{KBr} cm^{-1}: 1780 (β-lactam). NMR (90 MHz, d_6-DMSO) δ: 3.67 (2H, q, 2-CH_2), 3.89, 3.91 (3H×2, s×2, NCH_3 and OCH_3), 4.27 (2H, q, 3-CH_2), 5.09 (1H, d, 6-H), 5.71 (1H, dd, 7-H), 6.80 (1H, s, thiazole 5-H), 9.65 (1H, d, CONH). UV $\lambda_{max}^{0.1M\ phosphate\ buffer\ (pH\ 6.9)}$ nm (ε): 257 (1.84×10^4). $[\alpha]_D^{20}$ [*c* 1.0, 0.1 M phosphate buffer (pH 6.8)]: −33° (on the anhydrous basis).

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