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

III. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 7β-[2-AMINO-2-(2-AMINOTHIAZOL-4-YL)ACETAMIDO]CEPHALOSPORINS*

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New derivatives of 7β-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins having amino group at the 2-position of the 7-acyl moiety were synthesized in the hope that they would show improved antibacterial activity. Some of these compounds (**XXa, XXd, XVb**) showed improved activity against *Enterobacter cloacae*. Replacement of the annular amino group by a methyl group caused loss of activity.

In a previous paper[1] we reported the introduction of an alkyl and hydroxyl groups into the acyl moiety of 7β-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins and the changes in activity against some β-lactamase-producing strains of Gram-negative bacteria caused by these chemical modifications.

Among many cephalosporins, 2-aminoacyl derivatives are characterized[2] by acid stability, high potency against Gram-negative organisms as well as pharmacokinetic properties. These features prompted us to examine the effect that an amino group at the 2-position of the 7-acyl group might have biological activity.

Chemistry

Reaction of ethyl 4-bromo-2-ethoxyimino-3-oxobutyrate (**II**) obtained by bromination of ethyl 2-ethoxyimino-3-oxobutyrate (**I**)[3] with N-(2,2,2-trichloroethoxycarbonyl)thiourea followed by alkaline hydrolysis gave 2-ethoxyimino-2-[2-(2,2,2-trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid (**IVa**). Acylation of 7-aminodeacetoxycephalosporanic acid (7-ADCA) with the acid chloride (**IVb**) afforded the 7-acylated compound which was purified as its sodium salt (**V**). Simultaneous reduction of the ethoxyimine group and reductive removal of the amino-protecting group of **V** with zinc in formic acid gave the anticipated compound (**VI**) in good yield (Chart 1).

Similar treatment of 7-aminocephalosporanic acid (7-ACA, **XIIIa**) with **IVb** and conventional work up of the reaction mixture, however, resulted in a relatively low yield of the anticipated compound (**XVa**) probably due to reductive cleavage of the 3-substituent[4] and a nucleophilic attack of the free amino group at the 2-position of the acyl moiety on the β-lactam carbonyl group. Therefore another synthetic route, in which reductive reaction conditions and purification of the product in a form having a free amino group at the 2-position of the acyl moiety were avoided, was desirable.

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Thus 2-*t*-butyloxycarbonylamino-2-(2-chloroacetamidothiazol-4-yl)acetic acid (**XII**) was synthesized by the reactions shown in Chart 2 as an effective tool for the acylation of 7-aminocephalosporins. Thiourea and ethyl 4-chloro-2-hydroxyimino-3-oxobutyrate (**VII**)[5] gave ethyl 2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetate (**VIII**) as a mixture of geometric isomers. Although each isomer was separated (see Experimental), the following reductive reaction was carried out with this mixture to give ethyl 2-amino-2-(2-aminothiazol-4-yl)acetate (**IX**) which was purified and characterized as its 2-*t*-butyloxycarbonyl derivative (**X**). Hydrolysis after chloroacetylation of the annular amino group afforded the anticipated acid (**XII**).

Acylation of 7-aminocephalosporins (**XIII**) bearing various 3-substituents with **XII** through a mixed anhydride and subsequent removal of the chloroacetyl group with thiourea gave 2-*t*-butyloxycarbonyl derivatives (**XIV, XVII**). Treatment of these derivatives (**XIV, XVII**) with acetic acid containing hydrogen chloride successfully led to the anticipated compounds (**XV, XVIII**) (Chart 3).

XIVa was further subjected to nucleophilic displacement reaction with thiol compounds followed by removal of the amino-protecting group to afford 3-heterocyclic thiomethyl derivatives (**XX**).

In addition, acylation of **XIIIb** (7-ACT) with 2-*t*-butyloxycarbonylamino-2-(2-methylthiazol-4-yl)acetic acid (**XXI**), obtained from ethyl 2-hydroxyimino-2-(2-methylthiazol-4-yl)acetate[5] by the similar reactions shown in Chart 2 afforded 7β-[2-amino-(2-methylthiazol-4-yl)acetamidol-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**XXII**).

Chart 1.

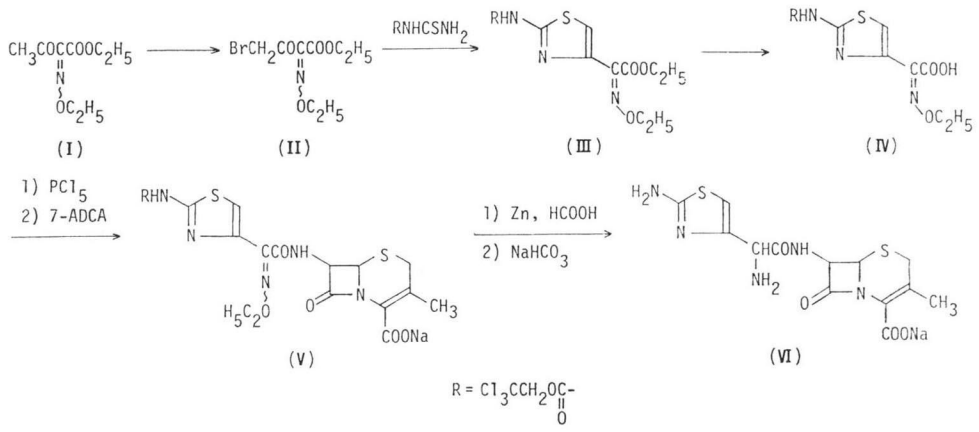


Chart 2.

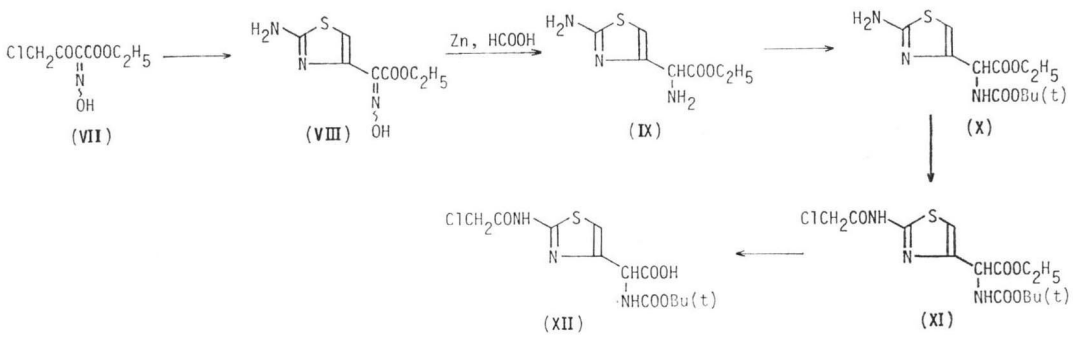
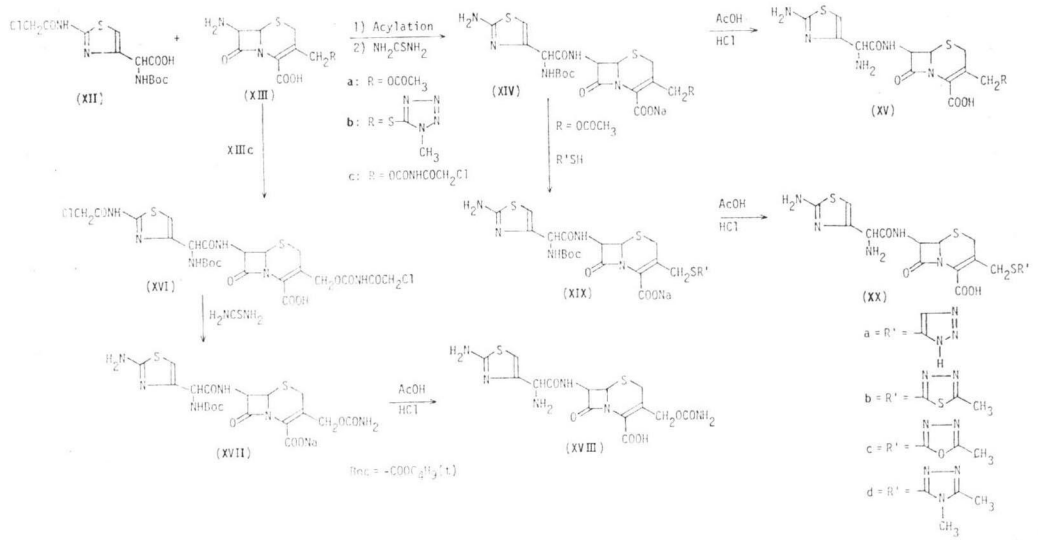


Chart 3.



Antibacterial Activity

The *in vitro* antibacterial activity of these new 2-aminoacyl derivatives against several bacteria, especially β-lactamase-producing strains, is exemplified in Table 1. All the 2-aminoacyl derivatives were tested without resolution of DL-mixtures.

The activity profile showed that some of the compounds (**XXa**, **XVb**, **XXd**) possess improved activity especially against *Enterobacter cloacae*. A similar trend in activity against *Proteus vulgaris* with that of 2-hydroxy- and 2-alkylacyl derivatives[1] is observed in 2-aminoacyl derivatives.

Replacement of the annular amino group by a methyl group caused loss in activity.

Experimental

Infrared spectra were measured in KBr disks with a Hitachi Type 215 spectrometer. NMR spectra were determined with a Varian HA-100 or T-60 spectrometer, tetramethylsilane being used as a standard. All melting points are uncorrected.

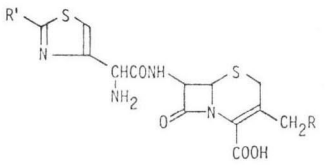
(*Z*)*-2-Ethoxyimino-2-[2-(2,2,2-trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid (**IVa**)

To a solution of ethyl 2-ethoxyimino-3-oxobutyrate (**I**)[3] (5.22 g) in CH_2Cl_2 (50 ml) was added dropwise Br_2 (4.40 g) under warming in a water bath (40°C). The water bath was removed after initiation of the reaction and dropwise addition of Br_2 was completed within 30 minutes. The reaction mixture was stirred for 1 hour at room temperature and washed with aqueous $Na_2S_2O_3$ and then with water. From the condensate of the dried organic layer was obtained crude ethyl 4-bromo-2-ethoxy-imino-3-oxobutyrate (**II**) as an oil (6.80 g). NMR ($CDCl_3$): δ 1.20~1.60 (6H, m, $CH_3\times2$), 4.10~4.60 (4H, m, $CH_2\times2$), 4.32 (2H, s, $BrCH_2$).

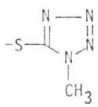
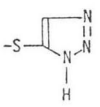
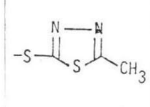
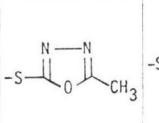
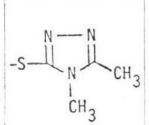
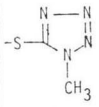
To a solution of the crude ester (**II**) (5.32 g) in EtOH (50 ml) were added N-(2,2,2-trichloroethoxycarbonyl)thiourea (5.03 g) and N,N-dimethylaniline (3.03 g). The mixture was stirred for 2 hours in a water bath (80°C) and concentrated under reduced pressure. The residue was dissolved in AcOEt and washed with 10% aqueous HCl and then with water. Evaporation of the dried extract gave crude ethyl (*Z*)-2-ethoxyimino-2-[2-(2, 2, 2-trichloroethoxycarbonylamino)thiazol-4-yl]acetate (**III**) as yellowish

* Assignment of stereochemistry will be described together with other related compounds in a forthcoming paper.

Table 1. *In vitro* antibacterial activity of aminoacyl derivatives (MIC: μg/ml).



(DL)

Compound		Cefotiam	Cefazolin	XVa	XVb	XVIII	XXa	XXb	XXc	XXd	XXII
	R			$OCOCH_3$		$OCONH_2$					
	R′			NH_2	NH_2	NH_2	NH_2	NH_2	NH_2	NH_2	CH_3
S. aureus 1840		1.56	0.78	6.25	12.5	6.25	3.13	6.25	25	12.5	25
E. coli T-7		3.13	100	3.13	3.13	6.25	3.13	6.25	25	3.13	100
S. marcescens TN 24		100	>100	100	50	>100	50	>100	>100	100	>100
P. vulgaris GN 4413		>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
E. cloacae TN 1282		100	>100	50	25	>100	25	100	>100	25	>100

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

Cefotiam: Generic name of 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thiomethyl]ceph-3-em-4-carboxylic acid.

solid (7.86 g). NMR ($CDCl_3$): δ 1.28, 1.38 (6H, t×2, CH_3×2), 4.29, 4.43 (4H, q×2, CH_2×2), 4.86 (2H, s, CH_2CCl_3), 7.20 (1H, s, thiazole 5-H).

To a solution of the crude ester (**III**) (2.0 g) in MeOH (40 ml) was added 1 N NaOH (20 ml) and the mixture was stirred for 2 hours at 50°C. After concentration water (50 ml) was added to the reaction mixture and the solution was washed with AcOEt. The aqueous layer was acidified (pH 2.0) with 10% aqueous HCl and the separated solid was collected on a filter by suction and washed with water to afford **IVa** as colorless crystals (1.20 g).

Anal. Calcd. for $C_{10}H_{10}Cl_3N_3O_5S$: C, 30.75; H, 2.58; N, 10.76.
Found: C, 30.87; H, 2.41; N, 10.66.

NMR (d_6-DMSO): δ 1.13 (3H, t, CH_3), 4.06 (2H, q, CH_2), 4.90 (2H, s, CH_2CCl_3), 7.40 (1H, s, thiazole 5-H).

Sodium 7β-[2-ethoxyimino-2-[2-(2, 2, 2-trichloroethoxycarbonylamino)thiazol-4-yl]acetamido-3-methylceph-3-em-4-carboxylate (**V**)

To a suspension of **IVa** (1.56 g) in CH_2Cl_2 (20 ml) was added PCl_5 (1.25 g). While being stirred for 1 hour at room temperature the reaction mixture became homogeneous. The mixture was concentrated and acetone (20 ml) was added to the concentrate to give a solution of the acid chloride. To an ice-cooled solution of 7-ADCA (857 mg) and $NaHCO_3$ (1.68 g) in a mixture of water (40 ml) and acetone (20 ml) was added dropwise the acetone solution of the acid chloride. The reaction mixture was, stirred for 2 hours at room temperature, concentrated under reduced pressure and the residue was washed with AcOEt. The aqueous layer was acidified (pH 2.0) with 10% aqueous HCl and extracted with AcOEt. The dried extract was concentrated and a part (573 mg) of the residue (2.04 g) was dissolved in a small amount of 5% aqueous $NaHCO_3$ and chromatographed on Amberlite XAD-2 (Rohm & Hass Co.) column. Elution with aqueous MeOH and lyophilization gave **V** as yellowish powder (233 mg).

Anal. Calcd. for $C_{18}H_{17}Cl_3N_5O_7S_2Na \cdot H_2O$: C, 34.49; H, 3.06; N, 11.17.
Found: C, 34.96; H, 3.43; N, 11.17.

NMR ($CDCl_3$+d_6-DMSO): δ 1.26 (3H, t, CH_3), 2.13 (3H, s, CH_3), 3.40 (2H, q, 2-CH_2), 4.23 (2H, q, CH_2CH_3), 4.86 (2H, s, CH_2CCl_3), 5.06 (1H, d, 6-H), 5.80 (1H, q, 7-H), 7.26, 7.83 (1H, s×2, thiazole 5-H).

Sodium 7β-[DL-2-amino-2-(2-aminothiazol-4-yl)acetamido]-3-methylceph-3-em-4-carboxylate (**VI**)

To an ice-cooled solution of **V** (1.46 g) in 90% formic acid (80 ml) was added gradually zinc dust (1.63 g) and the mixture was stirred for 1.5 hours. After filtration, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in water (200 ml) and H_2S was bubbled into this solution. After removal of ZnS by filtration, the reaction mixture was lyophilized to give a solid (1.15 g). This was dissolved in a small amount of 5% aqueous $NaHCO_3$ and chromatographed on an Amberlite XAD-2 column, with water as eluent, to afford **VI** as yellowish powder (614 mg).

Anal. Calcd. for $C_{13}H_{14}N_5O_4S_2Na \cdot 1.5H_2O$: C, 37.31; H, 4.10; N, 16.74.
Found: C, 37.81; H, 4.24; N, 16.69.

NMR (D_2O): δ 2.05 (3H, s, CH_3), 3.48 (2H, q, 2-CH_2), 5.13 (1H, s, CH), 5.18 (1H, d, 6-H), 5.79 (1H, d, 7-H), 6.99 (1H, s, thiazole 5-H).

Ethyl 2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetate (**VIII**)

A solution of ethyl 4-chloro-2-hydroxyimino-3-oxobutyrate (**VII**)[5] (19.3 g) and thiourea (8.0 g) in EtOH (200 ml) was refluxed for 2 hours. After the solvent had been distilled off the residue was dissolved in 10% aqueous HCl and washed with Et_2O. The aqueous layer was neutralized (pH 7.0) with $NaHCO_3$ and extracted with $CHCl_3$. From the washed and dried $CHCl_3$ layer was obtained **VIII** as a yellowish solid (6.43 g), mp 137~138°C (dec.).

Anal. Calcd. for $C_7H_9N_3O_3S$: C, 39.06; H, 4.21; N, 19.52.
Found: C, 39.64; H, 4.09; N, 19.62.

The NMR spectrum indicated that the product was a mixture of geometric isomers. Each geometric isomer* was isolated by column chromatography on silica gel using hexane-AcOEt as eluent.

* Assignment of stereochemistry will be described together with other related compounds in a forthcoming paper.

2-(2-Aminothiazol-4-yl)-(*E*)-2-hydroxyiminoacetate

Recrystallized from AcOEt-hexane, colorless crystals, mp 145.3°C.

Anal. Calcd. for $C_7H_9N_3O_3S$: C, 39.06; H, 4.21; N, 19.52.
Found: C, 38.81; H, 4.20; N, 19.62.

NMR (d_6-DMSO): δ 1.26 (3H, t, CH_3), 4.26 (2H, q, CH_2), 7.10 (2H, bs, NH_2), 7.50 (1H, s, thiazole 5-H), 12.5 (1H, s, OH).

2-(2-Aminothiazol-4-yl)-(*Z*)-2-hydroxyiminoacetate

Recrystallized from EtOH-hexane, yellowish crystals, mp 185.5°C (dec.).

Anal. Found: C, 39.28; H, 4.10; N, 19.63.

NMR (d_6-DMSO): δ 1.26 (3H, t, CH_3), 4.28 (2H, t, CH_2), 6.80 (1H, s, thiazole 5-H), 7.12 (2H, bs, NH_2), 11.6 (1H, s, OH).

Ethyl 2-amino-2-(2-aminothiazol-4-yl)acetate (**IX**)

To an ice-cooled solution of **VIII** (432 mg) in a mixture of 50% formic acid (10 ml) and EtOH (15 ml) was added zinc dust (300 mg) gradually with stirring. The reaction mixture was stirred for 3 hours under ice-cooling and concentrated under reduced pressure below 30°C to remove EtOH. The aqueous residue was neutralized (pH 7.0) with 1 N NaOH and extracted with AcOEt. From the washed and dried extract was obtained crude **IX** as an oil (130 mg). NMR (CF_3COOH): δ 1.04 (3H, t, CH_3), 4.18 (2H, q, CH_2), 5.35 (1H, s, CH), 6.90 (1H, s, thiazole 5-H).

Ethyl 2-(2-aminothiazol-4-yl)-2-(*t*-butyloxcarbonylamino)acetate (**X**)

IX (1.18 g) and 2-(*t*-butyloxycarbonylthio)-4,6-dimethylpyrimidine (1.41 g) were dissolved in DMF (10 ml). After being stirred for 40 hours at room temperature, the reaction mixture was poured into ice-water and extracted with AcOEt. The residue obtained by evaporation of the solvent from the extract was chromatographed on silica gel column to afford **X** as yellowish crystals (1.26 g), mp 143~144°C.

Anal. Calcd. for $C_{12}H_{19}N_3O_4S$: C, 47.83; H, 6.35; N, 13.94.
Found: C, 47.79; H, 6.27; N, 13.70.

NMR ($CDCl_3$): δ 1.22 (3H, t, CH_3), 1.40 (9H, s, *t*-C_4H_9), 1.96 (1H, d, NH), 4.22 (2H, q, CH_2), 5.20 (1H, d, CH), 5.83 (2H, bs, NH_2), 6.50 (1H, s, thiazole 5-H).

Ethyl 2-(*t*-butyloxycarbonylamino)-2-(2-chloroacetamidothiazol-4-yl)acetate (**XI**)

To a solution of **X** (1.26 g) in DMA (5 ml) was added chloroacetyl chloride (708 mg) and the mixture was stirred for 1 hour at room temperature. The reaction mixture was poured into water and extracted with AcOEt. From the extract, **XI** was obtained as yellowish crystals (1.43 g), mp 192~193°C.

Anal. Calcd. for $C_{14}H_{20}ClN_3O_5S$: C, 44.50; H, 5.34; N, 11.12.
Found: C, 44.87; H, 5.55; N, 10.94.

2-(*t*-Butyloxycarbonylamino)-2-(2-chloroacetamidothiazol-4-yl)acetic acid (**XII**)

To a solution of **XI** (920 mg) in EtOH (20 ml) was added a solution of KOH (681 mg) in water (1.4 ml) and the mixture was stirred for 15 minutes at room temperature. The reaction mixture was concentrated and the residue was dissolved in water. The aqueous solution was acidified (pH 2.0) with 1 N HCl and extracted with AcOEt. From the extract **XII** was obtained as yellowish crystals (690 mg), mp 169~170°C (dec.).

Anal. Calcd. for $C_{12}H_{16}ClN_3O_5S$: C, 41.21; H, 4.61; N, 12.01.
Found: C, 41.40; H, 4.68; N, 11.74.

Sodium 7β-[2-(2-aminothiazol-4-yl)-2-(*t*-butyloxycarbonylamino)acetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylate (**XIVb**)

To a cooled (−10°C) solution of **XII** (1.05 g) and Et_3N (303 mg) in THF (40 ml) was added a solution of isobutyl chloroformate (408 mg) in THF (1 ml) and the mixture was stirred for 70 minutes to give a mixed anhydride solution. This was added dropwise to an ice-cooled solution of 7-ACT (**XIIIb**) (1.02 g) and Et_3N (303 mg) in 50% aqueous THF (30 ml). The reaction mixture was stirred for 1 hour under ice-cooling and another 2 hours at room temperature and filtered. The filtrate was washed with AcOEt and the aqueous layer was acidified (pH 2.0) with 1 N HCl and extracted with AcOEt. The

residue (1.78 g) obtained from the extract was dissolved in a mixture of THF (15 ml) and EtOH (30 ml), and thiourea (660 mg) was added to this solution. After being stirred for 17 hours at room temperature the mixture was concentrated and the residue was dissolved in 5% aqueous $NaHCO_3$ and washed with AcOEt. The aqueous layer was acidified (pH 2.0) with 1 N HCl and the separated solid was collected on a filter by suction (595 mg). This was dissolved in a small amount of 5% aqueous $NaHCO_3$ and chromatographed on Amberlite XAD-2 column to afford, after lyophilization, **XIVb** as yellowish powder (311 mg).

Anal. Calcd. for $C_{20}H_{24}N_9O_6S_3Na \cdot 4H_2O$: C, 35.40; H, 4.75; N, 18.60.
Found: C, 35.03; H, 3.96; N, 18.16.

NMR (D_2O): δ 1.44 (9H, s, *t*-C_4H_9), 3.58 (2H, q, 2-CH_2), 4.03 (3H, s, N–CH_3), 4.19 (2H, q, 3-CH_2), 5.07 (1H, d, 6-H), 5.17 (1H, s, CH), 5.59 (1H, d, 7-H), 6.69 (1H, s, thiazole 5-H).

7β-[DL-2-Amino-2-(2-aminothiazol-4-yl)acetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**XVb**)

To a solution of **XIVb** (340 mg) in AcOH (5 ml) was added AcOH (2 ml) saturated with HCl. After 5 minutes, the separated solid was collected and washed with AcOH to give **XVb** as its hydrochloride, yellowish powder (142 mg).

Anal. Calcd. for $C_{15}H_{17}N_9O_4S_3 \cdot 2HCl \cdot 2H_2O$: C, 29.51; H, 4.12; N, 20.64.
Found: C, 30.62; H, 3.64; N, 20.09.

NMR (D_2O): δ 3.65 (2H, q, 2-CH_2), 3.93, 3.96 (3H, s×2, N–CH_3), 4.18 (2H, q, 3-CH_2), 5.34, 5.40 (1H, s×2, CH), 7.10, 7.16 (1H, s×2, thiazole 5-H).

Sodium 7β-[DL-2-(2-aminothiazol-4-yl)-2-(*t*-butyloxycarbonylamino)acetamido]cephalosporanate (**XIVa**)

Similar treatment of 7-ACA (**XIIIa**) (816 mg) with **XII** (1.04 g) as described for the preparation of **XIVb** gave **XIVa** as yellowish powder (80 mg).

Anal. Calcd. for $C_{20}H_{24}N_5O_8S_2Na \cdot 2H_2O$: C, 39.79; H, 5.00; N, 11.60.
Found: C, 39.80; H, 4.11; N, 11.12.

NMR (D_2O): δ 1.43 (9H, s, *t*-C_4H_9), 2.07 (3H, s, $COCH_3$), 3.50 (2H, q, 2-CH_2), 4.80 (2H, q, 3-CH_2), 5.10 (1H, d, 6-H), 5.16 (1H, s, CH), 5.75 (1H, d, 7-H), 6.72 (1H, s, thiazole 5-H).

7β-[DL-2-Amino-2-(2-aminothiazol-4-yl)acetamido]cephalosporanic acid (**XVa**)

Similar treatment of **XIVa** as described for the synthesis of **XVb** afforded **XVa** as its hydrochloride, colorless powder.

Anal. Calcd. for $C_{15}H_{16}N_5O_6S_2 \cdot 2HCl \cdot 2H_2O$: C, 33.65; H, 4.14; N, 13.08.
Found: C, 33.16; H, 3.80; N, 13.23.

NMR (D_2O): δ 2.15 (3H, s, $COCH_3$), 3.62 (2H, q, 2-CH_2), 4.99 (2H, q, 3-CH_2), 5.22 (1H, d, 6-H), 5.40 (1H, s, CH), 5.84 (1H, d, 7-H), 7.18 (1H, s, thiazole 5-H).

7β-[DL-2-Amino-2-(2-aminothiazol-4-yl)acetamido]-3-[(2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl]-ceph-3-em-4-carboxylic acid (**XXb**)

XIVa (2.11 g), 2-methyl-1,3,4-thiadiazole-5-thiol (0.53 g), $NaHCO_3$ (0.67 g) and triethylbenzylammonium bromide (20 mg) were dissolved in water (40 ml). After being stirred for 6 hours at 60°C, the reaction mixture was chromatographed on an Amberlite XAD-2 column to give **XIXb** (690 mg). This was dissolved in AcOH (8 ml) and to the solution was added AcOH (8 ml) saturated with HCl. The mixture was stirred for 15 minutes at room temperature and the separated solid was collected and washed with AcOH and then with Et_2O to give **XXb** as its hydrochloride (590 mg).

Anal. Calcd. for $C_{16}H_{17}N_7O_4S_4 \cdot 2HCl \cdot 3.5H_2O$: C, 30.24; H, 4.12; N, 15.43.
Found: C, 30.14; N, 3.90; N, 14.77.

NMR (D_2O): δ 2.65 (3H, s, CH_3), 3.69 (2H, bs, 2-CH_2), 5.15 (1H, d, 6-H), 5.45 (1H, s, CH), 5.70 (1H, d, 7-H), 7.20 (1H, s, thiazole 5-H).

XXa, **XXc** and **XXd** were obtained similarly from **XIVa**.

XXa

Anal. Calcd. for $C_{15}H_{16}N_8O_4S_3 \cdot 3HCl \cdot H_2O$: C, 30.23; H, 3.55; N, 18.80.
Found: C, 30.45; H, 3.32; N, 18.85.

NMR (D_2O): δ 5.10 (1H, d, 6-H), 5.38 (1H, s, CH), 7.16 (1H, s, thiazole 5-H), 7.95 (1H, s, triazole 4-H).

XXc

Anal. Calcd. for $C_{16}H_{17}N_7O_5S_2 \cdot 2HCl \cdot 2H_2O$: C, 32.43; H, 3.91; N, 16.55.
Found: C, 32.61; H, 3.84; N, 15.88.

NMR (D_2O): δ 2.45 (3H, s, CH_3), 3.65 (2H, bs, 2-CH_2), 4.17 (2H, bs, 3-CH_2), 5.11 (1H, d, 6-H), 5.40 (1H, s, CH), 5.66 (1H, d, 7-H), 7.17 (1H s, thiazole 5-H).

XXd NMR (D_2O): δ 2.76 (3H, s, CH_3), 3.80 (3H, s, N–CH_3), 5.25 (1H, d, 6-H), 5.50 (1H, s, CH), 5.75 (1H, d, 7-H), 7.30 (1H, s, thiazole 5-H).

7β-[DL-2-Amino-2-(2-aminothiazol-4-yl)acetamido]-3-carbamoyloxymethylceph-3-em-4-carboxylic acid (XVIII)

To a cooled (−10°C) solution of **XII** (2.09 g) and Et_3N (606 mg) in dry THF (80 ml) was added dropwise isobutyl chloroformate (816 mg) and the mixture was stirred for 70 minutes. A solution of 7-amino-3-(N-chloroacetylcarbamoyloxymethyl)ceph-3-em-4-carboxylic acid (**XIIIc**)[6] (2.09 g) and Et_3N (606 mg) in 50% aqueous THF (60 ml) was added to the above solution and the mixture was stirred for 1 hour under ice-cooling and another 2 hours at room temperature. THF was removed under reduced pressure and water (30 ml) was added to the aqueous residue. The aqueous solution was covered with AcOEt (100 ml) and acidified (pH 2.0) with 1 N HCl. After vigorous shaking, the AcOEt layer was separated and the aqueous layer was further extracted with AcOEt (100 ml). From the combined AcOEt layer was obtained crude 7β-[2-(t-butyloxycarbonylamino)-2-(2-chloroacetamidothiazol-4-yl)acetamido]-3-(N-chloroacetylcarbamoyloxymethyl)ceph-3-em-4-carboxylic acid (**XVI**) (3.5 g).

To a solution of the crude acid (**XVI**) (3.5 g) in THF (85 ml) were added thiourea (1.51 g) and $AcONa \cdot 3H_3O$ (2.8 g) and the mixture was stirred overnight at room temperature. The separated solid was collected by suction and washed with Et_2O. This was dissolved in water (15 ml) and adjusted to pH 7.0 with $NaHCO_3$. The solution was chromatographed on Amberlite XAD-2 with water as eluent to give, after lyophilization, **XVII** as colorless powder (582 mg). NMR (D_2O): δ 1.45 (9H, s, t-C_4H_9), 3.46 (2H, bd, 2-CH_2), 5.04 (1H, d, 6-H), 5.14 (1H, s, CH), 5.68 (1H, d, 7-H), 6.64 (1H, s, thiazole 5-H).

To a solution of **XVII** (380 mg) in AcOH (5 ml) was added AcOH (5 ml) saturated with HCl and the mixture was stirred for 15 minutes at room temperature. The separated solid was collected by suction and washed with AcOH and then with Et_2O to give **XVIII** as its hydrochloride (307 mg). IR: 1760 cm^{-1} (β-lactam).

Anal. Calcd. for $C_{14}H_{16}N_6O_6S_2 \cdot 3HCl \cdot 2H_2O$: C, 29.30; H, 4.04; N, 14.64.
Found: C, 29.42; H, 3.76; N, 14.92.

NMR (D_2O): δ 3.65 (2H, q, 2-CH_2), 4.98 (2H, q, 3-CH_2), 5.26 (1H, d, 6-H), 5.45 (1H, s, CH), 5.85 (1H, d, 7-H), 7.25 (1H, s, thiazole 5-H).

2-(t-Butyloxycarbonylamino)-2-(2-methylthiazol-4-yl)acetic acid (XXI)

Treatment of ethyl 2-hydroxyimino-2-(2-methylthiazol-4-yl)acetate[5] as described for the synthesis of **IX** from **VIII** gave ethyl 2-amino-2-(2-methylthiazol-4-yl)acetate as an oil. NMR ($CDCl_3$): δ 1.23 (3H, t, CH_3), 2.66 (3H, s, CH_3), 4.26 (2H, q, CH_2), 4.76 (1H, s, CH), 7.07 (1H, s, thiazole 5-H).

This was treated, without purification, as described for the conversion of **IX** to **X** to give ethyl 2-(t-butyloxycarbonylamino)-2-(2-methylthiazol-4-yl)acetate as an oil. NMR ($CDCl_3$): δ 1.24 (3H, t, CH_3), 1.50 (9H, s, t-C_4H_9), 2.75 (3H, s, CH_3), 4.28 (2H, q, CH_2), 5.44 (1H, d, CH), 5.91 (1H, d, NH), 7.10 (1H, s, thiazole 5-H).

The ester was hydrolyzed, without purification, as described for the synthesis of **XII** from **XI** to afford **XXI** as colorless crystals, mp 181 ~ 182°C.

Anal. Calcd. for $C_{11}H_{16}N_2O_4S$: C, 48.52; H, 5.92; N, 10.29.
Found: C, 48.58; H, 5.92; N, 10.04.

NMR (d_6-DMSO): δ 1.40 (9H, s, t-C_4H_9), 2.61 (3H, s, CH_3), 5.15 (1H, d, CH), 7.10 (1H, d, NH), 7.21 (1H, s, thiazole 5-H).

7β-[DL-2-Amino-2-(2-methylthiazol-4-yl)acetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**XXII**)

Treatment of 7-ACT (**XIIIb**) with **XXI** as described for the synthesis of **XVb** from **XII** and **XIIIb** gave **XXII** as its hydrochloride, yellowish powder. NMR (D_2O): δ 2.72 (3H, s, CH_3), 3.65, 3.73 (2H, q, 2-CH_2), 4.01, 4.05 (3H, s×2, N–CH_3), 5.11, 5.15 (1H, d×2, 6-H), 5.60, 5.75 (1H, d×2, 7-H), 5.50 (1H, s, –CH–), 7.75 (1H, s, thiazole 5-H).

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