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

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

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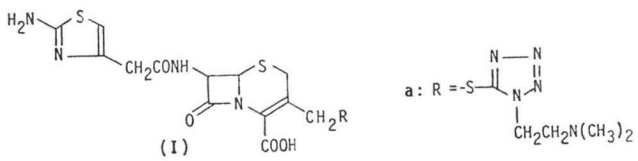
2-Alkyl- and 2-hydroxy derivatives of 7β-[2-aminothiazol-4-yl)acetamido]cephalosporins were synthesized to improve the antibacterial activity of the parent compounds especially against β-lactamase-producing organisms. Some of these compounds showed an increase in activity against *Serratia marcescens* (**Xb, XXIIIa**) and *Enterobacter cloacae* (**Xb, XIV**). The 2,2-dimethyl derivative (**XXVIIIb**) showed a definite loss of activity.

A tremendous number of new derivatives have been synthesized since the introduction of cephalosporin C, aimed at the discovery of a new compound which fullfils therapeutic needs. One of the research groups in our laboratories recently discovered[1] a facile synthesis and excellent antibiotic properties of 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[1-(2,2-dimethylaminoethyl)-1H-tetrazol-5-yl]-thiomethyl]ceph-3-em-4-carboxylic acid (**Ia**)** as a result of an extensive synthetic effort based on the speculation[2] that a new derivative with potent activity might be accessible.



Although 7β-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins (**I**) have potent broad spectrum activity, some Gram-negative bacteria, especially β-lactamase-producing strains, still remain resistant. Therefore, extensive chemical modifications have been done on these compounds (**I**) hoping to enhance resistance to β-lactamases and subsequently improve the antibacterial activity. Thus we took advantage of the chemical modifications obtained in earlier investigations in attempts to increase resistance to β-lactamases. One approach is to introduce substituent(s) to the methylene group of the 7-acyl moiety. Another is to introduce a methoxy group into the 7α-position, and finally variation of substituent(s) on the thiazole and the cephem nuclei.

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** Generic name: cefotiam (SCE-963).

In this paper the synthesis of 2-hydroxy- and 2-alkylacyl derivatives and their antibacterial activity will be described. Other new derivatives synthesized in this research program will be reported in a forthcoming paper.

Chemistry

The 2-hydroxyacyl derivatives (**X, XIV**) were synthesized by the sequence of reactions shown in Chart 1. By analogy with the reaction reported by BAGANZ *et al.*,[3] N-(2,2,2-trichloroethoxycarbonyl)thiourea (**II**) and tribromoacetone (**III**)[4] gave the formylthiazole compound (**IV**). Hydrolysis of the cyanohydrin compound (**Va**) to **VI** was successful only after it was converted into the acetyl derivative (**Vb**). The yields in deprotection of **IX** to afford 2-hydroxyacyl compounds (**X**) were rather low presumably because of reductive cleavage of the 3-substituents.[5] The 3-carbamoyloxymethyl derivative (**XIV**) was then synthesized by avoiding the reductive deprotection procedure. Considering various conventional protecting groups compatible with the chemical instability of β-lactams led to

Chart 1.

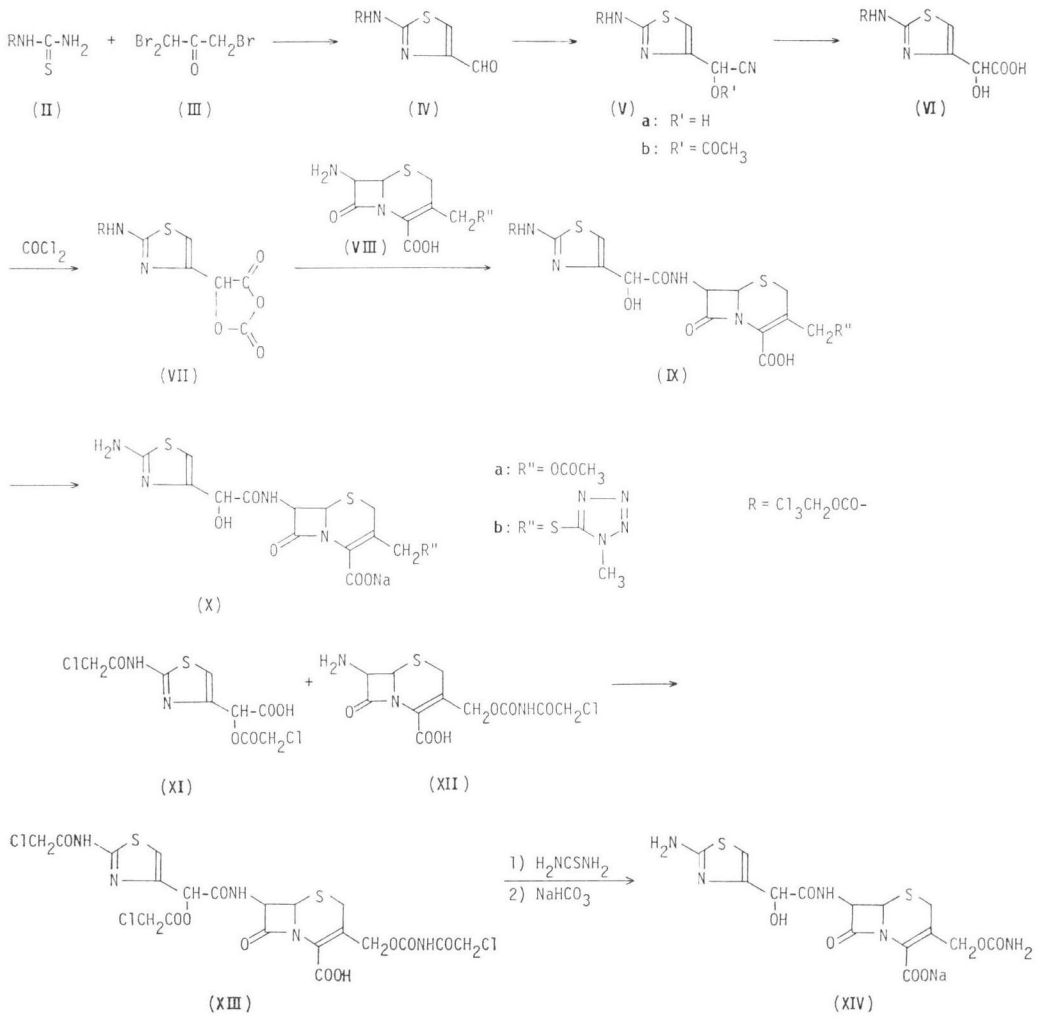


Chart 2.

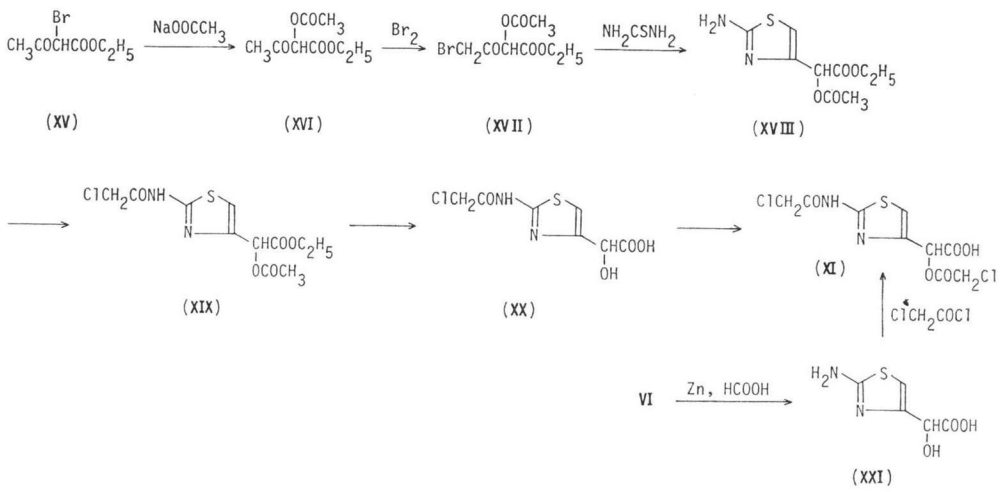
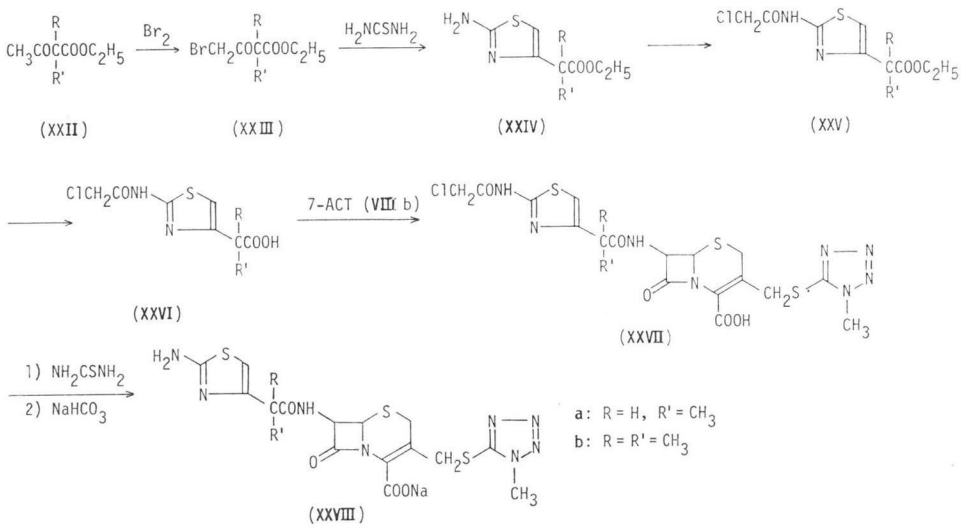


Chart 3.



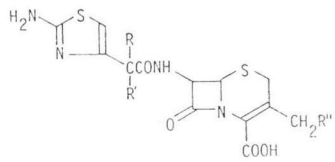
the selection of chloroacetyl group.[6] Thus, 7-amino-3-(N-chloroacetylcarbamoyloxymethyl)ceph-3-em-4-carboxylic acid (**XII**)[7] was acylated with a mixed anhydride of 2-(2-chloroacetamidothiazol-4-yl)-2-chloroacetoxyacetic acid (**XI**) which was obtained by the reactions shown in Chart 2. Removal of the chloroacetyl groups with thiourea followed by purification by column chromatography gave **XIV**.

The 2-alkyl derivatives (**XXVIII**) were obtained by the reactions shown in Chart 3. New carboxylic acids (**XXVI**) were synthesized by a sequence of conventional reactions. Acylation of 7-amino-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (7-ACT) (**VIIIb**) was carried out with mixed anhydrides derived from these acids and isobutyl chloroformate. Removal of the chloroacetyl group with thiourea followed by purification afforded the 2-alkylacyl derivatives (**XXVIII**).

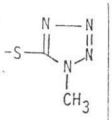
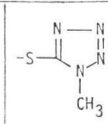
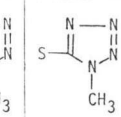
Antibacterial Activity

The *in vitro* antibacterial activity of these new compounds against several bacteria, especially β-lactamase-producing strains, is shown in Table 1. The 2-monosubstituted acyl derivatives were tested without resolution of the D, L-mixture.

Table 1. *In vitro* antibacterial activity of 2-hydroxy- and 2-alkylacyl derivatives.



(MIC: μg/ml)

	Cefotiam	Cefazolin	Xa	Xb	XIV	XXVIIIa	XXVIIIb
R			OH	OH	OH	CH_3	CH_3
R′			H	H	H	H	CH_3
R″			$OCOCH_3$ (DL)	 (DL)	$OCONH_2$ (DL)	 (DL)	
S. aureus 1840	1.56	0.78	3.13	1.56	3.13	3.13	50
E. coli T-7	3.13	100	6.25	1.56	3.13	25	>100
S. marcescens TN 24	100	100	>100	25	100	12.5	>100
P. vulgaris GN 4413	>100	>100	>100	>100	>100	>100	>100
E. cloacae TN 1282	100	>100	100	6.25	50	100	>100

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

From the Table it is apparent that the compound **Xb** has improved activity against *Serratia marcescens* and *Enterobacter cloacae*. A slight increase in activity is observed in the other compounds against *Serratia marcescens* (**XXVIIIa**) and *Enterobacter cloacae* (**XIV**), but these compounds have less activity against *Proteus vulgaris*. The dimethyl derivative (**XXVIIIb**) showed a definite loss of activity.

Experimental

Infrared spectra were measured in a KBr disk using a Hitachi Type 215 spectrophotometer. NMR spectra were done on a Varian HA-100 or T-60 spectrometer using tetramethylsilane as a standard. All melting points are uncorrected.

N-(2,2,2-Trichloroethoxycarbonyl)thiourea (**II**)

To a cooled (−20°C) solution of KSCN (25.5 g) in acetone (250 ml) was added dropwise 2,2,2-trichloroethoxycarbonyl chloride (50 g) under stirring. After the mixture was stirred for 30 minutes at room temperature, the separated solid (KCl) was filtered off. To the filtrate was added at −10°C MeOH solution of NH_3 (17% w/v, 26 g). After stirring for 45 minutes the solvent was distilled off under reduced pressure to give a gummy residue. Recrystallization from aqueous MeOH gave **II** as colorless crystals (21.8 g), mp 189~190°C.

Anal. Calcd. for $C_4H_5Cl_3N_2O_2S$: C, 19.10; H, 2.00; N, 11.14.
Found: C, 19.22; H, 1.96; N, 11.50.

N-(2,2,2-Trichloroethoxycarbonylamino)-4-formylthiazole (**IV**)

To a solution of **II** (10 g) and N,N-dimethylaniline (5 g) in EtOH (100 ml) was added 1,1,3-tribromoacetone[4] (12 g) under stirring. After refluxing for 2 hours the solvent was distilled off under reduced pressure. The residue was dissolved in AcOEt (150 ml) and dried over $MgSO_4$. A small quantity of $CHCl_3$ was added to the concentrated AcOEt layer and kept standing at room temperature. Separated solid was collected by suction to give **IV** (5 g). Recrystallization from $CHCl_3$ gave **IV** as crystals, mp 188~190°C.

Anal. Calcd. for $C_7H_5Cl_3N_2O_3S$: C, 27.69; H, 1.66; N, 9.23.
Found: C, 27.87; H, 1.69; N, 9.01.

NMR (d_6-DMSO): δ 5.05 (2H, s, CH_2O), 8.05 (1H, s, thiazole 5H), 9.80 (1H, s, CHO).

2-Hydroxy-2-[2-(2,2,2-trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid (**VI**)

To a suspension of **IV** (6 g) and KH_2PO_4 (5.2 g) in a mixture of water (30 ml) and DMF (30 ml) was added NaCN (2 g) and the mixture was stirred for 30 minutes at room temperature. AcOEt (200 ml) was added and the reaction mixture was washed several times with water. Evaporation of AcOEt after drying over $MgSO_4$ gave crude **Va** as an oil (6.8 g).

NMR ($CDCl_3$): δ 4.9 (2H, s, CH_2O), 5.7 (1H, s, $-\underset{\underset{OH}{|}}{C}HCN$), 7.2 (1H, s, thiazole 5-H).

Acetic anhydride (15 ml) was added to a solution of crude **Va** (6.8 g) in pyridine (6 ml) and the mixture was stirred under ice-cooling for 1 hour. After addition of Et_2O (100 ml), the reaction mixture was washed with water and dried over $MgSO_4$. Evaporation of the solvent gave crude **Vb** as an oil (6.9 g).

NMR[($CDCl_3$): δ 5.0 (2H, s, CH_2O), 7.3 (1H, s, thiazole 5-H), 6.6 (1H, s, –CHCN), 2.2 (3H, s, $COCH_3$).

A solution of crude **Vb** (6.9 g) in MeOH (60 ml) was saturated with hydrogen chloride under ice-cooling. After standing for 1 hour at room temperature, the solvent was distilled off to leave an oily residue. The residue was dissolved in MeOH (60 ml) and water (60 ml) and the solution was stirred for 1 hour at room temperature. Then NaOH (6 g) was added to the reaction mixture and stirring continued for 1 hour. The MeOH was distilled off and a small amount of insoluble matter was removed by filtration. After the filtrate was acidified with 10% HCl and extracted with AcOEt, the organic layer was again extracted with 5% aq. $NaHCO_3$. The aqueous layer was acidified with 10% aq. HCl and extracted with AcOEt. Evaporation of the solvent afforded **VI** (4.8 g). Recrystallization from $CHCl_3$ gave **VI** as a crystalline substance, mp 135~136°C.

Anal. Calcd. for $C_8H_7Cl_3N_2O_5S$: C, 27.48; H, 2.02; N, 8.01.
Found: C, 27.72; H, 2.05; N, 8.08.

NMR ($CDCl_3$): δ 4.9 (2H, s, CH_2O), 7.1 (1H, s, thiazole 5-H), 5.4 (1H, s, $-\underset{\underset{OH}{|}}{C}HCOOH$).

Sodium 7β-[2-(2-aminothiazol-4-yl)-2-hydroxyacetamido]-3-acetoxymethylceph-3-em-4-carboxylate (**Xa**)

To an ice-cooled solution of **VI** (11 g) in tetrahydrofuran (THF) (100 ml) phosgene was bubbled in for 20 minutes. After the excess phosgene was removed by bubbling nitrogen gas through the reaction mixture at 40°C, the solvent was distilled off under reduced pressure to give crude **VII** (13.5 g) as an oil IR: 1800, 1900 cm^{-1}.

To an ice-cooled suspension of 7-ACA (**VIIIa**) (10 g) in DMA (150 ml) was added the crude anhydride (**VII**) (13.5 g). After the mixture was stirred for 1.5 hours, AcOEt (200 ml) was added and insoluble matter was removed by filtration. The filtrate was extracted with 5% aq. $NaHCO_3$ and after acidification, the aqueous layer was extracted with AcOEt. Addition of a small amount of Et_2O to the oily residue obtained by evaporation of the solvent gave **IXa** as a powder (13 g).

NMR (CF_3COOD): δ 2.24 (3H, s, $COCH_3$), 3.70 (2H, q, 2-CH_2), 4.98 (2H, s, CH_2O), 5.22 (2H, s, 3-CH_2), 5.28 (1H, d, 6-H), 5.72 (1H, s, –CHCO), 5.88 (1H, d, 7-H), 7.48 (1H, s, thiazole 5-H).

To an ice-cooled soltion of **IXa** (3 g) in 90% formic acid (100 ml) was gradually added zinc powder (3 g) with stirring. After stirring for 1 hour the reaction mixture was filtered and the filtrate was concentrated under reduced pressure below 30°C. The residue was dissolved in water (100 ml) and H_2S

was bubbled into the solution to precipitate ZnS which was removed by filtration. The filtrate was concentrated to about 10 ml under reduced pressure below 30°C and the pH was adjusted to pH 7 with 1 N NaOH. Column chromatography of this solution through Amberlite XAD-2 (Rohm & Haas Co.) with water as eluent and lyophilization afforded **Xa** as colorless powder (750 mg).

Anal. Calcd. for $C_{15}H_{15}N_4O_7S_2Na \cdot 2H_2O$: C, 37.04; H, 3.94; N, 11.52.
Found: C, 36.70; H, 3.66; N, 11.86.

NMR (CF_3COOD): δ 2.24 (3H, s, $COCH_3$), 3.70 (2H, q, 2-CH_2), 5.23 (2H, s, 3-CH_2), 5.32 (1H, d, 6-H), 5.56 (1H, s, –CHCO), 5.85 (1H, d, 7-H), 6.92 (1H, s, thiazole 5-H).

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

Using **VIIIa** (1.3 g) in place of 7-ACA (**VIIIa**) in the above reaction **IXb** (995 mg) was obtained.

NMR (CF_3COOD): δ 3.99 (2H, q, 2-CH_2), 4.10 (3H, s, N–CH_3), 4.49 (2H, q, 3-CH_2), 4.96 (2H, s, CH_2O), 5.24 (1H, d, 6-H), 5.70 (1H, s, –CHCO), 5.85 (1H, d, 7-H), 6.76 (1H, s, thiazole 5-H).

Similar treatment of **IXb** (1.8 g) as described for the synthesis of **Xa** afforded **Xb** as colorless powder (84 mg).

Anal. Calcd. for $C_{15}H_{15}N_8O_5S_3Na \cdot 2H_2O$: C, 32.14; H, 3.78; N, 19.99.
Found: C, 32.53; H, 3.77; N, 19.50.

NMR (D_2O): δ 3.71 (2H, q, 2-CH_2), 4.14 (3H, s, N–CH_3), 4.29 (2H, q, 3-CH_2), 5.22 (1H, d, 6-H), 5.28 (1H, s, –CHCO), 5.75 (1H, d, 7-H), 6.84 (1H, s, thiazole 5-H).

Sodium 7β-[2-(2-aminothiazol-4-yl)-2-hydroxyacetamido]-3-carbamoyloxymethylceph-3-em-4-carboxylate (**XIV**)

(**a**) A mixture of ethyl 2-bromoacetoacetate (**XV**)[8] (104 g) and AcONa (60 g) in DMF (100 ml) was stirred for 4 hours at room temperature. After addition of water the reaction mixture was extracted with AcOEt. Evaporation of the dried AcOEt layer yielded crude **XVI**[9] as an oil (64.7 g). NMR ($CDCl_3$): δ 1.46 (3H, t, CH_2CH_3), 2.37 (3H, s, $OCOCH_3$), 2.50 (3H, s, CH_3CO), 4.56 (2H, q, CH_2CH_3), 5.61 (1H, s, –CH).

To an ice-cooled solution of the crude **XVI** (64.7 g) in CS_2 (200 ml) was added Br_2 (60 g) dropwise and the mixture was stirred for 2 hours. After additional stirring for 2 hours at room temperature the reaction mixture was washed with water and dried over $MgSO_4$, and the solvent was distilled off to give crude ester (**XVII**). Thiourea (50 g) was added to a solution of the crude ester (**XVII**) in EtOH (300 ml) and the mixture was stirred for 15 hours at room temperature and poured into water. The aqueous solution was washed with AcOEt and extracted with AcOEt after neutralization with $NaHCO_3$. From the dried AcOEt layer was obtained an oily residue. Recrystallization from acetone afforded ethyl 2-(2-aminothiazol-4-yl)-2-acetoxyacetate (**XVIII**) as colorless crystals (15.1 g), mp 153~154°C.

Anal. Calcd. for $C_9H_{12}N_2O_4S$: C, 44.25; H, 4.95; N, 11.46.
Found: C, 44.35; H, 4.94; N, 11.32.

NMR ($CDCl_3$+d_6-DMSO): δ 1.21 (2H, t, CH_2CH_3), 2.12 (3H, s, $OCOCH_3$), 4.16 (2H, q, CH_2CH_3), 5.76 (1H, s, –CH), 6.53 (1H, s, thiazole 5-H), 6.74 (2H, bs, NH_2).

To a solution of **XVIII** (2.44 g) in DMA (5 ml) was added chloroacetyl chloride (1.3 g). After stirring for 1 hour at room temperature the reaction mixture was poured into water and extracted with AcOEt. Evaporation of the dried AcOEt layer gave crude ethyl 2-(2-chloroacetamidothiazol-4-yl)-2-acetoxyacetate (**XIX**). This was dissolved in EtOH (30 ml) and a solution of NaOH (3.36 g) in water (10 ml) was added. After stirring for 30 minutes at room temperature, the mixture was poured into water. The aqueous layer was acidified (pH 2) with 1 N HCl and extracted with AcOEt. The residue obtained by evaporation of the dried AcOEt layer was treated with Et_2O to give 2-(2-chloroacetamidothiazol-4-yl)-2-hydroxyacetic acid (**XX**) (1.6 g), mp 161~162°C.

Anal. Calcd. for $C_7H_7ClN_2O_4S$: C, 33.54; H, 2.81; N, 11.17.
Found: C, 33.81; H, 2.71; N, 11.18.

NMR (d_6-DMSO): δ 4.33 (2H, s, $ClCH_2$), 5.04 (1H, s, –CH), 7.14 (1H, s, thiazole 5-H).

To a solution of **XX** (1.0 g) in DMA (4 ml) was added chloroacetyl chloride (0.8 ml). After stirring for 30 minutes at room temperature, the mixture was poured into water and extracted with AcOEt. The residue obtained by evaporation of the dried AcOEt layer was treated with Et_2O to

give crude 2-(2-chloroacetamidothiazol-4-yl)-2-chloroacetoxyacetic acid (**XI**) as a powder (0.86 g). The NMR spectrum confirmed the identity of the material with that described in section (**b**) below.

(**b**) To a solution of **VI** (3.0 g) in 90% formic acid (50 ml) was gradually added zinc dust (3.3 g) at room temperature and the mixture was stirred for 2 hours. After removal of insoluble matter, the solution was evaporated to dryness under reduced pressure to afford crude 2-(2-aminothiazol-4-yl)-2-hydroxyacetic acid (**XXI**). This was dissolved in DMA (10 ml) and chloroacetyl chloride (2.0 g) was added to this solution. After stirring for 1 hours at room temperature the reaction mixture was poured into water and extracted with AcOEt. Evaporation of the dried AcOEt layer gave crude 2-(2-chloroacetamidothiazol-4-yl)-2-chloroacetoxyacetic acid (**XI**) as an oil (980 mg). NMR ($CDCl_3$): δ 4.24, 4.27 (2H×2, s×2, $ClCH_2$×2), 6.18 (1H, s, –CH), 7.25 (1H, s, thiazole 5-H).

To a solution of **XI** (858 mg) in THF (20 ml) was added Et_3N (265 mg) and the mixture was cooled to −10°C. To this was added isobutyl chloroformate (356 mg) and the mixture was stirred for 1 hour under ice-cooling. To this mixed anhydride solution was added a solution of 7-amino-3-(N-chloroacetylcarbamoyloxymethyl)ceph-3-em-4-carboxylic acid (**XII**)[7] (915 mg) and Et_3N (265 mg) in 50% aq. THF (20 ml). After addition the mixture was stirred for 1 hour under ice-cooling and another 2 hours at room temperature. The mixture was poured into water and acidified (pH 2) with 1 N HCl and extracted with AcOEt. Evaporation of the dried AcOEt layer afforded crude 7β-[2-(2-chloroacetamidothiazol-4-yl)-2-chloroacetoxyacetamido]-3-(N-chloroacetylcarbamoyloxymethyl)ceph-3-em-4-carboxylic acid (**XIII**). This was added to a solution of thiourea (1.2 g) and $AcONa \cdot 3H_2O$ (2.6 g) in THF (5 ml) and the mixture was stirred for 12 hours at room temperature and poured into water. The mixture was washed with AcOEt, and the aqueous layer was lyophilized to give powdery substance, which was then dissolved in a small amount of water. Chromatography of this solution on Amberlite XAD-2 column using water as eluent and subsequent lyophilization afforded **XIV** as colorless powder (21 mg).

Anal. Calcd. for $C_{14}H_{14}N_5O_7S_2Na \cdot 1.5H_2O$: C, 35.15; H, 3.58; N, 14.64.
Found: C, 35.17; H, 3.39; N, 14.60.

NMR (D_2O): δ 3.46 (2H, q, 2-CH_2), 4.72 (2H, q, 3-CH_2), 5.11 (1H, d, 6-H), 5.13 (1H, s, –CH), 5.60 (1H, d, 7-H), 6.69 (1H, s, thiazole 5-H).

2-(2-Chloroacetamidothiazol-4-yl)-*n*-propionic acid (**XXVIa**)

Ethyl 4-bromo-2-methyl-3-oxobutyrate (**XXIIIa**)[10] (17.8 g) and thiourea (6.7 g) were dissolved in EtOH (50 ml) and the mixture was stirred for 1 hour at room temperature. The residue obtained after evaporation of the EtOH was dissolved in water and washed with Et_2O. After neutralization (pH 7.0~7.5) with $NaHCO_3$, the aqueous layer was extracted with AcOEt. Addition of Et_2O to the concentrated extract caused precipitation of ethyl 2-(2-aminothiazol-4-yl)-*n*-propionate (**XXIVa**) (5.3 g).

NMR ($CDCl_3$): δ 1.48 (3H, d, –$CHCH_3$), 1.22 (3H, t, CH_2CH_3), 3.72 (1H, q, –$CHCH_3$), 4.20 (2H, q, CH_2CH_3), 5.50 (2H, bs, NH_2), 6.30 (1H, s, thiazole 5-H).

To a solution of **XXIVa** (5.0 g) in DMA (18 ml) was added chloroacetyl chloride (3.38 g) dropwise under ice-cooling and the mixture was stirred for 10 minutes and then additionally for 50 minutes at room temperature. After addition of water (50 ml) the reaction mixture was extracted with AcOEt. Addition of Et_2O to the concentrated extract caused precipitation of crude ethyl 2-(2-chloroacetamidothiazol-4-yl)-*n*-propionate (**XXVa**) (5.24 g).

NMR ($CDCl_3$): δ 1.28 (3H, t, CH_2CH_3), 1.55 (3H, d, –$CHCH_3$), 3.84 (1H, q, –$CHCH_3$), 4.22 (2H, s, $ClCH_2$), 4.26 (2H, q, CH_2CH_3), 6.80 (1H, s, thiazole 5-H).

To a suspension of the crude **XXVa** (5.0 g) in EtOH (197 ml) was added under ice-cooling a solution of KOH (5.06 g) in water (10 ml) and the mixture was stirred for 1 hour at room temperature. After evaporation of EtOH and addition of water (100 ml) to the residue, the reaction mixture was washed with AcOEt. The aqueous layer was acidified (pH 2.0) with 10% HCl and extracted with AcOEt. Condensation of the dried extract afforded **XXVIa** as colorless crystals (3.58 g), mp 148~150°C. IR: 1700 cm^{-1} (COOH).

Anal. Calcd. for $C_5H_5ClN_2O_3S$: C, 38.64; H, 3.65; N, 11.26.
Found: C, 38.67; H, 3.75; N, 11.24.

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

To a solution of **XXVIa** (1.4 g) in dried THF (80 ml) was added Et_3N (606 mg) and the mixture was cooled (−10°C). Isobutyl chloroformate (816 mg) was added to this solution and stirred for 70 minutes at this temperature. To this was added a solution of Et_3N (627 mg) and **VIIIb** (2.04 g) in 50% aq. THF (60 ml) and the mixture was stirred for 1 hour under ice-cooling and then for 2 hours at room temperature. After evaporation of THF under reduced pressure and addition of water (30 ml), the reaction mixture was covered with a layer of AcOEt (250 ml) and acidified (pH 2.0) with 1 N HCl. The AcOEt layer was separated after vigorous shaking. From the AcOEt layer was obtained crude 7β-[2-(2-chloroacetamidothiazol-4-yl)-2-methylacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)-thiomethyl]ceph-3-em-4-carboxylic acid (**XXVIIa**) (3 g). This was dissolved in THF (70 ml) and thiourea (1.23 g) and $AcONa \cdot 3H_2O$ (2.19 g) were added to this solution. After stirring for 15 hours at room temperature, the separated solid was collected and washed with Et_2O. The solid was dissolved in water (20 ml) and neutralized (pH 7.0) with $NaHCO_3$. Chromatography of this solution on Amberlite XAD-2 column with water as eluent and lyophilization gave **XXVIIIa** as colorless powder (433 mg). IR: 1750 cm^{-1} (β-lactam).

Anal. Calcd. for $C_{16}H_{17}N_8O_4S_3Na \cdot 3H_2O$: C, 34.40; H, 4.15; N, 20.06.
Found: C, 34.12; H, 3.94; N, 19.70.

NMR (D_2O): δ 1.44 (3H, d, $-CHCH_3$), 3.51 (2H, q, 2-CH_2), 4.00 (3H, s, NCH_3), 5.00 (1H, d, 6-H), 5.55 (1H, d, 7-H), 6.41 (1H, s, thiazole 5-H).

2-(2-Chloroacetamidothiazol-4-yl)isobutyric acid (**XXVIb**)

To a solution of **XXIIIb**[11] (6.27 g) was added a solution of Br_2 (6.27 g) in CS_2 (10 ml) dropwise under ice-cooling. After stirring for 1 hour at room temperature $CHCl_3$ (50 ml) and water (50 ml) were added to the mixture. From the $CHCl_3$ lyaer was obtained crude 4-bromo-2,2-dimethylaceto-acetate (**XXIIIb**)[12] (8.9 g). The crude ester (8.9 g) and thiourea (3.14 g) were dissolved in EtOH (35 ml) and the solution was stirred for 1 hour at room temperature. The EtOH was distilled off and the residue was dissolved in water (100 ml) and washed with Et_2O. The aqueous layer was extracted with AcOEt after it was neutralized (pH 7.0~7.5) with $NaHCO_3$. Condensation of the dried extract gave crude ethyl 2-(2-aminothiazol-4-yl)isobutyrate (**XXIVb**) (4.25 g).

NMR ($CDCl_3$): δ 1.08 (3H, t, CH_2CH_3), 1.46 (6H, s, $CH_3 \times 2$), 4.10 (2H, q, CH_2CH_3), 5.58 (2H, bs, NH_2), 6.11 (1H, s, thiazole 5-H).

To a solution of the crude ester (**XXIVb**) (4.25 g) in DMA (15 ml) was added chloroacetyl chloride (2.68 g) dropwise under ice-cooling and the mixture was stirred for 50 minutes at room temperature. After addition of water (50 ml) the reaction mixture was extracted with a mixture (1:1) of AcOEt and THF. From the extract was obtained crude ethyl 2-(2-chloroacetamidothiazol-4-yl)isobutyrate (**XXVb**) (5.5 g).

NMR ($CDCl_3$): δ 1.23 (3H, t, CH_2CH_3), 1.60 (6H, s, $CH_3 \times 2$), 4.20 (2H, q, CH_2CH_3), 4.35 (2H, s, $ClCH_2$), 6.80 (1H, s, thiazole 5-H).

To a suspension of the crude ester (**XXVb**) (5.2 g) in EtOH (80 ml) was added a solution of KOH (4.5 g) in water (10 ml) under ice-cooling, and the mixture was stirred for 1 hour at room temperature and another 1 hour at 50°C. After evaporation of EtOH and addition of water (100 ml), the reaction mixture was washed with AcOEt. The aqueous layer was acidified (pH 2.0) with 10% aq. HCl and extracted with AcOEt. From the concentrated extract crystallized **XXVIb** (1.05 g), mp 170~171°C, was obtained. IR: 1700 cm^{-1} (COOH).

Anal. Calcd. for $C_9H_{11}ClN_2O_3S$: C, 41.15; H, 4.22; N, 10.66.
Found: C, 41.74; H, 4.34; N, 10.90.

Sodium 7β-[2-(2-aminothiazol-4-yl)-2,2-dimethylacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylate (**XXVIIIb**)

Similar treatment of **VIIIb** (1.25 g) with **XXVIb** (1.0 g) as described for the synthesis of **XXVIIIa** afforded **XXVIIIb** as colorless powder (445 mg). IR: 1760 cm^{-1} (β-lactam).

Anal. Calcd. for $C_{17}H_{19}N_8O_4S_3Na \cdot 3H_2O$: C, 35.66; H, 4.40; N, 19.57.
Found: C, 35.55; H, 4.21; N, 19.31.

NMR (D_2O): δ 1.53 (6H, s, $CH_3 \times 2$), 3.78 (2H, q, 2-CH_2), 4.03 (3H, s, NCH_3), 4.83 (1H, d, 6-H), 5.90 (1H, d, 7-H), 6.58 (1H, s, thiazole 5-H).

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