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Semisynthetic β-Lactam Antibiotics. IX.[1] Synthesis and Antibacterial Activity of 7-[2-(2-Aminothiazol-4-yl)-2-sulfoacetamido]-cephalosporanic Acid and Its Derivatives

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Novel 2-(2-aminothiazol-4-yl)-2-sulfoacetyl cephalosporins(**10a**—**c**)were synthesized by two routes: A, acylation of 7-aminocephalosporanic acid (**8a**) or its analogs (**8b**, **c**) with an active derivative of 2-(2-aminothiazol-4-yl)-2-sulfoacetic acid, and B, side chain sulfonation of γ-chloroacetoacetyl cephalosporins (**13a**, **b**) with SO_3-dioxane and subsequent thiazole ring formation by treatment with thiourea.

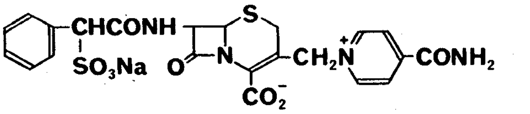
The cephalosporin with 4-carbamoylpyridiniomethyl at the 3-position (**10c**) showed a potent antipseudomonal activity but had poor activity against other gram-negative bacteria, while the cephalosporin with 1-methyltetrazol-5-ylthiomethyl at the 3-position (**10b**) showed a broad spectrum but caused no inhibition of *Pseudomonas aeruginosa*.

Keywords——cephalosporin; 7-[2-(2-aminothiazol-4-yl)-2-sulfoacetamido]cephalosporanic acid derivatives; 2-(2-aminothiazol-4-yl)-2-sulfoacetic acid; acylation; sulfonation; thiazole ring formation; antibacterial activity; antipseudomonal activity; cefsulodin; cefotiam

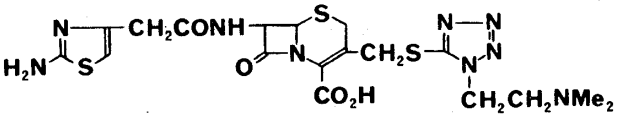
Introduction

Previous articles[2-4] from this laboratory have reported a series of semisynthetic cephalosporins characterized by a sulfoacyl side chain at the 7-position. Cefsulodin (**1**), a representative compound in this series, shows potent *in vivo* antipseudomonal activity comparable to that of gentamycin[5] and is now in clinical use in Japan and Europe.

From the viewpoint of expanding the antibacterial spectrum of **1**, special interest has been focused on the synthesis of cephalosporins bearing both a sulfo and a 2-aminothiazol-4-yl group on the 7-acyl side chain, since the brilliant debut of cefotiam,[6] a broad spectrum 2-(2-aminothiazol-4-yl)acetyl cephalosporin (**2**), in 1977, followed by its congeners.[7-11] This report deals with the synthesis and antibacterial activity of 7-(2-(2-aminothiazol-4-yl)-2-sulfoacetamido)cephalosporanic acid (**10a**) and its analogs with a modified substituent at the 3-position (**10b**, **c**).



1



2

Chemistry

2-(2-Aminothiazol-4-yl)-2-sulfoacetyl cephalosporins (**10a**—**c**) were synthesized by two routes; A, acylation of 7-aminocephalosporanic acid (**8a**) or its derivatives (**8b**, **c**) with an active derivative of 2-(2-aminothiazol-4-yl)-2-sulfoacetic acid (**5** or **7**) as illustrated in Chart 1, and B,

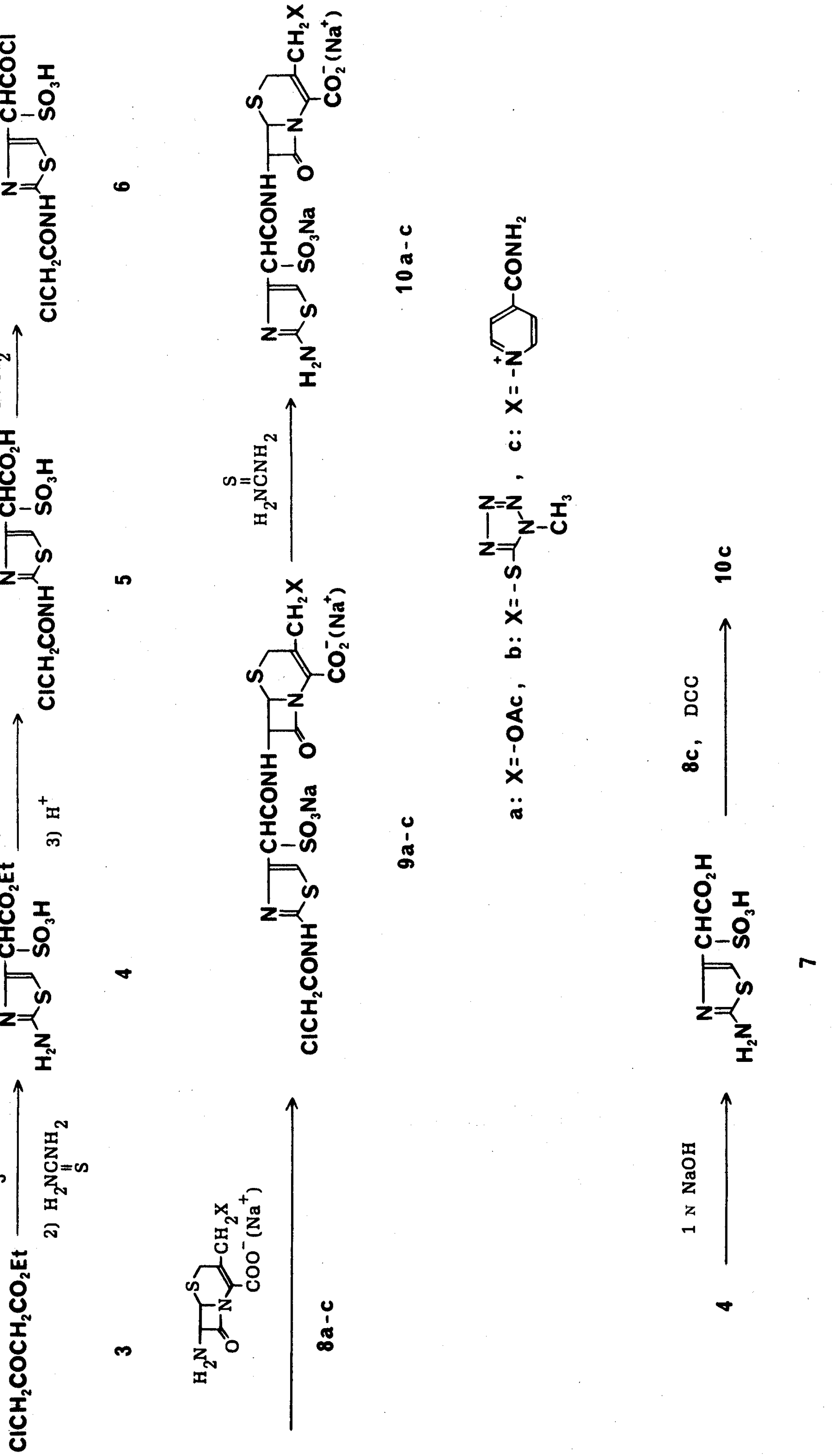

ClCH2COCH2CO2Et
3
1) SO3-dioxane
2) H2NCNH2 (S)
4
1) ClCH2COCl
2) 1 N NaOH
3) H+
5
SOCl2
6
8a-c
9a-c
H2NCNH2 (S)
10a-c
a: X=-OAc, b: X=-S-, c: X=-N+
CONH2
CH3
4
1 N NaOH
7
8c, DCC
10c

Chart 1

formation of the thiazole ring on preformed α-chloroaceto-α-sulfoacetyl cephalosporin, which was derived from γ-chloroacetoacetyl-cephalosporins (**13a**, **b**) as shown in Chart 2.

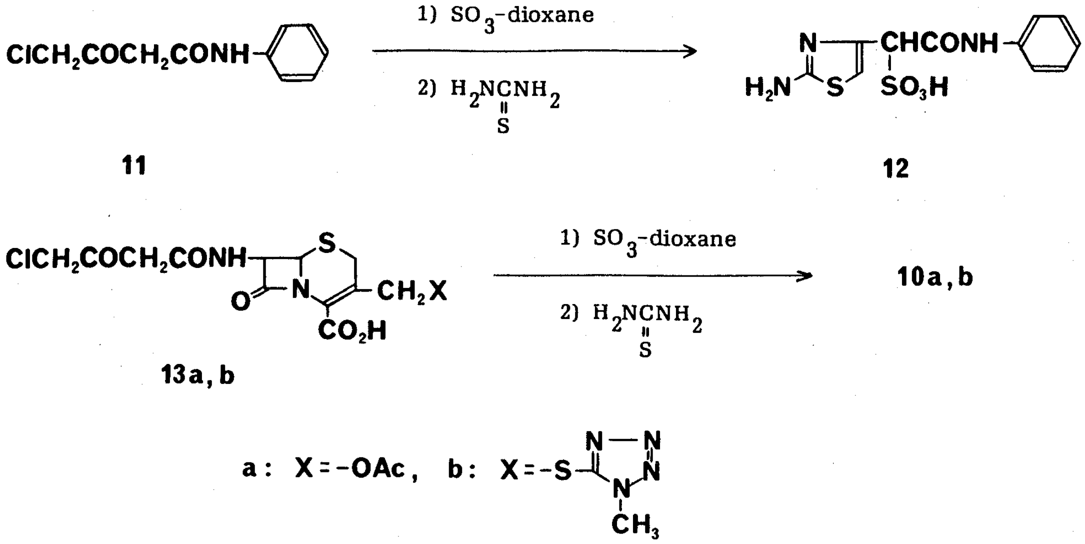


Chart 2

α-Sulfophenylacetic acid, an intermediate for the synthesis of both sulbenicillin and cefsulodin, has been synthesized by sulfonation of phenylacetic acid with SO_3-dioxane.[12] In a similar way, a number of 2-sulfoalkanoic acids and 2-sulfoarylalkanoic acids have been synthesized from the alkanoic and arylalkanoic acids.[4,13] This method, however, could not be applied to the sulfonation of 2-(2-aminothiazol-4-yl)acetic acid because of its lability to this reagent. In view of the general method for the synthesis of 2-aminothiazoles, which involves the reaction of α-haloketones with thiourea,[14] it seemed of interest to employ ethyl α-haloaceto-α-sulfoacetate for the preparation of ethyl 2-(2-aminothiazol-4-yl)-2-sulfoacetate (**4**). In fact, the present sulfonation method has been successfully applied for the preparation of α-chloroaceto-α-sulfoacetate from ethyl γ-chloroacetoacetate (**3**), and the subsequent cyclization reaction with thiourea gave the desired 2-(2-aminothiazol-4-yl)-2-sulfoacetoacetate (**4**) (Chart 1). Alkaline hydrolysis of compound **4** and its 2-chloroacetyl derivative gave the corresponding acids (**7** and **5**) which were isolated as colorless crystals. Their structures (**4**, **5** and **7**) were confirmed unequivocally by spectral data and the results of elemental analysis.

Route A requires the active derivative of the carboxyl group of **5** or **7**. When unprotected 2-(2-aminothiazol-4-yl)-2-sulfoacetic acid (**7**) was treated with $SOCl_2$ in ether, no smooth conversion to the acid chloride resulted, presumably due to its poor solubility in the solvent. To overcome this difficulty, suitable protection of the amino group of **7** was required. Thus, when 2-(2-chloroacetamidothiazol-4-yl)-2-sulfoacetic acid (**5**) was used, the conversion to the acid chloride (**6**) progressed smoothly. The reaction of 7-aminocephalosporanic acid (**8a**) or its derivatives (**8b**, **c**) with the sulfoacetyl chloride (**6**) in an aqueous solvent occurred smoothly and gave the *N*-protected cephalosporins (**9a**—**c**) in 50—55% yields. Removal of the chloroacetyl group with thiourea in the presence of sodium acetate and subsequent purification gave the desired 2-(2-aminothiazol-4-yl)-2-sulfoacetyl cephalosporins (**10a**—**c**) in quantitative yields. Another method in route A involved acylation of the 7-aminocephalosporanic acid derivative (**8c**) with sulfoacetic acid (**7**) by the conventional method using dicyclohexylcarbodiimide (DCC). The desired product **10c** was obtained in a relatively low yield.

The reaction from γ-chloroacetoacetanilide (**11**) to 2-(2-aminothiazol-4-yl)-2-sulfoacetanilide (**12**) was performed as a model reaction to examine whether route B (Chart 2) was promising for the synthesis of 2-(2-aminothiazol-4-yl)-2-sulfoacetyl cephalosporins (**10a**,

b). The result suggested the feasibility of this route, which involves side chain sulfonation of γ-chloroacetoacetyl cephalosporins and subsequent thiazole ring formation by treatment with thiourea. Cyclization by the reaction of γ-chloroacetoacetyl cephalosporins (**13**) with thiourea was first developed by Numata *et al.* and a number of 2-(2-aminothiazol-4-yl)acetyl cephalosporins, *e.g.*, **2** and **16**, have been synthesized.[6)] After sulfonation of the α-methylene moiety in the 7-γ-chloroacetoacetyl side chain of **13** by treatment with SO_3–dioxane, the resulting α-chloroaceto-α-sulfoacetamide was similarly converted to the desired cephalosporins (**10a**, **b**). The synthesis was achieved by one-pot reaction starting from **13a**, **b**, but the yields were relatively low (10—20%).

The structures of **10a**—**c** were readily deduced from spectral data and the results of elemental analysis. The nuclear magnetic resonance (NMR) spectra of **9a**—**c** exhibited absorption due to the single α-methine proton in the 7-acyl side chain as a symmetrical doublet (δ, ppm: 5.33 and 5.34 for **9a**; 5.46 and 5.48 for **9b**; 5.31 and 5.32 for **9c**), indicating that they were 1:1 mixtures of the diastereoisomers which differ in configuration at the α-position of the sulfoacyl side chain.

Antibacterial Activity

7-(2-(2-Aminothiazol-4-yl)-2-sulfoacetyl)cephalosporins (**10a**—**c**) were tested by the two-fold serial agar dilution method together with cefsulodin (**1**) and related compounds (**14**—**16**) as controls. The values given in Table I are the average minimum inhibitory concentration (MIC) values for six representative species; *P. aeruginosa, E. coli, P. vulgaris, P. mirabilis, K. pneumoniae* and *S. aureus,* which are routinely used as indicator strains in our Research Division to test for cephalosporin activity. The test samples used were 1:1 mixtures of the two diastereoisomers which differ in the configuration of the sulfoacyl side chain.

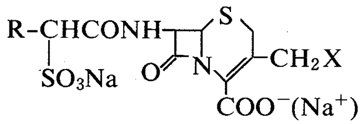
Based on data for **1** and its analogs (**14** and **15**), higher activity was expected with the corresponding (R)-isomer.[2)]

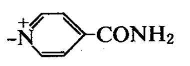
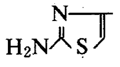
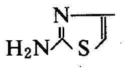
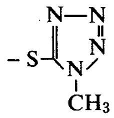
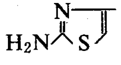
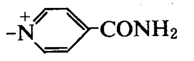
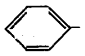
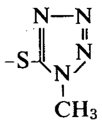
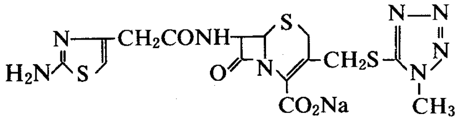
7-(2-(2-Aminothiazol-4-yl)-2-sulfoacetamido)cephalosporanic acid (**10a**) was less active against gram-positive and gram-negative bacteria, being similar to 7-(2-phenyl-2-sulfoacetamido)cephalosporanic acid (**14**). 2-(2-Aminothiazol-4-yl)-2-sulfoacetyl cephalosporin with a 1-methyltetrazol-5-ylthiomethyl group at the 3-position (**10b**) showed a broad spectrum which was the most striking effect produced by the modifications made in this study. Its inhibitory activities against *E. coli, K. pneumoniae* and *Proteus* sp. were potent and better than those of any of the known sulfoacyl cephalosporins.[2–4)] However, contrary to expectation, **10b** did not retain the antipseudomonal activity.

2-(2-Aminothiazol-4-yl)-2-sulfoacetyl cephalosporin with a 4-carbamoylpyridiniomethyl group at the 3-position (**10c**) showed antipseudomonal activity almost as potent as that of **1**. However, **10c** did not show improved antibacterial activity against other gram-negative bacteria and, therefore, its antibacterial spectrum remained narrow, being similar to that of **1**. Consequently, introduction of the 2-sulfo group into the 7-acyl group of a 2-(2-aminothiazol-4-yl)acetyl cephalosporin (**16**), a parent compound of cefotiam (**2**), or replacement of the phenyl group of **1** with a 2-aminothiazol-4-yl group resulted in no improvement in the antibacterial spectrum, and did not yield a cephalosporin possessing the advantages of both antipseudomonal cefsulodin and broad-spectrum cefotiam.

These results indicate that, in the cephalosporin molecule, mere coexistence of an assemblage of 2-aminothiazol-4-yl and 2-sulfo groups in the 7-acyl side chain does not insure broad-spectrum activity including potent antipseudomonal activity. Generally, in a series of α-sulfoacyl cephalosporins, the presence or absence of a positively charged substituent on the 3-methylene group has more influence upon their antibacterial properties than structure change in the α-substituent attached to the 7-sulfoacetyl side chain.[2,4)] This was true in the 2-aminothiazoly-2-sulfoacyl series, and variation of the 3-substituent caused the spectrum to change to either of the two extremes, the cefsulodin-type or the cefotiam-type spectrum. Further investigations in this area are under way.

TABLE I. Antibacterial Activities



				MIC μg/ml								
	R	X	Confign.[a]	*P. aeruginosa* IFO 3455	*P.aeruginosa* IFO 3080	*E. coli* NIHJ JC-2	*E. coli* O-111	*P. vulgaris* IFO 3988	*P. mirabilis* IFO 3849	*K.pneumoniae* DT	*S. aureus* FDA 209P	*S. aureus* 1840
1			(*R*)- (*RS*)-	0.78 1.56	1.56 3.13	50 100	50 50	100 >100	100 >100	50 100	3.13 6.25	6.25 12.5
10a		-OAc	(*RS*)-	>100	>100	1.56	0.78	6.25	12.5	3.13	50	100
10b			(*RS*)-	>100	>100	0.78	0.2	1.56	1.56	0.78	12.5	25
10c			(*RS*)-	1.56	1.56	25	12.5	50	25	25	6.25	12.5
14		-OAc	(*R*)- (*RS*)-	>100 >100	100 >100	100 >100	25 50	50 50	100 100	100 100	3.13 6.25	12.5 25
15			(*R*)- (*RS*)-	>100 >100	>100 >100	25 50	6.25 6.25	12.5 12.5	12.5 25	25 25	3.13 6.25	12.5 25
16				>100	>100	0.39	0.2	0.39	0.78	<0.1	0.39	0.78

[a] Configuration at asymmetric carbon in the side chain.

Experimental

All melting points are uncorrected. Infrared (IR) spectra were determined on a Hitachi EPI-S_2 spectrometer and NMR spectra with a Varian T-60 spectrometer using tetramethylsilane (TMS) as an internal standard in DMSO-d_6 or as an external standard in D_2O.

Material———Compounds **1** and **14** used in this study were prepared by the method described in the previous papers.[2,3] Compound **15** was prepared by acylation of **8b** with (*RS*)-α-sulfophenylacetyl chloride[16] and its (*R*)-isomer was prepared by the method described in a previous paper.[4] Compound **16** was a gift from Dr. Numata of this division. Compounds **8b**[17] and **8c**[18] were prepared individually from 7-aminocephalosporanic acid (**8a**) according to the known methods.

Minimum Inhibitory Concentrations———MIC's of the cephalosporins were determined by the agar dilution method. Nutrient agar was used as the assay medium. One loopful of a suspension containing about 1 mg/ml of test organism was incubated on the plate at 37 °C for 18 h.

Ethyl 2-(2-Aminothiazol-4-yl)-2-sulfoacetate (4)———Ethyl γ-chloroacetoacetate[15] (**3**, 19.75 g, 0.12 mol) was added dropwise to a stirred solution of SO_3–dioxane complex[12] (30.5 g, 0.18 mol) in CH_2Cl_2 (104.7 g) at 0—5 °C. The mixture was kept at 15—20 °C for 1 h under stirring and cooled to −10 °C. After dropwise addition of EtOH (300 ml), followed by addition of thiourea (13.7 g, 0.18 mol) at −5 to −10 °C, the mixture was allowed to come to room temperature and stirred overnight. A precipitate separated out after the reaction mixture had been cooled on an ice-bath. After work-up, recrystallization of the product from H_2O–EtOH (1:4) gave the desired sulfoacetate (20.5 g, 64%) as colorless needles. mp 256—258 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3340, 3100, 1743 (COOEt), 1654, 1603, 1588, 1208 ($-SO_2-$), 1190 ($-SO_2-$), 1053 ($-SO_3^-$). NMR (DMSO-d_6) δ: 1.20 (3H, t, J=7 Hz, CH_2CH_3), 4.13 (2H, q, J=7 Hz, CH_2CH_3), 4.89 (1H, s, –CH–), 6.91 (1H, s, =CH–), 8.90 (2H, br s, NH_2). *Anal.* Calcd for $C_7H_{10}N_2O_5S_2$: C, 31.57; H, 3.79; N, 10.52; S, 24.08. Found: C, 31.87; H, 3.85; N, 10.38; S, 24.23.

2-(2-Aminothiazol-4-yl)-2-sulfoacetic Acid (7)———Compound **4** (798 mg, 3 mmol) was added to an ice-cooled solution of 1 N NaOH (6.6 ml) with stirring and the mixture was allowed to stand for 15 min. Next, the mixture was neutralized with 1 N HCl (7 ml). The precipitate was collected by filtration and washed successively with ice-cooled H_2O, EtOH and Et_2O, which gave the desired product as colorless crystals (620 mg, 87%). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1732 (–COOH), 1628, 1603, 1576, 1230 ($-SO_2$), 1190 ($-SO_2-$), 1050 ($-SO_3^-$). NMR ($D_2O/NaHCO_3$) δ: 4.93 (1H, s, $-\underset{|}{C}HCO-$), 6.90 (1H, s, =CH–). *Anal.* Calcd for $C_5H_6N_2O_5S_2$: C, 25.21; H, 2.54; N, 11.76; S, 26.92. Found: C, 25.20; H, 2.69; N, 11.66; S, 27.11.

2-(2-Chloroacetamidothiazol-4-yl)-2-sulfoacetic Acid (5)———Chloroacetyl chloride (1.36 g, 12 mmol) was added dropwise to a solution of 2-sulfoacetate **4** (2.66 g, 10 mmol) and triethylamine (2.22 g, 22 mmol) in dimethylacetamide (6 ml) at 0—5 °C and the mixture was stirred at room temperature for 30 min. Addition of conc. HCl (1 ml) caused formation of a precipitate, which was removed by filtration. The filtrate, after being diluted with H_2O (60 ml) and washed with AcOEt (50 ml × 3), was concentrated to 20 ml *in vacuo*. This solution was made strongly basic with 7.5 N NaOH (8 ml) and left standing at 0—5 °C for 30 min. On acidification with conc. HCl, a colorless precipitate was deposited. After stirring for 1 h at 0—5 °C, this was collected by filtration and washed with ice-water, EtOH and Et_2O, successively. Recrystallization from H_2O–EtOH (1:4) gave the desired product (2 g, 64%) as colorless needles. mp 284—287 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1715 (–COOH), 1617, 1580 (–CONH–), 1247 ($-SO_2-$), 1043 ($-SO_3^-$). NMR (DMSO-d_6) δ: 4.32 (2H, s, $ClCH_2$), 4.93 (1H, s, $-\underset{|}{C}H-$), 7.31 (1H, s, =CH–). *Anal.* Calcd for $C_7H_7ClN_2O_6S_2 \cdot 0.5H_2O$: C, 25.97; H, 2.49; N, 8.65; S, 19.81; Cl, 10.95. Found: C, 26.37; H, 2.84; N, 8.70; S, 20.21; Cl, 10.87.

2-(2-Chloroacetamidothiazol-4-yl)-2-sulfoacetyl Chloride (6)———Thionyl chloride (3 ml) was added to a suspension of the sulfoacetic acid **5** (472 mg, 1.5 mmol) in Et_2O (3 ml). After 30 min stirring, dimethylformamide (2 drops) was added and stirring was continued for another 1 h at room temperature. Concentration of the mixture *in vacuo* gave the desired sulfoacetyl chloride as a brownish solid, which was used in the following reaction without further purification. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1761 (–COCl), 1718, 1585 (–CONH–), 1260, 1180 ($-SO_2-$), 1047 ($-SO_3^-$).

2-(2-Aminothiazol-4-yl)-2-sulfoacetanilide (12)———A stirred solution of SO_3–dioxane complex[12] (1.68 g, 10 mmol) in CH_2Cl_2 (4.4 ml) was treated with γ-chloroacetoacetanilide[15] (**11**, 2.12 g, 10 mmol) at 0 °C. After addition of 1,2-dichloroethane (10 ml), the mixture was brought to room temperature and stirred for 1 h. The mixture was cooled to −10 °C and CH_2Cl_2 (25 ml), EtOH (50 ml) and thiourea (1 g, 13.2 mmol) were added. The reaction mixture, after being stirred overnight at room temperature, gave a colorless precipitate. Filtration and washing with small amounts of EtOH and Et_2O gave 0.94 g (30%) of the desired product as colorless crystals. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1680 (–CONH–), 1645, 1602, 1557 (–CONH–), 1254 ($-SO_2-$), 1034 ($-SO_3^-$); NMR (DMSO-d_6) δ: 4.98 (1H, s, $-\underset{|}{C}HCO$), 6.79 (1H, s, =CH–), 6.98—7.67 (5H, m, Ph-H), 8.90 (1H, br s, NH).

Disodium 7-(2-(2-Chloroacetamidothiazol-4-yl)-2-sulfonatoacetamido)ceph-3-em-3-acetoxymethyl-4-carboxylate (9a, Route A)———A solution of 2-(2-chloroacetamidothiazol-4-yl)-2-sulfoacetylchloride (1.5 mmol) in dimethylacetamide (3 ml) was added to a solution of 7-aminocephalosporanic acid (**8a**, 408 mg, 1.5 mmol), 1 N NaOH (1.5 ml) and $NaHCO_3$ (302 mg, 3.6 mmol) in H_2O (6 ml), and the mixture was stirred at 0—5 °C for 20 min. After being adjusted to pH 6.5, the reaction mixture was chromatographed on a column of

Amberlite XAD-2 (100—200 mesh, 2.5 × 70 cm) using H_2O as an eluent; fractions were examined for ultraviolet (UV) absorption at 260 nm. The cephalosporin fraction was collected and concentrated *in vacuo*. Lyophilization gave the desired product as a slightly yellowish powder (480 mg, 52%). IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm}^{-1}$: 1760 ($\beta$-lactam), 1682 (–CONH), 1600 ($-COO^-$) 1555 (–CONH–), 1225 ($-SO_2-$), 1040 ($-SO_3^-$). NMR (D_2O) δ: 2.25 (3H, s, $OCOCH_3$), 3.42, 3.79 (2H, two sets of doublets, J=18 Hz, 2-CH_2), 4.50 (2H, s, $ClCH_2$), 4.92 (2H, q, 3-CH_2), 5.23 (1H, d, J=4.5 Hz, 6-CH), 5.33, 5.34 (1H, each s, $-\underset{|}{C}H-$), 5.61 (1H, d, J=4.5 Hz, 7-CH), 7.46 (1H, s, =CH–).

Disodium 7-(2-(2-Chloroacetamidothiazol-4-yl)-2-sulfonatoacetamido)ceph-3-em-3-(1-methyltetrazol-5-ylthiomethyl)-4-carboxylate (9b, Route A)———A solution of 2-(2-chloroacetamidothiazol-4-yl)-2-sulfoacetyl chloride (1.5 mmol) in dimethylacetamide (3 ml) was added to a solution of 7-aminoceph-3-em-3-(1-methyltetrazol-5-ylthiomethyl)-4-carboxylic acid (**8b**, 492 mg, 1.5 mmol), 1 N NaOH (1.5 ml) and $NaHCO_3$ (302 mg, 3.6 mmol) in H_2O (6 ml). The reaction mixture was stirred at 0—5 °C for 20 min and adjusted to pH 6.5. Purification by chromatography was performed in the manner described above. After work-up, the desired product was obtained as a slightly yellowish powder (540 mg, 54%). IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm}^{-1}$: 1760 ($\beta$-lactam), 1682 (–CONH–) 1595 ($-COO^-$), 1555 (–CONH–) 1213 ($-SO_2-$), 1042 ($-SO_3^-$). NMR (D_2O) δ: 3.57, 3.98 (2H, two sets of doublets, J=18 Hz, 2-CH_2), 4.21 (3H, s, N-CH_3), 4.40 (2H, q, 3-CH_2), 4.60 (2H, s, $ClCH_2$), 5.33 (1H, d, J=4.5 Hz, 6-CH), 5.46, 5.48 (1H, each s, $-\underset{|}{C}H-$), 5.93 (1H, d, J=4.5 Hz, 7-CH), 7.65 (1H, s, =CH–)

Sodium 7-(2-(2-Chloroacetamidothiazol-4-yl)-2-sulfonatoacetamido)ceph-3-em-3-(4-carbamoylpyridiniomethyl)-4-carboxylate (9c, Route A)———A dimethylacetamide solution (3 ml) of 2-(2-chloroacetamidothiazol-4-yl)-2-sulfoacetyl chloride (1.5 mmol) was added to a solution of 7-aminoceph-3-em-3-(4-carbamoylpyridiniomethyl)-4-carboxylate·2HCl (**8c**, 607 mg, 1.5 mmol), 1 N NaOH (3 ml) and $NaHCO_3$ (302 mg, 3.6 mmol) in H_2O (4.5 ml). The reaction mixture was stirred at 0—5 °C for 20 min and adjusted to pH 6.5. Purification by chromatography and subsequent work-up in the manner described above gave the desired product as a pale yellow powder. (440 mg, 45%). IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm}^{-1}$: 1767 ($\beta$-lactam), 1685 (–CONH–), 1605 ($-COO^-$), 1557 (–CONH–), 1213 ($-SO_2-$), 1040 ($-SO_3^-$). NMR (D_2O) δ: 3.25, 3.78 (2H, two sets of doublets, J=18 Hz, 2-CH_2), 4.45 (2H, s, $ClCH_2$), 5.30 (1H, d, J=4.5 Hz, 6-CH), 5.31 (1H, S, $-\underset{|}{C}H-$), 5.60 (2H, q, 3-CH_2), 5,86 (1H, d, J=4.5 Hz, 7-CH), 7.43 (1H, s, =CH–),8.48 (2H, d, pyridine ring-H), 9.25 (2H, d, pyridine ring-H).

Disodium 7-(2-(2-Aminothiazol-4-yl)-2-sulfonatoacetamido)ceph-3-em-3-acetoxymethyl-4-carboxylate (10a, Route A)———A solution of **9a** (184 mg, 0.3 mmol), thiourea (57 mg, 0.75 mmol) and AcONa (62 mg, 0.75 mmol) in H_2O (1 ml) was left overnight at room temperature. The reaction mixture was lyophilized and the residue was suspended in EtOH (20 ml) with stirring at room temperature for 1 h. The precipitate, after being collected by filtration, was chromatographed on a column of Amberlite XAD-2 (100—200 mesh, 1.5 × 100 cm) using H_2O as an eluent. The fraction of eluate containing the desired product, after being lyophilized, gave **10a** as a colorless powder (137 mg, 85%). IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm}^{-1}$: 1760 ($\beta$-lactam), 1672 (–CONH–), 1602 ($-COO^-$), 1520 (–CONH–), 1227 ($-SO_2-$), 1045 ($-SO_3^-$). NMR (D_2O) δ: 3.46, 3.84 (2H, two sets of doublets, J= 18 Hz, 2-CH_2), 4.93 (2H, q, 3-CH_2), 5.16 (1H, s, $-\underset{|}{C}H-$), 5.23, 5.26 (1H, each d, J=4.5 Hz, 6-CH), 5.81, 5.84 (1H, each d, J=4.5 Hz, 7-CH), 6.89 (1H, s, =CH–). *Anal.* Calcd for $C_{15}H_{14}N_4Na_2O_9S_3\cdot 2.5H_2O$: C, 30.98; H, 3.29; N, 9.64; S, 16.54. Found: C, 30.89; H, 3.53; N, 9.46; S, 16.84.

10a, Route B———7-(γ-Chloroacetoacetamido)cephalosporanic acid[6] (**13a**, 2.34 g, 7 mmol) was added in small portions to a stirred solution of SO_3–dioxane complex[12] (1.35 g, 8 mmol) in CH_2Cl_2 (3.5 ml) over a period of about 10 min at 0 °C. The mixture was brought to room temperature and stirred for 1 h. After being cooled at −10 °C, EtOH (30 ml), thiourea (547 mg, 7.2 mmol) and anhydrous CH_3COONa (1.13 g) were added and the mixture was stirred overnight at room temperature, then evaporated to dryness *in vacuo*. The residue was dissolved in H_2O (15 ml) and the solution was adjusted to pH 7.0 with 1 N NaOH, then chromatographed on an Amberlite XAD-2 column (100—200 mesh, 400 ml) with water as an eluent. The fractions of eluate containing the cephalosporin were combined and lyophilized. The desired product was obtained as a slightly yellowish powder (640 mg, 20%).

Disodium 7-(2-(2-Aminothiazol-4-yl)-2-sulfonatoacetamido)ceph-3-em-3-(1-methyltetrazol-5-ylthiomethyl)-4-carboxylate (10b, Route A)———This compound was synthesized as described for **10a** (route A) except for replacement of **9a** with **9b** (201 mg, 0.3 mmol) as the starting compound. Yield: 160 mg (90%) as a colorless powder. IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm}^{-1}$: 1762 ($\beta$-lactam), 1672 (–CONH–), 1597 ($-COO^-$), 1520 (–CONH–), 1210 ($-SO_2-$), 1045($-SO_3^-$). NMR (D_2O) δ: 3.57, 3.98 (2H, two sets of doublets, J=18 Hz, 2-CH_2), 4.17, 4.53 (2H, two sets of doublets, J=13Hz, 3-CH_2), 4.20 (3H, s, N-CH_3), 5.18, 5.19 (1H, each s, $-\underset{|}{C}H-$), 5.29 (1H, d, J=4.5 Hz, 6-CH), 5.84 (1H, d, J=4.5 Hz, 7-CH), 7.04 (1H, s, =CH–). *Anal.* Calcd for $C_{15}H_{14}N_8Na_2O_7S_4\cdot 4.75H_2O$: C, 26.57; H, 3.49; N, 16.52; S, 18.91. Found: C, 26.85; H, 3.23; N, 16.16; S, 18.52.

Route B———7-(γ-Chloroacetoacetamido)ceph-3-em-3-(1-methyltetrazol-5-ylthiomethyl)-4-carboxylic acid[6] (**13b**, 2.02 g, 4.5 mmol) was added in small portions to a stirred solution of SO_3–dioxane complex[12] (1.93 g, 11.5 mmol) in CH_2Cl_2 (5.1 ml) over a period of about 10 min at 0 °C. The mixture was allowed to come to room temperature and stirred for 1 h. Next, EtOH (25 ml), thiourea (0.41 g, 5.4 mmol) and anhydrous CH_3COONa (1.85 g) were added after the reaction mixture had been cooled to −10 °C, and the whole was stirred overnight at room temperature. Work-up in the manner described for **10a** (route B) gave the desired product as a slightly yellowish powder (270 mg, 10%).

Sodium 7-(2-(2-Aminothiazol-4-yl)-2-sulfonatoacetamido)ceph-3-em-3-(4-carbamoylpyridiniomethyl)-4-carboxylate (10c, Route A)——A mixed solution of **9c** (196 mg, 0.3 mmol), thiourea (57 mg, 0.75 mmol) and CH_3COONa (62 mg, 0.75 mmol) in H_2O (1 ml) was allowed to stand overnight at room temperature. Purification was performed in the manner described for **10a** (route A), yielding the desired product as a slightly yellowish powder (155 mg, 90%). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1763 (β-lactam), 1683 (–CONH–), 1608 ($-COO^-$), 1560 (–CONH–), 1517 (–CONH–), 1208 ($-SO_2-$), 1043 ($-SO_3^-$). NMR (D_2O) δ: 3.20, 3.73 (2H, two sets of doublets, $J=18$ Hz, 2-CH_2), 5.06 (1H, s, –CH–), 5.23 (1H, d, $J=4.5$ Hz, 6-CH), 5.56 (2H, q, 3-CH_2), 5.79 (1H, d, $J=4.5$ Hz, 7-CH), 6.89 (1H, s, =CH–) 8.41 (2H, d, pyridine ring-H), 9.15 (2H, d, pyridine ring-H). *Anal.* Calcd for $C_{19}H_{17}N_6NaO_8S_3 \cdot 3.25H_2O$: C, 35.93; H, 3.73; N, 13.23; S, 15.15. Found: C, 35.67; H, 3.80; N, 13.01; S, 15.45.

10c, DCC Method, Route A——The sulfoacetic acid (**7**, 238 mg, 1 mmol) and DCC (206 mg, 1 mmol) were added to an ice-cooled solution of 7-aminoceph-3-em-3-(4-carbamoylpyridiniomethyl)-4-carboxylate·2HCl (**8c**, 371 mg, 1 mmol) and $NaHCO_3$ (84 mg, 1 mmol) in H_2O (1 ml) and *N*,*N*-dimethylformamide (DMF) (4 ml). The mixture was allowed to react at room temperature for 4 h with stirring, and was neutralized with alkali after being diluted with H_2O(25 ml). The white precipitate that formed was removed by filtration and the filtrate was lyophilized.Chromatography in the usual way and subsequent work-up gave the desired product as a slightly yellowish powder (81 mg, 14%).

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