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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-carbamoylpyridiniummethyl 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 articles²⁻⁴⁾ 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 gentamycin⁵⁾ 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,

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-sulfoacetyl anilide (**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-carbamoylpyridinylmethyl 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-aminothiazol-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

	R	X	Confign. ^{a)}	MIC $\mu\text{g/ml}$								
				<i>P. aeruginosa</i> IFO 3455	<i>P. aeruginosa</i> IFO 3080	<i>E. coli</i> NIHJ JC-2	<i>E. coli</i> O-111	<i>P. vulgaris</i> IFO 3988	<i>P. mirabilis</i> IFO 3849	<i>K. pneumoniae</i> DT	<i>S. aureus</i> FDA 209P	<i>S. aureus</i> 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	>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-S₂ 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 D₂O.

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 chloride¹⁶⁾ 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 -chloroacetoacetate¹⁵⁾ (**3**, 19.75 g, 0.12 mol) was added dropwise to a stirred solution of SO₃-dioxane complex¹²⁾ (30.5 g, 0.18 mol) in CH₂Cl₂ (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 H₂O–EtOH (1:4) gave the desired sulfoacetate (20.5 g, 64%) as colorless needles. mp 256–258°C. IR $\nu_{\max}^{\text{KBr}}$ cm^{–1}: 3340, 3100, 1743 (COOEt), 1654, 1603, 1588, 1208 (–SO₂–), 1190 (–SO₂–), 1053 (–SO₃–). NMR (DMSO-*d*₆) δ : 1.20 (3H, t, *J*=7 Hz, CH₂CH₃), 4.13 (2H, q, *J*=7 Hz, CH₂CH₃), 4.89 (1H, s, –CH–), 6.91 (1H, s, =CH–), 8.90 (2H, br s, NH₂). Anal. Calcd for C₇H₁₀N₂O₅S₂: 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 1N NaOH (6.6 ml) with stirring and the mixture was allowed to stand for 15 min. Next, the mixture was neutralized with 1N HCl (7 ml). The precipitate was collected by filtration and washed successively with ice-cooled H₂O, EtOH and Et₂O, which gave the desired product as colorless crystals (620 mg, 87%). IR $\nu_{\max}^{\text{KBr}}$ cm^{–1}: 1732 (–COOH), 1628, 1603, 1576, 1230 (–SO₂–), 1190 (–SO₂–), 1050 (–SO₃–). NMR (D₂O/NaHCO₃) δ : 4.93 (1H, s, –CHCO–), 6.90 (1H, s, =CH–). Anal. Calcd for C₅H₆N₂O₅S₂: 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 H₂O (60 ml) and washed with AcOEt (50 ml $\times$ 3), was concentrated to 20 ml *in vacuo*. This solution was made strongly basic with 7.5N 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 Et₂O, successively. Recrystallization from H₂O–EtOH (1:4) gave the desired product (2 g, 64%) as colorless needles. mp 284–287°C. IR $\nu_{\max}^{\text{KBr}}$ cm^{–1}: 1715 (–COOH), 1617, 1580 (–CONH–), 1247 (–SO₂–), 1043 (–SO₃–). NMR (DMSO-*d*₆) δ : 4.32 (2H, s, ClCH₂), 4.93 (1H, s, –CH–), 7.31 (1H, s, =CH–). Anal. Calcd for C₇H₇ClN₂O₆S₂·0.5H₂O: 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 Et₂O (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 $\nu_{\max}^{\text{KBr}}$ cm^{–1}: 1761 (–COCl), 1718, 1585 (–CONH–), 1260, 1180 (–SO₂–), 1047 (–SO₃–).

2-(2-Aminothiazol-4-yl)-2-sulfoacetanilide (12)—A stirred solution of SO₃-dioxane complex¹²⁾ (1.68 g, 10 mmol) in CH₂Cl₂ (4.4 ml) was treated with γ -chloroacetoacetanilide¹⁵⁾ (**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 CH₂Cl₂ (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 Et₂O gave 0.94 g (30%) of the desired product as colorless crystals. IR $\nu_{\max}^{\text{KBr}}$ cm^{–1}: 1680 (–CONH–), 1645, 1602, 1557 (–CONH–), 1254 (–SO₂–), 1034 (–SO₃–); NMR (DMSO-*d*₆) δ : 4.98 (1H, s, –CHCO), 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), 1N NaOH (1.5 ml) and NaHCO₃ (302 mg, 3.6 mmol) in H₂O (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 $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1760 (β -lactam), 1682 ($-\text{CONH}-$), 1600 ($-\text{COO}^-$), 1555 ($-\text{CONH}-$), 1225 ($-\text{SO}_2-$), 1040 ($-\text{SO}_3^-$). 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, $-\dot{\text{C}}\text{H}-$), 5.61 (1H, d, $J=4.5$ Hz, 7-CH), 7.46 (1H, s, $=\text{CH}-$).

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), 1N NaOH (1.5 ml) and NaHCO_3 (302 mg, 3.6 mmol) in H_2O (6 ml). The reaction mixture was stirred at $0-5^\circ\text{C}$ 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 $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1760 (β -lactam), 1682 ($-\text{CONH}-$), 1595 ($-\text{COO}^-$), 1555 ($-\text{CONH}-$), 1213 ($-\text{SO}_2-$), 1042 ($-\text{SO}_3^-$). 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, $-\text{CH}-$), 5.93 (1H, d, $J=4.5$ Hz, 7-CH), 7.65 (1H, s, $=\text{CH}-$).

Sodium 7-(2-(2-Chloroacetamidothiazol-4-yl)-2-sulfonatoacetamido)ceph-3-em-3-(4-carbamoylpyridinio-methyl)-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-carbamoylpyridinio-methyl)-4-carboxylate $\cdot 2\text{HCl}$ (**8c**, 607 mg, 1.5 mmol), 1N NaOH (3 ml) and NaHCO_3 (302 mg, 3.6 mmol) in H_2O (4.5 ml). The reaction mixture was stirred at $0-5^\circ\text{C}$ 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 $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1767 (β -lactam), 1685 ($-\text{CONH}-$), 1605 ($-\text{COO}^-$), 1557 ($-\text{CONH}-$), 1213 ($-\text{SO}_2-$), 1040 ($-\text{SO}_3^-$). 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, $-\text{CH}-$), 5.60 (2H, q, 3- CH_2), 5.86 (1H, d, $J=4.5$ Hz, 7-CH), 7.43 (1H, s, $=\text{CH}-$), 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 $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1760 (β -lactam), 1672 ($-\text{CONH}-$), 1602 ($-\text{COO}^-$), 1520 ($-\text{CONH}-$), 1227 ($-\text{SO}_2-$), 1045 ($-\text{SO}_3^-$). 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, $-\text{CH}-$), 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, $=\text{CH}-$). Anal. Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_4\text{Na}_2\text{O}_9\text{S}_3 \cdot 2.5\text{H}_2\text{O}$: 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 acid⁶⁾ (**13a**, 2.34 g, 7 mmol) was added in small portions to a stirred solution of SO_3 -dioxane complex¹²⁾ (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 1N 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 $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1762 (β -lactam), 1672 ($-\text{CONH}-$), 1597 ($-\text{COO}^-$), 1520 ($-\text{CONH}-$), 1210 ($-\text{SO}_2-$), 1045 ($-\text{SO}_3^-$). 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, $J=13$ Hz, 3- CH_2), 4.20 (3H, s, N- CH_3), 5.18, 5.19 (1H, each s, $-\text{CH}-$), 5.29 (1H, d, $J=4.5$ Hz, 6-CH), 5.84 (1H, d, $J=4.5$ Hz, 7-CH), 7.04 (1H, s, $=\text{CH}-$). Anal. Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_8\text{Na}_2\text{O}_7\text{S}_4 \cdot 4.75\text{H}_2\text{O}$: 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 acid⁶⁾ (**13b**, 2.02 g, 4.5 mmol) was added in small portions to a stirred solution of SO_3 -dioxane complex¹²⁾ (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 $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1763 (β -lactam), 1683 ($-\text{CONH}-$), 1608 ($-\text{COO}-$), 1560 ($-\text{CONH}-$), 1517 ($-\text{CONH}-$), 1208 ($-\text{SO}_2-$), 1043 ($-\text{SO}_3-$). NMR (D_2O) δ : 3.20, 3.73 (2H, two sets of doublets, $J=18$ Hz, 2- CH_2), 5.06 (1H, s, $-\text{CH}-$), 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, $=\text{CH}-$), 8.41 (2H, d, pyridine ring-H), 9.15 (2H, d, pyridine ring-H). *Anal.* Calcd for $\text{C}_{19}\text{H}_{17}\text{N}_6\text{NaO}_8\text{S}_3 \cdot 3.25\text{H}_2\text{O}$: 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 $\cdot 2\text{HCl}$ (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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References and Notes

- 1) A part of this study has been presented in the following patent publication. H. Nomura, *et. al.*, Japan Patent Application, 103327 (1975), Japan Kokai Patent, 27791 (1977) [*Chem. Abstr.*, **87**, 39517v (1977)].
- 2) H. Nomura, T. Fugono, T. Hitaka, I. Minami, T. Azuma, S. Morimoto and T. Masuda, *J. Med. Chem.*, **17**, 1312 (1974).
- 3) H. Nomura, T. Fugono, T. Hitaka, I. Minami, T. Azuma, S. Morimoto and T. Masuda, *Heterocycles*, **2**, 67 (1974).
- 4) H. Nomura, I. Minami, T. Hitaka and T. Fugono, *J. Antibiot.*, **29**, 928 (1976).
- 5) Y. Kato, A. Saito, K. Ishikawa, E. Odagaki, H. Matsumiya, K. Sato, M. Tomizawa, I. Nakayama and Y. Kinoshita, *Chemotherapy*, **27** (S-2), 153 (1979).
- 6) a) M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi and T. Miyawaki, *J. Antibiot.*, **31**, 1252 (1978); b) M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi, T. Miyawaki, H. Akimoto, K. Naito and M. Kida, *J. Antibiot.*, **31**, 1262 (1978).
- 7) T. Kamimura, Y. Matsumoto, N. Okada, Y. Mine, M. Nishida, S. Goto and S. Kuwahara, *Antimicrob. Ag. Chemother.*, **16**, 540 (1979).
- 8) R. Bucourt, R. Heymes, A. Lutz, L. Penasse and J. Perronnet, *C. R. Acad. Sci. Ser. D*, **284**, 1847 (1977).
- 9) M. Ochiai, O. Aki, A. Morimoto, T. Okada and Y. Matsushita, *Chem. Pharm. Bull.*, **25**, 3115 (1977).
- 10) R. Reiner, U. Weiss, U. Brombacher, P. Lanz, M. Momtavan, A. Furlenmeier, P. Angehern and P. J. Probst, *J. Antibiot.*, **33**, 783 (1980).
- 11) P.B. Harper, S.M. Kirby and C.H. O'Callaghan, 19th Intersci. Conf. Antimicrob. Agents & Chemother., Boston, Mass., Abstract No. 559, 1979.
- 12) W.E. Truce and C.E. Olson, *J. Am. Chem. Soc.*, **75**, 1651 (1953).
- 13) S. Morimoto, H. Nomura, T. Fugono, I. Minami, T. Ishiguro and T. Masuda, *J. Antibiot.*, **26**, 146 (1973).
- 14) A. Blomquist and F. Baldwin, *J. Am. Chem. Soc.*, **70**, 29 (1948).
- 15) C.D. Hurd and J.L. Abernethy, *J. Am. Chem. Soc.*, **62**, 1147 (1940).
- 16) S. Morimoto, H. Nomura, T. Ishiguro, T. Fugono and K. Maeda, *J. Med. Chem.*, **15**, 1105 (1972).
- 17) J.M. Greene, Ger. Offen. 2312997 (1973) [*Chem. Abstr.*, **80**, 48020k (1974)].
- 18) E.M. Wilson and A.C.W. Curran, Ger. Offen. 1955475 (1970) [*Chem. Abstr.*, **73**, 25453v (1970)].