SYNTHETIC CEPHALOSPORINS

THE SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF 7-[2-(2-(AMINO-1,3,4-THIADIAZOL-5-YL)ACETAMIDO]-CEPHALOSPORINS

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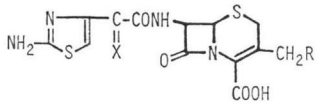
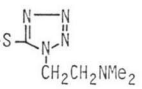
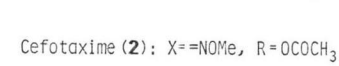
Synthesis and antibacterial activities of 7-[2-(2-amino-1,3,4-thiadiazol-5-yl)acetamido]-cephalosporins and their derivatives having the methoxyimino group at the 2-position of the 7-acyl moiety are described. These compounds are of interest as structural analogues of potent antibiotics, 7-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins. 7-[2-(2-Amino-1,3,4-thiadiazol-5-yl)acetamido]cephalosporins showed comparable activity with cefazolin. Introduction of methoxyimino group to the 7-side chain resulted in a lowering of activity.

Our previous publication has described that 6-[DL-2-amino-2-(thiazol-4-yl)acetamido]penicillanic acids were a new class of highly active antibiotics[1]. Recently, 7-[2-(2-aminothiazol-4-yl)acetamido]-cephalosporin derivatives, *e.g.*, **1** and **2**, have received considerable attention because of their potent broad-spectrum antibacterial activity[2]. These findings prompted us to examine the synthesis of the closely related 1,3,4-thiadiazole derivatives and attendant changes in activities. We have already reported unusual behavior of DL-α-(1,3,4-thiadiazol-2-yl)glycines, which were decarboxylated too rapidly to be isolated[3]. This paper deals with the synthesis and antibacterial activity of 7-[2-(2-amino-1,3,4-thiadiazol-5-yl) acetamido]-cephalosporins.



Cefotiam (**1**): X=H_2, R=





Cefotaxime (**2**): X=NOMe, R=$OCOCH_3$

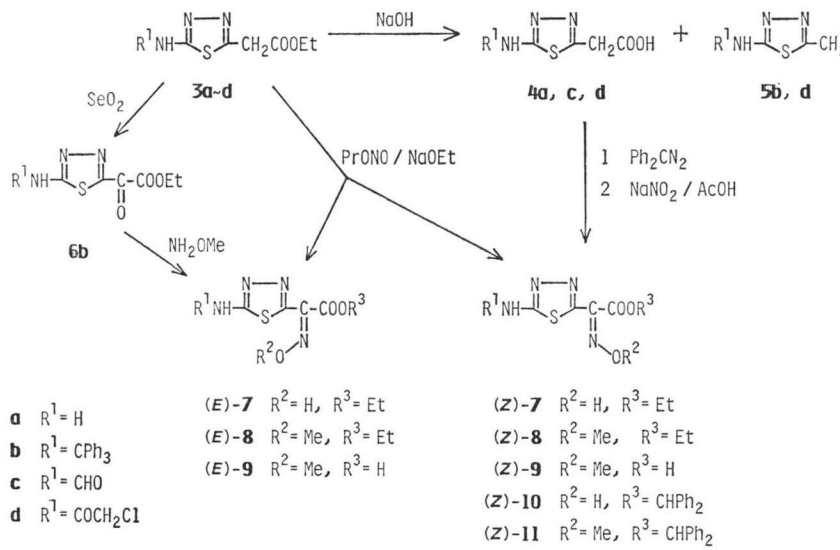
Chemistry

2-Amino-1,3,4-thiadiazol-5-ylacetic acid (**4a**) has been prepared by alkaline hydrolysis of the corresponding ester **3a**[4]. When the *N*-trityl derivative (**3b**) was hydrolyzed with aqueous potassium hydroxide and the reaction mixture was then acidified by hydrochloric acid at 0°C, spontaneous evolution of gaseous carbon dioxide occurred to give quantitatively **5b** (mp 197~198°C). In contrast to easy decarboxylation of the *N*-trityl acid (**4b**), the *N*-formyl derivative (**3c**) gave the desired acid **4c** in 71% yield by a similar treatment. The *N*-chloroacetyl derivative (**3d**) also afforded **4d** in 56% yield together with **5d**. The acid **4d** was more conveniently obtained in 85% yield by chloroacetylation of **4a**.

Next, introduction of methoxyimino group to the α-position of the ester **3** was examined. Oxidation of **3b** with selenium dioxide in boiling aqueous dioxane gave the α-ketoester **6b** in 33% yield. Treatment of **6b** with methoxyamine afforded (*E*)-α-methoxyimino ester, (*E*)-**8b**.

Alternatively, oximation of **3b** with propyl nitrite in the presence of sodium ethoxide gave quanti-

Scheme 1.



tatively a mixture of (*E*)-**7b** and (*Z*)-**7b**, which was then treated with diazomethane and the crude product was chromatographed on silica gel to give (*E*)-**8b** in 17.5% yield and (*Z*)-**8b** in 47% yield. In a similar manner, **3a** was converted to (*E*)-**8a** and (*Z*)-**8a** in 13% and 55% yields, respectively. Chloroacetylation of (*E*)-**8a** and (*Z*)-**8a** produced (*E*)-**8d** and (*Z*)-**8d** in 64% and 50% yields, respectively.

Hydrolysis of the *E* isomers, (*E*)-**8b** and (*E*)-**8d**, proceeded smoothly at 0°C with an equimolar amount of sodium hydroxide in aqueous tetrahydrofuran to give the acids, (*E*)-**9b** and (*E*)-**9d**. However, the *Z* isomers, (*Z*)-**8b** and (*Z*)-**8d** resisted to hydrolysis and were unchanged under the same conditions. When (*Z*)-**8b** or (*Z*)-**8d** was treated with an excess of sodium hydroxide at room temperature overnight until (*Z*)-**8b** or (*Z*)-**8d** disappeared, a complex mixture generated and the desired acid was not isolated. Similar resistance of (*Z*)-2-alkoxymino-2-arylacetic acid esters toward alkaline hydrolysis has been reported[5]. Our assignment of the *E* or *Z* configuration to the methoxyimino esters **8** was based on this finding.

In an effort to prepare the (*Z*)-methoxyimino acid, **4d** was converted to the benzhydryl ester with diphenyldiazomethane. Oximation of the benzhydryl ester was conveniently carried out with sodium nitrite in aqueous acetic acid to give only *Z* isomer (*Z*)-**10d** in 97% yield. Attempted *O*-methylation of (*Z*)-**10d** with diazomethane, methyl iodide or dimethyl sulfate was unsuccessful*. Thus, the chloroacetyl group of (*Z*)-**10d** was removed with thiourea and (*Z*)-**10a** was then treated with diazomethane to give (*Z*)-**11a**. After the 2-amino group was protected again with chloroacetyl chloride, treatment of (*Z*)-**11d** with trifluoroacetic acid gave the desired (*Z*)-methoxyimino acid (*Z*)-**9d**.

Condensation of the carboxylic acids, **4d**, (*E*)-**9b**, and (*Z*)-**9d**, with benzhydryl 7-amino-3-(1-methyl-1*H*-tetrazol-5-ylthiomethyl)ceph-3-em-4-carboxylate (**12**) was carried out using dicyclohexylcarbodiimide as condensing reagent (Scheme 2). Removal of the protective groups of **13**, **14** and **15** afforded the new cephalosporins (**16**~**19**).

* In a model experiment, **3d** was treated with diazomethane to give ethyl 2-(*N*-methylchloroacetylamino)-1,3,4-thiadiazol-5-ylacetate [mp 68~69°C, NMR ($CDCl_3$) δ 3.87 (N-CH_3)] in 25% yield and ethyl 2-chloroacetylimino-Δ^4-1,3,4-thiadiazolin-5-ylacetate [mp 88~89°C, NMR ($CDCl_3$) δ 3.95 (N-CH_3)] in 56% yield, indicating high nucleophilicity of the exocyclic and ring nitrogen atoms in 2-acylamino-1,3,4-thiadiazole.

Scheme 2.

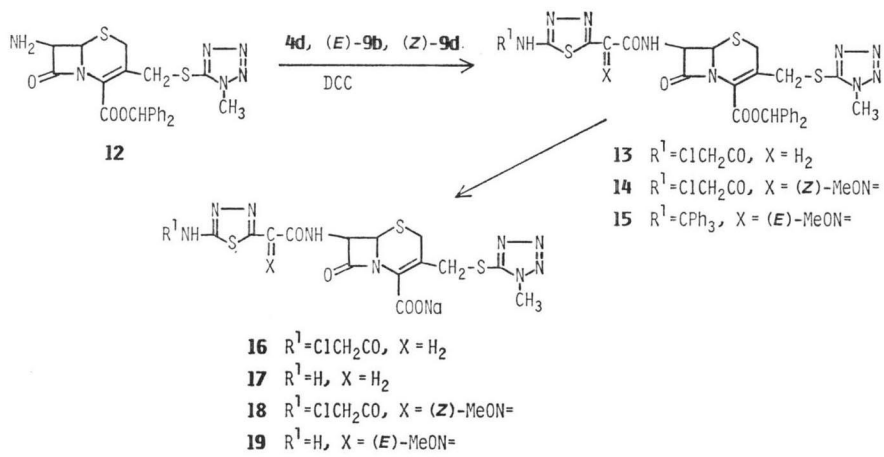


Table 1. *In vitro* minimum inhibitory concentrations (μg/ml).

Compound No.	*S. aureus*[a] Smith	*B. subtilis*[b] ATCC 8043	*E. coli* NIHJ JC-2	*E. coli* GN206	*K. pneumoniae*[c] GN69	*P. morganii* 1510	*P. rettgeri* GN624
16	0.39	0.2	1.56	100	3.12	3.12	100
17	0.39	0.1	3.12	100	3.12	3.12	100
18	3.13	6.25	100	25	100	100	50
19	6.25	6.25	50	25	25	100	50
Cefotiam (**1**)	0.2	0.2	0.05	3.12	1.56	6.25	25
Cefazolin	0.1	0.2	1.56	100	3.12	—	100

[a] *Staphylococcus aureus*, [b] *Bacillus subtilis*, [c] *Klebsiella pneumoniae*.

Antibacterial Activity

The minimum inhibitory concentrations (MIC) of the new cephalosporins were determined by the standard, two-fold, agar-dilution method. In Table 1, the MIC values of these compounds against several microorganisms are summarized and compared with the values for cefazolin and cefotiam (**1**). The compounds **16** and **17** had comparable activity in all respects with cefazolin, but when compared with cefotiam, did not show improved activity especially against the β-lactamase-producing strains, *Escherichia coli* GN 206 and *Proteus rettgeri* GN 624. Introduction of methoxyimino group to the side chain resulted in a lowering of activity.

Experimental

Melting points were uncorrected. IR spectra were recorded on a Jasco-IR-1 spectrometer. NMR spectra were determined with tetramethylsilane as an internal standard on a Hitachi R-600 or Jeol FX-100 spectrometer, chemical shifts being given in ppm unit.

Ethyl 2-Tritylamino-1,3,4-thiadiazol-5-ylacetate (**3b**)

A solution of trityl chloride (19.13 g) in CH_2Cl_2 (60 ml) was added at $-30°C$ to a stirred mixture of ethyl 2-amino-1,3,4-thiadiazol-5-ylacetate (**3a**, 10 g), Et_3N (6.94 g), CH_2Cl_2 (100 ml), and DMF (20 ml). The mixture was stirred at room temperature overnight, washed with cold 2.5% hydrochloric acid and brine, dried, and evaporated *in vacuo*. The residue was crystallized from ethanol to give **3b** as colorless crystals (20.6 g, 90%). mp 167~168°C; IR (Nujol) 3180, 1740 cm^{-1}.

Anal. Calcd. for $C_{25}H_{23}N_3O_2S$: C 69.90, H 5.39, N 9.78, S 7.46.
Found: C 69.10, H 5.29, N 9.54, S 7.45.

Ethyl 2-Formamido-1,3,4-thiadiazol-5-ylacetate (**3c**)

To a solution of acetic-formic anhydride prepared from HCOOH (14 ml) and Ac_2O (14 ml), **3a** (9.35 g) was added at once. The mixture was stirred for 12 hours at 30°C and evaporated *in vacuo*. The residue was crystallized from ethanol to give **3c** (7.65 g, 71%). mp 156~157°C; IR (Nujol) 3150, 1735, 1685 cm^{-1}.

Anal. Calcd. for $C_7H_9N_3O_3S$: C 39.07, H 4.21, N 19.52, S 14.90.
Found: C 39.09, H 4.21, N 19.51, S 14.62.

Ethyl 2-Chloroacetamido-1,3,4-thiadiazol-5-ylacetate (**3d**)

A solution of chloroacetyl chloride (7.16 g) in CH_2Cl_2 (10 ml) was added over a period of 45 minutes at 0°C to a stirred mixture of **3a** (10 g), pyridine (5.08 g), DMF (20 ml), and CH_2Cl_2 (100 ml). After being stirred for an additional hour at 0°C, the mixture was washed with $NaHCO_3$ solution and brine, dried, and evaporated *in vacuo*. The remaining solid was crystallized from ethanol to give **3d** (7.35 g, 52%). mp 186~187°C; IR (Nujol) 3150, 1735, 1690 cm^{-1}.

Anal. Calcd. for $C_8H_{10}N_3O_3SCl$: C 36.44, H 3.82, N 15.94.
Found: C 36.44, H 3.57, N 15.82.

2-Formamido-1,3,4-thiadiazol-5-ylacetic Acid (**4c**)

To a solution of **3c** (2.15 g) in methanol (40 ml), 1 N NaOH (20 ml) was added dropwise at 0°C. The mixture was stirred at room temperature for 3 hours, and concentrated *in vacuo*. The residue was diluted with water, washed with ether, and acidified at 0°C with 20% hydrochloric acid. The solid was collected by filtration and dried to yield **4c** (1.3 g, 70%). mp 150°C (dec.); IR (Nujol) 3240, 1690, 1550 cm^{-1}; NMR (DMSO-d_6) δ 4.09 (2H, s), 8.52 (1H, s), 10.60 (1H, s).

2-Chloroacetamido-1,3,4-thiadiazol-5-ylacetic Acid (**4d**)

a) By the use of the procedure described for **4c**, **3d** was hydrolyzed to give **4d** in 56% yield and **5d** in 19% yield.

4d: mp 200°C (dec.); IR (Nujol) 3200, 1690, 1570 cm^{-1}; NMR (DMSO-d_6) δ 4.06 (2H, s), 4.32 (2H, s).

5d: mp 254°C; IR (Nujol) 3160, 1695 cm^{-1}; NMR (DMSO-d_6) δ 2.64 (3H, s), 4.43 (2H, s).

b) A solution of chloroacetyl chloride (370 mg) in ether (0.5 ml) was added at 0°C to a suspension of **3a** (318 mg) in DMF (5 ml). The mixture was stirred at 0°C for 10 minutes and poured into ice-water (20 ml). The solid was collected by filtration, washed with water, and dried to give **4d** (400 mg, 85%).

Ethyl 2-Methoxyimino-2-(2-tritylamino-1,3,4-thiadiazol-5-yl)acetate [(*E*)-**8b**, (*Z*)-**8b**)]

a) A mixture of **3b** (10 g), SeO_2 (3.08 g), water (1 ml) and dioxane (120 ml) was refluxed for 15 minutes. The mixture was cooled, filtered and the filtrate was evaporated *in vacuo*. The residue was taken into CH_2Cl_2 (100 ml) and the solution was washed with $NaHCO_3$ solution, dried, and then evaporated. The oily residue was chromatographed on silica gel with benzene - ethyl acetate to give the glyoxalate **6b** (3.44 g, 33%) as a pale yellow solid. mp 216°C; IR (Nujol) 3190, 1740, 1695 cm^{-1}.

Anal. Calcd. for $C_{23}H_{21}O_3N_3S$: C 67.70, H 4.77, N 9.47.
Found: C 67.01, H 4.62, N 9.27.

A mixture of **6b** (443 mg), methoxyamine hydrochloride (100 mg), sodium acetate (98 mg), ethanol (4 ml), and CH_2Cl_2 (3 ml) was refluxed for 30 hours. The mixture was poured into ice-water and extracted with CH_2Cl_2. The extracts were dried and evaporated *in vacuo*. The oily residue was purified on a preparative TLC to give (*E*)-**8b** (98 mg, 21%). mp 188~189°C; IR (Nujol) 3230, 1742 cm^{-1}.

Anal. Calcd. for $C_{25}H_{22}N_4O_3S$: C 66.08, H 5.12, N 11.85, S 6.79.
Found: C 65.94, H 5.63, N 11.95, S 7.09.

b) To a solution of sodium ethoxide prepared from Na (670 mg) and absolute ethanol (125 ml), **3b** (12.5 g) and propyl nitrite (3.1 g) was added at once at 0°C. The mixture was stirred at 0°C for an additional hour, poured into ice-water and acidified with 20% hydrochloric acid. The solid was collected by filtration to give the hydroxyimino ester **7b** (9.9 g, 74%). IR (Nujol) 3260, 1730 cm^{-1}; NMR

($CDCl_3$) δ 1.31 (3H, t, J=7.0 Hz), 4.35 (2H, q, J=7.0 Hz), 7.10~7.70 (16H, m).

A solution of **7b** (3.1 g) in THF (60 ml) was treated at 0°C with a diazomethane - ether solution prepared from nitrosomethylurea (10 g). After 10 minutes, the excess diazomethane was decomposed with AcOH, and the mixture was evaporated *in vacuo*. The residue was chromatographed on silica gel with benzene - ethyl acetate to yield (*E*)-**8b** (556 mg, 18%) and (*Z*)-**8b** (1.42 g, 45%).

(*Z*)-**8b**: mp 258~259°C; IR (Nujol) 3250, 1720 cm^{-1}.

Anal. Calcd. for $C_{25}H_{22}N_4O_8S$: C 66.08, H 5.12, N 11.85, S 6.79.
Found: C 65.19, H 5.16, N 11.94, S 7.18.

Ethyl 2-(2-Amino-1,3,4-thiadiazol-5-yl)-2-methoxyiminoacetate [(*E*)-**8a**, (*Z*)-**8a**]

By the use of the procedure (method b) described for the synthesis of **8b**, **3a** was converted to (*E*)-**8a** and (*Z*)-**8a** in 13% and 54.5%.

(*E*)-**8a**: mp 148~149°C: IR (Nujol) 3360, 3260, 3080, 1735, 1630 cm^{-1}.

Anal. Calcd. for $C_7H_{10}N_4O_3S$: C 36.51, H 4.37, N 24.33, S 13.92.
Found: C 36.33, H 4.37, N 24.03, S 13.96.

(*Z*)-**8a**: mp 182~183°C; IR (Nujol) 3360, 3260, 3080, 1730, 1620 cm^{-1}.

Anal. Calcd. for $C_7H_{10}N_4O_3S$: C 36.51, H 4.37, N 24.33, S 13.92.
Found: C 36.75, H 4.48, N 24.03, S 13.98.

Ethyl 2-(2-Chloroacetamido-1,3,4-thiadiazol-5-yl)-2-methoxyiminoacetate [(*E*)-**8d**, (*Z*)-**8d**]

By the use of the procedure described for the synthesis of **3d**, (*E*)-**8a** and (*Z*)-**8a** were treated with chloroacetyl chloride to give (*E*)-**8d** and (*Z*)-**8d** in 64% and 50% yields, respectively.

(*E*)-**8d**: mp 203~204°C; IR (Nujol) 3150, 1740, 1700 cm^{-1}.

Anal. Calcd. for $C_6H_{11}N_4O_4SCl$: C 35.24, H 3.61, N 18.26, S 10.45.
Found: C 35.13, H 3.51, N 18.35, S 10.17.

(*Z*)-**8d**: mp >300°C; IR (Nujol) 3150, 1730, 1710 cm^{-1}; NMR (DMSO-d_6) δ 1.45 (3H, t, J=6.8 Hz), 4.02 (3H, s), 4.33 (2H, s), 4.56 (2H, q, J=6.8 Hz), 12.62 (1H, broad s).

(*E*)-2-Methoxyimino-2-(2-tritylamino-1,3,4-thiadiazol-5-yl)acetic Acid [(*E*)-**9b**]

To a solution of (*E*)-**8b** (472 mg) in THF (5 ml) and water (2 ml), 1 N NaOH (1 ml) was added dropwise under ice-cooling. The mixture was stirred for 4 hours at 0~5°C, and concentrated to 6 ml *in vacuo*. The solution was diluted with water (3 ml), washed with ether, and acidified with 5% hydrochloric acid. The solid was collected by filtration and dried to yield (*E*)-**9b** (302 mg, 68%). mp 179~180°C (dec.); IR (Nujol) 3220, 1650 cm^{-1}; NMR (DMSO-d_6) δ 4.00 (3H, s), 7.33 (16H, broad s).

2-(2-Chloroacetamido-1,3,4-thiadiazol-5-yl)-(*E*)-2-methoxyiminoacetic Acid [(*E*)-**9d**]

By the use of the procedure described above, (*E*)-**8d** was hydrolyzed to give (*E*)-**9d** in 45% yield. mp 163~164°C (dec.); IR (Nujol) 3150, 1700, 1580 cm^{-1}; NMR (DMSO-d_6) δ 4.27 (3H, s), 4.35 (2H, s), 11.10 (1H, broad s).

2-(2-Chloroacetatamino-1,3,4-thiadiazol-5-yl)-(*Z*)-2-methoxyiminoacetic Acid [(*Z*)-**9d**]

The compound (*Z*)-**11d** (140 mg) was treated at 0°C with anisole (0.15 ml) and CF_3COOH (1.5 ml). After 10 minutes, the mixture was evaporated *in vacuo* and the oily residue was triturated in diisopropyl ether to give a colorless solid of (*Z*)-**9d** (76 mg, 87%). mp 230°C; IR (Nujol) 3140, 1705, 1650 cm^{-1}; NMR (DMSO-d_6) δ 3.91 (3H, s), 4.45 (2H, s), 8.71 (1H, broad s).

Sodium 7-[2-(2-Amino-1,3,4-thiadiazol-5-yl)acetamido]-3-(1-methyl-1*H*-tetrazol-5-ylthiomethyl)-ceph-3-em-4-carboxylate (**17**)

To a solution of **4d** (500 mg) and **12** (1.13 g) in DMF (5 ml), dicyclohexylcarbodiimide (438 mg) was added at 0°C and the mixture was stirred for 3 hours under ice-cooling. The solid was filtered off, and the filtrate was diluted with ethyl acetate (30 ml), washed succesively with 2.5% hydrochloric acid, $NaHCO_3$ solution and brine, and then evaporated *in vacuo*. The residue was crystallized from diisopropyl ether to give **13** (950 mg, 60.5%). mp 156~157°C; IR (Nujol) 3420, 3350, 1790, 1770, 1640 cm^{-1}; NMR (DMSO-d_6) δ 3.72 (2 H, broad s), 3.86 (3H, s), 4.09 (2H, s), 4.12 (1H, d, J=12.8 Hz), 4.34 (1H, d, J=12.8 Hz), 4.41 (2H, s), 5.15 (1H, d, J=4.8 Hz), 5.77 (1H, dd, J=4.8 and 8.8 Hz), 6.86 (1H, s), 7.21~7.56 (10H, m), 9.06 (1H, d, J=8.8 Hz).

The compound **13** (900 mg) was treated at 0°C with anisole (10 ml) and CF_3COOH (20 ml). After 30 minutes at 0°C, the solution was evaporated *in vacuo* and the residue was triturated in diisopropyl ether to give **16** (550 mg, 85%) as white powder. mp 183°C (dec.); IR (Nujol) 3200, 1780, 1690, 1610 cm^{-1}; NMR (DMSO-d_6) δ 3.70 (2H, s), 3.92 (3H, s), 4.06 (2H, s), 4.25 (2H, broad s), 4.39 (2H, s), 5.06 (1H, d, J=4.8 Hz), 5.66 (1H, dd, J=4.8 and 8.8 Hz), 9.30 (1H, d, J=8.8 Hz).

To an ice-cooled solution of **16** (100 mg) in water (2.5 ml) containing $NaHCO_3$ (51 mg), sodium acetate (51 mg) and thiourea (28 mg) was added and the mixture was stirred for 12 hours at room temperature. The solution was cooled in an ice bath, ajusted to pH 7.2 with $NaHCO_3$ and extracted with ethyl acetate. The aqueous layer was chromatographed on a column of Diaion HP-20 with water - MeOH as eluent. The fractions were collected and lyophilized to give **17** (32 mg). IR (Nujol) 3400, 1760, 1655 cm^{-1}; NMR (D_2O) δ 3.70 (2H, s), 3.90 (3H, s), 4.05 (2H, s), 4.12 (1H, d, J=13 Hz), 4.35 (1H, d, J=13 Hz), 5.10 (1H, d, J=4.8 Hz), 5.68 (1H, d, J=4.8 Hz).

Sodium 7-[2-(2-Chloroacetamido-1,3,4-thiadiazol-5-yl)-(*Z*)-2-methoxyiminoacetamido]-3-(1-methyl-1*H*-tetrazol-5-ylthiomethyl)ceph-3-em-4-carboxylate (**18**)

To a solution of (*Z*)-**9d** (66 mg), **12** (126 mg) and 1-hydroxybenzotriazole (32 mg) in DMF (1.5 ml), a solution of dicyclohexylcarbodiimide (49 mg) in CH_2Cl_2 (0.5 ml) was added at once under ice-cooling. The mixture was stirred for 2 hours at 0~5°C and the solid was filtered off. The filtrate was diluted with ethyl acetate, washed with $NaHCO_3$ solution, dried and evaporated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2 - acetone (7: 1) to give **14** (121 mg), mp 94~95°C (dec.); IR (Nujol) 3160, 1780, 1710~1720, 1660 cm^{-1}; NMR ($CDCl_3$) δ 3.75 (2H, broad s), 3.85 (3H, s), 4.28 (4H, broad s), 4.45 (3H, s), 5.09 (1H, d, J=4.8 Hz), 6.04 (1H, dd, J=4.8 and 9.6 Hz), 6.95 (1H, s), 7.25~7.55 (11H, m).

The compound **14** was treated with CF_3COOH according to the procedure described above for **16** to yield the free acid of **18** in 94.5% yield. mp 211~213°C (dec.). The free acid (100 mg) was dissolved in THF (10 ml) and treated at 0°C with a solution of sodium 2-ethylhexanoate (30 mg) in THF (1 ml). The solid was collected by centrifugation, washed with ethyl acetate and dried to yield **18** (62 mg, 60%). IR (Nujol) 3300, 1770, 1690 cm^{-1}; NMR (D_2O) δ 3.70 (2H, s), 3.88 (3H, s), 3.90 (3H, s), 4.25 (1H, d, J=10.4 Hz), 4.35 (1H, d, J=10.4 Hz), 4.46 (2H, s), 5.20 (1H, d, J=4.4 Hz), 5.82 (1H, d, J=4.4 Hz).

Sodium 7-[2-(2-Amino-1,3,4-thiadiazol-5-yl)-(*E*)-2-methoxyiminoacetamido]-3-(1-methyl-1*H*-tetrazol-5-ylthiomethyl)ceph-3-em-4-carboxylate (**19**)

By the use of the procedure described above for **14**, (*E*)-**9b** was coupled with **12** to give **15** in 74% yield. mp 121~123°C (dec.); IR (Nujol) 3300, 1780, 1750, 1690 cm^{-1}.

The compound **15** (200 mg) was treated with CF_3COOH according to the procedure described above for **16** to yield the CF_3COOH salt. The salt was dissolved in a mixture of water (3 ml) and ethyl acetate (10 ml) and the mixture was ajusted to pH 7.2 with $NaHCO_3$. The aqueous layer was chromatographed on a column of Diaion HP-20 with water as eluent. The fractions were collected and lyophilized to give **19** (70 mg, 60%). IR (Nujol) 3300, 1775, 1690 cm^{-1}; NMR (D_2O) δ 3.66 (2H, s), 3.91 (3H, s), 4.05 (3H, s), 4.25 (2H, s), 5.08 (1H, d, J=4.8 Hz), 5.75 (1H, d, J=4.8 Hz).

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