SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 6- AND 7-[2-(5-CARBOXYIMIDAZOLE-4-CARBOXAMIDO)PHENYLACETAMIDO]-PENICILLINS AND CEPHALOSPORINS

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The synthesis and antibacterial activity of a series of β-lactam antibiotics having a 5-carboxyimidazole-4-carbonyl group attached through an amide linkage to ampicillin, cephaloglycin or their analogs are described. Some compounds of this series were found to possess high activity against *Pseudomonas* and other Gram-negative bacteria.

At present the serious problems in chemotherapy are infectious diseases caused by various Gram-negative bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Proteus* spp. and *Serratia marcescens, etc.* It is known that modification of the amino group of ampicillin, cephaloglycin or their various analogs have led to compounds with improved antibacterial activity.[1)]

In our laboratory we have prepared a series of penicillins and cephalosporins which incorporate, as part of the side chain, the 5-carboxyimidazole-4-carbonyl group, which is attached through an amide linkage to ampicillin, cephaloglycin or their analogs. Some of these compounds have shown good activity against the above-mentioned Gram-negative bacteria, especially against strains of *Pseudomonas aeruginosa*.

In addition, we have prepared 5-ethoxycarbonylimidazole derivatives of the corresponding compounds and compared their antibacterial activity with that of 5-carboxy derivatives.

A member of this series, AC-1370 (**12**) has been selected for further biological and clinical evaluations, because of its high activity *in vivo*.

This report describes the synthesis and the preliminary microbiological evaluations of this series of penicillins and cephalosporins.

Chemistry

The synthesis of this series of penicillins and cephalosporins was carried out as outlined in Chart 1.

In 1975, MITSUHASHI *et al.* reported imidazole-4,5-dicarboxylic acid (**1**) reacted with thionyl chloride to give a cyclic dimerization product (**2**), which reacted with amines to produce diamides of **1**.[2)] However, a method to prepare the monoamides of **1** has not yet been reported.

In our research for suitable synthetic methods of monoamides, we found that two carbonyl chloride groups of **2** are selectively hydrolyzed by treatment with water at 10~40°C to give a novel diimidazopyrazine derivative (**3**). Since both carbonyl imidazolide moieties in **3** are activated, the compound (**3**) reacts effectively with two equivalent moles of amines to produce the corresponding monoamides.

Applying this reaction to phenylglycyl substituted β-lactam derivatives, 5-carboxyimidazole-4-carbonyl-ampicillin (**5**) and -cephaloglycin (**6**) have been prepared. The reaction of **3** with ampicillin or cephaloglycin proceeds in both water and organic solvents such as formamide or dichloromethane in the

Chart 1.

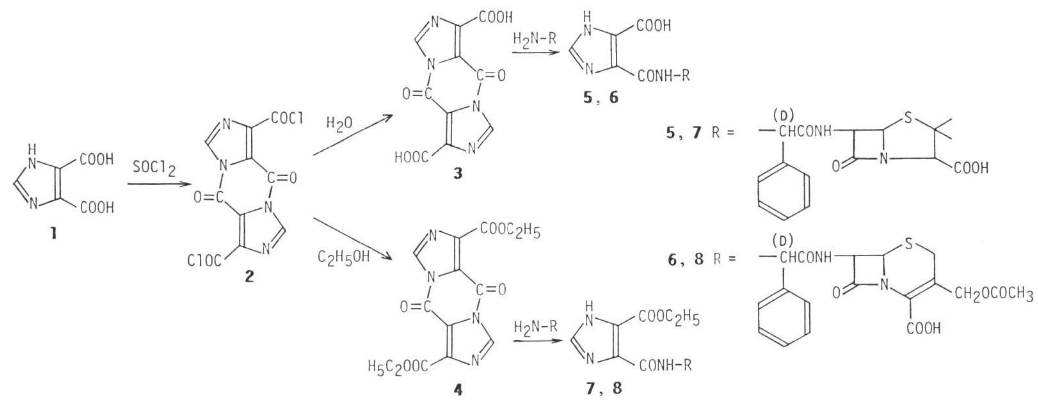
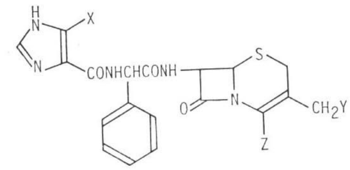
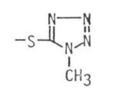
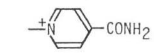
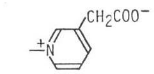
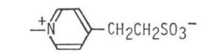
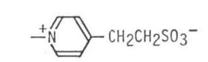


Table 1. C-3 analogs derived from the compound **6** or **8**.



Compound	X	Y	Z	Form
9	-COOH		-COOH	free acid
10	$-COO^-$		$-COO^-$	monopotassium salt
11	$-COO^-$		$-COO^-$	disodium salt
12 (AC-1370)	-COOH		$-COO^-$	monosodium salt
13	$-COOC_2H_5$		$-COO^-$	monosodium salt

presence of base at a low temperature (0~10°C).

A detailed report on the physical properties and further utilizations of **3** will be presented subsequently.

The synthesis of the 5-ethoxycarbonyl derivatives of the corresponding compounds, (**7**) and (**8**), can be performed in the same manner by using **4**, which was prepared by treating **2** with ethanol.

Nucleophilic displacement of the C-3 acetoxy group of **6** and **8** with the appropriate heterocyclic thiols and pyridine derivatives was achieved in the usual manner.[3,4,5] The prepared C-3 analogs (**9**~**13**) are presented in Table 1.

Antimicrobial Activity

The minimum inhibitory concentrations (MICs) of this series of penicillins and cephalosporins

against strains of clinically important bacteria are shown in Tables 2 and 3. The data show some compounds of this series have a broad spectrum of antibacterial activity, including good activity against *P. aeruginosa*.

In addition, the data also show the following relationships between structure and activity.

1) In the penicillin of this series the esterification of –COOH group on imidazole ring to $-CO-OC_2H_5$ reduced the activity against *P. aeruginosa*, but enhanced those against *Klebsiella pneumoniae* and *Enterobacter cloacae*. On the other hand, in the case of cephalosporins, this change showed a marked reduction in activity against Gram-negative bacteria in general.

2) The substituents at the C-3 position of cephalosporins showed a significant influence on anti-pseudomonal activity. The pyridinium groups enhanced the activity against *P. aeruginosa* in comparison with the acetoxy group or the 1-methyltetrazole thiol group.

3) With regard to the effect of substitution on the pyridinium ring the order of the activity is $-CH_2CH_2SO_3^- > -CH_2CO_2^- > -CONH_2$. The difference in the level of activity against Gram-negative bacteria among these compounds might be due to the difference in hydrophilicity or acidity, which were known to affect the penetrating ability of the molecule through the bacterial cell wall.[5,6]

Among all the compounds of this series, the compound **12** (AC-1370) showed the highest *in vitro* activity and it was selected for *in vivo* evaluations. The results of the *in vivo* tests are listed in Table 4 in comparison with cefoperazone. The data show AC-1370 has an excellent *in vivo* activity. The therapeutic activity of AC-1370 against *P. aeruginosa* was better than that of cefoperazone, contrary to the MIC value.

Table 2. Comparative *in vitro* activity (MIC, μg/ml) of the penicillins.

Organisms	**5**	**7**	Carbenicillin
Staphylococcus aureus 209P JC-1	3.13	1.56	0.78
Escherichia coli NIHJ JC-2	12.5	6.25	6.25
Klebsiella pneumoniae PCI 602	>100	12.5	50
Enterobacter cloacae ATCC 13047	100	12.5	6.25
Serratia marcescens IAM 1184	25	50	3.13
Proteus mirabilis IFO 3849	3.13	12.5	0.78
Proteus vulgaris OX-19	0.20	3.13	0.78
Pseudomonas aeruginosa IFO 3445	6.25	50	25
Psuedomonas aeruginosa ATCC 10145	12.5	100	50

Table 3. Comparative *in vitro* activity (MIC, μg/ml) of the cephalosporins.

Organisms	**6**	**8**	**9**	**10**	**11**	**12** (AC-1370)	**13**	Cefazolin	Cefoperazone
S. aureus 209P JC-1	3.13	1.56	3.13	6.25	12.5	6.25	12.5	0.20	1.56
E. coli NIHJ JC-2	6.25	50	6.25	6.25	3.13	1.56	100	0.78	0.10≧
K. pneumoniae PCI 602	0.39	0.78	0.39	1.56	0.39	0.20	3.13	0.78	0.10≧
E. cloacae ATCC 13047	12.5	100	12.5	6.25	3.13	1.56	>100	>100	0.10≧
S. marcescens IAM 1184	12.5	>100	12.5	12.5	3.13	1.56	>100	>100	0.78
P. mirabilis IFO 3849	6.25	100	25	25	3.13	1.56	100	12.5	0.78
P. vulgaris OX-19	6.25	1.56	0.20	3.13	0.78	0.20	1.56	12.5	0.10≧
P. aeruginosa IFO 3445	50	>100	100	12.5	3.13	3.13	>100	>100	3.13
P. aeruginosa ATCC 10145	100	>100	>100	25	6.25	6.25	>100	>100	6.25

Table 4. Comparative *in vivo* activity (ED_{50} values) in mice.

Organisms	Challenge dose (cells/mouse)	Drug	MIC (μg/ml)	ED_{50} (mg/kg)
E. coli 4	1.3×10^6	**12** (AC-1370)	0.39	2.25
		Cefoperazone	0.20	2.77
P. aeruginosa 61	4.6×10^3	**12** (AC-1370)	3.13	7.29
		Cefoperazone	1.56	41.2

A more detailed evaluation on AC-1370 will be presented in future reports.

Experimental

Infrared spectra were measured on a Shimadzu IR-430 spectrophotometer or Digilab STS-15E spectrophotometer (FT-IR). NMR spectra were measured on a Varian EM-390 (90 MHz) or T-60 (60 MHz) spectrometer using TMS or DSS as internal standard. MS spectra were measured on a JEOL DX-300. UV spectra were done on a Hitachi 220 double beam spectrophotometer. $[\alpha]_D$ was measured on a Perkin Elmer 241 polarimeter.

Minimum Inhibitory Concentrations

The MIC's of penicillins and cephalosporins were determined in two fold dilution by the agar dilution method. Overnight cultures of the test organisms in sensitivity test broth were diluted to a final concentration of 10^6 cells per ml and one loopful of suspension was inoculated onto a sensitivity test agar plate. The MIC was determined after incubation at 37°C for 18 hours.

Protection Tests

Protection tests were done in male ICR mice, weighing 19~21 g. Acute toxicity (LD_{50}) of AC-1370 (**12**) was 2.7 g/kg (i.v.) and 8.2 g/kg (s.c.) in the same mice. Mice were challenged by the intraperitoneal route with the test organisms suspended in 0.5 ml of 5% hog gastric mucin. Animals were treated by subcutaneous administration at 1 hour after challenge. Ten mice were used for each dose level. The 50% effective dose (ED_{50} in mg/kg) was calculated from the survival rate on 5 days by VAN DER WAERDEN method.

5,10-Dioxo-5*H*,10*H*-diimidazo[1,5-*a*: 1′,5′-*d*]pyrazine-1,6-dicarbonyl Dichloride (**2**)

By a modified procedure of the reported method,[2] **2** was prepared as follows. To a suspension of imidazole-4,5-dicarboxylic acid (**1**) (7.8 g, 50 mmole) in dry benzene (100 ml) containing DMF (4 ml), thionyl chloride (30 ml) was added and the mixture was stirred at 85°C for 6 hours under reflux. After evaporation of the solvent, dry benzene (50 ml) was added to residue and the mixture was concentrated *in vacuo* to give a solid material. To the residue, benzene (50 ml) was added and the mixture was stirred at room temperature for 30 minutes. The insoluble material was collected by filtration, washed with benzene, and dried to give 7.0 g (89%) of **2**.

Anal. Calcd. for $C_{10}H_2N_4O_4Cl_2$: C 38.36, H 0.64, N 17.90, Cl 22.65.
Found: C 38.03, H 0.74, N 17.81, Cl 22.37.

5,10-Dioxo-5*H*,10*H*-diimidazo[1,5-*a*: 1′,5′-*d*]pyrazine-1,6-dicarboxylic Acid Dihydrate (**3**)

A suspension of **2** (62.6 g, 200 mmole) in water (800 ml) was stirred at 40°C for 6 hours. An insoluble solid was collected by filtration and washed with water (100 ml) five times and then acetone (100 ml) five times. The material was dried under vacuum to give 60.8 g (97%) of **3**; mp 284°C (dec.), IR (Nujol): 3500, 1750, 1710, 1255, 930 cm^{-1}.

Anal. Calcd. for $C_{10}H_4N_4O_6\cdot2H_2O$: C 38.47, H 2.58, N 17.95.
Found: C 38.65, H 2.40, N 18.02.

5,10-Dioxo-5*H*,10*H*-diimidazo[1,5-*a*: 1′,5′-*d*]pyrazine-1,6-dicarboxylic Acid Diethylester (**4**)

A suspension of **2** (7.0 g, 22.4 mmole) in ethanol (150 ml) was stirred at room temperature overnight. An insoluble solid was collected by filtration, washed with ethanol and then ether, and dried under vacuum to give 7.0 g (94%) of **4**; FD-MS: M^+ at m/z 332 (mol. wt. 332).

Anal. Calcd. for $C_{14}H_{12}N_4O_6$: C 50.60, H 3.65, N 16.86.
Found: C 50.55, H 3.63, N 16.88.

(2*S*,5*R*,6*R*)-6-[(*R*)-2-(5-Carboxy-1*H*-imidazole-4-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid (**5**)

To an ice-cooled suspension of anhydrous ampicillin (5.6 g, 16 mmole) in dry CH_2Cl_2 (100 ml), triethylamine (6 ml) was added and the mixture was stirred for 30 minutes under ice-cooling. To this solution, **3** (2.2 g, 7 mmole) was added with stirring and cooling. After stirring overnight the reaction mixture was concentrated to give a solid material. This material was dissolved in water (50 ml). The solution was adjusted to pH 8 with 6% HCl, stirred for 10 minutes and washed with ethyl acetate (50 ml). The water phase was adjusted to pH 2 with 6% HCl and stirred for 20 minutes. The precipitate was collected by filtration, washed with water and dried under vacuum at 40°C. The solid thus obtained was suspended in a mixture of ethyl acetate - methanol (1 : 1) and the mixture was stirred for 20 minutes at 40°C. After insoluble material was removed by filtration, the solution was concentrated *in vacuo* to a volume of 50 ml and ether (500 ml) was added thereto. This mixture was kept overnight in a refrigerator. A precipitate was recovered, washed with petroleum ether and dried to give 5.9 g (74%) of **5**: IR (Nujol): 1773 cm^{-1} (β-lactam); NMR (DMSO-d_6+D_2O (trace)): δ 1.42 (3H, s, $-CH_3$), 1.52 (3H, s, $-CH_3$), 4.24 (1H, s, C_2-H), 5.41 (1H, d, C_5-H), 5.56 (1H, d, C_6-H), 5.90 (1H, s, Ph-C*H*–CO–), 7.43 (5H, m, Ph-H), 8.90 (1H, s, imidazole C_2-H).

Anal. Calcd. for $C_{21}H_{21}N_5O_7S \cdot 0.5H_2O$: C 50.79, H 4.47, N 14.11, S 6.46.
Found: C 50.77, H 4.51, N 14.00, S 6.35.

(6*R*,7*R*)-3-Acetoxymethyl-7-[(*R*)-[2-(5-carboxy-1*H*-imidazole-4-carboxamido)-2-phenyl]acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**6**)

Cephaloglycin (12.2 g, 30 mmole) was suspended in water (50 ml) and the pH of the suspension was adjusted carefully to 8.5 with 13.5% NaOH under ice-cooling. To this solution, **3** (9.36 g, 30 mmole) was added in limited amounts with stirring and cooling, maintaining the pH at 7.30 ~ 7.60 by adding 13.5% NaOH. After the reaction mixture was stirred for 40 minutes under ice-cooling, the pH of the mixture was adjusted to 6.3 with 6% HCl. After stirring for 10 minutes the precipitate was removed by filtration and the filtrate was adjusted to pH 2 with 6% HCl. The obtained solid was collected by filtration, washed with water, and dried under vacuum. The solid thus obtained was purified by a similar manner to that used for compound **5** to give 12.5 g (75%) of **6**; IR (Nujol): 1775 (β-lactam), 1740 ($-OCOCH_3$), 1240 cm^{-1} ($-OCOCH_3$); NMR (DMSO-d_6): δ 2.02 (3H, s, $-OCOCH_3$), 3.37, 3.52 (each 1H, two sets of doublet, C_4-H), 4.69, 4.97 (each 1H, two sets of doublet, C_3-CH_2–), 5.04 (1H, d, C_6-H), 5.8 (2H, m, C_7-H+Ph-C*H*–), 7.4 (5H, m, Ph-H), 8.01 (1H, s, imidazole C_2-H), 9.52 (2H, m, –CONH–).

Anal. Calcd. for $C_{23}H_{21}N_5O_9S \cdot 0.7H_2O$: C 49.67, H 4.07, N 12.60, S 5.76.
Found: C 49.66, H 3.98, N 12.77, S 5.66.

(2*S*,5*R*,6*R*)-3,3-Dimethyl-6-[(*R*)-2-(5-ethoxycarbonyl-1*H*-imidazole-4-carboxamido)-2-phenylacetamido]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid (**7**)

By a similar procedure to that used for compound **5**, anhydrous ampicillin (2.8 g, 8 mmole) was allowed to react with **4** (1.3 g, 4 mmole) to give 3.0 g (69%) of **7**; IR (Nujol): 1770 (β-lactam), 1730 cm^{-1} ($-CO_2C_2H_5$); NMR (DMSO-d_6); δ 1.33 (3H, t, $-COOCH_2CH_3$), 1.43 (3H, s, $-CH_3$), 1.57 (3H, s, $-CH_3$), 4.20 (1H, s, C_2-H), 4.37 (2H, q, $-COOCH_2CH_3$), 5.30 ~ 5.70 (2H, m, C_5-H and C_6-H), 5.98 (1H, d, Ph-C*H*–CO–), 7.40 (5H, m, Ph-H), 7.83 (1H, s, imidazole C_2-H), 9.30 (1H, d, –CONH–), 10.60 (1H, bd, –CONH–).

Anal. Calcd. for $C_{25}H_{25}N_5O_7S \cdot 1.3H_2O$: C 51.08, H 5.19, N 12.95, S 5.93.
Found: C 51.07, H 4.84, N 12.97, S 5.89.

(6*R*,7*R*)-3-Acetoxymethyl-7-[(*R*)-[2-(5-ethoxycarbonyl-1*H*-imidazole-4-carboxamido)-2-phenyl]-acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**8**)

By a similar procedure to that used for compound **5**, cephaloglycin (3.2 g, 7.9 mmole) was allowed to react with **4** (1.3 g, 4 mmole) to give 3.4 g (73%) of **8**; IR (Nujol): 1780 (β-lactam), 1740 ~ 1720 (ester), 1230 cm^{-1} ($-OCOCH_3$); NMR (DMSO-d_6+D_2O (trace)): δ 1.21 (3H, t, $-COOCH_2CH_3$), 2.02 (3H, s, $-OCOCH_3$), 3.10 (2H, q, $-COCH_2CH_3$), 3.38, 3.54 (each 1H, two sets of doublet, C_4-H), 4.70, 4.95

(each 1H, two sets of doublet, C_3-CH_2–), 5.03 (1H, d, C_6-H), 5.72 (1H, d, C_7-H), 5.83 (1H, s, Ph-C*H*–), 7.32 (5H, m, Ph-H), 8.00 (1H, s, imidazole C_2-H).

Anal. Calcd. for $C_{25}H_{25}N_5O_9S \cdot 1.2H_2O$: C 50.61, H 4.66, N 11.81, S 5.40.
Found: C 50.34, H 4.51, N 11.76, S 5.95.

(6*R*,7*R*)-7-[(*R*)-[2-(5-Carboxy-1*H*-imidazole-4-carboxamido)-2-phenyl]acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**9**)

To a suspension of **6** (0.77 g, 1.4 mmole) and 1-methyl-5-mercapto-1*H*-tetrazole (0.15 g, 1.3 mmole) in a pH 6.4 phosphate buffer (10 ml), 2 N NaOH was added to adjust to pH 6.4. After stirring for 24 hours at 60°C, water (20 ml) was added to the reaction mixture and the solution was adjusted to pH 7 with 2 N NaOH. The aqueous solution was washed with ethyl acetate (30 ml). To the aqueous layer ethyl acetate (50 ml) was added and the aqueous layer was brought to pH 2 with 6% HCl. After the insoluble material formed was removed by filtration, the organic layer was separated. The aqueous layer was extracted with ethyl acetate (50 ml). The combined extracts were washed with water, dried ($MgSO_4$) and evaporated *in vacuo* below 30°C. The residue was triturated with ether. The triturated material was isolated by filtration and dried to give 0.16 g (19%) of **9**; IR (Nujol): 1775 cm^{-1} (β-lactam); NMR (DMSO-d_6): δ 3.60 (2H, m, C_4-H), 3.87 (3H, s, tetrazole N-CH_3), 4.26 (2H, bs, C_3-CH_2–), 5.02 (1H, d, C_6-H), 5.78 (3H, m, C_7-H+Ph-C*H*–), 7.43 (5H, m, Ph-H), 8.03 (1H, s, imidazole C_2-H).

In spite of attempts of purification, this compound was not obtained in an analytically pure form and analyses (C, H, N, S) were within ±2.3%.

4-Carbamoyl-1-[[(6*R*,7*R*)-2-carboxy-7-[(*R*)-[2-(5-carboxy-1*H*-imidazole-4-carboxamido)-2-phenyl]-acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]pyridinium Hydroxide, Inner Salt, Monopotassium Salt (**10**)

To a solution of **6** (disodium salt, 0.59 g, 1 mmole) and isonicotinamide (0.24 g, 2 mmole) in water (5 ml), 2 N NaOH was added to adjust to pH 7. To this solution potassium iodide (8.3 g) was added and this solution was stirred for 2 hours at 70°C. The reaction mixture was chromatographed on Amberlite XAD-2 (450 ml). After isonicotinamide was eluted with water, the cephalosporin fraction, which was eluted with a mixture of water and methanol (1 : 1), was collected. After methanol was evaporated therefrom *in vacuo*, the resultant water solution was lyophilized to give 0.09 g (13%) of **10**; IR (Nujol): 1770 cm^{-1} (β-lactam); NMR (D_2O): δ 3.10 (2H, m, C_4-H), 5.10 (1H, d, C_6-H), 5.48 (2H, m, C_3-CH_2–), 5.67 (2H, m, C_7-H+Ph-C*H*–), 7.31 (5H, m, Ph-H), 7.73 (1H, s, imidazole C_2-H), 8.20 (2H, d, pyridinium $C_{3,5}$-H), 9.00 (2H, d, pyridinium $C_{2,6}$-H).

This compound was found to contain a small quantity of monosodium salt by elemental analyses as follows,

Anal. Calcd. for $C_{27}H_{22}N_7O_8SK_{0.78}Na_{0.22} \cdot 3H_2O$: C 46.71, H 4.07, N 14.13, S 4.62, K 4.39, Na 0.73.
Found: C 47.27, H 3.88, N 13.79, S 4.75, K 3.58, Na 0.58.

1-[(6*R*,7*R*)-2-Carboxy-7-[(*R*)-[2-(5-carboxy-1*H*-imidazole-4-carboxamido)-2-phenyl]acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl-3-carboxymethylpyridinium Hydroxide, Inner Salt, Disodium Salt (**11**)

By a similar procedure to that used for compound **10**, **6** (disodium salt, 1.17 g, 2 mmole) was allowed to react with 3-pyridylacetic acid (0.55 g, 4 mmole) in the presence of sodium iodide (8.3 g) to give 0.17 g (11%) of **11**; IR (Nujol): 1765 cm^{-1} (β-lactam); NMR (D_2O): δ 3.04, 3.49 (each 1H, two sets of doublet, C_4-H), 3.77 (2H, s, –C*H*$_2$COOH), 5.12 (1H, d, C_6-H), 5.30, 5.50 (each 1H, two sets of doublet, C_3-CH_2–), 5.63 (1H, s, Ph-C*H*–), 5.80 (1H, d, C_7-H), 7.50 (5H, m, Ph-H), 7.82 (1H, s, imidazole C_2-H), 7.90~8.90 (4H, m, pyridinium-H).

Anal. Calcd. for $C_{28}H_{22}N_6O_9SNa_2 \cdot 4.5H_2O$: C 45.10, H 4.20, N 11.27, S 4.30, Na 6.17.
Found: C 45.30, H 4.05, N 11.44, S 4.41, Na 6.1.

AC-1370: 1-[(6*R*,7*R*)-2-Carboxy-7-[(*R*)-[2-(5-carboxy-1*H*-imidazole-4-carboxamido)-2-phenyl]-acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl-4-(2-sulfoethyl)pyridinium Hydroxide, Inner Salt, Monosodium Salt (**12**)

To a suspension of **6** (7.13 g, 12.8 mmole) and 4-pyridineethanesulfonic acid (4.9 g, 26.3 mmole) in

water (30 ml), 2 N NaOH was added to dissolve them and adjust to pH 6.5. After sodium iodide (87.5 g) was added, the mixture was stirred at 65°C for 70 minutes. The reaction solution was cooled and added dropwise to acetone (330 ml) under ice-cooling and stirring. After the mixture was cooled overnight, a solid product was collected by filtration. This solid was dissolved in water and reprecipitated by adding to acetone. This reprecipitation was repeated once more using water and ethanol. Then the reprecipitate was dissolved in water (400 ml) at 30°C and 6 N HCl was added to adjust to pH 2. After being stirred for 30 minutes, a precipitate was removed by centrifugation. The mother liquor was adjusted to pH 4 with 2 N NaOH and concentrated to 80 ml *in vacuo* below 30°C. Ethanol (400 ml) was added to the residue and the precipitate was collected by filtration. The solid thus obtained was dissolved in water (40 ml) and the solution was adjusted to pH 3.3 with 2 N HCl. The solution was chromatographed on Amberlite XAD-7 (120 ml) with elution by water. The fraction containing the desired product was collected and concentrated *in vacuo*. After the concentrate was adjusted to pH 3.2 with 2 N HCl, acetone (300 ml) was added thereto under cooling and stirring. The precipitate was collected by filtration and dried under vacuum. The solid thus obtained was dissolved in water and lyophilized to give 2.7 g (28%) of **12**; IR (KBr) 1765 (β-lactam), 1044 cm^{-1} ($-SO_3^-$); $[\alpha]_D^{20}$ $-28.2°$ (*c* 0.5, H_2O)*, UV max (H_2O) 257 nm (ε 22400)*; FD-MS: $[M+H]^+$ at *m/z* 671 (mol. wt. 670, free form**); NMR (D_2O): δ 3.01, 3.46 (each 1H, two sets of doublet, J=18 Hz, C_4-H), 3.36 (4H, s, $-CH_2CH_2SO_3^-$), 5.09 (1H, d, J=5 Hz, C_6-H), 5.24, 5.44 (each 1H, two sets of doublet, J=15 Hz, C_3-CH_2–), 5.59 (1H, s, Ph-CH–), 5.73 (1H, d, J=5 Hz, C_7-H), 7.43 (5H, m, Ph-H), 7.97 (2H, d, J=7 Hz, pyridinium $C_{3,5}$-H), 8.65 (1H, s, imidazole C_2-H), 8.76 (2H, d, J=7 Hz, pyridinium $C_{2,6}$-H).

Anal. Calcd. for $C_{28}H_{25}N_6O_{10}S_2Na \cdot 3.6H_2O$: C 44.39, H 4.29, N 11.10, S 8.46, Na 3.03.
Found: C 44.02, H 4.01, N 11.16, S 8.91, Na 3.26.

1-[(6*R*,7*R*)-2-Carboxy-7-[(*R*)-[2-(5-ethoxycarbonyl-1*H*-imidazole-4-carboxamido)-2-phenyl]acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl-4-(2-sulfoethyl)pyridinium Hydroxide, Inner Salt, Monosodium Salt (**13**)

By a similar procedure to that used for compound **10**, **8** (1.7 g, 2.9 mmole) was allowed to react with 4-pyridineethanesulfonic acid (1.1 g, 6 mmole) in the presence of sodium iodide (12 g) to give 0.59 g (25%) of **13**; IR (Nujol): 1765 (β-lactam), 1210 ($-SO_2$–), 1045 cm^{-1} ($-SO_3^-$); NMR (D_2O): δ 1.33 (3H, t, $-COOCH_2CH_3$), 3.20 (2H, m, C_4-H), 3.36 (4H, s, $-CH_2CH_2SO_3^-$), 4.26 (2H, q, $-COOCH_2CH_3$), 5.06 (1H, d, C_6-H), 5.40 (2H, m, C_3-CH_2–), 5.66 (1H, s, Ph-CH–), 5.76 (1H, d, C_7-H), 7.42 (5H, m, Ph-H), 7.89 (1H, s, imidazole C_2-H), 8.00 (2H, d, pyridine $C_{3,5}$-H), 8.85 (2H, d, pyridine $C_{2,6}$-H).

Anal. Calcd. for $C_{30}H_{27}N_6O_{10}S_2Na \cdot 4.9H_2O$: C 44.64, H 4.61, N 10.42, S 7.95.
Found: C 45.04, H 4.64, N 10.42, S 7.10.

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* on the anhydrous basis.

** Free form of AC-1370 was prepared in the following manner: A water solution of monosodium salt was treated with a large excess of Dowex 50W (H^+ form) and after the resin was removed, the mother liquor was lyophilized.

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