[Chem. Pharm. Bull. 31(7)2200—2208(1983)]

Synthesis and Antibacterial Activity of 3-Acylamino-3-methoxy-2-azetidinone-1-sulfonic Acid Derivatives[1)]

TAISUKE MATSUO (the late), HIROTOMO MASUYA, TOHRU SUGAWARA, YASUHIKO KAWANO, NORIYOSHI NOGUCHI and MICHIHIKO OCHIAI*

Central Research Division, Takeda Chemical Ind., Ltd., Juso, Yodogawa-ku, Osaka 532, Japan

(Received November 12, 1982)

As a key intermediate for the synthesis of sulfazecin derivatives, 3-amino-3-methoxy-2-azetidinone (**24** or **26**) was synthesized from penicillins, and various new compounds, including sulfazecin, were synthesized by acylation and sulfonation of **24** or **26**. Some of these compounds (**33**, **36**) showed higher antibacterial activity than the corresponding 3-demethoxy derivatives against a β-lactamase-producing strain of *Escherichia coli*.

Keywords——sulfazecin; monocyclic β-lactam; sulfonation; acylation; 3-amino-3-methoxy-2-azetidinone; desulfurization; antibacterial activity

In the preceding paper[2)] the synthesis and antibacterial activity of 3-acylamino-2-azetidinone-1-sulfonic acid derivatives were reported, and it was demonstrated that some of the compounds synthesized showed potent antibacterial activity, especially against Gram-negative bacteria. Sulfazecin has a methoxy group at its 3α-position. In addition, it is well known that the introduction of a methoxy substituent at the 7α-position of cephalosporins increases stability to β-lactamases.[3)] Therefore, we thought it would be interesting to introduce a methoxy group into the 3-position of 3-acylamino-2-azetidinone-1-sulfonic acid derivatives. 3-Amino-3-methoxy-2-azetidinone (**24** or **26**), a key intermediate, was synthesized from penicillins, and various new compounds were synthesized by acylation and sulfonation of **24** or **26**. In this paper, the synthesis and antibacterial activity of some 3-acylamino-3-methoxy-2-azetidinone-1-sulfonic acids will be described.

As shown in Chart 1, the penicillin derivative **1** was converted into the thiazoline derivative **3** by applying the methoxylation reaction reported by Baldwin[4a)] or Koppel,[4b)] followed by treatment with trimethyl phosphite and then with triethylamine. The symmetrical disulfide derivative (**4**) was easily prepared by the treatment of **3** with iodine[5)] or by the treatment of **6**[2)] with *tert*-butyl hypochlorite-lithium methoxide. Desulfurization of **4** with Raney nickel afforded (3*R*)-3-methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-3-phenylacetamido-2-azetidinone (**9**). Similar treatment of the methylthio derivative (**8**), which was obtained from thiazoline derivative (**5**) by ring-opening[6)] and methoxylation reactions, also afforded **9**.

In order to remove the *N*-substituent, **9** was ozonized to yield the methoxalyl derivative (**10**). However, several attempts to remove this methoxalyl group by treatment with a catalytic amount of sodium methoxide in methyl alcohol under various conditions[7)] were unsuccessful. The only product in these reactions was a β-lactam-cleaved compound (**11**). This seemed to be due to the instability of the β-lactam ring of **10**, which showed an infrared (IR) absorption band at 1803 cm^{-1}. From a comparison of the 3-demethoxy compounds **13** (1765 cm^{-1}) and **14** (1738 cm^{-1}),[2)] the 3-benzyloxycarbonylamino group was expected to stabilize the β-lactam ring (Chart 2). We then investigated the reaction with the 3-benzyloxycarbonylamino

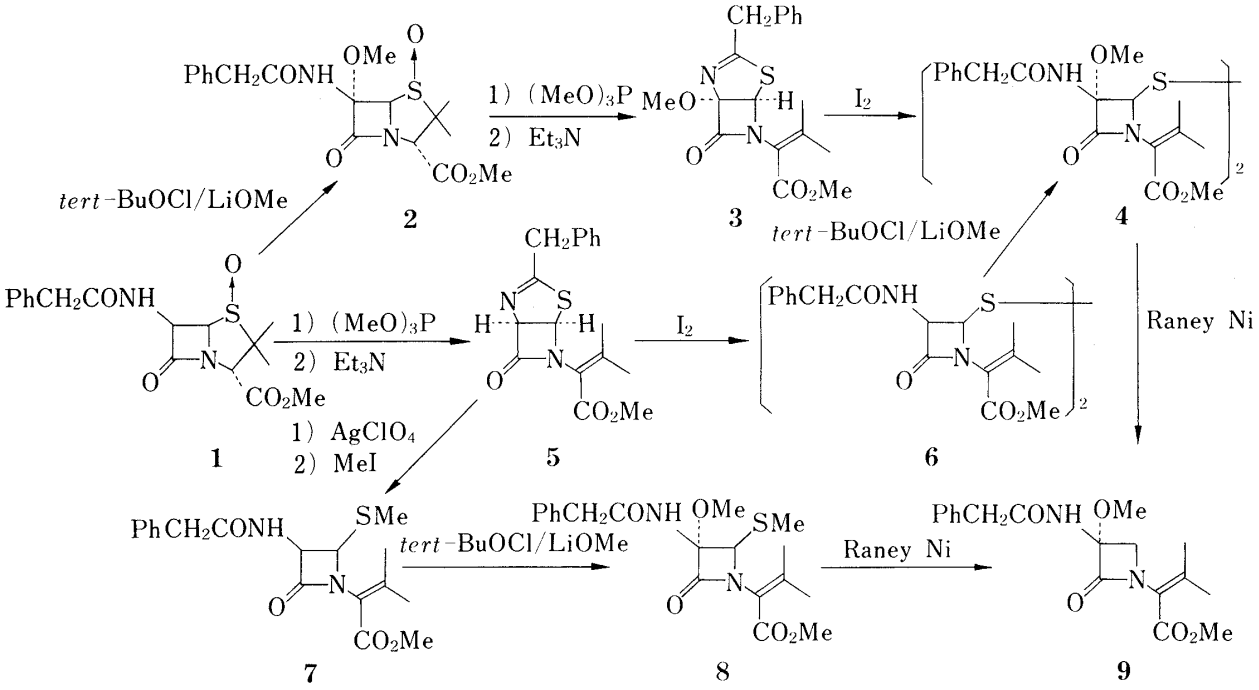


Chart 1

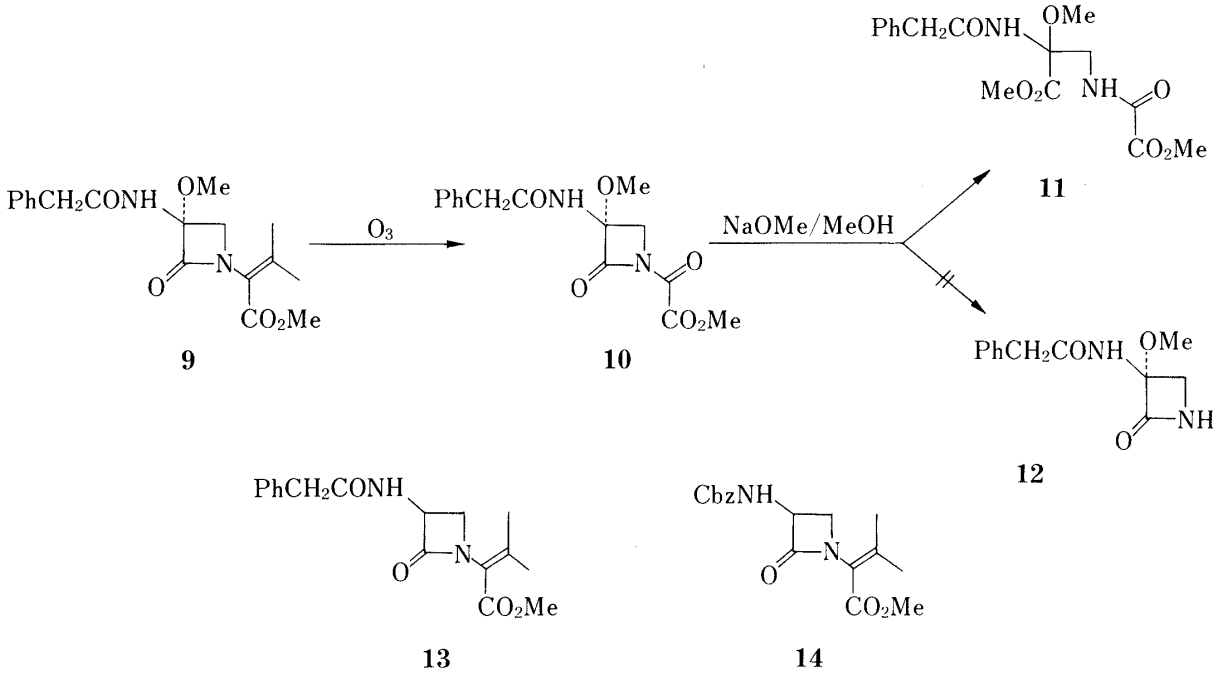


Chart 2

compound (**15**), which was prepared by a reaction similar to that reported by Morin *et al.*,[8] to obtain (3*R*)-3-benzyloxycarbonylamino-3-methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-2-azetidinone (**21**).

The methoxythiazoline derivative (**17**) could not be obtained by the treatment of **16**, which was prepared by a reaction similar to that reported by Koppel *et al.*,[4b] with trimethyl phosphite. Therefore, **21** was synthesized by the sequence of reactions shown in Chart 3.

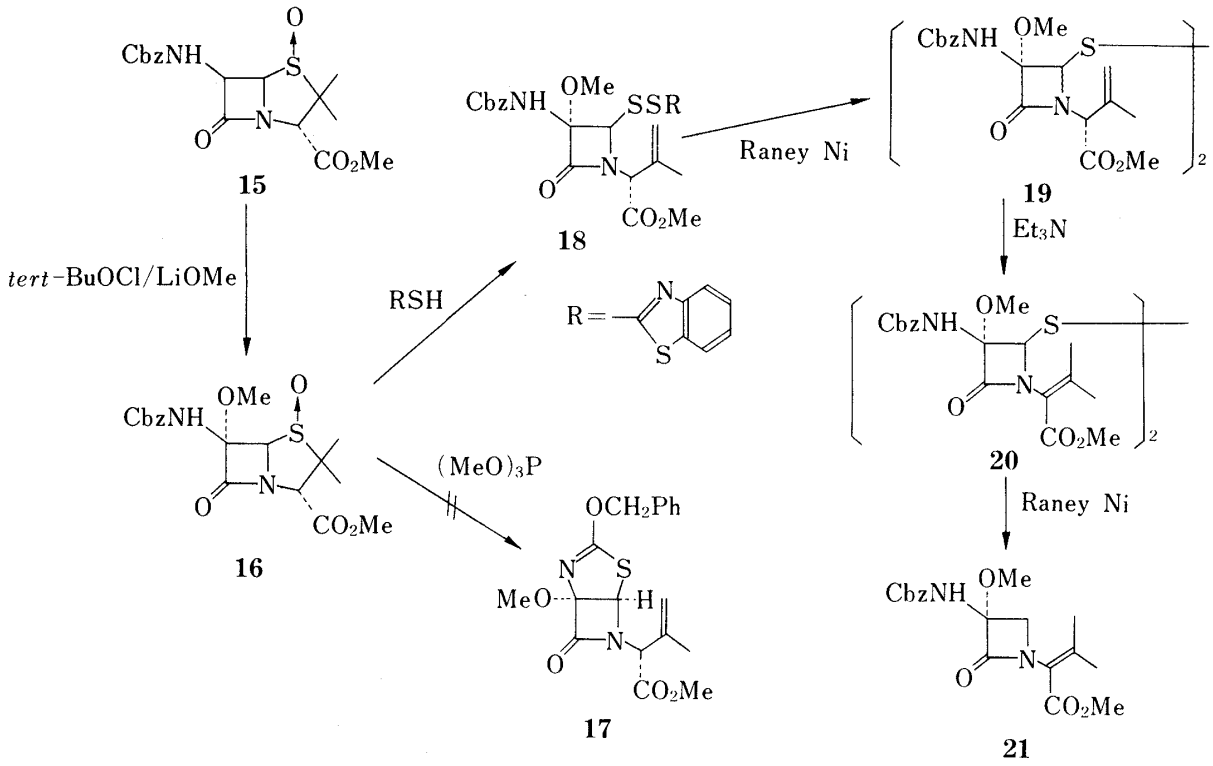
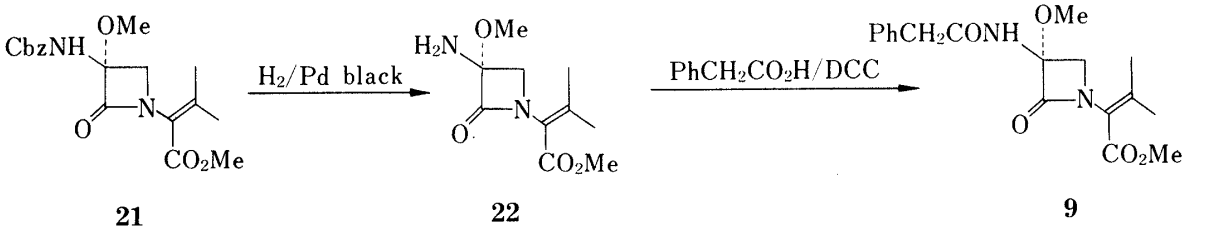
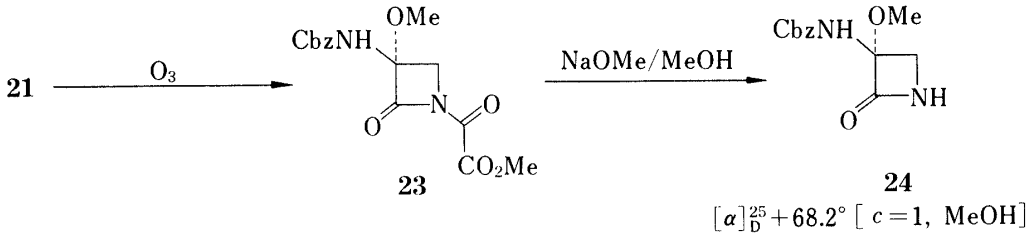


Chart 3





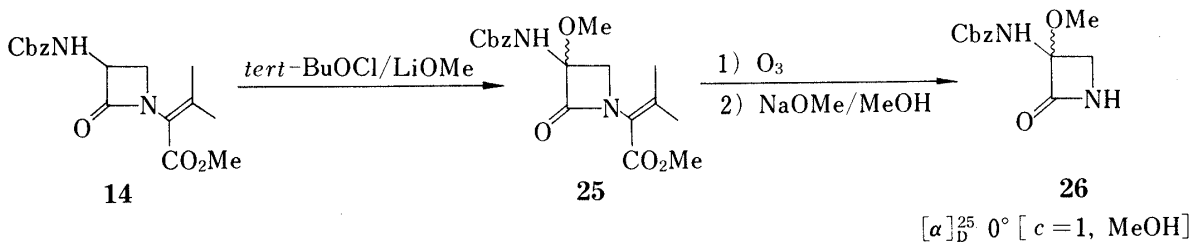


Chart 4

Retention of optical activity of **21** was confirmed by the following reactions (*cf.* Chart 4). Removal of the benzyloxycarbonyl group from **21** by catalytic hydrogenation, followed by treatment with phenylacetic acid and dicyclohexylcarbodiimide (DCC) afforded **9**, which was identical (including optical rotation) with the sample already obtained by the reactions shown in Chart 1.

Ozonolysis of **21**, followed by treatment with a methanol solution of a catalytic amount of sodium methoxide, provided (3*R*)-3-benzyloxycarbonylamino-3-methoxy-2-azetidinone (**24**). By similar reactions, the *dl*-derivative (**26**) was prepared starting from (3*S*)-3-benzyloxycarbonylamino-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-2-azetidinone (**14**),[2] as shown in Chart 4.

(3*R*)-3-(D-γ-Glutamyl-D-alanylamino)-3-methoxy-2-azetidinone-1-sulfonic acid (**31**, sulfazecin) was prepared by means of the sequence of reactions shown in Chart 5. In order to prevent racemization of the acyl groups,[9] stepwise condensation of the amino acid units was carried out by an application of Miyoshi's method.[10] Compound **28**, which was prepared from (3*R*)-3-amino-3-methoxy-2-azetidinone (**27**) and *N*-benzyloxycarbonyl-D-alanine activated with diphosgene-triethylamine,[10] was treated with palladium black and then with a D-glutamyl unit activated in the same manner as above to obtain **29**. The azetidinone derivative (**29**) was converted into the pyridinium salt (**30**) by sulfonation with sulfur trioxide-pyridine complex. Deprotection and subsequent purification by chromatography afforded sulfazecin (**31**). Comparison of the spectral data (nuclear magnetic resonance (NMR), IR and $[\alpha]_D$) and thin layer and paper chromatographic behavior of the synthetic sulfazecin with those of the natural product[11] established the identity.

Some (3*R*)- and (3*S*)-3-acylamino-3-methoxy-2-azetidinone-1-sulfonic acids were syn-

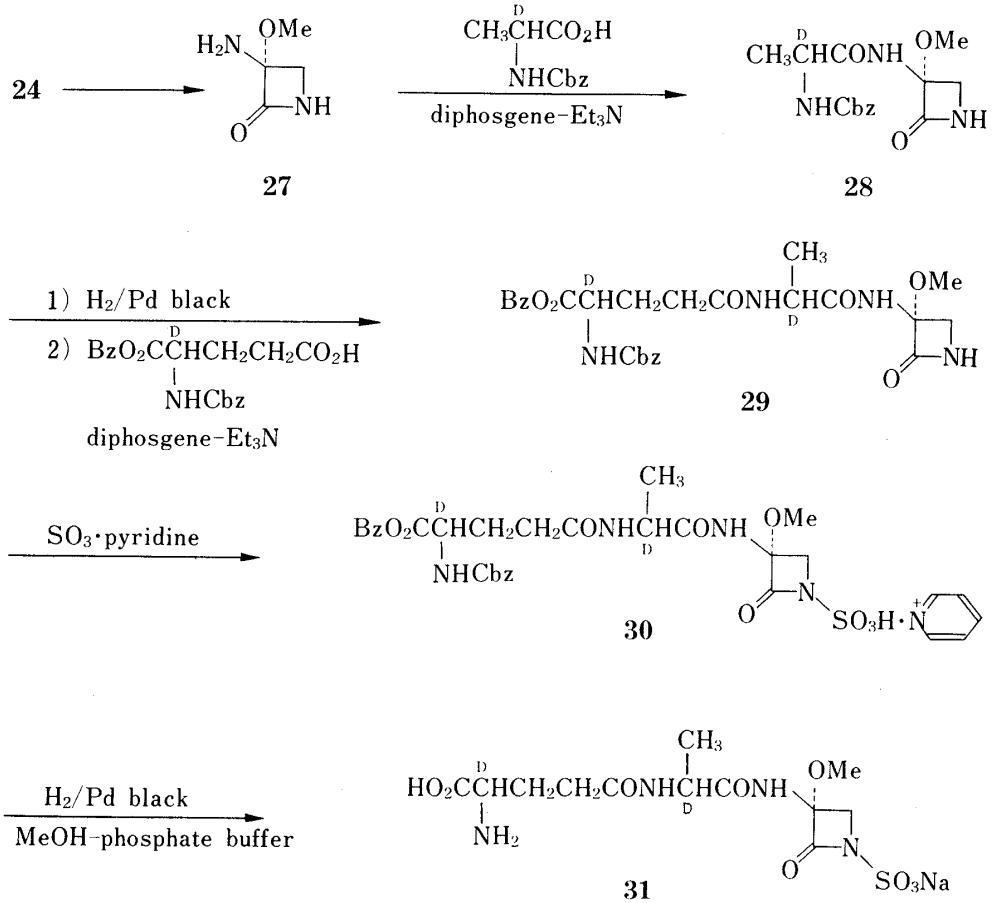
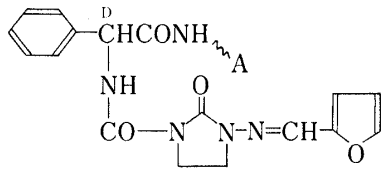


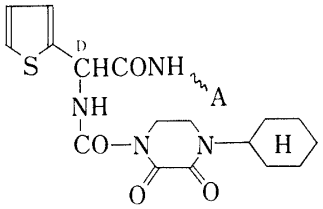
Chart 5

TABLE I. *In Vitro* Antibacterial Activity of 3-Acylamino-3-methoxy-2-azetidinone-1-sulfonic Acid Derivatives

(MIC: μg/ml)

Compound





Organism	A:	32	33	34	35	36	37
Escherichia coli O-111		3.13	6.25	25	0.2	3.13	25
Escherichia coli T-7		>100	12.5	50	>100	50	>100
Klebsiella pneumoniae DT		0.2	12.5	50	0.39	6.25	50

Inoculum size: 10^8 CFU/ml.

thesized by using the *dl*-derivative (**26**) as a starting material. A typical example is as follows. Removal of the benzyloxycarbonyl group from **26** gave the *dl*-3-amino-3-methoxy-2-azetidinone, which was acylated with an acid chloride possessing a chiral center[12] to yield a mixture of two diastereomers. These two diastereomers were easily separated by chromatography on silica gel (Table I, **33**, **34**, **36** and **37**). The configuration of the methoxy group of the product (**33** or **36**) was confirmed by comparison of the spectral data and thin layer chromatogram with those of the 3α-methoxy derivative obtained from the optically active 3-amino-3-methoxy-2-azetidinone (**24**) and the corresponding acid chloride.

The *in vitro* antibacterial activity of these new compounds against some bacteria is shown in Table I. The 3α-methoxy compounds (**33** and **36**) have improved activity against a β-lactamase-producing strain (*Escherichia coli* T-7). The 3β-isomers (**34** and **37**) showed lower activity than the corresponding 3α-isomers (Table I).

Experimental

All melting points were taken with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured on a Hitachi type 260-10 spectrophotometer. ^{1}H-NMR spectra were measured on a Varian HA-100 or T-60 spectrometer with tetramethylsilane as an internal standard. Abbreviations are as follows: s = singlet; br s = broad singlet; d = doublet; dd = doublet of doublets; t = triplet; q = quartet. The optical rotations were recorded with a JASCO DPI-181 digital polarimeter. Column chromatography was carried out on Kiesel G (0.05—0.2 mm, Merck).

Bis[(3*S*,4*R*)-3-methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-2-oxo-3-phenylacetamidoazetidin-4-yl] Disulfide (4)——(a) A solution of iodine (0.148 g) in tetrahydrofuran (THF) (0.5 ml) was added dropwise to a solution of the thiazoline derivative (**3**, 0.42 g) in THF (3 ml)–water (0.6 ml) at room temperature. The mixture was stirred for 3 h, then aqueous $Na_2S_2O_3$ solution was added. The reaction mixture was extracted with CH_2Cl_2 and the organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The crude product was chromatographed on silica gel. Elution with *n*-hexane–AcOEt (1 : 1) afforded **4** (0.3 g, 68.2%) as a foam. *Anal.* Calcd

for $C_{36}H_{42}N_4O_{10}S_2$: C, 57.28; H, 5.61; N, 7.42. Found: C, 57.44; H, 5.40; N, 7.41. $[\alpha]_D^{25}$ −71.2° ($c=1$, EtOH). IR ν_{max}^{KBr} cm^{-1}: 1775, 1725, 1700. ^{1}H-NMR ($CDCl_3$) δ: 2.16 (3H, s, CH_3), 2.27 (3H, s, CH_3), 3.20 (3H, s, OCH_3), 3.73 (2H, s, CH_2), 3.83 (3H, s, OCH_3), 5.20 (1H, s, C_4-H), 6.60 (1H, br s, NH), 7.37 (5H, s, aromatic-H).

(b) *tert*-Butyl hypochlorite (0.864 g) and LiOMe–MeOH solution (50.6 mg of lithium in 4 ml of MeOH) were added dropwise to a solution of the bisdisulfide derivative[2] (**6**, 2.31) in dry THF (30 ml) at −70 °C with stirring. The mixture was stirred for 20 min, then AcOH (0.5 ml) was added, and the resulting solution was concentrated to yield the residue, which was partitioned between AcOEt and water. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated. The crude product was chromatographed on silica gel. Elution with *n*-hexane–AcOEt (1 : 1) gave **4** (1.0 g, 40%).

(3*S*,4*R*)-3-Methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-4-methylthio-3-phenylacetamido-2-azetidinone (8)——*tert*-Butyl hypochlorite (0.28 ml) and LiOMe–MeOH solution (4.6 ml of 1.78 mmol solution) were added dropwise to a solution of the 4-methylthio derivative[13] (**7**, 0.8 g) in dry THF (20 ml) at −78 °C with stirring. The mixture was stirred for 1 h at −55 °C, then AcOH (0.46 ml) was added. The reaction mixture was poured into ice-water–AcOEt and extracted several times with AcOEt. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated to give the residue. The crude product was chromatographed on silica gel. Elution with *n*-hexane–AcOEt (2 : 3) provided **8** (0.502 g, 55.7%) as a foam. *Anal.* Calcd for $C_{19}H_{24}N_2O_5S$: C, 58.15; H, 6.16; N, 7.14. Found: C, 57.98; H, 6.23; N, 7.08. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1768, 1720, 1700 (br), 1650 (sh). ^{1}H-NMR ($CDCl_3$) δ: 2.06 (6H, s, CH_3), 2.28 (3H, s, CH_3), 3.52 (2H, s, CH_2), 3.76 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 5.08 (1H, s, CH), *ca.* 6.2 (1H, br s, NH), 7.37 (5H, s, aromatic-H).

(3*R*)-3-Methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-3-phenylacetamido-2-azetidinone (9)——(a) A mixture of **4** (0.28 g) and Raney nickel (2 ml) in AcOEt (5 ml) was stirred at 55 °C for 4 h. After removal of the catalyst, the filtrate was concentrated. The crude product was chromatographed on silica gel. Elution with *n*-hexane–AcOEt (2 : 1) gave **9** (0.15 g, 58.4%) as a viscous oil. $[\alpha]_D^{25}$ +98.2° ($c=1$, MeOH). IR ν_{max}^{neat} cm^{-1}: 3300, 1760, 1730, 1690. ^{1}H-NMR ($CDCl_3$) δ: 1.92 (3H, s, CH_3), 2.22 (3H, s, CH_3), 3.43 (3H, s, OCH_3), 3.62 (2H, s, CH_2), 3.70 (3H, s, OCH_3), 3.88 (1H, d, $J=6$ Hz, C_4-H), 4.03 (1H, d, $J=6$ Hz, C_4-H), 7.28 (1H, br s, NH), 7.31 (5H, s, aromatic-H).

(b) A mixture of **8** (0.393 g) and Raney nickel (4 ml) in dry THF (20 ml) was refluxed for 5 h. Treatment as described above afforded **9** (0.298 g, 89.5%). $[\alpha]_D^{24.5}$ +103.3° ($c=1$, MeOH).

(c) A mixture of **21** (0.36 g) and 10% Pd-C (0.2 g) in dry THF (8 ml) was stirred vigorously under a hydrogen gas stream. After 30 min, the catalyst was filtered off and washed with dry THF (10 ml), and the filtrate was concentrated until 5 ml of the solvent remained. Phenylacetic acid (0.136 g) and DCC (0.206 g) were added to the THF solution of **22** obtained above and the resulting reaction mixture was stirred at room temperature for 1 h. After removal of the insolule material, the filtrate was concentrated and then partitioned with AcOEt and water. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Treatment as described above afforded **9** (0.118 g, 32.9%). $[\alpha]_D^{22}$ +101.7° ($c=1$, MeOH).

(3*S*,4*R*)-3-Benzyloxycarbonylamino-3-methoxy-1-[(1*R*)-methoxycarbonyl-2-methylprop-2-enyl]-4-(benzothiazol-2-yl-dithio)-2-azetidinone (18) and Bis[(3*S*,4*R*)-3-benzyloxycarbonylamino-3-methoxy-1-[(1*R*)-methoxycarbonyl-2-methylprop-2-enyl]-2-oxoazetidin-4-yl] Disulfide (19)——(a) A mixture of **16** (15 g) and 2-mercaptobenzothiazole (6 g) in dry dioxane (100 ml) was refluxed for 3.5 h. After removal of the solvent, the resulting crude product was chromatographed on silica gel. Elution with *n*-hexane–AcOEt (2 : 1) afforded **18** (5.3 g, 25.9%), **19** (1.5 g, 10.5%) and the starting material **16** (28 g, 52%).

18: Foam, ^{1}H-NMR ($CDCl_3$) δ: 1.93 (3H, d, $J=0.5$ Hz, CH_3), 3.46 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 5.03 (2H, d, $J=4$ Hz, CH_2), 5.20 (1H, q, $J=0.5$ Hz, CH), 5.23 (2H, s, CH_2), 5.33 (1H, s, C_4-H), 7.13 (1H, s, NH), 7.3—8.0 (9H, m, aromatic-H).

19: Colorless plates, mp 131—133 °C (dec.). *Anal.* Calcd for $C_{36}H_{42}N_4O_{12}S_2$: C, 54.95; H, 5.38; N, 7.12. Found: C, 54.88; H, 5.41; N, 7.22. $[\alpha]_D^{24}$ −197.9° ($c=0.98$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3300, 1770, 1760, 1735 (sh), 1725, 1500. ^{1}H-NMR ($CDCl_3$) δ: 1.83 (3H, d, $J=0.5$ Hz, CH_3), 3.65 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 4.90 (2H, d, $J=4$ Hz, CH_2), 5.06 (1H, q, $J=0.5$ Hz, CH), 5.16 (2H, s, CH_2), 6.20 (1H, s, NH), 7.30 (5H, s, aromatic-H).

(b) A mixture of **18** (0.5 g) and Raney nickel (5 ml) saturated with hydrogen gas in THF (10 ml) was stirred at room temperature for 15 min. The catalyst was filtered off and the filtrate was concentrated to give the crude product. Purification was carried out as described above to obtain **19** (0.21 g, 59.8%).

Bis[(3*S*,4*R*)-3-benzyloxycarbonylamino-3-methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-2-oxoazetidin-4-yl] Disulfide (20)——Triethylamine (0.2 ml) was added to a cooled (4 °C) solution of **18** (17 g, containing a small amount of **19** on TLC) in CH_2Cl_2 (200 ml), and the reaction mixture was stirred at room temperature until the starting material was no longer detectable on TLC. Purification was carried out by chromatography on silica gel. Elution with *n*-hexane–AcOEt (2 : 1) provided (3*S*,4*R*)-3-benzyloxycarbonylamino-3-methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-4-(benzothiazol-2-yl-dithio)-2-azetidinone (5.3 g, 31.2%) and **20** (3.7 g, 31.0%) as foams. *Anal.* Calcd for $C_{36}H_{42}N_4O_{12}S_2$: C, 54.95; H, 5.38; N, 7.12. Found: C, 55.05; H, 5.22; N, 7.48. $[\alpha]_D^{24}$ −33.4° ($c=0.99$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1775, 1720, 1500. ^{1}H-NMR ($CDCl_3$) δ: 2.00 (3H, s, CH_3), 2.23 (3H, s, CH_3), 3.50 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 5.00 and 5.23 (2H, each d, $J=12$ Hz, CH_2), 5.03 (1H, s, C_4-H), 6.26 (1H, s, NH), 7.23 (5H, s, aromatic-H).

(3*R*)-3-Benzyloxycarbonylamino-3-methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-2-azetidinone (21) and Its *dl* Derivative (25)——(a) *tert*-Butyl hypochlorite (1.38 ml) and LiOMe–MeOH (0.133 g of lithium in 10 ml of MeOH) were added dropwise to a cooled (−50 °C) solution of (3*S*)-3-benzyloxycarbonylamino-1-(1-methoxy carbonyl-2-methylprop-1-enyl)-2-azetidinone (**14**, 2 g) in dry THF (68 ml). The reaction mixture was stirred for 1 h at −10 °C. After addition of AcOH (1.2 ml), the mixture was partitioned with AcOEt–cold water. The organic layer was washed with water, aqueous $Na_2S_2O_3$ solution and water successively, then dried over anhydrous Na_2SO_4. The solvent was evaporated off to yield the crude product, which was purified by chromatography on silica gel. Elution with *n*-hexane–AcOEt (2 : 1) afforded *dl*-**25** (1.85 g, 84.9%) as colorless needles (AcOEt–*n*-hexane). mp 77 °C. *Anal.* Calcd for $C_{18}H_{22}N_2O_6$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.55; H, 6.14; N, 7.68. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1761, 1723, 1140. ^{1}H-NMR ($CDCl_3$) δ: 1.91 (3H, s, CH_3), 2.22 (3H, s, CH_3), 3.53 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.8—4.4 (2H, m, C_4-H), 5.20 (2H, s, CH_2), 6.58 (1H, s, NH), 7.36 (5H, s, aromatic-H).

(b) A mixture of **20** (3 g) and Raney nickel (26 ml) saturated with hydrogen gas in EtOH (50 ml) was stirred at room temperature for 2 h. The catalyst was filtered off and washed with EtOH and the filtrate was concentrated to give the 3-amino derivative (0.97 g, 56.1%) as a pale brown oil. Benzyloxycarbonyl chloride (0.8 g) in CH_2Cl_2 (1 ml) was added dropwise to a solution of the 3-amino derivative obtained above and pyridine (0.37 g) in CH_2Cl_2 (4 ml). After being stirred at room temperature for 15 min, the reaction mixture was partitioned with AcOEt–water. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Purification was carried out by chromatography on silica gel; elution with *n*-hexane–CH_2Cl_2 (1 : 1) gave **21** (0.836 g, 31.7% from **20**) as a colorless viscous oil. IR ν_{max}^{KBr} cm^{-1}: 1760, 1718, 1510.

(3*R*)-3-Benzyloxycarbonylamino-3-methoxy-2-azetidinone (24) and Its *dl* Derivative (26)——(a) A solution of **25** (1.5 g) in dry CH_2Cl_2 (120 ml) was ozonized at −78 °C in an acetone-dry ice bath, until the solution turned bluish-green, at which time the ozone was replaced by a stream of dry nitrogen gas. After treatment with Me_2S (2.4 ml), the solution was allowed to come to room temperature over 1 h. The solution was evaporated to dryness and the residue was dissolved in MeOH (30 ml). A solution of a catalytic amount of NaOMe in MeOH was added to the methanol solution prepared above and the mixture was stirred at room temperature for 25 min. The reaction mixture was poured into ice-water–AcOEt, and the separated organic layer was washed with water and dried over anhydrous Na_2SO_4. The solvent was evaporated off to afford the *dl*-derivative (**26**, 1.0 g, 96.5%) as colorless prisms. mp 112—115 °C. *Anal.* Calcd for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.44; H, 5.45; N, 10.88. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1774, 1723. ^{1}H-NMR ($CDCl_3$) δ: 3.45 (3H, s, OCH_3), 3.58 and 3.76 (2H, each d, J=6 Hz, C_4-H), 5.14 (2H, s, CH_2), 6.74 (2H, br s, NH), 7.34 (5H, s, aromatic-H).

(b) A similar reaction using **21** afforded (3*R*)-3-benzyloxycarbonylamino-3-methoxy-2-azetidinone (**24**, 87.1%) as colorless prisms (AcOEt–*n*-hexane), mp 100—101 °C. *Anal.* Calcd for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.66; H, 5.52; N, 11.09. $[\alpha]_D^{23}$ +68.2° (c=1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3260, 1765, 1722 (sh), 1705. The ^{1}H-NMR spectrum was identical with that of **26**.

Sodium (3*R*)-3-(D-γ-Glutamyl-D-alanylamino)-3-methoxy-2-azetidinone-1-sulfonate (31)——(a) Diphosgene (1.89 g) in dry THF (4 ml) and Et_3N (2.1 g) in dry THF (4 ml) were added dropwise to a cooled (−40 °C) solution of *N*-benzyloxycarbonyl-D-alanine (4.46 g) in dry THF (35 ml). After 30 min, Et_3N (2.1 g) was added to this reaction mixture and the whole was stirred for an additional 30 min. (3*R*)-3-Amino-3-methoxy-2-azetidinone [**27**, prepared from **24** (2.5 g) in dry THF (25 ml) by hydrogenation with Pd black (1 g)] was added to the acid chloride solution prepared above at −40 °C with stirring. This reaction mixture was stirred at room temperature overnight, then the insoluble material was filtered off and the filtrate was concentrated. The crude product was chromatographed on silica gel. Elution with *n*-hexane–AcOEt (1 : 2) and then with AcOEt provided (3*R*)-3-(*N*-benzyloxycarbonyl-D-alanyl-amino)-3-methoxy-2-azetidinone (**28**, 0.905 g, 32%) as a foam. *Anal.* Calcd for $C_{15}H_{19}N_3O_5$: C, 56.07; H, 5.96; N, 13.08. Found: C, 56.22; H, 6.02; N, 12.91. $[\alpha]_D^{22}$ +79.5° (c=1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3270, 1755, 1680, 1520, 1245. ^{1}H-NMR (DMSO-d_6) δ: 1.22 (3H, d, J=7 Hz, CH_3), 3.32 (3H, s, OCH_3), 3.40 and 3.48 (2H, each d, J=7 Hz, C_4-H), 4.14 (1H, m, CH), 5.04 (2H, s, CH_2), 7.36 (5H, s, aromatic-H), 8.26 (1H, br s, NH), 8.98 (1H, d, J=7 Hz, NH).

(b) Diphosgene (0.238 g) was added dorpwise to a cooled (*ca.* −20 °C) solution of dimethylformamide (DMF) (0.205 g) in CH_2Cl_2 (10 ml) and the resulting solution was stirred at room temperature for 40 min. α-*O*-Benzyl-*N*-benzyloxycarbonyl-D-glutamic acid (0.747 g) and Et_3N (0.3 g) in CH_2Cl_2 (6 ml) were added dropwise to the solution prepared above at −78 °C. The mixture was stirred for 1.5 h at −20 °C, then Et_3N (0.26 g) was added (solution A). A mixture of the 2-azetidinone derivative (**28**, 0.58 g) prepared above (a), and Pd black (1 g) in THF (10 ml)–MeOH (10 ml) was stirred for 2 h under a hydrogen gas stream. The catalyst was filtered off and the filtrate was concentrated to give the residue, which was dissolved in THF (10 ml)–DMA (3 ml) (solution B). Solution A was added dropwise to solution B, and the reaction mixture was stirred at room temperature for 15 h. The insoluble material was filtered off and the filtrate was concentrated. The crude product was purified by chromatography on silica gel. Elution with CH_2Cl_2–AcOEt (1 : 1—1 : 2) gave (3*R*)-3-(*O*-benzyl-*N*-benzyloxycarbonyl-D-γ-glutamyl-D-alanylamino)-3-methoxy-2-azetidinone (**29**, 0.23 g, 23.6%) as colorless needles, mp 164—165 °C (dec.). *Anal.* Calcd for $C_{27}H_{32}N_4O_8$: C, 59.99; H, 5.97; N, 10.36. Found: C, 60.21; H, 5.69; N, 10.18. $[\alpha]_D^{25}$ +71° (c=1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3260, 1750, 1700, 1650, 1520, 1250, 1200. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, d, J=7 Hz, CH_3), 1.80—2.50 (2H, m, CH_2), 3.40 (3H, s, OCH_3), 3.53 and 3.66 (2H, each d, J=7 Hz, C_4-H), 4.30 (1H, m, CH), 4.60 (1H, m, CH), 5.03 (2H, s, CH_2), 5.10 (2H,

s, CH_2), 6.20 (1H, m, NH), 7.00 (1H, br s, NH), 7.30 (10H, s, aromatic-H), 8.40 (1H, br s, NH).

(c) A mixture of the 2-azetidinone derivative (**29**, 0.23 g) prepared above (b) and SO_3·pyridine complex (0.078 g) in DMF (1 ml) was stirred at room temperature. After 10 h, additional SO_3·pyridine complex (0.052 g) was added, and the reaction mixture was stirred for 30 h at room temperature. The solvent was evaporated off and the residue was washed with Et_2O, then dissolved in AcOEt–MeOH (5 : 1) solution. Purification was carried out by chromatography on silica gel to yield pyridinium (3*R*)-3-(*O*-benzyl-*N*-benzyloxycarbonyl-D-γ-glutamyl-D-alanylamino)-3-methoxy-2-azetidinone-1-sulfonate (**30**, 0.18 g, 60.4%) as a powder. $[\alpha]_D^{22}$ +79.6° (c=1.03, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1760, 1710, 1635, 1520, 1245, 1045. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (3H, d, J=7 Hz, CH_3), 1.92 (2H, m, CH_2), 2.22 (2H, m, CH_2), 3.30 (3H, s, OCH_3), 3.52 and 3.65 (2H, each d, J=7 Hz, C_4-H), 4.10 (1H, m, CH), 4.36 (1H, m, CH), 5.05 (2H, s, CH_2), 5.13 (2H, s, CH_2), 7.36 (5H, s, aromatic-H), 7.74 (1H, d, J=7 Hz, NH), 7.95 (1H, d, J=7 Hz, NH), 8.00, 8.52 and 8.90 (m, aromatic-H), 9.06 (1H, br s, NH).

(d) A mixture of the pyridinium 2-azetidinone-1-sulfonate derivative (**30**, 0.18 g) prepared above (c) and Pd black (0.1 g) in phosphate buffer (pH 5.7, 2 ml)–MeOH (6 ml) was stirred under a hydrogen gas stream for 50 min. After removal of the catalyst and MeOH, the aqueous solution was charged on an XAD-2 column and eluted with water. The fractions having antibacterial activity (checked by the *Proteus mirabilis* disk method) were lyophilized to give the crude product (0.107 g), which was further purified by chromatography on activated carbon (1 g) eluted with water and then with 20%-MeOH. The desired fractions having antibacterial activity (*Proteus milabilis* disk method) were lyophilized to obtain sulfazecin as the mono sodium salt[11] (**31**, 0.04 g, 37.4%). *Anal.* Calcd for $C_{12}H_{19}N_4NaO_9S \cdot H_2O$: C, 33.03; H, 4.85; H, 12.84. Found: C, 32.58; H, 4.99; N, 12.55. $[\alpha]_D^{24}$ +82.9° (c=0.55, H_2O). IR ν_{max}^{KBr} cm^{-1}: 1770, 1640, 1530, 1245, 1050, 632. ^{1}H-NMR (DMSO-d_6) δ: 1.23 (3H, s, CH_3), 2.02 (2H, m, CH_2), 2.31 (2H, m, CH_2), 3.31 (3H, s, OCH_3).

Sodium (3*S*)- and (3*R*)-[D-2-(3-Furfurylideneamino-2-oxoimidazolidin-1-yl)carbonylamino-2-phenylacetamido]-3-methoxy-2-azetidinone-1-sulfonates (33 and 34)——(a) A mixture of 3-benzyloxycarbonylamino-3-methoxy-2-azetidinone (**26**, *dl*-derivative, 0.501 g) and Pd black (0.3 g) in THF (20 ml) was stirred for 30 min under a hydrogen gas stream. The catalyst was filtered off, and the filtrate was concentrated to give the residue, which was dissolved in CH_2Cl_2 (20 ml). The acid chloride prepared from *N*-[(3-furfurylideneamino-2-oxoimidazolidin-1-yl)-carbonyl]-D-phenylglycine (0.856 g), PCl_5 (0.5 g) and Et_3N (0.243 g) in CH_2Cl_2 (15 ml) was added to the solution prepared above and propylene oxide (10 ml) at −15 °C. The mixture was stirred at the same temperature for 15 min, then pyridine (0.475 g) was added. The reaction mixture was stirred for a further 30 min, then poured into ice-water and extracted with $CHCl_3$. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by chromatography on silica gel [$CHCl_3$–AcOEt–MeOH = 4 : 5 : 1] to obtain the following two isomers: (3*S*)-3-[D-2-(3-Furfurylideneamino-2-oxoimidazolidin-1-yl)carbonylamino-2-phenylacetamido]-3-methoxy-2-azetidinone; 0.391 g (43%). Powder, *Anal.* Calcd for $C_{21}H_{22}N_6O_6 \cdot H_2O$: C, 53.39; H, 5.12; N, 17.79. Found: C, 53.60; H, 4.90; N, 17.86. IR ν_{max}^{KBr} cm^{-1}: 3280, 1760, 1720, 1670, 1475, 1410, 1230. ^{1}H-NMR (DMSO-d_6) δ: 3.08 (3H, s, CH_3), 3.42 and 3.56 (2H, each d, J=6 Hz, C_4-H), 3.79 (2H, s, CH_2), 5.62 (1H, d, J=7 Hz, CH), 6.5—7.9 (8H, m, aromatic-H), 7.73 (1H, s, –CH=N), 8.35 (1H, s, NH), 9.04 (1H, d, J=7 Hz, NH), 9.59 (1H, s, NH). (3*R*)-3-[D-2-(3-Furfurylideneamino-2-oxoimidazolidin-1-yl)carbonylamino-2-phenylacetamido]-3-methoxy-2-azetidinone; 0.141 g (15%). Powder, *Anal.* Calcd for $C_{21}H_{22}N_6O_6 \cdot H_2O$: C, 53.39; H, 5.12; N, 17.79. Found: C, 53.29; H, 4.74; N, 17.65. IR ν_{max}^{KBr} cm^{-1}: 3280, 1760, 1720, 1670, 1475, 1410, 1230. ^{1}H-NMR (DMSO-d_6) δ: 3.26 and 3.42 (2H, each d, J=6 Hz, C_4-H), 3.34 (3H, s, CH_3), 3.78 (2H, s, CH_2), 5.61 (1H, d, J=7 Hz, CH), 6.5—7.9 (8H, m, aromatic-H), 7.73 (1H, s, –CH=N), 8.23 (1H, s, NH), 8.98 (1H, d, J=7 Hz, NH), 9.54 (1H, s, NH).

(b) A mixture of the (3*S*)-isomer (0.228 g) and SO_3·pyridine complex (0.199 g) in DMF (2 ml) was stirred at room temperature for 1 d. Ethyl ether was added to the reaction mixture and the resulting oily substance was stirred with Dowex 50W resin (Na^+ form) in water. Purification was carried out by Amberlite XAD-2 column chromatography to obtain the (3*S*)-isomer of the title compound **34** (0.11 g, 40%) as a powder. *Anal.* Calcd for $C_{21}H_{21}N_6NaO_9S \cdot 3H_2O$: C, 41.31; H, 4.46; N, 13.77. Found: C, 41.75; H, 4.26; N, 13.68. IR ν_{max}^{KBr} cm^{-1}: 3300, 1770, 1720, 1670, 1475, 1420, 1270, 1235, 1050. ^{1}H-NMR (DMSO-d_6) δ: 3.34 (3H, s, OCH_3), 3.54 and 3.72 (2H, each d, J=6 Hz, C_4-H), 3.80 (2H, s, CH_2), 5.63 (1H, d, J=7 Hz, CH), 6.5—7.9 (8H, m, aromatic-H), 7.74 (1H, s, –CH=N–), 9.02 (1H, d, J=7 Hz, NH), 9.71 (1H, s, NH).

The corresponding (3*R*)-isomer (**33**) was obtained in a similar manner. Powder (32%), *Anal.* Calcd for $C_{21}H_{21}N_6NaO_9S \cdot 3H_2O$: C, 41.31; H, 4.46; N, 13.77. Found: C, 41.14; H, 4.78; N, 13.91. IR ν_{max}^{KBr} cm^{-1}: 3300, 1770, 1720, 1670, 1475, 1420, 1235, 1050. ^{1}H-NMR (DMSO-d_6) δ: 3.32 (3H, s, CH_3), 3.46, 3.55 (2H, each d, J=6 Hz, C_4-H), 3.80 (2H, s, CH_2), 5.59 (1H, d, J=7 Hz, CH), 6.5—7.9 (8H, m, aromatic-H), 7.74 (1H, s, –CH=N–), 9.86 (1H, d, J=7 Hz, NH), 9.65 (1H, s, NH).

(c) Starting from the optically active derivative (**24**), the (3*R*)-isomer (**33**) was prepared in 28% yield in the same manner as described above, (a) and (b). The IR and ^{1}H-NMR spectra were identical with those of **33** obtained in (b).

Sodium (3*S*)- and (3*R*)-3-[D-2-(4-Cyclohexyl-2,3-dioxo-1-piperazinecarbonylamino)-2-(2-thienyl)acetamido]-3-methoxy-2-azetidinone-1-sulfonates (36 and 37)——(a) Treatment as described for the preparation of **33** and **34** afforded the corresponding 2-azetidinone derivatives as a mixture of two diastereomers, which were separated by silica gel column chromatography (AcOEt) to afford the (3*S*)-isomer (38%) and (3*R*)-isomer (34%).

(3*R*)-Isomer: Powder, *Anal.* Calcd for $C_{21}H_{27}N_5O_6S$: C, 52.82; H, 5.70; N, 14.67. Found: C, 52.44; H, 5.61; N, 14.27. IR ν_{max}^{KBr} cm^{-1}: 2920, 1760, 1705, 1670, 1500, 1170. ^{1}H-NMR (DMSO-d_6) δ: 3.36 (3H, s, OCH_3), 3.39 and 3.47 (2H, each d, J=6 Hz, C_4-H), 5.87 (1H, d, J=7 Hz, CH), 6.9—7.6 (3H, m, thienyl-H), 8.30 (1H, s, NH), 9.67 (1H, s, NH), 9.69 (1H, d, J=7 Hz, NH).

(3*S*)-Isomer: Powder, *Anal.* Calcd for $C_{21}H_{27}N_5O_6S$: C, 52.82; H, 5.70; N, 14.67. Found: C, 52.43; H, 5.71; N, 14.54. IR ν_{max}^{KBr} cm^{-1}: 2920, 1760, 1705, 1670, 1500, 1170. ^{1}H-NMR (DMSO-d_6) δ: 3.18 (3H, s, OCH_3), 3.42 and 3.54 (2H, each d, J=6 Hz, C_4-H), 5.87 (1H, d, J=7 Hz, CH), 6.9—7.6 (3H, m, thienyl-H), 8.34 (1H, s, NH), 9.66 (1H, s, NH), 9.71 (1H, d, J=7 Hz, NH).

(b) Sulfonation and purification were carried out as described for the preparation of **33** and **34**.

(3*R*)-Isomer, **36** (70% yield): Powder, *Anal.* Calcd for $C_{21}H_{26}N_5NaO_9S_2 \cdot 2H_2O$: C, 40.97; H, 4.91; N, 11.38. Found: C, 40.93; H, 5.00; N, 11.11. IR ν_{max}^{KBr} cm^{-1}: 1765, 1710, 1675, 1505, 1250, 1175, 1050. ^{1}H-NMR (DMSO-d_6) δ: 3.34 (3H, s, OCH_3), 3.56 (2H, s, C_4-H), 5.86 (1H, d, J=7 Hz, CH), 6.9—7.5 (3H, m, thienyl-H), 9.75 (1H, d, J=7 Hz, NH), 9.77 (1H, s, NH).

(3*S*)-Isomer, **37** (68% yield): Powder, *Anal.* Calcd for $C_{21}H_{26}N_5NaO_9S \cdot 2.5H_2O$: C, 40.38; H, 5.00; N, 11.21. Found: C, 40.42; H, 4.84; N, 11.34. IR ν_{max}^{KBr} cm^{-1}: 1765, 1710, 1675, 1505, 1250, 1175, 1050. ^{1}H-NMR (DMSO-d_6) δ: 3.16 (3H, s, OCH_3), 3.55 and 3.71 (2H, each d, J=7 Hz, C_4-H), 5.89 (1H, d, J=7 Hz, CH), 6.9—7.6 (3H, m, thienyl-H), 9.71 (1H, d, J=7 Hz, NH), 9.79 (1H, s, NH).

(c) Starting from the optically active derivative (**24**), the (3*R*)-isomer (**36**) was prepared in 39% yield in the same manner as described above, (a) and (b). The IR and ^{1}H-NMR spectra were identical with those of **36** obtained in (b).

Acknowledgement The authors thank Dr. K. Morita of this Division for his advice and encouragement. Thanks are also due to Dr. M. Kondo for the biological evaluation.

References and Notes

1) Part of this paper was presented at the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April, 1982; Abstracts of Papers, p. 393.
2) T. Matsuo, T. Sugawara, H. Masuya, Y. Kawano, N. Noguchi and M. Ochiai, *Chem. Pharm. Bull.*, **31**, 1874 (1983).
3) J. A. Weber and J. L. Ott, "Structure–Activity Relationships among the Semisynthetic Antibiotics," ed. by D. Perlman, Academic Press, New York, 1977, pp. 182—183.
4) *a*) J. E. Baldwin, F. J. Urban, R. D. G. Cooper and F. L. Jośe, *J. Am. Chem. Soc.*, **95**, 2401 (1973); *b*) G. A. Koppel and R. E. Koehler, *ibid.*, **95**, 2403 (1973).
5) R. G. Micetich and R. B. Morin, *Tetrahedron Lett.*, **1976**, 973.
6) J. Elks, "Recent Advances in the Chemistry of β-Lactam Antibiotics," The Chemical Society, London, 1977, p. 243.
7) R. D. G. Cooper and F. L. Jośe, *J. Am. Chem. Soc.*, **94**, 1021 (1972).
8) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon and S. L. Andrews, *J. Am. Chem. Soc.*, **85**, 1896 (1963).
9) M. Bodanszky, Y. S. Klausner and M. A. Ondetti, "Peptide Synthesis," 2nd ed., John Wiley and Sons Inc., New York, 1976, p. 137.
10) M. Miyoshi, *Bull. Chem. Soc. Jpn.*, **46**, 212 (1973).
11) *a*) A. Imada, K. Kitano, K. Kintaka, M. Muroi and M. Asai, *Nature* (London), **289**, 590 (1981); *b*) M. Asai, K. Haibara, M. Muroi, K. Kintaka and T. Kishi, *J. Antibiot.*, **34**, 621 (1981).
12) *a*) I. Saikawa, S. Takano, C. Yoshida, O. Takashima, K. Momonoi, T. Yasuda, K. Kasuya and M. Komatsu, *Yakugaku Zasshi*, **97**, 980 (1977); *b*) H. B. Koenig, K. G. Metzger, M. Preiss and W. Schroeck, Jpn. Kokai Tokkyo Koho, 51-76438 [*Chem. Abstr.*, **86**, 29799*d* (1977)].
13) Ref. 6), p. 192.