[Chem. Pharm. Bull. 36(2) 469—480 (1988)]

Synthesis of 4-(Methoxyethyl) Monobactams by a Chemicoenzymatic Approach

HARUO YAMASHITA,[a] NOBUYOSHI MINAMI,[a] KYOICHI SAKAKIBARA,[a]
SUSUMU KOBAYASHI[b] and MASAJI OHNO*,[b]

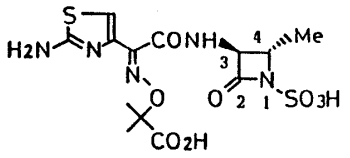
Department of Chemistry, Research Laboratories, Teikoku Hormone Mfg. Co., Ltd.,[a] Takatsu-ku, Kawasaki-shi, Kanagawa 213, Japan and Faculty of Pharmaceutical Sciences, University of Tokyo,[b] Hongo, Bunkyo-ku, Tokyo 113, Japan

(Received April 28, 1987)

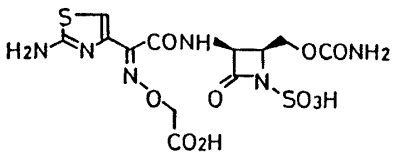
As a key intermediate for the synthesis of monobactam analogues, *cis*-3-benzyloxycarbonylamino-4-(2-hydroxyethyl)-2-azetidinone was synthesized from (4*S*)-4-methoxycarbonylmethyl-2-azetidinone, and converted into monobactams having a methoxyethyl group at the C-4 position of the β-lactam ring. Among the compounds synthesized, disodium (3*S*,4*R*)-3-[2-(2-aminothiazol-4-yl)-(*Z*)-2-carboxymethoxyiminoacetamido]-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate showed strong activity against a variety of gram-negative bacteria except *Pseudomonas aeruginosa*. Furthermore, the 4-methoxyethyl derivatives exhibited excellent stability to β-lactamases, and the syntheses of the corresponding *trans* isomer and 3α-methoxy derivatives are also described.

Keywords——monobactam; (4*S*)-4-methoxycarbonylmethyl-2-azetidinone; 3-benzyloxycarbonylamino-4-(2-hydroxyethyl)-2-azetidinone; 1-sulfo-2-azetidinone; antibacterial activity; oximination; stereoselective reduction

Monobactams are simple and unique monocyclic β-lactam antibiotics discovered independently by two groups at Takeda[1] and Squibb[2] in 1981. Subsequent chemical modifications at the C-3 amido side chain and C-4 substituents revealed that the introduction of alkyl groups into the C-4 position of the β-lactam ring, especially in a 3,4-*cis* configuration, significantly enhanced antibacterial activity against gram-negative bacteria. Aztreonam (**1**)[3] and carumonam (**2**),[4] synthesized at Squibb and Takeda respectively, have been selected as clinical candidates, and **1** is now in commercial use in Europe. These findings directed our interest to designing new monobactams starting from (4*S*)-4-methoxycarbonylmethyl-2-azetidinone (**3**), which is easily available by a chemicoenzymatic approach.[5] In this paper, we report the synthesis and antibacterial activity of 4-(2-methoxyethyl)-1-sulfo-2-azetidinone derivatives.



aztreonam (**1**)

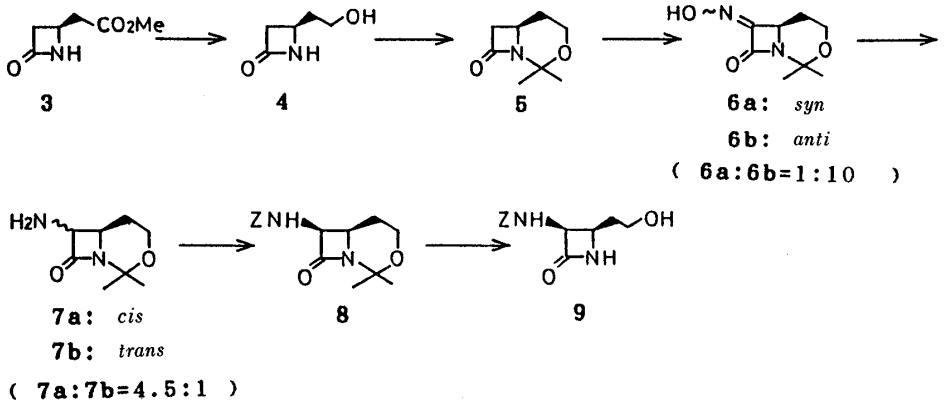


carumonam (**2**)

Chart 1

The first target compound was *cis*-3-benzyloxycarbonylamino-4-(2-hydroxyethyl)-2-azetidinone (**9**) starting from **3**, because the 4-(2-hydroxyethyl) group may be readily converted into a variety of other substituents. In preparing **9**, the most important step is the introduction of an amino group into the C-3 position of the β-lactam ring in a 3,4-*cis* manner. A two-step approach, the introduction of the oximino group and subsequent stereoselective reduction in the *cis* configuration[6] was studied (Chart 2). Reduction of **3** with lithium borohydride followed by simultaneous protection of the alcohol and lactam nitrogen with 2,2-

dimethoxypropane in the presence of acid gave the acetonide (**5**) in 71% overall yield. Introduction of an oximino group at the C-7 position of **5** by a modification of Kühlein's method[7] resulted in formation of the oxime (**6**) in 60% yield (*syn*:*anti*=1:10<). The stereochemistry was determined by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The signal of C_6-Hα of the major isomer appeared at 4.41 ppm, downfield by about 0.25 ppm from that of the minor isomer owing to the anisotropic deshielding effect of the hydroxy group of the oxime, supporting the view that the major isomer has the *anti* structure (**6b**).[8] Hydrogenation of the *anti* isomer (**6b**) was examined in detail using PtO_2, Pd–C and Rh_2O_3 as catalysts in various solvents. Among them, PtO_2 in ethyl acetate gave the most satisfactory result (*cis*:*trans*=4.5:1). The stereochemistry was determined by ^{1}H-NMR spectroscopy. The coupling constant of the major isomer was $J_{6,7}$=5 Hz and that of the minor isomer was 1.5 Hz, supporting the assigned stereochemistry.[6a] Similarly, hydrogenation of the *syn* isomer over PtO_2 in ethyl acetate gave a mixture of diastereomers (*cis*:*trans*=4—5:1). On the other hand, reduction with Al–Hg gave a 1:1 mixture of diastereomers. Then, the amino group of *cis* isomer (**7a**) was protected with a benzyloxycarbonyl group to give (6*R*,7*S*)-7-benzyloxycarbonylamino-2,2-dimethyl-3-oxa-1-azabicyclo[4.2.0]octan-8-one (**8**) in 91% yield. The treatment of **8** with hydrochloric acid in methanol provided (3*S*,4*R*)-3-benzyloxy-carbonylamino-4-(2-hydroxyethyl)-2-azetidinone (**9**) in good yield.

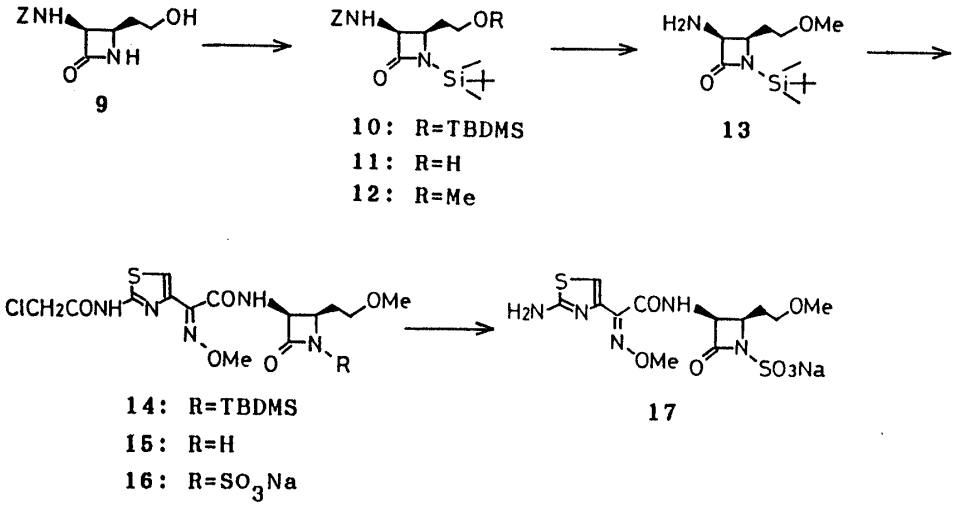


Z=benzyloxycarbonyl

Chart 2

The 4-(2-methoxyethyl)-1-sulfo-2-azetidinone derivative (**17**) was prepared from the key intermediate (**9**) through 4-(2-methoxyethyl)-2-azetidinone (**12**) (Chart 3). Disilylation of **9** with *tert*-butyldimethylchlorosilane and subsequent regioselective desilylation with hydrochloric acid in cold methanol gave the *N*-silylated alcohol (**11**) in 89% yield. Then, **11** was treated with diazomethane in the presence of boron trifluoride etherate to give the ether (**12**) in 80% yield. Hydrogenation of **12** and subsequent acylation with 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid[9] gave the 3-acylamino compound (**14**) in moderate yield. Removal of the silyl group in **14** was carried out by treatment with hydrochloric acid in methanol to afford 3-acylamino-2-azetidinone (**15**) in 95% yield. Sulfonation of **15** with sulfur trioxide pyridine complex (SO_3·Py) followed by the treatment with Dowex 50W (Na) gave the 1-sulfo derivative (**16**) in good yield. Finally, removal of the chloroacetyl group from **16** by treatment with sodium *N*-methyldithiocarbamate[9] afforded sodium *cis*-3-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (**17**). The structure of **17** was confirmed by its infrared (IR), ^{1}H-NMR and secondary ion mass spectra (SIMS).

The 4-(2-methoxyethyl)-1-sulfo-2-azetidinone derivative (**17**), obtained above, showed good antibacterial activity against a variety of gram-negative bacteria (Table I). However, **17**



TBDMS = *tert*-butyldimethylsilyl

Chart 3

showed less activity against *Pseudomonas aeruginosa* than aztreonam (**1**) and carumonam (**2**). We, therefore, introduced the same acyl groups as in **1** or **2** into the 3-position of **17** (Chart 4). Acylation with 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-(4-nitrobenzyloxycarbonylmethoxyimino)acetyl chloride[10] and 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-[1-methyl-1-(4-nitrobenzyloxycarbonyl)ethoxyimino]acetyl chloride[10] gave the corresponding 3-acylamino derivatives (**18a** and **18b**), respectively. Deprotection of each of **18** and subsequent sulfonation with $SO_3 \cdot Py$ in a similar manner to that described for the in preparation of **16** afforded 1-sulfo-2-azetidinones (**20a** and **20b**). After removal of the chloroacetyl groups, the 4-nitrobenzyl groups were removed by catalytic hydrogenation over 5% Pd–C in 50% methanol to give the deprotected products (**21a** and **21b**) in the form of disodium salts. Replacement of the methoxyimino group of **17** with the carboxymethoxyimino or 1-methyl-1-carboxyethoxyimino group resulted in a slight increase of the activity. In particular, the carboxymethoxyimino derivative (**21a**) showed excellent antibacterial activity against gram-negative bacteria. However, **21a** remained less active against *Pseudomonas aeruginosa* (Table I). These 4-(2-methoxyethyl) compounds (**17** and **21b**) also exhibited good stability against β-lactamases (Table II).

Moreover, in order to examine the effect of the 3,4-configuration and the 3α-methoxy group on the antibacterial activity, we synthesized the *trans* isomer of **17** (**31**) and the corresponding 3α-methoxy derivative (**38**) (Charts 5 and 6).

The *trans* isomer (**31**) was prepared starting from the chiral half ester (**22**). The half ester (**22**) was esterified with isobutylene in the presence of acid and subsequently saponified with sodium hydroxide in methanol to give the chiral *tert*-butyl ester (**23**) in 43% overall yield. Hydrogenation of **23** and subsequent cyclization by our β-lactam ring formation procedure[5] using triphenylphosphine-dipyridyl disulfide in acetonitrile afforded (4*R*)-*tert*-butoxycarbonylmethyl-2-azetidinone (**25**) in 48% yield. Reduction of **25** with lithium borohydride gave (4*R*)-4-(2-hydroxyethyl)-2-azetidinone (**26**), which was treated with 2,2-dimethoxypropane to give the acetonide (**27**). The introduction of the azido group at the C-7 position in **27** by use of *p*-toluenesulfonyl azide according to Kühlein's method[7] resulted in the stereoselective formation of the chemically more stable *trans* isomer (**28**) as colorless crystals in 70% yield. The $C_{6,7}$-*trans* relationship of **28** is evident from its small coupling constant ($J_{6,7} = 1.5$ Hz) in the ^{1}H-NMR spectrum.[6] Hydrogenolysis of **28** followed by acylation with benzyl *S*-4,6-dimethylpyrimidin-2-yl thiolcarbonate gave (6*S*,7*S*)-7-benzyloxycarbonylamino-2,2-di-

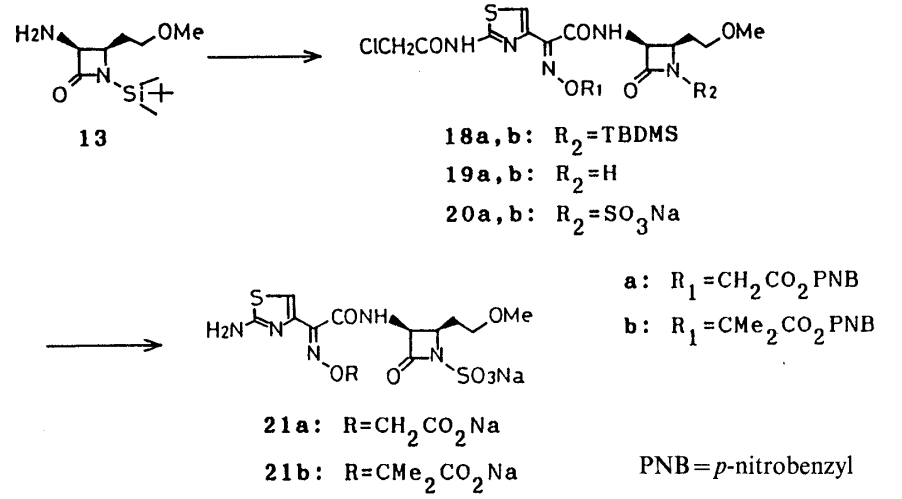


Chart 4

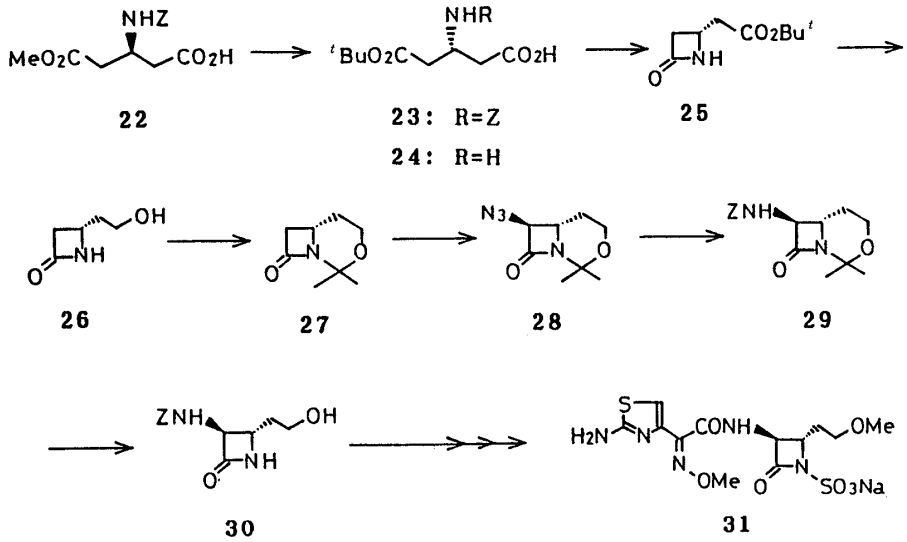


Chart 5

methyl-3-oxa-1-azabicyclo[4.2.0]octan-8-one (**29**) in 70% yield. The treatment of **29** with hydrochloric acid in methanol to remove the acetonide provided *trans*-3-acylamino-4-(2-hydroxyethyl)-2-azetidinone (**30**). The *trans*-2-azetidinone (**30**) was converted into sodium *trans*-3-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (**31**) by the same method as described in the preparation of *cis* isomer (**17**).

On the other hand, the 3α-methoxy derivative (**38**) was prepared from **12** (Chart 6). Deprotection of the silyl group in **12** with hydrochloric acid in methanol gave *cis*-3-benzyloxycarbonylamino-4-(2-methoxyethyl)-2-azetidinone (**32**) in good yield. Sulfonation of **32** with $SO_3 \cdot Py$ afforded colorless crystals of **33**, which was transformed to the tetra-*n*-butylammonium salt (**34**) by treatment with tetra-*n*-butylammonium hydroxide. Hydrogenation of **34** and subsequent acylation with 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid and dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole afforded 3-acylamino-1-sulfo-2-azetidinone (**35**). Introduction of a methoxy group at the C_3-α position of **35** was achieved by the acylimine method[11] with *tert*-butyl hypochlorite-lithium methoxide to give a mixture of 3α-methoxy derivatives (**36a** and **36b**). These compounds (**36a** and **36b**) were separated by HP-20 column chromatography after treatment with Dowex 50W (Na^+) to give **37a** and **37b** in 34% and 25% yields, respectively. Finally, removal of the

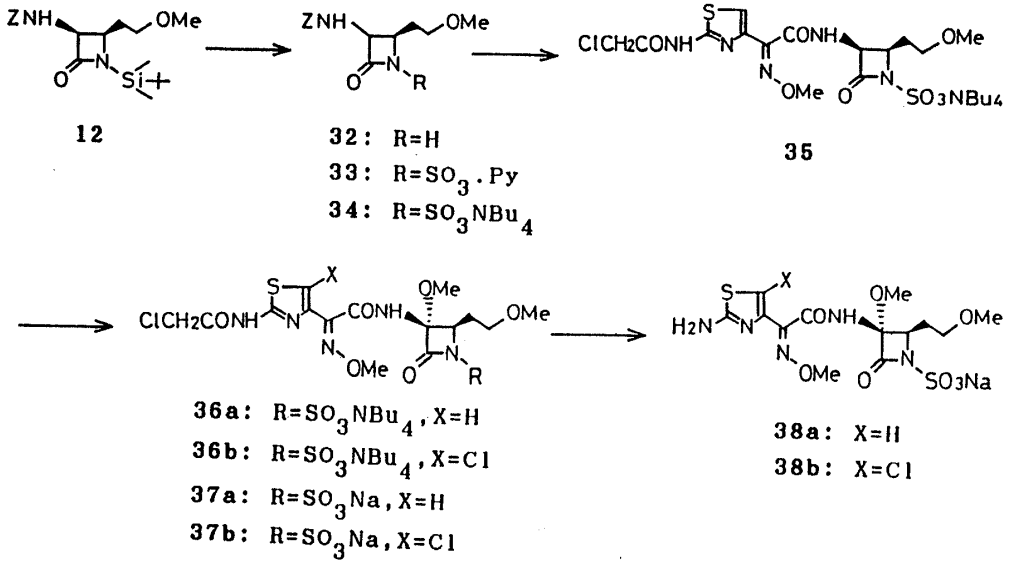


Chart 6

TABLE I. Antibacterial Activities of Monobactams Having a 2-Methoxyethyl Group at the C_4 Position of the β-Lactam Ring

(MIC: μg/ml)

Organism	17	21a	21b	31	38a	Aztreonam
E. coli NIHJ-JC-2	0.2	0.1	0.39	0.78	25	0.05
E. coli K-12 C600	0.2	0.1	0.2	0.39	12.5	0.1
K. pneumoniae PCI-602	0.012	0.025	0.2	0.1	3.12	0.006
S. marcescens IAM 1184	0.39	0.025	0.2	0.39	12.5	0.003
E. cloacae 963	0.39	0.05	0.39	0.2	12.5	0.05
P. vulgaris OX 19	0.025	0.025	0.025	0.39	3.12	0.012
P. mirabilis IFO 3849	0.025	0.012	0.025	0.2	6.25	0.012
P. aeruginosa IFO 3445	100	50	50	100	100	1.56
P. aeruginosa NCTC 10490	3.12	0.78	1.56	100	100	0.39

Minimal inhibitory concentrations (MICs) were determined by the agar dilution method using an inoculum of 10^6 CFU/ml.

TABLE II. Relative β-Lactamase Stability

Enzyme	Type	17	21b	Aztreonam
Ps. cepacia GN 11164	CXase	6.9[a)]	4.3	100
P. vulgaris GN 7919	CXase	1	1	100
K. oxytoca GN 10650	CXase	10.0	11.1	100

a) Relative stability is expressed as the relative hydrolysis rate with respect to aztreonam.

chloroacetyl groups in **37a** and **37b** with sodium *N*-methyldithiocarbamate afforded the deprotected 3α-methoxy derivatives (**38a** and **38b**, respectively).

The *in vitro* activity of the *trans* isomer (**31**) was slightly lower than that of the corresponding *cis* isomer (**17**). On the other hand, the introduction of the 3α-methoxy group resulted in a marked decrease in the antibacterial activity, contrary to our expectation (Table I).

Experimental

Melting points were measured on a Yamato MP-21 melting point apparatus and are uncorrected. IR spectra were obtained on a JASCO DS-402G spectrometer. ^{1}H-NMR spectra were recorded on a JEOL FX-100 spectrometer using tetramethylsilane or, for D_2O solution, sodium 3-(trimethylsilyl)propane sulfonate as an internal standard. Abbreviation are as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet. MS were measured with a Shimadzu GCMS-QP 1000 and SIMS were measured with a Hitachi M-80B mass spectrometer. Optical rotations were determined using a JASCO DIP-140 digital polarimeter. Elemental analyses were carried out on a Hitachi 026 CHN analyzer. Silica gel (Wakogel C-200) and Lobar column (Lichroprep Si 60) were used for column chromatography.

(4*R*)-4-(2-Hydroxyethyl)-2-azetidinone (4)——A solution of (4*S*)-4-methoxycarbonylmethyl-2-azetidinone (**3**)[5] (10 g, 70 mmol) in dimethoxyethane (DME, 60 ml) was added to a solution of lithium borohydride (1.52 g, 70 mmol) in DME (30 ml), and the resulting solution was stirred for 2 h at 45—50 °C. After addition of AcOH (25 ml) at 0—5 °C, the mixture was concentrated under reduced pressure. *n*-Heptane (100 ml) was added to the oily residue, and the mixture was concentrated again under reduced pressure. After repeating this procedure twice more, the residue was chromatographed on silica gel ($CHCl_3$: MeOH = 10 : 1) to give **4** (7.26 g, 90%) as a hygroscopic crystalline solid, mp 61—62 °C. *Anal.* Calcd for $C_5H_9NO_2$: C, 52.16: H, 7.88; N, 12.17. Found: C, 52.26; H, 7.58; N, 12.26. $[\alpha]_D^{22}$ +29.7° (*c* = 1, $CHCl_3$). IR (KBr) cm^{-1}; 3350, 1720. NMR ($CDCl_3$–CD_3OD): 1.65—2.05 (2H, m), 2.60 (1H, dd, *J* = 2.5 and 16 Hz), 3.08 (1H, dd, *J* = 4.5 and 16 Hz), 3.66 (2H, t, *J* = 6 Hz), 3.60—3.90 (1H, m).

(6*R*)-2,2-Dimethyl-3-oxa-1-azabicyclo[4.2.0]octan-8-one (5)——Boron trifluoride etherate (895 mg, 6.3 mmol) was added to a solution of **4** (7.26 g, 63 mmol) and 2,2-dimethoxypropane (19.7 g, 189 mmol) in anhydrous dichloromethane (CH_2Cl_2, 100 ml) at −20 °C. The mixture was stirred for 20 min at the same temperature, and then stirred for 3 h at room temperature. The mixture was poured into 1 M, pH 7, phosphate buffer (200 ml), and the separated organic layer was washed with brine, dried, and evaporated to give a residue. The oily residue was chromatographed on silica gel (ether : hexane = 3 : 1) to afford **5** (7.7 g, 79%) as a colorless oil. $[\alpha]_D^{22}$ +41.5° (*c* = 3, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 1740. NMR ($CDCl_3$): 1.42 (3H, s), 1.75 (3H, s), 1.50—2.10 (2H, m), 2.63 (1H, dd, *J* = 2 and 15 Hz), 3.04 (1H, dd, *J* = 5 and 15 Hz), 3.50—3.70 (1H, m), 3.86 (2H, dd, *J* = 2.5 and 8 Hz). MS *m/z*: 140 ($M^+ - CH_3$).

(6*R*)-7-Hydroxyimino-2,2-dimethyl-3-oxa-1-azabicyclo[4.2.0]octan-8-one (6)——A solution of 1.6 M *n*-butyllithium in hexane (13.75 ml, 22 mmol) was added to diisopropylamine (2.23 g, 22 mmol) in anhydrous tetrahydrofuran (THF, 15 ml), and the mixture was stirred for 20 min at −78 °C. A solution of **5** (1.54 g, 10 mmol) in anhydrous THF (10 ml) was then added dropwise, and the reaction mixture was stirred for 10 min at −78 °C. Next, trimethylchlorosilane (1.14 g, 10.5 mmol) was added dropwise. The whole was stirred for 30 min at −50 °C, isoamyl nitrite (2.34 g, 20 mmol) was added in one portion at −78 °C, and stirring was continued for 3 h at the same temperature. Then, the reaction was quenched by addition of saturated ammonium chloride solution (20 ml), and the mixture was allowed to warm to 0 °C. The product was extracted with EtOAc (100 ml), and the organic solution was washed with brine, dried and concentrated under reduced pressure. The residue was chromatographed on silica gel (ether : hexane = 1 : 1 → ether) to give the *anti* oxime (**6a**, 1.15 g) and the *syn* oxime (**6b**, 0.08 g) as colorless crystals. Yields, 62% and 4%, respectively.

6a: mp 130—134 °C. *Anal.* Calcd for $C_8H_{12}N_2O_3$: C, 52.34; H, 6.67; N, 15.19. Found: C, 52.16; H, 6.57; N, 15.21. $[\alpha]_D^{22}$ +55.1° (*c* = 2, $CHCl_3$). IR (KBr) cm^{-1}: 1750. NMR ($CDCl_3$): 1.49 (3H, s), 1.80 (3H, s), 1.80—2.20 (2H, m), 3.90 (2H, dd, *J* = 3 and 8 Hz), 4.41 (1H, dd, *J* = 5.5 and 10.5 Hz), 9.36 (1H, br s).

6b: mp 117—119 °C. *Anal.* Calcd for $C_8H_{12}N_2O_3$: C, 52.34; H, 6.67; N, 15.19. Found: C, 52.07; H, 6.69; N, 15.06. $[\alpha]_D^{22}$ −100.3° (*c* = 1, $CHCl_3$). IR (KBr) cm^{-1}: 1760, 1745. NMR ($CDCl_3$): 1.48 (3H, s), 1.80 (3H, s), 1.70—2.10 (2H, m), 3.89 (2H, dd, *J* = 3.5 and 7.5 Hz), 4.15 (1H, dd, *J* = 6 and 9.5 Hz), 9.92 (1H, br s).

(6*R*,7*S*) and (6*R*,7*R*)-7-Amino-2,2-dimethyl-3-oxa-1-azabicyclo[4.2.0]octan-8-one (7a and 7b)——A mixture of **6a** (1.16 g, 63 mmol) and platinum oxide (300 mg) in EtOAc (100 ml) was vigorously stirred for 8 h at room temperature under a hydrogen atmosphere. The catalyst was filtered off and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 20 : 1 → 10 : 1), and then the diastereomeric mixture was separated on a Lobar column with CH_2Cl_2–MeOH (20 : 1). The first eluate gave the *cis* isomer (**7a**, 0.81 g, 75%) as a colorless oil, which crystallized in a refrigerator, mp 53—55 °C. *Anal.* Calcd for $C_8H_{14}N_2O_2$: C, 55.82; H, 8.64; N, 15.90. Found: C, 56.25; H, 8.39, N, 16.36. $[\alpha]_D^{22}$ −16.6° (*c* = 2, $CHCl_3$). IR (KBr) cm^{-1}: 3390, 3355, 1750, 1720. NMR ($CDCl_3$): 1.40 (3H, s), 1.50—2.00 (2H, m), 1.64 (2H, s), 1.74 (3H, s), 3.65—4.00 (3H, m), 4.20 (1H, d, *J* = 5 Hz). MS *m/z*: 170 (M^+).

The second eluate gave the *trans* isomer (**7b**, 0.18 g, 16%) as a colorless oil, which crystallized in a refrigerator, mp 59—62 °C. $[\alpha]_D^{22}$ +51.6° (*c* = 1, $CHCl_3$). IR (KBr) cm^{-1}: 3400, 1730. NMR ($CHCl_3$): 1.43 (3H, s), 1.50—2.10 (2H, m), 1.70 (2H, s), 1.74 (3H, s), 3.30—3.50 (1H, m), 3.75—3.85 (2H, m), 3.88 (1H, d, *J* = 2.5 Hz). MS *m/z*: 170 (M^+).

(6*R*,7*S*)-7-Benzyloxycarbonylamino-2,2-dimethyl-3-oxa-1-azabicyclo[4.2.0]octan-8-one (8)——Benzyl *S*-4,6-dimethylpyrimidin-2-yl thiolcarbonate (*Z*-*S*) (3.3 g, 12 mmol) was added to a solution of **7a** (1.7 g, 10 mmol) in dioxane (30 ml), and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with

EtOAc (100 ml) and washed with successively with brine, 10% citric acid and brine, then dried and concentrated under reduced pressure. The solid residue was recrystallized from EtOAc–hexane to give **8** (2.77 g, 91%) as colorless crystals, mp 173—174 °C. *Anal.* Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.21. Found: C, 63.06, H, 6.58; N, 9.07. $[\alpha]_D^{22}$ +60.9° ($c=1$, MeOH). IR (KBr) cm^{-1}: 3300, 1760, 1695. NMR ($CDCl_3$): 1.38 (3H, s), 1.50—1.80 (2H, m), 1.72 (3H, s), 3.70—3.95 (3H, m), 4.95 (1H, dd, $J=5$ and 7.5 Hz), 5.10 (2H, s), 5.80 (1H, d, $J=7.5$ Hz), 7.31 (5H, s).

(3*S*,4*R*)-3-Benzyloxycarbonylamino-4-(2-hydroxyethyl)-2-azetidinone (9)——A 1 N HCl solution (20 ml) was added to a solution of **8** (3.27 g, 10.7 mmol) in MeOH (40 ml). The reaction mixture was stirred for 2 h at 40 °C, adjusted to pH 7 with $NaHCO_3$ at 0—5 °C, and concentrated under reduced pressure. The resulting solid residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 10 : 1) to give **9** (2.67 g, 94%) as colorless crystals, mp 177—179 °C. *Anal.* Calcd for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.60: Found: C, 58.91; H, 5.91; N, 10.60. $[\alpha]_D^{22}$ +41.8° ($c=1$, MeOH), IR (KBr) cm^{-1}: 3430, 3310, 1760, 1700. NMR (CD_3OD): 1.60—1.85 (2H, m), 3.61 (2H, t, $J=6.5$ Hz), 3.80—4.00 (1H, m), 4.99 (1H, d, $J=5$ Hz), 5.10 (2H, s), 7.31 (5H, s).

(3*S*,4*R*)-Benzyloxycarbonylamino-4-(2-hydroxyethyl)-1-*tert*-butyldimethylsilyl-2-azetidinone (11)——*tert*-Butyldimethylchlorosilane (3.16 g, 21 mmol) and triethylamine (2.13 g, 21 mmol) were added to an ice-cooled solution of **9** (1.85 g, 7 mmol) in dimethylformamide (DMF) (20 ml). After being stirred overnight at room temperature, the reaction mixture was diluted with ether (100 ml), washed with brine, dried and evaporated under reduced pressure to give crude **10** as an oil, which was used for the next reaction without further purification. Crude **10** was dissolved in MeOH (40 ml), and 1 N HCl (7 ml) was added at 0—5 °C. The mixture was stirred for 30 min at the same temperature, and adjusted to pH 7 with $NaHCO_3$. After removal of the solvent, the residue was chromatographed on silica gel (ether) to give **11** (2.36 g, 89%) as a colorless oil, which crystallized in a refrigerator, mp 84—85 °C. *Anal.* Calcd for $C_{19}H_{30}N_2O_4Si$: C, 60.29; H, 7.99; N, 7.40. Found: C, 60.38; H, 8.21; N, 7.38. $[\alpha]_D^{23}$ −19.7° ($c=1$, $CHCl_3$). IR (KBr) cm^{-1}: 3430, 3330, 1735, 1700. NMR ($CDCl_3$): 0.20 (3H, s), 0.28 (3H, s), 0.95 (9H, s), 1.70—2.15 (2H, m), 3.50—3.70 (2H, m), 3.90—4.05 (1H, m), 5.10 (2H, s), 5.15 (1H, dd, $J=5.5$ and 9.5 Hz), 6.15 (1H, d, $J=9.5$ Hz), 7.32 (5H, s).

The *trans* isomer of **11** was obtained in a similar manner as a colorless oil (83%). $[\alpha]_D^{22}$ +60.6° ($c=1.7$, $CHCl_3$). IR (neat) cm^{-1}: 3330, 1740, 1705. NMR ($CDCl_3$): 0.24 (6H, s), 0.95 (9H, s), 1.50—2.30 (2H, m), 3.45—3.95 (3H, m), 4.47 (1H, dd, $J=2$ and 5.5 Hz), 5.11 (1H, s), 5.85 (1H, d, $J=5.5$ Hz), 7.33 (5H, s). MS *m/z*: 378 (M^+), 363 ($M^+ - CH_3$), 321 ($M^+ - C_4H_9$).

(3*S*,4*R*)-3-Benzyloxycarbonylamino-4-(2-methoxyethyl)-1-*tert*-butyldimethylsilyl-2-azetidinone (12)——A solution of diazomethane in ether (large excess) was added to a stirred solution of **11** (830 mg, 2.2 mmol) and boron trifluoride etherate (31 mg, 0.22 mmol) in ether (30 ml) at 0—5 °C. After being stirred for 1 h at room temperature, the reaction mixture was poured into 1 M, pH 7, phosphate buffer (30 ml) and the organic solution was washed with brine, dried and concentrated under reduced pressure. The residue was chromatographed on silica gel (ether–hexane, 1 : 1) to give **12** (690 mg, 80%) as a colorless oil. $[\alpha]_D^{23}$ −24.5° ($c=1$, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 1740. NMR ($CDCl_3$): 0.17 (3H, s), 0.30 (3H, s), 0.95 (9H, s), 1.70—2.05 (2H, m), 3.15—3.45 (2H, m), 3.24 (3H, s), 3.90—4.00 (1H, m), 5.10 (2H, s), 5.18 (1H, dd, $J=5.5$ and 9.5 Hz), 6.10 (1H, d, $J=9.5$ Hz), 7.32 (5H, s). MS *m/z*: 392 (M^+).

The *trans* isomer of **12** was obtained in a similar manner as a colorless oil (53%). $[\alpha]_D^{22}$ +30.2° ($c=1.2$, $CHCl_3$). IR (KBr) cm^{-1}: 3300, 1740. NMR ($CDCl_3$): 0.24 (6H, s), 0.96 (9H, s), 1.45—1.85 (1H, m), 1.95—2.35 (1H, m), 3.26 (3H, s), 3.40—3.75 (3H, m), 4.38 (1H, dd, $J=2.5$ and 8 Hz), 5.10 (2H, s), 5.35 (1H, d, $J=8$ Hz), 7.33 (5H, s). MS *m/z*: 392 (M^+), 377 ($M^+ - CH_3$), 335 ($M^+ - C_4H_9$).

(3*S*,4*R*)-3-Amino-4-(2-methoxyethyl)-1-*tert*-butyldimethylsilyl-2-azetidinone (13)——A mixture of **12** (800 mg, 2 mmol) and 5% Pd–C (100 mg) in MeOH (15 ml) was stirred for 2 h at room temperature under a hydrogen atmosphere. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to give **13** (520 mg, quantitative) as a oil. $[\alpha]_D^{22}$ −85.5° ($c=0.6$, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 1725. NMR ($CDCl_3$): 0.22 (3H, s), 0.26 (3H, s), 0.96 (9H, s), 1.68 (2H, br s), 1.80—2.05 (2H, m), 3.32 (3H, s), 3.48 (2H, t, $J=6.5$ Hz), 3.65—3.85 (1H, m), 4.30 (1H, d, $J=5.5$ Hz). MS *m/z*: 258 (M^+).

(3*S*,4*R*)-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-(2-methoxyethyl)-1-*tert*-butyldimethylsilyl-2-azetidinone (14)——Diphosgene (50 mg, 0.25 mmol) was added to a solution of DMF (55 mg, 0.75 mmol) in CH_2Cl_2 (1 ml) at −20 °C and stirred for 30 min at room temperature. After the mixture had been cooled to −78 °C, 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (140 mg, 0.5 mmol) and triethylamine (51 mg, 0.5 mmol) in CH_2Cl_2 (2 ml) were added in one portion, and the resulting mixture was stirred for 1 h at −25—−20 °C. Then, the mixture was cooled to −78 °C again, and a solution of **13** (130 mg, 0.5 mmol) and triethylamine (51 mg, 0.5 mmol) in CH_2Cl_2 (2 ml) was added. The temperature of the reaction mixture was raised to 0 °C over 1 h. Stirring was continued for 30 min at 0 °C, then the reaction mixture was diluted with CH_2Cl_2 (10 ml) and water (10 ml). The organic layer was washed successively with saturated $NaHCO_3$ and brine, then dried and evaporated under reduced pressure. The residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 20 : 1) to afford **14** (126 mg, 49%) as colorless crystals, 156—158 °C. *Anal.* Calcd for $C_{20}H_{32}ClN_5O_5SSi$: C, 46.36; H, 6.23; N, 13.52. Found: C, 46.34; H, 6.24; N, 13.39. $[\alpha]_D^{21}$ −79.1 ($c=1$, $CHCl_3$). IR (KBr) cm^{-1}: 3400, 3260, 3180, 1720, 1675. NMR ($CDCl_3$): 0.24 (3H, s), 0.30 (3H, s), 0.96 (9H, s), 1.75—2.10 (2H, m), 3.30 (3H, s), 3.43 (2H, t, $J=7$ Hz), 3.90—4.10 (1H, m), 3.98 (3H, s), 4.21 (1H, d, $J=14$ Hz), 4.24 (1H, d, $J=14$ Hz), 5.44 (1H, dd, $J=5.5$ and 8 Hz), 7.08 (1H, s),

0.23 (1H, d, J=8 Hz).

The *trans* isomer of **14** was similarly synthesized as colorless crystals, mp 115—116 °C. Yield 50%. *Anal.* Calcd for $C_{20}H_{32}ClN_5O_5SSi$: C, 46.36; H, 6.32; N, 13.52. Found: C, 46.08; H, 6.29; N, 13.66. $[\alpha]_D^{22}$ −36.6° (c=1.1, $CHCl_3$). IR (KBr) cm^{-1}: 3285, 1760, 1695, 1660. NMR ($CDCl_3$): 0.23 (3H, s), 0.33 (3H, s), 0.93 (9H, s), 1.50—1.90 (1H, m), 2.10—2.50 (1H, m), 3.32 (3H, s), 3.50—3.70 (2H, m), 3.75—3.90 (1H, m), 3.94 (3H, s), 4.21 (2H, s), 5.08 (1H, dd, J= 2.5 and 9 Hz), 6.97 (1H, s), 8.60 (1H, d, J=9 Hz).

(3*S*,4*R*)-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-(2-methoxyethyl)-2-azetidione (15)——A 1 N HCl solution (1.3 ml) was added to a solution of **14** (350 mg, 0.67 mmol) in MeOH (5 ml), and the mixture was stirred for 4 h at room temperature. The resulting precipitate was collected by filtration and washed with MeOH and ether to give **15** (260 mg, 95%) as a colorless powder. *Anal.* Calcd for $C_{14}H_{18}ClN_5O_5S$: C, 41.64; H, 4.49; N, 17.34. Found: C, 41.69; H, 4.34; N, 17.22. $[\alpha]_D^{23}$ −7.1° (c=1, DMF). IR (KBr) cm^{-1}: 3280, 1750, 1690, 1670. NMR (DMSO-d_6): 1.50—1.90 (2H, m), 3.22 (3H, s), 3.36 (2H, t, J=7 Hz), 3.65—3.90 (1H, m), 3.87 (3H, s), 4.36 (2H, s), 5.18 (1H, dd, J=5 and 9 Hz), 7.42 (1H, s), 8.32 (1H, br s), 9.30 (1H, d, J=9 Hz).

The *trans* isomer of **15** was similarly prepared as a colorless powder (68%). *Anal.* Calcd for $C_{14}H_{18}ClN_5O_5S$: C, 41.64; H, 4.49; N, 17.34. Found: C, 41.25; H, 4.33; N, 17.30. $[\alpha]_D^{22}$ −55.5° (c=1, DMF). IR (KBr) cm^{-1}: 3285, 1760, 1695, 1660. NMR ($CDCl_3$): 1.95—2.20 (2H, m), 3.32 (3H, s), 3.45—3.65 (2H, m), 3.70—3.90 (1H, m), 3.96 (3H, s), 4.24 (2H, s), 4.77 (1H, dd, J=2 and 8 Hz), 6.32 (1H, br s), 7.19 (1H, s), 8.08 (1H, d, J=8 Hz).

Sodium (3*S*,4*R*)-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (16)——SO_3·Py (160 mg, 1 mmol) was added to a solution of **15** (80 mg, 0.2 mmol) in CH_2Cl_2 (2 ml) and DMF (2 ml), and the mixture was stirred for 3 h at 50 °C. The solvent was evaporated off under reduced pressure and the residue was chromatographed on silica gel ($CHCl_3$: MeOH : H_2O = 100 : 30 : 5) to give the sulfonated product, which was subsequently treated with Dowex 50W (Na^+) (2 ml) followed by lyophilization to give **16** (79 mg, 78%) as a colorless powder. **16** was used for the next reaction without further purification. $[\alpha]_D^{23}$ −22.1° (c=1, H_2O). IR (KBr) cm^{-1}: 1765, 1665. NMR (D_2O): 1.80—2.40 (2H, m), 3.36 (3H, s), 3.60 (2H, t, J=7 Hz), 4.02 (3H, s), 4.30—4.55 (1H, m), 4.42 (2H, s), 5.40 (1H, d, J=5.5 Hz), 7.48 (1H, s). SIMS m/z: 528 (M^++Na), 506 (M^++H).

The *trans* isomer of **16** was similarly prepared as a colorless powder (97%). $[\alpha]_D^{22}$ −25.0° (c=0.5, H_2O). IR (KBr) cm^{-1}: 1770, 1665. NMR (D_2O): 1.85—2.25 (1H, m), 2.30—2.65 (1H, m), 3.35 (3H, s), 3.67 (2H, t, J=6 Hz), 4.03 (3H, s), 4.15—4.40 (1H, m), 4.43 (2H, s), 4.81 (1H, d, J=2.5 Hz), 7.44 (1H, s).

Sodium (3*S*,4*R*)-3-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (17)——Sodium *N*-methyldithiocarbamate (20 mg, 0.15 mmol) was added to a solution of **16** (70 mg, 0.138 mmol) in water (2 ml) and the mixture was stirred for 2 h at room temperature, then diluted with water (3 ml) and washed with ether (5 ml). The aqueous solution was concentrated to a volume of about 1 ml, and the concentrate was chromatographed on HP-20 (20 ml). Elution with water and 10% EtOH, followed by lyophilization, gave **17** (47 mg, 80%) as a colorless powder. $[\alpha]_D^{22}$ −26.0° (c=1, H_2O). IR (KBr) cm^{-1}: 1760, 1660. NMR (D_2O): 1.60—2.40 (2H, m), 3.35 (3H, s), 3.58 (2H, t, J=7 Hz), 3.98 (3H, s), 4.30—4.50 (1H, m), 5.38 (1H, d, J=5.5 Hz), 6.96 (1H, s). SIMS m/z: 452 (M^++Na), 403 (M^++H).

The *trans* isomer of **17** was obtained in a similar manner as a pale yellow powder (77%). $[\alpha]_D^{22}$ −32.1° (c=0.5, H_2O). IR (KBr) cm^{-1}: 1765, 1660. NMR (D_2O): 1.85—2.25 (1H, m), 2.25—2.60 (1H, m), 3.34 (3H, s), 3.66 (2H, t, J=6 Hz), 3.98 (3H, s), 4.15—4.40 (1H, m), 4.79 (1H, d, J=2.5 Hz), 6.92 (1H, s). SIMS m/z: 452 (M^++Na).

(3*S*,4*R*)-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-(substituted oximino)acetamido-4-(2-methoxyethyl)-1-*tert*-butyldimethylsilyl-2-azetidinones (18a and 18b)——By using 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-(4-nitrobenzyloxycarbonylmethoxyimino)acetic acid[10] and 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-[1-methyl-1-(4-nitrobenzyloxycarbonyl)ethoxyimino]acetic acid[10] in place of 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid in the procedure described in the preparation of **14**, **18a** and **18b** were synthesized, respectively.

18a: A colorless oil. Yield 52%. $[\alpha]_D^{22}$ −24.4° (c=1, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 1745, 1685. NMR ($CDCl_3$): 0.21 (3H, s), 0.25 (3H, s), 0.94 (9H, s), 1.60—2.20 (2H, m), 3.24 (3H, s), 3.25—3.55 (2H, m), 3.75—4.05 (1H, m), 4.24 (2H, s), 4.85 (2H, s), 5.25 (2H, s), 5.39 (1H, dd, J=6 and 9 Hz), 7.38 (1H, s), 7.50 (2H, d, J=9 Hz), 8.15 (2H, d, J=9 Hz), 8.15 (1H, d, J=9 Hz).

18b: A colorless foam. Yield 58%. $[\alpha]_D^{22}$ −21.3° (c=1, $CHCl_3$). IR (KBr) cm^{-1}: 1735, 1675. NMR ($CDCl_3$): 0.19 (3H, s), 0.25 (3H, s), 0.93 (9H, s), 1.62 (6H, s), 1.65—2.05 (2H, m), 3.28 (3H, s), 3.30—3.60 (2H, m), 3.65—3.95 (1H, m), 4.25 (1H, d, J=15 Hz), 4.27 (1H, d, J=15 Hz), 5.09 (1H, dd, J=6 and 8 Hz), 5.22 (1H, d, J=13 Hz), 5.28 (1H, d, J=13 Hz), 7.29 (1H, s), 7.48 (2H, d, J=9 Hz), 8.03 (2H, d, J=9 Hz), 8.02 (1H, d, J=8 Hz).

(3*S*,4*R*)-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-(substituted oximino)acetamido]-4-(2-methoxyethyl)-2-azetidinones (19a and 19b)——Compounds **19a** and **19b** were prepared from **18a** and **18b**, respectively, by a method similar to that described for the preparation of **15**.

19a: A colorless powder. Yield 81%. *Anal.* Calcd for $C_{22}H_{23}ClN_6O_9S$: C, 45.33: H, 3.98; N, 14.42. Found: C, 45.34; H, 3.88; N, 14.24. $[\alpha]_D^{22}$ −5.4° (c=0.7, DMF). IR (KBr) cm^{-1}: 3265, 1755, 1665. NMR (DMSO-d_6): 1.45—1.95 (2H, m), 3.18 (3H, s), 3.39 (2H, t, J=6 Hz), 3.55—3.85 (1H, m), 4.34 (2H, s), 4.81 (2H, s), 5.19 (1H, dd, J=5 and 9 Hz), 5.33 (2H, s), 7.42 (1H, s), 7.65 (2H, d, J=9 Hz), 8.13 (2H, d, J=9 Hz), 8.28 (1H, br s), 9.29 (1H, d, J=9 Hz).

19b: A colorless powder. Yield 86%. *Anal.* Calcd for $C_{24}H_{27}ClN_6O_9S$: C, 47.18; H, 4.45; N, 13.75. Found: C,

47.13; H, 4.51; N, 13.82. $[\alpha]_D^{22}$ −8.5° (c=0.6, DMF). IR (KBr) cm^{-1}: 3270, 1745, 1670. NMR (DMSO-d_6): 1.50 (3H, s), 1.53 (3H, s), 1.55—1.85 (2H, m), 3.20 (3H, s), 3.40 (2H, t, J=6 Hz), 3.65—3.90 (1H, m), 4.35 (2H, s), 5.18 (1H, dd, J=6 and 9 Hz), 5.30 (2H, s), 7.35 (1H, s), 7.61 (2H, d, J=9 Hz), 8.03 (2H, d, J=9 Hz), 8.32 (1H, br s), 9.27 (1H, d, J=9 Hz).

Sodium (3*S*,4*R*)-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-(substituted oximino)acetamido]-4-(2-methoxyethyl)-2-azetidinone-1-sulfonates (20a and 20b)——Compounds **20a** and **20b** were synthesized from **19a** and **19b**, respectively, by a method similar to that described in preparation of **16**.

20a: A colorless powder. Yield 88%. $[\alpha]_D^{22}$ −11.9° (c=1, 50% EtOH). IR (KBr) cm^{-1}: 1760, 1670. NMR (DMSO-d_6): 1.60—2.30 (2H, m), 3.15 (3H, s), 3.39 (2H, t, J=6 Hz), 3.85—4.05 (1H, m), 4.35 (2H, s), 4.80 (2H, s), 5.14 (1H, dd, J=6 and 9 Hz), 5.34 (2H, s), 7.43 (1H, s), 7.66 (2H, d, J=8.5 Hz), 8.14 (2H, d, J=8.5 Hz), 9.37 (1H, d, J=9 Hz).

20b: A colorless powder. Yield 92%. $[\alpha]_D^{22}$ −13.8° (c=1.1, 50% EtOH). IR (KBr) cm^{-1}: 1760, 1680. NMR (DMSO-d_6): 1.51 (3H, s), 1.54 (3H, s), 1.65—2.20 (2H, m), 3.18 (3H, s), 3.45 (2H, t, J=6.5 Hz), 3.85—4.10 (1H, m), 4.34 (2H, s), 5.14 (1H, dd, J=5.5 and 8 Hz), 5.31 (2H, s), 7.33 (1H, s), 7.60 (2H, d, J=8.5 Hz), 8.03 (2H, d, J=8.5 Hz), 9.29 (1H, d, J=8 Hz).

Disodium (3*S*,4*R*)-3-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(carboxymethoxyimino)acetamido]-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (21a)——Sodium *N*-methyldithiocarbamate (23 mg, 0.18 mmol) was added to a solution of **20a** (62 mg, 0.09 mmol) in THF (1 ml) and water (1 ml), and the mixture was stirred for 1.5 h at room temperature, then concentrated under reduced pressure. The residue was dissolved in a mixture of water (5 ml) and ether (5 ml). The aqueous layer was washed with ether and concentrated to a volume of about 1 ml. The concentrate was subjected to chromatography on HP-20. Elution with water and 10% EtOH, followed by lyophilization, gave a colorless powder (34 mg). A solution of this powder (34 mg) and 5% Pd–C (20 mg) in 50% MeOH (4 ml) was stirred for 1 h at room temperature under a hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was treated with Dowex 50W (Na^+) (2 ml) for 15 min under ice-cooling. The resin was filtered off, and the filtrate was concentrated to a volume of about 1 ml under reduced pressure. The concentrate was chromatographed on HP-20 and eluted with water and 5% EtOH. Lyophilization of the 5% EtOH eluent gave **21a** (24 mg, 55%) as a colorless powder. $[\alpha]_D^{22}$ −20.5° (c=0.9, H_2O). IR (KBr) cm^{-1}: 1765, 1670. NMR (D_2O): 1.85—2.40 (2H, m), 3.34 (3H, s), 3.58 (2H, t, J=7 Hz), 4.30—4.60 (1H, m), 4.58 (2H, s), 5.39 (1H, d, J=5.5 Hz), 7.01 (1H, s). SIMS m/z: 518 (M^++Na), 496 (M^++H).

Disodium (3*S*,4*R*)-3-[2-(2-Aminothiazol-4yl)-(*Z*)-2-(1-methyl-1-carboxyethoxyimino)acetamido]-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (21b)——This compound was similarly synthesized from **20b** described above. A colorless powder. Yield 49%. $[\alpha]_D^{22}$ −2.4° (c=1, H_2O). IR (KBr) cm^{-1}: 1770, 1670. NMR (D_2O): 1.49 (6H, s), 1.90—2.40 (2H, m), 3.35 (3H, s), 3.65 (2H, t, J=6.5 Hz), 4.35—4.60 (1H, m), 5.39 (1H, d, J=5.5 Hz), 6.95 (1H, s). SIMS m/z: 546 (M^++Na), 524 (M^++H).

(3*R*)-3-Benzyloxycarbonylamino-4-*tert*-butoxycarbonylbutyric Acid (23)——Liquid isobutylene (40 ml) was added to a solution of (3*S*)-3-benzyloxycarbonylamino-4-methoxycarbonylbutyric acid (**22**) (14.76 g, 0.05 mol) and concentrated H_2SO_4 (2 ml) in CH_2Cl_2 (100 ml) under dry ice-acetone cooling. The mixture was sealed in a pressure bottle, shaken at room temperature for 72 h, and then poured into an excess of ice-cold aqueous $NaHCO_3$. Isobutylene and CH_2Cl_2 were removed under reduced pressure, the product was extracted with EtOAc (200 ml), and the organic layer was washed successively with saturated $NaHCO_3$ and brine, then dried and evaporated under reduced pressure. The oily residue was dissolved in MeOH (75 ml) and stirred for 2.5 h at room temperature after addition of 2 N NaOH (27 ml). The reaction mixture was adjusted to pH 7.0 with 2 N HCl and concentrated to about 30 ml. The concentrate was acidified with 10% citric acid and extracted with EtOAc. The extract was washed with brine, dried and concentrated under reduced pressure. The residue was triturated with hexane, and the resulting crystals were collected by filtration. Recrystallization from ether–hexane gave **23** (7.3 g, 43%) as colorless crystals, mp 102—107 °C. *Anal.* Calcd for $C_{17}H_{23}NO_6$: C, 60.52; H, 6.87; N, 4.15. Found: C, 60.21; H, 6.67; N, 4.24. $[\alpha]_D^{22}$ −1.3° (c=10, $CHCl_3$). IR (KBr) cm^{-1}: 3340, 1730, 1700. NMR ($CDCl_3$): 1.42 (9H, s), 2.58 (2H, d, J=6 Hz), 2.68 (2H, d, J=6 Hz), 4.15—4.55 (1H, m), 5.09 (2H, s), 5.68 (1H, d, J=8.5 Hz), 7.31 (5H, s), 9.12 (1H, br s).

(3*R*)-3-Amino-4-*tert*-butoxycarbonylbutyric Acid (24)——A solution of **23** (6.74 g, 20 mmol) and 5% Pd–C (350 mg) in 90% *tert*-BuOH (50 ml) was stirred for 6 h at room temperature under a hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was concentrated to dryness under reduced pressure. The resulting crystals were washed with ether, and recrystallized from MeOH–ether to give **24** as colorless crystals, 177—179 °C. *Anal.* Calcd for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 52.78; H, 8.34; N, 6.93. $[\alpha]_D^{22}$ +3.3° (c=1, H_2O). IR (KBr) cm^{-1}: 2980, 2930, 1730. NMR (D_2O): 1.48 (9H, s), 2.54 (1H, d, J=7 Hz), 2.55 (1H, d, J=6 Hz), 2.73 (2H, d, J=7 Hz), 3.70—4.00 (1H, m). MS m/z: 147 (M^++1−C_4H_9), 146 (M^+−C_4H_9).

(4*R*)-4-*tert*-Butoxycarbonylmethyl-2-azetidinone (25)——A solution of triphenylphosphine (4.72 g, 15 mmol) in CH_3CN (200 ml) was slowly added over a period of 3 h to a suspension of **24** (3.05 g, 15 mmol) and 2,2′-dipyridyl disulfide (3.64 g, 16.5 mmol) in CH_3CN (800 ml) at 80 °C. The reaction mixture was stirred for a further 2 h at the same temperature. The solvent was removed under reduced pressure, and benzene (30 ml) was added to the residue. After the removal of insoluble materials by filtration, the filtrate was concentrated, and the residue was

chromatographed on silica gel (ether) to give **25** (1.55 g, 55%) as colorless crystals, mp 83—84 °C. *Anal.* Calcd for $C_9H_{15}NO_3$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.32; H, 8.07; N, 7.63. $[\alpha]_D^{22}$ −46.1° ($c=1$, $CHCl_3$). IR (KBr) cm^{-1}: 3220, 1750, 1720. NMR ($CDCl_3$): 1.46 (9H, s), 2.50—2.75 (3H, m), 3.00—3.30 (1H, m), 3.75—4.05 (1H, m), 6.52 (1H, br s).

(4*S*)-4-(2-Hydroxyethyl)-2-azetidinone (26)——Compound **26** was synthesized from **25** by a method similar to that described for the preparation of **4**. A hygroscopic solid, mp 56—61 °C. Yield 78%. $[\alpha]_D^{22}$ −27.3° ($c=1$, $CHCl_3$). IR (KBr) cm^{-1}: 1735. NMR ($CDCl_3$): 1.70—2.00 (2H, m), 2.62 (1H, dd, $J=1.5$ and 15 Hz), 2.95—3.25 (1H, m), 3.35—3.90 (3H, m), 7.00 (1H, br s). MS *m/z*: 115 (M^+), 72 ($M^+ - CONH$).

(6*S*)-2,2-Dimethyl-3-oxa-1-azabicyclo[4.2.0]octan-8-one (27)——Compound **27** was synthesized from **26** by a method similar to that described for the preparation of **5**. A colorless oil. Yield 55%. $[\alpha]_D^{22}$ −28.1° ($c=1$, $CHCl_3$). IR (neat) cm^{-1}: 1750. NMR ($CDCl_3$): 1.40 (3H, s), 1.40—2.10 (2H, m), 1.73 (3H, s), 2.55 (1H, dd, $J=2$ and 15 Hz), 3.08 (1H, dd, $J=5$ and 15 Hz), 3.45—3.95 (3H, m). MS *m/z*: 140 ($M^+ - CH_3$).

(6*S*,7*S*)-7-Azido-2,2-dimethyl-3-oxa-1-azabicyclo[4.2.0]octan-8-one (28)——A 1.6 M solution of *n*-butyllithium (2.63 ml, 4.2 mmol) in hexane was added to a solution of diisopropylamine (425 mg, 4.2 mmol) in anhydrous THF (15 ml) at −78 °C under nitrogen. The mixture was stirred for 20 min at the same temperature, then a solution of **27** (640 mg, 4.12 mmol) in anhydrous THF (10 ml) was added dropwise. The reaction mixture was stirred for a further 20 min at −78 °C, and then a solution of *p*-toluenesulfonyl azide (830 mg, 4.2 mmol) in THF (5 ml) was added. After 10 min, trimethylchlorosilane (912 mg, 8.4 mmol) was added to the reaction mixture and the whole was gradually warmed to room temperature. After being stirred for 1 h at room temperature, the reaction mixture was diluted with EtOAc, and washed with brine, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel (ether : hexane = 2 : 1) to give **28** (570 mg, 70%) as colorless crystals, mp 106—108 °C. *Anal.* Calcd for $C_8N_{12}N_4O_2$: C, 48.97; H, 6.17; N, 28.56. Found: C, 49.02; H, 5.93; N, 28.38. $[\alpha]_D^{22}$ −197.7° ($c=1$, $CHCl_3$). IR (KBr) cm^{-1}: 2110, 1755, 1735. NMR ($CDCl_3$): 1.43 (3H, s), 1.50—2.10 (2H, m), 1.73 (3H, s), 3.45—3.65 (1H, m), 3.75—3.90 (2H, m), 4.22 (1H, d, $J=1.5$ Hz).

(6*S*,7*S*)-7-Benzyloxycarbonylamino-2,2-dimethyl-3-oxa-1-azabicyclo[4.2.0]octan-8-one (29)——Compound **29** was synthesized from **28** by a method similar to that described for the preparation of the *cis* isomer **8**. Colorless crystals, mp 138—141 °C. *Anal.* Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.21. Found: C, 63.14; H, 6.43; N, 9.25. $[\alpha]_D^{22}$ −0.5° ($c=1$, $CHCl_3$). IR (KBr) cm^{-1}: 3270, 1730. NMR ($CDCl_3$): 1.39 (3H, s), 1.70—2.10 (2H, m), 1.71 (3H, s), 3.45—3.65 (1H, m), 3.70—3.90 (2H, m), 4.37 (1H, dd, $J=2$ and 7 Hz), 5.10 (2H, s), 5.53 (1H, d, $J=7$ Hz), 7.33 (5H, s).

(3*S*,4*S*)-3-Benzyloxycarbonylamino-4-(2-hydroxyethyl)-2-azetidinone (30)——Compound **30** was synthesized from **29** by a method similar to that described for the preparation of the *cis* isomer **9**. Colorless crystals, mp 115—116 °C. *Anal.* Calcd for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.60. Found: C, 59.18; H, 5.96; N, 10.56. $[\alpha]_D^{22}$ −40.5° ($c=1$, MeOH). IR (KBr) cm^{-1}: 3330, 1750, 1700. NMR (CD_3OD): 1.75—2.00 (2H, m), 3.55—3.80 (3H, m), 4.33 (1H, d, $J=2$ Hz), 5.09 (2H, s), 7.32 (5H, s).

(3*S*,4*R*)-3-Benzyloxycarbonylamino-4-(2-methoxyethyl)-2-azetidinone (32)——A 2 N HCl solution (2 ml) was added to a solution of **12** (780 mg, 2 mmol) in MeOH (10 ml) and the mixture was stirred for 3 h at 40 °C. The mixture was adjusted to pH 7 with $NaHCO_3$ and concentrated under reduced pressure. The concentrate was diluted with EtOAc (30 ml) and brine, and the separated organic layer was washed with brine, dried, and evaporated under reduced pressure. The residue was crystallized from EtOAc–ether–hexane to give **32** (505 mg, 91%) as colorless crystals, mp 131 °C. *Anal.* Calcd for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.16; H, 6.51; N, 10.01. $[\alpha]_D^{22}$ +50.7° ($c=1$, $CHCl_3$). IR (KBr) cm^{-1}: 3300, 1780, 1735, 1695. NMR ($CDCl_3$): 1.65—1.90 (2H, m), 3.28 (3H, s), 3.35—3.60 (2H, m), 3.80—4.00 (1H, m), 5.08 (1H, dd, $J=5.5$ and 8 Hz), 5.11 (2H, s), 5.94 (1H, d, $J=8$ Hz), 6.29 (1H, br s), 7.33 (5H, s).

Pyridinium (3*S*,4*R*)-3-Benzyloxycarbonylamino-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (33)——A solution of **32** (460 mg, 1.65 mmol) and SO_3·Py (795 mg, 5 mmol) in CH_2Cl_2 (10 ml) and DMF (10 ml) was stirred for 5 h at 50 °C. After evaporation of the solvent under reduced pressure, the residue was dissolved in MeOH (5 ml). The resulting crystals were collected by filtration and washed with cold MeOH to give **33** (450 mg, 62%) as colorless crystals, mp 144—146 °C. *Anal.* Calcd for $C_{19}H_{22}N_3O_7S$: C, 52.29; H, 5.08; N, 9.63. Found: C, 52.04; H, 5.40; N, 9.63. $[\alpha]_D^{22}$ −15.6° ($c=1$, DMF). IR (KBr) cm^{-1}: 3325, 1755, 1695. NMR (DMSO-d_6): 1.50—2.35 (2H, m), 3.29 (2H, t, $J=7$ Hz), 3.75—4.00 (1H, m), 4.78 (1H, dd, $J=6$ and 9.5 Hz), 5.05 (2H, s), 7.34 (5H, s), 7.95—8.15 (3H, m), 8.02 (1H, d, $J=9.5$ Hz), 8.45—8.70 (1H, m), 8.85—9.00 (2H, m).

Tetra-*n*-butylammonium (3*S*,4*R*)-3-Benzyloxycarbonylamino-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (34)——A 10% tetra-*n*-butylammonium hydroxide solution (2.6 ml, 1 mmol) was added to a solution of **33** (450 mg, 1 mmol) in water (10 ml) at 0—5 °C. After being stirred for 20 min at the same temperature, the mixture was extracted three times with CH_2Cl_2 (25 ml). The combined organic solution was washed with brine, dried, and evaporated under reduced pressure. The oily residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 20 : 1) to give **34** as a colorless oil (500 mg, 83%). $[\alpha]_D^{22}$ −12.3° ($c=1$, $CHCl_3$). IR (neat) cm^{-1}: 1765, 1720, 1670. NMR ($CDCl_3$): 1.00 (12H, t, $J=7$ Hz), 1.20—1.85 (16H, m), 1.95—2.20 (2H, m), 3.15—3.55 (8H, m), 3.30 (3H, s), 3.40—4.60 (1H, m), 3.70—4.00 (1H, m), 4.25—4.45 (1H, m), 5.04 (1H, dd, $J=6$ and 9 Hz), 5.08 (2H, s), 6.52 (1H, d, $J=9$ Hz), 7.33 (5H, s). SIMS

m/z: 841 ($M^+ + NBu_3$).

Tetra-*n*-butylammonium (3*S*,4*R*)-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (35)——Compound **34** (460 mg, 0.77 mmol) was hydrogenated in DMF (10 ml) for 3 h over 5% Pd–C (50 mg) at room temperature under a hydrogen atmosphere. The catalyst was filtered off, then 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (215 mg, 0.77 mmol), *N*-hydroxybenzotriazole (104 mg, 0.77 mmol) and dicyclohexylcarbodiimide (160 mg, 0.77 mmol) were added to the filtrate at 0—5 °C. After being stirred overnight at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (10 ml) and the insoluble materials were filtered off and washed with EtOAc. The filtrate was washed with saturated $NaHCO_3$ and brine, dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 20 : 1) to give **35** (190 mg, 34%) as a foam. $[\alpha]_D^{22}$ −16.5° (*c* = 1, $CHCl_3$), IR (KBr) cm^{-1}: 1765, 1680. NMR ($CDCl_3$): 0.98 (12H, t, *J* = 7 Hz), 1.20—1.80 (16H, m), 2.00—2.40 (2H, m), 3.10—3.40 (8H, m), 3.27 (3H, s), 3.50—3.80 (2H, m), 3.97 (3H, s), 4.25—4.50 (1H, m), 4.34 (2H, s), 5.46 (1H, dd, *J* = 6 and 9 Hz), 7.13 (1H, s), 8.10 (1H, d, *J* = 9 Hz). SIMS *m/z*: 966 ($M^+ + NBu_4$).

Sodium (3*R*,4*R*)-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methoxy-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (37a)——A 1 N solution of lithium methoxide (0.8 ml, 0.8 mmol) in MeOH was added to a solution of **35** (280 mg, 0.386 mmol) in anhydrous THF (10 ml) at −78 °C. After being stirred for 20 min at the same temperature, *tert*-butyl hypochlorite (43 mg, 0.04 mmol) was added to the reaction mixture, and the mixture was stirred for 30 min at −78 °C. After additional stirring was continued for 2 h at −20 °C, the reaction was quenched by the addition of AcOH (0.2 ml), and the solvent was evaporated off under reduced pressure. The residue was dissolved in 30% acetone and treated with Dowex 50W (Na^+) (10 ml) for 1 h at room temperature. The resin was filtered off, and the filtrate was chromatographed on HP-20, and eluted with water, 10% and 20% EtOH. Lyophilization of the 10% eluent gave **37a** (69 mg, 34%) as a colorless powder. Lyophilization of the 20% eluent afforded sodium (3*R*,4*R*)-3-[2-(5-chloro-2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methoxy-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (**37b**) (54 mg, 25%) as a colorless powder.

37a: $[\alpha]_D^{22}$ +5.6° (*c* = 1, H_2O). IR (KBr) cm^{-1}: 1770, 1685. NMR (D_2O): 2.00—2.40 (2H, m), 3.35 (3H, s), 3.62 (3H, s), 3.62 (2H, t, *J* = 6 Hz), 4.05 (3H, s), 4.25 (1H, dd, *J* = 5 and 8.5 Hz), 4.40 (2H, s), 7.47 (1H, s).

37b: $[\alpha]_D^{22}$ +7.1° (*c* = 1, H_2O). IR (KBr) cm^{-1}: 1765, 1685. NMR (D_2O): 2.05—2.35 (2H, m), 3.35 (3H, s), 3.61 (3H, s), 3.61 (2H, t, *J* = 6 Hz), 4.06 (3H, s), 4.21 (1H, dd, *J* = 5 and 8.5 Hz), 4.37 (2H, s).

Sodium (3*R*,4*R*)-3-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methoxy-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (38a) and Sodium (3*R*,4*R*)-3-[2-(5-chloro-2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methoxy-4-(2-methoxyethyl)-2-azetidinone-1-sulfonate (38b)——Compounds **38a** and **38b** were synthesized from **37a** and **37b**, respectively, by a method similar to that described for the preparation of **17**.

38a: A colorless powder (73%). $[\alpha]_D^{22}$ +8.7° (*c* = 1, H_2O). IR (KBr) cm^{-1}: 1770, 1680. NMR (D_2O): 1.95—2.45 (2H, m), 3.35 (3H, s), 3.59 (3H, s), 3.60 (2H, t, *J* = 6.5 Hz), 4.01 (3H, s), 4.24 (1H, dd, *J* = 5 and 8 Hz), 6.96 (1H, s). SIMS *m/z*: 482 ($M^+ + Na$), 460 ($M^+ + H$).

38b: A colorless powder (81%). $[\alpha]_D^{22}$ +13.8° (*c* = 0.8, H_2O). IR (KBr) cm^{-1}: 1770, 1680. NMR (D_2O): 2.00—2.40 (2H, m), 3.36 (3H, s), 3.59 (3H, s), 3.60 (2H, t, *J* = 6.5 Hz), 4.02 (3H, s), 4.20 (1H, dd, *J* = 5 and 8.5 Hz). SIMS *m/z*: 516 ($M^+ + Na$), 494 ($M^+ + H$).

Acknowledgement We wish to express our thanks to Dr. M. Hamada of the Institute of Microbial Chemistry and Dr. S. Mitsuhashi of the Episome Institute for the biological tests, to Mr. K. Shizukuishi of the Application Laboratory, Naka Works, Hitachi, Ltd., for recording SIMS, and to Mr. K. Tsuneda of the Analytical Center, Teikoku Hormone Mfg. Co., Ltd., for recording the NMR and spectra, MS and elemental analyses. Thanks are also due to Drs. H. Mori and K. Yasuda, Research Laboratories, Teikoku Hormone Mfg. Co., Ltd., for helpful discussions.

References

1) A. Imada, K. Kitano, K. Kintaka, M. Muroi and M. Asai, *Nature* (London), **289**, 590 (1981).
2) R. B. Sykes, C. M. Cimarusti, D. P. Bonner, K. Bush, D. M. Floyd, N. H. Georgopapadakou, W. H. Koster, W. C. Liu, W. L. Parker, P. A. Principe, M. L. Rathnum, W. A. Slusarchyk, W. H. Trejo and J. S. Wells, *Nature* (London), **291**, 489 (1981).
3) R. B. Sykes, D. P. Bonner, K. Bush and N. H. Georgopapadakou, *Antimicrob. Agents Chemother.*, **21**, 85 (1982).
4) M. Sendai, S. Hashiguchi, M. Tomimoto, S. Kishimoto, T. Matsuo (deceased), M. Kondo and M. Ochiai, *J. Antibiot.*, **38**, 346 (1985).
5) *a*) M. Ohno, S. Kobayashi, T. Iimori, Y-F. Wang and T. Izawa, *J. Am. Chem. Soc.*, **103**, 2405 (1981); *b*) S. Kobayashi, T. Iimori, T. Izawa and M. Ohno, *ibid.*, **103**, 2406 (1981).
6) *a*) Y. Takahashi, H. Yamashita, S. Kobayashi and M. Ohno, *Chem. Pharm. Bull.*, **34**, 2732 (1986); *b*) T. Kametani, S-D. Chu, S-P. Huang and T. Honda, *Heterocycles*, **23**, 2693 (1985).

7) K. Kühlein and H. Jensen, *Justus Liebigs Ann. Chem.*, **1974**, 369.
8) J. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, Inc., New York, 1969, p. 226.
9) M. Ochiai, A. Morimoto, T. Miyawaki, Y. Matsushita, T. Okada, H. Natsugari and M. Kida, *J. Antibiot.*, **34**, 171 (1981).
10) S. Kishimoto, M. Sendai, S. Hashiguchi, M. Tomimoto, Y. Satoh, T. Matsuo (deceased), M. Kondo and M. Ochiai, *J. Antibiot.*, **36**, 1421 (1983).
11) G. A. Koppel and R. E. Koehler, *J. Am. Chem. Soc.*, **95**, 2403 (1973).