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

TAISUKE MATSUO (the late), HIROTOMO MASUYA, TOHRU SUGAWARA, YASUHIKO KAWANO, NORIYOSHI NOGUCHI and MICHIIKO 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 sulfonylation 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; sulfonylation; acylation; 3-amino-3-methoxy-2-azetidinone; desulfurization; antibacterial activity

In the preceding paper 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. 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 sulfonylation 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 methoxylolation reaction reported by Baldwin or Koppel, 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 or by the treatment of 6 with tert-butyl hypochlorite-lithium methoxide. Desulfurization of 4 with Raney nickel afforded (3R)-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 and methoxylolation 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 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), the 3-benzyloxy carbonylamino group was expected to stabilize the β-lactam ring (Chart 2). We then investigated the reaction with the 3-benzyloxy carbonylamino
compound (15), which was prepared by a reaction similar to that reported by Morin et al.\textsuperscript{80} to obtain (3R)-3-benzylxoycarbonylamino-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.,\textsuperscript{4b} with trimethyl phosphite. Therefore, 21 was synthesized by the sequence of reactions shown in Chart 3.

Chart 1

Chart 2
Chart 3

Chart 4
Retention of optical activity of 21 was confirmed by the following reactions (cf. Chart 4). Removal of the benzoxycarbonyl 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 (3R)-3-benzoxycarbonylamino-3-methoxy-2-azetidinone (24). By similar reactions, the dl-derivative (26) was prepared starting from (3S)-3-benzoxycarbonylamino-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-2-azetidinone (14), as shown in Chart 4.

(3R)-3-(dl-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, stepwise condensation of the amino acid units was carried out by an application of Miyoshi’s method. Compound 28, which was prepared from (3R)-3-amino-3-methoxy-2-azetidinone (27) and N-benzoxycarbonyl-d-alanine activated with diphosgene-triethylamine, 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 [a]D) and thin layer and paper chromatographic behavior of the synthetic sulfazecin with those of the natural product established the identity.

Some (3R)- and (3S)-3-acylamino-3-methoxy-2-azetidinone-1-sulfonic acids were syn-
Table I. In Vitro Antibacterial Activity of 3-Acylamino-3-methoxy-2-azetidinone-1-sulfonic Acid Derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tr>
<td></td>
<td>H</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>SO₃H</td>
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<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
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<tr>
<td></td>
<td>SO₂H</td>
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<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (µg/ml)</th>
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<tr>
<td><em>Escherichia coli</em> O-111</td>
<td>3.13 6.25 25</td>
</tr>
<tr>
<td><em>Escherichia coli</em> T-7</td>
<td>&gt;100 12.5 50</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> DT</td>
<td>0.2 12.5 50</td>
</tr>
</tbody>
</table>

Inoculum size: 10⁶ CFU/ml.

Thesized by using the dl-derivative (26) as a starting material. A typical example is as follows. Removal of the benzylxycarbonyl group from 26 gave the dl-3-amino-3-methoxy-2-azetidinone, which was acylated with an acid chloride possessing a chiral center 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. ¹H-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[(3S,4R)-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 Na₂S₂O₅ solution was added. The reaction mixture was extracted with CH₂Cl₂ and the organic layer was washed with water, dried over anhydrous Na₂SO₄, 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 C₂₀H₂₅N₆O₅S₂; C, 57.28; H, 5.61; N, 7.42. Found: C, 57.44; H, 5.40; N, 7.41. [α]D²₀ = -71.2° (c = 1, EtOH).
IR νmax/cm⁻¹: 1775, 1725, 1700. H-NMR (CDCl₃) δ: 2.16 (3H, s, CH₃), 2.27 (3H, s, CH₃), 3.20 (3H, s, OCH₃), 3.73 (2H, s, CH₂), 3.83 (3H, s, OCH₃), 5.20 (1H, s, C=H), 6.00 (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 bisdialkyl derivative (6.1, 2.3l 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 Na₂SO₄, and evaporated. The crude product was chromatographed on silica gel. Elution with n-hexane-AcOEt (1:1) gave 4 (1.0, 40%).

(3S,4R)-3-Methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-4-methylthio-3-phenylacetamido-2-azetidine (8) was obtained using a similar procedure as described above. A mixture of 4 (2.8 ml) and LiOMe-MeOH solution (6.8 ml of 1.78 mmol solution) were added dropwise to a solution of the 4-methylthio derivative (7) (0.8 g) in dry THF (20 ml) at −70°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 Na₂SO₄, 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 C₂₉H₂₅N₆O₅S₂: C, 58.15; H, 6.16; N, 7.14. Found: C, 57.98; H, 6.23; N, 7.08. IR νmax/cm⁻¹: 1768, 1720, 1700 (br), 1650 (sh). H-NMR (CDCl₃) δ: 2.06 (6H, s, CH₃), 2.28 (3H, s, CH₃), 3.52 (2H, s, CH₂), 3.76 (3H, s, OCH₃), 3.75 (3H, s, OCH₃), 5.08 (1H, s, CH), ca. 6.2 (1H, br s, NH), 7.37 (5H, s, aromatic-H).

(3R)-3-Methoxy-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-3-phenylacetamido-2-azetidine (9) was obtained using a similar procedure as described above. 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. [α]D²₀ = +98.2° (c = 1, MeOH).
IR νmax/cm⁻¹: 3300, 1760, 1730, 1690. H-NMR (CDCl₃) δ: 1.92 (3H, s, CH₃), 2.22 (3H, s, CH₃), 3.43 (3H, s, OCH₃), 3.62 (2H, s, CH₂), 3.70 (3H, s, OCH₃), 3.88 (1H, d, J = 6 Hz, C₆H), 4.03 (1H, d, J = 6 Hz, C₆H), 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%). [α]D²₀ = +103.3° (c = 1, MeOH).

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 insoluble material, the filtrate was concentrated and then partitioned with AcOEt and water. The organic layer was washed with water, dried over anhydrous Na₂SO₄, and evaporated. Treatment as described above afforded 9 (0.118 g, 32.9%). [α]D²₀ = +101.7° (c = 1, MeOH).

(3S,4R)-3-Benzoxycarbonylaminom-3-methoxy-1-(1R)-methoxy carbonyl-2-methylprop-2-enyl)-4-(benzothiazol-2-yl-dithio)-2-azetidinone (18) and Bis[(3S,4R)-3-benzyloxy carbonylaminom-3-methoxy-1-(1R)-methoxy carbonyl-2-methylprop-2-enyl]-2-oazazetidin-4-yl]] Disulfide (19) was obtained using a similar procedure as described above. 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, H-NMR (CDCl₃) δ: 1.93 (3H, d, J = 0.5 Hz, CH₃), 3.46 (3H, s, OCH₃), 3.76 (3H, s, OCH₃), 5.03 (2H, d, J = 4 Hz, CH₂), 5.20 (1H, q, J = 0.5 Hz, CH), 5.23 (2H, s, CH₂), 5.33 (1H, s, C₆H), 7.13 (1H, s, NH), 7.3−8.0 (9H, m, aromatic-H).

19: Colorless plates, mp 131−133°C (dec.). Anal. Calcd for C₃₆H₃₉N₅O₅S₄: C, 54.95; H, 5.38; N, 7.12. Found: C, 54.88; H, 5.41; N, 7.22. [α]D²₀ = −197.9° (c = 0.98, CHCl₃). IR νmax/cm⁻¹: 3300, 1770, 1760, 1735 (sh), 1725, 1500. H-NMR (CDCl₃) δ: 1.83 (3H, d, J = 0.5 Hz, CH₃), 3.65 (3H, s, OCH₃), 3.70 (3H, s, OCH₃), 4.90 (2H, d, J = 4 Hz, CH₄), 5.06 (1H, q, J = 0.5 Hz, CH), 5.16 (2H, s, CH₂), 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[(3S,4R)-3-benzyloxy carbonylaminom-3-methoxy-1-(1-methoxy carbonyl-2-methylprop-1-enyl)-2-oazazetidin-4-yl]] Disulfide (20) was obtained using a similar procedure as described above. A mixture of 17 (2 g) in dry dioxane (200 ml) was added to a cooled (4°C) solution of 20 (2 g, containing a small amount of 19 on TLC) in CH₂Cl₂ (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 20 (9.7 g, 30% yield). [α]D²₀ = −33.4° (c = 0.99, CHCl₃). IR νmax/cm⁻¹: 1780, 1750, 1500. H-NMR (CDCl₃) δ: 2.00 (3H, s, CH₃), 2.23 (3H, s, CH₃), 3.50 (3H, s, OCH₃), 3.70 (3H, s, OCH₃), 5.00 and 5.23 (2H, each d, J = 12 Hz, CH₂), 5.03 (1H, s, C₆H), 6.26 (1H, s, NH), 7.23 (5H, s, aromatic-H).
(3R)-3-Benzoxycarbonylamino-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 (3S)-3-benzoxycarbonylamino-1-(1-methoxycarbonyl-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 Na2S2O3 solution and water successively, then dried over anhydrous Na2SO4. 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. Calc'd for C14H15NO2S: C, 59.6; H, 6.12; N, 7.73. Found: C, 59.55; H, 6.14; N, 7.68. IR ν(CH3) cm⁻¹: 1761, 1723, 1140. 1H-NMR (CDCl3) δ: 1.91 (3H, s, CH3), 2.22 (3H, s, CH3), 3.53 (3H, s, OCH3), 3.73 (3H, s, OCH3) 3.8–4.4 (2H, m, C2-H), 5.20 (2H, s, CH2), 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. Benzoxycarbonyl chloride (0.8 g) in CH2Cl2 (1 ml) was added dropwise to a solution of the 3-amino derivative obtained above and pyridine (0.37 g) in CH2Cl2 (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 Na2SO4, and evaporated. Purification was carried out by chromatography on silica gel; elution with n-hexane-CH2Cl2 (1:1) gave 21 (0.836 g, 31.7% from 20) as a colorless viscous oil. IR νmax cm⁻¹: 3260, 1760, 1718, 1510.

(3R)-3-Benzoxycarbonylamino-3-methoxy-2-azetidinone (24) and Its dl Derivative (26)—(a) A solution of 25 (1.5 g) in dry CH2Cl2 (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 Me2S (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 Na2SO4. The solvent was evaporated off to afford the dl-derivative (26, 1.0 g, 96.6%) as colorless prisms. mp 122–115 °C. Anal. Calc'd for C14H14O2S: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.44; H, 5.45; N, 10.88. IR νmax cm⁻¹: 3420, 1774, 1723. 1H-NMR (CDCl3) δ: 3.45 (3H, s, OCH3), 3.58 and 3.76 (2H, each d, J = 6 Hz, C4-H), 5.14 (2H, s, CH2), 6.74 (2H, br s, NH), 7.34 (5H, s, aromatic-H).

(b) A similar reaction using 21 afforded (3R)-3-benzoxycarbonylamino-3-methoxy-2-azetidinone (24, 87.1%) as colorless prisms (AcOEt—n-hexane). mp 101–101 °C. Anal. Calc'd for C12H12O2S: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.46; H, 5.52; N, 11.09. [α]D20 +68.2° (c = 1, MeOH). IR νmax cm⁻¹: 3260, 1765, 1722 (sh), 1705. The 1H-NMR spectrum was identical with that of 26.

Sodium (3R)-(3S)-N-(o-Glutamyl-d-alanlylamino)-3-methoxy-2-azetidinone-1-sulfonate (31)—(a) Diphosgene (1.89 g) in dry THF (4 ml) and Et3N (2.1 g) in dry THF (4 ml) were added dropwise to a cooled (−40 °C) solution of N-benzoylcarbonyl-d-alanine (4.46 g) in dry THF (35 ml). After 30 min, Et3N (2.1 g) was added to this reaction mixture and the whole was stirred for an additional 30 min. (3R)-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 (3R)-3-(N-Benzoylcarbonyl-d-alanyl)-3-methoxy-2-azetidinone (28, 0.905 g, 32%) as a foam. Anal. Calc'd for C25H23NO5S: C, 56.07; H, 5.96; N, 13.08. Found: C, 56.22; H, 6.02; N, 12.91. [α]D20 +79.5° (c = 1, MeOH). IR νmax cm⁻¹: 3270, 1755, 1680, 1520, 1245. 1H-NMR (DMSO-δ6) δ: 1.22 (3H, d, J = 7 Hz, CH3), 3.32 (3H, s, OCH3), 3.40 and 3.48 (2H, each d, J = 7 Hz, C4-H), 4.14 (1H, m, CH3), 5.04 (2H, s, CH2), 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 dropwise to a cooled (ca. −20 °C) solution of dimethylformamide (DMF) (0.205 g) in CH2Cl2 (10 ml) and the resulting solution was stirred at room temperature for 40 min. α-D-Butyl-N-benzyl-N-benzoylcarbonyl-L-glutamic acid (0.747 g) and Et3N (0.3 g) in CH2Cl2 (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 Et3N (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 CH2Cl2—AcOEt (1.1:1 to 1:2) gave (3R)-3-(α-D-Benzy1-N-benzoylcarbonyl-D-glutamyl-d-alanlylamino)-3-methoxy-2-azetidinone (29, 0.23 g, 23% yield) as colorless needles, mp 164–165 °C (dec.). Anal. Calc'd for C15H18NO5S: C, 59.99; H, 5.97; N, 10.36. Found: C, 60.21; H, 5.69; N, 10.18. [α]D20 +71° (c = 1, MeOH). IR νmax cm⁻¹: 3260, 1750, 1700, 1650, 1520, 1250, 1200. 1H-NMR (CDCl3) δ: 1.50 (3H, d, J = 7 Hz, CH3), 1.80–2.50 (2H, m, CH2), 3.40 (3H, s, OCH3), 3.53 and 3.66 (2H, each d, J = 7 Hz, C4-H), 4.30 (1H, m, CH3), 4.60 (1H, m, CH3), 5.03 (2H, s, CH2), 5.10 (2H,
s, CH$_3$), 6.20 (1H, m, NH), 7.00 (1H, brs, NH), 7.30 (10H, s, aromatic-H), 8.40 (1H, brs, NH).

(c) A mixture of the 2-azetidinone derivative (29, 0.23 g) prepared above (b) and SO$_2$ pyridine complex (0.078 g) in DMF (1 ml) was stirred at room temperature. After 10 h, additional SO$_2$ 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$_2$O, then dissolved in AcOEt-MeOH (5:1) solution. Purification was carried out by chromatography on silica gel to yield pyridinium (3R)-3-(O-benzyl-N-benzylmethoxycarbonyl-γ-γ-glutamyl-b-alanyl-amino)-3-methoxy-2-azetidinone-1-sulfonate (30, 0.18 g, 60.4%) as a powder. [α]$_D^{22}$ +79.6$^\circ$ (c = 1.03, MeOH). IR v$_{max}^{KBr}$ 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, brs, NH).

(d) A mixture of the pyridinium 2-azetidinone-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 mirabilis disk method) were lyophilized to obtain sulfacetin as the mono sodium salt (31). C$_{12}$H$_{14}$Na$_2$O$_6$.H$_2$O: C, 33.03; H, 4.85; H, 12.84. Found: C, 32.58; H, 4.99; N, 12.55. [α]$_D^{22}$ +82.9$^\circ$ (c = 0.55, H$_2$O). IR v$_{max}^{KBr}$ 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 (3S)- and (3R)-[p-2-(3-Furfurylideneamino-2-oxoimidazolidin-1-y1)carbonylamino-2-phenylacetamidol]-3-methoxy-2-azetidinone-sulfonates (33 and 34) — A mixture of 3-benzylxycarbonylamino-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 2-azetidinone (0.174 g). IR v$_{max}^{KBr}$ cm$^{-1}$: 3280, 1760, 1720, 1670, 1475, 1410, 1230. 1H-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). (3S)-[p-2-(3-Furfurylideneamino-2-oxoimidazolidin-1-y1)carbonylamino-2-phenylacetamidol]-3-methoxy-2-azetidinone: 0.141 g (15%). Powder, Anal. Caled for C$_{12}$H$_{14}$Na$_2$O$_6$.H$_2$O: C, 33.39; H, 5.12; N, 17.79. Found: C, 53.30; H, 4.90; N, 17.86. IR v$_{max}^{KBr}$ 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 (3S)-isomer (0.228 g) and SO$_2$ 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 dried with Dowex 50W resin (Na$^+$ form) in water. Purification was carried out by Amberlite XAD-2 column chromatography to obtain the (3S)-isomer of the title compound 34 (0.11 g, 40%) as a powder. Anal. Caled for C$_{19}$H$_{20}$Na$_2$O$_6$.3H$_2$O: C, 41.31; H, 4.46; N, 13.77. Found: C, 41.75; H, 4.26; N, 13.68. IR v$_{max}^{KBr}$ cm$^{-1}$: 3300, 1770, 1720, 1670, 1475, 1420, 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 (3R)-isomer (33) was obtained in a similar manner. Powder (32%). Anal. Caled for C$_{19}$H$_{20}$Na$_2$O$_6$.3H$_2$O: C, 41.31; H, 4.46; N, 13.77. Found: C, 41.14; H, 4.78; N, 13.91. IR v$_{max}^{KBr}$ 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 (3R)-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 (3S)- and (3R)-[p-2-(4-Cyclohexyl-3-dioxy-1-piperazinylcarbonylamino)-2(2-thienyl)acetamidol]-3-methoxy-2-azetidinone-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 (3S)-isomer (38%) and (3R)-isomer (34%).
(3R)-Isomer: Powder, Anal. Calcd for C_{21}H_{27}N_{2}O_{6}S: C, 52.82; H, 5.70; N, 14.67. Found: C, 52.44; H, 5.61; N, 14.27. IR $\nu_{\text{max}}^\text{KBr}$ cm$^{-1}$: 2920, 1760, 1705, 1670, 1500, 1170. $^1$H-NMR (DMSO-d$_6$) $\delta$: 3.36 (3H, s, OCH$_3$), 3.39 and 3.47 (2H, each d, $J = 6$ Hz, C$_6$H), 5.87 (1H, d, $J = 7$ Hz, CH), 6.9–7.6 (3H, m, thiényl-H), 8.30 (1H, s, NH), 9.67 (1H, s, NH), 9.69 (1H, d, $J = 7$ Hz, NH).

(3S)-Isomer: Powder, Anal. Calcd for C$_{21}$H$_{27}$N$_2$O$_6$S: C, 52.82; H, 5.70; N, 14.67. Found: C, 52.43; H, 5.71; N, 14.54. IR $\nu_{\text{max}}^\text{KBr}$ cm$^{-1}$: 2920, 1760, 1705, 1670, 1500, 1170. $^1$H-NMR (DMSO-d$_6$) $\delta$: 3.18 (3H, s, OCH$_3$), 3.42 and 3.54 (2H, each d, $J = 6$ Hz, C$_6$H), 5.87 (1H, d, $J = 7$ Hz, CH), 6.9–7.6 (3H, m, thiényl-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.

(3R)-Isomer, 36 (70% yield): Powder, Anal. Calcd for C$_{21}$H$_{28}$N$_2$NaO$_6$S$_2$·2H$_2$O: C, 40.97; H, 4.91; N, 11.38. Found: C, 40.93; H, 5.00; N, 11.11. IR $\nu_{\text{max}}^\text{KBr}$ cm$^{-1}$: 1765, 1710, 1675, 1505, 1250, 1175, 1050. $^1$H-NMR (DMSO-d$_6$) $\delta$: 3.34 (3H, s, OCH$_3$), 3.56 (2H, s, C$_6$H), 5.86 (1H, d, $J = 7$ Hz, CH), 6.9–7.5 (3H, m, thiényl-H), 9.75 (1H, d, $J = 7$ Hz, NH), 9.77 (1H, s, NH).

(3S)-Isomer, 37 (68% yield): Powder, Anal. Calcd for C$_{21}$H$_{28}$N$_2$NaO$_6$S·2.5H$_2$O: C, 40.38; H, 5.00; N, 11.21. Found: C, 40.42; H, 4.84; N, 11.34. IR $\nu_{\text{max}}^\text{KBr}$ cm$^{-1}$: 1765, 1710, 1675, 1505, 1250, 1175, 1050. $^1$H-NMR (DMSO-d$_6$) $\delta$: 3.16 (3H, s, OCH$_3$), 3.55 and 3.71 (2H, each d, $J = 7$ Hz, C$_6$H), 5.89 (1H, d, $J = 7$ Hz, CH), 6.9–7.6 (3H, m, thiényl-H), 9.71 (1H, d, $J = 7$ Hz, NH), 9.79 (1H, s, NH).

(c) Starting from the optically active derivative (24), the (3R)-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.
13) Ref. 6, p. 192.