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Synthesis of Carumonam (AMA-1080) and a Related Compound Starting from (2*R*,3*R*)-Epoxysuccinic Acid[1)]

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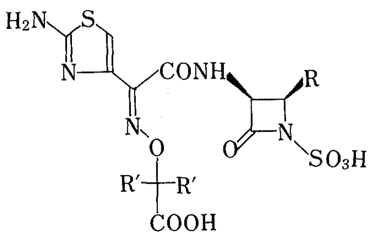
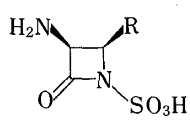
In the course of the chemical modification of sulfazecin, several 4-carbamoyl-2-azetidinone-1-sulfonic acid derivatives were synthesized with the aim of improving the antibacterial activity. Among those compounds, (3*S*,4*S*)-3-[2-(2-aminothiazol-4-yl)-(*Z*)-2-(1-carboxy-1-methylethoxyimino)acetamido]-4-carbamoyl-2-azetidinone-1-sulfonic acid (**2**) was found to have potent antibacterial activity, comparable to that of carumonam (**1**, AMA-1080; Ro 17-2301), against gram-negative bacteria. Efficient synthetic pathways to prepare **1** and **2** in large quantities were developed based on (2*R*,3*R*)-epoxysuccinic acid (**5**), an easily accessible fermentation product, as a starting chiral synthon.

Keywords——sulfazecin; AMA-1080; 4-carbamoyl-2-azetidinone-1-sulfonic acid; sulfonation; antibacterial activity; β-lactamase stability; structure–activity relationship; chiral sulfazecin-type derivative; (2*R*,3*R*)-epoxysuccinic acid

As reported in our previous communication,[2)] two sulfazecin-type derivatives, (3*S*,4*S*)-3-[2-(2-aminothiazol-4-yl)-(*Z*)-2-carboxymethoxyiminoacetamido]-4-carbamoyloxymethyl-2-azetidinone-1-sulfonic acid (**1**, AMA-1080; Ro 17-2301)[3)] and (3*S*,4*S*)-3-[2-(2-aminothiazol-4-yl)-(*Z*)-2-(1-carboxy-1-methylethoxyimino)acetamido]-4-carbamoyl-2-azetidinone-1-sulfonic acid (**2**), were found to exhibit strong antibacterial activity against gram-negative bacteria and high stability to β-lactamases produced by various bacterial species. In a preceding paper,[4)] the synthesis and structure–activity relationships of 4-(substituted methyl)-2-azetidinone-1-sulfonic acids including **1** were reported. In this paper, the synthesis and antibacterial activity of **2** and some related compounds, and efficient syntheses of the important chiral intermediates (**3** and **4**) for the preparation of **1** and **2** starting from (2*R*,3*R*)-epoxysuccinic acid (**5**)[5)] will be described.



1 : R=CH_2OCONH_2, R′=H
2 : R=$CONH_2$, R′=CH_3



3 : R=CH_2OCONH_2
4 : R=$CONH_2$

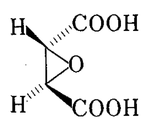


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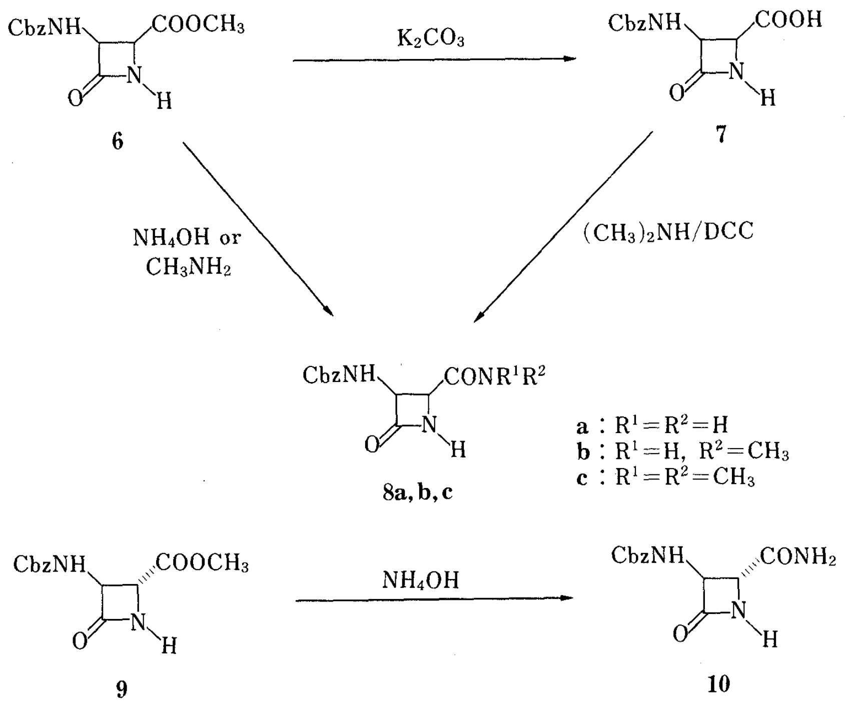
Chart 1

Synthesis and Antibacterial Activity of 4-Carbamoyl Derivatives

cis-3-Benzyloxycarbonylamino-4-methoxycarbonyl-2-azetidinone (**6**)[6)] was easily converted into the corresponding 4-carbamoyl and 4-(*N*-methylcarbamoyl) derivatives (**8a**, **b**) by treatment with ammonia–water and methylamine in tetrahydrofuran; however, a similar direct conversion of **6** into the 4-(*N*,*N*-dimethylcarbamoyl) compound (**8c**) was unsuccessful. Therefore, **6** was first hydrolyzed to give the 4-carboxy intermediate (**7**), which was then converted into **8c** by condensation with dimethylamine in the presence of *N*,*N*′-dicyclohexylcarbodiimide (Chart 2). The *trans* isomer (**10**) of **8a** was similarly prepared by treating *trans*-3-benzyloxycarbonylamino-4-methoxycarbonyl-2-azetidinone (**9**)[6] with ammonia–water.

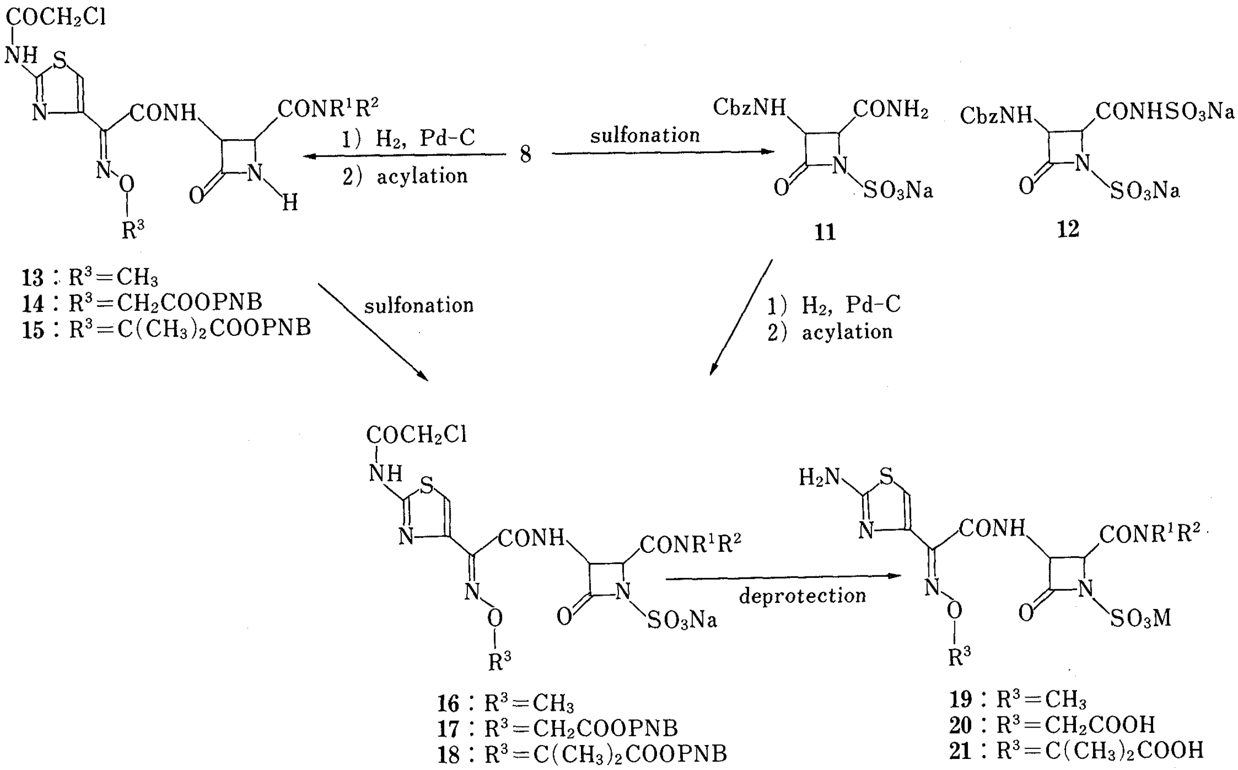


Cbz: carbobenzoxy

Chart 2

In the case of 4-carbamoyl compounds, sulfonation may take place at the carbamoyl moiety as well as on the *β*-lactam nitrogen. In order to examine this possibility, compound **8a** was treated with sulfur trioxide–*N*,*N*-dimethylformamide complex ($SO_3 \cdot DMF$) at 0—5 °C in *N*,*N*-dimethylformamide. Under these reaction conditions, a mixture of almost equal amounts of the monosulfo and disulfo compounds (**11** and **12**) was obtained. Therefore, sulfonation of **8a** was investigated in detail, and after various attempts, chemoselective sulfonation on the *β*-lactam nitrogen was achieved by treating a suspension of **8a** in dioxane with sulfur trioxide–pyridine complex ($SO_3 \cdot Py$) at room temperature. The reaction proceeded under heterogeneous conditions, and the desired product (**11**) was isolated in 87% yield as its sodium salt. Deprotection of **11** by hydrogenolysis and subsequent acylation with 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl chloride hydrochloride and its analogs gave 3-acylamino-2-azetidinone-1-sulfonic acid derivatives (**16a**, **17a** and **18a**). Meanwhile, the 4-(*N*-methylcarbamoyl) and 4-(*N*,*N*-dimethylcarbamoyl) compounds (**8b** and **8c**) were transformed to the protected sulfazecin-type derivatives (**16b**, **c**, **17b** and **18b**) by the routine sequence[6] comprising deprotection, acylation and sulfonation, as shown in Chart 3. The *N*-methylcarbamoyl moieties of **13b**, **14b** and **15b** were not affected by sulfonation. Conventional treatment[6] of compounds **16**—**18** afforded the deprotected 1-sulfo-2-azetidinones (**19**—**21**).

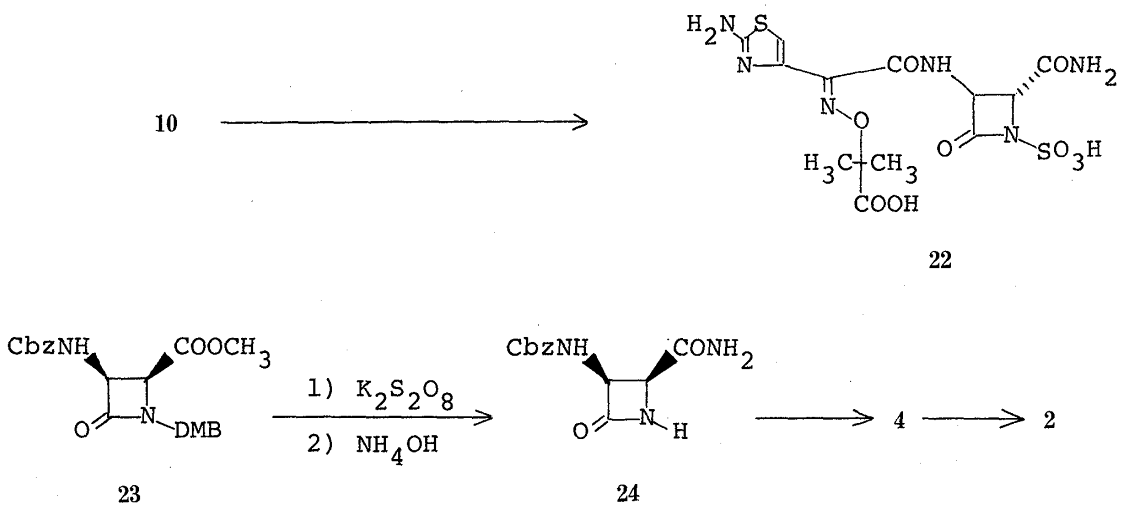
The *trans* isomer (**22**) and (3*S*,4*S*)-isomer (**2**) of **21a** were also synthesized in a similar



a : $R^1=R^2=H$, **b** : $R^1=H$, $R^2=CH_3$, **c** : $R^1=R^2=CH_3$

PNB : *p*-nitrobenzyl M=H or Na

Chart 3



DMB: 2,4-dimethoxybenzyl

Chart 4

manner to that described for the preparation of **21a** (Chart 4). The starting material (**23**) of the (3*S*,4*S*)-series was obtained by optical resolution of the corresponding racemic 3-amino compound and subsequent carbobenzoxylation.[2,4)]

The antibacterial activity of 1-sulfo-2-azetidinones described above is shown in Tables I and II. All compounds showed good to moderate activity against gram-negative bacteria. Introduction of a methyl group into the 4-carbamoyl moieties of **19a** and **20a** resulted in a

TABLE I. Antibacterial Activity[a] of (*Z*)-Methoxyimino Derivatives (**19a—c**) and (*Z*)-Carboxymethoxyimino Derivatives (**20a, b**)

Organism	MIC (μg/ml)				
	19a	**19b**	**19c**	**20a**	**20b**
E. coli NIHJ JC-2	0.2	0.78	3.13	0.2	0.39
E. coli T-7	0.2	1.56	6.25	0.2	0.78
E. cloacae IFO 12937	1.56	3.13	12.5	6.25	12.5
S. marcescens IFO 12648	0.2	0.78	3.13	0.2	0.39
P. vulgaris IFO 3988	0.39	0.78	3.13	0.2	0.39
P. aeruginosa IFO 3455	3.13	25	>100	1.56	6.25

a) Activity was determined by the agar dilution method using an inoculum of 10^8 CFU/ml.
MIC: minimum inhibitory concentration.

TABLE II. Antibacterial Activity[a] of (*Z*)-(1-Carboxy-1-methylethoxyimino) Derivatives (**21a, b**, **22** and **2**)

Organism	MIC (μg/ml)			
	21a	**21b**	**22**	**2**
E. coli NIHJ JC-2	0.78	6.25	1.56	0.2
E. coli T-7	0.78	12.5	3.13	0.39
E. cloacae IFO 12937	1.56	12.5	6.25	0.39
S. marcescens IFO 12648	0.39	3.13	1.56	0.2
P. vulgaris IFO 3988	0.39	1.56	0.78	0.2
P. aeruginosa IFO 3455	3.13	50	50	1.56

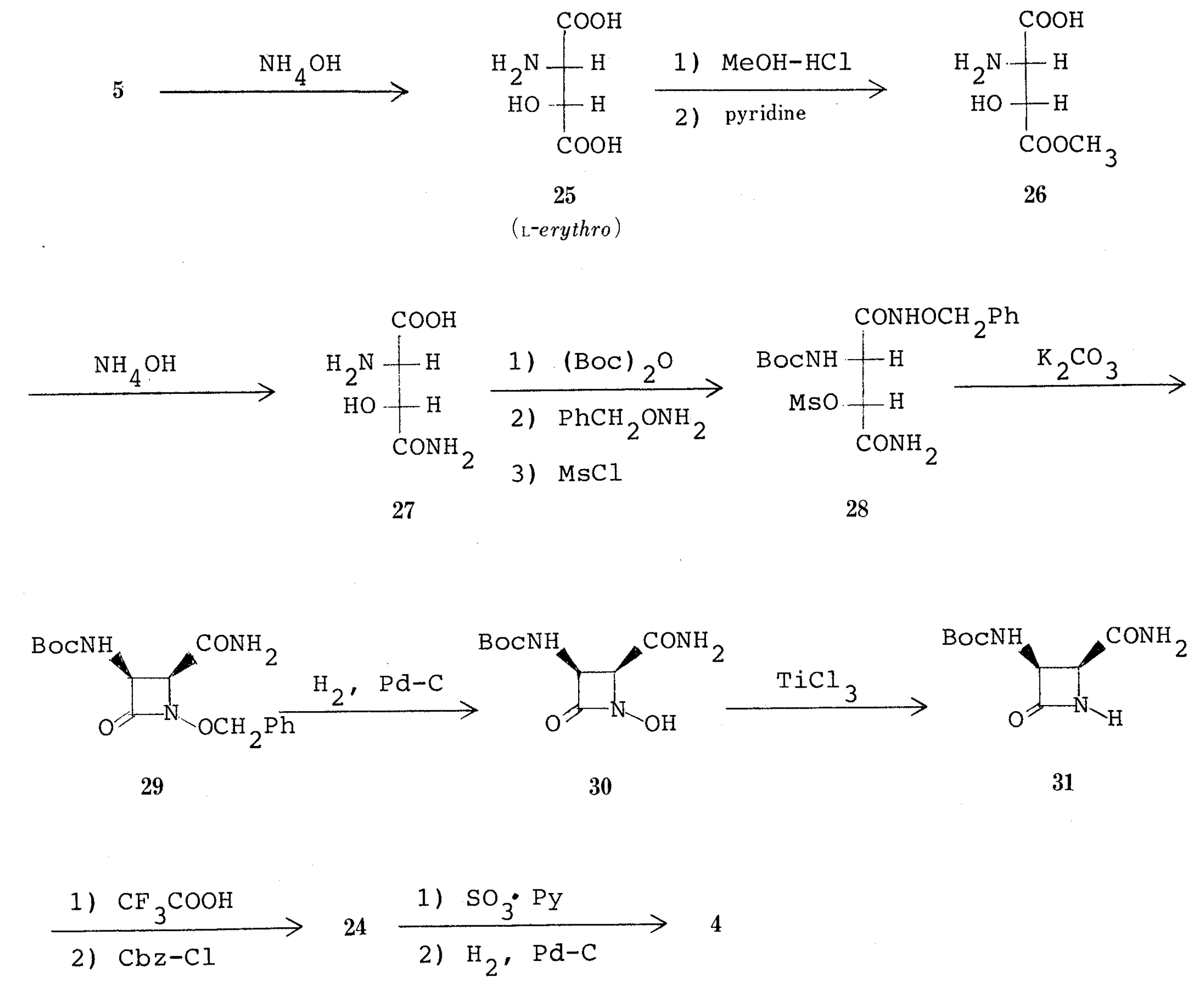
a) Activity was determined by the agar dilution method using an inoculum of 10^8 CFU/ml.

slight decrease of activity against all organisms tested, while a similar modification on **21a** and introduction of two methyl groups into the 4-carbamoyl moiety of **19a** brought about a significant diminution of the activity. The *trans* isomer (**22**) is less active than the corresponding *cis* isomer (**21a**) in analogy with the cases of other 4-substituted derivatives.[4,6] Among racemic compounds, **21a** showed the most promising activity against gram-negative bacteria including *Enterobacter cloacae* IFO 12937, a producer of Richmond 1a-type β-lactamase, and its (3*S*,4*S*)-isomer (**2**) was selected as a candidate for further biological evaluation.

Synthesis of (3*S*)-3-Amino-2-azetidinone-1-sulfonic Acids (3 and 4) Starting from (2*R*,3*R*)-Epoxysuccinic Acid (5)

The next target of our research was to develop efficient pathways leading to the key intermediates (**3** and **4**) to prepare the two sulfazecin-type candidates (**1** and **2**). When the carbon skeletons of **3** and **4** were taken into consideration, (2*R*,3*R*)-epoxysuccinic acid (**5**)[5] was expected to be a rational starting material for the practical syntheses of these chiral compounds.[1,7] Compound **5** obtained by fermentation was converted into pure *erythro*-3-hydroxy-L-aspartic acid (**25**), $[\alpha]_D^{24.5}$ $+55.6°$ ($c=0.9$, 1 N HCl),[10] by treatment with ammonia–water.[5] The synthetic routes to **4** and **3** are summarized in Charts 5 and 6.

erythro-3-Hydroxy-L-asparagine (**27**), prepared from **25** by the procedure of Singerman *et al.*,[11] was converted into *O*-benzyl (2*S*,3*R*)-2-(*tert*-butoxycarbonylamino)-3-carbamoyl-3-mesyloxypropanohydroxamate (**28**), which was then cyclized to (3*S*,4*S*)-1-benzyloxy-3-(*tert*-butoxycarbonylamino)-4-carbamoyl-2-azetidinone (**29**) in heated acetone containing potas-



Boc: *tert*-butoxycarbonyl

Chart 5

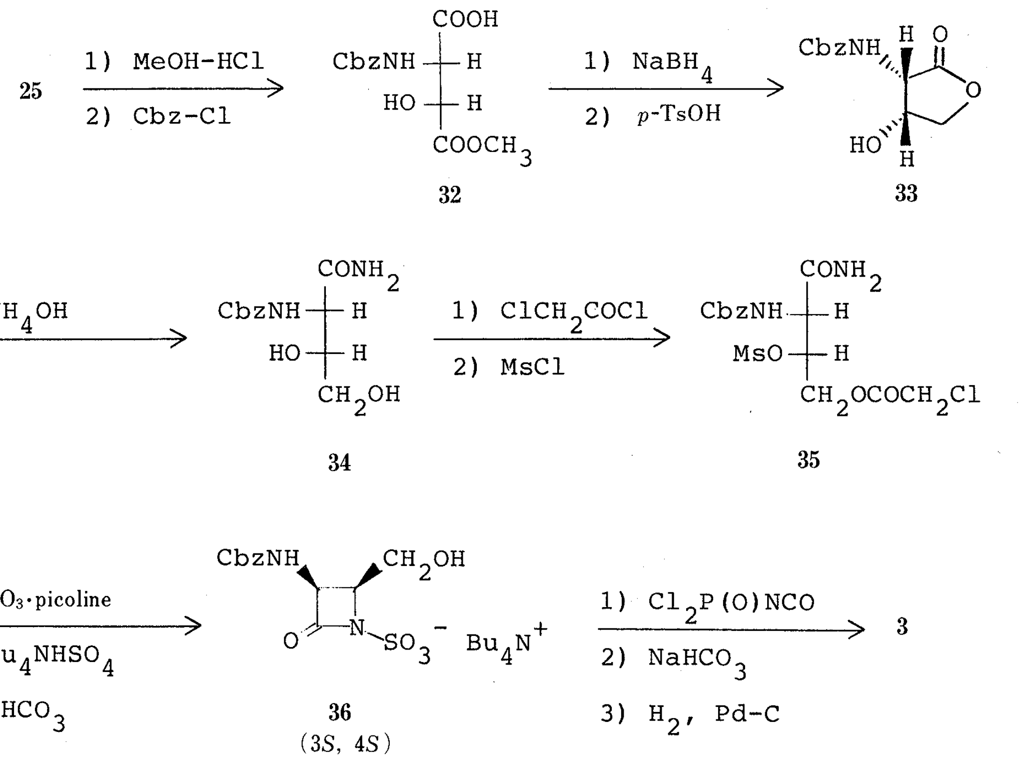


Chart 6

sium carbonate.[12)] Removal of the benzyloxy group from the 1-position was effected by the two-step procedure reported by Miller *et al.*[13)]: hydrogenolysis of the benzyl group afforded the 1-hydroxy compound (**30**), which was converted into the desired 1-unsubstituted-2-azetidinone (**31**) by treatment with 20% titanium trichloride solution. Deprotection of the 3-amino group and subsequent carbobenzoxylation gave (3*S*,4*S*)-3-benzyloxycarbonylamino-4-carbamoyl-2-azetidinone (**24**), which was identical with an authentic sample (Chart 4). After sulfonation and hydrogenation, (3*S*,4*S*)-3-amino-4-carbamoyl-2-azetidinone-1-sulfonic acid (**4**), the key intermediate for preparing **2**, was obtained as colorless crystals (Chart 5).

Esterification of **25** and subsequent carbobenzoxylation of the crude product gave the *N*-protected monoester (**32**), which was converted into the γ-lactone (**33**) in two steps. Ammonolysis of this lactone was conducted cleanly in a hexane suspension with ammonia–water to give (2*S*,3*R*)-2-benzyloxycarbonylamino-3,4-dihydroxybutanamide (**34**) in good yield. If this reaction was carried out in other solvents such as methanol, acetone or acetonitrile, epimerization took place to some extent and resulted in a decrease of the isolation yield. Conversion of **34** into (3*S*,4*S*)-3-amino-4-carbamoyloxymethyl-2-azetidinone-1-sulfonic acid (**3**), the key intermediate[4)] for preparing **1**, was achieved by the procedure reported by Wei *et al.*[14)] with a slight modification (Chart 6). Selective chloroacetylation of the primary hydroxy group was effected by treating **34** with chloroacetyl chloride in *N,N*-dimethylacetamide at -25—-20 °C, and the product was mesylated with mesyl chloride in 1,2-dimethoxyethane in the presence of triethylamine at the same temperature to give the mesylate (**35**). Sulfonation and subsequent cyclization[15)] gave the 4-hydroxymethyl intermediate (**36**), which was converted into **3** by carbamoylation with dichlorophosphoryl isocyanate[16)] followed by hydrogenolysis. The colorless crystals obtained were identical with an authentic sample of **3**[4)] prepared from **23**. Conversion of **3** into AMA-1080 (**1**) was carried out by the reported procedure.[4)] Thus, (2*R*,3*R*)-epoxysuccinic acid (**5**), easily accessible by fermentation, was proved to be a useful and versatile synthon for preparing chiral sulfazecin-type derivatives.

Experimental

Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a Hitachi 215 spectrometer. Proton-nuclear magnetic resonance (^{1}H-NMR) spectra were taken on a Varian T-60 (60 MHz) or a Varian EM-390 (90 MHz) spectrometer using 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; ABq, AB quartet. The optical rotations were recorded with a JASCO DPI-181 digital polarimeter.

***cis*-3-Benzyloxycarbonylamino-4-carboxy-2-azetidinone (7)**——A solution of K_2CO_3 (900 mg, 6.51 mmol) in water (9 ml) was added dropwise to a stirred solution of *cis*-3-benzyloxycarbonylamino-4-methoxycarbonyl-2-azetidinone (**6**)[6)] (1.40 g, 5.03 mmol) in MeOH (14 ml) at 0—5 °C, and the mixture was stirred for 2 h at the same temperature. After evaporation of the methanol under reduced pressure, the residue was washed with AcOEt. The aq. phase was acidified with 1 N HCl and extracted with AcOEt. The extract was washed with aq. NaCl, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting precipitate was collected by filtration to give **7** (1.10 g, 84%), which was used for the next reaction without further purification. Recrystallization from acetonitrile gave an analytical sample as colorless crystals, mp 156—157 °C. *Anal.* Calcd for $C_{12}H_{12}N_2O_5$: C, 54.54; H, 4.57; N, 10.60. Found: C, 54.55; H, 4.45; N, 10.77. IR ν_{max}^{KBr} cm^{-1}: 3300, 1770, 1700. ^{1}H-NMR (DMSO-d_6) δ: 4.27 (1H, d, $J=$ 6 Hz, C_4-H), 7.30 (5H, s, aromatic protons), 8.10 (1H, d, $J=9$ Hz, C_3-NH), 8.50 (1H, s, N_1-H).

***cis*-3-Benzyloxycarbonylamino-4-carbamoyl-2-azetidinone (8a)**——Ammonia–water (25—28%, 2.5 ml) was added to a solution of **6** (1.50 g, 5.39 mmol) in tetrahydrofuran (THF) (20 ml), and the mixture was vigorously stirred for 16 h at room temperature. After evaporation of the THF under reduced pressure, water (60 ml) was added to the residue. The resulting precipitate was collected by filtration and washed successively with water and ether to give **8a** (1.12 g, 79%) as colorless crystals, mp 236—237 °C (dec.). *Anal.* Calcd for $C_{12}H_{13}N_3O_4$: C, 54.75; H, 4.98; N, 15.96. Found: C, 54.93; H, 4.90; N, 15.65. IR ν_{max}^{KBr} cm^{-1}: 3400, 3300, 3200, 1760, 1670. ^{1}H-NMR (DMSO-d_6) δ: 4.14 (1H, d, $J=6$ Hz, C_4-H), 5.05 (2H, s, C$\underline{H}_2$Ph), 5.08 (1H, dd, $J=6$, 10 Hz, C_3-H), 7.36 (5H, s, aromatic protons).

***cis*-3-Benzyloxycarbonylamino-4-(*N*-methylcarbamoyl)-2-azetidinone (8b)**——Acetic acid (1 drop) and aq. methylamine solution (40%, 1.2 ml) were added to a solution of **6** (2.00 g, 7.19 mmol) in THF (20 ml), and the mixture was stirred for 5 h at 0—5 °C. The resulting precipitate was collected by filtration and washed with ether to give **8b** (1.57 g, 79%), which was used for the next reaction without further purification. Recrystallization from AcOEt–MeOH gave an analytical sample as colorless crystals, mp 207—208 °C. *Anal.* Calcd for $C_{13}H_{15}N_3O_4$: C, 56.30; H, 5.45; N, 15.15. Found: C, 56.17; H, 5.31; N, 15.23. IR ν_{max}^{KBr} cm^{-1}: 3270, 1770, 1700, 1660. ^{1}H-NMR (DMSO-d_6) δ: 2.56 (3H, d, J=5 Hz, CH_3), 4.13 (1H, d, J=5 Hz, C_4-H), 7.33 (5H, s, aromatic protons), 8.33 (1H, q, J=5 Hz, N$\underline{H}$$CH_3$).

***cis*-3-Benzyloxycarbonylamino-4-(*N*,*N*-dimethylcarbamoyl)-2-azetidinone (8c)**——A mixture of **7** (1.32 g, 5 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (4 g, 19.4 mmol) in THF (26 ml) was stirred for 10 min at 0—5 °C. Aqua dimethylamine solution (50%, 4 ml) was added, and the whole was stirred for 2 h at room temperature. The resulting precipitate was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate was extracted with AcOEt. The extract was washed successively with aq. $NaHCO_3$ and aq. NaCl, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on silica gel (140 g) with AcOEt–MeOH (10 : 1, v/v) to give **8c** (450 mg, 31%) as pale yellow crystals, mp 135—137 °C. *Anal.* Calcd for $C_{14}H_{17}N_3O_4$: C, 57.72; H, 5.88; N, 14.42. Found: C, 58.10; H, 6.22; N, 14.18. IR ν_{max}^{Nujol} cm^{-1}: 3290, 1790, 1770, 1730, 1700, 1650. ^{1}H-NMR (DMSO-d_6) δ: 2.77 (3H, s, NCH_3), 2.80 (3H, s, NCH_3), 4.53 (1H, d, J=5 Hz, C_4-H), 5.10 (2H, ABq, J=12 Hz, C$\underline{H}_2$Ph), 5.36 (1H, dd, J=5, 9 Hz, C_3-H), 7.10 (1H, d, J=9 Hz, C_3-NH), 7.30 (5H, s, aromatic protons), 7.60 (1H, s, N_1-H).

***trans*-3-Benzyloxycarbonylamino-4-carbamoyl-2-azetidinone (10)**——Compound **10** was prepared from *trans*-3-benzyloxycarbonylamino-4-methoxycarbonyl-2-azetidinone (**9**)[6] (3.0 g, 10.78 mmol) in a similar manner to that described for the preparation of **8a**. The product was extracted with $CHCl_3$–EtOH (3 : 1, v/v) from the reaction mixture and chromatographed on silica gel (180 g) with AcOEt–MeOH (8 : 1, v/v) to give **10** (970 mg, 34%) as a colorless powder, mp 179—184 °C. IR ν_{max}^{KBr} cm^{-1}: 3280, 1760, 1695, 1660. ^{1}H-NMR (DMSO-d_6) δ: 3.90 (1H, d, J= 3 Hz, C_4-H), 4.42 (1H, dd, J=3, 9 Hz, C_3-H), 5.04 (2H, s, C$\underline{H}_2$Ph), 7.36 (5H, s, aromatic protons), 8.04 (1H, d, J= 9 Hz, C_3-NH), 8.30 (1H, br s, N_1-H).

Sodium *cis*-3-Benzyloxycarbonylamino-4-carbamoyl-2-azetidinone-1-sulfonate (11) ——a) Sulfur trioxide–*N*,*N*-dimethylformamide complex ($SO_3 \cdot$DMF) (6.74 ml of 1.56 M DMF solution, 10.5 mmol) was added to a solution of **8a** (1.54 g, 5.84 mmol) in DMF (12 ml) at −70 °C, and the mixture was stirred for 3 h at 0—5 °C. The reaction mixture was treated with pyridine (0.85 ml) and diluted with ether (40 ml). The resulting precipitate was separated by decantation and washed with ether. A mixture of the precipitate and Dowex 50W (Na) (50 ml) in water (20 ml) was stirred for 2 h at room temperature. The resin was filtered off and the filtrate was concentrated under reduced pressure. The concentrate was chromatographed on Amberlite XAD-2 (200 ml). Elution with water and lyophilization of the eluate gave *cis*-3-benzyloxycarbonylamino-4-(*N*-sulfocarbamoyl)-2-azetidinone-1-sulfonic acid disodium salt (**12**) (1.12 g, 39%) as a colorless powder. *Anal.* Calcd for $C_{12}H_{11}N_3Na_2O_{10}S_2 \cdot 2.5H_2O$: C, 29.15; H, 2.85; N, 8.50. Found: C, 29.44; H, 3.10; N, 8.51. IR ν_{max}^{KBr} cm^{-1}: 1780, 1705 (br), 1635. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 4.55 (1H, d, J=6 Hz, C_4-H), 5.14 (1H, d, J=6 Hz, C_3-H), 5.17 (2H, ABq, C$\underline{H}_2$Ph), 7.48 (1H, s, aromatic protons).

Further elution and lyophilization of the eluate gave **11** (1.07 g, 47%) as a colorless powder. *Anal.* Calcd for $C_{12}H_{12}N_3NaO_7S \cdot 1.5H_2O$: C, 36.74; H, 3.85; N, 10.71. Found: C, 36.97; H, 3.64; N, 10.59. IR ν_{max}^{KBr} cm^{-1}: 1780, 1680. ^{1}H-NMR (DMSO-d_6) δ: 4.26 (1H, d, J=6 Hz, C_4-H), 4.98 (1H, dd, J=6, 9 Hz, C_3-H), 5.02 (2H, s, C$\underline{H}_2$Ph), 7.32 (5H, s, aromatic protons), 7.67 (1H, d, J=9 Hz, C_3-NH).

b) A suspension of **8a** (500 mg, 1.90 mmol) and sulfur trioxide–pyridine complex ($SO_3 \cdot$Py) (907 mg, 5.70 mmol) in dioxane (30 ml) was vigorously stirred for 5 h at room temperature. The solvent was evaporated off under reduced pressure. A mixture of the residue and Dowex 50W (Na) (25 ml) in water (20 ml) was stirred for 1 h at room temperature. The resin was filtered off and the filtrate was concentrated under reduced pressure. The concentrate was chromatographed on Amberlite XAD-2 (240 ml). Gradient elution with aq. EtOH (0→10%, v/v) and lyophilization of the eluate gave **11** (646 mg, 87%) as a colorless powder. This sample was identical with the monosulfo compound obtained above on the basis of ^{1}H-NMR and high performance liquid chromatography (HPLC) comparisons.

***cis*-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-(*N*-methylcarbamoyl)-2-azetidinone (13b)**——A mixture of **8b** (510 mg, 1.84 ml) and 5% Pd–C (200 mg) in EtOH (20 ml) was stirred for 1 h at room temperature under a hydrogen atmosphere, and then filtered. The filtrate was concentrated under reduced pressure, and the residue was dissolved in a mixture of THF (10 ml) and water (10 ml). 2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl chloride hydrochloride (CATAM-Cl·HCl)[17] (673 mg, 2.02 mmol) and $NaHCO_3$ (464 mg, 5.52 mmol) were added to the ice-cooled solution, and the mixture was stirred for 1 h at room temperature. After evaporation of the THF, the resulting precipitate was collected by filtration and washed successively with aq. $NaHCO_3$, water and ether to give **13b** (510 mg, 69%) as colorless crystals, mp >265 °C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3220, 1750, 1700, 1680. ^{1}H-NMR (DMSO-d_6) δ: 2.5 (3H, d, J=5 Hz, NCH_3), 3.76 (3H, s, OCH_3), 5.30 (1H, dd, J=5, 9 Hz, C_3-H), 7.28 (1H, s, thiazole-5-H), 7.80 (1H, q, J=5 Hz, N$\underline{H}$$CH_3$), 8.30 (1H, br s, N_1-H).

The corresponding 4-(*N*,*N*-dimethylcarbamoyl) compound (**13c**) was similarly synthesized.

13c: Pale yellow crystals (77%), mp 208—213 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3290, 3200, 1765, 1685, 1645. ^{1}H-NMR (DMSO-d_6) δ: 2.83 (3H, s, NCH_3), 2.93 (3H, s, NCH_3), 3.87 (3H, s, OCH_3), 4.36 (2H, s, $ClCH_2$), 4.71 (1H, d, J= 5 Hz, C_4-H), 5.48 (1H, dd, J=5, 9 Hz, C_3-H), 7.26 (1H, s, thiazole-5-H), 7.46 (1H, br s, N_1-H), 9.44 (1H, d, J=9 Hz,

C_3-NH).

By using 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-(4-nitrobenzyloxycarbonylmethoxyimino)acetyl chloride hydrochloride (CATAAN-Cl·HCl)[4] and 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-[1-methyl-1-(4-nitrobenzyloxycarbonyl)ethoxyimino]acetyl chloride hydrochloride (CATABN-Cl·HCl)[6] in place of CATAM-Cl·HCl in the procedure described above, **14b** and **15b** were obtained, respectively.

14b: Colorless crystals (79%), mp 281—283 °C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3280, 3100, 1760, 1710, 1670, 1650. ^{1}H-NMR (DMSO-d_6) δ: 2.58 (3H, d, J=5 Hz, NCH_3), 4.85 (2H, s, OCH_2CO), 7.50 (1H, s, thiazole-5-H), 7.70 and 8.20 (each 2H, d, J=9 Hz, aromatic protons), 7.95 (1H, q, J=5 Hz, N$\underline{H}$CH$_3$), 8.47 (1H, br s, N_1-H), 9.15 (1H, d, J=9 Hz, C_3-NH).

15b: Colorless crystals (68%), mp 181—183 °C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3260, 1750, 1660. ^{1}H-NMR (DMSO-d_6) δ: 1.50 (6H, s, 2×CH_3), 2.59 (3H, d, J=5 Hz, NCH_3), 4.26 (1H, d, J=5 Hz, C_4-H), 4.31 (2H, s, $ClCH_2$), 5.33 (2H, s, C$\underline{H}_2$Ph), 5.46 (1H, dd, J=5, 9 Hz, C_3-H), 7.40 (1H, s, thiazole-5-H), 8.43 (1H, br s, N_1-H), 8.80 (1H, d, J=9 Hz, C_3-NH).

Sodium *cis*-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-carbamoyl-2-azetidinone-1-sulfonate (16a)——A mixture of **11** (300 mg, 0.765 mmol) and 10% Pd–C (150 mg) in aq. THF (50%, v/v, 10 ml) was stirred for 30 min at room temperature under a hydrogen atmosphere. After removal of the catalyst by filtration, $NaHCO_3$ (154 mg, 1.84 mmol) and CATAM-Cl·HCl (280 mg, 0.841 mmol) were added to the ice-cooled filtrate, and the mixture was stirred for 1 h at 0—5 °C. The reaction mixture was concentrated under reduced pressure, and the concentrate was chromatographed on Amberlite XAD-2 (160 ml). Gradient elution with aq. EtOH (0→20%, v/v) and lyophilization of the eluate gave **16a** (416 mg, 100%) as a colorless powder. *Anal.* Calcd for $C_{12}H_{12}ClN_6NaO_8S_2 \cdot 3H_2O$: C, 26.45; H, 3.33; N, 15.43. Found: C, 26.33; H, 3.04; N, 15.29. IR ν_{max}^{KBr} cm^{-1}: 1770, 1680. ^{1}H-NMR (DMSO-d_6) δ: 3.90 (3H, s, OCH_3), 4.32 (2H, s, $ClCH_2$), 4.40 (1H, d, J=6 Hz, C_4-H), 5.33 (1H, dd, J=6, 10 Hz, C_3-H), 7.40 (2H, br s, $CONH_2$), 7.51 (1H, s, thiazole-5-H), 9.20 (1H, d, J=10 Hz, C_3-NH).

By using CATAAN-Cl·HCl and CATABN-Cl·HCl in place of CATAM-Cl·HCl in the procedure described above, **17a** and **18a** were obtained, respectively. Compound **17a** was used in the subsequent reaction without isolation.

18a: A colorless powder (60%). IR ν_{max}^{KBr} cm^{-1}: 1770, 1730, 1690. ^{1}H-NMR (DMSO-d_6) δ: 1.50 (6H, s, 2×CH_3), 4.34 (2H, s, $ClCH_2$), 4.38 (1H, d, J=6 Hz, C_4-H), 5.31 (2H, s, C$\underline{H}_2$Ph), 5.34 (1H, dd, J=6, 9 Hz, C_3-H), 7.54 (1H, s, thiazole-5-H), 7.62 and 8.07 (each 2H, d, J=9 Hz, aromatic protons), 8.98 (1H, d, J=9 Hz, C_3-NH).

Sodium *cis*-3-[2-(2-Chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-(*N*,*N*-dimethylcarbamoyl)-2-azetidinone-1-sulfonate (16c)——A solution of **13c** (333 mg, 0.8 mmol) in DMF (4 ml) was treated with SO_3·DMF (2.1 ml of 1.5 M DMF solution, 3.15 mmol) at −70 °C, and the mixture was stirred for 2 h at 0—5 °C. The reaction mixture was treated with pyridine (0.26 ml, 3.2 mmol) and diluted with ether. The resulting precipitate was separated by decantation, washed with ether and dissolved in water (20 ml). Dowex 50W (Na) (15 ml) was added, and the mixture was stirred for 1 h at room temperature. After removal of the resin by filtration, the filtrate was concentrated under reduced pressure. The concentrate was chromatographed on Amberlite XAD-2 (150 ml). Gradient elution with aq. EtOH (0→10%, v/v) and lyophilization of the eluate gave **16c** (325 mg, 73%) as a colorless powder. *Anal.* Calcd for $C_{14}H_{16}ClN_6NaO_8S_2 \cdot 2H_2O$: C, 30.30; H, 3.63; N, 15.14. Found: C, 30.40; H, 3.79; N, 15.11. IR ν_{max}^{KBr} cm^{-1}: 1780, 1680. ^{1}H-NMR (DMSO-d_6) δ: 2.83 (3H, s, NCH_3), 2.99 (3H, s, NCH_3), 3.87 (3H, s, OCH_3), 4.35 (2H, s, $ClCH_2$), 4.97 (1H, d, J=6 Hz, C_4-H), 5.34 (1H, dd, J=6, 9 Hz, C_3-H), 7.28 (1H, s, thiazole-5-H), 9.17 (1H, d, J=9 Hz, C_3-NH).

Compounds **16b**, **17b** and **18b** were similarly synthesized.

16b: A colorless powder (87%). IR ν_{max}^{KBr} cm^{-1}: 1770, 1650. ^{1}H-NMR (DMSO-d_6) δ: 2.68 (3H, d, J=5 Hz, NCH_3), 3.85 (3H, s, OCH_3), 5.35 (1H, dd, J=5, 9 Hz, C_3-H), 7.50 (1H, s, thiazole-5-H).

17b: A colorless powder (61%). *Anal.* Calcd for $C_{21}H_{19}ClN_7O_{12}S_2 \cdot 3H_2O$: C, 34.22; H, 3.41; N, 13.30. Found: C, 34.10; H, 3.27; N, 13.16. IR ν_{max}^{KBr} cm^{-1}: 1760, 1670. ^{1}H-NMR (DMSO-d_6) δ: 2.65 (3H, d, J=5 Hz, NCH_3), 4.31 (2H, s, $ClCH_2$), 4.41 (1H, d, J=5 Hz, C_4-H), 4.80 (2H, s, OCH_2CO), 9.16 (1H, d, J=9 Hz, C_3-NH).

18b: A colorless powder (75%). *Anal.* Calcd for $C_{23}H_{23}ClN_7O_{12}S_2 \cdot 2H_2O$: C, 36.92; H, 3.64; N, 13.10. Found: C, 36.80; H, 3.53; N, 13.15. IR ν_{max}^{KBr} cm^{-1}: 1780, 1670. ^{1}H-NMR (DMSO-d_6) δ: 1.52 (6H, s, 2×CH_3), 2.63 (3H, d, J=5 Hz, NCH_3), 4.33 (2H, s, $ClCH_2$), 4.43 (1H, d, J=5 Hz, C_4-H), 5.33 (2H, s, $COOCH_2$), 7.53 (1H, s, thiazole-5-H), 8.90 (1H, d, J=9 Hz, C_3-NH).

Sodium *cis*-3-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-4-carbamoyl-2-azetidinone-1-sulfonate (19a)——A mixture of **16a** (365 mg, 0.765 mmol) and sodium *N*-methyldithiocarbamate (197 mg, 1.56 mmol) in water (20 ml) was stirred for 1 h at room temperature. The reaction mixture was washed with ether and concentrated under reduced pressure. The concentrate was chromatographed on Amberlite XAD-2 (200 ml). Elution with water and lyophilization of the eluate gave **19a** (220 mg, 64%) as a colorless powder.

Compounds **19b, c** were similarly synthesized, and the results are shown in Table III.

***cis*-3-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(1-carboxy-1-methylethoxyimino)acetamido]-4-carbamoyl-2-azetidinone-1-sulfonic Acid (21a)**——A mixture of **18a** (280 mg, 0.401 mmol) and sodium *N*-methyldithiocarbamate (104 mg, 0.802 mmol) in water (20 ml) was stirred for 90 min at room temperature. The reaction mixture was washed with ether and concentrated under reduced pressure. The concentrate was chromatographed on Amberlite XAD-2 (80 ml).

TABLE III. Data for Compounds **19**, **20** and **21**

Compound	Yield (%)	IR (KBr) (cm^{-1})	NMR (DMSO-d_6) C$_3$-H (dd) J=6, 9 Hz	C$_4$-H (d) J=6 Hz	Th-H[a] (s)	Formula	Analysis (%) Calcd (Found) C	H	N
19a	64	1770	5.36	4.38	6.89	$C_{10}H_{11}N_6NaO_7S_2 \cdot 2.2H_2O$	26.46 (26.74	3.42 3.34	18.51 18.13)
19b	58	1770	5.28	4.35	6.86	$C_{11}H_{13}N_6NaO_7S_2 \cdot 2.2H_2O$	28.23 (28.47	3.75 3.67	17.96 17.79)
19c	85	1775	5.32	4.97	6.60	$C_{12}H_{15}N_6NaO_7S_2 \cdot 2.2H_2O$	29.90 (29.99	4.06 4.29	17.43 17.14)
20a	65[b]	1765	5.31	4.37	7.28	$C_{11}H_{12}N_6O_9S_2 \cdot 2H_2O$	27.97 (27.72	3.41 3.16	17.79 17.67)
20b	42	1770	5.33	4.39	7.25	$C_{12}H_{14}N_6O_9S_2 \cdot 2.2H_2O$	29.41 (29.66	3.78 3.83	17.15 16.75)
21a	37	1770	5.33	4.36	7.18	$C_{13}H_{16}N_6O_9S_2 \cdot 1.5H_2O$	31.77 (31.69	3.90 3.90	17.10 17.00)
21b	74	1770	5.33	4.40	7.16	$C_{14}H_{18}N_6O_9S_2 \cdot 2.5H_2O$	32.11 (32.39	4.42 4.30	16.05 15.89)

a) Thiazole-5-H. *b*) Overall yield from **11**.

Gradient elution with aq. EtOH (0→20%, v/v) and lyophilization of the eluate gave a colorless powder (210 mg). A suspension of the powder (200 mg) and 10% Pd–C (200 mg) in water (10 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 $NaHCO_3$ (27 mg) and washed with AcOEt. Dowex 50W (H) (15 ml) was added and the mixture was stirred for 90 min under ice-cooling. The resin was filtered off and the filtrate was concentrated under reduced pressure. The concentrate was chromatographed on Amberlite XAD-2 (80 ml). Gradient elution with aq. EtOH (0→15%, v/v) and lyophilization of the eluate gave **21a** (73 mg, 37%) as a colorless powder.

Compounds **20a**, **b** and **21b** were similarly synthesized, and the results are shown in Table III.

***trans*-3-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(1-carboxy-1-methylethoxyimino)acetamido]-4-carbamoyl-2-azetidinone-1-sulfonic Acid (22)**——According to method a) described for the synthesis of **11**, **10** was sulfonated to give the *trans* isomer of **11**, which was converted into **22** by a method similar to that described for the synthesis of the corresponding *cis* isomer (**21a**). Overall yield 12%. *Anal.* Calcd for $C_{13}H_{16}N_6O_9S_2 \cdot 2.5H_2O$: C, 30.65; H, 4.15; N, 16.50. Found: C, 30.61; H, 4.17; N, 16.46. IR ν_{max}^{KBr} cm^{-1}: 1760, 1660. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 1.61 (6H, s, 2×CH_3), 4.17 (1H, d, J=3 Hz, C_4-H), 4.84 (1H, d, J=3 Hz, C_3-H), 7.11 (1H, s, thiazole-5-H).

***erythro*-3-Hydroxy-L-aspartic Acid (25)**[5]——Ammonia–water (25—28%, 1.2 l) was added in portions to crystals of (2*R*,3*R*)-epoxysuccinic acid (**5**)[5] (79.2 g, 0.6 mol) under ice-cooling. The mixture was stirred for 48 h at 45—47 °C and concentrated under reduced pressure. The residue was dissolved in water (200 ml), and the solution was concentrated again under reduced pressure. The syrupy residue was dissolved in water (120 ml), and charcoal (1.2 g) was added to the solution. The mixture was stirred, and then filtered. After addition of conc. HCl (50 ml) to the filtrate, the mixture was cooled in a refrigerator overnight. The resulting precipitate was collected by filtration and washed with cold water (60 ml) to give **25** (37.1 g, 82%) as colorless crystals. *Anal.* Calcd for $C_4H_7NO_5$: C, 32.22; H, 4.73; N, 9.40. Found: C, 32.02; H, 4.84; N, 9.54. IR ν_{max}^{Nujol} cm^{-1}: 3460, 3210, 1690. $[\alpha]_D^{24.5}$ +55.6° (c=0.9, 1 N HCl).[10]

***β*-Methyl *erythro*-3-Hydroxy-L-aspartate (26)**[11]——Conc. HCl (20 ml) was added to a suspension of **25** (29.8 g, 0.2 mol) in MeOH (300 ml), and the mixture was heated under reflux for 8 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in MeOH. The solution was concentrated under reduced pressure, and the solid residue was dissolved in aq. EtOH (50%, v/v, 120 ml). Pyridine (15.8 g, 0.2 mol) was added to the stirred solution at 0—5 °C, and the mixture was cooled in a refrigerator overnight. The resulting crystals were collected by filtration and washed successively with aq. EtOH (50%, v/v) and EtOH to give **26** (23.8 g, 73%). Recrystallization of the product from aq. EtOH (50%, v/v) gave an analytical sample as colorless crystals, mp 226—229 °C (dec.). *Anal.* Calcd for $C_5H_9NO_5$: C, 36.81; H, 5.56; N, 8.59. Found: C, 36.57; H, 5.65; N, 8.54. IR ν_{max}^{Nujol} cm^{-1}: 3175, 1770, 1755, 1620. $[\alpha]_D^{25}$ +64.4° (c=1, 1 N HCl).

***erythro*-3-Hydroxy-L-asparagine (27)**[11]——Compound **26** (4.89 g, 30 mmol) was added to ammonia–water (25—28%, 20 ml) under ice-cooling, and the mixture was stirred for 15 h at room temperature. The reaction mixture was concentrated under reduced pressure and the solid residue was washed with cold water to give **27** (3.85 g, 87%). Recrystallization of the product from aq. EtOH (50%, v/v) gave an analytical sample as colorless crystals, mp 225—

230 °C. *Anal.* Calcd for $C_4H_8N_2O_4$: C, 32.44; H, 5.44; N, 18.91. Found: C, 32.50; H, 5.38; N, 18.94. IR ν_{max}^{KBr} cm^{-1}: 3360, 3180, 1690, 1670. ^{1}H-NMR (D_2O+DCl) δ: 4.82 (1H, d, $J=3$ Hz, C_2-H), 4.98 (1H, d, $J=3$ Hz, C_3-H). $[\alpha]_D^{23.5}$ +47.5° ($c=0.8$, H_2O).

***O*-Benzyl (2*S*,3*R*)-2-(*tert*-Butoxycarbonylamino)-3-carbamoyl-3-mesyloxypropanohydroxamate (28)**——Di-*tert*-butyl dicarbonate (15.66 g, 72 mmol) was added to a mixture of **27** (6.60 g, 45 mmol) and triethylamine (12.6 ml, 90 mmol) in aq. dioxane (50%, v/v, 135 ml), and the mixture was stirred for 4 h at room temperature and diluted with AcOEt (220 ml) and water (110 ml). After separation of the aq. phase, the organic phase was extracted with aq. NaCl (45 ml). The combined aq. phase was saturated with NaCl, acidified with aq. $KHSO_4$ (10%) and extracted with AcOEt–THF (2 : 1). The extract was washed with aq. NaCl, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was triturated with ether, and the resulting powder was collected by filtration to give *N*-(*tert*-butoxycarbonyl)-*erythro*-3-hydroxy-L-asparagine as a colorless powder (10.35 g). IR ν_{max}^{KBr} cm^{-1}: 3400, 3300, 1720, 1675, 1600. ^{1}H-NMR (DMSO-d_6) δ: 1.40 (9H, s, 3×CH_3), 4.10 (1H, d, $J=2.5$ Hz, C_3-H), 4.42 (1H, dd, $J=2.5$, 9 Hz, C_2-H), 6.22 (1H, d, $J=9$ Hz, C_2-NH). $[\alpha]_D^{25}$ +25.7° ($c=1.1$, DMSO).

A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (9.22 g, 48 mmol) in water (40 ml) was added to a mixture of the powder (9.92 g, 40 mmol) obtained above, $NaHCO_3$ (5.04 g, 60 mmol) and *O*-benzylhydroxylamine hydrochloride (9.60 g, 60 mmol) in water (400 ml), and the whole was stirred for 4 h at room temperature while the pH was maintained at 4—5 by occasional addition of 1 N HCl. The reaction mixture was saturated with NaCl and extracted with AcOEt–THF (4 : 1). The extract was washed successively with 1 N citric acid, aq. $NaHCO_3$ (3%) and aq. NaCl, dried over $MgSO_4$ and concentrated under reduced pressure. The solid residue was recrystallized from AcOEt to give *O*-benzyl (2*S*,3*R*)-2-(*tert*-butoxycarbonylamino)-3-carbamoyl-3-hydroxy-propanohydroxamate (7.72 g) as colorless crystals, mp 146—148 °C. IR ν_{max}^{KBr} cm^{-1}: 3310, 3220, 1690, 1670. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (9H, s, 3×CH_3), 4.77 (2H, s, C$\underline{H}_2$Ph), 7.38 (5H, s, aromatic protons). $[\alpha]_D^{25}$ +6.7° ($c=0.9$, DMSO).

Mesyl chloride (893 mg, 7.8 mmol) was added dropwise to a stirred solution of the crystals (2.12 g, 6 mmol) obtained above in pyridine (30 ml) at −20 °C under an argon atmosphere, and the mixture was stirred for 30 min at −20 °C and 20 h at 3—5 °C. The reaction mixture was diluted with AcOEt–THF (1 : 1), and adjusted to pH 2 with 6 N HCl under ice-cooling. After separation of the organic phase, the aq. phase was extracted with AcOEt. The combined organic phase was washed with aq. NaCl, dried over $MgSO_4$ and concentrated under reduced pressure. The solid residue was washed with ether to give **28** (1.84 g, 36% from **27**) as colorless crystals, mp 138—140 °C (dec.). *Anal.* Calcd for $C_{17}H_{25}N_3O_8S$: C, 47.32; H, 5.84; N, 9.74. Found: C, 47.59; H, 5.97; N, 9.75. IR ν_{max}^{KBr} cm^{-1}: 3310, 3230, 1690, 1670. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (9H, s, 3×CH_3), 3.13 (3H, s, SO_2CH_3), 4.72 (2H, s, C$\underline{H}_2$Ph), 4.96 (1H, d, $J=7.5$ Hz, C_3-H), 7.36 (5H, s, aromatic protons). $[\alpha]_D^{25}$ +1.3° ($c=1$, DMSO).

(3*S*,4*S*)-1-Benzyloxy-3-(*tert*-butoxycarbonylamino)-4-carbamoyl-2-azetidinone (29)——A mixture of **28** (3.24 g, 7.5 mmol) and K_2CO_3 (3.11 g, 22.5 mmol) in acetone (750 ml) was gradually heated to 50 °C and stirred for 35 min at the same temperature under an argon atmosphere. The reaction mixture was filtered through Celite, and the solid was washed with THF. The filtrate and washings were combined and concentrated under reduced pressure. The residue was dissolved in a mixture of AcOEt–THF (1 : 1) and water. The organic phase was separated, and the aq. phase was extracted with AcOEt–THF (3 : 1). The combined organic phase was washed successively with aq. NaCl, aq. $NaHCO_3$ and aq. NaCl, dried over $MgSO_4$ and concentrated under reduced pressure. The solid residue was washed with ether to give **29** (2.30 g, 91%) as colorless crystals, mp 232—235 °C (dec.). *Anal.* Calcd for $C_{16}H_{21}N_3O_5$: C, 57.30; H, 6.31; N, 12.53. Found: C, 57.40; H, 6.42; N, 12.51. IR ν_{max}^{KBr} cm^{-1}: 3330, 3170, 1795, 1700, 1665. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (9H, s, 3×CH_3), 4.47 (1H, d, $J=5$ Hz, C_4-H), 4.92 (1H, dd, $J=5$, 9 Hz, C_3-H), 4.99 (2H, s, C$\underline{H}_2$Ph), 6.95 (1H, d, $J=9$ Hz, C_3-NH), 7.40 (5H, s, aromatic protons). $[\alpha]_D^{23}$ +31.3° ($c=0.45$, DMSO).

(3*S*,4*S*)-3-(*tert*-Butoxycarbonylamino)-4-carbamoyl-1-hydroxy-2-azetidinone (30)——A mixture of **29** (671 mg, 2 mmol) and 10% Pd–C (140 mg) in MeOH (40 ml) was stirred for 25 min at room temperature under a hydrogen atmosphere and then filtered. The filtrate was concentrated under reduced pressure, and the residue was triturated with ether. The resulting powder was collected by filtration to give **30** (450 mg, 86%) as a colorless powder. *Anal.* Calcd for $C_9H_{15}N_3O_5 \cdot 0.5H_2O$: C, 42.52; H, 6.34; N, 16.53. Found: C, 42.79; H, 6.46; N, 16.34. IR ν_{max}^{KBr} cm^{-1}: 3330, 3190, 1780, 1690, 1665. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (9H, s, 3×CH_3), 4.32 (1H, d, $J=5$ Hz, C_4-H), 4.88 (1H, dd, $J=5$, 9 Hz, C_3-H), 6.90 (1H, d, $J=9$ Hz, C_3-NH). $[\alpha]_D^{25}$ +18.5° ($c=0.5$, MeOH).

(3*S*,4*S*)-3-(*tert*-Butoxycarbonylamino)-4-carbamoyl-2-azetidinone (31)——A solution of **30** (356 mg, 1.4 mmol) in a mixture of MeOH (12 ml) and 4.5 M aq. ammonium acetate (8 ml) was treated with a titanium trichloride solution (20%, 2 ml), and the mixture was stirred for 20 min at room temperature. After further addition of the titanium trichloride solution (20%, 1 ml), the mixture was stirred for 50 min at room temperature, diluted with aq. NaCl and extracted with AcOEt–THF (2 : 1). The extract was washed successively with aq. $NaHCO_3$ and aq. NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. The solid residue was recrystallized from EtOH to give **31** (146 mg, 46%) as colorless crystals, mp 188—190 °C (dec.). *Anal.* Calcd for $C_9H_{15}N_3O_4$: C, 47.16; H, 6.60; N, 18.33. Found: C, 46.85; H, 6.50; N, 17.95. IR ν_{max}^{KBr} cm^{-1}: 3330, 3200, 1760, 1690, 1665. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (9H, s, 3×CH_3), 4.08 (1H, d, $J=5$ Hz, C_4-H), 4.99 (1H, dd, $J=5$, 9 Hz, C_3-H), 6.72 (1H, d, $J=9$ Hz, C_3-NH), 7.30 (2H, br s, $CONH_2$), 8.30 (1H, br s, N_1-H). $[\alpha]_D^{24}$ +25.9° ($c=1.1$, DMSO).

(3*S*,4*S*)-3-Benzyloxycarbonylamino-4-carbamoyl-2-azetidinone (24)——a) (3*S*,4*S*)-3-Benzyloxycarbonylamino-1-(2,4-dimethoxybenzyl)-4-methoxycarbonyl-2-azetidinone (**23**)[4] (6.45 g, 15 mmol), $K_2S_2O_8$ (5.7 g, 21 mmol) and K_2HPO_4 (3.4 g, 19.5 mmol) were added to a mixture of acetonitrile (150 ml) and water (75 ml), and the whole was heated in an oil bath (95 °C) for 2 h with stirring. After evaporation of the acetonitrile under reduced pressure, the concentrate was extracted with AcOEt (2 × 100 ml). The extract was washed successively with aq. $NaHCO_3$ (2%, 200 ml) and aq. NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on silica gel (150 g) with AcOEt–hexane (1 : 1→1 : 3, v/v), and the eluate was concentrated under reduced pressure. Ether (40 ml) was added to the residue, and the resulting precipitate was collected by filtration. Recrystallization from a mixture of AcOEt (32 ml) and hexane (30 ml) gave (3*S*,4*S*)-3-benzyloxycarbonylamino-4-methoxycarbonyl-2-azetidinone (2.15 g, 50%) as colorless crystals, mp 125—126 °C. *Anal.* Calcd for $C_{13}H_{14}N_2O_5$: C, 56.11; H, 5.07; N, 10.07. Found: C, 56.16; H, 5.06; N, 9.89. IR ν_{max}^{Nujol} cm^{-1}: 3325, 3240, 1795, 1740, 1730, 1715. $[\alpha]_D^{24}$ +88.2° (*c*=1, $CHCl_3$).

A part of these crystals (1.0 g, 3.6 mmol) was dissolved in THF (13 ml). After addition of ammonia–water (25—28%, 1.6 ml), the mixture was stirred for 24 h at room temperature, and concentrated under reduced pressure. Water (60 ml) was added to the residue. The resulting precipitate was collected by filtration and washed successively with water and ether to give **24** (0.85 g, 89%) as colorless crystals, mp 238—241 °C (dec.). *Anal.* Calcd for $C_{12}H_{13}N_3O_4$: C, 54.75; H, 4.98; N, 15.96. Found: C, 54.38; H, 5.01; N, 15.71. IR ν_{max}^{KBr} cm^{-1}: 3410, 3310, 3220, 1770, 1750, 1730, 1670. ^{1}H-NMR (DMSO-d_6) δ: 4.14 (1H, d, *J*=5 Hz, C_4-H), 5.05 (2H, s, C$\underline{H}_2$Ph), 5.08 (1H, dd, *J*=5, 9 Hz, C_3-H), 7.35 (5H, s, aromatic protons), 7.55 (1H, d, *J*=9 Hz, C_3-NH). $[\alpha]_D^{24}$ +11.5° (*c*=0.9, DMSO).

b) Trifluoroacetic acid (5 ml) was added to an ice-cooled mixture of **31** (177 mg, 0.755 mmol) and anisole (0.4 ml) in dichloromethane (0.4 ml), and the whole was stirred for 50 min under ice-cooling. The reaction mixture was diluted with benzene (5 ml) and concentrated under reduced pressure. Acetone (5 ml) was added to the residue, and the mixture was adjusted to pH 7 with aq. $NaHCO_3$ (5%). Carbobenzoxy chloride (0.2 ml) was added to the mixture, and the whole was stirred for 2 h under ice-cooling, while the pH was maintained at 7 by occasional addition of aq. $NaHCO_3$ (5%). After further addition of carbobenzoxy chloride (0.1 ml), the mixture was stirred for 2 h at 0—5 °C with maintenance of the pH at 7. After evaporation of the acetone, the residue was diluted with water (10 ml). The resulting crystals were collected by filtration, washed successively with water and ether, and recrystallized from aq. EtOH (50%, v/v) to give **24** (108 mg, 53%) as colorless crystals, mp 242—245 °C (dec.). *Anal.* Calcd for $C_{12}H_{13}N_3O_4$: C, 54.75; H, 4.98; N, 15.96. Found: C, 54.45; H, 5.06; N, 15.93. $[\alpha]_D^{24}$ +13.1° (*c*=1, DMSO). These crystals were identical with the sample obtained in a) on the basis of thin-layer chromatography (TLC) and ^{1}H-NMR comparisons.

(3*S*,4*S*)-3-Amino-4-carbamoyl-2-azetidinone-1-sulfonic Acid (4)——Sulfur trioxide–pyridine complex (SO_3·Py, 2.54 g, 16.0 mmol) was added to a suspension of **24** (1.40 g, 5.32 mmol) in dioxane (84 ml), and the mixture was stirred for 15 h at 30—35 °C. After evaporation of the dioxane under reduced pressure, the residue was dissolved in water (150 ml). Dowex 50W (Na) (50 ml) was added to the solution, and the mixture was stirred for 1 h at room temperature. After removal of the resin by filtration, the filtrate was concentrated to 100 ml under reduced pressure. The concentrate was chromatographed on Amberlite XAD-2 (300 ml). Gradient elution with water and aq. EtOH (10%, v/v) and lyophilization of the eluate gave sodium (3*S*,4*S*)-benzyloxycarbonylamino-4-carbamoyl-2-azetidinone-1-sulfonate (2.01 g) as a colorless powder. IR ν_{max}^{KBr} cm^{-1}: 3300, 1770, 1680.

A part of this powder (1.52 g) was added to water (52 ml), and the suspension was adjusted to pH 2 with 1 N HCl. After addition of 10% Pd–C (1.52 g), the mixture was stirred for 30 min at room temperature under a hydrogen atmosphere, and adjusted again to pH 2 with 1 N HCl. The mixture was further stirred for 10 min under the same conditions. After removal of the catalyst by filtration, the filtrate was concentrated to 15 ml, and 1 N HCl (15.5 ml) was added to the cooled concentrate. The mixture was stirred for 30 min at 0—5 °C and concentrated to 2 ml under reduced pressure. The concentrate was allowed to stand at 0—5 °C for 30 min. The resulting precipitate was collected by filtration, washed with a small amount of cold water, and dried over P_2O_5 to give **4** (0.67 g, 78% from **24**) as colorless crystals. *Anal.* Calcd for $C_4H_7N_3O_5S$: C, 21.15; H, 3.99; N, 18.50. Found: C, 21.12; H, 4.10; N, 18.57. IR ν_{max}^{KBr} cm^{-1}: 3500, 3410, 3200, 1750, 1690, 1630. $[\alpha]_D^{25}$ −82.8° (*c*=0.64, DMSO).

(3*S*,4*S*)-3-[2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-4-carbamoyl-2-azetidinone-1-sulfonic Acid (2)——CATABN-Cl·HCl (1.60 g, 2.96 mmol) and $NaHCO_3$ (517 mg, 6.16 mmol) were added to a cooled solution of **4** (515 mg, 2.46 mmol) in aq. THF (50%, v/v, 20 ml), and the mixture was stirred for 1 h at 0—5 °C. Sodium *N*-methyldithiocarbamate (954 mg, 7.39 mmol) was added, and the whole was stirred for 2 h at room temperature. After evaporation of the THF, the residual solution was washed with ether and concentrated under reduced pressure. The concentrate was chromatographed on Amberlite XAD-2 (240 ml). Gradient elution with aq. EtOH (0→20%, v/v) and lyophilization of the eluate gave a powder (660 mg), which was dissolved in aq. THF (50%, v/v, 20 ml). After addition of 10% Pd–C (660 mg) to the solution, the mixture was stirred for 4 h ar room temperature under a hydrogen atmosphere. The catalyst was removed by filtration, and $NaHCO_3$ (88 mg) was added to the filtrate. The solution was washed with AcOEt, and Dowex 50W (H) (20 ml) was added. The mixture was stirred for 40 min at 0—5 °C and filtered to remove the resin. The filtrate was chromatographed on Amberlite XAD-2 (160 ml). Gradient elution with aq. EtOH (0→10%, v/v) and lyophilization of the eluate gave **2** (155 mg, 32%) as a colorless powder. *Anal.* Calcd for $C_{13}H_{16}N_6O_9S_2 \cdot 2.5H_2O$: C, 30.65; H, 4.15; N, 16.50. Found: C, 30.74; H, 4.27; N, 16.55. IR ν_{max}^{KBr} cm^{-1}: 3340, 1770, 1720, 1680, 1635. ^1H-NMR (DMSO-d_6) δ: 1.52 (6H, s, $2 \times CH_3$), 4.78 (1H, d, J=6 Hz, C_4-H), 5.33 (1H, dd, J=6, 9 Hz, C_3-H), 7.20 (1H, s, thiazole-5-H), 9.19 (1H, d, J=9 Hz, C_3-NH). $[\alpha]_D^{23}$ $-37.8°$ (c=1, water).

(2*S*,3*R*)-2-Benzyloxycarbonylamino-3-hydroxy-4-butanolide (33)——Compound **25** (17.89 g, 0.12 mol) was suspended in MeOH (180 ml), and conc. HCl (12 ml, 0.144 mol) was added dropwise to the suspension under ice-cooling. The mixture was stirred and heated under reflux for 9 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in water (360 ml), and $KHCO_3$ (40.85 g, 0.408 mol) was added to the solution. Subsequently, a solution of carbobenzoxy chloride (24.56 g, 0.144 mol) in dry ether (120 ml) was added dropwise to the stirred reaction mixture under ice-cooling over a period of 30 min. The whole was stirred vigorously for 3 h at room temperature, washed with AcOEt (120 ml) and acidified with conc. HCl (18 ml). The mixture was extracted twice with AcOEt (2 × 180 ml). The combined extract was washed with aq. NaCl (60 ml), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in THF (360 ml), and a solution of $NaBH_4$ (9.08 g, 0.24 mol) in aq. NaOH (0.5 N, 120 ml, 0.06 mol) was added dropwise to the stirred solution at 0—5 °C over a period of 30 min. The mixture was stirred for 4 h at room temperature. After addition of conc. HCl (30 ml, 0.36 mol) at 0—5 °C, the mixture was concentrated under reduced pressure. Dichloromethane (360 ml) and *p*-toluenesulfonic acid monohydrate (0.23 g, 1.2 mmol) were added to the solid residue, and the mixture was stirred and heated under reflux for 4 h. During the reaction course, the resulting water was removed by use of a water-trap. After evaporation of the solvent under reduced pressure, AcOEt (300 ml) and aq. $NaHCO_3$ (5%, 200 ml) were added to the residue. The organic phase was separated, washed successively with aq. $NaHCO_3$ (5%, 2 × 100 ml) and aq. NaCl, dried over Na_2SO_4, and then concentrated under reduced pressure. AcOEt (30 ml) and hexane (120 ml) were added to the residue, and the mixture was allowed to stand in a refrigerator overnight. The resulting precipitate was collected by filtration and washed with a cold mixture of AcOEt (10 ml) and hexane (40 ml) to give **33** (19.3 g, 64%) as colorless crystals, mp 128—131 °C. *Anal.* Calcd for $C_{12}H_{13}NO_5$: C, 57.41; H, 5.14; N, 5.57. Found: C, 57.37; H, 5.22; N, 5.58. IR ν_{max}^{KBr} cm^{-1}: 3420, 3300, 1750, 1690. ^1H-NMR (DMSO-d_6) δ: 4.08 (1H, d, J=10 Hz, C_4-H), 4.67 (1H, dd, J=4.5, 9 Hz, C_2-H), 5.07 (2H, s, $C\underline{H}_2$Ph), 5.64 (1H, d, J=4 Hz, OH), 7.35 (5H, s, aromatic protons), 7.37 (1H, d, J=9 Hz, CONH). $[\alpha]_D^{22}$ $+36.9°$ (c=0.5, AcOEt).

(2*S*,3*R*)-2-Benzyloxycarbonylamino-3,4-dihydroxybutanamide (34)——Cold ammonia–water (25—28%, 20 ml) was added to a stirred suspension of **33** (10.0 g, 39.8 mmol) in hexane (80 ml) under ice-cooling, and the mixture was stirred for 2 h at 0—5 °C. After further addition of ammonia–water (25—28%, 10 ml), the reaction mixture was stirred for 1 h at the same temperature. After evaporation of the hexane under reduced pressure, cold water (40 ml) was added to the residue, and the mixture was stirred for 10 min at 0—5 °C. The resulting precipitate was collected by filtration, washed with cold water (40 ml), and dried over P_2O_5 for 18 h at 50 °C *in vacuo* to give **34** (9.16 g, 86%) as colorless crystals, mp 169—172 °C. *Anal.* Calcd for $C_{12}H_{16}N_2O_5$: C, 53.73; H, 6.01; N, 10.44. Found: C, 53.81; H, 6.04; N, 10.44. IR ν_{max}^{KBr} cm^{-1}: 3380, 3300, 1660. ^1H-NMR (DMSO-d_6) δ: 4.07 (1H, dd, J=6, 9 Hz, C_2-H), 4.58 (1H, t, J=6 Hz, $CH_2O\underline{H}$), 4.85 (1H, d, J=5 Hz, $CHO\underline{H}$), 5.05 (2H, s, $C\underline{H}_2$Ph), 7.31 (5H, s, aromatic protons). $[\alpha]_D^{22}$ $+13.0°$ (c=0.5, DMSO).

(2*S*,3*R*)-2-Benzyloxycarbonylamino-4-chloroacetoxy-3-mesyloxybutanamide (35)——Chloroacetyl chloride (5.31 g, 47.0 mmol) was added dropwise to a stirred solution of **34** (9.00 g, 33.5 mmol) in *N*,*N*-dimethylacetamide (DMA, 36 ml) at −25—−20 °C over a period of 25 min. The mixture was stirred for 1 h at the same temperature, and then poured into cold water (108 ml). The mixture was extracted with AcOEt (2 × 150 ml), and the extract was washed successively with aq. $NaHCO_3$ (30 ml) and aq. NaCl (30 ml). The solution was dried over $MgSO_4$ and concentrated under reduced pressure. EtOH (27 ml) was added to the residue, and the mixture was allowed to stand in a refrigerator overnight. The resulting precipitate was collected by filtration, washed with cold EtOH (18 ml), and dried over P_2O_5 at 40 °C *in vacuo* to give colorless crystals (9.31 g), which were dissolved in 1,2-dimethoxyethane (122 ml). Triethylamine (5.58 g, 55.1 mmol) was added to the stirred solution at −25 °C, followed by addition of mesyl chloride (5.26 g, 45.9 mmol) at −25—−20 °C over a period of 15 min. The mixture was stirred for 1 h at the same temperature. AcOEt (190 ml) and aq. NaCl (80 ml) were added to the reaction mixture under ice-cooling. The organic phase was separated, washed with aq. NaCl (80 ml), dried over $MgSO_4$, and concentrated under reduced pressure. EtOH (30 ml) was added to the residue, and the mixture was allowed to stand in a refrigerator overnight. The resulting precipitate was collected by filtration and dried over P_2O_5 at room temperature *in vacuo* to give **35** (10.25 g, 72%) as colorless crystals, mp 139—141 °C. *Anal.* Calcd for $C_{15}H_{19}ClN_2O_8S$: C, 42.61; H, 4.53; N, 6.63. Found: C, 43.01; H, 4.51; N, 6.49. IR ν_{max}^{KBr} cm^{-1}: 3430, 3340, 1745, 1690, 1670. ^1H-NMR (DMSO-d_6) δ: 3.16 (3H, s, SO_2CH_3), 4.34 (2H, s, CH_2Cl), 4.55 (1H, dd, J=6, 9 Hz, C_2-H), 5.06 (2H, s, $C\underline{H}_2$Ph), 7.36 (5H, s, aromatic protons). $[\alpha]_D^{22}$ $+4.8°$ (c=1, MeOH).

(3*S*,4*S*)-3-Amino-4-carbamoyloxymethyl-2-azetidinone-1-sulfonic Acid (3)——Sulfur trioxide–picoline complex (5.73 g, 33.1 mmol) was added to a suspension of **35** (8.00 g, 18.9 mmol) in dichloromethane (35 ml), and the mixture was stirred for 2 h at room temperature. The reaction mixture was washed with aq. $KHSO_4$ (0.6 M, 34.8 ml), and the washings were re-extracted with dichloromethane (2 × 32 ml). The combined organic phase was extracted with aq. $NaHCO_3$ (2%, 3 × 70 ml). Tetrabutylammonium hydrogen sulfate (Bu_4NHSO_4, 8.35 g, 24.6 mmol) and dichloromethane (80 ml) were added to the combined extract, and the mixture was stirred for 10 min. The aq. phase was separated and re-extracted with dichloromethane (2 × 50 ml). The combined organic phase was concentrated under reduced pressure, and the residue was dissolved in dichloroethane (277 ml). The solution was added dropwise to a strirred solution of $KHCO_3$ (5.68 g, 56.7 mmol) in water (173 ml) at 60—65 °C over a period of 15 min. The mixture was stirred for 30 min at the same temperature, and then for 40 min at 65—70 °C. The aq. phase was separated and re-extracted with dichloromethane (2 × 150 ml). The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in dichloroethane (189 ml), and K_2CO_3 (1.31 g, 9.45 mmol) was added to the solution at 0—5 °C. A solution of dichlorophosphoryl isocyanate (5.34 g, 33.4 mmol) in dichloroethane (52.5 ml) was added dropwise to the stirred mixture at −20—−15 °C over a period of 10 min, and the whole was stirred for 2 h at the same temperature. A solution of $NaHCO_3$ (956 mg, 11.4 mmol) in water (142 ml) was added dropwise at 0—5 °C. The mixture was adjusted to pH 3.5—4.5 with aq. $NaHCO_3$ at room temperature, and stirred for 30 min with maintenance of the pH at 3.5—4.5 by occasional addition of aq. $NaHCO_3$. The mixture was then stirred for 5 h at 47—53 °C, and adjusted to pH 5.5 with aq. $NaHCO_3$ at 15—25 °C. After addition of Bu_4NHSO_4 (2.13 g, 6.28 mmol), the mixture was stirred for 20 min. The aq. phase was separated and re-extracted with dichloromethane (2 × 150 ml). The combined organic phase was concentrated under reduced pressure, and the residue was dissolved in EtOH (100 ml). After addition of Dowex 50W (H) (204 ml) and water (204 ml), the mixture was stirred for 30 min. The resin was filtered off and washed with water. The filtrate and washings were combined and concentrated to 250 ml under reduced pressure. The residual solution was washed with AcOEt (100 ml) and concentrated to 200 ml under reduced pressure. After addition of 10% Pd–C (3.2 g), the mixture was stirred for 1.5 h at room temperature under a hydrogen atmosphere. The catalyst was removed by filtration, and 1 N HCl (40.4 ml) was added to the filtrate. The solution was concentrated to 15 g under reduced pressure, and cooled in a refrigerator overnight. The resulting precipitate was collected by filtration, washed with cold aq. EtOH (50%, v/v, 4 ml), and dried to give **3** as colorless crystals. *Anal.* Calcd for $C_5H_9N_3O_6S \cdot 0.8H_2O$: C, 23.68; H, 4.21; N, 16.57. Found: C, 23.69; H, 3.94; N, 16.35. $[\alpha]_D^{22}$ −61.0 ° (c = 1, DMSO). The product **3** was shown to be identical with an authentic sample[4] by ^{1}H-NMR and HPLC comparisons.

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References and Notes

1) Part of this work was reported as a brief communication,[2] and presented (a) at the 2nd Symposium of the French-Japanese Society for Medicinal and Fine Chemistry, Montsoult, France, September 1982; (b) at the 4th Symposium on Medicinal Chemistry, Tokyo, November 1982; and (c) at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, U.S.A., October 1983.
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