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SYNTHESIS OF LACTIVICIN ANALOGUES*

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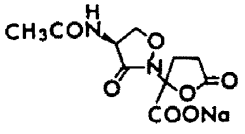
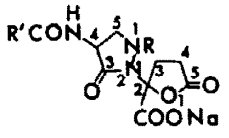
Abstract —— Aza analogues of lactivicin (1), a recently discovered novel antibiotic, were prepared by an application of our earlier convenient synthesis of 1 and its derivatives. A 1-unsubstituted pyrazolidinone derivative bearing 2-aminothiazol-4-yl-(Z)-methoxyiminoacetyl group exhibited *in vitro* antibacterial activity.

Lactivicin (1),[1] a new type of antibiotic having β-lactam-like activity, is a novel antibiotic isolated from *Empedobacter lactamgenus* YK-258 and *Lysobacter albus* YK-422. Although lacking a β-lactam ring in its structure, 1 has β-lactam-like biological activities; potent antibacterial activity, affinity to penicillin binding proteins and susceptibility to β-lactamases. This discovery will probably give further stimulus to the recent extensive investigations on preparing non-β-lactam antibiotics such as aza β-lactams,[2] γ-lactam analogues[3] and bicyclic pyrazolidinones.[4]

In an extension to our research program[5] on the chemical modification of 1 to develop new antibacterial compounds, we synthesized hitherto unreported aza analogues (2) of lactivicin.



1



2

An application of our earlier convenient synthesis of lactivicin and its derivatives[5a] to the synthesis of 2 by using pyrazolidinones in place of oxazolidinones was considered to be the most straightforward approach.

Synthesis of 1-substituted pyrazolidinone derivatives

Treatment of *N*-benzyloxycarbonyl-D,L-serine (Cbz-serine) methyl ester (3) with phosphorous pentachloride according to the method of Budovskii et al.[6] gave

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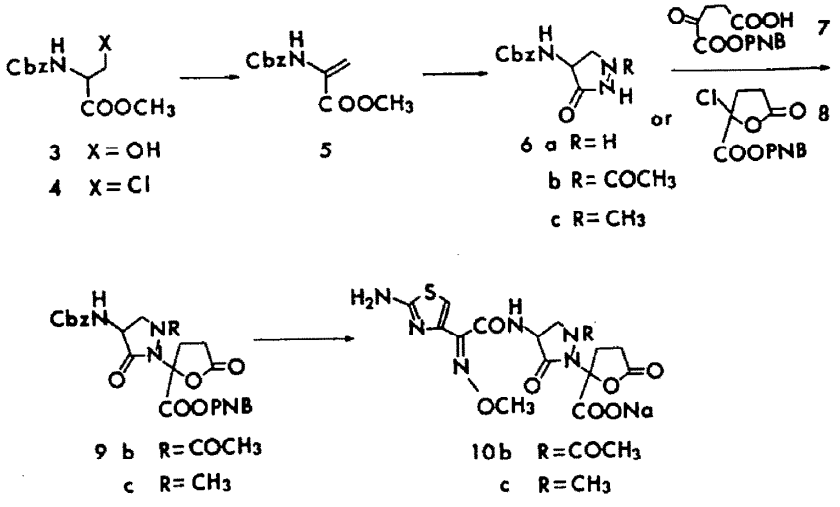
* This paper is dedicated to Professor Edward C. Taylor on the occasion of his 65th birthday.

the chloro compound 4. The acrylic acid derivative 5 obtained by the treatment of 4 with DBU was reacted with hydrazine hydrate without purification to give the pyrazolidinone 6a in 46% yield. Acetylation with acetic anhydride afforded 1-acetyl compound 6b in 76% yield. Similarly, 1-methyl compound 6c was prepared in 24% yield by reacting 5 with methylhydrazine. Treating 6b with 1-(4-nitrobenzyl) (PNB) 2-oxoglutarate (7)[5a] in the presence of DCC resulted in the recovery of the starting material 6b. Then the reaction of 6b in the presence of NaH with 4-nitrobenzyl 2-chloro-5-oxotetrahydrofuran-2-carboxylate (8)[5a] (obtained from 7 by the reaction of $SOCl_2$) afforded a key intermediate 9b* although in low yield (17%). The NMR and IR spectra confirmed the structure of 9b in which an absorption of γ-lactone at 1800 cm^{-1} (characteristic of lactivicin esters[1,5]) was observed.



Scheme 1

On the other hand, the reaction of 6c with 7 in the presence of DCC proceeded smoothly to give 9c in 57% yield. The difference of the chemical reactivity between 6b and 6c can be reasonably explained by the difference of nucleophilicity of the nitrogen at the 2-position as a result of the 1-substituent.

Conversion of 9b,c into 2-aminothiazol-4-yl-(Z)-methoxyiminoacetamido compounds (10b,c) was achieved by the hydrogenolytic removal of the protecting group from 9b,c in the presence of palladium-charcoal, followed by acylation with 2-(2-chloroacetamidothiazol-4-yl)-(Z)-2-methoxyiminoacetyl chloride (CATAM-Cl).[7] Subsequent removal of the chloroacetyl group with sodium *N*-methyldithiocarbamate gave desired 10b and 10c in 42 and 71% yields, respectively.

Synthesis of 1-unsubstituted pyrazolidinone derivatives

Synthesis of 1-unsubstituted pyrazolidinone derivatives 2 (R=H) was investigated. Before the introduction of the lactone moiety into the 2-position of the pyrazolidinone derivatives, the nitrogen at the 1-position has to be protected by an appropriate group.

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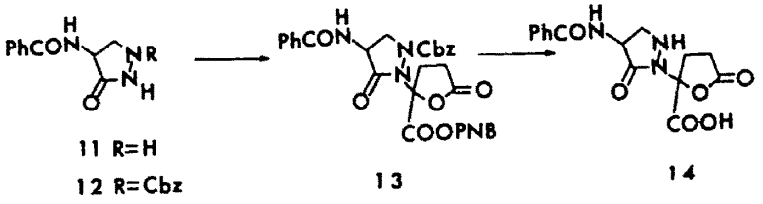
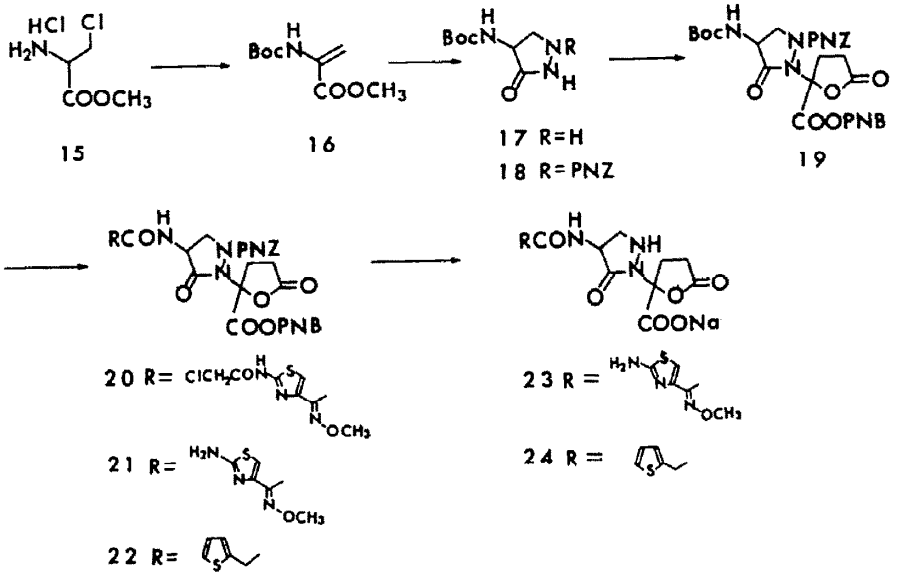
* Lactivicin and its ester exist as equilibrium mixtures of epimers (ca. 1:1) at the C-2 of the 5-oxotetrahydrofuran(γ-lactone) moiety,[1] therefore, 9b, and all the other new compounds having 5-oxotetrahydrofuran moiety described in this paper are considered also to be mixtures of C-2 epimers. However, clear information on the C-2 stereochemistry was not obtained from the NMR spectra.



11 R=H
12 R=Cbz
13
14

Scheme 2

4-Benzamidopyrazolidinone (11)[6] was prepared in a manner similar to that employed for the synthesis of 6a. Treatment of 11 with Cbz chloride in dimethylacetamide gave 12 in 64% yield. Condensation of 12 with 8 in the presence of a base gave rise to the γ-lactone compound 13 in 10% yield. Simultaneous hydrogenolytic removal of both the PNB and Cbz groups afforded 14 in 28% yield. These results indicate that the preparation of 1-unsubstituted pyrazolidinone derivatives is possible when an appropriate reductively removable protecting group is introduced into the 1-position. However, variation of the 4-acylamido group by this synthetic pathway needs repetition of this tedious procedure and, moreover, removal of the Cbz group can be difficult if the 4-acyl moiety has a sulfur containing group that causes catalysis poisoning.



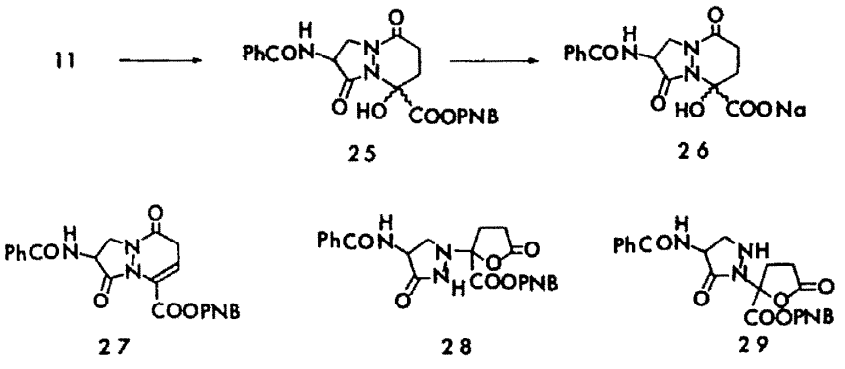
15
16
17 R=H
18 R=PNZ
19
20 R=
21 R=
22 R=
23 R=
24 R=

Scheme 3

For a more efficient synthesis of 1-unsubstituted pyrazolidinone derivatives (2, R=H) bearing various 4-acyl group, an alternative synthesis *via* 4-*tert*-butoxycarbonyl (Boc) amino-1-(4-nitrobenzyloxycarbonyl) (PNZ) pyrazolidinone (18) was investigated. Treatment of methyl 2-amino-3-chloropropionate·HCl (15),[8] prepared from D,L-serine, with di-*tert*-butyl dicarbonate in the presence of a base gave methyl 2-*tert*-butoxycarbonylaminopropenate (16) quantitatively. Pyrazolidinone 17 was prepared from 16 and hydrazine hydrate in 49% yield, which was then converted into 1-PNZ compound 18 in 75% yield. Condensation of 18 with 7 proceeded smoothly in the presence of DCC to afford 19 in 54% yield. In contrast, the reaction of 18 with 8 resulted in the recovery of 18. The Boc group was then

removed by treating 19 with trifluoroacetic acid. Subsequent acylation with CATAM-Cl (yield, 87%) and removal of the chloroacetyl group gave 21 in 85% yield. Simultaneous hydrogenolytic removal of both the PNB and PNZ groups in the presence of palladium-charcoal afforded the desired 23 in 62% yield.

Similarly, 4-(2-thienylacetamido) ester 22 was obtained from 19 in 54% yield. Hydrogenolysis of 22 gave 24 in 27% yield.



Scheme 4

Condensation of 1-unprotected 11 with 7 in the presence of DCC gave rise to neither 28 nor 29, but to bicyclic pyrazolidinone 25 in 54% yield. Attempted dehydration *via* the mesylate to give 27 was unsuccessful. The free acid obtained by removing the PNB group from 25 was isolated as its sodium salt (26).

Antibacterial activity

New pyrazolidinone derivatives (10b, 10c, 24 and 26) did not inhibit the growth of *Escherichia coli* 0-111 and *Streptococcus pyogenes* E-14 at 100 μg/ml (10^8 colony forming units/ml). However, 23 showed the minimum inhibitory concentration of 50 μg/ml against these microorganisms.

Acknowledgment The authors thank Dr. T. Iwahi for the biological evaluation.

EXPERIMENTAL

Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with a Hitachi 215 spectrophotometer. NMR spectra were taken on a Varian EM-390 (90 MHz) spectrometer with tetramethylsilan as an internal standard. Abbreviations are as follows: s=singlet; d=doublet; br=broad; dd=doublet of doublets; t=triplet; q=quartet. Extracted solutions were dried over sodium sulfate. Solvents were evaporated under reduced pressure.

Methyl 2-benzyloxycarbonylamino-3-chloropropionate (4)

N-Benzyloxycarbonyl-D,L-serine methyl ester (4.20 g, 16.6 mmol) was added to a stirred, cooled (0°C) solution of phosphorous pentachloride (3.80 g, 18 mmol) in $CHCl_3$ (15 ml). After being stirred for 4 h at 0°C, the solvent was evaporated and EtOAc was added to the residue. The organic layer was washed successively with sat. aq. $NaHCO_3$, H_2O, and sat. aq. NaCl, and dried. After evaporation of the solvent, the residue was subjected to chromatography on silica gel. Elution with EtOAc-hexane (1:1, v/v) afforded 4 (2.63 g, 58%) as a colorless liquid. IR ν_{max}

cm^{-1}: 1760, 1720. NMR ($CDCl_3$) δ : 3.78 (3H, s, OCH_3), 3.89 (2H, t, J=3Hz, CH_2Cl), 4.76 (1H, m, CHN), 5.12 (2H, s, OCH_2Ar), 5.70 (1H, br, NH), 7.35 (5H, s, ArH).

Methyl 2-benzyloxycarbonylaminopropenate (5)

Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.88 ml, 5.88 mmol) was added to a stirred, cooled (0°C) solution of 4 (1.45 g, 5.34 mmol) in $CHCl_3$ (5 ml) under an argon atmosphere. After being stirred for 1 h at 60°C, the solvent was evaporated and the residue was dissolved in EtOAc. Organic layer was washed successively with H_2O, sat. aq. NaCl, and dried. Evaporation of the solvent gave 5 (1.05 g, 84%) as a colorless oil. IR ν_{max} cm^{-1} : 1740, 1720. NMR ($CDCl_3$) δ : 3.79 (3H, s, OCH_3), 5.13 (2H, s, OCH_2Ar), 5.74 (1H, d, J=3Hz, CH=), 6.23 (1H, s, CH=), 7.33 (6H, s, NH, ArH).

4-Benzyloxycarbonylaminopyrazolidin-3-one (6a)

Hydrazine hydrate (0.24 ml, 4.95 mmol) was added at 0°C to the solution of 5 (1.05 g, 4.46 mmol) in EtOH (10 ml) and stirred for 30 min at room temperature, then 3 h at 90°C. After evaporation of the solvent, the residue was chromatographed on silica gel. Elution with EtOAc-MeOH (10:1, v/v) gave 6a (483 mg, 46%) as colorless crystals, mp 155-156°C. *Anal.* Calcd for $C_{11}H_{13}N_3O_3$: C, 56.16; H, 5.57; N, 17.86. Found: C, 56.13; H, 5.53; N, 17.58. IR ν_{max} cm^{-1} : 1710, 1680. NMR (DMSO-d_6) δ : 3.13 (1H, dd, J=9, 12Hz, CH), 3.70 (1H, dd, J=7.5, 12Hz, CH), 4.36 (1H, m, CHCO), 5.08 (2H, s, OCH_2Ar), 6.55 (1H, br, NH), 7.31 (5H, s, ArH).

1-Acetyl-4-benzyloxycarbonylaminopyrazolidin-3-one (6b)

Acetic anhydride (0.083 ml, 0.880 mmol) was added at 0°C to a stirred solution of 6a (208 mg, 0.884 mmol) in CH_2Cl_2 (16 ml) and the mixture was stirred for 30 min at room temperature. After evaporation of the solvent, 6b (186 mg, 76%) was obtained as colorless crystals, mp 193-195°C. *Anal.* Calcd for $C_{13}H_{15}N_3O_4$: C, 56.31; H, 5.45; N, 15.15. Found: C, 56.04; H, 5.36; N, 15.23. IR ν_{max} cm^{-1} : 1730, 1700, 1630. NMR (DMSO-d_6) δ : 2.01 (3H, s, $COCH_3$), 3.65 (1H, m, CH), 4.20-4.80 (2H, m, CH, CHCO), 5.07 (2H, s, OCH_2Ar), 7.31 (5H, s, ArH), 7.65 (1H, br, NH).

4-Benzyloxycarbonylamino-2-methylpyrazolidin-3-one (6c)

6c was prepared as a pale yellow oil in a manner similar to that described for the preparation of 6a by using methylhydrazine in place of hydrazine hydrate. Yield 24%. IR ν_{max} cm^{-1} : 1720-1680. NMR ($CDCl_3$) δ : 2.56 (3H, s, NCH_3), 3.05 (1H, dd, J=12, 12Hz, CH), 3.51 (1H, dd, J=7.5, 12Hz, CH), 4.65 (1H, m, CHCO), 5.07 (2H, s, OCH_2Ar), 6.28 (1H, br, NH), 7.31 (5H, s, ArH).

4-Nitrobenzyl 2-(1-acetyl-4-benzyloxycarbonylamino-3-oxopyrazolidin-2-yl)-5-oxotetrahydrofuran-2-carboxylate (9b)

A mixture of sodium hydride (60%) (34 mg, 0.85 mmol) and 6b (233 mg, 0.84 mmol) in DMF (12 ml) was stirred for 5 min at 0°C. A solution of 4-nitrobenzyl 2-chloro-5-oxotetrahydrofuran-2-carboxylate (8)[5a] in DMF (2 ml) was added dropwise to the mixture, which was stirred for further 1 h at 0°C. The mixture was extracted with EtOAc, and the organic layer was washed successively with H_2O and sat. aq. NaCl, and dried. After evaporation of the solvent, the residue was subjected to chromatography on silica gel. Elution with EtOAc-hexane (1:1 → 2:1) gave 9b (76 mg, 17%) as a colorless oil. IR ν_{max} cm^{-1} : 1800, 1760, 1740. NMR ($CDCl_3$) δ : 1.94 (3H, s, $COCH_3$), 2.40-3.01 (4H, m, CH_2CH_2), 4.13 (1H, dd, J=9, 12Hz, CH), 4.95 (2H, m, CHCO, CH), 5.08 (2H, s, OCH_2Ar), 5.32 (2H, s, OCH_2Ar), 7.31 (5H, s, ArH), 7.48 (2H, d, J=9Hz, ArH), 8.18 (2H, d, J=9Hz, ArH).

4-Nitrobenzyl 2-(4-benzyloxycarbonylamino-1-methyl-3-oxopyrazolidin-2-yl)-5-oxotetrahydrofuran-2-carboxylate (9c)

Dicyclohexylcarbodiimide (DCC) (260 mg, 1.260 mmol) was added at room temperature to a solution of 6c (210 mg, 0.842 mmol) and 1-(4-nitrobenzyl) 2-oxoglutarate 7[5a] (260 mg, 0.925 mmol) in CH_2Cl_2 (10 ml) under an argon atmosphere, and the mixture was stirred for 14 h. The precipitate was filtered off and the filtrate was concentrated. The concentrate was subjected to chromatography on silica gel. Elution with EtOAc-hexane (2:1) afforded 9c (248 mg, 57%) as a pale yellow oil. IR ν_{max} cm^{-1} : 1800, 1720. NMR (CDCl3) δ : 2.30-3.10 (4H, m, CH_2CH_2), 2.81 (3H, s, NCH_3), 3.10-3.60 (2H, m, CH_2), 4.90 (1H, m, CHCO), 5.08 (2H, s, OCH_2Ar), 5.30 (2H, s, OCH_2Ar), 7.31 (5H, s, ArH), 7.50 (2H, d, J=9Hz, ArH), 8.18 (2H, d, J=9Hz, ArH).

Sodium 2-[1-acetyl-4-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-oxopyrazolidin-2-yl]-5-oxotetrahydrofuran-2-carboxylate (10b)

A mixture of 9b (70 mg, 0.130 mmol) and 10% Pd-C (70 mg) in pH 7.0 phosphate buffer (5 ml) and EtOAc (4 ml) was stirred for 30 min at room temperature under a hydrogen atmosphere. After removal of the catalyst by filtration, aqueous layer was separated. $NaHCO_3$ and 2-(2-chloroacetamidothiazol-4-yl)-(Z)-2-methoxyiminoacetyl chloride hydrochloride (CATAM-Cl·HCl) (57 mg, 0.171 mmol) were added to the ice-cold aqueous solution, and the mixture was stirred for 40 min at 0°C. the reaction mixture was concentrated, and the aqueous layer was washed with EtOAc. A mixture of sodium *N*-methyldithiocarbamate, the aqueous solution, and THF (4 ml) was stirred for 1 h at room temperature. The reaction mixture was concentrated and the concentrate was chromatographed on HP-20, using H_2O and 5% EtOH as an eluent. The desired fractions were collected and lyophilized to give 10b (26 mg, 42%) as a colorless powder. Due to a limited quantity of the product, elemental analysis was not performed. IR ν_{max} cm^{-1} : 1780, 1660. NMR (D_2O) δ : 2.63 (3H, s, $COCH_3$), 2.90-3.50 (4H, m, CH_2CH_2), 4.43 (3H, s, OCH_3), 4.30-5.01 (2H, m, CH_2), 5.85 (1H, m, CHCO), 7.43 (1H, s, ArH).

Sodium 2-[4-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-1-methyl-3-oxopyrazolidin-2-yl]-5-oxotetrahydrofuran-2-carboxylate (10c)

10c was prepared from 9c as a colorless powder in the same manner as that described for the preparation of 10b. Yield 71%. *Anal.* Calcd for $C_{15}H_{17}N_6O_7SNa \cdot 4H_2O$: C, 34.62; H, 4.84; N, 16.15. Found: C, 34.57; H, 4.58; N, 15.87. IR ν_{max} cm^{-1} : 1780, 1710. NMR (D_2O) δ : 3.33 (3H, s, NCH_3), 2.90-3.60 (4H, m, CH_2CH_2), 4.30 (2H, m, CH_2), 4.43 (3H, s, OCH_3), 5.95 (1H, m, CHCO), 7.53 (1H, s, ArH).

4-Benzamido-1-benzyloxycarbonylpyrazolidin-3-one (12)

Benzyloxycarbonyl chloride (Cbz-Cl) (1.50 ml, 10.4 mmol) was added at 0°C to a solution of 4-benzamidopyrazolidin-3-one 11[6] (1.80 g, 8.8 mmol) in DMA (16 ml) under an argon atmosphere, and the mixture was stirred for 1 h at 0°C. The mixture was poured into water, and extracted with EtOAc. The organic layer was washed successively with H_2O and sat. aq. NaCl, and dried. After evaporation of the solvent, 12 (1.90 g, 64%) was obtained as colorless crystals, mp 207-208°C. *Anal.* Calcd for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.41; H, 5.12; N, 12.19. IR ν_{max} cm^{-1} : 1700, 1670. NMR (DMSO-d_6) δ : 3.62 (1H, m, CH), 4.25 (2H, m, CH, CHCO), 5.12 (2H, s, OCH_2Ar), 7.13-7.84 (10H, m, ArH), 8.81 (1H, br, NH).

4-Nitrobenzyl 2-(4-benzamido-1-benzyloxycarbonyl-3-oxopyrazolidin-2-yl)-5-oxotetrahydrofuran-2-carboxylate (13)

13 was prepared as a pale yellow oil from 12 in the same manner as that described for the preparation of 9b. Yield 10%. IR ν_{max} cm^{-1} : 1800, 1750, 1600. NMR ($CDCl_3$) δ : 2.40-3.19 (4H, m, CH_2CH_2), 3.98 (1H, m, CH), 4.60-4.98 (2H, m, CHCO, CH), 5.20 (2H, ABq, OCH_2Ar), 5.33 (2H, s, OCH_2Ar), 7.22-8.21 (14H, m, ArH).

2-(4-Benzamido-3-oxopyrazolidin-2-yl)-5-oxotetrahydrofuran-2-carboxylic acid (14)

A mixture of 13 (273 mg, 0.45 mmol) and 10% Pd-C (400 mg) in MeOH (8 ml) and pH 7.0 phosphate buffer (12 ml) was stirred under a hydrogen atmosphere for 2 h at room temperature. The catalyst was filtered off, and the filtrate was washed with EtOAc and concentrated under reduced pressure. The concentrate was subjected to chromatography on XAD-2 using H_2O and 20% EtOH as an eluent. The desired fractions were collected and lyophilized to give 14 (42 mg, 28%) as a colorless powder. *Anal.* Calcd for $C_{15}H_{15}N_3O_6 \cdot 1/4H_2O$: C, 53.26; H, 4.87; N, 12.34. Found: C, 53.33; H, 4.62; N, 12.43. IR ν_{max} cm^{-1} : 1780, 1750, 1640. NMR (D_2O) δ : 2.90-3.60 (4H, m, CH_2CH_2), 3.71 (1H, m, CH), 4.20 (1H, m, CH), 5.50 (1H, m, CH), 7.31-7.88 (5H, s, ArH).

Methyl 2-*tert*-butoxycarbonylaminopropenate (16)

Di-*tert*-butyl dicarbonate (4.80 ml, 20.9 mmol) was added at 0°C to a stirred solution of 15 (3.48 g, 20 mmol) and Et_3N (5.60 ml, 40.2 mmol) in $CHCl_3$ (50 ml), and the mixture was stirred for 30 min. DBU (2.90 ml, 19 mmol) was added dropwise to the mixture, and the mixture was stirred for 3 h at 0°C. After evaporation of the solvent, EtOAc and H_2O were added to the residue. The organic layer was separated and the aqueous layer was further extracted with EtOAc. The combined extracts were washed successively with H_2O and sat. aq. NaCl, and dried. After evaporation of the solvent, the residue was subjected to chromatography on silica gel. Elution with EtOAc-hexane (1:1) gave 16 (3.98 g, 99%) as a colorless oil. IR ν_{max} cm^{-1} : 1740, 1720. NMR ($CDCl_3$) δ : 1.53 (9H, s, *t*-Bu), 3.81 (3H, s, OCH_3), 5.69 (1H, d, J=3Hz, CH=), 6.14 (1H, s, CH=), 7.03 (1H, br, NH).

4-*tert*-Butoxycarbonylaminopyrazolidin-3-one (17)

17 was prepared from 16 in a manner similar to that described for the preparation of 6a, mp 168-172°C. Yield 49%. *Anal.* Calcd for $C_8H_{15}N_3O_3$: C, 47.75; H, 7.51; N, 20.88. Found: C, 47.90; H, 7.54; N, 20.59. IR ν_{max} cm^{-1} : 1680, 1540. NMR ($CDCl_3$-DMSO-d_6) δ : 1.45 (9H, s, *t*-Bu), 3.06 (1H, dd, J=12, 12Hz, CH), 3.63 (1H, dd, J=7.5, 12Hz, CH), 4.26 (1H, m, CH), 6.20 (1H, br, NH).

4-*tert*-Butoxycarbonylamino-1-(4-nitrobenzyloxycarbonyl)pyrazolidin-3-one (18)

18 was prepared from 17 in a manner similar to that described for the preparation of 12 by using 4-nitrobenzyloxycarbonyl chloride in place of Cbz-Cl, mp 162-163°C. Yield 75%. *Anal.* Calcd for $C_{16}H_{20}N_4O_7$: C, 50.53; H, 5.30; N, 14.73. Found: C, 47.90; H, 5.33; N, 14.64. IR ν_{max} cm^{-1} : 1700, 1600. NMR ($CDCl_3$-DMSO-d_6) δ : 1.40 (9H, s, *t*-Bu), 3.60 (1H, m, CH), 4.20-4.50 (2H, m, CH_2), 5.20 (2H, s, OCH_2Ar), 7.00 (1H, br, NH), 7.63 (2H, d, J=9Hz, ArH), 8.20 (2H, d, J=9Hz, ArH).

4-Nitrobenzyl 2-[4-*tert*-butoxycarbonylamino-1-(4-nitrobenzyloxycarbonyl)-3-oxopyrazolidin-2-yl]-5-oxotetrahydrofuran-2-carboxylate (19)

19 was prepared as a pale yellow oil from 18 in the same manner as that described for the preparation of 9c. Yield 54%. IR ν_{max} cm^{-1} : 1800, 1760-1710, 1600. NMR ($CDCl_3$) δ : 1.43 (9H, s, *t*-Bu), 2.60-3.20 (4H, m, CH_2CH_2), 3.63 (1H, dd, J=12, 12Hz, CH), 4.50-5.10 (2H, m, CHCO, CH), 5.30 (2H, ABq, OCH_2Ar), 5.33 (2H, s, OCH_2Ar), 7.50 (2H, d, J=9Hz, ArH), 7.51 (2H, d, J=9Hz, ArH), 8.23 (4H, d, J=9Hz, ArH).

4-Nitrobenzyl 2-[4-[2-(2-chloroacetamidothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-1-(4-nitrobenzyloxycarbonyl)-3-oxopyrazolidin-2-yl]-5-oxotetrahydrofuran-2-carboxylate (20)

Trifluoroacetic acid (0.61 ml) was added at 0°C to 19 (158 mg, 0.246 mmol) and allow to stand for 1 h. After evaporation of the excessive trifluoroacetic acid, the residue was dissolved in a mixture of THF (5 ml) and H_2O (5 ml). $NaHCO_3$ (63 mg, 0.750 mmol) and CATAM-Cl·HCl (83 mg, 0.250 mmol) were added at 0°C to the mixture, and the mixture was stirred for 1 h. The mixture was extracted with EtOAc, and the extracts were washed successively with H_2O and sat. aq. NaCl, and dried. After evaporation of the solvent, the residue was subjected to chromatography on silica gel. Elution with EtOAc-hexane (1:1 → 2:1) gave 20 (171 mg, 87%) as a pale yellow oil. IR ν_{max} cm^{-1} : 1800, 1750, 1670. NMR ($CDCl_3$) δ : 2.50-3.20 (4H, m, CH_2CH_2), 3.79 (1H, dd, J=12, 12Hz, CH), 4.00 (3H, s, OCH_3), 4.23 (2H, s, $ClCH_2CO$), 4.80-5.20 (2H, m, CHCO, CH), 5.26 (2H, s, OCH_2Ar), 7.33 (1H, s, ArH), 7.44 (2H, d, J=9Hz, ArH), 7.53 (2H, d, J=9Hz, ArH), 8.17 (2H, d, J=9Hz, ArH), 8.20 (2H, d, J=9Hz, ArH).

4-Nitrobenzyl 2-[4-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-1-(4-nitrobenzyloxycarbonyl)-3-oxopyrazolidin-2-yl]-5-oxotetrahydrofuran-2-carboxylate (21)

A stirred solution of 20 (171 mg, 0.213 mmol) in a mixture of THF (4.5 ml) and H_2O (4.5 ml) was treated with *N*-methyldithiocarbamate (41 mg, 0.317 mmol) at 0°C, and the mixture was stirred for 1 h at room temperature. The mixture was extracted with EtOAc, and the extracts were washed successively with H_2O and sat. aq. NaCl, and dried. After evaporation of the solvent, the residue was subjected to chromatography on silica gel. Elution with EtOAc gave 21 (131 mg, 85%) as a pale yellow foam. IR ν_{max} cm^{-1} : 1800, 1750, 1680. NMR ($CDCl_3$) δ : 2.50-3.20 (4H, m, CH_2CH_2), 3.80 (1H, m, CH), 3.85 (3H, s, OCH_3), 4.82 (1H, m, CH), 5.30 (1H, m, CH), 5.40 (2H, s, OCH_2Ar), 6.56 (2H, br, NH_2), 6.86 (1H, s, ArH), 7.59 (2H, d, J=9Hz, ArH), 7.66 (2H, d, J=9Hz, ArH), 8.15 (2H, d, J=9Hz, ArH), 8.20 (2H, d, J=9Hz, ArH).

4-Nitrobenzyl 2-[1-(4-nitrobenzyloxycarbonyl)-4-(2-thienylacetamido)-3-oxopyrazolidin-2-yl]-5-oxotetrahydrofuran-2-carboxylate (22)

22 was prepared from 19 in a manner similar to that described for the preparation of 20 by using thienylacetyl chloride in place of CATAM-Cl. Yield 54%. IR ν_{max} cm^{-1} : 1800, 1750, 1680. NMR ($CDCl_3$) δ : 2.50-3.20 (4H, m, CH_2CH_2), 3.50-3.80 (1H, m, CH), 3.79 (2H, s, CH_2Ar), 4.70-5.10 (2H, m, CH_2), 5.33 (2H, s, OCH_2Ar), 6.40 (1H, br, NH), 6.90-7.10 (2H, m, ArH), 7.35-7.70 (5H, m, ArH), 8.21 (4H, d, J=9Hz, ArH).

Sodium 2-[4-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-oxopyrazolidin-2-yl]-5-oxotetrahydrofuran-2-carboxylate (23)

A mixture of 21 (131 mg, 0.180 mmol), 10% Pd-C (130 mg), THF (6 ml), H_2O (3 ml), and pH 7.0 phosphate buffer (3 ml) was stirred for 2 h at room temperature

under a hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was concentrated, and washed with EtOAc. The aqueous layer was chromatographed on HP-20, using H_2O as an eluent. The desired fractions were collected and lyophilized to give 23 (50 mg, 63%) as a colorless powder. *Anal.* Calcd for $C_{14}H_{15}N_6O_7SNa \cdot 3.2H_2O$: C, 34.18; H, 4.38; N, 17.08. Found: C, 34.18; H, 4.13; N, 16.85. IR ν_{max} cm^{-1} : 1780, 1720, 1710. NMR (D_2O) δ : 2.80-3.60 (4H, m, CH_2CH_2), 3.70 (1H, m, CH), 4.20 (1H, m, CH), 4.46 (3H, s, OCH_3), 5.51 (1H, m, CH), 7.56 (1H, s, ArH).

2-[4-(2-Thienylacetamido)-3-oxopyrazolidin-2-yl]-5-oxotetrahydrofuran-2-carboxylic acid (24)

24 was prepared from 22 in the same manner as that described for the preparation of 14. Yield 30%. *Anal.* Calcd for $C_{14}H_{15}N_3O_6S \cdot 0.8H_2O$: C, 45.72; H, 4.55; N, 11.43. Found: C, 45.87; H, 4.78; N, 11.54. IR ν_{max} cm^{-1} : 1780, 1730. NMR (D_2O) δ : 2.80-3.60 (4H, m, CH_2CH_2), 3.70 (1H, m, CH), 4.20 (1H, m, CH), 4.30 (2H, m, CH_2Ar), 4.70 (1H, m, CH), 7.30-7.60 (2H, m, ArH), 7.70-7.90 (1H, m, ArH).

4-Nitrobenzyl 8-benzamido-2-hydroxyl-5,9-dioxo-1,6-diazabicyclo[4.3.0]nonane-2-carboxylate (25)

25 was prepared from 11 in the same manner as that described for the preparation of 9c. Yield 54%. IR ν_{max} cm^{-1} : 1750, 1720, 1650. NMR ($CDCl_3$) δ : 2.02-2.81 (4H, m, CH_2CH_2), 3.56-3.82 (1H, m, CH), 4.43-4.71 (1H, m, CH), 4.96-5.28 (1H, m, CHCO), 5.29 (2H, s, OCH_2Ar), 7.19-8.04 (9H, m, ArH). ^{13}C-NMR ($CDCl_3$) δ : 80.70 (s, C_2).

Sodium 8-benzamido-2-hydroxyl-5,9-dioxo-1,6-diazabicyclo[4.3.0]nonane-2-carboxylate (26)

26 was prepared from 25 in the same manner as that described for the preparation of 23. Yield 79%. *Anal.* Calcd for $C_{15}H_{14}N_3O_6Na \cdot 1.8H_2O$: C, 46.47; H, 4.58; N, 10.84. Found: C, 46.52; H, 4.30; N, 10.98. IR ν_{max} cm^{-1} : 1720, 1640. NMR (D_2O) δ : 1.99-2.72 (4H, m, CH_2CH_2), 3.60-3.91 (1H, m, CH), 4.22-4.58 (1H, m, CH), 4.89-5.09 (1H, m, CHCO), 7.21-7.84 (5H, m, ArH). ^{13}C-NMR (D_2O) δ : 85.00 (s, C_2).

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