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Medicinal Chemical Studies on Antiplasmin Drugs. VII.[1] Oxa Analogs of 4-Aminomethylcyclohexanecarboxylic Acid

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The two isomers of 5-aminomethyltetrahydro-2*H*-pyran-2-carboxylic acid (**9**) and those of 5-aminomethyl-1,4-dioxane-2-carboxylic acid (**18**) were synthesized from 2-ethoxycarbonyl-3,4-dihydro-2*H*-pyran-5-carboxylic acid chloride (**2**) and dimethyl 1,4-dioxane-2,5-dicarboxylate (**15**), respectively. From the aminoacetal (**19**) and dimethyl bis(hydroxymethyl)malonate (**21**), *trans*-2-aminomethyl-1,3-dioxane-5-carboxylic acid (**26A**) was synthesized. The configurations of these isomers were determined on the basis of their nuclear magnetic resonance spectra, and the preferred conformations of the isomers in aqueous solution were similarly deduced. No compound showed antiplasmin activity more potent than that of *trans*-4-aminomethylcyclohexanecarboxylic acid (**1A**).

Keywords——antiplasmin drug; 4-aminomethylcyclohexanecarboxylic acid; structure-activity relationship; aminomethyltetrahydro-2*H*-pyrancarboxylic acid; aminomethyldioxanecarboxylic acid; stereo isomer; conformation

It has already been shown[3,4] that replacement of the hydrogen at the cyclohexane ring carbon or the side chain of 4-aminomethylcyclohexanecarboxylic acid (AMCHA, **1**) by a methyl group reduces the antiplasmin activity. *trans* AMCHA (tranexamic acid, **1A**) has the highest activity among compounds in this series, and the difference of the conformational equilibrium of the methyl derivative of **1** from that of **1A** and/or the steric effect of the methyl group may be responsible for lowering the antiplasmin activity.

As an extension of our work on the structure-activity relationship, we were interested in replacing the cyclohexane ring of **1** with an oxa analog, namely, a tetrahydropyran or dioxane ring, since oxa analogs of **1A** are presumed to take almost entirely the chair and diequatorial form,[5,6] as dose **1A**,[3] and also they have no additional substituent which may hinder the interaction between the drug and the receptor site. This paper deals with the synthesis, assignment of configuration, and favored conformation in aqueous solution of the isomers of 5-aminomethyltetrahydro-2*H*-pyran-2-carboxylic acid (**9**), 5-aminomethyl-1,4-dioxane-2-carboxylic acid (**18**), and *trans*-2-aminomethyl-1,3-dioxane-5-carboxylic acid (**26A**).

5-Aminomethyltetrahydro-2*H*-pyran-2-carboxylic Acid (9)

Ammonolysis of 2-ethoxycarbonyl-3,4-dihydro-2*H*-pyran-5-carboxylic acid chloride (**2**)[7] at 9—10° afforded 5-carbamoyl-3,4-dihydro-2*H*-pyran-2-carboxylic acid (**3**) but not the corresponding 2,5-dicarboxamide.[7] Esterification of **3** using hydrochloric acid-methanol, followed by dehydration with tosyl chloride-pyridine, afforded methyl 5-cyano-3,4-dihydro-2*H*-pyran-2-carboxylate (**5**), which was hydrolyzed in an alkaline solution at room temperature to give 5-cyano-3,4-dihydro-2*H*-pyran-2-carboxylic acid (**6**). Hydrogenation of **6** over platinum in methanol in the presence of an equimolar amount of hydrochloric acid gave a mixture of amino

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2) Location: *1-16-13, Kitakasai, Edogawa-ku, Tokyo 132, Japan.*
3) S. Isoda and M. Hirata, *Chem. Pharm. Bull.*, **27**, 2735 (1979).
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6) E.L. Eliel, *Angew. Chem. Int. Ed. Engl.*, **11**, 739 (1972).
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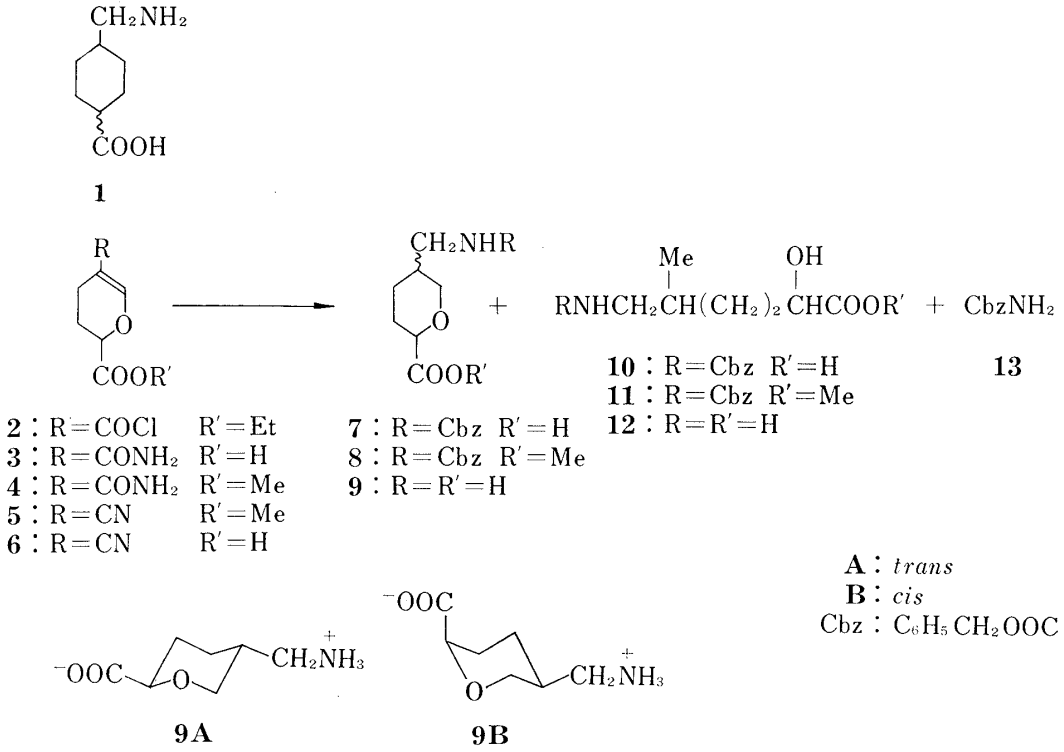

Chart 1

Only the 2*R* forms are shown.

acids. For separation, the mixture was acylated with benzyloxycarbonyl chloride and esterified with diazomethane. Chromatography of the product on silica gel gave benzyl carbamate (**13**) (yield 19%), methyl *trans*-5-benzyloxycarbonylaminomethyltetrahydro-2*H*-pyran-2-carboxylate (**8A**) (9%), its *cis* isomer (**8B**) (4%), and methyl 6-benzyloxycarbonylamino-2-hydroxy-5-methylhexanoate (**11**) (9%). Compound **13** was presumably a reaction product of benzyloxycarbonyl chloride with ammonia which was generated as a by-product in hydrogenation of the nitrile group.

Alkaline hydrolysis of **8A** and **8B**, followed by hydrogenation over palladium-carbon gave *trans*-5-aminomethyltetrahydro-2*H*-pyran-2-carboxylic acid (**9A**) and its *cis* isomer (**9B**), respectively. The assignment of *trans* or *cis* structure to these compounds was based on comparison of their nuclear magnetic resonance (NMR) spectra taken in deuterium oxide (D_2O). The C_2-proton signals of **9A** appeared at 3.80—4.05 ppm, overlapping with signals of the C_6-proton, whereas the C_2-proton signals of **9B** appeared at slightly lower field, 3.90—4.15 ppm, apart from the C_6-proton signals. The high field signals were ascribed to the axial hydrogen and the lower field signals to the equatorial hydrogen, by analogy with methyl *trans*- and *cis*-5-methyltetrahydro-2*H*-pyran-2-carboxylates whose stereochemistries have already been established.[5] The side chain methylene protons of both compounds, **9A** and **9B**, resonated at the same position, 3.16 ppm, indicating that they have an equatorial aminomethyl group. Thus, the aminomethyl-equatorial and carboxyl-equatorial form was deduced for **9A** in D_2O and the aminomethyl-equatorial and carboxyl-axial form for **9B** in D_2O.

The methyl ester **11** was hydrolyzed with an alkaline solution to give 6-benzyloxycarbonylamino-2-hydroxy-5-methylhexanoic acid (**10**), the structure of which was confirmed by its elemental analysis and NMR spectrum. The fission of the C–O bond of the vinyl ether[8] in the ring of **6** is responsible for the formation of **10**.

8) A.B. Woodward and W.E. Doering, *J. Am. Chem. Soc.*, **67**, 860 (1945).

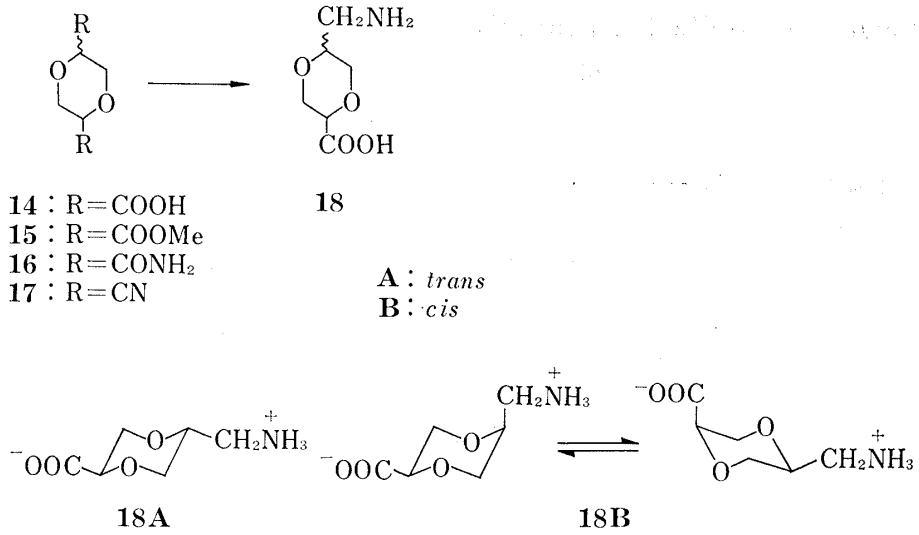

Chart 2

Only the 2*R* forms are shown.

5-Aminomethyl-1,4-dioxane-2-carboxylic Acid (18)

Dimethyl *trans*-1,4-dioxane-2,5-dicarboxylate (**15A**)[9] was treated with ammonia, and subsequent dehydration of the diamide (**16A**) with phosphoryl chloride-pyridine afforded *trans*-1,4-dioxane-2,5-dicarbonitrile (**17A**), which was hydrogenated over Raney Ni in ethanol until two mol of hydrogen had been absorbed, giving a hydrogenated mixture. The product was treated with hydrochloric acid-ethanol and the resulting imino ether was hydrolyzed under alkaline conditions to afford *trans*-5-aminomethyl-1,4-dioxane-2-carboxylic acid (**18A**). The structure of **18A** was confirmed by its elemental analysis and infrared (IR) spectrum, and the *trans* configuration was determined by conversion with nitric acid[9] to the dicarboxylic acid, which was identical with *trans*-1,4-dioxane-2,5-dicarboxylic acid (**14A**). The NMR spectrum of **18A** (D_2O) showed non-equivalent side chain methylene signals at 3.02 ppm and 3.33 ppm. The C_2-hydrogen signals at 4.28 ppm with $J_{2a,3a}$ (12Hz) and $J_{2a,3e}$ (3 Hz) indicate that the C_2-hydrogen is axial. From the C_{6e}-hydrogen signals at 4.07 ppm with $J_{6a,6e}$ (12 Hz) and $J_{5a,6e}$ (2 Hz) and the C_{6a}-hydrogen signals at 3.57 ppm with $J_{6a,6e}$ (12 Hz) and $J_{5a,6a}$ (10 Hz), the C_5-hydrogen, whose signals could not be assigned clearly due to overlapping with other signals, is deduced to be axial. Thus in D_2O **18A** is thought to take the aminomethyl-equatorial and carboxyl-equatorial form.

Dimethyl *cis*-1,4-dioxane-2,5-dicarboxylate (**15B**)[9] was allowed to react similarly to give *cis*-5-aminomethyl-1,4-dioxane-2-carboxylic acid (**18B**) as a hygroscopic powder. The NMR spectrum of **18B** (D_2O) showed unresolved multiplets at 3.1—3.3, 3.7—3.9, 3.95—4.2, and 4.2—4.35 ppm, indicating that **18B** exists as an equilibrium mixture of aminomethyl-axial and carboxyl-equatorial form and the opposite form, though the ratio could not be determined.

2-Aminomethyl-1,3-dioxane-5-carboxylic Acid (26)

Treatment of the aminoacetal (**19**) with benzyloxycarbonyl chloride in the presence of triethylamine gave the benzyloxycarbonylaminoacetal (**20**),[10] which was distilled under reduced pressure. The melting point of its 2,4-dinitrophenylhydrazone agreed with the reported value.[10] Heating **20** with dimethyl bis(hydroxymethyl)malonate (**21**)[11] in toluene

9) R.K. Summerbell and J.R. Stephens, *J. Am. Chem. Soc.*, **76**, 6401 (1954).
10) R. Mozingo and K. Folkers, "Chemistry of Penicillin," ed. by H.T. Clarke, Princeton Univ. Press, Princeton, 1949, p. 563 [*C.A.*, **44**, 9419 (1950)].

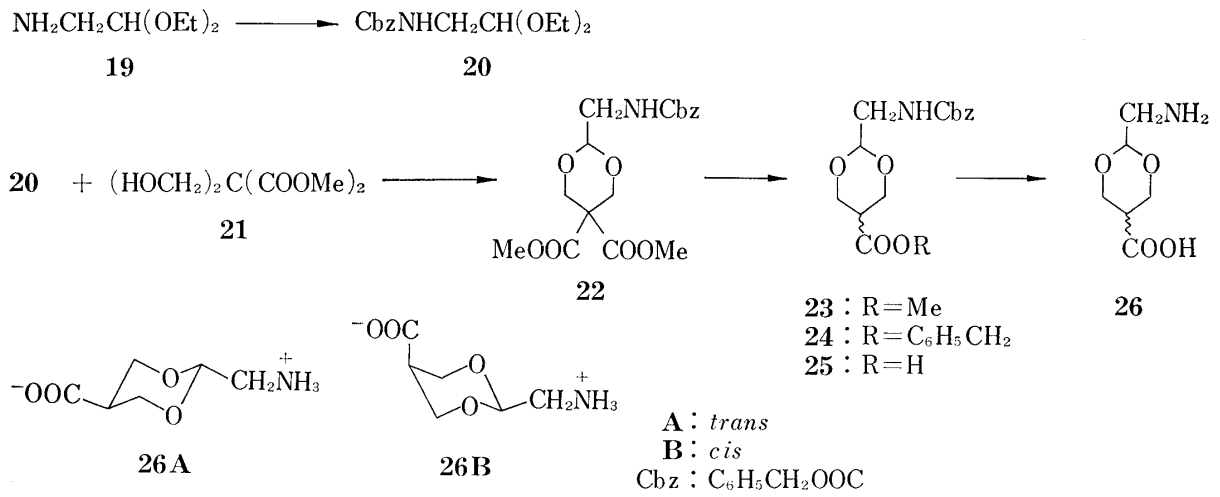

Chart 3

in the presence of *p*-toluenesulfonic acid with azeotropic removal of ethanol gave dimethyl 2-benzyloxycarbonylaminomethyl-1,3-dioxane-5,5-dicarboxylate (**22**). In the absence of *p*-toluenesulfonic acid **22** could not be obtained even when the reaction temperature was raised to 170—190°,[12] and the diethyl derivative of **22** could not be obtained because diethyl bis(hydroxymethyl)malonate[13] decomposed in refluxing toluene in the presence of *p*-toluenesulfonic acid. Treatment of **22** with sodium cyanide in dimethyl sulfoxide[14] gave methyl *trans*-2-benzyloxycarbonylaminomethyl-1,3-dioxane-5-carboxylate (**23A**), the corresponding *trans* benzyl ester (**24A**), and the *cis* methyl ester (**23B**). Hydrolysis of the benzyloxycarbonyl group of **22** and/or **23** followed by ester exchange of **23A** presumably leads to the formation of **24A**.

The configurations and conformations of **23A** and **23B** were determined on the basis of their NMR spectra. Both isomers are thought to exist in the benzyloxycarbonylaminomethyl-equatorial form, because the substituents at C_2 generally take equatorial orientations in the case of 2-substituted 1,3-dioxanes[6] and the chemical shift of the side chain methylene signals of **23A** (3.35 ppm) coincides with that of **23B** (3.36 ppm). The C_4- and C_6-hydrogen signals of **23A** in the region of 3.74—4.31 ppm, having $J_{4a,4e}$ (11 Hz), $J_{4a,5a}$ (12 Hz), and $J_{4e,5a}$ (5 Hz), and those of **23B** (3.80—4.80 ppm), which appeared as AB type signals having $J_{4a,4e}$ (11 Hz), $J_{4a,5e}$ (3 Hz), and $J_{4e,5e}$ (3 Hz) show that **23A** is in the benzyloxycarbonylaminomethyl-equatorial and methoxycarbonyl-equatorial form while **23B** is in the benzyloxycarbonylaminomethyl-equatorial and methoxycarbonyl-axial form. The C_5-hydrogen signals of **23A** appeared at 2.7—3.3 ppm as finely split signals and those of **23B** appeared at 2.35 ppm, higher field than those of **23A**. These findings are in agreement with the results obtained for 1,3-dioxane derivatives by Jones,[15] Pihlaja,[16] and Eliel.[17]

Treatment of **23A** with an alkaline solution and subsequent hydrogenation of the *trans* acid (**25A**) afforded *trans*-2-aminomethyl-1,3-dioxane-5-carboxylic acid (**26A**). The structure of **26A** was confirmed by its IR spectrum, NMR spectrum (D_2O) which indicated an amino-

11) A. Cohen and B.H. Brown, Brit. Patent 702766 (1954) [*C.A.*, **49**, 4710 (1955)].
12) F.F. Blicke and F.L. Schumann, *J. Am. Chem. Soc.*, **76**, 3153 (1954).
13) P. Block Jr., "Organic Syntheses," Coll. Vol. V, ed. by H.E. Baumgarten, John Wiley and Sons, Inc., New York, 1973, p. 381.
14) A.P. Krapcho, G.A. Glynn, and B.J. Grenon, *Tetrahedron Lett.*, **1967**, 215.
15) V.I.P. Jones and J.A. Ladd, *J. Chem. Soc.* (*B*), **1971**, 567.
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methyl-equatorial and carboxyl-equatorial *trans* form ($J_{4a,4e}$=11 Hz, $J_{4a,5a}$=11 Hz, and $J_{4e,5a}$=5 Hz), and its elemental analysis data.

On the other hand, treatment of **23B** with an alkaline solution at room temperature gave the *cis* acid (**25B**) together with isomerized **25A**. Separation of **25B** from **25A** was performed by fractional crystallization, and hydrogenation of **25B** gave a mixture of **26B** and **26A** in a ratio of about 2: 1. All attempts to isolate pure **26B** (recrystallization or column chromatography on cellulose) failed. The NMR spectrum of the mixture showed that **26B** takes the aminomethyl-equatorial and carboxyl-axial form.

Table I shows the antiplasmin activities of compounds obtained in this work. Replacement of the methylene in the cyclohexane ring of **1A** by oxygen(s) lowers the antiplasmin activity of **1A** to the level of **1B**. The reason for the lower antiplasmin activity of these oxa analogs may be that they have a slightly lower hydrophobicity than **1A**.

Table I. Antifibrinolytic Activity[a] of Oxa Analogs of AMCHA (**1**)

Compound No.	Relative activity[b]
9A	8.0×10^{-3}
9B	9.0×10^{-3}
18A	5.3×10^{-2}
18B	4.5×10^{-3}
26A	7.1×10^{-2}
1A	1.0
1B	3.2×10^{-2}

a) The inhibitory effects on fibrin clot lysis were determined accoriding to the method of Okamoto.[c]
b) Relative activities are assigned on a molar basis, taking the activity of **1A** as 1.0.[d]
c) S. Okamoto and U. Okamoto, *Keio J. Med.*, **11**, 105 (1962).
d) A. Okano, M. Inaoka, S. Funabashi, M. Iwamoto, S. Isoda, R. Moroi, Y. Abiko, and M. Hirata, *J. Med. Chem.*, **15**, 247 (1972).

Experimental

The following instruments were used. Melting points, a Yanagimoto MP-1 melting point apparatus; IR spectra, a Hitachi EPI-G2 or a Hitachi 285 spectrophotometer; NMR spectra, a Hitachi Perkin-Elmer R-20B or a Varian EM-360 spectrometer with tetramethylsilane (TMS) as an internal standard or with TMS in CCl_4 as an external standard when D_2O was used as a solvent. All melting points and boiling points are uncorrected.

Methyl 5-Carbamoyl-3,4-dihydro-2*H*-pyran-2-carboxylate (**4**)——Compound **2**[7] (120 g, 0.55 mol) was added to a mixture of c. NH_4OH (65.5 ml, 1.1 mol) and C_6H_6 (600 ml) at 9—10° over a 25 min period. After stirring for 1 hr at 9—10°, the precipitates were collected and recrystallized from H_2O to give **3** as colorless needles (42.5 g, 52%), mp 245—247° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3450, 3320, 3220, 1700, 1640, 1440, 1230, 1190, 1170, 755. NMR (DMSO-d_6) δ: 1.9—2.4 (4H, m, C_3-$\underline{H}_2$+C_4-$\underline{H}_2$), 4.67 (1H, m, C_2-$\underline{H}$), 6.90 (2H, bs, CON$\underline{H}_2$), 7.38 (1H, s, C_6-$\underline{H}$).

A suspension of **3** (24.0 g, 0.14 mol) in 16% HCl–MeOH (300 ml) was heated under reflux for 1 hr. The solvent was removed *in vacuo* and the residue was recrystallized from MeOH–H_2O to give **4** as colorless prisms (22.5 g, 87%), mp 174—176°. *Anal.* Calcd for $C_8H_{11}NO_3$: C, 51.88; H, 5.99; N, 7.56. Found: C, 51.60; H, 5.73; N, 7.60. IR ν_{max}^{KBr} cm^{-1}: 3420, 1735, 1665, 1580, 1220, 1190, 1170. NMR (DMSO-d_6) δ: 2.10—2.48 (4H, m, C_3-$\underline{H}_2$+C_4-$\underline{H}_2$), 3.84 (3H, s, COOC$\underline{H}_3$), 4.67 (1H, m, C_2-$\underline{H}$), 7.02 (2H, bs, CON$\underline{H}_2$), 7.43 (1H, s, C_6-$\underline{H}$).

Methyl 5-Cyano-3,4-dihydro-2*H*-pyran-2-carboxylate (**5**)——A mixture of **4** (18.5 g, 0.10 mol), *p*-TsCl (19.0 g, 0.10 mol) and pyridine (24.0 g, 0.30 mol) was heated at 80° until it became clear and the solution was concentrated *in vacuo*. The residue was extracted with ether. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was distilled under reduced pressure to give **5** as a colorless oil (11.5 g, 68%), bp 135—138° (3 mmHg). IR ν_{max}^{neat} cm^{-1}: 2210, 1755, 1635, 1440, 1210, 1180. NMR ($CDCl_3$) δ: 2.10—2.38 (4H, m, C_3-$\underline{H}_2$+C_4-$\underline{H}_2$), 3.77 (3H, s, COOC$\underline{H}_3$), 4.65 (1H, m, C_2-$\underline{H}$), 7.17 (1H, s, C_6-$\underline{H}$).

5-Cyano-3,4-dihydro-2*H*-pyran-2-carboxylic Acid (**6**)——A suspension of **5** (1.67 g, 10 mmol) in 2 N NaOH (5 ml, 10 mmol) was stirred at room temperature until it became clear, then 1 N HCl (10 ml, 10 mmol) was added to the solution. The solution was extracted with $CHCl_3$. The extract was dried over Na_2SO_4

and concentrated *in vacuo*. The residue was recrystallized from C_6H_6 to give **6** as colorless prisms (1.30 g, 85%), mp 95—96°. *Anal.* Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.14. Found: C, 55.08; H, 4.79; N, 8.83. IR ν_{max}^{KBr} cm^{-1}: 3070, 2200, 1720, 1625, 1435, 1230, 1200, 1180. NMR ($CDCl_3$) δ: 2.10—2.40 (4H, m, C_3-$\underline{H}_2$+C_4-$\underline{H}_2$), 4.60—4.80 (1H, m, C_2-$\underline{H}$), 7.17 (1H, s, C_6-$\underline{H}$), 9.38 (1H, s, COO$\underline{H}$).

Methyl 5-Benzyloxycarbonylaminomethyltetrahydro-2*H*-pyran-2-carboxylate (8) and Methyl 6-Benzyloxycarbonylamino-2-hydroxy-5-methyl Hexanoate(11)——A solution of **6** (3.06 g, 20 mmol) in 0.11 N HCl–MeOH (180 ml) was hydrogenated over PtO_2 (0.50 g) at room temperature and atmospheric pressure. After absorption of H_2 had stopped, the catalyst was filtered off, PtO_2 (1.0 g) was added to the filtrate and the solution was hydrogenated again. When the theoretical amount of H_2 had been absorbed, the catalyst was filtered off and the filtrate was concentrated *in vacuo* to give an oily residue (3.80 g). The residue was dissolved in 1 N NaOH (40 ml, 40 mmol) then benzyloxycarbonyl chloride (3.40 g, 20 mmol) and 1 N NaOH (20 ml, 20 mmol) were added all at once to the ice-cooled, stirred solution. The solution was stirred for 10 min under ice cooling and for 0.5 hr at room temperature, then 2 N HCl (30 ml, 60 mmol) was added and the acidic solution was extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated *in vacuo* to give an oily residue (4.50 g). The residue was dissolved in MeOH (50 ml) and treated with excess CH_2N_2 in ether. The solvent was removed *in vacuo* to give a pale yellow oil (4.60 g). The residue was chromatographed over silica gel (120 g). Compound **13** was obtained from the fractions eluted with C_6H_6, and **8** and **11** were obtained successively from the fractions eluted with C_6H_6–acetone (49: 1).

13 (0.56 g, 19%): mp 84—86° (reported[18] mp 86°). NMR ($CDCl_3$) δ: 4.50—5.35 (2H, m, N$\underline{H}_2$COO), 5.00 (2H, s, $C_6H_5C\underline{H}_2O$), 7.28 (5H, s, $C_6\underline{H}_5CH_2$).

8A (0.55 g, 9%): a colorless oil. IR ν_{max}^{neat} cm^{-1}: 3350, 1735, 1700, 1520, 1240, 1120. NMR ($CDCl_3$) δ: 1.15—2.32 (5H, m, C_3-$\underline{H}_2$+C_4-$\underline{H}_2$+C_5-$\underline{H}$), 3.18 (2H, t, J=7, NC$\underline{H}_2$CH), 3.67 (3H, s, overlapped with 2H, m, COOC$\underline{H}_3$+C_6-$\underline{H}_2$), 3.95—4.25 (1H, m, C_2-H), 5.02 (2H, s, $C_6H_5C\underline{H}_2O$), 7.38 (5H, s, $C_6\underline{H}_5CH_2$).

8B (0.22 g, 4%): a colorless oil. The IR and NMR spectra were almost identical with those of **8A**.

11 (0.50 g, 9%): a colorless oil. NMR ($CDCl_3$) δ: 0.89 (3H, d, J=6, C_6-C$\underline{H}_3$), 1.02—2.20 (5H, m, C_3-$\underline{H}_2$+C_4-$\underline{H}_2$+C_5-$\underline{H}$), 3.03 (2H, m, NC$\underline{H}_2$CH), 3.72 (3H, s, COOC$\underline{H}_3$), 3.85—4.30 (1H, m, OC$\underline{H}$COO), 5.04 (2H, s, $C_6H_5C\underline{H}_2O$), 7.32 (5H, s, $C_6\underline{H}_5CH_2$).

5-Aminomethyltetrahydro-2*H*-pyran-2-carboxylic Acid (9)——*trans* Isomer (**9A**): A suspension of **8A** (60 mg, 0.2 mmol) in 0.1 N NaOH (5 ml, 0.5 mmol) was stirred at room temperature for 2 hr, becoming a clear solution. After addition of 0.5 N HCl (1 ml, 0.5 mmol), the solution was extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated *in vacuo* to give an oily residue (50 mg). The residue was dissolved in 60% EtOH (17 ml) and hydrogenated over 10% Pd-carbon (30 mg) at room temperature and atmospheric pressure. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was recrystallized from H_2O–EtOH to give **9A** as colorless needles (16 mg, 50%), mp 251—252° (dec.). *Anal.* Calcd for $C_7H_{13}NO_3$: C, 52.81; H, 8.13; N, 8.80. Found: C, 52.85; H, 8.25; N, 8.69. IR ν_{max}^{KBr} cm^{-1}: 2900—2130, 1590, 1150, 1080. NMR (D_2O) δ: 1.40—2.28 (5H, m, C_3-$\underline{H}_2$+C_4-$\underline{H}_2$+C_5-$\underline{H}$), 3.16 (2H, bd, J=7, NC$\underline{H}_2$CH), 3.80—4.05 (3H, m, C_2-$\underline{H}$+C_6-$\underline{H}_2$).

cis Isomer (**9B**): Using the procedure described above, **8B** (220 mg, 0.7 mmol) gave **9B** as colorless prisms (80 mg, 72%), mp 171—173°. *Anal.* Calcd for $C_7H_{13}NO_3H_2O$: C, 47.44; H, 8.52; N, 7.91. Found: C, 47.87; H, 7.97; N, 7.56. IR ν_{max}^{KBr} cm^{-1}: 2930—1950, 1620, 1540, 1155, 1080. NMR (D_2O) δ: 1.40—2.25 (5H, m, C_3-$\underline{H}_2$+C_4-$\underline{H}_2$+C_5-$\underline{H}$), 3.16 (2H, bd, J=8, NC$\underline{H}_2$CH), 3.81 (2H, bd, J=3, C_6-$\underline{H}_2$), 3.90—4.15 (1H, m, C_2-$\underline{H}$).

6-Benzyloxycarbonylamino-2-hydroxy-5-methylhexanoic Acid (10)——A suspension of **11** (500 mg, 1.65 mmol) in 1 N NaOH (3 ml, 3 mmol) was stirred at room temperature for 2 hr, becoming a clear solution. After neutralization with 2 N HCl (1.5 ml, 3 mmol) the solution was extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from C_6H_6–hexane to give **10** as colorless needles (290 mg, 60%), mp 69—70°. *Anal.* Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 61.34; H, 7.11; N, 4.78. IR ν_{max}^{KBr} cm^{-1}: 3420, 3320, 1710, 1680, 1520, 1240, 1075. NMR ($CDCl_3$) δ: 0.88 (3H, d, J=6, C$\underline{H}_3$CH), 1.01—2.25 (5H, m, C_3-$\underline{H}_2$+C_4-$\underline{H}_2$+C_5-$\underline{H}$), 3.03 (2H, m, NC$\underline{H}_2$CH), 4.02—4.38 (1H, m, OC$\underline{H}$COO), 5.08 (2H, s, $C_6H_5C\underline{H}_2O$), 7.32 (5H, s, $C_6\underline{H}_5CH_2$), 7.53 (1H, m, OCN$\underline{H}$CH$_2$).

1,4-Dioxane-2,5-dicarboxamide (16)——*trans* Isomer (**16A**): A solution of **15A**[9] (4.08 g, 20 mmol) in 7% NH_3–EtOH (60 ml) was heated at 95° in a sealed tube for 41 hr. After cooling, deposited **16A** was collected (3.55 g, quantitative yield) mp 285°. IR ν_{max}^{KBr} cm^{-1}: 3390, 3200, 1660, 1115.

cis Isomer (**16B**): Using the method described above, **15B**[9] (4.08 g) gave **16B** (2.95 g, 85%), mp 270°. IR ν_{max}^{KBr} cm^{-1}: 3390, 3180, 1660, 1130.

1,4-Dioxane-2,5-dicarbonitrile (17)——*trans* Isomer (**17A**): $POCl_3$ (24.5 g, 0.16 mol) was added dropwise to a suspension of **16A** (14.2 g, 0.08 mol) in pyridine (140 ml) over a 15 min period. The mixture was stirred at room temperature for 1 hr, warmed at 70° for 2 hr, poured into ice-water and extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from MeOH to give **17A** as a white powder (5.95 g, 54%), mp 183—185°. *Anal.* Calcd for $C_6H_6N_2O_2$: C,

18) A. Berger, J. Noguchi, and E. Katchalski, *J. Am. Chem. Soc.*, **78**, 4483 (1956).

52.17; H, 4.38; N, 20.29. Found: C, 51.87; H, 4.37; N, 20.15. IR ν_{max}^{KBr} cm^{-1}: 2970, 2240, 1115. NMR (DMSO-d_6) δ: 4.07 (4H, d, J=2, C_3-$\underline{H}_2$+C_6-$\underline{H}_2$), 5.20 (2H, t, J=2, C_2-$\underline{H}$+C_5-$\underline{H}$).

cis Isomer (**17B**): Using the procedure described above, **16B** (7.83 g) gave **17B** (4.56 g, 73%), mp 91—93°. *Anal.* Calcd for $C_6H_6N_2O_2$: C, 52.17; H, 4.38; N, 20.29. Found: C, 51.99; H, 4.45; N, 20.44. IR ν_{max}^{KBr} cm^{-1}: 2930, 2240, 1115. NMR ($CDCl_3$) δ: 3.91 (2H, dd, J=12, J=4, C_3-a$\underline{H}$+C_6-a$\underline{H}$), 4.17 (2H, dd, J=12, J=5, C_3-e$\underline{H}$+C_6-e$\underline{H}$), 4.59 (2H, dd, J=5, J=4, C_2-$\underline{H}$+C_5-$\underline{H}$).

5-Aminomethyl-1,4-dioxane-2-carboxylic Acid (18)——*trans* Isomer (**18A**): A solution of **17A** (2.76 g, 20 mmol) in 0.3% NH_3–EtOH (1.0 l) was hydrogenated over Raney Ni (2.5 ml) at room temperature and atmospheric pressure. After absorption of H_2 (980 ml, 44 mmol) over a 22 hr period, the catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was treated with 16% HCl–EtOH (50 ml) and the solution was allowed to stand at room temperature for 48 hr. The solution was concentrated *in vacuo*, 0.7 N NaOH (120 ml, 84 mmol) was added to the residue and the solution was allowed to stand at room temperature overnight. The solution was applied to a column of Amberlite IR-120B (H^+ type, 75 ml). The column was washed with H_2O and the amino acid was eluted with 3 N NH_4OH. The effluent was evaporated to dryness *in vacuo* and the residue was recrystallized from H_2O–EtOH to give **18A** as colorless prisms (0.60 g, 18%), mp 290°. *Anal.* Calcd for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.50; H, 6.90; N, 8.57. IR ν_{max}^{KBr} cm^{-1}: 2970—2050, 1640, 1525, 1405, 1105. NMR (D_2O) δ: 3.02 (1H, dd, J=14, J=7, NC$\underline{H}$CH), 3.33 (1H, dd, J=14, J=4, NC$\underline{H}$CH), 3.57 (1H, dd, J=12, J=10, C_6-a$\underline{H}$), 3.65 (1H, dd, J=12, J=10, C_3-a$\underline{H}$), 4.07 (1H, dd, J=12, J=2, C_6-e$\underline{H}$), 4.22 (1H, dd, J=12, J=3, C_3-e$\underline{H}$), 4.28 (1H, dd, J=12, J=3, C_2-a$\underline{H}$), 3.4—4.1 (1H, m, C_5-a$\underline{H}$).

cis Isomer (**18B**): Using the procedure described above, **17B** (1.38 g, 10 mmol) gave crude **18B**, which was chromatographed on cellulose powder (150 g) using iso PrOH–H_2O (7: 3). The initial eluate was evaporated to dryness *in vacuo* and the residue was recrystallized from H_2O–EtOH–acetone to give **18B** as a hygroscopic powder (0.20 g, 12%), mp 258—261° (browning). *Anal.* Calcd for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.46; H, 6.73; N, 8.08. IR ν_{max}^{KBr} cm^{-1}: 3150, 2980—2120, 1610, 1520, 1125. NMR (D_2O) δ: 3.1—3.3 (m, C_5-$\underline{H}$+NC$\underline{H}_2$CH), 3.7—3.9 (m, C_3-$\underline{H}_2$), 3.95—4.2 (m, C_6-$\underline{H}_2$), 4.2—4.35 (m, C_2-$\underline{H}$).

Oxidation of 18A——A solution of **18A** (0.16 g) in c. HNO_3 (1.6 ml) and H_2O (0.8 ml) was boiled for 10 min. H_2O (0.2 ml) was added to the solution and it was boiled again for 10 min. The same procedure was repeated twice more. After cooling, the precipitate was collected and recrystallized from H_2O to give **14A** (0.025 g, 13%), mp 281—283°, which was identical with an authentic sample of **14A**[9] (mixed melting point and IR spectrum).

Benzyloxycarbonylaminoacetaldehyde Diethyl Acetal (20)——A solution of benzyloxycarbonyl chloride (85.0 g, 0.55 mol) in C_6H_6 (100 ml) was added dropwise to a stirred solution of **19** (66.5 g, 0.50 mol) and triethylamine (55.6 g, 0.55 mol) in C_6H_6 (300 ml) at 10—20° over a 40 min period. After the mixture had been stirred for 3 hr at room temperature, C_6H_6 was added to the solution and it was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was distilled under reduced pressure to give **20** as a colorless oil (88.5 g, 66%), bp 167—170° (1 mmHg) with slight decomposition. IR ν_{max}^{neat} cm^{-1}: 3350, 1730. NMR ($CDCl_3$) δ: 1.20 (6H, t, J=7, 2×C$\underline{H}_3$CH$_2$), 3.37 (2H, dd, J=11, J=6, NHC$\underline{H}_2$CH), 3.65, 3.68 (each 2H, q, J=7, 2×CH$_3$C$\underline{H}_2$O), 4.51 (1H, t, J=6, CH$_2$C$\underline{H}$<O/O), 5.10 (2H, s, $C_6H_5C\underline{H}_2O$), 7.33 (5H, s, $C_6\underline{H}_5CH_2$).

2,4-Dinitrophenylhydrazone: Recrystallization from pyridine–H_2O gave yellow prisms, mp 189—190° (reported[10] mp 186—187°).

Dimethyl 2-Benzyloxycarbonylaminomethyl-1,3-dioxane-5,5-dicarboxylate (22)——A solution of **20** (32.0 g, 0.12 mol), **21**[11] (23.0 g, 0.12 mol) and *p*-TsOH·H_2O (1.5 g) in toluene (500 ml) was refluxed and EtOH formed during the reaction was separated as the toluene azeotrope. After 2 hr, C_6H_6 was added to the solution and the extract was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed over silica gel (300 g) using C_6H_6 and subsequently C_6H_6–acetone (20: 1). The latter eluate was concentrated *in vacuo* to give **22** as a pale brown oil (26.5 g, 60%). IR ν_{max}^{neat} cm^{-1}: 3450, 1730, 1505, 1140. NMR ($CDCl_3$) δ: 3.33 (2H, dd, J=6, J=5, NHC$\underline{H}_2$CH), 3.72, 3.84 (each 3H, s, 2×COOC$\underline{H}_3$), 3.95 (2H, d, J=12, C_4-a$\underline{H}$+C_6-a$\underline{H}$), 4.60 (1H, t, J=5, C_2-$\underline{H}$), 4.75 (2H, d, J=12, C_4-e$\underline{H}$+C_6-e$\underline{H}$), 5.13 (2H, s, $C_6H_5C\underline{H}_2O$), 7.38 (5H, s, $C_6\underline{H}_5CH_2$).

Reaction of 22 with NaCN in DMSO——A solution of **22** (18.39 g, 0.05 mol) and NaCN (7.35 g, 0.15 mol) in DMSO (100 ml) was warmed at 100° for 1 hr. H_2O (300 ml) was added to the solution and the whole was extracted with C_6H_6. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (150 g) using C_6H_6–acetone (120: 1).

The first eluate was concentrated *in vacuo*, and the residue was recrystallized from C_6H_6–petroleum ether to give **24A** as colorless needles (77 mg, 0.4%), mp 85—86°. *Anal.* Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.48; H, 6.02; N, 3.60. IR ν_{max}^{KBr} cm^{-1}: 3340, 1730, 1715, 1690, 1535, 1150, 740, 700. NMR ($CDCl_3$) δ: 2.7—3.3 (1H, m, C_5-a$\underline{H}$), 3.36 (2H, dd, J=7, J=5, NHC$\underline{H}_2$CH), 3.78 (2H, dd, J=12, J=11, C_4-a$\underline{H}$+C_6-a$\underline{H}$), 4.33 (2H, dd, J=11, J=5, C_4-e$\underline{H}$+C_6-e$\underline{H}$), 4.54 (1H, t, J=5, C_2-a$\underline{H}$), 5.10 (4H, s, 2×$C_6H_5C\underline{H}_2O$), 7.34 (10H, s, 2×$C_6\underline{H}_5CH_2$).

The second eluate was concentrated *in vacuo* and the residue was recrystallized from C_6H_6–petroleum ether to give **23A** as colorless plates (2.16 g, 16%), mp 71—73°. *Anal.* Calcd for $C_{15}H_{19}NO_6$: C, 58.25; H,

6.19; N, 4.53. Found: C, 58.36; H, 6.22; N, 4.49. IR ν_{max}^{KBr} cm^{-1}: 3340, 1720, 1690, 1540, 1150. NMR ($CDCl_3$) δ: 2.7—3.3 (1H, m, C_5–aH), 3.35 (2H, dd, $J=6$, $J=5$, $NHCH_2CH$), 3.67 (3H, s, $COOCH_3$), 3.74 (2H, dd, $J=12$, $J=11$, C_4–aH+C_6–aH), 4.31 (2H, dd, $J=11$, $J=5$, C_4–eH+C_6–eH), 4.54 (1H, t, $J=5$, C_2–aH), 5.0—5.3 (1H, bs, $CONHCH_2$), 5.10 (2H, s, $C_6H_5CH_2O$), 7.35 (5H, s, $C_6H_5CH_2$).

The third eluate was concentrated *in vacuo* and the residue was recrystallized from C_6H_6–petroleum ether to give **23B** as colorless needles (1.83 g, 12%), mp 91—92°. *Anal.* Calcd for $C_{15}H_{19}NO_6$: C, 58.25; H, 6.19; N, 4.53. Found: C, 58.21; H, 6.29; N, 4.46. IR ν_{max}^{KBr} cm^{-1}: 3350, 1720, 1690, 1545, 1140. NMR ($CDCl_3$) δ: 2.35 (1H, m, C_5–eH), 3.36 (2H, dd, $J=6$, $J=5$, $NHCH_2CH$), 3.80—4.10 (2H, m, C_4–aH+C_6–aH), 3.82 (3H, s, $COOCH_3$), 4.52—4.80 (3H, m, C_2–aH+C_4–eH+C_6–eH), 4.90—5.20 (1H, bs, $CONHCH_2$), 5.13 (2H, s, $C_6H_5CH_2O$), 7.38 (5H, s, $C_6H_5CH_2$).

2-Benzyloxycarbonylaminomethyl-1,3-dioxane-5-carboxylic Acid (25)——*trans* Isomer (**25A**): A mixture of **23A** (1.24 g, 4 mmol) in MeOH (50 ml) and KOH (1.12 g, 20 mmol) in H_2O (1.5 ml) was refluxed for 3 hr. The solution was concentrated *in vacuo*, and the residue was acidified with c. HCl to pH 3.0 under ice cooling. The solution was extracted with $CHCl_3$. The extract was washed with saturated brine, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was recrystallized from EtOH–H_2O to give **25A** as colorless needles (0.49 g, 40%), mp 146—147°. *Anal.* Calcd for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.80; N, 4.74. Found: C, 57.27; H, 6.02; N, 4.54. IR ν_{max}^{KBr} cm^{-1}: 3340, 1700, 1690, 1545, 1150. NMR ($CDCl_3$) δ: 2.8—3.2 (1H, m, C_5–aH), 3.35 (2H, dd, $J=6$, $J=5$, $NHCH_2CH$), 3.74 (2H, dd, $J=12$, $J=11$, C_4–aH+C_6–aH), 4.33 (2H, dd, $J=12$, $J=5$, C_4–eH+C_6–eH), 4.54 (1H, t, $J=5$, C_2–aH), 5.11 (2H, s, $C_6H_5CH_2O$), 7.35 (5H, s, $C_6H_5CH_2$).

cis Isomer (**25B**): A mixture of **23B** (1.85 g, 6 mmol) in MeOH (10 ml) and KOH (1.10 g, 18 mmol) in H_2O (3.7 ml) was stirred at room temperature for 13 hr. The solution was concentrated *in vacuo* to 5 ml, H_2O (5 ml) was added, and the solution was concentrated again. The solution was acidified with c. HCl to pH 3.0 under ice cooling, and it was extracted with $CHCl_3$. The extract was washed with saturated brine, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was recrystallized from $CHCl_3$–C_6H_6 to give **25A** (453 mg, 22%), mp 146—148°. Ether was added to the mother liquor and the precipitated **25B** was collected as colorless needles (1.13 g, 64%), mp 111—112.5°. *Anal.* Calcd for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.80; N, 4.74. Found: C, 56.59; H, 5.99; N, 4.86. IR ν_{max}^{KBr} cm^{-1}: 3270, 1710, 1680, 1565, 1150. NMR ($CDCl_3$) δ: 2.38 (1H, m, C_5–eH), 3.36 (2H, dd, $J=6$, $J=5$, $NHCH_2CH$), 3.75—4.05 (2H, m, C_4–aH+C_6–aH), 4.45—4.75 (3H, m, C_2–aH+C_4–eH+C_6–eH), 5.11 (2H, s, $C_6H_5CH_2O$), 7.34 (5H, s, $C_6H_5CH_2$).

***trans*-2-Aminomethyl-1,3-dioxane-5-carboxylic Acid (26A)**——A solution of **25A** (326 mg, 1.11 mmol) in EtOH–H_2O (24 ml—10 ml) was hydrogenated over 10% Pd-carbon (60 mg) at room temperature and atmospheric pressure. H_2 (16 ml) was absorbed over a 2 hr period. The catalyst was filtered off and the filtrate was evaporated to dryness *in vacuo* below 50°. The residue was recrystallized from H_2O–EtOH to give **26A** as colorless columns (114 mg, 64%), mp 265—267° (dec.). *Anal.* Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69. Found: C, 44.40; H, 6.86; N, 8.75. IR ν_{max}^{KBr} cm^{-1}: 3300—2100, 1620—1540. NMR (D_2O) δ: 2.8—3.3 (1H, m, C_5–aH), 3.32 (2H, d, $J=4$, NCH_2CH), 4.01 (2H, dd, $J=11$, $J=11$, C_4–aH+C_6–aH), 4.50 (2H, dd, $J=11$, $J=5$, C_4–eH+C_6–eH), 5.08 (1H, t, $J=4$, C_2–aH).

Hydrogenation of 25B——Using the method described above, **25B** (100 mg, 0.33 mmol) gave crude **26.** It was recrystallized from H_2O–EtOH to give colorless prisms (32 mg, 60%), mp 233—235° (dec.); this material was found to be a mixture of **26A** and **26B** in a ratio of 1: 2 (NMR spectrum and TLC). NMR of **26B** (D_2O) δ: 2.3—2.5 (1H, m, C_5–eH), 3.17 (2H, d, $J=3$, NCH_2CH), 3.85—4.05, 4.10—4.30, 4.35—4.55, 4.60—4.70 (total 4H, each m, C_4–H_2+C_6–H_2), 5.10 (1H, t, $J=3$, C_2–aH).

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