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Medicinal Chemical Studies on Antiplasmin Drugs. III.[1] 4-Aminomethylcyclohexanecarboxylic Acid and Its Derivatives having a Methyl Group[2]

Sumiro Isoda and Miyoshi Hirata

Research Institute, Daiichi Seiyaku Co., Ltd.[3]

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To investigate the structure–activity relationship between two isomers of 4-aminomethylcyclohexanecarboxylic acid (AMCHA) in connection with their antiplasmin activity, their conformations in H_2O were simply estimated on the basis of conformational free energy ($-\Delta G°$). The nuclear magnetic resonance spectra of both isomers of 4-*tert*-butylcyclohexylmethylamine hydrochloride and of cyclohexylmethylamine hydrochloride gave a value of $-\Delta G° = 1.4$ kcal/mol for the $\overset{+}{N}H_3CH_2$-group. The biologically active *trans* AMCHA in H_2O exists, as in its crystal structure, largely in the equatorial $\overset{+}{N}H_3CH_2$—equatorial COO^- form, while about 79% of *cis* AMCHA exists as the axial $\overset{+}{N}H_3CH_2$—equatorial COO^- form, in contrast to the crystal structures of its hydrohalides. Further, 1-Me AMCHA, 4-Me AMCHA and 4-(1-aminoethyl)cyclohexanecarboxylic acid were synthesized. The stereoisomers of these compounds were separated and the configurations of the isomers were determined. No compound showed a more potent antiplasmin activity than *trans* AMCHA.

Keywords——antiplasmin drug; 4-aminomethylcyclohexanecarboxylic acid; conformational free energy difference; cyclohexylmethylamine; methyl substituent; stereoisomer

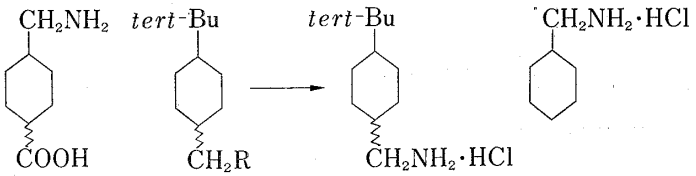
In the previous paper[1] Naito *et al.* reported the separation, the assignment of configurations, and the syntheses of two isomers of 4-aminomethylcyclohexanecarboxylic acid (AMCHA); the *trans* isomer (**1B**) showed more potent antiplasmin activity than the *cis* isomer (**1A**). It was reported[4] that the crystal structures of **1A** HBr and **1A** HCl both have equatorial aminomethyl and axial carboxyl groups, while those of **1B** and **1B** HBr have equatorial aminomethyl and carboxyl groups. In studies of the structure–activity relationship, the conformation in solution is more important than the crystal structure because the interaction between antiplasmin agents and the drug receptor occurs in solution. This paper describes simple conformational analyses of **1A** and **1B** in water, as well as the syntheses of AMCHA derivatives having a methyl group at C_1, C_4 or in the side chain.

Since both **1A** and **1B** are presumed to take zwitterionic forms in water, the conformational free energy difference $-\Delta G°$ for the $\overset{+}{N}H_3CH_2$– group was determined by nuclear magnetic resonance (NMR) spectroscopy. Both isomers of a reference compound, 4-*tert*-butylcyclohexylmethylamine hydrochloride (**4**) were prepared as shown in Chart 1.

In deuterium oxide at 34° the axial methylene (side chain) signal of **4A** appears at 3.14 ppm and the equatorial one of **4B** at 2.92 ppm, while the methylene (side chain) signal of cyclohexylmethylamine hydrochloride (**5**)[5] appears at 2.94 ppm. These data gave a value

1) Part II: T. Naito, A. Okano, S. Kadoya, T. Miki, M. Inaoka, R. Moroi, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **16**, 728 (1968).
2) A part of this work was presented at the 91st Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April 1971.
3) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo 132, Japan.*
4) S. Kadoya, F. Hanazaki, and Y. Iitaka, *Acta Cryst.*, **21**, 38 (1966); P. Groth and O. Hassel, *Acta Chem. Scand.*, **19**, 1709 (1965); P. Groth, *ibid.*, **22**, 143 (1968).
5) *a*) L. Ruzicka and W. Brugger, *Helv. Chim. Acta*, **9**, 399 (1926); *b*) N.J. Demjanov, *J. Chem. Soc.*, **1904**, 1214; *c*) O. Wallach, *Ann.*, **353**, 299 (1907).

of $-\Delta G^\circ$ for the $\overset{+}{N}H_3CH_2-$ group of 1.4 kcal/mol. Since $-\Delta G^\circ$=2.2 kcal/mol for $-COO^-$ in 50% EtOH,[6] and assuming additivity of $-\Delta G^\circ$ values,[7] the following $-\Delta G^\circ$ values were roughly estimated: $-\Delta G^\circ$=0.8 kcal/mol for **1A** and $-\Delta G^\circ$=3.6 kcal/mol for **1B**. Thus **1A** is presumed to exist in deuterium oxide as an equilibrium mixture of 21% (4e, 1a) form and 79% (4a, 1e) form, and **1B** almost entirely as the (4e, 1e) form. In fact the chemical shifts of the methylene (side chain) signals of **1A** and **1B**, 3.04 and 2.94 ppm, reflect the difference in conformation. It may be due to the deshielding effect of the $-COO^-$ group that the latter is shifted slightly to lower field to coincide with that of **5**.

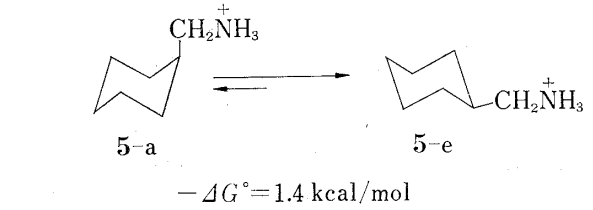


1 **2**: R=OTs **4** **5**

3: R=N_3

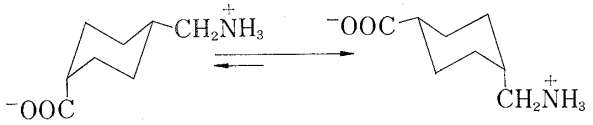
A=*cis*, B=*trans*

Chart 1



5-a **5**-e

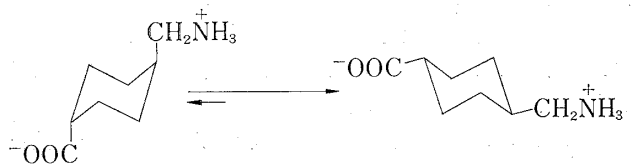
$-\Delta G^\circ$=1.4 kcal/mol



1A-4e, 1a **1A**-4a, 1e

21% 79%

$-\Delta G^\circ$=0.8 kcal/mol



1B-4a, 1a **1B**-4e, 1e

0.3% 99.7%

$-\Delta G^\circ$=3.6 kcal/mol

Fig. 1

These finding prompted us to attempt the synthesis of AMCHA derivatives, especially those having a methyl group in a position which might affect the spatial positions of the NH_2CH_2- and $-COOH$ groups of AMCHA. The compounds described below (**8**, **20**, **24**) were synthesized and their biological activities were evaluated.

4-Aminomethyl-1-methylcyclohexanecarboxylic Acid (1-Me AMCHA, 8)

Methyl 4-cyano-1-methyl-3-cyclohexenecarboxylate (**7**), which was prepared from methyl 1-methyl-4-oxocyclohexanecarboxylate (**6**)[8] *via* its cyanohydrin, was hydrogenated over Raney Ni. The hydrogenated product was hydrolyzed with 1 N NaOH to give a mixture of 1-Me AMCHA (**8**).

Addition of *p*-TsOH to an aqueous solution of **8** gave a slightly soluble salt. After decomposition of the precipitate by treatment with ion exchange resin, the lower-melting isomer α, mp 272—273° (dec.), was obtained. The higher-melting isomer β, mp 293—295° (dec.) was obtained by recrystallization of the above mixture of **8**. The purities of α and β were

6) E.L. Eliel, N.L. Allinger, S.J. Angyal, and G.A. Morrison, "Conformational Analysis," Interscience Publishers, New York, 1965, p. 441.
7) E.L. Eliel and C.A. Lukach, *J. Am. Chem. Soc.*, **79**, 5986 (1957).
8) M. Rubin and H. Wishinsky, *J. Am. Chem. Soc.*, **68**, 338 (1946).

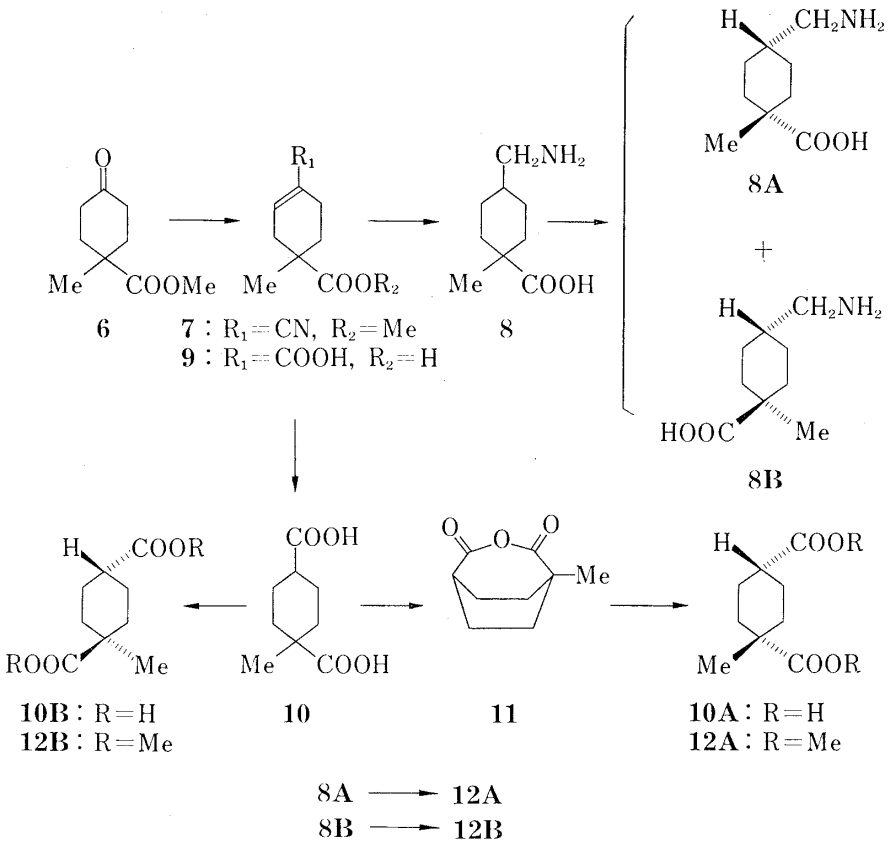


Chart 2

established by thin-layer chromatography. Neither α nor β gave the other isomer on heating aqueous solutions of each isomer.

The configuration of each isomer was determined in the following manner. The hydrolysis of **7** with 2 N NaOH and successive hydrogenation of **9** over Raney Ni gave the diacid (**10**). The mixture of **10** was refluxed with acetic anhydride as reported for cyclohexane-1,4-dicarboxylic acid.[9] Distillation of the reaction product gave the anhydride (**11**), mp 78—81°. The infrared (IR) spectrum showed carbonyl bands at 1780 and 1740 cm^{-1}, and the mass (MS) spectrum gave a molecular ion peak at *m/e* 168. Hydrolysis of **11** gave the *cis* diacid (**10A**), mp 190—195°. Esterification of **10A** with diazomethane gave the *cis* diester (**12A**), which gave a single peak at t_R 25.2 on a gas chromatogram. On the other hand, repeated recrystallization of the mixture of **10** gave another diacid (**10B**), mp 245—248°. Esterification of **10B** with diazomethane gave the *trans* ester (**12B**), which gave a single peak at t_R 26.1 on a gas chromatogram. Oxidation of α and β with potassium permanganate in 0.26 N Na_2CO_3 at room temperature and esterification of the oxidation products with diazomethane afforded **12A** and **12B**, respectively, which were identified by gas chromatography. Thus it was established that α is the 1-Me *cis* AMCHA (**8A**) and β is the 1-Me *trans* AMCHA (**8B**).

4-Aminomethyl-4-methylcyclohexanecarboxylic Acid (4-Me AMCHA, 20)

(1-Methyl-4-oxocyclohexyl)methyl acetate (**14**) was obtained by treatment of (8-methyl-1,4-dioxaspiro[4,5]decan-8-yl)methanol (**13**)[10] with AcOH. (4-Cyano-1-methyl-3-cyclohexenyl)methyl acetate (**15**) prepared from **14** *via* its cyanohydrin was hydrolyzed with c. H_2SO_4

9) R. Melachowski, *Ber.*, **67**, 1783 (1934).
10) P.C. MuKharji, P.K. SenGupta, and G.S. Sambamurti, *Tetrahedron*, **25**, 5287 (1969).

in EtOH to give the alcohol ester (**16**). The ester **16** was hydrogenated over Pt to give **17**, the NMR spectrum of which showed two sets of methyl (ester), methylene (ester) and methylene (alcohol) signals at δ 1.25, 4.11, 3.29 and 1.27, 3.46, 4.16 ppm, respectively, in a ratio of 4: 1. The azidomethyl ester (**19**), which was obtained from **17** *via* **18**, was also a mixture of *cis* and *trans* isomers. It was hydrogenated over Pd and the resulting product was hydrolyzed to give a mixture of 4-Me AMCHA (**20**). Recrystallization of the mixture from H_2O–EtOH gave the higher-melting isomer β, mp 251—255°. The mother liquor after the above recrystallization was heated at 200—215° in the presence of NaOH. The resulting mixture was recrystallized, and another isomer was isolated from the mother liquor as the *p*-tosylate. The *p*-tosylate was decomposed with ion exchange resin to give the lower-melting isomer α, mp 240—243.5° (dec.).

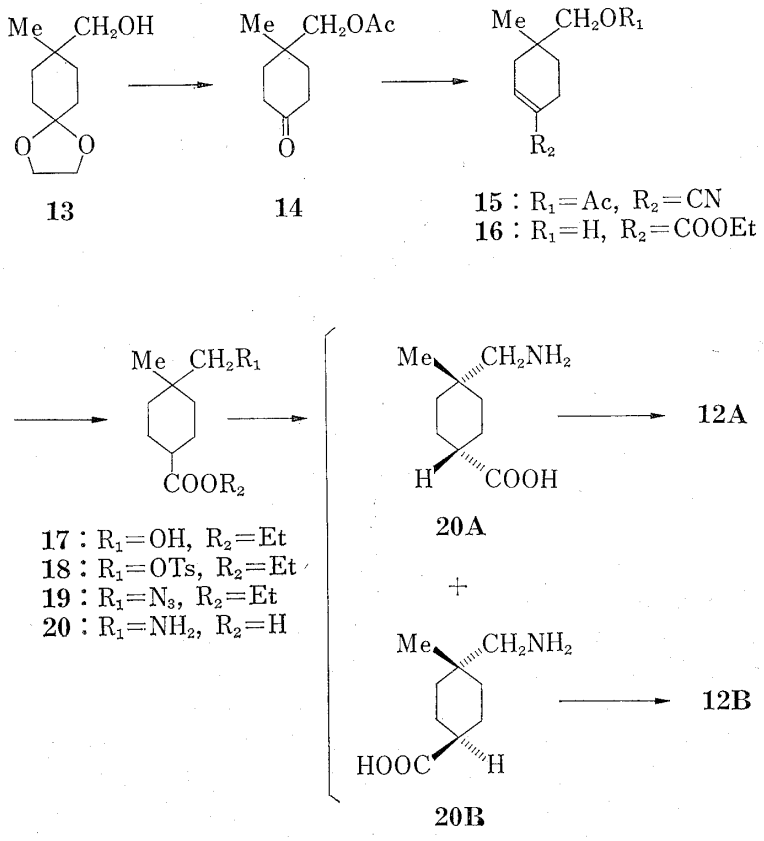


Chart 3

The configurations of α and β were confirmed by oxidation, esterification and gas chromatography as described for **8A** and **8B**. It was concluded that isomer α is the 4-Me *cis* AMCHA (**20A**) and β is the 4-Me *trans* AMCHA (**20B**).

4-(1-Aminoethyl)cyclohexanecarboxylic Acid (AECHA, 24)

Refluxing 4-acetylbenzonitrile (**21**)[11] in AcOH–HCl gave 4-acetylbenzoic acid (**22**), which was obtained in poor yield by the method of Langenbeck *et al.*[12] A solution of **22** in 10% NH_3–EtOH was warmed at 80° in an autoclave, and the resulting imino acid was hydrogenated over Raney Ni to give an amino acid having a benzene ring (**23**); on treatment with ninhydrin, its color changed from yellow to purple. The amino acid **23** was hydrogenated over PtO_2 in 1 N HCl to give a mixture of AECHA (**24**), which showed purple coloration with

11) L. Friedman and H. Shechter, *J. Org. Chem.*, **26**, 2522 (1961).
12) W. Langenbeck and J. Baltes, *Ber.*, **67**, 1204 (1934).

ninhydrin. Addition of 3,5-dinitrobenzoic acid to a 30% aq. EtOH solution of **24** gave a slightly soluble precipitate. After recrystallization and decomposition of the precipitate with ion exchange resin, the lower-melting isomer α, mp 243°, was obtained. On the other hand, another isomer was obtained as the *p*-tosylate after addition of *p*-TsOH to a solution of **24**. On decomposition of the salt with ion exchange resin, the higher-melting isomer β, mp 290° was obtained. On heating at 200° for 10 hr in 1 N NaOH solution, the isomer α changed to a mixture, from which the isomer β was obtained in 65% yield.

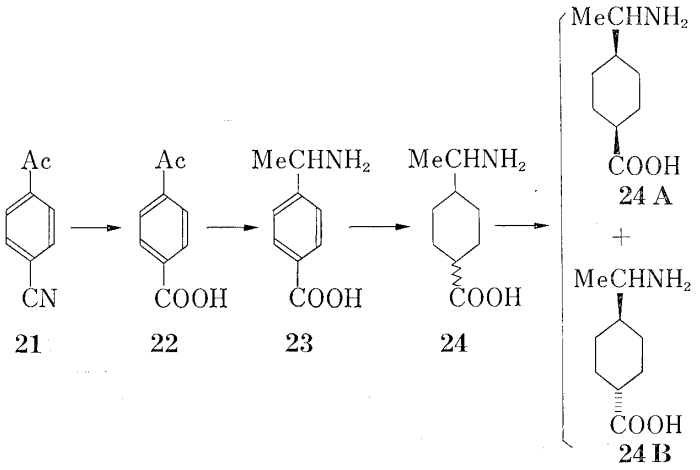


Chart 4

The configurations of α and β were confirmed by comparing the NMR spectra with those of **1A** and **1B**. The ring methylene signals of α were similar to those of **1A** and those of β were similar to those of **1B**; in addition, the methine (side chain) signal of α appeared at lower field than that of β. It was concluded that α is the *cis* AECHA (**24A**) and β is the *trans* AECHA (**24B**).

All the AMCHA derivatives prepared in this study were found to have lower antiplasmin activity than **1B**. The results suggest that none of the analogs prepared in this study satisfy the structural requirements of the receptor site as well as **1B**.

Experimental[13]

4-*tert*-Butylcyclohexylmethyl Azide (3)——*cis* Isomer (**3A**): A mixture of **2A**[14] (8.94 g, 27.5 mmol) and NaN_3 (1.95 g, 30 mmol) in DMF (20 ml) was warmed at 100° for 3 hr. After addition of isopropyl ether to the reaction mixture, the solution was washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was distilled under reduced pressure to give **3A** as a colorless oil (4.96 g, 92%), bp 140° (28 mmHg). IR ν_{max}^{neat} cm^{-1}: 2930, 2860, 2090. NMR ($CDCl_3$) δ: 0.85 (s), 0.4—1.9 (m), 3.32 (d, $J=8$).

trans Isomer (**3B**): Using the method described above, **2B**[14] (8.93 g) gave **3B** (5.05 g, 94%), bp 148° (33 mmHg). IR ν_{max}^{neat} cm^{-1}: 2940, 2860, 2100. NMR ($CDCl_3$) δ: 0.86 (s), 0.8—1.9 (m), 3.13 (d, $J=5$).

4-*tert*-Butylcyclohexylmethylamine Hydrochloride (4)——*cis* Isomer (**4A**): A solution of **3A** (0.59 g, 3 mmol) in EtOH (20 ml) was hydrogenated over 5% Pd–carbon (0.05 g) at room temperature and atmospheric pressure. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. Next, 20% HCl–EtOH (1 ml) was added to the residue. After removal of the solvent, the residue was washed with hexane to give **4A** (0.43 g, 70%). Recrystallization from EtOH–IPE gave **4A** as a white powder, mp 235—255° (ambiguous). *Anal.* Calcd. for $C_{11}H_{24}ClN$: C, 64.20; H, 11.76; N, 6.81. Found: C, 64.42; H, 11.54; N, 6.87. IR ν_{max}^{KBr} cm^{-1}: 2900—2480, 1910, 1565, 1490, 1360. NMR (D_2O) δ: 0.89 (s), 0.6—2.0 (m), 3.14 (d, $J=8$).

trans Isomer (**4B**): Using the method described above, **3B** (0.59 g) gave **4B** (0.42 g, 68%), mp 240—255° (ambiguous). *Anal.* Calcd. for $C_{11}H_{24}ClN$: C, 64.20; H, 11.76; N, 6.81. Found: C, 64.65; H, 11.63; N,

13) Melting points, which were measured on a Yanagimoto melting point apparatus, and boiling points are uncorrected. The infrared (IR) spectra were measured with a Hitachi 285 spectrophotometer. The nuclear magnetic resonance (NMR) spectra were obtained on a Hitachi R-20B spectrometer at 34° using tetramethylsilane (TMS) as an internal standard. TMS in CCl_4 was used as an external standard when D_2O was used as a solvent. Thin-layer chromatography (TLC) was performed on Silica Rider (Daiichi Kagaku) plates. The developing solvent was a mixture of *tert*-BuOH–H_2O (3:1). Amino acids were detected by ninhydrin coloration. Gas chromatography (GLC) was carried out on a Hitachi F6-D instrument equipped with a 45m stainless steel Golay column coated with BDS, at 145°. Mass (MS) spectra were obtained on a Hitachi RMS-4 mass spectrometer.

14) N. Mori, *Bull. Chem. Soc. Jpn.*, **34**, 1567 (1961).

6.90. IR ν_{max}^{KBr} cm^{-1}: 2900—2500, 2030, 1600, 1500, 1440, 1390, 1360. NMR (D_2O) δ: 0.87 (s), 1.0—2.0 (m), 2.92 (d, $J=6$).

Cyclohexylmethylamine Hydrochloride (5)——A solution of 1-cyclohexenylcarbonitrile[5a] (5.4 g, 50 mmol) in a mixture of c. NH_4OH (5 ml)–EtOH (100 ml) was hydrogenated over Raney Ni (5 ml) for 6 hr. The initial H_2 pressure was 4.5 kg/cm^2. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. Next, 20% HCl–EtOH (10 ml) was added to the residue. After removal of the solvent, the residue was recrystallized from EtOH–AcOEt to give **5** as a white powder (5.6 g, 75%), mp 205—260° (ambiguous), (reported mp *ca.* 254°,[5b] mp 210°[5c]). *Anal.* Calcd. for $C_7H_{16}ClN$: C, 56.17; H, 10.78; N, 9.36. Found: C, 56.23; H, 10.50; N, 9.38. IR ν_{max}^{KBr} cm^{-1}: 2930—2320, 2030, 1610, 1510, 1450, 1400. NMR (D_2O) δ: 1.0—2.0 (11H, m), 2.94 (2H, d, $J=6$).

Methyl 4-Cyano-1-methyl-3-cyclohexenecarboxylate (7)——A mixture of **6**[8] (10.2 g, 60 mmol), acetone cyanohydrin (6.2 g, 72 mmol) and triethylamine (2.4 g, 24 mmol) was allowed to stand at room temperature for 48 hr. The reaction mixture was concentrated under reduced pressure at 50°. An ice-cooled solution of the resulting residue in pyridine (19 g, 240 mmol) was treated dropwise with $POCl_3$ (9.2 g, 60 mmol). The mixture was stirred at room temperature for 0.5 hr, warmed at 80° for 3 hr, poured into ice-water, and extracted with $CHCl_3$. The extract was washed with 5% $NaHCO_3$ and H_2O successively, dried over Na_2SO_4, and evaporated down. The residue was distilled under reduced pressure to give 7 as a colorless oil (8.6 g, 80%), bp 125—129° (2 mmHg). IR ν_{max}^{neat} cm^{-1}: 2200, 1730, 1635. NMR ($CDCl_3$) δ: 1.24 (3H, s), 1.4—3.0 (m), 3.77 (3H, s), 6.61 (1H, m).

4-Aminomethyl-1-methylcyclohexanecarboxylic Acid (8)——A solution of 7 (5.9 g, 33 mmol) and c. NH_4OH (5 ml) in MeOH (100 ml) was hydrogenated over Raney Ni (5 ml) at room temperature and atmospheric pressure. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. A suspension of this residue in 1 N NaOH (60 ml) was stirred and heated at 95° for 3 hr, giving a homogeneous solution. The solution was applied to a column of Diaion SK-1A (H^+ type, 150 ml). The column was washed with H_2O, and the amino acid was eluted with 3 N NH_4OH (800ml). The effluent was concentrated *in vacuo* to give **8** as a white powder, (5.2 g, 93%), mp 248—260° (dec.). *Rf*: 0.09, 0.16.

***c*-4-Aminomethyl-*t*-1-methyl-*r*-1-cyclohexanecarboxylic Acid (8A)**——A solution of **8** (10.3 g, 60 mmol) in H_2O (120 ml) was treated with *p*-TsOH·H_2O (5.7 g, 30 mmol). The solution was allowed to stand at room temperature for 1 hr. The resulting precipitate was collected, and recrystallized from H_2O to give **8A**·*p*-TsOH as a white powder, (1.5 g, 15%), mp 241—243° (dec.). *Anal.* Calcd. for $C_{16}H_{25}NO_5S$: C, 55.47; H, 7.27; N, 4.04. Found: C, 55.74; H, 7.01; N, 4.02. A solution of this salt (1.5 g) in H_2O (100 ml) was freed from *p*-TsOH by passage down a column of Amberlite IR-45 (OH^- type, 10 ml). The eluted solution was evaporated to dryness *in vacuo*. The residue was recrystallized from H_2O–acetone to give **8A** as a white powder, (0.6 g, 6%), mp 272—273° (dec.). *Anal.* Calcd. for $C_9H_{17}NO_2$: C, 63.12; H, 10.01; N, 8.18. Found: C, 62.63; H, 9.94; N, 8.38. IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 2150, 1620, 1540. NMR (D_2O) δ: 1.11 (3H, s), 1.05—2.3 (m), 2.88 (2H, d, $J=6$). *Rf*: 0.16.

***t*-4-Aminomethyl-*t*-1-methyl-*r*-1-cyclohexanecarboxylic Acid (8B)**——EtOH (150 ml) was added to a solution of **8** (1.0 g, 6 mmol) in H_2O (25 ml). The solution was allowed to stand at room temperature overnight. The resulting precipitate was collected and recrystallized from H_2O–EtOH to give **8B** as a white powder, (0.10 g, 10%), mp 293—295° (dec.). *Anal.* Calcd. for $C_9H_{17}NO_2$: C, 63.12; H, 10.01; N, 8.18. Found: C, 63.08; H, 9.71; N, 8.33. IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 2100, 1610, 1520. NMR (D_2O) δ: 1.16 (3H, s), 1.60 (9H, b), 2.97 (2H, d, $J=6$). *Rf*: 0.06.

1-Methyl-3-cyclohexene-1,4-dicarboxylic Acid (9)——A solution of 7 (3.60 g, 20 mmol) in 2 N NaOH (20 ml) and EtOH (10 ml) was refluxed for 20 hr, then the solvent was evaporated off and 2 N HCl (25 ml) was added with ice cooling. The precipitate was collected, and a solution of this precipitate in 2 N NaOH (20 ml) was refluxed for 20 hr. After cooling, the solution was acidified to pH 3.0 with 2 N HCl. The precipitate was collected and recrystallized from 50% MeOH to give 9 as colorless prisms, (2.20 g, 60%), mp 235—242° (browning). *Anal.* Calcd. for $C_9H_{12}O_4$: C, 58.68; H, 6.57. Found: C, 58.89; H, 6.56. IR ν_{max}^{KBr} cm^{-1}: 3400, 2970, 1690, 1645. NMR (DMSO-d_6) δ: 1.13 (3H, s), 1.4—2.8 (m), 6.79 (1H, m).

1-Methylcyclohexane-1,4-dicarboxylic Acid (10)——A solution of **9** (0.92 g, 5 mmol) in 1 N NaOH (20 ml) was hydrogenated over Raney Ni (0.5 ml) at room temperature and atmospheric pressure. The catalyst was filtered off, and 2 N HCl (10 ml) was added to the filtrate. The solution was allowed to stand in an ice box for 10 hr. The precipitate was collected and recrystallized from 50% MeOH to give **10** as colorless prisms, (0.65 g, 70%), mp 194—195°. *Anal.* Calcd. for $C_9H_{14}O_4$: C, 58.06; H, 7.58. Found: C, 57.83; H, 7.26. IR ν_{max}^{KBr} cm^{-1}: 1690.

***t*-1-Methyl-*t*-4,*r*-1-cyclohexanedicarboxylic Acid (10B)**——The mixture **10** (7.5 g) was recrystallized from H_2O (400 ml). Repeated recrystallization gave **10B** as a white powder, (17 mg, 0.2%), mp 245—248°. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. NMR (DMSO-d_6) δ: 1.10 (s), 1.3—1.85 (m), 2.0—2.5 (m).

1-Methyl-3-oxabicyclo[3.2.2]nonane-2,4-dione (11)——A solution of **10** (1.0 g) in Ac_2O (10 ml) was refluxed for 5 hr. AcOH and Ac_2O was evaporated off under reduced pressure. The residue was sublimed under 2 mmHg at a bath temperature of 200—220°. The sublimate was sublimed again under 2 mmHg at 160° to give **11** as a white powder, (0.20 g, 22%), mp 78—81°. *Anal.* Calcd. for $C_9H_{12}O_3$: C, 64.27; H, 7.19.

Found: C, 64.80; H, 7.24. IR ν_{max}^{KBr} cm^{-1}: 1780, 1740. NMR ($CDCl_3$) δ: 1.32 (3H, s), 1.5—2.9 (m), 3.12 (1H, m). MS *m/e*: 168, 140, 125, 81.

***t*-1-Methyl-*c*-4,*r*-1-cyclohexanedicarboxylic Acid (10A)**——A suspension of **11** (3.8 g) in H_2O (50 ml) was allowed to stand at room temperature for 48 hr. The precipitate was collected and recrystallized twice from H_2O to give **10A** as a white powder, (1.8 g, 44%), mp 190—195°. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. NMR (DMSO-d_6) δ: 1.08 (s), 0.09—2.50 (m).

(1-Methyl-4-oxocyclohexyl)methyl Acetate (14)——A solution of **13**[10] (104.1 g, 0.56 mol) in AcOH (1 l) was refluxed for 12 hr. The mixture was distilled under reduced pressure to give **14** as a colorless oil, (96.2 g, 93%), bp 115—117° (4 mmHg). IR ν_{max}^{neat} cm^{-1}: 1730, 1710. NMR ($CDCl_3$) δ: 1.12 (3H, s), 1.72 (4H, m), 2.08 (3H, s), 2.39 (4H, t), 3.97 (2H, s).

(4-Cyano-1-methyl-3-cyclohexen-1-yl)methyl Acetate (15)——A solution of **14** (54.0 g, 0.293 mol), acetone cyanohydrin (29. 9g, 0.352 mol) and triethylamine (11.7 g, 0.117 mol) was allowed to stand at room temperature for 40 hr. The reaction mixture was concentrated under reduced pressure at 50°. On scratching, the residue solidified. It melted at 95—105°. IR ν_{max}^{KBr} cm^{-1}: 3430, 2200, 2150, 1700. $POCl_3$ (44.9 g, 0.293 mol) was added dropwise to an ice-cooled solution of the crude cyanohydrin in pyridine (92.7 g, 1.17 mol) over a 10-min period. The mixture was stirred at room temperature for 0.5 hr, warmed at 75° for 2 hr, poured onto ice, and extracted with $CHCl_3$. The extract was washed with H_2O, 1 N HCl, 5% $NaHCO_3$ and H_2O successively dried over Na_2SO_4 and concentrated *in vacuo*. The residue was distilled under reduced pressure to give **15** as a colorless oil, (45.4 g, 80%), bp 128—135° (3.5 mmHg). On scratching, the distillate solidified, and was recrystallized from ether as colorless prisms, mp 41—43°. *Anal.* Calcd. for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 67.99; H, 7.69; N, 7.24. IR ν_{max}^{KBr} cm^{-1}: 2200, 1735, 1635, 1240. NMR ($CDCl_3$) δ: 0.94 (3H, s), 2.07 (3H, s), 1.4—1.75 (m), 1.9—2.45 (m), 3.92 (2H, s), 6.56 (1H, m).

Ethyl 4-Hydroxymethyl-4-methyl-1-cyclohexenecarboxylate (16)——A solution of **15** (38.6 g, 0.20 mol) in c. H_2SO_4 (20 ml) and EtOH (200 ml) was refluxed for 7 days, poured onto ice, neutralized with K_2CO_3 and extracted with IPE. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was distilled under reduced pressure to give **16** as a colorless oil, (12.7 g, 39%), bp 125° (3 mmHg). IR ν_{max}^{neat} cm^{-1}: 3450, 1710, 1650. NMR ($CDCl_3$) δ: 0.91 (3H, s), 1.28 (3H, t, J=7), 1.15—1.8 (m), 1.95—2.4 (m), 3.35 (2H, s), 4.17 (2H, q, J=7), 6.94 (1H, m).

Ethyl 4-Hydroxymethyl-4-methylcyclohexanecarboxylate (17)——A solution of **16** (12.7 g, 64 mmol) in EtOH (40 ml) was hydrogenated over PtO_2 (0.2 g) in an autoclave at 50° for 4 hr. The initial pressure of H_2 was 90 kg/cm^2. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. The residue was distilled under reduced pressure to give **17** as a colorless oil, (12.1 g, 94%), bp 125° (3 mmHg). IR ν_{max}^{neat} cm^{-1}: 3400, 1720, 1030. NMR ($CDCl_3$) δ: 0.93 (3H, s), 1.25 (t, J=7), 1.27 (t, J=7), 1.5—2.5 (10H, m), 3.29 (s), 3.46 (s), 4.11 (q), 4.16 (q, J=7).

Ethyl 4-Azidomethyl-4-methylcyclohexanecarboxylate (19)——A stirred solution of **17** (15.0 g, 50 mmol) in pyridine (40 ml) was treated portionwise with *p*-TsCl (12.4 g, 65 mmol) over a 0.5 hr period with cooling (−7—−5°). After stirring for 1 hr at −5—−3°, the mixture was allowed to stand in an ice box overnight, poured onto ice and extracted with ether. The extract was washed with saturated brine, dried over Na_2SO_4 and concentrated. The residue was dissolved in dimethylformamide (DMF) (50 ml) and NaN_3 (4.23 g, 65 mmol) was added. The mixture was stirred at 120° for 5 hr, poured into H_2O and extracted with IPE. The extract was washed with saturated brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was distilled under reduced pressure to give **19** as a colorless oil, (6.60 g, 60%), bp 140—143° (12 mmHg). IR ν_{max}^{neat} cm^{-1}: 2080, 1725, 1040. NMR ($CDCl_3$) δ: 0.95 (3H, s), 1.23 (t, J=7), 1.27 (t, J=7), 1.1—2.4 (9H, m), 3.06 (s), 3.23 (s), 4.11 (2H, q, J=7).

4-Aminomethyl-4-methylcyclohexanecarboxylic Acid (20)——A solution of **19** (6.40 g, 28.4 mmol) in EtOH (100 ml) was hydrogenated over 10% Pd–carbon (3.0 g) at room temperature and atmospheric pressure. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. The residue was dissolved in 2 N NaOH (100 ml)–EtOH (20 ml) and refluxed for 3 hr. The mixture was concentrated *in vacuo*. H_2O (100 ml) was added to the residue, and the solution was applied to a column of Amberlite IR-120B (H^+ type, 200 ml). The column was washed with H_2O and the amino acid was eluted with 3 N NH_4OH (300 ml). The effluent was evaporated to dryness *in vacuo* to give **20** as a white powder, (3.0 g, 62%). *Rf*: 0.10, 0.15.

***t*-4-Aminomethyl-*c*-4-methyl-*r*-1-cyclohexanecarboxylic Acid (20B)**——The mixture of **20** (3.0 g) was recrystallized from H_2O–EtOH to give **20B** as a white powder, (0.58 g, 19%), mp 251—255° (dec.). *Anal.* Calcd. for $C_9H_{17}NO_2$: C, 63.12; H, 10.01; N, 8.18. Found: C, 63.15; H, 10.02; N, 8.62. IR ν_{max}^{KBr} cm^{-1}: 3450, 2950—2125, 1660, 1640, 1520. NMR (D_2O) δ: 1.07 (3H, s), 1.3—2.25 (m), 2.86 (2H, s). *Rf*: 0.10.

***c*-4-Aminomethyl-*t*-4-methyl-*r*-1-cyclohexanecarboxylic Acid (20A)**——The mother liquor of the previous recrystallization was evaporated down *in vacuo*. The residue was dissolved in 1 N NaOH (24 ml), then heated at 200—215° for 19 hr in an autoclave. The solution was applied to a column of Diaion SK#1 (H^+ type, 30 ml). The column was washed with H_2O, and the amino acid was eluted with 2 N NH_4OH. The effluent was concentrated *in vacuo*. The residue was recrystallized from H_2O–EtOH to give **20B** (1.05 g). This was isomerized as described above, and the recovered **20B** was isomerized again. All of the mother liquor was collected and evaporated down. The residue was dissolved in H_2O (3 ml) and *p*-TsOH·H_2O (0.50 g) was added. The precipitate was collected and recrystallized from H_2O to give **20A**·*p*-TsOH, mp

231—233°. This was dissolved in H_2O (20 ml), and the solution was applied to a column of SK#1 (H^+ type, 5 ml). The column was washed with H_2O, and the amino acid was eluted with 2 N NH_4OH. The effluent was evaporated to dryness *in vacuo*. The residue was recrystallized from H_2O–acetone to give **20A** as a white powder, (0.22 g), mp 240—243.5° (dec.). *Anal.* Calcd. for $C_9H_{17}NO_2$: C, 63.12; H, 10.01; N, 8.18. Found: C, 63.18; H, 9.95; N, 8.17. IR ν_{max}^{KBr} cm^{-1}: 3400, 2900—2150, 1620, 1530. NMR (D_2O) δ: 1.06 (3H, s), 1.25—2.3 (m), 3.08 (2H, s). *Rf*: 0.15.

4-Acetylbenzoic Acid (22)——A solution of **21**[11] (14.5 g, 0.1 mol) and AcOH (15 ml) in c. HCl (80 ml) was refluxed for 6 hr. After cooling, the reaction mixture was poured into ice-water. The precipitate was collected and recrystallized from H_2O to give **22**, (11.6 g, 71%), mp 197—200° (reported[12] mp 200—205°).

4-(1-Aminoethyl)benzoic Acid (23)——A suspension of **22** (32.8 g, 0.20 mol) and Raney Ni (10 ml) in 10% NH_3–EtOH (200 ml) was heated at 80° in an autoclave, and hydrogenated at 80 kg/cm^2 of H_2 for 16 hr. After cooling, H_2O (200 ml) was added to this reaction mixture, and the catalyst was filtered off. The filtrate was concentrated *in vacuo*, and the residue was dissolved in H_2O (4 l). The insoluble material was filtered off, and the filtrate was applied to a column of IR-SK1A (H^+ type, 350 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*. The residue was recrystallized from H_2O–EtOH–acetone to give **23**, (23.2 g, 70%), mp 289—293° (dec.). *Anal.* Calcd. for $C_9H_{11}NO_2$: C, 65.43; H, 6.71; N, 8.48. Found: C, 64.92; H, 6.65; N, 8.10. IR ν_{max}^{KBr} cm^{-1}: 2900—2300, 1610, 1520, 1390.

4-(1-Aminoethyl)cyclohexanecarboxylic Acid (24)——A solution of **23** (16.5g, 0.10 mol) in 1 N HCl (200 ml) was hydrogenated over PtO_2 (2.0 g) at room temperature and atmospheric pressure. The catalyst was filtered off, and the filtrate was freed from HCl by passage down a column of IR-45 (OH^- type, 200 ml). The solution was evaporated to dryness. The residue was recrystallized from H_2O–EtOH–acetone to give **24** (14.9 g, 87%), mp 239—240° (dec.). *Rf*: 0.12, 0.19.

***cis* 4-(1-Aminoethyl)cyclohexanecarboxylic Acid (24A)**——A solution of **24** (10.3 g, 0.06 mol) in 30% EtOH (100 ml) was treated with 3,5-dinitrobenzoic acid (12.7 g, 0.06 mol). The resulting precipitate was collected and recrystallized from 33% EtOH to give **24A**·3,5-dinitrobenzoate (12.5 g), mp 213—215° (dec.). *Anal.* Calcd. for $C_{16}H_{21}N_3O_8$: C, 50.13; H, 5.52; N, 10.96. Found: C, 50.27; H, 5.64; N, 11.01. A mixture of the salt (12.0 g) in 1 N HCl (50 ml) was heated at 50° for 30 min. After cooling, the precipitate was filtered off, and the filtrate was freed from acidic substances by passage down a column of IR-45 (OH^- type, 50 ml). The solution was evaporated to dryness. The residue was recrystallized from H_2O–acetone to give **24A** (5.0 g, 58%), mp 243° (dec.). *Anal.* Calcd. for $C_9H_{17}NO_2$: C, 63.12; H, 10.01; N, 8.18. Found: C, 63.41; H, 9.86; N, 8.32. IR ν_{max}^{KBr} cm^{-1}: 2930—2160, 1620, 1560, 1510, 1450, 1390. NMR (D_2O) δ: 1.32 (3H, d, J=7), 1.53 (9H, b), 2.45 (1H, m), 3.35 (1H, m). *Rf*: 0.19.

***trans* 4-(1-Aminoethyl)cyclohexanecarboxylic Acid (24B)**——A solution of **24** (11.5 g, 67 mmol) and *p*-TsOH·H_2O (12.8 g, 67 mmol) in H_2O (35 ml) was allowed to stand in an ice box overnight. The resulting precipitate was collected and recrystallized from *n*-PrOH to give **24B**·*p*-TsOH (2.0 g), mp 233—235°. *Anal.* Calcd. for $C_{16}H_{25}NO_5S$: C, 55.95; H, 7.34; N, 4.08; S 9.34. Found: C, 56.53; H, 7.38; N, 4.10; S, 9.35. A solution of the salt (2.0 g) in H_2O (50 ml) was freed from *p*-TsOH by passage down a column of IR-45 (OH^- type, 10 ml). The solution was evaporated to dryness. The residue was recrystallized from H_2O–EtOH–acetone to give **24B** (0.70 g, 6.0%), mp>290°. *Anal.* Calcd. for $C_9H_{17}NO_2$: C, 63.12; H, 10.01; N, 8.18. Found: C, 62.34; H, 9.99; N, 8.63. IR ν_{max}^{KBr} cm^{-1}: 2920—2200, 1630, 1560, 1520, 1380. NMR (D_2O) δ: 1.32 (3H, d, J=7), 1.1—2.3 (m), 3.26 (1H, m). *Rf*: 0.12.

Attempts to Isomerize 8——A solution of **8A** (50 mg) in 1 N NaOH (20 ml) was heated in an autoclave at 200° for 10 hr. The solution was passed through a column of SK-1A (NH_4^+ type) and the column was washed with H_2O. The eluted solution was evaporated to dryness *in vacuo* to give a white powder (45 mg). TLC showed that **8B** was not present in the residue. A solution of **8B** (20 mg) in 1 N NaOH (20 ml) was worked up as described above to give a white powder (20 mg). TLC showed that **8A** was not present in the residue.

Isomerization of 24A——A solution of **24A** (0.34 g, 2.0 mmol) in 1 N NaOH (10 ml) was heated in an autoclave at 200° for 10 hr. The resulting solution was passed through a column of IR-120B (NH_4^+ type) and the column was washed with H_2O. The eluted fraction was evaporated to dryness *in vacuo* and the residue was recrystallized from H_2O–EtOH–acetone to give **24B** (0.20 g, 60%), mp>290°. This was identical with an authentic sample (IR spectrum and TLC).

Dimethyl 1-Methylcyclohexane-1,4-dicarboxylate 12 from 8A, 8B, 20A, and 20B——A solution of amino acid (5 mg) in 0.26 N Na_2CO_3 (0.9 ml) was treated with $KMnO_4$ (18 mg). The solution was allowed to stand at room temperature overnight, then iso-PrOH was added and the precipitate was filtered off. The filtrate was freed from cations by passage down a column of SK#1 (H^+ type, 5 ml). The solution was evaporated to dryness *in vacuo*, and the residue was methylated with excess CH_2N_2 in ether. The solvent was removed, and the residue was gas chromatographed.

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