# Practical Syntheses of Optically Pure 1- and 3-Substituted Dihydroorotic Acids

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**Various optically pure 3-substituted dihydroorotic acids (9, 12) were synthesized by the *N*-alkylation and Mitsunobu reaction of optically active benzyl or *tert*-butyl dihydroorotate (6, 10), followed by deprotection of the ester group. Moreover, optically pure 1-methyl (16, 17) and 1-ethyl (19) dihydroorotic acids were obtained by an optical resolution method.**

**Keywords** dihydroorotic acid; benzyl dihydroorotate; substituted dihydroorotic acid; alkylation; Mitsunobu reaction; quinidine; optical resolution

(*S*)-Dihydroorotic acid (**5a**) [(*S*)-2,6-dioxo-4-hexahydropyrimidinecarboxylic acid] is a key intermediate in pyrimidine biosynthesis from L-carbamyl aspartic acid[1] and can be chemically prepared by cyclization of L-$N^{\alpha}$-ethoxycarbonyl asparagine (**4a**).[2] Moreover, this compound is easily convertible to hydantoin acetic acid by heating with hydrochloric acid (HCl)[2] and to ureidosuccinic acid by alkaline hydrolysis.[3] Recently, much attention has been paid to pyrimidine biosynthesis in connection with the search for anticancer agents and other pharmaceuticals.[4] From these viewpoints, dihydroorotic acid derivatives are biologically and chemically of interest.

Concerning the preparation of *N*-substituted dihydroorotic acids, only a few methods such as reduction of *N*-substituted orotic acids[5] and cyclization of DL-$N^{\alpha}$-alkyl-$N^{\alpha}$-ethoxycarbonylasparagine (**14**) under alkaline conditions[6] are available, and the corresponding optically active compounds have not been prepared. Therefore, we tried to find practical methods for synthesizing optically pure *N*-substituted dihydroorotic acids.

**3-Substituted Dihydroorotic Acids** At first, in order to obtain 3-methyldihydroorotic acid (**3**), we carried out the cyclization reaction of the optically active $N^{\alpha}$-ethoxycarbonyl compound (**1**) derived from L-$N^{\alpha}$-methylasparagine in the presence of sodium ethoxide (NaOEt) in ethanol (EtOH) under heating. Unfortunately, the desired *N*-methylated compound (**3**) was not obtained; instead, 3-methylhydantoin-5-acetic acid (**2**) was formed in 91% yield as shown in Chart 1. Then, we focused on *N*-alkylation of optically pure dihydroorotic acid.

**Reactions with Alkyl Halides** Recently, Johnston *et al.* reported that the reaction of methyl (*S*)-dihydroorotate with benzyl bromide in the presence of sodium methoxide gave only optically inactive methyl 3-benzylhydantoin-5-acetate without isolation of methyl 3-benzyldihydroorotate.[7] This result showed that dihydroorotic acid is apt to be converted into hydantoin acetic acid under alkaline conditions as well as by HCl hydrolysis.[2]

Then, as a starting material, we prepared optically pure benzyl (*S*)-dihydroorotate (**6a**) from (*S*)-dihydroorotic acid (**5a**)[2] with benzyl bromide in the presence of triethylamine and investigated 3-alkylation of **6a** under various mild conditions. When **6a** was allowed to react with methyl iodide using potassium carbonate as a base, with stirring overnight at room temperature, the 3-methylation smoothly proceeded in 86% yield. However, the optical rotation of the obtained benzyl 3-methydihydroorotate was almost zero and the proton nuclear magnetic resonance ($^1$H-NMR) spectrum in the presence of a shift reagent[8] showed a 1 : 1 mixture of two isomers. This result suggested that the *N*-methylation was accompanied with racemization.

Therefore, in order to prevent the racemization, we tried the methylation of **6a** using strong bases such as sodium hydride (NaH) or potassium *tert*-butoxide (*tert*-BuOK) under conditions of low temperature for a short time. The desired benzyl 3-methyldihydroorotate (**7a**) having high optical rotation, $[\alpha]_D$ +53.5° ($c$=1, DMF), was obtained in a good yield. The $^1$H-NMR spectrum using a shift reagent[8] showed the product to be a single isomer. Subsequently, **7a** was hydrogenated in the presence of 10% palladium on charcoal (Pd–C) catalyst to obtain 1-methyldihydroorotic acid (**3a**) showing $[\alpha]_D$ +49.2° ($c$=1, DMF). The structure

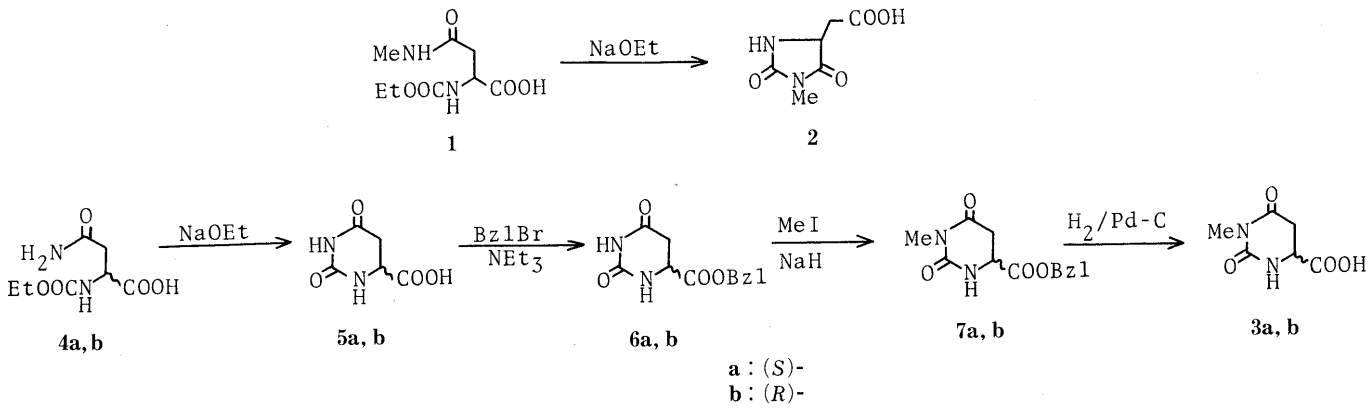


Chart 1

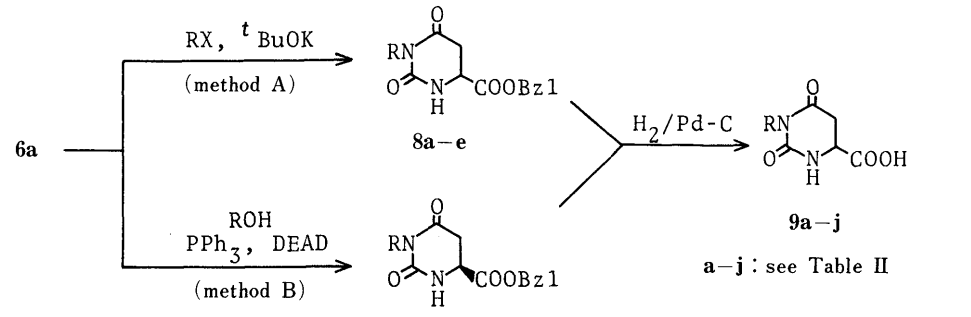


Chart 2

TABLE I. Physicochemical Data for Benzyl 3-Substituted Dihydroorotates (**8a—e**, **13a—e**)

| Compd. | R | Method[a] | Yield (%) | mp (°C) | $[\alpha]_D$ $c=0.5$ (DMF) | Formula | Analysis (%) Calcd | | | Analysis (%) Found | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | C | H | N | C | H | N |
| **8a** | Et | A | 54.1 | 129—130 | 0 | $C_{14}H_{16}N_2O_4$ | 60.86 | 5.84 | 10.14 | 60.98 | 5.79 | 10.02 |
| **8b** | *n*-Pr | A | 55.6 | 109—110 | 0 | $C_{15}H_{18}N_2O_4$ | 62.06 | 6.25 | 9.65 | 61.92 | 6.33 | 9.71 |
| **8c** | $CH_2=CHCH_2$ | A | 45.1 | 95—96 | +34.4° | $C_{15}H_{16}N_2O_4$ | 62.49 | 5.59 | 9.72 | 62.55 | 5.43 | 9.55 |
| **8d** | $MeOCH_2CH_2OCH_2$ | A | 48.2 | Syrup[b] | +38.4° | $C_{16}H_{20}N_2O_6$ | | | | | | |
| **8e** | $PhCH_2$ | A | 40.5 | 89—92 | +25.9° | $C_{19}H_{18}N_2O_4$ | 67.64 | 5.08 | 8.31 | 67.84 | 5.35 | 8.26 |
| **13a** | Et | B | 59.0 | 114—116 | +40.4° | $C_{14}H_{16}N_2O_4$ | 60.86 | 5.84 | 10.14 | 61.01 | 5.66 | 10.01 |
| **13b** | iso-Pr | B | 61.1 | 79—80 | +53.8° | $C_{15}H_{16}N_2O_4$ | 62.06 | 6.25 | 9.65 | 61.89 | 6.13 | 9.42 |
| **13c** | *n*-Bu | B | 69.4 | 70—71 | +41.0° | $C_{16}H_{20}N_2O_4$ | 63.14 | 6.62 | 9.21 | 63.20 | 6.58 | 9.38 |
| **13d** | $n$-$C_5H_{11}$ | B | 53.0 | 86—87 | +40.0° | $C_{17}H_{22}N_2O_4$ | 64.13 | 6.97 | 8.80 | 64.30 | 6.92 | 8.89 |
| **13e** | $n$-$C_{18}H_{37}$ | B | 39.2 | 77—80 | +20.5° | $C_{30}H_{48}N_2O_4$ | 71.96 | 9.66 | 5.59 | 72.06 | 9.61 | 5.75 |

*a*) Method A, reactions with alkyl halides; method B, Mitsunobu reaction. *b*) This compound was isolated by column chromatography using $CHCl_3$ as the eluent; MS *m/z*: 336 ($M^+$).

TABLE II. Physicochemical Data for 3-Substituted Dihydroorotic Acids (**9a—j**)

| 9 | R | Confi. | Yield (%) | mp (°C) | $[\alpha]_D$ $c=0.5$ (DMF) | Formula | Analysis (%) Calcd | | | Analysis (%) Found | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | C | H | N | C | H | N |
| **a** | Et | *RS* | 92.6 | 149—151 | 0 | $C_7H_{10}N_2O_4$ | 45.16 | 5.41 | 15.05 | 45.33 | 5.52 | 15.02 |
| **b** | *n*-Pr | *RS* | 94.2 | 146—148 | 0 | $C_8H_{12}N_2O_4$ | 48.00 | 6.04 | 13.99 | 48.31 | 6.08 | 14.08 |
| **c** | *n*-Pr (from **8c**) | *S* | 63.2 | 147—149 | +41.5° | $C_8H_{12}N_2O_4$ | 48.00 | 6.04 | 13.99 | 48.00 | 5.99 | 13.94 |
| **d** | $MeOCH_2CH_2OCH_2$ (as DCHA salt) | *S* | 61.0 | 136—138 | +11.3° | $C_{21}H_{37}N_3O_6$ | 58.99 | 8.72 | 9.83 | 58.59 | 8.75 | 9.67 |
| **e** | $PhCH_2$ | *S* | 77.1 | 193—195 | +24.8° | $C_{12}H_{12}N_2O_4$ | 58.06 | 4.87 | 11.29 | 57.97 | 4.83 | 11.23 |
| **f** | Et | *S* | 59.0 | 160—162 | +44.0° | $C_7H_{10}N_2O_4$ | 45.16 | 5.41 | 15.05 | 45.21 | 5.55 | 14.93 |
| **g** | iso-Pr | *S* | 57.8 | 154—156 | +58.4° | $C_8H_{12}N_2O_4$ | 47.99 | 6.04 | 14.00 | 47.85 | 5.93 | 14.05 |
| **h** | *n*-Bu | *S* | 78.3 | 131—132 | +38.8° | $C_9H_{14}N_2O_4$ | 50.46 | 6.59 | 13.08 | 50.33 | 6.48 | 13.04 |
| **i** | $n$-$C_5H_{11}$ | *S* | 66.1 | 139—140 | +36.2° | $C_{10}H_{16}N_2O_4$ | 52.62 | 7.07 | 12.28 | 52.57 | 7.05 | 12.39 |
| **j** | $n$-$C_{18}H_{37}$ | *S* | 90.0 | 125—126 | +15.4° | $C_{23}H_{42}N_2O_4 \cdot H_2O$ | 64.45 | 10.34 | 6.53 | 64.82 | 10.36 | 6.68 |

of **3a** was confirmed by the infrared (IR) and $^1$H-NMR spectra; the spectral patterns were quite different from those of 3-methylhydantoin-5-acetic acid (**2**).

In a similar way, optically pure (*R*)-3-methyldihydroorotic acid (**3b**) was obtained from (*R*)-dihydroorotic acid (**5b**)[2] (Chart 1).

It was confirmed that 3-methylation of **6a** or **6b** in the presence of NaH or *tert*-BuOK gave the desired 3-methyldihydroorotate without racemization or conversion to a hydantoin ring. Therefore, reactions of **6a** with various alkyl halides were carried out in a similar way (method A), and these results are summarized in Table I.

In the case of reactions using allyl bromide, methoxyethoxymethyl (MEM) chloride, and benzyl bromide as alkyl halides, the corresponding benzyl 3-substituted dihydroorotates (**8c—e**) with high optical rotation were obtained, and each was confirmed to be a single isomer by $^1$H-NMR spectroscopy using a shift reagent.[8] On the other hand, in the case of *N*-ethylation and *n*-propylation, the products (**8a** and **8b**) were completely racemized, which would be due to the lower reactivities of alkyl halides based on poor electrophilicity in comparison with **8c—e**. Subsequently, the benzyl ester compounds (**8a—e**) were converted to the corresponding 3-substituted dihydroorotic acids (**9a—e**) by hydrogenation (Table II). When the 3-allyl compound (**8c**) was hydrogenated, opti-

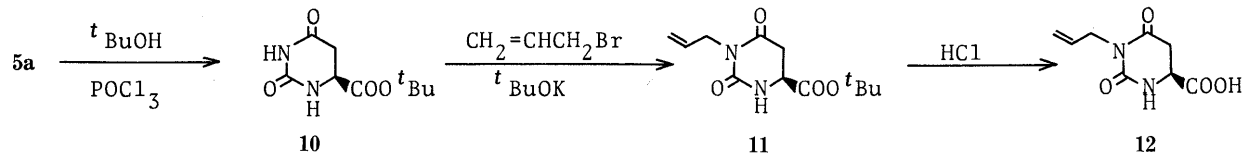

Chart 3

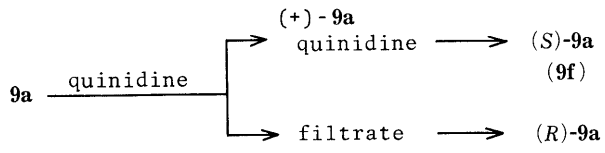

Chart 4

cally active 3-*n*-propyldihydroorotic acid (**9c**) was obtained in 63% yield accompanied with reduction of the allyl group.

In order to obtain optically pure 3-allyldihydroorotic acid, we examined an alternative method without the hydrogenation procedure. Namely, as shown in Chart 3, *tert*-butyl (*S*)-dihydroorotate (**10**) derived from **5a** was treated with allyl bromide using *tert*-BuOK in dimethylformamide (DMF) to afford the optically active 3-allyl compound (**11**) in 59% yield. Further, the product (**11**) was treated with HCl–dioxane solution to provide the 3-allyldihydroorotic acid (**12**) in a high yield.

**Optical Resolution Method** We also performed an optical resolution of racemic 3-ethyldihydroorotic acid (**9a**) obtained by the *N*-ethylation method. When **9a** was admixed with equimolar quinidine in EtOH, the quinidine salt of **9a** showing $[\alpha]_D$ +174.6° ($c$=0.5, MeOH) was isolated in 37% yield. Then, the product was treated with an ion exchange resin SK-1B ($H^+$ form) column to remove quinidine, and recrystallization of the product from ethyl acetate gave (+)-**9a** having $[\alpha]_D$ +43.0° ($c$=0.5, DMF) in 57% yield. On the other hand, from the filtrate of the quinidine salt of **9a**, (−)-**9a** having $[\alpha]_D$ −42.6° ($c$=0.5, DMF) was obtained in 20.8% by a similar treatment. The identity of the resulting (+)-**9a** and (−)-**9a** was confirmed by the IR and $^1$H-NMR spectra. The procedures are shown in outline in Chart 4.

Moreover, we carried out the optical resolution of the racemic 3-propyl compound (**9b**) to confirm the optical purity of (*S*)-3-propyl-dihydroorotic acid (**9c**) derived from the 3-allyl compound (**8c**) as described above. However, the expected resolution did not proceed successfully with quinidine, cinchonidine, (+)-α-methylbenzylamine as the resolving agent. Further investigations for *N*-alkylation without racemization were required, and we focused on neutral conditions.

**Mitsunobu Reaction** Mitsunobu *et al.* reported that *N*-alkylation by the reactions of various imide compounds with alcohols in the presence of triphenylphosphine ($PPh_3$) and diethyl azodicarboxylate (DEAD) proceeded in good yields.[9] We applied these reaction conditions (method B) to *N*-alkylation of benzyl (*S*)-dihydroorotate (**6a**), as shown in Chart 2. The desired 3-substituted products (**13a**—**e**) having high optical rotations were obtained by refluxing in dioxane followed by silica gel column chromatography in good yields, as summarized in Table I. Similarly, **13a**—**e** were hydrogenated to afford optically active 3-alkyldihydroorotic acids (**9f**—**j**) in high yields; these results are listed in Table II.

The 3-ethyl product (**9f**) thus obtained showed the same value of optical rotation as that of (+)-**9a** derived by optical resolution. It was confirmed that *N*-alkylation of **6a** using the Mitsunobu reaction (method B) proceeded without racemization.

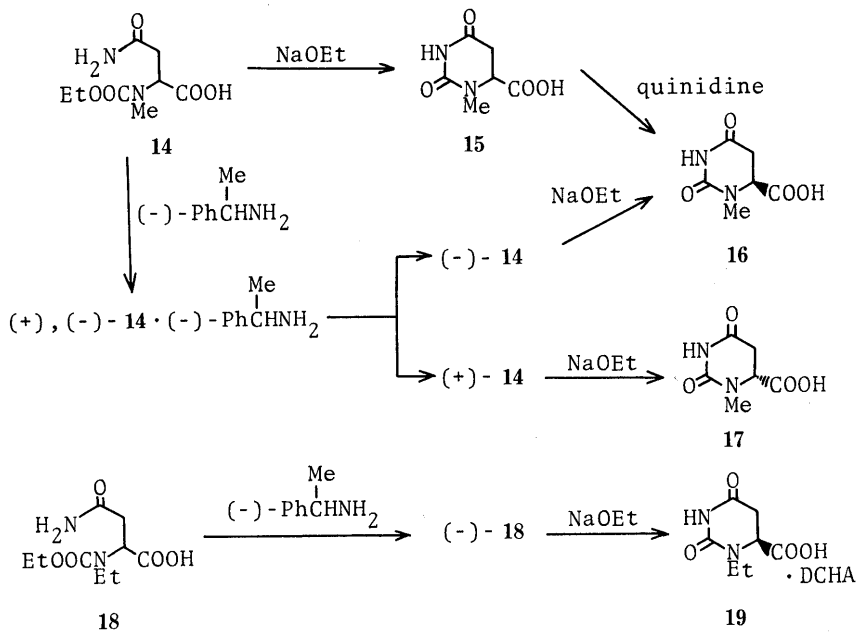

Chart 5

**1-Substituted Dihydroorotic Acids** It is known that 1-alkyldihydroorotic acids can be derived from the corresponding $N^{\alpha}$-alkyl-$N$-ethoxycarbonylasparagine in the presence of NaOEt in EtOH, but these compounds are racemic forms or the values[6a] of optical rotation were not reported.[6b] Therefore, at first the optical resolution of racemic 1-methyldihydroorotic acid (**15**)[6a] was carried out in a similar way to that described above. Namely, the (+)-**15** quinidine salt obtained by recrystallization from EtOH was treated with SK-1B ($H^+$ form) ion exchange resin to give (+)-**16** showing $[\alpha]_D$ +38.5° ($c$=0.5, DMF). Moreover, in order to investigate the optical purity of this product (**16**), the cyclization reaction of optically pure $N^{\alpha}$-methylasparagine derivative (−)-**14**, which was derived by the optical resolution of racemic **14** using (−)-α-methylbenzylamine, was performed under reflux EtOH in the presence of NaOEt. As a result, (*S*)-1-methyldihydroorotic acid having $[\alpha]_D$ +37.5° ($c$=0.5, DMF) was obtained in 66.7% yield; its spectral data were coincident with those of **16** derived by the optical resolution of **15**. On the other hand, (*R*)-1-methyldihydroorotic acid (**17**) was obtaineld from (+)-**14** by the same reaction in 55% yield, and the absolute value of the optical rotation was almost equal to that of **16**.

Similarly, (*S*)-1-ethyldihydroorotic acid (**19**) was prepared by the optical resolution of racemic $N^{\alpha}$-ethyl compound (**18**) followed by cyclization with NaOEt in EtOH as shown in Chart 5. These results showed that optically active $N^{\alpha}$-alkyl-$N^{\alpha}$-ethoxycarbonylasparagines would be converted to 1-alkyldihydroorotic acids without racemization or ring-transformation, differently from the case of $N^{\beta}$-alkyl derivatives (**1**) (Chart 1).

In conclusion, we have found that various new optically pure 1- or 3-substituted dihydroorotic acids can be prepared by optical resolution and $N$-alkylation or Mitsunobu reaction of benzyl or *tert*-butyl dihydroorotates followed by deprotection of the ester group. Investigation of the chemical properties and biological activities of these product are in progress and extensions of the scope of these procedures are also under consideration.

## Experimental

All melting points (mp) were measured by the use of a Yamato MP-21 melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR-420 spectrometer. $^1$H-NMR spectra were recorded on a Hitachi R-40 (90 MHz) spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were taken on a Hitachi M-60 spectrometer at an ionizing potential of 30 eV. Optical rotations were measured with a Perkin-Elmer 243 digital readout polarimeter using a 10 cm cell at 21 °C. For silica gel column chromatography, Kieselgel 60 (0.063—0.20 mm, E. Merck) was employed, and SK-1B (DIAION, Mitsubishi Chemical Ind. Ltd.) was utilized as an ion exchange resin with $H_2O$ as an eluent. TLC was done on precoated plates Kieselgel $60F_{254}$ (E. Merck). All organic solvent extracts were washed with brine and dried over anhydrous magnesium sulfate.

**3-Methylhydantoin-5-acetic Acid (2)** $N^{\alpha}$-Ethoxycarbonyl-$N^{\beta}$-methylasparagine (**1**, 1.4 g, 0.007 mol) was added to a solution of Na metal (0.33 g, 0.014 mol) in EtOH (20 ml) and the whole was refluxed for 20 h, then concentrated *in vacuo*. The residue was dissolved in $H_2O$ (20 ml) and the aqueous solution was charged on a column of ion exchange resin SK-1B ($H^+$ form, 100 ml). Fractions containing the desired product were concentrated *in vacuo* to afford **2** (1.1 g, 91%), mp 175—178 °C (lit.[10] mp 177 °C). IR (Nujol): 3300, 3000, 1770, 1730, 1700 cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.4—3.0 (2H, m, $CH_2$), 2.83 (3H, s, $CH_3$), 4.0—4.5 (1H, m, CH), 8.1 (1H, br, NH). MS $m/z$: 172 ($M^+$).

**Benzyl (*S*)-Dihydroorotate (6a)** Triethylamine (46.4 g, 0.46 mol) and benzyl bromide (6.28 g, 0.37 mol) were added to a suspension of (*S*)-dihydroorotic acid (**5a**,[2] 29.0 g, 0.184 mol) in DMF (300 ml) at room temperature and the whole was stirred for 2 d, then the mixture was concentrated *in vacuo*. $H_2O$ (200 ml) was added to the residue and the resulting precipitates were collected by filtration. Recrystallization from MeOH gave **6a** (27.0 g, 59%) as colorless prisms, mp 187—189 °C. $[\alpha]_D$ +54.7° ($c$=1, DMF). IR (Nujol): 3250, 3020, 1748, 1715, 1700 cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.6—3.2 (2H, m, $CH_2$), 4.2—4.4 (1H, m, CH), 5.17 (2H, s, $OCH_2$), 7.3—7.45 (5H, s, aromatic H), 7.93 (1H, br, N1-H), 10.16 (1H, s, N3-H). MS $m/z$: 248 ($M^+$). *Anal.* Calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 58.41; H, 4.75; N, 11.36.

In the same manner as described above, (*R*)-dihydroorotic acid (**5b**,[2] 14.5 g) was converted to benzyl (*R*)-dihydroorotate (**6b**, 12.1 g, 52%), mp 188—190 °C. $[\alpha]_D$ −53.8° ($c$=1, DMF). *Anal.* Calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 58.11; H, 4.77; N, 11.09. IR and $^1$H-NMR spectra of this product were coincident with those of **6a**.

***N*-Methylation of 6a Using $K_2CO_3$** $K_2CO_3$ (0.25 g, 1.8 mmol) was added to a solution of **6a** (0.4 g, 1.6 mmol) and methyl iodide (0.11 ml, 1.8 mmol) in DMF (5 ml) at room temperature. The mixture was stirred overnight and then filtered. The filtrate was evaporated *in vacuo* and the residue was extracted with AcOEt. The extract was washed and dried, then concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (98 : 2) as an eluent to give benzyl 3-methyldihydroorotate (360 mg, 86%), mp 137—139 °C. $[\alpha]_D$ 0° ($c$=1, DMF). IR (Nujol): 3250, 1725, 1710, 1685 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 2.6—3.2 (2H, m, $CH_2$), 3.1 (3H, s, $CH_3$), 4.1—4.3 (1H, m, CH), 5.2 (2H, s, $OCH_2$), 6.45 (1H, br, NH), 7.35 (5H, s, aromatic H). $^1$H-NMR [$CDCl_3$ + Eu(TFC)$_3$] $\delta$: 3.0—3.2 (2H, m, $CH_2$), 3.61 and 3.7 (each s, ratio 1 : 1, $CH_3$), 4.1—4.4 (1H, m, CH), 5.25 (2H, s, $OCH_2$), 6.65 and 6.8 (each br, ratio 1 : 1, NH), 7.4 (5H, s, aromatic H). MS $m/z$: 263 ($M^+$ + 1).

**Benzyl (*S*)-3-Methyldihydroorotate (7a)** NaH (61% dispersion in oil, 1.6 g, 0.04 mol) was added to a solution of **6a** (10.0 g, 0.04 mol) and methyl iodide (12.6 ml, 0.2 mol) in DMF (100 ml) at 25 °C. The mixture was stirred for 30 min at the same temperature, then AcOH (0.5 ml) was added, and the solvent was removed *in vacuo*. AcOEt (200 ml) and $H_2O$ (200 ml) were added to the residue and the precipitates were collected by filtration. Recrystallization from AcOEt afforded **7a** (6.4 g, 60%) as colorless prisms, mp 162—164 °C. $[\alpha]_D$ +53.5° ($c$=1, DMF). IR (Nujol): 3250, 1725, 1710, 1685 cm$^{-1}$. $^1$H-NMR [CDCl + Eu(TFC)$_3$] $\delta$: 3.0—3.2 (2H, m, $CH_2$), 3.7 (3H, s, $CH_3$), 4.1—4.4 (1H, m, CH), 5.25 (2H, s, $OCH_2$), 6.65 (1H, br, NH), 7.4 (5H, s, aromatic H). MS $m/z$: 263 ($M^+$ + 1). *Anal.* Calcd for $C_{13}H_{14}N_2O_4$: C, 59.53; H, 5.38; N, 10.68. Found: C, 59.52; H, 5.35; N, 10.73.

In the same manner as described above, **6b** (30.7 g) was converted to benzyl (*R*)-3-methyldihydroorotate (**7b**, 17.8 g, 55%), mp 164—165 °C. $[\alpha]_D$ −52.6° ($c$=1, DMF). *Anal.* Calcd for $C_{13}H_{14}N_2O_4$: C, 59.53; H, 5.38; N, 10.68. Found: C, 59.63; H, 5.35; N, 10.46. The IR, $^1$H-NMR, and MS spectra of **7b** were coincident with those of **7a**.

**(*S*)-3-Methyldihydroorotic Acid (3a)** As a catalyst, 5% Pd–C (0.4 g) was added to a solution of **7a** (4.0 g, 0.0153 mol) in EtOH (340 ml) and the mixture was subjected to catalytic hydrogenation under a hydrogen atmosphere for 2 h. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was recrystallized from MeOH to give **3a** (1.9 g, 73%) mp 214—216 °C. $[\alpha]_D$ +49.2° ($c$=1, DMF). IR (Nujol): 3300, 1753, 1715, 1680 cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.5—3.2 (2H, m, $CH_2$), 2.96 (3H, s, $CH_3$), 4.0—4.2 (1H, m, CH), 8.0 (1H, br, NH). MS $m/z$: 172 ($M^+$). *Anal.* Calcd for $C_6H_8N_2O_4$: C, 41.86; H, 4.69; N, 16.28. Found: C, 41.81; H, 4.69; N, 16.18.

In the same manner as described above, **7b** (4.0 g) was converted to (*R*)-3-methyldihydroorotic acid (**3b**, 2.0 g, 76.7%), mp 214—216 °C. $[\alpha]_D$ −49.2° ($c$=1, DMF). *Anal.* Calcd for $C_6H_8N_2O_4$: C, 41.86; H, 4.69; N, 16.28. Found: C, 41.99; H, 4.73; N, 16.00. The IR, $^1$H-NMR, and MS spectra of **3b** were coincident with those of **3a**.

***tert*-Butyl (*S*)-Dihydroorotate (10)** Phosphorus oxychloride (2.76 g, 0.018 mol) was added dropwise to a solution of **5a** (2.4 g, 0.015 mol), *tert*-butyl alcohol (8.9 g, 0.12 mol), and pyridine (9.5 g, 0.12 mol) in $CH_2Cl_2$ (400 ml) at −5 °C under stirring. After being stirred at room temperature for 1 h, the mixture was diluted with $H_2O$ (50 ml) and the organic layer was separated. The extract was washed with 20% aqueous citric acid solution and saturated $NaHCO_3$ solution, dried, and then concentrated *in vacuo*. The residue was crystallized from AcOEt to give **10** (1.0 g, 31%), mp 267—268 °C (dec.). $[\alpha]_D$ +56.8° ($c$=1, DMF). IR (Nujol): 3220, 3080, 1730—1700 (br). $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.42 (9H, s, $CH_3 \times 3$), 2.4—3.1 (2H, m, $CH_2$), 4.1—4.2 (1H, m, CH), 7.76 (1H, br, N3-H), 10.07 (1H, s, N1-H). MS $m/z$: 214 ($M^+$). *Anal.* Calcd for $C_9H_{14}N_2O_4$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.35; H, 6.59; N, 13.05.

***tert*-Butyl (*S*)-3-(2-Propenyl)dihydroorotate (11)** *tert*-BuOK (2.75 g, 0.0246 mol) was added to a solution of **10** (5.27 g, 0.0246 mol) and 2-propenyl bromide (8.94 g, 0.0739 mol) in DMF (80 ml) at 5 °C under stirring. The mixture was stirred at the same temperature for 1.5 h, AcOH (1 ml) was added, and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel with $Et_2O$ as an eluent and the product was recrystallized from iso-$Pr_2O$ to give **11** (3.7 g, 59%) as colorless prisms, mp 63—65 °C. $[\alpha]_D$ +42.6° ($c$=0.5, DMF). IR (Nujol): 3250, 3100, 1730, 1680 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.50 (9H, s, $CH_3 \times 3$), 2.6—3.2 (2H, m, $CH_2$), 4.0—4.2 (1H, m, CH), 4.3—4.45 (2H, m, $NCH_2$), 5.0—5.3 (2H, m, $=CH_2$), 5.6—6.1 (1H, m, =CH), 6.35 (1H br, NH). MS $m/z$: 254 ($M^+$). *Anal.* Calcd for $C_{12}H_{18}N_2O_4$: C, 56.68; H, 7.13; N, 11.02. Found: C, 56.80; H, 7.13; N, 11.09.

**(*S*)-3-(2-Propenyl)dihydroorotic Acid (12)** **11** (3.4 g, 0.0134 mol) was dissolved in 14% HCl–dioxane (50 ml) and the mixture was stirred overnight at room temperature, then concentrated *in vacuo*. The residue was triturated with iso-$Pr_2O$ and recrystallized from AcOEt to afford **12** (2.17 g, 82%) as colorless prisms, mp 160—161 °C. $[\alpha]_D$ +43.0° ($c$=0.5, DMF). IR (Nujol): 3270, 1720 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 2.6—3.3 (2H, m, $CH_2$), 4.0—4.3 (3H, m, $NCH_2$ and CH), 4.9—5.2 (2H, m, $=CH_2$), 5.5—6.0 (1H, m, =CH), 8.50 (1H, br, NH). MS $m/z$: 198 ($M^+$). *Anal.* Calcd for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.09, N, 14.14. Found: C, 48.75; H, 5.14; N, 14.29.

**Optical Resolution of 3-Ethyldihydroorotic Acid (9a)** Racemic acid **9a** (4.8 g, 0.026 mol) and quinidine (9.05 g, 0.028 mol) were dissolved in EtOH (100 ml) at 50 °C. The mixture was gradually cooled to room temperature and the precipitates that separated were collected by filtration. Recrystallization from EtOH gave the salt; (*S*)-isomer (+)-**9a**·quinidine (3.5 g), mp 199—201 °C. $[\alpha]_D$ +173.7° ($c$=0.5, DMF). IR (Nujol) 3210, 1710, 1675, 1620, 1595 $cm^{-1}$. The salt was dissolved in $H_2O$ (10 ml) and charged on a column of ion exchange resin (SK-1B, $H^+$ form, 100 ml). The fraction containing the desired product was concentrated *in vacuo* and the residual solid was recrystallized from AcOEt to give (*S*)-3-ethyldihydroorotic acid (0.72 g, 15%) as colorless prisms, mp 158—159 °C. $[\alpha]_D$ +43.0° ($c$=0.5, DMF). IR (Nujol) 3300, 1720, 1610 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 0.99 (3H, t, $J$=7 Hz, $CH_3$), 2.6—3.3 (2H, m, $CH_2$), 3.6 (2H, q, $J$=7 Hz, $NCH_2$), 3.9—4.2 (1H, m, CH), 7.9 (1H, d, $J$=6 Hz, NH). *Anal.* Calcd for $C_7H_{10}N_2O_4$: C, 45.16; H, 5.41; N, 15.05. Found: C, 45.26; H, 5.36; N, 15.16.

The combined EtOH mother liquor was concentrated *in vacuo* and the residual solid was washed with a small amount of EtOH, then filtered off by suction. $H_2O$ (50 ml) and 10% aqueous $Na_2CO_3$ solution (15 ml) were added to the filtrate and the mixture was extracted with $CHCl_3$. The aqueous layer was charged on a column of ion exchange resin; SK-1B ($H^+$ form, 100 ml) and the same work-up as described for (*S*)-**9a** gave (*R*)-**9a** (1.5 g, 31%), mp 160—161 °C. $[\alpha]_D$ −42.6° ($c$=0.5, DMF). *Anal.* Calcd for $C_7H_{10}N_2O_4$: C, 45.16; H, 5.41; N, 15.05. Found: C, 45.43; H, 5.42; N, 15.17. The IR and $^1$H-NMR spectra of this product were coincident with those of (*S*)-**9a**.

**Typical Procedure for Preparation of Benzyl 3-Substituted Dihydroorotates (8a—e and 13a—e)** Method A: *tert*-BuOK (2.24 g, 0.02 mol) was added to a solution of **6a** (5.0 g, 0.02 mol) and 2-propenyl bromide (7.26 g, 0.06 mol) in DMF (150 ml) at 5 °C under stirring. The mixture was stirred at the same temperature for 1.5 h, AcOH (0.5 ml) was added, and the solvent was removed *in vacuo*. The residue was extracted with AcOEt and the extract was washed, dried and then concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–AcOEt (15:1) as an eluent. Recrystallization from AcOEt–hexane gave benzyl (*S*)-3-(2-propenyl)dihydroorotate (**8c**, 2.6 g). In a similar way, other dihydroorotates (**8a—e**) were prepared. The yields and physicochemical data are listed in Tables I and III.

Method B: A mixture of **6a** (2.0 g, 0.0081 mol), $PPh_3$ (4.23 g, 0.016 mol), DEAD (4.2 g, 0.024 mol), and EtOH (1.47 g, 0.032 mol) in dioxane (100 ml) was refluxed for 4 h. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel with iso-$Pr_2O$–$Et_2O$ (1:4) as an eluent. Recrystallization from AcOEt–hexane gave benzyl (*S*)-3-ethyldihydroorotate (**13a**, 1.18 g). In a similar way, other dihydroorotates (**13a—e**) were prepared. The yields and physicochemical data are listed in Tables I and III.

**Typical Procedure for Preparation of 3-Substituted Dihydroorotic Acids (9a—j)** Hydrogen gas was bubbled into a mixture of **13a** (2.76 g, 0.01 mol) and 10% Pd–C (0.3 g) in EtOH (150 ml) at room temperature for 3 h. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was recrystallized from AcOEt to afford (*S*)-3-ethyldihydroorotic acid (**9f**, 1.1 g). Spectral data were identical with those of (*S*)-**9a** prepared by the optical resolution of **9a**. Other 3-substituted dihydroorotic acids (**9a—j**) were similarly prepared. The yields and physicochemical data are listed in Tables II and IV.

**Optical Resolution of 1-Methyldihydroorotic Acid (15)** Racemic acid **15**[6a] (1.5 g, 0.0087 mol) and quinidine were dissolved in EtOH (30 ml) and the mixture was kept standing overnight at room temperature. The precipitates that separated were collected by filtration and recrystallized twice from EtOH to give the salt; (*S*)-isomer (+)-**15** quinidine (0.94 g), mp 222—224 °C (dec.), $[\alpha]_D$ +201° ($c$=1, MeOH). IR (Nujol): 3200, 1690, 1610, 1590 $cm^{-1}$. The salt was dissolved in $H_2O$ (10 ml) and charged on a column of ion exchange resin (SK-1B, $H^+$ form, 100 ml). The fraction containing the desired product was concentrated *in vacuo* and the residual solid was recrystallized from EtOH to give (*S*)-1-methyldihydroorotic acid (0.25 g, 16.7%) as colorless needles, mp 219—220 °C. $[\alpha]_D$ +38.5° ($c$=0.5, DMF). IR (Nujol): 3200, 1735, 1720, 1640 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.4—3.3 (2H, m, $CH_2$), 2.9 (3H, s, $CH_3$), 4.2—4.4 (1H, m, CH), 10.2 (1H, br, NH). MS $m/z$: 172 ($M^+$). *Anal.* Calcd for $C_6H_8N_2O_4$: C, 41.86; H, 4.68; N, 16.27. Found: C, 41.72; H, 4.66; N, 16.10.

**Optical Resolution of $N^{\alpha}$-Methyl-$N^{\beta}$-ethoxycarbonylasparagine (14)** Racemic acid **14**[6a] (10.9 g, 0.05 mol) and α-(−)-methylbenzylamine (6.06 g, 0.05 mol) were dissolved in EtOH (30 ml) at 70—75 °C and the

TABLE III. IR and $^1$H-NMR Spectral Data for **8a—e** and **13a—e**

| No. | IR (Nujol)[a] $cm^{-1}$ | $^1$H-NMR ($CDCl_3$) $\delta$ (ppm) |
|---|---|---|
| **8a** | 3200, 3100, 1738, 1720, 1680 | 0.95 (3H, t, $J$=8 Hz), 2.6—3.2 (2H, m), 3.6 (2H, q, $J$=8 Hz), 4.1—4.3 (1H, m), 5.10 (2H, s), 7.2—7.4 (5H, m), 8.05 (1H, br) |
| **8b** | 3350, 1730, 1680 | 0.8 (3H, t, $J$=8 Hz), 1.1—1.6 (2H, m), 2.6—3.3 (2H, m), 3.6 (2H, t, $J$=8 Hz), 4.15—4.4 (1H, m), 5.2 (2H, s), 7.3—7.45 (5H, m), 5.15 (1H, br) |
| **8c** | 3200, 3100, 1738, 1720, 1685 | 2.8—3.1 (2H, m), 4.1—4.4 (3H, m), 4.9—5.2 (2H, m), 5.2 (2H, s), 5.5—6.0 (1H, m), 6.4 (1H, br), 7.2—7.4 (5H, m) |
| **8d** | 3250, 1735, 1695 | 2.9—3.1 (2H, m), 3.35 (3H, s), 3.4—3.8 (4H, m), 4.2—4.4 (1H, m), 5.2 (2H, s), 5.3 (2H, s), 6.3 (1H, br), 7.2—7.4 (5H, m) |
| **8e** | 3250, 1755, 1720, 1670 | 2.8—3.0 (2H, m), 4.0—4.3 (1H, m), 4.9 (2H, s), 5.15 (2H, s), 6.3 (1H, br), 7.1—7.5 (10H, m) |
| **13a** | 3210, 1738, 1720, 1680 | 1.1 (3H, t, $J$=7 Hz), 2.6—3.2 (2H, m), 3.75 (2H, q, $J$=7 Hz), 4.05—4.3 (1H, m), 5.15 (2H, s), 6.25 (1H, s), 7.2—7.4 (5H, m) |
| **13b** | 3410, 1740, 1725, 1675 | 1.3 and 1.35 (6H, each s), 2.6—3.2 (2H, m), 4.0—4.25 (1H, m), 4.55—4.95 (1H, m), 5.15 (2H, s), 6.1 (1H, br), 7.2—7.4 (5H, m) |
| **13c** | 3200, 1750, 1720, 1660 | 0.9 (3H, t, $J$=5 Hz), 1.1—1.7 (4H, m), 2.65—3.2 (2H, m), 3.6—3.9 (2H, t, $J$=7 Hz), 4.05—4.35 (1H, m), 5.2 (2H, s), 6.4 (1H, br), 7.25—7.5 (5H, m) |
| **13d** | 3225, 1740, 1725, 1675 | 0.95 (3H, t, $J$=5 Hz), 1.1—1.8 (6H, m), 2.65—3.2 (2H, m), 3.65—3.8 (2H, t, $J$=7 Hz), 4.05—4.3 (1H, m), 5.15 (2H, s), 6.2 (1H, br), 7.2—7.4 (5H, m) |
| **13e** | 3250, 1755, 1720, 1670 | 0.9 (3H, t, $J$=4 Hz), 1.1—1.8 (32H, m), 2.65—3.2 (2H, m), 3.7 (2H, t, $J$=7 Hz), 4.0—4.3 (1H, m), 5.15 (2H, s), 6.4 (1H, br), 7.25 (5H, s) |

*a*) The IR spectrum of **8d** was taken in film. *b*) $^1$H-NMR spectra of **8a** and **8b** were taken in DMSO-$d_6$.

TABLE IV. IR and $^1$H-NMR Spectral Data for **9a—j**

| No. | IR (Nujol) cm$^{-1}$ | $^1$H-NMR (DMSO-$d_6$)[a] $\delta$ (ppm) |
|---|---|---|
| **9a** | 3300, 1715, 1630 | 1.0 (3H, t, $J$=8 Hz), 2.7—3.25 (2H, m), 3.6 (2H, q, $J$=8 Hz), 3.9—4.15 (1H, m), 7.9 (1H, br) |
| **9b** | 3300, 1750, 1730, 1705, 1650 | 0.8 (3H, t, $J$=8 Hz), 1.2—1.7 (2H, m), 2.5—3.2 (2H, m), 3.6 (2H, t, $J$=8 Hz), 3.9—4.2 (1H, m), 7.9 (1H, br) |
| **9c** | 3300, 1730, 1710, 1655 | 0.8 (3H, t, $J$=8 Hz), 1.2—1.8 (2H, m), 2.6—3.3 (2H, m), 3.6 (2H, t, $J$=8 Hz), 3.95—4.2 (1H, m), 7.95 (1H, br) |
| **9d** | 3280, 1725, 1690, 1630 | 1.0—2.2 (22H, m), 2.7—2.9 (2H, m), 2.9—3.2 (2H, m), 3.25 (3H, s), 3.3—3.8 (5H, m), 5.05 (2H, s), 7.4 (1H, br) |
| **9e** | 3250, 1730, 1720, 1630 | 2.6—3.3 (2H, m), 4.0—4.2 (1H, m), 4.8 (2H, s), 7.2—7.35 (5H, m), 8.15 (1H, br) |
| **9g** | 3410, 3290, 1705, 1635 | 1.23 and 1.32 (6H, each s), 2.5—3.2 (2H, m), 3.95—4.2 (1H, m), 4.6—4.95 (1H, m), 7.9 (1H, br) |
| **9n** | 3300, 1730, 1715, 1650 | 0.95 (3H, t, $J$=4 Hz), 1.05—1.6 (4H, m), 2.5—3.1 (2H, m), 3.6 (2H, t, $J$=6 Hz), 3.95—4.2 (1H, m), 8.0 (1H, br) |
| **9i** | 3300, 1730, 1710, 1650 | 0.88 (3H, t, $J$=4 Hz), 1.1—1.7 (6H, m), 2.5—3.2 (2H, m), 3.68 (2H, t, $J$=7 Hz), 3.95—4.2 (1H, m), 7.95 (1H, br) |
| **9j** | 3400, 1720, 1660 | 0.86 (3H, t, $J$=4 Hz), 1.0—1.7 (32H, m), 2.7—3.2 (2H, m), 2.68 (2H, t, $J$=7 Hz), 3.9—4.2 (1H, m), 6.45 (1H, br), 6.75 (1H, br) |

a) $^1$H-NMR spectrum of **9j** was taken in DMSO-$d_6$+$CDCl_3$.

mixture was put in an ice box overnight. The precipitates that separated were collected by filtration to give the salt; (−)-**14**·(−)-α-methylbenzylamine (6.3 g), mp 171—173 °C (dec.). $[\alpha]_D$ −25.3° ($c$=1, MeOH). The salt was dissolved in $H_2O$ (20 ml) and charged on a column of ion exchange resin (SK-1B, $H^+$ form, 150 ml) to remove the amine. Recrystallization from AcOEt–$Et_2O$ gave (−)-**14** (2.8 g, 25.7%) as colorless prisms, mp 123—125 °C. $[\alpha]_D$ −77.6° ($c$=1, MeOH). IR (Nujol): 3400, 3310, 3210, 1720, 1690 (sh), 1650 cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.2 (3H, t, $J$=8 Hz, $CH_3$), 2.4—2.9 (2H, m, $CH_2$), 2.8 (3H, s, $NCH_3$), 4.0 (2H, q, $J$=8 Hz, $OCH_2$), 4.7 and 4.8 (1H, ABd, $J$=6 Hz, CH), 6.9 (1H, br, NH), 7.4 (1H, br, NH). *Anal.* Calcd for $C_8H_{14}N_2O_5$: C, 44.03; H, 6.47; N, 12.84. Found: C, 43.91; H, 6.48; N, 12.71.

The EtOH mother liquor was concentrated *in vacuo* and the residue was dissolved in $H_2O$ (20 ml) and then charged on a column of SK-1B ($H^+$ form, 150 ml). The same work-up as described above was performed to afford (+)-**14** (2.6 g, 23.9%), mp 120—121 °C. $[\alpha]_D$ +80.6° ($c$=0.5, MeOH). Spectral data were identical with those of (−)-**14**.

**(*S*)-1-Methyldihydroorotic Acid (16)** (−)-**14** (2.6 g, 0.012 mol) was added to a solution of Na metal (0.625 g, 0.027 mol) in EtOH (75 ml) at room temperature under stirring and the mixture was refluxed for 3 h. After being cooled, the mixture was evaporated *in vacuo* and the residue was dissolved in $H_2O$ (20 ml) and then charged on a column of SK-1B ($H^+$ form, 40 ml). The fraction containing the desired product was concentrated *in vacuo* and recrystallized from EtOH to give **16** (1.38 g, 66.7%), mp 219—221 °C, $[\alpha]_D$ +37.5° ($c$=0.5, DMF). This compound was identical with the product prepared by the optical resolution of racemic **15** as described above, in terms of the spectral data.

**(*R*)-1-Methyldihydroorotic Acid (17)** The same procedure as described for the synthesis of (*S*)-1-methyldihydroorotic acid (**16**) using (+)-**14** (2.6 g, 0.012 mol) gave **17** (1.14 g, 55%), mp 221—222 °C. $[\alpha]_D$ −37.0° ($c$=0.5, DMF). *Anal.* Calcd for $C_6H_8N_2O_4$: C, 41.86; H, 4.68; N, 16.27. Found: C, 42.01; H, 4.55; N, 16.23. The IR and $^1$H-NMR spectra were coincident with those of **16**.

**(−)-$N^2$-Ethoxycarbonyl-$N^2$-ethylasparagine (−)-18** In the same manner as described for the optical resolution of **14**, racemic **18**[6a] (10 g, 0.043 mol) was resolved using (−)-α-methylbenzylamine (5.22 g, 0.043 mol) in MeOH (20 ml) to give (−)-**18** (2.2 g, 24%), mp 150—152 °C. IR (Nujol) 3400, 3350, 3260, 3210, 1740, 1670, 1600 cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.1 and 1.2 (3H, each, 2t, $J$=8 Hz, $CH_3$×2), 2.4—3.0 (2H, m, 2H), 3.0—3.5 (2H, m, $NCH_2$), 4.05 (2H, q, $J$=8 Hz, $OCH_2$), 4.5 (1H, t, $J$=8 Hz, CH), 6.88 (1H, br, NH), 7.4 (1H, br, NH), MS $m/z$: 232 ($M^+$). *Anal.* Calcd for $C_9H_{16}N_2O_5$: C, 46.55; H, 6.94; N, 12.06. Found: C, 46.72; H, 7.03; N, 11.98.

**(*S*)-1-Ethyldihydroorotic Acid Dicyclohexylamine (DCHA) Salt (19)** (−)-**18** (2.2 g, 0.0095 mol) was added to a solution of Na metal (0.496 g, 0.0022 mol) in EtOH (63 ml) and the mixture was refluxed for 6 h. After being cooled, the mixture was evaporated *in vacuo* and the residue was dissolved in $H_2O$ (15 ml) and then charged on a column of SK-1B ($H^+$ form, 50 ml). The fraction containing the desired product was concentrated *in vacuo* and the residue was dissolved in EtOH (20 ml). DCHA (1.8 g) was added to the solution and the resulting precipitates were collected and then recrystallized from EtOH to give **19** (1.25 g, 35.8%) as colorless prisms, mp 149—153 °C. $[\alpha]_D$ +37.4° ($c$=0.5, MeOH). IR (Nujol): 3250, 1710, 1690, 1640 cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$+TFA) $\delta$: 1.1 (3H, t, $J$=8 Hz, $CH_3$). 1.0—2.2 (20H, m, $CH_2$×10), 2.9—3.3 (4H, m, $CH_2$, NCH×2), 3.4—3.9 (2H, m, $NCH_2$), 4.2—4.4 (1H, m, CH). *Anal.* Calcd for $C_{19}H_{33}N_3O_4$: C, 62.10; H, 9.05; N, 11.44. Found: C, 62.12; H, 9.25; N, 11.62.

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