Enzymatic Synthesis of 4-Methyl- and 4-Ethyl-L-glutamic Acids

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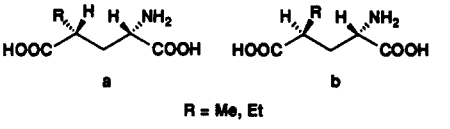
This paper reports the activity of glutamic oxalacetic aminotransferase (GOT) from pig heart and bacteria for racemic 4-hydroxy-, 4-methyl-, and 4-ethyl-2-ketoglutaric acids. These compounds are good substrates for GOT with V_{max} values of, respectively, 70, 90, and 130% relative to the natural substrate, 2-ketoglutaric acid. GOT displays high enantioselectivity toward 4-methyl-2-ketoglutaric acid and 4-ethyl-2-ketoglutaric acid; the synthesis of both diastereoisomers of the corresponding 4-substituted L-glutamic acids is thus possible.

Aminotransferases catalyze the transfer of the amino group from an amino acid to a keto acid, resulting in an equilibrium of two amino acids (one of them usually glutamic acid). They have been studied from both theoretical and practical points of view.[1] Numerous patents describe the utilization of bacterial aminotransferases for the production of natural amino acids, especially phenylalanine. The activity of aminotransferases for analogs of glutamic or 2-ketoglutaric acids has been reported,[2,3] but synthetic applications have not been described. As part of a program to evaluate the synthetic potential of aminotransferases, we have already described the enzymatic synthesis of 4-hydroxy-L-glutamic acids.[4] This paper describes the enzymatic synthesis of 4-methyl- and 4-ethyl-L-glutamic acids 1 and 2 (Chart I) as pure diastereoisomers, by transamination of the corresponding 2-ketoglutaric acids, catalyzed by glutamic oxalacetic aminotransferase (GOT, EC 2.6.1.1) from pig heart and bacteria (Scheme I).

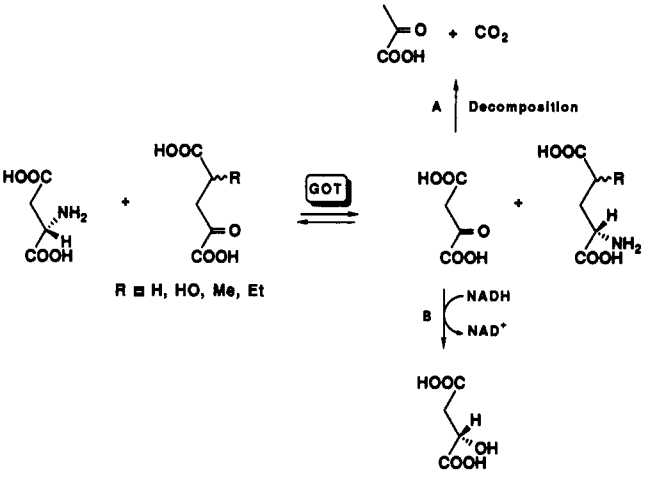
1 and 2 are natural products. 1 was isolated from various plants[5–8] and a fungus (*Mycena pura*).[9] Both diastereoisomers **1a** and **1b** are present in the fungus; the metabolite extracted from plants has been shown to have 2*S*,4*R* absolute configuration[10] and to derive from L-leucine by oxidation of one of the two methyl groups of the side chain.[11] **2a** was also isolated from plants;[12–14] its biosynthetic pathway is unknown.

Various chemical syntheses of 4-methylglutamic acids 1 have been described, generally by addition of an anion equivalent of glycine to ethyl methacrylate.[15–17] The use of a chiral auxiliary derived from proline afforded an enantiospecific synthesis.[18] An enzymatic synthesis of 4-methyl-L-glutamic acids 1 catalyzed by glutamate dehydrogenase was carried out,[19] yielding an equimolecular mixture of **1a** and **1b**.

Chart I. 4-Methyl- and 4-Ethyl-L-glutamic Acids 1 and 2



Scheme I. Reaction Catalyzed by Glutamic Oxalacetic Transaminase. A: Decomposition of Oxalacetic Acid during Synthesis. B: Reduction of Oxalacetic Acid by Malate Dehydrogenase for Analytical Assays



Results and Discussion

Analytical Study. We prepared several substituted 2-ketoglutaric acids: 4-hydroxy-2-ketoglutaric acid,[4] HOKG, 4-methyl-2-ketoglutaric acid,[20] MeKG, and 4-ethyl-2-ketoglutaric acid,[20] EtKG. We studied the kinetic parameters of the transamination according to Scheme I (path B): aspartic acid acts as an amino group donor, the oxalacetic acid product is reduced by malate dehydrogenase (MDH), and a decrease in absorbance due to the oxidation of NADH allows the reaction to be monitored.

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Table I.[a] Activity of GOT for Various Substituted 2-Ketoglutaric Acids

		KG	HOKG	MeKG	EtKG
pig heart	V_{rel}	100	70	90	130
GOT	K_m[b] (mM)	0.4	3.8	0.4	0.5
E. coli	V_{max}	100	8	93	50
GOT	K_m[b] (mM)	0.1	8.5	0.5	0.2
P. aeruginosa	V_{max}	100		87	31
GOT	K_m[b] (mM)	0.5		0.9	1.6

[a] Phosphate buffer 0.15 M, pH 7.5, 25 °C. [b] Apparent Michaelis constant, with aspartic acid concentration of 50 mM.

Table I gives the results for 2-ketoglutaric acid (KG), the natural substrate, and the three analogs in the transamination catalyzed by commercial pig heart GOT, *Escherichia coli*, or *Pseudomonas aeruginosa* enzymatic extract.

For HOKG, we observed the usual behavior for an analog of a natural substrate in an enzymatic reaction: a loss of stability of the enzyme–substrate complex (higher K_m value) and a decrease in the maximum rate. However, these values still indicate high activity, especially with pig heart GOT, which allowed the synthesis of 4-hydroxy-L-glutamic acids as a mixture of diastereoisomers.[10]

In contrast, MeKG and EtKG present behavior very close to that of the natural substrate, with K_m from 0.4 to 1.6 mM reflecting very close complementarity between active site and substrate. Racemates of these keto acids were used in this study, so that these values correspond to the more active of the two enantiomers. Given the high value of the ratio V_{max}/K_m observed, it seemed unlikely that the two enantiomers would act alike, and so we expected the enzyme to display some enantioselectivity in the transamination reaction. The V_{max}/K_m ratio for the other enantiomer (the less active substrate) must be very low: the enzymatic titration of a solution of MeKG or EtKG by a GOT/MDH coupled system[21] gave a concentration half the theoretical value.

Enzymatic Syntheses. For the synthesis, since transaminases catalyze an equilibrium, the equilibrium has to be shifted as far as possible to obtain a good yield. With GOT, when aspartic acid is used as an amino acid donor, the reaction product, oxalacetic acid, decomposes slowly into CO_2 and pyruvic acid, making the reaction irreversible (Scheme I, path A). The rate can be increased using cysteine sulfinate instead of aspartic acid: the keto acid product decomposes very rapidly into SO_2 and pyruvic acid.

The second method, using cysteine sulfinate, which simplifies the purification procedure, was used for the synthesis of glutamic acid analogs with commercial GOT in immobilized or soluble form and with *E. coli* GOT, where the enzyme was not isolated; we used resting cells of *E. coli* in suspension in the reaction mixture.

4-Methyl-L-glutamic Acid (1). Synthesis by Pig Heart GOT Catalysis. In the first assay, the synthesis was carried out on a 6 mmol scale with an equimolecular mixture of MeKG and cysteine sulfinic acid (ACS) and 300 units of immobilized GOT. After 24 h, no MeKG was detectable by enzymatic assay, suggesting a transamination yield of at least 50%. However, after purification by ion-exchange chromatography, 4-methyl-L-glutamic acid yield was only 32%.

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Analysis by HPLC shows the presence of two amino acids in an 80/20 ratio, identified as **1a** and **1b** by ^1H and ^{13}C NMR. Comparison with published spectra[19] indicates that the main product is **1a**: the ^1H NMR spectra are clearly distinct for each isomer. The signals due to the two protons at C_3 are of particular interest: these magnetically nonequivalent protons give well-separated multiplets with $\Delta\delta$ of 0.35 ppm for **1a** and 0.20 ppm for **1b**. Also, the proton at C_2 gives a four-line signal at 3.75 ppm for **1a** and a triplet at 3.82 ppm for **1b**. The broad band decoupled ^{13}C NMR spectra display the expected signals. The signals corresponding to $CH_3(4')$, C_3, and C_4 are distinct for **1a** and **1b** permitting easy determination of the diastereoisomeric ratio.

This synthesis leads to a mixture of **1a** and **1b**, showing that the enantioselectivity of the enzyme in these conditions is not absolute. The reaction does not go to completion, probably because the 4-methyl-L-glutamic acid formed acts as a competitive inhibitor. We considered that a reaction stopped before 50% conversion of the MeKG might give **1a** with higher diastereoisomeric purity. In addition, it would be useful to recover the unreacted keto acid and use it in a second transmination reaction yielding **1b**.

(2*S*,4*R*)- and (2*S*,4*S*)-4-Methyl-L-glutamic Acid (1a and 1b). Synthesis in Diastereoisomerically Pure Form by Pig Heart GOT Catalysis. The synthesis was carried out on a 0.6 mmol scale in three steps. In the first step, the reaction was stopped after 40% conversion of MeKG. The mixture was then treated with a cation-exchange resin, H^+ form, and eluted with water. The eluate contained the unreacted keto acid; 4-methyl-L-glutamic acid remained on the column. The eluate was used for the second step: the pH was adjusted to 7, cysteine sulfinic acid and GOT were added, and the mixture was incubated for 24 h and then treated as before on the cation-exchange resin column. The eluate was used in the same way for the third step. 4-Methyl-L-glutamate samples retained on the acidic resin in the three steps were eluted with 0.1 N ammonia and purified by chromatography on a OH^- form anion-exchange resin, giving, respectively, 25 mg of (2*S*,4*R*)-4-methyl-L-glutamic acid (**1a**) (26% yield, purity ≥98%), 30 mg of a mixture of **1a** and **1b** (31% yield), and 15 mg of (2*S*,4*S*)-4-methyl-L-glutamic acid (**1b**) (15% yield, purity ≥98%).

4-Ethyl-L-glutamic Acid (2). Synthesis by Pig Heart GOT Catalysis. The synthesis was carried out on a 0.6 mmol scale, with equimolecular amounts of EtKG and ACS and 100 units of soluble GOT. (Activity of the solution was maintained by addition of GOT.) After 24 h, less than 1% EtKG could be detected by enzymatic assay. After purification, 4-ethyl-L-glutamic acid was obtained in 36% yield and identified as **2a** (80%) and **2b** (20%) by ^1H and ^{13}C NMR spectroscopy.

The ^1H NMR spectra are clearly essentially identical to those reported in the literature.[14] The protons at C_3 give two multiplets centered at 1.95 and 2.25 ppm in **2a** and an undifferentiated multiplet at 2.00 ppm in **2b**. The proton at C_2 gives a doublet of doublets at 3.80 ppm for **2a** and a triplet at 3.65 ppm for **2b**. The same spectral differences also appear for **1a** and **1b** and enabled us to assign the 2*S*,4*R* configuration to the predominant product **2a**. The same configuration was assigned to natural 4-ethyl-L-glutamic acid isolated from plants on the basis of its CD spectrum.[14]

(2S,4R)- and (2S,4S)-4-Ethyl-L-glutamic Acid (2a and 2b). Synthesis in Diastereoisomerically Pure Form by Pig Heart GOT Catalysis. Since GOT displays the same enantioselectivity toward both 4-ethyl-2-ketoglutaric acid and 4-methyl-2-ketoglutaric acid, we followed the same sequential synthesis as described above for the production of diastereoisomerically pure 4-ethyl-L-glutamic acid. The synthesis was carried out on a 0.85 mmol scale leading to 50 mg of (2S,4R)-4-ethyl-L-glutamic acid **2a** (34% yield, purity ≥98%), 30 mg of a mixture of **2a** and **2b** (21% yield), and 30 mg of (2S,4S)-4-ethyl-L-glutamic acid **2b** (21% yield, purity ≥98%).

4-Methyl- and 4-Ethyl-L-glutamic Acid (1 and 2). Synthesis by *E. coli* GOT Catalysis: The synthesis was carried out on a 3 mmol scale using 3 g (wet weight) of cells. In the case of the transamination of MeKG, the reaction was stopped after 45% conversion. After the usual workup, (2S,4R)-4-methyl-L-glutamic acid (**1a**) was obtained with 96% purity in 35% yield. For EtKG, the (2S,4R)-ethyl-L-glutamic acid (**2a**) was obtained with 35% yield and no more than 95% purity though the reaction was stopped at 40% conversion.

Conclusion

In this paper we have shown that transamination of 4-substituted 2-ketoglutaric acids with cysteine sulfinic acid catalyzed by glutamic oxalacetic aminotransferase (GOT) provides a convenient route for 4-substituted L-glutamic acid synthesis. The keto acids are easily prepared by chemical synthesis. The enzymatic reaction needs only a small quantity of enzyme, and pig heart GOT is a cheap commercial enzyme. Moreover, *E. coli* provides an attractive source of GOT for large-scale synthesis. A convenient strategy affords the synthesis of both diastereoisomers of 4-methyl- and 4-ethyl-L-glutamic acids (1 and 2) in a diastereoisomerically pure form.

Experimental Section

General. Methylsuccinic acid and ethyl oxalate were purchased from Aldrich. Pig heart GOT, bovine heart malate dehydrogenase (MDH), and cysteine sulfinic acid (ACS) were obtained from Sigma. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O at 300 MHz. Mass spectra were provided by the Service Central d'Analyse du CNRS, Lyon. TLC of amino acids was performed on cellulose (Merck), using pyridine–1-butanol–water (6:4:5) and detection with ninhydrin. Amino acids were detected by precolumn derivatization with *o*-phthalaldehyde/ethanethiol reagent and fluorescence determination in HPLC analysis. All transaminations were carried out under nitrogen at 35 °C in 0.15 M phosphate buffer pH 7.5.

Bacterial Strain and Growth Conditions. *E. coli* ATCC 11303 strain was grown in a medium containing 0.5% succinate, 0.2% aspartate, 0.2% K_2HPO_4, 0.2% KH_2PO_4, 0.2% NaCl, and 0.01% $MgSO_4 \cdot 7H_2O$ at pH 7.5. The cells were harvested at the end of their growing phase by centrifugation (8000 g for 15 min) and washed once with 0.9% NaCl. Harvested bacteria were stored at −20 °C until used. *P. aeruginosa* ATCC 17504 strain was grown in Trypcase-soy broth medium (bioMerieux) at pH 7.3. The cells were harvested as above.

Bacterial Enzymatic Extract. A 1-g portion of resting cells was suspended in 5 mL of 0.015 M phosphate buffer (pH 7.5) containing 10 mmol of 2-mercaptoethanol. One volume of chloroform was added, and the mixture was vigorously stirred for 5 min before centrifugation (8000 g for 10 min). The aqueous layer was then used for determination of catalytic activity.

Enzymatic Assays. The GOT activity was determined by the spectrophotometric method described by Bergmeyer.[21] This method was suitable for all assays involving 4-substituted 2-ketoglutaric acids. MeKG or EtKG concentration was assayed at 340 nm in 1 mL of 0.15 M phosphate buffer (pH 7.5) containing 0.5 mL of aspartate (0.15 M), 0.1 mL of malate dehydrogenase (0.025 U/mL), 0.03 mL of NADH (0.14 M), 2 units of GOT, and 0.01 mL of the reaction mixture containing MeKG or EtKG.

Synthesis of Keto Acids. Ethylsuccinic acid was prepared according to Smith and Horwitz[22] by a two-step procedure from propionaldehyde and ethyl cyanoacetate: yield 60%, mp 99 °C (lit.[22] mp 98–100 °C); ^1H NMR ($CDCl_3$) δ 1.10 (t, 3 H, $CH_2(2'')$), 1.75 (m, 2 H, $CH_2(2')$), 2.55–2.75 (ddd, 2 H, $CH_2(1)$), 2.85 (m, 1 H, CH(2)).

Diethyl Methylsuccinate. A 20-g (0.15 mol) portion of methylsuccinic acid was esterified in 500 mL of EtOH (ethanol) saturated with HCl. The mixture was refluxed for 5 h and then concentrated in vacuo and distilled: yield 20 g (71%); bp 72 °C (1 mmHg); ^1H NMR ($CDCl_3$) δ 1.25 (m, 9 H, $COOCH_2CH_3$ and $CH_3(2)$), 2.40–2.75 (ddd, 2 H, $CH_2(1)$), 2.90 (m, 1 H, CH(2)), 4.15 (m, 4 H, $COOCH_2CH_3$); MS (*m/e*) 188 (M^+), 143, 115, 87, 73.

Diethyl Ethylsuccinate. Ethylsuccinic acid was esterified according to the procedure described above: yield 70%; bp 135 °C (12 mmHg); ^1H NMR ($CDCl_3$) δ 0.75 (t, 3 H, $CH_3(2'')$), 1.15 (m, 6 H, $COOCH_2CH_3$), 1.50 (m, 2 H, $CH_2(2')$), 2.25–2.55 (ddd, 2 H, $CH_2(1)$), 2.60 (m, 1 H, CH(2)), 4.00 (m, 4 H, $COOCH_2CH_3$); MS (*m/e*) 202 (M^+), 157, 129, 115, 87, 73.

4-Methyl-2-ketoglutaric acid (MeKG) was prepared according to Blaise and Gault[20] by condensation of diethyl methylsuccinate with ethyl oxalate followed by acid hydrolysis and decarboxylation of the triester: yield 60%; ^1H NMR (D_2O) δ 1.20 (d, 3 H, $CH_3(4')$), 2.62 (dd, 2 H, $CH_2(3)$), 2.88 (m, 1 H, CH(4)); ^{13}C NMR (D_2O) δ 18.91 (C4'), 38.17 (C3), 39.58 (C4), 178.64–183.44 (C1 and C5), 211.85 (C2).

4-Ethyl-2-ketoglutaric Acid (EtKG). To 10 mL of anhydrous ether, under N_2, were added sodium (0.06 mol), EtOH (0.06 mol), and then ethyl oxalate (0.06 mol) slowly. The mixture was stirred for 2 h at room temperature, and diethyl ethylsuccinate (0.06 mol) was added. After 48 h, the mixture was poured into 3 N H_2SO_4 and extracted four times with ether. The organic layer was washed with a 20% aqueous $KHCO_3$ solution, dried over $MgSO_4$, concentrated, and distilled in vacuo: bp 150 °C (1 mmHg). The triester was hydrolyzed and decarboxylated by heating in 4 N HCl at 100 °C for 6 h. The resulting 4-ethyl-2-ketoglutaric acid was recovered by concentration under reduced pressure: yield 50%; ^1H NMR (D_2O) δ 0.9 (t, 3 H, $CH_3(4'')$), 1.70 (m, 2 H, $CH_2(4')$), 2.40 (t, 2 H, $CH_2(3)$), 2.65 (m, 1 H, CH(4)); ^{13}C NMR (D_2O) δ 13.51 (C4''), 27.61 (C4'), 38.33 (C3), 45.72 (C4), 178.65–182.91 (C1 and C5), 194.13 (C2).

4-Hydroxy-2-ketoglutaric Acid (HOKG). An aqueous solution of the racemate was prepared by condensation of oxalacetic acid with glyoxylic acid in water at pH 7 and at room temperature, according to Ruffo et al.[23]

Synthesis of Amino Acids. 4-Methyl-L-glutamic Acids (1). Synthesis by Pig Heart GOT Catalysis. A 6.25 mmol (1 g) portion of MeKG was incubated in a final volume of 125 mL containing 6.25 mmol of cysteine sulfinic acid (ACS), pyridoxal phosphate (PLP) (5 mM), and 300 units of immobilized GOT. The reaction was monitored by enzymatic determination of residual MeKG. After 24 h, no more keto acid was detectable and the PAN gel containing 90% of the original enzymatic activity was separated by centrifugation. The supernatant was applied to a column of Dowex 50X1 (H^+) and eluted with 0.5 N ammonia to yield 250 mg of 4-methylglutamic acid 1.

4-Methyl-L-glutamic Acids (1a and 1b). Synthesis by Pig Heart GOT Catalysis. First Transamination. A 0.57 mmol portion of MeKG was incubated with 0.6 mmol of ACS, PLP (5 mM), and 100 units of soluble GOT in a final volume of 30 mL. MeKG concentration was monitored by enzymatic assay. The reaction was stopped after 40% conversion (5 h), and the mixture was applied to a 16- × 2-cm column of Dowex 50X1 (H^+). The first acidic eluate was recorded, and the resin was washed with one volume of water; both solutions (solution A) were kept for a second transamination. The column was washed with 200 mL of water, and then the amino acid was eluted with 0.1 N ammonia.

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The product was then purified on a 14- × 2-cm column of Dowex 3 weakly basic (OH^-) eluted with 1 N HCOOH. A total of 25 mg of (2*S*,4*R*)-4-methyl-L-glutamic acid (**1a**) was obtained.

Second Transamination. Solution A which still contained unreacted MeKG was incubated with ACS (10 mM), PLP (5 mM), and 100 units of GOT in a final volume of 75 mL. After 24 h, the reaction mixture was treated with Dowex H^+ and purified on a Dowex OH^- column as described above, and the acidic eluate (Solution B) was reused for a third transamination. A total of 30 mg of a mixture of **1a** and **1b** was obtained.

Third Transamination. Solution B which contained the second keto acid in an enantiomerically pure form was incubated with ACS (20 mM), PLP (5 mM), and 200 units of GOT in a final volume of 110 mL. After 115 h, the mixture was applied to a column of Dowex H^+ and purified on a Dowex OH^- column yielding 15 mg of (2*S*,4*S*)-4-methyl-L-glutamic acid **1b**.

4-Methyl-L-glutamic Acids (1). Synthesis by *E. coli* GOT Catalysis. A 3.1 mmol (0.5 g) portion of MeKG was incubated in a final volume of 150 mL containing 3 mmol of ACS, 2-mercaptoethanol (5 mM), PLP (5 mM), and 3 g of resting cells of *E. coli*. After 45% conversion (12 h), the reaction was stopped and the mixture was centrifuged. The same procedure as described above was used yielding 160 mg of 1.

1a: $[\alpha]^{25}_J$ = +24° (*c* 0.013, 6 N HCl) (lit.[18] $[\alpha]^{25}_J$ +21.4° (*c* 2.6, 6 N HCl)); mp 180 °C (lit.[19] mp 177 °C); ^{1}H NMR (D_2O) δ 1.25 (d, 3 H, CH_3(4′), *J* = 7 Hz), 1.90 (ddd, 1 H, CH_2(3), *J* = 5.5, 8, and 15 Hz), 2.25 (ddd, 1 H, CH_2(3), *J* = 4, 9, and 15 Hz), 2.65 (m, 1 H, CH(4), *J* = 5.5, 7, and 9 Hz), 3.75 (dd, 1 H, CH(2), *J* = 4 and 8 Hz); ^{13}C NMR (D_2O) δ 20.14 (C4′), 36.87 (C3), 39.36 (C4), 56.02 (C2), 178.78–185.03 (C1 and C5); HRMS(FAB) = 162 (M + H^+).

1b: $[\alpha]^{25}_J$ = +30° (*c* 0.013, 6 N, HCl) (lit.[18] $[\alpha]^{25}_J$ +29.2° (*c* 2.6, 6 N HCl)); mp 172 °C (lit.[19] mp 170 °C); ^{1}H NMR (D_2O) δ 1.25 (d, 3 H, CH_3(4′), *J* = 7 Hz), 1.95 (ddd, 1 H, CH_2(3), *J* = 7, 7.5, and 14 Hz), 2.15 (ddd, 1 H, CH_2(3), *J* = 7.5, 8, and 14 Hz), 2.70 (m, 1 H, CH(4), *J* = 7 and 8 Hz), 3.82 (t, 1 H, CH(2), *J* = 7.5 Hz); ^{13}C NMR (D_2O) δ 20.03 (C4′), 37.23 (C3), 39.58 (C4), 55.90 (C2), 178.02–184.96 (C1 and C5). HRMS(FAB) = 162 (M + H^+).

4-Ethyl-L-glutamic Acids (2). Synthesis by Pig Heart GOT Catalysis. A 0.6 mmol (0.1 g) portion of EtKG was incubated in a final volume of 15 mL containing 0.6 mmol of ACS, PLP (5 mM), and 100 units of soluble GOT. EtKG concentration was monitored by enzymatic assay. After 24 h, no more keto acid was detectable, and 35 mg of 4-ethylglutamic acid (**2**) was recovered as described above.

4-Ethyl-L-glutamic Acids (2a and 2b). Synthesis by Pig Heart GOT Catalysis. First Transamination. A 0.84 mmol portion of EtKG was incubated with 0.6 mmol of ACS, PLP (5 mM), and 100 units of soluble GOT in a final volume of 30 mL. EtKG concentration was monitored by enzymatic assay. The reaction was stopped after 40% conversion (8 h), and 50 mg of (2*S*,4*R*)-4-ethyl-L-glutamic acid (**2a**) was obtained as described above for **1a**.

Second Transamination. Solution A which still contained unreacted EtKG was incubated with ACS (8 mM), PLP (5 mM), and 100 units of GOT in a final volume of 60 mL. After 24 h, the reaction mixture was treated with Dowex H^+ and purified on a Dowex OH^- column as described above, and the acidic eluate (solution B) was reused for a third transamination. A total of 30 mg of a mixture of **2a** and **2b** was obtained.

Third Transamination. Solution B which contained the second keto acid in an enantiomerically pure form was incubated with ACS (20 mM), PLP (5 mM), and 200 units of GOT in a final volume of 100 mL. After 96 h, the mixture was applied to a column of Dowex H^+ and purified on a Dowex OH^- column yielding 30 mg of (2*S*,4*S*)-4-ethyl-L-glutamic acid (**2b**).

4-Ethyl-L-glutamic Acids (2). Synthesis by *E. coli* GOT Catalysis. A 3.4 mmol (0.6 g) portion of EtKG was incubated with 3 mmol of ACS, 2-mercaptoethanol (5 mM), PLP (5 mM), and 3 g of resting cells of *E. coli*. After 40% conversion (6 h), the reaction was stopped and the mixture was centrifuged. The same procedure as described above was used, yielding 168 mg of **2**.

2a: $[\alpha]^{25}_J$ = +40° (*c* 0.021, 6 N HCl); mp 179 °C; ^{1}H NMR (D_2O) δ 0.90 (t, CH_3(4″), *J* = 7 Hz), 1.65 (quintet, 2 H, CH_2(4′), *J* = 7 Hz), 1.95 (ddd, 1 H, CH_2(3), *J* = 4, 9, and 14 Hz), 2.25 (ddd, 1 H, CH_2(3), *J* = 4, 10, and 14 Hz), 2.55 (m, 1 H, CH(4), *J* = 4, 7, and 10 Hz), 3.80 (dd, 1 H, CH(2), *J* = 4 and 9 Hz); ^{13}C NMR (D_2O) δ 13.55 (C4″), 28.39 (C4′), 35.04 (C3), 46.47 (C4), 56.25 (C2), 177.81–186.71 (C1 and C5); HRMS(FAB) = 176 (M + H^+).

2b: $[\alpha]^{25}_J$ = +43° (*c* 0.007, 6 N HCl); mp 173 °C; ^{1}H NMR (D_2O) δ 0.90 (t, 3 H, CH_3(4″), *J* = 7 Hz), 1.60 (quintet, 2 H, CH_2(4′), *J* = 7 Hz), 2.00 (t, 2 H, CH_2(3), *J* = 7 Hz), 2.50 (quintet, 1 H, CH(4), *J* = 7 Hz), 3.65 (t, 1 H, CH(2), *J* = 7 Hz); ^{13}C NMR (D_2O) δ 13.71 (C4″), 28.41 (C4′), 36.02 (C3), 48.29 (C4), 56.73 (C2), 177.70–186.82 (C1 and C5); HRMS(FAB) = 176 (M + H^+).