# Synthesis of 4,4-Disubstituted L-Glutamic Acids by Enzymatic Transamination

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**Abstract:** The syntheses of optically pure 4,4-dimethyl-L-glutamic acid as well as (2*S*,4*R*)- and (2S,4S)-4-hydroxy-4-methylglutamic acids have been achieved by transamination of the corresponding 2-oxo-4,4-dimethyl- and *rac*-2-oxo-4-hydroxy-4-methylglutaric acids using glutamic oxalacetic transaminase (GOT).

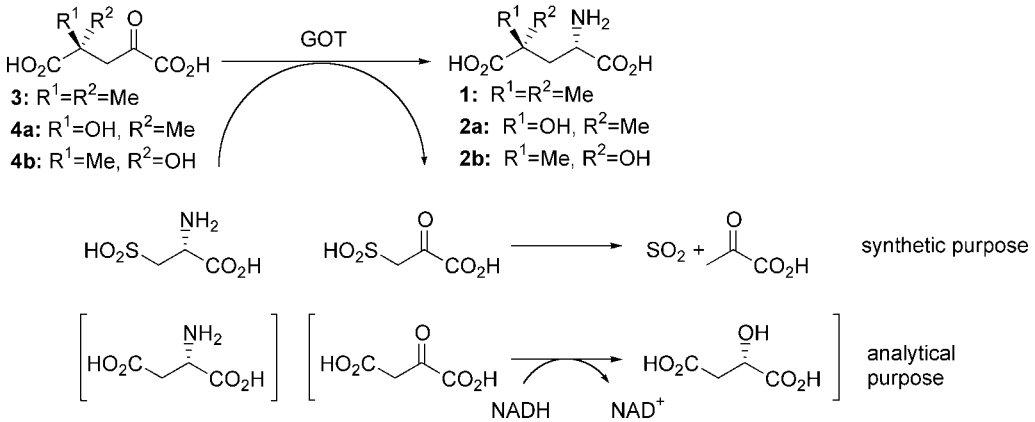
**Keywords:** amino acids; biotransformations; enzyme catalysis; glutamate analogues; transamination; transferases

## Introduction

Both synthetic and non-proteinogenic natural amino acids have been the subject of numerous studies for their enzyme inhibitory and antimetabolite properties. They have also been often incorporated into synthetic peptides.[1] Glutamic acid (Glu) analogues are of special interest because of their interaction with glutamate receptors in the central nervous system.[2] Even small changes in the structure of Glu can strongly influence the binding selectivity towards Glu receptors. For example, (4*S*)-4-methyl-L-glutamic acid interacts specifically with the metabotropic receptor Glu R1 as an agonist, whereas the (4*R*)-isomer is specific for the KA ionotropic receptor.[3] Moreover, since Glu or pyroglutamic acid are important chiral synthons,[4] analogues and specially analogues bearing an additional asymmetric center are of real interest.

For these reasons, numerous studies have been dedicated to the synthesis of natural and non-natural substituted glutamic acids in the last few years.[5] However, very few syntheses of 4,4-disubstituted glutamic acids have been published.[6]

Our interest for enzymes as tools in organic synthesis led us to consider the potential of aminotransferases for Glu analogues syntheses. Aminotransferases catalyze the reversible stereospecific transfer of the amino group from a donor amino acid to an acceptor keto acid (therefore providing a new amino acid and a new keto acid). Since Glu is a substrate of




**Scheme 1.** Glutamic oxaloacetic transaminase (GOT)-catalyzed syntheses of glutamic acid analogues.

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most transaminases, these enzymes appear appropriate for the synthesis of Glu analogues provided that a strategy can be defined to shift the equilibrium by removing the produced α-keto acid.[7]

We have already published the synthesis of 4-alkyl- and 4-hydroxyglutamic acids catalyzed by glutamic oxalacetic transaminase (GOT).[8] In these studies, cysteinesulfinic acid (CSA) was used as the amino acid donor for the transamination. This aspartic acid analogue is a good substrate for GOT and the keto acid produced, spontaneously decomposes into pyruvic acid and $SO_2$ which allows the equilibrium to be shifted (Scheme 1).

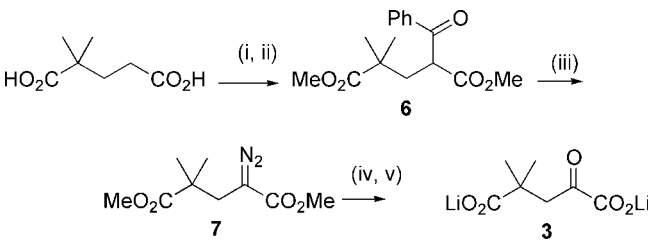
In this paper, we report the first enzymatic synthesis of 4,4-disubstituted L-Glu, namely 4,4-dimethyl- and 4-hydroxy-4-methyl-Glu (**1** and **2**), by transamination of the corresponding 4,4-disubstituted α-ketoglutaric acid, catalyzed by GOT (Scheme 1).

## Results and Discussion

### 4,4-Dimethyl-L-glutamic Acid (1)

To the best of our knowledge, **1** has never been isolated from natural sources. It was obtained by Koskinen and coworkers. as a by-product during the alkylation of protected L-glutamic acid,[6a] and by Bisset et al.[6b] in a protected form.

2-Oxo-4,4-dimethylglutaric acid, the required substrate for transamination, has not been described before. Having already prepared 2-oxo-4-methylglutaric acid by methylation of dimethyl 2,2-dimethoxyglutarate, we tried to achieve the dimethylation by using an excess of alkylating agent. However, even starting from the monomethyl intermediate, the yield of the desired dimethylated product was never higher than 4%. Since the dimethylation of a 2-oxoglutaric acid derivative appeared difficult, we turned our attention to the oxidation of commercial 2,2-dimethylglutaric acid *via* the formation of the α-diazo ester, as suggested by the work of Taber et al.[9] The synthesis was achieved according to Scheme 2.



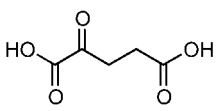
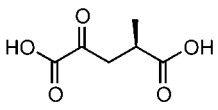
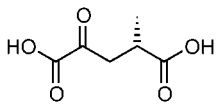
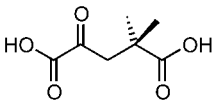
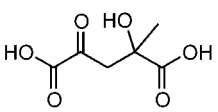
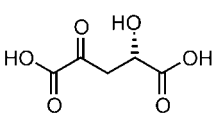

(i) $SOCl_2$ in MeOH;
(ii) $PhCO_2Me$, NaH in DME;
(iii) $p$-$NO_2PhSO_2N_3$, DBU in $CH_2Cl_2$
(iv) MCPBA in $CH_2Cl_2$;
(v) aqueous LiOH.

**Scheme 2.** Synthesis of lithium 2-oxo-4,4-dimethylglutarate

2,2-Dimethylglutaric acid was esterified in methanol in the presence of thionyl chloride to give **5** in 95% yield. Claisen reaction between **5** and methyl benzoate in the presence of NaH gave **6** (65% yield) which, in the presence of DBU, reacted with *p*-nitrobenzenesulfonyl azide[10] providing the α-diazo ester **7** with a nearly quantitative yield. The diazoester was oxidized by *m*-chloroperbenzoic acid,[11] leading to the α-keto diester **8** in 53% yield. Its hydrolysis was carried out in 93% yield by addition of the stoichiometric amount of LiOH in water to give **3**. In spite of the modest yield observed in the oxidation step, the lithium salt **3** was prepared with an overall yield of 31% from commercial 2,2-dimethylglutaric acid.

The activity of GOT for **3** was measured in phosphate buffer (pH 7.5) (Scheme 1) using aspartic acid as the donor amino acid. Oxalacetic acid produced in the transamination was reduced by NADH in the presence of malate dehydrogenase. The decrease in absorbance at 340 nm due to the oxidation of NADH allowed us to monitor the reaction. Compound **3** was found to be a substrate for GOT with a $K_m$ of 15 mM and a $k_{cat}$ of 4% relative to the natural substrate α-ke-

**Table 1.** Activity of glutamic oxaloacetic aminotransferase (GOT) towards α-ketoglutaric acid analogues.

| Entry | Substrate | $K_m$ (mM) | $(k_{cat})_{rel}$ (%) | $(k_{cat}/K_m)_{rel}$ (%) |
|---|---|---|---|---|
| 1 |  | 0.18 | 100 | 100 |
| 2 |  | 0.16 | 122 | 137 |
| 3 |  | 9 | 11 | 0.2 |
| 4 |  | 15 | 4 | 0.05 |
| 5 |  | 18 | 8 | 0.2 |
| 6 |  | 3.8 | 70 | 7 |

toglutaric acid (Table 1). These values can be compared with those observed for the (4*S*)- and (4*R*)-2-oxo-4-methylglutaric acids, respectively. The methyl group in the *R* position does not affect the catalysis by GOT, whereas for the *S*-isomer the affinity for the enzyme site as well as the reactivity are decreased. When both positions are substituted, the kinetic constants are, of course, in the same range as those of the S-isomer.

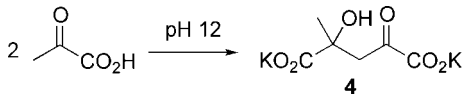
The transamination was carried out on a preparative scale in the presence of an excess of cysteinesulfinic acid (CSA) as the donor amino acid. 4,4-Dimethyl-Glu formed is easily purified by selective absorption on a Dowex $H^+$ resin. Excess of cysteinesulfinic acid is eluted first, and **1**, eluted with 1 N $NH_4OH$ was obtained with a 70% yield and a purity higher than 95% according to NMR spectra and HPLC analysis.

### 4-Hydroxy-4-methyl-L-glutamic Acid (2)

(4*S*)- and (4*R*)-4-hydroxy-4-methyl-L-glutamic acids are natural compounds, mainly occurring in plants. The (4*R*)-isomer is found in *Ledenbergia roseoaena*,[12] the (4*S*)-isomer in *Pandanus veichii*,[13] while both isomers are present in *Phyllitis scolopendrium*.[14] These compounds can be obtained by reduction of properly substituted isoxazolines[15] and we recently published a synthesis of the four stereoisomers (of the D- and L-series) based on this method.[16]

2-Oxo-4-hydroxy-4-methylglutaric acid (**4**) is formed by aldol condensation of pyruvic acid and is often present as impurities in commercial pyruvic acid or sodium pyruvate samples.[17] Recently, both stereoisomers of diethyl 2-oxo-4-hydroxy-4-methylglutarate were synthesized in 70 to 95% enantiomeric excess by catalytic asymmetric homo-aldol reaction of ethyl pyruvate.[18] Racemic **4** has been synthesized by base-catalyzed aldol condensation of pyruvic acid at pH 12 and purified by chromatography on a Sephadex G-15 column. However the yield was not indicated in this report.[17] We repeated this experiment with a few modifications: an aqueous solution of pyruvic acid was maintained at pH 12 for 15 minutes, then the solution was neutralized by addition of Dowex $H^+$ resin, and concentrated to give a solid. The solid was dissolved in a methanol/water mixture and precipitated by addition of diethyl ether, giving racemic potassium 2-oxo-4-hydroxy-4-methylglutarate (**4**) in 64% yield (Scheme 3). Analysis by NMR spectroscopy showed that **4** is about 90% pure. Attempts to purify this compound were unsuccessful, probably due to the easy retro-aldol reaction.

In spite of this low purity, we decided to study the transamination in the presence of GOT in the same conditions as described for 2-oxo-4,4-dimethylglutaric acid. We found a $K_m$ value of 18 mM and a $k_{cat}$ of




**Scheme 3.** Synthesis of potassium 2-oxo-4-hydroxy-4-methylglutarate.

8% relative to the natural substrate. These values can be compared to those of the C-4 monosubstitued oxoglutarates reported in Table 1. We already observed that GOT does not discriminate between the enantiomers of 4-hydroxy-2-oxoglutaric acid since (2*S*,4*R*)- and (2*S*,4*S*)-4-hydroxy-Glu are formed in equal amounts during the synthesis.[8a,8b] The $k_{cat}$ and $K_m$ values reported in entry 6 are for the racemic substrate. On the other hand, GOT prefers the (4*R*)-4-methyl isomer (entry 2), to the (4*S*)-4-methyl (entry 3),[8d] so that one can expect that the position of the hydroxy group is not determinant for activity and that the enantiomer of **4** that is the best substrate will have the methyl group in the same configuration as that in the (4*R*)-4-methyl compound. Therefore, GOT should present some enantioselectivity towards 4-hydro-4-methyl-2-oxoglutaric acid in favor of the (4*S*)-enantiomer.

The measured kinetic constant of the transamination reaction shows that GOT would be an efficient catalyst for synthetic purposes. The reaction was then achieved on a preparative scale with CSA as the donor amino acid. CSA was first added in deficit so that the conversion rate would be under 50%. In the conditions used (0.4 equivalent of CSA), only one isomer was produced. After purification by ion exchange chromatography, **2a** was obtained in 84% yield. When the transamination was carried out with an excess of CSA, the second isomer **2b** appeared, but it never exceeded 20% of the mixture. Careful purification of the mixture by ion exchange chromatography allowed us to obtain a pure sample of **2b.** The configurations of **2a** and **2b** were determined by comparison with $^1H$ NMR spectra of authentic samples previously synthesized by us.[16] The spectra of the two isomers in diluted NaOD solution are quite different, specially the protons at C3 with δ values of 1.74 and 2.31 ppm for the 2*S*,4*S*-configuration (**2a**) and 1.94 and 2.10 ppm for the 2*S*,4*R*-configuration (**2b**). Moreover, having in hands the four possible isomers of **2**, we can confirm the assignment of the configuration 2*S*,4*S* for **2a** and 2*S*,4*R* for **2b** by chiral gas chromatography after derivatization.[19] No traces of the 2*R*-isomers were detected, as expected due to the stereospecificity of the transaminase.

## Conclusion

GOT-catalyzed transamination constitutes a good method to synthesize 4,4-disubstituted L-glutamic

acids, such as 4,4-dimethyl-Glu and (4*S*)-4-hydroxy-4-methyl-Glu. The corresponding ketoglutaric acid analogues are quite easy to prepare. These results confirm that the *proR* hydrogen on position 4 of α-ketoglutaric acid can be substituted by an apolar methyl group without loss of the enzymatic activity, whereas the substitution of the *proS* hydrogen by a methyl or a hydroxy group results in a decrease in both the affinity for the enzymatic site (higher $K_m$) and the transamination rate ($V_{max}$).

## Experimental Section

### General

Chemicals were purchased from Aldrich or Fluka and enzymes are from Sigma. Chromatography were carried out on 220 – 400 mesh silica gel using the 'flash' methodology. Thin-layer chromatography was performed on Merck precoated silica gel 60 $F_{254}$ plates and spots were visualized with vanillin [vanillin (1 g) is dissolved in MeOH (60 mL) and conc. $H_2SO_4$ (0.6 mL)]. $^1$H (400 MHz), $^{13}$C (100 MHz) experiments were performed on a Bruker AC 400 spectrometer. CPV chromatography were performed on an apparatus with a chiral capillary column CHROMPACK (25 m × 0.25 mm) coated with Chirasil-L-valine.

### Dimethyl 2,2-Dimethylglutarate (5)

2,2-Dimethylglutaric acid (25.54 g, 0.16 mol) was dissolved in 150 mL of methanol. Thionyl chloride (25.5 mL, 0.35 mol) was added dropwise under stirring and the solution was refluxed during 2 h. Methanol was partially evaporated under vacuum and ethyl acetate was poured onto the residue. The organic phase was washed with saturated sodium bicarbonate solution until no more carbon dioxide evolved and then with brine. The organic phase was dried over $MgSO_4$ and concentrated under vacuum to give an oil; yield: 28.7 g (96%); $^1$H NMR ($CDCl_3$): δ = 1.18 (s, 6H, 2×$CH_3$), 1.88 (m, 2H, $CH_2$), 2.29 (m, 2H, $CH_2$), 3.67 (s, 6H, 2×$CO_2CH_3$); $^{13}$C NMR ($CDCl_3$): δ = 25.0 (2×2-$CH_3$), 30.1 (C3), 35.2 (C4), 41.8 (C2), 51.8 and 52 (2×$CO_2CH_3$), 173.9 ($C1_1$), 177.8 (C5) ppm.

### Dimethyl 2-Benzoyl-4,4-dimethylglutarate (6)

NaH (60% in mineral oil; 8.51 g, 210 mmol) was washed three times under argon with dry hexane and then suspended in 100 mL of dry 1,2-dimethoxyethane (DME). The mixture was cooled to 0 °C and **5** (10 g, 53 mmol) in 20 mL of DME was added dropwise under argon. The mixture was stirred at 0 °C during 10 min. Methyl benzoate (10 mL, 80 mmol) in 20 mL of dry DME was then added, followed by 10 drops of methanol. The reaction mixture was refluxed overnight. Excess of NaH was destroyed adding 1 N HCl until pH 4. Then the solution was extracted with ethyl acetate, the organic phase was dried over $MgSO_4$ and concentrated under vacuum. Chromatography of the residue eluting with cyclohexane/ethyl acetate (8:2) afforded a yellow liquid; yield: 9.2 g (65%); $^1$H NMR ($CDCl_3$): δ = 1.18 and 1.25 (2×s, 2×3H, 2×$CH_3$), 2.37 (d, 2H, *J* = 5 Hz, $CH_2$), 3.57 and 3.66 (2×s, 2×3H, 2×$CO_2CH_3$), 4.50 (t, 1H, *J* = 5 Hz, CH), 7.49 (dd, 2H, *J* = 9 Hz, 2×CH Ar *meta*), 7.62 (dd, 1H, *J* = 9 Hz, CH Ar *para*), 8.02 (d, 2H, *J* = 9 Hz, 2×CH Ar *ortho*); $^{13}$C NMR ($CDCl_3$): δ = 25.3 and 25.5 (2×4-$CH_3$), 38.6 (C3), 41.8 (C4), 50.6 (C2), 51.9 and 52.7 (2×$CO_2CH_3$), 128.8 (4×CH Ar *ortho* and *meta*), 133.6 (CH Ar *para*), 135.9 (C Ar), 170.3 (C1), 177.4 (C5), 194.8 (2-*C*O); IR (neat): ν = 2952.6, 1731.6, 1688.3, 1448.2, 1293.4, 1138.9, 690.0 $cm^{-1}$.

### Dimethyl 2-Diazo-4,4-dimethylglutarate (7)

To a solution of **6** (244 mg, 0.83 mmol) in 5 mL of dry $CH_2Cl_2$ under argon at 0 °C was added DBU (260 μL, 1.75 mmol) dropwise. The colorless solution turned to yellow. Then *p*-nitrobenzenesulfonyl azide (400 mg, 1.75 mmol) in 4 mL of dry $CH_2Cl_2$ was added and the mixture allowed to warm to room temperature. After stirring during 30 min, 4 mL of phosphate buffer (pH 7, 0.5 N) and then 4 mL of water were added. The solution was extracted with $CH_2Cl_2$, the organic phase dried over $MgSO_4$ and the solvent evaporated under vacuum. Chromatography of the residue eluting with dichloromethane/acetone (97:3) gave a yellow liquid; yield: 177 mg (99%); $^1$H NMR ($CDCl_3$): δ = 1.24 (s, 6H, 2×4-$CH_3$), 2.55 (s, 2H, $CH_2$), 3.68 and 3.76 (2×s, 2×3H, 2×$CO_2CH_3$); $^{13}$C NMR ($CDCl_3$): δ = 24.7 and 24.8 (2×4-$CH_3$), 33.7 (C3), 43.4 (C4), 52.0 (2×$CO_2CH_3$), 177.3 (C1 and C5); MS: *m/z* (relative intensity %) = 186 (31, $M^+ - N_2$), 171 (29), 127 (22), 123 (21), 105 (12), 95 (41), 73 (69), 59 (100, $CO_2Me$), 55 (37), 41 (72), 29 (36), 15 (29); IR (neat): ν = 2953.9, 2085.4, 1735.1, 1696.7, 1453.3, 1310.5, 1144.1 $cm^{-1}$.

### Dimethyl 2-Oxo-4,4-dimethylglutarate (8)

To a solution of **7** (130 mg, 0.61 mmol) in 5 mL of $CH_2Cl_2$ was added in portions *m*-chloroperbenzoic acid (175 mg, 0.61 mmol). After one hour of stirring at room temperature, the solvent was evaporated under vacuum. Pentane was then added to the residue and the *m*-chlorobenzoic acid formed was filtered. The filtrate was washed with diluted bicarbonate solution, dried over $MgSO_4$ and evaporated under reduced pressure. The liquid thus obtained was chromatographed on silica gel (eluent: cyclohexane/ethyl acetate, 9:1) to afford an oil; yield: 65 mg (53%); $^1$H NMR ($CDCl_3$): δ = 1.27 (s, 6H, 2×4-$CH_3$), 3.12 (s, 2H, $CH_2$), 3.65 (s, 3H, 5-$CO_2CH_3$), 3.87 (s, 3H, 1-$CO_2CH_3$); $^{13}$C NMR ($CDCl_3$): δ = 25.6 and 25.7 (2×4-$CH_3$), 40.7 (C4), 48.2 (C3), 52.2 and 53.1 (2×$CO_2CH_3$), 161.2 (C1), 177.0 (C5), 191.8 ($C2_2$); MS: *m/z* (relative intensity %) = 203 (4, $M^+$ + H), 171 (5), 143 (27), 115 (20), 83 (25), 73 (100), 59 (31), 41 (21), 29 (12), 15 (11); IR (neat): ν = 2957.5, 2874.5, 1758.1, 1731.9, 1434.3, 1388.8, 1257.4, 1166.4, 1060.3, 969.3 $cm^{-1}$.

### 2-Oxo-4,4-dimethylglutaric Acid Dilithium Salt (3)

Compound **8** (3.1 g, 15 mmol) was dissolved in 30 mL of water and the solution was cooled to 4 °C. Solid lithium hydroxide (74 mg, 30 mmol) was added in portions while maintaining a pH below 12. Acetone was then poured in until precipitation of the product occurred. The precipitate was filtered, washed with acetone and then with diethyl ether to obtain a white solid; yield: 2.6 g (93%); $^1$H NMR ($D_2O$): δ =

1.22 (s, 6H, 2×4-$CH_3$), 3.07 (s, 2H, $CH_2$); $^{13}$C NMR ($D_2O$): δ = 28.2 (2×4-$CH_3$), 44.0 (C4), 52.2 (C3), 173.3 (C1), 189.1 (C5), 207.4 (C2); IR (neat): ν = 2976.5, 1722.6, 1653.1, 1576.3 $cm^{-1}$.

### 2-Oxo-4-hydroxy-4-methylglutaric Acid Dipotassium Salt (4)

A solution of pyruvic acid (4 g, 45.4 mmol) in 50 mL of water was adjusted to pH 12 with a 6 N KOH solution. After 30 min of stirring, the pH is then adjusted to the neutrality by addition of a strong acidic ion exchange resin (Dowex 50WX8, 100 – 200 mesh) and the resin removed by filtration. The keto acid is dissolved in a hydro-methanolic solution (9:1, methanol/water) and then purified by two precipitations in diethyl ether to give a white solid; yield: 3.7 g (64%), that was used for transamination without further purification; spectroscopic data are in agreement with those reported by Margolis et al.[17]

### General Procedure for the Transamination of α-Keto Acids using Glutamic Oxalacetic Transaminase (GOT)

To a 1 mmol solution of the α-keto acid dilithium salt in 50 mL of distilled water was added 1 mmol of cysteinesulfinic acid monohydrate (CSA) and the pH was adjusted to 7.5 by addition of a 1 M NaOH solution. GOT (50 μL, 100 U) was then added and the mixture was stirred during 24 h at 30 °C. The product was purified through a 20-mL strong acidic resin column (Dowex 50WX8, 16 – 40 mesh, $H^+$ form) and eluted with 40 mL of a 1 M $NH_4OH$ solution. The alkaline fractions were evaporated under reduced pressure and below 40 °C (to avoid corresponding pyroglutamic acid formation) affording the amino acid as a zwitterion. The remaining starting material can be recycled by evaporating the acidic fractions from the resin up to 50 mL, adjusting the pH to 7.5 with a 1 M NaOH solution and starting a new transamination by adding the required quantity of enzyme.

### 4,4-Dimethyl-L-glutamic Acid (1)

The transamination was carried out as described above. Compound **1** was isolated in 70% yield after one recycling. $[\alpha]_{20}^{D}$ : +33.3° (*c*1, 0.1 N HCl); $^1$H NMR ($D_2O$): δ = 1.26 and 1.30 (2×s, 2×3H, 2×$CH_3$), 1.97 (dd, 1H, *J* = 2, 15 Hz, part of $CH_2$), 2.12 (dd, 1H, *J* = 9, 15 Hz, part of $CH_2$), 3.79 (dd, 1H, *J* = 2, 9 Hz, CH); $^{13}$C NMR ($D_2O$): δ = 26.1 and 31.1 (2×4-$CH_3$), 43.8 (C3), 45.9 (C4), 55.1 (C2), 178.5 (C1), 189.3 (C5); IR (neat): ν = 3500 – 2500, 1584.1, 1521.6, 1404.9 $cm^{-1}$; HRMS (electrospray): calcd. for $C_7H_{14}NO_4$ (M + H): 176.0923; found: 176.0923.

### (2*S*,4*S*)-4-Hydroxy-4-methyl-L-glutamic Acid (2a)

The transamination was carried out with only 0.4 equivalents of CSA and **2a** was isolated in 84% yield. $[\alpha]_{20}^{D}$: –3.7° (*c* 0.8, 0.5 N NaOH); HRMS (electrospray): calcd. for $C_6H_{12}NO_5$: 178.0715; found: 178.0717; $^1$H NMR ($D_2O$): δ = 1.50 (s, 3H, $CH_3$), 2.05 (dd, 1H, *J* = 11, 15 Hz, part of $CH_2$), 2.56 (dd, *J* = 2, 15 Hz, part of $CH_2$), 3.71 (dd, 1H, *J* = 2, 11 Hz, CH); $^{13}$C NMR ($D_2O$): δ = 29.5 (4-$CH_3$), 42.1 (C3), 55.2 (C2), 79.1 (C4), 177.0 (C1), 184.0 (C5) ppm; the $^1$H NMR spectral data recorded in NaOD are in agreement with those already published by us.[16] $^1$H NMR (NaOD): δ = 1.49 (s, 3H, $CH_3$), 1.89 (dd, 1H, *J* = 11, 15 Hz, part of $CH_2$), 2.43 (dd, *J* = 2, 15 Hz, part of $CH_2$), 3.55 (dd, 1H, *J* =2, 11 Hz, CH).

### (2*S*,4*R*)-4-Hydroxy-4-methyl-L-glutamic Acid (2b)

The transamination was carried out in the presence of 1.1 equivalent of CSA, and **2a** and **2b** were isolated with an overall yield of 74%. Analysis of the NMR spectrum of the mixture showed that the ratio **2a**/**2b** is around 80:20. Separation of these two diastereoisomers was realized as previously described for a mixture of the four isomers.[20] 300 mg of the **2a**/**2b** mixture were applied to a column (50 cm long and 1 cm diameter) of a strong basic resin (Dowex 2X8, 200 – 400 mesh, acetate form). The column was eluted with 0.5 M acetic acid and fractions of 5 mL collected. **2b** was eluted first in fractions 80 to 90, **2a** appeared in the fractions 91 to 105. The fractions were neutralized with NaOH and the amino acids isolated after the usual work-up. (application to a strong acidic resin and elution with 1 M $NH_4OH$). A pure sample of **2b** was obtained and characterized by comparison with an authentic sample already prepared by us.[16] $[\alpha]_{20}^{D}$: –7.8° (*c* 0.6, 0.5 N NaOH); $^1$H NMR ($D_2O$): δ = 1.51 (s, 3H, $CH_3$), 2.15 (dd, 1H, *J* = 9, 15 Hz, part of $CH_2$), 2.39 (d, *J* = 15 Hz, part of $CH_2$), 3.99 (d, 1H, *J* = 9 Hz, CH); $^1$H NMR (NaOD): δ = 1.50 (s, 3H, $CH_3$), 2.04 (dd, 1H, *J* =8, 15 Hz, part of $CH_2$), 2.23 (dd, *J* =5, 15 Hz, part of $CH_2$), 3.65 (dd, 1H, *J* = 5, 8 Hz, CH) ppm.

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