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Chemo-enzymatic synthesis of the four stereoisomers of 4-hydroxy-4-methylglutamic acid

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Abstract

A chemo-enzymatic synthesis of the four stereoisomers of 4-hydroxy-4-methylglutamic acid is described. As with other glutamate analogues these compounds can serve as probes for the investigation of glutamate receptors.

1. Introduction

Glutamic acid plays a crucial role in the central nervous system. As one of the excitatory amino acids, it is a neurotransmitter for the majority of synapses. Excitatory amino acid receptors appear not only to mediate normal synaptic transmission, but also to participate in the modification of synaptic connections associated with brain development and learning or memory process.[1,2] Moreover, excessive release of glutamic acid can result in neurodegenerative disorder such as Huntington's, Alzheimer's and Parkinson's diseases.[3] Two main types of glutamic receptors, called ionotropic and metabotropic, each with various subgroups, are known.[4,5] Glutamic acid analogues appear as important tools for topology investigation of these receptors and for possible therapeutic effects due to their specificities for a particular receptor. For example, (2*S*,4*S*)-4-methylglutamic acid interacts specifically with the metabotropic glutamic receptor mGluR1, as an agonist of glutamate, whereas (2*S*,4*R*)-4-methylglutamic acid has an exceptional selectivity for the KA ionotropic receptor.[6]

Various substituted glutamic acids have been described recently.[7] We have already published the syntheses of 4-methyl- and 4-hydroxy-L-glutamic acids by enzymatic transamination of the corresponding α-ketoacid.[8] 4-Hydroxy-4-methylglutamic acids have not been synthetised so far, although the isomers of the L series are natural compounds: the (2*S*,4*R*) is found in *Ledenbergia roseo-aena*,[9] the (2*S*,4*S*) in

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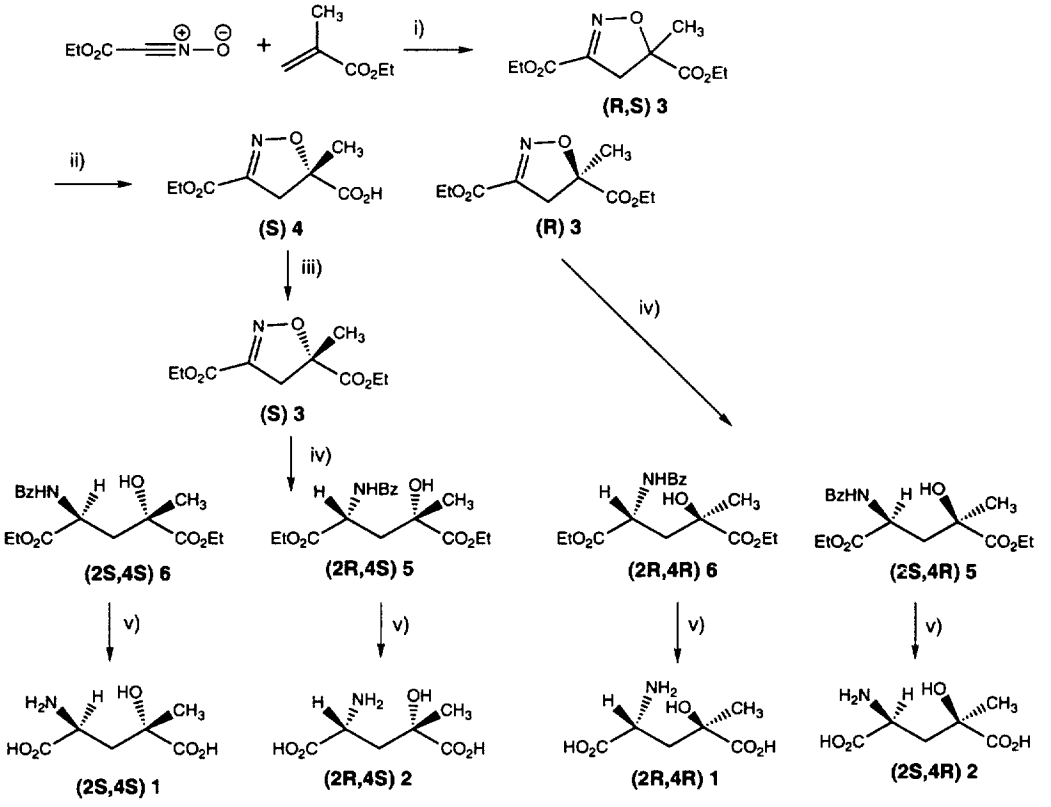
Pandanus veitchii[10] and both isomers in *Phyllitis scolopendrium*[11] and *Caylusea abyssinica*.[12] In this paper, we present the synthesis of the four stereoisomers of 4-hydroxy-4-methylglutamic acid **1** and **2**.

2. Results and discussion

The synthesis was carried out according to Scheme 1 and is based on the resolution of the dihydroisoxazole **3** by *Aspergillus oryzae* protease as described by Griengl et al.[13] Racemic **3** was easily obtained by cycloaddition of methacrylate and ethoxycarbonylnitrile oxide (generated in situ from ethyl chlorooximidoacetate), and then submitted to enzymatic hydrolysis. After 55% conversion, the remaining ester (*R*)-**3** was isolated in 44% yield and more than 97% ee and the hydrolysed compound, isolated in 55% yield and 77% enantiomeric excess (ee), was esterified and the ester re-submitted to the enzyme action. When the hydrolysis reached 80% of conversion, the acid was isolated and esterified to afford (*S*)-**3** in an overall yield of 40% and an enantiomeric excess of 96%.

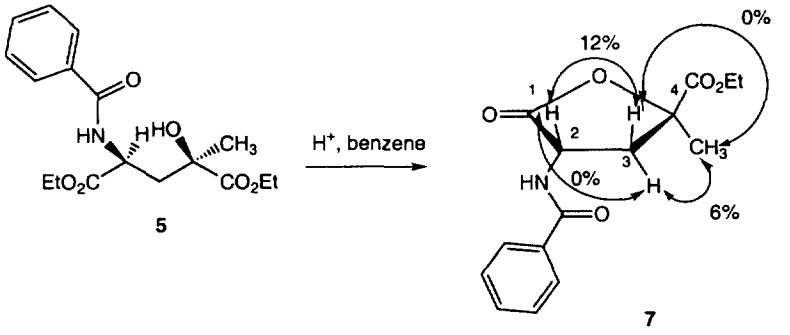


Scheme 1. Synthesis of 4-hydroxy-4-methyl glutamic acids: (i) lit.;[13] (ii) *Aspergillus oryzae* protease; (iii) a — $SOCl_2$, EtOH, Δ; b — *Aspergillus oryzae* protease; c — $SOCl_2$, EtOH, Δ; (iv) H_2/Raney Ni, $(Bz)_2O$; (v) HCl 6 N reflux, then LiOH

(*S*)- and (*R*)-**3** can lead to the desired amino acids as a mixture of separable diastereomers respectively (2*S*,4*S*)/(2*R*,4*S*) and (2*R*,4*R*)/(2*S*,4*R*) by reduction with hydrogen in the presence of Raney nickel. However, during this experiment, lactamisation occurred. This side reaction was avoided by trapping the amino acid as an *N*-acylated form, the reduction being carried out in the presence of a carboxylic anhydride. We first used acetic anhydride in order to obtain *N*-acetyl derivatives separable by the action

of acylase. However, acylase was inoperative on these compounds and chromatographic separation was also inefficient. Finally, the reduction was achieved in the presence of benzoic anhydride. The *N*-benzoyl compounds **5** and **6** were formed and have been separated by chromatography on silica gel.

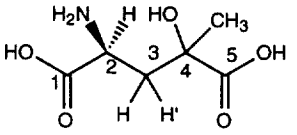
The relative configuration of carbons 2 and 3 was established by careful NMR study. First, since such an attribution is easier on compounds of restricted conformations, the stereoisomer **5** was transformed into the lactone **7** (Scheme 2) by heating to reflux in benzene in the presence of *para*-toluenesulfonic acid and removing the ethanol formed by azeotropic distillation. All nuclei are well differentiated in ^{1}H NMR spectroscopy, with chemical shifts of δ 1.73, 2.29, 3.10, 4.9 and 7.09 ppm respectively for C_4CH_3, C_3H, C_3H', C_2H, and NH. The stereochemical assignment was based on NOE experiments. Presaturation of the CH_3 (d 1.73 ppm) produced 6% enhancement of the signal at δ=2.29 ppm which was attributed to the proton in the *cis* position C_3H and 0% for the signal at 3.10 ppm attributed to the proton in the trans position, C_3H'. Presaturation of C_3H' produced 25% enhancement on the vicinal C_3H, 12% on C_2H and 0% on NH which is consistent with a *trans* configuration of a planar cycle as shown in Scheme 2. However, the close values observed (9 and 12 Hz) for the vicinal coupling constants for HC_2C_3H and HC_2C_3H' seem to indicate a deformation of the cycle which might decrease the difference in the distances between CH_3 and the protons on C_3. In order to examine if an ambiguity could remain at this level, we looked for the more stable conformation by molecular modelling. The dihedral angles $H'C_3C_2H$ and HC_3C_2H appeared to be 21° and 99° respectively, values consistent with the observed vicinal coupling constants. In this conformation, H is closer to the CH_3 group than H′. So, we can attribute the configuration (2*S*,4*R*) and (2*R*,4*S*) to the compounds **5** obtained in good yield by reduction of the enantiomerically pure dihydroisoxazoles **2**. Acidic hydrolysis of **5** or **6** leads to **2** or **1** contaminated by the corresponding lactone as indicated by the characteristic sharp IR band at 1795 cm^{-1}. Thus the mixture was submitted to hydrolysis in basic conditions (LiOH, pH 12). Then, after pH adjustment to 7 by HCl addition, the solution was poured on a Dowex anion exchange resin OH^- form, and the amino acids **1** and **2** were eluted with acetic acid solution and recovered by evaporation.



Scheme 2. Lactonisation of **5** and NOE experiments on **7**

^{1}H and ^{13}C NMR spectra of the four stereoisomers of 4-hydroxy-4-methylglutamic acids are presented in Table 1 and compared with literature data. Obviously there is a misfit between our results and literature for the relative configuration of the two stereogenic centers. Our attribution is based on the specificity of the protease of *Aspergillus oryzae* in the hydrolysis of **3**, which was clearly established by Griengl and coworkers by chemical correlation[13a] and by our NMR study of the lactone **7**. The previous attribution, by Alderweireldt et al.[10] was based on a ^{1}H NMR study of samples from natural sources. The protons in C2 and C3 of the two diastereomers have different coupling constants due to the existence of stabilised conformations. This can allow the attribution of the relative configuration of the

Table 1
^{13}C and ^{1}H NMR chemical shift, δ (ppm) for (2*S*,4*S*) and (2*S*,4*R*) 4-hydroxy-4-methylglutamic acid in 0.5 N NaOD in D_2O



^{13}C	2S,4S Ref.11	2S,4S found	2S,4R Ref.11	2S,4R found	^{1}H	2S,4S Ref.9	2S,4S found	2S,4R Ref.9	2S,4R found
C_1	183.8	183.7	182.5	184.1					
C_2	53.9	55.6	55.3	54.0	C_2-H	3.6 (J = 5.7 and 7.2 Hz)	3.39 (J = 3 and 10 Hz)	3.7 (J = 3 and 10 Hz)	3.47 (J = 5 and 8 Hz)
C_3	45.8	44.4	43.8	45.8	C_3-H C_3-H' Δδ	2.07 2.55 0.12 ($J_{H-H'}$= 14 Hz)	1.74 2.31 0.57 ($J_{H-H'}$= 14 Hz)	2.01 2.5 0.49 ($J_{H-H'}$= 15 Hz)	1.94 2.10 0.16 ($J_{H-H'}$= 14 Hz)
C_4	75.9	78.1	77.9	76.0					
C_5	184.1	184.0	183.7	184.3					
CH_3	26.8	27.5	27.4	27.0	CH_3	1.57	1.43	1.43	1.42

stereogenic centres provided that these conformations are known. In Alderweireldt's study, however, stabilised conformations were proposed without any evidence, on the basis of very simplistic and empirical arguments. A possible mistake in the attribution of configurations was already suspected by Kaas and Sorensen,[14] since the assignment of the configuration of 4-hydroxy-4-isobutylglutamic acid based on the comparison of its ^{1}H NMR spectra with those published for 4-hydroxy-4-methylglutamic acids[10] had to be changed after an X-ray structure determination.

3. Experimental section

3.1. General data

Melting points were determined on a Reichert hot-stage apparatus and are reported uncorrected. IR spectra were recorded on a Perkin–Elmer 801 spectrophotometer. Chromatography was carried out on 220–400 mesh silica gel using the 'flash' methodology. Thin-layer chromatography was obtained on Merck precoated silica gel 60 F_{254} plates and spots were visualised by UV at 254 nm. ^{1}H (400 MHz) and ^{13}C (100 MHz) experiments were performed on a AC 400 Brucker spectrometer. HPLC analyses were recorded on a Waters 660 liquid chromatograph at a flow rate of 0.3 ml/min, equipped with a Waters 440 UV detector (λ=220 nm) and a chiral column (Chiralcel OB, 4.6 mm×250 mm). The molecular modelling study was performed using the SYBYL 6.3 software package[15] on a Silicon Graphics R 8000 workstation. The structure was built within SYBYL and maximised by MAXIMIN 2 with the Tripos force field, in vacuo conditions, to provide reasonable standard geometries. The molecule was deemed to be minimised, by a conjugate gradient method, when a minimum of less than 0.021 kJ/mol for one iteration was reached. Semi-empirical AM1 calculations were performed using the MOPAC package[16] implemented in SYBYL and involved the singlet state. The conformational space of **7** was explored using

the SYBYL search facility. Torsion angles were defined and a grid search was performed allowing chosen bonds to rotate with a 360° or 180° revolution by 15° increments. The lowest energy conformers thus obtained were submitted to AM1 calculations to optimise their geometry.

3.2. *Diethyl 5-(RS)-methyl-4,5-dihydroisoxazole-3,5-dicarboxylate (±)-**3***

K. Faber and H. Griengl procedure[13] provided a 99% yield of **3**.

3.3. *Diethyl 5-(R)- and 5-(S)-methyl-4,5-dihydroisoxazole-3,5-dicarboxylate **3***

By a procedure described by the above mentioned authors, the resolution of **3** with protease from *Aspergillus oryzae* (Sigma type XXIII, 3.6 U/mg), was achieved. (*R*)-**3** was obtained in 44% yield (extent of conversion c=55%, ee>97%, T_R=105.96 min, E=35) and (*S*)-**4** in 55% yield (ee=77%).

This last compound was esterified with $SOCl_2$ in refluxing EtOH in quantitative yield, and after a usual workup was hydrolysed again with the enzyme (c=85%) in order to increase the enantiomeric purity. Thus, another esterification in the same conditions afforded a 43% overall yield of (*S*)-**3** (ee=96%, T_R=119.62 min).

3.4. *General procedure for the synthesis of the diethyl* N-*benzoyl-4-hydroxy-4-methylglutamates **5** and **6***

To a solution of 4.4 mmol of (*R*)-**3** (or (*S*)-**3**) in 80 ml of methanol were added 13.2 mmol (3 equiv.) of benzoic anhydride, 16 ml of phosphate buffer (0.1 N, pH 7) and 0.2 g of Raney nickel. The mixture was then vigourously stirred in a hydrogen atmosphere at room temperature until starting material was no longer revealed on TLC with cyclohexane:ethyl acetate (5:5). After filtration of the catalyst, the solution was evaporated and the residue was dissolved in 50 ml of ethyl acetate, washed twice with a 10% aqueous $NaHCO_3$ solution, dried over $MgSO_4$ and evaporated in vacuo. The diastereomers were separated and purified by chromatography on silica gel using diethyl ether:cyclohexane (6:4) as an eluent.

3.4.1. *Diethyl (2R,4R)-*N-*benzoyl-4-hydroxy-4-methylglutamate **6***

Oil (419 mg, 28%); ^{1}H NMR ($CDCl_3$) δ 1.15, 1.35 (each t, each 3H, each *J*=7 Hz, $2CH_2CH_3$), 1.43 (s, 3H, CH_3), 2.4 (dd, 1H, *J*=5, 14 Hz, CH_2), 2.75 (dd, 1H, *J*=5, 14 Hz, CH_2), 3.53 (s, 1H, OH), 3.83 (m, 1H, CH_2CH_3), 4.07 (m, 1H, CH_2CH_3), 4.25 (q, 2H, *J*=7 Hz, CH_2CH_3), 4.7 (q, 1H, *J*=5 Hz, CH), 7.11 (d, 1H, *J*=5 Hz, NH), 7.45 (t, 2H, *J*=8 Hz, Ar meta), 7.51 (t, 1H, *J*=8 Hz, Ar para), 7.80 (d, 2H, *J*=8 Hz, Ar ortho), ^{13}C NMR ($CDCl_3$) δ 14.1 ($2CH_2CH_3$), 29.7 (4-CH_3), 39.7 (C_3), 50.4 (C_2), 61.5, 62.1 ($2CH_2CH_3$), 73.3 (C_4), 127.0, 128.4, 131.7, 133.3 (6CH Ar), 166.8 (*C*OPh), 172.4, 177.2 ($2CO_2Et$).

3.4.2. *Diethyl (2S,4R)-*N-*benzoyl-4-hydroxy-4-methylglutamate **5***

Pasty solid (614 mg, 41%), ^{1}H NMR ($CDCl_3$) δ 1.15, 1.32 (each t, each 3H, *J*=7 Hz, $2CH_2CH_3$), 1.47 (s, 3H, CH_3), 2.3 (dd, 1H, *J*=5, 14 Hz, CH_2), 2.45 (dd, 1H *J*=12, 14 Hz, CH_2), 3.99 (m, 2H, CH_2CH_3), 4.25 (m, 3H, CH_2CH_3 and OH), 4.97 (m, 1H, CH), 7.05 (d, 1H, *J*=8 Hz, NH), 7.43 (t, 2H, *J*=8 Hz, Ar meta), 7.53 (t, 1H, *J*=8 Hz, Ar para), 7.80 (d, 2H, *J*=8 Hz, Ar ortho), ^{13}C NMR ($CDCl_3$) δ 14.0, 14.1 ($2CH_2CH_3$), 27.3 (4-CH_3), 41.3 (C_3), 49.8 (C_2), 61.9, 62.2 ($2CH_2CH_3$), 73.2 (C_4), 127.2, 128.7, 132.1, 133.4 (6CH Ar), 167.6 (*C*OPh), 172.2, 176.2 ($2CO_2Et$).

3.4.3. Diethyl (2S,4S)-N-benzoyl-4-hydroxy-4-methylglutamate 6

Oil (391 mg, 26%); spectroscopic data were identical to those of the diethyl (2*R*,4*R*)-*N*-benzoyl-4-hydroxy-4-methylglutamate **6**.

3.4.4. Diethyl (2R,4S)-N-benzoyl-4-hydroxy-4-methylglutamate 5

Pasty solid (587 mg, 40%); spectroscopic data were identical to those of the diethyl (2*S*,4*R*)-*N*-benzoyl-4-hydroxy-4-methylglutamate **5**.

3.5. General procedure for the synthesis of the 4-hydroxy-4-methylglutamic acids 1 and 2

The so obtained compounds (1.2–1.8 mmol) were dissolved in 6 N HCl (10 ml) and refluxed overnight. The solution was cooled to room temperature and evaporated under reduced pressure. The residue was then suspended in water (10 ml) and treated with LiOH until the solution maintained a permanently alkaline pH of 12. After neutralisation with HCl 1 N, the solution was passed through a strongly basic ion exchange column (Amberlite IRA-900, OH^- form). The amino acid was eluted with acetic acid 1 M. The solution thus obtained was evaporated under reduced pressure and traces of acetic acid were removed by azeotropic distillation with toluene.

3.5.1. (2R,4R)-4-Hydroxy-4-methylglutamic acid 1

Solid (215 mg, 98%), IR (KBr) ν 3175, 1740, 1715, 1630, 1595, 1550, 1490, 1410, 1340, 1270, 1185 cm^{-1}, ^1H NMR (NaOD) δ 1.43 (s, 3H, CH_3), 1.74 (dd, 1H *J*=10, 14 Hz, CH_2), 2.31 (dd, 1H, *J*=3, 14 Hz, CH_2), 3.39 (dd, 1H, *J*=3, 10 Hz, CH), ^{13}C NMR (NaOD) δ 27.5 (4-CH_3), 44.4 (C_3), 55.6 (C_2), 78.1 (C_4), 183.7 (C_1), 184.0 (C_5).

3.5.2. (2S,4R)-4-Hydroxy-4-methylglutamic acid 2

Solid (312 mg, 97%), IR (KBr) ν 3140, 1695, 1630, 1590, 1525, 1470, 1430, 1410, 1330, 1240, 1140 cm^{-1}, ^1H NMR (NaOD) δ 1.42 (s, 3H, CH_3), 1.94 (dd, 1H, *J*=8, 14 Hz, CH_2), 2.10 (dd, 1H, *J*=5, 14 Hz, CH_2), 3.47 (dd, 1H, *J*=5, 8 Hz, CH), ^{13}C NMR (NaOD) δ 27.0 (4-CH_3), 45.8 (C_3), 54.0 (C_2), 76.0 (C_4), 184.1 (C_1), 184.3 (C_5).

3.5.3. (2S,4S)-4-Hydroxy-4-methylglutamic acid 1

Solid (197 mg, 96%), spectroscopic data were identical to those of the (2*R*,4*R*)-4-hydroxy-4-methylglutamic acid **1**.

3.5.4. (2R,4S)-4-Hydroxy-4-methylglutamic acid 2

Solid (302 mg, 98%), spectroscopic data were identical to those of the (2*S*,4*R*)-4-hydroxy-4-methylglutamic acid **2**.

3.6. Assignment of the absolute configuration on the 2-carbon of the diethyl (2S,4R)-N-benzoyl-4-hydroxy-4-methylglutamate 5

They were achieved by considering the NOE effects on the corresponding lactone.

The diethyl (2*S*,4*R*)-*N*-benzoyl-4-hydroxy-4-methylglutamate **5** (0.3 g, 0.9 mmol) was dissolved in 10 ml of benzene. 17 mg (0.09 mmol, 0.1 equiv.) of *para*-toluenesulfonic acid was added and the mixture was refluxed overnight. The ethanol formed was then removed using a Dean–Stark system. After cooling to room temperature, the solution was transferred to a separatory funnel and washed with saturated

sodium bicarbonate solution. The organic layer was dried on $MgSO_4$ and evaporated in vacuo. Column chromatography on silica gel using cyclohexane:ethyl acetate (6:4) as an eluent gave 0.23 g (89%) of (2*S*,4*R*)-2-benzoylamino-4-ethoxycarbonyl-4-methylbutyrolactone **7** as a white solid (mp=89–90°C). IR ($CHCl_3$) ν 3432, 3002, 1792, 1742, 1668, 1484, 1307, 1140, 1095, 1020 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.34 (t, 3H, *J*=7 Hz, CH_2CH_3), 1.62 (s, 1H, OH), 1.76 (s, 3H, CH_3), 2.26 (t, 1H, *J*=13 Hz, CH_2), 3.17 (dd, 1H, *J*=9, 13 Hz, CH_2), 4.30 (m, 2H, CH_2CH_3), 4.88 (ddd, 1H, *J*=6, 9, 15 Hz, CH), 6.85 (d, 1H, *J*=6 Hz, NH), 7.43 (t, 2H, *J*=8 Hz, Ar meta), 7.52 (t, 1H, *J*=8 Hz, Ar para), 7.78 (d, 2H, *J*=8 Hz, Ar ortho), ^{13}C NMR ($CDCl_3$) δ 14.1 (CH_2CH_3), 23.7 (4-CH_3), 39.9 (C_3), 50.1 (C_2), 62.6 (CH_2CH_3), 82.1 (C_4), 127.2, 128.6, 132.1 (6CH Ar), 167.5 (*C*OPh), 171.1, 175.0 (2*C*O_2Et).

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