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A new access to alkyl-α-ketoglutaric acids, precursors of glutamic acid analogues by enzymatic transamination. Application to the synthesis of (2*S*,4*R*)-4-propyl-glutamic acid

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Abstract

4-Alkyl-2-alkylidene-glutaric acids are easily obtained by a Claisen–Johnson rearrangement, providing a short access to 4-alkyl-α-ketoglutaric acids. These compounds are substrates of the glutamic oxalacetic transaminase (GOT), allowing the enzymatic synthesis of biologically important analogues of L-glutamate. A new analogue, (2*S*,4*R*)-4-propyl-glutamic acid, is described. © 1999 Elsevier Science Ltd. All rights reserved.

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Glutamic acid, one of the most important neurotransmitters in the central nervous system, has been a subject of interest for years, and many modified structures have been described in relation to this biological function.[1a] Among these analogues, 4-alkyl-L-glutamates are of special interest, since it has been shown that (4*R*)-4-methyl-L-glutamate is a potent and selective agonist of the kainate receptor,[1b] while the (4*S*) isomer is an agonist of one of the glutamate metabotropic receptors.[1c] α-Ketoglutaric acid (α-KG) is also a compound of biological importance, associated to glutamate in various steps of the primary metabolism: α-KG is a precursor of glutamate in the glutamate dehydrogenase-catalysed reaction and a product in reactions catalysed by aminotransferases where glutamate is the amino group donor. Moreover, we have already shown that substituted α-ketoglutaric acids can be used as starting materials for the enzymatic synthesis of glutamic acid analogues.[2] However, only a few α-ketoglutaric acid analogues have been synthesised so far.

4-Methyl and 4-ethyl-α-KG have been obtained by condensation of the corresponding alkylsuccinic diester onto diethyloxalate, this reaction leading to a mixture of 3- and 4-alkyl-α-KG.[3] 4-Methyl-α-KG was also prepared from diethyl 3-methyl-3-bromomethyl-oxaloacetate,[4] and by direct alkylation of protected diethyl α-KG.[5] We recently improved this last synthesis including an enzymatic resolution step,[6] but we observed that alkylation is restricted to the introduction of a methyl substituent.

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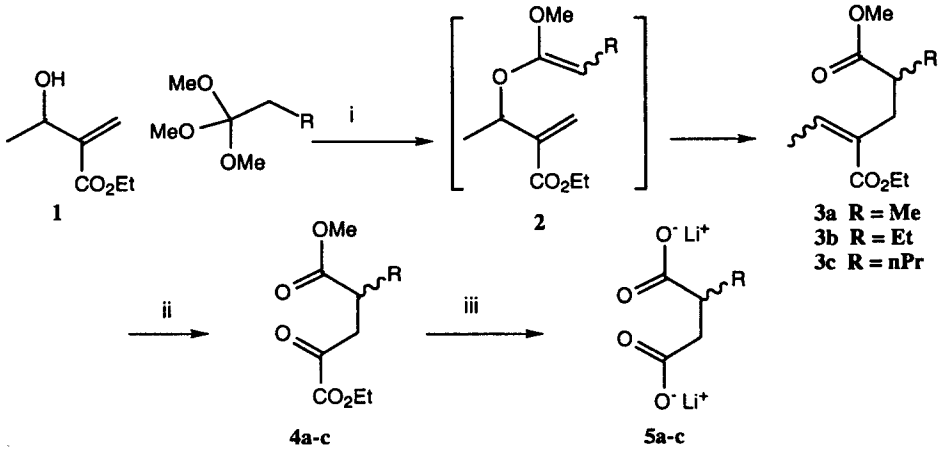
When looking for general ways to synthesise α-ketoacids, potential substrates of transaminases, we found that methylidene or ethylidene groups are good precursors of the keto function through simple ozonolysis. In this paper, we describe an easy access to α-ethylidene-alkyl glutaric acids and their transformation into α-KG analogues. One of them (**5c**) is a new compound, and led by glutamic oxalacetic transaminase (GOT) catalysis to (2*S*,4*R*)-4-propyl-glutamic acid, which had not been previously obtained, although the 4*S* isomer is accessible from the known (2*S*,4*S*)-4-propyl-pyroglutamic acid.[7]

1. Synthesis of 4-alkyl-α-ketoglutaric acids

The synthesis was achieved according to Scheme 1.



Scheme 1. (i) R-CH_2-COOH (0.05 equiv.) 1.5 h reflux in benzene, 70% yield; (ii) O_3, CH_2Cl_2, −78°C, then Me_2S, 80% yield; (iii) aqueous LiOH, pH 10, 100% yield

Compound **1** is easily obtained by the Baylis–Hillman reaction from acetaldehyde and ethyl acrylate.[8] This allylic alcohol can lead to diethyl 2-ethylidene-4-methyl-glutarate **3a** by the Claisen–Johnson rearrangement in the presence of trimethyl propanoic orthoester via the enol ether **2** which is not isolated.[9] We carried out this reaction in a 71% yield and obtained the (*Z*) and (*E*) diastereomers **3a** in the ratio 80:20 according to ^{1}H NMR spectra. In the same way, we obtained **3b** and **3c** in comparable overall yields and stereomeric ratio. Ozonolyses of **3a–c** were performed in methylene chloride at −78°C and the ozonides reduced by addition of dimethylsulphide to afford **4a–c** in 80% yield.[10] Final hydrolysis was carried out in the presence of LiOH at a pH less than 10, then the reaction mixture was neutralised by addition of a cationic exchange resin (H^+) and lyophilised to provide **5a–c** quantitatively.[11]

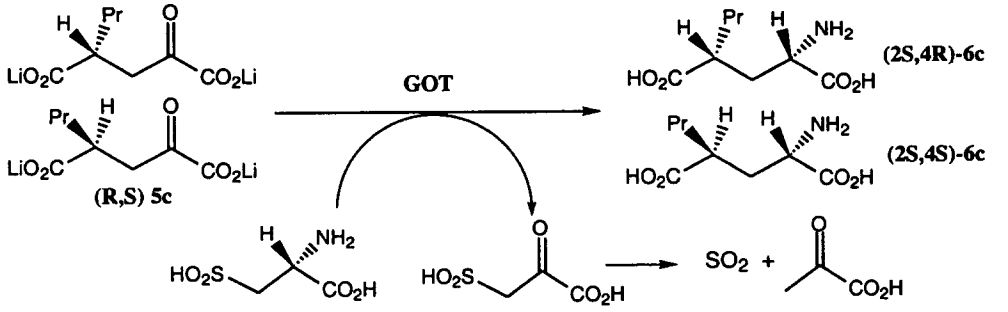
One of the advantages of the Claisen rearrangement is the possible preservation of the chirality resulting from the cyclic structure of the transition state.[12] Starting from enantiomerically pure **1**, it should be possible to have access to **4** in good enantiomeric excess. Such a transfer of chirality from **2** is more often investigated through the allylic system rather than through the vinylic one. Moreover, in our case, the configuration of the vinylic double bond which influences the transfer cannot be controlled. In spite of these unfavourable conditions, but due to the simplicity of our synthesis, we carried out this reaction. Optically pure **1** was easily prepared by enzymatic resolution,[13] and was submitted to the Claisen–Johnson rearrangement in order to obtain **3a**. Enantiomeric excess of (*Z*) and (*E*) **3a** were determined by ^{1}H NMR spectroscopy. Indeed, the signals of the methyl group at C_4 are well differentiated

for the *Z* and *E* isomers and are split in the presence of $Eu(hfc)_3$. The enantiomeric excess of the *Z* isomer is 20%, whereas it is higher than 95% for the *E* isomer. However, this isomer being the minor one, we did not try to isolate it. In this rearrangement, the configuration of the double bond and the configuration of the asymmetric centre created are related. So, each of the starting materials (*Z* or *E*) will lead to *Z* and *E* products of inverse configuration. Therefore, since only three of the four posssible stereomers **3** are formed, one of the precursor (*Z*) or (*E*) **2a** leads by the Claisen rearrangement to enantiomerically pure (*E*) **3a**. This observation encourages us to go on with this study, trying to control the vinylic double bond configuration using another variant of the Claisen rearrangement.

2. Enzymatic transamination

Glutamic oxalacetic transaminase (GOT), which is involved in the biosynthesis of amino acids, catalyses a reversible transamination between glutamate and oxaloacetate leading to α-KG and aspartate. We already showed that GOT accepts as substrates **5a** and **5b**, providing an efficient synthesis of 4-methyl and 4-ethyl-L-glutamic acids.[2] We observed in the present work that, although bearing a more hindered substituent at C_4, 4-propyl-α-ketoglutaric acid **5c** is also a substrate of GOT.

The reaction was carried out according to Scheme 2.



Scheme 2.

In this reaction, cysteine sulphinic acid (ACS), an analogue of aspartic acid, is the amino group donor. The ketoacid produced spontaneously decomposes into SO_2 and pyruvic acid so that the equilibrium is shifted toward the synthesis of the glutamic acid analogue **6c**, easily recovered by ion exchange chromatography.

When the synthesis was carried out in the presence of excess ACS, a mixture of (2*S*,4*R*)- and (2*S*,4*S*)-diastereomers of **6c** were obtained, in a 75:25 ratio and a 68% yield. These compounds were easily identified by analysis of their NMR spectra and comparison with the already known 4-ethyl and 4-methyl-glutamates. The chemical shifts of the C_3 protons are different in the (2*S*,4*R*) isomers and very close in the (2*S*,4*S*), which allowed us to attribute the former configuration to the major isomer produced here. This is in agreement with the already observed enantioselectivity of GOT towards 4-methyl and 4-ethyl-ketoglutarates.

When the reaction was carried out in the presence of only 33% of the stoichiometric amount of ACS, (2*S*,4*R*)-**5c** was obtained in more than 98% purity with a yield of 62%.[14]

In conclusion, we have described a new easy access to 4-alkyl-α-ketoglutarates. This method seems to be applicable to other analogues. The compounds obtained in this work are good substrates of GOT which tolerates hindered substituents in position 4. The enzyme displays a quite satisfactory enantioselectivity

and appears to be a convenient catalyst for the synthesis of diastereomerically pure analogues of L-glutamate.

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10. NMR spectral data for **4c**: ^{1}H NMR (400 MHz, $CDCl_3$) δ=0.91 (3H, CH_3 (propyl)); 1.35 (3H, CH_3 (ester)); 1.52 (2H, CH_2 (propyl)); 1.65 (2H, CH_2 (propyl)); 2.95 (2H, C_3H and C_4H); 3.30 (1H, C_3H); 3.68 (3H, CH_3 (ester)); 4.32 (2H, CH_2 (ester)). ^{13}C NMR (100 MHz, $CDCl_3$) δ=13.78 (CH_3 (propyl)); 20.01 (CH_2 (propyl)); 33.74 (CH_2 (propyl)); 39.46 (CH_3 (ester)); 40.56 (CH_2 (ester)); 51.72 (C_2); 62.38 (C_3); 160.4 (C_5); 175.06 (C_1); 192.55 (C_2).
11. NMR spectral data for **5c**: ^{1}H NMR (400 MHz, D_2O) δ=0.90 (3H, CH_3); 1.35 (2H, CH_2); 1.54 (2H, CH_2); 2.72 (1H, C_4H); 2.90 (1H, C_3H); 3.05 (1H, C_3H). ^{13}C NMR (100 MHz, D_2O) δ=16.08 (CH_3); 22.69 (CH_2); 37.14 (CH_2); 44.95 (C_4); 45.62 (C_3); 172.60 (C_5); 166.83 (C_1); 207.97 (C_2).
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14. Synthesis of **6c**: 70 mL of a solution containing **5c** (300 mg; 1.5 mmol), cysteine sulphinic acid (85 mg; 0.5 mmol), pyridoxal phosphate (0.5 μmol) was adjusted to pH 7. GOT (50 units) was added and the mixture was stirred for 24 hours at room temperature. Then the solution was applied to a Dowex 50X (H^+) resin column (10×2 cm). The resin was washed with water, and the amino acid eluted with 0.1N NH_4OH. The solution was concentrated in vacuo and the product was purified by fixation on anionic ion exchange resin (Dowex3 weakly basic (OH^-)) and elution with 1N HCOOH yielding after lyophilisation 59 mg (62%) of **6c**. F=135–137°C. $[\alpha]_D^{25}$=20.1 (c 0.9, 1N HCl). ^{1}H NMR (400 MHz, D_2O) δ=0.98 (3H, CH_3); 1.40 (2H, CH_2); 1.40 (2H, CH_2); 1.55 (2H, CH_2) 1.55 (1H, CH_2 (propyl)); 1.65 (1H, CH_2 (propyl)); 1.97 (1H, C_4H); 2.20 (1H, C_3H); 2.49 (1H, C_3H); 3.78 (1H, C_2H). ^{13}C NMR (100 MHz, D_2O) δ=15.70 (CH_3); 22.45 (CH_2); 35.57 (CH_2); 36.92 (CH_2); 47.30 (C_4); 55.76 (C_2); 117.06 (C_1); 186.30 (C_5).