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Synthesis of Naturally-Occurring 4-Alkylideneglutamic Acids

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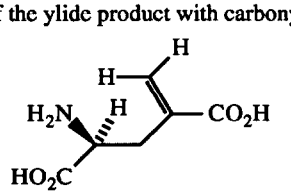
Key words : amino acid; synthesis; alkylideneglutamate; Grignard; natural products

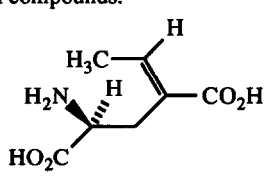
Abstract : The enaminone (**4**), prepared from (2S)-pyroglutamic acid, has been reacted with Grignard reagents to afford a variety of (E)-alkylidene derivatives (**8**). Three of these have been converted to the 4-alkylideneglutamic acids, (**1**), (**2**) and (**3**), which are identical to known natural products, (**2**) and (**3**) being the E-isomers.

In addition to the amino acids found in proteins, nearly one thousand naturally occurring non-proteinogenic amino acids have now been discovered.[1,2] There has recently been enormous interest in these and in unnatural synthetic amino acids[3] for their enzyme inhibitory and antimetabolite properties and their ability to impart protease resistance and unique conformational inducing properties when incorporated into proteins. Substituted glutamic acids are of particular interest because of their interaction with glutamate receptors in the CNS and their involvement in many other biological processes. We now report a method of synthesis of optically pure derivatives of (E)-4-alkylidenepyroglutamate and 4-alkylideneglutamate which is general and which has given access to three naturally-occurring non-proteinogenic amino acids, among other products.

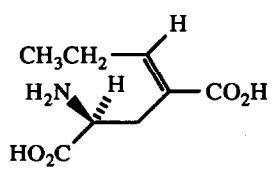
4-Methyleneglutamic acid (**1**) was first isolated as a product of germinated peanuts[4] and was then found in a variety of other plants.[5-13] It has been shown to exhibit strong CNS inhibitory action,[14] and, in peptides, to inhibit the vitamin K mediated γ-carboxylation of glutamic acid, which is important in the blood clotting process.[15] 4-Ethylideneglutamic acid (**2**) is also a widespread amino acid in plants,[8-11,13,16] and 4-propylideneglutamic acid (**3**) has been found in the fungus *Mycena pura*.[9] Synthesis of compounds (**1**) and (**2**) has previously been achieved by reaction of substituted aziridines with stabilised Wittig reagents followed by reaction of the ylide product with carbonyl compounds.[17,18]



(**1**)

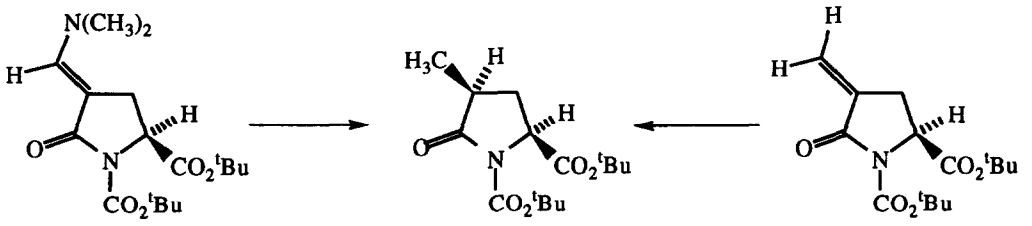


(**2**)



(**3**)

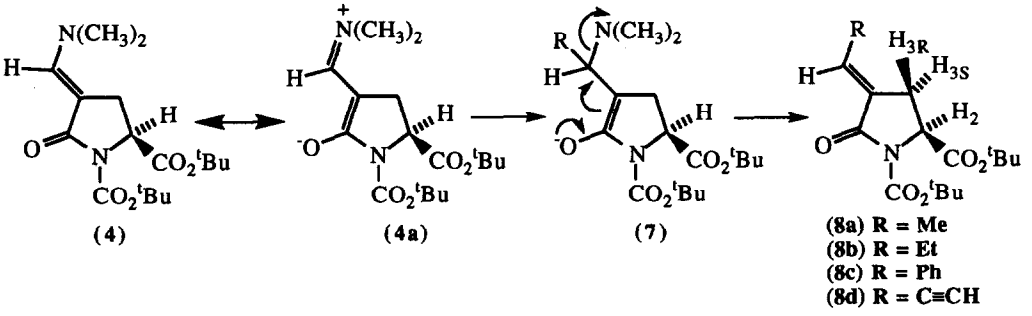
Our interest in the stereospecific synthesis of amino acids[19] led us to consider 4-alkylidenepyroglutamic acids as possible synthetic intermediates since we had shown in our synthesis of stereospecifically deuteriated leucine,[20] that the enaminone (**4**) and the enone (**5**) could be reduced stereospecifically to yield the *cis*-4-methylpyroglutamate derivative (**6**). We therefore reasoned that a general synthesis of 4-alkylidenepyroglutamic acids might allow access to stereospecifically alkylated derivatives of glutamic acid and proline. In developing such a general synthesis we have prepared the three 4-alkylideneglutamic acid natural products (**1**), (**2**) and (**3**).



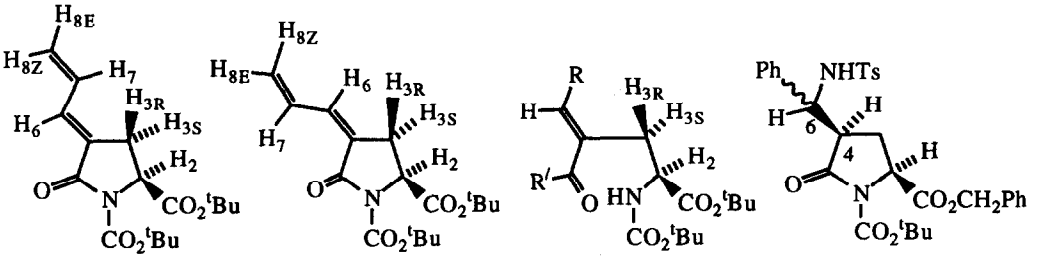
(4) (6) (5)

One approach to 4-alkylidenepyroglutamic acid derivatives (**8**) would be to react the enaminone (**4**) directly with Grignard reagents, since other vinylogous amides have been converted to alkylidene derivatives in this way.[21,22] The danger of 1,2-attack to give ring opened products was real, since reaction of protected pyroglutamates with Grignard reagents had been shown to proceed with ring opening to yield chain elongated amino acids.[23,24] However, should 1,4-attack proceed as with other vinylogous amides,[21,22] the intermediate (**7**) might not undergo elimination until the magnesium salt was quenched and so ring opening would be prevented even if excess Grignard reagent were used.

When the enaminone (**4**)[20] was reacted with 1.1 equivalents of methyl magnesium bromide in Et_2O at -78°C, the (E)-ethylidene derivative (**8a**)† was obtained in 40% yield. Increasing the amount of Grignard reagent to 2.5 equivalents caused the yield to increase to 71%. Only a very small amount of a byproduct, which was identified from its spectroscopic properties as the methyl ketone (**9, R = R′ = Me**), obtained by ring opening, was obtained. In keeping with other work,[23,24] none of the alcohol which would result from attack of the Grignard reagent on the ketone (**9, R = R′ = Me**) was obtained and no reaction with the ester group was observed. The (E)-stereochemistry of the product (**8a**) was assigned on the basis of the observed nuclear Overhauser enhancement to H_{3R} when the C_7 methyl group was irradiated, H_{3R} andH_{3S} being assigned on the basis of the nOe between H_2 and H_{3S}.



(4) (4a) (7) (8a) R = Me; (8b) R = Et; (8c) R = Ph; (8d) R = C≡CH

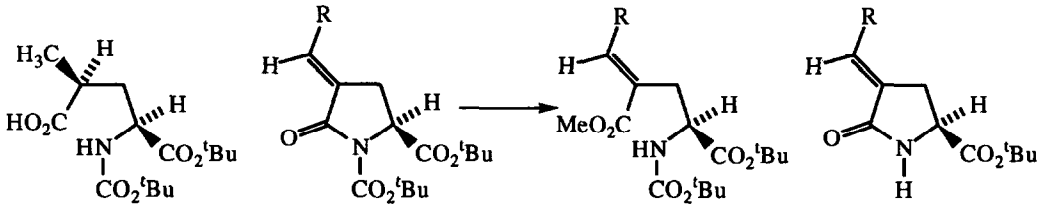


(8e) (8f) (9) (10)

Reaction of the enaminone (**4**) with EtMgBr, PhMgBr and HC≡CMgBr gave the products (**8b**)†, (**8c**)† and (**8d**) in yields of 56%, 78% and 59% respectively. (E)-Stereochemistry could be assigned to (**8b**) by observation of an nOe between the methylene of the ethyl group and H_{3S}, and to (**8c**) by an nOe between the aromatic protons and both H_{3R} and H_{3S}. The stereochemistry of the single isomer of (**8d**) was assigned by analogy, as no useful nOe could be observed. A small amount of the byproduct (**9, R = R′ = Ph**) was obtained in the reaction with phenylmagnesium bromide. When the enaminone (**4**) was treated with vinylmagnesium bromide, the major product, obtained in 47% yield, was evidently the (E)-isomer (**8e**)†, since an nOe was observed between H_7 and both H_{3R} and H_{3S}. There was also a very small amount (1.6%) of the (Z)-isomer (**8f**)† which showed an nOe between H_6 and H_{3R}.

The reaction proved remarkably stereospecific, the (E)-isomer being the preferred product in each case. It was felt that this was not necessarily due to initial attack of the Grignard reagent being stereospecific, since we showed[25,26] in our work on imine addition[19] that base catalysed elimination of both (6R) and (6S) isomers of the adduct (**10**) yielded the benzyl ester analogue of the (E)-benzylidene derivative (**8c**) as the only isomer. Presumably in elimination of dimethylamine from the intermediate (**7**), steric interaction of the alkyl group, R, and the imide carbonyl group must play an important role in determining the stereochemistry of the product.

Although reaction to yield the ring opened ketones (**9**) was not a problem in our synthesis, we were interested in the possibility of preparing these compounds in reasonable yields. The 4-ethylidene derivative (**8a**) was therefore reacted with MeMgBr at room temperature when the ketone (**9, R = R′ = Me**) was obtained in good yield. The fact that no 1,4-addition was observed in this reaction, would suggest that the resonance form (**4a**) is important in achieving the original addition to the enaminone to yield the 4-alkylidenepyroglutamate derivative (**8**). This might therefore suggest that the reaction be regarded as 1,2-attack on the iminium form (**4a**) rather than 1,4-attack on the enaminone form (**4**).



(**11**) (**8**) (**12**) (**13**)

Since 4-methyleneglutamic acid, 4-ethylideneglutamic acid and 4-propylideneglutamic acid were known to be natural products, a synthesis of these compounds required methods of ring opening of the pyroglutamates (**8**). In previous studies, we had found that the saturated pyroglutamate (**6**) could be converted to the acid (**11**) by treatment with aqueous LiOH in THF,[20] but this was not successful with the less electrophilic carbonyl group in the unsaturated compounds (**8**). When (**5**),[20] (**8, R = Me**) and (**8, R = Et**) were reacted with LiOMe in THF at -40 °C, however, good yields of the esters (**12, R = H**),† (**12, R = Me**)† and (**12, R = Et**)† were obtained. In addition to the ring opening reaction of the ethylidene and propylidene compounds, deprotection of the pyroglutamate was observed and (**13, R = Me**) and (**13, R = Et**) were obtained in small amounts as byproducts. The protected esters (**12**) were hydrolysed to the amino acids (**1**), (**2**) and (**3**) using 6N HCl. The acid (**2**) exhibited an nOe at both H_3 protons when the C-7 methyl group was irradiated, and so the double bond had maintained the (E)-stereochemistry in both the ring opening reaction and the deprotection step.

The sample of synthetic 4-methyleneglutamic acid (**1**), $[\alpha]_D$ +13.2° (c 1, 3N HCl), {lit.[13] +13.2° (c 2, 3N HCl)} was identical to a sample obtained from *Lilium maximowiczii* by Kasai.[13,27] The synthetic sample of 4-ethylideneglutamic acid (**2**), $[\alpha]_D$ +27° (c 0.67, 3N HCl) {lit.[13] +29.4° (c 1.4, 6N HCl)} was identical in all respects with samples isolated from *Tulipa gesueriana* by Kasai,[13,27] and from *Mycena pura* by Hatanaka.[9,28]

The sample of 4-propylideneglutamic acid (**3**) obtained synthetically was identical to a sample isolated from *Mycena pura* by Hatanaka,[9,28] and the synthesis defined the double bond geometry as (E).

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27. We thank Prof. T. Kasai, Faculty of Agriculture, Hokkaido University, Japan for his kindness in providing us with a sample of this compound.
28. We thank Prof. S. Hatanaka, Department of Biology, Universty of Tokyo, Japan for his kindness in providing us with a sample of this compound.

† These compounds had the expected analytical and spectroscopic properties and an acceptable specific rotation.

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