Tetrahedron Letters, Vol. 34, No. 34, pp. 5455-5458, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Synthesis of (2S,3S)-[3-$^2H_1$]- and (2S,3R)-[2,3-$^2H_2$]-Proline

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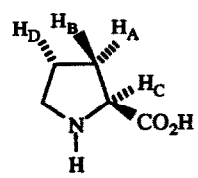
Key Words : proline; amino acid; pyroglutamate; isotopic labelling; bio-organic

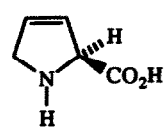
Abstract : Discovery of conditions for intramolecular trapping of a ketene intermediate has led to an effective synthesis of samples of the amino acid proline which are stereospecifically labelled on the β-carbon. Since samples of stereospecifically labelled pyroglutamic acid derivatives are prepared in the route, other stereospecifically labelled, biologically important amino acids may be accessed by the synthesis.

The amino acid proline (**1**) and its analogues are constituents of antibiotics and provide conformational constraint in proteins. Investigation of the stereochemistry of metabolic reactions of proline[1-6] led to early syntheses of stereospecifically labelled samples of proline. Samples labelled in the 4-position were prepared by reduction of tosylates of 4-hydroxyprolines with $LiAl^3H_4$ or $LiAl^2H_4$.[1] For stereospecific labelling at C-3, catalytic tritiation of 3,4-dehydroproline (**2**) yielded (2S,3S,4R)-[3,4-$^3H_2$]-proline (**1**, $H_D = H_A = {}^3H$) [2,3] and catalytic reduction of the protected [3-^{3}H]-2,3-dehydroproline derivative (**3**), followed by deprotection and resolution, gave (2S,3R)-[3-$^3H_1$]-proline (**1**, $H_B = {}^3H$).[5] Recent NMR spectroscopic studies have revealed that the samples of (2S,3S,4R)-[3,4-$^3H_2$]-proline (**1**, $H_D = H_A = {}^3H$) were not, in fact, entirely labelled only in the positions indicated,[7] and it was also known[5] that the sample of (2S,3R)-[3-^{3}H]-proline (**1**, $H_B = {}^3H$) exhibited some scrambling of the label.

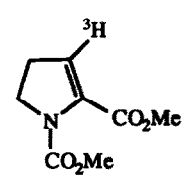
It is evident that an improved synthesis of samples of proline stereospecifically labelled at C-3 would be of considerable benefit to stereochemical studies of the metabolic reactions of proline. We therefore wish to report a simple and effective synthesis of samples of proline which are labelled with deuterium in the 3-*pro-R* and 3-*pro-S* positions respectively. This synthesis has the added advantage of proceeding *via* stereospecifically labelled derivatives (**4**) of pyroglutamic acid. Since we have shown that leucine[8] and other amino acids[9] can be accessed from pyroglutamic acid, the synthesis of a variety of useful stereospecifically labelled amino acids can, therefore, in principle be completed .



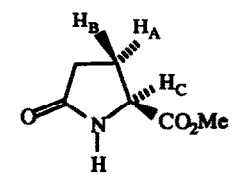
(**1**)



(**2**)



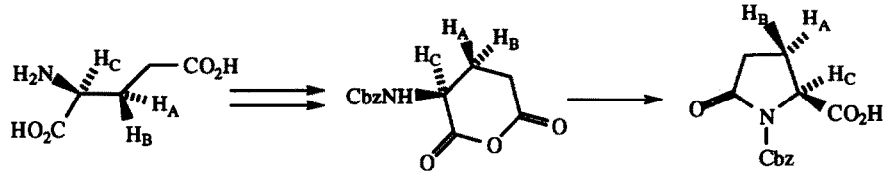
(**3**)



(**4**)

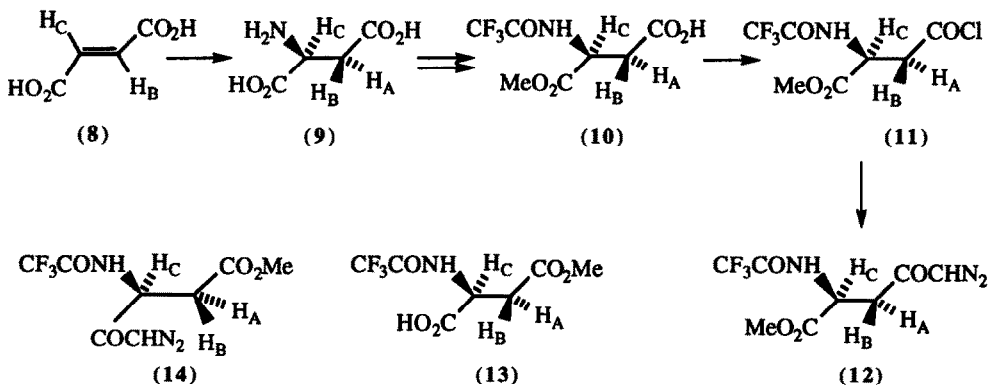
As we had already completed a synthesis of stereospecifically labelled samples of glutamic acid,[10] cyclisation of these seemed to provide a useful route to stereospecifically labelled samples of pyroglutamic acid

and thence of proline. We therefore used the method of Gibian and Klieger[11] to convert our samples of glutamic acid to the pyroglutamates (**7**) *via* the anhydride (**6**).[12] The ^{1}H-NMR spectra of these compounds indicated that, although the labels at C-3 were intact in the products, the procedure was accompanied by a small amount of epimerisation at C-2 in the rearrangement of the anhydride (**6**) to the acid (**7**). The synthesis was also lengthy. At this point, reinvestigation of our original synthesis of glutamic acid in connection with studies on the biosynthesis of carbapenam antibiotics, suggested a more direct solution to the problem.



(**5**) (**6**) (**7**)

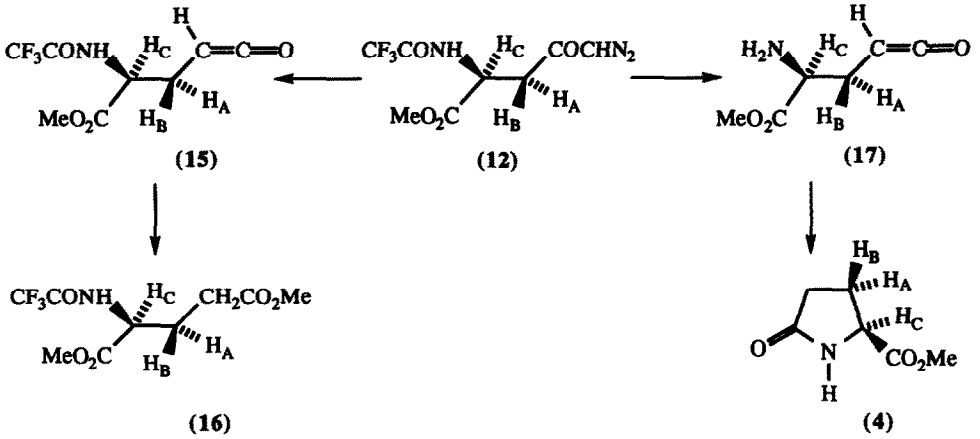
In connection with metabolic studies, we have devised general syntheses of stereospecifically labelled L- and D-amino acids. As the first step in the synthesis of the L-amino acids[10,13,14] and some β-amino acids,[15,16] we used the well known[6,17] *anti*- addition of ammonia to the double bond of fumaric acid (**8**) to obtain samples of (2S,3R)-[3-^{2}H$_1$]- and (2S,3S)-[2,3-^{2}H$_2$]-aspartic acids, (**9**, $\mathbf{H_A} = {}^2\mathbf{H}$) and (**9**, $\mathbf{H_B} = \mathbf{H_C} = {}^2\mathbf{H}$) respectively. Yields were of the order of 25 - 50% from this reaction and it was not always easy to obtain the product free of inorganic salts. Subsequent to our work, Woodard[18] reported that *E. coli* immobilised on polyacrylamide gel could be used to effect this synthesis, and we have obtained the labelled aspartates (**9**, $\mathbf{H_A} = {}^2\mathbf{H}$) and (**9**, $\mathbf{H_B} = \mathbf{H_C} = {}^2\mathbf{H}$) in excellent yield and purity using this method.[19] The samples of aspartic acid were converted to the diazoketones (**12**) by the route outlined in the scheme below. Since reaction of the aspartates (**9**) with trifluoroacetic anhydride, followed by methanol treatment of the resultant protected anhydride was not entirely regiospecific, giving *ca.* 80 % of the esters (**10**), and 20 % of the isomers (**13**), we originally purified the compounds in our synthesis by recrystallisation of the acid chlorides (**11**) from benzene. We have now found it more convenient to carry the synthesis through on mixtures of regioisomers and to separate the diazoketones (**12**) from their regioisomers (**14**) by recrystallisation from dichloromethane and petroleum ether.



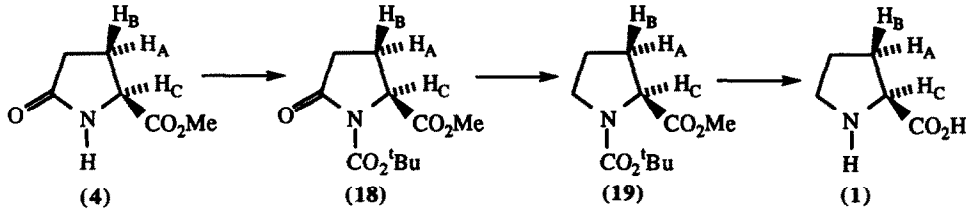
(**8**) (**9**) (**10**) (**11**)

(**14**) (**13**) (**12**)

Photolysis of the diazoketones (**12**) in redistilled methanol gave the desired protected dimethyl glutamates (**16**) and we have shown[10] that this reaction proceeds with retention of stereochemistry at the migrating primary chiral centre in the Wolff rearrangement. However, when photolysis of the unlabelled diazoketone was carried out in methanol which had previously been dried by distillation from magnesium methoxide or calcium hydride, a new product was often obtained. This was identified as methyl pyroglutamate (**4**) by spectroscopic comparison with an authentic sample. When the reaction was repeated using the labelled diazoketones, stereospecifically labelled samples of methyl pyroglutamate were obtained with no loss of chirality.



The most reasonable explanation for the new course of the reaction was that the solution had become slightly alkaline leading to hydrolysis of the trifluoroacetate. The resultant intermediate (**17**) would then undergo intramolecular nucleophilic addition of the amine to the ketene function, yielding the pyroglutamate (**4**). In neutral conditions the unhydrolysed trifluoroacetamide moiety would not be nucleophilic enough to compete with methanol for the ketene in the intermediate (**15**) and so the protected dimethyl glutamate (**16**) would be formed. To test this hypothesis, the diazoketone (**12**) was hydrolysed to the corresponding free amino acid by adaptation of the method of Weygand[20] and DeWald[21] using 1N aqueous NaOH in methanol. Photolysis of the crude amino acid diazoketone in methanol gave (2S)-pyroglutamic acid. Unfortunately when this sequence of reactions was conducted using the stereospecifically labelled samples of diazoketone (**12**), the 3R and 3S deuterium labels (but not the C-2 label) were exchanged. Photolysis in methanol containing sodium methoxide caused exchange of labels both at C-2 and at C-3. Eventually conditions were found which reliably gave the stereospecifically labelled methyl pyroglutamates (**4**). Photolysis in methanol containing 1 equivalent of 6% w/v aqueous sodium bicarbonate gave the labelled esters (**4**, $H_A = {}^2H$) and (**4**, $H_B = H_C = {}^2H$) in good crude yield. These were converted to the corresponding *tert*-butoxycarbonyl protected derivatives (**18**, $H_A = {}^2H$) and (**18**, $H_B = H_C = {}^2H$) by reaction with di-*tert*-butyldicarbonate and DMAP in acetonitrile and the products were purified by flash chromatography on silica gel.



There are several methods in the literature for converting pyroglutamic acid derivatives to proline. We were unsuccessful in reducing pyroglutamic acid itself using the method of Montiero,[22] but reduction of the methyl *tert*-butoxycarbonylpyroglutamates (**18**) was achieved in good yield using borane dimethyl sulphide. The ^1H NMR spectra of the products (**19**) were complicated by the well known[23] conformational isomerism found in N-acylproline derivatives but, when the products were deprotected using refluxing 6N HCl, good yields of the labelled prolines (**1**) were obtained. The ^1H NMR spectra of these compounds, shown in the Figure, indicated stereospecific labelling, and so synthesis of (2S,3S)-[3-$^2H_1$]-proline (**1**, $H_A = {}^2H$) and (2S,3R)-[2,3-$^2H_2$]-proline (**1**, $H_B = H_C = {}^2H$) had been achieved.

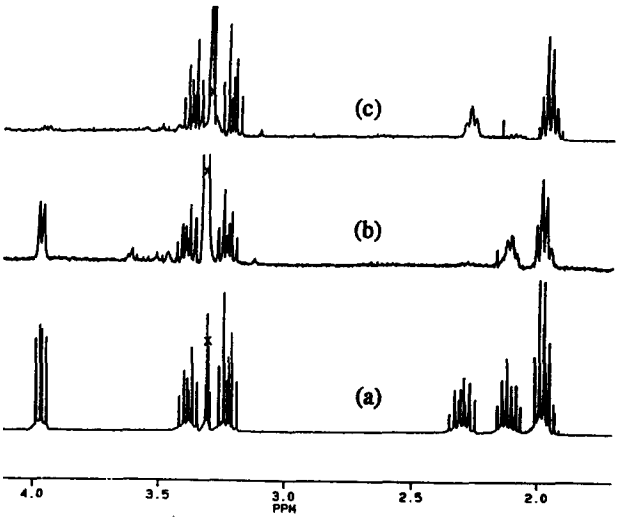


Figure 360 MHz ^1H NMR Spectrum in $C^2H_3O^2H$ of (a) (2S)-proline (**4**) (b) (2S,3S)-[3-$^2H_1$]-proline (**4**, $H_A = {}^2H$); and (c) (2S,3R)-[2,3-$^2H_2$]-proline (**4**, $H_B = H_C = {}^2H$)

Acknowledgements We thank the S.E.R.C. for a studentship (to P.D.).

References and Notes

1. Fujita, Y.; Gottlieb, A.; Peterkofsky, B.; Udenfriend, S. and Witkop, B., *J. Am. Chem. Soc.*, 1964, **86**, 4709 - 4716.
2. Ebert, P.S. and Prockop, D.J.; *Biochem. Biophys. Res. Commun.*, 1962, **8**, 305 - 309.
3. Prockop, D.J.; Ebert, P.S. and Shapiro, B.M., *Arch. Biochem. Biophys.*, 1964, **106**, 112 - 122.
4. Salzman, L.A.; Weissbach, H. and Katz, E.; *Proc. Natl. Acad. Sci., U.S.A.*, 1965, **54**, 542 - 546.
5. Purvis, M.B.; LeFevre, J.W.; Jones, V.L.; Kingston, D.G.I.; Biot, A.M. and Gosselé, F., *J. Am. Chem. Soc.*, 1989, **111**, 5931 - 5935.
6. Young, D.W. *Topics in Stereochemistry*, 1993, **21**, *in press.*
7. Altman, L.J. and Silberman, N., *Anal. Biochem.*, 1977, **79**, 302 - 309.
8. August, R.A.; Khan, J.A.; Moody, C.M. and Young, D.W., *Tetrahedron Letters*, 1992, **33**, 4617 - 4620.
9. Moody, C.M. and Young, D.W., *Tetrahedron Letters*, 1993, **34**, 4667 - 4670.
10. Field, S.J. and Young, D.W., *J. Chem. Soc., Perkin Trans. 1*, 1983, 2387 - 2392.
11. Gibian, M. and Klieger, E., *Annalen der Chemie*, 1961, **640**, 145 - 156.
12. August, R.A., *D. Phil. Thesis.*, Sussex, 1987.
13. Gani, D. and Young, D.W., *J. Chem. Soc., Perkin Trans. 1*, 1983, 2393 - 2398.
14. Gani, D.; Young, D.W.; Carr, D.M.; Poyser, J.P. and Sadler, I.H., *J. Chem. Soc., Perkin Trans. 1*, 1983, 2811 - 2814.
15. Gani, D. and Young, D.W., *J. Chem. Soc., Perkin Trans. 1*, 1985, 1355 - 1362.
16 Gani, D.; Hitchcock, P.B. and Young, D.W., *J. Chem. Soc., Perkin Trans. 1*, 1985, 1363 - 1372.
17. Young, D.W. in *Isotopes in the Physical and Biomedical Sciences*, vol. 1, *Labelled Compounds (Part B)*, eds. Buncel, E. and Jones, J.R., Elsevier, Amsterdam, 1991, pp 341 - 427.
18. Lee, K.M.; Ramalingam, K.; Son, J.K. and Woodard, R.W., *J. Org. Chem.*, 1989, **54**, 3195 - 3198.
19. *E. coli* strain B (ATCC 11303) was grown for us by Dr. R. Eady and colleagues of the AFRC Nitrogen Fixation Laboratory, University of Sussex.
20. Weygand, F.; Bestmann, H.J. and Klieger, E., *Chem. Ber.*, 1958, **91**, 1037 - 1040.
21. DeWald, H.A. and Moore, A.M., *J. Am. Chem. Soc.*, 1958, **80**, 3941 - 3945.
22. Montiero, H.J., *Synthesis*, 1974, 137.
23. Hondrelis, J.; Lonergan, G.; Voliotis, S. and Matsoukas, J., *Tetrahedron*, 1990, 46, 565 - 576.

(Received in UK 1 June 1993; accepted 25 June 1993)