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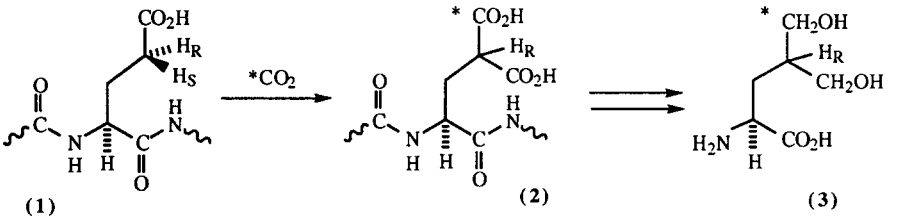
Two Independent Syntheses of (2S,4S)- and (2S,4R)-[5,5-$^2H_2$]-5,5′-Dihydroxyleucine

Xavier Durand, Piétrick Hudhomme, Jeffrey A. Khan and Douglas W. Young*

School of Chemistry and Molecular Sciences, University of Sussex, Falmer, Brighton, BN1 9QJ, U.K.

Abstract : Two independent stereoselective syntheses have been used to prepare samples of (2S,4S)-[5,5-$^2H_2$]-5,5′-dihydroxyleucine **(3b)** and the (2S,4R)-diastereoisomer **(3a)**. The first synthesis uses (2S)-pyroglutamic acid as starting material and provides the (2S,4S)-isomer **(3b)**, while the second starts with (2S,4R)-4-hydroxyproline and provides the (2S,4R)-isomer **(3a)**.

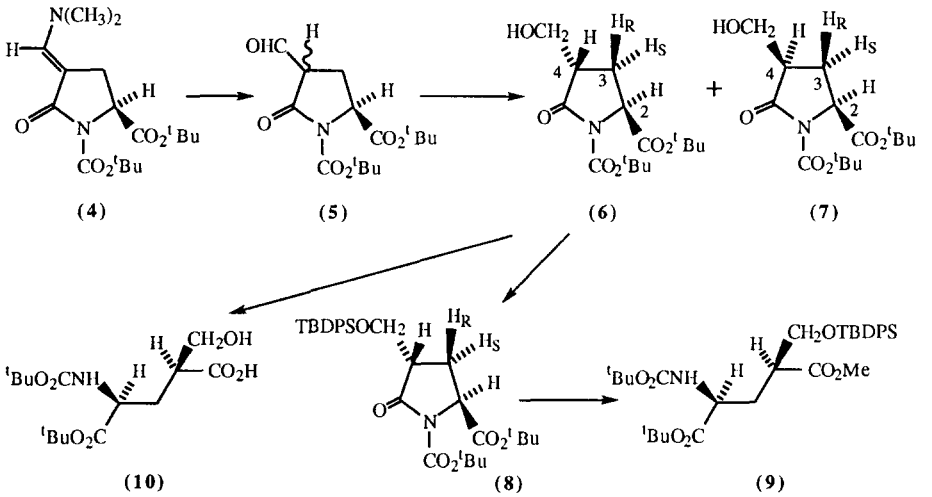
The unusual amino acid γ-carboxyglutamic acid was discovered in 1974 in normal prothrombin[1,2] and was later discovered to be present in other proteins of the blood clotting cascade. Residues of this amino acid occur at the amino terminal end of these proteins and arise through post-translational modification of glutamate residues in precursor proteins as shown in Scheme 1. The enzyme responsible for the γ-carboxylation of glutamate residues is unusual, being linked to oxidation of vitamin K hydroquinone to vitamin K epoxide,[3] and inhibitors are potential anti-thrombotic compounds. The mechanism of the reaction has excited much speculation[4] and elucidation of the stereochemistry of the process is of interest in mechanistic studies and in the design of inhibitors. Marquet, Azerad *et al.* have shown[5-8] that the hydrogen, 4-H_S, is abstracted in the carboxylation process and, more recently, using a pentapeptide containing (4S)-4-fluoroglutamate[9] they have implied that the *4-pro-R* carboxyl group in the non-fluorinated product is derived from CO_2, suggesting inversion of stereochemistry in the enzyme catalysed reaction. In our own studies in this area, we noted that reduction of peptide bound γ-carboxyglutamate with diborane followed by hydrolysis had been reported[10] to yield 5,5′-dihydroxyleucine **(3)** so that, if ^{13}C-labelled carbon dioxide were used in the enzyme catalysed reaction, we might obtain a sample of **(3)** labelled in one of the diastereotopic CH_2OH groups. Thus synthesis of a sample of this compound labelled in an unambiguously defined manner and comparison of the ^{13}C-NMR spectra would define the stereochemistry of the enzymic reaction. Since deuterium labelling will define the resonances in the ^{13}C NMR spectrum, we opted to synthesise samples of 5,5′-dihydroxyleucine **(3)** labelled with deuterium in one of the diastereotopic CH_2OH groups so that the synthesis would define the chirality of the labelled compound.



Scheme 1

For our first synthesis of stereospecifically labelled dihydroxyleucine **(3)**, we chose the enaminone **(4)** as the starting point. This had been useful in our synthesis of stereospecifically labelled leucine,[11] various non-proteinogenic amino acids[12-14] and glutamate antagonists.[15] We hydrolysed this compound at pH 4.5 in methanolic HCl until the UV spectrum indicated that the chromophore due to the enaminone at λ_{max} 313 nm had been replaced by that due to the enolate of aldehyde **(5)** at λ_{max} 257 nm. We then added $NaB(CN)H_3$, keeping the pH constant at 4.5, until this chromophore was no longer present in the UV spectrum. The reaction had

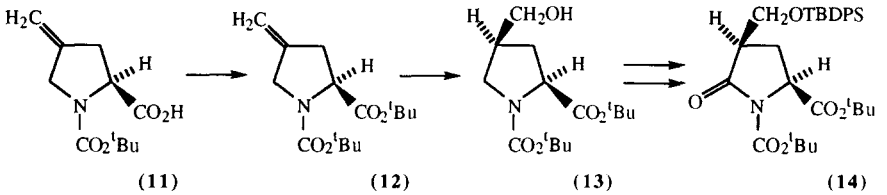
proceeded stereoselectively due to the *trans*- isomer of the intermediate aldehyde (**5**) being the thermodynamically more stable diastereoisomer, and the product was a mixture of the *trans*- and *cis*- alcohols (**6**)† and (**7**)† respectively in a ratio of 5 : 2.[16] The stereochemistry of the products was confirmed by NMR spectroscopic experiments, the *cis* isomer (**7**) showing nOe of *both* H-2 *and* H-4 with H-3$_S$; and the *trans* isomer (**6**) showing separate enhancements between H-4 and H-3$_R$ and H-2 and H-3$_S$. Although a good yield of the mixture was obtained, separation of the isomers in useful amounts proved an obstacle to progress at this stage. We were, however, able to obtain sufficient quantities of the *trans*- isomer (**6**) to investigate further steps in the synthesis. On preparation of the TBDPS derivative (**8**),† subsequent ring opening using LiOH in tetrahydrofuran proved difficult and, although ring opening to the methyl ester (**9**)† was possible using Et_3N/MeOH, it was evident from the ^{1}H NMR spectrum that *ca.* 20% epimerisation had occurred. Use of MeO^{2}H in this reaction gave a product with a ^{2}H-NMR spectrum which indicated deuteriation at C-4 but not at C-2. The unprotected alcohol (**6**) could be ring opened in good yield to give the single diastereoisomer (**10**)† using LiOH/THF but attempted reduction of the carboxyl group to a deuteriated alcohol (**3**) was unsuccessful.



Scheme 2

Although the synthesis outlined in Scheme 2 above was stereoselective, the problems encountered encouraged us to seek an alternative route. We were encouraged by an early report[17,18] that hydroboronation of 4-methyleneproline derivatives with disiamylborane followed by oxidation with aqueous hydrogen peroxide gave *cis*-4-hydroxymethylproline derivatives in good yield. N-*tert*-Butoxycarbonyl-4-methylene(2S)proline (**11**) was therefore prepared by the method of Herdewijn *et al.*[19] from (2S,4R)-4-hydroxyproline *via* the protected 4-ketone and Wittig reaction. This was converted to the *tert*-butyl ester (**12**)† in 84% yield using (Boc)$_2$O, DMAP and triethylamine. Reaction with disiamylborane followed by oxidation using aqueous hydrogen peroxide gave the protected alcohol (**13**).† The ^{1}H NMR spectrum was complicated by the well known[20] rotational isomerism of proline amides and urethanes and so an estimate of the stereospecificity of the reaction could not be made at this stage. To proceed to ring opened compounds, it was necessary to convert the proline derivative to a pyroglutamic acid derivative. This had been achieved for simpler compounds using ruthenium tetroxide[21] and we decided to protect the alcohol as the TBDPS derivative before attempting this step. Protection was achieved in 79% yield using TBDPSCl and imidazole in DMF. The protected compound was reacted with RuO_2/$NaIO_4$ to yield the pyroglutamic acid derivative (**14**)† in 40% yield together with a 20% yield of a product in which the protecting group had been oxidised to ^{t}Bu(Ph)Si(OH)O-. The ^{1}H-NMR spectra of the pyroglutamic acid derivatives were no longer complicated and we were able to show that the hydroboronation step had given a mixture containing *ca.* 80% of the *cis*-isomer (**13**) and *ca.* 20% of the corresponding *trans*-isomer. The stereochemistry of the products was confirmed by nOe studies in the ^{1}H NMR spectra of the products (**14**) and (**8**). Interestingly, when the

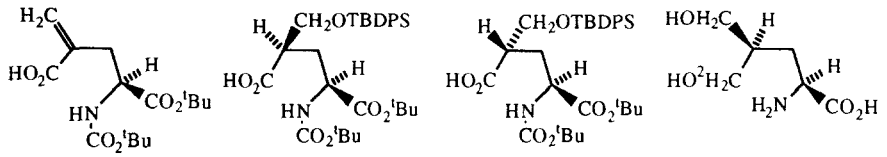
hydroboronation step was accomplished using $BH_3.Me_2S$, the ratio of the isomers **(14)** : **(8)** in the final product was *ca.* 3 : 2. Most usefully, we now found that the TBDPS derivatives **(14)** and **(8)**, unlike the corresponding alcohols, could be separated chromatographically in excellent yield. We were, therefore, able to obtain the mixed TBDPS ethers from the two different syntheses and separate them in synthetically useful quantities.



(11) **(12)** **(13)** **(14)**

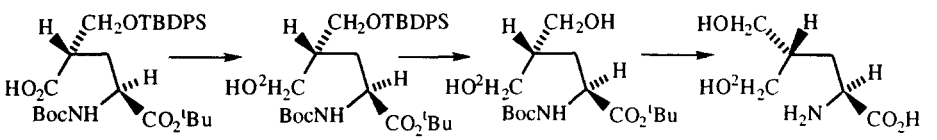
Scheme 3

We now had two useful syntheses, one yielding principally the *trans* compound **(8)** and the other yielding principally the *cis* compound **(14)**. Ring opening and further elaboration should thus allow us to complete the synthesis of the separate samples of (2S, 4S)- and (2S,4R)-[5,5-$^2H_2$]-5,5′-dihydroxyleucine to assign the ^{13}C-NMR spectrum. However when the ether **(14)** was reacted with LiOH in THF or CH_3CN, although the desired product **(16)** was obtained, the yield was less than 20%. Unexpectedly the main product was the olefin **(15)** from elimination of the TBDPS ether. We evidently needed to alter the ratio of basicity to nucleophilicity in our reagent for the ring opening reaction, and so we used lithium hydroperoxide in aqueous THF. With the *trans*-lactam **(8)** this gave a clean product **(17)**† in 65% yield.



(15) **(16)** **(17)** **(3a)**

To prepare our target labelled samples of dihydroxyleucine, we now proceeded as outlined in Scheme 4. The acid **(17)** was first converted to the mixed anhydride with *iso*butyl chloroformate and this was reduced with NaB^2H_4 in ^2H_2O to give the labelled alcohol **(18)**† in 80% yield after purification. Deprotection was now effected in two stages, first by reacting the compound **(18)** with ammonium fluoride in methanol to obtain the diol **(19)** in 95% yield. The final hydrolysis of the diol **(19)**† using trifluoroacetic acid was complicated by cyclisation of the product to diastereoisomeric lactones but these could be hydrolysed to the sodium salt of the acid **(3b)** with sodium hydroxide. Use of $NaBH_4$ in the synthesis gave the unlabelled diol **(3)** and the ^1H- and ^{13}C- NMR spectra were in keeping with those reported[22] for a sample obtained by an alternative synthesis.



(17) **(18)** **(19)** **(3b)**

Scheme 4

The diastereoisomerically labelled alcohol **(3a)** was prepared by the same route using the lactam **(14)** as starting material. The ^{13}C-NMR spectra of the products in $NaO^2H/^2H_2O$ are shown in Figure 1. The proximity of the ^{13}C shifts of the hydroxymethylene groups, and the deuterium isotope shift, caused overlap of the C^2H_2OH with the CH_2OH resonance in the spectrum of the (2S, 4R)-isomer **(3a)** as shown in Figure 1(a) but the spectrum could be assigned by addition of unlabelled diol **(3)** to the sample, as in Figure 1(c). The spectrum of the (2S,4S)-isomer **(3b)**, shown in Figure 1(b), was unambiguous. From the spectra, it is evident that the higher field absorption can be assigned to the *4-pro-S* hydroxymethyl group and the lower field absorption to the

4-pro-R hydroxymethyl group.

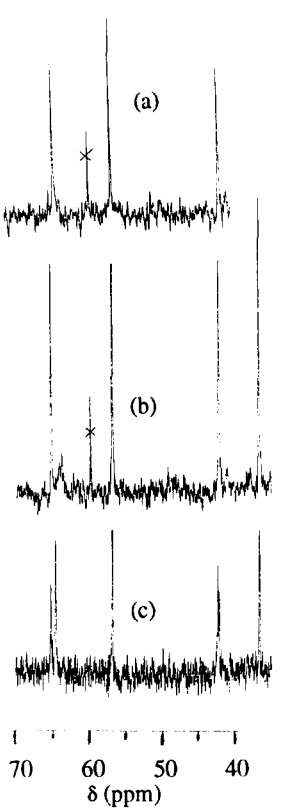


Figure 1: ^{13}C-NMR spectra in 10% $NaO^2H/^2H_2O$ of :
(a) (2S,4R)-[5,5-$^2H_2$]-5,5′-dihydroxyleucine (**3a**)
(b) the (2S,4S) isomer (**3b**)
(c) (**3a**) mixed with unlabelled 5,5′-dihydroxyleucine (**3**)

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References and Notes

1. Stenflo, J.; Fernlund, P.; Egan, W.; Roepstorff, P., *Proc. Natl. Acad. Sci., U.S.A.*, 1974, **71**, 2730 - 2733.
2. Nelsestuen, G. L.; Zytkovicz, T. H.; Howard, J. B., *J. Biol. Chem.*, 1974, **249**, 6347 - 6350.
3. Vermeer, C., *Biochem. J.*, 1990, **266**, 625 - 636 *and refs cited.*
4. Naganathan, S.; Hershline, R; Ham, S. W.; Dowd, P., *J. Am. Chem. Soc.*, 1993, **115**, 5839 - 5840 *and refs cited .*
5. Dubois, J.; Gaudry, M.; Bory, S.; Azerad, R.; Marquet, A., *J. Biol. Chem.*, 1983, **258**, 7897 - 7899.
6. Decottignies-Le-Maréchal, P.; Ducrocq, C.; Marquet, A.; Azerad, R., *C. R. Acad. Sci. Paris*, 1984, **298**, II, 343 - 346.
7. Decottignies-Le-Maréchal, P.; Ducrocq, C.; Marquet, A.; Azerad, R., *J. Biol. Chem.*, 1984, **259**, 15010 - 15012.
8. Ducrocq, C.; Righini-Tapie, A.; Azerad, R.; Green, J. F.; Friedman, P. A.; Beaucourt, J.; Rousseau, B., *J. Chem. Soc., Perkin Trans. 1*, 1986, 1323 - 1328.
9. Dubois, J.; Dugave, C.; Fourès, C.; Kaminsky, M.; Tabet, J.; Bory, S.; Gaudry, M.; Marquet, A., *Biochemistry*, 1991, **30**, 10506 - 10512.
10. Zytkovicz, T. H.; Nelsestuen, G. L., *J. Biol. Chem.*, 1975, **250**, 2968 - 2972.
11. August, R. A.; Khan, J. A.; Moody, C. M.; Young, D. W., *Tetrahedron Lett.*, 1992, **33**, 4617 - 4620.
12. Moody, C. M.; Young, D. W., *Tetrahedron Lett.*, 1993, **34**, 4667 - 4670.
13. Moody, C. M.; Starkmann, B. A.; Young, D. W., *Tetrahedron Lett.*, 1994, **35**, 5485 - 5488.
14. Moody, C. M.; Young, D. W., *Tetrahedron Lett.*, 1994, **35**, in press.
15. Bowler, A. N.; Doyle, P. M.; Young, D. W., *J. Chem. Soc., Chem. Commun.*, 1991, 314 - 316.
16. We first noted this reaction using the N-Cbz benzyl ester corresponding to (**6**) (August, R. A., *D. Phil Thesis*, Sussex, 1987.). The process has recently been independently noted for a reduced ester by Katoh, T.; Nagata, Y.; Arai, K.; Minami, J.; Terashima, S., *Tetrahedron Lett.*, 1993, **34**, 5743 - 5746.
17. Bethell, M.; Kenner, G. W., *J. Chem. Soc.*, 1965, 3850 - 3854.
18. Bethell, M.; Bigley, D. B.; Kenner, G. W., *Chem. and Ind.*, 1963, 653 - 654.
19. Herdewijn, P.; Claes, P. J.; Vanderhaeghe, H., *Canad. J. Chem.*, 1982, **60**, 2903 - 2907.
20. Hondrelis, J.; Lonergan, G.; Voliotis, S.; Matsoukas, J., *Tetrahedron*, 1990, **46**, 565 - 576.
21. Yoshifuji, S.; Tanaka, K.; Kawai, T.; Nitta, Y., *Chem. Pharm. Bull.*, 1985, **34**, 5515 - 5521.
22. Dubois, J.; Fourès, C.; Bory, S.; Falcou, S.; Gaudry, M.; Marquet, A., *Tetrahedron*, 1991, **47**, 1001 - 1012.

† These compounds had the expected analytical and spectroscopic data.

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