Pergamon

Tetrahedron Letters, Vol. 35, No. 30, pp. 5485-5488, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01036-6

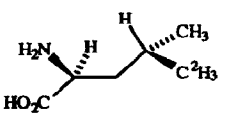
Synthesis of (2S,4S)-5-Fluoroleucine

Claire M. Moody, Bernard A. Starkmann and Douglas W. Young*

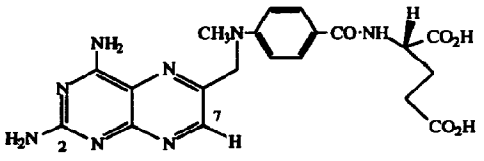
School of Chemistry and Molecular Sciences, University of Sussex, Falmer, Brighton, BN1 9QJ, U. K.

Abstract : (2S,4S)-5-Fluoroleucine, has been prepared by stereospecific synthesis using (2S)-pyroglutamic acid as a chiral template. Protection was required in the synthesis to prevent cyclisation to a proline derivative during the fluorination step.

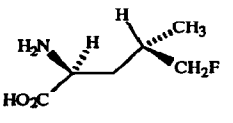
Strategically fluorinated amino acids have been used as inhibitors of a variety of enzymes, including those catalysing reactions mediated by the coenzyme pyridoxal phosphate.[1] Although ^{19}F-NMR spectroscopy is very sensitive with large ^{19}F-^{1}H coupling constants, the use of fluorinated amino acids incorporated into proteins for the elucidation of protein structure has not been well exploited. We have recently completed a synthesis of (2S,4R)-[5,5,5-$^{2}H_3$]-leucine (**1**)[2] and, by incorporating this amino acid into the enzyme dihydrofolate reductase, we have been able to use ^{1}H-NMR spectroscopy to identify the prochiral methyl groups in twelve of the thirteen leucine residues in a complex of the enzyme with the anti-cancer drug methotrexate (**2**).[3] This has allowed spacial interactions within the enzyme and between the enzyme and the drug to be identified by nOe measurements. For example, the 4-pro-R methyl group of Leu-19 and the 4-pro-S methyl group of Leu-27 come close to H-7 of methotrexate (**2**) and the 4-pro-R methyl group of Leu-4 is close to the 2-NH_2 group of methotrexate (**2**).[3] As ^{19}F-NMR spectroscopy would give additional information on protein folding and protein-protein and protein-drug interactions, we have decided to synthesise a sample of leucine in which one of the hydrogen atoms in one of the prochiral methyl groups has been replaced by a fluorine atom. As these methyl groups are important in hydrophobic interactions, incorporation of such a compound into a protein will allow investigation of the way in which such substitution might alter the conformation of the protein and also provide information on the structure of the protein itself.



(1)



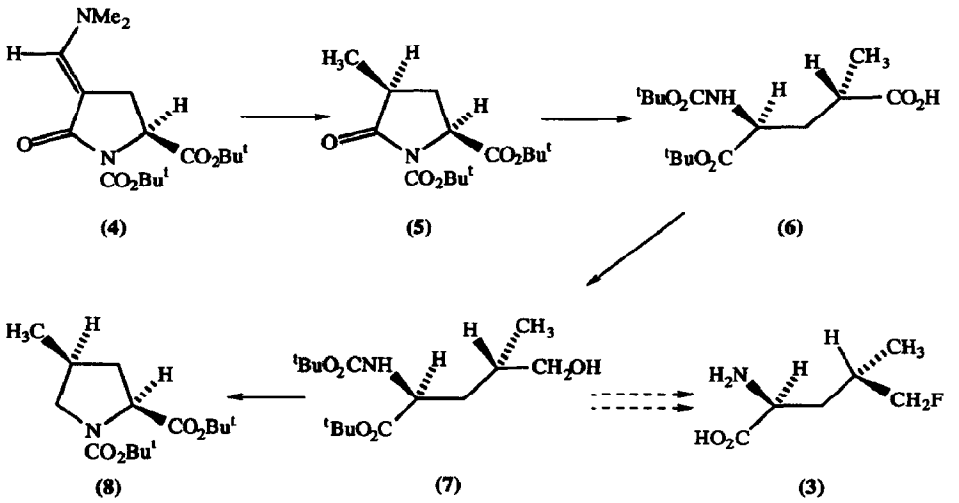
(2)



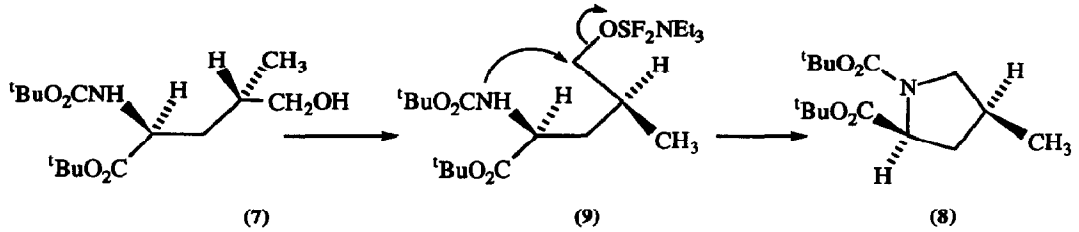
(3)

Having devised a synthesis of the labelled sample (**1**) of leucine, it seemed that (2S,4S)-5-fluoroleucine might be readily accessed by simple modification of this synthesis. The second chiral centre, C-4, in the synthesis of (2S,4R)-[5,5,5-$^{2}H_3$]-leucine (**1**) was introduced stereospecifically using the protected enaminone (**4**) of (2S)-pyroglutamatic acid. On catalytic hydrogenation, complete asymmetric induction was achieved, hydrogen adding from the less hindered side of the molecule to yield the *cis*-methyl derivative (**5**) which could be hydrolysed to the acid (**6**).[2] It seemed to us that this acid might provide a starting point for the synthesis of the

desired product (3). Thus reduction of the acid (6) to the alcohol (7), fluorination using (diethylamino)sulfur trifluoride (DAST), and deprotection might be expected to provide the desired compound.

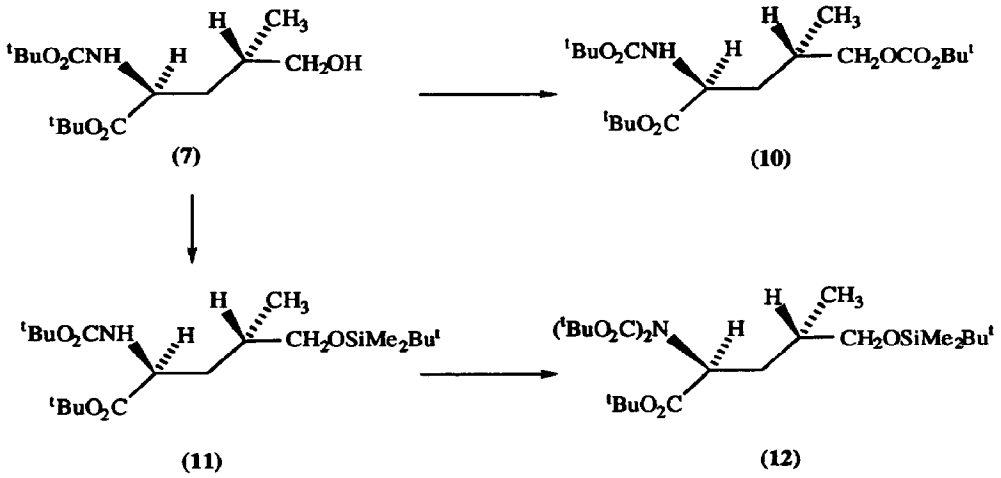


In the event, the alcohol (7) was readily prepared as a colourless oil, $[\alpha]_D^{28}$ -3.1° (c 2.1, $CHCl_3$), in 84% yield from the acid (6) by conversion to the mixed anhydride with *iso*-butyl chloroformate and reduction with $NaBH_4$. However, reaction of the alcohol (7) with DAST at -40 °C gave a colourless oil, $[\alpha]_D^{21}$ -5.2° (c 1, $CHCl_3$), in 63% yield with spectral characteristics which suggested that it was the protected *cis*-4-methylproline derivative (8). An authentic sample of this compound was therefore prepared independently in 65% yield by reduction of the pyroglutamate derivative (5) with borane dimethylsulfide in tetrahydrofuran. The product of the DAST reaction was spectroscopically identical to this authentic sample and so the reaction had yielded the *cis*-4-methylproline derivative (8) rather than the desired protected 4-fluoroleucine. It was evident that the urethane nitrogen in the intermediate (9) formed from the alcohol (7) and DAST was sufficiently nucleophilic to compete with the fluoride ion, so that intramolecular cyclisation yielded the proline derivative (8).

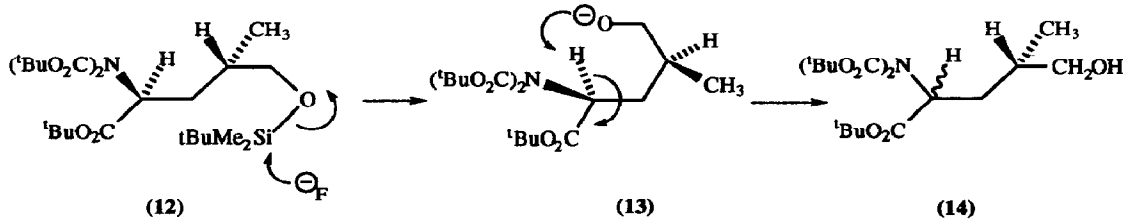


Since the cyclisation reaction might be prevented if a second protecting group were present on nitrogen, we investigated the possibility of preparing the bis-urethane, in the first instance without protecting the primary alcohol group. Since no reaction was observed under the mild conditions recommended for exhaustive urethanylation by Gunnarson,[4] we reacted the alcohol (7) with di-*tert*-butyldicarbonate and DMAP in dioxan at 100 °C overnight. The product, a colourless oil, $[\alpha]_D^{22}$ -3.3° (c 2.2, $CHCl_3$), was obtained in 58% yield and evidently contained a second *tert*-butoxycarbonyl group. The presence of an NH proton in the ^{1}H-NMR

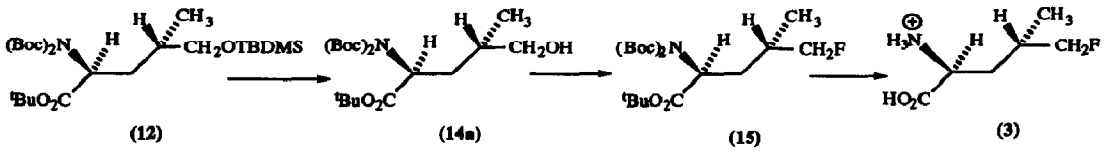
spectrum and the shift to lower field of the CH_2O protons, however, suggested that the N,O-diacylated product (**10**) had been obtained. This was confirmed by the other spectral data. The alcohol function was therefore protected by reaction with *tert*-butyldimethylsilyl chloride, triethylamine and DBU in dichloromethane. The silyl ether (**11**), a colourless oil, $[\alpha]_D^{21}$ -5.2° (c 1.0, $CHCl_3$), was obtained in quantitative yield. In order to achieve perurethanylation of the product (**11**), it was necessary to heat it to 100 °C in dioxan with triethylamine and DMAP whilst adding excess di-*tert*-butyldicarbonate dropwise over two hours. The fully protected product (**12**) was then obtained as a colourless oil, $[\alpha]_D^{22}$ -13.3° (c 1.0, $CHCl_3$), in 95 % yield.



When deprotection of the silyl ether was attempted using tetrabutylammonium fluoride in tetrahydrofuran at room temperature, the product alcohol (**14**), obtained in 51% yield, was shown by its ^1H and ^{13}C NMR spectra to be a 1:1 mixture of diastereoisomers. This was ascribed to the fact that when the oxide anion (**13**) is produced by fluoride ion deprotection, it can act as a base for the intramolecular removal of the C-2 hydrogen, as shown below. This intramolecular deprotonation would lead to epimerisation at C-2.



In the hope of quenching the intermediate anion (**13**) before it caused epimerisation, the silyl ether (**12**) was stirred at room temperature with tetrabutylammonium fluoride in tetrahydrofuran in the presence of acetic acid . The product was obtained from this reaction as a white solid, m.p. 54 - 56 °C, $[\alpha]_D^{25}$ -23.3° (c 0.6, $CHCl_3$), in 94% yield. Both ^1H- and ^{13}C-NMR spectra indicated that the product was a single diastereoisomer of the N, N-di-*tert*-butoxycarbonyl alcohol (**14a**).



We were now in a position to attempt to replace the alcohol group with fluorine using an N-diprotected substrate to prevent intramolecular cyclisation. Although reaction with DAST at -40 °C for one hour gave mixtures, a 65% yield of the desired product (**15**) was obtained by leaving the alcohol (**14a**) with excess DAST in the presence of triethylamine overnight at room temperature. This compound was a colourless oil, $[\alpha]_D^{28}$ -17.6° (c 0.34, $CHCl_3$), which could be deprotected to yield (2S,4S)-5-fluoroleucine hydrochloride (**3**), m.p. 185 - 186 °C, $[\alpha]_D^{28}$ -2° (c 0.2, 3N HCl), in 86% yield by stirring in 6N HCl for four days at room temperature. The ^1H NMR spectra, shown in the Figure, were very pH dependent, the geminal ^{19}F-^1H coupling constant being 47 Hz. The ^{19}F NMR spectrum showed the single fluorine absorption at δ -225.76 ppm from $CFCl_3$ in $C^2H_3O^2H$ / ^2HCl. This was a triplet of doublets, $J_{F,H-5}$ = 47 Hz, $J_{F,H-4}$ = 18 Hz and the mass spectrum (+ve FAB) had an ion at m/z 150 (M^++1).

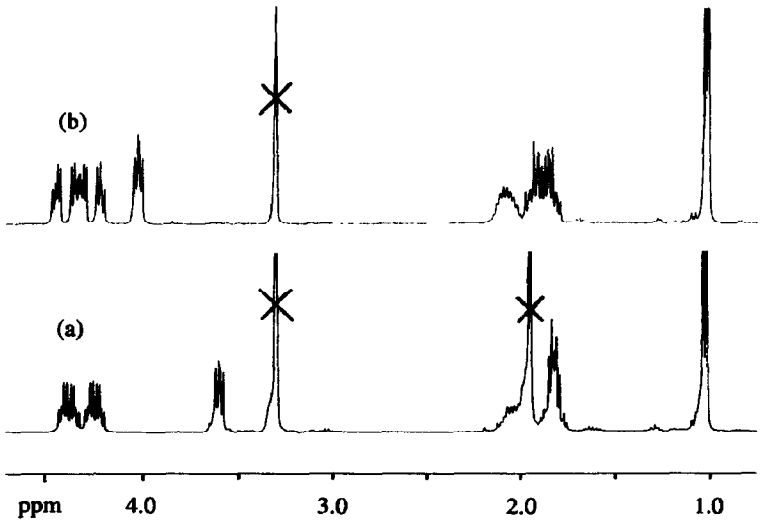


Figure: ^1H NMR spectra at 360 MHz of (2S,4S)-5-fluoroleucine (**3**), (a) in $C^2H_3O^2H$; and (b) in $C^2H_3O^2H/^2HCl$

Acknowledgements

We thank the SERC for studentships (to C. M. M. and B. A. S.).

References

1. (a) Walsh, C., *Adv. in Enzymol.*, 1983, **55**, 197 - 289; (b) Walsh, C. T., *Ann. Rev. Biochem.*, 1984, **53**, 493 - 535; (c) Welch, J. T., *Tetrahedron*, 1987, **43**, 3125 - 3197; (d) Palfreyman, M. G., Bey, P. and Sjoerdsma, A., *Essays in Biochem.*, 1987, **23**, 28 - 81; (e) Zembower, D. E., Gilbert, J. A. and Ames, M. M., *J. Med. Chem.*, 1993, **36**, 305 - 313 and references cited therein.
2. August, R. A.; Khan, J. A.; Moody, C. M. and Young, D. W., *Tetrahedron Letters*, 1992, **32**, 4617 - 4620.
3. Ostler, G.; Soteriou, A.; Moody, C. M.; Khan, J. A.; Birdsall, B.; Carr, M. D.; Young, D. W. and Feeney, J., *FEBS Letters*, 1993, **318**, 177 - 180.
4. Gunnarsson, K. and Ragnarsson, U., *Acta Chem. Scand.*, 1990, **44**, 944 - 951.

(*Received in UK* 14 *April* 1994; *revised* 23 *May* 1994; *accepted* 27 *May* 1994)