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Stereospecific Synthesis of (2S,4R)-[5,5,5-$^2H_3$]-Leucine

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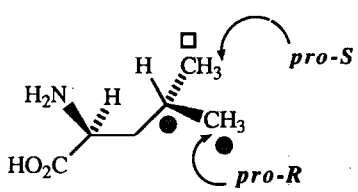
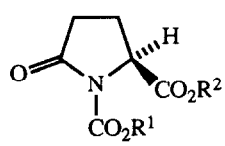
Key Words : leucine; stereospecific synthesis; amino acid; isotopic labelling; bio-organic

Abstract : The first fully stereospecific synthesis of a sample of the amino acid (2S)-leucine labelled in only one of the diastereotopic methyl groups has been achieved using (2S)-pyroglutamic acid as a chiral template.

The importance of amino acids having hydrophobic side chains in determining tertiary structure has led to an interest in studying the nature of these interactions in proteins by nmr spectroscopic techniques. Leucine residues are important to the tertiary structure of proteins by being involved in hydrophobic interactions. Assignment of the resonances of the diastereotopic methyl groups in the side chains of valine and leucine residues in proteins will, therefore, allow protein three dimensional structure to be defined more exactly. Recently Wuthrich and his colleagues have developed the method of biosynthetic fractional ^{13}C-labelling to assign the resonances of the diastereotopic methyl groups of valine and leucine residues in the ^{13}C- and ^1H-nmr spectra of cyclosporin A,[1] the DNA binding domain of the 434 repressor[2] and other proteins.[3] This method relies on the biosynthetic pathway causing the 4-*pro-R* methyl and C-4 carbon atoms of leucine to be labelled contiguously so that they will show coupling, whereas the 4-*pro-S* methyl carbon atom, which arises by reductoisomerase catalysed rearrangement, will not be coupled to the C-4 carbon atom.



(1)



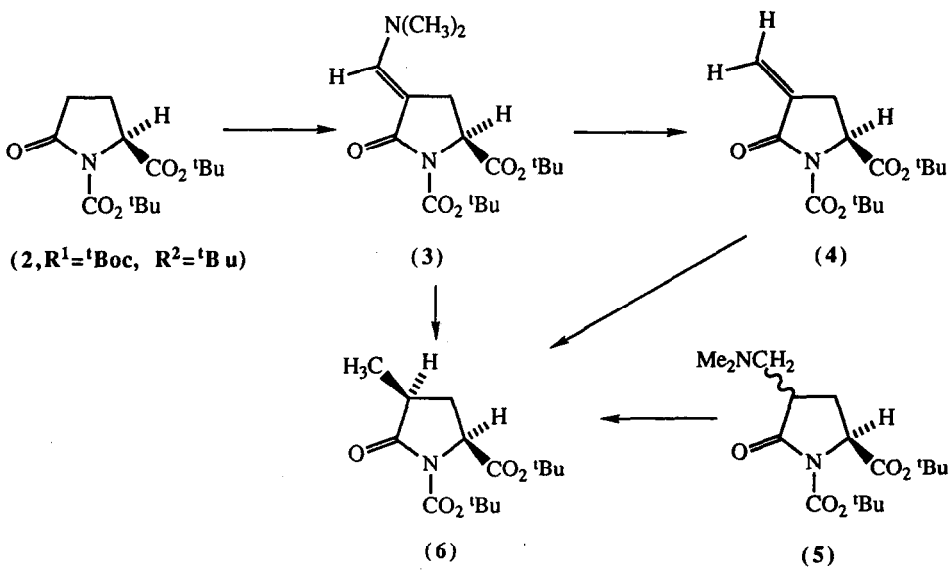
(2)

Because of our interest in aspects of binding of drugs at the active site of dihydrofolate reductase and in the binding shown in leucine zipper proteins, we decided that we could use nmr spectroscopy to observe structural interactions by incorporating samples of leucine which were labelled in one of the diastereotopic methyl groups into these proteins. This required stereospecific synthesis of reasonable quantities of leucine labelled in only one of these methyl groups. Although [5-^{13}C]-, [5-^{14}C]- and [5-^3H]-leucines have been synthesised by a non-stereospecific synthesis involving resolution,[4-6] by homologation of labelled valine[7] and by biosynthetic methods,[8,9] a fully stereospecific chemical synthesis has not, until now, been achieved. In order to obtain sufficient quantities of diastereotopically labelled leucine, we decided that a fully stereospecific synthesis would

have to be developed, and we opted to use (2S)-pyroglutamic acid, (**1**, $R^1 = R^2 = H$), as a starting point for this synthesis. This compound not only had the desired stereochemistry at C-2, but the 1,3-relationship of C-2 and C-4 in the ring offered the possibility of inducing chirality at C-4. Further, the presence of the ring would allow stereochemical assignments to be made unambiguously by n.O.e techniques before ring-opening to the acyclic precursors of leucine.

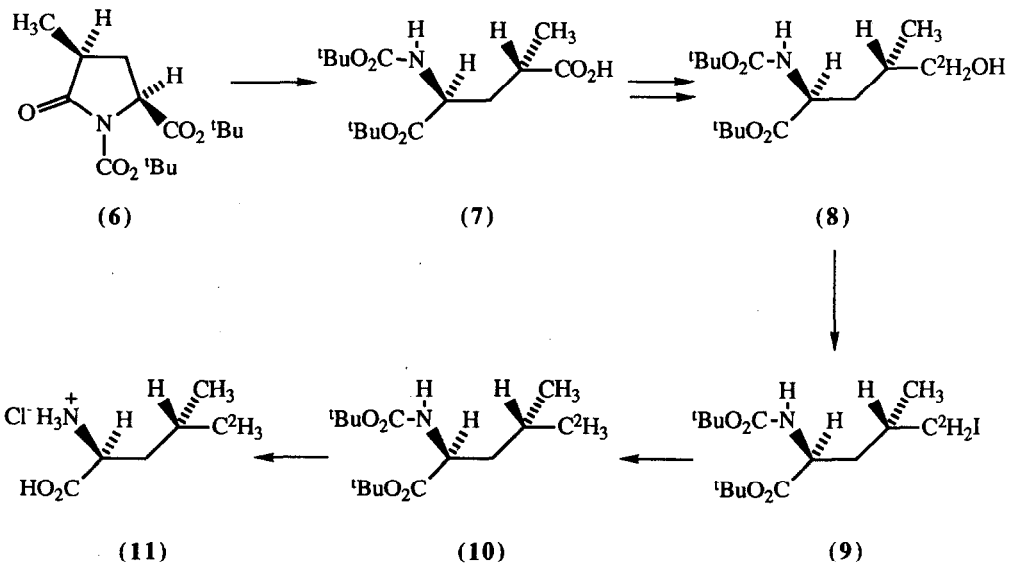
In initial studies, we prepared benzyl N-carbobenzyloxy-(2S)-pyroglutamate, (**2**, R^1 = Cbz, $R^2 = PhCH_2$), from (2S)-glutamic acid by an existing five step sequence.[10,11] This method proved cumbersome, and we had evidence from studies using samples of (2S)-glutamic acid stereospecifically labelled at C-3 with deuterium that the stereochemical integrity of the α-centre was not fully maintained throughout the process. We therefore developed a more direct method of protecting (2S)-pyroglutamic acid by esterification followed by preparation of the N-urethane using di-*tert*-butyldicarbonate and DMAP in acetonitrile. In this way, the benzyl and *tert*-butyl esters, (**2**, $R^1 = {}^t$Boc, $R^2 = PhCH_2$)† and (**2**, $R^1 = {}^t$Boc, $R^2 = {}^t$Bu),† were prepared, although it was found that better yields of the *tert*-butyl ester, (**2**, $R^1 = {}^t$Boc, $R^2 = {}^t$Bu), could be obtained by hydrogenolysis of the benzyl ester, (**2**, $R^1 = {}^t$Boc, $R^2 = PhCH_2$), followed by esterification *via* the mixed anhydride using di-*tert*-butyldicarbonate, DMAP and NEt_3 in acetonitrile.

The protected pyroglutamate, (**2**, $R^1 = {}^t$Boc, $R^2 = {}^t$Bu), was converted to the enaminone (**3**)† in 91% yield using Bredereck's reagent[12] and this was reduced to the exomethylene derivative (**4**)† in 77% yield by reduction using DIBAL in tetrahydrofuran. We next created the second chiral centre by catalytic hydrogenation of (**4**). This occurred stereospecifically to give *tert*-butyl (2S,4S)-N-*tert*-butoxycarbonyl-4-methylpyroglutamate (**6**)† as the sole product. In early attempts at reduction of the enaminone (**3**) with DIBAL, a mixture of diastereoisomeric amines (**5**) was obtained as a byproduct, and we found that this could be converted to the desired stereochemically pure product (**6**) by catalytic hydrogenation using 10% Pd-C (50% w/w) in isopropanol. This suggested that catalytic reduction had involved prior elimination of dimethylamine from each diastereoisomer to give the exomethylene compound (**4**) which would be further reduced to one diastereoisomeric product (**6**). We therefore hydrogenated the enaminone (**3**) directly in ethyl acetate using 10% Pd-C as catalyst and obtained the *cis*-4-methyl derivative (**6**) in 78% yield. The *cis* nature of the product was confirmed by observation of a 3% nuclear Overhauser effect between H-2 and H-4 in the ^{1}H-nmr spectrum.



† These compounds were optically active and had the expected analytical and spectroscopic data.

The pyroglutamate template had now served its purpose, yielding a stereochemically pure product (**6**) with two chiral centres, and it was now necessary to effect ring opening to obtain the desired acyclic amino acid. The *tert*-butyl ester was essential for this step as it was necessary to maintain regioselectivity between the α- and γ-carboxylic functions in the remainder of the synthesis and other esters were found to suffer hydrolysis or transesterification on attempted ring-opening. We have shown[13] that ring opening of related compounds using NEt_3 / methanol was accompanied by epimerisation at C-4 as evidenced by the ^{2}H-nmr spectrum indicating incorporation of deuterium at this position when CH_3O^2H was used instead of CH_3OH in this reaction. Conversion to the acyclic series was finally achieved without loss of stereochemical integrity in 94% yield by reaction with 1N aq. LiOH in tetrahydrofuran. The resultant protected 4-methylglutamic acid (**7**)† was then converted to the labelled alcohol (**8**)† in an overall yield of 75% by conversion to the mixed anhydride with isobutyl chloroformate and NEt_3 in tetrahydrofuran at -40°C followed by reduction with NaB^2H_4 in ^2H_2O / tetrahydrofuran. The alcohol (**8**) was converted to the iodide (**9**)† using methyltriphenoxyphosphonium iodide in HMPA and reduction with $NaB(CN)^2H_3$ *in situ* at 70°C gave the protected labelled leucine (**10**)† in 73% yield. Deprotection was achieved by hydrolysis in 6N aqueous hydrochloric acid at room temperature and the product (2S,4R)-[5,5,5-$^2H_3$]-leucine hydrochloride (**11**) was obtained in 87% yield.



The ^{13}C-nmr spectrum of the resultant labelled sample of leucine, shown in Figure 1, allowed assignment of the methyl absorptions. The methyl absorptions in the ^{1}H-nmr spectrum were, however, too close for direct assignment, but this could be achieved indirectly using two dimensional ^{13}C-^{1}H shift correlation, as shown in Figure 2. The signals due to the *4-pro-R* methyl group were to lower field in both ^{1}H- and ^{13}C-nmr spectra and those due to the *4-pro-S* methyl group were to higher field. The α-isotope shift in the ^{13}C-nmr spectrum for ^{13}C-$^2H_3$ was in the expected range[14] as was the β-isotope shift for C-4.[14] The latter absorption was accompanied by a small absorption for ^{13}C-C-H$^2H_2$ and this small amount of dideuteriated compound was confirmed to be in the *4-pro-R* methyl group by a DEPT experiment.

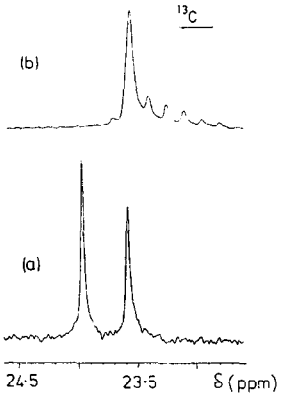


FIGURE 1

Part of the ^{1}H-decoupled ^{13}C-nmr spectrum in 20% ^{2}HCl / $^{2}H_2O$ at 125.8 MHz of:
(a) (2S)-leucine hydrochloride;
(b) (2S,4R)-[5,5,5-$^{2}H_3$]-leucine hydrochloride (**11**)

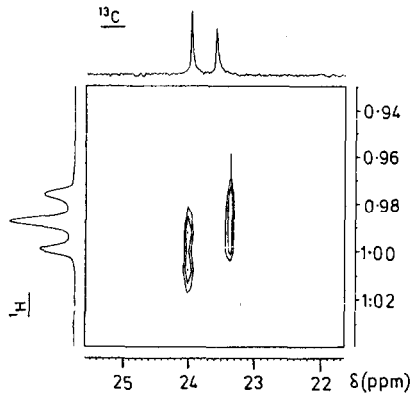


FIGURE 2

Two dimensional ^{1}H-^{13}C shift correlation of leucine hydrochloride in 20% ^{2}HCl in $^{2}H_2O$.

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