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Stereoselective Deuterium-Labelling of Diastereotopic Methyl and Methylene Protons of L-Leucine

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Abstract: An asymmetric synthesis of regio- and stereoselectively deuterium-labelled L-leucine was examined using 4-hydroxy-L-proline as a chiral template. An enol triflate of 4-oxoproline prepared from 4-hydroxyproline was effectively methylated by a Gilman reagent to afford a 4-methyl-3,4-dehydroproline derivative. Then, a catalytic deuteration of the dehydroproline followed by RuO_4-oxidation gave a deuterated 4-methylpyroglutamic acid derivative that could easily be converted to (*2S,3S,4R*)-leucine-3,4,5,5,5-d_5 via a base-promoted ring opening and a reductive deuteration of the terminal carboxyl moiety. This strategy was also applied to the stereoselective synthesis of (*2S,3S,4S*)-[3,4,5,5,5-D_5]leucine.

The amino acid L-leucine has been recognized as an important residue which participates in the inter- and intramolecular hydrophobic interactions based on its lipophilic side chain in polypeptides. Therefore, determination of the three-dimensional structure of its side chain is important. In order to clarify the exact conformation of the side chain by NMR spectroscopy, a stereospecific assignment of the diastereotopic methyl and methylene protons is essential. An effective method for the above assignment is believed to be an isotopic displacement of the diastereotopic groups.[1] Obviously, a scheme for synthesizing L-leucine stereoselectively labelled with stable isotopes must be devised. It is also evident that the availability of such samples would be of considerable benefit to the studies on enzymatic reactions including the leucine framework.[2]

Regarding the stereoselective labelling of the diastereotopic methyl group, there have been several reports concerning the introduction of deuterium or carbon-13 into the methyl group;[3] however, a fully stereoselective chemical synthesis was limited to the procedure of D. W. Young *et al.*[4] On the other hand, the stereoselective synthesis of L-leucine labelled with deuterium in only one of the prochiral methylene protons had not been achieved. In this respect, we recently reported the preparation of L-leucine stereoselectively labelled with deuterium in the C-3 position based on a combination of chemical and enzymatic methods or an asymmetric synthesis using a chiral template.[5] In our continuing work on synthesizing amino acids stereoselectively labelled with stable isotopes for NMR analysis,[5-7] we here report the first example of stereoselective deuterium-labelling of both the diastereotopic methyl and methylene protons within L-leucine.

Recently, we envisioned 4-hydroxy-L-proline as a chiral template for the synthesis of deuterium-labelled amino acids. Our previous paper demonstrated the stereoselective synthesis of (*2S,3S,4R,5S*)-[3,4,5-D_3]proline using the template.[7] Scheme 1 shows the synthetic course of (*2S,3S,4R*)-[3,4,5,5,5-D_5]leucine.

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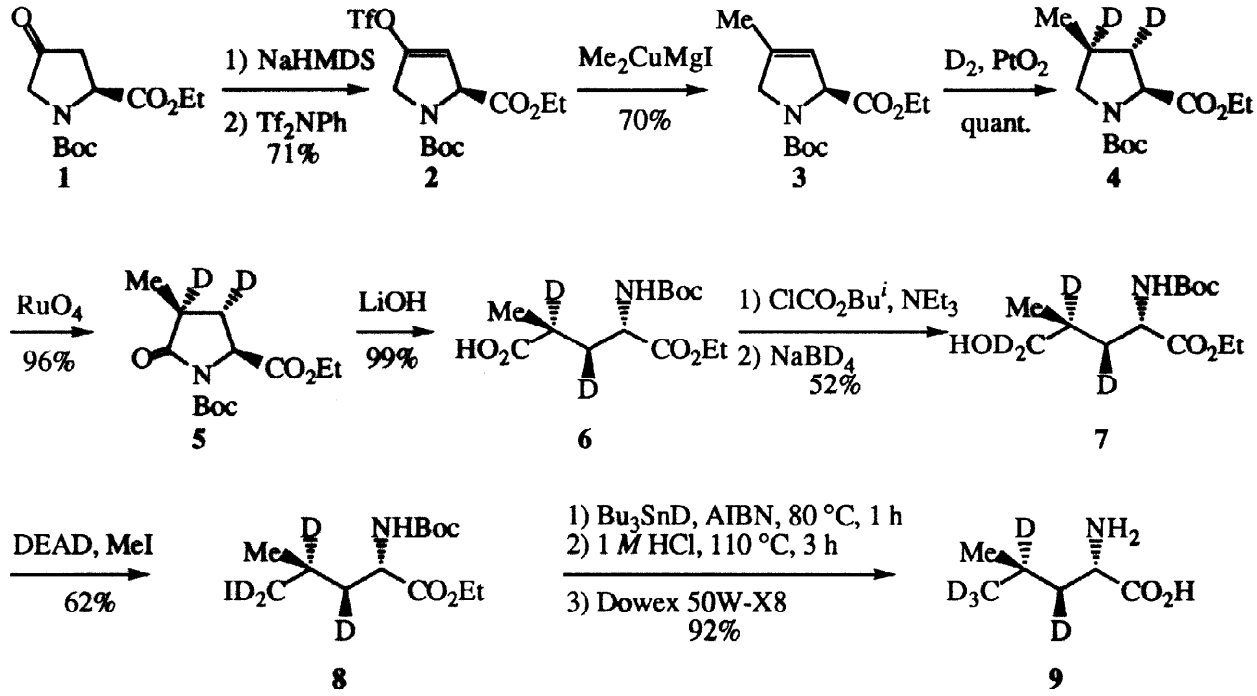
The starting 4-oxo-L-proline ethyl ester (**1**) was easily prepared from 4-hydroxy-L-proline according to the reported procedure for the synthesis of the corresponding methyl ester with minor modification.[8] The regioselective enolization of the ketone **1** toward C-3 was accomplished using sodium bis(trimethylsilyl)amide (NaHMDS) in THF at -78 °C and the resulting enolate was trapped by *N*-phenyl bis(trifluoromethanesulfonimide) to give the vinyl triflate **2** in 71% yield.



Scheme 1

We next examined the cross-coupling reaction of the triflate **2** with organocuprate reagents. When the triflate **2** was treated with a conventional Gilman reagent, Me_2CuLi, prepared from CuI and MeLi·LiI, a direct analysis of the reaction mixture by ^1H NMR spectroscopy revealed production of the desired 4-methyl-3,4-dehydroproline derivative **3**[9] along with a considerable amount of non-methylated olefin. Among the Gilman-type reagents tested, Me_2CuMgI was found to be the most effective in affording the methylated olefin **3** in 70% yield.

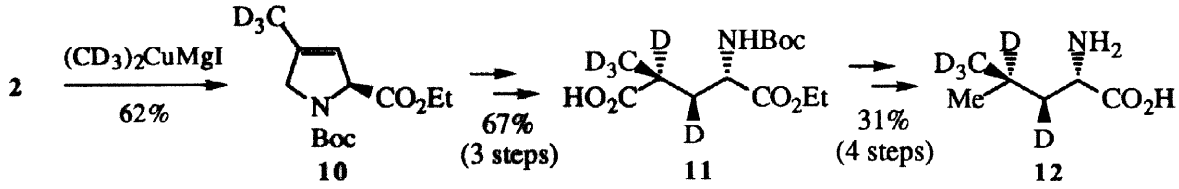
Catalytic deuteration of the olefin **3** was performed in MeOD using deuterium gas at medium pressure (5 kgf/cm^2). The use of PtO_2 as a catalyst proved advantageous, resulting in quantitative formation of the 3,4-dideuterated 4-methylproline derivative **4**. In this case, Pd on carbon was not suitable, causing the considerable H-D scrambling which had also been encountered in our previous work.[5,7] The stereoselectivity was determined by ^1H NMR spectroscopy on the final deuterated leucine because of the expected complexity of the spectrum of the proline derivative **4** due to conformational isomerism.[10]

In order to obtain the leucine derivative, the 4-methylproline derivative **4** was converted to the 4-methylpyroglutamic acid derivative **5** using RuO_4, prepared *in situ* from RuO_2 and $NaIO_4$,[11] in 96% yield. The synthetic pathway to the labelled leucine from the pyroglutamate **5** was similar to that of Young *et al.*[4] Thus, the pyroglutamate **5** was treated with 1 *M* LiOH to give the 4-methylglutamic acid derivative **6** in 99%

yield leaving other functional groups intact. Although Young *et al.* asserted that the *t*-butyl ester was essential to avoid further saponification of the ester at C-2, the ethyl ester also worked well in our case.

The chemoselective reduction of the terminal carboxyl moiety into the labelled alcohol **7** was carried out using $NaBD_4$ via the corresponding mixed anhydride with isobutyl chloroformate in 52% yield. The deoxygenative deuteration of the hydroxyl function within the alcohol **7** to generate the CD_3 moiety was effected by iodination with the diethyl azodicarboxylate (DEAD)-MeI-PPh_3 system followed by radical deuteration of the iodide **8** so formed by Bu_3SnD in the presence of AIBN. The crude protected leucine was directly subjected to deprotection to furnish the desired (*2S,3S,4R*)-[3,4,5,5,5-D_5]leucine (**9**) in 92% yield based on the iodide **8**. The optical purity at the C-2 position was determined to be 93%ee by HPLC analysis using chiral stationary phase column (MCIGEL CRS10W). The relative configuration was confirmed by the 400 MHz ^1H NMR spectrum of the leucine **9** on comparison with that of unlabelled leucine (Figure 1).

Since Young's procedure[4] employed the Bredereck reagent for introduction of a *pro-S* methyl fragment, it is difficult to label the methyl group with deuterium or carbon-13. In contrast, our protocol described here utilized the Me_2CuMgI as the source of the *pro-S* methyl moiety; therefore, the introduction of labelling atom(s) into the methyl group is feasible using commercially available CD_3I, $^{13}CH_3I$, or the corresponding Grignard reagents. We therefore carried out the stereoselective deuterium-labelling of the *pro-S* methyl group leading to (*2S,3S,4S*)-[3,4,5,5,5-D_5]leucine.



Scheme 2

As shown in Scheme 2, $(CD_3)_2CuMgI$ was employed as a methylating agent in the cross-coupling reaction with the triflate **2** to give the deuterated 4-methyldehydroprolinate **10** in 75% yield. After the conversion of the prolinate **10** into the deuterated glutamate **11** (67% yield for 3 steps), a selective reduction of the terminal carboxyl moiety into the methyl group was accomplished by use of sodium borohydride and tributylstannane in the reduction steps followed by deprotection to furnish (*2S,3S,4S*)-[3,4,5,5,5-D_5]leucine (**12**) in 31% yield based on the glutamate **11**. The optical purity at C-2 (97%ee) and the relative configuration were ascertained by HPLC and ^1H NMR (Figure 1) analyses, respectively.

In summary, we have completed the stereoselective synthesis of L-leucine in which both the diastereotopic methyl and methylene protons were chirally labelled with deuterium. In addition, the stereoselective deuterium-labelling of either prochiral methyl group was achieved. For more concise conformational analysis of the larger molecule by use of NMR technique, introduction of carbon-13 into C-3 and C-5 is now under investigation.

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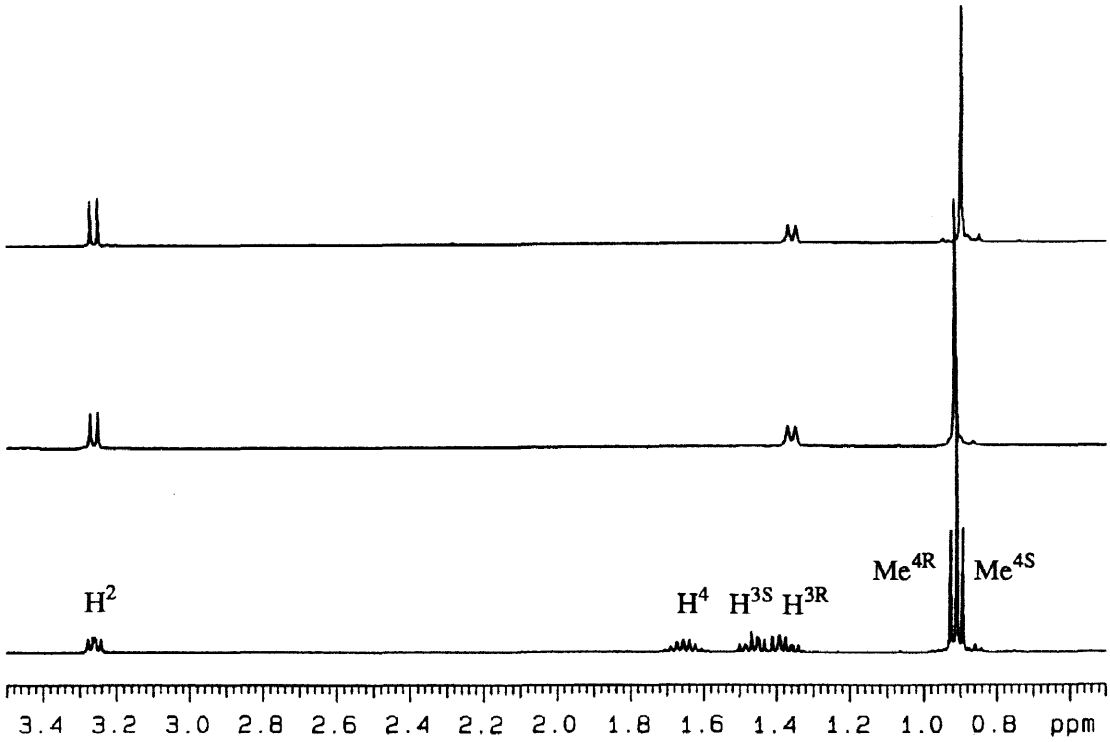

Figure 1. 400 MHz ^{1}H NMR Spectra of Deuterated Leucine 9 (top), 12 (middle), and Unlabelled Leucine (bottom) in 5% NaOD/D_2O.

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