

## Chemo-enzymatic Synthesis of Isotopically Labelled L-Valine, L-Isoleucine and *allo*-Isoleucine

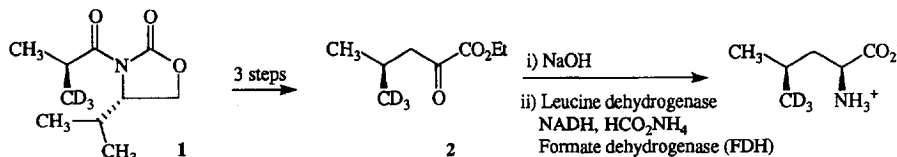
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**Abstract** : Syntheses of (3*R*)-[4,4,4-D<sub>3</sub>]-L-valine, [<sup>15</sup>N]-L-isoleucine and [<sup>15</sup>N]-*allo*-isoleucine from homochiral 2-alkylated carboxylic acids are described. The approach involves a one-carbon homologation of the carboxylic acid to give the corresponding β-substituted α-keto ester which is converted directly to the α-amino acid in a one-pot procedure involving two enzyme catalysed reactions (*Candida cylindracea* lipase to hydrolyse the ester and leucine dehydrogenase to catalyse the reductive amination of the ketone). This strategy may be simply adapted for the selective labelling of each site of the L-amino acid.

Isotopically labelled amino acids in enantiomerically pure form are important tracers for the elucidation of various metabolic pathways and are valuable for structural studies of proteins. The design of efficient strategies for the synthesis of labelled amino acids is a rapidly expanding field. Recently we have described a chemo-enzymatic approach for the synthesis of L-leucine in which either of the diastereotopic methyl groups may be selectively labelled with carbon-13 or deuterium.<sup>1</sup> The method was based on the use of chiral enolates, derived from Evans oxazolidinone imides<sup>2</sup>, to selectively introduce the isotopic label into either prochiral methyl group. Cleavage of the oxazolidinone **1**, followed by a two carbon homologation gave the α-keto ester **2**. Saponification of the ester then reductive amination of the resultant α-keto acid catalysed by leucine dehydrogenase afforded (2*S*, 4*R*)-[5,5,5-D<sub>3</sub>]-leucine in 85% yield from the ester **2** (Scheme 1).



Scheme 1

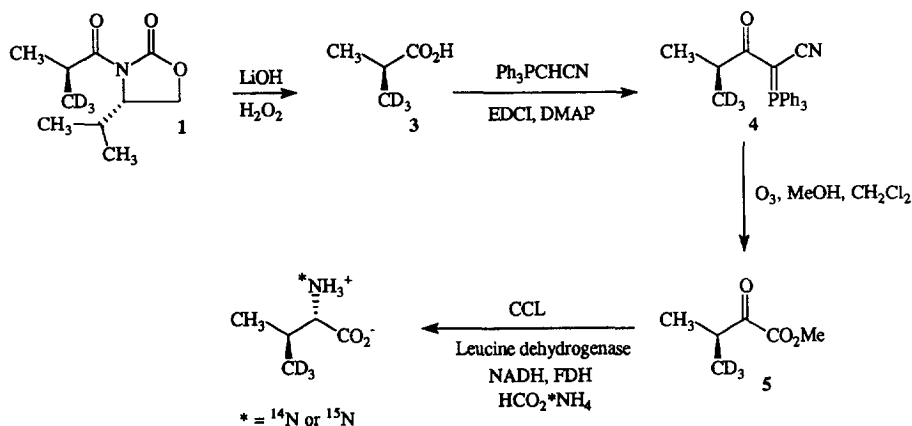
Since 3-methyl-2-oxobutanoic acid is also a good substrate for leucine dehydrogenase<sup>3</sup>, we reasoned that if after cleavage of the chiral auxiliary a one carbon homologation to the α-keto ester **5** (Scheme 2) could be achieved without racemisation, then we would have a synthetic route to L-valine selectively labelled in either

diastereotopic methyl group. Several methods have been reported for the enantioselective synthesis of isotopically labelled valine, for example from aspartic acid<sup>4,5</sup> and via resolution with  $\beta$ -methylaspartase<sup>6</sup>. We now report a more general approach which has the advantage that it enables the stereoselective labelling of either diastereotopic methyl group with carbon-13 or deuterium as well as labelling at C-2 and/or C-3. The strategy may be extended to the synthesis of further amino acids with an asymmetric centre at C-3 e.g. [<sup>15</sup>N]-L-isoleucine and [<sup>15</sup>N]-L-*allo*-isoleucine which complements existing routes to these compounds<sup>4,7,8</sup>.

## Results and Discussion

### *Isotopically labelled L-valine.*

The oxazolidinone **1** was cleaved under standard conditions with lithium hydroxide and hydrogen peroxide<sup>9</sup> to give (*R*)-[3,3,3-D<sub>3</sub>]-2-methylpropanoic acid **3** in 78% yield (Scheme 2). Several methods are known for conversion of carboxylic acids to  $\alpha$ -keto esters including a Pummerer-like rearrangement<sup>10</sup>, formation of Weinreb's amide followed by displacement with ethyl vinyl ether<sup>11</sup> and subsequent ozonolysis and the reaction of acyl halides with cyanide followed by hydrolysis.<sup>12</sup> However, we required a method which would not cause racemisation  $\alpha$ - to the carbonyl group and we favoured an approach described recently by Wasserman<sup>13</sup>. Treatment of the carboxylic acid **3** with (cyanomethylene)triphenylphosphorane in the presence of EDCl/DMAP gave the  $\beta$ -keto cyanophosphorane **4**. Oxidative cleavage of **4** with ozone in MeOH/CH<sub>2</sub>Cl<sub>2</sub> gave methyl (*R*)-[4,4,4-D<sub>3</sub>]-3-methyl-2-oxobutanoate **5** in 63% yield over the two steps.



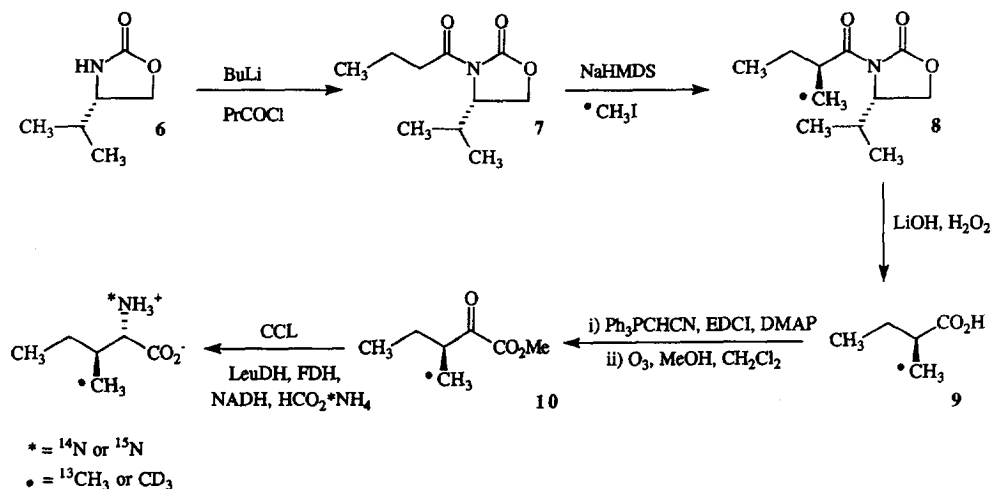
Scheme 2

The next stage of the synthesis required hydrolysis of the ester **5** without racemisation at C-3. Previously we have converted  $\alpha$ -keto esters to  $\alpha$ -keto acids with sodium hydroxide<sup>14</sup>, however these conditions led to racemisation of **5**. A milder method is to use the lipase isolated from *Candida cylindracea*. Since the reaction conditions required for the lipase catalysed hydrolysis are compatible with those used in the reductive

amination of the  $\alpha$ -keto acid catalysed by leucine dehydrogenase, the ester **5** was directly converted in one-pot to (2*S*, 3*R*)-[4,4,4- $D_3$ ]-valine in 80% yield after purification by ion exchange chromatography. The  $^1\text{H-NMR}$  spectrum of L-valine showed doublets ( $J$  7Hz) at  $\delta$ 0.94 and  $\delta$ 0.89 assigned to the *pro-R* and *pro-S* methyl groups whereas for (2*S*, 3*R*)-[4,4,4- $D_3$ ]-valine only the doublet (at  $\delta$ 0.94) was apparent confirming that no racemisation had occurred at C-3. Adding nitrogen-15 labelled ammonium formate to the enzyme catalysed reaction afforded (2*S*, 3*R*)-[ $^{15}\text{N}$ , 4,4,4- $D_3$ ]-valine in 70% yield from the ester **5**. This approach may be extended to the synthesis of [2- $^{13}\text{C}$ ]-L-valine using the labelled acylated chiral auxiliary described previously<sup>1</sup> or indeed to a combination of isotopes ( $^{15}\text{N}$ , 2- $^{13}\text{C}$ ,  $^{13}\text{C}$  and/or  $^2\text{H}$  in either diastereotopic methyl group).

#### Isotopically labelled L-isoleucine.

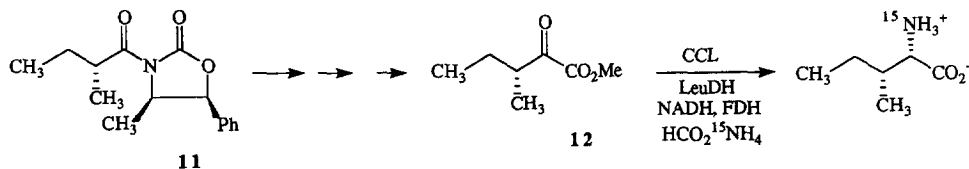
L-isoleucine has an asymmetric centre at C-3 and the above strategy has been successfully applied to the synthesis of [ $^{15}\text{N}$ ]-L-isoleucine. Coupling (cyanomethylene)triphenylphosphorane with (*S*)-3-methylbutyric acid **9**<sup>15</sup> in the presence of EDCI/DMAP followed by ozonolysis (Scheme 3) afforded the  $\alpha$ -keto ester **10** in 75% overall yield. Conversion of the ester **10** to [ $^{15}\text{N}$ ]-L-isoleucine was achieved in 74% yield in a one-pot reaction with lipase and leucine dehydrogenase in the presence of [ $^{15}\text{N}$ ]-ammonium formate. Since carbon-13 or deuterium labelled  $\alpha$ -keto ester **10** may be prepared using the chiral auxiliary derived from (*S*)-valinol **6**, this chemo-enzymatic approach to the synthesis of L-isoleucine is versatile enabling the selective incorporation of several isotopic labels (Scheme 3). Thus reaction of **6** with butyl lithium followed by butyryl chloride gave the oxazolidinone **7** in quantitative yield. Alkylation of the sodium enolate of **7** with methyl iodide ( $^{13}\text{CH}_3\text{I}$  or  $\text{CD}_3\text{I}$ ) gave an 18:1 ratio of the oxazolidinone **8** and its diastereoisomer<sup>2</sup>. The chiral auxiliary was then removed with lithium hydroxide and hydrogen peroxide to give (*S*)-2-methylbutanoic acid **9**.



Scheme 3

*Isotopically labelled L-allo-isoleucine.*

[<sup>15</sup>N]-L-allo-isoleucine was prepared using an analogous approach to that for [<sup>15</sup>N]-L-isoleucine but starting from the chiral auxiliary derived from (+)-norephedrine<sup>2</sup>. Hydrolytic cleavage of the chiral auxiliary in **11** afforded (*R*)-3-methylbutyric acid in 98% yield. The one carbon homologation of the acid gave the α-keto ester **12** which was successfully hydrolysed and reductively aminated in one-pot using the enzyme catalysed procedure giving [<sup>15</sup>N]-allo-isoleucine in 74% yield (Scheme 4). To the best of our knowledge this is the first reported synthesis of [<sup>15</sup>N]-allo-isoleucine and the approach can be readily adapted to introduce carbon-13 and deuterium.



Scheme 4

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**References and Notes.**

- Kelly, N. M.; Reid, R. G.; Willis, C. L. and Winton, P. L., *Tetrahedron Lett.*, 1995, **36**, 8315.
- Evans, D. A.; Bartroli, J. and Shih, T. L., *J. Am. Chem. Soc.*, 1981, **103**, 2127; Evans, D. A.; Ennis, M. D. and Mathre, D. J., *J. Am. Chem. Soc.*, 1982, **104**, 1737.
- Kelly, N. M.; O'Neill, B. C.; Probert, J.; Reid, R. G.; Stephen, R.; Wang, T.; Willis, C. L. and Winton, P. L., *Tetrahedron Lett.*, 1994, **35**, 6533.
- Humphrey, J. M.; Hart, J. A. and Chamberlin, A. R., *Bioorg. Med. Chem. Lett.*, 1995, **5**, 1315.
- Baldwin, J. E.; Adlington, R. M.; Crouch, N. P.; Mellor, L. C.; Morgan, N.; Smith, A. M. and Sutherland, J. D., *Tetrahedron*, 1995, **51**, 4089.
- Baldwin, J. E.; Adlington, R. M.; Crouch, N. P.; Turner, N. J.; and Schofield, C. J., *J. Chem. Soc., Chem Commun.*, 1989, 1141.
- D'Aniello, F.; Mann, A.; Taddei, M. and Wermuth, C.-G., *Tetrahedron Lett.*, 1994, **35**, 7775.
- Oppolzer, W.; Tamura, O. and Deerberg, J., *Helv. Chim. Acta*, 1992, **75**, 1965; Yamamoto, Y.; Ito, W. and Maruyama, K., *J. Chem. Soc., Chem. Commun.*, 1985, 1131.
- Evans, D. A.; Britton, T. C. and Ellman, J. A., *Tetrahedron Lett.*, 1987, **28**, 6141.
- Iriuchijima, S. and Ogawa, M., *Synthesis*, 1982, 41
- Angelastro, M. R.; Peet, N. P. and Bey, P., *J. Org. Chem.*, 1989, **54**, 3913.
- Chelintzev, V. V. and Schmidt, W. N., *Ber. Dtsch. Chem. Ges.*, 1929, **62B**, 2210
- Wasserman, H. H. and Ho, W.-B., *J. Org. Chem.*, 1994, **59**, 4364
- Bentley, J. M.; Wadsworth, H. J. and Willis, C. L., *J. Chem. Soc., Chem Commun.*, 1995, 231
- (*S*)-2-Methylbutanoic acid is commercially available from Aldrich Chemical Company.

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