Pergamon

Tetrahedron Letters, Vol. 38, No. 10, pp. 1837-1840, 1997
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0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00164-0

Enantioselective Syntheses of α-Amino-β-Hydroxy Acids, [^{15}N]-L-*allo*threonine and [^{15}N]-L-threonine

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Abstract : The enantioselective synthesis of [^{15}N]-L-*allo*threonine from ethyl (*S*)-lactate *via* methyl (*S*)-3-methoxymethoxy-2-oxobutanoate **15** is described. The stereogenic centre at C-2 was established by a one-pot, dual enzyme catalysed hydrolysis of the ester (by a lipase) and reductive amination of the ketone of **15** (with leucine dehydrogenase) to give, after deprotection, [^{15}N]-(2*S*,3*S*)-2-amino-3-hydroxybutanoic acid as a single diastereomer in 93% yield. [^{15}N]-L-Threonine was prepared by an analogous strategy from methyl (*R*)-lactate using phenylalanine dehydrogenase in the reductive amination step. This approach may be simply adapted for the incorporation of deuterium and carbon-13.
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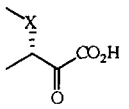
Isotopically labelled amino acids are important for a range of studies in bioorganic chemistry including metabolic investigations and to facilitate the elucidation of the 3-dimensional structure of proteins and peptides by NMR spectroscopy. These studies are often limited by the cost and availability of suitably labelled compounds and so there has been considerable effort recently directed towards developing methods for the enantioselective synthesis of amino acids incorporating deuterium, carbon-13 and nitrogen-15.[1] We have prepared a series of [^{15}N]-L-amino acids (including ala, *allo*isoleu, isoleu, leu, phe, ser and val) from α-keto acids in good yields and excellent enantioselectivities using commercially available amino acid dehydrogenases to catalyse the reductive amination step and [^{15}N]-ammonium formate as the source of isotopic label.[2,3] One potential drawback of this approach as a general method for the synthesis of further isotopically labelled proteinogenic amino acids, particularly those with heteroatoms in the side-chain, is that the enzymes of this superfamily have been reported to have limited substrate specificities.[4] However, since we have shown that both (*R*)- and (*S*)-3-methylpentanoic acids **1** and **2** are good substrates for leucine dehydrogenase, enabling efficient syntheses of [^{15}N]-L-*allo*isoleucine and [^{15}N]-L-isoleucine respectively,[3] we reasoned on steric grounds that (*S*)- and (*R*)-3-methoxy-2-oxobutanoic acids **3** and **4** should also be substrates for the dehydrogenase giving access, after deprotection, to [^{15}N]-L-*allo*threonine **5** and [^{15}N]-L-threonine **6** respectively. In this paper, results from investigations on the use of amino acid dehydrogenases for the preparation of isotopically labelled α-amino-β-hydroxy acids are described.



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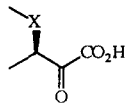

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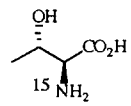

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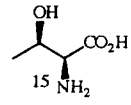

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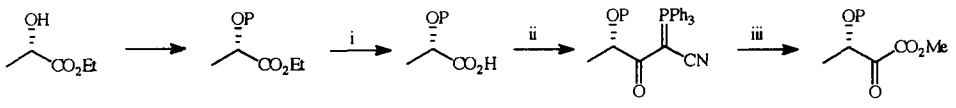
Results and Discussion

Methyl (*S*)-3-methoxy-2-oxobutanoate **10** was prepared as shown in Scheme 1. Ethyl (*S*)-lactate was protected as the methyl ether **7** by reaction with methyl iodide and sodium hydride. Hydrolysis of the ester with NaOH led to epimerisation at C-2 whereas with LiOH the required homochiral (*S*)-2-methoxypropionic acid **8** was obtained in 75% yield.



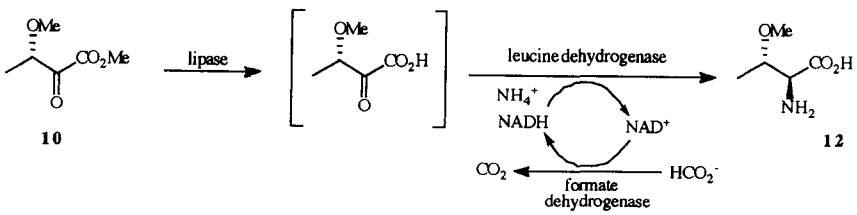
7 P = Me **8** P = Me **9** P = Me **10** P = Me **14** P = CH_2Ph **15** P = CH_2OMe

Reagents: i) LiOH, MeOH, H_2O; ii) Ph_3PCHCN, EDCI, DMAP, CH_2Cl_2; iii) O_3, MeOH, CH_2Cl_2

Scheme 1

Many methods are known for the conversion of carboxylic acids to α-keto esters.[5] However,we required a strategy which would maintain the stereochemical integrity of the molecule and we favoured an approach involving ozonolysis of a β-ketocyanophosphorane originally described by Wasserman and Ho.[6] Treatment of the protected acid **8** with (cyanomethylene)triphenylphosphorane and EDCI/DMAP gave **9** which on ozonolysis in $MeOH/CH_2Cl_2$ gave the required α-keto ester **10**. A similar 4 stage approach was used to prepare the enantiomer **11** from methyl (*R*)-lactate.

β-Substituted-α-keto esters can be hydrolysed to the corresponding acids with a lipase from *Candida cylindracea*.[3] Since the reaction conditions are compatible with those required for the leucine dehydrogenase catalysed reductive amination of the ketone, **10** was converted directly in a one-pot process to (2*S*,3*S*)-2-amino-3-methoxybutanoic acid **12** in 73 % yield (Scheme 2). The ^{1}H-NMR spectrum confirmed that a single diastereomer was obtained.



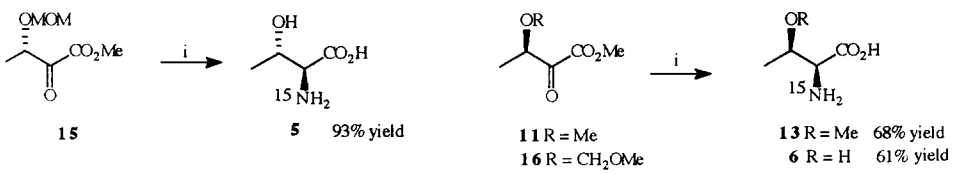
10 **12**

Scheme 2

The (2*S*,3*R*)-diastereomer **13** was prepared efficiently using the same procedure (Scheme 3). Unfortunately repeated attempts to deprotect the methyl ether in **12** for example with 6M HCl, $BF_3.Et_2O$, or BF_3/PrSH, failed to give L-*allo*threonine and simply returned the ether.

Wasserman has reported[6] that hydroxyl groups in the side-chain of a carboxylic acid must be protected prior to coupling with the ylide, therefore an alternative protecting group was required for lactic acid and the use of a benzyl ether was investigated. Methyl (*S*)-3-benzyloxy-2-oxobutanoate **14** was prepared in 4 steps from ethyl (*S*)-lactate via the approach shown in Scheme 1 using sodium hydride and benzyl bromide to protect the alcohol. Although the ester was cleanly hydrolysed using the lipase, the resultant (*S*)-3-benzyloxy-2-oxobutanoic acid was recovered unchanged when incubated with either leucine dehydrogenase or phenylalanine dehydrogenase. These results demonstrate the problem of the relatively narrow substrate specificity range of these enzymes.

Therefore it was apparent that a less bulky protecting group than the benzyl ether was required in the synthesis of [^{15}N]-L-*allo*threonine **5** from ethyl (*S*)-lactate and the methoxymethyl ether proved ideal. Methyl (*S*)-3-methoxymethoxy-2-oxobutanoate **15** was incubated with the lipase and leucine dehydrogenase in the presence of [^{15}N]ammonium formate (Scheme 3). The MOM protecting group was removed with 2M HCl and after purification by ion exchange chromatography, [^{15}N]-(2*S*,3*S*)-2-amino-3-hydroxybutanoic acid **5** was isolated in 93% yield from keto ester **15**.[7] When phenylalanine dehydrogenase was used to catalyse the reductive amination step [^{15}N]-L-*allo*threonine was obtained in 53% yield from **15**. A similar strategy was adopted for the synthesis of [^{15}N]-L-threonine from methyl (*R*)-lactate. In this case it was found that (*R*)-3-methoxymethoxy-2-oxobutanoic acid was a better substrate for phenylalanine dehydrogenase than for leucine dehydrogenase giving, after deprotection, [^{15}N]-(2*S*,3*R*)-2-amino-3-hydroxybutanoic acid **6** in 61% yield from keto ester **16** and with complete stereocontrol.



15 **5** 93% yield **11** R = Me **16** R = CH_2OMe **13** R = Me 68% yield **6** R = H 61% yield

Reagents: i) CC-Lipase, amino acid dehydrogenase, FDH, NADH, $^{15}NH_4HCO_2$ then 2M HCl

Scheme 3

This chemo-enzymatic approach to the synthesis of L-threonine and L-*allo*threonine has the further advantage that it may be simply adapted for the incorporation of carbon-13 or deuterium. For example, treatment of diethyl oxalate with [^{13}C]methylmagnesium iodide at -78°C in a mixture of THF and diethyl ether gave ethyl [3-^{13}C]pyruvate in 85% yield.[2] Saponification of the ester with sodium hydroxide followed by reduction of the ketone using lactate dehydrogenase from *Bacillus stearothermophilus* [9] as the catalyst gives [3-^{13}C]-(*S*)-lactic acid for use as the starting material in the synthesis of [4-^{13}C]-L-*allo*threonine. Using lactate dehydrogenase from *Staphyllococcus epidermidis*,[10] isotopically labelled (*R*)-lactate may be prepared as the precursor to [4-^{13}C]-L-threonine.

Acknowledgements. *The University of Bristol is thanked for a Scholarship (for AS).*

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7. *Typical procedure:* A mixture of keto ester (1 mmol) and *Candida cylindracea* lipase, 10000 eU) in aqueous phosphate buffer (30 ml) was maintained at pH 7.5 by the addition of 1M NaOH until 1 equivalent of base had been added. Ammonium formate (1.2 mmol) was then added and the solution deoxygenated by bubbling through a stream of nitrogen for 1 h. Dithiothreitol (2 μl), leucine (or phenylalanine) dehydrogenase (1 eU), formate dehydrogenase (10 mg) and NADH (11 mg) were added to the reaction mixture and the pH maintained at 7.0 by addition of 1M HCl until 1 equivalent of acid had been added. The reaction mixture was concentrated *in vacuo* and the resulting residue dissolved in 2M HCl (10ml) and heated under reflux for 1 h. After removal of the solvent, the amino acid was purified on a Dowex 50W ion exchange column using concentrated ammonia solution as the eluent.

All products gave the expected NMR and MS spectral data. [^{15}N]-L-*allo*threonine $[\alpha]_D$ + 8.6, c 1.0, H_2O; (lit[8] unlabelled material $[\alpha]_D$ + 9.0, c 1.0, H_2O); [^{15}N]-L-threonine $[\alpha]_D$ -27.2, c 2.0, H_2O; (lit[8] unlabelled material $[\alpha]_D$ -28.3, c 6.0, H_2O).

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(*Received in UK* 31 *December* 1996; *accepted* 24 *January* 1997)