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SYNTHESIS OF PROBES FOR THE ACTIVE SITE OF LEUCINE DEHYDROGENASE

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Abstract. A series of novel 3-substituted 2-oxobutanoic acids were prepared and incubated with leucine dehydrogenase giving in one case both a kinetic resolution at C-3 and reductive amination of the ketone. This is the first example of an amino acid dehydrogenase catalysed kinetic resolution and reductive amination.

Amino acids incorporating stable isotopes are valuable for a range of studies in bioorganic chemistry and in particular to investigate the 3-dimensional structure of proteins by NMR spectroscopy. The incorporation of nitrogen-15 labelled amino acids into peptides and proteins is of particular value as it increases the dispersion of the signals and so assists the assignment of the complex NMR spectra. Hence the design of efficient synthetic strategies towards selectively labelled α-amino acids is a rapidly expanding field.[1]

Amino acid dehydrogenases have been used by several groups for the synthesis of a range of [^{15}N]-L-amino acids (including ala, glu, isoleu, leu, phe and val) *via* reductive amination of the corresponding 2-oxo acids.[2] In addition, non-proteinogenic L-amino acids with hydrophobic aliphatic and aromatic side-chains have been prepared using leucine and phenylalanine dehydrogenases respectively.[3] However, the enzymes of this superfamily have relatively limited substrate specificities for 2-oxo acids with heteroatoms in the side-chains.[4] A thorough understanding of the mechanism for discrimination between the different amino acid substrates would enhance our ability to alter specifically these enzymes for use as catalysts in synthesis. We now describe our investigations with leucine dehydrogenase which have led to the first example of both the kinetic resolution and reductive amination of a racemic 2-oxo acid by an amino acid dehydrogenase leading to the syntheses of 3-substituted-2-aminobutanoic acids including L-threonine and L-*allo*threonine.

Results and Discussion

In a recent communication we have outlined the synthesis of [^{15}N]-L-threonine and [^{15}N]-L-*allo*threonine using amino acid dehydrogenases to establish the stereogenic centre at C-2.[5] Interestingly we found that (*S*)-3-methoxymethoxy-2-oxobutanoic acid ***S*-1** was a better substrate for leucine dehydrogenase (*Bacillus* species purchased from Sigma) than phenylalanine dehydrogenase (*Sporosarcina* species) whereas the reductive amination of the enantiomer ***R*-1** proceeded more efficiently with phenylalanine dehydrogenase than with leucine dehydrogenase (scheme 1). The

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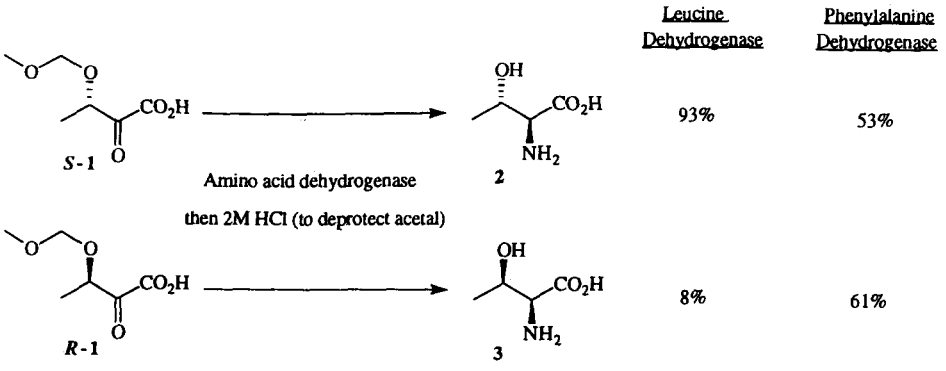
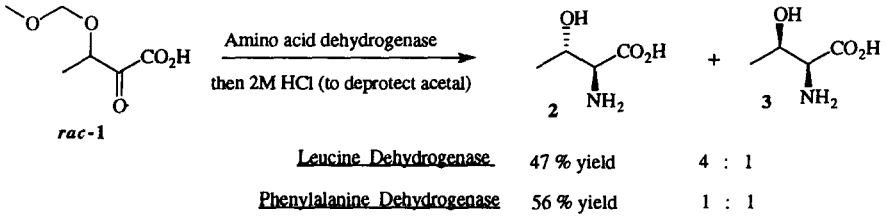


Scheme 1

difference in the yield of L-*allo*threonine and L-threonine (93% versus 8% respectively) with leucine dehydrogenase indicated that this enzyme exhibited a marked preference for the (*S*)-enantiomer. Although there are many examples of esterase and lipase catalysed kinetic resolutions,[6] an amino acid dehydrogenase catalysed resolution has not been reported previously.

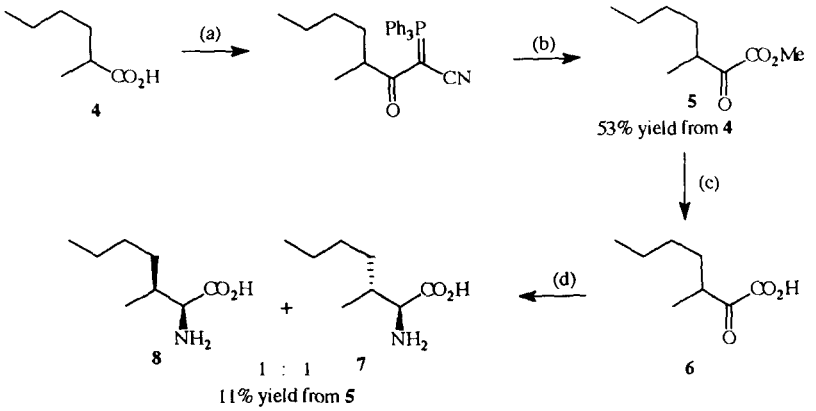
To confirm this observation a racemic mixture of the α-keto acid ***rac*-1** was prepared and incubated with leucine dehydrogenase (scheme 2). Removal of the protecting group and purification by ion exchange chromatography gave L-*allo*threonine **2** and L-threonine **3** in a 4 : 1 ratio (by ^{1}H NMR spectroscopy). In contrast, reductive amination of ***rac*-1** with phenylalanine dehydrogenase gave after deprotection, the two amino acids **2** and **3** in a 1 : 1 ratio.



Scheme 2

Kula and coworkers[3b] have found that leucine dehydrogenase accepts 2-oxo acids with hydrophobic side chains up to eight C-atoms. Hence to investigate if leucine dehydrogenase would accept a 2-oxo acid with a branched hydrocarbon side chain of the same length as the MOM-protected substrate **1**, methyl (*rac*)-3-methyl-2-oxoheptanoate **5** was prepared. The synthetic approach involved the one carbon homologation of commercially available (*rac*)-2-methylhexanoic acid **4** *via* ozonolysis of a β-ketocyanophosphorane[7] as shown in scheme 3. Lipase catalysed hydrolysis of **5** followed by reductive amination of the resultant 2-oxo acid **6** with leucine dehydrogenase gave (2*S*,3*R*)- and (2*S*,3*S*)-2-amino-3-methylheptanoic acids **7** and **8** in only 11% yield and with no resolution at the C-3 position.

It has been suggested that the enzyme superfamily of amino acid dehydrogenases are related by tertiary structure, nucleotide binding and catalytic mechanism with the main differences being in the side chain pockets where subtle point mutations discriminate between substrates giving each



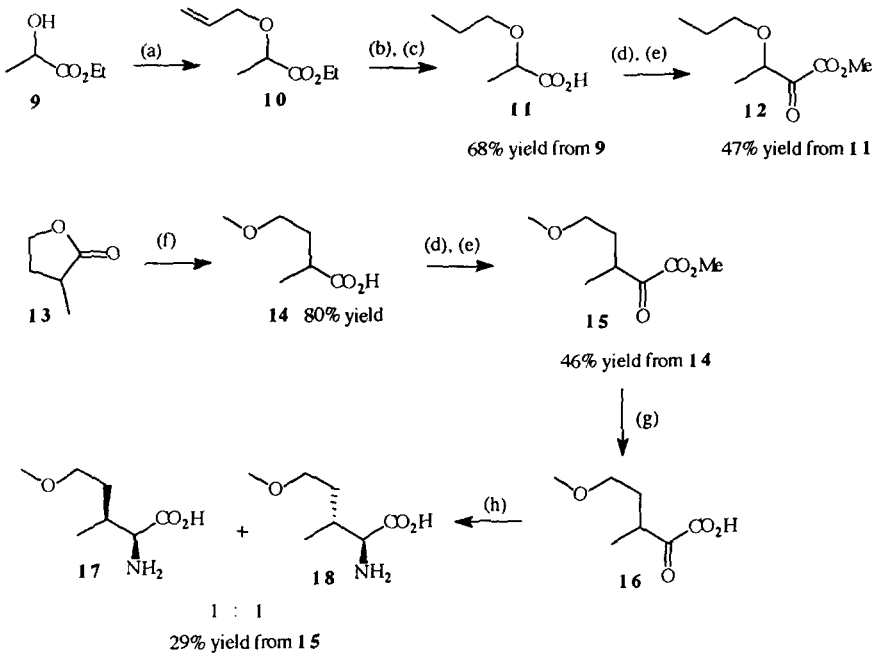
Reagents: (a) EDCI, DMAP, Ph_3PCHCN; (b) O_3, MeOH, CH_2Cl_2; (c) *Candida rugosa* lipase; (d) leucine dehydrogenase, formate dehydrogenase, NADH, HCO_2NH_4

Scheme 3

enzyme its characteristic specificity.[8] The X-ray crystal structure of leucine dehydrogenase from *Bacillus sphericus* has recently been reported by Rice and coworkers on the open conformation of this protein.[9] It has been tentatively suggested[9] that the hydrophobic side-chain of leucine is bounded by Leu40, Gly41, Ala131, Val291 and Val294 and the shape and character of the pocket is in keeping with preferred substrates for this enzyme having branched hydrocarbon side-chains.

One explanation for the resolution of ***rac*-1** is an electronic interaction of either (or both) side-chain oxygen atoms in the substrate with, as yet, unidentified polar amino acid residue(s) in the side-chain binding pocket. To investigate whether the enzyme required both side chain oxygen atoms, two further 2-oxobutanoates **12** and **15** were prepared each containing a single oxygen atom in the C-3 side-chain (scheme 4). Methyl (*rac*)-3-propyloxy-2-oxobutanoate **12** was prepared by an analogous approach to that used for the synthesis of **5**. Attempts to protect alcohol **9** with base and propyl iodide gave very poor yields of the required ether. However, propyl ether **11** was prepared in 68% yield by a three step process involving reaction of ethyl lactate with sodium hydride and allyl bromide followed by catalytic hydrogenation of the resultant allyl ether **10** and finally hydrolysis of the ester. Methyl 5-methoxy-3-methyl-2-oxopentanoate **15** was prepared in 3 steps from (*rac*)-α-methyl-γ-butyrolactone **13** using sodium methoxide to open the γ-lactone ring to acid **14**[10] followed by a one carbon homologation.

Although the 2-oxo acid formed from lipase catalysed hydrolysis of **12** was not a substrate for leucine dehydrogenase, the isomer, 5-methoxy-3-methyl-2-oxopentanoic acid **16** gave a 29% yield of a 1 : 1 mixture of diastereomers **17** and **18** (scheme 4). From these results we can conclude that for the combined kinetic resolution and reductive amination of 3-substituted-2-oxobutanoic acids, two oxygen atoms are required in the side-chain and that selective replacement of the oxygen atoms by methylene units not only destroys the preference of the enzyme for one enantiomer but also significantly reduces the ability of leucine dehydrogenase to catalyse the reductive amination of the 2-oxo acid. To fully understand the significance of these results, further structural information on the binding of the substrate in the active site of the enzyme is required.



Reagents: (a) Allyl bromide, NaH, THF; (b) H_2, Pd on C, EtOH; (c) LiOH, MeOH, H_2O
(d) EDCI, DMAP, Ph_3PCHCN; (e) O_3, MeOH, CH_2Cl_2; (f) Na, MeOH;
(g) *Candida rugosa* lipase; (h) leucine dehydrogenase, formate dehydrogenase, NADH, HCO_2NH_4.

Scheme 4

Acknowledgements

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References and Notes

1. Kelly, N. M.; Sutherland, A.; Willis, C. L., *Nat. Prod. Rep.*, **1997**, *14*, 205.
2. See for example: (a) Cappon, J.J.; Baart, J.; van der Walle, G. A. M.; Raap, J.; Lugtenburg, J., *Recl. Trav. Chim. Pays-Bas*, **1991**, *110*, 158. (b) Kragl, U.; Godde, A.; Wandrey, C.; Kinzy, W.; Cappon, J. J.; Lugtenburg, *Tetrahedron: Asymmetry*, **1993**, *4*, 1193. (c) Kelly, N. M.; O'Neill, B. C.; Probert, J.; Reid, G.; Stephen, R.; Wang, T.; Willis, C. L.; Winton, P., *Tetrahedron Lett.*, **1994**, *35*, 6533. (d) Ogrel, A.; Vasilenko, I. A.; Lugtenburg, J.; Raap, J., *Recl. Trav. Chim. Pays-Bas*, **1994**, *113*, 369.
3. (a) Asano, Y.; Yamada, A.; Kato, Y.; Yamaguchi, K.; Hibino, Y.; Hirai, K.; Kondo, K., *J. Org. Chem.*, **1990**, *55*, 5567. (b) Krix, G.; Bommarius, A. S.; Drauz, K.; Kottenhahn, M.; Schwarm, M.; Kula, M.-R., *J. Biotech.*, **1997**,*53*, 29.
4. (a) Bradshaw, C. W.; Wong, C.-H.; Hummel, W.; Kula, M.-R., *Bioorganic Chemistry*, **1991**, *19*, 29. (b) Yonaha, K; Soda, K, *Adv. in Biochem. Eng./ Biotech.*, **1986**, *33*, 95.
5. Sutherland, A.; Willis, C. L., *Tetrahedron Lett.*, **1997**, *38*, 1837.
6. For a review see: Turner, N. J., *J. Prod. Rep.*, **1994**, *11*, 1.
7. Wasserman, H. H.; Ho, W.-B., *J. Org. Chem.*, **1994**, *59*, 4364.
8. (a) Britton, K. L.; Baker, P. J.; Engel, P.C.; Rice, D. W.; Stillman, T. J., *J. Mol. Biol.*, **1993**, *234*, 938. (b) Hummel, W.; Kula, M.-R., *Eur. J. Biochem*, **1989**, *184*, 1. (c) Nagata, S.; Tanizawa, K.; Eski, N.; Sakamoto, Y.; Ohshima, T.; Tanaka, H.; Soda, K.; *Biochemistry*, **1988**, *27*, 9056.
9. Baker, P. J.; Turnbull, A. P.; Sedelnikova, S. E.; Stillman, T. J.; Rice, D. W., *Structure*, **1995**, *3*, 693.
10. Granduillot, J. C.; Rouessac, F., *Tetrahedron*, **1991**, *47*, 5133.