Enantioselective Syntheses of (*S*)- and (*R*)-3-Hydroxypyrrolidin-2-ones *via* Lactate Dehydrogenase Catalysed Reductions of 4-Benzyloxycarbonylamino-2-oxobutanoic Acid

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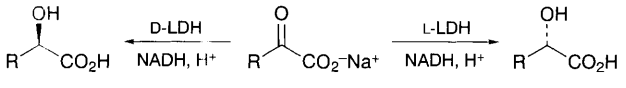
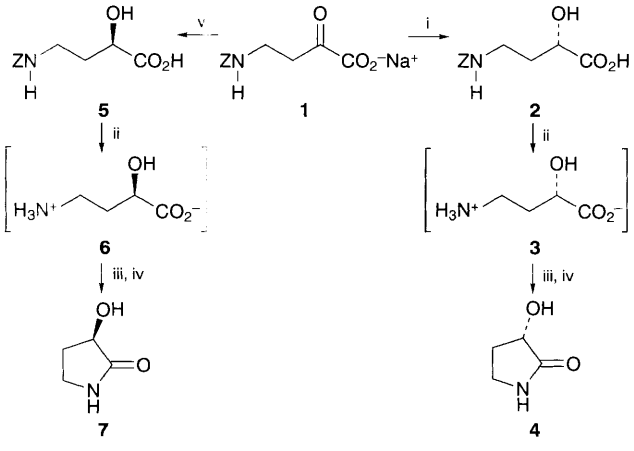
The first examples of the *BS*- and *SE*-lactate dehydrogenase catalysed reductions of an α-keto acid incorporating a nitrogen containing function in the side chain are described: (*S*)- and (*R*)-benzyloxycarbonylamino-2-hydroxybutanoic acids were prepared in good yield and excellent enantioselectivities and were converted to the (*S*)- and (*R*)-3-hydroxypyrrolidin-2-ones respectively.

Transformations based upon enzymatic catalysis are providing an increasingly valuable component of the methodology of enantioselective synthesis. The reduction of α-keto acids catalysed by lactate dehydrogenases (LDHs) has been established as a valuable method for the synthesis of homochiral (*S*)- and (*R*)-2-hydroxy acids (Scheme 1). L-LDHs from various mammalian and bacterial sources are commercially available and have been found to accommodate a diversity of structural variations in the side chain of the α-ketoacids including saturated[1] or unsaturated[2] hydrocarbon side chains, halides[3] and oxygen containing functions.[4] Here we describe the first examples of L- and D-LDH catalysed reductions of an α-keto acid with a nitrogen containing function in the side chain and the conversion of the enzyme products to homochiral 3-hydroxypyrrolidin-2-ones.

The sodium salt of 4-benzyloxycarbonylamino-2-oxobutanoic acid **1** was selected to investigate the tolerance of LDHs to a nitrogen-containing α-keto acid. It was chosen since the expected product, (*S*)-2-hydroxy acid **2** is an excellent precursor to (*S*)-4-amino-2-hydroxybutanoic acid **3** which is one of the most potent known inhibitors of the neurotransmitter 4-aminobutanoic acid and it exhibits anticancer activity.[5] In addition the (*S*)- and (*R*)-2-hydroxy acids **2** and **5** may be converted to the homochiral 3-hydroxypyrrolidin-2-ones **4** and **7** respectively which are valuable building blocks to more complex molecules (Scheme 2).



Scheme 1

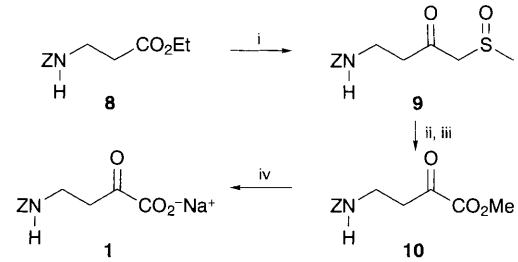


Scheme 2 *Reagents and conditions*: i, *BS*-LDH, NADH, H+; ii, H_2, 5% Pd on C; iii, $(Me_3Si)_2SiNH$, TMSCl, xylene, heat; iv, TFA, THF–H_2O (20 : 1); v, *SE*-LDH, NADH, H+ Z = $PhCH_2OC(:O)$

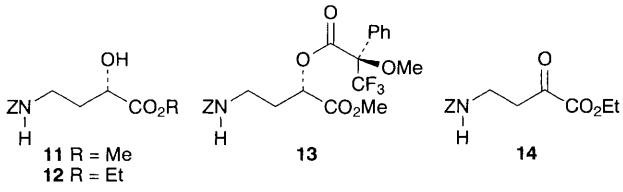
The enzyme substrate **1** was prepared from methyl 4-benzyloxycarbonylamino-2-oxobutanoate **10** which in turn, was synthesised from *N*-benzyloxycarbonyl-β-alanine ethyl ester **8** following a 3 stage literature procedure (Scheme 3).[6] Reaction of **8** with dimsylsodium followed by a Pummerer type rearrangement of the resultant β-keto-sulfoxide **9** gave the methyl ester **10** which was carefully saponified using sodium hydroxide to give the required salt **1**.

Initially we incubated **1** with lactate dehydrogenase from *Bacillus stearothermophilus* (*BS*-LDH) which is commercially available and is ideally suited for use as a synthetic catalyst because it is thermostable and has a broad substrate specificity. Lactate dehydrogenases are nicotinamide coenzyme dependent and the *BS*-LDH catalysed reduction of **1** was successfully carried out on a 1 mmol scale using the formate–formate dehydrogenase (FDH) protocol developed by Shaked and Whitesides[7] to recycle the NADH *in situ*. The reaction was complete within 40 h giving the (*S*)-2-hydroxy acid **2** in 91% yield and >99% ee (Scheme 2).†

The enantiomeric purity of **2** was determined by formation of the methyl ester **11** using ethereal diazomethane which was then converted to the (*R*)-(+)-MTPA (α-methoxy-α-trifluoromethylphenylacetate)[8] derivative **13** and comparison of the ^1H and ^{19}F NMR spectra (500 MHz) with a racemic standard prepared *via* reduction of **1** with sodium borohydride. The signals attributed to the individual diastereoisomers were well resolved allowing the minor component to be detected in quantities as low as 0.5%.‡ The assignment of the absolute configuration of the (*S*)-2-hydroxy acid **2** was made by analysis of the ^1H NMR spectrum of the (*R*)-(+)-MTPA derivative **13** according to the correlation methods of Mosher[9] and



Scheme 3 *Reagents and conditions*: i, Me_2SO, NaH; 61%; ii, NBS; iii, MeOH, H+, 52%; iv, NaOH, 95%



Yamaguchi.[10] This assignment was confirmed by conversion of **2** to the known (*S*)-4-amino-2-hydroxybutanoic acid **3**[10] by catalytic hydrogenolysis of the Z protecting group to give **3**, mp 201 °C, $[\alpha]_D$ −28.58 (*c* = 1.016, H_2O), lit[11] mp 203–206 °C, $[\alpha]_D$ −28.2 (*c* = 1.22, H_2O). Cyclisation of the γ-amino-α-hydroxy acid **3** under the conditions of Srairi and Maurey[12] gave (*S*)-3-hydroxypyrrolidin-2-one **4** in 72% overall yield from **2**.

The use of *BS*-LDH for the preparation of the (*S*)-2-hydroxy acid **2** compares favourably with two previous approaches for the reduction of the α-keto ethyl ester **14** using baker's yeast which were reported to give the corresponding (*S*)-2-hydroxy ester **12** in 47% yield (49% ee)[6] and 54% yield (88% ee).[13]

In order to prepare the enantiomeric (*R*)-2-hydroxy acid **5**, LDH from *Staphylococcus epidermidis* (*SE*-LDH) was used as the catalyst. *SE*-LDH is commercially available and inexpensive. Several (*R*)-2-hydroxy acids have been prepared previously in high optical purity and good yield from α-keto acids using this enzyme.[14] The *SE*-LDH catalysed reduction of **1** was successfully carried out using an analogous procedure to that used for the *BS*-LDH reaction described above. The reaction was more sluggish than in the case of *BS*-LDH but, on a 1 mmol scale, was complete within 7 days giving the (*R*)-2-hydroxy acid **5** in 95% yield and >99% ee (Scheme 2). Again the enantiomeric excess was determined by formation of the (*R*)-(+)-MTPA derivative of the methyl ester and comparison of the ^1H and ^{19}F NMR spectra with those of a racemic standard. Reductive cleavage of the CBz protecting group in **5** followed by cyclisation of **6** gave (*R*)-3-hydroxypyrrolidin-2-one **7** in 70% yield. Using this approach to prepare **7**, the sample gave $[\alpha]_D$ +121.7 (*c* = 2.07, $CHCl_3$) in accord with the literature value[15] of $[\alpha]_D$ +121.9 (*c* = 0.7, $CHCl_3$).

In conclusion it is apparent that the sodium salt of the Z-protected amino acid **1** is a good substrate for *BS*-LDH giving the (*S*)-2-hydroxy acids **2** in excellent yield and enantioselectivity. The *SE*-LDH catalysed reduction of **1** is slower but gives the (*R*)-2-hydroxy acid **5** in good yield and optical purity. The homochiral α-hydroxy acids **2** and **5** were each converted in good yield to the 3-hydroxypyrrolidin-2-ones **4** and **7** respectively.

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Footnotes

† *Typical procedure for the lactate dehydrogenase catalysed reduction.*— 4-Benzyloxycarbonylamino-2-oxobutanoic acid salt **1** (0.26 g, 1 mmol) and sodium formate (65 mg, 1 mmol) in aqueous tris buffer (50 ml) were deoxygenated by bubbling through nitrogen gas for 0.5 h. Dithiothreitol (1 ml l^{-1}, 2 μl), LDH from *Bacillus stearothermophilus* (5 mg), NADH (5 mg) and formate dehydrogenase (5 mg) were added and the mixture was stirred at room temp. under a nitrogen atmosphere for 40 h. The pH was maintained between 6.5 and 7.0 by the addition of dilute hydrochloric acid. When no further change in pH was observed the water was removed *in vacuo*. Saturated brine (10 ml) and concentrated hydrochloric acid were added and the mixture was extracted with ethyl acetate (3 × 30 ml). The combined organic phases were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give (*S*)-4-benzyloxycarbonylamino-2-hydroxybutanoic acid **2** which crystallised from ethyl acetate–light petroleum (0.22 g, 91%). mp 72 °C (lit[17] mp 77–78 °C); $[\alpha]_D$ +4.9 (*c* = 4.6, $CHCl_3$, 25 °C), lit[17] $[\alpha]_D$ +5.7 (*c* = 1, $CHCl_3$); (Found: C, 56.5; H, 6.0; N, 5.4 $C_{12}H_{15}O_5N$ requires C, 56.9; H, 5.9; N, 5.5%); ^1H NMR δ 1.88 and 2.04 (2 × m, CH_2CHOH), 3.37 (m, CH_2N), 4.26 (m, *CH*OH), 5.07 (s, CH_2Ph), 5.45 (m, N*H*) and 7.37 (m, C_6H_5); *m/z* (CI) 254 (M + 1^+, 1.5%), 210 (10), 146 (20), 102 (23), 91 (100) and 79 (30).

The reaction was repeated using LDH from *Staphylococcus epidermidis* and was complete after 7 days to give (*R*)-4-benzyloxycarbonylamino-2-hydroxybutanoic acid **5** in 95% yield mp 70.5 °C (from ethyl acetate–light petroleum), (lit[17] mp 76.5–78 °C, from EtOH); $[\alpha]_D$ −4.1 (*c* = 10.0 in $CHCl_3$), lit[17] $[\alpha]_D$ −5.0 (*c* = 1, $CHCl_3$); Found: C, 56.8; H, 6.1; N, 5.5 $C_{12}H_{15}O_5N$ requires C, 56.9; H, 5.9; N, 5.5%).

‡ *Selected NMR data* for (*RS*) diastereoisomer of **13**: ^{19}F NMR, δ −71.73; ^1H NMR, δ 3.63 (s, CO_2Me), 3.42 (s, OMe). For (*RR*) diastereoisomer of **13**; ^{19}F NMR, δ −71.41; ^1H NMR, δ 3.68 (s, CO_2CH_3), 3.58 (s, OCH_3).

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