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## Synthesis of Optically Pure (R)-2-Hydroxy Acids using D-Lactate Dehydrogenase

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Several (*R*)-2-hydroxy acids have been prepared in high optical purities and good yields using D-lactate dehydrogenase (*Staphylococcus epidermidis*) as the catalyst.

Optically active 2-hydroxy acids, and simple derivatives thereof, are a versatile class of molecules which have been extensively exploited as chiral building blocks<sup>1,2</sup> and reagents<sup>3–6</sup> in asymmetric synthesis. Among procedures currently available for their preparations, including chemical methods,<sup>7,8</sup> fermentation<sup>9,10</sup> and enzymatic catalysis,<sup>11–16</sup> those using cell-free enzymes as the catalysts provide higher optical purity. For example, the procedure using rabbit muscle L-lactate dehydrogenase (RMLDH)<sup>14</sup> can provide a broad range of (*S*)-2-hydroxy acids in greater than 99% enantiomeric excess (e.e.). We herein report a complementary procedure using *Staphylococcus epidermidis* D-lactate dehydrogenase (SELDH) as the catalyst which provides products of opposite configuration in optically pure forms.

SELDH is commercially available and inexpensive. To the best of our knowledge, it has never been explored as a catalyst in organic synthesis. The objective of this work was to explore the range of substrates accepted by SELDH at a rate useful in organic synthesis and illustrate practical synthetic procedures using SELDH. The syntheses of four different (R)-2-hydroxy acids using SELDH as the catalyst are now described, including (R)-2-hydroxybutanoic acid **2a**, (R)-cyclopropaneglycolic acid **2b**, (R)-phenyllactic acid **2c** and (R)-2-hydroxypentanoic acid **2d**.

The syntheses were performed on a gram scale using compounds **1a–1d** as the substrates and formate/formate dehydrogenase (FDH) as the NADH-regenerating system<sup>17</sup> (Scheme 1). In typical experiments, the enzymes (SELDH and FDH) enclosed in dialysis tubing were submerged in an aqueous solution (100 ml; pH 7.5) containing substrate (1 equiv.), formate (1.1 equiv.), NADH (0.005–0.01 equiv.),



Scheme 1 SELDH-catalysed synthesis of (R)-2-hydroxy acids

Tris [(HOCH<sub>2</sub>)<sub>3</sub>CNH<sub>2</sub>; 0.1 equiv.] and mercaptoethanol (0.01 equiv.). Reactions were carried out at 25 °C under nitrogen. The pH was kept between 7.4 and 7.6 by the controlled addition of 1.0 mol dm<sup>-3</sup> HCl. The reaction was stopped when the theoretical amount of hydrochloric acid had been added. The mother liquor, after usual work-up,<sup>14</sup> provided the pure products as white solids.

The results from these syntheses are summarized in Table 1. The yields ranged from 80 to 92%. The absolute configuration and optical purities of the products were determined on the basis of the analysis of <sup>1</sup>H NMR spectra of the (*R*)-(+)-MTPA [ $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenylacetyl] derivatives;<sup>18,19</sup> all the enzymatically reduced products have the *R* configuration and high optical purities (>99% e.e.).

This procedure thus uses enzymes that are commercially available and stable under the given conditions; the enzymes can be recycled three times without losing a significant amount of activity. It employs dialysis tubing for manipulation of the enzymes, so that the soluble enzymes can be used without being immobilised on a polymer. It provides the products in excellent optical purities and good yields, and is a complementary route to optically pure 2-hydroxy acids.

To illustrate the use of (*R*)-2-hydroxy acids as chiral synthons, we synthesized (*R*)-cyclopropyloxirane  $\{5, [\alpha]_D^{25}\}$ 



Scheme 2 Reagents and conditions: i, BH<sub>3</sub>, tetrahydrofuran, -10 to 25 °C, 24 h, 100%; ii, p-MeC<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>Cl, pyridine, -5 °C, 2 h, 85%; iii, Na, (CH<sub>2</sub>OH)<sub>2</sub>, -10 °C, 1 h, 45%

Table 1 SELDH-catalysed reductions of 2-oxo acids to (R)-2-hydroxy acids [RCH(OH)CO<sub>2</sub>H]

R	Product	Conditions							
		Scale (mmol)	SELDH <sup>a</sup> (units)	FDH <sup>b</sup> (units)	NADH (mmol)	Formate (mmol)	Time (days)	Yield (%)	E.e. (%) <sup>c</sup>
Et	2a	40	100	25	0.2	44.0	6.0	86	>99
cPr	2Ь	15	1000	25	0.1	16.5	4.0	86	>99
$PhCH_2$	2c	10	1700	25	0.1	11.0	3.4	80	>99
Prn	2d	15	d	d	0.1	16.5	$12.0^{e}$	92	>99

<sup>*a*</sup> One unit of SELDH reduces 1.0 mmol of pyruvate to (*R*)-lactate per min in the presence of NADH at pH 7.0 and 25 °C. <sup>*b*</sup> FDH, formate dehydrogenase. One unit of FDH liberates 1 mmol of CO<sub>2</sub> from formate per min in the presence of NAD at pH 7.5 and 25 °C. <sup>*c*</sup> Enantiomeric excess, determined by <sup>1</sup>H NMR analysis of (*R*)-(+)-MTPA esters.<sup>18 d</sup> The enzymes recovered from the reaction for the preparation of **2c** were used. <sup>*e*</sup> The longer reaction time may be due to the lower reactivity of the substrate.

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<sup>+</sup> <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 2.73 (m, 2H), 2.53 (dd, J 3.6 and 4.5 Hz, 1 H), 0.85 (m, 1 H), 0.52 (m, 2 H) and 0.36 (m, 2 H). These data are in good agreement with those for racemic 5.20

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