Synthesis of Enantiomerically Pure α-Hydroxyaldehydes from the Corresponding α-Hydroxycarboxylic Acids: Novel Substrates for *Escherichia coli* Transketolase

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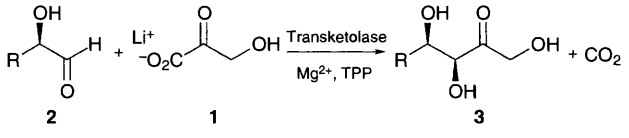
Enantiomerically pure (*R*)-α-hydroxyaldehydes (>95% ee) are prepared from the corresponding α-hydroxyesters by silyl protection, reduction with diisobutylaluminium hydride, and finally deprotection under acidic conditions; subsequent coupling of these aldehydes with lithium hydroxypyruvate, catalysed by *Escherichia coli* transketolase, leads to novel optically pure triols.

Transketolase [E.C. 2.2.1.1] catalyses the transfer of a two-carbon ketol unit, $C(O)CH_2OH$, stereospecifically to the carbonyl terminus of a variety of aldehydes, especially carbohydrates *e.g.* ribose-5-phosphate and erythrose-4-phosphate.[1] The reaction has synthetic utility when the lithium salt of β-hydroxypyruvic acid **1** is employed as the donor substrate since CO_2 is evolved and the reaction becomes essentially irreversible[2] (Scheme 1). A wide range of α-hydroxy-, α-oxo and α-unsubstituted aldehydes have been employed as acceptor substrates in carbon–carbon bond syntheses using transketolase from yeast,[3,4,5] spinach[3] and *Escherichia coli*.[6a] Condensation of **1** with α-hydroxylated aldehydes **2** of *R*-configuration yields (3*S*,4*R*) vicinal diols **3** of high optical purity.

As part of an ongoing study into the development of a biocatalytic process for asymmetric carbon–carbon bond formation on a large scale,[6b] we were interested in establishing a general synthetic route to (*R*)-α-hydroxylated aldehydes **2** in enantiomerically pure form. Many of the best substrates for transketolase are α-hydroxyaldehydes,[3–6a] but relatively few general and efficient syntheses of these compounds, in unprotected form, have been reported in the literature.[4,5,7] Since a large number of methods exist for the preparation of α-hydroxy acids in optically active form (*e.g.* diazotisation of α-amino acids,[8] asymmetric reduction of α-keto acids[9]) we set out to develop a method based on these as precursors (Scheme 2). Here we report that α-hydroxy acids can be converted to the corresponding unprotected α-hydroxy aldehydes in good yields and with no loss of optical activity. Furthermore, we show that the aldehydes can be used as substrates for *E. coli* transketolase.

As outlined in Scheme 2, the chiral α-hydroxy acids were converted to the methyl esters **4a–c** followed by silylation (**5a–c**) and then reduction with DIBAL-H to give the silyl protected aldehydes **6a–c** which were not purified but simply deprotected in the work-up (acidic conditions for TMS; TBAF for TBDMS) to furnish the α-hydroxyaldehydes **2a–2c**.† The choice of TBDMS or TMS protection was based on a consideration of the volatility of the respective intermediates. ^1H chiral shift NMR using $Eu(hfc)_3$ confirmed that all of the relevant intermediates for the synthesis of (*R*)-**2a** and (*R*)-**2c** possessed ee >95%. The optical purities of (*R*)-**2a** and (*R*)-**2c** were determined by conversion to the corresponding diethylacetals **7a** and **7c** followed by ^1H chiral shift NMR as above and in both cases were shown to be >95%. The diethylacetals **7** represent a convenient method of storing the α-hydroxyaldehydes since they are stable and can be simply hydrolysed back under acidic conditions. It is significant that for (*R*)-**2c**, which contains a labile α-H atom, there was no evidence of either racemisation or rearrangement to the α-ketoalcohol.



2 **1** **3**

Scheme 1 Transketolase-catalysed condensation between lithium hydroxypyruvate **1** and an (*R*)-α-hydroxyaldehyde **2**

The aldehyde products **2a–c** were isolated as clear, very viscous oils which rapidly oligomerised when treated with D_2O, the resulting waxy solids proving difficult to handle. The ^1H NMR spectra of the aldehydes in solvents other than D_2O showed a complex mixture of signals which could not be readily assigned; in D_2O the spectra consisted mainly of a mixture of aldehyde and its hydrate, with the time taken to reach equilibrium in the solution varying from several hours (**2b**) to more than 7 days (**2c**). These observations clearly have implications for the use of α-hydroxyaldehydes as substrates in enzyme catalysed reactions.

Condensation of the aldehydes (*R*)-**2a** and (*R*)-**2c** with lithium hydroxypyruvate in the presence of transketolase was performed either in glycylglycine buffer at pH 7.0, or in unbuffered medium using a pH stat (pH maintained at 7.0 with 1 mol dm^{-3} HCl) (Table 2). Both (*R*)-**2a** and (*R*)-**2c** yielded single diastereoisomer products (as judged by ^1H and ^{13}C NMR analysis) in good yields.‡ In one reaction of (*R*)-**2a**, which was not taken to completion, a 13% yield of the unreacted aldehyde was isolated, converted to the diethyl acetal, and shown by ^1H chiral shift NMR to be enantiomerically pure, demonstrating that there is no racemisation of the substrate under the conditions of the biotransformation. The specificity of the *E. coli* transketolase for α-hydroxyaldehydes of (*R*)-configuration was established by the observation that (±)-**2a** yielded the triol **3a** as a single diastereoisomer and by the fact that (*S*)-**2c**, prepared from (*S*)-mandelic acid, failed to undergo the enzyme catalysed coupling reaction.

In conclusion, a method for the synthesis of enantiomerically pure α-hydroxyaldehydes has been developed, utilising readily available α-hydroxy acids as the precursors. Condensation of these (*R*)-α-hydroxyaldehydes with lithium hydroxypyruvate using transketolase from *E. coli* generates novel [3*S*,4*R*] chiral triols.

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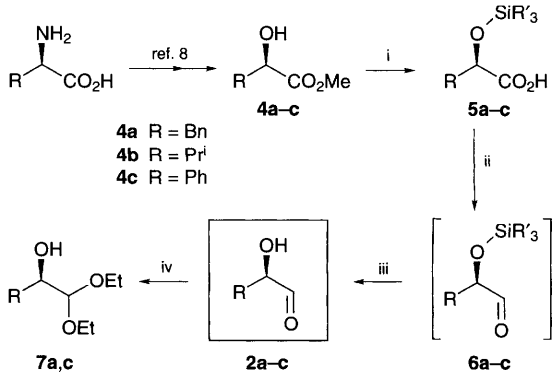


Scheme 2 Synthesis of chiral α-hydroxyaldehydes. *Reagents and conditions*: i, TMSCl or TBDMSCl/imidazole (see Table 1); ii, DIBAL-H/toluene/−78 °C; iii, deprotection (see Table 1); iv, EtOH/Amberlyst-15

Table 1 Synthesis of α-hydroxyaldehydes **2a–c**

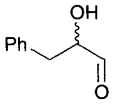
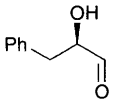
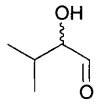
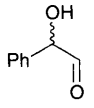
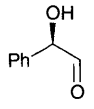
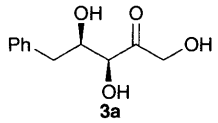
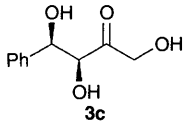
Aldehyde	Precursor	Protecting group	Deprotection conditions	Yield(%) (i)	(ii)–(iii)	(iv)	ee (%)
 (±)-**2a**	DL-Phenylalanine	TMS	AcOH/H_2O/THF	58	69	81	—
 (*R*)-**2a**	D-Phenylalanine	TMS	AcOH/H_2O/THF	82	95	63	>95
 (±)-**2b**	DL-Valine	TBDMS	TBAF/THF	73	7§	—	—
 (±)-**2c**	DL-Phenylglycine	TMS	AcOH/H_2O/THF	77	56	65	—
 (*R*)-**2c**	(*R*)-Mandelic acid	TMS	AcOH/H_2O/THF	8	64	7	>95

Table 2 Preparative biotransformation results

Aldehyde	Conditions	Yield of product(s)	Product
(*R*)-**2a**	(i) Buffer (ii) pH stat	(i) 44% (ii) 54%	 **3a**
(*R*)-**2c**	pH stat	44%	 **3c**

shift NMR studies, and to Drs Mark Smith and Gail Morris for helpful discussions.

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Footnotes

† *Example procedure: Preparation of* (R)-*2-hydroxy-3-phenylpropanal* (R)-**2a**: Methyl (*R*)-3-phenyl-2-(trimethylsilyloxy)propanoate **5a** (1.087 g, 4.31 mmol) was dissolved in toluene (25 cm^3), cooled to −78 °C with an acetone-dry ice bath, and the reaction vessel flushed with nitrogen. Diisobutylaluminium hydride (DIBAL-H) (1.5 mol dm^{-3} in toluene; 5.5 cm^3, 8.25 mmol) was added dropwise, and the mixture stirred at −78 °C for 1 h. The mixture was quenched at −78 °C by cautious addition of aq. HCl (2 mol dm^{-3}; 25 cm^3), extracted with ethyl acetate (3 × 20 cm^3), and the combined organic extracts dried ($MgSO_4$) and evaporated *in vacuo*. The resulting crude (*R*)-α-(trimethylsilyloxy)aldehyde **6a** was redissolved in 10 cm^3 of a 3 : 1 : 1 mixture of acetic acid : water : THF, and stirred for 17 h. After removal of the solvents *in vacuo*, the residue was redissolved in ethyl acetate (2 cm^3) and purified by flash chromatography on silica gel [petroleum (40/60)–ethyl acetate (2 : 1) as eluent]. The aldehyde (*R*)-**2a** was obtained as a clear, very viscous oil: 612 mg (4.08 mmol, 95%); ^1H NMR (250 MHz; D_2O) δ 2.81 (1 H, dd, *J* 9.5 Hz, 14.0, PhC*H*H), 3.11 (1 H, dd, *J* 3.2 Hz, 14.0, PhCH*H*), 3.88 (1 H, m, α-CH), 5.02 [1 H, d, *J* 4.9 Hz, C*H*$(OD)_2$] and 7.47 (5 H, m, Ph); ^{13}C NMR (126 MHz; D_2O) δ 37.8 (CH_2), 75.2 (α-CH), 91.8 [$CH(OD)_2$], 126.9, 129.0, 129.9 (aromatic CH) and 138.9 (aromatic quaternary).

‡ *Biotransformation of* (R)-*2-hydroxy-3-phenylpropanal* (R)-**2a** *in unbuffered medium.* The product (*3S,4R*)-*1,3,4-trihydroxy-5-phenylpentan-2-one* **3a** was obtained as a pale yellow viscous oil: 450 mg (54%); $[\alpha]_D^{23}$ + 26.7° (*c* 1.07, $CHCl_3$); ^1H NMR (300 MHz; $CDCl_3$) δ 2.69 (1 H, s, OH), 2.90 (2 H, m, $PhCH_2$), 3.31 (1 H, s, OH), 3.68 (1 H, d, *J* 6.3 Hz, OH), 4.16 [2 H, m, C*H*(OH)C*H*(OH)], 4.43 (2 H, AB-quartet, CH_2OH) and 7.31 (5 H, m, Ph); ^{13}C NMR (62 MHz; $CDCl_3$) δ 39.7 (CH_2), 66.5 [C(O)CH_2OH], 73.3 (CH), 76.8 (CH), 127.0, 128.8, 129.5 (aromatic CH), 137.3 (aromatic quaternary) and 211.6 (C=O).

§ Owing to the volatility of the intermediates in this instance, the protecting group was changed from TMS to TBDMS, but this protecting group proved difficult to remove. Deprotection with tetrabutylammonium fluoride (TBAF) produced a number of side products by TLC, the α-hydroxyaldehyde (±)-**2b** being isolated in low yield. Enzyme assay according to the procedure described in reference 6*a* showed aldehyde **2b** to be a substrate, with a relative rate of 5% that of an equiv. concentration of glycoaldehyde.

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