Stereospecific formation of *R*-aromatic acyloins by *Zymomonas mobilis* pyruvate decarboxylase

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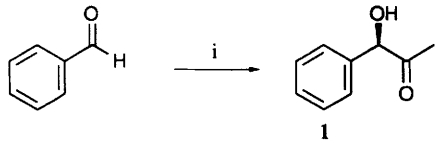
Recombinant pyruvate decarboxylase from *Zymomonas mobilis* catalysed the formation of *R*-aromatic acyloins of high optical purity from aromatic aldehydes and either pyruvate or acetaldehyde. The results are contrasted with those obtained with aliphatic acyloins and compared with those obtained with the pyruvate decarboxylase of *Saccharomyces* sp.

The first report of an unnatural biological acyloin product was published over 70 years ago. It described the transformation of benzaldehyde into 1-hydroxy-1-phenylpropan-2-one **1** ('phenylacetylcarbinol', PAC) using fermenting brewer's yeast (Scheme 1).[1] The PAC **1** was later shown to have the *R* configuration.[2]



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Scheme 1 *Reagent:* i, brewer's yeast

In 1930, PAC found its industrial application[3] in the production of (−)-ephedrine. It has been demonstrated only recently that pyruvate decarboxylase (PDC) is the enzyme responsible for the formation of acyloins in yeast.[4–6] The PDC isolated from *Zymomonas mobilis* has also been shown to catalyse the formation of PAC **1**.[4]

A study of the substrate- and stereo-specificity of the PDCs from yeast (*Saccharomyces*, sp.) and *Z. mobilis* showed that the yeast enzyme catalysed the formation of a series of *R*-aromatic acyloins of high optical purity.[7] We describe here the stereochemistry of the acyloin condensations catalysed by the *Z. mobilis* enzyme with aromatic aldehydes and studies with heterocyclic and aliphatic aldehydes.

Recombinant *Z. mobilis* PDC was purified to homogeneity from an *Escherichia coli* DH1 strain transformed with the plasmid pLOI295 harbouring the gene encoding the *Z. mobilis* ATCC 31821 PDC. Active PDC comprised 27% of the soluble cell protein of this over-producing strain, permitting the isolation of large quantities of the enzyme.

A range of aromatic aldehydes **2** were found to be suitable substrates for the condensation with 'active acetaldehyde' generated from pyruvate using the *Z. mobilis* enzyme (Scheme 2 and Table 1). The formation of products was monitored by direct ^{1}H NMR spectroscopy of the reaction mixtures. The products were isolated by simple extraction using ethyl acetate and characterised by ^{1}H NMR spectrospcopy and GC–MS. In general, the *Z. mobilis* PDC gave lower yields of the aromatic acyloins **3** than the yeast enzyme.[7] This may be attributable to the higher K_M for benzaldehyde for the *Z. mobilis* enzyme.[4] However, the relative reactivities of the aldehydes **2** were found to be remarkably similar. For example, **2b** and **2e** were, respectively, the most and least reactive aromatic aldehydes tested with the two enzymes. Chiral analysis of the acyloins **3** was carried out using GLC.[7] The optical purity of the products

2 **3**

Ar = **a** = Ph
b = 2-fluorophenyl
c = 3-fluorophenyl
d = 4-fluorophenyl
e = 2-chlorophenyl
f = 3-chlorophenyl
g = 4-chlorophenyl

Scheme 2 *Reagent:* i, *Z. mobilis* PDC

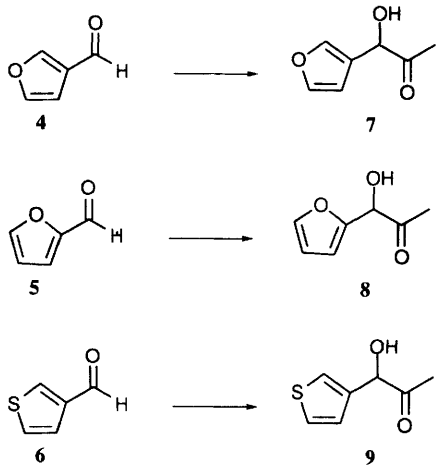
Table 1 Optical purity and yield of the *R*-aromatic acyloins **3** formed from the aldehydes **2** and pyruvate using *Z. mobilis* PDC[a]

Optical purity Aldehyde, **2**	Acyloin, **3**	Yield (%)[b]	Optical purity (% ee)[c]
a	**a**	15	98
b	**b**	60	98
c	**c**	30	97
d	**d**	35	98
e	**e**	5	98
f	**f**	20	98
g	**g**	35	98

[a] Reaction mixtures contained aldehydes **2a–g** (20 mmol dm^{-3}), sodium pyruvate (100 mmol dm^{-3}) and *Z. mobilis* PDC (2.06 U) and were incubated for 23 h. Other constituents were as described in the Experimental section. [b] Yields were determined by ^{1}H NMR spectroscopy of the reaction mixtures. [c] Optical purity was determined by chiral GLC using a Lipodex A column.

was high (Table 1). It was apparent from the elution order of the chiral products **3** that the predominant enantiomer was consistently identical with that obtained with *Saccharomyces cerevisiae*[7] and with the isolated yeast enzyme. Accordingly the *R* configuration could be assigned to the products.

Yeast PDC has been shown to accept heterocyclic aldehydes as well as aromatic aldehydes.[6] A number of heterocyclic aldehydes were tested for their ability to form acyloins with pyruvate using the *Z. mobilis* enzyme. 3-Furaldehyde **4**, 2-furaldehyde **5** and thiophene-3-aldehyde **6** were found to be poor substrates (Scheme 3), although chiral analysis using GLC indicated that the corresponding products **7**, **8** and **9**,

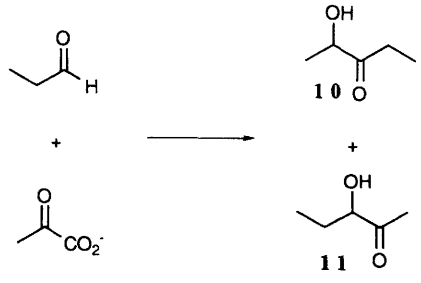


Scheme 3

respectively, were formed with optical purities between 80 and 90% ee and that the predominant isomers were identical with those obtained with the yeast enzyme. No product was detected with thiophene-2-aldehyde.

It was reported previously that both the yeast and *Z. mobilis* enzymes catalyse the formation of acetoin from acetaldehyde alone.[8] It is now found that the *Z. mobilis* enzyme can catalyse this reaction at a rate four orders of magnitude greater than yeast PDC. The ability to form aromatic acyloins from the corresponding aldehydes with 'active acetaldehyde' generated from acetaldehyde itself was tested with the *Z. mobilis* PDC. The *R*-aromatic acyloins **3a–d** were indeed produced from aldehydes **2a–d** (Scheme 2) with high optical purity (98% ee). The rate of reaction was several-fold lower than that obtained with pyruvate. No reaction was observed with the yeast enzyme. This is the first demonstration of such a biotransformation and exposes an important difference between the two PDCs.

The production of acetoin by PDCs has been known for some time.[9] The substrate specificity of the acyloin condensation with respect to the acceptor aldehyde appeared to be rather specific for acetaldehyde and aromatic aldehydes. Of a number of other aliphatic aldehydes tested, including dialdehydes, only propanal (100 mmol dm^{-3}) gave any acyloin product with pyruvate. This relatively poor substrate gave a mixture of 2-hydroxypentan-3-one **10** and 3-hydroxypentan-2-one **11** in roughly equal amounts, indicating that propanal was probably capable of condensing with 'active acetaldehyde' and generating 'active propanal' itself (Scheme 4). No propionin was detected suggesting that propanal did not condense with itself.

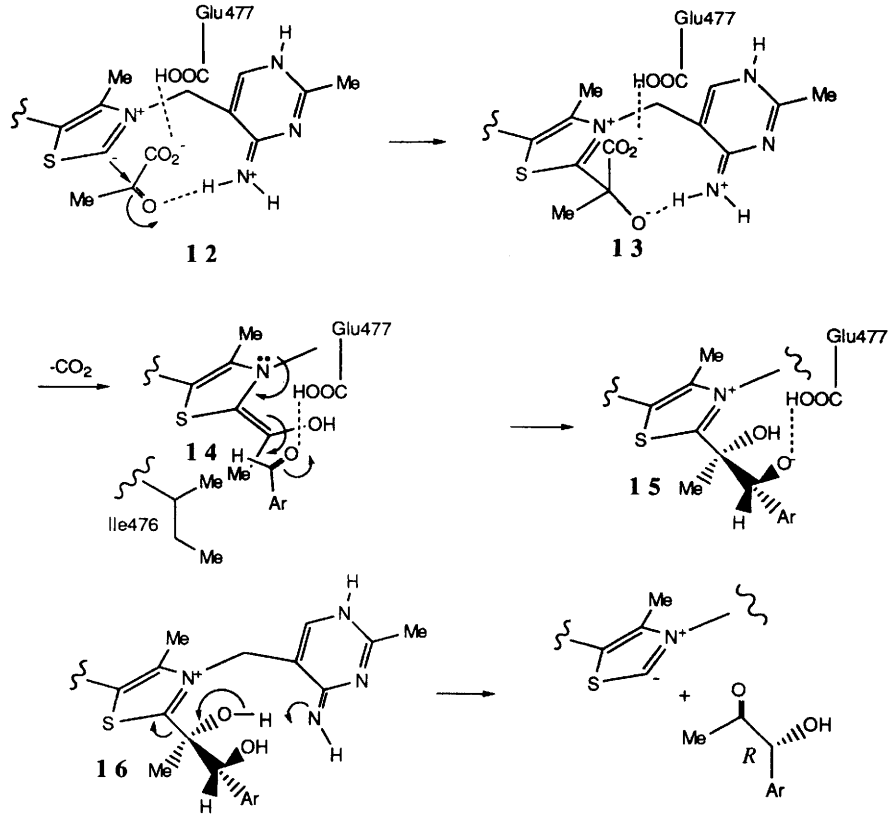


Scheme 4

The formation of lactaldehyde from glyoxylate and acetaldehyde using the PDC from *Z. mobilis* has been demonstrated.[8] Interestingly, the condensation of 'active formaldehyde' generated from glyoxylate is highly specific for acetaldehyde and no acyloins have been detected using aromatic aldehydes. A number of additional 2-oxo acids failed to give acyloins. For example, 3-hydroxypyruvate is quantitatively decarboxylated by *Z. mobilis* PDC to glycolaldehyde. However, rather surprisingly, no acyloins are produced with either aliphatic or aromatic aldehydes.

These studies have clearly demonstrated that the *Z. mobilis* PDC is capable of forming a series of *R*-aromatic acyloins from the corresponding aldehydes utilising either pyruvate or acetaldehyde to generate 'active acetaldehyde'. The high stereoselectivity of the reaction is common to the enzymes from *Z. mobilis* and yeast. This is in sharp contrast to the results obtained with the production of acetoin, from pyruvate and acetaldehyde, and lactaldehyde, from glyoxylate and acetaldehyde.[8] *Z. mobilis* and yeast PDC produce these acyloins with predominantly opposite absolute configurations with moderate to low enantioselectivity. These results have been interpreted in terms of different Boltzmann distributions between the bound forms of the electrophile, acetaldehyde.[8] It appears that acetaldehyde binds to the yeast enzyme in predominantly the same orientation as that for benzaldehyde, giving the *R*-acyloin as the major product. By contrast, the *Z. mobilis* PDC binds acetaldehyde, but not benzaldehyde, in predominantly the reverse orientation, giving *R*-aromatic acyloins, and (*S*)-acetoin and (*S*)-lactaldehyde. It was deduced that the binding of benzaldehyde is more sterically demanding than the binding of acetaldehyde and that the active sites of both enzymes can accommodate only one productive orientation of the binding of benzaldehyde. This conclusion was borne out by molecular modelling studies.

Yeast (*Saccharomyces* sp.) PDC[10] and *Z. mobilis* PDC[11] are homologous. The X-ray crystal structure of a *Saccharomyces* PDC is available.[12] Using this structure, an analysis of the acyloin condensation reaction was carried out using the programme QUANTA. The first step in the catalytic mechanism is attack of the anion of thiamin diphosphate (TDP) on the ketonic carbonyl group of pyruvate (**12**; Scheme 5). To avoid the high energy that would be associated with a dianionic species, the developing negative charge on the oxygen atom is stabilised by interaction with the amino group of the (probably) protonated pyrimidine ring of TDP (**13**; Scheme 5). The adduct also may be stabilised by interaction between the carboxylate ion and the carboxyl group of Glu477. This interaction is only stabilising if the carboxy group of Glu477 is unionised, which would require a perturbation of the pK_a by several units from its normal value in aqueous solution. However, such perturbations have ample precedent.[13] According to this analysis, the adduct **13** has the *S* configuration. It should be noted that this absolute configuration is imposed by the chiral conformation of the achiral TDP. This chiral conformation, in turn, is imposed by the architecture of the active site. The bond to the carboxylate group in the adduct **13** is almost perfectly placed (perpendicular to the plane of the thiazolium ring) to facilitate decarboxylation to the enamine intermediate **14** (the so-called 'active acetaldehyde'). The *E* configuration of the enamine **14** is correlated with, and is a consequence of, the *S* configuration of the adduct **13**. Glu477 appears to play a dual role in this mechanism since it is ideally placed to stabilise also the developing oxyanion during formation of the acyloin carbon–carbon bond (**14** ⟶ **15**; Scheme 5). This is initiated by approach of the aldehyde to the enamine. The orientation of the carbonyl group oxygen atom of the aldehyde is always fixed and is determined by the position of Glu477 relative to the bond-forming α-carbon atom of the enamine **14**. With benzaldehyde, this approach is restricted to the arrangement that leads to the *R*-acyloin (attack on the *si*-face of the aldehyde group). The approach that could lead to *S*-acyloin (*re*-face attack) is prevented by the side chain of Ile476, which completely obstructs the phenyl group (Scheme 5 and Fig. 1). However, the smaller methyl group of acetaldehyde can bind so as to present either the *re*- or the *si*-face to the enamine intermediate, with little difference in computed binding energies. These observations explain the strict control of product stereochemistry with



Scheme 5

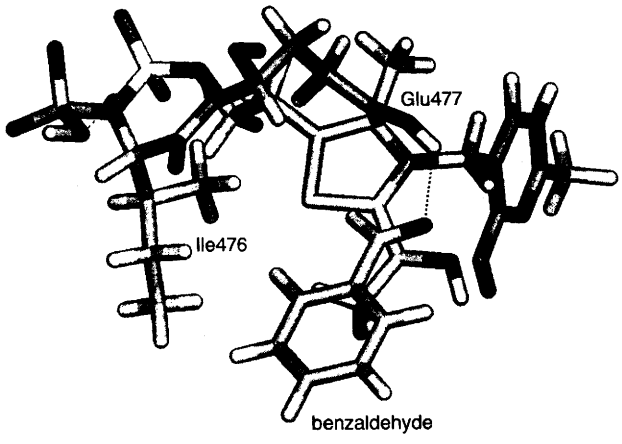


Fig. 1 QUANTA-minimised system comprising the active site of yeast PDC with non-covalently bound TDP-carbanion-enamine intermediate and with docked benzaldehyde (compare with **14**, Scheme 5). The benzaldehyde presents the *si*-face for C–C bond formation. The alternative docking mode with the *re*-face presented for C–C bond formation is prevented by steric interference from the side chain of Ile476.

aromatic acyloins and the relaxed control of stereochemistry in the formation of acetoin.[7]

Following protonation of the oxyanion, product release is initiated by deprotonation of the α-hydroxy group, with regeneration of the TDP anion. Although these modelling studies were based on the crystal structure of yeast PDC, the essential amino acid residues at the active site are very similar in *Z. mobilis* PDC. The foregoing explanation for the high stereoselectivity in acyloin formation from aromatic aldehydes and the relaxed stereoselectivity with acetaldehyde is almost certainly valid for the *Z. mobilis* enzyme as well as the enzyme from *Saccharomyces*. A full description of these modelling studies will be reported shortly. There are many examples of the synthetic value of yeast-catalysed acyloin condensations.[14] The use of the isolated PDCs has many advantages over whole cells, particularly in terms of the lack of unwanted side reactions and ease of product isolation. The recombinant *Z. mobilis* PDC appears not to be as efficient a catalyst as the yeast enzyme in this regard, but it can be isolated in large quantities and is capable of utilising acetaldehyde rather than pyruvate in the acyloin condensation.

Experimental

PDC from yeast was purchased from the Sigma Chemical Co. Ltd. NMR spectra were obtained using Perkin-Elmer 220 MHz R34, Bruker 250 MHz AFC250 and Bruker 400 MHz WH400 spectrometers. NMR coupling constants (J) are quoted in Hz. GC–MS spectra were determined using a Kratos MS80 spectrometer. Protein separations were carried out using the PhastSystem and FPLC equipment, respectively, from Pharmacia LKB Biotechnology. Analytical and preparative high pressure liquid chromatography (HPLC) separations were carried out using Gilson 302 and 305/306 solvent delivery systems, respectively. GLC was carried out using a Shimadzu GC-14A gas chromatograph and a Shimadzu Chromatopac C-R5A integrator. Spectrophotometric enzyme and protein assays were monitored using a Pye Unicam SP1800 spectrophotometer and scanning measurements were obtained using a Philips PU8720 spectrophotometer. Mps were uncorrected. TLC plates were developed with a solution of Tetrazolium Blue (0.25%, w/v) in methanol–water (1:1, v/v) containing NaOH (3 mol dm^{-3}). Acyloins appeared as purple spots on a pale yellow background.

Preparation of enzymes

Escherichia coli strain DH1 (with no detectable endogenous PDC activity) was transformed with pLOI295[15] using the method described by Chung *et al.*[16] Recombinants were selected as described by Ingram *et al.*[15] Transformed *E. coli* cells were grown at 37 °C in Luria broth[17] containing glucose (2 g dm^{-3}) and ampicillin (0.1 g dm^{-3}). Wild-type *Z. mobilis* ATCC 31821 was grown at 30 °C in broth containing yeast extract (10 g

dm^{-3}), KH_2PO_4 (0.3 g dm^{-3}) and glucose (2 g dm^{-3}). Solid media contained agar (15 g dm^{-3}) and glucose (20 g dm^{-3}). Cells were prepared on a gram scale from anaerobic 2 and 20 dm^{-3} fermentation broths using 10% (v/v) inocula. The PDC activity was determined by the method of Ullrich.[18] One unit (U) of enzyme activity was defined as the formation of 1 mmol min^{-1} of acetaldehyde. Protein was determined using the Bio-Rad protein assay. Transformed *E. coli* cells were harvested in the late exponential phase of growth by centrifugation. The cells were disrupted by three passes through a French pressure cell and cell debris was removed by centrifugation. The cell-free supernatant was heat treated (60 °C; 10 min). Heat precipitated protein was removed by centrifugation. Material of molecular weight below 10 000 was diluted 5-fold by repeated ultrafiltration and dilution with pH 7.0 bistrispropane buffer (20 mmol dm^{-3}) containing thiamin diphosphate (1 mmol dm^{-3}) and $MgCl_2$ (1 mmol dm^{-3}). This step was necessary for the efficient binding of the PDC to the ion exchange resin. [As an alternative to the ultra-filtration step, dodecyl β-D-glucopyranose could be added to a concentration of 50% (w/w with respect to protein) to the same effect.] The protein was applied to an FPLC Mono Q HR10/10 anion exchange column [or preferably a DEAE (diethylaminoethyl) Fast-Flow Sepharose column], previously equilibrated with pH 7.0 bistrispropane buffer (20 mmol dm^{-3}) and eluted with the same buffer using a NaCl gradient. The maximum *Z. mobilis* PDC activity was eluted with 0.11 mol dm^{-3} NaCl. Finally, the material was applied to an FPLC Superdex 200 gel filtration column previously equilibrated with pH 6.0 sodium citrate buffer (20 mmol dm^{-3}) containing NaCl (50 mmol dm^{-3}), thiamin diphosphate (1 mmol dm^{-3}) and $MgCl_2$ (10 mmol dm^{-3}) and eluted with the same buffer. The purified *Z. mobilis* PDC (15–20% overall yield) appeared to be homogeneous, with a molecular weight of 245 000, on examination with native polyacrylamide gel electrophoresis (PAGE) with Coomassie Blue staining (lit., 200 000,[19] 240 000,[20] 219 700[21]). The identity of the stained protein was confirmed by using the activity stain for PDC as described by Zehender *et al.*[22] on a duplicate gel. The subunit molecular weight of the enzyme was determined to be 65 000 (lit., 56 500,[19] 59 000[20]) by denaturing PAGE. The pI of the native enzyme was 5.00 (lit.,[19] 4.87). The PDC was purified up to 5-fold and material of specific activity 150 and 186 U mg^{-1} was obtained (lit.,[19,20] 120–181 U mg^{-1}). *Z. mobilis* PDC was stable when stored at −20 °C with glycerol (50%, v/v). The wild-type *Z. mobilis* PDC purified by the above procedure from the *Z. mobilis* strain CP4 ATCC 31821 wild-type strain exhibited identical chromatographic and electrophoretic properties to the recombinant enzyme. Commercial yeast PDC (12 U mg^{-1}) was purified (23 U mg^{-1}) by gel filtration chromatography as described above except that the eluent contained dithiothreitol (0.5 mmol dm^{-3}).

PDC-catalysed reactions

The reaction mixtures contained pH 6.0 sodium citrate buffer (0.1 mol dm^{-3}), sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS; 11.45 mmol dm^{-3}), thiamin diphosphate (15 mmol dm^{-3}), $MgSO_4$ (0.1 mmol dm^{-3}) and recombinant *Z. mobilis* PDC. The substrates were added last to give a final reaction volume of 1 cm^3. The reaction mixtures were incubated at 30 °C. Semi-preparative reactions (5 cm^3) were identical with the analytical scale reactions. These reaction mixtures were added to the 2,4-dinitrophenylhydrazine reagent as described below. The lactaldehyde derivative was purified by silica gel chromatography and HPLC as described below. The R_F (TLC), ^1H NMR and mass spectra of this derivative were indistinguishable from those obtained with authentic material.

GLC analysis of acetoin

Acetoin was analysed by GLC using a BP20 column (25 m × 0.22 mm; film 0.25 mm; S.G.E. Ltd.). The injector, column and flame ionisation detector temperatures were 200, 72 and 250 °C, respectively. Using hydrogen as the carrier gas (linear flow rate 40 cm s^{-1}), the retention time for acetoin was 6.8 min. Reaction mixtures were extracted with ethyl acetate containing heptan-2-one (1:1500, v/v) as internal standard. The BP20 column was also used to determine GC–MS.

Lactaldehyde 2,4-dinitrophenylhydrazone

(*RS*)- and (*R*)-Lactaldehyde were synthesised by the method of Zagalak *et al.*[23] from DL- and L-threonine, respectively; δ_H(220 MHz, H_2O, DSS) 1.17 (3 H, d, *J* 6.7, Me) and 3.69 (1 H, m, C*H*OH). Lactaldehyde was derivatised with 2,4-dinitrophenylhydrazine under acidic conditions at ambient temperature, avoiding the formation of the osazone, and the product was partially purified by flash chromatography. Pure product was obtained after preparative reverse phase HPLC. [Dynamax 60A (8 mm C_{18}) column (25 cm × 10 mm) with guard column (5 cm × 10 mm), Rainin Instrument Co. Inc.] with an eluent of water–methanol (35:65, v/v) at a flow rate of 3 cm^3 min^{-1}. Eluted compounds were detected by UV absorbance at 254 nm. Lactaldehyde 2,4-dinitrophenylhydrazone eluted at 12.1 min, mp 155–156 °C ($CHCl_3$) (lit.,[24,25] 155–158 °C) (Found: M^+, 254.0658. $C_9H_{10}N_4O_5$ requires *M*, 254.0651); λ_{max}(MeOH)/nm 354 (ε/dm^3 mol^{-1} cm^{-1} 6.4 × 10^6), 253sh (3.3 × 10^6) and 225 (4.1 × 10^6); ν_{max}($CHCl_3$)/cm^{-1} 3020, 1802, 1619, 1522, 1340, 1161, 1128, 1092, 1073 and 1035; δ_H(400 MHz, $CDCl_3$, $SiMe_4$) 1.46 (3 H, d, *J* 6.69, Me), 2.50 (1 H, s, OH), 4.62 (1 H, m, C*H*OH), 7.56 (1 H, d, *J* 3.94, NCH), 7.91 (1 H, d, *J* 9.52, 6-ArH), 8.32 (1 H, dd, *J* 9.60 and 2.54, 5-ArH), 9.12 (1 H, d, *J* 2.58, 3-ArH) and 11.08 (1 H, s, NH); δ_C(400 MHz, $CDCl_3$, $SiMe_4$) 20.9 (Me), 66.9 (CHOH), 116.3 (Ph), 123.3 (Ph), 130.0 (Ph) and 152.5 (CN); *m/z* (CI, NH_3) 272 ($M + NH_4^+$, 39%), 255 ($M + H^+$, 38), 254 (M^+, 17) and 237 (39). The R_F (TLC), ^1H NMR and mass spectra of (*R*)-lactaldehyde 2,4-dinitrophenylhydrazone were indistinguishable from those obtained with the racemic material.

Chiral analysis: lactaldehyde

The optical purity of lactaldehyde 2,4-dinitrophenylhydrazone was determined by HPLC [Chiralcel OB column (25 cm × 4.6 mm), Baker, Daicel Chemical Industries Ltd.] with propan-2-ol–hexane (1:4) as eluent. Eluted compounds were detected by UV absorbance at 254 nm. (*RS*)-Lactaldehyde 2,4-dinitrophenylhydrazone (t_R/min 55 and 68) and (*R*)-lactaldehyde 2,4-dinitrophenylhydrazone (t_R 68) were eluted at a flow rate of 0.5 cm^3 min^{-1}. The lactaldehyde derivatives obtained from the preparative enzyme-catalysed reactions exhibited the same retention times as the synthetic material. Co-injections were performed with synthetic racemic material. Identical results were obtained when the eluted compounds were detected by absorbance at 350 nm.

Acetoin

Acetoin optical purity was determined by chiral GLC [Lipodex A, OV1700 and 50% hexa-(2,3,6-*O*-pentyl)-α-cyclodextrin column (25 m × 0.25 mm)]. The injector, column and flame ionisation detector temperatures were 200, 20 and 250 °C, respectively. Using hydrogen as the carrier gas (linear flow rate/cm s^{-1} 40), the t_R values for the acetoin enantiomers were 9.7 and 10.3 min. (*R*)-Acetoin was eluted first.[26] Co-injections with authentic racemate were performed.

Aromatic and heterocyclic acyloins

The optical purity of aromatic and heterocyclic acyloins was determined by chiral GLC as described above except that the column temperatures were as indicated below. The enantiomers of the authentic racemates of the following compounds were well resolved using this column: 1-hydroxy-1-phenylpropan-2-one **3a** (t_R 44.72 and 45.65 min^{-1}, column temperature 70 °C); 1-(2-fluorophenyl)-1-hydroxypropan-2-one **3b** (20.08 and 20.39,

78 °C); 1-(3-fluorophenyl)-1-hydroxypropan-2-one **3c** (19.40 and 19.93, 93 °C); 1-(4-fluorophenyl)-1-hydroxypropan-2-one **3d** (37.39 and 37.83, 78 °C); 1-(2-chlorophenyl)-1-hydroxypropan-2-one **3e** (44.59 and 45.28, 85 °C); 1-(3-chlorophenyl)-1-hydroxypropan-2-one **3f** (93.63 and 94.97, 85 °C); 1-(4-chlorophenyl)-1-hydroxypropan-2-one **3g** (166.54 and 169.21, 78 °C); 1-(2-furyl)-1-hydroxypropan-2-one **8** (15.19 and 15.58, 65 °C); 1-(3-furyl)-1-hydroxypropan-2-one **7** (13.23 and 14.45, 75 °C) and 1-hydroxy-1-(3-thienyl)propan-2-one **9** (51.36 and 51.77, 75 °C) were well resolved using this column. In each case, except for **3b** and **3e**, the *R* enantiomer was eluted first. Co-injections with authentic racemates [7] were performed.

Characterisation of biological acyloins: aliphatic acyloins.—(a) Acetoin (3-hydroxybutan-2-one), δ_H(250 MHz, $CDCl_3$, $SiMe_4$) 1.39 [3 H, d, *J* 7.11, CH_3CH(OH)], 2.20 (3 H, s, CH_3CO) and 4.24 [1 H, d, *J* 7.11, CH(OH)]; δ_H(220 MHz, H_2O, DSS) 1.37 [3 H, d, *J* 7.5, CH_3CH(OH)] and 2.24 (3 H, s, CH_3CO); *m/z* (EI) 88 (M^+, 4%), 73 (2), 59 (1), 57 (1), 45 (100) and 43 (96). (b) Lactaldehyde (2-hydroxypropanal), δ_H(220 MHz, H_2O, DSS) 1.17 [3 H, d, *J* 6.7, CH_3(hydrate)] and 3.69 [1 H, m, CH(OH)(hydrate)]. (c) 2-Hydroxypentan-3-one **10**, δ_H(250 MHz, $CDCl_3$, $SiMe_4$) 1.12 (3 H, t, *J* 7.17, CH_3CH_2), 1.38 [3 H, d, *J* 7.03, CH_3CH(OH)], 2.53 (2 H, m, CH_2) and 4.25 [1 H, q, *J* 7.03, CH(OH)]; *m/z* (EI) 102 (M^+, 1%), 59 (12), 57 (37), 45 (100) and 43 (69). (d) 3-Hydroxypentan-2-one **11**, δ_H(250 MHz, $CDCl_3$, $SiMe_4$) 0.94 (3 H, t, *J* 7.40, CH_3CH_2), 1.62 (2 H, m, CH_2), 2.19 (3 H, s, CH_3CO) and 4.17 [1 H, dd, *J* 4.01 and 6.86, CH(OH)]; *m/z* (EI) 102 (M^+, 1%), 59 (71), 57 (11), 45 (22) and 43 (100).

Aromatic and heterocyclic acyloins

1-(2-Fluorophenyl)-1-hydroxypropan-2-one 3b. (Found: M^+, 168.0593. $C_9H_9FO_2$ requires *M*, 168.058 65), $[\alpha]_D$ −186 (*c* 0.56 in MeOH); δ_H(400 MHz; $CDCl_3$) 2.01 (3 H, s, Me), 5.33 [1 H, s, C*H*(OH)], 7.0 (1 H, m, 2′-H), 7.05 (1 H, m, 5′-H) and 7.20 (2 H, m, 4′,6′-H); δ_C(90 MHz; $CDCl_3$) 24.83 (CH_3), 73.49 [*C*H(OH)], 115.74 (C-3′), 124.70 (C-5′), 125.14 (C-1′), 128.65 (C-4′), 130.30 (C-6′), 160.22 (C-2′) and 206.13 (CO); *m/z* (EI) 168 (M^+, 0.2%), 151 (5), 126 (50), 125 (100), 124 (49), 123 (80), 105 (51), 97 (80), 95 (68), 77 (74), 75 (67) and 45 (36).

1-(3-Fluorophenyl)-1-hydroxypropan-2-one 3c. (Found: M^+, 168.0579. $C_9H_9FO_2$ requires *M*, 168.058 65), $[\alpha]_D$ −221 (0.14 in MeOH) (for material of 95% ee); δ_H(400 MHz; $CDCl_3$) 2.10 (3 H, s, Me), 5.08 [1 H, s, C*H*(OH)], 7.03 (3 H, m, 2′,4′,6′-H) and 7.34 (1 H, m, 5′-H); δ_C(90 MHz; $CDCl_3$) 25.06 (CH_3), 79.39 [CH(OH)], 114.11 (C-4′), 115.65 (C-2′), 122.91 (C-6′), 125.14 (C-1′), 130.48 (C-5′), 142.96 (C-3′) and 206.20 (CO); *m/z* (EI) 168 (M^+, 18%), 125 (95), 123 (92), 107 (11), 97 (100), 95 (92), 77 (76), 75 (76), 74 (24), 69 (33) and 45 (58).

1-(4-Fluorophenyl)-1-hydroxypropan-2-one 3d. (Found: M^+, 168.0589. $C_9H_9FO_2$ requires *M*, 168.058 65), $[\alpha]_D$ −197 (*c* 0.69 in MeOH) (for material of 97% ee); δ_H(400 MHz, $CDCl_3$) 2.07 (3 H, s Me), 5.07 [1 H, s, CH(OH)], 7.25 (2 H, m, 2′,6′-H) and 7.07 (2 H, m, 3′,5′-H); δ_C(90 MHz; $CDCl_3$) 24.92 (Me), 79.10 [CH(OH)], 115.72 (C-3′,5′), 128.93 (C-2′,6′), 133.85 (C-1′), 162.63 (C-4′) and 207.02 (CO); *m/z* (EI) 168 (M^+, 2%), 166 (2), 125 (99), 123 (100), 97 (97), 95 (99), 77 (54), 75 (66) and 45 (25).

1-(2-Chlorophenyl)-1-hydroxypropan-2-one 3e. [Found: $(M + NH_4)^+$ 202.0635. $C_9H_{13}{}^{35}ClNO_2$ requires $(M + NH_4)$, 202.0635] $[\alpha]_D$ −207 (*c* 0.34 in MeOH); δ_H(400 MHz, $CDCl_3$) 2.16 (3 H, s, Me), 5.62 [1 H, d, *J* 4.0, CH(OH)] and 7.30 (4 H, m, ArH); δ_C(90 MHz; $CDCl_3$) 25.17 (Me), 127.44 (C-5′), 128.79, 129.79, 129.97 (C-3′,4′,6′), 133.31 (C-2′), 135.53 (C-1′) and 206.18 (CO) [N.B. the signal attributable to CH(OH) was not observed owing to overlap with solvent peaks]; *m/z* (EI) 186 (M^+, 0.2), 184 (0.5), 149 (36), 143 (73), 141 (100), 139 (64), 113 (58), 111 (32), 77 (93) and 43 (66).

1-(3-Chlorophenyl)-1-hydroxypropan-2-one 3f. (Found M^+, 184.0286, 186.0259. $C_9H_9ClO_2$ requires *M*, 184.0291, 186.0261), $[\alpha]_D$ could not be determined. The product was difficult to separate from the chlorobenzyl alcohol byproduct. Repeated flash chromatography was necessary which caused significant loss of optical purity: δ_H(400 MHz, $CDCl_3$) 2.05 (s, 3 H, Me), 5.05 [1 H, s, CH(OH)], 7.31 (1 H, m, 2′-H), 7.20 (1 H, m, 5′-H) and 7.26 (2 H, m, 4′,6′-H); δ_C(90 MHz; $CDCl_3$) 25.14 (Me), 79.40 [CH(OH)], 125.40 (C-6′), 127.37 (C-4′), 128.87 (C-2′), 130.18 (C-5′), 134.90 (C-3′), 139.78 (C-1′) and 206.13 (CO); *m/z* (EI) 186 (M^+, 2%), 184 (M^+, 8%), 143 (52), 141 (100), 139 (67), 113 (67), 111 (42), 77 (97) and 43 (77).

1-(4-Chlorophenyl)-1-hydroxypropan-2-one 3g. (Found M^+, 184.0300, 186.0261. $C_9H_9ClO_2$ requires *M*, 184.0291, 186.0261), $[\alpha]_D$ −158 (*c* 0.58 in MeOH); δ_H(400 MHz, $CDCl_3$) 2.07 (3 H, s, Me), 5.08 [1 H, s, CH(OH)], 7.35 (2 H, m, 2′,6′-H), 7.27 (2 H, m, 3′,5′-H); δ_C(90 MHz; $CDCl_3$) 25.10 (Me), 79.28 [CH(OH)], 128.56 (C-3′,5′), 129.11 (C-2′,6′), 134.55 (C-4′), 136.36 (C-1′) and 206.49 (CO); *m/z* (EI) 186 (M^+, 0.5%), 184 (1.8), 143 (20), 141 (76), 139 (46), 111 (30), 77 (100) and 43 (35).

Molecular modelling

All calculations were performed on a Silicon Graphics Iris Indigo work station. QUANTA (version 4.0, Molecular Simulations Inc., Burlington, MA, USA) was used for construction and analysis of all modelled structures. Energy minimisations were performed using CHARMm (subprogram used within QUANTA) and the Adopted Basis Newton-Raphson Minimization Algorithm. Electrostatic interactions were calculated by applying a distance-dependent dielectric term (value of dielectric constant: 4). Non-bonded interactions were calculated with a cut-off distance of 12 Å. The non-bonded atom list was updated every 25 minimisation steps. A switching smoothing function was applied to the van der Waals and electrostatic terms between 8 and 10 Å distance.

Each yeast PDC monomer contains a single polypeptide chain. Two monomers are tightly associated to form a dimer with approximate 2-fold symmetry. A 2-fold rotation of the dimer about one axis generates another dimer, which contacts the first to form a complete PDC tetramer. The TDPs are situated at the interface between monomers within each tightly associated dimer, with two sites per dimer and four per tetramer. The computer model of PDC contains one dimer, which is represented by all carbon and heteroatoms of the two polypeptide chains (A- and B-chain) and the two bound TDP molecules. Pre-defined residue topology files were used by the HBUILD function of CHARMm to add all hydrogen atoms (polar and non polar). In general, aspartic acid and glutamic acid residues have not been protonated; the only exception is Glu477. Protonation of histidine residues was decided according to hydrogen bonding pattern and solvent accessibility: His97 (uncharged, proton on N-3), His481 (uncharged, proton on N-1), His92, 114, 115, 126, 225, 310, 313, 495, 510 (charged, both N protonated). All arginine and lysine residues were protonated. Some CHARMm parameters had to be corrected in the following cases:

The 4′-amino group was held in the plane of the pyrimidine ring by applying an additional dihedral constraint [harmonic force constant: 50 kcal mol^{-1} (209.2 kJ mol^{-1})]. The CH_3–C(α)–OH group of the carbanion–enamine intermediate was held in the plane of the thiazolium ring by applying an additional dihedral constraint [harmonic force constant: 50 kcal mol^{-1} (209.2 kJ mol^{-1})].

In all CHARMm calculations of minimum-energy conformations all enzyme atoms with the exception of a few residues were fixed in their positions as found in the crystal structure. The residues allowed to move were TDP (or a corresponding derivative) excluding the diphosphate group, Glu477(A), Asp28(B), His115(B), and any docking substrate or water molecule. In general, starting conformations of intermediates were modelled by modifying the minimised conformation of the preceding intermediate.

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