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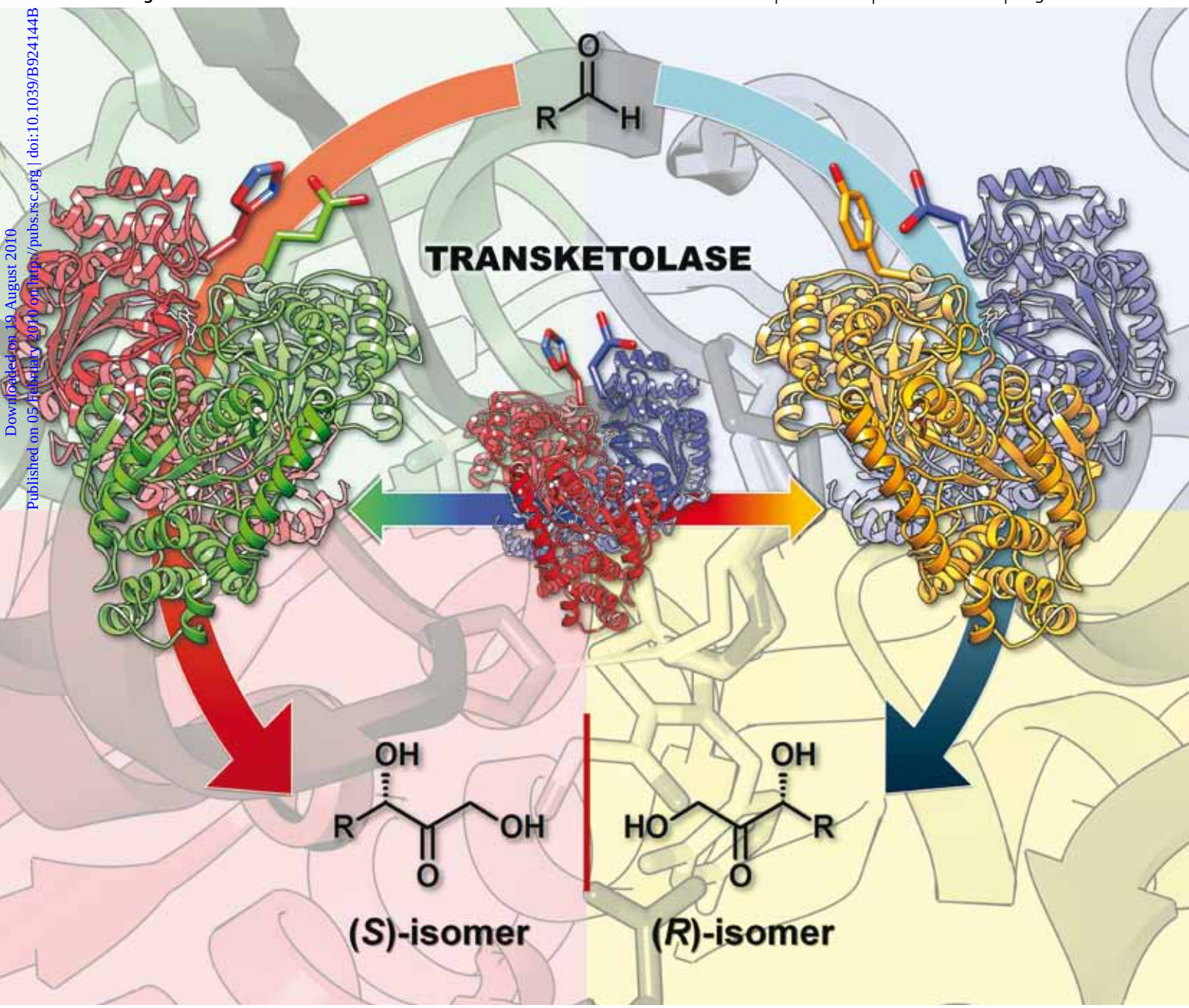

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PERSPECTIVE
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Synthesis of 3-substituted indoles *via* reactive alkylideneindolenine intermediates

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Non-α-hydroxylated aldehydes with evolved transketolase enzymes†

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Transketolase mutants previously identified for use with the non-phosphorylated aldehyde propanal have been explored with a series of linear and cyclic aliphatic aldehydes, and excellent stereoselectivities observed.

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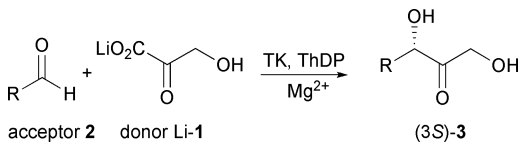
Introduction

The use of biocatalysis as a sustainable, atom efficient strategy in organic synthesis is of increasing importance, and is particularly attractive due to the high stereoselectivities that can be achieved.[1] Transketolase (TK) (EC 2.2.1.1) is an essential thiamine diphosphate (ThDP) dependent enzyme, which provides a link between the glycolytic and pentose phosphate pathways.[2] *In vivo* it catalyses the reversible transfer of a ketol unit to D-ribose-5-phosphate or D-erythrose-4-phosphate.[2] The TK reaction is made irreversible using the donor β-hydroxypyruvate (HPA **1**),[3] which has been used with acceptors **2** such as α-hydroxyaldehydes where it is stereospecific for the (2*R*)-hydroxyaldehyde to give (*S*)-α,α′-dihydroxyketones **3** (Scheme 1).[4,5] The substrate tolerance of TK towards a range α-hydroxyaldehydes has led to interest in industrial applications.[6] *E. coli* TK, which has been overexpressed,[7] shows increased specific activity towards **1** compared to yeast and spinach TKs.[8]



Scheme 1 Formation of α,α′-dihydroxy ketones (3*S*)-**3** using TK.

α,α′-Dihydroxyketone functionalities (**3**) are present in a range of natural products and are also important compounds for further conversion into other synthons, including ketosugars and 2-amino-1,3-diols.[4d,9,10] TK shows high specificity towards the donor substrates but is more tolerant towards the acceptor aldehyde: several non-α-hydroxylated aldehydes have been used but lower relative rates of reaction (5–35% compared to hydroxylated aldehydes) were noted.[5]

With a view to enhancing the use of TK in synthetic applications with a wider range of aldehydes, we used saturation mutagenesis that was targeted to the TK active site residues. Mutants with improved activity towards glycolaldehyde (Scheme 1, R = CH_2OH), and enhanced specificity to propanal **2a** (R = CH_2CH_3) such as D469T, were identified.[11] In addition, when propanal was used with wild-type (WT) TK, the *ee* of the product **3a** (R = CH_2CH_3) was only 58% (Table 1) and therefore chiral assays were developed to identify mutants with improved stereoselectivities.[12,13] Notable variants leading to high stereoselectivities were D469E (90% *ee*, 3*S*-isomer) and H26Y (88% *ee*, 3*R*-isomer), which remarkably with a single point active site mutation reversed the stereoselectivity.[12]

The D469E mutant TK has also been reported to reduce the acceptance of glycolaldehyde and formaldehyde,[14] and the D469 residue has been highlighted as a key residue involved in enantioselection with α-hydroxylated aldehydes: a yeast TK structure with the adjunct erythrose-4-phosphate, indicated it hydrogen bonds to the C-2 hydroxy group of 2-hydroxylated aldehydes in the active site.[15] In view of the interesting substrate tolerances exhibited by the TK mutants, a more systematic study was carried out using linear and cyclic aliphatic aldehydes, with the aim of understanding substrate tolerance and limitations with selected mutants.

Results and discussion

Stereoselectivity of *E. coli* WT TK

Linear aliphatic aldehydes (C_4–C_8) and cyclopropane-, cyclopentane- and cyclohexanecarboxaldehyde were selected for use with WT-TK and TK mutants to determine the influence of chain length and ring size on reaction selectivities. Initially racemic α,α′-dihydroxyketones **3b–3i** were prepared for chiral assay development. The commercially available aldehydes **2b–2i** were converted into **3b–3i** in yields of 2–35% using *N*-methylmorpholine and the previously described TK biomimetic reaction in water (Scheme 2).[16] In general, lower yields were observed with the more lipophilic aldehydes, which may reflect poor substrate solubilities in water.

Methods were established for the determination of *ee*s in **3** *via* monobenzoylation at the primary alcohol of **3g–3i** and chiral HPLC. Compounds **3b–3f** required dibenzoylation for satisfactory peak resolution by chiral HPLC. Then WT-TK and **1** were reacted with **2b–2f** (C_4–C_8) to determine product stereoselectivities and yields (Table 1). As well as establishing *ee*s *via* derivatisation and chiral HPLC, the selected ketodiols **3b** and **3d** were coupled to (*S*)-MTPACl to give the corresponding Mosher's esters: application of

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† Electronic supplementary information (ESI) available: Colorimetric assay plates for the cyclic aldehydes. See DOI: 10.1039/b924144b

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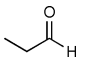
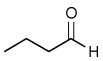
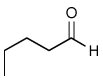
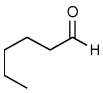
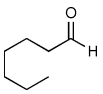
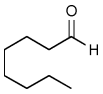
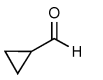
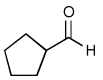
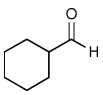
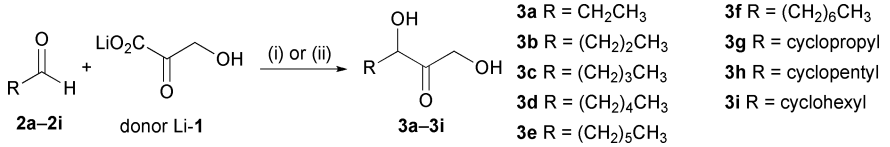
Table 1 Stereoselectivities and yields for WT-TK and TK mutant reactions using linear aliphatic and cyclic aldehydes

	Aldehyde	Product	WT-TK *ee* (yield)	D469E *ee* (yield)	D469T *ee* (yield)	D469K *ee* (yield)	D469L *ee* (yield)	H26Y *ee* (yield)
2a		**3a**[12]	58% (3*S*) (36%)[12]	90% (3*S*) (70%)[12]	64% (3*S*)[12] (68%)[17]	—	12% (3*S*) (nd)	88% (3*R*) (63%)[12]
2b		**3b**	75% (3*S*) (36%)	98% (3*S*) (44%)	—	—	—	92% (3*R*) (16%)
2c		**3c**	84% (3*S*) (16%)	97% (3*S*) (58%)	—	—	—	84% (3*R*) (7%)
2d		**3d**	85% (3*S*) (25%)	97% (3*S*) (47%)	—	—	—	84% (3*R*) (12%)
2e		**3e**	74% (3*S*) (7%)	86% (3*S*) (14%)	—	—	—	78% (3*R*) (4%)
2f		**3f**	66% (3*S*) (<3%)	86% (3*S*) (18%)	—	—	—	83% (3*R*) (21%)
2g		**3g**	72% (1*S*) (<3%)	>99% (1*S*) (10%)	99% (1*S*) (10%)	99% (1*S*) (<3%)	99% (1*S*) (<3%)	no reaction
2h		**3h**	0% (<3%)	>99% (1*S*) (40%)	99% (1*S*) (30%)	25% (1*S*) (10%)	no reaction	30% (1*R*) (<3%)
2i		**3i**	0% (<3%)	97% (1*S*) (10%)	99% (1*S*) (<3%)	25% (1*S*) (<3%)	no reaction	no reaction



Scheme 2 *Reagents and conditions*: (i) *N*-methylmorpholine, pH 8, H_2O; (ii) TK, ThDP, Mg^{2+}, pH 7.

a recently reported NMR method indicated the major enantiomers formed were (3*S*)-**3b** and (3*S*)-**3d**.[13]

As in previous work using propanal, WT-TK leads to the formation of the (3*S*)-isomer as the major enantiomer, and the *ee*s are given in Table 1: by analogy, the major isomers of **3c**, **3e** and **3f** were assigned as (3*S*). Interestingly, the degree of stereoselectivity of the transformation varied with the length of the aliphatic chain of the aldehyde, reaching a maximum of 84% and 85% *ee* for pentanal and hexanal (Fig. 1), which are similar in size to the *in vivo* substrates of TK. In addition, increasing lipophilicity of the aldehyde acceptor resulted in lower conversion yields, probably due to decreasing aqueous solubilities.

WT-TK was then used with the cyclic aldehydes **2g–2i**. The conversion yields were low (<3%) and for the cyclopropane analogue **3g** the *ee* was 72%. The major isomer formed was determined using the modified Mosher's ester method as again the (1*S*)-isomer.[13] However, for the cyclopentane and cyclohexane analogues **3h** and **3i**, racemic products were formed. The substrate

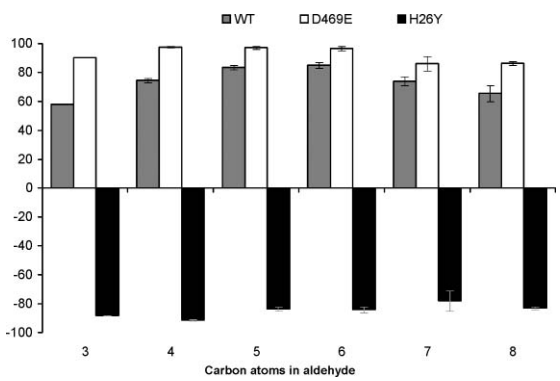


Fig. 1 Variation in stereoselectivities for linear aliphatic aldehydes using WT-TK, D469E-TK and H26Y-TK.

cyclopropane carboxaldehyde may be able to adopt a similar conformation to butanal in the active site leading to a similar

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level of stereoselectivity in the product; however, the larger-ringed cyclic analogues cannot.

Molecular modelling: WT TK

With a view to rationalising the stereoselectivities with WT-TK and the linear aliphatic aldehydes, which demonstrated a clear experimental relationship between chain length and *ee* in the product, molecular docking experiments were carried out using the X-ray crystal structure of WT-TK (PDB ID: 1QGD).[18] Initially, in order to validate the method, direct docking of D-erythrose-4-phosphate, a natural substrate of TK, was carried out using AutoDock.[19] The predominant conformations obtained were compared with an existing X-ray crystal structure of a noncovalent complex of *E. coli* TK with the aldose (PDB ID: 1NGS) and co-factor.[20] Most docked conformations coincided with the conformation of the ligand in the crystal structure in terms of hydrogen bonding and consequently orientation. Despite the dynamic nature of proteins, the conformation generated *in silico* reproduced the experimental findings suggesting a molecular docking method would be a useful model to adopt.

Direct docking of the linear aliphatic aldehydes (C_3–C_8) was performed into the TK active site containing a modelled ThDP-enamine intermediate and the results compared, firstly in terms of cluster populations, and then the hydrogen bonding and orientation. The conformations obtained populated one cluster preferentially in all cases, except for butanal, which occupied two energetically similar conformations. For all the C_3–C_8 aldehydes, docks were obtained in which the aldo oxygen atom is bound to two histidine residues (His26 and His261), bringing the substrates into close proximity with the coenzyme for nucleophilic attack.

The longer C_5–C_8 aldehydes docked with the aliphatic chain bound to a hydrophobic region around the lip of the entrance to the ThDP-containing cavity (Fig. 2, magenta model). This presented neither face of the aldehyde preferentially towards the ThDP enamine intermediate. Interestingly, the smallest aliphatic chain (C_3) of **2a** docked in an alternative orientation with the aliphatic chain occupying the narrow entrance to the cofactor containing cavity (Fig. 2, white model), marginally rendering the Re-face of the carbonyl exposed to the ThDP-enamine Cα atom.

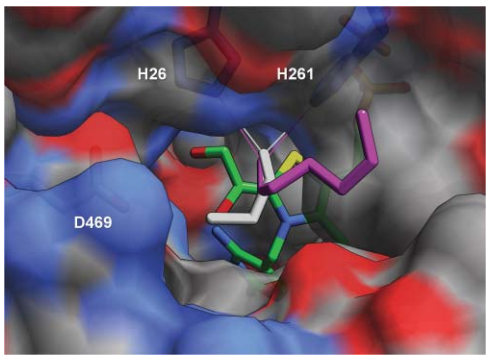

Fig. 2 Aldehydes **2a** (grey) and **2d** (magenta) docked into the TK active site containing the modelled ThDP-enamine intermediate (green). A surface plot is shown with D469, H26 and H261 highlighted as sticks beneath the surface.

This is reflected in the low *ee* (58%) in favour of the (3*S*)-isomer product for reaction with **2a**. The slightly larger C_4 aldehyde was found to dock in two different conformations, with the first similar to that for C_3, and the second similar to the C_5–C_8 aldehydes. This transition of docking conformations at C_4 in the series from C_3 to C_8 mirrors the observed increase in *ee* from 58% for C_3, through 75% for C_4 to 84% and 85% for C_5 and C_6 respectively. However, the increased *ee* resulting from the alternative conformations adopted by the C_4–C_8 aldehydes is not easily rationalised. One possibility is that as the aldo O-atom moves closer to H26 and H261 during the reaction, and the Van der Waals contacts between the aliphatic chains and the lipophilic region of the active site are weakened, allowing the chain to move further into the active site and more freedom to rotate the carbonyl with the *Re*-face of the carbonyl exposed to the ThDP-enamine Cα atom. The decrease in *ee* observed for C_7 and C_8 may be due to an increase in the Van der Waals interactions with the active-site wall and therefore, less flexibility in the re-orientation of the substrate.

Finally docking of the cyclic aldehydes **2g–2i** was carried out; however, they docked preferentially in a lip at the entrance to the active site in non-productive conformations, which could explain the low yielding reactions observed. Only the cyclopropyl analogue **2g** docked in a similar conformation to propanal **2a**, which gave **3g** in 72% *ee*. Modelling with the mutant TKs was not performed as we do not have the crystal structures of the mutants at present, and modelling both the protein mutation and the substrate binding simultaneously would be less informative.

Stereoselectivity of selected single-point TK mutants

In previous mutant screening studies using propanal as the acceptor, two active-site single-point mutant TKs exhibited marked improved and reversed stereoselectivity, Asp469Glu (D469E) and His26Tyr (H26Y). Variant D469E had led to the formation of (3*S*)-**3a** in 90% *ee*, while H26Y gave (3*R*)- **3a** in 88% *ee* (Table 1).[12] With the aim of understanding substrate tolerances, and enhancing and reversing stereoselectivities compared to WT-TK with the C_4–C_8 linear aldehydes, reactions were carried out using these high performing mutants: others screened with propanal were not explored due to the lower stereoselectivities achieved. D469E gave products in 14–58% isolated yield, with decreasing yield upon increasing chain length, but enhanced yields compared to WT-TK. Notably, high stereoselectivities (97–98% *ee*) were observed when using butanal, pentanal and hexanal, and the highest *ee*s were when using aldehydes of a similar size to *in vivo* substrates for TK. As shown in Fig. 1, the greatest enhancement in *ee* between WT and D469E was for the C_3 and C_4 aldehydes. The use of H26Y gave products in generally lower yields (4–21%), with the highest (3*R*)-stereoselectivities observed in **3b** using butanal (92% *ee*), decreasing to 78% *ee* for **3e**. The reversal in *ee* was observed across the whole series of linear aldehydes used, although a smaller change in *ee* was observed in going from C_3 to C_8 (Fig. 1). The lower yields with some longer chain length analogues reflect a lower turnover of substrate and may be due to steric interactions in the active site.

The selection of TK-mutants for use with cyclic aldehydes was less clear, and very low yields were observed using WT-TK. A recently developed tetrazolium red-based colorimetric assay was therefore used with the three substrates **2g–2i** against the D469X library, which had led to the identification of several improved mutants when using propanal.[12,21] From this, three

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mutants were selected, which were able to accept one or more of the cyclic aldehydes, D469T, D469K and D469L. These were used together with mutants D469E and H29Y, which gave high selectivities with the linear series. The results indicated that high stereoselectivities could be achieved with D469E: the cyclopropane and cyclopentane adducts, **3g** and **3h**, were formed in >99% *ee*, and cyclohexane analogue **3i** formed in 97% *ee* (by HPLC) (Table 1). In all cases, the major isomer was the (1*S*)-product. When using D469T similar results were observed, with all products **3g-3i** formed in 99% *ee* (the (1*S*)-isomer). Similar yields were observed with both mutants, with **3g** and **3i** formed in 3–10% yields, while the cyclopentane product was formed in a higher 30–40% yield. This possibly reflects the comparable ring size of **2h** and the natural substrate, ribose-5-phosphate (furanose form). Mutants D469K and D469L gave the cyclopropane product in 99% *ee* in low yields, but when using **2h** and **2i** either low *ee*s (D469K) or no reaction (D469L) were observed. The use of H26Y-TK gave no product with the cyclopropane and cyclohexane aldehydes, but when using cyclopentanecarboxaldehyde, **3h** was formed in 30% *ee*, and the major isomer was the (1*R*)-product. Overall, these results suggest that when using cyclic substrates D469E and D469T may be suitable mutants to achieve bioconversions. The smallest cyclic substrate **2g** appeared to have greater activity with a larger range of mutants (leading to high *ee*s), presumably because it causes less steric problems. Most mutants gave (1*S*)-products, other than the low *ee* for the (1*R*)-isomer observed with H26Y-TK. The generally lower yields for the cyclic series may reflect poor substrate solubilities and/or product inhibition.

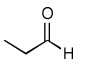
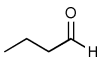
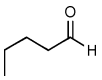
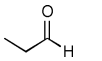
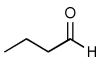
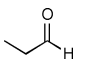
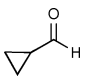
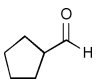
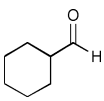
Initial relative rates were also determined for selected reactions using D469E, H26Y and D469T for the cyclic series (Table 2). Relative activities are given compared to propanal for each particular mutant: for comparison the wild-type specific activity towards glycolaldehyde was previously reported as 0.65 μmol $mg^{-1}min^{-1}$.[11]

Previous work highlighted that when using propanal, higher relative activities for D469E, H26Y and D469T compared to WT were observed. Here, relative rates decreased with increasing aldehyde size in all cases, although was most marked for H26Y. Interestingly, butanal had a higher initial rate with D469E than pentanal, although the isolated yield for the reaction was higher for the pentanal-derived product **3c**. This may be due to product inhibition by **3b**, limiting the overall reaction. For the cyclic aldehydes and D469T, increasing ring size also had less of an effect on initial rates, than increasing the linear chain length with D469E, although it decreased slightly as the ring size increased. This may result from lower conformational flexibilities of cyclic substrates, compared to aliphatic substrates, resulting in a smaller and more favourable entropy loss on binding. The small increase in conformational flexibility as the ring size increases may also lead to a less favourable loss of entropy upon binding.

Conclusions

Ideally, for use in synthesis, enzymes should have good substrate tolerance and demonstrate high stereoselectivities. In this work transketolase mutants able to accept lipophilic longer chain and cyclic aldehydes have been identified, which gives significant potential for further synthetic applications. With several substrate/mutant combinations very high stereoselectivities were observed, with moderate to low conversion yields. Future work is under way to investigate strategies to improve yields with lipophilic aldehyde acceptors.

Table 2 Initial relative rates for selected reactions with the aldehyde indicated

Aldehyde	Mutant	Initial rate/ mM h^{-1}	TK/ mg mL^{-1}	Spec. activity/ μmol mg^{-1} min^{-1}	Initial rel. rate[a]
	D469E	8.14	0.45	0.30	1
	D469E	2.14	0.45	0.08	0.3
	D469E	0.89	0.45	0.03	0.1
	H26Y	8.00	1.01	0.13	1
	H26Y	0.41	1.01	0.007	0.05
	D469T	12.5	0.30	0.69	1
	D469T	7.45	0.30	0.41	0.6
	D469T	4.47	0.30	0.25	0.4
	D469T	3.46	0.30	0.19	0.3

[a] Relative rates are given for substrates compared to propanal with the selected mutant.

Experimental

General methods

Unless otherwise noted, solvents and reagents were reagent grade from commercial suppliers (Sigma-Aldrich) and used without further purification. Dry CH_2Cl_2 was obtained using anhydrous alumina columns.[22] All moisture-sensitive reactions were performed under a nitrogen or argon atmosphere using oven-dried glassware. Reactions were monitored by TLC on Kieselgel 60 F_{254} plates with detection by UV, potassium permanganate and phosphomolybdic acid (PMA) [PMA hydrate (12 g) and ethanol (250 ml)] stains. Flash column chromatography was carried out using silica gel (particle size 40-63 μm). ^{1}H NMR and ^{13}C NMR spectra were recorded at the field indicated using Bruker AMX300 MHz, AMX400 Avance-500 MHz and Avance-600 MHz machines. Coupling constants are measured in Hertz (Hz) and unless otherwise specified, NMR spectra were recorded at 298 K. Mass spectra were recorded on a Thermo Finnegan MAT 900XP and Micro Mass Quattro LC electrospray mass spectrometers VG ZAB 2SE. Infrared spectra were recorded on

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a Shimadzu FTIR-8700 and Perkin Elmer Spectrum 100 FTIR spectrometer. Optical rotations were recorded on a Perkin Elmer model 343 polarimeter at 589 nm, quoted in deg cm^2 g^{-1} and conc (*c*) in g/100 mL. Chiral HPLC analysis was performed on a Varian Prostar instrument equipped with a Chiracel AD chiral column (Daicel; Chiral Technologies Europe, France) 25 cm × 0.46 cm.

Lithium hydroxypyruvate was synthesised as previously described.[2] 1,3-Dihydroxypentan-2-one **3a** was prepared as previously described.[12]

Synthesis of racemic α,α′-dihydroxyketones. The corresponding aldehyde (3.00 mmol) was added to a solution of Li-**1** (336 mg, 3.00 mmol) and *N*-methylmorpholine (330 μL, 3.00 mmol) in water (60 mL) at pH 8 (adjusted with 10% HCl). The reaction was stirred for 24–48 h at rt and monitored by TLC analysis. Upon concentration *in vacuo*, the crude material was dry loaded and purified using flash silica chromatography.

Chiral HPLC analysis of 3b–3i to determine *ee*s. Compounds **3b–3f** were dibenzoylated (monobenzylated compounds were not separable by chiral HPL columns used) and the products analysed by chiral HPLC to determine *ee*s. Ketodiols **3g–3i** were monobenzoylated at the primary alcohol for chiral HPLC analysis. HPLC analysis was carried out using a Chiralpak AD column and the hexane–2-propanol solvent system given.

Monobenzoylation procedure. To the ketodiol (30 μmol) in CH_2Cl_2 (5 mL) triethylamine (1.5 eq, 45 μmol) and benzoyl chloride (1.5 eq, 45 μmol) were added. The reaction was stirred for 2 h at rt, quenched by the addition of saturated $NaHCO_3$ solution (5 mL), and the organic phase was dried ($MgSO_4$). Upon removal of the solvent *in vacuo*, the resulting monobenzoylated material was dissolved in hexane–2-propanol (1 : 1) to a final concentration of 1 mg mL^{-1}.

Dibenzoylation procedure. To the ketodiol (30 μmol) in CH_2Cl_2 (5 mL) triethylamine (5 eq, 150 μmol) and benzoyl chloride (5 eq, 150 μmol) were added. The reaction was stirred for 2 h at rt, quenched by the addition of saturated $NaHCO_3$ solution (5 mL), and the organic phase was dried ($MgSO_4$). Upon removal of the solvent *in vacuo*, the resulting dibenzoylated material was dissolved in hexane–2-propanol (1 : 1) to a final concentration of 1 mg mL^{-1}.

1,3-Dihydroxyhexan-2-one (3b). The reaction was carried out for 24 h and the product purified using flash silica chromatography (EtOAc–hexane, 1 : 1) to give **3b** as a white powder (47 mg, 12%). Mp 109–112 °C (EtOAc); ν_{max}(neat)/cm^{-1} 3413, 2960, 2935, 2874, 1719; ^{1}H NMR (500 MHz; $CDCl_3$) δ 4.49 (1H, d, *J* 20.1, C*H*OH), 4.38 (1H, d, *J* 20.1, CH*H*OH), 4.31 (1H, dd, *J* 8.0 and 3.9, C*H*OH), 2.70 (br, O*H*), 1.75 (1H, m, 4-*H*H), 1.57 (1H, m, 4-H*H*), 1.46 (2H, m, CH_2CH_3), 0.95 (3H, t, *J* 7.3, CH_3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 211.9 (*C*-2), 74.8 (*C*-3), 65.6 (*C*-1), 36.3 (*C*-4), 18.1 (*C*-5), 13.8 (*C*-6); *m*/*z* (CI) 133 (MH^+, 100%), 115 ([MH – H_2O]$^+$, 30), 85 (47); Found (HRCI) MH^+ 133.08611. $C_6H_{12}O_3$ requires 133.08647. Racemic **3b** was dibenzoylated and HPLC analysis (97 : 3, 1 mL min^{-1}) gave retention times of 20.4 min (*R*-isomer) and 24.5 min (*S*-isomer).

1,3-Dihydroxyheptan-2-one (3c). The reaction was carried out for 24 h and the product purified using flash silica chromatography (EtOAc–hexane, 1 : 1) to give **3c** a white powder (152 mg, 35%). Mp 110–125 °C (EtOAc); ν_{max}(neat)/cm^{-1} 3430, 3263, 2956, 2929, 2872, 1720; ^{1}H NMR (500 MHz; $CDCl_3$) δ 4.49 (1H, d, *J* 19.4, C*H*OH), 4.39 (1H, d, *J* 19.4, CH*H*OH), 4.31 (1H, dd, *J* 7.9 and 3.9, C*H*OH), 2.89 (br, O*H*), 1.80 (1H, m, 4-*H*H), 1.20–1.75 (5H, m, 4-H*H*, $(CH_2)_2CH_3$), 0.91 (3H, t, *J* 6.6, CH_3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 211.7 (*C*-2), 75.0 (*C*-3), 65.6 (*C*-1), 34.0 (*C*-4), 26.8 (*C*-5), 22.5 (*C*-6), 13.9 (*C*-7); *m*/*z* (CI) 147 (MH^+, 100%), 129 ([MH – H_2O]$^+$, 35), 85 (94); Found (HRCI) MH^+ 147.10288. $C_7H_{14}O_3$ requires 147.10212. Racemic **3c** was dibenzoylated and HPLC analysis (97 : 3, 1 mL min^{-1}) gave retention times of 16.6 min (*R*-isomer) and 20.0 min (*S*-isomer).

1,3-Dihydroxyoctan-2-one (3d). The reaction was carried out for 24 h and the product purified using flash silica chromatography (EtOAc–hexane, 1 : 1) to give **3d** as a white powder (11 mg, 3%). Mp 106–110 °C (EtOAc); ν_{max}(neat)/cm^{-1} 3406, 2956, 2925, 2858, 1720; ^{1}H NMR (500 MHz; $CDCl_3$) δ 4.49 (1H, d, *J* 19.4, C*H*OH), 4.38 (1H, d, *J* 19.4, CH*H*OH), 4.31 (1H, dd, *J* 7.9 and 3.9, C*H*OH), 2.89 (br, O*H*), 1.77 (1H, m, 4-*H*H), 1.58 (1H, m, 4-H*H*) 1.20–1.34 (6H, m, 5-H_2, 6-H_2, 7-H_2), 0.89 (3H, t, *J* 6.9, CH_3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 211.7 (*C*-2), 75.0 (*C*-3), 65.6 (*C*-1), 34.3 (*C*-4), 31.6 (*C*-5), 24.4 (*C*-6), 22.5 (*C*-7), 14.0 (*C*-8); *m*/*z* (CI) 161 (MH^+, 100%); Found (HRCI) MH^+ 161.11823. $C_7H_{14}O_3$ requires 161.11777. Racemic **3d** was dibenzoylated and HPLC analysis (97 : 3, 1 mL min^{-1}) gave retention times of 15.5 min (*R*-isomer) and 19.9 min (*S*-isomer).

1,3-Dihydroxynonan-2-one (3e). The reaction was carried out for 24 h and the product purified using flash silica chromatography (EtOAc–hexane, 1 : 1) to give **3e** as a white powder (22 mg, 4%). Mp 104–107 °C (EtOAc); ν_{max}(neat)/cm^{-1} 3406, 2955, 2925, 2857, 1718; ^{1}H NMR (500 MHz; $CDCl_3$) δ 4.49 (1H, d, *J* 19.4, C*H*OH), 4.38 (1H, d, *J* 19.4, CH*H*OH), 4.30 (1H, dd, *J* 7.8, 3.9, C*H*OH), 2.96 (br s, O*H*), 1.78 (1H, m, 4-H*H*), 1.57 (1H, m, 4-H*H*), 1.27–1.50 (8H, m, 5-H_2, 6-H_2, 7-H_2, 8-H_2), 0.88 (3H, t, *J* 6.9, CH_3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 211.7 (*C*-2), 75.0 (*C*-3), 65.6 (*C*-1), 34.3 (*C*-4), 31.7 (*C*-5), 29.0 (*C*-6), 24.7 (*C*-7), 22.6 (*C*-8), 14.1 (*C*-9); *m*/*z* (CI) 175 (MH^+, 100%), 139 (62), 113 (97), 97 (100); Found (HRCI) MH^+ 175.13360. $C_9H_{19}O_3$ requires 175.13342. Racemic **3e** was dibenzoylated and HPLC analysis (97 : 3, 1.2 mL min^{-1}) gave retention times of 11.6 min (*R*-isomer) and 15.4 min (*S*-isomer).

1,3-Dihydroxydecan-2-one (3f). The reaction was carried out for 24 h and the product purified using flash silica chromatography (EtOAc–hexane, 1 : 1) to give **3f** as a white powder (2%, 3 mg). Mp 100–103 °C (EtOAc); ν_{max}(neat)/cm^{-1} 3420, 2959, 2928, 2873, 1721; ^{1}H NMR (500 MHz; $CDCl_3$) δ 4.49 (1H, d, *J* 19.4, C*H*OH), 4.38 (1H, d, *J* 19.4, CH*H*OH), 4.31 (1H, m, C*H*OH), 2.93 (br s, O*H*), 1.77 (1H, m, 4-H*H*), 1.20–1.61 (11H, m, 4-H*H*,5-H_2, 6-H_2, 7-H_2, 8-H_2, 9-H_2), 0.88 (3H, t, *J* 7.0, CH_3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 211.7 (*C*-2), 75.0 (*C*-3), 65.6 (*C*-1), 34.3 (*C*-4), 31.8 (*C*-5), 29.3 (*C*-6), 29.1 (*C*-7), 24.7 (*C*-8), 22.7 (*C*-9), 14.1 (*C*-10); *m*/*z* (CI) 189 (MH^+, 100%), 153 (93), 127 (97); Found (HRCI) MH^+ 189.14971. $C_{10}H_{21}O_3$ requires 189.14907. Racemic **3f** was dibenzoylated and HPLC analysis (97 : 3, 1.2 mL min^{-1}) gave retention times of 10.5 min (*R*-isomer) and 13.9 min (*S*-isomer).

1-Cyclopropyl-1,3-dihydroxy-2-propanone (3g). The reaction was carried out for 48 h and the product purified using flash silica chromatography (EtOAc) to give **3g** as an oil (0.013 g, 10%). R_f 0.50 (EtOAc). ν_{max}(KBr)/cm^{-1} 3380, 3007, 2924, 1724; ^{1}H NMR (300 MHz; $CDCl_3$) δ 4.50 (1H, d, *J* 19.3, C*H*OH), 4.37

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(1H, d, *J* 19.3, CH*H*OH), 4.30 (1H, d, *J* 4.2, C*H*OH), 2.19 (1H, m), 1.30–1.80 (4H, m, 2 × CH_2); ^{13}C NMR (75 MHz; $CDCl_3$) δ 211.4 (*C*-2), 77.0 (*C*HOH), 65.9 (*C*H_2OH), 43.0, 29.1, 25.8 and 25.6; *m*/*z* (CI) 161 (MH^+, 100%); *m*/*z* (FTMS) found [M + NH_4]$^+$ 148.0969. $C_6H_{14}O_3N$ requires 148.0968. Racemic **3g** was monobenzoylated and HPLC analysis (97 : 3, 1 mL min^{-1}) gave retention times of 31.1 min (*S*-isomer) and 33.6 min (*R*-isomer).

1-Cyclopentyl-1,3-dihydroxy-2-propanone (3h). The reaction was carried out for 48 h and the product purified using flash silica chromatography (EtOAc–hexane, 1 : 1) to give **3h** as a white solid (0.119 g, 25%). R_f 0.21 (EtOAc–hexane, 1 : 1). Mp 110–112 °C (EtOAc–hexane, 1 : 1); ν_{max}(KBr)/cm^{-1} 3411, 2953, 2870, 1718; ^{1}H NMR (300 MHz; $CDCl_3$) δ 4.50 (1H, d, *J* 19.4, C*H*HOH), 4.36 (1H, d, *J* 19.4, CH*H*OH), 4.30 (1H, m, C*H*OH), 3.04 (2H, s, 2 × O*H*), 2.18 (1H, m), 1.31–1.75 (8H, m, 4 × CH_2); ^{13}C NMR (75 MHz; $CDCl_3$) δ 211.5 (*C*-2), 76.9 (*C*HOH), 65.8 (*C*H_2OH), 42.9, 29.0, 25.7 and 25.6; *m*/*z* (FTMS) found [M + NH_4]$^+$ 176.1281. $C_8H_{18}O_3N$ requires 176.1281. Racemic **3h** was monobenzoylated and HPLC analysis (97 : 3, 1 mL min^{-1}) gave retention times of 32.1 min (*R*-isomer) and 34.8 min (*S*-isomer).

1-Cyclohexyl-1,3-dihydroxy-2-propanone (3i). The reaction was carried out for 48 h and the product purified using flash silica chromatography (EtOAc–hexane, 1 : 1) to give **3i** as a white solid (0.088 g, 17%). R_f 0.21 (EtOAc–hexane, 1 : 1). Mp 115–118 °C (EtOAc–hexane, 1 : 1); ν_{max}(KBr)/cm^{-1} 3429, 2940, 1712; ^{1}H NMR (300 MHz; CD_3OD) δ 4.45 (1H, d, *J* 19.3, C*H*HOH), 4.34 (1H, d, *J* 19.3, CH*H*OH), 3.96 (1H, d, *J* 4.4, C*H*OH), 1.45–1.87 (6H, m), 1.16–1.30 (5H, m); ^{13}C NMR (75 MHz; CD_3OD) δ 214.3 (*C*-2), 80.7 (*C*HOH), 67.4 (*C*H_2OH), 43.0, 30.3, 27.7, 27.3 (signals superimposed); *m*/*z* (CI) 173 (MH^+, 100%), 155 (45), 137 (86), 95 (100); Found (HRCI) MH^+ 173.11736. $C_9H_{17}O_3$ requires 173.11722. Racemic **3i** was monobenzoylated and HPLC analysis of the product (97 : 3, 1 mL min^{-1}) gave retention times of 17.0 min (*R*-isomer) and 18.5 min (*S*-isomer).

Biocatalytic synthesis of 3b-3i

TK Cell-free lysate preparation. Cells from a single colony of *Escherichia coli* XL10 transformed with the plasmid containing the appropriate TK mutant gene were inoculated in 10 mL of sterile Luria-Bertani medium containing ampicillin (150 mg mL^{-1}). The culture was incubated overnight (14 h) with orbital shaking at 200 rpm and 37 °C. The overnight culture was inoculated in 90 mL of fresh medium and incubated until the absorbance at 600 nm was greater than 4. The culture was pelleted by centrifugation at 4000 rpm for 20 min at 4 °C and then resuspended in cold phosphate buffer (5 mM, pH 7) to a final suspension of 1 g cell paste/10 mL buffer. The suspension was sonicated for 3 min in total at 4 °C, and centrifuged at 4500 rpm for 20 min at 4 °C. The supernatant cell-free lysate was aliquoted and stored at –20 °C until needed.

TK conversions. ThDP (22 mg, 48 μmol) and $MgCl_2 \cdot 6H_2O$ (39 mg, 180 μmol) were dissolved in H_2O (10 mL) and the pH adjusted to 7 using 0.1 M NaOH. To this stirred solution, at 25 °C, was added TK clarified lysate (2 mL) and the mixture stirred for 20 min. In another flask, Li-**1** (110 mg, 1.00 mmol) and the aldehyde (1.00 mmol) were dissolved in H_2O (8 mL) and the pH adjusted to 7 with 0.1 M NaOH. Following the 20 min enzyme/cofactor pre-incubation, the **1**–aldehyde mixture was added to the enzyme solution and the mixture stirred at rt for 24 h. During this time, the pH was maintained at 7.0 by addition of 1 M HCl using a pH stat (Stat Titrino, Metrohm) and the reactions were followed by TLC analysis. Silica was added and the reaction mixture concentrated to dryness, dry loaded onto a flash silica gel column, and purified as before.

Determination of initial rates for selected reactions

Linear series. Data for selected compounds was determined as detailed below. Aliquots of the biotransformation reaction (200 μL) were collected and diluted with 0.1% TFA (200 μL) and stored at –20 °C after various reaction times (0, 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 12, 24 and 48 h). Samples were centrifuged at 13,000 rpm for 5 min and the supernatant transferred to a clean microcentrifuge tube. The samples were diluted accordingly for the absorbance to be within the range of the calibration curve and then analysed by HPLC. Separation of the biotransformation components was achieved by HPLC (Dionex, UK) using a Bio-Rad Amines HPX-87H reverse phase column (300 × 7.8 mm) and 0.2% TFA in water as the mobile phase at 60 °C. Detection of the products was carried out *via* UV absorption at 210 nm. Retention times for **3a**, **3b**, and **3c** were 15.4, 18.5 and 23.4 min, respectively.

Cyclic series. Data was determined *via* application of the colorimetric assay[21] as poor separation was observed using HPLC methods. D469T TK lysate, 60 μL was incubated with 100 μL cofactor solution (TPP (2.4 mM), and $MgCl_2$ (9 mM)) for 20 min at 20 °C. Li-**1** (7.4 mg, 50 mmol) and aldehyde (50 mmol) were added to the reaction mixture. Aliquots (50 μL) were taken at hourly intervals and the reaction was quenched with 50 μL methanol. The aliquots were transferred onto a microwell plate containing 10 mg MP-Carbonate resin (Biotage AB) and incubated at 20 °C for 3 h. 50 μL of the quenched reaction sample was transferred without resin into a microwell plate. The colour assay was performed as described previously:[21] 50 μL of each concentration was diluted with 100 μL of water. Automated injection of 20 μL of tetrazolium red solution (0.2% of 2,3,5-tripheyltetrazolium chloride in methanol) and 10 μL 3 M NaOH (aq) with shaking by FLUOstar Optima plate reader (BMG Labtechnologies GmbH), was followed by immediate measurement at OD_{485nm}.

Protein concentrations were determined using determined using a combined Bradford and SDS-PAGE densitometry method as previously described.[11a]

TK formation of 1,3-dihydroxyhexan-2-one (3b). WT-TK gave **3b** (47 mg, 36%) in 75% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}). D469E-TK gave **3b** (58 mg, 44%) in 98% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}); $[\alpha]_D^{20}$ –16.3 (*c* 1.4, CH_3OH). H26Y-TK gave **3b** (21 mg, 16%) in 92% *ee* (3*R*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}); $[\alpha]_D^{20}$ +14.2 (*c* 1.6, CH_3OH). The absolute stereochemistry of **3b** generated using D469E-TK was determined using the Mosher's derivatisation method.[13]

(2*R*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-hydroxy-2′-oxo-hexyl ester. The reaction was carried out under anhydrous conditions. Triethylamine (20 μL, 0.13 mmol) and (*S*)-MTPA chloride (5 μL, 0.03 mmol) were added to a stirred solution of **3b** from the D469E-TK reaction (0.018 g, 0.07 mmol) in CH_2Cl_2 (2 mL) and the reaction was stirred for 2 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography

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(hexane–EtOAc, 10 : 1) to afford the Mosher's derivative as a colourless oil (4.6 mg, 43%). ^{1}H NMR (600 MHz; $CDCl_3$) δ 7.64 (2H, m, Ph), 7.45 (3H, m, Ph), 5.21 (d, *J* 18.0, C*H*HO (2*R*,3′*R*-trace)), 5.11 (1H, d, *J* 18.0, C*H*HO (2*R*,3′*S*)), 5.07 (1H, d, *J* 18.0, CH*H*O (2*R*,3′*S*)), 4,96 (1H, d, *J* 18.0, CH*H*O (2*R*,3′*R*-trace)), 4.33 (1H, m, C*H*OH), 3.66 (3H, s, OCH_3), 2.90 (1H, d, *J* 5.4, O*H*), 1.79 (1H, m, C*H*H), 1.46–1.65 (3H, m, CH*H* and CH_2), 0.97 (3H, t, *J* 7.0, CH_2CH_3); ^{13}C NMR (150 MHz; $CDCl_3$) δ 204.3 (*C*-2′), 166.2 (C=O ester), 131.7, 129.9, 128.5, 127.5, 123.0 (q, J_{CF} 290, CF_3), 75.1 (*C*HOH), 67.0 (CH_2OH), 55.8 (OCH_3), 36.0, 29.7, 13.8 (CH_2CH_3); ^{19}F NMR (282 MHz; $CDCl_3$) δ -72.2; *m*/*z* (ES+) 371 (MNa^+, 100%); Found (HRES) MNa^+ 371.1096. $C_{16}H_{19}O_5F_3Na$ requires 371.1106.

TK formation of 1,3-dihydroxyheptan-2-one (3c). WT-TK gave **3c** (24 mg, 16%) in 84% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}). D469E-TK gave **3c** (84 mg, 58%) in 97% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}); $[\alpha]_D^{20}$ −38.5 (*c* 1.4, CH_3OH). H26Y-TK gave **3c** (10 mg, 7%) in 84% *ee* (3*R*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}); $[\alpha]_D^{20}$ +18.6 (*c* 1.4, CH_3OH).

TK formation of 1,3-dihydroxyoctan-2-one (3d). WT-TK gave **3d** (40 mg, 25%) in 85% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}). D469E-TK gave **3d** (75 mg, 47%) in 97% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}); $[\alpha]_D^{20}$ −32.5 (*c* 1.4, CH_3OH). H26Y-TK gave **3d** (20 mg, 12%) in 84% *ee* (3*R*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}); $[\alpha]_D^{20}$ +16.0 (*c* 1.4, CH_3OH). The absolute stereochemistry of **3d** generated using D469E-TK was determined using the Mosher's derivatisation method.[13]

(2*R*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-hydroxy-2′-oxo-octyl ester. The reaction was carried out under anhydrous conditions. To a stirred solution of **3d** from the D469E-TK reaction (0.015 g, 0.06 mmol) in CH_2Cl_2 (2 mL) was added triethylamine (20 µL, 0.13 mmol) and (*S*)-MTPA chloride (5 µL, 0.03 mmol) and the reaction was stirred for 2 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (hexane–EtOAc, 10 : 1) to afford the Mosher's derivative as a colourless oil (3.2 mg, 28%). ^{1}H NMR (600 MHz; $CDCl_3$) δ 7.64 (2H, m, Ph), 7.45 (3H, m, Ph), 5.20 (d, *J* 16.8, C*H*HO (2*R*,3′*R*-trace)), 5.10 (1H, d, *J* 16.8, C*H*HO (2*R*,3′*S*)), 5.07 (1H, d, *J* 16.8, CH*H*O (2*R*,3′*S*)), 4,96 (1H, d, *J* 16.8, CH*H*O (2*R*,3′*R*-trace)), 4.32 (1H, dd, *J* 8.0 and 3.9, C*H*OH), 3.66 (3H, s, OCH_3), 2.85 (1H, br s, O*H*), 1.05–1.80 (8H, m, 4 × CH_2), 0.89 (3H, t, *J* 7.3, CH_2CH_3); ^{13}C NMR (150 MHz; $CDCl_3$) δ 204.3 (*C*-2′), 166.2 (C=O ester), 131.7, 129.9, 128.5, 127.5, 75.3 (*C*HOH), 67.0 (CH_2OH), 55.8 (OCH_3), 33.9, 32.0, 29.5, 22.7, 14.0 (CH_2CH_3); ^{19}F NMR (282 MHz; $CDCl_3$) δ -72.2; *m*/*z* (ES+) 399 (MNa^+, 100%); Found (HRES) MNa^+ 399.1338. $C_{18}H_{23}O_5F_3Na$ requires 399.1395.

TK formation of 1,3-dihydroxynonan-2-one (3e). WT-TK gave **3e** (13 mg, 7%) in 74% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.2 mL min^{-1}). D469E-TK gave **3e** (25 mg, 14%) in 86% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.2 mL min^{-1}); $[\alpha]_D^{20}$ −22.7 (*c* 1.1, CH_3OH). H26Y-TK gave **3e** (6 mg, 4%) in 78% *ee* (3*R*-isomer) by HPLC (97 : 3, 1.2 mL min^{-1}); $[\alpha]_D^{20}$ +36.7 (*c* 0.9, CH_3OH).

TK formation of 1,3-dihydroxydecan-2-one (3f). WT-TK gave **3f** (3 mg, 2%) in 66% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.2 mL min^{-1}). D469E-TK gave **3f** (34 mg, 18%) in 86% *ee* (3*S*-isomer) by HPLC (97 : 3, 1.2 mL min^{-1}); $[\alpha]_D^{20}$ −13.3 (*c* 1.1, CH_3OH). H26Y-TK gave **3f** (39 mg, 21%) in 83% *ee* (3*R*-isomer) by HPLC (97 : 3, 1.2 mL min^{-1}); $[\alpha]_D^{20}$ +9.1 (*c* 0.6, CH_3OH).

TK formation of 1-cyclopropyl-1,3-dihydroxy-2-propanone (3g). WT-TK gave **3g** (3 mg, 2%) in 72% *ee* (1*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}). D469E-TK gave **3g** (13 mg, 10%) in >99% *ee* (1*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}); $[\alpha]_D^{20}$ +70.0 (*c* 0.3, $CHCl_3$). H26Y-TK gave no reaction. D469T-TK gave **3g** (14 mg, 10%) in 99% *ee* (1*S*-isomer), D469K-TK gave **3g** (3 mg, 2%) in 99% *ee* (1*S*-isomer), and D469L gave **3g** (2 mg, 2%) in 99% *ee* (1*S*-isomer). The absolute stereochemistry of **3g** generated using D469E-TK was determined using the Mosher's derivatisation method.[13]

(2*S*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-cyclopropyl-3′-hydroxy-2′-oxo-propyl ester. The reaction was carried out under anhydrous conditions. To a stirred solution of **3g** from the D469E-TK reaction (0.010 g, 0.08 mmol) in CH_2Cl_2 (1 mL) was added triethylamine (34 µL, 0.25 mmol) and (*R*)-MTPA chloride (10 µL, 0.04 mmol) in CH_2Cl_2 (2 mL) and the reaction was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (hexane–EtOAc, 4 : 1) to afford the Mosher's derivative as a colourless oil (0.013 g, 87%). R_f 0.45 (hexane–EtOAc; 1 : 1); $[\alpha]_D^{20}$ +45.0 (*c* 0.2, EtOH); ν_{max}(KBr)/cm^{-1} 3420, 2930, 2855, 1734; ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.63 (2H, m, Ph), 7.43 (3H, m, Ph), 5.37 (1H, d, *J* 15.0, C*H*HO (2*S*,3′*S*)), 5.17 (d, *J* 18.0, CH*H*O (2*S*,3′*R*-trace)), 5.05 (d, *J* 18.0, C*H*HO (2*S*,3′*R*-trace)), 4.95 (1H, d, *J* 15.0, C*H*HO (2*S*,3′*S*)), 4.33 (1H, m, C*H*OH), 3.60 (3H, s, OCH_3), 3.09 (1H, d, *J* 4.3 Hz), 1.03 (1H, m), 0.71 (2H, m), 0.55 (2H, m); ^{13}C NMR (125 MHz; $CDCl_3$) δ 203.0 (*C*-2′), 166.3 (C=O ester), 131.8, 129.9, 128.5, 127.6, 78.1 (*C*HOH), 66.9 (CH_2OH), 55.8 (OCH_3), 14.7, 2.9 and 2.8; ^{19}F NMR (282 MHz; $CDCl_3$) δ -72.3; *m*/*z* (FTMS) found $[M + NH_4]^+$ 364.1364. $C_{16}H_{21}F_3O_5N$ requires 364.1366.

TK formation of 1-cyclopentyl-1,3-dihydroxy-2-propanone (3h). WT-TK gave **3h** (2 mg, 1%) as a racemate. D469E-TK gave **3h** (63 mg, 40%) in >99% *ee* (1*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}); $[\alpha]_D^{20}$ +33.0 (*c* 0.5, $CHCl_3$). H26Y-TK gave **3h** (3 mg, 2%) in 30% *ee* (1*R*-isomer), D469T-TK gave **3h** (47 mg, 30%) in 99% *ee* (1*S*-isomer) and D469K-TK gave **3h** (16 mg, 10%) in 25% *ee* (1*S*-isomer). The absolute stereochemistry of **3h** generated using D469E-TK was determined using the Mosher's derivatisation method.[13]

(2*S*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-cyclopentyl-3′-hydroxy-2′-oxo-propyl ester. The reaction was carried out under anhydrous conditions. Triethylamine (34 µL, 0.25 mmol) and (*R*)-MTPA chloride (10 µL, 0.04 mmol) in CH_2Cl_2 (2 mL) were added to a stirred solution of **3h** from the D469E-TK reaction (0.010 g, 0.05 mmol) in CH_2Cl_2 (1 mL) and the reaction was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (hexane–EtOAc, 4 : 1) to afford the Mosher's derivative as a colourless oil (0.018 g, 78%). R_f 0.50 (hexane–EtOAc; 4 : 1); $[\alpha]_D^{25}$ −60.0 (*c* 0.1, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3429, 2930, 2855, 1733; ^{1}H NMR (600 MHz; $CDCl_3$) δ 7.62 (2H, m, Ph), 7.43 (3H, m, Ph), 5.20 (1H, d, *J* 16.9, C*H*HO (2*S*,3′*S*)), 4.92 (1H, d, *J* 16.9, C*H*HO (2*S*,3′*S*)), 4.31 (1H, d, *J* 3.6, C*H*OH), 3.65 (3H, s, OCH_3), 2.91 (1H, m), 2.23 (1H, m), 1.27–1.85 (10H, m, 5 × CH_2), no (2*S*,3′*R*)

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detected; ^{13}C NMR (150 MHz; $CDCl_3$) δ 204.0 (*C*-2′), 166.3 (C=O ester), 131.9, 130.0, 128.6, 127.6, 123.2 (q, J_{CF} 287, *C*F_3), 84.7 (q, J_{CF} 27, *C*CF_3), 77.2 (*C*HOH), 67.4 (*C*H_2OH), 55.9 (O*C*H_3), 42.9, 29.1, 25.8 (signals superimposed); ^{19}F NMR (282 MHz; $CDCl_3$) δ -72.2; *m/z* (FTMS) found $[M + NH_4]^+$ 392.1678. $C_{18}H_{25}F_3O_5N$ requires 392.1679.

(2*R*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-cyclopentyl-3′-hydroxy-2′-oxo-propyl ester. The reaction was carried out under anhydrous conditions. Triethylamine (34 μL, 0.25 mmol) and (*S*)-MTPA chloride (10 μL, 0.04 mmol) in CH_2Cl_2 (2 mL) were added to a stirred solution of **3h** from the D469E-TK reaction (0.010 g, 0.05 mmol) in CH_2Cl_2 (1 mL), and the reaction was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (hexane–EtOAc, 4 : 1) to afford the Mosher's derivative as a colourless oil (0.020 g, 87%). R_f 0.50 (hexane–EtOAc; 4 : 1); $[\alpha]_D^{25}$ +30.0 (*c* 0.1, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3430, 2930, 1732; ^{1}H NMR (600 MHz; $CDCl_3$) δ 7.63 (2H, m, Ph), 7.43 (3H, m, Ph), 5.08 (1H, d, *J* 16.9, C*H*HO (2*R*,3′*S*)), 5.05 (1H, d, *J* 16.9, C*H*HO (2*R*,3′*S*)), 4.31 (1H, t, *J* 4.2, C*H*OH), 3.64 (3H, s, OCH_3), 2.90 (1H, d, *J* 4.2, O*H*), 2.23 (1H, m), 1.34–1.78 (10H, m, 5 × CH_2), no (2*R*,3′*R*) detected; ^{13}C NMR (150 MHz; $CDCl_3$) δ 204.0 (*C*-2′), 166.3 (C=O ester), 131.9, 130.0, 128.6, 127.6, 123.2 (q, J_{CF} 287, *C*F_3), 84.7 (q, J_{CF} 27, *C*CF_3), 77.0 (*C*HOH), 67.4 (*C*H_2OH), 55.9 (O*C*H_3), 42.8, 29.1, 25.8 (signals superimposed); ^{19}F NMR (282 MHz; $CDCl_3$) δ –72.2.

TK formation of 1-cyclohexyl-1,3-dihydroxy-2-propanone (3i). WT-TK gave **3i** (1 mg, 1%) as a racemate. D469E-TK gave **3i** (17 mg, 10%) in 97% *ee* (1*S*-isomer) by HPLC (97 : 3, 1.0 mL min^{-1}); $[\alpha]_D^{20}$ +33.0 (*c* 0.5, $CHCl_3$). H26Y-TK gave no reaction. D469T-TK gave **3i** (5 mg, 3%) in 99% *ee* (1*S*-isomer) and D469K-TK gave **3i** (5 mg, 3%) in 25% *ee* (1*S*-isomer). The absolute stereochemistry with D469E-TK was determined using the Mosher's derivatisation method.[13]

(2*S*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-cyclohexyl-3′-hydroxy-2′-oxo-propyl ester. The reaction was carried out under anhydrous conditions. To a stirred solution of **3i** from the D469E-TK reaction (0.010 g, 0.05 mmol) in CH_2Cl_2 (1 mL) was added triethylamine (34 μL, 0.25 mmol) and (*R*)-MTPA chloride (10 μL, 0.04 mmol) in CH_2Cl_2 (2 mL) and the reaction was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (hexane–EtOAc, 4 : 1) to afford the Mosher's derivative as a colourless oil (0.018 g, 78%). R_f 0.45 (hexane–EtOAc; 4 : 1); $[\alpha]_D^{20}$ –26.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.63 (2H, m, Ph), 7.43 (3H, m, Ph), 5.19 (1H, d, *J* 17.0, C*H*HO (2*S*,3′*S*)), 5.07 (d, *J* 17.0, C*H*HO (2*S*,3′R-trace)), 5.03 (d, *J* 17.0, CH*H*O (2*S*,3′*R*-trace)), 4.90 (1H, d, *J* 17.0, CH*H*O (2*S*,3′*S*)), 4.16 (1H, dd, *J* 5.2, 3.3, C*H*OH), 3.65 (3H, s, OCH_3), 2.80 (1H, d, *J* 5.2, O*H*), 1.11–1.76 (11H, m); ^{13}C NMR (125 MHz; $CDCl_3$) δ 204.2 (*C*-2′), 166.2 (C=O ester), 131.9, 129.9, 128.5, 127.5, 79.7 (*C*HOH), 67.8 (*C*H_2OH), 55.8 (O*C*H_3), 41.9, 29.6, 26.4, 25.9 (signal overlap), 25.5; ^{19}F NMR (282 MHz; $CDCl_3$) δ –72.2; *m/z* (FTMS) found $[M + NH_4]^+$ 406.1831. $C_{19}H_{27}F_3O_5N$ requires 406.1836.

Modelling of substrate binding in WT-TK. The open source Autodock software[19] was used for all substrate docking models in the *E. coli* TK structure 1qgd.pdb with a cubic grid in the active site of sides 80 Å. Defaults were used for docking each substrate except for the following: the maximum number of energy evaluations was increased to 1 million, the number of genetic algorithm runs was increased from 10 to 200, and the grid spacing used was 0.375 Å. The enamine-ThDP intermediate structure obtained in yeast TK[23] was first docked into the *E. coli* TK, prior to docking of the aldehyde substrates.

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