DOI: 10.1002/adsc.200700550

α,β-Unsaturated Aldehydes as Substrates for Asymmetric C–C Bond Forming Reactions with Thiamin Diphosphate (ThDP)-Dependent Enzymes

Anabel Cosp,[a,e] Carola Dresen,[b,e] Martina Pohl,[c] Lydia Walter,[b] Caroline Röhr,[d] and Michael Müller[b,*]

[a] Institute of Biotechnology 2, Research Center Jülich, 52425 Jülich, Germany
[b] Institute of Pharmaceutical Sciences, Albert-Ludwigs-Universität Freiburg, Albertstr. 25, 79104 Freiburg, Germany
Fax: (+49)-761-203-6351; e-mail: michael.mueller@pharmazie.uni-freiburg.de
[c] Institute of Molecular Enzyme Technology, Heinrich-Heine University Duesseldorf, Research Center Jülich, 52426 Jülich, Germany
[d] Institute of Inorganic and Analytical Chemistry, Albert-Ludwigs-Universität Freiburg, Albertstr. 21, 79104 Freiburg, Germany
[e] These authors contributed equally to this work

Received: November 21, 2007; Published online: March 17, 2008

Supporting information for this article is available on the WWW under http://asc.wiley-vch.de/home/.

Abstract: The enzymes benzaldehyde lyase (BAL) from *Pseudomonas fluorescens,* benzoylformate decarboxylase (BFD) from *Pseudomonas putida* and pyruvate decarboxylase (PDC) from *Saccharomyces cerevisiae* provide different C–C bond forming possibilities of α,β-unsaturated aldehydes with aliphatic and aromatic aldehydes. Structure elucidation and determination of the absolute configuration of the products, which were obtained with high regio- and stereoselectivity were carried out. Selective 1,2-reactivity with yields of 75% and >98% *ee*, for one single isomer (**A**) were obtained, by choosing the suitable enzyme in combination with the appropriate substrates. By varying enzymes or substrates the regioisomeric hydroxy ketones **C**, with up to >99% *ee*, can be obtained. The application of these new chiral building blocks in the synthesis of natural products or biological active substances is considerably facilitated by applying the different ThDP-dependent enzymes as catalysts.

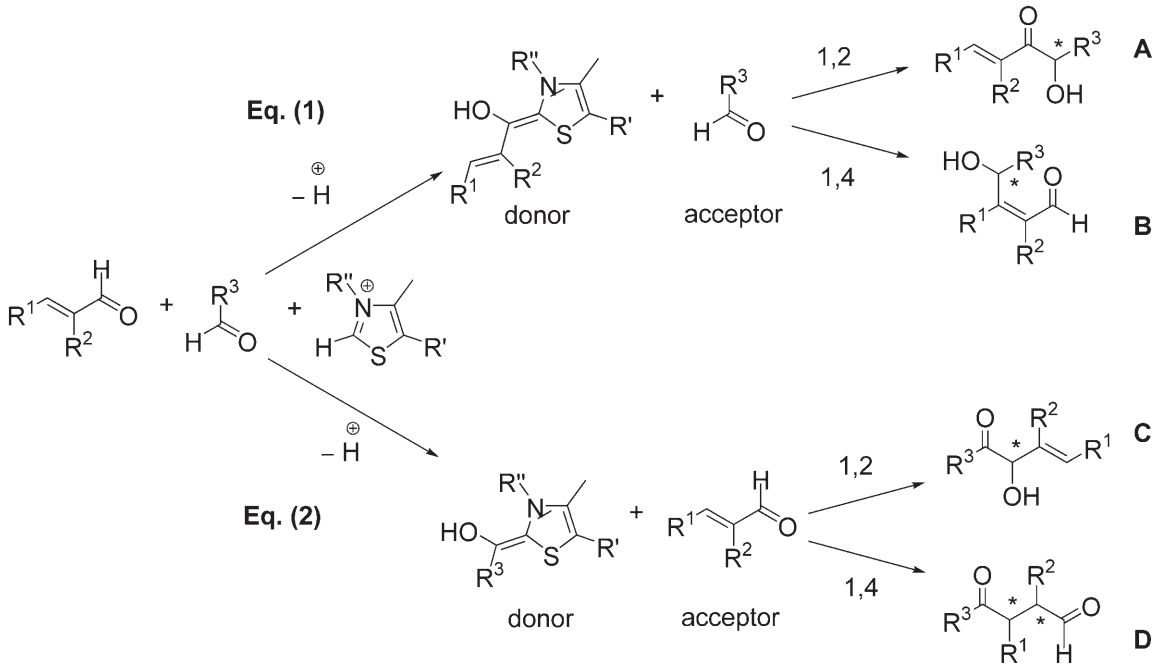
Abbreviations: BAL, benzaldehyde lyase; BFD, benzoylformate decarboxylase; PDC, pyruvate decarboxylase; His, hexahistidine; 2-HPP, 2-hydroxy-1-phenylpropan-1-one; PAC, phenylacetylcarbinol; NTA, nitrilotriacetic acid; ThDP, thiamin diphosphate; wt, wild-type.

Keywords: benzaldehyde lyase; benzoylformate decarboxylase; biocatalysts; pyruvate decarboxylase; stereochemistry

Introduction

Thiamin diphosphate (ThDP)-dependent enzymes are well-known to catalyse a broad range of asymmetric reactions.[1] Enzymes of this class like benzaldehyde lyase (BAL) from *Pseudomonas fluorescens*,[2] benzoylformate decarboxylase (BFD) from *Pseudomonas putida*[3] and pyruvate decarboxylase (PDC) from *Saccharomyces cerevisiae*[4] enable various modes of C–C bond forming reactions. Besides the physiological reactivity, several of these enzymes can catalyse, for example, the C–C bond formation between two aldehydes, giving chiral 2-hydroxy ketones with high enantioselectivity. The reactions of aromatic aldehydes as donor and aliphatic aldehydes as acceptor give rise to 2-hydroxy-1-phenyl-1-propanone derivatives with *S* absolute configuration in the case of BFD catalysis[5] and *R* for BAL.[6] If aromatic aldehydes are employed as donor and acceptor, (*R*)-benzoins are optained with both enzymes.[7,8] (*R*)-Phenylacetylcarbinol is formed in the PDC-catalysed reaction of decarboxylated pyruvate with benzaldehyde as acceptor aldehyde.[4,9]

Here we describe the use of α,β-unsaturated aldehydes as substrates for C–C bond ligation in BAL-, BFD- and PDC-catalysed reactions. These substrates might act as donor and as acceptor for the 1,2- (**A**, **C**) and the 1,4-addition (**B**, **D**) (Scheme 1).



Scheme 1. ThDP-dependent catalytic reaction possibilities of α,β-unsaturated aldehydes.

In reactions according to Equation (1) the α,β-unsaturated aldehyde is bound to ThDP and reacts as donor resulting in the formation of hydroxy ketone **A** or hydroxy enone **B**. In reactions according to Equation (2) the α,β-unsaturated aldehyde is attacked by an "active aldehyde" and reacts as acceptor resulting in the formation of hydroxy ketone **C** or 1,4-diketone **D**.

The aim of the work presented here was to evaluate the regio- and stereoselectivity of the enzymes BAL, BFD and PDC when using α,β-unsaturated aldehydes in combination with a second aldehyde – pyruvate in case of PDC – as substrates. The resulting enantioenriched hydroxy ketones **A** and **C**, which are difficult to synthesise by other means, are valuable building blocks for organic synthesis.[10] These structural moieties are found in diverse natural products.[11] Moreover, they can be easily converted into other building blocks by functional group transformations, such as reduction of or nucleophilic addition to the carbonyl function and oxidative cleavage of or addition to the double bond.[12,13]

Hydroxy ketones of type **A** and **C** have been synthesised among others by direct oxidation of enolates, although with low levels of stereoinduction,[14] by treatment of chiral hydroxy acids with a vinyllithium reagent,[15] by addition of 1-lithio-1-methoxyallene to a chiral ketone followed by hydrolysis,[16] by addition of an umpoled α,β-unsaturated acyl anion to aldehydes,[17] or by aldol reaction between aldehydes and protected hydroxy ketones in the presence of sodium hydride and butanol.[18] α,β-Unsaturated-α′-acetoxy ketones have been submitted to lipase-catalysed racemic resolution.[19]

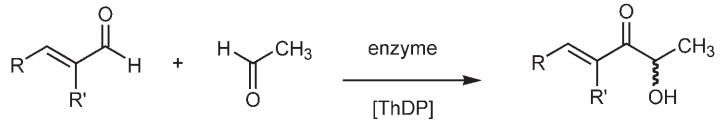
It is our aim to extend the donor-acceptor concept developed[8] and to broaden the scope of reactivity by using aromatic and aliphatic α,β-unsaturated aldehydes as substrates in ThDP-dependent enzymatic transformations.

Results and Discussion

α,β-Unsaturated Aldehydes as Donor Substrates [Scheme 1, Equation (1)]

We started evaluating the reaction between different α,β-unsaturated aldehydes (**1a–i**) and acetaldehyde (**2**) catalysed by BAL and wild-type BFD (BFDwt). In addition to the wild-type enzymes the variant BFD-H281A,[20] which showed a broader substrate range in earlier studies, was used (Table 1).[8] We assumed that in the presence of acetaldehyde the α,β-unsaturated aldehydes **1a–i** would act as donors in this kind of condensation, in a similar fashion as aromatic aldehydes do. Nevertheless, the ambident nature of the umpoled α,β-unsaturated aldehydes[21] could cause some regioselectivity problems since the electrophilic addition of acetaldehyde can occur in a 1,2- or 1,4-fashion. In fact, only one regioisomer aris-

Table 1. Reaction of the α,β-unsaturated aldehydes **1a–i** and acetaldehyde (**2**) catalysed by BAL, BFDwt or BFD-H281A.



1a – i **2** **3a – i**

Entry	R	R′	Aldehyde	Enzyme	Product	Isolated Yield	*ee*	Abs. config.
1	Ph	H	**1a**	BAL	(*R*)-**3a**	80%[a]	80%	*R*
2				BFDwt	**3a**	<1%	-	-
3				BFD-H281A		<1%	-	-
4	Ph	CH_3	**1b**	BAL	(*R*)-**3b**	75%[a]	> 96%	*R*
5				BFDwt	**3b**	<1%	-	-
6				BFD-H281A		<1%	-	-
7	Ph	Br	**1c**	BAL	(*R*)-**3c**	<1%	-	-
8				BFDwt	**3c**	<1%	-	-
9				BFD-H281A		<1%	-	-
10	Furyl	H	**1d**	BAL	(*R*)-**3d**	48%[a]	50%	*R*
11	-$(CH_2)_4$-		**1e**	BAL	(*R*)-**3e**	23%[a]	> 98%	*R*
12				BFDwt	(*S*)-**3e**	39%[b]	94%	*S*
13				BFD-H281A		<1%	-	-
14	H	CH_2CH_3	**1f**	BAL	**3f**	41%[c]	-	-
15	$(CH_2)_2CH_3$	H	**1g**	BAL	**3g**	80%[d]	87%	n.d.[e]
16				BFDwt		79%[d]	24%	n.d.[e]
17				BFD-H281 A		63%[d]	27%	n.d.[e]
18	$(CH_2)_4CH_3$	H	**1h**	BAL	**3h**	63%[d]	77%	n.d.[e]
19				BFDwt		26%[d]	23%	n.d.[e]
20				BFD-H281A		<1%	-	-
21	CH_2CH_3	CH_3	**1i**	BAL	**3i**	71%[d]	>98%	-
22				BFDwt		<1%	-	-

[a] Reaction performed in preparative scale (100 mL) with 5–10 mM α,β-unsaturated aldehyde (**1a**, **b**, **d**, **e**), 450–500 mM **2** and 150–180 mg lyophilised powder containing 30% of pure BAL along with phosphate buffer, ThDP and $MgSO_4$.
[b] Reaction performed in 15 mL reaction volume.
[c] According to GC-MS. Reaction performed on an analytical scale (1.5 mL).
[d] Conversion (mol%) according to NMR. Reaction performed on an analytical scale (1.5 mL).
[e] According to chiral phase HPLC the same absolute configuration.

ing from the attack at carbon C-1 of compounds **1a–i** was observed resulting solely in the formation of products **3** (Table 1).

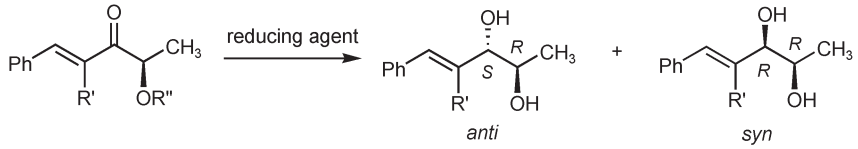
BAL catalyses the reaction of **2** and several α,β-unsaturated aldehydes **1** to give products of type (*R*)-**3**, with yields ranging from very good (entries 1 and 4) to poor (entry 11), and enantioselectivities spanning from excellent (entries 4 and 11) to moderate (entries 1 and 10). All tested unsaturated aldehydes, except α-bromocinnamaldehyde (**1c**, entry 7) were accepted as a donor substrate by this enzyme. This should not be greatly affected by variation of the substrate ratio, as shown for other substrate combinations in BAL catalysis. Nevertheless, influence of substrate ratio on donor/acceptor properties are well known for other ThDP-dependent enzymes.[22]

With BFDwt as a catalyst cyclohex-1-enecarbaldehyde (**1e**) was converted to (*S*)-**3e** with moderate yield and good enantioselectivity (entry 12).[23] Moreover, hexenal (**1g**) and octenal (**1h**) were also donor substrates for BFDwt in the presence of acetaldehyde (entry 16 and 19). The only substrate for BFD-H281A in the ligation with acetaldehyde was hexenal (**1g**) with moderate conversion to give **3g** with poor enantioselectivity (entry 17).

The enantioselectivity of the products obtained was determined by chiral phase HPLC and preparation of the Mosher ester derivative by reaction of (*R*)-**3b** [or (*S*)-**3e**, respectively] with Mosher's acid chloride.

The absolute configuration was assigned according to Mosher's method,[24] showing that *R*-configured products arise from BAL catalysis (see entries 1, 4, 10 and 11) while the *S* enantiomer is gained in one case with BFDwt as a catalyst (entry 12). Surprisingly, using hexenal (**1g**, entry 16) and octenal (**1h**, entry 19), both BAL and BFDwt yielded products with the same absolute configuration as was determined by chiral phase HPLC.

Table 2. Reduction of (*R*)-**3a**, (*R*)-**3b**, (*R*)-**4a**, (*R*)-**4b** to the corresponding diols.



(*R*)-**3a** R' = H, R" = H
(*R*)-**3b** R' = CH_3, R" = H
(*R*)-**4a** R' = H, R" = OAc
(*R*)-**4b** R' = CH_3, R" = OAc

5a, R' = H
5b, R' = CH_3

6a, R' = H
6b, R' = CH_3

Entry	Substrate	Reductant	Yield of diol	*anti*:*syn*
1	(*R*)-**3a** (R′ = H, R″ = H)	$NaBH_4$	84%	50:50
2		$Zn(BH_4)_2$	70%	78:22
3	(*R*)-**4a** (R′ = H, R″ = OAc)	$PhMe_2SiH$/TBAF	46%[a]	12:88
4	(*R*)-**3b** (R′ = CH_3, R″ = H)	$Zn(BH_4)_2$	80%	93:7
5	(*R*)-**4b** (R′ = CH_3, R″ = OAc)	$PhMe_2SiH$/TBAF	65%[a]	7:93

[a] Yield over two steps.

These results were confirmed by chiroptical correlations. The optical rotation of (*R*)-**4a** (*ee* = 80%), the *O*-acetylated derivative of (*R*)-**3a**, was determined as $[\alpha]_D^{25}$: + 18.6° ($CHCl_3$), while the optical rotation of the known *E*-(*S*)-2-acetoxy-5-phenyl-4-penten-3-one [(*S*)-**4a**] (*ee* > 95%) is described to be $[\alpha]_D^{25}$: −29° ($CHCl_3$).[12]

Diastereoselective reduction of the carbonyl function of (*R*)-**3a** and (*R*)-**3b** should give access to *anti*-diols (**5a** or **5b**) or *syn*-diols (**6a** or **6b**), respectively (Table 2).

For the preparation of the *anti*-diols **5a** and **5b** hydroxy ketones **3a** and **3b** were reduced with $Zn(BH_4)_2$, since this reductant is known to transform 2-hydroxy ketones into the *anti*-diols predominantly.[25] Accordingly, the corresponding *anti*-diols were isolated with good yields and moderate (**5a**) to good (**5b**) diastereoselectivities (Table 2). In contrast to $Zn(BH_4)_2$, chelation with $NaBH_4$ is not sufficient to induce high stereocontrol.

Comparison of the optical rotations of **5a** and **5b** with literature data[26,27] corroborated the (2*R*,3*S*) configuration of the predominant *anti*-diols, confirming the stereochemistry at C-2 of compounds **3a** and **3b** to be *R*.

Diastereoselective *syn* reduction of (*R*)-**4a** and (*R*)-**4b** was performed using the fluoride ion-catalysed hydrosilane reduction, a methodology developed by Hiyama and co-workers.[18,19] After acetylating (*R*)-**3a** and (*R*)-**3b** and treating the resulting α-acetoxyenones (*R*)-**4a** and (*R*)-**4b** with the aforementioned reductant the *syn*-diols **6a** and **6b** were obtained with moderate to good yields (Table 2).

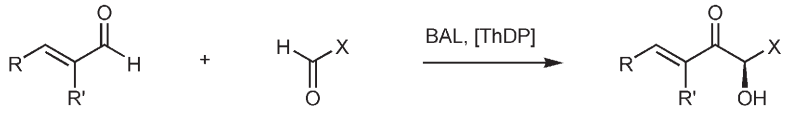
Since BAL proved to be a powerful biocatalyst for the carboligation reaction of α,β-unsaturated aldehydes as a donor and acetaldehyde as an acceptor, we turned our attention to extend the scope of this reaction to other acceptor substrates. The α-heteroatom-substituted acetaldehydes[28,29] **8**, **9** are promising substrates as the putative products **11**, **12** of the reaction would open a wide range of further transformations towards more complex structures. The use of formaldehyde (**7**)[30] seemed attractive, because the elongated products are not easy to obtain selectively by chemical methods.[31]

The reactions were run on an analytical scale and the results are summarised in Table 3. The product yields arising from the condensation with formaldehyde are very high (entries 1, 4, 10 and 13) with the noticeable exception of the condensation with **1d** (entry 7). Compounds **10a**, **10b** and **10e** were synthesised on a preparative scale and isolated in 51%, 82% and 56% yield, respectively.

Acetaldehyde derivatives **8**, **9** behave differently depending on which unsaturated aldehyde is used as a donor substrate. For instance, cinnamaldehyde (**1a**), α-methylcinnamaldehyde (**1b**), furylacrolein (**1d**), and 2-methylenebutanal (**1f**) gave poor to moderate yields (entries 2, 3, 5, 6, 8, 9, 14, 15), whereas the carbocyclic aldehyde **1e** offers good results with respect to yield (entries 11 and 12).

The BAL-catalysed condensation of **1e** with **8** or **9**, (entries 11 and 12, Table 3) were performed on a 50-mL scale (Scheme 2). Moderate yields (24% of **11e**, and 29% of **12e**) were obtained. The *ee* measured by preparation of the Mosher ester derivative was 90% for **11e** and 93% for **12e**, showing that these kinds of reactions occur with good stereochemical control. According to the analysis of the proton NMR of the Mosher ester derivatives, the absolute configuration of the new created stereocentre is *R*.

Table 3. Reaction between α,β-unsaturated aldehydes **1a**, **1b**, **1d–f** and formaldehyde (**7**) or acetaldehyde derivatives **8** and **9** catalysed by BAL.



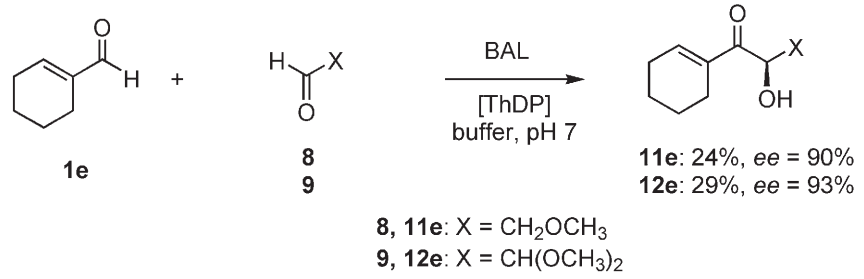
1 a, **b**, **d – f**

7 X = H
8 X = CH_2OCH_3
9 X = $CH(OCH_3)_2$

10a, **b**, **d – f** X = H
11a, **b**, **d – f** X = CH_2OCH_3
12a, **b**, **d – f** X = $CH(OCH_3)_2$

Entry	R	R′	Aldehyde	X	Product	Conversion[a]
1	Ph	H	**1a**	H	**10a**	92%
2				CH_2OCH_3	**11a**	10%
3				$CH(OCH_3)_2$	**12a**	60%
4	Ph	CH_3	**1b**	H	**10b**	95%
5				CH_2OCH_3	**11b**	25%
6				$CH(OCH_3)_2$	**12b**	<1%
7	Furyl	H	**1d**	H	**10d**	<1%
8				CH_2OCH_3	**11 d**	<1%
9				$CH(OCH_3)_2$	**12 d**	<1%
10	-$(CH_2)_4$-		**1e**	H	**10e**	>99%
11			CH_2OCH_3		**11e**	80%
12			$CH(OCH_3)_2$		**12e**	76%
13	H	CH_2CH_3	**1f**	H	**10f**	79%
14				CH_2OCH_3	**11 f**	58%
15				$CH(OCH_3)_2$	**12 f**	<1%

[a] According to GC-MS. Reactions performed on an analytical scale (1.5 mL) with 10 mM α,β-unsaturated aldehyde (**1a–f**), 60 mM **7**, **8** or **9** and 1 mg lyophilised powder containing 30% of pure BAL along with phosphate buffer, ThDP and $MgSO_4$.



1e

8
9

11e: 24%, *ee* = 90%
12e: 29%, *ee* = 93%

8, 11e: X = CH_2OCH_3
9, 12e: X = $CH(OCH_3)_2$

Scheme 2. Reaction of cyclohex-1-enecarbaldehyde (**1e**) and the acetaldehyde derivatives **8** and **9** catalysed by BAL.

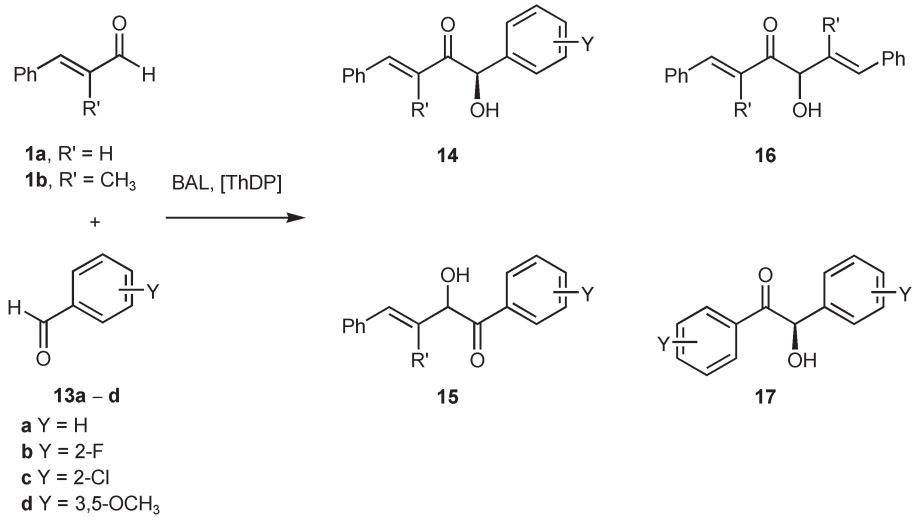
In summary, α,β-unsaturated aldehydes act as donors in the presence of acetaldehyde, acetaldehyde derivatives, or formaldehyde under BAL or BFDwt catalysis, giving rise to condensation products of type **3**, **10**, **11**, and **12**. The acceptor aldehyde adds in all cases to the carbonyl carbon of the α,β-unsaturated aldehydes (1,2-addition).

To further broaden the scope of this condensation reaction the aliphatic acceptor aldehyde has been replaced by substituted aromatic aldehydes. Up to 4 different compounds (each of them in two possible enantiomeric forms) are feasible, if selective 1,2-addition is assumed (Table 4): **14** and **15** obtained by cross-condensation, **16** and **17** arising from self-condensation.

Remarkably, the BAL-catalysed reaction of **1a** or **1b** with different aromatic aldehydes (**13a–d**) produces only 2 of the 4 possible products, namely those of type **14** and **17**. Products **15**, **16** for which the α,β-unsaturated aromatic aldehydes would have acted as the acceptor substrate,[32] were not observed under the conditions tested.

The yields of the cross-condensation product **14** are moderate as summarised in Table 4. The *ee* and absolute configurations were determined by analysis of the proton NMR of the Mosher ester derivatives.

Table 4. Reaction between α,β-unsaturated aromatic aldehydes **1a**, **b** and benzaldehyde derivatives **13a–d** catalysed by BAL.[a]



Entry	α,β-Unsaturated aldehyde	Aromatic aldehyde	Yield of **14**	Yield of **17**	*ee*	Absolute Configuration
1	**1a**, R′ = H	**13a**, Y = H	**14a**: < 1%	**17a**: < 1%	-	-
2		**13b**, Y = 2-F	**14b**: 56%	**17b**: 8%	-	-
3		**13c**, Y = 2-Cl	**14c**: 40%	**17c**: 18%	-	-
4		**13d**, Y = 3,5-CH_3O	**14d**: 33%	**17d**: 20%	**14d**: 72%	**14d**: *R*
5	**1b**, R′ = CH_3	**13a**, Y = H	**14e**: < 1%	**17a**: 50%	-	-
6		**13b**, Y = 2-F	**14f**: < 1%	**17b**: < 1%	-	-
7		**13c**, Y = 2-Cl	**14g**:[b] 43%	**17c**:[b] 17%	**14g**: 99%	**14g**: *R*
8		**13d**, Y = 3,5-CH_3O	**14h**: < 1%	**17d**: 25%	-	-

[a] Reactions performed on a preparative scale (50 mL) with 10 mM α,β-unsaturated aldehyde (**1a**, **b**), 10 mM aromatic aldehyde (**13a–d**) and 30 mg lyophilised powder containing 30% BAL.
[b] Reaction performed in 30 mL reaction volume with 15 mg lyophilised powder containing 30% BAL.

Thus, we have shown that the donor-acceptor concept described for aromatic aldehydes,[8] can be extended to the BAL-catalysed condensation of α,β-unsaturated aldehydes like **1a, b** with substituted benzaldehydes like **13a–d**.

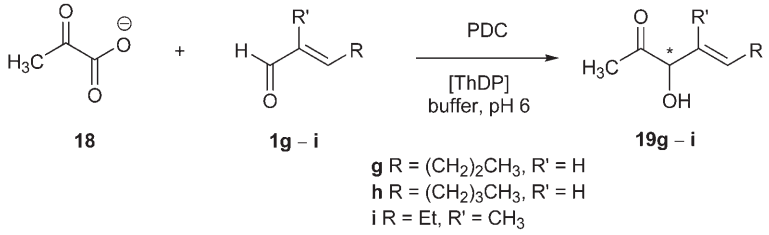
α,β-Unsaturated Aldehydes as Acceptor Substrates [Scheme 1, Equation (2)]

Pyruvate decarboxylase (PDC)[4] is known to catalyse the reaction between benzaldehyde (**13a**) (acceptor substrate) and pyruvate (**18**) or acetaldehyde, respectively, (activated acetaldehyde as donor substrate) to yield (*R*)-phenylacetylcarbinol [(*R*)-PAC].[33] We conducted analoguous transformations by reacting α,β-unsaturated aldehydes and pyruvate in the presence of PDC (Table 5). Substrates **1a** and **1g–i** were expected to act as the acceptor substrates. Although the ambident nature of **1** could also give rise to 1,4-addition products[34] in PDC-catalysed reactions only 1,2-regioselectivity was observed (see below).

Servi et al. reported that commercially available (Sigma) purified PDC from *Saccharomyces cerevisae* (*Sc*PDC) was able to condense benzaldehyde (**13a**) with pyruvate (**18**), but not α,β-unsaturated aldehydes.[35] However, it is known that baker's yeast catalyses the transformation depicted in Scheme 3.[36,37] This transformation is thought to occur as follows: pyruvate, obtained by degradation of glucose, is condensed with the α,β-unsaturated aldehyde (**1a–c**) catalysed by PDC to the hydroxy ketone **19** which is reduced *in situ* to the isolated diol *ent*-**5a–c** by means of an alcohol dehydrogenase (Scheme 3).[38]

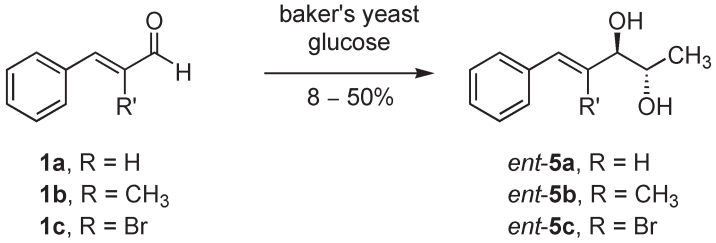
Crude extract of *E. coli* cells overexpressing the PDC gene from *Zymomonas mobilis* (*Zm*PDC) was used to test the type of reactivity depicted in Scheme 3. Compounds **1a–c** were investigated as possible acceptors versus pyruvate (**18**) as donor. Formation of (*R*)-phenylacetylcarbinol arising from the condensation of pyruvate (**18**) and benzaldehyde (**13a**) served as a positive control. Although the crude extract catalyses the (*R*)-PAC-production, no condensation products were detected with **1a**, **1b**, or **1c**. Thus,

Table 5. Reaction of α,β-unsaturated aldehydes **1g–i** and pyruvate (**18**) catalysed by *Sc*PDC.



Entry	R	R′	Aldehyde	Product	Conversion[a]	*ee*[b]
1	$(CH_2)_2CH_3$	H	**1g**	**19g**	58	>98%
2	$(CH_2)_4CH_3$	H	**1h**	**19h**	50	>98%
3	Et	Me	**1i**	**19i**	<2%	n.d.

[a] Conversion (mol%) according to NMR. Reaction performed on an analytical scale (1.5 mL).
[b] According to chiral phase HPLC.



Scheme 3. Baker's yeast-catalysed transformation of α,β-unsaturated aldehydes.[36,37]

it can be assumed that *Zm*PDCwt is not suitable as catalyst to perform this kind of transformations.

As already stated, baker's yeast is known to transform α,β-unsaturated aldehydes **1a–c** to chiral diols *ent*-**5a–c** (Scheme 3),[35,36] and PDC from *Saccharomyces cerevisiae* (*Sc*PDC) is thought to be one of the enzymes responsible for this transformation. *E. coli* BL21(DE3) expression strain was transformed with the pET22bPDC1 vector and enzyme expression was induced with IPTG. The cell-free extract was used for the following tests. The putative reaction between different α,β-unsaturated aldehydes (**1a** and **1g–i**) and pyruvate was assayed (Table 5). Again, the reaction between pyruvate (**18**) and benzaldehyde (**13a**) to give (*R*)-PAC served as positive control experiment.

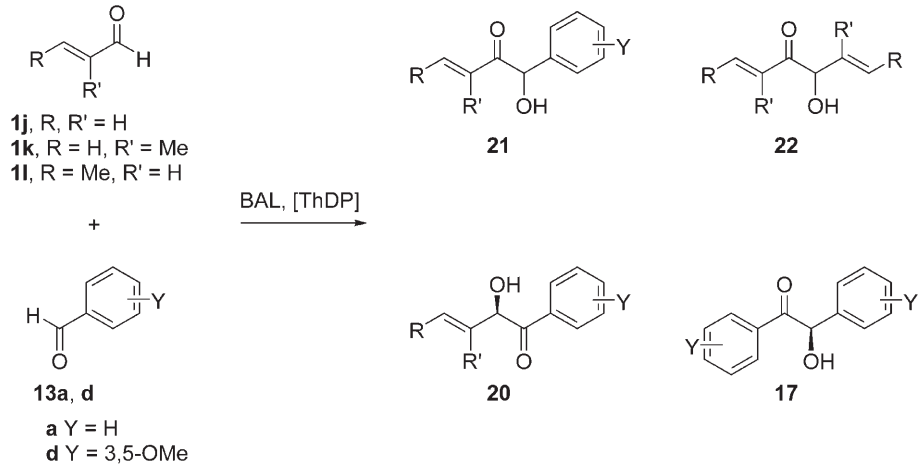
Whereas cinnamaldehyde (**1a**) and 2-methyl-2-pentenal (**1i**) were poor substrates for *Sc*PDC, the α,β-unsaturated aldehydes hexenal (**1g**) and octenal (**1h**) act as acceptor substrates with *Sc*PDC. The resulting hydroxy enones **19g** and **19h** (**C**) are formed with excellent enantioselectivity (>98% *ee*). Under BAL catalyses, using the same substrates, the α,β-unsaturated aldehydes **1g** and **1h** act as donors with acetaldehyde and give access to the isomeric hydroxy enones **3g** and **3h** (**A**).

Until now all tested α,β-unsaturated aldehydes **1a**, **1b** and **1d–i** act as donor, if BAL is the catalyst. Acetaldehyde (**2**), α-heteroatom-substituted acetaldehydes (**8** and **9**), and formaldehyde (**7**) as well as additional aromatic aldehydes were the acceptor substrates. All of them resulted in the hydroxy enones of type **A**.

To find a possibility for gaining access to the isomeric hydroxy enone **C**, we tested the reactivity of the small α,β-unsaturated aliphatic aldehydes acrolein (**1j**), methylacrolein (**1k**) and crotonaldehyde (**1l**), with aromatic aldehydes such as benzaldehyde (**13a**) and 3,5-dimethoxybenzaldehyde (**13b**) with BAL as biocatalyst (Table 6).

The reactions with 3,5-dimethoxybenzaldehyde (**13b**) as donor gave the best conversion in all cases tested, with methylacroleine (**1k**) showing the best acceptor capacity. The enantiomeric excess >99% of **20e** was determined by chiral phase HPLC and by comparison of the Mosher esters prepared by reaction with (*R*)- and (*S*)-Mosher's acid chloride, respectively. The absolute configuration was assigned according to the Mosher's method,[24] showing the *R* configuration of **20e**. Single crystal X-ray structure analysis was carried out (Figure 1). Measurements of the torsion angle O2–C2–C1–O1 resulted in 12.5(2)° and the angle O1–H2′–O2 was calculated to be 117(2)°. The distance between O2 and H2′ was calculated to be 0.98(2) Å and between O1 an H2′ 1.99(2) Å, showing that a hydrogen bond between the carbonyl oxygen

Table 6. Reaction of benzaldehyde derivatives **13a**, **d** and α,β-unsaturated aliphatic aldehydes **18j**, **k**, **l** catalysed by BAL.



Entry	Aldehyde	Aldehyde	Product	Conversion[a]	*ee*
1	**13a**	**1j**	**20a**	1%	n.d.
2		**1k**	**20b**[b]	12%	>98%
3		**1l**	**20c**	2%	n.d.
4	**13d**	**1j**	**20d**	36%	n.d.
5		**1k**	**20e**[c]	55%	>99%
6		**1l**	**20f**	25%	n.d.

[a] According to GC-MS.
[b] Reaction performed on a semipreparative scale (12 mL).
[c] Reaction performed on a preparative scale (100 mL) with 20 mM **13b**, 200 mM **1k** and 80 mg lyophilised powder containing 30% BAL.

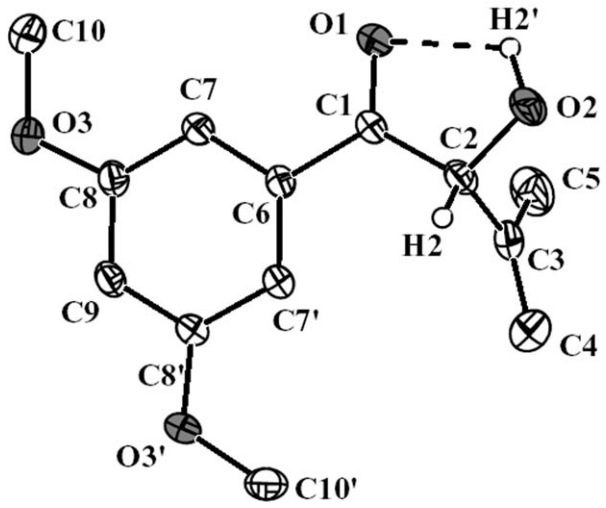


Figure 1. Molecular structure of **20e** showing the conformation.

and the hydroxy hydrogen is present, typical for 2-hydroxy ketones. The single crystal X-ray structure analysis presents the rigid conformation of **20e**. In combination with the UV and circular dichroism of **20e** compared with those of other (*R*)-configured 2-hydroxy ketones[8] (Figure 2) the results of the Mosher method are confirmed.

Conclusions

In summary, we have demonstrated, that α,β-unsaturated aldehydes are accepted as substrates by BAL, BFD and *Sc*PDC.

In BAL and BFDwt catalysis α,β-unsaturated aromatic aldehydes act as donors when combined with various aldehydes such as formaldehyde, acetaldehyde, acetaldehyde derivatives or benzaldehyde derivatives, affording highly functionalised condensation products with high regio- and stereoselectivity. Nevertheless, the same α,β-unsaturated aldehyde can act as acceptor substrate as well, if other enzyme or substrate combinations are used.

Moreover, one enzyme can use selectively α,β-unsaturated aldehydes as donor and as acceptor substrates, which is shown through experiments with BAL. Small α,β-unsaturated aliphatic aldehydes react regio- and stereoselectively as acceptors in the BAL-catalysed reaction with an aromatic aldehyde as donor.

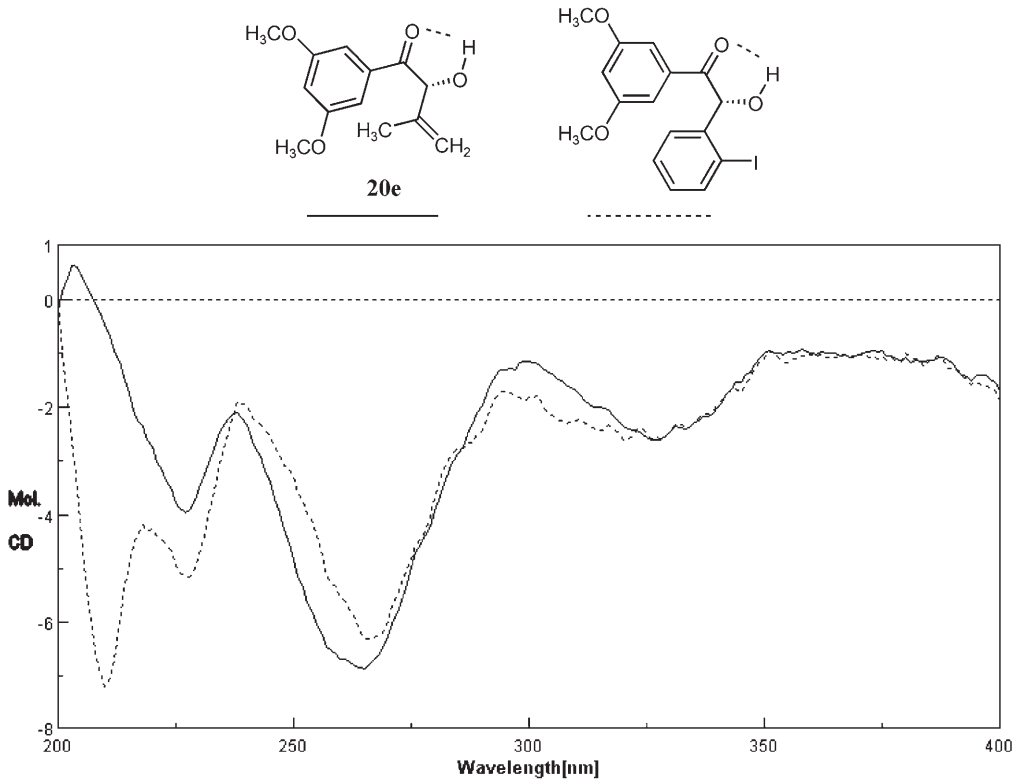


Figure 2. CD spectra of (*R*)-1-(3,5-dimethoxyphenyl)-2-hydroxy-3-methylbut-3-en-1-one (**20e**, solid line) in comparison to (*R*)-2-(2-iodophenyl)-1-(3,5-dimethoxyphenyl)-2-hydroxyethanone[8] (dashed line).

In the BAL- and BFD-catalysed reactions with acetaldehyde α,β-unsaturated aliphatic aldehydes react selectively as donor. In PDC-catalysed transformations with pyruvate as substrate, which forms by decarboxylation the activated acetaldehyde, the same α,β-unsaturated aliphatic aldehydes react selectively as acceptor.

In all cases the 1,2-regioselectivity is observed soley. Thus, if α,β-unsaturated aldehydes are submitted to C–C bond forming reactions with different ThDP-dependent enzymes, 1,2-addition products can be chemically and stereoselectively synthesised. Enzyme engineering should improve the scale-up potential with these non-physiological substrates. This opens the possibility for new retrosynthetic disconnections in synthetic strategies. In future, the access to chiral building blocks like **A** and **C** for the synthesis of natural products or biological and pharmacological active substances should be considerably facilitated. Furthermore, the still unexplored possibility of reactions towards products **B** and **D** demonstrates the potential of diversity-oriented approaches in biocatalytic syntheses.

Experimental Section

General Remarks

The characterisation data are available in the Supporting Information.

Representative Example for the Synthesis of (*R*)-2-Hydroxy-4-penten-3-ones (*R*)-3

(*R*)-2-Hydroxy-5-phenylpent-4-en-3-one [(*R*)-3a]: Cinnamaldehyde (**1a**) (126 μL, 132 mg, 1 mmol) and acetaldehyde (**2**) (2.5 mL, 45 mmol) were added into a mixture of DMSO (20 mL) and buffer A (75 mL) under an N_2 atmosphere. After addition of BAL (50 mg lyophilised powder in 5 mL buffer A) the reaction mixture was slowly stirred at 25 °C. Reaction process was monitored by GC-MS. Additional amounts of BAL were added, after 24 h (25 mg lyophilised powder in 2 mL buffer A), after 3 days (50 mg lyophilised powder in 5 mL buffer A) and after 4 days (25 mg lyophilised powder in 2 mL buffer A). After 7 days, the mixture was extracted with ethyl acetate (3×125 mL), the organic layer was washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate, 5:1) afforded (*R*)-**3a** as a pale yellow oil; yield: 139 mg (80%); *ee* = 80%.

(*R*)-2-Hydroxy-4-methyl-5-phenylpent-4-en-3-one [(*R*)-3b]: Reaction of α-methylcinnamaldehyde (**1b**) (140 μL, 146 mg, 1 mmol) with **2** under the same procedure as above afforded after purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 5:1) (*R*)-**3b** as a pale yellow oil; yield: 143 mg (75%); *ee* > 96% [(*R*)- and (*S*)-Mosher ester of (*R*)-**3b**, NMR] .

(*R*)-5-(Furan-2-yl)-2-hydroxypent-4-en-3-one [(*R*)-3d]: 3-(Furan-2-yl)acrylaldehyde (**1d**) (123 mg, 1 mmol) and acetaldehyde (**2**) (2.8 mL, 50 mmol) were added into a mixture of DMSO (20 mL) and buffer A (75 mL) under an N_2 atmosphere. After addition of BAL (100 mg lyophilised powder in 3 mL buffer A) the reaction mixture was slowly stirred at 25 °C. Reaction process was monitored by GCMS. Additional amounts of BAL were added, after 24 h (50 mg lyophilised powder in 2 mL buffer A) and after 3 days (30 mg lyophilised powder in 1 mL buffer A). After 5 days, the mixture was extracted with ethyl acetate (3 × 125 mL), the organic layer was washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 5:1) afforded (*R*)-**3d** as a brown oil; yield: 79 mg (48%); *ee* = 50%.

(*R*)-3-Cyclohexenyl-2-hydroxypropan-3-one [(*R*)-3e]: Reaction of cyclohexen-1-carbaldehyde (**1e**) (51 μL, 49 mg, 0.45 mmol) with **2** under the same conditions as above afforded after purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 5:1) (*R*)-**3e** as a colourless oil; yield: 16 mg (23%); *ee* > 98% (HPLC).

(*S*)-3-Cyclohexenyl-2-hydroxypropan-3-one [(*S*)-3e]: Cyclohexen-1-carbaldehyde (**1e**) (17 μL, 16 mg, 0.15 mmol) and acetaldehyde (**2**) (420 μL, 7.5 mmol) were added into a mixture of DMSO (3 mL) and buffer B (10 mL) under N_2 atmosphere. After addition of BFDwt (11 mg lyophilised powder in 2 mL buffer B) the reaction mixture was slowly stirred at 30 °C. Reaction process was monitored by GCMS. Additional amounts of BFDwt were added, after 1 and 2 days. After 12 days, the mixture was extracted with ethyl acetate (3 × 30 mL), the organic layer washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 5:1) afforded (*S*)-**3e**; yield: 9 mg (39%); *ee* = 94% (Lit.: *ee* = 94%)[5].

2-Hydroxyoct-4-en-3-one (3g), 2-hydroxydec-4-en-3-one (3h) and 2-hydroxy-4-methylhept-4-en-3-one (3i): 1 mg lyophilised powder of BAL was incubated in 1.5 mL of buffer A with 5% MTBE in presence of 30 mM hexenal (**1g**), octenal (**1h**) or 2-methyl-2-pentenal (**1i**) and 450 mM acetaldehyde (**2**) at 25 °C and 300 rpm. After 48 h the reactions were stopped and extraction with $CDCl_3$ was performed.

1 mg lyophilised powder of BFDwt or BFD-H281 A was incubated in 1.5 mL of buffer B with 5% MTBE in presence of 30 mM hexenal (**1g**), octenal (**1h**) or 2-methyl-2-pentenal (**1i**) and 450 mM acetaldehyde (**2**) at 25 °C and 300 rpm. After 42 h the reactions were stopped and extraction with $CDCl_3$ was performed.

(*R*)-3-Oxo-5-phenylpent-4-en-2-yl acetate [(*R*)-4a]: To a stirred solution of (*R*)-**3a** (16.8 mg, 0.095 mmol) in anhydrous pyridine (1 mL) acetyl chloride (23 μL, 0.32 mmol) was added at room temperature under N_2 atmosphere. After 3 h saturated NH_4Cl solution was added and the mixture extracted with $CHCl_3$ (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and brine and dried over Na_2SO_4. Evaporation of the solvent afforded (*R*)-**4a**; yield: 20 mg (98%); *ee* = 80%.

(*R*)-4-Methyl-3-oxo-5-phenylpent-4-en-2-yl acetate [(*R*)-4b]: (*R*)-**3b** (40.9 mg, 0.22 mmol) was submitted to the same acylating reaction conditions as for (*R*)-**3a** affording (*R*)-**4b**; yield: 43 mg (86%).

(2*R*,3*S*)-5-Phenylpent-4-ene-2,3-diol (5a): A 0.145 M solution of $Zn(BH_4)_2$ in Et_2O (1 mL, 0.145 mmol) was added to a stirred solution of (*R*)-**3a** (33.5 mg, 0.19 mmol) in anhydrous Et_2O at 0 °C under N_2 atmosphere. After 1 hour diluted acetic acid (25% (v/v), 1 mL) was added and the mixture was extracted with ethyl acetate (3 × 10 mL). The organic layer was washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, CH_2Cl_2/MTBE = 5:1) afforded **5a**; yield: 24 mg (70%); ($dr_{a:s}$ = 78:22 according to ^{1}H NMR from the crude product).

(2*R*,3*S*)-4-Methyl-5-phenylpent-4-ene-2,3-diol (5b): (*R*)-**3b** was submitted to identical reductive reaction conditions as for (*R*)-**3a**. Work-up, evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, CH_2Cl_2/ethyl acetate = 9:1) afforded **5b**; yield: 32.2 mg (80%); ($dr_{a:s}$ = 93:7 according to ^{1}H NMR from the crude product).

(2*R*,3*R*)-5-Phenylpent-4-ene-2,3-diol (6a): To a hexamethylphosphoric triamide (545 μL) solution of (*R*)-**4a** (23.3 mg, 0.107 mmol) and $PhMe_2SiH$ (20.5 μL, 0.13 mmol) was added TBAF (1 M in THF, 5.2 μL, 0.005 mmol) at 0 °C and the mixture was stirred for 2.5 h at this temperature. NaOH (1 M in MeOH, 2 mL) was added and the reaction mixture stirred for 30 min. After treatment with H_2O (2 mL), the solution was extracted with Et_2O (3 × 15 mL) and the organic layer was dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, CH_2Cl_2/MTBE = 6:1) afforded **6a**; yield: 9 mg (47%); ($dr_{a:s}$ = 12:88 according to ^{1}H NMR).

(2*R*,3*R*)-4-Methyl-5-phenylpent-4-ene-2,3-diol (6b): To a hexamethylphosphoric triamide (600 μL) solution of (*R*)-**4b** (40.4 mg, 0.17 mmol) and $PhMe_2SiH$ (33 μL, 0.21 mmol) was added TBAF (1 M in THF, 8.5 μL, 0.0085 mmol) at 0 °C and the mixture was stirred for 4 h at this temperature. The reaction was monitored by TLC and since no reaction was observed, $PhMe_2SiH$ (33 μL, 0.21 mmol) and TBAF (1 M in THF, 8.5 μL, 0.0085 mmol) were added. After 16 h at 0 °C, NaOH (1 M in MeOH, 2 mL) was added and the reaction mixture stirred for 30 min. After treatment with H_2O (2 mL), the solution was extracted with Et_2O (3 × 15 mL) and the organic layer was dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, petroleum ether/ethyl acetate = 2:1) afforded **6b**; yield: 25 mg (76%); ($dr_{a:s}$ = 7:93 according to ^{1}H NMR).

Reaction Conditions for 10–12a, 10–12b, 10–12d, 10–12e, 10–12f

1 mg lyophilised powder of BAL was incubated in 1.5 mL of buffer A with 20% DMSO in presence of 10 mM donor aldehyde **1a**, **b**, **d–f** and 60 mM formaldehyde (**7**), methoxy-

acetaldehyde (**8**) or dimethoxyacetaldehyde (**9**) at 23 °C and 300 rpm. Conversion was controlled by GC-MS.

1-Hydroxy-4-phenylbut-3-en-2-one (10a): Cinnamaldehyde (**1a**) (63 μL, 66 mg, 0.5 mmol) and formaldehyde (**7**) (37% in H_2O, 225 μL, 3 mmol) were added into a mixture of DMSO (10 mL) and buffer A (38 mL) under N_2 atmosphere. After addition of BAL (10 mg lyophilised powder in 2 mL buffer A) the reaction mixture was slowly stirred at room temperature. Reaction process was monitored by GC-MS. Additional amounts of BAL were added after 1 day (10 mg lyophilised powder in 2 mL buffer A). After 5 days, the mixture was extracted with ethyl acetate (3×50 mL), the organic layer was washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 5:1) afforded **10a** as a pale yellow oil; yield: 42 mg (51%).

1-Hydroxy-3-methyl-4-phenylbut-3-en-2-one (10b): Reaction of methylcinnamaldehyde (**1b**) (70 μL, 73 mg, 0.5 mmol) with formaldehyde (**7**) under the same conditions as above (**10a**) afforded after purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 5:1) **10b**, as a pale yellow oil; yield: 73 mg (82%).

2-Cyclohexenyl-1-hydroxyethanone (10e): Reaction of cyclohex-1-enecarbaldehyde (**1e**) (57 μL, 55 mg, 0. 5 mmol) with formaldehyde (**7**) was performed under the same conditions as above (**1a** to **10a**), but with a reaction time of 11 days. After purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 5:1) **10e** was obtained as a colourless oil; yield: 39 mg (56%).

(*R*)-3-Cyclohexenyl-2-hydroxy-1-methoxypropan-3-one [(*R*)-11e]: Cyclohex-1-enecarbaldehyde (**1e**) (57 μL, 55 mg, 0. 5 mmol) and methoxyacetaldehyde[39] (**8**) (0.45 M, 3.37 mL, 1.5 mmol) were added into a mixture of DMSO (10 mL) and buffer A (38 mL) under an N_2 atmosphere. After addition of BAL (30 mg lyophilised powder in 2 mL buffer A) the reaction mixture was slowly stirred at room temperature. Reaction process was monitored by GC-MS. After 7 days, the mixture was extracted with ethyl acetate (3×50 mL), the organic layer washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 5:1) afforded (*R*)-**11e** as a colourless oil; yield: 22 mg (24%); *ee* according to (*R*)-Mosher ester of (*R*)-**11e**: 90% (GC-MS, NMR).

(*R*)-3-Cyclohexenyl-2-hydroxy-1,1-dimethoxypropan-3-one [(*R*)-12e]: Cyclohex-1-enecarbaldehyde (**1e**) (57 μL, 55 mg, 0. 5 mmol) and dimethoxyacetaldehyde (**9**) (226 μL, 1.5 mmol) were added into a mixture of DMSO (10 mL) and buffer A (38 mL) under an N_2 atmosphere. After addition of BAL (30 mg lyophilised powder in 2 mL buffer A) the reaction mixture was slowly stirred at room temperature. Reaction process was monitored by GCMS. After 7 days, the mixture was extracted with ethyl acetate (3×50 mL), the organic layer washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, from isohexane/ethyl acetate = 10:1 to isohexane/ethyl acetate = 5:1) afforded (*R*)-**12e** as a colourless oil; yield: 31 mg (29%); *ee* according to (*S*)-Mosher ester of (*R*)-**12e**: 93% (GC-MS, NMR).

1-(2-Fluorophenyl)-1-hydroxy-4-phenylbut-3-en-2-one (14b): Cinnamaldehyde (**1a**) (63 μL, 66 mg, 0.5 mmol) and 2-fluorobenzaldehyde (**13b**) (53 μL, 62 mg, 0.5 mmol) were added into a mixture of DMSO (10 mL) and buffer A (38 mL) under an N_2 atmosphere. After addition of BAL (30 mg lyophilised powder in 2 mL buffer A) the reaction mixture was slowly stirred at 28 °C. Reaction process was monitored by GC-MS. After 42 h, the mixture was extracted with ethyl acetate (3×50 mL), the organic layer washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 10:1) afforded **14b** (yield: 72 mg, 56%) and **17b** (yield: 10 mg, 8%).

1-(2-Chlorophenyl)-1-hydroxy-4-phenylbut-3-en-2-one [14c]: Cinnamaldehyde (**1a**) (63 μL, 66 mg, 0.5 mmol) and 2-chlorobenzaldehyde (**13c**) (57 μL, 70 mg, 0.5 mmol) were added into a mixture of DMSO (10 mL) and buffer A (38 mL) under an N_2 atmosphere. After addition of BAL (30 mg lyophilised powder in 2 mL buffer A) the reaction mixture was slowly stirred at room temperature. Reaction process was monitored by GC-MS. After 2 days, the mixture was extracted with ethyl acetate (3×50 mL), the organic layer washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 10:1) afforded 52 mg of a mixture of cinnamaldehyde (**1a**), 2,2′-dichlorobenzoin (**17c**) and **14c** (according to ^{1}H NMR) and 35 mg of a mixture of **1a**, **17c** and **14c** (according to ^{1}H NMR). The calculated yield of **14c** is 40% (according to ^{1}H NMR).

(*R*)-1-(3,5-Dimethoxyphenyl)-1-hydroxy-4-phenylbut-3-en-2-one [(*R*)-14d]: Cinnamaldehyde (**1a**) (63 μL, 66 mg, 0.5 mmol) and 3,5-dimethoxybenzaldehyde (**13d**) (83 mg, 0.5 mmol) were added into a mixture of DMSO (10 mL) and buffer A (38 mL) under an N_2 atmosphere. After addition of BAL (30 mg lyophilised powder in 2 mL buffer A) the reaction mixture was slowly stirred at room temperature. Reaction process was monitored by GC-MS. After 24 h, the mixture was extracted with ethyl acetate (3×50 mL), the organic layer washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 5:1) afforded (*R*)-**14d** as a pale yellow oil (yield: 50 mg, 33%) and **17d** (yield: 34 mg, 20%); *ee* according to (*R*)-Mosher ester of (*R*)-**14d**: 72% (GC-MS, NMR)

(*R*)-1-(2-Chlorophenyl)-1-hydroxy-3-methyl-4-phenylbut-3-en-2-one [(*R*)-14g]: Methylcinnamaldehyde (**1b**) (42 μL, 44 mg, 0.3 mmol) and 2-chlorobenzaldehyde (**13c**) (34 μL, 42 mg, 0.5 mmol) were added into a mixture of DMSO (6 mL) and buffer A (22 mL) under an N_2 atmosphere. After addition of BAL (15 mg lyophilised powder in 2 mL buffer A) the reaction mixture was slowly stirred at room temperature. Reaction process was monitored by GC-MS. After 7 days, the mixture was extracted with ethyl acetate (3×50 mL), the organic layer washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, isohexane/ethyl acetate = 10:1) afforded (*R*)-**14g** as a pale yellow oil (yield: 37 mg, 43%) and **17c** (yield:14 mg, 17%); *ee* according to (*R*)-and (*S*)-Mosher ester of (*R*)-**14g**: 99% (GC-MS, NMR)

Reaction Conditions for 19g–i

20 μL *Sc*PDC (cell-free extract, decarboxylase activity: 173 U/mL) were incubated in 1.5 mL of 0.1 M citrate buffer, pH 6.2, containing 1.5 mM ThDP, 20 mM $MgSO_4$ with 10% ethanol in the presence of 30 mM hexenal (**1g**), octenal (**1h**) or 2-methylpentenal (**1i**), respectively, and 50 mM sodium pyruvate (**2**) at 25 °C and 300 rpm. After 20 h the reactions were stopped by extraction with $CDCl_3$.

Reaction Conditions for 20a–f

1 mg lyophilised powder of BAL was incubated in buffer A (1.5 mL) with 5% MTBE in presence of 10 mM benzaldehyde (**13a**) or 3,5-dimethoxybenzaldehyde (**13d**) and 100 mM acrolein (**1j**), methylacrolein (**1k**) or crotonaldehyde (**1l**), respectively, at 30 °C and 300 rpm. After 50 h the reactions were stopped by extraction with ethyl acetate.

(*R*)-1-(3,5-Dimethoxyphenyl)-2-hydroxy-3-methylbut-3-en-1-one [(*R*)-20e]: 3.5-Dimethoxybenzaldehyde (**13d**) (332 mg, 2 mmol) and methylacrolein (**1k**) (1.6 mL, 20 mmol) were added into a mixture of MTBE (5 mL) and buffer A (90 mL) under an N_2 atmosphere. After addition of BAL (40 mg lyophilised powder in 5 mL buffer A) the reaction mixture was slowly stirred at 30 °C. Reaction process was monitored by GCMS. Additional amount of BAL (40 mg lyophilised powder in 5 mL buffer A) was added after 8 h. After 48 h, the mixture was extracted with ethyl acetate (3 × 125 mL), the organic layer was washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by column chromatography (SiO_2, cyclohexane/ethyl acetate = 5:1) afforded (*R*)-**20e**; yield: 160 mg (34%); *ee* > 99%. After crystallisation from diethyl ether clear crystals were obtained; mp 96.1 °C.

Crystal Structure of (*R*)-20e

Crystals suitable for X-ray analysis were obtained by recrystallisation from diethyl ether at 4 °C. Crystallographic data for (*R*)-1-(3,5-dimethoxyphenyl)-2-hydroxy-3-methylbut-3-en-1-one, (*R*)-**20e**, $C_{16}O_4H_{16}$: $M_r = 236.26$, orthorhombic, space group $P\ 2_12_12_1$, $a = 7.272(3)$ Å, $b = 10.510(4)$ Å, $c = 15.941(6)$ Å; $V = 1218.3(8)$ Å^3, $Z = 4$, $\rho_{calcd.} = 1.288$ g cm^{-3}, $\mu = 0.10$ mm^{-1}, $R_1 = 0.0340$, $wR_2 = 0.1144$, GOF = 1.063. The intensity data (5154 reflections, $R_{int} = 0.0346$) were collected on a Bruker AXS CCD diffractometer with graphite monochromated $Mo_{k\alpha}$ radiation ($\lambda = 0.71070$ Å).

The structure was solved by direct methods with SHELXS-97.[40] In the subsequent full-matrix least-squares refinements using SHELXL-97[41] based on 2117 observed reflections (all data) and 162 variable parameters all non-hydrogen atom positions were refined anisotropically, the calculated H-atom positions were treated using the riding model. CCDC 668323 contains the supplementary crystallographic data which can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam,ac,uk/data_request/cif, or from The Cambridge Crystallographic Data Centre 12 Union Road, Cambridge CB21EZ, UK; fax: (+44)-1223–336–033; or deposit@ccdc.cam.ac.uk.

Selected bond lengths (in Å) for (*R*)-**20e**: O1–C1 1.219(2); O2–C2 1.426(2); O3′–C8′ 1.369(2); O3′–C10′ 1.428(2); O3–C8 1.3723 (0.0021); O3–C10 1.420(2); C1–O1 1.219(2); C1–C6 1.502(2); C1–C2 1.519(3); C2–C3 1.527(3); C3–C4 1.317(3); C3–C5 1.485(3); C6–C7′ 1.394(2); C6–C7 1.396(3); C7–C8 1.391(2); C7′–C8′ 1.3995(2); C8–C9 1.397(2); C8′–O3′ 1.369(2); C8′–C9 1.377(3).

Acknowledgements

We express grateful thanks to Dr. Stephan König in Halle for providing the pET22bPDC1 plasmid. Dr. Marion Wendorff and Dr. Thorsten Eggert from the IMET are thanked for transforming the plasmid in E. coli BL21(DE3) cells and expression of ScPDC. We thank Volker Brecht for numerous NMR measurements.

References

[1] M. Pohl, B. Lingen, M. Müller, *Chem. Eur. J.* **2002**, *8*, 5289–5295; M. Pohl, G. A. Sprenger, M. Müller, *Curr. Opin. Biotechnol.* **2004**, *15*, 335–342.

[2] E. Janzen, M. Müller, D. Kolter-Jung, M. M. Kneen, M. J. McLeish, M. Pohl, *Bioorg. Chem.* **2006**, *34*, 345–361; A. S. Demir, M. Pohl, E. Janzen, M. Müller, *J. Chem. Soc. Perkin Trans. 1* **2001**, 633–635.

[3] R. Wilcocks, O. P. Ward, S. Collins, N. J. Dewdney, Y. P. Hong, E. Prosen, *Appl. Environ. Microbiol.* **1992**, *58*, 1699–1704.

[4] M. Pohl, *Adv. Biochem. Eng. Biotechnol.* **1997**, *58*, 15–43.

[5] H. Iding, T. Dünnwald, L. Greiner, A. Liese, M. Müller, P. Siegert, J. Grötzinger, A. S. Demir, M. Pohl, *Chem. Eur. J.* **2000**, *6*, 1483–1495.

[6] A. S. Demir, O. Sesenoglu, E. Eren, B. Hosrik, M. Pohl, E. Janzen, D. Kolter, R. Feldmann, P. Dünkelmann, M. Müller, *Adv. Synth. Catal.* **2002**, *344*, 96–103.

[7] A. S. Demir, T. Dünnwald, H. Iding, M. Pohl, M. Müller, *Tetrahedron: Asymmetry* **1999**, *10*, 4769–4774.

[8] P. Dünkelmann, D. Kolter-Jung, A. Nitsche, A. S. Demir, P. Siegert, B. Lingen, M. Baumann, M. Pohl, M. Müller, *J. Am. Chem. Soc.* **2002**, *124*, 12084–12085.

[9] H. Iding, P. Siegert, K. Mesch, M. Pohl, *Biochim. Biophys. Acta Prot. Struct. Mol. Enzymol.* **1998**, *1385*, 307–322.

[10] C. Rodriguez-Escrich, A. Olivella, F. Urpi, J. Vilarrasa, *Org. Lett.* **2007**, *9*, 989–992; M. Rodriquez, S. Terracciano, E. Cini, G. Settembrini, I. Bruno, G. Bifulco, M. Taddei, L. Gomez-Paloma, *Angew. Chem.* **2006**, *118*, 437–441; *Angew. Chem. Int. Ed.* **2006**, *45*, 423–427.

[11] F. A. Davis, B. C. Chen, *Chem. Rev.* **1992**, *92*, 919–934; D. B. Stierle, A. A. Stierle, B. Ganser, *J. Nat. Prod.* **1997**, *60*, 1207–1209; M. Clericuzio, M. Mella, P. Vita-Finzi, M. Zema, G. Vidari, *J. Nat. Prod.* **2004**, *67*, 1823–1828; K. H. Jang, B. H. Lee, B. W. Choi, H. S. Lee, J. Shin, *J. Nat. Prod.* **2005**, *68*, 716–723.

[12] T. Hiyama, K. Kobayashi, M. Fujita, *Tetrahedron Lett.* **1984**, *25*, 4959–4962.

[13] M. Fujita, T. Hiyama, *J. Org. Chem.* **1988**, *53*, 5405–5415; W. C. Still, J. H. Mcdonald, *Tetrahedron Lett.* **1980**, *21*, 1031–1034; J. Mulzer, B. List, J. W. Bats, *J.*

Am. Chem. Soc. **1997**, *119*, 5512–5518; Y. Ichikawa, H. Egawa, T. Ito, M. Isobe, K. Nakano, H. Kotsuki, *Org. Lett.* **2006**, *8*, 5737–5740.
[14] A. B. Smith, B. D. Dorsey, M. Ohba, A. T. Lupo, M. S. Malamas, *J. Org. Chem.* **1988**, *53*, 4314–4325.
[15] W. Choy, L. A. Reed, S. Masamune, *J. Org. Chem.* **1983**, *48*, 1137–1139.
[16] C. Palomo, M. Oiarbide, J. M. Garcia, A. Gonzalez, A. Lecumberri, A. Linden, *J. Am. Chem. Soc.* **2002**, *124*, 10288–10289.
[17] P. Tebben, M. Reggelin, D. Hoppe, *Tetrahedron Lett.* **1989**, *30*, 2919–2922; F. Pierre, D. Enders, *Tetrahedron Lett.* **1999**, *40*, 5301–5305; S. Harada, T. Taguchi, N. Tabuchi, K. Narita, Y. Hanzawa, *Angew. Chem.* **1998**, *110*, 1796–1798; *Angew. Chem. Int. Ed.* **1998**, *37*, 1696–1698.
[18] T. Ishikawa, T. Aikawa, E. Ohata, T. Iseki, S. Maeda, T. Matsuo, T. Fujino, S. Saito, *J. Org. Chem.* **2007**, *72*, 435–441.
[19] W. Adam, M. T. Diaz, C. R. Saha-Möller, *Tetrahedron: Asymmetry* **1998**, *9*, 791–796.
[20] E. S. Polovnikova, M. J. McLeish, E. A. Sergienko, J. T. Burgner, N. L. Anderson, A. K. Bera, F. Jordan, G. L. Kenyon, M. S. Hasson, *Biochemistry* **2003**, *42*, 1820–1830.
[21] J. M. Fang, M. Y. Chen, W. J. Yang, *Tetrahedron Lett.* **1988**, *29*, 5937–5938; S. Hünig, M. Schäfer, *Chem. Ber.* **1993**, *126*, 177–189; S. Hünig, M. Schäfer, W. Schweeberg, *Chem. Ber.* **1993**, *126*, 205–210.
[22] D. Gocke, C. L. Nguyen, M. Pohl, T. Stillger, L. Walter, M. Müller, *Adv. Synth. Catal.* **2007**, *349*, 1425–1435.
[23] T. Dünnwald, A. S. Demir, P. Siegert, M. Pohl, M. Müller, *Eur. J. Org. Chem.* **2000**, 2161–2170.
[24] G. R. Sullivan, J. A. Dale, H. S. Mosher, *J. Org. Chem.* **1973**, *38*, 2143–2147; J. M. Seco, E. Quinoa, R. Riguera, *Tetrahedron: Asymmetry* **2000**, *11*, 2781–2791.
[25] T. Nakata, T. Tanaka, T. Oishi, *Tetrahedron Lett.* **1983**, *24*, 2653–2656.
[26] R. Bernardi, C. Fuganti, P. Grasselli, G. Marinoni, *Synthesis* **1980**, 50–52.
[27] C. Fuganti, P. Grasselli, S. Servi, *J. Chem. Soc. Perkin Trans. 1* **1983**, 241–244.
[28] A. S. Demir, O. Sesenoglu, P. Dünkelmann, M. Müller, *Org. Lett.* **2003**, *5*, 2047–2050.
[29] A. S. Demir, O. Reis, *Tetrahedron* **2004**, *60*, 3803–3811.
[30] A. S. Demir, P. Ayhan, A. C. Igdir, A. N. Duygu, *Tetrahedron* **2004**, *60*, 6509–6512.
[31] J. H. Teles, J. P. Melder, K. Ebel, R. Schneider, E. Gehrer, W. Harder, S. Brode, D. Enders, K. Breuer, G. Raabe, *Helv. Chim. Acta* **1996**, *79*, 61–83.
[32] Both protons of the olefinic moiety of compounds **14** appear as doublets, whereas for **15** and **16** one olefinic proton would be a doublet of doublets.
[33] V. Kren, D. H. G. Crout, H. Dalton, D. W. Hutchinson, W. Konig, M. M. Turner, G. Dean, N. Thomson, *J. Chem. Soc. Chem. Commun.* **1993**, 341–343.
[34] T. Kitazume, N. Ishikawa, *Chem. Lett.* **1984**, 1815–1818; K. Faber, *Biotransformations in Organic Chemistry*, Springer, Berlin, **2000**, 5th edn.
[35] R. Cardillo, S. Servi, C. Tinti, *Appl. Microbiol. Biotechnol.* **1991**, *36*, 300–303.
[36] C. Fuganti, P. Grasselli, *Chem. Ind.* **1977**, 983–983.
[37] G. Bertolli, G. Fronza, C. Fuganti, P. Grasselli, L. Majori, F. Spreafico, *Tetrahedron Lett.* **1981**, *22*, 965–968.
[38] H. Ohta, K. Ozaki, J. Konishi, G. Tsuchihashi, *Agric. Biol. Chem.* **1986**, *50*, 1261–1266; R. Csuk, B. I. Glänzer, *Chem. Rev.* **1991**, *91*, 49–97.
[39] A solution of 1,1,2-trimethoxyethane (465 μL, 3.6 mmol) in aqueous HCl (0.5 M, 6.5 mL) was stirred for 1 hour at 50 °C and then cooled down to room temperature. Aqueous NaOH (2 M, 1.6 mL) was added until pH 7.
[40] G. M. Sheldrick, *SHELXS-97, Program for the Solution of Crystal Structures*, University of Göttingen **1997**.
[41] G M. Sheldrick, *SHELXL-97, Program for the Refinement of Crystal Structures*, University of Göttingen **1997**.

