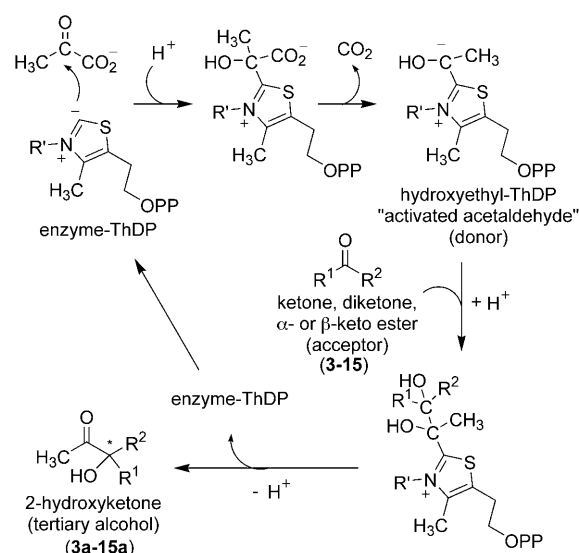


Enantioselective Intermolecular Aldehyde–Ketone Cross-Coupling through an Enzymatic Carboligation Reaction**

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Thiamine diphosphate (ThDP)-dependent enzymes are well-established catalysts in the field of asymmetric synthesis.^[1] One of the model reactions catalyzed by these enzymes is the asymmetric C–C bond-formation reaction between two aldehyde substrates that leads to the formation of 2-hydroxyketones in high enantioselectivities.^[2] Exchange of one of the aldehyde substrates in this reaction for a ketone^[3] would offer the opportunity for the catalytic asymmetric formation of chiral tertiary alcohols, which are important structural units in natural products and bioactive agents.^[4,5] During the last decade, different organocatalysts have been developed for the asymmetric aldehyde–ketone cross-coupling reaction,^[6] and intramolecular variants of this reaction have been reported in the literature.^[7,8] Most recently, Enders and Henseler described the direct intermolecular cross-coupling between aldehydes and trifluoromethyl ketones using a bicyclic triazolium salt as the catalyst.^[9] The asymmetric intermolecular non-enzymatic coupling reaction with ketone acceptors should be more difficult, owing to the lower electrophilicity of the ketone carbonyl group, and the increased steric hindrance compared with aldehyde substrates, and has not yet been reported in the literature. Herein, we present an asymmetric intermolecular aldehyde–ketone carboligation reaction using a ThDP-dependent enzyme as the catalyst (Scheme 1).

Branched-chain sugars are important bioactive carbohydrates and are widely represented in nature. Using feeding experiments, it can be shown that the two-carbon branch of several of these sugar derivatives is derived from pyruvate. In 1972, Grisebach and Schmid postulated the participation of



Scheme 1. YerE-catalyzed formation of tertiary alcohols.

ThDP in this carboligation reaction.^[10] In the biosynthetic pathway of yersinirose A, a two-carbon branched-chain 3,6-di(deoxy)hexose that is found in the O-antigen of *Yersinia pseudotuberculosis* O:VI, the ThDP-dependent flavoenzyme YerE catalyzes the decarboxylation of pyruvate and the transfer of the activated acetaldehyde onto the carbonyl function of CDP-3,6-di(deoxy)-4-keto-D-glucose (CDP = cytidine-5'-diphosphate).^[11,12] The enzymatic activity of YerE was confirmed by incubation of the protein with the enzymatically prepared physiological substrate (starting from CDP-D-glucose). The isolated product CDP-4-aceto-3,6-dideoxygalactose was confirmed by NMR spectroscopy.

To analyze the substrate range of the enzyme, we amplified the gene *yerE* from the chromosomal DNA of *Y. pseudotuberculosis* O:VI using a polymerase chain reaction. The gene was cloned into the pQE-60 expression vector and the recombinant protein was produced in *Escherichia coli* BL21(DE3) cells. The overexpressed C-terminal His-tagged YerE protein was purified to homogeneity by affinity chromatography using an Ni-NTA purification system (see the Supporting Information).

The amino acid sequence of YerE is similar to that of the large subunit of *E. coli* acetohydroxyacid synthases (EaHAS) (31 % identity). HAS successfully catalyzed the ThDP-dependent formation of (*S*)-acetolactate, and (*S*)-acetohydroxybutyrate, the first step in the biosynthesis of branched-chain amino acids, by the decarboxylation of pyruvate and condensation of the activated acetaldehyde with either a second molecule of pyruvate or with 2-

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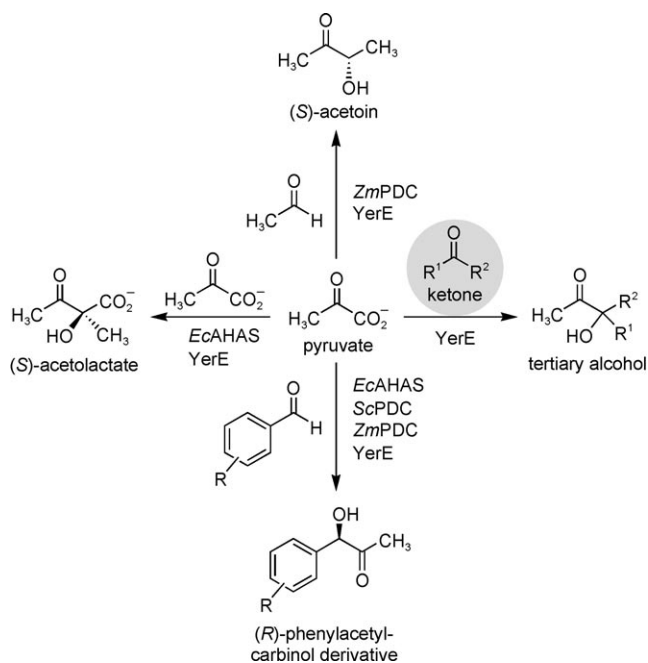
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oxobutyrate.^[13] Chipman et al.^[13] reported the carboligation activity of AHAS in the synthesis of (*R*)-phenylacetylcarbinol, (*R*)-PAC, a reaction that was first demonstrated for pyruvate decarboxylases (PDC) from *Saccharomyces cerevisiae* (ScPDC) and *Zymomonas mobilis* (ZmPDC) (Scheme 2).^[2] Chipman et al. also identified a variant of



Scheme 2. Carbolication reactions catalyzed by the ThDP-dependent enzyme YerE.

AHAS II from *E. coli* which suppressed the formation of acetolactate in favor of (*R*)-PAC.^[14] Our investigation into the substrate range of YerE revealed that this protein catalyzed the formation of (*S*)-acetolactate as well as (*R*)-PAC (Scheme 2).^[15] Furthermore, *ortho*-substituted benzaldehydes, which are poor substrates for EcAHAS I and ZmPDC catalysts,^[16,17] were efficiently converted into their corresponding (*R*)-2-hydroxyketones, such as **1a** (1-(2-chlorophenyl)-1-hydroxypropan-2-one) and **2a** (1-hydroxy-1-(4-hydroxyphenyl)-propan-2-one), in high enantiomeric excess (Table 1). YerE also catalyzed a carbolication reaction to afford (*S*)-acetoin, using pyruvate and acetaldehyde as substrates.^[18] This transformation is also catalyzed by ZmPDC (Scheme 2).^[19]

As well as these transformations, which to some extent are known to be inherent to ThDP-dependent enzymes, and according to the retro-biosynthetic strategy, we reasoned that YerE might activate non-sugar ketones in cross-coupling reactions. Enzymatic aldehyde–ketone cross-couplings have not yet been reported, although many different combinations of enzymes and substrates have been tested.^[20] By testing putatively successful aldehyde–ketone combinations with the YerE catalyst, we observed the conversion of various cyclic or acyclic ketones as acceptor substrates (Scheme 1).

We examined the tolerance of the procedure for different acceptor substrates and found that small cyclic aliphatic

Table 1: Substrate range of YerE.^[a]

Acceptor		Conversion [%] ^[b] after 20–25 h	Yield ^[c] [%]	ee ^[e] [%]
	1	88	60	94 (<i>R</i>)
	2	79	69	96 (<i>R</i>)
	3	55	—	— ^[f]
	4	47	39	9
	5	97	34	84 ^[h]
	6	48	24	91
	7	37	26	78 (<i>R</i>)
	8	20	14	96
	9	27	9	87
	10	31	25	< 5
	11	32 ^[d]	— ^[g]	22
	12	58 ^[d]	34	84
	13	26	18	63 (<i>R</i>)
	14	42	23	30 ^[i]
	15	> 99	— ^[j]	30 ^[i]

[a] All transformations were performed at 25 °C using 20 mM of the acceptor, 50 mM of pyruvate, and a reaction volume of 40 or 50 mL. [b] Conversion determined by ¹H NMR spectroscopy. [c] Yield of isolated product. [d] Conversion determined by GC–MS. [e] Enantiomeric excess determined by chiral-phase HPLC or by chiral-phase GC. [f] Achiral product. [g] The starting material and the product could not be separated by column chromatography on silica gel. [h] The low yield was probably due to the formation of an azeotrope. [i] Enantiomeric excess determined by ¹H NMR spectroscopy using a chiral lanthanide shift reagent. [j] No quantifiable yield was isolated owing to the volatility of the compound.

compounds like cyclohexanone (**3**) and methylated cyclohexanones were successfully converted under these conditions. In the next step, we synthesized tetrahydro-2H-pyran-3-one (**5**),^[21] as this compound was expected to successfully react with YerE; quantitative conversion of **5** confirmed this assumption. The enantiomeric excess of the product (84 %) was determined by chiral-phase GC with a chemically synthesized racemic reference. This represents the first example of an asymmetric aldehyde–ketone carboligation reaction that is catalyzed by a ThDP-dependent enzyme, YerE.

After establishing that cyclic ether **5** was a suitable acceptor substrate for YerE, we investigated the tolerance of the carboligation reaction for open-chain acceptor ketones that contained an ether or thioether moiety (Table 1).^[22] The products were formed in moderate (**7** and **9**) to excellent enantiomeric excesses (**6** and **8**), although the tertiary alcohol formed from thioether substrate **10** was almost racemic. Therefore, exchange of the ether oxygen in substrate **6** for a sulfur atom (**10**) led to a strong decrease in the stereoselectivity of the enzyme. A crystal structure of the protein with bound substrates (currently under investigation) might help to explain this observation at the molecular level.

Next, we investigated the applicability of cyclic and open-chain 1,2-diketones as acceptor substrates (Table 1). In these cases, the activated acetaldehyde is transferred to only one of the carbonyl moieties. An interesting compound in this context is cyclohexane-1,2-dione (**11**) because it has been successfully employed in the hydrolytic C–C bond ring-cleavage reaction using ThDP-dependent flavoenzyme cyclohexane-1,2-dione hydrolase (CDH).^[23] This reaction is completely different from the carboligation reaction discussed herein, and demonstrates the diversity of ThDP-dependent enzyme-catalyzed transformations.

Furthermore, we found that ketones containing an α - or β -ketoesters (**14**, **15**) could also undergo carboligation using YerE as the biocatalyst (Table 1). When ethyl 4,4,4-trifluoro-3-oxobutyrates, an analogue of **14**, was used as the acceptor substrate, 52 % conversion was achieved (determined by ¹H NMR spectroscopy). However, aryl ketones, α,β -unsaturated, and α -branched ketones were not compatible substrates with YerE under these conditions.

Control experiments using the cell lysate, which were transformed with the pQE-60 vector without *yerE* insertion after application of the same expression conditions, did not show any activity with respect to the investigated reaction; therefore, after expression of YerE, the crude extract obtained after cell lysis was used for the preparative biotransformations (see the Supporting Information). All transformations were performed on a mmol scale, and the tertiary alcohols (**4a–10a**, **12a–14a**) were isolated in yields of up to 40 % (Table 1). After extraction of the reaction solution with organic solvent, GC–MS analysis of the organic layer showed only the desired tertiary alcohol reaction product as well as some residual acceptor substrate. The aldehyde–ketone cross-coupling products were obtained using pyruvate as the acetaldehyde synthon because the 2-ketoacid was the best donor substrate for enzyme YerE. Nevertheless, it was also possible to apply acetaldehyde directly into this reaction.

We also successfully incorporated an acetaldehyde functionality into the crossed-ligation product using equimolar concentrations of [2-¹³C]pyruvate and non-labeled acetaldehyde in the presence of [1-¹³C]cyclohexanone. The NMR analysis revealed a 4:1 ratio of the products afforded from the pyruvate to that formed from conversion of acetaldehyde (see the Supporting Information).

For the determination of the absolute configuration, two of the enzymatic products (**7a** and **13a**) were crystallized. The crystallographic data of both compounds were obtained using single-crystal X-ray diffraction analysis. In both cases, all molecules in the unit cell had an *R* configuration (Figure 1). To confirm that this configuration could be assigned as the major enantiomer, the crystals were subsequently analyzed by chiral-phase HPLC.

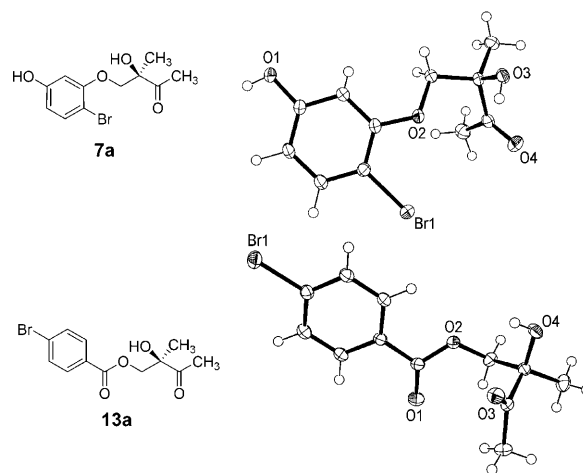


Figure 1. ORTEP structures of **7a** and **13a**. Ellipsoids set at 50 % probability level.^[24]

In summary, we have successfully performed asymmetric intermolecular crossed aldehyde–ketone coupling reactions using the ThDP-dependent enzyme YerE as catalyst. The substrate tolerance of the enzyme is very broad and includes cyclic and open-chain ketones, as well as diketones and α - and β -ketoesters as acceptor substrates. Several enzymatic products were isolated on the preparative scale, and the absolute configurations of two products were determined by single-crystal structure analysis.

This enzymatic transformation offers a simple entry to the preparation of enantioenriched tertiary alcohols that contain an α -acetyl moiety; these alcohols are valuable building blocks for asymmetric synthesis: in the formation of 1,2-diols (reduction), vicinal amino alcohols (reductive amination), or two contiguous tertiary alcohols (nucleophilic addition).

We propose that the identification of homologues of YerE would be a good starting point for the identification of similar activities. Elucidation of the three-dimensional structure of YerE will offer some information concerning the catalytic mechanism of the protein, because all other well-known ThDP-dependent enzymes like BAL, BFD or PDCs, which have been intensively studied with respect to their carboligation activity, did not accept ketones as acceptor substrates.

The design of structural variants of YerE is intended to improve the stereoselectivity of the protein. Furthermore, suppression of the formation of the acetolactate side product to improve the yield of the desired carboligation product is under investigation.

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- [1] M. Pohl, G. A. Sprenger, M. Müller, *Curr. Opin. Biotechnol.* **2004**, *15*, 335.
- [2] M. Pohl, B. Lingen, M. Müller, *Chem. Eur. J.* **2002**, *8*, 5288.
- [3] The enzymatic formation of acetolactate from the 2-ketoacid pyruvate has already been demonstrated (see Ref. [13]) but no ketones were used as substrates.
- [4] C. García, V. S. Martín, *Curr. Org. Chem.* **2006**, *10*, 1849.
- [5] H. Kourist, P. Dominguez de María, U. T. Bornscheuer, *ChemBioChem* **2008**, *9*, 491.
- [6] D. Enders, T. Balensiefer, O. Niemeier, M. Christmann, *Enantioselective Organocatalysis*, Wiley-VCH, Weinheim, **2007**, p. 331.
- [7] D. Enders, O. Niemeier, T. Balensiefer, *Angew. Chem.* **2006**, *118*, 1491; *Angew. Chem. Int. Ed.* **2006**, *45*, 1463.
- [8] H. Takikawa, Y. Hachisu, J. W. Bode, K. Suzuki, *Angew. Chem.* **2006**, *118*, 3572; *Angew. Chem. Int. Ed.* **2006**, *45*, 3492.
- [9] D. Enders, A. Henseler, *Adv. Synth. Catal.* **2009**, *351*, 1749.
- [10] H. Grisebach, R. Schmid, *Angew. Chem.* **1972**, *84*, 192; *Angew. Chem. Int. Ed. Engl.* **1972**, *11*, 159.
- [11] H. Chen, Z. Guo, H.-W. Liu, *J. Am. Chem. Soc.* **1998**, *120*, 11796.
- [12] S. O. Mansoorabadi, C. J. Thibodeaux, H.-W. Liu, *J. Org. Chem.* **2007**, *72*, 6329.
- [13] D. Chipman, Z. Barak, J. V. Schloss, *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol.* **1998**, *1385*, 401.
- [14] S. Engel, M. Vyazmensky, S. Geresh, Z. Barak, D. M. Chipman, *Biotechnol. Bioeng.* **2003**, *83*, 833.
- [15] The absolute configuration of (*S*)-acetolactate was determined by CD spectroscopy; see: A. Baykal, S. Chakraborty, A. Dodoo, F. Jordan, *Bioorg. Chem.* **2006**, *34*, 380. The absolute configuration of (*R*)-PAC was determined by chiral-phase HPLC analysis with the (*R*)-PAC formed by ScPDC. The absolute configurations of (*R*)-**1a** and (*R*)-**2a** were determined by optical rotation; see Refs. [16] and [17].
- [16] S. Engel, M. Vyazmensky, D. Berkovich, Z. Barak, D. M. Chipman, *Biotechnol. Bioeng.* **2004**, *88*, 825.
- [17] S. Bornemann, D. H. G. Crout, H. Dalton, V. Kren, M. Lobell, G. Dean, N. Thomson, M. M. Turner, *J. Chem. Soc. Perkin Trans. 1* **1996**, 425.
- [18] The absolute configuration of (*S*)-acetoin was also determined by CD spectroscopy; see: A. Baykal, S. Chakraborty, A. Dodoo, F. Jordan, *Bioorg. Chem.* **2006**, *34*, 380.
- [19] S. Bornemann, D. H. G. Crout, H. Dalton, D. W. Hutchinson, G. Dean, N. Thomson, M. M. Turner, *J. Chem. Soc. Perkin Trans. 1* **1993**, 309.
- [20] We have tested the recombinant enzymes benzaldehyde lyase (from *Pseudomonas fluorescens*; protein ID (NCBI): AAG02282.1), benzoylformate decarboxylase (from *Pseudomonas putida*; protein ID (NCBI): AAC15502.1), pyruvate decarboxylase (from *Saccharomyces cerevisiae*; protein ID (NCBI): CAA39398.1 and from *Zymomonas mobilis*; protein ID (NCBI): AAA27696.2), and PigD (from *Serratia marcescens*; C. Dresen, *PhD Thesis*, University of Freiburg, **2008**) in combinations with aromatic, aliphatic and olefinic methyl- and trifluoromethyl ketones. No traces of aldehyde–ketone cross-coupling products were found.
- [21] Two-step synthesis: 1) hydroboration of 3,4-dihydro-2*H*-pyran, according to: H. C. Brown, J. V. N. Vara Prasad, S.-H. Zee, *J. Org. Chem.* **1985**, *50*, 1582; 2) oxidation of tetrahydro-2*H*-pyran-3-ol, according to: A. J. Mancuso, D. Swern et al., *Synthesis* **1981**, 165.
- [22] Compounds **7–10** were prepared by Williamson ether synthesis using the phenols or thiophenol and chloroacetone as reactants.
- [23] S. Fraas, A. K. Steinbach, A. Tabbert, J. Harder, U. Ermler, K. Tittmann, A. Meyer, P. M. H. Kroneck, *J. Mol. Catal. B* **2009**, *61*, 47.
- [24] CCDC 752572 (**7a**), and CCDC 752573 (**13a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.