Asymmetric Intermolecular Crossed Aldehyde-Ketone Condensation through 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 example reactions catalyzed by various types of these enzymes are asymmetric C-C-bond formations of two aldehydes which lead to the formation of 2-hydroxyketones with high enantioselectivity.[2] The exchange of one of the aldehydes in this reaction with 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 for asymmetric crossed aldehyde-ketone benzoin reaction were developed.[6] Intramolecular variants of this reaction are already described in the literature.[7,8] Most recently, Enders and Henseler described the direct intermolecular cross-benzoin type reaction of aldehydes with trifluoromethyl ketones using a bicyclic triazolium salt as catalyst.[9] Due to the lower electrophilicity of the carbonyl group of ketones and the increased steric hindrance

compared to aldehydes, the asymmetric intermolecular non-enzymatic coupling reaction with ketones as acceptors seems to be more difficult and has not been successful yet. Here we present the first example of an asymmetric intermolecular crossed aldehyde-ketone carboligation reaction using a ThDP-dependent enzyme as catalyst (Scheme 1).

The class of branched-chain sugars are important bioactive carbohydrates which are widely represented in nature. It could be shown by feeding experiments that the two carbon branch of several of these sugar derivatives is derived from pyruvate. Already in 1972, Grisebach and Schmid postulated the participation of ThDP in this carboligation reaction.[10] In the biosynthesis pathway of yersiniose A, which is a two carbon branched-chain 3,6-dideoxyhexose 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 to the carbonyl function of CDP-3,6-dideoxy-4-keto-D-glucose.[11,12] The enzymatic activity of YerE was confirmed by incubation of the protein with the enzymatically – starting from CDP-D-glucose – prepared physiological substrate. The obtained product CDP-4-aceto-3,6-dideoxygalactose was determined by NMR spectroscopy.

To analyze the substrate range of the enzyme, we amplified the gene *yerE* from chromosomal DNA of *Y. pseudotuberculosis* O:VI by 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 Ni-NTA (Supporting information).

The amino acid sequence of YerE exhibits a high similarity to that of the large subunit of *E. coli* acetohydroxyacid synthases (*Ec*AHAS) (31% identity). AHAS catalyze the ThDP-dependent formation of (*S*)-acetolactate and (*S*)-acetohydroxybutyrate, the first step in the biosynthesis of branched-chain amino acids, by decarboxylation of pyruvate and condensation of the activated acetaldehyde with either a second molecule of pyruvate or with 2-oxobutyrate.[13] Chipman *et al*. discovered another carboligation activity of AHAS, the synthesis of (*R*)-phenylacetylcarbinol [(*R*)-PAC], a reaction that was first demonstrated for the pyruvate decarboxylases from *Saccharomyces cerevisiae* (*Sc*PDC) and *Zymomonas mobilis* (*Zm*PDC) (Scheme 2).[2] They also identified a variant of the AHAS II from *E. coli* with suppressed acetolactate formation in favour of the (*R*)-PAC formation.[14] Our investigation of the substrate range of YerE revealed that this protein is able to catalyze the formation of (*S*)-acetolactate as well as of (*R*)-PAC (Scheme 2).[15] Beside benzaldehyde also *ortho*-substituted benzaldehydes, which are poor substrates for *Ec*AHAS I and *Zm*PDC,[16,17] are converted efficiently and the corresponding (*R*)-2-hydroxyketones like **1a** (1-(2-chlorophenyl)-1-hydroxy-propan-2-one) and **2a** (1-hydroxy-1-(4-hydroxy-phenyl)-propan-2-one) are formed with high enantiomeric excess (Table 1). Another carboligation activity of YerE is the synthesis of (*S*)-acetoin when pyruvate and acetaldehyde are used as substrates.[18] This transformation is also catalyzed by *Zm*PDC (Scheme 2).[19]

In addition to these different 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 posses the ability to activate non-sugar ketones for cross-benzoin condensations. Enzymatic aldehyde-ketone cross-benzoin condensations have not been observed so far, although many different enzyme-substrate combinations have been tested already.[20] By testing putatively successful aldehyde-ketone combinations using YerE as the catalyst, the conversion of cyclic and acyclic ketones as acceptor substrates were demonstrated (Scheme 1).

We conducted an extended examination of the acceptor substrate range of the enzyme and detected a conversion of small cyclic aliphatic compounds like cyclohexanone (**3**) and methylated cyclohexanones. In the next step we synthesized tetrahydro-2*H*-pyran-3-one (**5**)[21] as this compound was supposed to be a substrate of YerE. The quantitative conversion of **5** confirmed our assumption. The enantiomeric excess of the product (84%) was determined by chiral phase GC with the chemically synthesized racemic reference. This represents the first example of an asymmetric crossed aldehyde-ketone carboligation reaction catalyzed by the ThDP-dependent enzyme YerE.

After establishing the cyclic ether **5** as a suitable acceptor substrate, through substrate variation different open-chain acceptor ketones with an ether or thioether moiety were identified (Table 1).[22] The enantiomeric excess of the products spans from excellent (entries **6** and **8**) to moderate (entry **7** and **9**) while the tertiary alcohol formed from the ketone with the thioether moiety (**10**) is almost racemic. According to this the formal exchange of the ether oxygen in the acceptor substrate **6** against sulfur (**10**) leads to a strong decrease in the stereoselectivity of the enzyme. The crystal structure of the protein with bound substrates (in progress) might help to explain this observation on molecular level.

Another class of acceptor substrates are cyclic and open-chain 1,2-diketones (Table 1). In the case of these substrates the activated acetaldehyde is transferred to only one of the carbonyl functions. An interesting compound in this context is cyclohexane-1,2-dione (**11**) because it is also a substrate for the ThDP-dependent flavoenzyme cyclohexane-1,2-dione hydrolase (CDH). The reaction catalyzed by the latter protein, the hydrolytic C-C-bond ring cleavage of the cyclic diketone,[23] is completely different from the carboligation reaction discussed here and demonstrates once more the diversity of ThDP-dependent enzyme-catalyzed transformations.

Furthermore, our investigation of the substrate range revealed that ketones with an ester moiety can also be converted using the biocatalyst YerE. These compounds can be structured like the acceptor ketone **13**, synthesized from 4-bromobenzoyl chloride and hydroxyacetone, or belong to the group of α- or β-ketoesters (**14, 15**) (Table 1). Using ethyl 4,4,4-trifluoro-3-oxobutyrate, an analogue of **14**, as an acceptor substrate 52% conversion (determined by NMR) was achieved, showing that trifluoromethyl ketones can be used advantageously as substrates, too. In contrast to this arylketones as well as α,β-unsaturated and α-branched ketones seemed to be no substrates for the enzyme YerE.

Since control experiments using the lysate of the cells, transformed with the pQE-60 vector without insert after applying the same expression conditions, do not show any activity with respect to the investigated reaction, the crude extract received after cell lysis was used for

the preparative biotransformations (Supporting information). All transformations shown in Table 1 were performed on a mmol scale and the tertiary alcohols (**4a-10a, 12a-14a**) were isolated in yields of up to 40%. After extraction of the reaction solution with an organic solvent, the analysis of the organic layer (GC-MS) showed beside residual acceptor substrate only the desired tertiary alcohol as reaction product. The crossed aldehyde-ketone coupling products shown here were obtained using pyruvate as acetaldehyde synthon because the 2-ketoacid was found to be the best donor substrate for the enzyme YerE. Nevertheless, it is also possible to apply acetaldehyde directly in this reaction. By using equimolar concentrations of $[2-^{13}C]$-pyruvate and non-labeled acetaldehyde in the presence of $[1-^{13}C]$-cyclohexanone, we indeed could demonstrate the incorporation of acetaldehyde into the crossed ligation product. The NMR-analysis revealed a 4:1 ratio of the product received from pyruvate to that one formed by conversion of acetaldehyde (Supporting information).

For 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 of the measured crystal were (*R*)-configured (Figure 1). To assure that this configuration can be assigned to the major enantiomer, the crystals used for X-ray structure analyses were subsequently analyzed by chiral phase HPLC.

In summary, we have introduced asymmetric intermolecular crossed aldehyde-ketone coupling reactions using the ThDP-dependent enzyme YerE as catalyst. The substrate range 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 in preparative scale and for two of them the absolute configuration was determined by single crystal structure analysis.

The presented enzymatic transformations offer a simple entry for the preparation of so far only difficult accessible compounds. These enantioenriched tertiary alcohols with an α-acetyl moiety could be used as valuable building blocks for asymmetric synthesis, resulting in the formation of 1,2-diols (reduction), vicinal amino alcohols (reductive amination) or two contiguous tertiary alcohols (nucleophilic addition).

In addition, we propose that the identification of YerE homologous enzymes (and variants) will be a good starting point for the identification of similar activities. Elucidation of the threedimensional structure of YerE (ongoing) 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 so far. Based on the structure the design of YerE variants is intended to improve the stereoselectivity of the protein. Another aspect is the suppression of the acetolactate formation to channel most of the pyruvate respectively acetaldehyde to the synthesis of the desired crossed carboligation product.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

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3. The enzymatic formation of acetolactate from the 2-ketoacid pyruvate has already been demonstrated (cf. [13]) but no ketones were used as substrates.

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15. Absolute configuration of (*S*)-acetolactate was determined through CD-spectroscopy according to Jordan *et al*., Bioorg Chem. 2006; 34:380. [PubMed: 17083961] . Absolute configuration of (*R*)-PAC was determined through chiral HPLC-analysis with the (*R*)-PAC formed by *Sc*PDC. Absolute configuration of (*R*)-**1a** and (*R*)-**2a** was determined through optical rotation according to Crout *et al*., J Chem Soc Perkin Trans I. 1996; 5:425. and Chipman *et al*., (ref. [16])

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18. Absolute configuration of (*S*)-acetoin was also determined through CD-specroscopy according to Jordan *et al*., Bioorg Chem. 2006; 34:380. [PubMed: 17083961]

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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*; Dresen C. PhD thesis. University Freiburg2008) in combinations with aromatic, aliphatic and olefinic methyl- and trifluoromethyl ketones. No hint for the formation of aldehyde-ketone cross-benzoin condensation products was found.

21. Two step synthesis. First step: hydroboration of 3,4-dihydro-2*H*-pyran according to Brown *et al*., J Org Chem. 1985; 50:1582.; second step: oxidation of tetrahydro-2*H*-pyran-3-ol according to Swern *et al*., Synthesis. 1981; 81:165.

22. Compounds **7-10** were prepared by Williamson ether synthesis using the corresponding phenols respectively thiophenol and chloroacetone as reactants. Fraas S, Steinbach AK, Tabbert A, Harder J, Ermler U, Tittmann K, Meyer A, Kroneck PMH. J Mol Cat B: Enzym. 2009; 61:47.
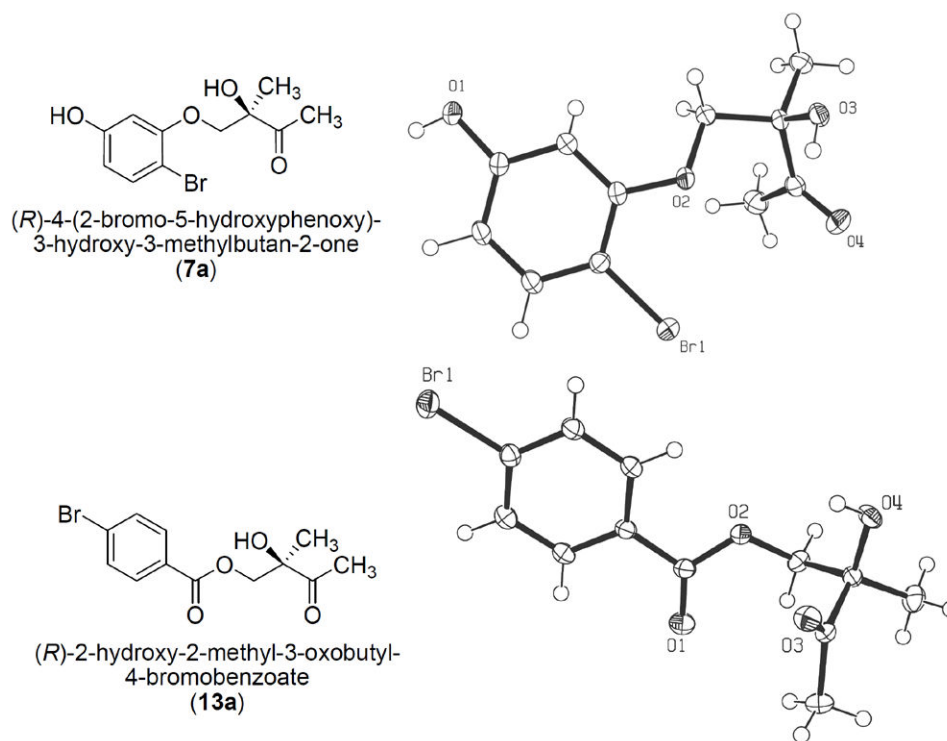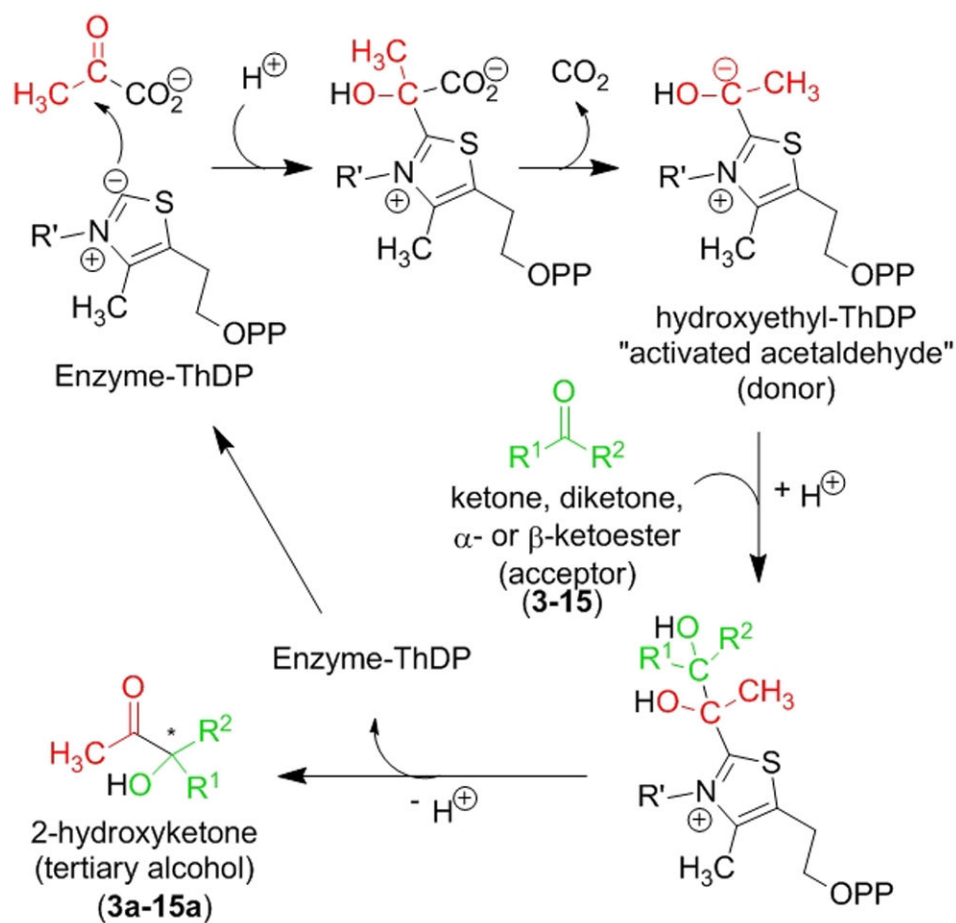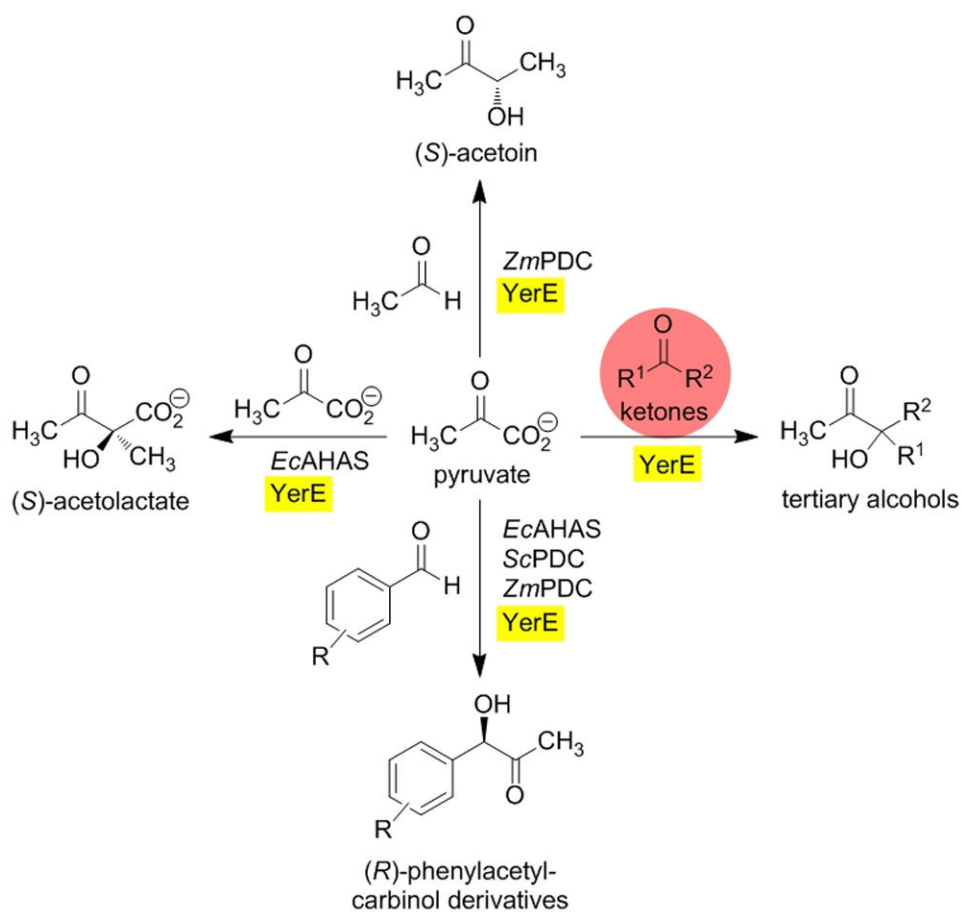
Figure 1.
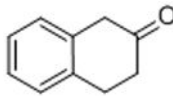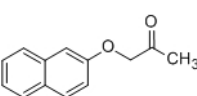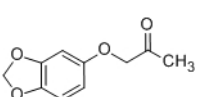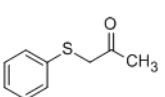X-ray crystal structure of **7a** and **13a**. Ellipsoids include 50% of the electron density.
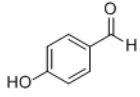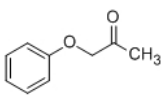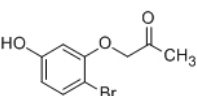
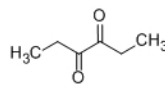**Scheme 1.**
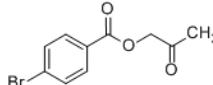YerE-catalyzed formation of tertiary alcohols.
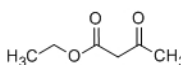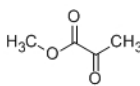
Scheme 2.
Carboligation reactions catalyzed by the ThDP-dependent enzyme YerE.

Table 1

Substrate range of YerE.[a]

Substrate (acceptor)		Conversion [%][b] after 20-25 h	Isolated Yield [%]	ee[d] [%]
	1	88	60	94 (R)
	2	79	69	96 (R)
	3	55	--	--[e]
	4	47	39	9
	5	97	34	84[g]
	6	48	24	91
	7	37	26	78 (R)
	8	20	14	96
	9	27	9	87
	10	31	25	<5
	11	32[c]	--[f]	22

Substrate (acceptor)		Conversion [%][b] after 20-25 h	Isolated Yield [%]	ee[d] [%]
	12	58[c]	34	84
	13	26	18	63 (R)
	14	42	23	30[h]
	15	> 99	--[i]	30[h]

[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 ^{1}H-NMR.

[c] Conversion determined by GC-MS.

[d] Enantiomeric excess (ee) determined by chiral phase HPLC or by chiral phase GC.

[e] Achiral product.

[f] No separation of educt and product by column chromatography on silica gel.

[g] Low isolated yield probably due to azeotrope formation.

[h] ee determined by ^{1}H-NMR-spectroscopy with a chiral lanthanide shift reagent.

[i] No quantification achieved due to the volatility of the compound.