Enantioselective Synthesis of (*S*)-2-Hydroxypropanone Derivatives by Benzoylformate Decarboxylase Catalyzed C−C Bond Formation

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Chiral 2-hydroxypropanone derivatives **5a–v**, **8a–d**, and **10a**, **b** were formed by benzoylformate decarboxylase (BFD) catalyzed C–C bond formation. A donor aldehyde and acetaldehyde as an acceptor were carboligated in aqueous buffer solution with remarkable ease in high chemical yield and good to high optical purity. The substrate range of this thiamin diphosphate dependent enzyme was examined to employ this benzoin condensation type reaction in stereoselective synthesis. The observed dependence of the enantiomeric excess on the substitution pattern could be exploited to design substrates resulting in high selectivity. Best substrates with regard to optical purity were *meta*-substituted benzaldehyde derivatives. To enable a general and convenient applicability of the BFD-catalyzed C–C bond formation, analytical batch experiments were scaled up to give (*S*)-2-hydroxy ketones in good to high yields on a preparative scale. Further, the solubility of some of the organic substrates in aqueous solution was increased by the use of cyclodextrin or buffer/DMSO mixtures.

Introduction

The advantage of enzymes in bioorganic chemistry is based on their stereo- and regioselective manner of catalysis.[1] Recently, the class of thiamin diphosphate (ThDP) dependent enzymes was demonstrated to be of increasing interest.[2] These enzymes, e.g. α-keto acid decarboxylases[3] and transketolases,[4] catalyze both the cleavage and the formation of C−C bonds.

We focussed our interest on the evaluation of the synthetic potential of benzoylformate decarboxylase (BFD, EC 4.1.1.7) from *Pseudomonas putida*, with regard to the ability to catalyze carboligation. BFD is accessible as wild-type (BFD-wt) or as recombinant enzyme (BFD-His), the latter elongated with a hexahistidine tail at the C-terminus. BFD-His is especially easily available by expression in *Escherichia coli* and a one-step purification protocol using simple chromatography on an Ni-NTA-agarose resin.[5]

The common feature of α-keto acid decarboxylases is their ability to form a ThDP-bonded carbanionic intermediate **II** upon decarboxylation of **I** (Scheme 1). As a side reaction, which constitutes a hitherto unknown physiological function of BFD, the resulting "active aldehyde" species **II** (donor aldehyde) undergoes a benzoin condensation type reaction with a second (acceptor) aldehyde leading to symmetrical or unsymmetrical chiral 2-hydroxy ketones. The formation of **II** does not require previous decarboxylation (path **A**, Scheme 1), thus **II** can also be obtained by reversible addition of the donor aldehyde itself to thiamin diphosphate (path **B**, Scheme 1).[5] The cofactor ThDP is regenerated at the end of the reaction cycle. Since ThDP is not covalently bound to the enzyme, it has to be added in low concentration (0.5 mmol·L^{-1}) in order to ensure maximum activity of the holo enzyme.

Benzoylformate decarboxylase from *P. putida* is involved in the mandelate biosynthetic pathway,[6] and has recently been introduced as a catalyst for the enantioselective formation of (*S*)-2-hydroxy-1-phenylpropan-1-one [**5a**, (*S*)-2-HPP] starting from benzoylformate (**1**) and acetaldehyde (**4**),[7] or from benzaldehyde (**3a**) and acetaldehyde (**4**),[3,5] respectively, as well as for the formation of (*R*)-benzoin (**6**).[3,8] This biotransformation represents a versatile stereoselective alternative to the classical benzoin condensation[9] yielding unsymmetrical acyloins and symmetrical benzoins. Several chemical methods were developed using achiral[10] and chiral[11] thiazolium and triazolium salts to catalyze this C−C bond-forming reaction, leading to symmetrical benzoins. Apart from this reaction type, several methods have been developed for the preparation of such symmetrical and even unsymmetrical optically active compounds.[12] In general, 2-hydroxy ketones are important structural subunits in many biologically active natural products, and they are also important building blocks for stereoselective synthesis.[13]

In a previous paper we described an easy access to pure BFD and developed a continuous process using an enzyme membrane reactor for the C−C bond-forming reaction.[5] As a main result, we observed a hitherto unknown depend-

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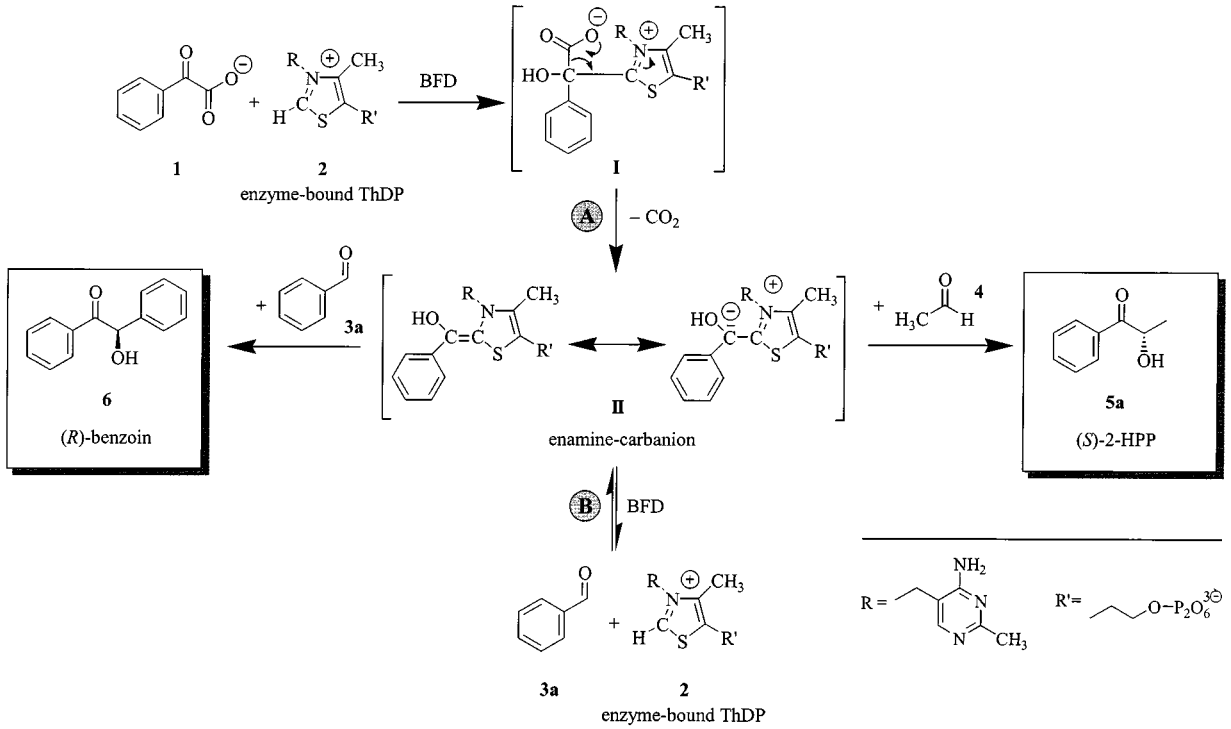


Scheme 1. Reaction mechanism of BFD-mediated formation of (*S*)-2-HPP (**5a**) and (*R*)-benzoin (**6**); the "active aldehyde" (enamine carbanion **II**) is a common intermediate of the decarboxylation of benzoylformate (**1**) and the carboligation with an acceptor aldehyde (here acetaldehyde or benzaldehyde)

ence of the enantioselectivity of the BFD-catalyzed reaction on the benzaldehyde concentration, which was found in the formation of (*S*)-2-HPP. Herein we report the enzymatically catalyzed synthesis of unsymmetrical 2-hydroxy ketones with regard to a broad applicability in stereoselective synthesis on a preparative scale. Another aim of this work was to give a detailed survey of the substrate and product range of the BFD-catalyzed C−C bond formation, considering the effects of substituents of the donor and acceptor aldehydes on enzyme activity and enantioselectivity. Further, we demonstrate that the substrate range of BFD can be extended to hydrophobic aldehydes with very low solubility in aqueous buffer solution by the use of solubilizers.

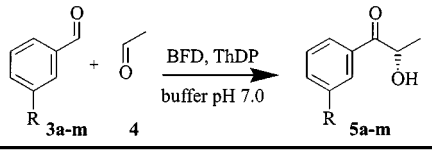
Results and Discussion

As demonstrated in Table 1, the enantioselectivity of BFD-catalyzed carboligation of different benzaldehyde derivatives and acetaldehyde was significantly influenced by the steric demand of substituents on the aromatic ring. This effect was most pronounced in *meta*-substituted benzaldehydes. Best results with regard to enantiopurity were obtained using 3-alkoxy- and 3-phenyloxy-substituted substrates (**3f**−**k**). BFD converts a vast variety of substituted benzaldehyde derivatives in the presence of acetaldehyde to the corresponding 2-hydroxypropanones. However, the low solubility in aqueous buffer solution of many of these compounds was the principal problem. Particularly, the enzyme activity for the respective substrates decreased (Table 1) since the substrate concentration in aqueous solution was too low to allow substrate saturation of the active site.

BFD displayed a similar broad substrate and product range in the case of *para* substitution. Surprisingly, both, the enzyme activity and *ee* decreased relative to *meta*-substituted substrates (Table 2). Besides steric effects electronic factors influence these parameters as well. Substituents with strong electron-withdrawing effects (e.g. CN) resulted in a conspicuous decrease of enatioselectivity (*ee* of **5t**: 74%). This −*M* effect has a negative effect on the role of the substrate to act as an electron donor, especially in the *para* position to the aldehyde moiety. *meta*-Cyanobenzaldehyde (**3m**) did not show such a distinct effect (*ee* of **5m**: 95%). *ortho*-Substituted benzaldehyde derivatives, except 2-fluorobenzaldehyde (**3u**) which gave the corresponding chiral 2-hydroxy ketone **5u** in adequate yield (Table 3), were not accepted by BFD due to steric hindrance.

Products obtained using heteroaromatic aldehydes are shown in Table 4. Differently substituted aldehydes **7a**−**c** of the furan series, thiophene- (**7d**) and pyrrol-2-carbaldehyde were tested as substrates for BFD-catalyzed C−C bond formation with acetaldehyde (**4**). Starting from unsubstituted furan-2-carbaldehyde (**7a**) we obtained the desired 2-hydroxy ketone **8a** in 45% *ee*. The enzyme activity was found to be 56% based on the standard benzaldehyde and acetaldehyde system. To prove the favorable influence of sterically demanding substituents with respect to stereoselectivity, as was observed using *meta*-substituted benzaldehydes (cf. Table 1), we introduced an isopropyl substitu-

Table 1. BFD-mediated carboligation of *meta*-substituted benzaldehyde derivatives **3a−m** and acetaldehyde to the corresponding (*S*)-2-hydroxypropanone derivatives **5a−m**



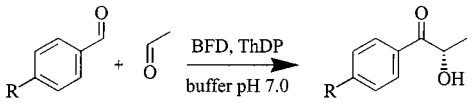
3a-m **4** **5a-m**

aldehyde	R	2-hydroxy ketone	conversion / %[a, b]	ee / %[a, c]
3a	H	**5a**	99	92
3b	F	**5b**	100	87
3c	Cl	**5c**	94	94
3d	Br	**5d**	68	96
3e	Me	**5e**	99	97
3f	OMe	**5f**	94	96
3g	OEt	**5g**	91	97
3h	O*i*Pr	**5h**	62	>99
3i	OPh	**5i**	12	>99
3j	OBzl	**5j**	[d]	>99
3k	OAc	**5k**	80	>99
3l	OH	**5l**	62	92
3m	CN	**5m**	95	92

R = NO_2: no product formation observed

[a] Determined by HPLC. − [b] Unconverted starting material was recovered. − [c] Absolute (*S*)-configuration was determined by comparison of HPLC data and with regard to mechanistic aspects of BFD catalysis.[5] − [d] Complete conversion with concentration of **3j** << 1 mmol·L^{-1}.

Table 2. BFD-mediated carboligation of *para*-substituted benzaldehyde derivatives **3n−t** and acetaldehyde to the corresponding (*S*)-2-hydroxypropanone derivatives **5n−t**



3n-t **4** **5n-t**

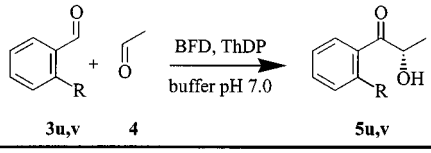
aldehyde	R	2-hydroxy ketone	conversion / %[a, b]	ee / %[a, c]
3n	F	**5n**	69	87
3o	Cl	**5o**	85	82
3p	Br	**5p**	42	83
3q	Me	**5q**	65	88
3r	OMe	**5r**	23	92
3s	OH	**5s**	11	86
3t	CN	**5t**	89	74

R = NO_2, NMe_2, NHAc: no product formation observed

[a] Determined by HPLC. − [b] Unconverted starting material was recovered. − [c] Absolute (*S*) configuration was determined by comparison of HPLC data and with regard to mechanistic aspects of BFD catalysis.[5]

ent on the furan ring. As expected, a significant increase in *ee* (73%) was observed by exchanging the less demanding substrate for the more demanding 5-isopropyl-substituted furan derivative **7c**. Surprisingly, a further increase in enantioselectivity to 86% *ee* was obtained using 5-methylfuran-2-carbaldehyde (**7b**). The slight depression of enantioselectivity in the case of substrate **7c** relative to the sterically

Table 3. BFD-mediated carboligation of *ortho*-substituted benzaldehyde derivatives **3u,v** and acetaldehyde to the corresponding (*S*)-2-hydroxypropanone derivatives **5u,v**



3u,v **4** **5u,v**

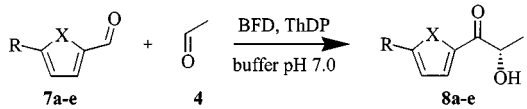
aldehyde	R	2-hydroxy ketone	conversion / %	ee / %
3u	F	**5u**	91[a, b]	87[d]
3v	Me	**5v**	4[b, c]	n.d.

R = Cl, Br, OMe, OH, NO_2: no product formation observed

[a] Determined by HPLC. − [b] Unconverted starting material was recovered. − [c] Determined by GCMS. − [d] Absolute (*S*) configuration was determined by comparison of HPLC data and with regard to mechanistic aspects of BFD catalysis.[5]

less demanding **7b** is in contrast to the trend of enantioselectivity we found with *meta*-substituted benzaldehydes. Obviously, electronic effects become more important with the furan carbaldehydes. The enzyme activity decreased while the enantioselectivity increased with an enlarged steric demand of the substrates assigned to the BFD-catalyzed carboligation (vide supra). Analogously, thiophene-2-carbaldehyde (**7d**), which is a sterically more demanding substrate than **7a**, gave the corresponding 2-hydroxy ketone **8d** in 83% *ee* (50% conversion). Pyrrole-2-carbaldehyde was not a donor substrate for BFD-catalyzed reaction in the presence of acetaldehyde.

Table 4. BFD-mediated carboligation of heteroaromatic carbaldehydes **7a−d** and acetaldehyde to the corresponding (*S*)-2-hydroxypropanone derivatives **8a−d**



7a-e **4** **8a-e**

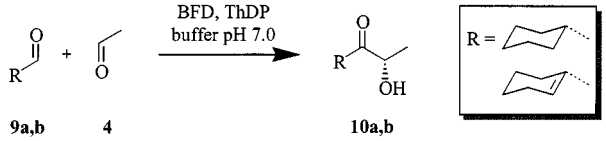
aldehyde	X	R	2-hydroxy ketone	conversion / %[a, b]	ee / %[a, c]
7a	O	H	**8a**	56	45
7b	O	Me	**8b**	32	86
7c	O	*i*Pr	**8c**	49	73
7d	S	H	**8d**	50	83

X = NH, R = H: no product formation observed

[a] Determined by HPLC. − [b] Unconverted starting material was recovered. − [c] Absolute (*S*) configuration was determined by comparison of HPLC data and with regard to mechanistic aspects of BFD catalysis.[5]

Further, the condensation of an aliphatic substrate, cyclohexanecarbaldehyde (**9a**), with acetaldehyde was proven (Table 5). However, the enzyme activity and optical purity of product **10a** was only moderate, which was probably due to the absence of favorable interactions with aromatic amino acid residues in the active site of BFD.[5] Cyclohex-1-enecarbaldehyde (**9b**) resembles the aromatic substrates

Table 5. BFD-mediated carboligation of nonaromatic carbaldehydes **9a**,**b** and acetaldehyde to the corresponding (*S*)-2-hydroxypropanone derivatives **10a**,**b**



9a,b **4** **10a,b**

aldehyde		2-hydroxy ketone	conversion / %	ee / %
9a	cyclohexane carbaldehyde	**10a**	21[a,c]	61[a]
9b	cyclohex-1-ene carbaldehyde	**10b**	50[b,c]	94[b]

[a] Determined by GC. – [b] Determined by HPLC. – [c] Unconverted starting material was recovered. – [d] Absolute (*S*) configuration was determined by comparison of HPLC data and with regard to mechanistic aspects of BFD catalysis.[5]

more closely than the aliphatic ones with regard to enzyme activity and *ee* of product **10b**.

In contrast to the large variety of aldehydes which can be used as donor substrates, BFD does not tolerate a modification of the methyl group of acetaldehyde (acceptor) in the mixed benzoin type condensation.[5] However, benzaldehyde, benzaldehyde derivatives, and heteroaromatic aldehydes are also suitable acceptor aldehydes.[8]

The ratio of donor/acceptor substrate applied to the enzyme reaction remarkably influences the product selectivity. A ratio of at least 1:50, whereby the donor aldehyde benzaldehyde is applied at an absolute concentration of 10 mmol·L^{-1} and acetaldehyde at 0.5 mol·L^{-1}, results in the formation of (*S*)-2-HPP as sole product. The competitive formation of the corresponding benzoin derivative (cf. Scheme 1) becomes more relevant using a minor proportion of acetaldehyde or, more significantly, by omitting acetaldehyde from the reaction mixture (Figure 1). Whereas the use of aromatic acceptor aldehydes resulted in the corresponding (*R*)-benzoin derivatives[8] all 2-HPP derivatives examined so far show an absolute (*S*) configuration (vide infra).

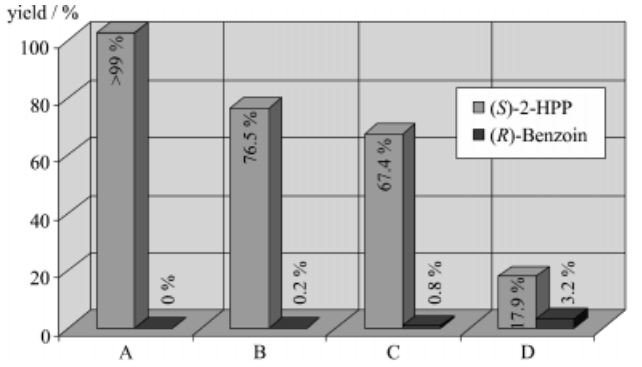


Figure 1. Comparison of the competitive formation of products (*S*)-2-HPP and (*R*)-benzoin formed by BFD-catalyzed C–C bond formation, showing a dependence on the substrate concentration; (**A**) benzaldehyde 1 mmol·L^{-1}, acetaldehyde 500 mmol·L^{-1}; (**B**) benzaldehyde 10 mmol·L^{-1}, acetaldehyde 500 mmol·L^{-1}; (**C**) benzaldehyde 25 mmol·L^{-1}, acetaldehyde 500 mmol·L^{-1}; (**D**) benzaldehyde 25 mmol·L^{-1}, acetaldehyde 20 mmol·L^{-1}

The competing formation of (*S*)-2-HPP and (*R*)-benzoin can easily be shifted towards (*S*)-2-HPP using a continuous stirred tank reactor (CSTR, enzyme membrane reactor) which we reported in a previous paper.[5] Additionally, this kind of reactor takes the concentration-dependent enantioselectivity into account, which we found for the formation of (*S*)-2-HPP.[5] During the BFD-catalyzed reaction of benzaldehyde and acetaldehyde in a batch reactor, the benzaldehyde concentration in the reaction mixture decreased significantly over time, especially when using large amounts of the donor aldehyde. A CSTR works under product efflux conditions, i.e. despite a high substrate concentration at the inlet, a low concentration in the reactor itself is maintained.[14] However, this kind of reactor is unsuitable for common chemical practice. We therefore developed a simple and efficient procedure for the preparative scale synthesis, to enable a broad applicability of BFD. Applying the donor aldehyde in a concentration of 10 mmol·L^{-1} in a simple batch reactor, the competitive formation of benzoin and, additionally, the concentration-dependent enantioselectivity which was observed for the

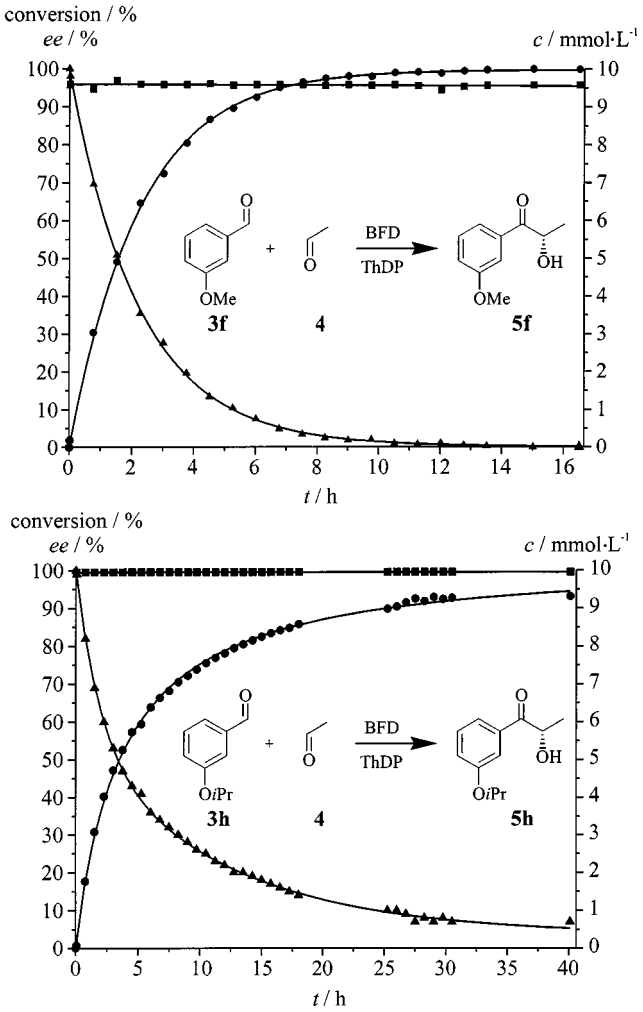


Figure 2. Progress of BFD-mediated carboligation of **3f** and acetaldehyde yielding **5f** (top) and of **3h** and acetaldehyde yielding **5h** (bottom); concentration of **3f**, **h** (–▲–), of **5f**, **h** (–●–), and *ee* of **5f**, **h** (–■–) are shown

formation of (*S*)-2-HPP,[5,15] can be neglected if benzaldehyde derivatives are used as acyl donors. 3-Methoxybenzaldehyde (**3f**; 10 mmol·L^{-1}) as well as 3-isopropoxybenzaldehyde (**3h**) can be transformed to the desired products **5f** and **5h** in high yields, as pointed out in Figure 2. The high optical purity (**5f**: 96% *ee*; **5h**: >99% *ee*) was kept constant during the reaction course.

We developed a convenient batch process for water-soluble substrates (solubility ≥ 5 mmol·L^{-1}) by scaling up the enzyme-catalyzed C−C bond formation leading to the corresponding chiral 2-hydroxy ketones in preparative yield and high *ee*. Compounds **5f** (**5h**) were prepared in 97% (91%) yield and 96% *ee* (> 99% *ee*) on a 500-mg (200-mg) scale. The batch process could be performed several times, in a repetitive way. Using this technique, the product solution was filtered off at adequate conversion (> 90%) retaining the enzyme by an ultrafiltration membrane (cut-off 10000 Da). Subsequently, a further reaction was started by the addition of fresh substrate solution to recovered BFD. The optical purity of **5f** and **5h**, as well as that of all other enzymatically prepared 2-hydroxy ketones described herein was determined by HPLC using a chiral-phase column packed with Chiralpak AD or Chiralcel OB (Daicel) (Figure 3).[16] As a standard for the HPLC resolution, racemic 2-hydroxy ketones ***rac*-5f** and ***rac*-14** were synthesized by "Umpolung" of the aldehyde with trimethylsilyl cyanide[17] and subsequent condensation with acetaldehyde.[18] The absolute configuration of enzymatically prepared (*S*)-2-HPP (**5a**) was assigned by comparison of the optical rotation with literature values.[5,7a,19] The (*S*) configuration of the new acyloins formed was determined by comparison of the CD spectra to (**5a**).

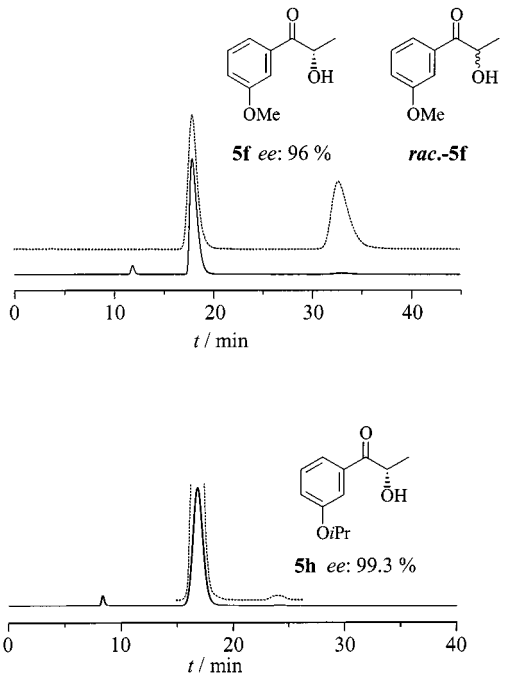


Figure 3. HPLC resolution of **5f** (top, solid line), ***rac*-5f** (top, dashed line), and **5h** (bottom, solid line; magnification: dashed line) on Chiralcel OB (isohexane/2-propanol = 9:1, flow 0.75 mL·min^{-1}, 25 °C)

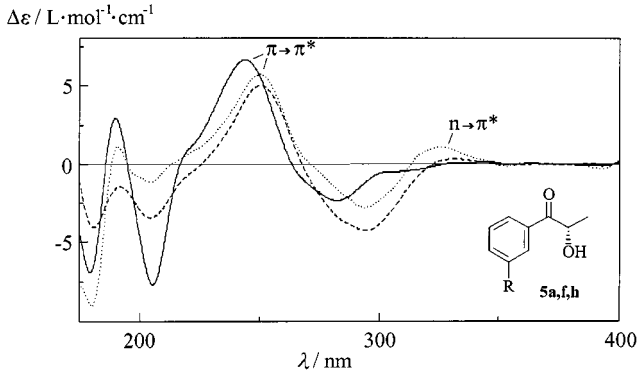


Figure 4. CD spectra of (*S*)-2-hydroxy-1-phenylpropan-1-one (**5a**, *ee* = 92%, solid line), (*S*)-2-hydroxy-1-(3-methoxyphenyl)propan-1-one (**5f**, *ee* = 96%, dotted line), and (*S*)-2-hydroxy-1-(3-isopropoxyphenyl)propan-1-one (**5h**, *ee* > 99%, dashed line)

Although several studies have been published dealing with the relationship between structure and chiroptical properties of tetralones and tetralone-like structures,[20] no general assignment of the absolute configuration of acyclic alkyl aryl ketones is accessible so far, which is due to the high flexibility of the side chain. Nevertheless, assuming that the conformation of **5f**, **5h**, and **14** in solution is not influenced by *meta*-alkoxy substituents, the absolute (*S*) configuration can be deduced from the similar CD spectrum of the known (*S*)-2-HPP (**5a**). As depicted in Figure 4−6 all of them show a positive Cotton effect for the n→π* transition as well as for the π→π* transition (Table 6). The π→π* transitions in the CD spectra correlate with the wavelength found for the UV absorption maxima for all 2-hydroxy ketones.[21] According to this assignment, as well as by comparison with HPLC data and mechanistic reflections, all enzymatically formed 2-hydroxy ketones described in this study are considered to have (*S*) configuration.

Table 6. Comparison of the π→π* and n→π* transitions of the CD spectra for **5a**,**f**,**h**, **12**, and **14**; because of the agreement of the CD curves, an absolute configuration of (*S*) is concluded for all enzymatically synthesized 2-hydroxypropanones

2-Hydroxy ketone	π → π*/nm (Cotton effect)	n → π*/nm (Cotton effect)	Absolute configuration
5a	247 (+)	n.d.	(*S*)
5f	252 (+)	325 (+)	(*S*)
5h	252 (+)	332 (+)	(*S*)
12	249 (+)	346 (+)	(*S*)
14	265 (+)	334 (+)	(*S*)

It is noteworthy that the formation of benzoins with (*R*) configuration from aromatic aldehydes as sole substrates[8] is explained by the same model,[5] which is based on the X-ray structure[22] of BFD.

Due to its low solubility in aqueous buffer (ca. 5 mmol·L^{-1}) 3-isopropoxybenzaldehyde (**3h**) points out the limitation of the general batch process for those substrates. Further decrease of maximum substrate concentration in the case of even more hydrophobic aromatic aldehydes is

impractical owing to the high substrate dilution for synthesis on a preparative scale. However, analytical experiments indicated conversion of hydrophobic substrates by BFD. Carboligation of 2-naphthaldehyde (**11**, $<<$ 1 mmol·L^{-1}) and acetaldehyde yielded (*S*)-2-hydroxy-1-(2-naphthyl)propan-1-one (**12**) with an *ee* of 89% on an analytical scale. We therefore envisaged a launch of a preparative batch synthesis using poorly soluble hydrophobic aldehydes for the biotransformation in aqueous buffer solution.

In contrast to lipases (for instance), which act at aqueous/lipid interfaces in their natural environment and can be advantageously employed with biphasic media in the presence of water-immiscible solvents,[23] BFD is inactivated by such heterogeneous systems. Adoption of the presented batch process is possible even with hydrophobic substrates, if a solubility promotor like heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD)[24] or a cosolvent are applied to improve their solubility in homogeneous aqueous media. Cyclodextrins, consisting of six (α), seven (β), or eight (γ) D-glucose units ligated by α-1,4-glycosidic linkages, form an apolar cavity which is able to enclose hydrophobic guest molecules.[25] This strategy was successfully applied to the reduction of hydrophobic ketones by alcohol dehydrogenases, for example.[26] We also obtained good results performing the BFD-catalyzed C−C bond formation in the presence of DM-β-CD (10 mmol·L^{-1}). Comparative experiments carried out with benzaldehyde and acetaldehyde in the presence of, and without DM-β-CD, respectively, indicated a decrease in enzyme activity by about 50% on addition of this solubilizer. This can be explained by the low free substrate concentration in the buffer solution which is caused by the complex-forming torus-shaped host molecule. Generally, the carboligation of 2-naphthaldehyde (**11**) with acetaldehyde to give **12** can be performed using **11** in a concentration of 10 mmol·L^{-1} and a twofold excess of DM-β-CD in aqueous buffer solution (Figure 5). No activity was observed with 1-naphthaldehyde (**15**), either in the absence or in the presence of solubility promoters (see below).

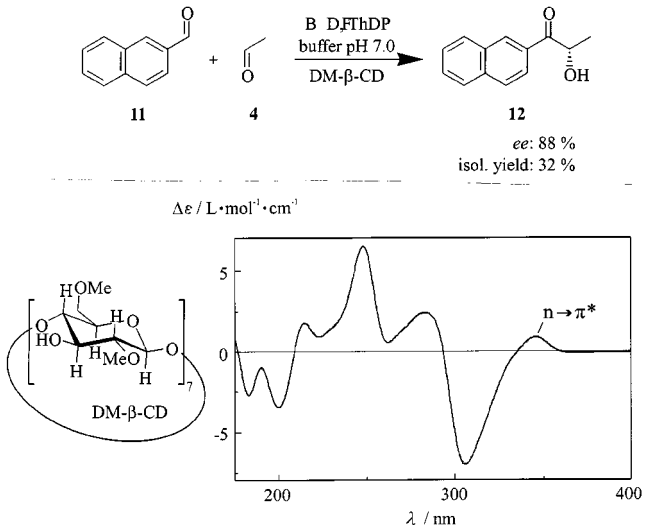


Figure 5. Enzymatic synthesis of (*S*)-2-hydroxy-1-(2-naphthyl)propan-1-one (**12**) in the presence of DM-β-CD as solubilizer, and CD spectrum of **12**; unconverted starting material **11** was recovered

Alternatively, biotransformation of hydrophobic aldehydes is also possible in the presence of water-miscible organic solvents. The best results with regard to increased solubility of hydrophobic substrates together with the slightest loss of ligase activity of BFD were obtained by addition of DMSO. Experiments with the standard system (10 mmol·L^{-1} benzaldehyde, 0.5 mol L^{-1} acetaldehyde) indicated the general applicability of this cosolvent. However, BFD-wt and BFD-His differ in a significant manner: Whereas the wild-type enzyme tolerates a ratio of 30% DMSO (v/v) in aqueous buffer, BFD-His responds in a more sensitive way to DMSO.[27] In the latter case, a ratio of about 10% DMSO (v/v) is applicable. Under these conditions, the enzyme activity decreased slightly to 70% using the standard system. The differences in stability are probably a consequence of different purities of the enzyme preparations. The conversion of 3,5-dimethoxybenzaldehyde (**13**) (10 mmol·L^{-1}) catalyzed by BFD-His in 10% DMSO/buffer solution (v/v) resulted in 2-hydroxy ketone **14** (Figure 6) which was isolated in 40% yield (97% *ee*) on a 100-mg scale.

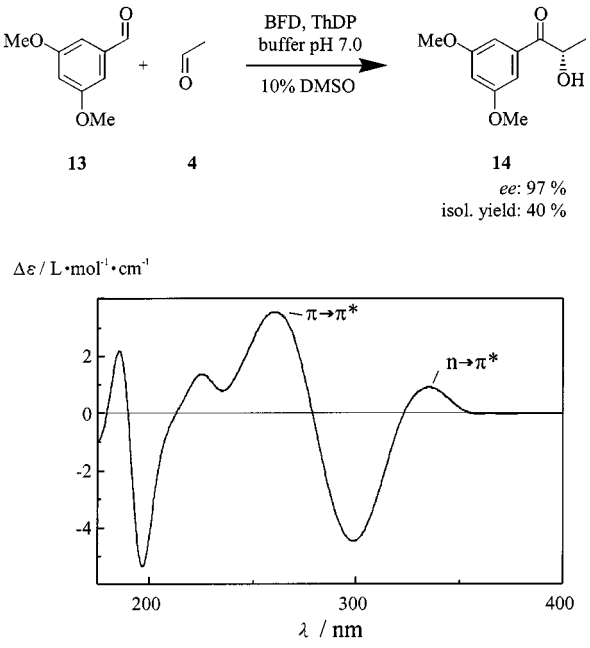
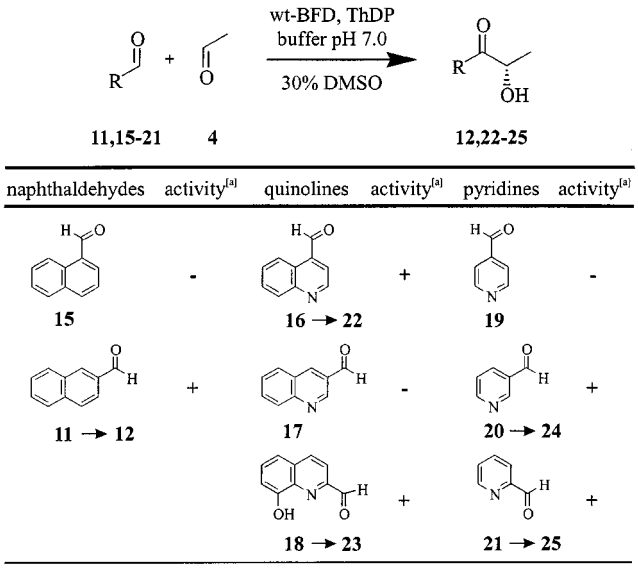


Figure 6. Enzymatic synthesis of (*S*)-2-hydroxy-1-(3,5-dimethoxyphenyl)propan-1-one (**14**), and CD spectrum of **14**; unconverted starting material **13** was recovered

With regard to the simplicity, the use of DMSO as a cosolvent represents an efficient and reproducible method for BFD-catalyzed biotransformations leading to a broad applicability for the synthesis of new chiral 2-hydroxypropanone derivatives, even from highly lipophilic substrates. Fortunately, added DMSO did not interfere with the workup which could be carried out by standard procedures. These results encouraged us to expand the substrate range of BFD to less soluble substrates. Even some quinoline- and pyridinecarbaldehydes were subjected to BFD-catalyzed (BFD-wt) carboligation in 30% DMSO/buffer solution

(v/v) (Table 7). Pyridine-2- (**21**) and pyridine-3-carbaldehyde (**20**) were converted into the corresponding 2-hydroxy ketones **24** and **25**, whereas in the case of pyridine-4-carbaldehyde (**19**) no product formation was observed. Using quinolinecarbaldehydes **16**−**18** as substrates the relationship between substitution pattern and product formation was inverted relative to the pyridinecarbaldehydes. Surprisingly, 2- and 4-substituted quinolines lead to the desired 2-hydroxypropanones **22** and **23**, whereas quinoline-3-carbaldehyde (**17**) was not a substrate for BFD [cf. pyridine-3-carbaldehyde (**20**) gave the desired product, whereas pyridine-4-carbaldehyde (**19**) did not act as substrate]. 2-Naphthaldehyde (**11**) gave the desired product in good yield, whereas the 1-substituted regioisomer **15** did not behave as a donor substrate in the presence of acetaldehyde. This agreed with the results obtained with sterically hindered *ortho*-substituted benzaldehyde derivatives (vide supra).

Table 7. Comparison of regioisomers of naphthaldehyde, quinolinecarbaldehydes and pyridinecarbaldehydes with regard to the BFD-mediated carboligation with acetaldehyde



[a] Product formation was proved by GCMS, unconverted starting material was recovered.

Further studies on the optical purity and the absolute configuration of the resulting 2-hydroxy ketones depicted in Table 7 and a preparative application of the quinoline derivatives are under investigation.

Conclusion

BFD has been established as a biocatalyst for enantioselective benzoin-type condensation comprising an expandable applicability for stereoselective synthesis. The enzymatically catalyzed C−C bond formation yielding unsymmetrical 2-hydroxy ketones represents a superior alternative to the conventional asymmetric benzoin condensation using chiral auxiliaries. The biocatalyst (BFD-His) is available with remarkable ease from the crude extract of *E. coli* cells carrying the BFD-gene in a one-step purification protocol.[5] We have demonstrated the applicability in preparative-scale synthesis leading to highly enantiomerically enriched and optically pure 2-hydroxypropanones in good to high yields. The simple batch-reactor technique engaged herein offers a convenient and easy method for stereoselective synthesis in organic chemistry, if the competitive formation of homocoupling products (cf. benzoins)[8] and concentration-dependent enantioselectivity (cf. formation of 2-HPP)[5] can be neglected. The use of cyclodextrin or DMSO as cosolvent enables a further broadening of the substrate range by employing hydrophobic substrates. In summary, our results open the way for the application of BFD in organic synthesis, by offering enantiopure unsymmetrical and symmetrical[8] 2-hydroxy ketones as general starting materials.

Experimental Section

Abbreviations: BFD, benzoylformate decarboxylase; CSTR, continuous stirred tank reactor; DM-β-CD, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin; His, hexahistidine; 2-HPP, 2-hydroxy-1-phenylpropan-1-one (2-hydroxypropiophenone); MOM, methoxymethyl; NTA, nitrilotriacetic acid; ThDP, thiamin diphosphate; wt, wild-type.

General Remarks: All reagents used were of analytical grade. Solvents were dried by standard methods when necessary. Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) was obtained from Wacker, Germany. 5-Isopropylfurfural (**7c**)[28] and 3-isopropoxybenzaldehyde (**3h**)[29] were synthesized according to published procedures. BFD was used as wild-type enzyme (BFD-wt) from *P. putida* or as a recombinant enzyme elongated with a hexahistidine tail (BFD-His) expressed in *E. coli*. Purification of the enzymes is described elsewhere.[5] BFD activity is related to the catalytic decarboxylase activity which was determined in a coupled photometric assay;[5] 1 unit is defined as the amount of enzyme that decarboxylates 1 μmol of benzoylformate per min at 30 °C in potassium phosphate buffer (50 mmol·L^{-1}, pH = 6.0). Enzymatic syntheses were performed in standard buffer consisting of potassium phosphate (50 mmol·L^{-1}, pH = 7.0) containing $MgSO_4$ (0.5 mmol·L^{-1}) and ThDP (0.5 mmol·L^{-1}). TLC was carried out on aluminium sheets precoated with silica gel 60F_{254} (Merck). Detection was performed by UV light (λ = 254 nm). Preparative column chromatography was carried out on silica gel 60 (mesh size 40−63 μm). NMR spectra were recorded with an AMX 300 (Bruker Physik AG, Germany). Chemical shifts δ are reported in ppm relative to C*H*Cl$_3$ (^{1}H: δ = 7.26) and *C*DCl$_3$ (^{13}C: δ = 77.0) as internal standard. GCMS was carried out with an HP 6890 series GC system fitted with an HP 5973 mass-selective detector [Hewlett Packard; column HP-5MS, 30 m × 250 μm; T_{GC}(injector) = 250 °C, T_{MS}(ion source) = 200 °C, time program (oven): $T_{0\,min}$ = 60 °C, $T_{3\,min}$ = 60 °C, $T_{14\,min}$ = 280 °C (heating rate 20 °C·min^{-1}), $T_{19\,min}$ = 280 °C]. HRMS (EI) was performed with an A.E.I. MS 50 and elemental analysis with a Vario EL (Heraeus) at the Analytical Department, Chemische Institute der Universität Bonn. HPLC was performed with an HP series 1100, Hewlett Packard, fitted with a diode-array detector, and equipped with a chiral-phase column Chiralpak AD (Daicel Ltd., 250 × 4 mm, equipped

with a precolumn, 80 × 4 mm; isohexane/2-propanol = 85:15, flow 0.75 mL·min^{-1}, 20 °C) or Chiralcel OB (Daicel Ltd., 250 × 4 mm, equipped with a precolumn, 80 × 4 mm; isohexane/2-propanol = 9:1, flow 0.75 mL·min^{-1}, 25 °C). Optical rotations were measured with a polarimeter 241 (Perkin−Elmer) and CD spectra were recorded with a Jasco J-720 spectrophotometer using 2,2,2-trifluoroethanol as solvent. UV spectra were recorded with an Ultrospec 2000 UV/Vis spectrophotometer of Pharmacia Biotech, Sweden. Melting points were measured with a Büchi B-540 heating unit and are not corrected.

Analytical-Scale Synthesis of (*S*)-2-Hydroxypropanone Derivatives Depicted in Table 1−5: Enzymatic syntheses were carried out in 1.5-mL batches. The donor aldehyde substrates were employed in a concentration of 10 mmol·L^{-1} or with less soluble substrates as saturated solution in standard buffer. After addition of acetaldehyde (44 μL, 0.5 mol·L^{-1}) the reaction was started at room temp. by adding BFD (6.75 U). Conversion was stopped after 20 h by extracting the reaction mixture with trichloromethane (150 μL) followed by phase separation by centrifugation (13,000 rpm). 2-Hydroxy ketones were analyzed by GCMS and chiral-phase HPLC. Analytical data of 2-hydroxy ketones, which are not noted, have been published elsewhere.[5]

(*S*)-2-Hydroxy-1-(3-phenoxyphenyl)propan-1-one (5i): Conv.: 12%. − *ee* > 99%. − HPLC (Chiralcel OB): $R_t(S)$ = 16.5 min. − GCMS: R_t = 12.1 min. − MS (70 eV, EI); *m/z* (%): 242 (22) [M$^+$], 197 (100) [M$^+$ − C_2H_5O], 169 (18) [M$^+$ − $C_3H_5O_2$], 93 (7.3) [$C_6H_5O^+$], 77 (16) [$C_6H_5^+$].

(*S*)-1-(3-Benzyloxyphenyl)-2-hydroxypropan-1-one (5j): Conv.: not determined. − *ee* > 99%. − HPLC (Chiralcel OB): $R_t(S)$ = 34.1 min. − GCMS: R_t = 12.9 min. − MS (70 eV, EI); *m/z* (%): 256 (4.1) [M$^+$], 211 (29) [M$^+$ − C_2H_5O], 91 (100) [$C_7H_7^+$].

(*S*)-2-Hydroxy-1-(5-methylfuran-2-yl)propan-1-one (8b): Conv.: 32%. − *ee* = 86%. − HPLC (Chiralpak AD; isohexane/2-propanol = 98:2, flow: 0.75 mL·min^{-1}, 20 °C): $R_t(S)$ = 29.2 min; $R_t(R)$ = 37.3 min. − GCMS: R_t = 7.4 min. − MS (70 eV, EI); *m/z* (%): 154 (19) [M$^+$], 109 (100) [M$^+$ − C_2H_5O], 95 (9.3) [$C_5H_3O_2^+$], 81 (7.3) [$C_5H_5O^+$], 53 (17) [$C_4H_5^+$].

(*S*)-2-Hydroxy-1-(5-isopropylfuran-2-yl)propan-1-one (8c): Conv.: 49%. − *ee* = 73%. − HPLC (Chiralpak AD; isohexane/2-propanol = 98:2, flow: 0.75 mL·min^{-1}, 10 °C): $R_t(S)$ = 23.6 min; $R_t(R)$ = 26.1 min. − GCMS: R_t = 8.3 min. − MS (70 eV, EI); *m/z* (%): 182 (4.7) [M$^+$], 137 (100) [M$^+$ − C_2H_5O], 123 (20) [M$^+$ − C_2H_5O, − CH_2], 109 (22) [M$^+$ − $C_3H_5O_2$], 95 (24) [$C_5H_3O_2^+$], 81 (11) [$C_5H_5O^+$], 66 (12) [$C_5H_6^+$], 53 (8.8) [$C_4H_5^+$].

Competitive Formation of (*S*)-2-HPP (5a) and (*R*)-Benzoin (6): Enzymatic conversions were carried out in 1.5-mL batches as described for 2-hydroxy ketones depicted in Table 1−5 using benzaldehyde and acetaldehyde in various amounts. Conversion was stopped after 18 h by extracting the reaction mixture with trichloromethane (150 μL). Product mixtures were analyzed by chiral-phase HPLC.

HPLC (Chiralcel OB; isohexane/2-propanol = 95:5, flow: 0.75 mL·min^{-1}, 20 °C): benzaldehyde, R_t = 11.6 min. − 2-HPP, $R_t(S)$ = 20.6 min; $R_t(R)$ = 44.4 min. − Benzoin, $R_t(S)$ = 37.4 min; $R_t(R)$ = 50.3 min.

i. Benzaldehyde (0.16 mg, 1 mmol·L^{-1}), acetaldehyde (44 μL, 0.5 mol·L^{-1}). − (*S*)-2-HPP: > 99%. − *ee* = 90.8%. − (*R*)-Benzoin: not detected.

ii. Benzaldehyde (1.6 mg, 10 mmol·L^{-1}), acetaldehyde (44 μL, 0.5 mol·L^{-1}). − (*S*)-2-HPP: 76.5%. − *ee* = 89.2%. − (*R*)-Benzoin: 0.2%. − *ee* > 99%.

iii. Benzaldehyde (4.0 mg, 25 mmol·L^{-1}), acetaldehyde (44 μL, 0.5 mol·L^{-1}). − (*S*)-2-HPP: 67.4%. − *ee* = 88.0%. − (*R*)-Benzoin: 0.8%. − *ee* > 99%.

iv. Benzaldehyde (1.6 mg, 10 mmol·L^{-1}), acetaldehyde (8.8 μL, 100 mmol·L^{-1}). − (*S*)-2-HPP: 88.6%. − *ee* = 89.8%. − (*R*)-Benzoin: 0.4%.

v. Benzaldehyde (1.6 mg, 10 mmol·L^{-1}), acetaldehyde (4.4 μL, 50 mmol·L^{-1}). − (*S*)-2-HPP: 72.9%. − *ee* = 89.5%. − (*R*)-Benzoin: 0.7%. − *ee* > 99%.

vi. Benzaldehyde (1.6 mg, 10 mmol·L^{-1}), acetaldehyde (0.9 μL, 10 mol·L^{-1}). − (*S*)-2-HPP: 14.2%. − *ee* = 89.9%. − (*R*)-Benzoin: 1.6%. − *ee* > 99%.

vii. Benzaldehyde (4.0 mg, 25 mmol·L^{-1}), acetaldehyde (1.8 μL, 20 mol·L^{-1}). − (*S*)-2-HPP: 17.9%. − *ee* = 88.2%. − (*R*)-Benzoin: 3.2%. − *ee* > 99%.

Reaction Control of (*S*)-2-Hydroxy-1-(3-methoxyphenyl)propan-1-one (5f) Formation: Enzymatic conversion was carried out in a 1.5-mL batch as described for 2-hydroxy ketones depicted in Table 1−5 using 3-methoxybenzaldehyde (**3f**) (2 mg, 10 mmol·L^{-1}) and acetaldehyde (44 μL, 0.5 mol·L^{-1}). Aliquots (20 μL) withdrawn at intervals of 45 min were extracted with isohexane (10 μL). The organic phase (10 μL) was directly injected into the HPLC column (Chiralcel OB).

Reaction Control of (*S*)-2-Hydroxy-1-(3-isopropoxyphenyl)propan-1-one (5h) Formation: Formation of **5h** was carried out according to the procedure for **5f** using a solution of 3-isopropoxybenzaldehyde (**3h**) (1.6 mg, 5 mmol·L^{-1}) and acetaldehyde (44 μL, 0.5 mol·L^{-1}).

Preparative-Scale Synthesis of (*S*)-2-Hydroxy Ketones

(*S*)-2-Hydroxy-1-phenylpropan-1-one (5a): Compound **5a** was prepared as described in a previous paper.[5] − *ee* = 92%. − $[\alpha]_D^{25}$ = −79.2 (*c* = 0.016 in trichloromethane). − CD: λ_{max} (Δε) = 179 (−7.0), 190 (+2.9), 204 (−7.9), 247 (+6.7), 282 (−2.4) nm. − UV: λ_{max} (ε) = 201 (12951), 247 (12393), 280 (1221) nm.

(*S*)-2-Hydroxy-1-(3-methoxyphenyl)propan-1-one (5f): 3-Methoxybenzaldehyde (**3f**) (408 mg, 3.0 mmol, 10 mmol·L^{-1}) was dissolved in standard buffer (300 mL). After addition of acetaldehyde (8.5 mL, 0.15 mol, 0.5 mol·L^{-1}), the reaction was started by adding BFD (120 U) and the reaction mixture was allowed to stand at room temp. Conversion was monitored by HPLC by extracting analytical samples (150 μL) with isohexane or trichloromethane (150 μL) followed by phase separation by centrifugation (13000 rpm). If conversion stagnated, one more portion of BFD (40 U) was added. After 40 h, the reaction mixture was filtered using an ultrafiltration membrane (Amicon YM10, cut-off 10000 Da) in order to remove proteins. The filtrate was extracted with dichloromethane (3 × 60 mL), the organic layer dried with Na_2SO_4 and the solvent removed in vacuo to dryness, yielding pure **5f** as a viscous yellowish oil (530 mg, 97%). − *ee* = 96%. − $[\alpha]_D^{25}$ = −65.2 (*c* = 1.13 in trichloromethane). − CD: λ_{max} (Δε) = 180 (−8.9), 191 (+1.1), 204 (−1.2), 252 (+5.6), 294 (−2.8), 325 (+1.1) nm. − UV: λ_{max} (ε) = 216 (12396), 252 (6128), 307 (1689) nm. − HPLC (Chiralcel OB): $R_t(S)$ = 17.8 min; $R_t(R)$ = 32.7 min. − ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 1.45 (d, 3 H, ^3J = 6.8 Hz, CH_3), 3.78 (d, 1 H, ^3J = 6.8 Hz, OH), 3.89 (s, 3 H, OCH_3), 5.14 ("quint", 1 H, ^3J = 6.8 Hz, C*H*OH), 7.15 (d"t", 1 H, ^3J = 8.0 Hz, ^4J =

1.8 Hz, ar-H), 7.39 ("t", 1 H, ^3J = 8.0 Hz, ar-H), 7.45 ("t", 1 H, ^4J = 1.8 Hz, ar-H), 7.47 (m, 1 H, ar-H). − ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): δ = 22.8 (CH_3), 55.9 (OCH_3), 69.8 (CHOH), 113.4, 120.7, 121.5, 130.3 (CH), 135.0, 160.4 (C_q), 202.7 (CO). − GCMS: R_t = 9.3 min. − MS (70 eV, EI); *m/z* (%): 180 (22) [M^+], 135 (100) [$M^+ - C_2H_5O$], 107 (32) [$M^+ - C_3H_5O_2$], 77 (25) [$C_6H_5^+$]. − HRMS [M^+]; *m/z* calcd. for $C_{10}H_{12}O_3$: 180.0787; found 180.0782. − $C_{10}H_{12}O_3$ (180.2): calcd. C 66.65, H 6.71; found C 66.27, H 6.64.

(*S*)-2-Hydroxy-1-(3-isopropoxyphenyl)propan-1-one (5h): Compound **5h** was prepared according to the procedure for **5f** using a solution of 3-isopropoxybenzaldehyde (**3h**) (164 mg, 1.0 mmol, 5 mmol·L^{-1}) and acetaldehyde (5.7 mL, 0.1 mol, 0.5 mol·L^{-1}) in standard buffer (200 mL). Reaction was complete after 72 h (conv. 96%). After drying in vacuo, pure **5h** was obtained as a viscous yellowish oil (190 mg, 91%). − *ee* = 99.3%. − $[\alpha]_D^{25}$ = −54.9 (*c* = 1.45 in trichloromethane). − CD: λ_{max} (Δε) = 181 (−4.0), 193 (−1.5), 203 (−3.5), 252 (+4.9), 295 (−4.1), 332 (+0.3) nm. − UV: λ_{max} (ε) = 218 (19512), 252 (8619), 307 (2119) nm. − HPLC (Chiralcel OB): R_t(*S*) = 16.8 min; R_t(*R*) = 24.2 min. − ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 1.36 [d, 6 H, ^3J = 6.1 Hz, CH(C*H*$_3$)$_2$], 1.43 (d, 3 H, ^3J = 6.8 Hz, CH_3), 3.77 (d, 1 H, ^3J = 6.8 Hz, OH), 4.62 [sept, 1 H, ^3J = 6.1 Hz, C*H*(CH$_3$)$_2$], 5.12 ("quint", 1 H, ^3J = 6.8 Hz; C*H*OH), 7.12 (dm, 1 H, ^3J = 8.0 Hz, ar-H), 7.38 ("t", 1 H, ^3J = 8.0 Hz, ar-H), 7.45 (m, 2 H, ar-H). − ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): δ = 22.1 [CH(*C*H$_3$)$_2$], 22.6 (CH_3), 69.6, 70.5 [*C*H(CH$_3$)$_2$, CHOH], 115.3, 121.0, 122.05, 130.1 (CH), 134.8, 158.5 (C_q), 202.5 (CO). − GCMS: R_t = 9.8 min. − MS (70 eV, EI); *m/z* (%): 208 (25) [M^+], 163 (100) [$M^+ - C_2H_5O$], 121 (99) [$M^+ - C_2H_5O - C_3H_6$], 95 (32) [$C_5H_3O_2^+$], 77 (17) [$C_6H_5^+$], 65 (19) [$C_5H_5^+$]. − HRMS [M^+]; *m/z* calcd. for $C_{12}H_{16}O_3$: 208.1100; found 208.1102. − $C_{12}H_{16}O_3$ (208.3): calcd. C 69.21, H 7.74; found C 69.09, H 7.74.

(*S*)-2-Hydroxy-1-naphthalen-2-ylpropan-1-one (12): 2-Naphthaldehyde (**11**) (156 mg, 1.0 mmol, 10 mmol·L^{-1}) was dissolved in a solution of DM-β-CD (2.66 g, 2.0 mmol, 20 mmol·L^{-1}) in standard buffer (100 mL). After addition of acetaldehyde (2.8 mL, 50 mmol, 0.5 mol·L^{-1}), the reaction was started by treatment with BFD (400 U). The reaction was stopped at 34% conversion (72 h) and worked up according to the procedure for **5f**. Compound **12** was purified by column chromatography (isohexane/ethyl acetate = 10:1; R_f = 0.12) yielding a colorless solid (64 mg, 32%). − M.p. 58 °C. − *ee* = 88%. − $[\alpha]_D^{25}$ = −106.2 (*c* = 1.16 in trichloromethane). − CD: λ_{max} (Δε) = 182 (−2.9), 189 (−1.0), 200 (−3.8), 215 (+1.9), 223 (+1.0), 249 (+6.9), 262 (+0.7), 284 (+2.5), 306 (−7.5), 346 (+0.8) nm. − UV: λ_{max} (ε) = 207 (16715), 249 (34359), 292 (8029), 341 (1490) nm. − HPLC (Chiralpak AD): R_t(*R*) = 19.3 min; R_t(*S*) = 24.4 min. − ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 1.53 (d, 3 H, ^3J = 6.5 Hz, CH_3), 3.90 (d, 1 H, ^3J = 6.5 Hz, OH), 5.35 ("quint", 1 H, ^3J = 6.5 Hz; C*H*OH), 7.56−7.69 (m, 2 H, ar-H), 7.89−8.04 (m, 4 H, ar-H), 8.45 (s, 1 H, ar-H). − ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): δ = 23.0 (CH_3), 69.8 (CHOH), 124.4, 127.6, 128.3, 129.3, 129.5, 130.1, 130.9 (CH), 131.0, 132.8, 136.4 (C_q), 202.8 (CO). − GCMS: R_t = 11.2 min. − MS (70 eV, EI); *m/z* (%): 200 (13) [M^+], 155 (100) [$M^+ - C_2H_5O$], 127 (97) [$M^+ - C_3H_5O_2$], 77 (14) [$C_6H_5^+$]. − HRMS [M^+]; *m/z* calcd. for $C_{13}H_{12}O_2$: 200.0837; found 200.0839. − $C_{13}H_{12}O_2$ (200.2): calcd. C 77.98, H 6.04; found C 77.81, H 5.97.

(*S*)-2-Hydroxy-1-(3,5-dimethoxyphenyl)propan-1-one (14): 3,5-Dimethoxybenzaldehyde (**13**) (166 mg, 1 mmol, 5 mmol·L^{-1}) was dissolved in DMSO (20 mL) whereupon standard buffer (180 mL) and acetaldehyde (5.6 mL, 0.1 mol, 0.5 mol·L^{-1}) were added. After addition of BFD (400 U) the reaction mixture was allowed to stand at room temp. The reaction was stopped at 45% conversion (72 h) and the product isolated according to the procedure for **5f**. Compound **14** was purified by column chromatography (isohexane/ethyl acetate = 7:1; R_f = 0.13) yielding a viscous yellowish oil (84 mg, 40%). − *ee* = 97%. − $[\alpha]_D^{25}$ = −60.0 (*c* = 0.54 in trichloromethane). − CD: λ_{max} (Δε) = 184 (+2.3), 196 (−5.5), 224 (+1.4), 234 (+0.8), 265 (+3.6), 298 (−4.5), 334 (+0.9) nm. − UV: λ_{max} (ε) = 215 (15094), 265 (4151), 317 (1472) nm. − HPLC (Chiralpak AD): R_t(*S*) = 16.4 min; R_t(*R*) = 18.5 min. − ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 1.45 (d, 3 H, ^3J = 6.6 Hz, CH_3), 3.78 (d, 1 H, ^3J = 6.6 Hz, OH), 3.86 (s, 6 H, OCH_3), 5.10 ("quint", 1 H, ^3J = 6.6 Hz; C*H*OH), 6.69 (t, 1 H, ^4J = 2.3 Hz, ar-H), 7.02 (d, 2 H, ^4J = 2.3 Hz, ar-H). − ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): δ = 22.8 (CH_3), 56.1 (OCH_3), 69.9 (CHOH), 106.4, 106.9 (CH), 135.6, 161.4 (C_q), 202.7 (CO). − GCMS: R_t = 10.6 min. − MS (70 eV, EI); *m/z* (%): 210 (28) [M^+], 165 (100) [$M^+ - C_2H_5O$], 137 (36) [$M^+ - C_3H_5O_2$], 122 (99) [$M^+ - C_3H_5O_2 - CH_3$], 77 (14) [$C_6H_5^+$]. − HRMS [M^+]; *m/z* calcd. for $C_{11}H_{14}O_4$: 210.0892; found 210.0893. − $C_{11}H_{14}O_4$ (210.2): calcd. C 62.85, H 6.71; found C 62.96, H 6.71.

rac-2-Hydroxy-1-(3-methoxyphenyl)propan-1-one (rac-5f)

i. 3-Methoxybenzaldehyde (**3f**) (6.8 g, 50 mmol) was added slowly to a mixture of trimethylsilyl cyanide (5.0 g, 55 mmol) and anhydrous ZnI_2 (catalytic amount). The reaction mixture was heated at 95 °C for 2 h. The resulting product was fractionated in vacuo yielding pure (3-methoxyphenyl)trimethylsilanyloxyacetonitrile (11.3 g, 96%) as a colorless liquid. − B.p.$_{0.025\ mbar}$ 86 °C. − ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 0.28 [s, 9 H, Si(CH$_3$)$_3$], 3.84 (s, 3 H, OCH_3), 5.50 (s, 1 H, CH), 6.92 (dm, 1 H, ^3J = 7.9 Hz, ar-H), 7.04 (s, 1 H, ar-H), 7.06 (d, 1 H, ^3J = 7.9 Hz, ar-H), 7.32 ("t", 1 H, ^3J = 7.9 Hz, ar-H). − ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): δ = 0.0 [Si(CH$_3$)$_3$], 55.6 (OCH_3), 63.8 (*C*HCN), 112.1, 115.1, 118.8 (CH), 119.4 (CN), 130.3 (CH), 138.0, 160.3 (C_q). − GCMS: R_t = 9.75 min. − MS (70 eV, EI); *m/z* (%): 235 (50) [M^+], 220 (100) [$M^+ - CH_3$], 205 (8.1) [$M^+ - 2\ CH_3$], 146 (38) [$M^+ - OSi(CH_3)_3$], 135 (24) [$M^+ - HCN - Si(CH_3)_3$].

ii. To a solution of LDA (22 mmol) in dry THF (20 mL) (3-methoxyphenyl)trimethylsilanyloxyacetonitrile (4.7 g, 20 mmol) in THF (4 mL) was added at −78 °C. The solution was stirred for 30 min at −78 °C whereupon dry acetaldehyde (1.1 mL, 20 mmol) was added at this temperature. The reaction mixture was warmed to room temp. within 8 h and subsequently quenched with saturated NH_4Cl solution (40 mL). After an additional 10 min of stirring at room temp., the mixture was extracted with diethyl ether (3 × 30 mL), the organic layer dried with Na_2SO_4 and concentrated to dryness. The resulting crude TMS ether of ***rac*-5f** was stirred in a mixture of hydrochloric acid (2 N, 40 mL) and methanol (20 mL) for 15 h. After addition of water (30 mL), the crude product was extracted with diethyl ether, the organic layer washed with aqueous NaOH (1 N, 20 mL) and dried with Na_2SO_4. Compound ***rac*-5f** was purified by column chromatography (dichloromethane/ethyl acetate = 30:1; R_f = 0.38) yielding a viscous yellowish oil (2.2 g, 60%). Analytical data are in accordance with enzymatically prepared **5f**. − HPLC (Chiralcel OB): R_t(*S*) = 17.8 min; R_t(*R*) = 32.7 min.

***rac*-2-Hydroxy-1-(3,5-dimethoxyphenyl)propan-1-one (*rac*-14)**

i. ***rac*-14** was prepared according to the procedure for ***rac*-5f** using 3,5-dimethoxybenzaldehyde (**13**) (2.4 g, 14.5 mmol) and trimethylsilyl cyanide (1.6 g, 16 mmol) to give (3,5-dimethoxyphenyl)trimethylsilanyloxyacetonitrile as a colorless liquid (3.1 g, 82%). − B.p.$_{0.1\ mbar}$ 130 °C. − ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 0.27 [s, 9 H, Si(CH$_3$)$_3$], 3.80 (s, 6 H, OCH_3), 5.42 (s, 1 H, CH), 6.48

(s, 1 H, ar-H), 6.65 (s, 2 H, ar-H). − GCMS: R_t = 10.9 min. − MS (70 eV, EI); *m/z* (%): 265 (67) [M^+], 250 (100) [$M^+ - CH_3$], 235 (6.8) [$M^+ - 2\,CH_3$], 176 (29) [$M^+ - OSi(CH_3)_3$], 165 (10) [$M^+ - HCN - Si(CH_3)_3$].

ii. LDA (11 mmol), (3,5-dimethoxyphenyl)trimethylsilanyloxyacetonitrile (2.7 g, 10 mmol) and dry acetaldehyde (0.6 mL, 10 mmol) were used for the C−C coupling reaction according to procedure (ii) for ***rac*-5f**. Column chromatography (isohexane/ethyl acetate = 7:1; R_f = 0.13) resulted in a viscous yellowish oil (1.2 g, 55%). Analytical data are in accordance with enzymatically prepared **14**. − HPLC (Chiralpak AD): $R_t(S)$ = 16.3 min; $R_t(R)$ = 18.4 min.

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