

## Development of a Donor–Acceptor Concept for Enzymatic Cross-Coupling Reactions of Aldehydes: The First Asymmetric Cross-Benzoin Condensation

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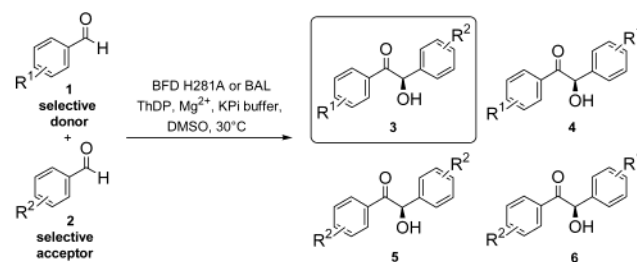
In earlier publications, we showed that enantiopure benzoin s can be efficiently obtained by use of the thiamin diphosphate (ThDP)-dependent enzymes benzaldehyde lyase (BAL)<sup>1</sup> or benzoylformate decarboxylase (BFD).<sup>2</sup> In the present contribution we present successful and unprecedented results leading to a donor–acceptor concept for the asymmetric synthesis of mixed benzoin s, that is, benzoin s with nonidentical aromatic moieties, using an enzyme-catalyzed benzoin condensation. An asymmetric variant of this cross-coupling has not yet been described in the literature.<sup>3</sup>

The synthesis of mixed benzoin s is of interest in many respects as they are versatile building blocks in organic and pharmaceutical chemistry.<sup>4</sup> In addition to other synthetic routes the racemic compounds can be synthesized selectively by means of a cyanide-catalyzed cross-benzoin condensation.<sup>5</sup> This selectivity prompted various scientists to subdivide the aromatic aldehydes into donors, adding the cyanide ion, and acceptors.<sup>6</sup>

Although we do not agree completely with the authors' statements<sup>6c</sup> we were inspired by this idea of selective donors and acceptors so that in an initial experiment 2-chlorobenzaldehyde (**2a**), 2-methylbenzaldehyde (**2b**), 2-methoxybenzaldehyde (**2c**), and subsequently the ortho-substituted benzaldehyde derivatives **2d–f** were reacted with benzaldehyde in the presence of different ThDP-dependent enzymes. The three substrates **2a–c** were chosen because of their inability to form symmetrical benzoin s through the wild-type BFD-catalyzed reaction,<sup>2a</sup> assuming that they still might serve as acceptor substrates although they are not accepted as donors by this enzyme. Here, the enzymes BFD H281A<sup>7</sup> and BAL were identified as potent catalysts for asymmetric cross-carboligation (Table 1). The absolute configuration of compound **3a** was determined to be *R*, as expected (*ee* > 99%).<sup>8</sup> This compound has also been synthesized on a preparative scale using BFD H281A as well, with pleasing results concerning conversion and selectivity. Remarkably, the 2,2'-disubstituted benzoin s **5a–c** or the mixed benzoin s substituted in 2-position **6a–c** were not generated in the BFD H281A-catalyzed reactions while **5d,f** and **6d,f** were not formed in the BAL-catalyzed reactions, showing that **2a–d,f** react selectively as acceptors in the presence of the respective biocatalyst.

On the basis of these observations, we focused on the identification of selective donor substrates and discovered that an appropriate screening lead us to a broad variety of selectively reacting benzaldehyde derivatives. This screening was carried out by reacting 2-chlorobenzaldehyde with putative donors in the presence of BFD

**Table 1.** Combined Enzyme–Substrate Screening



	1 [R <sup>1</sup> ]	2 [R <sup>2</sup> ]	enzyme	3 [%]	4 [%]	5 [%]	6 [%]
<b>a</b>	H	2-Cl	BFD H281A	90	10	—	—
			BAL	70	16	14	—
<b>b</b>	H	2-Me	BFD H281A	34	66	—	—
			BAL	39	30	12	19
<b>c</b>	H	2-MeO	BFD H281A	40	60	—	—
			BAL	66	25	10	—
<b>d</b>	H	2,6-F <sub>2</sub>	BFD H281A	—	—	—	—
			BAL	88	12	—	—
<b>e</b>	H	2,3,5-F <sub>3</sub>	BFD H281A	53	46	1	—
			BAL	67	24	9	—
<b>f</b>	H	2,3,4,5,6-F <sub>5</sub>	BFD H281A	—	—	—	—
			BAL	90	10	—	—

**Table 2.** Mixed Benzoin s Synthesized Chemoselectively and Asymmetrically on a Preparative Scale<sup>a</sup>

3	donor 1 [R <sup>1</sup> ]	acceptor 2a [R <sup>2</sup> ]	enzyme	conversion [%]	selectivity <sup>b</sup> [%]	ee <sup>c</sup> [%]
<b>g</b>	3-CN	2-Cl	BFD H281A	>99	>99	90 <sup>d</sup>
<b>h</b>	4-Br	2-Cl	BFD H281A	90	95	95
<b>i</b>	4-CF <sub>3</sub>	2-Cl	BFD H281A	75	>99	93
<b>j</b>	3,4-CH <sub>2</sub> O <sub>2</sub>	2-Cl	BAL	98	83	>99
<b>k</b>	3,4,5-(CH <sub>3</sub> O) <sub>3</sub>	2-Cl	BAL	82	97	>99
<b>l</b>	3,5-(CH <sub>3</sub> O) <sub>2</sub>	2-Cl	BAL	>99	95	>99

<sup>a</sup> Experimental procedure: BFD H281A or BAL, ThDP, Mg<sup>2+</sup>, KPi buffer, DMSO, 30 °C, 24–48 h. <sup>b</sup> The selectivity is defined as the percent ratio of product in relation to the sum of all benzoin s obtained. <sup>c</sup> Determined by HPLC analysis. <sup>d</sup> Compound **3g** exhibits a strong racemization tendency.

H281A and BAL, respectively. Table 2 presents those examples of selectively obtained mixed benzoin s that are likewise synthesized on a preparative scale (0.2–1.5 g) also with good-to-excellent conversion and chemoselectivity. Obviously, reactions catalyzed by these enzymes obey a donor–acceptor selectivity. Notably, the selectivity exhibited by the enzymes is different to the extent that BAL has a much broader substrate range concerning aromatic aldehydes with sterically demanding substituents, while BFD H281A shows a higher selectivity with aromatic aldehydes with

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**Table 3.** Preliminary Results of the Permutative Donor–Acceptor Screening Using BAL as Catalyst<sup>a</sup>

3	donor 1 [R <sup>1</sup> ]	acceptor 2 [R <sup>2</sup> ]	conversion [%]	selectivity <sup>b</sup> [%]
<b>m</b>	3-CN	2,6-F <sub>2</sub>	>99	71
<b>n</b>	4-Br	2,6-F <sub>2</sub>	98	66
<b>o</b>	4-CF <sub>3</sub>	2,6-F <sub>2</sub>	>99	78
<b>p</b>	3,4-CH <sub>2</sub> O <sub>2</sub>	2,6-F <sub>2</sub>	97	72
<b>q</b>	3,4,5-(CH <sub>3</sub> O) <sub>3</sub>	2,6-F <sub>2</sub>	93	84
<b>r</b>	3,5-(CH <sub>3</sub> O) <sub>2</sub>	2,6-F <sub>2</sub>	>99	96
<b>s</b>	3-CN	2,3,5-F <sub>3</sub>	89	66
<b>t</b>	4-Br	2,3,5-F <sub>3</sub>	>99	90
<b>u</b>	4-CF <sub>3</sub>	2,3,5-F <sub>3</sub>	94	78
<b>v</b>	3,4-CH <sub>2</sub> O <sub>2</sub>	2,3,5-F <sub>3</sub>	40	94
<b>w</b>	3,4,5-(CH <sub>3</sub> O) <sub>3</sub>	2,3,5-F <sub>3</sub>	>99	83
<b>x</b>	3,5-(CH <sub>3</sub> O) <sub>2</sub>	2,3,5-F <sub>3</sub>	98	92
<b>y</b>	3-CN	2,3,4,5,6-F <sub>5</sub>	nc <sup>c</sup>	–
<b>z</b>	4-Br	2,3,4,5,6-F <sub>5</sub>	>99	97
<b>Γ</b>	4-CF <sub>3</sub>	2,3,4,5,6-F <sub>5</sub>	76	96
<b>Π</b>	3,4-CH <sub>2</sub> O <sub>2</sub>	2,3,4,5,6-F <sub>5</sub>	>99	>99
<b>Σ</b>	3,4,5-(CH <sub>3</sub> O) <sub>3</sub>	2,3,4,5,6-F <sub>5</sub>	>99	92
<b>Ω</b>	3,5-(CH <sub>3</sub> O) <sub>2</sub>	2,3,4,5,6-F <sub>5</sub>	>99	>99

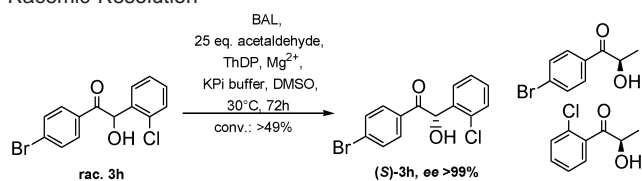
<sup>a</sup> The selectivity was determined by gaschromatography. <sup>b</sup> The selectivity is defined as the percent ratio of product in relation to the sum of all benzoinz obtained. <sup>c</sup> nc = no conversion.

small substituents. Thus, the complementary substrate ranges of different enzymes enable the synthesis of a large diversity of mixed benzoinz.

Evidence that the aldehydes used do not serve as selective acceptors only in the presence of one special donor, and vice versa, could be provided by an additional test series combining identified selective donors with selective acceptors in the presence of BAL (Table 3). In most of these attempts the mixed benzoin **3** was obtained with high-to-excellent selectivity.

To obtain access to the (*S*)-enantiomer of the mixed benzoinz, we employed the kinetic racemic resolution via C–C bond cleavage established for the BAL-catalyzed (*S*)-benzoin formation (Scheme 1).<sup>1a</sup> In doing so the enantiopure mixed (*S*)-benzoin (**S**)-**3h** was obtained with more than 49% conversion (ee > 99%). Thus, both enantiomers of the mixed benzoinz are accessible through this enzyme-catalyzed reaction.

#### Scheme 1. Generation of the Mixed (*S*)-Benzoin (**S**)-**3h** by Kinetic Racemic Resolution



In summary, we have shown that mixed benzoinz can be synthesized enantioselectively through an enzymatic cross-benzoin condensation by ThDP-dependent enzymes taking advantage of the aldehydes donor–acceptor behavior. This one-step synthesis starting from cheap and commercially available aldehydes represents an outstanding improvement in comparison to the costly and tedious synthesis based on the conversion of chiral cyanohydrines with phenyl-Grignard derivatives.<sup>4a–c,9</sup> Essential for the realization of this concept was a successful implementation of both enzymatic

and chemical mechanistic concerns. Moreover, we are convinced that the concept presented here should be transferable to reactions that proceed in a comparable manner, for example Tishchenko reactions<sup>10,11</sup> or pinacol couplings.<sup>11,12</sup> In this case, our work, stimulated by classic organic chemistry and carried out in the field of enzymatic synthesis, would lead us to an advanced insight into general chemical concerns.

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**Supporting Information Available:** Experimental data for the compounds **3a**, **3h**, **3j**, and **3II** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (8) Since symmetric benzoinz generated through wild-type BAL- and wild-type BFD-catalyzed reactions are of *R*-configuration, we assumed that the mixed benzoinz **3** possess *R*-configuration, too.<sup>1,2</sup> To scrutinize the absolute configuration of the mixed benzoinz, (*R*)-2'-chlorobenzoin **3a** was prepared nonenzymatically starting from (*R*)-(2-chlorophenyl)-2-trimethylsilyloxy-acetonitrile<sup>9a</sup> by means of a Grignard reaction with phenylmagnesium bromide.
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