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Enzyme-Catalyzed Trans-Benzoin Condensation

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Abstract: Benzaldehyde lyase (BAL) is an enzyme that is used in the C-C bond cleavage and formation which was isolated first from *Pseudomonas fluorescens* Biovar I. It requires thiamine diphosphate (ThDP) and Mg(II) ions as cofactors. In this work, BAL was used as an enzymatic catalyst for the trans-benzoin condensation reaction between racemic benzoins and benzyloxyacetaldehyde to form unsymmetrical benzoin products with moderate enantiomeric excesses. (*S*)-benzoin derivatives remained unreacted at the end of the reaction. In this enzymatic trans-benzoin condensation, benzyloxyacetaldehyde acted as acceptor and different variety of racemic benzoin derivatives were used as donor and (*R*)-2-hydroxy-1-phenylpropanone derivatives were synthesized up to 66% ee.

Keywords: Benzaldehyde lyase, benzoin condensation, enzymatic asymmetric catalysis.

Note: *This article is dedicated to Prof. Dr. Ayhan S. Demir (1950-2012).*

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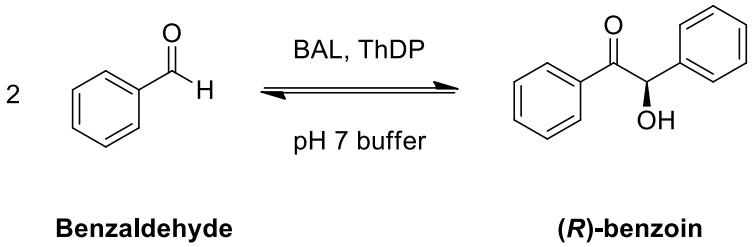
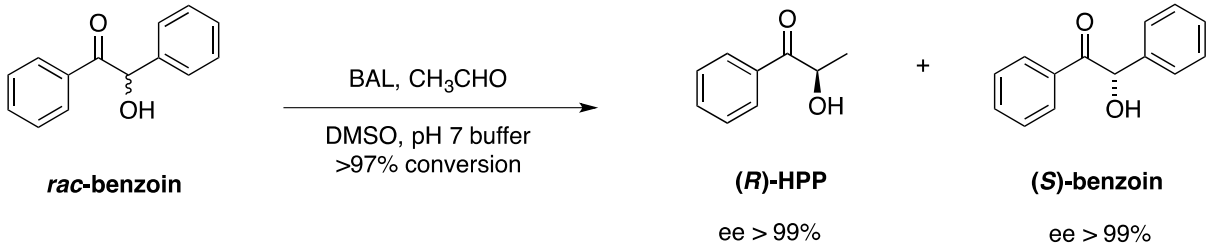
INTRODUCTION

Benzaldehyde lyase was first isolated from *Pseudomonas fluorescens* Biovar I strain which was found in a cellulose factory growing on lignin-degradation products. It was realized that BAL was using a chiral benzoin derivative as an energy source by cleaving the acyloin linkage to give benzaldehyde derivatives (1). BAL can also catalyze the benzoin formation reaction rather than cleavage especially from benzaldehyde to yield (*R*)-benzoin with high chemical yield and enantioselectivity (ee>99%) (Scheme 1). These high selectivities and yields of BAL-catalyzed benzoin condensation reactions make it very attractive for industrial processes. In benzoin condensation reaction catalyzed by BAL, substituted benzaldehyde derivatives were used as donors. Formaldehyde, acetaldehyde and its derivatives were acted as acceptors (2).



Scheme 1. Benzoin condensation of benzaldehyde catalyzed by BAL to give (*R*)-benzoin.

Demir *et al.* reported various examples of asymmetric benzoin condensation catalyzed by BAL (3, 4). One significant example of BAL-catalyzed reactions was the trans-benzoin condensation where the cleavage of C-C bond and the formation of C-C bond occurred at the same time to give chiral unsymmetrical benzoin derivatives. (R)-benzoin was used as a benzaldehyde source and reaction with acetaldehyde in the presence of BAL as a catalyst produced (R)-2-hydroxy-1-phenylpropanone, ((R)-HPP) in an optically pure form, quantitatively (5). Furthermore, Demir *et al* also showed (6) a single example of trans benzoin condensation using *rac*-benzoin as an aromatic aldehyde source to react with acetaldehyde to produce (R)-HPP with perfect enantioselectivity (Scheme 2). However, the scope of this reactions remained unexplored.



Scheme 2. Trans-benzoin condensation of *rac*-benzoin with acetaldehyde.

In this work, trans-benzoin condensation of various racemic benzoin derivatives with benzyloxyacetaldehyde catalyzed by BAL to produce unsymmetrical benzoin products were studied. The obtained HPP derivatives are important precursors in the synthesis of asymmetric triol derivatives.

MATERIALS AND METHODS

Production of Benzaldehyde lyase

The cells of *E. coli SG13009/BALHis* that contain the overexpressed enzyme were supplied from Institute of Biotechnology, Research Centre Jülich. Hexahistidine tagged BAL was obtained from recombinant *E.Coli SG13009* cells. Expression of BAL was performed based on the literature (6). The recombinant *E.coli* strains were grown on LB agar that contains 100 µg/mL ampicillin and 35 µg/mL chloroamphenicol incubated in an oven overnight at 37 °C. Firstly, to produce our enzyme, cells were taken from LB agars by sterile loop and transported to a sterile 10 mL LB medium contains 20 µL ampicillin and 20 µL chloroamphenicol. This medium was inoculated for 12 hours at 37 °C. In the precultivation part, growing time range of the bacteria is critical because cells begin to die at some point. In this part, a 500 mL Erlenmeyer flask was used for 100 mL of LB (90 mL distilled water + 10 mL growth cell) medium containing 100 µL ampicillin and chloroamphenicol inoculation ratio 1/1000. It was incubated and grown for 6 hours at 37 °C, then 100 mL transferred to the production medium that contains 1500 mL of LB medium is shaken with 180 rpm. Four hours after the inoculation of the microorganism, production of enzyme was initiated with addition of isopropyl-β-D-thiogalactopyranosid (IPTG). After the induction, enzyme production was started and continued for 12 hours, cell pellets were collected by using centrifugation. In order to break the cell walls to release our enzyme, the cell pellets which are taken from -20 °C were melted to room temperature and sonicated. Finally, removal of water from cells by lyophilization was made with a freeze dryer.

Activity assay

According to the literature (3), one unit (U) of activity is described as the quantity of enzyme that catalyzes the formation of 1 µmol benzoin (1.5 mM) from benzaldehyde in potassium phosphate buffer (50 mM, pH 7) that contains $MgSO_4$ (2.5 mM), ThDP (0.15 mM) and DMSO (20%, v/v) in 1 minute at 30 °C. To measure the activity of our enzyme, a set of reactions with the same concentration of benzaldehyde was prepared with commercially available benzaldehyde. At appropriate time intervals, samples were withdrawn to measure the amount of benzoin. Then, the standard curve was drawn by using HPLC analysis results to measure the activity of BAL.

For activity experiment, to a set of 2.5 µL benzaldehyde, 0.5 mL DMSO and 1.5 mL phosphate buffer containing ThDP and $MgSO_4$ was added. The process was initiated with the addition of 5 mg crude enzyme. At 10 minute intervals, one sample was withdrawn by adding chloroform and it was centrifuged. Finally, organic layer was collected and analyzed with HPLC to determine the activity.

General procedure for racemic synthesis of self-benzoin condensation products

The synthesis of racemic benzoin derivatives was done based on the literature procedure (7). A solution of sodium cyanide (2 mmol, 0.098 g) in H_2O (2 mL) was added to a stirred solution of a benzaldehyde derivative (10 mmol) in EtOH (10 mL). The mixture was then refluxed. The progress of reaction was monitored by TLC using hexane/ethyl acetate as eluent. The solvent was then removed by evaporation under reduced pressure. The residue was washed with water and diethyl ether. The product was purified by using flash column chromatography technique.

Synthesis of 3,3′-difluorobenzoin (1)

General procedure stated above starting from 3-fluorobenzaldehyde was applied and the pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (48% yield, white solid). TLC: hexane/ethyl acetate = 80:20. R_f = 0.60.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.67-7.58 (m, 1H), 7.55-7.50 (m, 1H), 7.41-7.29 (m, 2H), 7.08-7.03 (m, 2H), 6.98-6.88 (m, 2H), 5.83 (s, 1H), 4.43 (br s, 1H, OH).

Synthesis of 3,3′-dibromobenzoin (2)

General procedure stated above starting from 3-bromobenzaldehyde was applied and the pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (60% yield, yellow oil). TLC: hexane/ethyl acetate = 80:20. R_f = 0.75

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 (t, *J* = 1.8 Hz, 1H), 7.78 (t, *J* = 7.5 Hz, 1H), 7.66 (dd, *J* = 8.01, 1.0 Hz, 1H), 7.48 (t, *J* = 1.7 Hz, 1H), 7.42 (d, *J* = 7.7 Hz, 1H), 7.31-7.17 (m, 3H), 5.87 (s, 1H), 4.45 (br s, 1H, OH).

Synthesis of 3,3′-dimethoxybenzoin (3)

The general procedure stated above, starting from 3-methoxybenzaldehyde, was applied and pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (45% yield, light yellow solid). TLC: hexane/ethyl acetate = 80:20. R_f = 0.65.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50-7.44 (m, 2H), 7.33-7.20 (m, 2H), 7.09-7.02 (m, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.86-6.79 (m, 2H), 5.90 (s, 1H), 4.55 (br s, 1H, OH), 3.79 (s, 3H), 3.76 (s, 3H).

Synthesis of 4,4′-difluorobenzoin (4)

General procedure stated above starting from 4-fluorobenzaldehyde was applied and the pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (68% yield, light yellow solid). TLC: hexane/ethyl acetate = 80:20. R_f = 0.63.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09-8.03 (m, 1H), 7.89-7.82 (m, 2H), 7.26-7.20 (m, 1H), 7.03-6.89 (m, 4H), 5.83 (s, 1H).

Synthesis of 3,3'-dimethylbenzoin (5)

General procedure stated above starting from *m*-tolualdehyde was applied and the pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (42% yield, yellow oil). TLC: hexane/ethyl acetate = 80:20. R_f = 0.62.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 7.6 Hz, 1H), 7.21-7.17 (m, 2H), 7.13 (d, *J* = 5.8 Hz, 2H), 7.06 (t, *J* = 6.4 Hz, 1H), 5.90 (d, *J* = 5.5 Hz, 1H), 4.54 (d, *J* = 6.0 Hz, 1H, OH), 2.34 (s, 3H), 2.29 (s, 3H).

Synthesis of 4,4'-dimethylbenzoin (6)

General procedure stated above starting from *p*-tolualdehyde was applied and the pure product was obtained after crystallization with ethyl acetate and washed with ethyl acetate (55% yield, light yellow oil). TLC: hexane/ethyl acetate = 80:20. R_f = 0.50.

^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.70 (d, *J* = 8.1 Hz, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 7.04-6.90 (m, 4H), 5.78 (s, 1H), 2.12 (s, 3H), 2.08 (s, 3H).

General procedure of enzymatic trans-benzoin condensation of benzoin and benzyloxyacetaldehyde

To a mixture of *rac*-benzoin derivative (0.25 mmol) and benzyloxyacetaldehyde (0.25 mmol) in 2.5 mL DMSO (25 vol%) 7.5 mL (75 vol%) MOPS buffer (50 mM, pH 7) that contains 0.15 mM ThDP and 2.5 mM $MgSO_4$ was transferred. The reaction was initiated with the addition of BAL (0.2 U) at 37 °C (120 rpm). Every 24 hours 0.2 U of BAL was added. The reaction was controlled with TLC and concluded after 72 h. The reaction mixture was extracted with diethyl ether (8 x 40 mL) and the combined organic layers were washed with brine and dried over $MgSO_4$, and the solvent was concentrated *in vacuo* to yield the product which was further purified by using flash column chromatography.

Synthesis of (R)-3-(benzyloxy)-2-hydroxy-1-(3-methoxyphenyl)-propan-1-one (8)

General procedure described above starting from *m*-anisoin and benzyloxyacetaldehyde, provided the pure product obtained as white oil (43% yield). TLC: hexane/ethyl acetate = 10:1. R_f = 0.28.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40-7.35 (m, 2H), 7.32-7.26 (m, 1H), 7.18-7.12 (m, 3H), 7.10-7.02 (m, 3H), 5.10 (br s, 1H), 4.44 (d, *J* = 12.3 Hz, 1H),), 4.36 (d, *J* = 12.3 Hz, 1H), 3.90 (br s, 1H, OH), 3.78 (s, 3H), 3.74 (dd, *J* = 10.3, 3.8 Hz, 1H), 3.68 (dd, *J* = 10.3, 4.4 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 199.5, 159.9, 137.6, 135.3, 129.8, 128.3, 127.6, 127.5, 121.0, 120.5, 112.8, 73.9, 73.4, 72.6, 55.5.

IR: 3444, 2959, 2922, 2859, 1684, 1596, 1581, 1452, 1258, 1094, 1014 cm^{-1}

$[\alpha]_D^{22}$= +3.22° (*c* = 0.026 g/mL, $CHCl_3$).

HRMS $C_{17}H_{18}O_4$ ($M+Na^+$): Calcd. 309.1103, found 309.1104.

Synthesis of (R)-3-(benzyloxy)-2-hydroxy-1-(p-tolyl)-propan-1-one (9)

General procedure described above starting from 4,4'-dimethylbenzoin and benzyloxyacetaldehyde, provided the pure product obtained as yellow oil (44% yield). TLC: hexane/ethyl acetate = 10:1. R_f = 0.21.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84-7.70 (m, 2H), 7.23-7.14 (m, 5H), 7.07-7.04 (m, 2H), 5.16-5.10 (m, 1H), 4.44 (d, *J* = 12.3 Hz, 1H), 4.38 (d, *J* = 12.3 Hz, 1H), 3.94 (d, *J*= 6.6 Hz, 1H, OH), 3.74 (dd, *J* = 10.3, 3.1 Hz, 1H), 3.66 (dd, *J* = 10.3, 4.5 Hz, 1H), 2.36 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.0, 144.0, 136.6, 132.9, 130.4, 128.5, 127.7, 127.3, 126.5, 72.6, 72.4, 71.8, 20.7.

IR: 3457, 2957, 2919, 2855, 1680, 1606, 1452, 1258, 1093, 1017 cm^{-1}.

$[\alpha]_D^{22}$= +6.80° (*c* = 1.7 x 10^{-3} g/mL, $CHCl_3$).

HRMS $C_{17}H_{18}O_3$ ($M+Na^+$): Calcd. 293.1154, found 293.1158.

Synthesis of (R)-3-(benzyloxy)-2-hydroxy-1-phenylpropan-1-one (10)

General procedure described above starting from commercially available *rac*-benzoin and benzyloxyacetaldehyde, provided the pure product obtained as white powder (24% yield). TLC: hexane/ethyl acetate = 50:10. R_f = 0.67.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.92-7.88 (d, *J*= 7.1 Hz, 2H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 2H), 7.25-7.20 (m, 3H), 7.15-7.09 (m, 2H), 5.24-5.18 (m, 1H), 4.52 (d, *J* = 12.3 Hz, 1H), 4.44 (d, *J* = 12.3 Hz, 1H), 3.96 (d, *J* = 6.5 Hz, 1H, OH), 3.82 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.75 (dd, *J* = 10.3, 4.4 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 199.5, 137.6, 134.0, 133.9, 128.8, 128.6, 128.3,

127.6, 127.5, 73.8, 73.4, 72.5.

IR: 3445, 3017, 2926, 1685, 1618, 1559, 1508, 1496, 1214, 1099 cm^{-1}.

$[\alpha]_D^{22}$= -0.0264° (*c* = 1.0 x 10^{-4} g/mL, $CHCl_3$).

HRMS $C_{16}H_{16}O_3$ ($M+Na^+$): Calcd. 279.0997, found 279.0999.

Synthesis of (R)-3-(benzyloxy)-2-hydroxy-1-(4-methoxyphenyl)-propan-1-one (11)

General procedure described above starting from commercially available *p*-anisoin and benzyloxyacetaldehyde, provided the pure product obtained as white oil (43% yield). TLC: hexane/ethyl acetate = 10:1. R_f = 0.24.

1H NMR (400 MHz, $CDCl_3$) δ: 7.83 (d, *J* = 8.9 Hz, 2H), 7.20-7.15 (m, 3H), 7.09 (dd, *J* = 8.3, 5.9 Hz, 2H), 6.89-6.83 (d, *J*= 8.9 Hz, 2H), 5.10 (br s, 1H), 4.45 (d, *J* = 12.3 Hz, 1H), 4.39 (d, *J* = 12.3 Hz, 1H), 3.94 (br s, 1H, OH), 3.81 (s, 3H), 3.73 (dd, *J*= 9.6, 3.4 Hz, 1H), 3.65 (dd, *J*= 9.6, 4.7 Hz, 1H).

13C NMR (100 MHz, $CDCl_3$) δ: 197.7, 164.2, 137.7, 131.0, 129.0, 128.3, 127.5, 126.8, 114.0, 73.4, 73.3, 73.0, 55.6.

IR: 3444, 2923, 2856, 1671, 1598, 1572, 1510, 1454, 1256, 1172, 1101, 1025 cm^{-1}.

$[\alpha]_D^{22}$= +19.26° (*c* = 0.0185 g/mL, $CHCl_3$).

HRMS $C_{17}H_{18}O_4$ ($M+Na^+$): Calcd. 309.1103, found 309.1106.

HPLC conditions of chiral α-hydroxy ketones

(*R*)-3-(benzyloxy)-2-hydroxy-1-(3-methoxyphenyl)-propan-1-one (**8**)

Enantiomerically enriched compound **8** was obtained in 36% *ee*. The enantiomeric excess was verified by using chiral HPLC analysis (AD-H Column, hexane:*i*-PrOH / 90:10, flow rate 0.75 mL/min, λ= 254 nm), tR= 21.36 min (minor enantiomer), tR= 26.39 min (major enantiomer).

(*R*)-3-(benzyloxy)-2-hydroxy-1-(*p*-tolyl)-propan-1-one (9)

Enantiomerically enriched compound **9** was obtained in 62% *ee*. The enantiomeric excess was verified by using chiral HPLC analysis (AD-H Column, hexane:*i*-PrOH / 95:5, flow rate 0.75 mL/min, λ= 254 nm), tR= 31.75 min (minor enantiomer), tR= 33.46 min (major enantiomer).

(*R*)-3-(benzyloxy)-2-hydroxy-1-phenylpropan-1-one (10)

Enantiomerically enriched compound **10** was obtained in 36% *ee*. The enantiomeric excess was verified by using chiral HPLC analysis (AD-H Column, hexane:*i*-PrOH / 90:10, flow rate 0.75 mL/min, λ= 254 nm) tR= 19.49 min (minor enantiomer), tR= 22.75 min (major enantiomer).

(*R*)-3-(benzyloxy)-2-hydroxy-1-(4-methoxyphenyl)-propan-1-one (11)

Enantiomerically enriched compound **11** was obtained in 63% *ee*. The enantiomeric excess was verified by using chiral HPLC analysis (AD-H Column, hexane:*i*-PrOH / 90:10, flow rate 0.75 mL/min, λ= 254 nm), tR= 33.87 min (minor enantiomer), tR= 35.76 min (major enantiomer).

General procedure for racemic cross benzoin condensation of benzaldehyde derivative and benzyloxyacetaldehyde with thiazolium catalyst (8)

Firstly, to the derivatives of benzaldehyde (0.3 mmol) and benzyloxyacetaldehyde (0.5 mmol), dry THF (1 mL) and 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium bromide was added as a catalyst (0.05 mmol); subsequently Cs_2CO_3 (0.05 mmol) was transferred into the reaction medium. After 20 hours, the reaction mixture was extracted with EtOAc (10 mL x 3), dried over Na_2SO_4 and solvent was removed under reduced pressure. The residue was afforded after flash column chromatography (EtOAc/Hexane =1:10) as an oil. This procedure was adapted from the literature (8).

Synthesis of racemic-3-(benzyloxy)-2-hydroxy-1-(3-bromophenyl)-propan-1-one (rac-12)

General procedure described above starting from 3-bromobenzaldehyde and benzyloxyacetaldehyde, provided the pure product obtained as yellow powder (21% yield). TLC hexane/ethyl acetate = 90:10. R_f = 0.67.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18 (t, *J* = 1.7 Hz, 1H), 7.98-7.96 (m, 2H), 7.74- 7.70 (m, 1H), 7.32-7.28 (m, 1H), 7.19-7.16 (m, 4H), 5.07 (t, *J* = 3.8 Hz, 1H), 4.44 (d, *J* = 12.3 Hz, 1H), 4.35 (d, *J* = 12.3 Hz, 1H), 3.71 (dd, *J* = 10.3, 3.6 Hz, 1H), 3.69 (dd, *J* = 10.3, 4.1 Hz, 1H).

Synthesis of racemic-3-(benzyloxy)-2-hydroxy-1-(m-tolyl)-propan-1-one (rac-13)

General procedure described above starting from *m*-tolualdehyde and benzyloxyacetaldehyde, provided the pure product obtained as white oil (23% yield). TLC hexane/ethyl acetate = 90:10. R_f = 0.73.

H NMR (400 MHz, $CDCl_3$) δ: 7.68-7.59 (m, 3H), 7.35 (d, *J* = 7.6 Hz, 2H), 7.16 (m, 2H), 7.06 (dd, J = 6.8, 2.5 Hz, 2H), 5.13 (t, *J* = 3.8 Hz, 1H), 4.44 (d, *J* = 12.3 Hz, 1H), 4.37 (d, *J* = 12.3 Hz, 1H), 3.71 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.67 (dd, *J* = 10.3, 4.4 Hz, 1H), 2.33 (s, 3H).

Synthesis of racemic 3-(benzyloxy)-2-hydroxy-1-(3-fluorophenyl)-propan-1-one (rac-14)

The general procedure described above, starting from 3-fluorobenzaldehyde and benzyloxyacetaldehyde, provided the pure product obtained as a white powder (36% yield). TLC: hexane/ethyl acetate = 90:10. R_f = 0.63.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.83 (d, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.37 (tt, *J* = 7.9, 5.5 Hz, 2H), 7.27-7.19 (m, 3H), 7.06 (dd, *J* = 7.9, 5.1 Hz, 2H), 5.08 (t, *J* = 3.7 Hz, 1H), 4.45 (d, *J* = 12.3 Hz, 1H), 4.36 (d, *J* = 12.3 Hz, 1H), 3.73 (dd, *J* = 9.8, 3.5 Hz, 1H), 3.69 (dd, *J* = 10.3, 4.2 Hz, 1H).

RESULTS AND DISCUSSION

BAL was expressed in *E. coli* based on the procedures described before. Crude lysate was used without purification as catalyst after determination of its activity in benzoin condensation reactions based on literature. One unit activity (U) of BAL is defined as the amount of the enzyme that is used for the formation of 1 μmol of benzoin in pH 7 buffer containing 50 mM phosphate, 2.5 mM $MgSO_4$, 0.15 mM ThDP and DMSO (20%, v/v) in 1 minute at 30 °C.

For this measurement, a set of reactions with the same concentration of benzaldehyde was treated with BAL and every 10 minutes, a small sample was taken from the reaction mixture. The amount of benzoin that was formed during the reaction was determined by HPLC based on the calibration curve that was drawn by using commercial benzoin samples. The activity of BAL that was used in this work found to be 0.2 U.

For the synthesis of racemic benzoin derivatives, standard benzoin condensation reactions were used by using NaCN as catalyst and water/ethanol mixture as solvent. In Figure 1, the synthesized *rac*-benzoin derivatives are given with their yields. Racemic benzoin and 4,4'-dimethoxybenzoin were commercially available and obtained from Sigma-Aldrich.

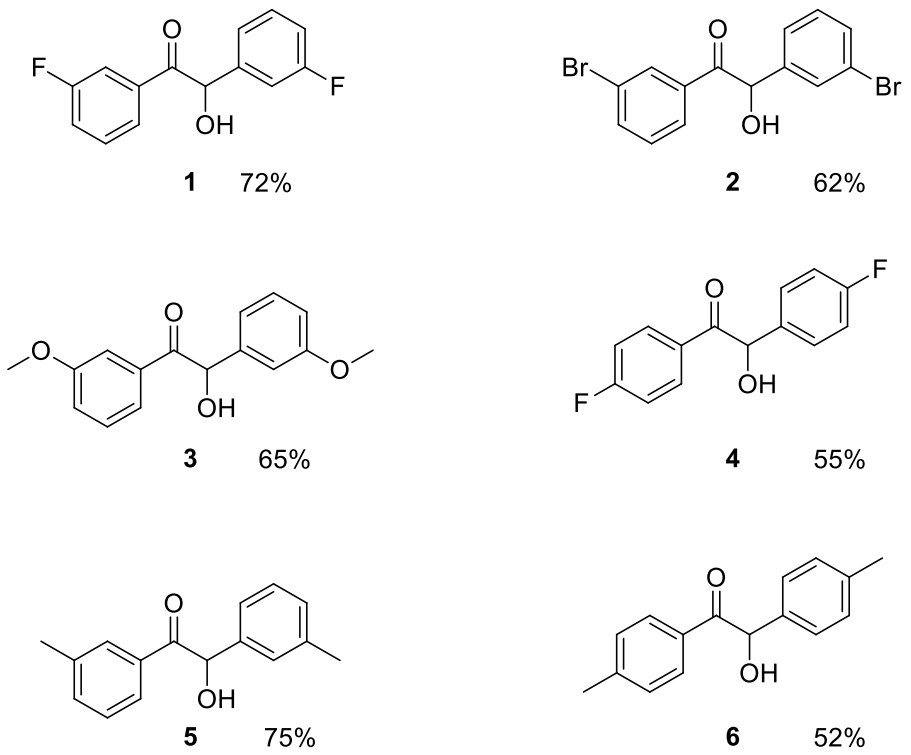
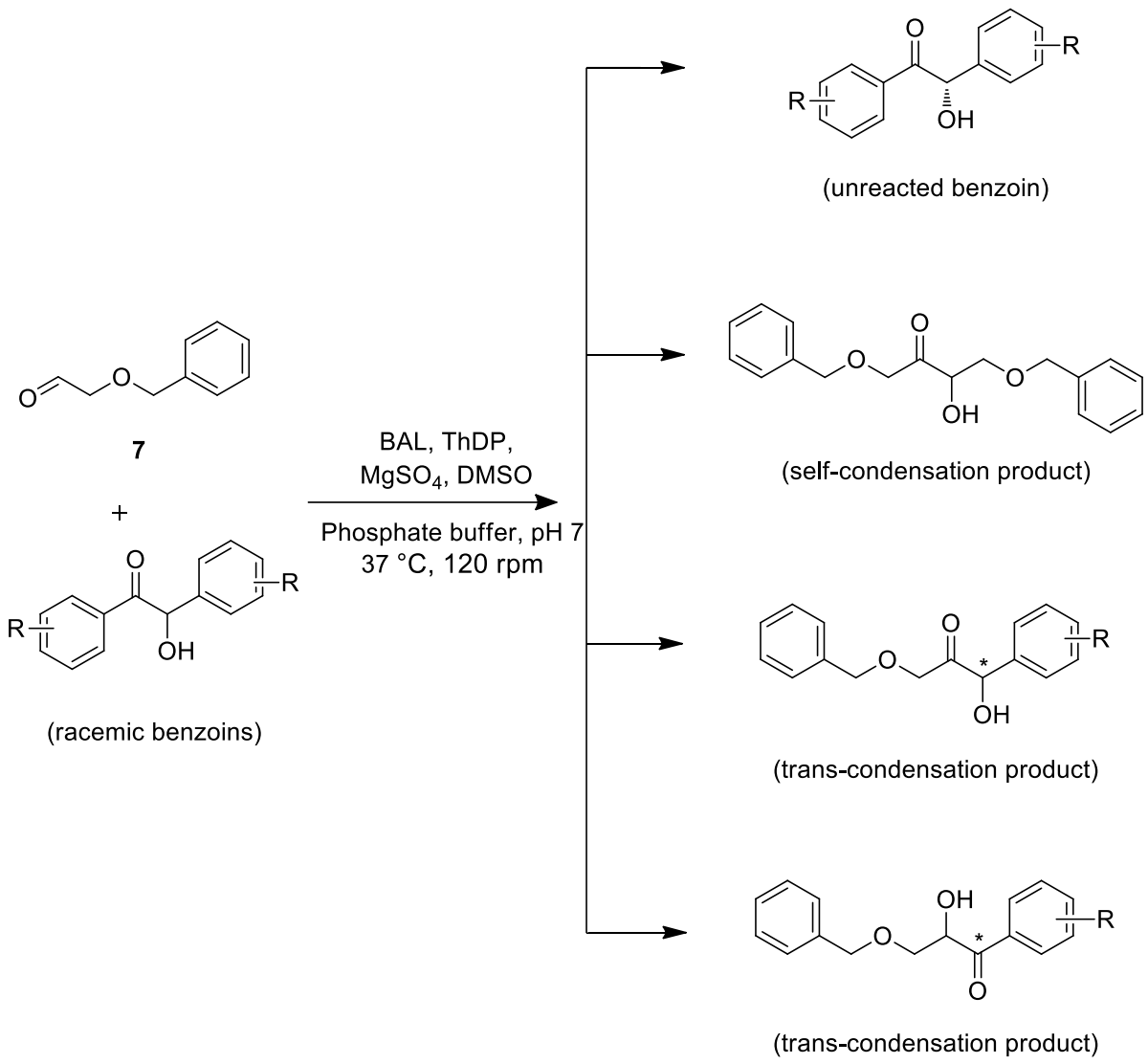


Figure 1. The structure and the yield of the *rac*-benzoin derivatives that were used in trans-benzoin condensation reactions.

These six synthesized and two commercially available *rac*-benzoin derivatives were used in the enzymatic trans-benzoin condensation reactions. For this purpose, each *rac*-benzoin derivative and benzyloxyacetaldehyde (**–**) were dissolved in DMSO and then added to pH 7 buffer containing MOPS (50 mM), ThDP and $MgSO_4$. After the temperature of the reaction mixture was set to 37 °C, BAL was added (0.2 U) and it was shaken with a constant speed at 120 rpm. The reaction was monitored by TLC, and BAL was added daily. After 72 hours, the reaction was stopped and the aqueous phase was extracted by diethyl ether three times. Then, the combined etheric phases were washed with brine and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the crude product was purified by flash column chromatography using ethyl acetate-hexane solvent mixture as eluent.



Scheme 3. All possible prodeucts of enzymatic-trans benzoin condensation reaction.

From the trans-benzoin condensation reaction of *rac*-banzoin and benzyloxyacetaldeyde, theoretically four different products can be formed. One of them is the unreacted (*S*)-benzoin derivative. Two of them are trans-benzoin products where benzyloxyacetaldeyde acts as a donor and acceptor. Another of them is the self-benzoin condensation of benzyloxyacetaldeyde. However, practically only (*S*)-benzoin and one trans-benzoin products, where benzyloxyacetaldehyde acted as an acceptor, was observed (Scheme 3).

Among eight different rac-benzoin derivatives, four of them were able to be purified, characterized, and their enantiomeric excess were determined by chiral HPLC (9). 4,4'-dimethoxybenzoin and 4,4'-dimethylbenzoin gave the corresponding trans-benzoin products with the highest enantiomeric excesses (63 and 62% ee, respectively). Benzoin and 3,3'-dimethoxybenzoin resulted trans-benzoin products with relatively low enantiomeric excesses (35 and 36% ee, respectively) (Figure 2).

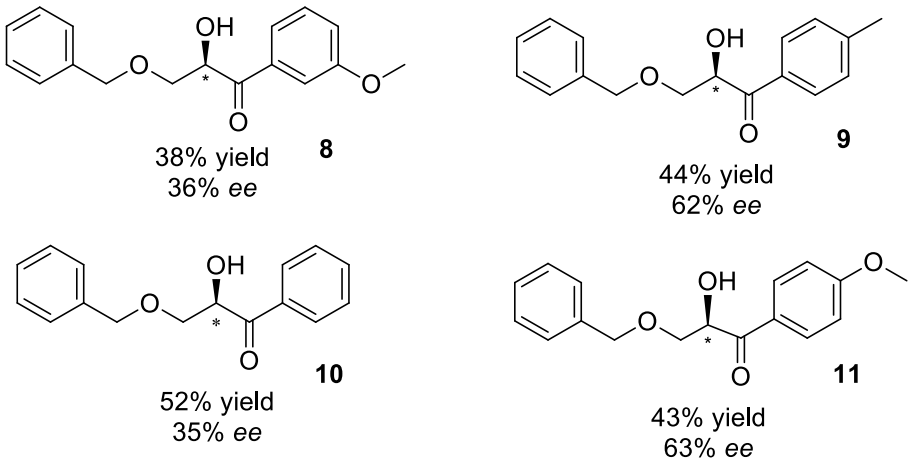


Figure 2. Yield and enantiomeric excess of purified enzymatic trans-benzoin reaction products.

The trans-benzoin products that were formed from 3,3'-difluoro-, 3,3'-dibromo- and 3,3'-dimethylbenzoin derivatives were detected by ^{1}H NMR spectroscopy in their crude mixture. However, they could not be separated from other benzoin products due to structural similarities. Enantiomeric excesses of these products were able to be determined by chiral HPLC (9) by comparing the retention time of racemic benzoin derivatives (***rac*-12, *rac*-13, *rac*-14**) obtained from cross-benzoin condensation reaction of corresponding aldehyde derivatives and benzyloxyacetaldehyde. 4,4'-difluorobenzoin derivative gave a complex mixture of products that were not possible to determine enantiomeric excess of the trans-benzoin product (Figure 3).

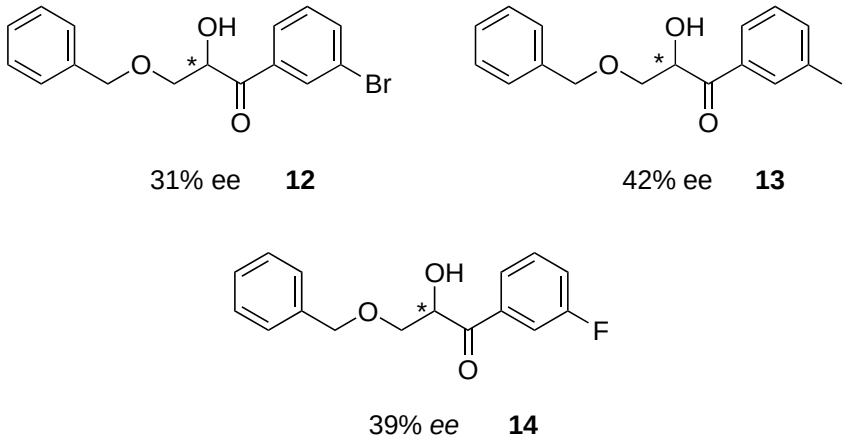


Figure 3. Enantiomeric excesses of crude enzymatic trans-benzoin reaction products.

In conclusion, various racemic benzoin derivatives were reacted with benzyloxyacetaldehyde with BAL as a catalyst to obtain enantiomerically enriched trans-benzoin products. These products are important precursors in the synthesis of chiral polyalcohols. After removal of benzyl

protection and reduction of carbonyl group, they can be converted into corresponding 1,2,3-triols.

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9. See supporting information for the HPLC chromatograms used to determine enantiomeric excesses.