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COMMUNICATION

Benzaldehyde lyase catalyzed enantioselective self and cross condensation reactions of acetaldehyde derivatives†

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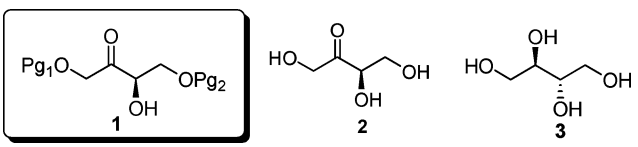
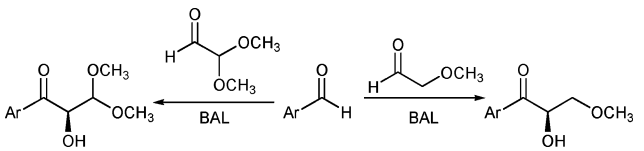
Flexible protected 1,3,4-trihydroxy-2-butanone is synthesized in high enantiomeric excesses by using asymmetric homo- and cross- acyloin coupling of aliphatic aldehydes catalyzed by benzaldehyde lyase.

Synthetically useful asymmetric cross acyloin reactions with functionalized aliphatic aldehydes, such as **1**, have a broad range of application, for which no general and efficient enantioselective system is currently available. The α,α′-dihydroxy ketone subunits, as shown in **2**, can be found in many biologically important compounds and are used for the synthesis of a wide range of compounds, such as ketosugars and aminodiols.[1] 1,3,4-Trihydroxy-2-butanone (erythrulose) (**2**) (Scheme 1) is one of the trioses and tetroses that are widely used as a self-tanning substance in cosmetic and dermatological formulations.[2] Erythritols (**3**) are used as sweeteners, and its mannosyl derivatives form a promising class of biosurfactants.[3]



Scheme 1 Polyhydroxy compounds.

Lyases are an important class of enzymes that achieve several important reactions, including C–C bond formations.[4] The thiamine diphosphate dependent enzyme, benzaldehyde lyase (BAL, EC 4.1.2.38),[5] has been shown to catalyze a broad range of C–C bond formation reactions.[6] BAL has been used extensively to achieve aromatic-aromatic,[7] aromatic-aliphatic,[7–9] and non-functionalized aliphatic-aliphatic acyloin derivatives.[10] Recently, BAL has been used in asymmetric carboligation reactions using α,β-unsaturated aldehydes.[11] BAL is believed to need aromatic aldehydes as donors while acceptors can be aromatic and aliphatic aldehydes.[12] For the first time, we published the aromatic-aliphatic cross coupling reaction with functionalized acetaldehyde derivatives as shown in Scheme 2.[8] Recently, this enzyme has been used



Scheme 2 BAL catalyzed cross coupling reactions of aromatic aldehydes with mono- & dimethoxy acetaldehydes.

in the asymmetric condensation reactions of aliphatic aldehydes in low enantioselectivities. However, the products that were formed lacked functionality, as the groups on the molecule cannot be easily converted to other functionalities, and aliphatic aldehydes were only used in self condensation reactions.[10]

Up to now, only phosphate buffer has been used for BAL mediated reactions, and the improvement of the enantioselectivity of the targeted reaction has been attempted by using increased amounts of enzyme. However, the screening of reaction parameters such as the solvent and buffer for the improvement of the desired activity is an important strategy.

By searching for the ability of BAL for the cross aliphatic acyloin condensation study, BAL was used for the self and cross condensation reactions of functionalized acetaldehyde where aliphatic aldehydes were used as donors. A new two phase system was developed for efficient aliphatic acyloin reactions.

The self-condensation of benzyloxyacetaldehyde was firstly achieved with the classical methodology where the substrate was dissolved in potassium phosphate buffer (pH 7, containing $MgSO_4$ and ThDP) containing 20% DMSO. By the addition of BAL (50 U), the reaction was started at 37 °C. The same amount of enzyme was added on a daily basis. After 4 days, the reaction was terminated. The ee of the self-condensation product of benzyloxyacetaldehyde was 50%. Various solvents, such as dimethyl formamide (DMF), toluene, ether, benzene, acetonitrile, and tetrahydrofuran (THF) were tested to increase the selectivity. With acetonitrile and THF, product formation could not be achieved. It was found that when toluene was used with phosphate buffer (two-phase reaction), the condensation product was obtained with 61% ee and 54% yield. A further improvement was achieved with employing diisopropyl ether where a 75% ee and 71% yield were attained.

The reaction was finally optimized with the selection of the proper buffer. The buffer type has also been shown to be effective on both enantioselectivity and the yield of the reaction, as seen in Table 1. Several buffers (Good buffers[12] such as MES, MOPS,

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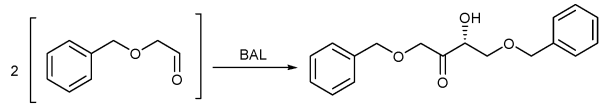
Table 1 Effect of the buffer type on enantiomeric excess and conversion

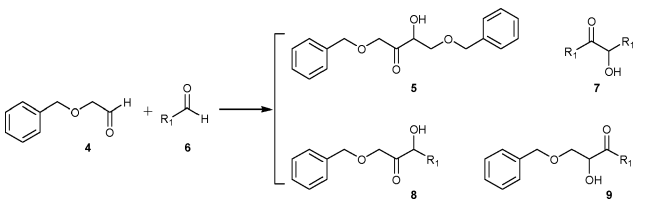


Buffer[a]	% Conversion	% ee[b]
Phosphate[a]	71	75
MES[b]	88	93
MOPS[a]	91	95
HEPES[c]	85	87
Tris[a]	73	91

[a] Self condensation reactions were performed at specific pH values according to the buffer type; (a) pH 7, (b) pH 6.5, (c) pH 8 and diisopropyl ether was used as cosolvent. [b] The ee value was determined with a Chiralpak® OA column, 80 : 20/hexane : isopropanol, 0.8 mL min^{-1}, 220 nm, R_t: 12.036 min for (*S*), 13.934 min for (*R*).

HEPES, and other previously used buffers such as phosphate and tris) were screened for increasing the enantioselectivity and yield. Good buffers were employed at varying pH values to observe the effect of pH together with the buffer type. However, the pH was found to have a negligible effect on the enantioselectivity and yield. Although MES buffer was previously shown to be inappropriate for BAL[13] with the findings of lower residual activity, and *via* regular enzyme addition, the lower residual activity limitation could be overcome.

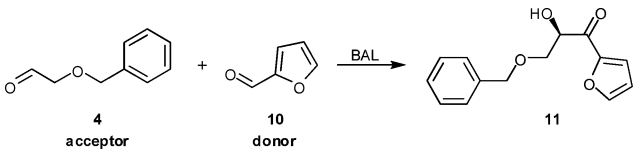
After the optimization of the reaction conditions, cross condensation reactions of benzyloxyacetaldehyde with 2-furan-2-carbaldehyde and dimethoxyacetaldehyde were performed. When two different aldehydes are reacted under the catalysis of BAL in a cross condensation manner, four products can be expected (Scheme 3); two self-condensation (**5** & **7**) and two cross condensation products (**8** & **9**).



Scheme 3 Carboligation of benzyloxyacetaldehyde, **4** with different aldehydes.

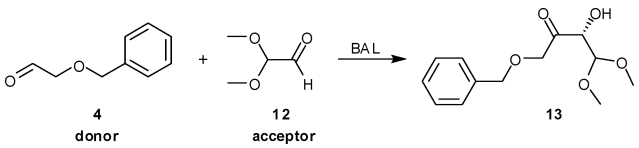
When benzyloxyacetaldehyde is reacted with furan-2-carbaldehyde under BAL catalysis at a 1 : 1 ratio in DIPE (diisopropyl ether)-MOPS in a two phase system, only one cross condensation product [3-(benzyloxy)-1-(furan-2-yl)-2-hydroxypropan-1-one, **11**] was obtained with 90% ee, Scheme 4. Furan-2-carbaldehyde acted as donor while benzyoxyacetaldehyde was used by the enzyme as an acceptor. Self-condensation products (**5** & **7**) were only detectable when one of the substrates was used in excess amounts.

The preference for acceptor and donor changed when benzyloxyacetaldehyde was reacted with dimethoxyacetaldehyde, Scheme 5. This substrate acted as a donor in the reaction with dimethoxyacetaldehyde while it was not accepted as a donor when furan-2-carbaldehyde was employed as a cosubstrate. Similar to



Scheme 4 BAL mediated cross condensation of **4** with **10**.

the previous cross condensation reaction, only one cross condensation product [1-(benzyloxy)-3-hydroxy-4,4-dimethoxybutan-2-one, **13**] was observed in the reaction medium out of four theoretical products in 93% ee (Scheme 3). The self-condensation products (**5** & **7**) were again only detectable when one of the substrates was used in excess amounts. In all cases, (*R*)-configured products are obtained.



Scheme 5 BAL mediated cross condensation of **4** with **12**.

The changing of the reaction conditions and co-solvents change the substrate spectrum and selectivity. It seems that the aromatic aldehydes are the preferred substrate as donor aldehyde when they are present. It has been shown that aliphatic aldehydes are both suitable donors and acceptors. In this case, the active side differs regarding the aliphatic aldehydes whether to be a donor or acceptor. This interaction could be the summary of the steric and electronic interaction. The orientation of the carbonyl moiety should be the same due to the results of the absolute configuration of the product. These results also show that the substrate spectra of these C–C-ligating enzymes is broader than described in the literature so far, so that further interesting substrates could be expected to be applied in this attractive carboligating reaction. More studies are essential for the explanation of the mechanism.

With this novel system, it is possible to synthesize **1** with different protecting groups, which also makes it possible to have both enantiomers of the products in one reaction after further modifications.

Experimental

General

NMR spectra were recorded with Brucker-Spectrospin DPX-400, Ultra Shield, High Performance Digital FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard and deuterated chloroform ($CDCl_3$) as a solvent. Mass spectra were recorded with Thermo Quest GC-MS equipped with a Phenomenex Zebron capillary GC column (60 m length, 0.25 mm ID, 0.25 μm film thickness). Flash column chromatography was performed by using Merck Silica Gel 60 (particle size: 40–63 μm, 230–400 mesh ASTM). Enantiomeric excess values were determined by an Agilent 1100 series HPLC device using a Chiralpak OA column, Chiralpak OD column and Chiralpak OJ column.

The *E.coli* BL21(DE3)pLysS strain purchased from Invitrogen® was used as a host to produce the recombinant enzyme (BAL_{HIS}). Enzyme production was performed in the

New Brunswick BioFlo110 Fermentor, equipped with pH and temperature probes as well stirring rate controls. The purification of the hexa-histidine tagged enzyme was performed with an Ni^{2+}-NTA affinity column (Invitrogen®).

Preparation of benzaldehyde lyase

E.coli BL21(DE3)pLysS carrying pUC19-BAL_{HIS} construct was used for BAL (EC. 4.1.2.38) production. The cultures were maintained on LB agar slants. The cells from the newly prepared slants were inoculated into the preculture Luria broth (LB) where it was grown for 8 h at 37 °C, and then transferred to the production (LB) medium with an inoculation ratio of 1/10 (1.65 L in a 2 L fermentor). 6 h after the induction with isopropyl-β-D-thiogalactopyranoside (IPTG), cells were harvested by centrifugation. The enzyme was either used as a crude form without purification (the pelleted cells were transferred to a Petri dish and lyophilized for 36 h) or as a purified enzyme.

One unit (U) of BAL activity is defined as the amount of enzyme that catalyzes the cleavage of 1 μmol benzoin in potassium phosphate buffer (50 mM, pH 7) containing $MgSO_4$ (2.5 mmol L^{-1}), ThDP (0.15 mmol L^{-1}), and DMSO (20 vol-%) at 30 °C per minute at 30 °C.

General procedure for the BAL catalyzed self condensation of benzyloxyacetaldehyde

Benzyloxyacetaldehyde **4** (150 mg, 1 mmol) was dissolved in 10 mL diisopropylether (25 vol-%), and then 30 mL (75 vol-%) MOPS buffer (50mM, pH 7) containing 0.15 mM THDP and 2.5 mM $MgSO_4$ was added to this solution. The reaction was started with the addition of BAL (50 U) at 30 °C (120 rpm). BAL was added (50 U) on a daily basis. The reaction was monitored with TLC and GC-MS and concluded after 96 h. The reaction mixture was extracted with chloroform (3 × 50 mL) and the combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified with flash column chromatography.

General procedure for BAL catalyzed cross condensation reactions of benzyloxyacetaldehyde

Benzyloxyacetaldehyde **4** (150 mg, 1 mmol) and the corresponding aldehyde (1 mmol) were dissolved in 10 mL diisopropylether (25 vol-%) then 30 mL (75 vol-%) MOPS buffer (50mM, pH 7) containing 0.15 mM THDP and 2.5 mM $MgSO_4$ was added to this solution. The reaction was started with the addition of BAL (50 U) at 30 °C (120 rpm). BAL was added (50 U) on a daily basis. The reaction was monitored with TLC and GC-MS and concluded after 96 h. The reaction mixture was extracted with chloroform (3 × 50 mL) and the combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified with flash column chromatography.

(*R*)-1,4-Bis(benzyloxy)-3-hydroxybutan-2-one (5)

Yellowish oil, $[\alpha]_D^{25}$: −1.3 (c 0.9, CH_2Cl_2) 95% ee, Chiralpak® OA column, 80 : 20/hexane: isopropanol, 0.8 mL min^{-1}, 220 nm, R_t: 12.036 min for (*S*), 13.934 min for (*R*).^{1}H-NMR (400 MHz, 2.5/1: $CDCl_3/CCl_4$): δ = 3.35 (s, 1H, OH), 3.72 (dd, *J* = 3.7, 10.1 Hz, 1H), 3.8 (dd, *J* = 3.7, 10.1 Hz, 1H), 4.21 (d, *J* = 17.2 Hz, 1H), 4.28 (d, *J* = 17.2 Hz, 1H), 4.58–4.30 (m, 5H), 7.37–7.09 (m, 10H). ^{13}C-NMR (100 MHz, 2.5/1: $CDCl_3/CCl_4$): δ ppm 70.86, 73.21, 73.53, 73.63, 75.07, 127.80, 127.93, 128.12, 128.45, 128.54, 132.75, 134.12, 204.90. HRMS for $C_{18}H_{20}NaO_4$ (M + Na^+): calcd. 323.1252, found: 323.1263.

(*R*)-3-(Benzyloxy)-1-(furan-2-yl)-2-hydroxypropan-1-one (11)

Yellow oil, $[\alpha]_D^{25}$: +70.3 (c 0.4, $CHCl_3$)90% ee, Chiralpak® OD column, 98 : 2/hexane: isopropanol, 1 mL min^{-1}, 254 nm, R_t: 68.688 min for (*S*), 75.499 min (*R*). ^{1}H NMR (400 MHz, 2.5/1: $CDCl_3/CCl_4$): 3.72 (dd, *J* = 3.26, 10.21 Hz, 1H), 3.81 (dd, *J* = 3.26, 10.21 Hz, 1H), 4.38 (d, *J* = 12.3 Hz, 1H), 4.48 (d, *J* = 12.3 Hz, 1H), 4.82 (t, *J* = 3.57 Hz, 1H), 6.47 (m, 1H), 7.06–7.09 (m, 2H), 7.11–7.19 (m, 3H), 7.24 (d, 1H), 7.47 (m, 1H). ^{13}C NMR (100 MHz, 2.5/1: $CDCl_3/CCl_4$): 72.26, 75.92, 95.07, 111.36, 117.70, 126, 127, 128, 137, 145.52, 145.59, 149.59, 186.32. HRMS for $C_{14}H_{14}NaO_4$ (M + Na^+): calcd. 269.0790, found: 269.0784.

(*R*)-1-(Benzyloxy)-3-hydroxy-4,4-dimethoxybutan-2-one (13)

Yellow oil, $[\alpha]_D^{25}$: −14.5 (c 0.4, $CHCl_3$). 93% ee, Chiralpak® OJ column, 90 : 10/hexane: isopropanol, 0,8 mL min^{-1}, 220 nm, R_t: 18.833 min for (*S*), 65.387 min (*R*). ^{1}H-NMR (400 MHz, 2.5/1: $CDCl_3/CCl_4$): δ = 3.37 (s, 6H), 4.18 (d, *J* = 18.2 Hz, 2H), 4.35 (m, 2H), 4.49 (dd, *J* = 11.8 Hz, 1H), 4.49 (dd, *J* = 11.8 Hz, 1H), 7.22–7.26 (m, 5H). ^{13}C-NMR (100 MHz, 2.5/1: $CDCl_3/CCl_4$): δ ppm 54.69, 55.971, 72.482, 72.561, 104.397, 127.009, 127.281, 127.465, 136.201, 205.274. HRMS for $C_{13}H_{18}NaO_5$ (M + Na^+): calcd. 277.1052, found: 277.1045.

Conclusions

BAL catalyzed the cross acyloin reactions with functionalized acetaldehydes with the same and different protecting groups that were achieved in high yields and enantioselectivities. All of the products obtained in this study can be employed as chiral synthons, as they are amenable to further modifications.

Acknowledgements

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