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Enzyme catalyzed hydroxymethylation of aromatic aldehydes with formaldehyde. Synthesis of hydroxyacetophenones and (*S*)-benzoins

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Abstract—Benzaldehyde lyase from the *Pseudomonas Fluorescens* catalyzed reaction of aromatic aldehydes with formaldehyde providing 2-hydroxy-1-arylethan-1-one in high yields via an acyloin linkage. Kinetic resolution of *rac*-benzoins with formaldehyde providing (*S*)-benzoins and 2-hydroxy-1-arylethan-1-one via C–C bond cleavage and a bond formation reaction.

1. Introduction

Selective hydroxymethylation of aromatic aldehydes with formaldehyde leading to terminal hydroxymethyl functionality represents a potentially useful strategy for the one carbon extension of carbonyls in order to obtain hydroxy ketones. This method could have many advantages compared to other methods that lead to hydroxy ketones. Formaldehyde is a versatile reagent and is one of the most highly reactive C1 electrophiles in organic synthesis.[1] Dry gaseous formaldehyde that is required for many reactions has some disadvantages because it must be generated before use from solid polymer paraformaldehyde by way of thermal depolymerization, in which it easily self-polymerizes.[2] On the other hand, commercial formaldehyde solution, which is an aqueous solution containing 37% formaldehyde and 8–10% methanol is cheap, easy to handle, and stable even at room temperature. However, the use of this reagent is strongly restricted due to the existence of a large amount of water. For example, the titanium tetrachloride promoted hydroxymethylation reaction of silyl enol ethers was carried out using trioxane as a HCHO source under strict anhydrous conditions.[3] Aqueous formaldehyde solution could not be used because $TiCl_4$ and the silyl enol ether reacted with water rather than HCHO. Kobayashi et al. showed that Lanthanide triflates are able to function as Lewis acids in aqueous media and catalyze the aldol reaction of silyl enol ether with formaldehyde.[4]

Thiamine pyrophosphate (vitamin B_1) is a coenzyme which participates in a number of important biochemical reactions involving the formation and breaking of carbon–carbon bonds immediately adjacent to a carbonyl group (acyloins, α-diketones, α-keto acids).[5] Among the reactions catalyzed by thiazolium salts, the acyloin condensation, the intermolecular condensation of two molecules of aldehyde to produce an α-hydroxy ketone, is of much interest as a convenient method for carbon–carbon bond formation.[6] Inoue et al.[7] found that the condensation of formaldehyde (paraformaldehyde) with benzaldehyde and furfural catalyzed by 3-ethylbenzothiazolium bromide in the presence of triethylamine in dry ethanol or dioxane at 60 °C gave 2-hydroxy-1-phenyl ethanone and 2-hydroxy-1-(2-furyl)-ethanone in 17 and 6% yields, respectively.

2-Hydroxy-1-arylethan-1-ones are valuable synthetic intermediates for the preparation of a range of compounds of biological interest and pharmaceutical products such as the substituted 2-amino-1-arylethanols.[8] Benzaldehyde lyase (BAL, EC 4.1.2.38) from *Ps. fluorescens Biovar I* was first reported by Gonzáles and Vicuña.[9a,b] They showed that this strain can grow on benzoin as a sole carbon and energy source due to the ability of BAL to catalyze the cleavage of the acyloin linkage of benzoin yielding benzaldehyde. Furthermore, we have recently reported on benzaldehyde lyase (BAL), a novel thiamin diphosphate (ThDP) dependent enzyme from *Ps. Fluorescens Biovar I*, which is able to perform the enantioselective formation of (*R*)- and (*S*)-benzoins and (*R*)-2-hydroxypropiophenone ((*R*)-2-HPP) derivatives via C–C bond cleavage and C–C bond formation. (*R*)-2-HPP derivatives are formed in preparative scale by benzaldehyde lyase (BAL)-catalyzed C–C bond formation from aromatic aldehydes and acetaldehyde,

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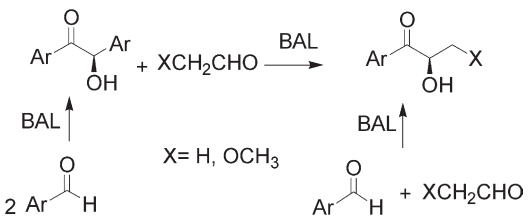


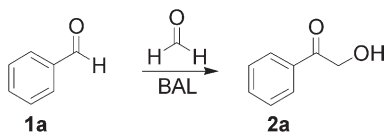
Scheme 1.

methoxy- and dimethoxyacetaldehyde in buffer/DMSO solution with remarkable ease in high chemical yields and high optical purity[9c–g] (Scheme 1).

We assumed that under the influence of a BAL catalyst, enzymatic coupling of aromatic aldehydes with formaldehyde would be a simple method for the synthesis of hydroxyacetophenones (HAP). We report herein, a new and efficient procedure for the direct hydroxymethylation of aromatic aldehydes with formaldehyde in one step, and kinetic resolution of *rac*-benzoins with formaldehyde to obtain (*S*)-benzoins and 2-hydroxy-1-arylethan-1-one via C–C bond cleavage and bond formation reaction in an aqueous medium. Carboligation with formaldehyde may be a new and efficient way to obtain important hydroxyacetophenone derivatives.

2. Results and discussion

As shown in Scheme 2, for the carboligation of aromatic aldehydes with formaldehyde, benzaldehyde (**1a**) was dissolved in a potassium phosphate buffer (pH 7.0, containing $MgSO_4$ and ThDP) containing 20% DMSO and formaldehyde solution. After the addition of BAL, the reaction was shaken and kept at 37 °C. The reaction was monitored by TLC and GC-MS using a commercially available authentic sample. After 3 days, no more change was observed and the purification of the crude product by column chromatography provided 2-hydroxy-1-phenylethan-1-one (**2a**) in a 94% yield (Table 1). We evaluated the influences of varying BAL and substrate concentrations, reaction time, and benzaldehyde substituents on the yield and range of products formed during reactions. Maximum yields were obtained with excess amounts of formaldehyde added at fixed time intervals. We observed that the benzaldehyde/formaldehyde ratio is very important for the product distribution, since excess formaldehyde resulted in high yield formation of **2a**, whereas a 1:1 ratio of benzaldehyde/formaldehyde gave mixture of (R)-benzoin and **2a**. Temperature had little influence on the reaction outcomes. A slight increase in yield was observed by passing nitrogen gas through the reaction solution at the onset of the reaction.



Scheme 2.

Table 1. Synthesis of 2-hydroxy-1-arylethan-1-one derivatives (Scheme 2)

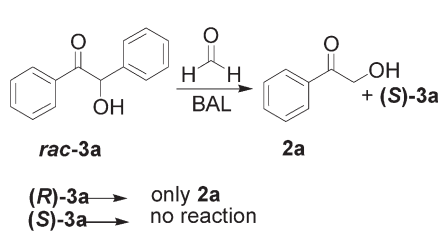
1	ArCHO	2	
		Yield (%)[a]	Mp (Lit. mp) °C
a	Ph	94	86–89[b]
b	4-MeC_6H_4	94	83–84 (83)[10]
c	4-MeOC_6H_4	91	105–106 (106–107)[10]
d	3-MeOC_6H_4	92	48–50 (50)[10]
e	2-MeOC_6H_4	68	81–83 (83)[10]
f	3-MeO-4-OHC_6H_3	51	177–179 (177–178)[11]
g	3-BrC_6H_4	83	104–106 (105–107)[c]
h	2-ClC_6H_4	77	Semisolid[8d]
i	4-ClC_6H_4	88	120–123 (122–123)[12]
j	4-OHC_6H_4	89	165–167 (165–167)[13]
k	2-furanyl	77	82–83 (84–86)[7]
l	4-pyridinyl	<5[d]	
m	3-pyridinyl	<5[d]	
n	2-FC_6H_4	<5[d]	
o	2,4-$F_2C_6H_3$	15	Semisolid (90–93)[14]
p	Indole-3-carbaldehyde	No reaction	

[a] Isolated yields (the yields are based on ArCHO). All compounds are known and all analytical data are in agreement with the previously reported data.
[b] Commercially available compound.
[c] Imperial chemical industries, US 4489074.
[d] Detected by GC-MS.

This carboligation reaction was carried out using the above described conditions with a wide range of aromatic aldehydes and heteroaromatic aldehydes, and the corresponding acyloin derivatives **2a**–**k** were obtained in high yields as summarized in Table 1. No acyloin formation was observed in the absence of the enzyme.

As shown in Table 1, BAL has the ability to bind a broad range of different aromatic and heteroaromatic aldehydes to C2-ThDP prior to ligation. The yield of the reaction depends on the structure of the aldehyde. Fluorine substitution on the 2- and 2,4-positions on the ring decreased the yield of the reaction. Pyridine carboxaldehyde also furnished a low yield. The steric and electronic demands of the substituent play a role in the yield of the reaction.

In our previous reports,[9c–g] we showed that BAL is also able to accept benzoin as a substrate to catalyze C–C bond cleavage followed by carboligation in the presence of acetaldehyde. Accordingly, (*R*)-benzoin was reacted with BAL in the presence of formaldehyde; the reaction was monitored by TLC and LC-MS (with the appropriate chiral column). Addition of formaldehyde provided 2-hydroxy-1-phenylethan-1-one (**2a**) in high yield (Scheme 3). As anticipated, the same reaction starting from (*S*)-benzoin failed. Repeating this reaction with *rac*-benzoin provided 2-hydroxy-1-phenylethan-1-one (**2a**) and (*S*)-benzoin ((*S*)-**3a**) (in an enantiomerically pure form) after the separation of the products by column chromatography. In order to obtain the full conversion of (*R*)-benzoin into HAP



Scheme 3.

Table 2. Synthesized (*S*)-benzoins and 2-HAP derivatives

rac. **3**		(*S*)-**3a–f**[9e]		**2** (Yield (%))
	Ar	Yield (%)[a]	ee (%)[b]	
a	Ph	41	>98[c]	**a** 40
b	4-ClC_6H_4	38	>98[d]	**i** 34
c	4-$MeOC_6H_4$	42	>98[e]	**c** 40
d	2-$MeOC_6H_4$	38	>98[f]	**e** 43
e	4-MeC_6H_4	39	96[g]	**b** 43
f	2-furanyl	37	93[h]	**k** 36

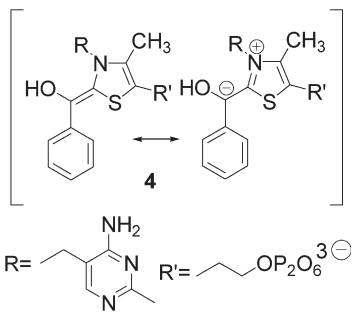
[a] Formaldehyde is used in excess amounts and yields are based on benzoin. All compounds are known and all analytical data are in agreement with the previously reported data.[9e]
[b] The ee value is measured immediately after work-up.
[c] $[\alpha]_D^{22}$=+112.1 (*c* 1.5, CH_3COCH_3), Chiralpak AD, UV detection at 254 nm, 90:10 hexane/2-propanol, flow 0.8 mL/min. R_t(*R*)=27.8 min; R_t(*S*)=35.1 min.
[d] $[\alpha]_D^{20}$=+26.2 (*c* 0.1, CH_3OH), Chiralpak AD, UV detection at 254 nm, eluent: *n*-hexane/2-propanol=90:10, flow 0.8 mL/min. R_t(*R*)=26.3 min.; R_t(*S*)=31.1 min.
[e] $[\alpha]_D^{20}$=+88.6 (*c* 1.0, CH_3OH), Chiralpak AD, UV detection at 254 nm, 75:25 hexane/2-propanol, flow 0.95 mL/min. R_t(*R*)=25.7 min.; R_t(*S*)=31.2 min.
[f] $[\alpha]_D^{20}$=+123.0 (*c* 1.0, $CHCl_3$), Chiralpak AD, UV detection at 254 nm, 98:2 hexane/2-propanol, flow 0.90 mL/min. R_t(*R*)=31.8 min.; R_t(*S*)=42.7 min.
[g] $[\alpha]_D^{20}$=+148.2 (*c* 0.8, CH_3OH), Chiralpak AD, UV detection at 254 nm, eluent: *n*-hexane/2-propanol=90:10, flow 0.8 mL/min. R_t(*R*)=30.4 min; R_t(*S*)=35.8 min.
[h] $[\alpha]_D^{20}$=+26.4 (*c* 0.2, CH_3OH), Chiralpak AD, UV detection at 254 nm, 90:10 hexane/2-propanol, flow 0.8 mL/min. R_t(*S*)=22.4 min; R_t(*R*)=28.3 min.

derivatives, formaldehyde has to be used in excess and should be added to the reaction mixture at fixed time intervals. Some representative examples of (*S*)-benzoins **3a–f** and 2-HAP derivatives are shown in Table 2.

Since structural information about the enzyme is still missing, a structure-based discussion of the observed stereocontrol is not yet possible. From mechanistic considerations it is more likely that the enamine-carbanion intermediate **4** is the active species in the BAL-catalyzed carboligation (Scheme 4). No reaction was observed with BAL by using 2-hydroxy-1-phenylethan-1-one with and without formaldehyde.

3. Conclusion

The method described herein presents the enzyme-catalyzed hydroxymethylation of aromatic aldehydes with formaldehyde via acyloin linkage in high yield. In addition, starting from *rac*-benzoins and formaldehyde 2-hydroxy-1-arylethan-1-one and the corresponding (*S*)-benzoins (enzymatic kinetic resolution via C–C bond cleavage) are obtained in high enantiomeric excess via C–C bond cleavage and carboligation reactions. During the cleavage of the benzoin linkage, only (*R*)-benzoin is accepted as a substrate. The reaction functions in an organic-aqueous medium, and overcomes the solubility problem with organic substrates.



Scheme 4.

The products are obtained in high yields starting from simple, easily available aromatic aldehydes and benzoins.

4. Experimental

Enzymatic syntheses were performed in standard buffer consisting of potassium phosphate (50 mM, pH 7.0) containing $MgSO_4$ (2.5 mM) and ThDP (0.15 mM). NMR spectra were recorded on a Bruker DPX 400. Chemical shifts δ are reported in ppm relative to $CHCl_3$ (^{1}H: δ=7.27), $CDCl_3$ (^{13}C: δ=77.0) and CCl_4 (^{13}C: δ=96.4) as internal standards. Column chromatography was conducted on silica gel 60 (40–63 μm). TLC was carried out on aluminum sheets precoated with silica gel $60F_{254}$ (Merck), and the spots were visualized with UV light (λ=254 nm). Enantiomeric excesses were determined by HPLC and LC-MS analysis using a Thermo Finnigan Surveyor equipped with an appropriate chiral phase column, as described in the footnotes of the Tables. Optical rotations were measured with an Autopol IV automatic polarimeter.

Hexahistidine tagged BAL was obtained as described previously.[9c,d] One unit of activity is defined as the amount of enzyme which catalyses the cleavage of 1 μmol benzoin in 1 min at 30 °C.

4.1. General procedure for the synthesis of 2-hydroxy-1-arylethan-1-ones from aromatic aldehydes: representative example: 2-hydroxy-1-(4-hydroxyphenyl)-ethan-1-one 2j

4-Hydroxybenzaldehyde (122 mg, 1 mmol) was dissolved in a mixture of DMSO (10 mL) and potassium phosphate buffer (40 mL, 50 mM, pH 7.0, containing $MgSO_4$ (2.5 mM) and ThDP (0.15 mM)). To this solution was added formaldehyde solution (8 mmol, 0.64 mL 37% solution). After the addition of BAL (40 U), the reaction was allowed to stand at 37 °C. Every 24 h, 30–40 U of BAL and 8 mmol of formaldehyde solution were added. After 4 days (checked by TLC), the reaction mixture was filtered and extracted with dichloromethane (3×50 mL). After

drying the collected organic phase over $MgSO_4$, removal of the solvent under reduced pressure gave the crude product, which was then purified by flash column chromatography (EtOAc:hexane 1:3) to give 135 mg (89%) of the desired compound 2-hydroxy-1-(4-hydroxyphenyl)ethan-1-one. Mp 165–167 (Lit.[13] 165–167); ^{1}H NMR ($CDCl_3$): δ 4.03 (br s,OH, 1H), 4.67 (s, 2H), 6.79 (d, J=8.4 Hz, 2H), 7.72 (d, J=8.4 Hz, 2H).

4.2. General procedure for the synthesis of (*S*)-benzoins and 2-hydroxy-1-arylethan-1-one from *rac*. benzoins: representative example: (*S*)-2-hydroxy-1,2-bis(4-methylphenyl)ethan-1-one ((*S*)-3e) and 2-hydroxy-1-(4-methylphenyl)-1-one (2b)

Rac-**3e** (240 mg, 1 mmol) was dissolved in a mixture of DMSO (20 mL) and potassium phosphate buffer (80 mL, 50 mM, pH 7.0, containing $MgSO_4$ (2.5 mM) and ThDP (0.15 mM)). To this solution was added 4 mmol formaldehyde solution. After the addition of BAL (40 U), the reaction was allowed to stand at room temperature. After 24 h, 20–50 U of BAL and 4 mmol of formaldehyde solution were added. This was repeated every 24 h until no more (*R*)-benzoin was observed. After 6 days, only (*S*)-**3e** and **2b** were detected (HPLC). The mixture was extracted with dichloromethane (250 mL) and the organic layer washed with water (25 mL) and brine (25 mL) and dried over $MgSO_4$. Evaporation of the solvent and separation of the crude products by column chromatography afforded (*S*)-**3e** and **2b**.

4.2.1. Compound (*S*)-3e. Colorless solid; yield: 93 mg, 39%; mp 88 °C [Lit.[15], mp 89 °C]; $[\alpha]_D^{20}$=+148.2 (*c* 0.8, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.83 (d, J=8.1 Hz, 2H), 7.18-7.22 (m, 4H), 7.16 (d, J=8.1 Hz, 2H), 5.88 (d, J=5.8 Hz, 1H), 4.52 (d, J=5.8 Hz, 1H), 2.36 (s, 3H), 2.30 (s, 3H).

4.2.2. Compound 2b. Yellow solid; yield: 129 mg, 43%; mp 84 °C [Lit.[10], mp 83 °C]; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.83–7.81 (m, 2H), 7.32–7.28 (m, 2H), 4.86 (s, 2H), 3.52 (br s, 1H), 2.43 (s, 3H).

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