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Homoallylic Alcohols *via* a Chemo-Enzymatic One-Pot Oxidation–Allylation Cascade

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Abstract: A chemo-enzymatic one-pot, two-step transformation of (hetero)-benzylic and cinnamic alcohols to yield the elongated homoallylic *sec*-alcohols in water in up to 96% isolated yield has been developed. The sequence comprised an enzymatic alcohol oxidation using galactose oxidase from *Fusarium* sp. NRRL 2903 to furnish the corresponding aldehydes, which were subjected directly to allylation *via* indium(0)-mediated Barbier-type coupling with allyl bromide or by addition of allylboronic acid pinacol ester.

Keywords: Barbier-type coupling; biocatalysis; boronic acids; cascade reaction; galactose oxidase; indium

The low-valent metal-mediated coupling of aldehydes with allylic bromides represents an elegant method for the synthesis of homoallylic alcohols.[1] These compounds can be subjected to various synthetic transformations and are thus precursors for chiral chromanes,[2] lactones[3] or urea derivatives.[4] Additionally, they serve as synthons for Prins cyclizations[5] and are used in natural product synthesis.[6] Based on these facts, extensive studies of the Barbier-type coupling reaction between allylic bromides and carbonyl compounds have been conducted.[1,7] Among the catalysts used, indium(0) has emerged as a metal with high potential, especially when it comes to using mild reaction conditions (room temperature) and aqueous reaction media.[3] Alternatively, a metal-free method based on allylboronic esters in organic media is available.[17]

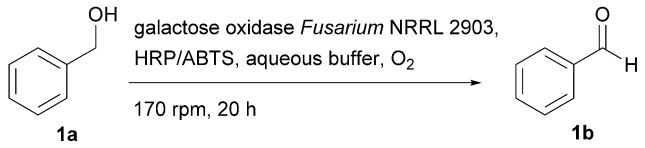
One drawback of these protocols is the requirement for aldehyde precursors, which exhibit limited stabilities. Hence, it has been recently proposed to form the aldehyde by chemical *in-situ* oxidation of the corresponding alcohol and to transform the carbonyl intermediate immediately to the target compounds.[8]

In order to exploit the potential of O_2-dependent biooxidation, we envisaged to employ an alcohol oxidase as a 'green' substitute for the oxidation step. The groups of Arnold and Turner have demonstrated the high potential of galactose oxidase from *Fusarium* NRRL 2903 for the biocatalytic oxidation of alcohols.[9–11] Galactose oxidase is a copper-dependent enzyme which in nature oxidizes the *prim*-C-6-OH group of D-galactose to the corresponding aldehyde at the expense of molecular oxygen.[12,13] Reports in the biochemical literature indicate that in addition to the 'natural' substrate galactose, also benzylic alcohols are oxidized.[9,10,14] Although the enzyme is biochemically well characterized, examples for its use in preparative biotransformations are limited.[9,14]

His-tagged galactose oxidase was epressed in *E. coli* BL21(DE3) and Ni-affinity chromatography was used for protein purification. The optimization of reaction conditions was performed using benzyl alcohol as test substrate in the presence of horseradish peroxidase (HRP) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), (ABTS) for the removal of hydrogen peroxide.

Initial tests were performed using purified apoenzyme, which was reconstituted by addition of the Cu(II) cofactor. Since some loss of activity was observed during this procedure indicative for incomplete reconstitution of the holoenzyme, lyophilized whole cell preparations were employed (see Supporting Information). The key parameters for the oxidation re-

Table 1. Optimization of the enzymatic oxidation.



Entry	Conditions[a]	Substrate conc. [M]	Conv. [%][b]
1	–	0.1	9
2	O_2 (4 bar)	0.1	45
3	150 mM P_i buffer	0.1	69
4	Cu(II) (0.2 mM)	0.1	97
5	Cu(II) (10 mM)	0.2	>99

[a] *Standard conditions:* Substrate, galactose oxidase (whole-cell preparation, 20 mg mL^{-1}), horseradish peroxidase (0.150 mg mL^{-1}), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 0.150 mg mL^{-1}), phosphate buffer (0.5 mL, pH 7.0, 100 mM), 170 rpm, 20 h, atmospheric air pressure.

[b] Conversions were determined *via* GC-FID analysis.

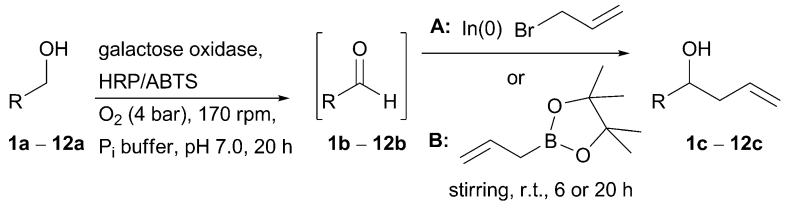
action are summarized in Table 1 (details are given in the Supporting Information).

Modification of the standard conditions by applying oxygen pressure caused a dramatic (five-fold) increase in the conversion, which reached an optimum at 4 bar (Table 1, entry 2); beyond this level, the conversion dropped. In addition, increased buffer capacity had a positive effect (entry 3). Finally, the addition of Cu(II) allowed the reaction to reach completion, even at increased substrate concentrations of up to 0.2 M (entries 4 and 5). Extreme values of 1 M led to reduced conversions (41% conv.). The published pH optimum of 7.0 could be confirmed.[11]

Since the enzymatic oxidation step was performed in aqeous buffer, also the indium-mediated coupling was transferred from water to buffer medium. A modest drop in reaction rate could be fully compensated by extension of the reaction time from 5 to 20 h. Different methods of agitation were found to have a profound effect on the reaction rate. Since shaking did not give any conversion, magnetic stirring was applied, which most likely causes the mechanical removal of the indium oxide passivating layer shielding the catalytically active In(0) species. In line with this hypothesis, it was observed that prolonged exposure of indium to air caused decreased reaction rates. While a three-month-old (untreated) batch of indium gave a conversion of 53%, etching of the same catalyst with hydrochloric acid (5 M), followed by washing with water and acetone to remove traces of acid[15] led to complete conversion within one hour. Based on these observations, indium was either used freshly or treated with hydrochloric acid prior to use. In addition to the indium properties, enhanced substrate concentrations were found to improve the rate of the Barbier-type coupling, which showed an optimum at 0.2 M, which is identical to that of the enzymatic oxidation step. Additionally, attempts were undertaken to use indium in catalytic amounts. Thus, allylboronic acid pinacol ester was used in presence of 5 mol% indium(0), as this method had been already shown to work for similar ketone derivatives.[16] However, careful blank experiments revealed that the allylboronic ester readily adds to the aldehyde in the absence of metal catalyst. To avoid possible indium contaminations, which would cause false positives, the reaction was conducted in new glassware, using a new stirring bar and fresh reagents. Perfect conversions were observed without metal catalyst in both water and buffered aqueous media.[17]

With these data in hand, we attempted to couple both steps to a one-pot cascade, where the aldehyde formed *in situ* was immediately coupled to the allyl moiety. Unfortunately, the enzymatic oxidation was completely inhibited in presence of both allylating reagents and no aldehyde intermediate was formed. One explanation could be a copper-catalyzed Ullmann-type aryl ether formation involving the catalytically important phenolic moieties of Tyr272 and/or Tyr495 in the active site of galactose oxidase (pdb 1GOF).[12,18] In order to prove this assumption, we analyzed the amino acid sequence of galactose oxidase for tyrosine modification in the presence and absence of allylation reagents by liquid chromatography-tandem MS of enzymatic digests of the protein with three different proteases to ensure high sequence coverage. We unambiguously identified allylated Tyr495 in two distinct peptides (IVRAYHSIS and IVRAYHSISL) when galactose oxidase was incubated with In(0)/allyl bromide. In contrast, unmodified Tyr495 was detected in the peptide AYHSISLLLPDGR in untreated enzyme. Tyr272 was not detected in any of the samples suggesting that the native cross-link between Tyr272 and Cys228 was not broken in any of the conditions (see Supporting Information).

Since galactose oxidase was incompatible with both allylation reagents, the reactions were performed in tandem in a one-pot fashion (Scheme 1). We were pleased to see that the cascade reaction proceeded smoothly when In(0) and allyl bromide or the allylboronic ester were added after the enzymatic oxidation was completed without any work-up/separation steps being required inbetween (Table 2). The majority of substrates were transformed to the corresponding homoallylic alcohols with good to excellent conversion (**1a**, **3a**–**6a**, **8a**, **10a**–**12a**). Remarkably, the presence of unprotected phenolic groups was nicely tolerated (**5a–7a**). Although unprotected aminobenzyl alcohols, such as *ortho*- and *meta*-aminobenzyl alcohol were enzymatically oxidized, complex product mixtures were obtained in the cascade process. Interestingly, the heteroaromatic substrate **9a** was only ally-



Scheme 1. Alcohol oxidation/C–C coupling cascade.

lated using In(0)/allyl bromide, whereas a complex product mixture was obtained with the boronic ester.

However, some substrates could not be oxidized, particularly those bearing substituents in the *ortho*-position of the aryl moiety, such as the *o*-Cl (**2a**), *o*-Br and *o*-nitro analogues (data not shown). However, oxygen-containing substituents in *ortho*-position [hydroxy (**5a**) or methoxy (**8a**)] gave excellent results. Although the *para*-hydroxy derivative (**7a**) was nicely converted, product **7c** decomposed during work-up. The origin for the modest conversions with substrate **11c** were investigated employing reused cells, which gave comparable results. Thus the diminished reaction rate was not caused by enzyme deactivation, but due to reduced catalytic activity towards this substrate,

Table 2. One-pot alcohol oxidation/C–C coupling cascade.[a]

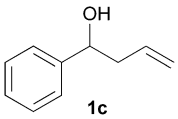
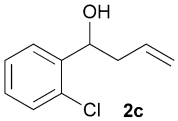
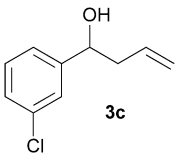
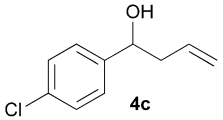
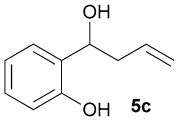
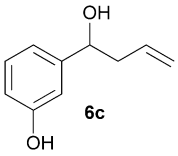
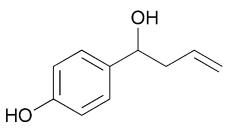
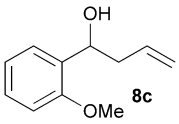
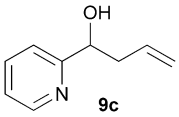
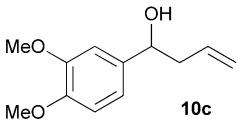
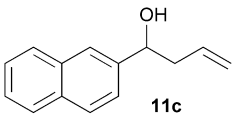
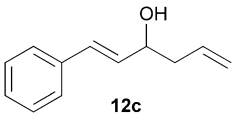
Product	Method	Conv. [%][b] Oxidation[c]	Overall[d]
 **1c**	A	>99	>99
	B	>99	>99
 **2c**	A	n.c.	–
	B	n.c.	–
 **3c**	A	>99	97
	B	>99	>99
 **4c**	A	>99	88
	B	>99	>99
 **5c**	A	>99	>99
	B	91	90
 **6c**	A	>99	83
	B	>99	>99
 **7c**	A	product mixture	
	B	88[e]	81[e]

Table 2. (Continued)

Product	Method	Conv. [%][b] Oxidation[c]	Overall[d]
 **8c**	A	>99	>99
	B	>99	>99
 **9c**	A	>99	51
	B	product mixture	
 **10c**	A	>99	51
	B	>99	87
 **11c**	A	78	77
	B	54	53
 **12c**	A	91	90
	B	98	94

[a] *Reaction conditions:* phosphate buffer (100 mM, pH 7.0), substrate (0.2 M), galactose oxidase (whole-cell preparation, 20 mg mL^{-1}), horseradish peroxidase (0.150 mg mL^{-1}), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 0.150 mg mL^{-1}), step 2: Method A: In powder (1.2 equiv.), allyl bromide (2 equiv.), Method B: allylboronic acid pinacol ester (1.2 equiv.).

[b] Conversions were determined *via* GC-FID.

[c] A sample of 20 μL was taken to determine the conversion of the oxidation step.

[d] Overall conversion of the one-pot procedure.

[e] Product decomposition during work-up.

most likely due to steric hindrance. Fortunately, the 'slim' cinnamic alcohol (**12a**) was smoothly converted.

The recovery of the whole-cell biocatalyst by simple centrifugation allows one to circumvent enzyme deactivation caused by the allylation reagents in the second step. Depending on the substrate concentration, three to five oxidation cycles could be performed before the activity started to decline.

In conclusion, we have established a one-pot, two-step procedure for the chemo-enzymatic synthesis of homoallylic alcohols. All reactions were conducted at room temperature in aqueous buffer without requirement of cosolvents. The cascade starts from a benzylic alcohol, which is enzymatically oxidized by galactose oxidase in a clean fashion at the expense of O_2 to furnish the corresponding aldehyde, which is coupled to an allyl moiety derived from allyl bromide/In(0) or from an allylboronic ester in a metal-free variant. A special feature of this method is the functional group tolerance, which includes unprotected phenolic hydroxy groups and – in the case of In(0)/allyl bromide – basic heteroaromatic moieties. Overall, the reaction sequence is carried out under mild (aqueous) conditions and avoids the handling of moisture-sensitive and flammable organometallics.

Experimental Section

General Procedure for the One-Pot Preparation of Homoallylic Alcohols 1c–12c

Whole cells of *E. coli* containing overexpressed galactose oxidase (40 mg lyophilized dry weight) were rehydrated in phosphate buffer (2 mL, 100 mM, pH 7.0, 10 mM $CuSO_4 \cdot 5H_2O$) by shaking at 30 °C and 120 rpm in a horizontal position for 20 min. The rehydrated cells were transferred to a 10-mL round-bottom flask equipped with a magnetic stirring bar. Horseradish peroxidase (0.300 mg in 60 μL buffer), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (0.300 mg in 60 μL buffer) and substrate **1a–12a** (method **A**: 0.4 mmol; method **B**: 0.2 mmol) was added, the reaction mixture was placed in an oxygen-pressurizing apparatus (see Supporting Information) using a rack for round-bottom flasks. After the apparatus had been primed with oxygen (technical grade) for 1–2 min, the cylinder was closed and pressurized to 4 bar. The reaction mixture was shaken at room temperature and 170 rpm for 20 h. After careful depressurization, the flask was removed and subjected to the allylation step.

*Method **A**:* Indium powder (54 mg, 0.48 mmol, 100 mesh) and allyl bromide (70 μL, 96 mg, 0.8 mmol) were added, the flask was closed with a glass stopper and the mixture was stirred at room temperature for 20 h.

*Method **B**:* Allylboronic acid pinacol ester (45 μL, 40 mg, 0.24 mmol) was added, the flask was closed with a glass stopper and the mixture was stirred at room temperature for 6 h.

For work-up, the mixture was treated with saturated NH_4Cl solution (10 mL; saturated K_2CO_3 solution in case of compound **9c**) to clarify the solution and products were extracted with EtOAc (3×20 mL). Phase separation was conducted in 50-mL Sarstedt tubes, which were centrifuged in order to facilitate phase seperation. The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified using column chromatography (silica gel, petroleum ether/EtOAc) to furnish the products in >95% NMR purity and >99% GC-MS purity.

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