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Laccase-Mediated Deprotection of *para*-Methoxyphenyl (PMP)-Protected Amines

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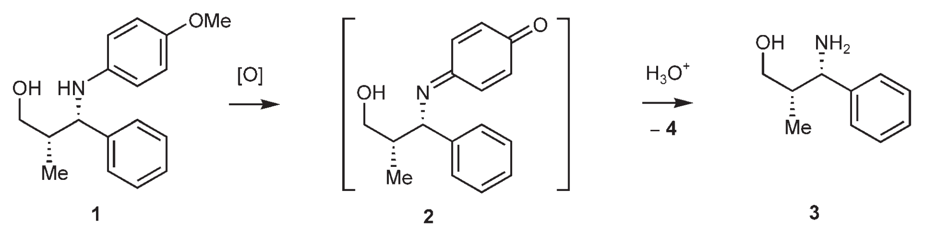
Abstract: A novel enzymatic procedure for the oxidative deprotection of *p*-methoxyphenyl (PMP)-protected amines is described. By using laccases (E.C. 1.10.3.2) under mildly acidic conditions, a variety of PMP-protected amines were successfully deprotected. The rate of deprotection was solvent- and pH-dependent, and the reaction scope could be increased by using so-called mediators.

Keywords: biocatalysis; enzymatic deprotection; green chemistry; laccase; *para*-methoxyphenyl protecting group; organocatalysis

The *para*-methoxyphenyl (PMP) group is being increasingly used as a nitrogen protecting group for amines[1–3] and imines.[4] In particular, in proline-catalyzed asymmetric Mannich reactions, the PMP protecting group appeared to be a crucial element for reaching high enantioselectivities.[5] Until recently, industrial application of the PMP protecting group has been thwarted by a lack of cheap and practical deprotection methods. In most cases, ceric ammonium nitrate (CAN) has been used for deprotection, which is expensive and highly toxic.[6] Phenyl iodoacetate[7] and also electrochemical deprotection[8] have been proposed instead, but both approaches do not provide viable alternatives for industrial application. Recently, however, we demonstrated that the cheap oxidants trichloroisocyanuric acid (TCCA) and periodic acid both can be efficiently applied for PMP deprotection of amines.[9] In conjunction with this latter research and the growing need for green and sustainable catalytic deprotection strategies, we herewith report an oxidative enzymatic method for the effective removal of the PMP group from nitrogen atoms.

Having observed from our recent work[9] that a rather large variety of oxidative reagents can be employed for PMP removal, we reasoned that this transformation might also be executed with oxidative enzymes. In view of the many applications of laccases (E.C. 1.10.3.2) in oxidative transformations,[10] we envisioned that they are potential candidates for selective PMP removal as well. Laccases are multi-copper oxidases found in several trees and fungi, catalyzing the oxidation of various types of substances with concomitant reduction of oxygen to water, avoiding the formation of the hazardous hydrogen peroxide.

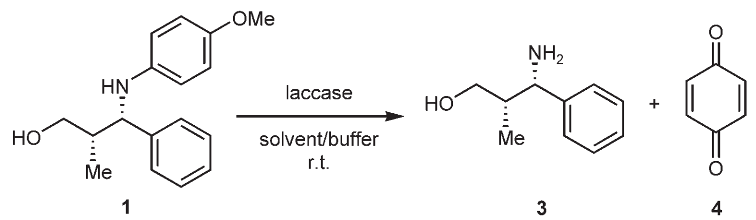
Our investigation into the applicability of laccases for PMP group removal commenced with developing an HPLC assay to screen laccases by monitoring the conversion of starting material, that is, the *N*-PMP-protected amine into the desired free amine. As a benchmark substrate, we chose the protected 1,3-amino alcohol **1** (Scheme 1).[11] In a first attempt, addition of 4 mg of laccase T (from *Trametes versicolor*) to 1 mL of a solution of **1** (1.0 mg) in an acetonitrile/aqueous buffer (pH 5.0) mixture immediately altered the color of the reaction mixture from colorless to purple, indicating enzymatic activity. Subjection of the crude reaction mixture to the HPLC assay clearly demonstrated the formation of the free amine **3** and benzoquinone (**4**, Scheme 1). After this promising first result, a more thorough screen of reaction conditions was carried out in which co-solvent and pH were varied. Two commercially available laccases were employed without modification in lyophilized form, namely laccase T and laccase AB (from *Agaricus bisporus*).



Scheme 1. Deprotection of the PMP group.

Because of the generally low solubility of organic substrates in aqueous solution, we decided to investigate the use of co-solvents from the start. We considered this especially important, since earlier studies revealed that laccases are often deactivated by organic solvents.[12] Thus, a series of experiments was carried out in a phosphate buffer (50 mM, pH 5)/co-solvent mixture, thereby varying the co-solvent. The conver-

Table 1. Screening of the reaction parameters.[a]



Entry	Laccase	Solvent	Vol% buffer	pH	*t* [h]	Conversion to **3** [%][b]	Entry	Laccase	Solvent	Vol% buffer	pH	*t* [h]	Conversion to **3** (%)[b]
1	T	MeCN	60	5	24	32	31	T	MeCN	80	7	18	23
2	T	MeCN	70	5	24	46	32	T	MeCN	80	8.5	18	0
3	T	MeCN	80	5	24	46	33	T	MeCN	60	3	4	42
4	T	MeCN	90	5	24	73	34	T	MeCN	70	3	4	68
5	T	THF	60	5	24	27	35	T	MeCN	80	3	4	77
6	T	THF	70	5	24	47	36	T	MeCN	90	3	4	79
7	T	THF	80	5	24	79	37	T	MeCN	60	3	20	53
8	T	THF	90	5	24	51	38	T	MeCN	70	3	20	76
9	T	EtOAc	60	5	24	0	39	T	MeCN	80	3	20	80
10	T	EtOAc	70	5	24	12	40	T	MeCN	90	3	20	85
11	T	EtOAc	80	5	24	21	41	T	THF	60	3	4	19
12	T	EtOAc	90	5	24	58	42	T	THF	70	3	4	48
13	T	toluene	60	5	24	11	43	T	THF	80	3	4	58
14	T	toluene	70	5	24	23	44	T	THF	90	3	4	70
15	T	toluene	80	5	24	44	45	T	THF	60	3	20	31
16	T	toluene	90	5	24	44	46	T	THF	70	3	20	72
17	AB	MeCN	80	1	18	5	47	T	THF	80	3	20	75
18	AB	MeCN	80	2	18	2	48	T	THF	90	3	20	82
19	AB	MeCN	80	3	18	4	49	T	DMSO	60	3	18	84
20	AB	MeCN	80	4	18	24	50	T	DMSO	70	3	18	85
21	AB	MeCN	80	5	18	25	51	T	DMSO	80	3	18	89
22	AB	MeCN	80	6	18	20	52	T	DMSO	90	3	18	91
23	AB	MeCN	80	7	18	15	53	T	MeOH	60	3	18	75
24	AB	MeCN	80	8.5	18	13	54	T	MeOH	70	3	18	78
25	T	MeCN	80	1	18	0	55	T	MeOH	80	3	18	82
26	T	MeCN	80	2	18	8	56	T	MeOH	90	3	18	87
27	T	MeCN	80	3	18	82	57	T	MTBE	60	3	18	47
28	T	MeCN	80	4	18	74	58	T	MTBE	70	3	18	64
29	T	MeCN	80	5	18	61	59	T	MTBE	80	3	18	72
30	T	MeCN	80	6	18	37	60	T	MTBE	90	3	18	87

[a] *Conditions*: laccase T or AB (4 mg) was added to a solution of **1** (1 mg) in the buffer/solvent mixture (1 mL), room temperature.

[b] Conversions were determined using HPLC (Inertsil ODS-3 column) on crude samples taken from the reaction mixture.

sions of substrate **1** into the free amine **3**, as determined by HPLC, are depicted in Table 1 (entries 1–16). These results led us to conclude that the laccase-mediated PMP deprotection proceeds faster in homogeneous systems (e.g., MeCN, THF, MeOH, DMSO), but that the use of a biphasic system (e.g., EtOAc, toluene, MTBE) is also applicable. Additionally, it must be noted that higher amounts of co-solvent, although rendering the substrate more soluble, significantly decrease the overall reaction rate through enzyme deactivation. Considering these initial results, we continued with evaluation of the pH dependence of the reaction in a buffer mixture/MeCN (4:1) using both laccases T and AB (entries 17–32). Clearly, the conversions increased with lower pH values, albeit that too acidic conditions (pH < 3) led to enzyme deactivation. Having concluded that 3 was the optimum pH value and laccase T the more suited enzyme, a series of industrially viable solvents was evaluated in varying ratios with the buffer solution (entries 33–60). No real solvent limitations were identified in these experiments, although the use of acetonitrile and DMSO gave slightly better results. In addition, there was no clear difference between mono- and biphasic systems both giving high conversions to the desired product.

Subjection of protected amino alcohol **1** to laccase AB gave a maximum conversion of 25% (phosphate buffer pH 5/MeCN 4:1, entry 21). Since the scope of laccases is known to be enlarged by invoking so-called mediators,[13] we investigated the influence of mediators (Figure 1) on the efficiency of this particular reaction. Mechanistically the mediator, after being oxidized by the laccase, is responsible for the conversion of the substrate into the oxidized form. Since the reduced mediator can be reoxidized by the enzyme, both laccase and mediator can be used in a catalytic fashion using oxygen as the stoichiometric oxidant thereby producing water as the by-product.

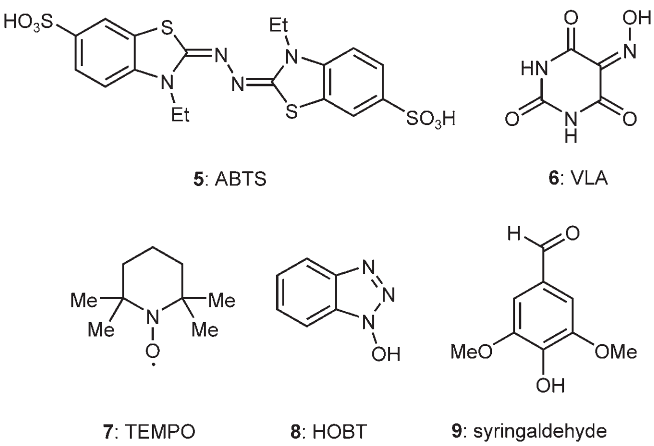
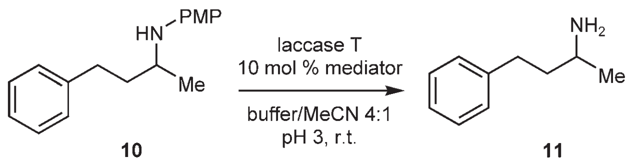


Figure 1. Mediators.

The best result was obtained with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, **5**) as the mediator [84% conversion to **3** (instead of 25% in entry 21), 18 h, r.t., 1 equiv. of ABTS]. For each mediator, a blank reaction without enzyme was also conducted. In addition to increasing the conversion, it was also found that the substrate scope could be extended by using mediators. For instance, *N*-PMP protected 4-phenylbutan-2-amine (**10**) could not be enzymatically deprotected as such with laccases T and AB, which could be due to the fact that this is not a benzylic amine. Gratifyingly, introduction of any of the mediators **5–9** led to high conversions to the corresponding amine **11** (Table 2).

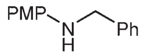
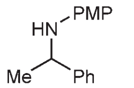
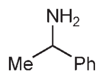
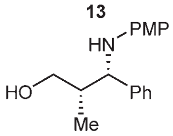
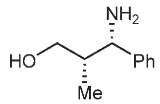
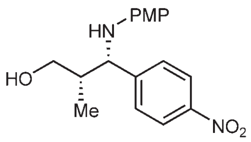
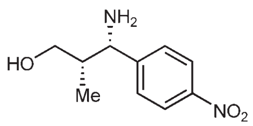
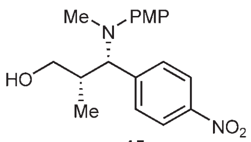
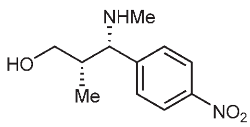
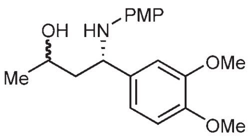
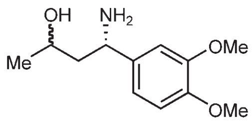
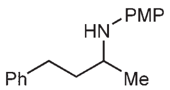
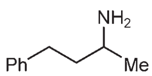
Table 2. Influence of the mediators.



Entry	Mediator	Conversion (18 h)	Conversion (48 h)
1	-	0	-
2	**5**	43	87
3	**6**	50	88
4	**7**	53	80
5	**8**	30	75
6	**9**	71	83

To validate the screening results, we performed a series of preparative experiments using the initial substrate **1** as well as substrates **10**, **13**-**16** with laccases T and AB (Table 3). Surprisingly, subjection of PMP-protected benzylamine **12** to these enzymes did not produce benzylamine (**17**) itself. Instead, formation of benzaldehyde was observed, suggesting oxidation at the benzylic position, followed by hydrolysis of the resulting imine. In all other cases, however, the corresponding free amines **11**, **18–21** were isolated after work-up in reasonable to good yields and excellent purity. The isolated yields are generally lower than the HPLC conversions, which reflects the difficulty to separate the emulsions formed in the extraction step. Moreover, the high dilution of the reaction mixtures (1 mg/mL) might contribute to the low yield of the products. Finally, realizing that higher concentrations are required to render processes industrially viable, we repeated one of the deprotection experiments in a 10 mg/mL suspension to obtain comparable results (63% yield *vs.* 67% in entry 5). Further concentration of the reaction mixture (100 mg/mL) led to a considerably lower reaction rate, although a clean conver-

Table 3. Preparative deprotections using laccase T and AB.[a]

Entry	*N*-PMP amine	Laccase	Product (Yield)[c]
			
1	**12**	T	**17** (0%)
2[b]	**12**	AB	**17** (0%)
			
3	**13**	T	**18** (31%)
4[b]	**13**	AB	**18** (56%)
			
5	**1**	T	**2** (67%)
6[b]	**1**	AB	**2** (71%)
			
7	**14**	T	**19** (64%)
8[b]	**14**	AB	**19** (74%)
			
9	**15**	T	**20** (89%)
10[b]	**15**	AB	**20** (52%)
			
11	**16**	T	**21** (34%)
12[b]	**16**	AB	**21** (41%)
			
13[b]	**10**	T	**11** (30%)
14[b]	**10**	AB	**11** (47%)

[a] *Conditions*: laccase T or AB (4 mg) was added to a solution of **1** (1 mg) in the buffer/solvent mixture (1 mL), room temperature.
[b] Conversions were determined using HPLC (Inertsil ODS-3 column) on crude samples taken from the reaction mixture.[a] *Conditions*: PMP-protected amine (0.92 mmol), laccase (120 mg), MeCN/buffer mixture (1:4, pH 3, 200 mL), room temperature.
[b] Reaction conducted in the presence of ABTS (10 mol%).
[c] Products were isolated as HCl salts.

sion to the desired product was still observed. Alternative ways to attain rapid deprotection reactions in a more concentrated fashion, such as the use of elevated temperatures or other organic co-solvents to increase the solubility of the substrates or portionwise addition of the substrate, are currently under investigation.

In conclusion, the increasing use of the *p*-methoxyphenyl (PMP) group in organic synthesis requires cost-efficient, environmentally friendly and scalable deprotection procedures to render these processes industrially viable. We have developed a novel enzymatic oxidative deprotection procedure for *N*-PMP protected benzylic amines involving laccases, which is effective at a pH below 4. Additionally, we have found that the use of mediators leads to an extension of the substrate scope and an increase in reaction rate. Studies to elucidate the exact mechanism and further exploration of the scope and limitations of laccase-mediated oxidative deprotection reactions are currently ongoing.

Experimental Section

Commercially available laccase T from *Trametes versicolor* was purchased from Jülich Fine Chemicals in two batches as a light brown lyophilized powder. The activity of the first batch (used in the experiments as shown in Table 1, entries 1–32) was 24 U/mg (using syringaldazin). The activity of the second batch (used in the experiments as shown in Table 1, entries 33–60, Table 2 and Table 3) was 1.2 U/mg (using syringaldazin). Laccase AB from *Agaricus bisporus* was commercially available from Jülich Fine Chemicals as a brown lyophilized powder [activity 7.9 U/mg (using catechol)].

Representative Procedure for the Laccase-Mediated PMP Deprotection

To a solution of **1** (250 mg, 0.92 mmol) in THF (40 mL) and phosphate buffer (160 mL, pH 3, 50 mM) was added laccase T (120 mg) and if required ABTS (10 mol%). The resulting suspension was stirred for 20 h at room temperature, acidified to pH 1 with 5 N HCl and filtrated over Celite. The filtrate was washed with CH_2Cl_2 (3×100 mL). The resulting aqueous phase was subsequently brought to pH 10.5 *via* addition of 5 N NaOH and extracted with EtOAc (4×75 mL). The combined organic fractions were brought to pH 1 *via* addition of HCl/EtOAc, dried (Na_2SO_4) and concentrated under reduced pressure to afford **2**·HCl as a white solid; yield: 124 mg (67%); $[\alpha]_D^{20}$: −24.3 (*c* 0.18, MeOH); ^{1}H NMR (400 MHz, D_2O): δ=1.12 (d, J=6.9 Hz, 3H), 2.36 (m, 1H), 3.37 (dd, J=5.4, 11.4 Hz, 1H), 3.42 (dd, J=5.4, 11.4 Hz, 1H), 4.33 (d, J=8.2 Hz, 1H), 7.50 (m, 5H); ^{13}C NMR (75 MHz, D_2O): δ=12.3, 37.9, 57.8, 62.6, 126.9, 128.7, 128.7, 134.6; IR: ν=3330, 3112, 2975, 2871, 1489, 1473, 1026, 706, 558 cm^{-1}; HRMS (ESI$^+$): m/z=166.1248, calcd. for $C_{10}H_{16}NO$: 166.1232; anal. calcd. for $C_{10}H_{16}ClNO$: C 59.55, H 8.00, N 6.94; found: C 58.05, H 7.87, N 6.84.

Spectroscopic data for new compounds are available in the Supporting Information.

Acknowledgements

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[13] Mediators are usually small organic molecules, which act as 'electron shuttles' between the enzyme and the substrate to overcome a limited substrate scope. Hence, they might make the necessity of a fit of the substrate in the active site of the enzyme redundant; see, e.g.: H. P. Call, I. Mucke, *J. Biotechnol.* **1997**, *53*, 163–202.

