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Chemoselective oxidative N-debenzylation of aryl halogenated amines using the Laccase LAC97/TEMPO system

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ABSTRACT

The N-debenzylation of aryl-halogenated amines using the Laccase LAC97/TEMPO system is reported, demonstrating selective N-debenzylation with no dehalogenation of the phenyl ring, which is observed with conventional debenzylation methods such as palladium-catalysed hydrogenation. This reaction was performed under ambient conditions in acidic buffer with 5 % DMSO as co-solvent in an open-to-air vessel. The versatility and robustness of the system was demonstrated on substrates harbouring different halogen moieties. The limitations of the Laccase LAC97/TEMPO system were also studied, highlighting the formation of by-products due to overoxidation on selected substrates. A series of substrates with various halogens were studied. High conversion (78–90 %) in 6 h and full conversion was achieved within 24 h using HPLC to monitor the reaction. Overall, this system is presented as a chemoselective N-debenzylation alternative for aryl-halogenated substrates.

Introduction

The use of protecting groups is a common technique in organic synthesis to prevent unwanted side reactions by masking the reactive group. The benzyl group (Bn or $PhCH_2$-) is a common protecting group of amines [1]. It is one of the most robust protective groups that is stable under both acidic and basic conditions. Deprotection of the N-benzyl groups was conventionally performed via palladium-catalysed hydrogenation using Pd/C catalyst and H_2 gas, where the amine is deprotected via reduction along with the release of the by-product toluene [2–5]. Other non-palladium debenzylation methods exist, by utilizing a range of oxidizing agents. O-Benzyl groups have been shown to be deprotected by ozone [6] or NBS [7], while N-benzyl groups were shown to be deprotected using ceric ammonium nitrate (CAN) [8] or diisopropyl azodicarboxylate (DIAD) [9]. In the majority of cases, deprotection of these benzyl groups require stoichiometric amounts of toxic reagents as well as harsh conditions such as elevated temperatures which can be hazardous on a larger scale and also generate large quantities of waste.

Laccase (EC 1.10.3.2) is a multicopper oxidase enzyme class capable of a myriad of oxidative reactions including for example, oxidising phenolic compounds by using molecular oxygen as the oxidant thus forming water as the sole by-product [10,11]. Laccase is considered as a “green catalyst” due to its ability to work in water at ambient temperature and uses clean air as the oxidant. However the substrate scope of Laccase oxidation is limited by i) its copper-bound active site within the enzyme that might not be accessible, and ii) its low oxidative potential of 0.4–0.8 V [12]. The combination of a mediator – a known substrate of Laccase while being a redox active species, such as 2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO) [13] in a so-called Laccase-Mediator system (LMS) addresses both limitations of Laccase transformations, enabling the oxidation of a wider range of functional groups such as alcohols and amines [14,15] (See Fig. 1). There has been an increase in the use of Laccase and LMS in oxidation reactions in the past decade, such as the removal of the para-methoxyphenyl (PMP) protection group [16,17], trimerization of indole [18], dimerization of aromatic amines [19], formation of imine [20], and many more reactions. A recent article by Martinez-Montero and co-workers described the use of the Laccase/TEMPO system for the deprotection of N-benzyl protecting groups, utilizing an oxidative approach for the de-protection [21]. The authors developed conditions that resulted in complete N-deprotection under mild conditions.

One of the biggest limitations for N-debenzylation using palladium-

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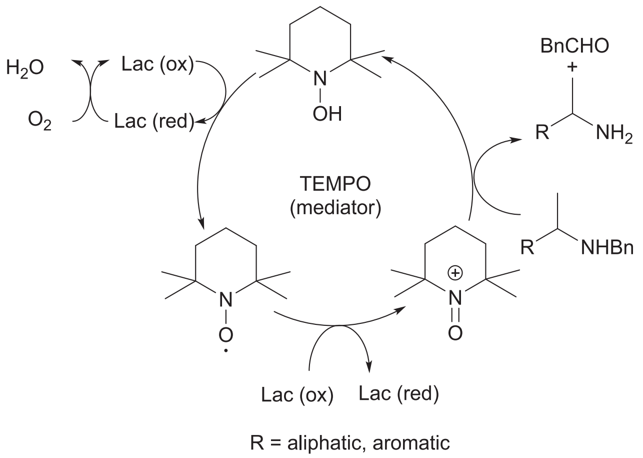


Fig. 1. Catalytic cycle of LMS with TEMPO as the mediator for the N-debenzylation reaction.

catalysed hydrogenation, besides employing potentially explosive H_2, is the undesired and inevitable cleavage of the halogens for aryl-halogenated compounds via reductive elimination [22]. This side reaction restricts molecule design that can utilize N-benzyl protecting groups and certain synthesis pathways, and the substrate almost never contains any aryl-halogen bonds prior to the removal of the benzyl group from the amine. As the N-debenzylation reaction using the Laccase/TEMPO system was carried out under oxidative condition in contrast to reductive condition in palladium-catalysed hydrogenation, we strongly believed that the Laccase/TEMPO system would circumvent the dehalogenation in compounds that contain aryl-halogen bonds (Scheme 1), yet it was noted that none of the substrates used in Martinez-Montero's article include any halogens or aryl-halogen bonds to verify this advantage. Our previous experience of benzyl alcohol oxidation using the same system with aryl-halogenated substrates also showed that they are compatible. By showcasing the N-debenzylation reaction on aryl-halogenated amines without causing dehalogenation, this article hopes to bring a novel perspective on the utility and advantage of the Laccase/TEMPO system on N-benzyl group deprotection, potentially enabling more efficient design of synthetic pathways, all the while using a mild and straightforward procedure.

Results and discussion

Initially, N-benzyl-1-(4-bromophenyl)ethylamine (**1**) was selected as a model substrate (Scheme 2), with both the substrate and the product reference for HPLC analysis synthesized from commercially available 4-bromoacetophenone (**3**).

Surprisingly the substrate **1** remained fully protected even after 24 h under reported conditions with Tris.HCl buffer and the free amine product **2** was not detected by HPLC analysis (Table 1, entry 1) after multiple attempts. This was most likely due to Tris.HCl buffer not being able to buffer the pH change at pH 5.0 (Tris.HCl buffer range between pH 7.0 – 9.2) and this resulted in a drastic increase of pH when the substrate stock was added. This might explain why the reaction did not proceed with the enzyme inhibited or denatured in basic pH. In contrast to Martinez-Montero and co-workers, by changing the buffer to pH 5.0 50 mM acetate buffer with 5 % (v/v) DMSO, the N-debenzylation proceeded successfully with a good conversion rate (Table 1, entry 2), however formation of a significant amount of by-product was also observed. Encouraged by the high conversion rate using acetate buffer, a variety of parameters were adjusted and investigated. Attempts at

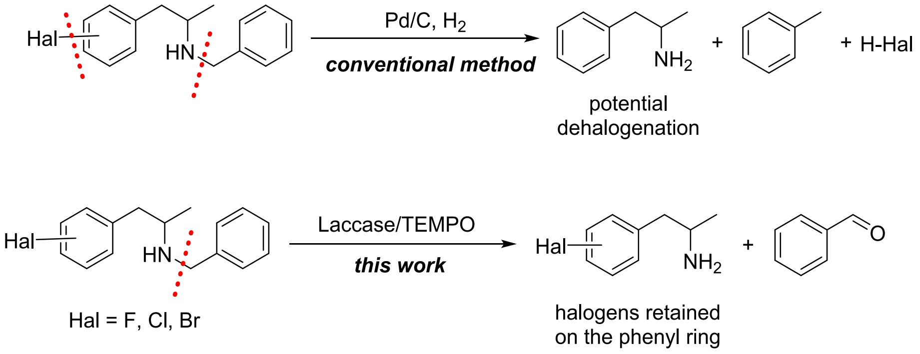
Table 1
Buffer and reaction condition development using 1 as the model substrate.

Entry	Buffer system	Conversion (%)[a]	
		6 h	24 h
1	pH 5 200 mM Tris.HCl	0	0
2	pH5 50 mM acetate (5 % DMSO)	65	100
3[b]	pH5 50 mM acetate (5 % DMSO)	83	86
4	pH4 200 mM acetate (5 % DMSO)	71	100

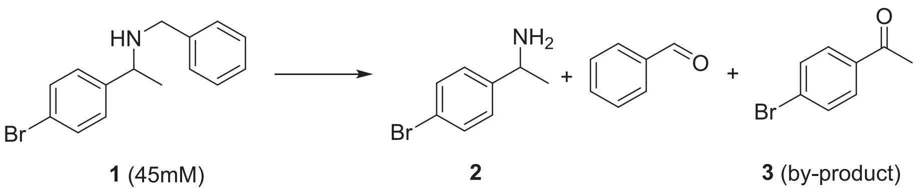
Reaction conditions: **1** (0.45 mmol, 45 mM), Laccase *Almac LAC97* (32.8 U), TEMPO (33 mol%), 30 °C, 250 rpm stirring via stir bar, open-to-air, 24 h.

[a] Conversion based on **1** depletion measured by HPLC area.

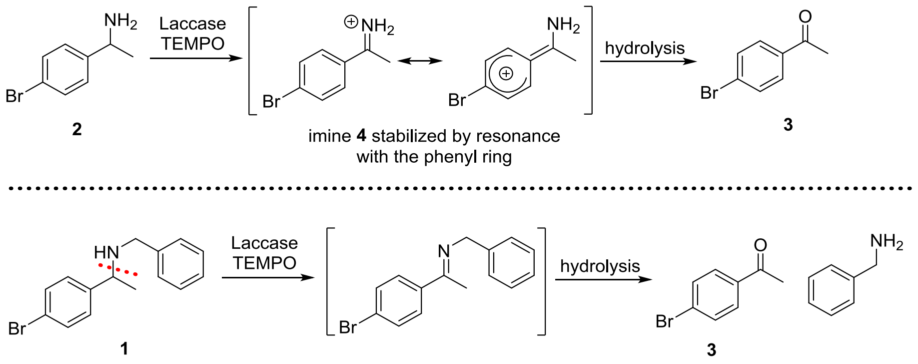
[b] Reaction temperature increased to 35 °C.



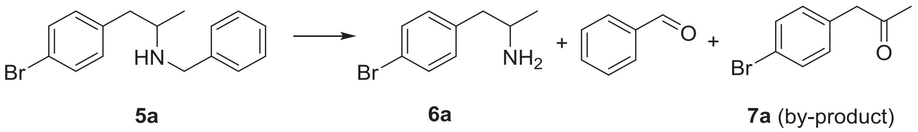
Scheme 1. Chemoselectivity of N-debenzylation of aryl-halogenated amines using the Laccase/TEMPO system in contrast to the conventional method such as palladium-catalysed hydrogenation.



Scheme 2. N-debenzylation reaction of **1** using the Laccase/TEMPO system. Reaction condition: **1** (0.45 mmol, 45 mM), Laccase *Almac LAC97* (32.8 U), TEMPO (33 mol%), pH 5.0, 200 mM Tris.HCl buffer (10 mL), 30 °C, 250 rpm stirring via stir bar, open-to-air, 24 h.



Scheme 3. Two potential pathways that led to the formation of by-product **3**.

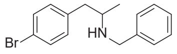
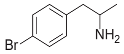
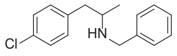
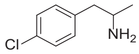
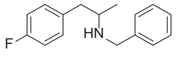
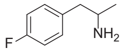
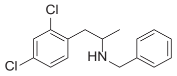
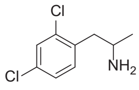
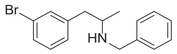
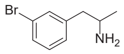
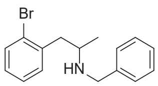
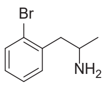


Scheme 4. N-debenzylation reaction of **5a** using the Laccase/TEMPO system. Reaction conditions: Laccase *Almac LAC97* (32.8 U), TEMPO (33 mol%), pH 4.0 200 mM acetate buffer (9.5 mL), 5 % DMSO v/v (0.5 mL), 30 °C, 250 rpm stirring via stir bar, open-to-air, 24 h.

increasing temperature to drive the reaction to completion while limiting overoxidation led to lower conversion rates. While the conversion rate improved between 0 to 6 h, the reaction stalled and did not reach completion even at 24 h, possibly due to enzyme denaturation (Table 1, entry 3). Subsequently, the reaction temperature was maintained at 30 °C for further parameter investigations. Throughout the development, solubility tests revealed that the pH of the reaction played an important role on the solubility of **1** as anticipated. When using 50 mM buffer, the pH of the reaction gradually increased from the initial pH 5.0 to pH 6.5 at the 24 h timepoint, with a formation of precipitate observed and attributed to reactants. Adjustment of the buffer pH to 4.0 ensured that all materials were dissolved prior to sample preparation and increasing buffer concentration from 50 mM to 200 mM limited the change in pH over time (for the solubility test, see ESI†). With this buffer concentration and pH, the reaction reached 71 % at 6 h and full conversion at 24 h (Table 1, entry 4). Pleasingly, there was no trace of dehalogenation of the substrate or product indicated by the absence of peaks on HPLC chromatograms corresponding to the dehalogenated compounds.

A by-product was observed during HPLC monitoring of the reaction was later confirmed to be 4-bromoacetophenone (**3**), consistent with Martinez-Montero's results. It was speculated that the by-product **3** was formed through two potential pathways, illustrated in Scheme 3. Based on a previous article by Galletti and co-workers [20], the amine group of product **2** which is benzylic can undergo overoxidation to the imine **4** that is subsequently resonance stabilized, and is eventually hydrolysed to form the by-product **3.** This is supported by a further overoxidation experiments which confirmed that the by-product **3** was formed from product **2** under the typical conditions of the Laccase/TEMPO reaction (for details of the overoxidation test, see ESI†). Alternatively, the amine substrate **1** can also be oxidised from a different side resulting in the release of by-product **3** and benzylamine. Confirming which pathway the by-product was formed by proved difficult, as benzylamine co-eluted closely with DMSO, and the imine was hydrolysed under HPLC analysis conditions. The formation of the by-product **3** persisted throughout the development work and the rate of its formation increased as the rate of N-debenzylation increased. A workaround for this issue involved additional reactants, which will be discussed in more detail later. After several unsuccessful attempts at changing the reaction conditions, it was surmised that the formation of by-product **3** could not be limited by adjusting buffer and reaction conditions alone. The focus was shifted to substrates where the amine was not conjugated (i.e. not benzylic to) the phenyl ring, to determine if the overoxidation issue persisted.

Table 2
Substrate scope of chemoselective N-debenzylation of amine with various aryl-halides.

Entry	Substrate	Product	Conversion (%)[a,b]	
			6 h	24 h
1	 **5a**	 **6a**	80[c]	99
2	 **5b**	 **6b**	85[c]	>99
3	 **5c**	 **6c**	79[c]	>99
4	 **5d**	 **6d**	90[c]	>99
5	 **5e**	 **6e**	84[c]	>99
6	 **5f**	 **6f**	78	>99

Reaction conditions: Substrate **5** (0.45 mmol, 45 mM), Laccase *Almac LAC97* (32.8 U), TEMPO (33 mol%), pH 4.0 200 mM acetate buffer (9.5 mL), 5 % DMSO v/v (0.5 mL), 30 °C, 250 rpm stirring via stir bar, open-to-air, 24 h.

[a] Conversion based on substrate **5** depletion measured by HPLC.

[b] >99 % conversion was indicated by the disappearance of **5** in HPLC analysis.

[c] Trace amount (<2 %) of ketone by-product **7** detected between 6 h and 24 h.

To ascertain if the oxidative deprotection could be deployed, N-

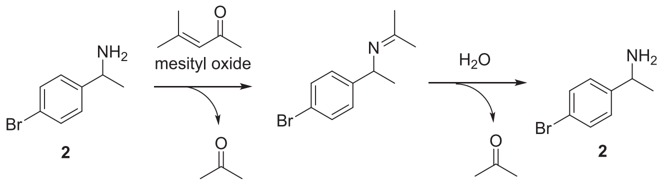


Fig. 2. Proposed imine formation with mesityl oxide to suppress overoxidation of the free amine **2**.

benzyl-1-(4-bromophenyl)propan-2-amine (**5a**) was selected as a model substrate (Scheme 4). The optimized reaction conditions from the previous substrate **1** were applied to this new model substrate **5a** (Table 1, entry 4). Similarly, both the substrate and product reference for HPLC analysis were synthesized from commercially available 4-bromophenylacetone (**7a**).

With this modification, the rate of N-debenzylation of **5a** surpassed that of **1**, achieving 80 % conversion in 6 h and almost complete conversion (99 %) at 24 h (Table 2, entry 1). There were trace amounts (<2 %) of the ketone by-product **7a** detected by HPLC analysis at 24 h, which supported speculation of the stabilization of the benzylic imine **4** led to significant overoxidation. Encouraged by the satisfactory results from **5a**, a focused screen against substrates **5a-5f** was conducted with a selection of halogen moieties substituted on the aromatic ring. The conversion rates determined for substrates **5a-5f** were similar with all substrates achieving approx. 80 % conversion in 6 h (Table 2, entry 1–3), with the 4-Cl being the best of the para monosubstituted substrate (Table 2, entry 1–3). *Ortho*- and meta-substituted substrates were also tested with comparable conversion rates (Table 2, entry 5–6). The system also tolerated an amine **5d** with two chloro-moieties attached (Table 2, entry 4), which achieved the highest conversion rate of 90 % in 6 h. There was a trace amount (<2 %) of ketone by-product detected at 24 h (detailed HPLC analysis is in the supporting information). In all cases, no sign of dehalogenation, indicated by extra peaks in the HPLC analysis was detected, and the concentration of product **6** detected in the HPLC analysis matched well with the depletion of the substrate **5**. Using the same optimized method and condition, the N-debenzylation of substrate **5a** was also carried out using Laccase *Trametes versicolor* (LTv) from SigmaAldrich, performing slightly wose than *LAC97*. However, it was possible that this could be caused by the difference in the optimum condition between LTv and *LAC97* (see S.I. for more detail). Overall, the chemoselective N-debenzylation reaction using the Laccase/TEMPO system was successful with only trace amounts of by-product being detected.

Using the benzylic amine substrate **1**, additional screening experiments were conducted to investigate the addition of selected additives to try to limit the overoxidation of **2** thus obtaining a cleaner product. Attempts at using Amberlite® 120H resin to capture the free amine product were unsuccessful, as the substrate amine was rapidly captured which resulted in little to no conversion, and addition of resin halfway through the reaction had no impact on the overoxidation. Attempts were made using mesityl oxide to limit the overoxidation while allowing the N-debenzylation reaction to proceed. Mesityl oxide has been used elsewhere for the protection of primary amines by forming a temporary imine (Fig. 2), however this imine was cleaved by the presence of water [23]. Although the N-debenzylation was conducted in buffer, it was hoped that the temporary formation of an imine moiety would slow down the overoxidation and allow quenching of the reaction thus maximizing the amount of product in the reaction mixture. Indeed, the addition of 1 equivalence of mesityl oxide successfully reduced the ratio of product **2** to by-product **3** from the initial 3:1 to 50:1 at 24 h (Table 3, entry 2). No other peaks were detected in the HPLC analysis. While this modification may have alleviated the by-product formation of N-debenzylation of **1**, it also slowed down the reaction and prevented it from reaching complete conversion at 24 h.

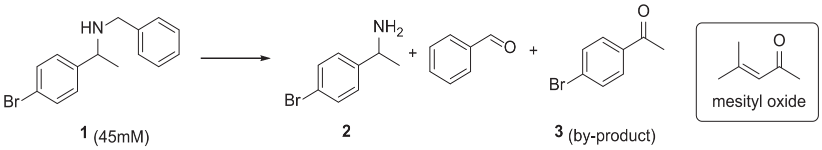
Conclusions

In conclusion, we have demonstrated the utility of the Laccase/TEMPO system in performing N-debenzylation of aryl-halogenated amines preventing dehalogenation under mild reaction conditions. In all substrates tested, the HPLC analysis indicated no evidence of dehalogenation even after 24 h. This system was compatible with substrates with various and numerous halogens attached to an aromatic ring. For the substrates screened herein, the reaction achieved full conversion or almost full conversion at 8 h, with only trace amounts of ketone by-product detected after 24 h. The limitation of this system is the presence of two benzylic position for the amine. This caused overoxidation of the free amine product leading to the formation of a significant amount of ketone by-product. This can be alleviated using additives such as mesityl oxide, but with slower reaction rates. The reduced solubility of the halogenated substrates was overcome by adjusting the buffer conditions and with the addition of the co-solvent DMSO. Overall, this chemoselective N-debenzylation is a useful alternative to the conventional N-debenzylation methods that utilize transition metal such as Pd/C with H_2.

CRediT authorship contribution statement

Hong Ann Gan: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Sean Reidy:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Noreen Morris:** Supervision, Funding acquisition, Conceptualization. **Megan Smyth:** Writing – review & editing, Validation, Supervision, Resources, Project administration. **Guang Xing Wang:** Validation, Supervision, Resources, Conceptualization. **Thomas S. Moody:** Supervision, Resources, Conceptualization.

Table 3
Addition of 1.0 equiv. mesityl oxide to suppress overoxidation of the free amine product 2.



Entry	Conditions[a]	Conversion (%)[b]		**2**:**3** ratio at 24 h
		6 h	24 h	
1	Without mesityl oxide	71	100	3.1: 1
2	1.0 equiv, mesityl oxide	68	91	50.6: 1

[a] All other conditions are the same as Table 1, entry 4.

[b] Conversion based on substrate **1** depletion measured by HPLC area.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2024.155298.

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