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Integrated Electro-Biocatalysis for Amine Alkylation with Alcohols

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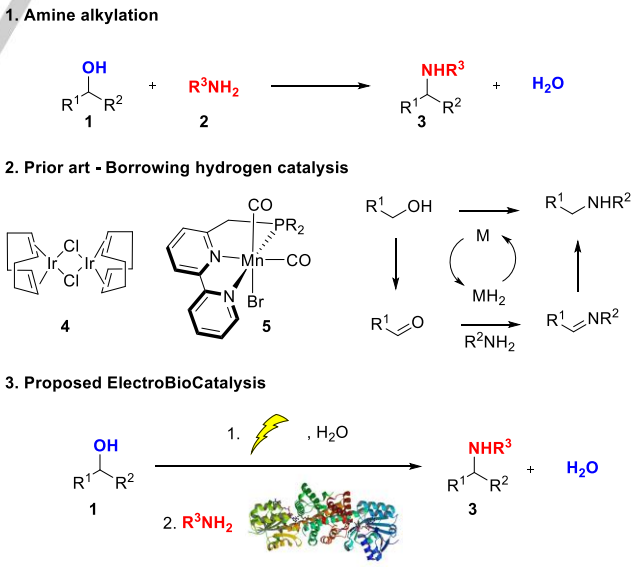
Abstract: The integration of electro and bio-catalysis offers new ways of making molecules under very mild, environmentally benign conditions. We show that TEMPO mediated electro-catalytic oxidation of alcohols can be adapted to work in aqueous buffers, with minimal organic co-solvent, enabling integration with biocatalytic reductive amination using the *Ad*RedAm enzyme. The combined process offers a new approach to amine alkylation with native alcohols, a key bond formation in the chemical economy that is currently achieved *via* precious metal-catalyzed hydrogen-borrowing technologies. The electrobio transformation is effective for primary and secondary alcohols undergoing coupling with allyl, propargyl, benzyl, and cyclopropyl amines, and has been adapted for use with solid-supported *Ad*RedAm for ease of operation.

The integration of different catalysis regimes can create new ways of building molecules, stimulating different disconnections with enhanced process sustainability and efficiency. Biocatalysis, in particular, offers exciting potential for integrated catalysis as the requisite reaction conditions (aqueous, mild temperatures and dilute concentrations) have historically dictated separate processing from other reactions in a synthetic sequence.[1] Recent developments in merging biocatalysis with transition metal,[2] photoredox,[3] and organo-[4] catalytic transformations have challenged this traditional model and created some highly innovative transformations that could not have been achieved under a single catalysis regime.

Our interest in developing new integrated biocatalysis systems[5] motivated us to examine electro-catalysis as a possible partner technology. Electro-synthesis is undergoing a vigorous renaissance in the literature,[6] as chemists seek to exploit its enormous potential for sustainable synthesis, using cheap and potentially renewable energy for redox transformations. Electro-bio processes have been extensively studied in areas such as fuel cells, biosensors, waste remediation, and co-factor recycling[7] but the analogous integration has yet to be widely explored for chemical synthesis.[8]

We chose to study the direct coupling of simple alcohols (**1**) and amines (**2**) as a putative electro-biocatalytic process (Scheme 1.1). This transformation is key to the future chemical economy as it creates a valuable C-N bond from readily available feedstocks, with excellent atom economy, in principle generating water as the only by-product. The low native reactivity of alcohols precludes direct amination using classical stoichiometric methods, but the reaction can be achieved using hydrogen-borrowing catalysis.[9] This richly innovative chemo-catalysis method takes transfer-hydrogenation metal complexes, initially from the precious platinum group (*e.g.* Ir complex **4**) but more recently encompassing the earth-abundant first row transition metals (*e.g.* Mn complex **5**),[9d] and sets up an oxidation / condensation / reduction cycle (Scheme 1.2). Sophisticated pincer ligands are usually required, and the reaction proceeds at high temperature (T > 100 °C). Biocatalytic hydrogen borrowing has also been achieved for amine-alcohol coupling although the inherent reversibility of the systems limits the conversions that can be obtained when employing oxidoreductases.[10] An electro-oxidation integrated with a biocatalytic reductive amination could offer a simple alternative, with potential for mild, sustainable reaction conditions and high conversions.

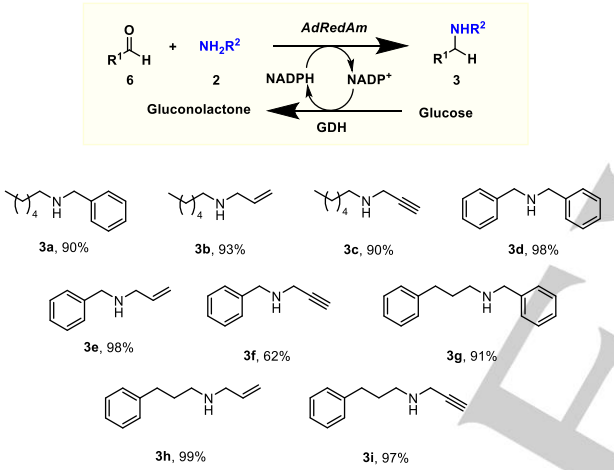


Scheme 1. Integrated electro-biocatalysis

We initially focused on the biocatalytic reductive amination component. Enzymatic reductive amination has recently been

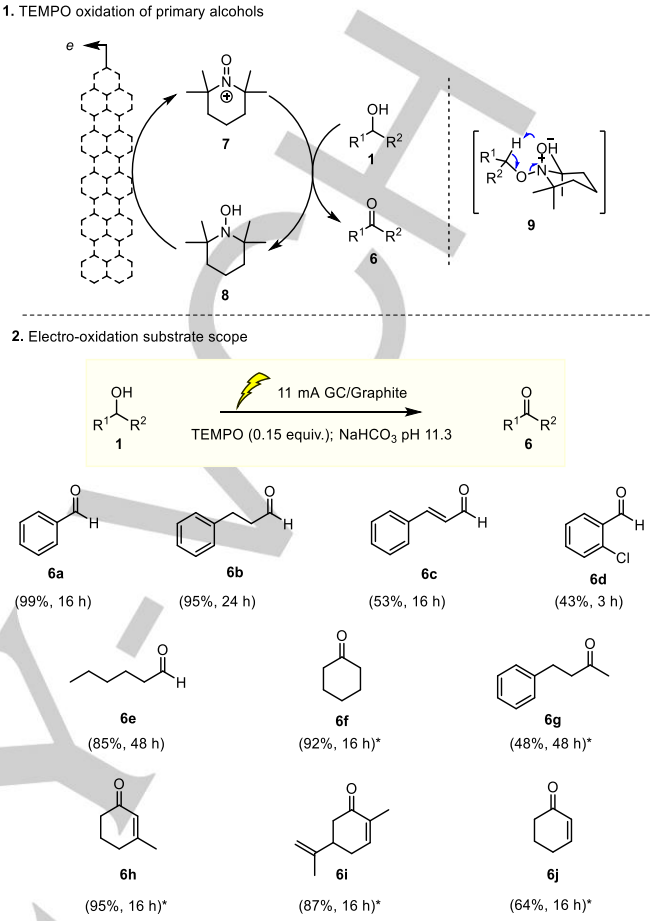
demonstrated for ketones using NAD(P)H- dependent imine reductases (IREDs).[11] In most of the cases excess amine is needed in order to get good conversions, as IREDs' principal role is to catalyze the reduction of pre-formed imines.[12] The Turner laboratory has recently discovered the fungal reductive aminases (RedAms), a sub-group of IREDs able to efficiently catalyze the reductive amination of a range of ketones at low ketone to amine ratio, which suggests that they catalyse imine formation in addition to imine reduction.[13] We selected the reductive aminase *Ad*RedAm, from *Ajellomyces dermatidis*, as the best candidate for integrative studies, due to its ability to participate in enzymatic cascades, high substrate tolerance and potential for preparative scale reactions.

Based on the previously reported activity of *Ad*RedAm with a range of ketones, [14] we investigated the ability of this enzyme to also perform the reductive amination of aliphatic aldehyde substrates including benzaldehyde, phenylpropionaldehyde and butyraldehyde (Scheme 2). We were pleased to observe excellent conversions for coupling these aldehydes with allyl, benzyl, and propargyl amines into the secondary amines **3a-3i**, under aqueous conditions containing 5% (v/v) DMSO at pH 7.4.[15]



Scheme 2. AdRedAm reductive amination of aldehydes. Reaction Conditions: aldehyde (10mM), amine (2 equiv.), glucose (4 equiv.), $NADP^+$ (1mM), AdRedAm (1mg/mL), GDH (0.5 mg/mL), 5% (v/v) DMSO, TRIS buffer pH 7.4, 250 rpm, 30 °C, 24 hour. Percentage conversions determined by GC.

Turning to the electro-catalytic reaction, we were attracted to the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) electro-oxidation system as a scalable, catalytic and environmentally benign method for the oxidation of alcohols.[16] The active species in TEMPO-mediated oxidation is oxoammonium **7**, which is electrochemically generated from TEMPO through an anodic single electron transfer process and is able to oxidize alcohol **1** to the corresponding aldehyde or ketone **6** (Scheme 3.1). During this process, hydroxylamine **8** is generated and rapidly re-oxidized to the TEMPO radical in the presence of oxygen. Intermediate **9** has been proposed for the interaction of oxoammonium **7** and the alcohol **1** in basic conditions.[16b] Literature examples for the electrolytic TEMPO-mediated oxidation of alcohols generally require the employment of organic solvents in ratios between 30 and 95 %, in part due to the fact that TEMPO is sparingly soluble in polar electrolytic media.[6a] As our *Ad*RedAm reductive amination tolerated a maximum of 5% (v/v) DMSO before showing loss of activity, this divergence in reaction media was clearly an important challenge to solve for the development of an integrated process.



Scheme 3. 1. TEMPO-mediated electrochemical alcohol oxidation. 2. Substrate tolerance of TEMPO-mediated electro-oxidation in water. Reaction conditions: Alcohol (20 mM); 0.1 M $NaHCO_3$ aqueous buffer solution pH 11.3; 5% (v/v) DMSO, rt. Percentage conversions determined by GC. * 0.2 equiv of TEMPO used.

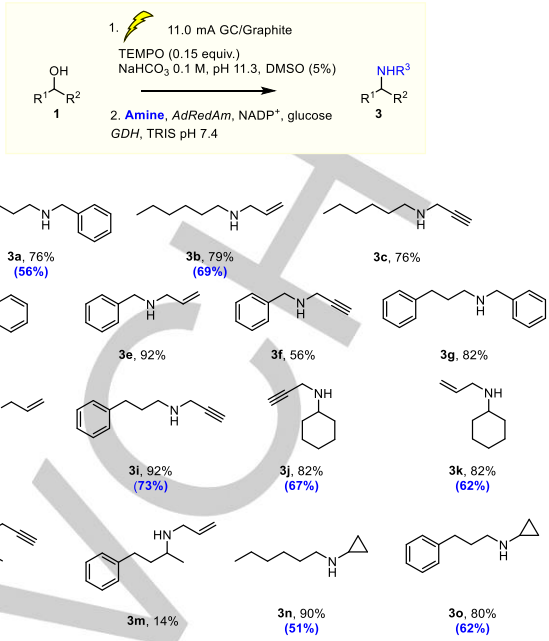
Optimization of the electro-oxidation was conducted using a commercially available ElectraSyn device fitted with an undivided cell (5.5 cm^2 per electrode for a liquid volume of 10 mL, total current of 11 mA). A study of TEMPO electro-oxidation in aqueous buffers was carried out with respect to electrode materials, equivalency, common additives, pH [17] and reaction time dependence (see supporting information), establishing optimal conditions of 0.15 equivalents of TEMPO, pH 11.3 using $NaHCO_3$ buffer with a glassy carbon (GC) working electrode and graphite counter electrode. A range of primary and secondary alcohols were then successfully oxidized under these aqueous electrocatalytic conditions (Scheme 3.2). Highest conversions were obtained for aromatic primary alcohols **6a** and **6b**. The system allows the presence of halogen groups in the aromatic ring, although the reaction should be stopped earlier to avoid the generation of several by-products by halogen group reaction at the cathode in our undivided cell. (**6d**) Primary aliphatic alcohols such as 1-hexanol (**6e**) provided lower conversions, which is consistent with the literature reports.[18] Our system also allowed the electro-oxidation of secondary aliphatic alcohols, although the addition of 0.2 equivalents of TEMPO were needed (**6f-6j**). It is

remarkable that only traces of the corresponding carboxylic acids were observed during the oxidation of linear aliphatic alcohols (**1c** and **1e**). Overall the reaction was very clean with excellent specificity, using the cheap GC as the WE and proceeding under aqueous conditions with no requirement for additional bases or electrolytes (e.g. tetraalkylammonium salts).

Initial investigations into integrating the electro/bio catalysis involved taking aliquots of the ketones and aldehyde products direct from the electro-oxidation and adding them to solutions of aqueous buffer containing allyl amine, *Ad*RedAm, GDH, glucose and cofactor $NADP^+$. Unfortunately, the conversions were very low, suggesting a pH and media incompatibility – the very basic conditions for optimal electrosynthesis in carbonate buffer (pH 11) were not compatible with enzymatic reductive amination in TRIS media (optimal pH 7.8). We re-examined the biotransformation in a series of buffers at different pH values (see supporting information, Scheme S4), and saw substantial deterioration in activity in alkaline media (*e.g.* 50% conversion using $NaHCO_3$ buffer pH 11.3). However, encouraging results were obtained in buffer mixtures, with a conversion of 89% at pH 8.0 in 1:2 mixture of $NaHCO_3$ / TRIS system. We were delighted to find that this medium was effective for an integrated electrobio process; 1-hexanol undergoing electro-catalytic TEMPO oxidation at pH 11.3 followed by addition to *AdRedAm* in aq TRIS buffer for amination (overall pH 8.0) affording a good 76% conversion. The reaction conditions were general for the primary alcohol and amine components previously examined in Schemes 2 and 3, delivering the secondary amine products **3a** – **3p** in good to excellent conversion (Scheme 4). The integrated reaction was also effective for cyclohexanol as a secondary substrate (**3j** and **3k**), but 1-phenylbutan-3-ol was problematic, affording low yields of the reductively aminated products **3l** and **3m**. We were also able to introduce cyclopropylamine as a secondary alkyl amine component, efficiently transforming into **3n** and **3o** with aldehyde substrates.

Improving the scale of the process required some optimization around the 20 mL reaction volume of the Electrasyn apparatus (see supporting information). Alcohol concentrations above 50mM started to generate significant quantities of pinacol condensation products, and we established 40mM alcohol as optimal. The reaction mixture (20 mL) containing the electrochemically generated oxidation product and TEMPO at pH 11.3 could then be added direct to a 40 mL solution containing the enzymatic system, cofactor, glucose and amine in TRIS buffer at pH 7.4. After 24 hours at 30 degrees and 250 rpm the corresponding amines **3a**, **3b**, **3h** – **3k**, **3n** and **3o** were isolated in good overall yields (51-73%).

Finally, we turned our attention to strengthening the processivity of the method through enzyme immobilization, a fundamental consideration for industrial biotransformations where work-up and catalyst re-use are critical. Our previous work had identified the increased stability of RedAm enzymes on a functionalised pore glass support,[19] so we decided to immobilize our enzymatic system (*Ad*RedAm and GDH) on EziG Amber support. Pleasingly, we observed successful re-use in three cycles for the 3-phenyl-propan-1-ol substrate, forming amine **3h** in 70%, 70%, and 57% in three consecutive cycles. A fourth cycle gave diminished yields (<20%).



Scheme 4. Reaction Conditions: 1) Electrolytic oxidation: alcohol (20mM), TEMPO (0.15 equiv), $NaHCO_3$ 0.1 M pH 11.3, 5% (v/v) DMSO, rt, 18 hours; 2) Reductive amination: amine (2 equiv.), glucose (4 equiv.), NADP+ (1mM), AdRedAm (1mg/mL), GDH (0.5 mg/mL), TRIS buffer solution pH 7.4, 250 rpm, 30 °C, 24 hours. Isolated yields in blue.

In summary we have developed an integrated electrobio-catalytic approach to amine alkylation. The process harnesses the versatility of two powerful catalysis systems: TEMPO electro-oxidation and *AdRedAm* biocatalytic reductive amination, merging them *via* the development of a single compatible aqueous reaction medium with minimal organic solvent. The reaction proceeds under exceptionally mild conditions, with no requirements for precious metals and accompanying ligands. Further applications of electrobio-catalysis for chemical synthesis are underway in our laboratories.

Keywords: biocatalysis • electrocatalysis • amines • reductive amination •

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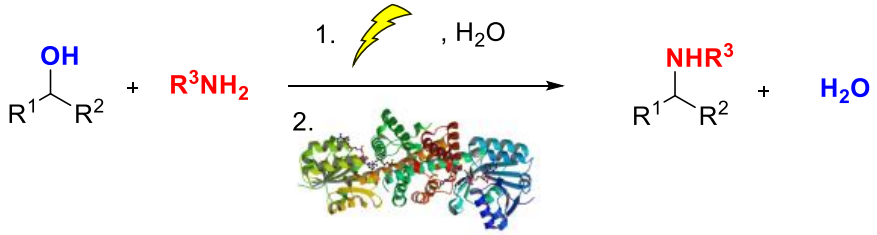
Entry for the Table of Contents



An integrated electrobiocatalytic amine alkylation reaction is reported that proceeds under very mild, environmentally benign conditions. Using a combination of aqueous TEMPO electrocatalysis and *Ad*RedAm biocatalytic reductive amination, a range of primary and secondary alcohols can be coupled with allyl, propargyl, benzyl, and cyclopropyl amines in a single operation.

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