Cofactor-free biocatalytic hydrogenation of nitro compounds for synthesis of amines

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Abstract

We report a new paradigm for chemoselective hydrogenation of nitro compounds to amines, under mild, aqueous conditions. Hydrogenase enzyme releases electrons from H_2 to a carbon black support which facilitates nitro-group reduction. For 30 nitroarenes we demonstrate full conversion (isolated yields 78 – 96%), with products including pharmaceuticals benzocaine, procainamide and mesalazine, and 4-aminophenol – precursor to acetaminophen (paracetamol). We also showcase gram-scale synthesis of procainamide with 90% isolated yield. We demonstrate potential for extension to aliphatic substrates. The catalyst is highly selective for reduction of the nitro group over other unsaturated bonds, tolerant to a wide range of functional groups, and exhibits excellent stability in reactions lasting up to 72 hours and full reusability over 5 cycles, indicating scope for direct translation to fine chemical manufacturing.

Main

Efficient and sustainable routes to synthesis of amines remain in high demand for the production of pharmaceuticals (Fig. 1A) and agrochemicals as well as other areas of chemical manufacturing. This has led to a wide range of developments in selective methods for amine synthesis.[1–3] The reduction of nitro-groups is a common synthetic route to amines, and is a key target for greener synthetic protocols because the available routes are dominated by use of stoichiometric reductants or precious-metal hydrogenations which often lack functional group selectivity (Fig. 1B).[4–7] Recent developments have focused on organocatalysts, or the more abundant first row transition metals as either heterogeneous or homogeneous hydrogenation catalysts (including for transfer hydrogenations using formic acid, hydrazine or $NaBH_4$), and have led to some improvements in functional group tolerance.[4,8,9] Although biocatalytic reduction of unsaturated bonds is often viewed as an environmentally friendly and more selective alternative to metal-catalyzed hydrogenations in the pharmaceutical sector, biocatalytic strategies are still in the early stages of development for the 6-electron reduction of nitro groups. Nitro reductions by flavin-containing nitroreductase enzymes typically rely upon multiple equivalents of glucose to recycle the costly redox cofactor, NAD(P)H, and often fail to progress beyond the *N*-hydroxylamine intermediate, although this can be mitigated by photocatalysis[10] or addition of a co-catalyst such as V_2O_5.[11–13] Electrochemical reduction of nitro compounds to give the corresponding amine at a carbon electrode surface in an aqueous electrolyte has been reported.[14] It has been noted recently that nitro

group hydrogenations at palladium on carbon (Pd/C) heterogeneous catalyst may actually proceed *via* a redox mechanism whereby H_2 oxidation occurs at active sites on the Pd and provides electrons for reduction of the nitro compound at the carbon support.[15] However the presence of Pd nanoparticles under H_2 can lead to a range of unwanted side reactions such as hydrogenation of other unsaturated bonds or dehalogenation, while sulfur-containing substituents may poison the precious metal catalyst.[4,5] In contrast, hydrogenases are able to split H_2 at a buried active site which is inaccessible to larger organic substrates, and release electrons, *via* a chain of iron-sulfur clusters, to the protein surface.[16] This suggested to us the possibility of exploiting hydrogenase on carbon as a selective site-separated biocatalyst system for nitro hydrogenations, with no requirement for expensive redox cofactors (Fig. 1C).

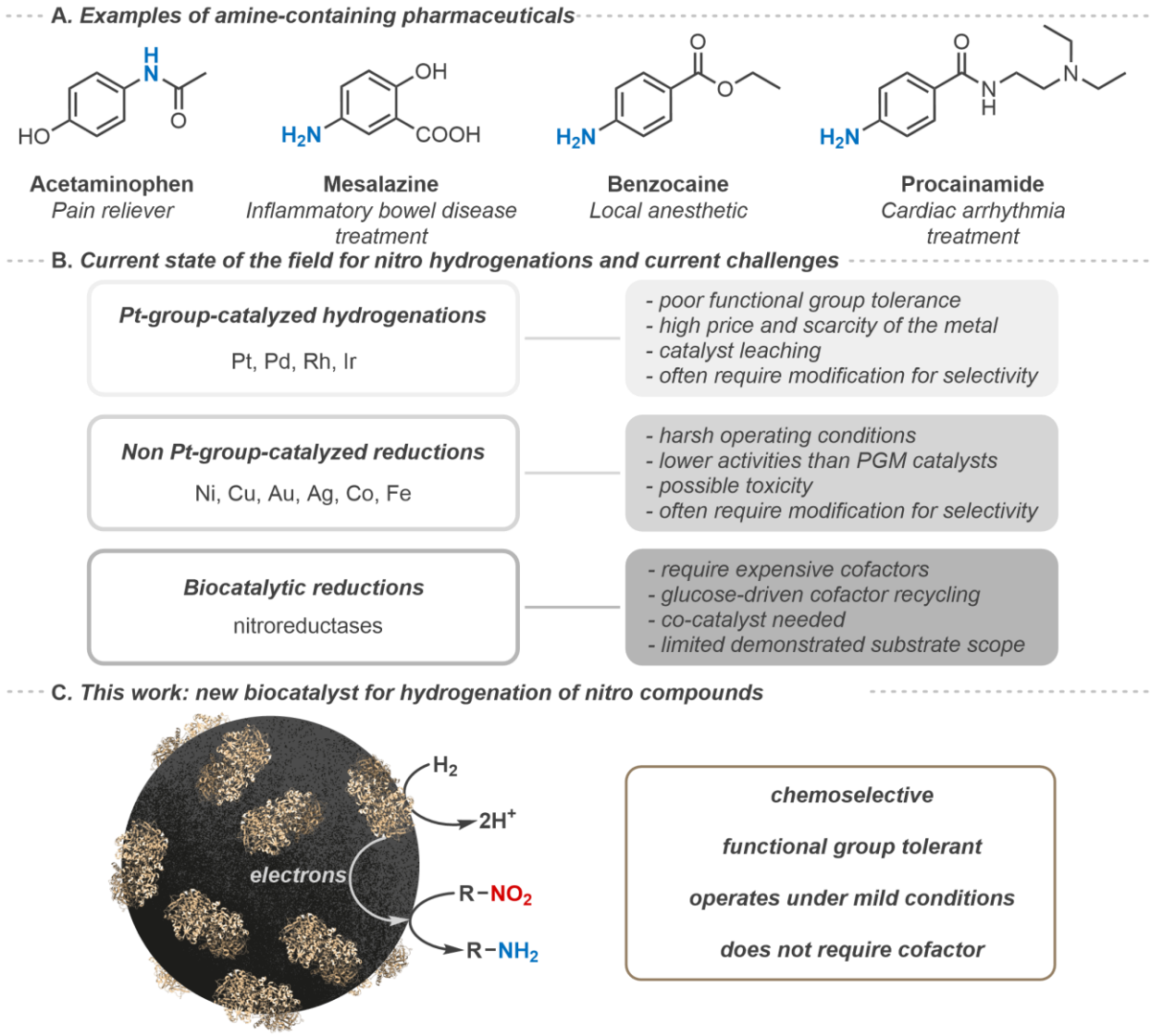

Figure 1. A. Examples of amine pharmaceuticals relevant to this work. **B.** Currently-used methods for the reduction of nitro compounds to generate amines. **C.** Hydrogenase enzyme (gold) immobilized on the surface of carbon black particle as a catalyst for efficient chemoselective synthesis of amines (this work).

We have shown previously that the nickel-iron enzyme, hydrogenase, adsorbs readily onto a carbon black support and is able to channel electrons from H_2 oxidation into the carbon where they can be taken up by a co-adsorbed reductase enzyme for a coupled reduction, such as the conversion of NAD^+ to NADH by an NAD^+ reductase moiety.[17–19] For this study we select the robust and O_2-tolerant nickel-iron (NiFe) hydrogenase 1 from *Escherichia* (*E.*) *coli* (Hyd-1), which is purified readily following modest over-expression in the native host organism.[20]

The onset potential for reduction of nitrobenzene (**1**) (Fig. 2A, Fig. S48), a model aromatic nitro compound, on a graphite electrode in aqueous medium at pH 6.0 commences at -0.113 V vs the standard hydrogen electrode (SHE; all subsequent potentials are quoted vs this reference). At pH 6.0, 1 bar H_2, the potential of the proton/dihydrogen couple, $E'(2H^+/H_2)$ is -0.355 V. The fact that the onset of nitrobenzene reduction is positive of $E'(2H^+/H_2)$ means that reduction of the nitroaromatic compound by H_2 is thermodynamically feasible, i.e. the overall reaction has a negative free energy change. Hyd-1 has a small over-potential relative to $E'(2H^+/H_2)$,[21] with the onset potential for H_2 oxidation lying at -0.296 V (Fig. 2B). We therefore hypothesized that a catalyst comprising Hyd-1 immobilized on carbon black particles (Hyd-1/C) should be able to carry out the hydrogenation of nitrobenzene, where the reduction of the nitro group would occur at the carbon surface, akin to an electrochemical half reaction, using electrons supplied from H_2 oxidation by the hydrogenase as shown in Figure 1C.

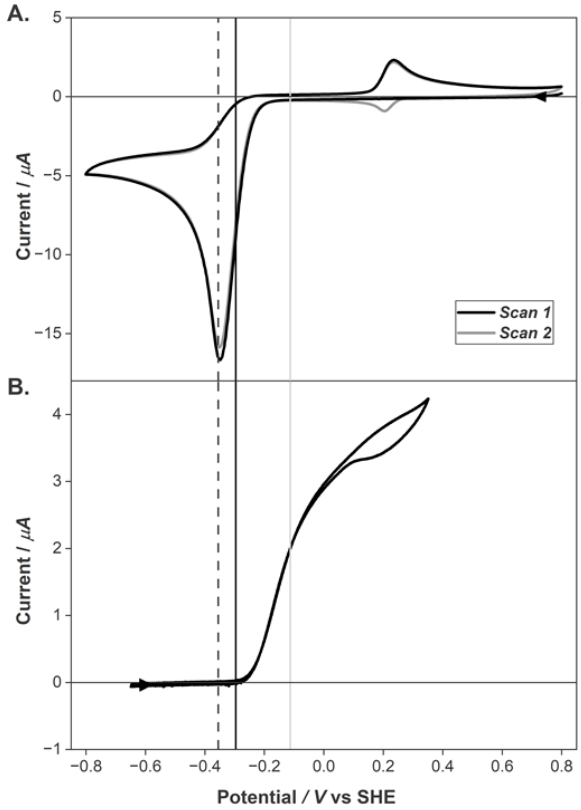

Figure 2. Onset potential for nitrobenzene reduction and H_2 oxidation on a carbon electrode. Cyclic voltammograms for **A**: nitrobenzene at a stationary graphite electrode under a N_2 atmosphere, scan rate 10 mV/s; and **B**: a film of Hyd-1 adsorbed onto the electrode under H_2 with electrode rotation at 3000 rpm, scan rate 1 mV/s. Potentials are quoted vs the standard hydrogen electrode, SHE. Dashed vertical line: potential of the $2H^+/H_2$ couple at the experimental conditions, $E'(2H^+/H_2)$; solid black vertical line: measured onset potential for H_2 oxidation by Hyd-1; solid gray vertical line: measured onset for nitrobenzene reduction. Conditions: 25 °C, 1 bar H_2, pH 6.0.

We therefore tested the feasibility of nitrobenzene hydrogenation using a Hyd-1/C catalyst. Hyd-1 was immobilized by direct adsorption onto carbon black which we have previously shown is suitable for direct electron-exchange with Hyd-1.[22,23] After 12 hours of reaction under H_2 flow, we observed full conversion of 10 mM nitrobenzene to aniline (**1a**) with no side products. Control experiments confirm that both Hyd-1 and carbon particles are necessary for this reactivity (Fig. S3). These results encouraged us to explore a wide range of aromatic nitro compounds to understand the substrate scope, functional group tolerance, and chemoselectivity of the Hyd-1/C catalyst, as summarized in Figure 3. All nitrobenzene derivatives shown in Figure 3 were fully hydrogenated to the corresponding amine by Hyd-1/C at 1 bar H_2. Some substrates required 10% v/v% of MeCN as a co-solvent to overcome the solubility issues, and for some the length of reaction time or catalyst loading were increased to facilitate full conversion (Section IV of the Supplementary Materials). These results demonstrate the high tolerance of this biocatalyst system to different substituents on the aromatic ring.

Halogenated substrates **8**-**10**, and **15**-**16** were selected to test the ability of Hyd-1/C to hydrogenate the nitro-group without dehalogenation. Promisingly, complete, selective conversion to the amine products with no loss of the halogen substituent (Cl, Br, I) was observed in all cases (Fig. S13-S15, S20-S21). Additionally, no side reduction was observed for substrate **17**, which is often the case for the reduction of benzylic alcohols and their derivatives using Pd/C (Fig. S22).[24] Thiolate moieties are known to poison precious metal-based catalysts, such as Pd/C,[25] but full conversion of substrate **25** was achieved in 24 hours using the Hyd-1/C catalyst (Fig. S30).

Selectivity of Hyd-1/C for hydrogenation of the nitro group was demonstrated with substrates **18**, **21**, **26**-**28** for which full conversion of the nitro group to the amine was observed, with no evidence for reduction of ketone, aldehyde, alkene, alkyne or nitrile groups (Fig. S23, S26, S31-33). Substrate **28** required higher catalyst loading and pH 8.0 to suppress the side reaction of alkyne hydration.

Sterically hindered substrates **2**, **5**, **8**, **11** and **29** and substrates with the bulky *tert*-butyl group in the *ortho*- or *para*- position (**23** and **24**), were fully converted to the corresponding aniline derivatives, although some required higher catalyst loadings and/or extended reaction time (Fig. 3 and Fig. S7, S10, S13, S16, S34, S28, S29).

Substrates with two nitro groups (**11**-**13**) were found to be reduced completely to the corresponding di-amine using the Hyd-1/C catalyst under the experimental conditions employed (Fig. S16-S18).

We next demonstrated the use of the catalyst on nitro compounds which are precursors to pharmaceuticals. Reduction of substrate **7** gives 4-aminophenol, which requires only a simple acetylation of the amine to form the widely-used pain reliever, acetaminophen (paracetamol). Hydrogenation of substrate **20** produces the local anesthetic, benzocaine. Reduction of substrate **30** generates the important drug molecule, mesalazine, which is used to treat inflammatory bowel disease and features in the World Health Organisation List of Essential Medicines.[26]

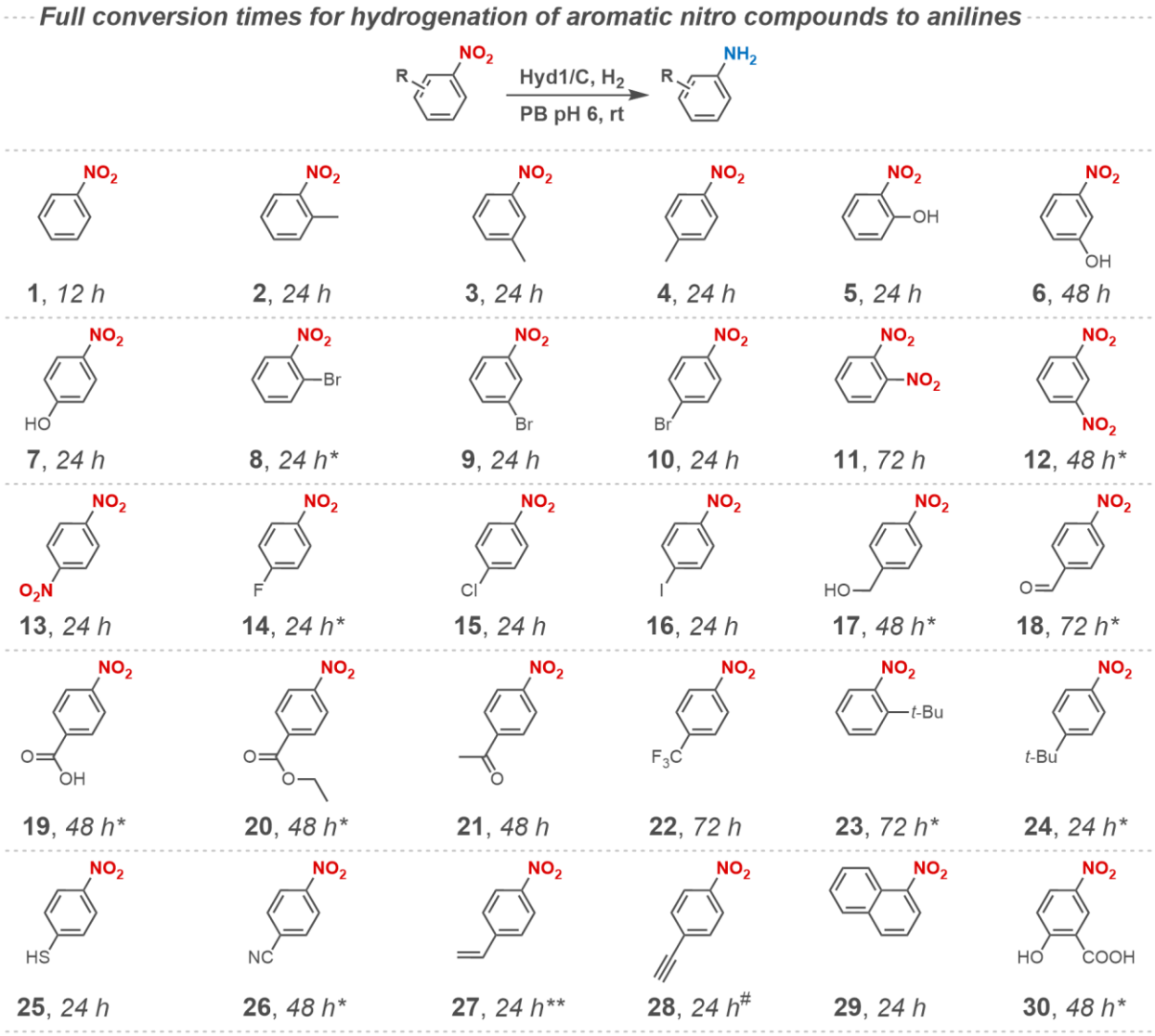

Figure 3. Substrate scope of hydrogenation reactions achieved with the Hyd-1/C catalytic system at 10 mM concentration of substrate, 2 mL reaction volume, 0% or 10% v/v % of MeCN in sodium phosphate buffer (PB, 50 mM, pH 6.0, unless stated otherwise), room temperature, 1 bar H_2. *Double catalyst loading. **Quadruple catalyst loading. #Double catalyst loading, pH 8.0.

Having demonstrated Hyd-1/C as a versatile catalyst for nitroarene reductions, we now focus on reaction-scale-up and isolation of products, summarized in Figure 4. For most of the substrates, the corresponding amines were isolated by simple extraction with organic solvent without any further purification (see Section V of the Supplementary Materials) with 78 – 96% yield (Fig. 4A). For the highest yielding product, **29a**, this represents 2.22×10^5 turnovers of Hyd-1 during the 24-hour reaction. Some loss of product is likely to occur during the workup due to the relatively small scale of these reactions. For chemically unstable products (**18a** and **25a**) the yields were determined by ^{1}H-NMR spectroscopy with the use of an internal standard.

To demonstrate scalability of the biocatalytic system for a pharmaceutically-relevant product, we chose reduction of *N*-(2-(diethylamino)ethyl)-4-nitrobenzamide (**31**) to procainamide (**31a**) – medication for treatment of cardiac arrhythmia.[27] The precursor **31** was synthesized in one step from the commercially available 4-nitrobenzoyl chloride and *N*,*N*-diethylethylenediamine (Fig. 4C) and then was subsequently hydrogenated using Hyd-1/C to yield 1.10 g of **31a** with 96%

purity (90% yield). This clearly indicates scalability, and potential applicability of this system in the production of fine chemicals and their precursors.

To understand aspects of the mechanism of nitro hydrogenation we undertook further experiments with **1** as a model substrate. Figure 4B presents ^{1}H-NMR traces of the reaction progress of nitrobenzene hydrogenation, carried out at ambient H_2 pressure, in the presence of the Hyd-1/C catalyst. After 30 minutes, signals corresponding to the *N*-phenylhydroxylamine (**1b**) intermediate were observed. By 1 hour, the starting nitro compound **1** was fully consumed, giving **1b** with traces of **1a**. By 12 hours, complete conversion to the aniline was achieved. These results indicate that the reduction of nitrobenzene using Hyd-1/C proceeds *via* four-electron reduction to the *N*-phenylhydroxylamine intermediate. This is also corroborated by closer examination of the electrochemistry of nitrobenzene in aqueous solvent. Cyclic voltammograms of **1** (Fig. 2A) reveal that after the first cathodic sweep, a new, reversible redox couple, centered at *ca* +0.21 V, appears on the return and subsequent sweeps. By comparison with analytical standards (Fig. S44–45), this new wave is assigned to the two-electron oxidation of **1b** to nitrosobenzene, which is subsequently re-reduced on the reverse sweep, providing further evidence that **1b** is indeed produced at the potential of the nitrobenzene reduction wave. Together these results support a mechanism for the Hyd-1/C catalyst whereby nitrobenzene is first reduced by four electrons to the *N*-phenylhydroxylamine derivative, which can be subsequently reduced to the aniline. This is further evidenced in a catalyst recycling experiment, made possible because of the heterogeneous nature of our biocatalytic system (Fig. S43). Full conversion of nitrobenzene to aniline was recorded over five cycles of recovery/re-use, representing a total turnover number (TTN) for Hyd-1 of 1.16×10^{6}. In subsequent re-use cycles, the percentage of *N*-phenylhydroxylamine intermediate increased gradually, although the starting material **1** was still fully consumed over 13 cycles in total.

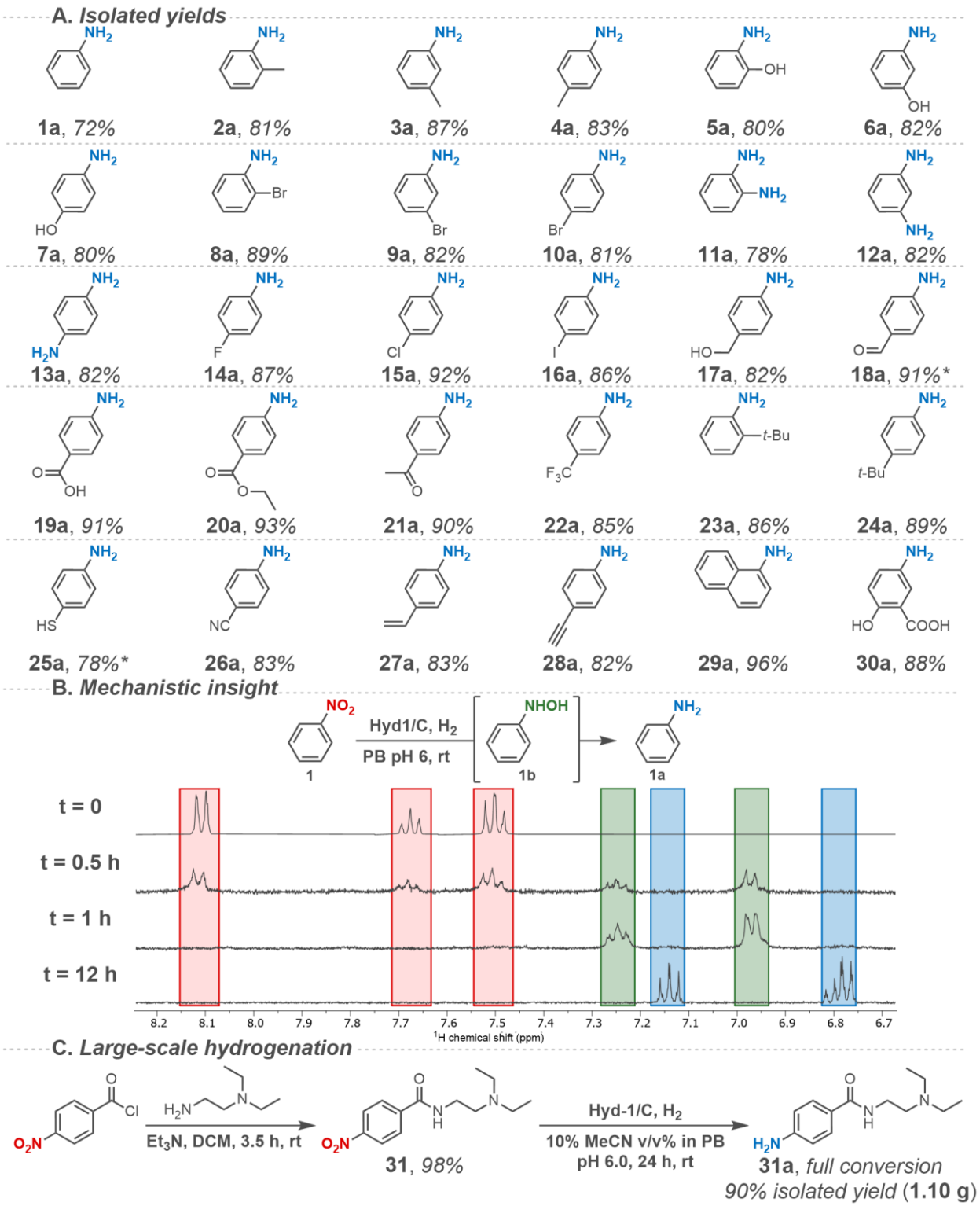

Figure 4. A. Isolated yields (%) for **1a–30a**. Conditions: 10 mM substrate, 2 mL reaction volume, 0% or 10% v/v % of MeCN in PB (50 mM, pH 6.0, unless stated otherwise), room temperature, 1 bar H_2. *^{1}H-NMR yields. **B.** ^{1}H-NMR traces of hydrogenation of **1** at indicated time points. Conditions: Hyd-1/C, 10 mM **1**, PB (50 mM, pH 6.0), room temperature, 1 bar H_2. Traces for **1**, **1b**, and **1a** are labelled with red, green, and blue, respectively. **C.** Synthesis of substrate **31** and its hydrogenation on a gram-scale. Conditions: Hyd-1/C, 10 mM **31**, 500 mL reaction volume**,** 10% MeCN v/v% in PB (50 mM, pH 6.0), room temperature, 1 bar H_2.

Figure S48 shows the onset potentials for the reduction of a selection of nitroarenes under aqueous conditions. All have an onset potential for reduction which is positive of $E'(2H^+/H_2)$, and also positive of the onset for H_2 oxidation by Hyd-1. We also tested the aliphatic nitro compound, 1-nitrohexane (**32**), which has a more negative onset potential of -0.313 V. Attempts to reduce **32** using the Hyd-1/C catalyst gave no product (Fig. S77). These data allow us to set a tentative lower limit for the potential of nitro substrates which could be hydrogenated by Hyd-1/C without application of more forcing conditions.

We hypothesized that replacing Hyd-1 with another NiFe hydrogenase from *E. coli*, Hydrogenase 2 (Hyd-2), which shows no overpotential for H_2 oxidation, i.e. operating at $E'(2H^+/H_2)$,[21] might allow us to extend this catalytic approach to more challenging substrates. We were therefore encouraged to test whether Hyd-2/C was able to hydrogenate the aliphatic nitro compound **32**. In a 48 hour reaction, **32** was fully converted to the hexylamine (**32a**) over the Hyd-2/C catalyst (Fig. S78), demonstrating that there is scope for expanding the 'hydrogenase on carbon' catalyst concept to aliphatic nitro compounds.

Conclusions

Here we present an easy-to-use, highly versatile catalyst for the synthesis of amines *via* hydrogenation of aromatic nitro compounds under mild conditions. The catalyst comprises a carbon black supported NiFe hydrogenase (Hyd-1) which enables use of H_2 at atmospheric pressure as an atom-efficient reductant, without need for a co-catalyst or cofactor. We confirmed tolerance of the biocatalytic system to a wide range of functional groups by hydrogenating various derivatives of nitrobenzene to the corresponding aniline, with isolated yields from 78% to 96%. The catalyst is fully recyclable over 5 reaction cycles, and is applicable for a gram-scale hydrogenation, as demonstrated with procainamide synthesis. We further show that this catalytic approach can be extended to hydrogenation of aliphatic nitro compounds by utilizing another NiFe hydrogenase (Hyd-2), which we confirm by hydrogenation of 1-nitrohexane as a model substrate. Overall, this work represents a valuable addition to the suite of approaches available for amine synthesis.

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Author Contributions

Conceptualization of the research was contributed by K.A.V., J.S.R., H.A.R., S.E.C, T.S., and D.S. Supervision of research was performed by K.A.V, H.A.R., S.E.C., J.S.R., T.S. and D.S.

Experimental investigations were conducted by J.S.R, S.E.C., G.S., T.S., D.S. and T.C.L. Writing the original draft was contributed by K.A.V. and D.S. Revisions to the manuscript were carried out by all authors. Funding acquisition was contributed by K.A.V. and H.A.R.

Competing interests

The authors declare their competing financial interests: a patent has been filed related to the catalyst system described in this manuscript (WO2023218206) and might afford royalties to the authors. This patent is licensed to HydRegen. K.A.V, H.A.R. and S.E.C. are founders of HydRegen.

Supplementary materials

Experimental procedures and characterization data are provided in the supplementary materials. Correspondence and requests for materials should be addressed to K.A.V.

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