RESEARCH ARTICLE doi.org/10.1002/adsc.202400460

Advanced Synthesis & Catalysis

Very Important Publication

Merging Organolithium Chemistry and Stereoselective Biocatalysis: Transformation of Aromatic Nitriles into Chiral Alcohols

Nicolás Ríos-Lombardía,[a,*] Gloria Morís-Menéndez,[a] Iván Lavandera,[b] Vicente Gotor-Fernández,[b,*] and Joaquín García-Álvarez[a,*]

[a] Laboratorio de Química Sintética Sostenible (QuimSinSos), Organic and Inorganic Chemistry Department, Instituto de Química Organometálica Enrique Moles, University of Oviedo. Avenida Julián Clavería 8, 33006 Oviedo, Asturias (Spain)
E-mail: rioslombardian.fuo@uniovi.es; garciajoaquin@uniovi.es

[b] Organic and Inorganic Chemistry Department, Instituto de Química Organometálica Enrique Moles, University of Oviedo. Avenida Julián Clavería 8, 33006 Oviedo, Asturias (Spain)
E-mail: vicgotfer@uniovi.es

Manuscript received: April 22, 2024; Revised manuscript received: May 29, 2024;
Version of record online: July 15, 2024

Supporting information for this article is available on the WWW under https://doi.org/10.1002/adsc.202400460. Complete experimental details for bioreduction protocols, spectroscopy data for alcohols **3a–j**, analytical data, and copies of chiral GC/HPLC chromatograms $^{1}H/^{13}C\{^{1}H\}$ spectra for alcohols **3a–j** are shown as Supporting Information (SI) to complement the results shown in the main manuscript.

Abstract: The combination of RLi-mediated organic transformations (under air and at room temperature) with a subsequent stereoselective biocatalytic reaction is for the first time presented. Most of the previous asymmetric chemoenzymatic routes have been limited to the concomitant or sequential combination of transition metals/organocatalysts with enzymes. However, the use of polar organometallic reagents (RLi) in the design of these stereoselective hybrid protocols has been totally neglected, as far as we are concerned. Thus, in this work, the combination of organolithium chemistry and asymmetric biocatalysis is described for the first time in a one-pot fashion. The chemoenzymatic approach converts a series of nitriles into chiral alcohols consisting of two steps, where the key item was the finding of suitable conditions to adapt the reactivities of organolithiums and alcohol dehydrogenases (ADHs) in the same recipient. The organolithium addition occurred with total chemoselectivity (no side reactions were observed) under neat conditions and room temperature leading to the corresponding imines, which were hydrolyzed using a buffer, and adjusting the pH for the subsequent ADH action. Commercial and made in house overexpressed ADHs allowed to produce chiral alcohols with excellent selectivities and good overall yields. The different behavior displayed for the reductive enzymes in the presence of diethyl ether clearly influenced in the decision to let the organolithium solvent evaporate under open-air conditions before developing the bioreduction step. Our results demonstrate the importance of the fine orchestration of the reaction conditions for the development of efficient hybrid chemoenzymatic cascades without the need of intermediate isolation/purification steps or compartmentalization of the different synthetic systems.

Keywords: Alcohol dehydrogenase; Bioreduction; Chemoenzymatic synthesis; Nitriles; Organolithium chemistry

Introduction

Since their discovery by Schlenk and Holz back in 1917,[1] organolithium (RLi) reagents have played a pivotal role as indispensable synthetic tool in organic chemistry, with a variety of application across various domains of organic synthesis, including anionic polymerization protocols, metalation reactions, lithium-halogen exchange processes, or Pd-catalyzed C–C coupling protocols, among others.[2–5] Consequently, it is not surprising that over 95% of methodologies employed in the total syntheses of natural products rely on RLi reagents in at least one step.[6] In fact, the attractiveness of organolithium chemistry for synthetic organic chemists is related to its: *i*) high and rich reactivity; *ii*) commercially availability (usually these reagents are sold as hydrocarbons or ethereal solutions); and *iii*) affordable prices. However, their inherent high reactivity also presents undesired drawbacks, usually related with the tendency of RLi compounds to react with organic solvents and secondary moieties, thus imposing the use of low temperatures (from 0 to −78 °C). Moreover, and due to the high polarity of the C–Li bond, which implies other side reactions such as hydrolysis/oxidations, the use of these polar organometallic compounds relies on the employment of: *i*) rigorously dried, aprotic and toxic *Volatile Organic Solvents* (*VOCs*); and *ii*) inert atmosphere (usually nitrogen or argon), to prevent side reactions in the presence of moisture or oxygen.

However, recent innovations from our own research group and others have demonstrated that, in contrast with the aforementioned conventional wisdom, polar *s*-block organometallic RLi reagents can be used: *i*) under air; *ii*) in sustainable, protic, non-toxic and non-dried solvents [like *deep eutectic solvents* or even water]; and *iii*) at room temperature and under air/moisture.[7–14] Consequently, this paradigm shift in organolithium chemistry illustrates the capability of RLi reagents to operate under conditions compatible with other catalysts, such as enzymes, thus opening new avenues to the design of chemoenzymatic cascades.[15–24]

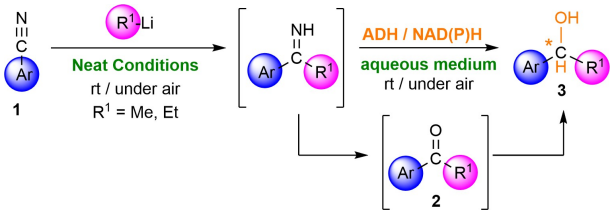
The traditional challenge in transformations involving organolithium compounds and enzymes is that they typically rely on tedious multistep and energy/time consuming protocols in which intermediate isolation/purification steps or compartmentalization of the different synthetic systems is required.[25] In some cases, these hybrid reactions RLi/enzyme have been successfully conducted sequentially in a one-pot manner. Unfortunately, such examples are scarce and mostly involve the combination of laccases with RLi compounds,[26,27] with no reported instances to date of synergistic combination between RLi reagents and enzymes for the development of multi-step stereoselective processes. Consequently, and trying to finish with this discontinuity in the design of stereoselective chemoenzymatic protocols, we decided to focus our interest in exploring the potential combination of organolithium reagents with alcohol dehydrogenases (ADHs), enzymes capable of catalyzing prochiral ketone bioreduction processes,[28–31] that have previously demonstrated high compatibility with a wide array of chemical reagents and catalysts.[32] Encouraged by previous results achieved by our research group in the stoichiometric addition of RLi to nitriles for their chemoselective and fast conversion into the corresponding prochiral ketones (working under neat conditions, at room temperature and in the absence of protecting atmosphere),[33–40] we have devised the chemoenzymatic sequence presented in Scheme 1.

Therefore, herein, we demonstrate that fine-tuning of the different synthetic parameters (reaction medium, stoichiometry, concentration) involved in our hybrid tandem protocol, allowed us to design an operationally simple and straightforward two-step chemoenzymatic cascade that transforms nitriles into the desired secondary alcohols with total stereoselectivity. This unpreceded combination is achieved by merging the chemoselective, fast and quantitative addition of aliphatic organolithium reagents (MeLi or EtLi) into different nitriles (under neat and bench-type reaction conditions) with a subsequent bioreduction of the *in-situ* generated prochiral ketones (promoted by the corresponding ADHs). Our results demonstrate the importance of catalyst choreography for the development of efficient hybrid chemoenzymatic cascades without the need of intermediate isolation/purification steps or compartmentalization of the different synthetic systems employed.

Results and Discussion

Conversion of Aromatic Nitriles 1 a–h into the Corresponding Acetophenone-Type Ketones 2 a–h

Initially, we decided to revisit the capability of MeLi to promote the quantitative conversion of benzonitrile (**1 a**) into the corresponding prochiral ketone [acetophenone (**2 a**) in this case].[34] For this purpose, **1 a** was



Scheme 1. Envisioned chemoenzymatic cascade to transform nitriles into chiral alcohols using organolithium chemistry and enzyme catalysis under aerial conditions.

directly treated with a commercially-available 1.6 M ethereal solution of MeLi in the absence of any external *VOC* solvent (this is by using directly the diethyl ether present in the MeLi solution as solvent), in the presence of air/moisture and at room temperature. Here, it is important to mention that by employing only one equivalent of MeLi, benzonitrile (**1 a**, 0.5 mmol) was quantitatively converted into ketone **2 a** under bench-type reaction conditions (room temperature and under air) after simple treatment of the obtained reaction mixture with a Tris·HCl buffer 50 mM pH 7.5, which is the reaction medium required for the second step of our chemoenzymatic protocol, this is, the bioreduction promoted by the ADH.

Next, the scope of suitable and commercially-available nitriles was explored by employing a series of benzonitriles **1 b–h** bearing different pattern substitutions (methyl, methoxy and chloride), and including all possible positions in the aromatic ring (*ortho*, *meta* or *para*). Reactions developed under air conditions occurred with good to excellent conversions (87- > 99%), exclusively observing the formation of prochiral ketones **2 b–h** as reaction products after the aforementioned hydrolytic treatment with a Tris·HCl buffer (Figure 1). Here, it is important to mention that we consistently observed complete chemoselectivity in the addition of MeLi into nitriles **1 b–h**, even when operating at room temperature, in the presence of air/moisture, and utilizing a 1:1 stoichiometry. This was a pivotal finding taking into account the potential competing processes that can take place during the addition of RLi reagents into nitriles **1 b–h** under bench-type reaction conditions (no side products were observed in the crude reaction mixtures, only unreacted starting nitriles **1 b–h**). These undesired side processes could include: *i*) the metalation of the benzylic position in nitriles **1 b–d** (*i. e.*, lateral lithiation); *ii*) directed-*ortho*-metalation processes in nitriles containing MeO substituents (**1 e–g**); and *iii*) Li-halogen exchange process in nitrile **1 h**.

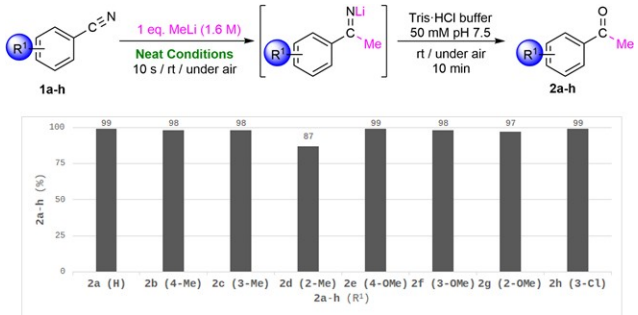


Figure 1. Conversions obtained after addition of 1 eq. of MeLi (1.6 M solution in diethyl ether) to benzonitriles **1 a–h** to produce the desired prochiral ketones **2 a–h** under air conditions.

Conversion of Acetophenone (2 a) into Enantiopure 1-Phenylethanol (3 a): Parametrization Studies

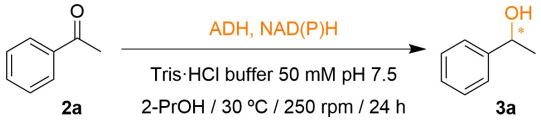
Once we set up the best conditions for the quantitative conversion of the starting nitriles **1 a–h** into the desired prochiral ketones **2 a–h**, we questioned ourselves whether ADHs could be exploited to promote the stereoselective bioreduction of the *in-situ* obtained ketones **2 a–h** (*vide supra*). At this point, acetophenone (**2 a**) was selected as benchmark substrate for an initial bioreduction study, searching for adequate reactions to accomplish the cascade approach in a two-step fashion (Table 1). Stereocomplementary enzymes were selected from previous reports, including lyophilized cells of *Escherichia coli* heterologously expressing ADH from *Rhodococcus ruber* (*E. coli*/ADH-A; capable of working without the need of an external cofactor)[41–43] to produce (*S*)-alcohols,[44] and *Lactobacillus brevis* ADH (*E. coli*/*Lb*ADH)[45–47] and commercial evo.1.1.200[48] to reach the (*R*)-enantiomers.[44] In all cases, isopropanol (2-PrOH) was selected as simple and inexpensive co-substrate for cofactor recycling purposes under coupled-substrate approach. Due to the possibility to work at high substrate concentrations in the first step, the initial parameter considered for optimization was the substrate concentration assaying an interval from 100 to 300 mM of **2 a** (entries 1–3). A notable decrease of the conversion was observed at the highest ketone concentrations in the reactions with ADH-A (52–78%) due to the reversible nature of the cofactor recycling system used. To increase the conversion values, the use of a higher loading of enzyme was considered, although no significant improvements were attained (entries 4 and 5). Gratifyingly, the influence of 2-PrOH was superior (entries 6–8), achieving a 94% conversion at 20% vol of the co-solvent. Remarkably, all the bioreductions catalyzed by ADH-A occurred with complete stereoselectivity towards the alcohol (*S*)-**3 a**.

To achieve the synthesis of the alcohol (*R*)-**3 a**, different trends were observed depending on the ADH source. On one hand, lyophilized cells of *E. coli*/*Lb*ADH led to similar conversion values (90–93%, entries 9–11) at different 2-PrOH volumes (10–20%), observing a significant decrease in the selectivity at higher co-solvent loadings (85–99% *ee*). On the other hand, the beneficial effect of increasing the 2-PrOH amount was noticed for evo.1.1.200 in terms of reactivity (80–91% conversion), isolating (*R*)-**3 a** in all cases with the same 97% *ee* (entries 12–14).

Parametrization and Scope of the Hybrid Chemoenzymatic Cascade: Conversion of Nitriles 1 a–i into Enantiopure Alcohols 3 a–j

Once individually studied both reaction steps, the foreseeable limitations to be addressed for the develop-

Table 1. Bioreduction of acetophenone (**2 a**) at different concentrations varying the amount of enzyme and the 2-PrOH content.



Entry	ADH	[**2 a**] (mM)	ADH:**2 a** (w/w)	2-PrOH (% vol)	*c* (%)[d]	*ee* **3 a** (%)[e]
1[a]	ADH-A	100	2:1	6	78	>99 (*S*)
2[a]	ADH-A	200	2:1	6	66	>99 (*S*)
3[a]	ADH-A	300	2:1	6	52	>99 (*S*)
4[a]	ADH-A	100	6:1	6	80	>99 (*S*)
5[a]	ADH-A	200	6:1	6	60	>99 (*S*)
6[a]	ADH-A	100	2:1	10	87	>99 (*S*)
7[a]	ADH-A	100	2:1	15	89	>99 (*S*)
8[a]	ADH-A	100	2:1	20	94	>99 (*S*)
9[b]	*Lb*ADH	100	2:1	10	93	99 (*R*)
10[b]	*Lb*ADH	100	2:1	15	92	92 (*R*)
11[b]	*Lb*ADH	100	2:1	20	90	85 (*R*)
12[c]	evo.1.1.200	100	2:1	10	80	97 (*R*)
13[c]	evo.1.1.200	100	2:1	15	86	97 (*R*)
14[c]	evo.1.1.200	100	2:1	20	91	97 (*R*)

[a] Reaction conditions: In a 2.0 mL Eppendorf tube, ADH (2:1 to 6:1 w/w enzyme:substrate), acetophenone (**2 a**, 100–300 mM), 2-PrOH (6–20% vol) and Tris·HCl buffer 50 mM pH 7.5 were added and the mixture shaken at 250 rpm and 30 °C for 24 h.
[b] For entries 9–11: Tris·HCl buffer 50 mM (containing 1 mM $MgCl_2$ and 1 mM NADPH) pH 7.5 was used.
[c] For entries 12–14: Tris·HCl buffer 50 mM (containing 1 mM $MgCl_2$ and 1 mM NADH) pH 7.5 was used.
[d] Conversion values measured by chiral GC analyses (see SI). Only product **3 a** was observed as the reaction product.
[e] Enantiomeric excess values determined by chiral GC, previously derivatized as acetate (see SI).

ment of a one-pot chemoenzymatic approach were identified. These are, the highly basic pH derived from the formation of lithium hydroxide in the reaction medium after the first step, and the presence of an organic solvent in which the organolithium compound is dissolved (diethyl ethyl in our case). In fact, both issues influenced the ADH action, so different strategies were considered to assemble both steps and developed a straightforward cascade. Interestingly, the presence of Et_2O affected differently to the three tested ADHs (Table 2). ADH-A behaves properly in the presence of this organic solvent (entries 1–3), while for the anti-Prelog ADHs, a complete loss of *Lb*ADH activity was observed (entries 4–6), whereas the presence of Et_2O caused a decrease in the stereoselectivity displayed by evo.1.1.200 (entries 7–9).

Combining both types of chemistry involved in our chemoenzymatic protocol (RLi and ADH) also requires the selection of an aqueous medium necessary to hydrolyze the imine intermediate towards the ketone, at the same time leading to an adequate scenario (in terms of substrate concentration and pH) for the bioreduction experiment. Thus, starting from 0.32 mmol of nitrile **1 a** and an equimolar amount of MeLi (1.6 M solution in Et_2O), the first step led to completion. Then, a 50 mM Tris·HCl buffer pH 7.5 (960 µL) and 2-PrOH (640 µL) were added, requiring the extra addition of concentrated HCl (40 µL) due to the highly alkaline pH achieved after the organolithium addition. Under these conditions, a pH 8–9 was attained, conducing to an approximate 200 mM intermediate **2 a** concentration. Two equal aliquots of the sample were taken to perform the reaction with *E. coli*/ADH-A and evo.1.1.200 at a resulting 100 mM ketone concentration (Figure 2 and Table 3), leading to high global conversions (92–94%, entries 1 and 5) and good selectivities, specially for *E. coli*/ADH-A (86->99% *ee*). Other substrates were also tested under similar conditions, finding that *meta*-chloro (**1 h**, entry 3) and

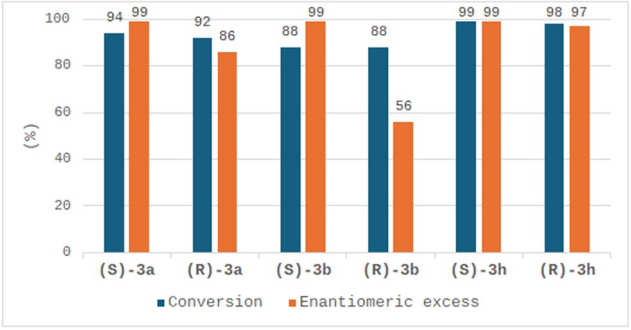
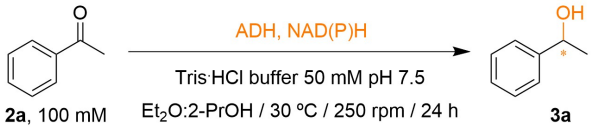


Figure 2. MeLi addition and bioreduction sequence using nitriles **1 a**,**b**,**h**. Blue: conversion values into optically active alcohols **3 a**,**b**,**h**; Orange: enantiomeric excess values of the mentioned alcohols.

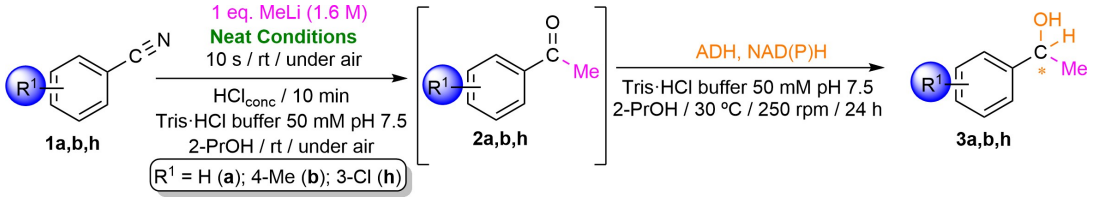
Table 2. Study of the effect of diethyl ether in the enzymatic activity in the bioreduction step.



Entry	ADH	Et_2O (% vol)	2-PrOH (% vol)	*c* (%)[d]	*ee* **3 a** (%)[e]
1[a]	ADH-A	10	20	94	>99 (*S*)
2[a]	ADH-A	0	20	94	>99 (*S*)
3[a]	ADH-A	0	10	89	>99 (*S*)
4[b]	*Lb*ADH	10	20	<1	–
5[b]	*Lb*ADH	0	20	90	85 (*R*)
6[b]	*Lb*ADH	0	10	93	99 (*R*)
7[c]	evo-1.1.200	10	20	92	86 (*R*)
8[c]	evo-1.1.200	0	20	91	97 (*R*)
9[c]	evo-1.1.200	0	10	80	97 (*R*)

[a] Reaction conditions: In a 2.0 mL Eppendorf tube, ADH (2:1 w/w), acetophenone (**2 a**, 100 mM), 2-PrOH (10–20% vol), Et_2O (0–10% vol) and Tris·HCl buffer 50 mM pH 7.5 were added and the mixture shaken at 250 rpm and 30 °C for 24 h.
[b] For entries 4–6: Tris·HCl buffer 50 mM (containing 1 mM $MgCl_2$, 1 mM NADPH) pH 7.5 was used.
[c] For entries 7–9: Tris·HCl buffer 50 mM (containing 1 mM $MgCl_2$, 1 mM NADH) pH 7.5 was used.
[d] Conversion values measured by chiral GC analyses (see SI).
[e] Enantiomeric excess values measured by chiral GC, previously derivatized as acetate (see SI).

Table 3. Optimization of the sequential stereoselective synthesis of chiral alcohols **3 a**,**b**,**h** from nitriles **1 a**,**b**,**h**.



Entry	Compound	First step		Second step	
		c (%)[d]	ADH	*c* (%)[d]	*ee* **3 a**,**b**,**h** (%)[e]
1[a]	**1 a**	>99	ADH-A	94	>99 (*S*)
2[a]	**1 b**	96	ADH-A	88	>99 (*S*)
3[a]	**1 h**	>99	ADH-A	>99	>99 (*S*)
4[b]	**1 a**	>99	*Lb*ADH	<1	—
5[c]	**1 a**	>99	evo-1.1.200	92	86 (*R*)
6[c]	**1 b**	96	evo-1.1.200	88	56 (*R*)
7[c]	**1 h**	>99	evo-1.1.200	98	97 (*R*)

[a] Reaction conditions: An open vial was charged with the nitrile compound **1 a**,**b**,**h** (0.32 mmol) and a commercial solution of methyl lithium (1.6 M in diethyl ether, 0.32 mmol, 200 µL) was added under vigorous stirring. After 10 s of stirring, the reaction was quenched with Tris·HCl buffer 50 mM pH 7.5 (960 µL). Subsequently, 2-PrOH (640 µL) and HCl conc (40 µL) were added to obtain a final mixture with pH 8. Then, 164 µL of this crude, ADH (2:1 w/w enzyme:substrate) and Tris·HCl buffer 50 mM pH 7.5 (128 µL) were added in a 2.0 mL Eppendorf tube and the mixture was shaken at 250 rpm and 30 °C for 24 h.
[b] For entry 4: 1 mM $MgCl_2$ and 1 mM NADPH were used in the bioreduction step.
[c] For entries 5–7: 1 mM $MgCl_2$ and 1 mM NADH were used in the bioreduction step.
[d] Conversion values measured by chiral GC analyses (see SI).
[e] Enantiomeric excess values determined by chiral GC, previously derivatized as acetate (see SI).

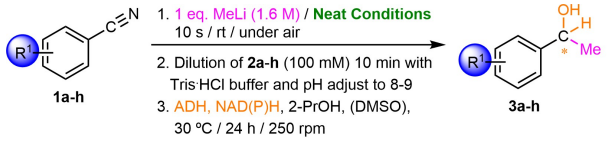
para-methyl (**1 b**, entry 2) substitutions provided excellent results with *E. coli*/ADH-A, while the *para*-methyl (**1 b**, entry 6) and *meta*-chloro (**1 h**, entry 7) led to lower conversions and/or stereoselectivity for evo.1.1.200.

The chemoenzymatic sequence was next attempted, also in telescoped fashion, but including ether evapo-

ration prior developing the enzymatic step. This was simply addressed by adding the buffer and stirring the resulting mixture in an open-air tube for a few minutes. Thus, the presence of the organic solvent was excluded, avoiding a total or partial enzyme inactivation, and requiring less amount of 2-PrOH (10% vol) to obtain a homogenous mixture. The results are summarized in Table 4, highlighting the high product formation in most cases (>57% global conversion). The poorest results from a synthetic point of view were attained with the 2-substituted compounds [2-methyl (**3 d**) and 2-methoxy (**3 g**)], which especially resulted to be not good substrates for *E. coli*/*Lb*ADH (entries 4, 5, 10 and 11). Only, the reduction of the intermediate concentration (25 mM) led to better conversion values into (*R*)-**3 d** (22%) and (*R*)-**3 g** (28%). The addition of DMSO as co-solvent (5% vol) was also attempted to improve the solubility of the ketone intermediate, although only slight conversion improvements were achieved in specific cases (entries 5, 7, 9 and 11).

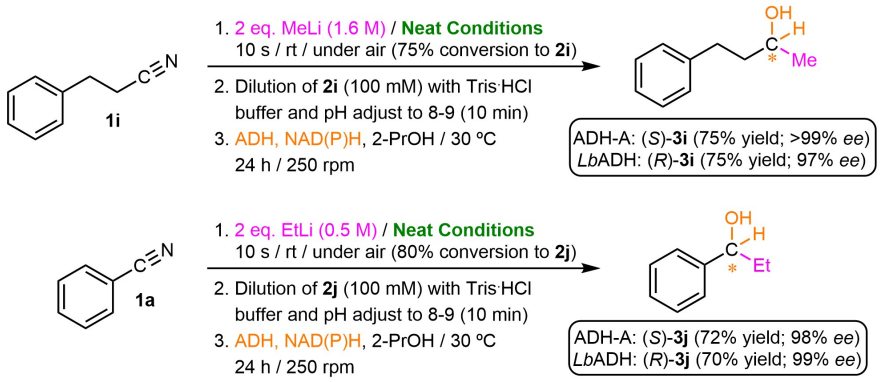
Next, and to have a full picture of our chemoenzymatic protocol for the enantioselective synthesis of secondary alcohols, we decided to extend our studies to (Scheme 2): *i*) a bulkier substrate such as phenylpropionitrile (**1 i**); and *ii*) propiophenone prochiral ketone, obtained by reaction of benzonitrile (**1 a**) with other organolithium reagents such as ethyl lithium, that moreover is not commercialized in an ethereal solution (0.5 M benzene/cyclohexane solution). For the synthesis of **3 i** (Scheme 2 top), different trials were made to produce the intermediate **2 i** in good conversion (75%), requiring in this case two equivalents of MeLi since the reactions with 1 and 1.5 equivalents did not reach a notable extension (48% and 70%, respectively). Then, after dilution of the sample including the evaporation of the ethereal solvent in the open-air tube, the bioreductions with both *E. coli*/ADH-A and *E. coli*/*Lb*ADH proceeded smoothly with a global 75% yield for the two-step approach with excellent stereoselectivity for both enantiomers (97->99% *ee*). In a similar manner and for the case of **3 j** (Scheme 2 bottom), the use of ethyl lithium in a benzene/cyclohexane solution, allowed the production of propiophenone (**2 j**) as intermediate, which was also efficiently reduced with *E. coli*/ADH-A and *E. coli*/*Lb*ADH producing the desired chiral alcohol **3 j** in

Table 4. Chemoenzymatic sequence for the transformation of nitriles **1 a–h** into chiral alcohols **3 a–h**.



Entry	**2 a–h** (%)[a]	DMSO (5%)	(*S*)-**3 a–h** (%)[b,c]	(*R*)-**3 a–h** (%)[b,c]
1	>99 (**2 a**)	No	89 (>99)	89 (>99)
2	98 (**2 b**)	No	80 (>99)	80 (>99)
3	98 (**2 c**)	No	86 (>99)	89 (97)
4	87 (**2 d**)	No	95 (99)	<1 (n.d.)
5	87 (**2 d**)	Yes	99 (>99)	<1 (n.d.)
6	99 (**2 e**)	No	58 (96)	54 (97)
7	99 (**2 e**)	Yes	57 (97)	58 (96)
8	98 (**2 f**)	No	93 (>99)	40 (97)
9	98 (**2 f**)	Yes	89 (99)	44 (92)
10	97 (**2 g**)	No	94 (>99)	14 (n.d.)
11	97 (**2 g**)	Yes	94 (>99)	<1 (n.d.)
12	>99 (**2 h**)	No	96 (>99)	95 (99)

[a] Conversion into **2 a–h** after reaction with MeLi (1 eq.) determined by GC analyses (see SI).
[b] Overall conversion into **3 a–h** after two-step sequence including bioreduction of ketone intermediates (100 mM) with ADH-A for (*S*)-alcohols and *Lb*ADH for (*R*)-alcohols.
[c] Enantiomeric excess of alcohols **3 a–h** in parentheses (see SI).
n.d: Not determined.



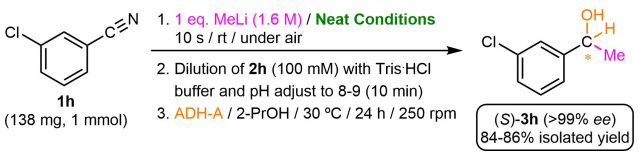
Scheme 2. Expanding the methodology of our chemoenzymatic protocol to other nitriles towards the synthesis of chiral alcohols **3 i** and **3 j**.

good overall yield (70–72%) and excellent enantiopurity for both enantiomers (98–99% *ee*).

Finally, the scale-up of the one-pot chemoenzymatic approach was developed using 3-chlorobenzonitrile (**1 h**) as substrate (Scheme 3). Two independent strategies were followed, the first one without allowing to evaporate the ethereal MeLi solution and using 20% vol of 2-PrOH, thus yielding 86% of enantiopure (*S*)-**3 h** after an extraction protocol. Alternatively, the evaporation in an open-air tube was considered using in this case 10% vol of 2-PrOH and leading to a global 84% isolated yield into the desired chiral alcohol, thus showing that our methodology is amenable for a possible semi-preparative synthetic application.

Conclusions

In summary, we have designed an unprecedented chemoenzymatic approach consisting of two steps developed in one-pot fashion capable to transform inexpensive nitriles into valuable chiral alcohols, working under bench-type reaction conditions (room temperature and in the absence of protecting atmosphere), which are traditionally prohibited for RLi reagents. Our sequence starts with the formation of the corresponding ketone intermediates using commercially available organolithium reagents under neat conditions, to later adapt the medium to the bioreduction reaction by adding the corresponding buffer, ADH, cofactor (except for ADH-A)[43] and 2-PrOH as co-substrate. By using our methodology, a series of (*S*)- and (*R*)-alcohols were obtained depending on the enzyme of choice with good to excellent yields and enantiomeric excess. Also, it is important to mention the high chemoselectivity of our synthetic protocol, which is compatible with a variety of functional groups in the starting nitrile, without observing any undesired side reaction typically associated with the use of highly-polar RLi reagents (*i.e.*, Li/halogen interchange reactions; *ortho*-metalations; or metalations at the activated benzylic positions), even working under bench-type reaction conditions. Thus, our study represents the first example of a sequential multistep synthesis combining organolithium chemistry and biocatalysis for the synthesis of enantioenriched molecules, paving the way for the disclosing of other chemoenzymatic sequences involving different substrates and enzyme classes. Overall, our findings emphasize the importance of fine orchestration of all the synthetic tools involved in the development of efficient hybrid chemoenzymatic cascades.



Scheme 3. Semi-preparative conversion of 3-chlorobenzonitrile (**1 h**) into the desired enantiopure alcohol **3 h** by using our chemoenzymatic protocol which merges chemoselective and fast addition of MeLi and *E. coli*/ADH-A catalyzed bioreduction protocol.

Experimental Section

Nitrile compounds **1 a–i** and organolithium solutions such as methyl lithium (1.6 M in diethyl ether) and ethyl lithium (0.5 M in cyclohexane/benzene) were purchased from Sigma-Aldrich. Lyophilized *E. coli* cells heterologously overexpressing ADHs from *Lactobacillus brevis* (*Lb*ADH) and *Rhodococcus ruber* (ADH-A) were obtained as previously reported in the literature.[41–43,45–48] These enzymatic preparations showed an activity of approximately 0.5–1 U/mg. The commercial evo-1.1.200 ADH was obtained from Evoxx Technologies GmbH. Nicotinamide cofactors (NADPH and NADH) were acquired from Sigma-Aldrich. All other reagents and solvents were of the highest quality available.

^{1}H, $^{13}C\{^{1}H\}$ and DEPT NMR experiments were recorded on a Bruker DPX-300 MHz (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometer (see section VII; SI). Optical rotations were measured using a Perkin-Elmer 241 polarimeter, and values were quoted in units of 10^{-1} deg cm^2 g^{-1} (see section V.4; SI). Thin-layer chromatography (TLC) analyses were conducted with Merck Silica Gel 60 F254 precoated plates and visualized with UV, and potassium permanganate stain. Column chromatographies were performed using silica gel 60 (230–240 mesh). Gas chromatography (GC) analyses were performed on a Hewlett Packard 6890 Series II chromatograph [CP-ChiraSil-DEX CB (25 m×0.25 nm×0.25 µm) column was used, see sections V.1 and V.2; ESI]. HPLC analyses were performed with Hewlett Packard 1100 LC liquid chromatograph (OJ–H column was used, see section V.3; SI).

General Procedure for the Telescoped RLi Addition and ADH-Catalyzed Bioreduction with Evaporation of Et_2O

An open vial was charged with the nitrile compound (**1 a–i**, 0.32 mmol) and the corresponding organolithium reagent (MeLi in 1.6 M solution in Et_2O or 2 equiv. of EtLi in a 0.5 M solution in benzene/cyclohexane; 0.20 and 1.28 mL, respectively) was added under vigorous stirring. After 10 s of stirring, the reaction was quenched by addition of Tris·HCl buffer 50 mM pH 7.5 (1.28 mL), and the organic solvent from the organolithium solution (Et_2O or cyclohexane/benzene) was allowed to evaporate. Subsequently, 2-PrOH (320 µL) and an aqueous solution of HCl concentrated (40 µL) were added to obtain a final mixture with pH 8 for 10 min. Then, an aliquot of this crude (164 µL), ADH (8 mg) and Tris·HCl buffer 50 mM pH 7.5 (164 µL, containing NADPH and $MgCl_2$ for *E. coli*/*Lb*ADH and NADH and $MgCl_2$ for evo-1.1.200) were added in a 2.0 mL Eppendorf tube. The reaction was shaken at 250 rpm and 30 °C for 24 h. Then, the mixture was extracted with EtOAc (2×500 µL), the organic layers separated by centrifugation (90 s, 13000 rpm), combined and finally dried over Na_2SO_4.

Conversions were determined directly by GC analysis (see Table S1, SI). In the case of chiral substrates, the enantiomeric excess values of the resulting alcohols were measured by chiral GC (see Table S2 for **3a–f,h–j**; SI) or HPLC (see Table S3 for **3g**; SI) after derivatization as the corresponding acetates. The identity of resulting alcohols **3a–j** was confirmed by ^{1}H-NMR analyses after comparison with reported spectra (see sections IV and VII; SI).

General Procedure for the Telescoped MeLi Addition and ADH-Catalyzed Bioreduction without Evaporation of Et_2O

An open vial was charged with the nitrile compound **1a,b,h** (0.32 mmol) and the corresponding organolithium reagent (MeLi in 1.6 M solution in Et_2O, 200 µL) was added under vigorous stirring. After 10 s of stirring, the reaction was quenched by addition of Tris·HCl buffer 50 mM pH 7.5 (960 µL) for 10 min. Subsequently, 2-PrOH (640 µL) and an aqueous solution of HCl concentrated (40 µL) were added to obtain a final mixture with pH 8. Then, an aliquot of this crude (164 µL), ADH (8 mg) and Tris·HCl buffer 50 mM pH 7.5 (127 µL) containing NADPH and $MgCl_2$ for *E. coli*/*Lb*ADH and NADH and $MgCl_2$ for evo-1.1.200 were added in a 2.0 mL Eppendorf tube. The reaction was shaken at 250 rpm and 30 °C for 24 h. Then, the mixture was extracted with EtOAc (2×500 µL), the organic layers separated by centrifugation (90 s, 13000 rpm), combined and finally dried over Na_2SO_4. Conversions were determined directly by GC analysis (see Table S1; SI). In the case of chiral substrates, the enantiomeric excess values of the resulting alcohols were measured by chiral GC after derivatization as the corresponding acetates (see Table S2; SI). The identity of resulting alcohols **3a,b,h** was confirmed by ^{1}H-NMR analyses after comparison with reported spectra (see sections IV and VII; SI).

One-Pot Sequential Process using 3-Chlorobenzonitrile (1 h) with Evaporation of Et_2O

A commercial solution of MeLi (1.6 M in diethyl ether, 625 µL, 1 equiv.) was added to an open vial containing the nitrile compound **1h** (138 mg, 1.00 mmol). The mixture was vigorously stirred for 10 s, and after this time the reaction was quenched by addition of Tris·HCl buffer 50 mM pH 7.5 (4.00 mL) for 10 min. Then, Et_2O from the organolithium reagent was allowed to evaporate. Subsequently, 2-PrOH (1.00 mL) and an aqueous solution of HCl concentrated (125 µL) were added to obtain a final mixture with pH 8. Then, *E. coli*/ADH-A (274 mg) and Tris·HCl buffer 50 mM pH 7.5 (5.00 mL) were added and the reaction mixture was shaken at 250 rpm and 30 °C for 24 h. Then, the mixture was extracted with EtOAc (3×20 mL), the organic layers separated, combined and finally dried over Na_2SO_4 yielding enantiopure (*S*)-**3h** (> 99% *ee*) in 84% isolated yield after filtration on silica gel (20% EtOAc/hexane).

One-Pot Sequential Process using 3-Chlorobenzonitrile (1 h) without Evaporation of Et_2O

A commercial solution of MeLi (1.6 M in diethyl ether, 625 µL, 1 eq) was added to an open vial containing the nitrile compound **1h** (138 mg, 1.00 mmol). The mixture was vigorously stirred for 10 s, and after this time the reaction was quenched by addition of Tris·HCl buffer 50 mM pH 7.5 (3.00 mL) for 10 min. Subsequently, 2-PrOH (2.00 mL) and an aqueous solution of HCl concentrated (125 µL) were added to obtain a final mixture with pH 8. Then, *E. coli*/ADH-A (274 mg) and Tris·HCl buffer 50 mM pH 7.5 (4.00 mL) were added and the reaction mixture was shaken at 250 rpm and 30 °C for 24 h. Then, the mixture was extracted with EtOAc (3×20 mL), the organic layers separated, combined and finally dried over Na_2SO_4 yielding enantiopure (*S*)-**3h** (> 99% *ee*) in 86% isolated yield after filtration on silica gel (20% EtOAc/hexane).

Author Contributions

N.R.-L. and J.G.-A. formulated the project. Core experiments were performed by N.R.-L. and G.M.-M.. I.L., V.G.-F. and J.G.-A. conceptualized the work and secured the funding. V.G.-F. and J.G.-A. wrote the first draft of the article. All authors discussed the results and revised the manuscript. All authors have given approval to the final version of the present manuscript.

Acknowledgements

N.R.-L., G.M.-M. and J.G.-A. thank MCIN/AEI/10.13039/501100011033 (projects numbers RED2018-102387-T and PID2020-113473GB-I00) for financial support. I.L. and V.G.-F. gratefully acknowledge financial support from the Spanish Ministry of Science and Innovation (PID2022-137893OB-I00 project and the Asturian Regional Government (AYUD/2021/51542). Technical support from the Scientific-Technical Services (University of Oviedo) is acknowledged. Prof. Wolfgang Kroutil (University of Graz, Austria) is also acknowledged for providing us with the heterologously overexpressed alcohol dehydrogenases in E. coli cells.

References

[1] W. Schlenk, J. Holtz, *Ber. Dtsch. Chem. Ges.* **1917**, *50*, 262–274.
[2] J. Clayden, *Organolithiums: Selectivity for Synthesis*, Pergamon, Oxford, UK, **2002**.
[3] *The Chemistry of Organolithium Compounds*; Eds. Z. Rappoport, I. Marek, Wiley, Chichester, UK, **2005**.
[4] V. Capriati, F. M. Perna, A. Salomone, *Dalton Trans.* **2014**, *43*, 14204–14210.
[5] R. Luisi, V. Capriati, *Lithium Compounds in Organic Synthesis: From Fundamentals to Applications*, John Wiley & Sons, Weinheim, Germany, **2014**.
[6] U. Wietelmann, J. Klett, *Z. Anorg. Allg. Chem.* **2018**, *644*, 194–204.

[7] For previous revisions in this field the interested readers are also encouraged to consult references 8–14.
[8] J. García-Álvarez, *Eur. J. Inorg. Chem.* **2015**, *2015*, 5147–5157.
[9] J. García-Álvarez, E. Hevia, V. Capriati, *Eur. J. Org. Chem.* **2015**, *2015*, 6779–6799.
[10] J. García-Álvarez, E. Hevia, V. Capriati, *Chem. Eur. J.* **2018**, *24*, 14854–14863.
[11] E. Hevia, *Chimia* **2020**, *74*, 681–688.
[12] T. X. Gentner, R. E. Mulvey, *Angew. Chem.* **2021**, *133*, 9331–9348; *Angew. Chem. Int. Ed.* **2021**, *60*, 9247–9262.
[13] F. M. Perna, P. Vitale, V. Capriati, *Curr. Opin. Green Sustain. Chem.* **2021**, *30*, 100487.
[14] S. E. García-Garrido, A. Presa Soto, E. Hevia, J. García-Álvarez, *Eur. J. Inorg. Chem.* **2021**, *2021*, 3116–3130.
[15] For recent reviews in this field, see references 16–24.
[16] X. Huang, M. Cao, H. Zhao, *Curr. Opin. Chem. Biol.* **2020**, *55*, 161–170.
[17] Y. Zhou, S. Wu, U. T. Bornscheuer, *Chem. Commun.* **2021**, *57*, 10661–10674.
[18] Y. Liu, P. Liu, S. Gao, Z. Wang, P. Luan, J. González-Sabín, Y. Jing, *Chem. Eng. J.* **2021**, *420*, 127659.
[19] C. Ascaso-Alegre, J. Mangas-Sánchez, *Eur. J. Org. Chem.* **2022**, *2022*, e202200093.
[20] L. Bering, J. Thompson, J. Micklefield, *Trends Chem.* **2022**, *4*, 392–408.
[21] H. Gröger, F. Gallou, B. H. Lipshutz, *Chem. Rev.* **2023**, *123*, 5262–5296.
[22] S. González-Granda, J. Albarrán-Velo, I. Lavandera, V. Gotor-Fernández, *Chem. Rev.* **2023**, *123*, 5297–5346.
[23] S. González-Granda, L. Escot, I. Lavandera, V. Gotor-Fernández, *Angew. Chem.* **2023**, *135*, e202217713; *Angew. Chem. Int. Ed.* **2023**, *62*, e202217713.
[24] L. Z. Hessefort, L. J. Hastad, K. R. Merker, L. P. T. Ramos, K. F. Biegasiewicz, *ChemBioChem* **2023**, *24*, e20230034.
[25] For a recent example, see: L. Castoldi, L. Ielo, P. Hoyos, M. J. Hernáiz, L. De Luca, Á. R. Alcántara, W. Holzer, V. Pace, *Tetrahedron* **2018**, *74*, 2211–2217.
[26] M. Ramos-Martín, R. Lecuna, L. Cicco, P. Vitale, V. Capriati, N. Ríos-Lombardía, J. González-Sabín, A. Presa-Soto, J. García-Álvarez, *Chem. Commun.* **2021**, *57*, 13534–13537.
[27] D. Arnodo, M. Ramos-Martín, L. Cicco, V. Capriati, N. Ríos-Lombardía, J. González-Sabín, A. Presa Soto, J. García-Álvarez, *Org. Biomol. Chem.* **2023**, *21*, 4414–4421.
[28] For recent reviews on the use ADHs for the reduction of prochiral ketones into the corresponding chiral secondary alcohols, see references 29–31.
[29] Z. Li, H. Yang, J. Liu, Z. Huang, F. Chen, *Chem. Rec.* **2021**, *21*, 1611–1630.
[30] L. Qiao, Z. Luo, H. Chen, P. Zhang, A. Wang, R. A. Sheldon, *Chem. Commun.* **2023**, *59*, 7518–7533.
[31] A. Chadha, S. K. Padhi, S. Stella, S. Venkataraman, T. Saravanan, *Org. Biomol. Chem.* **2024**, *22*, 228–251.
[32] For a recent review on the design of chemoenzymatic protocols based on ADHs, see: G. de Gonzalo, C. E. Paul, *Curr. Opin. Green Sustain. Chem.* **2021**, *32*, 100548.
[33] M. J. Rodríguez-Álvarez, J. García-Álvarez, M. Uzelac, M. Fairley, C. T. O'Hara, E. Hevia, *Chem. Eur. J.* **2018**, *24*, 1720–1725.
[34] D. Elorriaga, F. Carrillo-Hermosilla, B. Parra-Cadenas, A. Antiñolo, J. García-Álvarez, *ChemSusChem* **2022**, *15*, e202201348.
[35] B. Parra-Cadenas, I. Fernández, F. Carrillo-Hermosilla, J. García-Álvarez, D. Elorriaga, *Chem. Sci.* **2024**, *15*, 5929–5937.
[36] For a selection of our works in the field of organolithium reagents under bench-type conditions and in green solvents that have made an impact in multidisciplinary general chemistry, see references 37–40.
[37] C. Vidal, J. García-Álvarez, A. Hernán-Gómez, A. R. Kennedy, E. Hevia, *Angew. Chem.* **2014**, *126*, 6079–6083; *Angew. Chem. Int. Ed.* **2014**, *53*, 5969–5973.
[38] C. Vidal, J. García-Álvarez, A. Hernán-Gómez, A. R. Kennedy, E. Hevia, *Angew. Chem.* **2016**, *128*, 16379–16382; *Angew. Chem. Int. Ed.* **2016**, *55*, 16145–16148.
[39] F. F. Mulks, L. J. Bole, L. Davin, A. Hernán-Gómez, A. R. Kennedy, J. García-AÁlvarez, E. Hevia, *Angew. Chem.* **2020**, *132*, 19183–19188; *Angew. Chem. Int. Ed.* **2020**, *59*, 19021–19026.
[40] M. Fairley, L. J. Bole, F. F. Mulks, L. Main, A. R. Kennedy, C. T. O'Hara, J. García-Álvarez, E. Hevia, *Chem. Sci.* **2020**, *11*, 6500–6509.
[41] W. Stampfer, B. Kosjek, C. Moitzi, W. Kroutil, K. Faber, *Angew. Chem.* **2002**, *114*, 1056–1059; *Angew. Chem. Int. Ed.* **2002**, *41*, 1014–1017.
[42] K. Edegger, C. C. Gruber, T. M. Poessl, S. R. Wallner, I. Lavandera, K. Faber, F. Niehaus, J. Eck, R. Oehrlein, A. Hafner, W. Kroutil, *Chem. Commun.* **2006**, 2402–2404.
[43] C. E. Paul, I. Lavandera, V. Gotor-Fernández, W. Kroutil, V. Gotor, *ChemCatChem* **2013**, *5*, 3875–3881.
[44] This is true when the large moiety attached to the carbonyl group has the preference with regards to the smaller one following the Cahn-Ingold-Prelog priority rules.
[45] M. Wolberg, W. Hummel, C. Wandrey, M. Müller, *Angew. Chem.* **2000**, *112*, 4476–4478; *Angew. Chem. Int. Ed.* **2000**, *39*, 4306–4308.
[46] S. Leuchs, L. Greiner, *Chem. Biochem. Eng. Q.* **2011**, *25*, 267–281.
[47] C. Rodríguez, W. Borzęcka, J. H. Sattler, W. Kroutil, I. Lavandera, V. Gotor, *Org. Biomol. Chem.* **2014**, *12*, 673–681.
[48] R. C. Simon, E. Busto, N. Richter, F. Belaj, W. Kroutil, *Eur. J. Org. Chem.* **2014**, *2014*, 111–121.