

# Strengthening the Combination between Enzymes and Metals in Aqueous Medium: Concurrent Ruthenium-Catalyzed Nitrile Hydration - Asymmetric Ketone Bioreduction

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**Abstract:** A dual ruthenium/ketoreductase catalytic system has been developed for the conversion of  $\beta$ -ketonitriles into optically active  $\beta$ -hydroxyamides through an unprecedented hydration/bioreduction cascade process in aqueous medium working in concurrent mode. The ketoreductase-mediated ketone reduction took place with exquisite stereoselectivity and it was simultaneous to the nitrile hydration promoted by the ruthenium catalyst. The overall transformation occurred: (i) employing commercially and readily available catalytic systems (ii) under mild reaction conditions, (iii) with high degree of conversion and excellent stereoselectivity, and (iv) without the need to isolate intermediates and with high final product yields. This genuine process demonstrates the benefits of combining metal and enzymatic catalysis to tackle the limitations arising from each field.

## Introduction

The development of one-pot multistep catalytic processes is an emerging trend within the synthetic chemistry to produce high added-value chemicals in a more efficient and sustainable manner.<sup>[1]</sup> To this end, by mimicking natural biosynthetic pathways in living organisms, chemists have successfully implemented several biocatalytic reactions into non-natural synthetic cascades in both *in vivo* and *in vitro* approaches.<sup>[2]</sup> Similarly, a plethora of synthetic steps mediated by chemical catalysts, mainly transition-metal catalysts, have been coupled in organic media.<sup>[3]</sup> Now, another turn of the screw in

constructing catalytic networks consists of combining chemical and biological catalysts in aqueous media, the natural environment of enzymes. Although these hybrid systems are still in their infancy, the wider available pool of water-compatible metal catalysts<sup>[4]</sup> along with the advances in protein engineering to produce more stable enzymes have enabled some remarkable examples of this kind.<sup>[5]</sup> Nevertheless, when planning such a chemo-enzymatic strategy a critical pitfall is the coexistence of both catalysts leading to: a) reciprocal poisoning, b) degradation because of additives, cofactors, or cosolvents, and c) incompatibility of reaction conditions.<sup>[6]</sup> As a result, most of these hybrid reactions run sequentially or, alternatively, the catalysts are site-isolated by compartmentalization or encapsulation techniques.<sup>[6c,7]</sup> Actually, the number of concurrent chemoenzymatic processes with concomitant action of metal and enzyme are still very scarce,<sup>[8]</sup> and include the pioneering dynamic kinetic resolution (DKR) processes combining a racemizing metal catalyst with an enzymatic transformation,<sup>[8a]</sup> ruthenium-catalyzed olefin metathesis coupled with monoamine oxidases and P-450 enzymes,<sup>[8c,f]</sup> a simultaneous ruthenium-catalyzed isomerization of allylic alcohols-bioreduction,<sup>[8e]</sup> and the oxidation of cyclic amines to lactams by a monoamine oxidase and CuI/H<sub>2</sub>O<sub>2</sub>.<sup>[8d]</sup>

In this context, two different approaches have been recently reported for converting  $\beta$ -ketonitriles into  $\beta$ -hydroxyamides by cascade processes in aqueous media combining a nitrile hydration and a ketone reduction (Scheme 1). In one case, a dual enzymatic system consisting of a ketoreductase (KRED) and a nitrile hydratase from whole cells of *R. rhodochrous* grown in the presence of DEPA (an inhibitor of amidase activity) was used.<sup>[9]</sup> In this asymmetric version, the first step is the KRED catalyzed dynamic reductive kinetic resolution of the ketone to a  $\beta$ -hydroxynitrile in very high enantio- and diastereomeric ratio. As the  $\beta$ -hydroxynitrile is formed, the nitrile hydratase catalyzes the water addition enabling the corresponding  $\beta$ -hydroxyamides with excellent stereoselectivity (route a).<sup>[10]</sup> On the other hand, a concomitant hydration/transfer hydrogenation from  $\beta$ -ketonitriles using a single ruthenium(II) complex (route b) was recently reported.<sup>[11]</sup>

Despite the merit of these concurrent cascade processes, some issues remained challenging; for route a (enzymatic) the amidase activity was not completely inhibited and the resulting mixture of  $\beta$ -hydroxyamide and  $\beta$ -hydroxyacid required chromatographic purification which decreased the yield,<sup>[12]</sup> while for route b (metal-catalyzed) the process was performed as a non-asymmetric version, and suffered from harsh reaction

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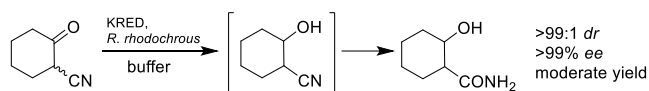
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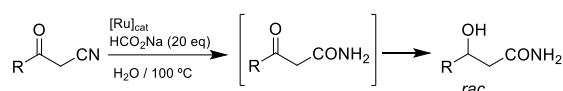
conditions (100 °C and excess of sodium formate) to achieve carbonyl reduction efficiently. On the light of this background, we envisioned a hybrid catalytic system combining an enzymatic ketone reduction with a ruthenium-catalyzed nitrile hydration, to exploit the synergistic effect of each catalysis area and bypass the drawbacks just mentioned.

#### Previous work:

##### a) Enzymatic *in vivo/in vitro* cascade

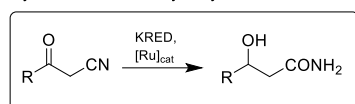


##### b) Metal-catalyzed tandem process



#### This work:

##### Hybrid metal-biocatalytic system

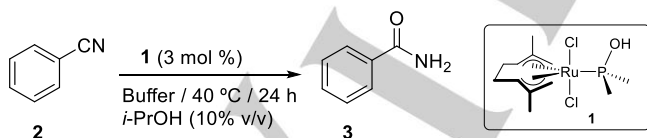


**Challenges:**  
 -Compatibility of catalysts  
 -Process orthogonality  
 -Stereoselectivity

**Scheme 1.** One-pot tandem processes for converting  $\beta$ -ketonitriles into  $\beta$ -hydroxyamides.

## Results and Discussion

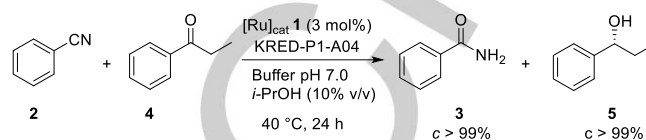
The first aspect explored was the identification of a medium that balances the requirements for each catalytic reaction type. While the ruthenium-catalyzed hydration of nitriles is usually performed in pure water,<sup>[13]</sup> the bioreduction requires an aqueous medium consisting of a phosphate buffer pH 7.0 (125 mM KPi, including 1.25 mM MgSO<sub>4</sub>, and 1.0 mM NADP<sup>+</sup>) and *i*-PrOH (10% v/v) for recycling the cofactor and driving the equilibrium towards the product. Thus, after assessing a series of ruthenium(II) and (IV) complexes for the hydration of a typical substrate, benzonitrile (**2**), in the medium required for the KRED, complex **1** emerged as the best candidate (Scheme 2).<sup>[14]</sup> The treatment of **2** (200 mM) with 3 mol% of **1**, at 40 °C for 24 h, led to benzamide (**3**) with >95% of conversion (*c*).<sup>[15]</sup>



**Scheme 2.** Ru(IV) complex **1** catalyzed hydration of benzonitrile (**2**) in the reaction medium of KREDs.

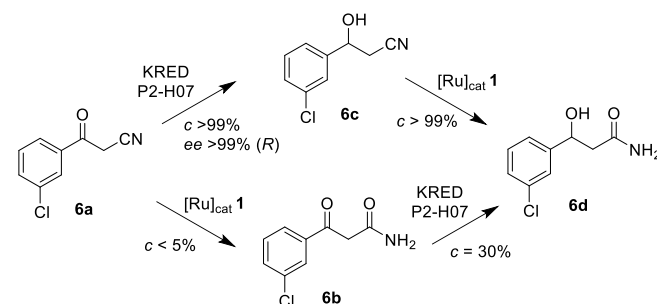
Next, the focus was on the compatibility of both catalytic systems. Accordingly, two model compounds, namely benzonitrile (**2**) and propiophenone (**4**) which are substrates for each catalyst respectively, were put together and subjected to the simultaneous action of a ketoreductase (KRED-P1-A04) and the ruthenium complex **1** in the previous buffer at 200 mM

concentration (Scheme 3). Pleasantly, both hydration and reduction reactions occurred with conversions >99% after 24 h at 40 °C, and the enantiomeric excess (*ee*) measured for the resulting (*R*)-1-phenylpropan-1-ol (**5**) was >99%. Seeing as the perfect coexistence of catalysts and tolerance of their reaction conditions, these findings reveal a compatibility window for the two synthetic steps, and pave the way for a new tandem catalytic process.<sup>[16]</sup>



**Scheme 3.** Compatibility test for the catalytic systems: hydration of benzonitrile (**2**) and reduction of propiophenone (**4**) catalyzed by the Ru complex **1** and KRED-P1-A04, respectively.

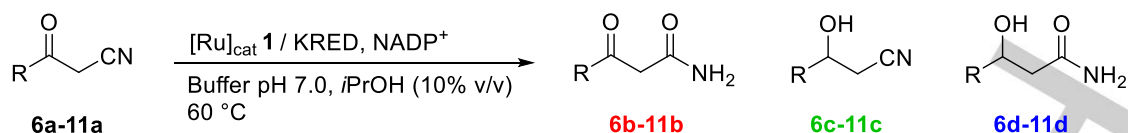
Anticipated as an important challenge in the present work is the level of orthogonality between the two processes.<sup>[17]</sup> Specifically, the KRED could reduce not only the starting  $\beta$ -ketonitrile but also the hypothetical  $\beta$ -ketoamide delivered if the nitrile hydration by the ruthenium complex takes place first. As a result, the optical purity of the target  $\beta$ -hydroxyamide would depend on the stereoselectivity of the KRED towards the two species. Nevertheless, selectivity could be envisaged if one of the catalysts was much more reactive than the other, which remains active towards the formed intermediate. With these premises, we selected  $\beta$ -ketonitrile **6a** and assessed the scope of the reduction by using a commercial kit of KREDs (Codex<sup>®</sup> KRED Screening Kit)<sup>[18]</sup> and two enzymes overexpressed in *E. coli* with opposite stereoselectivity, namely the (*R*)-selective ADH from *Lactobacillus kefir*<sup>[19]</sup> and the (*S*)-selective from *Rhodococcus ruber*.<sup>[20]</sup> Likewise, the reduction of the respective  $\beta$ -ketoamide **6b** was also considered. In general, both purified/overexpressed KREDs displayed higher activity towards **6a** and it was possible to identify biocatalysts which gave rise to both (*R*)- and (*S*)-enantiomers with *c* >99% and *ee* >99%. On the contrary, KREDs showed poor activity towards **6b**,<sup>[21]</sup> and only a few hits joined high conversion and high enantioselectivity. As an example, KRED-P2-H07 showed an excellent activity with **6a**, but very poor with **6b** (Scheme 4). Simultaneously, we also checked the activity of Ru complex **1** in the hydration of **6a** and **6c** at 40 °C. While **6c** was completely converted into the  $\beta$ -hydroxyamide **6d**, compound **6a** was recovered mostly unaltered.



**Scheme 4.** Testing pathways for the tandem bioreduction-nitrile hydration of  $\beta$ -ketonitrile **6a**. All reactions in buffer medium at 40 °C and 200 mM.

Accordingly, a preliminary concurrent tandem catalysis was essayed on  $\beta$ -ketonitrile **6a** (200 mM) by the combined action of KRED-P2-H07 and complex **1** (3 mol%) at 40 °C, working together from the beginning. As expected, the starting **6a** disappeared but a 79:21 mixture of enantiomerically pure (*R*)-**6c** and (*R*)-**6d** was obtained, which shows a high efficiency of the enzyme and a loss of activity of the metallic catalyst during the process. Because the hydration effectiveness of the complex **1** is significantly improved at higher temperatures (60-100 °C),<sup>[14]</sup> the hydrations of **6a** and **6c** were tested in buffer medium at 60 °C, conditions where KREDs also maintain their catalytic activity. As in both cases the conversions >99% into amide were observed, the concurrent approach was designed by using a biocatalyst displaying matched stereopreference towards the  $\beta$ -ketonitrile/ $\beta$ -ketoamide pair. Gratifyingly, KRED-P2-C11 met these requirements catalyzing the formation of (*R*)-**6c** and (*R*)-**6d** with high conversions and *ee* >99%. After thorough medium engineering, optimal conditions consisted of carrying out the reaction at 60 °C, as well as at 100 mM substrate concentration. In this way, (*R*)-**6d** was finally obtained from **6a** with 97% of conversion and *ee* >99% with metal and biocatalyst coexisting throughout the entire process (Table 1, entry 1). Moreover, although the hydration reaction was parametrized under argon atmosphere (see Table S1 in the SI),<sup>[11]</sup> the concurrent process yielded identical results without the need for an inert atmosphere. Likewise, and in order to assess the possible promiscuous activity of one or either catalyst for the alternative reaction, blank runs accomplished with  $\beta$ -ketonitrile **6a** i) in the absence of complex **1** and ii) in the absence of KRED, yielded the  $\beta$ -hydroxynitrile **6c** or the  $\beta$ -ketoamide **6b** as the sole product, respectively (*c* >99%).

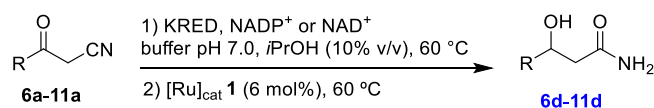
Encouraged by the success of this dual catalytic system, we extended the methodology to  $\beta$ -ketonitriles **7a-11a** (Table 1). Thereby, by using the appropriate KRED and a higher catalyst loading (6 mol%),<sup>[22]</sup> it was possible to reach the (*R*)-enantiomer of most  $\beta$ -hydroxyamides (**6d-10d**) as well as (*S*)-**11d** in enantiopure form and high degree of conversion (entries 1-7). Although it was challenging to find KREDs meeting the requirements to prepare the hydroxyamide antipodes, the examples compiled in Table 1 are a valuable proof of concept of the successful simultaneous action of enzymes and metal catalysts. Moreover, it is worth highlighting the excellent outcome of the process in several cases, which allowed us to isolate the resulting  $\beta$ -hydroxyamides with very high yield (92-94% for entries 1, 2, and 4). Particularly, the null or very low activity of the overexpressed enzymes towards  $\beta$ -ketoamides, with the exception of **9b** (see Tables S2-S13 in the SI), precluded their use in concurrent processes. Nonetheless, the simultaneous catalytic activity of **1** and *Lactobacillus kefir* on the  $\beta$ -ketonitrile **9a** rendered the target  $\beta$ -hydroxyamide (*R*)-**9d** in enantiopure form and conversion close to 90% (entry 5).

**Table 1.** Orthogonal tandem ruthenium catalyzed nitrile hydration-enzymatic reduction in aqueous medium by combining **1** and KREDs.<sup>[a]</sup>

Entry	Substrate	Enzyme	t (h)	<b>a</b> (%) <sup>[b]</sup>	<b>b</b> (%) <sup>[b]</sup>	<b>c</b> (%) <sup>[b]</sup>	<b>d</b> (%) <sup>[b]</sup>	ee for <b>d</b> (%) <sup>[c],[d]</sup>
1		P2-C11	16	-	-	-	>99	>99 (R)
2		P2-C11	16	3	-	-	97	>99 (R)
3		P2-C11	18	-	9	17	74	>99 (R)
4		P2-G03	18	-	-	-	>99	>99 (R)
5		<i>L. kefir</i>	24	-	11	2	87	>99 (R)
6		P2-G03	18	-	-	5	95	>99 (R)
7		P1-B10	18	-	7	10	83	>99 (S)

[a] Reaction conditions: **6a-11a** (100 mM) in KPi buffer 125 mM (1.25 mM MgSO<sub>4</sub>, 1.0 mM NADP<sup>+</sup>) pH 7.0, *i*-PrOH (10% v/v), complex **1** (6 mol%), KRED (100% w/w, mg of enzyme powder per mg of substrate) and stirring at 60 °C. For entry 5, **9a** (100 mM) in KPi buffer 50 mM (1.0 mM MgCl<sub>2</sub>, 1.0 mM NADP<sup>+</sup>) pH 7.0, *i*-PrOH (10% v/v), complex **1** (6 mol%), *L. kefir* (33 U/mg of substrate) and stirring at 60 °C [b] Ratio of starting material (**a**), β-ketoamide (**b**), β-hydroxynitrile (**c**), and β-hydroxyamide (**d**) was measured by NMR and HPLC. [c] Measured by chiral HPLC. [d] Absolute configuration established as detailed in the SI.

For those cases with mismatched KRED stereoselectivity towards the β-ketonitrile/β-ketoamide pair or incomplete conversion, a practical solution would be a sequential one-pot/two step process. Although less elegant than the concurrent one with both steps running simultaneously, the stepwise approach offers essentially the same advantages and low ecological footprint.<sup>[23]</sup> Actually, the only setting would be the addition of the ruthenium complex **1** once the bioreduction was completed. Concerning the order of the steps, this was established in view of the superior activity displayed by KREDs towards β-ketonitriles with respect to β-ketoamides. Accordingly, the reduction of β-ketonitriles **6a-11a** was accomplished in the conditions of the previous screening by choosing stereoselective KREDs. Once the enzymatic step was complete at 60 °C (18-24 h), complex **1** (6 mol%) was added and the reaction mixture stirred overnight at the same temperature. As a result, both enantiomers of **6d-11d** were isolated in essentially pure form (*c* >99%) and *ee* >99%. Table 2 collects some representative examples, including the counterpart of those missing in Table 1.

**Table 2.** Sequential enzymatic reduction-ruthenium catalyzed nitrile hydration in aqueous medium by combining **1** and KREDs.<sup>[a]</sup>

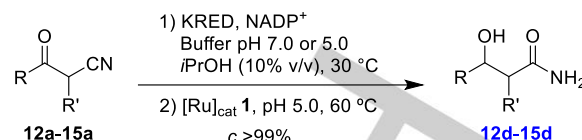
Entry	Substrate	KRED	Overall Conv. (%) <sup>[b]</sup>	ee (%) <sup>[c],[d]</sup>
1	<b>6a</b>	P3-H12	>99	>99 (S)
2	<b>6a</b>	<i>R. ruber</i>	97	>99 (S)
3	<b>7a</b>	P3-B03	>99	>99 (S)
4	<b>8a</b>	P3-H12	>99	>99 (S)
5	<b>9a</b>	P1-H08	>99	>99 (S)
6	<b>9a</b>	<i>L. kefir</i>	>99	>99 (R)
7	<b>10a</b>	P1-B10	>99	>99 (S)



8	<b>10a</b>	<i>L. kefir</i>	80	>99 ( <i>R</i> )
9	<b>10a</b>	<i>R. ruber</i>	85	>99 ( <i>S</i> )
10	<b>11a</b>	P2-G03	>99	>99 ( <i>R</i> )
11	<b>11a</b>	<i>L. kefir</i>	75	>99 ( <i>R</i> )

[a] Reaction conditions: For purified enzymes,  $\beta$ -ketonitrile (100 mM), KRED (100% w/w), *i*-PrOH (10% v/v), KPi buffer 125 mM (1.25 mM MgSO<sub>4</sub>, 1.0 mM NADP<sup>+</sup>) and shaking at 250 rpm and 60 °C; For *L. kefir* reactions,  $\beta$ -ketonitrile (100 mM), *L. kefir* (33 U/mg of substrate), *i*-PrOH (10% v/v), KPi buffer 50 mM (1.0 mM MgCl<sub>2</sub>, 1.0 mM NADP<sup>+</sup>) pH 7.0 and shaking as above; For *R. ruber* reactions,  $\beta$ -ketonitrile (100 mM), *R. ruber* (11 U/mg of substrate), *i*-PrOH (10% v/v), KPi buffer 50 mM (1.0 mM NADP<sup>+</sup>) pH 7.0 and shaking as above. After 24 h, complex **1** (6 mol%) was added and the mixture stirred at 60 °C. [b] Conversion for the first step was complete in all cases. [c] Measured by chiral HPLC. [d] Absolute configuration established as detailed in the SI.

Additionally, both cyclic and acyclic  $\beta$ -ketonitriles bearing a stereogenic carbon (**12a-15a**) were also considered (Table 3). The main feature of these compounds relies on the lability of their chiral center which is prone to racemize in the reaction medium, hence triggering a dynamic reductive kinetic resolution (DYRKR).<sup>[24]</sup> This fact along with the proven poor activity of the KREDs towards the corresponding  $\beta$ -ketoamides **12b-15b**,<sup>[18]</sup> precluded the option of a concurrent process and turned our attention towards the sequential protocol. The first step –KRED reduction– was carried out at 30 °C applying the conditions already reported for **13a-15a**.<sup>[10]</sup> Moreover, epimerization of the intermediate  $\beta$ -hydroxynitrile **12c** at 60 °C was observed, so the bioreduction step of **12a** should also be run at lower temperature. With these premises, the reduction of **12a**, **14a**, and **15a** were carried out with the selected KRED (Table 3) at pH 7.0 and 30 °C and quantitatively afforded the *syn*-**12c** and *cis*-**14c-15c** stereoisomers in >99:<1 *dr* and >99% *ee*. To obtain the analogous **13c** with high diastereoselectivity, the bioreduction of **13a** was conducted at pH 5.0. Finally, after proving that the Ru complex **1** catalyzed the hydration of **13c** more efficiently at pH 5.0 than 7.0, the Ru-catalyzed second step was carried out at this pH value in all cases. No epimerization of any chiral center was observed in the second step, being the optically active  $\alpha$ -substituted  $\beta$ -hydroxyamides **12d-15d** isolated with the same *dr* and *ee* (Table 3) than the corresponding  $\beta$ -hydroxynitrile intermediate.<sup>[25]</sup> As a result, and by means of the two synthetic approaches developed herein (concurrent or sequential mode), the joint action of a ruthenium complex and a ketoreductase allowed to address the gaps of the prior methodologies which were based on enzymatic<sup>[10]</sup> or metal catalysis alone.<sup>[11]</sup> Specifically, the reduction step was now smoothly accomplished by the enzyme, leading to optically pure hydroxy-compounds, while the metal complex promoted a highly efficient nitrile hydration enabling the  $\beta$ -hydroxyamides without tedious purification.

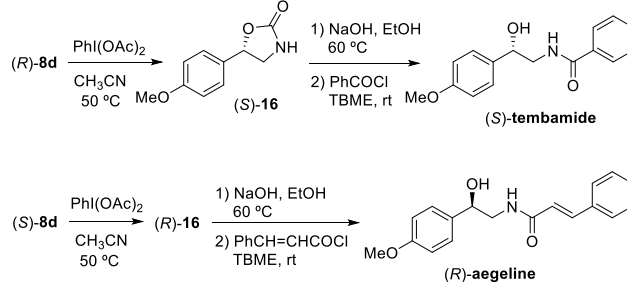


**Table 3.** A sequential one-pot dynamic reductive kinetic resolution – hydration of  $\alpha$ -substituted  $\beta$ -ketonitriles **12a-15a**.<sup>[a]</sup>

Entry	Substrate	KRED	Product <sup>[b]</sup>	<i>dr</i> <sup>[c]</sup>	<i>ee</i> (%) <sup>[c]</sup>
1		P3-H12	(2 <i>R</i> ,3 <i>S</i> )- <b>12d</b>	>99:<1	>99
2		P2-D11	(1 <i>S</i> ,2 <i>S</i> )- <b>13d</b>	15:85	>99
3		P1-B12	(1 <i>S</i> ,2 <i>R</i> )- <b>13d</b>	80:20	>99
4		P1-B12	(1 <i>S</i> ,2 <i>R</i> )- <b>14d</b>	>99:<1	>99
5		P1-A04	(1 <i>R</i> ,2 <i>S</i> )- <b>14d</b>	>99:<1	>99
6		P1-A04	(1 <i>R</i> ,2 <i>S</i> )- <b>15d</b>	>99:<1	>99

[a] Reaction conditions: **12a-15a** (100 mM), KPi buffer 125 mM (1.25 mM MgSO<sub>4</sub>, 1.0 mM NADP<sup>+</sup>) pH 7.0 (for **12a**, **14a**, and **15a**) or pH 5.0 (for **13a**), *i*-PrOH (10% v/v), KRED (100% w/w) and 30 °C. Once completed the reduction, addition of **1** (6 mol%) and conc. H<sub>3</sub>PO<sub>4</sub> until pH 5.0, and stirring at 60 °C. [b] Absolute configuration established as detailed in the SI. [c] Diastereomeric ratio (*dr*) and *ee* measured by chiral HPLC or GC.

By last, to illustrate the usefulness of this methodology the chemoenzymatic syntheses of the naturally occurring alkaloids (*S*)-(+)-tembamide (active against HIV)<sup>[26]</sup> and (*R*)-(-)-aegeline (hypoglycemic activity) were designed.<sup>[27]</sup> Starting from 3-(4-methoxyphenyl)-3-oxopropanenitrile (**8a**), the sequential one-pot reaction of KRED-P2-C11 or KRED-P3-H12 and complex **1** (6 mol%) gave access to the key enantiopure precursors (*R*)-**8d** and (*S*)-**8d**, respectively, in very high yields (93%). Then, a Hofmann rearrangement of these  $\beta$ -hydroxyamides with iodobenzene diacetate in acetonitrile at 50 °C gave the oxazolidinones (*S*)-**16** or (*R*)-**16** through an intramolecular attack of the hydroxyl group to the isocyanate intermediate (Scheme 5). Finally, basic hydrolysis of each enantiomer of **16** and subsequent treatment of the resulting  $\beta$ -amino alcohol with the required acid chloride enabled the isolation of (*S*)-(+)-tembamide<sup>[28]</sup> or (*R*)-(-)-aegeline in 77% and 73% overall yield, respectively, and >99% *ee* in both cases.



**Scheme 5.** Synthesis of (S)-(+)-tembamide and (R)-(-)-aegeline.

## Conclusions

In summary, we have developed a concurrent tandem-type cascade in aqueous medium which provides enantiopure  $\beta$ -hydroxyamides from  $\beta$ -ketonitriles by the combination of a Ru(IV)-catalyzed nitrile hydration with a biocatalytic reduction. Likewise, a complementary sequential cascade was applied to  $\beta$ -ketonitriles bearing a chiral center. In this case, the obtained products exhibited very high diastereo- and enantiomeric excesses as a result of a selective DYRKR. The genuine mutual compatibility of catalysts reported herein, a frequent recalcitrant challenge, is an excellent proof of concept of the practical value of chemoenzymatic one-pot processes for synthetic chemists. Although immense advances are being made in areas such as immobilization and compartmentalization, it is no less true that metal catalysis and biocatalysis are not antagonistic areas and can efficiently coexist in a synergic fashion. The key to the success is probably related with the use of ruthenium(IV) catalysts, which show a compatibility with enzymes far superior to that observed with their ruthenium(II) congeners. Finally, the enantiopure alkaloids (S)-(+)-tembamide and (R)-(-)-aegeline were efficiently prepared by means of a simple synthetic sequence in very high yields.

## Experimental Section

### General procedure for the conversion of $\beta$ -ketonitriles 6a-11a into $\beta$ -ketoamides 6d-11d in a concurrent fashion

In a 2.0 mL eppendorf tube, the corresponding  $\beta$ -ketonitrile (100 mM), KRED (100% w/w; mg of enzyme powder per mg of substrate), complex **1** (3.0 mol% for **6a**, 6.0 mol% for **7a-11a**), *i*-PrOH (10% v/v) and 125 mM phosphate buffer (also containing 1.25 mM MgSO<sub>4</sub> and 1.0 mM NADP<sup>+</sup>) pH 7.0 were added. The resulting reaction mixture was shaken at 250 rpm and 60 °C for 16-24 h. After this time, the mixture was extracted with ethyl acetate (2 × 500  $\mu$ L), centrifugated (90 s, 13000 rpm), and the combined organic layers were finally dried over Na<sub>2</sub>SO<sub>4</sub>. The degree of conversion was measured by <sup>1</sup>H-NMR. The enantiomeric excess of the corresponding product was determined by chiral HPLC or GC.

### General procedure for the conversion of $\beta$ -ketonitriles 6a-11a into $\beta$ -ketoamides 6d-11d in a sequential fashion

In a 2.0 mL eppendorf tube, the corresponding  $\beta$ -ketonitrile (100 mM), KRED (100% w/w; mg of enzyme powder per mg of substrate), *i*-PrOH (10% v/v) and 125 mM phosphate buffer pH 7.0 (also containing 1.25 mM MgSO<sub>4</sub>, 1.0 mM NADP<sup>+</sup>) were added. The resulting reaction mixture was shaken at 250 rpm and 60 °C for 24 h. After this time, complex **1** (6.0 mol %) was added and the reaction was left stirring overnight at 60 °C. Then, the mixture was extracted with ethyl acetate (2 × 500  $\mu$ L), the organic layers were separated by centrifugation (90 s, 13000 rpm), combined, and finally dried over Na<sub>2</sub>SO<sub>4</sub>. The degree of conversion was measured by <sup>1</sup>H-NMR and it was complete in all cases. The enantiomeric excess of the corresponding product was determined by chiral HPLC or GC.

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**Keywords:** One-pot processes • concurrent tandem catalysis • metal catalyst • chiral  $\beta$ -hydroxyamides • tembamide

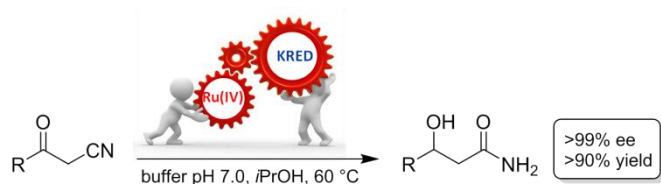
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**Metals and enzymes, union is strength:** A chemoenzymatic orthogonal tandem protocol for the highly enantioselective synthesis of  $\beta$ -hydroxyamides from  $\beta$ -ketonitriles in water is reported, establishing a fruitful cooperation between metal catalysts and enzymes for filling in the gaps from previous methodologies.

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