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 β-ketonitriles into optically active β-hydroxamides 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.[1] 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.[2] Similarly, a plethora of synthetic steps mediated by chemical catalysts, mainly transition-metal catalysts, have been coupled in organic medium.[3] Now, another turn of the screw in constructing catalytic networks consists of combining chemical and biological catalysts in aqueous medium, the natural environment of enzymes. Although these hybrid systems are still in their infancy, the wider available pool of water-compatible metal catalysts[4] along with the advances in protein engineering to produce more stable enzymes have enabled some remarkable examples of this kind.[5] Nevertheless, when planning such a chemo-enzymatic strategy a critical pitfall is the coexistence of both catalytic leading to: a) reciprocal poisoning, b) degradation because of additives, cofactors, or cosolvents, and c) incompatibility of reaction conditions.[6] As a result, most of these hybrid reactions run sequentially or, alternatively, the catalysts are site-isolated by compartmentalization or encapsulation techniques.[6c,7] Actually, the number of concurrent chemoenzymatic processes with concomitant action of metal and enzyme are still very scarce,[6d] and include the pioneering dynamic kinetic resolution (DKR) processes combining a racemization metal catalyst with an enzymatic transformation,[8a] ruthenium-catalyzed olefin metathesis coupled with monoamine oxidases and P-450 enzymes,[8c,6l] a simultaneous ruthenium-catalyzed isomerization of aliphatic alcohols-bioreduction,[8d] and the oxidation of cyclic amines to lactams by a monoamine oxidase and CuI/H2O2.[8d]

In this context, two different approaches have been recently reported for converting β-ketonitriles into β-hydroxamides 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 nitrite hydratase from whole cells of R. rhodochrous grown in the presence of DEPA (an inhibitor of amidase activity) was used.[9] In this asymmetric version, the first step is the KRED catalyzed dynamic reductive kinetic resolution of the ketone to a β-hydroxynitrile in very high enantio- and diastereomeric ratio. As the β-hydroxynitrile is formed, the nitrite hydratase catalyzes the water addition enabling the corresponding β-hydroxamides with excellent stereoselectivity (route a).[10] On the other hand, a concomitant hydration/transfer hydrogenation from β-ketonitriles...
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using a single ruthenium(II) complex (route b) was recently reported.[11]

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 β-hydroxyamide and β-hydroxyacid required chromatographic purification which decreased the yield,[12] while for route b (metal-catalyzed) the process was performed as a non-asymmetric version, and suffered from harsh reaction 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.

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,[13] the bioreduction requires an aqueous medium consisting of a phosphate buffer pH 7.0 (125 mM KPi, including 1.25 mM MgSO4, and 1.0 mM NADP+) 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).[14] 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).[15]

| Scheme 1. One-pot tandem processes for converting β-ketonitriles into β-hydroxyamides. |

| 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.[16]

Anticipated as an important challenge in the present work is the level of orthogonality between the two processes.[17] Specifically, the KRED could reduce not only the starting β-ketonitrile but also the hypothetical β-ketoamide delivered if the nitrile hydration by the ruthenium complex takes place first. As a result, the optical purity of the target β-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 β-ketonitrile 6a and assessed the scope of the reduction by using a commercial kit of KREDs (Codex®, KRED Screening Kit)[18] and two enzymes overexpressed in E. coli with opposite stereoselectivity, namely the (R)-selective ADH from Lactobacillus kefir[19] and the (S)-selective from Rhodococcus ruber.[20] Likewise, the reduction of the respective β-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[21] 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 β-hydroxyamide 6d, compound 6a was recovered mostly unaltered.
Accordingly, a preliminary concurrent tandem catalysis was essayed on β-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),[14] 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 β-ketonitrile/β-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),[13] 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 β-ketonitrile 6a i) in the absence of complex 1 and ii) in the absence of KRED, yielded the β-hydroxynitrile 6c or the β-ketoamide 6b as the sole product, respectively (c >99%).

Encouraged by the success of this dual catalytic system, we extended the methodology to β-ketonitriles 7a-11a (Table 1). Thereby, by using the appropriate KRED and a higher catalyst loading (6 mol%),[22] it was possible to reach the (R)-enantiomer of most β-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 β-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 β-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 β-ketonitrile 9a rendered the target β-hydroxyamide (R)-9d in enantiopure form and conversion close to 90% (entry 5).
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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 approach offers economical advantages and low ecological footprint.58 Actually, the only setting would be the addition of the ruthenium complex once the bioreduction was accomplished in the 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.

![Image of Table 1](https://example.com/table1.png)

Table 1. Orthogonal tandem ruthenium catalyzed nitrile hydration-enzymatic reduction in aqueous medium by combining 1 and KREDs.54

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

[a] Reaction conditions: 6a-11a (100 mM) in KPi buffer 125 mM (1.25 mM MgSO₄, 1.0 mM NADP⁺) pH 7.0, iPrOH (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 MgCl₂, 1.0 mM NADP⁺) pH 7.0, iPrOH (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.
he isolation of RS smoothly accomplished by the enzyme, leading to optically pure catalysis alone. (concurrent or sequential mode), the joint action of a ruthenium by means of the two synthetic approaches developed herein corresponding step, being the optically active proving that the Ru complex bioreduction of the analogous pH 7.0 and 30 ºC and quantitatively afforded the temperature. With these premises, the reduction of bioreduction step of intermediate already reported for KREDs towards the corresponding medium, hence trigger. The main feature of these compounds relies on the lability of stereogenic carbon (12a-15a) were also considered (Table 3). This fact along with the proven poor activity of the – hydroxynitrile intermediate. Specifically, the reduction step was now accomplished with the same (11 U/mg of substrate), reaction of KRED (100% w/w), – PrOH (10% v/v), KPi buffer 50 mM (1.0 mM NADP+) pH 7.0 and shaking as above; For R. ruber reactions, – ketonitrile (100 mM), L. kefir (33 U/mg of substrate), – PrOH (10% v/v), KPi buffer 50 mM (1.0 mM NADP+) pH 7.0 and shaking as above; For R. ruber reactions, – ketonitrile (100 mM), R. ruber (11 U/mg of substrate), – PrOH (10% v/v), KPi buffer 50 mM (1.0 mM NADP+) pH 7.0 and shaking as above. Above 24 h, complex 1 (6 mol%) was added and the mixture stirred at 60 ºC. [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 β-ketone derivatives 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).[24] This fact along with the proven poor activity of the KREDs towards the corresponding β-ketoamides 12b-15b,[18] 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 ºC applying the conditions already reported for 13a-15a.[10] Moreover, epimerization of the intermediate β-hydroxy nitrile 12c at 60 ºC 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 ºC 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 β-substituted β-hydroxyamides 12d-15d isolated with the same dr and ee (Table 3) than the corresponding β-hydroxy nitrile intermediate.[25] 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[19] or metal catalysis alone.[11] 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 β-hydroxyamides without tedious purification.

By last, to illustrate the usefulness of this methodology the chemoenzymatic syntheses of the naturally occurring alkaloids (S)-(+)‐tembamide (active against HIV)[25] and (R)‐(–)‐aegeline (hypoglycemic activity) were designed.[26] 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 β-hydroxyamides with iodo benzene diacetate in acetonitrile at 50 ºC 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 β-amino alcohol with the required acid chloride enabled the isolation of (S)-(+)‐ tembamide[25] or (R)‐(–)‐aegeline in 77% and 73% overall yield, respectively, and >99% ee in both cases.
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Conclusions

In summary, we have developed a concurrent tandem-type cascade in aqueous medium which provides enantiopure β-hydroxyamides from β-ketonicnitriles by the combination of a Ru(IV)-catalyzed nitrile hydration with a biocatalytic reduction. Likewise, a complementary sequential cascade was applied to β-ketonicnitriles bearing a chiral center. In this case, the obtained products exhibited very high diastereo- and enantioemic 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 synergetic 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 β-ketonicnitriles 6a-11a into β-ketoamides 6d-11d in a concurrent fashion

In a 2.0 mL eppendorf tube, the corresponding β-ketonicnitrile (100 mM), KRED (100% w/w; mg of enzyme powder per mg of substrate), compound phosphate buffer (also containing 1.25 mM MgSO4 and 1.0 mM NADP+) pH 7.0 were added. The resulting reaction mixture was shaken at 250 rpm and 60 °C for 24 h. After this time, the mixture was extracted with ethyl acetate (2 × 500 μL), centrifuged (90 s, 13000 rpm), and the combined organic layers were finally dried over Na2SO4. The degree of conversion was measured by 1H-NMR. The enantiomeric excess of the corresponding product was determined by chiral HPLC or GC.

Acknowledgements

We are indebted to the MINECO of Spain (CTQ2013-40591-P and CTQ2016-75986-P) and the Gobierno del Principado de Asturias (Project GRUPIN14-006) for financial support. E. Liardo acknowledges funding from the European Union’s Horizon 2020 MSCA ITN-EID program (grant agreement No 634200). The authors also thank Dr. Martin Schürmann (InnoSyn) and Prof. Harald Gröger (Bielefeld University) for the generous gift of the KRED of Rhodococcus ruber and Lactobacillus kefir, respectively.

Keywords: One-pot processes • concurrent tandem catalysis • metal catalyst • chiral β-hydroxyamides • tembamide

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Three ruthenium complexes, known for their ability to hydrate nitriles under mild conditions, were considered, i.e., the bis(allyl)-ruthenium(V) complex [RuCl₂(η⁵-C₅H₅)(PMe₂O)] (1; C₅H₅N = 2,7-dimethyl-octa-2,6-diene-1,8-diy) and the arené ruthenium(II) derivatives [RuCl₂(η⁵-p-cymene)(PR₃)] (R = Me (1'), 4-C₅H₄F (1'')). a) E. Tomás-Mendivil, F. J. Suárez, J. Díez, V. Cadierno, Chem. Commun. 2014, 50, 9661-9664. b) E. Tomás-Mendivil, V. Cadierno, M. I. Menéndez, R. López, Chem. Eur. J. 2015, 21, 16874-16886. c) E. Tomás-Mendivil, P. J. González-Liste, J. Borge, P. Crochet, V. Cadierno, Catal. Sci. Technol. 2016, 6, 4398-4409. d) E. Tomás-Mendivil, J. Francos, R. González-Fernández, P. J. González-Liste, J. Borge, V. Cadierno, Dalton Trans. 2016, 45, 13590-13603. e) R. González-Fernández, P. Crochet, V. Cadierno, M. I. Menéndez, R. López, Chem. Eur. J. 2017, 23, 15210-15221. A parametric study of the catalytic activity of these ruthenium complexes in the hydration of 2 is shown in Table S1 in the SI.

The concentration was lowered from the reported 330 mM (ref. 14a) to 200 mM, to fit the maximum concentration tolerated by KREDs for an efficient performance.

The stability of the KRED was also evaluated in the presence of growing percentages of the metal complex 1 (up to 20% mol). As a result, the enzymatic activity remained unaltered in all the cases.

Orthogonal tandem catalysis is defined as a one-pot sequence of reactions involving two or more functionally distinct catalytic mechanisms, promoted by two or more different catalysts that are present from the outset. See: T. L. Lohr, T. J. Marks, Nat. Chem. 2015, 7, 477-482.

The Codex® KRED Screening Kit (Codexis, Reedwood City, USA) contains 24 ketoreductases. For the full panel of enzymatic screenings, see section 3 in the SI.

We have previously reported coupled chemoenzymatic one-pot/two step reactions in aqueous media: a) N. Rios-Lombardia, C. Vidal, M. Cocina, F. Moris, J. García-Álvarez, J. González-Sabin, Chem. Commun. 2015, 51, 10937-10940. b) M. J. Rodríguez-Álvarez, N. Rios-Lombardia, S. Schumacher, D. Pérez-Iglesias, F. Moris, V. Cadierno, J. García-Álvarez, J. González-Sabin, ACS Catal. 2017, 7, 7753-7759.

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Metals and enzymes, union is strength: A chemoenzymatic orthogonal tandem protocol for the highly enantioselective synthesis of β-hydroxyamides from β-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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