Preparation of chiral β-hydroxytriazoles in one-pot chemoenzymatic bioprocesses catalyzed by *Rhodotorula mucilaginosa*

Celeste Aguirre-Pranzoni[a], Rodrigo D. Tosso[a], Fabricio R. Bisogno[b], Marcela Kurina-Sanz[a,*], Alejandro A. Orden[a,*]

[a.] *INTEQUI-CONICET, Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis, Almirante Brown 1455, D5700HGC, San Luis, Argentina*

[b.] *INFIQC-CONICET, Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina*

**Corresponding authors. Tel.: +54 0266-4520300 ext. 3153.*

E-mail addresses: pranzonica@gmail.com (C. Aguirre-Pranzoni), rdtosso@unsl.edu.ar (R. D. Tosso) fbisogno@fcq.unc.edu.ar (F. R. Bisogno), marcelakurina@gmail.com (M. Kurina-Sanz), aaorden@unsl.edu.ar (A. A. Orden).

Abstract

Chemoenzymatic strategies for the preparation of enantiopure β-hydroxytriazoles were designed. These and other related compounds are particularly relevant because of their antitubercular bioactivities and as β-adrenergic receptor blockers. The ability of *Rhodotorula mucilaginosa* LSL to stereoselectively reduce prochiral ketones coupled to copper(I)-catalyzed azide-alkyne cycloaddition was exploited. The reactions were performed in aqueous medium and at room temperature. *R. mucilaginosa* LSL offered the advantage of internal redox cofactor recycling. Notably, the biocatalyst was compatible with all the chemicals required namely sodium azide, copper sulfate and alkynes and showed a broad substrate scope reducing small-bulky and bulky-bulky ketones stereoselectively. Considering this, two one-pot processes were assessed to synthesize enantiopure (*R*)-β-hydroxytriazoles. A one-pot, three-step sequential transformation allowed obtaining enantiopure products up to 65% yield starting from α-chloro arylketones. On the other hand, with α-bromo arylketones, a one-pot cascade process furnished the same products in *ca* 80% isolated yield.

Keywords: (*R*)-β-hydroxytriazoles; divergent-convergent process; biocatalysis; bulky-bulky ketones; bioreduction; stereoselectivity.

1. **Introduction**

The combination of sustainable tools with efficient processes is continuously searched [1,2]. Particularly, one-pot procedures minimize time, number of unit operations and costs of complex molecule synthesis. On the other hand, biocatalysts have gained place as promising tools in organic chemistry. A good example is the industrial synthesis of atorvastatin using a ketoreductase (KRED) and halohydrin dehalogenase (HHdH) in its synthetic route [3] Some biocatalysts have been tailored by protein engineering and designed for the synthesis of bioactive compounds [4,5].

The asymmetric reduction of prochiral ketones is a good strategy to obtain enantiopure *sec*-alcohols, which can be used as building blocks for the synthesis of chiral pharmaceutical intermediates and fine chemicals. For this purpose, bioreductions mediated by ketoreductases (KRED) are widely used. There is a huge number of KRED described in literature that shows high stereoselectivity with a broad substrate scope [6] although most of them are limited to ketones bearing small-bulky substituents [7]. So far, only a few biocatalysts having the ability of reducing bulky-bulky ketones have been reported [8–11]. Nevertheless, the screening of new KRED possessing the ability of accommodating bulky substrates is still necessary for the expansion of the enzymatic toolbox.

Azole compounds have been extensively studied as potential targets for drug discovery since they possess a broad range of biological properties, including antimicrobial, antiviral, antiepileptic, anti-HIV [12]. In particular, 1,2,3-triazoles are active against tuberculosis [13–15]. Besides, β-hydroxytriazole derivatives, specifically the *R* enantiomers, have been reported as potential β-adrenergic receptor blockers [16,17].

One of the most practical and reliable chemical methods to synthesize 1*H*-1,2,3-triazoles is the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) under mild conditions via click chemistry [18]. Interesting examples for the preparation of enantiopure β-hydroxytriazoles derivatives by chemoenzymatic approaches can be surveyed in the literature, being ADHs the most employed enzymes in these multi-step procedures. In all these strategies, the stereocenter was defined before the CuAAC reaction [19–24]. In a recent report, a bioreduction of a β-ketotriazole was performed by a

strain of *Penicillium citrinum* affording the corresponding (*S*)-β-hydroxytriazole in high enantiomeric excess (ee) values [25].

Previously, we reported a novel wild type biocatalyst, *Rhodotorula mucilaginosa* LSL, isolated from a soil sample of a landfarming site, capable of carrying out the stereoselective reduction of a series of arylketones [26]. In the present work, we took advantage of the robustness and broad substrate scope of *R. mucilaginosa* LSL to design chemoenzymatic processes with the aim of preparing (*R*)-β-hydroxytriazoles.

2. Results and Discussion

In a first approach, we synthesized (*R*)-β-hydroxytriazoles (**7b**-**10b**) with excellent ee values in a three-step sequential procedure starting from the commercially available 2-halo-1-phenylethanones (**1a**-**4a**). Thus, these compounds were treated with sodium azide and then the α-azidoketones (2 mM) were stereoselectively reduced to (*R*)-2-azido-1-arylethanols (**5b** and **6b**) by whole cells of wild-type *R. mucilaginosa* LSL. The biocatalyst was used either as resting or lyophilized cells in phosphate buffer (30 mL, pH 6.5) and sucrose (1.2 g) at room temperature, without the addition of external redox cofactors, showing exquisite stereoselectivity, as previously reported [26]. Then, the purified azidoarylethanols (*R*)-**5b** and (*R*)-**6b** were subjected to CuAAC with either phenylacetylene or propargyl alcohol catalyzed by *in situ* formed Cu(I) [Cu(II)/ascorbate system] in a mixture of water-dimethylsulfoxide (DMSO) at room temperature (Figure 1). In every case, the products were recovered in *ca* 40% final isolated yield (see Table S3, SI).

Alternatively, we assessed the bioreduction of the β-ketotriazoles **7a**-**10a** assuming that these bulky-bulky ketones could be accepted as substrates for the KRED of *R. mucilaginosa* LSL. A complete conversion to the corresponding (*R*)-β-hydroxytriazoles in 24 h was achieved and the stereoselectivity was excellent with ee values higher than 98% (Figure 2). Taking into account the instability of the β-hydroxyazides [27] and based on these results, we decided to invert the last two steps of the synthetic route. Following this methodology, the overall yield increased reaching 60% of the isolated products (see Table S3, SI). In this manner, it was demonstrated that *R. mucilaginosa* LSL accepts α-azidoketones **5a**-**6a** (small-bulky substrates) and β-ketotriazoles **7a**-**10a** (bulky-bulky substrates) (table 1) with the same stereopreference (*R*).

Encouraged by these results and in order to improve the yields of the (*R*)-β-hydroxytriazoles, we designed one-pot strategies. On the one hand, by starting from α-chloroketones, a one-pot, three-step sequential procedure was developed. In the first step, both compounds **5a** and **6a** were obtained by azidation of α-chloroacetophenones. Immediately after completion of the reaction, the azides were subjected to CuAAC reaction by adding the corresponding alkynes and the Cu(I) *in situ* forming reagents. Once the β-ketotriazoles were formed, *R. mucilaginosa* LSL lyophilized cells were added and incubated under non-sterile conditions giving the complete and stereoselective reduction of the bulky-bulky substrates after 24 h (Figure 3). Notably, the copper species present in the reaction medium did not affect the enzymatic machinery responsible of catalyzing the ketones reduction and the redox cofactor regeneration. Afterwards, only one purification procedure was necessary to yield the (*R*)-β-hydroxytriazoles with a slight improvement in the isolated yield (see Table S3, SI).

On the other hand, we tried to develop a one-pot cascade strategy starting from α-chloroketones and all the chemicals and the lyophilized biocatalyst. In this process, chlorine was substituted by the azide nucleophile in S_N2 reaction and, in competition the α-chloroketones were bioreduced to the corresponding chlorohydrins. This divergence furnished two different products resulting in a low conversion to the desired products (data not shown). Alternatively, we envisaged the use of α-bromoketones in aqueous medium at pH 4.5. This reaction condition was selected to avoid the formation of by-products such as epoxides, as previously reported [26]. Under this condition, bromine was substituted by the azide nucleophile in S_N2 reaction and, in parallel a small amount of the α-bromoketones were bioreduced to the corresponding bromohydrins (*R*)-**3b** and (*R*)-**4b**. Since bromide is a better leaving group than chloride, the bromohydrins were also reactive in S_N2 reaction and were transformed to the β-hydroxyazides. The formation of all these intermediates was confirmed by TLC and GC-FID (data not shown). Consequently, these products converged efficiently to the synthesis of the (*R*)-β-hydroxytriazoles **7b-10b** (Figure 4) in up to 80% isolated yield (see Table S3, SI). This increase in the yield could be attributable to *i)* the immediate consumption of the unstable azide compounds as soon as they are formed, *ii)* the complete substitution of the bromine by the azide even in the bromohydrin intermediate, *iii)* the ability of *R. mucilaginosa* LSL to reduce the bromoketones,

the azidoketones as well as the bulky-bulky β-ketotriazoles, and *iv)* the avoidance of intermediates isolation steps.

3. Conclusions

The capability of *R. mucilaginosa* LSL to stereoselectively reduce small-bulky and bulky-bulky ketones was demonstrated. This biocatalyst can operate as resting and lyophilized cells without the addition of external cofactors resulting in a valuable tool for green chemistry endeavors. The KRED responsible of these biotransformations are promising to be further investigated. Based on these facts, we designed efficient one-pot chemoenzymatic strategies to synthesize (*R*)-β-hydroxytriazoles. The carbonyl reductase activity of the yeast was not impaired despite all the chemicals present in the reaction medium. By a one-pot divergent-convergent process with the appropriate substrate, we could obtain the desired products in high isolated yields in a very simple, green, cost-effective and easy-to-handle fashion.

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