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Coupling biocatalysis and click chemistry: one-pot two-step convergent synthesis of enantioenriched 1,2,3-triazole-derived diols†

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A fully convergent one-pot two-step synthesis of different chiral 1,2,3-triazole-derived diols in high yields and excellent enantio- and diastereoselectivities has been achieved under very mild conditions in aqueous medium by combining a single alcohol dehydrogenase (ADH) with a Cu-catalysed 'click' reaction.
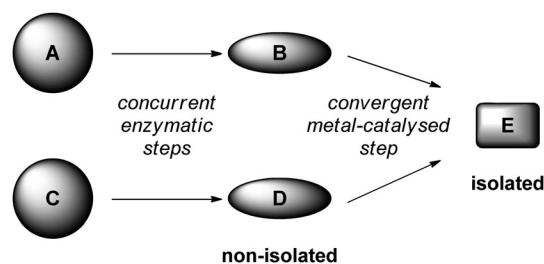
Nowadays, the selective synthesis of enantioenriched derivatives for fine chemicals must go in hand with the design of sustainable processes.[1] Hence, final targets must be obtained with excellent purities, but minimising the use of harmful solvents, reagents and also purification steps involved in a synthetic pathway.[2] In this sense, the employment of water as reaction medium,[3] recyclable catalysts[4] and the design of one-pot multi-step sequential or concurrent synthesis[5] are highly desired. In the last few years enzymes have been elegantly employed together with organo- or metal-catalyst(s), achieving (chemoenzymatic) one-pot multi-step synthesis of interesting compounds.[6] Besides lipase-based dynamic processes,[7,8] most of the examples deal with the concurrent use of oxidoreductases with organo-[9] or metal-[10] catalysts. For instance, alcohol dehydrogenases,[11] responsible for the stereoselective reduction of carbonylic compounds, have been successfully combined with proline-[9a] or Zn-catalysed[10b] aldol reactions to obtain enantioenriched 1,3-diols, and with Pd-based catalysts for Wacker–Tsuji,[10a] Heck,[10cj] Suzuki,[10d,f,i] or Suzuki–Miyaura[10h] reactions. In these examples, the non-enzymatic transformation occurred first to afford the non-isolated (di)ketone which further underwent ADH-catalysed reduction.

1,2,3-Triazoles are very important pharmacophores that can exert multiple biological activities,[12] e.g., hydroxylated derivatives have been described as potent β-adrenergic receptor blockers.[13a] Besides,

triazole-containing structures are versatile ligands in coordination chemistry,[13b] and some related complexes have displayed antitumor activities.[13c] In this sense, the copper(I)-catalysed version of the Huisgen cycloaddition in water between an alkyne and an azido compound to form them, is perhaps the most outstanding example of the so-called 'click' chemistry.[14] A Cu(I) salt must be added into the reaction medium, but in the last few years, the in situ formation of this species by reduction of Cu(II) salts with e.g. ascorbate has been mostly employed. Albeit more scarcely studied, comproportionation of a Cu(II) salt with Cu(0) is gaining more relevance due to availability and economic issues.[15] Noteworthily, there are few examples in which biocatalysis and click chemistry have been combined in one-pot procedures, although both protocols can be perfectly compatible. In this sense, the kinetic resolution of aromatic epoxides by halohydrin dehalogenase (Hhe)-catalysed azidolysis has been reported, affording the corresponding β-azido alcohols that subsequently reacted with alkynes to achieve chiral 1,2,3-triazole alcohols.[16] Yields of these processes could be increased by introducing the bioreduction of an α-halo ketone as a first step.[17]

Herein, we envisaged a one-pot two-step fully convergent strategy in which, starting from two achiral compounds, a pair of suitable chiral precursors could be stereoselectively formed and then assembled by a 'click' reaction, giving rise to a single compound bearing two chiral centres (Scheme 1).

The synthesis of the enantioenriched 1,2,3-triazole-derived diols was performed by the concurrent stereoselective bioreduction of an α-azido- and an alkynyl ketone, followed by cycloaddition catalysed

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Scheme 1 One-pot two-step fully convergent strategy combining stereoselective enzymatic and metal-catalysed transformations.
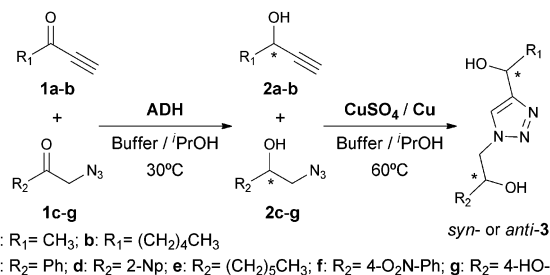
Scheme 2 Chemoenzymatic protocol to synthesise enantioenriched 1,2,3-triazole-derived diols.

by Cu(I) *in situ* formed *via* comproportionation of Cu(II) and Cu(0) (Scheme 2).

Several practical issues were considered in advance: (a) both ketones should be quantitatively reduced so, after the cycloaddition step, only diols must be formed, avoiding hydroxy ketones or diketones that would make the purification more complex; (b) alkynyl ketones may decompose in aqueous media,[18] so the bioreduction step must also be quick and clean; (c) the selectivity of the ADH-catalysed process must be high to avoid the formation of diastereoisomers at the end of the sequence; (d) the cycloaddition step must take place in the presence of the enzyme and the hydroalcoholic medium without the loss of the performance; and (e) it would be highly desirable that the Cu(0)-precatalyst source could be easily recycled with no stirring impairment.

In a first set of experiments, we studied the influence of different alkynones (**1a–b**) and α-azido ketones (**1c–g**) bearing aliphatic or aromatic (phenyl or 2-naphthyl) groups with *e.g.* nitro or hydroxy moieties on ADH-catalysed reduction using Prelog ADHs over-expressed in *E. coli* (ADH-A from *Rhodococcus ruber*,[19] ADH-T from *Thermoanaerobium* sp.,[20] and TesADH from *Thermoanaerobacter ethanolicus*[21]) or commercially available anti-Prelog enzymes (LBADH from *Lactobacillus brevis*[22] or LKADH from *Lactobacillus kefir*[23]). In ADH-catalysed bioreductions, Tris·HCl buffer is usually a suitable medium, but it was observed that alkynones were not stable, so we turned to phosphate buffer (50 mM) pH 7.5 at 30 °C and 250 rpm, in which these derivatives remained stable for longer periods. Under these conditions, the reduction of all ketones was tested using ADHs (Table 1), showing in some cases quantitative conversions (*c*) and excellent stereoselectivities (ee).

Especially ADH-A as a Prelog representative enzyme and LBADH as an anti-Prelog counterpart rendered quantitative conversions and excellent ee with several substrates. Furthermore, they were able to accept both alkynones and α-azido ketones, being excellent candidates to achieve the desired one-pot process. In a second stage, simultaneous bioreduction of a 1:1 mixture of alkynone–azido derivatives was tried to study if any interference could exist, either between reactants or with the ADH. Pleasingly, both substrates were perfectly reduced with the same values of stereoselectivity and no cross-inhibition was detected.

Later, the Cu-catalysed 'click' reaction was studied to find out suitable conditions. In this regard, the source of Cu(I) was firstly studied using racemic alcohols, *rac*-**2a** and *rac*-**2c**, as model substrates to afford a mixture of *syn*- and *anti*-**3ac** diols. CuSO₄–ascorbic acid in a H₂O:^{t}BuOH 1:1 v/v^{-1} mixture was tried at room temperature for 24 h, but the conversion was not complete. At this point,

Table 1 Stereoselectivities in selected bioreductions of alkynones **1a–b** and α-azido ketones **1c–g** employing ADHs (*t* = 24 h)^a

Ketone^b	*E. coli*/ADH-A	*E. coli*/ADH-T	*E. coli*/TesADH	LBADH	LKADH
1a	96 (*S*)	96 (*S*)	76 (*S*)	64 (*R*)	—
1b^c	98 (*R*)	90 (*R*)	96 (*R*)	>99 (*S*)	99 (*S*)
1c^c	>99 (*R*)	>99 (*R*)	—	>99 (*S*)	98 (*S*)
1d^c	>99 (*R*)	—	—	—	—
1e^c	99 (*R*)	99 (*R*)	—	99 (*S*)	99 (*S*)
1f^c	>99 (*R*)	>99 (*R*)	—	>99 (*S*)	—
1g^c	>99 (*R*)	—	—	—	—

^{a} For experimental details, see ESI. ^{b} In all cases where an ee value appears, the conversion was quantitative. For incomplete reactions, see *c* and ee in Tables S1 and S2 in ESI. ^{c} Change in Cahn–Ingold–Prelog (CIP) priority.

we envisaged a system that could be simple, economic and environmentally friendly to obtain Cu(I) *via* comproportionation using a Cu wire with a catalytic amount of CuSO₄. Thus, a copper wire was rolled on a magnetic bar (see ESI†), allowing at the same time stirring, easy recovery and its reuse along several cycles. Different solvent mixtures were tried (H₂O:^{t}BuOH 1:1 v v^{-1}; H₂O:^{i}PrOH 1:1 v v^{-1}; H₂O:^{i}PrOH 95:5 v v^{-1}) at 60 °C for 24 h, obtaining in all cases the triazole diols with quantitative conversions, but since the enzymatic step took place with ^{i}PrOH (5% v v^{-1}),[24] we chose the last setting to perform the sequential chemoenzymatic transformation. Microwave heating was also tried, but due to the formation of by-products, *e.g.* 1,5-regioisomers,[25] this methodology was not further investigated. After four cycles, the Cu wire got passivated, but it could be recovered by simple washing with HCl 2 N for 5 min.

In the final stage, the one-pot fully convergent chemoenzymatic approach was achieved furnishing the 1,2,3-triazole-derived diols in good overall yields and excellent selectivities (Fig. 1). After performing the bioreduction in phosphate buffer at 30 °C with 2-propanol for 24 h, the copper wire and CuSO₄ were added into the reaction mixture, and it was heated up at 60 °C for 24 h. While with alkynone **1b** *syn*-**3** diols were obtained, in the case of substrate **1a**,
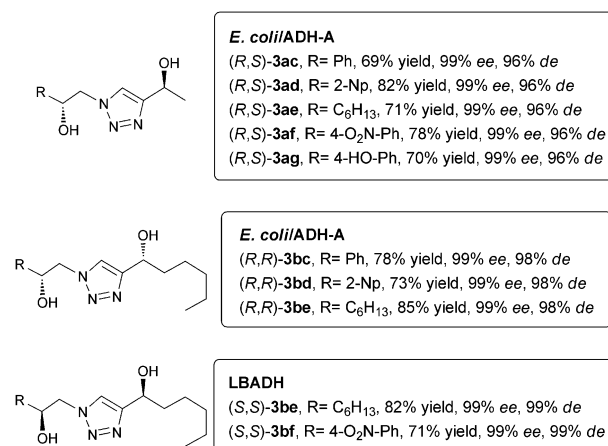

Fig. 1 Examples of 1,2,3-triazole-derived diols synthesised using the chemoenzymatic approach. ee values correspond to the major diastereoisomer obtained. Isolated yields (69–85%) are relative to the diastereoisomeric mixture of the final diols.

since the enzyme recognised the ethynyl group as the 'big' moiety,[26] anti-**3** diols were synthesised.

In the last years, the combination of bio- and metal-catalysis has emerged as a potent tool to achieve the synthesis of novel derivatives in a 'one-pot' fashion, thus avoiding the time-consuming and yield-lowering isolation of intermediates. In this sense, biocatalysed redox processes and copper(ı)-catalysed [3+2] cycloadditions can be perfectly compatible in an environmentally benign medium like water. Herein a one-pot two-step fully convergent[27] synthesis of different chiral 1,2,3-triazole-derived diols in high yields and excellent enantio- and diastereoselectivities under very mild conditions has been developed. Hence, starting from two prochiral ketones and using a single ADH with ^{i}PrOH in a first step, and then applying a catalytic amount of $CuSO_4$ and a Cu-wrapped stirring bar, a triazole core bearing two chiral centres could be easily synthesised. Moreover, the Cu(0)-precatalyst could be recycled, easily removed, and remained active after several cycles. The possibility of the enzymatic recycling via immobilisation[28] could also afford a more efficient and cost-effective method. This system also has the advantage that by simple selection of the enzyme, the chirality of the final compounds can be finely tuned.

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