DOI: 10.1002/adsc.201000502

# Combining Designer Cells and Click Chemistry for a One-Pot Four-Step Preparation of Enantiopure β-Hydroxytriazoles

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Received: June 29, 2010; Published online: September 1, 2010

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.201000502.

**Abstract:** The multistep catalytic process using designer cells, either added as freshly prepared suspensions or as stable lyophilized powder, and click reaction can be performed in one pot. The sequence of four reactions allows the production of both enantiomers of β-hydroxytriazoles with high enantiomeric excess.

**Keywords:** azides; biocatalysis; click chemistry; designer cells; halohydrin dehalogenase; reaction cascade

The synthetic application of designer cells, that is, engineered microbial cells which overexpress one or more synthetically useful biocatalysts, attracts special attention. Recent reports, in which two genes encoding enzymes involved in reductive biotransformations were cooverexpressed in such "tailor-made" host cells, describe their use in the stereoselective reduction of ketones,[1] α-halo ketones,[2] α-hydroxy ketones[3] and α-keto esters[4] or reductive amination of α-keto esters.[5] In all of these cases one of the cloned enzymes is responsible for the biocatalytic transformation of the substrate, while the other one oxidizes a sacrificial electron donor (such as glucose, methanol or formate) thus regenerating the cofactor (NADP or NADPH). The main advantages of such designer cells include low cost, as there is no need for expensive isolated enzymes and cofactors, and the higher stability of the biocatalysts in the cell environment.

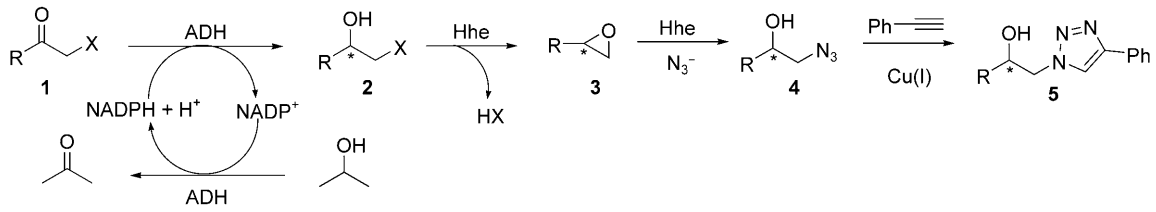
The outstanding possibilities offered by the use of designer cells for the one-pot catalysis of a set of subsequent reactions can be further broadened through combination with a bioorthogonal catalytic process that takes place in the same reaction vessel. We have recently reported the possibility of combining a biocatalytic process, in which an isolated enzyme is used for the enantioselective introduction of an azide moiety into a substrate, with a subsequent Cu(I)-catalyzed [2+3]-dipolar cycloaddition ("click" reaction) of the product with acetylenes.[6]

The combination of the multienzymatic cell cascade and the bioorthogonal click reaction might offer a valuable method for the one-pot transformation of simple, prochiral substrates into enantiopure, highly functionalized products. The triazole group which is formed by the cycloaddition is a bioisostere of the peptide bond[7] and thus an important pharmacophore. Specifically, β-hydroxytriazoles have been reported to be β-adrenergic receptors blockers.[8]

We report here a new, simple, one-pot procedure for the preparation of enantiopure β-hydroxytriazoles **5** from simple α-halo ketones **1** (Scheme 1), which combines the use of designer cells and click reactions.

The biocatalytic cascade incorporates four processes, which transform the prochiral α-halo ketones **1** into enantiopure β-hydroxy azides **4**. In the first reaction the ketone **1** is stereoselectively reduced to β-halo alcohol **2** by an alcohol dehydrogenase (ADH).[9] In this process NADPH is used as a cofactor. The recycling of NADPH is provided by the same enzyme in a reaction which transforms isopropyl alcohol, a sacrificial electron donor, into acetone. Compound **2**

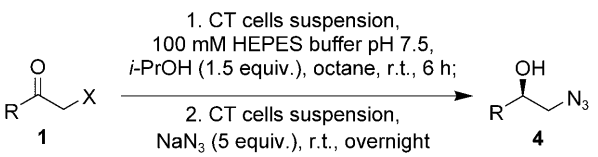



**Scheme 1.** Multicatalytic cascade leading to enantiopure β-hydroxytriazoles.

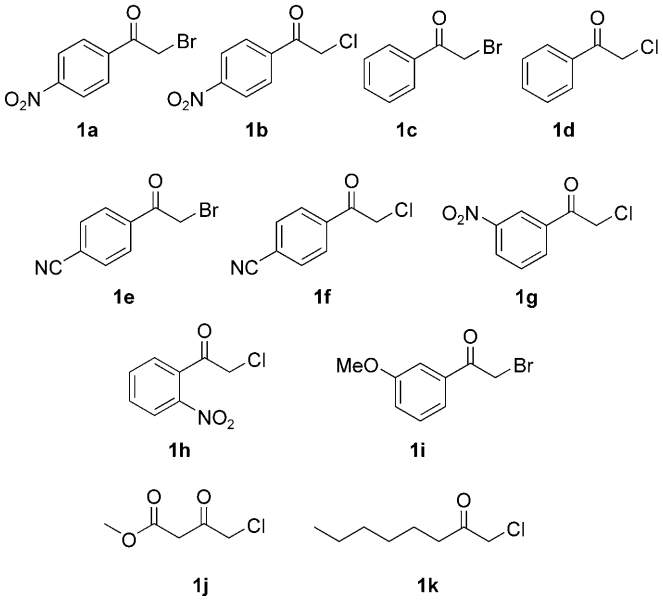
serves as a substrate for halohydrin dehalogenase (Hhe), which catalyzes the ring-closure towards epoxide **3** and subsequent ring-opening of **3** by azide anion, forming compound **4**, the final product of the biocatalytic cascade. Azido alcohol **4** is in the same reaction vessel converted to β-hydroxytriazole **5** in a Cu(I)-catalyzed [2+3]-dipolar cycloaddition reaction. We have decided to use the enzyme-catalyzed highly regioselective azidolysis of the epoxide ring (Scheme 1, **3** to **4**), reported by us earlier,[10] as the central reaction of this system, allowing for the introduction of an azide into the substrates. A similar reaction was explored for the incorporation of azide using isolated enzymes and cofactor addition by Kroutil and co-workers[11] and by researchers from Codexis for the incorporation of cyanide for producing a statin intermediate.[12]

We aimed at engineering cells that overexpress both enzymes used in the cascade, thus making the process cheaper and applicable on a larger scale. Two such constructs were made, using combinations of ketoreductases (dehydrogenases) expressing opposite stereoselectivity. In both cases cells of *E. coli* strain MC1061 were used as the host and the enzymes were cloned on a single pBad-vector. The first construct (CT cells) expressed AdhT, an *R*-selective alcohol dehydrogenase from *Thermoanaerobacter* sp.[13] and HheC, the *R*-selective halohydrin dehalogenase from *Agrobacterium radiobacter* AD1.[14] The second type of cells (BL cells) expressed AdhL, the *S*-selective alcohol dehydrogenase from *Lactobacillus brevis*[15] and HheBGP1, the halohydrin dehalogenase from *Mycobacterium* sp. GP1 that displays low enantioselectivity.[14] Between the genes a linker region was added containing a translational enhancer and a ribosome binding site, so each transcribed gene was independently translated.

We studied the applicability of the engineered cells on a model process (Scheme 2), in which CT cells were used for the transformations of compound **1a** (Scheme 3). The reaction was carried out at room temperature in a two-phase system composed of HEPES buffer, octane as the organic solvent, and isopropyl alcohol for regeneration of the nicotinamide cofactor of the reductase. After 6 h of incubation with a suspension of freshly thawed CT cells we observed high, though not full, conversion of the starting material and the formation of the epoxide **3a**. No halo alcohol **2a** was observed, indication that under these conditions the conversion of **2a** to **3a** is faster than the formation of **2a**. After 6 h a new portion of cells was added, followed by sodium azide (5 equiv.). Overnight incubation resulted in full conversion of the starting material **1a** and formation of β-azido alcohol **4a**. Compounds **2a** and **3a** were no longer observed in the reaction mixture.[16]




**Scheme 2.** Whole cell-catalyzed conversion of β-halo ketones **1** to β-azido alcohols **4**.




**Scheme 3.** Compounds studied in the biocatalytic reaction cascade.

Having confirmed the applicability of the CT-type designer cells in the model reaction, we studied the scope of the whole-cell biocatalytic cascade on a larger set of compounds (Scheme 3, Table 1). Transformation of compound **1a** (Table 1, entry 1) led to **4a**

**Table 1.** Conversion of halo ketones **1a**–**1k** to azido alcohols with CT cells.

| Entry | Substrate | Conversion of **1** | Isolated yield of **4** | *ee* of **4** |
|---|---|---|---|---|
| 1 | **1a** | full | 42% (**4a**) | 96% (*R*) |
| 2 | **1b** | full | 44% (**4a**) | 96% (*R*) |
| 3 | **1c** | full | –[a] | – |
| 4 | **1d** | full | –[a] | – |
| 5 | **1e** | full | 39% (**4e**) | 98% (*R*) |
| 6 | **1f** | full | 35% (**4e**) | 99% (*R*) |
| 7 | **1g** | full | –[a] | – |
| 8 | **1h** | <5% | – | – |
| 9 | **1i** | <5% | – | – |
| 10 | **1j** | full | 49% (**4j**) | 98% (*R*) |
| 11 | **1k** | full | 70% (**4k**) | 99% (*R*) |

[a] The formation of the expected product was not observed.

which was isolated with 42% yield and very high enantiomeric excess. For compound **1b**, an analogue of **1a**, with chloride instead of bromide as the leaving group, the results were virtually identical (Table 1, entry 2). Conversion of α-haloacetophenones **1c** and **1d** (Table 1, entries 3 and 4) did not result in the formation of the expected products.

Encouraged by the good results obtained with substrates having electron-withdrawing groups in the *para* position of the phenyl ring (**1a** and **1b**), we tested the cyano-substituted compounds **1e** and **1f**. Nearly enantiopure product **4e** was obtained with 35–39% yield (Table 1, entries 5 and 6). We investigated further the influence of the position of the ring-substituent on the outcome of the cascade. Compounds with nitro substituents in *ortho* and *meta* positions were either not converted (Table 1, entry 8) or the expected product was not observed (Table 1, entry 7).

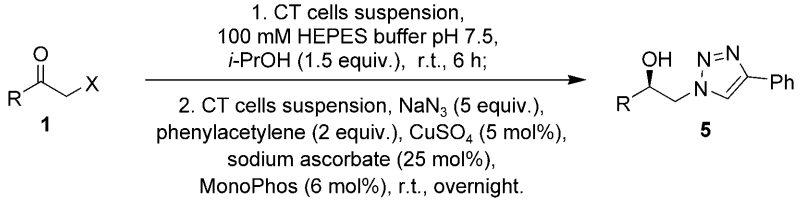
Substrate **1i**, with an electron-donating methoxy substituent, was also not converted. Two additional aliphatic substrates were tested. Very high enantioselectivities were obtained for both the ester **1j** and the aliphatic compound **1k**.

In general, the isolated yields of the azido alcohols (35–70%) obtained in this one-pot, three-reaction process correspond to those obtained with isolated enzymes (38–61%).[11] In view of the high enantiomeric excess of the products and the simple reaction setup (see also the paragraph below, covering the use of lyophilized cells) we envisage this new one-pot, whole cell cascade to be a valuable tool for the stereoselective preparation of an important class of chiral building blocks.

Having demonstrated the possibility to convert the halo ketones **1** into enantiopure azido alcohols **4** using the whole-cell cascade of biocatalytic transformations, we studied the possibility of combining it with a click reaction in a one-pot process (Scheme 4). Therefore, the addition of sodium azide in the second step of the cascade is followed after 30 min by the introduction of a model [2+3] cycloaddition partner (phenylacetylene), a copper(I) source ($CuSO_4$ and sodium ascorbate) and MonoPhos, a ligand that has recently been shown to efficiently enhance the rate of the click reaction.[17] We used only substrates that were within the substrate scope of the designer cells; chlorine-substituted compounds **1b** and **1f** were also not included, as they gave the same results as their bromo analogues **1a** and **1g**. Furthermore, we observed that the addition of octane as an organic solvent results in a reduction of reactivity, probably due to the extraction of phenylacetylene into the organic phase. Therefore octane was not used.[18] The results of the reactions performed under one-pot conditions, combining the whole-cell reaction cascade and the click reaction, are presented in Table 2.

The conversion of substrate **1a** provided the expected enantiopure triazole **5a** with 41% isolated yield after flash chromatography (Table 2, entry 1). This result corresponds perfectly to the one obtained without the subsequent cycloaddition, where product was isolated with 42% yield (Table 1, entry 1), indicating that in the case of compound **1a** the introduction of the fifth transformation in the same reaction vessel does not affect the efficiency of the process. The same conclusions may be drawn for compounds **1e** and **1k** (Table 2, entries 2 and 4). The isolated yield of the four-reaction cascade was significantly lower when compared to that of the whole-cell biocatalytic cascade without click reaction for compound **1j** (Table 2, entry 3).

Encouraged by these results, we decided to explore the possibility of obtaining the other enantiomer of β-hydroxytriazoles **5**, using the BL cells, in which the *Lactobacillus brevis* ketoreductase, which has an opposite enantiopreference,[19] was expressed. Since the




**Scheme 4.** One-pot whole cell catalysis and click reaction for the conversion of α-halo ketones **1** to β-hydroxytriazoles **5**.

**Table 2.** Conversion of halo ketones to triazoles in a one-pot whole-cell biocatalytic cascade with subsequent click reaction.

| Entry | Substrate | Cells | Isolated yield of **5** | *ee* of **5** |
|---|---|---|---|---|
| 1 | **1a** | CT | 41% (**5a**) | 99% (*R*) |
| 2 | **1e** | CT | 36% (**5e**) | 99% (*R*) |
| 3 | **1j** | CT | 18% (**5j**) | 99% (*R*) |
| 4 | **1k** | CT | 65% (**5k**) | 99% (*R*) |
| 5 | **1k** | BL | 53% (**5k**) | 97% (*S*) |

halohydrin dehalogenase HheB used in this construct prefers aliphatic compounds,[12] we chose compound **1k** as the substrate for this transformation. Product (*S*)-**5k** was obtained in pure form with 53% yield and with high enantiomeric excess (Table 2, entry 5), thus expanding the synthetic possibilities offered by this one-pot, four-transformations methodology.

Finally, to fully exploit the possibilities offered by low cost and easy preparation of the biocatalyst, we studied the application of lyophilized cells instead of frozen cells. Such dried, whole-cell catalysts may be stored and transported as a powder after preparation, without any microbiological work, which increases their value to the synthetic community. Thus we tested the changes in activity and selectivity of the lyophilized CT cells in time, on a model substrate – compound **1k** (Table 3). Immediately after freeze-drying with phosphate buffer, lyophilized cells (Table 3, entry 2) perform as well as the freshly prepared cells (Table 3, entry 1), both in terms of isolated yield and enantiopurity of the product. The same result was obtained when we used lyophilized cells that were stored for 3 weeks at 4 °C (Table 3, entry 3), which is a common storage temperature for chemical catalysts. Even after 10 weeks of storage the cells could be used in catalysis, and the enantiopure product could be isolated with a moderate yield of 50% (Table 3, entry 4).

**Table 3.** The use of lyophilized cells in the one-pot whole-cell biocatalytic cascade and the click reaction.

| Entry | CT Cells | Storage time at 4 °C | Isolated yield of **5k** | *ee* of **5k** |
|---|---|---|---|---|
| 1 | freshly prepared | – | 65% | 99% (*R*) |
| 2 | lyophilized | – | 63% | 99% (*R*) |
| 3 | lyophilized | 3 weeks | 63% | 99% (*R*) |
| 4 | lyophilized | 10 weeks | 50% | 99% (*R*) |

We thus have demonstrated the synthetic applicability of a system which combines five reactions in one container, making use of the compatibility of biocatalytic processes and the bioorthogonality of the click reactions. The use of designer cells lowers the cost of the catalyst and avoids problems connected with the use of isolated enzymes, giving comparable results in terms of the product yield and enantiomeric excess. Due to the built-in cofactor regeneration system and the possibility of storage and easy use of lyophilized cells, these biocatalysts which catalyze a cofactor-dependent redox reaction and lyase reactions, can be as conveniently used as hydrolytic enzymes, which have found widespread application in organic synthesis.

## Experimental Section

### General Procedure for Preparation of β-Hydroxytriazoles (5) from α-Halo Ketones (1)

In a 50-mL flask were placed HEPES buffer (9 mL, 0.1 M, pH 7.5, KOH, 1 mM $MgSO_4$, 1 mM $ZnSO_4$), *i*-PrOH (0.2 mL, 1.5 M), halo ketone **1** (0.2 mmol) and freshly thawed cells (4 mL). The mixture was stirred at room temperature for 6 h. Then thawed cells (4 mL) were added together with $NaN_3$ (1 mmol in 200 μL water). After 30 min phenylacetylene (44 μL, 0.4 mmol) and pre-formed click catalyst (10 μmol $CuSO_4$ and 50 μmol Na-ascorbate dissolved in 1 mL water, 11 μmol MonoPhos added, all stirred for 10 min at room temperature) were added and the reaction mixture was stirred overnight at room temperature. After completion of the reaction the mixture was extracted using $CHCl_3$ (3 times 25 mL). The combined organic layers were dried ($MgSO_4$) and the volatiles were evaporated under reduced pressure. The β-hydroxytriazoles **5** were purified using flash chromatography ($SiO_2$, pentane/$Et_2O$ = 1/1–pentane/EtOAc = 4/6).

### Supporting Information

Full experimental procedures, preparation of cells and the analytical data are presented in the Supporting Information.

## Acknowledgements

*The project is financially supported by the Netherlands Ministry of Economic Affairs and the B-Basic partner organizations (www.b-basic.nl) through B-Basic, a public-private NWO-ACTS program (ACTS = Advanced Chemical Technologies for Sustainability) (W.S.) and IBOS (C.T. and F.B.), and through NWO-STW (C.P.P).*

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