One pot 'click' reactions: tandem enantioselective biocatalytic epoxide ring opening and [3 + 2] azide alkyne cycloaddition†‡

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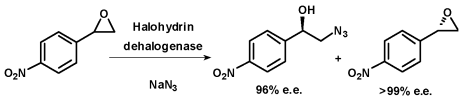
Halohydrin dehalogenase (HheC) can perform enantioselective azidolysis of aromatic epoxides to 1,2-azido alcohols which are subsequently ligated to alkynes producing chiral hydroxy triazoles in a one-pot procedure with excellent enantiomeric excess.

The discovery that copper catalyzes the 1,3-dipolar cycloaddition of azides and alkynes to form 1,4-disubstituted triazoles has significantly contributed to the popularization of 'click' chemistry and its subsequent application to the areas of drug discovery, polymer chemistry, medicinal and biological sciences, amongst others.[1] Recently, efforts have been pursued to involve the copper catalyzed azide alkyne cycloaddition (CuAAC) in one-pot multicomponent reactions.[2] The bioorthogonality of the azide-alkyne cycloaddition makes it uniquely suited to one-pot procedures. An attractive possibility to execute a tandem reaction is the combination of azide induced ring opening of epoxides with the copper catalyzed 1,3-dipolar cycloaddition of azides and alkynes. Starting from enantiomerically pure epoxides, it has been shown that sodium azide triggered ring opening followed by the click reaction can occur in one pot with PEG-400 as a solvent, with retention of the enantiomeric excess of the starting material.[3] Another approach demonstrated the possibility of enzymatically reducing α-azidoacetophenone derivatives in an enantioselective manner to their azido alcohol counterparts. As both the acetophenones and the resulting alcohols contain the azide functionality, the alcohol must first be isolated prior to attachment to an alkyne *via* copper catalysis.[4]

We have previously reported on the biocatalytic azidolysis of aromatic epoxides using HheC, the halohydrin dehalogenase from *Agrobacterium radiobacter*.[5] This enzyme catalyzes azidolysis of substituted styrene oxides to their corresponding chiral 1,2-azido alcohols in a highly enantioselective ($E > 200$) and β-regioselective manner (Scheme 1).



Scheme 1 Biocatalytic azidolysis of aromatic epoxides.[5]

The halohydrin dehalogenase has been cloned, and brought to overexpression, lending it potential for industrial scale production, and making it a versatile method for synthetic organic chemists.[6,7]

The use of isolated enzymes can be advantageous in several respects, particularly for simplification of product separation, and to avoid the potential for undesired byproduct formation.[4] We constructed a variant of HheC with cysteine 153 mutated into serine.[8] This increases the enzyme's stability towards oxidation and removes the need for addition of β-mercaptoethanol.

We envisioned that this exquisite selectivity could be combined with the copper catalyzed [3 + 2] cycloaddition of azides and alkynes to produce optically pure triazoles. These products are particularly interesting, not only due to the presence of the 1,2,3-triazole moiety which has proven to be a promising pharmacophore[9] but also as β-adrenergic receptor blocker analogues and potential imaging agents.[10]

We demonstrate herein the first example of a one-pot tandem biocatalytic enantioselective epoxide ring opening and click reaction to produce optically pure hydroxy triazoles. This method uses inexpensive and readily available racemic epoxides and allows for the subsequent click reaction to occur in one pot, thus limiting the experimental steps and proceeding in a more environmentally friendly fashion. Both traditional copper(I) catalyzed and copper free click reactions give excellent results.

The investigation was initiated by exploration of the reactivity and selectivity of the enzyme in the presence of the click additives, namely, $CuSO_4 \cdot 5H_2O$, the reducing agent sodium ascorbate and the MonoPhos ligand used to enhance the rate of the azide-alkyne cycloaddition as recently demonstrated.[11] In anticipation of the one-pot reaction, the buffer used to store the enzyme, 10 mM Tris-HCl (pH 7.5, 1.0 mM EDTA, 10% glycerol), had to be changed due to the presence of EDTA which has the ability to chelate copper thereby inhibiting catalysis. Potassium phosphate buffer was chosen as a substitute (pH 7.5, 50 mM). Styrene oxide **1** was used as the initial substrate.

We were pleased to find that the alteration of buffer had no apparent impact on the enantioselectivity of the conversion of

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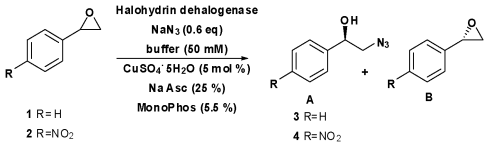
‡ Electronic supplementary information (ESI) available: General experimental procedures, ^1H and ^{13}C NMR data for all compounds, full characterization for all new compounds. See DOI: 10.1039/b919434g

Table 1 Enzymatic activity in "click" conditions



	R	Concentration[a]	Solvent[b]	Additives[c]	Conv.[d]	ee A (%)	ee B (%)	E
1	H	2.0	Buffer	No	47%	>99	89	>200
2	H	2.0	Buffer	Yes	26%	>99	35	>200
3	NO_2	2.0	Buffer	No	46%	>99	83	>200
4	NO_2	2.0	Buffer	Yes	50%[e]	97	98	>200
5	NO_2	4.0	Buffer	No	17%	>99	20	>200
6	NO_2	25.0	Water	Yes	<1%	n.d.	n.d.	n.d.
7	NO_2	25.0	Water	No	<1%	n.d.	n.d.	n.d.
8	NO_2	50.0	Buffer	No	<1%	n.d.	n.d.	n.d.

[a] Epoxide concentration (mM). [b] Reactions were conducted either in 50.0 mM potassium phosphate buffer (pH = 7.5) or in distilled water. [c] Includes copper(II) sulfate pentahydrate, sodium ascorbate and MonoPhos. [d] Conversion at 16 h. Max. conv. 50%. [e] Conversion after 24 h.

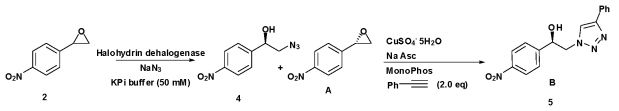
epoxide to azido alcohol **3** giving the product with 99% ee (Table 1, entry 1) albeit with slightly lower conversion overnight (47% of the available 50% conversion for a kinetic resolution). The same reaction was performed in the presence of $CuSO_4{\cdot}5H_2O$ (5 mol%), sodium ascorbate (25 mol%) and MonoPhos (5.5 mol%).[12] Fortunately, the presence of these additives proved to have no effect on the optical purity of **3**, but we found that the rate decreased considerably in their presence (Table 1, entry 2).

The conversion of 2-(4-nitrophenyl)oxirane **2** to its corresponding azido alcohol **4** in potassium phosphate buffer was also investigated (Table 1, entry 3). Nearly full conversion was achieved overnight with >99% and 83% ee in the azide and the epoxide, respectively. The same reaction in the presence of the additives (Table 1, entry 4) showed full conversion to **4** after 24 h with 97% ee and 98% ee for the azide and the epoxide, respectively. A higher substrate concentration (4.0 mM) proved to have a detrimental effect upon the conversion while retaining the perfect enantioselectivity of the transformation (Table 1, entry 5).

When the enzymatic conversion to azido alcohol was attempted in water, no conversion was detected (Table 1, entry 6). In the absence of click additives, the same result was observed (Table 1, entry 7). The poor result is thus attributed to the inability of the enzyme to perform under these buffer-free conditions. High substrate concentration (50 mM) also proved too challenging for the enzyme, and no trace of **4** was detected (Table 1, entry 8).

Having established the ability of the reaction to proceed in potassium phosphate buffer in the presence of the necessary additives with essentially unaltered selectivity, it was possible to attempt the one-pot ring opening and subsequent click reaction. In the first attempt, with 5 mol% of catalyst, after 24 h, the triazole product **5** could be detected with 99% ee, and the remaining epoxide with 75% ee (Table 2, entry 1). Thus, the first step of the cascade maintains its high level of selectivity, and the click reaction proceeds at such a rate that

Table 2 Optimization of one pot enzymatic and click reactions



	Concentration	Cu (mol%)	Time/h	Conv.[b]	ee A(%)	ee B(%)	E
1	2.0 mM	5	24	43%	75	>99	>200
2	4.0 mM	5	24	34%	51	97	109
3	4.0 mM	5	67	39%	62	97	124
4	4.0 mM	1	24	n.d.[a]	85	99	>200
5	50.0 mM	5	67	24%	23	42	2.8

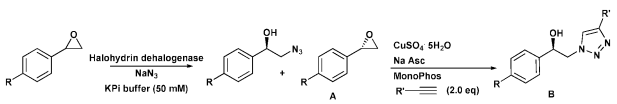
[a] Azido alcohol remaining. [b] Max. conversion 50%.

with 5 mol% of copper, no azido alcohol remained in the reaction mixture. There appears to be a slight effect on the efficiency of the overall process, either on the part of the phenylacetylene, or due to a process within the catalysis of the click reaction, on the rate of the ring opening, as only 43% conversion occurs after 24 h. The same experiment at a higher concentration showed a slight drop in the ee of **4** (97%) and lower conversion (Table 2, entry 2).

Repetition of the experiment with a longer reaction time gave a slight increase in conversion (Table 2, entry 3). Reducing the amount of catalyst to 1 mol% also gave excellent results, 99% ee for **5** and 85% ee for the remaining epoxide (Table 2, entry 4). Comparing entries 2 and 4 it can be ascertained that the concentration of the catalytic additives affects the ee. With 1 mol% of copper the effective copper complex concentration is lowered (from 0.20 mM to 0.04 mM) and the ee rises while the starting substrate concentration is kept constant at 4.0 mM. However, detection of azido alcohol in the reaction mixture indicates that with 1 mol% of catalyst the cycloaddition slows to the extent that it becomes the rate limiting step in the cascade. Of particular interest is a reaction performed at 50 mM concentration (Table 2, entry 5). As aforementioned, no trace of **4** had been detected when the enzymatic transformation was attempted at such a high substrate concentration (Table 1, entry 8). However, in the presence of click additives and phenylacetylene, after 24 h, 24% conversion to triazole **5** was detected. Although the enantioselectivity was lower (42%) in the product than usual, it is significant that the occurrence of the second reaction appears to promote formation of azido alcohol in the first.§

A selection of substrates was made for further investigation. In the instance of styrene oxide as a substrate, the results were even more satisfying. At 4.0 mM substrate concentration triazole was detected with >99% ee, and conversion from epoxide to product was 44%, with 78% ee for the epoxide (Table 3, entry 1). Reducing the catalyst loading from 5 to 3% again proved insufficient, as azido alcohol **3** remained in the reaction mixture (Table 3, entry 2). We also tested propiolic acid as an alkyne substrate (Table 3, entry 4). Interestingly, the triazole product was detected with nearly racemic distribution. We hypothesized that the presence of the acid could impact the functionality of the enzyme. Thus the corresponding ester, ethyl propiolate, was tested as well (Table 3, entry 5). Indeed, the resulting triazole showed a dramatic improvement in ee to 80% but the conversion of epoxide remained low after 24 h.

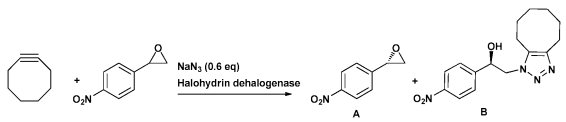
Table 3 Substrate scope



	R^a	R'	Cu (mol%)	Conv. (%)[b]	ee A(%)	ee B (%)	E
1	H	Ph	5	44	78	99	>200
2	H	Ph	3	n.d.[c]	78	98	>200
3	NO_2	Ph	5	34	51	97	109
4	NO_2	COOH	5	n.d.	n.d.	2	n.d.
5	NO_2	COOMe	5	20[d]	20	80	10

[a] 4.0 mM substrate concentration. [b] After 24 h. Max. conversion 50%. [c] Azido alcohol remaining in the reaction mixture. [d] Enzyme added in two portions (at 0 h and 12 h).

Table 4 Copper free cycloaddition of cyclooctyne



	Time/h	Conversion (%)[b]	ee A (%)	ee B (%)	E
1	24	20	24	96	83
2	48[a]	32	47	96	78

[a] Enzyme added in two portions (half at $t = 0$ h, half at $t = 24$ h). [b] Max. conversion 50%.

We can conclude from these observations that not only is the epoxide important as the substrate undergoing enzymatic conversion, but the choice of accompanying acetylene is equally relevant with regards to both rate and selectivity.

We also attempted the more biologically interesting copper free click reaction. Cyclooctyne was chosen as a model substrate.[13] After 24 h analysis by HPLC revealed the triazole with 96% ee, and the epoxide with 24% ee, indicating 20% conversion (Table 4, entry 1).[14] We repeated the reaction over 48 h, adding the enzyme in two portions (half at the start of the reaction, and the other half after 24 h) to ensure constant enzymatic activity. This resulted in a significant increase in the ee of the epoxide (47%) along with improved conversion.

The scope of the one-pot ring opening click reaction can therefore be extended to include the variety of strained cyclic cyclooctyne derivatives that have been developed recently.[15]

In conclusion, we have developed a methodology to enzymatically catalyze azidolysis of aromatic epoxides in an enantioselective fashion to the corresponding azido alcohols, and in the same pot, click the resulting azides to alkynes. The reaction conditions are very mild, proceeding in aqueous solution with neutral pH at room temperature. The one-pot nature of the process allows for a simpler, faster and more environmentally friendly reaction, work-up and purification. We have demonstrated that biocatalysis is compatible with one-pot multicomponent reactions. This transformation can be promoted either through copper catalysis or by ring strain, opening the possibility for a wide variety of applications.

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Notes and references

§ The possibility of enzyme inhibition by the azidoalcohol product at higher concentrations cannot be excluded.

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