Highly Enantioselective Propargylic Hydroxylations Catalyzed by Chloroperoxidase

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Chiral propargylic alcohols are important building blocks for the enantioselective synthesis of complex molecules, in particular, biologically active compounds.[1] There are two general strategies for the asymmetric synthesis of propargylic alcohols: (a) enantioselective alkynylation of aldehydes[2] and (b) stoichiometric or catalytic reduction of acetylenic ketones.[3] However, the most direct procedure for the preparation of optically pure propargylic alcohols would be the asymmetric hydroxylation of alkynes. Until now an effective and versatile catalyst for this approach has not been available.[4]

Chloroperoxidase (CPO) is the most versatile and efficient oxidation catalyst in the heme enzyme family.[5] Since the discovery of CPO more than three decades ago,[6] a large number of CPO-catalyzed reactions have been described. CPO catalyzes halogenation,[6,7] the oxidation of alcohols to aldehydes,[8] aldehydes to acids,[9] dealkylations of alkylamines,[10] dimerization of phenols,[11] and oxidation of amines to nitroso compounds.[12] In particular, CPO catalyzes several enantioselective oxidation reactions: epoxidation of alkenes,[13] allylic and benzylic hydroxylations,[9] and sulfoxidations.[14] There are very few reports concerning the enzymatic oxidation of acetylenes.[15] We now report a new oxidation reaction catalyzed by CPO, stereoselective propargylic hydroxylations.

Table 1. Enantioselective Propargylic Hydroxylation Catalyzed by CPO[a]

entry	R	R′	ee (%)	yield[f] (%)	config[h]
1	CH_3	CH_3	57	7[g]	R
2	CH_2CH_3	CH_3	91[b]	26	R
3	CH_2 CH_2CH_3	CH_3	87[c]	30	R
4	$CH_2(CH_2)_2CH_3$	CH_3	78[c]	8	R
5	Ph	CH_3	86[c]	15	R[i]
6	CH_2OAc	CH_3	95[c,d]	52	R
7	CH_2Br	CH_3	94[e]	65	R
8	CH_2CH_2OAc	CH_3	83[c]	26	R
9	CH_2CH_2Br	CH_3	94[d,e]	25	R
10	CH_2OAc	CH_2CH_3	87[d]	8	N.D.[j]
11	$COCH_3$	CH_3	no reaction		

[a] Hydrogen peroxide as the terminal oxidant. [b] Determined by GLC on a β-CDX column. [c] Determined by conversion to corresponding trifluoroacetate and GLC analysis using a chiral G-TA column. [d] Determined by ^{1}H NMR analysis of the corresponding Mosher (MTPA) ester. [e] Determined by GLC on a chiral G-TA column. [f] Unless otherwise specified, the isolated yields are after flash chromatography and are not optimized. [g] Determined by GLC analysis. [h] Determined by hydrogenation (Pd/C, MeOH) followed by correlation to (*R*)-mono or diols commercially available (Aldrich) or previously reported. [i] Determined by comparison of the optical rotation with the known compound. [j] Not determined.

Under appropriate conditions[16] CPO catalyzes the enantioselective hydroxylation of a variety of unfunctionalized and functionalized alkynes yielding chiral propargylic alcohols. Both hydrogen peroxide and *tert*-butyl hydroperoxide (TBHP) can serve as the terminal oxidant in this reaction. When hydrogen peroxide is used as the oxidant, it is added slowly to the reaction mixture to minimize the potent catalase activity of CPO. When TBHP is used, the oxidation is started by adding 2 equiv of TBHP directly to the reaction medium.

Table 1 records the results obtained with a variety of alkynes. To assess the relative utilization of the different alkynes, all of the reactions contained 1 equiv of alkyne, 2 equiv of oxidant, and identical amounts of enzyme. All product yields and ee's are reported for 2-h reaction periods. The extent of the conversion of an alkyne to product in a 2-h reaction is controlled by the rate

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(16) General procedure: A mixture of alkyne (1.0 mmol) and CPO (1.1×10^{-3} mmol) was stirred vigorously in 10.0 mL of 10 mM Na citrate buffer adjusted to pH 5.5. H_2O_2 was provided to the system via a syringe pump in a continuous and slow addition fashion (1.7 M, 10 μL/min.). When *t*-BuOOH served as the terminal oxidant, it was directly added to the reaction medium (2 mmol). The reaction vial was capped, and the reaction was stirred for 2 h at room temperature, after which $Na_2S_2O_3$ was added and the mixture was extracted twice with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, and the products were purified by flash chromatography using pentane/ether as eluent. Quantitative gas chromatography was carried out with decane or dodecane as an internal standard. For further details, see the Supporting Information.

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of hydroxylation of the alkyne, the rate of inactivation of CPO, the loss of the chiral alcohol due to overoxidation, and the loss of oxidation equivalents via the catalase side reaction. Obviously in most cases the yield of product could be increased by the addition of more enzyme and oxidant. In the case of unfunctionalized acetylenes, the results in Table 1 record the effect of carbon chain length on enantioselectivity and product yield. When the smallest alkyne (entry 1) serves as a substrate, both the enantiometric excess and the yield of the propargylic alcohol are very moderate (ee 57%, yield 7%). However, the enantioselectivity and yield dramatically increase for an acetylene having an additional carbon atom (entry 2, ee 91%, yield 26%). CPO proved to be progressively less stereoselective toward alkynes possessing additional carbons (ee 87 and 78%, respectively for entries 3 and 4), and yields also decreased from 30 to 8%. The modest ee for entry 1 must be due to the small difference in asymmetry between the two substituents on the prochiral propargylic carbon. As the carbon chain lengthens, the asymmetry becomes more apparent and the enantioselectivity increases, but only to a point. Obviously steric requirements at the active site of CPO severely limit the entry of the larger acetylenic substrates. These size restrictions are similar to those noted in the CPO epoxidation of *cis*-alkenes and 2-methyl-1-alkynes.[13] CPO also hydroxylates the conjugated phenyl acetylene (entry 5) with good selectivity, but the yield is very modest in this case.

Very surprisingly, when an electron-withdrawing group (acetoxy or bromo) is attached to the methyl group remote from the prochiral progargylic carbon (entries 6 and 7), the enantioselectivity and the yields show remarkable enhancements (ee 95%, yield 52%, and ee 94%, yield 65%, respectively). When these functional groups are more remote from the triple bond (entries 8 and 9), the ee still remains high for entry 9 (94%), but there is a significant decrease for entry 8 (from 95 to 83%). In both cases the yields are significantly lower (from 52 to 26% for entry 8, and 65 to 25% for entry 9). Furthermore, the presence of an ethyl group adjacent to the prochiral propargylic carbon (entry 10) promotes high stereoselectivity; however the yield decreases dramatically from 52 to 8%. These results suggest that the active site of CPO is very sterically constrained.

The mechanism of the propargylic hydroxylation catalyzed by CPO is ambiguous. However, since radical intermediates can be stabilized by electron-withdrawing groups,[17] it is tempting to speculate that the propargylic hydroxylations go via a free radical intermediate. This hypothesis differs from the mechanism proposed for CPO benzylic hydroxylation reactions.[9]

It is interesting to note that the terminal oxidant has a significant effect on the enantioselectivity of propargylic hydroxylation reactions (Table 2). In all cases higher stereoselectivity is obtained when H_2O_2 serves as a terminal oxidant rather than TBHP although the yields of the alcohols are only modestly affected. In particular, entries 2 and 4 give lower ee's with TBHP as oxidant. When TBHP serves as the oxidant (2 equiv of TBHP), the ee of the propargylic alcohol (entry 2) decreases progressively as the incubation time lengthens (ee 65% for 1 h, 50% for 2 h). A remarkably large amount of α,β-ynone is produced with entry 2, especially in a 2-h incubation period (14% yield). A significant amount of α,β-ynone also was isolated from the reaction medium of entry 3 (20% after 2 h incubation with 2 equiv of TBHP). In these cases, even with less TBHP (1 equiv or less), the production of α,β-ynones is still pronounced and the ee's of propargylic alcohols are much lower than with H_2O_2. Since H_2O_2 is provided to the system via a syringe pump in a continuous and slow addition process (10 μL/min for 1.0 mmol of substrates),[16] hydrogen peroxide is maintained at a very low concentration during the propargylic hydroxylations. This not only protects the enzyme from inactivation by H_2O_2, but also avoids further oxidation of the propargylic alcohols to α,β-ynones. Thus, in some cases the maintenance of low concentrations of oxidant in the reaction is essential for achieving high stereoselectivity.

Table 2. Effect of Terminal Oxidant on Enantioselectivity

			ee (%)	
entry	R	R′	H_2O_2	TBHP
1	CH_2CH_3	CH_3	91	86[a]
2	Ph	CH_3	86	50[b,c]
3	CH_2CH_2OAc	CH_3	83	76[b,c]
4	CH_2OAc	CH_2CH_3	87	43[b]
5	CH_2Br	CH_3	94	88[d]
6	CH_2CH_2Br	CH_3	94	90[c,d]

[a] Determined by GLC on a β-CDX column. [b] Determined by conversion to trifluoroacetate and GLC analysis using a chiral G-TA column. [c] Determined by ^1H NMR analysis of the corresponding mosher (MTPA) ester. [d] Determined by GLC on a chiral G-TA column.

In all cases the chiral product which is produced is of the R-configuration. Different substituents at the prochiral propargylic carbon have no significant effect on the stereo-orientation of substrates in the active site of CPO. These results are similar to the enantioselective epoxidation of alkenes by CPO.[13]

In contrast to P450 cytochromes, CPO is very readily available, it utilizes H_2O_2 or TBHP instead of oxygen as the terminal oxidant, and it does not require a cofactor.[5] Although selective hydroxylations of allylic or benzylic C−H groups are a quite common feature for the cytochrome P450 family of enzymes, asymmetric propargylic hydroxylations are rare. The X-ray crystal structure shows that CPO possesses a unique active-site environment.[18] The proximal heme iron ligand is a cysteine thiolate and a glutamate residue hydrogen bonded to a histidine functions as an acid−base catalyst distal to the heme. The thiolate ligand is surrounded by a positive electrostatic environment which serves to increase the oxidation potential of this heme enzyme.[5c,18] This unique heme environment in CPO undoubtedly plays a significant role in promoting its unusual propargylic hydroxylation activity.

Herein, we have reported highly enantioselective propargylic hydroxylation reactions catalyzed by CPO. In all cases the carbon chain length of the substrate plays a significant role in the overall enantioselectivity and yields of the reaction. CPO prefers acetylenes containing short, straight carbon chains and produces products with high ee and in good yields. These results indicate that CPO shows considerable promise for the production of chiral propargylic alcohols. The potential for modification of the active site of CPO by random and/or site-directed mutagenesis provides an exciting approach for broadening the substrate specificity and improving the selectivity of the propargylic hydroxylations.

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Supporting Information Available: Experimental procedures for the preparation of chiral propargylic alcohols, GLC behavior, and the physical data of products (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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