C-8), 125.90 and 126.06 (Phen C-5 and C-6), 127.35 and 128.55 (Phen C-4a and C-7a), 135.86 and 136.42 (Phen C-4 and C-7), 145.01 and 145.48 (Phen C-1b and C-10b), 149.86 (Phen C-9) and 158.99 (Phen C-2); FDMS m/z 294 (MH^+).

Kinetic Studies. CTABr and Brij 35 micellar solutions were prepared in *N*-ethylmorpholine–HBr buffer pH = 7.00. Each kinetic run was initiated by injecting an acetonitrile solution (0.01 M) of substrate ester into a 1-cm cuvette containing 2 mL of buffered micellar solution and the desired concentrations of metal ion and ligand. Pseudo-first-order rate constants for the hydrolysis of substrate ester were determined by monitoring the release of *p*-nitrophenolate at 400 nm, under the conditions of excess of catalyst over substrate. Reactions were generally followed for at least 10 half-lives. Pseudo-first-order rate constants were obtained from linear plots of ln ($A_\infty - A_t$) vs time for at least 3 half-lives. Kinetic runs carried out in triplicate gave rate constants with uncertainty of less than 3%.

The apparent second-order rate constants ($k_{a,obs}$) were calculated from $k_{a,obs} = (k_{complex} - k_s)/[complex]_0$, where $k_{complex}$ and k_s refer to the observed pseudo-first-order rate constant for the hydrolysis of the ester substrates in the presence and absence of ligand–metal–ion complex, respectively.

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Supplementary Material Available: Proton NMR spectra for compounds 1–7 (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Chloroperoxidase-Catalyzed Asymmetric Synthesis: Enantioselective Reactions of Chiral Hydroperoxides with Sulfides and Bromohydration of Glycals

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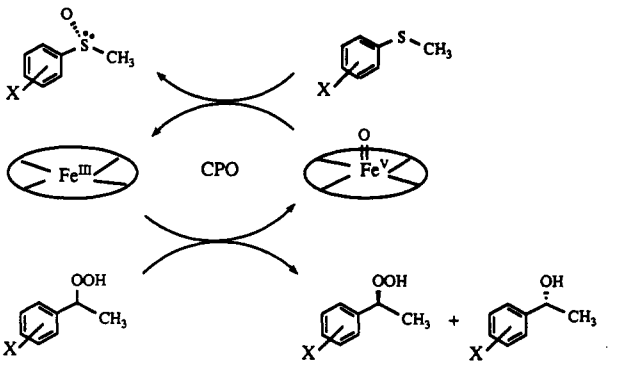
This paper describes the use of chloroperoxidase (CPO) from *Caldariomyces fumago* in the oxidation of sulfides to prepare (*R*)-sulfoxides with excellent ee (97–100%) and yield (66–92%) using H_2O_2 as oxidant. When racemic 1-phenylethyl hydroperoxides were used in the oxidation of sulfides, the corresponding (*R*)-alcohol generated from the oxidant and the unreacted (*S*)-hydroperoxide were recovered with high enantiomeric purity. The enantioselectivity in the enzymatic asymmetric oxidation was found to depend on the concentrations of the substrate and enzyme. Chloroperoxidase was also used in the regioselective bromohydration of certain saccharide glycals with KBr and H_2O_2 to give the corresponding 2-deoxy-2-bromo saccharides.

Introduction

Metal-assisted catalytic asymmetric oxidation is a subject of current interest in synthetic organic chemistry. Several practical methods based on biological[1] or abiological[2] catalysts have been developed. We report here the enantioselective asymmetric oxidation of sulfides catalyzed by chloroperoxidase (CPO, EC 1.11.1.10) from *Caldariomyces fumago* using hydrogen peroxide or chiral hydroperoxides as oxidation reagents. In the latter case, the enzyme is highly selective for the (*R*)-enantiomer of chiral hydroperoxides, generating (*R*)-alcohols and unreacted (*S*)-hydroperoxides. We also report the enzyme-catalyzed selective bromohydration of certain glycals to give 2-deoxy-2-bromo saccharides.

Scheme I. Chloroperoxidase (CPO) Catalyzation of Enantioselective Oxidation of Aryl Methyl Sulfides with Chiral Hydroperoxides



Chloroperoxidase from *Caldariomyces fumago* is a heme-containing glycoprotein.[3] Recent studies on the

(1) Fu, H.; Newcomb, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 5878 and references cited therein. For a general reference, see: Davies, H. G.; Greene, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in preparative chemistry*; Academic Press: New York, 1989; pp 169–219. Abushanab, E.; Reed, D.; Suzuki, F.; Sih, C. J. *Tetrahedron Lett.* **1978**, *37*, 3415. Katopodis, A. G.; Smith, H. A.; May, S. W. *J. Am. Chem. Soc.* **1988**, *110*, 897. Baldwin, J. E.; Abraham, E. P. *Nat. Prod. Rep.* **1988**, *5*, 129. Buist, P. H.; Marecak, D. M. *J. Am. Chem. Soc.* **1992**, *114*, 5073. Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P. *J. Chem. Soc., Chem. Commun.* **1992**, 357.

(2) For examples of practical use, see: Zhang, W.; Loebach, J. L.; Wilson, S. R.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1990**, *112*, 2801. Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765 and references cited therein. Rebiere, F.; Samuel, O.; Ricard, L.; Kagan, H. B. *J. Org. Chem.* **1991**, *56*, 5991. Phillips, M. L.; Berry, D. M.; Panetta, J. A. *J. Org. Chem.* **1992**, *57*, 4047. Zhao, S. H.; Samuel, O.; Kagan, H. B. *Tetrahedron* **1987**, *43*, 4135. Davis, F. A.; Reddy, R. T.; Han, W.; Carroll, P. J. *J. Am. Chem. Soc.* **1992**, *114*, 1428. For a review, see: Kogan, H. B.; Minoun, M.; Mark, C.; Schuring, V. *Angew. Chem., Int. Ed. Engl.* **1979**, 485.

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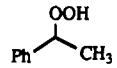
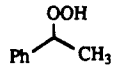
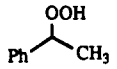
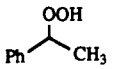
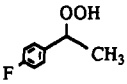
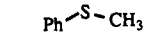
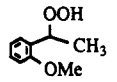
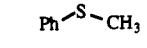
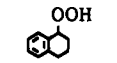
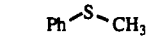
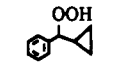
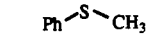
Table I. CPO-Catalyzed Oxidation of Aryl Methyl Sulfide Using 1-Arylethyl Hydroperoxide as Oxidant[a]

entry	oxidants[b]	sulfides[c]	conversn (%) (sulfides)	conversn (%) (peroxides)	ee (%) ((R)-sulfoxides)	ee (%) ((S)-peroxides)	ee (%) ((R)-alcohol)
1			34	46	86	62	71
2			ND[d]	64	70	89	50
3			21	38	76	24	39
4			66	71	61	91	38
5			ND[d]	45	58	56	68
6			ND[d]	ND[d]	13	ND[d]	17
7			50	ND[d]	0	0	0
8			50	ND[d]	0	ND[d]	ND[d]

[a] All reactions were conducted on a 0.5 mmol scale. [b] The hydroperoxides were prepared according to literature (Davis, A. G.; Feld, R. *J. Chem. Soc. London* **1965**, Part I, 665). The identities and absolute configurations of the corresponding alcohols were confirmed by comparison with the authentic compounds purchased from Aldrich. [c] The sulfides were prepared according to literature (Wargner, R. B.; Zook, H. D. *Synthetic Organic Chemistry*; Wiley: New York, 1965; pp 787–796). The identities and the absolute configurations of the corresponding sulfoxides were confirmed by comparison with the literature (Pitchen, P.; Dunach, M. N.; Kagan, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 8188). [d] Not determined.

coordination status of the active-site iron complex[4–10] indicate that a Cys-thiol group is bound to a high-spin five-coordinate ferric ion center. In the absence of halogen anion, the enzyme catalyzes a variety of reactions, such as the epoxidations of olefins,[11,12] N-demethylation of *N,N*-dimethylaniline,[13] and the oxidation of primary alcohols to aldehydes.[14] In the presence of halogen anions, it catalyzes the halogenation of a wide spectrum of organic molecules[15] and N-oxidation of aniline.[16] Recent mechanistic studies suggest that the "iron–oxo" species is the reactive intermediate.[12]

Table II. Kinetic Parameters for Chloroperoxidase Reactions[a]

substrate	K_m (μM)	V_{max} (μmol mg^{-1} min^{-1})	V_{max}/K_m (L^{-1} mg^{-1} min^{-1})
$PhSCH_3$	94	67.2	7.2×10^{-1}
$PhCH(OOH)CH_3$	104	17.8	1.7×10^{-1}

[a] Kinetic parameters for reactions with $PhSCH_3$ were obtained with the concentration of $PhCH(OOH)CH_3$ fixed at 200 μM, and those with $PhCH(OOH)CH_3$ were obtained with the concentration of $PhSCH_3$ fixed at 25 μM. The initial velocity was determined (in 50 mM citrate buffer, pH 5.0) based on the consumption of the sulfide (ϵ = 9643 M^{-1} cm^{-1}), and the data were processed with Enzfitter on an IBM PS-2 to give V_{max} and K_m values.

The chloroperoxidase-catalyzed halogenation is not stereoselective, indicating that the halogenation does not occur in the active site.[15b] It was, however, recently reported that oxidations of some sulfides by CPO using *tert*-butyl hydroperoxide as oxidant gave sulfoxides with enantiomeric excess ranging from 19% to 91%.[17a,b] However, the sulfoxide obtained from these reactions was a result of both enzyme-catalyzed and chemical oxidations. In our asymmetric oxidation process, we were able to separate the nonenzymatic reaction, and high optically pure sulfoxides were obtained.[17c] We also observed for the first time a double asymmetric induction using chiral hydroperoxides as oxidants. In all cases, the enzyme selectively accepts (*R*)-hydroperoxides as substrates and oxi-

(4) Bangcharoenpaurpong, O.; Champion, P. M.; Hall, K. S.; Hager, L. P. *Biochemistry* **1986**, *25*, 2374.
(5) (a) Champion, P. M.; Munck, E.; Debrunner, P.; Hollenberg, P.; Hager, L. P. *Biochemistry* **1973**, *12*, 426. (b) Champion, P. M.; Chang, R.; Munck, E.; Debrunner, P.; Hager, L. P. *Biochemistry* **1975**, *14*, 4159.
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(7) Hollenberg, P. F.; Hager, L. P.; Blumberg, W. E.; Peisach, J. *J. Biol. Chem.* **1980**, *255*, 4801.
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(12) Ortiz de Montellano, P. R.; Choe, Y. S.; Depillis, G.; Catalano, C. E. *J. Biol. Chem.* **1987**, *262*, 11641.
(13) (a) Kedderix, G. L.; Koop, D. R.; Hollenberg, P. F. *J. Biol. Chem.* **1980**, *255*, 10174. (b) Kedderis, G. L.; Hollenberg, P. F. *J. Biol. Chem.* **1983**, *258*, 12413.
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(15) (a) Geigert, J.; Neileman, S. L.; Dalietos, D. J. *J. Biol. Chem.* **1983**, *258*, 2273. (b) Ramakrishnan, K.; Oppenhuizen, M. E.; Saunders, S.; Fisher, J. *Biochemistry* **1983**, *22*, 3271. (c) Yamada, H.; Itoh, N.; Izumi, Y. *J. Biol. Chem.* **1985**, *260*, 11962. (d) Geigert, J.; Neileman, S. L.; Dalietos, D. J.; Dewitt, S. K. *Appl. Envir. Microbiol.* **1983**, *45*, 366.
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(17) (a) Colonna, S.; Gaggero, N.; Manfredi, A.; Casella, L.; Gulloti, M. *J. Chem. Soc., Chem. Commun.* **1988**, 1452. (b) Colonna, S.; Gaggero, N.; Manfredi, A.; Casella, L.; Gulloti, M.; Carrea, G.; Pasta, P. *Biochemistry* **1990**, *29*, 10465. (c) After submission of our paper, the following paper appeared: Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P. *Tetrahedron: Asymmetry* **1992**, *3*, 95. The authors describe the improvement of enantioselectivity using H_2O_2 and *tert*-butyl hydroperoxide.

Table III. CPO-Catalyzed Oxidation of Phenyl Methyl Sulfide by 1-Phenylethyl Hydroperoxide[a]

ratio (sulfide: hydroperoxide)	conversn (%) based on sulfide	ee (%) sulfoxide	ee (%) alcohol	ee (%) hydroperoxide
1:1	48	91	80	74
1:2	80	98	37	6
1:1	94	99	ND[b]	ND[b]

[a] All reactions were conducted in the presence of 4.0×10^{-4} M sulfide and 4.0×10^{-4} M or 8.0×10^{-4} M hydroperoxide at 4 °C in a 0.05 M citrate buffer (pH 5). The amount of CPO used was 600 units as defined by SIGMA. [b] Not determined.

dizes the sulfides to (*R*)-sulfoxides with a concurrent formation of (*S*)-hydroperoxides and (*R*)-alcohols (Scheme I).

We have recently found that the enzyme also catalyzes the halohydration of glycals with a very high regio- and stereoselectivity to give 2-deoxy-2-halo hexoses.[18] This interesting reaction has been extended in this report to sialic acid and oligosaccharide glycals.

Results and Discussion

Enantioselective Reactions between Sulfides and Chiral Hydroperoxides. In the oxidation of phenyl methyl sulfide or aryl methyl sulfide catalyzed by CPO using racemic 1-phenylethyl hydroperoxide or 1-arylethyl hydroperoxide as oxidant, it was found that the enzyme selectively accepts (*R*)-hydroperoxides as substrates and oxidizes the sulfides to (*R*)-sulfoxides with a concurrent formation of (*S*)-hydroperoxides and (*R*)-alcohols (Table I) from the racemic hydroperoxides. When the concentrations of both substrates were higher than 8.3×10^{-3} M, the oxidation process was, however, a combination of chemical and enzymatic reaction since about 15% of sulfoxide was obtained in a control experiment in the absence of CPO. Thus, the ee's for the products were relatively low. The enzyme also catalyzes the oxidation of sulfoxide to sulfone at a slower rate. To eliminate these undesirable reactions, we first determined the kinetic parameters (Table II) of the enzymatic reaction with phenyl methyl sulfide and 1-phenylethyl hydroperoxide and established a K_m value of 94 μM for the sulfide and 104 μM for the hydroperoxide. These affinity constants led us to conduct the reaction at lower substrate concentrations to minimize nonenzymatic reactions. Indeed, when the reaction was conducted at 4.0×10^{-4} M for both substrates, the enzymatic reaction was still very significant but no chemical oxidation was observed without CPO catalysis. Therefore, we are able to obtain the (*R*)-enantiomer of phenyl methyl sulfoxide with an ee as high as 99% (Table III). The other reactions listed in Table I were not optimized; however, a similar approach could be utilized to improve the enantiomeric excess. We also obtained (*R*)-1-phenylethanol and unreacted (*S*)-hydroperoxide at the same time with good enantiomeric excess. When the ratio of hydroperoxide/sulfide increased, the ee for the hydroperoxide decreased dramatically while that for the sulfoxide remained the same.

Encouraged by the fact that sulfoxides with high ee can be obtained under low substrate concentration, we then conducted the oxidation of several sulfides on mmol scales (>100 mg) using hydrogen peroxide as oxidant. To maintain a low concentration for both sulfide and hydrogen peroxide in the reaction mixture, 0.025 mmol of sulfide and 0.03 mmol of hydrogen peroxide were added to 100 mL of a buffer containing 200 μL of CPO every 1.5 h via a capillary pipet. In this manner, all of the sulfoxide products were obtained with ee ranging from 97% to 100% in 66–92% isolated yield (Table IV). The kinetic parameters for these sulfides were obtained as shown in Table V with the concentration of hydrogen peroxide fixed at 50 μM.

Table IV. CPO-Catalyzed Oxidation of Sulfides Using Hydrogen Peroxide as Oxidant[a]

sulfides[b]	yield (%)	ee (%)	absolute config
$PhSCH_3$	90	99	*R*
p-$ClC_6H_4SCH_3$	87	97	*R*
p-$FC_6H_4SCH_3$	86	97	*R*
p-$CH_3C_6H_4SCH_3$	92	99	*R*
p-$MeOC_6H_4SCH_3$	66	100	*R*

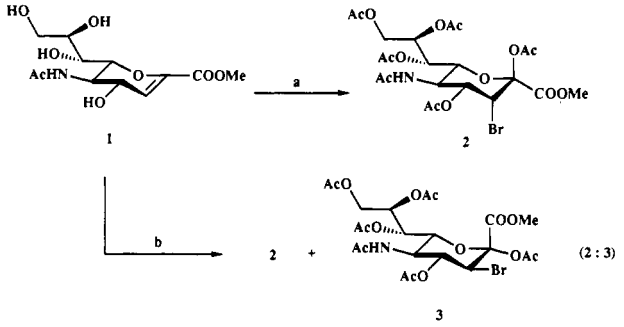
[a] All reactions were conducted on 1 mmol scales in 100 mL of citrate buffer (0.05 M, pH 5) with a slow addition of 0.025 mmol of sulfide and 0.03 mmol of H_2O_2 to the reaction mixture every 1.5 h at 4 °C. The amount of CPO used was 1200 units (200 μL) as defined by SIGMA. [b] The sulfides were prepared according to literature (Wargner, R. B.; Zook, H. D. *Synthetic Organic Chemistry*; Wiley: New York, 1965; pp 787–796). The identities and the absolute configurations of the corresponding sulfoxides were confirmed by comparison with the literature (Pitchen, P.; Dunach, M. N.; Kagan, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 8188).

Table V. Kinetic Parameters for Chloroperoxidase Reactions Using H_2O_2 as Oxidant[a]

substrate	K_m (μM)	V_{max} (μmol mg^{-1} min^{-1})	V_{max}/K_m (L^{-1} mg^{-1} min^{-1})
$PhSCH_3$	122	25.2	2.1×10^{-1}
p-$ClC_6H_4SCH_3$	814	69.1	8.5×10^{-2}
p-$FC_6H_4SCH_3$	366	33.0	9.0×10^{-2}
p-$CH_3C_6H_4SCH_3$	284	22.2	7.8×10^{-2}

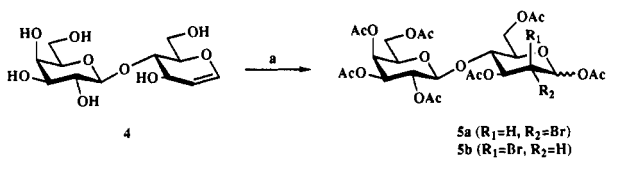
[a] Kinetic parameters for sulfides were obtained with the concentration of H_2O_2 fixed at 50 μM. The initial velocity was determined (in 50 mM citrate buffer, pH 5.0) based on the consumption of the sulfide (for $PhSCH_3$, ϵ = 9643 M^{-1} cm^{-1} at 250 nm; for p-$ClC_6H_4SCH_3$, ϵ = 10531 M^{-1} cm^{-1} at 256 nm; for p-$FC_6H_4SCH_3$, ϵ = 6668 M^{-1} cm^{-1} at 248 nm; for p-$CH_3C_6H_4SCH_3$, ϵ = 9388 M^{-1} cm^{-1} at 252 nm), and the data were processed with Enzfitter on an IBM PS-2 to give V_{max} and K_m values.

Scheme II[a]



[a] Key: (a) (1) CPO/KBr/H_2O_2, pH 3, (2) Ac_2O/Py, (3) Cs_2CO_3/CH_3I, 65%; (b) (1) NBS/CH_3CN/H_2O, (2) and (3) are the same as above, 74%.

Scheme III[a]

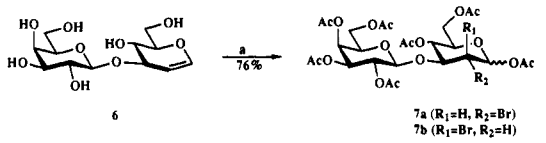


[a] Key: (a) condition 1 (enzymatic): (1) CPO/KBr/H_2O_2, pH 3, (2) Ac_2O/Pyr, 71%, **5a:5b** = 1:1; condition 2 (chemical): (1) NBS/CH_3CN/H_2O, (2) Ac_2O/Pyr, 78%, **5a:5b** = 1:2.5.

Chloroperoxidase-Catalyzed Bromohydration of Glycals. The CPO-catalyzed bromohydration of 2,3-

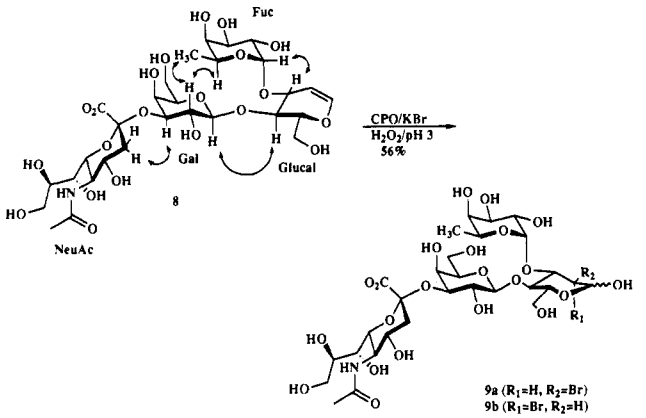
(18) Liu, K. K.-C.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 3748.

Scheme IV[a]



[a] Key: (a) same as condition 1 in Scheme III.

Scheme V



dehydrosialic acid **1** is regio- and stereospecific, giving 3-deoxy-3-bromosialic acid exclusively in 65% isolated yield. Chemical bromohydration of **1** with *N*-bromosuccinimide, however, gave a mixture of **2** and **3**. The enzymatic halohydration was also carried out using glycals **4**, **6**, and **8** as substrates to give products **5**, **7**, and **9**, each as a 1:1 mixture of 2-axial and 2-equatorial bromo derivatives. These bromo oligosaccharides may be useful as bioactive saccharides. For example, we have found that the sialyl Lewis X terminal glycal **8** is conformationally similar to the parent molecule sialyl Lewis x,[19] especially in the binding domain area consisting of NeuAc-Gal-Fuc residues (see the conformational presentation of **8** and NOE's in Scheme V) and thus may bind to the cell adhesion molecule ELAM-1. The halohydration products **9a** and **9b** may share the same conformation due to the strong exo-anomeric effect.[19] Work is in progress to evaluate the biological activities of these bromo saccharides.

In summary, we have demonstrated that CPO-catalyzed oxidation of sulfides is highly enantioselective, and three chiral compounds can be prepared simultaneously with high ee when chiral hydroperoxides are used as oxidants. The undesirable chemical reactions in CPO oxidation of sulfides can be minimized considerably by reducing the concentration of the substrates. To our knowledge, this is the first demonstration that chiral hydroperoxides can be used in the asymmetric oxidation with a concurrent formation of (*R*)-alcohols and (*S*)-hydroperoxides. This general strategy would be applied to the oxidation of other heteroatom-containing compounds with different racemic hydroperoxides as oxidants. Selenides and dialkyl sulfides are not suitable for this oxidation system, because selenoxides are not chirally stable in water and a significant chemical oxidation of dialkyl sulfides occurs in the system. The CPO catalyzed halohydration of glycals provides a new practical procedure for the synthesis of halogenated saccharides.

(19) For the synthesis of 8 and sialyl Lewis x and its conformation, see: Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, S.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.*, in press. ROESY cross peaks were observed between Fuc-H_1 and glucal-H_3, Fuc-CH_3 and Gal-H_2, Gal-H_2 and Fuc-H_5, NeuAc-H_{3ax} and Gal-H_3, and Gal-H_1 and glucal-H_4.

Experimental Section

Materials and Methods. Chloroperoxidase from *Caldariomyces fumago* was purchased from Sigma and used as received. All of the sulfides were either purchased from Aldrich or prepared according to the literature procedure.[20] 1-Phenylethyl hydroperoxide and 1-arylethyl hydroperoxides were prepared by a modified literature procedure.[21] Enantiomeric excess for sulfoxides and alcohols was determined with HPLC using a chiralcel OB column (0.46 × 25 cm) and a UV detector (254 nm). K_m and V_{max} were determined on a UV–vis spectrophotometer. The reaction progress was also monitored with the spectrophotometer to follow the disappearance of the sulfides.

Small-Scale Oxidation of Sulfides Using 1-Phenylethyl Hydroperoxide and Other Racemic Hydroperoxides as Oxidants. In a typical reaction, 100 μL of CPO (600 U, Sigma) and 0.5 mmol of sulfide were added to 50 mL of 0.05 M citrate buffer (pH 5). The mixture was stirred at 4 °C for 15 min. A racemic hydroperoxide (0.5 mmol) was added over a period of 2 h. The reaction progress was monitored by HPLC and stopped at a desired conversion. The mixture was extracted with $CHCl_3$, and the organic layer was combined and dried over $MgSO_4$. The solvent was removed under vacuum, and the products were isolated by silica gel column chromatography (ethyl acetate:hexane = 1:10–1:0). The ee of sulfoxides and alcohols were determined on HPLC using a chiralcel OB column and a UV detector (254 nm). The mobile phase for sulfoxides was hexane/2-propanol (7/3), and the retention time was as follows: phenyl methyl sulfoxide ((*S*)-enantiomer = 16.3 min, (*R*)-enantiomer = 24.8 min) and *p*-methoxyphenyl methyl sulfoxide ((*S*)-enantiomer = 23.6 min, (*R*)-enantiomer = 37.3 min). The mobile phase for alcohols was hexane/2-propanol (9/1) for 1-phenylethanol and other 1-arylethanols (entries 1–4, 6) and 49/1 for entry 5. The retention time was as follows: entry 1, (*S*)-enantiomer = 12.3 min and (*R*)-enantiomer = 15.9 min; entry 5, (*S*)-enantiomer = 27.0 min and (*R*)-enantiomer = 29.6 min; entry 6, (*S*)-enantiomer = 13.2 min and (*R*)-enantiomer = 19.0 min; entry 7, (*S*)-enantiomer = 10.4 min and (*R*)-enantiomer = 14.1 min. The ee's were determined by comparing the relative intensities of the two enantiomers. To determine the ee for 1-arylethyl hydroperoxides, the hydroperoxides were reduced to the corresponding alcohols by $LiAlH_4$ reduction, and the ee's of the alcohols were then determined with the HPLC method as described above.

Preparative-Scale Oxidation of Phenyl Methyl Sulfide Using 1-Phenylethyl Hydroperoxide as Oxidant. In a typical reaction, 40 mL of 0.05 M citrate buffer (pH 5) and 100 μL of CPO (600 U, Sigma) were used. The mixture was stirred at 4 °C for 5 min. Phenyl methyl sulfide (2 mg for 1 equiv, 4 mg for 2 equiv) in 1 mL of acetonitrile was then added, and the mixture was stirred for another 10 min. To this solution was added 1-phenylethyl hydroperoxide (2.2 mg for 1 equiv), and the mixture was stirred at 4 °C for ~3 h. The reaction progress was monitored by measuring the disappearance of phenyl methyl sulfide at 250 nm on a spectrophotometer. The reaction was stopped when the conversion approached the desired value. The mixture was then extracted with $CHCl_3$ (30 mL × 4). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The products were isolated by silica gel column chromatography (ethyl acetate:hexane = 1:10–1:0). The ee values for phenyl methyl sulfoxide, 1-phenylethyl hydroperoxide, and 1-phenylethanol were determined on HPLC as described above.

Oxidation of Sulfides Using Hydrogen Peroxide as Oxidant. In a typical reaction, to 100 mL of 0.05 M citrate buffer (pH 5) was added 200 μL of CPO (1200 U, Sigma). The mixture was stirred at 4 °C for 5 min. To this mixture were added 0.5 mL of the sulfide solution (1 mmol of sulfide dissolved in 10 mL of CH_3CN and 10 mL of buffer) and 0.5 mL of a hydrogen peroxide solution (1.2 mmol of 50% H_2O_2 dissolved in 20 mL of buffer) via a capillary pipet every 1.5 h. The reaction mixture was stirred at 4 °C overnight after the addition of the substrate was complete. The mixture was then extracted with $CHCl_3$ (80 mL × 5), and the organic layer was combined and dried over $MgSO_4$. The sulfoxide was isolated on a silica gel column using

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ethyl acetate as eluant. The enantiomeric excess was determined on HPLC using a chiralcel OB column (0.46 × 25 cm) and a UV detector (254 nm). The mobile phase was hexane/2-propanol = 7/3 for all sulfoxides. The retention time was the following: phenyl methyl sulfoxide ((*S*)-enantiomer = 16.3 min, (*R*)-enantiomer = 24.8 min); *p*-chlorophenyl methyl sulfoxide ((*S*)-enantiomer = 15.9 min, (*R*)-enantiomer = 20.2 min); *p*-fluorophenyl methyl sulfoxide ((*S*)-enantiomer = 16.3 min, (*R*)-enantiomer = 21.0 min); *p*-methylphenyl methyl sulfoxide ((*S*)-enantiomer = 13.4 min, (*R*)-enantiomer = 23.8 min); *p*-methoxyphenyl methyl sulfoxide ((*S*)-enantiomer = 23.6 min, (*R*)-enantiomer = 37.3 min).

Determination of Kinetic Parameters for CPO. To determine the kinetic parameters, 3.1 mg of phenyl methyl sulfide and 5.5 mg of 1-phenylethyl hydroperoxide were dissolved in 100 mL of citric acid buffer (0.05 M, pH 5.0) independently. This gave a stock concentration of 250 μM for sulfide and 400 μM for hydroperoxide, respectively. The K_m and V_{max} values of phenyl methyl sulfide were determined with the concentration of 1-phenylethyl hydroperoxide fixed at 200 μM. The concentration of sulfide ranged from 20 to 100 μM. In each assay, 500 μL of a peroxide solution was added to a quartz cuvette. After a certain amount of sulfide solution was added, the volume was adjusted to 999 μL with a buffer solution. When the substrates were well mixed, 60 units of enzyme was injected to start the reaction. The disappearance of sulfide, which has a maximum absorption of 250 nm with ϵ = 9643, was then monitored for 15 min to calculate the initial rate. After all the initial rates were acquired, the data were processed with Enzfitter on an IBM PS-2 computer to give V_{max} and K_m values.

The K_m and V_{max} values for hydroperoxide were determined in a similar way with the sulfide concentration fixed at 25 μM. The concentration of hydroperoxide ranged from 30 to 200 μM.

The K_m and V_{max} values for phenyl methyl sulfide, *p*-chlorophenyl methyl sulfide, *p*-fluorophenyl sulfide, and *p*-methylphenyl methyl sulfide were determined with hydrogen peroxide concentration fixed at 50 μM and the sulfide concentration ranging from 10 to 80 μM. The disappearance of sulfide was monitored for 3 min on the spectrophotometer using the following ϵ values (M^{-1} cm^{-1}) and wavelengths (nm): $PhSCH_3$, 9643 and 250; *p*-$ClPhSCH_3$, 10531 and 256; *p*-$FPhSCH_3$, 6668 and 248; *p*-CH_3PhSCH_3, 9388 and 252.

General Procedure for Chloroperoxidase-Catalyzed Bromohydration. To a reaction mixture containing 20 mL of citrate buffer (pH 3), 1 mmol of glycal, 5 mmol of potassium bromide, and 1170 units of the enzyme was added 600 μL of H_2O_2 (30%). The reaction was continued for 3 h at room temperature. The solvent was removed under reduced pressure, and methanol was added to the residue. The insoluble material was filtered off, and the solvent was removed under reduced pressure. The residue was purified with C8-reversed-phase silica gel column chromatography to yield the bromohydration product. The products were converted to peracetates by pyridine and acetic anhydride in the presence of a catalytic amount of 4-(dimethylamino)pyridine and purified by silica gel column chromatography for characterization.

Methyl 5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3-bromo-3,5-dideoxy-β-D-erythro-L-*manno*-2-nonulopyranosate (2). According to the general procedure, the product was prepared from 2,3-dehydrosialic acid[22] and converted to the peracetate, followed by esterification with methyl iodide in the presence of an equimolar amount of cesium carbonate to obtain **2** (41 mg, 65%).

^{1}H-NMR ($CDCl_3$) δ: 1.95, 2.04, 2.05, 2.10, 2.19, 2.20 (3 H, s, each, OAc and NHAc), 3.83 (3 H, s, $COOCH_3$), 4.11 (1 H, ddd, J = 8.7, 10.6, 10.7 Hz, H-5), 4.22 (1 H, dd, J = 6.4, 12.5 Hz, H-9), 4.32 (1 H, dd, J = 1.8, 10.6 Hz, H-6), 4.57 (1 H, dd, J = 5.15 (1 H, ddd, J = 2.4, 5.5, 6.4 Hz, H-8), 5.31 (1 H, dd, J = 1.8, 5.5 Hz, H-7), 5.43 (1 H, d, J = 8.7 Hz, NH), 5.67 (1 H, dd, J = 3.8, 10.7 Hz, H-4). HRMS: calcd for $C_{22}H_{30}NO_{14}BrCs$ (M + Cs^+) 743.9904/746, found 743.9900/746.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1,4)-1,3,6-tri-*O*-acetyl-2-bromo-2-deoxy-D-glucopyranose (5a) and -D-mannopyranose (5b). According to the general procedure, a 1:1 mixture of **5a** and **5b** (155 mg, 71%) was obtained from Galβ-(1,4)glucal **4** (96.5 mg). The ratio of **5a** and **5b** was determined from the integral ratio of the anomeric protons of **5a** and **5b**. **5a** and **5b** were obtained as α:β monomeric mixtures: **5a** (α:β = 1:3), **5b** (α:β = 2:1).

^{1}H-NMR of the mixture of **5a** and **5b**. ^{1}H-NMR ($CDCl_3$) δ: 1.96, 1.97, 1.98, 1.981, 2.03, 2.04, 2.05, 2.06, 2.070, 2.074, 2.075, 2.08, 2.12, 2.13, 2.132, 2.15, 2.16, 2.166 (-OCH_3), 3.84 (dd, J = 1.6, 9.0 Hz, H-2 of Glu of β anomer of **5a**), 3.73–4.22 (m), 4.40 (dd, J = 2.2, 3.8 Hz, H-2 of α anomer of **5b**), 4.43–4.47 (m), 4.46 (dd, J = 1.0, 7.6 Hz), 4.55 (d, J = 8.0 Hz), 4.57 (dd, J = 1.6, 4.0 Hz, H-2 of α anomer of **5b**), 4.59 (d, J = 8.0 Hz), 4.93 (dd, J = 3.5, 5.1 Hz), 4.96 (dd, J = 3.5, 4.96 Hz), 4.99 (dd, J = 3.5, 10.5 Hz, H-3′ of β anomer of **5a**, 5.03 (dd, J = 3.8, 8.8 Hz), 4.07–5.12 (m), 5.16 (dd, J = 8.0, 10.5 Hz), 5.20–5.26 (m), 5.35–5.38 (m), 5.70 (d, J = 1.6, H-1 of β anomer of **5b**), 5.76 (d, J = 9.0 Hz, H-1 of β anomer of **5a**), 6.26 (d, J = 2.2 Hz, H-1 of α anomer of **5b**), 6.30 (d, J = 3.4 Hz, H-1 of α anomer of **5a**). ^{13}C-NMR ($CDCl_3$) δ: 20.52, 20.61, 20.65, 20.75, 20.80, 20.84, 20.88, 20.93, 46.27, 47.95, 48.13, 51.26, 60.77, 60.91, 61.08, 61.49, 61.67, 61.79, 62.04, 66.56, 66.62, 66.71, 66.75, 68.97, 69.03, 69.09, 69.14, 69.30, 70.68, 70.72, 70.75, 70.78, 70.82, 70.86, 70.91, 70.94, 70.98, 71.16, 71.73, 73.44, 73.68, 73.80, 74.13, 74.29, 76.32, 76.61, 89.77, 90.34, 92.98, 93.13, 100.84, 101.19, 101.45, 168.42, 168.45, 168.53, 168.92, 169.14, 169.22, 169.36, 169.59, 169.65, 170.08, 170.13, 170.16, 170.29, 170.36, 170.46. HRMS of **5a** and **5b**: calcd for $C_{26}H_{35}O_{17}BrCs$ (M + Cs^+) 831.0112/833, found 831.0112/833.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1,3)-1,4,6-tri-*O*-acetyl-2-bromo-2-deoxy-D-glucopyranose (7a) and -D-mannopyranose (7b). According to the general procedure, a 1:1 mixture of **7a** and **7b** (66 mg, 76%) was obtained from Galβ-(1,3)glucal **6** (38.6 mg). **7a** and **7b** were isolated by silica gel column chromatography (ACOEt/*n*-hexane (5/2)) as α/β anomeric mixtures: **7a** (α:β = 1:10), **7b** (α:β = 12:5).

β anomer of **7a**. ^{1}H-NMR ($CDCl_3$) δ: 1.98, 2.04, 2.07, 2.08, 2.09, 2.15, 2.17 (3 H, each, s, OAc × 7), 3.78 (1 H, ddd, J = 1.8, 4.6, 9.7 Hz, H-5 of Glc), 3.85 (1 H, t, J = 9.5 Hz, H-2 of Glc), 3.89 (1 H, t, J = 7 Hz, H-5 of Gal), 3.96 (1 H, t, J = 10.0 Hz, H-3 of Glc), 4.07 (1 H, m, H-6 of Gal), 4.09 (1 H, dd, J = 1.8, 12.4 Hz, H-6 of Glc), 4.13 (1 H, dd, J = 7.0, 11.1 Hz, H-6 of Gal), 4.25 (1 H, dd, J = 4.6, 12.4 Hz, H-6 of Glc), 4.89 (1 H, d, J = 7.7 Hz, H-1 of Gal), 4.97 (1 H, t, J = 9.6 Hz, H-4 of Glc), 5.03 (1 H, dd, J = 3.4, 10.0 Hz, H-3 of Gal), 5.13 (1 H, dd, J = 7.7, 10.0 Hz, H-2 of Gal), 5.36 (1 H, d, J = 3.4 Hz, H-4 of Gal), 5.75 (1 H, d, J = 9.0 Hz, H-1 of Glc). HRMS: calcd for $C_{26}H_{35}O_{17}BrCs$ (M + Cs^+) 831.0112/833, found 831.0112/833. ^{13}C-NMR ($CDCl_3$) δ: 20.55, 20.64, 20.68, 20.71, 20.75, 20.96, 50.11, 60.93, 61.62, 66.73, 68.53, 68.96, 70.62, 70.82, 72.87, 77.21, 81.30, 92.86, 101.61, 168.77, 169.03, 169.35, 170.13, 170.20, 170.36, 170.67.

α anomer of **7b**. ^{1}H-NMR ($CDCl_3$) δ: 4.24 (1 H, bd, J = 3.0 Hz, H-2 of Man), 4.55 (1 H, d, J = 8.0 Hz, H-1 of Gal), 5.01 (1 H, dd, J = 3.4, 10.5 Hz, H-3 of Gal), 5.18 (1 H, dd, J = 7.8, 10.5 Hz, H-2 of Gal), 5.32 (1 H, t, J = 8.7 Hz, H-4 of Man), 5.39 (1 H, dd, J = 1.0, 3.4 Hz, H-4 of Gal), 6.30 (1 H, d, J = 3.0 Hz, H-1 of Man).

β anomer of **7b**. ^{1}H-NMR ($CDCl_3$) δ: 4.45 (1 H, dd, J = 2.0, 3.5 Hz, H-2 of Man), 4.55 (1 H, d, J = 8.0 Hz, H-1 of Gal), 5.01 (1 H, dd, J = 3.4, 10.5 Hz, H-3 of Gal), 5.20 (1 H, dd, J = 7.8, 10.5 Hz, H-2 of Gal), 5.30 (1 H, t, J = 7.4 Hz, H-4 of Man), 5.39 (1 H, dd, J = 1.0, 3.4 Hz, H-4 of Gal), 5.80 (1 H, d, J = 2.0 Hz, H-1 of Man). HRMS: calcd for $C_{26}H_{35}O_{17}BrCs$ (M + Cs^+) 831.0112/833, found 831.0110/833. ^{13}C-NMR δ: 20.57, 20.65, 20.76, 20.87, 20.95, 21.01, 21.04, 48.21, 60.98, 61.19, 62.01, 62.61, 66.75, 66.82, 68.43, 68.52, 70.71, 70.88, 71.05, 72.03, 73.36, 74.74, 75.93, 77.23, 90.08, 92.80, 100.10, 100.24, 168.23, 169.13, 169.22, 169.73, 170.18, 170.40.

Sialyl α(1,3)Galβ(1,4)[Fucα(1,3)]-2-bromo-2-deoxy-D-glucopyranose (9a) and -2-bromo-2-deoxy-D-mannopyranose (9b). According to the general procedure, a 1:1 mixture of **9a** and **9b** (3.5 mg, 56%) was obtained from NeuAca(2,3)Galβ(1,4)-[Fucα(1,3)]glucal[19] (5.5 mg). The ratio of **9a** and **9b** was determined from the integrated ratio of the methyl protons of fucose.

^{1}H-NMR of the mixture of **9a** and **9b**. ^{1}H-NMR (D_2O) δ: 1.17 (d, J = 6.0 Hz, CH_3 of Fuc), 1.18 (d, J = 6.0 Hz, CH_3 of Fuc), 1.80 (t, J = 12.7 Hz, H-3ax of NeuAc), 2.02 (s, NHAc), 2.75 (dd, J =

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5.0, 12.7 Hz, H-3eq of NeuAc), 3.45–4.13 (m), 4.48 (d, J = 8.0 Hz, H-1 of Gal), 4.49 (d, J = 8.0 Hz, H-1 of Gal), 5.0–5.04 (m), 5.18–5.22 (m), 5.38–4.42 (m).

General Procedure for Bromohydration with NBS. To a solution of 1 mmol of glucal in a mixture of 3.6 mL of CH_3CN–1.5 mL of H_2O was added 1 mmol of NBS at room temperature. The reaction was continued for 3 h at the same temperature. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel column chromatography. The products were converted to peracetates by pyridine and acetic anhydride in the presence of a catalytic amount of 4-(dimethylamino)pyridine and purified by silica gel column chromatography for characterization.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1,4)-1,3,6-tri-*O*-acetyl-2-bromo-2-deoxy-D-glucopyranose (5a) and -D-mannopyranose (5b). According to the general procedure, a 1:2.5 mixture of **5a** and **5b** (30 mg, 78%) was obtained from Galβ-(1,4)glucal 4 (17 mg). The ratio of **5a** and **5b** was determined from the integrated ratio of the anomeric protons. **5a** and **5b** were obtained as α:β anomeric mixtures: **5a** (α:β = 3.5), **5b** (α:β = 5:2).

Methyl 5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3-bromo-3,5-dideoxy-β-D-*erythro*-L-*manno*-2-nonulopyranosonate (2) and Methyl 5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3-bromo-3,5-dideoxy-α-D-*erythro*-L-*gluco*-2-nonulopyranosonate (3). Chemical bromohydration was carried out according to the general procedure, and the products were converted to peracetate, followed by esterification with methyl iodide in the presence of an equimolar amount of secium carbonate to obtain a mixture of **2** and **3** (155 mg, 74%). The production ratio of **2** and **3** was determined from the integral ratio of methyl ester protons.

^{1}H-NMR spectra of **2** and **3** were in good agreement with a previous report.[25]

Compound **3**. ^{1}H-NMR ($CDCl_3$) δ: 1.90, 2.03, 2.08, 2.10, 2.12, 2.15, (3 H, s, each OAc and NHAc), 3.78 (3 H, s, $COOCH_3$), 4.04 (1 H, dd, J = 6.0, 12.4 Hz, H-9), 4.09 (1 H, d, J = 10.0 Hz, H-3ax), 4.33 (1 H, ddd, J = 10.0, 10.6, 10.7 Hz, H-5), 4.36 (1 H, dd, J = 2.4, 12.4 Hz, H-9′), 5.10 (1 H, ddd, J = 2.4, 6.0, 6.1 Hz, H-8), 5.25 (1 H, dd, J = 2.5, 10.7 Hz, H-6), 5.30 (1 H, dd, J = 10.0, 10.6 Hz, H-4), 5.38 (1 H, dd, J = 2.5, 6.1 Hz, H-7), 5.90 (1 H, d, J = 10.0 Hz, NH).

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Enol Oxalacetic Acid Exists in the *Z* Form in the Crystalline State and in Solution†

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Because of inconsistencies in the literature regarding the structure of the enol form of oxalacetic acid (OAA), this subject has been reinvestigated. It was approached initially by attempting to determine the crystal structure of enol OAA directly by X-ray crystallography. This approach failed due to the poor quality of enol OAA crystals. The subject was then approached indirectly as follows. First, the structure of enol di-*tert*-butyl OAA in the crystalline state was determined to be *Z* by X-ray crystallography. Second, the solution structure of enol di-*t*-butyl OAA was determined to also to be *Z* by ^{1}H and ^{13}C-NMR studies combined with the results from X-ray crystallography of enol di-*tert*-butyl OAA. Third, the solution structure of enol OAA was determined to be *Z* by ^{13}C-NMR studies using enol di-*tert*-butyl OAA as a reference. Fourth, the structure of enol OAA in the crystalline state was determined to be *Z* by ^{1}H-NMR studies using freshly dissolved enol OAA solutions at low temperature. Finally, the structure of enol OAA in aqueous solution was inferred to be *Z* on the basis of is activity as a substrate for fumarase A. The previous reports in the literature of crystalline (*E*)-enol OAA are in error.

Introduction

Oxalacetic acid (OAA) is an important primary metabolite. It can exist in the keto, enol (which could be cis(*E*) or trans(*Z*)), and hydrated *gem*-diol forms each of which can also exist in several ionization states. A number of investigators have attempted to identify the particular forms of OAA present in the crystalline state and in solution. Although there is general agreement on the nature of the equilibrium between the keto, enol, and hydrated *gem*-diol forms in solution,[1-3] there is little known about the structure of the enol in solution and considerable disagreement over the structure of the enol of OAA in the crystalline state.[4-10]

The earliest studies on the various forms of OAA were those of Wohl and co-workers who reported that crystalline OAA existed as the enol. Depending on the concentration of H_2SO_4 in the mother liquor, they claimed that the (*Z*)-enol melting at 182 °C or the (*E*)-enol melting at 152 °C would crystallize out of solution.[4,5]

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