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Asymmetric sulfoxidation catalyzed by a vanadium bromoperoxidase: Substrate requirements of the catalyst

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Abstract

An investigation of the catalytic effect of vanadium bromoperoxidase (VBrPO, from *Corallina officinalis*) on the oxidation of a series of prochiral sulfides by hydrogen peroxide, revealed that substrates having a *cis*-positioned carboxyl group are oxidized rapidly, giving the sulfoxide in >95% e.e. The pH-rate profile shows a typical sharp sigmoidal curve, indicative of a deprotonation event at around pH 6.4. The corresponding, non-protolytic, methyl esters were not catalyzed by the enzyme. Rapid loss of stereoselectivity was found to occur when VBrPO-catalyzed oxidation was carried out in the presence of bromide ions. This has been interpreted as being due to the intervention of a competing reaction involving oxidation of bromide and the subsequent formation of a bromosulfonium ion intermediate.

Keywords: Asymmetric synthesis; Enzyme reactions; Sulfoxides; Vanadium.

Introduction

Chiral sulfoxides are very useful as synthons in asymmetric synthesis. A chiral sulfoxide group will not only provide a stereogenic centre, but may also induce chirality in successive reaction steps [1-3]. The Andersen synthesis and the use of chiral oxaziridines [4,5] or catalysts, like the Jacobsen catalyst [6] and particularly the titanium complex in the modified Sharpless oxidation, are some general methods which can be applied in order to obtain chiral sulfoxides [7]. In addition, a number of biocatalysts have been found to catalyze sulfoxidation with high stereospecificity, but often with limited generality [8-11]. Several heme-containing

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peroxidases, however, have been found to be useful catalysts in a diverse range of oxidative reactions [12] and have been utilized in asymmetric oxidation of sulfides. Chloroperoxidase from the fungus *Caldariomyces fumago* (CPO) belongs to those which have been more thoroughly investigated and shows high catalytic activity as well as enantioselectivity towards several small sulfides of diversified structure [13-16].

Recently, we discovered that another class of peroxidases, namely the vanadium-dependent bromoperoxidases, also could be used in asymmetric sulfoxidation [17]. Vanadium-dependent bromoperoxidase from the alga *Corallina officinalis* [18] (VBrPO) was shown for a series of bicyclic, rigid sulfides to selectively catalyze oxidation to the (*S*)-enantiomer of the corresponding sulfoxides. This enantiopreference is the opposite to what is obtained by CPO [16]. VBrPO showed high catalytic activity and e.g. 2,3-dihydrobenzo[*b*]thiophene (**1**) was oxidized quantitatively in 98% e.e.

The VBrPO's [19] have been considered to possess relatively high stability towards elevated temperature and presence of co-solvents [20]. This is an interesting feature when considering the usefulness of a biocatalyst in organic synthesis.

In this contribution we have mainly focused on the versatility and the substrate requirements of VBrPO, by studying the oxidation of a wide range of sulfides. The effect on rate and enantioselectivity by the presence of different alcohols has also been examined.

Prior to our previous study, showing VBrPO to be an effective catalyst for asymmetric sulfoxidation, vanadium-dependent peroxidases had only been utilized in halogenation or halide-assisted oxidation reactions [21,22]. Therefore, the mechanistic question concerning the competition between sulfide and halide ion oxidation by VBrPO, was addressed and a series of sulfoxidation reactions in the presence of halide ions was investigated.

Results and Discussion

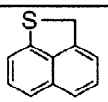
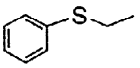
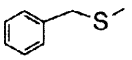
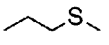
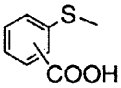
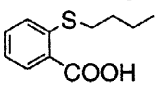
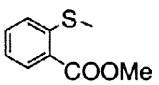
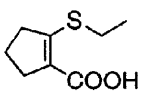
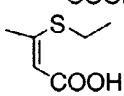
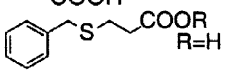
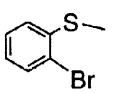
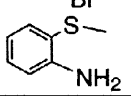
Our previous study on VBrPO-catalyzed oxidation of sulfides showed 2,3-dihydrobenzo[*b*]thiophene to be an excellent substrate for VBrPO whereas methyl p-tolyl sulfide (**2**) was not accepted [17]. To further explore the substrate requirements of the active site of VBrPO, oxidation of a series of sulfides in the presence of VBrPO and with hydrogen peroxide as terminal oxidant was carried out, Table 1. The reactions were carried out with continuous addition of hydrogen peroxide during a relatively long reaction time (16 h) for the purpose of keeping a constant, low concentration of hydrogen peroxide, a prerequisite to obtain high activity and selectivity of VBrPO [17].

Since **1** was successfully oxidized by VBrPO, the structurally related sulfide, 2*H*-naphtho[1,8-*b,c*]thiophene (**3**) was tried as a substrate. Only a very low yield was obtained however, and the e.e. could not be determined. Neither was **3** oxidized by CPO.

Like compound **2**, ethyl phenyl sulfide (**4**) was not accepted as a substrate. Extension with a methylene group on the other side of the sulfur atom to benzyl methyl sulfide (**5**) had a more positive effect. However, the rate of the VBrPO-catalyzed oxidation of **5** was not significantly

higher than the background reaction, explaining the low e.e. In the background reactions, for which the resulting yields are given within brackets in Table 1, identical experimental conditions (except for the absence of enzyme) were used. However, these background values, representing the contribution of the uncatalyzed reaction, are somewhat overestimated, since in the presence of enzyme the hydrogen peroxide concentration is reduced due to its consumption in the catalyzed reaction.

Table 1. Results obtained in VBrPO-catalyzed oxidation of a series of sulfides[a]

substrate	no.	co-solvent	VBrPO (u)	yield[b] (%)	e.e. (%)	absolute config.
	**3**	4% 1-propanol	1	1.6 (0)	n.d.	-
		30% *tert*-butanol	"	0.7 (0.7)	-	-
	**4**	0.5% 1-propanol	"	7 (5)	-	-
	**5**	"	"	57 (55)	11	(+)-S
	**6**	"	"	86 (90)	-	-
	**7**	"	"	29 (22)	-	-
 *ortho*	**8**	-	"	76 (21)	93	(-)-S
		5% 1-propanol	"	80 (20)	97	"
meta	**9**	-	"	20 (21)	-	-
para	**10**	-	"	16	2	-
	**11**	5% 1-propanol	"	34 (27)	15	(-)-S
		"	5	70 (31)	34	"
	**12**	2.5% 1-propanol	1	2.4 (2.3)	-	-
		16% 1-propanol	"	1.2 (1.2)	-	-
	**13**	-	"	70 (43)	86	(-)-[c]
		"	3	89	98	"
	**14**[d]	-	1	77 (41)	93	(-)-[c]
		"	3	94	97	"
 R=H	**15**	5% 1-propanol	"	39 (42)	-	-
R=Me	**16**	1% 1-propanol	"	12 (12)	-	-
	**17**	0.5% 1-propanol	1	0.6 (0.5)	-	-
	**18**	0.5% 1-propanol	"	16 (20)	-	-

[a] Hydrogen peroxide was added continuously during the reaction, which was carried out for 16 h at pH 6.5 and 25°C in phosphate buffer.

[b] Values within brackets correspond to yields obtained in the absence of enzyme.

[c] The absolute configuration is to be elucidated.

[d] **14** was contaminated by 8% of the corresponding (*E*)-isomer.

Reduction of the reaction time for **5** from 16 h to 3 h in an attempt to suppress the uncatalyzed reaction did not lead to any improvement. The absolute configuration of the benzyl methyl sulfoxide enantiomer in excess was found to be S. This assignment was made on the basis of the elution order found in the LC-analysis by comparison with the enantiomeric composition obtained from the CPO-catalyzed reaction, known to give the (R)-enantiomer in excess [13].

The dialkyl sulfides, **6** and **7**, which, like compounds **2**, **4** and **5**, have been oxidized in high yield and e.e. by CPO, also showed a non-substrate behavior towards VBrPO. These discouraging results led us to try some charged substrates in the form of acid-functionalized sulfides.

2-(Methylthio)benzoic acid (**8**) was found to be oxidized in high yield and high e.e. to the (*S*)-sulfoxide. A slight increase in both e.e. and yield was obtained in the presence of 5% 1-propanol, possibly due to an enhanced solubility of **8** in the phosphate buffer. An attempt to oxidize **8** by CPO was made for comparison, but turned out to be unsuccessful. Extension of the alkyl side-chain, like in **11**, gave a reduced reaction rate and consequently also a reduced e.e. An improved product yield can easily be obtained, however, by increasing the VBrPO concentration, as shown in Table 1. The (S)-configuration of the favoured product enantiomer in the oxidation of both **8** and **11** was determined from the known elution order in the LC-analysis [23]. These enantiomerically enriched sulfoxides should be potentially useful as synthons in asymmetric synthesis since the carboxylate group constitutes a site of further reaction.

The methyl ester **12** of compound **8** was totally rejected as substrate by VBrPO and a similar result was found for compounds **9** and **10** which both have the carboxylic acid substituent located to the *meta* and *para* position, respectively.

To further investigate the interaction between **8** and VBrPO, the pH-profile of the reaction was generated. Figure 1, which shows the initial rate versus pH for the oxidation of **1** and **8**, displays a typical sigmoid profile and a point of inflexion at ca. pH 6.4 for **8**. The corresponding pH-dependence of the non-protolytic sulfide **1**, on the other hand, gave a flat pH-profile with a maximum at pH 6. The pH-profile of **8** most likely reflects the preferential oxidation of the anionic form of **8**. The pKa of **8** has been determined to 3.67 in water, but with a large shift to 8.84 in 95% DMSO [24]. Consequently, a pKa of **8** increased to 6.4 within the active site of VBrPO should be possible. The preferred oxidation of the dissociated form of **8** might be due to an interaction with a positive residue in the vicinity of the vanadate complex in VBrPO.

A preliminary X-ray analysis of VBrPO has been reported [25], but the refinement is insufficient to permit any conclusion about the structure of the active site. Recently, however, a crystal structure of vanadium chloroperoxidase from the fungus *Curvularia inaequalis* (VClPO) was reported [26].

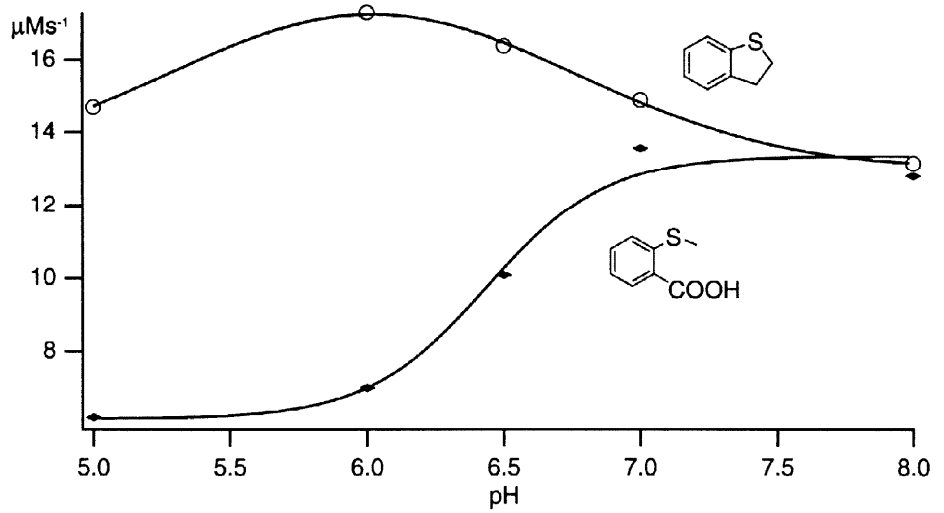


Figure 1. The pH-dependences of the initial rates in VBrPO-catalyzed oxidation of **1** and **8**, respectively, showing a sigmoidal curvature for **8**, most likely due to a preferred oxidation of the dissociated form of **8**, whereas a broad optimum around pH 6 is observed for the non-protolytic **1**.

VClPO shows sequence analogy with VBrPO's, particulary in the active site, and structural similarities are proposed [21]. The vanadate unit is covalently bound to a histidine and the oxygens are hydrogen bonded to several positively charged amino acids, resulting in a trigonal bipyramidal coordination geometry. In addition, two histidines, an aspartate and an arginine constitute part of the active site of VBrPO. The arginine is a likely candidate for interaction with the anionic **8**. A similar interaction between dissociated **9** and **10** might result in a non-productive position of the metylthio substituent relative to the peroxovanadate intermediate, explaning the non-substrate behaviour. In the methyl ester case this ionic interaction will vanish, explaining the poor results in the oxidation of **12**.

However, since the pH-region, in which the rate enhancement for the oxidation of **8** (Fig. 1) is found, coincides with the pKa of histidine, an effect caused by deprotonation of a histidine in the active site cannot be excluded. It has been suggested that the histidine close to vanadium (His 404 in VClPO) acts as an acid-base site (pKa 5.7-6.5) crucial for regulating the activation of the peroxovanadium complex [27], which might influence the kinetic behaviour of a negatively charged substrate.

Two other acid-functionalized sulfides, **13** and **14**, with a locked conformation similar to **8** were examined. Both were oxidized in high yield and e.e. which indicates some generality in the preference of the carboxylate positioned *ortho* or *cis* relative to the sulfur atom. Furthermore, **14** contained a minor amount of corresponding (*E*)-isomer (8%), which was found to be totally unaffected during the VBrPO-catalyzed oxidation. A determination of the absolute configuration of the enantiomers obtained in excess in the oxidation of **13** and **14** is underway. The negative signs of optical rotation, as well as an elution order in the LC-

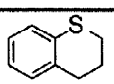
analysis, consistent with that of the oxidation product of **8**, however, clearly indicate the (S)-enantiomer to be favoured also in these reactions.

3-Benzylthiopropanoic acid (**15**) and its corresponding methyl ester (**16**) were not accepted as substrates by VBrPO. Although the three bonds separating the sulfur atom from the carbonyl group would allow **15** to adopt the preferred *cis*-conformation, this is energetically disfavoured and together with the large steric requirement of the benzyl group it might lead to a decreased affinity for the active site of VBrPO and explain why no oxidation takes place.

Thioanisole with either a bromo (**17**) or an amino substituent (**18**) in the *ortho*-position appeared as non-substrates. The pKa of the corresponding sulfoxide of **18** has been determined to 2.53 [28] and the pKa of **18** will not be much different. Consequently **18** can be considered as uncharged under these conditions.

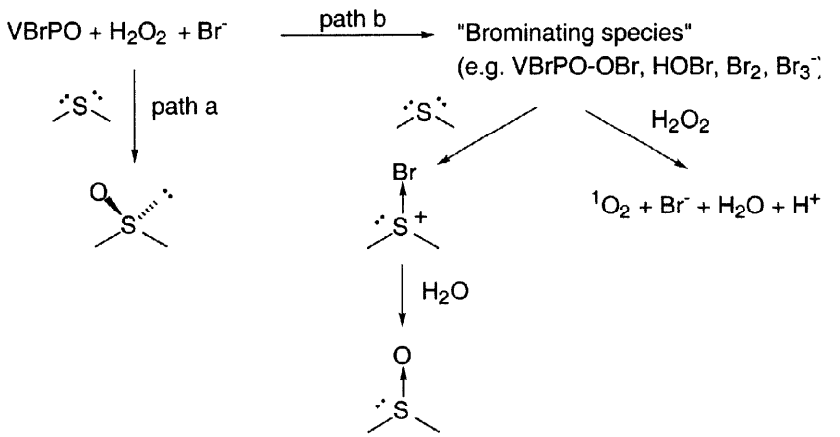
Many of the sulfides investigated showed limited solubility in the phosphate buffer and the possibility to add co-solvents, without affecting the catalytic activity, was explored. A high stability of the enzyme in the presence of different co-solvents, e.g. alcohols, has been demonstrated earlier by examination of the catalytic activity in bromination reactions [20]. The influence on sulfoxidation might be somewhat different and the enantioselectivity has to be considered as well. Results obtained from the oxidation of 1-thiochroman in the presence of different alcohols are presented in Table 2. The presence of 0.5% 1-propanol has no effect on the reaction [17]. Generally, VBrPO showed a higher tolerance towards the branched alcohols as compared to the primary, and the same holds for CPO [29].

Table 2. The influence on VBrPO catalyzed sulfoxidation by addition of co-solvent

substrate	co-solvent	yield (%)	e.e. (%)
	0.5% 1-propanol	18.9	63.3
	5% 1-propanol	14.3	45.2
	14% 1-propanol	15.3	32.2
	27% 1-propanol	11.1	18.0
	0.5% 2-propanol	18.9	63.3
	17% 2-propanol	14.0	44.0
	5% *tert*-butanol	15.3	66.1
	17% *tert*-butanol	16.8	62.0
	27% *tert*-butanol	23.6	52.4
	41% *tert*-butanol	2.8	52

The hydrogen peroxide concentration has been found to significantly affect the catalytic activity of VBrPO during sulfoxidation [17]. VBrPO can catalyze halide-assisted disproportionation of hydrogen peroxide during oxidation of bromide in the absence of a secondary substrate prone to bromination [30]. No formation of molecular oxygen could be

detected during sulfoxidation and thereby the possibility of a catalase activity in VBrPO has been excluded [17]. Interestingly, a complete inhibition of the oxygen evolution has been observed by addition of **1** during the oxidation of bromide. The question has been addressed, whether this is a consequence of a predominating catalysis of sulfoxidation (path a) over the bromide oxidation (path b), or if **1** is acting as a secondary substrate to be brominated (Scheme 1).



Scheme 1

To be able to answer that question, the product formation in the VBrPO-catalyzed oxidation of **1** in the presence of bromide or chloride ions was analyzed, Figure 2. The quantitative yield was unaffected by the addition of halide ions, but the enantioselectivity was radically decreased in the presence of bromide ions.

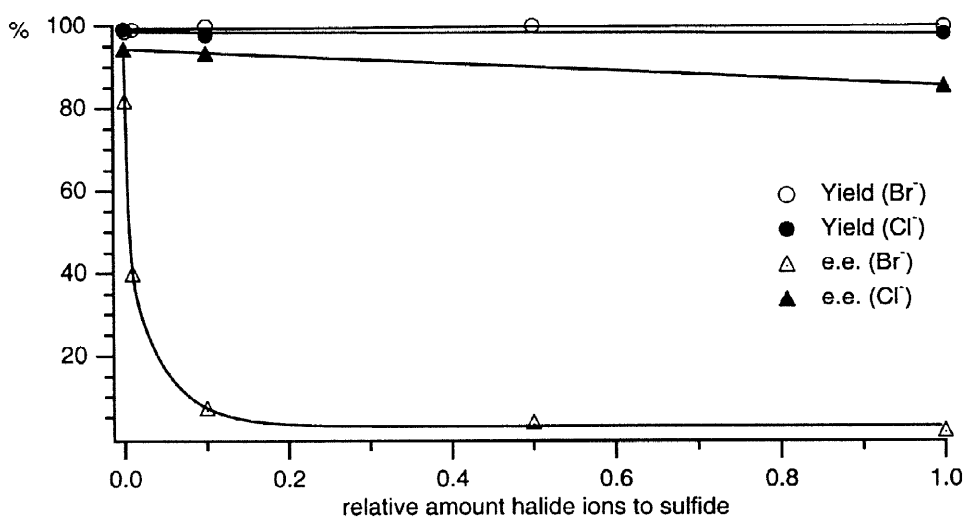


Figure 2. The influence of halide addition on yield and e.e. in the VBrPO-catalyzed oxidation of **1**.

This can only be explained by a favoured oxidation of bromide and with a subsequent step of sulfide bromination, leading to racemic sulfoxide *via* a rapid halogen exchange in a bromosulfonium ion. The formation of optically active sulfoxide at low bromide ion concentration will be due to either asymmetric sulfoxidation (path a) or a slow halogen exchange in the bromosulfonium ion. The enantioselectivity was much less influenced by the presence of chloride ions, due to the low capability of VBrPO to oxidize more electronegative halides than bromide [31].

Conclusion

Although VBrPO shows restricted applicability in its acceptance of substrates, sulfides bearing a carboxylate functionality in a *cis*-position to the sulfur atom were found to be oxidized with high enantioselectivity. A preferred oxidation of the deprotonated form of the acid functionalized sulfides due to interaction to a positively charged residue in the active site of VBrPO is suggested.

Branched alcohols seem to be preferred when the addition of co-solvents is necessary to increase the solubility of the substrate.

By studies of the competition between VBrPO-catalyzed oxidation of sulfide versus halide, we have shown that the oxidation of halide completely predominates in the case of bromide but not chloride.

Experimental

General Procedures

High-performance liquid chromatography (HPLC) was performed using a Varian 9012Q pump and a Varian 9050 detector (λ=230 nm). Data processing was performed with the use of a Varian Star 4.5 program. The columns and stationary phases used were as follows: Microsorb-MV™ of C18-type (4.6x250 mm; Rainin Instrument Co., Inc.); Kromasil™ CHI-TBB (4.6x250 mm; EKA Chemicals Co.); Chiralcel OD (4.6x250 mm; Daicel Chemical Industries, Ltd.) and BSA (4.6x50 + 4.6x150 mm) [32]. Straight phase chromatography was run with a flow of 1.5 ml/min and reversed phase chromatography with 1.2 ml/min. Gas chromatography was operated with 2 ml H_2/min and with injector and detector temperatures of 150°C and 200°C, respectively. The columns used were a 24 m (1m for compound **3**) *oktakis*-(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E) column (Macherey-Nagel GmbH) and a 10 m *heptakis*-(6-*O*-*tert*-butyl-dimethylsilyl-2,3-*O*-acetyl)-β-cyclodextrin column (both obtained from Professor W. König, University of Hamburg). Optical rotation was measured by use of a Perkin Elmer Polarimeter 341LC and melting points were obtained with a Büchi Melting Point B-545 instrument. Additional equipment has been described previously [15,16]. To be noted, the septum in

the lid covering the reaction tube during VBrPO oxidation had to be covered with an inert teflon surface to prevent diffusion of volatile sulfides, e.g. **6**.

VBrPO was obtained from Exeter University, UK (see acknowledgement) and for further details regarding preparation and activity measurements, together with determination of hydrogen peroxide concentration, see reference [17].The preparation of 2H-naphtho[1,8-*b*,*c*]thiophene-2-one [33], **8** [34], **11** [34], **13-14** [35,36], **15** [37] and the sulfoxides corresponding to **8** [34] and **11** [34], **15-16** [37] have been performed previously. Methyl iodide, diethylene glycol diethyl ether, **4-7** and **18** were obtained from Lancaster. *m*-Chloroperbenzoic acid (MCPBA) and **17** were from Fluka and *m*- and *p*-thiosalicylic acid were from Toronto Research Chemicals, Inc. Aldrich was the supplier of boron trifluoride-diethyl ether complex, sodium borohydride, hydrogen peroxide, peracetic acid and N-nitroso-N-methyl-*p*-toluenesulfonamide whereas sodium metaperiodate and aluminium oxide were obtained from Merck. Chloroform-*d* was from Dr. Glaser AG and other solvents were of analytical or HPLC grade.

Synthetic Procedures

*2H-Naphtho[1,8-*b,c*]thiophene, 3:* Reduction of 2*H*-naphtho[1,8-*b*,*c*]thiophene-2-one was carried out according to the literature [38]. Pale pink crystals, showing a m.p. of 85.7-87.2°C (lit.: 83-85°C [38]), were obtained in 37% yield. The purity was 99.5% according to GC-analysis. NMR: $\delta(CDCl_3)$: 4.77 (2H, s), 7.24 (1H, d), 7.32 (1H, t), 7.34 (1H, d), 7.41 (1H, d), 7.43 (1H, t), 7.59 (1H, d).

(Z)-3-Ethylthio-2-butenoic acid, 14: This compound was found to contain 8% of related (*E*)-isomer and the white crystals of **14** were recrystallized twice in aqueous ethanol [35]; however without improvement of the purity. M.p. 111-111.5°C (lit: 90-91°C ((*E*)-isomer) and 114-115°C (**14**) [35]). NMR of **14**: $\delta(CDCl_3)$: 1.33 (3H, t), 2.27 (3H, s), 2.60 (2H, q), 5.82 (1H, s); and of the (*E*)-isomer: $\delta(CDCl_3)$: 1.34 (3H, t), 2.39 (3H, s), 2.83 (2H, q), 5.52 (1H, s).

m- and p-Methylthiobenzoic acid: **9** and **10** were prepared via alkylation of thiosalicylic acid with methyl iodide [34]. The melting points of **9** and **10** were 122-123°C (lit.: 126-127°C) and 192-193°C (lit.: 191-192°C), respectively [39]. NMR of **9**: $\delta(CDCl_3)$: 2.55 (3H, s), 7.39 (1H, t), 7.49 (1H, d), 7.89 (1H, d), 7.98 (1H, s); and of **10**: $\delta(CDCl_3)$: 2.55 (3H, s), 7.28 (2H, d), 8.05 (2H, d).

Methyl esters: Compounds **12** and **16** were obtained from the corresponding acids by reaction with diazomethane. The m.p. of **12** was 65-66°C (lit.: 63-65°C [40]). NMR of **16** (oil) [41]: $\delta(CDCl_3)$: 2.56 (2H, t), 2.69 (2H, t), 3.68 (3H, s), 3.74 (2H, s), 7.2-7.4 (5H, m).

Sulfoxides: Racemic sulfoxides were prepared as reference materials for the chromatographic evaluation. Compounds **4-7** and **17** were all oxidized by means of sodium periodate (1.1 equiv.) in aqueous ethanol. Peracetic acid (0.9 equiv.) was used for the oxidation of **9**, **10**, **13** and **14** with acetic acid as solvent. The resulting sulfoxides, except the oxide of **14** (oil), were recrystallized from acetic acid. The oxides of **13** and **14** were further purified by flash-chromatography on silica gel with 0.3% formic acid in acetone as the mobile phase. The corresponding oxide of **12** [34] was obtained by esterification of corresponding acid by means of diazomethane. Compound **3** was oxidized by means of MCPBA according to the literature [38] in 50% yield and 95% purity (GC). M.p.:114.6-115.8°C (lit.: 113-115°C [38]).

Enzymatic oxidation

Standard solutions of sulfides were prepared by dissolving the sulfide in the required volume of alcohol, or when possible in phosphate buffer directly. The only exception was **13** which was added as a solid due to insufficient solubility. Sulfide (5 μmol) was mixed with one unit of VBrPO (stock solution [17]) and 50 mM phosphate buffer pH 6.5 to a final volume of 560 μl and the mixture was stirred and thermostated to 25°C. Hydrogen peroxide (0.137 M in phosphate buffer) was added continuously by an autoinjector. The final reaction mixture after the addition of 1.1 equiv. of hydrogen peroxide (i.e. after 16 h.) had a total volume of 600 μl. Saturated sodium sulfite solution was added (0.2 ml) to reduce any remaining hydrogen peroxide and the solution was extracted with 3x1 ml of dichloromethane. The organic phase was dried ($MgSO_4$), filtered, and then analyzed by gas chromatography or evaporated prior to analysis by straight phase liquid chromatography. When reversed phase liquid chromatography was required for the determination of yield, due to incomplete transfer of the analyte to the organic phase, a small aliquot was taken from the reaction mixture prior to extraction.

When the substrate contained a carboxyl group, addition of saturated sodium sulfite was followed by addition of 2 drops of 6 M sulfuric acid to facilitate the extraction.

The buffers used in the study of the pH-dependence were all 50 mM and based on phosphate (pH 6.5-8.0), citrate (pH 5.0) or a mixture of the two (pH 6.0). The initial rates were determined by carrying out the experiments as described, including the addition rate of hydrogen peroxide, but by quenching the reactions at either 0.5, 1, 2 or 4 h.

The influence of co-solvents and halides on the VBrPO-catalyzed reaction was studied using buffers and hydrogen peroxide solutions with the appropriate alcohol or halide concentrations and after adjustment of the pH.

An estimation of background oxidation in the absence of enzyme, using the same reaction conditions and time (16 h), was performed in most cases.

The conditions used for the CPO-catalyzed oxidations, carried out for comparison, were as described previously [15,16].

Analytical procedures

Chiral gas and liquid chromatography were utilized to determine both chemical and optical yield with high accuracy using the synthetic sulfoxide racemates as references. Relative response factors between sulfides and sulfoxides were determined. Control extractions were performed to ascertain the full recovery of sulfides and sulfoxides from the aqueous phase. It was found that the acid functionalized compounds, except **11** and **15** and their oxidation products, were not fully recovered from the aqueous phase and therefore the yield of these protolytic sulfoxides was determined by reversed phase liquid chromatography prior to extraction.

GC analyses of product formation from substrates **3-4** and **6-7** were performed with the γ-cyclodextrin column, on which **7** was only partially separated, however. The reactions involving compounds **5**, **12** and **17** were analyzed using the β-cyclodextrin column.

Reversed phase liquid chromatography was performed on the C18 column and the product yields during oxidation of **8-10** and **14** were determined with a mobile phase consisting of 8% acetonitrile in 20 mM phosphate buffer, pH 6.8-7.1. A mobile phase gradient from 20 mM phosphate buffer, pH 6.9, to 15% acetonitrile in 20 mM phosphate buffer, pH 7.3, was necessary for analysis of **13**.

Determination of e.e. of the sulfoxides resulting from the oxidation of **8**, **11**, **13**, **14** and **18** was performed on the Kromasil CHI-TBB phase with a mobile phase consisting of 0.05% of formic acid in cyclohexane containing 70% or 50% of *tert*-butyl methyl ether for **8** and **11**, respectively, and 6% of 2-propanol for **13** and **14**. The (-)-sulfoxide obtained in excess from these four acids ((*S*)-configuration in the case of **8** and **11** [34]) was consistently the second eluting enantiomer from this column. The mobile phase used for analysis of the reaction with **18** consisted of 4% 2-propanol in cyclohexane. Baseline separation of the sulfoxide enantiomers corresponding to **9** and **10** could not be obtained on the columns available and therefore the product mixtures were treated with diazomethane. The resulting methyl esters of the sulfoxides were then analyzed by gas chromatography on the β-cyclodextrin column, which gave baseline resolution of the enantiomers. The Chiralcel OD column was used with 7% of 2-propanol in hexane as the mobile phase to determine both chemical and optical yield from the oxidation of **16**. The chemical yield from the oxidation of **15** was determined with a mobile phase composed of 20% of 2-propanol and 0.05% of formic acid in hexane, whereas a BSA column and a mobile phase consisting of 20 mM phosphate buffer, pH 5.2, was required for the separation of the sulfoxide enantiomers and determination of the e.e.

Determination of absolute configuration

The absolute configuration of the enantiomer obtained in excess when **5** was oxidized by VBrPO, was determined from the chromatographic elution order found, using the (-)-(*R*)-sulfoxide (prepared with CPO as the catalyst [13]) as a reference. The elution order of the enantiomers of the sulfoxides corresponding to **8** and **11** has been determined previously [23]. A negative optical rotation at 589 nm (ethanol) was observed for both of the oxidation products of **13** and **14**.

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