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Probing the scope of the sulfoxidation activity of vanadium bromoperoxidase from *Ascophyllum nodosum*

Hilda B. ten Brink,[a] Herbert L. Holland,[b] Hans E. Schoemaker,[c] Hester van Lingen [a] and Ron Wever [a,*]

[a] *E.C. Slater Institute, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands*
[b] *Department of Chemistry, Brock University, St. Catharines, Ontario, L2S 3A1, Canada*
[c] *DSM Research, Bio-Organic Chemistry, PO Box 18, 6160 MD Geleen, The Netherlands*

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Abstract

It has been shown previously that the vanadium bromoperoxidase from *Ascophyllum nodosum* mediates the production of (*R*)-methyl phenyl sulfoxide with 91% enantiomeric excess from the corresponding sulfide in the presence of hydrogen peroxide. Investigation of the sulfoxidation activity of this enzyme shows that activating substituents at the *para*-position of the aromatic ring of methyl phenyl sulfide positively influence the selectivity of the reaction, whereas strongly electron-withdrawing groups cancel the catalyzed sulfoxidation reaction. The first evidence is presented that the vanadium bromoperoxidase catalyzes the sulfoxidation of racemic non-aromatic cyclic thioethers with high kinetic resolution.

1. Introduction

Several different catalytic methods for the production of chiral sulfoxides have been studied in great detail over the last two decades due to the growing demand for these chiral auxiliaries in asymmetric synthesis, in particular for the preparation of biologically active compounds.[1,2] In addition to chemically based reactions[3,4] great interest has developed for both whole-cell[5–9] and enzymatic[10–19] approaches, as more satisfying results are obtained using biological procedures. A great variety of organic sulfides have been converted to their corresponding sulfoxides using fungi, including *Helminthosporium* species NRRL 4671 and *Mortierella isabellina*, and moderate to high yields and enantiomeric purities were obtained.[5–9] In addition, the conversion of prochiral organic sulfides to the corresponding chiral sulfoxides has been shown to be catalyzed by various isolated enzymes,[10–13] including a large number of different heme peroxidases,[14–17] showing in general rather low enantioselectivities and turnovers. The

* Corresponding author. Tel: +31-20-525-5110; fax: +31-20-525-5124; e-mail: a311rw@chem.uva.nl

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heme chloroperoxidase from *Caldariomyces fumago* is an exception as this enzyme was shown to be capable of catalyzing oxygen transfer reactions with very high enantioselectivity and versatility.[18,19] However, the potential application of this class of peroxidases in asymmetric organic synthesis is hindered, primarily due to the gradual oxidative inactivation of these enzymes by their natural co-substrate, hydrogen peroxide, during catalysis.[20,21]

Recently, it was shown that another class of peroxidases, which contain a vanadate ion in the active site as the prosthetic group, is capable of selectively mediating the transformation of aromatic organic sulfides to the corresponding sulfoxides.[22,23] Previously, these vanadium haloperoxidases were thought to oxidize only halides (X^-) in the presence of hydrogen peroxide yielding hypohalous acid (HOX), a highly reactive intermediate, which in turn reacts with an electrophilic acceptor, if present, to form a halogenated compound. In contrast to the high selectivity of the sulfoxidation reactions catalyzed by these enzymes, mediation of selective halogenation reactions has not been established for these enzymes except for one observation of regioselective bromohydration of certain cinnamyl substrates.[24] Moreover, as expected, the presence of halides cancels the selectivity of the sulfoxidation reactions entirely.[22,25]

The vanadium haloperoxidases are named according to their oxidizing ability; e.g. bromoperoxidases are capable of oxidizing both iodide and bromide, whereas chloroperoxidases can also oxidize chloride. The vanadium bromoperoxidases originate primarily from seaweeds[26] and the vanadium chloroperoxidases are mainly found in terrestrial fungi.[27] One fundamental feature shared by this group of enzymes is their high stability, as the vanadium haloperoxidases were shown to remain fully active in the presence of organic solvents and high concentrations of aggressive oxidants, in addition to their stability towards elevated temperatures.[28–31]

In previous studies it has been demonstrated that vanadium bromoperoxidases are capable of catalyzing the sulfoxidation of small organic sulfides with very high enantioselectivity.[22,23,25] The bromoperoxidase from the brown seaweed *Ascophyllum nodosum* produces the (*R*)-enantiomer of methyl phenyl sulfoxide (91% ee) under slightly acidic conditions, while the bromoperoxidase from the red seaweeds *Corallina pilulifera* produces the (*S*)-enantiomer of this sulfoxide (55% ee).[22] The bromoperoxidase from the red seaweed *Corallina officinalis* was observed to convert organic sulfides structurally resembling indenes to the (*S*)-enantiomer of the corresponding sulfoxides showing high selectivities (91% ee).[23] In addition, it has been reported that small sulfides, possessing a *cis*-positioned carboxyl group with respect to the sulfur atom, are rapidly converted to the corresponding sulfoxide with selectivities exceeding 95% ee in the presence of vanadium bromoperoxidase from *C. officinalis*.[25] Remarkably, methyl phenyl sulfide and many derivatives resembling this sulfide are hardly oxidized by this enzyme.[23,25]

Presumably, the high selectivity of the reaction is due to the direct transfer of oxygen from the peroxide bound to the vanadium in the active site to the organic sulfide. X-Ray crystallography data have shown for the vanadium chloroperoxidase from the fungus *Curvularia inaequalis* that such a peroxo-enzyme intermediate, with the peroxide coordinated side-on to the vanadium, is formed during halide oxidation catalysis.[32] The sulfide might bind near or in the active site to facilitate the oxygen transfer from the bound peroxide to the substrate. The presence of a binding site for organic substrates in the bromoperoxidase from *A. nodosum* was suggested previously.[33]

The sulfoxidation activity of vanadium bromoperoxidase from *A. nodosum* in enantioselective sulfoxidation reactions is described here, and the electronic influence of *para*-substituents on the aromatic ring of methyl phenyl sulfide on the conversion and selectivity of the enzymatic oxidation is examined. In addition, the ability of this enzyme to oxidize a broad range of sulfides of various size and substitution patterns was probed. It is shown for the first time that the vanadium bromoperoxidase from *A. nodosum* mediates the sulfoxidation of racemic non-aromatic cyclic thioethers with high kinetic resolution.

2. Results and discussion

2.1. Electronic effects

Substrate specificity in the sulfoxidation catalyzed by vanadium bromoperoxidase from the brown seaweed *A. nodosum* was studied using several organic sulfides in the presence of hydrogen peroxide. First we investigated the electronic effects on vanadium bromoperoxidase sulfoxidation activity using *para*-substituted phenyl methyl sulfides. The conversion, enantiomeric excess (ee) and configuration of the resulting sulfoxides are reported in Table 1. The incubation period lasted for 3 days in order to assure sufficient conversion to be able to analyze the products by NMR. A relatively low enzyme concentration had to be used, compared to the heme peroxidase mediated sulfoxidations, as the quantity of enzyme isolated and purified from the seaweed is rather low at present.[34,35] Attempts are being made to develop a recombinant expression system, as has been successfully done for the vanadium chloroperoxidase from the fungus *C. inaequalis*.[36]

Table 1
Sulfoxidation of *para*-substituted methyl phenyl sulfides by vanadium bromoperoxidase from *A. nodosum* at pH 5.3

substituent		conversion[a] (%)	ee (%)	configuration
NH_2	**1a**	51 (38)	89	*R*
OCH_3[b]	**b**	n.d.	78	*R*
CH_3[b]	**c**	18 (n.d.)	82	*R*
H	**d**	71 (93)	76	*R*
Br	**e**	21 (14)	47	*R*
Cl	**f**	97 (35)	54	*R*
F	**g**	81 (99)	54	*R*
CN	**h**	> 1 (<1)	n.d.	-
NO_2	**i**	7 (3)	racemic	-

a. Conversion is defined as the relative amount of sulfoxide produced from the available sulfide
b. Results obtained in a previous study[21]
n.d. Not determined
The values in parenthesis are the conversions obtained in the absence of enzyme

Evidently, activating substituents on the *para*-position of the aromatic ring, such as -NH_2 or -OCH_3, result in an increase in the selectivity of the sulfoxidation of the phenyl methyl sulfide from 76% ee for **1d** to up to 89% ee for **1a**. By contrast, strong electron withdrawing groups, such as -CN **1h** and -NO_2 **1i** have a dramatic effect on the selectivity and conversion of the reaction. Sulfides containing these substituents are found to be hardly converted and the product, if detected, was found to be racemic.

A similar tendency was found when lactoperoxidase was used in the conversion of these sulfides.[37] In contrast, the results obtained using the heme chloroperoxidase[18,19] and horseradish peroxidase[38] in these sulfoxidations show that the enantioselectivity of these enzymes is not influenced by electronic effects. However, these results should be interpreted with care. When the conversion and enantiomeric excesses are determined for the sulfoxide produced by heme peroxidases, the yields of the racemic product formed in the non-enzymatic sulfoxidation reactions are subtracted from the yields of the enzymatic product. Consequently, the reported enantioselectivities in the sulfoxidations catalyzed by horseradish peroxidase are artificially high, and cannot be compared with the selectivities found in this and previous studies on vanadium peroxidase mediated sulfoxidation.[22,23,25] In addition, early reports on the sulfoxidation activity of the heme chloroperoxidase using *tert*-butyl hydroperoxide show *para*-substitution dependency similar to the results obtained in this contribution.[14]

Table 1 shows that the conversion observed after 3 days of incubation for the various substrates is

almost similar in the presence or absence of enzyme. The latter non-enzymatic process produces a racemic mixture. However, the relative contribution of the two processes to the determined conversion and selectivity of the reaction is difficult to assess. For the sulfoxidation reaction in the presence of enzyme the initial rate of conversion in the non-enzymatic reaction may closely parallel that of the enzyme-catalyzed reaction, however as the substrates are consumed, the contribution of the enzymatic reaction becomes more important.[22] Therefore, higher selectivities are obtained when sulfides are used, which are more readily oxidizable for the enzyme, such as **1a** to **1d**. In the absence of enzyme, only the non-enzymatic sulfoxidation reaction consumes the substrates and determines the rate of conversion. Correction of the determined selectivity by the contribution of the non-enzymatic reaction would lead to extremely high and unlikely selectivities for the enzyme-catalyzed reaction.

For **1d** and **1g** it is observed that the conversion of the non-enzymatic reaction exceeds that of the enzymatic one. It is difficult to explain why a higher conversion is found in the non-enzymatic sulfoxidation of methyl phenyl sulfide than in the enzymatic reaction. It is possible that hydrogen peroxide is gradually non-productively consumed in the presence of the vanadium enzyme due to impurities present in the enzyme preparation, as has been seen before.[22] As a consequence of this hydrogen peroxide consumption the conversion of the sulfide does not reach completion in the enzymatic reaction.

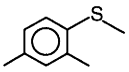
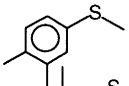
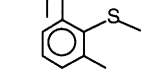
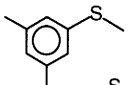
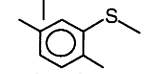
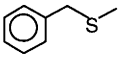
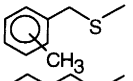
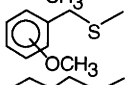
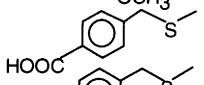
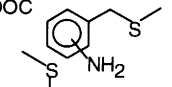
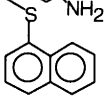
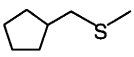
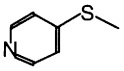
From Table 1 it can also be inferred that the apparent selectivity of the vanadium bromoperoxidase mediated sulfoxidation of methyl phenyl sulfide **1d** is only moderate, 76% ee, compared to the results of previous studies where the enzyme converted the sulfide with 91% ee.[22] The low selectivity of the reaction is attributed to a larger contribution of the non-enzymatic sulfoxidation reaction under the reaction conditions used in the present study. Previous studies[22,23,25] show that vanadium bromoperoxidases exhibit high enantioselectivity, however the enantioselectivity and versatility of the heme chloroperoxidase from *C. fumago* in the biotransformation of sulfides is still better.[18,19] We believe that the difference in enantioselectivity between the two enzymes is due to the low turnover frequency, which is only 1 min^{-1} for the vanadium bromoperoxidase[21] and at least 10^3 min^{-1} for heme chloroperoxidase.[19,20,39] As a result of the long incubation times required to convert the sulfide into the sulfoxide the non-enzymatic reaction has a relatively large contribution and results, as a consequence, in a decreased selectivity of the vanadium bromoperoxidase formed methyl phenyl sulfoxide. Other heme peroxidases, including horseradish peroxidase and lactoperoxidase, are observed to catalyze the conversion of methyl phenyl sulfide with lower selectivity, at best 80% ee with lactoperoxidase.[21] The lower selectivity is also due to oxidative inactivation which also greatly suppresses the turnover frequency during catalysis.[21,37,40] Further investigations are being conducted in order to optimize the sulfoxidation activity of the vanadium bromoperoxidase from the brown seaweed.

2.2. Substrate screening

Several substituted methyl phenyl sulfides, benzyl methyl sulfides and non-aromatic organic sulfides were used as substrates in the sulfoxidation reaction catalyzed by vanadium bromoperoxidase in order to investigate the scope of the vanadium enzyme from the brown seaweed. Some results are presented in Table 2. The reactions were conducted at room temperature over a long reaction time (3 days) due to low enzyme availability in order to ensure sufficient conversion of all substrates for ^{1}H NMR analysis. Hydrogen peroxide was added in five sequential steps of 12 h apart during the reaction to decrease the non-stereoselective contribution of the non-enzymatic reaction and to obtain higher selectivity.

From Table 2 it can be inferred that substitution at the *para*-position of the aromatic ring is important for controlling selectivity for both the dimethyl substituted phenyl methyl sulfides and the substituted

Table 2
Sulfoxidation of several organic sulfides catalyzed by vanadium bromoperoxidase from *A. nodosum* at pH 5.3

sulfide		conversion[a] (%)	ee (%)	configuration
	**2**	98 (>99)	63	*R*
	**3**	50 (51)	81	*R*
	**4**	90 (84)	46	*R*
	**5**	83 (25)	11	*R*
	**6**	31 (56)	29	*R*
	**7**	90 (83)	6	*R*
	**8a**	*ortho* 87 (>99)	-	racemic
	b	*para* 97 (98)	27	*R*
	**9a**	*ortho* 23 (41)	18	*R*
	b	*para* 82 (38)	16	*R*
	**10**	10 (28)	60	*R*
	**11a**	*ortho* 49 (76)	14	*R*
	b	*meta* 15 (58)	66	*R*
	c	*para* 83 (86)	9	*R*
	**12**	6 (3)	25	*R*
	**13**	49 (23)[b]	27	*R*
	**14**	26 (10)	69	*R*

a. Conversion is defined as the relative amount of sulfoxide produced from the available sulfide
b. In addition 39% sulfone was formed
n.d. Not determined

benzyl methyl sulfides. *para*-Substituted dimethyl phenyl methyl sulfides **2** and **3** were observed to be converted with nearly similar or higher selectivity [62% ee and 81% ee (*R*), respectively], than the unsubstituted phenyl methyl sulfide **1d** [76% ee (*R*)] under identical reaction conditions.

Unsubstituted benzyl methyl sulfoxide **7** is produced by the vanadium enzyme with very low enantioselectivity [6% ee (*R*)]. However, introducing a substituent on the aromatic ring results in a more selective conversion. Thus, introduction of activating groups such as -OCH_3 and -CH_3 at the *para*-position of benzyl methyl sulfide slightly increases the selectivity of the sulfoxidation reaction [16% ee and 27% ee (*R*), respectively], and the presence of a carboxyl group elevates the selectivity to 60% ee (*R*). No trend can be detected for the benzyl methyl sulfides concerning the electronic influence of the *para*-substituents. An amino substituent at the *ortho*- or *para*-position, **11a** and **11c**, hardly increases the selectivity of the conversion compared with the unsubstituted substrate, but substitution at the *meta*-position results in a dramatic increase in stereoselectivity [66% ee (*R*)]. The presence of a nitro group,

a strong electron-withdrawing group, on the aromatic ring was found to cancel the selectivity of the reaction completely (results not shown).

The majority of aromatic sulfides where the distance between the aromatic group and the sulfur in aromatic sulfides is greater than two carbon atoms or those bearing an alkyl group other than -CH_3 could not be converted by the vanadium bromoperoxidase, indicating that sulfides exceeding benzyl methyl sulfides in size are not converted by the enzyme. In contrast, the vanadium bromoperoxidase is capable of catalyzing larger sulfides, which are sterically more constrained with respect to the enzyme than **1d**, as the catalyzed sulfoxidation of the naphthalene derivative **12** resulted in a small amount (6%) of the corresponding sulfoxide with 25% ee (*R*).

The enzyme also selectively oxidizes small cyclic non-aromatic sulfides, bearing a cyclohexyl or a cyclopentyl group (**13**), to the corresponding sulfoxides with enantioselectivities ranging from approximately 10% to 27% ee (*R*). With the exception of cyclopentylmethyl methyl sulfide (conversion yields 39% of sulfone in both the catalyzed and non-enzymatic reaction) all the organic sulfides studied were only oxidized to the corresponding sulfoxide.

In previous studies[24] it has been shown that a carboxyl group at the *ortho*-position of several aromatic and at the *cis*-position, with respect to the sulfur atom, of non-aromatic sulfides is a prerequisite for selectivity in the biotransformations mediated by vanadium bromoperoxidase from *C. officinalis*. However, the vanadium enzyme from *A. nodosum* does not show such a strong preference. Moreover, substituents at the *ortho*-position of aromatic sulfides do not greatly affect the selectivity of the reaction (Table 2). We believe that for the latter, vanadium bromoperoxidase substituents at the *meta*-position are more relevant for selectivity as can be seen from the results of the sulfoxidation of sulfide **3** and **11b**. However, more experiments with *meta*-substituted aromatic sulfides have to be performed to prove this assumption.

Evidently, our data suggest that the vanadium bromoperoxidase from the brown seaweed exhibits a more versatile substrate requirement than the enzyme from the red seaweed *C. officinalis*, as it is not restricted to carboxyl substituted sulfides. However, the selectivities of the transformations are still relatively low due to the reaction conditions. Optimization of the sulfoxidation reactions by continuous addition of low concentrations of hydrogen peroxide,[21,41] elevation of the reaction temperature and reduction of the incubation period by increasing the enzyme concentration, may result in higher enantioselectivities and conversions.

2.3. Kinetic resolution

In the oxidation of racemic sulfides **15** and **16**, containing either a five-member or six-member cyclic ether group, respectively, by the bromoperoxidase from *A. nodosum* to the corresponding sulfoxide, it was observed that the enzyme exhibits a clear preference towards one of the stereoisomers of **15** and **16**. The results are depicted in Fig. 1. As two separate selective processes occur simultaneously in the sulfoxidation catalyzed by the vanadium bromoperoxidase, both kinetic resolution and asymmetric catalysis, it is difficult to define the selectivity of the reaction. Therefore the excess of one isomer over another is defined in terms of ratios. In the sulfoxidation of racemic sulfide **15** by vanadium bromoperoxidase it was found that the stereoisomers of this sulfide were converted with a ratio of approximately 98 to 2, which means that predominantly one stereoisomer is converted to the corresponding sulfoxide. A less distinct preference was observed in the enzymatic sulfoxidation of racemic sulfide **16**, as a ratio of approximately 10 to 1 was found for the conversion of the two stereoisomers of this sulfide. From the results presented in Fig. 1, E values of approximately 10 and 30 (for the conversion of **16** and **15**, respectively) can be

calculated.[44,45] At present the configuration favored by the vanadium bromoperoxidase has not been determined.

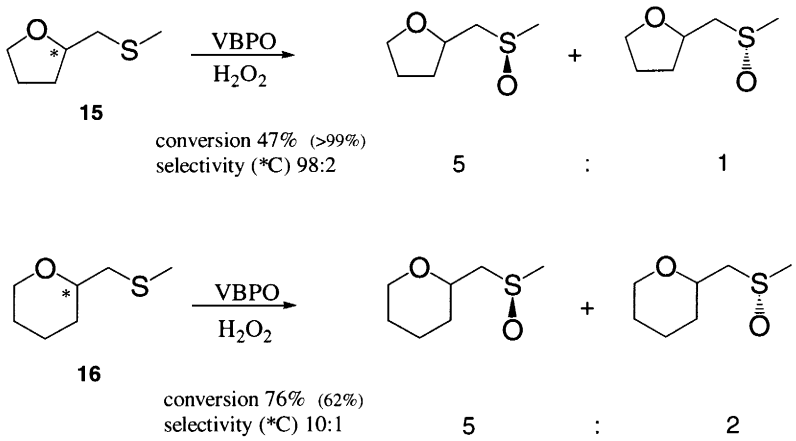


Fig. 1. Sulfoxidation of racemic non-aromatic cyclic thioethers by vanadium bromoperoxidase from *A. nodosum* at pH 5.3. The values in parentheses are the conversions obtained in the absence of enzyme

The sulfoxides produced from the racemic sulfides **15** and **16** consist of two pairs of diastereoisomers (in $CDCl_3$, 300 MHz, **15**: δ (ppm)=2.66 and 2.70 (3H, CH_3) and **16**: δ (ppm)=2.61 and 2.65 (3H, CH_3)), which can be separately observed by NMR analysis. Upon addition of (*S*)-(+)-α-methoxyphenyl acetic acid[43] all four isomers can be observed (in $CDCl_3$, 300 MHz, **15**: δ (ppm)=2.667, 2.684, 2.693 and 2.703 (3H, CH_3) and **16**: δ (ppm)=2.630, 2.650, 2.680 and 2.692 (3H, CH_3)). For comparison the sulfoxidation reactions were also conducted in the absence of enzyme yielding racemic mixtures of the products, as expected. NMR analysis of the products formed in the enzymatic and non-enzymatic reaction did not indicate overoxidation to sulfone. The ratio between the major diastereoisomers of the sulfoxide formed in the conversion of racemic sulfide **15** was found to be 5 to 1. Presumably, the major product formed in this enzymatic conversion is the (*R*)-enantiomer of the sulfoxide corresponding to the favored isomer of racemic sulfide **15** (Fig. 1). The ratio between the products formed in the enzymatic sulfoxidation of the less favored isomer of **15** could not be determined. Similar results were obtained for the enzyme-catalyzed sulfoxidation of **16** yielding a slightly lower selectivity for the formation of the major product, as the ratio between the major diastereoisomers of the sulfoxide produced was determined to be 5 to 2. The absolute configurations of the major products formed in the enzymatic sulfoxidation of sulfides **15** and **16** are probably identical, however the absolute configurations still have to be elucidated.

Nevertheless, these results establish that vanadium peroxidases not only catalyze sulfoxidation reactions through asymmetric catalysis starting with a prochiral substrate as has been shown before,[22,23,25] but also distinguish the most suitable substrate from a mixture of isomers in the sulfoxidation reaction. Therefore these vanadium enzymes are also capable of mediating sulfoxidation reactions based upon kinetic resolution with high selectivity. This feature of the vanadium peroxidases has not been reported before.

Surprisingly, the VBPO from *A. nodosum* is the only asymmetric vanadium-dependent catalyst converting organic sulfides bearing a phenyl group to the (*R*)-enantiomer of the corresponding sulfoxide at present. Several chiral Schiff-base ligated vanadium(V) peroxo-complexes have been shown to mediate the formation of the (*S*)-sulfoxides from (substituted) methyl phenyl sulfide with moderate selectivities of up to 78% ee.[46,47] In addition, the VBPOs isolated from the red seaweeds *C. pilulifera* and *C. officinalis* were observed to catalyze the production of the (*S*)-sulfoxide from methyl phenyl sulfide derivatives.[22,25]

Unfortunately it is not yet possible to compare the active sites of the VBPOs, because there are no X-ray structures available of these enzymes, although preliminary X-ray analysis has been reported several years ago for both the VBPO from *A. nodosum* and *C. officinalis*.[48,49] We believe, however, that the environment of the active site of these enzymes will differ substantially. In spite of the primary sequence analogy between the vanadium haloperoxidases in the active site,[36] the presence or absence of a particular amino acid in the active site may determine the sulfoxidation activity of the enzyme. This has been shown for horseradish peroxidase, because mutating phenylalanine 41 to a leucine was observed to remarkably increase both the rate and enantioselectivity of the reaction, while opposite effects were obtained by mutating to threonine.[50] The development of a recombinant expression system for the VBPO from *A. nodosum* will allow us to produce various vanadium enzymes by site-directed mutagenesis in order to study the involvement of the active site amino acids on the sulfoxidation activity and eventually create new enantioselective enzymes.

3. Experimental

3.1. General procedures

The following sulfides were purchased from Sigma Aldrich; 4-aminophenyl methyl sulfide **1a**, methyl *p*-methoxy phenyl sulfide **1b**, methyl *p*-tolyl sulfide **1c** and methyl phenyl sulfide **1d**. Hydrogen peroxide was purchased from Merck and the concentration was determined to be 9.2 M by spectrophotometric analysis at 240 nm. The synthesis and characterization of sulfides **1e**–**1i** and **2**–**14** and the corresponding sulfoxides have been performed at Brock University in the group of Prof. H. Holland and have been reported previously.[7,8,51–53] Substrates **15** and **16** were prepared by reaction of the corresponding halides with sodium thiomethylate as previously described.[7,51,52] NMR spectra were recorded at 300 MHz (routine ^{1}H) with a Bruker AC300 using $CDCl_3$ as solvent. The vanadium bromoperoxidase was isolated from the brown seaweed *A. nodosum* and purified as described before.[21,34,35]

3.2. Enzymatic sulfoxidation

The sulfoxidation of different organic sulfides was carried out using the following reaction conditions: in 10 ml of 100 mM sodium citrate/NaOH buffer, pH 5.3, the organic sulfide (2.5 mM) was incubated with hydrogen peroxide (final concentration 2.5 mM) and vanadium bromoperoxidase from *A. nodosum* (1 μM) for 3 days at room temperature. The sulfides were added dissolved in methanol (100 mM stock solution) and therefore the reaction mixtures contained 2.5% methanol, however at this concentration this does not influence the sulfoxidation reaction. The hydrogen peroxide was added in five equal sequential steps (of 0.5 mM) with approximately 12 h between each addition during the reaction in order to minimize the contribution of the non-enzymatic reaction between the sulfide and hydrogen peroxide. The non-enzymatic reaction was carried out under the same conditions.

After three days the samples were quenched with 2 ml of a saturated sodium sulfite solution and extracted with 2×25 ml of dichloromethane. The organic fractions were pooled, evaporated and dissolved in approximately 1 ml of $CDCl_3$. The conversion and enantiomeric excess of the enzymatic and non-enzymatic reactions were determined by NMR analysis at 300 MHz. The conversion of the reaction was calculated from the integrals of the remaining sulfide and produced sulfoxide. Enantiomeric ratios were determined in the presence of either (*S*)-(+)-α-methoxyphenylacetic acid (MPAA reagent) or (*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine (Kagan reagent).[42,43]

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