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Enzyme-catalysed oxidation of 1,2-disulfides to yield chiral thiosulfinate, sulfoxide and *cis*-dihydrodiol metabolites

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Enantioenriched and enantiopure thiosulfinates were obtained by asymmetric sulfoxidation of cyclic 1,2-disulfides, using chemical and enzymatic (peroxidase, monooxygenase, dioxygenase) oxidation methods and chiral stationary phase HPLC resolution of racemic thiosulfinates. Enantiomeric excess values, absolute configurations and configurational stabilities of chiral thiosulfinates were determined. Methyl phenyl sulfoxide, benzo[*c*]thiophene *cis*-4,5-dihydrodiol and 1,3-dihydrobenzo[*c*]thiophene derivatives were among unexpected types of metabolites isolated, when acyclic and cyclic 1,2-disulfide substrates were used as substrates for *Pseudomonas putida* strains. Possible biosynthetic pathways are presented for the production of metabolites from 1,4-dihydrobenzo-2,3-dithiane, including a novel *cis*-dihydrodiol metabolite that was also derived from benzo[*c*]thiophene and 1,3-dihydrobenzo[*c*]thiophene.

Introduction

The enzyme-catalysed oxidation of achiral sulfides, to yield chiral sulfoxides, has been of interest for many years, as sulfoxidation occurs readily during drug metabolism and as it can provide an alternative route to enantiopure chiral building blocks of value in chemical synthesis and the pharmaceutical industry. Enzymes involved in chiral sulfoxidations include peroxidases, peroxygenases, monooxygenases, and dioxygenases.[1a-i] The asymmetric sulfoxidation of monosulfide substrates has been extensively studied, as most of the resulting sulfoxide metabolites are both thermally and configurationally stable compounds, *i.e.* having a relatively high racemization barrier. Sulfoxides derived from thiophenes, [2a-c] and 1,2-sulfides[3a-c] are, however, generally less stable and have much lower barriers to racemization.

The value of arene dioxygenases, as biocatalysts for the synthesis of chiral sulfoxides, has been reported.[2c,4a-g,5a-c] The advantages of these enzymes include their abilities to: (i) catalyse sulfoxidation of a wide range of alkylaryl sulfides, (ii) produce either enantiomer in a stereoselective manner and (iii) decrease the possibility of further oxidation to sulfones. While dioxygenase-catalysed sulfoxidation is the preferred metabolic step for alkylaryl sulfides, competition from benzylic hydroxylation and arene *cis*-dihydroxylation may occur, particularly when using benzyl-substituted dialkyl sulfides.[5a]

In Scheme 1a is shown alternative sulfoxide enantiomers ($\mathbf{2}_R$ and $\mathbf{2}_S$) being produced by enantioselective toluene dioxygenase (TDO)- and naphthalene dioxygenase (NDO)-catalysed oxidations of methylphenyl sulfide **1**, without further oxidation to sulfone.[4d] Dioxygenase-catalysed sulfoxidation of alkylaryl sulfides occurs much faster than dialkyl sulfides, while the sulfoxidation of dialkyl sulfides using NDO is faster than TDO.

The differences in regio- and stereo-selectivity, observed during TDO- and NDO-catalysed sulfoxidation of an alkylaryl sulfide, in comparison with a dialkyl sulfide, was exemplified, when the acyclic 1,3-disulfide **3** was used as substrate (Scheme 1b).[5a] The enantiopure alkylaryl sulfoxide enantiomer $\mathbf{4}_S$ was produced from methylsulfanyl methyl phenyl sulfide **3**, by TDO and NDO biocatalysts. The dialkyl sulfoxide $\mathbf{5}_S$, with a lower *ee* value, was obtained only when using NDO as biocatalyst.

The monosulfoxidation of bicyclic alkylaryl 1,4-disulfides, *e.g.* compounds **6a** and **6b** (Scheme 1c), was also found to occur in an enantiocomplementary manner, when using either TDO or NDO to yield the corresponding sulfoxide enantiomers, $\mathbf{7a}_R$ or $\mathbf{7a}_S$ and $\mathbf{7b}_R$ or $\mathbf{7b}_S$. [5b] No evidence was obtained of further or alternative arene dioxygenase-catalysed oxidations of alkylaryl 1,3-disulfides **3**, or 1,4-disulfides **6a** or **6b**, to give the corresponding disulfoxides, sulfones or *cis*-dihydrodiols.[5b]

The earlier successful dioxygenase-catalysed sulfoxidation of monosulfides,[4a-g] 1,3-disulfides,[4a,5a,5b] 1,4-disulfides,[5b] and 1,5-disulfides,[5b] prompted our preliminary investigation of the enzymatic sulfoxidation of 1,2-disulfides, using similar conditions.[5c] This more comprehensive study now reports chemical resolution and asymmetric synthesis routes to several previously unavailable enantiopure thiosulfinates; their absolute configurations and chemical and configurational stabilities were determined prior to their attempted enzymatic synthesis *via* peroxidase-, monooxygenase- and dioxygenase-catalysed oxidations. Results obtained from enzyme-catalysed oxidation reactions of acyclic and cyclic 1,2-disulfides and related substrates, including potential thiol, monosulfide and monosulfoxide intermediates, have led to possible new metabolic pathways for 1,2-disulfides being proposed.

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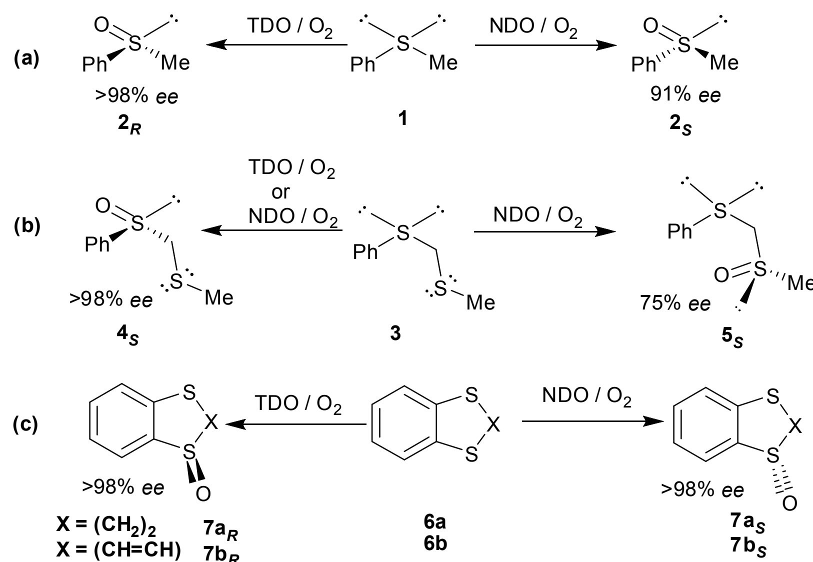


Scheme 1 TDO- and NDO-catalysed sulfoxidations of (a) monosulfide **1**, (b) 1,3-disulfide **3** and (c) 1,4-disulfides **6a** and **6b**.

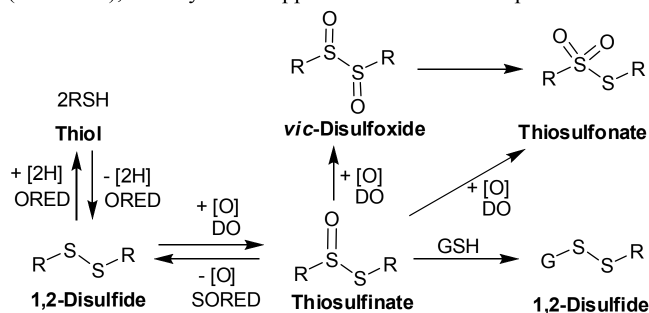
Results and discussion

Enzyme-catalysed oxidation of monosulfides can provide a route to many enantiopure sulfoxides,[1a-i] of interest to the pharmaceutical industry, *e.g.* omeprazole (Fig.1). However, few reports have appeared on the enzymatic oxidations of 1,2-disulfides, to yield enantiopure 1,2-disulfide-S-monoxides (thiosulfinates) despite (i) the important role that the S-S bond plays in many protein structures and natural products, *e.g.* gliotoxin and (ii) the established potential of some naturally occurring chiral thiosulfinates medicinal chemistry, *e.g.* racemic allicin and leinamycin having an (*S*) thiosulfinate configuration.

The instability of 1,2-disulfides, *in vivo*, often results from cleavage of the S-S bond *via* a reversible reduction-oxidation process with the corresponding thiols (Scheme 2). It is evident that thiol-disulfide oxido-reductase activity (ORED) could present a problem during biotransformations, using bacterial whole cell systems, *e.g. Pseudomonas putida*. Further instability problems might also be encountered with thiosulfinates, formed *via* dioxygenase (DO)-catalysed sulfoxidation of 1,2-disulfides, that could: (i) spontaneously disproportionate to yield 1,2-disulfide and thiosulfonate derivatives,[3c] (ii) undergo further enzymatic oxidation to yield unstable *vic*-disulfoxides, which rearrange spontaneously to stable thiosulfonates (Scheme 2),[6a-d] (iii) undergo sulfoxide reductase (SORED)-catalysed deoxygenation, when using *P. putida* cells and (iv) act as reactive oxygen species, under conditions of oxidative stress and interact with biological thiols, *e.g.* glutathione (GSH, Scheme 2).[7a-c]

In addition to their chemical instability, some thiosulfinates have also been found to racemize, at ambient temperature, and their configurational stabilities have been reported to vary with their structure and the solution pH or solvent used.[3c,8a-c]

Despite the potential problems of both decomposition and racemization occurring, several reports of the production of enantiomerically enriched thiosulfinates, by chemical asymmetric oxidation of the corresponding 1,2-disulfides, have appeared. Low enantiomeric excess (*ee*) values for thiosulfinate products were reported, when using chiral peroxyacids (<10 % *ee*) [3a,8a] or chiral oxaziridines (2-14% *ee*) [8b] as oxidants. Higher enantiopurity values were observed when hydroperoxide or other peroxide oxidizing agents were used in the presence of chiral catalysts, including *t*-BuO_2H-Ti(O-^{i}Pr)$_4$-DET (13-52% *ee*) [8c] or H_2O_2-VO(acac)$_2$-imine ligands (98% *ee*).[8d,8e] With the availability of more stable acyclic thiosulfinates as single enantiomers, from chemical asymmetric synthesis, *e.g.* R = *t*-Bu (Scheme 2), their synthetic applications have been reported.[8e,8f]



Scheme 2 Enzyme-catalysed metabolism of 1,2-disulfides.

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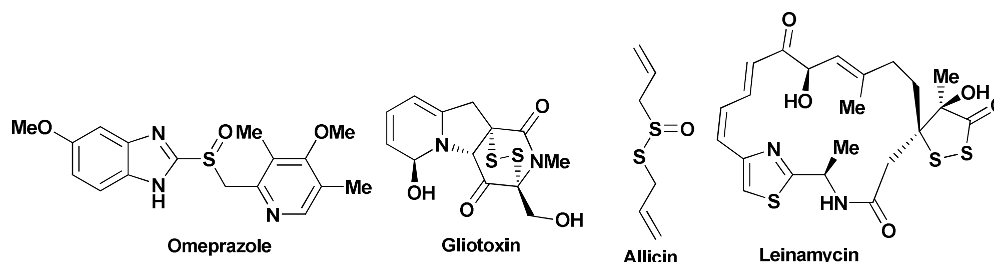


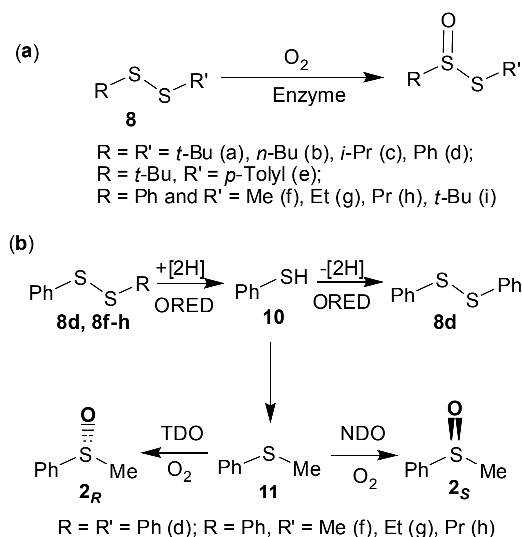
Figure 1 Structures of omeprazole, gliotoxin, allicin and leinamycin.

Although the structures of thiosulfinate metabolites, obtained from early enzymatic sulfoxidation studies of naturally occurring 1,2-disulfides, *e.g.* diallyl and dipropyl disulfides,[9a-d] and of anthropogenic 1,2-disulfides, *e.g.* 1,2-dithianes,[9e] were determined, their stereochemistries were not assigned.[9a-e] The first evidence that the enzyme-catalysed oxidation of unnatural 1,2-disulfides can yield enantiomerically enriched thiosulfinates, as isolable metabolites, was reported in a seminal study by Colonna *et al* (Scheme 3a).[10] Thus, cyclohexanone monooxygenase (CYMO)-catalysed sulfoxidation of dialkyl 1,2-disulfides **8a-c** and alkylaryl 1,2-disulfide **8e** resulted in the formation of the corresponding thiosulfinates **9a** (97% *ee*), **9b** (70% *ee*), **9c** (22% *ee*) and **9e** (44% *ee*). This study[10] established the feasibility of enantioselective synthesis of thiosulfinates, *via* enzyme-catalysed oxidation of 1,2-disulfides, with absolute configurations being assigned to metabolites **9a** and **9c** (Scheme 3a).

(i) Biotransformations of acyclic alkylaryl 1,2-disulfides 8f-i and diaryl disulfide 8d using *P. putida* strains expressing TDO or NDO

The successful arene dioxygenase-catalysed sulfoxidations of alkylaryl 1,3-, 1,4-, 1,5-disulfides,[4a,5a,5b] and dialkyl 1,3-disulfides,[5a] to yield enantiopure monosulfoxides prompted our preliminary communication,[5c] using whole cell systems expressing the same TDO and NDO enzymes but with the alkylaryl 1,2-disulfides **8f-h** as potential substrates. These acyclic 1,2-disulfides were initially selected, based on: (i) their similarity in size to alkylphenyl sulfides previously biotransformed to the corresponding enantiopure monosulfoxides, without evidence of further oxidation to sulfones,[4d] (ii) the availability of these, and other 1,2-disulfides and the corresponding thiosulfinates, either from commercial sources or from literature synthesis routes, (iii) the strong preference shown by TDO or NDO enzymes for alkylaryl sulfide substrates rather than dialkyl[5a] or diaryl sulfides.[4d]

The racemic thiosulfinates **9d**, **9f-i** (Scheme 3a) were obtained by reported methods involving treatment of benzenesulfinyl chloride with the appropriate thiols. The biotransformations of disulfides **8d**, **8f-h** were studied, using whole cell cultures of *P. putida*, as either a mutant strain (UV4, expressing TDO) or a wild-type strain (NCIMB 8859, expressing NDO). Following the extraction and separation of metabolites by PLC, diphenyl disulfide **8d** was consistently isolated as the major identified bioproduct (from substrates **8f-h**) or recovered (from substrate **8d**), using TDO (2-34% yield) or NDO (2-10% yield, Table 1). Methylphenyl sulfoxide, metabolite **2**, was similarly isolated from disulfides **8d**, **8f-h** with high *ee* values but in low yield (2-11%). Chiral stationary phase HPLC (CSP-HPLC) analysis (Chiralcel-OD, IPA/hexane) was employed, to determine the enantiopurity of the methylphenyl sulfoxide obtained using either TDO (**2_R**, 85-97% *ee*) or NDO (**2_S**, 94-97% *ee*). Despite employing either wild type or mutant *P. putida* strains, under the previously reported sulfoxidation conditions,[4a,5a,5b] no evidence of thiosulfinate metabolites was found. Possible metabolic pathways, to explain the absence of the expected thiosulfinates **9d**, **9f-h** and presence of metabolites **2** and **8d**, are shown in Scheme 3b.



Scheme 3 Enzyme-catalysed reactions of 1,2-disulfides **8d** and **8f-h** to yield 1,2-disulfide **8d** and monosulfoxides **2_R** or **2_S**.

Table 1 Yield, *ee* and absolute configuration (AC) of chiral metabolite **2** and yield of bioproduct **8d** obtained from biotransformation of substrates **8d**, **8f-h** and **10** using *P. putida* UV4 (TDO) and *P. putida* NCIMB 8859 (NDO).

Substrates **8d**, **8f-h**,**10**	Enzyme type	Metabolites			
		MeSOPh **2**			PhSSPh **8d**
		% Yield	% *ee*	AC	% Yield
8d	TDO	2	97	*R*	10
8d	NDO	1	97	*S*	2
8f	TDO	6	92	*R*	2
8f	NDO	11	94	*S*	10
8g	TDO	3	91	*R*	28
8g	NDO	1	95	*S*	5
8h	TDO	4	85	*R*	34
8h	NDO	1	97	*S*	4
10	TDO	1	97	*R*	6
10	NDO	[a]	-	-	3

[a] Sulfoxide **2** not observed

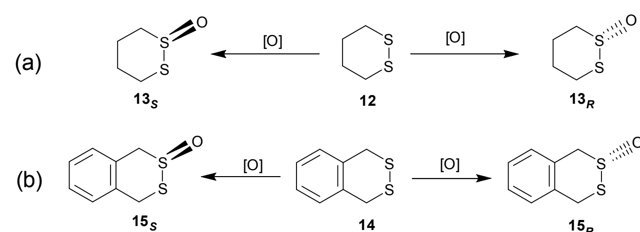
Several types of ORED enzymes have been reported to catalyse the reductive cleavage of 1,2-disulfides to yield thiols and the reverse oxidative S-S bond formation reaction. It is probable that a similar type of enzyme, and also a thiol S-methyl transferase (TSMT),[11a] were present in the *P. putida* whole cells. The ORED enzymes thus catalysed the formation of thiophenol **10** and diphenyl disulfide **8d**, while TSMT catalysed the formation of methylphenyl sulfide **11** from S-methylation of thiol **10**. The reduction of 1,2-disulfides to thiols, and thiol S-methylation, had been observed earlier during eukaryotic (animal) metabolism.[11b] As shown in Scheme 1a, sulfide **1** had previously been biotransformed to either sulfoxide enantiomer using TDO ($\mathbf{2}_R$, >98% *ee*) or NDO ($\mathbf{2}_S$, 91% *ee*).[4d] Although thiophenol **10** was not detected as a metabolite from substrates **8d**, **8f-h**, its addition as a substrate to *P. putida* UV4 and *P. putida* NCIMB 8859 and the resulting formation of diphenyldisulfide **8d**, albeit in very low yield (3-6%, Table 1), provided support for a mechanism involving a thiol:disulfide oxido-reductase enzyme (Scheme 3b). Methylphenyl sulfoxide $\mathbf{2}_R$ (97% *ee*) was also detected a metabolite of thiol **10**, as a result of sequential TSMT-catalysed S-methylation and TDO-catalysed sulfoxidation. The generally very low yields of extracted metabolites $\mathbf{2}_S$, $\mathbf{2}_R$ and **8d**, and our inability to isolate the thiol intermediate **10**, was assumed to be due to its formation and further metabolism including oxidation to water-soluble metabolites.

(ii) Thermal and configurational stability studies of chiral thiosulfinate enantiomers obtained by chemical asymmetric synthesis and CSP-HPLC resolution methods

In our preliminary study,[5c] it was suspected that the absence of the expected thiosulfinate products **9d**, **9f-h**, among metabolites from dioxygenase-catalysed sulfoxidation of the corresponding 1,2-disulfides **8d**, **8f-h** (Scheme 3b), was a result of the instability of substrates and/or bioproducts under the biotransformation or work-up conditions. Further studies showed that these acyclic 1,2-disulfides were thermally stable, at the higher temperatures (50-130 °C) used during their purification by distillation, and were also chemically stable for extended periods, under aqueous conditions at ambient temperature. It was, however, postulated that enzyme-catalysed reductive cleavage of the S-S bond had occurred, during the first biotransformation step of 1,2-disulfides **8d**, **8f-h**, using *P. putida* UV4 and *P. putida* NCIMB 8859 cultures to yield thiol **10** as a metabolite (Scheme 3b).

While the possibility of a non-enzymatic cleavage of the S-S bond in 1,2-disulfides occurring cannot be excluded, it was considered more likely that the much weaker thiosulfinate S-SO bond (*ca*. 47 kcal/mol) would cleave during the biotransformation or work-up. Thiosulfinates are known to readily disproportionate into 1,2-disulfides and thiosulfonates and react with other thiols (Scheme 2). To investigate this possibility, the stability of 1,2-thiosulfinates **9d**, **9f-i** was examined by NMR analysis at ambient temperature. While they showed no sign of decomposition in D_2O solution over 24 h, the stability of some thiosulfinates was found to be solvent dependent. Thus, thiosulfinate **9f**, possibly the least stable member of the thiosulfinates **9d**, **9f-i**, remained unchanged in D_2O after 72 h. However, in $CDCl_3$ solution, over the same period, 85% of it was found to have disproportionated, spontaneously, to yield a mixture (1:1) of 1,2-disulfide **8f** and the corresponding thiosulfonate. The total disappearance of substrates **8f-h**, and absence of the corresponding more stable thiosulfonates during the biotransformation, furnished further evidence that thiosulfinates **9f-h** were not formed.

It was anticipated that six-membered ring 1,2-disulfides would be more stable than the corresponding acyclic compounds **8a-i**, as reversible S-S bond ring cleavage and closure could occur. Furthermore, earlier results obtained, using 1,2-dithiane **12** as substrate with rabbit liver microsomes, revealed that cytochrome P-450 monooxygenase-catalysed sulfoxidation had occurred, to yield the isolable thiosulfinate metabolite **13** (Scheme 4a, [O] = P-450, O_2).[9e] Since thiosulfinate metabolites **9f-h** could not be isolated from dioxygenase-catalysed oxidation of the corresponding acyclic precursors **8f-h**, the cyclic 1,2-disulfides **12** and **14** were synthesised and utilized as potential alternative substrates. They proved to be stable in aqueous solution over a 24 h period; chemical oxidation using sodium periodate in aqueous MeOH (Schemes 4a and 4b, [O] = $NaIO_4$) yielded the corresponding racemic cyclic thiosulfinates **13** and **15**. The earlier liver microsomal metabolism study of 1,2-dithiane **12**[9e] did not address the questions of enantiopurity, absolute configuration or configurational stability of the resulting thiosulfinate metabolite **13**, therefore particular emphasis was placed on these aspects during this programme.



Scheme 4 Sulfoxidation of (a) 1,2-disulfide **12** to yield thiosulfinates $\mathbf{13}_S$ or $\mathbf{13}_R$ and (b) 1,2-disulfide **14** to yield thiosulfinates $\mathbf{15}_S$ **or** $\mathbf{13}_R$.

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Table 2 Yields, *ee* values and absolute configurations (AC) of thiosulfinates **13** and **15** obtained by chemical and enzyme-catalysed asymmetric sulfoxidation of 1,2-disulfides **12** and **14**.

Substrates Disulfide	Chemical and enzymatic oxidants	Products			
		Thiosulfinate	% Yield	% *ee*	AC
12	Kagan[a]	**13**	35	18	*R*
12	Oxaziridine[b]	**13**	89	52	*S*
12	CYMO/O_2	**13**	8	22	*R*
12	CPO/H_2O_2	**13**	100	96	*S*
14	Kagan[a]	**15**	69	13	*R*
14	Oxaziridine[b]	**15**	95	20	*S*
14	NDO/O_2	**15**	11	9	*S*
14	CPO/H_2O_2	**15**	59	32	*S*

[a] t-BuO_2H-Ti(O-^{i}Pr)$_4$-DET Sulfoxide; [b] (1*R*)-(10-camphorsulfonyl) oxaziridine

Thiosulfinates were reported to undergo racemization more readily than normal sulfoxides and several mechanisms were proposed.[3a-c] The pyramidal sulfur atom in chiral alkylaryl or diaryl sulfoxides is generally assumed to racemize *via* a thermal inversion process ($\Delta G^{\neq}$ = *ca*. 40 kcal mol^{-1}). An alternative mechanism was initially proposed for racemization of chiral diaryl thiosulfinates ($\Delta G^{\neq}$ = *ca*. 23 kcal mol^{-1}) involving an internal displacement at the sulfenyl sulfur atom.[8a,12a] The possibility of thiosulfinate racemization being catalysed by traces of a sulfenic acid,[12b] and other acids or nucleophiles,[3b] was later suggested. Configurational stability of chiral thiosulfinates was dependent on both substituent size and solvent. The presence of bulky substituents, *e.g.* *tert*-butyl, was found to increase the barrier to racemization. In this context, S-*tert*-butyl-*tert*-butanethiosulfinate **9a** was found to be configurationally stable ($\Delta G^{\neq}$ = >23 kcal mol^{-1}), after heating in refluxing benzene for 8 h. [3a,3b]

To establish configurational stability of the cyclic thiosulfinates **13** and **15**, enantiomerically enriched samples were first produced by chemical asymmetric synthesis. Application of the Kagan asymmetric oxidation method (t-BuO_2H-Ti(O-^{i}Pr)$_4$-DET), to acyclic 1,2-disulfides, had earlier been found to yield thiosulfinates with a maximum *ee* value of *ca*. 50%.[8c] When the Kagan oxidation method was applied to cyclic 1,2-disulfides **12** and **14** (Scheme 4a and 4b), the corresponding thiosulfinates were produced in moderate yield but of relatively low enantiopurity, based on CSP-HPLC analysis (**13**, 35% yield, 18% *ee*; **15**, 69% yield, 13% *ee*, Table 2). Use of a chiral oxaziridine oxidant, [O] = (-)-(1*R*)-(10-camphorsulfonyl)oxaziridine, resulted in an improvement in both yields and enantiopurity values of the thiosulfinates (**13**, 89% yield, 52% *ee*; **15**, 95% yield, 20% *ee*).

An alternative chemical approach, to obtaining enantiopure thiosulfinates **13** and **15**, involved a semi-preparative CSP-HPLC separation of enantiomers from their racemates. The analytical CSP-HPLC system used earlier for *ee* determination of sulfoxide **2** (Chiralcel OD, IPA/hexane) was examined as a possible method for separation of the individual thiosulfinate enantiomers $\mathbf{13}_S/\mathbf{13}_R$ ($\alpha = 1.1$) and $\mathbf{15}_S/\mathbf{15}_R$ ($\alpha = 1.6$). It was, subsequently, found that an alternative CSP-HPLC analytical column, (*R*,*R*)-Whelk-O1, provided a much better separation of enantiomers $\mathbf{13}_S/\mathbf{13}_R$ ($\alpha = 1.4$), and $\mathbf{15}_S/\mathbf{15}_R$ ($\alpha = 2.8$), using *tert*-butylmethyl ether (*t*-BME) or *t*-BME/hexane as eluant. It was then possible to process larger quantities (20 mg per injection) of the corresponding racemates ($\mathbf{13}_S/\mathbf{13}_R$ and $\mathbf{15}_S/\mathbf{15}_R$), using a semi-preparative version of the Whelk-O1 CSP-HPLC column, which gave a base-line separation of the enantiomers. This approach also proved successful for the isolation of single enantiomers of acyclic thiosulfinate $\mathbf{9i}_S/\mathbf{9i}_R$ ($\alpha = 1.7$) whose stability was improved by the presence of a bulky *tert*-butyl substituent. No evidence of decomposition of the thiosulfinate enantiomers was observed when using any of the CSP-HPLC solvent systems.

Furthermore, each of these enantiomers ($\mathbf{13}_S$, $\mathbf{13}_R$, $\mathbf{15}_S$, $\mathbf{15}_R$, $\mathbf{9i}_S$, $\mathbf{9i}_R$) was found to be configurationally stable, over a period of 24 h at ambient temperature, in *t*-BME and mixtures of *t*-BME/hexane or IPA/hexane. However, in more polar solvents, under the same conditions, varying degrees of racemization were found. Thus, CSP-HPLC analysis 24 h after dissolution in a mixture of H_2O/MeOH (1:1), showed a decrease in the *ee* values for each of the thiosulfinate enantiomers $\mathbf{13}_S$ or $\mathbf{13}_R$ (100 → 17%), $\mathbf{15}_S$ or $\mathbf{15}_R$ (100 → 86%) and $\mathbf{9i}_S$ or $\mathbf{9i}_R$ (100 → 83%). Efforts were made to minimize the degree of racemization occurring, during biotransformation, isolation and stereochemical analysis of thiosulfinate metabolites.

Absolute configurations of the enantiomers of cyclic thiosulfinates **13** and **15** were assigned using X-ray crystallography and electronic circular dichroism (ECD) spectroscopy. The axial conformation of the oxygen atom, and (1*S*) absolute configuration, present in an enantiopure crystalline sample of (+)-1,4-dihydrobenzo-2,3-dithian-2-oxide $\mathbf{15}_S$, $[\alpha]_D$ + 250 (*c* 0.4, $CHCl_3$), was established unequivocally by the X-ray crystal structure, reported but not shown, in our preliminary communication.[5c] The crystalline thiosulfinate $\mathbf{9i}_S$, $[\alpha]_D$ - 144 (*c* 0.4, $CHCl_3$), obtained by CSP-HPLC separation of enantiomers, was also assigned an (*S*) absolute configuration by X-ray crystallography.[5c] The absolute configurations of cyclic thiosulfinate enantiomers $\mathbf{13}_S$ and $\mathbf{13}_R$, were assigned by a comparison of their ECD spectra with the corresponding spectra of cyclic thiosulfinate enantiomers $\mathbf{15}_S$ and $\mathbf{15}_R$ of known configurations. Based on the similarity between the ECD spectra of the early eluted enantiomer $\mathbf{15}_S$, $[\alpha]_D$ + 250 (*c* 0.40, $CHCl_3$), and the early eluted enantiomer $\mathbf{13}_S$, $[\alpha]_D$ + 341 (*c* 0.43, $CHCl_3$), an identical (*S*) absolute configuration was assigned in each case.

(iii) Asymmetric sulfoxidation of cyclic 1,2-disulfides 12 and 14 to yield thiosufinates 13 and 15 using cyclohexanone monooxygenase (CYMO) and chloroperoxidase (CPO)

Following our unsuccessful efforts to obtain the acyclic thiosulfinates **9d**, **9f-h** from the 1,2-disulfides **8d**, **8f-h**, using whole cells from *P. putida* strains expressing arene dioxygenases and other enzymes, the biotransformation of 1,2-disulfide substrates **12**, **14** and **8i** with pure enzymes was investigated (Table 2). It was anticipated that the more stable thiosulfinate bioproducts **13**, **15** and **9i**, if formed, would survive longer under the shorter aqueous biotransformation and work-up conditions and less racemisation would occur. A preparation of cyclohexanone monooxygenase, isolated from *Acinetobacter*

calcoaceticus NCIB9871, had been used earlier by Colonna *et al*, to catalyse the sulfoxidation of 1,2-disulfides **8a-c**. In each case, the corresponding enantioenriched thiosulfinates **9a** (90% yield), **9b** (4% yield) and **9c** (4% yield) were obtained as metabolites.[10] When 1,2-disulfides **12**, **14** and **8i** were evaluated as potential substrates, under identical conditions (CYMO, NADPH, tris buffer), only thiosulfinate $\mathbf{13}_R$ was isolated, but in relatively low yield (8%) and enantiopurity (22% *ee*) (Table 2, Scheme 4a, [O] = CYMO, O_2). The less water-soluble substrates **14** and **8i** were recovered without being metabolized by CYMO.

The earlier success of purified chloroperoxidase, in catalysing the asymmetric sulfoxidation of monosulfides to sulfoxides,[13a-g] encouraged us to study its potential in the oxidation of cyclic 1,2-disulfides **12** and **14**, under similar conditions to those used earlier by Allenmark *et al* (CPO, H_2O_2, citrate buffer).[13b,13d] The biotransformation of 1,2-dithiane **12** yielded the corresponding (+)-(*S*)-1,2-dithiane-1-oxide $\mathbf{13}_S$ in almost quantitative yield and excellent enantiopurity (Table 2, 96% *ee*, Scheme 4a, [O] = CPO, H_2O_2). The possibility of racemic thiosulfinate being produced *via* hydrogen peroxide oxidation, and a kinetic resolution process, by further oxidation to a disulfoxide or thiosulfonate, being partly responsible for the stereoselectivity, was excluded by: (a) the isolated yield of the product and (b) the results obtained from a time course study. It showed, unequivocally, that the enantiopurity of thiosulfinate metabolite $\mathbf{13}_S$ remained consistently high (96% *ee*), throughout the period required for completion of the biotransformation (40 minutes), and confirmed that a highly stereoselective CPO-catalysed asymmetric synthesis process was responsible. Following our preliminary report,[5c] Klibanov and Dzyuba also used cyclic 1,2-disulfide **12** as a substrate for horseradish peroxidase (HRP).[14] Thiosulfinate $\mathbf{13}_S$ was again formed preferentially (Scheme 4a, [O] = HRP, H_2O_2) with stereoselectivity being solvent-dependent, *e.g.* E = 7.20 in aqueous buffer and E = 1.8 in neat methanol.[14]

CPO-catalysed sulfoxidation of the less water-soluble cyclic 1,2-disulfide **14**, under the conditions used for compound **12**, was also successful but the resulting (+)-(*S*)-thiosulfinate **15** was obtained in lower yield (59%) and enantiopurity (32% *ee*) along with recovered substrate (Table 2, Scheme 4b, [O] = CPO, H_2O_2). This experiment, when repeated using 20% *tert*-butyl alcohol as co-solvent, to improve the solubility and uptake of the 1,2-disulfide substrate **14**, resulted in an increased *ee* value (47%). When acyclic 1,2-disulfide **8i** was used as substrate for CPO, in common with results obtained using the acyclic 1,2-disulfides **8d** and **8f-h** with TDO or NDO, it did not yield a thiosulfinate.

The (*S*) absolute configuration of cyclic thiosulfinates $\mathbf{13}_S$ and $\mathbf{15}_S$, obtained by CPO-catalysed asymmetric sulfoxidation of the corresponding 1,2-disulfides **12** and **14**, was predominant (Schemes 4a,4b, [O] = CPO, H_2O_2). This appeared to be at variance with the almost exclusive formation of the (*R*)- cyclic sulfoxides enantiomers found earlier when using cyclic monosulfide substrates, *e.g.* 2,3-dihydrobenzo[*b*]thiophene (99% *ee*), thiochroman (96% *ee*) and 1-thiochroman-4-one (95% *ee*) under identical biotransformation conditions.[13b] This apparent reversal in preferred absolute configurations was accounted for by a change in Sequence Rule priorities between the latter monosulfoxides and thiosulfinate metabolites $\mathbf{13}_S$ and $\mathbf{15}_S$.

(iv) Dioxygenase-catalysed oxidation of 1,4-dihydrobenzo-2,3-dithiane 14 using *P. putida* NCIMB 8859

Addition of the bicyclic dialkyl 1,2-disulfide **14** as substrate, to whole cell cultures of *P. putida* NCIMB 8859, resulted in NDO-catalysed sulfoxidation to give the corresponding (+)-(*S*)-thiosulfinate $\mathbf{15}_S$ (Scheme 4b, [O] = NDO, O_2) in lower yield (11%) and enantiopurity (9% *ee*), compared with the earlier results obtained using CPO (Scheme 4b, [O] = CPO, H_2O_2, 58% yield and 47% *ee*). The monocyclic dialkyl 1,2-disulfide **12** was not biotransformed into thiosulfinate **13**, when the arene hydroxylating dioxygenases, NDO (*P. putida* NCIMB 8859) or TDO (*P. putida* UV4), were used.

Based on this limited enantioselectivity study of enzymatic asymmetric sulfoxidation of 1,2-disulfides **12** and **14**, to yield the corresponding thiosulfinates **13** and **15**, the two purified peroxidase enzymes (CPO and HRP) gave better results than the monooxygenase enzyme (CYMO) or whole cells expressing arene dioxygenases (NDO and TDO).

Although it was not possible to obtain thiosulfinate **15**, as a single enantiomer, by the enzyme-catalysed sulfoxidation methods described herein, a complementary kinetic resolution approach was developed in our laboratory. This alternative method involved a sulfoxide reductase-catalyzed deoxygenation process;[15a,15b] enantioselective deoxygenation of racemic thiosulfinate **15**, yielded mainly the residual (*S*) enantiomer ($\mathbf{15}_S$, >95% *ee*), when using purified dimethyl sulfoxide reductase from *Citrobacter braaki* DMSO 11.

(v) Biotransformation of 1,4-dihydrobenzo-2,3-dithiane 14, benzo[*c*]thiophene 19, 1,3-dihydrobenzo[*c*]thiophene 20 and 1,3-dihydrobenzo[*c*]thiophene sulfoxide 21 using *P. putida* UV4

The biotransformation of bicyclic 1,2-disulfide substrate **14**, with *P. putida* UV4, yielded three metabolites but the expected thiosulfinate **15** was not found. PLC separation of the metabolites, followed by analysis (MS and NMR spectroscopy) and comparison with literature data, led to the identification of major bioproduct, 2-thiophthalide **16** (17% yield), and a monohydroxylated derivative, 6-hydroxy-2-thiophthalide **17**, as a very minor product (1% yield) (Scheme 5). A more polar minor (2% yield) chiral metabolite, *cis*-dihydrodiol **18**, was also isolated and identified, based upon NMR and CD spectroscopic analysis.

Bioproduct **18**, contained a benzene *cis*-tetrahydrodiol ring of similar structure to (1*R*,2*S*)-diol metabolite **24**, obtained using 1,3-cyclohexadiene as substrate with *P. putida* UV4.[16a] *cis*-Diol **18** was a particularly novel metabolite, as virtually all previous TDO-catalysed *cis*-dihydroxylation products, from a carbocyclic arene ring, were *cis*-dihydrodiols, *e.g.* metabolite **23** obtained from biotransformation of benzo[*b*]thiophene **22** using *P. putida* UV4.[16b] Metabolite **18** had similar structural features to *cis*-tetrahydrodiol **24**, which was much more stable than the corresponding benzene *cis*-dihydrodiol. However, *cis*-diol **18** was unstable and readily aromatized, as found with all arene *cis*-dihydrodiol metabolites including diol **23** (Fig.2).[16b]

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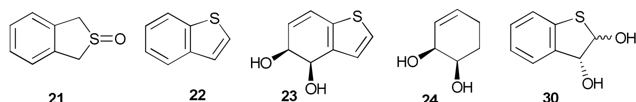
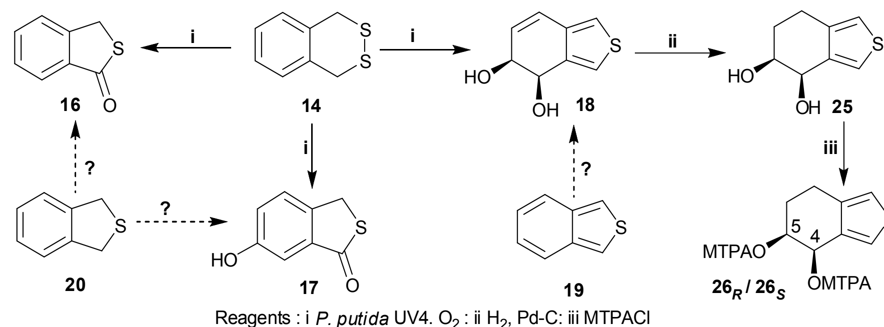


Figure 2 Structures of sulfoxide **21**, benzo[*b*]thiophene **22**, and *cis*-diol metabolites **23**, **24** and **30.**

To prevent aromatization of metabolite **18**, and to determine its enantiopurity and absolute configuration, a partial hydrogenation (Pd-C, H_2 in EtOAc) was carried out, to give the more stable cyclohexane *cis*-diol derivative **25**. Formation of the corresponding diastereoisomeric diMTPA ester derivatives, $\mathbf{26_R}$ (using [*R*]-MTPA) and $\mathbf{26_S}$ (using [*S*]-MTPA) respectively, followed by NMR analysis, indicated that both *cis*-diol **25** and its *cis*-diol precursor **18** were enantiopure (Scheme 5, >98% *ee*). Earlier studies had shown that di-MTPA esters of *cis*-



Scheme 5 Bioproducts **16-18** obtained from 1,2-disulfide **14** as substrate with *P. putida* UV4 and derivatives **25** and $\mathbf{26_R}$ / $\mathbf{26_S}$.

tetrahydrodiols, obtained from partial hydrogenation of the corresponding *cis*-dihydrodiol metabolites of polycyclic arenes and heteroarenes, could be used to determine both their enantiopurity values and absolute configurations.[17] Using this method, based on the smaller difference in chemical shift values, observed between H-4 and H-5 ($\mathbf{26_R}$, $\Delta\delta$ 0.76 ppm) when using (+)-(*R*)-MTPA, compared with (-)-(*S*)-MTPA ($\mathbf{26_S}$, $\Delta\delta$ 0.88 ppm), *cis*-diol **25** was provisionally assigned a (4*R*,5*S*) configuration. The structure, enantiopurity (>98% *ee*) and (4*R*,5*S*) absolute configuration of the crystalline *cis*-diol **25**, and of *cis*-dihydrodiol precursor **18** ($[\alpha]_D$ + 169 ($CHCl_3$), was confirmed unequivocally by X-ray crystallography.[5c]

Benzo[*b*]thiophene **22** is a very stable compound, formed during partial combustion of fossil fuels, prior to its release into the atmosphere and ultimately its biodegradation in the environment.[16b] Conversely, benzo[*c*]thiophene **19** is much less stable, has not been detected in the environment, and to our knowledge, had not been used, previously, as a substrate for biotransformation studies or identified as a metabolite. It was thus surprising to find that all three bioproducts **16-18**, obtained from 1,2-disulfide **14** appeared to be possible metabolites of benzo[*c*]thiophene **19** (Scheme 5), or precursors including 1,3-dihydrobenzo[*c*]thiophene **20** and 1,3-dihydrobenzo[*c*]thiophene-2-oxide **21** (Scheme 6); this unexpected observation led to further studies of their biosynthetic origins.

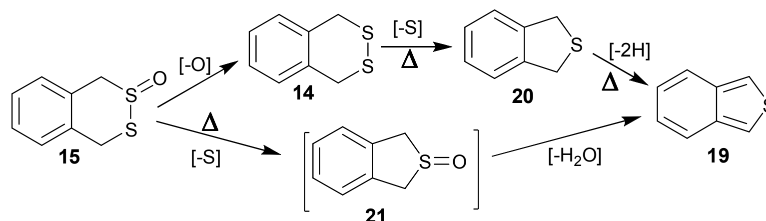
When a pure sample of thiosulfinate **15** was analysed by GC-MS, three decomposition compounds were detected. Using authentic samples, these were identified as 1,2-disulfide **14**, 1,3-dihydrobenzo[*c*]thiophene **20** and benzo[*c*]thiophene **19** (Scheme 6). Benzo[*c*]thiophene **19** was obtained by dehydration of 1,3-dihydrobenzo[*c*]thiophene-2-oxide **21**, using the literature method.[18] GC-MS analysis of a pure sample of 1,2-disulfide **14**, under identical conditions, again yielded the decomposition products **19** and **20**.

The previously reported thermal deoxygenation and disproportionation reactions of thiosulfinates, to yield 1,2-disulfides (*cf.* Scheme 2) lent support for the initial step in the proposed reaction sequence followed during GC-MS analysis of thiosulfinate **15** (Scheme 6). Thermal disproportionation of thiosulfinate **15** to yield 1,2-disulfide **14** followed by thermal extrusion of a sulfur atom at the elevated temperature of the GC injection port (200° C), could generate 1,3-dihydrobenzo[*c*]thiophene **20**. Catalytic thermal dehydrogenation of intermediate **20** could then yield benzo[*c*]thiophene **19**. A shorter alternative sequence, involving thermal extrusion of a sulfur atom from thiosulfinate **15** and dehydration of the resulting sulfoxide **21**, could also account for the formation of benzo[*c*]thiophene **19**. Further evidence for the formation of compounds **14**, **19** and **20** as thermal decomposition products of thiosulfinate **15**, was found as the latter compounds were not detected by GC-MS analysis of compound **15** (or **14**) when the GC injection port temperature was reduced to 150 ^{o}C.

Metabolites **16**, **17** and **18** were isolated in a relatively low combined yield (*ca.* 20%), after the complete biotransformation of 1,2-disulfide **14** in *P. putida* UV4. It is probable that reductive cleavage of the disulfide bond, found earlier with 1,2-disulfides **8f-h**, followed by oxidation of the resulting thiols to yield water-soluble bioproducts, was partly responsible for the low yield of extracted metabolites. Although benzo[*c*]thiophene **19** was a possible metabolite of disulfide **14**, and a potential precursor of *cis*-dihydrodiol **18** as shown in Scheme 5, it was neither detected during, nor isolated following, the biotransformation **(**Scheme 7**)**.

A metabolic pathway involving the extrusion of a sulfur atom from 1,2-disulfide **14** to yield monosulfide **20**, as proposed during GC-MS analysis, although considered less likely to occur during the biotransformation process, was initially considered (Scheme 7). In this context, potential intermediates **19-21** involved in the biosynthesis of the unusual *cis*-dihydrodiol **18**, were then added separately as substrates to *P. putida* UV4 whole cells. Addition of 1,3-dihydrobenzo[*c*]thiophene **20** yielded *cis*-diol **18** as the main isolated metabolite (6% yield), along with 2-thiophthalide

16 (1% yield) and 6-hydroxy-2-thiophthalide **17** (1% yield). The sulfoxide substrate **21** was deoxygenated to yield sulfide **20** (6% yield) and thiophthalide **16** (3% yield). When benzo[*c*]thiophene **19** was used as a substrate, the only identified metabolite was the corresponding *cis*-dihydrodiol **18** (8% yield). Although the isolated yields of compounds **18-21** were very low,



Scheme 6 Thermal decomposition products **14**, **20** and **19** identified during GC-MS analysis of thiosulfinate **15**.

based on these observations, the formation of *cis*-diol **18** from the 1,2-disulfide **14** was in principle consistent with the metabolic sequence: **14** → **20** → **21** → **19** → **18** (Scheme 7).

The formation of metabolites **16**, **17** and **18** from 1,2-disulfide **14** suggested that, in addition to TDO, other enzymes were also involved. *P. putida* UV4 was already known to have a capacity for sulfoxide deoxygenation, catalysed by a sulfoxide reductase enzyme (SORED).[15a] This could account for the formation of sulfide **20** from sulfoxide **21**. The TDO enzyme in *P. putida* UV4 was also shown to catalyse benzylic hydroxylation,[19a-d] particularly in preference to sulfoxidation of dialkylsulfides.[5a] Thus, TDO-catalysed benzylic hydroxylation of methylbenzyl sulfide was previously found to occur as an initial step but ultimately resulting in an S dealkylation reaction and formation of an aldehyde intermediate.[19e]

These earlier observations provide a precedent for the possible formation of thioacetal intermediate **27** from monosulfide **20**. A reversible ring opening process of cyclic thioacetal **27**, could then yield aldehyde itermediate **28** (Scheme 7). A similar reversible ring opening/closure reaction was also involved in the spontaneous *cis-trans* isomerization of the *cis*-dihydrodiol metabolite **30** (Fig. 2) formed from TDO-catalysed dihydroxylation of the heterocyclic ring of benzo[*b*]thiophene **22** under similar conditions.[16]

TDO-catalysed oxidation of aldehyde **28** and spontaneous thiolactonization of the resulting carboxylic acid **29** could then yield the isolated thiolactone **16.** A similar benzylic hydroxylation could also occur on 1,2-disulfide **14**. When followed by ORED-catalysed cleavage of the S-S bond and decomposition of the resulting acyclic thioacetal intermediate to give aldehyde **28** and thioacetal **27** this provides an alternative three step route to intermediate **28** (Scheme 7).

The metabolism of 1,2-disulfide **14**, to yield *cis*-dihydrodiol **18** could also proceed *via* thioacetal **27** and the trihydroxylated intermediate **31** (Scheme 7). A precedent was provided by a TDO-catalysed monol→ triol → diol metabolic pathway discovered, during earlier biotransformations of 1,2-dihydronaphthalene[19d] and 2,3-dihydrobenzo[*b*]furan,[5b] using *P.putida* UV4. In these examples, monohydroxylation yielded the corresponding isolable benzylic alcohols, which were further metabolized, *via* transient triol intermediates, that in turn spontaneously dehydrated to yield the corresponding *cis*-dihydrodiol derivatives of naphthalene[19d] and benzo[*b*]furan.[5b]

Benzylic hydroxylation of dialkyl sulfide **20** and *cis*-dihydroxylation catalysed by TDO could thus yield the trihydroxylated intermediate **31** *via* the thioacetal **27**. Dehydration of compound **31** could yield either the *cis*-diol metabolite **18** or the thioacetal intermediate **32**. A similar three-step metabolic sequence to that proposed for the conversion of thioacetal **27** into thiolactone **16** (**27** → **28** → **29** → **16**) could also apply to the formation of thiolactone **17** from thioacetal **32** (**32** → **33** → **34** → **17**).

Scheme 7 indicates that two metabolic pathways could be involved in the formation of compound **18** from 1,2-disulfide **14** *i.e.* (i) **14** → **20** → **21** → **19** → **18** and (ii) **14** → **28** → **27** → **31** → **18**. However, based on earlier studies with *P. putida* UV4 which indicated (a) a reluctance of TDO to catalyse sulfoxidation of a dialkyl sulfide, *e.g.* **20** → **21**, and (b) a lack of precedent for the extrusion of a sulfur atom from a 1,2-disulfide to yield a monosulfide *e.g.* **14** → **20**, sequence (ii) is currently the preferred option.

Conclusion

The oxidative metabolism of cyclic and acyclic 1,2-disulfide substrates, to yield thiosulfinates and other bioproducts, was studied, using monooxygenase (CYMO), dioxygenase (TDO, NDO) and peroxidase (CPO) enzymes. Enantioenriched samples of thiosulfinates were obtained, by chemical and enzymatic asymmetric synthesis and enantiopure samples by semi-preparative CSP-HPLC. Their chemical and configurational stabilities, and absolute configurations, were investigated by NMR, ECD spectroscopy and X-ray crystallography. Enzymatic asymmetric sulfoxidation of cyclic 1,2-disulfides **12** and **14** yielded thiosulfinates **13** (22-96% *ee*) and **15** (9-47% *ee*).

The presence of TDO in whole cells of *P. putida* UV4, shown earlier to catalyse sulfoxidation, benzylic hydroxylation, dealkylation, aldehyde oxidation and arene ring *cis*-dihydroxylation, was responsible for the majority of metabolites derived from 1,2-disulfides. Other redox enzymes, expressed in *P. putida* UV4 whole cells, were assumed to catalyse: (a) the reversible reductive cleavage of S-S bonds, (b) thiol S-methylation, (c) and sulfoxide deoxygenation. A combination of these enzymes was required to rationalize the unexpectedly wide range of metabolites formed from 1,2-disulfide substrates. Possible biosynthetic mechanisms were proposed, for the formation of the novel benzo[*c*]thiophene *cis*-dihydrodiol **18** and 1,3-dihydrobenzo[*c*]thiophene derivatives **16** and **17**, isolated from 1,4-dihydrobenzo-2,3-dithiane **14**.

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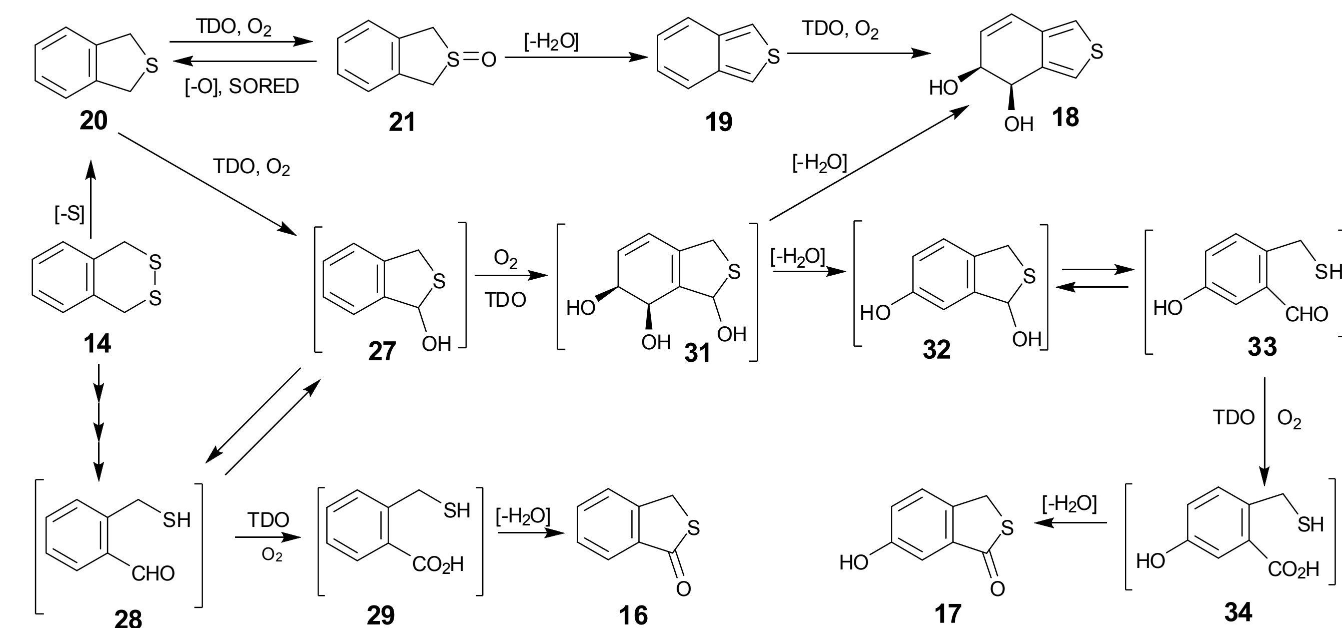

Scheme 7 Possible biosynthetic pathways for metabolites **16-18** during the biotransformation of 1,2-disulfide **14** in *P. putida* UV4.

cis-Dihydrodiol **18** was formed as a biotransformation product, when 1,2-disulfide **14**, cyclic monosulfide **20** and benzo[*c*]thiophene **19** were each used as substrates with *P. putida* UV4 whole cells.

Experimental

Melting Points (m.p.) were reported in degrees Celsius using a Reichert block and are uncorrected. Infrared Spectra (IR) were recorded either on a Bio-Rad 185 FT-IR spectrometer coupled to a PC running the Bio-Rad Win-IR software package or a Perkin-Elmer Spectrum RX1 FT-IR spectrometer. ^{1}H Nuclear Magnetic Resonance (NMR) spectra were recorded at 300 MHz on either General Electric QE 300 or Brüker Avance DPX-300 equipment, and at 500 MHz on either General Electric GN 500 or Brüker Avance DPX-500 instruments. Coupling constants are quoted in Hertz. Chemical shift values are reported in δ (ppm) downfield from TMS and coupling constants (J) are reported in Hz. ^{13}C NMR spectra were recorded at 75 MHz on either a General Electric QE 300 or a Brüker Avance DPX-300 instrument, and at 125 MHz on either a General Electric GN 500 or a Brüker Avance DPX-500 instrument. Mass spectra were recorded at 70 eV, on a VG Autospec Mass Spectrometer, using a heated inlet system. Accurate molecular weights were determined by the peak matching method, with perfluorokerosene as the standard.

Flash column chromatography and preparative layer chromatography (PLC) were performed on Merck Kieselgel type 60 (250-400 mesh) and $PF_{254/366}$ plates respectively. Merck Kieselgel type $60F_{254}$ analytical plates were employed for TLC. Chiral Stationary Phase-High Performance Liquid Chromatography (CSP-HPLC) analyses were run using a Beckman System Gold 128 solvent module attached to a 168 UV detector coupled to a PC running "Gold Nouveau Chromatography Data System" version 1.72 integration software. Columns and solvent systems used were as individually specified. Optical rotations ($[\alpha]_D$) were determined on a Perkin-Elmer automatic precision polarimeter Model 241. Concentrations were measured in g/100 cm^3 using the specified solvent at a wavelength of 589 nm (sodium D line) and ambient temperature. Electronic circular dichroism spectra were recorded using a Jasco J-720 instrument and acetonitrile solvent as specified (concentration approximately 0.1 mg/cm^3), $\Delta\varepsilon$ in mol^{-1} dm.

The acyclic 1,2-disulfides **8d** (Sigma-Aldrich) and **8f** (Acros), racemic methylphenyl sulfoxide **2**, (1*R*)-(-)-(10-camphorsulfonyl) oxaziridine and chloroperoxidase *Caldariomyces fumago* (Sigma-Aldrich) were obtained commercially. The other acyclic 1,2-disulfides **8g-i**,[20a-d] and cyclic 1,2-disulfides **12**[20e,f] and **14**,[20g] were synthesised using literature procedures. Other substrates including benzo[*c*]thiophene **19**,[21a] 1,3-dihydrobenzo[*c*]thiophene **20**,[21a,b] 1,3-dihydrobenzo[*c*] thiophene sulfoxide **21**,[21c] and metabolite 2-thiophthalide **16**[21d,e] were also obtained by following the literature methods and were found to have identical characteristics (Supplementary information).

The CSP-HPLC enantiomeric separations of thiosulfinates **9i, 13** and **15** were carried out using a semi-preparative Whelk-O1 (*R*,*R*) column (25 cm x 4.6 mm) with a mobile phase flow rate of 1.0 cm^3/min and a UV detector set at 254 nm. The chemically synthesised racemic thiosulfinate samples were dissolved in the mobile phase and injected as 1 cm^3 samples. The optical rotations, separation factors, elution sequence and ECD spectra of the thiosulfinate enantiomers $\mathbf{9i}_R$, $\mathbf{9i}_S$, $\mathbf{13}_R$, $\mathbf{15}_R$ and $\mathbf{15}_S$ are shown in Table 3.

Table 3: Chiral stationary phase high performance liquid chromatography (CSP-HPLC) separation of racemic thiosulfinates 9i, 13 and 15 into enantiomers using a Whelk-O1(*R*,*R*) column.

Thiosulfinate	Optical rotations $[\alpha]_D$ ($CHCl_3$)	Separation factor (α)	Elution order (min.)	ECD spectra (MeCN)	
				λ (nm)	$\Delta\varepsilon$
$9i_R$	+150 (*c* 0.88)	1.5[a]	10.3	289 226 200	-2.30 -5.40 +16.15
$9i_S$	-144 (*c* 0.44)	1.5[a]	13.2	290 224 200	+2.02 +5.74 -16.06
13_R	-338 (*c* 0.27)	1.4[a]	36.6	250 218 208 188	-2.77 -2.75 +3.99 -3.32
13_S	+341 (*c* 0.43)	1.4[a]	31.6	250 218 208 198	+1.75 +1.77 +3.32 +2.34
15_R	-246 (*c* 0.40)	2.8[b]	41.3	258 221 197	-5.39 -6.32 +16.95
15_S	+250 (*c* 0.40)	2.8[b]	17.3	258 221 197	+14.5 +17.6 -46.8

[a] 50% *tert*-butyl methyl ether/hexane; [b] 100% *tert*-butyl methyl ether

Biotransformation of 1,2-disulfides 8d, 8f-h by *Pseudomonas putida* UV4 and *Pseudomonas putida* NCIMB 8859

Biotransformations with *Pseudomonas putida* UV4 and *Pseudomonas putida* NCIMB 8859 were conducted following the procedures used earlier for other monosulfide and 1,3-disulfide sulfoxidations.[4a,d] In each case, methyl phenyl sulfoxide **2** and 1,2-disulfide **8d** were obtained as the only identified metabolites (Table 1). The enantiopurity and absolute configuration of the methyl phenyl sulfoxide product **2** was determined by CSP-HPLC (Chiralcel OD Column, 25 cm x 4.6 mm, 0.8 cm^3/min, 10% *iso*-propyl alcohol (IPA)/hexane), α = 1.25, 16.4 min, early major *R* peak, 20.5 min, late minor *S* peak).

Chemical and enzyme-catalysed sulfoxidation of 1,2-disulfides 12 and 14 to yield the corresponding thiosulfinates 13 and 15

(a) Asymmetric sulfoxidation of 1,2-disulfides 12 and 14 using *tert*-BuO_2H-Ti(*iso*-PrO)$_4$-DET

General procedure. Titanium (IV) *iso*-propoxide (0.44 cm^3, 1.47 mmol) and (+)-diethyl tartrate (0.50 cm^3, 2.94 mmol) were dissolved in dry CH_2Cl_2 (75 cm^3) under nitrogen atmosphere at room temperature. Water (0.026 cm^3, 1.47 mmol) was added through a septum and the mixture stirred until the solution was homogeneous (*ca*. 0.5 h). 1,2-Disulfide (1.47 mmol) was added and the solution cooled to -20 ^oC followed by the addition of *tert*-butyl hydroperoxide (0.32 cm^3, 1.76 mmol). The reaction mixture was monitored by TLC for the disappearance of the starting material. On completion of the reaction, water (0.26 cm^3, 14.7 mmol) was added, the solution stirred for 1 h at -20 ^oC and for another 1 h at room temperature. The reaction mixture was filtrated after treatment with a small amount of alumina. The filtrate was stirred for 1 h with an equivalent volume of 5% sodium hydroxide solution and then for another 1 h with brine solution. The organic phase was separated, dried (Na_2SO_4), filtered and evaporated to give the crude product.
Oxidation of 1,2-dithiane **12** (1.0 g, 8.33 mmol) and purification of the crude product by flash chromatography (CH_2Cl_2) gave an excess of (-)-(*R*)-1,2-dithiane-2-oxide **13_R** (0.40 g, 35%); *ee* 18% by CSP-HPLC (Chiralcel OD Column, 25 cm x 4.6 mm, 0.5 cm^3/min, 2.5% IPA/hexane, α = 1.1, 27.4 min, early *R* major peak, 28.6 min, late *S* minor peak).

Oxidation of 1,4-dihydrobenzo-2,3-dithiane **14** (0.20 g 1.19 mmol) and purification of the crude product by flash chromatography (30% EtOAc/hexane) furnished an excess of (-)-(*R*)-1,4-dihydro-2,3-benzodithiane-2-oxide **15_R** (0.151 g, 69%); *ee* 13% by CSP-HPLC (Chiralcel OD Column, 25 cm x 4.6 mm, 0.5 cm^3/min, 2.5% IPA/hexane, α = 1.6, 29.7 min, early *S* minor peak, 45.1 min, late *R* major peak).

(b) Asymmetric oxidation of 1,2-disulfides 12 and 14 by (1*R*)-(-)-(10-camphorsulfonyl)oxaziridine to yield the corresponding thiosulfinates 13 and 15

General procedure. (1*R*)-(-)-(10-Camphorsulfonyl)oxaziridine (0.2-0.4 mmol) was added to a stirring solution of 1,2-disulfide (0.05 g, 0.2-0.4 mmol) in $CHCl_3$ (25 cm^3) at ambient temperature. The reaction mixture was monitored by TLC for the disappearance of the starting material. On completion of the reaction, removal of the solvent under reduced pressure yielded the crude product.
Oxidation of 1,2-dithiane **12** (0.050 g, 0.42 mmol) yielded (+)-(*S*)-1,2-dithiane-1-oxide **13_S**, which was purified by PLC (CH_2Cl_2); (0.050 g, 89%); *ee* 52% by CSP-HPLC (Chiralcel OD Column 25 cm x 4.6 mm, 0.5cm^3/min, 2.5% IPA/hexane, α = 1.1, 27.4 min, early *R* minor peak, 28.6 min, late *S* major peak).
Oxidation of 1,4-dihydrobenzo-2,3-dithiane **14** (0.050 g, 0.20 mmol) yielded (+)-(*S*)-1,4-dihydro-2,3-benzodithiane-2-oxide **15_S**, which was purified by PLC (3% MeOH/$CHCl_3$); (0.052 g, 95%); *ee* 20% by CSP-HPLC (Chiralcel OD Column, 25 cm x 4.6 mm, 0.5cm^3/min, 25% IPA/hexane, α = 1.6, 29.7 min, early *S* major peak, 45.1 min, late *R* minor peak).

(b) Enzymatic oxidation of 1,2-disulfides to yield the corresponding thiosulfinates and other metabolites

(i) Cyclohexanone monooxygenase (CYMO)-catalysed sulfoxidation of 1,2-dithiane 12

(-)-(*R*)-1,2-dithiane-1-oxide 13_R. Biotransformation of 1,2-dithiane **12** (0.025 g, 0.21 mmol, 18 h) by CYMO (70 units) with NADPH (0.175 g) and tris buffer (0.05 M, pH 8.6) followed by ethyl acetate extraction of the aqueous biotransformed material yielded (-)-(*R*)-1,2-dithiane-1-oxide **13_R** (2.2 mg, 8%); *ee* 22% by CSP-HPLC, Table 3, ($[\alpha]_D$ -69.5 (*c* 0.18, $CHCl_3$). The 1,2-disulfide **14** was not biotransformed under the same conditions.

(ii) Chloroperoxidase (CPO)-catalysed sulfoxidation of 1,2-dithianes 12 and 14

General procedure. 1,2-Disulfide (25 μmol) and chloroperoxidase (*Caldariomyces fumago* 30 units) were stirred magnetically in 2.9 cm^3 of a 0.1 M citrate ion buffer (pH 5.0) and maintained at 25 ^oC, using a thermostatically controlled water

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bath. Hydrogen peroxide (0.113 cm^3, 50 μmol, 0.44 M) was injected, using a continuous addition syringe pump over a period of 55 min. The reaction mixture was quenched after a further 10 min, by adding saturated sodium sulfite solution (2 cm^3). The mixture was extracted with CH_2Cl_2 (3 x 2 cm^3), the extract dried (Na_2SO_4) and filtered. Removal of the solvent gave the crude product, which was analysed by CSP-HPLC.

(+)-(*S*)-1,2-Dithiane-1-oxide 13*S*. Biotransformation of 1,2-dithiane **12** (3 mg, 25 μmol, 65 min) by CPO followed by CH_2Cl_2 extraction of the biotransformed material yielded (+)-(*S*)-1,2-dithiane-1-oxide **13*S*** (3.4 mg, 100%); *ee* 96% by CSP-HPLC (Table 3).

(+)-(*S*)-1,4-Dihydrobenzo-2,3-dithiane-2-oxide 15*S*. Biotransformation of 1,4-dihydrobenzo-2,3-dithiane **14** (4.2 mg, 25 μmol, 65 min) by CPO followed by CH_2Cl_2 extraction of the biotransformed material yielded (+)-(*S*)-1,4-dihydrobenzo-2,3-dithian-2-oxide **15*S*** (2.7 mg, 59%); *ee* 32% by CSP-HPLC (Table 3). When the above biotransformation was repeated using *tert*-butyl alcohol as co-solvent (0.9 cm^3) with citrate buffer (2.00 cm^3), (+)-(*S*)-oxide **15*S*** of 47% *ee* was obtained.

(iii) Naphthalene dioxygenase-catalysed sulfoxidation of 1,4-dihydrobenzo-2,3-dithiane 14 by *Pseudomonas putida* NCIMB 8859

(+)-(*S*)-1,4-Dihydrobenzo-2,3-dithiane-2-oxide 15*S*. Biotransformation of 1,4-dihydrobenzo-2,3-dithiane **14** (0.10 g, 0.60 mmol) by *P. putida* NCIMB 8859 followed by ethyl acetate extraction of the centrifuged culture medium yielded (+)-(*S*)-1,4-dihydrobenzo-2,3-dithiane-2-oxide **15*S*** as the only identified metabolite (12 mg, 11%) after purification by PLC (30% EtOAc/hexane); *ee* 9% by CSP-HPLC (Table 3). The monocyclic 1,2-disulfide **12** was not biotransformed under the same conditions.

(iv) Biotransformation of 1,4-dihydrobenzo-2,3-dithiane 14 by *Pseudomonas putida* UV4

Biotransformation of 1,4-dihydrobenzo-2,3-dithiane **14** (0.20 g, 1.19 mmol) by *P. putida* UV4 followed by the usual work up yielded 2-thiophthalide **16**, 6-hydroxy-2-thiophthalide **17** and (+)-(4*R*,5*S*)-4,5-dihydroxy-4,5-dihydrobenzo[*c*]thiophene **18**, after PLC separation (3% MeOH/$CHCl_3$).

2-Thiophthalide 16. White solid (31 mg, 17%); m.p. 55-56 °C ($CHCl_3$/hexane) (*lit.*,[21d,e] m.p. 56-57 °C); R_f 0.69 (3% MeOH/$CHCl_3$); (Found: C, 63.9; H, 4.0. C_8H_6OS requires C, 64.0; H, 4.0%); (Found: M^+, 150.0141. C_8H_6OS requires 150.0139); δ_H (500 MHz, $CDCl_3$) 4.48 (2 H, s, CH_2), 7.46-7.49 (1 H, td, $J_{5,4}$=$J_{5,6}$ 7.5, $J_{5,7}$ 0.4, 5-H), 7.54 (1 H, d, $J_{7,6}$ 7.7, 7-H), 7.61-7.64 (1 H, td, $J_{6,5}$=$J_{6,7}$ 7.5, $J_{6,4}$ 1.2, 6-H), 7.83 (1 H, d, $J_{4,5}$ 7.8, 4-H); δ_C (125 MHz, $CDCl_3$) 34.6, 123.9, 126.4, 128.0, 133.2, 135.9, 147.0, 197.8; *m/z* (EI) 150 (M^+, 100%), 122 (81), 121 (96), 105 (18), 89 (24), 78 (40), 63 (30), 51 (20); ν_{max} (KBr)/cm^{-1} 3082, 2922, 2850, 1686, 772.

6-Hydroxy-2-thiophthalide 17. An oil (1.9 mg, 1%); R_f 0.20 (3% MeOH/$CHCl_3$); (Found: M^+, 166.0092. $C_8H_6O_2S$ requires 166.0089); δ_H (500 MHz, $CDCl_3$) 4.39 (2 H, s, CH_2), 5.80 (1 H, br s, OH), 7.14-7.17 (1 H, dd, $J_{5,4}$ 8.4, $J_{5,7}$ 2.6 5-H), 7.24-7.25 (1 H, d, $J_{7,5}$ 2.5, 7-H), 7.38-7.40 (1 H, d, $J_{4,5}$ 8.4, 4-H), saturation at δ 4.39 gave a 0.8% nOe at δ 7.38-7.40; δ_C (125 MHz, $CDCl_3$) 34.1, 109.1, 121.5, 127.1, 137.4, 139.1, 156.1, 197.7; *m/z* (EI) 166 (M^+, 100%), 150 (20), 138 (35), 137 (63), 121 (13), 121 (15), 91 (27), 85 (28), 71 (43), 57 (54), 43 (43), 28 (26); ν_{max} (KBr)/cm^{-1} 3421, 3033, 2920, 2852, 1652, 1482, 1109, 720.

(+)-(4*R*,5*S*)-4,5-Dihydro-4,5-dihydroxybenzo[*c*]thiophene 18. White crystalline solid (3.8 mg, 2%); m.p. 114-116 °C ($CHCl_3$/hexane); R_f 0.06 (3% MeOH/$CHCl_3$); $[\alpha]_D$ +169 (*c* 0.23, $CHCl_3$); (Found: M^+, 168.0253. $C_8H_8O_2S$ requires 168.0251); δ_H (500 MHz, $CDCl_3$) 2.35 (2 H, br s, 2 x OH), 4.35-4.39 (1 H, td, $J_{5,4}$=$J_{5,6}$ 4.3, $J_{5,7}$ 1.1, 5-H), 4.76-4.77 (1 H, d, $J_{4,5}$ 4.5, 4-H), 5.95-5.98 (1 H, dd, $J_{6,7}$ 9.7, $J_{6,5}$ 4.0, 6-H), 6.59 - 6.61 (1 H, d, $J_{7,6}$ 9.8, 7-H), 7.07-7.08 (1 H, d, $J_{1,3}$ 2.7, 1-H), 7.34-7.35 (1 H, d, $J_{3,1}$ 2.7, 3-H); δ_C (125 MHz, $CDCl_3$) 69.1, 69.2, 121.9, 124.1, 125.0, 128.7, 129.6, 138.9; *m/z* (EI) 168 (M^+, 75%), 150 (59), 139 (42), 122 (100), 111 (35), 96 (27), 77 (42), 55 (31), 45 (53), 39 (37), 29 (29); ECD (MeCN): 253 nm $\Delta\varepsilon$ + 1.11, 239 nm $\Delta\varepsilon$ + 2.17, 219 nm $\Delta\varepsilon$ + 4.07, 192 nm $\Delta\varepsilon$ - 4.25; ν_{max} (KBr)/cm^{-1} 3421, 3032, 2924, 2853, 1651, 1099, 797.

(+)-(4*R*,5*S*)-4,5,6,7-Tetrahydro-4,5-dihydroxybenzo[*c*]thiophene 25. A solution of (+)-(4*R*,5*S*)-4,5-dihydro-4,5-dihydroxybenzo[*c*]thiophene **18** (9 mg, 0.054 mmol) containing Pd/C (10%, 5 mg) in ethyl acetate (5 cm^3) was stirred overnight under a hydrogen atmosphere. The catalyst was removed by filtration, the filtrate dried (Na_2SO_4) and concentrated under reduced pressure. The crude product obtained was purified by flash chromatography (EtOAc) to yield (+)-(4*R*,5*S*)-4,5,6,7-tetrahydro-4,5-dihydroxybenzo[*c*]thiophene **25** as a white crystalline solid (9 mg, 99%); m.p. 128-129 °C ($CHCl_3$/hexane); $[\alpha]_D$ + 19.6 (*c* 0.43, $CHCl_3$); (Found: M^+, 170.0409. $C_8H_{10}O_2S$ requires 170.0402); δ_H (500 MHz, $CDCl_3$) 1.82-1.88 (1 H, dtd, $J_{6eq,6ax}$ 13.3, $J_{6eq,5}$=$J_{6eq,7ax}$ 6.6, $J_{6eq,7eq}$ 2.7, 6-H_{eq}), 2.07-2.14 (1 H, dddd, $J_{6ax,6eq}$ 13.4, $J_{6ax,7ax}$ 8.1, $J_{6ax,5}$ 7.8, $J_{6ax,7eq}$ 5.6, 6-H_{ax}), 2.32 (2 H, br s, 2 x OH), 2.66 - 2.72 (1 H, dt, $J_{7ax,7eq}$ 16.6, $J_{7ax,6ax}$=$J_{7ax,6eq}$ 6.9, 7-H_{ax}), 2.89-2.95 (1 H, dt, $J_{7eq,7ax}$ 16.6, $J_{7eq,6ax}$=$J_{7eq,6eq}$ 6.4, 7-H_{eq}), 4.06 (1 H, dt, $J_{5,6ax}$=$J_{5,6eq}$ 8.8, $J_{5,4}$ 3.3, 5-H), 4.76 (1 H, d, $J_{4,5}$ 3.5, 4-H), 6.91-6.92 (1 H, d, $J_{1,3}$ 3.0, 1-H), 7.36-7.37 (1 H, d, $J_{3,1}$ 3.0, 3-H), saturation at δ 4.76 gave a 1.1% nOe at δ 4.06 and a 0.4% nOe at δ 7.36-7.37; δ_C (125 MHz, $CDCl_3$) 21.4, 26.9, 68.2, 69.9, 119.7, 124.0, 136.6, 139.5; *m/z* (EI) 170 (M^+, 43%), 152 (65), 126 (100), 125 (96), 97 (38), 45 (32); ECD (MeCN): 235 nm $\Delta\varepsilon$ + 0.47, 221 nm $\Delta\varepsilon$ + 0.85, 209 nm $\Delta\varepsilon$ + 2.37; ν_{max} (KBr)/cm^{-1} 3447, 3105, 2924, 2847, 1651, 1120, 669.

DiMTPA esters 26 of *cis*-diol 25. (+)-(*R*)-MTPA gave ester **26*R***; typical signals: δ_H (500 MHz, $CDCl_3$) 5.50 (5-H), 6.26 (4-H); (-)-(*S*)-MTPA furnished ester **26*S***; typical signals: δ_H (500 MHz, $CDCl_3$) 5.54 (5-H), 6.42 (4-H).

(v) Biotransformation of benzo[*c*]thiophene 19 by *Pseudomonas putida* UV4

Biotransformation of benzo[*c*]thiophene **19** (0.134 g, 1.00 mmol) by *P. putida* UV4 followed by ethyl acetate extraction of the centrifuged medium yielded (+)-(4*R*,5*S*)-4,5-dihydro-4,5-dihydroxybenzo[*c*]thiophene **18** (14 mg, 8%) as the only metabolite after purification by PLC (3% MeOH/$CHCl_3$). The sample of compound **18** was indistinguishable from the sample obtained from the 1,2-disulfide **14**.

(vi) Biotransformation of 1,3-dihydrobenzo[*c*]thiophene 20 by *Pseudomonas putida* UV4

Biotransformation of 1,3-dihydrobenzo[*c*]thiophene **20** (0.20 g, 1.47 mmol) by *P. putida* UV4 followed by the usual work up and PLC (2% MeOH/$CHCl_3$) separation of the crude bioproduct yielded 2-thiophthalide **16** (3 mg, 1.4%), 6-hydroxy-2-

thiophthalide **17** (2.4 mg, 1%) and (+)-(4*R*,5*S*)-4,5-dihydro-4,5-dihydroxybenzo[*c*]thiophene **18** (15 mg, 6%). *cis*-Dihydrodiol **18** was found to have an identical *ee* value (>98%) and absolute configuration to the sample isolated from 1,2-disulfide **14**.

(vii) Biotransformation of 1,3-dihydrobenzo[*c*]thiophene sulfoxide 21 by *Pseudomonas putida* UV4

Biotransformation of 1,3-dihydrobenzo[*c*]thiophene sulfoxide **21** (0.20 g, 1.32 mmol) by *P. putida* UV4 followed by the usual work up yielded 1,3-dihydrobenzo[*c*]thiophene **20** (11 mg, 6%) and 2-thiophthalide **16** (7 mg, 3.6%) after separation by PLC (2% MeOH/$CHCl_3$).

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References

1 (*a*) H. L. Holland, *Chem. Rev.*1988, **88**, 473; (*b*) H. L. Holland, *Nat. Prod. Rep.*, 2001, **18**, 171; (*c*) S. G. Burton, *Trends. Biotechnol.*, 2003, **21**, 543; (*d*) I. Fernandez, N. Khiar, *Chem. Rev.*, 2003, **103**, 3651; (*e*) C. M. Thomas, T. R. Ward, *Chem. Soc. Rev.*, 2005, **34**, 337; (*f*) E. Aranda, M. Kinne, M. Kluge, R. Ullrich, M. Hofrichter, *Appl. Microbiol. Biotechnol.*, 2009, **82**, 1057; (*g*) E. Wojaczynska, J. Wojaczynski, *Chem. Rev.*, 2010, **110**, 4303; (*h*) G. E. O'Mahony, P. Kelly, S. E. Lawrence, A. R. Maguire, *Arkivoc*, 2011, (i), 1; (*i*) C-S. Jiang, W. E. G. Muller, H. C. Schroder, Y-W. Guo, *Chem. Rev.*, 2012, **112**, 2179.

2 (*a*) W. L. Mock, *J. Am. Chem. Soc*., 1970, **92**, 7610; (*b*) W. S. Jenks, N. Matsunaga, M. Gordon, *J. Org. Chem.*, 1996, **61**, 1275; (*c*) D. R. Boyd, N. D. Sharma, B. T. McMurray, S. A. Haughey, C. C. R. Allen, J. T. G. Hamilton, W. C. McRoberts, R. A, More O'Ferrall, J. Nikodinovic-Runic, L. A. Coulombel, K. E. O'Connor, *Org. Biomol. Chem*., 2012, **10**, 782.

3 (*a*) W. E. Savige, A. Fava, *J. Chem. Soc. Chem. Commun.*, 1965, 417; (*b*) M. Mikolajczyk, J. Drabowicz, *J. Am. Chem. Soc.*, 1978, **100**, 2510; (*c*) T. Takata, T. Endo in *The Chemistry of Sulfinic Acids, Esters and Their Derivatives*, S. Patai. Ed.; John Wiley and Sons; Chichester; 1990, 527.

4 (*a*) J. R. Cashman, L. D. Olsen, D. R. Boyd, R. A. S. McMordie, R. Dunlop, H. Dalton, *J. Amer. Chem. Soc.,* 1992, **114**, 8772; (*b*) K. Lee, J. M. Brand, D. T. Gibson, *Biochem. Biophys. Res. Commun.,* 1995, **212**, 9; (*c*) C. C. R. Allen, D. R. Boyd, H. Dalton, N. D. Sharma, S. A. Haughey, R. A. S. McMordie, B. T. McMurray, G. N. Sheldrake, K. Sproule, *J. Chem. Soc., Chem. Commun*, 1995, 119; (*d*) D. R. Boyd, N. D. Sharma, S. A. Haughey, M. A. Kennedy, B. T. McMurray, G. N. Sheldrake, C. C. R. Allen, H. Dalton, K. Sproule. *J. Chem. Soc., Perkin Trans 1*, 1998, 1929; (*e*) D. R. Boyd, N. D. Sharma, V. Ljubez, B. E. Byrne, S. D. Shepherd, C. C. R. Allen, L. A. Kulakov, M. L. Larkin, H. Dalton, *Chem. Commun.,* 2002, 1914; (*f*) A. J. Fin, O. Pavlyuk, T. Hudlicky, *Collect. Czech. Chem. Commun.*, 2005, **70**, 1709; (*g*) W. Adam, F. Heckel, C. R. Saha-Moller, M. Taupp. J-M. Meyer, P. Schreier, *Appl. Environ. Microbiol.*, 2005, **71**, 2199.

5 (*a*) D. R. Boyd, N. D. Sharma, S. A. Haughey, J. F. Malone, A. W. T. King, B. T. McMurray, A. Alves-Areias, C. C. R. Allen, R. Holt, H. Dalton, *J. Chem. Soc., Perkin Trans.1*, 2001, 3288; (*b*) D. R. Boyd, N. D. Sharma, S. Haughey, M. A. Kennedy, J. F. Malone, S. D. Shepherd, C. C. R. Allen, H. Dalton, *Tetrahedron*, 2004, **60**, 549; (*c*) D. R. Boyd, N. D. Sharma, M. A. Kennedy, S. D. Shepherd, J. F. Malone, A. Alves-Areias, R. Holt, S. G. Allenmark, M. A. Lemurell, H. Dalton, H. Luckarift, *Chem. Commun.,* 2002, 1452.

6 (*a*) F. Freeman, *Chem. Rev.* 1984, **84**, 117; (*b*) P. L. Folkins, D. N. Harpp, *J. Am. Chem. Soc.*, 1993, **115**, 3066; (*c*) A. Ishii, M. Nakabayashi, J. Nakayama, *J. Am. Chem. Soc.*, 1999, **121**, 7959; (*d*) D. D. Gregory, W. S. Jenks, *J. Phys. Chem. A*, 2003, **107**, 3414.

7 (*a*) G. I. Giles, K. M. Tasker, C. Jacob, *Gen. Physiol. Biophys*., 2002, **21**, 65; (*b*) K. C. Gates, *Chem. Res.Toxicol.*, 2000, **13**, 953; (*c*) K. Keerthi, A. Rajapakse, D. Sun, K. C. Gates, *Biorg. Med. Chem*., 2013, **21**, 235.

8 (*a*) J. L. Kice, G. B. Large, *J. Am. Chem. Soc.*, 1968, **90**, 4069; (*b*) F. A. Davis, R. H. Jenkins, S. B. Awad, O. D. Stringer, W. H. Watson, J. Galloy, *J. Am. Chem. Soc.*, 1982, **104**, 5412; (*c*) C. Nemecek, E. Dunach, H. B, Kagan, *New. J. Chem.*, 1986, **10**, 761; (*d*) G. Liu, J. A. Ellman, *J. Am. Chem. Soc.*, 1997, **119**, 9913; (*e*) D. A. Cogan, G. Liu, K. Kim, B. J. Backes, J. A. Ellman, *J. Am. Chem. Soc.*, 1998, **120**, 8011; (*f*) D. J. Weix, J. A. Ellman, *Org. Synth*., 2005, **82**, 157.

9 (*a*) E. Block, S. Ahmad, J. L. Catalfamo, M. K. Jain, R. Apitz-Castro, *J. Am. Chem. Soc.*, 1986, **108**, 7045; (*b*) C. Teyssier, L. Guenot, M. Suschetet, M-H. Siess, *Drug.Metab. Dispos*., 1999, **27**, 835; (*c*) C. Teyssier, M-H. Siess, *Drug.Metab. Dispos*., 2000, **28**, 648; (*d*) D. P. Ilic, V. D. Nikolic, L. B. Nikolic, M. Z. Stankovic, L. P. Stanojevic, M. D. Cakic, *Physics Chemistry and Technology*, 2011, **9**, 9; (*e*) D. Fukushima, Y. H. Kim, T. Iyanagi, A. Oae, *J. Biochem*, 1978, **83**, 1019.

10 S. Colonna, N. Gaggero, G. Carrea, P. Pasta, V. Alphand, R. Furstoss, *Chirality*, 2001, **13**, 40.

11 (*a*) C.M. Remy, *Biochim. Biophys. Acta,* 1967, **138**, 258; (*b*) T. Fujita, Z. Suzuoki, S. Kozuka, S. Oae, *J. Biochem*, 1973, **74**, 723.

12 (*a*) P. Koch, A. Fava, *J. Am. Chem. Soc*., 1968, **90**, 3867; (*b*) E. Block, J. O'Connor, *J. Am. Chem. Soc*., 1974, **96**, 3629.

13 (*a*) S. Colonna, N. Gaggero, L. Carella, G. Carrea, P. Pasta, *Tetrahedron: Asymmetry*, 1992, **3**, 95; (*b*) S. Allenmark, M. A. Andersson, *Tetrahedron: Asymmetry*, 1996, **7**, 1089; (*c*) S. Colonna, N. Gaggero, G. Carrea, P. Pasta, *J. Chem. Soc. Chem. Commun.,* 1997, 439; (*d*) S. Allenmark, M. Andersson, *Chirality*, 1998, **10**, 264; (*e*) S. Colonna, N. Gaggero, C. Richelmi, P. Pasta, *Trends Biotechnol.,* 1999, **17**, 163; (*f*) S. Colonna, S. Del Sordo, N. Gaggero, G. Carrea, P. Pasta, *Heterat.Chem.,* 2002, **13**, 467; (*g*) H. L. Holland, F. M. Brown, D. LoZada, B. Mayne, W. R. Szerminski, A. J. Van Vliet, *Can. J. Chem*., 2002, **80**, 633.

14 S. V. Dzyuba, A. M. Klibanov, *Biotechnol. Lett.,* 2003, **25**, 1961.

15 (*a*) D. R. Boyd, N. D. Sharma, A. W. T. King, S. D. Shepherd, C. C. R. Allen, R. Holt, H. Luckarift, H. Dalton, *Org. Biomol. Chem*., 2004, **2**, 554; (*b*) H. R. Luckarift, H. Dalton, N. D. Sharma, D. R. Boyd, R. A. Holt, *Appl. Microbiol. Biotechnol.*, 2004, **65**, 678.

16 (*a*) D. R. Boyd, N. D. Sharma, N. I. Bowers, I. N. Brannigan, M. R. Groocock, J. F. Malone, G. McConville, C. C. R. Allen, *Adv. Synth. Catal.,* 2005, **347**, 1081; (*b*) D. R. Boyd, N. D. Sharma, I. A. Brannigan, T. A. Evans, S. A. Haughey, B. T. McMurray, J. F. Malone, P. B. A. McIntyre, P. J. Stevenson, C. C. R. Allen, *Org. Biomol. Chem*., 2012, **10**, 7292.

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17 D. R. Boyd, N. D. Sharma, R. Boyle, R. A. S. McMordie, J. Chima, H. Dalton. *Tetrahedron Lett.*, 1992, **33**, 1241.

18 R. P. Kreher, J. Kalishko, *Chem. Ber*., 1991, **124**, 645.

19 (*a*) D. R. Boyd, N. D. Sharma, T. A. Evans, M. Groocock, J. F. Malone, P. J. Stevenson, H. Dalton, *J. Chem. Soc., Perk. Trans. 1*, 1997, 1879; (*b*) N. I. Bowers, D. R. Boyd, N. D. Sharma, P. A. Goodrich, M. R. Groocock, A. J. Blacker, P. Goode, H. Dalton, *J. Chem. Soc. Perkin Trans 1*, 1999, 1453; (*c*) D. R. Boyd, N. D. Sharma, N. I. Bowers, R. Boyle, J. S. Harrison, K. Lee, T. D. H. Bugg, D. T. Gibson, *Org. Biomol. Chem*., 2003, **1**, 1298; (*d*) D. R. Boyd, N. D. Sharma, N. A. Kerley, R. A. S. McMordie, G. N. Sheldrake, P. Williams, H. Dalton, *J. Chem. Soc., Perkin Trans. 1*, 1996, 67; (*e*) D. R. Boyd, N. D. Sharma, N. I. Bowers, J. Duffy, J. F. Malone, J. S. Harrison, H. Dalton, *J. Chem. Soc., Perk. Trans. 1*, 2000, 1345.

20 (*a*) G. Derbesy, D. N. Harpp, *Tetrahedron Lett*., 1994, **35**, 5381; (*b*) A. K. Bhattacharya, A. G. Hortmann, *J. Org. Chem*., 1978, **43**, 2728; (*c*) D. N. Harpp, D. K. Ash, T. G. Back, J. G. Gleason, *Tetrahedron Lett*., 1970, 3551; (*d*) W. G. Bentrude, T. Kawashima, B. A. Keys, M. Garroussian, W. Heide, D. A. Wedgaertner, *J. Am. Chem. Soc*., 1987, **109**, 1227; (*e*) N. A. Noureldin, M. Caldwell, J. Hendry, D. G. Lee, *Synthesis*, 1998, 1587; (*f*) D. N. Harpp, S. J. Bodzay, T. Aida, T. H. Chan, *Tetrahedron Lett*., 1986, **27**, 441; (*g*) B. Milligan, J. M. Swan, *J. Chem. Soc*., 1965, 2901.

21 (*a*) M. Yamato, Y. Takeuchi, K. Hattori, K. Hashigaki, *Synthesis*, 1982, 1014; (*b*) M. P. Cava, N. M. Pollack, O. A. Mamer, M. J. Mitchell, *J. Org. Chem.*, 1971, **36**, 3932; (*c*) B. A. Kellogg, R. S. Brown, R. S. McDonald, *J. Org. Chem.*, 1994, **59**, 4652; (*d*) D. R. Storm, D. E. Koshland Jr., *J. Am. Chem. Soc.*, 1972, **94**, 5815.

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