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PAPER

Bacterial dioxygenase- and monooxygenase-catalysed sulfoxidation of benzo[*b*]thiophenes†

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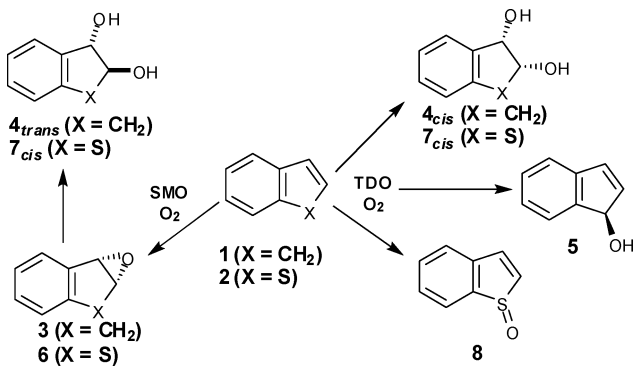
Asymmetric heteroatom oxidation of benzo[*b*]thiophenes to yield the corresponding sulfoxides was catalysed by toluene dioxygenase (TDO), naphthalene dioxygenase (NDO) and styrene monooxygenase (SMO) enzymes present in *P. putida* mutant and *E. coli* recombinant whole cells. TDO-catalysed oxidation yielded the relatively unstable benzo[*b*]thiophene sulfoxide; its dimerization, followed by dehydrogenation, resulted in the isolation of stable tetracyclic sulfoxides as minor products with *cis*-dihydrodiols being the dominant metabolites. SMO mainly catalysed the formation of enantioenriched benzo[*b*]thiophene sulfoxide and 2-methyl benzo[*b*]thiophene sulfoxides which racemized at ambient temperature. The barriers to pyramidal sulfur inversion of 2- and 3-methyl benzo[*b*]thiophene sulfoxide metabolites, obtained using TDO and NDO as biocatalysts, were found to be *ca.*: 25–27 kcal mol^{-1}. The absolute configurations of the benzo[*b*]thiophene sulfoxides were determined by ECD spectroscopy, X-ray crystallography and stereochemical correlation. A site-directed mutant *E. coli* strain containing an engineered form of NDO, was found to change the regioselectivity toward preferential oxidation of the thiophene ring rather than the benzene ring.

Introduction

Monooxygenase- and dioxygenase-catalysed oxidations of the isosteric substrates indene **1**,[1a–c,2a–f] benzo[*b*]thiophene (B[*b*]T) **2**,[3a–l] and substituted derivatives, have previously been studied in these and other laboratories. The stereoselective oxidation product from indene **1**, was the (1*S*,2*R*)-epoxide **3** (up to 97% *ee*, Scheme 1) when using SMO from a *Pseudomonas putida* strain (CA-3) and derived *E. coli* recombinant strains.[1a–c]

Earlier studies [2a–f] have also shown that biocatalytic asymmetric *cis*-dihydroxylation of the alkene bond in substrate **1**, using different *P. putida* strains, can give the corresponding (1*S*,2*R*)-dihydrodiol $\mathbf{4}_{cis}$ with variable degrees of stereoselectivity (20–>98% *ee*) depending on the type of dioxygenase used. *Rhodococcus* strains have also been reported to yield epoxide **3**, *trans*-indandiol $\mathbf{4}_{trans}$ and *cis*-indandiol $\mathbf{4}_{cis}$ among bioproducts obtained *via* oxygenase-catalysed oxidation of indene **1**.[2e,2f] In addition, asymmetric benzylic hydroxylation of indene **1** to yield (1*R*)-indenol **5** of variable enantiopurity (up to >98% *ee*) was also observed using different *P. putida* dioxygenases (Scheme 1).[2a–d] Thus epoxide **3**, *cis*-diol $\mathbf{4}_{cis}$, and benzylic alcohol **5**, can each be formed by oxygenase-catalysed asymmetric oxidation of indene **1** with high *ee* values (≥97%) according to the type of oxygenase enzyme selected.



Scheme 1 Isolated and potential products resulting from enzyme–catalysed oxidation of the five-membered rings in indene **1** and benzo[*b*]thiophene **2**.

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The prevalence of B[*b*]T **2** and derivatives in the environment, *e.g.* in crude oil, coal and their combustion products, had prompted earlier studies of their bacterial biotransformation.[3a–l] However, with the exception of our preliminary reports from this study, obtained with B[*b*]T substrates and TDO from *Pseudomonas putida* UV4,[3e,i,j] most of the earlier studies[3a–d,3f–h] on prokaryotic metabolism of B[*b*]T **2** and methyl substituted benzo[*b*]thiophene (MB[*b*]T) derivatives were conducted on a relatively small scale. After extraction (EtOAc) of aqueous culture medium from biotransformations and concentration of the extract, the bioproducts were generally detected using GC-MS or GC-FTIR analysis of the crude mixture.[3a–d,3f–h] The GC analytical method resulted in partial decomposition of some metabolites, *e.g.* B[*b*]T sulfoxides (B[*b*]T-1-oxides), within the injection port,[3d] and precluded the full structural and stereochemical characterization (*ee* and absolute configuration) of individual metabolites from the mixture of bioproducts.

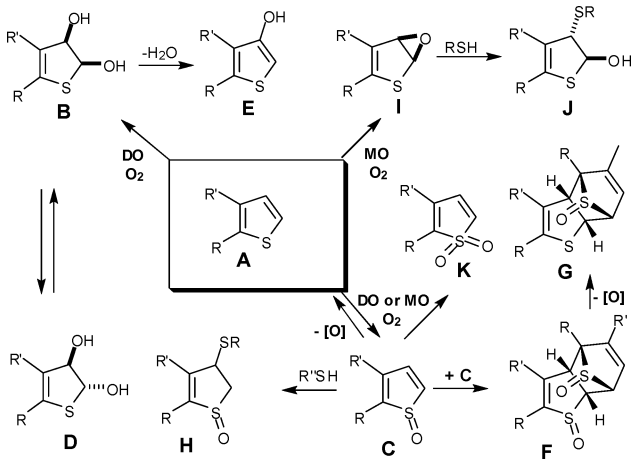
The major objectives of this more comprehensive study following from our earlier communications[3e,i,j] are: (i) to fully characterize our previously reported and newly isolated TDO-catalysed sulfoxidation products from B[*b*]T **2** and MB[*b*]T substrates, (ii) to compare the range and stereochemistry of bioproducts obtained using these substrates with both dioxygenase (TDO, NDO) and monooxygenase (SMO) enzymes and (iii) to determine the *ee* values and absolute configurations of chiral sulfoxide metabolites from three B[*b*]T substrates and to study their thermally-induced racemization.

Results and discussion

The biocatalytic oxidation products **3–5**, derived earlier from indene **1**, were of sufficient stability to be isolated and fully characterized structurally and stereochemically.[1a–c,2a–f] This contrasted with our preliminary observations from the oxygenase-catalysed oxidation of the isosteric substrate **2**.[3e,3i,3j] Thus it was found that: (i) the initially formed minor sulfoxide metabolite **8**, when obtained using TDO, was too unstable to be detected and isolated, (ii) the major *cis*-diol metabolite **7** formed using TDO was prone to spontaneous equilibration with the *trans* isomer in $CDCl_3$,[3e] (iii) the potential arene oxide metabolite **6**, using SMO enzyme (*cf.* the indene epoxide metabolite **3**) was not detected. Arene oxide **6** has only been characterized by ^{1}H-NMR analysis (THF-d_8) of the chemically synthesised crude sample which rapidly decomposed to an isomeric ketone during attempted purification.[4,5]

TDO-catalysed oxidation (TO) of monocyclic thiophenes **A**, using *P. putida* UV4 whole cells (Scheme 2), has recently been found to occur at both the sulfur atom (sulfoxidation) to yield the unstable monosulfoxides **C** and at the 2,3-bond (*cis*-dihydroxylation) to give *cis*-diols **B** as initial metabolites.[6] Under acidic conditions the equilibrating mixture of *cis*-diols **B** and *trans*-diols **D** was also found to dehydrate yielding hydroxythiophenes **E**. Due to the spontaneous dimerization of the transient monocyclic thiophene monosulfoxides **C**, and their disproportionation products, thiophenes **A** and sulfones **K**, and disulfoxide adducts **F** were isolated as metabolites. A sulfoxide reductase-catalysed deoxygenation of stable disulfoxides **F** also gave the corresponding monosulfoxides **G**.[5]

CYP-450 monooxygenase-catalysed sulfoxidation (MO) of monocyclic thiophenes **A** was again found to yield the unstable



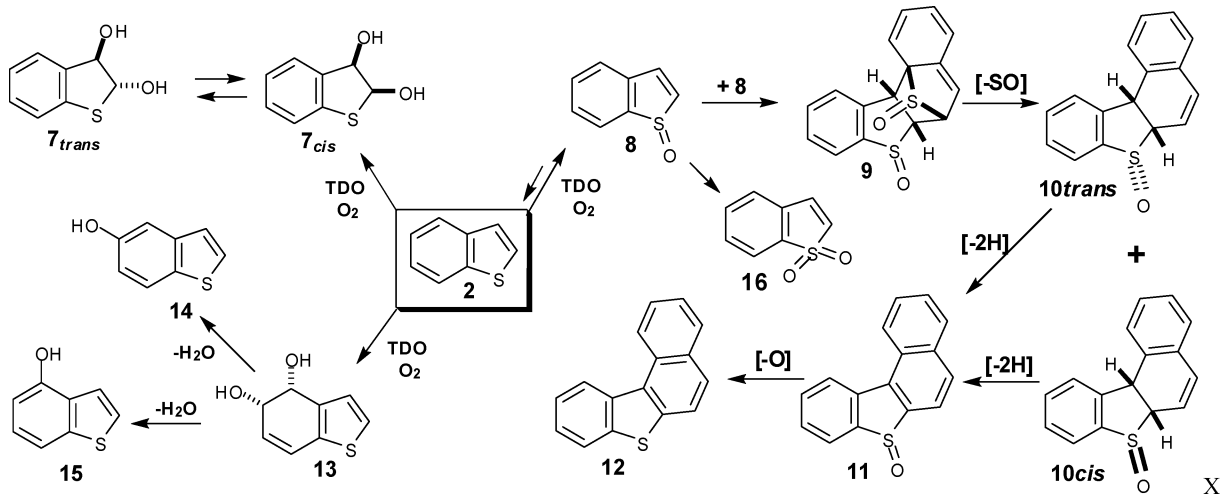
Scheme 2 Oxygenase-catalysed oxidative pathways of monocyclic thiophenes (R and R′ = H, alkyl or aryl groups and halogen atoms).

sulfoxides **C** followed by dimerization to yield disulfoxides **F** and, after chemical deoxygenation, monosulfoxide adducts **G**.[7] The elusive monosulfoxides **C** were also found to form sulfoxide–thioether adducts **H** with thiols. The isolation of *trans*-hydroxythioether metabolites **J**, has been postulated to result from the CYP-450 monooxygenase-catalysed formation of unstable thiophene arene oxide metabolites **I** followed by nucleophilic attack of thiols (*e.g.* glutathione).[7] Due to their instability, arene oxide derivatives of monocyclic thiophenes have not yet been isolated or detected.[4]

The metabolic steps shown in Scheme 2 could, in principle, be applicable to mono- and bi-cyclic thiophenes. Preliminary communications of this study showed that the major biotransformation pathway of benzo[*b*]thiophene **2** using *P. putida* UV4, resulted in TDO-catalysed *cis*-dihydroxylation of the thiophene ring to yield *cis*-diol $\mathbf{7}_{cis}$, which equilibrated with *trans*-diol $\mathbf{7}_{trans}$ (Scheme 3).[3e,i,j] The initial biotransformation of B[*b*]T **2** was carried out over an extended period (24 h) followed by column chromatography of the crude mixture of bioproducts; it yielded mainly dihydrodiols $\mathbf{7}_{cis}/\mathbf{7}_{trans}$ and phenols **14** and **15** as minor bioproducts (Scheme 3) with little evidence of sulfoxidation products. It was assumed that phenol **14** and **15** were derived from dehydration of the carbocyclic *cis*-dihydrodiol **13** which was much less stable than the heterocyclic dihydrodiols $\mathbf{7}_{cis}/\mathbf{7}_{trans}$. When the biotransformation was repeated on a larger scale using a shorter biotransformation period (7 h), the heterocyclic ring dihydrodiols $\mathbf{7}_{cis}/\mathbf{7}_{trans}$ were again the dominant metabolites along with phenols **14** and **15**. However, additional products, including the less stable carbocyclic *cis*-dihydrodiol **13** and the tetracyclic sulfoxidation products, **10–12** were also isolated.

A comparison of the relative yields of bioproducts, obtained from biotransformation of B[*b*]T, 2-MB[*b*]T and 3-MB[*b*]T with several dioxygenases (TDO, NDO_1, NDO_2, NDO_3) and a monooxygenase enzyme (SMO), that demonstrated a strong preference for the sulfoxidation pathway is shown in Table 1. As this study is primarily focused on oxygenase-catalysed heteroatom oxidation and the structure, stereochemistry and reactivity of the resulting sulfoxides, only the relative proportions of other metabolites have been shown (Table 1).

A discussion of the full structural characterization, enantiopurity, absolute configuration and synthetic applications of



Scheme 3 Dioxygenase-catalysed oxidation of B[*b*]T **2**.

Table 1 Relative yields of bioproducts obtained using substrates **2**, **17** and **22**

		Bioproducts detected (Relative % yield)			
Enz.	B[*b*]T	B[*b*]TSO[a]	BC/TD[b]	BCD[c]	Other bioproducts
TDO[d]	**2**		**7** (63)	**13** (10)	**10** (5), **11** (5), **12** (2), **14** (8), **15** (7)
NDO_1[e]	**2**		**7** (85)		**11** (15)
NDO_2[f]	**2**				**10** (50), **11** (50)
NDO_3[g]	**2**		**7** (85)		**11** (15)
SMO[h]	**2**	**8** (>95)			
TDO[d]	**17**	**18** (7)	**19** (10)	**20** (83)	
NDO_1[e]	**17**	**18** (10)		**20** (90)	
NDO_2[f]	**17**	**18** (90)			**21** (10)
NDO_3[g]	**17**	**18** (40)	**19** (60)		
SMO[h]	**17**	**18** (100)			
TDO[d]	**22**			**24** (100)	
NDO_1[e]	**22**	**23** (90)	**24** (10)		
NDO_2[f]	**22**	**23** (39)			**26** (33), **27** (28)
NDO_3[g]	**22**	**23** (100)			
SMO[h]	**22**	[i]			

[a] B[*b*]TSO (B[*b*]T sulfoxides). [b] BC/TD (B[*b*]T) heterocyclic ring *cis* or *trans* diols). [c] BCD (B[*b*]T carbocyclic *cis*-diols). [d] *P. putida* UV4. [e] *P. putida* 9816/11. [f] *P. putida* NCIMB 8859. [g] *E. coli* F352V. [h] *E.coli* BL21 (DE3)-pRSET-styAB. [i] No sulfoxide formed.

cis-dihydrodiols derived from both the carbocyclic and heterocyclic ring of benzo[*b*]thiophenes isolated during this work, and from the corresponding benzo[*b*]furan (B[*b*]F) substrates, will be presented in a later publication.[7]

The biotransformation of B[*b*]T **2** in *P. putida* UV4 resulted in a strong preference for TDO-catalysed oxidation to occur within the electron-rich thiophene ring of the substrate with *cis*-hydroxylation and sulfoxidation accounting for *ca.*: 75% of the identified metabolites (**7**$_{cis}$ **10**$_{cis}$, **10**$_{trans}$, **11**, **12**).

The equilibrating *cis*/*trans* diol mixture **7**$_{cis}$/ **7**$_{trans}$ was isolated as the major product (63% relative yield). However, the stable tetracyclic derivatives **10–12**, resulting from dimerization of the initial transient B[*b*]T sulfoxide (**8** → **9**), accounted for a minor, but significant, proportion (12%) of the isolated metabolites (Table 1). Although the initially formed sulfoxide **8** was not detected during the earlier biotransformations of B[*b*]T **2**, recent studies using LC-TOFMS analysis showed it to be an identifiable metabolite formed during the early stages of the biotransformation. However it could not be isolated from among the mixture of metabolites (**8–15**, Scheme 3).

An early attempt to synthesise an authentic sample of sulfoxide **8** by MCPBA oxidation of B[*b*]T **2** gave mainly the B[*b*]T sulfone (B[*b*]T-1,1-dioxide) **16** (60% isolated yield) along with small amounts (<1%) of each of the tetracyclic sulfoxides **10**$_{cis}$, **10**$_{trans}$ and **11** and benzo[*b*]naphtho[2,1-*d*]thiophene **12**. Formation of the last product suggests that a degree of spontaneous deoxygenation had occurred, possibly *via* disproportionation. A later attempt to obtain the unstable sulfoxide **8**, under milder conditions, using dimethyldioxirane as oxidant in acetone-d_6 solvent at ambient temperature, provided direct ^{1}H-NMR evidence of its formation in solution.

The biosynthetic sequence involved in the formation of the tetracyclic metabolites **10–12** from B[*b*]T **2** has not yet been rigorously established. However, the earlier formation and dimerization of a wide range of monocyclic thiophene sulfoxides, using *P. putida* UV4, to give a series of stable racemic disulfoxides (compound

F, R = H and R′ = H; R′ = H and R = Me, Et, Cl, Br, Ph, Scheme 2), whose structure and stereochemistry was confirmed by X-ray crystallography and NMR spectroscopy, provide a credible precedent.[6]

Although disulfoxide **9** was not isolated, a fully deoxygenated derivative has recently been detected by GC-MS analysis following a whole cell biotransformation of B[*b*]T **2** using a *Sphingomonas* strain (XLDN2-5).[3l] Our inability to detect disulfoxide **9** as a metabolite of B[*b*]T **2**, or as a product from chemical oxidation, may be due to the spontaneous formation of the stereoisomeric tetracyclic metabolites $\mathbf{10}_{cis}$ and $\mathbf{10}_{trans}$.

The extrusion of sulfur monoxide from tetracyclic disulfoxide **9** to yield $\mathbf{10}_{cis}$ and $\mathbf{10}_{trans}$ would result in the reformation of a benzene ring while a similar reaction of a bicyclic disulfoxide (*e.g.* **F**, R and R′ = H, Scheme 2) formed from a monocyclic thiophene sulfoxide (*e.g.* **C**, R and R′ = H) would produce a thiophene ring. The larger increase in resonance energy resulting from formation of the tetracyclic monosulfoxides $\mathbf{10}_{cis}$ and $\mathbf{10}_{trans}$ compared with the bicyclic monosulfoxides (*e.g.* **G**, R and R′ = H, Scheme 2) may be an important factor in the instability of disulfoxide **9**.

In our preliminary communication,[3j] the possibility of a cycloaddition reaction occurring between the substrate B[*b*]T **2** and the B[*b*]T sulfoxide metabolite **8** was discussed. However, in light of more recent results showing that: (i) *P. putida* UV4 contains a sulfoxide reductase enzyme with an ability to catalyse the mono-deoxygenation of monocyclic thiophene sulfoxide dimers (F, Scheme 2) [8] and (ii) both benzo[*b*]naphtho[2,1-*d*]thiophene **12** and its monosulfoxide **11** can be formed as minor products from MCPBA oxidation of B[*b*]T **2** followed by abiotic deoxygenation, the metabolic sequence **8** → **9** → $\mathbf{10}_{cis}/\mathbf{10}_{trans}$ → **11** → **12**, shown in Scheme 3, is proposed.

In common with the disulfoxides, formed by dimerization of monocyclic thiophene sulfoxides (*e.g.* compound **F** → **G**, R and R′ = H, R = H and R′ = Me, Scheme 2),[8] the *trans* isomer $\mathbf{10}_{trans}$ was formed in preference (3 : 1) to the *cis* diastereoisomer $\mathbf{10}_{cis}$. The separated tetracyclic sulfoxide metabolites $\mathbf{10}_{trans}$ ($[\alpha]_D$ +65) and **11** ($[\alpha]_D$ −13), whose absolute configurations were not determined, had very low enantiopurity values (8% *ee* and 3% *ee* respectively by chiral stationary phase HPLC analysis).

(−)-Benzo[*b*]naphtho[2,1-*d*]thiophene-7-oxide **11** appeared to be configurationally stable, *i.e.* showed no evidence of spontaneous racemization at ambient temperature. This would be expected for a sulfoxide inversion barrier ($\Delta G^{\ddagger}$) greater than 23 kcal mol^{-1} and was in accord with the calculated value of $\Delta G^{\ddagger}$ = 32.3 kcal mol^{-1} for dibenzo[*b*]thiophene sulfoxide.[9a] The low enantiopurity values (<10% *ee*) observed for the tetracyclic sulfoxides $\mathbf{10}_{trans}$ and **11** could result from several metabolic sequences. These include: (i) an initial enzyme-catalysed asymmetric sulfoxidation (**2** → **8**), (ii) a sulfoxide reductase-catalysed kinetic resolution involving a mono-deoxygenation step (**8** → **2** or **11** → **12**), (iii) a combination of these pathways allied to partial racemization of sulfoxide **2**.

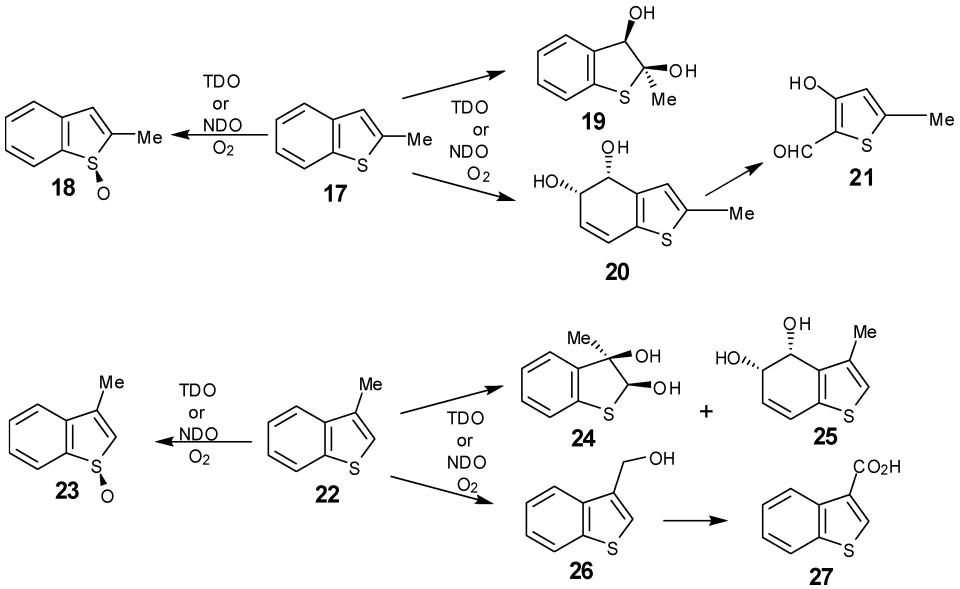
Whole cells of *P. putida* UV4, a source of TDO, are known to catalyse a wide range of aromatic dihydroxylations and sulfoxidations.[3e,3i,3j,10a–c] The relative yields of the B[*b*]T metabolites obtained using three bacterial strains each containing naphthalene dioxygenase (NDO) are shown in Table 1. *P. putida* 9816/11(NDO_1, an inducible mutant strain) and *P. putida* NCIMB 8859 (NDO_2, a wild-type strain), each contain NDO and can catalyse both vicinal dihydroxylations and sulfoxidations of aromatic substrates.[10a,10c] However, the wild-type strain cannot accumulate *cis*-dihydrodiols due to the presence of a *cis*-diol dehydrogenase which catalyses their further biotransformation into catechols. In contrast with TDO, NDO can only catalyse a very limited range of arene *cis*-dihydroxylations *e.g.* mainly biaryls and polycyclic arenes.[11a–c] A site-directed mutant strain, *E. coli* F352V, containing the same NDO gene (NDO_3) as *P. putida* 9816/4, but with the phenylalanine amino acid, Phe 352, at the active site replaced by valine,[11a] was also used in the biotransformation of B[*b*]T substrates. Earlier studies had shown that the regioselectivity of *cis*-dihydroxylation found using NDO_1 from *P. putida* 9816/11 on biaryl substrates (*e.g.* biphenyl and 2-phenyl pyridine), polycyclic arenes (*e.g.* naphthalene, phenanthrene), and polycyclic azaarenes (*e.g.* benzo[*h*]quinoline and phenanthridine) could be redirected in some cases, when *E. coli* F352V (NDO_3) was used.[11a–c] Evidence of a similar change in regioselectivity was sought during NDO_3-catalysed sulfoxidation or *cis*-dihydroxylation of polycyclic thiaarenes when using B[*b*]T and MB[*b*]T substrates.

The oxidation of B[*b*]T **2** using *P. putida* 9816/11 (NDO_1), *P. putida* NCIMB 8859 (NDO_2) and *E. coli* F352V (NDO_3) only showed evidence of oxidation within the electron-rich thiophene ring giving bioproducts **7**, **10** or **11** (Table 1). As anticipated, no *cis*-dihydrodiol products were observed using the wild-type strain (*P. putida* NCIMB 8859) and only the tetracyclic adducts, **10** and **11** derived from B[*b*]T **2** were detected, albeit in very low isolated yield (<5%). The heterocyclic dihydrodiol $\mathbf{7}_{cis}/\mathbf{7}_{trans}$ was formed as the major product (85%) along with the tetracyclic sulfoxide **11** (15%) using both NDO_2 and NDO_3 (Table 1). As expected from earlier *cis*-dihydroxylations, using *E. coli* F352V (NDO_3),[11a–c] the isolated yields were much lower compared with those obtained using *P. putida* 9816/11 (NDO_1).

Following earlier bacterial metabolism studies of B[*b*]T **2**, GC-MS analysis showed the presence of both sulfoxide **8** and sulfone **16**.[3g,3h,3l] A similar GC-MS approach was initially employed in the current study using the recombinant strain *E.coli* BL21 (DE3)-pRSET-styAB, a source of styrene monooxygenase (SMO).[12a–c] This method again showed the presence of B[*b*]T-1-oxide **8** as the major metabolite (>95%), and B[*b*]T-1,1-dioxide **16** as a minor product (<5%). This metabolic profile was confirmed by reverse phase LC-TOFMS analysis (Table 1). The relatively pure sample of B[*b*]T sulfoxide **8** thus obtained, enabled a full structure characterization to be carried out.

Sulfone **16** could have been formed from sulfoxide **8** by: (a) decomposition of B[*b*]T sulfoxide **8** in the GC injection port (as reported for 2-MB[*b*]T sulfoxide **18**,[3d] or (b) a disproportionation reaction (with B[*b*]T **2** as the other product) during the work up procedure involving extraction, concentration and purification.

We anticipated that it would be quite difficult to isolate and determine either the enantiopurity or absolute configuration of sulfoxide **8** due to its thermal instability and possible spontaneous racemisation, as predicted from an earlier calculation of its barrier to inversion ($\Delta G^{\ddagger}$ *ca.*: 24 kcal mol^{-1} at 25 °C).[9a] To circumvent these potential difficulties, the cooled (*ca.*: 4 °C) aqueous culture medium, containing the bioproducts from a biotransformation of B[*b*]T **2**, using SMO [whole cells of *E. coli* BL21 (DE3)-pRSET-styAB], was concentrated at ice bath temperature and the concentrate quickly extracted (EtOAc) to minimize the exposure of bioproducts at ambient temperature. The solvent was removed at a similar temperature, the residue dried at 0 °C under high



Scheme 4 Dioxygenase-catalysed oxidation of B[*b*]T **17** and **22**.

vacuum and stored below –20 °C. The ^{1}H-NMR spectrum of the crude bioproduct indicated the presence of sulfoxide **8** as the major metabolite (*ca.*: 97%) and sulfone **16** as the minor product (*ca.*: 2%) with trace amounts of substrate **2**. After recording the optical rotation (sign and magnitude) of a portion of the crude product in $CHCl_3$ solution, the sample was carefully recovered and purified by PLC (EtOAc–hexane, 1 : 1). The purified, dried and weighed sample was then used to calculate the optical rotation ($[\alpha]_D$ –235, $CHCl_3$) of sulfoxide **8**. The $[\alpha]_D$ value of the purified sulfoxide **8** recorded may not be the maximum possible. It registered a rapid decrease and after keeping the solution at room temperature for a few hours the sample was found to have an extremely low value ($[\alpha]_D$ –3). ^{1}H- and ^{13}C-NMR spectral data of this sample, recorded every hour, confirmed the purity of sulfoxide **8** (≥97%), indicating that a rapid racemisation without significant decomposition had occurred.

When the sample of B[*b*]T sulfoxide **8** was allowed to remain at ambient temperature for several days, it was again found to have decomposed into a mixture of achiral disproportionation and racemic dimerization products. This observation suggests that the enantiomeric excess found in bioproducts $\mathbf{10}_{trans}$ ($[\alpha]_D$ +65) and **11** ($[\alpha]_D$ –13), obtained using *P. putida* UV4, is consistent with either an asymmetric synthesis and partial racemization of sulfoxide **8** and/or a sulfoxide reductase-catalysed kinetic resolution of the racemized dimer **9**.

The effects of methyl substituent location around the B[*b*]T ring, on the regioselectivity of oxygenase-catalysed oxidation, were also investigated using substrates **17** and **22** (Scheme 4, Table 1). Both TDO- and NDO_1-catalysed oxidations of 2-methylbenzo[*b*]thiophene (2-MB[*b*]T) **17** showed a marked preference for *cis*-dihydroxylation of the carbocyclic ring to yield *cis*-dihydrodiol **20** (83% and 90% relative yields respectively, Table 1). This result was consistent with a 2-methyl substituent inhibiting attack in the thiophene ring at either the sulfur atom or the substituted 2,3-bond. While the heterocyclic *cis*-diol **19** was also found as a minor metabolite using TDO (10%), it was not observed using NDO_1 from *P. putida* 9816/11. Sulfoxide **18** was only obtained as a minor metabolite (<10%) from these TDO- and NDO_1-catalysed oxidations. Conversely, oxidation catalysed by NDO_2 from the wild-type strain, *P. putida* NCIMB 8859, gave no evidence of *cis*-dihydroxylation and, as expected, yielded mainly the sulfoxide **18** (90%) and a minor achiral metabolite (10%) that was tentatively assigned as 3-hydroxy-5-methyl-thiophene-2-carboxaldehyde **21**, based on ^{1}H-NMR and MS data. Further evidence in support of this assignment was available from the similar characteristics of 3-hydroxythiophene-2-carboxaldehyde, a metabolite isolated from the bacterial biotransformation of the parent B[*b*]T **2**.[3f] It was assumed that bioproduct **21** originated from the initially formed but undetected carbocyclic *cis*-dihydrodiol **20** and ring opening of the derived catechol.

Significantly, when NDO_3 was used as biocatalyst, a marked change in regioselectivity, compared with NDO_1, was observed. A comparison of relative yields showed that oxidation occurred exclusively within the thiophene ring to give *cis*-diol **19** (60%) and 2-MB[*b*]T sulfoxide **18** (40%) using NDO_3, while oxidation catalysed by NDO_1 occurred mainly in the carbocyclic ring to give *cis*-dihydrodiol **20** (90%).

The sulfoxide metabolite **18** proved to be relatively stable in comparison to the parent sulfoxide **8** but was only found as a minor metabolite from TDO (7%), or NDO_1-catalysed oxidation (10%, Table 1). To separate sulfoxide **18** from the *cis*-dihydrodiol metabolites **19** and **20** derived from 2-MB[*b*]T **17**, flash column chromatography was used. Although sulfoxide **18** had been detected earlier as a *P. putida* metabolite, using GC-MS and GC-FTIR analysis,[3c] it had not been isolated and structurally/stereochemically assigned prior to the preliminary report of this study.[3i,j] The recrystallized sulfoxide **18** isolated as a minor metabolite using TDO, proved to be a single enantiomer ($[\alpha]_D$ –476, $CHCl_3$) based on ^{1}H-NMR analysis in the presence of (+)-(*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol. The (1*R*) absolute configuration, established for (–)-2-MB[*b*]T sulfoxide **18** by X-ray crystallography in our earlier communication,[3i] showed a

characteristic electronic circular dichroism (ECD) spectrum. Sulfoxide **18**, obtained in higher relative (90%) and isolated yields (26%), using *P. putida* NCIMB 8859 (NDO_2), had the opposite (1*S*) configuration ($[\alpha]_D$ +267, $CHCl_3$), and a lower *ee* value (56%).

In contrast to the results obtained using dioxygenase enzymes, 2-MB[*b*]T **17** was converted in quantitative yield to the corresponding sulfoxide **18** using SMO [*E. coli* BL21(DE3)]. For preparative purposes, the SMO-catalysed oxidation appeared to be the most promising enzymatic route but stereochemical analysis of the isolated metabolite **18** indicated that, stereoselectivity during sulfoxidation of 2-MB[*b*]T **17** was relatively low ($[\alpha]_D$ +15, $CHCl_3$; 3% *ee*). Although chemically stable, sulfoxide **18**, in common with sulfoxide **8**, was found to have limited configurational stability. Thus, a significant degree of racemisation (pyramidal sulfur inversion) of sulfoxides **8** and **18** was found to occur over a period of 24 h at 25 °C in $CHCl_3$ solution with the former racemizing faster. Kinetic studies of the thermal racemization of enantiopure (−)-(1*R*)- 2-MB[*b*]T sulfoxide **18** in $CHCl_3$ solution were conducted using a thermostatically controlled polarimeter cell. The rate constants (*k*) for racemization at 25 °C and 50 °C were 2.45×10^{-6} and 7.11×10^{-5} s^{-1} respectively, giving similar values for the free energy of activation ($\Delta G^{\ddagger}$ = 25.1 kcal mol^{-1}) and activation energy (E_a = 25.7 kcal mol^{-1}).

The biotransformation of 3-MB[*b*]T **22** using either TDO or SMO did not produce the expected sulfoxide metabolite **23** (Table 1). However, the other bacterial strains (NDO_1-NDO_3) yielded sulfoxide **23** as the major or sole identified metabolite. NDO_1 present in the mutant strain *P. putida* 9816/11 yielded mainly sulfoxide **23** (90%) and a small proportion of the heterocyclic ring *cis*-dihydrodiol **24** (10%). When the wild type strain *P. putida* NCIMB 8859 was used, as anticipated, no *cis*-diols were detected but the sulfoxide **23** (39%, ($[\alpha]_D$ −181, $CHCl_3$; 41% *ee*) was obtained as one of the three major bioproducts. The inversion barrier ($\Delta G^{\ddagger}$) for thermal racemisation of 3-MB[*b*]T sulfoxide **23** was found to be 26.4 kcal mol^{-1}.

Utilization of the *E. coli* F352V strain (NDO_3) with 3-MB[*b*]T substrate **22** resulted in the detection of sulfoxide **23** as the sole isolated metabolite but the very low isolated yield obtained using NDO_3 precluded any rigorous stereochemical analysis. Earlier reports showed how regioselectivity, during NDO-catalysed carbocyclic arene *cis*-dihydroxylation, could be redirected towards alternative C=C bonds, when using NDO_3.[11a–c] The present study now indicates that the NDO_3 enzyme, present in the *E. coli* F352V site directed mutant strain, catalyses preferential oxidation of the heterocyclic ring (and the sulfur atom in particular) rather than the carbocyclic ring when using MB[*b*]T substrates **17** and **22**.

The ECD spectra of sulfoxides (−)-**18** and (−)-**23** obtained using TDO were very similar and thus confirmed that the [1*R*] absolute configuration was common to both. The other two achiral bioproducts, **26** and **27**, resulting from NDO-catalysed oxidation of the methyl group had been reported as bioproducts from bacterial metabolism of 3-MB[*b*]T **22**.[3h] On account of its thermal and configurational instability, only a qualitative ECD spectrum of (−)-sulfoxide **8** metabolite (using SMO) was recorded in a cooled MeCN solution. The ECD spectrum showed similar Cotton effects to those found for (−)-(1*R*)-sulfoxides **18** and (−)-**23**. Based on this observation (−)-B[*b*]T sulfoxide **8** was tentatively assigned a similar (1*R*) absolute configuration.

It was not possible to obtain an accurate value for the barrier to racemization of B[*b*]T sulfoxide **8**, due to its reduced configurational and thermal stability. However, concomitant observations using polarimetry and ^{1}H-NMR spectroscopy allowed a crude estimate from the faster rate of racemization ($t_{1/2}$ value of *ca.* 180 min at 25 °C, $k = 6.4 \times 10^{-6}$ s^{-1}). This was entirely consistent with the calculated lower barrier to racemization $\Delta G^{\ddagger}$ 23.9 kcal mol^{-1})[9a] compared with those found experimentally for sulfoxides **18** ($k = 2.45 \times 10^{-6}$ s −1, $\Delta G^{\ddagger}$ = 25.1 kcal mol^{-1}) and **23** ($\Delta G^{\ddagger}$ = 26.4 kcal mol^{-1}).

An earlier value for the barrier to pyramidal inversion of 2,5-di-*tert*-octylthiophene sulfoxide, obtained experimentally at 25 °C by NMR spectroscopy, ($\Delta G^{\ddagger}$ = 14.8 kcal mol^{-1}),[9b] compared favourably with that obtained by semiempirical calculation of the inversion barrier for 2,5-dimethylthiophene ($\Delta H^{\ddagger}$ = 13.3 kcal mol^{-1}).[9c] These[9b] and later *ab initio* calculations[9a] led to the prediction that the barriers to pyramidal inversion for sulfoxides **8** ($\Delta H^{\ddagger}$ = 23.9 kcal mol^{-1}) [9a] and **18** ($\Delta H^{\ddagger}$ = 19.4 kcal mol^{-1})[9c] would be significantly higher than those calculated for thiophene sulfoxide ($\Delta H^{\ddagger}$ = 11.2 kcal mol^{-1}) [9a] as a result of 2,3-annelation. The higher inversion barriers ($\Delta G^{\ddagger}$) obtained experimentally for sulfoxides **8** (< 25 kcal mol^{-1}), **18** (25.1 kcal mol^{-1}) and **23** (26.4 kcal mol^{-1}) are in general accord with the calculated values. As the normal barriers for racemisation and pyramidal inversion of sulfoxides are generally in the range $\Delta G^{\ddagger}$ = 37–42 kcal $mol,^{-1}$ the calculated and experimental values obtained for monocyclic and bicyclic thiophene sulfoxides are evidently much lower. These markedly reduced pyramidal inversion barriers for thiophene sulfoxides have been ascribed to maximal cyclic π-conjugation and aromatic stabilization of the planar transition state during inversion.[11b]

Conclusion

The biotransformations of B[*b*]T substrates **2**, **17** and **22** using TDO, NDO and SMO enzymes were found to yield the corresponding chiral sulfoxides **8**, **18**, **23** and derived products. The unstable B[*b*]T sulfoxide **8** from B[*b*]T **2** was isolated and characterized following a biotransformation using SMO. Dimerization of sulfoxide **8** followed by sulfoxide reductase-catalysed deoxygenation is proposed to account for the formation of the tetracyclic metabolites **10–12** using TDO.

The regioselectivity observed during biocatalysed oxidation of B[*b*]T substrates **2**, **17**, **22**, examined using the oxygenase enzymes TDO, NDO_1, NDO_2, NDO_3 and SMO, was found to be dependent on the substituent pattern and enzyme type. The site-directed mutant strain *E. coli* F352V (NDO_3) generally showed a strong preference (85–100%) for oxidation of the thiophene ring over the benzene ring compared to TDO and NDO_1.

The first example of an enantiopure thiophene sulfoxide, metabolite (−)-**18**, along with enantioenriched sulfoxides (−)-**8** and (−)-**23**, were found to racemize at ambient temperature. Absolute configurations of the (−)-B[*b*]T sulfoxides were assigned as (1*R*) on the basis of ECD spectroscopy (**8**, **18** and **23**) and X-ray crystallography (**18**).

The barriers to pyramidal inversion were determined for the (1*R*)-sulfoxides (−)-**18** and (−)-**23** ($\Delta G^{\ddagger}$ = 25–27 kcal $mol.^{-1}$) and compared with previously calculated barriers. The inversion barrier for (−)-sulfoxide **8** appeared to be slightly lower ($\Delta G^{\ddagger}$ < 25 kcal $mol.^{-1}$).

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400, DPX-300 and DRX-500 instruments. Chemical shifts (δ) are reported in ppm relative to $SiMe_4$ and coupling constants (J) are given in Hz. Mass spectra were run 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. The benzo[*b*]thiophene substrates (**2**, **17**, **22**), were available from commercial sources and from earlier studies.[3e,i,j,5,9a] A PerkinElmer 341 polarimeter was used for optical rotation ($[\alpha]_D$) measurements at ambient temperature (23–25 °C). Enantiomeric excess values were determined by chiral stationary phase HPLC using Chiralcel OJ or OD-H columns (method A), by ^{1}H-NMR analysis in the presence of the chiral resolving reagent (+)-(*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol (method B) or by comparison of optical rotation values (method C). Absolute configurations were determined using ECD spectroscopy and spectra were recorded using spectroscopic grade acetonitrile and a JASCO J-720 instrument.

Liquid chromatography-time of flight mass spectrometry (LC-TOFMS) analyses were conducted using an Agilent 1100 series HPLC coupled to an Agilent 6510 Q-TOF (Agilent Technologies, USA). Separation was performed using a reverse phase column (Agilent Eclipse Plus C18, 5 µm, 150 × 2.1 mm) together with the corresponding guard column (5 µm, 12.5 × 2.1 mm). The mobile phase consisted of 95% methanol containing 0.1% formic acid in channel A, and 5% methanol containing 0.1% formic acid in channel B. The system was programmed to perform an analysis cycle consisting of 100% B for 1 min, followed by gradient elution from 100% to 5% B over a 14 min period, hold at 5% B for 10 min, return to initial conditions over 2 min and then hold these conditions for a further 8 min. The flow rate was 0.20 ml min^{-1} and the injection volume was 5 µl. MS experiments were carried out using ESI in positive ion mode with the capillary voltage set at 4.0 kV. The desolvation gas was nitrogen set at a flow rate of 8 L min^{-1} and maintained at a temperature of 350 °C.

Shake flask and fermenter scale (50 L) biotransformations were carried out using whole cells of *P. putida* UV4 (TDO), *P. putida* NCIMB 8859 (NDO_1), *P. putida* 9816/11 (NDO_2), and *E. coli* F352V (NDO_3) using methods described earlier.[10a–c,13a–c] SMO-catalysed sulfoxidations were conducted using cell suspensions of the *E.coli* BL21(DE3)-pRSET-styAB strain expressing styrene monooxygenase from *Pseudomonas putida* CA-3 as previously described,[1c] using B[*b*]T **2** and 2-MB[*b*]T **17** as substrates.

Oxidation of benzo[*b*]thiophene 2

(a) Using *m*-chloroperoxybenzoic acid (MCPBA). To a stirred solution of benzo[*b*]thiophene **2** (10.0 g, 77 mmol) in methylene chloride (200 ml) maintained at 0 °C was added, in small portions, MCPBA (16.8 g, 81 mmol). The mixture was stirred overnight at ambient temperature. The chlorobenzoic acid was filtered from the reaction mixture, the filtrate washed successively with Na_2SO_3 solution, $NaHCO_3$ solution, water and then dried (Na_2SO_4). The crude product obtained after evaporating the solvent was separated into two fractions by flash chromatography ($CHCl_3$). The earlier major fraction on evaporation gave sulfone **16** and the minor fraction on PLC (Et_2O : pentane, 1 : 1) further separated into four minor products $\mathbf{10}_{trans}$, $\mathbf{10}_{cis}$, **11** and **12**.

*Benzo[*b*]thiophene-1,1-dioxide* ***16***[14a]. White crystalline solid (7.63 g, 60%); m.p. 141–143 °C (from MeOH), (lit.[14a] m.p. 142–143 °C); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.73 (1 H, d, $J_{3,2}$ 6.9, 3-H), 7.23 (1 H, d, $J_{2,3}$ 6.8, 2-H), 7.37 (1 H, dd, $J_{4,5}$ 7.8, $J_{4,6}$ 1.8, 4-H), 7.53–7.58 (2 H, m, 6-H), 7.73 (1 H, dd, $J_{7,6}$ 7.6, $J_{7,5}$ 1.3, 7-H); *m/z* 166 ($M^{+\bullet}$, 52%), 137 (100).

*Benzo[*b*]naphtho[2,1-*d*]thiophene* ***12***[3g,14b]. White solid (0.018 g); m.p. 98–100 °C (MeOH), (lit.[14b] m.p. 102–103 °C); R_f 0.95; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.48–7.53 (1 H, m, Ar–H), 7.56–7.63 (2 H, m, Ar–H), 7.72–7.77 (1 H, m, Ar–H), 7.88–7.94 (2 H, m, Ar–H), 8.00–8.04 (2 H, m, Ar–H), 8.87 (1 H, d, $J_{5,6}$ 8.2, 5-H), 9.02 (1 H, d, $J_{6,5}$ 8.3, 6-H); *m/z* 234 ($M^{+\bullet}$, 100%).

trans-*6a,11b-Dihydronaphtho[2,1-*d*]benzo[*b*]thiophene-7-oxide* $\mathbf{10}_{trans}$[3l]. White solid (0.045 g); m.p. 155–156 °C (EtOAc/pentane); R_f 0.42; (Found: C, 76.0; H 4.7; S, 12.5 $C_{16}H_{12}OS$ requires C, 76.2, H 4.8; S, 12.7%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.51 (1 H, d, $J_{11b,6a}$ 7.2, 11b-H) 4.74–4.76 (1 H, m, 6a-H), 6.23 (1 H, dd, $J_{6,5}$ 9.9, $J_{6,6a}$ 3.0, 6-H), 6.76 (1 H, dd, $J_{5,6}$ 9.9, $J_{5,6a}$ 2.2, 5-H), 7.13–7.17 (2 H, m, Ar–H), 7.27–7.30 (3 H, m, Ar–H), 7.39–7.46 (2 H, m, Ar–H), 7.79 (1 H, d, $J_{8,9}$ 7.4, 8-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 44.64, 63.97, 117.91, 126.54, 126.70, 128.55, 128.67, 128.69, 128.74, 129.49, 132.00, 132.44, 132.64, 132.78, 142.25, 143.58; *m/z* 252 ($M^{+\bullet}$, 40%), 235 (100).

*Benzo[*b*]naphtho[2,1-*d*]thiophene-7-oxide* ***11***[14c]. White crystalline solid (0.026 g); m.p. 142–144 °C (Et_2O/pentane); (lit.[14c] 147–148 °C); R_f 0.40; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.50–7.55 (1 H, m, Ar–H), 7.61–7.72 (3 H, m, Ar–H), 7.96–8.08 (4 H, m, Ar–H), 8.45 (1 H, d, $J_{5,6}$ 8.2, 5-H), 8.74 (1 H, d, $J_{6,5}$ 8.3, 6-H); *m/z* 250 ($M^{+\bullet}$, 14%), 234 (100).

cis-*6a,11b-Dihydronaphtho[2,1-*d*]benzo[*b*]thiophene-7-oxide* $\mathbf{10}_{cis}$[3l]. White solid (0.015 g); m.p. 156–157 °C (EtOAc/pentane); R_f 0.36; (Found: M^+, 252.0609 $C_{16}H_{12}OS$ requires 252.0593); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.60–4.63 (1 H, m, 6a-H), 5.24 (1 H, d, $J_{11b,6a}$ 6.7, 11b-H) 5.87 (1 H, dd, $J_{6,5}$ 9.7, $J_{6,6a}$ 2.4, 6-H), 6.47 (1 H, dd, $J_{5,6}$ 9.8, $J_{5,6a}$ 2.5, 5-H), 7.08–7.14 (2 H, m, Ar–H), 7.30–7.46 (5 H, m, Ar–H), 7.86 (1 H, d, $J_{8,9}$ 6.9, 8-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 45.84, 69.16, 119.20, 126.59, 126.73, 128.08, 128.28, 128.53, 128.71, 129.13, 131.15, 132.62, 132.45, 132.60, 143.25, 143.63; *m/z* 252 ($M^{+\bullet}$, 48%), 235 (100).

(b) Using dimethyl dioxirane (DMD). A sample of benzo[*b*]thiophene **2** (*ca.* 0.1 g) was treated, in an ice cold bath, with an excess of pre-dried d_6-acetone solution of DMD (0.085 M), prepared using the literature method.[17] The reaction mixture was stirred, monitored by TLC (EtOAc : hexane, 1 : 1), and was allowed to warm to room temperature. When most of the starting compound had been consumed, the products of the reaction mixture were examined, *in situ*, by NMR spectroscopy and showed mainly the presence of racemic benzo[*b*]thiophene-1-oxide **8**. Removal of the solvent under vacuum and storage of crude product at ambient temperature resulted in a mixture of products.

*(±)-Benzo[*b*]thiophene-1-oxide* ***8***. ^{1}H-NMR (300 MHz, CD_3COCD_3) δ 7.30–7.35 (2 H, m, 5-H, 7-H), 7.38 (1 H, d, $J_{3,2}$

5.5, 3-H), 7.60 (1 H, d, $J_{2,3}$ 5.4, 2-H), 7.82–7.85 (1 H, m, 6-H), 7.90–7.93 (1H, m, 4-H); ^{13}C-NMR (125 MHz, CD_3COCD_3) δ 122.98, 124.21, 124.53, 124.78, 124.85, 127.20, 140.26, 140.45.

Data also given for (–)-(*R*)-sulfoxide **8** in section (c, iii).

(c) Oxygenase-catalysed sulfoxidations of benzo[*b*]thiophenes 2, 17, 22.

*(i) Biotransformation of benzo[*b*]thiophene **2** with* P. putida *UV4*. A biotransformation with *P. putida* UV4 and substrate **2** (13 g, 100 mmol) followed by flash column chromatography (50% Et_2O in pentane) yielded pure samples of benzo[*b*]naphtho[2,1-*d*]thiophene **12** (0.180 g, R_f 0.95), 4-hydroxy-benzo[*b*]thiophene **15** (0.520 g, R_f 0.88), 5-hydroxy-benzo[*b*]thiophene **14** (0.705 g, R_f 0.85), *cis*/*trans*-2,3-dihydroxy-2,3-dihydrobenzo[*b*]thiophene **7** (4.9 g, R_f 0.50),[7] and *cis*-4,5-dihydroxy-4,5-dihydrobenzo[*b*]thiophene **13** (0.785 g, R_f 0.20).[7] Further purification of unresolved fractions by multiple elution PLC (50% Et_2O in pentane) yielded three other bioproducts $\mathbf{10}_{trans}$, $\mathbf{10}_{cis}$, and **11** which were identified by direct comparison with authentic samples synthesised earlier by chemical oxidation. Metabolites 5-hydroxybenzo[*b*]thiophene **14** and 4-hydroxybenzo[*b*]thiophene **15** were found to have similar properties to those reported in the literature.[14c] Full spectroscopic and other characterization data for compounds **14**, **15** and **7** will be given in the separate report.[7] The samples of $\mathbf{10}_{trans}$, $\mathbf{10}_{cis}$**and 11** showed identical spectra to the racemic products obtained by chemical oxidation in Section (a).

(+)-*trans*-6a,11b-Dihydronaphtho[2,1-*d*]benzo[*b*]thiophene-7-oxide $\mathbf{10}_{trans}$. (0.245 g); R_f 0.42; $[\alpha]_D$ +65 (*c* 0.47, EtOH); 8% *ee* (Method A, Chiralcel OJ, 20% EtOH/hexane).

(±)-*cis*-6a,11b-Dihydronaphtho[2,1-*d*]benzo[*b*]thiophene-7-oxide $\mathbf{10}_{cis}$. (0.080 g); R_f 0.36.

(–)-Benzo[*b*]naphtho[2,1-*d*]thiophene-7-oxide 11. (0.390 g); R_f 0.40; $[\alpha]_D$ –13 (*c* 0.52, $CHCl_3$); 3% *ee* (Method A, Chiralcel OJ, 20% EtOH/hexane).

*(ii) Biotransformation of benzo[*b*]thiophene 2 with NDO.* Biotransformation of substrate **2** was carried out using *P. putida* 9816/11 (NDO_1), *P. putida* NCIMB 8859 (NDO_2) and *E. coli* F352V (NDO_3) in two litre shake flasks for comparative study under standard conditions (*ca.* 0.4 g substrate/500 ml culture medium). The metabolites **7**, **10** and **11** present in the crude aqueous extract were identified by LC-TOFMS. The aqueous biotransformed material was extracted with EtOAc and the crude mixture of bioproducts obtained after removal of EtOAc was separated by PLC. The separated bioproducts **7**, **10** and **11** were identified by direct comparison with authentic samples (Table 1).

*(iii) Biotransformation of benzo[*b*]thiophene **2** with SMO [*E.coli *BL21(DE3)-pRSET-styAB]*. Substrate **2** (*ca.* 0.1 g) in 500 ml of cell suspension of *E.coli* BL21(DE3)-pRSET-styABO (5 g l^{-1}) in 50 mM phosphate buffer (pH 7.4) containing 1% (w/v) glucose was biotransformed as previously described.[1c] The cellular debris was removed by centrifugation and the clear aqueous portion was stored in the fridge. LC-TOFMS analysis of the aqueous medium showed benzo[*b*]thiophene sulfoxide **8** to be the dominant metabolite (>95%) with only traces of benzo[*b*]thiophene-1,1-dioxide **16** (<5%) detected. A small sample of the cooled aqueous portion was then extracted (EtOAc) and the extract analysed by GC-MS. It showed the presence of benzo[*b*]thiophene-1-oxide **8** as the major product (*ca.* 97%) along with minor amounts of benzo[*b*]thiophene-1,1-dioxide **16** (*ca.* 2%) and trace amounts of benz[*b*]thiophene **2**.

(–)-(1*R*)-Benzo[*b*]thiophene-1-oxide 8. Light yellow oil (purified by PLC); (0.078 g, 70%); R_f 0.28 (EtOAc–hexane, 1 : 1); (Found $M^{+\bullet}$ 150.0146. C_8H_6SO requires 150.0139); $[\alpha]_D$ –235 (*c* 1.25, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.08 (1 H, d, *J* 6.3, 3-H), 7.22 (1 H, d, *J* 6.3, 2-H), 7.46–7.54 (3 H, m, 5-H, 6-H and 7-H), 7.92 (1H, d, *J* 7.3 4-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 125.42, 126.64, 129.46, 132.45, 135.27, 137.54, 137.93, 145.67.

*(iv) Biotransformation of 2-methylbenzo[*b*]thiophene **17** with* P. putida *UV4*. Substrate **17** (6.0 g) was metabolised with *P. putida* UV4 and the bioproducts were extracted (EtOAc) from the aqueous phase. The crude mixture of products obtained after removal of EtOAc was purified by flash chromatography (75% Et_2O in pentane → Et_2O) to afford (+)-2-methyl-*cis*-2,3-dihydroxy-2,3-dihydro-benzo[*b*]thiophene **19** (0.2 g, 3%, R_f 0.4, 50% Et_2O/pentane), (+)-2-methyl-*cis*-4,5-dihydroxy-4,5-dihydrobenzo[*b*]thiophene **20** (1.8 g, 25%, R_f 0.23, 50%, Et_2O/pentane) [7] and (–)-2-methylbenzo[*b*] thiophene-1-oxide **18** (0.15 g, 2%).

(–)-(1*R*)-2-Methylbenzo[*b*]thiophene-1-oxide 18.[15] white crystalline solid (0.057 g, 51%); R_f 0.24 ($CHCl_3$); m.p. 80–82 °C (from $CHCl_3$/hexane), (lit.[15] m.p. 80–81 °C); $[\alpha]_D$ –476 (*c* 0.41, $CHCl_3$); >99% *ee* (Method B); ν_{max} (KBr) 1060 cm^{-1} (S=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.36 (3 H, d, *J* 1.5, Me), 6.78 (1 H, q, *J* 1.5, 3-H), 7.32–7.46 (3 H, m, 5-H, 6-H and 7-H), 7.82 (1 H, d, *J* 7.0, 4-H); *m*/*z* 164 ($M^{+\bullet}$, 96%), 147 (70), 121 (100); ECD: 332 nm ($\Delta\varepsilon$ –7.0), 301 nm ($\Delta\varepsilon$ +5.8), 270 nm ($\Delta\varepsilon$ –4.5), 224 nm ($\Delta\varepsilon$ –35.0).

*(v) Biotransformation of 2-methylbenzo[*b*]thiophene **17** using* P. putida *8859*. Substrate **17** (0.2 g) on biotransformation using *P. putida* 8859 gave a mixture of two metabolites. Separation by PLC (1% MeOH in $CHCl_3$) afforded of 3-hydroxy-5-methylthiophene-2-carboxaldehyde **21** and the more polar (+)-(1*S*)-2-methylbenzo[*b*]thiophene-1-oxide **18**.

3-Hydroxy-5-methylthiophene-2-carboxaldehyde 21. (0.006 g, 3%); R_f 0.57; ν_{max} (neat) 1610 cm^{-1} (C=O) and 3330 cm^{-1} (OH); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.51 (3 H, s, Me), 6.5 (1 H, s, 4-H), 9.47 (1 H, s, CHO); *m*/*z* 142 ($M^{+\bullet}$, 87%), 141 (100).

(+)-(1*S*)-2-Methylbenzo[*b*]thiophene-1-oxide 18. (0.057 g, 26%); $[\alpha]_D$ +267 (*c* 0.81, $CHCl_3$); 56% *ee* (methods B and C).

*(vi) Biotransformation of 2-methylbenzo[*b*]thiophene **17** with SMO [*E.coli *BL21(DE3)-pRSET-styAB]*. Substrate **17** (0.02 g) was metabolized using SMO. The NMR spectrum of the crude bioproduct showed the presence of metabolite **18** only and a trace amount of starting substrate. Purification by PLC (EtOAc : hexane, 1 : 1) furnished sulfoxide **18** (0.019 g, 94%); $[\alpha]_D$ +15 (*c* 0.8, $CHCl_3$); 3% *ee* (method C).

*(vii) Biotransformation of 3-methylbenzo[*b*]thiophene **22** with* P. putida *UV4*. Substrate **22** (7.5 g) when biotransformed using *P. putida* UV4 yielded a single bioproduct, (+)-3-methyl-(4*R*,5*S*)-*cis*-4,5-dihydroxy-4,5-dihydrobenzo[*b*]thiophene **25** (4.1 g, 45%) whose characterization will be reported elsewhere.[7]

*(viii) Biotransformation of 3-methylbenzo[*b*]thiophene **22** with* P. putida *NCIMB 8859*. Substrate **22** (0.3 g) yielded a crude mixture of three metabolites on biotransformation with *P. putida* NCIMB 8859. Separation by PLC (Et_2O) gave 3-hydroxymethylbenzo[*b*]thiophene **26**, benzo[*b*]thiophene-3-carboxylic acid **27** and (–)-(*R*)-2-methylbenzo[*b*]thiophene-1-oxide **23**.

3-Hydroxymethylbenzo[*b*]thiophene 26.[3h] (0.02 g, 6%); R_f 0.64; ν_{max} (neat) 3350 cm^{-1} (OH); (Found $M^{+\bullet}$ 164.0292. C_9H_8OS requires 164.0296); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.91 (2 H, m, CH_2) 7.35–7.42 (3 H, m, Ar–H), 7.82–7.87 (2 H, m, Ar–H); *m*/*z* 164 (M^+, 100%), 147 (83), 135 (84).

Benzo[*b*]thiophene-3-carboxylic acid 27.[3h] (0.021 g, 5%); R_f 0.38 (Et_2O); ν_{max} (neat) 1674 cm^{-1} (C═O); (Found M^+ 178.0087 $C_9H_6O_2S$ requires 178.0088); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.40–7.53 (2 H, m, Ar–H), 7.89 (1 H, d, *J* 7.9, Ar–H), 8.55 (1 H, s, 2-H), 8.62 (1 H, d, *J* 8.1, Ar–H); *m*/*z* 178 ($M^{+\bullet}$, 100%), 161 (58).

(–)-(1*R*)-3-Methylbenzo[*b*]thiophene-1-oxide 23. (0.023 g, 8%); R_f 0.18 ($CHCl_3$); m.p. 75–77 °C (from $CHCl_3$/hexane), (lit.[15] m.p. 77 °C); $[\alpha]_D$ –181 (*c* 0.8, $CHCl_3$); 41% *ee* (method B); ν_{max} (KBr) 1050 cm^{-1} (S═O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.28 (3 H, d, *J* 1.5, Me), 6.77 (1 H, q, *J* 1.4, 2-H), 7.43–7.59 (3 H, m, Ar–H), 7.90 (1 H, d, *J* 7.4, Ar–H); *m*/*z* 164 ($M^{+\bullet}$, 30%), 147 (22), 135 (100); ECD: 325 nm ($\Delta\varepsilon$ –8.0), 296 nm ($\Delta\varepsilon$ +3.8), 267 nm ($\Delta\varepsilon$ –1.8), 225 nm ($\Delta\varepsilon$ –26.2).

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