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PAPER

Toluene dioxygenase-catalyzed *cis*-dihydroxylation of benzo[*b*]thiophenes and benzo[*b*]furans: synthesis of benzo[*b*]thiophene 2,3-oxide†

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Enzymatic *cis*-dihydroxylation of benzo[*b*]thiophene, benzo[*b*]furan and several methyl substituted derivatives was found to occur in both the carbocyclic and heterocyclic rings. Relative and absolute configurations and enantiopurities of the resulting dihydrodiols were determined. Hydrogenation of the alkene bond in carbocyclic *cis*-dihydrodiols and ring-opening epimerization/reduction reactions of heterocyclic *cis*/*trans*-dihydrodiols were also studied. The relatively stable heterocyclic dihydrodiols of benzo[*b*]thiophene and benzo[*b*]furan showed a strong preference for the *trans* configuration in aqueous solutions. The 2,3-dihydrodiol metabolite of benzo[*b*]thiophene was utilized as a precursor in the chemoenzymatic synthesis of the unstable arene oxide, benzo[*b*]thiophene 2,3-oxide.

Introduction

Benzo[*b*]thiophene, B[*b*]T, **1a**, and methylated derivatives (MB-[*b*]Ts) including **1b** and **1c** are present in fossil fuels (*e.g.* crude oil, coal and shale). As they are liberated into the atmosphere through combustion, this has prompted a number of studies into the fate of these thiaarenes in the environment (Scheme 1).[1a–i] Earlier reports, from these laboratories,[2a–d] have shown that the corresponding sulfoxides **2a–c** and their derivatives were formed *via* mono- and di-oxygenase-catalyzed heteroatom oxidations of the corresponding B[*b*]T substrates **1a–c**.

The concomitant formation of *cis*-diols **3a–c**, **5a**$_{cis}$ and **5b**$_{cis}$, along with sulfoxides **2a–c**, from biocatalytic oxidation of the carbocyclic and heterocyclic rings of B[*b*]T substrates **1a–c** using several dioxygenase enzymes, was briefly mentioned in our recent paper.[2a] The results from this comprehensive study of the sulfoxide metabolites **2a–c** (and their decomposition products), obtained using B[*b*]T **1a** and the MB[*b*]T substrates **1b** and **1c** with bacterial monooxygenase or dioxygenase enzymes, were presented initially.[2a] However, in the interest of brevity neither the characterization nor reactivity of the corresponding dihydrodiols **3a–c**, **5a** and **5b** were discussed. Full structural and stereochemical data of the *vic*-dihydroxylation bioproducts **3a–c**, **5a**$_{cis}$/**5a**$_{trans}$, **5b**$_{cis}$, and **5d**$_{cis}$/**5d**$_{trans}$ (from 5-methylbenzo[*b*]thiophene, 5MB[*b*]-T **1d**), and the derived products **7–12** are presented in this paper.

Although benzo[*b*]furans, B[*b*]Fs, have only been detected as minor products, during combustion processes (*e.g.* coal gasification, coke production and tobacco smoke),[1i] many members of this family occur in the environment as secondary plant metabolites[3] (*e.g.* furanocoumarins and furanoquinolines) and undergo further biodegradation. In comparison with B[*b*]Ts, the biotransformation of B[*b*]F **1e**, and methyl substituted benzo[*b*]furans, MB[*b*]Fs, **1f** and **1g** has, to date, received relatively little attention (Scheme 1). Our earlier studies of arene dioxygenase-catalyzed *cis*-dihydroxylation of bicyclic aromatic heterocycles[2b,c,4a,b] included only one member, compound **1e**, of the B[*b*]F family as a substrate. This study allows a comparison of the results obtained using three B[*b*]F substrates **1e–g** with the corresponding B[*b*]T substrates **1a–d**.

Most of the earlier reports, on the bacterial biotransformations of B[*b*]T substrates from other laboratories, were based largely on GC-MS analyses of bioproducts.[1b–i] While the approach was successful in detecting the more stable products, it did not permit a full structural or stereochemical analysis. This was particularly relevant to the thermally unstable metabolites, including those with a propensity to racemize or epimerize spontaneously.

In this study, we report (i) full structural characterizations of the *vic*-dihydrodiol metabolites from B[*b*]T substrates (withheld from our recent report[2a]) which were not included in earlier communications,[2b,c,4a] (ii) new dihydrodiols from methyl substituted B[*b*]T **1d** and B[*b*]F substrates **1f** and **1g**, (iii) results of solvent-dependent *cis*/*trans* equilibration studies of heterocyclic 2,3-dihydrodiol metabolites and (iv) examples of the reactivity of *cis*-dihydrodiols, including regioselective hydrogenation, aromatization and other reactions of value, *e.g.* in the chemoenzymatic synthesis of B[*b*]T 2,3-oxide.

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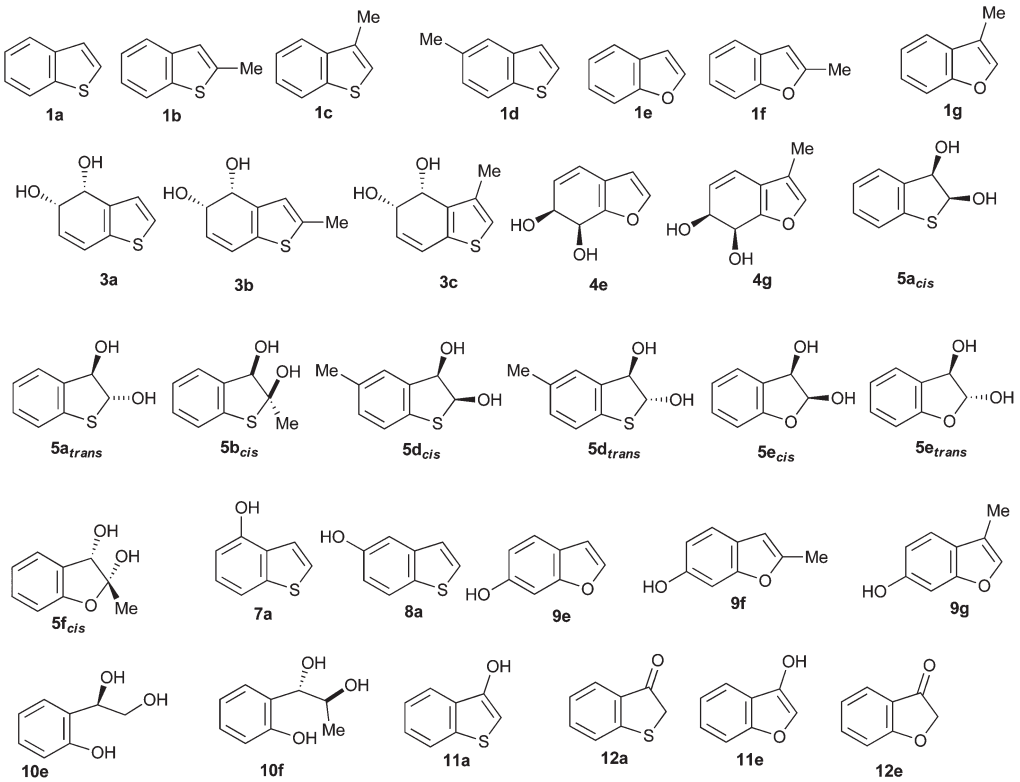
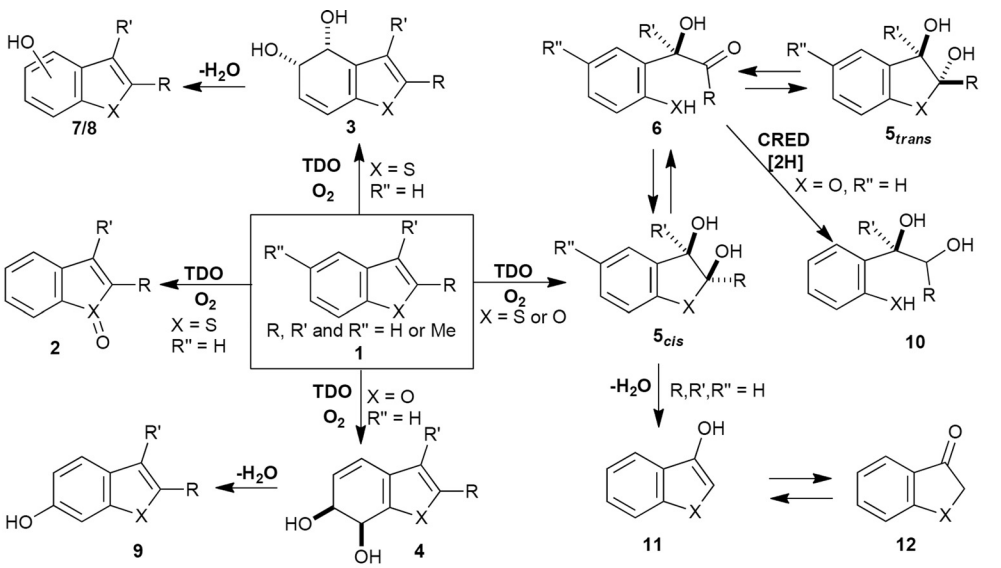


Results and discussion

(a) Structural and stereochemical assignments of *cis*-dihydrodiol metabolites and derivatives from TDO-catalyzed oxidation of carbocyclic rings in substrates 1a–c, 1e and 1g

The biotransformation of substrates **1a–g** was carried out using whole cells of *Pseudomonas putida* UV4, a constitutive mutant strain containing toluene dioxygenase (TDO). Under previously reported conditions,[2a] where heteroatom oxidation (sulfoxidation) was encountered, *cis*-dihydroxylation was also found to occur in both the carbocyclic and heterocyclic rings. The regioselectivity was dependent on the nature of the heteroatom and position of the methyl group (Table 1). Characterization of the corresponding sulfoxide bioproducts **2a–d** and their derivatives,



Scheme 1 Metabolites from TDO-catalyzed *cis*-dihydroxylation (**3–5**) and heteroatom oxidation (**2**) of B[*b*]T or B[*b*]F substrates (**1**) and derived products (**7–12**).

Table 1 Relative ratios (%) of *cis*-dihydrodiols **3a–c**, **4e**, **4g** and *cis* or *trans*-diols **5a**, **5b**, **5d–f** from TDO-catalyzed *cis*-dihydroxylation of substrates **1a–g**

Substrate	Carbocyclic *cis*-diol[a] (%)	Heterocyclic *cis* or *trans*-diol[a] (%)	Phenol products[a,b]
1a	**3a** (0, 12)[c]	**5a** (94, 71)[c]	**7a** (3, 8)[c], **8a** (3, 9)[c]
1b	**3b** (89)	**5b** (11)	
1c	**3c** (100)		
1d		**5d** (100)	
1e	**4e** (44, 35)[c]	**5e** (0, 39)[c]	**9e** (15, 7)[c], **10e** (34, 19)[c]
1f		**5f** (67)	**9f** (25), **10f** (8)
1g	**4g** (75)		**9g** (25)

[a] Relative ratio excludes sulfoxidation bioproducts. [b] Bioproducts resulting from further reactions of *cis*- and *cis*/*trans*-dihydrodiols. [c] Results from two biotransformations carried out under slightly different conditions.

obtained after chromatographic separation from the corresponding *cis*-dihydrodiol metabolites **3a–c** and **5a–d**, was reported earlier.[2a]

(i) Structural assignments for *cis*-diols 3a–c, 4e and 4g. Carbocyclic ring dihydroxylation of the parent substrate B[*b*]T **1a** occurred, exclusively, at the 4,5-bond to yield *cis*-dihydrodiol **3a**, along with several phenol derivatives, *e.g.* **7a** and **8a**, which were separable by PLC (Table 1). A similar pattern of 4,5 bond regioselectivity was found for MB[*b*]T substrates **1b** and **1c** to give the corresponding *cis*-diols **3b** and **3c**. However, the methyl group of 5MB[*b*]T **1d** appeared to block dihydroxylation at the 4,5 bond.

Biotransformation of the parent B[*b*]F substrate **1e** also resulted in carbocyclic *cis*-dihydroxylation, exclusively at the 6,7 bond to give *cis*-dihydrodiol **4e** and the derived phenol **9e**. Substrate 3MB[*b*]F **1g** yielded diol **4g** and phenol **9g** products. The corresponding 6,7-*cis*-dihydrodiol metabolite of 2MB[*b*]F **1f** was not isolated. Isolation of phenol **9f** suggested that this presumed relatively unstable carbocyclic *cis*-dihydrodiol precursor had been formed but decomposed during the course of biotransformation or workup procedure. This change of regioselectivity indicates that, during *cis*-dihydroxylation of B[*b*]T (4,5 bond oxidation) and B[*b*]F substrates (6,7 bond oxidation), proximity to an aromatic heteroatom is less important than the type of heteroatom present, when bound within the TDO active site. A different trend was observed during TDO-catalyzed *cis*-dihydroxylation of quinoline and isoquinoline, where carbocyclic ring oxidation at the 5,6 and 7,8 bonds occurred.[5]

The chiral *cis*-dihydrodiol metabolites **3a–c**, **4e** and **4g** were found to be enantioenriched and showed the characteristic NMR spectral patterns and coupling constants reported for carbocyclic *cis*-diol metabolites from other bicyclic arene substrates, *e.g.* naphthalene, quinoline, and isoquinoline.[5] To date, the only member of the carbocyclic *cis*-dihydrodiol family from either B[*b*]T or B[*b*]F substrates to have been structurally and stereochemically characterized was *cis*-dihydrodiol **4e**.[4b] This bioproduct, along with carbocyclic *cis*-diols **3a–c** and **4g**, was relatively unstable and readily aromatized upon heating or treatment with acid. Thus, the isolated phenolic products **7a**, **8a**, **9e–g** were assumed to result from decomposition of the corresponding *cis*-dihydrodiol metabolites **3a**, **4e–g**.

Table 2 Relative (J_{cis}) and absolute configurations (Ab. config.) of *cis*-dihydrodiols (**3a**, **3b**, **3c**, **4e** and **4g**) and *cis*-tetrahydrodiols (**3a′**, **3b′**, **3c′**, **4e′** and **4g′**)

cis-Dihydrodiol	J_{cis} (Hz)[a]	Ab. config.	*cis*-Tetrahydrodiol	J_{cis} [a] (Hz)	Ab. config.
3a	$J_{4,5}$ 5.6	(+)-(4*R*,5*S*)	**3a′**	$J_{4,5}$ 3.6	(−)-(4*R*,5*S*)
3b	$J_{4,5}$ 5.7	(+)-(4*R*,5*S*)	**3b′**	$J_{4,5}$ 3.6	(−)-(4*R*,5*S*)
3c	[b]	(+)-(4*R*,5*S*)	**3c′**	$J_{4,5}$ 3.7	(−)-(4*R*,5*S*)
4e	[b]	(−)-(6*S*,7*S*)	**4e′**	$J_{6,7}$ 3.9[c]	(−)-(6*S*,7*S*)
4g	[b]	(−)-(6*S*,7*S*)	**4g′**	$J_{6,7}$ 4.2	(−)-(6*S*,7*S*)

[a] Vicinal coupling constants. [b] Superimposed ^{1}H-NMR signals. [c] Ref. 4*b*.

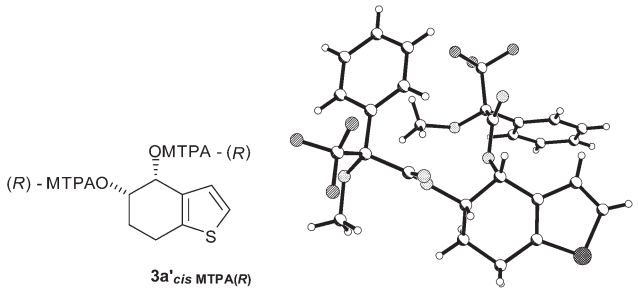


Fig. 1 X-ray crystal structure of **3a′**$_{cis\text{MTPA}(R)}$.

(ii) Absolute configurations/ee values of diols 3a–c, 4e and 4g. In view of the instability of carbocyclic *cis*-dihydrodiols **3a–c**, **4e** and **4g**, the relative and absolute configurations and enantiopurity values (% ee) were determined after their catalytic partial hydrogenation (H_2, Pd–C). Hydrogenation of the 6,7 alkene bonds of *cis*-diols **3a–c** and the 4,5 bonds of *cis*-diols **4e**, **4g** yielded the corresponding stable *cis*-tetrahydrodiol derivatives **3a′–c′**, **4e′** and **4g′** with typical vicinal coupling constants ($J_{4,5}$ or $J_{6,7}$) within the range 3.6–4.2 Hz (Table 2). These values were consistent with those previously found for *cis*-tetrahydrodiols derived from other polycyclic arenes.[5]

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The absolute configuration of carbocyclic *cis*-dihydrodiol metabolite **3a** was established as (4*R*,5*S*) by X-ray crystallographic analysis of diMTPA ester derivative **3a′**$_{cis\text{MTPA}(R)}$, formed using *cis*-tetrahydrodiol **3a′** and the acid chloride from (*R*)-MTPA (Fig. 1). The cyclohexene ring adopted a half-chair conformation with the OMTPA groups *equatorial* on C-5 and

pseudoaxial on C-4. The dihedral angle between the *cis*-OMTPA substituents was −51°.

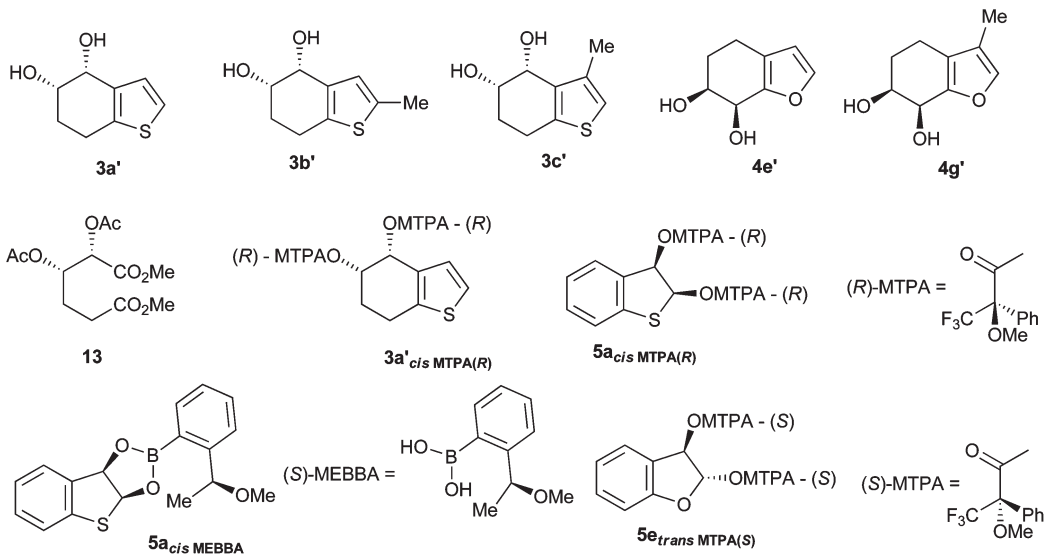
The very similar ECD spectra, *i.e.* strong negative Cotton effects in the region 205–209 nm and weak negative Cotton effects about 250 nm, observed for *cis*-tetrahydrodiols **3a′** and **3b′** indicated that the corresponding *cis*-dihydrodiol metabolites **3a** and **3b** had identical (4*R*,5*S*) absolute configurations (see ESI†). A similar absolute configuration for *cis*-diol **3c** was assigned, after comparison of the ECD spectrum of its *cis*-tetrahydrodiol **3c′** with that of *cis*-tetrahydrodiol **3a′**. An alternative approach, involving acetylation (Ac_2O/pyridine), oxidative cleavage (RuO_2/$NaIO_4$) of the corresponding diacetate, and dimethyl ester formation **13** (CH_2N_2) of the resulting dicarboxylic acid, was adopted for the absolute configuration assignment of *cis*-tetrahydrodiols **3c′** and **4g′**. This stereochemical correlation sequence relating these compounds to (2*S*,3*S*)-dimethyl(2,3-diacetoxy)adipate **13**, similar to that used earlier for assigning the absolute configuration of compound **4e′**,[4b] confirmed that *cis*-dihydrodiols **3c** and **4g** had (4*R*,5*S*) and (6*S*,7*S*) absolute configurations respectively.

As expected from earlier biotransformation studies of bicyclic azaarene substrates, *e.g.* quinoline, isoquinoline, quinoxaline and quinazoline,[5] the TDO-catalyzed *cis*-dihydroxylation of the carbocyclic rings in bicyclic thiaarenes **1a–c** and oxaarenes **1e** and **1g** was, consistently, found to yield the corresponding *cis*-dihydrodiols **3a–c**, **4e** and **4g** having identical absolute configurations (non-benzylic *S*) and ee values (>98%).

Spontaneous epimerization with the corresponding *trans* isomers (**5**$_{cis}$ ⇌ **6** ⇌ **5**$_{trans}$, Scheme 1) involved ring opening/closure *via* undetected isomeric aldehyde or ketone intermediates **6** (mutarotation). Initial epimerization studies were conducted on the 2,3-diol **5a** metabolite from the parent B[*b*]T **1a**. Similar results were observed for 2,3-diols **5d** and **5e** derived from 5MB[*b*]T **1d** and B[*b*]F **1e** respectively.

(i) Structure and relative stereochemistry of diols 5a, 5b, 5d–f. ^{1}H-NMR ($CDCl_3$) analysis showed that the initially formed *cis*-dihydrodiols **5a**$_{cis}$, **5d**$_{cis}$, and **5e**$_{cis}$ had larger vicinal coupling constants ($J_{2,3cis}$ 4.1–5.1 Hz) compared with the *trans*-isomers, **5a**$_{trans}$, **5d**$_{trans}$, and **5e**$_{trans}$ ($J_{2,3trans}$ < 1.0–1.7 Hz, Table 3). Application of the GOESY method to *cis*-dihydrodiols gave larger NOE values (2.3–3.5%) between H-2 and H-3 atoms and smaller NOE values (0.8–1.2%) for the corresponding *trans*-diols.

It was found that the equilibrium position attained by metabolites **5a**, **5d** and **5e** favoured the *trans*-epimer (90–100%) in polar solvents, *e.g.* D_2O or CD_3OD (Table 3). A similar *trans* preference (80–92%), in polar solvents and spontaneous equilibration process for *cis*- ($J_{2,3cis}$ 4.1–5.1 Hz) and *trans*-diol metabolites ($J_{2,3trans}$ 1.0 Hz), had been observed earlier with 2,3-diol metabolites derived from monocyclic thiophenes.[6] Indeed, the TDO-catalyzed dihydroxylation of substrates **1a**, **1d** and **1e** resulted in *trans*-diols **5a**$_{trans}$, **5d**$_{trans}$ and **5e**$_{trans}$ being the predominant (or only) epimers present in the aqueous culture medium.



(b) Structural and stereochemical assignments of *cis*-dihydroxylation products and derivatives from the heterocyclic rings of substrates 1a, 1b, 1d–f

Due to their configurational instability, characterization of TDO-catalysed dihydroxylation products **5**, formed by oxidation of the heterocyclic rings of the corresponding substrates **1**, was more challenging, compared with the carbocyclic ring derived *cis*-dihydrodiols **3** and **4**. The labile cyclic thioacetal, acetal or ketal groups were present in the initially formed *cis*-dihydrodiols **5**$_{cis}$. This "*trans*-dihydroxylation" of arenes was evidently the combined result of an initial *cis*-dihydroxylation followed by a rapid epimerization process. In a less polar solvent, *e.g.* $CDCl_3$, the *cis* configuration was found to be dominant for diols **5a**, **5d** and **5e** (60–80%) both in this study and in our earlier analysis of monocyclic thiophene 2,3-diols.[6] A steric preference for the *cis* configuration is expected in non-hydroxylic solvents, where favourable intramolecular H-bonding can occur.

Recrystallization of dihydrodiol **5a**, from MeOH, yielded a pure sample of *trans*-isomer **5a**$_{trans}$. The NMR spectrum of this

Table 3 Relative configurations (J_{cis} or J_{trans}), absolute configurations (Ab. config.), enantiomeric excess values (% ee), equilibrium ratios of *cis*-dihydrodiols **5a**$_{cis}$, **5b**$_{cis}$, **5d**$_{cis}$–**f**$_{cis}$ and *trans*-dihydrodiols **5a**$_{trans}$, **5d**$_{trans}$ and **5e**$_{trans}$ (*cis*/*trans* ratio)

cis-Dihydrodiol	$J_{2,3cis}$ [a] (Hz)	(% ee)	Ab. config.	*trans*-Dihydrodiol	$J_{2,3trans}$ [a] (Hz)	Ab. config.	*cis*/*trans* ratio
5a$_{cis}$	4.1	60[b], 63[c] >98[b,c]	(2*S*,3*R*)	**5a**$_{trans}$	1.6	(2*R*,3*R*)	80 : 20[a], 0 : 100[d]
5b$_{cis}$	[e]	9[b,c]	[f]				100 : 0[a]
5d$_{cis}$	4.3	>98[c]	(2*S*,3*R*)	**5d**$_{trans}$	<1.0	(2*R*,3*R*)	78 : 22[a], 0 : 100[d]
5e$_{cis}$	5.1	55[b,c]	(2*S*,3*R*)	**5e**$_{trans}$	<1.0	(2*R*,3*R*)	60 : 40[a], 55 : 45[g]
							10 : 90[h], 5 : 95[i]
5f$_{cis}$	[e]	80[c]	(2*R*,3*S*)				100 : 0[a]

[a] $CDCl_3$. [b] ^{1}H-NMR analysis of diMTPA esters. [c] ^{1}H-NMR analysis of MEBBA derivatives. [d] CD_3OD. [e] Absent. [f] Not determined. [g] d_6-benzene. [h] D_2O. [i] d_5-pyridine.

sample confirmed that only the *trans*-epimer ($J_{2,3}$ 1.6 Hz; $[\alpha]_D$ −97) was present at equilibrium in D_2O. Conversely, recrystallization of dihydrodiol **5a** from a mixture of CH_2Cl_2–hexane yielded a pure sample of *cis*-isomer **5a**$_{cis}$ ($J_{2,3}$ 4.1 Hz; $[\alpha]_D$ +117 in $CHCl_3$), which equilibrated rapidly in $CDCl_3$ solution. Pure samples of the individual epimeric metabolites **5e**$_{cis}$ and **5e**$_{trans}$, derived from B[*b*]F **1e**, could not be detected or isolated using similar recrystallization methods.

TDO-catalyzed dihydroxylation of the 2,3 bond in 2-methyl substituted substrates **1b** and **1f**, each, yielded a single diol product whose NMR spectrum and MS data were consistent with the corresponding *cis*-diol structures, **5b**$_{cis}$ and **5f**$_{cis}$. The TDO-catalyzed *cis*-dihydroxylation of alkyl substituted bonds in the carbocyclic arene rings is unusual but a precedent was found earlier using benzocyclobutene as a substrate.[9b] It was not possible to use the relative magnitude of vicinal coupling constants ($J_{2,3}$), or relative NOE values for H-2 and H-3, as an indicator of *cis* or *trans* configurations. Both ^{1}H- and ^{13}C-NMR spectra showed only the presence of single epimers, *i.e.* diols **5b**$_{cis}$ and **5f**$_{cis}$ which lack proton H-3.

Recrystallization of diol **5b**$_{cis}$ had earlier yielded a racemic sample suitable for X-ray crystal structure analysis. This confirmed the relative stereochemistry of diol **5b** as *cis*.[2c] While, in principle, the monothioacetal diol **5b**$_{cis}$ could epimerize to its *trans*-isomer **5b**$_{trans}$, it was expected that the process would be slower and that the *cis*-epimer would be dominant at equilibrium, due to steric interactions between the vicinal Me and OH groups. For similar reasons, it is assumed that the heterocyclic ring diol **5f** would also have a *cis* configuration.

A marked difference in stabilities was found between carbocyclic *cis*-dihydrodiols **3a–c**, **4e** and **4g** and the heterocyclic *cis*/*trans* diols **5a**, **5b** and **5d–f**. This resulted in the isolation of a range of phenols, *e.g.* **7a**, **8a**, **9e–g**, from the mixture of bioproducts of the corresponding substrates **1a**, **1e–g**. No evidence of spontaneous aromatization of heterocyclic dihydrodiols **5a**, **5b**, **5d–f** to yield the corresponding phenols, *e.g.* **11a** and **11e** (or their keto tautomers **12a** and **12e**), was found during the biotransformation. These observations were supported by NMR analysis of products formed by acid-catalyzed (TFA) dehydration of the representative heterocyclic diol **5a**, in $CDCl_3$ solution over several hours, at room temperature. The major product (95%) found was ketone **12a** whose structure was confirmed by chemical synthesis using the literature method.[7a] A crystalline sample of ketone **12a** showed the characteristic NMR signal for H-2 (δ_H 3.8) in $CDCl_3$ solution. Rapid tautomerization resulted in the formation of enol **11a** (δ_H 6.5 for H-2), as the minor component (5%) at equilibrium, in $CDCl_3$ solution. The equilibrium ratio of tautomers **11a** : **12a** was found to favour the enol tautomer (2 : 1) in CD_3OD solution as reported earlier.[7b] Similar TFA treatment of dihydrodiol **5e**, at room temperature for an extended period (24 h), showed no evidence of decomposition, but dehydration was found to occur at an elevated temperature (60 °C, 4 h), giving keto tautomer **12e** (δ_H 4.7 for H-2) exclusively. It is noteworthy that the dehydration products (phenols **7–9**) derived from the less stable carbocyclic dihydrodiols **3a**, **4e**, and **4g** exhibited increased aromatic character compared with the dehydration products (keto/enols **11** and **12**), formed from the more stable heterocyclic diols, *e.g.* **5a** and **5e**.

Early biotransformation studies of B[*b*]F **1e**, using *P. putida* UV4,[2b] provided no direct evidence of the heterocyclic dihydrodiol **5e** (Table 1). Isolation of the phenolic acyclic diol **10e**, however, suggested that it could have resulted from ring opening and reduction of aldehyde **6e**. This premise was confirmed by a subsequent time-course study which showed that while dihydrodiol **5e** was the major metabolite (45%), after a biotransformation period of 12 h, its proportion decreased to zero over a 24 h biotransformation period with the concomitant formation of phenolic diol **10e** (35% after 24 h). Dihydrodiol **5e** was successfully intercepted and isolated, when the biotransformation was terminated after 12 h (19% isolated yield, Table 1).

Addition of metabolite **5e** as a substrate, to *P. putida* UV4, or treatment with $NaBH_4$, both resulted in its total conversion to phenolic diol **10e** (55% ee, determined by the cyclic boronate method[5,6]). The absolute configuration of (−)-phenolic diol **10e** was established as (1*R*), in our earlier study[2b] of B[*b*]F **1e** metabolites. A similar phenolic diol **10f**, isolated as a very minor metabolite of 2MB[*b*]F **1f**, was found to be a single enantiomer (>98% ee). It was assigned as a *syn* diastereoisomer, by comparison of its ^{1}H NMR spectrum with that reported in the literature.[8] This result clearly shows that a carbonyl reductase (CRED) enzyme, capable of catalyzing stereoselective aldehyde and ketone reductions, is present in *P. putida* UV4 cultures. A similar ring opening and aldehyde reduction sequence of B[*b*]T dihydrodiol **5a** did not appear to have occurred, since the corresponding exocyclic thiophenolic diol was not detected within the mixture of bioproducts.

(ii) Absolute configurations and ee values of diols 5a, 5b, 5d–f. As the instability of carbocyclic *cis*-dihydrodiols **3a–c**, **4e** and **4g** led to their decomposition, during the attempted direct

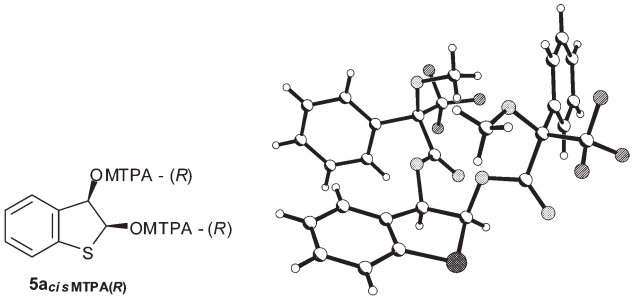


Fig. 2 X-ray crystal structure of $\mathbf{5a}_{cis\mathbf{MTPA}(R)}$.

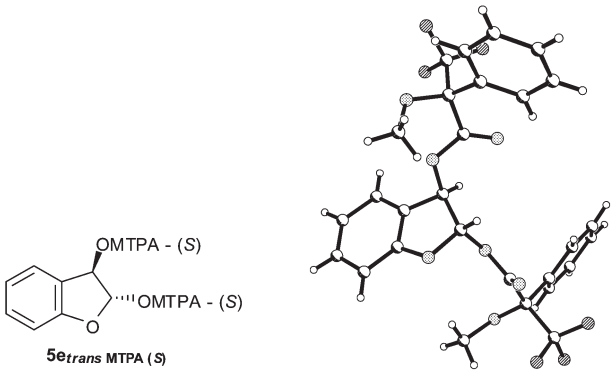


Fig. 3 X-ray crystal structure of $\mathbf{5e}_{trans\mathbf{MTPA}(S)}$.

formation of their diMTPA esters, the diMTPA esters from the stable *cis*-tetrahydrodiols **3a′–c′**, **4e′** and **4g′** were utilized for the determination of enantiopurity. The heterocyclic 2,3-diols **5a**, **5b**, **5d–f**, as indicated, were found to be much more resistant to aromatization and could be converted, directly, to the corresponding diMTPA esters. Treatment of dihydrodiols $\mathbf{5a}_{cis}/\mathbf{5a}_{trans}$ with the acid chloride of (+)-(*R*)-MTPA, in pyridine solution, gave a mixture (40 : 60) of the corresponding diMTPA esters $\mathbf{5a}_{cis\mathbf{MTPA}(R)}$ and $\mathbf{5a}_{trans\mathbf{MTPA}(R)}$. ^{1}H-NMR spectral analysis of the diMTPA esters showed that the dihydrodiol metabolites $\mathbf{5a}_{cis}/\mathbf{5a}_{trans}$ had been formed as single enantiomers (>98% ee, Table 3). PLC separation of the diMTPA esters, followed by recrystallization, provided a suitable sample of ester $\mathbf{5a}_{cis\mathbf{MTPA}(R)}$ for X-ray crystallographic analysis (Fig. 2). The analysis confirmed the relative configuration as *cis* and the absolute configuration as (2*S*,3*R*). In the crystal, the heterocyclic ring adopts an envelope conformation with C-2 0.38 Å *above* the plane of the ring (as viewed in Fig. 2). The OMTPA group on C-2 is *axial* and on C-3 *pseudoequatorial*. The dihedral angle between the *cis*-related oxygen atoms at C-2 and C-3, shown in Fig. 2, is +28°. When the biotransformation of B[*b*]T **1a** was repeated at a later date, formation of the diMTPA esters, surprisingly, revealed that dihydrodiols $\mathbf{5a}_{cis}/\mathbf{5a}_{trans}$ were of lower enantiopurity (60% ee).

An alternative approach, to the determination of ee values of dihydrodiols, involved forming cyclic boronate derivatives, using (−)-(*S*)-2-(1-methoxyethyl)-benzene boronic acid (MEBBA). This method had earlier been successfully used for *cis*/*trans*-dihydrodiol metabolites of monocyclic thiophenes,[6] and was again applied to dihydrodiols $\mathbf{5a}_{cis}/\mathbf{5a}_{trans}$. The MEBBA derivative $\mathbf{5a}_{cis\mathbf{MEBBA}}$ was formed, exclusively, from the equilibrating epimeric mixture.

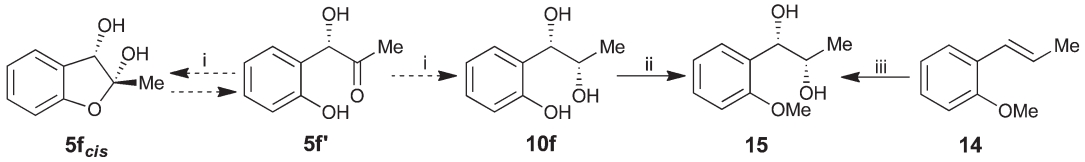
Analysis of the ^{1}H-NMR spectrum of boronate $\mathbf{5a}_{cis\mathbf{MEBBA}}$ gave an ee value of 63% which was in good agreement with the value obtained, using the diMTPA method, for the later sample of dihydrodiols $\mathbf{5a}_{cis}/\mathbf{5a}_{trans}$ (60%, Table 3). Formation of the corresponding cyclic MEBBA derivatives of dihydrodiols **5b** and **5f** provided further evidence that both existed exclusively as *cis*-diol epimers $\mathbf{5b}_{cis}$ and $\mathbf{5f}_{cis}$ at equilibrium.

The ee values for heterocyclic dihydrodiols **5b** and **5d–f**, determined using the MEBBA derivatives, covered a remarkably wide range (<10 to >98% ee, Table 3). The different ee values, found among the heterocyclic dihydrodiols in Table 3, could have resulted from variable enantioselectivity occurring during the TDO-catalyzed dihydroxylation. However, in view of the consistent formation of the carbocyclic *cis*-diols **3a–c**, **4e** and **4g**, as single enantiomers, an alternative explanation based on the partial racemization of the single chiral centre, present in the elusive ring-opened intermediate **6**, should be considered (Scheme 1). Tautomerization of the aldehyde or ketone group in compound **6**, to form a conjugated achiral enol, could explain this partial racemization of the thiophene and furan ring dihydrodiols **5a**, **5b**, **5e** and **5f**.

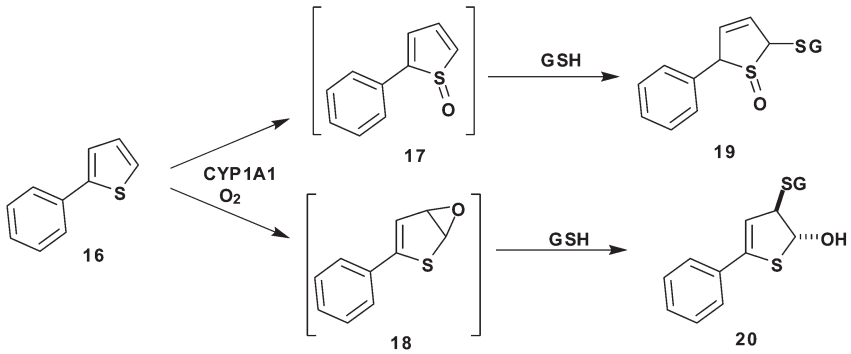
Reaction of the acid chloride from (−)-(*S*)-MTPA with the metabolite $\mathbf{5e}_{trans}/\mathbf{5e}_{cis}$ yielded four diastereoisomers from which the major (85%) *trans* isomers were separated by PLC. Fractional crystallization of the *trans* diastereoisomers yielded a sample of a single diastereoisomer. X-ray crystal structure analysis showed it to be the (2*S*,3*R*)-diMTPA ester $\mathbf{5e}_{trans\mathbf{MTPA}(S)}$ (Fig. 3). This confirmed the *trans* configuration and provided unequivocal evidence of a (2*R*,3*R*) absolute configuration for the precursor diol $\mathbf{5e}_{trans}$ (Table 3). This result is in accord with the established (1*R*) configuration of the derived phenolic diol **10e** which was found to be a further metabolite of diols $\mathbf{5e}_{cis}/\mathbf{5e}_{trans}$. In the crystal of $\mathbf{5e}_{trans\mathbf{MTPA}(S)}$, the heterocyclic ring adopts an envelope conformation with C-2 0.27 Å *below* the plane of the ring. This fold is smaller, and in the opposite sense, to that observed in $\mathbf{5a}_{cis\mathbf{MTPA}(R)}$. It thus retains the *axial* conformation for the oxygen atom on C-2, as $\mathbf{5e}_{trans\mathbf{MTPA}(S)}$ and $\mathbf{5a}_{cis\mathbf{MTPA}(R)}$ have opposite *absolute* configurations at C-2. It follows that the OMTPA group on C-3 in $\mathbf{5e}_{trans\mathbf{MTPA}(S)}$ is *pseudoaxial*. The dihedral angle between the *trans*-related oxygen atoms at C-2 and C-3, shown in Fig. 3, is −153°.

The preference for *axial* substituents at C-2 in solution, even when the bulky OMTPA group is absent, is supported by the crystal structure of *cis*-diol $\mathbf{5b}_{cis}$[2c] which also has an envelope conformation for the heterocyclic ring. In the (2*S*,3*R*) enantiomer, within the diol racemate $\mathbf{5b}_{cis}$, C-2 lies *above* the plane of the ring by 0.59 Å and 0.61 Å in the two crystallographically independent molecules. As in $\mathbf{5a}_{cis\mathbf{MTPA}(R)}$, the oxygen atom on C-2 in diol $\mathbf{5b}_{cis}$ is *axial* and on C-3 *pseudoequatorial*. The dihedral angles between the *cis*-related oxygen atoms at C-2 and C-3, in the two independent molecules of diol $\mathbf{5b}_{cis}$, are +50° and +53°. The preference for *axial* substituents at C-2 appears to be analogous to the anomeric effect found in sugars and substituted tetrahydropyrans or thianes.

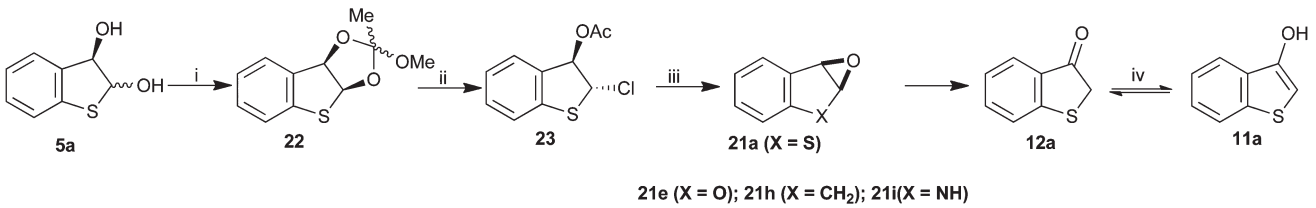
Electronic circular dichroism (ECD) spectra of dihydrodiols $\mathbf{5a}_{cis}/\mathbf{5a}_{trans}$ and $\mathbf{5d}_{cis}/\mathbf{5d}_{trans}$, in acetonitrile solvent, proved to be very similar and provided confirmation that they had identical absolute configurations (Table 3 and also ESI†). The relative



Scheme 2 Absolute configuration assignment of metabolites **5f**$_{cis}$ and **10f**.



Scheme 3 Monooxygenase-catalyzed sulfoxidation and epoxidation of 2-phenylthiophene (**16**) to yield sulfoxide (**17**) and arene oxide (**18**) metabolites and their glutathione adducts (**19** and **20**).[13]



Scheme 4 Synthesis of B[*b*]T-2,3-oxide **21a** and derived products, **11a** and **12a**.

configuration of the 2-methyl substituted diol metabolites, from substrates **1b** and **1f**, was established as *cis* by X-ray crystallography (**5b**$_{cis}$)[2c] and MEBBA derivative formation (**5f**$_{cis}$). The crude sample of heterocyclic ring metabolite **5b**$_{cis}$ was found to have a very low ee value (9%, Table 3), which on recrystallization yielded a racemic sample.[2c] X-ray crystallography of the sample[2c] therefore proved only the relative configuration.

The assignment of absolute configuration to *cis*-diol **5f**$_{cis}$ was carried out indirectly through stereochemical correlation to the phenolic diol metabolite **10f** (Scheme 2). It was assumed to be formed *via* ring opening of the diol **5f**$_{cis}$, to yield the corresponding undetected acyclic ketone **5f'**. Treatment of phenolic diol metabolite **10f** with diazomethane yielded the corresponding methyl ether **15** ([α]$_D$ +22, EtOH, Scheme 2). A sample of methyl ether **15**, with an [α]$_D$ +26 (EtOH), was independently synthesized by *cis*-dihydroxylation of *trans*-1-(2′-methoxyphenyl)prop-1-ene **14** using the chiral dihydroxylating reagent, AD-mix-α. Based on the reported *cis*-dihydroxylation of *trans*-β-methylstyrene by AD-mix-α, which produced mainly the (*S*,*S*) enantiomer (97% ee) of the corresponding diol,[10] it was assumed that methyl ether **15** would have a similar configuration. This resulted in a (2*R*,3*S*) configuration being assigned to *cis*-diol metabolite **5f**$_{cis}$. As the configuration at C-3 is opposite to that found for the other heterocyclic *cis* diols, *e.g.* **5a**$_{cis}$, **5d**$_{cis}$ and **5e**$_{cis}$, this assignment is tentative; it is based only on the assumption that the Sharpless mnemonic for AD-mix-α dihydroxylations[10] is applicable to the *ortho* substituted phenyl alkene **14**.

(c) Synthesis of B[*b*]T 2,3-oxide 21a from 2,3-dihydroxy-2,3-dihydrobenzo[*b*]thiophene 5a$_{cis}$/5a$_{trans}$

Dansette *et al.*[13] have proposed that monooxygenases, *e.g.* the cytochrome P450, CYP1A1, can catalyze the concomitant sulfoxidation and epoxidation of thiophene rings. Thus, 2-phenylthiophene **16** was biotransformed into the unstable thiophene oxide **17** and arene oxide **18** (Scheme 3). Evidence for the presence of these transient intermediates was obtained by trapping them as stable glutathione adducts **19** and **20**. This report,[13] allied to our earlier isolation of the unstable B[*b*]T sulfoxide **2a**,[2a] prompted the current synthesis of the previously unknown B[*b*]T 2,3-arene oxide **21a**.

Dioxygenase-catalyzed oxidation of indene **1h**,[11a–e] B[*b*]T **1a** and B[*b*]F **1e**, using TDO, has been shown to produce the corresponding *cis*-diols **5h**$_{cis}$,[11a–e] **5a**$_{cis}$, **5e**$_{cis}$ and sulfoxide **2a**[2a] as the initial metabolites (Scheme 1). Using styrene monooxygenase (SMO) as a biocatalyst, the oxidation of indene **1h** was found to form, exclusively, indene 1,2-epoxide **21h** (Scheme 4).[12a–c] The formation of indigo as a metabolite of indole **1i** (R = R′ = R″ = H, X = NH, Scheme 1), when using either monooxygenase or dioxygenase biocatalysts, is also consistent with an epoxidation (SMO-catalyzed) to yield arene oxide **21i**, or a dihydroxylation (TDO-catalyzed) to give *cis*-dihydrodiol **5i**$_{cis}$ (R = R′ = R″ = H, X = NH, Scheme 1) as the initial metabolite. Both oxidation products **21i** and **5i** are assumed to be transient metabolites and would spontaneously decompose to yield indoxyl, which in turn undergoes autoxidation to yield indigo.[12a–c] In this context, the question of whether the SMO enzyme could similarly catalyze the epoxidation of B[*b*]T **1a** to yield metabolite **21a**, albeit as a very minor metabolic pathway compared to the formation of unstable sulfoxide **2a**,[2a] was also addressed in this study.

Earlier publications[14a–c] had shown that the relatively stable carbocyclic K-region *cis*-dihydrodiols of polycyclic aromatic hydrocarbons, and their *ortho*-ester derivatives, can be used as precursors in the synthesis of the corresponding K-region arene oxides (*e.g.* from phenanthrene, pyrene, chrysene, benz[*a*]anthracene, benzo[*c*]phenanthrene, benzo[*a*]pyrene and benzo[*e*]-pyrene). Adapting this *ortho*-ester approach to the relatively stable heterocyclic ring dihydrodiol **5a**$_{cis}$/**5a**$_{trans}$, a chemoenzymatic synthesis of B[*b*]T 2,3-oxide **21a** was developed (Scheme 4).

Diol epimers **5a**$_{cis}$/**5a**$_{trans}$ were converted into an inseparable mixture of dioxolane stereoisomers **22** (77 : 27). The mixture was reacted, without further purification, with trimethylsilyl chloride to yield *trans*-chloroacetate **23**. In common with the *ortho*-ester procedure, used earlier for the synthesis of K-region arene oxides of PAHs, [14a–c] dioxolane mixture **22** and the relatively unstable *trans*-chloroacetate intermediates **23** were characterized mainly by ^{1}H-NMR spectroscopy and mass spectrometry. A sample of chloroacetate **23**, obtained by rapid purification using PLC, was treated with NaOMe in THF-d_8 solvent to yield the desired unpurified arene oxide **21a** in a yellow solution. The NMR spectra and MS data of the crude arene oxide **21a** were fully consistent with its structure. As expected, arene oxide **21a** was found to be very unstable and during attempted PLC purification, prior to $[\alpha]_D$ measurement, it rapidly isomerized to give the keto tautomer **12a** which was found to equilibrate, spontaneously, to phenol **11a** in MeOH solution.

Acid-catalyzed dehydration of diols **5a**$_{cis}$/**5a**$_{trans}$ and isomerization of the arene oxide **21a** both yielded tautomers **11a** and **12a**. Neither LC-TOFMS nor NMR spectroscopic analyses showed any evidence for the presence of arene oxide **21a** or the expected decomposition products **11a** and **12a**, among the metabolites of B[*b*]T **1a**, following biotransformation using *E. coli* BL21 (DE3) as the SMO source. While SMO-catalyzed oxidation of indene **1h** followed an epoxidation pathway, exclusively, in this study sulfoxidation was observed as the only SMO-catalyzed oxidation pathway during B[*b*]T **1a** metabolism.

A direct single step synthesis of methyl substituted 2,3-oxide derivatives of B[*b*]Fs and *N*-acylindoles has been reported by Adam *et al*. using dimethyldioxirane (DMD) as an oxidant.[15a–d] Our efforts to synthesise B[*b*]T 2,3-oxide using DMD oxidation yielded only the corresponding sulfoxide and derivatives.[2a] No attempt was made to synthesize the parent 2,3-oxide from B[*b*]F **1e** *via* the *ortho*-ester method, as the corresponding *cis*/*trans* dihydrodiols **5e**$_{cis}$/**5e**$_{trans}$ were only available in lower yields and were less stable than diols **5a**$_{cis}$/**5a**$_{trans}$. To date, the corresponding 2,3-arene oxides of indole (**21i**) and B[*b*]F (**21e**) have not been reported; they are expected to be much less stable than the isolated 2,3-dimethyl substituted 2,3-oxides of B[*b*]F[15a–c] and *N*-acylindoles.[15d]

Conclusions

The TDO-catalyzed dihydroxylation of a series of B[*b*]T **1a–d** and B[*b*]F **1e–g** substrates yielded (i) five carbocyclic *cis*-diols **3a–c**, **4e**, **4g**, (ii) eight heterocyclic *cis*-diols **5a**$_{cis}$, **5b**$_{cis}$, **5d**$_{cis}$–**f**$_{cis}$, **5a**$_{trans}$, **5d**$_{trans}$ and **5e**$_{trans}$ and (iii) two acyclic diols **10e** and **10f**. The structures, ee values and absolute configurations of these chiral metabolites were determined by a combination of X-ray crystallography, ECD spectroscopy and stereochemical correlation methods. The heterocyclic ring *cis*-diols **5a**$_{cis}$, **5d**$_{cis}$, and **5e**$_{cis}$ were assumed to be the initial metabolites and were found to equilibrate spontaneously (mutarotation), preferring the *trans* configuration **5a**$_{trans}$, **5d**$_{trans}$, and **5e**$_{trans}$ in aqueous solution.

The chemoenzymatic synthesis of the unstable heterocyclic arene oxide B[*b*]T 2,3-oxide **21a** has been achieved in three steps from the corresponding *cis*/*trans*-diol bioproduct **5a**$_{cis}$/**5a**$_{trans}$. No evidence for this transient metabolite was found, during the biotransformation of B[*b*]T **1a**, using SMO as a biocatalyst. It is probable that the mutarotation process of the initially formed 2,3-*cis*-diol metabolites of B[*b*]T **1a** and B[*b*]F **1e** (*via* TDO biocatalysis), and formation of a heterocyclic 2,3-oxide (*via* SMO biocatalysis), in the five membered heterocyclic rings of B[*b*]T and B[*b*]F also occurs during TDO- and SMO-catalyzed biotransformations of other five-membered aromatic heterocyclic ring systems, *e.g.* indole **1i**.

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. IR spectra were recorded on a Perkin-Elmer Model 983G instrument, coupled to a Perkin-Elmer 3700 data station, in potassium bromide (KBr) disks unless otherwise stated. High resolution mass spectra (HRMS) were determined by the peak matching method, with perfluorokerosene as the standard. A PerkinElmer 341 polarimeter was used for optical rotation ($[\alpha]_D$) measurements. ECD spectra were recorded in spectroscopic grade acetonitrile using a JASCO J-720 instrument. 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. Enantiomeric excess values (% ee) for diols were determined by formation of MTPA (Method A) and

MEBBA (Method B) derivatives using previously described methods.[4] X-ray crystal structure analysis of diMTPA esters was carried out using a Siemens P3 diffractometer. All B[*b*]T and B[*b*]F substrates were available from commercial sources.

Biotransformations, with *Pseudomonas putida* UV4 whole cells, as a source of TDO, were conducted employing the reported conditions.[2a,6] The relative yields of diols and derived products are given in Table 1. Separation of bioproducts was achieved by flash column chromatography and PLC on silica gel. R_f values were recorded using either Et_2O : hexane (1 : 1, eluent A) or EtOAc : hexane (55 : 45, eluent B).

Biotransformations of benzo[*b*]thiophene 1a, 2-methyl benzo[*b*]-thiophene 1b, 3-methyl benzo[*b*]thiophene 1c, benzo[*b*]furan 1e and 3-methyl benzo[*b*]furan 1g using *P. putida* UV4 to yield the corresponding carbocyclic *cis*-diol and phenol bioproducts

(i) Metabolites from benzo[*b*]thiophene 1a

*(+)-(4*R,*5*S*)*-cis-*4,5-Dihydroxy-4,5-dihydrobenzo[*b*]*thiophene* ***3a***. Yield (0.79 g, 6%); mp (dec.) 62–76 °C (from $CHCl_3$–hexane); R_f 0.20 (eluent A); $[\alpha]_D$ +98.0 (*c* 0.57, $CHCl_3$) (Found: C, 57.5; H, 4.6; S, 18.9. $C_8H_8O_2S$ requires C, 57.1; H, 4.8; S, 19.1%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.35 (2 H, br s, 2 × OH), 4.47 (1 H, m, 5-H), 4.71 (1 H, d, $J_{4,5}$ 5.6, 4-H), 5.91 (1 H, dd, $J_{6,7}$ 9.8, $J_{6,5}$ 3.3, 6-H), 6.51 (1 H, dd, $J_{7,6}$ 9.8, $J_{7,5}$ 1.6, 7-H), 7.13 (1 H, d, $J_{3,2}$ 5.0, 3-H), 7.20 (1 H, d, $J_{2,3}$ 4.9, 2-H); LRMS (EI): *m*/*z* 168 (M^+, 100%), 150 ($M^+ - H_2O$, 66.8); >98% ee (Method A).

*4-Hydroxybenzo[*b*]thiophene* ***7a***. Yield (0.52 g, 4%); mp 74–76 °C (from Et_2O–hexane) (lit.[16] 76–78 °C); R_f 0.88 (eluent A); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.19 (1 H, br s, OH), 6.71 (1 H, d, $J_{5,6}$ 7.3, 5-H), 7.19 (1 H, dd, $J_{6,7}$ 7.6, $J_{6,5}$ 7.3, 6-H), 7.35 (1 H, d, $J_{2,3}$ 5.6, 2-H), 7.47 (2 H, dd, $J_{7,6}$ 7.5, $J_{3,2}$ 5.7, 7-H, 3-H); LRMS (EI): *m*/*z* 150 (M^+, 100%), 121 (25).

*5-Hydroxybenzo[*b*]thiophene* ***8a***. Yield (0.71 g, 5%); mp 70–71 °C (from Et_2O–hexane) (lit.[16] 72–77 °C); R_f 0.85 (eluent A); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.95 (1 H, dd, $J_{6,7}$ 8.7, $J_{6,4}$ 2.4, 6-H), 7.23 (1 H, d, $J_{3,2}$ 5.6, 3-H), 7.27 (1 H, d, $J_{4,6}$ 2.5, 4-H), 7.47 (1 H, d, $J_{2,3}$ 5.6, 2-H), 7.74 (1 H, d, $J_{7,6}$ 8.6, 7-H); LRMS (EI): *m*/*z* 150 (M^+, 100%), 121 (14).

(ii) Metabolite from 2-methyl benzo[*b*]thiophene 1b

*(+)-2-Methyl-(4*R,*5*S*)*-cis-*4,5-dihydroxy-4,5-dihydrobenzo[*b*]*thiophene* ***3b***. Yield (1.80 g, 25%); mp (dec.) 96–102 °C (from $CHCl_3$–hexane); R_f 0.23 (eluent A); $[\alpha]_D$ +125.0 (*c* 0.55, MeOH) (Found: C, 54.3; H, 5.8; S, 16.4. $C_9H_{10}O_2S{\cdot}H_2O$ requires C, 54.0; H, 6.0; S, 16.0%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.38 (1 H, br d, $J_{OH,4}$ 6.5, OH), 2.46 (1 H, s, Me), 2.59 (1 H, d, $J_{OH,5}$ 4.9, OH), 4.39–4.44 (1 H, m, 5-H), 4.59 (1 H, dd, $J_{4,5}$ 5.7, $J_{4,OH}$ 7.9, 4-H), 5.80 (1 H, dd, $J_{6,7}$ 9.7, $J_{6,5}$ 3.2, 6-H), 6.38 (1 H, dd, $J_{7,6}$ 9.9, $J_{7,5}$ 1.9, 7-H), 6.78 (1 H, s, 2-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 15.3, 67.8, 69.6, 121.1, 125.5, 126.9, 132.1, 136.5, 139.6; LRMS (EI): *m*/*z* 182 (M^+, 55%), 164 (100); ν_{max} (KBr) 3250 cm^{-1} (OH).

(iii) Metabolite from 3-methyl benzo[*b*]thiophene 1c

*(+)-3-Methyl-(4*R,*5*S*)*-cis-*4,5-dihydroxy-4,5-dihydrobenzo[*b*]*thiophene* ***3c***. Yield (4.1 g, 45%); mp (dec.) 83–89 °C (from $CHCl_3$–hexane); R_f 0.2 (eluent A); $[\alpha]_D$ +103.0 (*c* 0.46, MeOH) (Found: C, 58.9; H, 5.5; S, 17.6. $C_9H_{10}O_2S$ requires C, 59.3; H, 5.5; S, 17.6%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.72 (1 H, br s, OH), 2.29 (3 H, s, Me), 2.79 (1 H, br s, OH), 4.56–4.60 (2 H, m, 4-H, 5-H), 5.80 (1 H, d, $J_{7,6}$ 9.8, 7-H), 6.42 (1 H, dd, $J_{6,7}$ 9.8, $J_{6,5}$ 2.5, 6-H), 6.81 (1 H, s, 2-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 13.5, 65.2, 71.1, 120.2, 120.3, 129.2, 134.8, 135.8, 137.0; LRMS (EI): *m*/*z* 182 (M^+, 50%), 164 ($M^+ - H_2O$, 100); ν_{max} (KBr) 3255 cm^{-1} (OH); >98% ee (Method A).

(iv) Metabolites from benzo[*b*]furan 1e

*(−)-(6*S,*7*S*)*-cis-*6,7-Dihydroxy-6,7-dihydrobenzo[*b*]*furan* ***4e***. Yield (1.9 g, 15%); mp (dec.) 60–61 °C (from $CHCl_3$–hexane) (lit.,[4b] 61–63 °C); R_f 0.39 (eluent B); $[\alpha]_D$ −33.0 (*c* 0.77, MeOH) (lit.,[4b] −34.7, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.99 (2 H, br s, OH), 4.57–4.65 (2 H, m, 6-H, 7-H), 6.57 (1 H, dd, $J_{5,4}$ 9.6, $J_{5,6}$ 2.1, 5-H), 6.24 (1 H, dd, $J_{4,5}$ 9.6, $J_{4,6}$ 2.5, 4-H), 6.29 (1 H, d, $J_{3,2}$ 1.8, 3-H), 7.35 (1 H, d, $J_{2,3}$ 1.8, 2-H); >98% ee (Method A).

6-Hydroxybenzofuran ***9e***. Yield (0.034 g, 3%); R_f 0.83 (eluent B). It was found to be spectrally identical to an authentic sample.[4b]

(v) Metabolite from 2-methyl benzo[*b*]furan 4g

*6-Hydroxy-2-methylbenzo[*b*]furan* ***9f***. Yield (0.168 g, 3%); mp 48–49 °C (Et_2O–hexane); R_f 0.85 (eluent B) (Found: C, 72.8; H, 5.6. $C_9H_8O_2$ requires C, 73.0; H, 5.4%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.41 (3 H, s, Me), 6.27 (1 H, s, 3-H), 6.72 (1 H, dd, $J_{5,4}$ 8.3, $J_{5,7}$ 2.2, 5-H), 6.91 (1 H, d, $J_{7,5}$ 1.9, 7-H), 7.28 (1 H, d, $J_{4,5}$ 8.2, 4-H); LRMS (EI): *m*/*z* 148 (M^+, 100%), 91(56), 77(83); ν_{max} (KBr): 3498 cm^{-1} (OH).

(vi) Metabolites from 3-methylbenzo[*b*]furan 1g

*(−)-(6*S,*7*S*)*-cis-*6,7-Dihydroxy-6,7-dihydro-3-methyl benzo[*b*]-furan* ***4g***. Yield (0.60 g, 12%); mp (dec.) 82–84 °C (from Et_2O–hexane); R_f 0.41 (eluent B); $[\alpha]_D$ −48 (*c* 0.78, $CHCl_3$); HRMS (Found: M^+ 166.06384. $C_9H_{10}O_3$ requires 166.06389); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.06 (3 H, s, Me), 4.58–4.62 (2 H, m, 6-H, 7-H), 5.75 (1 H, d, $J_{5,4}$ 10.3, 5-H), 6.34 (1 H, dd, $J_{4,5}$ 10.0, $J_{4,6}$ 2.3, 4-H), 7.08 (1 H, s, 2-H); LRMS (EI): *m*/*z* 166 (M^+, 44%), 148 (100), 123 (74); ν_{max} (KBr): 3468 (OH); >98% ee (Method A).

6-Hydroxy-3-methyl benzofuran ***9g*** *(from* cis*-diol* ***4g****)*. Yield (0.175 g, 4%); mp 90–91 °C ($CHCl_3$–hexane); R_f 0.88 (eluent B) (Found: C, 72.5; H, 5.2. $C_9H_8O_2$ requires C, 73.0; H, 5.4%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.16 (3 H, s, Me), 6.80 (1 H, dd, $J_{5,4}$ 8.7, $J_{5,7}$ 2.6, 5-H), 6.92 (1 H, d, $J_{7,5}$ 2.5, 7-H), 7.29 (1 H, d, $J_{4,5}$ 8.7, 4-H), 7.37 (1 H, s, 2-H); LRMS (EI): *m*/*z* 148 (M^+, 100%), 147 (82); ν_{max} (KBr): 3550 cm^{-1} (OH).

Heterocyclic ring *cis*/*trans* diol bioproducts 5a, 5b, 5d–f and derivatives $5a_{cis}$MTPA(*R*), $5e_{trans}$MTPA(*S*), 10a, 10e, 10f, 11a from substrates 1a, 1b, 1d–f using *P. putida* UV4

(i) Metabolites from benzo[*b*]thiophene 1a

*2,3-Dihydroxy-2,3-dihydrobenzo[*b*]thiophene* ***5a****cis*/***5a****trans*. The isomeric mixture of diols $\mathbf{5a}_{cis}/\mathbf{5a}_{trans}$ was obtained by flash chromatography (Et_2O : hexane, 1 : 1); yield (4.86 g, 37%); R_f 0.5 (eluent A) (Found: C, 57.1; H, 4.8; S, 19.0. $C_8H_8O_2S$ requires C, 57.1; H, 4.8; S, 19.1%); LRMS (EI): *m*/*z* 168 (M^+, 86%), 150 (12), 139 (100); 60–63% ee (Methods A and B).

*(+)-(2*S*,3*R*)-2,3-Dihydroxy-2,3-dihydrobenzo[*b*]thiophene* ***5a**$_{cis}$*. Mp 99–100 °C (from CH_2Cl_2–hexane); $[\alpha]_D$ +116.8 (*c* 0.41, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.13 (1 H, d, $J_{3,2}$ 4.1, 3-H), 5.58 (1 H, d, $J_{2,3}$ 4.1, 2-H), 7.13–7.25 (3 H, m, 4-H, 5-H, 6-H), 7.40 (1 H, d, $J_{7,6}$ 7.3, 7-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 78.8, 82.0, 123.6, 125.5, 125.7, 129.8, 136.7, 138.4; ν_{max} (KBr): 3412 cm^{-1} (OH).

*(+)-(2*S*,3*R*)-bis-[(*R*)-2'-Methoxy-2'-phenyl-2'-trifluoromethylacetoxy]-2,3-dihydrobenzo[*b*]thiophene* ***5a**$_{cisMTPA(R)}$*. To a mixture (40 : 60) of *cis* : *trans*-diols **5a**$_{cis}$/**5a**$_{trans}$ (0.050 g, 0.3 mmol) in anhydrous pyridine (0.5 ml) was added (+)-MTPA chloride (0.154 g, 0.66 mmol). The reaction mixture was left overnight at room temperature. Excess of pyridine was removed under high vacuum by addition of an equivalent volume of toluene and the mixture concentrated under high vacuum. Purification of the crude product by PLC (two elutions using 10% Et_2O : hexane) afforded diMTPA ester (+)-**5a**$_{cisMTPA(R)}$ as a white crystalline compound (0.029 g, 16%); mp 123–125 °C (from Et_2O–hexane); R_f 0.59; $[\alpha]_D$ +243 (0.78, $CHCl_3$) (Found: C, 56.3; H, 3.8; S, 5.8. $C_{28}H_{22}O_6F_6S$ requires C, 56.0; H, 3.7; S, 5.4%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.07 (3 H, s, OMe), 3.35 (3 H, s, OMe), 6.32 (1 H, d, $J_{3,2}$ 4.8, 3-H), 6.72 (1 H, d, $J_{2,3}$ 4.9, 2-H), 6.82–7.49 (14 H, m, Ar-H); LRMS (EI): *m*/*z* 600 (M$^+$, 8%), 189 (74), 57 (100); ν_{max} (KBr): 1758 cm^{-1} (C=O).

Crystal data for ***5a**$_{cisMTPA(R)}$*. $C_{28}H_{22}F_6O_6S$, M = 600.5, monoclinic, a = 10.186(3), b = 12.692(4), c = 10.984(3) Å, β = 93.75 (2)°, U = 1417.0(7) Å^3, T = 293(2) K, space group $P2_1$ (no. 4), Mo-Kα radiation, λ = 0.71073 Å, Z = 2, F(000) = 616, D_x = 1.407 g cm^{-3}, μ = 0.194 mm^{-1}, Siemens P3 diffractometer, ω scans, 3.7° < 2θ < 55.1°, measured/independent reflections: 3595/3423, direct methods solution, full-matrix least squares refinement on F_o^2, anisotropic displacement parameters for non-hydrogen atoms; all hydrogen atoms located in a difference Fourier synthesis but included at positions calculated from the geometry of the molecule using the riding model, with isotropic vibration parameters. R_1 = 0.063 for 1474 data with $F_o > 4\sigma(F_o)$, 373 parameters, wR_2 = 0.122 (all data), GoF = 1.03, $\Delta\rho_{min,max}$ = −0.17/0.21 e Å^{-3}. CCDC 871979. The absolute configuration is established as (2*S*,3*R*) from the anomalous scattering arising from the sulfur atom (Flack parameter, x = 0.05(17)) and independently from the known absolute configuration of the (*R*)-MTPA group.

*(−)-(2*R*,3*R*)-2,3-Dihydroxy-2,3-dihydrobenzo[*b*]thiophene* ***5a**$_{trans}$*. Mp 97–98 °C (from MeOH); $[\alpha]_D$ −97.0 (*c* 0.48, MeOH) and $[\alpha]_D$ −98.3 (*c* 0.57, D_2O); ^{1}H-NMR (300 MHz, D_2O) δ 5.19 (1 H, d, $J_{3,2}$ 1.6, 3-H), 5.58 (1 H, d, $J_{2,3}$ 1.6, 2-H), 7.23–7.26 (1 H, m, 4-H), 7.34–7.36 (2 H, m, 5-H, 6-H), 7.46 (1 H, d, $J_{7,6}$ 7.2, 7-H); ^{13}C-NMR (125 MHz, CD_3OD) δ 84.7, 90.0, 123.7, 125.6, 127.3, 130.7, 140.2, 141.3; ν_{max} (KBr): 3256 cm^{-1} (OH).

(ii) Metabolite from 2-methyl benzo[*b*]thiophene 1b

*2-Methyl-2,3-dihydroxy-2,3-dihydrobenzo[*b*]thiophene* ***5b**$_{cis}$*. Yield (0.20 g, 3%); mp 87–89 °C (from CH_2Cl_2–hexane); R_f 0.4 (eluent A); HRMS (Found: M$^+$ 182.0394. $C_9H_{10}O_2S$ requires 182.0402); $[\alpha]_D$ −2.9 (*c* 0.51, $CHCl_3$) and $[\alpha]_D$ +5.0 (*c* 0.51, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.84 (3 H, s, Me), 2.48 (1 H, d, $J_{OH,3}$ 9.9, OH), 3.27 (1 H, s, OH), 4.78 (1 H, d, $J_{3,OH}$ 9.9, 3-H), 7.12–7.16 (1 H, m, Ar-H), 7.22–7.28 (2 H, m, Ar-H), 7.38 (1 H, d, *J* 7.7, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 25.7, 82.4, 94.5, 123.4, 125.9 (×2), 129.7, 137.9, 138.8; LRMS (EI): *m*/*z* 182 (M$^+$, 30%), 139 (100); ν_{max} (KBr): 3300 cm^{-1} (OH); 9% ee (Methods A and B).

(iii) Metabolites from 5-methyl benzo[*b*]thiophene 1d

*5-Methyl 2,3-dihydroxy-2,3-dihydrobenzo[*b*]thiophene* ***5d**$_{cis}$*/***5d**$_{trans}$*. The isomeric mixture of diols **5d**$_{cis}$/**5d**$_{trans}$ was obtained by flash chromatography (Et_2O : hexane, 3 : 1); yield (2.9 g, 79%); R_f 0.5 (eluent B) (Found: C, 59.0; H, 5.6; S, 17.3. $C_9H_{10}O_2S$ requires C, 59.3; H, 5.5; S, 17.6%); LRMS (EI): *m*/*z* 182 (M$^+$, 51%), 166 (100); ν_{max} (KBr): 3408 cm^{-1} (OH); >98% ee (Method B).

*(+)-(2*S*,3*R*)-5-Methyl-2,3-dihydroxy-2,3-dihydrobenzo[*b*]thiophene* ***5d**$_{cis}$*. Mp 126–128 °C (from $CHCl_3$); $[\alpha]_D$ +135.6 (*c* 0.54, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.34 (3 H, s, Me), 2.69 (1 H, br s, OH), 2.87 (1 H, br s, OH), 5.11 (1 H, d, $J_{3,2}$ 4.3, 3-H), 5.60 (1 H, d, $J_{2,3}$ 4.7, 2-H), 7.06–7.27 (3 H, m, Ar-H).

*(−)-(2*R*,3*R*)-5-Methyl-2,3-dihydroxy-2,3-dihydrobenzo[*b*]thiophene* ***5d**$_{trans}$*. Mp 126–128 °C (from MeOH); $[\alpha]_D$ −80.0 (*c* 0.45, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ 2.29 (3 H, s, Me), 4.98 (1 H, bs, 3-H), 5.48 (1 H, bs, 2-H), 7.02–7.04 (2 H, m, 6-H, 7-H), 7.18 (1H, s, 4-H).

(iv) Metabolites from benzo[*b*]furan 1e

*2,3-Dihydroxy-2,3-dihydrobenzo[*b*]furan* ***5e**$_{cis}$*/***5e**$_{trans}$*. The isomeric mixture of diols **5e**$_{cis}$/**5e**$_{trans}$ was obtained as a gum by flash chromatography (EtOAc : hexane, 2 : 3); yield (2.2 g, 17%); $[\alpha]_D$ −14.0 (*c* 0.76, $CHCl_3$), $[\alpha]_D$ −34.0 (*c* 0.84, H_2O); HRMS (Found: M$^+$ 152.0470. $C_8H_8O_3$ requires 152.0473); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (*cis*/*trans* 60 : 40), 4.99 (1H, s, 3-H$_{trans}$), 5.00 (1 H, d, $J_{3,2}$ 5.1, 3-H$_{cis}$), 5.73 (1 H, s, 2-H$_{trans}$), 5.75 (1 H, d, $J_{2,3}$ 5.1, 2-H$_{cis}$), 6.80–6.98 (2 H, m, 5-H, 7-H), 7.25 (1 H, ddd, $J_{6,7} = J_{6,5}$ 8.1, $J_{6,4}$ 2.1, 6-H), 7.37 (1 H, dd, $J_{4,5}$ 7.8, $J_{4,6}$ 2.0, 4-H); ν_{max} (neat): 3472 cm^{-1} (OH); ^{1}H-NMR (500 MHz, C_6D_6) (*cis*/*trans*, 55 : 45) δ 4.35 (1 H, d, $J_{3,2}$ 5.1, 3-H$_{cis}$), 4.78 (1 H, s, 3-H$_{trans}$), 5.39 (1 H, d, $J_{2,3}$ 5.2, 2-H$_{cis}$), 5.49 (1 H, s, 2-H$_{trans}$), 6.66–7.10 (4H, m, ArH); ^{1}H-NMR (500 MHz, D_2O) (*cis*/*trans*, 10 : 90) δ 5.04 (1 H, s, 3-H$_{trans}$), 5.18 (1 H, d, $J_{3,2}$ 5.3, 3-H$_{cis}$), 5.77 (1 H, d, $J_{2,3}$ 0.8, 2-H$_{trans}$), 5.89 (1 H, d, $J_{2,3}$ 5.2, 2-H$_{cis}$), 6.78–7.22 (2 H, m, ArH), 7.25–7.52 (2 H, m, ArH); ^{13}C-NMR (125 MHz, $CDCl_3$) (*cis* isomer) δ 70.5, 100.5, 110.5, 121.5, 125.6, 126.2, 130.9, 157.1; (*trans* isomer) 77.7, 106.8, 110.7, 121.7, 126.0, 126.3, 131.1, 158.6; LRMS (EI): *m*/*z* 152 (M$^+$, 58%), 134 (44), 121 (100); 55% ee (Methods A and B).

*(+)-(2*S*,3*R*)-bis-[(*S*)-2'-Methoxy-2'-phenyl-2'-trifluoromethylacetoxy]-2,3-dihydrobenzo[*b*]furan* ***5e**$_{transMTPA(S)}$*. To a mixture of *cis*/*trans*-diols **5e**$_{cis}$/**5e**$_{trans}$ (0.016 g, 0.1 mmol) in anhydrous pyridine (0.2 ml) was added (−)-MTPA chloride (0.056 g, 0.22 mmol). The reaction mixture was shaken gently and then left overnight at room temperature. Excess of pyridine was removed from the mixture under high vacuum to yield an oily residue. ^{1}H-NMR analysis of this crude product showed that (a) it was mainly (95%) composed of a mixture of *trans* diMTPA esters **5e**$_{transMTPA(S)}$ and (b) the starting mixture of diols **5e**$_{cis}$/**5e**$_{trans}$ was estimated to be of 55% ee. From PLC (6% Et_2O in hexane) of the oily residue, a mixture of (2*S*,3*R*)- and (2*R*,3*S*)-diMTPA diastereoisomers of diol **5e**$_{trans}$ (the major component)

was separated (0.012 g, 30%, R_f 0.42); *trans*-isomer **5e**$_{transMTPA(S)}$, the major (85%) compound in this separated mixture, was then crystallized out as a white solid; mp 116–118 °C (from CH_2Cl_2–hexane); $[\alpha]_D$ +68 (0.73, $CHCl_3$) (Found: C, 57.4; H, 3.4. $C_{28}H_{22}O_7F_6$ requires C, 57.5; H, 3.8%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.47 (3 H, s, OMe), 3.51 (3 H, s, OMe), 6.20 (1 H, s, 3-H), 6.73 (1 H, d, $J_{2,3}$ 0.7, 2-H), 7.00 (1H, d, J 8.1, Ar-H), 7.05 (1 H, m, Ar-H), 7.37–7.50 (12 H, m, Ar-H); LRMS (EI): m/z 584 (M^+, 14%), 351 (37), 189 (100); ν_{max} (KBr): 1733 cm^{-1} (C═O).

Crystal data for **5e**$_{transMTPA(S)}$. $C_{28}H_{22}F_6O_7$, M = 584.5, orthorhombic, a = 6.783(2), b = 16.637(6), c = 23.581(9) Å, U = 2661.1(16) Å^3, T = 293(2) K, space group $P2_12_12_1$ (no. 19), Cu-Kα radiation, λ = 1.54178 Å, Z = 4, $F(000)$ = 1200, D_x = 1.459 g cm^{-3}, μ = 1.150 mm^{-1}, Siemens P3 diffractometer, ω scans, 6.5° < 2θ < 110.2°, 1961 independent reflections, direct methods solution, full-matrix least squares refinement on F_o^2, anisotropic displacement parameters for non-hydrogen atoms; all hydrogen atoms located in a difference Fourier synthesis but included at positions calculated from the geometry of the molecule using the riding model, with isotropic vibration parameters. R_1 = 0.069 for 1364 data with $F_o > 4\sigma(F_o)$, 373 parameters, wR_2 = 0.206 (all data), GoF = 1.02, $\Delta\rho_{min,max}$ = −0.27/0.28 e Å^{-3}. CCDC 871980. The absolute configuration is established as (2*S*,3*R*) relative to the known absolute configuration of the (*S*)-MTPA group.

*(1*R*)-1,2-Dihydroxy-1-(2'-hydroxyphenyl)ethane* ***10e***. The most polar fractions obtained from purification by flash chromatography (EtOAc : hexane, 2 : 3) yielded phenolic diol **10e** (1.05 g, 8%); mp 68–69 °C (from CH_2Cl_2–hexane) (lit.,[4b] mp 69–71 °C); R_f 0.3 (eluent B); $[\alpha]_D$ −23.4 (*c* 0.89, MeOH) (lit.,[4b] −24, MeOH); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.83 (2 H, m, 2-H, 2-H), 4.95 (1 H, dd, $J_{1,2a}$ 5.0, $J_{1,2b}$ 7.5, 1-H), 6.82–6.89 (2 H, m, 3′-H, 5′-H), 7.01 (1 H, d, J 7.5, 4-H), 7.19 (1H, dd, $J_{6,5}$ 7.8, $J_{6,4}$ 1.4, 6′-H).

(v) Metabolite from 2-methyl benzo[*b*]furan 1f

*(+)-(2*R*,3*S*)-2,3-Dihydroxy-2,3-dihydro-2-methyl[*b*]benzofuran* ***5f***$_{cis}$. Diol **5f**$_{cis}$ was isolated as a gum, using flash chromatography (5–25% EtOAc in hexane) followed by PLC (10% EtOAc in hexane) (0.444 g, 8%); $[\alpha]_D$ +206 (*c* 1.1, $CHCl_3$); HRMS (Found: 166.0632. $C_9H_{10}O_3$ requires 166.0630); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.12 (3 H, s, Me), 5.23 (1 H, s, 3-H), 6.85 (1 H, d, J 8.2, 4-H), 6.95 (1 H, m, 5-H), 7.23 (2 H, m, 6-H, 7-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 23.5, 77.5, 116.7, 118.0, 120.2, 121.5, 129.5, 131.1, 155.0; LRMS (EI): m/z 166 (M^+, 4%), 148 (100), 91 (58); ν_{max} (KBr): 3540 cm^{-1} (OH); 80% ee (Method B).

*(+)-(1*S*,2*S*)-1,2-Dihydroxy-1-(2'-hydroxyphenyl)propane* ***10f***. Phenolic diol **10f**, the most polar bioproduct, was obtained as a yellow gum, after purification by PLC (EtOAc : hexane, 9 : 1) (0.02 g, 1%); $[\alpha]_D$ +13.3 (*c* 0.8, EtOH); HRMS (Found: M^+ 168.0789. $C_9H_{12}O_3$ requires 168.0787); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.11 (3 H, d, $J_{Me,2}$ 6.4, Me), 4.05 (1 H, dq, $J_{2,1}$ 7.9, $J_{2,Me}$ 6.5, 2-H), 4.90 (1 H, d, $J_{1,2}$ 7.9, 1-H), 6.85 (2 H, m, 3′-H, 5′-H), 7.01 (1 H, ddd, $J_{4',3'} = J_{4',4}$ 7.6, $J_{4',6'}$ 1.6, 4′-H), 7.20 (1 H, dd, $J_{6',5'}$ 7.3, $J_{6',4'}$ 1.6, 6′-H); ^{13}C-NMR (125 MHz, $CDCl_3$) 19.3, 70.3, 80.3, 117.4, 119.7, 124.3, 129.0, 129.5, 155.7; LRMS (EI): m/z 168 (M^+, 13%), 123 (100), 95 (31); ν_{max} (CH_2Cl_2): 3480, 3468 cm^{-1} (OH); >98% ee (Method A).

Enantiomeric excess values and absolute configurations of diol metabolites 3a–c, 4e, 4g and 10f. The enantiopurity of *cis*-dihydrodiol **4e**, obtained from *P. putida* UV4 biotransformation of benzo[*b*]furan **1e**, had been determined as >98% ee,[4b] following partial hydrogenation (10% Pd–C, EtOAc) to yield the corresponding *cis*-tetrahydrodiol **4e′**, formation of the diMTPA ester derivative and analysis of its ^{1}H-NMR spectrum. A similar approach was used for *cis*-dihydrodiols **3a–c** and **4g**. Formation of diMTPA esters of the corresponding *cis*-tetrahydrodiols **3a′–c′** and **4g′** and their ^{1}H-NMR analyses showed that each of the *cis*-dihydrodiol precursors was a single enantiomer (>98% ee).

*(−)-(4*R*,5*S*)-4,5-Dihydroxy-4,5,6,7-tetrahydrobenzo[*b*]thiophene* ***3a′*** *(from diol* ***3a****)*. Yield (0.067 g, 66%); mp 135–137 °C (from $CHCl_3$–hexane); $[\alpha]_D$ −56.0 (*c* 0.77, $CHCl_3$); HRMS (Found: M^+ 170.0408. $C_8H_{10}O_2S$ requires 170.0408); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.91–1.99 (1 H, m, 6-H), 2.01–2.13 (1 H, m, 6′-H), 2.72–2.83 (1 H, m, 7-H), 2.93–3.02 (1 H, m, 7′-H), 3.97–4.02 (1 H, m, 5-H), 4.69 (1 H, d, $J_{4,5}$ 3.6, 4-H), 7.01 (1 H, d, $J_{3,2}$ 5.1, 3-H), 7.13 (1 H, d, $J_{2,3}$ 5.1, 2-H); LRMS (EI): m/z 170 (M^+, 22%), 126 (100); ν_{max} (KBr): 3250 cm^{-1} (OH).

*(−)-(4*R*,5*S*)-bis-[(*R*)-2'-Methoxy-2'-phenyl-2'-trifluoromethylacetoxy]-4,5,6,7-tetrahydrobenzo[*b*]thiophene* ***3a′***$_{cisMTPA(R)}$. A solution of *cis*-tetrahydrodiol **3a′** (0.06 g, 0.35 mmol) in anhydrous pyridine (*ca*. 0.5 ml) was treated with (+)-MTPA chloride (0.194 g, 0.77 mmol) and the mixture left at room temperature overnight. Excess of pyridine was removed under high vacuum and the residue purified by PLC (10% Et_2O in hexane) to afford crystals of bis-(*R*)-MTPA ester **3a′**$_{cisMTPA(R)}$ (0.83 g, 39%); mp 111–112 °C (MeOH–$CHCl_3$); $[\alpha]_D$ −53.0 (*c*. 0.66, $CHCl_3$) (Found: C, 55.9; H, 3.9; S, 5.0. $C_{28}H_{24}O_6F_6S$ requires C, 55.8; H, 4.0; S, 5.3%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.24–2.29 (1 H, m, 6-H), 2.37–2.45 (1 H, m, 6′-H), 2.98–3.03 (2 H, m, 7-H, 7′-H), 3.28 (3 H, s, OMe), 3.50 (3 H, s, OMe), 5.50–5.53 (1H, m, 5-H), 6.20 (1 H, d, $J_{4,5}$ 2.6, 4-H), 6.95 (1 H, d, $J_{3,2}$ 5.3, 3-H), 7.12 (1 H, d, $J_{2,3}$ 5.3 2-H), 7.23–7.54 (10 H, m, Ar-H); LRMS (EI): m/z 602 (M^+, 3%), 189 (100); ν_{max}(KBr): 1756 cm^{-1} (C═O).

Crystal data for **3a′**$_{cisMTPA(R)}$. $C_{28}H_{24}F_6O_6S$, M = 602.5, orthorhombic, a = 10.026(4), b = 12.876(4), c = 21.444(7) Å, U = 2768.3(17) Å^3, T = 293(2) K, space group $P2_12_12_1$ (no. 19), Mo-Kα radiation, λ = 0.71073 Å, Z = 4, $F(000)$ = 1240, D_x = 1.446 g cm^{-3}, μ = 0.198 mm^{-1}, Siemens P3 diffractometer, ω scans, 3.7° < 2θ < 55.1°, 3593 independent reflections, direct methods solution, full-matrix least squares refinement on F_o^2, anisotropic displacement parameters for non-hydrogen atoms; all hydrogen atoms located in a difference Fourier synthesis but included at positions calculated from the geometry of the molecule using the riding model, with isotropic vibration parameters. R_1 = 0.061 for 1853 data with $F_o > 4\sigma(F_o)$, 373 parameters, wR_2 = 0.136 (all data), GoF = 1.02, $\Delta\rho_{min,max}$ = −0.24/0.25 e Å^{-3}. CCDC 871978. The absolute configuration is established as (4*R*,5*S*) from the anomalous scattering arising from the sulfur atom (Flack parameter x = 0.2(2)) and independently from the known absolute configuration of the (*R*)-MTPA group.

*(−)-2-Methyl-(4*R*,5*S*)-4,5-dihydroxy-4,5,6,7-tetrahydrobenzo[*b*]-thiophene* ***3b′*** *(from diol* ***3b****)*. Yield (0.06 g, 55%); mp

155–157 °C (from $CHCl_3$–hexane) (Found: C, 58.2; H, 6.5; S, 17.7. $C_9H_{12}O_2S$ requires C, 58.7; H, 6.5; S, 17.4%); $[\alpha]_D$ −57.6 (*c* 0.83, MeOH); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.58 (2 H, br s, OH), 1.92–2.10 (2 H, m, 6-H, 6′-H), 2.42 (3 H, s, Me), 2.72–2.77 (1 H, m, 7-H), 2.84–2.92 (1 H, m, 7′-H), 3.94–4.00 (1 H, m, 5-H), 4.62 (1 H, d, $J_{4,5}$ 3.6, 4-H), 6.66 (1 H, s, 3-H); LRMS (EI): *m*/*z* 184 (M^+, 22%), 166 (13), 140 (100).

*(−)-3-Methyl-(4*R,5S*)-4,5-dihydroxy-4,5,6,7-tetrahydrobenzo[*b*]-thiophene* ***3c′*** *(from diol* ***3c****).* Yield (0.33 g, 65%); mp 158–160 °C (from $CHCl_3$–hexane); $[\alpha]_D$ −101.0 (*c* 0.45, MeOH) (Found: C, 58.2; H, 6.8; S, 17.1. $C_9H_{12}O_2S$ requires C, 58.7; H, 6.5; S, 17.4%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.91–1.99 (2 H, m, 6-H, 6′-H), 2.26 (3 H, s, Me), 2.78–2.84 (1 H, m, 7-H), 2.91–2.97 (1 H, m, 7′-H), 3.90–3.97 (1 H, m, 5-H), 4.62 (1 H, d, $J_{4,5}$ 3.7, 4-H), 6.76 (1 H, s, 2-H); LRMS (EI): *m*/*z* 184 (M^+, 19%), 166 (14), 140 (100).

*(−)-3-Methyl-(6*S,7S*)-6,7-dihydroxy-4,5,6,7-tetrahydrobenzo[*b*]-furan* ***4g′*** *(from diol* ***4g****).* Yield (0.164 g, 81%); an oil; $[\alpha]_D$ −76.0 (*c* 0.74, MeOH) (Found: C, 64.1; H, 7.4. $C_9H_{12}O_3$ requires C, 64.3; H, 7.2%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.92 (2 H, m, 5-H), 2.13 (3 H, s, Me), 2.47 (1 H, m, 4-H), 2.73 (1 H, m, 4-H′), 4.06 (1 H, m, 6-H), 4.68 (1 H, d, $J_{7,6}$ 4.2, 7-H), 7.10 (1 H, s, 2-H); LRMS (EI): *m*/*z* 168 (M^+, 23%), 124 (100); ν_{max} (neat): 3546 cm^{-1} (OH).

*(+)-(1*S,2S*)-1,2-Dihydroxy-1-(2′-methoxyphenyl)propane* ***15****.* To a stirred mixture of *tert*-butyl alcohol (5 ml), H_2O (5 ml) and AD-mix-α (1.4 g) was added methanesulphonamide (0.095 g) and the mixture cooled to 0 °C. *trans*-1-(2′-Methoxyphenyl) prop-1-ene **14** (0.150 g, 1 mmol) was added and vigorous stirring, at 0 °C, continued for 16 h. The reaction mixture was treated with sodium sulphite (1.0 g), stirred for another 0.5 h at ambient temperature, and diluted with EtOAc (20 ml). The organic layer was separated and the remaining aqueous phase extracted with EtOAc. The extract was washed with water, dried (Na_2SO_4), and concentrated to yield the crude diol **15**. Purification by PLC (EtOAc : hexane, 2 : 3) gave diol **15** as a white solid (0.156 g, 86%); mp 69–70 °C (from $CHCl_3$–hexane); $[\alpha]_D$ +26.0 (*c* 0.9, EtOH) (Found: C, 66.3; H, 7.7. $C_{10}H_{14}O_3$ requires C, 65.9; H, 7.7%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.07 (3 H, d, $J_{Me,2}$ 6.3, Me), 3.86 (3 H, s, OMe), 3.99 (1 H, dq, $J_{2,1}$ 7.1, $J_{2,Me}$ 6.4, 2-H), 4.57 (1 H, d, $J_{1,2}$ 7.4, 1-H), 6.86–7.00 (2 H, m, 3-H, 5-H), 7.25–7.31 (2 H, m, 4-H, 6-H); LRMS (EI): *m*/*z* 182 (M^+, 34%), 137 (100); ν_{max} (CH_2Cl_2): 3502 cm^{-1} (OH).

*(+)-(1*S,2S*)-1,2-Dihydroxy-1-(2′-methoxyphenyl)propane* ***15*** *(from diol* ***10f****).* A sample of the phenolic diol metabolite **10f** (0.01 g) was methylated by treating it at 0 °C with an excess of freshly prepared solution of ethereal diazomethane. Purification by PLC (5% MeOH in $CHCl_3$) gave the methoxy derivative **15** (0.009 g, 83%), $[\alpha]_D$ +22.0 (*c* 0.4, EtOH). This sample was spectrally indistinguishable from the sample of diol **15** synthesized from alkene **14**.

Synthesis of benzo[*b*]thiophene-2,3-oxide 21a. The synthesis of compounds **22**, **23** and **21a** was carried out using an ortho-ester procedure, similar to that reported earlier for other types of arene oxides employing THF-d_8 solvent in the final step.[14a–c]

*2-Methoxy-2-methyl-3a,8b-dihydrobenzo[*b*]thiophene-1,3-dioxole* ***22****.* A mixture of diol $\mathbf{5a}_{cis}$/$\mathbf{5a}_{trans}$ (0.45 g, 2.68 mmol), dry benzene (30 ml), benzoic acid (0.030 g) and trimethyl *ortho*-acetate (1.36 ml, 10.7 mmol) was gently refluxed under nitrogen until the starting diol was consumed. The cooled reaction mixture was dried (Na_2CO_3), filtered and the filtrate concentrated under reduced pressure. The crude diastereoisomeric mixture of dioxoles **22**, obtained as a pale yellow oil (0.57 g, 95%), was used without purification in the next step due to its instability on silica gel. HRMS (EI) (Found: M^+ 224.0512. $C_{11}H_{12}O_3S$ requires 224.0507); LRMS (EI): *m*/*z* 224 (M^+, 30%), 151 (38), 150 (76), 122 (100), 121 (83).

Major diastereoisomer: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.62 (3, s, Me), 2.93 (3 H, s, OMe), 5.88 (1 H, d, $J_{8b,3a}$ 6.5, 8b-H), 6.18 (1 H, d, $J_{3a,8b}$ 6.5, 3a-H), 7.09–7.18 (2 H, m, 4-H, 6-H), 7.21–7.28 (1 H, m, 5-H), 7.36 (1 H, d, *J* 7.4, 7-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 19.9, 48.4, 84.4, 84.9, 120.2, 122.0, 122.9, 124.3, 128.1, 134.8, 137.3.

Minor diastereoisomer: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.46 (3H, s, Me), 3.33 (3H, s, OMe), 5.89 (1H, d, $J_{8b,3a}$ 6.0, 8b-H), 6.24 (1H, d, $J_{3a,8b}$ 6.0, 3a-H), 7.04–7.40 (4H, m, 4-H, 5-H, 6-H, 7-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 22.0, 47.5, 84.6, 85.3, 120.5, 122.0, 123.2, 124.6, 128.5, 134.2, 137.3.

*2-Chloro-2,3-dihydrobenzo[*b*]thiophen-3-yl acetate* ***23****.* To a cooled (0 °C) and stirred solution of dioxole isomers **22** (0.5 g, 2.23 mmol) in dried CH_2Cl_2 (20 ml) containing Et_3N (0.1 ml) was added, dropwise, a solution of chlorotrimethylsilane (0.56 ml, 4.46 mmol) in CH_2Cl_2 (5 ml) over a period of 15 min. After leaving the stirred reaction mixture for a further 10 min, the solvent was removed under reduced pressure to give crude chloroacetate **23** as an oil (yield *ca.* 70%). A small sample (0.1 g) of the crude product was rapidly purified by PLC (10% EtOAc in hexane) to furnish chloroacetate **23** as a light yellow oil, R_f 0.62; $[\alpha]_D$ −421.6 (*c* 0.45, $CHCl_3$); HRMS (Found: M^+ 228.0003. $C_{10}H_9O_2SCl$ requires 228.0011); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.07 (3 H, s, Me), 5.67 (1 H, s, 2-H), 6.32 (1 H, s, 3-H), 7.21 (1 H, ddd, $J_{5,4} = J_{5,6}$ 7.4, $J_{5,7}$ 1.2, 5-H), 7.32 (1 H, dm, $J_{7,6}$ 7.8, 7-H), 7.36–7.41(1 H, m, 6-H), 7.53 (1 H, dm, $J_{4,5}$ 7.4, 4-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 21.1, 70.1, 85.1, 123.2, 126.0, 128.0, 131.2, 134.0, 140.4, 170.2; LRMS (EI): *m*/*z* 228 (M^+, 5%), 168 (47), 150 (100). ν_{max} (CH_2Cl_2): 1742 cm^{-1} (C═O), 1225 (C–O), 753 (aromatic).

*Benzo[*b*]thiophene-2,3-oxide* ***21a****.* Chloroacetate **23** (0.050 g, 0.22 mmol) was dissolved in THF-d_8 (1 ml) under nitrogen and sodium methoxide (0.024 g, 0.44 mmol) was added to the solution. The mixture was stirred for 2 h at 0 °C, filtered and the resulting yellow filtrate submitted immediately for NMR and mass spectrometry analyses. HRMS (EI) (Found: M^+ 150.0134. C_8H_6OS requires 150.0139); ^{1}H-NMR (400 MHz, THF-d_8) δ 4.80 (1 H, d, *J* 3.0, 3-H), 5.47 (1 H, d, J_2 3.0, 2-H), 7.11 (1 H, ddd, *J* 7.5, 6.3, 2.0, 6-H), 7.23–7.27 (2 H, m, 4-H, 5-H), 7.59 (1 H, ddd, *J* 7.5, 1.2, 0.7, 7-H); ^{13}C-NMR (100 MHz, THF-d_8) δ 62.8, 67.1, 124.4, 125.2, 128.0, 130.3, 136.1, 143.8; LRMS (EI): *m*/*z* 150 (M^+, 74%), 121 (89), 43 (100).

*2,3-Dihydrobenzo[*b*]thiophen-3-one* ***12a****.* Attempts to purify arene oxide **21a** led to its decomposition to yield ketone **12a**, which on purification by PLC (10% EtOAc in hexane) gave a light pink coloured solid. Crystallization from hexane furnished white needles; mp 64–66 °C (hexane) (lit.[7a] mp 65–66 °C); R_f 0.55 (10% EtOAc in hexane); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.80 (2 H, s, CH_2), 7.22 (1 H, ddd, $J_{5,4}$ 8.0, $J_{5,6}$ 7.2, $J_{5,7}$ 1.0, 5-H), 7.44 (1 H, dd, $J_{7,6}$ 8.0, $J_{7,5}$ 1.0, 7-H), 7.56 (1 H, ddd, $J_{6,7}$

8.0, $J_{6,5}$ 7.2, $J_{6,4}$ 1.3, 6-H), 7.79 (1 H, dd, $J_{4,5}$ 8.0, $J_{4,6}$ 1.3, 4-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 39.4, 124.7, 124.9, 126.8, 131.1, 135.8, 154.3, 200.2; LRMS (EI): *m/z* 150 (M^+, 100%); ν_{max} (KBr): 1691 cm^{-1} (C=O). The ketone **12a** was found to equilibrate spontaneously with the phenol tautomer **11a** in CD_3OD solution at ambient temperature over a period of 24 h (**11a** : **12a**, 2 : 1).

*3-Hydroxybenzo[*b*]thiophene* ***11a***. ^{1}H-NMR (400 MHz, CD_3OD) δ 6.34 (1H, s, 2-H), 7.29–7.33 (2H, m, 4-H, 7-H), 7.70–7.76 (2H, m, 5-H, 6-H).

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