TOLUENE DIOXYGENASE-MEDIATED OXIDATION OF BROMO(METHYLSULFANYL)BENZENES. ABSOLUTE CONFIGURATION OF METABOLITES AND EVALUATION OF CHEMO- AND REGIOSELECTIVITY TRENDS

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A series of 2-, 3-, and 4-bromo-1-(methylsulfanyl)benzenes was subjected to whole-cell fermentation with *Escherichia coli* JM 109 (pDTG601), a recombinant strain that expresses toluene dioxygenase. New metabolites were isolated and their structure and absolute configuration determined by chemical and spectral methods. Experimental details are provided for all new compounds and directing trends in the enzymatic oxidation of substituted aromatics are discussed along with projected applications of these metabolites in asymmetric synthesis.
Keywords: Biotransformations; Toluene dioxygenase; Enzymatic sulfur oxidations; Whole-cell fermentations; Dienediols; Dioxygenations; Chiral building blocks; Diols; Asymmetric synthesis.

Recombinant strains of *Escherichia coli* that overexpress several types of aromatic dioxygenases have been used to generate a wide variety of *cis*-dienediols from aromatic substrates. Since Gibson's 1968 discovery[1] of this remarkable transformation, over 300 homochiral metabolites have been identified[2]; many of these have been employed in asymmetric syntheses of complex molecules[2b,3]. The most commonly used of these strains, *E. coli* JM 109 (pDTG601)[4], is used in whole-cell fermentations to generate useful amounts of the chiral synthons. With good substrates such as monosubstituted arenes, yields of 10 g l^{-1} or higher are common[5]. The enzyme toluene dioxygenase (TDO) exhibits remarkable tolerance for functionality and substitution pattern on the aromatic ring while maintaining specificity in regio- and stereochemistry of the oxidation. Furthermore, with sulfur-containing compounds[6,7b], the oxidation produces homochiral dienediols in many cases without oxidation of divalent sulfur. Like the dearomatization of the aromatic ring, this process has no equivalent in traditional synthetic chemistry.

With our ongoing commitment to increasing the pool of available chiral metabolites with varied functional content, we were interested in the fermentation of sulfur-containing compounds such as the bromo(methylsulfanyl)benzenes. We reported the oxidation of *p*-bromothioanisole **1** to diol **2** [8], whose corresponding sulfone **3** offers unique opportunities for application in asymmetric synthesis, Fig. 1. Sulfones such as **3**, prepared by chemical oxidation of thioether **2** and equipped with olefin-containing tethers, offer options for selective and tandem reactions such as radical or Heck-type cyclizations, nucleophilic additions, cycloadditions of the vinyl (dienyl) sulfone, and intramolecular cycloadditions. Both the sulfur functionality and the *cis*-diol unit may be removed following the transfer of chirality to other stereogenic centers. Diol **2** thus serves as an equivalent for a "prochiral" butadiene. In this paper we report the outcome of enzymatic oxidation of the series of bromothioanisoles and provide absolute configuration of all metabolites.

RESULTS AND DISCUSSION

Several dihydroarene *cis*-diols containing sulfur have been reported: those derived from thiophene[7a], several benzothiophenes[7a,7b,7d], benzothiopyran[7c], aryldithianes[7e], halogenated thioanisoles[6,8], and other alkyl aryl sulfides[7]. Most of these compounds yielded the corresponding diols either exclusively or along with the corresponding chiral sulfoxides. Interestingly, thioanisole itself is oxidized exclusively to its (*R*)-sulfoxide by the enzyme toluene dioxygenase with high enantioselectivity[9]; the *S*-isomer is available by fermentation with the enzyme naphthalene dioxygenase (NDO)[7e,10].

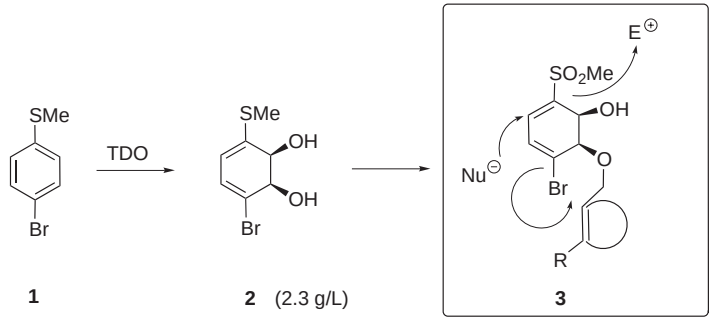


FIG. 1
Oxidation of 4-bromo-1-(methylsulfanyl)benzene and indication of further reactive options

Boyd proposed the working model for predicting and explaining the absolute stereochemistry and regiochemistry of aromatic ring dioxygenation[2c] as well as the chemoselectivity of sulfur vs ring oxidation[6], Fig. 2. It is thought that the larger of the two substituents on the arene that remains distal to the iron–oxygen complex, and in almost all cases the observed regiochemistry of dioxygenation is 2,3 with respect to the larger group. The (2*R*,3*S*)-configuration shown in Fig. 2 is also almost exclusive; the only exceptions are benzoic acids, which display 1,2-regiochemistry with the opposite configuration of the diol. The prediction is more difficult with sulfur-containing compounds; Boyd invoked positioning of alkyl aryl sulfides in the active site to account for mono- vs dioxygenation[6]. Despite the lower predictability of the fate of sulfides during enzymatic oxidation, the fact that some of them undergo oxidative dearomatization without concomitant formation of sulfoxides is quite astonishing.

We began our study with 4-bromo-1-(methylsulfanyl)benzene, which proved to be a good substrate that led to the formation of diol **2** in a yield of 2.3 g l^{-1} (15-l fermentor). The compound reduced electrochemically[11] to the unstable dienediol **4**, previously prepared by Boyd[12] in low yield from diol **8** by displacement of the halide with sodium methylthiolate, Scheme 1.

Compound **4** cannot be prepared directly from thioanisole, and the reduction of **2** is thus a more efficient method of access than the low-yielding chemical conversion of **8**. It can be purified by chromatography and can be stored for up to a week in dilute solution at –20 °C; the crystalline compound decomposes to its phenolic derivative within 20 min at room temperature.

The proof of absolute configuration was performed as shown in Scheme 1, and the optical purity was established by ^{19}F NMR analysis of the Mosher ester **7**, as reported in our preliminary communication[8]. Thus the oxidation of **1** proceeded in agreement with Boyd's predictive model, pre-

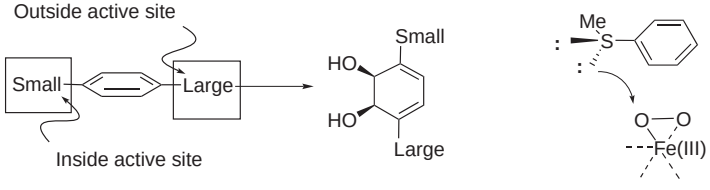


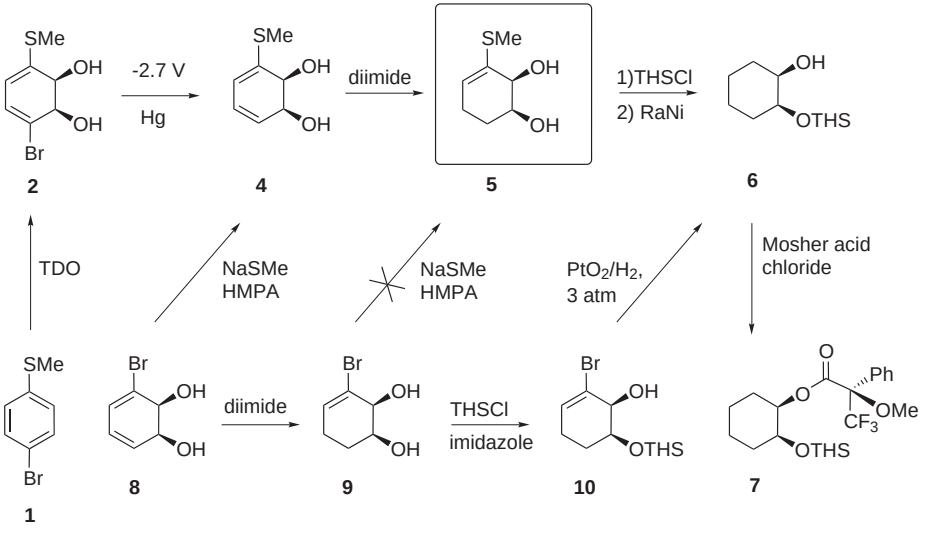
FIG. 2
Predictive models for arene vs side chain oxidation and directing effects

sumably because the methylsulfanyl group (A_{value} 1.04) is slightly larger in spherical volume than the bromine atom (A_{value} 0.67)[13].

We observed an interesting rearrangement during an attempted conversion of **9** to **5** via the addition–elimination method reported by Boyd. Whereas diol **4** can be obtained from **8**, albeit in low yield, treatment of **9** with sodium methanethiolate did not produce **5**, but rather its regioisomer **11**, presumably through the intermediacy of either **12** or the epoxy alcohol **14**, as shown in Fig. 3.

The oxidation of 2-bromo-1-(methylsulfanyl)benzene (**15**) provided diol **17** and (*R*)-sulfoxide **16** in a ratio of 2.5:1 and a yield of 0.117 and 0.047 g l^{-1}, respectively (Scheme 2). In the case of 3-bromo-1-(methylsulfanyl)benzene, the fermentation yielded almost exclusive oxidation of sulfur; the (*R*)-sulfoxide **20** (0.130 g l^{-1}) was formed in preference to diol **21** (0.007 g l^{-1}) in a 18:1 ratio. The chiral sulfoxides prepared by whole-cell fermentation of aromatic thioethers of both the ortho[6] and meta isomers[14] are known. They were found to be 93 and 34% optically pure, respectively, based on comparison of optical rotation values with those reported in the literature[15].

The proof of absolute configuration for the corresponding diols was performed as shown in Scheme 2. The electrochemical reduction protocol employed for conversion of compound **2** to its dehalogenated derivative **4** was



SCHEME 1

initially attempted for the diol derived from 2-bromo-1-(methylsulfanyl)-benzene without success and the starting material was recovered unchanged. Dehalogenation of the ortho isomer **17** was finally accomplished by first reducing the unsubstituted olefin with potassium azodicarboxylate/acetic acid to compound **18**, protecting the diol functionality as its acetonide, and converting compound it via BuLi metallation and quench to **22**, which was obtained by treatment of **5** with 2,2-dimethoxypropane (DMP). The optical purity of 2-bromo-1-(methylsulfanyl)benzene diol **17** was found to be in excess of 95% based on comparison of optical rotation value for compound **22** prepared from 4-bromo-1-(methylsulfanyl)benzene relative to the value obtained from **22** prepared from 2-bromo-1-(methylsulfanyl)benzene diol **17**, ($[\alpha]_D^{22}$ +20.3 (*c* 0.3, $CHCl_3$)). Given the small amounts of material used in the measurement of optical rotations, these values are in good agreement and provide the proof of absolute configuration for diol **17**.

In the case of the meta isomer, a set of mild conditions for the removal of the bromine functionality was found in the use of the Pd-catalyzed formate dehalogenation procedure reported by Heck[16]. The low yield of the meta-substituted dienediol from fermentation coupled with the relatively low conversion of the debromination reaction necessitated the use of ex-

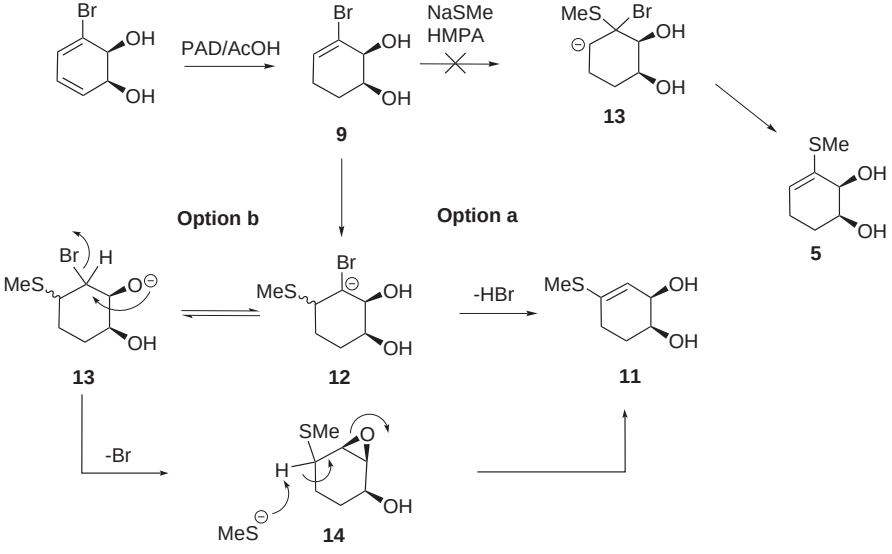
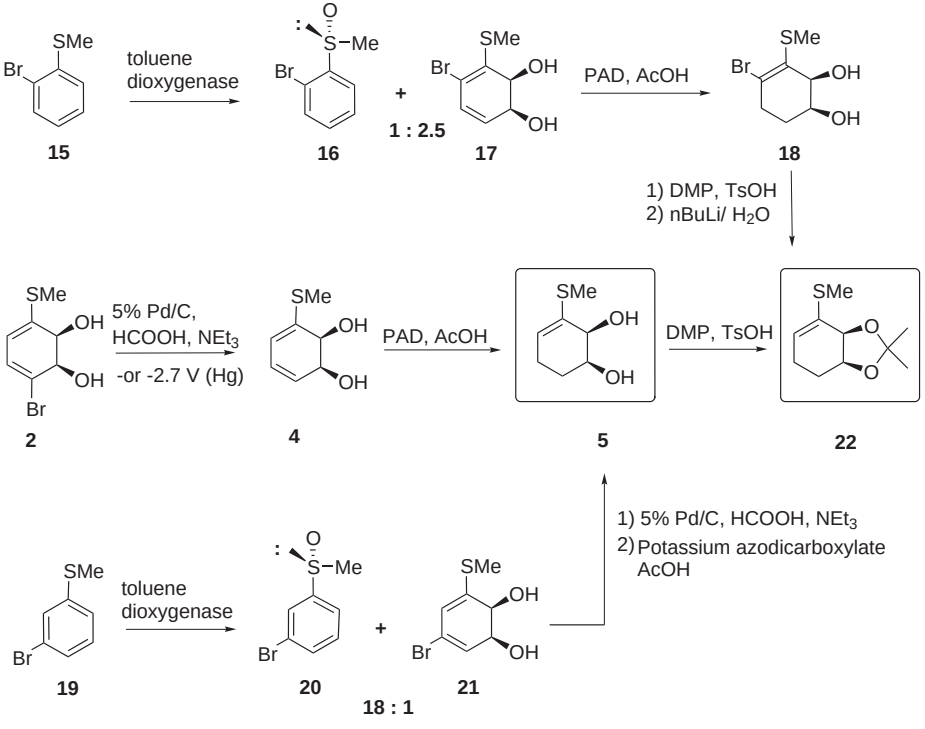


FIG. 3
Divergent modes of addition–elimination of compound **9**

tremely small amounts of material for optical rotation comparison. The sign of optical rotation of diol **5** prepared from 4-bromo-1-(methylsulfanyl)benzene matched that for diol **5** prepared from 3-bromo-1-(methylsulfanyl)-benzene, thus confirming the absolute configuration of diol **21** shown in Scheme 2. Optical purity was shown to be identical in all cases to that of the standard derived from bromocyclohexadienediol **8**.

In all three cases, the regio- and stereochemistry of the enzymatic oxidation was found to be in agreement with the model proposed by Boyd. The yields and chemoselectivities observed for this series of substrates are also in agreement with expectations for processing of substituted arenes by TDO. The highest yields are usually obtained for mono- or para-substituted compounds and the lowest with meta substitution.

The exception to this rule is the oxidation of *m*-dibromobenzene which yielded[5] 3 g l^{-1}. Biotransformations of both 2-bromo-1-(methylsulfanyl)-benzene and its meta isomer were carried out in 1-l shake flask and 8-l

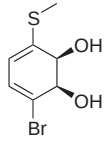
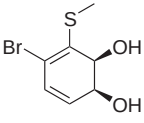
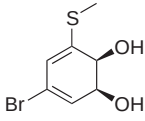


SCHEME 2

fermentor for comparison of both yield and product distribution with respect to diol and sulfoxide formation (Table I).

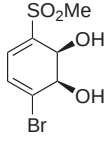
In the case of the ortho isomer, the diol was consistently produced with a 2.5:1 preference for the sulfoxide in shake flask fermentations and a 3.5:1 ratio favoring the sulfoxide in large-scale fermentation. Interestingly, we were unable to detect the minor product in large-scale transformations of 3-bromo-1-(methylsulfanyl)benzene; the only oxidation product was that resulting from sulfoxidation; however, the meta-substituted dienediol was isolated in small amounts from small-scale fermentations, albeit in low yield. The stability of the meta-, para-, and ortho-substituted dienediols is similar to that of the diol derived from bromobenzene; these compounds can be stored at –20 °C in dilute solution for up to one month without significant decomposition.

TABLE I
Comparison of yield and distribution of products from shake-flask and fermentor transformations

	Fermentor		Shake flask	
	sulfoxide mg/l	diol mg/l	sulfoxide mg/l	diol mg/l
 **2**	0	2300	–[a]	–[a]
 **17**	50	175	47	117
 **21**	150	0	130	7

[a] Not performed.

The explanation of trends in oxidation may lie in the precise fit of the substrate in the active site; in the case of sulfur-substituted substrates such positioning may also explain the preference for the observed monooxygenation. Nevertheless, the metabolites obtained from halogenated (methylsulfanyl)benzenes carry two differentiated functionalites, which features may be exploited in asymmetric synthesis, especially in the case of the para isomer which yields preparatively useful amounts of the homochiral diol. This diol was converted chemically to sulfone **23**, which is stable and amenable to the type of transformation shown in Fig. 1. We will report further results in applications of this metabolite to asymmetric synthesis in due course.



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EXPERIMENTAL

All non-hydrolytic reactions were carried out under argon atmosphere. Glassware used for moisture-sensitive reactions was flame-dried under vacuum and subsequently purged with argon. THF was distilled from potassium/benzophenone. Methylene chloride and acetonitrile were distilled from calcium hydride. Electrochemistry supplies and apparatus were purchased from EG&G Princeton Applied Research. Flash column chromatography was performed using Kieselgel 60 (230–400 mesh). Analytical thin-layer chromatography was performed using silica gel 60-F_{254} plates. Melting points were measured on a Thomas–Hoover melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin–Elmer FT-IR 1600 Series Spectrum One instrument and were recorded as neat samples. ^{1}H and ^{13}C NMR spectra (δ, ppm; *J*, Hz) were obtained on a 300-MHz Bruker instrument. Specific rotation measurements are given in 10^{-1} deg cm^2 g^{-1} and were recorded on a Perkin–Elmer 341 Polarimeter. Ultraviolet spectroscopy was performed using a Perkin–Elmer 8452 A diode array spectrophotometer. Large-scale fermentation was performed in a 15-l B. Braun Biostat C-15 fermentor. All biological media was purchased through Sigma–Aldrich Canada. Combustion analyses were performed by Atlantic Microlabs, Norcross, Georgia, USA, and ISAR Laboratories, Guelph, Ontario, Canada.

General Biotransformation Procedure

Small-Scale Fermentation with E. coli JM 109 pDTG601

Growth of colonies. Agar plates consisted of bactotryptone (10 g l^{-1}), yeast extract (5 g l^{-1}), NaCl (5 g l^{-1}), agar (30 g l^{-1}) and ampicillin (100 mg l^{-1}). *E. coli* JM 109 pDTG601 cells were streaked onto a plate and were incubated at 35 °C for 12–24 h. Single colonies were selected for the preculture preparations.

Preparation of preculture. Luria Bertani (LB) liquid medium consisted of bactotryptone (10 g l^{-1}), yeast extract (5 g l^{-1}), NaCl (5 g l^{-1}) and ampicillin (100 mg l^{-1}). LB medium (3 ml) was inoculated with a single colony of *E. coli* JM 109 (pDTG601) and was grown at 35 °C on an orbital shaker (200 rpm) for 6 h.

Fernbach flask preparation. LB liquid medium consisted of bactotryptone (10 g l^{-1}), yeast extract (5 g l^{-1}), NaCl (5 g l^{-1}), glucose (5 g l^{-1}) and ampicillin (100 mg l^{-1}). LB medium (500 ml) was inoculated with 1 ml of *E. coli* JM 109 (pDTG601) preculture medium and it was grown at 35 °C on an orbital shaker (180 rpm) for 5 h. A chemical inducer, isopropyl-1-thio-β-D-galactopyranoside (IPTG) (10 mg l^{-1}), was added via sterile filter and the cells were grown for additional 7 h at 35 °C on an orbital shaker (200 rpm).

Substrate addition. The supernatant was separated from the cells by centrifugation at 7000 rpm for 15 min. The supernatant was decanted and the cell pellet was resuspended in 500 ml of 0.1 M phosphate buffer consisting of KH_2PO_4 (6.8 g l^{-1}), K_2HPO_4 (8.7 g l^{-1}) and glucose (2 g l^{-1}). The aromatic substrate (400 mg l^{-1}) was added as a solution in isopropyl alcohol. Product formation was monitored by thin-layer chromatography (silica gel, hexane–ethyl acetate, 1:1).

Product isolation. After 5 h of incubation with substrate the pH of the culture medium was adjusted with 6 M NaOH to 8.5, and a cell pellet was obtained by centrifugation at 7000 rpm and 4 °C for 20 min. The supernatant liquid was extracted with acid-free ethyl acetate, prepared by stirring with a saturated solution of Na_2CO_3 and separation of the organic from the aqueous layer[17]. The extract was dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude material was purified by crystallization or flash column chromatography (silica gel deactivated with 10% distilled water) immediately after concentration of the solvent in order to minimize decomposition of the unstable dienediols.

Large-scale fermentations were carried out in a 15-l (8-l working volume) B. Braun Fermentor according to a published procedure[5].

Extraction of Products

Dienediols obtained from large-scale (15-l fermentation) were extracted from the aqueous fermentation broth into base-washed ethyl acetate either by standard manual extraction, or alternatively, by continuous extraction. Large-scale manual extraction requires up to 20 l of ethyl acetate, whereas the use of a rotary-evaporator-driven continous extractor facilitates the extraction of up to 9 l of aqueous broth using as little as 3 l of ethyl acetate. The dienediols derived from small-scale fermentations (1-l) were extracted manually. Progress of either manual or continuous extraction was monitored by thin layer chromatographic analysis of the aqueous layer.

(1*S*,2*R*)-3-Bromo-6-(methylsulfanyl)cyclohexa-3,5-diene-1,2-diol (**2**)

The biooxidation of 4-bromo-1-(methylsulfanyl)benzene was performed according to the general procedure for large-scale fermentation[5]. 4-Bromo-1-(methylsulfanyl)benzene (35 g) was added as a slurry in a minimal amount of DMSO to a 15-l fermentor containing a growing culture of *E. coli* JM 109 (pDTG601) over a 2-h period. After stirring the media for an additional 2 h, the cell broth was separated from the cells by centrifugation. The broth was then extracted twice with equal volumes of ethyl acetate (20 l) until TLC analysis of the aqueous layer revealed that the extraction was complete. The combined organic layers were

washed twice with 10% (by volume) of saturated sodium carbonate solution to remove any residual phenol which may be present as a result of partial aromatization. The organic extracts of the fermentation broth were concentrated in vacuo and diol **2** precipitated by addition of pentane. Recrystallization from ethyl acetate–pentane provided the title compound as a white solid (18 g, 2.25 g l^{-1}): m.p. 59–63 °C; $[\alpha]_D^{19}$ –10.0 (*c* 1.08, $CHCl_3$); R_F 0.26 (hexanes–ethyl acetate, 1:1). IR (film): 3197, 2921, 1626, 1548, 1416, 1340, 1306, 1036. ^{1}H NMR (300 MHz, $CDCl_3$): 6.37 (d, *J* = 6.1, 1 H); 5.35 (d, *J* = 6.3, 1 H); 4.43 (dd, *J* = 9.0, 3.2, 1 H); 4.32 (dd, *J* = 7.5, 1.4, 1 H); 2.63 (d, *J* = 9, 1 H); 2.26 (s, 3 H); 2.24 (d, *J* = 7.6, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): 142.8, 127.8, 120.3, 112.7, 73.6, 72.5, 14.4. MS (EI), *m/z* (%): 238 (8), 236 (8), 220 (100), 218 (98), 205 (41), 203 (39), 177 (24), 175 (24), 157 (20), 142 (25), 109 (33), 96 (24), 45 (41). HRMS (EI) calculated for $C_7H_9BrO_2S$ (M^+): 235.9501, found: 235.9506.

(1*S*,2*S*)-4-Bromo-3-(methylsulfanyl)cyclohexa-3,5-diene-1,2-diol (**17**)

Diol **17** was prepared according to the general procedure for shake-flask fermentation described above. 2-Bromophenyl methyl sulfide (0.6 g, 2.97 mmol) was added to the *E. coli* JM 109 (pDTG601) culture, and product formation was monitored by TLC (R_F 0.3; hexanes–ethyl acetate, 1:1) and UV/VIS (λ_{max} 288 nm, 100X dilution, distilled H_2O as blank). The resulting yellow-white crystalline material was purified by column chromatography (CH_2Cl_2–MeOH, 96:4), and the title compound (0.117 g, 16%) was isolated as off-white solid: m.p. 70 °C; $[\alpha]_D^{22}$ +130 (*c* 1.0, MeOH), $[\alpha]_D^{22}$ +123 (*c* 1.0, MeOH)]; R_F 0.2 (CH_2Cl_2–MeOH, 96:4; positive $KMnO_4$ test on TLC). IR (film): 3258, 2924, 2871, 1259, 1101, 1095, 1005. ^{1}H NMR (300 MHz, $CDCl_3$): 5.9 (dd, *J* = 9.83, 2.56, 1 H); 5.5 (d, *J* = 9.7, 1 H); 4.4 (s, 1 H); 4.1 (d, *J* = 5.3, 1 H); 2.5 (s, 1 H); 2.3 (s, 3 H); 2.1 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): 135.9, 129.8, 129.0, 116.6, 70.1, 68.7, 15.0. MS (EI), *m/z* (%): 238 (46.1), 236 (46.4), 220 (68.4), 218 (67.4), 111 (100), 109 (69.1), 81 (87.3). HRMS (EI) calculated for $C_7H_9BrO_2S$ (M^+): 235.9506, found: 235.9501.

(1*S*,2*S*)-5-Bromo-3-(methylsulfanyl)cyclohexa-3,5-diene-1,2-diol (**21**)

3-Bromophenyl methyl sulfide (0.4 g, 1.97 mmol) was added to a growing culture of *E. coli* JM 109 (pDTG601) according to the general procedure for shake-flask transformation described above. Product formation was monitored by TLC (R_F 0.5; hexane–ethyl acetate, 1:1). A yellow oily material was purified by column chromatography on 10% deactivated silica gel (hexane–ethyl acetate, 1:1), and the title compound was isolated as off-white solid (7 mg, 1.6%). M.p. 87–89 °C; $[\alpha]_D^{23}$ +26.6 (*c* 0.88, acetone); R_F 0.5 (hexane–ethyl acetate, 1:1). IR (film): 3350, 3019, 2917, 1603, 1551, 1435, 1384, 1216, 1112, 1043. ^{1}H NMR (400 MHz, $CDCl_3$): 6.0 (d, *J* = 2.7, 1 H); 5.4 (s, 1 H); 4.1 (m, *J* = 9.8, 6.2, 4.4, 2 H); 2.2 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): 146.7, 124.8, 118.9, 116.3, 70.7, 69.5, 13.0. MS (EI), *m/z* (%): 238 (10.2), 236 (10.3), 220 (100), 218 (97.2), 205 (42.2), 203 (40.8), 177 (18.8), 175 (18.4), 111 (40.5), 95 (25.1). HRMS (EI) calculated for $C_7H_9BrO_2S$ (M^+): 235.9507, found: 235.9509.

(1*S*,2*S*)-3-(Methylsulfanyl)cyclohexa-4,6-diene-1,2-diol (**4**)

*a) Preparation from diol **2** by electrochemical reduction*. Electrolysis was performed in a 150-ml beaker with a mercury pool cathode and Ag/Ag^+ (silver wire in a solution of 0.1 M $AgNO_3$ in acetonitrile) as reference electrode. A Pt anode was placed in a chamber separated

from the rest of the cell by a sintered-glass frit. Dienediol **2** (237 mg, 1 mmol) was dissolved in a minimum amount of acetonitrile and transferred to the beaker containing 100 ml of 0.15 M Et_4NBr solution in acetonitrile (previously degassed under positive pressure of argon for 20 min) followed by tetrabutylammonium hydroxide (1 ml of 40% aqueous solution). Electrolysis was performed at –2.75 V. A charge of 2 F mol^{-1} was delivered over approximately a 1-h period; progress of the reaction was monitored by TLC. Following electroreduction, the crude reaction mixture was decanted, and 10 ml of saturated sodium carbonate solution was added to neutralize any phenolic residue formed as a byproduct of the reaction. The organic solvent was evaporated under reduced pressure, and the residue diluted with 20 ml of distilled water. The aqueous layer was extracted with 5 × 50 ml of diethyl ether. The combined ethereal layers were washed with 2 × 3 ml of saturated sodium carbonate, 10 ml of brine and dried over anhydrous $MgSO_4$. After filtration to remove the drying agent, the solvent was evaporated under reduced pressure, and the crude product was immediately purified by flash chromatography on deactivated silica gel (hexanes–ethyl acetate, 6:4). Purification afforded 31.1 mg (20%) of the title compound as a white crystalline solid. The crystalline product decomposes completely to its phenolic derivative at room temperature in less than 30 min; however, in dilute dichloromethane solution the decomposition is significantly slowed and the dienediol survives for several days. M.p. 61–62 °C; $[\alpha]_D^{24}$ +81.3 (*c* 0.27, MeOH) (lit.[18] $[\alpha]_D$ +37 (*c* 0.7, MeOH)); R_F 0.4 (hexanes–ethyl acetate, 30:70). IR (film): 3247, 2915, 2858, 1551, 1545, 1431, 1420, 1321, 1292, 1106. ^{1}H NMR (300 MHz, CD_3OD): 5.99 (dq, *J* = 5.5, 2, 1 H); 5.72 (dd, *J* = 9.5, 3.6, 1 H); 5.58 (d, *J* = 5.6, 1 H); 4.25 (m, 1 H); 4.02 (d, *J* = 5.5, 1 H); 2.31 (s, 3 H). ^{13}C NMR (100 MHz, CD_3OD): 141.7, 125.4, 124.5, 114.0, 71.3, 69.1, 13.0. Note: Compound **4** has also been prepared in low yield from homochiral diol **9** by a displacement reaction mediated by NaSMe/HMPA [12,18]. Spectral data for this compound are consistent with the data reported here with the exception of the higher optical rotation than that reported in the literature[18].

*b) Preparation from diol **2** by Heck reduction*[16]. To a thick-walled reaction vessel equipped with magnetic stirring bar and Teflon seal was added dienediol **2** (300 mg, 1.27 mmol, 1 equiv.), 5% palladium on activated carbon (60 mg), and freshly distilled triethylamine (500 µl, 3.8 mmol, 3 equiv.). The reaction vessel was flushed with argon before addition of formic acid (120 µl, 2.8 mmol, 2.2 equiv.). The vessel was sealed and the reaction mixture stirred at room temperature for 1 h and then heated at 55 °C for 2 h. The crude mixture was filtered through Celite and washed with 5 ml of methanol. The crude product, which is unstable, was used directly in the subsequent reaction without further purification.

c) Preparation from diol 21 by Heck reduction[16]. To a thick-walled reaction vessel equipped with magnetic stirring bar and Teflon seal was added dienediol **21** (25 mg, 0.11 mmol, 1 equiv.), 5% palladium on activated carbon (15 mg), and freshly distilled triethylamine (87 µl, 0.63 mmol, 5.7 equiv.). The reaction vessel was flushed with argon before addition of formic acid (8 µl, 0.46 mmol, 4 equiv.). The vessel was sealed and the reaction mixture was stirred at room temperature for 10 min and then heated at 55 °C for 1 h. The crude mixture was filtered through Celite and washed with 5 ml of methanol. The crude product, which is unstable, was used directly in the subsequent reaction without further purification.

(1*S*,2*S*)-3-(Methylsulfanyl)cyclohex-5-ene-1,2-diol (**5**)

*a) Preparation of **5** by diimide reduction of diene **4***. The crude product (1 mmol) from electroreduction was chromatographed on 10% deactivated silica (hexanes–ethyl acetate,

30:70). Fractions containing the pure dienediol (TLC analysis) were transferred to a round-bottomed flask containing 10 ml methanol, and the hexanes–ethyl acetate mixture was evaporated with continuous addition of methanol. When the solvent volume was reduced to approximately 30 ml, the flask was placed in an ice/salt bath. Potassium azodicarboxylate (580 mg, 3.0 mmol, 3 equiv.) was added in portions to the methanolic solution. Upon complete addition of the PAD reagent, the yellow slurry was stirred for 10 min. Acetic acid (400 μl, 7 mmol, 7 equiv.) in 15 ml methanol was added dropwise over a 2-h period to the slurry at 0 °C. The reaction mixture was allowed to warm to room temperature overnight. The pH of the mixture was brought to neutral by addition of 4 ml of saturated solution of sodium hydrogencarbonate. Methanol was removed under reduced pressure and the residue diluted with 5 ml of distilled water followed by 20 ml of ethyl acetate. The layers were separated, and the aqueous layer extracted three times with 20-ml portions of ethyl acetate. The combined organic layers were dried (anhydrous $MgSO_4$) and the solvent removed under reduced pressure to afford the title compound as a white crystalline solid, 37 mg (16% yield over two steps). The crude product may be further purified by recrystallization from methylene chloride/pentane. M.p. 91–93 °C; $[\alpha]_D^{24}$ –104 (*c* 0.75, $CHCl_3$); R_F 0.4 (hexanes–ethyl acetate, 30:70). IR (film): 3249, 2944, 2913, 2890, 2829, 1623, 1434, 1356, 1327, 1100. ^{1}H NMR (300 MHz, $CDCl_3$): 5.57 (t, *J* = 4, 1 H); 4.05 (s, 1 H); 3.82 (d, *J* = 4.2, 1 H); 2.55 (d, *J* = 4.1, 1 H); 2.40 (s, 1 H); 2.19 (s, 3 H); 2.15–2.10 (m, 1 H); 1.85–1.60 (m, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): 134.6, 124.8, 69.4, 69.1, 25.7, 24.8, 15.1. MS (EI), *m/z* (%): 160 (50), 142 (22), 127 (31), 116 (100), 95 (47), 87 (58), 68 (40), 55 (30), 45 (54). HRMS (EI) calculated for $C_7H_{12}O_2S$ (M^+): 160.0554, found: 160.0558. For $C_7H_{12}O_2S$ (160.2) calculated: 52.47% C, 7.55% H; found: 52.74% C, 7.39% H.

b) An alternative procedure for the reduction of unstable dienediol ***4*** *from the Heck reduction product* ***4****.* A 50-ml round-bottomed flask fitted with an addition funnel was charged with the crude debrominated material dissolved in 5 ml of methanol. The reaction flask was placed in an ice bath and potassium azodicarboxylate (730 mg, 3.8 mmol, 3 equiv.) added in two portions. After 10 min, a solution of acetic acid (493 μl, 8.89 mmol, 7 equiv.) in 10 ml of methanol was added to the yellow slurry over a 45-min period. The reaction mixture, whose yellow color gradually faded to tan, was allowed to warm overnight. The reaction mixture was neutralized with a saturated solution of sodium hydrogencarbonate. After neutralization, the volume of the reaction mixture was reduced to approximately half under reduced pressure. The contents of the reaction flask were extracted with several portions of ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Removal of the organic solvent under reduced pressure provided a tan solid (57 mg, 28% over two steps), whose spectral data were consistent with that previously published[8].

c) Preparation from diol ***4*** *(prepared by Heck reduction of m-bromothioanisole diol* ***21****).* To a 25-ml round-bottomed flask equipped with an addition funnel and magnetic stirring bar was added the crude debrominated material dissolved in 5 ml of methanol. The reaction flask was placed in an ice bath and potassium azodicarboxylate (73 mg, 0.38 mmol, 3 equiv.) added in two portions. After 10 min, a solution of acetic acid (53 μl, 0.88 mmol, 7 equiv.) in 3 ml of methanol was added to the yellow slurry over 30 min. The reaction mixture was allowed to warm to room temperature overnight and neutralized with a saturated solution of sodium hydrogencarbonate. After neutralization, the volume of the reaction mixture was reduced to approximately half under reduced pressure. The content of the reaction flask was extracted with several portions of ethyl acetate. The combined organic

layers were washed with brine and dried over anhydrous magnesium sulfate. Removal of the organic solvent under reduced pressure gave the crude diol **5**, which was purified by flash column chromatography to furnish 1 mg of the title compound, ca. 6% yield over 2 steps): $[\alpha]_D^{21}$ –35 (*c* 0.1, $CHCl_3$) (lit.[8] $[\alpha]_D^{24}$ –104 (*c* 0.75, $CHCl_3$)).

(1*R*,2*S*)-2-[(Hexyldimethylsilyl)oxy]cyclohexan-1-ol (**6**)

a) Preparation from vinyl bromide ***10*** *[19,20] (derived from homochiral bromodienediol)[12].* A hydrogenation flask was charged with bromide **10** (1.0 g, 2.9 mmol, 1 equiv.), triethylamine (0.40 ml, 2.9 mmol, 1 equiv.), platinum oxide (Adams catalyst, 65 mg, 0.29 mmol, 0.10 equiv.) and 5 ml of methanol. The flask was placed in a Parr hydrogenation apparatus, slowly evacuated, and the headspace filled with hydrogen gas. The flask was shaken for 2 h under 3 atm hydrogen. When the reaction was deemed complete by TLC analysis, the catalyst was removed by filtration through a short plug of Celite and sand. The solvent was evaporated, and the residue diluted with 1 ml water, 10 ml ethyl acetate, and extracted. The organic layer was washed with water (2 × 2 ml), saturated $NaHCO_3$ (2 × 2 ml), brine (2 × 2 ml), and dried (anhydrous $MgSO_4$). Removal of the solvent provided the title compound as a clear and colorless oil (735 mg, 98% crude yield). $[\alpha]_D^{23}$ +10.6 (*c* 1.0, $CHCl_3$); R_F 0.55 (hexanes–ethyl acetate, 9:1). IR (neat): 3580, 3487, 2938, 2866, 1463, 1447, 1377, 1252, 1078. ^{1}H NMR (300 MHz, $CDCl_3$): 3.62 (dt, *J* = 7, 3, 1 H); 3.51 (m, 1 H); 2.05 (bs, 1 H); 1.7–1.3 (m, 8 H); 0.77 (dd, *J* = 6.8, 2, 6 H); 0.72 (s, 6 H); 0 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$): 74.4, 73.0, 36.6, 32.9, 32.5, 24.4, 22.7, 22.5, 21.0, 20.9, 0, –0.5. MS (EI), *m/z* (%): 173 (19), 155 (2), 129 (2), 105 (6), 81 (13), 75 (35), 55 (4), 41 (6). HRMS (EI) calculated for $C_8H_{17}O_2Si$ (M^+): 173.10195, found: 173.09933.

b) Preparation of ***10*** *from diol* ***5***. A 5-ml round-bottomed flask was charged with diol **5** (75 mg, 0.46 mmol, 1 equiv.), imidazole (41 mg, 0.61 mmol, 1.3 equiv.), and 400 µl of anhydrous dimethylformamide. The flask was cooled to –30 °C, then hexyldimethylsilyl chloride (87 µl, 0.49 mmol, 1.05 equiv.) was added. The mixture was stirred at –30 °C for 1 h and then the reaction flask was placed in a freezer (–18 °C) for 10 h. The mixture was diluted with 30 ml of ether, washed with 8 × 1 ml of aqueous solution of 5% citric acid, 2 × 1 ml brine, and the ethereal layer dried over anhydrous $MgSO_4$. The drying agent was filtered off, and the solvent was removed under reduced pressure to afford the crude silyl ether (120 mg, 90% crude yield) which was used directly in the subsequent reaction without further purification.

Commercially available Raney nickel 2400® was activated by swirling with 10% NaOH solution and decanting the supernatant. The procedure was repeated with distilled water until neutral pH was attained (about 12 washes). The nickel was finally washed with absolute ethanol three times. The THS-protected thioether (60 mg, 0.199 mmol) was introduced into a 25-ml round-bottomed flask equipped with a magnetic stirring bar and reflux condenser. The reaction flask was then charged with freshly activated Raney Ni (spatula tip) and 5 ml absolute ethanol. The reaction flask was heated to maintain steady reflux overnight. Reaction progress was monitored by TLC and the reaction deemed complete after 11 h at reflux temperature. The reaction mixture was filtered through a plug of Celite and washed with copious amounts of hot methanol. The washings were passed through a plug of silica gel to remove residual impurities. The product was obtained as yellow oil, 15 mg (29% yield). ^{1}H and ^{13}C NMR spectral data matched those of compound **6**, prepared by hydrogenation of protected diol **10**. The optical rotation value was, however, lower in the case of the product

obtained from Raney Ni treatment $[\alpha]_D^{25}$ +3.02 (*c* 1.0, $CHCl_3$) compared with $[\alpha]_D^{23}$ +10.6 (*c* 1.0, $CHCl_3$). We attributed this discrepancy to high-molecular-weight impurities observed in the mass spectrum but apparently transparent in ^1H and ^{13}C NMR spectra, as ^{19}F NMR analysis of the corresponding Mosher ester derivatives showed a single diastereomer in both cases.

(1*S*,2*S*)-4-(Methylsulfanyl)cyclohex-3-ene-1,2-diol (**11**)

To a flame-dried flask containing a magnetic stirring bar was added bromide **9** (1.0 g, 5.18 mmol, 1 equiv.), sodium methanethiolate (725 mg, 10.4 mmol, 2 equiv.), and 3 ml of HMPA under argon atmosphere. The reaction flask was heated to an external temperature of 65 °C for a period of 16 h. The crude reaction mixture was taken up in 50 ml of diethyl ether and washed with water (10 × 1 ml) and brine (2 × 1 ml). The ethereal layer was dried (anhydrous $MgSO_4$), the drying agent filtered off, and the solvent removed under reduced pressure to provide a brown oil which was purified by silica gel flash chromatography (hexanes–ethyl acetate, 30:70). The title compound was isolated as a crystalline white solid (24%). M.p. 83 °C; $[\alpha]_D^{24}$ –74 (*c* 0.75, $CHCl_3$); R_F 0.32 (hexanes–ethyl acetate, 30:70). IR (film): 3247, 2914, 1432, 1294, 1123, 1100, 996. ^1H NMR (400 MHz, $CDCl_3$): 5.25 (d, J = 4.1, 1 H); 4.12 (m, 1 H); 3.78 (m, 1 H); 2.75–2.4 (bs, 2 H); 2.30–2.05 (m, 2 H); 2.17 (s, 3 H); 1.85–1.65 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$): 141.1, 116.2, 68.9, 67.3, 28.3, 26.7, 14.4. MS (EI), *m/z* (%): 160 (39), 142 (64), 127 (31), 116 (72), 109 (30), 101 (100), 85 (24), 69 (49), 53 (22), 41 (41). HRMS (EI) calculated for $C_7H_{12}O_2S$ (M^+): 160.0565, found: 160.0556. For $C_7H_{12}O_2S$ (160.2) calculated: 52.47% C, 7.55% H; found: 53.02% C, 7.82% H.

(1*S*,2*S*)-4-Bromo-3-(methylsulfanyl)cyclohex-3-ene-1,2-diol (**18**)

Diol **17** (0.63 g, 3.0 mmol) was dissolved in 35 ml MeOH, and the round-bottomed flask containing the solution was subsequently placed into an ice/NaCl bath. Potassium azodicarboxylate (1.8 g, 9.0 mmol) was then added in portions to the methanolic solution. The resulting yellow slurry was stirred for 15 min, at which point acetic acid (1.2 ml, 21 mmol) in 25 ml MeOH was added dropwise via addition funnel over a period of 3 h. The reaction mixture was allowed to warm to room temperature overnight. The pH of the reaction mixture was brought to slightly alkaline by adding 20 ml of saturated $NaHCO_3$ solution. Methanol was removed under reduced pressure and the residue diluted with ethyl acetate. The layers were separated and the aqueous layer was extracted with several portions of ethyl acetate. The combined organic layers were washed with a saturated NaCl solution, dried over anhydrous MgSO4, filtered, and solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (CH_2Cl_2–MeOH, 96:4) yielding the title compound as white solid, 0.321 g (51%). M.p. 135 °C; $[\alpha]_D$ –104.3 (*c* 0.5, acetone); R_F 0.2 (CH_2Cl_2–MeOH, 96:4). IR (film): 3401, 2977, 2400, 1604, 1521, 1476, 1424, 1216, 669. ^1H NMR (300 MHz, $CDCl_3$): 4.3 (s, 1 H); 3.9 (s, 1 H); 2.4 (s, 3 H); 2.3 (s, 3 H); 1.9 (m, 2 H); 1.8 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$): 134.7, 121.4, 69.1, 68.7, 36.4, 26.9, 13.4. MS (EI), *m/z* (%): 240 (17.5), 238 (18.4), 196 (30.0), 194 (30.1), 149 (100). HRMS (EI) calculated for $C_7H_{11}BrO_2S$ (M^+): 237.9663, found: 237.9661. For $C_7H_{11}BrO_2S$ (239.1) calculated: 35.16% C, 4.64% H; found: 35.48% C, 4.30% H.

(3a*S*,7a*S*)-2,2-Dimethyl-4-(methylsulfanyl)-3a,6,7a-tetrahydro-1,3-benzodioxole (**22**)

a) Preparation from diol ***5***. Diol **5** (55 mg, 0.35 mmol, 1 equiv.) was transferred to a 10-ml round-bottomed flask and dissolved in 1 ml of acetone. 2,2-Dimethoxypropane (250 μl, 2.0 mmol, 5.7 equiv.) was added followed by 1 crystal of 4-methylbenzene-1-sulfonic acid. The solution was stirred at room temperature for 24 h before the reaction was quenched with 1 ml of a saturated sodium hydrogencarbonate solution. The cloudy mixture was diluted with 15 ml ethyl acetate and 2 ml of distilled water. The layers were separated and the aqueous layer back-extracted with fresh ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. The solvent was removed under vacuum to provide a tan oil (24 mg) which was purified by flash column chromatography (10% ethyl acetate in hexanes) to afford the title compound as a clear oil, 16 mg (23%). $[\alpha]_D^{22}$ +20.1 (*c* 0.8, $CHCl_3$); R_F 0.38 (10% ethyl acetate in hexanes). IR (film): 2984, 2924, 1624, 1438, 1379. ^{1}H NMR (300 MHz, $CDCl_3$): 5.56 (s, 1 H); 4.40 (s, 1 H); 4.29 (s, 1 H); 2.20 (s, 3 H); 2.20 (m, 1 H); 2.0–1.8 (m, 2 H); 1.75–1.60 (m, 1 H); 1.36 (s, 3 H); 1.30 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): 134.0, 123.8, 109.2, 74.5, 74.1, 28.1, 26.6, 25.9, 22.0, 14.9. HRMS (EI) calculated for $C_{10}H_{16}O_2S$: 200.0871, found: 200.0864. For $C_{10}H_{16}O_2S$ (200.3) calculated: 59.97% C, 8.05% H; found: 60.11% C, 7.78% H.

b) Preparation from compound ***18***. Diol **18** (0.020 g, 0.084 mmol, 1 equiv.) was dissolved in 2,2-dimethoxypropane (DMP) (1 ml, 8.4 mmol, 10 equiv.), to which one crystal of 4-methylbenzene sulfonic acid was then added. The reaction mixture was stirred at room temperature under a positive pressure of argon until the complete consumption of the starting material (ca. 1 h). Reaction progress was monitored by TLC (R_F 0.9; hexane–ethyl acetate, 1:1). DMP was removed under reduced pressure and 0.5 ml of saturated $NaHCO_3$ was then added. Organic material was extracted into ethyl acetate, washed with a saturated solution of NaCl and dried over anhydrous $MgSO_4$. The organic solvent was removed under reduced pressure and residual white-yellow solid dried under high vacuum. The protected diol was used directly in the subsequent reaction without further purification.

The crude material from the previous reaction was dissolved in 1 ml of anhydrous THF in a flame-dried 25-ml round-bottomed flask and cooled to –60 °C. 1.6 M butyllithium (195 μl in hexanes, 0.25 mmol, 3 equiv.) was added dropwise and the reaction mixture stirred at –60 °C under positive pressure of argon for 45 min. Reaction progress was monitored by TLC. The reaction mixture was quenched with 0.5 ml of distilled water and 1 ml saturated aqueous solution of ammonium chloride. The mixture was extracted with ether several times and the ethereal layer then washed with brine and dried over anhydrous $MgSO_4$. The drying agent was removed by filtration and the solvent removed under reduced pressure to provide a crude oil which was purified by flash column chromatography (hexanes–ethyl acetate, 9:1), affording the title compound **22** as a clear, colorless oil (3 mg, 18%). $[\alpha]_D$ +20.3 (*c* 0.3, $CHCl_3$). IR (film): 3368, 2984, 2924, 1625, 1576, 1439, 1368, 1219. ^{1}H NMR (400 MHz, $CDCl_3$): 5.5 (s, 1 H); 4.4 (s, 1 H); 4.2 (s, 1 H); 2.2 (s, 3 H); 2.2 (m, 1 H); 1.9 (m, 2 H); 1.7 (m, 2 H); 1.35 (s, 3 H); 1.31 (s, 3 H).

(+)-(*R*)-2-Bromophenyl Methyl Sulfoxide (**16**)

Sulfoxide **20** was prepared according to the general procedure for shake-flask fermentation described above. 2-Bromophenyl methyl sulfide (0.6 g, 2.97 mmol) was added to the 12-h-old culture of *E. coli* JM 109 (pDTG601) and product formation was monitored by TLC (R_F 0.4; hexane–ethyl acetate, 1:1). Yellow-white crystalline material was purified by column

chromatography (CH_2Cl_2–MeOH, 96:4) and 2-bromophenyl methyl sulfoxide (0.047 g, 7%) was isolated as brown oil. The other two isolated products were starting material (0.070 g, 0.346 mmol) and 4-bromo(methylsulfanyl)cyclohexa-3,5-diene-1,2-diol (0.210 g, 0.890 mmol). $[\alpha]_D^{23}$ +32.5 (*c* 1.8, $CHCl_3$) (lit.[6] $[\alpha]_D$ +35 (*c* 1.8, $CHCl_3$)); R_F 0.4 (CH_2Cl_2–MeOH, 96:4; positive $KMnO_4$ test on TLC). IR (film): 3400, 3059, 2996, 1447, 1093, 1058, 755. ^{1}H NMR (400 MHz, $CDCl_3$): 7.9 (dd, *J* = 7.6, 1.3, 1 H); 7.5 (t, *J* = 17.2, 8.6, 2 H); 7.2 (td, *J* = 7.8, 1.4, 1 H); 2.7 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): 144.3, 131.9, 131.3, 127.7, 124.7, 117.4, 40.9. MS (EI), *m/z* (%): 220 (81.6), 218 (84.4), 205 (100), 203 (98.7), 96 (33.8). HRMS (EI) calculated for C_7H_7OSBr (M^+): 217.9401, found: 217.9398.

(+)-(*R*)-3-Bromophenyl Methyl Sulfoxide (**20**)

3-Bromophenyl methyl sulfide (0.4 g, 1.97 mmol) was added to the fermentation of *E. coli* JM 109 (pDTG601) culture according to the general procedure described above. Product formation was monitored by TLC. Yellow oily material was purified by column chromatography (hexane–ethyl acetate, 1:1) and 3-bromophenyl methyl sulfoxide (0.130 g, 0.6 mmol, 28%) was isolated as yellow oil. $[\alpha]_D^{23}$ +39.8 (*c* 1.2, acetone) (lit.[14] $[\alpha]_D$ +116.3 (*c* 1.2, acetone)); R_F 0.2 (hexane–ethyl acetate, 1:1). IR (film): 3440, 3054, 2998, 2912, 2856, 2094, 1959, 1885, 1709, 1643, 1567, 1460, 1402, 1080, 784. ^{1}H NMR (400 MHz, $CDCl_3$): 7.8 (s, 1 H); 7.6 (td, *J* = 9.2, 1.1, 2 H); 7.4 (t, *J* = 15.7, 7.9, 1 H); 2.6 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): 150.3, 133.9, 131.5, 126.5, 123.2, 122.9, 43.7. MS (EI), *m/z* (%): 220 (83.4), 218 (83.3), 205 (100), 203 (97.6), 177 (10.4), 175 (12.0), 157 (24.8), 155 (25.4), 139 (21.0), 108 (22.2), 96 (23.0), 50 (44.4). HRMS (EI) calculated for C_7H_7BrOS (M^+): 217.9401, found: 217.9396.

(1*S*,2*R*)-3-Bromo-6-(methylsulfanyl)cyclohexa-4,6-diene-1,2-diol (**23**)

Dienediol **2** (500 mg, 2.1 mmol) was suspended in 10 ml of 50% aqueous acetone. To 0.6 ml of 0.3 M aqueous ammonium molybdate solution was added 4.6 ml of 30% aqueous hydrogen peroxide, and the resulting yellow solution was immediately added to the suspension of dienediol. Within minutes the reaction mixture became homogenous. The progress of the reaction was monitored by TlC and deemed complete after 10 min at which time the reaction was quenched by addition of 3 ml of saturated solution of sodium hydrogensulfite. The organic solvent was removed under reduced pressure and the aqueous portion extracted with ethyl acetate (5 × 20 ml). The extract was dried (anhydrous $MgSO_4$) and the solvent removed in vacuo to provide the title compound as a yellow crystalline solid (450 mg, 79% yield). M.p. 100–103 °C; $[\alpha]_D^{21}$ –30.1 (*c* 1.0, MeOH); R_F 0.37 (hexanes–ethyl acetate, 30:70). IR (film): 3409, 3008, 2946, 2929, 2816, 1634, 1562, 1371, 1294, 1201, 1138. ^{1}H NMR (300 MHz, $CDCl_3$): 6.90 (d, *J* = 6.3, 1 H); 6.60 (d, *J* = 4.6, 1 H); 5.05 (bs, 2 H); 4.70 (d, *J* = 6, 1 H); 4.45 (d, *J* = 4.2, 1 H); 3.08 (s, 3 H). ^{13}C NMR (100 MHz, CD_3OD): 139.8, 132.7, 124.3, 108.5, 72.7, 66.2, 44.2. MS (EI), *m/z* (%): 270 (13), 268 (15), 252 (30), 250 (29), 237 (16), 235 (16), 221 (13), 219 (13), 189 (42), 173 (27), 171 (29), 109 (77), 81 (100). HRMS (EI) calculated for $C_7H_9BrO_4S$ (M^+): 267.9404, found: 267.9397.

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REFERENCES AND NOTES

1. Gibson D. T., Koch J. R., Schuld C. L., Kallio R. E.: *Biochemistry* **1968**, *7*, 3795.
2. For a compilation of metabolites and their structures, see: a) Johnson R. A.: *Org. React.* **2004**, *63*, 117; b) Hudlický T., Gonzalez D., Gibson D. T.: *Aldrichim. Acta* **1999**, *32*, 35; c) Boyd D. R., Sheldrake G. N.: *Nat. Prod. Rep.* **1998**, *15*, 309.
3. a) Hudlický T., Reed J. W. in: *Advances in Asymmetric Synthesis* (A. Hassner, Ed.), p. 271. JAI Press, Greenwich, CT 1995; b) Carless H. A. J.: *Tetrahedron: Asymmetry* **1992**, *3*, 795; c) Widdowson D. A., Ribbons D. W., Thomas S. D.: *Janssen Chim. Acta* **1990**, *8*, 3; d) Sheldrake G. N. in: *Chirality and Industry* (A. N. Collins, G. N. Sheldrake and J. Crosby, Eds), p. 127. John Wiley and Sons, Ltd., Chichester, U.K. 1992; e) Brown S. M., Hudlický T. in: *Organic Synthesis: Theory and Applications* (T. Hudlicky, Ed.), Vol. 2, p. 113. JAI Press, Greewich, CT 1993; f) Grund A. D.: *SIMS News* **1995**, *45*, 59; g) Hudlický T.: *Chem. Rev.* **1996**, *96*, 3; h) Hudlický T., Entwistle D. A., Pitzer K. K., Thorpe A. J.: *Chem. Rev.* **1996**, *96*, 1195; i) Hudlický T. in: *Green Chemistry: Designing Chemistry for the Environment* (P. T. Anastas and T. C. Williamson, Eds), Vol. 626, p. 180. ACS Symp. Ser., 1996; j) Hudlický T. in: *Green Chemistry: Frontiers in Benign Chemical Syntheses and Processes* (P. T. Anastas and T. C. Williamson, Eds), Chap. 10, p. 166. Oxford University Press, Oxford, U.K. 1998.
4. Zylstra G., Gibson D. T.: *J. Biol. Chem.* **1989**, *264*, 14940.
5. For indication of yields of more commonly used diols as well as a full description of medium-scale fermentation, see: Endoma M. A., Bui V. P., Hansen J., Hudlický T.: *Org. Process Res. Dev.* **2002**, *6*, 525.
6. Boyd D. R., Sharma N. D., Byrne B. E., Haughey S. A., Kennedy M. A., Allen C. C. R.: *Org. Biomol. Chem.* **2004**, *2*, 2530.
7. a) Boyd D. R., Sharma N. D., Brannigan I. F., Haughey S. A., Malone J. F., Clarke D. A., Dalton H.: *Chem. Commun.* **1996**, 2361; b) Boyd D. R., Sharma N. D., Boyle R., McMurray B. T., Evans T. A., Malone J. F., Dalton H., Chima J., Sheldrake G. N.: *J. Chem. Soc., Chem. Commun.* **1993**, 49; c) Boyd D. R., Sharma N. D., Boyle R., Evans T. A., Malone J. F., McCombe K. M., Dalton H., Chima J.: *J. Chem. Soc., Perkin Trans. 1* **1996**, 1757; d) Boyd D. R., Sharma N. D., Hempenstall F., Kennedy M. A., Malone J. F., Allen C. C. R.: *J. Org. Chem.* **1999**, *64*, 4005; e) Alien C. C. R., Boyd D. R., Dalton H., Sharma N. D., Haughey S. A., McMordie R. A. S., McMurray B. T., Sheldrake G. N., Sproule K.: *J. Chem. Soc., Chem. Commun.* **1995**, 119.
8. Finn K. J., Cankař P., Jones R. T. B., Hudlický T.: *Tetrahedron: Asymmetry* **2004**, *15*, 2833.
9. The optical purity was found to be greater than 90% based on comparison of optical rotation values for methyl phenyl sulfoxide found in the literature: Holland H. L., Brown F. H., Larsen B. G.: *Biorg. Med. Chem. Lett.* **1994**, 647.
10. Lee K., Brand J. M., Gibson D. T.: *Biochem. Biophys. Res. Commun.* **1995**, *212*, 9.
11. Hudlický T., Claeboe C. D., Brammer L. E., Koroniak L., Butora G., Ghiviriga I.: *J. Org. Chem.* **1999**, *64*, 4909.

12. Boyd D. R., Hand M. V., Sharma N. D., Chima J., Dalton H., Sheldrake G. N.: *J. Chem. Soc., Chem. Commun.* **1991**, 1630.
13. Eliel E. L., Wilen S. H., Doyle M. P.: *Basic Organic Stereochemistry*, p. 443. John Wiley & Sons, Inc., New York 2001.
14. Capozzi M., Cardellicchio C., Naso F., Tortorella P.: *J. Org. Chem.* **2000**, *65*, 2843.
15. Optical puritiy of 2-bromophenyl methyl sulfoxide: 93%, $[\alpha]_D^{23}$ +32.5 (*c* 1.8, $CHCl_3$) (lit.[6] $[\alpha]_D$ +35 (*c* 1.8, $CHCl_3$)); optical purity of 3-bromophenyl methyl sulfoxide: 34%, $[\alpha]_D^{23}$ +39.8 (*c* 1.2, acetone) (lit.[14] $[\alpha]_D$ +116.3 (*c* 1.2, acetone)).
16. Cortese N. A., Heck R. F.: *J. Org. Chem.* **1977**, *42*, 3491.
17. The basic washing step was necessary since trace amounts of acid have been known to catalyze rapid and extremely exothermic dehydration and aromatization of *cis*-cyclohexadienediols.
18. Boyd D. R., Sharma N. D., Byrne B., Hand M. V., Malone J. F., Sheldrake G. N., Blacker J., Dalton H.: *J. Chem. Soc., Perkin Trans. 1* **1998**, 1935.
19. Bui V. P., Nguyen M., Hansen J., Baker J., Hudlicky T.: *Can. J. Chem.* **2002**, *80*, 708.
20. Hudlický T., Oppong K., Duan C., Stanton C., Laufensweiler M. J., Natchus M. G.: *Biorg. Med. Chem. Lett.* **2001**, *11*, 627.