Bacterial dioxygenase-catalysed dihydroxylation and chemical resolution routes to enantiopure *cis*-dihydrodiols of chrysene

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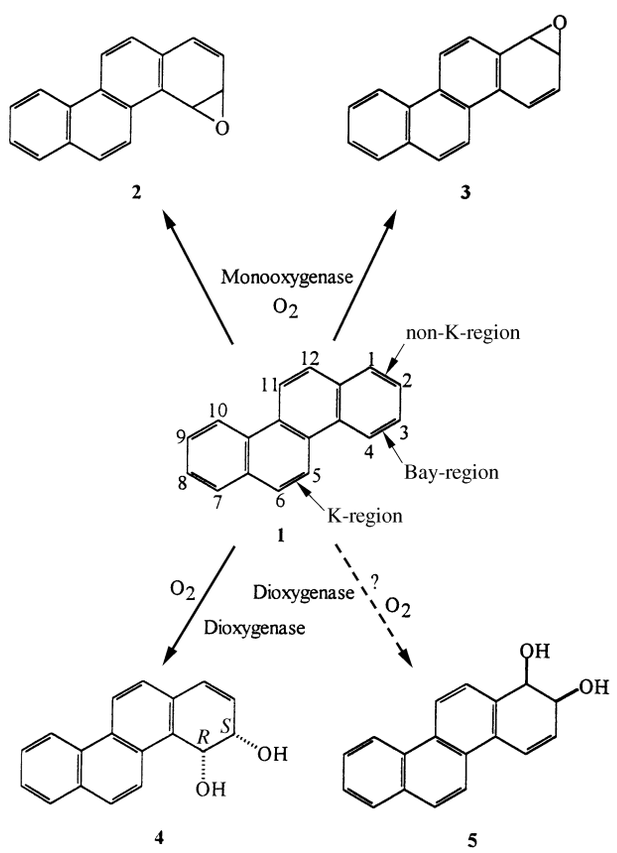
Biotransformation of the environmental pollutant chrysene 1 by resting cells of a mutant strain (B8/36) of the soil bacterium *Sphingomonas yanoikuyae* produces (+)-*cis*-3,4-dihydroxy-3,4-dihydrochrysene 4 which has been assigned (3*S*,4*R*) absolute configuration by stereochemical correlation with (−)-(3*S*,4*R*)-*cis*-3,4-dihydroxy-1,2,3,4-tetrahydrochrysene 6. Both *cis*-3,4-diol 6 and *cis*-1,2-dihydroxy-1,2,3,4-tetrahydrochrysene 12 are obtained in enantiopure form after chromatographic separation of the individual bis(2-methoxy-2-phenyl-2-trifluoromethylacetyl) (bis-MTPA) diastereoisomers of compound 6 and the MTPA diastereoisomers of bromohydrin 19, respectively, followed by hydrolysis. A new general synthetic route to *cis*-dihydrodiols, from the corresponding *cis*-tetrahydrodiol cyclic carbonates, is used to obtain both racemic and enantiopure forms of the bay-region diol 4, and the non-bay region diol 5. ^{1}H NMR and CD spectra of the *cis*- and *trans*-dihydrodiols of chrysene are described.

Introduction

Chrysene **1** is widely distributed in the environment due to the incomplete combustion of fossil fuels and was the first member of the ubiquitous polycyclic aromatic hydrocarbon (PAH) series to be detected in soil samples.[1] Predominant metabolites of chrysene **1** from mammalian liver preparations are its *trans*-1,2- and *trans*-3,4-dihydrodiols which have been shown to be formed from the corresponding arene oxides (*e.g.* **3** and **2**, Scheme 1).[2,3] Mutagenicity and tumour studies[4–6] have provided evidence that a metabolically formed[2,7,8] bay-region 1,2-diol 3,4-epoxide is responsible for most of the carcinogenic activity of the hydrocarbon. Synthetic routes to the arene oxides **2**[9] and **3**,[10] the corresponding *trans*-dihydrodiols and diol epoxides, in both racemic and enantiomerically enriched forms, have been reported.[2,7–12]

Earlier studies have demonstrated the ability of mutant strains of soil bacteria, *e.g. Pseudomonas putida* and *Sphingomonas yanoikuyae*[13] (formerly identified as a *Beijerinckia* sp[14]) to accumulate *cis*-dihydrodiol metabolites of bicyclic,[15,16] tricyclic,[17–19] tetracyclic[20,21] and pentacyclic[20] members of the PAH series. In addition, the initial reaction in the degradation of pyrene by a *Mycobacterium* sp. involves the formation of *cis*- and *trans*-dihydrodiols.[22] Dioxygenase-catalysed asymmetric dihydroxylation of PAHs may, in principle, yield regioisomeric *cis*-dihydrodiol metabolites. In practice, however, few strains have been reported that accept PAH substrates larger than naphthalene.[23] The biphenyl dioxygenase system present in the *Sphingomonas yanoikuyae* mutant strain B8/36 has, however, been found to biotransform larger PAH substrates including anthracene,[18] phenanthrene,[19] benz[*a*]anthracene[20,21] and benzo[*a*]-pyrene[20] largely to bay-region regioisomers of enantiopure *cis*-dihydrodiol metabolites. The bacterial biphenyl dioxygenase system catalyses *cis*-dihydroxylation of arenes with optimal regioselectivity in the sequence: bay-region bonds ≫ non-K region bonds > K-region bonds. Chrysene **1**, a symmetrical tetracyclic PAH containing duplicate bay regions, non-K-regions and K-regions should thus, in principle, be a good substrate for regioselectivity studies. Despite its early detection and prevalence as an environmental pollutant,[1] the metabolism of chrysene **1** by procaryotic organisms has received relatively little attention.[24,25]



Scheme 1

Table 1 ^{1}H NMR spectra [500 MHz, $(CD_3)_2C{=}O$ (A) and CD_3OD (B)] of the *cis*- and *trans*-dihydrodiols of chrysene. Assignments are based on decoupling[a]

	Methine protons		Olefinic protons		Hydroxy protons	
Dihydrodiols (solvent)	4-H	3-H	1-H	2-H	3-OH	4-OH
cis-3,4-Dihydroxy-[b] **4** (A)	5.37	4.67	6.59	6.00	4.24	4.01
	$J_{1,2}$ 9.6, $J_{1,3}$ 2.8, $J_{2,3} = J_{2,4}$ 1.7, $J_{3,4}$ 5.1				$J_{3,OH3}$ 7.8, $J_{4,OH4}$ 5.2	
cis-3,4-Dihydroxy-[b] **4** (B)	5.37	4.65	6.64	6.08	—	—
	$J_{1,2}$ 9.8, $J_{1,3}$ 2.9, $J_{2,3} = J_{2,4}$ 1.8, $J_{3,4}$ 5.1					
trans-3,4-Dihydroxy- (A)	5.44	4.41	6.78	6.26	3.91	4.12
	$J_{1,2}$ 9.4, $J_{1,3}$ 1.6, $J_{2,3}$ 5.4, $J_{3,4}$ 1.5				$J_{3,OH3}$ 6.4, $J_{4,OH4}$ 6.2	
trans-3,4-Dihydroxy- (B)	5.49	4.43	6.90	6.29	—	—
	$J_{1,2}$ 9.5, $J_{1,3}$ 0, $J_{2,3}$ 5.5, $J_{3,4}$ 1.8					
	1-H	2-H	4-H	3-H	1-OH	2-OH
cis-1,2-Dihydroxy- **5** (A)	4.79	4.41	7.46	6.30	—	—
	$J_{1,2}$ 5.5, $J_{2,3}$ 4.0, $J_{3,4}$ 9.9					
cis-1,2-Dihydroxy- **5** (B)	4.77	4.42	7.45	6.26	—	—
	$J_{1,2}$ 5.1, $J_{2,3}$ 4.4, $J_{3,4}$ 10.3					
trans-1,2-Dihydroxy- (A)	4.89	4.49	7.32	6.21	4.69	4.34
	$J_{1,2}$ 11.4, $J_{2,3} = J_{2,4}$ 2.4, $J_{3,4}$ 10.3				$J_{1,OH1}$ 5.5, $J_{2,OH2}$ 4.3	
trans-1,2-Dihydroxy- (B)	4.90	5.51	7.40	6.26	—	—
	$J_{1,2}$ 10.6, $J_{2,3}$ 2.6, $J_{2.4}$ 2.2, $J_{3,4}$ 10.3					

[a] Aromatic regions of the *cis*- and *trans*-dihydrodiols are almost identical (± <0.05) unless noted otherwise: 3,4-dihydrodiols, 8.25 (5-H), 7.88 (6-H), 7.96 (7-H), 7.61 (8-H), 7.67 (9-H), 8.91 (10-H, *cis*-isomer), 8.78 (10-H, *trans*-isomer), 8.77 (11-H) and 7.50 (12-H) with $J_{5,6}$ 9.3, $J_{7,8}$ 8.0, $J_{7,9}$ 1.0, $J_{8,9}$ 6.9, $J_{9,10}$ 8.0, $J_{8,10}$ 1.0, $J_{11,12}$ 8.6; 1,2-dihydrodiols, 8.20 (5-H), 7.86 (6-H), 7.96 (7-H), 7.65 (8-H), 7.70 (9-H), 8.81 (10-H), 8.77 (11-H), 7.89 (12-H, *cis*-isomer), 8.00 (12-H, *trans*-isomer). [b] The bacterial metabolite and the synthetic product had identical spectra.

Chrysene **1** was examined as a substrate for the biphenyl dioxygenase system present in the *Sphingomonas yanoikuyae* B8/36 mutant strain. Based upon earlier studies using other PAH substrates, it was anticipated that formation of the bay-region *cis*-3,4-dihydrodiol **4** would again be preferred over the non-bay-region *cis*-1,2-dihydrodiol **5** (Scheme 1). The second goal of these studies has been the synthesis of these dihydrodiols in optically pure form.

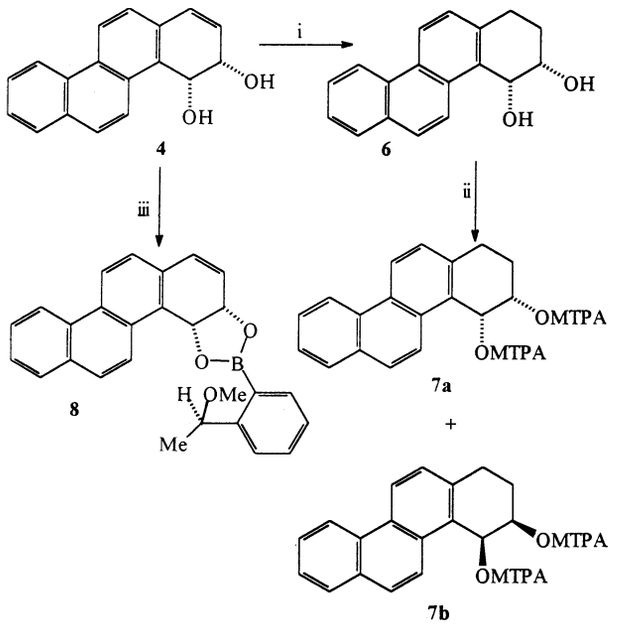
Results and discussion

Metabolism of chrysene **1** by *m*-xylene-induced cells of *Sphingomonas yanoikuyae* strain B8/36, was examined under similar conditions to those used for biotransformation of other PAHs.[17–21] Chromatography of an ethyl acetate extract of the incubation mixture on silica gel provided a dihydrodiol fraction which was examined by HPLC on a DuPont Zorbax Sil column (0.95 × 25 cm) eluted with 2.0% methanol and 15% ethyl acetate in hexane at 12.5 cm^3 min^{-1}. Under these conditions, synthetic (see later) chrysene *cis*-5,6-(K-region), *cis*-3,4-(bay-region) and *cis*-1,2-(non-K-region) dihydrodiols are readily separated (R_t 3.76, 4.84 and 5.35 min, respectively). Only a single metabolite, co-chromatographic with the chrysene *cis*-3,4-dihydrodiol **4**, was detected. Its UV spectrum is practically identical to that of *trans*-3,4-dihydroxy-3,4-dihydrochrysene but markedly different from those of *trans*-1,2-dihydroxy-1,2-dihydrochrysene and *trans*-5,6-dihydroxy-5,6-dihydrochrysene.[11] Preparative chromatography allowed isolation of the metabolite in *ca.* 1% yield. The very low yield of the metabolite was presumably due to the low solubility of chrysene **1** in the aqueous culture medium.

^{1}H NMR spectra of the *cis*-1,2- and *cis*-3,4-dihydrodiols of chrysene (**5** and **4**) are compared to their corresponding *trans* isomers[11] in Table 1. The pattern of coupling constants is consistent with that observed previously;[17–21] both *cis* and *trans* non-bay-region dihydrodiols prefer to have their benzylic hydroxy group (C-1 in chrysene) quasi-equatorial. For the *trans*-1,2-dihydrodiol, $J_{1,2}$ is close to its maximal value, indicating a strong preference for the quasi-equatorial orientation of the hydroxy groups (quasi-axial H-1 and H-2), and, as expected for quasi-axial H-2, $J_{2,4}$ is substantial (2.4 Hz). In the *cis*-1,2-dihydrodiol **5**, H-2 exhibits no measurable homoallylic coupling with H-4; thus H-2 is quasi-equatorial, OH-2 quasi-axial and OH-1 quasi-equatorial as in the *trans* isomer. In contrast, for the bay-region *trans*-3,4-dihydrodiol, small values of $J_{3,4}$ are diagnostic of a preference for the conformation with the hydroxy groups quasi-axial (H-3 and H-4 quasi-equatorial). However, a small but significant homoallylic coupling $J_{1,3}$ suggests that this preference is not absolute, and that there is some contribution to the conformational equilibrium from the other conformer, in which H-4 is quasi-axial (hydroxy groups quasi-equatorial). In the *cis*-3,4-dihydrodiol, OH-4 in the bay region remains largely quasi-axial and thus OH-3 must be quasi-equatorial; the corresponding quasi-axial orientation of H-3 results in a much larger value (2.8–2.9 Hz) for $J_{1,3}$.

Enantiopurity of the *cis*-dihydrodiol metabolite **4** ($[a]_D$ +112) was determined as >98% by ^{1}H NMR analysis following reaction with (−)- and (+)-2-(1-methoxyethyl)phenylboronic acid (MPBA)[26] to yield the corresponding boronate **8**. (3*S*,4*R*) Absolute configuration of the metabolite was established by catalytic hydrogenation to yield the previously characterized (−)-(3*S*,4*R*)-*cis*-3,4-dihydroxy-1,2,3,4-tetrahydrochrysene **6** ($[a]_D$ −45 observed, lit.,[7–9] −43). ^{1}H NMR analyses of the bis-[methoxy(trifluorophenyl)acetic] acid (MTPA) ester **7a** and boronate **8** (Scheme 2) were consistent with the stereochemical assignment and estimation of optical purity.[26,27] CD spectra of the *cis*- and *trans*-dihydrodiols of chrysene are compared in Fig. 1. These spectra are presumed to be derived primarily from the skew sense of the vinyl group relative to the phenanthrene chromophore with little contribution from the chiral methanol centres.[28] Although one might have expected that the CD spectra of *cis*-(3*S*,4*R*)- and *trans*-(3*R*,4*R*)-dihydrodiols (both with axial benzylic hydroxy groups) would be similar, they are quite different. It is inferred that small changes in the torsion angle between the vinyl group and the phenanthrene chromophore markedly alter the CD spectra of these as well as the 1,2-dihydrodiols.

Synthetic routes to the *cis*-dihydrodiols **4** and **5** have now been developed. Racemic *cis*-3,4-tetrahydrodiol **6** was synthesized by osmylation of 1,2-dihydrochrysene which was available from earlier studies.[9,11] Racemic *cis*-tetrahydrodiol **6** was treated with 1,1′-carbonyldiimidazole (CDA) in benzene to yield the cyclic tetrahydrocarbonate **9** in 85% yield (Scheme 3).



Scheme 2 *Reagents:* i, Pd/C, H_2; ii, MTPACl–pyridine; iii, MPBA

Benzylic bromination of compound **9**, using *N*-bromosuccinimide (NBS) in CCl_4, yielded an isomeric mixture of bromocarbonates **10a** and **10b** (89% yield), which was, in turn, dehydrobrominated using 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), to give the dihydrocarbonate **11** (93% yield). Hydrolysis, using an aqueous methanolic solution of potassium carbonate, gave the racemic *cis*-dihydrodiol **4** (55% yield) in an overall yield of *ca.* 40% from the tetrahydrodiol **6**. The use of cyclic carbonate esters of *cis*-tetrahydrodiols constitutes an effective new approach to the synthesis of *cis*-dihydrodiols.

Conversion of the racemic *cis*-tetrahydrodiol **6** to the corresponding bis-MTPA esters **7a** and **7b** using (+)-MTPA chloride in pyridine provided both a measure of diol enantiopurity (Scheme 2), and a method for the resolution of diol enantiomers. The bis-MTPA esters **7a** and **7b**, derived from a racemic sample of diol **6**, were thus separated by PLC on silica gel into the less polar **7b** (R_f 0.26 $[\alpha]_D$ +49) and more polar **7a** (R_f 0.21, $[\alpha]_D$ +43) diastereoisomers. Hydrolysis of the diesters **7b** and **7a** yielded the corresponding *cis*-tetrahydrodiol enantiomers (+)-(3*R*,4*S*)-**6** ($[\alpha]_D$ +46) and (−)-(3*S*,4*R*)-**6** ($[\alpha]_D$ −45) respectively. (+)-(3*R*,4*S*)-*cis*-Tetrahydrodiol **6** ($[\alpha]_D$ +46) was treated in a similar manner to the racemic sample to provide, in sequence, the cyclic tetrahydrocarbonate **9** (83% yield, $[\alpha]_D$ −306), an isomeric mixture of bromo carbonates **10a** and **10b** (93% yield, $[\alpha]_D$ −150), the dihydro carbonate **11** (89% yield, $[\alpha]_D$ −467) and the (−)-(3*R*,4*S*)-*cis*-dihydrodiol **4** (52% yield, $[\alpha]_D$ −111). The *cis*-dihydrodiol enantiomer **4** ($[\alpha]_D$ +111) which corresponds to the metabolite was synthesised using the alternative *cis*-tetrahydrodiol enantiomer (−)-**6** ($[\alpha]_D$ −45) following an identical reaction sequence. The derived synthetic and metabolic *cis*-tetrahydrodiol samples were indistinguishable spectrally. The synthetic sequence and absolute configurations of products from (3*S*,4*R*)-*cis*-tetrahydrodiol **6** are shown in Scheme 3.

Conversion of the racemic *cis*-1,2-tetrahydrodiol **12** to *cis*-1,2-dihydrodiol **5** was achieved in an overall yield of *ca.* 15% using a bromination–dehydrobromination sequence on the corresponding cyclic carbonates (**12** ⟶ **13** ⟶ **14** ⟶ **15** ⟶ **5**, Scheme 4). In view of the lower overall yield obtained for *cis*-dihydrodiol **5**, compared with dihydrodiol **4**, using the cyclic carbonate method, we also examined the use of acetate as a blocking group for the hydroxy groups as had been done previously in the synthesis of *cis*-dihydrodiols from benzo[*a*]anthracene.[21] The sequence parallels that shown in Scheme 4 (**12** ⟶ **13** ⟶ **14** ⟶ **15** ⟶ **5**), except that vicinal *cis*-

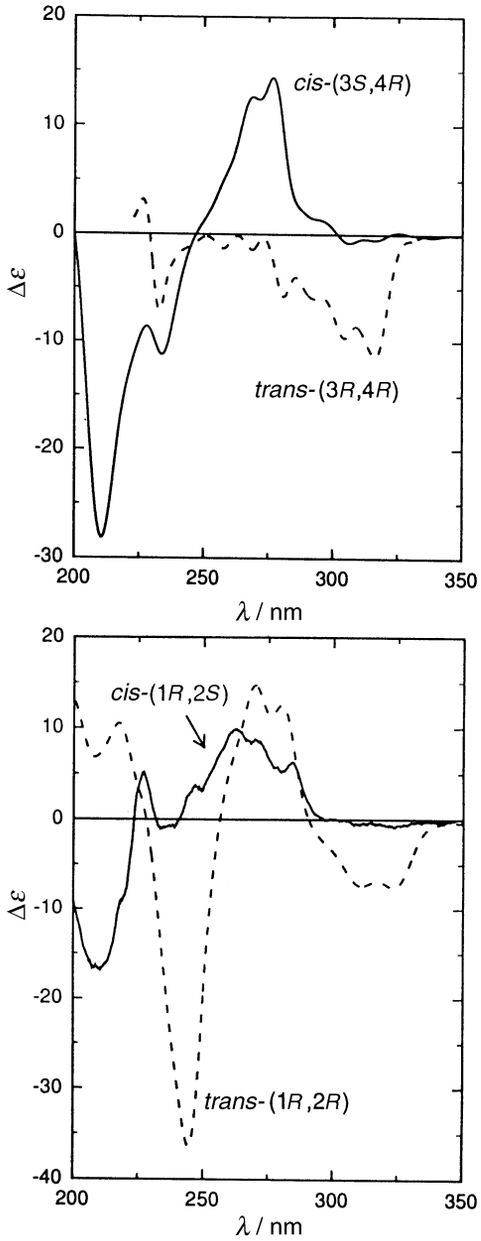
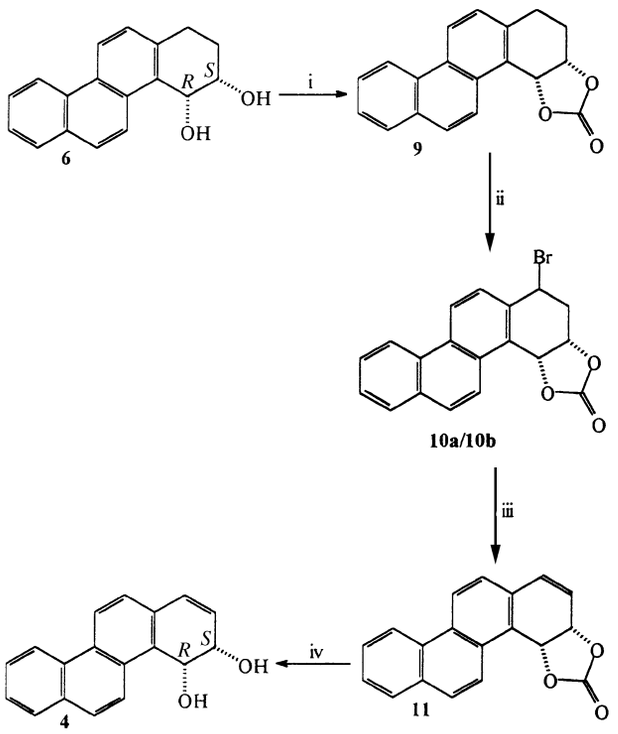


Fig. 1 Circular dichroism spectra of one enantiomer [(*R*) absolute configuration at the benzylic hydroxyl position] of each of the isomeric chrysene dihydrodiols [methanol, $l = 0.2$ cm for the *cis*-(3*S*,4*R*) and *trans*-(1*R*,2*R*); $l = 1.0$ cm for the *cis*-(1*R*,2*S*) and *trans*-(3*R*,4*R*) isomers]. Observed ellipticities were converted to $\Delta\varepsilon$ ($dm^3\ mol^{-1}\ cm^{-1}$) by use of sample concentrations determined by weight (*cis*-3,4-dihydrodiol) or from their absorption spectra and the following UV extinction coefficients in methanol: *trans*-3,4-dihydrodiol, 64 200 (277 nm),[11] *cis*-1,2-dihydrodiol, 66 100 (220 nm) (see Experimental section); *trans*-1,2-dihydrodiol, 69 500 (220 nm) [this work, determined for the (1*S*,2*S*)-enantiomer]. Selected $\Delta\varepsilon$ values are as follows: *cis*-(3*S*,4*R*), 14.3 (277 nm); *trans*-(3*R*,4*R*), −11.0 (316 nm); *cis*-(1*R*,2*S*), 9.6 (261 nm) and *trans*-(1*R*,2*R*), −36.2 (244 nm).

diacetates replace the cyclic carbonate. Attempted direct dehydrogenation of the racemic diacetate **16**, obtained in 98% yield from **12**, in refluxing benzene or dioxane was unsuccessful. Benzylic bromination of the diacetate **16** using NBS in CCl_4 produced an isomeric mixture (9:1) of two bromodiacetates **17a** and **17b** (98% yield). Attempted dehydrobromination with DBN or 1,8-diazabicyclo[5.3.0]undec-7-one (DBU) was also unsuccessful. However, dehydrobromination of the mixture of bromodiacetates **17** to the diacetate **18** was successfully achieved (85% yield) in refluxing xylene in the presence of sodium hydrogen carbonate and triethylamine. In the absence of triethylamine, complete aromatization took place. Ammonolysis of diacetate **18** with ammoniacal methanol provided



Scheme 3 *Reagents:* i, $(Imid)_2CO$–benzene; ii, NBS–CCl_4; iii, DBN–THF; iv, K_2CO_3–MeOH–THF–H_2O

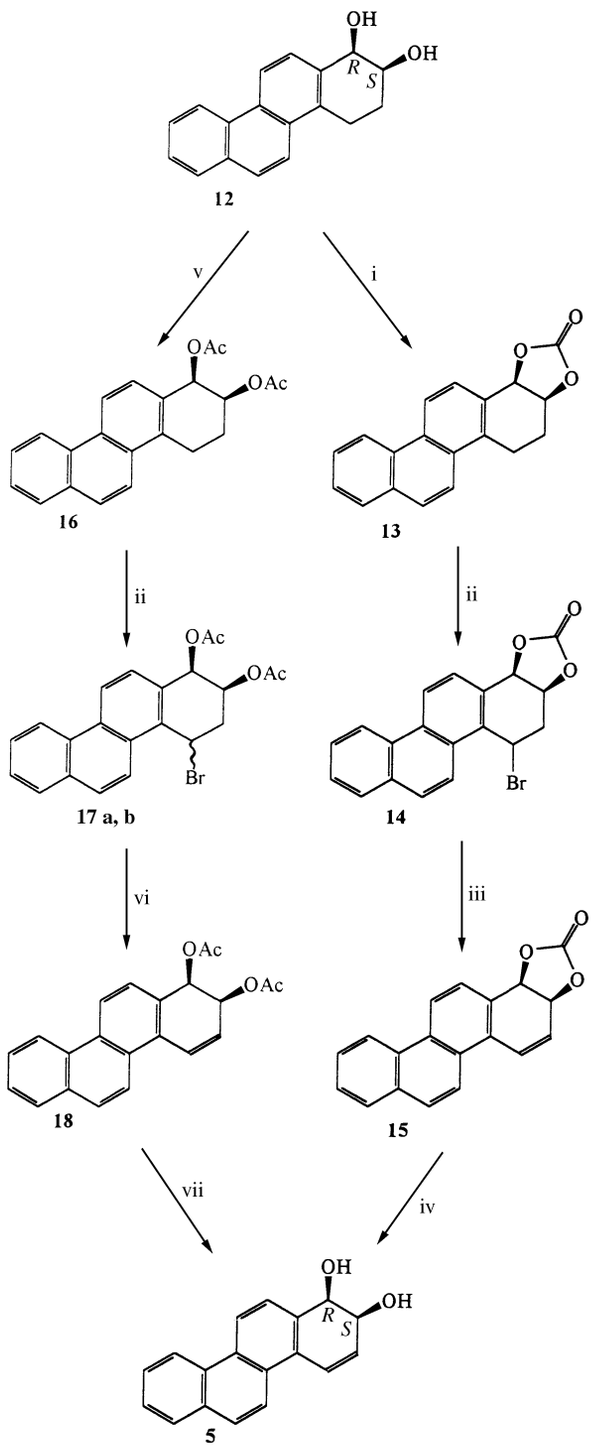
the desired *cis*-1,2-dihydrodiol **5** (94% yield). Overall yield for the four steps (**12** ⟶ **16** ⟶ **17** ⟶ **18** ⟶ **5**) from *cis*-tetrahydrodiol **12** using acetates as blocking groups was *ca.* 75%.

Enantiomerically pure samples of *cis*-tetrahydrodiol **12** were previously obtained from hydrolysis of 1,2-epoxy-1,2,3,4-tetrahydrochrysene and from silver acetate treatment of the *trans*-2-bromo-1-acetate derivative, both of which were derived from the corresponding bromohydrin **19** (resolved *via* the bromoMTPA ester).[10] In view of the low yields available from the latter approach, the alternative method shown in Scheme 5 (based upon an earlier report[29] where a bromohydrin was converted to a diol) was adopted. The enantiopure (+)-(1*R*,2*R*)-*trans*-bromohydrin **19** ($[\alpha]_D$ +27, available from chromatographic resolution and hydrolysis of the corresponding MTPA esters[10]) was converted to the (−)-(1*R*,2*S*)-*cis*-tetrahydrodiol **12** *via* the bromoester **20**, the dioxolane mixture **21a** and **21b**, and the ester mixture **22a** and **22b** in a total yield of *ca.* 70%. Conversion of the (1*R*,2*S*)-*cis*-tetrahydrodiol **12** ($[\alpha]_D$ −29) to (1*R*,2*S*)-*cis*-dihydrodiol **5** ($[\alpha]_D$ +74) was carried out in a similar manner to that described for the racemic compounds using the sequence **12** ⟶ **13** ⟶ **14** ⟶ **15** ⟶ **5** shown in Scheme 4.

Despite the advantage of having synthetic standards of three possible *vicinal cis*-dihydrodiols of chrysene **1**, only the *cis*-dihydrodiol **4** could be detected by HPLC on examination of several crude extracts obtained by biotransformation. Preliminary experiments have however provided tentative evidence of a trace metabolite that appears to be the product from two dihydroxylation processes and further efforts to isolate and characterize this metabolite are in progress. The virtually exclusive formation of the 3*S*,4*R*-enantiomer of *cis*-dihydrodiol **4**, confirms that the bay region is a dominant feature in controlling both the stereoselectivity and the regioselectivity of dioxygenase-catalysed *cis*-dihydroxylation of arenes.

Experimental

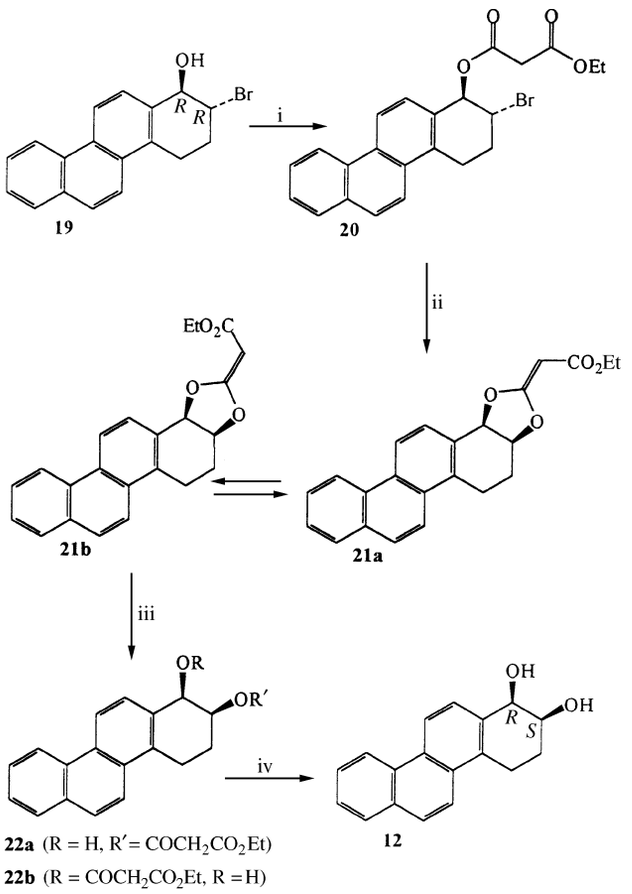
^{1}H NMR spectra were measured at 300 MHz in $CDCl_3$ solvent unless otherwise indicated. Chemical shifts (δ) are reported in



Scheme 4 *Reagents:* i, $(Imid)_2CO$–benzene; ii, NBS–CCl_4; iii, DBN–THF; iv, K_2CO_3–MeOH–THF; v, $(Ac)_2O$–pyridine; vi, $NaHCO_3$–Et_3N–xylene; vii, NH_3–MeOH–THF–H_2O

ppm relative to TMS and coupling constants (J) in Hz. ^{1}H NMR spectra of optically active compounds were identical to their racemic counterparts in all cases. Optical rotations ($[\alpha]_D$) were measured at ambient temperature *ca.* 20 °C and are given in units of 10^{-1} deg cm^2 g^{-1}. Standard work-up consisted of drying of the pooled organic phase over anhydrous $MgSO_4$ and concentration *in vacuo*. Light petroleum refers to the fraction with bp 40–60 °C.

Racemic samples of *cis*-3,4-dihydroxy-1,2,3,4-tetrahydrochrysene **6** and *cis*-1,2-dihydroxy-1,2,3,4-tetrahydrochrysene **12** were either obtained from the corresponding dihydrochrysene using the literature procedures[9,10,30] or were prepared by dihydroxylation (OsO_4) of the corresponding dihydrochrysenes[6] and synthesis of tetrahydrodiol **12** is reported herein.



Scheme 5 *Reagents:* i, Ethyl malonyl chloride; ii, NaH–THF; iii, HCl–THF; iv, Et_3N–THF

Biotransformation of chrysene 1 and identification of the *cis*-dihydrodiol metabolite 4

A small scale biotransformation was carried out by incubating *m*-xylene-induced cells of *Sphingomonas yanoikuyae* strain B8/36 (A600 = 5.0) suspended in 250 cm^3 0.05 M potassium phosphate buffer (pH 7.2) containing 0.5% pyruvate and 0.05% (w/v) chrysene (solution in 0.2% DMF). The reaction mixture was incubated in the dark at 30 °C with shaking (220 rpm) for 20 h. The cells were subsequently removed by centrifugation and after saturation with sodium chloride the supernatant was extracted with ethyl acetate (3 × 125 cm^3). The dried ethyl acetate extract (Na_2SO_4) was concentrated and the light brown semi-solid (~0.005 g) was purified by PLC on silica-gel using $CHCl_3$–MeOH (92:8), and by HPLC (see Results and discussion) to yield *cis*-dihydrodiol metabolite **4** (0.0025 g); mp 241–243 °C (decomp.) as colourless needles ($CHCl_3$–MeOH); $[\alpha]_D$ +112 (*c* 0.5, THF) (Found: M^+, 262.0998. $C_{18}H_{14}O_2$ requires *M*, 262.0994); λ_{max}(methanol)/nm 227 (ε/dm^3 mol^{-1} cm^{-1} 28 460), 278 (57 650), 307 (11 200) and 320 (9010). See Table 1 for ^{1}H NMR data.

The enantiopurity of the dihydrodiol metabolite **4** (0.0005 g) was established by treatment with (−)-(*S*)- and (+)-(*R*)-2-(1-methoxyethyl)phenylboronic acid (0.00034 g) in chloroform solution. The boronate reaction product 4-[2-(1-methoxyethyl)-phenyl]-2a,5a-dihydrochryseno[3,4-*d*][1,3,2]dioxaborole **8** was filtered through a small bed of sodium sulfate, concentrated to dryness and the residue dissolved in $CDCl_3$ for ^{1}H NMR analysis.[26] The diagnostic OMe signal of the boronate ester **8**, formed with (−)-(*S*)-MPBA (δ_H 3.11) was shifted upfield ($\Delta\delta$ −0.14) from that of the (+)-(*R*)-MPBA ester, suggesting an (*R*)-configuration for the benzylic C-4 position. Thus the *cis*-dihydrodiol metabolite **4** was predicted to have (3*S*,4*R*) absolute configuration. Since only one OMe signal was observed in the ^{1}H NMR spectrum of either the (*R*)- or the (*S*)-MPBA ester **8**, the *cis*-dihydrodiol metabolite **4** was assumed to be enantiopure.

The absolute configuration of the (+)-*cis*-dihydrodiol metabolite **4** (0.004 g, $[\alpha]_D$ +112, in 2 cm^3 of methanol) was established by catalytic hydrogenation (5% Pd/C, 0.002 g, 3 h, 1 atm pressure) to the (−)-*cis*-tetrahydrodiol **6** known to have (3*S*,4*R*) absolute configuration;[7,9] white crystalline solid (0.0035 g, $[\alpha]_D$ −45, *c* 0.5, THF), mp 192–196 °C. The enantiopure *cis*-tetrahydrodiol **6** was found to have identical spectral characteristics to a racemic sample. The bis-MTPA ester **7a**, formed from (−)-*cis*-tetrahydrodiol **6** ($[\alpha]_D$ −45), using (+)-MTPA chloride in pyridine containing a trace of DMAP, showed only two OMe signals at δ 3.34 and 3.57 confirming that the *cis*-tetrahydrodiol derivative **6** and the parent *cis*-dihydrodiol metabolite **4** were both enantiopure.

(±)-1,2,2a,5a-Tetrahydrochryseno[3,4-*d*][1,3]dioxol-4-one 9

To a refluxing mixture of racemic *cis*-tetrahydrodiol **6** (0.120 g, 0.45 mmol) in dry benzene (40 cm^3), 1,1′-carbonyldiimidazole (0.180 g, 1.10 mmol) was added in portions over a period of 8 h, under nitrogen, and heating was continued overnight. After cooling, the benzene layer was washed with water (2 × 35 cm^3), the aqueous layer was subsequently back extracted with benzene (2 × 60 cm^3). Standard work-up provided a crude product which was purified by PLC using $CHCl_3$–light petroleum (30:70). Cyclic carbonate **9** (0.112 g, 85%) was obtained as colourless crystals, mp 220 °C (CH_2Cl_2–pentane) (Found: C, 78.7; H, 4.8. $C_{19}H_{14}O_3$ requires C, 78.6; H, 4.9%); δ_H 2.09–2.18 (1 H, m, 2-H), 2.30–2.36 (1 H, m, 2-H), 2.80–2.89 (1 H, m, 1-H), 3.1–3.2 (1 H, m, 1-H), 5.19–5.25 (1 H, m, 2a-H), 6.3 (1 H, d, $J_{2a,5a}$ 7.4, 5a-H), 7.39 (1 H, d, $J_{12,13}$ 8.8, 13-H), 7.53–7.64 (2 H, m, 9-H and 10-H), 7.82 (1 H, d, $J_{6,7}$ 9.2, 7-H), 7.84 (1 H, dd, $J_{8,9}$ 7.8, $J_{8,10}$ 1.3, 8-H), 7.96 (1 H, d, $J_{6,7}$ 9.2, 6-H), 8.62 (1 H, d, $J_{11,10}$ 9.0, 11-H), 8.64 (1 H, d, $J_{12,13}$ 9.0, 12-H); ν_{max}(KBr)/cm^{-1} 1800 (C=O); *m/z* (EI) 290 (M^+, 100%).

(±)-1-Bromo-1,2,2a,5a-tetrahydrochryseno[3,4-*d*][1,3]dioxol-4-one 10a, 10b

The racemic cyclic carbonate **9** (0.100 g, 0.34 mmol), NBS (0.064 g, 0.36 mmol) and AIBN (0.005 g), in a mixture of CH_2Cl_2 (10 cm^3) and carbon tetrachloride (25 cm^3) was irradiated with a heating lamp and stirred under nitrogen at 50–60 °C for 60 min. ^{1}H NMR analysis of an aliquot indicated that carbonate **9** had been consumed giving two stereoisomers **10a**, **10b** in a ratio of 90:10. Succinimide was removed by filtration from the reaction mixture, and the solvent evaporated to leave a pale brown oil (0.112 g, 89%). The pure, major stereoisomer **10a** was obtained by preparative TLC using $CHCl_3$–light petroleum (50:50), and recrystallization of a small portion of the purified product; mp 240 °C ($CHCl_3$–pentane); δ_H 2.40–2.50 (1 H, m, 2-H), 2.84–2.92 (1 H, m, 2-H), 5.53–5.57 (1 H, m, 2a-H), 5.60 (1 H, dd, $J_{1,2} = J_{1,2'}$ 3.3, 1-H), 6.29 (1 H, d, $J_{2a,5a}$ 7.0, 5a-H), 7.56–7.67 (3 H, m, 9-H, 10-H and 13-H), 7.86–7.90 (2 H, m, 7-H, 8-H), 8.01 (1 H, d, $J_{6,7}$ 9.2, 6-H), 8.61 (1 H, d, $J_{12,13}$ 7.6, 12-H) and 8.74 (1 H, d, $J_{10,11}$ 8.7, 11-H); ν_{max}(KBr)/cm^{-1} 1788 (C=O).

(±)-2a,5a-Dihydrochryseno[3,4-*d*][1,3]dioxol-4-one 11

DBN (0.2 cm^3, 1.6 mmol) was added dropwise to a stirred solution of racemic stereoisomers **10a**, **10b** (0.100 g, 0.27 mmol) in dry THF (20 cm^3) at 0 °C under nitrogen. The resulting mixture was stirred for a further 12 h at room temperature. Water (15 cm^3) was added, and the THF was removed under reduced pressure. The aqueous layer was extracted with CH_2Cl_2 (3 × 40 cm^3). Purification by PLC using $CHCl_3$–light petroleum (25:75) gave compound **11** (0.072 g, 93%); white crystals, mp 168–178 °C (decomp., from $CHCl_3$–hexane) (Found: C, 79.2; H, 3.9. $C_{19}H_{12}O_3$ requires C, 79.1; H, 4.2%); δ_H 5.68–5.72 (1 H, ddd, $J_{2a,5a}$ 9.6, $J_{2,2a}$ 3.0, $J_{1,2a}$ 1.1, 2a-H), 5.96 (1 H, dd, $J_{1,2}$ 9.4, $J_{2,2a}$ 3, 2-H), 6.45 (1 H, d, $J_{1,2}$ 9.4, 1-H), 6.70 (1 H, d, $J_{2a,5a}$ 9.4, 5a-H), 7.39 (1 H, d, $J_{12,13}$ 8.5, 13-H), 7.57–7.65 (2 H, m, 9-H, 10-H),

7.82 (1 H, d, $J_{6,7}$ 9.4, 7-H), 7.86 (1 H, d, $J_{1,2}$ 9.26, 8-H), 7.93 (1 H, d, $J_{6,7}$ 9.3, 6-H), 8.59 (1 H, d, $J_{10,11}$ 7.9, 11-H) and 8.70 (1 H, d, $J_{12,13}$ 8.5, 12-H); ν_{max}(KBr)/cm^{-1} 1794 (C=O); *m/z* (EI) 288 (M^+, 9%) and 244 ($M^+ - CO_2$, 3).

(±)-*cis*-3,4-Dihydroxy-3,4-dihydrochrysene 4

To a stirred mixture of THF (2 cm^3), methanol (5 cm^3), water (1 cm^3) and triethylamine (1 cm^3) was added the racemic cyclic carbonate **11** (0.070 g, 0.24 mmol) in THF (1 cm^3) at room temperature. The reaction was monitored by TLC using $CHCl_3$–methanol as eluent (95:5). On completion (12 h), the reaction mixture was concentrated under reduced pressure and extracted thoroughly with ethyl acetate (4 × 10 cm^3). Purification by PLC using 0.1% triethylamine in $CHCl_3$–methanol (95:5) and crystallization gave pure *cis*-3,4-dihydroxy-3,4-dihydrochrysene **4** (0.035 g, 55%); colourless crystals, mp 180–183 °C (decomp., from CH_2Cl_2–hexane) (Found: M^+, 262.10128. $C_{18}H_{14}O_2$ requires *M*, 262.09937); *m/z* (EI) 262 (M^+, 43%) and 244 ($M^+ - H_2O$, 100). The ^{1}H NMR spectrum of the synthetic sample of racemic *cis*-dihydrodiol **4** was identical to that of metabolite **4**.

(3*S*,4*R*)- and (3*R*,4*S*)-*cis*-3,4-Bis[(*R*)-2-methoxy-2-phenyl-2-trifluoromethylacetoxy]-1,2,3,4-tetrahydrochrysene 7a and 7b

(+)-MTPA chloride (2.1 g, 8.3 mmol) was added slowly to a stirred solution of racemic *cis*-tetrahydrodiol **6** (1 g, 3.78 mmol) and DMAP (0.08 g, 0.65 mmol) in dry pyridine (15 cm^3) at 0 °C, and stirring was continued (24 h) at room temperature. Saturated aqueous sodium hydrogen carbonate (20 cm^3) was added, and the reaction mixture was extracted with CH_2Cl_2 (3 × 50 cm^3). After standard work-up followed by column chromatography on silica gel using diethyl ether–light petroleum (10:90), the diastereoisomeric mixture **7a**, **7b** was obtained as a viscous oil (2.24 g, 85%). Separation of the two diastereoisomers was achieved by means of PLC using diethyl ether–light petroleum (5:95) as eluent.

(+)-*cis*-(3*R*,4*S*)-Bis[(*R*)-2-methoxy-2-phenyl-2-trifluoromethylacetoxy]-1,2,3,4-tetrahydrochrysene 7b. Less polar isomer (R_f 0.26), 0.963 g, 43%; mp 183–186 °C ($CHCl_3$–pentane); $[\alpha]_D$ +49 (*c* 1.2, $CHCl_3$) (Found: C, 65.3; H, 4.3. $C_{38}H_{30}F_6O_6$ requires C, 65.5; H, 4.3%); δ_H 2.10 (2 H, m, 2-H), 3.19 (4 H, m, 1-H and OMe), 3.76 (3 H, s, OMe), 5.61–5.68 (1 H, m, 3-H), 6.76–7.98 (17 H, m, 4-H and Ar-H) and 8.66–8.72 (2 H, m, Ar-H).

(+)-*cis*-(3*S*,4*R*)-Bis[(*R*)-2-methoxy-2-phenyl-2-trifluoromethylacetoxy]-1,2,3,4-tetrahydrochrysene 7a. More polar isomer (R_f 0.21), 1.07 g, 48%; mp 166–168 °C ($CHCl_3$–pentane); $[\alpha]_D$ +43 (*c* 0.6, $CHCl_3$) (Found: C, 65.3; H, 4.1. $C_{38}H_{30}F_6O_6$ requires C, 65.5; H, 4.3%); δ_H 2.20 (1 H, m, 2-H), 2.40 (1 H, m, 2-H), 3.24–3.29 (2 H, m, 1-H), 3.34 (3 H, s, OMe), 3.57 (3 H, s, OMe), 5.62–5.68 (1 H, m, 3-H), 7.03–8.03 (17 H, m, 4-H and Ar-H) and 8.62–8.66 (2 H, m, Ar-H).

(+)-(3*R*,4*S*)- and (−)-(3*S*,4*R*)-*cis*-3,4-Dihydroxy-1,2,3,4-tetrahydrochrysene 6

The bis-MTPA ester **7b** (0.500 g, 0.72 mmol, $[\alpha]_D$ +49), dissolved in THF (30 cm^3), was treated with methanolic 1 M sodium hydroxide (15 cm^3) and stirred at room temperature for 24 h. Saturated aqueous ammonium chloride (10 cm^3) was added and the solution was concentrated *in vacuo*. Water (10 cm^3) was added and the product was extracted into ethyl acetate. Standard work-up provided the (+)-(3*R*,4*S*)-tetrahydrodiol **6** (0.151 g, 80%), mp 190–196 °C (from $CHCl_3$–methanol); $[\alpha]_D$ +46.4 (*c* 0.53, THF) (lit.,[9] mp 196–198 °C, $[\alpha]_D$ +43, THF). The bis-MTPA ester, **7a** $[\alpha]_D$ +43, was treated in an identical manner to yield (−)-(3*S*,4*R*)-*cis*-3,4-dihydroxy-1,2,3,4-tetrahydrochrysene **6**, $[\alpha]_D$ −45 (*c* 0.5, THF).

(−)-*cis*-(2a*R*,5a*S*)- and (+)-(2a*S*,5a*R*)-1,2,2a,5a-Tetrahydrochryseno[3,4-*d*][1,3]dioxol-4-one 9

Using a similar method for the conversion of racemic diol **6** to carbonate **9**, a sample of enantiopure diol (+)-**6** (0.100 g, 0.37 mmol, $[\alpha]_D$ +46.4) provided the (−)-*cis*-(2a*R*,5a*S*) cyclic carbonate **9** (0.100 g, 83%), mp 216 °C (CH_2Cl_2–pentane); $[\alpha]_D$ −306 (*c* 1.2, THF). Similar treatment of (−)-*cis*-tetrahydrodiol **6** ($[\alpha]_D$ −45) gave (+)-(2a*S*,5a*R*)-cyclic carbonate **9**, $[\alpha]_D$ +304 (*c* 0.53, THF). Both enantiomers of carbonate **9** showed identical spectral characteristics to the racemate.

(−)-(2a*R*,5a*S*)- and (+)-(2a*S*,5a*R*)-1-Bromo-1,2,2a,5a-tetrahydrochryseno[3,4-*d*][1,3]dioxol-4-one 10a and 10b

Bromination of carbonate **9** (0.08 g, 0.27 mmol, $[\alpha]_D$ −306) with NBS (0.05 g, 0.29 mmol) using the procedure described for the conversion of racemic diol to carbonate **10** provided the (−)-(2a*R*,5a*S*) benzylic bromide mixture **10a**, **10b** (0.094 g, 93%), mp 232 °C ($CHCl_3$–pentane); $[\alpha]_D$ −150 (*c* 0.15, $CHCl_3$) which was spectrally indistinguishable from the racemic sample. Similarly the (+)-enantiomer of carbonate **9** yielded the (+)-(2a*S*,5a*R*) benzylic bromide mixture **10a**, **10b**.

(−)-(2a*R*,5a*S*)- and (+)-(2a*S*,5a*R*)-2a,5a-Dihydrochryseno-[3,4-*d*][1,3]dioxol-4-one 11

Dehydrobromination of the bromo carbonate mixture **10a**, **10b** (0.094 g, 0.25 mmol, $[\alpha]_D$ −150), as described for the conversion of the racemic sample gave the (2a*R*,5a*S*)-enantiomer of carbonate **11** (0.065 g, 89% yield), mp 180 °C ($CHCl_3$–pentane); $[\alpha]_D$ −467 (*c* 0.71, $CHCl_3$) which was spectrally indistinguishable from the racemic sample. The (+)-(2a*S*,5a*R*)-enantiomer of the bromo carbonate mixture **10a**, **10b**, similarly yielded the (2a*S*,5a*R*)-enantiomer of carbonate **11**, $[\alpha]_D$ +462 (*c* 0.6, $CHCl_3$).

(−)-(3*R*,4*S*)- and (+)-(3*S*,4*R*)-*cis*-3,4-Dihydroxy-3,4-dihydrochrysene 4

A modification of the described procedure for hydrolysis of racemic carbonate **11** to *cis*-diol **4** was applied to each enantiomer. Thus, a solution of (−)-cyclic carbonate **11**, $[\alpha]_D$ −467, (0.030 g, 0.10 mmol) and potassium carbonate (0.001 g) in a mixture of THF (2 cm^3), methanol (3 cm^3) and water (0.5 cm^3), was stirred for 48 h at room temperature. After dilution with water (10 cm^3) the organic solvent was removed under vacuum and the residual aqueous solution was extracted with ethyl acetate. The extract was dried (Na_2SO_4), the solvent was removed under vacuum and the residue was purified by PLC on silica gel using $CHCl_3$–methanol (95:5) to yield (−)-(3*R*,4*S*)-3,4-dihydroxy-3,4-dihydrochrysene **4** (0.014 g, 52%), $[\alpha]_D$ −111 (*c* 0.33, THF), mp 240–242 °C. Similarly an enantiopure sample of carbonate **11** (0.060 g, 0.20 mmol, $[\alpha]_D$ +462) was converted into (+)-(3*S*,4*R*)-*cis*-3,4-dihydroxy-3,4-dihydrochrysene **4** (0.049 g, 90%), mp 240–242 °C ($CHCl_3$–methanol); $[\alpha]_D$ +111 (*c* 0.48, THF). The ^{1}H NMR and CD spectra of the chemically synthesised (+)-enantiomer of diol **4** were identical to the metabolite **4**.

(±)-3a,4,5,13b-Tetrahydrochryseno[1,2-*d*][1,3]dioxol-2-one 13

Using a similar procedure to that reported for the conversion of *cis*-diol **6** to carbonate **9**, *cis*-1,2-dihydroxy-1,2,3,4-tetrahydrochrysene **12** (0.210 g, 0.79 mmol) provided the cyclic carbonate **13**. This was purified by flash chromatography on silica gel using $CHCl_3$–pentane (30:70) and crystallization to yield compound **13** (0.184 g, 80%), mp 200–220 °C (CH_2Cl_2–pentane) (Found: C, 78.2; H, 4.8. $C_{19}H_{14}O_3$ requires C, 78.6; H, 4.9%); δ_H 2.17–2.19 (1 H, m, 4-H), 2.46–2.52 (1 H, m, 4-H), 3.33 (2 H, m, 5-H), 5.25–5.28 (1 H, m, 3a-H), 5.90 (1 H, d, $J_{3a,13b}$ 7.6, 13b-H), 7.62–7.73 (3 H, m, 9-H, 10-H and 13-H), 7.83 (1 H, dd, $J_{8,9}$ 7.0, $J_{8,10}$ 1.8, 8-H), 7.87 (1 H, d, $J_{6,7}$ 9.2, 7-H), 8.00 (1 H, d, $J_{6,7}$ 9.2, 6-H), 8.68–8.73 (2 H, m, 11-H, 12-H); ν_{max}(KBr)/cm^{-1} 1778 (C=O); *m/z* (EI) 290 (M^+, 100%).

(±)-5-Bromo-3a,4,5,13b-tetrahydrochryseno[1,2-*d*][1,3]dioxol-2-one 14

A mixture of the cyclic carbonate **13** (0.170 g, 0.58 mmol), NBS (0.105 g, 0.59 mmol) and AIBN (0.005 g) in carbon tetra-

chloride (25 cm^3) was maintained at 60 °C using a heat lamp under an atmosphere of nitrogen until a precipitate of succinimide formed (*ca.* 1 h). Activated charcoal (0.020 g) was added and the reaction mixture was stirred, filtered and evaporated to dryness to give a mixture of bromo carbonate diastereoisomers **14** (0.174 g, 81%) as a light brown oil. The product **14** was analysed by ^{1}H NMR spectroscopy and used immediately in the next step without purification; δ_H 2.30–2.39 (1 H, m, 4-H), 3.01–3.09 (1 H, m, 4-H′), 5.50–5.58 (1 H, m, 3a-H), 5.88 (1 H, d, $J_{3a,13a}$ 7.6, 13b-H), 6.05 (1 H, dd, $J_{4,5} = J_{4',5}$ 3.2, 5-H), 7.60–7.73 (3 H, m, 9-H, 10-H, 13-H), 7.89–8.01 (3 H, m, 6-H, 7-H, 8-H), 8.65 (1 H, d, $J_{12,13}$ 8.3, 12-H), 8.77 (1 H, d, $J_{10,11}$ 8.7, 11-H).

(±)-3a,13b-Dihydrochryseno[1,2-*d*][1,3]dioxolan-2-one 15

Dehydrobromination of the bromo carbonate **14** (0.170 g, 0.46 mmol) using the method previously described for compound **10** gave the title compound **15** (0.126 g, 93%). Purification by flash chromatography on silica gel using CH_2Cl_2–light petroleum (30:70) and subsequent crystallization of the product provided cyclic carbonate **15**, mp 248–258 °C (decomp.) (from CH_2Cl_2–methanol) (Found: C, 78.90; H, 4.03. $C_{19}H_{12}O_3$ requires C, 79.14; H, 4.19%); δ_H 5.58–5.61 (1 H, ddd, $J_{3a,13b}$ 8.8, $J_{3a,4}$ 3.3, $J_{3a,5}$ 1.8, 3a-H), 5.89 (1 H, d, $J_{3a,13b}$ 8.8, 13b-H), 6.05 (1 H, dd, $J_{4,5}$ 10.3, $J_{4,3a}$ 3.2, 4-H), 7.49 (1 H, d, $J_{5,4}$ 10.6, 5-H), 7.59–7.64 (3 H, m, 9-H, 10-H, 13-H), 7.79 (1 H, d, $J_{7,6}$ 9.3, 7-H), 7.85 (1 H, dd, $J_{8,9}$ 8.0, $J_{8,10}$ 1.8, 8-H), 8.02 (1 H, d, $J_{6,7}$ 9.3, 6-H), 8.62 (1 H, d, $J_{12,13}$ 8.6, 12-H), 8.66 (1 H, d, $J_{10,11}$ 8.6, 11-H); ν_{max}(KBr)/cm^{-1} 1796 (C=O); *m/z* (EI) 244 ($M^+ - CO_2$, 23%).

(±)-*cis*-1,2-Dihydroxy-1,2-dihydrochrysene 5

cis-Dihydrodiol **5** was obtained by treatment of cyclic carbonate **15** (0.050 g, 0.17 mmol) with potassium carbonate (0.01 g) in THF (2 cm^3), methanol (3 cm^3) and water (1 cm^3) using the method described for hydrolysis of carbonate **11**. Purification by PLC using $CHCl_3$–methanol (95:5) and crystallization gave pure *cis*-1,2-dihydroxy-1,2-dihydrochrysene **5** (0.01 g, 24%), colourless crystals, mp 245–246 °C (decomp.) (from CH_2Cl_2–methanol) (Found: M^+, 262.09717. $C_{18}H_{14}O_2$ requires *M*, 262.09937); *m/z* (EI) 262 (M^+, 1.5%), 244 ($M^+ - H_2O$, 100). See Table 1 for ^{1} NMR data.

(±)-*cis*-1,2-Dihydroxy-1,2,3,4-tetrahydrochrysene 12

A solution of 3,4-dihydrochrysene (0.178 g), osmium tetraoxide (0.217 g) in dry pyridine (15 cm^3) was stirred at room temperature for 30 min. Sodium hydrogen sulfite (0.300 g) and water (5 cm^3) were added, and the mixture was stirred for 1 h. Work-up provided colourless prisms, mp 227 °C (acetone) (Found: C, 81.85; H, 6.1. $C_{18}H_{16}O_2$ requires C, 81.8; H, 6.1%); ^{1}H NMR data are shown in Table 1; *m/z* (EI) 264 (M^+, 60%), 246 ($M^+ - H_2O$, 60). A similar approach provided racemic *cis*-tetrahydrodiol **6**.

(±)-*cis*-1,2-Diacetoxy-1,2,3,4-tetrahydrochrysene 16

A mixture of the *cis*-1,2-tetrahydrodiol **12** (0.132 g), acetic anhydride (0.3 cm^3) and dry pyridine (1 cm^3) was allowed to stand at room temperature in the dark for 18 h. Evaporation *in vacuo* gave a crystalline residue which was taken up in $CHCl_3$. Standard work-up provided colourless crystals of diacetate **16** (0.170 g, 98%) which were recrystallized from diethyl ether to give colourless leaflets, mp 218–219 °C (Found: C, 75.8; H, 5.8. $C_{22}H_{20}O_4$ requires C, 75.85; H, 5.8%); δ_H(500 MHz, $[CD_3]_2CO$–CD_3OD) 2.22 (1 H, m, 3_{ax}-H), 2.85 (1 H, m, 3_{eq}-H), 3.15 (1 H, m, 4_{ax}-H), 3.45 (1 H, dt, $J_{4eq,4ax}$ 17.2, $J_{3ax,4eq} = J_{3eq,4eq}$ 6.2, 4_{eq}-H), 4.10 (1 H, m, 2-H), 4.82 (1 H, d, $J_{1,2}$ 3.7, 1-H), 7.62 (1 H, dd, $J_{7,8}$ 8.1, $J_{8,9}$ 8.2, 8-H), 7.69 (1 H, dd, $J_{8,9}$ 8.2, $J_{9,10}$ 8.4, 9-H), 7.78 (1 H, d, $J_{11,12}$ 8.4, 12-H), 7.88 (1 H, d, $J_{5,6}$ 9.15, 6-H), 7.98 (1 H, dd, $J_{7,8}$ 8.1, $J_{7,9}$ 0.8, 7-H), 8.05 (1 H, d, $J_{5,6}$ 9.2, 5-H), 8.79 (1 H, d, $J_{11,12}$ 8.8, 11-H) and 8.42 (1 H, br d, $J_{10,9}$ 8.4, 10-H); *m/z* (EI) 348 (M^+, 20%), 288 ($M^+ - CH_3COOH$, 10), 246 ($M^+ - 60 - CH_2CO$, 50).

(±)-*cis*-1,2-Diacetoxy-4-bromo-1,2,3,4-tetrahydrochrysene 17a, 17b†

A mixture of CCl_4 (50 cm^3), *N*-bromosuccinimide (0.025 g), *cis*-1,2-tetrahydroacetate **16** (0.050 g) and α,α′-azoisobutyronitrile was maintained at ~50 °C with a heat lamp for 30 min while a stream of N_2 was passed through the solution. The reaction mixture was cooled and filtered, and the CCl_4 was removed under reduced pressure to yield colourless crystals (0.060 g, 98%). The product was shown to be a mixture of two isomeric bromides at C-4 in a ratio of 9:1 as detected by HPLC and NMR analysis. A portion of the above mixture was separated by HPLC on a DuPont Zorbax Sil column (9.5 × 250 mm) using 6% EtOAc in hexane at a flow rate of 15 cm^3 min^{-1} (detected at 300 nm) to give a major cis-1R*,2S*-*diacetoxy*-4S*-*bromo*-1,2,3,4-*tetrahydrochrysene* **17a** (k' = 5.17) as colourless prisms, mp 132 °C (diethyl ether–light petroleum) (Found: C, 61.3; H, 4.5. $C_{22}H_{19}O_4Br$ requires C, 61.2; H, 4.5%); δ_H(500 MHz, $CDCl_3$) 2.62 (1 H, td, $J_{2,3eq} = J_{3eq,4}$ 3.8, $J_{3eq,3ax}$ 14.1, 3_{eq}-H), 2.90 (1 H, ddd, $J_{2,3ax}$ 12.2, $J_{3ax,4}$ 3.7, $J_{3ax,3eq}$ 14.1, 3_{ax}-H), 5.90 (1 H, td, $J_{1,2} = J_{2,3eq}$ 3.8, $J_{2,3ax}$ 12.2, 2-H), 6.25 (1 H, t, $J_{4,3eq}$ 3.8, $J_{4,3ax}$ 3.7, 4-H), 6.56 (1 H, d, $J_{1,2}$ 3.8, 1-H), 7.50 (1 H, d, $J_{11,12}$ 8.5, 12-H), 7.67 (2 H, m, 8-H and 9-H), 7.92 (1 H, br d, $J_{7,8}$ 8.0, 7-H), 7.95 (1 H, d, $J_{6,5}$ 9.2, 6-H), 8.15 (1 H, d, $J_{5,6}$ 9.2, 5-H), 8.65 (1 H, br d, $J_{9,10}$ 7.9, 10-H) and 8.73 (1 H, d, $J_{11,12}$ 8.5, 11-H) and a minor cis-1R*,2S*-*diacetoxy*-4R*-*bromo*-1,2,3,4-*tetrahydrochrysene* **17b** (k' = 5.67), as colourless prisms, mp 126 °C (diethyl ether–light petroleum) (Found: M^+, 428.0449 and 426.0467. $C_{22}H_{19}O_4Br$ requires *M*, 428.0473 and 426.0489); δ_H(500 MHz, $CDCl_3$) 2.91 (1 H, ddd, $J_{2,3ax}$ 3.5, $J_{3ax,4}$ 7.0, $J_{3ax,3eq}$ 15.3, 3_{ax}-H), 2.21 (1 H, ddd, $J_{3eq,4}$ 3.9, $J_{2,3eq}$ 8.0, $J_{3ax,3eq}$ 15.3, 3_{eq}-H), 5.43 (1 H, dt, $J_{2,3eq}$ 8.0, $J_{1,2} = J_{2,3ax}$ 3.5, 2-H), 6.13 (1 H, dd, $J_{4,3eq}$ 3.9, $J_{4,3ax}$ 7.0, 4-H), 6.24 (1 H, d, $J_{1,2}$ 3.5, 1-H), 7.55 (1 H, d, $J_{11,12}$ 8.5, 12-H), 7.70 (2 H, m, 8-H and 9-H), 7.95 (2 H, m, 6-H and 7-H), 8.15 (1 H, d, $J_{5,6}$ 9.2, 5-H), 8.65 (1 H, br d, $J_{9,10}$ 7.9, 10-H) and 8.73 (1 H, d, $J_{11,12}$ 8.5, 11-H). Since both the isomers gave the expected dihydrodiol diacetate **18** by the following dehydrobromination, the isomeric mixture was used without purification.

(±)-*cis*-1,2-Dihydroxy-1,2-dihydrochrysene 5

A mixture of the above isomeric bromides **17a**, **17b** (0.05 g), sodium hydrogen carbonate (0.25 g), triethylamine (0.10 cm^3) and xylene (75 cm^3) was refluxed under stirring for 2 h. The mixture was filtered and the filtrate was evaporated to leave a yellow oil which was purified by HPLC on a DuPont Zorbax Sil column (9.5 × 250 mm) using 1% EtOAc in CH_2Cl_2 at a flow rate of 12 cm^3 min^{-1}. Evaporation of the major fraction (>95%, k' = 5.33) afforded the objective dihydrodiol diacetate **18** as colourless needles (0.035 g, 83%), mp 155–157 °C (diethyl ether) (Found: C, 76.2; H, 5.3. $C_{22}H_{18}O_4$ requires C, 76.3; H, 5.2%); δ_H(500 MHz, $CDCl_3$) 2.07 (3 H, s, OCOMe), 2.15 (3 H, s, OCOMe), 5.80 (1 H, dd, $J_{2,3}$ 3.9, $J_{1,2}$ 4.8, 2-H), 6.22 (1 H, dd, $J_{3,4}$ 10.2, $J_{2,3}$ 3.85, 3-H), 6.29 (1 H, d, $J_{1,2}$ 4.8, 1-H), 7.51 (1 H, d, $J_{4,3}$ 10.2, 4-H), 7.62 (1 H, m, 8-H), 7.67 (1 H, m, 9-H), 7.69 (1 H, d, $J_{11,12}$ 8.5, 12-H), 7.82 (1 H, d, $J_{5,6}$ 9.3, 6-H), 7.80 (1 H, dd, $J_{7,8}$ 9.0, $J_{7,9}$ 1.0, 7-H), 8.09 (1 H, d, $J_{5,6}$ 9.3, 5-H), 8.67 (1 H, d, $J_{11,12}$ 8.5, 11-H) and 8.69 (1 H, br d, $J_{9,10}$ 8.0, 10-H); *m/z* (EI) 346 (M^+, 10%), 286 ($M^+ - CH_3COOH$, 15) and 244 ($M^+ - 60 - CH_2CO$, 100).

The *cis*-1,2-dihydrodiol diacetate **18** (0.030 g in 20 cm^3 of NH_3 saturated MeOH and 3 cm^3 of THF, room temperature 10 h) was then hydrolysed to the free racemic dihydrodiol **5**. Standard work-up and trituration with diethyl ether gave diol **5** as colourless prisms (0.025 g, 94%), mp 245–246 °C (decomp.);

† In compound **17**, *cis* refers to the relative stereochemistry of the two acetoxy substituents.

for ^{1}H NMR data, see Table 1 (Found: C, 82.4; H, 5.35. $C_{18}H_{14}O_2$ requires C, 82.4; H, 5.4%); *m/z* (EI) 262 (M^+, 65%), 244 ($M^+ - H_2O$, 40) 231 ($M^+ - H_2O - OH$, 30), 216 (100); λ_{max}(MeOH)/nm 220 (ε/dm^3 mol^{-1} cm^{-1} 66 100).

(−)-(1*R*,2*R*)-*trans*-2-Bromo-1-(ethoxycarbonylacetoxy)-1,2,3,4-tetrahydrochrysene 20

A solution of optically pure (+)-(1*R*,2*R*)-*trans*-2-bromo-1-hydroxy-1,2,3,4-tetrahydrochrysene **19** {0.300 g, 92 mmol, $[\alpha]_D$ +27 (*c* 0.2, $CHCl_3$), resolved as the bromoMTPA ester} in dry diethyl ether (20 cm^3) was added to a stirred solution of ethyl malonyl chloride (0.11 cm^3, 1.0 mmol) and pyridine (0.5 cm^3) in dry diethyl ether (40 cm^3) under nitrogen at room temperature. The mixture was refluxed for 1 h and concentrated under reduced pressure to yield crude *trans*-bromo ester **20** (0.397 g, 98%). A portion of the crude product (0.05 g) was purified by PLC using CH_2Cl_2–light petroleum (50:50); mp 106 °C (from CH_2Cl_2–pentane); $[\alpha]_D$ −32.3 (*c* 1.0, $CHCl_3$) (Found: C, 62.35; H, 4.6. $C_{23}H_{21}BrO_4$ requires C, 62.7; H, 4.8%); δ_H 1.24 (3 H, t, *J* 7.1, OCH_2CH_3), 2.45–2.59 (1 H, m, 3-H), 2.60–2.63 (1 H, m, 3′-H), 3.38–3.42 (2 H, m, 4-H), 3.44 (2 H, s, $COCH_2$), 4.41–4.21 (2 H, q, *J* 7.1, OCH_2CH_3), 4.62–4.64 (1 H, m, 2-H), 6.37 (1 H, d, $J_{1,2}$ 4, 1-H), 7.54 (1 H, d, $J_{11,12}$ 8.7, 12-H), 7.58–7.69 (2 H, m, 8-H, 9-H), 7.83 (1 H, d, $J_{5,6}$ 9.3, 6-H), 7.90 (1 H, d, $J_{7,8}$ 7.5, 7-H), 7.97 (1 H, d, $J_{5,6}$ 9,3, 5-H), 8.60 (1 H, d, $J_{11,12}$ 8.7, 11-H) and 8.68 (1 H, d, $J_{9,10}$ 9.0, 10-H); ν_{max}(KBr)/cm^{-1} 1752 and 1724 (C=O); *m/z* (EI) 442 [M(^{81}Br)$^+$, 8%] and 440 [M(^{79}Br)$^+$, 8].

(*E*)/(*Z*)-2-(Ethoxycarbonylmethylidene)-3a,4,5,13b-tetrahydrochryseno[1,2-*d*][1,3]dioxole 21a and 21b

A solution of crude *trans*-bromo ester **20** (0.390 g, 0.88 mmol, $[\alpha]_D$ −32.3) in dry THF (25 cm^3) was added to a stirred suspension of sodium hydride (60% dispersion in oil, 10 mmol) in dry THF (30 cm^3) under nitrogen at 0 °C. Stirring was continued for 12 h at room temperature. Water (1 cm^3) was added to destroy excess sodium hydride, and the reaction mixture was filtered through a pad of $MgSO_4$. The organic filtrate was concentrated under reduced pressure to provide as a blue–green oil **21a** and **21b** (0.340 g, 97%). The ^{1}H NMR spectrum indicated the presence of a mixture of *E*/*Z* isomers which decomposed during attempted purification by PLC using silica gel. The *E*/*Z* isomers were distinguishable from both the vinyl signals (δ 4.44 and 4.49) and the benzylic signals (δ 5.76, $J_{3a,13b}$ 7.0, 5.96, $J_{3a,13b}$ 7.2); the crude product mixture was used directly in the next step.

(1*R*,2*S*)-2-(Ethoxycarbonylacetoxy)-1-hydroxy-1,2,3,4-tetrahydrochrysene 22a and (1*R*,2*S*)-1-(ethoxycarbonylacetoxy)-2-hydroxy-1,2,3,4-tetrahydrochrysene 22b

Dilute hydrochloric acid (1 M, 10 cm^3) was added to a solution of the ketene acetal mixture **21a** and **21b** (0.310 g, 0.86 mmol) in THF (45 cm^3), and the resulting mixture was stirred at room temperature for 30 min. This gave a mixture of monoethyl malonyl esters **22a** and **22b** of the enantiopure diol. Purification by flash chromatography (silica gel, CH_2Cl_2) and crystallization gave a mixture of **22a** and **22b** in a ratio of (29:71) (0.246 g, 76%), light brown crystals, mp 95–106 °C (from CH_2Cl_2–pentane); $[\alpha]_D$ −6.9 (*c* 0.30, $CHCl_3$) (Found: M^+, 378.14634. $C_{23}H_{22}O_5$ requires *M*, 378.14671); δ_H 1.12–1.26 (6 H, m, CH_3^a and CH_3^b), 2.10–2.19 (4 H, m, 3-H^a and 3-H^b), 3.11–3.53 (OH, m, $OCCH_2^a$, $OCCH_2^b$, 4-H^a and 4-H^b), 4.07–4.23 (5 H, m, $OCH_2^aCH_3$, $OCH_2^bCH_3$ and 2-H^b), 5.03 (1 H, d, $J_{1,2}$ 3.5, 1-H^a), 5.35–5.38 (1 H, m, 2-H^a), 6.29 (1 H, d, $J_{1,2}$ 3.5, 1-H^b), 7.52–7.91 (12 H, m, Ar-H^a and Ar-H^b) and 8.51–8.64 (4 H, m, Ar-H^a and Ar-H^b); ν_{max}(KBr)/cm^{-1} 3485 (OH), 1725 (C=O); *m/z* (EI) 378 (M^+, 21%) and 246 ($M^+ - HCO_2CH_2CO_2C_2H_5$, 100).

(−)-(1*R*,2*S*)-*cis*-1,2-Dihydroxy-1,2,3,4-tetrahydrochrysene 12

The monoethyl malonyl ester mixture **22a**, **22b** (0.240 g, 0.63 mmol, $[\alpha]_D$ −6.9) was dissolved in THF (20 cm^3) and methanol (5 cm^3). Triethylamine (2 cm^3) was added with stirring over 10 min, and the solution was left for 14 h at room temperature. Most of the solvent was removed *in vacuo*, and $CHCl_3$ (25 cm^3) was added. Standard work-up and purification by PLC using CH_2Cl_2–methanol (90:10), provided *cis*-tetrahydrodiol **12** (0.164 g, 97%), mp 169 °C (from $CHCl_3$–methanol); $[\alpha]_D$ −29 (*c* 0.4, THF) (lit.,[10] mp 166–168 °C, $[\alpha]_D$ −26).

(+)-(3a*S*,13b*R*)-3a,13b-Dihydrochryseno[1,2-*d*][1,3]dioxol-2-one 15

The bromination–dehydrobromination sequence used in the conversion of racemic compound **13** to carbonates **14** and **15**, was applied to the conversion of the (3a*S*,13b*R*)-enantiomer of cyclic tetrahydrocarbonate **13** (0.150 g, 0.51 mmol, $[\alpha]_D$ +160, THF) to produce the (3a*S*,13b*R*)-dihydrocarbonate **15**. Crystallization provided a pure sample of compound **15** (0.117 g, 79%), white crystals, mp 248–258 °C (decomp.) (from CH_2Cl_2–methanol); $[\alpha]_D$ +237 (*c* 0.52, pyridine).

(+)-*cis*-(1*R*,2*S*)-1,2-Dihydroxy-1,2-dihydrochrysene 5

Using identical conditions to those used for the hydrolysis of racemic compounds, the (+)-cyclic carbonate **15** (0.110 g, 0.38 mmol, $[\alpha]_D$ +237) was hydrolysed. Purification by PLC using $CHCl_3$–methanol (95:5) and crystallization provided pure (+)-(1*R*,2*S*)-1,2-dihydroxy-1,2-dihydrochrysene **5** (0.022 g, 22%), colourless crystals, mp 193–196 °C (from CH_2Cl_2–methanol); $[\alpha]_D$ +74 (*c* 0.50, THF). See Table 1 for ^{1}H NMR data.

Acknowledgements

This work was supported by the BBSRC (NDS), the Queen's University of Belfast (R. A.) and US Public Health Service grant GM29909 from the National Institute of General Medical Sciences (D. T. G.). S. M. R. was supported by predoctoral fellowships from the Center for Biocatalysis and Bioprocessing at the University of Iowa and training grant T32GM08365 from the National Institute of General Medical Sciences, US PHS.

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Paper 6/06686K
Received 10*th October* 1996
Accepted 11*th February* 1997