Nucleophilic substitution of protected β-bromoethyl cyclohexadiene-*cis*-diol as an alternative to direct microbial oxidation of β-functionalized phenethyl substrates

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(5*S*,6*R*)-1-(2-Bromoethyl)-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 6 is subjected to nucleophilic substitution reaction using C-, N-, O- and S-based nucleophiles. The yields of the products are compared with those obtained by direct microbial oxidation of the corresponding aromatic substrates. The enantiomeric excess and isolated yield for each compound are reported.

Introduction

The utility of optically pure cyclohexadiene-*cis*-diol metabolites derived from aromatic compounds *via* biocatalysis has been amply demonstrated in many syntheses of complex molecules.[1] The enzyme toluene dioxygenase, responsible for this unique transformation, recognizes a wide variety of substrates but maintains surprising specificity with respect to the regiochemistry and absolute stereochemistry of the *cis*-diol unit. As evidenced by examination of the structures of more than 200 known metabolites,[2] there are some limitations of this transformation: for example, single ring aromatics substituted with two or more functionalities tend to be processed by the enzyme in far lower yields than monofunctional substrates. Similarly, the presence of certain functionalities (*e.g.* OH, NH_2) slows down or even inhibits the recognition and the subsequent oxidation of such substrates.

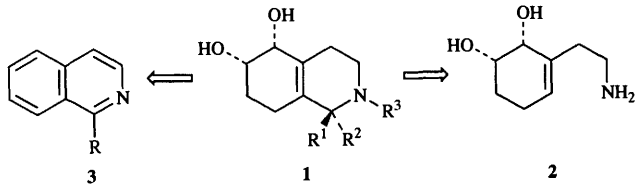
In connection with a project concerned with the synthesis of enantiomerically pure isoquinoline derivatives of type **1**, we first considered the availability of such compounds *via* direct enzymatic oxygenation of isoquinoline **3** with either naphthalene or toluene dioxygenase-carrying organisms (Fig. 1). Such compounds have been reported in the literature by Boyd,[3a] who also noted that the oxidations are not enantiospecific and the diols derived from **3** are more unstable to manipulations than other known metabolites.[3b]

Limitations such as these prompted us to investigate a supplemental chemical strategy[4] in order to obtain cyclohexadiene-*cis*-diols, corresponding to oxidation products of various β-substituted phenethyl substrates, that would be useful in natural product synthesis and that could be converted, in the case of amine **2**, to isoquinoline **1**, based on adjustment of procedures reported by Grewe for cyclization of β-cyclohexenyl ethyl amines.[3c]

Here we report a comparison between direct oxidation of various β-substituted phenethyl derivatives and their synthesis from the protected 2-bromoethylcyclohexadiene-*cis*-diol **6** which is produced in exceptionally high space/time yields from phenylethyl bromide **4**.[5] The availability of bromoethyl acetonide **6** allowed the direct synthesis of standard samples of the presumed metabolites and therefore offered the unique opportunity to investigate the previously unknown bio-oxidation of β-substituted phenethyl series of substrates.

Results and discussion

The aromatic substrates **4**, **7a–k** were either commercially available (**4**, **7a–c**, **e**, **h** and **j**), or synthesized by simple nucleophilic substitution of bromoethylbenzene (**7d**, **f**, **g**, **i** and **k**).



3 1 2

R, R^1, R^2, R^3 = H, Me, Allyl Alkoxycarbonyl

Fig. 1 Biocatalytic design of the synthesis of *cis*-5,6-dihydroxy-1,2,3,4,5,6,7,8-octahydroisoquinoline **1**

Shake-flask experiments with each aromatic compound were conducted using *E. coli* JM 109 (pDTG601).[6] The isolated diols **8** were converted to the corresponding acetonides **9** and compared to the standards, prepared from bromoethyl acetonide **6**.

Acetonide **6** was converted to the series of compounds **9a**, **b**, **d**, **f–i**, and **k** (Scheme 1) by stirring **6** in DMF in the presence of the appropriate nucleophile.[7] The alcohol **9c** and primary amine **9e** were synthesized by reduction of the corresponding acetate **9b** and azide **9d**, with lithium aluminium hydride and triphenylphosphine, respectively.[8,9]

Yields shown in Table 1 are representative of a three-step synthesis of **9a–k** starting from β-bromoethylbenzene by either route. Metabolites **9** are usually obtained in higher overall yield by the indirect method from acetonide **6**. In extreme cases (**9e–h**), this is the only way to obtain the desired compound. On the other hand, the enantiomeric purity of protected diol derivatives **9**, obtained *via* bio-oxidation of aromatic substrates **7**, was higher than that determined for products of substitution of acetonide **6**, which had constant ee of 96%.

The hydroxy derivative **9c** was obtained by $LiAlH_4$ reduction rather than base catalysed hydrolysis of **9b**, since the latter induced rapid elimination to produce the corresponding triene. Similar difficulties were encountered in the case of diethylamino derivative **9f** and phthalimido derivative **9g**, upon attempted column chromatography on silica gel and work-up at room temperature, respectively.

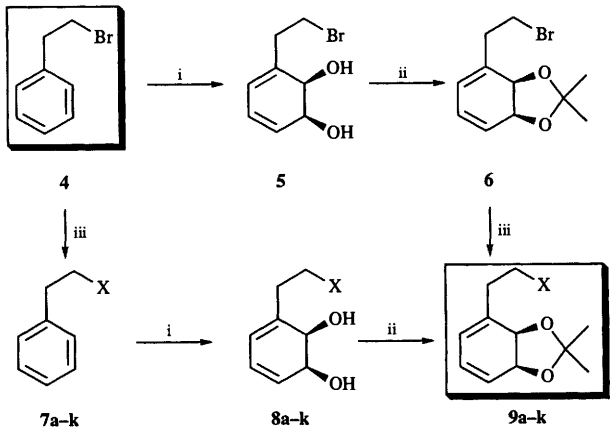
Isothiocyanato derivative **9j** was not available by direct nucleophilic substitution of bromoethyl acetonide **6** with thiocyanate ion, and instead, as expected,[7] **9i** was the sole product.

Finally, the phenyl sulfanyl derivative **9k** synthesized by substitution was identical to the metabolite from bio-oxidation indicating that dihydroxylation of the sulfur-substituted aromatic ring did not take place.

Table 1 Comparison of isolated overall yields of substituted phenethyl diol acetonides **9a–k** obtained *via* substitution and biotransformation

		Substitution route		Biotransformation route	
Entry	X	Yield (%)[a]	$[\alpha]_D^{28}$ (96% ee)	Yield (%)[b]	$[\alpha]_D^{28}$ (% ee)[c]
6[d]	Br	—		67	—
9a	CN	53	+68.8	22	+69.0 (96)
9b	OAc	36	+73.9	11	+78.4 (99)
9c	OH	16	+39.2	4	+37.2 (94)
9d	N_3	53	+118.4	40	+127.8 (100)
9e	NH_2	49	+88.9	0[e]	—
9f	NEt_2	52	+74.1	0[e]	—
9g	phthalimide	41	+213.2	0[e]	—
9h	SH	28	+178.6	0[e]	—
9i	SCN	31	+105.1	27	+101.0 (94)
9j	NCS	—	—	4	+99.3 (—)
9k	SPh	44	+106.3	16	+110.9 (98)

[a] Yield (%) of oxidation of bromoethylbenzene (67%) multiplied by the yield (%) of the substitution reaction. [b] Yield (%) of oxidation multiplied by yield (%) of protection (set at 95%). [c] Calculated based on the %ee of **6**. [d] The %ee of this compound was 96% (see ref. 4). [e] Non-substrates.



a X = CN
b X = OAc
c X = OH
d X = N_3
e X = NH_2
f X = NEt_2
g X = $N(CO)_2Ph$
h X = SH
i X = SCN
j X = NCS
k X = SPh

Scheme 1 *Reagents and conditions:* i, (**7e–h** not substrates) JM 109 (*E. coli*); ii, DMP, *p*TSA, CH_2Cl_2, room temp.; iii, (preparation of **7d, f, g, i, k, 9a, b, d, f–i, k**), Nu^-, DMF, room temp.; (preparation of **9c**) NaOAc, DMF, room temp.; LAH, THF; (preparation of **9e**) NaN_3, DMF, room temp.; PPh_3, THF, H_2O; compound **9j** could not be obtained *via* nucleophilic substitution

Conclusion

Preparation of the series of compounds **9** was achieved in greater overall yields using nucleophilic substitution of bromoethylbenzene diol acetonide. Compounds of type **7**, where X = NH_2, NEt_2, *N*-phthalimido and SH have been shown not to be substrates for toluene dioxygenase and the indirect route to these compounds represents an important enhancement of the available pool of metabolites. The synthesis of functionalized β-phenethyl substrates provides a greater variety of available synthons and may become useful as means of access to the aforementioned isoquinoline derivatives for alkaloid synthesis.

Experimental

General

NMR spectra were determined in $CDCl_3$ on a Gemini 300, VXR 300 or Bruker WP-270 MHz spectrometer. Coupling constants (*J*) are given in Hertz. ^{13}C multiplicities were determined by APT experiments. IR spectra were obtained on a Perkin-Elmer 1600 Series instrument. Anhydrous DMF was purchased from Aldrich. All other solvents were dried according to standard procedures. Flash column chromatography was performed on Fisher silica gel (grade 60, 200–425 mesh). Melting points were observed on a Thomas Hoover Unimelt apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer model 341 polarimeter. Mass spectral data were obtained on a Finnigan Mat 95 Q instrument, using chemical ionization or fast atom bombardment. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Spectral characteristics of acetonides **9a–d, j** and **k**, prepared *via* bio-oxidation of β-substituted ethylbenzenes **7**, followed by protection, were found to be identical to those prepared by nucleophilic substitution of bromoethylcyclohexadienediol acetonide **6**.

The synthesized dienes are prone to decomposition, and no satisfactory combustion analysis data could be obtained for most of them, although they are suitable for immediate further use.

Synthesis of the bio-oxidation substrates 7d,f,g,i and k

(2-Azidoethyl)benzene 7d. A solution of phenethyl bromide **4** (1 g, 5.4 mmol) and NaN_3 (1.05 g, 16.2 mmol) in DMF (5 ml) was stirred at room temperature, and the progress of the reaction was monitored by TLC. After 12 h the reaction mixture was diluted with water (5 ml), extracted with EtOAc (3 × 10 ml) and dried (Na_2SO_4). Evaporation of the solvent and concentration *in vacuo* afforded **7d** (742 mg, 93%) as an oil, which gave a satisfactory ^1H NMR spectrum, identical to that in the literature;[10] $\delta_H(CDCl_3)$ 2.82 (2 H, t, *J* 7.1, CH_2), 3.42 (2 H, t, *J* 7.1, CH_2), 7.21 (5 H, m, ArH).

[2-(*N*,*N*-Diethylamino)ethyl]benzene 7f. Diethylamine (1.26 ml, 12.2 mmol) was added to a stirred solution of phenethyl bromide **4** (1.11 ml, 8.1 mmol) in DMF (10 ml). After overnight stirring at room temperature, 10% aq. HCl (5 ml) was added to the reaction mixture and extracted with diethyl ether (2 × 10 ml). The aqueous layer was made alkaline (1 M NaOH) and extracted with EtOAc (2 × 10 ml). The combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo* to give **7f** (1.12 g, 78%) as a yellow oil, whose ^1H NMR spectrum was

identical to that in the literature;[11] $\delta_H(CDCl_3)$ 1.08 (6 H, t, *J* 7.2, 2 CH_3), 2.62 (4 H, q, *J* 7.2, 2 CH_2), 2.73 (4 H, m, 2 CH_2), 7.25 (5 H, m, ArH).

(2-Phthalimidoethyl)benzene 7g. Potassium phthalimide (2 g, 10.8 mmol) was added to stirred solution of phenethyl bromide **4** in DMF (10 ml). After 12 h, the mixture was diluted with water (5 ml) and the crude product was filtered off. Recrystallization from EtOH afforded **7g** (1.08, 61%) as a white solid, mp 130–131 °C (lit.,[12] 131–132 °C); $\delta_H(CDCl_3)$ 2.99 (2 H, t, *J* 7.8, CH_2), 3.92 (2 H, t, *J* 7.8, CH_2N), 7.26 (5 H, m, ArH), 7.67 (2 H, m, ArH), 7.80 (2 H, m, ArH); $\delta_C(CDCl_3)$ 34.7, 39.3, 123.2, 126.7, 128.6, 128.9, 132.2, 133.9, 138.0, 169.0.

(2-Thiocyanatoethyl)benzene 7i. Potassium thiocyanate (1.57 g, 16.2 mmol) was added to a stirrred solution of phenethyl bromide **4** (1 g, 5.4 mmol) in DMF (5 ml). After 12 h the reaction mixture was diluted with water (5 ml) and extracted with EtOAc (2 × 10 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to afford **7i** (750 mg, 85%) as an oil, the ^1H NMR spectrum of which was identical to that published in the literature;[13a,b] $\delta_H(CDCl_3)$ 3.00 (4 H, m, 2 CH_2), 7.17 (5 H, m, ArH).

(2-Phenylsulfanylethyl)benzene 7k. A mixture of thiophenol (0.67 ml, 6.48 mmol), potassium hydroxide (362 mg, 6.48 mmol) and phenethyl bromide **4** (1 g, 5.4 mmol) in DMF (10 ml) was stirred for 6 h. Water (5 ml) was added and the mixture was extracted with EtOAc (2 × 10 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated. Chromatography on silica gel (1 : 1 hexanes–EtOAc) afforded **7k** as a yellow oil (856 mg, 74%), whose ^1H NMR spectrum was identical with the literature;[14] $\delta_H(CDCl_3)$ 2.80 (2 H, t, *J* 7.6, CH_2), 3.22 (2 H, t, *J* 7.6, CH_2), 7.26 (5 H, m, ArH).

General procedure for shake-flask experiments[15]

Screening for acceptable substrates. JM 109 (pDTG601) cells were grown at 30 °C in mineral salts broth (MSB) solution supplemented with 0.2% glucose, 1 mM thiamine hydrochloride, 50 mg l^{-1} ampicillin and 10 mg l^{-1} isopropyl β-D-thiogluco-pyranoside (IPTG). Biotransformations were carried out at 35 °C in 0.10 M KPO_4 buffer at pH 7.0, maintained by manual addition of 10 M NaOH.

The preculture solution was prepared (100 ml solution containing 1 g tryptone, 500 mg yeast extract, 500 mg NaCl supplemented with 5 mg ampicillin). This was divided into four 250 ml fernbachs and autoclaved at 120 °C for 20 min. Each fernbach was inoculated with JM 109 (pDTG601) in cryovials stored at −78 °C and placed in the rotary shaker at 120 rpm (30 °C) overnight. MSB solution (2 l) supplemented with glucose (4 g), thiamine hydrochloride (740 mg), ampicillin (100 mg) and IPTG (20 mg) was divided into four 2.8 l fernbachs. The precultures that were grown overnight were transferred to these fernbachs and replaced in the orbital shaker at 30 °C and allowed to grow for another 20 h. The culture media were centrifuged at 5000 rpm at 10 °C for 15 min. The cells were resuspended to a final O.D. of 5.0 (λ_{640}) in 50 ml 0.10 M KPO_4 buffer (pH 7.0) supplemented with 0.2% glucose and 0.1% substrate. The pH of the biotransformation was checked every hour and kept at 7.0. The production of the diol was observed by TLC and UV absorbance of the broth in the region of 260 nm. Biotransformations were run for 20 h after which the cells were removed by centrifugation.

Scale-up of bio-oxidation. Those substrates that gave positive results from screening were taken on to this step. In an attempt to make enough of the diol metabolites, higher cell densities were obtained by enriching the culture media. The preculture concentration was increased to three times more concentrated. Similarly, the glucose supplement was increased to five times higher while the rest of the culture medium components were taken as similar to the small-scale oxidations. Biotransformations were carried out in 300 ml KPO_4 buffer with an O.D. of 10 (λ_{640}).

Isolation of the diols. The microbial oxidation broth (300 ml) was centrifuged at 7000 rpm for 20 min, the supernatant solution was decanted and was extracted with EtOAc (3 × 100 ml). The extract was dried with Na_2SO_4 and concentrated using a rotary evaporator. The crude extract was chromatographed on silica gel (1 : 1 hexanes–EtOAc) to afford the desired diols **8a–d, i–k** as white solids or very viscous creamish oils.

(5*S*,6*R*)-1-(2-Cyanoethyl)-5,6-dihydroxycyclohexa-1,3-diene 8a. R_f 0.24 (1 : 1 hexanes–EtOAc); mp 43–44 °C; $[\alpha]_D^{28}$ +96.8 (*c* 1.7, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3388, 3037, 2932, 2850, 2242, 1643, 1425, 1075; $\delta_H(CDCl_3)$ 2.64 (4 H, m, 2 CH_2), 3.57 (2 H, exch, 2 OH), 4.14 (1 H, m, CH), 4.31 (1 H, m, CH), 5.90 (2 H, m, 2 C=CH), 6.02 (1 H, dd, *J* 5.1 and 9.5, C=CH); $\delta_C(CDCl_3)$ 16.8 (CH_2), 29.9 (CH_2), 68.5 (CH), 69.9 (CH), 119.9 (C), 121.8 (CH), 124.8 (CH), 125.8 (CH), 137.7 (C); *m*/*z* (CI+) 69 (10%), 107 (20), 148 (100), 165 (10) (HRMS: calc. for $C_9H_{11}NO_2$, 165.0789. Found, 165.085).

(5*S*,6*R*)-1-(2-Acetoxyethyl)-5,6-dihydroxycyclohexa-1,3-diene 8b. R_f 0.15 (1 : 1 hexanes–EtOAc); $[\alpha]_D^{28}$ +40.7 (*c* 2.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3398, 3044, 2915, 1734, 1648, 1380, 1241, 1037; $\delta_H(CDCl_3)$ 2.03 (3 H, s, CH_3), 2.54 (2 H, t, *J* 6.5, CH_2), 2.73 (2 H, exch, 2 OH), 4.26 (4 H, m, CH_2O), 5.73 (1 H, d, *J* 5.2, C=CH), 5.82 (1 H, dd, *J* 3.7 and 9.5, C=CH), 5.90 (1 H, dd, *J* 5.2 and 9.5, C=CH); $\delta_C(CDCl_3)$ 20.9 (CH_3), 33.2 (CH_2), 62.9 (CH_2), 68.2 (CH), 69.9 (CH), 121.5 (CH), 124.6 (CH), 127.9 (CH), 137.5 (C), 171.4 (C); *m*/*z* (CI+) 93 (10%), 121 (100), 138 (90), 163 (20), 180 (40), 198 (20) (HRMS: calc. for $C_{10}H_{14}O_4$, 198.0892. Found, 198.0950).

(5*S*,6*R*)-1-(2-Hydroxyethyl)-5,6-dihydroxycyclohexa-1,3-diene 8c. R_f 0.26 (10 : 1 CH_2Cl_2–MeOH); mp 66–67 °C; $[\alpha]_D^{28}$ +45.4 (*c* 2.2, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3376, 3039, 2927, 1666, 1643, 1402, 1075, 1036; $\delta_H(CD_3OD)$ 2.45 (2 H, m, CH_2), 3.70 (2 H, m, CH_2O), 3.98 (1 H, d, *J* 5.8, CH), 4.22 (1 H, m, CH), 5.74 (1 H, dd, *J* 3.5 and *J* 9.5, C=CH), 5.78 (1 H, d, *J* 5.3, C=CH), 5.88 (1 H, d, *J* 5.3, C=CH); $\delta_C(CD_3OD)$ 40.7 (CH_2), 64.5 (CH_2), 72.8 (CH), 73.3 (CH), 124.9 (CH), 127.7 (CH), 131.7 (CH), 142.2 (C); *m*/*z* (CI+) 156 (5%), 122 (10), 121 (100), 93 (10) (HRMS: calc. for $C_8H_{12}O_3$, 156.0786. Found, 156.0782).

(5*S*,6*R*)-1-(2-Azidoethyl)-5,6-dihydroxycyclohexa-1,3-diene 8d. R_f 0.23 (1 : 1 hexanes–EtOAc); $[\alpha]_D^{28}$ +92.9 (*c* 2.2, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3376, 3038, 2926, 2092, 1647, 1256, 1073; $\delta_H(CDCl_3)$ 2.50 (2 H, m, CH_2), 3.15 (2 H, exch, 2 OH), 3.45 (2 H, t, *J* 6.4, CH_2N), 4.07 (1 H, m, CH), 4.26 (1 H, m, CH), 5.79 (1 H, d, *J* 3.4, C=CH), 5.81 (1 H, dd, *J* 3.4 and 9.6, C=CH), 5.94 (1 H, dd, *J* 5.1 and 9.6, C=CH); $\delta_C(CDCl_3)$ 33.2 (CH_2), 49.9 (CH_2), 68.5 (CH), 69.8 (CH), 121.8 (CH), 124.6 (CH), 128.0 (CH), 137.6 (C); *m*/*z* (CI+) 152 (10%), 154 (100), 155 (10), 164 (10), 181 (10) (HRMS: calc. for $C_8H_{11}N_3O_2$, 181.0851. Found, 181.0850).

(5*S*,6*R*)-1-(2-Thiocyanatoethyl)-5,6-dihydroxycyclohexa-1,3-diene 8i. R_f 0.35 (1 : 2 hexanes–EtOAc); $[\alpha]_D^{28}$ +91.8 (*c* 1.5, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3385, 3045, 2920, 2150, 1650, 1400, 1230, 1160, 990, 800; $\delta_H(CDCl_3)$ 2.71 (2 H, m, CH_2S), 3.14 (2 H, m, CH_2), 3.19 (2 H, exch, 2 OH), 4.07 (1 H, d, *J* 5.6, CH), 4.24 (1 H, s, CH), 5.84 (2 H, m, 2 C=CH), 5.93 (1 H, dd, *J* 5.2 and 9.6, C=CH); $\delta_C(CDCl_3)$ 32.6 (CH_2), 34.4 (CH_2), 68.0 (CH), 69.7 (CH), 112.5 (C), 122.5 (CH), 124.6 (CH), 128.1 (CH), 136.9 (C); *m*/*z* (CI+) 166 (10%), 140 (10), 138 (100) [HRMS: calc. for $C_9H_{11}NO_2$ (M^+ − S), 166.0868. Found, 166.0804].

(5*S*,6*R*)-1-(2-Isothiocyanatoethyl)-5,6-dihydroxycyclohexa-1,3-diene 8j. R_f 0.18 (1 : 1 hexanes–EtOAc); $[\alpha]_D^{28}$ +161.8 (*c* 1.0, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3365, 3039, 2927, 2837, 2174, 2095, 1643, 1340, 1075; $\delta_H(CDCl_3)$ 2.17 (1 H, exch, OH), 2.45 (1 H, exch, OH), 2.62 (2 H, m, CH_2), 3.69 (2 H, m, CH_2N), 4.14 (1 H, s, CH), 4.25 (1 H, s, CH), 5.90 (3 H, m, 3 C=CH); $\delta_C(CDCl_3)$ 34.6 (CH_2), 43.9 (CH_2), 68.0 (CH), 70.2 (CH), 97.8 (C), 122.8 (CH), 125.1 (CH), 128.1 (CH), 136.8 (C); *m*/*z* (CI+) 153 (100%), 121 (70) [HRMS: calc. for $C_9H_{11}NSO_2$ (M^+ − OH), 180.0813. Found, 180.0816].

(5*S*,6*R*)-1-(2-Phenylsulfanylethyl)-5,6-dihydroxycyclohexa-1,3-diene 8k. R_f 0.26 (1:1 hexanes–EtOAc); $[\alpha]_D^{28}$ +80.8 (*c* 4.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3376, 3044, 2926, 2851, 1648, 1584, 1477, 1434, 1074; $\delta_H(CDCl_3)$ 2.13 (1 H, exch, OH), 2.22 (1 H, exch, OH), 2.59 (2 H, m, CH_2), 3.11 (2 H, m, CH_2S), 4.09 (1 H, m, CH), 4.27 (1 H, m, CH), 5.76 (1 H, d, *J* 5.2, C=CH), 5.85 (1 H, dd, *J* 3.3 and 9.2, C=CH), 5.95 (1 H, dd, *J* 5.2 and 9.2, C=CH), 7.27 (5 H, m, ArH); $\delta_C(CDCl_3)$ 32.2 (CH_2), 33.4 (CH_2), 68.5 (CH), 69.9 (CH), 120.9 (CH), 124.7 (CH), 125.9 (CH), 127.8 (CH), 128.7 (CH), 129.1 (CH), 139.5 (C); *m*/*z* (CI+) 248 (10%), 230 (100), 121 (30) (HRMS: calc. for $C_{14}H_{16}SO_2$, 248.0871. Found, 248.087).

Typical procedure for acetonide protection of diols 8

The cyano diol **8a** (15 mg, 0.09 mmol) was dissolved in dry CH_2Cl_2 (5 ml) and DMP (0.5 ml, 4.0 mmol) was added followed by a catalytic amount of *p*-TsOH. The solution was stirred for 5 min at room temperature and diluted with 1 M NaOH (1 ml). The CH_2Cl_2 layer was separated, dried with Na_2SO_4, solvent was removed on the rotary evaporator and the residual oil was dried *in vacuo* to afford 15 mg (93%) of product.

Typical procedure for nucleophilic substitution of bromoethylbenzene diol acetonide

The solution of acetonide **6** (1.10 g, 4.26 mmol) and NaCN (1 g, 20.4 mmol) in DMF (5 ml) was stirred at room temperature. The reaction was monitored by TLC. At the completion of the reaction (typically 12 h), water (5 ml) was added and the mixture was extracted with EtOAc (3 × 10 ml). The extract was dried with Na_2SO_4 and concentrated on a rotary evaporator. The crude product was chromatographed on silica gel (3:1 hexanes–EtOAc) to afford **9a** (667 mg, 76%) as an oil.

(5*S*,6*R*)-1-(2-Cyanoethyl)-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 9a. Chromatography on silica gel (3:1 hexanes–ethyl acetate) afforded **9a** in 76% yield; R_f = 0.48 (3:2 hexanes–EtOAc); $[\alpha]_D^{28}$ +68.8 (*c* 0.48, MeOH) (Found: C, 70.31; H, 7.40; N, 6.88. Calc. for $C_{12}H_{15}NO_2$: C, 70.24; H, 7.32; N, 6.88%); ν_{max}(neat)/cm^{-1} 3005, 3000, 2950, 2900, 2250, 1430, 1375, 1200, 1050, 875; $\delta_H(CDCl_3)$ 1.38 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 2.60 (4 H, m, 2 CH_2), 4.55 (1 H, d, *J* 8.9, CH), 4.69 (1 H, dd, *J* 3.8 and 8.9, CH), 5.87 (2 H, m, 2 C=CH), 6.00 (1 H, m, C=CH); $\delta_C(CDCl_3)$ 24.7 (CH_3), 26.6 (CH_3), 29.7 (C), 70.9 (CH), 72.8 (CH), 118.9 (C), 120.4 (CH), 123.8 (CH), 123.9 (CH), 133.8 (C); *m*/*z* (CI^+) 204 (4%), 190 (6), 176 (10), 148 (100), 121 (20), 107 (80), 82 (30), 59 (50) [HRMS (FAB): calc. for $C_{12}H_{16}NO_2$, 206.1181. Found, 206.1196].

Compound **9a** from microbial oxidation: $[\alpha]_D^{28}$ +69.0 (*c* 0.7, MeOH).

(5*S*,6*R*)-1-(2-Acetoxyethyl)-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 9b. Chromatography on silica gel (3:1 hexanes–EtOAc) afforded **9b** in 54% yield; R_f 0.53 (3:1 hexanes–EtOAc); $[\alpha]_D^{28}$ +73.9 (*c* 1.1, MeOH); ν_{max}(neat)/cm^{-1} 3005, 3000, 2900, 1750, 1375, 1250, 1050, 875; $\delta_H(CDCl_3)$ 1.39 (3 H, s, CH_3), 1.40 (3 H, s, CH_3), 2.04 (3 H, s, $COCH_3$), 2.56 (2 H, m, CH_2), 4.28 (2 H, m, CH_2O), 4.58 (1 H, d, *J* 8.8, CH), 4.67 (1 H, dd, *J* 3.8 and 8.8, CH), 5.80 (2 H, m, 2 C=CH), 5.99 (1 H, m, C=CH); $\delta_C(CDCl_3)$ 20.7 (CH_3), 24.9 (CH_3), 26.8 (CH_3), 32.9 (C), 62.3 (CH_2), 71.0 (CH), 73.2 (CH), 105.3 (C), 120.2 (CH), 123.4 (CH), 124.2 (CH), 134.3 (C), 170.7 (C); *m*/*z* (CI^+) 163 (20%), 149 (20), 122 (25), 121 (100), 120 (50), 107 (10), 93 (15), 79 (15), 59 (25) (HRMS: calc. for $C_{13}H_{19}O_4$, 239.1238. Found, 239.1246).

Compound **9b** from microbial oxidation: $[\alpha]_D^{28}$ +78.4 (*c* 0.8, MeOH).

(5*S*,6*R*)-1-(2-Azidoethyl)-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 9d. Chromatography on silica gel (95:5 hexanes–EtOAc) afforded **9d** in 56% yield. The neat compound was kept at 0 °C because it undergoes facile dimerization at room temperature; R_f 0.24 (95:5 hexanes–EtOAc); $[\alpha]_D^{28}$ +118.4 (*c* 1.57, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2986, 2934, 2100, 2099, 1458, 1376, 1257, 1158, 1044, 886; $\delta_H(CDCl_3)$ 1.39 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 2.4 (2 H, m, CH_2), 3.49 (2 H, t, *J* 7.2, CH_2N), 4.56 (1 H, d, *J* 9, CH), 4.67 (1 H, dd, *J* 9 and 3.9, CH), 5.87 (2 H, dd, *J* 3.6 and 6, 2 C=CH), 5.99 (1 H, m, C=CH); $\delta_C(CDCl_3)$ 24.9, 26.8, 33.3, 49.3, 71.0, 73.0, 103.1, 105.4, 120.8, 123.5, 124.3, 134.2; dimer *m*/*z* (FAB) 443 (15%), 154 (85), 136 (100), 107 (95), 91 (90) (HRMS: calc. for $C_{22}H_{31}O_4N_6$, 443.2407. Found, 443.2352).

Compound **9d** from microbial oxidation: $[\alpha]_D^{28}$ +128.7 (*c* 1.0, $CHCl_3$).

(5*S*,6*R*)-1-(2-Sulfanylethyl)-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 9h. Extraction with diethyl ether followed by chromatography on silica gel (95:5 hexanes–EtOAc) afforded **9h** as a yellow oil in 42% yield; R_f 0.12 (95:5 hexanes–EtOAc); $[\alpha]_D^{28}$ +178.6 (*c* 1.1, MeOH); ν_{max}(neat)/cm^{-1} 3517, 3045, 2984, 2932, 1602, 1370, 1210, 1031, 869; $\delta_H(CDCl_3)$ 1.39 (3 H, s, CH_3), 1.40 (3 H, s, CH_3), 1.62 (1 H, s, CH), 2.64 (2 H, m, CH_2), 2.91 (2 H, t, *J* 7.7, CH_2S), 4.55 (1 H, d, *J* 8.5, CH), 4.66 (1 H, dd, *J* 3.8 and 8.5, CH), 5.78 (1 H, d, *J* 5.6, C=CH), 5.83 (1 H, dd, *J* 3.8 and 9.8, C=CH), 6.00 (1 H, dd, *J* 5.6 and 9.8, C=CH); $\delta_C(CDCl_3)$ 24.8 (CH_3), 26.7 (CH_3), 33.3 (CH_2), 36.5 (CH_2), 70.9 (CH), 73.0 (CH), 105.2 (C), 119.7 (CH), 123.0 (CH), 124.3 (CH), 135.8 (C); *m*/*z* (CI^+) 211 (70%), 153 (90), 131 (10), 121 (100), 59 (10) [HRMS (FAB): calc. for $C_{11}H_{17}SO_2$, 213.0950. Found, 213.0940].

(5*S*,6*R*)-1-(2-Thiocyanatoethyl)-5,6-(propane-2,2-diyldioxy)-cyclohexa-1,3-diene 9i. Chromatography on silica gel (95:5 hexanes–EtOAc) afforded **9i** in 46% yield; R_f 0.16 (95:5 hexanes–EtOAc); $[\alpha]_D^{28}$ +105.1 (*c* 1.75, MeOH); ν_{max}(neat)/cm^{-1} 3047, 2986, 2934, 2153, 1663, 1604, 1434, 1376, 1210, 1158, 1036, 959, 871, 712; $\delta_H(CDCl_3)$ 1.37 (3 H, s, CH_3), 1.38 (3 H, s, CH_3), 2.73 (2 H, m, CH_2), 3.17 (2 H, m, CH_2S), 4.52 (1 H, d, *J* 8.8, CH), 4.68 (1 H, dd, *J* 3.85 and 8.8, CH), 5.86 (2 H, m, 2 C=CH), 5.99 (1 H, m, C=CH); $\delta_C(CDCl_3)$ 25.9, 27.9, 33.3, 35.7, 72.0, 73.9, 106.5, 113.17, 122.7, 125.0, 125.2, 134.4, 151.8; *m*/*z* (CI^+) 238 (15%), 180 (20), 121 (100), 93 (25), 59 (15) (HRMS: calc. for $C_{12}H_{16}NSO_2$, 238.0902. Found, 238.0904).

Compound **9i** from microbial oxidation: $[\alpha]_D^{28}$ +101.0 (*c* 1.1, MeOH).

(5*S*,6*R*)-1-(2-Isothiocyanatoethyl)-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 9j. R_f 0.18 (95:5 hexanes–EtOAc); $[\alpha]_D^{28}$ +99.3 (*c* 0.6, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3010, 2985, 2918, 2184, 2100, 1455, 1370, 1210, 1157, 1025; $\delta_H(CDCl_3)$ 1.39 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 2.63 (2 H, m, CH_2), 3.81 (2 H, m, CH_2N), 4.58 (1 H, d, *J* 8.8, CH), 4.69 (1 H, dd, *J* 3.9 and 8.8, CH), 5.86 (1 H, d, *J* 5.8, C=CH), 5.90 (1 H, dd, *J* 3.9 and 9.5, C=CH); $\delta_C(CDCl_3)$ 24.8 (CH_3), 26.8 (CH_3), 34.7 (CH_2), 43.4 (CH_2), 70.9 (CH), 72.9 (CH), 105.4 (C), 121.7 (CH), 123.8 (CH), 124.2 (CH), 132.9 (C); *m*/*z* (FAB) 136 (80%), 107 (35), 91 (25) (HRMS: calc. for $C_{12}H_{16}NSO_2$, 238.0912. Found, 238.0915).

(5*S*,6*R*)-1-(2-Hydroxyethyl)-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 9c. $LiAlH_4$ (18 mg, 0.46 mmol) was suspended in dry THF (5 ml) at 0 °C. Acetate diol acetonide **9b** was added to this and the suspension was stirred for 12 h. The reaction mixture was worked up by adding water (18 μl), followed by 10% NaOH (18 μl) then water (54 μl). The precipitate was filtered off and washed with EtOAc. The washing was combined with the filtrate, this solution was concentrated *in vacuo* and the residue was chromatographed on silica gel (2:1 EtOAc–hexanes) to afford **9c** (38.5 mg, 47%); R_f 0.34 (1:1 hexanes–EtOAc); $[\alpha]_D^{28}$ +39.2 (*c* 0.2, $CHCl_3$); ν_{max}/(neat) cm^{-1} 3423, 3046, 2985, 2933, 2888, 1659, 1603, 1371, 1210, 1158, 1040; $\delta_H(CDCl_3)$ 1.40 (6 H, s, 2 CH_3), 1.9 (1 H, s, OH), 2.52 (2 H, m, CH_2), 3.75 (2 H, m, CH_2O), 4.52 (1 H, d, *J* 8.5, CH), 4.74 (1 H, dd, *J* 3.3 and 8.5, CH), 5.78 (1 H, dd, *J* 3.3 and 9.6, C=CH), 5.83 (1 H, d, *J* 5.8, C=CH), 5.95 (1 H, dd, *J* 5.8 and 9.6, C=CH); $\delta_C(CDCl_3)$ 24.7 (CH_3), 26.5 (CH_3), 38.1 (CH_2),

61.0 (CH_2), 71.6 (CH), 72.9 (CH), 104.9 (C), 121.6 (CH), 123.6 (CH), 123.7 (CH), 134.4 (C); *m/z* (FAB) 191 (15%), 177 (20), 167 (20), 149 (100), 85 (60) (HRMS: calc. for $C_{11}H_{16}O_3$, 196.1100. Found 196.1052).

Compound **9c** from microbial oxidation: $[\alpha]_D^{28}$ +37.2 (*c* 0.39, MeOH).

(5*S*,6*R*)-1-(2-Aminonethyl)-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 9e. The azide **9d** (65 mg, 0.29 mmol) and PPh_3 (116 mg, 0.44 mmol) were mixed together. THF (5 ml) was added followed by water (0.02 ml). After 18 h the reaction was complete. Concentration and chromatography on silica gel (7:3:0.3 EtOAc–EtOH–NH_4OH) gave a yellow viscous oil (53 mg, 92%); R_f 0.38 (7:3:0.3 EtOAc–EtOH–NH_4OH); $[\alpha]_D^{28}$ + 88.9 (*c* 2.4, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3361, 3045, 2985, 2934, 1572, 1483, 1379, 1209, 1041; δ_H($CDCl_3$) 1.38 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.54 (2 H, s, NH_2), 2.37 (2 H, m, CH_2), 2.88 (2 H, m, CH_2N), 4.51 (1 H, d, *J* 8.5, CH), 4.67 (1 H, dd, *J* 3.8 and 8.5, CH), 5.78 (1 H, d, *J* 5.5, C=CH), 5.80 (1 H, dd, *J* 3.8 and 9.6, C=CH), 5.96 (1 H, *J* 5.5 and 9.6, C=CH); δ_C($CDCl_3$) 24.9 (CH_3), 26.8 (CH_3), 38.2 (CH_2), 39.7 (CH_2), 71.4 (CH), 73.1 (CH), 105.2 (C), 120.3 (CH), 123.3 (CH), 124.2 (CH), 135.6 (C); *m/z* (POS FAB NBA) 196 (55%), 176 (10), 121 (75), 89 (30) (HRMS: calc. for $C_{11}H_{18}NO_2$, 196.1337. Found, 196.1336).

(5*S*,6*R*)-1-[2-(*N*,*N*-Diethylamino)ethyl]-5,6-(propane-2,2-diyldioxycyclohexa-1,3-diene 9f. The diol acetonide **6** (210 mg, 0.81 mmol) was dissolved in DMF (5 ml), diethylamine (0.10 ml, 0.98 mmol) was added and the reaction mixture was stirred until the starting material had completely disappeared (TLC). Water (5 ml) was added to the reaction mixture and the organic material was extracted with EtOAc (3 × 10 ml). The amine was back-extracted with 5% HOAc (2 × 5 ml). The aqueous solution was made alkaline (1 M NaOH), extracted with EtOAc (3 × 10 ml), dried and concentrated to yield a thin yellow oil (158 mg, 78%). Attempted chromatography on silica gel, or prolonged reaction time, led to contamination with the product of elimination, (5*S*,6*R*)-1-vinyl-5,6-(propane-2,2-diyldioxy)-cyclohexa-1,3-diene. R_f 0.71 (7:3 hexanes–EtOAc); $[\alpha]_D^{28}$ +74.1 (*c* 0.29, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3046, 2970, 2933, 2802, 1602, 1379, 1208, 1159, 1043; δ_H($CDCl_3$) 1.16 (6 H, t, *J* 7.3, CH_2-CH_3), 1.48 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 2.48 (2 H, m, CH_2), 2.70 (4 H, q, *J* 7.3, CH_2CH_3), 2.81 (2 H, m, CH_2N), 4.65 (1 H, d, *J* 8.6, CH), 4.74 (1 H, dd, *J* 3.9 and 8.6, CH), 5.84 (1 H, d, *J* 5.6, C=CH), 5.88 (1 H, dd, *J* 3.9 and 9.5, C=CH), 6.06 (1 H, dd, *J* 5.6 and 9.5, C=CH); δ_C($CDCl_3$) 11.7 (CH_3), 25.04 (CH_3), 26.8 (CH_3), 30.4 (CH_2), 46.7 (CH_2), 50.8 (CH_2), 71.2 (CH), 73.5 (CH), 105.1 (C), 119.1 (CH), 122.6 (CH), 124.6 (CH), 137.1 (C); *m/z* (CI+) 252 (15%), 194 (10), 86 (100) (HRMS: calc. for $C_{15}H_{26}NO_2$, 252.1964. Found, 252.1969).

(5*S*,6*R*)-1-[2-(*N*-Phthalimido)ethyl]-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 9g. The reaction was quenched with ice–water and the resulting solution was extracted with diethyl ether (3 × 10 ml). The organic layer was washed with saturated NaCl (2 × 3 ml), dried with $MgSO_4$ and the solvent was evaporated on the rotary evaporator. The residue was chromatographed on silica gel (5:1 hexanes–EtOAc) to afford **9g** as an oil (173 mg, 61%); R_f 0.43 (1:1 hexanes–EtOAc); $[\alpha]_D^{28}$ +213.2 (*c* 0.87, $CHCl_3$) (Found: C, 70.31; H, 7.40; N, 6.88. $C_{19}H_{19}NO_4$ requires C, 70.24; H, 7.32; N, 70.14%); ν_{max}(neat)/cm^{-1} 3468, 2985, 2935, 2891, 2249, 1612, 1465, 1299, 970; δ_H($CDCl_3$) 1.28 (3 H, s, CH_3), 1.33 (3 H, s, CH_3), 2.56 (2 H, m, CH_2), 3.75 (1 H, m, CH_2N), 3.92 (1 H, m, CH_2N), 4.61 (1 H, dd, *J* 8.8 and 3.3, CH), 4.65 (1 H, d, *J* 8.8, CH), 5.63 (1 H, d, *J* 5.5, C=CH), 5.71 (1 H, dd, *J* 9.6 and 3.3, C=CH), 5.80 (1 H, dd, *J* 9.6 and 5.4, C=CH), 7.64 (2 H, dd, *J* 2.9 and 5.6, ArH), 7.75 (2 H, dd, *J* 2.9 and 5.6, ArH); δ_C($CDCl_3$) 24.9 (CH_3), 26.6 (CH_3), 32.4 (CH_2), 36.0 (CH_2), 70.9 (CH), 72.3 (CH), 105.1 (C), 120.6 (CH), 122.9 (CH), 123.5 (CH), 123.9 (CH), 131.9 (C), 133.7 (CH), 134.4 (C), 167.9 (C); *m/z* (CI+) 280 (10%), 268 (100), 250 (25), 160 (10) (HRMS: calc. for $C_{19}H_{20}NO_4$, 326.1393. Found, 326.1391).

(5*S*,6*R*)-1-(2-Phenylsulfanylethyl)-5,6-(propane-2,2-diyldioxy)cyclohexa-1,3-diene 9k. The acetonide **6** (32.9 mg, 0.13 mmol) was added to a solution of potassium hydroxide (90 mg, 1.61 mmol) and thiophenol (0.10 ml, 1.0 mmol) in DMF (5 ml). The reaction was complete after 6 h of stirring at room temperature (TLC). Water (5 ml) was added and the product was extracted with EtOAc (3 × 5 ml). Flash chromatography on silica gel (95:5 hexanes–EtOAc as eluent) afforded the desired sulfide (23.7 mg, 64.5%) as an oil; R_f 0.27 (95:5 hexanes–EtOAc); $[\alpha]_D^{28}$ +106.3 (*c* 1.51, MeOH); ν_{max}(neat)/cm^{-1} 3049, 2985, 2928, 1583, 1458, 1375, 1211, 1158, 1027, 739; δ_H($CDCl_3$) 1.47 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 2.68 (2 H, m, CH_2), 3.22 (2 H, t, *J* 7.8, CH_2), 4.65 (1 H, d, *J* 8.8, CH), 4.73 (1 H, m, CH), 5.80 (2 H, m, CH_2), 7.19 (1 H, m, CH); δ_C($CDCl_3$) 26.9, 31.6, 33.5, 71.1, 73.2, 94.2, 105.4, 119.8, 123.2, 124.5, 125.9, 128.9, 129.1, 136.4; *m/z* (CI+) 289 (10%), 264 (15), 231 (30), 231 (100), 121 (30) (HRMS: calc. for $C_{17}H_{21}SO_2$, 289.1262. Found, 289.1240).

Compound **9k** from microbial oxidation: $[\alpha]_D^{28}$ +110.9 (*c* 1.1, MeOH).

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