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PAPER

Toluene dioxygenase mediated oxidation of halogen-substituted benzoate esters†

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A series of *ortho*-, *meta*-, and *para*- halogen-substituted methyl benzoate esters was subjected to enzymatic dihydroxylation *via* the whole-cell fermentation with *E. coli* JM109 (pDTG601A). Only *ortho*-substituted benzoates were metabolized. Methyl 2-fluorobenzoate yielded one diol regioselectively whereas methyl 2-chloro-, methyl 2-bromo- and methyl 2-iodobenzoates each yielded a mixture of regioisomers. Absolute stereochemistry was determined for all new metabolites. Computational analysis of these results and a possible rationale for the regioselectivity of the enzymatic dihydroxylation is advanced.

Introduction

The oxidation of aromatic compounds by toluene dioxygenase (TDO) yields *cis*-dihydrodiols, over 400 of which are known.[1] In 1968 Gibson isolated the first stable arene-*cis*-diol from a fermentation of *Pseudomonas putida* grown in the presence of *para*-chlorotoluene.[2] He later developed a mutant strain that lacked the requisite enzymes to process *cis*-dihydrodiols, allowing the accumulation of these metabolites in the cell broth.[3] The genes encoding TDO were later cloned into a strain of *Escherichia coli* producing the recombinant organism JM109 (pDTG601A), in which the protein synthesis is initiated by isopropylthiogalactose (IPTG) and thus no aromatic inducer is required.[4] This aspect greatly simplifies the identification of any new metabolites as the diol derived from the inducer will be absent from the product mixtures.

The first applications of *cis*-dihydrodiols in organic synthesis were the synthesis of polyphenylene by the ICI group[5] and the preparation of pinitol from the *meso*-diol derived from benzene accomplished by Ley some 20 years after Gibson's disclosure.[6] In 1988 our group published the formal synthesis of a prostaglandin from the diol derived from toluene.[7] Since then, many new metabolites have been discovered, and *cis*-dihydrodiols have enjoyed widespread use in organic synthesis.[8]

In 2009 we reported the microbial oxidation of several benzoate esters **1** in order to probe the limits of substrate size.[9] It was found that TDO oxidized methyl, ethyl, allyl, and propargyl benzoates to their corresponding diols **2** in yields of about 1 g L^{-1}, whereas *n*Pr, and *i*Pr, esters were found to be poor substrates and *n*Bu, and *t*Bu benzoate were not metabolized (Fig. 1). The diol derived from the oxidation of ethyl benzoate was recently used in several generations of synthesis of oseltamivir.[10] In this disclosure, we report the metabolism of halogen-substituted methyl benzoates, provide computational study and rationale for the observed selectivities, and offer further applications for the use of new metabolites.

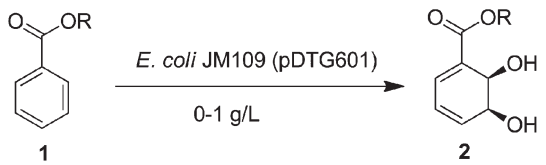

Fig. 1 Microbial dihydroxylation of benzoate esters.

Results and discussion

In a recent paper[9] we detailed the results of the microbial dihydroxylation of benzoate esters **1** and the application of dienediols **2** in the preparation of pseudo-sugars. To further extend the applicability of diols derived from benzoate esters, we subjected methyl halobenzoates to microbial dihydroxylation studies to determine the effect of the halogen substituent on the outcome of bio-oxidation. From the standpoint of synthesis, the halogen group provides an additional reactive functionality that can be further exploited in various radical or transition metal-catalyzed coupling protocols for accessing new optically pure *cis*-diols not

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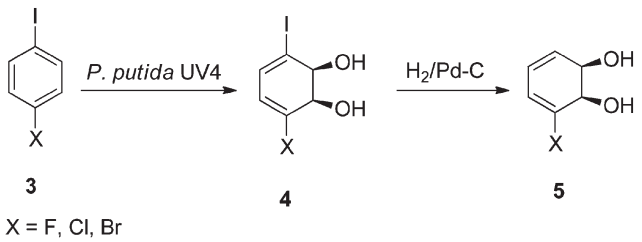
available by enzymatic dihydroxylation of the corresponding aromatic substrates.

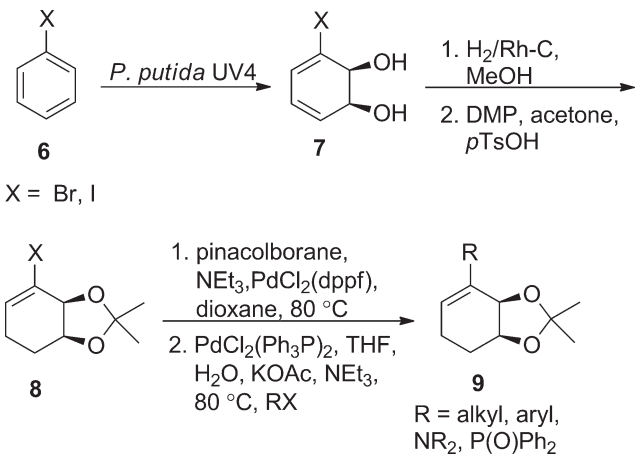
The required methyl halobenzoate esters were prepared from the corresponding commercially available halobenzoic acids by treatment with sodium carbonate and dimethyl sulfate in acetone. The methyl halobenzoate esters were incubated in Fernbach shake flasks (2 L) with *E. coli* JM109 (pDTG601A), grown to an optimum optical density in a 15 L Biostat C fermentor. After incubation for six hours, the broth was extracted with EtOAc and analyzed by TLC and ^{1}H NMR. If a new metabolite was detected, preparative-scale fermentation was undertaken in a 15 L Biostat fermentor as previously described.[11] We found that *meta*- or *para*- substituted benzoate esters, unlike their dihalo- or halo-alkyl counterparts, were not metabolized by TDO.

The utility of the iodine atom as a directing group for bio-oxidation and its subsequent reductive removal has been illustrated in the work of Boyd and co-workers.[12] A series of dihalogenated arene substrates (Scheme 1) was oxidized using *P. putida* UV4. Removal of iodine in the presence of bromine or fluorine was achieved by means of catalytic hydrogenation as shown below for the *para* isomer. The corresponding *ortho* and *meta* isomers were also investigated. This study provided access to *ent*-halo diols, which are not accessible from the corresponding halobenzenes using bacterial dioxygenases.



Scheme 1

Boyd and co-workers[13] have recently demonstrated the versatility of bromo- and iodobenzene-derived diols as starting materials in the preparation of a wide array of other diols either not accessible by fermentation or produced in low yields by bio-oxidation (Scheme 2). The bio-oxidation of bromo- or iodobenzene **6** afforded the enantiomerically pure diols **7**, and selective



Scheme 2

hydrogenation of the less hindered alkene catalyzed by rhodium on carbon under a hydrogen atmosphere provided tetrahydrodiols, which were protected as acetonides **8**. Substitution of Br or I with boronates followed by coupling with carbon, nitrogen, or phosphorus nucleophiles afforded coupled products of type **9**.

The coupling strategy employed by Boyd provides access to a wide variety of optically pure intermediates. Similarly useful would be an approach to diols derived from disubstituted aromatics especially ones containing functionalities of different size, using the Charton steric parameter as an indicator of substituent size. Such substrates are processed according to a model proposed by Boyd *et al.*[8g,14] where the larger of the substituents "directs" the oxidation.

There are 15 known *cis*-dihydrodiols of type **11**, derived from *o*-, *m*-, and *p*-alkyl-/or halo-iodobenzenes, Fig. 2.[12,15] In all cases, the iodine atom "directed" the regiochemistry of dihydroxylation.

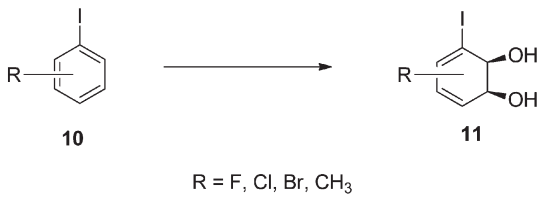


Fig. 2 Metabolites of iodobenzenes.

Nineteen *cis*-dihydrodiols derived from various isomers of alkyl- or halo-benzoic acids have been reported; however, there is no clear trend in regiochemical preferences of dihydroxylation. Most of these substrates give *ipso*[16] diols of type **14** (only a few exceptions provide diols of type **13**).[17]

There are, surprisingly, only seven metabolites derived from various alkyl benzoate esters, as shown in Fig. 1.[9,16a,18] In contrast to the number of metabolites available from the disubstituted arenes, disubstituted alkyl benzoate dihydroxylation has not yet been reported even though the oxidation of substituted benzoic acids is known (Fig. 3).[16,17] We therefore decided to pursue the current study to seek functionalized benzoate-derived diols *via* subsequent coupling protocols for providing additional building blocks not available directly by the enzymatic dihydroxylation.

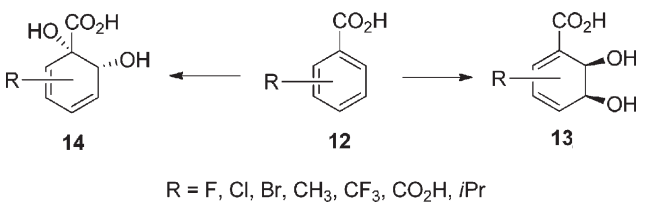
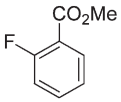
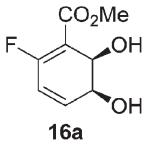
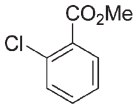
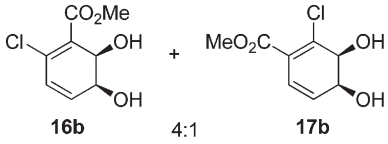
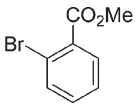
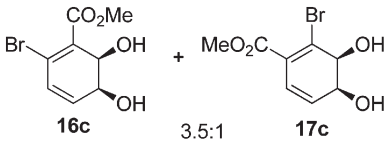
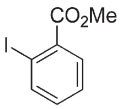
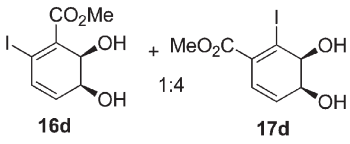


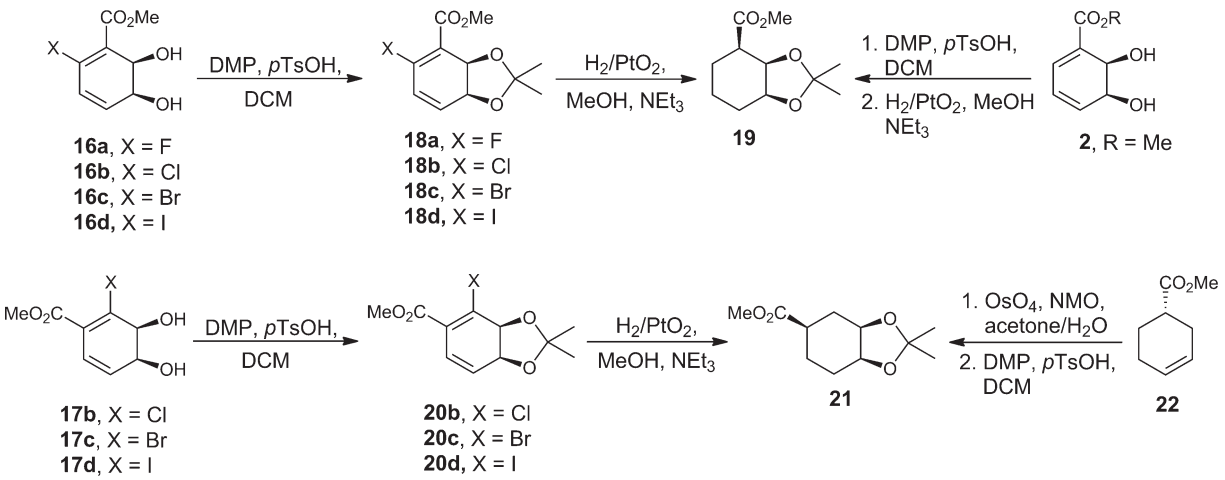
Fig. 3 Metabolites of substituted benzoic acids.

Ortho-halogen substituted methyl benzoate esters **15** gave diols of type **16** or **17**, as shown in Table 1. Methyl 2-fluorobenzoate **15a** furnished a single diol of type **16**, whereas methyl 2-chloro-, methyl 2-bromo- and methyl 2-iodobenzoate gave mixtures of **16** and **17**. These observations are in agreement with Boyd's rules for predicting the regio- and stereochemistry of dihydroxylation by TDO.[8g,14]

Table 1 Microbial dihydroxylation of methyl halobenzoate substrates

Starting material	Conversion (%)	Yield (g L^{-1})	Products[a] (ratio)
 **15a**	49.6	0.50	 **16a**
 **15b**	60.2	0.47	 **16b** 4:1 **17b**
 **15c**	13.3	0.20	 **16c** 3.5:1 **17c**
 **15d**	10.0	0.15	 **16d** 1:4 **17d**

[a] Ratio was obtained using integration of ^{1}H NMR peaks corresponding to olefinic signals in the crude mixture.



Scheme 3

Yields of diols derived from *ortho*-substituted benzoates are given in Table 1, along with the ratios of regioisomers. The stability of diols **16** and **17** above was found to depend strongly on the nature of the X group. The most electronegative substituent, fluorine, confers the highest stability while the least electronegative substituent, iodine, contributes to lower stability. In fact, the diols **16d** and **17c** could not be isolated (these undergo a facile dehydration to the corresponding phenols upon concentration in the rotary evaporator, even at low temperatures) and hence were characterized as the corresponding acetonide derivatives instead. Even the acetonides had a limited stability at room temperature and had to be stored at low temperatures.

For substrates **15a**, **15b** and **15c** the major diol product was the result of methyl carboxylate directing the dihydroxylation. This trend was reversed with substrate **15d** where the iodine atom directed the regiochemistry of the dihydroxylation.

With the exception of methyl 2-fluorobenzoate diol **16a**, these diols readily underwent dehydration at room temperature. They are stable in crystalline form at −78 °C or in pH 8 phosphate buffer at 0 °C. They are less stable than diols **2** derived from non-halogenated alkyl benzoate substrates **1**, for example, methyl benzoate or ethyl benzoate.[9]

In order to determine the relative and absolute stereochemistry, the new metabolites were protected as acetonides followed by hydrogenation as shown below (Scheme 3). The products obtained from diols **16** were matched with the fully hydrogenated diol **19** derived from the known diol **2**[9] obtained previously from methyl benzoate. Similarly, diols **17** were converted to

ester **21**, which was derived from known compound **22**[19] in two steps. These experiments confirmed the relative as well as absolute stereochemistry of the new metabolites as drawn.

Hydrogenation of acetonides **18a**, **18b**, **18c** and **18d** from type **16** diol resulted in a product that was identical in all properties to ester **19**, prepared from the known methyl benzoate diol **2**. Similarly, hydrogenation of acetonide **20b**, **20c** and **20d** resulted in the formation of ester **21** (except for the sign of optical rotation), derived from methyl cyclohexenylcarboxylate ester **22**. The starting ester **22** was obtained in racemic form *via* Diels–Alder reaction of butadiene sulfone and methyl acrylate in toluene at 130 °C performed for two days in a sealed tube. Resolution was achieved using porcine pancreatic lipase (PPL) in phosphate buffer at pH 7.0. Dihydroxylation of **22** followed by acetonide formation afforded the desired standard compound *cis* (relative stereochemistry of ester to acetonide) **21** as a 3 : 4 mixture with its *trans* diastereomer (relative stereochemistry of ester to acetonide), which was separated by silica gel column chromatography.

With respect to potential applications of diols derived from halobenzoates, Porco and co-workers[20] recently determined the absolute stereochemistry of the natural product kibdelone C by way of its total synthesis. A key intermediate, **24**, was prepared in 13 steps starting from commercially available material **23** as shown below (Fig. 4). We have shown[21] that diol **16d** provided a good choice for access to this key intermediate: two *versus* the thirteen steps originally reported. Applications of the metabolites derived from halobenzoates to the synthesis of additional kibdelone derivatives are now in progress with collaboration.

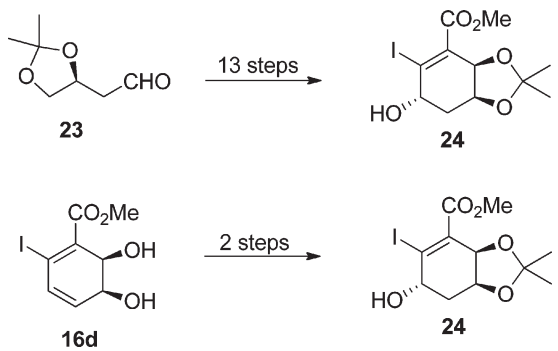


Fig. 4 A short route for the preparation of Kibledone C intermediate **24**.

Computational studies

The Boyd empirical model, based on Charton steric parameters (ν), cannot satisfactorily explain the observed regioselectivity in TDO-catalysed *cis*-dihydroxylation of the *ortho*-substituted benzoates **15a–15d**. The size of the substituents, according to Charton steric parameters, are as follows: COOMe (ν = 1.39) > I (ν = 0.78) > Br (ν = 0.65) > Cl (ν = 0.55) > F (ν = 0.27). This indicates that in all examples the COOMe should be the larger stereodirecting group; however, in the case of iodine derivative **15d**, the iodine atom is clearly dominant over the ester group. As Boyd claims for the case of nonsymmetrical substituents, other considerations, *e.g.*, substituent length and conformation in the vicinity of the active site, should also be taken into consideration.[8g,14]

The mechanism of the enzymatic dihydroxylation of arenes is unknown. Various propositions have been advanced[22] but none has been proven by experiment. The original proposition by Gibson[23] for dioxetane intermediates is, to date, the only one partially subjected to experimental investigation through studies of the kinetic isotope effects in dehydrogenation to cathecols.[24] We have briefly investigated mechanistic options involving radical intermediates but have not arrived at any reasonable mechanistic postulates.[25] Other options include high oxidation state iron peroxides, iron oxo species, and singlet oxygen cycloadditons. Because the actual mechanism is not known, it is logical that all possibilities are equivalent in merit until proven otherwise. For this reason, the computational studies that follow are based on the assumption that dioxetanes are the intermediates in the reaction, whether formed by singlet oxygen cycloaddition or internal redox reactions of triplet species. The results of these computational studies follow.

Methyl benzoates 15a–15d. Taking into account Boyd's observations, we studied the geometry and electrostatic effects of methyl benzoates **15a–15d**. The resulting data were employed in the calculation of energies of proposed dioxetane intermediates. Two rotamers of benzoates **15a–15d** were studied (Fig. 5).

In all cases except for **15a**, the $COOCH_3$ group is rotated out of the plane of benzene ring. As seen from Table 2, this angle varies from 17 to 31°, reducing π-interaction of $COOCH_3$ group with the aromatic ring. For benzoates **15a–15c**, the *anti*-conformer is more stable, but for iodine derivative **15d** the *syn* arrangement is preferred. Quantitatively, the stability difference between *syn* and *anti* is significant only for **15a**. In the case of Cl-, Br- and I-substituted benzoates (**15b–15d**) the barrier for interconversion (1.3–1.5 kcal mol^{-1}), is small (Table 2). Even one weak hydrogen-bond formation can supply enough energy for a conformational change, and the $COOCH_3$ group can easily adopt conformation, as required by the active center of enzyme.

Electrostatic effects can also play a role in substrate docking. Table 2 shows the atomic charges calculated by natural

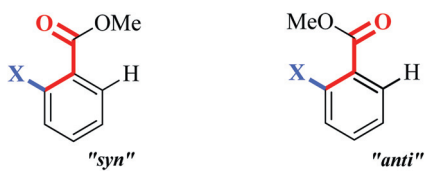


Fig. 5 Rotamers of **15a–15b**.

Table 2 Properties of **15a–15d**

		Out-of-plane angle [°]	$E_{relative}$ [kcal mol^{-1}]	Barrier heights [kcal mol^{-1}]	NPA charge on X [*e*]
15a	*syn*	0.0	1.17	3.9	−0.355
	anti	0.0	0.00		−0.363
15b	*syn*	25.5	0.21	1.5	−0.005
	anti	24.2	0.00		−0.018
15c	*syn*	23.0	0.13	1.3	+0.088
	anti	26.2	0.00		+0.071
15d	*syn*	17.4	0.00	1.3	+0.216
	anti	30.0	0.19		+0.192

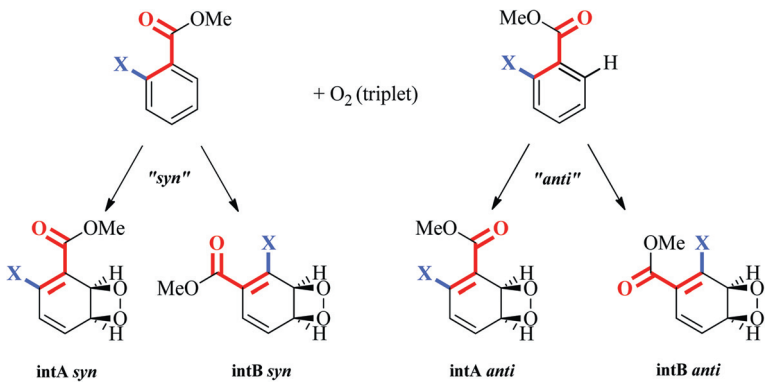
population analysis (NPA)[26] for halogen atoms in *syn* and *anti* conformations. The charges of the oxygen atoms in the acetyl group are negative in each case, but the charge of halogen is variable. This can have a significant influence on the orientation of the benzoate during docking in the active site of the enzyme. This preference can be "matched" or "mismatched" with the preference based on the orientation effect of the functional group (electronic or steric). As seen in Table 2, the charge on the halogen is practically the same in either the *syn* and *anti* conformation.

Formation of dioxetane intermediates: electronic effects

We wished to assess the combined orientational effect of X and COOMe substituents on the specific type of reaction, *i.e.* formation of dioxetane intermediate[25a] (see Scheme 4). Each formed regioisomer of dioxetane intermediate can be in two rotameric forms, *syn* and *anti.* If the COOMe group is regiodirecting, the intermediate must have the **A** type structure, if halogen is in this role, the intermediate is of **B** type. Calculated reaction energies for formations of intermediates **intA** and **intB** in *syn* and *anti* conformations by reaction of benzoate with triplet (ground state) oxygen are listed in Table 3. The idea behind this is that relative energies of these intermediates are related to barrier heights for formation of diols (Hammond's postulate) and thus can serve as a rough guide to assess effect of different halogen substituents.

It is not known whether singlet oxygen or another reactive oxygen species (ROS) takes part in this reaction; however, our calculations were based on oxygen in its ground (triplet) state as we do not know the exact character of the reacting species (singlet oxygen, superoxide anion, peroxy or hydroperoxy radical, or any metal-bound species that may exist in the active site). The proper theoretical description of singlet oxygen is technically more complicated, but for the present study only the relative preference for **A** and **B** in different halo-benzoates is important. We also assume that the intermediate of the oxidation is the dioxetane, as originally proposed by Gibson. This assumption is valid for the comparisons made in the computational study but may not accurately describe the actual intermediates in the enzymatic reaction.

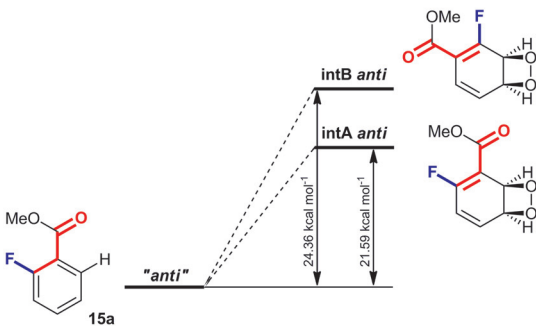
From the energy values in Table 3 we may conclude that the most pronounced orientational effect exists in the case of **15a**. The energy difference between **intA** and **intB** is 3.4 kcal mol^{-1}. In the case of **15d** it drops to just 0.5 kcal mol^{-1}. If there is another orientational effect (steric, electrostatic *etc.*) that competes with this preference for formation of intermediate **intA**,



Scheme 4

Table 3 Reaction energies for formation of intermediates **intA** ("actE A") and **intB** ("actE B")

		"actE" A [kcal mol^{-1}]	"actE" B [kcal mol^{-1}]
15a	*syn*	21.24	24.57
	anti	21.59	24.36
15b	*syn*	22.06	24.18
	anti	22.01	23.48
15c	*syn*	21.73	23.64
	anti	21.95	23.04
15d	*syn*	21.95	23.28
	anti	22.17	22.30



it can easily change this preference for iodine, but not for fluorine. This effect will be very similar for Cl and Br substituents. If we would consider the reacting species to be singlet oxygen (instead of triplet) in the lowest delta state, we can add its experimental energy: +22.5 kcal mol^{-1} (relative to triplet O_2) to the left-hand side of reactions for the formation of intermediates **A** and **B** and thus reduce the reaction energy by this amount. Resulting energies are then close to zero, indicating that these reactions are now almost thermally neutral, *i.e.* reaction enthalpy is ~0 kcal mol^{-1}.

Conclusions

We have identified seven new metabolites from the TDO-catalyzed dihydroxylation of *o*-halo benzoates. In general, benzoate esters are converted to diols in significantly lower yields than halobenzenes, and substituted benzoate esters are somewhat reluctant substrates. However, all of these compounds are accessible and can be prepared in multi-gram amounts by fermentation.

Ab initio calculations were shown to be in good qualitative agreement with the experimental results for microbial dihydroxylation of *ortho*-substituted halo benzoates. The most pronounced effect was observed in the case of fluorobenzoate **15a**, where TDO oxidation is regioselective. Practically no difference was noted between chloro and bromo derivatives (**15b** and **15c**) in both the theoretical calculations and the experimental results. Preference for the formation of **B** type diol **17d** in the case of the iodo derivative can be partially explained by Boyd's rules. In addition the positive charge (δ+) of iodine in **15d** can influence the orientation of the benzoate during docking to the active site of the enzyme.

The new metabolites will find widespread use in the preparation of optically pure diols not otherwise available from enzymatic oxidation of the corresponding arenes. The short preparation of the intermediate in the kibledone C synthesis lends credence to the above investigation. Although the precise mechanism of the enzymatic dihydroxylation remains unsolved, there is an increase in the power of prediction in investigations of new arene substrates.

Experimental section

Inoculum was obtained from viable cells stored −78 °C in cryovials. They were grown in suitable media as previously described.[11] Substrate was fed in 1 g increments over the course of ~3 h with metabolites being harvested in the usual manner. All non-aqueous reactions were conducted in an argon atmosphere using standard Schlenk techniques for the exclusion of moisture and air. Methylene chloride was distilled from calcium hydride, THF and toluene were dried over sodium/benzophenone. Analytical thin layer chromatography was performed on Silicycle 60 A° 250 mm TLC plates with F-254 indicator. Flash column chromatography was performed using Kieselgel 60 (230–400 mesh). Melting points were recorded on a Hoover Unimelt apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer One FT-IR spectrometer. Optical rotation was measured on a Perkin-Elmer 341 polarimeter at a wavelength of 589 nm. ^1H and ^{13}C spectra were recorded on a 300 MHz and 600 MHz Bruker spectrometer. All chemical shifts are referenced to TMS or residual undeuterated solvent. Data of proton spectra are reported as follows: chemical shift in ppm (multiplicity: singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m)), coupling constants [Hz], integration). Carbon spectra were recorded with complete proton decoupling and the chemical shifts are reported in ppm (δ) relative to solvent resonance as internal standard. Mass spectra and high resolution mass spectra were performed by the analytical division at Brock University.

Computational details and methods

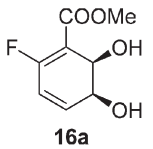
For calculations of molecular structures and energetics DFT theory was used with B3LYP[27] hybrid functional. This is an obvious choice for organic molecules, because it supplies good results and has been used with enough examples that a critical assessment of its performance is available. What is less obvious is the selection of appropriate basis sets, as we need basis set which can describe, with comparable precision, all halogens, including iodine. From relatively few possibilities we choose SBKJC[28] basis set with relativistic pseudopotentials, augmented with 1p and 2d polarization functions.[29] All reported energies correspond to structures, fully optimized at the above level. All calculations were done using GAUSSIAN03 program package.[30]

General procedure for acetonide formation

A catalytic amount of toluenesulfonic acid was added to a stirred solution of diol (2 mmol) and dimethoxypropane (10 mmol) in CH_2Cl_2 (10 mL). The reaction was monitored by TLC on silica gel (1 : 1 EtOAc–hexanes). When all the starting material was consumed, the reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 1.0 M NaOH (5 mL) and dried over anhydrous $MgSO_4$. The filtrate was concentrated by rotary evaporation and further purified by column chromatography on silica gel (1 : 1 EtOAc–hexanes) to afford the acetonide as an oil (80–95% yield).

General procedure for hydrogenation

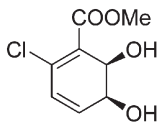
To a solution of acetonide (0.20 mmol) in MeOH (1 mL) was added PtO_2 (catalytic, 10% w/w) and NEt_3 (0.20 mmol). The flask was evacuated and filled with H_2 at atmospheric pressure. After the reaction was judged complete by TLC (8–12 h), the suspension was filtered through Celite; concentrated in the rotary evaporator and purified by column chromatography on silica gel using mixture of hexanes–EtOAc 4 : 1) as eluent. The eluent was concentrated to give an oil (50–61% yield).



16a

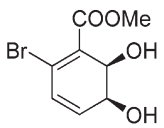
(5*S*,6*R*)-Methyl 2-fluoro-5,6-dihydroxycyclohexa-1,3-dienecarboxylate (16a). mp 74–76 °C (EtOAc); $[\alpha]_D^{20}$ +73.2 (*c* 1.05, MeOH); R_f = 0.15 (1 : 1 hexanes–ethyl acetate); IR (film) 3558,

3025, 1694, 1439, 1401, 1040 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.33 (m, 1H), 5.94 (ddd, J = 10.2, 8.3, 2.6 Hz, 1H), 4.71 (t, J = 6.2 Hz, 1H), 4.55 (m, 1H), 3.83 (s, 3H), 3.17 (brs, 1H), 3.09 (brs, 1H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.0 (d, J = 2.2 Hz), 163.2 (d, J = 281.0 Hz), 143.1 (d, J = 12.1 Hz), 119.6 (d, J = 36.2 Hz), 106.2 (d, J = 2.2 Hz), 69.06 (s), 67.0 (d, J = 6.6 Hz), 52.2 (s) ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ −92.6 (s) ppm; MS (EI) m/z (%): 188 (15), 133 (44), 119 (49), 102 (100), 91 (37), 90 (46), 86 (28), 74 (16), 46 (27); HRMS (EI) calcd for $C_8H_9FO_4$ (M^+): 188.0485; found: 188.0484; MS (FAB) m/z (%): 189 (24) $[M + H]^+$, 188 (16) $[M]^+$, 171 (100), 139 (27), 59 (16); HRMS (FAB) calcd for $C_8H_9FO_4$ (M+): 188.0485; found: 188.0479; Anal. calcd for $C_8H_9FO_4$: C, 51.07; H, 4.82; found: C, 51.18; H, 4.76.



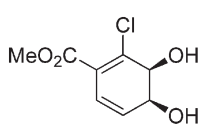
16b

(3*S*,4*S*)-Methyl 2-chloro-3,4-dihydroxycyclohexa-1,5-dienecarboxylate (16b). mp 107–109 °C (pentene–ethyl acetate); $[\alpha]_D^{20}$ = +36.06 (c 1.0, $CHCl_3$); R_f = 0.18 (1 : 1 hexanes–ethyl acetate); IR (KBr) 3398, 3459, 1698, 1317, 1057, 762 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.18 (ddd, J = 10.0, 2.4, 1.2 Hz, 1H), 6.01 (dd, J = 10.0, 2.4 Hz, 1H), 4.64 (ddd, J = 6.0, 4.7, 1.2 Hz, 1H), 4.52 (ddt, J = 8.7, 6.0, 2.4 Hz, 1H), 3.86 (s, 3H), 2.76 (d, J = 9.6 Hz, 1H), 2.60 (d, J = 4.7 Hz, 1H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.32, 138.78, 138.52, 127.50, 123.79, 68.56, 67.72, 52.27 ppm; MS (EI) m/z (%) $[M]^+$: 204 (14), 173 (23), 172 (54), 155 (50), 146 (32), 145 (36), 144 (100), 143 (80), 139 (27), 99 (27), 81 (41), 53 (25), 51 (21); HRMS (EI) calcd for $C_8H_9ClO_4$: 204.0189; found: 204. 0190.



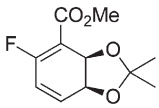
16c

(5*S*,6*R*)-Methyl 2-bromo-5,6-dihydroxycyclohexa-1,3-dienecarboxylate (16c). mp 106–109 °C ($CHCl_3$); $[\alpha]_D^{20}$ = +29.40 (c 1.0, DCM); R_f = 0.18 (1 : 1 hexanes–ethyl acetate); IR (KBr) 3402, 1703, 1437, 1314, 1234, 1048 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.17 (dd, J = 10.0, 2.5 Hz, 1H), 6.04 (ddd, J = 10.0, 2.5, 1.3 Hz, 1H), 4.57 (m, 1H), 4.49 (m, 1H), 3.85 (s, 3H), 3.00 (brd, J = 7.9 Hz, 1H), 2.97 (brs, 1H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.61, 137.54, 129.97, 128.04, 127.31, 68.37, 68.14, 52.28 ppm; MS (EI) m/z (%): 248 (9), 218 (38), 216 (47), 190 (82), 189 (53), 188 (85), 187 (48), 109 (71), 108 (31), 81 (100), 65 (79), 59 (45), 53 (54); HRMS (EI) calcd for $C_8H_9BrO_4$ (M^+): 247.9684; found: 247.9679.



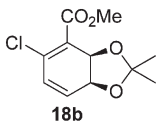
17b

(5*S*,6*R*)-Methyl 2-chloro-5,6-dihydroxycyclohexa-1,3-dienecarboxylate (17b). $[\alpha]_D^{20}$ = +86.6 (c 1.0, $CHCl_3$); R_f = 0.25 (1 : 1 hexanes–ethyl acetate); IR (KBr) 3422, 2959, 1721, 1578, 1444, 1270, 758 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.35 (d, J = 9.8 Hz, 1H), 6.03 (dd, J = 9.8, 3.0 Hz, 1H), 4.44 (m, 1H), 4.31 (d, J = 6.0 Hz, 1H), 3.83 (s, 3H), 2.50 (vbrs, 2H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.77, 140.46, 127.77, 125.09, 124.00, 72.47, 67.36, 52.34 ppm; MS (EI) m/z (%) $[M–H_2O]^+$: 188 (15), 186 (43), 157 (32), 155 (100), 99 (14); HRMS (EI) calcd for $C_8H_9ClO_4–H_2O$ $[M - H_2O]^+$: 188.0084; found: 188.0077.



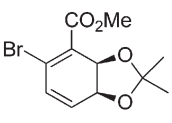
18a

(3aR,7aS)-Methyl 5-fluoro-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (18a). $[\alpha]_D^{20}$ +23.8 (c 1.2, $CHCl_3$); R_f = 0.67 (1 : 1 hexanes–ethyl acetate); IR (film) ν 3020, 2932, 2254, 1706, 1613 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.25 (m, 1H), 5.98 (m, 1H), 5.11 (t, J = 7.5 Hz, 1H), 4.87 (m, 1H), 3.86 (s, 3H), 1.46 (s, 3H), 1.42 (s, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.9, 163.7, 161.9, 138.1, 119.8, 119.5, 106.4, 103.9, 72.0, 70.9, 52.2, 26.8, 25.2, 1.03 ppm; MS (EI) m/z (%): 228 (1.7), 213, (80.0), 197 (11.3), 181 (10.8), 171 (49.8), 139 (42.0), 127 (20.8); HRMS (EI) calcd for $C_{11}H_{13}FO_4$: 228.0798; found: 228.0807.



18b

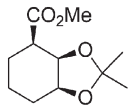
(3aR,7aS)-Methyl 5-chloro-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (18b). $[\alpha]_D^{20}$ +141.4 (c 1.5, CH_2Cl_2); R_f = 0.80 (1 : 1 hexanes–ethyl acetate); IR (film) ν 2989, 2952, 1726, 1644, 1587 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.08 (m, 2H), 5.07 (d, J = 8.1 Hz, 1H), 4.79 (dd, J = 8.1, 2.1 Hz, 1H), 3.86 (s, 3H), 1.43 (s, 3H), 1.40 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 137.0, 132.5, 127.5, 122.1, 106.5, 71.6, 71.0, 52.2, 26.7, 25.3 ppm; MS (EI) m/z (%): 244 (2.3), 231, (16.4), 229 (49.6), 213 (11.3), 197 (14.0), 187 (50.1), 186 (17.9), 157 (17.9), 155 (49.4); HRMS (EI) calcd for $C_{11}H_{13}ClO_4$: 244.0495; found: 244.00502.



18c

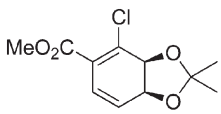
(3aR,7aS)-Methyl-5-bromo-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (18c). $[\alpha]_D^{20}$ +285.8 (c 1.1, CH_2Cl_2); R_f = 0.72 (1 : 1 hexanes–ethyl acetate); IR (film) ν 2988, 2951, 1725, 1639, 1582, 1434 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.17 (d, J = 0.72 Hz, 1H), 5.94 (dd, J = 9.87, 3.36 Hz, 1H), 4.99 (d, J = 8.04 Hz, 1H), 4.75 (m, 1H), 3.82 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 131.3, 129.9, 125.8, 125.7, 106.5,

72.1, 70.6, 52.2, 26.7, 25.2 ppm; MS (EI) *m*/*z* (%): 275 (24), 273 (25), 233 (41), 231 (45), 201 (28), 199 (28), 108 (33); HRMS (EI) calcd for $C_{11}H_{13}BrO_4$: 287.9997; found: 287.9994.



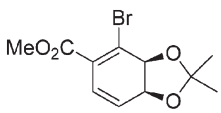
19

(3aR,4R,7aS)-Methyl 2,2-dimethylhexahydrobenzo[*d*][1,3]dioxole-4-carboxylate (19). $[\alpha]_D^{20}$ +10.4 (*c* 1.2, CH_2Cl_2); R_f = 0.29 (6 : 1 hexanes–ethyl acetate); IR (film) *ν* 2987, 2950, 1742 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 4.55 (dd, *J* = 5.4 Hz, *J* = 3.6 Hz, 1H), 4.19 (dt, *J* = 7.8 Hz, *J* = 5.4 Hz, 1H), 3.74 (s, 3H), 2.62 (m, 1H), 1.77 (m, 5H), 1.50 (m, 1H), 1.35 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 172.9, 108.5, 74.1, 73.3, 51.9, 43.0, 27.8, 27.7, 25.8, 20.1, 19.4 ppm; MS (EI) *m*/*z* (%): 199 (100), 157 (17), 139 (10), 125 (58), 97 (29), 79 (59); HRMS (EI) calcd for $C_{11}H_{18}O_4$: 214.1205; found: 214.1201.



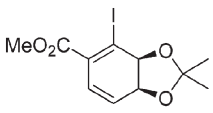
20b

(3aS,7aS)-Methyl 4-chloro-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-5-carboxylate (20b). $[\alpha]_D^{20}$ +92.6 (*c* 1.0, CH_2Cl_2); R_f = 0.80 (1 : 1 hexanes–ethyl acetate); IR (film) *ν* 2990, 2953, 1735, 1654, 1584, 1436, 1374, 1249 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 6.32 (d, *J* = 9.9 Hz, 1H), 5.97 (dd, *J* = 9.9, 3.6 Hz, 1H), 4.74 (dd, *J* = 8.4, 3.6 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 3.81 (s, 3H), 1.41 (s, 3H), 1.39 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 165.0, 137.9, 124.5, 123.8, 123.5, 107.1, 75.8, 71.8, 52.3, 26.7, 25.0 ppm; MS (EI) *m*/*z* (%): 244 (20), 213 (20), 229 (35), 186 (60), 155 (40), 59 (60), 43(100); HRMS (EI) calcd for $C_{11}H_{13}ClO_4$: 244.0495; found: 244.05024.



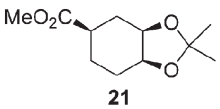
20c

(3aS,7aS)-Methyl 4-bromo-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-5-carboxylate (20c). $[\alpha]_D^{20}$ +94.0 (*c* 0.2, CH_2Cl_2); R_f = 0.72 (1 : 1 hexanes–ethyl acetate); IR (film) *ν* 3436, 2918, 1732, 1435 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 6.29 (d, *J* = 9.6 Hz, 1H), 6.08 (dd, *J* = 9.6, 3.9 Hz, 1H), 4.83 (d, 8.4 Hz, 1H), 4.74 (dd, *J* = 8.4, 3.9 Hz, 1H), 3.87 (s, 3H), 1.47 (s, 3H), 1.27 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 165.7, 129.0, 127.9, 124.5, 123.7, 106.7, 76.8, 71.8, 52.4, 26.8, 25.1 ppm; MS (EI) *m*/*z* (%): 288 (10.6), 233 (56.0), 232 (40.6), 231 (58.8), 230 (36.8), 201 (27.1), 199 (26.6), 189 (11.3), 187 (11.8), 185 (12.5), 183 (14.4); HRMS (EI) calcd for $C_{11}H_{13}BrO_4$: 288.0002; found: 287.9997.



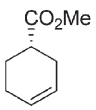
20d

(3aS,7aS)-Methyl 4-iodo-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-5-carboxylate (20d). $[\alpha]_D^{20}$ = +32.4 (*c* 3.3, CH_2Cl_2); R_f = 0.35 (4 : 1 hexanes–ethyl acetate); IR (film) 3444, 2987, 2951, 1730, 1372, 1243, 1062 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 6.22 (d, *J* = 9.9 Hz, 1H), 6.10 (dd, *J* = 9.9, 4.2 Hz, 1H), 4.83 (d, *J* = 8.1 Hz, 1H), 4.63 (dd, *J* = 8.1, 4.2 Hz, 1H), 3.85 (s, 3H), 1.45 (s, 3H), 1.43 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 166.2, 133.2, 124.9, 123.8, 107.9, 106.6, 79.6, 71.0, 52.4, 26.8, 25.2 ppm; MS (EI) *m*/*z* (%): 337 (6.7), 336 (41.0), 319 (10.7), 279 (89.8), 278 (97.6), 247 (44.5), 231 (17.8), 220 (12.3), 152 (100.0), 151 (48.4), 127 (13.6); HRMS (EI) calcd for $C_{11}H_{13}IO_4$ (M^+): 335.9864; found: 335.9859.



21

(3aR,5R,7aS)-Methyl 2,2-dimethylhexahydrobenzo[*d*][1,3]dioxole-5-carboxylate (21). $[\alpha]_D^{20}$ +32.8 (*c* 4.4, CH_2Cl_2); R_f = 0.27 (4 : 1 hexanes–ethyl acetate); IR (film) *ν* 3453, 2987, 2952, 1731, 1638, 1436 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 4.17 (m, 1H), 4.10 (m, 1H), 3.70 (s, 3H), 2.25 (m, 2H), 2.10 (m, 1H) 1.77 (m, 4H), 1.60 (m, 2H), 1,52 (s, 3H),1.35 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 175.2, 108.3, 73.7, 72.4, 51.8, 39.4, 31.5, 28.3, 26.3, 26.1, 22.6 ppm; MS (EI) *m*/*z* (%): 199 (100), 125 (13), 97 (30), 78 (52), 69 (11); HRMS (EI) calcd for $C_{11}H_{18}O_4$: 199.0973; found: 199.0970.



22

(S)-Methyl cyclohex-3-enecarboxylate(22)

Preparation of racemic ***22***. A solution of butadiene sulfone (8.0 g, 64.4 mmol), methyl acrylate (3.7 g, 42.8 mmol) and hydroquinone (100 mg, catalytic), in toluene (100 mL) was heated to 110 °C in sealed tube for 2 d. The reaction mixture was allowed to reach room temperature and was concentrated using rotary evaporation of afford a dark brown viscous residue. The residue was chromatographed on silica gel using hexanes as eluent to afford the racemic ester **22** as a colorless oil (4.55 g, 75.6% yield).

Resolution of ester ***22***. A mixture of racemic ester 19 (500 mg, 3.57 mmol) and Porcine Pancreatic Lipase (50 mg) in 0.10 M phosphate buffer (50 mL) was shaken in an orbital shaker at 20 °C. The pH was maintained at 7.0 by the addition of 0.10 M NaOH. At the end of 6.5 h, the enzyme was filtered through a plug of Celite. The filtrate was extracted with EtOAc (3 × 20 mL). The organic extracts were combined and dried over anhydrous Na_2SO_4. The filtrate was concentrated by rotary evaporation and further purified by column chromatography on silica gel (6 : 1 hexanes–EtOAc) to afford ester **22** (148 mg, 29% yield) as a yellowish oil. 89% ee [$\alpha]_D^{20}$ −63.5 (*c* 1.0, $CHCl_3$)) **lit**[19] $[\alpha]_D^{20}$ −82.2 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) *δ* 5.68 (s, 2H), 3.70 (s, 3H), 2.59 (m, 1H), 2.26 (m, 2H), 2.06 (m, 2H), 1.98 (m, 1H) 1.72 (m, 1H) ppm.

*Conversion of **22** to **21**.* A catalytic amount of OsO_4 was added to a stirred solution of ester **19** (100 mg, 0.72 mmol), *N*-methyl morpholine *N*-oxide (82 mg, 0.72 mmol) in acetone–water (2 mL/0.6 mL). The resulting solution was stirred for 1 h at room temperature, and then it was quenched with 15% $NaHSO_3$ solution (1 mL). The reaction mixture was diluted with EtOAc (20 mL) and water (5 mL). The layers were separated and the aqueous layer was extracted further with EtOAc (2 × 10 mL). The combined organic extracts were dried over anhydrous $MgSO_4$. The filtrate was concentrated *via* rotary evaporation and was used as crude in the next step.

To the crude mixture of diol (from the previous step) dissolved in CH_2Cl_2 (10 mL) was added dimethoxypropane (2.0 mL, 16.3 mmol) followed by a catalytic amount of *p*TsOH. The reaction mixture was stirred at room temperature for 30 min. Then it was diluted with 1 N NaOH (2 mL), the two layers were separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 5 mL). The organic layers were combined and dried over anhydrous $MgSO_4$. The filtrate was concentrated by rotary evaporation and further purified by column chromatography on silica gel (4 : 1 hexanes–EtOAc) to afford the desired product **21** (25 mg, 16.3% yield, over two steps) as a yellowish oil. 88% ee $[\alpha]_D^{20}$ −25.1 (*c* 1.0, CH_2Cl_2)) compound **21** from **20b–20d** $[\alpha]_D^{20}$ +32.8 (*c* 4.4, CH_2Cl_2).

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