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Processing of *o*-Halobenzoates by Toluene Dioxygenase. The Role of the Alkoxy Functionality in the Regioselectivity of the Enzymatic Dihydroxylation Reaction

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Supporting Information

ABSTRACT: In order to investigate the relationship between the size of a substituent on the aromatic substrate and its directing effect on the dihydroxylation, a series of 2-halobenzoates was synthesized and subjected to metabolism by toluene dioxygenase in preparative-scale fermentation cultures of *Escherichia coli* JM109 (pDTG601A). Larger ester substituents were shown to have a greater directing effect on the dihydroxylation reaction. Furthermore, significant increases in regioselectivity were observed using propargyl substituents, relative to the use of any other ester substituent. The selectivity and the product ratios are reported for *o*-fluoro-, *o*-chloro-, *o*-bromo-, and *o*-iodobenzoate esters (methyl, ethyl, *n*-propyl, allyl, and propargyl). Experimental and spectral data, as well as absolute stereochemistry, are provided for all new compounds.

INTRODUCTION

The *cis*-dihydroxylation of aromatic substrates by the enzyme toluene dioxygenase (TDO) has been utilized to produce a wide variety of chiral diols that have been used in the synthesis of many natural products.[1−6] In wild-type *Pseudomonas putida* bacteria, aromatic dioxygenases and related enzymes are involved in a cascade, which processes benzene, naphthalene, biphenyl, and other aromatic compounds as carbon and energy sources. In 1968, Gibson first reported the bio-oxidative degradation of benzene and other simple derivatives, as well as the isolation of the first stable *cis*-arene-dihydrodiol from a fermentation of aromatics with *Pseudomonas putida*.[7,8] In order to allow for the build-up of diols of type **1** in the fermentation culture, in 1970 Gibson developed a blocked mutant strain (*Pp*39D) lacking the enzymes responsible for their further degradation to catechol **2** and, eventually, to acetate.[9] The gene encoding toluene dioxygenase was later cloned into a recombinant strain of *Escherichia coli* (JM109 pDTG601A), which facilitates large-scale fermentation cultures and provides a convenient method for the induction of enzyme transcription, Figure 1. Unlike the blocked mutant, in which the protein synthesis must be initiated by a known aromatic inducer, the recombinant organism contains the inducer, isopropylthiogalactose (IPTG), on the plasmid.[10] Thus, the use of the recombinant strain leads only to the metabolite derived from the substrate, and the isolation is not complicated by the need for the separation of the diols originating from the aromatic inducer.

There are more than 400 arene-dihydrodiols of type **1** known to date, and the substrate scope was comprehensibly reviewed up to 2004 by Johnson.[11] New metabolites continue to be isolated, and the diversity of the structures available from the enzymatic dihydroxylation has led to a widespread use of *cis*-dihydrodiols in organic synthesis.[1−6,11−18] The first application of the dihydrodiol metabolites in synthesis was reported by Ley in 1987 who published the synthesis of racemic pinitol using *cis*-1,2-dihydroxycyclohexa-3,5-diene derived from benzene.[19] This event led us and many other groups to pursue applications of these metabolites to the total synthesis of natural products.[20]

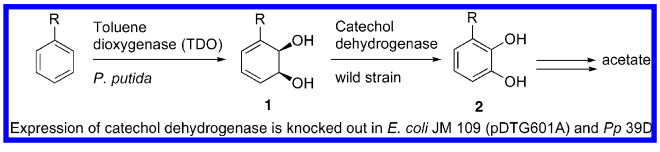

Figure 1. Comparison of degradation pathways of aromatics by wild-type organisms (*P. putida*), blocked mutants (*Pp* 39D), and engineered strains (*E. coli* JM109 pDTG601A).

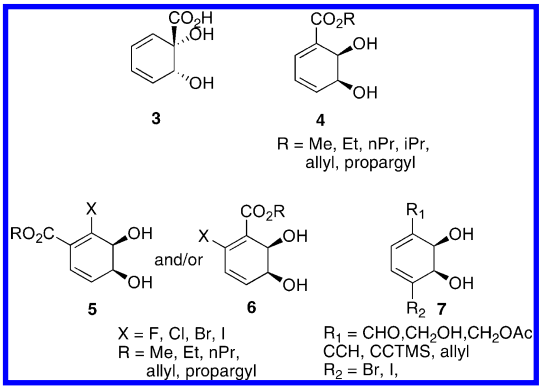

Figure 2. Examples of *cis*-dihydrodiols derived from benzoic acids, benzoate esters, and para-substituted arenes.

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To date the metabolites derived from single-ring aromatics comprise the largest group of compounds numbering into the hundreds.[11] Of the more than 400 compounds of this type only about 12 have been used in total synthesis. On the basis of a model proposed by Boyd[13b] it is possible to predict whether a single-ring arene might be a good substrate for TDO. Metabolites from fused aromatics and biphenyls are also known but are less numerous.

A far smaller group of metabolites is composed of diols derived from benzoic acids, benzoate esters, or *p*-disubstituted arenes, as shown in Figure 2. The *ipso*-diol **3** was first identified by Reiner and Hegeman as a metabolite of benzoic acid, produced by a mutant strain of *Alcaligenes eutrophus* which was blocked in benzoic acid catabolism.[21] This compound is also generated from benzoic acid by *R. eutrophus* B9 and has been used in synthesis.[22] There are limited reports on the use of metabolites derived from benzoate esters.

In 2009 we published a brief study on steric and functionality limits of esters in the production of diols of type **4**.[23] At that time, only the diol derived from methyl benzoate had been described.[24] Several new metabolites were identified and, subsequently, we have used the diol produced from ethyl benzoate in a short synthesis of oseltamivir (Tamiflu).[25] For metabolites of type **5** and **6** only the methyl *o*-halobenzoates have been previously reported.[26] Finally, a group of para-substituted arenes was investigated recently and led to several diol metabolites of type **7**, Figure 2, that could potentially be used in an approach to tetrodotoxin.[27]

In 2011 diol **9a**, Figure 3, was identified as a minor product derived from dihydroxylation of methyl *o*-iodobenzoate[26] and

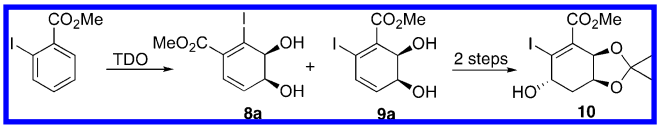


Figure 3. Chemoenzymatic preparation of the key fragment for the synthesis of kibdelone C.

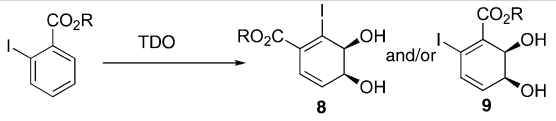
used in a short synthesis of ester **10**[28] employed by Porco in the total synthesis of kibdelone C.[29] [In Porco's original synthesis of kibdelone C the key intermediate **10** was synthesized in 13 steps.[29]] Methyl *o*-iodobenzoate produced a 4:1 mixture of **8a** and **9a** (as might be expected from the Boyd model[13b]), and this observation led to a study of methyl *o*-halobenzoates where such selectivity trend was reversed with methyl *o*-chlorobenzoate.[26]

The short chemoenzymatic preparation of the key intermediate **10** allowed for the more efficient synthesis of kibdelone C and derivatives through a fruitful collaboration.[30] However, the dihydroxylation of methyl *o*-iodobenzoate produced a mixture of regioisomers in which the desired metabolite **9a** was only a minor product.[26] The manipulation of the ratio of dihydroxylation products through the use of substituents of varying sizes should be possible, in line with the results of the previous investigations. In this study, we report the effect of altering both the halogen substituent and the alkoxy functionality of the ester in 2-halobenzoates on the ratio of regioisomeric products produced by the dihydroxylation of such compounds by toluene dioxygenase.

RESULTS AND DISCUSSION

In order to examine the effect of steric bulk of the halogen versus ester substituents on the dihydroxylation of 2-halobenzoates, a series of five esters was prepared for each 2-halobenzoate. The substrates were prepared from commercially available 2-halobenzoic acids. Substrates were incubated in a preparative-scale fermentation culture of *E. coli* JM109 (pDTG601A) in a 15-L Biostat C fermentor as has been previously described.[31] The crude fermentation extract was immediately analyzed by ^{1}H NMR spectroscopy in order to determine the ratio of regioisomeric products. As shown in Table 1, the enzymatic dihydroxylation of 2-iodobenzoates by

Table 1. Results of the enzymatic dihydroxylation of various 2-iodobenzoates by toluene dioxygenase



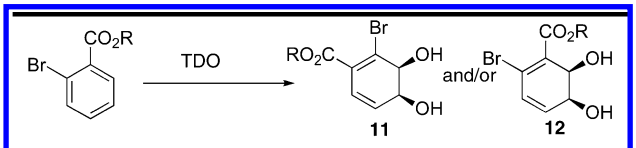
Entry	R =	Yield (g/L)	Ratio 8:9*
8 a/ 9a	-methyl	0.33[26]	4:1[26]
8b / 9b	-ethyl	0.04	1:1
8c / 9c	-*n*-propyl	trace	1:2
8d / 9d	-allyl	0.01	1:4
8e / 9e	-propargyl	0.07	1:12

*Isomeric ratios obtained by ^{1}H NMR spectroscopic analysis of crude fermentation extract.

toluene dioxygenase yielded a mixture of regioisomers (**8** and **9**, Table 1) as was previously observed with methyl 2-iodobenzoate.[26] As might be predicted by the Boyd model,[13b] increasing the size of the ester substituent resulted in a greater directing effect on the dihydroxylation.

As shown in Table 2, 2-bromobenzoates displayed a similar regioselectivity trend. Significantly increased yields were obtained with ethyl and propargyl 2-bromobenzoates, relative to the use of their iodo analogues.

Table 2. Results of the enzymatic dihydroxylation of various 2-bromobenzoates by toluene dioxygenase



Entry	R =	Yield (g/L)	Ratio 11:12*
11a / 12a	-methyl	0.40[26]	1:4[26]
11b / 12b	-ethyl	0.30	1:3
11c / 12c	-*n*-propyl	trace	1:4
11d / 12d	-allyl	0.02	1:10
11e / 12e	-propargyl	0.35	2:98

*Isomeric ratios obtained by ^{1}H NMR spectroscopic analysis of crude fermentation extract.

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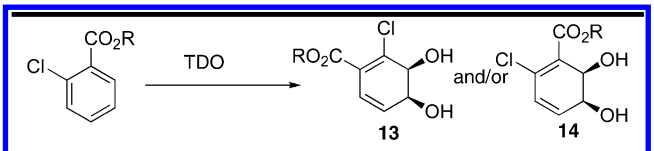
With 2-chlorobenzoate substrates, increased regioselectivities in favor of isomer **14** were observed with the yields comparable to those of 2-bromobenzoates, as shown in Table 3.

Table 3. Results of the enzymatic dihydroxylation of various 2-chlorobenzoates by toluene dioxygenase

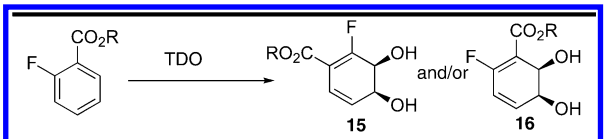


Entry	R =	Yield (g/L)	Ratio 13:14*
13a / 14a	-methyl	0.33[26]	1:4[26]
13b / 14b	-ethyl	0.04	1:3.5
13c / 14c	-*n*-propyl	trace	1:20
13d / 14d	-allyl	0.02	1:7
13e / 14e	-propargyl	0.30	3:97

*Isomeric ratios obtained by ^{1}H NMR spectroscopic analysis of crude fermentation extract.

Consistent with previous findings,[26] 2-fluorobenzoates yielded only one regioisomer upon dihydroxylation by toluene dioxygenase, irrespective of the size of the ester substituent, Table 4. Furthermore, significantly higher yields were obtained using 2-fluorobenzoates, relative to the use of the other halogenated substrates.

Table 4. Results of the enzymatic dihydroxylation of various 2-fluorobenzoates by toluene dioxygenase



Entry	R =	Yield (g/L)	Ratio 15:16*
15a / 16a	-methyl	0.50[26]	- 100[26]
15b / 16b	-ethyl	0.46	-100
15c / 16c	-*n*-propyl	0.02	-100
15d / 16d	-allyl	0.20	-100
15e / 16e	-propargyl	0.60	-100

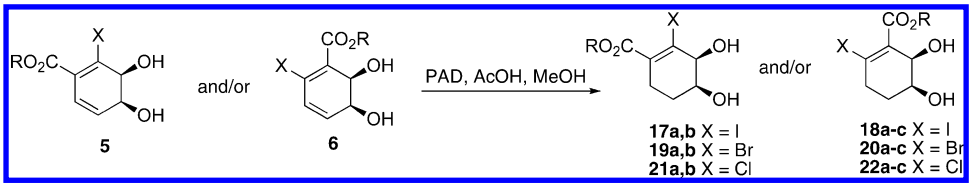
*Isomeric ratios obtained by ^{1}H NMR spectroscopic analysis of crude fermentation extract.

In addition to the considerations of Boyd's model[13b] for directing effects in dihydroxylation (i.e., the relationship of isomeric ratio to the size of the substituents on the aromatic substrate), an interesting trend emerged when examining the isomeric ratios associated with the propargyl compounds. In the case of iodo, bromo, and chloro compounds, the propargyl ester substituent was shown to be a much stronger directing group than any other. Attempts to quantify Boyd's model by only considering *A*-values of substituents leads to some confusion: for example, the *A*-values of the halogens are 0.25−0.42, 0.53−0.64, 0.48−0.67, and 0.47−0.61 for fluorine, chlorine, bromine, and iodine, respectively.[32,33] Thus, other factors need to be considered to justify any regioselectivity trends associated with the steric bulk of the halogen atoms. For esters, the *A*-values should roughly correspond to the *A*-values of the alkyl group of the alkoxide as the carboxylates remain constant. Thus, a comparison of methyl, ethyl, *n*-propyl, allyl, and the propargyl group could, in principle, be made on the basis of the difference between terminal substituents; i.e., H for methyl ester, CH_3 for ethyl ester, ethyl, vinyl, and acetylene for *n*-propyl, allyl, and propargyl esters, respectively. These values are: Me = 1.7, Et = 1.8, vinyl =1.5, and acetylene 0.4. Clearly the *A*-values cannot be used to explain the directing effects. Another metric to consider would be the Taft[34] and Charton[35] steric parameter values (E_s and ν values, respectively) for both halogens and ester alkyl groups. Unlike the *A*-values, these follow a logical trend: F = 0.27; Cl = 0.55; Br = 0.65; I = 0.78. For ester alkyl groups these are: H = 0; Me = 0.52; Et = 0.56; *n*Pr = 0.68, (C_2H_3 = 1.51).[35] These values would correspond to Me, Et, *n*Pr, *n*Bu, and allyl esters, respectively (the value for alkynyl is not available). While there is no clear linear relationship that would explain the reversal of directing trends between the "apparent size" of the halogen versus that of the ester, at least some of the trends may be rationalized by using the internally consistent ν values for halogens and for the alkyl groups. For the esters it is clear that propargyl groups, regardless of apparent size with respect to that of other alkoxy groups, override the directing effects of halogens, as evidenced from the ratios of the two regioisomers obtained in all cases.

In the case of the iodo, bromo, and chloro substrates, the regioisomeric mixtures of diols proved to be very difficult to separate. This problem was exacerbated by the relative instability of these compounds at room temperature, particularly when being taken to dryness. In order to facilitate isolation and characterization, a portion of the crude regioisomeric mixture was subjected to partial reduction with potassium azodicarboxylate (PAD) in the presence of acetic acid (Scheme 1). Preparative high pressure liquid chromatography (HPLC) was employed to separate the regioisomeric mixtures, which facilitated partial characterization of the diene diol compounds, and full characterization of their reduced counterparts. This derivatization was not necessary in the case of the fluoro compounds, as these were significantly more stable.

In order to facilitate the confirmation of absolute stereochemistry in the new metabolites, all of the fluorinated

Scheme 1. Enzymatic dihydroxylation of 2-halobenzoates and subsequent derivation to facilitate isolation and characterization [note that 17c, 19c, and 21c are not produced in sufficient quantities to allow full characterization]



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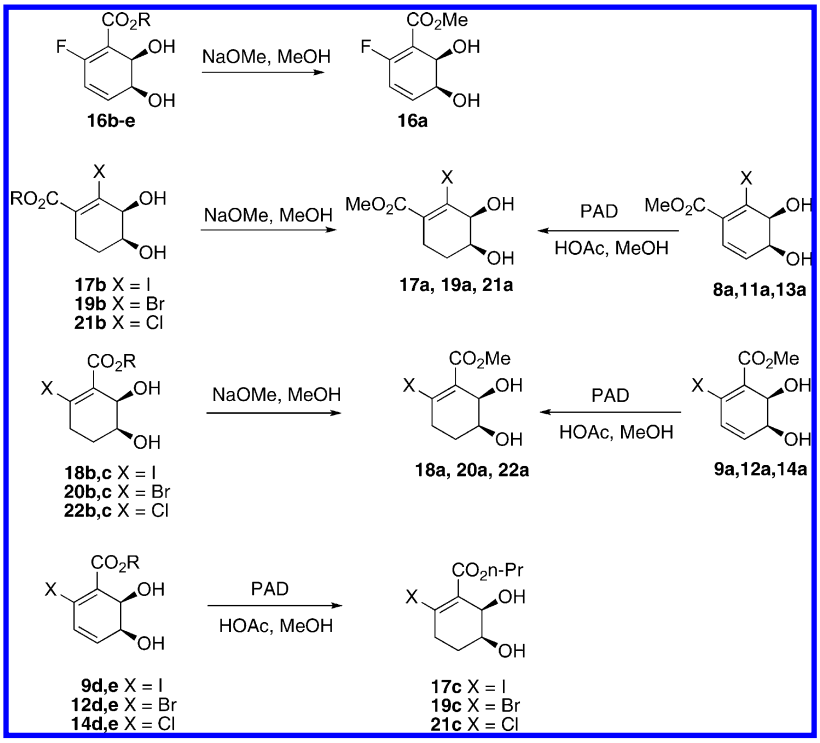
Scheme 2. Determination of absolute stereochemistry of the metabolites [note that 17c, 19c, and 21c are not produced in sufficient quantities to allow full characterization]



derivatives were converted to the corresponding methyl ester **16a**, (Scheme 2), the absolute stereochemistry of which has been previously published.[26] In the case of the iodo, bromo, and chloro compounds, their reduced derivatives (**17b**, **18b–c**, **19b**, **20b–c**, **21b**, **22b–c**) were converted to their corresponding methyl esters (**17a**, **18a**, **19a**, **20a**, **21a**, **22a**) in order to confirm absolute stereochemistry (Scheme 2). The partially reduced methyl ester metabolites (**17a**, **18a**, **19a**, **20a**, **21a**, **22a**) were matched by ^{1}H NMR, melting point, and $[\alpha]_D$ to their corresponding compounds prepared from metabolites (**8a**, **9a**, **11a**, **12a**, **13a**, **14a**) previously published,[26] Scheme 2.

CONCLUSION

The original hypothesis, that the directing effect of halogen and ester substituents on the dihydroxylation of aromatic substrates roughly follows the corresponding *A*-values of the functional groups was not confirmed. The *A*-values alone cannot be used to rationalize the ratios of the regioisomers produced. The Charton ν values for halogens and for the alkyl groups of the esters show a logical and linear trend with respect to the "apparent size" of functionalities. They may certainly be used to explain their *relative directing effects* but not necessarily their *mutual directing effects*. We have shown that significantly greater levels of regioselectivity, are obtained when propargylic ester substituents are used for any of the halobenzoate substrates. At this time, and without additional information, we cannot explain the origin of the selectivity apparent for only propargylic esters, nor can we explain the difference in observed yields for some of the substrates.

EXPERIMENTAL SECTION

Viable cells were prepared in a 15-L Biostat C fermentor as previously described.[24] Substrates were fed in 1-g increments over 3 h, with a total of 15 g being added into the media before centrifugation and extraction with ethyl acetate. All diene diol products were subjected to immediate reduction with potassium azodicarboxylate, or stored at −78 °C. Where possible, diene diol products were isolated by preparative HPLC (60:40 v/v methanol/water) and recrystallized from ethyl acetate and pentane. Reduced diol products were also isolated by preparative HPLC (60:40 v/v methanol/water) and recrystallized from ethyl acetate and pentane.

General Procedure for Reduction with Potassium Azodicarboxylate. To a stirred solution of diene diol (2.5 mmol) and potassium azodicarboxylate (PAD) (7.5–15.0 mmol) in MeOH (4 mL), glacial acetic acid was added (17.5–37.5 equiv) dropwise at −15 °C. The reaction was allowed to warm to room temperature over 14 h and then was quenched by the addition of a saturated aqueous solution of Na_2CO_3 (7–15 mL of a saturated aqueous solution), concentrated under reduced pressure, and extracted with ethyl acetate (5 × 5 mL). The combined organic layers were washed with brine (1 × 7 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude material was recrystallized from ethyl acetate/pentane.

General Procedure for the Transesterification of PAD-Reduced Metabolites. To a stirred solution of an ester (0.5 mmol) in MeOH (5 mL) was added NaOMe until the solution was observed to be just basic by pH paper. The reaction mixture was allowed to stir at room temperature until the starting material was consumed by TLC. The product was purified by flash column chromatography (3:2 v/v ethyl acetate/hexanes) and recrystallized from ethyl acetate/pentane.

*Ethyl (3S,4S)-3,4-dihydroxy-2-iodocyclohex-1,5-dienecarboxylate (**8b**):* off-white solid; mp 105–107 °C (pentane); $[\alpha]_D^{20} = -72.4$ (c = 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.19 (d, J = 9.8 Hz, 1H), 6.13 (dd, J = 9.6, 3.7 Hz, 1H), 4.44

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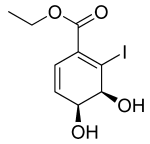
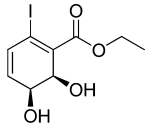
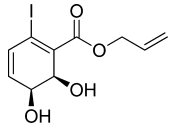


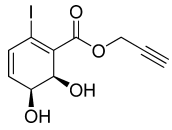
(br d, 1H), 4.39 (br d, 1H), 4.32 (q, J = 7.2 Hz, 2H), 1.37 (t, J = 7.1 Hz, 3H).



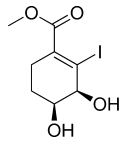
*Ethyl (5S,6R)-5,6-dihydroxy-2-iodocyclohex-1,3-dienecarboxylate (**9b**):* off-white solid; mp 107−110 °C (pentane); $[\alpha]_D^{20}$ = 67.1 (c = 0.45, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.47 (d, J = 9.3 Hz, 1H), 5.86 (d, J = 9.4 Hz, 1H), 4.55 (m, 1H), 4.48 (m, 1H), 4.34 (m, 2H), 1.39 (t, J = 7.1 Hz, 3H).



*Allyl (5S,6R)-5,6-dihydroxy-2-iodocyclohex-1,3-dienecarboxylate (**9d**):* off-white solid; mp 63−66 °C (pentane); $[\alpha]_D^{20}$ = 68.0 (c = 0.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (dd, J = 9.0, 2.3 Hz, 1H), 6.02 (m, 1H), 5.88 (ddd, J = 9.8, 1.9, 1.3 Hz, 1H), 5.43 (ddd, J = 17.2, 1.5, 1.4 Hz, 1H), 5.33 (dd, J = 10.4, 1.2 Hz, 1H), 4.79 (m, 2H), 4.58 (d, J = 5.6 Hz, 1H), 4.51 (br d, 1H).

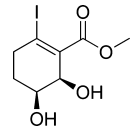


*Propargyl (5S,6R)-5,6-dihydroxy-2-iodocyclohex-1,3-dienecarboxylate (**9e**):* off-white solid; mp 70−72 °C (pentane); $[\alpha]_D^{20}$ = 77.1 (c = 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (dd, J = 9.8, 2.4 Hz, 1H), 5.89 (ddd, J = 9.8, 1.8, 1.0 Hz, 1H), 4.87 (m, 1H), 4.59 (t, J = 5.9 Hz, 1H), 4.50 (m, 2H), 2.56 (t, J = 2.4 Hz, 1H).

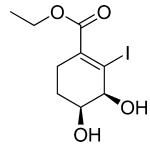


*Methyl (3S,4S)-3,4-dihydroxy-2-iodocyclohex-1-enecarboxylate (**17a**):* white solid; mp 78−80 °C (pentane) [lit.[36] mp 80−81 °C (hexanes)]; R_f = 0.23 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −32.5 (c = 0.1, $CHCl_3$) [lit.[36] $[\alpha]_D^{20}$ = −34.5 (c = 1.1, $CHCl_3$)]; IR (KBr, cm^{-1}) 3259, 2949, 1722, 1635, 1433, 1347, 1094; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.33 (br s, 1H), 4.02 (m, 1H), 3.83 (s, 3H), 2.73 (s, 1H), 2.65 (dt, J = 18.0, 5.7 Hz, 1H), 2.35 (dt, J = 18.0, 5.7 Hz, 1H), 1.97 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 136.8, 108.5, 68.4, 67.1, 52.3, 41.0, 28.3; HRMS calcd for $C_8H_{11}IO_4^+$ 297.9702, found 297.9713.

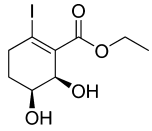
*Methyl (5S,6R)-5,6-dihydroxy-2-iodocyclohex-1-enecarboxylate (**18a**):* white solid; mp 83−85 °C (pentane), mp 84−86 °C (pentane) [lit.[36] mp 85−86 °C (hexanes)]; R_f = 0.27



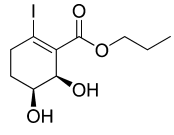
(3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −37.4 (c = 0.2, $CHCl_3$), $[\alpha]_D^{20}$ = −38.0 (c = 0.2, $CHCl_3$) [lit.[36] $[\alpha]_D^{20}$ = −39.5 (c = 1.1, $CHCl_3$)]; IR (KBr, cm^{-1}) 3268, 2950, 1727, 1634, 1432, 1236, 1093; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.50 (br s, 1H), 3.94 (m, 1H), 3.86 (s, 3H), 3.00 (m, 2H), 2.77 (m, 2H), 1.97 (m, 1H), 1.74 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 140.4, 103.2, 74.8, 68.2, 52.4, 27.2, 25.3; HRMS calcd for $C_8H_{11}IO_4^+$ 297.9702, found 297.9707.



*Ethyl (3S,4S)-3,4-dihydroxy-2-iodocyclohex-1-enecarboxylate (**17b**):* off-white solid; mp 108−109 °C (pentane); R_f = 0.46 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −32.6 (c = 0.75, $CHCl_3$); IR (KBr, cm^{-1}) 3270, 2940, 1704, 1593, 1463, 1253; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.32 (br d, 1H), 4.30 (q, J = 7.1 Hz, 2H), 4.02 (m, 1H), 2.64 (dt, J = 18.1, 5.7 Hz, 1H), 2.39 (dt, J = 17.9, 6.7 Hz, 1H), 1.94 (m, 2H), 1.37 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.96,140.70, 102.69, 74.75, 68.19, 61.67, 27.21, 25.26, 14.06; HRMS calcd for $C_9H_{13}IO_4^+$ 311.9859, found 311.9847.

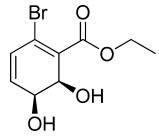


*Ethyl (5S,6R)-5,6-dihydroxy-2-iodocyclohex-1-enecarboxylate (**18b**):* off-white solid; mp 106−108 °C (pentane); R_f = 0.42 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −31.6 (c = 0.45, $CHCl_3$); IR (KBr, cm^{-1}) 3282, 2950, 1714, 1642, 1461, 1241; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.52 (br d, 1H), 4.36 (q, J = 7.1 Hz, 2H), 3.95 (m, 1H), 3.00 (dt, J = 19.1, 5.4 Hz, 1H), 2.75 (dt, J = 18.6, 6.9 Hz, 1H), 1.98 (m, 1H), 1.77 (m, 1H), 1.37 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.98, 136.67, 108.49, 68.38, 67.05, 61.73, 41.14, 28.37, 14.12; HRMS calcd for $C_9H_{13}IO_4^+$ 311.9859, found 311.9859.

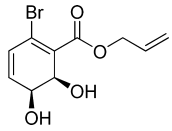


*n-Propyl (5S,6R)-5,6-dihydroxy-2-iodocyclohex-1-enecarboxylate (**18c**):* off-white solid; mp 70−72 °C (pentane); R_f = 0.43 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −42.6 (c = 0.5, $CHCl_3$); IR (KBr, cm^{-1}) 3271, 2962, 2879, 1715, 1606, 1462, 1231; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.50 (br d, 1H), 4.23 (td, J = 6.5, 1.9 Hz, 2H), 3.94 (m, 1H), 3.01 (dt, J = 19.1, 5.5 Hz, 1H), 2.77 (dt, J = 19.0, 7.1 Hz, 1H), 1.97 (m, 1H), 1.77 (m, 2H), 1.74 (m, 2H), 1.04 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.01, 136.73, 108.49, 68.43, 67.33, 67.07, 41.22, 28.37, 21.87, 10.63; HRMS calcd for $C_{10}H_{15}IO_4^+$ 326.0015, found 326.0015.

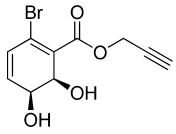
dx.doi.org/10.1021/op400343c | Org. Process Res. Dev. 2014, 18, 801−809



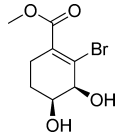
*Ethyl (5S,6R)-5,6-dihydroxy-2-bromocyclohex-1,3-diene-carboxylate (**12b**):* white solid; mp 78−80 °C (pentane); $[\alpha]_D^{20}$ = 46.1 (*c* = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.19 (dd, *J* = 9.9, 2.3 Hz, 1H), 6.06 (ddd, *J* = 9.8, 1.9, 1.2 Hz, 1H), 4.59 (m, 1H), 4.51 (m, 1H), 4.35 (m, 2H), 1.38 (t, *J* = 7.1 Hz, 3H).



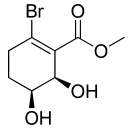
*Allyl (5S,6R)-5,6-dihydroxy-2-bromocyclohex-1,3-diene-carboxylate (**12d**):* white solid; mp 59−61 °C (pentane); $[\alpha]_D^{20}$ = 52.3 (*c* = 0.7, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.20 (dd, *J* = 9.9, 2.4 Hz, 1H), 6.08 (ddd, *J* = 8.7, 1.9, 1.4 Hz, 1H), 6.00 (m, 1H), 5.43 (ddd, *J* = 17.3, 2.3, 1.5 Hz, 1H), 5.31 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.78 (m, 2H), 4.63 (d, *J* = 5.9 Hz, 1H), 4.54 (br d, 1H).



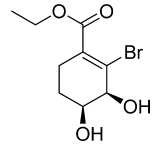
*Propargyl (5S,6R)-5,6-dihydroxy-2-bromocyclohex-1,3-di-enecarboxylate (**12e**):* white solid; mp 83−85 °C (pentane); $[\alpha]_D^{20}$ = 65.6 (*c* = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.22 (dd, *J* = 2.5, 9.8 Hz, 1H), 6.10 (ddd, *J* = 9.8, 1.9, 1.1 Hz, 1H), 4.88 (t, *J* = 2.6 Hz, 2H), 4.64 (dd, *J* = 6.0, 1.2 Hz, 1H), 4.54 (m, 1H), 2.55 (t, *J* = 2.4 Hz, 1H).



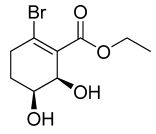
*Methyl (3S,4S)-3,4-dihydroxy-2-bromocyclohex-1-enecar-boxylate (**19a**):* white solid; mp 91−93 °C (pentane) [lit.[36] mp 92−93 °C (pentane)]; R_f = 0.42 (2:1 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −30.6 (*c* = 0.7, $CHCl_3$), [lit.[36] $[\alpha]_D^{20}$ = −32.4 (*c* = 0.7, $CHCl_3$)]; IR (KBr, cm^{-1}) 3270, 2951, 1728, 1431, 1254, 1092, 756; ^1H NMR (300 MHz, $CDCl_3$) δ 4.29 (br s, 1H), 4.01 (br s, 1H), 3.81 (s, 3H), 2.56−2.71 (m, 1H), 2.31−2.37 (m, 1H), 1.85−1.96 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 134.4, 124.8, 72.2, 68.2, 52.3, 26.4, 25.2; HRMS calcd for $C_8H_{11}BrO_4^+$ 249.9841, found 249.9852.



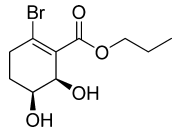
*Methyl (5S,6R)-5,6-dihydroxy-2-bromocyclohex-1-enecar-boxylate (**20a**):* white solid; mp 95−97 °C (pentane), mp 98−100 °C (pentane) [lit.[36] mp 98−100 °C (pentane)]; R_f = 0.36 (2:1 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −43.5 (*c* = 0.7, $CHCl_3$), $[\alpha]_D^{20}$ = −52.8 (*c* = 0.7, $CHCl_3$) [lit.[36] $[\alpha]_D^{20}$ = −44.9 (*c* = 0.7, $CHCl_3$)]; IR (KBr, cm^{-1}) 3237, 2953, 1731, 1430, 1217, 1099, 1037; ^1H NMR (300 MHz, $CDCl_3$) δ 4.53 (s, 1H), 3.90−3.93 (m, 1H), 3.86 (s, 3H), 2.87−2.90 (m, 1H), 2.60−2.71 (m, 1H), 2.05−2.08 (m, 1H), 1.85−1.86 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 132.5, 130.7, 68.5, 67.0, 52.3, 36.8, 27.1; HRMS calcd for $C_8H_{11}BrO_4^+$ 249.9841, found 249.9832; Anal. Calcd for $C_8H_{11}BrO_4$: C, 38.27; H, 4.42. Found: C, 38.33; H, 4.34.



*Ethyl (3S,4S)-3,4-dihydroxy-2-bromocyclohex-1-enecar-boxylate (**19b**):* white solid; mp 90−92 °C (pentane); R_f = 0.26 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −52.9 (*c* = 1.0, $CHCl_3$); IR (KBr, cm^{-1}) 3255, 2950, 1708, 1461, 1252, 1094; ^1H NMR (300 MHz, $CDCl_3$) δ 4.31 (br d, 1H), 4.29 (q, *J* = 7.1 Hz, 2H), 4.01 (m, 1H), 2.61 (dt, *J* = 17.8, 6.0 Hz, 1H), 2.34 (dt, *J* = 17.9, 6.6 Hz, 1H), 1.95 (m, 1H), 1.86 (m, 2H), 1.35 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.19, 134.72, 124.11, 72.22, 68.33, 61.55, 26.52, 25.15, 14.07; HRMS calcd for $C_9H_{12}BrO_4^+$ (M − H species) 262.9919, found 262.9925; Anal. Calcd for $C_9H_{13}BrO_4$: C, 40.78; H, 4.94. Found: C, 40.57; H, 4.87.



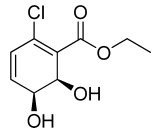
*Ethyl (5S,6R)-5,6-dihydroxy-2-bromocyclohex-1-enecar-boxylate (**20b**):* white solid; mp 87−89 °C (pentane); R_f = 0.24 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −59.8 (*c* = 1.0, $CHCl_3$); IR (KBr, cm^{-1}) 3269, 2955, 1721, 1622, 1462, 1364, 1241, 1042; ^1H NMR (300 MHz, $CDCl_3$) δ 4.52 (t, *J* = 3.8 Hz, 1H), 4.32 (q, *J* = 7.2 Hz, 2H), 3.91 (m, 1H), 2.84 (dt, *J* = 19.0, 5.5 Hz, 1H), 2.63 (dt, *J* = 19.0, 7.3 Hz, 1H), 2.01 (m, 1H), 1.81 (m, 1H), 1.36 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.80, 131.69, 131.02, 68.51, 67.03, 61.67, 35.80, 27.06, 14.11; HRMS calcd for $C_9H_{13}BrO_4^+$ 263.9997, found 263.9996; Anal. Calcd for $C_9H_{13}BrO_4$: C, 40.78; H, 4.94. Found: C, 40.71; H, 4.92.



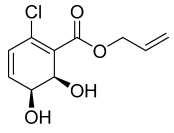
*n-Propyl (5S,6R)- 5,6-dihydroxy-2-bromocyclohex-1-ene-carboxylate (**20c**):* white solid; mp 82−85 °C (pentane); R_f = 0.27 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −49.8 (*c* = 0.6, $CHCl_3$); IR (KBr, cm^{-1}) 3383, 2966, 1714, 1657, 1462, 1426, 1392, 1337, 1268, 1092, 1040, 913; ^1H NMR (300 MHz, $CDCl_3$) δ 4.53 (t, *J* = 3.8 Hz, 1H), 4.23 (t, *J* = 6.8 Hz, 2H), 3.92 (m, 1H), 2.86 (dt, *J* = 19.0, 5.4 Hz, 1H), 2.65 (dt, *J* = 19.1, 7.2 Hz, 1H), 2.03 (m, 1H), 1.82 (m, 1H), 1.77 (q, *J* = 7.2 Hz, 2H), 1.02 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.81, 132.27, 130.78, 68.59, 67.30, 67.04, 35.88, 27.08, 21.89, 10.60; HRMS calcd for $C_{10}H_{15}BrO_4^+$ 278.0148, found 278.0169.

*Ethyl (5S,6R)-5,6-dihydroxy-2-chlorocyclohex-1,3-diene-carboxylate (**14b**):* white solid; mp 78−80 °C (pentane);

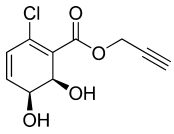
dx.doi.org/10.1021/op400343c | *Org. Process Res. Dev.* 2014, 18, 801−809



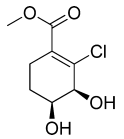
$[\alpha]_D^{20}$ = 46.1 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.18 (ddd, J = 9.8, 1.9, 1.0 Hz, 1H), 6.01 (dd, J = 9.8, 2.4 Hz, 1H), 4.64 (dd, J = 6.1, 1.4 Hz, 1H), 4.53 (m, 1H), 4.34 (m, 2H), 1.38 (t, J = 7.20 Hz, 3H).



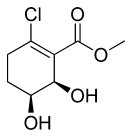
*Allyl (5S,6R)-5,6-dihydroxy-2-chlorocyclohex-1,3-dienecarboxylate (**14d**):* white solid; mp 61−63 °C (pentane); $[\alpha]_D^{20}$ = 33.8 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.16 (d, J = 10.7 Hz, 1H), 5.99 (dd, J = 10.1, 2.6 Hz, 1H), 5.95 (m, 1H), 5.39 (dd, J = 17.2, 1.4 Hz, 1H), 5.27 (dd, J = 10.5, 1.5 Hz, 1H), 4.74 (m, 2H), 4.62 (br d, 1H), 4.50 (br d, 1H).



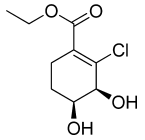
*Propargyl (5S,6R)-5,6-dihydroxy-2-chlorocyclohex-1,3-dienecarboxylate (**14e**):* white solid; mp 66−69 °C (pentane); $[\alpha]_D^{20}$ = 29.5 (c = 0.65, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.22 (dt, J = 9.8, 1.8 Hz, 1H), 6.03 (dd, J = 2.5, 9.9 Hz, 1H), 4.87 (t, J = 2.0 Hz, 2H), 4.68 (d, J = 5.9 Hz, 1H), 4.55 (br d, 1H), 2.54 (t, J = 2.51 Hz, 1H).



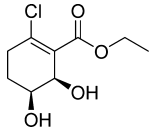
*Methyl (3S,4S)-3,4-dihydroxy-2-chlorocyclohex-1-enecarboxylate (**21a**):* white solid; mp 91−93 °C (pentane) [lit.[36] mp 92−94 °C (pentane)]; R_f = 0.23 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −59.7 (c = 0.2, $CHCl_3$) [lit.[36] $[\alpha]_D^{20}$ = −62.3 (c = 0.4, $CHCl_3$)]; IR (KBr, cm^{-1}) 3400, 2953, 1728, 1627, 1435, 1344, 1259, 1143, 1091, 985, 931; ^1H NMR (300 MHz, $CDCl_3$) δ 4.24 (br d, 1H), 4.00 (m, 1H), 3.82 (s, 3H), 2.64 (dt, J = 18.1, 5.7 Hz, 1H), 2.38 (dt, J = 18.0, 6.4 Hz, 1H), 1.94 (m, 1H), 1.83 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.86, 133.96, 130.65, 71.10, 68.07, 52.23, 25.42, 25.15; HRMS calcd for $C_8H_{11}ClO_4^+$ 206.0346, found 206.0350; Anal. Calcd for $C_8H_{11}ClO_4$: C, 46.50; H, 5.37. Found: C, 46.69; H, 5.44.



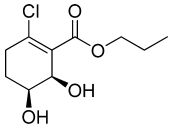
*Methyl (5S,6R)-5,6-dihydroxy-2-chlorocyclohex-1-enecarboxylate (**22a**):* off-white solid; mp 74−75 °C (pentane), mp 74−76 °C (pentane) [lit.[36] mp 75−77 °C (pentane)]; R_f = 0.18 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −65.9 (c = 0.8, $CHCl_3$), $[\alpha]_D^{20}$ = −66.5 (c = 1.0, $CHCl_3$) [lit.[36] $[\alpha]_D^{20}$ = −68.5 (c = 1.0, $CHCl_3$)]; IR (KBr, cm^{-1}) 3228, 2956, 1731, 1430, 1336, 1245, 1101, 1040, 967, 931; ^1H NMR (300 MHz, $CDCl_3$) δ 4.56 (br d, 1H), 3.87 (m, 4H), 2.69 (dt, J = 19.0, 5.3 Hz, 1H), 2.51 (dt, J = 19.0, 7.3 Hz, 1H), 2.02 (m, 1H), 1.82 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.69, 142.58, 127.30, 67.88, 67.22, 52.25, 33.39, 26.00; HRMS calcd for $C_9H_{13}ClO_4^+$ 206.0346, found 206.0352; Anal. Calcd for $C_9H_{13}ClO_4$: C, 46.50; H, 5.37. Found: C, 46.63; H, 5.40.



*Ethyl (3S,4S)-3,4-dihydroxy-2-chlorocyclohex-1-enecarboxylate (**21b**):* white solid; mp 68−70 °C (pentane); R_f = 0.30 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −62.3 (c = 0.4, $CHCl_3$); IR (KBr, cm^{-1}) 3268, 2952, 1726, 1624, 1464, 1368, 1289, 1254, 1094, 940; ^1H NMR (300 MHz, $CDCl_3$) δ 4.29 (q, J = 7.0 Hz, 1H), 4.25 (s, 2H), 4.02 (s, 1H), 2.65 (dt, J = 18.0, 5.9 Hz, 1H), 2.38 (dt, J = 18.0, 6.5 Hz, 1H), 1.95 (m, 1H), 1.83 (m, 1H), 1.35 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.46, 133.32, 131.05, 71.10, 68.09, 61.40, 25.41, 25.18, 14.10; HRMS calcd for $C_9H_{13}ClO_4^+$ 220.0402, found 220.0505.



*Ethyl (5S,6R)-5,6-dihydroxy-2-chlorocyclohex-1-enecarboxylate (**22b**):* white solid; mp 74−76 °C (pentane); R_f = 0.33 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −72.3 (c = 0.45, $CHCl_3$); IR (KBr, cm^{-1}) 3271, 2958, 1717, 1624, 1461, 1424, 1365, 1241, 1097, 1043; ^1H NMR (300 MHz, $CDCl_3$) δ 4.54 (s, 1H), 4.36 (q, J = 7.11 Hz, 2H), 3.86 (s, 1H), 2.67 (dt, J = 19.0, 5.4 Hz, 1H), 2.49 (dt, J = 18.7, 7.3 Hz, 1H), 1.99 (m, 1H), 1.79 (m, 1H), 1.33 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.29, 142.24, 127.39, 67.89, 67.14, 61.50, 33.34, 26.03, 14.09; HRMS calcd for $C_9H_{13}ClO_4^+$ 220.0402, found 220.0405; Anal. Calcd for $C_9H_{13}ClO_4$: C, 48.99; H, 5.94. Found: C, 48.77; H, 5.91.



*n-Propyl (5S,6R)-5,6-dihydroxy-2-chlorocyclohex-1-enecarboxylate (**22c**):* white solid; mp 60−63 °C (pentane); R_f = 0.34 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −65.6 (c = 0.3, $CHCl_3$); IR (KBr, cm^{-1}) 3420, 3304, 2964, 1714, 1661, 1552, 1521, 1392, 1344, 1271, 1094, 1056, 1009, 928, 795; ^1H NMR (300 MHz, $CDCl_3$) δ 4.58 (m, 1H), 4.24 (t, J = 6.72 Hz, 2H), 3.90 (m, 1H), 2.73 (dt, J = 18.8, 5.7 Hz, 1H), 2.55 (dt, J = 18.4, 7.3 Hz, 1H), 2.03 (m, 1H), 1.85 (m, 1H), 1.77 (q, J = 7.05 Hz, 2H), 1.02 (t, J = 7.47 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.39, 142.99, 127.10, 68.01, 67.18, 67.12, 33.62, 26.13, 21.91, 10.58; HRMS calcd for $C_{10}H_{15}ClO_4^+$ 234.0659, found 234.06609.

*Methyl (5S,6R)-5,6-dihydroxy-2-fluorocyclohexa-1,3-dienecarboxylate (**16a**):* white solid; mp 72−73 °C (pentane), mp 73−75 °C (pentane), mp 73−76 °C (pentane), mp 74−77 °C

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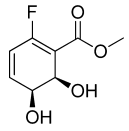
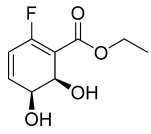
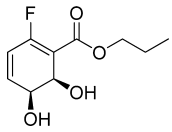


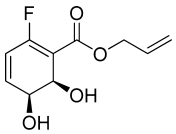
(pentane) [lit.[26] value: mp 74−76 °C (ethyl acetate)]; R_f = 0.15 (1:1 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = +68.2 (*c* = 1.1, MeOH), +69.4 (*c* = 1.0, MeOH), +70.2 (*c* = 0.9, MeOH), +72.8 (*c* = 1.0, MeOH) [lit.[26] value: $[\alpha]_D^{20}$ = +73.2 (*c* = 1.05, MeOH)]; IR (KBr, cm^{-1}) 3558, 3025, 1694, 1439, 1401, 1040; ^{1}H 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 (br s, 1H), 3.09 (br s, 1H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 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); HRMS calcd for $C_8H_9FO_4^+$ 188.0485, found 188.0484; Anal. Calcd for $C_8H_9FO_4$: C, 51.07; H, 4.82; found: C, 51.18; H, 4.76.



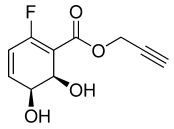
*Ethyl (5S,6R)-5,6-dihydroxy-2-fluorocyclohex-1,3-dienecarboxylate (**16b**):* white solid; mp 70−72 °C (pentane); R_f = 0.24 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −11.6 (*c* = 0.65, $CHCl_3$); IR (KBr, cm^{-1}) 3418, 2986, 1693, 1601, 1407, 1260, 1138, 1096, 1045, 992, 832; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34 (m, 1H), 5.96 (ddd, *J* = 10.6, 8.3, 2.7 Hz, 1H), 4.73 (t, *J* = 6.2 Hz, 1H), 4.56 (m, 1H), 4.31 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.60 (d, *J* = 2.4 Hz), 162.98 (d, *J* = 279.3 Hz), 142.90 (d, *J* = 12.1 Hz), 119.71 (d, *J* = 36.0 Hz), 106.54 (d, *J* = 3.2 Hz), 69.01 (s), 67.10 (d, *J* = 6.1 Hz), 61.28 (s), 14.22 (s); HRMS calcd for $C_9H_{11}FO_4^+$ 202.0641, found 202.0647; Anal. Calcd for $C_9H_{11}FO_4$: C, 53.47; H, 5.48. Found: C, 53.20; H, 5.51.



*n-Propyl (5S,6R)-5,6-dihydroxy-2-fluorocyclohex-1,3-dienecarboxylate (**16c**):* white solid; mp 52−54 °C (pentane); R_f = 0.30 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −11.3 (*c* = 0.55, $CHCl_3$); IR (KBr, cm^{-1}) 3390, 2970, 1693, 1600, 1407, 1266, 1137, 1095, 1042, 993, 938, 829; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34 (m, 1H), 5.96 (ddd, *J* = 10.6, 8.2, 2.6 Hz, 1H), 4.73 (t, *J* = 6.4, 1H), 4.57 (br d, 1H), 4.21 (m, 2H), 1.74 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.77 (d, *J* = 3.3 Hz), 164.81 (s), 161.09 (s), 142.87 (d, *J* = 12.0 Hz), 119.74 (d, *J* = 36.0 Hz), 106.51 (d, *J* = 2.3 Hz), 68.95 (s), 67.12 (d, *J* = 11.4 Hz), 66.86 (s), 21.96 (s), 10.42 (s); HRMS calcd for $C_{10}H_{13}FO_4^+$ 216.0798, found 216.0801.



*Allyl (5S,6R)-5,6-dihydroxy-2-fluorocyclohex-1,3-dienecarboxylate (**16d**):* white solid; mp 56−58 °C (pentane); R_f = 0.32 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = −5.4 (*c* = 0.35, $CHCl_3$); IR (KBr, cm^{-1}) 3418, 1694, 1651, 1600, 1404, 1259, 1138, 1095, 1044, 994, 930, 829; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (m, 1H), 5.95 (m, 2H), 5.40 (ddd, *J* = 17.2, 2.3, 1.4 Hz, 1H), 5.28 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.75 (m, 3H), 4.57 (br d, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.28 (t, *J* = 2.9 Hz), 161.47 (s), 143.24 (d, *J* = 25.9 Hz), 131.71 (s), 119.67 (d, *J* = 35.8 Hz), 118.35 (s), 106.31 (d, *J* = 2.1 Hz), 69.07 (s), 67.04 (d, *J* = 6.2 Hz), 65.73 (s); HRMS calcd for $C_{10}H_{11}FO_4^+$ 214.0641, found 214.0642.



*Propargyl (5S,6R)-5,6-dihydroxy-2-fluorocyclohex-1,3-dienecarboxylate (**16e**):* white solid; mp 82−84 °C (pentane); R_f = 0.27 (3:2 v/v ethyl acetate/hexanes); $[\alpha]_D^{20}$ = +13.4 (*c* = 1.7, $CHCl_3$); IR (KBr, cm^{-1}) 3296, 2949, 1703, 1654, 1599, 1405, 1256, 1135, 1095, 1045, 991, 828; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (m, 1H), 5.95 (ddd, *J* = 10.6, 8.3, 2.8 Hz, 1H), 4.82 (d, *J* = 2.5, 2H), 4.72 (t, *J* = 6.3 Hz, 1H), 4.56 (br d, 1H), 2.51 (t, *J* = 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.83 (s), 164.63 (d, *J* = 2.6 Hz), 162.08 (s), 143.85 (d, *J* = 12.7 Hz), 119.58 (d, *J* = 35.8 Hz), 105.83 (d, *J* = 2.1 Hz), 75.27 (s), 69.14 (s), 66.88 (d, *J* = 5.7 Hz), 52.52 (s); HRMS calcd for $C_9H_{13}ClO_4^+$ 212.0485, found 212.0482. Anal. Calcd for $C_9H_{13}BrO_4$: C, 56.61; H, 4.28. Found: C, 56.87; H, 4.42.

■ ASSOCIATED CONTENT

Supporting Information

Copies of NMR spectra for compounds (**8b**, **9b**, **9d**, **9e**, **12b**, **12d**, **12e**, **14b**, **14d**, **14e**, **16a**, **16b**, **16c**, **16d**, **16e**, **17a**, **17b**, **18a**, **18b**, **18c**, **19a**, **19b**, **20a**, **20b**, **20c**, **21a**, **21b**, **22a**, **22b**, **22c**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Hudlicky, T.; Reed, J. W. *Synlett* **2009**, 685.
(2) Hudlicky, T.; Reed, J. W. *Chem. Soc. Rev.* **2009**, *38*, 3117.
(3) Boyd, D. R.; Sharma, N. D. *J. Mol. Catal. B: Enzym.* **2002**, *19*−*20*, 31.
(4) Boyd, D. R.; Bugg, T. D. H. *Org. Biomol. Chem.* **2006**, *4*, 181.

(5) Banwell, M. G.; Edwards, A. J.; Harfoot, K. A.; Jolliffe, K. A.; McLeod, M. D.; McRae, K. J.; Stewart, S. G.; Vogtle, M. *Pure Appl. Chem.* **2003**, *75*, 223.
(6) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *32*, 35.
(7) Gibson, D. T.; Koch, J. R.; Schuld, C. L.; Kallio, R. E. *Biochemistry* **1968**, *7*, 3795.
(8) Gibson, D. T.; Koch, J. R.; Kallio, R. E. *Biochemistry* **1968**, *7*, 2653.
(9) Gibson, D. T.; Hensley, M.; Yoshioka, H.; Mabry, T. J. *Biochemistry* **1970**, *9*, 1626.
(10) Zylstra, G. J.; Gibson, D. T. *J. Biol. Chem.* **1989**, *264*, 14940.
(11) Johnson, R. A. *Org. React.* **2004**, *63*, 117.
(12) Johnson, R. A. *Microbial Arene Oxidations.* Organic Reactions, Vol. *63*; Wiley: New York, 2002.
(13) (a) Boyd, D. R.; Sharma, N. D.; Allen, C. C. R. *Curr. Opin. Biotechnol.* **2001**, *12*, 564. (b) Boyd, R. D.; Sheldrake, N. G. *Nat. Prod. Rep.* **1998**, *15*, 309.
(14) Carless, H. A. J. *Tetrahedron Asymmetry* **1992**, *3*, 795.
(15) Brown, S. M.; Hudlicky, T. In *Organic Synthesis: Theory and Applications*; Hudlicky, T., Ed.; JAI Press: Greenwich, CT, 1993.
(16) Reed, J. W.; Hudlicky, T. In *Advances in Asymmetric Synthesis*; Hassner, A., Ed.; JAI Press: Greenwich, CT, 1995.
(17) Widdowson, D. A.; Ribbons, D. W.; Thomas, S. D. *Janssen Chim. Acta* **1990**, *8*, 3.
(18) Duchek, J.; Adams, D. R.; Hudlicky, T. *Chem. Rev.* **2011**, *111*, 4223.
(19) Ley, S. V.; Sternfeld, F.; Taylor, S. *Tetrahedron Lett.* **1987**, *28*, 225.
(20) Today there are several groups active worldwide in this discipline: Hudlicky (Brock University), Banwell (Australian National University), Gonzalez (University of Montevideo), Seoane (University of Montevideo), Boyd (Belfast), Lewis (Bath).
(21) Reiner, A. M.; Hegeman, G. D. *Biochemistry* **1971**, *10*, 2530.
(22) (a) Adams, D. R.; van Kempen, J.; Hudlicky, J. R.; Hudlicky, T. *Heterocycles* **2014**, *88*, 1255. (b) Griffen, J.; White, J.; Kociok-Kohn, G.; Lloyd, M.; Wells, A.; Arnot, T.; Lewis, S. *Tetrahedron* **2013**, *69*, 5989. (c) Ali Khan, M.; Mahon, M. F.; Lowe, J. P.; Stewart, A. J. W.; Lewis, S. E. *Chem.—Eur. J.* **2012**, *18*, 13480. (d) Palframan, M. J.; Kociok-Kohn, G.; Lewis, S. E. *Chem.—Eur. J.* **2012**, *18*, 4766. (e) Adams, D. R.; Aichinger, C.; Collins, J.; Rinner, U.; Hudlicky, T. *Synlett* **2011**, *5*, 725. (f) Palframan, M. J.; Kociok-Kohn, G.; Lewis, S. E. *Org. Lett.* **2011**, *13*, 3150. (g) Griffen, J. A.; Le Coz, A. M.; Kociok-Kohn, G.; Ali Khan, M.; Stewart, A. J. W; Lewis, S. E. *Org. Biomol. Chem.* **2011**, *9*, 3920. (h) Pilgrim, S.; Kociok-Kohn, G.; Lloyd, M. D.; Lewis, S. E. *Chem. Commun.* **2011**, *47*, 4799. (i) Mihovilovic, M.; Fischer, T.; Leisch, H. *Monatsh. Chem.* **2010**, *141*, 699. (j) Charest, M. G.; Lerner, C. D.; Brubaker, J. D.; Siegel, D. R.; Myers, A. G. *Science* **2005**, 395. (k) Mihovilovic, M. D.; Leisch, H. G.; Mereiter, K. *Tetrahedron Lett.* **2004**, *45*, 7087. (l) Parker, M. H.; Maryanoff, B. E.; Reitz, A. B. *Synlett* **2004**, *2004*, 2095. (m) Myers, A. G.; Siegel, D. R.; Buzard, D. J.; Charest, M. G. *Org. Lett.* **2001**, *3*, 2923. (n) Jenkins, G. N.; Ribbons, D. W.; Widdowson, D. A.; Slawin, A. M. Z.; Williams, D. J. *J. Chem. Soc., Perkin Trans. 1* **1995**, *20*, 2647.
(23) Fabris, F.; Collins, J.; Sullivan, B.; Leisch, H.; Hudlicky, T. *Org. Biomol. Chem.* **2009**, *7*, 2619.
(24) (a) Blacker, A.; Booth, R.; Davies, G.; Sutherland, J. *J. Chem. Soc., Perkin Trans. 1* **1995**, *1*, 2861. (b) Blacker, A.; Brown, S. M.; Bowden, M. C. *Hydroxy Derivatives of Cyclohexadiene*. Int. Pat. WO/1993/17994, September 16, 1993.
(25) (a) Werner, L.; Machara, A.; Sullivan, B.; Carrera, I.; Moser, M.; Adams, D. R.; Hudlicky, T.; Andraos, J. *J. Org. Chem.* **2011**, *76*, 10050. (b) Werner, L.; Machara, A.; Hudlicky, T. *Adv. Synth. Catal.* **2010**, *352*, 195. (c) Sullivan, B.; Carrera, I.; Drouin, M.; Hudlicky, T. *Angew. Chem., Int. Ed.* **2009**, *48*, 4229.
(26) Semak, V.; Metcalf, T. A.; Endoma-Arias, M. A. A.; Mach, P.; Hudlicky, T. *Org. Biomol. Chem.* **2012**, *10*, 4407.
(27) Trant, J. F.; Froese, J.; Hudlicky, T. *Tetrahedron: Asymmetry* **2013**, *24*, 184.
(28) Endoma-Arias, M. A. A.; Hudlicky, T. *Tetrahedron Lett.* **2011**, *52*, 6632.
(29) Sloman, D. L.; Bacon, J. W.; Porco, J. A. *J. Am. Chem. Soc.* **2011**, *133*, 9952.
(30) Winter, D.; Endoma-Arias, M. A.; Hudlicky, T.; Beutler, J.; Porco, J. *J. Org. Chem.* **2013**, *78*, 7617.
(31) Endoma, M. A.; Bui, V. P.; Hansen, J.; Hudlicky, T. *Org. Process Res. Dev.* **2002**, *6*, 525.
(32) Juaristi, E. *Conformational Behavior of Six-Membered Rings: Analysis, Dynamics, and Stereoelectronic Effects*; Wiley: New York,1995; pp 200−217.
(33) Eliel, E. L.; Wilen, S. H., Mander, L. N. *Stereochemistry of Organic Compounds*; Wiley: New York, 1994; pp 696−697.
(34) Taft, R. W. *J. Am. Chem. Soc.* **1952**, *74*, 3120.
(35) (a) Charton, M. *J. Org. Chem.* **1976**, *41*, 2217. (b) Charton, M. *J. Am. Chem. Soc.* **1975**, *91*, 1552.
(36) Endoma-Arias, M. A. A.; Froese, J. Unpublished observations. 2012.