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Lipase-catalyzed resolution of 1,3-dioxolane derivatives: synthesis of a homochiral intermediate for antifungal agents

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Abstract—Dioxolane alcohol (±)-**1** and the corresponding acid (±)-**2** were kinetically resolved into their respective enantiomers by lipase-catalyzed hydrolysis and esterification reactions. Various alcohols were tested to resolve the acid (±)-**2**, and the desired (2*R*,4*R*)-isomer of the acid was obtained with >96% ee as the best result. Halogenated solvents such as methylene chloride and 1,2-dichloroethane were found to raise the reactivity and selectivity of the system. After recrystallization, the purity of the desired (2*R*,4*R*)-acid could be increased to over 98% ee.

1. Introduction

Azoles such as itraconazole, ketoconazole and voriconazole are important anti-fungal agents. These chiral compounds are stereoisomeric, and each isomer displays different biological activities. Thus, single enantiomer pharmaceuticals are increasingly attractive owing to their biological importance.[1] For instance, itraconazole is marketed as a racemic mixture and only one isomer displays valuable biological activity, inhibiting the cytochrome P450-dependent synthesis of ergosterol. This pharmaceutical has been found to cause nausea, vomiting, anorexia, headache and dizziness as side effects. Many efforts have been made to reduce these side effects, but the best way is to prescribe the drug as a single enantiomer. The dioxolanes **1** and **2** are important precursors of itracoanazole and ketoconazole.[2] These compounds have two stereogenic centers and four stereoisomers, respectively. The chemical synthesis of the racemic mixture of itraconazole is well known by the method described in a previous report.[3] Among the four isomers, *cis*-isomers are separated from *trans*-ones by selective crystallization of the benzoyl ester derivatives. Thus, efforts to obtain the *cis*-(2*R*,4*S*)-isomer of dioxolane have been focused on resolving its enantiomer. There are some reports to get its pure enantiomer by the chemical or the enzymatic method.[4,5] Herein, we report our results from the enzymatic resolution of racemic *cis*-dioxolane (±)-**1** and its acid analog (±)-**2** (Fig. 1).

2. Results and discussion

To resolve *cis*-dioxolane (±)-**1**, it was transesterified with vinyl acetate as acyl donor using several lipases such as *Candida rugosa*, *Mucor miehei*, *Aspergillus niger*, *Candida antarctica* B, *Pseudomonas cepacia*, *Pseudomonas fluorescens* and esterases such as pig liver, bovine cholesterol. Unfortunately, using these enzymes, **1** was resolved poorly, and good enantiomeric purities were not obtained. The alternative approach, involving hydrolysis of the ester derivatives with the same biocatalysts, also failed to give good resolution. Recently, Servi et al.[6] reported the resolution of dioxolane derivatives using various enzymes. Among their results, the dioxolane **1** was efficiently resolved in 99% ee at 10% conversion using lipase from *A. niger*. However, this method is unfavorable in economic terms. Whilst searching for economical and efficient enzymatic methods, a report was found which stated that changing the acyl moiety from an acetyl group to a longer chain carboxylic acid residues afforded improved enantioselectivity in the resolution of several primary alcohols.[7] At first, butanoic acid ester **4**, was hydrolyzed with four enzymes and resolved in moderate enantiopurity when

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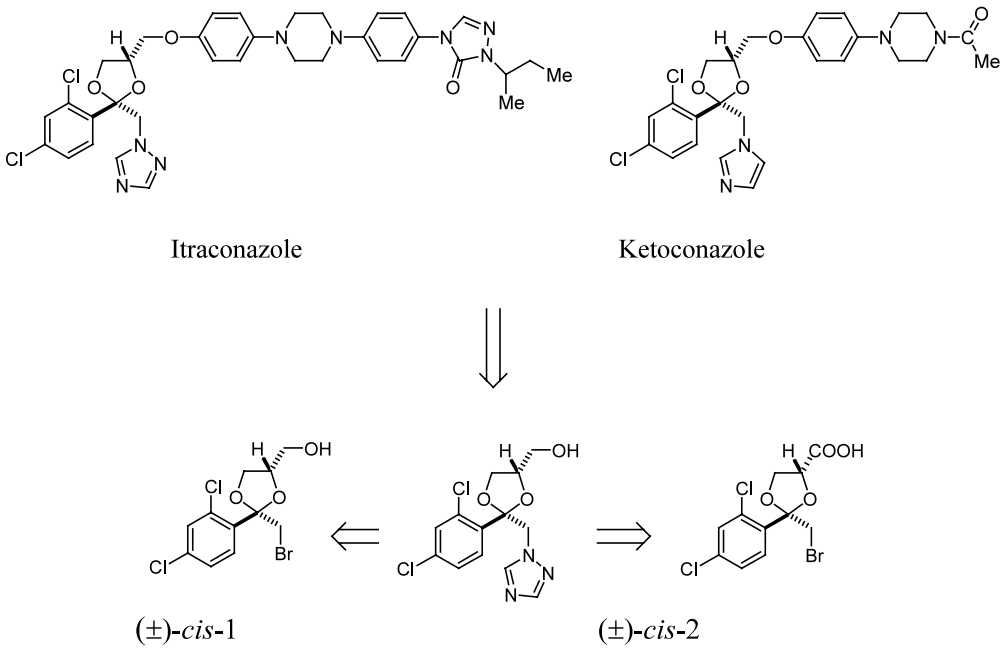
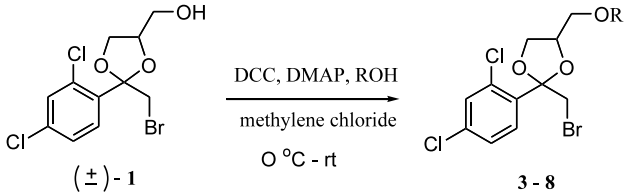


Figure 1. Precursors for enzymatic resolution.

using the lipase from *P. cepacia*. Therefore, we investigated the effect of different acyl moieties on the resolution of (±)-**1** shown in Scheme 1 by preparing **3**–**8** as substrates.

The substrate (±)-**1** was coupled with straight chain alkanoic acids using *N*,*N*-dicyclohexyl carbodiimide. The resulting products **3**–**8** were obtained in 80–90% yields and purified by column chromatography. The hydrolysis reactions of **3**–**8** with PCL were then performed as shown in Scheme 2 and the results are shown in Table 1.

In this reaction, the absolute stereochemistry of the resolved products was identified by the specific rotation. Of the straight chain alkanoic acid esters, the hexanoic acid ester **7** was well resolved in 94% ee at 85% conversion. This level of enantiomeric purity was not sufficient



$R = CO(CH_2)_nCH_3$

3 n = 0
4 n = 1
5 n = 2
6 n = 3
7 n = 4
8 n = 5

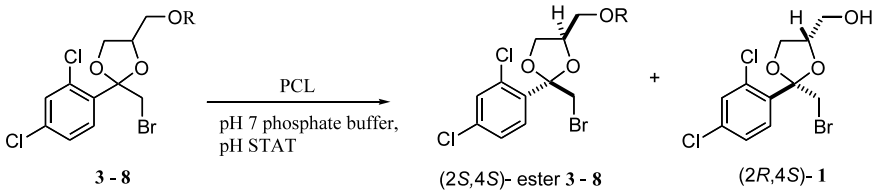
Scheme 1. Synthesis of the ester derivatives of (±)-**1** for enzymatic hydrolysis.

and the conversion was too high. To find the optimum conditions, the reaction was performed in the presence of additives, such as KCl and 18-crown-6. These additives are known to change the enzyme structure and positively affect the efficiency and selectivity of the resolution. Our results are shown in Tables 2 and 3.

Both of the additives increased the enantiomeric purity and accelerated the rate of hydrolysis in accordance with the amounts used. The ee of the unreacted ester increased to 99% at 75–85% conversion. However, these results are not satisfactory in terms of the yields, and unfortunately the enantiomerically pure ester is not the desired isomer. We attempted the esterification of (±)-**1** with the same aliphatic acids, but their resolutions were not adequate for our purposes either. Therefore, we tried to use dioxolane acid (±)-**2** as the other modified substrate. Acid **2** was synthesized by oxidation of the alcohol **1** using Jones' reagent.

In the resolution of (±)-**2** via enzyme catalyzed esterification, various alcohols were used, since we previously reported the results on a similar reaction and substrate.[8] Of the enzymes screened, lipase B from *C. antarctica* (CAL-B) was the most effective for the resolution of (±)-**2**. The reaction conditions are shown in Scheme 3.

Firstly, the effect of the alcohol chain length on the enantioselectivity of the CAL-B-catalyzed esterification was examined and the results obtained are shown in Table 4. We are interested in the remaining pure acid since it is the correct isomer for the synthesis of the anti-fungal pharmaceuticals. The use of methanol and hexanol led to the highest enantioselectivity. Methanol is better than hexanol in terms of the yield. It is also notable that if we want to obtain the unreacted enantiopure ester, we can control the conversion using methanol, ethanol and butanol.



Scheme 2. Enzymatic hydrolysis for the resolution of (±)-*cis*-dioxolane derivatives.

Table 1. Influence of alkyl chain length on enantioselectivity of PCL-catalyzed hydrolyses

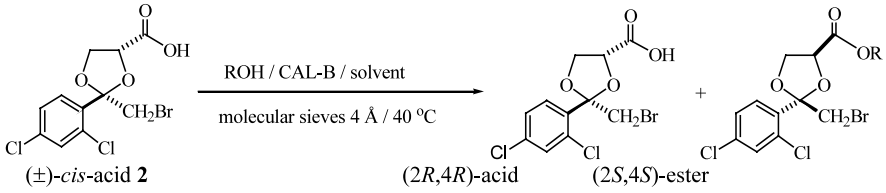
Substrate (n)	Time (h)	Conversion (%)	Alcohol ee (%)	Ester ee (%)	*E*
0	1.5	50	81	43	14
	3	60	40	48	3.6
1	1.5	49	44	43	3.8
	3	52	33	56	3.3
2	1	62	33	56	3.3
	3	79	14	61	2.2
3	0.5	50	56	60	6.4
	2	68	45	83	6.4
4	1	68	47	70	5.5
	2	85	6	94	2.5
5	0.5	56	23	43	2.3
	1.5	87	11	64	2.1

Table 2. Effect of KCl additive on the resolution of (±)-**7**

KCl additive (g)	Time (min)	Conversion (%)	Alcohol ee (%)	Ester ee (%)	*E*
0.7	60	84	20	99	5.8
1.4	50	88	17	99	5.3
2.1	35	79	32	87	4.9
2.8	23	68	31	78	4.1
4.2	20	57	49	57	5

Table 3. Effect of crown ether additive on the resolution of (±)-**7**

Crown ether additive (g)	Time (min)	Conversion (%)	Alcohol ee (%)	Ester ee (%)	*E*
0.7	100	87	20	99	5.8
1.4	50	79	17	93	3.7
2.1	30	74	35	99	9.1
2.8	10	61	58	99	18
4.2	10	53	42	9	2.7



Scheme 3. General procedure for esterification of acid with various alcohols.

Next, we studied the effect of solvents on the enantioselectivity as shown in Table 5.

The choice of the solvent significantly affects the reaction rate and enantioselectivity. With halogenated solvents, such as methylene chloride, chloroform and 1,2-dichloroethane, the esterification reaction gave adequate results (95% ee at about 60% conversion) though the rate of the reaction was very low. The other solvents were found to be inadequate for our substrate.

Table 4. The effect of alcohol chain length on the enantioselectivity of CAL-B-catalyzed esterification

Alcohol	Time (h)	Conversion (%)	Acid ee (%)	Ester ee (%)	*E*
Methanol	11	35	35	95	54
	48	63	95	82	37
Ethanol	11	26	23	95	48
	48	58	90	90	58
Butanol	11	34	24	96	61
	48	55	86	93	76
Hexanol	48	76	95	80	32
Octanol	48	57	54	81	16

Table 5. Solvent effects on the enantioselectivity of CAL-B-catalyzed esterification

Solvent	Time (h)	Conversion (%)	Acid ee (%)	Ester ee (%)	*E*
Methylene chloride	117	68	89	91	63
1,2-Dichloroethane	236	61	95	77	27
Chloroform	115	76	95	24	4.9
Acetonitrile	120	38	36	97	93
Diisopropyl ether	17	65	53	55	5.7
Tetrahydrofuran	108	8	5	98	104
n-Hexane	17	97	68	47	5.4

Table 6. The effect of temperature on the conversion rate of CAL-B-catalyzed esterification using MeOH

Temperature (°C)	Time (h)	Conversion (%)	Acid ee (%)	Ester ee (%)	*E*
40	20	6	3	38	2.3
	51	29	26	78	10
	237	61	95	77	27
50	19	45	65	98	194
	31	50	76	96	112
	75	65	95	95	145
60	19	43	58	97	118
	56	57	89	93	82
	75	62	96	92	94

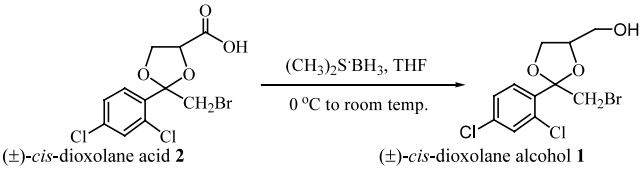
To increase the reaction rate, the temperature was varied within the working range of the enzyme (CAL-B is known to be stable at temperatures up to 60°C). The results are shown in Table 6.

As expected, the reaction rate increased with the temperature in a linear relationship. At 60°C, the desired acid was obtained in 96% ee at 62% conversion within 75 h. Under these conditions, the enzyme was not deactivated during three recycles. The resolved acid **2** was recrystallized from *n*-hexane/ethyl acetate, the enantiomeric purity finally attained being 98% ee. The acid **2** from the resolved ester was more easily crystallized than the desired isomer. Thus we could not obtain product with >99% ee.

(±)-*cis*-Acid **2** was then quantitatively reduced to the corresponding alcohol **1** using borane–dimethylsulfide complex reagent as shown in Scheme 4.

3. Conclusions

We have examined the resolution of the racemic dioxolane alcohol (±)-**1** and acid (±)-**2** as the intermediate for an azole antifungal agent. Among the various enzymes, *P. cepacia* and *C. antarctica* lipase B recognized these substrates well. In particular, CAL-B was efficient for esterification in view of the reaction rate and enantioselectivity and the faster reacting isomer



(±)-*cis*-dioxolane acid **2** (±)-*cis*-dioxolane alcohol **1**

Scheme 4. The conversion of acid **2** to alcohol **1** using borane–dimethyl sulfide complex.

was the undesired one. However, the desired remaining acid was easily recrystallized allowing its enantiomeric purity to be increased. The product will be coupled with the appropriate fragment of itraconazole. These resolving conditions will also be applied to functionally similar fine chemicals and pharmaceutical.

4. Experimental

4.1. General

The organic solvents, methanol, *n*-hexane and 2-propanol were all HPLC-grade and purchased from J. T. Baker. Trifluoroacetic acid (TFA) was obtained from Aldrich. Lipase PS 'AMANO' (PCL) was obtained from Amano Phamarceutical Co., Ltd. *Candida antartica* lipase B was obtained from NOVOZYME. The IR spectra were measured on a Perkin–Elmer 16FPC FT-IR grating infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, at 300 MHz, respectively, on a Varian Gemini-300 spectrometer; chemical shifts being expressed in ppm with reference to Me_4Si, coupling constants (J) in Hz. Mass spectra were obtained on a Hewlett–Packard 5890 series II; a Hewlett–Packard 5972 series mass selective detector GC–MS spectrometer equipped with HP-5 cross-linked 5% phenyl methyl silicon fused silica capillary column (25 m×0.20 mm I.D.). The optical rotations were determined with an AUTOPOL III (Rudolph Research, Flanders, NJ) recording polarimeter in MeOH. A YOUNG-LIN high performance liquid chromatograph (Korea), equipped with a YOUNG-LIN M930 Pump; a YOUNG-LIN M720 Absorbance Detector were used. The computer programs used were Autochro 2.0 plus for chromatographic analysis. A chiralcel OD column (0.46 cm I.D.×25 cm) packed with a cellulose derivative coated on silica gel used (Daicel Chemical Industries). TLC was carried out on Merck glass plates precoated with silica gel 60F-254. Column chromatography was performed by Merck 70–230 or 230–400 mesh silica gel. Enzymatic hydrolysis was carried out on 718 STAT Titrino (Metrohom, Switzerland).

4.2. (±)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-methanol, 1

Methanolic KOH solution (1.2N, 50 ml) was added to (±)-*cis*-[2-(bromomethyl)-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-yl]methyl benzoate (0.97 g, 2.16 mmol). The solution was stirred at rt for 4 h. After checking by TLC, methanol was evaporated in vacuo, diluted with water and extracted in ether then dried with $MgSO_4$. The oily residue was purified by column chromatography on silica gel (*n*-hexane: ethyl acetate = 4:1). The product was obtained as an oil (806 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (m, 2H), 3.93 (m, 2H), 4.11 (m, 1H), 4.19 (m, 2H), 7.24 (dd, J = 8.4, 2.4 Hz, 1H), 7.43 (d, J = 2.1 Hz, 1H), 7.64 (dd, J = 9.3 Hz, 8.4 Hz, 1H).

4.3. (±)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(acetoxymethyl)-1,3-dioxolane, 3

1,3-Dicyclohexylcarbodiimide (0.985 g, 4.77 mmol, 1.2 equiv.) and 4-(dimethylamino)pyridine (48.6 mg, 0.40 mmol, 0.1 equiv.) was added to stirred solution of acetic acid (273 μl, 4.77 mmol, 1.2 equiv.) in dried methylene chloride (20 ml) at 0°C. After 30 min, (±)-*cis*-2-(bromomethyl)-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-methanol (1.360 g, 3.98 mmol) in dried methylene chloride (5 ml) was added dropwise to a solution at 0°C for 5 min. The mixture was stirred at rt for 2 h. The reaction mixture was diluted with ethyl acetate then stirred for 10 min. The DCU side product was removed by filtering the mixture and the filtrate was collected and evaporated. Water was added to the mixture and the product was extracted into ethyl acetate. The organic extract was washed with satd $NaHCO_3$, brine and dried over anhydrous $MgSO_4$ then evaporated in vacuo. The residue was purified by column chromatography over silica gel (*n*-hexane:ethyl acetate = 10:1). The product was obtained as an oil (1.63 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (s, 3H), 3.87 (dd, J = 11.4 Hz, 2H), 3.97 (m, 2H), 4.28 (m, 3H), 7.24 (dd, J = 8.4 Hz, J = 2.1 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H).

4.4. (±)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(propionyloxymethyl)-1,3-dioxolane, 4

Compound **4** was synthesized as previously described for compound **3** and the product was obtained as an oil (1.304 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (t, J = 7.5 Hz, 3H), 2.37 (q, J = 7.6 Hz, 2H), 3.84 (dd, J = 11.3 Hz, J = 1.4 Hz, 2H), 3.92 (m, 2H), 4.29 (m, 3H), 7.24 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H), 7.40 (d, J = 2.2 Hz, 1H), 7.59 (d, J = 8.5 Hz, 1H).

4.5. (±)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(butyryloxymethyl)-1,3-dioxolane, 5

Compound **5** was synthesized as previously described for compound **3** and the product (1.27 g, 69%) was obtained as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.1 Hz, 3H), 1.15 (s, J = 7.4 Hz, 2H), 2.32 (t, J = 7.6 Hz, 2H), 3.84(dd, J = 11.3 Hz, J = 1.4 Hz, 2H), 3.91 (m, 2H), 4.26 (m, 3H), 7.24 (dd, J = 8.6 Hz, J = 2,0 Hz, 1H), 7.36 (d, J = 2.2 Hz, 1H), 7.59 (d, J = 8.5 Hz, 1H).

4.6. (±)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(valeryloxymethyl)-1,3-dioxolane, 6

Compound **6** was synthesized as previously described for compound **3** and the product (1.53 g, 83%) was obtained as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.1 Hz, 3H), 1.15 (s, J = 7.4 Hz, 2H), 2.32 (t, J = 7.6 Hz, 2H), 3.84 (dd, J = 11.3 Hz, J = 1.4 Hz, 2H), 3.91 (m, 2H), 4.26 (m, 3H), 7.24 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H), 7.36 (d, J = 2.2 Hz, 1H), 7.59 (d, J = 8.5 Hz, 1H).

4.7. (±)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(hexanoyloxymethyl)-1,3-dioxolane, 7

Compound **7** was synthesized as previously described for compound **3** and the product was obtained as an oil (1.8 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, $J=5.6$ Hz, 3H), 1.32 (m, 4H), 2.36 (t, $J=7.6$ Hz, 2H), 3.84 (dd, $J=11.3$ Hz, $J=1.4$ Hz, 2H), 3.94 (m, 2H), 4.29 (m, 3H), 7.24 (dd, $J=8.6$ Hz, $J=2.0$ Hz, 1H), 7.36 (d, $J=2.2$ Hz, 1H), 7.59 (d, $J=8.5$ Hz, 1H).

4.8. (±)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(heptanoyloxymethyl)-1,3-dioxolane, 8

Compound **8** was synthesized as previously described for compound **3** and the product was obtained as an oil (1.68 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.78 (t, $J=7.1$ Hz, 3H), 1.21 (m, 6H), 1.53 (m, 2H), 2.28 (t, $J=7.1$ Hz, 2H), 3.72 (dd, $J=11.3$ Hz, 1.4 Hz, 2H), 3.83 (m, 2H), 4.22 (m, 3H), 7.17 (dd, $J=8.6$ Hz, $J=2.0$ Hz, 1H), 7.33 (d, $J=2.2$ Hz, 1H), 7.53 (d, $J=8.5$ Hz, 1H).

4.9. Enzymatic hydrolysis of 1,3-dioxolane esters, 3–8

Each of the 1,3-dioxolane esters **3**–**8** (40 mg) was dissolved in phosphate buffer (pH 6.99, 7 ml) and lipase from *P. cepacia* (4 mg, 0.1 mass equiv.) was added to the solution. A pH stat regulated the addition of 0.02N NaOH solution at rt. To maintain the pH at 7.0. 1 ml of solution was taken periodically from the reactor and extracted with ethyl acetate, then the organic layer was washed with satd $NaHCO_3$, and brine. Then dried with anhydrous $MgSO_4$, filtered, and evaporated in vacuo. The slurry was dissolved in 1 ml of MeOH for HPLC analysis. (chiralcel OD, *n*-hexane:*i*-PrOH = 95:5 v/v%, 230 nm, 1 ml/min).

4.10. Enzymatic hydrolysis of 1,3-dioxolane ester, 7 (KCl and crown ether additive effects)

1,3-Dioxolane ester **7** (40 mg, 0.12 mmol) was dissolved in phosphate buffer (pH 7.0) (7 ml), lipase from *P. cepacia* (4 mg, 0.1 mass equiv.) and surfactant (KCl, Crown ether) (10, 20, 30, 40, 60 w/v of buffer) was added to the solution. A pH stat regulated the addition of 0.02N NaOH solution at rt. To maintain the pH at 7.0. 1 ml of solution was taken periodically from the reactor and extracted with ethyl acetate, then organic layer was washed with satd $NaHCO_3$, and brine. The solution was then dried with anhydrous $MgSO_4$, filtered and evaporated in vacuo. The slurry was dissolved in 1 ml of MeOH for HPLC analysis. (chiralcel OD, *n*-hexane:*i*-PrOH = 95:5 v/v%, 230 nm, 1 ml/min).

4.11. (±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid, 2

Jones' reagent (6 ml) was added to a stirred solution of (±)-*cis*-2-(bromomethyl)-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-methanol (545 mg, 1.60 mmol) in acetone (5 ml) at 0°C. The mixture was stirred at rt. The solution was concentrated to a small volume in vacuo, satd $NaHCO_3$ was added to the residue, then the aqueous layer was washed with diethyl ether. The water layer was made acidic with concentrated HCl and the product was extracted with ethyl acetate. The extract was washed with brine, dried with anhydrous $MgSO_4$, evaporated in vacuo to leave an oily residue and crystallized with ethyl acetate/*n*-hexane. White crystal, *cis*-2-bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (440 mg, 77%), was recrystallized in ethyl acetate/*n*-hexane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.26 (m, 3H), 4.43 (dd, $J=8.9$ Hz, $J=4.6$ Hz, 1H), 4.67 (dd, $J=6.2$ Hz, $J=3.9$ Hz 1H), 7.17 (dd, $J=8.6$ Hz, $J=2.0$ Hz, 1H), 7.33 (d, $J=2.2$ Hz, 1H), 7.53 (d, $J=8.5$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.547 68.509 74.061 109.741 127.048 130.021 131.446 132.886 133.486 136.091 174.699; IR (KBr) 2894, 1712, 1586, 1268, 1042 cm^{-1}.

4.12. Enzymatic esterification of (±)-*cis*-2-bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (alcohol chain length effect)

(±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (10 mg, 0.03 mmol) was dissolved in 1,2-dichloroethane (5 ml) and ROH (0.12 mmol, 4 equiv.) and molecular sieves 4 Å (30 mg) was added to the solution. CAL-B (10 mg, 1 mass equiv.) was added to the solution, and then the mixture was shaken in an incubator at 40°C. Alcohols (ROH) used: methanol, ethanol, 1-butanol, 1-hexanol, 1-octanol.

4.13. Enzymatic esterification of (±)-*cis*-2-bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (solvent effect)

(±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (10 mg, 0.03 mmol) was dissolved in each solvent (5 ml) and MeOH (0.24 mmol, 8 equiv.) and 4 Å molecular sieves (30 mg) was added to the solution. CAL-B (10 mg, 1 mass equiv.) was added to the solution, and then the mixture was shaken in a shaking incubator at 40°C. Solvents used: 1,2-dichloroethane, methylene chloride, chloroform, acetonitrile, tetrahydrofuran, diisopropyl ether, *n*-hexane.

4.14. Enzymatic esterification of (±)-*cis*-2-bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (temperature effect)

(±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (10 mg, 0.03 mmol) was dissolved in 1,2-dichloroethane (5 ml) and MeOH (0.24 mmol, 8 equiv.) and molecular sieves 4 Å (30 mg) was added to the solution. CAL-B (10 mg, 1 mass equiv.) was added to the solution, and then each mixture was shaken in an incubator at 40, 50 and 60°C.

4.15. (±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid methyl ester

(±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (100 mg, 0.292 mmol) in toluene (10 ml) was stirred at rt. MeOH (59.1 μl, 1.46 mmol, 5 equiv.) and *p*-toluenesulfonic acid (6 mg, 0.03 mmol, 0.1 equiv.) was added to a solution. The mixture was heated to reflux temperature for 1 h. The mixture was quenched by water. The product was extracted with ether, the organic layer was washed with satd $NaHCO_3$ and brine. The extract was dried with anhydrous $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography (*n*-hexane:ethyl acetate = 10:1) (yield = 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.79 (s, 3H), 4.04 (m, 3H), 4.35 (dd, J = 8.7 Hz, J = 3.9 Hz, 1H), 4.63 (dd, J = 7.2 Hz, J = 3.6 Hz 1H), 7.23 (dd, J = 8.1 Hz, J = 2.1 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H).

4.16. (±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid ethyl ester

(±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (200 mg, 0.56 mmol) in toluene (10 ml) was stirred at rt. EtOH (160 μl, 2.8 mmol, 5 equiv.) and *p*-toluenesulfonic acid (13 mg, 0.06 mmol, 0.1 equiv.) was added to a solution. The mixture was heated to reflux temperature for 1 h. The mixture was quenched with water. The product was extracted with ether, the organic layer was washed with satd $NaHCO_3$, brine. The extract was dried with anhydrous $MgSO_4$ and evaporated in vacuo. The residue (244 mg) was purified by column chromatography (*n*-hexane:ethyl acetate = 10:1) (yield = 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, J = 7.16 Hz, 3H), 4.02 (m, 4H), 4.31 (m, 4H), 4.63 (dd, J = 7.3 Hz, J = 3.9 Hz 1H), 7.26 (dd, J = 8.4 Hz, J = 2.1 Hz, 1H), 7.42 (d, J = 2.1 Hz, 1H), 7.56 (d, J = 8.6 Hz, 1H).

4.17. (±)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-1,3-dioxalane-4-methanol, 1

(±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid **2** (325 mg, 1 mmol) in tetrahydrofuran (3 ml) was stirred at rt. Borane–dimethylsulfide complex in THF solution (2.0 M, 1.5 ml, 1.5 mmol, 1.5 equiv.) was added to a solution at 0°C. The mixture was stirred at 0°C to rt for 2 h. The excess hydride was carefully destroyed with water. The product was extracted with ethyl acetate, the organic layer was washed with satd $NaHCO_3$, brine. The extract was dried with anhydrous $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography (*n*-hexane: ethyl acetate = 4:1) (yield = 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (m, 2H), 3.93 (m, 2H), 4.11 (m, 1H), 4.19 (m, 2H), 7.24 (dd, J = 8.4, 2.4 Hz, 1H), 7.43 (d, J = 2.1 Hz, 1H), 7.64 (dd, J = 9.3 Hz, 8.4 Hz, 1H).

4.18. Enzymatic esterification of (±)-*cis*-2-bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (optimized conditions)

(±)-*cis*-2-Bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid (10 g, 28.1 mmol) was dissolved in 1,2-dichloroethane (500 ml) and MeOH (4.6 ml, 112.4 mmol, 4 equiv.), and molecular sieves 4 Å (10 g) was added to the solution. CAL-B (10 g, 1 mass equiv.) was added to the solution and then each mixture was stirred at 60°C. The mixture was filtered and the filtrate was concentrated to a small volume in vacuo. The residue was treated with satd $NaHCO_3$ and the resulting aqueous layer was washed with ether. The organic layer was washed with brine. The extract was dried with anhydrous $MgSO_4$ and evaporated in vacuo. The water layer was made acidic with concentrated HCl, the product was extracted with ethyl acetate. The extract was washed with brine, dried with anhydrous $MgSO_4$, evaporated in vacuo to leave an oily residue and recrystallized with ethyl acetate/*n*-hexane to afford a white crystalline solid of (2*R*,4*R*)-*cis*-2-bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-carboxylic acid.

Unreacted (2*R*,4*R*)-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.26 (m, 3H), 4.43 (dd, J = 8.9 Hz, J = 4.6 Hz, 1H), 4.67 (dd, J = 6.2 Hz, J = 3.9 Hz 1H), 7.17 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H), 7.33 (d, J = 2.2 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.547 68.509 74.061 109.741 127.048 130.021 131.446 132.886 133.486 136.091 174.699; IR (KBr) 2894, 1712, 1586, 1268, 1042 cm^{-1}; GC/MSD retention time (min) 12.52, *m*/*z* 50, 59, 67, 75, 89, 99, 109, 123, 136, 145, 159, 173, 189, 197, 205, 215, 223, 240, 253, 275 (100), 311; $[\alpha]_D^{26}$ = +28 (*c* 1, MeOH, ee = 98%).

(2*S*,4*S*)-Ester product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.79 (s, 3H), 4.04 (m, 3H), 4.35 (dd, J = 8.7 Hz, J = 3.9 Hz, 1H), 4.63 (dd, J = 7.2 Hz, J = 3.6 Hz 1H), 7.23 (dd, J = 8.1 Hz, J = 2.1 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H).

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