Enantioselective Synthesis of (−)- and (+)-Conduritol F *via* Enzymatic Asymmetrization of *cis*-Cyclohexa-3,5-diene-1,2-diol

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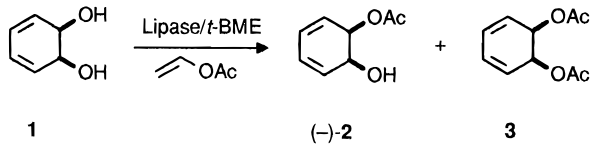
Introduction

Conduritols A−F (5-cyclohexene-1,2,3,4-tetrols)[1] are a class of cyclitols of great relevance in the synthesis of inositol derivatives, such as the antidiabetic (+)-pinitol[2] and the putative insulin mimics D-*myo*-inositol 1,4,5-triphosphate[3] and 6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-1-*O*-dihydrogenphosphoryl-D-*myo*-inositol.[4] Moreover, conduritols themselves and their epoxides are inhibitors of D-glucosidases.[5] Preparation of these tetrols is not always simple, and this is particularly true for those existing as couples of enantiomers (conduritol B, C, E, and F), the synthesis of which from achiral precursors obviously requires a resolution step.[6] To overcome this difficulty stereocontrolled syntheses have been developed, starting from chiral materials, in many instances cyclic *cis*-glycols obtained by microbial oxidation of aromatic substrates mediated by *Pseudomonas putida*, and thus (−)-C, (+)-C, (−)-E, (+)-E, and (−)-F conduritols have been synthesized.[7] Lipase-mediated enantiotoposelective esterification or hydrolysis of *meso*-compounds derived from 2-cyclohexene-1,4-diol has been used to obtain chiral intermediates valuable for the synthesis of conduritols with the desired stereochemistry.[8] As part of a project aimed at the evaluation of the synthetic value[9] of (1*R*,2*S*)-1-acetoxy-2-hydroxycyclohexa-3,5-diene, (−)-**2**, prepared by enantiotoposelective esterification of "benzene *cis*-glycol" **1** catalyzed by lipase,[10] we report the synthesis of homochiral F-conduritol enantiomers via OsO_4-catalyzed dihydroxylation of this hydroxy ester.

Results and Discussion

Enzymatic Preparation of Both Enantiomers of 1-Acetoxy-2-hydroxycyclohexa-3,5-diene. In the course of a study directed to the asymmetrization of cyclic *meso*-diols through the enantiotoposelective esterification mediated by lipases in organic medium,[11] we observed that *Mucor miehei* lipase (immobilized, Lipozyme IM) in *tert*-butyl methyl ether (*t*-BME) promotes the esterification of *cis*-1,2-dihydroxycyclohexa-3,5-diene (**1**) to afford monoester (−)-**2**.[10] In an attempt to optimize the reaction



1 (−)-**2** **3**

we screened several lipases (lipases from *Candida cylindracea*, *C. antarctica*, *Aspergillus niger*, *Rhizopus javanicus*, *Pseudomonas cepacia* and porcine pancreas). Among them, only those from *P. cepacia* and *C. antarctica* (immobilized, Novozym 235) were active in the esterification of **1** in anhydrous *t*-BME with vinyl acetate as the acyl donor. The results, summarized in Table 1, show that *P. cepacia* lipase is the most effective. With this enzyme, conversion reached 90% after 2 h incubation and the reaction mixture contained (−)-**2** (86%) with ee 90% (entry 1), along with minor amounts (4%) of diester **3**. The ee of the desired product could be improved up to >98% by prolonging the reaction time beyond the complete conversion of the substrate (entry 2), due to kinetic amplification;[12] in this case about 10% of diester **3** is formed. *Mucor miehei* lipase gave comparable results in terms of chemical and optical yields, but the reaction rate was lower (entries 3 and 4), while *C. antarctica* lipase was definitely the worst (entry 5), yielding a monoester with low enantiomeric excess which did not improve by prolonging the reaction time (entry 6). Therefore *P. cepacia* lipase was used in a gram scale preparation to give homochiral (−)-**2** in high yield (90%).

Table 1. Esterification of 1 with Vinyl Acetate in *tert*-Butyl Methyl Ether[a]

entry	lipase source	time, h	**1**, %[b]	(−)-**2**, %[b]	ee, %[c]	stereo-preference[d]	**3**, %[b]
1	*P. cepacia*	2	10	86	90	*R*	4
2		3	−	89	>98		11
3	*M. miehei*[e]	4.5	14	82	91	*R*	4
4		6	−	93	95		7
5	*C. antarctica*[f]	2	49	51	68	*R*	−
6		5	−	89	70		11

[a] Reaction conditions: lipase 10 mg/mL, substrate 10 mg/mL, vinyl acetate 1 equiv, 40 °C, 300 rpm. [b] Determined by ^{1}H-NMR spectrum of the reaction mixture. [c] Determined by chirospecific GC analysis of the corresponding tetrahydro derivative. [d] Assigned according ref 10. [e] Immobilized (Lipozyme® IM). [f] Immobilized (Novozym® 435).

Monoester (−)-**2** is very sensitive to the action of water, acids, and heat, undergoing loss of acetic acid to afford phenol. Extensive degradation also occurs on silica gel and chromatographic purification is only possible on Si Diol. The corresponding propanoate and benzoate, obtained by biocatalyzed asymmetrization of **1** using vinyl propanoate or vinyl benzoate, respectively, proved to be as unstable as (−)-**2**.

Since the lipases that catalyze the esterification of **1** all give the same monoester, (−)-**2**, to obtain the opposite

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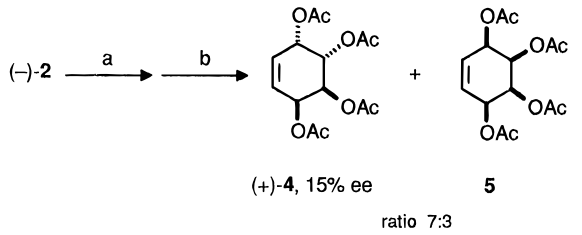
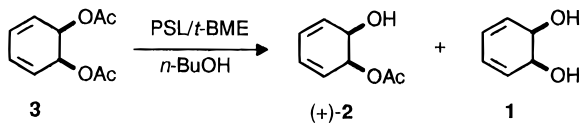
Scheme 1[a]



[a] Reaction conditions: (a) NMMO; 1% OsO_4, CH_2Cl_2/*t*-BME, rt; (b) Ac_2O, Py, rt.

enantiomer we resorted to the alcoholysis of diester **3** prepared by chemical esterification of **1**. Therefore, when **3** was treated with *n*-butanol in the presence of *P. cepacia* lipase using *t*-BME as solvent after 24 h, a monoester (+)-**2** with 77% yield and ee 90% was recovered.



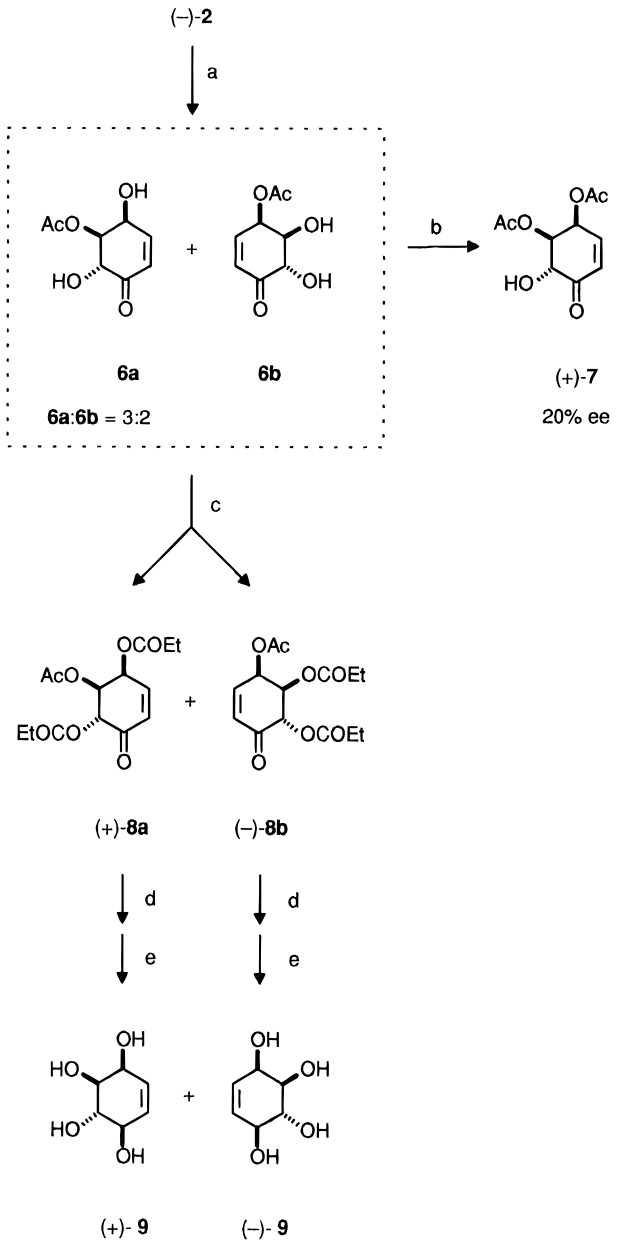
Osmylation of (−)-2. Dihydroxylation of 1,2-dihydroxycyclohexa-3,5-diene derivatives with OsO_4 in stoichiometric[7a] or catalytic amounts in the presence of *N*-methylmorpholine *N*-oxide[13] (NMMO) has been used successfully in the preparation of conduritol isomers. We studied the dihydroxylation of (−)-**2** in the presence of OsO_4 in catalytic amounts to assess the influence of the substrate chirality on the regio- and stereoselectivity of the reaction outcome.

Monoester (−)-**2** by treatment with NMMO in the presence of catalytic amounts of OsO_4, under the same conditions described by Carless for the dihydroxylation of **1**, after 15 h gave a complex mixture containing four monoacetyl tetrols, as evidenced by ^{1}H NMR analysis. Complete acetylation of the crude reaction products afforded conduritol E tetraacetate (+)-**4** of low ee (15%) and *meso*-conduritol D tetraacetate **5** in a 7:3 ratio (see Scheme 1). This shows that (−)-**2** behaves, as regards the *syn*/*anti* stereoselectivity, the same as **1**.[13]

In the hope of a more stereoselective course[14] we then considered catalytic osmylation in the presence of H_2O_2 (Milas' reagent)[15] as a possible alternative for attaining *cis*-dihydroxylation, although it is known that the reaction products may undergo further oxidation to an α-ketol.[16]

When (−)-**2** was subjected to this reaction, it did not give the corresponding tetrahydroxycyclohexene monoacetates, but two trihydroxycyclohexenone monoacetates in 3:2 ratio, as deduced from ^{1}H-NMR analysis. Chemical acetylation of the crude reaction mixture yielded 4,5,6-triacetoxycyclohex-2-enone [(+)-**7**] with 20% ee [^{1}H-NMR analysis in the presence of Eu(hfc)$_3$], corresponding to the diastereomeric ratio of the parent ketones (see Scheme 2). This indicates that in this case overoxidation is complete.

Scheme 2[a]



[a] Reaction conditions: (a) 2% OsO_4 in *t*-BuOH/H_2O_2, 0 °C; (b) Ac_2O Py, rt; (c) $(EtCO)_2O$/Py, rt; (d) $NaBH_4$/$CeCl_3$, 0 °C; (d) K_2CO_3/MeOH.

The presence of an axial−axial coupling (J = 11.2 Hz) between H-5 and H-6 in the spectrum of (+)-**7** allows exclusion of their *cis* relation, thus defining the relative stereochemistry at C-6. It is therefore apparent that OsO_4 attacks the diene system in (−)-**2** with complete preference *anti* with respect to the preexisting oxygenated functions. The presence of the acetyl group originates a partial regioselectivity, so that the hydroxylation of the C_5 over the C_3 double bond is preferred, yielding the two diastereoismers **6a** and **6b**. These cannot be separated by chromatography without partial loss of optical purity due to intramolecular transacetylation. Therefore, the reaction mixture was treated with propanoic anhydride to afford the mixed esters (+)-**8a** and (−)-**8b,** which were purified by column chromatography on Si gel.

Reduction of (+)-**8a** with $NaBH_4$ in the presence of $CeCl_3$ occurred stereoselectively, the attack of the hydride

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anion to the carbonyl carbon taking place exclusively *syn* with respect to the ester at C-6, thus giving, after treatment with K_2CO_3 in MeOH, the single tetrol derivative (+)-**9** [(+)-conduritol F] with ee >95%. An analogous procedure applied to (−)-**8b** gave the enantiomer (−)-**9**.

Conclusions

In brief, enantiotoposelective esterification and alcoholysis assisted by lipase are convenient ways to obtain both enantiomers of 1-acetoxy-2-hydroxycyclohexa-3,5-diene with high chemical and optical yields. This compound is a valuable starting material for the synthesis of chiral conduritol derivatives. In particular, dihydroxylation of (−)-**2** with H_2O_2 in the presence of catalytic amounts of OsO_4 is highly stereoselective and moderately regioselective, yielding the two monoacetates **6a** and **6b**. Treatment of the crude reaction mixture with propanoic anhydride afforded the mixed esters (+)-**8a** and (−)-**8b** that were separated by conventional column chromatography and each subjected to $NaBH_4$ reduction followed by alkaline hydrolysis to give (+)-**9** and (−)-**9**, respectively. Compounds (+)-**8a** and (−)-**8b** have structural features that prelude their use as starting material for the preparation of other conduritol derivatives.

Experimental Section

All chemicals were purchased from Aldrich and used as received. Vinyl acetate was distilled prior to use. Solvents were dried according to literature procedures. Column chromatography was performed on silica gel or Si Diol; analytical TLC was carried out on Merck silica gel 60-F_{254} precoated glass plates and compounds were visualized by spraying with molybdophosphoric acid. Lipases from *C. cylindracea*, *P. cepacia* (PSL), *R. javanicus*, and *A. niger* were from Amano International Enzyme Co. Lipase from porcine pancreas was obtained from Sigma. Novozym 435 (immobilized lipase from *Candida antarctica*) and Lipozyme IM (immobilized lipase from *Mucor miehei*) are registred marks from Novo Nordisk. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ at 250.13 and 62.9 MHz, respectively. Chemical shifts are in ppm (δ) downfield from Me_4Si . (*R*)-(−)-1-(9-Anthryl)-2,2,2-trifluoroethanol (Pirkle's alcohol) or europium(III) tris-[3-(heptafluoropropyl)hydroxymethylene]-(+)-camphorate were used as chiral shift reagents to determine enantiomeric ratios. Optical rotations were measured on a DIP 135 JASCO instrument. GC analyses were performed on Chiraldex G-DA (dialkyl γ-cyclodextrin) capillary column.

General Procedure for Enzymatic Esterification of *cis*-1,2-Dihydroxycyclohexa-3,5-diene (1). For a typical enzymatic esterification, the lipase of choice (10 mg) and vinyl acetate (0.02 mL) were added to a solution of **1** (10 mg) in *t*-BME (1 mL), and the suspension was stirred at 40 °C and 300 rpm. Substrate conversion was determined by ^{1}H-NMR at regular time intervals. The enantiomeric excess of (−)-**2** was determined by ^{1}H-NMR in the presence of Pirkle's alcohol or by chirospecific GC of the corresponding tetrahydro derivative.[11]

Enzymatic Preparation of (−)-(1*R*,2*S*)-1-Acetoxy-2-hydroxycyclohexa-3,5-diene [(−)-(2)]. PSL (0.5 g) was added to a solution of **1** (0.5 g, 4.46 mmol) and vinyl acetate (1 mL) in *t*-BME (50 mL), and the mixture was shaken at 40 °C and 300 rpm. After 3 h the reaction was stopped by filtering off the enzyme and the solvent evaporated under a stream of N_2. Conversion of substrate and ratio of the products were determined by ^{1}H-NMR analysis. After purification on Si Diol with hexane/CH_2Cl_2 5:95, (−)-**2** (580 mg, 85%, >98% ee) ([α]$_D$ −215° (*c* 1, C_6H_6)) was recovered.

Enzymatic Preparation of (+)-(1*S*,2*R*)-1-acetoxy-2-hydroxycyclohexa-3,5-diene [(+)-(2)]. PSL (0.5 g) was added to a solution of **3** (0.5 g, 2.55 mmol) and *n*-butanol (0.5 mL) in *t*-BME (50 mL), and the resulting suspension was shaken at 40 °C and 300 rpm. After 24 h the enzyme was removed by filtration and the reaction mixture, containing **(+)-2** (77%) and **3** (23%), processed as above to afford **(+)-2** (290 mg, 74%, 90% ee) ([α]$_D$ +197° (*c* 0.48, C_6H_6)).

Osmylation of (−)-2 with NMMO. A solution (10 mL) of NMMO (320 mg, 2.73 mmol) and OsO_4 (7 mg, 0.027 mmol) in CH_2Cl_2 was added to a solution of (−)-**2** (400 mg, 2.6 mmol) in *t*-BME (40 mL). After 15 h of stirring at rt, the reaction was stopped by evaporation of solvent. Conventional acetylation (Ac_2O/Py) at room temperature of the residue followed by chromatographic purification (SiO_2, acetone−CH_2Cl_2 1:9) afforded (+)-**4** (510 mg, 63%) and **5** (220 mg, 27%). The enantiomeric excess of (+)-**4** was determined as 15% after hydrolysis to (+)-conduritol E ([α]$_D$ +50° (*c* 1, H_2O), lit.[7c] [α]$_D$ +330° (*c* 4.5, H_2O)).

Osmylation of (−)-(2) with *t*-BuOH/H_2O_2. To a solution of (−)-**2** (0.95 g, 6.17 mmol, >98% ee) in *t*-BME (95 mL) were successively added 5 mL of a 6.3% solution of H_2O_2 in *t*-BuOH prepared according to the Milas procedure[15] and 6 mL of a 0.5% solution of OsO_4 in *t*-BuOH. The mixture was stirred at 0 °C until TLC analysis showed the nearly complete disappearance of (−)-**2** (ca. 12 h), then the reaction was quenched by filtration on a Sep-pack cartridge and the solvent evaporated under reduced pressure. ^{1}H-NMR analysis of the residue showed the presence of two monoesters (**6a** and **6b**) in a 3:2 ratio, as calculated from the integrated areas of the resonances for the carboxymethine (δ 5.01 and 5.63, respectively).

Half of the osmylation mixture was submitted to conventional acetylation followed by chromatography (SiO_2, hexane−Et_2O 1:1) to afford **(+)-(4*S*,5*S*,6*R*)-4,5,6-triacetoxycyclohex-2-enone** (+)-**7:** (720 mg, 86%, 20% ee), [α]$_D$ +13° (*c* 1, $CHCl_3$); ^{1}H-NMR δ 2.06 (3H, s), 2.14 (3H, s), 2.18 (3H, s), 5.43 (1H, dd, J = 11.2 and 3.9 Hz), 5.75 (1H, d, J = 11.2 Hz), 5.80 (1H, dd, J = 5.9 and 3.9 Hz), 6.27 (1H, d, J = 9.9 Hz), 6.92 (1H, dd, J = 9.9 and 5.9 Hz); ^{13}C-NMR δ 20.04, 20.56, 20.62, 65.27, 68.62, 72.01, 131.98, 140.93, 169.60, 169.88, 169.92, 190.93.

The second half of the crude osmylation product was treated with propanoic anhydride/pyridine in CH_2Cl_2. After 10 h of stirring at rt, the solution was extracted with 1 N HCl and taken to dryness under reduced pressure, and the residue was subjected to column chromatography (Si gel, hexane−Et_2O 60:40) to give (+)-**8a** (445 mg, 48%) and (−)-**8b** (295 mg, 32%).

(+)-(4*S*,5*S*,6*R*)-5-Acetoxy-4,6-dipropyloxycyclohex-2-enone [(+)-8a]: [α]$_D$ +118°, (*c* 0.3, $CHCl_3$); ^{1}H-NMR δ 1.18 (3H, t, J = 7.6 Hz), 1.19 (3H, t, J = 7.6 Hz), 2.05 (3H, s), 2.44 (4H, m), 5.44 (1H, dd, J = 11.2 and 3.9 Hz), 5.77 (1H, d, J = 11.2 Hz), 5.84 (1H, dd, J = 5.9 and 3.9 Hz), 6.28 (1H, d, J = 10 Hz), 6.93 (1H, dd, J = 5.9 and 10 Hz); ^{13}C-NMR δ 9.07, 9.13, 20.55, 27.26, 27.38, 65.09, 68.73, 71.84, 132.00, 140.97, 169.59, 173.42, 173.52, 191.11.

(−)-(4*R*,5*R*,6*S*)-4-Acetoxy-5,6-dipropyloxycyclohex-2-enone [(−)-8b]: [α]$_D$ −82°, (*c* 0.2, $CHCl_3$); ^{1}H-NMR δ 1.13 (3H, t, J = 7.6 Hz), 1.18 (3H, t, J = 7.5 Hz), 2.14 (3H,s), 2.51 (4H, m), 5.46 (1H, dd, J = 3.8 and 11.1 Hz), 5.78 (1H, d, J = 11.1 Hz), 5.82 (1H, dd, J = 5.9 and 3.8 Hz), 6.28 (1H, d, J = 10 Hz), 6.93 (1H, dd, J = 10 and 5.9); ^{13}C-NMR δ 8.88, 9.06, 20.66, 27.24, 27.28, 65.34, 68.50, 71.84,132.04, 140.88, 169.94, 173.01, 173.39, 191.12.

(+)-Conduritol F [(+)-9]. $NaBH_4$ (46 mg) was added portionwise to a stirred solution of **(+)-8a** (150 mg, 0.5 mmol) in 0.1 M $CeCl_3$ (8 mL, 0.8 mmol) in methanol.[17] After 15 min at 0 °C the resulting mixture was poured in water and extracted with AcOEt. The organic phase was taken to dryness and K_2CO_3 was then added to the residue dissolved in MeOH. Hydrolysis proceeded smoothly to give (+)-**9** (66 mg, 90%), the ^{1}H-NMR data of which were in agreement with the literature values[6] ([α]$_D$ +68.5° (*c* 0.13, CH_3OH); lit.[6] [α]$_D$ +70.8° (*c* 0.13, CH_3OH)).

(−)-Conduritol F [(−)-9]. A solution of (−)-**8b** (150 mg, 0.5 mmol) was treated as above to give (−)-**9** (64 mg, 88%) ([α]$_D$ −68.7° (*c* 0.2, CH_3OH); lit.[6] [α]$_D$ −71.7° (*c* 0.75, CH_3OH)).

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Innovative". The authors thank Amano Co and NOVO Nordisk for a gift of *P. cepacia* lipase and *M. miehei* lipase (Lipozyme IM) respectively.

Supporting Information Available: ^{13}C NMR spectra of compounds (+)-**2**, (+)-**7**, (+)-**8a**, and (-)-**8b** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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