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# Chemoenzymatic synthesis of enantiopure isopropyl (3*R*)- and (3*S*)-3-hydroxycyclohex-1-ene-1-carboxylates and their reduction to isomers of isopropyl 3-hydroxy-cyclohexane-1-carboxylate

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**Abstract**—Reduction of an α,β-unsaturated cyclic ketone with *Geotrichum candidum* affords the corresponding (*S*)-allylic alcohol, while enantiospecific oxidation of the corresponding racemic alcohols leaves the (*R*)-enantiomer unchanged, giving access to both enantiomeric forms. Subsequent chemical reduction of the double bond of these homochiral allylic alcohol allows all isomers of the corresponding cyclohexanols to be obtained. 

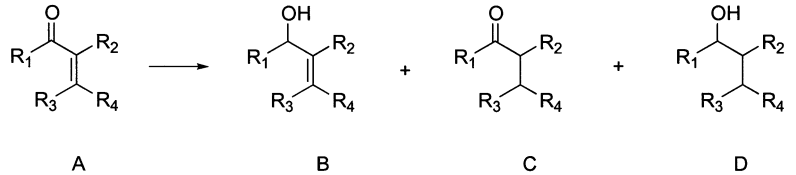
## 1. Introduction

γ-Hydroxy-α,β-unsaturated carboxylic acids are versatile synthons and occur widely in natural products. However, their preparation in enantiopure form is not easy and many syntheses start from the chiral pool. For example, quinic and shikimic acids are commonly used to obtain such compounds in the cyclohexane series. Indeed selective reduction of the corresponding carbonyl compounds to afford allylic alcohols is difficult since it is usually associated with reduction of the carbon–carbon double bond.

Some examples of biotransformation of α,β-unsaturated ketones A have been described. As the carbonyl group and the carbon–carbon double bond may be reduced by microorganisms, three possible products (B, C and D) may be obtained in various yields, depending on the substituents $R_1$, $R_2$, $R_3$ and $R_4$ (Scheme 1).

Veschambre et al. have studied the reduction of these compounds by the fungus *Beauveria sulfurescens*. In the cases of aldehydes[1,2] ($R_1 = H$), the carbonyl group is reduced more rapidly or at the same rate as the carbon–carbon double bond, and the major product is unsaturated alcohol B or saturated alcohol D; generally, the double bond in compound B is not reduced. On the contrary, in the case of ketones[3–5] ($R_1 \neq H$) reduction of the carbon–carbon double bond is faster than reduction of the carbonyl group and all three products B, C and D can be observed. However, if $R_3$ and $R_4$ are not hydrogens, reduction of the carbon–carbon double bond does not occur. The enzyme responsible for this reduction is an enoate reductase and can be induced in fungus *B. sulfurescens*.[6]

To synthesise optically active 3-oxocycloalkane 1-carboxylic acids **2a** and **2b**, we studied[7] the microbial reduction of the corresponding isopropyl 3-oxocycloalken-1-carboxylates **1a** and **1b**, respectively. The reduction of the carbon–carbon double bond was achieved using the fungus *Geotrichum candidum*. However, **1a** and **1b** were not transformed in the same




**Scheme 1.**

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manner. The reduction of **1a** gave the (1*S*,3*S*)-3-hydroxycyclohexane-1-carboxylate **4a**, while the reduction of **1b** afforded mainly the (1*S*)-3-oxocyclopentane-1-carboxylate **2b** (Scheme 2). The optically active saturated ketones were used in the synthesis of cyclic analogues of glutamate **3a** and **3b**.

Furthermore, we noticed[8] during the bioreduction of **1a** the additional formation in varying ratios of 3-hydroxycyclohex-1-ene-1-carboxylate **5a**. In order to explain this phenomenon we investigated the biotransformation of **1a**,**b**, **2a**,**b**, racemic 3-hydroxycyclohex-1-ene-1-carboxylate **5a** and 3-hydroxycyclopent-1-ene-1-carboxylate **5b** derivatives. We report herein the preparation of optically active allylic alcohols and their use in the synthesis of all isomers of the corresponding saturated alcohols.

## 2. Results

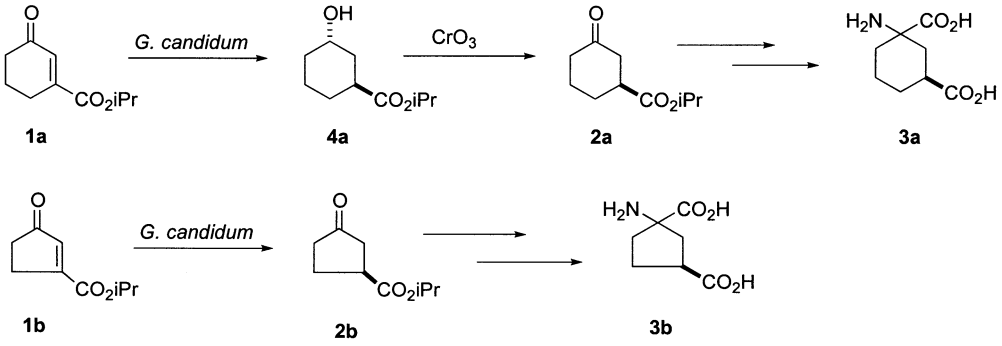
### 2.1. Synthesis of substrates

The ketones **1a** and **1b** were prepared from cycloalkanecarboxylic acids, as described previously[9,10] using isopropanol instead of methanol in the synthesis of **6a** and **6b**. Allylic alcohols can be obtained by $NaBH_4$ reduction[11] of the corresponding ketones **1a** and **1b**. While **5b** was prepared in the presence of $CeCl_3$[12] at −20°C, the reduction of **1a** to (±)-**5a** was obtained without $CeCl_3$ at −25°C (Scheme 3).

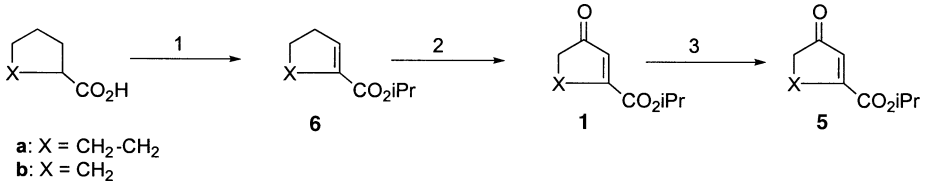
### 2.2. Biotransformation

Biotransformations were performed after full growth of the fungus *G. candidum* (60 h) in culture media or in phosphate buffer pH 7. Substrates were added in Tween 80–ethanol solution to obtain final concentrations of 1–1.6 g $L^{-1}$. The reaction rates were determined by GC analysis of the crude incubation extracts and the enantiomeric excesses of the cyclohexenols were measured after derivatization to their (*S*)-*O*-acetyllactyl esters and analysis by GC.[13]

**2.2.1. Reduction of 1a**. A kinetic study of the biotransformation of **1a** (Table 1) showed that it was rapidly reduced to two products, isopropyl 3-hydroxycyclohex-1-ene-1-carboxylate (−)-**5a** and isopropyl (1*S*,3*S*)-3-




**Scheme 2.**




**Scheme 3.** (1) a. $SOCl_2$, b. $Br_2$, c. *i*PrOH; (2) $CrO_3$, (3) **1a**: $NaBH_4$, −25°C, **1b**: $NaBH_4$, $CeCl_3$, −20°C.

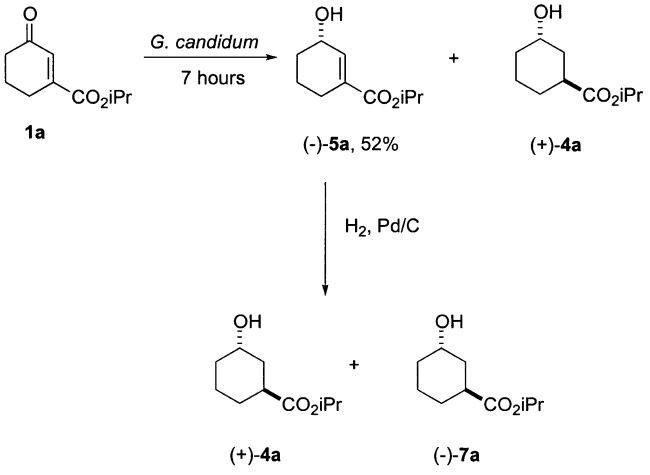
**Table 1.** Reduction of **1a** and **1b** by *G. candidum*. Percentage of total area of GC peak, OV 1701 capillary column

| Time (h) | **1a** (%) | **5a** (%) | **4a** (%) | **1b** (%) | **2b** (%) | **4b**+**7b** (%) |
|---|---|---|---|---|---|---|
| 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| 1.5 | 77 | 17 | 6 | 45 | 54 | 1 |
| 3 | 37 | 46 | 17 | –[a] | –[a] | –[a] |
| 6.5 | 6 | 55 | 39 | 7 | 85 | 8 |
| 24 | 3 | 20 | 77 | 2 | 47 | 51 |
| 36 | 0 | 0 | 100 | –[a] | –[a] | –[a] |

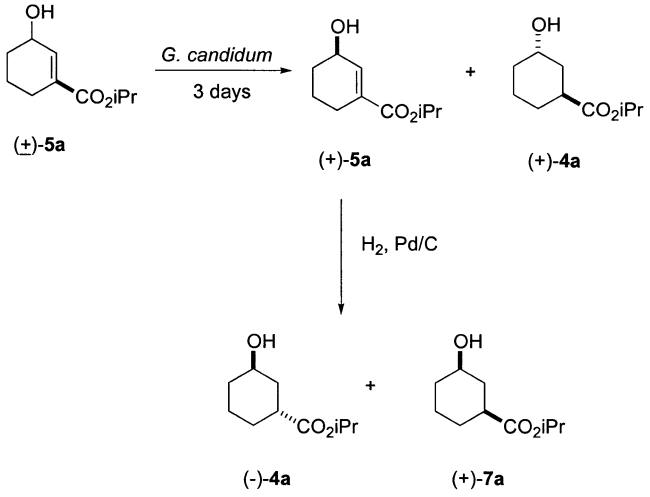
[a] Not measured.

hydroxycyclohexane-1-carboxylate (+)-**4a**. At first (−)-**5a** was predominant with a maximum obtained after 7 h incubation (55%, GC analysis). Then it was consumed and (+)-**4a** was the sole product obtained. In order to obtain (−)-**5a** on preparative scale, the biotransformation was stopped after 7 h incubation and afforded (−)-**5a** in 52% chemical yield and 90% e.e., $[\alpha]_D^{20}$ −36 (*c* 1, MeOH). The purified unsaturated alcohol (−)-**5a** was incubated in the presence of *G. candidum* and (+)-**4a** was obtained as the sole product.

The configuration of (−)-**5a** was established by chemical reduction into the known isopropyl 3-hydroxycyclohexane-1-carboxylate (+)-**4a** and (−)-**7a** obtained by reduction of racemic ketone[7] **1a** using *G. candidum* or another fungus *Rhizoppus arrhizus*. Catalytic hydrogenation of (−)-**5a** in ethyl acetate afforded a mixture of *trans*/*cis*-diastereoisomers in 4:6 ratio (Scheme 4). After separation on a silica gel column, comparison of their optical activity with literature (**4a**: $[\alpha]_D^{20}$ +9.4 (*c* 1, MeOH) lit.[7] $[\alpha]_D^{20}$ +10 (*c* 1, MeOH) for (1*S*,3*S*)-3-hydroxycyclohexane-1-carboxylate, **7a**: $[\alpha]_D^{20}$ −4 (*c* 1, MeOH) lit.[7] $[\alpha]_D^{20}$ −5 (*c* 2, MeOH)) for (1*R*,3*S*)-isomer) indicated a (3*S*)-configuration for (−)-**5a**. The enantiomeric excesses of (+)-**4a** (90%) and (−)-**7a** (90%) were determined by GC after derivatization as their (*S*)-*O*-acetyllactyl esters.




**Scheme 4.**



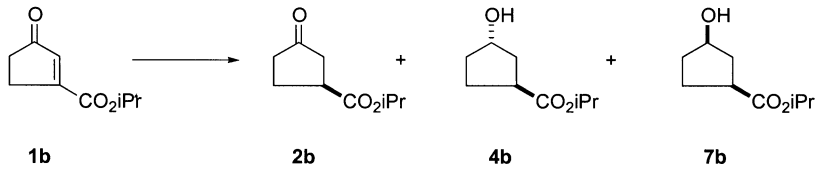

**Scheme 5.**

**2.2.2. Resolution of (±)-3-hydroxycyclohex-1-ene-1-carboxylate 5a**. The biotransformation of (±)-**5a** afforded isopropyl (1*S*,3*S*)-3-hydroxycyclohexane-1-carboxylate (+)-**4a** and left the (1*R*)-hydroxy ester unreacted (Scheme 5). (+)-**5a** was obtained with 96% e.e. after 72 h incubation in 22% yield (50% max.). During the transformation, the formation of isopropyl 3-oxocyclohex-1-ene 1-carboxylate (2–3%) was observed (GC analysis). Catalytic hydrogenation of (+)-**5a** afforded a diastereomeric mixture of isopropyl 3-hydroxycyclohexane-1-carboxylates (−)-**4a** and (+)-**7a**. After chromatographic separation on silica gel (−)-**4a** ($[\alpha]_D^{20}$ −10 (*c* 1, MeOH)) and (+)-**7a** ($[\alpha]_D^{20}$ +4.4 (*c* 1, MeOH)) were obtained in 55 and 30% yields, respectively.

**2.2.3. Reduction of ketone 1b**. The reduction of the 3-oxocyclopent-1-ene-1-carboxylate **1b** rapidly afforded the (1*S*)-keto ester **2b**,[7,8] which was slowly reduced to the diastereomeric 3-hydroxy esters **4b** and **7b** (Table 1). No formation of allylic alcohol **5b** was observed. Good stereospecificity was observed in the carbon–carbon double bond reduction (e.e. of **2b**: 85%) but it was lower in the reduction of the carbonyl group (both diastereoisomers **4b** and **5b** were obtained) (Scheme 6). As the reduction of the carbonyl group was slow, a mixture of ketone **2b** and alcohols **4b** and **7b** was obtained.

The reduction of (±)-**2b** took place with low stereospecificity, and reduction occurred with approximately equal attack of both faces of the carbonyl group to give a near racemic mixture of diastereomers.

**2.2.4. Resolution of (±)-3-hydroxycyclopent-1-ene-1-carboxylate 5b**. The biotransformation of (±) 3-hydroxycyclopent-1-ene-1-carboxylate (±)-**5b** rapidly gave the (1*S*)-keto ester **2b**, which was slowly reduced to afford



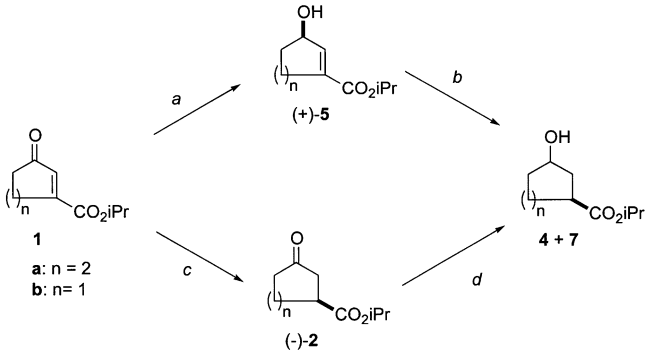

**Scheme 6.** Reduction of **1b** by *G. candidum*.

a mixture of **4b** and **7b**. During this transformation, small amounts of 3-oxocyclopent-1-ene-1-carboxylate **1b** were detected. After 14 h, 50% of **5b** remained unreacted and presented an optical activity (Scheme 7). Its enantiomeric excess was not determined by chromatographic methods because none of the conditions, using chiral column or chiral derivatizing agent, gave an analytical separation of enantiomers. To show that the oxidation of 3-hydroxycyclopent-1-ene-1-carboxylate to the corresponding 3-oxocyclopentane-1-carboxylate **2b** was enantioselective, we studied the biotransformation of **5b′**. The enantiomeric excess of the remaining **5b′** obtained after 50% conversion (48 h incubation) was measured by NMR analysis. The deuterium NMR spectrum of the racemic **5b′** in a chiral solvent[14] exhibited four signals, two for each enantiomer, whereas only two signals were observed in the spectrum of **5b′** after biotransformation, indicating the high enantiomeric purity of the biotransformed compound. The configuration of **5b** was not determined, but by comparison with the cyclohexene derivative it was assumed to be R.

## 3. Discussion

Only carbon–carbon double bonds which are activated by an electron-withdrawing substituent such as the carbonyl group (aldehyde or ketone), carboxyl group or nitro group are reduced.[15] Allenic alcohols are also reduced into β-ethylenic compounds by proliferating *S. cerevisae* cells.[16] In the compounds **1a** and **1b**, two substituents are able to activate the carbon–carbon double bond, the carbonyl group and the carboxylate group whereas only the carboxylate group activates the carbon–carbon double bond in compounds **5a** and **5b**.

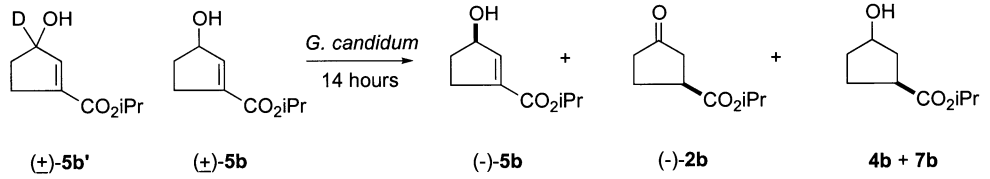
The formation of cyclohexenol **5a** and cyclopentanone **2b** from the respective cycloalkenones **1a** and **1b** suggests two pathways for the production of cycloalkanol **4a**, **4b** and **7b** (Scheme 8). In the cyclohexane series, the reduction of the carbonyl group is fast (steps *a* and *d*) and occurs more rapidly than reduction of the carbon–carbon double bond. Thus, we observe exclusive formation of the intermediate **5a**. No accumulation of **2a** takes place because it is rapidly reduced after its formation. In the cyclopentane series, the reduction of the carbon–carbon double bond is the faster biotransformation, and cyclopentanone is the sole observable intermediate. We have shown that the reduction of (−)-**2b** and (±)-**2b** is very slow.




**Scheme 8.**

The carbon–carbon double bond in compound **5** is conjugated with the carboxylate group and this activation could explain the reduction of the double bond. To verify this hypothesis, we incubated compounds **6a** and **6b** in the presence of *G. candidum* but no transformation was observed. This suggests that **5** was not transformed following step *b*. The fact that microorganisms are unable to reduce such compounds has been described during the biotransformation of ethyl 4,4-dimethoxy-3-methylcrotonate[17] and the biotransformation of γ-cyano-α,β-unsaturated.[18] In both cases the formation of α,β-ethylenic alcohol was observed, suggesting that microorganisms did not reduce (or reduce only slowly) the carbon–carbon double bond of the unsaturated hydroxy ester (former case) or of unsaturated cyano-alcohol derivatives (latter case). However, reduction of the carbon–carbon double bond of a γ-hydroxy-α,β-unsaturated carboxylic ester by *G. candidum* has been reported.[17] The biotransformation of (*E*)-3-(1′,3′-dioxolan-2′-yl)-2-buten-1-ol into (*S*)-2-methyl-γ-butyrolactone involved reduction of the carbon–carbon double bond of 2-methyl–4-hydroxybut-2-enoic acid.

In order to explain the disappearance of (−)-**5a** after its formation from **1a**, we studied the bioconversion of racemic cycloalkenols **5a** and **5b**. The transformation of the (*S*)-enantiomers by oxidation to cycloalkenones **1a** and **1b**, respectively, was observed. These were rapidly reduced to **2a** (and **5a**) and **2b**, respectively, and only a few percent each of **1a** and **1b** could be detected by GC analysis. The reduction of **1a** afforded cyclohexenol (*S*)-**5a**, which was re-oxidised in **1a**. Step *a* was reversible while step *b* did not occur. Owing to the reversibility of the oxidation of (*S*)-**5a**, the transformation of (±)-**5a** into enantiopure (*R*)-**5a** was slow. However, rapid oxidation of (*S*)-**5b** and no reduction of **2b** (reverse reaction) explained the short time to obtain the




**Scheme 7.**

resolution of (±)-**5b**. These remarks suggest that the oxidoreduction reactions displayed in the cyclohexane series are not catalysed by the same enzyme. We have already demonstrated the presence of some oxidoreductases with different stereospecificities in *G. candidum*.[19,20] The potential of this fungus has been exploited by us[21] and others[22] in asymmetric syntheses and in the deracemisation of hydroxy compounds.

The reaction time to obtain the transformation of 50% of **5b′** (40 h) is longer than the reaction time necessary for **5b** (14 h). This isotope effect shows that limiting step of the process is the abstraction of hydride during the oxidation reaction.

The stereospecificity of the reduction of the carbonyl group is high (e.e. of **5a**: 90%). The oxidation of racemic **5a** and **5b** is enantiospecific; only (*S*)-enantiomers react, and (*R*)-**5a** and (*R*)-**5b** are obtained in 96% and >98% enantiomeric excesses, respectively. The e.e. of (*R*)-**5b** is very high because the intermediate ketone **2b** is not reduced in (*S*)-**5b**.

## 4. Conclusion

We have synthesised (3*S*)- and (3*R*)-3-hydroxycyclohex-1-ene-carboxylate using the same microorganism via the asymmetric reduction of the corresponding ketone for the former and an enantioselective oxidative process for the latter. In order to improve the yield it may be useful to control the activities of desired and undesired enzymes. Among them, the carbon–carbon double bond reductase that transforms the substrate in the reductive process or the intermediate in the resolution process. From these enantiomers, we subsequently obtained all isomers of 3-hydroxycyclohexane carboxylate in optically active form.

## 5. Experimental

### 5.1. Material and methods

All chemicals used in the synthetic procedure were reagent grade or better. $^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ with a Bruker WM250 instrument at 250.13 and 62.9 MHz, respectively, and chemical shifts, calibrated on the $CHCl_3$–$CDCl_3$ resonance, were reported in ppm downfield from $(CH_3)_4Si$. Optical rotations were measured using a Perkin Elmer 241C spectropolarimeter. GC analyses were performed on a Hewlett Packard 4890 chromatograph with a flame ionization detector and helium as carrier gas equipped with a OV1701 (Pierce, 15 m×0.20 mm) capillary column or on a Shimadzu GC-8A chromatograph with a flame ionization detector and helium as carrier gas equipped with a BP 20 (SGE, 25 m×020 mm) capillary column.

**5.1.1. Biotransformations**. *Geotrichum candidum* LCP 98-4202 was grown at 27°C with orbital shaker (200 rpm) in a liquid medium containing (per litre), corn steep liquor 10 g, glucose 30 g, $KH_2PO_4$ 1 g, $K_2HPO_4$ 2 g, $NaNO_3$ 2 g, KCl 0.5 g, $MgSO_4$, $7H_2O$ 0.5 g, $FeSO_4$, $7H_2O$ 0.02 g. The bioconversions were performed in medium culture by addition of the substrate in ethanol–Tween 80 (8:2) solution onto 65 h old culture and incubation was continued under the same conditions.

### 5.2. Isopropyl cyclohex-1-ene-1-carboxylate 6a

To 1-bromo-cyclohexane carboxylic acid chloride [obtained as described[9] from cyclohexanecarboxylic acid (26.4 g, 0.2 mol), thionyl chloride (18 mL, 0.248 mol), red phosphorus (0.325 g) and bromine (12.8 mL)] was added dropwise isopropanol (81 mL). During the addition the temperature was maintained below 25°C and the mixture was heated under reflux for 15 min. After cooling, ice-water (80 mL) was added. The mixture was extracted with ether and the organic phase was washed with 1 M $Na_2S_2O_3$, with saturated $NaHCO_3$ and brine. After the solvent was dried and removed, the residue was distilled to yield 41.67 g (81%) of isopropyl 1-bromo-cyclohexane carboxylate, bp 80–82°C (2 mmHg). A solution of isopropyl 1-bromo-cyclohexane carboxylate (41 g, 0.167 mol) and quinoline (32 mL) was heated at 120°C under nitrogen for 2 h. The two-phase system was treated with 20% aqueous HCl (200 mL) and extracted with ether. The organic phase was washed with 10% aqueous HCl, saturated $NaHCO_3$, brine and dried over $Na_2SO_4$. The solvent was removed under reduced pressure and the residue distilled to afford **6a** (26.7 g, 95%) bp 63–65°C (1.5 mmHg).

### 5.3. Isopropyl 3-oxo-cyclohex-1-ene-1-carboxylate 1a

To a stirred solution of **6a** (10 g, 59.5 mmol) in $CH_2Cl_2$ (105 mL) was added dropwise a solution of $CrO_3$ (15.93 g) in acetic anhydride (12 mL) and glacial acetic acid (25 mL) over 45 min. The cooled mixture was neutralised with concentrated KOH (82 mL) and water (150 mL) was added. The two-phase system was extracted with ether, and the organic phase was washed with saturated $NaHCO_3$, brine and dried over $Na_2SO_4$. Removal of solvent and distillation of the residue gave unsaturated ketone **1a** (3.81 g, 35%), bp 82–84°C (0.6 mmHg). $^1$H NMR ($CDCl_3$), $\delta$ ppm, $J$ Hz: 1.2 (6H, d, $J=6.38$, $(CH_3)_2$CH-), 1.5–2.7 (6H, m, $H_2$C), 5.5 (1H, sext, $J=6.38$, C*H*OR), 6.6 (1H, s, C*H*=C). $^{13}$C NMR, $\delta$ ppm, 19.08 ($C_5$), 21.68 (*C*H$_3$), 24.19 ($C_6$) 37.67 ($C_4$), 69.30 (*C*H$(CH_3)_2$, 132.64 ($C_2$) 149.63 ($C_1$), 165.95 (*C*OO), 200.30 ($C_3$).

### 5.4. Isopropyl cyclopent-1-ene 1-carboxylate 6b

Using the same protocol as for **6a**, cyclopentanecarboxylic acid (19 mL, 0.175 mol) gave isopropyl 1-bromo-cyclopentane carboxylate (34.52 g, 84%), bp 55–58°C (1 mmHg). Treatment with quinoline afforded **6b** (16.5 g, 74%), bp 46–49°C (1.5 mmHg). $^1$H NMR ($CDCl_3$), $\delta$ ppm, $J$ Hz: 1.2 (6H, d, $J=6.38$, $(CH_3)_2$-CH-), 1.83–1.95 (2H, m, $H_2$C(4)), 2.35–2.55 (4H, m, $H_2$C(3) and (6)), 5.0 (1H, sext, $J=6.38$, C*H*OR), 6.68

(1H, s, C*H*=C). $^{13}$C NMR, $\delta$ ppm, 21.83 (*C*H$_3$), 23.07 (C$_4$), 31.30 and 33.24 (C$_3$ and C$_5$), 67.16 (*C*H(CH$_3$)$_2$), 137.16 (C$_1$), 143.00 (C$_2$), 164.92 (COO).

### 5.5. Isopropyl 3-oxocyclopent-1-ene-1-carboxylate 1b

Oxidation of **6b** (8 g, 52 mmol) as described above afforded **1b** (2.53 g, 29%), bp 66–69°C (6 mmHg). $^1$H NMR (CDCl$_3$), $\delta$ ppm, *J* Hz: 1.2 (6H, d, *J*=6.38, (C*H*$_3$)$_2$CH-), 2.43–2.49 (2H, m, *H*$_2$C), 2.76–2.82 (2H, m, H$_2$C), 5.01 (1H, sext, *J*=6.38, C*H*OR), 6.66 (1H, s, C*H*=C). $^{13}$C NMR, $\delta$ ppm, 21.67 (*C*H$_3$), 27.42 (C$_5$) 35.52 (C$_4$) and 69.38 (*C*H(CH$_3$)$_2$), 137.78 (C$_2$), 163.80 and 164.83 (C$_1$) and (COO), 209.12 (C$_3$).

### 5.6. (±)-Isopropyl 3-hydroxycyclohex-1-ene-1-carboxylate 5a

To a solution of **1a** (2 g, 11 mmol) in methanol (60 mL) cooled at −25°C was added NaBH$_4$ (208 mg, 0.5 equiv.). The reaction was monitored by GC analysis. Water (10 mL) was added and the mixture was acidified with 5N aqueous HCl solution. After removal of methanol, the aqueous phase was saturated with NaCl and extracted with ether (three times). The organic phase was washed with saturated NaHCO$_3$, brine and dried over Na$_2$SO$_4$. The solvent was evaporated and the product was purified by flash chromatography (CH$_2$Cl$_2$/AcOEt, 9:1) to yield **5a** (1.3 g, 60%). IR (CHCl$_3$) 3600 cm$^{-1}$, 1700 cm$^{-1}$. $^1$H NMR (CDCl$_3$), $\delta$ ppm, *J* Hz: 1.2 (6H, d, *J*=6.37, (C*H*$_3$)$_2$CH-), 1.5–1.9 (4H, m, CH$_2$), 2.2 (2H, m, CH$_2$), 4.3 (1H, m, C*H*OH), 5–5.1 (1H, sext, *J*=6.37, C*H*OR), 6.8 (1H, br s, C*H*=C). $^{13}$C NMR, $\delta$ ppm, 19.08 (C$_5$), 21.82 (CH$_3$), 24.19 (C$_6$) 31.19 (C$_4$), 65.98 (*C*H(CH$_3$)$_2$, 67.90 (C$_3$), 133.08 (C$_2$), 139.00 (C$_1$), 166.77 (COO). Anal. calcd for C$_{10}$H$_{16}$O$_3$: C, 65.19; H, 8.75. Found: C, 65.13; H, 8.75%.

(*S*)-*O*-acetyllactate derivatives were obtained as described[13] and injected on a OV 1701 capillary column, 150°C (7 min) then 150–185°C (3°C/min), retention times (*S*)-**5a** 20.7 min, (*R*)-**5a** 20.9 min.

### 5.7. (±)-Isopropyl 3-hydroxycyclopent-1-ene-1-carboxylate 5b

To a cooled solution (−20°C) of **1b** (700 mg, 4.16 mmol) and CeCl$_3$ (1.55 g, 1 equiv.) in methanol (28 mL) was added NaBH$_4$ (78 mg, 0.5 equiv.) and the mixture stirred for 30 min. A few drops of a 5N aqueous HCl solution were added followed by the addition of H$_2$O (20 mL). The mixture was saturated with NaCl and extracted with AcOEt (three times). The organic phase was washed with brine and dried over Na$_2$SO$_4$. Removal of solvent and flash chromatography (CH$_2$Cl$_2$/AcOEt, 7:3) yielded **5b** (500 mg, 71%). IR (CHCl$_3$) 3600 cm$^{-1}$, 1700 cm$^{-1}$. $^1$H NMR (CDCl$_3$), $\delta$ ppm, *J* Hz: 1.2 (6H, d, *J*=6.37, (C*H*$_3$)$_2$CH-), 1.65–1.8 (1H, m, CH$_2$), 2.2–2.45 (1H, m, CH$_2$), 2.55–2.75 (1H, m, CH$_2$), 4.8 (1H, br s, C*H*=C)), 4.9–5.04 (1H, sext, *J*=6.37, C*H*OR). $^{13}$C NMR, $\delta$ ppm, 21.75 (CH$_3$), 29.84 (C$_5$) 33.39 (C$_4$), 67.91 (*C*H(CH$_3$)$_2$) 67.90 (C$_3$), 139.12 (C$_1$), 142.73 (C$_2$), 164.87 (C=O).

### 5.8. (−)-(*S*)-Isopropyl 3-hydroxycyclohex-1-ene-1-carboxylate 5a

A solution of isopropyl 3-oxo-1-cyclohexene-1 carboxylate **1a** (1.6 g, 8.8 mmol) in EtOH–Tween 80 (5 mL) was added to culture of *G. candidum* (1 L) and the mixture was incubated for 8 h. The suspension was filtered and the filtrate was saturated with sodium chloride, filtered again through Celite and extracted with AcOEt (three times). The organic phase was concentrated to 150 mL, washed with brine and dried over MgSO$_4$. After evaporation of the solvent, the crude residue was purified by flash chromatography (cyclohexane/AcOEt, 8:2) to give (−)-(*S*)-**5a** (830 mg, 52%, 90% e.e.). $[\alpha]_D^{20}$ −36 (*c* 1, MeOH).

### 5.9. (1*S*,3*S*)- and (1*R*,3*S*)-Isopropyl 3-hydroxycyclohexane-1-carboxylate 4a and 7a

(−)-(*S*)-Isopropyl 3-hydroxycyclohex-1-ene-1 carboxylate **5a** (492 mg, 2.67 mmol) in ethyl acetate (20 mL) was hydrogenated at 1 atm over Pd–C (10%, 70 mg) for 3 h. The mixture was filtered on Celite and the solvent was evaporated. The diastereomeric products were purified by flash chromatography (dichloromethane–ethyl acetate, 85:15) to give (1*S*,3*S*)-**4a** (135 mg, 27%) and (1*R*,3*S*)-**7a** (205 mg, 42%).

(1*S*,3*S*)-**4a**: $^1$H NMR (CDCl$_3$), $\delta$ ppm, *J* Hz: 1.2 (6H, d, *J*=6.38, (C*H*$_3$)$_2$CH-), 1.5–1.6 (4H, m, CH$_2$), 1.8–1.9 (2H, m, CH$_2$), 2.7 (1H, m, C*H*(CH$_3$)$_2$), 4.1 (1H, m, C*H*OH), 5.0 (1H, sext, *J*=6.38, C*H*OR). $^{13}$C NMR, $\delta$ ppm, 19.8 (C$_5$), 21.8 (CH$_3$), 28.19 (C$_2$) 32.19 (C$_6$), 35.5 (C$_1$), 38.1 (C$_4$), 66.2 (C$_3$), 67.30 (*C*H(CH$_3$)$_2$, 175.37 (COO). $[\alpha]_D^{20}$ +9.4 (*c* 1, MeOH). (*S*)-*O*-Acetyllactate derivatives[13] were injected on a OV 1701 capillary column 150°C (7 min) then 150–185°C (3°C/min), retention times (1*R*,3*R*) 18.3 min, (1*S*,3*S*) 18.5 min (90% e.e.).

(1*R*,3*S*)-**7a**: $^1$H NMR (CDCl$_3$), $\delta$ ppm, *J* Hz: 1.2 (6H, d, *J*=6.37, (C*H*$_3$)$_2$CH-), 1.5–1.9 (4H, m, CH$_2$), 2.2 (2H, m, CH$_2$), 4.3 (1H, m, C*H*OH), 5–5.1 (1H, sext, *J*=6.37, C*H*OR), 6.8 (1H, br s, C*H*=C). $^{13}$C NMR, $\delta$ ppm, 19.08 (C$_5$), 21.82 (CH$_3$), 24.19 (C$_6$) 31.19 (C$_4$), 65.98 (*C*H(CH$_3$)$_2$, 67.90 (C$_3$), 133.08 (C$_2$), 139.00 (C$_1$), 166.77 (COO). $[\alpha]_D^{20}$ −4 (*c* 1, MeOH). (*S*)-*O*-Acetyllactate derivatives were injected on BP 20 capillary column 130°C–185°C (3°C/min), retention times (1*R*,3*S*) 28.9 min, (1*S*,3*R*) 29.3 min (90% e.e.).

### 5.10. (+)-(*R*)-Isopropyl 3-hydroxycyclohex-1-ene-1-carboxylate 5a

A solution of (±)-isopropyl 3-hydroxy-1-cyclohexene-1 carboxylate **5a** (1.2 g, 6.5 mmol) in EtOH–Tween (5 mL) solution was added to a 1 L culture of *G. candidum* and the incubation was carried on for 3 days. The suspension was filtered. The filtrate was saturated with NaCl and filtered again with Celite then extracted with AcOEt (three times). The organic phase was concentrated to 150 mL, washed with brine and dried over MgSO$_4$. After evaporation of the solvent, the crude

residue was chromatographed ($CH_2Cl_2$/AcOEt, 8:2) to give (+)-**5a** (258 mg, 22%, 96% e.e.). $[\alpha]_D^{20}$ +37 (*c* 1, MeOH) and (1*S*,3*S*)-isopropyl 3-hydroxy cyclohexane 1-carboxylate **4a** (249 mg, 21%) $[\alpha]_D^{20}$ +9 (*c* 1, MeOH).

### 5.11. (1*R*,3*R*)- and (1*S*,3*R*)-Isopropyl 3-hydroxycyclohexane-1-carboxylate 4a and 7a

(+)-(*R*)-Isopropyl 3-hydroxycyclohex-1-ene-1 carboxylate **5a** (368 mg, 2.67 mmol) in ethyl acetate (20 mL) was hydrogenated at 1 atm over Pd–C (10%, 40 mg) for 6 h. The mixture was filtered on Celite and the solvent was evaporated. The diastereomeric products were purified by flash chromatography (dichloromethane–ethyl acetate, 85:15) to give (1*R*,3*R*)-**4a** (190 mg, 52%, 96% e.e.) ($[\alpha]_D^{20}$ −10 (*c* 1, MeOH) and (1*S*,3*R*)-**7a** (125 mg, 34%, 96% e.e.). ($[\alpha]_D^{20}$ +4.4 (*c* 1, MeOH).

### 5.12. (+)-(*R*)-Isopropyl 3-hydroxycyclopent-1-ene-1-carboxylate 5b

As described for (+)-(*R*)-**5a**, (±)-isopropyl 3-hydroxy-1-cyclopentene-1-carboxylate **5b** (0.5 g, 2.9 mmol) was incubated in 500 mL culture medium of *G. candidum* for 14 h. The crude extract was chromatographed (cyclohexane/AcOEt, 8:2) and gave (1*S*)-3-oxocyclopentane-1-carboxylate **2b** (151 mg, 30%; >98% e.e., $^2$H NMR) $[\alpha]_D^{20}$ −15 (*c* 1, $CHCl_3$), 253 mg of a mixture of alcohols **4b**, **7b** and **5b** and 42 mg of **5b**, $[\alpha]_D^{20}$ +62 (*c* 0.7, $CHCl_3$).

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