

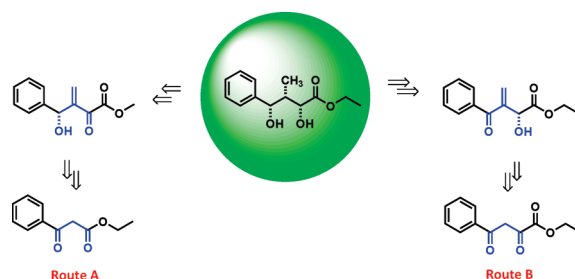
Chemoenzymatic Synthesis of α -Hydroxy- β -methyl- γ -hydroxy Esters: Role of the Keto–Enol Equilibrium To Control the Stereoselective Hydrogenation in a Key Step

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α -Hydroxy- β -methyl- γ -hydroxy esters not only are found in many natural products and potent drugs but also are useful intermediates in organic synthesis due to their highly functionalized skeleton that can be further manipulated and applied in the synthesis of many compounds with remarkable biological activities. This work was based on a chemoenzymatic approach to obtain these molecules with three contiguous stereogenic centers in a highly enantio- and diastereoselective way. Two distinct linear routes were proposed in which the key steps in both routes consisted of initial stereocontrolled ketoester bioreduction followed by unsaturated carbonyl bioreduction or reduction with Pd–C. Other key reactions in the synthesis include a Wasserman protocol for chain homology and a Mannich-type olefination with maintenance of enantiomeric excess for all intermediates during the sequence. Whereas route A gave exclusively the skeleton with 3*R*,4*R*,5*S* configuration (99% ee and 11.5% global yield after 7 steps), route B gave the skeleton with 3*R*,4*R*,5*S* and 3*R*,4*S*,5*R* configurations (dr 1:12, 98% ee and 20% global yield after 5 steps).

Introduction

Unsaturated carbonyl compounds are prochiral substrates that can provide densely functionalized molecules with two or more consecutive stereogenic centers. Asymmetric reductive products such as optically pure α -hydroxy- β -methyl- γ -hydroxy esters are important building blocks for

the synthesis of a variety of bioactive molecules¹ used as pharmaceutical intermediates² and are found in natural products.³ In our continuing efforts toward the synthesis

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of such useful intermediates, we developed two novel chemo-enzymatic routes for the preparation of enantio and diastereomerically pure α -hydroxy- β -methyl- γ -hydroxy esters.⁴ Our study has focused on a biocatalytic approach to create the first chiral center from wild microbial whole cell reduction of β -ketoesters and α,γ -ketoesters. The second and third asymmetric centers were obtained by controlling the absolute configuration of the adjacent stereogenic center through a heterogeneous catalytic hydrogenation with Pd–C of the double bond in the enol tautomer in route A and by biocatalytic reduction of the exomethylene group followed by reduction with $n\text{Bu}_4\text{NBH}_4$ in route B (Schemes 1 and 5). Other key reactions in the synthesis include such classical organic reactions as Claisen condensation, a Wasserman chain homologation, a Mannich-type olefination with maintenance of stereo integrity of the involved intermediates, and Pd–C catalyzed hydrogenation to obtain a diastereo- and enantioselective product by the reduction of the appropriate enol formed in the keto–enol equilibrium.

Results and Discussion

Our synthetic approach for route A is shown in Scheme 1. The ethyl benzoylacetate **1** was bioreduced, giving (*S*)-ethyl 3-hydroxyphenylpropanoate **2**, which was produced on a multigram scale⁵ (9.0 g) with 80% yield and 99% ee mediated by the yeast *Pichia kluyveri* CCT 3365 immobilized on alginate beads in a reactor (400 mL working volume) with mechanical stirring and temperature control (30 °C). The details were discussed in a recent publication.⁶

Protection of the β -hydroxy ester **2** as the TBS **3** ether (TBSCl, imidazole, DMF, rt, 2 h, 90% yield and 99% ee),⁷ followed by alkaline ester hydrolysis of **3** (LiOH/H₂O/EtOH in 80% yield and 99% ee) gave the corresponding acid **4**. Extension of **4** through the Wasserman protocol ([Ph₃PCH₂CN]Cl, EDCI and DMAP followed by ozonolysis in methanol and dichloromethane)⁸ furnished the required (*S*)-2-oxo ester **5** in 50% yield (after 2 steps) with preservation of the stereochemical integrity of the molecule. The direct Mannich-type α -methylenation of **5** under optimized conditions described previously⁹ yielded **6** in 85% yield and

99% ee after 2 h. Then, the chemical regio- and diastereoselective reduction of the α,β -unsaturated system **6** was investigated in order to examine the substrate specificities of the oxidoreductase yeasts. Initially a screening of biocatalysts to reduce **6** was carried out mediated by several yeasts cells in growing conditions (Scheme 2).

Unfortunately, all of the yeasts evaluated (*Saccharomyces cerevisiae*, *Pichia kluyveri* (CCT 3365), *Pichia stipitis* (CCT 2617), *Pichia canadensis* (CCT 2636), *Rhodotorula minuta* (CCT 1751), *Rhodotorula glutinis* (CCT 2182)) were not able to reduce **6** to **7**, **8**, or even **9** under several experimental conditions. These results can be rationalized by the high sterical hindrance caused by the TBS protecting group that could reduce or hinder the interaction between substrate and enzyme at the active site. To overcome these difficulties, we exchanged biocatalysts for traditional chemical methods. A catalytic hydrogenation of the C=C of (*S*)-**6** mediated by Pd–C in methanol under 1 atm gave after 16 h the methylhydroxy product **7** in 45% yield. The reduction step of this sequence proceed with modest diastereoselectivity giving a mixture of *syn:anti* isomers (ca. 3:1). Surprisingly, when the reaction was left more than 16 h, just one diastereoisomer, **8a**, was formed with excellent stereoselectivity (70% yield, > 99% de, ¹H NMR spectroscopic analysis (300 MHz); > 99% ee). Alternatively, a one-pot, two-step tandem catalytic hydrogenation in a round-bottom flask of (*S*)-**6** with Pd–C in methanol suspension under 1 atm after 15 h gave only one diastereoisomer, **8a**, with > 99% ee. These unique high diastereoselectivities could be attributed to the favorable keto–enol equilibrium when methanol is used as a solvent leading to epimerization of C-3 and subsequent reaction of **7** in the enolic form (Scheme 3).¹⁰ To our knowledge, there are no reports of hydrogenation of an enol intermediate in a keto–enol equilibrium using Pd–C (low pressure and room temperature), although there are reports using hydrogenation of enol acetates and silyl enol ethers from cyclic ketones¹¹ and with enolate ion.¹²

Although enol **7** can exist as two geometric isomers, *Z* and *E*, it is well-known that in Pd-catalyzed reactions, the overall reaction is not controlled by the adsorption step but by the step in which the hydrogen is transferred from the catalyst surface to the molecule on the opposite side of the larger substituent.¹³ In order to inspect these two isomeric structures, density functional theory calculations were performed

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(5) The limitation of scale here is of practical use. We limited the reaction scale to the use of a 400 mL stirred-tank reactor, 1.0 g of **1** per batch with no loss in yield and ee. See ref⁶ for schematic representation of the bioreactor and a photo of the used platform.

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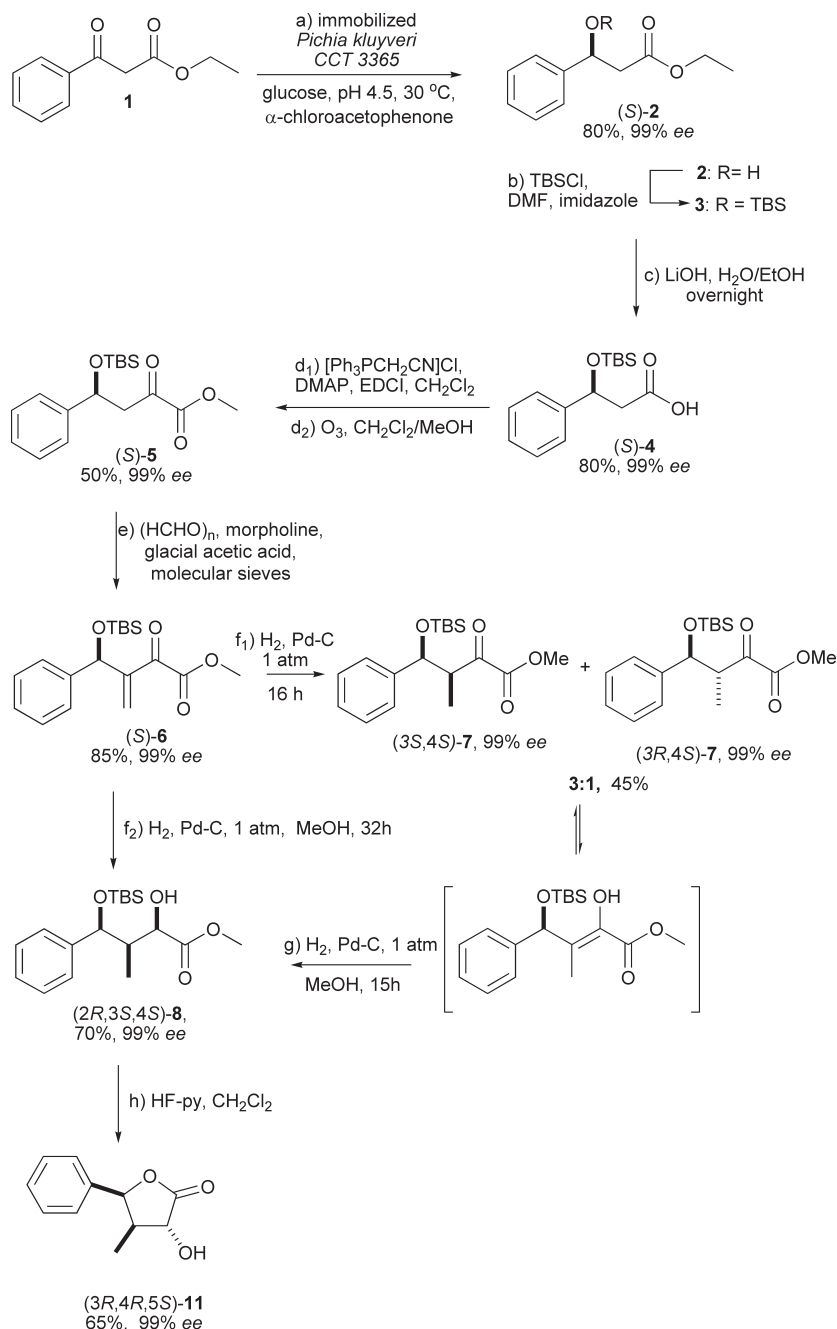
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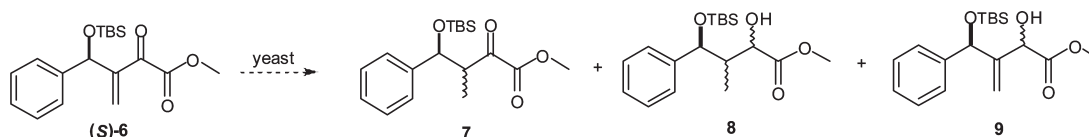
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SCHEME 1. Synthetic Route A



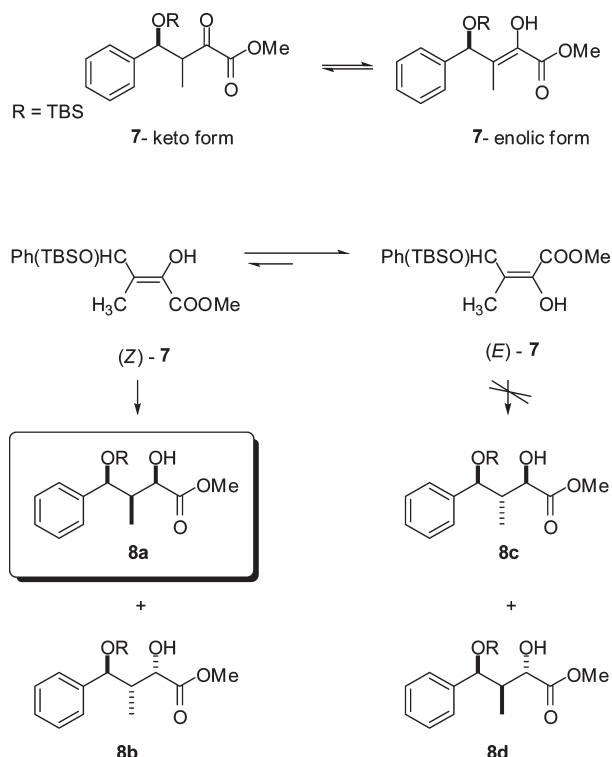
SCHEME 2. Possible Products from Yeast Reduction of (S)-6



with B3LYP/6-31G++(d,p).¹⁴ Those calculations have shown that tautomer (*E*)-7 (Scheme 3) is 1.55 Kcal/mol more stable when compared with (*Z*)-7 and that both sides of the

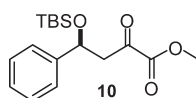
C=C bond of enol (*E*)-7 should be blocked by the voluminous -OTBS and -Ph groups (the dihedral angle between C-OTBS groups and C=C bonds is -127.3°) leaving no room for approximation of the hydrogen adsorbed on Pd-C to either side of the C=C bond. Examination of the calculated structure of enol (*Z*)-7 reveals that this enol is stabilized by an

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SCHEME 3. Keto–Enol Equilibrium of 7 Leading to 8a with High Stereoselectivity


internal hydrogen bond of 1.72 Å between the OH and OTBS groups. This internal hydrogen bond results in a distortion of the dihedral angle between C–OTBS and C=C bonds to 26.1°, bringing the OTBS nearer to the plane of the C=C bond and therefore leaving room for the approximation of hydrogen adsorbed on Pd–C to this side. This model justifies the exclusive formation of **8a** (see Figure 1). The high diastereoselectivity obtained demonstrates that the OTBS group acts not only as an effective protective group but also to assist the vicinal chiral center of the substrate to stereoselectively drive the reduction of C=C bond of the enol.

To emphasize the relevance of the keto–enol equilibrium, we repeated the reaction using the less enolizable ketoester **10** under the same reaction conditions (Pd–C, H₂, 1 atm, MeOH, 32 h), as a model to verify the selectivity of carbonyl reduction. The result showed no C=O reduction, and the starting material **10** was quantitatively recovered. This result shows the role of the enol tautomer for the reduction of the ketone in the above conditions.



The relative stereochemistry of **8a** was determined as 2,3-*syn*-3,4-*syn* through ¹H NMR chemical shift and coupling constant analysis. As the absolute configuration of C-4 was determined in the first step and was preserved since then and knowing the relative stereochemistry of these three contiguous centers, the absolute configuration of **8a** could be established as 2*R*,3*S*,4*S*. To confirm these results the desilylation of **8a** was performed with HF–pyridine in CH₂Cl₂ with

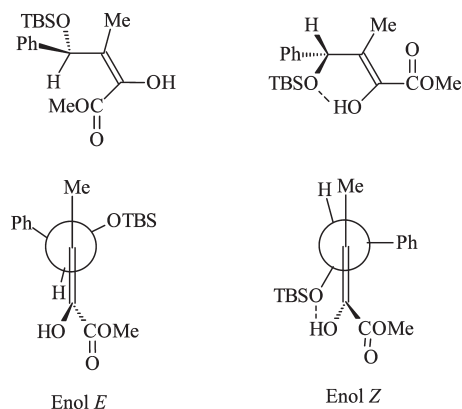


FIGURE 1. Conformation of both enols *E*-7 and *Z*-7.

concomitant molecule lactonization to give **11**. The data for NOE are shown in Scheme 4. Chemical shifts and coupling constants from ¹H NMR experiments in addition to GC–MS retention times and ¹³C NMR data are consistent with the formation of lactone **11** from compound **8a**. These data were compared with the literature,¹⁵ and the absolute configuration of **11** was established as 3*R*,4*R*,5*S*-**11**.¹⁶

The second strategy to obtain the desired α-hydroxy-β-methyl-γ-hydroxy esters intermediates, route B, is shown in Scheme 5. The first step was the Claisen condensation of acetophenone with diethyloxalate, which proved to be straightforward. A nearly quantitative yield of the 2,4-dioxo ester **12** was obtained by addition of diethyl oxalate followed by acetophenone to a freshly prepared NaOEt suspension in THF in 92% yield.¹⁷

The bioreduction of **12** was performed according to the Fadnavis protocol,^{10a} and the desired product **13** was achieved in 80% yield and 99% ee.¹⁸ The Mannich type α-methylenation previously described was applied to *R*-**13**,

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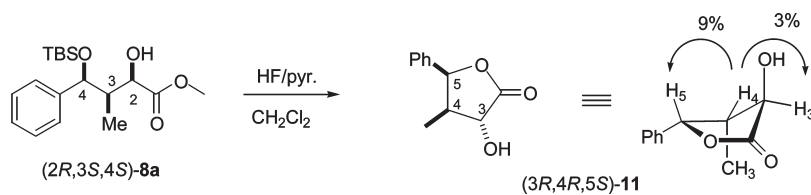
(16) Data of relative and absolute configuration of compounds **8a** and **11** were compared with data of their respective enantiomers shown in ref ¹⁵. These comparisons could be done because GC–MS retention times and ¹H NMR and ¹³C NMR data for the enantiomeric compounds are the same, and the first chiral center therein was established accurately in the first step and afterwards remained unchanged assisted by OTBS protecting group throughout route. The same comparison procedure was used for compound **18**.

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(18) Some difficulties were found in scaling up bioreduction of **12** due to formation of aggregates of yeast and water in the highly volatile diethyl ether. The vigorous stirring and a bath reactor coupled to a condenser was essential to minimize these issues.

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SCHEME 4. Relative and Absolute Configuration of 11 Determined by NMR



SCHEME 5. Synthetic Route B

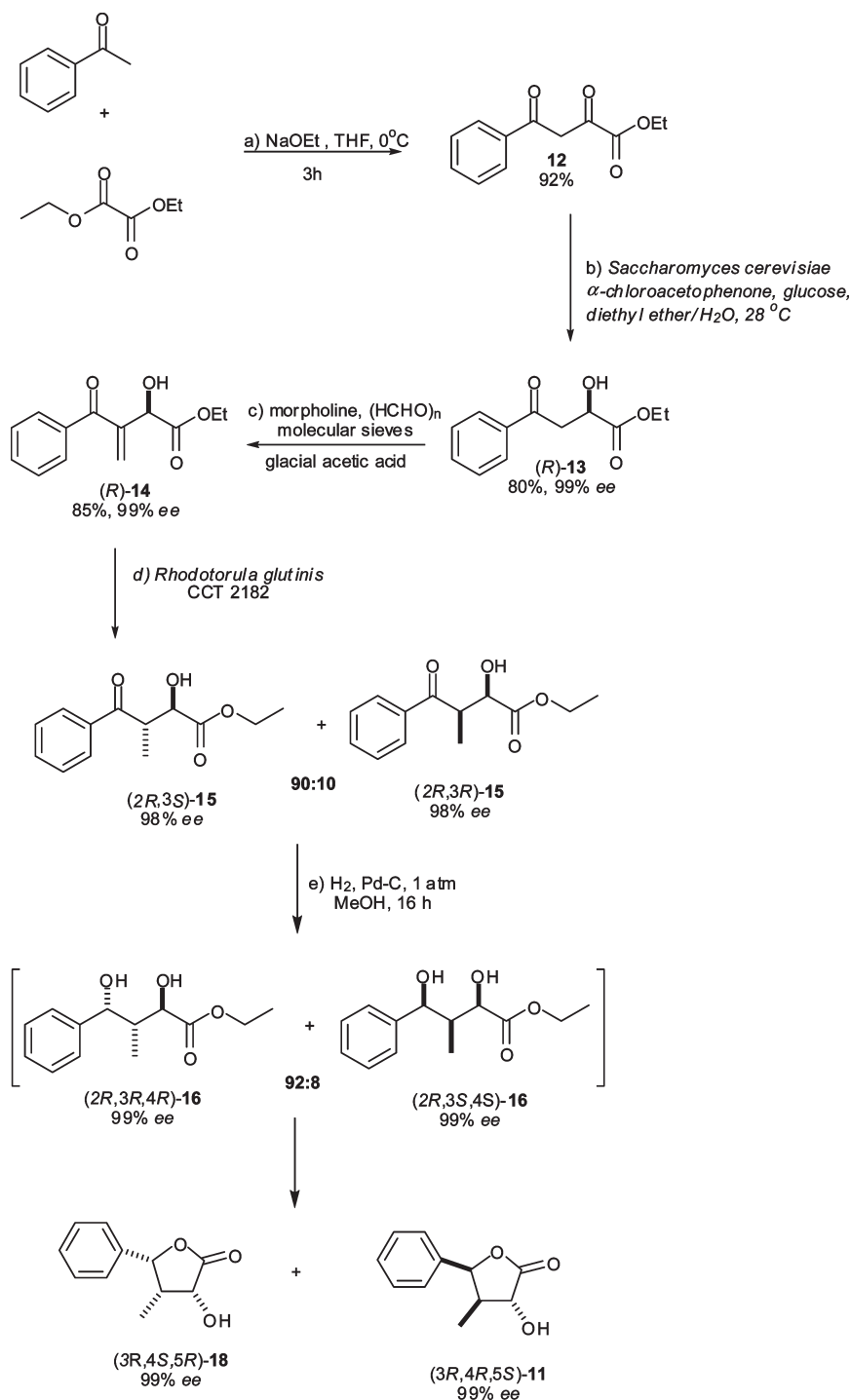
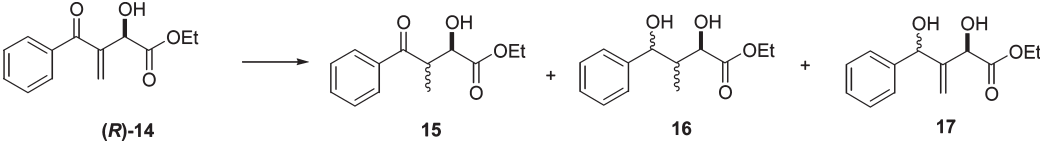
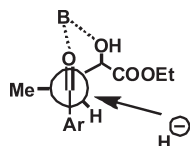


TABLE 1. Microbial Reduction of (*R*)-**14** Using Different Yeasts


yeast ^a	yield (%)	15 <i>anti:syn</i> (ee %) ^c	16 and/or 17	time (h)
<i>Saccharomyces cerevisiae</i> ^b	—	—	—	48
<i>Candida parapsilosis</i> (CCT 3438)	73	77:23 (99)	—	24
<i>Trichosporum cutaneum</i> (CCT 1903)	75	74:26 (97)	—	30
<i>Rhodotorula glutinis</i> (CCT 2182)	76	85:15 (99)	—	24

^aGrowing cells, YM medium, (*R*)-**14** 0.4 mmol (100 mg) in ethanol 1 mL, orbital stirring, 28 °C. ^bCommercial lyophilized yeast. ^cPercentage of enantiomeric excess of each diastereoisomer; enantiomeric and diastereoisomeric excess were determined by HPLC.

SCHEME 6. Cram Chelation Model of **15**

yielding *R*-**14** in 80% and 99% ee with preserved stereochemical integrity. The asymmetric biocatalytic reduction of **14** could provide two chiral centers. Although the stereoselectivity achieved in this kind of reduction is excellent, the chemoselectivity of whole cell bioreductions with respect to C=C versus C=O bond reduction is often poor as a result of the presence of competing alcohol dehydrogenases, since the enoate reductases and the alcohol dehydrogenases depend on the same nicotinamide cofactor.¹⁹ Thus, several yeast strains were evaluated for the bioreduction of **14** in order to find the most reactive ones (Table 1).

In all cases, the bioreduction of *R*-**14** afforded exclusively **15** in good yields, with diastereoselectivity and excellent enantioselectivity.²⁰ It is well-known that in α,β -unsaturated ketone systems, the double bond is reduced preferably.²¹ Only in rare cases, when α,β -unsaturated ketone resonance is destabilized by electron-withdrawing groups bonded to double bonds, the C=O is reduced first, which justifies the absence of **17**. The **14** γ -carbonyl is not electrophilic enough to favor a NADH/NADPH type hydride attack. Thus formation of **16** is not observed. The bioreduction of *R*-**14** was carried out on a multigram scale by the yeast *Rhodotorula glutinis* CCT 2182, giving **15** in 90:10 diastereoisomeric ratio favoring the isomer *anti*. As the γ -carbonyl of **15** was not reduced by any of the employed yeasts, a chemical reduction with *n*Bu₄NBH₄ was carried out. Reduction of **15** gave lactones **18** (white solid) and **11** (yellow oil) in 92:8 (*trans:cis*) selectivity (anti-Felkin products) as a result of favored five-membered ring formation. The high 1,3-*anti* selectivity can be explained by the Cram chelation model due to the OH group in the β position (Scheme 6).

The configuration of **11** was already established in route A. The relative configuration of **18** was determined as 3,4-*cis*-4,5-*cis* which is supported by ¹H NMR experiments and increments in NOE signals of NOESY1D

experiments as shown in Scheme 7, which are in agreement with the literature.¹⁵

Further determination of the absolute configuration of **18** was obtained using the chiral auxiliary reagent (*S*)-MPA (α -methoxyphenylacetic acid)²² (Scheme 8) and comparison of these data with the literature.¹⁵ The anisotropic effect of the aromatic ring of (*S*)-MPA over the methyl group was observed through the 0.61 ppm difference in the chemical shift toward TMS, proving that both groups are synperiplanar. Thus, it has been possible to designate the absolute configuration as (3*R*,4*S*,5*R*)-**18**.

Conclusions

In summary, we have achieved the syntheses of α -hydroxy- β -methyl- γ -hydroxy esters, which are a versatile chiral synthon possessing three stereogenic centers, through two distinct chemoenzymatic routes with excellent stereoselectivity. Notable features of these approaches include biocatalytic reductions and an outstanding stereo- and regioselective Pd–C hydrogenation due to the keto–enol equilibrium, in accordance with a model obtained by theoretical calculations. The synthetic strategies used to obtain these skeletons were efficient and provide the desired products in good overall yield. Furthermore, the biocatalytic pathways were performed on a multigram scale, and both syntheses are amenable to scale-up to produce important chiral synthons. In principle, these approaches are readily applicable for the preparation of more complex organic structures of industrial interest.

Experimental Section

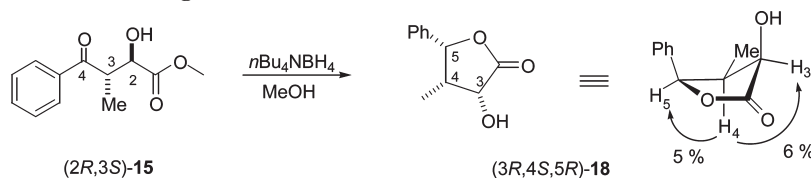
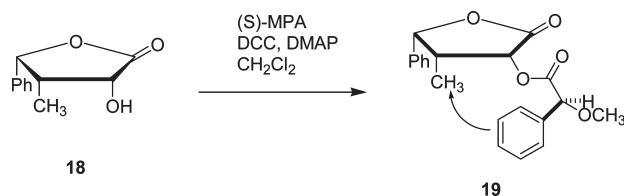
Ethyl (*S*)-(–)-3-hydroxy-3-phenylpropanoate, (*S*)-2**.** A solution of sodium alginate (150 mL, 2%) was added to a suspension of *Pichia kluyveri* CCT 3365 (23 g wet biomass) in distilled water (50 mL, pH 6.5). This mixture was extruded using syringe nozzles with inner diameters of 0.8 mm into a solution of CaCl₂ (0.2 mol/L) to produce beads with 2 mm diameters. After 20 min, the beads were filtered and washed with water to remove the excess of CaCl₂. The beads were suspended in distilled water (300 mL, pH 6.5) containing glucose (6 g) and α -chloroacetophenone (40 mg) in a 400 mL working volume bioreactor and stirred at 300 rpm at 30 °C. After activation of the yeast for 2 h, substrate **1** (5.2 mmol in 3.0 mL of ethanol) was added. The reaction was monitored by GC–MS, and after 24 h the beads were filtered and washed with ethyl acetate. The bead-free

(20) This bioreduction is easily scaled up, and as described previously, the limitation of scale is of practical use (a 400 mL stirred-tank reactor).

(21) Faber, K. *Bioreductions in Organic Chemistry*, 4th ed.; Springer-Verlag: Berlin, 2000.

(22) For a revision about absolute configuration assembly through NMR, see: Riguera, R.; Seco, J. M.; Quiñoá, E. *Chem. Rev.* **2004**, *104*, 17–117.

SCHEME 7. Assignment of Relative Configuration of 18

SCHEME 8. Absolute Configuration Assembly of 18 by ^1H NMR Employing (S)-MPA

mixture was extracted with ethyl acetate, dried over anhydrous MgSO_4 , and filtered, and the solvent was evaporated. The crude material was purified by flash chromatography, eluting with 10% ethyl acetate in hexane (to recover acetophenone side product) and 20% ethyl acetate in hexane (to recover **2** with 80% yield). Colorless oil; $^{23}\text{ee} = 99\%$, $[\alpha]_{\text{D}}^{20} = -20.0$ (c 2.05, MeOH); IR (neat) ν_{max} (cm^{-1}) 3420, 1727, 1452; MS m/z (%) 194 (M^+), 120 (14), 107 (100) and 77 (59); ^1H NMR (300.1 MHz, CDCl_3) δ 1.25 (t, 3H, $J = 7.3$ Hz), 2.70 (dd, 2H, $J = 4.4$ and $J = 8.4$ Hz), 4.16 (q, 2H, $J = 7.1$ Hz), 5.11 (t, 1H, $J = 4.4$ Hz), 7.31 (m, 5H); ^{13}C NMR (75.0 MHz, CDCl_3) δ 14.1 (CH_3), 43.4 (CH_2), 60.7 (CH_2), 70.2 (CH), 125.5 (CH), 127.5 (CH), 128.2 (CH), 142.3 (C_0), 172.1 (C_0).

Ethyl (S)-(-)-3-(tert-butylidimethylsilyloxy)-3-phenylpropanoate, (S)-3. A solution containing (S)-**2** (2.7 g, 14 mmol) previously dried in a vacuum for 30 min, *tert*-butyldimethylsilyl chloride (3.7 g, 24 mmol), imidazole (2.9 g, 42 mmol) and anhydrous DMF (15 mL) was stirred for 3 h under Ar atmosphere at room temperature. Then this mixture was dissolved in hexane (50 mL) and washed with water (3×50 mL). The combined organic phases were dried over MgSO_4 and evaporated. The residue was purified by DSC (hexane/EtOAc 20%) affording (S)-**3** with 90% yield and 99% ee as a colorless oil. $^{24}[\alpha]_{\text{D}}^{20} = -26.7$ (c 1.02, MeOH); IR (neat) ν_{max} (cm^{-1}) 1738, 1258, 1090; MS m/z (%) 293 (4), 103 (35) and 75 (100); ^1H NMR (300.1 MHz, CDCl_3) δ -0.09 (s, 3H), 0.02 (s, 3H), 0.86 (s, 9H), 1.25 (t, 3H, $J = 7.3$ Hz), 2.54 (dd, 1H, $J = 4.0$ and $J = 10.2$ Hz), 2.73 (dd, 1H, $J = 4.9$ and $J = 9.1$ Hz), 4.15 (m, 2H), 5.15 (dd, 1H, $J = 4.3$ Hz), 7.30 (m, 5H); ^{13}C NMR (75.0 MHz, CDCl_3) δ -4.6 (CH_3), -5.1 (CH_3), 14.3 (CH_3), 18.1 (C_0), 25.7 (CH_3), 46.6 (CH_2), 60.5 (CH_2), 72.2 (CH), 125.7 (CH), 127.4 (CH), 128.1 (CH), 144.0 (C_0), 171.0 (C_0).

(S)-(-)-3-(tert-Butyldimethylsilyloxy)-3-phenylpropanoic Acid, (S)-4. Compound (S)-**3** (2.43 g, 8 mmol) was dissolved in ethanol (10 mL) and cooled to 0 °C. Lithium hydroxide monohydrate (1.37 g, 32 mmol) was dissolved in water (5 mL) and slowly added to the (S)-**3** solution. This mixture was magnetically stirring with gradual warming. After 15 h, the solvent was evaporated. The residue was dissolved in acidified water (pH 4.0) and washed with hexane (3×10 mL). The combined organic phases were dried over MgSO_4 and evaporated to afford (S)-**4** with 80% yield and

99% ee. (S)-**4** was pure enough for further manipulation. Colorless oil; $^{25}[\alpha]_{\text{D}}^{20} = -16.8$ (c 0.90, MeOH); IR (neat) ν_{max} (cm^{-1}) 3300–2500, 1713, 1436; ^1H NMR (300.1 MHz, CDCl_3) δ -0.87 (s, 3H), 0.02 (s, 3H), 1.01 (s, 9H), 2.81 (dd, 1H, $J = 4.0$ and $J = 10.9$ Hz), 2.99 (dd, 1H, $J = 5.8$ and $J = 9.1$ Hz), 5.50 (dd, 1H, $J = 4.0$ Hz and $J = 4.0$ Hz), 7.51 (m, 5H); ^{13}C NMR (75.0 MHz, CDCl_3) δ -4.5 (CH_3), -5.2 (CH_3), 18.2 (C_0), 25.6 (CH_3), 46.0 (CH_2), 72.0 (CH), 125.7 (CH), 127.5 (CH), 128.3 (CH), 143.5 (C_0), 176.7 (C_0).

Methyl (S)-(-)-4-(tert-butylidimethylsilyloxy)-2-oxo-4-phenylbutanoate, (S)-5. (Cyanomethylene)triphenylphosphonium chloride (4.17 g, 12 mmol) was dissolved in water (25 mL) and dichloromethane (25 mL). Sodium hydroxide (1.26 g, 32 mmol) in water (6 mL) was slowly added. After 20 min, the two layers were separated. The organic layer was dried (MgSO_4) and added to a solution of (S)-**4** (1.94 g, 7 mmol), DMAP (0.2 g) and EDCI (2.13 g, 11 mmol) in dichloromethane (50 mL) at 0 °C under a nitrogen atmosphere. After 0.5 h, the reaction mixture was warmed to room temperature and left stirring for 15 h. Water (50 mL) was added, and the two layers separated. The organic layer was dried (MgSO_4) and concentrated in vacuum, resulting in a yellow paste. The yellow paste was dissolved in dichloromethane (10 mL) and methanol (20 mL) and cooled to -78 °C. Ozone was then bubbled through the solution until the reaction mixture turned colorless-gray (around 2 h). The reaction was followed by TLC. Nitrogen was then passed through the solution to remove ozone excess. The reaction mixture was warmed to room temperature and concentrated in vacuum. Purification by DSC, eluting with hexane/EtOAc 25%, gave (S)-**5** as a pale yellow oil 8a (0.87 g, 50% over two steps). $^{20}[\alpha]_{\text{D}}^{20} = -48.0$ (c 1.50, MeOH); IR (neat) ν_{max} (cm^{-1}) 1755, 1732, 1254; MS m/z (%) 159 (100), 131 (31), 89 (50), 75 (85); ^1H NMR (300.1 MHz, CDCl_3) δ -0.85 (s, 3H), 0.02 (s, 3H), 1.11 (s, 9H), 3.10 (dd, 1H, $J = 4.0$ and $J = 11.0$ Hz), 3.59 (dd, 1H, $J = 5.3$ and $J = 9.1$ Hz), 4.11 (s, 3H), 5.40 (dd, 1H, $J = 4.0$ Hz and $J = 4.0$ Hz), 7.51 (m, 5H); ^{13}C NMR (75.0 MHz, CDCl_3) δ -4.1 (CH_3), -4.6 (CH_3), 18.6 (C_0), 26.3 (CH_3), 50.6 (CH_2), 53.3 (CH_3), 72.0 (CH), 126.3 (CH), 128.1 (CH), 128.8 (CH), 144.1 (C_0), 161.5 (C_0), 192.3 (C_0).

Methyl (S)-(-)-4-(tert-butylidimethylsilyloxy)-2-oxo-3-methylene-4-phenylbutanoate, (S)-6. The reaction was carried out under a nitrogen atmosphere. The ketoester **5** (1.0 mmol) and a freshly prepared solution of morpholine (0.0264 g, 0.3 mmol) in glacial acetic acid (5.0 mL) were mixed in a 25 mL double-necked round-bottomed flask with a coiled reflux condenser flask. Molecular sieves (4 Å) and *p*-formaldehyde (0.29 g, 9.0 mmol) were then added, and the mixture was stirred and heated (reflux). After 2 h, the reaction mixture was cooled to room temperature and neutralized with solid NaHCO_3 followed by repeated (3 times) extractions with equal volumes of ethyl acetate. The combined organic extracts were washed with brine and water and dried over anhydrous MgSO_4 followed by evaporation under reduced pressure. The product was filtered through a chromatographic column filled with silica gel. Pale yellow oil; $^{9a}[\alpha]_{\text{D}}^{20} = -56.0$ (c 0.92, MeOH); IR (neat) ν_{max} (cm^{-1}) 1742, 1684, 1472, 1255; MS m/z (%) 319 (M^+), 277 (17), 171 (100), 115 (59), 89 (76), 75 (79); ^1H NMR (300.1 MHz, CDCl_3) δ -0.87 (s, 3H), 0.03 (s, 3H), 0.98 (s, 9H), 3.98 (s, 3H), 5.70 (s, 1H), 6.39 (s, 1H), 6.61 (s, 1H), 7.49 (m, 5H); ^{13}C NMR (75.0 MHz, CDCl_3) δ -4.6 (CH_3), -4.9 (CH_3), 18.4 (C_0), 25.9 (CH_3), 52.8 (CH_3), 71.5 (CH), 126.1

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(CH), 127.1 (CH), 128.0 (CH), 131.5 (CH₂), 142.3 (C₀), 148.3 (C₀), 163.7 (C₀), 186.7 (C₀).

Methyl 4-(*tert*-butyldimethylsilyloxy)-2-oxo-3-methyl-4-phenylbutanoate (syn:anti mixture), 7. Pd-C (5%, 0.005 mmol) was dissolved in anhydrous methanol (2 mL) and stirred under a H₂ atmosphere (1 atm). After 30 min, 0.01 mmol of (*S*)-**6** dissolved in anhydrous methanol was added to the mixture. The reaction was maintained under magnetic stirring, at room temperature and under a H₂ atmosphere (1 atm) for 16 h to obtain (*S*)-**7** and 15 h more to obtain (*S*)-**8a**. The mixture was filtered with filter paper, and the solvent was evaporated. The product were purified by column chromatography (hex/EtOAc 20%) affording a colorless oil in 70% yield; HPLC (Chiralcel OJ, hexane/*i*PrOH = 9:1, 0.1% trifluoroacetic acid, flow rate = 0.4 mL/min, λ = 254 nm) t_R = 44.2 min (3*S*,4*S*)-**7**; t_R = 45.1 min (3*R*,4*S*)-**7**; IR (neat) $\nu_{\max}$ (cm⁻¹) 1730, 1283, 1090; MS m/z (%) 173 (100), 117 (16), 105 (14), 89 (59), 73 (67), 59 (40); ¹H NMR (300.1 MHz, CDCl₃) 7-*syn* δ 0.20 (s, 9H), 0.91 (s, 6H), 1.10 (d, 3H, J = 6.6 Hz), 3.68 (m, 1H), 3.87 (s, 3H), 5.03 (d, 1H, J = 5.1 Hz), 7.27 (m, 5H); 7-*anti* δ 4.11 (s, 3H) and 4.72 (d, 1H, J = 9.2 Hz); ¹³C NMR (75.0 MHz, CDCl₃) 7-*syn* δ -4.5 (2 CH₃), 12.9 (CH₃), 26.2 (3 CH₃), 53.3 (CH₃), 50.5 (CH), 75.5 (CH), 142.5 (C₀), 162.0 (C₀), 195.9 (C₀); 7-*anti* δ -4.7 (2 CH₃), 13.8 (CH₃), 26.2 (3 CH₃), 51.3 (CH₃), 53.3 (CH), 79.3 (CH), 143.2 (C₀), 164.9 (C₀), 197.6 (C₀). HRMS 336.17569. C₁₈H₂₈O₄Si requires 336.17572.

Methyl (2*R*,3*S*,4*S*)-4-(*tert*-butyldimethylsilyloxy)-2-hydroxy-3-methyl-4-phenylbutanoate, 8a. Colorless oil; $[\alpha]_D^{20}$ -32.4 (*c* 0.60, MeOH); IR (neat) $\nu_{\max}$ (cm⁻¹) 3530, 1738, 1410, 1253; MS m/z (%) 163 (34), 115 (21), 107 (31), 91 (31), 75 (100); ¹H NMR (300.1 MHz, CDCl₃) δ 0.29 (s, 9H), 1.12 (s, 6H), 1.26 (d, 3H, J = 7.0 Hz), 2.45 (m, 1H), 3.05 (d, 1H, J = 4.4 Hz), 4.08 (s, 3H), 4.86 (d, 1H, J = 8.4 Hz), 7.59 (m, 5H); ¹³C NMR (75.0 MHz, CDCl₃) δ -4.5 (CH₃), -3.9 (CH₃), 10.7 (CH₃), 26.4 (3 CH₃), 53.0 (CH₃), 71.8 (CH), 77.1 (CH), 127.4 (2 CH), 127.9 (CH), 128.5 (2 CH), 143.8 (C₀), 175.7 (C₀). HRMS 338.19134. C₁₈H₃₀O₄Si requires 338.19129.

(3*R*,4*R*,5*S*)-3-Hydroxy-4-methyl-5-phenyl-dihydrofuran-2-one, 11. A HF-pyridine solution (0.8 mL) was added to **8a** (15 mg, 0.04 mmol) dissolved in anhydrous methylene chloride (5 mL), under magnetic stirring, Ar atmosphere at 0 °C with gradual warming. The reaction was monitored by GC-MS. The reaction was neutralized with solid NaHCO₃. The solvent was evaporated and the residue purified by column chromatography (hex/EtOAc 15%) affording **11** in 65% yield as a colorless oil. ¹⁵ IR (neat) $\nu_{\max}$ (cm⁻¹) 3362, 1776, 1455, 1096; MS m/z (%) 192 (M⁺), 148 (80), 115 (10), 105 (30), 91 (100), 77 (25); ¹H NMR (300.1 MHz, CDCl₃) δ 0.87 (d, 3H, J = 7.0 Hz), 2.75-2.96 (m, 1H), 4.22 (d, 1H, J = 9.9 Hz), 5.63 (d, 1H, J = 8.1 Hz), 7.13 (m, 2H), 7.31-7.43 (m, 3H), 7.38-7.45 (m, 2H); ¹³C NMR (75.0 MHz, CDCl₃) δ 13.3 (CH₃), 42.1 (CH), 72.2 (CH), 82.4 (CH), 125.7 (CH), 128.5 (CH), 128.6 (CH), 135.5 (C₀), 177.5 (C₀).

Ethyl 2,4-Dioxo-4-phenylbutanoate, 12. A freshly prepared sodium ethoxide solution (Na 0.02 mol in 10 mL of ethanol at 0 °C) was added to acetophenone (1.0 g, 0.009 mol) and diethyl oxalate (2.7 g, 0.02 mol) dissolved in anhydrous THF, under a Ar atmosphere. This solution was stirred at 0 °C for 2 h and quenched by addition of cooled 2 mol/L HCl (120 mL, 0 °C) and extracted with EtOAc. The combined organic layers were dried over MgSO₄ and filtered, and the solvent was evaporated. The residue, a yellow paste, ^{17c} was pure enough for further manipulation, 92% yield. Mp 34-36 °C; IR (neat) $\nu_{\max}$ (cm⁻¹) 3125, 1742, 1600, 1615, 1451; MS m/z (%) 220 (M⁺), 147 (100), 105 (38), 120 (5), 77 (20); ¹H NMR (300.1 MHz, CDCl₃) δ 1.42 (t, 3H, J = 7.3 Hz), 2.40 (q, 2H, J = 6.9); 7.05 (s, 1H); 7.45-7.62 (m, 3H), 8.01 (d, 2H, J = 8.0 Hz); ¹³C NMR (75.0 MHz, CDCl₃) δ 14.4 (CH₃), 63.1 (CH₂), 98.4 (CH), 128.0 (CH), 129.0 (CH), 134.2 (CH), 135.3 (C₀), 162.6 (C₀), 170.3 (C₀), 191.1 (C₀).

Ethyl (*R*)-(-)-2-Hydroxy-4-oxo-4-phenylbutanoate, 13. Yeast cells (20 g) were suspended in sodium citrate buffer (0.1 mol/L,

pH 4.5, 50 mL) containing glucose (5 g). The cells were allowed to activate for 3 h, and diethyl ether was added (200 mL), followed by the addition of α -chloroacetophenone (150 mg) dissolved in diethyl ether (10 mL). The contents were vigorously stirred on a magnetic stirrer at 28 °C for 3 h in a bath reactor coupled to a condenser. Substrate **12** (1.1 g, 45 mmol) dissolved in diethyl ether (50 mL) was added, and the contents were stirred. After 48 h, the cells were filtered with Celite. The aqueous phase was acidified with 6 mol/L HCl (10 mL) and extracted with EtOAc (3 $\times$ 50 mL). The combined organic layer was washed with brine, dried over anhydrous MgSO₄, and concentrated by rotatory evaporation to give a viscous yellow oil. This oil was dissolved in ethanolic HCl (50 mL) and stirred at room temperature overnight. Ethanol was then evaporated, and the residual oil was purified by column chromatography using hexane/EtOAc 25%, affording **13**, a white solid ^{17a,d} with low melting point in 80% yield and 99% ee. Mp 36 to 38 °C; $[\alpha]_D^{20}$ = -5.5 (*c* 1.0, CHCl₃); HPLC (Chiralcel OJ, hexane/*i*PrOH = 9:1, 0.1% trifluoroacetic acid, flow rate = 0.7 mL/min, λ = 254 nm) t_R = 27.4 min (*S*)-**13**; t_R = 29.6 min (*R*)-**13**; IR (neat) $\nu_{\max}$ (cm⁻¹) 3489, 1738, 1685; MS m/z (%) 222 (M⁺), 149 (19), 120 (20), 105 (100), 77 (48); ¹H NMR (300.1 MHz, CDCl₃) δ 1.28 (t, 3H, J = 7.1 Hz), 3.50 (dd, 2H, J = 7.0), 4.30 (q, 2H, J = 7.1 Hz), 4.64-4.62 (m, 1H), 7.40-7.60 (m, 3H), 7.95 (d, 2H, J = 7.1 Hz); ¹³C NMR (75.0 MHz, CDCl₃) δ 14.1 (CH₃), 42.1 (CH₂), 61.8 (CH₂), 67.2 (CH), 128.1 (CH), 128.7 (CH), 128.8 (CH), 133.5 (C₀), 173.8 (C₀), 197.2 (C₀).

Ethyl (*R*)-(-)-2-Hydroxy-3-methylene-4-oxophenylbutanoate, 14. The reaction was carried out under a nitrogen atmosphere. The ketoester **13** (1.0 mmol) and a freshly prepared solution of morpholine (0.0264 g, 0.3 mmol) in glacial acetic acid (5.0 mL) were mixed in a 25 mL double-necked round-bottomed flask with a coiled reflux condenser flask. Molecular sieves (4Å) and *p*-formaldehyde (0.29 g, 9.0 mmol) were then added, and the mixture was stirred and heated (reflux). After 2 h, the reaction mixture was cooled to room temperature and quenched with solid NaHCO₃ followed by repeated (3 times) extractions with equal volumes of ethyl acetate. The combined organic extracts were washed with brine and water and dried over anhydrous MgSO₄ followed by evaporation under reduced pressure. The product was purified by DSC chromatography (hexane/EtOAc 70%) affording **14**, a pale yellow oil, ^{9a} in 85% yield. $[\alpha]_D^{20}$ = -21.0 (*c* 2.4, MeOH); IR (neat) $\nu_{\max}$ (cm⁻¹) 3489, 1739, 1659, 1597; MS m/z (%) 234 (M⁺), 205 (6), 132 (15), 149 (16), 120 (5), 105 (100), 77 (34); ¹H NMR (300.1 MHz, CDCl₃) δ 1.26 (t, 3H, J = 8.0 Hz), 4.18 (q, 2H, J = 7.0), 5.34 (s, 1H), 5.82 (s, 1H), 6.19 (s, 1H), 7.40-7.60 (m, 3H), 7.79 (d, 2H, J = 7.0 Hz); ¹³C NMR (75.0 MHz, CDCl₃) δ 15.2 (CH₃), 62.3 (CH₂), 71.7 (CH), 127.3 (CH₂), 128.4 (CH), 128.5 (2 CH), 129.6 (2 CH), 132.9 (C₀), 144.9 (C₀), 172.5 (C₀), 197.2 (C₀). HRMS 234.04671. C₁₃H₁₄O₄ requires 234.08921.

Ethyl (2*R*)-2-Hydroxy-3-methyl-4-oxo-4-phenylbutanoate, 15. (*syn/anti* mixture) (General procedure) Yeast medium (125 mL) was autoclaved for 15 min at 121 °C and 1.5 atm. After cooling, 3 mL of yeast inoculum was added, and the reaction was kept at 30 °C in an orbital shaker for 48 h. Then, **14** (1 mmol) was dissolved in ethanol (1 mL) and added to the growing medium. The reaction was monitored by GC-MS. At the end of reaction, the cells were centrifuged and the aqueous phase extracted with EtOAc. The combined organic layers were dried with MgSO₄, and the solvent was evaporated affording a pale yellow oil. ¹⁵ The residue was purified by TLC (hexane/EtOAc 65%). HPLC (Chiralcel OJ, hexane/*i*PrOH = 4:1, flow rate = 0.4 mL/min, λ = 254 nm) t_R = 19.9 min (2*R*,3*R*)-**15**; t_R = 21.4 min (2*R*,3*S*)-**15**; IR (neat) $\nu_{\max}$ (cm⁻¹) 3484, 1736, 1683, 1596; MS m/z (%) 236 (M⁺), 163 (16), 105 (100), 77 (32); ¹H NMR *syn* (300.1 MHz, CDCl₃) δ 1.26 (t, 3H, J = 7.0 Hz), 1.29 (d, 3H, J = 7.0 Hz), 3.28 (s, 1H), 3.93 (dq, 1H, J = 4.2 and 7.0 Hz), 4.25 (q, 2H, J = 7.0

Hz), 4.58 (m, 1H), 7.40–7.65 (m, 3H), 7.90–8.05 (m, 2H); *anti* δ 1.20 (t, 3H, $J = 7.1$ Hz), 1.36 (d, 3H, $J = 7.3$ Hz), 3.61 (d, 1H, OH, $J = 8.3$ Hz), 3.98 (dq, 1H, $J = 4.6$ and 7.3 Hz), 4.10–4.25 (m, 2H), 4.39 (dd, 1H, $J = 4.6$ and 8.3 Hz), 7.40–7.65 (m, 3H), 7.90–8.00 (m, 2H); ^{13}C NMR *syn* (75.0 MHz, CDCl_3) δ 12.1 (CH_3), 14.0 (CH_3), 44.3 (CH_2), 61.9 (CH), 71.6 (CH), 128.4 (CH), 128.7 (CH), 133.3 (CH), 135.7 (C_0), 173.1 (C_0), 201.6 (C_0); *anti* δ 14.0 (CH_3), 14.1 (CH_3), 44.0 (CH_2), 61.5 (CH), 73.1 (CH), 128.3 (CH), 128.7 (CH), 133.4 (CH), 135.9 (C_0), 173.1 (C_0), 201.6 (C_0).

(3*R*,4*S*,5*R*)-3-Hydroxy-4-methyl-5-phenyl-dihydrofuran-2-one, 18. (*cis/trans* mixture) Compound **15** (1.0 mmol) was dissolved in anhydrous ethanol (2 mL) under magnetic stirring and cooled to -78°C . Then, $n\text{Bu}_4\text{NBH}_4$ (1.0 mmol) was slowly added, and the temperature was increased to -23°C . After 1 h, acetone (5 mL) and saturated NaHCO_3 solution were added. The aqueous phase was extracted with EtOAc (3×10 mL), and the combined organic layers dried over MgSO_4 and concentrated under vacuum. The residue was purified by TLC (45% hexane/EtOAc) affording the diastereoisomeric mixture (3*R*,4*S*,5*R*)-**18** (white solid) (3*R*,4*R*,5*R*)-**11** (colorless oil) in 98:2 ratio. The relative stereochemistries of **11** and **18** were determined by ^1H NMR, NOESY1D and literature comparison.¹⁵ White solid, MP $150\text{--}151^\circ\text{C}$; IR (neat) ν_{max} (cm^{-1}) 3443, 1758, 1414, 1294,; MS m/z (%) 192 (M^+), 148 (80) 133 (15), 115 (10), 105 (30), 91 (100), 77 (25); ^1H NMR (300.1 MHz, CDCl_3) δ 0.65 (d, 3H, $J = 7.3$ Hz), 2.75 (s, 1H), 2.98–3.08 (m, 1H), 4.79 (d, 1H, $J = 6.8$ Hz), 5.57 (d, 1H, $J = 4.6$ Hz), 7.25–7.38 (m, 5H); ^{13}C NMR (75.0

MHz, CDCl_3) δ 7.4 (CH_3), 41.1 (CH); 72.1 (CH); 80.2 (CH); 125.2 (CH); 128.2 (CH); 128.6 (CH); 135.1 (C_0); 177.0 (C_0).

(3*R*,4*S*,5*R*,2'*S*)-4-Methyl-2-oxo-5-phenyl-tetrahydrofuran-3-yl (2'-methoxy-2'-phenylacetate), 19. Anhydrous methylene chloride (1.5 mL) was added to **18** (10 mg, 0.05 mmol), and then DMAP and (*S*)-MPA (4 mg, 0.05 mmol) were added to this solution. The mixture was cooled to 0°C , and DCC (10 mg, 0.005 mmol) was added. The reaction was maintained under magnetic stirring with gradual warming. After 28 h the mixture was filtered, and the solvent evaporated. The residue was purified by column chromatography (20% hexane/EtOAc) and yielded a white solid.¹⁵ IR (neat) ν_{max} (cm^{-1}) 3445, 1798, 1756, 1455; MS m/z (%) 121 (100), 122 (9), 105 (9), 91 (12), 77 (19). ^1H NMR (300.1 MHz, CDCl_3) δ 0.10 (d, 3H, $J = 7.3$ Hz), 2.96–3.02 (m, 1H), 3.45 (s, 3H), 4.96 (s, 1H), 5.55 (d, 1H, $J = 5.1$ Hz), 5.77 (d, 1H, $J = 6.9$ Hz), 7.25–7.45 (m, 10H).

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Supporting Information Available: General experimental and compound characterization data. This material is available free of charge via the Internet at <http://pubs.acs.org>.