Mixed Aromatic Acyloin Condensations with Recombinant Benzaldehyde Lyase: Synthesis of α-Hydroxydihydrochalcones and Related α-Hydroxy Ketones

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Abstract: Recombinant benzaldehyde lyase (BAL), expressed and purified from *E. coli* strain JM-109, was used to catalyze the condensation of a series of methoxybenzaldehydes and phenylacetaldehyde in the synthesis of α-(*R*)-hydroxydihydrochalcones. Enantiomerically pure 1-hydroxy-1,3-diphenylpropan-2-ones and *o*-anisoin were also obtained as products of the BAL reaction. The *R* absolute configurations of chiral centers were determined by CD spectroscopy. α-(*R*)-Hydroxydihydrochalcones and 1-hydroxy-1,3-diphenylpropan-2-ones are valuable synthons for chemoenzymatic syntheses of flavonoids. This is the first synthesis of α-(*R*)-hydroxydihydrochalcones by a microbial enzyme.

Keywords: acyloin condensation, benzaldehyde lyase, chemoenzymatic synthesis, flavonoid, α-hydroxydihydrochalcone

Introduction

Flavonoids are a diverse group of natural products that play important roles in plant growth and development. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these polyphenolic compounds.[1] These properties make flavonoids interesting targets for chemical and enzyme synthetic processes.

Enzymes are now widely used for both the biotransformation and synthesis of natural products.[2] These biocatalysts are useful reagents because of their inherent abilities to catalyze highly regio- or stereospecific reactions under mild reaction conditions.[3] Practically every known type of synthetic reaction finds its counterpart in enzyme-mediated catalysis. Reactions that form carbon-carbon bonds are among these useful processes.

Benzaldehyde lyase (BAL, EC 4.1.2.38), a thiamine pyrophosphate (TPP) dependent enzyme from *Pseudomonas fluorescens* Biovar I, catalyzes cleavage of the carbon-carbon α-hydroxy ketone bond of benzoin to form two benzaldehydes.[4] BAL also catalyzes the reverse acyloin condensation of benzaldehydes resulting in the synthesis of (*R*)-benzoins.[5] Chiral substituted acyloins are useful intermediates in organic synthesis because they are bifunctional and contain a stereogenic center amenable to synthetic manipulation.[6] Although BAL has been successfully used in the synthesis of benzoins and precursors of pharmaceuticals with antifungal properties,[5,7] little has been reported on the abilities of the biocatalyst to catalyze mixed aromatic acyloin condensations between benzaldehydes and phenylacetaldehyde derivatives.

Enzymatic condensations between benzaldehydes and phenylacetaldehydes could afford chiral α-hydroxydihydrochalcones, a rare group of C_6-C_3-C_6 plant metabolites that share biogenetic and synthetic precursor relationships with flavonoids and other compounds.[8,9] We envisioned the possibility of forming α-hydroxydihydrochalcones such as **3** enzymatically (Scheme 1) and subsequent chemical demethylation and dehydration in a simple, chemoenzymatic synthesis of chalcones. In this study, we describe the products formed when recombinant BAL catalyzes mixed acyloin condensations between methoxy-substituted benzaldehydes and phenylacetaldehyde.

Results and Discussion

Benzoylformate decarboxylase and pyruvate decarboxylase are TPP-containing enzymes that catalyze acyloin condensations.[3,10,11] However, while they both may accept benzaldehydes as one reactant in forming acyloins, the second reaction component is obligatory, and not amenable to wide-scale synthetic use in the synthesis of chalcone-like compounds. Pyruvate decarboxylase, for example, condenses an acetaldehyde equivalent with many acceptor aldehydes in the synthesis of acyloins.[11b] Although benzoylformate decar-

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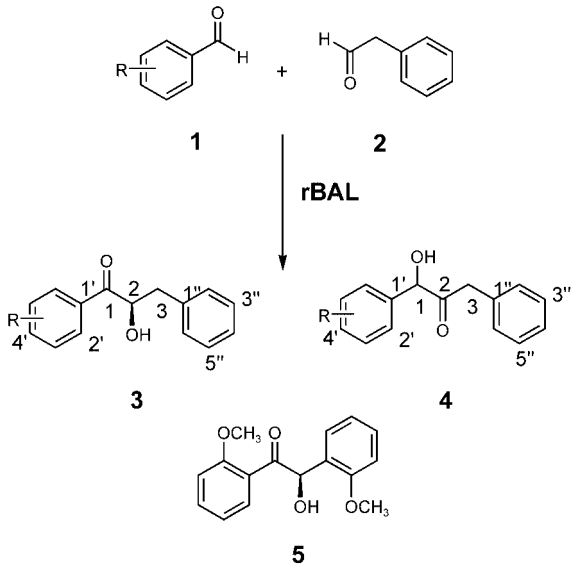

R	
2-MeO	a
2,3-DiMeO	b
2,4-DiMeO	c
2,5-DiMeO	d
3,5-DiMeO	e

Scheme 1. BAL-catalyzed phenylacetaldehyde and benzaldehyde condensation. Enzyme reactions were conducted as described in the Experimental Section.

boxylase binds a broad range of different aldehydes, the best results with respect to enantiomeric excess (ee) of acyloin products appears to occur with *meta*-substituted benzaldehydes.[5,10]

Aldehyde substrate requirements for BAL acyloin synthesis appear to be more relaxed. Thus, many types of substituted benzaldehydes can be coupled in acyloin syntheses. However, little use of BAL to couple dissimilar aromatic aldehydes has been reported.[12] While 2-hydroxybenzaldehyde is a poor substrate with BAL,[13] the corresponding 2-methoxybenzaldehydes do condense to form acyloins.[7] Thus, we examined BAL-catalyzed, mixed acyloin reactions with phenylacetaldehyde and variously substituted methoxybenzaldehydes (Scheme 1).

Preparation of the biocatalyst was relatively straightforward. The $pBAL_{HIS}$ plasmid was introduced into *E. coli* JM109 by heat shock treatment. Selection of colonies of *E. coli* containing the plasmid was rendered simply by cultivation of competent cells on medium containing ampicillin. Growth of plasmid-containing colonies by shake flask culture and induction of BAL synthesis by isopropylthio-β-D-galactoside (IPTG) gave cells that were enriched in BAL. Hexahistidine tagged BAL was purified from cell free extracts by nickel agarose chromatography, and frozen for later use. The enzyme prepared this way was stable, and demonstrated a specific activity of 0.15 U/mg protein.

BAL reactions between phenylacetaldehyde (**2**) and several mono- and dimethoxybenzaldehydes (**1a – e**) usually gave mixtures of products, **3**, **4** and **5**. Although products like **3** and **4** have been prepared by multistep chemical processes,[9,14] and a product like **4** was formed in trace quantities using phenylpyruvate decarboxylase as biocatalyst,[15] this is the first report of a biocatalytic synthesis of α-hydroxydihydrochalcones (**3**).

Before selecting final reaction conditions, we initially conducted BAL reactions to obtain sufficient amounts of products for structure analysis. ^{1}H NMR spectra of **3a – e** were characterized by doublets of doublets between 5.15 and 5.3 ppm for carbinol methine protons, and doublets of doublets centered at about 3.1 and 2.4 ppm for geminally split methylene group protons. UV spectra contained absorption bands for conjugated carbonyls between 305 – 330 nm, significantly shifted *vs.* the carbonyl absorptions for non-conjugated **4a – e**. CD spectra exhibited weak negative Cotton effects in the 315 – 340 nm region, slightly positive between 290 and 306 nm, and negative between 248 and 264 nm. These types of CD spectra are characteristic for α-(*R*)-hydroxydihydrochalcones.[8,16] Since BAL is known to catalyze the synthesis of *R*-acyloins,[5,7] we conclude that all of the **3a – e** acyloins synthesized by BAL were of *R*-absolute configuration.

In general, ^{1}H NMR spectra of **4a – e** contained singlets at about 5.5 ppm for the α-hydroxymethine protons, doublets at about 3.7 ppm from geminal couplings of the two, methylene group protons, and readily assigned signals for methoxy and aromatic protons. UV spectra contained absorptions between 270 – 300 nm typical to unconjugated carbonyl moieties, and between 230 – 220 nm for aromatics. The enantiomeric purities of **4a – e** were established by chiral HPLC over a Chiracel OJ column, known to resolve racemic alcohols. To test the capabilities of such a column, racemic anisoin was well resolved into two peaks at Rv of 38.5 mL and 40.2 mL. The peak eluting at 38.5 mL was determined to be that of *R*-anisoin by using *R*-anisoin synthesized by BAL. All of the compounds evaluated by chiral HPLC gave single peaks with estimated % ee of greater than 99%. CD spectra for **4a – e** showed negative Cotton effects between 280 and 305 nm and weakly positive ones between 229 and 231 nm. These observations suggest that **4a – e** are also of *R*-absolute configurations. Since acyloin products formed from BAL-catalyzed condensation of benzaldehydes are always of *R* absolute configuration,[5,7] they likely form by attack of the TPP bound donor aldehyde to the *Si* face of the acceptor aldehyde. Mechanistically, BAL would be similar to benzoylformate decarboxy-

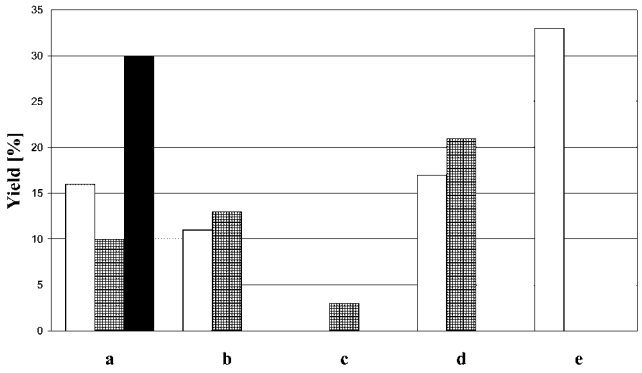

Figure 1. Effects of the benzaldehyde substitutions on BAL catalyzed reaction yields and products. Compound **3** □, **4** ⊡, **5** ■. Methoxy substitutions for **a**, **b**, **c**, **d**, and **e** are from Scheme 1. The yields are the averages of three determinations for each bar.

lase, a TPP-dependent enzyme that also forms *R*-benzoin.[11a]

Beginning with previously reported conditions,[5] we evaluated the influences on the reaction of varying BAL and substrate concentrations, reaction time, and benzaldehyde substituents on the yield and range of products formed during condensation reactions. Maximum yields were obtained with 80 units of enzyme/L with equimolar amounts of 2′-methoxybenzaldehyde and phenylacetaldehyde added initially. Temperature had little influence on reaction outcomes and HPLC analysis showed that maximum yields were obtained within 72 h. The yield of products was independent of the benzaldehyde:phenylacetaldehyde ratio. Although second and third quantities of BAL and phenylacetaldehyde were added initially to obtain sufficient amounts of products for structure analysis, we later found that this was not necessary. Attempts to improve the selectivity of the BAL reaction for **3a** or **4a** *vs.* **5** were unsuccessful.

A donor-acceptor concept for enzymatic cross-coupling reactions of aldehydes with BAL has been proposed.[12] Some aldehydes act as both donors and acceptors while others can selectively act as only donor or acceptor. Four different types of products were expected from BAL mediated condensations of benzaldehydes with phenylacetaldehyde (Scheme 1). Interestingly, no phenylacetaldehyde condensation products were ever observed, while **3**, **4** and **5** were formed to varying degrees depending on the structures of benzaldehyde reactants. With **1a** and **2**, BAL reaction yields were good, and **3a**, **4a** and **5** were all produced (Figure 1). Ratios of products were unaltered with time in this or any of the other reactions summarized in Scheme 1, indicating that the results were a function of the substituents on benzaldehyde, and not on substrate or product inhibition of the biocatalyst. In general, lower yields and fewer products were obtained using disubstituted benzaldehydes (**1b – e**). With disubstituted benzaldehydes, no acyloins such as **5** were produced. 2,3-Dimethoxybenzaldehyde (**1b**) gave only **3b** and **4b**, and, 2,5-dimethoxybenzaldehyde (**1d**) gave only **3d** and **4d**. With 2,4-dimethoxybenzaldehyde (**1c**) only **4c** was synthesized, albeit in lower yield than obtained in other reactions. On the other hand 3,5-dimethoxybenzaldehyde (**1e**) gave only **3e** and in good yield. Benzaldehydes like the 2,6-dimethoxy-, 2,3,4-trimethoxy-, 2,4,5-trimethoxy- and 2,4,6-trimethoxy-derivatives were poor substrates for BAL acyloin condensations. The results with BAL condensations are similar to those observed with other TPP-dependent enzymes, where steric and electronic effects dictate stereochemical outcomes of catalysis.[5,17]

Conclusion

BAL-catalyzed condensations of benzaldehydes and phenylacetaldehyde in the synthesis of α-*R*-hydroxydihydrochalcones and 1-hydroxy-1,3-diphenylpropan-2-ones. The relaxed specificity of BAL enabled the synthesis of a variety of acyloin products. In general, trisubstituted benzaldehydes were poor substrates, while disubstituted benzaldehydes, and especially those substituted in the *meta* position gave fewer products in better yields.

Experimental Section

Chemicals

Anisoin and phenylacetaldehyde were obtained from Aldrich, Milwaukee, WI; *o*-anisaldehyde was obtained from TCI America, Portland, OR; 2,3-dimethoxybenzaldehyde, 2,6-dimethoxybenzaldehyde, 3,5-dimethoxybenzaldehyde, 2,3,4-trimethoxybenzladehyde, and 2,4,6-trimethoxybenzaldehyde were obtained from Acros Organics, Springfield, NJ; 2,4-dimethoxybenzaldehyde, 2,5-dimethoxybenzaldehyde, and 2,4,5-trimethoxybenzaldehyde were obtained from ICN, Aurora, OH; thiamine pyrophosphate chloride was obtained from Sigma Chemicals, St. Louis, MO.

Chromatography

TLC was performed on silica gel GF_{245} (Merck) layers of 0.5 mm of thickness for analysis and 1 mm thickness for preparative layer chromatography. Plates were developed using hexanes:ethyl acetate (7:3 v/v). The developed plates were visualized under 254 and 360 nm UV light before being sprayed with 2,4-dinitrophenylhydrazine. 2,4-Dinitrophenylhydrazine was prepared by dissolving 3 g of 2,4-dinitrophenylhydrazine in 25% (v/v) H_2SO_4 in EtOH.

HPLC was performed with a Shimadzu LC-6A dual pumping system connected to a Shimadzu SPD-6AV UV/vis detector and a Shimadzu SCL-6B system controller (Kyoto,

Japan). Separations were carried out over an Econosil C18 column (250 mm × 4.6 mm ID, 10 μm particle size, Alltech, Deerdfield IL). The mobile phases consisted of acetonitrile:-water (10:90) containing 5% of formic acid (A) and acetonitrile:water (90:10) containing 5% formic acid (B). The gradient was 0–100% B over 65 min at a flow rate of 1 mL/min. UV absorbances were recorded at 280 nm. Chiral HPLC was carried out with a Chiralcel OJ, (250 × 4.6 mm ID, 10 μm particle size, Diacel Chemical Industries, Exton, PA) linked to a Chiralcel OJ guard column (50 × 4.6 mm), at a flow rate of 0.5 mL/min with *n*-hexane:ethanol (90:10, v/v).

Spectral Analysis

UV spectra were determined with a Shimadzu UV-210PC, UV-vis scanning spectrophotometer (Shimadzu Corporation, Kyoto, Japan). ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AMX-600 high field spectrometer (Bruker Instruments, Billerica, MA) equipped with an IBM Aspect-2000 processor, operating at 600 and 150.94 MHz for ^{1}H and ^{13}C, respectively. All NMR spectra were obtained in methanol-d_4 using TMS as internal standard, with chemical shifts expressed in parts per million (δ) and coupling constants (*J*) in Hertz. HMBC and HMQC experiments were carried out using a Bruker AMX-600 high field spectrometer. CD spectra were determined with an Olis CD spectrophotometer (Bogart, GA). Optical rotations were measured with a Jasco P-1020 polarimeter (Easton, MD).

Preparation of BAL Biocatalyst

$pBAL_{HIS}$ plasmid was kindly donated by Dr. Michael Müller from the Institute of Biotechnology from Julich, Germany.[5] The plasmid was introduced into *E. coli* JM109 by heat shock.[18] 100 μL of thawed high efficiency competent *E. coli* JM 109 cells (Promega Corporation, Madison, WI), were mixed with 20 ng of the $pBAL_{HIS}$, the mixture was left on ice for 10 min and then incubated for 45 sec in a 42 °C water bath. After the incubation the tubes were returned to ice for 2 min, then 900 μL of SOC medium were added and the mixture was left for 1 h, at 37 °C, and 250 rpm. At the end of the incubation 150 μL were plated in Luria-Bertani (LB)[19] medium containing 100 μg/mL ampicillin. The plates were incubated at 37 °C for 12 h, after the incubation a single colony was picked into 5 mL of LB/ampicillin (100 μg/mL) medium and growth overnight, 30 °C, 250 rpm. 100 μL of the resulting fermentation were inoculated in 50 mL of LB/ampicillin medium and grown overnight. The last fermentation was used to inoculate 450 mL of LB/ampicillin medium, growth at 30 °C, and 250 rpm for 1 h or until the optical density (595 nm) was 0.5–0.7. After this time, the expression was induced by the addition of 1 mM final concentration of IPTG. Cells were harvested after further 27 h by centrifugation 2,400 × *g* and stored at −70 °C.

The hexahistidine-tagged BAL was purified using a nickel nitriloacetic acid (Ni-NTA) agarose column (Qiagene, Valencia, CA) as reported previously.[20] All the following steps were performed in 50 mM in potassium phosphate buffer, pH 7, containing $MgSO_4$ (2.5 mM), TPP (0.15 mM) and 15% PEG 400 (v/v). 28 g of cells were thawed and disrupted by sonication (Sonifier cell disruptor 350, Branson Sonic Co. Danbury, CT). The lysate was cleared by centrifugation at 13,000 × *g*. Pre-equilibrated Ni-NTA resin (7 mL) was added to the cleared lysate and incubated at 4 °C, while being shaken at 200 rpm for 1 h. After this time, the lysate-Ni-NTA mixture was placed into a column and fractions were collected. Unbound proteins were removed by washing four times with buffer. Subsequently, weakly bound proteins were eluted with 10 mM imidazole in 50 mM potassium phosphate buffer (pH 7). The elution of His-tagged, bound enzyme was achieved by washing the column with 250 mM imidazole in 50 mM phosphate buffer (pH 7.6).

One mL reactions containing BAL in 50 mM phosphate buffer (pH 7.0) and 1.5 mM anisoin were monitored at 280 nm to measure the formation of anisaldehyde. One unit (U) of BAL activity was defined as the amount of enzyme that catalyzed the cleavage of 1 μmol anisoin to anisaldehyde per min at 30 °C.

Enzymatic Acyloin Condensations

Phenylacetaldehyde (0.046 mL, 0.35 mmol) and methoxybenzaldehydes **1a – e** (0.4 mmol), were dissolved in a mixture of dimethyl sulfoxide (0.8 mL), and potassium phosphate buffer [3.2 mL, 50 mM, pH 7, containing $MgSO_4$ (2.5 mM) and TPP (0.15 mM)]. After addition of BAL (0.32 U) the reaction mixture was allowed to stand at 25 °C for 24 h before further BAL (0.32 U) and phenylacetaldehyde (0.046 mL, 0.35 mmol) were added. After 72 h the pH was adjusted to pH 2 with 5 N HCl and extracted three times with 4 mL of ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate, and vacuum concentrated to viscous oils. Products were purified from crude extracts by preparative TLC.

(*R*)-1-(2′-Methoxyphenyl)-2-hydroxy-3-phenylpropanone (**3a**): yield: 16%; ee >99%; $[\alpha]_D^{25}$: 7.9 (*c* 0.02, MeOH); UV (*c* 0.04, MeOH): λ_{max} (log ε) = 211 (4.5), 250 (3.63), 307 nm (3.11); CD (*c* 0.3 MeOH): $[\theta]_{234} = 0$, $[\theta]_{250} = -287$, $[\theta]_{268} = 0$, $[\theta]_{296} = 66.3$; ^{1}H NMR (methanol-d_4, 600 MHz, HMBC): δ = 7.7 (1H, dd, *J* = 7.7/1.7 Hz, H-6′), 7.6 (1H, dt, *J* = 7.6/1.8 Hz, H-4′), 7.4 (2H, dt, *J* = 8.2/1.7 Hz, H-3″,5‴), 7.25 (2H, d, *J* = 8.2 Hz, H-2″,6″), 7.15 (1H, d, *J* = 7.6 Hz, H-3′), 7.1 (1H, t, *J* = 8.5 Hz, H-4″), 7.04 (1H, dt, *J* = 7.7/1.7 Hz, H-5′), 7.10 (1H, dd, *J* = 8/1.6 Hz, H-6′), 5.23 (1H, dd, *J* = 8.5/3.8 Hz, H-2), 3.8 (3H, s, 2′-OMe), 3.06 (1H, dd, *J* = 14/3.8 Hz, H-3a), 2.07 (1H, dd, *J* = 8.5/14 Hz, H-3b); ^{13}C NMR (methanol-d_4, 150.94 MHz, HMQC): δ = 139 (C1″), 127.5 (C2″), 129.5 (C3″), 130 (C4″), 129 (C5″), 127 (C6″), 42 (C3), 79 (C2), 204 (C1), 125 (C1′), 159 (C2′), 115 (C3′), 132 (C4′), 122 (C5′), 132 (C6′), 58 (2′OMe); HRMS: *m/z* $(M - H_2O)^+ = 238.0993$, calculated for $C_{16}H_{14}O_2$: 238.0993.

(*R*)-1-(2′,3′-Dimethoxyphenyl)-2-hydroxy-3-phenylpropanone (**3b**): yield: 11%; ee >99%; $[\alpha]_D^{25}$: −4.7 (*c* 0.02, MeOH); UV (*c* 0.02, MeOH): λ_{max} (log ε) = 211 (4.5), 254 (3.66), 309 nm (3.09); CD (*c* 0.005 MeOH): $[\theta]_{220} = 0$, $[\theta]_{248} = -16016$, $[\theta]_{274} = 0$, $[\theta]_{296} = 8408$, $[\theta]_{314} = 0$; $[\theta]_{328} = -6864$; ^{1}H NMR (methanol-d_4, 600 MHz, HMBC): δ = 7.22 (2H, dt, *J* = 8.2/1.7 Hz, H-3″,5″), 7.25 (1H, dd, *J* = 8/1.7 Hz, H-4′), 7.16 (2H, d, *J* = 8.2 Hz, H-2″,6″), 7.18 (1H, t, *J* = 8.5 Hz, H-4″), 7.14 (1H, t, *J* = 8 Hz, H-5′), 7.10 (1H, dd, *J* = 8/1.6 Hz, H-6′), 5.15 (1H, dd, *J* = 8.5/3.8 Hz, H-2), 3.9 (3H, s, 3′-OMe), 3.87 (3H, s, 2′-OMe), 3.06 (1H, dd, *J* = 14/3.8 Hz, H-3a), 2.07 (1H, dd, *J* = 8.5/14 Hz, H-3b); ^{13}C NMR (methanol-d_4, 150.94 MHz, HMQC): δ = 139 (C1″),127.5 (C2″), 129.5 (C3″), 130.5 (C4″), 129.5 (C5″), 127.5 (C6″), 41.2 (C3), 78 (C2), 202 (C1), 132 (C1′), 147.3 (C2′), 154.5 (C3′), 117.5

(C4′), 125.5 (C5′), 122.2 (C6′), 56 (3′OMe), 63 (2′OMe); HRMS: *m/z* $(M)^+ = 286.1190$, calculated for $C_{17}H_{18}O_4$: 286.1205.

(*R*)-1-(2′,5′-Dimethoxyphenyl)-2-hydroxy-3-phenylpropanone (**3d**): yield: 17%; ee >99%; $[\alpha]_D^{25}$: 7.9 (*c* 0.38, MeOH); UV (*c* 0.09, MeOH): λ_{max} (log ε) = 209 (4.5), 252 (3.69), 334 nm (3.35); CD (*c* 0.006, MeOH): $[\theta]_{240} = 0$, $[\theta]_{254} = -13346$, $[\theta]_{306} = 2860$, $[\theta]_{322} = 0$, $[\theta]_{338} = -3336$, $[\theta]_{358} = 0$, $[\theta]_{364} = 438$; ^{1}H NMR (methanol-d_4, 600 MHz, HMBC): δ = 7.23 (2H, dt, *J* = 7.4/1.6 Hz, H-3″,5″), 7.215 (1H, d, *J* = 3.2 Hz, H-6′), 7.18 (2H, d, *J* = 8.5 Hz, H-2″,6″), 7.179 (1H, t, *J* = 8.5 Hz, H-4″), 7.146 (1H, dd, *J* = 8.5/3.2 Hz, H-4′), 7.09 (1H, d, *J* = 8.5 Hz, H-3′), 5.3 (1H, dd, *J* = 8.4/3.6 Hz, H-2), 3.9 (3H, s, 2′-OMe), 3.77 (3H, s, 5′-OMe), 3.08 (1H, dd, *J* = 14/3.6 Hz, H-3a), 2.67 (1H, dd, *J* = 8.35/14 Hz, H-3b); ^{13}C NMR (methanol-d_4, 150.94 MHz, HMQC): δ = 139 (C1″), 130.3 (C2″), 129 (C3″), 127.5 (C4″), 129 (C5″), 130.3 (C6″), 41.5 (C3), 78.8 (C2), 204 (C1), 127 (C1′), 154 (C2′), 114.5 (C3′), 122 (C4′), 155.5 (C5′), 115.5 (C6′), 56.8 (2′OMe), 56.2 (5′OMe); HRMS: *m/z* $(M)^+ = 286.1204$, calculated for $C_{17}H_{18}O_4$: 286.1205.

(*R*)-1-(3′,5′-Dimethoxyphenyl)-2-hydroxy-3-phenylpropanone (**3e**): yield: 33%; ee >99%; $[\alpha]_D^{25}$: −20.4 (*c* 0.017, MeOH); UV (*c* 0.03, MeOH): λ_{max} (log ε) = 211 (4.5), 266 (3.89), 321 nm (3.41); CD (*c* 0.0002, MeOH): $[\theta]_{230} = 0$, $[\theta]_{234} = 54340$, $[\theta]_{242} = 0$, $[\theta]_{264} = -257400$, $[\theta]_{285} = 0$; $[\theta]_{300} = 181610$, $[\theta]_{320} = 0$, $[\theta]_{334} = -157300$; ^{1}H NMR (methanol-d_4, 600 MHz, HMBC): δ = 7.23 (2H, dt, *J* = 7.4/1.6 Hz, H-3″,5″), 7.186 (1H, t, *J* = 8.5 Hz, H-4″), 7.18 (2H, d, *J* = 8.5 Hz, H-2″,6″), 7.059 (2H, d, *J* = 2.3 Hz, H-2′,6′), 6.72 (1H, t, *J* = 2.3 Hz, H-4′), 5.23 (1H, dd, *J* = 8.4/3.6 Hz, H-2), 3.8 (6H, s, 3′,5′-OMe), 3.11 (1H, dd, *J* = 14/3.6 Hz, H-3a), 2.87 (1H, dd, *J* = 8.35/14 Hz, H-3b); ^{13}C NMR (methanol-d_4, 150.94 MHz, HMQC): δ = 139 (C1″),127.2 (C2″), 129.5 (C3″), 130.5 (C4″), 129.5 (C5″), 127.5 (C6″), 42 (C3), 75.3 (C2), 202 (C1), 137 (C1′), 107.3 (C2′), 162.5 (C3′), 106.5 (C4′), 162.5 (C5′), 107.3 (C6′), 57 (3′OMe), 57 (5′OMe); HRMS: *m/z* $(M)^+ = 286.1198$, calculated for $C_{17}H_{18}O_4$: 286.1205.

1-Hydroxy-1-(2′-methoxyphenyl)-3-phenylpropanone (**4a**): yield: 10%; ee >99%; $[\alpha]_D^{25}$: −167 (*c* 0.25, MeOH); UV (*c* 0.017, MeOH): λ_{max} (log ε) = 220 (4.6), 257 nm (3.9); CD (*c* 0.1, MeOH): $[\theta]_{210} = 0$, $[\theta]_{228} = 990$, $[\theta]_{248} = 0$, $[\theta]_{290} = -2014$; ^{1}H NMR (methanol-d_4, 600 MHz, HMBC): δ = 7.34 (1H, dt, *J* = 7.5/1.7 Hz, H-4′), 7.28 (1H, dd, *J* = 7.5/1.7 Hz, H-6′), 7.5 (2H, dt, *J* = 7.6/1.7 Hz, H-3″,5″), 7.17 (1H, dt, *J* = 7.5/1.7 Hz, H-4″), 7.01 (1H, d, *J* = 7.5 Hz, H-3′), 6.99 (2H, d, *J* = 7.5 Hz, H-2″,6″), 6.96 (1H, t, *J* = 7.5 Hz, H-5′), 5.5 (1H, s, H-1), 3.8 (3H, s, 2′-OMe), 3.68 (1H, d, *J* = 15 Hz, H-3a), 3.66 (1H, d, *J* = 15 Hz, H-3b); ^{13}C NMR (methanol-d_4, 150.94 MHz, HMQC): δ = 137 (C1″),131 (C2″), 128 (C3″), 126 (C4″), 128 (C5″), 131 (C6″), 56 (C3), 75 (C1), 208 (C2), 128 (C1′), 159 (C2′), 112 (C3′), 132 (C4′), 123 (C5′), 130 (C6′), 55.7 (2′-Me); HRMS: *m/z* $[M - (H_2O_2)]^+ = 222.1043$, calculated for $C_{16}H_{14}O$: 222.1044.

1-Hydroxy-1-(2′,3′-dimethoxyphenyl)-3-phenylpropanone (**4b**): yield: 13%; ee >99%; $[\alpha]_D^{25}$: −123 (*c* 0.5, MeOH); UV (*c* 0.017, MeOH): λ_{max} (log ε) = 208 (4.5), 275 nm (3.41); CD (*c* 0.004, MeOH): $[\theta]_{210} = 0$, $[\theta]_{226} = 91000$, $[\theta]_{242} = 0$, $[\theta]_{278} = -122200$, $[\theta]_{292} = -117000$; ^{1}H NMR (methanol-d_4, 600 MHz, HMBC): δ = 7.23 (2H, t, *J* = 7.6 Hz, H-3″,5″), 7.17 (1H, t, *J* = 7.4 Hz, H-4″), 7.07 (1H, t, *J* = 8 Hz, H-5′), 7.05 (2H, d, *J* = 8 Hz, H-2″,6″), 7.03 (1H, dd, *J* = 8/1.6 Hz, H-4′), 6.87 (1H, dd, *J* = 7.6/1.6 Hz, H-6′), 5.45 (1H, s, H-1), 3.87 (3H, s, 3′-OMe), 3.82 (3H, s, 2′-OMe), 3.71 (1H, d, *J* = 15 Hz, H-3a), 3.62 (1H, d, *J* = 15 Hz, H-3b); ^{13}C NMR (methanol-d_4, 150.94 MHz, HMQC): δ = 136 (C1″),131 (C2″), 129 (C3″), 127 (C4″), 129 (C5″), 131 (C6″), 56 (C3), 75 (C1), 208 (C2), 124 (C1′), 147 (C2′), 155 (C3′), 115 (C4′), 124 (C5′), 122 (C6′), 55.7 (2′,3′-Me); HRMS: *m/z* $(M)^+ = 286.1209$, calculated for $C_{17}H_{18}O_4$: 286.1205.

(α)-1-Hydroxy-1-(2′,4′-dimethoxyphenyl)-3-phenylpropan-2-one (**4c**): yield: 3%; ee >99%; $[\alpha]_D^{25}$: −82 (*c* 0.2, MeOH); UV (*c* 0.018, MeOH): λ_{max} (log ε) = 207 (4.5), 275 nm (3.5); CD (*c* 0.004, MeOH): $[\theta]_{210} = 0$, $[\theta]_{238} = 27646$, $[\theta]_{258} = 0$, $[\theta]_{288} = -42900$; ^{1}H NMR (methanol-d_4, 600 MHz, HMBC): δ = 7.22 (2H, t, *J* = 7.2 Hz, H-3″,5″), 7.16 (1H, d, *J* = 8 Hz, H-6′), 7.17 (1H, dt, *J* = 8.5/2.3 Hz, H-4″), 7.01 (2H, d, *J* = 8.5 Hz, H-2″,6″), 6.56 (1H, d, *J* = 2.3 Hz, H-3′), 6.54 (1H, dd, *J* = 8.5/2.3 Hz, H-5′), 5.4 (1H, s, H-1), 3.81 (3H, s, 4′-OMe), 3.77 (3H, s, 2′-OMe), 3.7 (1H, d, *J* = 15 Hz, H-3a), 3.67 (1H, d, *J* = 15 Hz, H-3b); ^{13}C NMR (methanol-d_4, 150.94 MHz, HMQC): δ = 137 (C1″),130.5 (C2″), 129.4 (C3″), 131.4 (C4″), 129.4 (C5″), 130.5 (C6″), 45.6 (C3), 75.3 (C1), 209 (C2), 120 (C1′), 158 (C2′), 106.3 (C3′), 165 (C4′), 99.6 (C5′), 127.7 (C6′), 55.7 (2′,4′-OMe); HRMS: *m/z* $(M)^+ = 286.1206$, calculated for $C_{17}H_{18}O_4$: 286.1205.

1-Hydroxy-1-(2′,5′-dimethoxyphenyl)-3-phenylpropanone (**4d**): yield: 21%; ee >99%; $[\alpha]_D^{25}$: −110 (*c* 0.23, MeOH); UV (*c* 0.018, MeOH): λ_{max} (log ε) = 207 (4.5), 296 nm (3.45); CD (*c* 0.004, MeOH): $[\theta]_{210} = 0$, $[\theta]_{224} = 20020$, $[\theta]_{250} = 0$, $[\theta]_{304} = -45760$; ^{1}H NMR (methanol-d_4, 600 MHz, HMBC): δ = 7.21 (2H, t, *J* = 7.4 Hz, H-3″,5″), 7.17 (1H, t, *J* = 7.4 Hz, H-4″), 7.02 (2H, d, *J* = 7.4 Hz, H-2″,6″), 7.95 (1H, d, *J* = 8.5 Hz, H-3′), 6.88 (1H, dd, *J* = 8.5/3.2 Hz, H-4′), 6.86 (1H, d, *J* = 3.2 Hz, H-6′), 5.5 (1H, s, H-1), 3.79 (3H, s, 2′-OMe), 3.72 (3H, s, 5′-OMe), 3.7 (1H, d, *J* = 15 Hz, H-3a), 3.67 (1H, d, *J* = 15 Hz, H-3b); ^{13}C NMR (methanol-d_4, 150.94 MHz, HMQC): δ = 131 (C1″), 130.7 (C2″), 129.3 (C3″), 127.8 (C4″), 129.3 (C5″), 130.7 (C6″), 45.8 (C3), 75.2 (C1), 208 (C2), 129 (C1′), 152 (C2′), 113.5 (C3′), 115.8 (C4′), 155 (C5′), 115.7 (C6′), 56.5 (2′,6′-OMe); HRMS: *m/z* $(M)^+ = 286.1206$, calculated for $C_{17}H_{18}O_4$: 286.1205.

(*R*)-2-Hydroxy-1-(2′-methoxyphenyl)-2-(2″-methoxyphenyl)-ethanone (**5**): yield: 30%; ee >99%; $[\alpha]_D^{25}$: −122[7] (*c* 0.15, $CHCl_3$); ^{1}H NMR (methanol-d_4, 600 MHz): δ = 7.49 (1H, dd, *J* = 7.6/1.8 Hz, H-6′), 7.36 (1H, dt, *J* = 7.6/1.8 Hz, H-4′), 7.18 (1H, dd, *J* = 7.6/1.8 Hz, H-4″), 7.16 (1H, dt, *J* = 7.6/1.8 Hz, H-6″), 6.9 (1H, d, *J* = 7.6 Hz, H-3′), 6.89 (1H, t, *J* = 7.6 Hz, H-5′), 6.83 (1H, d, *J* = 7.6/1.8 Hz, H-3″), 6.83 (1H, t, *J* = 7.6/1.8 Hz, H-5″), 6.17 (1H, s, H-α), 3.7 (3H, s, 2′-OMe), 3.67 (3H, s, 2″-OMe); ^{13}C NMR (methanol-d_4, 150.94 MHz): δ = 127 (C1″), 158 (C2″), 110 (C3″), 130 (C4″), 122 (C5″), 131 (C6″), 75 (C2), 208 (C1), 127 (C1′), 159 (C2′), 112 (C3′), 134 (C4′), 123 (C5′), 130 (C6′), 58 (2″-OMe), 58 (2′-Me); HRMS: *m/z* $(M)^+ = 272.1043$, calculated for $C_{16}H_{16}O_4$: 272.1049.

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