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**PAPER**

# Enzymatic diastereo- and enantioselective synthesis of α-alkyl-α,β-dihydroxyketones†

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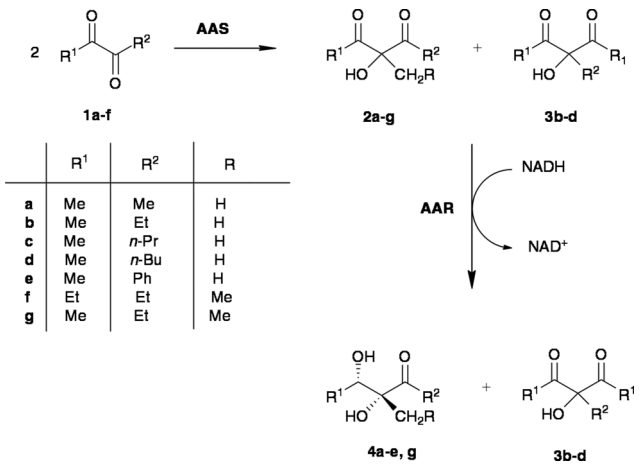
An enzymatic strategy for the preparation of optically pure α-alkyl-α,β-dihydroxyketones is reported. Homo- and cross-coupling reactions of α-diketones catalyzed by acetylacetoin synthase (AAS) produce a set of α-alkyl-α-hydroxy-β-diketones (30–60%, ee 67–90%), which in turn are reduced regio-, diastereo-, and enantioselectively to the corresponding chiral α-alkyl-α,β-dihydroxyketones (60–70%, ee >95%) using acetylacetoin reductase (AAR) as catalyst. Both enzymes are obtained from *Bacillus licheniformis* and used in a crude form. The relative *syn* stereochemistry of the enantiopure α,β-dihydroxy products is assigned by NOE experiments, whereas their absolute configuration is determined by conversion of the selected 3,4-dihydroxy-3-methyl-pentan-2-one to the natural product (+)-citreodiol.

## Introduction

Enantiopure tertiary alcohols are very valuable building blocks for the synthesis of many different natural products and pharmaceuticals. As a consequence, many efforts have been devoted to the development of chemical[1] and enzymatic[2] strategies towards these valuable structures. Various biocatalysts, such as epoxide hydrolases,[3] halohydrin dehalogenases,[4] hydroxynitrile lyases,[5] lipases, and esterases[6] have been widely used to this purpose. Surprisingly, the use of thiamine diphosphate (ThDP)-dependent enzymes in this field appears to be scanty. In 2010 Müller and co-workers described the utilization of YerE, a recombinant ThDP-dependent flavoenzyme, for umpolung couplings of pyruvate with different ketone acceptors to give enantioenriched tertiary alcohols.[7] In the same year, we reported a parallel study in which we demonstrated that α-diketones may serve as acyl anion equivalents when ThDP-dependent acetylacetoin synthase (AAS) from *Bacillus licheniformis*[8] is used as catalyst in the homo- and cross-couplings of α-diketones **1** (Scheme 1).

By the disclosed carboligation reaction, which is virtually an intermolecular aldehyde–ketone coupling, we were able to prepare a set of chiral α-alkyl-α-hydroxy-β-diketones **2** (30–45%) with good enantioselectivity (ee 67–91%) together with the regioisomeric prochiral derivatives **3**[9] (Scheme 1). Tertiary alcohols **2** and **3** are promising building blocks for asymmetric synthesis because they display a structure with two additional carbonyl groups which




**Scheme 1** Synthesis of the *syn*-α-alkyl-α,β-dihydroxy-ketones **4a–e,g**.

can be selectively elaborated. In this regard, the enantio- and stereoselective reduction of α-hydroxy-β-diketones **2** towards α,β-dihydroxy-ketones **4** is an opportunity (Scheme 1).

On the other hand, enantiopure diols of type **4** have already proven to be valuable precursors for the synthesis of chiral polysubstituted tetrahydrofurans[10] and natural products.[11] Indeed, various dehydrogenases have been successfully employed for the asymmetric reduction of β-diketones,[12] but only few of them showed activity toward α-disubstituted substrates.[13]

Herein we report the highly regio-, diastereo-, and enantioselective reduction of α-hydroxy-β-diketones **2**, obtained by the previously described AAS-based strategy, to *syn*-α-alkyl-α,β-dihydroxy-ketones **4** using *B. licheniformis* acetylacetoin reductase (AAR) as catalyst.[14] A crucial issue, in the present work, is the stereochemical assignment of the resulting α,β-dihydroxyketones **4** that we addressed by means of NOE experiments and by

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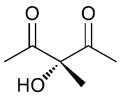
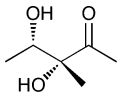
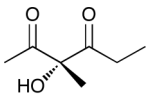
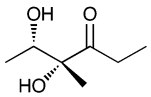
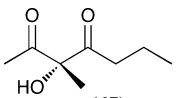
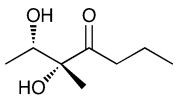
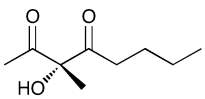
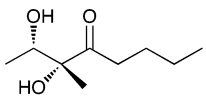
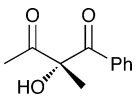
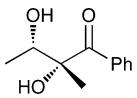
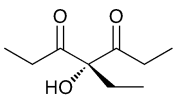
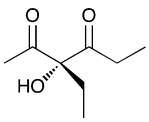
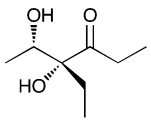
† Electronic supplementary information (ESI) available: copies of $^{1}H$ and $^{13}C$ NMR spectra. See DOI: 10.1039/c1ob05928a

**Table 1** AAR-mediated reduction of compounds **2a–g**

| Entry | α-Hydroxy-β-diketone (ee%)[a] | Time (h) | α,β-Dihydroxyketone yield%[b] (ee%)[a] |
|---|---|---|---|
| 1 |  **2a** | 3 |  **4a** 60 (>95) |
| 2 |  **2b** (70) | 3 |  **4b** 61[c] (>95) |
| 3 |  **2c** (67) | 4 |  **4c** 65[c] (>95) |
| 4 |  **2d** (72) | 4 |  **4d** 70[c] (>95) |
| 5 |  **2e** (76) | 5 |  **4e** 63 (>95) |
| 6 |  **2f** | 12 | — |
| 7 |  **2g** (91) | 12 |  **4g** 63 (>95) |

[a] Determined by chiral GC-analysis (see experimental section). [b] Isolated yield. [c] Isolated yield based on α-hydroxy-β-diketone **2**.

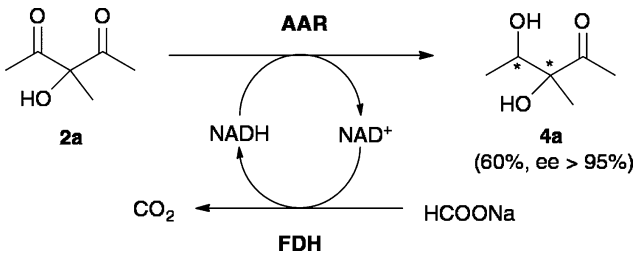
the stereoconservative conversion of a selected product into the natural compound (+)-citreodiol.

## Results and discussion

We recently synthesised a number of α-alkyl-α-hydroxy-β-diketones **2** and **3** by coupling reactions of α-diketones **1** using acetylacetoin synthase from *Bacillus licheniformis* (Scheme 1 and Table 1 for complete structures).[9] More precisely, compounds **2a–f** were obtained by homo-couplings of the corresponding diketones **1a–f**, while **2g** was prepared by cross-coupling of **1a** as donor and diketone **1f** as acceptor. In the case of unsymmetrically substituted diketones **1b–d**, the formation of chiral β-diketones **2b–d** was accompanied by the generation of variable amounts (24–45%) of the regioisomeric prochiral derivatives **3b–d**.[15]

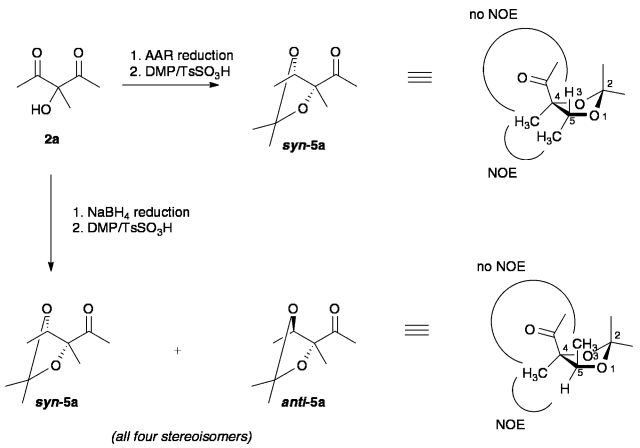
With diketones **2** and **3** in hand, we could begin our investigation on their asymmetric reduction to acyl diols through selective reduction of one carbonyl group. A previous explorative mechanistic study showed the capability of bacterial AAR to promote the selective reduction of acetylacetoin **2a** to acetylbutanediol **4a** on an analytical scale.[16] This evidence prompted us to optimize a preparative procedure for the same transformation and investigate AAR activity on all diketones **2** and **3**. Accordingly, *B. licheniformis* was grown on high sucrose concentration broth to induce a high expression level of AAR,[17] thus allowing the use of the cell free extract in its crude form. The enzymatic reduction of **2a** was carried out in phosphate buffer at pH 6.5 in the presence of sodium formate (5 equiv.) and formate dehydrogenase (FDH) for NADH recycling (Scheme 2).

After 3 h shaking, 3-methyl-3,4-dihydroxy-2-pentanone (acetylbutanediol) **4a** was recovered in 60% yield as a single diastereoisomer and high enantiomeric purity (ee >95%), as detected by $^1$H NMR and chiral GC analyses. It is worth noting that the use of excess sodium formate for reaction rate acceleration was possible due to the complete inactivity of AAR on **4a**. Enzyme selectivity was further confirmed by extending




**Scheme 2** Acetylacetoin reductase (AAR)-based reduction of acetylacetoin **2a**.

the reaction time and/or adding excess of enzyme. The relative stereochemistry of compound **4a** was next investigated by NOE experiments performed on the isopropylidene derivative **5a**, which was obtained by treating **4a** with dimethoxypropane (DMP) under acidic conditions (Scheme 3).

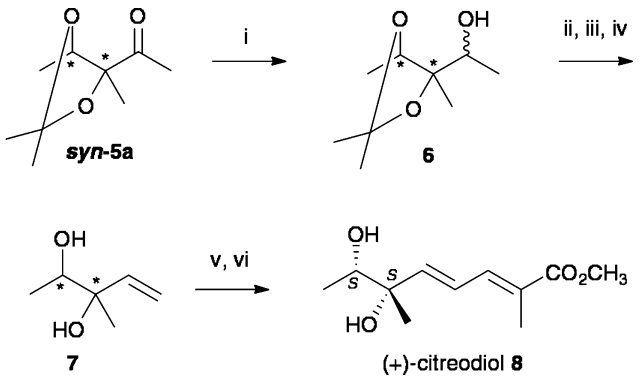



**Scheme 3** Synthesis of *syn*- and *anti*-**5a** and NOE signals.

These measurements showed the presence of a signal between the $C_4$- and $C_5$-methyl groups whereas no NOE was observed between the $C_5$-hydrogen and $C_4$-methyl group directly bound to the dioxolane ring. These results were in accordance with a *syn* stereochemistry that was unequivocally confirmed by performing the same NOE measurements on the diastereomeric *anti*-**5a**. The mixture of *syn*- and *anti*-**5a** was readily prepared by reduction of **2a** with $NaBH_4$ and treatment of the resulting diols with DMP (Scheme 3).

As expected, opposite results were detected in the NOE measurements of *anti*-**5a**, namely the presence of a signal between $C_5$-hydrogen and $C_4$-methyl group and lack of signal between $C_4$- and $C_5$-methyl groups. On the other hand, an optimized chiral GC analysis of the *syn*/*anti*-**5a** mixture displays all the four stereoisomers, one of which corresponds in retention time to the one obtained from the enzymatic reaction. Overall, these results confirmed the essentially complete regio-, diastereo-, and enantioselectivity of the AAR-catalyzed reduction of **2a** (de >95%, ee >95%).

In order to assign the absolute configuration of the two chiral centres of **4a**, the isopropylidene derivative *syn*-**5a** was transformed to (+)-citreodiol **8**, the antipode of a metabolite of *Penicillium citreoviride* B (IFO 6200), which is related to the inhibitor of ATP-synthesis and ATP-hydrolysis citreoviridine[11] (Scheme 4).




**Scheme 4** Synthesis of (+)-citreodiol **8**. *Reagents and conditions*: (i) $NaBH_4$, $Et_2O$, MeOH; (ii) $Tf_2O$, pyridine, $CH_2Cl_2$; (iii) DBU, $CH_2Cl_2$; (iv) *p*-TsOH, $CH_2Cl_2$, MeOH; (v) Hoveyda-Grubbs 2nd gen. catal., acrolein, $CH_2Cl_2$, THF, reflux; (vi) $H_3C(P(C_6H_5)_3)CCOOCH_3$, benzene, reflux.

The synthesis began with the reduction of *syn*-**5a** with sodium borohydride to afford the corresponding alcohol **6** (85% yield). This compound was next converted to the allyl diol **7** in 40% yield *via* a three-step one-pot procedure. This involved the initial activation of **6** with triflic anhydride ($Tf_2O$), followed by elimination to the corresponding terminal alkene and isopropylidene removal promoted by 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) and *p*-toluenesulfonic acid (*p*-TsOH), respectively. The next two steps were run in a single flask as well. Hence, cross-metathesis of pure **7** with acrolein promoted by Hoveyda-Grubbs 2nd generation catalyst was followed by Wittig olefination with the 2-(triphenylphosphoranylidene)-2-propanoic acid methyl ester[18] to give (+)-citreodiol **8** in 67% yield (two steps). The spectral data, and optical rotation of **8** ($[\alpha]_D$ = +6.8, *c* 0.7; lit.[19] $[\alpha]_D$ = +4.3, *c* 1.0) were consistent with those reported for (+)-citreodiol, thus allowing us to confirm the *syn* stereochemistry of **4a** and establish the 3(*R*) and 4(*S*) absolute configurations of its chiral centers. Importantly, this stereochemical assignment is in agreement with the (*S*)-stereospecificity previously observed for AAR-catalyzed reductions.[14]

Encouraged by the excellent stereochemical outcome detected in the reduction of **2a**, we next examined the substrate scope of the proposed enzymatic reduction by applying the optimized procedure to the previously synthesized α-hydroxy-β-diketones **2** and **3**. The results of this investigation are summarized in Table 1.

Due to their difficult purification, chiral diketones **2b–d** were subjected to the AAR-catalyzed reduction together with the corresponding regioisomers **3b–d** (entries 2–4). In spite of this, α-methyl-α,β-dihydroxy-ketones **4b–d** were obtained in satisfactory yields (61–70%) and excellent diastereo- and enantiomeric excesses (de >95%; ee >95%). It is worth noting that prochiral diketones **3b–d** were unreactive under these conditions. However, this outcome can be exploited as a method for the kinetic resolution of **2**/**3** regioisomeric mixtures, though the recovery by chromatography of symmetric diketones **3b–d** was complicated by their intrinsic instability on silica.[20] The enzymatic reduction of pure diketone **2e** proceeded smoothly in 5 h furnishing the diol **4e** in a 63% yield with high stereoselectivity (entry 5). By contrast, no reduction product was obtained with symmetric diketone **2f** (entry 6). The optically pure α,β-dihydroxy-ketone **4g** was finally obtained in



**Table 2** Absolute configuration and optical rotation of compounds **4**, **5** and **2**

| | Compound | ee (%) | $[a]_D$ |
|---|---|---|---|
| **4a** | 3*R*,4*S*-dihydroxy-3-methyl-2-pentanone | >95 | −10.7 |
| **5a** | 4*R*,5*S*-1-(2,2,4,5-tetramethyl-1,3-dioxolan-4-yl)-ethanone | >95 | +46.9 |
| **4b** | 4*R*,5*S*-dihydroxy-3-methyl-2-hexanone | >95 | −8.6 |
| **5b** | 4*R*,5*S*-1-(2,2,4,5-tetramethyl-1,3-dioxolan-4-yl)-propanone | >95 | +5.2 |
| **4c** | 2*S*,3*R*-dihydroxy-3-methyl-4-heptanone | >95 | −10.0 |
| **5c** | 4*R*,5*S*-1-(2,2,4,5-tetramethyl-1,3-dioxolan-4-yl)-butanone | >95 | +46.5 |
| **4d** | 2*S*,3*R*-dihydroxy-3-methyl-4-octanone | >95 | −8.2 |
| **5d** | 4*R*,5*S*-1-(2,2,4,5-tetramethyl-1,3-dioxolan-4-yl)-pentanone | >95 | +46.4 |
| **4e** | 2*R*,3*S*-dihydroxy-2-methyl-1-phenyl-1-butanone | >95 | +8 |
| **5e** | 4*R*,5*S*-1-(2,2,4,5-tetramethyl-1,3-dioxolan-4-yl)-benzophenone | >95 | +8.1 |
| **4g** | 4*R*,5*S*-dihydroxy-4-ethyl-3-hexanone | >95 | +6.6 |
| **5g** | 4*R*,5*S*-1-(4-ethyl-2,2,5-trimethyl-1,3-dioxolan-4-yl)-propanone | >95 | +51.9 |
| **2b** | 3*R*-hydroxy-3-methyl-2,4-hexandione | 70 | +15[a] |
| **2c** | 3*R*-hydroxy-3-methyl-2,4-heptanedione | 67 | +3[a] |
| **2d** | 3*R*-hydroxy-3-methyl-2,4-octandione | 72 | +7.3 |
| **2e** | 2*R*-hydroxy-2-methyl-1-phenyl-1,3-butandione | 76 | +13[a] |
| **2g** | 3*R*-hydroxy-3-ethyl-1,3-hexandione | 91 | +49.8[a] |

[a] The optical rotations are reported in ref. 15



good yield (72%) by the optimized procedure although with a longer reaction time (12 h; entry 7).

A common feature of the AAR-catalyzed reduction of chiral diketones **2b–e** and **2g** was the complete selectivity of the enzyme toward the corresponding major (*R*)-enantiomers. The minor (*S*)-enantiomers of **2b–e** and **2g** were, in fact, recovered unaltered in the crude mixtures after reduction as determined by chiral GC analysis. Moreover, on the basis of the above substrate study it can be pointed out that: (i) AAR exhibits complete specificity for the reduction of acetyl group in α-hydroxy-α-methyl-β-diketones **2**; (ii) the presence of substituents bulkier than a methyl group on the quaternary carbon of **2** inhibits the catalysis, as demonstrated by the unreactivity of **2f** and the lower reaction rate observed for **2g** reduction; (iii) as already mentioned, the enzyme exhibits a high selectivity with respect to the configuration of the α-quaternary carbon.

As previously described for **4a**, the relative *syn* stereochemistry of the reduction products **4b–e** and **4g** was confirmed by their conversion to the corresponding dioxolanes **5b–e** and **5g**. In all 1,3-dioxolanes, in fact, a NOE was observed between $C_5$-methyl group and $R^1$-hydrogens, while no NOE interaction was detected between $C_5$- and $R^1$-hydrogens. The absolute configuration of **4b–e** and **4g** was finally assigned by assuming that the AAR-mediated hydride attack on the acetyl group of **2b–e** and **2g** occurs at the *Re*-face furnishing a methylcarbinol moiety with (*S*)-configuration. As a consequence it is possible to deduce the (*R*) configuration of the α-quaternary chiral centre. The absolute configurations and the optical rotation of the newly synthesized chiral products are summarized in Table 2.

## Conclusions

In summary, we have shown that the combined use of the two enzymes AAS and AAR, readily available from the same bacterium *B. licheniformis*, allows the preparation of enantiopure α-alkyl-α,β-dihydroxyketones starting from inexpensive and commercially available α-diketones. The high enantiopurity of the products is mainly due to the high stereospecificity of AAR either for the conversion of acetyl groups to (*S*)-methyl carbinols or with respect to the (*R*)-configuration of the α-quaternary carbon. This last feature is particularly interesting as it is known that dehydrogenases' stereoselectivity for the α-stereocenter is often modest. Most of the enantiomerically pure ketodiols herein synthesized are new compounds and their complete characterization including their stereochemical assignment is duly reported. Finally, the present investigation has also allowed us to determine the absolute configuration of the chiral diketone precursor.

## Experimental

### General information

Diketones **1a–f**, formate dehydrogenase (FDH) from *Candida boidinii*, nicotinamide adenine dinucleotide (NADH), thiamine diphosphate (ThDP) and sodium formate were purchased from Sigma-Aldrich. Products **2a**, **2e**, **2f**, **2g** and the mixtures of regioisomers **2b–3b** (ratio 1.2 : 1) and **2c–3c** (ratio 2.2 : 1) were obtained as described.[15] TLC were run on precoated silica pates (thickness 0.25 mm, Merck), and silica gel (Fluka, Kieselgel 60, 70–230 mesh) was used for column chromatography. ESI mass spectra were obtained using a LCQ Duo (ThermoQuest, San Jose, CA, USA), in positive-ion mode by introducing the sample as $10^{-6}$ M solution in methanol. Instrumental parameter: capillary voltage, spray voltage 4.6 kV, capillary temperature 200 °C, mass scan range was from *m*/*z* 100 to 1000 amu for 30000 ms scan time; $N_2$ was used as sheath gas. NMR spectra were recorded on a Varian Gemini 300 spectrometer. Chemical shifts are given in parts per million from $Me_4Si$ as internal standard. Optical rotations were measured on a Perkin–Elmer Model 241 polarimeter.

### GC analyses

Gas chromatographic analyses were performed on a Carlo Erba 6000, equipped with a FID detector and a fused capillary column Megadex 5 (25 m X 0.25 mm) containing dimethyl-*n*-pentyl-β-cyclodextrin on OV 1701 (from Mega snc), helium as carrier gas (80 kPa).

For AAR reduction of **2a**: 90–200 °C (2 °C $min^{-1}$), retention time (min): **2a**, 7.1; **4a**, 15.5.

For $NaBH_4$ reduction of **2a**: 90–200 °C (2 °C $min^{-1}$), retention time (min): **2a**, 7.1; **4a**, 15.5 and 15.9.

For AAR reduction of **2b**: temp 80–100 °C (1.5 °C $min^{-1}$), 100–200 °C (10 °C $min^{-1}$) retention time (min): **3b**, 10.7; **2b**, 12.5 and 12.8; **4b**, 19.8.

For AAR reduction of **2c**: temp 80–100 °C (1.5 °C $min^{-1}$), 100–200 °C (10 °C $min^{-1}$) retention time (min): **3c**, 14.6; **2c**, 15.9 and 16.0; **4c**, 21.0.

For AAR reduction of **2d**: temp 80–100 °C (1.5 °C $min^{-1}$), 100–200 °C (10 °C $min^{-1}$) retention time (min): **3d**, 17.5; **2d**, 18.3 and 18.4; **4d**, 22.3.

For AAR reduction of **2e**: temp 100–200 °C (5 °C $min^{-1}$), retention time (min): **2e**, 17.1 and 17.2; **4e**, 16.5.

For AAR reduction of **2g**: temp 80–200 °C (1.5 °C $min^{-1}$), retention time (min): **2g**, 16.0 and 16.3; **4g**, 33.6.

For the synthesis of dioxolane **5a–e** and **5g**: temp 60–200 °C (2 °C $min^{-1}$) retention time (min): **5a** from enzymatic reduction, 16.1; ***syn*-5a** from $NaBH_4$ reduction, 15.5 and 16.1; ***anti*-5a** from $NaBH_4$ reduction, 17.1 and 17.4; **5b**, 21.1; **5c**, 26.3; **5d**, 30.9; **5e**, 52.8; **5g**, 29.6.

### Enzymes preparation

*B. licheniformis* was cultured in two different media in order to obtain crude AAS or AAR respectively. For the preparation of AAS, the medium was composed of meat extract (10 g $L^{-1}$), polypeptone (10 g $L^{-1}$), NaCl (5 g $L^{-1}$) and 3-hydroxy-2-butanone (5 g $L^{-1}$), while in order to obtain BSDR sucrose (40 g $L^{-1}$), peptone (20 g $L^{-1}$), yeast extract (10 g $L^{-1}$) $Na_2HPO_4{\cdot}6H_2O$ (6.8 g $L^{-1}$), $K_2SO_4$ (2.6 g $L^{-1}$) were present in the medium. The culture conditions and the cells' treatment were the same for both the enzymes: the bacteria was grown for 48 h at 38–40 °C and 110 rpm, then the cells were harvested by centrifugation (6000 rpm, 20 min) and washed with 150 mM NaCl solution (50 mL). Wet cells obtained from the two different cultures (2.0 g) were suspended in 50 mM phosphate buffer at pH 6.5 containing EDTA (0.1 mM) and β-mercaptoethanol (1 mM) (20 mL), the suspensions were treated at high pressure (1280 bar) with a French press and then centrifuged (9000 rpm, 20 min, 10 °C). The cell free extracts (16 mL) were used without further purification.

### 3-Hydroxy-3-methyl-2,4-heptanedione (2d) and 3-butyl-3-hydroxy-2,4-pentanedione (3d)

The AAS cell free extract (16 mL) was added to a solution of diketone **1d** (384 mg, 3 mmol), ThDP (17 mg, 39 μmol) and magnesium sulfate (15 mg, 125 μmol) in 50 mM phosphate buffer at pH 6.5 (50 mL). The reaction was gently shaken at 30 °C for 14 h and then heated (80 °C, 20 min). After removing the precipitate by centrifugation (9000 rpm, 20 min) the solution was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with saturated $NaHCO_3$ (40 mL) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated to afford 168 mg (0.98 mmol, 65%) of a mixture of compounds **2d** and **3d** in a 3 : 1 ratio. Pure samples of **2d** and **3d** were obtained by flash chromatography on silica gel (*n*-hexane–AcOEt 15 : 1 as eluent).

### 3-Hydroxy-3-methyl-2,4-octanedione (2d)

Colourless oil (48%, ee 72%). $[\alpha]_D^{20}$ +7.3 (*c* 0.2, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 0.93 (t, 3H, *J* = 7.5 Hz, $CH_3$) 1.22–1.36 (m, 2H, $CH_2$) 1.48–1.62 (m, 2H, $CH_2$) 1.56 (s, 3H, $CH_3$) 2.25 (s, 3H, $CH_3CO$) 2.51 (dt, 1H, J = 17.5 and 7.5 Hz, $CH_2CO$) 2.70 (dt, 1H, J = 17.5 and 7.5 Hz $CH_2CO$) 4.75 (br s, 1H, OH). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 13.8, 22.5, 22,7, 24.6, 25.5, 36.5, 87.6, 207.4, 209.6; ESI $[MNa]^+$ *m*/*z* 195.1. Anal calcd for $C_9H_{16}O_3$: C, 62.77%; H, 9.36%. Found: C, 62.69%; H, 9.29%.

### 3-Hydroxy-3-butyl-2,4-pentanedione (3d)

Colourless oil (15%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 0.91 (t, 3H, *J* = 7.5 Hz, $CH_3$) 1.10–1.40 (m, 4H, $2CH_2$) 1.95–2.05 (m, 2H, $CH_2$) 2.25 (s, 6H, $2CH_3CO$) 4.65 (br s, 1H, OH). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 13.8; 22.7, 25.2, 25.3, 29.4, 29.7, 36.1, 91.0, 207; ESI $[MNa]^+$ *m*/*z* 195.1. Anal calcd for $C_9H_{16}O_3$: C, 62.77%; H, 9.36%. Found: C, 62.71%; H, 9.32%.

### General procedure for the enzymatic reduction of α-alkyl-α-hydroxy-β-diketones 2a–g

To a solution of $NAD^+$ (10 mg, 15 μmol), sodium formate (335 mg, 5 mmol), formate dehydrogenase (1.0 mg, 10 U) and AAR cell free extract (15 mL) in phosphate buffer 50 mM at pH 6.5 (35 mL) containing EDTA (0.1 mM) and 1 mM 2-mercapto-ethanol, the selected diketones **2** (1.0 mmol) were added. For compounds **2b–d**, the mixtures **2b**/**3b**, **2c**/**3c** and **2d**/**3d** were used in the proper amount to have 1.0 mmol of the diketone **2**. The mixture was gently shaken overnight at 30 °C and than warmed up at 80 °C for 20 min. The proteins were removed by centrifugation (9000 rpm, 20 min) and the supernatant was extracted with ethyl acetate (3 × 30 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated to afford a pale yellow oil that was purified by column chromatography on silica gel using cyclohexane–ethyl acetate 2 : 1 as eluent.

### 3*R*,4*S*-Dihydroxy-3-methyl-2-pentanone (4a)

Colourless oil (79 mg, 60%, ee >95%). $[\alpha]_D^{20}$ −10.7 (*c* 1.4, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 1.25 (s, 3H, $CH_3$) 1.27 (d, 3H, *J* = 7.5 Hz, $CH_3$) 1.98 (d, 1H, *J* = 9.0 Hz, OH) 2.30 (s, 3H, $CH_3CO$) 4.02 (br s, 1H, OH) 4.05 (m, 1H, CHOH). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 13.8, 21.8, 24.0, 71.3; 81.6, 211.7, ESI $[MNa]^+$ *m*/*z* 155.2. Anal calcd for $C_6H_{12}O_3$: C, 54.53%; H, 9.15%. Found: C, 54.38%; H, 9.21%.

### 4*R*,5*S*-Dihydroxy-4-methyl-3-hexanone (4b)

Colourless oil (89 mg, 61%, ee >95%). $[\alpha]_D^{20}$ −8.6 (*c* 1.5, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 1.13 (t, 3H, *J* = 7.0 Hz, $CH_3$) 1.25 (s, 3H, $CH_3$) 1.27 (d, 3H, *J* = 7.0 Hz, $CH_3$) 2.05 (br s, 1H, OH) 2.60 (dq, 1H, J = 7.0 and 18.0 Hz, $CH_2CO$) 2.68 (dq, 1H, J = 7.0 and 18.0 Hz, $CH_2CO$) 4.05 (m, 1H, OH) 4.10 (br s, 1H, OH). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 7.6, 16.9, 21,7, 29.2, 71.1, 81.0, 214.3; ESI $[MNa]^+$ *m*/*z* 169.2. Anal calcd for $C_7H_{14}O_3$: C, 57.51%; H, 9.65%. Found: C, 57.45%; H, 9.51%.

**2*S*,3*R*-Dihydroxy-3-methyl-4-heptanone (4c)**

Colourless oil (104 mg, 65%, ee >95%). $[\alpha]_D^{20}$ −10.0 (*c* 0.8, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 0.94 (t, 3H, *J* = 7.0 Hz, $CH_3$) 1.23 (s, 3H, $CH_3$) 1.27 (d, 3H, *J* = 7.0 Hz, $CH_3$) 1.68 (sex, 2H, *J* = 7.0 Hz, $CH_2$) 2.10 (br s, 1H, OH) 2.53 (dt, 1H, J = 7.0 and 18.0 Hz, $CH_2CO$) 2.60 (dt, 1H, J = 7.0 and 18.0 Hz, $CH_2CO$) 4.05 (m, 1H, CHOH) 4.10 (br s, 1H, OH); $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 13.6, 16.8, 16.9, 21.6, 37.8, 71.0, 81.0, 213.6; ESI $[MNa]^+$ *m*/*z* 183.0. Anal calcd for $C_8H_{16}O_3$: C, 59.97%; H, 10.07%. Found: C, 60.01%; H, 10.11%.

**2*S*,3*R*-Dihydroxy-3-methyl-4-octanone (4d)**

Colourless oil (122 mg, 70%, ee >95%). $[\alpha]_D^{20}$ −8.2 (*c* 1.0, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 0.94 (t, 3H, *J* = 7.0 Hz, $CH_3$) 1.25 (s, 3H, $CH_3$) 1.28 (d, 3H, *J* = 7.0 Hz, $CH_3$) 1.28–1.42 (m, 2H, CH2) 1.58–1.70 (m, 2H, $CH_2$) 1.88 (br s, 1H, OH) 2.58 (dt, 1H, J = 7.0 and 18.0 Hz, $CH_2CO$) 2.65 (dt, 1H, J = 7.0 and 18.0 Hz, $CH_2CO$) 4.05 (m, 1H, CHOH), 4.08 (br s, 1H, OH). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 13.8, 17.0, 21.7, 22.3, 25.5, 35.6, 71.0, 81.0, 213,6; ESI $[MNa]^+$ *m*/*z* 198.1. Anal calcd for $C_9H_{18}O_3$: C, 62.04%; H, 10.41%. Found: C, 61.99%; H, 10.13%

**2*R*,3*S*-Dihydroxy-2-methyl-1-phenyl-1-butanone (4e)**

Yellow pale oil (122 mg, 63%, ee >95%). $[\alpha]_D^{20}$ +8.0 (*c* 0.4, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 1.30 (d, 1H, *J* = 7.0 Hz, $CH_3$) 1.54 (s, 1H, $CH_3$) 2.10 (br s, 1H, OH) 4.22 (br s, 1H, OH) 4.38 (m, 1H, CHOH) 7.43–8.05 (m, 5H, Ph). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 16.8, 23.1, 71.4, 81.4, 128.5, 129.3, 132.8, 204.8; ESI $[MNa]^+$ *m*/*z* 217.1. Anal calcd for $C_{11}H_{14}O_3$: C, 68.02%; H, 7.27%. Found: C, 68.15%; H, 7.23%.

**4*R*,5*S*-Dihydroxy-4-ethyl-3-hexanone (4g)**

Colourless oil (115 mg, 72%, ee >95%). $[\alpha]_D^{20}$ +6.6 (*c* 0.5, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 0.75 (t, 3H, *J* = 7.0 Hz, $CH_3$) 1.15 (t, 3H, *J* = 7.0 Hz, $CH_3$) 1.27 (d, 3H, *J* = 7.0 Hz, $CH_3$) 1.65–1.75 (m, 2H, $CH_2$) 2.54 (dq, 1H, J = 7.0 and 18.0 Hz, $CH_2CO$) 2.68 (dq, 1H, J = 7.0 and 18.0 Hz, $CH_2CO$) 4.05 (m, 1H, CHOH) 4.13 (br s, 1H, OH). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 7.4, 7.5, 17.5, 27.8, 29.5, 71,2, 84.5, 213.8; ESI $[MNa]^+$ *m*/*z* 183.1 Anal calcd for $C_8H_{16}O_3$: C, 59.97%; H, 10.07%. Found: C, 60.11%; H, 10.15%.

**Reduction of acetylacetoin (2a) with $NaBH_4$**

Acetylacetoin **2a** (130 mg, 1.0 mmol) was dissolved in diethyl ether–methanol 5 : 1 (8 mL), the solution was cooled (ice bath) and $NaBH_4$ (38 mg, 1.0 mmol) was added. The ice bath was removed and the mixture was maintained at r. t. The reaction was monitored by TLC (cyclohexane–ethyl acetate 1 : 1) and when the conversion was complete 1 M HCl (5 mL) was added. The organic layer was separated and the aqueous solution was extracted with diethyl ether (2 × 10 mL). The combined organic phases were dried ($Na_2SO_4$) and the solvent was removed under vacuum. The products were purified by short column chromatography (silica gel, cyclohexane–ethyl acetate 2 : 1 as eluent) to obtain the mixture of *syn*- and *anti*-**4a** (110 mg, 83%).

The $^1$H NMR spectrum of the mixture allows us to identify the signals of *anti*-2,3-dihydroxy-3-methyl-pentan-2-one (**4a**): $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 1.08 (d, 3H, *J* = 7.5 Hz, $CH_3$),1.45 (s, 3H, $CH_3$), 1.85 (d, 1H, *J* = 14 Hz, OH), 2.25 (s, 3H, $CH_3CO$), 3.80 (br s, 1H, OH), 3.90 (dq, 1H, J = 7.0 and 10 Hz, CHOH). The diastereomeric mixture of *syn* and *anti*-**4a** was dissolved in dichloromethane (5 mL) and treated successively as described in the general procedure for the synthesis of the corresponding dioxolane. Column chromatography (silica, cyclohexane–diethyl ether 10 : 1 as eluent) afforded the mixture of *syn* and *anti*-**5a** (130 mg, 92%). The signals of 1-(2,2,4,5-tetramethyl-1,3-dioxolan-4-yl)-ethanone (*anti*-**5a**) were identified from $^1$H NMR spectrum of the mixture: $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 1.16 (d, 3H, *J* = 7.0 Hz, $CH_3$), 1.38 (s, 3H, $CH_3$), 1.44 (s, 3H, $CH_3$), 1.58 (s, 3H, $CH_3$), 2.24 (s, 3H, $CH_3CO$), 4.14 (q, 1H, *J* = 7.0 Hz, CH-OR).

**General procedure for the synthesis of 1,3-dioxolane derivatives 5**

To a solution of the selected α-alkyl-α,β-dihydroxy-ketones **4** (0.5 mmol) in $CH_2Cl_2$ (2 mL), 2,2-dimethoxypropane (312 mg, 0.37 mL, 3 mmol) and *p*-toluenesulfonic acid (5 mg, 0.03 mmol) were added. After 2 h at room temperature the mixture was diluted with a saturated solution of $NaHCO_3$ (10 mL) and extracted with dichloromethane (3 × 10 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated. The residue was chromatographed (silica gel, cyclohexane–diethyl ether 10 : 1 as eluent).

**4*R*,5*S*-1-(2,2,4,5-Tetramethyl-1,3-dioxolan-4-yl)-ethanone (*syn*-5a)**

Colourless oil (69 mg, 80%, ee >95%). $[\alpha]_D^{20}$ +46.9 (*c* 1.3, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 1.20 (s, 3H, $CH_3$) 1.28 (d, 3H, *J* = 7.0 Hz, $CH_3$) 1.40 (s, 3H, $CH_3$) 1.50 (s, 3H, $CH_3$) 2.30 (s, 3H, $CH_3CO$) 4.14 (q, 1H, CHOC). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 14.5, 18.7, 25.5, 25.9, 28.4, 74.4, 87.5, 108.3, 211.7; ESI $[MNa]^+$ *m*/*z* 195.1. Anal calcd for $C_9H_{16}O_3$: C, 62.77%; H, 9,36%. Found: C, 62.41%; H, 9.53%.

**1-(2,2,4,5-Tetramethyl-1,3-dioxolan-4-yl)-propanone (5b)**

Colourless oil (79 mg, 85%, ee >95%). $[\alpha]_D^{20}$ +5.2 (*c* 1.0, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 1.03 (t, 3H, *J* = 7.0 Hz, $CH_3$) 1.2 (s, 3H, $CH_3$) 1.28 (d, 3H, *J* = 7.0 Hz, $CH_3$) 1.39 (s, 3H, $CH_3$) 1.48 (s, 3H, $CH_3$) 2.65–2.80 (m, 2H, $CH_2CO$), 4.08 (q, *J* = 7.0 Hz, CHOR). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 7.2, 14.5, 19.1, 26.0, 28.4, 30.6, 74.6, 87.6, 108.2, 214.0; ESI $[MNa]^+$ *m*/*z* 209.3 Anal calcd for $C_{10}H_{18}O_3$: C, 64,49%; H, 9,74%. Found: C, 64.14%; H, 9.66%.

**1-(2,2,4,5-Tetramethyl-1,3-dioxolan-4-yl)-buranone (5c)**

Colourless oil (87 mg, 87%, ee >95%). $[\alpha]_D^{20}$ +46.5 (*c* 1.5, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 0.93 (t, 3H, *J* = 7.0 Hz, $CH_3$) 1.21 (s, 3H, $CH_3$) 1.28 (d, 3H, *J* = 7.0 Hz, $CH_3$) 1.40 (s, 3H, $CH_3$) 1.50 (s. 3H, $CH_3$) 1.60 (sex, 2H, *J* = 7.0 Hz, $CH_2$) 2.68 (t, 2H, $CH_2CO$) 4.10 (q, 1H, *J* = 7.0 Hz, CHOR). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 13.7, 14.5, 16.4, 19.0, 25.9, 28.4, 39.2, 74.5, 87.5, 108.1, 213.4; ESI $[MNa]^+$ *m*/*z* 223.0. Anal calcd for $C_{11}H_{20}O_3$: C, 65,97%; H, 10,07%. Found: C, 65.62%; H, 9.96%.

**1-(2,2,4,5-Tetramethyl-1,3-dioxolan-4-yl)-pentanone (5d)**

Colourless oil (92 mg, 86%, ee >95%). $[\alpha]_D^{20}$ +46.4 (*c* 0.8, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 0.92 (t, 3H, *J* = 7.0 Hz, $CH_3$) 1.20 (s, 3H, $CH_3$) 1.29 (d, 3H, *J* = 7.0 Hz, $CH_3$), 1.30–1.36 (m, 2H, $CH_2$), 1.38 (s, 3H, $CH_3$) 1.47 (s, 3H, $CH_3$) 1.60–1.40 (m, 2H, $CH_2$) 2.70 (t, 2H, *J* = 7.0 Hz, $CH_2CO$) 4.10 (q, 1H, *J* = 7.0 Hz, CHOR). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 13.9, 14.5, 19.1, 22.3, 25.1, 25.9, 28.4, 37.0, 74.5, 87.5, 108.1, 213.5; ESI $[MNa]^+$ *m*/*z* 237.0. Anal calcd for $C_{12}H_{22}O_3$: C, 67,26%; H, 10,35%. Found: C, 67.17%; H, 10.51%.

**1-(2,2,4,5-Tetramethyl-1,3-dioxolan-4-yl)-benzophenone (5e)**

Colourless oil (106 mg, 91%, ee >95%). $[\alpha]_D^{20}$ +8.1 (*c* 0.8, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 1.20 (s, 3H, $CH_3$) 1.43 (d, 3H, *J* = 7.0 Hz, $CH_3$) 1.48 (s, 3H, $CH_3$) 1.55 (s, 3H, $CH_3$) 4.56 (q, 1H, *J* = 7.0 Hz, CHOR) 7.40–8.25 (m, 5H, Ph). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 15.5, 21.7, 25.8, 28.5, 75.7, 87.5, 108.0, 128.1, 130.4, 132.9, 134.7, 202.5; ESI $[MNa]^+$ *m*/*z* 257.0. Anal calcd for $C_{14}H_{18}O_3$: C, 71,77%; H, 7,74%. Found: C, 71.32%; H, 7.81%.

**1-(4-Ethyl-2,2,5-trimethyl-1,3-dioxolan-4-yl)-propanone (5g)**

Colourless oil (85 mg, 85%, ee >95%). $[\alpha]_D^{20}$ +51.9 (*c* 0.5, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$: 0.81 (t, 3H, *J* = 7.5 Hz, $CH_3$) 1.02 (t, 3H, *J* = 7.0 Hz, $CH_3$) 1.30 (d, 3H, *J* = 7.0 Hz, $CH_3$) 1.42 (s, 3H, $CH_3$), 1.40–1.55 (m, 1H, $CH_2$), 1.49 (s, 3H, $CH_3$), 1.82–1.95 (m, 1H, $CH_2$), 2.27 (dq, J = 7.0 and 19.0 Hz, $CH_2CO$) 2.64 (dq, J = 7.0 and 19.0 Hz, $CH_2CO$) 3.97 (q, 1H, *J* = 7.0 Hz, CHOC). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta_C$: 7.0, 7.7, 13.8, 25.0, 26.2, 28.4, 32.4, 75.0, 90.9, 108.4, 214.4; ESI $[MNa]^+$ *m*/*z* 223.1, Anal calcd for $C_{11}H_{20}O_3$: C, 65,97%; H, 10,07%. Found: C, 70.12%; H, 10.13%.

**Synthesis of 1-(2,2,4,5-tetramethyl-1,3-dioxolan-4-yl)-ethanol (6)**

Compound **5a** (150 mg, 0.87 mmol) was dissolved in diethyl ether–methanol 8 : 1 (5 mL), the solution was cooled (ice bath) and $NaBH_4$ (10 mg, 0.26 mmol) was added. After the addition the mixture was stirred at room temperature for 1 h and then diluted with 1 M HCl (5 mL). The organic phase was separated and the aqueous layer was extracted with diethyl ether (2 × 10 mL). The combined organic phases were dried (anhydrous $Na_2SO_4$), evaporated and the residue was purified by column chromatography (silica gel, cyclohexane–ethyl acetate 2 : 1 as eluent) affording an equimolecular mixture of (4*R*,5*S*,6*S*)- and (4*R*,5*S*,6*R*)-**6** diastereomers (136 mg, 90%): $^1$H NMR (300 MHz, $CDCl_3$) selected data: $\delta$ 1.04, 1.08, 1.35, 1.36, 1.43 and 1.44 (6 s, 6 × 3H, 6$CH_3$), 3.58 and 3.75 (2q, 2 × 1H, *J* = 6.5 Hz, CHOH), 4.14 and 4.24 (2q, 2 × 1H, *J* = 6.5 Hz, CHOR). ESI $[MNa]^+$ *m*/*z* 197.1.

**Synthesis of 3-methyl-1-penten-3,4-diol (7)**

Compound **6** (150 mg, 0.86 mmol) and pyridine (153 μL, 1.9 mmol) were dissolved in dichloromethane (4 mL). To the stirred solution trifluoromethanesulfonic anhydride (173 μL, 1.03 mmol) was added at room temperature. After 30 min DBU (0.64 mL, 4.3 mmol) was added and after a further 30 min the mixture was diluted with 1 M HCl (5 mL). The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 5 mL). The combined organic phases were dried (anhydrous $Na_2SO_4$) and evaporated under nitrogen. The crude 2,2,4,5-tetramethyl-4-vinyl-1,3-dioxolane [selected data $^1$H-NMR ($CDCl_3$) $\delta_H$: 3.96 (dd, 1H, *J* = 6.5 Hz, CHOR), 5.15 (dd, 1H, J = 1.5 and 11.0 Hz, $CH_2$), 5.33 (dd, 1H, J = 1.5 and 17.5 Hz, $CH_2$), 5.88 (dd, 1H, J = 11.0 and 17.5 Hz, CH)] was dissolved in anhydrous methanol (7 mL) and acidified with *p*-toluenesulfonic acid. The reaction was stirred at room temperature and checked by TLC (eluent: cyclohexane–ethyl acetate 1.5 : 1). When the conversion was complete silica gel was added to the mixture in order to absorb the product and the solvent was removed under reduced pressure. Short column chromatography of the residue using cyclohexane–ethyl acetate 2 : 1 as eluent afforded product **7** slightly contaminated (45 mg, 45%): $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.18 (d, 3H, *J* = 6.5 Hz, $CH_3$), 1.26 (s, 3H, $CH_3$), 3,68 (q, 1H, *J* = 6.5 Hz, CHOH), 5.22 (dd, 1H, J = 1.3 and 11.0 Hz, $CH_2$), 5.38 (dd, 1H, J = 1.3 and 17.0 Hz, $CH_2$), 5.92 (dd, 1H, J = 11.0 and 17.0 Hz, CH), $^{13}$C NMR (300 MHz, $CDCl_3$): $\delta$ 16.5, 21.5, 72.8, 75.6, 114.3, 142.73; ESI $[MNa]^+$ *m*/*z* 139.2.

**Synthesis of (+)-citreodiol (8)**

The diol **7** (45 mg, 0.39 mmol) was dissolved in anhydrous dichloromethane–tetrahydrofuran 4 : 1 (10 mL) with acrolein (0.22 mL, 3.1 mmol) and Hoveyda-Grubbs 2nd generation catalyst (23 mg, 7 mol%). The solution was heated to reflux for 6 h and then the solvent was removed under reduced pressure. The residue was dissolved in benzene (8 mL) together with methyl-2-(triphenylphosphoranylidene)-propanoate (0.3 g, 0.86 mmol) and the mixture was refluxed for 6 h. After evaporation, the residue was purified by column chromatography (silica gel, cyclohexane–ethyl acetate 2 : 1 as eluent) affording citreodiol **8** as a colourless oil: $[\alpha]_D^{20}$ +6.8 (*c* 0.7, $CHCl_3$), lit[19] +4.3 (*c* 1.0; $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.20 (d, 3H, *J* = 6.5 Hz, $CH_3$), 1.30 (s, 3H, $CH_3$), 2.0 (s, 1H, $CH_3$), 3.75 (m, 1H, CHOH), 3.77 (s, 3H, $CH_3$), 6.12 (d, 1H, *J* = 15 Hz, CH), 6.70 (dd, 1H, J = 11.0 and 15.0 Hz, CH), 7.20 (1H, *J* = 11.0 Hz, CH), $^{13}$C NMR (300 MHz, $CDCl_3$): $\delta$ 12.9, 17.0, 22.0, 52.0, 73.1, 75.7, 76.6, 124.8, 127.6, 137.5, 145.2, 168.9. 13.8, 17.0, 21.7, 22.3, 25.5, 35.6, 71.0, 81.0, 213.6. ESI $[MNa]^+$ *m*/*z* 237.3.

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