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A One-Pot Two-Step Enzymatic Pathway for the Synthesis of Enantiomerically Enriched Vicinal Diols

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Dedicated to Professor Franco Cozzi on the occasion of his 70th birthday.

Enantiomerically enriched 1,2-diols are prominent compounds that find numerous applications in organic chemistry. They are privileged building blocks for the synthesis of APIs (Active Pharmaceutical Ingredients), broadly used as chiral ligands in asymmetric catalysis, and efficient auxiliaries employed to control the stereochemical outcome of total synthesis. Among the number of strategies developed for the preparation of these molecules, enzyme mediated reactions have gained a crucial role in the toolbox of organic chemists for their high efficiency and sustainability. Herein we describe a one-pot two-step protocol designed by combining a thiamine diphosphate (ThDP)-dependent lyase and a NADH-dependent reductase. The ThDP-dependent acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR) is exploited to produce enantioenriched α-hydroxyketones through the benzoin-type condensation of methylacetoin with either aldehydes or activated ketones. The enantioenriched α-hydroxyketones undergo the selective reduction into the corresponding 1,2-diols in the same reaction mixture due to the addition of NAD^+ and of the NADH-dependent acetylacetoin reductase (AAR). Sodium formate was selected as the sacrificial reductive reactant to generate and recycle *in situ* the precious NADH by formate-dehydrogenase. Unprecedented reported details on the cloning and expression of the AAR are reported as well.

Introduction

A plethora of protocols have been reported to synthetize enantiomerically enriched 1,2-diols due to their applications in production of active pharmaceutical ingredients (APIs), chiral catalysis, and total syntheses of natural products.[1] Chemical transformation of optically pure intermediates is one of the strategies that includes the reductions of acyloins or diketones,[2] and the hydrolysis of epoxides.[3] Besides, the osmium tetroxide catalyzed synthesis of cis vicinal diols disclosed by Sharpless in the late 80s paved the way to the catalytic asymmetric synthesis in the production of enantiorich 1,2-diols.[4] Even though excellent results were achieved so far, some issues had to be addressed. The main methods employed do not often reach the high level of enantioselectivity required by the pharmaceutical industry. Furthermore, the use of toxic metals is discouraged nowadays for the more pressing challenge of sustainability. Enzymatic reactions have received an increasing attention in sustainability.[5] Many enantiomerically enriched 1,2-diols have been produced by kinetic resolution of their racemates by lipases[6] or by stereoselective hydrogenation of the corresponding diketones with NAD(P)H-dependent reductase.[7] An emerging enzymatic strategy combines the building of the carbonious skeleton through benzoin-type reactions promoted by Thiamine diphosphate (ThDP)-dependent carboligases with the reduction of the resulting acyloins conduced with NAD(P)H-dependent reductases.[8] The amplification of enantioselectivity due to consecutive transformations mediated by both enzymes raises the optical purity of desired 1,2-diols. Furthermore, the benzoin reaction allows the preparation of substrates that are not often commercially available.

Combining two consecutives reactions promoted by two crude enzymes, a thiamine diphosphate (ThDP)-dependent carboligase and a NADH-dependent reductase, both extracted from *Bacillus licheniformis*, we recently proposed a methodology for the preparation of enantioenriched tertiary α-β-dihydroxyketones.[8e] The ThDP-dependent carboligase was then identified as the acetoin dichlorophenolindophenol oxidoreductase (Ao:DCPIO OR) and, after cloning and over-

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expression, has been successfully employed to create a broad library of aromatic and aliphatic acyloins either with secondary and tertiary carbinol stereogenic centers (compounds **3**, Scheme 1).[9]

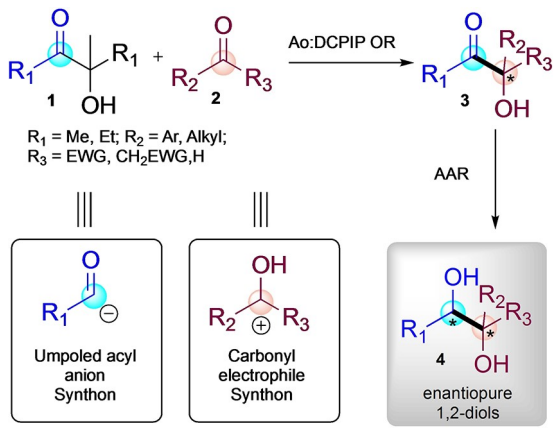
Herein we report the unprecedented cloning and over-expression of the NADH-dependent reductase previously named acetylacetoin reductase (AAR)[8e] as well as its combined use with the Ao:DCPIP OR into a one-pot two-step procedure for the synthesis of enantiomerically enriched *vic*-diols (Scheme 1).

Results and Discussion

In modern biocatalysis, the availability of pure recombinant enzymes is a prerequisite for the development of reproducible and scalable enzymatic synthetic procedures. For this reason, our starting efforts have been devoted to the identification of the gene encoding for the NADH-dependent reductase that, in virtue of its substrate specificity, we previously named acetylacetoin reductase (AAR).[8e]

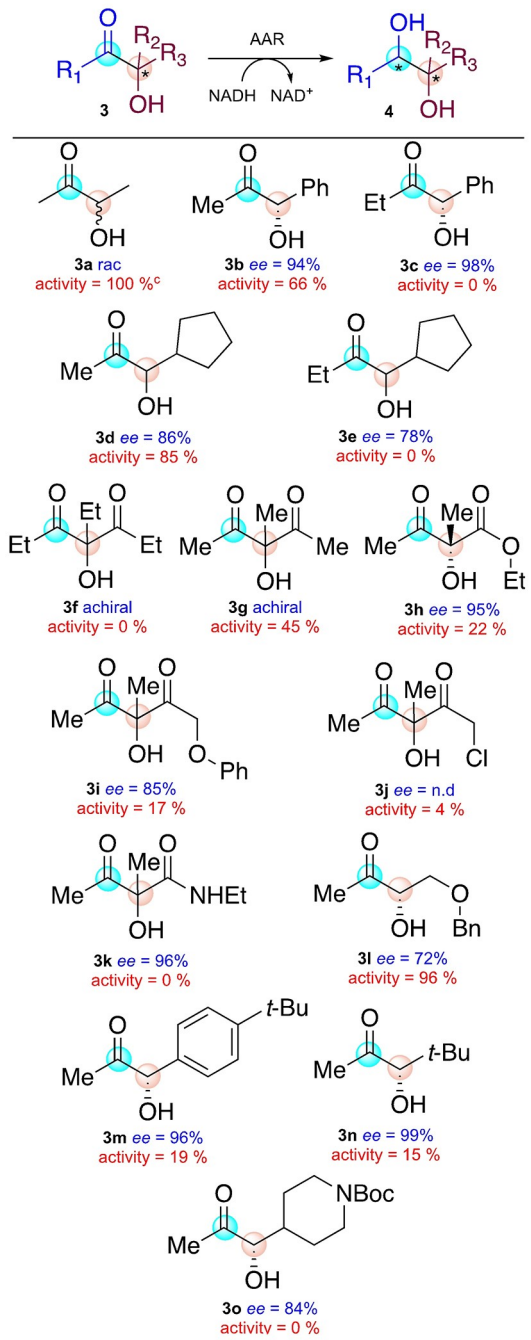
The enzyme has been purified to homogeneity through two chromatographic steps (see supporting information S3). The aminoacid sequences of the purified wild-type (*wt*) AAR was deduced from the HRMS (ESI-Q-TOF) analysis of the peptides obtained from the trypsinolysis of the enzyme separated by UPLC. The sequences were used to identify and clone the corresponding coding sequence from the *B. licheniformis* genome. The predicted polypeptide is 257 aa long with a putative molecular weight of 28 kDa. The cloned sequences (Accession number MW265947) appeared identical to other putative diacetyl reductases (i.e. ARC67779) identified in the genomes of other *B. licheniformis* strains but never characterized in biochemical terms. Cloning, screening, expression and His-tag purification were performed as described for the Ao:DCPIP OR.[9c]

The activity of the recombinant AAR was tested on a range of secondary and tertiary α-hydroxyketones (**3b–o**, Table 1) obtained through the benzoin-type condensation of the methylacetoin or of its upper homologue (Scheme 1, **1** with R^1 = Me or Et, respectively) with aldehydes or activated ketones **2** (Scheme 1). The reaction rates were measured following the disappearance of NADH (absorbance at 340 nm) from a solution of the cofactor and the substrate, after the addition of the enzyme. Table 1 shows the results expressed as a percentage



Scheme 1. Combined use of Ao:DCPIP OR and AAR in the synthesis of *vic*-diols.

Table 1. Reductive activity of the AAR[a] on representative acyloins obtainable by Ao:DCPIP OR catalyzed cross-coupling.[b]



[a] experimental conditions: (see experimental section: Measure of the AAR activity) substrate **3** (5 mM), NADH (0.2 mM), Na phosphate buffer pH 6.5 (50 mM), purified AAR solution (0.5 μL). [b] see reference.[9] [c] Activity of freshly prepared AAR measured on substrate **3a** was 24 U/mg.

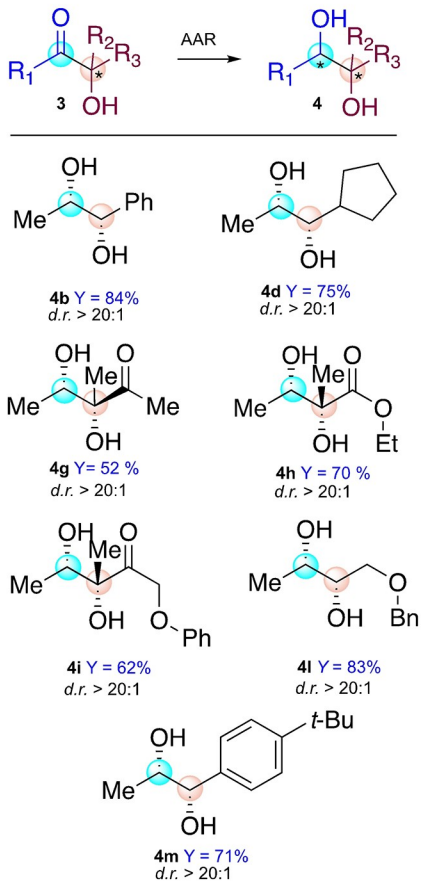
with respect to the reaction rate measured with acetoin (**3a**) as the substrate. The results obtained with the couples of homologue compounds **3b–c** and **3d–e** suggested that AAR does not accept as substrates acyloins with ethyl ketone groups. The inactivity on the substrate **3f** supported this hypothesis as well. On the contrary, AAR displayed an appreciable activity on most of the substrates bearing a methyl ketone moiety, although the structure of the substituents of the carbinol group strongly affected the reaction rate. In general, the substrates with a tertiary carbinol center (**3f–k**) were reduced slowly with respect to those with a secondary carbinol group (**3b,d,l–n**). Furthermore, if compared with the good activity exhibited with the ester **3h**, the complete inactivity on the amide **3k**, which is an isostere of **3h**, suggested that the presence of a nitrogen atom could be detrimental for the catalysis. The nitrogen-containing **3o** was not reduced as well. Although, the hindrance of the *N*-Boc-4-piperidyl group has to be considered.

The best substrates (activity > 15% of that measured with acetoin) were reacted on a preparative scale (20 mg) adding to the reaction mixture sodium formate (5 equiv.) and formate dehydrogenase (FDH) for the recycling of NADH. Table 2 reports the results of the preparative reactions. The first substrate taken into account was the acetylacetoin **3g** since the expected product **4g** was already obtained by using the *wt* AAR,[8e] hence, the formation of the same product, would give further evidence about the coincidence of the recombinant with the *wt* enzyme. As expected, the product isolated after column chromatography (52% yield, Table 2) was the only *syn*-(3*R*,4*S*)-**4g** as demonstrated by the ^{1}H NMR and chiral phase GC analyses.[8e] Worthy to note, despite the excess of sodium formate, only one of the two methyl ketone groups of **3g** was reduced, confirming the same behavior of the *wt* AAR.[8e]

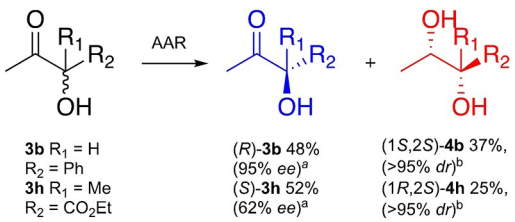
The next substrate examined was the (*S*)-phenylacetil carbinol (PAC) **3b**, which reduction afforded the *syn*-(1*S*,2*S*)-1-phenylpropane-1,2-diol (PPD) **4b** with an 84% yield (Table 2). The high *ee* of the substrate **3b** (94%, Table 1), together with the narrow (*S*)-stereospecificity of the AAR, oriented the formation of a highly enantiomerically enriched product, as demonstrated by the complete absence of signals attributed to the *anti*-diasteroisomer, in the ^{1}H NMR spectrum of the crude reaction mixture.[10] The relevance of the different stereoisomers of PPD, either as building blocks or therapeutic agents,[1] prompted us to attempt the concurrent synthesis of the *syn*-(1*S*,2*S*) and the *anti*-(1*S*,2*R*) diastereoisomers of **4b** through the enzymatic reduction of the *rac*-**3b**, in turn obtained by cross-benzoin coupling of benzaldehyde and acetaldehyde promoted by the Rovis catalyst.[11] Interestingly, the reduction of the (*R*)-**3b** did not take place (see supporting information S8), making the reaction a further enzymatic approach for the kinetic resolution of *rac*-PAC[12] (Scheme 2).

The substrate **3h** was subsequently reduced affording the *syn*-diastereoisomer **4h** (relative configuration determined by comparing ^{1}H NMR spectrum of isolated product with the spectrum reported in literature).[13] Since the (*R*)-**3h** employed as substrate was enantiomerically enriched (95% ee), the (2*R*,3*S*) absolute configuration could been reasonably assigned

Table 2. Results of the preparative reductions of representative secondary and tertiary acyloins with AAR.[a]



[a] experimental conditions: substrate **3** (14 mM), Na phosphate buffer pH 6.5 (50 mM)(10 mL), NAD^+ (0.9 mM), sodium formate (70 mM), FDH (4 mg) and AAR (1 U, 16 μL). The yields refer to products isolated by column chromatography.



Scheme 2. Kinetic resolution of racemic substrates **3b** and **3h**.

to the *syn*-**4h**. Having available from previous work[14] a sample of *rac*-**3h**, we verified the eventually specificity of AAR, with respect to the configuration of the carbinol. As matter of fact, the enantiomer (*R*)-**3h** has been consumed, giving the resulting *vic*-diols (2*S*,3*R*)-**4h** in 25% of yield and 95% of ee and resolving partially the starting carbinol **3h** (Yield = 52%, ee = 62%) (Scheme 2).

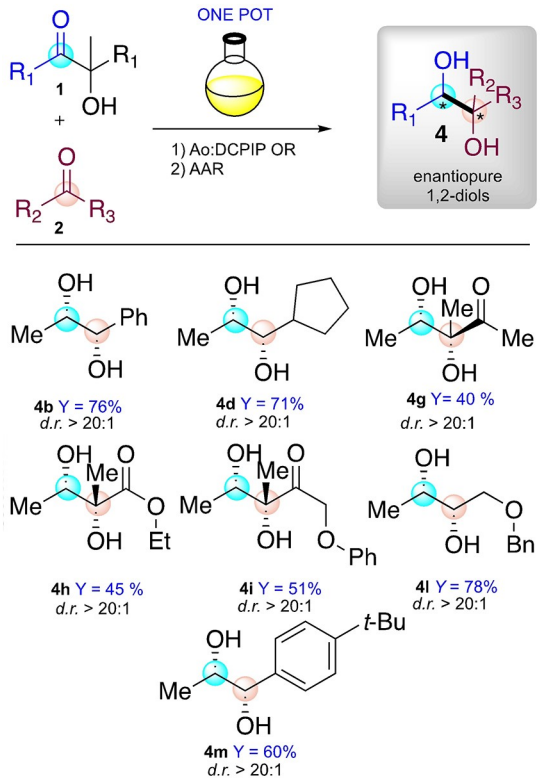
For what concern the other substrates, compound **3l**, is reduced with a very good rate by AAR (96% with respect to reduction rate of acetoin, Table 1), and converted to the

corresponding *syn*-1-*O*-benzyl-(2*R*,3*S*)-butane-1,2,3-triol **4l** (83% yield, Table 2) as deduced by comparing its ^{1}H NMR spectrum with those reported in literature for the *syn*- and *anti*-stereoisomer.[15] The reduction of substrates **3m**, afforded a single diastereoisomer (71% yield, Table 2) as well. By observing the ^{1}H NMR spectrum of the *syn*- and *anti*-**4m** mixture, obtained by reduction of (*S*)-**3m** with $NaBH_4$ (see supporting information S16), it was possible to note that the diagnostic signals of the enzymatically produced **4m** (δ 4.34 and 3.88 ppm), were very similar to those of the *syn*-**4b** (d 4.38 and 3.87 ppm) while, the additional set of signals of the diastereomeric mixture, due to the *anti*-**4m** (δ 4.63 and 4.13 ppm), resembled that of the *anti*-**4b** (δ 4.6 and 3.9 ppm).[8d] This suggested that also in this case the enzymatic reduction afforded the *syn*-diastereoisomer which, in virtue of the optical purity of the substrate **3m** [96% ee (*S*)] as well as of the (*S*)-specificity of the AAR towards secondary carbinols, could be reasonably identified as the *syn*-(1*S*,2*S*)-**4m**. Finally, the reduction of the substrates **3d** and **3i**, afforded single diasteroisomers as well. On the basis of the above discussion were identified as the *syn*-(1*S*,2*S*)-**4d** (75% yield) and *syn*-(2*R*,3*S*)-**4i** (62% yield), respectively.

In developing a one-pot procedure, it was not possible to simultaneously apply the Ao:DCPIP OR and AAR into a real cascade process because of the reductive activity of the AAR on some substrates of the benzoin reaction [the donor **1** and acceptors **2g–i**, (Table 3)]. For this reason, a one-pot two-step process was implemented where the AAR, FDH, sodium formate and NAD^+ were added after the formation of the hydroxyketones **3**. In order to simplify the multi-step processes, the use of immobilized enzymes was considered as well. The Ao:DCPIP OR was been already successfully immobilized onto aminopropyl-activated mesoporous silica by means of glutaraldehyde as the bifunctional linker.[16] By following the same strategy, a silica-bound AAR was obtained as well. Unfortunately, the heterogeneous AAR did not show a long-term stability comparable with that of the immobilized Ao:DCPIPOR (see supporting information S6). For this reason, we preferred to employ the immobilized Ao:DCPIP OR in the first step and the soluble AAR in the second one of the process, respectively.

In order to explore the designed procedure, cyclopentane carboxaldehyde **2d** was chosen as the model substrate, in virtue of the easy detection of the corresponding derivatives **3d** and **4d** by GC analysis. The first enzymatic step was performed adding the immobilized Ao:DCPIP OR (70 mg) to a solution of **2d** (20 Mm) and **1a** (1.3 equiv.) in phosphate buffer at pH 6.5 (12 mL) containing DMSO (8% v/v) and catalytic amount ThDP and $MgSO_4$ (0.4 and 0.9 Mm, respectively) to prevent the cofactor leaching. The mixture was gently shaken at 30 °C and the conversion was monitored by GC analysis. When the coupling reaction was complete (**2d** conversion to **3d** >90%), the silica supported Ao:DCPIP OR was removed by centrifugation and a solution containing AAR (1 U, 16 μL), FDH (5 U, 3 mg) and NAD^+ (6 mg) in 50 mM Na phosphate buffer at pH 6.5 (1 mL) was added to the mixture. The reaction was gently shaken at 30 °C following the conversion of **3d** to **4d** by GC analysis. After extraction with ethyl acetate, the residue was chromatographed on silica gel, resulting in the pure diol **4d** with a 71% yield. This was considered an appreciable result by taking into account the yields reported for the two separated steps (82% for the preparation of **3d**[9a] and 75% for **4d** (Table 2)].

Table 3. results of the one-pot two-step enzymatic synthesis of *vic*-diols.[a]



[a] experimental conditions: *step 1* - donor **1** (30 mM), acceptor **2** (20 mM), $MgSO_4$ (0.9 mM), ThDP (0.4 mM), Na phosphate buffer pH 6.5 (50 mM), DMSO (8% v/v), immobilized Ao:DCPIP OR (5.4 mg·mL^{-1}). *step 2* - AAR (U, mL), FDH (5 U, 3 mg), NAD^+ (6 mg). The yields refer to products isolated by column chromatography.

After these encouraging results, the procedure was extended to the preparation of all of the products previously obtained starting from the isolated hydroxyketones **3**. Table 3 resumes the results of this study. The yields, which range from 40 to 80%, can be positively evaluated by considering those reported for the preparation of the substrates **3**,[9] and for their reduction to the diols **4** (Table 2). This is particularly true for the products **4f** and **4g** for which, a significant loss of product during the purification of the intermediates **3f**[17] and **3g**[9c] has been reported.

Conclusion

The unprecedented cloning and overexpression of the AAR has made available amounts of enzyme suitable for the developing of a new synthetic methodology. A set of α-hydroxyketones arising from the Ao:DCPIP OR catalyzed cross-benzoin coupling of methylacetoin with various aldehydes or activated ketones,

has been accepted as substrates by the recombinant AAR and reduced to the corresponding enantiomerically enriched *syn*-1,2-diols. The sequential performance of the two enzymatic reactions into a one-pot two-step procedure, furnished improved yields and thanks to the by-passing of the intermediates isolation resulted into a more environmental benign, time saving, and less energy-consuming process with respect to the two separated steps. Furthermore, the possibility to employ the AAR to promote the kinetic resolution of significant racemic α-hydroxyketones, has been highlighted as well. The new recombinant enzyme (AAR) and the multi-enzyme synthetic procedure herein described contribute to expand the biocatalytic toolbox for the preparation of optically active *vic*-diols.

Experimental Section

General information – All commercially available reagents were used as received without further purification, unless otherwise stated. Liquid aldehydes were freshly distilled before use. The hydroxyketones **3** were obtained as previously reported.[9] Formate dehydrogenase form *Candida boidinii* (0.45 U/mg) was from Fluka. Reactions were monitored by TLC on silica gel 60 F254 with detection by charring with phosphomolybdic acid. Flash column chromatography was performed on silica gel 60 (230–400 mesh) or on Florisil (60–100 mesh). ^{1}H and ^{13}C NMR spectra were recorded on 300 and 400 MHz spectrometers at room temperature using $CDCl_3$ as solvent. Chemical shifts (δ) are reported in ppm relative to residual solvent signals. High-resolution mass spectra (HRMS) were recorded in positive ion mode with an Agilent 6520 HPLC-Chip Q/TOF-MS nanospray system using a time-of-flight, quadrupole or hexapole unit to produce spectra. Optical rotations were measured at 20±2 °C in the stated solvent; $[\alpha]^{20}_D$ values are given in 10^{-1} deg cm^2 g^{-1}. GC analyses were performed using a Thermo Focus gas chromatograph equipped with a flame ionization detector and a Megadex 5 column (25 m×0.25 mm), with the temperature programs as specified. Purified Ao:DCPIP OR was obtained as previously described.[9c] The *wt* AAR was purified as described in the supporting information (S3). The pure enzyme was sequenced, cloned and expressed in *E. coli* as reported in the supporting Information (S4–S5).

Measure of the AAR activity (Table 1) - The enzyme assay was carried out at 20 °C by following the conversion of NADH to NAD$^+$ (decrease of absorbance at 340 nm) into 1 mL solution of each of the substrates **3** (5 mM) and NADH (0.2 mM) in 50 mM Na phosphate buffer at pH 6.5 after the addition of the AAR (0.5 μL). One unit (U) of enzyme activity is defined as the amount of enzyme able to reduce 1 μmol of acetoin **3a** in one minute, under the above assay conditions.

General procedure A: reduction of substrates 3 on preparative scale (Table 2) – The substrate **3** (0.15 mmol) was dissolved in DMSO (1 mL) and the solution was diluted with 50 mM Na phosphate buffer pH 6.5 (10 mL) containing NAD$^+$ (6 mg, 0.01 mmol) and sodium formate (51 mg, 0.75 mmol). Formate dehydrogenase (1.8 U, 4 mg) and AAR (1 U) were added to the solution and the resulting mixture was kept at 30 °C, under gently shaking, for ten hours. After this time, the reaction mixture was saturated with NaCl (about 1.0 g) and extracted with ethyl acetate (3×4 mL). The combined organic layers were dried (Na_2SO_4), evaporated under reduced pressure and chromatographed on silica gel with cyclohexane – ethyl acetate 2:1 as eluent.

General procedure B: one-pot synthesis of the *vic*-diols 4 (Table 3) - a mixture of methylacetoin **1** (31 mg, 0.3 mmol) and one of the substrates **2** (0.2 mmol) dissolved in DMSO (1 mL), was diluted with Na phosphate buffer 50 mM at pH 6.5 (12 mL) containing $MgSO_4$ (0.9 mM) and ThDP (0.4 mM). The immobilized Ao:DCPIP OR (70 mg) was added and the reaction mixture was gently shaken at 30 °C for 48 h. After that, the immobilized enzyme was removed by centrifugation and a solution of AAR (1 U), FDH (1.8 U, 4 mg), sodium formate (51 mg, 0.75 mmol) and NADH (6 mg, 0.01 mmol) in 50 mM Na phosphate buffer pH 6.5 (1.5 mL), was added to the reaction mixture. The reaction was gently shaken at 30 °C for ten hours and after that NaCl (1.0 g) was added and the solution was extracted with ethyl acetate (3×5 mL). The combined organic layers were dried (Na_2SO_4), evaporated under reduced pressure and chromatographed on silica gel with cyclohexane – ethyl acetate 2:1 as eluent.

4b Colorless oil, 84% yield (from general procedure A), 76% yield (from general procedure B), dr >20:1, $[\alpha]^{20}_D$ = +42.0 (c 0.3, $CHCl_3$), lit.: +54.3 (*c* 1.9, $CHCl_3$).[8d] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.28 (m, 5H, Ar), 4.38 (d, J=7.3 Hz, 1H, C*H*OH), 3.97–3.75 (m, 1H, C*H*OH), 2.63 (m, 2H, O*H*), 1.07 (d, J=6.3 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.05, 128.59, 128.23, 126.88, 79.57, 72.30, 18.81. HRMS (ESI) m/z calcd for $C_9H_{13}O_2^+$: 153.0910 [M+H]$^+$; found: 153.0921.

4d Colorless oil, 75% yield (from general procedure A), 71% yield (from general procedure B), dr>20:1, $[\alpha]^{20}_D$ = +32.3 (c 0.9, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85–3.51 (m, 1H, C*H*OH), 3.23 (m, 1H, C*H*OH), 2.23-1.89 (m, 3H, C*H*, O*H*), 1.84–1.27 (m, 8H, CH_2), 1.22 (d, J=6.4 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, CD_3OD) δ 75.10, 65.51, 38.39, 25.33, 23.48, 21.62, 21.52, 16.11. HRMS (ESI) m/z calcd for $C_8H_{17}O_2^+$: 145.1223 [M+H]$^+$; found: 145.1231.

4g Colorless oil, 52% yield (from general procedure A), 40% yield (from general procedure B), dr>20:1, $[\alpha]^{20}_D$ = −11.3 (c 0.9, $CHCl_3$), lit.: −10.7 (c 1.4, $CHCl_3$).[8e] ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (m, 1H, C*H*OH), 4.02 (s, 1H, O*H*), 2.30 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 1.27 (d, 3H, J=7.5 Hz), 1.25 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 211.7, 81.6, 71.3, 24.0, 21.8, 13.8. HRMS (ESI) m/z calcd for $C_6H_{13}O_3^+$: 133.0859 [M+H]$^+$; found: 133.0851.

4h Colorless oil, 70% yield (from general procedure A), 45% yield (from general procedure B), dr > 20:1, $[\alpha]^{20}_D$ = +23.8 (c 1.0, $CHCl_3$).^{1}H NMR (300 MHz, $CDCl_3$) δ 4.28 (q, J=7.1 Hz, 2H, CH_2), 3.95 (dq, J=8.9, 6.4 Hz, 1H, C*H*OH), 3.38 (s, 1H, O*H*), 1.98 (d, J=9.1 Hz, 1H, O*H*), 1.32 (s, 3H, CH_3), 1.32 (t, J=7.1 Hz, 3H, CH_3), 1.23 (d, J=6.4 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.26, 71.58, 62.20, 21.68, 16.71, 14.12. HRMS (ESI) m/z calcd for $C_7H_{15}O_4^+$: 163.0965 [M+H]$^+$; found: 163.0957.

4i Colorless oil, 62% yield (from general procedure A), 51% yield (from general procedure B), dr>20:1, $[\alpha]^{20}_D$ = +25.0 (c 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48–7.14 (m, 2H, Ar), 7.09-6.80 (m, 3H, Ar), 3.98 (dq, J=6.4, 4.0 Hz, C*H*OH), 3.92 (s, 2H, CH_2), 2.66 (s, 1H, O*H*), 2.49 (d, J=4.0 Hz, 1H, O*H*), 1.24 (s, 3H, CH_3) 1.21 (d, J=6.4 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.50, 129.62, 121.35, 114.61, 73.99, 73.58, 70.59, 19.33, 17.10. HRMS (ESI) m/z calcd for $C_{11}H_{17}O_3^+$: 197.1172 [M+H]$^+$; found: 197.1167.

4l Colorless oil, 83% yield (from general procedure A), 78% yield (from general procedure B), dr>20:1, $[\alpha]^{20}_D$ = +8.5 (c 1.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.25 (m, 5H, Ar), 4.59 (d, J=11.8 Hz, 1H, CH_2), 4.53 (d, J=11.8 Hz, 1H, CH_2), 3.83 (m, 1H, C*H*OH), 3.71 – 3.46 (m, 3H, CH_2 and C*H*OH), 2.61 (d, J=5.4 Hz, 1H, OH), 2.56 (d, J=3.7 Hz, 1H, OH), 1.20 (d, J=6.4 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.53, 128.53, 127.96, 127.78, 73.95, 73.65, 72.36, 68.50, 19.26. HRMS (ESI) m/z calcd for $C_{11}H_{17}O_3^+$: 197.1172 [M+H]$^+$; found: 197.1177.

4m Colorless oil, 71% yield (from general procedure A), 60% yield (from general procedure B), dr > 20:1, $[\alpha]^{20}_{D}$ + 37.0 (c 0.7, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.22 (m, 4H, Ar), 4.37 (dd, J = 7.3, 3.3 Hz, 1H, C*H*OH), 4.00–3.77 (m, 1H, C*H*OH), 2.43 (d, J = 3.4 Hz, 2H, O*H*), 1.32 (s, 9H, t-Bu), 1.08 (d, J = 6.3 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.25, 138.11, 126.57, 125.54, 79.33, 72.22, 34.65, 31.41, 18.88. HRMS (ESI) m/z calcd for $C_{13}H_{21}O_2^+$: 209.1536 [M + H]$^+$; found: 209.1529.

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Conflict of Interest

The authors declare no conflict of interest.

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