

Article

Chemoenzymatic Stereodivergent Synthesis of All the Possible Stereoisomers of the 2,3-Dimethylglyceric Acid Ethyl Ester

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Abstract: 2,3-dihydroxy-2-methylbutyric acid, also known as 2,3-dimethylglyceric acid, constitutes the acyl and/or the alcoholic moiety of many bioactive natural esters. Herein, we describe a chemoenzymatic methodology which gives access to all the four possible stereoisomers of the 2,3-dimethylglyceric acid ethyl ester. The racemic ethyl α -acetolactate, produced by the *N*-heterocycle carbene (NHC)-catalyzed coupling of ethyl pyruvate and methylacetoin was employed as the starting material. The racemic mixture was resolved through (*S*)-selective reductions, promoted by the acetylacetonate reductase (AAR) affording the resulting ethyl (2*R*,3*S*)-2,3-dimethylglycerate; the isolated remaining (*S*)-ethyl α -acetolactate was successively treated with baker's yeast to obtain the corresponding (2*S*,3*S*) stereoisomer. *syn*-2,3-Dimethylglyceric acid ethyl ester afforded by reducing the *rac*- α -acetolactate with NaBH₄ in the presence of ZnCl₂ was kinetically resolved through selective acetylation with lipase B from *Candida antarctica* (CAL-B) and vinyl acetate to access to (2*S*,3*R*) stereoisomer. Finally, the (2*R*,3*R*) stereoisomer, was prepared by C3 epimerization of the (2*R*,3*S*) stereoisomer recovered from the above kinetic resolution, achieved through the TEMPO-mediated oxidation, followed by the reduction of the produced ketone with NaBH₄. The resulting 2,3-dimethylglycerate enriched in the (2*R*,3*R*) stereoisomer was submitted to stereospecific acetylation with vinyl acetate and CAL-B in order to separate the major stereoisomer. The entire procedure enabled conversion of the racemic α -acetolactate into the four enantiopure stereoisomers of the ethyl 2,3-dihydroxy-2-methylbutyrate with the following overall yields: 42% for the (2*R*,3*S*), 40% for the (2*S*,3*S*), 42% for the (2*S*,3*R*) and 20% for the (2*R*,3*R*).

Keywords: biocatalysis; stereodivergent synthesis; asymmetric synthesis; natural compounds



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1. Introduction

The curative effects of traditional pharmaceuticals are frequently related to the activity of secondary metabolites produced by plants or microorganisms. Because of the low concentration of such substances within natural sources, many efforts are devoted to the identification of their chemical structure in order to develop synthetic strategies which could allow their therapeutic exploitation. Knowing the precise structure is also of pivotal importance to understand the mechanisms of action and to design derivatives with best pharmacological performance [1,2]. Many bioactive natural products are chiral compounds produced by living organisms as single stereoisomers whose artificial enantiomers often result less active, if not noxious [3,4]. From an economic and environmental point of view, a sustainable industrial synthesis of these metabolites should require highly efficient and selective reactions so as to reduce the number of steps, simplify the purification procedures and consequently reduce waste formation and energy costs [5]. From this perspective, biocatalysis nowadays offers a broad range of easily accessible enzymes to

perform challenging reactions with excellent results in terms of yield and selectivity [6–8]. Moreover, thanks to the recent advances in bioinformatic and protein engineering which have expanded the biocatalytic toolbox [9], some exquisite examples of total enzymatic syntheses have been recently reported [10–12]. Moving in this field, we recently highlighted that the combined use of a thiamine diphosphate (ThDP)-dependent lyase and a NADH-dependent dehydrogenase enables the preparation of enantiopure 1-substituted-1,2-propanediols [13]. Some of the compounds obtained in this work are secondary metabolites or metabolite moieties, produced by living organisms [14–16]. One representative example is 2,3-dihydroxy-2-methylbutanoic acid, also known as 2,3-dimethylglyceric acid, whose different stereoisomeric forms are contained in a number of bioactive natural esters (Figure 1).

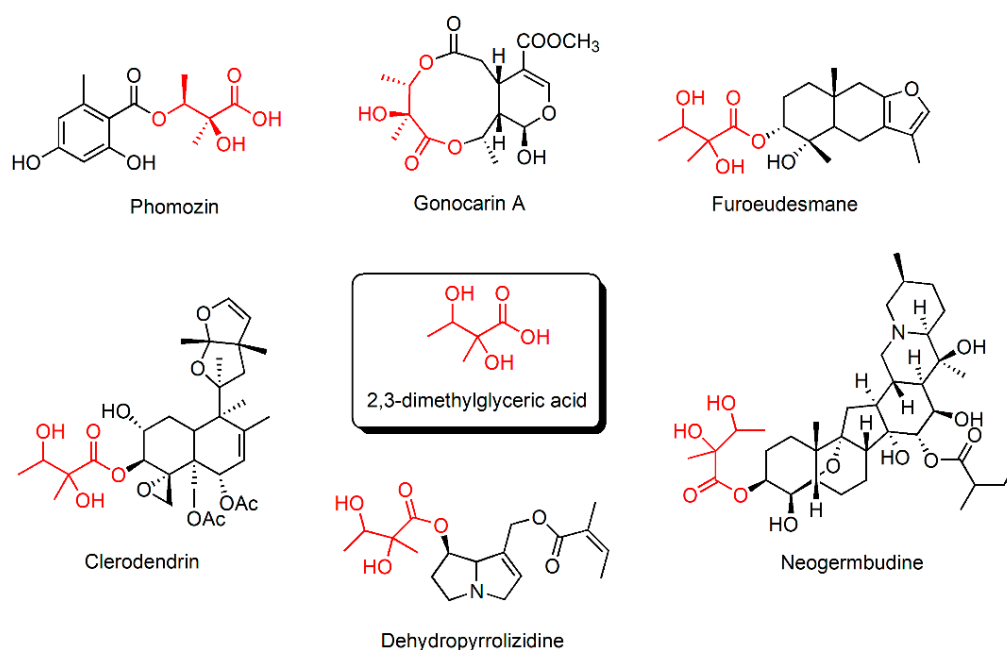


Figure 1. Representative examples of natural products bearing the 2,3-dimethylglyceryl moiety in their structures.

For instance, the phytotoxin phomozin, responsible for the stem cankering of sunflower during infection by *Phomopsis helianthi* is an ester of orsellinic acid and (2*S*,3*S*)-2,3-dimethylglyceric acid [17,18]. In gonocarins A and B, two secoiridoids from *Gonocaryum calleryanum*, the 3-hydroxyl group and the carboxylic group of 2,3-dimethylglyceric acid, are involved in ester linkages with complementary functional groups of the secoiridoid part, resulting in macrocyclic lactones with anti-inflammatory activity [19,20]. In some clerodane diterpenoids [21–23] and furoeudesmane sesquiterpenes [24] with feeding stimulating and antifeedant activity, respectively, the alcoholic terpenoids part is esterified with 2,3-dimethylglyceric acid. Likewise, pyrrolizidine [25] and dehydropyrrolizidine alkaloids pointed out as potential hepatotoxic metabolites [26] or antitumor prodrugs [27] show ester linkages with 2,3-dimethylglyceric acid. Furthermore, the steroidal alkaloids proteroveratrines B and C [28] and neogermbudine [29] are worth mentioning. As for the above clerodanes and furoeudesmanes, these plant metabolites also show a 2,3-dimethylglycerate ester in position 3, which seems to be responsible for their documented neurotoxic activity [29,30]. It is worth noting that most the above studies report only the relative stereochemistry of the dimethylglycerate fragment (except for phomozin, where the absolute stereochemistry was ascertained) [17]. This means that the stereoselective access to all the four stereoisomers of the 2,3-dimethylglyceric acid, would allow the determination of the absolute configuration of the above bioactive natural products, making it possible to design asymmetric total synthetic pathways. The literature reports only a few examples

of stereoselective preparation of 2,3-dimethylglyceric acid esters. The racemic *syn* and *anti* methyl esters were prepared by OsO₄ oxidation of the corresponding *trans*- and *cis*-2-methyl-2-butenates, respectively [20]. Although it uses inexpensive substrates, this route relies on the use of a toxic oxidant and does not show enantioselectivity. On the other hand, the enantioselective preparation of the (2*R*,3*R*)- and (2*S*,3*S*)-2,3-dimethylglycerate ethyl esters via addition of the sterically hindered 2-*t*-butyl-5-methyl-2-phenyl-1,3-dioxolan-4-one lithium enolate to acetaldehyde reported by Greiner et al. [18] has a low atom economy because of the large amount of unrecoverable waste produced by employing the chiral auxiliary [17]. More recently, an iron(II) complex was exploited as catalyst along with aqueous H₂O₂ oxidant as a green alternative to the OsO₄ for the enantioselective *cis*-hydroxylation of the phenyl *trans*-2-methyl-2-butenate yielding the corresponding (2*S*,3*R*) diol with 87% yield and >99% *ee* [31]. Finally, as above mentioned, we recently reported the enzymatic synthesis of the (2*R*,3*S*)-2,3-dimethylglyceric acid ethyl ester through the enzymatic reduction of the ethyl (*R*)- α -acetolactate previously prepared by benzoin-type condensation of methylacetoin and ethyl pyruvate catalyzed by a thiamine diphosphate-dependent lyase [13]. Inspired by this last work, we herein report a stereodivergent chemoenzymatic strategy for the preparation of all the four stereoisomers of the 2,3-dimethylglyceric acid ethyl ester starting from cheap and safe reagents, using easily available biological and chemical catalysts.

2. Results and Discussion

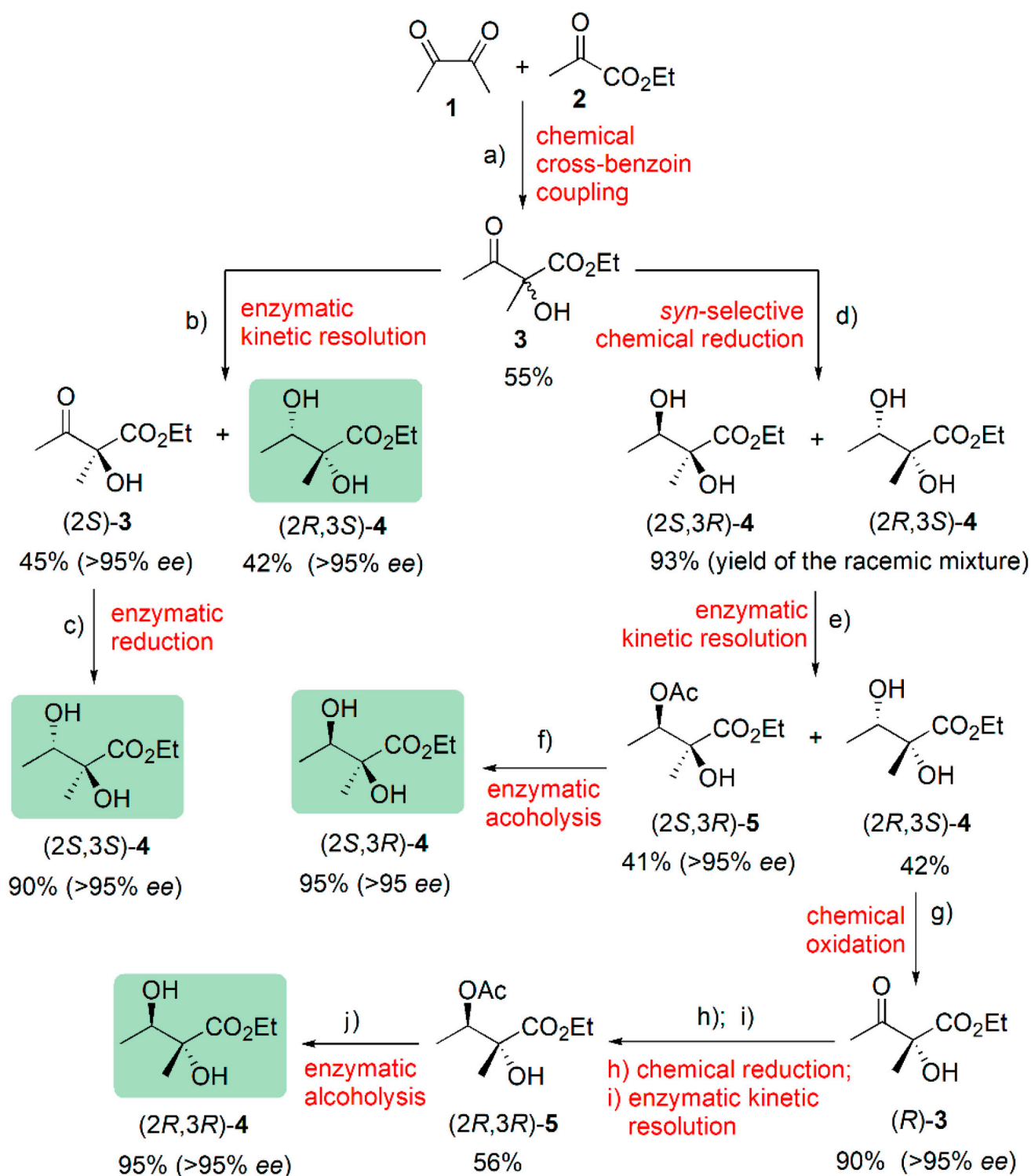
Within a previous study we reported the enzymatic synthesis of optically pure ethyl ester of the (2*R*,3*S*)-2,3-dimethylglyceric acid [13]. Searching in the literature for characterization data, we realized the biological relevance of the different stereoisomers of this acid as well as the limited number of stereoselective synthetic routes for this compound. Moved from these observations, we envisaged the racemic ethyl α -acetolactate (Scheme 1, compound 3) as the potential starting point for a stereodivergent synthesis leading to all the four possible stereoisomers of ethyl 2,3-dimethylglycerate (Scheme 1, product 4). The starting compound 3, can be easily produced from the cross-benzoin type coupling of 2,3-butanedione and ethyl pyruvate (Scheme 1, compounds 1 and 2, respectively) promoted by the *N*-heterocycle carbene (NHC) catalysts generated in situ by treating the thiamine hydrochloride with trimethylamine [32] (Scheme 1, reaction a).

2.1. Enzymatic Kinetic Resolution of the Racemic α -Acetolactate: Synthesis of Ethyl (2*R*,3*S*)-2,3-Dimethylglycerate (2*R*,3*S*)-4

Taking into account the recently highlighted preference of the NADH-dependent acetylacetoin reductase (AAR) for the (*R*) enantiomer of ethyl α -acetolactate [13], we engaged the kinetic resolution of 3 through the enantioselective reduction of the carbonyl group. The optimized reaction performed in 50 mM phosphate buffer at pH 6.5 in the presence of sodium formate (5 equivalents) and formate dehydrogenase (FDH) for the NADH recycle, afforded the expected (2*R*,3*S*)-4 in 42% isolated yield (>95%, *ee*; *d.r.* 92%) (Scheme 1, reaction b).

2.2. Baker's Yeast Catalyzed Reduction of the (*R*)- α -Acetolactate (2*S*)-3: Synthesis of Ethyl (2*S*,3*S*)-2,3-Dimethylglycerate (2*S*,3*S*)-4

The enantiopure (2*S*)-3 recovered from the above reaction mixture (45% yield, > 95% *ee*), could have been enantioselectively reduced in order to obtain the (2*S*,3*S*) or to the (2*S*,3*R*) stereoisomers of 4. To the best of our knowledge, the (*R*)-selective enzymatic reduction of 3 was never reported in the literature, while it is known that whole cells of baker's yeast (BY) are able to reduce racemic 3, giving a 50/50 diastomeric mixture of (2*S*,3*S*)- and (2*R*,3*S*)-4 [33]. Following this example, we treated an aqueous solution of (*S*)-3 with BY in the presence of glucose. After 6 h at 30 °C, the expected (2*S*,3*S*)-4 (*ee* > 95%) was obtained in 90% isolated yield (Scheme 1, reaction c).



Scheme 1. Overall synthetic pathway for the four stereoisomers of the ethyl 2,3-dimethylglycerate. Reaction conditions: (a) **1** (1.0 mmol), **2** (3.0 mmol), thiamine hydrochloride (1.0 mmol), Et₃N (2.0 mmol), ethanol (50 mL), 22 °C, 10 h. (b) AAR (10 U), *rac*-**3** (1.0 mmol), sodium formate (6 mmol), FDH (738 U), 50 mM phosphate buffer pH 6.5 (15 mL), 30 °C, 10 h. (c) (*2S*)-**3** (0.45 mmol), glucose (0.5 g), BY (0.9 g), water (50 mL), 30 °C for 6 h. (d) *rac*-**3** (1.0 mmol), ZnCl₂ (1.0 mmol), NaBH₄ (1.0 mmol), Et₂O-EtOH 3:1 (10 mL), 4 °C, 2h. (e) *rac-syn*-**4** (0.93 mmol), CAL-B (720 U), vinyl acetate (4 mL), 22 °C, 6 h. (f) (*2S,3R*)-**5** (0.42 mmol), CAL-B (180 U), EtOH-cyclohexane 1:30 (3 mL), 22 °C, 10 h. (g) (*2R,3S*)-**4** (1.0 mmol), 10% aq. NaHCO₃ (3.5 mL), TEMPO (0.1 mmol), KBr (0.1 mmol), NaOCl (1.5 mL, available Cl₂ 10%), CH₂Cl₂ (10 mL), 0 °C (20 min), 22 °C (30 min). (h) (*R*)-**3** (0.9 mmol), NaBH₄ (1.0 mmol), Et₂O-MeOH 3:1 (10 mL), 0 °C, 2h. (i) As for step (e), (j) (*2R,3R*)-**5** (0.5 mmol), CAL-B (270 U), EtOH-cyclohexane 1:30 (3.5 mL), 60 °C, 3 h.

2.3. Chemoenzymatic Synthesis of the Enantiopure ethyl (2*S*,3*R*)-2,3-Dimethylglycerate (2*S*,3*R*)-4

Once we paved the way for the synthesis of two stereoisomers of **4**, we moved to investigate a route to obtain the other two stereoisomers, namely the *syn*-(2*S*,3*R*)- and the *anti*-(2*R*,3*R*)-**4**. We started exploring the effect of coordinating metals on the reduction of racemic **3** with NaBH₄. While the reaction conducted in diethyl-ether/ethanol in the absence of strong coordinating metals afforded a *syn/anti* mixture (*d.r.* 30/70), the addition of ZnCl₂ (1 equiv.) to the reaction mixture, led to the diastereoselective formation of the only *syn*-**4** (Scheme 1, reaction d) due to a chelation control [34]. The so-obtained racemic *syn*-**4** contains one of the two desired stereoisomers, namely (2*S*,3*R*)-**4**. In order to avoid troublesome and inconvenient chromatographic separation, we studied the enzymatic acylation as an approach for the kinetic resolution of this racemate. We did not find in literature, examples of the enzymatic acylations conducted on the compound **4**. On the contrary, the kinetic resolution of the less hindered ethyl 3-hydroxybutyrate was successfully performed by using *Candida antarctica* lipase B (CAL-B) as the catalyst, and vinyl acetate as the acylating agent [35]. Therefore, we extended this approach to the racemic *syn*-**4**. Taking into account that CAL-B is notoriously inactive toward tertiary alcohols [36], it remained to be verified how the hindrance of the quaternary center should have affected the rate and the stereoselectivity of the acetylation of the hydroxyl group on position 3. Fortunately, the reaction performed with CAL-B (20% *w/w*) in vinyl acetate without additional solvents led, after 4 h, to the complete conversion of the (2*S*,3*R*)-**4** to the corresponding 3-*O*-acetyl derivative (2*S*,3*R*)-**5** (Scheme 1, reaction e). We also verified that prolonged reaction time (10 h) did not reduce the enantiomeric excess. The product was isolated in 42% yield by column chromatography, and at the same time, the unreacted (2*R*,3*S*)-**4** was recovered (41% yield, >95% *ee*). The acetyl derivative **5** was then dissolved in cyclohexane and reacted with ethanol (3 equivalent) in the presence of CAL-B (20% *w/w*). Thus, the desired (2*S*,3*R*)-**4** was isolated in pure form (95% yield, >95% *ee*) simply by filtering out the enzyme and evaporating the solvents (Scheme 1, reaction f).

2.4. Synthesis of the Ethyl (2*R*,3*R*)-2,3-Dimethylglycerate (2*R*,3*R*)-4 by Chemoenzymatic C3 Epimerization of the (2*R*,3*S*)-4

In order to complete the set of stereoisomers avoiding coproduct waste, we investigate the possibility of invert the C3 configuration of the *syn* (2*R*,3*S*)-**4** recovered from the above kinetic resolution. The first attempt consisted of the tosylation of the secondary hydroxyl group, followed by the S_N2 displacement of the sulfonyloxy moiety with triethylammonium acetate, as reported by a known procedure [37]. This approach, never applied before to esters of 2,3-dimethylglyceric acid, smoothly furnished the tosylated derivative but failed in the following S_N2 step, giving the ethyl (2*R*,3*R*)-2,3-epoxy-2-methylbutyrate as the main product (65%) (for more details see Supplementary Materials, S7 and S8). Hence, we envisaged the oxidation of the (2*R*,3*S*)-**4** followed by the reduction of the resulting hydroxyketone with NaBH₄ as a reasonable route for the partial conversion of the (2*R*,3*S*)-**4** into its diastereoisomer (2*R*,3*R*)-**4** since, as reported above, the reduction of **3**, conducted in the absence of strong chelating metals, leads to the *anti* diol as the main product. Thus, the hydroxyketone (2*R*)-**3** was obtained by treating (2*R*,3*S*)-**4** with sodium hypochlorite in the presence of catalytic amount of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) as described by Anelli's procedure [38] (Scheme 1, reaction g). The crude (2*R*)-**3**, was then dissolved in Et₂O/MeOH (3:1) and treated with NaBH₄ at 0 °C to afford the expected mixture of (2*R*,3*R*)- and (2*R*,3*S*)-**4** in a 70/30 *d.r.* (Scheme 1, reaction h) which was kinetically resolved through the selective acetylation of the *anti* diastereoisomer with CAL-B and vinyl acetate (Scheme 1, reaction i). After chromatographic separation, the acetyl derivative (2*R*,3*R*)-**5**, obtained in 56% yield, was converted to the desired (2*R*,3*R*)-**4** by enzymatic alcoholysis (Scheme 1, reaction j). Although it took four steps, this route allowed to convert the (2*R*,3*S*)-**4** co-produced during the preparation of the (2*S*,3*R*)-**4** to the stereoisomer (2*R*,3*R*)-**4** with an overall yield of 48%.

3. Materials and Methods

3.1. General Information

All commercially available reagents were used as received without further purification, unless otherwise stated. Formate dehydrogenase from *Candida boidinii* (0.45 U/mg) was purchased from Fluka. The *Candida antarctica* lipase B Lipozyme 435[®] (CAL-B) was obtained from Novozymes. The recombinant acetylacetoin reductase (AAR) was obtained as described [13]. The baker's yeast was purchased from Lesaffre Italia. 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). ¹H and ¹³C NMR spectra were acquired at room temperature on spectrometers operating at 300 and 400 MHz; CDCl₃ was employed as a solvent. The chemical shifts (δ) are given in ppm by taking as reference the solvent signal. High-resolution mass spectrometry (HRMS) analyses were performed in positive ion mode on an Agilent 6520 HPLC-Chip Q/TOF-MS nanospray system equipped with a time-of-flight, quadrupole or hexapole unit as analyzer. Optical rotation values were acquired at 20 ± 2 °C in CHCl₃ as solvent; [α]²⁰_D values are given in 10⁻¹ deg cm² g⁻¹. GC analyses were performed using a flame ionization detector and a Megadex 5 column (25 m × 0.25 mm). The samples, free from solvents (about 1 mg), were dissolved in trifluoroacetic anhydride (0.1 mL) and the solution was kept at room temperature for 20 min. After dilution with dichloromethane (1.0 mL), 1.0 μL of the resulting solution was injected. The products were detected using the following temperature program: from 80 °C, 10 °C min⁻¹ up to 200 °C. For retention times, see Supplementary Materials (S10–S16).

3.2. AAR Activity Assay

The enzyme activity was measured by following the disappearance of NADH (decrease of absorbance at 340 nm) during the reduction of racemic **3** as follows. To a solution of NADH (0.2 mM) and racemic **3** (5 mM) in 50 mM phosphate buffer at pH 6.5 (1 mL) the AAR was added and the change in absorbance at 340 nm was monitored for 3 min. One activity unit (U) is defined as the enzyme amount needed to reduce 1 μmol of (S)-**3** in one minute, under the above reaction conditions.

3.3. Synthesis of Racemic Ethyl α-Acetolactate **3**

The racemic ethyl α-acetolactate **3** was obtained through a slightly modified known procedure [32]. Briefly, the 2,3-butanedione **1** (86 mg, 1.0 mmol) was added to a stirred solution of thiamine hydrochloride (337 mg, 1.0 mmol), Et₃N (279 mL, 2.0 mmol) and ethyl pyruvate **2** (348 mg, 3.0 mmol) in ethanol (50 mL). The reaction mixture was stirred at room temperature for 10 h and then partially evaporated, diluted with water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel with cyclohexane-ethyl acetate 3:1 as the eluent in order to obtain the pure ethyl α-acetolactate **3** as a colorless oil (88 mg, 0.56 mmol), 55% yield. ¹H NMR (300 MHz, CDCl₃) δ 4.25 (q, *J* = 7.1 Hz, 2H, CH₂), 4.16 (s, 1H, OH), 2.27 (s, 3H, CH₃), 1.59 (s, 3H, CH₃), 1.29 (t, *J* = 7.1 Hz, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ 205.16, 171.66, 81.29, 62.91, 24.48, 22.09, 14.30. HRMS (ESI) *m/z* calcd for C₇H₁₂O₄⁺: 161,0808 [M + H]⁺; found: 161,0804.

3.4. Synthesis of Ethyl (2R,3S)-2-Methyl-2,3-Dihydroxybutyrate (2R,3S)-**4**

The acetylacetoin dehydrogenase AAR (1 mL, 10 U) was added to a solution of racemic **3** (160 mg, 1.0 mmol), sodium formate (0.4 g, 6 mmol), and formate dehydrogenase (1 mg, 738 U) in 50 mM phosphate buffer pH 6.5 (15 mL). The reaction mixture was gently shaken at 30 °C for 10 h and then extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was chromatographed on silica gel with cyclohexane-ethyl acetate 3:1 as the eluent. The unreacted (2S)-acetolactate (2S)-**3** was eluted first (72 mg, 0.45 mmol), 45% yield, > 95% *ee*. The ethyl (2R,3S)-2-methyl-2,3-dihydroxybutyrate (2R,3S)-**4** eluted last and

after solvent evaporation appeared as a colorless oil (68 mg, 0.42 mmol), 42% yield, > 95% *ee*, 92:8 *d.r.*, $[\alpha]_{\text{D}}^{20} = +1.2$ (c 2.0, CHCl₃). ¹H NMR (300 MHz, CDCl₃) δ 4.24 (q, *J* = 7.1 Hz, 2H, CH₂), 3.92 (q, *J* = 6.4 Hz, 1H, CHOH), 3.51 (br s, 1H, OH), 2.33 (br s, 1H, OH), 1.29 (s, 3H, CH₃), 1.28 (t, *J* = 7.1 Hz, 3H, CH₃), 1.20 (d, *J* = 6.4 Hz, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ 176.59, 77.52, 71.95, 62.47, 21.96, 16.96, 14.43. (100 MHz, CDCl₃). HRMS (ESI) *m/z* calcd for C₇H₁₄O₄⁺: 163.0965 [M + H]⁺; found: 163.0957.

3.5. Synthesis of Ethyl (2*S*,3*S*)-2-Methyl-2,3-Dihydroxybutyrate (2*S*,3*S*)-4

The (2*S*)-3 (72 mg, 0.45 mmol) recovered from the above kinetic resolution was dissolved into water (50 mL). Glucose (0.5 g) and baker's yeast (0.9 g) were added, and the mixture was gently shaken at 30 °C for 6 h. After that, the mixture was centrifuged (14,000 rpm, 10 min) in order to remove the yeast cells and the resulting clarified solution was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure, affording (2*S*,3*S*)-2-methyl-2,3-dihydroxybutyrate (2*S*,3*S*)-4 as a colorless oil (65 mg, 0.40 mmol), 90% yield, >95% *ee*, $[\alpha]_{\text{D}}^{20} = +15.9$ (c 1.3, CHCl₃). ¹H NMR (300 MHz, CDCl₃) δ 4.24 (q, *J* = 7.1 Hz, 2H, CH₂), 3.79 (q, *J* = 6.4 Hz, 1H, CHOH), 3.52 (br s, 1H, OH), 2.43 (br s, 1H, OH), 1.42 (s, 3H, CH₃), 1.29 (t, *J* = 7.1 Hz, 3H, CH₃), 1.14 (d, *J* = 6.4 Hz, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ 175.79, 77.38, 72.51, 62.43, 22.63, 17.96, 14.46. HRMS (ESI) *m/z* calcd for C₇H₁₄O₄⁺: 163.0965 [M + H]⁺; found: 163.0959.

3.6. Synthesis of Ethyl (2*S*,3*R*)-2-Methyl-2,3-Dihydroxybutyrate (2*S*,3*R*)-4

The racemic α -acetolactate **3** (160 mg, 1.0 mmol) and ZnCl₂ (136 mg, 1.0 mmol) were dissolved in diethyl ether-ethanol 3:1 (10 mL). The solution was cooled into an ice-bath and NaBH₄ (38 mg, 1.0 mmol) was added in four portions within 20 min. The reaction was monitored by TLC until the disappearance of the substrate **3** and then quenched with acetone (0.2 mL). The mixture was partially evaporated, diluted with brine (5 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain the crude *syn*-4 (150 mg, 0.93 mmol), *d.r.* > 95% (by ¹H-NMR analysis). The racemic *syn*-4 was dissolved in vinyl acetate (4 mL) and the lipase CAL-B (80 mg, 720 U) was added to the solution. The mixture was gently shaken at room temperature and the reaction course was monitored by gas chromatographic analysis. After 6 h, the lipase was filtered out, and the resulting solution was concentrated under reduced pressure. The residue was chromatographed on silica gel with cyclohexane-ethyl acetate 3:1 as the eluent in order to separate the unreacted (2*R*,3*S*)-4 (66 mg, 0.41 mmol), 41% yield, from the ethyl (2*S*,3*R*)-3-(acetyloxy)-2-hydroxy-2-methylbutyrate **5** (86 mg, 0.42 mmol), 42% yield. ¹H NMR (300 MHz, CDCl₃) δ 5.11 (q, *J* = 6.5 Hz, 1H, CHOAc), 4.27 (q, *J* = 7.1 Hz, 2H, CH₂), 3.37 (s, 1H, OH), 2.09 (s, 3H, Ac), 1.39 (s, 3H, CH₃), 1.31 (t, *J* = 7.1 Hz, 3H, CH₃), 1.19 (d, *J* = 6.5 Hz, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ 175.19, 170.80, 76.23, 73.95, 62.80, 22.56, 21.45, 15.11, 14.46. The compound (2*S*,3*R*)-5 (86 mg, 0.42 mmol) was dissolved in ethanol-cyclohexane 1:30 (3 mL) and the lipase CAL-B (20 mg, 180 U) was added to the solution. The mixture was gently shaken at room temperature and the reaction course was monitored by gas chromatographic analysis. After 10 h, the lipase was filtered out, and the resulting solution was concentrated under reduced pressure to give the (2*S*,3*R*)-2-methyl-2,3-dihydroxybutyrate (2*S*,3*R*)-4 (65 mg, 0.40 mmol), 95% yield, > 95% *ee*, $[\alpha]_{\text{D}}^{20} = -1.6$ (c 3.6, CHCl₃). ¹H and ¹³C NMR consistent with those above reported for compound (2*R*,3*S*)-4. HRMS (ESI) *m/z* calcd for C₇H₁₄O₄⁺: 163.0965 [M + H]⁺; found: 163.0963.

3.7. Synthesis of Ethyl (2*R*,3*R*)-2-Methyl-2,3-Dihydroxybutyrate (2*R*,3*R*)-4

The (2*R*,3*S*)-4 (162 mg, 1.0 mmol) was dissolved in CH₂Cl₂ (10 mL) containing 10% aqueous NaHCO₃ (3.5 mL), TEMPO (15.6 mg, 0.1 mmol), KBr (12 mg, 0.1 mmol). The mixture was warmed to 0 °C, and sodium hypochlorite (1.5 mL, available Cl₂ 10%) was added. The mixture was stirred at 0 °C for 20 min, then warmed to room temperature

and stirred for additional 30 min. After that, the reaction was quenched by adding 0.1 N aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (20 mL) and extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were washed with saturated NH_4Cl (20 mL) and brine (20 mL), and then evaporated. The crude compound (*R*)-**3** (144 mg, 0.9 mmol), 90% yield, >95% *ee*, was dissolved in diethyl ether-methanol 3:1 (10 mL). The solution was cooled to 0 °C and NaBH_4 (38 mg, 1.0 mmol) was added in four portions within 20 min. The reaction was monitored by TLC until the disappearance of the substrate **3** and then quenched with acetone (0.2 mL). The mixture was partially evaporated, diluted with brine (10 mL) and extracted with ethyl acetate (3×10 mL). The combined organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain a diastereomeric mixture of (2*R*,3*R*)- and (2*R*,3*S*)-**4** (*d.r.* 70:30) which was submitted to enzymatic acetylation as described for the racemic *syn*-**4** in paragraph 3.6. After solvent evaporation, the residue was chromatographed on silica gel with cyclohexane-ethyl acetate 5:1 as the eluent in order to obtain the acetyl derivative (2*R*,3*R*)-**5**, (103 mg, 0.50 mmol), 56% yield. ^1H NMR (300 MHz, CDCl_3) δ 5.12 (q, $J = 6.4$ Hz, 1H, CHOAc), 4.29–4.13 (m, 2H, CH_2), 3.25 (br s, 1H, OH), 2.00 (s, 3H, Ac), 1.35 (s, 3H, CH_3), 1.28 (d, $J = 6.4$ Hz, 3H, CH_3), 1.26 (t, $J = 7.1$ Hz, 3H, CH_3). ^{13}C NMR (101 MHz, CDCl_3) δ 175.08, 169.80, 75.95, 74.12, 62.13, 21.72, 20.94, 14.09, 13.26. The acetyl derivative (2*R*,3*R*)-**5** was dissolved in ethanol-cyclohexane 1:30 (3.5 mL) and the lipase CAL-B (30 mg, 270 U) was added to the solution. The mixture was gently shaken at 60 °C and the reaction course was monitored by gas chromatographic analysis. After 3 h, the lipase was filtered out, and the resulting solution was concentrated under reduced pressure to give the ethyl 2-methyl-2,3-dihydroxybutyrate (2*R*,3*R*)-**4** (76 mg, 0.47 mmol), 95% yield, >95% *ee*, $[\alpha]_{\text{D}}^{20} = -10.3$ (c 1.8, CHCl_3). ^1H and ^{13}C NMR consistent with those above reported for compound (2*S*,3*S*)-**4**. HRMS (ESI) m/z calcd for $\text{C}_7\text{H}_{14}\text{O}_4^+$: 163.0965 $[\text{M} + \text{H}]^+$; found: 163.0971.

4. Conclusions

The herein reported chemoenzymatic methodology allows accessing to the ethyl esters of the four possible stereoisomers of the biologically relevant 2,3-dimethylglyceric acid. The products were obtained as pure enantiomers (*ee* > 95%) with good overall yields (from 20 to 42%). All the coproducts of the kinetic resolution steps are employed as intermediates for the preparation of one of the other enantiomers, minimizing waste production. All the reagents and the catalysts employed are commercially available, other than AAR, whose gene sequence and cloning procedure are, however, known. In conclusion, this study contributes to demonstrate how a synergistic integration of chemical and biocatalytic approaches could be a winning strategy for the asymmetric synthesis of stereochemically dense products.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/catal11121440/s1>, Figure S1. ^1H - and ^{13}C -NMR spectra of compound **3**; Figure S2. ^1H - and ^{13}C -NMR spectra of *syn*-**4** [(2*R*,3*S*)-**4** and (2*S*,3*R*)-**4**]; Figure S3. ^1H - and ^{13}C -NMR spectra of *anti*-**4** [(2*S*,3*S*)-**4** and (2*R*,3*R*)-**4**]; Figure S4. ^1H - and ^{13}C -NMR spectra of (2*S*,3*R*)-**5**; Figure S5. ^1H - and ^{13}C -NMR spectra of (2*R*,3*R*)-**5**; Figure S6. ^1H -NMR of the *anti/syn* mixture of **4**; Figure S7. Synthesis and ^1H -NMR of compound **6**; Figure S8. Synthesis and ^1H -NMR of compound **7**; Figure S9. Chiral phase GC for the trifluoroacetyl derivative of (2*R*,3*S*)-**4**; Figure S10. Chiral phase GC for the trifluoroacetyl derivative of (2*S*,3*R*)-**4**; Figure S11. Chiral phase GC for the trifluoroacetyl derivative of (2*S*,3*S*)-**4**; Figure S12. Chiral phase GC for the trifluoroacetyl derivative of (2*R*,3*S*)-**4**; Figure S13. Chiral phase GC for the trifluoroacetyl derivative of (2*R*)-**3**; Figure S14. Chiral phase GC for the trifluoroacetyl derivative of (2*S*)-**3**.

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