Enzymatic Baeyer–Villiger Oxidation of Bicyclic Diketones

Gianluca Ottolina,[a,*] Gonzalo de Gonzalo,[a] Giacomo Carrea,[a] Bruno Danieli[b]

[a] Istituto di Chimica del Riconoscimento Molecolare, CNR ,Via Mario Bianco 9, 20131 Milano, Italy
Fax: (+39)-02-2850-0036, e-mail: gianluca.ottolina@icrm.cnr.it
[b] Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, Via Venezian 21, 20133 Milano, Italy

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Abstract: Cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* was employed for the Baeyer–Villiger oxidation of racemic bicyclic diketones such as the Wieland–Miescher and the Hajos–Parrish diketones and of some of their derivatives. The corresponding lactones were produced in a highly regio- and enantioselective manner. The reactions were carried out using a crude enzyme preparation in aqueous buffer, at room temperature, and the recycling of the expensive coenzyme NADPH was conducted with a second ancillary enzymatic system. The enzymatic process was simple and easy to handle, thus providing a very practical tool to access enantiopure lactones.

Keywords: Baeyer–Villiger oxidation; diketones; enzyme catalysis; lactones; monooxygenase

Introduction

The enzymatic Baeyer–Villiger oxidation is an interesting methodology aiming at obtaining optically active lactones and esters in environmental friendly processes, avoiding the conventional use of peracids or peroxides and heavy-metal catalysts. Among the various Baeyer–Villiger monooxygenases, the most studied to date is the flavoprotein cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* NCIMB 9871,[1] and many examples of its substrate selectivity and enantioselectively to oxidize ketones, sulfides, dithioacetals and amines,[2] sometimes on a preparative scale,[3] have been reported. The reaction takes place in aqueous buffer using atmospheric O_2 as the oxidant and whole cells or isolated enzyme as the catalyst. In the latter case, the oxidation has to be coupled with a second enzymatic reaction for the regeneration of the expensive cofactor NADPH.

The use of enzymes in synthesis has two major hurdles to overcome, their availability and their scarce substrate versatility. Until some years ago the number of Baeyer–Villiger monooxygenases was limited and only recently is their accessibility from different sources increasing.[2f] On the other hand, despite the fact that many molecules with different skeletons have been tested, still only few studies on substrates with more than one reactive group have been conducted. For instance, CHMO converted 3- and 4-thiacyclohexanones exclusively into the lactone products with no detectable sulfide oxidation,[4] and the same happened with 4-methylamine-cyclohexanone, where the lactone was formed with no *N*-oxidation.[5]

The chemical Baeyer–Villiger oxidation of bicyclic and polycyclic ketones has been employed quite often to generate the corresponding lactones, since these structures are frequently found in natural products or are key intermediates in synthesis.[6] However, the presence of two (or more) non-equivalent keto groups in the molecule raised problems of selectivity with the formation of mixtures of products.[7]

This difficulty could, in principle, be surmounted by exploring the recognized chemo-, regio- and enantioselectivity of enzymes and in this paper we have investigated the ability of CHMO to carry out the Baeyer–Villiger oxidation of two representative bicyclic diketones, the Wieland–Miescher[8] (**1**) and the Hajos–Parrish[9] (**12**) diketones and of some of their derivatives, which are building blocks for the synthesis of many natural products.[10]

Results and Discussion

The enzymatic Baeyer–Villiger oxidations were carried out using as the biocatalyst a crude preparation of recombinant CHMO from *Escherichia coli* TOP10 pQR239[11] and were coupled to an ancillary enzymatic reaction in order to regenerate NADPH. As the NADPH regeneration system, glucose 6-phosphate and glucose 6-phosphate dehydrogenase (G6PDH, Scheme 1) were employed.[12]

Oxidation of the racemic Wieland–Miescher ketone (*rac*-**1**) with the crude enzyme preparation gave the lactone (*S*)-**2** in 35% yield and in enantiomerically pure form, as demonstrated by chiral HPLC analysis. Oxygen insertion at the C1–C8a carbon-carbon bond of (*S*)-**1**

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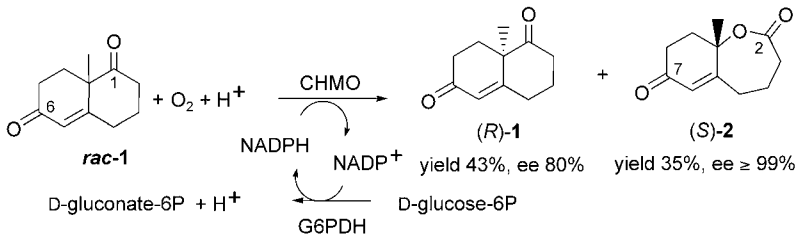
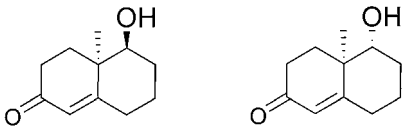


Scheme 1.

was inferred by NMR analysis, which revealed the absence of both the -OCH_2- group (which rules out oxygen insertion at the C1–C2 or C6–C7 carbon-carbon bond) and the -OCHC- fragment (which rules out oxygen insertion at the C5–C6 carbon-carbon bond). This oxygen insertion is in agreement with previous studies by Walsh and Chen which have shown that CHMO is not able to oxidize α,β-unsaturated ketones.[4] The absolute configuration of the lactone product was deduced by the enzymatic oxidation of the enantiopure ketone (*S*)-**1** which gave the lactone (*S*)-**2**. The unreacted starting material, recovered in 43% yield (Scheme 1), was shown to be the ketone (*R*)-**1**, ee 80%, by HPLC comparison with an authentic sample. In addition, careful inspection of the chiral HPLC profile of this reaction revealed the presence of small amounts of by-products, about 5% altogether, that were identified as the alcohols derived by reduction of the saturated carbonyl. Since under this enzymatic conditions, the only product obtained from (*S*)-**1** was the lactone (*S*)-**2**, these alcohols could only derive by a slow enzymatic side reaction on the (*R*)-**1** ketone. Actually, the incubation of (*R*)-**1** with a crude preparation of CHMO gave a small amount of alcohols (4a*R*,5*S*)-**3** and (4a*R*,5*R*)-**4** identified by comparison with authentic samples obtained *via* $NaBH_4$ reduction.[13]



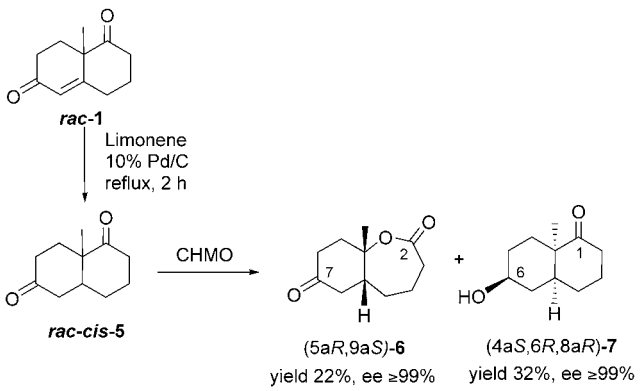
(4a*R*,5*S*)-**3** (4a*R*,5*R*)-**4**

The formation of the two alcohols **3** and **4** was due to a contaminating dehydrogenase activity, present in the crude preparation of CHMO, which came from the *E. coli* strain where the enzyme was overexpressed.[11] In fact, when *rac*-**1** was treated with a crude preparation obtained from the same *E. coli* strain, but that did not contain the CHMO gene, only the alcohols were formed in small amounts. It should be mentioned that when a purified preparation of CHMO devoid of contaminating dehydrogenase was employed, only the lactone (*S*)-**2** was formed.

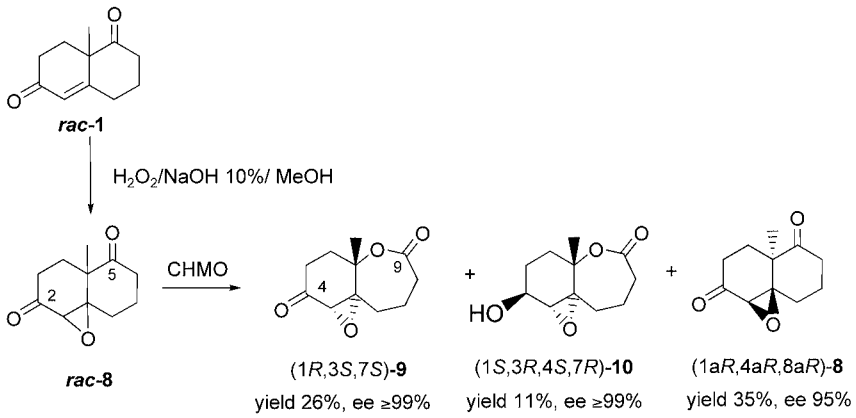
Furthermore, by pushing the conversion of *rac*-**1** close to 50% in lactone, the remaining ketone (*R*)-**1** was obtained with an ee of 96% (yield 40%). Therefore, due to the characteristics of this enzymatic preparation, it is possible to discriminate between the (*S*) enantiomer of **1** that is oxidized to the lactone, and the (*R*) enantiomer, which remains almost unaffected. In principle, this might be an alternative methodology for the preparation of (*R*)-**1** which, in analogy with the chemical synthesis of (*S*)-**1**, could be accessible *via* proline-enamine Robinson annulation reaction of 1-methylcyclohexane-1,3-dione with methyl vinyl ketone, employing the expansive D-proline [(*R*)-(+)-proline] as catalyst and tedious repeated crystallization at low temperature.[14]

Starting from the Wieland–Miescher ketone (*rac*-**1**), racemic 8a–methylhexahydronaphthalene-1,6-dione **5** was easily obtained by selective hydrogen transfer employing limonene and 10% Pd/C. The stereoselectivity for this type of hydrogenation has been demonstrated to be almost exclusively in favor of the formation of the *cis*-isomer.[15] The treatment of *rac-cis*-**5** with crude CHMO gave the enantiopure lactone (5a*R*,9a*S*)-**6** (yield 22%, ee ≥99%, Scheme 2).

Oxygen insertion at the C1–C8a carbon-carbon bond was deduced by the absence of $-OCH_2-$signals in the NMR spectra. It should be emphasized that only one out of four possible regioisomers was formed by enzymatic oxidation. Besides the lactone, the second reaction product was an alcohol isolated with 32% yield and ee ≥99%, identified as (4a*S*,6*S*,8a*R*)-**7** and derived



Scheme 2.



Scheme 3.

by the complete reduction of (4a*S*,8a*R*)-**5** by contaminant dehydrogenase(s). It should be mentioned that the rate of reduction of (4a*S*,8a*R*)-**5** by dehydrogenase(s) was even faster than that of oxidation of (4a*R*,8a*S*)-**5** by CHMO. The absolute stereochemistry of products **6** and **7** was inferred by conducting the enzymatic reaction with the single enantiomers, which were obtained by chemical reduction of the respective optically active ketones **1**.

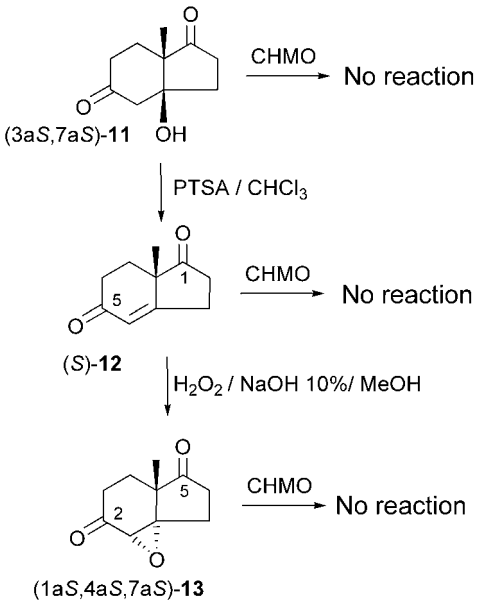
Hydrogen peroxide oxidation of *rac*-**1** gave the ketoepoxide *rac*-**8**, which is formed through an *anti* attack to the conjugated double bond with respect to the methyl group (Scheme 3).[16]

The enzymatic treatment of *rac*-**8** gave the enantiopure lactone (1*R*,3*S*,7*S*)-**9**, again through the selective Baeyer–Villiger oxidation of the carbonyl in position 5. The unreacted starting material was recovered, as the (1a*R*,4a*R*,8a*R*)-**8** enantiomer, in high optical purity (ee 95%). (1a*R*,4a*R*,8a*R*)-**8** and (1a*S*,4a*S*,8a*S*)-**8**, prepared from the corresponding enantiomers of **1**, confirmed that only the latter was oxidized by CHMO, leaving the other enantiomer unreacted. As depicted in Scheme 3, another reaction product was isolated and identified as the alcohol **10** by usual spectroscopic analysis. In fact the ^{1}H NMR spectrum reveals H-4 as a singlet at 3.05 ppm due to an almost orthogonal relationship to H-3. This oxymethine proton is in turn a dd for the vicinal coupling to the C-2 protons which resonate in the 1.27–1.40 and 1.80–2.10 ppm zones, respectively, and are connected to the same carbon at 28.52 ppm. Time course experiments showed the slow formation of **10** by reduction of **9** due to contaminant dehydrogenase(s).

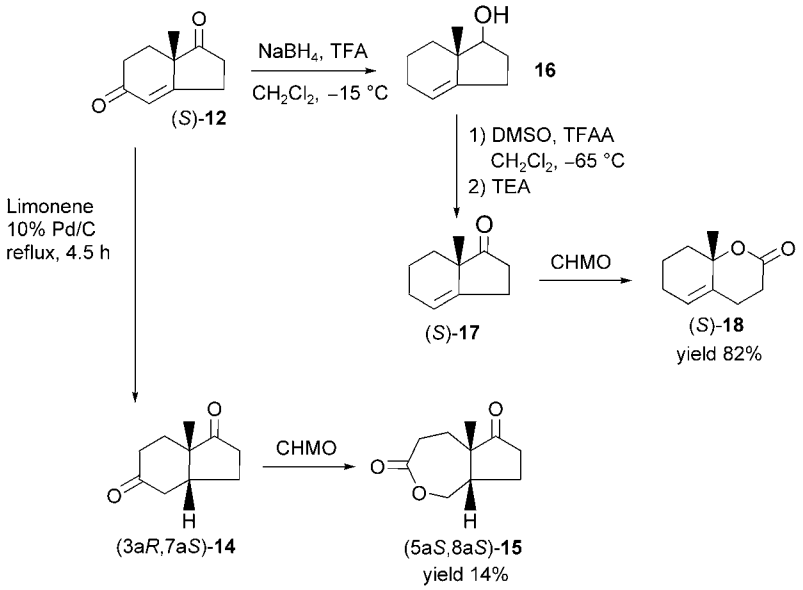
The Hajos–Parrish ketone **12** permitted us to gain some information on the difference in enzyme reactivity between the diketoperhydronaphthalene and the diketoperhydroindene structures. Starting from the commercially available enantiopure ketol (3a*S*,7a*S*)-**11**, compound (*S*)-**12** and its epoxide derivative (1a*S*,4a*S*,7a*S*)-**13** were easily prepared and then treated with CHMO (Scheme 4).

Neither ketone (*S*)-**12** nor (1a*S*,4a*S*,7a*S*)-**13** and (3a*S*,7a*S*)-**11** were affected by CHMO (Scheme 4), that was quite surprising since the structurally closely related compounds **1** and **8** with the same absolute configuration were oxidized by CHMO to lactones.

Taken all together, these results suggest that among the compounds investigated in this work the structure able to better fit the active site of CHMO is that of diketoperhydronaphthalene in the (*S*) configuration. The fact that the diketoperhydroindene derivatives with the same absolute configuration do not react is hardly compatible with the hypothesis that substrates cannot be accommodated in the active site.[17] A possible explanation might be that the five-membered ring does not possess the conformational mobility endowed with the six-membered ring that allows the latter to satisfy the stereoelectronic requirements essential for the subsequent migration in the Criegee intermediate. In fact,



Scheme 4.



Scheme 5.

the saturated ketone (3a*R*,7a*S*)-**14**, obtained by reduction of (*S*)-**12** with limonene and 10% Pd/C,[18] was oxidized by CHMO to lactone (5a*S*,8a*S*)-**15** in low yield (Scheme 5), as demonstrated by NMR analysis, that showed the presence of the signals of two doublets of doublets for the oxymethylene of the $-OCH_2-CH-$fragment.

Other subtle conformational and sterical factors might play an important role in stirring the reaction outcome. Indeed, it is interesting to note that ketone (*S*)-**17**, obtained from (*S*)-**12** *via* $NaBH_4$ reduction to **16**[19] and subsequent oxidation,[20] now proved to be a substrate for CHMO, forming lactones (*S*)-**18** (yield 82%) by insertion of the oxygen at the C1–C7a carbon-carbon bond (Scheme 5).

Conclusion

CHMO proved to be able to catalyze the Baeyer–Villiger oxidation of some racemic diketones in a highly regio- and enantioselective manner, yielding lactones with very high optical purity. In fact the ee of the products was $\geq$99% which points to an enantiomeric ratio for CHMO higher than one hundred. The lactones obtained might be valuable synthons for fine and medicinal chemistry because of their degree of complexity and proper functionalization. As already seen with other substrates (monoketones, sulfides, organic sulfites),[2] enzyme selectivity is quite dependent on substrate structure (e.g., see the different regiopreference shown with substrate **5** compared to substrate **14**). A marked dependence on substrate structure was also observed for the catalytic efficiency of the enzyme, which sometimes was totally inactive with compounds closely similar to those that were accepted as substrates (e.g., **1** and **5** versus **12** and **14**).

The reactions were carried out using a crude enzyme preparation obtained from *E. coli* cells in which the gene of CHMO had been overexpressed. It was chosen to utilize a crude biocatalyst because it was more convenient and required less man-power to obtain it. These are important requisites in view of possible future large-scale applications of CHMO. However, it should be mentioned that the crude enzyme contained contaminating dehydrogenase activities which, as in the case of **5**, completely reduced one of the enantiomers to a keto alcohol. Since the keto alcohol was optically pure, the result might even be considered positive, should one need this product. Anyway, as shown in the case of compound **1**, this drawback can be easily overcome by using CHMO purified by conventional methods.

Experimental Section

General Remarks

Melting points (uncorrected) were determined with a Reichert-Köfler apparatus. IR spectra were recorded on a Jasco FTIR 610. Optical rotations were determined on a Perkin Elmer 141 polarimeter. Flash chromatography: silica gel 60 (70–230 mesh, Merck). Chiral HPLC analysis: Jasco HPLC instrument (model 880-PU pump, model 870-UV/VS detector) equipped with a Chiracel OD (Daicel) chiral column. ^1H and ^{13}C NMR spectra at 300 MHz and 72.5 MHz were recorded in $CDCl_3$ on a Bruker AC-300. Mass spectra were recorder on a GC-MS-EI (Finnigan-Thermo). All chiral compounds were purchased from Aldrich-Fluka.

Enzymatic Oxidation of Wieland–Miescher Ketone (1)

The Wieland–Miescher ketone (**1**; 100 mg, 0.56 mmol) was magnetically stirred at room temperature in potassium phosphate buffer (50 mM, pH 8.7, 22 mL), containing NADP (2.78 mg, 3.6 μmol), glucose 6-phosphate (188 mg, 0.62 mmol), CHMO (10 U) and G6PDH (50 U). The reaction was stopped after 26 hours and worked up. The crude product was purified by flash chromatography (eluent ethyl acetate-light petroleum ether, 2:1) to give (*R*)-(−)-**1**; yield: 43 mg (43%); ee 80%; $[\alpha]_D^{25}$: −67.0 (*c* 2, benzene) and (*S*)-(+)-**2**; yield: 39 mg (35%); ee ≥99%; white powder; $[\alpha]_D^{25}$: +90.3 (*c* 1.5, chloroform); mp 140–143 °C; IR (nujol): ν = 1720, 1659 cm^{-1}; MS: *m*/*z* = 194 (M^+, 9), 176 (21), 122 (36), 108 (71), 93 (90); ^{1}H NMR: δ = 1.64 (3 H, s), 1.83–1.93 (1 H, m), 2.12–2.22 (1 H, m), 2.25–2.32 (1 H, m), 2.43–2.74 (7 H, m) 5.9 (1 H, s); ^{13}C NMR δ = 196.35, 171.95, 127.00, 111.19, 81.23, 35.40, 35.28, 32.50, 28.97, 26.08, 23.52; HPLC: column Chiracel OD Diacel, λ_{245}, flow rate 1 mL/min, eluent: 93% light petroleum ether, 7% *i*-PrOH; R_t: (4a*R*,5*R*)-**4** 12.5 min, (4a*R*,5*S*)-**3** 14.0 min, (*S*)-(+)-**1** 16.8 min, (*R*)-(−)-**1** 18.4 min, (*S*)-(+)-**2** 42.0 min.

Compounds (4a*R*,5*S*)-**3** and (4a*R*,5*R*)-**4** were confirmed by comparison with authentic samples.[13]

Chemical Oxidation of Wieland–Miescher Ketone (1)

The Wieland–Miescher ketone (**1**; 70 mg, 0.39 mmol) was magnetically stirred at room temperature in dichloromethane (7.8 mL) containing mCPBA (406 mg, 1.18 mmol). The reaction was stopped after 48 hours and worked up. The crude product was purified by flash chromatography (eluent ethyl acetate-light petroleum ether, 2:1) to give **2**; yield: 72 mg (95%). HPLC: column Chiracel OD Diacel, λ_{245}, flow rate 1 mL/min, eluent: 93% light petroleum ether, 7% *i*-PrOH; R_t: (*S*)-(+)-2 42.0 min, (*R*)-(−)-2 54.2 min.

Enzymatic Oxidation of *rac-cis*-5

Compound *rac-cis*-**5**[15] (110 mg, 0.61 mmol) was magnetically stirred at room temperature in potassium phosphate buffer (50 mM, pH 8.7, 24.4 mL), containing NADP (3 mg, 3.9 μmol), glucose 6-phosphate (204 mg, 0.67 mmol), CHMO (11 U) and G6PDH (55 U). The reaction was stopped after 26 hours and worked up. The crude product was purified by flash chromatography (eluent ethyl acetate-light petroleum ether, 1:1) to give 29 mg of starting material **5** (ee 4%), $[\alpha]_D^{25}$: −0.3 (*c* 1, chloroform), 27 mg of **6** and 36 mg of **7**.

(5a*R*,9a*S*)-9a-Methylhexahydrobenzo[*b*]oxepine-2,7-dione (6): Yield: 22%; white powder; $[\alpha]_D^{25}$: +16.0 (*c* 1.5, chloroform); ee ≥99%; mp 70–73 °C; IR (nujol): ν = 1716 cm^{-1}; MS: *m*/*z* = 196 (M^+, 3), 178 (5), 168 (5), 125 (37), 99 (100); ^{1}H NMR: δ = 1.22 (3 H, s), 1.65–1.70 (3 H, m), 1.75–1.85 (4 H, m), 2.67–2.74 (3 H, m), 2.85–2.95 (3 H, m); ^{13}C NMR: δ = 210.02, 174.18, 80.15, 43.13, 41.22, 41.00, 37.20, 37.07, 29.62, 24.40, 17.43.

(4a*S*,6*S*,8a*R*)-6-Hydroxy-8a-methyloctahydronaphthalen-1-one (7): Yield: 32%; oil; $[\alpha]_D^{25}$: +47.5 (*c* 2, chloroform); ee ≥ 99%; IR (nujol): ν = 3474, 1687 cm^{-1}; MS: *m*/*z* = 182 (M^+, 5), 164 (58), 121 (100), 93 (88),79 (56); ^{1}H NMR: δ = 0.96 (1 H, dt, *J* = 13.7, 4.0 Hz), 1.22 (3 H, s), 1.24–1.43 (3 H, m), 1.47–1.55 (2 H, m), 1.68–1.75 (1 H, dq, *J* = 19.03, 2.65 Hz), 1.78–1.86 (1 H, dquint, *J* = 12.45, 3.65 Hz), 1.88–2.00 (3 H, m), 2.19–2.34 (3 H, m), 3.6 (1 H, m); ^{13}C NMR δ = 211.00, 70.88, 48.40, 43.53, 38.36, 37.82, 32.94, 32.72, 26.57, 26.27, 22.06.

Enzymatic Oxidation of *rac*-8

Compound *rac*-**8**[16] (120 mg, 0.61 mmol) was magnetically stirred at room temperature in potassium phosphate buffer (50 mM, pH 8.7, 25 mL), containing NADP (3 mg, 3.9 μmol), glucose 6-phosphate (207 mg, 0.67 mmol), CHMO (12 U) and G6PDH (60 U). The reaction was stopped after 24 hours and worked up. The crude product was purified by flash chromatography (eluent ethyl acetate-light petroleum ether, 3:1) to give 42.5 mg of starting material (1a*R*,4a*R*,8a*R*)-**8**, 33.5 mg of **9** and 14 mg of **10**.

(1a*R*,4a*R*,8a*R*)-8: Yield: 35%; $[\alpha]_D^{25}$: +188.2 (*c* 2, chloroform); ee 95%; HPLC: column Chiracel OD Diacel, λ_{215}, flow rate 1 mL/min, eluent: 90% light petroleum ether, 10% *i*-PrOH; R_t: (1a*S*,4a*S*,8a*S*)-**8** 10.0 min and (1a*R*,4a*R*,8a*R*)-(+)-**8** 11.4 min.

(1*R*,3*S*,7*S*)-7-Methyl-2,8-dioxatricyclo[5.5.0.0^{1,3}]dodecane-4,9-dione (9): Yield: 26%; oil; IR (nujol): ν = 1713, 1289 cm^{-1}; ee ≥99%; MS: *m*/*z* = 182 (2), 154 (4), 139 (15), 111 (42), 99 (100); ^{1}H NMR: δ = 1.54 (1 H, dt, *J* = 14.9, 4.1 Hz), 1.65 (3 H, s), 1.84 (1 H, dd, *J* = 11.7, 7.8 Hz), 1.95–2.05 (2 H, m), 2.28 (1 H, ddd, *J* = 13.6, 11.7, 5.8 Hz), 2.5–2.65 (3 H, m), 2.73 (1 H, ddd, *J* = 13.5, 9.1, 3.8 Hz), 3.02 (1 H, ddd, *J* = 13.5, 8.1, 3.6 Hz), 3.23 (1 H, s); ^{13}C NMR: δ = 206.50, 172.06, 83.05, 71.40, 63.94, 35.96, 33.69, 30.62, 29.14, 23.16, 19.46; HPLC: column Chiracel OD Diacel, λ_{215}, flow rate 1 mL/min, eluent: 95% light petroleum ether, 5% *i*-PrOH; R_t: (1*R*,3*S*,7*S*)-**9** 45.4 min.

(1*S*,3*R*,4*S*,7*R*)-4-Hydroxy-7-methyl-2-oxatricyclo[5.5.0.0^{1,3}]dodecan-9-one (10): Yield: 11%; white powder; mp 83–85 °C; $[\alpha]_D^{25}$: −18.3 (*c* 1, chloroform); ee ≥99%; IR (nujol): ν = 3420, 1704, 1295 cm^{-1}; MS: *m*/*z* = 212 (M^+, 4), 194 (3), 165 (4), 111 (38), 97 (100); ^{1}H NMR: δ = 1.27–1.40 (2 H, m), 1.63 (1 H, ddd, *J* = 9.95, 1.8, 1.53 Hz), 1.7 (3 H, s), 1.8–2.1 (4 H, m), 2.43 (1 H, ddd, *J* = 14.9, 11.85, 4.1 Hz), 2.74 (1 H, ddd, *J* = 15.82, 12.62, 2.95 Hz), 2.95 (1 H, dd, *J* = 13.0, 6.6 Hz), 3.05 (1 H, s), 4.08 (1 H, dd, *J* = 9.7, 7.06 Hz).

Chemical Oxidation of *rac*-8

Compound *rac*-**8**[16] (25 mg; 0.13 mmol) was magnetically stirred at room temperature in dichloromethane (2.5 mL) and mCPBA was added portionwise over 3 hours (100 mg; 0.38 mmol). After 24 hours the reaction mixture was quenched by the addition of sodium dithionite and worked up. The crude product was purified by flash chromatography (eluent ethyl acetate-light petroleum ether, 3:1) to give racemic **9**; yield: 8 mg (30%). HPLC: column Chiracel OD Diacel, λ_{215}, flow rate 1 mL/min, eluent: 95% light petroleum ether, 5% *i*-PrOH; R_t: (1*S*,3*R*,7*R*)-**9** 43.4 min, (1*R*,3*S*,7*S*)-**9** 45.4 min.

Enzymatic Oxidation of (3a*R*,7a*S*)-14

Compound (3a*R*,7a*S*)-**14**[18] (76 mg; 0.45 mmol) was magnetically stirred at room temperature in potassium phosphate buffer (50 mM, pH 8.7, 12.5 mL), containing NADP (2.3 mg;

3 μmol), glucose 6-phosphate (153 mg, 0.5 mmol), CHMO (7.6 U) and G6PDH (38 U). The reaction was stopped after 24 hours and worked up. The crude product was purified by flash chromatography (eluent ethyl acetate-light petroleum ether, 3:1) to give **15**; yield: 11.3 mg (14%); white powder; $[\alpha]_D^{25}$: +59.3 (*c* 0.7, chloroform); mp 76–79 °C; IR (nujol): $\nu = 1733\ cm^{-1}$; MS: *m/z* = 182 (M^+, 20), 154 (15), 123 (48), 68 (100); ^1H NMR δ = 1.17 (3 H, s), 1.55 (1 H, ddd, *J* = 11.45, 9.5, 2.0 Hz), 1.85–1.95 (1 H, m), 1.98 (1 H, ddd, *J* = 11.45, 10.3, 2.0 Hz), 2.1–2.25 (1 H, m; 1 H, m, X part of an ABX system), 2.3–2.5 (2 H, m), 2.56 (1 H, ddd, *J* = 14.8, 9.4, 2.0 Hz), 2.66 (1 H, ddd, *J* = 14.8, 10.3, 2.0 Hz), 4.18 (1 H, dd, *J* = 13.5, 4.2 Hz, B part of an ABX system), 4.37 (1 H, dd, *J* = 13.5, 1.8 Hz, A part of an ABX system); ^{13}C NMR: δ = 210.50, 172.51, 67.10, 45.95, 44.50, 34.89, 29.39, 26.74, 21.64, 20.63.

Enzymatic Oxidation of (S)-17

Compound **17**[20] (47 mg, 0.31 mmol) was magnetically stirred at room temperature in potassium phosphate buffer (25 mM, pH 8.7, 12.5 mL), containing NADP (1.6 mg, 2 μmol), glucose 6-phosphate (105 mg, 0.34 mmol), CHMO (4.7 U) and G6PDH (24 U). The reaction was stopped after 8 hours and worked up. The crude product was purified by flash chromatography (eluent ethyl acetate-light petroleum ether, 3:1) to give **18**; yield: 42.5 mg (82%); oil; MS: *m/z* = 166 (M^+, 39), 151 (40), 123 (29), 106 (53); 91 (100); ^1H NMR: δ = 1.64 (3 H, s), 1.5–1.8 (4 H, m), 1.8–2.2 (2 H, m), 2.25–2.75 (4 H, m), 4.83 (1 H, bs); ^{13}C NMR: δ = 172.32, 127.21, 111.30, 80.53, 31.26, 29.70, 28.83, 26.31, 23.00, 19.71.

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