Tetrahedron: *Asymmetry* 24 (2013) 973–981

Contents lists available at SciVerse ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Enantiodivergence in the reduction of α-methyl and α-halomethyl enones by microorganisms

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ARTICLE INFO

Article history:
Received 7 May 2013
Accepted 5 July 2013

ABSTRACT

Enones (*Z*)-3-methyl-(*Z*)-3-chloromethyl- and (*Z*)-3-bromomethyl-4-R-3-buten-2-one (R = *n*-pentyl, phenyl, 2′- and 4′-chlorophenyl, 3′- and 4′-nitrophenyl, 4′-methoxyphenyl) were synthesized and subjected to reduction by the microorganisms *Saccharomyces cerevisiae* and *Geotrichum candidum*. Whereas the bioreduction of 3-methy-4-R-3-buten-2-ones afforded the corresponding (*S*)-4-R-3-methybutan-2-ones, the bioreduction of 3-chloromethyl- and 3-bromomethyl-4-R-3-buten-2-ones afforded the corresponding (*R*)-4-R-3-methybutan-2-ones.

1. Introduction

The bioreduction of activated C=C bonds is a powerful tool in asymmetric organic synthesis and has been extensively studied over recent years; both whole cell preparations and isolated enoate reductases have been employed for this purpose.[1] Among activated olefins, those bearing a halogen at the α- or β-position with respect to the electron withdrawing group show interesting behavior over the course of their bioreductions.

Generally, α-haloenones tend to afford the corresponding halohydrins after sequential C=C and C=O reductions.[2–4] On the other hand, bioreductions of β-haloenones show a distinct behavior due to the acidity of the α-hydrogen in the β-haloketone intermediate, which facilitates elimination of hydrogen halides.

Elimination reactions in the bioreduction of β-haloketones have been known for several years—for instance, it has been shown that in the bioreduction of β-chloropropiophenone, it undergoes spontaneous elimination to afford a methyl vinyl ketone, whose C=C bond is reduced to produce propiophenone.[5] More recently, examples of β-elimination have been demonstrated in β-haloenals[6,7] and methyl trichloropropenoate and related esters.[8] In these examples, dehydrohalogenations occur spontaneously after the first C=C bond reduction, producing an α,β-unsaturated carbonyl compound that undergoes further C=C reduction. In contrast to spontaneous dehydrohalogenations of β-haloketones, in cases where reductive dehalogenation occurs in α-haloketones, mechanisms other than elimination have been proposed to account for the carbon–halogen bond cleavage.[9–11]

For synthetic organic chemists, an interesting feature of these reduction-elimination cascades is that the exchange of a C–H bond for a C–X bond at the β-position of a carbonyl can be used as a substrate-based strategy to control the stereochemistry of the C=C bond reduction.[6,7] For example, the reduction of 2-chloromethyl- and 2-methylcinnamaldehydes mediated by baker's yeast has been shown to afford the same alcohol, but with opposite stereochemistries.[6] Thus, this strategy can overcome the drawback of needing stereocomplementary biocatalysts when both enantiomers of a given compound are needed. Herein we report the bioreductions of structurally related α-methylenones and α-halomethylenones to produce both stereoisomers of enantiomerically enriched α-methylketones.

2. Results and discussion

Substrates **2a**–**g** and **3a**–**g** were prepared in two steps from aldehydes and 3-buten-2-one, following procedures similar to those described elsewhere for both the Morita–Baylis–Hillman (MBH) reactions[12,13] and reactions with concentrated HCl or HBr.[14] Substrates **4a**–**g** were prepared from aldehydes and butanone in aldol condensations, following a procedure described elsewhere[15] (Scheme 1).

Substrate **2a** was subjected to preliminary bioreduction experiments to evaluate the capability of microorganisms producing **6a** in high ee (Table 1). A range of products were observed over the course of the reactions: along with the expected intermediate **5a** and its bioreduction product **6a**, the racemic MBH adduct **1a** and its isomer **7**[16] were also observed in some cases (Table 1).

Experiments performed in the absence of microorganisms led to the extensive formation of **1a** and **7**, suggesting that solvolysis of **2a** competes with the bioreduction and prevails when the microorganism is not capable of reducing **2a**. It was observed that the MBH adduct **1a** was not further reduced by any of the microorganisms screened.

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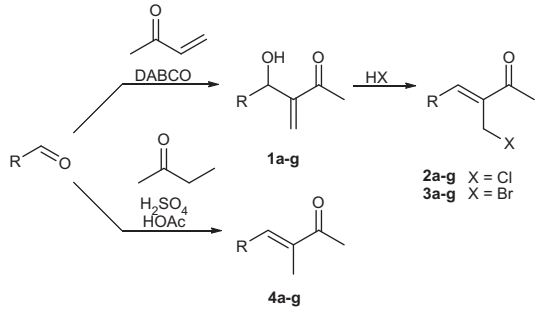
http://dx.doi.org/10.1016/j.tetasy.2013.07.007



a: R = C_6H_5; **b**: R = 2-Cl-C_6H_4; **c**: R = 4-Cl-C_6H_4; **d**: R = 3-NO_2-C_6H_4; **e**: R = 4-NO_2-C_6H_4; **f**: R = 4-MeO-C_6H_4; **g**: R = *n*-C_5H_{11}

Scheme 1. Preparation of substrates **2a**–**g**, **3a**–**g**, and **4a**–**g**.

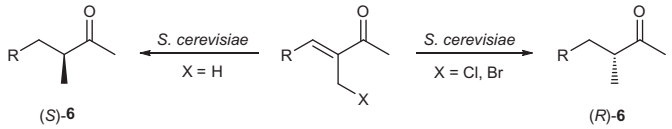
It should be noted that, in general, the C=O bond reduction was significantly slower than the C=C bond reduction, which means it was possible to isolate ketone **6a** free from the corresponding alcohol. Moreover, even though intermediate **5a** did not accumulate in the reaction medium, the production of (*R*)-**6a** from intermediate **5a** is in agreement with the stereochemical outcome of a previously reported bioreduction of **5a** by *S. cerevisiae*.[18]

We found that *S. cerevisiae*, *Rhodotorula glutinis*, and *Geotrichum candidum* showed the best conversion to (*R*)-**6a**, although only *S. cerevisiae* and *G. candidum* afforded (*R*)-**6a** in high ee. Thus, commercial lyophilized *S. cerevisiae* and *G. candidum* were used for subsequent reductions of α-chloromethylenones **2a**–**g**, α–bromomethylenones **3a**–**g,** and α-methylenones **4a**–**g**. The results are summarized in Tables 2 and 3.

In general, enones bearing an α-halomethyl group produced the corresponding α-methylketones (*R*)-**6a**–**g**. On the other hand, the reduction of α-methylenones **4a**–**g** gave the corresponding α-methylketones (*S*)-**6a**–**g**. Generally, *S. cerevisiae* gave products in better yields and ee than *G. Candidum*, which did not reduce several substrates.

An explanation for the observed stereochemical outcome of these reactions takes into account the mechanism of action of enzymes of the Old Yellow Enzyme (OYE) family. These enzymes, present in *S. cerevisiae*,[21–24] catalyze the transfer of a formal hydride from a flavin mononucleotide ($FMNH_2$) to the β-carbon of the activated double bond. This step is followed by a proton abstraction by the α-carbon, constituting an overall formal *trans*-hydrogen addition to the double bond.[25] According to a recently published work,[25,26] the stereochemistry of these reactions can be attributed to a 'flipped' binding mode of enones **4a**–**g** and **5a**–**g** to the active site of OYEs, as envisaged in Figure 1. Whereas the C=C bond reduction of **4a**–**g** affords (*S*)-**6a**–**g**, the

Table 2
Bioreduction of enones by *S. cerevisiae*[a]

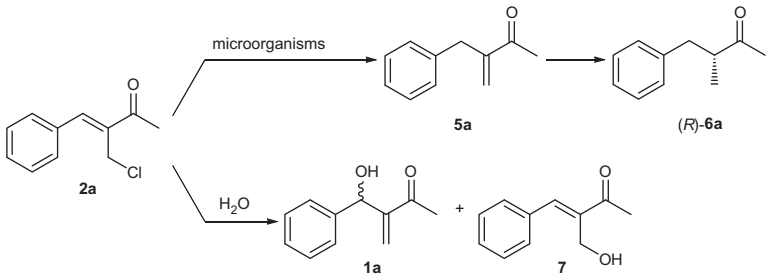


a: R = C_6H_5; **b**: R = 2-Cl-C_6H_4; **c**: R = 4-Cl-C_6H_4; **d**: R = 3-NO_2-C_6H_4; **e**: R = 4-NO_2-C_6H_4; **f**: R = 4-MeO-C_6H_4; **g**: R = *n*-C_5H_{11}

Substrate	R	X	*t* (h)	Yield (%)[b]	ee (%)[c]
2a	Ph	Cl	24	42	96 (*R*)
3a		Br	8	53	95 (*R*)
4a		H	24	59	68 (*S*) [71]
2b	2-Cl-C_6H_4	Cl	16	62	>99 (*R*)
3b		Br	16	54	>99 (*R*)
4b		H	40	44	96 (*S*)
2c	4-Cl-C_6H_4	Cl	16	57	92 (*R*)
3c		Br	6	70	94 (*R*)
4c		H	24	60	80 (*S*)
2d	3-NO_2-C_6H_4	Cl	24	40	83 (*R*)
3d		Br	24	40	78 (*R*)
4d		H	48	37	93 (*S*) [>95]
2e	4-NO_2-C_6H_4	Cl	24	38	82 (*R*)
3e		Br	24	52	80 (*R*)
4e		H	24	61	63 (*S*)
2f	4-MeO-C_6H_4	Cl	4	—	—
3f		Br	4	—	—
4f		H	24	63	53 (*S*) [61]
2g	*n*-C_5H_{11}	Cl	12	16	92 (*R*)
3g		Br	12	25	95 (*R*)
4g		H	8	38	91 (*S*)

[a] Yeast type II from *S. cerevisiae*, Sigma.
[b] In cases where no yield is given, only the starting material and/or degradation products were recovered.
[c] *ee* Values between brackets refer to bioreductions reported in Ref. [19,20].

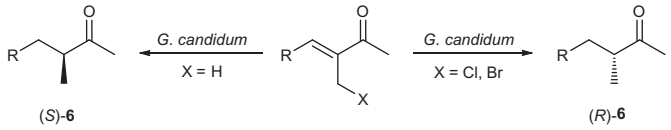
Table 1
Reaction mixture composition in the bioreduction of (*Z*)-3-chloromethyl-4-phenyl-3-buten-2-one **2a** mediated by microorganisms



Microorganism	*t* (h)	**2a** (%)	**5a** (%)	**1a** + **7** (%)	**6a** (ee) (%)
Saccharomyces cerevisiae[a]	16	0	2	12	86 (96)
Saccharomyces cerevisiae[b]	24	0	8	14	78 (94)
Saccharomyces boulardii	72	14	10	60	16 (85)
Rhodotorula glutinis	10	0	3	0	97 (59)
Candida albicans	72	4	0	95	1 (n.d.)[c]
Geotrichum candidum	6	0	9	0	91 (87)
Curvularia lunata	72	6	0	92	2 (n.d.)[c]

[a] Yeast type II from *S. cerevisiae*, Sigma.
[b] *S. cerevisiae* CCT 3019 obtained from Fundação André Tosello.[17]
[c] Not determined.

Table 3
Bioreduction of enones by *G. candidum*



a: R = C_6H_5; **b**: R = 2-Cl-C_6H_4; **c**: R = 4-Cl-C_6H_4; **d**: R = 3-NO_2-C_6H_4; **e**: R = 4-NO_2-C_6H_4; **f**: R = 4-MeO-C_6H_4; **g**: R = *n*-C_5H_{11}

Substrate	R	X	*t* (h)	Yield (%)[a]	ee (%)
2a	Ph	Cl	6	66	87 (*R*)
3a		Br	6	57	84 (*R*)
4a		H	96	—	—
2b	2-Cl-C_6H_4	Cl	96	—	—
3b		Br	96	—	—
4b		H	96	—	—
2c	4-Cl-C_6H_4	Cl	10	50	86 (*R*)
3c		Br	6	66	87 (*R*)
4c		H	96	13	54 (*S*)
2d	3-NO_2-C_6H_4	Cl	16	48	71 (*R*)
3d		Br	12	42	74 (*R*)
4d		H	48	53	42 (*S*)
2e	4-NO_2-C_6H_4	Cl	10	44	72 (*R*)
3e		Br	6	48	71 (*R*)
4e		H	24	51	33 (*S*)
2f	4-OMe-C_6H_4	Cl	4	—	—
3f		Br	4	—	—
4f		H	96	16	17 (*S*)
2g	*n*-C_5H_{11}	Cl	96	—	—
3g		Br	96	—	—
4g		H	96	—	—

[a] In cases where no yield is given, only the starting material and/or degradation products were recovered.

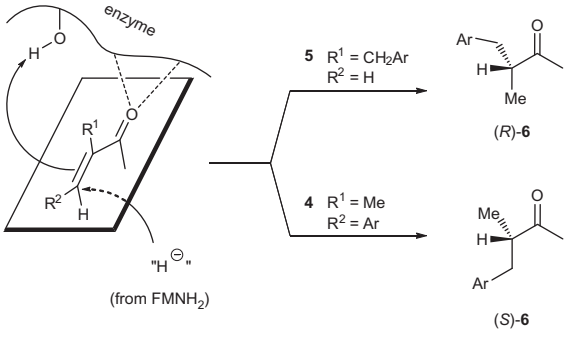


Figure 1. 'Flipped' binding mode of enones **4a**–**g** and **5a**–**g** to the active site of OYEs as an explanation for the enantiodivergence of the enone reductions.

reduction of intermediate **5a**–**g** produced from **2a**–**g** and **3a**–**g** affords (*R*)-**6a**–**g** by the same 'flipped' binding mode.

A particular reactivity was observed for *p*-anisaldehyde-derived enones **2f** and **3f**. Whereas **4f** was reduced to the corresponding (*S*)-α-methylketone, both **2f** and **3f** afforded the corresponding MBH adduct **1f** after only 4 h of reaction. This is an indication that both **2f** and **3f** undergo rapid solvolysis, possibly due to the electron-donating effect of the methoxy group at the *para*-position; the MBH adduct formed **1f** was not further biotransformed by either microorganism.

The bioreduction of **2a** by *S. cerevisiae* was carried out with up to 10 mmol (1.94 g) of substrate. The bioreduction product (*R*)-**6a**, upon treatment with an alkaline solution of sodium hypobromite[27] was converted into the carboxylic acid (*R*)-**8a** (Scheme 2), with a slight loss of enantiomeric purity. Acid (*R*)-**8a** has been used as a chiral building block in the synthesis of zaragozic Acids A and C C-1 side-chains.[28]

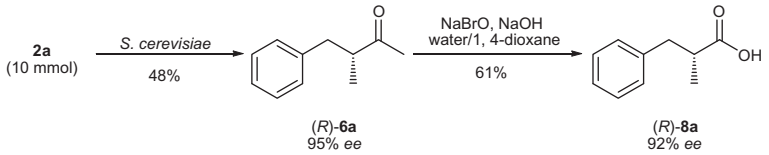
3. Conclusion

The yeast *Saccharomyces cerevisiae* was used to reduce enones **4a**–**g** to produce α-methylketones (*S*)-**6a**–**g** in 63–96% ee. When α-chloromethylenones **2a**–**e** and **2g** and α-bromomethylenones **3a**–**e** and **3g** were subjected to the same bioreduction conditions, a reduction-elimination-reduction sequence afforded the α-methylketone antipodes (*R*)-**6a**–**e** and (*R*)-**6g** in 78–99% ee. The *p*-anisaldehyde-derived enones **2f** and **3f** underwent rapid solvolysis, possibly due to the electron-donating effect of the methoxy group; the solvolysis product **1f** was not further bioreduced. The fungus *Geotrichum candidum* was also able to reduce some of the substrates in the same manner as *S. cerevisiae* although enantiomeric excesses were generally poorer.

4. Experimental

4.1. General

Benzaldehyde, 2-chlorobenzaldehyde, 3-nitrobenzaldehyde, 4-nitrobenzaldehyde, hexanal, *p*-anysaldehyde, 3-buten-2-one, DABCO, bromine, and Amberlite® XAD7HP were purchased from Aldrich. Hydrobromic acid, 1,4-dioxane, and 4-chlorobenzaldehyde were purchased from Merck. Glacial acetic acid, sodium hydroxide, and hydrochloric acid were purchased from Synth. Benzaldehyde and *p*-anysaldehyde were distilled before use; all other reagents were used as received. Thin-layer chromatography (TLC) analyses were performed with precoated aluminum sheets (silica gel 60 Merck), and column chromatography was carried out on silica (200–400 mesh, Merck). Detection was performed by UV inspection (254 nm) for all compounds except for **6g**, which was detected using a 2,4-DNP/H_2SO_4 stain. ^{1}H NMR spectra were recorded at 250, 400, 500, or 600 MHz, and ^{13}C NMR spectra were recorded at 62.5, 100, 125, or 150 MHz. Chemical shifts are reported in ppm relative to tetramethylsilane ($\delta = 0$) in $CDCl_3$, and coupling constants (*J*) are reported in Hz. GC/MS analyses were obtained on an Agilent 6890 Series GC System with a Hewlett-Packard 5973 Mass Selective Detector (70 eV) using a HP-5MS fused silica capillary column (crosslinked 5% phenyl ethyl siloxane, 30 m × 0.25 m ID × 0.25 μm film thickness) and helium as a carrier gas (1 mL min^{-1}). The split ratio was 1:50. The injector temperature was kept 270 °C and detector was kept at 280 °C. The column temperature was held at 60 °C for 3 min, increased to 280 °C at a rate of 25 °C min^{-1}, and then kept at 280 °C for 5 min. The 1 μL of a compound solution (1 mg mL^{-1}) in ethyl acetate was injected and the retention times (min) are reported for all compounds. Chiral GC/FID analyses were obtained on an Agilent 6850 Series GC System, using a Hydrodex



Scheme 2. Preparation of (*R*)-**8a**.

chiral capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness, Methods A and B) or a Chirasil-Dex CB μ-cyclodextrin (30 m × 0.25 mm ID × 0.25 μm film thickness, Method C). Hydrogen was used as a carrier gas (1 mL min^{-1}), the injector temperature was 200 °C, and the detector temperature was 220 °C. Three different methods were employed for the analysis of the bioreduction products. Method A: the column temperature was kept at 80 °C for 3 min, increased to 180 °C at a rate of 3 °C min^{-1}, and then kept at 180 °C for 20 min. Method B: the column temperature was kept at 80 °C for 3 min, increased to 180 °C at a rate of 1 °C min^{-1}, and then kept at 180 °C for 20 min. Method C: the column temperature was kept at 80 °C for 3 min, increased to 130 °C at a rate of 1 °C min^{-1}, then to 180 °C at a rate of 10 °C min^{-1} and kept at 180 °C for 1 min. In all cases, 1 μL of a compound solution (1 mg mL^{-1}) in ethyl acetate was injected; the retention times (min) are reported for all compounds. IR spectra were recorded on a FT-IR BOMEM MB-100 from Hartmann & Braun. Melting points were recorded on a Metler Toledo MP50 Melting Point System. Optical rotation measurements were recorded on a Perkin Elmer 341 polarimeter with a sodium lamp. GC/EI-HRMS analysis of **2g** was performed on a GCT Premier (Waters) GC–MS/TOF using a HP5-MS column (30 m × 0.25 mm ID × 0.25 μm film thickness). The injector temperature was kept at 260 °C and the detector was kept at 280 °C. The column temperature was held at 100 °C for 1 min and then increased to 280 °C at a rate of 10 °C min^{-1}. One μL of a compound solution (1 mg mL^{-1}) in ethyl acetate was injected. ESI-HRMS analysis of **3g** was performed on a Waters Xevo Q-Tof.

4.2. Microorganisms

Saccharomyces cerevisiae CCT 3019, *Saccharomyces boulardii* CCT 3007, *Rhodotorula glutinis* CCT 2182, *Geotrichum candidum* CCT 1205, *Candida albicans* CCT 0776, and *Curvularia lunata* CCT 5628 were purchased from André Tosello Research Foundation.[17] Yeast type II from *Saccharomyces cerevisiae* was purchased from Sigma (Lot BCBG5157V).

4.3. Growth conditions

Microorganisms were grown in a medium containing 10 g L^{-1} of sucrose, 5 g L^{-1} of malt extract, 5 g L^{-1} of yeast extract and 3 g L^{-1} of peptone for 24 h, in an orbital shaker at 30 °C and 180 rpm. Cells were harvested by centrifuging at 3500 rpm for 10 min in the case of *S. cerevisiae*, *S. boulardii*, *R. glutinis,* and *C. albicans*; *G. candidum* and *C. lunata* cells were harvested by filtration.

4.4. General procedure for the Morita–Baylis–Hillman reaction between 3-buten-2-one and aldehydes

3-Buten-2-one (1.82 g, 26 mmol) and aldehyde (20 mmol) were added to a 25 mL round-bottom flask. Next, DABCO (448 mg, 4 mmol) was added, and the mixture was stirred for 48 h. Then, 10% HCl (10 mL) was added to the flask, and the mixture was extracted with ethyl acetate (50 mL). The mixture was washed with 10% HCl, 10% $NaHCO_3$, brine, and then dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by column chromatography.

4.4.1. 4-Hydroxy-3-methylidene-4-phenylbutan-2-one 1a

Following the general procedure for the Morita–Baylis–Hillman reaction, **1a** was obtained in 51% yield as a colorless oil. Retention time on GC/MS: 9.825 min; EI-MS *m*/*z* (%): 176 (23) [M^+], 175 (100), 161 (14), 158 (24), 131 (13), 116 (15), 115 (53), 105 (42), 79 (29), 78 (10), 77 (54), 55 (13), 51 (18); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.32 (s, 3H), 3.18 (d, 1H, *J* = 5.00 Hz), 5.61 (d, 1H, *J* = 5.00 Hz), 5.97 (s, 1H), 6.18 (s, 1H), 7.27–7.35 (m, 5H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 26.5, 72.8, 126.5, 126.6, 127.7, 128.4, 141.6, 150.0, 200.3.

4.4.2. 4-(2′-Chlorophenyl)-4-hydroxy-3-methylidenebutan-2-one 1b

Following the general procedure for the Morita–Baylis–Hillman reaction, **1b** was obtained in 58% yield as a colorless solid. Mp 39.9-41.3 °C; Retention time on GC/MS: 10.644 min; EI-MS *m*/*z* (%): 210/212 (12/4) [M^+], 209/211 (62/22), 176 (12), 175 (100), 173 (43), 149 (14), 141 (31), 139 (52), 131 (12), 116 (12), 115 (32), 111 (21), 103 (14), 77 (58), 75 (23), 70 (16), 55 (17), 51 (19); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.39 (s, 3H), 3.49 (d, 1H, *J* = 4.25 Hz), 5.67 (s, 1H), 5.99 (d, 1H, *J* = 4.25 Hz), 6.16 (s, 1H), 7.20–7.59 (m, 4H);^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.3, 69.0, 127.0, 127.5, 128.2, 128.9, 129.4, 132.6, 138.5, 138.5, 200.7.

4.4.3. 4-(4′-Chlorophenyl)-4-hydroxy-3-methylidenebutan-2-one 1c

Following the general procedure for the Morita–Baylis–Hillman reaction, **1c** was obtained in 63% yield as a colorless oil. Retention time on GC/MS: 10.939 min; EI-MS *m*/*z* (%): 210/212 (14/4) [M^+], 209/211 (88/29), 195 (26), 176 (11), 175 (100), 157 (13), 149 (18), 141 (43), 140 (16), 139 (63), 129 (11), 116 (10), 115 (39), 114 (10), 113 (29), 111 (34), 103 (11), 99 (10), 78 (10), 77 (78), 75 (30), 74 (12), 70 (21), 55 (22), 51 (20); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.34 (s, 3H), 3.09 (d, 1H, *J* = 5.00 Hz), 5.58 (d, 1H, *J* = 5.00 Hz), 5.97 (s, 1H), 6.20 (s, 1H), 7.25–7.31 (m, 4H);^{13}C NMR ($CDCl_3$, 150 MHz) δ 26.4, 72.2, 126.9, 127.9, 128.5, 133.4, 140.1, 149.7, 200.3.

4.4.4. 4-Hydroxy-3-methylidene-4-(3′-nitrophenyl)butan-2-one 1d

Following the general procedure for the Morita–Baylis–Hillman reaction, **1d** was obtained in 68% yield as a colorless solid. Mp 81.2–81.7 °C (lit.[29] 77–78 °C); Retention time on GC/MS: 12.173 min; EI-MS *m*/*z* (%): 221 (6) [M^+], 220 (44), 206 (25), 205 (15), 204 (100), 203 (34), 202 (25), 177 (16), 175 (15), 174 (43), 162 (18), 161 (14), 160 (19), 159 (10), 152 (12), 151 (15), 150 (85), 134 (12), 132 (21), 131 (43), 130 (11), 129 (10), 128 (19), 116 (20), 115 (41), 114 (13), 106 (13), 105 (37), 104 (49), 103 (27), 102 (19), 78 (24), 77 (67), 76 (60), 75 (24), 74 (17), 70 (31), 63 (18), 55 (35), 53 (10), 52 (10), 51 (36); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.36 (s, 3H), 3.40 (d, 1H, *J* = 5.50 Hz), 5.68 (d, 1H, *J* = 5.50 Hz), 6.09 (s, 1H), 6.29 (s, 1H), 7.51 (t, 1H, *J* = 8.00 Hz), 7.73 (d, 1H, *J* = 8.00 Hz), 8.12 (d, 1H, *J* = 8.00 Hz), 8.22 (s, 1H);^{13}C NMR ($CDCl_3$, 125 MHz) δ 26.4, 72.1, 121.4, 122.6, 127.8, 129.3, 132.7, 144.0, 148.3, 149.0, 200.1.

4.4.5. 4-Hydroxy-3-methylidene-4-(4′-nitrophenyl)butan-2-one 1e

Following the general procedure for the Morita–Baylis–Hillman reaction, **1e** was obtained in 72% yield as a colorless solid. Mp 71.1–74.5 °C (lit.[29] 76–77 °C); Retention time on GC/MS: 12.335 min; EI-MS *m*/*z* (%): 220 (16), 206 (16), 205 (13), 204 (100), 202 (11), 177 (11), 175 (13), 174 (76), 150 (35), 132 (11), 131 (14), 116 (14), 115 (19), 105 (15), 104 (27), 103 (15), 78 (14), 77 (33), 76 (25), 75 (12), 63 (10), 55 (21), 51 (18); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.35 (s, 3H), 3.51 (d, 1H, *J* = 5.40 Hz), 5.69 (d, 1H, *J* = 5.40 Hz), 6.07 (s, 1H), 6.28 (s, 1H), 7.55 (d, 1H, *J* = 9.00 Hz), 8.17 (d, 1H, *J* = 9.00 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 26.3, 72.0, 123.6, 127.3, 127.7, 147.3, 149.1, 149.1, 200.1.

4.4.6. 4-Hydroxy-4-(4′-methoxyphenyl)-3-methylidenebutan-2-one 1f

Following the general procedure for the Morita–Baylis–Hillman reaction, **1f** was obtained in 18% yield as a colorless solid. Mp 45.3–

46.0 °C; Retention time on GC/MS: 11.227 min; EI-MS *m*/*z* (%): 206 (22) [M$^+$], 205 (100), 191 (13), 188 (13), 175 (63), 146 (12), 145 (54), 137 (38), 135 (52), 131 (10), 115 (12), 109 (27), 108 (37), 103 (11), 102 (10), 94 (27), 92 (12), 91 (10), 78 (11), 77 (39), 65 (14), 55 (12); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.31 (s, 3H), 3.13 (d, 1H, *J* = 4.75 Hz), 3.78 (s, 3H), 5.56 (d, 1H, *J* = 4.75 Hz), 5.99 (s, 1H), 6.16 (s, 1H), 6.85 (d, 2H, *J* = 8.75 Hz), 7.25 (d, 2H, *J* = 8.75 Hz);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 26.5, 55.2, 72.2, 113.8, 126.1, 127.9, 133.8, 150.3, 159.1, 200.3.

4.4.7. 4-Hydroxy-3-methylidenenonan-2-one 1g

Following the general procedure for the Morita–Baylis–Hillman reaction, **1g** was obtained in 52% yield as a colorless oil. Retention time on GC/MS: 8.753 min; EI-MS *m*/*z* (%): 109 (33), 99 (100), 95 (12), 85 (11), 81 (10), 70 (10), 67 (15), 55 (24); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.85–0.89 (m, 3H), 1.29–1.61 (m, 9H), 2.36 (s, 3H), 4.42 (t, 1H, *J* = 1.25 Hz), 6.00 (s, 1H), 6.10 (s, 1H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 14.0, 22.6, 25.6, 26.5, 31.7, 36.3, 71.5, 125.5, 150.5, 200.8.

4.5. General procedure for the reaction of Morita–Baylis–Hillman adducts 1a–f with concentrated HCl or HBr

In a 10 mL round-bottom flask, 36% HCl or 48% HBr (4 mL) was added to the Morita–Baylis–Hillman adduct (2 mmol) with vigorous stirring. The mixture was stirred for 1 minute (in some cases, a solid mass formed in the first few seconds, and the reaction mixture had to be shaken manually), and then poured into water (50 mL), using ethyl acetate to dissolve the solid mass when necessary. The aqueous phase was extracted with ethyl acetate (3 × 50 mL), the organic layer was washed with 10% $NaHCO_3$, brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by column chromatography.

4.5.1. 3-Chloromethyl-4-phenyl-3-buten-2-one 2a

Following the general procedure for the reaction of **1a** with concentrated HCl, **2a** was obtained in 73% yield as a colorless solid. Mp 46.1–46.7 °C; Retention time on GC/MS: 10.631 min; EI-MS *m*/*z* (%): 194/196 (29/10) [M$^+$], 193/195 (40/16), 159 (10), 158 (17), 143 (10), 116 (28), 115 (100), 89 (11), 63 (10); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.50 (s, 3H), 4.45 (s, 2H), 7.39–7.61 (m, 5H), 7.69 (s, 1H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 25.9, 37.6, 129.0, 129.7, 129.9, 134.2, 137.1, 143.6, 197.4.

4.5.2. 3-Bromomethyl-4-phenyl-3-buten-2-one 3a

Following the general procedure for the reaction of **1a** with concentrated HBr, **3a** was obtained in 82% yield as a colorless solid. Mp 45.9–46.5 °C (lit.[30] 52.5–53.0 °C); Retention time on GC/MS: 11.093 min; EI-MS *m*/*z* (%): 159 (67), 158 (22), 116 (37), 115 (100), 89 (14), 63 (13); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.51 (s, 3H), 4.36 (s, 2H), 7.39–7.64 (m, 5H), 7.64 (s, 1H);^{13}C NMR ($CDCl_3$, 150 MHz) δ 25.2, 26.0, 129.0, 129.7, 129.8, 134.3, 137.4, 142.9, 197.3

4.5.3. 3-Chloromethyl-4-(2′-chlorophenyl)-3-buten-2-one 2b

Following the general procedure for the reaction of **1b** with concentrated HCl, **2b** was obtained in 80% yield as a colorless solid. Mp 51.5–52.0 °C; Retention time on GC/MS: 11.258 min; EI-MS *m*/*z* (%): 193/195 (100/34), 149 (25), 115 (41); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.53 (s, 3H), 4.33 (s, 2H), 7.36–7.74 (m, 4H), 7.82 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.1, 37.4, 127.2, 129.9, 130.2, 130.9, 132.7, 134.3, 138.4, 140.1, 197.2.

4.5.4. 3-Bromomethyl-4-(2′-chlorophenyl)-3-buten-2-one 3b

Following the general procedure for the reaction of **1b** with concentrated HBr, **3b** was obtained in 79% yield as a colorless solid. 61.5–62.5 °C; Retention time on GC/MS: 11.674 min; EI-MS *m*/*z* (%): 237/239 (70/68), 193 (23), 158 (30), 151 (11), 150 (14), 149 (23), 116 (12), 115 (100), 114 (21), 113 (17), 89 (15), 88 (11), 87 (10), 75 (13), 63 (19), 62 (10); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.53 (s, 3H), 4.23 (s, 2H), 7.33–7.79 (m, 5H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 24.6, 26.1, 127.2, 129.7, 129.9, 130.8, 132.8, 134.4, 138.8, 139.3, 197.0.

4.5.5. 3-Chloromethyl-4-(4′-chlorophenyl)-3-buten-2-one 2c

Following the general procedure for the reaction of **1c** with concentrated HCl, **2c** was obtained in 82% yield as a colorless solid. Mp 86.6–87.3 °C (lit.[31] 87–89 °C); Retention time on GC/MS: 11.631 min; EI-MS *m*/*z* (%): 228/230 (23/17) [M$^+$], 227/229 (14/13), 195 (19), 194 (10), 193 (57), 192 (13), 177 (12), 157 (10), 151 (26), 150 (26), 149 (63), 139 (11), 116 (12), 115 (100), 114 (23), 113 (16), 89 (14), 87 (10), 75 (17), 74 (10), 63 (19); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.50 (s, 3H), 4.41 (s, 2H), 7.43–7.56 (m, 4H), 7.63 (s, 1H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 25.9, 37.3, 129.3, 131.0, 132.6, 136.1, 137.5, 142.1, 197.1.

4.5.6. 3-Bromomethyl-4-(4′-chlorophenyl)-3-buten-2-one 3c

Following the general procedure for the reaction of **1c** with concentrated HBr, **3c** was obtained in 87% yield as a colorless solid. Mp 97.8–98.4 °C (lit.[32] 99–100 °C); Retention time on GC/MS: 12.060 min; EI-MS *m*/*z* (%): 195 (24), 194 (11), 193 (79), 192 (11), 151 (12), 150 (25), 149 (25), 139 (11), 116 (13), 115 (100), 114 (22), 113 (15), 89 (15), 75 (14), 63 (19); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.50 (s, 3H), 4.32 (s, 2H), 7.43–7.58 (m, 5H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 24.8, 26.0, 129.3, 131.0, 132.7, 136.0, 137.8, 141.3, 197.0.

4.5.7. 3-Chloromethyl-4-(3′-nitrophenyl)-3-buten-2-one 2d

Following the general procedure for the reaction of **1d** with concentrated HCl, **2d** was obtained in 84% yield as a colorless solid. 133.3–133.8 °C (lit.[33] 130–132 °C); Retention time on GC/MS: 12.762 min; EI-MS *m*/*z* (%): 239 (13) [M$^+$], 224 (26), 204 (15), 188 (19), 180 (11), 179 (13), 161 (25), 151 (11), 150 (13), 149 (28), 143 (18), 116 (14), 115 (100), 114 (25), 113 (11), 89 (22), 65 (10), 63 (23); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.55 (s, 3H), 4.40 (s, 2H), 7.70 (t, 1H, *J* = 8.00 Hz), 7.72 (s, 1H), 7.95 (d, 1H, *J* = 8.00 Hz), 8.29 (d, 1H, *J* = 8.00 Hz), 8.43 (s, 1H);^{13}C NMR ($CDCl_3$, 125 MHz) δ 26.0, 36.8, 124.3, 124.4, 130.2, 135.0, 135.8, 139.3, 140.3, 148.6, 196.7.

4.5.8. 3-Bromomethyl-4-(3′-nitrophenyl)-3-buten-2-one 3d

Following the general procedure for the reaction of **1d** with concentrated HBr, **3d** was obtained in 92% yield as a colorless solid. 127.0–127.9 °C; Retention time on GC/MS: 13.233 min; EI-MS *m*/*z* (%): 207 (18), 205 (10), 204 (100), 162 (14), 161 (14), 158 (51), 116 (14), 115 (82), 114 (21), 89 (26), 88 (11), 82 (11), 79 (10), 77 (11), 75 (11), 63 (21); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.55 (s, 3H), 4.29 (s, 2H), 7.65 (s, 1H), 7.70 (t, 1H, *J* = 8.25 Hz), 7.97 (d, 1H, *J* = 8.25 Hz), 8.28 (d, 1H, *J* = 8.25 Hz), 8.44 (s, 1H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 23.8, 26.1, 124.1, 124.3, 130.2, 134.8, 135.9, 139.3, 139.7, 148.6, 196.6.

4.5.9. 3-Chloromethyl-4-(4′-nitrophenyl)-3-buten-2-one 2e

Following the general procedure for the reaction of **1e** with concentrated HCl, **2e** was obtained in 82% yield as a colorless solid. 126.6–129.6 °C (decomp.; lit.[31] 134–136 °C); Retention time on GC/MS: 12.880 min; EI-MS *m*/*z* (%): 239/241 (3/1) [M$^+$], 224 (37), 223 (10), 222 (73), 203 (10), 192 (24), 188 (11), 162 (12), 161 (28), 158 (14), 157 (15), 150 (13), 143 (33), 116 (13), 115 (100), 114 (16), 103 (10), 89 (19), 63 (19); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.54 (s, 3H), 4.38 (s, 2H), 7.71 (s, 1H), 7.75 (d, 2H, *J* = 8.75 Hz),

8.33 (d, 2H, J = 8.75 Hz);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 26.1, 36.8, 124.1, 130.3, 139.7, 140.2, 140.5, 148.1, 196.7.

4.5.10. 3-Bromomethyl-4-(4′-nitrophenyl)-3-buten-2-one 3e

Following the general procedure for the reaction of **1e** with concentrated HBr, **3e** was obtained in 65% yield as a colorless solid. Mp 132.4–133.6 °C (decomp.; lit.[32] 137 °C); Retention time on GC/MS: 13.359 min; EI-MS m/z 283/285 (2/2) [M$^+$], 207 (14), 204 (23), 162 (43), 161 (16), 159 (13), 158 (100), 145 (11), 143 (13), 116 (15), 115 (67), 114 (14), 89 (21), 77 (13), 63 (20), 51 (12); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.55 (s, 3H), 4.28 (s, 2H), 7.65 (s, 1H), 7.77 (d, 2H, J = 8.75 Hz), 8.34 (d, 2H, J = 8.75 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 23.9, 26.1, 124.1, 130.2, 139.3, 140.1, 140.7, 148.0, 196.6.

4.5.11. 3-Chloromethyl-4-(4′-methoxyphenyl)-3-buten-2-one 2f

Following the general procedure for the reaction of **1f** with concentrated HCl, **2f** was obtained in 69% yield as a colorless solid. Mp 77.6–78.7 °C (lit.[14] 65–67 °C); Retention time on GC/MS: 12.088 min; EI-MS m/z 224/226 (11/4) [M$^+$], 189 (25), 188 (60), 173 (13), 147 (16), 146 (41), 145 (100), 131 (21), 130 (16), 115 (21), 103 (18), 102 (40), 77 (14); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.48 (s, 3H), 3.86 (s, 3H), 4.49 (s, 2H), 6.99 (d, 2H, J = 8.75 Hz), 7.59 (d, 2H, J = 8.75 Hz), 7.64 (s, 1H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 25.8, 38.0, 55.4, 114.5, 126.6, 132.0, 135.0, 143.8, 161.1, 197.3.

4.5.12. 3-Bromomethyl-4-(4′-methoxyphenyl)-3-buten-2-one 3f

Following the general procedure for the reaction of **1f** with concentrated HBr, **3f** was obtained in 82% yield as a colorless solid. Mp 80.9–82.0 °C (lit.[14] 85–86 °C); Retention time on GC/MS: 12.502 min; EI-MS m/z 189 (53), 188 (62), 173 (15), 147 (19), 146 (45), 145 (100), 131 (23), 130 (15), 115 (21), 103 (21), 102 (39), 82 (12), 80 (14), 77 (16), 63 (10), 51 (10); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.48 (s, 3H), 3.87 (s, 3H), 4.40 (s, 2H), 7.01 (d, 2H, J = 8.75 Hz), 7.59 (s, 1H), 7.61 (d, 2H, J = 8.75 Hz);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 25.9, 55.4, 114.6, 126.7, 132.1, 135.2, 143.1, 161.1, 197.2.

4.5.13. 3-Chloromethyl-3-nonen-2-one 2g

Following the general procedure for the reaction of **1g** with concentrated HCl, **2g** was obtained in 68% yield as a colorless oil that decomposes rapidly on standing at room temperature. Retention time on GC/MS: 9.483 min; EI-MS m/z 173 (11), 153 (30), 123 (12), 119 (14), 110 (20), 109 (100), 103 (19), 97 (11), 96 (10), 95 (42), 82 (12), 81 (29), 79 (14), 77 (10), 69 (16), 67 (44), 65 (10), 55 (24), 54 (18), 53 (23); IR (film) 3478, 3411, 2958, 2930, 2860, 1715, 1675, 1639, 1620, 1435, 1380, 1353, 1292, 1265, 1175, 1074, 1023, 967, 882, 728; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.89–1.64 (m, 9H), 2.35 (s, 3H), 2.39 (q, 2H, J = 7.50 Hz), 4.31 (s, 2H), 6.85 (t, 1H, J = 7.50 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 13.9, 22.4, 25.5, 28.2, 29.2, 31.6, 35.5, 138.3, 149.1, 196.9; EI-HRMS (M$^+$) calcd: 188.0968; found: 188.0975.

4.5.14. 3-Bromomethyl-3-nonen-2-one 3g

Following the general procedure for the reaction of **1g** with concentrated HBr, **3g** was obtained in 80% yield as a colorless oil that decomposes rapidly on standing at room temperature. Retention time on GC/MS: 9.967 min; EI-MS m/z (%): 153 (100), 152 (12), 135 (18), 123 (11), 111 (14), 110 (16), 109 (99), 107 (13), 97 (24), 96 (15), 95 (96), 93 (18), 85 (17), 82 (14), 81 (40), 79 (24), 77 (14), 69 (28), 68 (10), 67 (61), 65 (13), 57 (16), 55 (32), 54 (27), 53 (35), 51 (10); IR (film) 3471, 3409, 2956, 2929, 1705, 1672, 1638, 1620, 1465, 1430, 1378, 1352, 1255, 1217, 1154, 977; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.89–1.59 (m, 9H), 2.35 (s, 3H), 2.36 (q, 2H, J = 7.25 Hz), 4.20 (s, 2H), 6.82 (t, 1H, J = 7.25 Hz);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 14.9, 22.4, 22.6, 25.5, 28.0, 29.3, 31.6, 138.4, 148.8, 196.6; ESI$^+$-HRMS ((M+H)$^+$) calcd: 233.0541/235.0521; found: 233.0540/235.0512.

4.6. General procedure for the aldol condensation between butanone and aldehydes

Glacial acetic acid (10 mL), aldehyde (10 mmol), and butanone (20 mmol for the preparation of **4a**–**f**, and 100 mmol for **4g**) were added to a 50 mL round bottom flask. Under stirring, sulfuric acid (2 mL) was added dropwise after which the reaction was stirred at room temperature for 16 hours. Next, the reaction mixture was neutralized with 10% NaOH and extracted with diethyl ether (3 × 50 mL). The organic layer was washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by column chromatography.

4.6.1. 3-Methyl-4-phenyl-3-buten-2-one 4a

Following the general procedure for the aldol condensation, **4a** was obtained in 39% yield as a pale yellow solid. Mp 34.9–36.7 °C (lit.[34] 35–37 °C); Retention time on GC/MS: 9.454 min; EI-MS m/z (%): 160 (70) [M$^+$], 159 (100), 145 (37), 118 (10), 117 (90), 116 (26), 115 (99), 91 (32); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.06 (s, 3H), 2.46 (s, 3H), 7.30–7.42 (m, 5H), 7.52 (s, 1H);^{13}C NMR ($CDCl_3$, 125 MHz) δ 12.9, 25.9, 128.5, 128.6, 129.7, 135.9, 137.8, 139.7, 200.3.

4.6.2. 4-(2′-Chlorophenyl)-3-methyl-3-buten-2-one 4b

Following the general procedure for the aldol condensation, **4b** was obtained in 47% yield as a pale yellow oil. Retention time on GC/MS: 10.184 min; EI-MS m/z (%): 160 (12), 159 (100), 116 (24), 115 (36); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.93 (s, 3H), 2.49 (s, 3H), 7.26–7.46 (m, 5H), 7.62 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 12.9, 25.9, 126.5, 129.6, 129.7, 130.5, 134.0, 134.4, 136.6, 139.2, 200.1.

4.6.3. 4-(4′-Chlorophenyl)-3-methyl-3-buten-2-one 4c

Following the general procedure for the aldol condensation, **4c** was obtained in 58% yield as a colorless solid. Mp 49.4–50.0 °C (lit.[35] 50–51 °C); retention time on GC/MS: 10.591 min; EI-MS m/z (%): 194/196 (30/11) [M$^+$], 193 (28), 181 (14), 179 (40), 176 (10), 159 (52), 151 (27), 141 (20), 139 (13), 116 (71), 115 (100), 89 (14), 75 (12), 63 (14); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.03 (s, 3H), 2.45 (s, 3H), 7.26–7.41 (m, 5H), 7.45 (s, 1H);^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.0, 25.9, 128.7, 131.0, 134.3, 134.5, 138.2, 138.2, 200.0.

4.6.4. 3-Methyl-4-(3′-nitrophenyl)-3-buten-2-one 4d

Following the general procedure for the aldol condensation, **4d** was obtained in 59% yield as a colorless solid. Mp 74.9–75.4 °C; Retention time on GC/MS: 11.744 min; EI-MS m/z (%): 205 (17) [M$^+$], 204 (11), 190 (44), 188 (31), 145 (11), 116 (68), 115 (100), 89 (11), 63 (10); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.07 (s, 3H), 2.49 (s, 3H), 7.53 (s, 1H), 7.618.27 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.1, 26.0, 123.1, 124.2, 129.6, 135.3, 136.4, 137.6, 140.1, 148.3, 199.6.

4.6.5. 3-Methyl-4-(4′-nitrophenyl)-3-buten-2-one 4e

Following the general procedure for the aldol condensation, **4e** was obtained in 47% yield as a colorless solid. Mp 93.1–93.6 °C; Retention time on GC/MS: 11.899 min; EI-MS m/z (%): 205 (8) [M$^+$], 190 (32), 189 (12), 188 (79), 159 (10), 158 (31), 145 (10), 144 (10), 117 (10), 116 (100), 115 (96), 89 (12), 63 (11); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.06 (s, 3H), 2.49 (s, 3H), 7.52 (s, 1H), 7.56 (d, 2H, J = 8.75 Hz), 8.27 (d, 2H, J = 8.75 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.2, 26.0, 123.7, 130.3, 136.5, 140.7, 142.5, 147.3, 199.6.

4.6.6. 4-(4′-Methoxyphenyl)-3-methyl-3-buten-2-one 4f

Following the general procedure for the aldol condensation, **4f** was obtained in 46% yield as a colorless oil. Retention time on GC/MS: 11.064 min; EI-MS m/z (%): 190 (99) [M$^+$], 189 (65), 176 (13), 175 (100), 159 (50), 147 (58), 146 (20), 136 (11), 135 (19), 132 (25), 131 (29), 128 (10), 121 (12), 117 (16), 116 (17), 115

(53), 107 (12), 104 (16), 103 (33), 102 (11), 91 (47), 89 (12), 78 (21), 77 (34), 63 (15), 51 (15); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.07 (s, 3H), 2.44 (s, 3H), 3.84 (s, 3H), 6.94 (d, 2H, J = 8.75 Hz), 7.41 (d, 2H, J = 8.75 Hz), 7.47 (s, 1H);^{13}C NMR ($CDCl_3$, 125 MHz) δ 12.9, 25.8, 55.3, 114.0, 128.5, 131.6, 135.9, 139.6, 159.9, 200.2.

4.6.7. 3-Methyl-3-nonen-2-one 4g

Following the general procedure for the aldol condensation, **4g** was obtained in 36% yield as a colorless oil. Retention time on GC/MS: 8.094 min; EI-MS m/z (%): 154 (16) [M$^+$], 139 (49), 111 (99), 110 (11), 109 (10), 97 (10), 85 (34), 83 (22), 82 (11), 72 (23), 70 (10), 69 (98), 67 (15), 55 (100), 53 (14); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.88–0.94 (m, 3H), 1.25–1.51 (m, 6H), 1.77 (s, 3H), 2.24 (q, 2H, J = 7.25 Hz), 2.30 (s, 3H), 6.63 (t, 1H, J = 7.25 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.1, 14.0, 22.5, 25.4, 28.4, 29.1, 31.6, 137.6, 144.0, 200.0.

4.7. Preparation of 3-methylidene-4-phenylbutan-2-one 5a

An authentic sample of **5a** was prepared following a literature procedure.[15] 4-Phenylbutan-2-one (2.96 g, 20 mmol), 37% aqueous formaldehyde (2 mL), and morpholine (86 μL) were added to glacial acetic acid (10 mL) in a 25 mL round-bottom flask. The mixture was refluxed for 24 h, and then cooled to room temperature and neutralized with 10% aqueous NaOH. The product was extracted with EtOAc (3 × 20 mL), and the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by column chromatography to afford **5a** in 7% yield as a colorless oil. Retention time on GC/MS: 8.822 min; EI-MS m/z (%): 160 (41) [M$^+$], 159 (48), 145 (41), 117 (69), 116 (71), 115 (100), 91 (47), 89 (11), 65 (16), 63 (11), 51 (12); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.34 (s, 3H), 3.59 (s, 2H), 5.64 (s, 1H), 6.08 (s, 1H), 7.15–7.29 (m, 5H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 26.0, 36.7, 126.2, 126.4, 128.5, 129.2, 139.2, 148.7, 199.1.

4.8. General procedure for the bioreduction of enones by *Geotrichum candidum*

Geotrichum candidum cells (10 g of wet cells) were added to distilled water at room temperature (50 mL) in a 125 mL Erlenmeyer flask. The substrate (0.5 mmol) was adsorbed in filter paper (approximately 2 cm^2 per mg of substrate) and added in pieces to the suspension. The reaction was stirred in an orbital shaker at 30 °C and 180 rpm and monitored by GC/MS. After the appropriate amount of time, the reaction mixture was extracted with ethyl acetate (3 × 100 mL), the organic phase was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure. The product was purified by column chromatography. The ee was obtained by chiral GC/FID analysis and the results are provided in Table 3.

4.9. General procedure for the bioreduction of enones by *Saccharomyces cerevisiae*

Yeast from *Saccharomyces cerevisiae*, type II (5.0 g of lyophilized cells), was added to distilled water at 40–42 °C (50 mL) in a 125 mL Erlenmeyer flask. The substrate (0.5 mmol) was adsorbed in filter paper (approximately 2 cm^2 per mg of substrate) and added in small pieces to the cell suspension. The reaction was stirred in an orbital shaker at 30 °C and 180 rpm and monitored by GC/MS. After the appropriate amount of time, the reaction mixture was extracted with ethyl acetate (3 × 100 mL), the organic phase was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure. The product was purified by column chromatography. The ee value was obtained by chiral GC/FID analysis and the results are provided in Table 2.

When the reaction was performed with 10 mmol of **2a**, the reaction mixture was extracted with 100 g of Amberlite® XAD7HP for 2 h, and then the resin was extracted with 3 × 200 mL of ethyl acetate. The solvent was removed under reduced pressure and the residue was purified by column chromatography to afford (*R*)-**6a** in 48% yield and 95% ee.

4.9.1. (*R*)- and (*S*)-3-Methyl-4-phenylbutan-2-one (*R*)- and (*S*)-6a

The reduction of **2a**, following the general procedure for the bioreduction of enones by *Saccharomyces cerevisiae*, afforded (*R*)-**6a** as a colorless oil. $[\alpha]_D^{25} = -20.6$ (c 1.00, $CHCl_3$); lit.[15] $[\alpha]_D^{23} = +40.7$ (c 0.9, $CHCl_3$) for the (*S*)-isomer; Retention time on GC/MS: 8.610 min; Retention time on GC/FID (Method A): 21.90 min ((*R*)-isomer), 22.27 min ((*S*)-isomer); EI-MS m/z (%): 162 (27) [M$^+$], 147 (22), 119 (13), 117 (10), 91 (100); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.09 (d, 3H, J = 7.50 Hz), 2.08 (s, 3H), 2.56 (dd, 1H, J = 7.50, 13.5 Hz), 2.77 (sx, 1H, J = 7.50 Hz), 3.00 (dd, 1H, J = 7.50, 13.5 Hz), 7.13–7.31 (m, 5H);^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.2, 28.9, 38.9, 48.8, 126.3, 128.4, 128.9, 139.7, 212.1. Compound (*R*)-**6a** was also obtained from the reduction of **3a** by *S. cerevisiae* and from the reduction of **2a** and**3a** by *G. candidum*. Its enantiomer, (*S*)-**6a**, was obtained from the reduction of **4a** by *S. cerevisiae*.

4.9.2. (*R*)- and (*S*)-4-(2′-Chlorophenyl)-3-methyl-butan-2-one (*R*)- and (*S*)-6b

The reduction of **2b**, following the general procedure for the bioreduction of enones by *Saccharomyces cerevisiae*, afforded (*R*)-**6b** as a colorless oil. $[\alpha]_D^{25} = -22.5$ (c 0.94, $CHCl_3$); lit.[15] $[\alpha]_D^{21} = +44.9$ (c 1.1, $CHCl_3$) for the (*S*)-isomer; Retention time on GC/MS: 9.637 min; Retention time on GC/FID (Method A): 26.49 min ((*R*)-isomer), 26.78 min ((*S*)-isomer); EI-MS m/z (%): 196 [M$^+$], 181 (14), 161 (70), 127 (35), 125 (100), 117 (12), 115 (24), 91 (10), 89 (13); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.10 (d, 3H, J = 7.20 Hz), 2.12 (s, 3H), 2.66 (dd, 1H, J = 7.20, 13.6 Hz), 2.97 (sx, 1H, J = 7.20 Hz), 3.13 (dd, 1H, J = 7.20, 13.6 Hz), 7.15–7.38 (m, 4H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 16.1, 28;9, 36.5, 46.5, 126.7, 127.9, 129.6, 131.6, 134.1, 137.4, 211.8. Compound (*R*)-**6b** was also obtained from the reduction of **3b** by *S. cerevisiae*. Its enantiomer, (*S*)-**6b**, was obtained from the reduction of **4b** by *S. cerevisiae*.

4.9.3. (*R*)- and (*S*)-4-(4′-Chlorophenyl)-3-methyl-butan-2-one (*R*)- and (*S*)-6c

The reduction of **2c**, following the general procedure for the bioreduction of enones by *Saccharomyces cerevisiae*, afforded (*R*)-**6c** as a colorless oil. $[\alpha]_D^{25} = -25.0$ (c 1.60, $CHCl_3$); lit.[15] $[\alpha]_D^{23} = +27.7$ (c 0.94, $CHCl_3$) for the (*S*)-isomer; Retention time on GC/MS: 9.905 min; Retention time on GC/FID (Method A): 30.35 min ((*R*)-isomer), 30.66 min ((*S*)-isomer); EI-MS m/z (%): 196/198 (24/8) [M$^+$], 181 (22), 153 (10), 127 (34), 125 (100), 89 (12); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.09 (d, 3H, J = 7.20 Hz), 2.08 (s, 3H), 2.53 (dd, 1H, J = 7.20, 13.6 Hz), 2.78 (sx, 1H, J = 7.20 Hz), 2.97 (dd, 1H, J = 7.20, 13.6 Hz), 7.07–7.27 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.3, 28.9, 38.1, 48.7, 128.5, 130.3, 132.1, 138.2, 211.7. Compound (*R*)-**6c** was also obtained from the reduction of **3c** by *S. cerevisiae* and from the reduction of **2c** and **3c** by *G. candidum*. Its enantiomer, (*S*)-**6c**, was obtained from the reduction of **4c** by *S. cerevisiae* and by *G. candidum*.

4.9.4. (*R*)- and (*S*)-3-Methyl-4-(3′-nitrophenyl)butan-2-one (*R*)- and (*S*)-6d

The reduction of **2d**, following the general procedure for the bioreduction of enones by *Saccharomyces cerevisiae*, afforded (*R*)-**6d** as a colorless oil. $[\alpha]_D^{25} = -4.9$ (c 1.50, $CHCl_3$); lit.[15] $[\alpha]_D^{23} = +3.9$ (c 1.1, $CHCl_3$) for (*S*)-isomer; Retention time on GC/MS: 11.228 min; Retention time on GC/FID (Method B):

91.58 min ((*R*)-isomer), 92.08 min ((*S*)-isomer); EI-MS *m*/*z* (%): 192 (8), 148 (38), 147 (100), 136 (48), 120 (33), 118 (15), 117 (42), 115 (32), 91 (22), 90 (32), 89 (23), 77 (14), 63 (12); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.15 (d, 3H, J = 7.00 Hz), 2.12 (s, 3H), 2.66 (dd, 1H, J = 7.20, 13.6 Hz), 2.97 (sx, 1H, J = 7.20 Hz), 3.13 (dd, 1H, J = 7.20, 13.6 Hz), 7.15–7.38 (m, 4H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 16.5, 28.8, 38.0, 48.4, 121.5, 123.7, 129.3, 135.4, 141.9, 148.4, 210.8. Compound (*R*)-**6d** was also obtained from the reduction of **3d** by *S. cerevisiae* and from the reduction of **2d** and **3d** by *G. candidum*. Its enantiomer, (*S*)-**6d**, was obtained from the reduction of **4d** by *S. cerevisiae* and by *G. candidum*.

4.9.5. (*R*)- and (*S*)-3-Methyl-4-(4′-nitrophenyl)butan-2-one (*R*)- and (*S*)-6e

The reduction of **2e**, following the general procedure for the bioreduction of enones by *Saccharomyces cerevisiae*, afforded (*R*)-**6e** as colorless crystals of mp 52.5–55.6 °C (lit.[36] 64 °C); $[\alpha]_D^{25} = -10.7$ (*c* 1.45, $CHCl_3$); lit.[15] $[\alpha]_D^{23} = +10.9$ (*c* 1.1, $CHCl_3$) for the (*S*)-isomer; Retention time on GC/MS: 11.433 min; Retention time on GC/FID (Method A): 45.88 min ((*R*)-isomer), 46.27 min ((*S*)-isomer); EI-MS *m*/*z* (%): 207 (53) [M$^+$], 192 (40), 166 (10), 165 (100), 148 (29), 136 (32), 120 (24), 118 (22), 117 (26), 116 (13), 115 (33), 106 (13), 91 (22), 90 (25), 89 (26), 78 (18), 77 (14), 63 (11); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.15 (d, 3H, J = 7.00 Hz), 2.13 (s, 3H), 2.68 (dd, 1H, J = 7.00, 13.5 Hz), 2.88 (sx, 1H, J = 7.00 Hz), 3.13 (dd, 1H, J = 7.00, 13.5 Hz), 7.33 (d, 2H, J = 8.50 Hz), 8.14 (d, 2H, J = 8.50 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.6, 28.8, 38.3, 48.4, 123.7, 129.9, 146.6, 147.8, 210.8. Compound (*R*)-**6e** was also obtained from the reduction of **3e** by *S. cerevisiae* and from the reduction of **2e** and **3e** by *G. candidum*. Its enantiomer, (*S*)-**6e**, was obtained from the reduction of **4e** by *S. cerevisiae* and by *G. candidum*.

4.9.6. (*S*)-4-(4′-Methoxyphenyl)-3-methyl-butan-2-one (*S*)-6f

The reduction of **4f**, following the general procedure for the bioreduction of enones by *Saccharomyces cerevisiae*, afforded (*S*)-**6f** as a colorless oil. $[\alpha]_D^{25} = +12.1$ (*c* 1.59, $CHCl_3$); lit.[15] $[\alpha]_D^{23} = +42.1$ (*c* 1.1, $CHCl_3$) for the (*S*)-isomer; Retention time on GC/MS: 10.203 min; Retention time on GC/FID (Method A): 32.02 min ((*R*)-isomer), 32.22 min ((*S*)-isomer); EI-MS *m*/*z* (%): 192 (11) [M$^+$], 121 (100); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.07 (d, 3H, J = 6.80 Hz), 2.08 (s, 3H), 2.51 (dd, 1H, J = 6.80, 13.6 Hz), 2.79 (sx, 1H, J = 6.80 Hz), 2.93 (dd, 1H, J = 6.80, 13.6 Hz), 3.78 (s, 3H) 6.80–7.09 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.2, 28.9, 38.1, 49.0, 55.2, 113.8, 129.9, 131.7, 158.1, 212.4. Compound (*S*)-**6f** was also obtained, in low ee, from the reduction of **4f** by *G. candidum*.

4.9.7. (*R*)- and (*S*)-3-Methylnonan-2-one (*R*)- and (*S*)-6g

The reduction of **4g**, following the general procedure for the bioreduction of enones by *Saccharomyces cerevisiae*, afforded (*S*)-**6g** as a colorless oil. $[\alpha]_D^{25} = +6.0$ (*c* 1.01, $CHCl_3$); lit.[18] $[\alpha]_D^{20} = -17.1$ (*c* 3.2, $CHCl_3$) for the (*R*)-isomer. Retention time on GC/MS: 7.522 min; Retention time on GC/FID (Method A): 14.11 min ((*R*)-isomer), 14.34 min ((*S*)-isomer); EI-MS *m*/*z* (%): 156 (2) [M$^+$], 85 (10), 72 (100), 71 (16), 57 (25), 55 (10); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.85–0.90 (m, 3H), 1.08 (d, 3H, J = 7.00 Hz), 1.09–1.30 (m, 11H), 2.13 (s, 3H), 2.36–2.57 (m, 1H);^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 14.1, 16.2, 22.6, 27.2, 28.0, 29.4, 31.7, 33.0, 47.3, 212.9. Its enantiomer, (*R*)-**6g** was obtained from the reduction of **2g** and **3g** by *S. cerevisiae*.

4.10. Preparation of (*R*)-2-methyl-3-phenylpropanoic acid (*R*)-8a

A solution of sodium hypobromite was prepared by dropwise addition of bromine (681 μL, 13.2 mmol) to a solution of sodium hydroxide (52 mmol) in water (20 mL) in an ice/salt bath. After all the bromine was consumed, the bright yellow solution was diluted with 1,4-dioxane (5 mL), and added dropwise to a cooled (cold water bath) solution of (*R*)-**6a** (95% ee, 648 mg, 4 mmol) in dioxane (50 mL) and water (10 mL). The mixture was stirred for 2 h. Next, most of the dioxane/water mixture was evaporated under reduced pressure, the residue was diluted in water (40 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The aqueous phase was acidified, and then extracted with CH_2Cl_2 (3 × 20 mL). The organic phase from the second extraction was dried over Na_2SO_4 and evaporated under reduced pressure to afford (*R*)-**8a** in 61% yield, and 92% ee as a colorless oil. $[\alpha]_D^{25} = -20.8$ (*c* 0.87, $CHCl_3$); lit.[37] $[\alpha]_D^{25} = -22.7$ (*c* 1.02, $CHCl_3$) for the (*R*)-isomer. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.18 (d, 3H, J = 7.00 Hz), 2.67 (dd, 1H, J = 8.00, 13.5 Hz), 2.77 (sx, 1H, J = 7.00 Hz), 3.08 (dd, 1H, J = 6.50, 13.5 Hz), 7.17–7.31 (m, 5H);^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.5, 39.3, 41.2, 126.4, 128.4, 129.0, 139.0, 182.5.

4.11. Methyl 2-methyl-3-phenylpropanoate (methyl ester of 8a)

A solution of diazomethane in diethyl ether was added to a sample of acid **8a** in diethyl ether. The organic solvent was evaporated to afford methyl ester of **8a** as a colorless oil. Retention time on GC/MS: 8.317 min; EI-MS *m*/*z* (%): 178 (16) [M$^+$], 119 (15), 118 (47), 117 (14), 91 (100), 65 (10); Retention time on GC/FID (Method C): 28.41 min ((*R*)-isomer), 28.76 min ((*S*)-isomer); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.15 (d, 3H, J = 6.50 Hz), 2.66 (dd, 1H, J = 7.50, 13.5 Hz), 2.74 (sx, 1H, J = 6.50 Hz), 3.03 (dd, 1H, J = 6.50, 13.5 Hz), 3.63 (s, 3H), 7.14–7.29 (m, 5H);^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.8, 39.7, 41.4, 51.6, 126.3, 128.4, 129.0, 139.4, 176.6.

Acknowledgements

This work was supported by FAPESP and CNPq.

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