Regio- and Stereo-selective Bioreduction of Diketo-*n*-butylphosphonate by Baker's Yeast[+†]

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A regio- and stereo-selective reduction of diketo-*n*-butylphosphonates by baker's yeast was reported. The chemical yield and *ee* value of these reactions are highly dependent on the structure of substrates. The resulting optical active hydroxyalkanephosphonates can be used as chirons for the synthesis of polyfunctional organophosphorus compounds. As useful building block, a series of α,β-unsaturated ketones bearing chiral hydroxy group in addition to trifluoromethyl moiety was prepared via the Horner-Wadsworth-Emmons (HWE) reaction of the biotransformation products.

Keywords biotransformation, baker's yeast, diketo-*n*-butylphosphonate, regio- and stereo-selective reduction

Introduction

Baker's yeast (*Saccharomyces cerevisiae*) is well recognized as a valuable stereoselective reagent in biotransformations of organic molecules. The asymmetric reduction of carbonyl groups with this microbe has been studied extensively. Moreover, in the course of reduction of diketocompounds in which the carbonyl groups are located either in the vicinity or separated by one or more methylene groups, stereoselectivity is usually associated with regioselectivity in such case. As a result, either just one or both carbonyl groups may be reduced.[1-3] Although the reduction of acyclic 1,2-diketones and 1,3-diketones by baker's yeast is well documented, to the best of our knowledge, there are no reports related to corresponding phosphonates. On the other hand, chiral hydroxyalkylphosphonic acids have received much attention due to their unique physiological activities as well as their ability to mimic the hydroxy- or aminoalkanecarboxylic acids.[4] In addition to this, it is much interesting to prepare non-racemic 3(or 4)-hydroxy-2-ketoalkylphosphonates and to look at their behaviour in Horner-Wadsworth-Emmons (HWE) reaction.[5]

As a part of our study on the biotransformation of organic compounds,[6,7] the bioreductions of 2,3-diketoalkylphosphonates and 2,4-diketoalkylphosphonates by baker's yeast were studied. In addition, optically active 3(or 4)-hydroxy-2-keto-alkylphosphonates can be regarded as useful chirons for the preparation of optically active α,β-unsaturated ketones.

Results and discussion

The 2,3-diketo-*n*-propylphosphonate (**1**) was prepared by reaction of methylphosphonate and pyruvic acid as detailed in the literature.[8] Bioreduction of 2,3-diketo-*n*-propylphosphonate (**1**) with baker's yeast in the usual manner, that is in aqueous medium was failed due to the instability of the substrate in water.

However, the reduction of diethyl 2,3-diketo-*n*-propylphosphonate (**1**), mediated by dried baker's yeast proceeded smoothly in organic solvents containing a small amount of water. Hexane, petroleum ether, ethyl acetate and isopropyl ether were used as organic solvent (Scheme 1).

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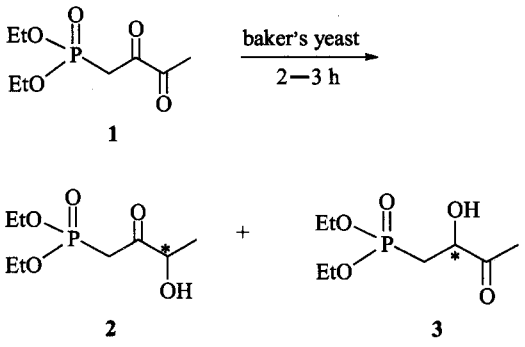
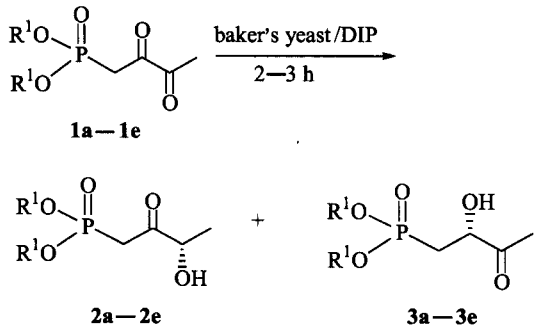
Scheme 1



Scheme 2



As shown in Table 1, a small amount of water (0.6 mL of water per gm of yeast) was required for the reaction. The water/yeast/ solvent ratio and the solvent polarity have shown remarkable influence on the reactivity of the system. It is important to point out that in diisopropyl ether, the time required for such biotransformation is significantly short. Diisopropyl ether could increase the turnover rate markedly. Two isomers, 2-keto-3-hydroxy-*n*-butylphosphonate (**2**) and 2-hydroxy-3-keto-*n*-propylphosphonate (**3**) were formed, and the former was the major product. These two compounds were easily separated by column chromatography.

Table 1 Reduction of **1** with baker's yeast in organic solvent[a]

Organic solvent	Conversion rate (%)	Time (h)
Hexane	30	5
Petroleum ether	30	5
Ethyl acetate	100	3
Diisopropyl ether	100	0.5

[a] Reaction condition: baker's yeast 2 g, organic solvent 20 mL, water 1.2 g, substrate 0.5 mmol.

As shown in Table 2, a series of 2,3-diketo-*n*-butylphosphonates (**1a—1e**) was reduced with dried bakers' yeast in diisopropyl ether containing a small amount of water. The reduction proceeded smoothly to give the corresponding 2-keto-3-hydroxy-*n*-butylphosphonates (**2a—2e**) and 2-hydroxy-3-keto-*n*-butylphosphonates (**3a—3e**) in moderate yields and high *ee* value. The major products 2-keto-3-hydroxy-*n*-butyl-phosphonates can be used in HWE reaction.

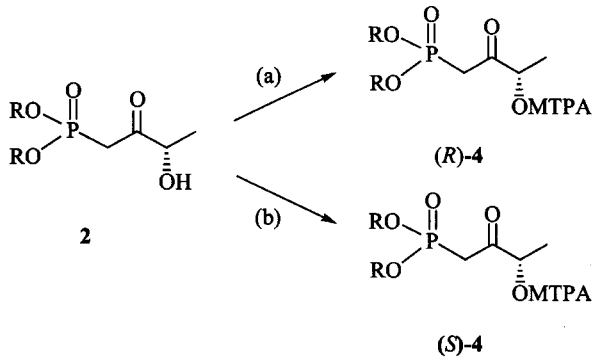
The absolute configuration of the resulting 2-keto-3-hydroxy-*n*-butylphosphonates (**2a—2e**) was preliminarily assigned on the basis of $\Delta\delta$ values in ^{1}H NMR spectra of their α-methoxy-α-trifluoromethylphenyl-acetic acid (MTPA) esters using the modified Mosher's method (Scheme 3).[9] As shown by us, this bioreduction with baker's yeast obeys Prelog's rule.[10]

Table 2 Reduction of **1a—1e** with baker's yeast in diisopropyl ether system[a]

Substrate	R^1	Compound **2**			Compound **3**	
		Yield (%)	*ee*[b] (%)	Config.[b]	Yield (%)	*ee*[b] (%)
1a	Me	44	91	—	—	—
1b	Et	58	90	*S*	17	55
1c	*i*-Pr	53	81	—	23	34
1d	*n*-Pr	49	83	*S*	15	55
1e	*n*-Bu	42	87	*S*	21	45

[a] Reaction condition: baker's yeast 10 g, organic solvent 100 mL, water 6 mL, substrate 2—5 mmol. [b] the *ee* value and absolute configuration were determined according to Mosher's methods.[9]

Scheme 3



(a) (*R*)-(+)-MTPA, DCC, DMAP, CH_2Cl_2, r.t., 1—2 days.
(b) (*S*)-(-)-MTPA, DCC, DMAP, CH_2Cl_2, r.t., 1—2 days.

Table 3 $\Delta\delta$ values in ^{1}H NMR spectra of compound **4**

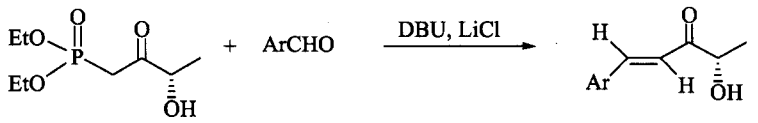
Compound	δ_S (CH_3CHOH)	δ_R (CH_3CHOH)	$\Delta\delta$ ($\delta_S - \delta_R$)	Config.
4b	1.55	1.48	0.07 (>0)	*S*
4d	1.56	1.52	0.04 (>0)	*S*
4e	1.53	1.49	0.04 (>0)	*S*

The optically active 2-keto-3-hydroxy-*n*-butylphosphonates can be used as chirons in HWE reaction to give chiral α,β-unsaturated ketones (Scheme 4). Under mild HWE reaction using DBU as base and LiCl at room temperature,[11] a series of α,β-unsaturated ketones **5** containing optical active hydroxy group on α'-position was prepared conveniently.

The 2,4-diketoalkylphosphonates (**6**) was prepared by reaction of 2-keto-*n*-propylphosphonate and ethyl acetate or ethyl trifluoro acetate according to the literature method (Scheme 5),[12] and the substrates **6** thus obtained are stable in aqueous medium.

2,4-Diketoalkylphosphonates can be reduced to optically pure hydroxyalkylphosphonates. When R was methyl group, the reduction proceeded smoothly to give the corresponding 4-hydroxy-2-keto-*n*-pentylphosphonate (**7a**) and 4-keto-2-hydroxy-*n*-pentylphosphonate (**8a**) in moderate yields, the former was the major product, and the *ee* value was 55%, as estimated by Mosher's methods.[9] Meanwhile, these two isomers were easily separated by column chromatography. However, when R was trifluoromethyl group, it is interesting to note that a regio and stereo selective reduction occurred. The reduction proceeded smoothly to give the corresponding 5,5,5-trifluoro-4-hydroxy-2-keto-*n*-pentylphosphonate (**7b**) in 41% yield and 91% *ee*. The nonracemic **7b** was important as synthetic chiron that was directly subjected to HWE reaction to give chiral α,β-unsaturated ketones. Under mild HWE reduction using DBU as base at room temperature, thus a series of compounds **9** bearing chiral hydroxy group in addition to trifluoromethyl moiety was prepared conveniently.

Scheme 4

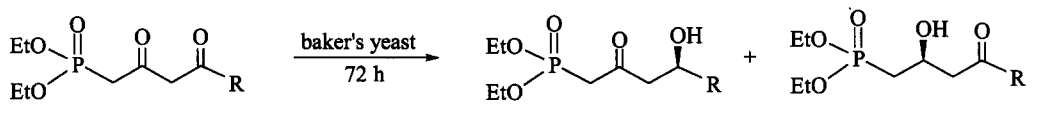


2 **5**

a: Ar = 4-NO_2Ph
b: Ar = 2,4-Cl_2Ph
c: Ar = Ph

a: Ar = 4-NO_2Ph, Yield 66%
b: Ar = 2,4-Cl_2Ph, Yield 44%
c: Ar = Ph, Yield 56%

Scheme 5

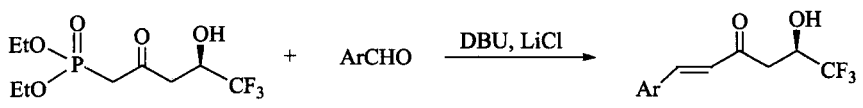


6a, 6b **7a, 7b** **8a**

a: R = Me, Yield 43%
b: R = CF_3, Yield 41%

a: R = Me, Yield 5%

Scheme 6



7b **9a—9c**

a: Ar = 4-MePh
b: Ar = 4-NO_2Ph
c: Ar = 4-ClPh

a: Ar = 4-MePh, Yield 66%
b: Ar = 4-NO_2Ph, Yield 71%
c: Ar = 4-ClPh, Yield 68%

The chiral 5,5,5-trifluoro-4-hydroxy-2-keto-*n*-pentylphosphonate (**7b**) thus obtained underwent Horner Emmons olefination as expected. Consequently a series of *trans*-6,6,6-trifluoro-5-hydroxy-1-substituted phenyl-1-hexen-3-ones (**9a**—**9c**) was prepared by reaction of 5,5,5-trifluoro-4-hydroxy-2-keto-*n*-pentylphosphonate (**7b**) with substituted benzaldehyde under mild condition in 66%—71% yields. The absolute configuration of 6,6,6-trifluoro-5-hydroxy-1-(4-methyl-phenyl)-1-hexen-3-one (**9a**) was illustrated by X-ray diffraction analyses (Fig. 1). Details of crystal data and structure refinement for compound **9a** are listed in Table 3.

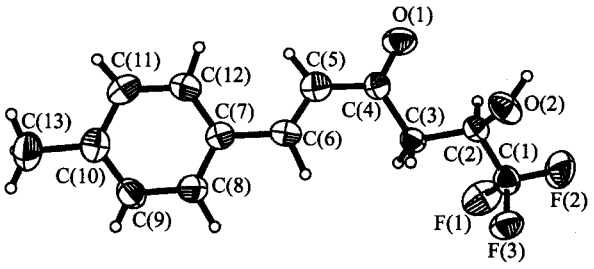


Fig. 1 X-Ray structure of compound **9a**.

In summary, a regio and stereoselective reduction of 2,3-diketo-*n*-butyl-phosphonates and 2,4-diketo-*n*-butylphosphonates was observed, and the chemical yield and *ee* value of the reaction were influenced significantly by the substituents. The optically active hydroxy phosphonates can be usesd as chirons in the synthesis of polyfunctional compounds or in the HWE reaction.

Experimental

IR spectra were recorded on a Shimadzu IR-440 spectrometer. EI mass spectra (MS) were run on an HP-5989A mass spectrometer. ^1H and ^{13}P NMR spectra were recorded on a Bruker AMX-330 (300 MHz) spectrometer in $CDCl_3$ solution and chemical shifts (δ) were reported in ppm downfield relative to TMS (internal standard) and 80% phosphoric acid (external standard) in phosphorus spectra. Baker's yeast was purchased from Sigma Co.. X-Ray data were collected on a Rigaku AFC7R single crystal diffractometer.

Spots in TLC monitoring were visualized by dipping the plate into a solution of 24 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 1 g of $Ce(SO_4)_2\cdot 4H_2O$ in 500 mL of 10% H_2SO_4 in water, followed by heating with a hot gun.

Table 4 Crystal data and structure refinement for compound **9a**

Molecular formula	$C_{13}H_{13}F_3O_2$
Formula weight	258.23
Crystal system	Monoclinic
Space group	$F2(1)$
Unit cell dimensions	$a = 0.86860(11)$ nm, $\alpha = 90°$
	$b = 0.52806(7)$ nm, $\beta = 107°$
	$c = 1.43882(19)$ nm, $\gamma = 90°$
V	0.63056(14) nm^3
Z	2
Density (calculated)	1.360 mg/mm^3
Absorption coefficient	0.120 mm^{-1}
$F(000)$	268
θ (°)	1.48 to 28.36
Reflections collected	3889
Independent reflections	2584/(R(int) = 0.0320)
Absorption correction	Sadabs
Range of relative transm. factors	1.0000, 0.6700
Final R indices $I > 2\sigma$ (I)	$R_1 = 0.0388$, $wR_2 = 0.0574$
Goodness-of-fit on F	0.740
Largest difference peak and hole	1.70×10^{-4} and -1.08×10^{-4} e/nm^3

General procedure for the biotransformation of diketo-n-alkylphosphonate with baker's yeast

A mixture of baker's yeast (10 g) in diisopropyl ether (100 mL), and water (6 mL) was stirred for 10 min, then diketoalkylphosphonate (**1a**—**1e**) (1.70—4.50 mmol) was added and the mixture was shaken at 30 ℃. The process of the reduction was monitored by TLC. Finally the mixture was filtered and the filtrate was concentrated under diminished pressure.

Synthesis of **2a**

Compound **1a** (330 mg, 1.70 mmol) was added to a stirred mixture of baker's yeast in diisopropyl ether containing a small amount of water as described on the general procedure, and the mixture was shaken at 30 ℃. After 50 min, the reaction was completed as monitored by TLC. Upon work-out as described, the product **2a** was separated by silica gel column chromatography using petroleum ether/ethyl acetate (3/1) as eluent.

Dimethyl 2-keto-3-hydroxybutylphosphonate (**2a**) Colorless liquid, yield 145 mg (44%), *ee* 91%, $[\alpha]_D^{20}$ 11.7 (*c* 0.7, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ: 4.35 (q, J = 7.1 Hz, 1H, C**H**OH), 3.82 (d, J = 4 Hz, 6H, 2 × OCH_3), 3.40—3.25 (m, 2H, PCH_2), 2.82 (OH), 1.38 (d, J = 7.0 Hz, 3H, $CHCH_3$). ^{31}P NMR (160 MHz, $CDCl_3$) δ: 23.955; IR (film) ν_{max}: 3374, 1720, 1201, 1038, 832 cm^{-1}; MS *m/z* (rel. intensity): 179 (M^+ − OH, 1.99), 151 (36.75), 124 (100), 109 (46.63), 94 (91.87), 79 (58.90), 45 (24.65). Anal. calcd for $C_6H_{13}O_5P$ (196.14): C 36.74, H 6.68; found C 36.53, H 6.76.

Syntheses of **2b** *and* **3b**

Compound **1b** (1.0 g, 4.50 mmol) was added to a stirred mixture of baker's yeast in diisopropyl ether containing a small amount of water as described on the general procedure, and the mixture was shaken at 30 ℃. After 50 min, the reaction was completed as monitored by TLC. Upon work-out as described, the products **2b** and **3b** were separated by silica gel column chromatography using petroleum ether/ethyl acetate (1/1) as eluent.

Diethyl 2-keto-3(S)-hydroxy-n-butylphosphonate (**2b**) Colorless liquid, yield 580 mg (58%), *ee* 90%, $[\alpha]_D^{20}$ 17 (*c* 0.7, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ: 4.30 (q, J = 7.0 Hz, 1H, C**H**OH), 4.19—4.13 (m, 4H, 2 × OCH_2CH_3), 3.85 (OH), 3.33 (m, 2H, PCH_2), 1.51—1.33 (m, 9H, 2 × OCH_2CH_3 + $CHCH_3$); ^{31}P NMR (160 MHz, $CDCl_3$) δ: 21.110; IR (film) ν_{max}: 3353 (OH), 2986, 2936, 1720 (s), 1242, 1026, 972, 811 cm^{-1}; MS *m/z* (rel. intensity): 207 (M^+ − OH, 0.71), 197 (2.73), 179 (17.36), 152 (49.45), 125 (100), 97 (43.01). Anal. calcd for $C_8H_{17}O_5P$: C 42.86, H 7.64; found C 42.84, H 7.32.

Diethyl 2-hydroxy-3-keto-n-butylphosphonate (**3b**) Colorless liquid, yield 170 mg (17%), *ee* 57%, $[\alpha]_D^{20}$ − 4.6 (*c* 0.85, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ: 4.50—4.42 (m, 1H, C**H**OH), 4.26—4.17 (m, 4H, 2 × OCH_2CH_3), 3.30 (OH), 2.46—2.02(m, 2H, PCH_2), 2.33 (s, 3H, $COCH_3$), 1.40 (t, J = 7.1 Hz, 6H, 2 × OCH_2CH_3); IR (film) ν: 3318 (OH), 2987, 2943, 1720 (s), 1395, 1357, 1226 (s), 1029 (s) cm^{-1}; MS *m/z* (rel. intensity): 224 (M^+, 0.54), 179 (12.91), 152 (76.77), 125 (100), 97 (69.88), 79 (33.03), 43 (18.63). Anal. calcd for $C_8H_{17}O_5P$: C 42.86, H 7.64; found C 43.04, H 7.61.

Syntheses of **2c** *and* **3c**

Compound **1c** (500 mg, 2 mmol) was added to a stirred mixture of baker's yeast in diisopropyl ether containing a small amount of water as described on the general procedure, and the mixture was shaken at 30 ℃. After 45 min, the reaction was completed as monitored by TLC. Upon work-out as described, the products **2c** and **3c** were separated by silica gel column chromatography using petroleum ether/ethyl acetate (2/3) as eluent.

Diisopropyl 2-keto-3-hydroxy-n-butylphosphonate (**2c**) Colorless liquid, yield 264 mg (53%), *ee* 85%, $[\alpha]_D^{20}$ 14 (*c* 0.9, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$) δ: 4.77—4.66 (m, 2H, 2 × $OCH(CH_3)_2$), 4.32 (q, J = 7.0 Hz, 2H, 2 × C**H**OH), 3.73 (OH, 1H), 3.44—3.20 (m, 2H, PCH_2), 1.40 (d, J = 7.0 Hz, 3H, $CHCH_3$), 1.35 (d, J = 4 Hz, 12H, 2 × $OCH(CH_3)_2$); ^{31}P NMR (160 MHz, $CDCl_3$) δ: 19.129; IR (film) ν: 3367 (OH), 2983, 2937, 1721 (s), 1388, 1377, 1234, 1105, 997 cm^{-1}; MS *m/z* (rel. intensity): 253(M^+ + 1, 0.50), 209 (5.17), 180 (19.01), 151 (59.28), 123 (60.06), 96 (100),

43 (44.12). Anal. calcd for $C_{10}H_{21}O_5P$: C 47.62, H 8.39; found C 47.81, H 8.28.

Diisopropyl 2-hydroxy-3-keto-n-butylphosphonate (**3c**) Colorless liquid, yield 113 mg (23%), *ee* 33%, $[\alpha]_D^{20}$ −4 (*c* 1, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$) δ: 4.77—4.66 (m, 2H, 2 × OC**H**$(CH_3)_2$), 4.44—4.38 (m, 1H, C**H**OH), 2.29 (s, 3H, $COCH_3$), 2.40—1.98 (m, 2H, PCH_2), 1.78 (d, J = 2 Hz, 12H, 2 × OCH(CH_3)$_2$); ^{31}P NMR (160 MHz, $CDCl_3$) δ: 26.943. Anal. calcd for $C_{10}H_{21}O_5P$: C 47.62, H 8.39; found C 47.77, H 8.32.

Syntheses of **2d** *and* **3d**

Compound **1d** (500 mg, 2 mmol) was added to a stirred mixture of baker's yeast in diisopropyl ether containing a small amount of water as described on the general procedure, and the mixture was shaken at 30 ℃. After 45 min, the reaction was completed as monitored by TLC. Upon work-out as described, the products **2d** and **3d** were separated by silica gel column chromatography using petroleum ether/ethyl acetate (2/3) as eluent.

Di-n-propyl 2-keto-3(S)-hydroxy-n-butylphosphonate (**2d**) Colorless liquid, yield 245 mg (49%), *ee* 83%, $[\alpha]_D^{20}$ 9.2 (*c* 0.6, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$) δ: 4.31 (q, J = 7.5 Hz, 1H, C**H**(OH)CH_3), 4.02—3.97 (m, 4H, 2 × OC**H**$_2$$CH_2CH_3$), 3.52—3.18 (m, 2H, PCH_2), 1.69—1.62 (m, 4H, 2 × OCH_2C**H**$_2$$CH_3$), 1.35 (d, J = 7.2 Hz, 3H, CH(OH)C**H**$_3$), 0.90 (t, J = 9.2 Hz, 6H, 2 × OCH_2CH_2C**H**$_3$); IR (film) ν: 2971, 2883, 1730 (s), 1464, 1392, 1372, 1249 (s), 1002 (s), 894 cm^{-1}; MS *m/z* (rel. intensity): 253 (M^+ + 1, 0.50), 180 (9.06), 151 (13.51), 139 (62.65), 123 (41.07), 97 (100). Anal. calcd for $C_{10}H_{21}O_5P$: C 47.62, H 8.39; found C 47.38, H 8.85.

Di-n-propyl 2-hydroxy-3-keto-n-butylphosphonate (**3d**) Colorless liquid, yield 122 mg (25%), *ee* 55%, $[\alpha]_D^{20}$ −6.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ: 4.50—4.25 (m, 1H, C**H**OH), 4.15—4.01 (m, 4H, 2 × OC**H**$_2$$CH_2CH_3$), 2.46—2.06 (m, 2H, PCH_2), 2.32 (s, 3H, $COCH_3$), 1.78—1.68 (m, 4H, 2 × OCH_2C**H**$_2$$CH_3$), 1.04—0.94 (m, 6H, 2 × OCH_2CH_2C**H**$_3$); IR (film) ν: 3325, 2972, 1721 (s), 1226 (s), 1097, 1004 (s), 863, 504 cm^{-1}; MS *m/z* (rel. intensity): 253 (M^+ + 1, 1.83), 209 (15.04), 167 (14.96), 151 (8.04), 125 (100), 107 (12.54), 43 (50.12). Anal. calcd for $C_{10}H_{21}O_5P$: C 47.62, H 8.39; found C 47.95, H 8.81.

Syntheses of **2e** *and* **3e**

Compound **1e** (500 mg, 1.77 mmol) was added to a stirred mixture of baker's yeast in diisopropyl ether containing a small amount of water as described on the general procedure, and the mixture was shaken at 30 ℃. After 1 h, the reaction was completed as monitored by TLC. Upon work-out as described, the products **2e** and **3e** were separated by silica gel column chromatography using petroleum ether/ethyl acetate (2/3) as eluent.

Di-n-butyl 2-keto-3(S)-hydroxy-n-butylphosphonate (**2e**) Colorless liquid, yield 285 mg (57%), *ee* 86%, $[\alpha]_D^{20}$ 6 (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$) δ: 4.29 (q, J = 9.2 Hz, 1H, C**H**(OH)CH_3), 4.10—4.04 (m, 4H, 2 × OC**H**$_2$$CH_2CH_2CH_3$), 3.80—3.40 (OH), 3.39—3.21 (m, 2H, PCH_2), 1.70—1.61 (m, 4H, 2 × OCH_2C**H**$_2$$CH_2CH_3$), 1.45—1.36 (m, 4H, 2 × OCH_2CH_2C**H**$_2$$CH_3$), 1.37 (d, J = 9 Hz, 3H, CHC**H**$_3$), 0.93 (t, J = 10 Hz, 6H, 2 × $OCH_2CH_2CH_2$C**H**$_3$); ^{31}P NMR (160 MHz, $CDCl_3$) δ: 18.555; IR (film) ν: 3369, 2963, 2937, 2877, 1720, 1466, 1242 (s), 1025 (s), 834, 526 cm^{-1}; MS *m/z* (rel. intensity): 281 (M^+ + 1, 1.56), 237 (5.74), 181 (10.77), 125 (100), 97 (5.99), 41 (29.07). Anal. calcd for $C_{12}H_{25}O_5P$: C 51.42, H 8.99; found C 51.44, H 8.84.

Di-n-butyl 2-hydroxy-3-keto-n-butylphosphonate (**3e**) Colorless liquid, yield 132 mg (27%), *ee* 45%, $[\alpha]_D^{20}$ −9.0 (*c* 0.6, CH_3OH); ^{31}P NMR (160 MHz, $CDCl_3$) δ: 28.891; IR (film) ν: 3319 (OH), 2963, 2876, 1721 (s), 1395, 1356, 1223 (s), 1026 (s) cm^{-1}; MS *m/z* (rel. intensity): 281 (M^+ + 1, 14.85), 237 (6.44), 181 (9.62), 151 (14.13), 125 (100), 97 (26.77), 41 (16.50). Anal. calcd for $C_{12}H_{25}O_5P$: C 51.42, H 8.99; found C 51.54, H 8.78.

Conversion of 2-keto-3-hydroxy-n-butylphosphonate (**2**) *to the corresponding α-methoxy-α-trifluoromethylphenylacetic acid* (MTPA) *esters*

General procedure To a stirred solution of (*R*)

[or (*S*)]-α-methoxy-α-trifluoromethylphenyl-acetic acid (0.1 mmol) in anhydrous CH_2Cl_2 (1 mL) was added 2-keto-3-hydroxy-*n*-butylphosphonate (**2**) (0.1 mmol) and DMAP (2—3 mg). The dicyclohexylcarbodiimide (DCC) (0.1 mmol) was added to the reaction mixture at 0 ℃, and then stirred for 8 h at 0 ℃. Precipitated urea was then filtered off. CH_2Cl_2 (20 mL) was added, and the solution was washed twice with saturated $NaHCO_3$ solution, and then dried with $MgSO_4$. The solvent was removed by evaporation and the ester was purified by silica gel column chromatography using petroleum ether/ethyl acetate (2/1) as eluent.

(*R*)-MTPA-(*S*)-**2b** [(*R*)-**4b**] Colorless liquid, yield 36 mg (83%), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58—7.55 (m, 2H, PhH), 7.43—7.42 (m, 3H, PhH), 5.50—5.45 (m, 1H, C**H**CH_3), 4.17—4.09 (m, 4H, 2 × OC**H**$_2$$CH_3$), 3.57 (s, 3H, OCH_3), 3.14—3.03 (m, 2H, PCH_2), 1.55 (d, J = 7 Hz, 3H, CHC**H**$_3$), 1.34—1.29 (m, 6H, 2 × OCH_2C**H**$_3$).

(*S*)-MTPA-(*S*)-**2b** [(*S*)-**4b**] Colorless liquid, yield 31.5 mg (74%), ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.62—7.59 (m, 2H, PhH), 7.45—7.41 (m, 3H, PhH), 5.49—5.42 (m, 1H, C**H**CH_3), 4.21—4.14 (m, 4H, 2 × OC**H**$_2$$CH_3$), 3.62 (s, 3H, OCH_3), 3.27—3.12 (m, 2H, PCH_2), 1.48 (d, J = 7 Hz, 3H, CHC**H**$_3$), 1.31 (t, J = 6 Hz, 6H, 2 × OCH_2C**H**$_3$); ^{19}F NMR (282 MHz, $CDCl_3$) δ: 5.491.

(*R*)-MTPA-(*S*)-**2d** [(*R*)-**4d**] Colorless liquid, yield 27 mg (58%), ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.62—7.60 (m, 2H, PhH), 7.46—7.43 (m, 3H, PhH), 5.50—5.43 (m, 1H, C**H**CH_3), 4.10—4.05 (m, 4H, 2 × OC**H**$_2$$CH_2CH_3$), 3.63 (s, 3H, OCH_3), 3.29—3.15 (m, 2H, PCH_2), 1.75—1.68 (m, 4H, 2 × OCH_2C**H**$_2$$CH_3$), 1.52 (d, J = 6.6 Hz, 3H, CHC**H**$_3$), 0.98—0.94 (m, 6H, 2 × OCH_2CH_2C**H**$_3$).

(*S*)-MTPA-(*S*)-**2d** [(*S*)-**4d**] Colorless liquid, yield 31 mg (67%), ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.58—7.57 (m, 2H, PhH), 7.46—7.42 (m, 3H, PhH), 5.51—5.44 (m, 1H, C**H**CH_3), 4.05—4.00 (m, 4H, 2 × OC**H**$_2$$CH_2CH_3$), 3.64 (s, 3H, OCH_3), 3.32—3.10 (m, 2H, PCH_2), 1.76—1.69 (m, 4H, 2 × OCH_2C**H**$_2$$CH_3$), 1.56 (d, J = 6 Hz, 3H, CHC**H**$_3$), 0.98—0.93 (m, 6H, 2 × OCH_2CH_2C**H**$_3$).

(*R*)-MTPA-(*S*)-**2e** [(*R*)-**4e**] Colorless liquid, yield 33 mg (67%), ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.62—7.56 (m, PhH, 2H), 7.48—7.40 (m, PhH, 3H), 5.47 (q, J = 7.1 Hz, 1H, C**H**CH_3), 4.20—4.04 (m, 4H, 2 × OC**H**$_2$$CH_2CH_2CH_3$), 3.56 (s, 3H, OCH_3), 3.25—3.03 (m, 2H, PCH_2), 1.73—1.66 (m, 4H, 2 × OCH_2C**H**$_2$$CH_2CH_3$), 1.53 (d, J = 7.1 Hz, 3H, CHC**H**$_3$), 1.44—1.30 (m, 4H, 2 × OCH_2CH_2C**H**$_2$$CH_3$), 0.92 (t, J = 7.3 Hz, 6H, 2 × $OCH_2CH_2CH_2$C**H**$_3$).

(*S*)-MTPA-(*S*)-**2e** [(*S*)-**4e**] Colorless liquid, yield 37 mg (73%), ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.63—7.61 (m, 2H, PhH), 7.44—7.42 (m, 3H, PhH), 5.48—5.40 (m, 1H, C**H**CH_3), 4.17—4.06 (m, 4H, 2 × OC**H**$_2$$CH_2CH_2CH_3$), 3.32 —3.14 (m, 2H, PCH_2), 1.72—1.64 (m, 4H, 2 × OCH_2C**H**$_2$$CH_2CH_3$), 1.49 (d, J = 7.0 Hz, 3H, CHC**H**$_3$), 1.46—1.36 (m, 4H, 2 × OCH_2CH_2C**H**$_2$$CH_3$), 0.94 (t, J = 7.3 Hz, 6H, 2 × $OCH_2CH_2CH_2$C**H**$_3$).

Preparation of α,β-unsaturated ketones via the HWE *reaction*

To a stirred solution of 0.5 mmol of 2-keto-3-hydroxybutylphosphonate (**2b**) in 2 mL of anhydrous CH_3CN was added 21 mg (0.5 mmol) of anhydrous LiCl and 0.5 mmol of DBU at 20 ℃. The mixture was stirred for 30 min, and the benzaldehyde (0.5 mmol) was added to the reaction mixture, which was then stirred for 8—20 h at 0 ℃. The solvent was removed by evaporation and the product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1/1) as eluent.

Trans-4(*S*)-*hydroxy*-1-(4-*nitro-phenyl*)-*pent*-1-*en*-3-*one* (**5a**) Yellow solid, yield 74 mg (66%); $[\alpha]_D^{20}$ 6 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.26(d, J = 7 Hz, 2H, ArH), 7.78 (d, J = 16 Hz, 1H, ArCH=), 7.73(d, J = 7 Hz, 2H, ArH), 6.96 (d, J = 16 Hz, 1H, CHCO), 4.57 (q, J = 7.1 Hz, 1H, C**H**CH_3), 3.50 (OH), 1.48 (d, J = 7.1 Hz, 3H, CHC**H**$_3$); IR (film) ν: 3467, 3109, 1686, 1618, 1514, 1345, 1066, 840, 799, 754 cm^{-1}; MS *m/z* (rel. intensity): 193 (M^+ − OH, 2.98), 176 (100), 160 (52.90), 130 (74.39), 102 (65.67), 45 (40.06). Anal. calcd for $C_{11}H_{11}NO_4$: C 59.72, H 5.01, N 6.33; found C 59.82, H 5.24, N 6.14.

Trans-4(*S*)-*hydroxy*-1-(2,4-*dichloro-phenyl*)-*pent*-1-*en*-3-*one* (**5b**) Yellow solid, yield 54 mg (44%), $[\alpha]_D^{20}$ 4 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.08 (d, J = 16 Hz, 1H, ArCH=), 7.60—7.30 (m, 3H, ArH), 6.80 (d, J = 16 Hz, 1H,

CHCO), 4.57 (q, J = 6.9 Hz, 1H, C**H**CH_3), 1.45 (d, J = 6.9 Hz, 3H, CH**C**H_3); IR (KBr) ν: 3361, 2928, 1692, 1610, 1585, 1471, 1103, 1066 (s), 1052, 867 cm^{-1}; MS m/z (rel. intensity): 245 (M^+, 0.51), 199 (100), 171 (30.92), 136 (32.15), 89 (42.00).

Trans-4 (S)-*hydroxy*-1-*phenyl*-*pent*-1-*en*-3-*one* (**5c**) Colorless liquid, yield 49 mg (56%), $[\alpha]_D^{20}$ 1 (c 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.77(d, J = 16 Hz, 1H, PhC**H** =), 7.65—7.58 (m, 2H, PhH), 7.50—7.32 (m, 3H, PhH), 6.85 (d, J = 16 Hz, 1H, CHCO), 4.58 (q, J = 7 Hz, 1H, C**H**CH_3), 1.48 (d, J = 7 Hz, 3H, CH**C**H_3); IR (KBr) ν: 3447, 2980, 1684, 1614, 1450, 1068, 763, 689 cm^{-1}; MS m/z (rel. intensity): 159 (M^+ - OH, 5.79), 131 (100), 103 (38.77), 77 (20.91), 51 (9.07), 43 (10.51).

General procedure for the reaction of baker's yeast

Baker's yeast (9 g) was suspended in water (90 mL), then diketoalkylphosphonate **6a** or **6b** (1 mmol) was added and the mixture was shaken at 30 ℃ about 72 h, while being monitored by TLC. The biomass was removed and extracted with ethyl ether, and with chloroform (20 mL × 3). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvents removed under reduced pressure. The product was separated by silica gel column chromatography using petroleum ether/acetone (1/1) as eluent.

Diethyl 4-*hydroxy*-2-*keto*-*n*-*pentylphosphonate* (**7a**) Colorless liquid, yield 94 mg (42.5%), $[\alpha]_D^{20}$ 11 (c 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ: 4.40—4.33 (m, 1H, C**H**OH), 4.11—4.04 (m, 4H, 2× POC**H**$_2$CH$_3$), 3.10 (d, J = 21 Hz, 2H, PC**H**$_2$), 2.75—2.70 (m, 2H, COC**H**$_2$), 2.32—2.17 (OH), 1.34—1.23 (m, 6H, 2× POCH$_2$C**H**$_3$), 1.17 (d, J = 2.4 Hz, 3H, CHC**H**$_3$); IR (film) ν: 3404, 2980, 1714, 1446, 1394, 1372, 1249, 1164, 1026 cm^{-1}; MS m/z (rel. intensity): 221 (M^+ - OH, 7.50), 195 (10.85), 179 (16.26), 139 (25.80), 125 (100), 97 (55.50), 43 (43.62). Anal. calcd for $C_9H_{19}O_5P$: C 45.38, H 8.04; found C 45.19, H 8.18.

Diethyl 2-*hydroxy*-4-*keto*-*n*-*pentylphosphonate* (**8a**) Colorless liquid, yield 11 mg (5%), ^1H NMR (300 MHz, $CDCl_3$) δ: 5.40—5.33 (m, 1H, CHO**H**), 4.68—4.63 (m, 1H, C**H**OH), 4.10—4.01 (m, 4H, 2× POC**H**$_2$CH$_3$), 2.70 (2H, COC**H**$_2$), 2.18—1.85 (m, 2H, PC**H**$_2$), 2.10 (s, 3H, COC**H**$_3$), 1.33—1.19 (m, 6H; 2× POCH$_2$C**H**$_3$). Anal. calcd for $C_9H_{19}O_5P$: C 45.38, H 8.04; found C 45.01, H 8.09.

Diethyl 5,5,5-*trifluoro*-4-*hydroxy*-2-*keto*-*n*-*pentyl*-*phosphonate* (**7b**) Colorless liquid, yield 110 mg (41%), $[\alpha]_D^{20}$ 3 (c 0.9, EtOH), *ee* 91%; ^1H NMR (300 MHz, $CDCl_3$) δ: 4.79—4.78 (OH), 4.52—4.40 (m, 1H, C**H**OH), 4.23—4.07 (m, 4H, 2× POC**H**$_2$CH$_3$), 3.24 (d, J = 15 Hz, 2H, PC**H**$_2$), 3.09—2.84 (m, 2H, COC**H**$_2$), 1.41—1.22 (m, 6H, 2× POCH$_2$C**H**$_3$); ^{19}F NMR (282 MHz, $CDCl_3$) δ: -2.75 (d, J = 7.03 Hz, CF_3); MS m/z (rel. intensity): 275 (M^+ - OH, 11.70), 265 (6.19), 223 (19.89), 179 (22.48), 123 (100), 109 (18.16), 97 (15.14); HRMS calcd for $C_9H_{16}F_3O_5P$ 292.0687, found 292.0687.

Preparation of α,β-unsaturated ketones via the HWE *reaction*

To a stirred solution of diethyl 5,5,5-trifluoro-4-hydroxy-2-keto-*n*-pentylphosphonate (**7b**) (0.5 mmol) in anhydrous CH_3CN (2 mL) was added anhydrous LiCl (21 mg, 0.5 mmol) and DBU (0.5 mmol) at 20 ℃. The mixture was stirred for 30 min, and the benzaldehyde (0.5 mmol) was added to the reaction mixture, which was then stirred for 8—20 h at 0 ℃. The solvent was removed by evaporation and the product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1/1) as eluent.

Trans-6,6,6-*trifluoro*-5-*hydroxy*-1-(4-*methylphenyl*)-1-*hex*-3-*one* (**9a**) White solid, yiled 85 mg (66%). Single crystals suitable for X-ray diffraction were recrystallized from $CHCl_3$. $[\alpha]_D^{20}$ 25 (c 0.74, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.65 (d, J = 15 Hz, 1H, ArC**H** =), 7.58—7.22 (m, 4H, ArH), 6.70 (d, J = 15 Hz, 1H, COC**H** =), 4.62 (m, 1H, C**H**OH), 3.73 (OH), 3.07 (d, J = 4 Hz, 2H, COC**H**$_2$), 2.39 (s, 3H, ArC**H**$_3$); IR (KBr) ν: 3446, 1664, 1605, 1316, 1268, 1183, 1167, 1118, 1103, 803 cm^{-1}; MS m/z (rel. intensity): 258 (M^+, 22.75), 243 (43.01), 145 (100), 115 (33.35), 87 (54.67); HRMS calcd for $C_{13}H_{13}F_3O_2$ 258.0868, found 258.0868.

Trans-6,6,6-*trifluoro*-5-*hydroxy*-1-(4-*nitrophenyl*)-1-*hex*-3-*one* (**9b**) Yellow solid, yield 102 mg (71%), m.p. 74—76 ℃, $[\alpha]_D^{20}$ 17 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.28 (d, J = 8.7 Hz, 2H, ArH), 7.73 (d, J = 8.9 Hz, 2H, ArH), 7.65 (d, J = 16 Hz, 1H, ArCH=), 6.88 (d, J = 16 Hz, 1H, COCH=), 4.64 (m, 1H, CHOH), 3.40 (OH), 3.10 (m, 2H, $COCH_2$); IR (KBr) ν: 3315, 1670, 1611, 1594, 1534, 1352 (s), 1118, 859, 745, 672 cm^{-1}; MS *m/z* (rel. intensity): 289 (M^+, 0.65), 272 (14.18), 242 (2.87), 176 (100), 130 (25.85), 102 (22.89), 90 (9.17), 76 (9.08); HRMS calcd for $C_{12}H_{10}F_3NO_4$ 289.0562, found 289.0532.

Trans-6,6,6-*trifluoro*-5-*hydroxy*-1-(4-*chlorophenyl*)-1-*hex*-3-*one* (**9c**) White solid, yiled 94 mg (68%), m. p. 104—106 ℃, $[\alpha]_D^{20}$ 5 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.57 (d, J = 16 Hz, 1H, COCH=), 7.52—7.38 (m, 4H, ArH), 6.98 (d, J = 16 Hz, 1H, ArCH=), 4.61 (m, 1H, CHOH), 3.55 (OH), 3.12—3.00 (m, 2H, $COCH_2$); IR (KBr) ν: 3433, 1689, 1667, 1614, 1319, 1274, 1167, 1093, 983, 811, 681, 489 cm^{-1}; MS *m/z* (rel. intensity): 278 (M^+, 23.40), 258 (4.74), 243 (21.45), 165 (100), 137 (29.01), 87 (54.53); HRMS calcd for $C_{12}H_{10}ClF_3O_2$ 278.0321, found 278.0317.

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