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Enzymatic resolution of ethyl 3-hydroxy-2(1′substituted-methylidene)-butyrate by *Pseudomonas cepacia* lipase catalyzed acetylation

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Abstract—Enzymatic resolution of a series of enantiomerically pure ethyl 3-hydroxy-2(1′substituted-methylidene)-butyrates was performed using *Pseudomonas cepacia* lipase (EC 3.1.1.3) as a catalyst. Optically active ethyl 3-hydroxy-2(1′substituted-methylidene)-butyrates, as well as their acetates, were obtained from this reaction in good yield and excellent enantiomeric excess.

1. Introduction

Enantiomerically pure allylic alcohols constitute versatile starting materials in organic synthesis. The kinetic enzymatic resolution of this class of compounds by lipase catalyzed acetylation[1] has been reported under different conditions and in the presence of different lipases.[2] The resolution of polyfunctionalyzed derivatives such as γ-hydroxy-α,β-unsaturated esters, via irreversible acylations in hexane mediated by *Pseudomonas* sp. Amano has been already reported in the literature.[3] The same enzyme promoted enantioselective esterifications of β-hydroxy-α-methylene esters (Fig. 1).

Over the course of our research, we required the title compounds in an enantiomerically pure form. Our substrates are particular for the presence of the double bond, whose configuration could affect the enzymatic activity, and also for the presence of the carboxylic function. We decided to investigate the resolution of a series of substituted racemic alcohols by using an enzymatic acetylation reaction (Scheme 1).

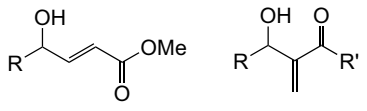
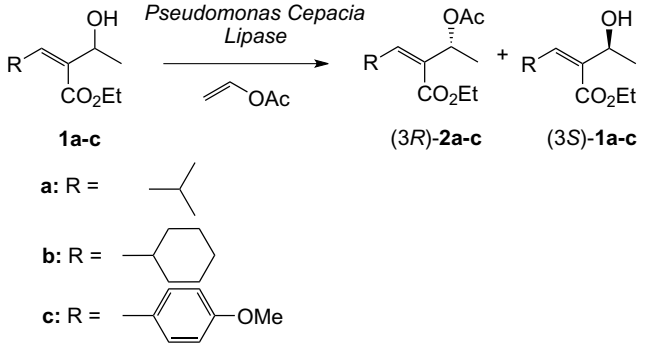


Figure 1. Polyfunctionalized allylic alcohols resolved via enzymatic irreversible acylation.



Scheme 1. Ethyl 3-hydroxy-2(1′substituted-methylidene)-butyrate enzymatic irreversible acylation reaction.

2. Results and discussion

The racemic alcohols were prepared in an extremely simple manner by treating 10 equiv of aldehyde and 1 equiv of ethyl acetoacetate in the presence of 0.15 equiv of piperidine under microwave assisted conditions for 7.5 min (Scheme 2). Microwave assisted organic synthesis[4] is in fact a rapidly expanding area of research that offers the opportunity to strongly reduce reaction times from hours to minutes. After the usual work up, a 30/70 mixtures of *E* and *Z* ketones were obtained in good yields.

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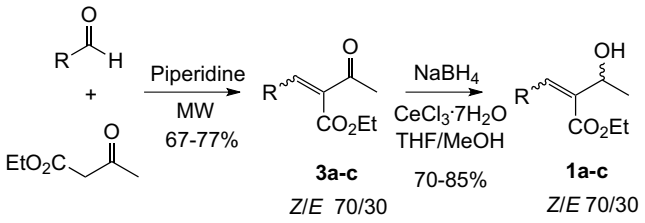
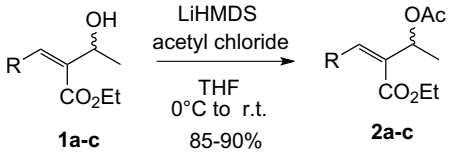


Scheme 2. Synthesis of racemic alcohols **1a**–**c**.

The mixture of ketones was subjected to reduction in the presence of 1 equiv of $CeCl_3$ and 1.1 equiv of $NaBH_4$ in THF/MeOH 9:1 (Scheme 2).[5] The corresponding *Z*/*E* alcohols **1a**–**c** were obtained in excellent yields and were separated by flash chromatography.

In order to select the most useful enzyme for the kinetic resolution of these compounds, a series of screening experiments were carried out with various lipases. Therefore, the lipases from *Candida cylindracea*, *Mucor miehei*, *Aspergillus niger*, and *Rhizopus nivens* were tested via irreversible acylation by treating the substrates with 4 equiv of vinyl acetate and 0.5 mass equiv of enzyme in diethyl ether at rt for several days.[7]

Finally, the use of *Pseudomonas cepacia* proved to be very successful for the resolution of *Z*-**1a**–**c** alcohols, affording the acetylated compounds in good yields and high ee, although with lengthy reaction times. However, any effort to resolve the allylic alcohols *E*-**1a**–**c** under these conditions failed.[1] The lipase from *P. cepacia* (EC 3.1.1.3) is a well-known catalyst in organic synthesis, used both for the kinetic resolution of a racemic mixture of secondary alcohols, as well as in hydrolysis and transesterification.[8] The racemic alcohols **1a**–**c** were subjected to catalyzed enzymatic acetylation employing vinyl acetate in diethyl ether at 40 °C (Table 1). No advantages could be observed under different reaction conditions, by using vinyl acetate both as the acyl donor and solvent or performing the resolution at higher temperatures. Monitoring of the reaction progress by GC–MS allowed for the determination of the conversion. Samples of racemic acetates **2a**–**c** were prepared as reference compounds, via acetylation of racemic alcohols **1a**–**c**. Although several different basic conditions were tested for the reaction with acetyl chloride (TEA, NaH, K_2CO_3), good results could be obtained only when the reaction was performed in the presence of 1.5 equiv of LiHMDS (Scheme 3).



Scheme 3.

After the required conversion in the lipase catalyzed reaction was achieved, the enzyme was filtered off and the mixture subjected to the usual work up. The ^{1}H NMR of the crude material showed a 1:1 ratio of alcohol/acetate, based on the peak area of the hydrogen adjacent to the secondary alcohol and its corresponding acetate (a quartet at 4.50–4.60 ppm for alcohols **1a**–**c** and a quartet at 5.60–5.70 ppm for acetate **2a**–**c**, Fig. 2). The resulting acetates and the remaining alcohols were separated by flash chromatography.

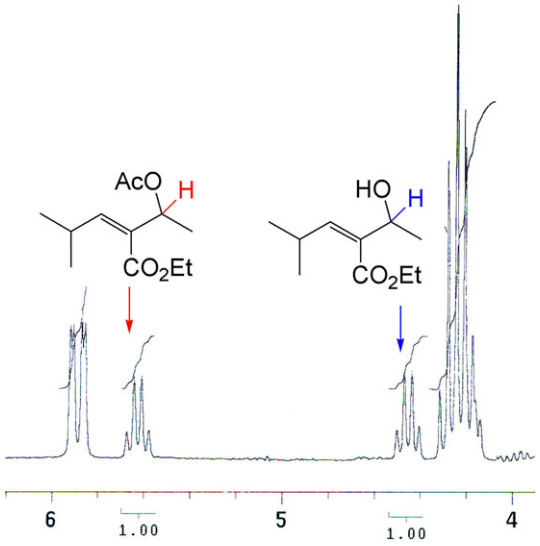


Figure 2. ^{1}H NMR of the crude enzymatic reaction.

Table 1. Biocatalytic resolution of allylic alcohols[a] **1a**–**c**

Entry	Substrate	Products	Conversion[b] (%)	Isolated yield[c] (%)	$[\alpha]_D$	ee[d]	*E*[e]
1	**1a**	(3*S*)-**1a**	50	45	−11.0	>99	>1000
		(3*R*)-**2a**		34	+36.5	>99	
2	**1b**	(3*S*)-**1b**	47	45	−8.7	88	37
		(3*R*)-**2b**		42	+27.2	>99	
3	**1c**	(3*S*)-**1c**	50	36	−4.0	>99	>1000
		(3*R*)-**2c**		40	+44.3	>99	

[a] The substrate at 0.2 M concentration in diethyl ether was stirred with 0.5 mass equiv of enzyme, and 5 equiv of vinyl acetate for five days at 40 °C.
[b] As monitored by GC–MS and by ^{1}H NMR integrals.
[c] Determined on isolated and purified products.
[d] Determined by HPLC with a chiral column (see Section 4).
[e] Values determined using Sih's method, from the extent of conversion and enantiomeric excess of the recovered substrate as described and checked via values determined from the enantiomeric excess of the product (Ref. 6).

The racemic alcohols were analytically resolved into their enantiomers by chiral HPLC and were compared with the alcohols that were derived from enzymatic resolution. Representative chromatograms of racemic **1a** and (−)-**1a** are reported in Figure 3a and b.

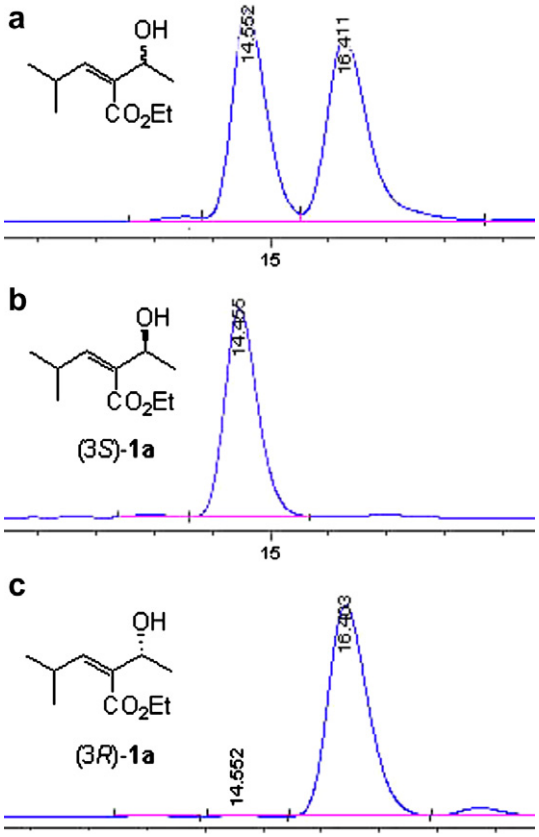
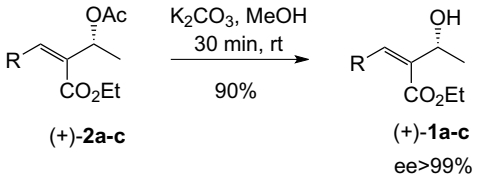


Figure 3. HPLC chromatograms of the racemic *Z*-**1a** in comparison with the enantiomerically pure alcohols obtained by enzymatic resolution.

Since the analytical resolution of acetates could not be easily achieved, acetates (+)-**2a**–**c** were hydrolyzed to the corresponding alcohols. For this purpose, samples of the optically active acetates **2a**–**c** were treated with K_2CO_3 in methanol (Scheme 4). The enatiomeric excesses of (+)-**1a**–**c** were determined via HPLC by comparison with the corresponding racemates (Fig. 3c).



Scheme 4.

The stereoselectivity of the lipase prepared from *P. cepacia* have been widely studied via models based on a classification of the relative size of the substituents (small and large) at the stereocenter of the secondary alcohol.[9] The Kazlauskas' rule to predict which enantiomer of the secondary alcohol reacts faster, has received reliable application. On the basis of the data reported in the literature, we have attributed the (*S*)-configuration to the allylic alcohols and the (*R*)-configuration to their corresponding acetates. In fact in our case, the methyl group is unequivocally identified as the smaller substituent in comparison to the large one (Fig. 4).

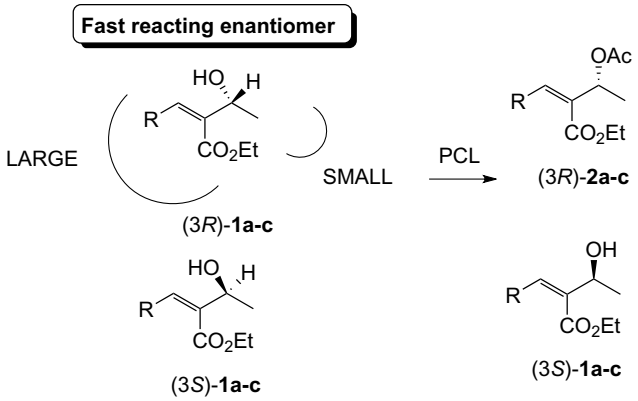


Figure 4. Stereochemical assignments on the basis of Kazlauskas' model.

3. Conclusion

In conclusion, optically active ethyl 3-hydroxy-2(1′substituted-methylidene)-butyrates, as well as their acetates, were obtained in excellent enantiomeric excess via lipase catalyzed enzymatic resolution. *P. cepacia* lipase was shown to be the most efficient biocatalyst, allowing us to obtain these useful intermediates in a high yield and in an enantiomerically pure form.

4. Experimental

4.1. General methods

All chemicals were purchased from commercial suppliers and used without further purification. Flash chromatography was performed on silica gel (230–400 mesh). NMR Spectra were recorded with 200 or 300 MHz spectrometers. Chemical shifts were reported as δ values (ppm) relative to the solvent peak of $CDCl_3$ set at $\delta = 7.27$ (^{1}H NMR) or $\delta = 77.0$ (^{13}C NMR). GC–MS analysis were performed on HP-5 (crosslinked 5% Ph Me silicone, 30 m × 0.32 mm × 0.25 μm thickness) using an injection program (initial temperature 50 °C for 2′, then 10 °C/min up to 280 °C) in scan mode acquisition. Microwave assisted reactions were performed with a Milestone Mycrosynth multimode apparatus, keeping irradiation power fixed and monitoring internal reaction temperature with a Built-in ATC-FO advanced fiber optic automatic temperature control. The reactions were performed in an open vessel, equipped with a refrigerator connected to a fume hood. The enantiomeric excesses of alcohols were determined by HPLC analyses performed an HP1100 instrument with UV–vis detector and equipped with Chiralcel OD column (25 × 0.46 cm) or Chiralcel OF column (25 × 0.46 cm), eluted with *n*-hexane/2-propanol. Optical rotations were

measured in a Perkin Elmer 343 polarimeter using a 1 dm cuvette and are referenced to the Na-D line value.

4.2. Synthesis of ketones 3a–c

Ethyl acetoacetate (10 mmol, 1.3 g, 1.26 mL) was diluted in the aldehyde (10 equiv, 100 mmol) and piperidine (0.15 equiv, 1.5 mmol, 0.15 mL) was added in one portion. The mixture was subjected to microwave irradiation (Power 500 W) for 7.5 min and then diluted with ethyl acetate (20 mL) and washed twice with 0.1 M HCl (20 mL). The two phases were separated, the organic layer dried over Na_2SO_4, and the solvent removed under reduced pressure. Compounds *Z*-**3a**–**c** and *E*-**3a**–**c** were separated by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10 as eluant).

4.3. Characterization of ketones 3a–c

4.3.1. *Z*-3a. Yellow oil; isolated yield 61%; IR (film) ν/cm^{-1} 2967, 2936, 2872, 1731, 1698, 1670, 1467, 1321, 1258, 1212, 1036. ^1H NMR (200 MHz, $CDCl_3$) δ 1.07 (d, 6H, $J = 6.6$ Hz), 1.31 (t, 3H, $J = 7.4$ Hz), 2.30 (s, 3H), 2.65 (m, 1H), 4.29 (q, 2H, $J = 7.4$ Hz), 6.60 (d, 1H, $J = 10.6$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.1, 21.9 (2), 26.8, 29.6, 61.2, 134.9, 154.0, 166.6, 195.4; GC–MS rt 11.74 min, *m*/*z*: 184(2), 138(100), 123(60), 110(15), 96(57), 81(24), 67(32), 55(26).

4.3.2. *E*-3a. Yellow oil; isolated yield 26%; IR (film) ν/cm^{-1} 2965, 2933, 2872, 1705, 1638, 1467, 1367, 1239, 1200, 1051. ^1H NMR (200 MHz, $CDCl_3$) δ 1.02 (d, 6H, $J = 6.6$ Hz), 1.30 (t, 3H, $J = 7.4$ Hz), 2.37 (s, 3H), 2.62 (m, 1H), 4.24 (q, 2H, $J = 7.4$ Hz), 6.69 (d, 1H, $J = 10.6$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 13.9, 21.6, 21.8, 26.5, 29.4, 61.1, 134.8, 154.0, 166.5, 197.9; GC–MS rt 11.45 min, *m*/*z*: 184(2), 138(100), 123(55), 110(15), 96(64), 81(26), 67(31), 55(19).

4.3.3. *Z*-3b. Yellow oil; isolated yield 55%; IR (film) ν/cm^{-1} 2981, 2928, 2852, 1731, 1698, 1673, 1635, 1448, 1380, 1305, 1210, 1150, 1035. ^1H NMR (200 MHz, $CDCl_3$) δ 1.21–1.43 (m, 5H), 1.34 (t, 3H, $J = 7.0$ Hz), 1.60–1.77 (m, 5H), 2.31 (s, 3H), 2.37 (m, 1H), 4.30 (q, 2H, $J = 7.0$ Hz), 6.63 (d, 1H, $J = 9.8$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.1, 25.1(2), 25.3, 26.7, 31.7, 31.8, 39.1, 61.0, 135.2, 152.5, 166.6, 197.9. GC–MS rt 16.60 min, *m*/*z*: 224(2), 178(100), 163(60), 149(20), 135(90), 121(29), 107(33), 95(27), 79(30), 67(21), 55(25).

4.3.4. *E*-3b. Yellow oil; isolated yield 23%; IR (film) ν/cm^{-1} 2927, 2853, 2360, 2342, 1701, 1654, 1637, 1448, 1274, 1253. ^1H NMR (200 MHz, $CDCl_3$) δ 1.14–1.26 (m, 5H), 1.27 (t, 3H, $J = 7.0$ Hz), 1.66–1.77 (m, 5H), 2.38 (s, 3H), 2.42 (m, 1H), 4.26 (q, 2H, $J = 7.0$ Hz), 6.72 (d, 1H, $J = 10.6$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.1, 25.0 (2), 25.6, 31.3, 31.9 (2), 38.3, 61.0, 133.9, 152.8, 164.7, 201.3. GC–MS rt 16.40 min, *m*/*z*: 224(5), 182(100), 163(9), 135(27), 107(42), 79(27), 67(20), 55(12).

4.3.5. *Z*-3c. Yellow oil; isolated yield 50%; IR (film) ν/cm^{-1} 2980, 2936, 2841, 1726, 1655, 1601, 1513, 1452, 1260, 1222, 1176, 1028. ^1H NMR (300 MHz, $CDCl_3$) δ 1.33 (t, 3H, $J = 7.2$ Hz), 2.44 (s, 3H), 3.88 (s, 3H), 4.40 (q, 2H, $J = 7.2$ Hz), 6.93 (d, 2H, $J = 7.2$ Hz), 7.46 (d, 2H, $J = 7.2$ Hz), 7.50 (s, 1H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.0, 26.2, 55.2, 61.4, 114.2(2), 125.0, 131.6(2), 132.1, 140.9, 161.6, 168.1, 194.6. GC–MS rt 20.30 min, *m*/*z*: 248(100), 233(80), 217(21), 203(30), 189(8), 174(24), 161(55), 137(18), 117(9), 89(12).

4.3.6. *E*-3c. Yellow oil; isolated yield 21%; IR (film) ν/cm^{-1} 2979, 2935, 2841, 2739, 1700, 1601, 1577, 1512, 1315, 1258, 1174, 1026. ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, 3H, $J = 7.2$ Hz), 2.39 (s, 3H), 3.89 (s, 3H), 4.27 (q, 2H, $J = 7.2$ Hz), 7.00 (d, 2H, $J = 7.2$ Hz), 7.61 (s, 1H), 7.83 (d, 2H, $J = 7.2$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.1, 26.5, 55.4, 61.6, 114.4(2), 125.5, 131.7(2), 132.3, 140.3, 161.5, 168.3, 194.6; GC–MS rt 20.20 min, *m*/*z*: 248(100), 233(88), 217(26), 203(35), 189(12), 174(29), 161(76), 137(31), 117(17), 103(10), 89(31).

4.4. Synthesis of racemic alcohols 1a–c

To a stirred solution of compound *Z*-**3a**–**c** and *E*-**3a**–**c** (5 mmol) in THF/MeOH 9:1 (10 mL) at room temperature, $CeCl_3{\cdot}7H_2O$ (1 equiv, 5 mmol, 1.86 g) and $NaBH_4$ (1.1 equiv, 5.5 mmol, 0.2 g) were added in one portion. The solution was monitored by TLC and quenched after disappearance of the starting ketone by the addition of water (5 mL). After removal of THF and MeOH under reduced pressure, the residue was diluted with ethyl acetate (10 mL) and washed twice with water (5 mL). The two phases were separated, the organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Racemic alcohols *Z*-**1a**–**c** or *E*-**1a**–**c** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20 as eluant).

4.5. Synthesis of racemic acetates 2a–c

To a solution of compound *Z*-**1** (1 mmol) in dry THF (5 mL), under an inert atmosphere at 0 °C, LiHMDS (1.5 equiv, 1.5 mL 1 M solution in THF) was added dropwise. The solution was stirred for 30 min and then acetyl chloride (1.5 equiv, 1.5 mmol, 0.1 mL) was added in one portion and the ice bath was removed. After 1 h, the mixture was quenched with water (5 mL) and THF removed under reduced pressure. The residue was diluted with ethyl acetate (10 mL) and washed twice with water (5 mL). The two phases were separated, the organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure. Racemic acetates *Z*-**2a**–**c** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10 as eluant).

4.6. Lipase catalyzed resolution of alcohols 1a–c

To a solution of racemic alcohol **1** (5 mmol) in diethyl ether (25 mL) at 40 °C, vinyl acetate (4 equiv, 4 mmol, 0.37 mL) and lipase from *P. cepacia* (46 U/mg, 0.2 mass equiv) were added. The progress of the reaction was assessed every 12 h by GC–MS. The reaction was stopped by filtration of the enzyme and elimination of solvent and by-products

under reduced pressure. Enantiomerically pure alcohols (3*S*)-*Z*-**1a**–**c** and acetates (3*R*)-*Z*-**2a**–**c** were separated by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10 then 80/20 as eluant).

4.7. Hydrolysis of enantiomerically pure acetates 2a–c to enantiomerically pure alcohols 1a–c

Acetate (3*R*)-*Z*-**2** (0.5 mmol) was stirred in MeOH (5 mL) in the presence of K_2CO_3 (1 equiv, 0.5 mmol, 70 mg) for 30 min at room temperature. The reaction was quenched by the addition of 0.1 M HCl (5 mL). After removal of MeOH under reduced pressure, the residue was diluted with ethyl acetate (10 mL) and washed twice with water. The two phases were separated, the organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure. Enantiomerically pure alcohols (3*R*)-*Z*-**1a**–**c** were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20 as eluant).

4.8. Characterization of alcohols 1a–c

4.8.1. *Z*-1a. Yellow oil; IR (film) ν/cm^{-1} 3437, 2964, 2933, 2870, 1717, 1467, 1372, 1228, 1178, 1159. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (d, 6H, $J = 6.6$ Hz), 1.24 (m, 6H), 3.10 (m, 1H), 4.23 (q, 2H, $J = 6.6$ Hz), 4.42 (q, 1H, $J = 6.2$ Hz) 5.87 (d, 1H, $J = 10.0$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.1, 22.4, 22.5, 28.2, 60.3, 69.2, 133.1, 146.9, 167.7. GC–MS rt 11.62 min, *m*/*z*: 186(2), 171(36), 144(21), 125(100), 115(20), 97(61), 79(35), 67(25), 55(24).

(3*S*)-*Z*-**1a**: HPLC on Chiralcel OF (97.5/2.5 *n*-hexane/2-propanol, flow 1.0 mL/min): rt 14.5 min, $[\alpha]_D^{25} = -11.0$ (*c* 1.0, $CHCl_3$).

(3*R*)-Z-**1a**: HPLC on Chiralcel OF (97.5/2.5 *n*-hexane/2-propanol, flow 1.0 mL/min): rt 16.4 min, $[\alpha]_D^{25} = +12.4$ (*c* 1.0, $CHCl_3$).

4.8.2. *E*-1a. Yellow oil; IR (film) ν/cm^{-1} 3452, 2963, 2871, 1693, 1641, 1466, 1368, 1261, 1176, 1190. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.03 (d, 3H, $J = 6.6$ Hz), 1.06 (d, 3H, $J = 6.6$ Hz), 1.24 (t, 3H, $J = 7.2$ Hz), 1.42 (d, 3H, $J = 6.6$ Hz), 2.77 (m, 1H), 4.22 (q, 2H, $J = 7.2$ Hz), 4.72 (m, 1H), 6.52 (d, 1H, $J = 10.2$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.1, 22.2(2), 23.8, 27.1, 60.4, 65.0, 131.7, 149.2, 167.5. GC–MS rt 11.80 min, *m*/*z*: 186(2), 171(64), 143(27), 125(100), 115(11), 97(46), 79(20), 67(18), 55(15).

4.8.3. *Z*-1b. Yellow oil; IR (film) ν/cm^{-1} 3438, 2926, 2851, 1714, 1448, 1373, 1303, 1263, 1208, 1178, 1153. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01–1.36 (m, 5H), 1.25 (t, 3H, $J = 7.0$ Hz), 1.25 (d, 3H, $J = 6.6$ Hz), 1.60–1.73 (m, 5H), 2.30 (br s, 1H), 2.75 (m, 1H), 4.24 (q, 2H, $J = 7.0$ Hz), 4.41 (q, 1H, $J = 6.6$ Hz), 5.88 (d, 1H, $J = 10.0$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.0, 22.5, 25.4, 25.7, 25.9, 32.4, 32.5, 37.9, 60.2, 69.2, 133.4, 145.6, 167.7. GC–MS rt 16.40 min, *m*/*z*: 226(2), 208(38), 179(33), 162(100), 147(28), 133(51), 119(37), 107(20), 99(29), 91(36), 81(87), 67(38), 55(34).

(3*S*)-*Z*-**1b**: HPLC on Chiralcel OF (97.5/2.5 *n*-hexane/2-propanol, flow 1.0 mL/min): rt 17.7 min, $[\alpha]_D^{25} = -8.7$ (*c* 1.0, $CHCl_3$).

(3*R*)-*Z*-**1b**: HPLC on Chiralcel OF (97.5/2.5 *n*-hexane/2-propanol, flow 1.0 mL/min): rt 19.3 min, $[\alpha]_D^{25} = +9.4$ (*c* 1.0, $CHCl_3$).

4.8.4. *E*-1b. Yellow oil; IR (film) ν/cm^{-1} 3423, 2926, 2852, 1691, 1448, 1368, 1263. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.11–1.25 (m, 5H), 1.29 (t, 3H, $J = 7.4$ Hz), 1.39 (d, 3H, $J = 6.6$ Hz), 1.44–1.73 (m, 5H), 2.34 (m, 1H), 4.19 (q, 2H, $J = 7.4$ Hz), 4.70 (q, 1H, $J = 6.6$ Hz) 6.50 (d, 1H, $J = 9.8$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.1, 23.9, 25.4 (2), 25.7, 32.1(2), 37.0, 60.6, 65.1, 132.1, 147.8, 167.7. GC–MS rt 16.81 min, *m*/*z*: 226(2), 211(100), 179(18), 165(68), 143(55), 129(38), 119(35), 105(19), 91(32), 81(70), 67(45), 55(43).

4.8.5. *Z*-1c. Yellow oil; IR (film) ν/cm^{-1} 3448, 2977, 2934, 1718, 1607, 1511, 1458, 1300, 1254, 1193, 1031. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15 (t, 3H, $J = 7.0$ Hz), 1.41 (d, 3H, $J = 6.6$ Hz), 3.78 (s, 3H), 4.17 (q, 2H, $J = 7.0$ Hz), 4.58 (q, 1H, $J = 6.6$ Hz), 6.84 (d, 2H, $J = 8.8$ Hz), 6.88 (s, 1H), 7.26 (d, 2H, $J = 8.8$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 13.9, 22.4, 55.2, 60.8, 70.2, 113.6(2), 127.8, 129.9 (2), 132.2, 135.5, 159.6, 167.6. GC–MS rt 19.60 min, *m*/*z*: 250(21), 232(34), 203(25), 189(75), 159(100), 144(66), 128(21), 115(57), 89(20), 77(18).

(3*S*)-*Z*-**1c**: HPLC on Chiralcel OD (98/2 *n*-hexane/2-propanol, flow 1.5 mL/min): rt 24.8 min, $[\alpha]_D^{25} = -4.0$ (*c* 1.0, $CHCl_3$).

(3*R*)-*Z*-**1c**: HPLC on Chiralcel OD (98/2 *n*-hexane/2-propanol, flow 1.5 mL/min): rt 27.1 min, $[\alpha]_D^{25} = +4.8$ (*c* 1.0, $CHCl_3$).

4.8.6. *E*-1c. Yellow oil; IR (film) ν/cm^{-1} 3442, 2976, 2929, 1717, 1607, 1512, 1253, 1178. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21 (t, 3H, $J = 7.2$ Hz), 1.48 (d, 3H, $J = 7.2$ Hz), 1.90 (br s, 1H), 3.81 (s, 3H), 4.09 (q, 1H, $J = 7.2$ Hz), 4.23 (q, 1H, $J = 7.2$ Hz), 6.91 (s, 1H), 6.93 (d, 2H, $J = 8.4$ Hz), 7.33 (d, 2H, $J = 8.4$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 13.8, 22.3, 55.2, 65.0, 70.2, 113.7(2), 127.6, 128.6, 130.0, 133.0, 135.4, 159.1, 168.0. GC–MS rt 19.70 min, *m*/*z*: 250(11), 232(32), 203(25), 189(42), 159(100), 144(68), 128(22), 121(25), 115(60), 89(17), 77(12).

4.9. Characterization of acetates 2a–c

4.9.1. (3*R*)-*Z*-2a. Yellow oil; IR (film) ν/cm^{-1} 2963, 1644, 1466, 1241, 1178, 1097. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (d, 6H, $J = 6.6$ Hz), 1.34 (m, 6H), 2.08 (s, 3H), 3.15 (m, 1H), 4.26 (q, 2H, $J = 7.2$ Hz), 5.65 (q, 1H, $J = 6.6$ Hz) 5.91 (d, 1H, $J = 9.9$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.1, 19.8, 21.2, 22.4, 22.5, 28.2, 60.4, 69.9, 130.5, 147.9, 169.9,170.5. GC–MS rt 12.89 min, *m*/*z*: 213(2), 185(24), 168(35), 143(100), 123(57), 95(83), 79(54), 67(35), 55(28). $[\alpha]_D^{25} = +36.5$ (*c* 1.0, $CHCl_3$).

4.9.2. (3*R*)-*Z*-2b. Yellow oil; IR (film) ν/cm^{-1} 2926, 2852, 1690, 1448, 1368, 1303, 1263, 1222, 1155, 1064. ^1H NMR (200 MHz, $CDCl_3$) δ 0.89–1.25 (m, 5H), 1.26 (t, 3H, $J = 7.0$ Hz), 1.33 (d, 3H, $J = 6.6$ Hz), 1.55–1.77 (m, 5H), 2.02 (s, 3H), 2.80 (m, 1H), 4.19 (q, 2H, $J = 7.0$ Hz), 5.60 (q, 1H, $J = 6.6$ Hz), 5.88 (d, 1H, $J = 10.0$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.1, 19.9, 21.2(2), 25.5(2), 26.0, 32.4, 32.6, 37.8, 60.2, 69.9, 130.8, 146.7, 166.4, 170.1. GC–MS rt 17.40 min, *m*/*z*: 268(2), 225(18), 208(55), 179(63), 162(100), 151(10), 143(95), 133(57), 119(35), 105(33), 91(41), 81(84), 67(38), 55(31). $[\alpha]_D^{25} = +27.2$ (*c* 1.0, $CHCl_3$).

4.9.3. (3*R*)-*Z*-2c. Yellow oil; IR (film) ν/cm^{-1} 2981, 2936, 1735, 1718, 1618, 1607, 1511, 1458, 1370, 1254, 1178, 1055, 1029. ^1H NMR (200 MHz, $CDCl_3$) δ 1.17 (t, 3H, $J = 7.4$ Hz), 1.48 (d, 3H, $J = 6.6$ Hz), 2.05 (s, 3H), 3.79 (s, 3H), 4.17 (q, 2H, $J = 7.4$ Hz), 5.68 (q, 1H, $J = 6.6$ Hz), 6.77 (s, 1H), 6.82 (d, 2H, $J = 9.2$ Hz), 7.23 (d, 2H, $J = 9.2$ Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.2, 20.2, 21.5, 55.5, 61.1, 71.7, 113.9(2), 127.6, 129.8(2), 130.4, 133.6, 160.0, 168.2, 170.2 GC–MS rt 20.60 min, *m*/*z*: 292(3), 249(6), 232(21), 203(49), 187(19), 159(100), 144(62), 128(22), 115(59), 89(14), 77(12). $[\alpha]_D^{25} = +44.3$ (*c* 1.0, $CHCl_3$).

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References

1. Morgan, B.; Oehlschlager, A. C.; Stokes, T. M. *J. Org. Chem.* **1992**, *57*, 3231–3236.
2. (a) Ghanem, A. *Tetrahedron* **2007**, *63*, 1721–1754; (b) Faber, K. In *Biotransformations in Organic Chemistry: A Textbook*; Springer: Berlin, 2004; (c) Schmidt, R. D.; Verger, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 1608–1633.
3. (a) Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129–6139; (b) Burgess, K.; Henderson, I. *Tetrahedron: Asymmetry* **1990**, *1*, A7–A13; (c) Burgess, K.; Cassidy, J.; Henderson, I. *J. Org. Chem.* **1991**, *56*, 2050–2058; (d) Burgess, K.; Jennings, L. *J. Org. Chem.* **1990**, *55*, 1138–1139; (e) Kalaritis, P.; Regenye, R. W.; Partridge, J. J.; Coffen, D. L. *J. Org. Chem.* **1990**, *55*, 812–815; (f) Chadha, A.; Manohar, M. *Tetrahedron: Asymmetry* **1995**, *6*, 651–652.
4. (a) Kappe, C. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250–6284; (b) Kappe, C. O.; Stadler, A. In *Microwaves in Organic and Medicinal Chemistry*; Mannhold, R., Kubinyi, H., Folkers, G., Eds.; Methods and Principles in Medicinal Chemistry; Wiley-VCH: Weinheim, 2005; Vol. 25, (c) Hayes, B. L. *Aldrichim. Acta* **2004**, *37*, 66–76; (d) Hayes, B. L.. *Microwave Synthesis: Chemistry at the Speed of Light*, 1st ed.; CEM: Matthews, NC, 2002.
5. Luche, J.-C. *J. Am. Chem. Soc.* **1978**, *100*, 2226–2227.
6. Chen, C.-S.; Wu, S.-H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812–2817.
7. Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114–120.
8. Ferraboschi, P.; Santaniello, E. *Meth. Biotech.* **2001**, *15*, 291–305.
9. (a) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665; (b) Schulz, T.; Pleiss, J.; Schmidt, R. D. *Protein Sci.* **2000**, *9*, 1053–1062; (c) Zuegg, J.; Hönig, H.; Schrag, J. D.; Cygler, M. *J. Mol. Catal. B: Enzym.* **1997**, 83–98; (d) Rotticci, D.; Orrenius, C.; Hult, K.; Norin, T. *Tetrahedron: Asymmetry* **1997**, *8*, 359–362; (e) Shimizu, M.; Kawanami, H.; Fujisawa, T. *Chem. Lett.* **1992**, 107–110.