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Kinetic resolution of aliphatic acyclic β-hydroxyketones by recombinant whole-cell Baeyer–Villiger monooxygenases—Formation of enantiocomplementary regioisomeric esters

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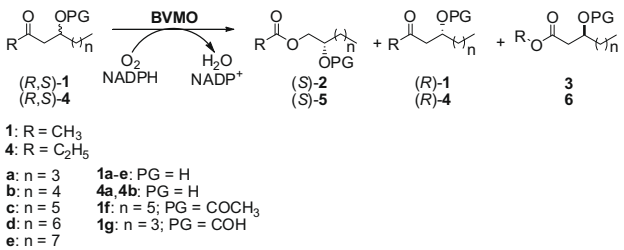
ABSTRACT

A set of various linear aliphatic β-hydroxyketones was investigated as substrates in the enzymatic kinetic and regioselective Baeyer–Villiger oxidation catalyzed by 12 Baeyer–Villiger monooxygenases from different bacterial origin. Excellent enantioselectivities (E >100) could be observed with 4-hydroxy-2-ketones. After acyl migration, the ester undergoes hydrolysis followed by the formation of optically active 1,2-diols. Furthermore, resolution of 5-hydroxy-3-ketones gave access to the 'abnormal' esters, which broadens applicability of these enzymes in organic chemistry. Additionally, it was noticed, that several substrates were converted by different enzymes in an enantiocomplementary way and with high optical purities.

As a powerful methodology in synthesis to break carbon–carbon bonds in an oxygen insertion process,[1] the Baeyer–Villiger oxidation is an appealing reaction in organic synthesis.[2] The transformation of ketones into the corresponding esters or lactones, respectively, gives access to valuable intermediates in organic chemistry on both bulk and fine chemical scale. The enzyme-mediated Baeyer–Villiger oxidation has received increasing attention in recent years.[1,3] Key advantages are chemo-, regio- and stereoselectivity combined with utilization of molecular oxygen as primary oxidant compared to metal-based mediated catalytic strategies.[4,5] Such biocatalytic strategies offer sustainable access to chiral compounds of pharmaceutical, nutritional and industrial interest.[6] During the last years, the number of recombinantly available Baeyer–Villiger monooxygenases (BVMOs) has increased, leading to an enzyme platform representing novel substrate specificities and complementary properties. The exploitation of whole-cell biotransformations using recombinant expression hosts for such NAD(P)H-dependent flavoenzymes[7] is currently of particular popularity in both laboratory and industrial scale applications, as the operational aspects are simple and facile, hence, enabling straight forward utilization in synthetic chemistry.[8]

Recently, we have reported on the first BVMO-catalyzed kinetic resolution of aliphatic acyclic ketones with racemic 4-hydroxy-2-ketones serving as model substrates.[9] We discovered that a BVMO from *Pseudomonas fluorescens* DSM 50106 ($BVMO_{Pfl}$)[10] oxidizes 4-hydroxy-2-octanone-, decanone- and dodecanone with moderate enantioselectivities, giving access to both optically active acetates and hydroxyketones (Scheme 1). Reports on biooxygenations of linear ketones by BVMOs are remarkably limited; only few precendences for the conversion of aryl-aliphatic compounds bearing the carbonyl group in a side chain have been published for arylketone accepting enzymes.[11,12]

In this study we enlarge the number of aliphatic acyclic β-hydroxyketones including 4-hydroxy-2-nonanone and -undecanone as well as the structurally more demanding compounds 5-hydroxy-3-nonanone and -decanone (synthesized after Kourouli



Scheme 1. Kinetic resolution of β-substituted linear ketones using recombinant BVMOs.

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et al.[13]). Furthermore, we extended the toolbox of linear-ketone accepting BVMOs by eleven additional enzymes of various bacterial origin: cyclohexanone monooxygenase ($CHMO_{Acineto}$) from *Acinetobacter calcoaceticus* NCIMB 9871[14] and seven other cyclohexanone monooxygenases ($CHMO_{Arthro}$ from *Arthrobacter* sp.,[15] $CHMO_{Brachy}$ from *Brachymonas* sp.,[16] $CHMO_{Brevi1}$ and $CHMO_{Brevi2}$ from *Brevibacterium* sp.,[17] $CHMO_{Rhodo1}$ and $CHMO_{Rhodo2}$ from *Rhodococcus* sp.[15] and $CHMO_{Xantho}$ from *Xanthobacter* sp. ZL5[18]), as well as cyclopentanone monooxygenase (CPMO) from *Comamonas* sp. NCIMB 9872,[19] 4-hydroxyacetophenone monooxygenase from *Pseudomonas putida* JD1 ($HAPMO_{PpJD1}$)[20] and alkylketone-converting BVMO from *P. putida* KT2440[21] ($BVMO_{PpKT2440}$).

BVMO-mediated oxidations were initially accomplished on screening scale using 24-well plastic dishes in parallel format.[22] On the basis of screening results, individual biotransformations were conducted as time-course experiments with selected biocatalysts in order to investigate the relationship between time, conversion and enantiomeric excess in detail.[23] Enantioselectivity values (*E*; determined by computer fitting)[24] and regioisomeric excess (% re)[25] were calculated to evaluate the efficiency of kinetic resolution and the regioselectivity of the oxygen insertion process.

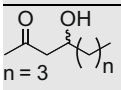
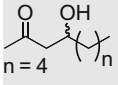
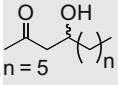
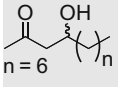
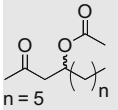
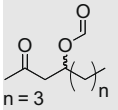
With the exception of $CHMO_{Brevi1}$ and $HAPMO_{PpJD1}$ ketones **1a**–**e** were almost fully converted (*c* >70%) in most cases to the product esters **2a**–**e** within 24 h at 24 °C (data not shown) and short- and middle-chain ketones **1a**–**c** seem to be preferred. This is consistent with the discovered clustering of BVMOs into two distinct groups, the cyclohexanone- and cyclopentanone-type monooxygenases.[26] $CHMO_{Acineto}$ and $BVMO_{Pfl}$ showed best results with conversions between 40% and 60% and ee_P >80% for **1a**–**d**, while for the other BVMOs it seemed that the 'better-fitting' enantiomer is only converted with a slightly higher velocity than the 'less-fitting' enantiomer. Surprisingly, $CHMO_{Acineto}$ and $BVMO_{Pfl}$ showed only low activity against **1e**. The longer chain is possibly too bulky for BVMO active sites. Substrate **1e** was therefore excluded from further experiments.

We also found that in the case of a protection of the free hydroxyl group prior to the biotransformation (incorporation of acetyl **1f** and formyl protecting groups **1g**), substrate acceptance of CHMO-type enzymes decreased significantly. However, CPMO-type BVMOs (CPMO and $CHMO_{Brevi2}$) now display good kinetic resolutions providing access to enantiocomplementary esters compared to the above biooxygenations of the hydroxyl-substrates.

Similar results could be obtained using 5-hydroxy-3-ketones as substrates. Ketones **4a** and **4b** were almost fully oxidized (*c* >70%), but again $CHMO_{Brevi1}$ showed no and $CHMO_{Brevi2}$ only very low conversion. Conversion of **4a** by CPMO stopped at 53% suggesting a higher enantioselectivity compared to **1a**–**d**. Interestingly, the formation of two regioisomers could be observed with $BVMO_{Pfl}$. Either the more substituted or the less substituted carbon center undergoes migration, leading consequently to 'normal' esters **5a** and **5b** and 'abnormal' esters **6a** and **6b** ester (Scheme 1). Such regiodivergent biooxygenations have been observed in previous studies on cyclic systems.[27–30] A mechanistic relationship has been proposed for this behavior based on stereoelectronic effects.[31]

In order to optimize enantiomeric excesses and enantioselectivities of products **2a**–**d**, **5a** and **5b** within the kinetic resolution process, time-course experiments were performed to stop conversion close to 50% (for optimized resolution results). For these experiments selected biocatalysts were used that showed satisfactory results in pre-screening experiments as described above: $CHMO_{Acineto}$

Table 1
Microbial Baeyer–Villiger oxidations of β-hydroxy-2-ketones **1a**–**d** and β-protected ketones **1f** and **1g** using recombinant whole-cells of *E. coli* expressing BVMOs from different bacterial origin

Substrate	BVMO	*t* (h)	*c*[a] (%)	ee_S[b] (%)	ee_P[b] (%)	abs. conf.[c]	*E*[d]
 **1a**	$CHMO_{Acineto}$	8	49	91	96	(*S*)	156
	CPMO	3	53	64	56	(*S*)	7
	$BVMO_{Pfl}$	24	46	85	>99	(*S*)	>200
 **1b**	$CHMO_{Acineto}$	20	49	96	>99	(*S*)	>200
	$CHMO_{Xantho}$	10	47	84	95	(*S*)	103
	CPMO	2	44	48	62	(*S*)	7
	$BVMO_{Pfl}$	8	48	89	98	(*S*)	>200
 **1c**	$CHMO_{Acineto}$	20	50	92	93	(*S*)	90
	CPMO	2	44	39	50	(*S*)	4
	$BVMO_{Pfl}$	6	41	68	96	(*S*)	100
 **1d**	$CHMO_{Acineto}$	30	20	13	52	(*S*)	4
	$CHMO_{Arthro}$	20	55	62	50	(*S*)	6
	$CHMO_{Rhodo2}$	10	18	17	80	(*S*)	10
	CPMO	2	65	37	20	(*S*)	2
 **1f**	$CHMO_{Acineto}$	24	<5	n.d.	n.d.	n.a.	n.d.
	CPMO	24	25	45	>99	(*R*)	>200
	$CHMO_{Brevi2}$	20	23	41	91	(*R*)	31
 **1g**	$CHMO_{Acineto}$	24	42	25	33	(*S*)	3
	$CHMO_{Xantho}$	24	57	73	66	(*S*)	10
	CPMO	24	50	99	99	(*R*)	>200
	$CHMO_{Brevi2}$	24	28	27	>99	(*R*)	>200

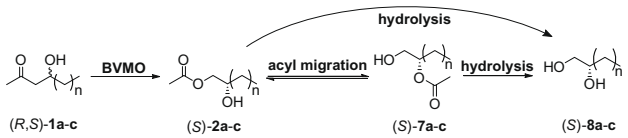
Reactions were monitored over 48 h. Syntheses of **2a** and **2c** as standards for GC analysis were performed enzymatically by esterification of 1,2-diols with vinyl acetate by immobilized *Candida antarctica* lipase B as described by Kirschner and Bornscheuer.[9] Syntheses of **2b** and **2d** was accomplished as described in Ref. 2. Spectral data are shown in Ref. 33. Syntheses of **1f** and **1g** were performed from β-hydroxyketones according standard procedures.
n.d. = not determined; n.a. = not applicable.

[a] Conversion calculated from % ee_S (enantiomeric excess of substrate) and % ee_P (enantiomeric excess of product).

[b] % ee_S and % ee_P were determined by chiral phase GC and calculated according to Chen et al.[34]

[c] Absolute configurations of hydroxyalkyl acetates **2a**–**d** were determined by comparison with (*R*)-2-hydroxydecyl acetate ((*R*)-**2e**), which was synthesized from (*R*)-1,2-decandiol by using lipase-catalyzed transesterification. Since **2a**–**d** are structurally related (homologue series) the same configuration is assumed for **2a**–**c**.[9]

[d] Enantioselectivity values were determined by computer fitting of GC data from % ee_S and % ee_P.



Scheme 2. Kinetic resolution of **1a–c** followed by direct ester hydrolysis or via acyl migration and ester bond cleavage releasing optically active 1,2-diols **8a–c**.

and CPMO as two 'benchmark' catalysts, reflecting the key features of the two family clusters among BVMOs, CHMO$_{Xantho}$, CHMO$_{Rhodo2}$, CHMO$_{Brachy}$, CHMO$_{Arthro}$, CHMO$_{Brevi1}$ and BVMO$_{Pfl}$. Results obtained from biotransformations with **1a–d** are compiled in Table 1.

All investigated enzymes oxidized the (*S*)-enantiomer of **1a–d,** whereas biotransformations of **1a–c** with CHMO$_{Acineto}$ and BVMO$_{Pfl}$ lead to very high enantioselectivities. Even after 48 h the (*R*)-hydroxyketone is not converted at all and reaction rate is stopped at 50%. In comparison, CPMO oxidized ketones very fast (less than 3 h for 50% conversion), but showed nearly no selectivity. The highest *E*-value of 7 could be achieved for **2a** and **2b**. Investigating the effect of chain-length indicated that enantioselectivity decreases (compare CHMO$_{Acineto}$ in Table 1) from **1a** with eight carbon atoms to **1d** with eleven carbon atoms. Tentatively, the chain-length and therefore the increase in hydrophobicity are criteria for selectivity.

Interestingly, kinetic resolution of 4-hydroxy-2-ketones can be used to generate two different chemical species, which additionally differ in their configuration. Besides the residual optically active hydroxyketones (*R*)-**1a–d**, optically active hydroxyalkyl esters (*S*)-**2a–d** are formed, which in case of **2a**, **2b** and **2c** undergo acyl migration to form optically pure acetates of 1,2-diols **7a–c** (Scheme 2).

The equilibrium between **2a–c** and **7a–c** is shifted towards the production of the acylated 1,2-diol after 24 h reaction time followed by hydrolysis of the ester bond yielding in enantiomerically pure 1,2-diols **8a–c**. Since acyl migration proceeds without any conformational changes, all 1,2-diols possess the same configuration as the hydroxyalkyl acetates. The maximum amount of generated 1,2-diol was 55% after 48 h using **1c** as starting material and BVMO$_{Pfl}$ as biocatalyst (compare Fig. 1).

Indeed, this feature makes this biotransformation especially useful and complementary to bioreductions by highly regio- and stereospecific ketoreductases.[35,36] The alternative kinetic resolution of 1,2-diols using lipases or esterases proceeds so far with low selectivity.[37–39]

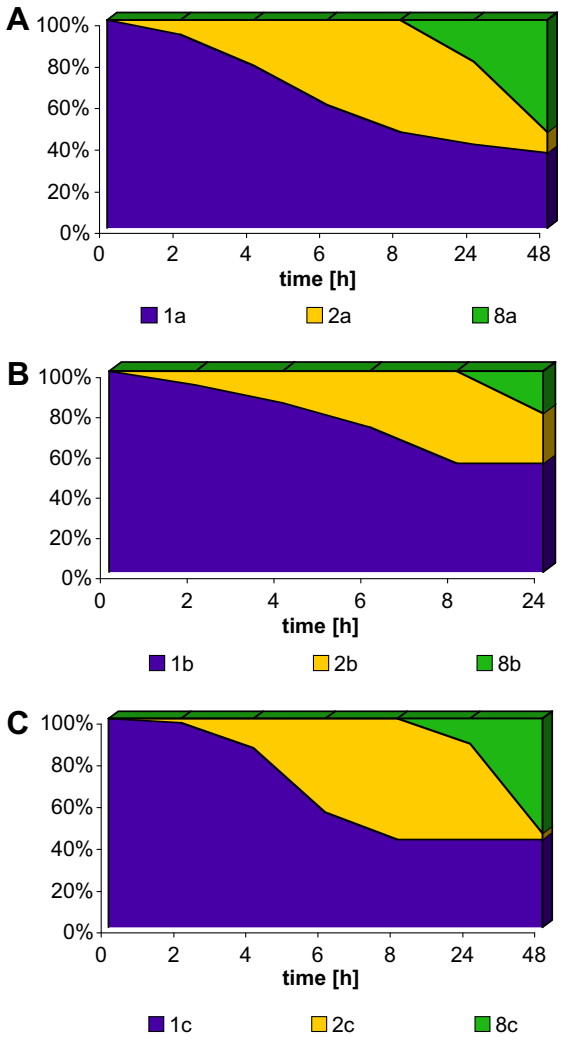
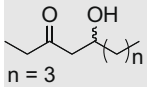
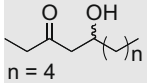


Figure 1. Enzymatic formation of 1,2-diols **8a–c** via kinetic resolution of 4-hydroxy-2-ketones **1a–c** at 24 °C after 24 and 48 h with (A) **1a** and CHMO from *A. calcoaceticus* NCIMB 9871, (B) **1b** an CHMO from *A. calcoaceticus* NCIMB 9871 and (C) **1c** and BVMO from *P. fluorescens* DSM 50106, both enzymes expressed in *E. coli*.

Oxidation of **4a** and **4b** showed different results (summarized in Table 2). In this case, enantioselectivity among utilized BVMOs is opposite compared to **1a–d**. While for CHMO$_{Acineto}$ and BVMO$_{Pfl}$

Table 2
Microbial Baeyer–Villiger oxidations of β-hydroxy-3-ketones **4a** and 4**b** using recombinant whole-cells of *E. coli* expressing BVMOs from different bacterial origin

Substrate		BVMO	*t* (h)	*c*[a] (%)	ee$_S$[b] (%)	ee$_P$[b] (%)	*E*[c]	re[d] (%)
 n = 3	**4a**	CHMO$_{Acineto}$	10	46	75	88	35	–
		CHMO$_{Xantho}$	10	53	81	72	15	–
		CHMO$_{Brachy}$	8	58	>99	70	40	–
		CPMO	24	50	>99	96	>200	–
		BVMO$_{Pfl}$	10	32	25	53	4	64
 n = 4	**4b**	CHMO$_{Acineto}$	8	40	46	69	8	–
		CHMO$_{Xantho}$	8	44	61	78	14	–
		CHMO$_{Brachy}$	6	42	57	75	12	–
		CPMO	10	48	81	87	35	–
		BVMO$_{Pfl}$	24	55	>99	82	74	40

Reactions were monitored over 48 h. For syntheses of product standards see Ref. 32. Spectral data are shown in Ref. 33.
[a] Conversion calculated from % ee$_S$ (enantiomeric excess of substrate) and % ee$_P$ (enantiomeric excess of product).
[b] % ee$_S$ and % ee$_P$ were determined by chiral phase GC and calculated according to Chen et al.[34]
[c] Enantioselectivity values were determined by computer fitting of GC data from % ee$_S$ and % ee$_P$.
[d] Percent regioisomeric excess in favor of 'abnormal' ester determined by chiral phase GC.

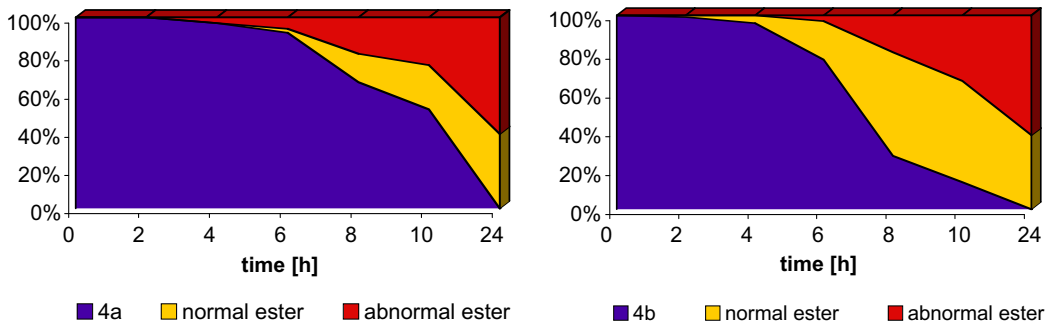


Figure 2. Regioselective Baeyer–Villiger oxidation of 5-hydroxy-3-ketones **4a** and **4b** using engineered *E. coli* cells expressing BVMO from *P. fluorescens* DSM 50106.

E-values are between 4 and 40, CPMO now showed a very high and even reverse selectivity for **4a** (*E* >200). Thus, implementing $CHMO_{Brachy}$ and CPMO for kinetic resolution of **4a** gives access to both enantiomers of **5a** in high optically purity. This fact makes this reaction a powerful and interesting tool in organic chemistry.

Furthermore, it could be observed that the location of the keto group in the molecule influences regioselectivity of the enzymes. While the methyl ester ('abnormal' product) was formed only up to 10% (data not shown) for 4-hydroxy-2-ketones this fact changed using 5-hydroxy-3-ketones. $BVMO_{Pfl}$ even generated 64% or 40% in favor of the 'abnormal' ester in case of **4a** and **4b**, respectively (Fig. 2). This is the only option to synthesize 'abnormal' esters enzymatically to our knowledge until now. This observation underscores the powerful capabilities of BVMOs and not only broadens their synthetic applicability, but also provides a synthetically useful alternative to already established chemical reactions in organic chemistry.

Based on our observations, aliphatic open-chain β-hydroxyketones are good substrates for most of the BVMOs recombinantly available so far, especially for those previously described as cycloketone converting enzymes. The possibility to synthesize enantiopure 1,2-diols enzymatically is noteworthy, since these compounds are of special interest for example, in organic industry for the synthesis of polyesters but also in medical treatment as antimicrobial agents.[40] Furthermore, 1,2-diols are predominantly synthesized chemically, so far; thus, kinetic resolution of 4-hydroxy-2-ketones using BVMOs can accomplish a considerable contribution for environmental protection.

The fact that some BVMOs are capable to generate the 'abnormal' Baeyer–Villiger product with high enantioselectivity offers new possibilities for the synthesis of natural products. Together with the enantiocomplementary conversion of ketones by different enzymes it displays the potential of the natural diversity to provide suites of catalysts for chemical operations. Currently, further studies on the regioselectivity of BVMOs are in progress in our laboratories.

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21. Rehdorf, J.; Kirschner, A.; Bornscheuer, U. T. *Biotechnol. Lett.* **2007**, *29*, 1393.
22. *Typical procedure for screening experiments*—Precultures were inoculated with a single colony from a plate and incubated at 37 °C overnight (in case of CHMO from *Xanthobacter* sp. at 30 °C) in an orbital shaker in a baffled Erlenmeyer flask. LB_{amp} ($LB_{amp/chl}$ for HAPMO from *P. putida* JD1) was inoculated with 1% of the overnight preculture and incubated at 37 or 30 °C, respectively, until OD_{600nm} reached 0.5–0.7. Then, protein expression was induced with either IPTG (0.1 mM final concentration) or in case of BVMO from *P. putida* KT2440 with L-rhamnose (0.2% final concentration). Protein expression was performed at 24 °C for 2 h. Screening was performed in 24-well plastic plates (*Greiner BioOne company*). 1 mL bacterial culture was transfered into each well (max. volume of 2 mL) and substrate was added (2.5 mM final concentration). Biotransformations were carried out at 24 °C and analyzed after 24 h. Therefore, samples were extracted with ethyl acetate supplemented with 1.2 mM of an internal standard (benzoic acid methylester) and dried over sodium sulfate.
23. *Typical procedure for time-course experiments*—Biotransformations were performed as described for screening experiments, but here samples were taken at certain time intervals (2, 4, 6, 8, 10, 24, 30 and 48 h), extracted and analyzed via GC.
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25. Percent regioisomeric excess (% re) is defined as the percentage of the major regioisomer (both enantiomers) minus the percentage of the minor regioisomer (both enantiomers).
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32. *Preparative scale biotransformations and enzymatic synthesis of **2b**, **2d**, **5a**, **5b**, **6a** and **6b***—Baffled Erlenmeyer flask with LB_{amp} (500 mL) was inoculated with an overnight bacterial culture, IPTG was added at OD 0.6 (0.1 mM final concentration) together with substrate (50 mg). Flasks were shaken at 24 °C for 24 h, then biomass was removed by centrifugation, the aqueous phase was saturated with sodium chloride and extracted five times with ethyl acetate. Organic layers were combined, dried over sodium sulfate and concentrated. Esters were purified by silica gel chromatography.
33. *Physical and spectral data of* **4a**: Obtained from 3-oxoheptanoate and *n*-valeraldehyde as a colorless liquid (685 mg, 84%); δ_H (300 MHz, $CDCl_3$) 0.90 (t, *J* = 6.9 Hz, 3H), 1.07 (t, *J* = 6.6 Hz, 3H), 1.23–1.55 (m, 6H), 2.42–2.58 (m, 2H), 2.59 (q, *J* = 3 Hz, 2H), 4.0–4.07 (m, 1H); δ_C (50 Hz, $CDCl_3$) 7.5 (q), 14.0 (q), 22.6 (t), 27.6 (t), 36.1 (t), 36.8 (t), 48.6 (t), 67.7 (d), 212.8 (s). Physical and spectral data of **4b**: obtained from 3-oxoheptanoate and capronaldehyde as a colorless liquid (220 mg, 60%); specific rotation of sample obtained in enzyme mediated kinetic resolution: $[\alpha]_D^{20} = -34.8$ (*c* 0.34, $CHCl_3$; ee = 93%); δ_H (300 MHz, $CDCl_3$) 0.89 (t, *J* = 7 Hz, 3H), 1.06 (t, *J* = 7.4 Hz, 3H), 1.25–1.52 (m, 8H), 2.48–2.67 (m, 2H), 2.55 (q, *J* = 4 Hz, 2H), 4.0–4.07 (m, 1H); δ_C (50 MHz, $CDCl_3$) 7.9 (q), 14.1 (q), 22.7 (t), 24.8 (t), 31.4 (t), 32.1 (t), 36.2 (t), 48.6 (t), 67.6 (d), 211.6 (s). Physical and spectral data of **2b**: colorless liquid (15 mg, 30%); δ_H (200 MHz, $CDCl_3$) 0.85 (t, *J* = 8 Hz, 3H), 1.29-1.45 (m, 8H), 2.09 (s, 3H), 3.83-3.95 (m, 1H), 3.98-4.1 (m, 2H); δ_C (50 MHz, $CDCl_3$) 14.1 (q), 21.0 (q), 22.7 (t), 25.1 (t), 31.9 (t), 33.4 (t), 68.9 (d), 70.0 (t), 171.4 (s). Physical and spectral data of **2d:** colorless liquid (20 mg, 40%); δ_H (200 MHz, $CDCl_3$) 0.86 (t, *J* = 9 Hz, 3H), 1.26–1.49 (m, 12H), 2.1 (s, 3H), 3.81–3.90 (m, 1H), 4.0–4.18 (m, 2H); δ_C (50 MHz, $CDCl_3$) 14.3 (q), 21.1 (q), 22.8 (t), 25.5 (t), 29.3 (t), 29.7 (t), 31.9 (t), 33.5 (t), 68.9 (d), 70.1 (t), 171.4 (s). Physical and spectral data of **5a**: colorless liquid (24 mg, 48%); δ_H (200 MHz, $CDCl_3$) 0.9 (t, *J* = 10 Hz, 3H), 1.14 (t, *J* = 8 Hz, 3H), 1.25–1.44 (m, 6H), 2.29 (q, 18 Hz, 2H), 3.74–3.87 (m, 1H), 4.0–4.36 (m, 2H); δ_C (50 MHz, $CDCl_3$) 9.4 (q), 14.1 (q), 23.1 (t), 27.2 (t), 27.6 (t), 32.2 (t), 70.9 (d), 71.2 (t), 171.4 (s). Physical and spectral data of **6a**: colorless liquid (23 mg, 46%); specific rotation of sample obtained in enzyme mediated kinetic resolution: $[\alpha]_D^{20}$ = -18.2 (c 0.43, $CHCl_3$; ee = 93%); δ_H (200 MHz, $CDCl_3$) 0.91 (t, *J* = 11 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.31–1.46 (m, 6H), 2.32–2.56 (m, 2H), 3.89–4.0 (m, 1H), 4.12–4.23 (q, *J* = 7.4 Hz, 2H); δ_C (50 MHz, $CDCl_3$) 12.9 (q), 13.2 (q), 21.6 (t), 26.6 (t), 35.2 (t), 40.3 (t), 59.7 (d), 67.0 (t), 172.1 (s). Physical and spectral data of **5b**: colorless liquid (17 mg, 34%); specific rotation of sample obtained in enzyme mediated kinetic resolution: $[\alpha]_D^{20}$ = +2.2 (*c* 0.74, $CHCl_3$; ee = 93%); δ_H (200 MHz, $CDCl_3$) *J* = 0.91 (t, 6 Hz, 3H), 1.15 (t, *J* = 7.6 Hz, 3H), 1.24–1.45 (m, 8H), 2.35 (q, *J* = 5.4 Hz, 2H), 3.8–3.92 (m, 1H), 3.91–4.19 (m, 2H); δ_C (50 MHz, $CDCl_3$) 9.4 (q), 14.1 (q), 22.7 (t), 24.8 (t), 27.6 (t), 32.1 (t), 32.5 (t), 70.8 (d), 71.2 (t), 174,1 (s). Physical and spectral data of **6b**: colorless liquid (25 mg, 50%); specific rotation of sample obtained in enzyme mediated kinetic resolution: $[\alpha]_D^{20} = -20.5$ (*c* 0.69, $CHCl_3$; ee >99%); δ_H (200 MHz, $CDCl_3$) 0.88 (t, *J* = 6.4 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.31–1.43 (m, 8H), 2.4–2.48 (m, 2H), 3.95–4.03 (m, 1H), 4.06–4.19 (m, 2H). δ_C (50 MHz, $CDCl_3$) 14.0 (q), 14.2 (q), 21.0 (t), 22.6 (t), 25.1 (t), 31.7 (t), 36.5 (t), 41.3 (t), 60.7 (d), 173.1 (s).
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