Stereodivergent Preparation of Valuable γ- or δ-Hydroxy Esters and Lactones through One-Pot Cascade or Tandem Chemoenzymatic Protocols

Alba Díaz-Rodríguez,‡ Wioleta Borzęcka,‡ Iván Lavandera,* and Vicente Gotor*

Departamento de Química Orgánica e Inorgánica, Universidad de Oviedo, Instituto Universitario de Biotecnología de Asturias, C/Julián Clavería 8, 33006 Oviedo, Spain

e-mail: lavanderaivan@uniovi.es (I.L.) and vgs@uniovi.es (V.G.)

‡ Both authors equally contributed to this work.

Supporting Information (page S1 of S15)

Table of contents

1. General considerations

Ethyl 5-oxohexanoate (**1b**), methyl levulinate (**1c**), ethyl levulinate (**1d**), methyl 4-oxo-4-phenylbutyrate (**1f**), δ-hexalactone (**3a**), γ-valerolactone (**3c**), and γ-phenyl-γ-butyrolactone (**3e**) were obtained from Alfa Aesar, Fluka, Acros or Aldrich, and were used as supplied unless otherwise indicated. Methyl 5-oxohexanoate (**1a**),[1] methyl 5-oxo-5-phenylpentanoate (**1e**),[2] and methyl 4-(4-bromophenyl)-4-oxobutanoate (**1g**)[3] were synthesized by simple esterification of the corresponding carboxylic acids.

In addition to those specified above, the following abbreviations, designations and formulas are used throughout the Supporting Information: 2-PrOH= isopropanol; Na_2SO_4= sodium sulfate; K_2CO_3 = potassium carbonate; Et_2O= diethyl ether.

2. Experimental procedures

2.1. General procedure for the synthesis of racemic hydroxy esters 2a-g

To a solution of the corresponding keto ester **1a-g** (4.0 mmol) in methanol (5 mL), sodium borohydride (1.2 mmol, 4.8 equiv.) was added portionwise at 0˚C. The reaction mixture was allowed to warm up to room temperature. When the reduction was completed (according to the TLC) the solvent was carefully evaporated under reduced pressure. Further purification was not necessary for **2f**, and flash chromatography column was necessary (1% MeOH/CH_2Cl_2) for the preparation of **2a**, **2b**, **2d**, **2e** and **2g** obtaining the hydroxy esters with yields >90%.

These compounds exhibited physical and spectral data in agreement with those reported: **2a**,[4] **2b**,[5] **2d**,[6] **2e**,[7] **2f**,[8] and **2g**.[8,9]

2.2. General procedure for the synthesis of racemic lactones 3e-g

To the corresponding solution of hydroxy ester **2e-g** (4.0 mmol) in methanol (5 mL), a solution of HCl 1 M was added (5 mL). The mixture was stirred overnight at room temperature. Then, the solvent was carefully evaporated under reduced pressure 10 mL of H_2O were added and the solution was extracted with Et_2O (3 x 10 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated and further purification was not necessary (isolated yields: >90%).

These compounds exhibited physical and spectral data in agreement with those reported: **3e**,[10] **3f**,[11] and **3g**.[11]

2.3. ADH-catalyzed reductions

2.3.1 Protocol using ADH from **Rhodococcus ruber** *(***E. coli***/ADH-A)*

In a 10 mL vial, *E. coli*/ADH-A (50 mg) was resuspended in Tris-HCl buffer (50 mM, pH 5.0-9.0, 2.28 mL, 1 mM NADH), 2-PrOH (120 μL, 5% v v^{-1}) and the corresponding keto ester (50 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with Et_2O (3 x 2 mL). The organic layers were combined and dried over

Na_2SO_4. The solvent was carefully evaporated and the conversion of the corresponding hydroxy ester or lactone was determined by NMR and the *ee* by GC or HPLC.

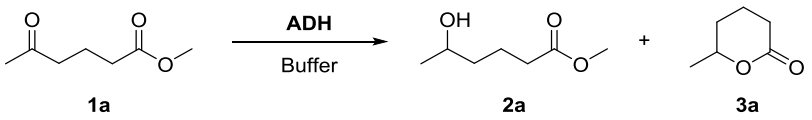
2.3.2. Protocol using ADH from Sphingobium yanoikuyae *(*E. coli*/SyADH)*

In a 10 mL vial, *E. coli*/SyADH (50 mg) was resuspended in Tris-HCl buffer 50 mM pH 9.0 (570 μL, 1 mM NADPH), 2-PrOH (120 μL, 5% v v^{-1}) and the corresponding keto ester (50 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with Et_2O (3 x 2 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was carefully evaporated and the conversion of the corresponding hydroxy ester or lactone was determined by NMR and the *ee* by GC or HPLC.

2.3.3. Protocol using ADH from Thermoanaerobacter ethanolicus *(*E. coli*/TeSADH)*

In a 10 mL vial, *E. coli*/TeSADH (50 mg) was resuspended in Tris-HCl buffer (50 mM, pH 9.0, 2.28 mL, 1 mM NADPH), 2-PrOH (120 μL, 5% v v^{-1}) and the corresponding keto ester (50 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with Et_2O (3 x 2 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was carefully evaporated and the conversion of the corresponding hydroxy ester or lactone was determined by NMR and the *ee* by GC or HPLC.

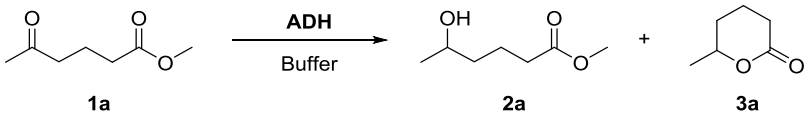
Table S1. Enzymatic screening with keto ester **1a** at pH 9.0 and 30 ºC (*t*= 24 h).



enzyme	**2a**		**3a**	
	***c* (%)**[a]	***ee* (%)**[b]	***c* (%)**[a]	***ee* (%)**[b]
LBADH	82	>99 (*R*)	18	>99 (*R*)
ADH-A	81	>99 (*S*)	19	>99 (*S*)
E. coli/RasADH	32	n.d.	2	n.d.
E. coli/SyADH	73	88 (*S*)	27	88 (*S*)
E. coli/TeSADH	76	99 (*S*)	24	99 (*S*)

[a] Measured by NMR. [b] Measured by chiral GC.

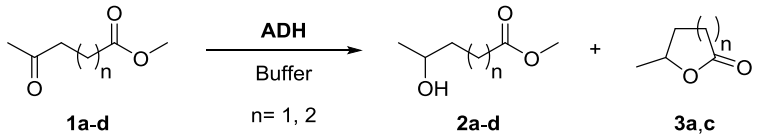
Table S2. pH effect in the bioreduction of keto ester **1a** at 30ºC (*t*= 24 h).



pH	**LBADH**				**ADH-A**			
	2a		**3a**		**2a**		**3a**	
	***c* (%)**[a]	***ee* (%)**[b]	***c* (%)**[a]	***ee* (%)**[b]	***c* (%)**[a]	***ee* (%)**[b]	***c* (%)**[a]	***ee* (%)**[b]
5.0	47	>99 (*R*)	<3	n.d.	45	>99 (*S*)	<3	n.d.
7.5	91	>99 (*R*)	<3	n.d.	68	>99 (*S*)	<3	n.d.
9.0	82	>99 (*R*)	18	>99 (*R*)	81	>99 (*S*)	19	>99 (*S*)

[a] Measured by NMR. [b] Measured by chiral GC.

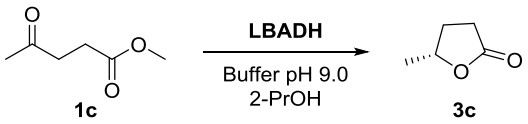
Table S3. Substrate structure effect in the bioreduction of keto esters **1a-d** at pH 9.0 and 30 ºC (*t*= 24 h).



1	**LBADH**				**ADH-A**			
	2		**3**		**2**		**3**	
	***c* (%)**[a]	***ee* (%)**[b]	***c* (%)**[a]	***ee* (%)**[b]	***c* (%)**[a]	***ee* (%)**[b]	***c* (%)**[a]	***ee* (%)**[b]
a	82	>99 (*R*)	18	>99 (*R*)	81	>99 (*S*)	19	>99 (*S*)
b	95	>99 (*R*)	5	>99 (*R*)	79	>99 (*S*)	7	>99 (*S*)
c	<3	n.d.	>97	>99 (*R*)	<3	n.d.	>97	99 (*S*)
d	35	>99 (*R*)	65	>99 (*R*)	24	99 (*S*)	76	99 (*S*)

[a] Measured by NMR. [b] Measured by chiral GC.

2.4. Synthesis of (R)-GVL (3c) with LBADH at higher substrate concentrations



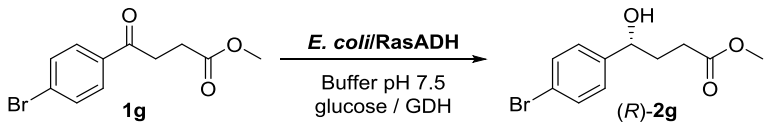
In a 10 mL vial, LBADH (3 U) was dissolved in Tris-HCl buffer (50 mM, pH 9.0, 2.16 mL, 1 mM $NADP^+$, 1 mM $MgCl_2$), 2-PrOH (240 μL, 10% v v^{-1}) and **1c** (100-200 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with Et_2O (3 x 2 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was carefully evaporated and the conversion of the reaction was determined by NMR and the *ee* of **3c** by chiral GC.

Table S4. Conversions and *ee* in the LBADH-catalyzed reduction of **1c** at different substrate concentrations.

conc. (mM)	**ratio[a] (*ee*)[b]**		
	1c	**(*R*)-2c**	**(*R*)-3c**
50	<3	<3	>99 (>99)
100	6	<3	94 (98)
200	21	<3	79 (95)

[a] Measured by NMR. [b] Measured by chiral GC.

2.5. Enzymatic reduction of 1g with **E. coli***/RasADH at different substrate concentrations*



2.5.1. Protocol using 50 mM substrate concentration

In a 10 mL vial, *E. coli*/RasADH (25 mg) was added to Tris-HCl buffer (50 mM, pH 7.5, 1.14 mL, 1 mM NADPH), with glucose (100 mM), GDH (20 U), 2-PrOH (60 µL, 5% v v^{-1}), and **1g** (50 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with Et_2O (3 x 1 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated and the conversion of the corresponding alcohol was determined by NMR and the *ee* by chiral HPLC.

2.5.2. Protocol using 100 mM substrate concentration

In a 10 mL vial, *E. coli*/RasADH (25 mg) was dissolved in Tris-HCl buffer (50 mM pH 7.5, 540 µL, 1 mM NADPH), with glucose (200 mM), GDH (20 U), 2-PrOH (60 µL, 10% v v^{-1}), and **1g** (100 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with Et_2O (3 x 0.5 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated and the conversion of the corresponding alcohol was determined by NMR and the *ee* by chiral HPLC.

2.5.3. Protocol using 200 mM substrate concentration

In a 10 mL vial, *E. coli*/RasADH (25 mg) was added to Tris-HCl buffer (50 mM, pH 7.5, 540 µL, 1 mM NADPH) and treated with glucose (400 mM), GDH (40 U), 2-PrOH (60 µL, 10% v v^{-1}), and **1g** (200 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with Et_2O (3 x 10 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated and the conversion of the corresponding alcohol was determined by NMR and the *ee* by chiral HPLC.

Table S5. Conversions and *ee* in the RasADH-catalyzed reduction of **1g** at different substrate concentrations.

conc. (mM)	ratio[a] (*ee*)[b]		
	1g	(***R***)-**2g**	(***R***)-**3g**
50	<3	99 (94)	<3
100	<3	98 (91)	<3
200	32	68 (91)	<3

[a] Measured by NMR. [b] Measured by chiral HPLC.

2.6. General procedures for the Suzuki coupling of 3g and phenylboronic acid

Protocol A: The corresponding brominated lactone **3g** (0.081 mmol) and phenylboronic acid (0.131 mmol) were dissolved in a solution of Tris-HCl buffer 50 mM pH 7.5 (4 mL) containing 2-PrOH (400 μL, 10% v v^{-1}). $Pd(PPh_3)_2Cl_2$ (2.4 mg, 0.004 mmol) was then added and the mixture was shaken at 45ºC for 24 h. The reaction mixture was extracted with EtOAc (3 x 10 mL). The organic layers were combined and dried over Na_2SO_4. The crude residue was purified by column chromatography (20% EtOAc/hexane) isolating the corresponding lactone **4g** in 80% yield. Spectral data for **4g** were consistent with those reported in the literature.[11a]

Protocol B: The corresponding brominated lactone **3g** (0.081 mmol) and phenylboronic acid (0.131 mmol) were dissolved in 2-PrOH (4 mL). To the stirred solution, K_2CO_3 was added (23 mg, 0.166 mmol) and then $Pd(PPh_3)_2Cl_2$ (2.4 mg, 0.004 mmol, 5%). The reaction mixture was shaken at 45ºC for 24 h. Then the purification and isolation steps were the same as for protocol A obtaining a comparable yield.

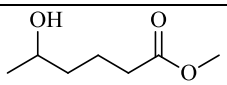
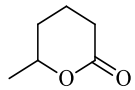
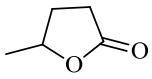
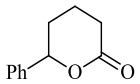
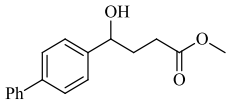
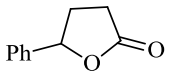
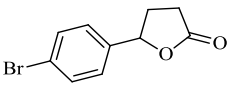
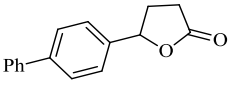
Protocol C: The corresponding brominated lactone **3g** (0.081 mmol) and phenylboronic acid (0.131 mmol) were dissolved in 2-PrOH (4 mL) in a microwave sealed tube. To the stirred solution K_2CO_3 (23 mg, 0.166 mmol) and then $Pd(PPh_3)_2Cl_2$ (2.4 mg, 0.004 mmol, 5%) were added. The reaction mixture was irradiated in a sealed tube at 85ºC for 30 min using a standard microwave apparatus. Then the purification and isolation steps were the same as for protocol A obtaining a comparable yield.

3. Analytical data

3.1. GC data

The following chiral GC columns were used for determining the *ee* of hydroxy esters and/or lactones: **A**: Restek RT-GAMMA DEXsa (30 m x 0.25 mm x 0.25 µm, 12.2 psi N_2); **B**: Restek RT-BetaDEXse (30 m x 0.25 mm x 0.25 µm, 12.2 psi N_2); **C**: Hewlett Packard HP1 (30 m x 0.32 mm x 0.25 µm, 12.2 psi N_2; and **D**: Varian Chirasil Dex CB (25 m x 0.25 mm x 0.25 µm, 12.2 psi N_2).

Table S6. Analytical separation of hydroxy esters and lactones by chiral GC.

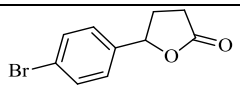
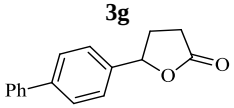
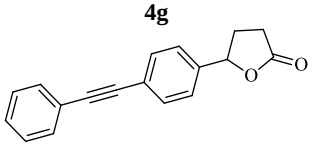
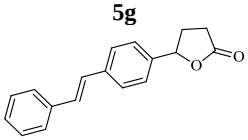
compound	column	method[a]	retention time (min)	
			R	***S***
 **2a**	A	120/25/20/180/2	22.2	22.8
 **3a**	A	120/50/20/200/2	45.8	46.0
 **3c**	B	32/5/20/200/5	12.3	12.4
 **3e**	B	110/0/3/140/10/3/170/10/3/200/2	47.1	47.4
 **2f**	C	90/3/10/220/5	16.1	
 **3f**	B	110/0/3/140/10/3/170/10/10/200/2	35.7	36.7
 **3g**	D	90/3/10/220/5	18.1	18.2
 **4g**	C	90/3/10/220/5	18.7	

[a] GC program: initial temp. (°C)/ time (min)/ slope (°C/min)/ temp. (°C)/ time (min)/ slope (°C/min)/ final temp. (°C)/ time (min)/slope (°C/min)/ final temp. (°C)/ time (min).

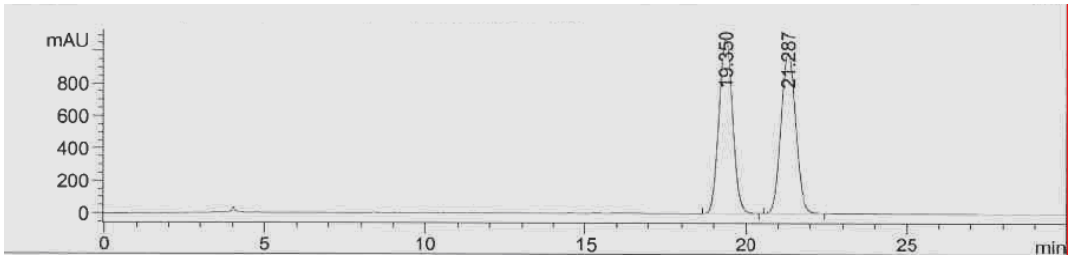
3.2. HPLC data

The following HPLC column was used **D**: Chiralpak IC detected at 210 nm (25 cm x 4.6 mm I.D, Daicel Chemical Ind. Ltd.) using 70:30 (*n*-hexane/2-PrOH) as eluent, 30ºC and 0.8 mL/min.

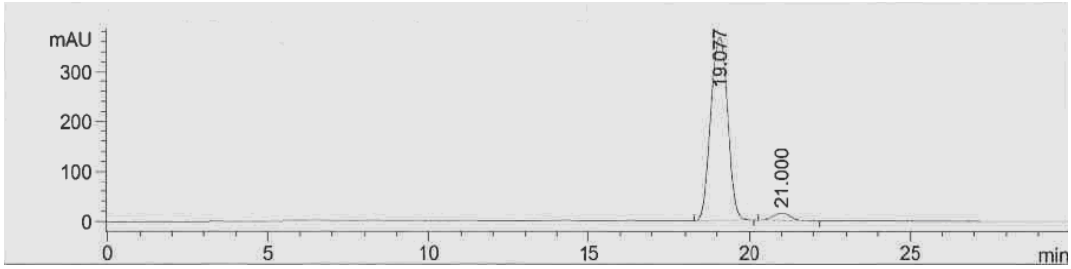
Table S7. Analytical separation of hydroxy esters and lactones by chiral HPLC.

compound	retention time (min)
 **3g**	13.9 (*R*), 14.7 (*S*)
 **4g**	19.4 (*R*), 21.3 (*S*)
 **5g**	14.3(*R*), 15.6(*S*)
 **6g**	22.4 (*R*), 23.4 (*S*)

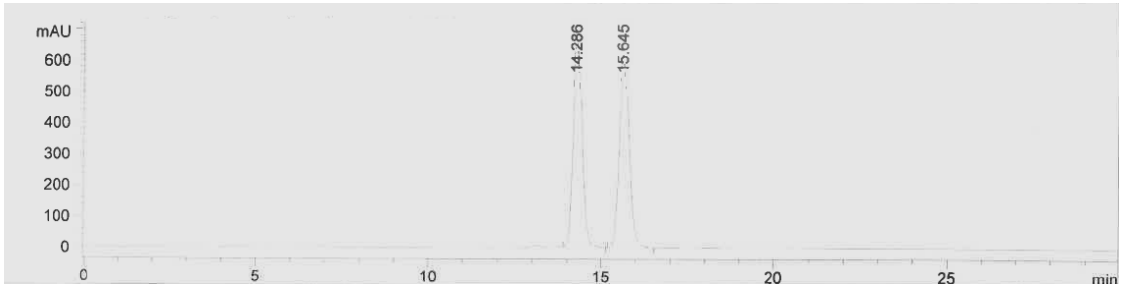
rac-**4g**



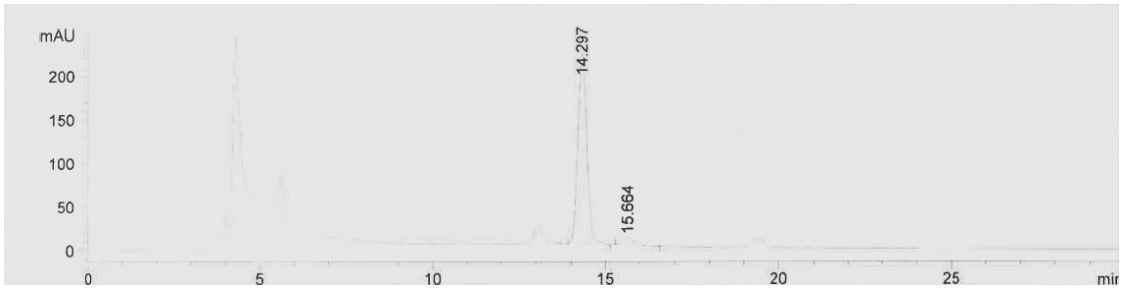
(*R*)-**4g**



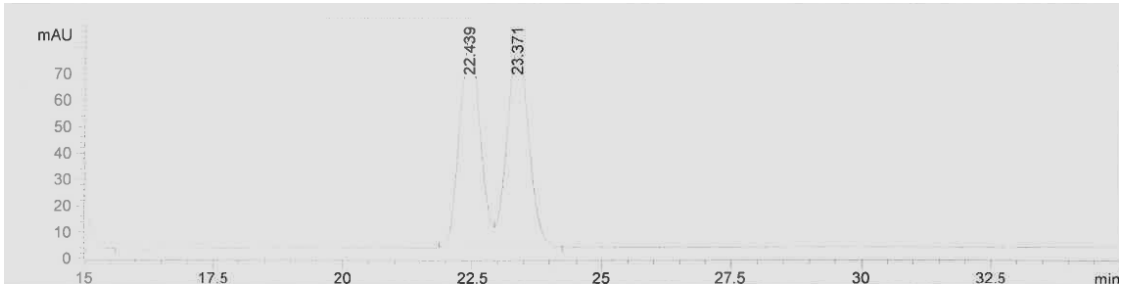
rac-**5g**



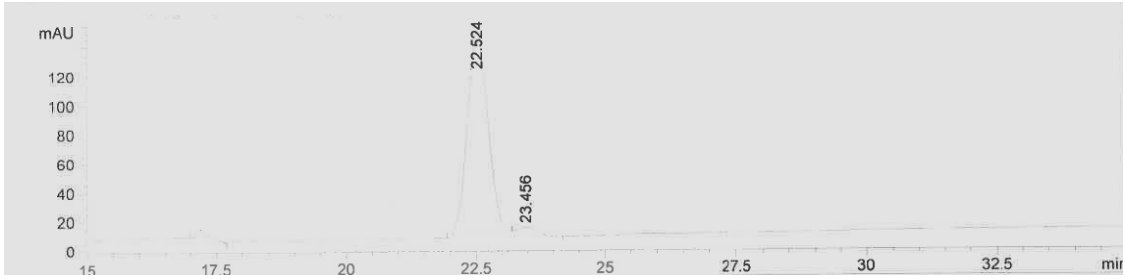
(*R*)-**5g**



rac-**6g**



(*R*)-**6g**



4. References

1. Boers, R. B.; Gast, P.; Hoff, A. J.; De Groot, H. J. M.; Lugtenburg, J. *Eur. J. Org. Chem.* **2002**, 189-202.

2. Jo, E.-A.; Jun, C.-H. *Eur. J. Org. Chem.* **2006**, 2504-2507.

3. Sierra, M. A.; del Amo, J. C.; Mancheno, M. J.; Gómez-Gallego, M. *J. Am. Chem. Soc.* **2001**, *123*, 851-861.

4. Hernández, A. S.; Thaler, A.; Castells, J.; Rapoport, H. *J. Org. Chem.* **1996**, *61*, 314-323.

5. Rios, M. Y.; Salazar, E.; Olivo, F. H. *J. Mol. Catal. B: Enzym.* **2008**, *54*, 61-66.

6. Taylor, S. K.; Atkinson, R. F.; Almli, E. P.; Carr, M. D.; Van Huis, T. J.; Whittaker, M. R. *Tetrahedron: Asymmetry* **1995**, *6*, 157-164.

7. Yus, M.; Torregrosa, R.; Pastor, I. M. *Molecules* **2004**, *9*, 330-348.

8. Gutman, A. L.; Zuobi, K.; Bravdo, T. *J. Org. Chem.* **1990**, *55*, 3546-3552.

9. Kallemeyn, J. M.; Mulhern, M. M.; Ku, Y.-Y. *Synlett* **2011**, 535-538.

10. Díaz-Rodríguez, A.; Lavandera, I.; Kanbak-Aksu, S.; Sheldon, R. A.; Gotor, V.; Gotor-Fernández, V. *Adv. Synth. Catal.* **2012**, *354*, 3405-3408.

11. (a) Dohi, T.; Takenaga, N.; Goto, A.; Maruyama, A.; Kita, Y. *Org. Lett.* **2007**, *9*, 3129-3132. (b) Murphy, S. K.; Dong, V. M. *J. Am. Chem. Soc.* **2013**, *135*, 5553-5556.

12. Høg, S.; Wellendorph, P.; Nielsen, B.; Frydenvang, K.; Dahl, I. F.; Bräuner-Osborne, H.; Brehm, L.; Frølund, B.; Clausen, R. P. *J. Med. Chem.* **2008**, *51*, 8088-8095.

5. Spectral data