Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Experimental

General

(*S*)-(-)-Lactic acid sodium salt (Fluka), (*D*)-(-)- and (*L*)-(+)-3-cyclohexylalanine (Fluka), (*R*)-(-)- and (*S*)-(+)-2-hydroxy-3-methylbutanoic acid (**3**) (Acros), (*R*)-(+)- and (*S*)-(-)-3-phenyllactic acid (**5**) (Aldrich) and (*S*)-Mandelic acid (**7**) (Aldrich) were purchased, (*R*)-(-)- and (*S*)-(+)-2-hydroxy-4-phenylbutanoic acid ethylester were donated by Ciba, (*R*)-*o*-chloromandelic acid (**8**) was a gift from R. Gaisberger (Graz). (*S*)-2-Hydroxyhexanoic acid (**2**) was prepared by lipase-catalysed kinetic resolution in analogy to a known procedure.[1] *Lactobacillus paracasei* DSM 20207 was obtained from the DSMZ (Braunschweig, Germany).

TLC plates were run on silica gel Merck 60 (F_{254}) and compounds were visualized by spraying with Mo-reagent [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (100 g/L), $Ce(SO_4)_2\cdot 4H_2O$(4 g/L) in H_2SO_4 (10%)]. Compounds were purified by flash chromatography on silica gel Merck 60 (230-400 mesh). Optical rotation values were measured on a Perkin-Elmer polarimeter 341 at 589 nm (Na-line) in a 1dm cuvette and are given in units of 10 deg cm^2 g^{-1}. NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 360 at 360 (^1H) and 90 (^{13}C) MHz. Chemical shifts are reported relative to TMS (δ 0.00) as internal standard in ppm, coupling constants (*J*) are given in Hz.

Conversion and enantiomeric excesses were determined *via* GC or HPLC on a chiral stationary phase. HPLC analyses were carried out on a Jasco HPLC-system (pumps PU-980, multi-wave-length-detector MD-910, autosampler AS-950, degasser CMA/260), using a Chiralpak AD column (column A, Daicel, 0.46cm x 25cm). GC analyses were performed on a Varian 3800 gas chromatograph equipped with FID, using a CP-Chirasil-DEX CB column (column B, 25 m, 0.32 mm, 0.25 μm film) or an Astec Chiraldex B-TA (column C, 30 m, 0.25 mm). H_2 was used as carrier gas.

General procedure for biocatalytic racemisation

Lactobacillus paracasei DSM 20207 was grown in medium #11 as suggested by DSMZ at 30°C in flask cultures without shaking. After 55h, cells were harvested by centrifugation (18.000 x g), lyophilized and stored at +4°C. For the biotransformation, 50mg whole lyophilized cells were rehydrated in 0.5ml aqueous BIS-TRIS buffer (50mM, 10^{-2}M $MgCl_2$, pH 6) for 1h at 42°C with shaking at 150rpm. Substrate (5mg) was added followed by shaking of the reaction mixture at 42°C at 150rpm for 24 h. Then the reaction mixture was acidified with 2M HCl (1 drop) and the cells

were removed by centrifugation. The supernatant was extracted with Et_2O and the organic phase was dried with Na_2SO_4. The determination of conversion and enantiomeric excess was carried out by GC or HPLC on a chiral stationary phase. For HPLC-determination, the organic phase was evaporated under reduced pressure and the residue was dissolved in the HPLC-eluent (without trifluoroacetic acid).

*Synthesis of substrates (R)- and (S)-**4***

(L)-3-Cyclohexylalanine (0.117g, 0.68mmol) was dissolved in H_2SO_4 (4ml, 1M). The stirred solution was cooled to 0°C and $NaNO_2$ (0.189g, 2.73mmol) was added in small portions. The reaction was allowed to warm to rt and stirring was continued overnight. After diluting the mixture with water (4 mL) the aqueous phase was extracted with EtOAc (3x, 5ml), the combined organic layers were washed with sat. aqueous $NaHCO_3$, dried and evaporated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to give (*S*)-3-cyclohexyl-2-hydroxypropionic acid (*S*)-**4** as white crystals (53mg, 45%, e.e. >99%). R_f (p.e./EtOAc, 1:1) = 0.2; mp = 150° C; ^{1}H NMR (360MHz, $CDCl_3$) δ = 0.88-1.01 (2H, m, C<u>H</u>$_2$), 1.18-1.28 (4H, m, cyclohexyl), 1.51-1.71 (6H, m, cyclohexyl), 1.83-1.86 (1H, d, J = 15.0 Hz, cyclohexyl), 4.19 (1H, dd, J_1 = 3.5 Hz, J_2 = 9.5 Hz, C<u>H</u>-OH); ^{13}C NMR (90MHz; $CDCl_3$) δ = 25.9, 26.2, 26.3, 32.1, 33.6, 33.8, 41.9 (CH_2), 68.0 (CH-OH), 176.3 (COOH); $[\alpha]_D^{20}$ –7.3 (*c* 0.6, MeOH). The same procedure was used for the synthesis of substrate (*R*)-**4**. $[\alpha]_D^{20}$ +7.5 (*c* 0.6, MeOH).

*Synthesis of substrates (R)- and (S)-**6***

(*S*)-2-Hydroxy-4-phenylbutanoic acid ethyl ester (20.8g, 0.1mol) was stirred in NaOH (100ml, 1N) at rt until a clear solution was obtained. The solution was heated to 70°C, acidified with HCl (4N) to pH 1 and slowly cooled to 0°C while crystallization of acid 5 proceeded. After stirring for 30min at 0° C the crystals were filtered and the filter cake was washed twice with cold water (2 x 20ml). The product was dried at 40°C in a vacuum oven to give (*S*)-2-hydroxy-4-phenylbutanoic acid (**6**) as white crystals (16.2g, 90%, e.e. >99 %). $[\alpha]_D^{20}$ +8.1 (*c* 1.0, EtOH). The same procedure was used for the synthesis of substrate (*R*)-**6**. $[\alpha]_D^{20}$ -8.2 (*c* 1.0, EtOH).

*Derivatization of **1** and **3** to the corresponding methyl esters for GC-analysis*

A solution of CH_2N_2 in Et_2O (prepared from *N*-methylnitrosourea) was added to the samples until a yellow color persisted. The mixture was directly subjected to gas chromatography.

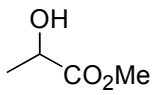
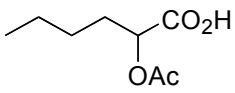
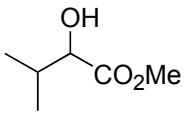
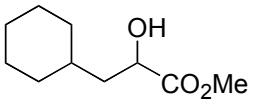
*Derivatization of **2** to the corresponding O-acetyl derivative for GC-analysis*

A sample of **2** (5mg) in CH_2Cl_2 was acetylated with acetic anhydride (5 drops) and a few crystals of *p*-dimethylaminopyridine (DMAP) were added. After 15min of stirring at rt, the reaction mixture was quenched with water. The organic layer was separated and after drying over Na_2SO_4 directly used for GC analysis.

*Derivatization of **4** to the corresponding methyl ester for GC-analysis*

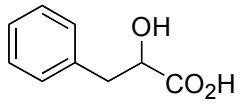
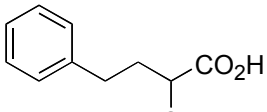
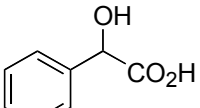
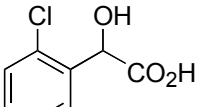
A sample of **4** (5 mg, 0.029 mmol) in methanol (0.4 mL) was esterified with H_2SO_4 (conc, 1 drop) at 55°C for 1h. Water was added (0.1 mL) to the reaction mixture and the product was extracted with CH_2Cl_2. After drying over Na_2SO_4, this solution was directly used for GC analysis.

Table 2: GC data using a chiral stationary phase.

Compound	Column	Conditions[a]	t_R [min]	
			R	*S*
	B	A	5.7	7.0
	B	D	7.3	7.5
	C	B	7.8	8.3
	C	C	8.4	8.9

[a] Conditions: A) 12.0 psi H_2 at 45° C, hold for 6 min, heat rate 15° C min^{-1} to 160° C; hold for 3min. B) 14.5 psi H_2 at 60° C, hold for 6 min, heat rate 15° C min^{-1} to 100° C, heat rate 30° C min^{-1} to 160° C, hold for 2min. C) 14.5 psi H_2 at 100° C, heat rate 5° C min^{-1} to 170°C, hold for 1min. D) 14.5 psi H_2 at 100° C, hold for 6 min, heat rate 15° C min^{-1} to 160°C, hold for 3min.

Table 3: HPLC data using a chiral stationary phase.

Compound	Column[a]	t_R [min]	
		R	*S*
 **5**	A	20.8	24.7
 **6**	A	20.8	21.8
 **7**	A	27.5	25.0
 **8**	A	28.9	23.4

[a] Conditions: heptane/*i*-propanol/trifluoroacetic acid (90/10/0.1), flow 0.5 ml min^{-1}.

References and notes

1 W. Adam, M. Lazarus, A. Schmerder, H.-U. Humpf, C. R. Saha-Möller, P. Schreier, *Eur. J. Org. Chem.* 1998, 2013.