SUPPORTING INFORMATION

Preparation of sterically demanding 2,2-disubstituted-2-hydroxy acids by enzymatic hydrolysis

Andrea Pinto*[a], Immacolata Serra[a], Diego Romano[a], Martina Contente[a], Francesco Molinari[a], Fabio Rancati[b], Roberta Mazzucato[b], Laura Carzaniga*[b]

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1. Optimization of the biotransformation

Optimization of the enzymatic hydrolysis of **1e** using commercial PLE was carried out using the sequential experimental design Multisimplex. The conditions of the sequential experimental trials were selected employing the Multisimplex® 2.0 software. Two response variables were chosen for the optimization, namely productivity (expressed as mmol of product/mg of enzyme used) and enantioselectivity (expressed as enantiomeric ratio, E).

	Control variables				
	Substrate (mM)	Enzyme mg/mL)	DMSO (%)	pH	T (°C)
Reference value	2.5	7.5	5	7.0	30
Step size	1	3	1	1	5
Minimum	2.0	2.0	0	4.0	20
Maximum	50.0	10.0	15	9.0	50

Table S1. Control variables and initial levels considered for the optimization.

Each trial was performed in triplicate and the mean value was introduced as response for further optimization. The results of the sequential experiments aimed at the optimization of initial rate and E are reported in Fig. S1.

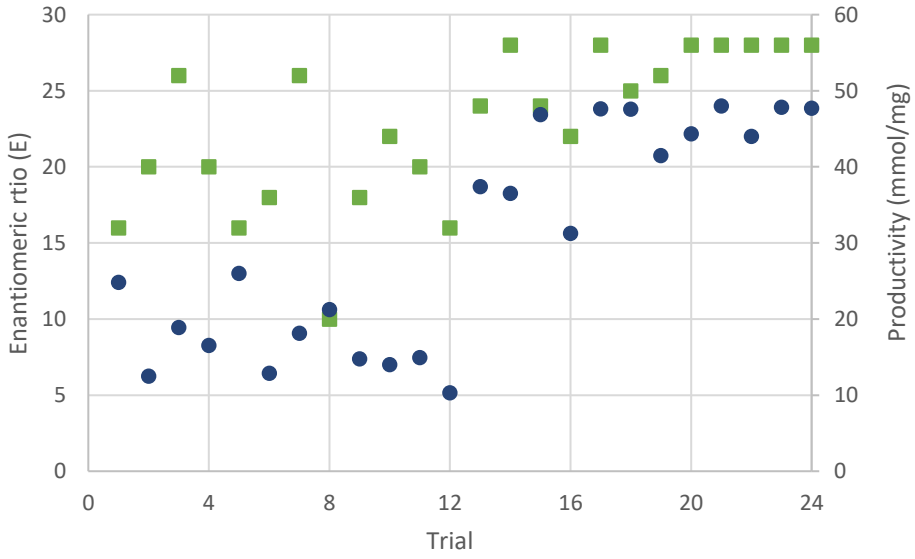

Figure S1. Sequential optimization of the PLE-catalysed hydrolysis of **1e**. Experimental responses (■ enantiomeric ratio, ● productivity (expressed as mmol of product formed/mg of enzyme) as

function of sequential trials.

The simultaneous highest enantioselectivity and productivity were achieved in trials 17, 21, 23 and 24; after trial 20 the optimum for E was always achieved. The optimized conditions corresponding to the trial 16 ([S] 3.5 mg/mL (8 mM); [Enz] 5.0 mg/mL; solvent 0.1 M phosphate buffer/DMSO 8% pH 7.0 at 25 °C) were chosen for further experiments since the lowest amount of enzyme was used to produce the highest amount of product with the highest enantioselectivity.

2. NMR spectra

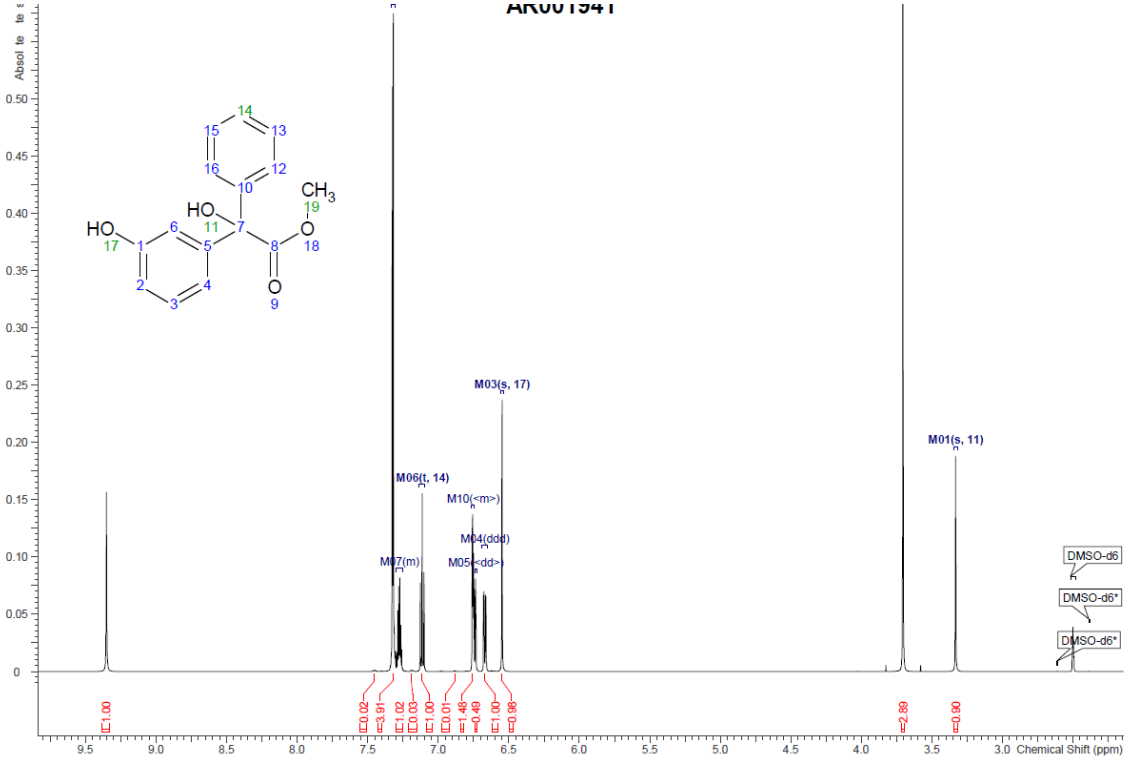

Figure S2. ^{1}H NMR spectrum of 1a.

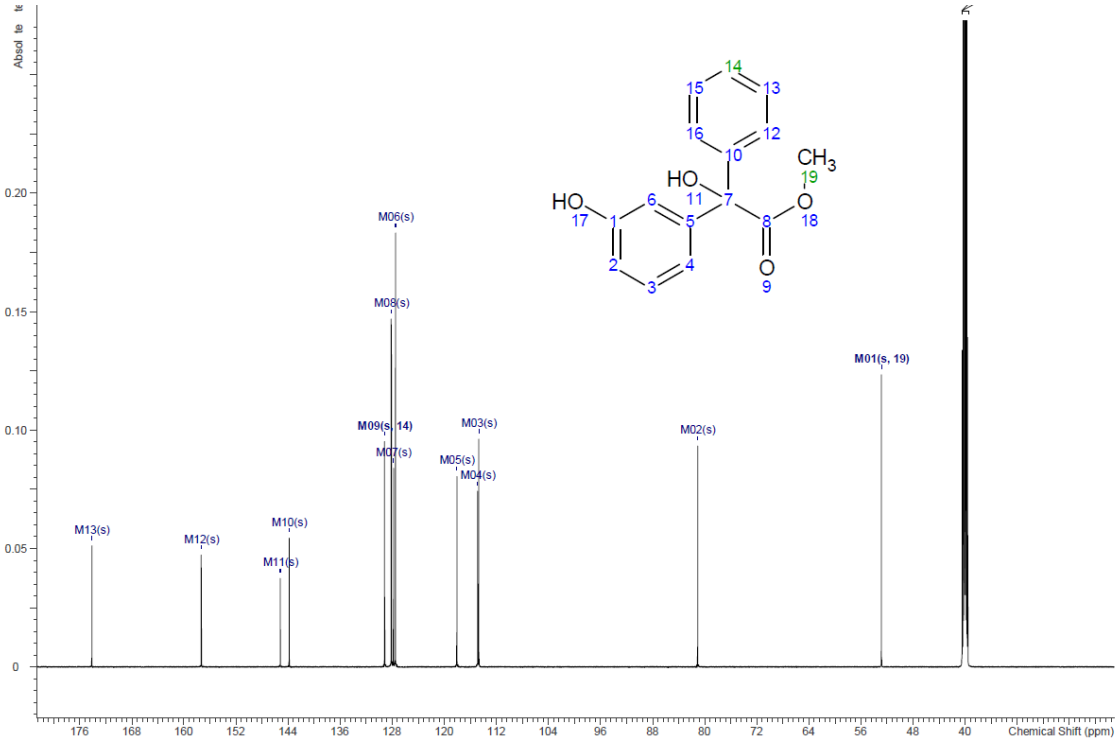

Figure S3. ^{13}C NMR spectrum of 1a.

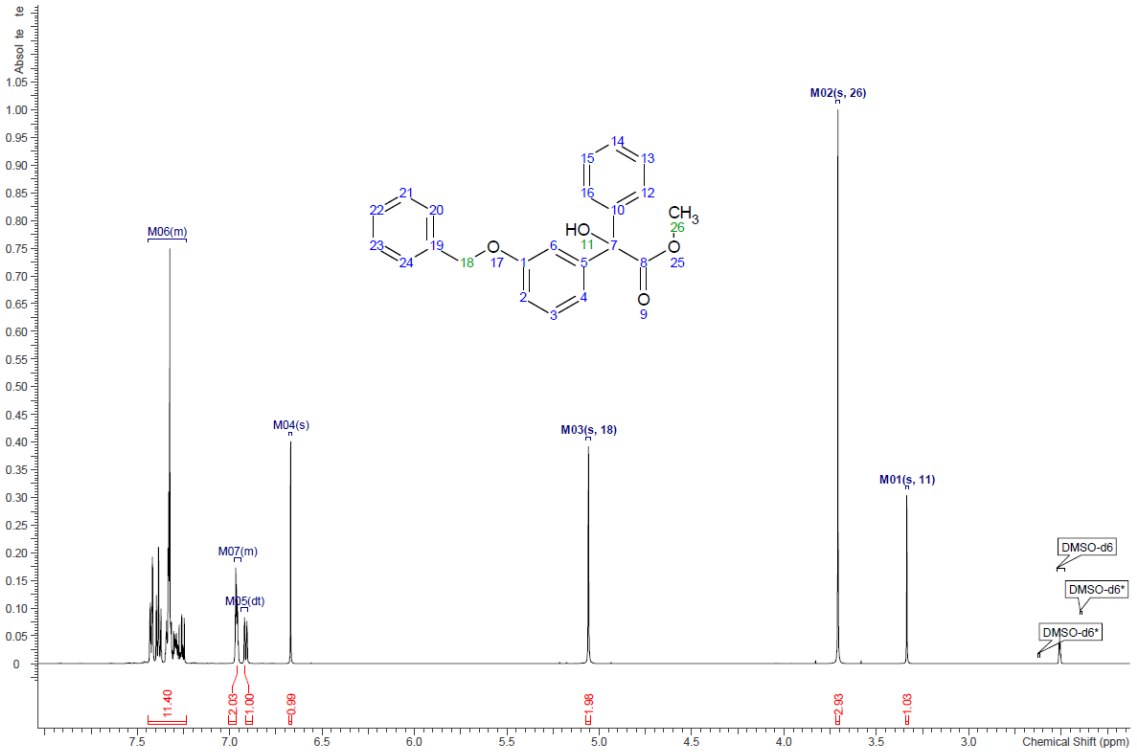

Figure S4. ^{1}H NMR spectrum of 1b.

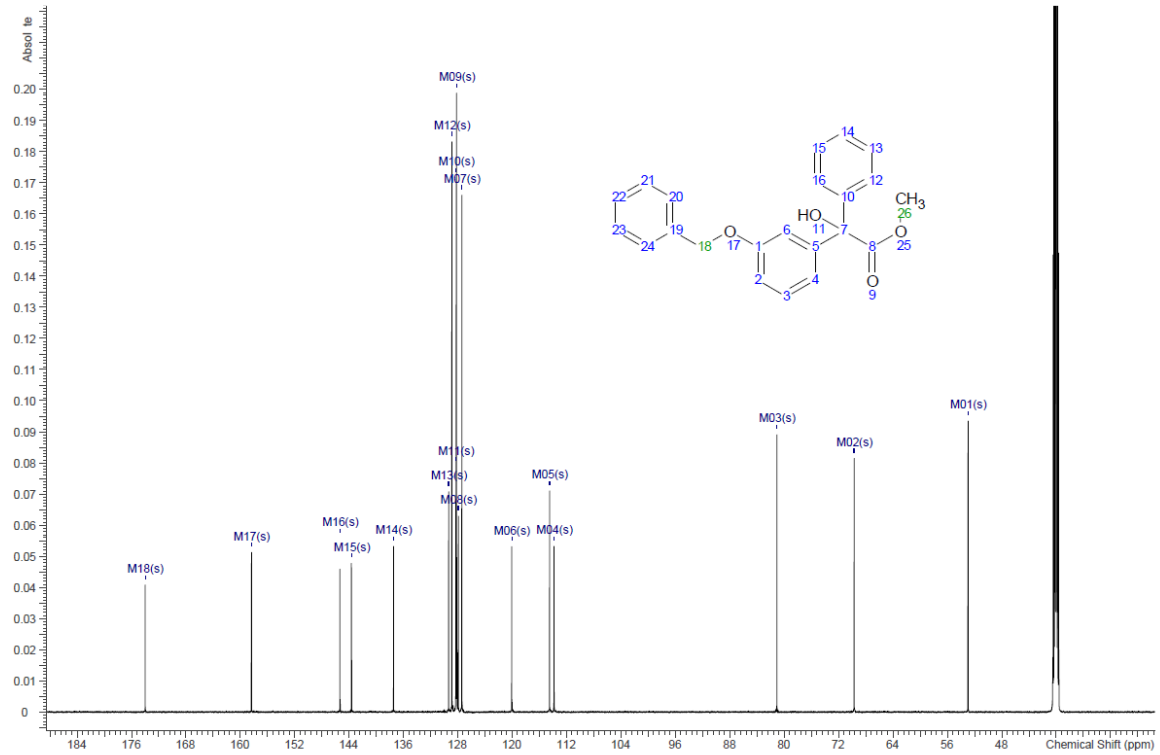

Figure S5. ^{13}C NMR spectrum of 1b.

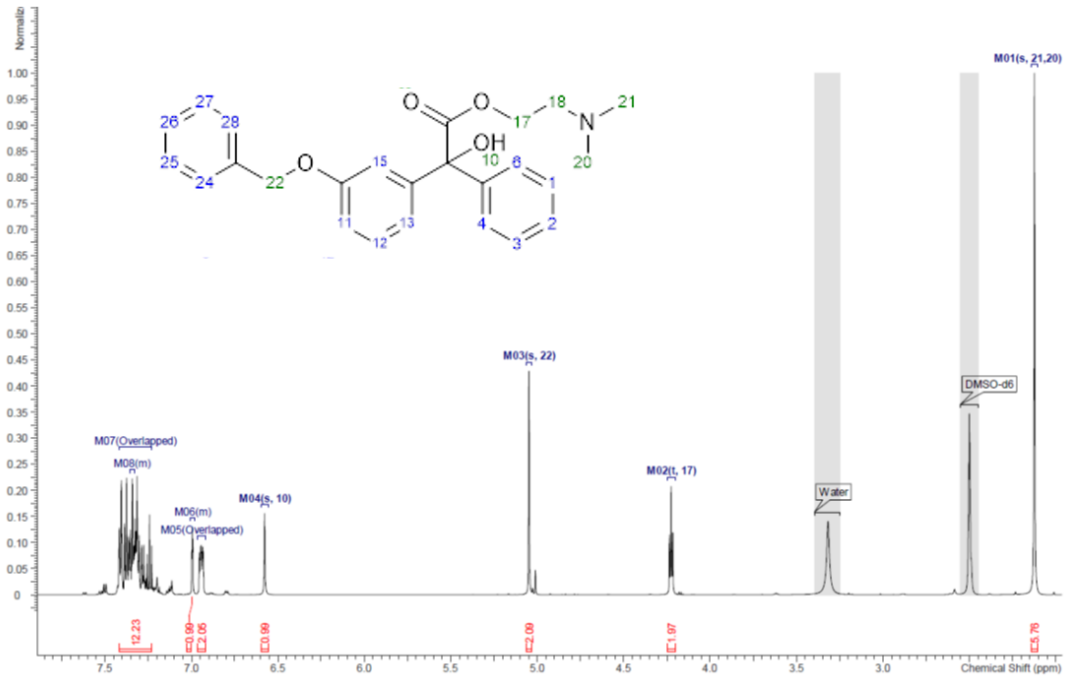

Figure S6. ^{1}H NMR spectrum of 1c.

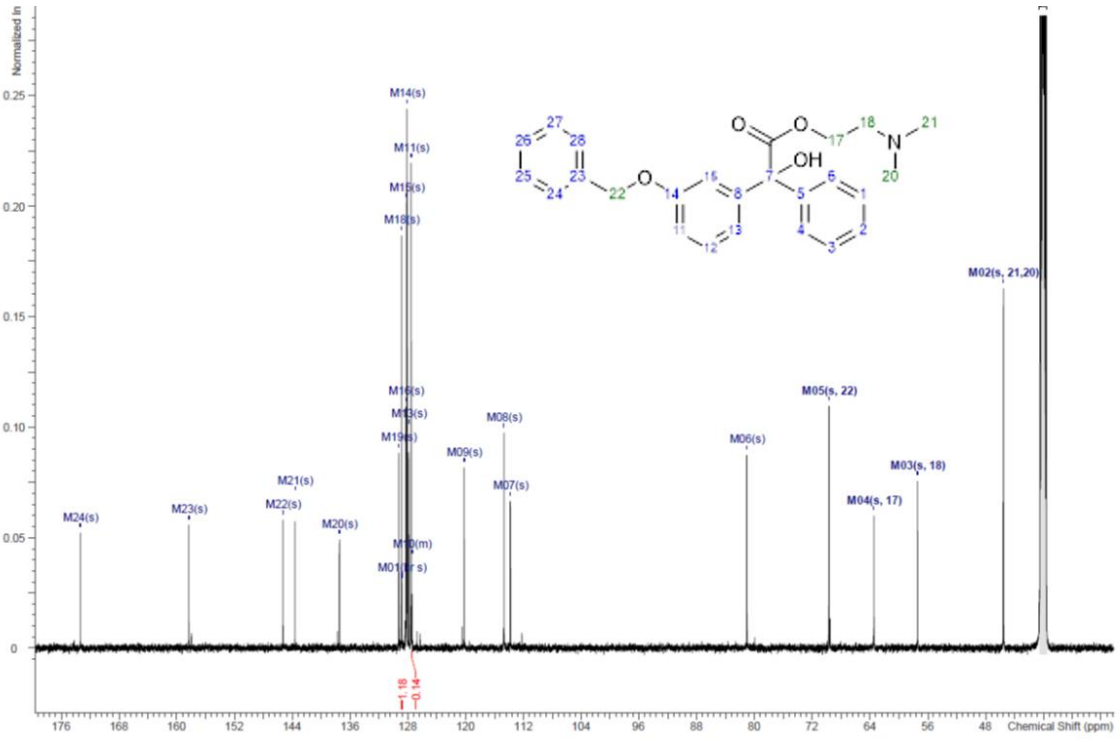

Figure S7. ^{13}C NMR spectrum of 1c.

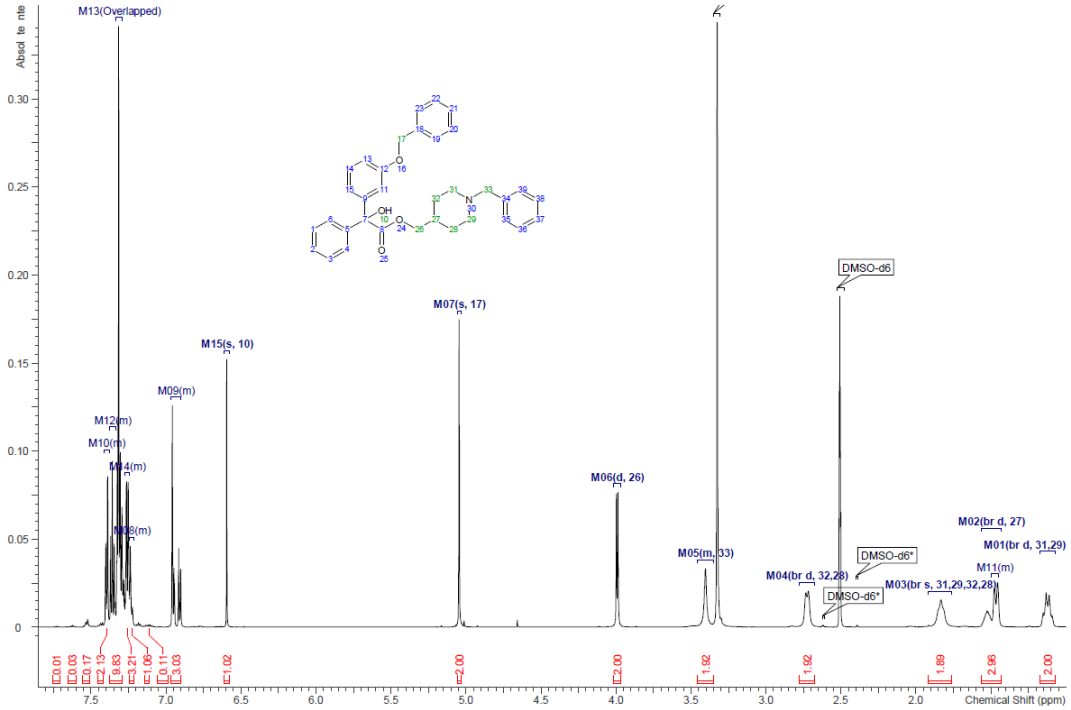

Figure S8. ^{1}H NMR spectrum of 1d.

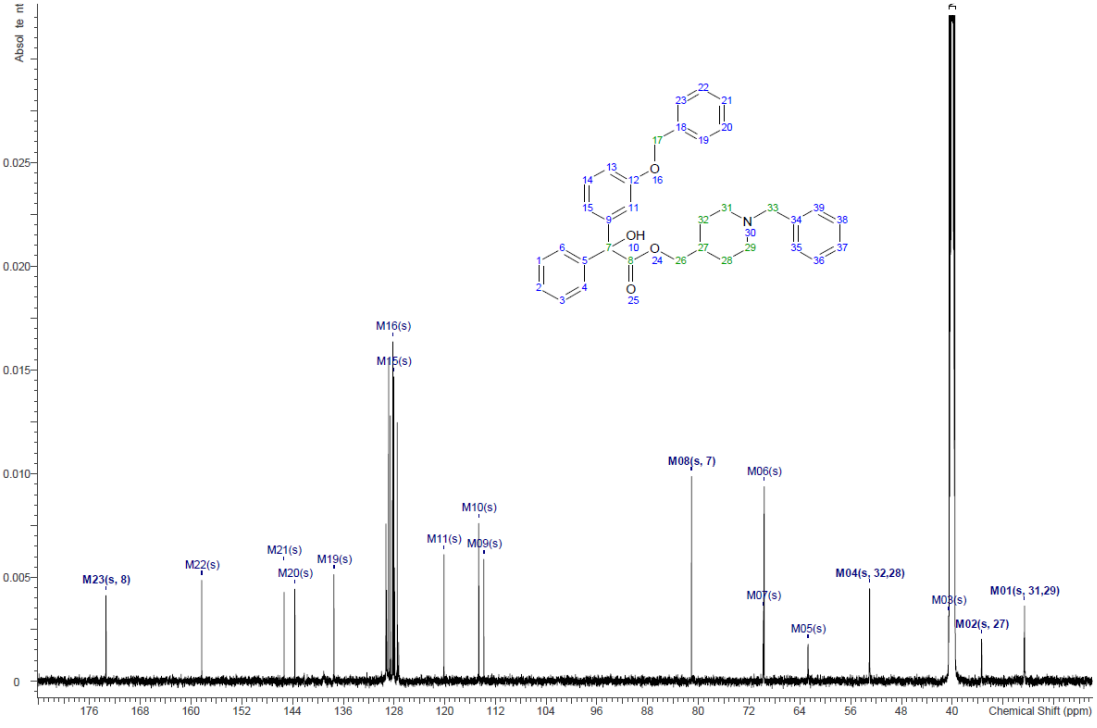

Figure S9. ^{13}C NMR spectrum of 1d.

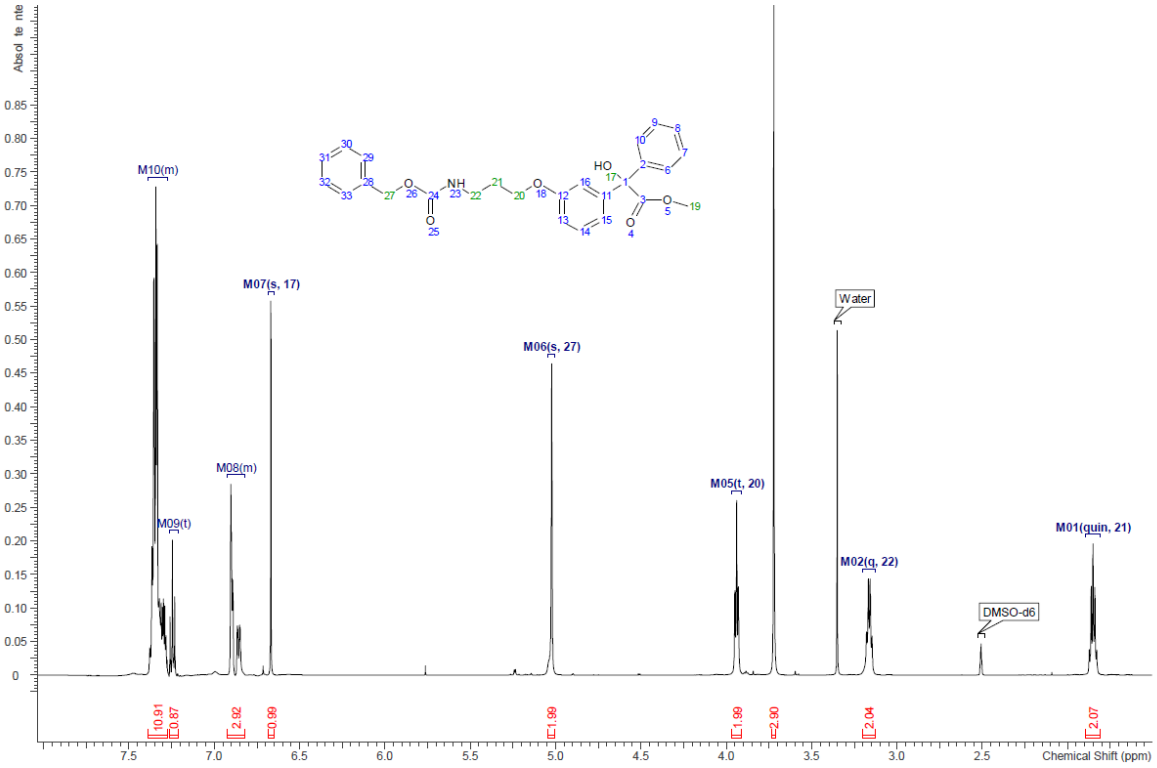

Figure S10. ^{1}H NMR spectrum of 1e.

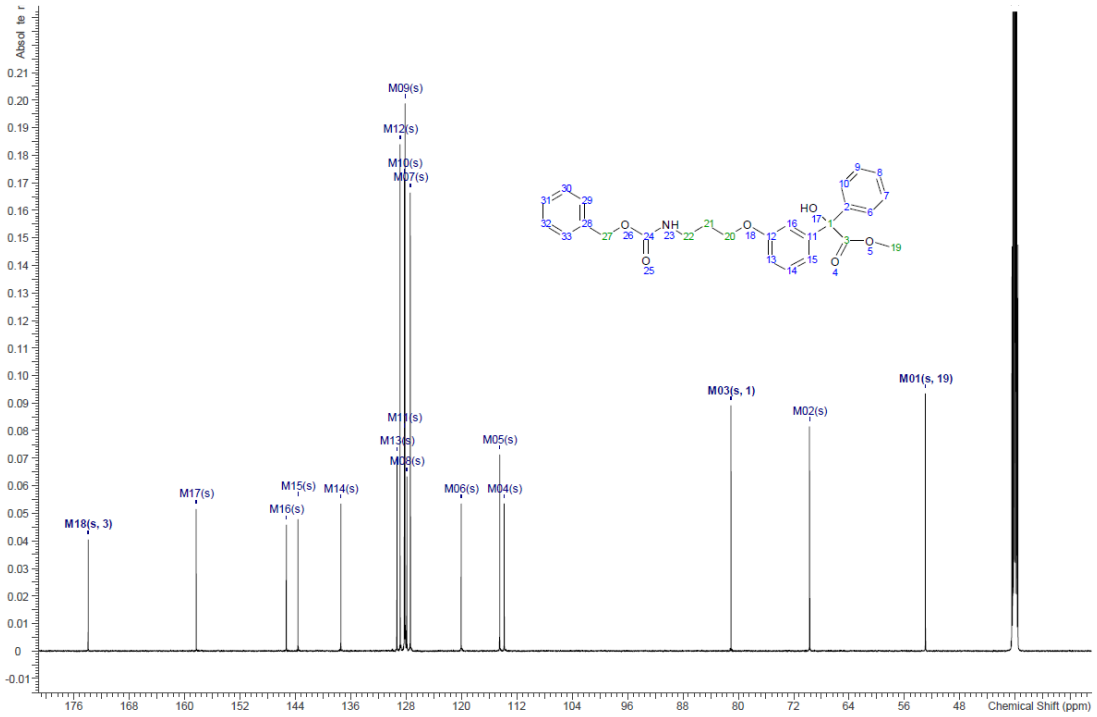

Figure S11. ^{13}C NMR spectrum of 1e.

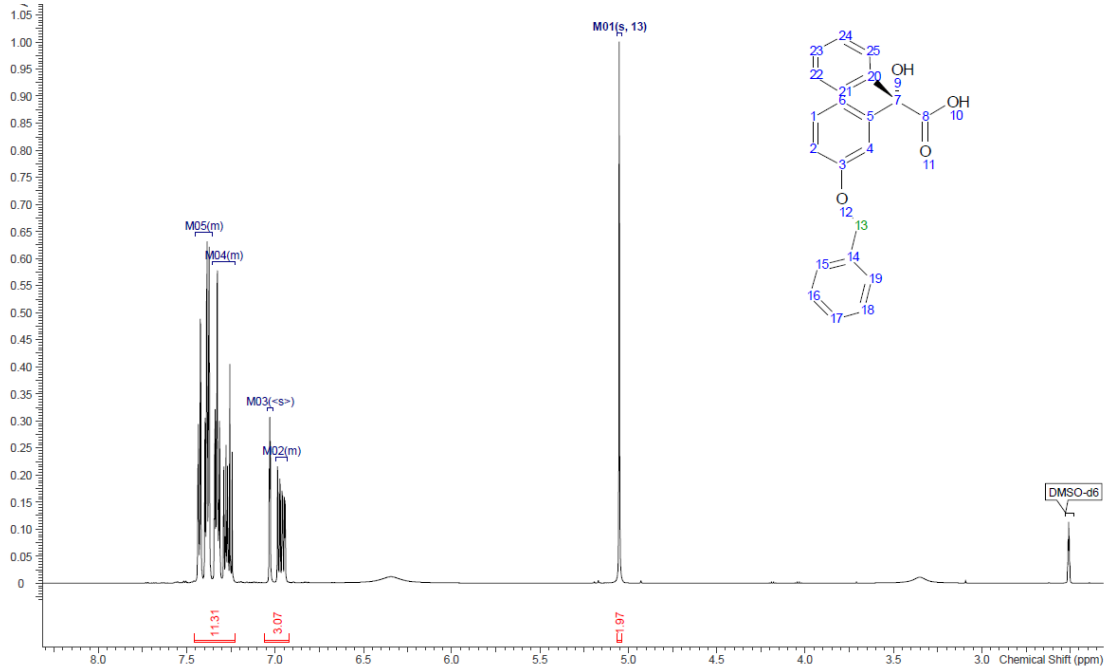

Figure S12. ^{1}H NMR spectrum of (*S*)-2a.

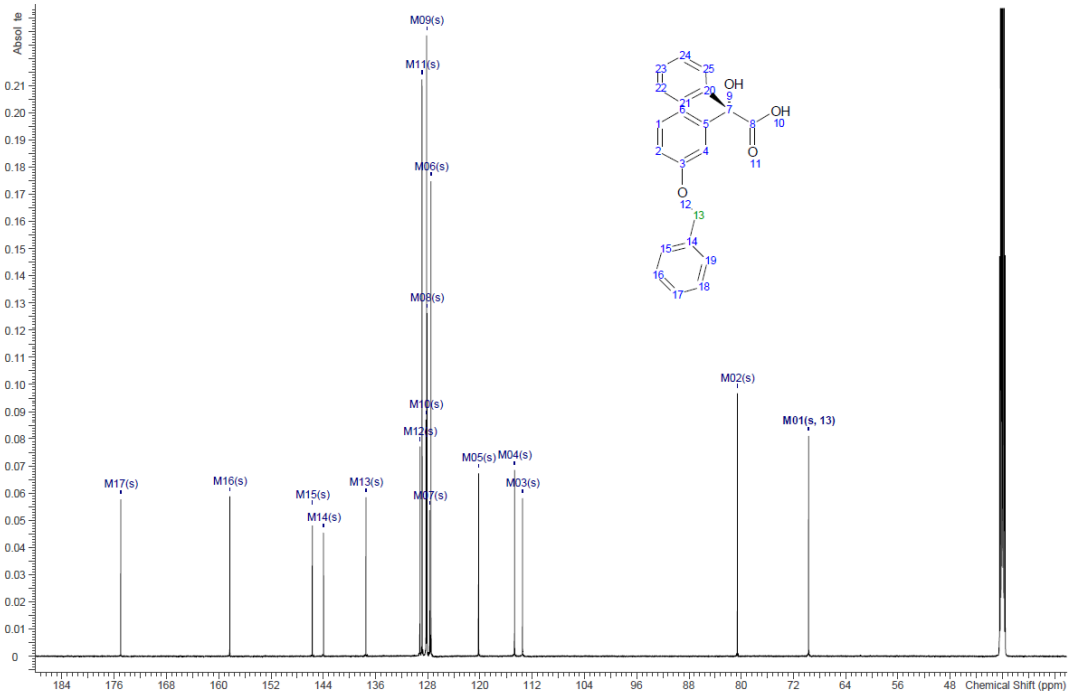

Figure S13. ^{13}C NMR spectrum of (*S*)-2a.

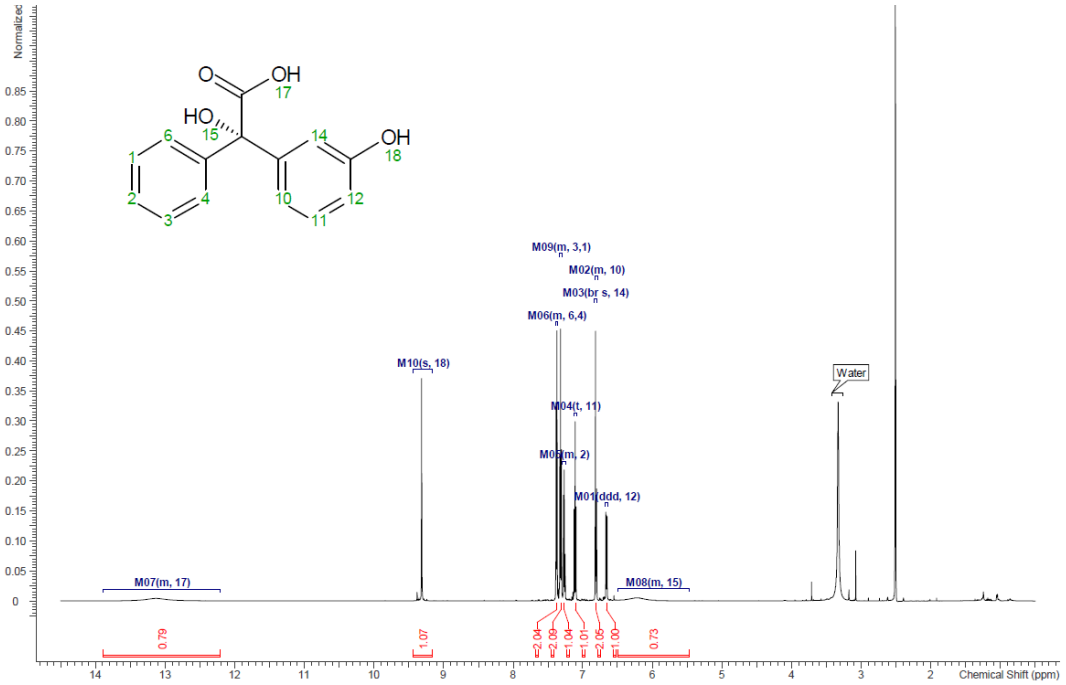

Figure S14. ^{1}H NMR spectrum of (*S*)-2b.

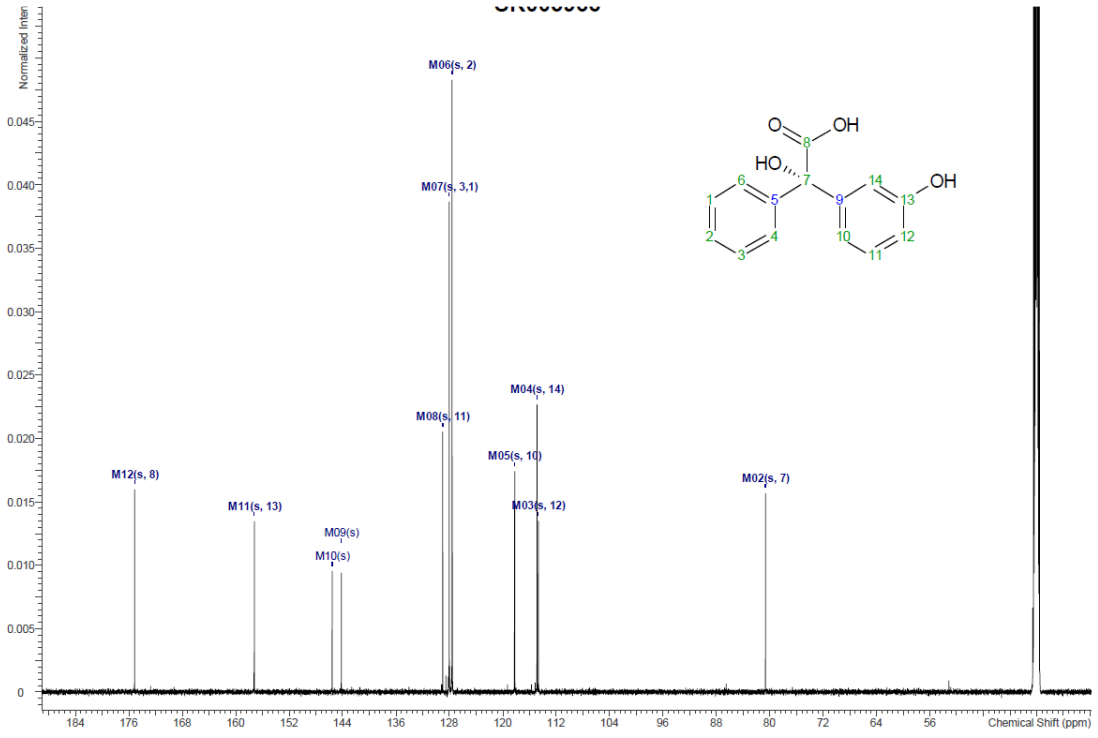

Figure S15. ^{13}C NMR spectrum of (*S*)-2b.

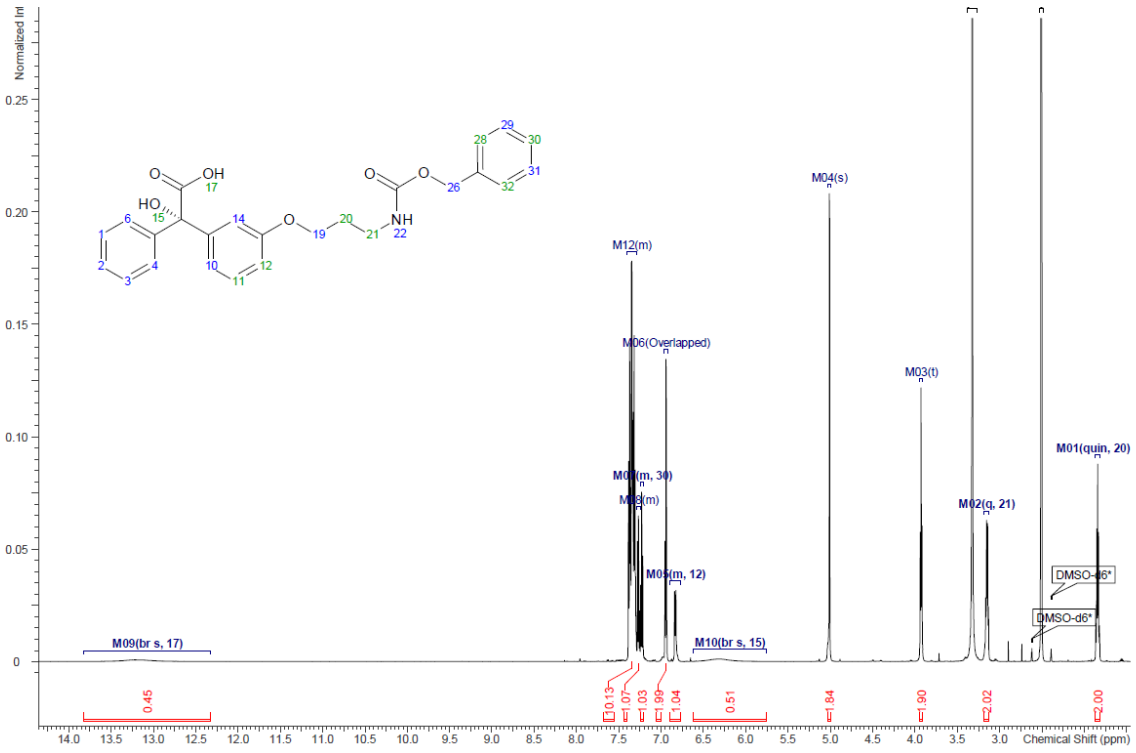

Figure S16. ^{1}H NMR spectrum of (*S*)-2e.

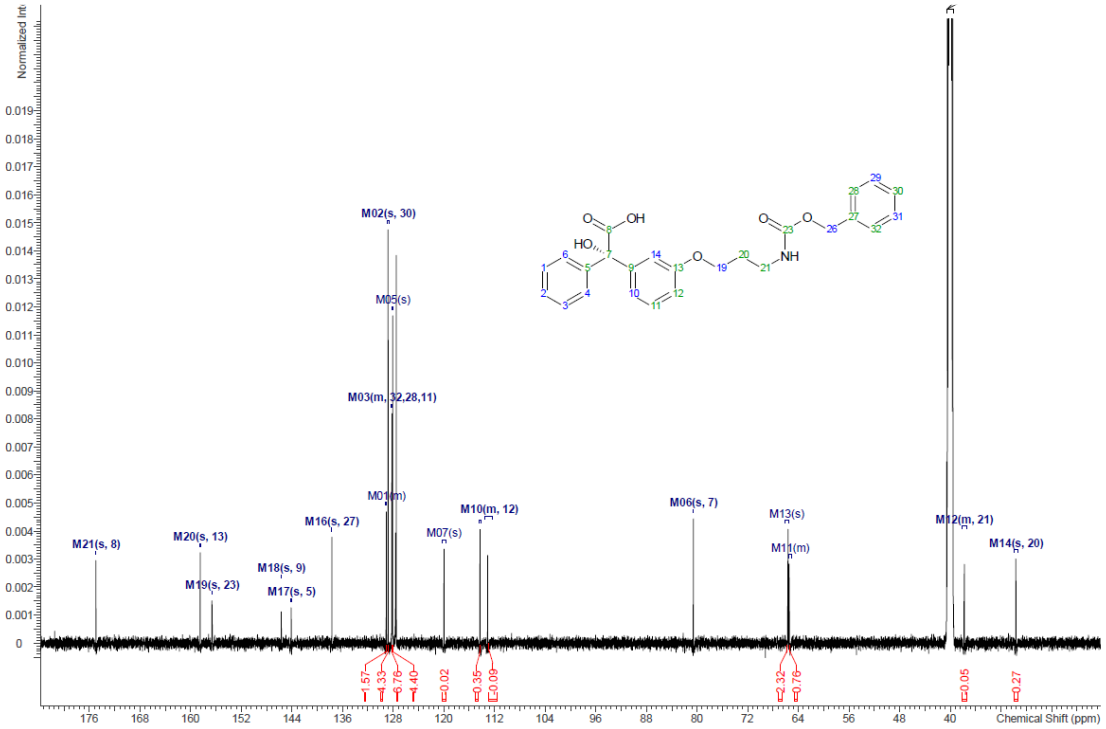

Figure S17. ^{13}C NMR spectrum of (*S*)-2e.

3. HPLC chromatograms

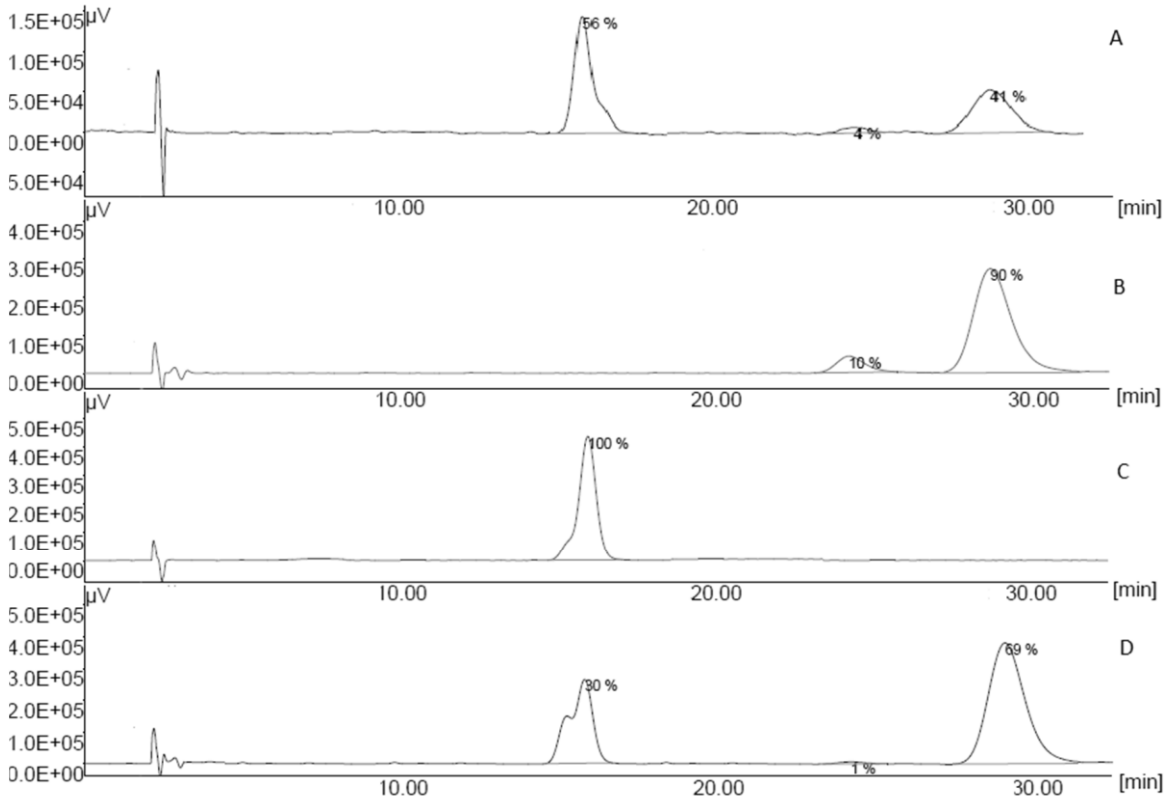

Figure S18. Chiral HPLC of the hydrolysis of 1e to 2e catalysed by PLE.

A: chromatogram of the reaction corresponding to the optimized conditions, as described in entry 2 of Table 5.

B: chromatogram of the product (2e, 80% ee) isolated from the preparative hydrolysis of racemic 1e (first reaction of Scheme 2)

C: chromatogram of enantiomerically enriched 1e obtained by methylation with MeOH/$SOCl_2$ of enantiomerically enriched 2e (second reaction of Scheme 2)

D: chromatogram of the enzymatic hydrolysis of enantiomerically enriched 1e to give enantiomerically pure 2e (third reaction of Scheme 2 in the manuscript)