Electronic Supplementary Material (ESI) for Green Chemistry
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Electronic Supporting Information

Contents:

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A) Analysis and quantification of the reaction products (GC):

According to our procedure a sample was taken at certain moments in time and analyzed with chiral gas chromatography (GC) on a 25 m WCOT fused silica CP-Chirasil-DEX CB (Agilent) capillary column. As a carrier gas H_2 was used. The GC had an FID detector and analysis of the peaks was performed with Chemstation software. The temperature program of the oven starts at 85 °C, holding this temperature for 5 minutes. Then a linear temperature ramp of 25°C/min is applied untill 150 °C is reached. The oven temperature is kept at 150°C for 5 minutes, after which the temperature is increased a with a ramp of 25 °C/min, until 200 °C is reached and kept for 5 minutes. A chromatogram is depicted in figure S7. The conversion of the α-hydroxy ester was determined by quantification of the released alcohol. The conversions Conv.(L) and Conv.(D) in Table 1-3 express respectively the conversion of the L- and the D-lactate enantiomer. In the applied aqueous conditions, only the corresponding α-hydroxy acid was produced (together with the alcohol); no side products such as dimers of lactic acid and higher oligomers were detected with both GC and NMR (see NMR-spectrum S1-S4). This corresponds with the observations of Vu *et al.*, who reported that significant oligomerization of lactic acid only occurs in aqueous solutions having more than 20 wt% of lactic acid.[1] L- and D-lactate signals were quantified and product ee's (ee_p) were determined according to the classic formula: ([L-LA] – [D-LA])/([L-LA] + [D-LA]). The enantiomers of *n*PrLA could not be separated on the chiral GC. Therefore separate reactions in time were performed to determine the conversion. After reaction, *n*PrLA was partly extracted with toluene and transesterified with MeOH at 70 °C, using amberlyst wet 15 (Acros). Next, the L/D-ratio and the corresponding ee_p was determined from the produced MLA. The volume productivity (in $g.L^{-1}h^{-1}$) was defined as the weight acid (in g) released per L per hour plus the equimolar amount of ester, because two valuable product streams are created containing one enantiomer in acid form and the other enantiomer as ester. The molar enantiomeric purity on product side is defined as the ratio of the amount of the preferred lactic acid enantiomer produced to the total amount of lactic acid formed. Consequently, this determines the purity of the corresponding lactide isomer.

Figure S1: ^{13}C-NMR spectrum in dmso-d6 of the *C. rugosa* lipase catalyzed hydrolysis of MLA at 0.55 M, 45 °C and 3 hours of reaction (raw reaction mixture diluted in dmso-d6). Figure S2, S3 and S4 refer to a more detailed zoom.

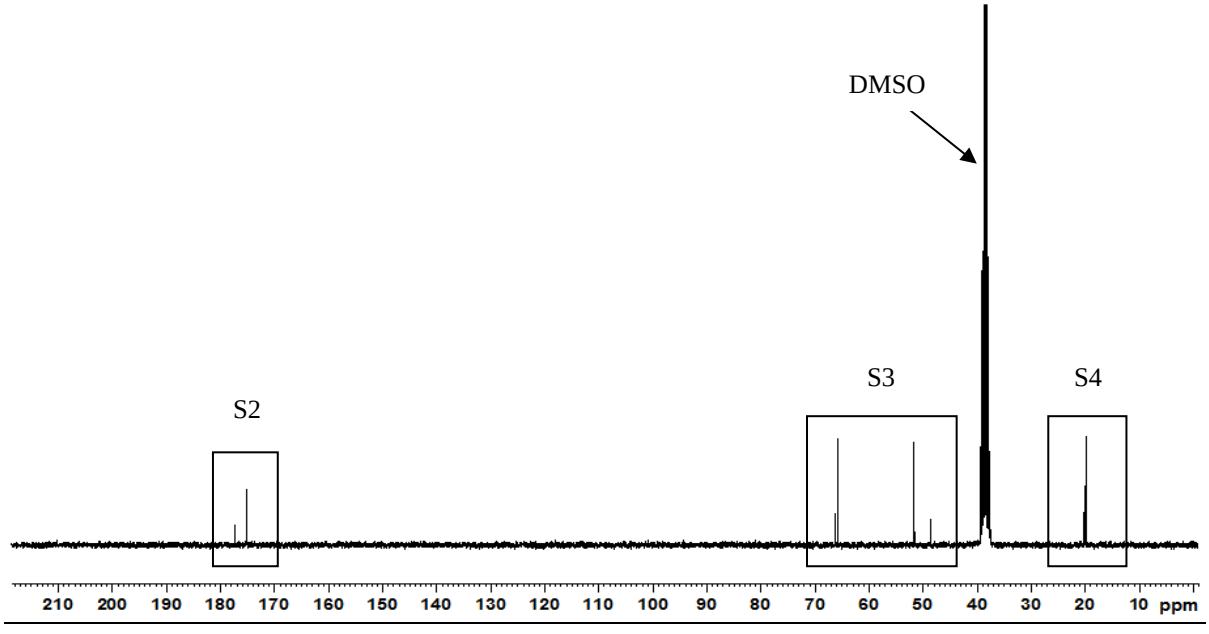

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Figure S2: Spectrum of figure S1, zoomed in at the carbonyl area.

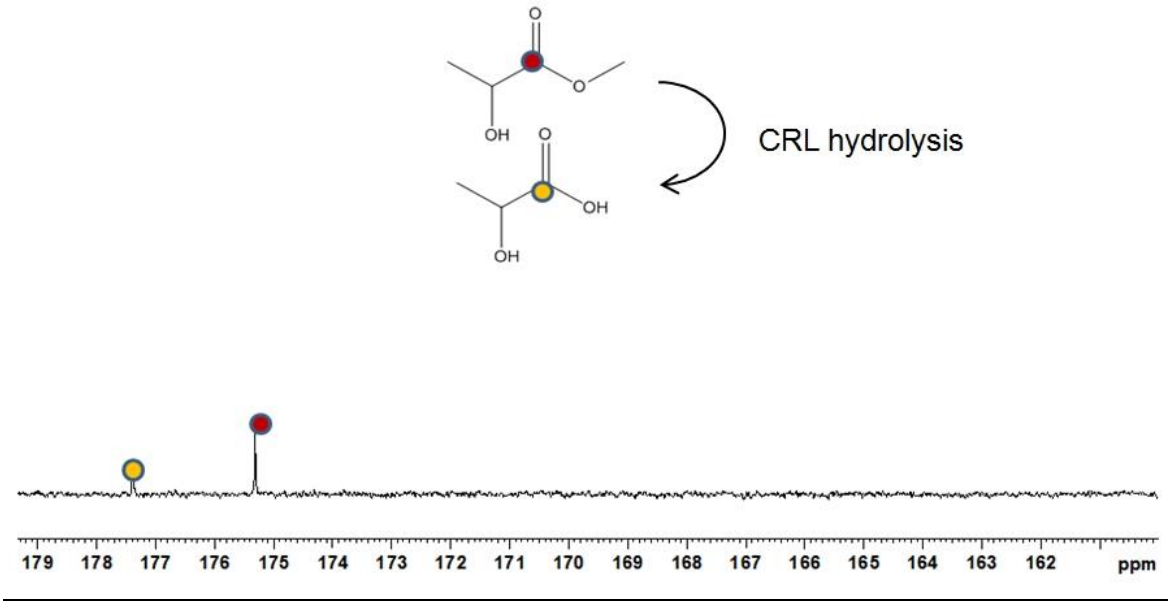

Figure S3: Spectrum of figure S1, zoomed in at the alkyl to α-carbon region. The signal between 48-49 ppm, is originating from MeOH

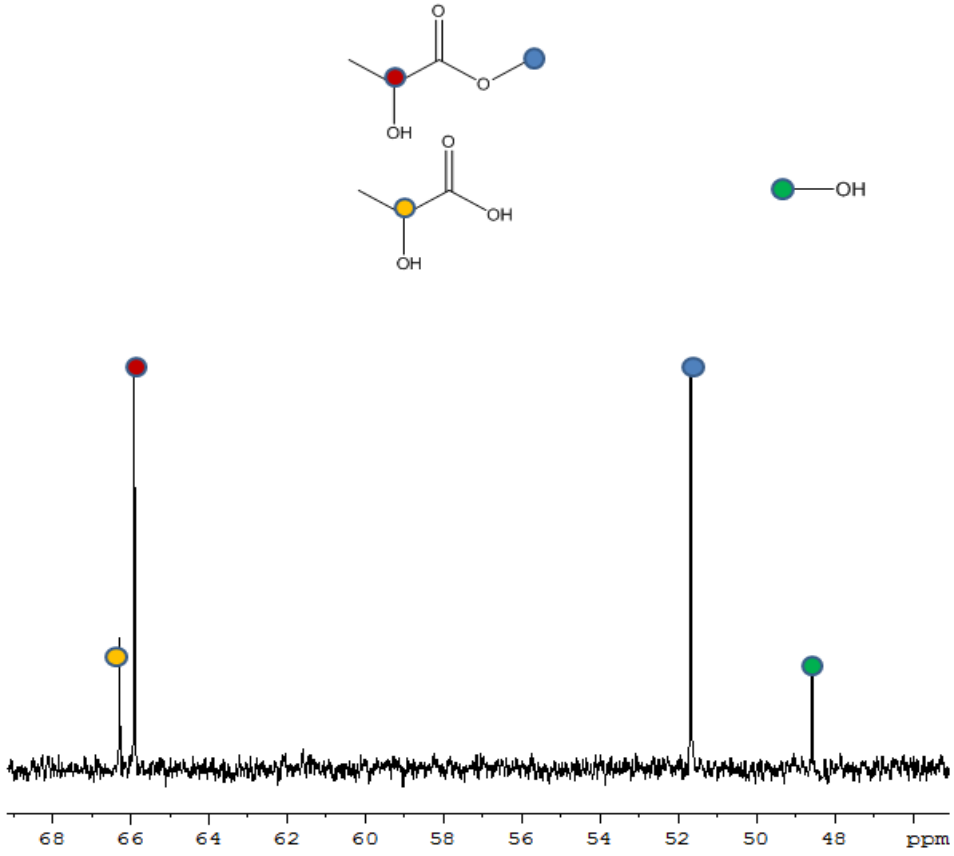

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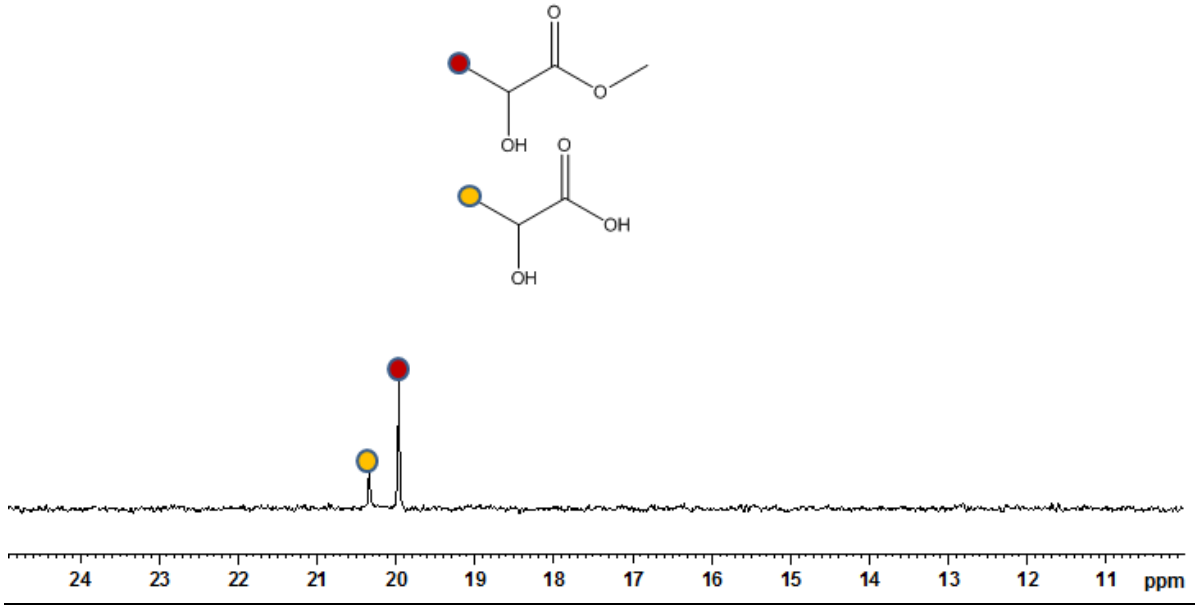
Figure S4: Spectrum of figure S1, zoomed in at the methyl area.



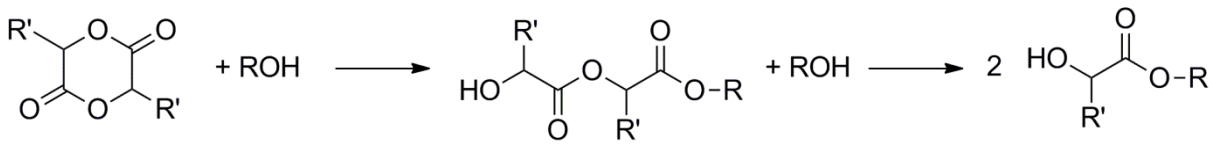
Interpretation of figure S1-S4: Only carbon signals originating from MLA, LA or free methanol were detected in the spectra, indicating that no side reactions occur.

B) Synthesis of α-hydroxybutyric acid esters:

The methyl ester of α-hydroxybutyric acid (MHBA) was prepared by combining an equal quantity of the enantiomers of α-hydroxy butyric acid in a round bottom flask with acidic amberlyst 15 (wet) ion exchange resin (Acros) and an excess amount of the corresponding alcohol. The reaction was carried out at 90 °C. The mixture was allowed to react over weekend to completeness and was analyzed with GC and ^{1}H-NMR. Finally the alcohol was removed with a rotary evaporator.

C) Synthesis of alkyl lactates and glycolates:

EtLA, *n*PrLA, methyl glycolate (MGA), ethyl glycolate (EtGA) and propyl glycolate (*n*PrGA) were syntesized by combining respectively lactide (the cyclic dimer of LA), provided by Purac, or glycolide (the cyclic dimer of GA) with an excess amount of the respective alcohol and amberlyst 15 (wet) ion exchange resin (Acros). The mixture was allowed to react to completeness at 130 °C and analyzed with GC and ^{1}H-NMR. The alcohol was then removed with a rotary evaporator.



As can be seen, the lactate or glycolate is formed by an initial opening of the cyclic dimer (R'=H for glycolide; R' = CH_3 for lactide) by the alcohol and a subsequent depolymerisation of the dimer by a second alcohol molecule. The chromatograms in figure S5 show the conversion of lactide into ethyl lactate. Reference alkyl lactate samples were also generously provided by Purac.

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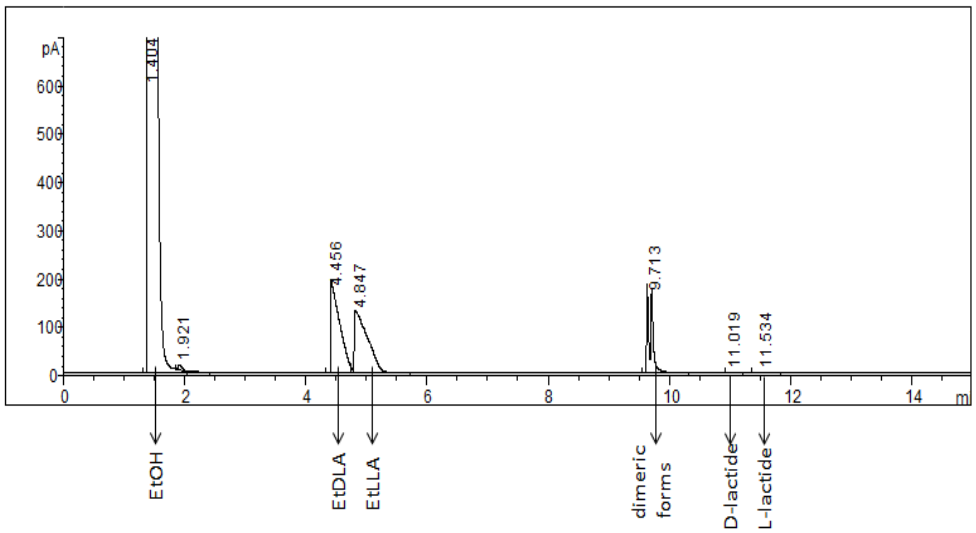
Figure S5: Chromatograms of the conversion of racemic lactide into ethyl lactate (EtLA).

i)



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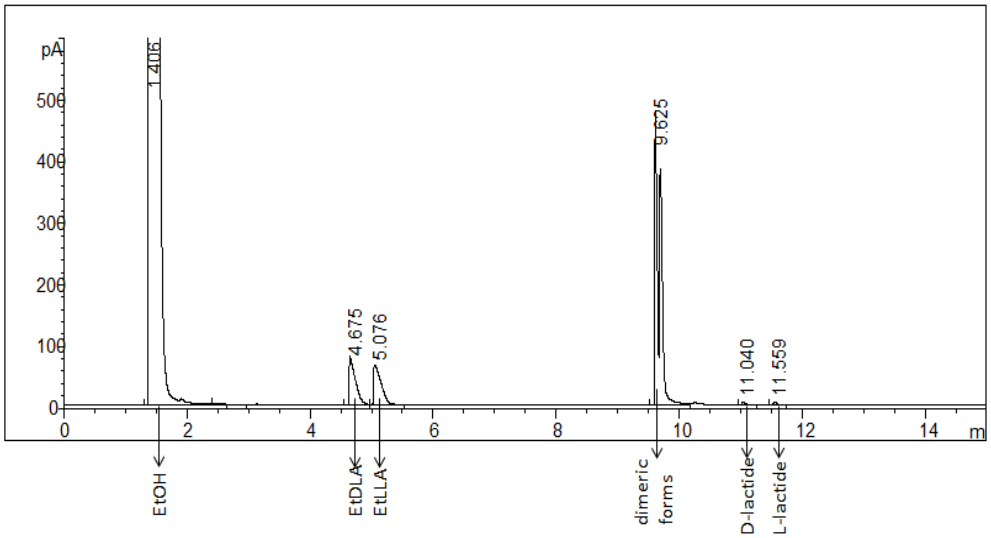
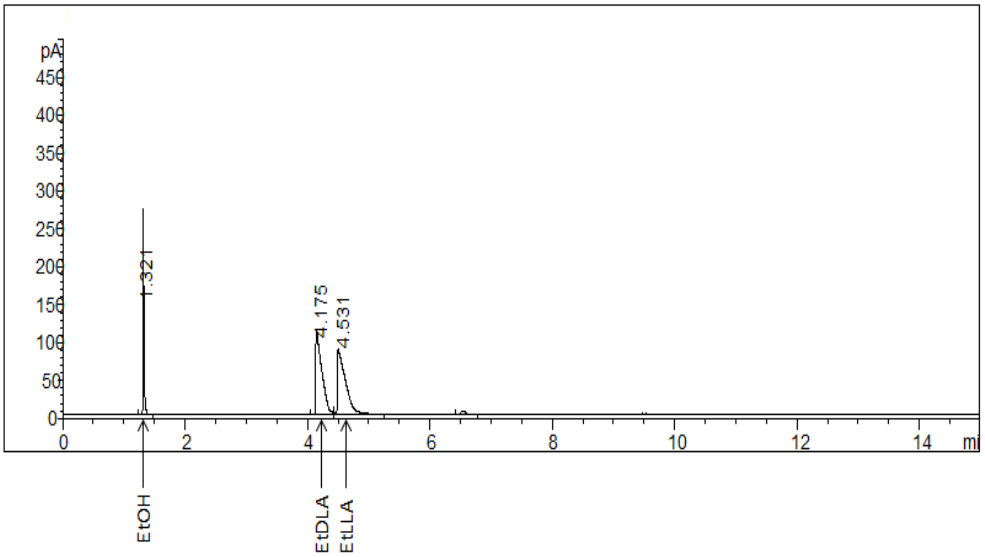


iii)



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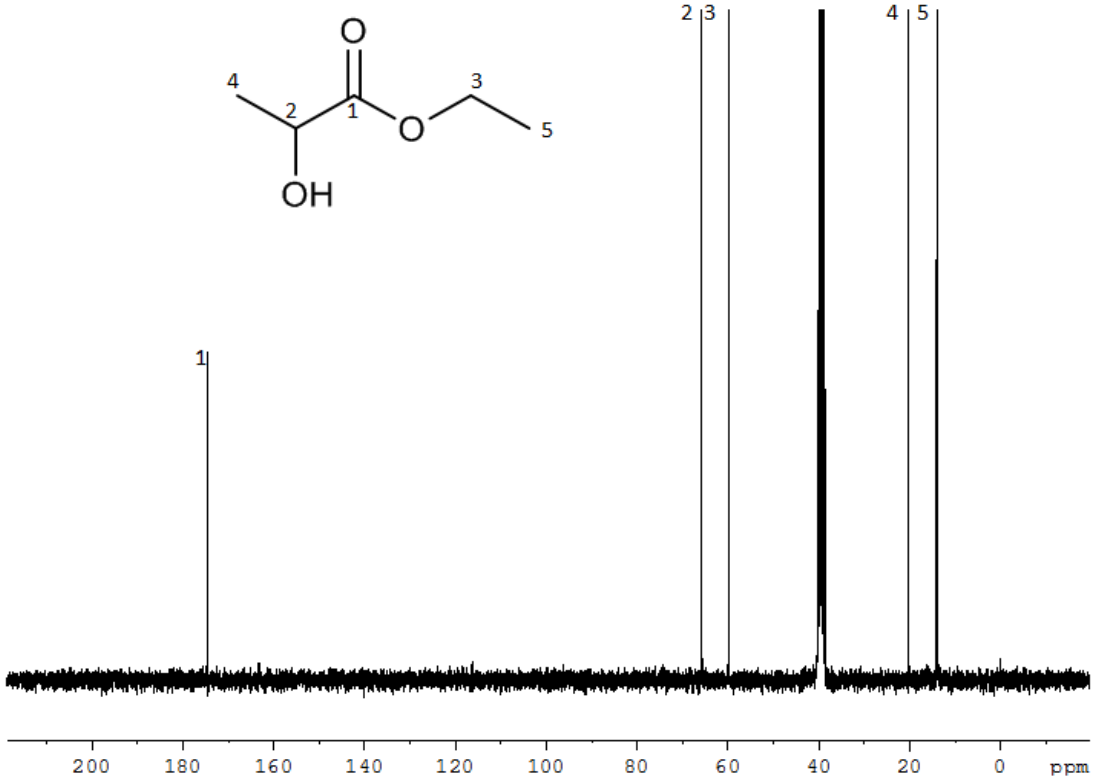
Interpretation of figure S5: Initially the ring opening of racemic lactide occurs (rapidly) by alcoholysis of the cyclic dimer (i). Next the corresponding dimer is further converted by a slower second alcoholysis step (ii), until full conversion into racemic ethyl lactate (iii).

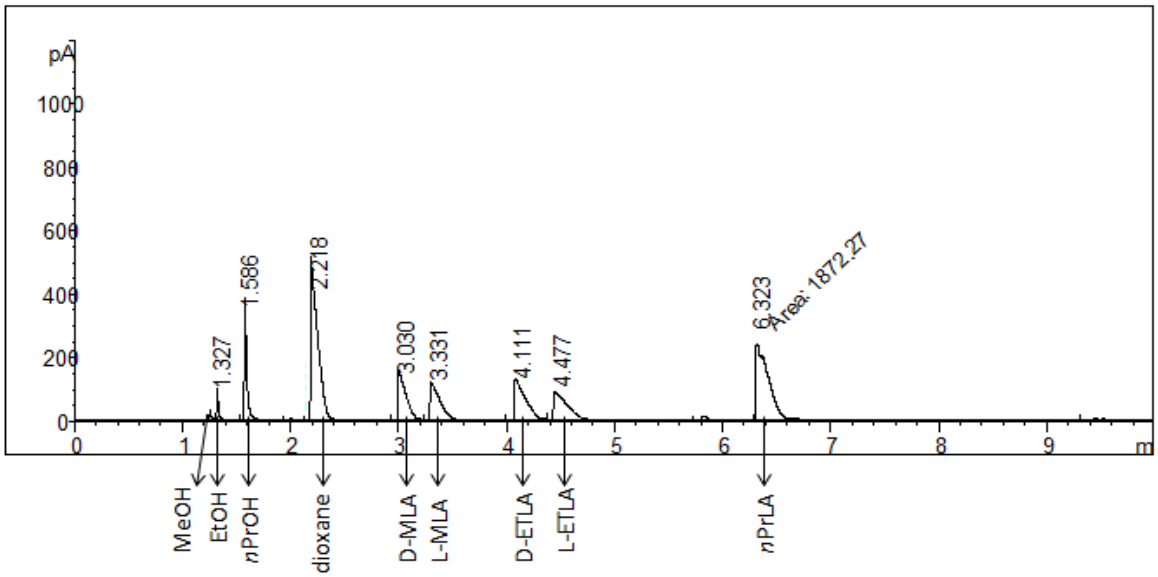
Figure S6: ^{13}C-NMR spectrum in DMSO-*d*6 of the synthesized EtLA. Ethanol was evaporated under a mild dry N_2-flush. Synthesize according to the above mentioned procedure.



Interpretation figure S6: The 5 chemical shifts of the different carbons of ethyl lactate are shown. The multiplet signal around 40 ppm is originating from DMSO-*d*6. .

D) Typical chromatogram and separation characteristics:

Figure S7: Chromatogram of the enantiomers of MLA, EtLA and *n*PrLA.



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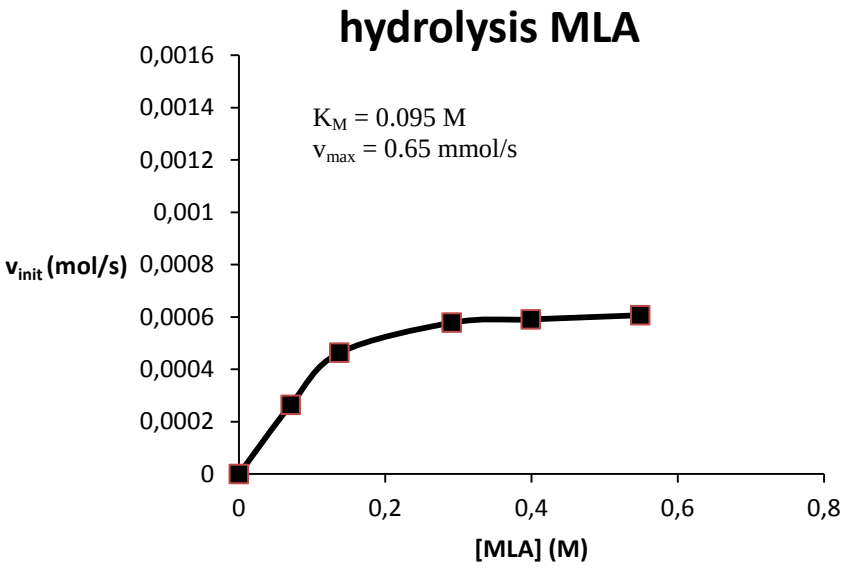
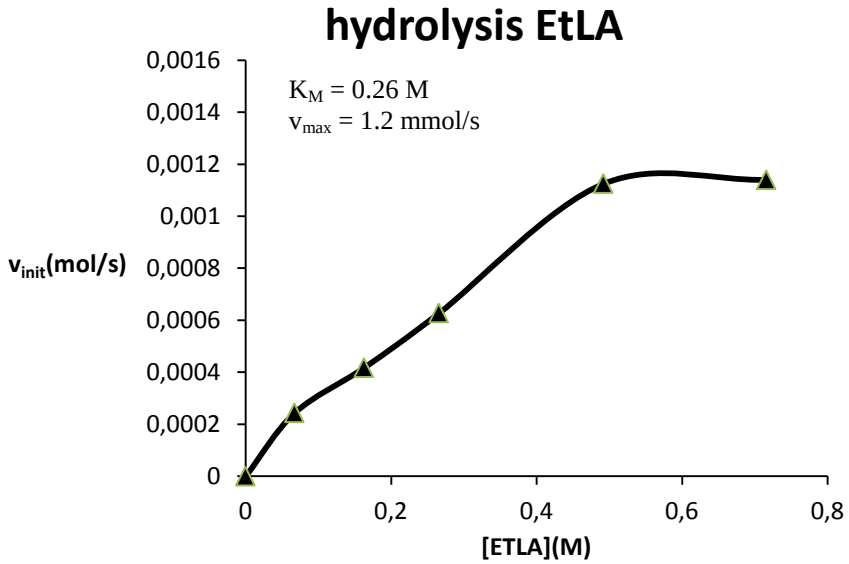
E) Determination of kinetic parameters

General procedure: *C. rugosa* lipase, water and 0.1 M pH = 7.2 phosphate buffer were first combined in a crimp cap vial and heated and magnetically stirred for 15 minutes in a heated copper block, to assure the mixture is already at the fixed temperature (45 °C) before introducing the substrate. Next a fixed amount of MLA, EtLA or *n*PrLA was introduced in the vial. After a short time (in the very initial part of the reaction kinetics) a sample was taken and immediately injected in the GC. Different concentrations for the three substrates were evaluated. The obtained values were depicted in a Michaelis Menten plot. K_M and v_{max} values were estimated from these plots.

Figure S9: Michaelis Menten plots for MLA, EtLA and *n*PrLA using 0.08 g of CRL.





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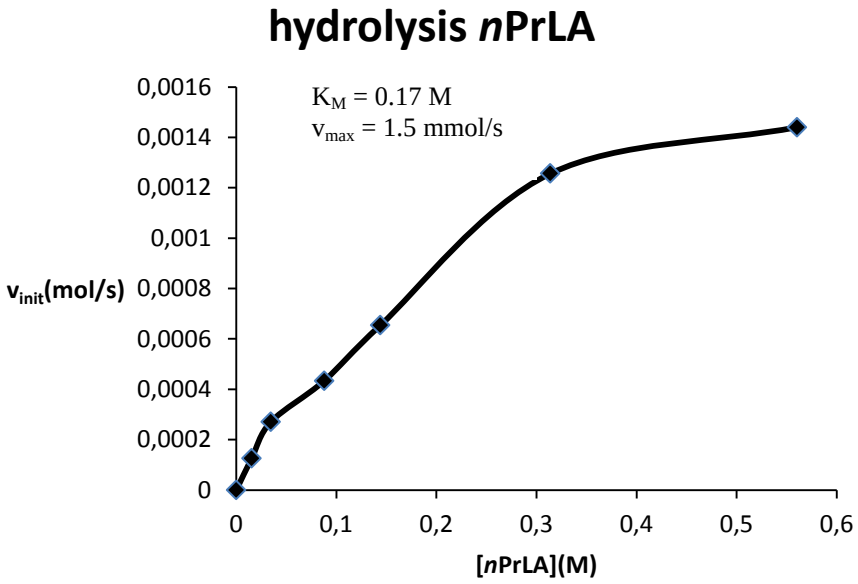

F) Reported enhancement of productivity and stability of lipases

Enhancement of v_{max} (and subsequent volume productivity) by protein engineering

Reaction	**v_{max} improvement**	**Reference**
amide hydrolysis	6.9-fold	[10]
amide hydrolysis	28-fold	[10]
olive oil hydrolysis	10-fold	[17]

The use of protein engineering (for example directed evolution) resulting in more active enzymes (typically factor 10) is an interesting strategy towards higher productivity values for the enzymatic hydrolysis.

Stability of immobilized *Candida rugosa* lipase in hydrolysis reactions

Reaction	**$kg_{product}kg_{enzym}^{-1}$[a]**	**Time on stream[b]**	**Reference**
rice bran oil hydrolysis	647	270	[7]
p-nitrophenol butyrate hydrolysis	1446	720	[8]
olive oil hydrolysis	193	4	[18]
naproxen methyl ester hydrolysis	12634	84	[9]

[a] Catalyst productivity as a measure for stability in combination with activity
[b] Time on stream as a measure for stability

An immobilization strategy potentially increase the catalyst productivity with a factor of 100-1000, and is therefore the main strategy towards the economic viability of the process, next to the enzyme activity enhancement and enzyme production cost reduction.

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G) Economic feasibility study of the enantioselective hydrolysis of racemic lactates for stereoselective polylactic acid applications with *Candida rugosa* lipase

In line with our manuscript "Bridging racemic lactate esters with stereoselective polylactic acid using commercial lipase catalysis" we have assessed the technical economical viability of the proposed methodology for upscaling. The purpose is to obtain enantiopure lactate fractions, which can then be separately processed to enantiopure poly (L) and poly (D)lactic acid with existing processes. Recent literature proposes novel heterogeneous catalysed synthesis routes towards racemic lactates as opposed to the costly fermentation route. This racemates both contain L- and D-lactic acid, but require enantioseparation. The question that arises is if a lipase catalysed kinetic resolution can be carried out on an medium sized commodity plastic scale in an industrial profitable way.

→ For this study we have following **boundary conditions**:

- The price of the starting material (sucrose): 0.7 euro kg^{-1}
- The crucial cost factor in an enzyme based process is the enzyme price due to the mild conditions and low technological barrier.
- The current price of L-lactic acid: 1.2 euro kg^{-1} (literature value confirmed by our industrial partner)

→ We define **economic parameters a** and **b**:

a = 500 / 375 / 250: **the cost of the enzyme in euro kg^{-1}**. The current sales price of crude *Candida rugosa* lipase is 500 euro kg^{-1}. We vary this parameter to two lower values as the sales price never equals the production price. In a joint venture concept the production price is a more realistic value. Literature and information gathered from industry learned that a factor 2 of can exist between the production and sales price.[2] Therefore, 250 euro kg^{-1} was chosen as the lowest possible price and 375 euro kg^{-1} as an intermediate value.

b = 1.7/ 3.5 / 5.3: **the added value of the product in euro kg^{-1}** (separated L- and D-lactates) with respect to the starting source sucrose. The current price of L-lactic acid is about 1.2 euro/kg). The price of stereocomplex PLA, which depends on separated equimolar L- and D-lactic acid streams was expected to be 2 to 5 times the price of commercial PLLA.[3] Therefore we assumed the price of separate L- and D-lactic acid to be 2 to 5 times the price of L-lactid acid. Based on this assumption, three specific cases were born: 2*1.2 euro kg^{-1} (lower price estimation), 3.5*1.2 euro kg^{-1} (medium price estimation) and 5*1.2 euro kg^{-1} (high price estimation). Subtracting the cost of sucrose (0.7 euro kg^{-1}) from these values renders the added value of the product for three cases of parameter b.

→ With these parameters **three scenarios were calculated:**

- **worst** case: high enzyme price, and low added value of the product = **high a, low b**
- **realistic** case: intermediate enzyme price and added value of the product = **medium a and b.**
  The boundaries of the **grey zone** are defined by a high and a low enzyme price respectively, while maintaining a medium added value.
- **opportunistic** case scenario: low enzyme price and high added value of the product = **low a, high b**

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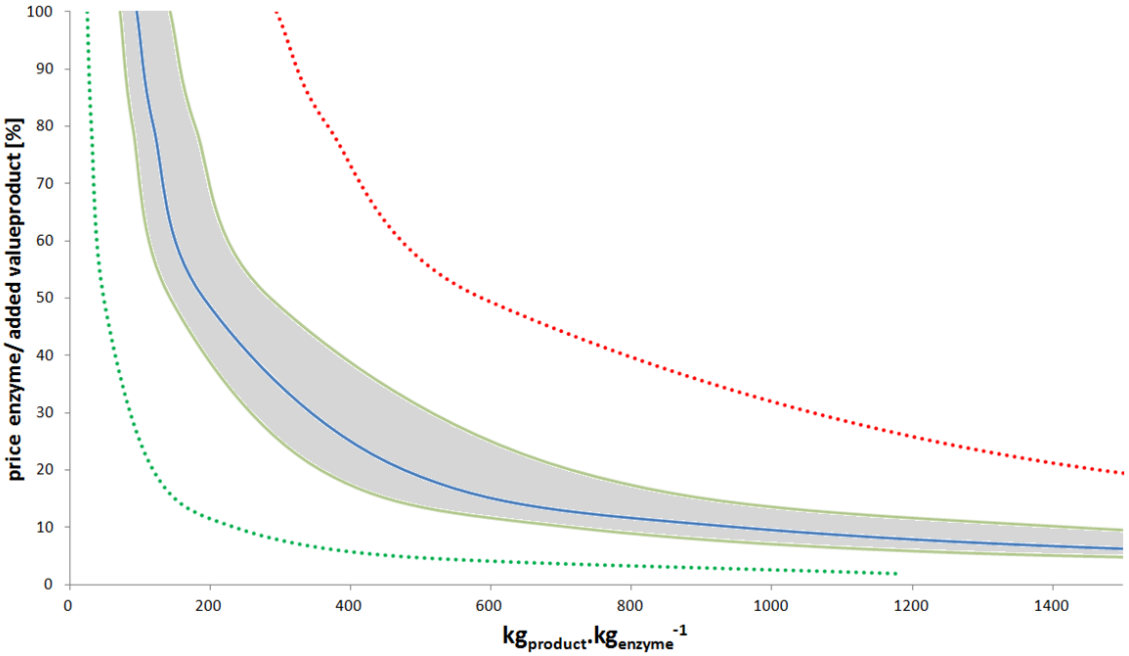
Figure S10: Economic viability **plot** and mathematical **equations**



Mathematical equation of the curves: $Y = \frac{a}{b} \times \frac{100}{x}$		
Scenario	**a**	**b**
worst case scenario	500	1.7
realistic scenario	375	3.5
opportunistic scenario	250	5.3

→ The graph represents the % of the enzyme price with respect to the added value of the product versus the catalyst productivity (expressed in kg product per kg enzyme) and illustrates the economic feasibility. The red and green curves respectively represent the worst and opportunistic scenarios with the black line in between as the most realistic case and the grey zone as a variation on the latter.
From this figure and supported by literature we can **conclude**:

- **Catalyst productivity expressed as $kg_{product}.kg_{enzyme}^{-1}$ is the most important parameter** in the viability of the envisioned synthesis route. **Immobilization of the enzyme** is generally an insignificant cost and it is the main strategy towards high catalyst productivities and stabilities and thus for evaluationg economic feasibility of such process.[4, 5]

- Taking into account the industrial price of the enzyme, the economics of the envisioned process are certainly industrially feasible. At catalyst productivities between 300-400 $kg_{product}.kg_{enzyme}^{-1}$ the contribution of the enzyme cost to the added value of the product is already below 30 % in the **realistic** scenario (intersection). **This range of productivity values are common in biocatalytic processes.** [4, 6] More specific, productivities between **600 and 12,000 $kg_{product}.kg_{enzyme}^{-1}$ are reported in literature for hydrolysis reactions with immobilized *Candida rugosa* lipase in similar conditions** as the ones in our manuscript.[7, 8, 9]

- Apart from immobilisation, **protein engineering of the lipase can improve the activity** (and thus also the catalyst productivity) drastically in hydrolysis reactions, for example with a factor of 5 and further enhances the feasibility.[10] In the graph, this means that one can advance much further to the right of the solid blue line and find an intersection well beneath 30 % on the Y-axis and thus below the blue dotted line.

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- **Progress in recombinant DNA technology and fermentation technology can drastically improve lipase production**. [6, 11, 12, 13, 14] This will further stimulate the production of CRL at lower prices, which would move the plot in the direction of the green scenario.

H) Effect molar enantiomeric purity of lactate streams and corresponding stereocomplex properties

The effect of the molar enantiomeric purity (Pur.) of the lactates (and thus the homopolymers) on a corresponding PLA stereocomplex, is illustrated in the table.[15] Note that the melting point (T_m) of commercial PLLA is around 165°C. The T_m of stereocomplexed PLA generally decreases with a lower optical purity, but shifts to higher temperatures in comparison with the separate homopolymers of the same purity. [15, 16]

Pur.[%]	T_m homopolymer	T_m stereocomplex
93	155°C-185°C	195°C-263°C
91	150°C-173°C	188°C-248°C
83	135°C-158°C	180°C-228°C
76	125°C-147°C	175°C-214°C

I) Commercial lipase preparations used

Commercial lipase preparations were purchased from Sigma-Aldrich:
Crude lipase from *Candida rugosa* (L-1754, Type VII, ≥700 unit/mg solid)
Immobilized lipase B from *Candida antarctica* (on acrylic resin) (L-4777)
Crude lipase from *Porcine pancreas* (L-3126, Type II, 100-400 units/mg protein)
Amano Lipase PS, from *Burkholderia cepacia*
Lipozyme, immobilized from *Mucor miehei*

J) Additional information on crude *Candida rugosa* lipase preparations

Candida rugosa produces at least 7 different lipases (isoenzymes). The isoenzymes differ in amino acid sequence, isoelectric points and degree of glycosylation.[19, 20, 21] The enantioselectivity of the several isoforms is differing, although they have generally the same enantiopreference. Five of these isoenzymes (Lip1- Lip5) are biochemically characterized.[19] The crystal structure of Lip1-Lip3 is also known and available from the Protein Data Bank (PDB).[22, 23, 24] Commercial preparations of CRL mainly contain Lip1-Lip3. Crude CRL from Sigma-Aldrich contains almost 90% of Lip1 and only a small amount of Lip3.[25] The main impurity in such commercial lipase samples is lactose, which acts as water reservoir.[26]

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