Electronic Supplementary Information (ESI):

An insight into the solvent effect on the positional selectivity of the immobilized lipase from *Burkholderia cepacia* in 1,3-diolein synthesis

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Experimental section

Biological and chemical materials

The lipase from *Burkholderia cepacia* (PSL-C, immobilized on ceramics, 35.8 U g^{-1}, one unit was defined as the amount of enzyme which catalyzed the esterification of 1 mmol oleic acid with glycerol per minute at 50 °C) was a gift from Amano Enzyme China Ltd.. 1-Monoolein, 2-monoolein, 1,2-diolein, 1,3-diolein and triolein were purchased from Sigma-Aldrich. All other chemicals and reagents were of the highest purity commercially available.

General procedure for 1,3-diolein preparation

In a typical experiment, the PSL-C-catalyzed esterification of oleic acid with glycerol proceeded

in a 50 mL flask on a rotary shaker at 200 rpm at 50 °C. The reaction mixtures contained oleic acid, glycerol, solvent and PSL-C. Certain amount of 4 Å molecular sieves was employed to remove the generated water online. Samples were withdrawn, centrifuged to obtain the upper layer and analyzed by HPLC and GC.

Analysis of the samples

1-Monoolein, 2-monoolein, 1,2-diolein, 1,3-diolein and triolein contents in the reaction mixture were analyzed using the methods described by us previously.[1] Shimadzu 20A HPLC system (Shimadzu, Kyoto, Japan) with a low temperature evaporative light scattering detector was employed. External standards of 1-monoolein, 2-monoolein, 1,2-diolein, 1,3-diolein and triolein were used to prepare calibration solutions at eight different concentrations. 2 μL sample and 1 mL acetone were precisely measured and mixed thoroughly. 20 μL of the aforementioned mixture was injected. The stationary and mobile phases were a C18 column (5 μm, 250 mm × 4.6 mm) (Dikma Technology, PLATISIL ODS, China) and a gradient elution program (Table 1s) by acetonitrile and dichloromethane at 1.5 mL min^{-1}, respectively. The column temperature and drift pipe temperature were controlled at 40 °C and 70 °C, respectively, and the nitrogen pressure was controlled at 320 kPa. The retention times were 3.75 min, 4.54 min, 23.12 min, 23.90 min, and 42.91 min for 2-monoolein, 1-monoolein, 1,3-diolein, 1,2-diolein and triolein, respectively. The oleic acid and glycerol concentrations were detected using the methods described by Du.[2] The oleic acid content was analyzed by GC-14B gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a FFAP capillary column (0.32 mm × 25 m) and FID detector. The column temperature was kept at 150 °C for 0.5 min, raised to 250 °C at 15 °C min^{-1}, and maintained at this

temperature for 10 min. The temperatures of the injector and detector were set at 245 and 250 °C, respectively. The glycerol concentration was measured by HPLC using a SCL-10A system (Shimadzu, Kyoto, Japan) equipped with a refractive index detector and an Aminex HPX-87H column thermostated at 65 °C. Elution was carried out with a 5 mM H_2SO_4 mobile phase at a flow rate of 0.8 mL min^{-1}. All reported data are averages of experiments performed at least in duplicate.

Table 1s Gradient elution program

Time (min)	Flow rate (mL min^{-1})	Acetonitrile-acetic acid (99.85:0.15) (V/V, %)	Dichloromethane (V/V, %)
0	1.50	100	0
4	1.50	100	0
12	1.50	90	10
25	1.50	90	10
30	1.50	70	30
35	1.50	70	30
45	1.50	20	80
55	1.50	20	80
60	1.50	100	0
65	1.50	100	0

Rate constants identified[1]

For investigating the kinetic behaviors during PSL-C-catalyzed synthesis of 1,3-diolein in these solvent systems, the synthetic scheme shown in Scheme 1 was adopted in which oleic acid and the byproduct water was neglected for simplification. The esterification reaction rates would follow second-order kinetics with respect to the concentration of each substrate involved in the respective reaction step;[3,4] the mass transfer limitation in the reaction system could be neglected. Based on

the above assumptions, the differential equations characterizing the whole reaction process were listed as follows and in the equations, OA, 1-MO, 2-MO, 1,3-DO, 1,2-DO, TO and Gly referred to oleic acid, l-monoolein, 2-monoolein, 1,3-diolein, 1,2-diolein, triolein and glycerol, respectively.

$$\frac{d[OA]}{dt} = k_2[1-MO] + k_{10}[2-MO] + k_4[1,3-DO] + (k_6 + k_{12})[1,2-DO] + (k_8 + k_{14})[TO] - ((k_1 + k_9)[Gly] + (k_3 + k_{11})[1-MO] + k_5[2-MO] + k_7[1,2-DO] + k_{13}[1,3-DO])[OA]$$

$$\frac{d[1-MO]}{dt} = k_1[OA][Gly] + k_4[1,3-DO] + k_{12}[1,2-DO] + k_{16}[2-MO] - (k_2 + k_5 + (k_3 + k_{11})[OA])[1-MO]$$

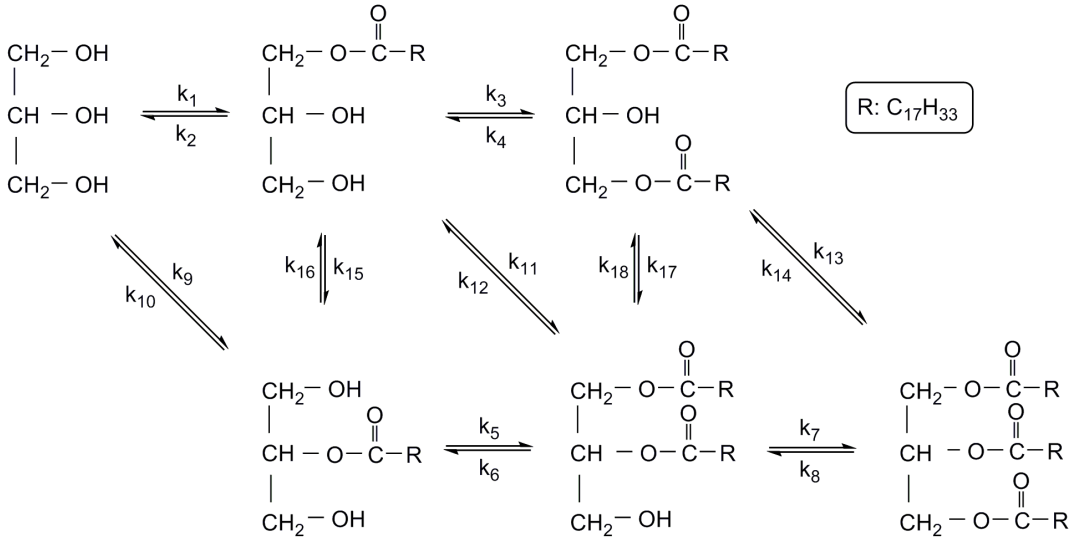
$$\frac{d[2-MO]}{dt} = k_6[1,2-DO] + k_9[OA][Gly] + k_{15}[1-MO] - (k_5[OA] + k_{10} + k_{16})[2-MO]$$

$$\frac{d[1,3-DO]}{dt} = k_3[1-MO][OA] + k_{14}[TO] + k_{18}[1,2-DO] - (k_4 + k_{13}[OA] + k_{17})[1,3-DO]$$

$$\frac{d[1,2-DO]}{dt} = (k_5[2-MO] + k_{11}[1-MO])[OA] + k_8[TO] + k_{17}[1,3-DO] - (k_6 + k_7[OA] + k_{12} + k_{18})[1,2-DO]$$

$$\frac{d[TO]}{dt} = (k_7[1,2-DO] + k_{13}[1,3-DO])[OA] - (k_8 + k_{14})[TO]$$

$$\frac{d[Gly]}{dt} = k_2[1-MO] + k_{10}[2-MO] - (k_1 + k_9)[OA][Gly]$$



Scheme 1

References

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