

The synthesis method of deep eutectic solvents

The hydrogen bond acceptor and hydrogen bond donor are mixed in a certain molar ratio, stirred in a water bath at 100 °C to a colorless transparent solution, and allowed to stand at room temperature.

Table S1 Hydrolysis of CLA-EE by different enzymes^a

	CALB	G50	MAS1	MH	SD	AYS
Hydrolysis (%)	52.0±2.3	5.7±1.0	27.2±1.5	11.1±2.1	28.7±1.9	24.1±1.3
Purity (%)	40.3±1.1	63.4±1.7	59.9±1.6	54.1±1.0	32.7±0.5	86.4±0.6

^aLipozyme CALB, *Candida antarctica* lipase B; G50, *Penicillium camembertii*; MAS1, marine *Streptomyces* sp. MAS1 lipase; Lipase PS Amano SD, *Burkholderia cepacia*; Lipase AYS, *Candida rugosa* lipase;
Purity (%) : Purity of c9, t11-CLA

Table S2 Number of phases in different systems

	PEG400	PEG600	PEG2000	BMIM[BF ₄]	BMIM[PF ₆]	Ethanol
(NH ₄) ₂ SO ₄	III	III	III	III	III	II
Na ₂ SO ₄	III	III	III	III	III	II

III: three-phase; II: two-phase;

Table S3 The effect of selective hydrolysis of CLA-EE isomers and the distribution behavior of lipases in different systems

	PEG600/K ₂ HPO ₄	PEG2000/K ₂ HPO ₄	PEG2000/Na ₂ SO ₄	BMIM[PF ₆]/Na ₂ SO ₄	CG/K ₂ HPO ₄
K	6.9±1.3	0.8±0.0	0.4±0.0	0.3±0.0	2.6±0.4
Y (%)	84.8±2.5	36.9±2.6	24.6±0.7	55.6±1.8	68.1±3.2
Hydrolysis (%)	—	—	24.7±1.3	31.9±0.2	-
Purity (%)	—	—	57.8±0.2	87.4±0.6	-

Purity (%) : Purity of c9, t11-CLA; CG: Choline chloride: glycerin=1:1, 30% CG, 25% K₂HPO₄;

Table S4 Effect of pH on selective hydrolysis of CLA-EE isomers in TLPS.

	pH 4	pH 5.2*	pH 6	pH 7	pH 8	pH 9
Hydrolysis (%)	44.6±2.4	45.1±2.1	44.8±1.0	35.0±2.6	27.6±1.9	5.1±0.5
Purity (%)	90.7±0.3	90.9±0.3	91.0±0.3	90.1±0.3	88.8±0.4	—

*Initial pH of reaction system

Table S5 Diameter and specific surface of oil phase droplets dispersed middle phase, bottom phase in TLPS and water, and middle phase droplets dispersed in the bottom phase.

continuous phase	Water ¹	Middle phase ¹	Bottom phase ¹	Bottom phase ²
Droplet (μm)	69.800	20.709	28.559	6.697
SS ($\text{m}^2 \text{g}^{-1}$)	0.086	0.29	0.21	0.896

SS: specific surface; ¹: the dispersed phase was the oil phase of TLPS; ²: the dispersed phase was the middle phase of TLPS.

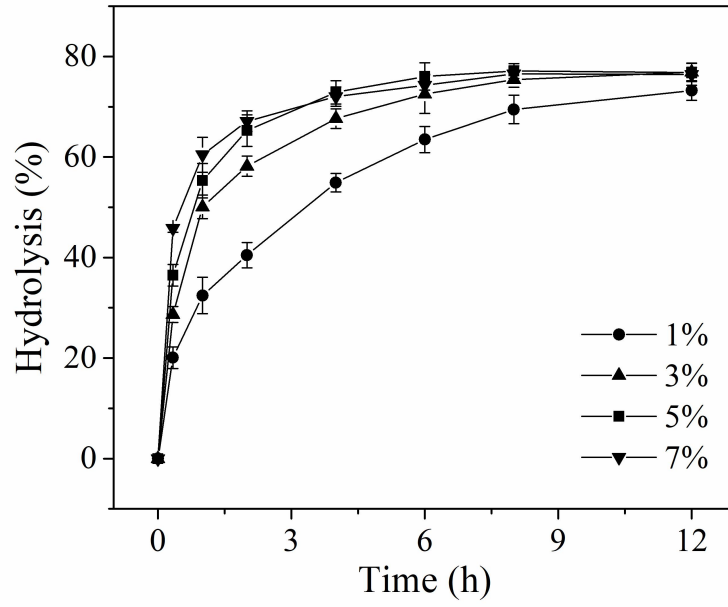


Figure S1 Effect of the amount of enzyme added on the selective hydrolysis of CLA-EE isomers in TLPS.

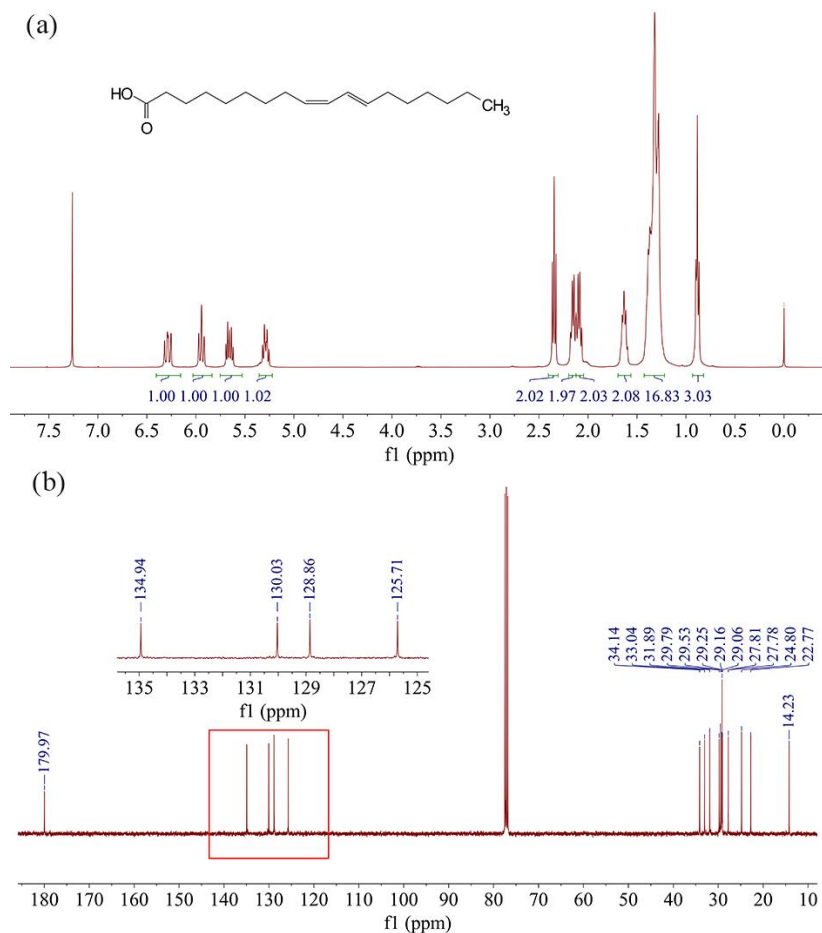


Figure S2 (a) The ^1H NMR spectra of product c9, t11-CLA; (b) The ^{13}C NMR spectra of product c9, t11-CLA; ^1H NMR (400 MHz, CDCl_3 , TMS) δ 6.29 (dd, $J = 15.0, 10.9$ Hz, 1H), 5.94 (t, $J = 10.9$ Hz, 1H), 5.66 (dt, $J = 14.6, 7.0$ Hz, 1H), 5.35 – 5.22 (m, 1H), 2.35 (t, $J = 7.5$ Hz, 2H), 2.15 (q, $J = 6.8$ Hz, 2H), 2.09 (q, $J = 7.8, 7.3$ Hz, 2H), 1.63 (p, $J = 7.4$ Hz, 2H), 1.43 – 1.22 (m, 16H), 0.88 (t, $J = 6.7$ Hz, 3H).

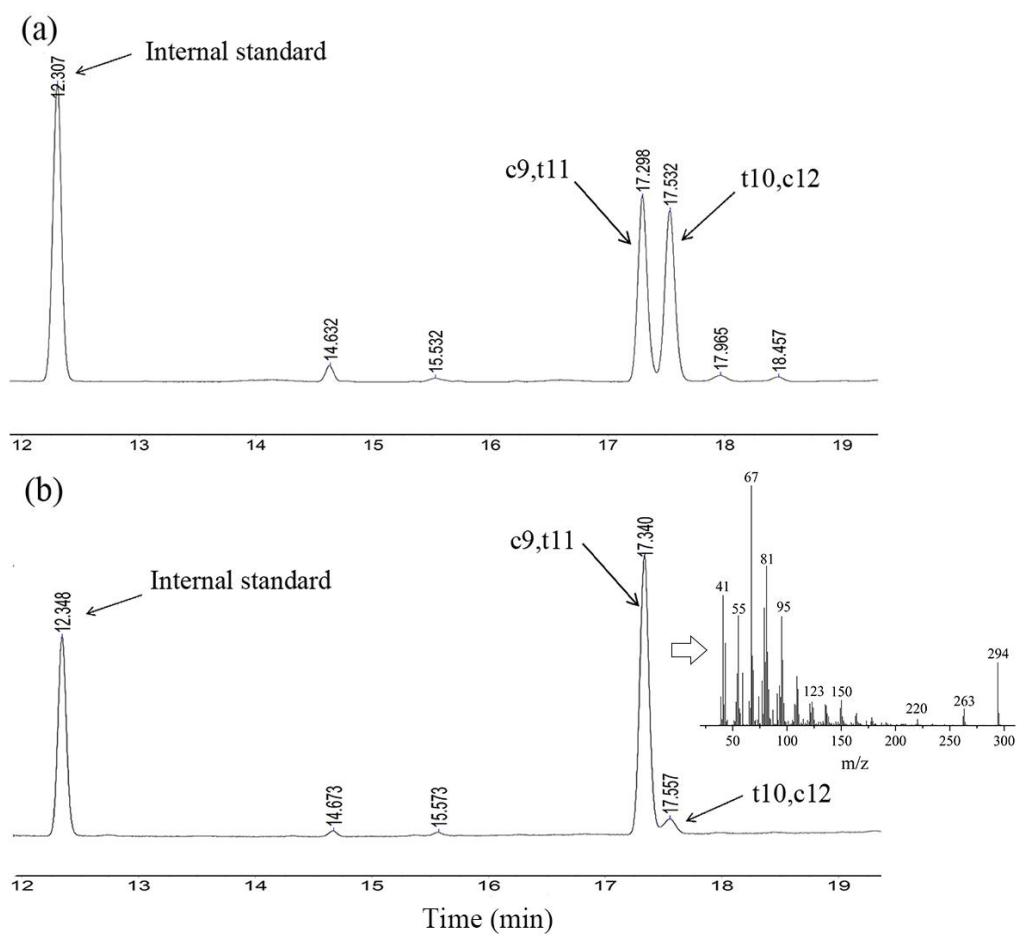


Figure S3 The GC-MS analysis of CLA methyl ester. (a) CLA mixture; (b) product.