Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Supporting information

Water-dependent kinetics guide complex lipase-mediated synthesis of biolubricants in a water activity control reactor

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Supplementary material

Part I Intrinsic kinetics of enzymatic reactions

I. Notation

TMP	Trimethylol propane	mmol/$g_{substrate}$
FA	Fatty acid	mmol/$g_{substrate}$
MAP	Mono-substituted TMP esters	mmol/$g_{substrate}$
DAP	Di-substituted TMP esters	mmol/$g_{substrate}$
TAP	Tri-substituted TMP esters	mmol/$g_{substrate}$
W	Water	mmol/$g_{substrate}$
[E]	Free enzyme concentration	mmol/$g_{substrate}$
[ET]	Total enzyme concentration	mmol/$g_{substrate}$
$[A \times E \times B]$	Ternary substrate A-enzyme-substrate B complex	mmol/$g_{substrate}$
[A]	Compound A concentration	mmol/$g_{substrate}$
k_i	Kinetic constant	
K_i	Equilibrium constant	$g_{substrate}^2$/mmol^2
V_i	Apparent rate constants	$g_{substrate}^2/(g_{enzyme}$•mmol•min)

II. Esterification/hydrolysis processes

In our works, three assumptions were made in order to simplify the kinetic model. Firstly, the reaction rates for different enzymatic complexes follow pseudo-equilibrium relationships because the concentration of the enzyme used was much lower than the concentration of substrates. Secondly, although the reaction rates might be controlled by external or internal mass transfer for lipase powder, the mass transfer limitation could be neglected as suggestion from the literature[1] when investigating the free enzyme system. Thirdly, there might be substrates or products inhibitions on the enzyme, but these effects were negligible.

Then, the following esterification/hydrolysis equations are proposed:

$$TMP + FA + E \underset{k_2}{\overset{k_1}{\Leftrightarrow}} TMP \times FA \times E \underset{k_4}{\overset{k_3}{\Leftrightarrow}} MAP \times E \times W \underset{k_6}{\overset{k_5}{\Leftrightarrow}} MAP + E + W$$

$$MAP + FA + E \underset{k_8}{\overset{k_7}{\Leftrightarrow}} MAP \times FA \times E \underset{k_{10}}{\overset{k_9}{\Leftrightarrow}} DAP \times E \times W \underset{k_{12}}{\overset{k_{11}}{\Leftrightarrow}} DAP + E + W$$

$$DAP + FA + E \underset{k_{14}}{\overset{k_{13}}{\Leftrightarrow}} DAP \times FA \times E \underset{k_{16}}{\overset{k_{15}}{\Leftrightarrow}} TAP \times E \times W \underset{k_{18}}{\overset{k_{17}}{\Leftrightarrow}} TAP + E + W$$

III. The reaction rates for each compound can be described as following:

$$\frac{d[TAP]}{dt} = -k_{18}[TAP][E][W] + k_{17}[TAP \times E \times W] \qquad (Seq1)$$

$$\frac{d[DAP]}{dt} = k_{11}[DAP \times E \times W] - k_{12}[DAP][E][W] + k_{14}[DAP \times FA \times E] - k_{13}[DAP][E][FA]$$

$$\frac{d[MAP]}{dt} = k_5[MAP \times E \times W] - k_6[MAP][E][W] + k_8[MAP \times FA \times E] - k_7[MAP][E][FA]$$

$$\frac{d[TMP]}{dt} = -k_1[TMP][FA][E] + k_2[TMP \times FA \times E] \qquad (Seq4)$$

$$\frac{d[FA]}{dt} = -k_1[TMP][FA][E] + k_2[TMP \times FA \times E] - k_7[MAP][FA][E] + k_8[MAP \times FA \times \ldots \times FA \times E] \qquad (Seq5)$$

$$\frac{d[W]}{dt} = -k_6[MAP][W][E] + k_5[MAP \times W \times E] - k_{12}[DAP][W][E] + k_{11}[DAP \times W \times E \ldots \qquad (Seq6)$$

IV. Following pseudo-equilibrium relationships, the concentration of the different enzymatic complexes was approximated to be zero.

$$\frac{d[TAP \times E \times W]}{dt} = 0 \quad = -(k_{16} + k_{17})[TAP \times E \times W] + k_{18}[TAP][E][W] + k_{15}[DAP \times FA \times E]$$

$$\frac{d[DAP \times FA \times E]}{dt} = 0 \quad = -(k_{14} + k_{15})[DAP \times FA \times E] + k_{13}[DAP][E][FA] + k_{16}[TAP \times E \times W]$$

$$\frac{d[DAP \times E \times W]}{dt} = 0 \quad = -(k_{10} + k_{11})[DAP \times E \times W] + k_{12}[DAP][E][W] + k_{9}[MAP \times FA \times E]$$

$$\frac{d[MAP \times FA \times E]}{dt} = 0 \quad = -(k_{8} + k_{9})[MAP \times FA \times E] + k_{7}[MAP][E][FA] + k_{10}[DAP \times E \times W]$$

$$\frac{d[MAP \times E \times W]}{dt} = 0 \quad = -(k_{4} + k_{5})[MAP \times E \times W] + k_{6}[MAP][E][W] + k_{3}[TMP \times FA \times E]$$

$$\frac{d[TMP \times FA \times E]}{dt} = 0 \quad = -(k_{2} + k_{3})[TMP \times FA \times E] + k_{1}[TMP][E][FA] + k_{4}[MAP \times E \times W]$$

V. the total enzyme concentration (ET) is the sum of the concentrations of the complexes and the free enzyme:

$$[ET] = [E] + [TAP \times E \times W] + [DAP \times E \times FA] + [DAP \times E \times W] + [MAP \times E \times W] + \quad (Seq13)$$

It is worth pointing out that for convenience the enzyme total concentration was expressed in terms of enzyme weight and substrate weight[2], whose values are already known at the beginning of reactions.

VI. Several algebraic manipulations of the resulting equations give the following expressions of reaction rates:

$$[TMP \times FA \times E] = \frac{k_1(k_4 + k_5)}{k_2k_4 + (k_2 + k_3)k_5} \times [TMP][E][FA] + \frac{k_4k_6}{k_2k_4 + (k_2 + k_3)k_5} \times [MAP][E][W]$$

$$[MAP \times E \times W] = \frac{k_1k_3}{k_2k_4 + (k_2 + k_3)k_5} \times [TMP][E][FA] + \frac{(k_2 + k_3)k_6}{k_2k_4 + (k_2 + k_3)k_5} \times [MAP][E][W]$$

$$[MAP \times FA \times E] = \frac{k_7(k_{10} + k_{11})}{k_8k_{10} + (k_8 + k_9)k_{11}} \times [MAP][E][FA] + \frac{k_{10}k_{12}}{k_8k_{10} + (k_8 + k_9)k_{11}} \times [DAP][E][W]$$

$$[MAP \times E \times W] = \frac{k_7k_9}{k_8k_{10} + (k_8 + k_9)k_{11}} \times [MAP][E][FA] + \frac{(k_8 + k_9)k_{12}}{k_8k_{10} + (k_8 + k_9)k_{11}} \times [DAP][E][W]$$

$$[DAP \times FA \times E] = \frac{k_{13}(k_{16} + k_{17})}{k_{14}k_{16} + (k_{14} + k_{15})k_{17}} \times [DAP][E][FA] + \frac{k_{16}k_{18}}{k_8k_{10} + (k_8 + k_9)k_{11}} \times [DAP][E][W]$$

$$[DAP \times E \times W] = \frac{k_{13}k_{15}}{k_{14}k_{16} + (k_{14} + k_{15})k_{17}} \times [DAP][E][FA] + \frac{(k_{14} + k_{15})k_{18}}{k_8k_{10} + (k_8 + k_9)k_{11}} \times [DAP][E][W]$$

VII. The constants K_i and V_i are defined as functions of the constants k$_i$.

$$K_1 = \frac{k_1(k_3 + k_4 + k_5)}{k_2k_4 + (k_2 + k_3)k_5}; \qquad K_2 = \frac{k_6(k_2 + k_3 + k_4)}{k_2k_4 + (k_2 + k_3)k_5}$$

$$K_3 = \frac{k_7(k_9 + k_{10} + k_{11})}{k_8 k_{10} + (k_8 + k_9)k_{11}}; \qquad K_4 = \frac{k_{12}(k_8 + k_9 + k_{10})}{k_8 k_{10} + (k_8 + k_9)k_{11}}$$

$$K_5 = \frac{k_{13}(k_{15} + k_{16} + k_{17})}{k_{14} k_{16} + (k_{14} + k_{15})k_{17}}; \quad K_6 = \frac{k_{18}(k_{14} + k_{15} + k_{16})}{k_{14} k_{16} + (k_{14} + k_{15})k_{17}}$$

$$V_1 = \frac{k_1 k_3 k_5}{k_2 k_4 + (k_2 + k_3)k_5}; \qquad V_2 = \frac{k_2 k_4 k_6}{k_2 k_4 + (k_2 + k_3)k_5}$$

$$V_3 = \frac{k_7 k_9 k_{11}}{k_8 k_{10} + (k_8 + k_9)k_{11}}; \qquad V_4 = \frac{k_8 k_{10} k_{12}}{k_8 k_{10} + (k_8 + k_9)k_{11}}$$

$$V_5 = \frac{k_{13} k_{15} k_{17}}{k_{14} k_{16} + (k_{14} + k_{15})k_{17}}; \quad V_6 = \frac{k_{14} k_{16} k_{18}}{k_{14} k_{16} + (k_{14} + k_{15})k_{17}}$$

Hence, the reactions rates could be expressed as the concentrations of total enzyme and the TMP, MAP, DAP, TAP, FA, W.

$$[E] = \frac{a[ET]}{\begin{pmatrix} 1 + K_1[TMP][FA] + K_2[MAP][W] + K_3[MAP][F] \\ + K_4[DAP][W] + K_5[DAP][F] + K_6[TAP][W] \end{pmatrix}} \qquad (Seq20)$$

$$\frac{d[TAP]}{dt} = \frac{a[ET](V_5[DAP][FA] - V_6[TAP][W])}{\begin{pmatrix} 1 + K_1[TMP][FA] + K_2[MAP][W] + K_3[MAP][FA] \\ + K_4[DAP][W] + K_5[DAP][FA] + K_6[TAP][W] \end{pmatrix}} \qquad (Seq21)$$

$$\frac{d[DAP]}{dt} = \frac{a[ET](V_3[MAP][FA] - V_4[DAP][W] - V_5[DAP][FA] + V_6[TAP][W])}{\begin{pmatrix} 1 + K_1[TMP][FA] + K_2[MAP][W] + K_3[MAP][FA] \\ + K_4[DAP][W] + K_5[DAP][FA] + K_6[TAP][W] \end{pmatrix}} \qquad (Seq22)$$

$$\frac{d[MAP]}{dt} = \frac{a[ET]\big(V_1[TMP][FA] - V_2[MAP][WA] - V_3[MAP][FA] + V_4[DAP][WA]\big)}{\begin{pmatrix} 1 + K_1[TMP][FA] + K_2[MAP][W] + K_3[MAP][F] \\ + K_4[DAP][W] + K_5[DAP][FA] + K_6[TAP][W] \end{pmatrix}} \quad (Seq23)$$

$$\frac{d[TMP]}{dt} = \frac{a[ET]\big(- V_1[TMP][FA] + V_2[MAP][W]\big)}{\begin{pmatrix} 1 + K_1[TMP][FA] + K_2[MAP][W] + K_3[MAP][FA] \\ + K_4[DAP][W] + K_5[DAP][FA] + K_6[TAP][W] \end{pmatrix}} \quad (Seq24)$$

$$\frac{d[FA]}{dt} = \frac{a[ET]\begin{pmatrix} - V_1[TMP][FA] + V_2[MAP][W] - V_3[MAP][FA] \\ + V_4[DAP][W] - V_5[DAP][FA] + V_6[TAP][W] \end{pmatrix}}{\begin{pmatrix} 1 + K_1[TMP][FA] + K_2[MAP][W] + K_3[MAP][FA] \\ + K_4[DAP][W] + K_5[DAP][FA] + K_6[TAP][W] \end{pmatrix}} \quad (Seq25)$$

$$\frac{d[W]}{dt} = \frac{a[ET]\begin{pmatrix} V_1[TMP][FA] - V_2[MAP][W] + V_3[MAP][FA] \\ - V_4[DAP][W] + V_5[DAP][FA] - V_6[TAP][W] \end{pmatrix}}{\begin{pmatrix} 1 + K_1[TMP][FA] + K_2[MAP][W] + K_3[MAP][FA] \\ + K_4[DAP][W] + K_5[DAP][FA] + K_6[TAP][W] \end{pmatrix}} \quad (Seq26)$$

Part II Supplementary Figures and Tables

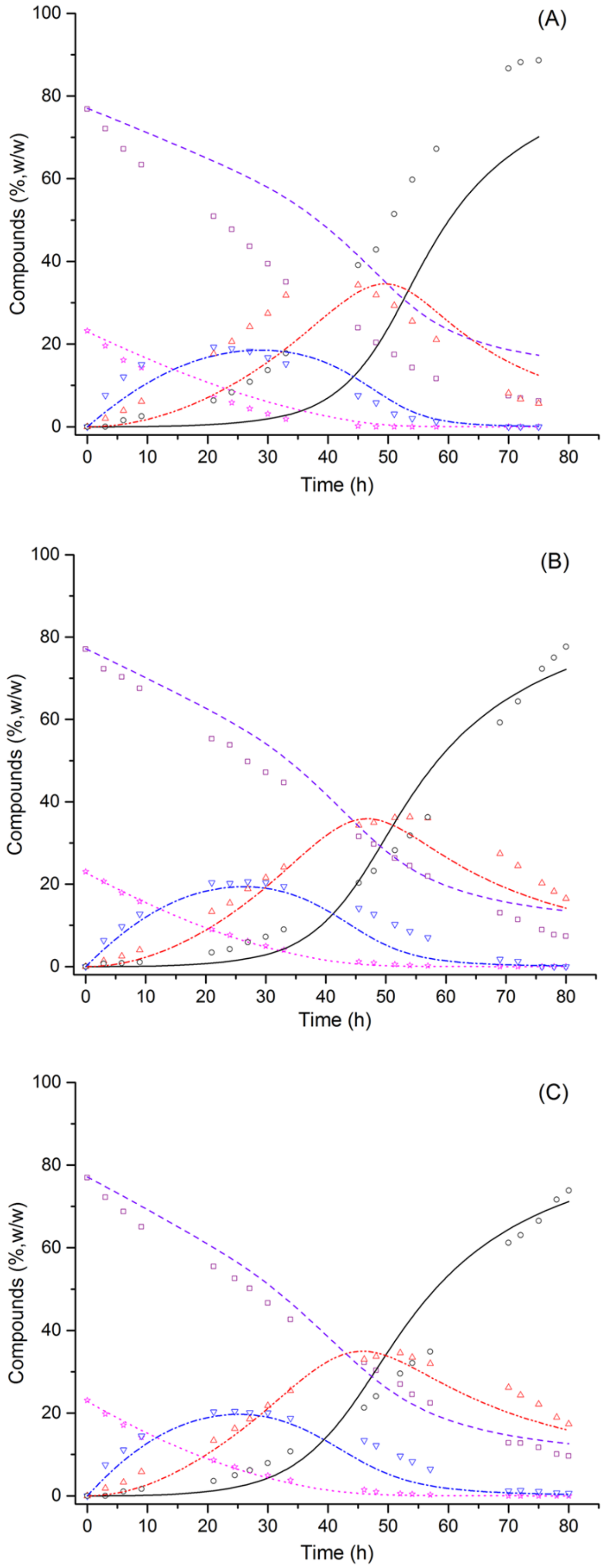

Fig. S1 Fitting of the kinetic model without water balance to the experimental data when water activity was controlled at (A) 0.25, (B) 0.35 and (C) 0.45. ○: TAP; △: DAP; ▽: MAP; ☆: TMP;

□: FA.

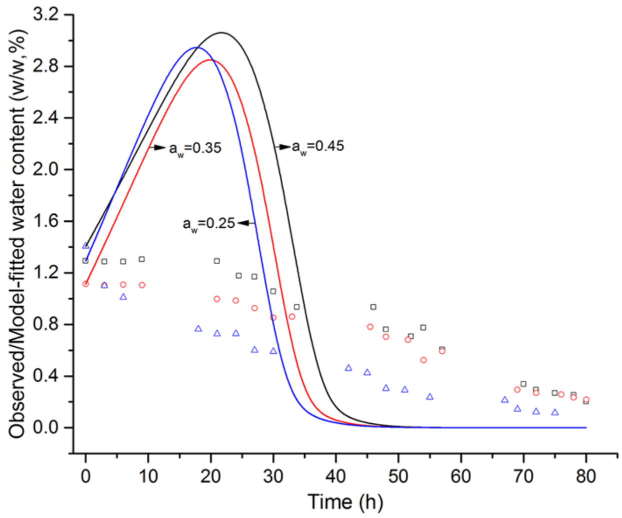

Fig. S2 Comparison of observed (symbols) and model-fitted (solid line, without water balance) water content in medium when water activity was controlled at 0.25 (△, blue), 0.35 (○, red) and 0.45 (□, black).

Table S1 Correlation efficient between observed and model-fitted (within water balance) data for each compound when water activity was controlled at different values.

Compounds	a_w=0.25	a_w=0.35	a_w=0.45
[TAP]	0.999	0.999	0.999
[DAP]	0.990	0.995	0.996
[MAP]	0.999	0.995	0.999
[TMP]	0.999	1.000	0.999
[FA]	0.999	1.000	1.000
[W]	0.963	0.983	0.967

Table S2 Correlation efficient between observed and model-fitted (without water balance) data for each compound when water activity was controlled at different values.

Compounds	a_w=0.25	a_w=0.35	a_w=0.45
[TAP]	0.978	0.973	0.989
[DAP]	0.818	0.967	0.955
[MAP]	0.927	0.941	0.959
[TMP]	0.983	0.979	0.990
[FA]	0.964	0.989	0.979
[W]	0.655	0.772	0.797

Part III Spectra of products

1. 1H and 13C of tri-substituted TMP esters

Tri-substituted TMP esters (Light yellow liquid, 97%, w/w) was purified by molecular distillation and the yield of the whole process was around 75%.

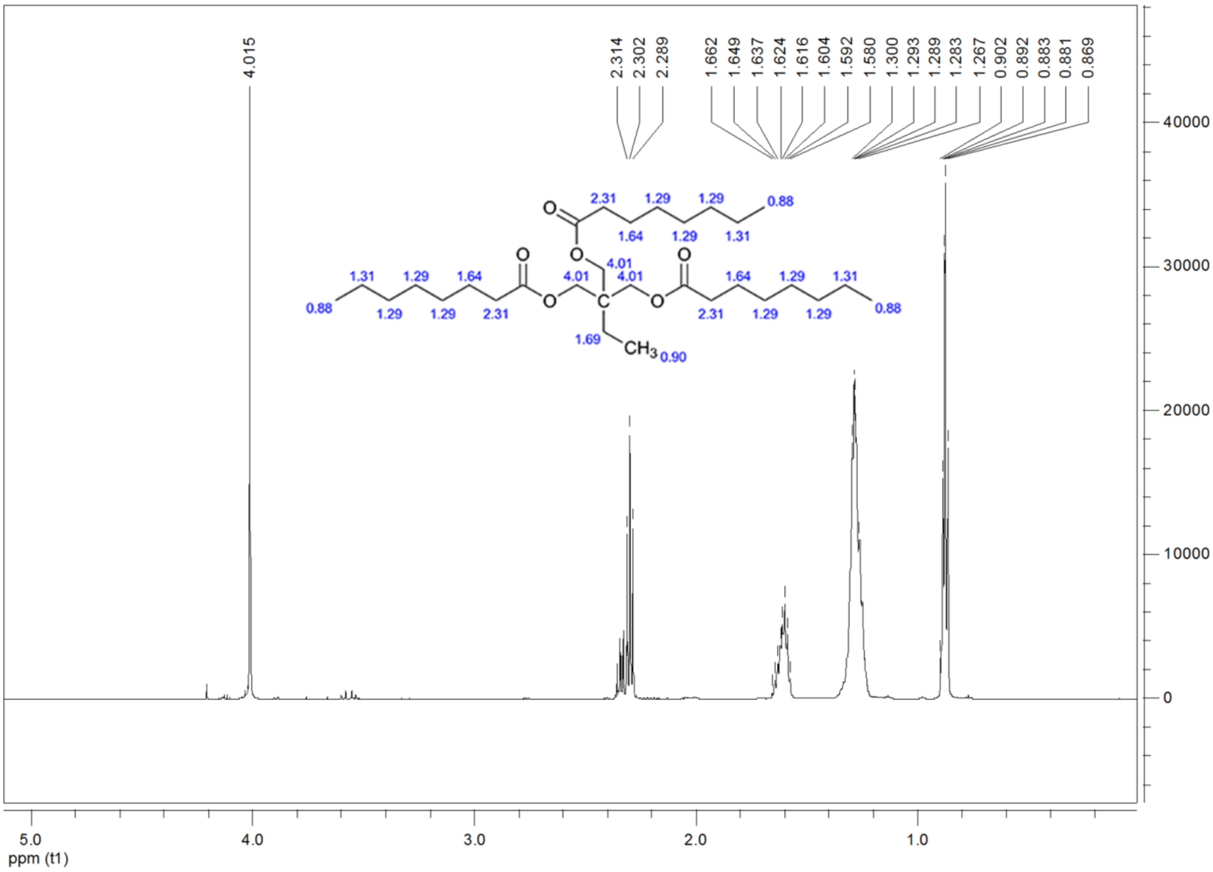

Fig. S3 1H NMR (600M, $CDCl_3$) spetra of tri-substituted TMP esters

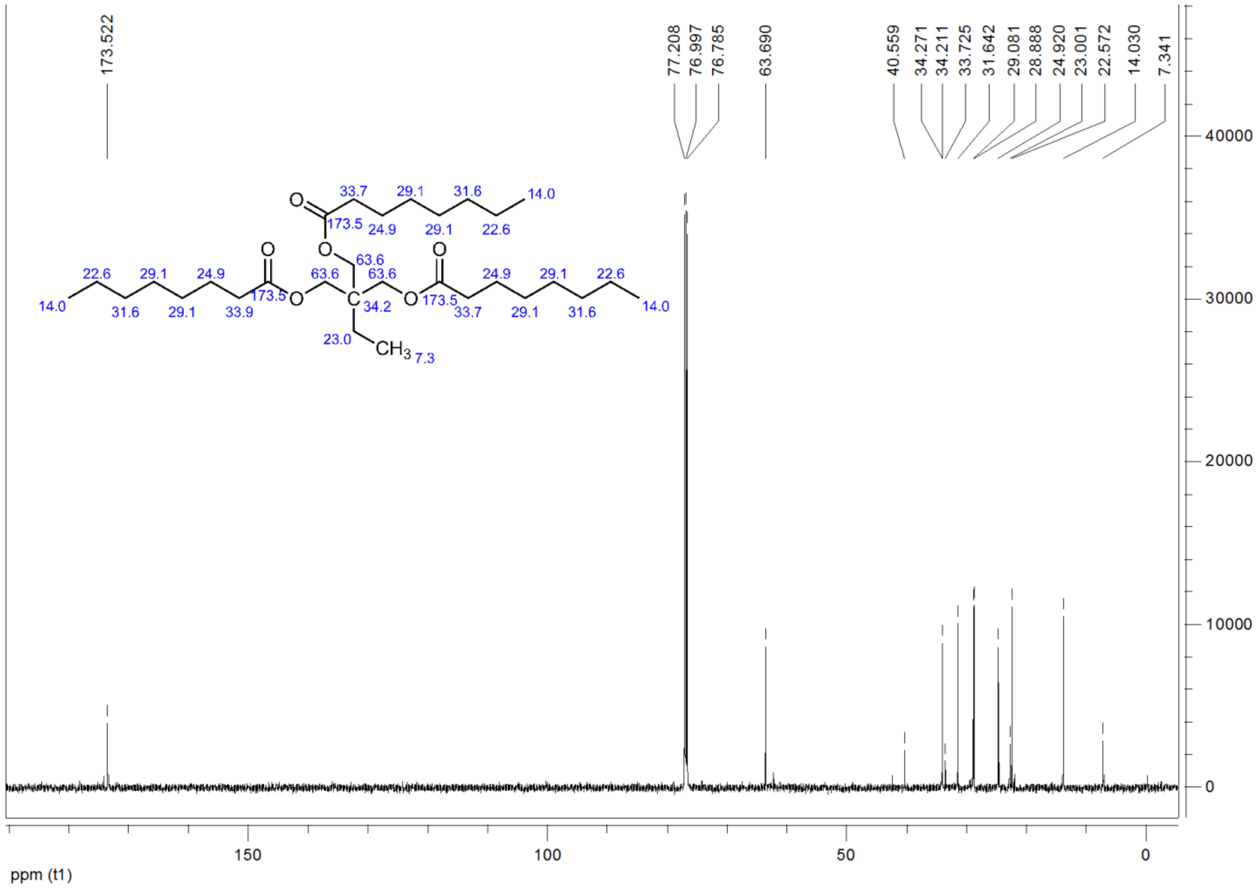

Fig. S4 13C NMR spetra (600M, $CDCl_3$) of tri-substituted TMP esters.

2. MS spectra

GC-MS conditions have been described in previous works[3].

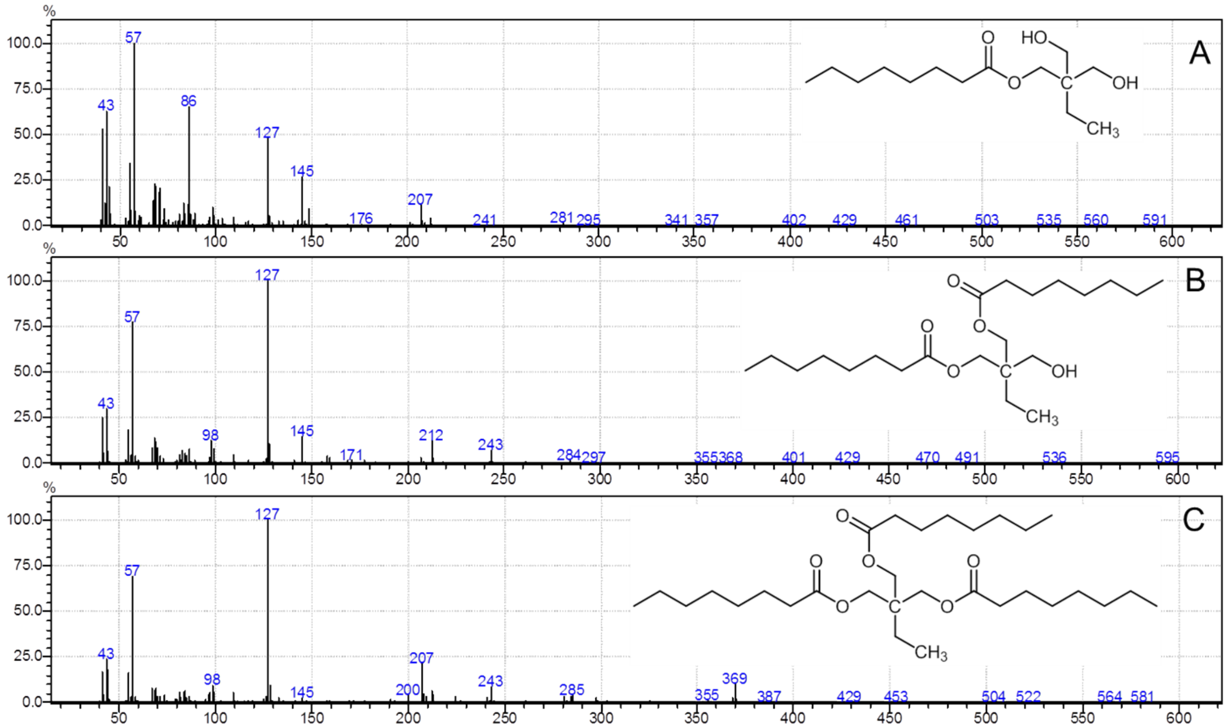

Fig. S5. MS spectra of mono-, di- and tri-substituted TMP esters.

3. GC chromatogram

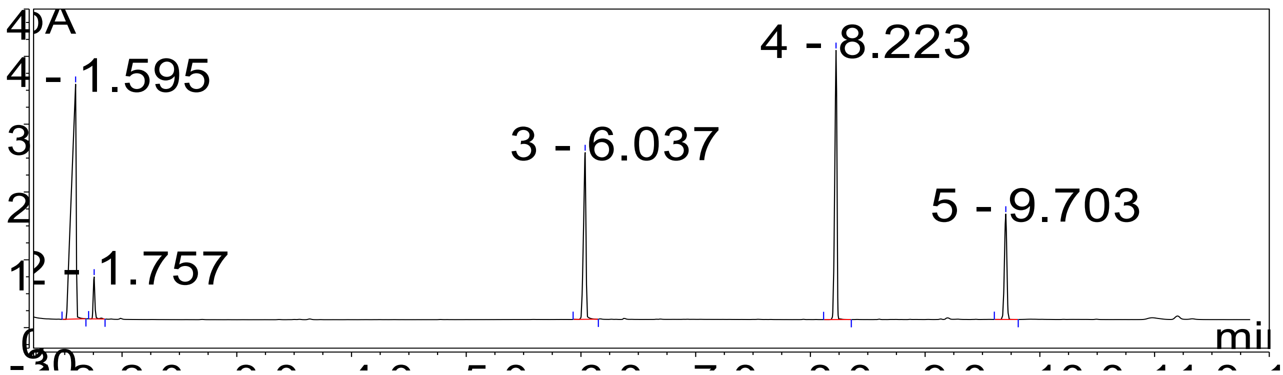

Fig. S6. GC chromatogram of substrates and products. (1) caprylic acid, 1.595 min; (2) TMP, 1.757 min; (3) mono-substituted TMP esters, 6.037 min; (4) di-substituted TMP esters, 8.223 min; (5) tri-substituted TMP esters, 9.703 min.

Reference

1. S. Al-Zuhair, A. Dowaidar and H. Kamal, *Biochem. Eng. J.*, 2009, **44**, 256-262.

2. F. Voll, R. L. Krüger, F. de Castilhos, V. Cabral, J. Ninow and M. L. Corazza, *Biochem. Eng. J.*, 2011, **56**, 107-115.

3. Y. Tao, B. Chen, L. Liu and T. Tan, *J. Mol. Catal. B: Enzym.*, 2012, **74**, 151-155.