Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

Kinetics of prebiotic depsipeptide formation from the ester-amide exchange reaction

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I. Illustrative examples of the exchange reaction

a. Simple example

The first example is the reaction between $P^O_{1,1}$ (parent oligomer) and $P^N_{0,0}$ (nucleophilic attacker) to form $P^O_{0,0}$ (ejection product) and $P^O_{0,2}$ (growth product).

Ejection of $P^O_{0,0}$ from parent oligomers $P^O_{1,1}$

As shown in Scheme S1, the parent oligomer in this case has two possible sequences ($C^{1+1}_1 = 2$), but only one of them leads to $P^O_{0,0}$ by the ejection reaction.

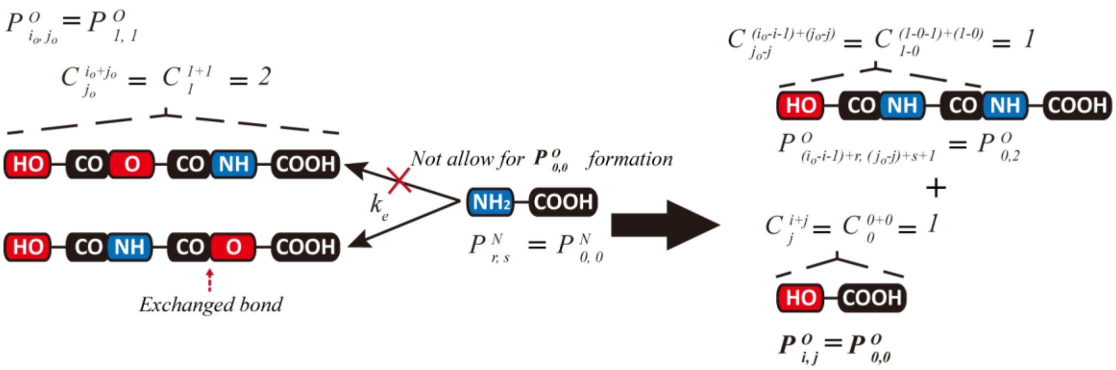
$$P^O_{i_o,j_o} + P^N_{r,s} \xrightarrow{k_e} P^O_{(i_o-i-1)+r,(j_o-j)+s+1} + P^O_{i,j}$$

$$\Rightarrow P^O_{1,1} + P^N_{0,0} \xrightarrow{k_e} P^O_{0,2} + P^O_{0,0}$$

To calculate the possibility of forming $P^O_{0,0}$ by the ejection reaction, we first count the numbers of ester and amide bonds in the two products that are originated from the parent oligomer. The ejection product does not have any ester or amide bond and it can only be arranged in one way ($C^{0+0}_0 = 1$). The other product, $P^O_{0,2}$, has two amide bonds, but one of them is made from the exchange reaction. Only one amide bond is from the parent oligomer, so this segment has one possible sequence ($C^{(1-0-1)+(1-0)}_{1-0} = 1$). Therefore, only one sequence of the parent oligomer allows this reaction to happen. Averagely speaking, half of the ejection reaction produces $P^O_{0,0}$.

$$\frac{dP^O_{0,0}}{dt} = \frac{C^{(1-0-1)+(1-0)}_{1-0} C^{0+0}_0}{C^{1+1}_1} k_e P^O_{1,1} P^N_{0,0}$$

$$= \frac{1 \cdot 1}{2} k_e P^O_{1,1} P^N_{0,0}$$



Scheme S1 The ejection formation of $P^O_{0,0}$ from $P^O_{1,1}$ by a $P^N_{0,0}$.

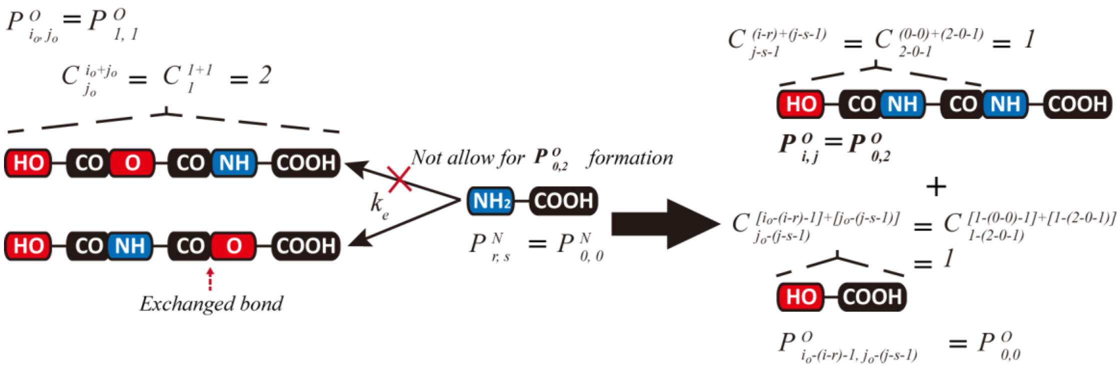
Growth of $P^O_{0,2}$ with new amide bond from $P^O_{1,1}$

In the second case, we consider the growth rate of the oligomer $P^O_{0,2}$ with a newly attached amide bond for the same reaction (Scheme S2).

$$P^X_{i_o,j_o} + P^N_{r,s} \xrightarrow{k_e} P^X_{i,j} + P^O_{i_o-(i-r)-1,j_o-(j-s-1)}$$
$$\Rightarrow P^O_{1,1} + P^N_{0,0} \xrightarrow{k_e} P^O_{0,2} + P^O_{0,0}$$

Again, we need to calculate the numbers of ester and amide bonds in the two products that belong to the parent oligomer. The product with the nucleophile has two amide bonds, but the segment from the parent oligomer has only one amide bond, so it has one possible arrangement ($C^{(0-0)+(2-0-1)}_{2-0-1} = 1$). Because one amide bond of the parent oligomer has been used to the product with the nucleophile and its ester bond has been consumed by the exchange reaction, the ejection product contains zero linkage and has only one sequence ($C^{[1-(0-0)-1]+[1-(2-0-1)]}_{1-(2-0-1)} = 1$). Therefore, only half of the reaction from $P^O_{1,1}$ and $P^N_{0,0}$ yields $P^O_{0,2}$.

$$\frac{dP^O_{0,2}}{dt} = \frac{C^{(0-0)+(2-0-1)}_{2-0-1} C^{[1-(0-0)-1]+[1-(2-0-1)]}_{1-(2-0-1)}}{C^{1+1}_{1}} k_e P^O_{1,1} P^N_{0,0}$$
$$= \frac{1 \cdot 1}{2} k_e P^O_{1,1} P^N_{0,0}$$



Scheme S2 The growth of $P^O_{0,2}$ from $P^O_{1,1}$ by a $P^N_{0,0}$.

b. Advanced example

The second example is a more complex case. It is the reaction between $P^O_{2,2}$ (parent oligomer) and $P^N_{0,1}$ (nucleophilic attacker) to form $P^O_{0,1}$ (ejection product) and $P^O_{1,3}$ (growth product).

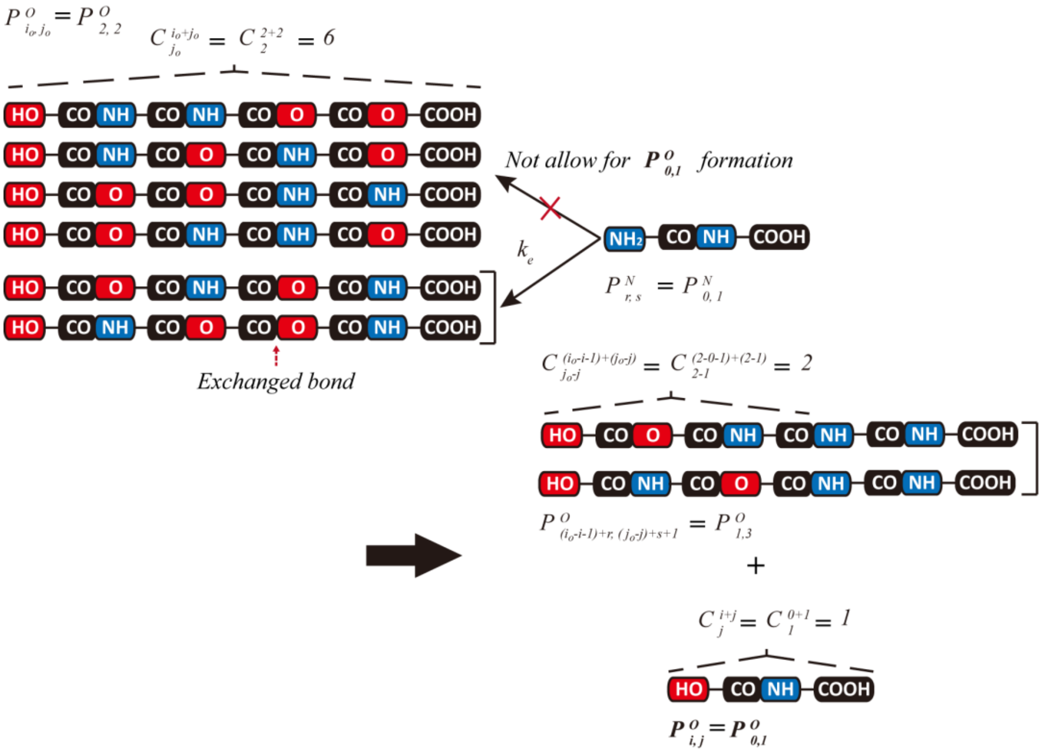
Ejection of $P^O_{0,1}$ from parent oligomers $P^O_{2,2}$

As shown in Scheme S3, the parent oligomer in this case has six possible sequences ($C_2^{2+2}=6$), but only two of them can form $P_{0,0}^O$ by the exchange reaction.

$$P_{i_o,j_o}^O + P_{r,s}^N \xrightarrow{k_e} P_{(i_o-i-1)+r,(j_o-j)+s+1}^O + P_{i,j}^O$$
$$\Rightarrow P_{2,2}^O + P_{0,1}^N \xrightarrow{k_e} P_{1,3}^O + P_{0,1}^O$$

To calculate the possibility of forming $P_{0,1}^O$ by the ejection reaction, we count the numbers of ester and amide bonds in the two products that are originated from the parent oligomer. The ejection product has one amide bond and it can only be arranged in one way ($C_1^{0+1}=1$). The other product, $P_{1,3}^O$, has one ester bond and three amide bonds, but one of amide is made from the exchange reaction and another amide bond comes for the nucleophilic attacker. Only one ester bond and one amide bond are from the parent oligomer and this segment has two possible sequences ($C_{2-1}^{(2-0-1)+(2-1)}=2$). Therefore, two sequences of the parent oligomer allow this reaction to happen. Averagely speaking, one-third of the ejection reaction produces $P_{0,1}^O$.

$$\frac{dP_{0,1}^O}{dt} = \frac{C_{2-1}^{(2-0-1)+(2-1)} C_1^{0+1}}{C_2^{2+2}} k_e P_{2,2}^O P_{0,1}^N$$
$$= \frac{2\cdot 1}{6} k_e P_{2,2}^O P_{0,1}^N$$



Scheme S3 The ejection formation of $P_{0,1}^O$ from $P_{2,2}^O$ by a $P_{0,1}^N$.

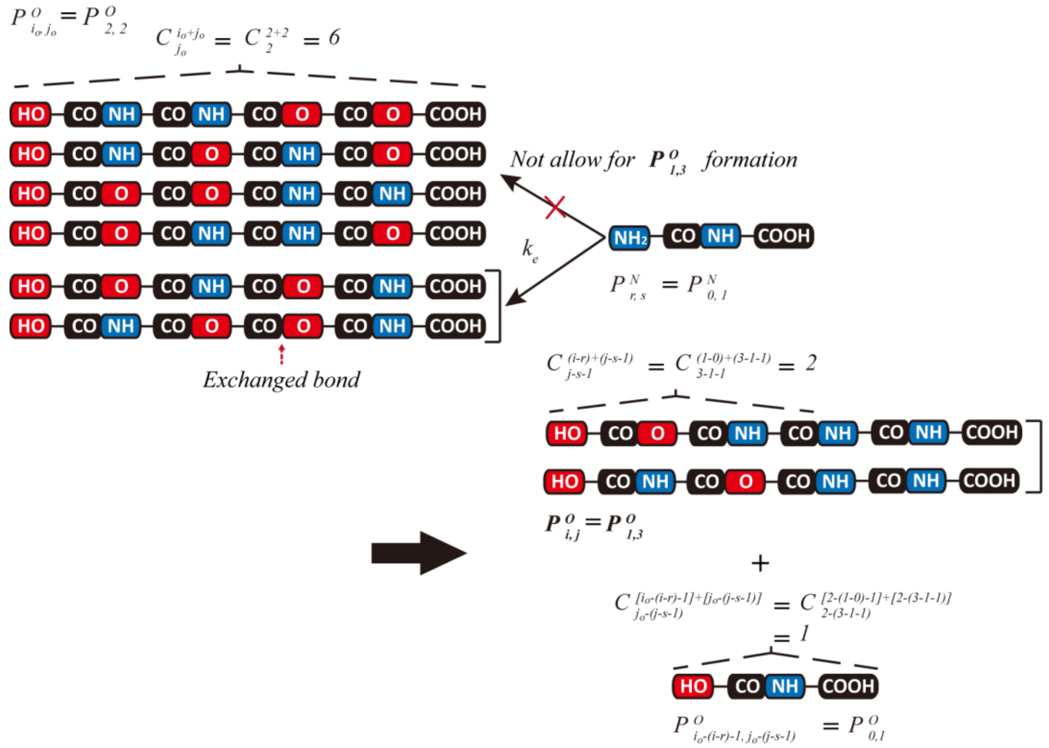
Growth of $\boldsymbol{P^{O}_{1,3}}$ with new amide bond from $\boldsymbol{P^{O}_{2,2}}$

Here, we consider the growth rate of the oligomer $P^{O}_{1,3}$ with a newly attached amide bond for the same reaction (Scheme S4).

$$P^{X}_{i_o,j_o}+P^{N}_{r,s}\xrightarrow{k_e}P^{X}_{i,j}+P^{O}_{i_o-(i-r)-1,j_o-(j-s-1)}$$
$$\Rightarrow P^{O}_{2,2}+P^{N}_{0,1}\xrightarrow{k_e}P^{O}_{1,3}+P^{O}_{0,1}$$

Here, the product with the nucleophile has one ester bond and three amide bonds, but the segment from the parent oligomer has only one ester and one amide bond. Therefore, it has two possible arrangements ($C^{(1-0)+(3-1-1)}_{3-1-1}=2$). Because two bonds of the parent oligomer have been used in the growth product and one ester bond is consumed by the exchange reaction, the ejection product has one amide bond and one possible sequence ($C^{[2-(1-0)-1]+[2-(3-1-1)]}_{2-(3-1-1)}=1$). Therefore, only one-third of the reaction from $P^{O}_{2,2}$ and $P^{N}_{0,1}$ yields $P^{O}_{1,3}$.

$$\frac{dP^{O}_{1,3}}{dt}=\frac{C^{(1-0)+(3-1-1)}_{3-1-1}C^{[2-(1-0)-1]+[2-(3-1-1)]}_{2-(3-1-1)}}{C^{2+2}_{2}}k_eP^{O}_{2,2}P^{N}_{0,1}$$
$$=\frac{2\cdot 1}{6}k_eP^{O}_{2,2}P^{N}_{0,1}$$



Scheme S4 The growth of $P^{O}_{1,3}$ from $P^{O}_{2,2}$ by a $P^{N}_{0,1}$.

II. The design of the closed reactor

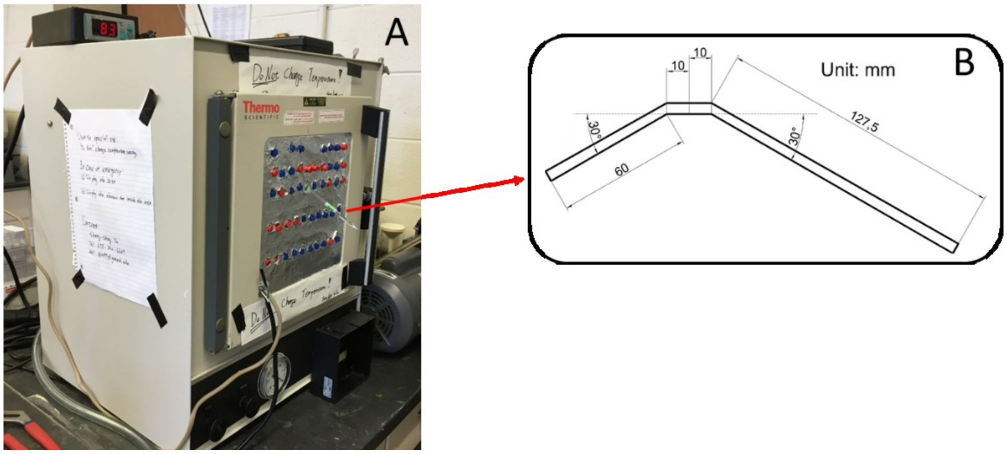


Fig. S1 The closed reactor. (A) The oven used to perform the reaction. (B) The blue print of the closed tube reactor.

III. HPLC-UV/MS analysis

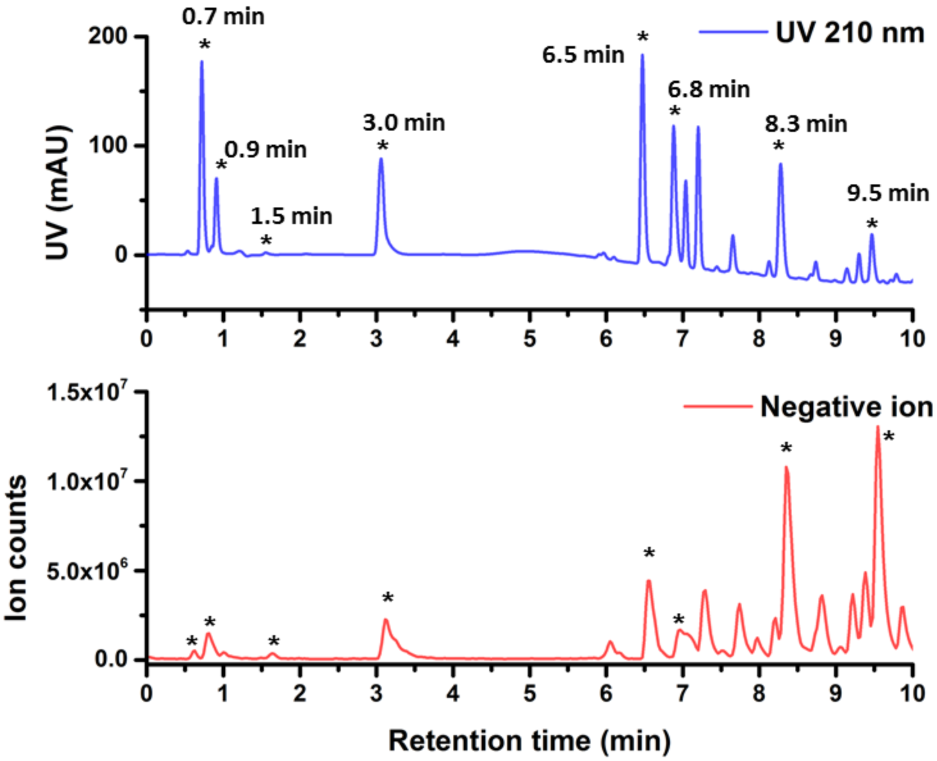


Fig. S2 HPLC-UV/MS analysis of the V/LA depsipeptide mixture. The sample was prepared from 33.34 mM valine and 100 mM lactic acid, 300 µl solution. The solution was dried in the closed reactor for 24 hours.

Table S1. UV response factor at 210 nm

Compound	UV response factor, k [µmole/mAU*min]
1LA	2.64×10^{-5}
2LA	8.29×10^{-6}
3LA	5.53×10^{-6}
4LA	4.15×10^{-6}
5LA	3.32×10^{-6}
1V-1LA	8.29×10^{-6}
1LA-1V	2.48×10^{-6}

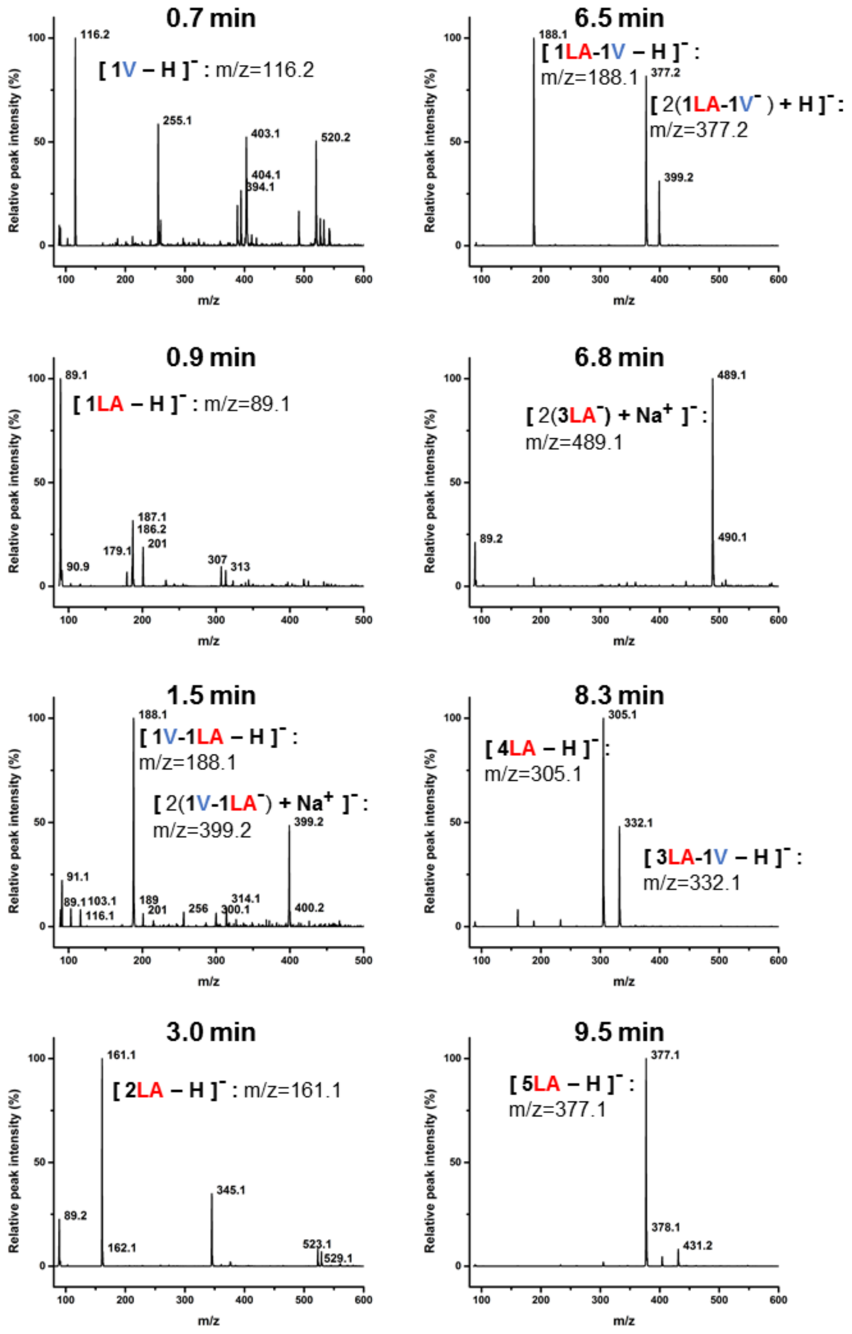


Fig. S3 Mass spectra of species after HPLC separation.

IV. Kinetic of ester hydrolysis and water evaporation

a. Ester hydrolysis

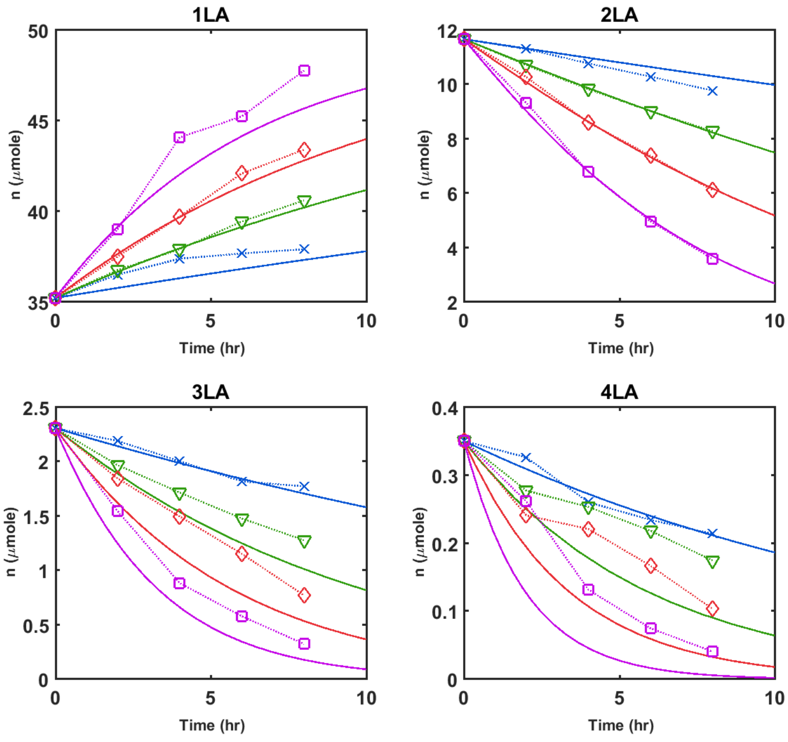


Fig. S4 Monomer and oligomer distributions versus time profile in the hydrolysis stage. The reaction temperatures are denoted as different colors: purple (95 °C), red (85 °C), green (75 °C) and blue (65 °C). Solid lines are the model predictions. The experimental data points are represented by □, ◇, ▽ and × symbols, respectively.

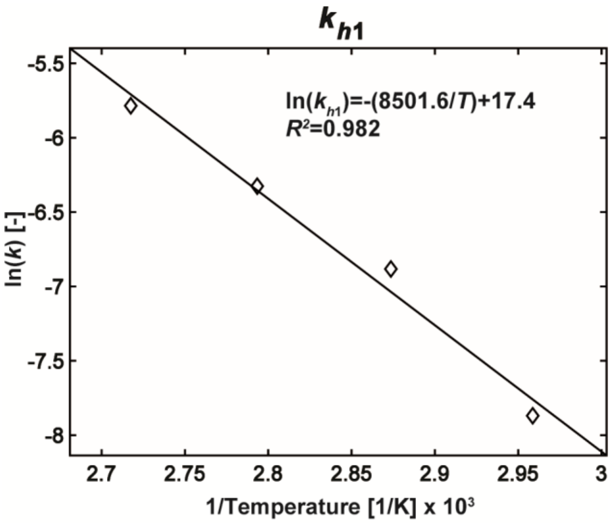


Fig. S5 Arrhenius plot of the hydrolysis rate constants evaluated from the lactic acid hydrolysis experiments.

b. Water evaporation

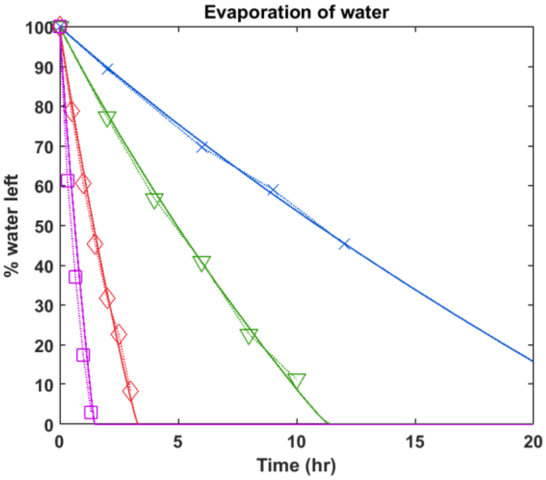


Fig. S6 The amount of water left in the drying side of the reaction at different temperatures are denoted as different colors: purple (95 °C), red (85 °C), green (75 °C) and blue (65 °C). Solid lines are the model predictions. The experimental data points are represented by □, ◇, ▽ and ╳ symbols, respectively.

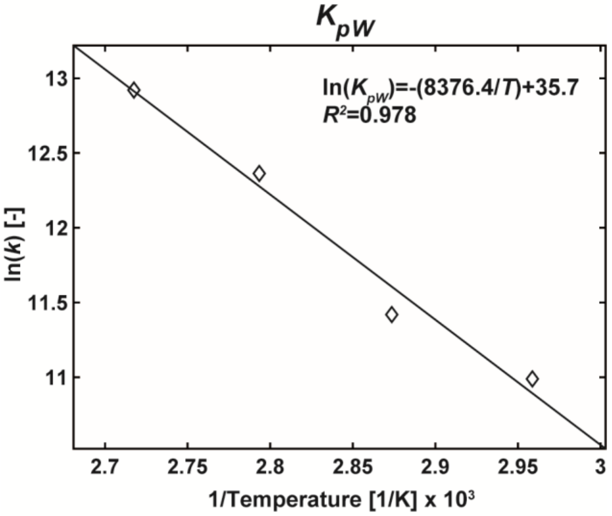
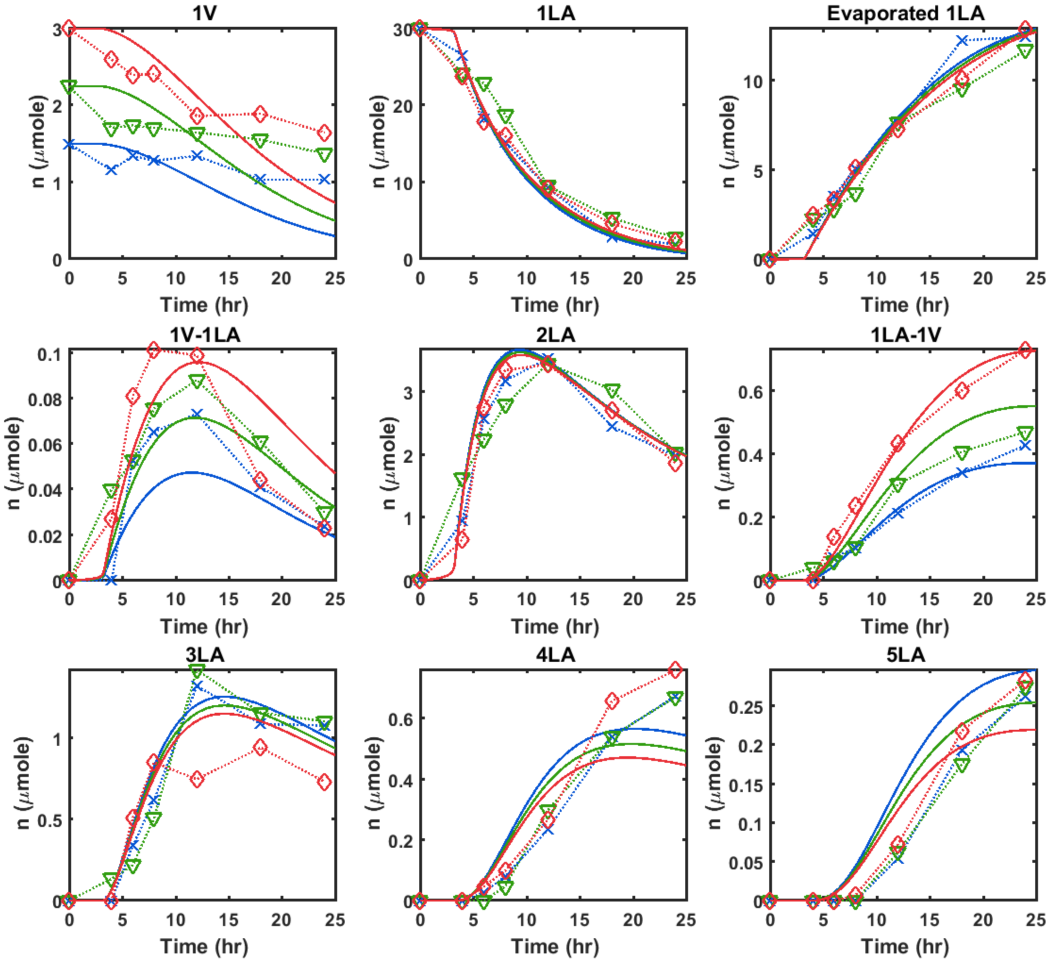


Fig. S7 Arrhenius plot of the water evaporation rate constants.

V. Kinetics of V/LA copolymerization with different monomer ratios


1V

1LA

Evaporated 1LA

1V-1LA

2LA

1LA-1V

3LA

4LA

5LA

Fig. S8 Experimental results and model prediction for low valine loading. The results for different V/LA ratios are denoted as different colors: blue (5/100), green (7.5/100) and red (10/100). Solid lines are the model predictions. The experimental data points are represented by the same colors and by the ×, ▽ and ◇ symbols, respectively. All simulations were calculated by using the rate constants obtained from the 10/100 experiment.

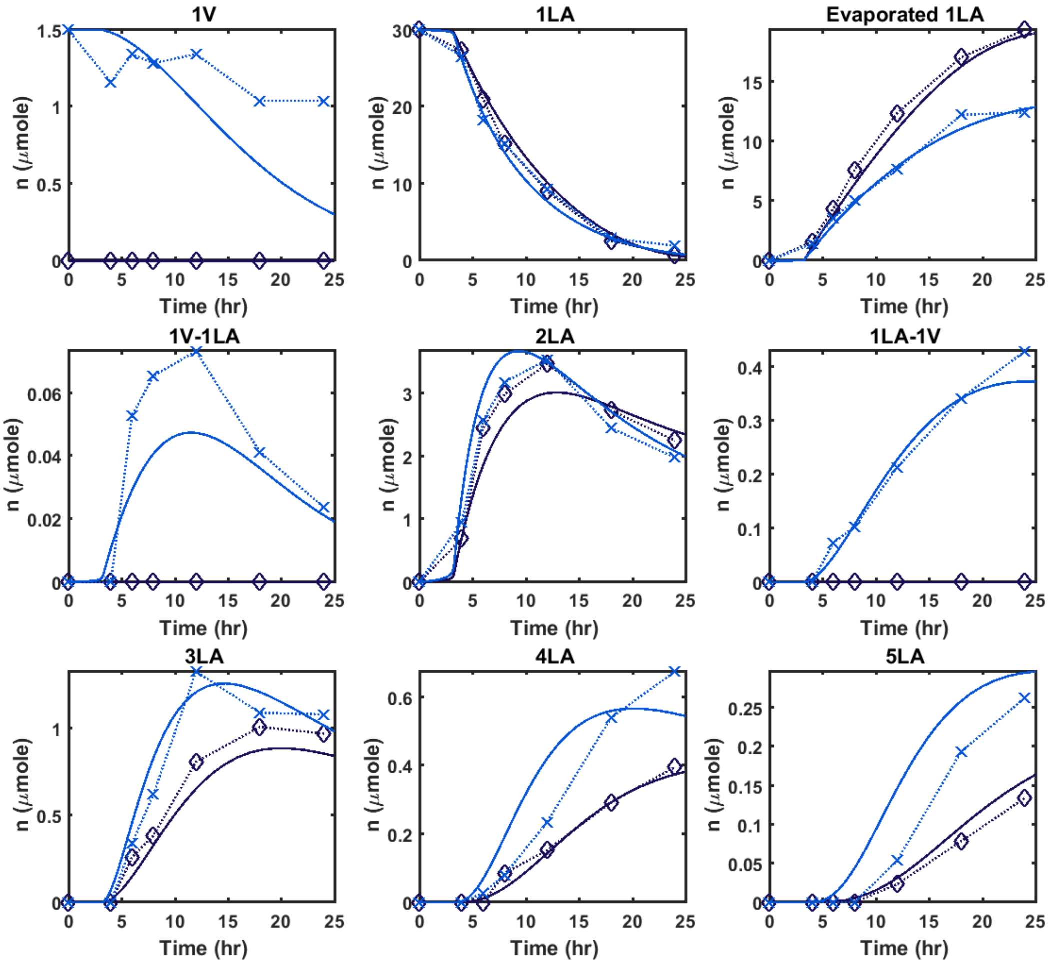


Fig. S9 Experimental results and model prediction for the 0/100 and 5/100 experiment. The results for different V/LA ratios are denoted as different colors: black (0/100) and blue (5/100). Solid lines are the model predictions. The experimental data points are represented by the same colors and by the $\diamond$ and $\times$ symbols, respectively. Optimized rate constants from the data was used for the model prediction of the 0/100 experiment. The simulation for 5/100 ratio experiment was calculated by using the rate constants obtained from the 10/100 experiment.

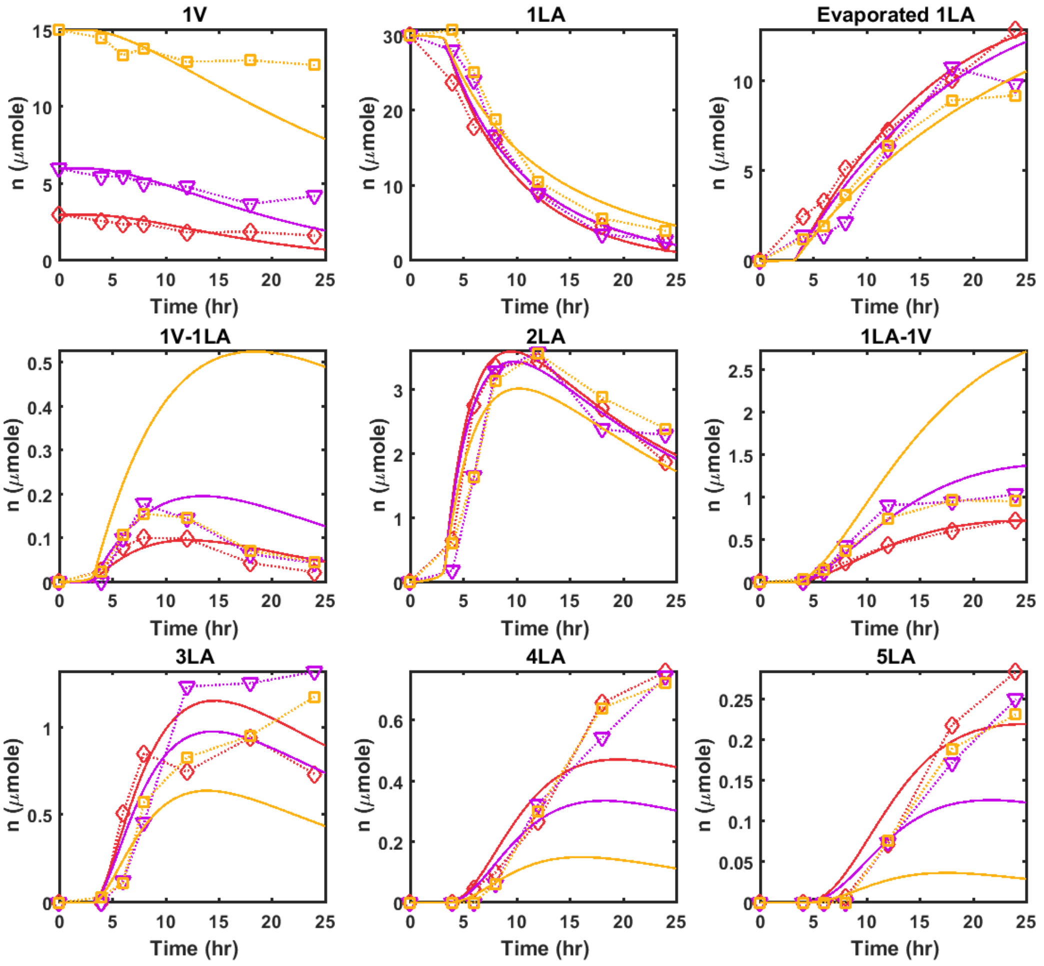


Fig. S10 Experimental results and model prediction for high valine loading. The results for different V/LA ratios are denoted as different colors: red (10/100), purple (20/100) and orange (50/100). Solid lines are the model predictions. The experimental data points are represented by the same colors and by the ◇, ▽ and □ symbols, respectively. All simulations were calculated by using the rate constants obtained from the 10/100 experiment.

VI. Flux analysis

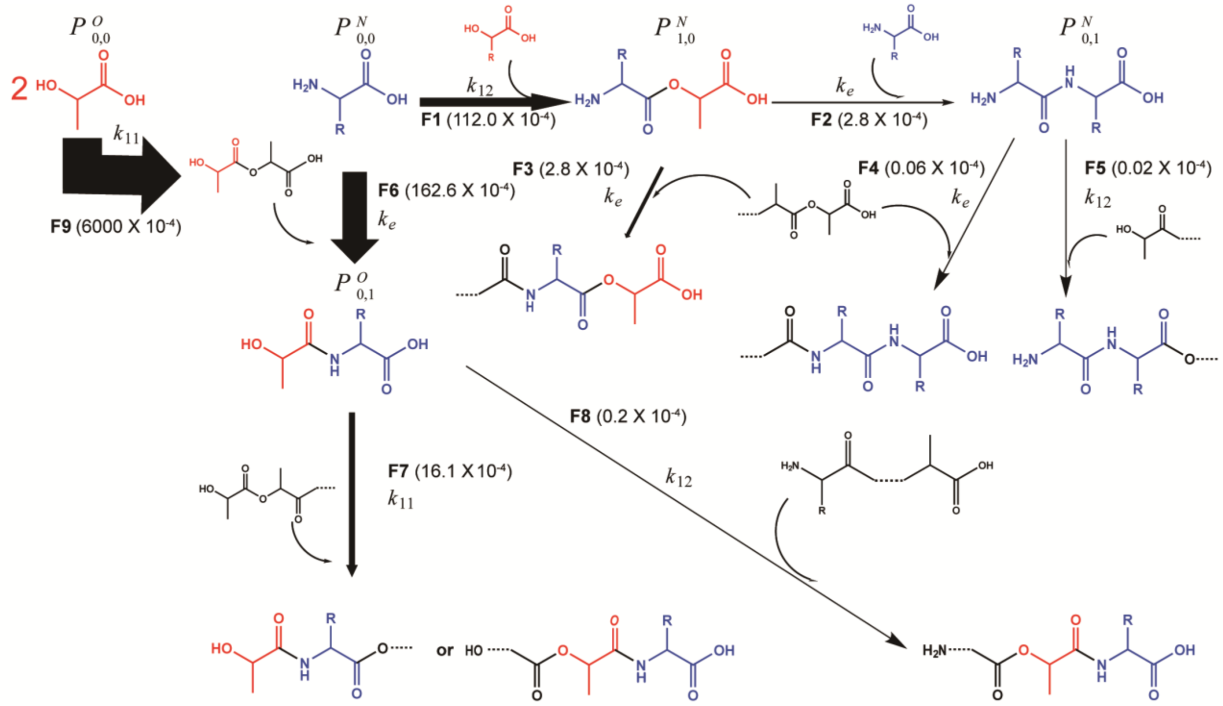


Fig. S11 Flux analysis for the reactions in 6 hours. The average fluxes (μmole/hr) were calculated from the simulation of 6 hr reaction of 10/100 V/LA mixture at 85 °C. The width of each arrow indicates the relative magnitudes of each flux. Only fluxes of forward reactions are included because the fluxes of hydrolysis and reverse reactions are negligible in the dry state.

VII. Nomenclature

$P_{i,j}^{O}$	Hydroxy-terminated oligomers with *i* ester bonds and *j* amide bonds (µmole)
$P_{i,j}^{N}$	Amine-terminated oligomers with *i* ester bonds and *j* amide bonds (µmole)
V	System volume (µL)
W	Water (µmole)
k_{11}	Rate constant for the esterification between two hydroxy-terminated oligomers ($L \cdot mol^{-1} \cdot h^{-1}$)
k_{12}	Rate constant for the esterification between hydroxy-terminated and amine-terminated oligomers ($L \cdot mol^{-1} \cdot h^{-1}$)
k_{h1}	Rate constant for the hydrolysis of ester ($L \cdot mol^{-1} \cdot h^{-1}$)
k_e	Rate constant for the exchange reaction ($L \cdot mol^{-1} \cdot h^{-1}$)
K_{pLA}	Rate constant for the evaporation of lactic acid ($\mu mole \cdot cm^{-1} \cdot h^{-1}$)
K_{pw}	Rate constant for the evaporation of water ($\mu mole \cdot cm^{-1} \cdot h^{-1}$)
$R_{v,w}$	Evaporation of water ($\mu mole \cdot h^{-1}$)
$N_{w,z}$	Flux of water ($\mu mole \cdot h^{-1} \cdot cm^{-2}$)
z_1	Liquid height (cm)
z_2	Length of tube inside oven (cm)
x_w	Liquid mole fraction of water
$y_{w,1}$, $y_{w,2}$	Gas mole fraction water at (1) liquid-gas interface and (2) the top of high temperature region
S_A	Cross-section area (cm^2)
T_H	Drying temperature (^0C)
P_{air}	Partial pressure of air (kPa)
P_w^*	Vapor pressure of water (kPa)
$R_{v,LA}$	Evaporation rate of lactic acid ($\mu mole \cdot h^{-1}$)
x_{LA}	Mole fraction of lactic acid in liquid