Supporting Information for

Michael addition of pyrimidine derivatives with acrylates Catalyzed by lipase TL IM from *Thermomyces lanuginosus* in a Continuous-Flow Microreactor

Li-Hua Du,[*a] Hui-Min Ling[a], Xi-Ping Luo,[a, b]

[a]*College of Pharmaceutical Science, ZheJiang University of Technology, Hangzhou， 310014, China*

[b]*Department of Environmental Science and Technology, ZheJiang A&F University, Hangzhou, 311300, China.*

* Corresponding author. Fax: +86 571 88320903. E-mail: orgdlh@gmail.com; orgdlh@zjut.edu.cn.

Materials

Unless otherwise stated, all chemicals were obtained from commercial sources and used without further purification. Lipozyme TLIM from *Thermomyces lanuginosus* was purchased from Novo Nordisk. Pyrimidine, acrylates, and all other chemicals were of the highest purity commercially available and without further purification. Harvard apparatus PHD 2000 syringe pumps were purchased from Harvard apparatus.

Thin-Layer Chromatography

Analytical TLC was performed on silica gel 60 plates (Merck) using methanol/ chloroform/ethyl acetate (1: 4:10, by vol) as eluent. Spots were detected by ultraviolet irradiation at 254 nm.

High Performance Liquid Chromatography (HPLC)

The reaction was monitored by HPLC analysis using a reversed-phase Shim-Pack VP-ODS column (4.6×150 mm) and a UV detector (265 nm). MeOH-H_2O (40:60) was used as eluant at 1.0 mL min^{-1}. The yield was defined as the ratio between the

molar concentration of *N*-substituted pyrimidine derivatives and the initial molar concentration of pyrimidine derivatives used.

Experimental setup

The enzymatic Michael addition reaction of pyrimidine derivatives with acrylates was performed in microreactor. The equipment configuration that was used for the enzymatic Michael addition of pyrimidine derivatives with acrylates starting from 5-fluorouracil and methyl acrylate is described in Figure 1. Harvard Apparatus PHD 2000 syringe pumps were used to deliver reagents from syringes to the reactor. On the syringe pump, a 10 mL syringe with the 5-fluorouracil solution and a 10 mL syringe with methyl acrylate in DMSO were mounted. Lipozyme TL IM were filled in silica gel tubing (inner diameter ID = 2.0 mm, length = 1 m). The temperature of this reaction was controlled by water bath, just immersed the tubing in water and control the temperature of water. Streams **1** and **2** were mixed together at a flow rate of 10.4 μL min^{-1} in a Y-mixer at 50 °C and the resulting stream (20.8 μL min^{-1}) was connected to a sample vial which was used to collect the final mixture.

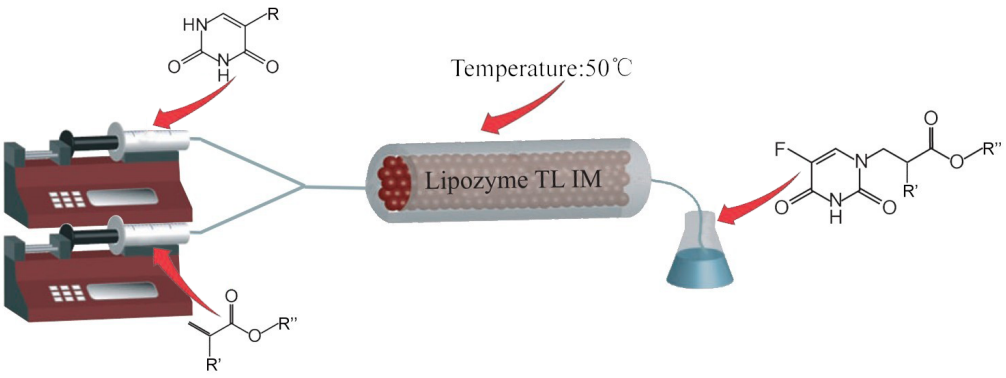


Figure 1. Microreactor setup for the continuous-flow Michael addition reaction of pyrimidine derivatives with acrylates catalyzed by Lipozyme TL IM from *Thermomyces lanuginosus*.

General Procedure for Michael addition reaction in Continuous Flow Microreactors.

Method B: 1.0 mmol of the 5-fluorouracil was dissolved in 10 mL DMSO (feed 1, ~0.1 M) and 5.0 mmol methyl acrylate were dissolved in 10 mL DMSO (feed 2; ~0.5 M). Lipozyme TL IM (0.80 g) were filled in silica gel tubing (inner diameter ID= 2.0 mm, length = 1m). Streams **1** and **2** were mixed together at a flow rate of. 10.4 μL min^{-1} in a Y-mixer at 50 °C and the resulting stream (20.8 μL min^{-1}) was connected to a sample vial which was used to collect the final mixture. The final mixture was then evaporated, and the oily residue was submitted to column chromatography on silica gel (200-300 mesh). The products were eluted with a gradient of methanol/chloroform/ethyl acetate (1:4:10, by vol). The purification was monitored by TLC. The fractions containing the main products were pooled, the solvent evaporated, and the residue analyzed by ^{1}H NMR.

General Procedure for Michael addition reaction under Shaker Conditions.

Method A: 5-fluorouracil (0.25 mmol) and methyl acrylate (1.25 mmol) were added to 5 mL DMSO. The biocatalyst lipozyme TL IM (40 mg/mL，0.20 g) was then added and the suspension maintained at 50 °C for 24 h ~ 48 h under shaker conditions. The reactions were performed in the presence of 3 Å molecular sieves. Aliquots were withdrawn at different times, analyzed by TLC and HPLC. When the conversion of 5-fluorouracil to methyl 3-(1'-fluorouracil) propionate reached the maximum value (determined by TLC and HPLC), the mixture was cooled and filtered. The DMSO was evaporated under reduced pressure (60 ºC, 0.5 mmHg), and the oily residue was submitted to column chromatography on silica gel (200–300 mesh). The products

were eluted with a gradient of hexane/ethyl acetate (1:1, by vol). The purification was monitored by TLC. The fractions containing the main products were pooled, the solvent evaporated, and the residue analyzed by ^{1}H NMR.

In order to examine the reproducibility of the method, we repeated the reaction five times, the result are illustrate in Figure S1.

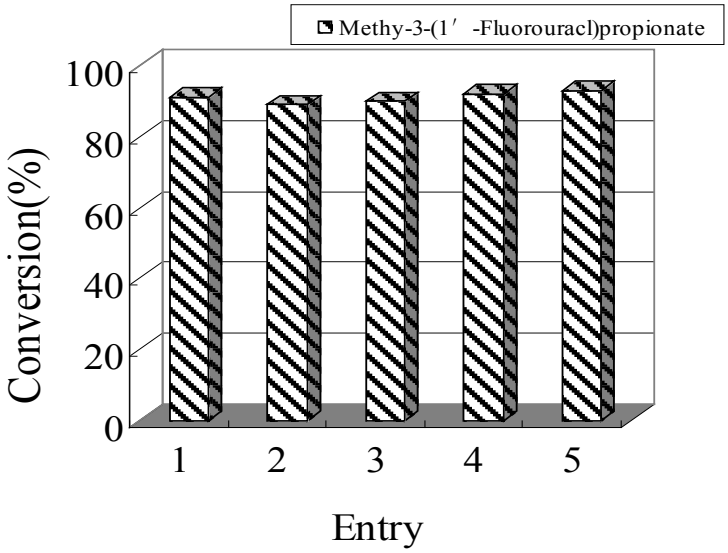


Figure S1. The reproducibility of the reaction on the conversion of methyl 3-(1'-fluorouracil)propionate catalysed by Lipozyme TL IM in a flow microreactor.

The structure of the Michael acceptor and donor can affect the results of the enzymatic Michael reaction. With respect to the acceptor structure, the longer alcohol chain, the lower the yield. Figure S2. shows the effect of the length of the alcohol chain on acceptor structure in the Michael addition reaction. When fluorouracil was used as Michael donor, the yields of the adducts decreased when the acrylate had a long alcohol chain, such as methyl acrylate and butyl methacrylate.

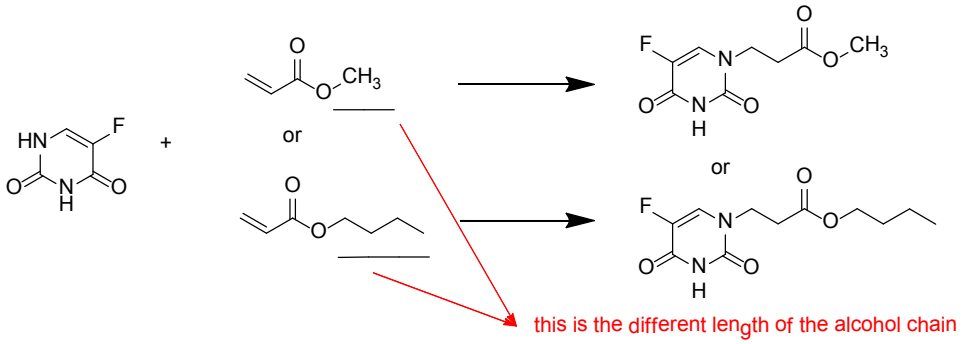
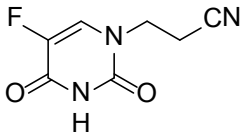


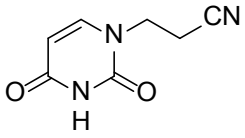
Figure S1. The effect of the alcohol chain on the Michael addition.

Experimental Procedures for Examples Described in Table 1



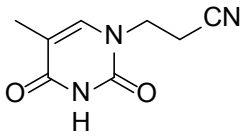
3-(1'-Fluorouracil) propionitrile **(3a)*:*** White crystals; Mp: 213-215 ^{o}C, ^{1}HNMR (500MHz, DMSO-d_6): δ = 8.09 (br s, 1 H, O=CNHC=O), 7.28 (s, 1H, FC=CH), 4.00 (t, 2H, J = 6.18Hz, NCH_2), 2.87 (t, 2H, J = 6.18Hz, CH_2CN). ^{13}CNMR (500MHz,$CDCl_3$): δ = 157.53, 157.38 (C4), 149.43 (C2), 140.60, 138.75 (C6), 130.39, 130.64 (C5), 118.22 (CN), 42.80 (NCH_2), 16.75 ($\underline{C}H_2CN$).

ESI-MS: m/z = 184 (M+1).

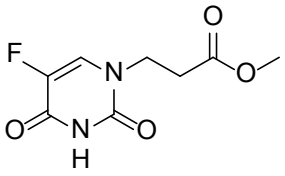


3-(1'-Uracil) propionitrile **(3b)**[1]***:*** White crystals; Mp: 230-231 ^{o}C, ^{1}HNMR (500 MHz,DMSO-d_6): δ = 11.38 (br s, 1 H, O=CNHC=O), 7.68 (d, 1H, J = 7.80 Hz, O=CNCH=), 5.61 (d, 1 H, J = 7.85 Hz, O=CCH=), 3.94 (t, 2H, J = 6.58 Hz, NCH_2), 2.90 (t, 2H, J = 6.50 Hz, CH_2CN). ^{13}CNMR (500 MHz,$CDCl_3$): δ = 164.64 (C4), 150.80 (C2), 145.75 (C6), 118.31 (CN), 101.56 (C5), 45.27 (NCH_2), 16.76 ($\underline{C}H_2CN$).

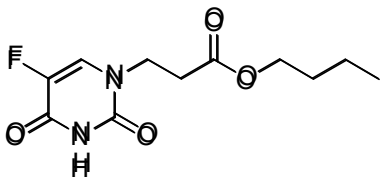
ESI-MS: *m/z* = 166 (M+1).



3-(1'-Thymine) propionitrile **(3c)**[1]***:*** White crystals; Mp: 188-190 °C, ^{1}HNMR (500 MHz, DMSO-d_6): δ = 11.37 (br s, 1H, O=CNHC=O), 7.57 (s, 1H, O=CNCH=), 3.90 (t, 2H, *J* = 6.55 Hz, NCH_2), 2.90 (t, 2H, *J* = 6.58 Hz, CH_2CN), 1.76 (s, 3H, $=CCH_3$). ^{13}CNMR (500 MHz, DMSO-d_6): δ = 164.27 (C4), 150.69 (C2), 140.92 (C6), 118.28 (CN), 108.81 (C5), 42.98 (NCH_2), 16.77 ($\underline{C}H_2CN$), 11.92 (CH_3 thymine). ESI-MS: *m/z* = 180 (M+1).

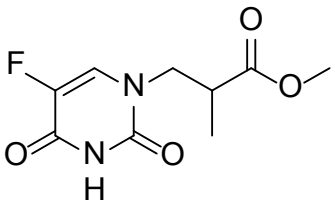


Methyl 3-(1'-Fluorouracil) propionate **(3d)**[2]***:*** White crystals; Mp: 139-142 °C, ^{1}HNMR (500 MHz, DMSO-d_6): δ = 11.78 (br s, 1H, O=CNHC=O), 8.06 (d, 1H, *J* = 6.85 Hz, FC=CH), 3.85 (t, 2 H, *J* = 6.93 Hz, NCH_2), 3.61 (s, 3H, OCH_3), 2.71 (t, 2H, *J* = 6.90 Hz, CH_2C=O). ^{13}CNMR (500 MHz, DMSO-d_6): δ = 171.11 (CO), 157.50, 157.30 (C4), 149.43 (C2), 140.19, 138.38 (C6), 130.59, 130.32 (C5), 51.53 (OCH_3), 44.04 (NCH_2), 32.23 ($\underline{C}H_2CO$). ESI-MS: *m/z* = 217 (M+1).

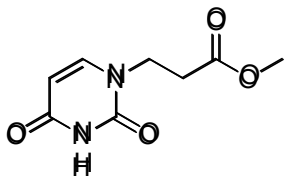


n-Butyl 3-(1'-Fluorouracil) propionate **(3e):** yellow liquid oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ = 10.70 (br s, 1H, O=CNHC=O), 7.50 (d, 1H, *J* = 5.85 Hz, FC=CH), 4.00

(t, 2H, J = 6.73 Hz, NCH_2), 3.88 (t, 2H, J = 5.98 Hz, OCH_2), 2.69 (t, 2H, J = 5.98 Hz, $O{=}CCH_2$), 1.51 (m, 2H, $OCH_2\underline{CH_2}$), 1.25 (m, 2H, $\underline{CH_2}CH_3$), 0.82 (t, 3H, J = 7.43 Hz, $-CH_3$). ^{13}CNMR (500 MHz, $CDCl_3$): δ = 171.23 (CO), 157.67, 157.47 (C4), 149.58 (C2), 140.72, 138.85 (C6), 130.07, 129.81 (C5), 64.82 (OCH_2), 45.18 (NCH_2), 34.50 ($\underline{C}H_2CO$), 30.24 ($\underline{C}H_2CH_2CO$), 18.78, 18.65 ($\underline{C}H_2CH_3$), 13.59, 13.35 (CH_3). ESI-MS: m/z = 259 (M+1).

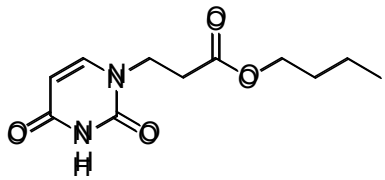


Methyl 3-(1'-Fluorouracil)isobutyrate **(3f):** White crystals; Mp: 139-142 ^{o}C, ^{1}HNMR (500 MHz, $CDCl_3$): δ = 9.44 (br s, 1H, O=CNHC=O), 7.27 (s, 1H, FC=CH), 3.96 (dd, 1H, J_1 = 4.40 Hz, J_2 = 13.85 Hz, NCH_2), 3.69 (s, 3H, OCH_3), 3.06 (dd, 1H, J_1 = 4.50 Hz, J_2 = 13.85 Hz, NCH_2), 3.06 (q, 1H, J = 4.05 Hz, O=CCH), 1.26 (d, 3H, J = 2.45 Hz, $CH\underline{CH_3}$). ^{13}CNMR (500 MHz,$CDCl_3$): δ = 174.83 (CO), 157.24, 157.04 (C4), 149.53 (C2), 140.95, 139.05 (C6), 130.11, 129.86 (C5), 52.32 (OCH_3), 38.64 (NCH_2), 29.70 ($\underline{C}HCH_3$), 15.15 ($CH\underline{C}H_3$). ESI-MS: m/z = 231 (M+1).

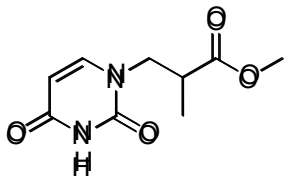


Methyl 3-(1'-Uracil) propionate **(3g)[3]:** White crystals; Mp: 131-132 ^{o}C, ^{1}HNMR (500 MHz, $CDCl_3$): δ = 9.76 (br s, 1H, O=CNHC=O), 7.39 (d, 1H, J = 8.00 Hz, O=CNCH=), 5.67 (d, 1H, J = 7.95 Hz, O=CCH=), 4.00 (t, 2H, J = 5.90 Hz, NCH_2), 3.70 (s, 3H, OCH_3), 2.79 (t, 2H, J = 5.95 Hz, $CH_2C{=}O$). ^{13}CNMR (500 MHz, $CDCl_3$):

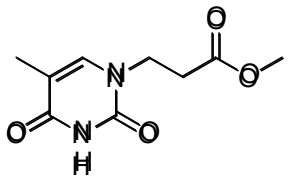
δ = 171.82 (CO), 164.04 (C4), 150.89 (C2), 145.80 (C6), 101.76 (C5), 52.08 (OCH_3), 45.27 (NCH_2), 32.73 ($\underline{C}H_2CO$). ESI-MS: *m/z* = 199 (M+1).



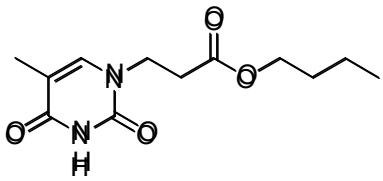
n-Butyl 3-(1'-Uracil) propionate **(3h)**[3]***:*** yellow liquid oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ = 10.00 (br s, 1H, O=CNHC=O), 7.36 (d, 1H, *J* = 7.90 Hz, O=CNCH=), 5.61 (d, 1H, *J* = 7.85 Hz, O=CCH=), 4.05 (t, 2H, *J* = 6.73 Hz, OCH_2), 3.95 (t, 2H, *J* = 6.00 Hz, NCH_2), 2.74 (t, 2H, *J* = 5.98 Hz, $O=CCH_2$), 1.56 (m, 2H, $OCH_2\underline{CH_2}$), 1.32 (m, 2H, $CH_3\underline{CH_2}$), 0.88 (t, 3H, *J* = 7.38 Hz, CH_3). ^{13}CNMR (500 MHz, $CDCl_3$): δ = 172.02 (CO), 164.54 (C4), 150.74 (C2), 145.50 (C6), 101.76 (C5), 56.08 (OCH_3), 45.25 (NCH_2), 34.73 ($\underline{C}H_2CO$), 31.25 ($\underline{C}H_2CH_2CO$), 18.64 ($\underline{C}H_2CH_3$), 13.50 (CH_3). ESI-MS: *m/z* = 241 (M+1).



Methyl 3-(1'-Uracil) isobutyrate **(3i)*:*** White crystals; Mp: 138-140 ^{o}C, ^{1}HNMR (500 MHz, $CDCl_3$): δ = 9.60 (br s, 1H, O=CNHC=O), 7.27 (d, 1H, *J* = 7.85 Hz, O=CNCH=), 5.64 (d, 1H, *J* = 7.9 Hz, O=CCH=), 3.95 (dd, 1H, J_1 = 4.40 Hz, J_2 = 13.85 Hz, NCH_2), 3.73 (dd, 1H, J_1 = 4.50 Hz, J_2 = 13.85 Hz, NCH_2), 3.69 (s, 3H, OCH_3), 3.08 (m, 1H, O=CCH), 1.27(d, 3 H, *J* = 2.45Hz, $CHCH_3$). ^{13}CNMR (500 MHz, $CDCl_3$): δ = 171.42 (CO), 163.54 (C4), 150.78 (C2), 145.56 (C6), 101.56 (C5), 51.08 (OCH_3), 43.25 (NCH_2), 30.56 ($\underline{C}HCH_3$), 17.15 ($CH\underline{C}H_3$). ESI-MS: *m/z* = 213 (M+1).



Methyl 3-(1'-Thymine) propionate **(3j)**[3]***:*** White crystals; Mp: 127-128 ^{o}C, ^{1}HNMR (500 MHz, $CDCl_3$): δ = 10.00 (br s, 1H, O=CNHC=O), 7.28 (s, 1H, O=CNCH=), 3.94 (t, 2 H, *J* = 6.08 Hz, NCH_2), 3.67 (s, 3H, OCH_3), 2.76 (t, 2H, *J* =6 .05 Hz, O=CCH_2), 1.87 (s, 3H, =CCH_3). ^{13}CNMR (500 MHz, $CDCl_3$): δ = 17.22 (CO), 164.37 (C4), 151.69 (C2), 141.68 (C6), 108.81 (C5), 52.35(OCH_3), 42.88 (NCH_2), 33.05 ($\underline{C}H_2CO$), 11.92 (CH_3 thymine). ESI-MS: *m/z* = 213 (M+1).



n-Butyl 3-(1'-Thymine) propionate **(3k)**[3]***:*** White crystals; Mp: 90-92 ^{o}C, ^{1}HNMR (500 MHz, $CDCl_3$): δ = 9.75 (br s, 1H, O=CNHC=O), 7.28 (s, 1H, O=CNCH=), 4.08 (t, 2H, *J* = 6.73 Hz, OCH_2), 3.96 (t, 2H, *J* = 6.07 Hz, NCH_2), 2.76 (t, 2H, *J* = 6.05 Hz, O=CCH_2), 1.89 (s, 3H, =CCH_3), 1.59 (m, 2H, $OCH_2\underline{CH_2}$), 1.34 (m, 2H, $\underline{CH_2}CH_3$), 0.91 (t, 3H, *J* = 7.40 Hz, $CH_2\underline{CH_3}$). ^{13}CNMR (500 MHz, $CDCl_3$): δ = 171.82 (CO), 163.87 (C4), 151.50 (C2), 141.89 (C6), 1110.81 (C5), 62.06 (OCH_3), 42.78 (NCH_2), 33.45 ($\underline{C}H_2CO$), 32.24 ($\underline{C}H_2CH_2CO$), 18.05 ($\underline{C}H_2CH_3$), 13.50 (CH_3), 11.62 (CH_3 thymine). ESI-MS: *m/z* = 255 (M+1).

Notes and references

[1] S. Boncel and K. Walczak, *Lett. Org. Chem*, 2006, **3**, 534.

[2] J. M. Xu, C. Qian, B. K. Liu and X. F. Lin, *Tetrahedron.*2007, **63**, 986.

[3] Y. Cai, X. F. Sun, N. Wang and X. F. Lin, *Synthesis*, 2004, **5**, 671.