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Michael addition of pyrimidine derivatives with acrylates catalyzed by lipase TL IM from *Thermomyces lanuginosus* in a continuous-flow microreactor†

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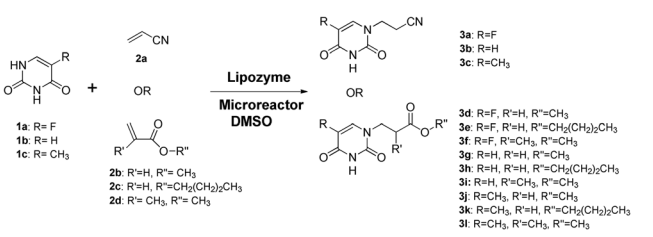
Lipase-catalyzed Michael addition of pyrimidine derivatives to acrylates in a continuous-flow microreactor is described. The influence of the structure of the Michael acceptor and the corresponding donor on the enzymatic addition was also investigated. The important features of this method include mild reaction conditions, short reaction times (30 min) and high yields.

Microreactors are a relatively new technology for performing safer, more efficient, and more selective reactions.[1–4] The improved performance is attributed to more rapid heat transfer and mixing as a result of the increased surface-area to volume ratio.[2] The advantages of microreactors have been substantiated by a growing number of examples over the past decade.[5–13] In recent years, microreactors containing immobilized enzymes have attracted considerable attention, as a consequence of their many potential industrial applications.[14–23] Important advantages over conventional batch reactions for biocatalytic reactions include high throughput, reduced reaction time, and high conversion efficiency.

The Michael addition reaction is among the most fundamental types of reactions in organic synthesis. Michael additions are generally promoted by harsh bases or strong acids, which could lead to environmentally hazardous residues and undesirable by-products.[24–29] To avoid these problems, various types of catalysts have been developed. The most common catalysts reported for Michael additions are transition metals or lanthanide catalysts.[30] Enzymes provide a alternative to these inorganic catalysts. Some natural lipases[31–33] and proteases[34–36] have been applied in Michael-type addition reactions to form carbon–nitrogen and carbon–sulfur bonds. However, most of these methods led to lower conversion yields and longer reaction times. Our interest in microreactors prompted us to ask if a Michael addition of pyrimidine derivatives to acrylates catalyzed by lipase TL IM from *Thermomyces lanuginosus* would work when performed in a continuous-flow microreactor. We report here, for the first time, the production of lipase-catalyzed Michael additions of pyrimidine derivatives (uracil, 5-fluorouracil and thymine) to acrylates (methyl acrylate, acrylonitrile, butyl acrylate and methyl methacrylate) in a continuous-flow microreactor (Scheme 1). The aim of this paper is to investigate, under a continuous-flow microreactor, the effect of the donor structure (uracil, 5-fluorouracil and thymine) and the acceptor (methyl acrylate, acrylonitrile, butyl acrylate and methyl methacrylate) on the reaction yield. These reactions were catalyzed by Lipozyme TL IM from *Thermomyces lanuginosus* using DMSO as a solvent.

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 Fig. 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 was filled in silica gel tubing (inner diameter ID = 2.0 mm, length = 1 m). The temperature of this reaction was controlled by a water bath by immersion of tubing in water and control the temperature of water. Streams **1** and **2** were



Scheme 1 Michael addition of pyrimidine derivatives with acrylates catalyzed by lipase TL IM from *Thermomyces lanuginosus* in a continuous-flow microreactor.

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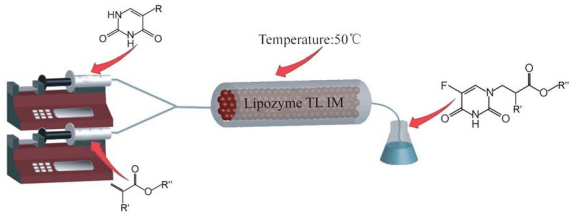

Fig. 1 Microreactor setup for the continuous-flow Michael addition reaction of pyrimidine derivatives with acrylates catalyzed by Lipozyme TL IM from *Thermomyces lanuginosus*.

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 structure of the Michael acceptor and donor can affect the results of the enzymatic Michael reaction. Fig. 2 summarizes the donor structure effect on the Michael addition in microreactors. These results indicate that 5-fluorouracil was the most reactive substrate. In fact, conversion yields were approximately 96, 88 and 80%, respectively, for 5-fluorouracil, uracil and thymine. The high conversion yield obtained with 5-fluorouracil can be explained as an effect of an electron-withdrawing group on the donor structure. An electron-withdrawing group on the donor will increase its reactivity, and an electron-donating group will have the opposite effect. Under the same condition, the Michael reaction of uracil and methyl acrylate was more rapid than that using thymine as the donor, while using 5-fluorouracil as the donor lowered the reaction rate.

We have also investigated the acceptor structure effect on Michael additions and found the longer alcohol chain, the lower the yield. Using 5-fluorouracil as the donor, the decrease of yields was detected with the increase of the alcohol chain. The steric effect of the side chain in the acceptor also affects its reactivity. The yield of adduct detected by HPLC was less than 10% in the reaction of 5-fluorouracil and methyl methacrylate (Fig. 3).

Following the standard procedure given above, the effect of molar ratio (donor–acceptor) on the enzymatic Michael reaction of pyrimidine derivatives with acrylates in a microreactor was

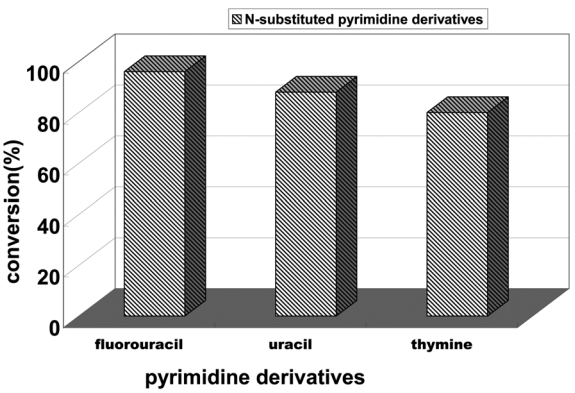

Fig. 2 Donor structure effect on the conversion of the methyl acrylate reaction with *N*-substituted pyrimidine derivatives carried out in microreactors using *Thermomyces lanuginosus* lipase.

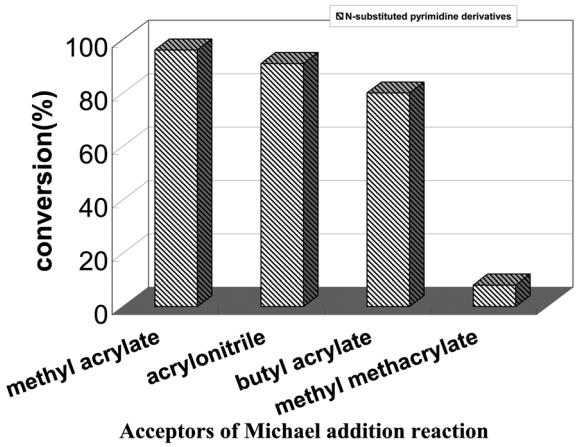

Fig. 3 Acceptor structure effect on the Michael addition performance with 5-fluorouracil carried out in microreactors using from *Thermomyces lanuginosus* lipase.

investigated in ratios from 1 : 1 to 1 : 7 for pyrimidine–acrylate. Fig. 4 shows the strong effect of this parameter on the enzymatic Michael addition reaction; the best result can be obtained with the ratio of donor–acceptor = 1 : 5.

Furthermore, the influence of the reaction time/flow rate on the conversion of methyl 3-(1′-uracil)propionate was also studied. Fig. 5 shows that the best conversion of methyl 3-(1′-uracil) propionate was observed at a residence time of 30 minutes and a flow rate of 20.8 μL min^{-1}.

Finally, to explore the scope and limitations of this new high-speed Michael addition of pyrimidine derivatives to acrylates in a continuous-flow microreactor, three pyrimidine derivatives, 5-fluorouracil (**1a**), uracil (**1b**), thymine (**1c**), and four acrylates (**2a–d**) were subjected to the general reaction conditions, using both a single-mode shaker reactor and a continuous flow/microreactor processing. For the shaker experiments, reaction times needed to be about 24 h or more to obtain ideal conversion (Method A). Using lipase-catalyzed Michael addition of pyrimidine derivatives with acrylates under continuous-flow conditions, 12 adducts have synthesized in parallel in a single experiment at the same flow rate

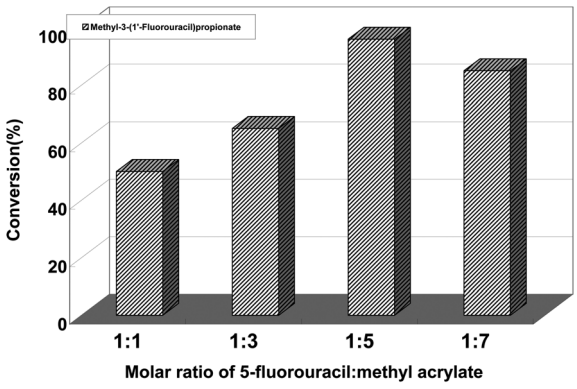

Fig. 4 The influence of Michael donor–acceptor on the enzymatic Michael addition reaction in a microreactor.

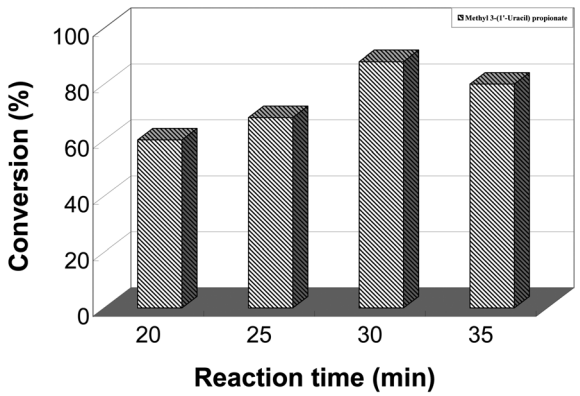

Fig. 5 The influence of reaction time on the conversion of methyl 3-(1′-uracil)propionate in microreactor.

(Method B). The results were better with flow/microreactor processing than with the single-mode shaker (Table 1, entry 1–12). Importantly, applying continuous flow/microreactor processing, resulted in a conversion of to *N*-substituted pyrimidine derivatives of 80% or more. This allows us to reduce the reaction time and simplify the purification of products.

In conclusion, we have demonstrated that Michael addition of pyrimidine derivatives with acrylates can be carried out with unprecedented efficiency using a flow microreactor approach. The large surface-area-to-volume ratios of the catalyst, Lipozyme® TL IM adsorbed on silica particles is the key to the success of this protocol. The adsorbed catalyst permits the substrate pyrimidine derivatives and acrylates to make efficient contact and react within the microreactor environment. The salient features of this method include mild reaction conditions (50 °C), short reaction times (30 min) and high yields that make our methodology a valuable contribution to the field of *N*-substituted pyrimidine derivatives synthesis. The method of enzymatic synthesis in a microreactor environment described here may have general applications to synthetic organic chemistry by enzymatic catalysis in the future. Michael additions of imidazole, purine, amine and other nitrogen nucleophiles to α,β-ethylenic compounds catalyzed by lipase TL IM from *Thermomyces lanuginosus* in a continuous-flow microreactor are in progress.

Table 1 Shaker and continuous flow synthesis of pyrimidine derivatives to acrylates catalyzed by Lipozyme TL IM from *Thermomyces lanuginosus*

Entry	Product[a]	Method[b]	Time	Conversion[c] [%]
1	**3a**	B	30 min	91
		A	24 h	70
2	**3b**	B	30 min	75
		A	24 h	65
3	**3c**	B	30 min	80
		A	24 h	71
4	**3d**	B	30 min	96
		A	24 h	92
5	**3e**	B	30 min	80
		A	24 h	78
6	**3f**	B	30 min	8
		A	24 h	10
7	**3g**	B	30 min	88
		A	24 h	85
8	**3h**	B	30 min	72
		A	24 h	70
9	**3i**	B	30 min	<5
		A	24 h	<5
10	**3j**	B	30 min	80
		A	36 h	75
11	**3k**	B	30 min	68
		A	36 h	65
12	**3l**	B	30 min	<5
		A	48 h	<5

[a] Reactions and the structure of the products **3a–3l** see Scheme 1. [b] Method A: Shaker reactor, DMSO 5 mL 0.2 g Lipozyme TL IM (40 mg mL^{-1}), 24 h. Method B: continuous flow microreactor, 10.4 μL min^{-1} feed **1** (0.1 M solution of pyrimidine derivatives in 10 mL DMSO) and 10.4 μL min^{-1} feed **2** (0.5 M solution of acrylates in 10 mL DMSO) at 50 °C (residence time 30 min), Lipozyme TL IM 0.80 g. [c] Isolated yield.

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