RSC Advances

This article can be cited before page numbers have been issued, to do this please use: L. Du, B. Cheng, W. Yang, L. Xu and X. Luo, *RSC Adv.*, 2016, DOI: 10.1039/C6RA05983J.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

View Article Online
DOI: 10.1039/C6RA05983J

Journal Name

COMMUNICATION

Markovnikov addition of imidazole derivatives with vinyl esters Catalyzed by lipase TL IM from *Thermomyces lanuginosus*/K_2CO_3 in a Continuous-Flow Microreactor

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Li-Hua Du,[*a] *Bing-Zhuo Cheng,*[a] *Wen-Jun Yan,*[a] *Liang-Liang Xu,*[a] *and Xi-Ping Luo*[a, b]

In this work, a simple and efficient method for Markovnikov addition of imidazole derivatives to vinyl esters catalyzed by lipozyme TL IM/K_2CO_3 in a continuous-flow microreactor was described. The effects of the mixed catalysts, molar ratio and the structure of reactants on the reaction performance were experimentally studied. The attractive features of this process are shorter reaction times (30 min) and excellent yields compared to that in shaker reactors.

Biocatalysis as an efficient and green tool for the synthesis of pharmaceutical, industrial and agricultural chemicals and intermediates has received great attention.[1-6] Compared with chemical catalysts, enzymes have a high catalytic activity and high selectivities under mild reaction conditions.[7-8] Although these features have brought about an increasingly interests in the field of organic synthesis, chemical catalytic routes are still preferred due to the ease of implementation and the short reaction time compared with enzymatic biotransformation. To overcome these, some novel approaches have been proposed to improve the efficiency of the conversion processes.[9-11] Among others, microreactors as a relatively new technology for performing safer, more-efficient, and more-selective reactions have attracted considerable attention due to their large surface-area-to-volume ratios[12] that allow precise reaction control through rapid heat transfer and mixing.[13-16] Catalytic reactions have been widely studied in microreactors, and, in most cases, there is an increasing interest for the enzymatic synthesis in microreactors.[14-15,17-22] Utilizing immobilized enzyme catalysts in microreactor is recognized as the most prioritized consideration of key green engineering areas for chemicals synthesis.[23-29]

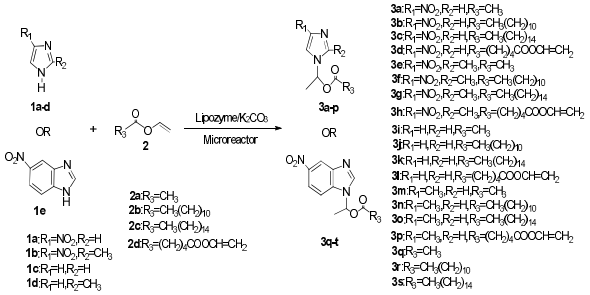
The addition reaction is among the most fundamental types of reactions in organic synthesis. However, there were only scarce reports about enzymes which are able to catalyze general addition reactions. Among them, the Michael addition has been most extensively studied.[30-32] Ling have reported enzymatic Michael addition reaction in a microreactor.[33] In order to enrich enzymatic addition reactions in microreactors, exploration of enzymes with new activities in microreactors becomes particularly fascinating and remains a great challenge.

The Markovnikov addition is one type of useful carbon-carbon, oxygen-carbon or nitrogen-carbon bond forming reaction. It is especially important to synthesize bioactive N-heterocycle derivatives with a nitrogen-carbon linkage which could be achieved by an addition reaction. Traditionally, aza-Markovnikov additions always be performed under harsh chemical conditions such as bases, acids and strong heating to promote the reaction.[34] In many cases, the yields and selectivities are far from satisfactory due to the occurrence of several side reactions. Enzyme catalysts with high regioselectivity and stereoselectivity have gained recognition as favorable, environmentally benign alternatives. X. F. Lin and co-workers used penicillin G acylase from *Escherichia coli* catalyzing the Markovnikov addition of allopurinol to vinyl ester.[35] Then they report another two zinc-binding acylases, D-aminoacylase from *Escherichia coli* and acylase "Amano" from *Aspergillus oryzae* to catalyze the Markovnikov addition.[36] Lipases were most used as carboxylic acid esterases, thioesterases, peptidases, dehalogenases, epoxide hydrolases and halo peroxidases, etc. and were scarcely used to catalyze general addition reactions especial the Markovnikov addition reation. We report here for the first time, the novel Markovnikov addition of imidazole derivatives to vinyl esters catalyzed by lipozyme TL IM/K_2CO_3 in a continuous-flow microreactor (Scheme 1). Under the catalysis of the mixed catalyst lipozyme TL IM/K_2CO_3, a number of pentacyclic N-heterocycles, imidazole and 6-nitro-benzimidazole were successfully added to a series of vinyl esters in moderate to high yields. The N-heterocycle derivatives obtained are usually pharmacologically active and may be applied as potential therapeutic alternatives. [37] The influence of the structure of the imidazole derivatives or vinyl esters to the enzymatic reactions

[a]*College of Pharmaceutical Science, Zhe Jiang University of Technology, Hangzhou, 310014, China*
[b]*Department of Environmental Science and Technology, Zhe Jiang A&F University, Hangzhou, 311300, China.*
Corresponding author. Fax: +86 571 88320903. E-mail: orgdlh@gmail.com; orgdlh@zjut.edu.cn.
† Electronic Supplementary Information available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

View Article Online
DOI: 10.1039/C6RA05983J

in a continuous-flow microreactor has been systematically evaluated. This mixed catalyst lipozyme TL IM/K_2CO_3 catalyzed Markovnikov addition in microreactor is of practical significance in expanding the application of lipases and exploring new reactions in microreactor.



Scheme 1. Markovnikov addition of imidazole derivatives with vinyl esters in DMSO catalyzed by lipase TLIM from *Thermomyces lanuginosus*/K_2CO_3 in a Continuous-Flow Microreactor.

The enzymatic Markovnikov addition of imidazole derivatives with vinyl esters starting from 4-nitroimidazole and vinyl acetate is described in Figure 1. In this equipment, Harvard Apparatus PHD 2000 syringe pumps were used to deliver reagents from syringes to the reactor. One syringe (10 mL) with the 4-nitroimidazole solution and the other syringe (10 mL) with vinyl acetate were mounted in DMSO respectively. Lipozyme TL IM (catalyst reactivity: 260 IUN/g) and K_2CO_3 were fully mixed and then filled in silica gel tubing. A water bath was applied to control the temperature of this reaction by immersion of tubing in water. In this process, streams **1** and **2** flowing into tubing at the same rate of 10.4 μL min^{-1} and then turned at the resulting stream (20.8 μL min^{-1}) when they mixed together in a Y-mixer at 50℃. Finally, a sample vial is needed for collecting the final mixture.

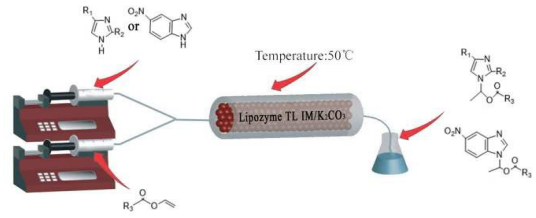


Figure 1. Experimental setup for Markovnikov addition of imidazole derivatives with vinyl esters in the continuous flow microreactor catalyzed by lipase TL IM/K_2CO_3.

Firstly, we investigated the effect of K_2CO_3 in the mixed catalysts lipase TL IM/K_2CO_3 on the Markovnikov addition performance and found that the amount of K_2CO_3 in the mixed catalysts can greatly affect the results of the reaction. Figure 2 summarized the effect of the K_2CO_3 in the mixed catalysts lipase TL IM/K_2CO_3 on the Markovnikov addition reaction in microreactors. These results indicate that with 4.5% (m/m) K_2CO_3 can give the best conversion. In fact, conversion yields were of about of 78% and 75%, respectively, when the reaction catalyzed by 100% lipase TL IM and 100% K_2CO_3.

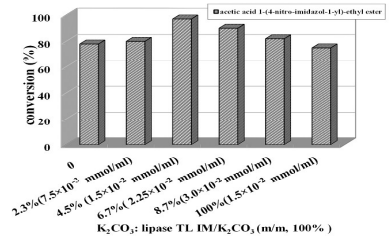


Figure 2. The influence of K_2CO_3 on the enzymatic Markovnikov addition reaction in a microreactor.

Structure of Markovnikov addition acceptor and donor can affect the results of the enzymatic Markovnikov addition reaction. The effect of different substituted groups on the imidazole ring was examined, as shown in Figure 3. Addition of 4-nitroimidazole (1a) to vinyl acetate (2a) afforded a much higher yield (97%, entry 1) than imidazole (52%, entry 9) in a shorter time, indicating that an electron-withdrawing group improves the addition reactivity of the N-heterocycle. Oppositely, the additions of 4-methylimidazole proceeded more slowly (entries 13−16). Under the same condition, the markovnikov addition of 2-methyl-4-nitroimidazole and vinyl acetate was more rapid than that using 4-methylimidazole as the donor, while lower than that using 4-nitroimidazole as the donor.

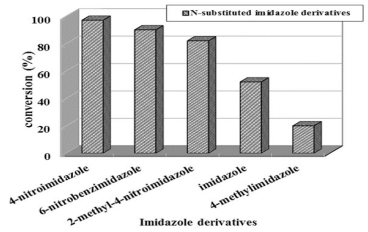


Figure 3. The effect of different substituted groups on the imidazole ring on the conversion of the vinyl acetate reaction with N-substituted imidazole derivatives carried out in microreactors using *Thermomyces lanuginosus* lipase/K_2CO_3.

We have also investigated the acceptor structure effect on Markovnikov additions and found the longer the vinyl esters carboxyl group chain, the lower the conversion. Using 4-nitroimidazole as the donor, the decrease of yields was detected with the increase of carboxyl group chain. The conversion yield was less than 60% in the reaction of 4-nitroimdazole and vinyl palmitate (Figure 4).

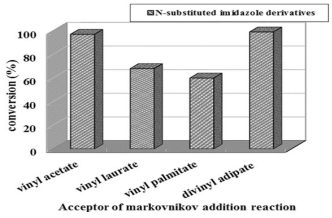


Figure 4. Acceptor structure effect on the Markovnikov addition performance with 4-nitroimidazole carried out in microreactors.

Having obtained favorable results given above, we then examined the effect of molar ratio (donor/acceptor) on the enzymatic Markovnikov addition reaction in a microreactor.

View Article Online
DOI: 10.1039/C6RA05983J

We choose 4-nitroimidazole and vinyl acetate as the model reaction and changed the molar ratio from 1:1 to 1:8. As we can see from the Figure 5, with the increase of the vinyl acetate, the reaction conversion increase too, the best result could be obtained when molar ratio reached to 1:6. Then if we continued to increase the amount of vinyl acetate, the reaction conversation will decrease. That maybe be explained as the occurrence of some side reaction, and then affect the conversion of the object product. So the best molar ratio (donor/acceptor) is 1:6 when the the enzymatic Markovnikov addition reaction performed in a microreactor.

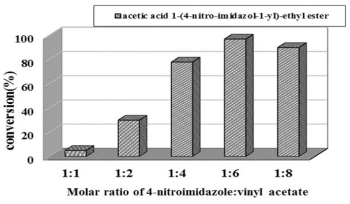


Figure 5. The influence of donor/acceptor on the enzymatic Markovnikov addition reaction in a microreactor.

Furthermore, the influence of the reaction time/flow rate on the conversion of acetic acid 1-(4-nitro-imidazol-1-yl)-ethyl ester was also studied. Figure 6 shows that the best conversion of acetic acid 1-(4-nitro-imidazol-1-yl)-ethyl ester was observed at a residence time of 30 minutes and a flow rate of 20.8 μL min^{-1}.

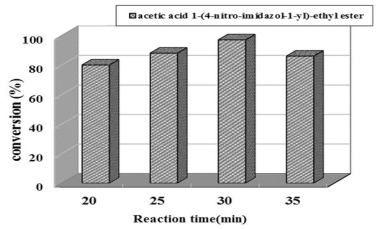


Figure 6. The influence of reaction time on the conversion of acetic acid 1-(4-nitro-imidazol-1-yl)-ethyl ester in microreactor.

Finally, to explore the scope and limitations of this new high-speed Marknoikov addition of imidazole derivatives to vinyl esters in a continuous-flow microreactor, five imidazole derivatives 4-nitroimidazole (**1a**), 2-methyl-4-nitroimidazole (**1b**), imidazole (**1c**), 4-methyl-imidazole (**1d**), benzimidazole (**1e**) and four vinyl esters (**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 Marknoikov addition of imidazole derivatives with vinyl esters under continuous-flow conditions, 20 adducts were synthesized in parallel in a single experiment at the same flow rate (Method **B**). The results were better with flow/microreactor processing than with the single-mode shaker (Table 1, entry 1-20). Importantly, applying continuous flow/microreactor processing, yielded a conversion of to *N*-substituted imidazole derivatives of 80% or more. This allows us to reduce the reaction time and simplify the purification of products.

Table 1. Shaker and continuous flow synthesis of imidazole derivatives to vinyl esters catalyzed by Lipozyme TL IM from *Thermomyces lanuginosus*/ K_2CO_3.

Entry	Product[a]	Method[b]	Time	Conversion[%][c]
1	**3a**	**B**	30 min	97
		A	24 h	85
2	**3b**	**B**	30 min	68
		A	24 h	65
3	**3c**	**B**	30 min	60
		A	24 h	55
4	**3d**	**B**	30 min	99
		A	24 h	90
5	**3e**	**B**	30 min	82
		A	24 h	75
6	**3f**	**B**	30 min	70
		A	24 h	65
7	**3g**	**B**	30 min	65
		A	24 h	60
8	**3h**	**B**	30 min	92
		A	24 h	85
9	**3i**	**B**	30 min	52
		A	24 h	8
10	**3j**	**B**	30 min	25
		A	24 h	10
11	**3k**	**B**	30 min	8
		A	24 h	<5
12	**3l**	**B**	30 min	68
		A	24 h	46
13	**3m**	**B**	30 min	20
		A	24 h	8
14	**3n**	**B**	30 min	7
		A	24 h	<5
15	**3o**	**B**	30 min	<5
		A	24 h	<5
16	**3p**	**B**	30 min	15
		A	24 h	8
17	**3q**	**B**	30 min	90
		A	24 h	70
18	**3r**	**B**	30 min	65
		A	24 h	60
19	**3s**	**B**	30 min	35
		A	24 h	30
20	**3t**	**B**	30 min	95
		A	24 h	82

[a] Reactions and the structure of the products **3a-3t** see scheme 1.

[b] Method A: Shaker reactor, DMSO 5 mL , 0.2 g Lipozyme TL IM (40 mg/mL), K_2CO_3 (1.5×10^{-2} mmol/mL), 24 h. Method B: continuous flow microreactor, 10.4 uL min^{-1} feed **1** (0.1 M solution of imidazole derivatives in 10 mL DMSO) and 10.4 uL min^{-1} feed **2** (0.6 M solution of vinyl esters in 10 mL DMSO) at 50℃(residence time 30 min), Lipozyme TL IM 0.8 g, K_2CO_3 (1.5×10^{-2} mmol/mL) 41.4 mg.

[c] isolated yield.

In conclusion, we have demonstrated that Markovnikov addition of imidazole derivatives with vinyl esters can be carried out with unprecedented efficiency using a flow microreactor approach. The large surface-area-to-volume ratios of the catalyst, the mixed catalysts lipase TL IM/K_2CO_3 adsorbed on silica particles is key to the success of this protocol. The adsorbed catalyst permits the substrate imidazole derivatives and vinyl esters to make efficient contact and react within the microreactor environment. The salient features of this method include mild reaction conditions (50 ^{o}C), short

View Article Online
DOI: 10.1039/C6RA05983J

reaction times (30 min) and high yields that make our methodology a valuable contribution to the field of N-substituted imidazole 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. Markovnikov additions of triazole, purine, pyrimidine and other nitrogen nucleophiles to vinyl esters catalyzed by lipase TL IM from *Thermomyces lanuginosus*/K_2CO_3 in a continuous-flow microreactor are in progress.

The authors would like to thank National Natural Science Foundation of China (no.21306172), the Natural Science Foundation of Zhejiang Province (LY13B020010 and LY13C160008), the Science and Technology Research Program of Zhejiang Province (2014C32094), the State forestry administration 948 Foundation (2014-4-29) as well as the Natural Science Foundation of Zhejiang University of Technology (116004029) for financial support.

Notes and references

1 D. J. Pollard and J. M. Woodley, *Trends Biotechnol*, 2007, **25**, 66.
2 M. S. Thomsen and B. Nidetzky, *Biotechnol. J.*, 2009, **4**, 98.
3 J. Sukumaran and U. Hanefeld, *Chem. Soc. Rev.,* 2005, **34,** 530.
4 X. W. Feng, C. Li, N. Wang, K. Li, W. W. Zhang, Z. Wang and X. Q. Yu, *Green Chem.*, 2009, **11**, 1933.
5 W. A. Greenberg, A. Varvak, S. R. Hanson, K. Wong, H. J. Huang, P. Chen and M. J. Burk, *Proc. Natl. Acad. Sci. U. S. A.,* 2004, **101**, 5788.
6 H. J. Lamble, M. J. Danson, D. W. Hough and S. D. Bull, *Chem. Commun.*, 2005, 124.
7 R. A. Sheldon, *Adv. Synth. Catal.*, 2007, **349**, 1289.
8 N. Ran, L. Zhao, Z. Chen and J. Tao, *Green Chem.*, 2008, **10**, 361.
9 H. Freund and K. Sundmacher, *Chem. Eng. Process,* 2008, **47**, 2051.
10 P. Lutze, R. Gani and J. M. Woodley, *Chem. Eng. Process*, 2010, **49**, 547.
11 A. Stankiewicz and J. Moulijn, *Chem. Eng. Prog.*, 2000, **96**, 22.
12 V. Hessel and H. LÖöwe, *Chem. Eng. Technol.,* 2005, **28**, 267.
13 A. Nagaki, H. Kim and J. Yoshida, *Angew. Chem.,* 2008, **120**, 7951.
14 A. Nagaki, H. Kim and J. Yoshida, *Angew. Chem. Int. Ed.,* 2008, **47**, 7833.
15 A. Nagaki, N. Takabayashi, Y. Tomida and J. Yoshida, *Org. Lett.,* 2008, **10**, 3937.
16 J. M. J. vander Linden, P. W. Hilberink, C. M. P. Kronenburg and G. J. Kemperman, *Org. Process Res. Dev.,* 2008, **12**, 911.
17 J. Yoshida, A. Nagaki and T. Yamada, *Chem. Eur. J.,* 2008, **14**, 7450.
18 H. E. Schoemaker, D. Mink and M. G. Wubbolts, *Science,* 2003, **299**, 1694.
19 P. Fernandes, *Int. J. Mol. Sci.,* 2010, **11**, 858.
20 B. P. Mason, K. E. Price, J. L. Steinbacher, A. R. Bogdan and D. T. McQuade, *Chem. Rev.,* 2007, **107**, 2300.
21 D. R. Patil, D. G. Rethwisch and J. S. Dordick, *Biotechnol. Bio eng.*, 1991, **37**, 639.
22 M. Miyazaki and H. Maeda, *Trends. Biotechnol.,* 2006, **24**, 463.
23 A. Polyzoidis, T. Altenburg, M. Schwarzer, S. Loelbeche and S. Kaskel, *Chem. Eng. J.,* 2016, **283**, 971.
24 Y. Asanomi, H. Yamaguchi, M.Miyazaki and H. Maeda, *Molecules*, 2011, **16**, 6041.
25 C. J. Gonzalez, P. Poechlauer, Q. B. Broxterman and B. Yang, *Org. Process. Res. Dev.*, 2011, **15**, 900.
26 J. Wang, S. S. Gu, H. S. Cai, L. Q. Yang and X. Y. Wu, *Bioresour. Techno*., 2013, **149**, 367.
27 S. Ueno, T. Hirai, S. Sato, M. Biyani, H. Kuramochi, R. Iizuka, T. Funatsu and T. Ichiki, *J. Photopolym. Sci. Technol.*, 2015, **28**, 719.
28 I. Iliuta, A. Garnier and M. C. Iliuta, *Ind. Eng. Chem. Res.* 2015, **54**, 7787.
29 M. R. F. Cerqueira, M. S. F. Santos, R. C. Matos, I. G. R. Gutz and L. Angnes, *Microchemical Journal*, 2015, **118**, 231.
30 J. F. Cai, Z. Guan and Y. H. He, *J. Mol. Catal. B: Enzym.,* 2011, **71**, 108.
31 J. F. Cai, Z. Guan and Y. H. He, *J. Mol. Catal. B: Enzym.,* 2011, **68**, 240.
32 J. M. Xu, F. Zhang, Q. Wu, Q. Y. Zhang and X. F. Lin, *J. Mol. Catal. B: Enzym*., 2007, **49**, 50.
33 L. H. Du, H. M. Ling and X. P. Luo, *RSC. Adv,* 2014, **4**, 7770.
34 B. V. Timokhin, A. I. Golubin, O. V. Vysotskaya, V. A. Kron, L. A. Oparina, N. K. Gusarova and B. A. Trofimov, *Chem. Heterocycl. Com.,* 2002, **38**, 981.
35 W. B. Wu, N. Wang, J. M. Xu, Q. Wu and X. F. Lin, *Chem. Commun.,* 2005, 2348.
36 W. B. Wu, J. M. Xu, Q. Wu, D. S. Lv and X. F. Lin, *Adv. Synth. Catal.,* 2006, **348**, 487.
37 A. Tanitame, Y. Oyamada, K. Ofuji, M. Fujimoto, N. Iwai, Y. Hiyama, K. Suzuki, H. Ito, H. Terauchi, M. Kawasaki, K. Nagai, M.Wachi and J. Yamagishi, *J. Med. Chem.,* 2004, **47**, 3693.

View Article Online
DOI: 10.1039/C6RA05983J

Markovnikov addition of imidazole derivatives with vinyl esters Catalyzed by lipase TL IM from *Thermomyces lanuginosus*/K_2CO_3 in a Continuous-Flow Microreactor

Li-Hua Du,[*a] *Bing-Zhuo Cheng*[a], *Wen-Jun Yang*[a], *Liang-Liang Xu*[a] *and Xi-Ping Luo*[a, b]

In this work, a simple and efficient method for Markovnikov addition of imidazole derivatives to vinyl esters catalyzed by lipozyme TL IM/K_2CO_3 in a continuous-flow microreactor was described. The effects of the mixed catalysts, molar ratio and the structure of reactants on the reaction performance were experimentally studied. The attractive features of this process are shorter reaction times (30 min) and excellent yields compared to that in shaker reactors.