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α-糜蛋白酶催化合成β-脲基巴豆酸酯

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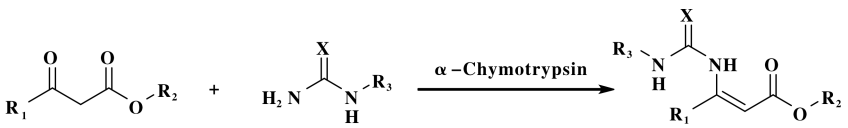
摘要：β-脲基巴豆酸酯是合成 6-甲基嘧啶二酮衍生物及 3,4-二氢嘧啶二酮衍生物的重要中间体，在生物制药中有着广泛的应用. 由于酶具有绿色、安全、高效的催化特性，采用 α-糜蛋白酶为生物催化剂，以 N,N-二甲基甲酰胺为反应介质，37 ℃条件下，通过 1,3-二羰基化合物和脲的缩合反应，合成了一系列β-脲基巴豆酸酯类化合物.

关键词：α-糜蛋白酶；β-脲基巴豆酸酯；生物催化；酶的非专一性
中图分类号：TQ314；O643.3　　**文献标志码**：A

β-脲基巴豆酸酯是合成 6-甲基嘧啶二酮衍生物及 3,4-二氢嘧啶二酮衍生物的重要中间体，6-甲基嘧啶二酮衍生物具有选择性抗肿瘤、抗病毒、抗结核和抗真菌活性[1-2]，而 3,4-二氢嘧啶二酮衍生物具有钙通道阻断[3]、抗菌[4]和抗病毒[5]等生物活性. 但关于β-脲基巴豆酸酯合成的报道却很少，虽有报道用氯化锑[6]、三氟甲磺酸锌[7]和盐酸[8]等作催化剂合成该类物质，但反应条件较苛刻，所以寻找温和的合成方法仍具有积极意义. 酶作为生物体内大量生化反应的催化剂，其绿色、安全、高效的催化性能已得到化学家们的广泛关注，并将酶的非专一性运用到多种有机合成反应中且取得了较好的效果[9-12]，例如水解酶的天然功能是催化化学键的断裂，然而近些年来的研究表明，水解酶同样可催化化学键的形成[13-14]，并已成功应用于 C—C[15-16]键、C-杂[17-18]原子键的构建及氧化[19]、过氧化[20]、聚合[21]等多种类型的化学反应中. 于是，我们以 α-糜蛋白酶为生物催化剂，通过 1,3-二羰基化合物和脲的缩合反应，合成了一系列β-脲基巴豆酸酯类化合物，反应过程如图 1 所示.



I　　II　　IIIa~i

R_1=CH_3, CH_2CH_3, $(CH_2)_2CH_3$; R_2=CH_3, CH_2CH_3; R_3=H, CH_3; X=O, S

图 1 β-脲基巴豆酸酯的合成

Fig.1 Synthesis of β-uramino crotonic ester

1 实验部分

1.1 试剂与仪器

乙酰乙酸乙酯(AR，98%)、丙酰乙酸甲酯(AR，98%)、丁酰乙酸乙酯(AR，97%)、尿素(AR，99%)、硫脲(AR，99%)、N-甲基脲(AR，98%)、氘代 DMSO(氘代率99.9% +体积分数0.03% TMS)和 α-糜蛋白酶(800 usp U/mg)，上海阿拉丁生化科技股份有限公司；其它试剂均为国产 AR. 所有试剂使用前未经任何处理.

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WRS-2A 型微机熔点仪，上海仪电物理光学仪器有限公司(使用前温度未经校正，起始温度为 60 ℃，升温速率为 1 ℃/min)；Bruker AV II-600 型核磁共振波谱仪，瑞士 Bruker 公司，扫场范围为^{1}H：−1～14 ppm，^{13}C：−10～230 ppm. LTQ-XL 型线性离子阱质谱仪，美国 Thermo-Fisher 公司，配有 Xcalibur 型数据处理系统，甲醇(美国 Fisher 公司)，质谱扫描范围 *m/z* 50-500；喷雾电压为 1.0 kV，金属毛细管温度 150 ℃，ESI 喷雾气(N2)压力 1.0 MPa，载气(N2)压力为 0.2 MPa；萃取剂通过进样泵进样，流速 3 μL/min；串联质谱分析时，母离子的隔离宽度 1.0 μm，碰撞时间 30 ms，碰撞能量 20%；其他参数由 LTQ-MS 系统自动优化.

1.2 β-脲基巴豆酸酯的合成

在 10 mL 具塞锥形瓶中，加入 3 mmol 乙酰乙酸乙酯、1 mmol 尿素、20 mg α-糜蛋白酶和 3 mL N,N-二甲基甲酰胺，于 37 ℃恒温摇床(200 r/min)中反应 48 h，经硅胶填充的吸附柱色谱分离($V_{petroleum\ ether}$ ∶ $V_{ethyl\ acetate}$ = 2 ∶ 1)得到目标产物，并用^{1}H NMR 和 HR-MS 进行了结构表征，产率为柱色谱分离产率.

2 结果与讨论

2.1 催化剂对酶促反应的影响

催化剂是影响反应的关键因素，所以研究了酶的种类对反应的影响，以乙酰乙酸乙酯和尿素作为模板反应来研究各种酶的催化效率，在相同的反应条件下，只有 α-糜蛋白酶能催化乙酰乙酸乙酯和尿素反应，得到 33%的β-脲基巴豆酸酯，结果见表 1. 因此，选择 α-糜蛋白酶作为最佳催化剂进行后续研究.

表 1 不同酶对模板反应的催化效果[a]

Table 1 The catalytic effect of various enzymes for the model reaction[a]

Entry	Enzyme	Yield/%[b]
1	Pancreatin from porcine pancreas (8 U/mg)	None
2	Alkaline protease (200 U/mg)	None
3	Trypsin from porcine pancreas (2500 U/mg)	None
4	Papain from papaya latex (800 U/mg)	None
5	β-Dextranase (200 U/mg)	None
6	Lipase (30-90 U/mg)	None
7	Lipase B from *Candida antarctica* (100 U/mg)	None
8	Cellulase from *Aspergillus niger* (50 U/mg)	None
9	α-Chymotrypsin (800 U/mg)	33
10	Denatured α-chymotrypsin[c]	None
11	Blank control	None

a. Reaction conditions: 20 mg enzyme, solvent: DMF, Temp.: 37 ℃, reaction time: 48 h; b. Yields of pure products isolated by chromatography; c. At 100 ℃, deal α-chymotrypsin with 8 mol/L urea for 8 h.

2.2 溶剂对酶促反应的影响

溶剂是影响化学反应的重要因素，因此又考察了溶剂对模板反应的影响. 由表 2 可知，α-糜蛋白酶在 DMF 和 DMSO 等非质子溶剂催化活性较好，最高可取得 33%的产率；而在甲醇、乙醇和水等质子溶剂无产物生成，这可能是溶剂影响了酶的活性和稳定性所致，而溶剂 Log P 值对反应的影响并无明显的规律性，最终选择 DMF 为溶剂进行后续研究.

2.3 底物摩尔比对酶促反应的影响

选用乙酰乙酸乙酯和尿素为模板反应底物，首先考察了底物摩尔比对反应效果的影响，结果见表 3. 尿素过量时反应效果较差，而随着乙酰乙酸

乙酯用量的增加产率不断提高，当乙酰乙酸乙酯：尿素=3∶1 时产率取得最大值 33%，之后，再增加乙酰乙酸乙酯用量产率反而降低. 所以选择了乙酰乙酸乙酯：尿素=3∶1 为最佳底物摩尔比.

表 2 溶剂对模板反应产率的影响[a]

Table 2 Effect of solvent on the yield of model reaction[a]

Solvent	Log P	Yield/%[b]
MeOH	−0.27	None
EtOH	0.07	None
H_2O	−	None
DMF	−0.6	33
DMSO	−1.49	20
MeCN	0.17	Trace

a. Reaction conditions: 20 mg α-chymotrypsin, Temperature: 37 ℃, reaction time: 48 h; b. Yields of pure products isolated by chromatography.

表 3 底物摩尔比对模板反应产率的影响[a]

Table 3 Effect of molar ratio on the yield of model reaction[a]

Ethylacetoacetate:Urea	Yield/%[b]
1∶1	26
1∶2	17
1∶3	Trace
2∶1	27
3∶1	33
4∶1	32

a. Reaction conditions: 3 mL DMF, 20 mg α-chymotrypsin, Temperature: 37 ℃, reaction time: 48 h; b. Yields of pure products isolated by chromatography.

2.4 温度对酶促反应的影响

温度不仅影响化学反应速率，也是影响酶活性的重要因素，因此又考察了温度对模板反应的影响，结果见表 4. 随着温度升高反应产率也逐渐升高，当温度为 37 ℃时产率取得最大值，之后再升高温度产率反而降低. 因为酶是生物催化剂，过高的温度会使酶蛋白变性失活，从而影响其催化性能，导致反应产率降低. 因此，最终选择 37 ℃作为最佳反应温度.

表 4 温度对模板反应产率的影响[a]

Table 4 Effect of temperature on the yield of model reaction[a]

Temperature/℃	Yield/%[b]
20	5
30	15
37	33
40	33
50	29

a. Reaction conditions: 3 mmol ethyl acetoacetate, 1 mmol urea, 3 mL DMF, 20 mg α-chymotrypsin, reaction time: 48 h;

b. Yields of pure products isolated by chromatography.

2.5 催化剂用量对酶促反应效果的影响

最后又对催化剂用量进行了优化，结果见表 5. 不加催化剂时，没有产物生成，当用 5 mg 的 α-糜蛋白酶作催化剂时，取得了 7%的产率；当酶用量增加至 20 mg 时，产率达到了最大值. 因此，最终选择 20 mg 为最佳酶用量.

表 5 催化剂用量对模板反应产率的影响[a]

Table 5 Effect of enzyme loading on the yield of model reaction[a]

Enzyme/mg	Yield/%[b]
0	Trace
5	7
10	17
15	30
20	33
25	33

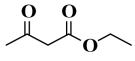
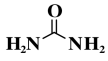
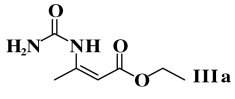
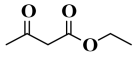
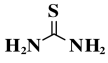
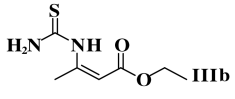
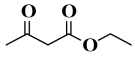
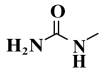
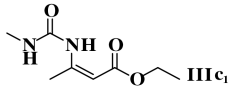
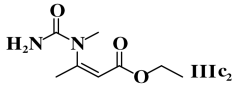
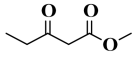
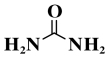
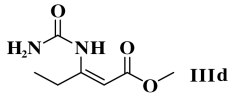
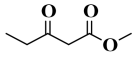
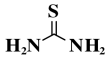
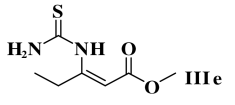
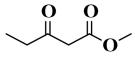
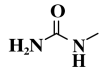
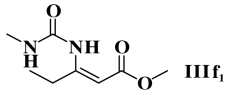
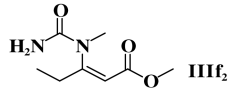
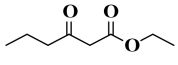
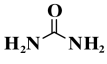
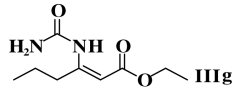
a. Reaction conditions: 3 mmol ethyl acetoacetate, 1 mmol urea, 3 mL DMF, 0-25 mg α-chymotrypsin, Temperature: 37 ℃, reaction time: 48 h; b. Yields of pure products isolated by chromatography.

2.6 β-脲基巴豆酸酯类化合物的合成

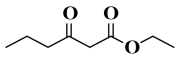
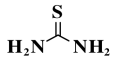
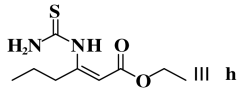
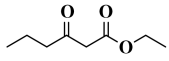
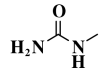
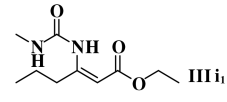
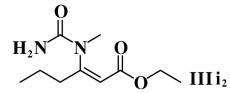
在确定了酶促反应的最佳条件后，又选取了一系列β-二羰基化合物和脲进行反应，以考察该方法的底物普适性. 由表 6 实验数据可知，α-糜蛋白酶可以催化多种二羰基化合物和脲的缩合反应合成β-脲基巴豆酸酯类化合物；同时也可以看出二羰基化合物和脲的结构对反应产率有较大影响. 硫脲的反应效果较尿素和甲基脲好，且产率受二羰基化合物结构影响较小(表 6，序号 2，5，8)；当二羰基化合物与甲基脲反应时，区域选择性较好，分别只得到了 $IIIc_1$、$IIIf_1$和 $IIIi_1$(表 6，序号 3，6，9)；丁酰乙酸乙酯与脲反应时的产率相对较低，可能是受空间效应的影响(表 6，序号 7，8).

表 6 α-糜蛋白酶催化合成β-脲基巴豆酸酯[a]

Table 6α-Chymotrypsin-catalyzed synthesis of β-uramino crotonic esters[a]

Entry	I	II	III	Yield/%[b]
1			 IIIa	33
2			 IIIb	59
3			 $IIIc_1$	27
			 $IIIc_2$	0
4			 IIId	35
5			 IIIe	63
6			 $IIIf_1$	34
			 $IIIf_2$	0
7			 IIIg	15

续表 6

Entry	I	II	III	Yield/%[b]
8			 III h	61
9			 III i$_1$	9
			 III i$_2$	0

a. Reaction conditions: 3 mmol β-ketoester, 1 mmol urea(thiourea), 3 mL DMF, 20 mg α-chymotrypsin, Temperature: 37 ℃, reaction time: 48 h; b. Yields of pure products isolated by chromatography.

IIIa: 白色固体. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.14 (s, 1H), 6.80 (s, 2H), 4.75 (s, 1H), 4.06 (q, J = 7.1 Hz, 2H), 2.24 (s, 3H), 1.18 (t, J = 7.1 Hz, 3H). ESI -HRMS: m/z, [$C_7H_{12}N_2O_3$+H]$^+$计算值: 173.085, 测定值: 173.091.

IIIb: 白色固体. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.09 (s, 1H), 6.90 (s, 2H), 5.45 (s, 1H), 4.06 (q, J = 7.1 Hz, 2H), 2.24 (s, 3H), 1.18 (t, J=7.1 Hz, 3H). ESI -HRMS: m/z, [$C_7H_{12}N_2O_2S$+H]$^-$计算值: 187.062, 测定值: 187.053.

IIIc$_1$: 白色固体. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.87 (s, 1H), 7.73 (s, 1H), 4.77 (s, 1H), 4.06 (q, J = 7.1 Hz, 2H), 2.77 (s, 3H), 2.30 (s, 3H), 1.19 (t, J = 7.1 Hz, 3H). ESI -HRMS: m/z, [$C_8H_{14}N_2O_3$+ H]$^+$计算值: 187.100, 测定值: 187.108.

IIId: 白色固体. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.13 (s, 1H), 6.80 (s, 2H), 4.75 (s, 1H), 3.79 (s, 3H), 2.84 (q, J = 7.1 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H). ESI -HRMS: m/z, [$C_7H_{12}N_2O_3$+H]$^+$计算值: 173.085, 测定值: 173.092.

IIIe: 白色固体. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.13 (s, 1H), 7.10 (s, 2H), 4.75 (s, 1H), 3.79 (s, 3H), 2.84 (q, J = 7.1 Hz, 2H), 1.19 (t, J = 7.1 Hz, 3H). ESI -HRMS: m/z, [$C_7H_{12}N_2O_2S$+H]$^+$计算值: 188.062, 测定值: 188.059.

IIIf$_1$: 白色固体. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.65 (s, 1H), 6.95 (s, 1H), 4.75 (s, 1H), 3.79 (s, 3H), 2.84 (q, J = 7.1 Hz, 2H), 2.75 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H). ESI -HRMS: m/z, [$C_8H_{14}N_2O_3$ + H]$^+$计算值: 187.100, 测定值: 187.107.

IIIg: 白色固体. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.14 (s, 1H), 6.80 (s, 2H), 4.75 (s, 1H), 4.05 (q, J = 7.1 Hz, 2H), 2.01 (t, J = 7.2 Hz, 2H), 1.36 (m, 2H), 1.18 (t, J = 7.1 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H). ESI -HRMS: m/z, [$C_9H_{16}N_2O_3$+ H]$^+$ 计算值: 201.116, 测定值: 201.123.

IIIh: 白色固体. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.14 (s, 1H), 7.10 (s, 2H), 4.75 (s, 1H), 4.05 (q, J = 7.1 Hz, 2H), 2.01 (t, J = 7.2 Hz, 2H), 1.36 (m, 2H), 1.18 (t, J = 7.1 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H). ESI -HRMS: m/z, [$C_9H_{16}N_2O_2S$ + H]$^-$计算值: 215.093, 测定值: 215.034.

IIIi: 白色固体. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.25 (s, 1H), 6.91 (s, 1H), 4.75 (s, 1H), 4.05 (q, J = 7.1 Hz, 2H), 2.76 (s, 3H), 2.01 (t, J = 7.2 Hz, 2H), 1.36 (m, 2H), 1.18 (t, J = 7.1 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H). ESI -HRMS: m/z, [$C_{10}H_{18}N_2O_3$+ H]$^+$计算值: 215.132, 测定值: 215.139.

2.7 反应机理的推测

α-糜蛋白酶属于肽酶 S1 族的丝氨酸蛋白酶, 由 3 个通过二硫键链接的多肽链组成, A 链

(Cys1-Leu13)，B 链(Ile16-Tyr146)和 C 链(Ala149-Asn245)，A 链是 13 个残基的短链，而 B 和 C 链分别含有 131 和 97 个残基[22]. 催化三联体由 His57、Asp102 和 Ser195 组成[23-24]. 参照相关文献[25]，推测出了 α-糜蛋白酶催化合成 β-脲基巴豆酸酯类化合物的可能机理. 如图 2 所示，His57 先夺取脲氨基上的质子，使脲作为亲核试剂进攻被 Ser195 通过氢键活化了的羰基，生成一个过渡态的化合物，之后再通过 Ser195 发生脱水生成 β-脲基巴豆酸酯.

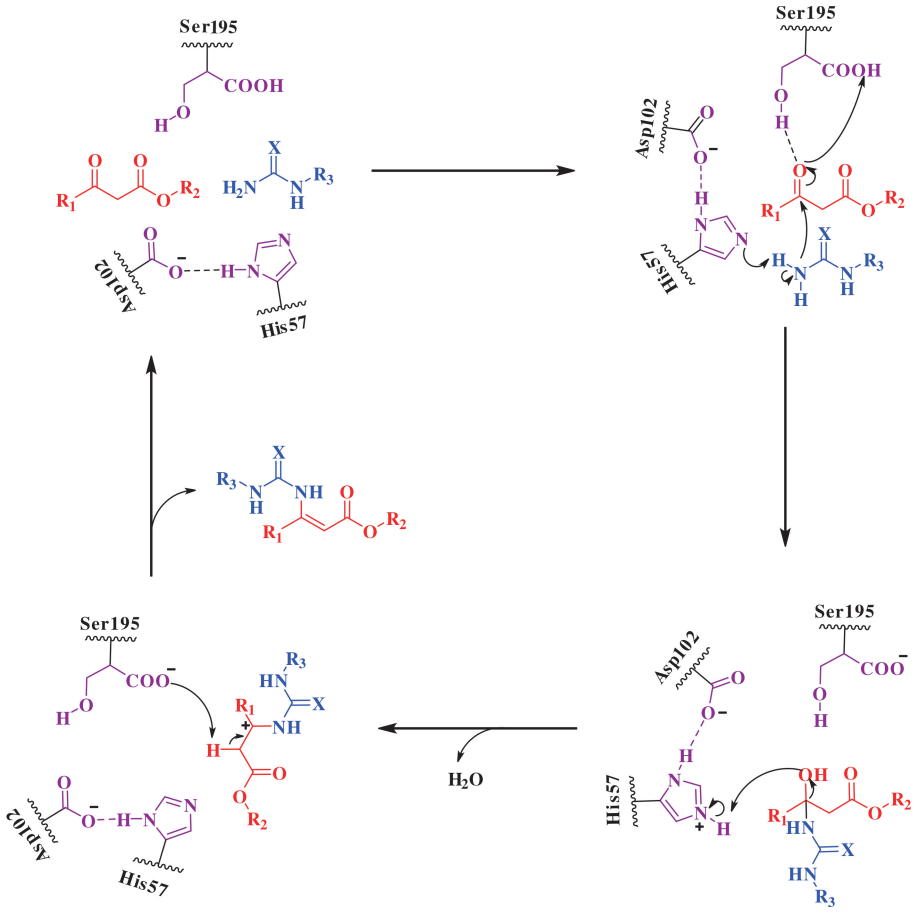


图 2 α-糜蛋白酶催化合成 β-脲基巴豆酸酯的可能机理

Fig.2 Proposed mechanism for α-chymotrypsin-catalyzed synthesis of β-uramino crotonic ester

3 结论

我们建立了一种生物催化合成 β-脲基巴豆酸酯类化合物的新方法，并对可能的催化机理进行了推测；该方法反应条件较为温和，符合绿色化学的基本理念；但该方法的产率还较低，底物适用范围也有待拓展. 该研究进一步拓展了酶非专一性的应用范围，对推动生物催化在有机合成中的应用具有积极意义.

参考文献：

[1] Gilberg E, Stumpfe D, Bajorath J. Towards a systematic assessment of assay interference: Identification of extensively tested compounds with high assay promiscuity[J]. *F1000research*, 2017, **6**: 1505-1515.

[2] Mak J Y, Xu W, Reid R C, *et al*. Stabilizing short-lived Schiff base derivatives of 5-aminouracils that activate mucosal-associated invariant T cells[J]. *Nat Commun*, 2017, **8**: 14599-14612.

[3] Haline G O A, Tatiani B L, Aline L de O, *et al*. Facts, presumptions, and myths on the solvent- free and catalyst- free Biginelli reaction. What is catalysis for? [J]. *J Org Chem*, 2014, **79**(8): 3383-3397.

[4] Nandi G C, Samai S, Singh M S. Biginelli and Hantzsch-type reactions leading to highly functionalized dihydropyri-

midinone, thiocoumarin, and pyridopyrimidinone frameworks via ring annulation with β-oxodithioesters[J]. *J Org Chem*, 2010, **75**(22): 7785-7795.

[5] Simón L, Goodman J M. A model for the enantioselectivity of imine reactions catalyzed by BINOL- phosphoric acid catalysts[J]. *J Org Chem*, 2011, **76**(6): 1775-1788.

[6] Schneider P, Stutz K, Kasper L, *et al*. Target profile prediction and practical Evaluation of a biginelli- type dihydropyrimidine compound library[J]. *Pharm*, 2011, **4**(9): 1236-1247.

[7] Majee A, Kundu S K, Santra S, *et al*. ChemInform abstract: An improved procedure of miyashita protocol for the preparation of ureidomethylene derivatives of 1,3-dicarbonyl compounds[J]. *Cheminform*, 2014, **45**(20): 124-126.

[8] Ehsan S, Khan B. Microwave synthesis of β-uramino crotonic ester and its derivatives[J]. *Asian J Chem*, 2011, **23**(7): 3202-3204.

[9] a. Humble M S, Berglund P. Biocatalytic promiscuity[J]. *Eur J Org Chem*, 2011, **2011**(19): 3391-3401.

b. Lu Jia-wei(卢佳伟), Li You-ran(李由然), Shi Gui-yang(石贵阳). The modification of polyacrylonitrile hollow membrane by polyethyleneimine to immobilize lipase(聚乙烯亚胺改性聚丙烯腈中空膜固定脂肪酶)[J]. *J Mol Catal*(*China*)(分子催化), 2018, **32**(1): 79-89.

c. Lin Hui-ying(林惠颖), Xin Jia-ying(辛嘉英), Li Chun-yu(李春雨), *et al*. Progress on isolation and purification of particulate methane monooxygenase(颗粒性甲烷单加氧酶分离纯化方法的研究进展)[J]. *J Mol Catal*(*China*)(分子催化), 2018, **32**(1): 90-98.

d. Zheng Li-na(郑丽娜), Xin Jia-ying(辛嘉英), Wang Yan(王 艳), *et al*. Research progress on the influence of microwave on enzyme-catalyzed reactions and the microwave effect(微波对酶催化反应的影响及其微波效应的研究进展)[J]. *J Mol Catal*(*China*)(分子催化), 2017, **31**(6): 567-574.

[10] a. Busto E, Gotor-Fernandez V, Gotor V. Hydrolases: Catalytically promiscuous enzymes for non-conventional reactions in organic synthesis[J]. *Chem Soc Rev*, 2010, **39**: 4504-4523.

b. Li Chun-yu(李春雨), Xin Jia-ying(辛嘉英), Lin Hui-ying(林惠颖), *et al*. Study on functionalized gold nanoparticles of methanobactin by copper ion coordination uesd as simulated peroxidase(铜离子配位甲烷氧化菌素功能化纳米金模拟过氧化物酶的研究)[J]. *J Mol Catal*(*China*)(分子催化), 2017, **31**(5): 480-485.

[11] Xin Jia-ying(辛嘉英). Lipase-catalyzed double kinetic resolution for the prepartion of high enantiopurity (*S*)-naproxen(脂肪酶催化二次动力学拆分制备高光学纯度(*S*)-萘普生)[J]. *J Mol Catal* (*China*)(分子催化), 2015, **29**(1): 90-95.

[12] Wang Yan(王 艳), Xin Jia-ying(辛嘉英), Yu Jia-qi(于佳琪). One step esterification synergy resolution synthesis (*S*)-naproxen starch ester by lipase in solvent system(脂肪酶催化一步酯化协同拆分合成 *S*-萘普生淀粉酯)[J]. *J Mol Catal* (*China*)(分子催化), 2015, **29**(5): 476-481.

[13] Hua X, Xing Y, Zhang X. Enhanced promiscuity of lipase-inorganic nanocrystal composites in the epoxidation of fatty acids in organic media[J]. *ACS Appl Materinter*, 2016, **8**(25): 16257-16261.

[14] Lin B, Su H, Ma G, *et al*. Theoretical study of the hydrolysis mechanism of dihydrocoumarin catalyzed by serum paraoxonase 1 (PON1): Different roles of Glu53 and His115 for catalysis[J]. *Rsc Adv*, 2016, **6**(65): 60376-60384.

[15] Wu W B, Xu J M, Wu Q. Promiscuous acylases-catalyzed markovnikov addition of n-heterocycles to vinyl esters in organic media[J]. *Adv Synth Catal*, 2006, **348**(4/5): 487-492.

[16] Xie Z B, Wang N, Zhou L H, *et al*. Lipase-catalyzed stereoselective cross-aldol reaction promoted by water[J]. *ChemCatChem*, 2013, **5**(7): 1935-1940.

[17] Lou F W, Liu B K, Wu Q. Candida antarctica lipase B (CAL-B)-catalyzed carbon-sulfur bond addition and controllable selectivity in organic media[J]. *Adv Synth Catal*, 2008, **350**(13): 1959-1962.

[18] Guan Z, Fu J P, He Y H. Biocatalytic promiscuity: lipase-catalyzed asymmetric aldol reaction of heterocyclic ketones with aldehydes[J]. *Cheminform*, 2012, **53**(37): 4959-4961.

[19] Zhang X D, Gao N, Guan Z, *et al*. Enzyme-catalyzed asymmetric domino aza-Michael/aldol reaction for the synthesis of 1,2-dihydroquinolines using pepsin from porcine gastric mucosa[J]. *Chin Chem Lett*, 2016, **27**(6): 964-968.

[20] Jiang, Ling, Hongwei. Enzymatic promiscuity: Escherichia coli bioh esterase-catalysed aldol reaction and knoevenagel reaction[J]. *Chem Res Chinese U*, 2014, **30**(2): 289-292.

[21] Xu Jian-ming(许建明), Lin Xian-fu(林贤福). Catalytic promiscuity of enzyme and its new progress in organic synthesis(酶的催化多功能性及其在有机合成中的新

进展)[J]. *Chin J Org Chem*(有机化学), 2007, **27**(12): 1473-1478.

[22] Kumar A, Venkatesu P. Overview of the stability of α-chymotrypsin in different solvent media[J]. *Chem Rev*, 2012, **112**(7): 4283-4307.

[23] Liu Y, Liu R. The interaction of α-chymotrypsin with one persistent organic pollutant (dicofol): Spectroscope and molecular modeling identification[J]. *Food Chem Toxicol*, 2012, **50**(9): 3298-3305.

[24] Blow D M, Birktoft J J, Hartley B S. Role of a buried acid group in the mechanism of action of chymotrypsin [J]. *Nature*, 1969, **221**(5178): 337-340.

[25] Xie Z B, Sun D Z, Jiang G F, *et al*. Facile synthesis of bis(indolyl) methanes catalyzed by α-chymotrypsin[J]. *Molecules*, 2014, **19**(12): 19665-19677.

α-Chymotrypsin-catalyzed Synthesis of β-Uramino Crotonic Ester

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Abstract: β-Uramino crotonic esters are important intermediate for the synthesis of 6-methylpyrimidinedione derivatives and 3,4-dihydropyrimidinedione derivatives, and they have a wide range of applications in biopharmaceuticals. Enzymes have green, safe and highly efficient catalytic properties, so a series of β-uramino crotonic esters was synthesized via the α-chymotrypsin-catalyzed condensation reaction between 1,3-dicarbonyl compounds and ureas at 37 ℃ and in DMF medium.

Key words: α-chymotrypsin; β-uramino crotonic ester; biocatalysis; promiscuity