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One-Pot Synthesis of Spirooxindole Derivatives Catalyzed by Lipase in the Presence of Water

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Abstract: A facile one-pot synthesis route to spirooxindole derivatives was developed by combining the three types of catalytic activities of lipase from porcine pancreas (PPL) in the presence of water, i.e., the Knoevengel condensation, Michael addition and cyclization. PPL showed excellent catalytic activity and have a good adaptability to different substrates in the reaction. All the reactions go smoothly and provide spirooxindole derivatives with high yield under the mild conditions. This lipase-catalyzed multistep conversion method has provided a new strategy to synthesize spirooxindole derivatives and expanded the application of biocatalysts.

Keywords: enzymatic reactions; lipase catalysis; multistep conversions; one-pot synthesis; spirooxindole derivatives

Introduction

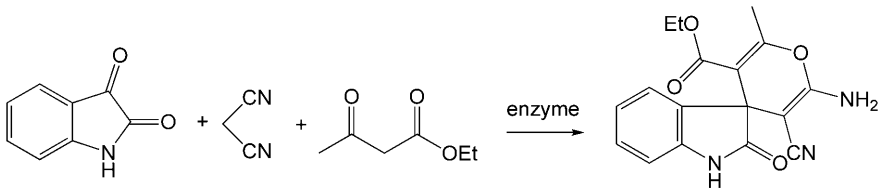
Spirooxindoles are commonly occurring heterocyclic ring systems and are found in many natural products and pharmaceuticals.[1] A range of compounds carrying the indole system exhibit antibacterial and antifungal activities.[2] The significant biological activity and recent synthetic advances of these natural products encouraged the development of biologically promising analogues that would be more efficacious and selective than the original natural products.[3] The privileged spirooxindole skeletons have high potential for the development of some medicinal agents.[4]

In recent years, numerous efficient transformations have been developed for the construction of these heterocyclic skeletons.[5] Carreira and co-workers have developed a reliable methodology to access the pyrrolidinyl-spirooxindole structure starting from imines and spirocyclopropyl oxindoles.[6] Williams accessed both enantiomers of the target molecule and prepared analogues of the natural products in the synthesis of spirotryprostatin B.[7] Gong and co-workers described an enantioselective organocatalytic approach for the rapid synthesis of spiro[pyrrolidin-3,3′-oxindole] derivatives in the presence of a chiral phosphoric acid.[8] Much attention has been forced on environmentally friendly biosynthesis in this field.

We have been interested in the study of enzymatic reactions and especially the enzyme-catalyzed synthesis of hetero-yclic compounds recently.[9] Several enzymes have fully been exhibited great advantages and potential in organic transformations such as aldol reaction, Michael addition, Diels–Alder reaction, Knoevenagel reaction, and Mannich reaction.[10–14] However, natural enzymes that are capable of catalyzing multiple reactions are very scarce, and only a few single-enzyme multistep conversions have been reported.[15] Here we report the unprecedented lipase (PPL)-catalyzed one-pot multiple reaction for synthesizing spirooxindole derivatives in the presence of water.

Results and Discussion

In initial research, we tested the reaction of isatin **1a**, malononitrile **2a**, and ethyl acetoacetate **3a** as model substrates with various additives in the presence of



Scheme 1. Model reaction.

water. Compounds **1a**, **2a**, and **3a** (0.5 mmol each) were added to a solution (0.5 mL water, 3 mL EtOH) containing 30 mg lipase from porcine pancreas (PPL) at 30 °C for 3 h. A single product was obtained in 92% isolated yield (Scheme 1), and the results are shown in Table 1.

A series of commercially available hydrolytic enzymes have been investigated in order to find the most suitable enzyme for the envisaged one-pot synthesis of spirooxindole derivatives (Table 1). It was found that only a trace of product was formed even after 12 h (entry 1, Table 1) without any additives. In contrast, we found that several enzymes displayed observable activities for this reaction, whereby, PPL displayed an especially significant role in catalytic activity (entries 6, 7, and 11, Table 1). When the reactions were incubated with denatured PPL, or some similar amino acid, no product was observed (entries 2–5, 10, and 12, Table 1). AL-AK showed moderate catalytic activity for this reaction (entry 11, Table 1). All the results suggest that the tertiary structure and the specific spatial conformation of PPL are responsible for the one-pot synthesis of spirooxindole derivatives reaction. So PPL was chosen as the catalyst for this reaction.

Table 1. Optimization of the reaction conditions.[a]

Entry	Catalyst	Amount [mg]	Temp. [°C]	Time [h]	Yield [%][b]
1	no enzyme	–	30	12	trace
2	CYS[c]	30	30	3	trace
3	ARG[c]	30	30	3	trace
4	LYS[c]	30	30	3	trace
5	ASN[c]	30	30	3	trace
6	AL-M[c]	30	30	3	82
7	AL-A[c]	30	30	3	84
8	AYL[c]	30	30	3	trace
9	AL-AK[c]	30	30	3	44
10	CAL[c]	30	30	3	trace
11	PPL[c]	30	30	3	92
12	PPL[d]	30	30	3	trace
13	PPL	10	30	3	58
14	PPL	20	30	3	78
15	PPL	40	30	3	94
16	PPL	30	20	3	57
17	PPL	30	40	3	91
18	PPL	30	50	3	93
19	PPL	30	30	1	43
20	PPL	30	30	2	81
21	PPL	30	30	4	93

[a] *Reaction conditions:* isatin (0.5 mmol), malononitrile (0.50 mmol), ethyl acetoacetate (0.5 mmol), enzyme, EtOH (3 mL), water (0.5 mL), 30 °C.

[b] Isolated yields.

[c] CYS: cysteine; ARG: arginine; LYS: lysine; ASN: asparagine; AL-M: Amano lipase M from *Mucor javanicus*; AL-A: Amano lipase A from *Aspergillus niger*; AYL: lipase AY30; AL-AK: Amano lipase AK from *Pseudomonas fluorescens*; CAL: lipase acrylic resin from *Candida antarctica*; PPL: lipase from porcine pancreas.

[d] PPL predenatured with urea at 100 °C for 10 h.

To optimize the reaction conditions, such as temperature, concentration of catalyst, and reaction time, some further experiments were performed. The results are shown in Table 1. It was found that with the temperature ranging from 20 °C to 50 °C, the best yield of the desired product was obtained at 40 °C (entries 11 and 16–18, Table 1). Therefore, 30 °C is the most suitable temperature for this reaction. When the amount of the PPL was increased from 10 mg to 20 mg, 30 mg, 40 mg, the corresponding yields were 58% to 78%, 92%, and 94%, respectively (entries 11 and 13–15, Table 1). The outcomes display that 30 mg PPL is sufficient to push this reaction forward. Larger amounts of the catalyst did not improve the yields. Beside, it can be easily seen from the Table 1 that this reaction was complete after 3 h (entries 11 and 19–21, Table 1).

The reaction medium has been recognized to be one of the most important factors influencing the enzymatic reaction. We screened the reaction in various organic solvents in the presence of water and the results are shown in Table 2. It can be obsserved that ethanol was the most efficient medium to promote this reaction, and the spirooxindole product was obtained in yield of 92%. Other solvents, such as *n*-hexane, CH_2Cl_2, $CHCl_3$, and acetone did not promote the product formation (entries 1–4 and 9) and other polar solvents (entries 6–8) led to moderate yields. The best yield of this reaction was obtained in ethanol as a solvent, which maybe attribute to the protin nature of the solvent and that it accelerates the reaction compared to other solvents.

Table 2. PPL-catalyzed the reaction of isatin (**1a**), malononitrile (**2a**), and ethyl acetoacetate (**3a**) in different solvents.[a]

Entry	Solvent	Time [h]	Yield [%][b]
1	*n*-hexane	3	trace
2	CH_2Cl_2	3	trace
3	$CHCl_3$	3	trace
4	acetone	3	trace
5	ethanol	3	92
6	THF	3	16
7	CH_3CN	3	23
8	DMF	3	31
9	water	3	trace

[a] *Reaction conditions:* isatin (0.5 mmol), malononitrile (0.5 mmol), ethyl acetoacetate (0.5 mmol), PPL (30 mg), solvent (3 mL), and water (0.5 mL), shaken at 160 rpm at 30 °C.

[b] Isolated yields.

In previous studies of enzymatic promiscuity, water has been considered as an important factor in enzymatic activity leading to an acceleration of the enzyme-catalyzed reaction.[16] Therefore, we designed some experiments to optimize the percentage of water in water/ethanol binary mixtures for this reaction. The effect of the water concentration on the considered lipase-catalyzed one-pot synthesis of spirooxindoles reaction is illustrated in Figure 1. As apparent, when the water content was between 10–20%, we got the desired product with the highest yields of more than 90%. However, once the water content surpassed 30%, the yield of spirooxindole product declined sharply due to the insolubility of the substrates. A 23% yield of spirooxindole product was obtained when using the pure solvent. All these results indicated obviously that water was essential in this promiscuous biocatalysis reaction.

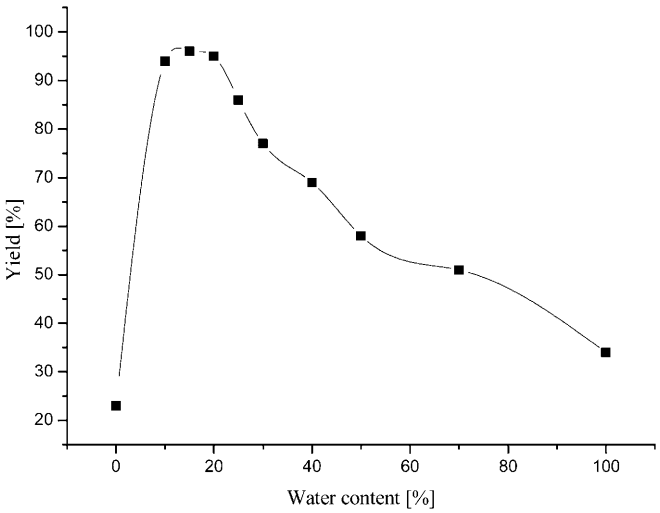


Figure 1. Influence of water on the PPL-catalyzed one-pot synthesis of spirooxindoles. *Reaction conditions:* isatin (0.5 mmol), malononitrile (0.5 mmol), ethyl acetoacetate (0.5 mmol), PPL (30 mg), ethanol (3 mL), and water (0.5 mL), was shaken at 160 rpm at 30 °C for 3 h; deionized water from 0% to 100% [water/ethanol, v/v]; product yield was determined by HPLC.

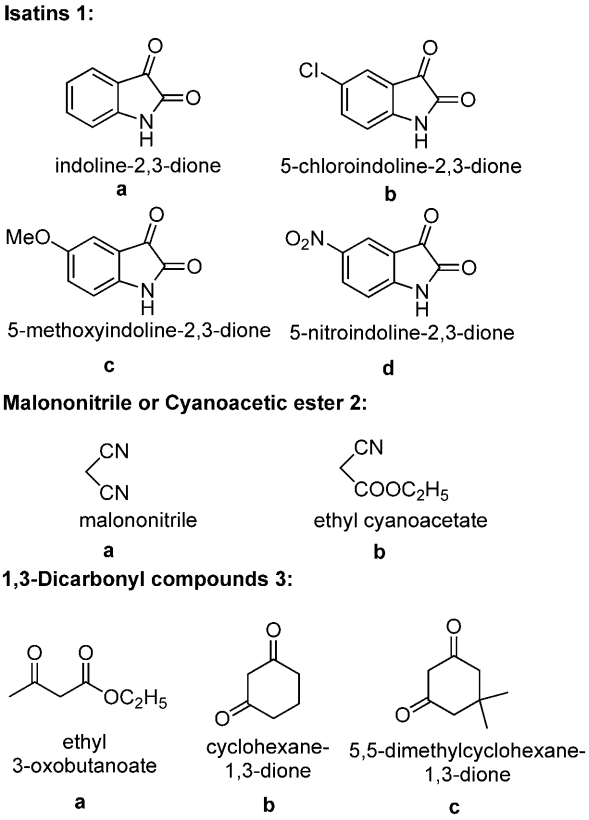
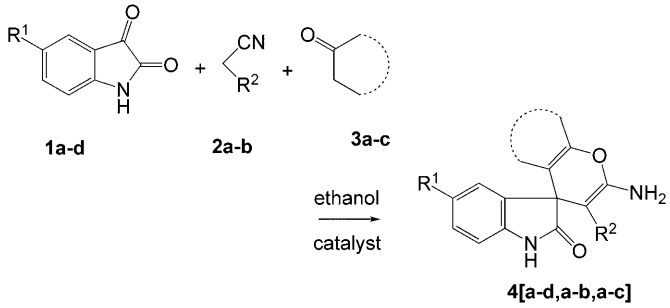


Figure 2. The structures of the different substrates.

With the optimal conditions in hand, we have employed various different isatins, 1,3-dicarbonyl compounds, and malononitrile or cyanoacetic ester as substrates in order to investigate the generality for the scope of this new catalystic method (Figure 2). The results are summarized in Table 3. Gratifyingly, it was found that nearly all of the corresponding products were obtained in good yields. However, the reaction with cyanoacetic ester offered a lower yield than that with malononitrile, which is probably due to the lower reactivity of cyanoacetic ester. Although many substrates and conditions were applied in this reaction to measure the enantioselectivity, there is no obvious optical activity and we will undertake related further research to improve it.

The experiments clearly showed an increased reaction rate when the synthesis of spirooxindole reaction was catalyzed by the PPL as compared to other enzymes. Based on the mechanism of lipase-catalyzed[11] carbon-carbon formation which has been widely accepted, we propose a tentative mechanism for the synthesis of spirooxindole derivative **4** as shown in

Table 3. Synthesis of different spirooxindole derivatives **4** catalyzed by PPL.



Entry	Products **4**	Yield [%][b]	mp [°C]
1	**4[a,a,a]**	92	260–262
2	**4[a,a,b]**	93	277–278
3	**4[a,a,c]**	94	284–285
4	**4[a,b,a]**	87	169–170
5	**4[a,b,b]**	85	280–283
6	**4[a,b,c]**	88	269–271
7	**4[b,a,a]**	93	263–265
8	**4[b,a,b]**	95	288–290
9	**4[b,a,c]**	95	286–288
10	**4[b,b,a]**	85	213–215
11	**4[b,b,b]**	83	270–272
12	**4[b,b,c]**	86	271–272
13	**4[c,a,a]**	93	230–233
14	**4[c,a,b]**	95	282–283
15	**4[c,a,c]**	93	287–289
16	**4[c,b,b]**	88	240–242
17	**4[c,b,c]**	82	246–248
18	**4[d,a,a]**	91	247–249
19	**4[d,a,b]**	94	>300
20	**4[d,a,c]**	95	>300

[a] *Reaction conditions:* isatins (1 mmol), malononitrile or cyanoacetic ester (1 mmol), 1,3-dicarbonyl compounds (1 mmol), PPL (30 mg), solvent (5 mL), and water (1 mL), shaken at 160 rpm at 30 °C.

[b] Isolated yields.

Scheme 2. Firstly, **1a** and **2a** form **7** quickly by Knoevenagel condensation, while the 1,3-dicarbonyl compound is pre-activated by the lipase. After the transfer of a proton, intermediate **7** is convered to **8**. Secondly, intermediate **6**, which is also pre-activated by the lipase, is attacked by **8**, which leads to a new C–C bond formation *via* Michael addition, and then **10** is obtained. Thirdly, after enolization and addition to the cyano group, the intermediate **12** is formed, and the desired product **4** is obtained *via* isomerization.

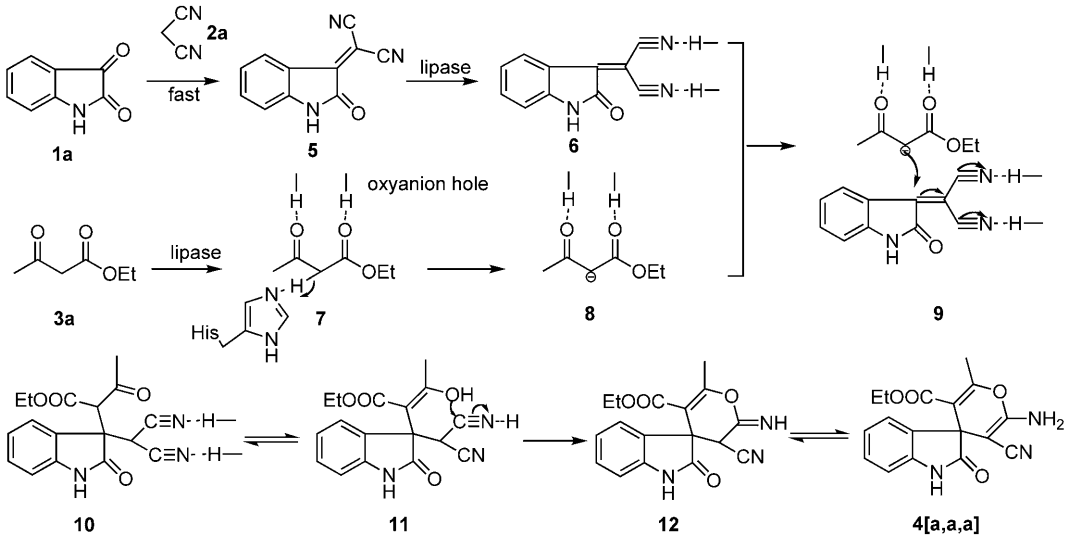
Conclusions

In conclusion, we herein report an unprecedented lipase (PPL)-catalyzed one-pot reaction for the synthesis of spirooxindole derivatives in the presence of water. This reaction can be carried out under mild conditions and covers a great range of substrates with excellent yields of spirooxindole products. This novel protocol provides an efficient, easy to separate, environmental friendly synthetic route for synthesis of spirooxindole derivatives, and further extends the application of enzymes in pharmaceutical industry.

Experimental Section

Materials and General Methods

Commercial reagents were used without further purification unless otherwise indicated. All solvents were distilled prior to use. Reactions were performed in oven-dried glassware. Analytical TLC was performed on Merck precoated TLC (silica gel 60 F254) plates. Melting points were recorded on an X4-Data microscopic melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet 380 FT-IR spectrophotometer using KBr discs. ESI-MS were ac-



Scheme 2. Proposed mechanism.

quired on a Bruker Esquire 3000 plus spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz and 100 MHz in DMSO-d_6 using TMS as internal standard. A C18 column was used in the HPLC experiments with MeOH/water = 85:15 (v/v), 0.8 mL min^{-1} and UV detection at 254 nm. All the compounds were characterized (IR, ^{1}H, ^{13}C NMR and massd spectroscopy). All enzymes were purchased from Acros, Alfa, Aldrich and TCI and used directly: Amano lipase M from *Mucor javanicus* (EC, 3.1.1.3), Amano lipase A from *Aspergillus niger* (EC 3.1.1.3), lipase AY30 (EC 3.1.1.3), Amano lipase AK from *Pseudomonas fluorescens* (EC 3.1.1.3), lipase acrylic resin from *Candida antarctica* (EC 3.1.1.3), lipase from porcine pancreas (EC 3.1.1.3).

General Procedure for Synthesis of Spirooxindole Derivatives

A suspension of isatin **1[a-d]** (1 mmol), malononitrile (or cyanoacetic ester) **2[a-b]** (1 mmol), 1,3-dicarbonyl compounds **3[a-c]** (1 mmol), and 30 mg PPL in 5 mL EtOH in the presence of water (1 mL) was shaken at 30 °C and 160 rpm for 3 h. After completion of the reaction as confirmed by TLC, the precipitated product was filtered and washed with water and cooled EtOH to afford the pure **4**.

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