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Biocatalytic one-pot three-component synthesis of 3,3'-disubstituted oxindoles and spirooxindole pyrans using α -amylase from hog pancreas†

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α -Amylase from hog pancreas displayed catalytic promiscuity in three-component reaction for the synthesis of 3,3'-disubstituted oxindoles and spirooxindole pyrans. The reactions between isatins, malononitrile and active methyl or active methylene compounds (acetone, nitromethane, indole, acetylacetone, 4-hydroxycoumarin and dimedone) offered corresponding products *via* Knoevenagel/Michael reactions or Knoevenagel/Michael/cyclization reactions in one pot with high to excellent yields of up to 98% under mild reaction conditions. The α -amylase showed a broad spectrum of adaptability to various substrates. A possible mechanism of the α -amylase catalyzed three-component reaction was proposed.

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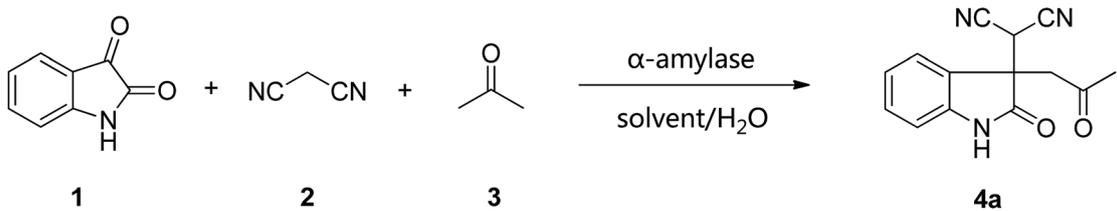
Introduction

As a green, economically feasible, environmentally friendly, mild, and burgeoning approach in organic chemistry, biocatalysis demonstrates a powerful potential in organic synthesis.¹ Enzymes, as typical representatives of biocatalysts, are widely employed in organic synthesis as green catalysts and have received more and more attention. However, there are limited kinds of enzymes in nature and only some of them can be available commercially.² Thus, it is crucial to expand the application scope of existing enzymes. Enzymatic promiscuity, which means not only natural substrates but non-natural substrates can be catalyzed by the single active site of a given enzyme, has been investigated in the past decade,^{3–7} having greatly enriched the reaction types of enzyme catalysis. Some elegant works on the enzyme catalytic promiscuity have been described.^{8,9} For example, a number of hydrolases were found to have the ability to catalyze aldol,^{1,2,10–15} Michael,^{16–21} Henry,^{22–24} Mannich²⁵ and Knoevenagel^{26–28} reactions, and even some domino reactions.²⁹ Nevertheless, promiscuous enzyme catalyzed multicomponent reactions are still rather rare although there are a few examples reported.^{30–32} Hence, in order to expand the application of enzyme in multicomponent reactions, and to further understand enzymatic promiscuity, it is still a formidable and significant task to discover enzyme-catalyzed multicomponent reactions.

The oxindoles are the core structural motifs which are present in many natural products and biologically active compounds.^{33–37} A large number of compounds based on the structure of indoles show pharmaceutical activity and bioactivity.^{38–40} 3,3'-Disubstituted oxindoles and spirooxindoles containing a quaternary carbon center and diverse functional groups are the powerful synthetic intermediates which provide a shortcut to synthesize complex and potentially biologically active compounds.⁴⁰ Because of those promising advantages, more and more chemists try to achieve the target of developing green and easy methods to synthesize 3,3'-disubstituted oxindole and spirooxindole frameworks.^{41–50} Spirooxindole pyrans as a type of heterocyclic spirooxindoles have attracted considerable attention, and some synthetic protocols have been reported for the construction of these compounds. For example, the Michael/cyclization reactions of isatylidenemalononitriles with α -keto esters or 1,3-dicarbonyl compounds, or three-component Knoevenagel/Michael/cyclization reactions of isatins, malononitrile (cyanoacetic ester) and 1,3-dicarbonyl compounds were conveniently employed to synthesize spirooxindole pyrans. The rosin-derived tertiary amine–thiourea⁴⁶ and cupreine⁴⁸ were used as catalysts to provide spirooxindole pyrans. L-proline⁵¹ as well as an enzyme, lipase from porcine pancreas (PPL),⁴⁷ were also applied to catalyze this type of reactions, in which spirooxindole pyrans were prepared as racemates. Moreover, some methods have been developed for the enantioselective construction of 3,3'-disubstituted oxindoles *via* the Michael reaction of ketones to isatylidenemalononitriles catalyzed by a quinidine derived primary amine with (*R*)-binol-phosphoric acid as a co-catalyst,⁵² or a cinchona-based chiral primary amine with L-camphorsulfonic acid as a co-catalyst.⁵³ These 3,3'-disubstituted oxindoles could be further converted to spirooxindole dihydropyrans by reduction/cyclization reactions.

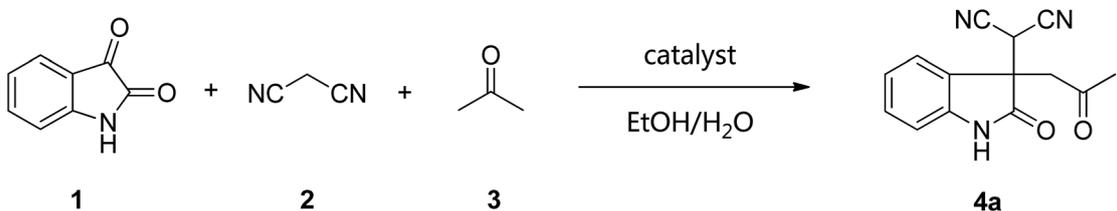
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† Electronic supplementary information (ESI) available. CCDC 1053771 and 1053772. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4ra16825a

Table 1 Solvent screening^a


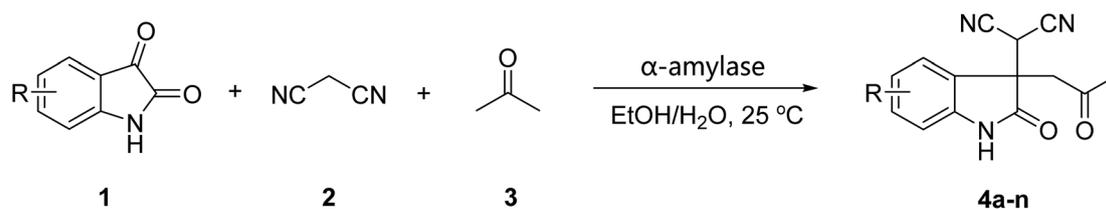
Entry	Solvent	Time [d]	Yield ^b [%]
1	Ethanol	2	55
2	DMF	2	54
3	DMSO	2	53
4	Methanol	2	53
5	1,4-Dioxane	2	52
6	Acetonitrile	4	43
7	THF	4	29
8	Methyl <i>tert</i> -butyl ether	4	27
9	Chloroform	4	24
10	Ethyl acetate	4	21
11	Butyl acetate	4	18
12	1,2-Dichloroethane	4	18
13	Toluene	4	8

^a Reaction conditions: isatin (0.25 mmol), malononitrile (0.25 mmol), acetone (2.5 mmol), α -amylase (738 U), solvent (0.90 mL), and deionized water (0.10 mL) at 25 °C. ^b Yield of the isolated product.

Table 2 Control experiments^a


Entry	Catalyst	Time [d]	Yield ^b [%]	Natural activity ^c [U mg ⁻¹]
1	α -Amylase	2	55	24.6
2	None	4	Trace	—
3	α -Amylase (pretreated with urea) ^d	3	21	4.9
4	Urea (200 mg)	3	No reaction	—
5	α -Amylase (pretreated with 250 mM Cu ²⁺) ^e	3	Trace	1.1
6	CuSO ₄ (40 mg)	3	No reaction	—
7	α -Amylase (pretreated with 250 mM Ag ⁺) ^f	3	Trace	1.1
8	AgNO ₃ (42 mg)	3	No reaction	—
9	α -Amylase (pretreated with miglitol) ^g	3	33	18.0

^a Unless otherwise noted, the reaction conditions: isatin (0.25 mmol), malononitrile (0.25 mmol), acetone (2.5 mmol), α -amylase (738 U), ethanol (0.90 mL), and deionized water (0.10 mL) at 25 °C. ^b Yield of the isolated product. ^c Unit definition (U mg⁻¹): one unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C. ^d α -Amylase (738 U) in urea solution (3.3 M) [urea (200 mg) in deionized water (1 mL)] was stirred at 25 °C for 24 h, and then water was removed under reduced pressure before use. ^e α -Amylase (738 U) in Cu²⁺ solution (250 mM) [CuSO₄ (40 mg) in deionized water (1 mL)] was stirred at 25 °C for 24 h, and then water was removed under reduced pressure before use. ^f α -Amylase (738 U) in Ag⁺ solution (250 mM) [AgNO₃ (42 mg) in deionized water (1 mL)] was stirred at 25 °C for 24 h, and then water was removed under reduced pressure before use. ^g α -Amylase (738 U) in miglitol solution [miglitol (50 mg) in deionized water (1 mL)] was stirred at 25 °C for 24 h, and then water was removed under reduced pressure before use.

Table 3 Substrate scope for the synthesis of 3,3'-disubstituted oxindoles using acetone^a

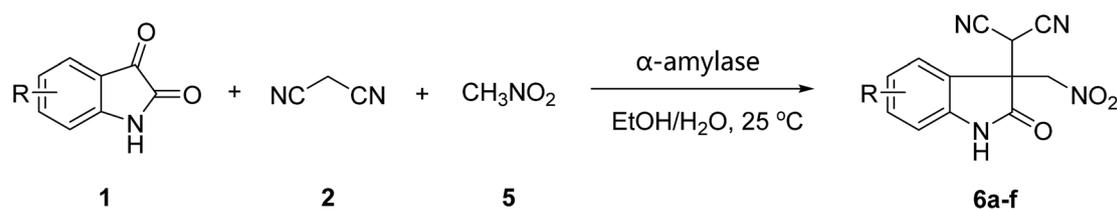
Entry	R	Product	Time [d]	Yield ^b [%]
1	1-H	4a	2	74
2	1-Me	4b	2	54
3	4-Cl	4c	3	58
4	4-Br	4d	3	43
5	5-F	4e	2	78
6	5-Cl	4f	1	81
7	5-I	4g	2	72
8	5-NO ₂	4h	2	95
9	5-CF ₃ O	4i	3	95
10	5-Me	4j	2	89
11	5-MeO	4k	3	62
12	6-Br	4l	2	91
13	7-Cl	4m	1	77
14	4,6-2Br	4n	2	60

^a Reaction conditions: isatin (0.25 mmol), malononitrile (0.25 mmol), acetone (7.5 mmol), α -amylase (246 U), ethanol (0.90 mL), and deionized water (0.10 mL) at 25 °C. ^b Yield of the isolated product.

Herein we report a one-pot three-component reaction between isatin, malononitrile and active methyl or active methylene compounds (acetone, nitromethane, indole, acetylacetone, 4-hydroxycoumarin and dimedone) for the synthesis of 3,3'-disubstituted oxindoles and spirooxindole pyrans *via* Knoevenagel/Michael reactions or Knoevenagel/Michael/cyclization reactions using α -amylase from hog pancreas as a catalyst.

Results and discussion

Initially, the three-component reaction of isatin, malononitrile and acetone was used as a model reaction to investigate the optimal conditions for the α -amylase catalyzed synthesis of 3,3'-disubstituted oxindole (4a). Since reaction medium is one of the most important factors influencing enzymatic reactions,

Table 4 Substrate scope for the synthesis of 3,3'-disubstituted oxindoles using nitromethane^a

Entry	R	Product	Time [d]	Yield ^b [%]
1	1-Me	6a	2	91
2	5-F	6b	4	90
3	5-CF ₃ O	6c	4	88
4	5-Me	6d	3	96
5	6-Br	6e	3	56
6	7-Cl	6f	3	89

^a Reaction conditions: isatin (0.25 mmol), malononitrile (0.25 mmol), nitromethane (7.5 mmol), α -amylase (246 U), ethanol (0.90 mL), and deionized water (0.10 mL) at 25 °C. ^b Yield of the isolated product.

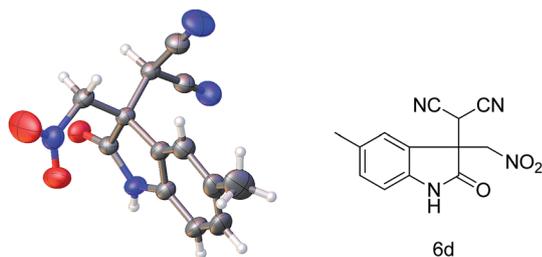


Fig. 1 X-ray crystal structure of 6d.

different solvents were investigated to obtain the suitable medium for this reaction (Table 1). Generally, the α -amylase-catalyzed reaction in polar solvents (such as ethanol, methanol, DMF, DMSO, 1,4-dioxane and acetonitrile) gave the higher yields and the reaction proceeded faster (Table 1, entries 1–6) than in the non- or low-polar solvents (such as methyl *tert*-butyl ether, chloroform, ethyl acetate, butyl acetate, 1,2-dichloroethane, toluene) (Table 1, entries 8–13). Based on the results we obtained, ethanol was thought to be the best solvent for this reaction, through a combination of environment protection, ability to dissolve a large number of substrates and commercial price.

Next, a series of control experiments to confirm the specific catalytic effect of α -amylase were carried out. The reaction with α -amylase gave the product in a yield of 55% after 2 days (Table 2, entry 1). In the absence of α -amylase, only a trace amount of product was observed even after 4 days (Table 2, entry 2). The urea pretreated α -amylase still promoted the reaction but the yield was much lower, and only 21% yield was received after 3 days (Table 2, entry 3). Meantime, to compare the natural activity with the non-natural activity of the α -amylase, the enzymatic assay of α -amylase in natural reaction (hydrolyzing starch) was also conducted. It showed that the urea pretreated

α -amylase lost its most natural activity (the activity reduced from 24.6 U mg⁻¹ to 4.9 U mg⁻¹) (Table 2, entry 3). It indicated that the urea treatment caused a great degree of denaturation of the enzyme, which accordingly decreased its catalytic ability in the unnatural reaction. The blank experiment suggested that urea alone did not have any catalytic effect on the reaction (Table 2, entry 4). Moreover, metal ions Cu²⁺ and Ag⁺ were used as denaturation agents to pretreat the α -amylase, respectively, and the reaction with the metal ion pretreated enzyme only gave a trace amount of product detected on TLC (Table 2, entries 5 and 7). The enzymatic assay of α -amylase in starch hydrolyzing reaction showed that the metal ion nearly completely disrupted natural activity of the enzyme (Table 2, entries 5 and 7). The product failed to form when using Cu²⁺ or Ag⁺ alone as catalyst (Table 2, entries 6 and 8). The above control experiments with denaturation agents, urea and metal ions, pretreated enzyme suggested that the native fold of the enzyme was not only responsible for its natural activity but also for its unnatural activity. In addition, miglitol as a specific competitive inhibitor of amylase was also used to pretreat the enzyme. The reaction with miglitol pretreated α -amylase only gave the product in a low yield of 33% after 3 days (Table 2, entry 9), suggesting that the active site of the enzyme has close relation with the promiscuous reaction. Meanwhile, the activity of α -amylase in starch hydrolyzing reaction was also obviously decreased after miglitol treatment (the activity reduced from 24.6 U mg⁻¹ to 18.0 U mg⁻¹) (Table 2, entry 9). The above results indicated that the α -amylase promoted the observed three-component reaction, and similar to the natural reaction, the unnatural reaction may also take place on the active site of the enzyme.

Next, the effect of various parameters (mole ratio of substrates, reaction temperature, enzyme loading, water content in the system, and reaction time) on the α -amylase catalyzed model three-component reaction was investigated (for details, please see the ESI†). The optimized reaction conditions

Table 5 Substrate scope for the synthesis of 3,3'-disubstituted oxindoles using indole^a

Entry	R	Product	Time [d]	Yield ^b [%]
1	1-H	8a	5	90
2	5-I	8b	5	83
3	5-NO ₂	8c	5	97
4	5-CF ₃ O	8d	5	92
5	7-Cl	8e	5	95

^a Reaction conditions: isatin (0.25 mmol), malononitrile (0.25 mmol), indole (0.38 mmol), α -amylase (246 U), ethanol (0.90 mL), and deionized water (0.10 mL) at 25 °C. ^b Yield of the isolated product.

were found to consist of the following: mole ratio of substrates (isatin: malononitrile: acetone) of 1 : 1 : 30, temperature of 25 °C, enzyme loading of 246 U, water content in the system of 10% [water/(water + ethanol), in vol] (%).

To verify the generality of the α -amylase catalyzed three-component reaction for the synthesis of 3,3'-disubstituted oxindoles, various substituted isatins were used as substrates to carry out the reaction. Basically, a wide range of substituted isatins could effectively participate in the reaction with malononitrile and acetone to give the corresponding products (Table 3). The product yields ranged from moderate to high depending on the position and electronic property of substituents. 1-Methyl isatin was also able to be used as a substrate, giving the desired product in a yield of 54% (Table 3, entry 2). Isatins with 4-substituents showed low reactivity probably attributed to the steric effects (Table 3, entries 3 and 4). The electronic feature of 5-substituents on isatin had some effect on the yield. The reactions using isatins with an electron-withdrawing group in 5-position gave the products in good yields up to 95% (Table 3, entry 8). On the contrary, the isatin with an electron-donating group in 5-position provided product in a lower yield (Table 3, entry 11). The 6- or 7-substituted isatins performed well in the reaction giving products in good yields (Table 3, entries 12 and 13). In addition, a 4,6-disubstituted isatin was also used for the reaction, and a moderated yield was obtained (Table 3, entry 14). Unfortunately, there was no enantiomeric excess of the products observed by the chiral HPLC analysis.

When nitromethane was used to replace the role of acetone, the reaction could proceed smoothly to afford the desired products in satisfied yields (Table 4). Generally, the reactions with nitromethane gave higher yields than those with acetone.



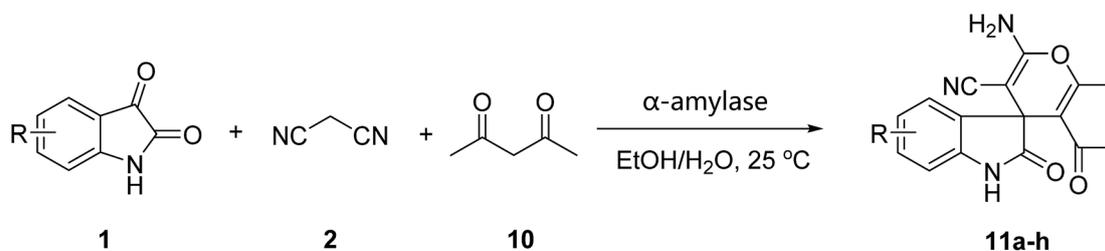
Scheme 1 Conversion of 3,3'-disubstituted oxindoles (4) to spirooxindole dihydropyrans (9).

Isatin bearing either electron-withdrawing or electron-donating groups performed very well except 6-Br substituted isatin. Among the tested reactions, the best yield of 96% was obtained (Table 4, entry 4). Unfortunately, there was no enantiomeric excess of the products observed by the chiral HPLC analysis. To the best of our knowledge, so far the products listed in Table 4 have not been reported yet; the products **6a-f** were all new compounds. To confirm the structure, product **6d** as a representative example was determined by single-crystal X-ray analysis (Fig. 1).

Indole could be used as a substrate to replace acetone in this α -amylase catalyzed three-component reaction (Table 5). The corresponding 3,3'-disubstituted oxindoles were synthesized *via* Knoevenagel reaction between isatin and malononitrile followed by Friedel-Crafts alkylation of indole in one pot. Excellent yields up to 97% were obtained with various substituted isatins (Table 5). To the best of our knowledge, α -amylase catalyzed Friedel-Crafts alkylation has not been reported yet. The products **8a-e** were all new compounds. No enantiomeric excess of the products observed by the chiral HPLC analysis.

As described above, α -amylase catalyzed three-component reactions of various isatins, malononitrile and the compounds

Table 6 Substrate scope for the synthesis of spirooxindole pyrans using acetylacetone^a



Entry	R	Product	Time [d]	Yield ^b [%]
1	1-H	11a	2	72
2	1-Me	11b	2	61
3	5-NO ₂	11c	4	50
4	5-Cl	11d	2	74
5	5-I	11e	4	80
6	5-Me	11f	3	83
7	5-MeO	11g	2	84
8	7-Cl	11h	2	75

^a Reaction conditions: isatin (0.25 mmol), malononitrile (0.25 mmol), acetylacetone (7.5 mmol), α -amylase (246 U), ethanol (0.90 mL), and deionized water (0.10 mL) at 25 °C. ^b Yield of the isolated product.

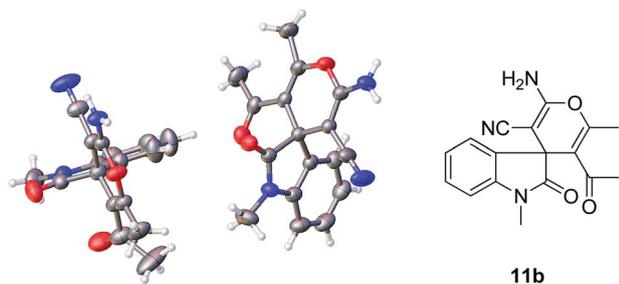


Fig. 2 X-ray crystal structure of **11b**.

containing an active methyl or methylene group provided a series of multi-functionalized 3,3'-disubstituted oxindoles. Among them, the products **4a–n** could be easily converted to spirooxindole dihydropyrans (**9**) by reduction/cyclization reactions according to the literature⁵⁴ (Scheme 1).

Moreover, several compounds which exist in the form of enols such as acetylacetone, 4-hydroxycoumarin and dimedone were used to prepare spirooxindole pyrans. When acetylacetone was used as a substrate to replace acetone in this α -amylase catalyzed three-component reaction, highly functionalized spirooxindole pyrans were prepared. The reaction with various substituted isatins proceeded smoothly to form the products in yields ranged from 50% to 84% (Table 6). The electronic feature of substituents on the 5-position of isatin had some effect on the yield. The isatins with electron-withdrawing group on the 5-position afforded lower yields than those with electron-donating group on the 5-position (Table 6, entries 3–7). There was no enantiomeric excess of the products observed by the

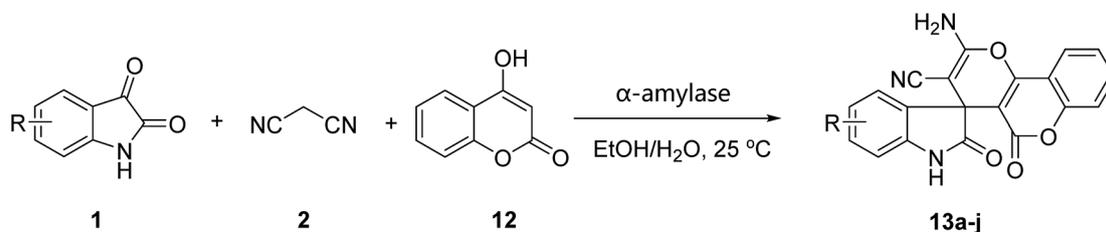
chiral HPLC analysis. To confirm the structure of the spirooxindole pyrans, product **11b** as a representative example was determined by single-crystal X-ray analysis (Fig. 2).

4-Hydroxycoumarin could be used as a substrate in the α -amylase catalyzed three-component reaction to react with malononitrile and different substituted isatins (Table 7). In general, the presence of substituents with different electronic and steric properties in various positions of isatin did not have a significant effect on the formation of spirooxindole pyrans. All provided good to excellent yields, however no enantiomeric excess was observed by chiral HPLC analysis. The reactions with 4-hydroxycoumarin were faster than those with acetylacetone probably because the carbonyl of 4-hydroxycoumarin can exist in the form of an enol due to conjugation with the benzene ring while the acetylacetone does not fully exist in the form of an enol.

Another active methylene compound dimedone was also used to explore the scope of the α -amylase catalyzed three-component reaction for the synthesis of spirooxindole pyrans (Table 8). To our surprise, all the reactions were completed within 24 hours. The reactions with all the tested isatins, except 1-Me substituted isatin (Table 8, entry 2), gave products in good to excellent yields efficiently. However, no enantiomeric excess was observed by chiral HPLC analysis.

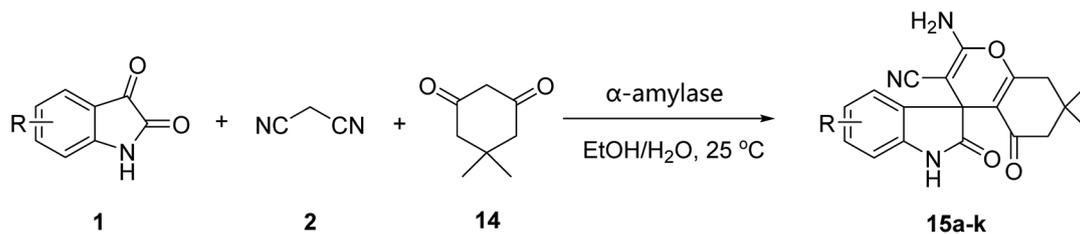
Finally, in order to explore the mechanism of α -amylase catalyzed three-component reaction, some comparison experiments were conducted (Scheme 2). To verify if the first-step Knoevenagel reaction is spontaneous or not, the reaction of isatin and malononitrile was carried out without catalyst, which gave the Knoevenagel adduct (**16**) in 95% isolated yield after 5 minutes. It showed that the reaction between isatin and

Table 7 Substrate scope for the synthesis of spirooxindole pyrans using 4-hydroxycoumarin^a



Entry	R	Product	Time [d]	Yield ^b [%]
1	1-H	13a	2	55
2	1-Me	13b	2	88
3	5-NO ₂	13c	2	80
4	5-F	13d	2	96
5	5-Cl	13e	1	80
6	5-I	13f	2	93
7	5-Me	13g	2	77
8	5-MeO	13h	2	90
9	6-Br	13i	2	90
10	7-Cl	13j	2	86

^a Reaction conditions: isatin (0.25 mmol), malononitrile (0.25 mmol), 4-hydroxycoumarin (0.25 mmol), α -amylase (246 U), ethanol (0.90 mL), and deionized water (0.10 mL) at 25 °C. ^b Yield of the isolated product.

Table 8 Substrate scope for the synthesis of spirooxindole pyrans using dimedone^a

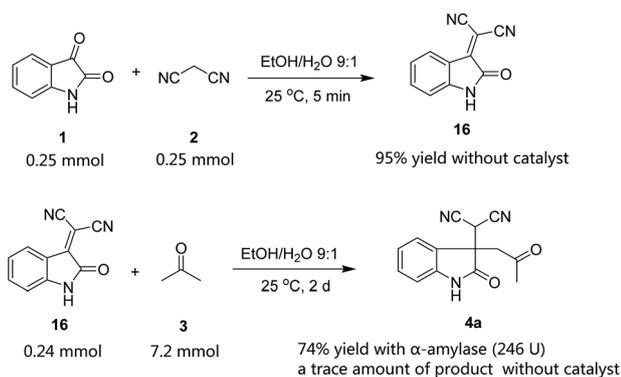
Entry	R	Product	Time [h]	Yield ^b [%]
1	1-H	15a	3	98
2	1-Me	15b	3	47
3	4-Cl	15c	18	63
4	5-NO ₂	15d	18	82
5	5-F	15e	3	92
6	5-Cl	15f	18	88
7	5-I	15g	3	81
8	5-Me	15h	3	75
9	5-MeO	15i	3	85
10	6-Br	15j	3	76
11	7-Cl	15k	18	87

^a Reaction conditions: isatin (0.25 mmol), malononitrile (0.25 mmol), dimedone (0.25 mmol), α -amylase (246 U), ethanol (0.90 mL), and deionized water (0.10 mL) at 25 °C. ^b Yield of the isolated product.

malononitrile spontaneously form the Knoevenagel adduct (**16**). Then, the Michael addition of intermediate (**16**) with acetone was conducted for 2 days. In the absence of catalyst, the reaction only gave a trace amount of product **4a**, but in the presence of α -amylase, the reaction gave product **4a** in a good yield of 74%. The results indicated that α -amylase catalyzed the Michael addition between Knoevenagel adduct (**16**) and acetone.

According to the literature,^{55–58} α -amylase from hog pancreas contains 496 amino acid residues. The aspartic acid and glutamic acid, as catalytic amino acid residues in the active site, are located in 197 and 233 respectively. For the natural activity of the α -amylase, the Asp197 acts as a nucleophile and the Glu233 acts as a proton donor. Moreover, from the results of the control experiments and the comparison of natural activity with the unnatural reaction above, it could be inferred that similar to the natural reaction, the observed unnatural reaction may also took

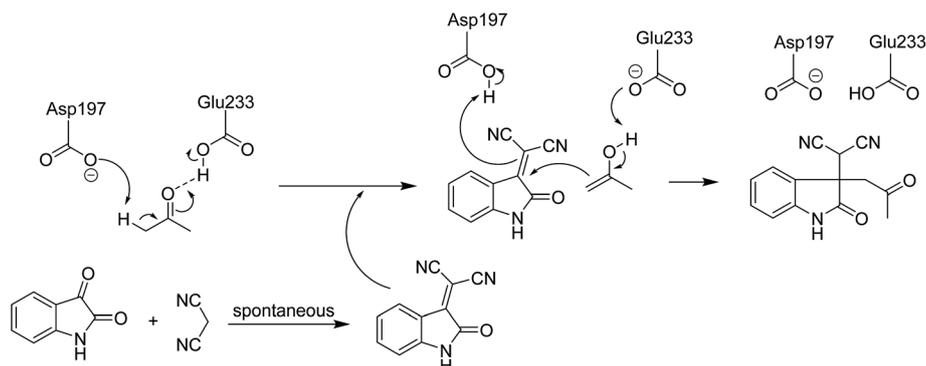
place on the active site of the enzyme. Thus, we tried to speculate the mechanism of α -amylase catalyzed three-component reaction. The proposed mechanism for the synthesis of 3,3'-disubstituted oxindoles was demonstrated using the reaction of isatin, malononitrile and acetone (Scheme 3). Firstly, Asp197 in the α -amylase acts as a base to take off an acid proton from the acetone, and Glu233 provides a proton to the carbonyl of acetone to form the enol. Secondly, the enol reacts with the spontaneously formed Knoevenagel adduct *via* a Michael addition with the assistance of Asp197 and Glu233 affording the 3,3'-disubstituted oxindole. The proposed mechanism for the synthesis of spirooxindole pyrans was demonstrated using the reaction of isatin, malononitrile and acetylacetone (Scheme 4). It includes the enolization of acetylacetone, the Michael addition of the enol to the Knoevenagel adduct, the cyclization and tautomerization.



Scheme 2 Comparison experiments.

Conclusion

α -Amylase from hog pancreas was found to display catalytic promiscuity in three-component reaction for the synthesis of 3,3'-disubstituted oxindoles and spirooxindole pyrans in ethanol/water mixed solvents. The reaction conditions including solvent, molar ratio of substrates, temperature, enzyme loading, water content and reaction time were investigated. The reactions between isatin, malononitrile and active methyl or active methylene compounds (acetone, nitromethane, indole, acetylacetone, 4-hydroxycoumarin and dimedone) offered corresponding products *via* Knoevenagel/Michael reactions or Knoevenagel/Michael/cyclization reactions in one pot with high to excellent yields of up to 98%



Scheme 3 The speculated mechanism for the synthesis of 3,3'-disubstituted oxindole.

under mild reaction conditions. The α -amylase showed a broad spectrum of adaptability to various substrates. Some control experiments were performed and the comparison of natural activity with the unnatural reaction was conducted, suggesting this unnatural reaction may take place on the active site of the α -amylase. A possible mechanism of the α -amylase catalyzed three-component reaction was speculated.

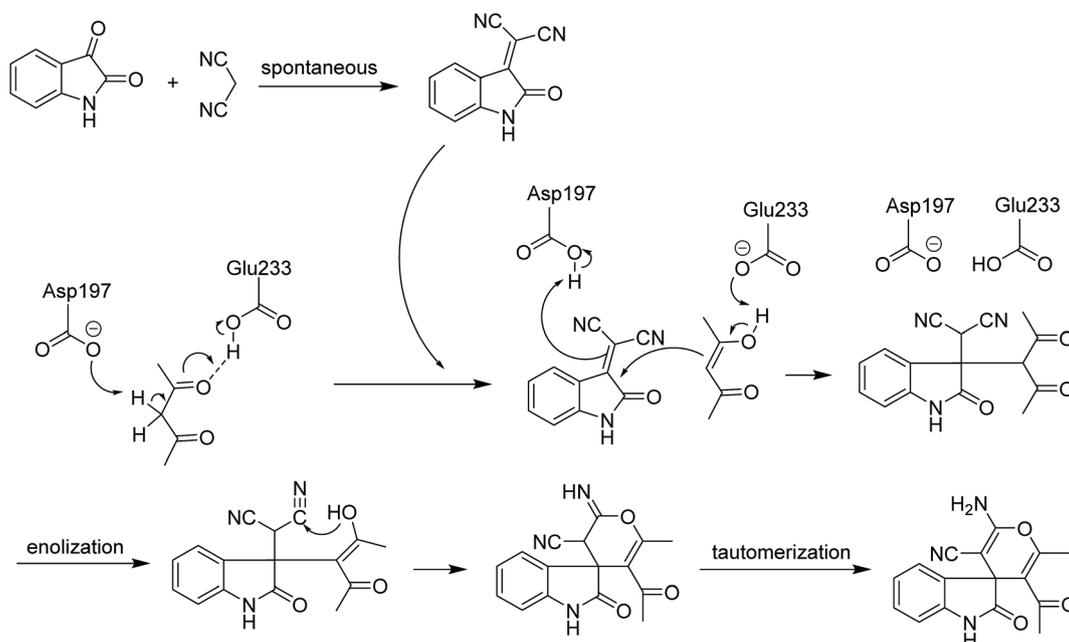
General method for the α -amylase catalyzed synthesis of 3,3'-disubstituted oxindoles (products 4a–n, 6a–f and 8a–e)

To a mixture of isatin (0.25 mmol), malononitrile (0.25 mmol), α -amylase (246 U), acetone (or nitromethane) (7.5 mmol) [or indole (0.38 mmol)] and deionized water (0.10 mL) was added ethanol (0.90 mL). The resultant mixture was stirred for the specified time

at 25 °C, and monitored by thin-layer chromatography. The reaction was terminated by filtering off the enzyme. Ethyl acetate was employed to wash the residue on the filter paper to ensure that the products were all dissolved in the filtrate. The filtrate was dried over anhydrous Na_2SO_4 , and the organic solvents were then removed under reduced pressure. The crude products were purified by silica gel column chromatography with petroleum ether/ethyl acetate as the eluent.

General method for the α -amylase catalyzed synthesis of spirooxindole pyrans (products 11a–h, 13a–j and 15a–k)

To a mixture of isatin (0.25 mmol), malononitrile (0.25 mmol), α -amylase (246 U), acetylacetone (7.5 mmol) [or 4-hydroxyl-coumarin (0.25 mmol) or dimedone (0.25 mmol)] and deionized



Scheme 4 The speculated mechanism for the synthesis of spirooxindole pyran.

water (0.10 mL) was added ethanol (0.90 mL). The resultant mixture was stirred for the specified time at 25 °C, and monitored by thin-layer chromatography. After completion of the reaction the precipitated product was filtered and washed with water (5 mL × 4) and cooled EtOH (5 mL × 2) to afford the pure products.

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