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Efficient Synthesis of Pyrrole Disulfides Catalyzed by Lipase in Ethanol

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Abstract: Disulfides, as fundamental scaffolds, are widely present in peptides, natural products, and pharmaceutical molecules. However, traditional synthesis of disulfides often involves the utilization of toxic reagents or environmentally unfriendly reaction conditions. In this work, a green and efficient method was developed for synthesizing pyrrole disulfides using β-ketothioamides and ethyl cyanoacetate as substrates, with lipase serving as a catalyst. Under the optimal conditions (β-Ketothioamides (1 mmol), ethyl cyanoacetate (1 mmol), PPL (200 U), and EtOH (5 mL)), lipase leads to the formation of pyrrole disulfides in yields of up to 88% at 40 °C. The related mechanism is also speculated in this paper. This approach not only presents a new application of lipase in enzyme catalytic promiscuity, but also offers a significant advancement in the synthetic pathway for pyrrole disulfides and aligns with the current mainstream research direction of green chemistry, contributing to the further development of environmentally friendly biocatalytic processes.

Keywords: lipase; catalytic promiscuity; pyrrole disulfide; β-ketothioamides; cyanoacetate

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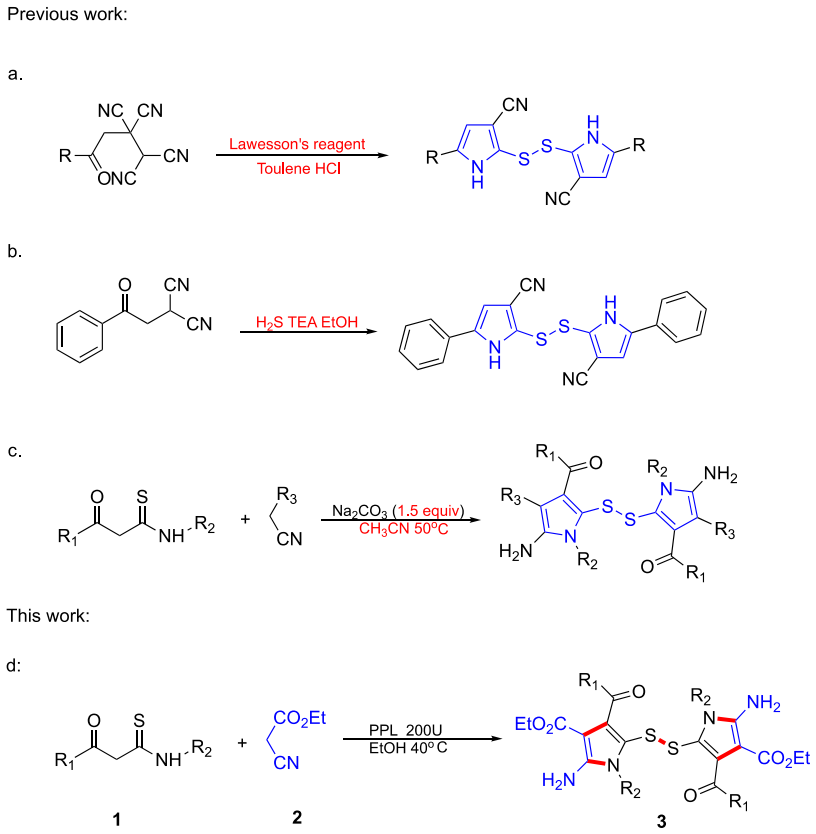
1. Introduction

β-Ketothioamides (KTAs) are versatile intermediates in organic synthesis possessing carbonyl and thioamide functional groups. These functionalities make them intriguing and valuable for various synthesis transformations. On the one hand, the carbonyl group in KTAs renders them susceptible to nucleophilic attack, allowing them to act as substrates for nucleophilic addition and leading to the formation of many heterocyclic compounds [1]. On the other hand, the thioamide functional group in KTAs undergoes isomerization to generate thiol [2], allowing KTAs to participate in synthesis. Multiple heterocycles, such as thiazoles [3,4], piperidines [5], pyran [6] and fused heterocycles [7,8], have been efficiently constructed. Such heterocycles form the core structures of numerous bioactive molecules and pharmaceutical formulations [9,10].

Pyrrole disulfides represent a fusion between pyrrole (a pentagonal heterocyclic organic compound) and disulfide functional groups and have relevance across various disciplines, such as organic synthesis [11], material science [12,13], and biochemistry [14,15]. The typical synthesis of pyrrole disulfides often involves the use of malodorous and toxic hydrogen sulfide gas or environmentally unfriendly reaction conditions. In 2020, Hussein's group reported a method to synthesize an unreported pyrrole disulfide using Lawesson's reagent and tetracyanide at 40 °C, achieving a yield of over 92% (Scheme 1a) [16]. In 2022, El-Remaily and co-workers synthesized pyrrole disulfides by reacting hydrogen sulfide with 2-(2-oxo-2-phenylethyl) malononitrile in ethanol (Scheme 1b) [17]. In the same year, Li et al. designed an environmentally friendly synthesis method using KTAs

and ethyl cyanoacetates as substrates, sodium carbonate as the base (1.5 equiv.), and acetonitrile as the reaction solvent to generate the target product via an umpolung reaction (Scheme 1c) [18]. Despite these advancements, the synthesis routes for pyrrole disulfides are yet to be fully developed. Additional sustainable and environmentally benign strategies need to be devised.



Scheme 1. Previous works and lipase-catalyzed synthesis of pyrrole disulfides (Reprinted/adapted with permission from Refs. [16–18]). (**a**): Hussein's work, Modather F. Hussein, 2020 [16], (**b**): El-Remaily's work, Mahmoud Abd El Aleem Ali Ali El-Remaily [17], 2022, (**c**): Li's work, Ming Li, 2022 [18]).

Enzymes are large biological molecules capable of catalyzing chemical reactions within living organisms. Their presence and activity ensure the normal functioning of life. Enzymes display a high degree of specificity toward the reactions they catalyze, a characteristic termed as "specificity". However, when studied outside their natural context, some enzymes can catalyze a variety of different chemical reactions or act on multiple substrates, such as hemoproteins [19,20], Baeyer–Villiger monooxygenase [21], transaminases [22] and laccase [23]. This phenomenon is referred to as "catalytic promiscuity". The ability of enzymes to catalyze unnatural reactions is undeniably a substantial discovery for the advancement of organic catalytic synthesis. Lipases are a class of enzymes ubiquitously found in nature with a vast reservoir of resources and types, making them one of the most extensively studied hydrolases. Their broad substrate compatibility, high stability under extreme conditions, excellent enantioselectivity, and environmentally benign characteristics have garnered widespread attention from scholars. In addition to catalyzing natural reactions such as hydrolysis, lipases possess exceptional abilities to catalyze nonnative reactions [24–26]. Lipases have successfully catalyzed chemical reactions such as the Michael addition [27], Knoevenagel reaction [28], Aldol reaction [29], Mannich reaction [30], and oxidation reactions [31]. Our group conducted a number of studies on lipase-catalyzed unnatural reactions in various solvents, including organic solvents [32], ionic liquids [33],

and supercritical carbon dioxide [34]. The results fully illustrated the feasibility and strong potential of lipase-catalyzed organic synthesis.

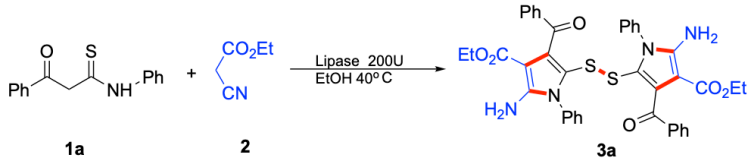
Compared with traditional chemical synthesis, the enzymatic strategy exhibits more potent catalytic capabilities and aligns significantly with the principles of green chemistry. Building upon the achievements from previous works and motivated by our interest in the high-performance capabilities of KTAs, our group investigated the lipase-catalyzed synthesis of pyrrole disulfides under mild conditions using KTAs (**1a**) and ethyl cyanoacetate (**2**) as substrates. (Scheme 1d). To the best of our knowledge, this study provides the first example of the biocatalytic synthesis of pyrrole disulfides.

2. Results and Discussion

2.1. Effect of Lipase Source

In the initial stages, we chose KTA (1a) and ethyl cyanoacetate (2) as template substrates to screen for the optimal reaction conditions. We also evaluated the catalytic abilities of lipases from various sources. As depicted in Table 1, all the selected lipases could afford the desired product **3a** (entries 1–6). Among them, PPL demonstrated the most superior catalytic performance with a yield of 88%. The other lipases exhibited markedly different catalytic effects, which could be attributed to the variations in their protein structure. Control experiments were also conducted by employing inactivated PPLs and bovine serum albumin (entries 7–9) as catalysts, and no product formation was observed. Consistent results were also found when the catalyst was absent in the reaction (entry 10). These findings highlighted the crucial role of the active site of lipase in catalyzing this reaction. Compared with PPL, the use of sodium carbonate as the catalyst resulted in a moderate yield of the desired product **3a** (entry 11). Compared to previous study, when PPL was employed as the catalyst, higher yields were achieved under lower temperature, undoubtedly demonstrating the catalytic potential of lipase [18]. This finding indicated that the enzymatic method is efficient for the synthesis of pyrrole disulfides.

Table 1. Lipase-catalyzed synthesis of pyrrole disulfide (**3a**) [a].



1a **2** **3a**

Entry	Enzyme [b]	Yield (%)
1	PPL	88
2	Cal-B	68
3	Novozym 435	77
4	CSL	40
5	CRL	48
6	MML	55
7	BSA [c]	N.D. [d]
8	PPL [e]	N.D.
9	PPL [f]	N.D.
10	None	N.D.
11	Na_2CO_3 [g]	80

[a] Reaction conditions: **1a** (1 mmol), **2** (1 mmol), EtOH (5 mL), lipase (200 U), 40 °C, 24 h. [b] PPL (Porcine pancreatic lipase); Cal-B (*C. antarctica* lipase B); Novozym 435 (a commercial immobilized Cal-B); CSL (*Candida* sp. lipase); CRL (*C. rugosa* lipase); MML (*Mucor miehei* Lipase). [c] BSA (Bovine serum albumin). [d] No detected. [e] PPL was denatured by heating it to 100 °C for 24 h in water. [f] PPL was denatured by treating PPL with phenylmethanesulfonyl fluoride (PMSF). [g] Na_2CO_3 (1.5 mmol).

2.2. Effect of Solvents

In enzymatic reactions, the reaction solvent and temperature are two crucial factors that significantly influence the outcome [35,36]. As shown in Table 2, the reaction sol-

vent can influence the reaction extent by affecting the enzyme's conformation and the substrates' solubility. Compared with nonpolar solvents, polar solvents generally exhibit better catalytic effects. High yields could be obtained (entry 1–6) when polar solvents such as acetonitrile, ethanol, and N, N-dimethylformamide were utilized as reaction mediums. Conversely, solvents such as dichloromethane, toluene, and hexane resulted in yields lower than expected (entry 7–9). These observations demonstrated the importance of polar solvents in maintaining the catalytic efficiency of enzymes. However, even though water is a polar solvent, the yield of the desired product was relatively low due to the limited solubility of the substrates in water (entry 1). Therefore, water cannot be considered as the optimal solvent. Considering that ethanol aligns more closely with green chemistry principles than acetonitrile [37], we adopted ethanol as the optimal solvent in this reaction.

Table 2. Effect of solvents on the enzymatic synthesis of pyrrole disulfide (**3a**) [a].

Entry	Solvent	Yield (%)
1	Water	73
2	N, N-dimethylformamide	68
3	Dimethyl sulfoxide	57
4	Ethanol	88
5	Acetonitrile	89
6	Ethyl Acetate	80
7	Dichloromethane	37
8	Toluene	36
9	n-Hexane	27

[a] Reaction conditions: **1a** (1 mmol), **2** (1 mmol), solvent (5 mL), PPL (200 U), 40 °C, 24 h.

2.3. Effect of Temperature

The effect of temperature on this reaction was investigated. In general, increasing the temperature enhances the likelihood of collisions between substrate molecules and the enzyme. However, excessive temperature can lead to enzyme deactivation, thereby hampering the catalytic process [38]. Considering the thermostability and catalytic efficiency of lipase, we assessed its catalytic activity across a temperature range from 20 °C to 80 °C to understand the effect of temperature on this reaction (Figure 1). The results were consistent with our expectations. Below 40 °C, the catalytic efficiency increased with the temperature. The yield increased slightly with the increase in temperature from 40 °C to 60 °C. However, beyond 60 °C, the yield declined due to lipase inactivation. Based on the above results, we determined that the optimal reaction temperature is 40 °C.

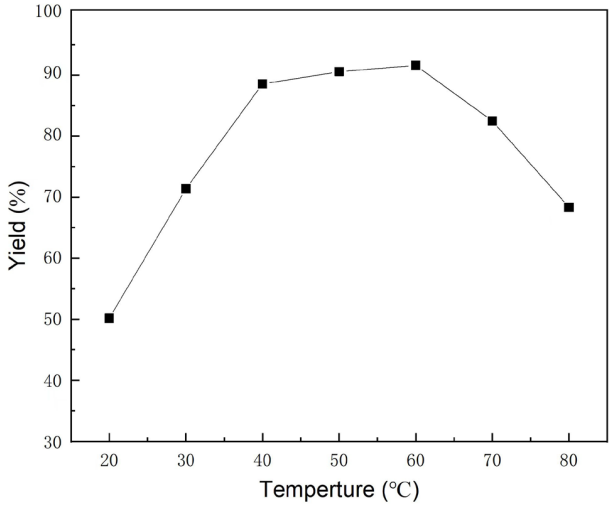


Figure 1. Effect of temperature on the enzymatic synthesis of pyrrole disulfide (**3a**). (Reaction conditions: **1a** (1 mmol), **2** (1 mmol), EtOH (5 mL), PPL (200 U), 24 h).

2.4. Effect of Lipase Dosage

The dosage of the biocatalyst has a significant impact on the reaction. Considering the economy and efficiency of this enzymatic method, we investigated the effect of various amounts of PPL from 0 to 350 U (Figure 2). The change was most noticeable when the enzyme dosage was in the range of 0–150 U. When the enzyme dosage surpassed 150 U, the change rate of yield diminished with the increase in enzyme dosage. The highest yield was achieved at a dosage of 200 U; further increase in enzyme dosage did not result in any significant improvement in yield. Given the cost of lipase, the dosage of 200 U is sufficient for the reaction.

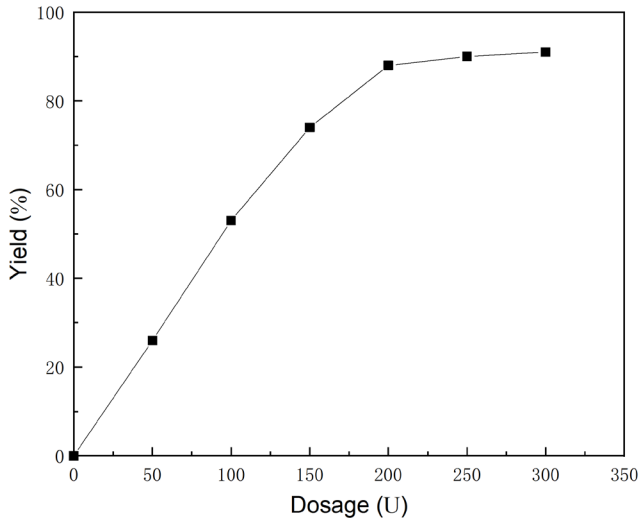


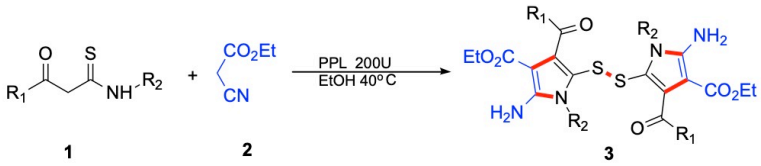
Figure 2. Effect of enzyme dosage on the enzymatic synthesis of pyrrole disulfide (**3a**). (Reaction conditions: **1a** (1 mmol), **2** (1 mmol), EtOH (5 mL), PPL, 40 °C, 24 h).

2.5. Substrate Scope

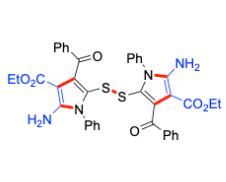
After determining the optimal reaction conditions, we investigated the substrate scope of the reaction. As depicted in Table 3, the reaction showed good compatibility with various substituents (R_1) on the phenyl ring, regardless of whether they were electron-withdrawing (**3b**–**3d**) or electron-donating (**3e**–**3g**) groups. The corresponding pyrrole disulfides were obtained with satisfactory yields ranging from 70% to 90%. Similarly, favorable results were obtained with various substituents (R_2) on the other phenyl ring (**3h**–**3n**), and the corresponding yields ranged from 72% to 87%. Even when the phenyl ring was replaced with a methyl group, a yield of 75% was still achieved (**3o**). We further explored the substrates with substituents at the R_1 and R_2 positions (**3p**–**3t**), and the results remained commendable. Whether the functional groups on R_1 and R_2 were electron-withdrawing or electron-donating, lipase-catalyzed product yields manifested varying degrees of superiority compared to those obtained through the chemical method [18]. These findings demonstrated the broad substrate applicability of PPL in catalyzing the synthesis of pyrrole disulfides, with satisfactory yields for products containing various types of functional group substitutions. To demonstrate the practical feasibility of the lipase-catalyzed synthesis of pyrrole disulfides from KTAs and ethyl cyanoacetates, we designed a corresponding decagram-scale reaction. In a 200 mL round-bottom flask containing 50 mL of ethanol, 2.55 g of substrate **1a** and 1.13 g of ethyl cyanoacetate were added. PPL was employed as the catalytic agent (2000 U), and the reaction proceeded at 40 °C for 24 h. After the completion of the reaction was confirmed through TLC, purification was carried out, resulting in a product yield of 2.95 g corresponding to an 81% yield. This value is higher than that reported for chemical synthesis (70% yield). These findings motivated us to improve the catalytic performance of lipase by enzyme engineering and directed evolution techniques. Immobilization is an efficient strategy that leads to a significant

enhancement in enzyme stability, catalytic efficiency, and recyclability. Currently, we are also investigating methods for immobilizing PPL to further enhance the efficiency of this enzymatic system and will continue to report our findings in due course [39,40].

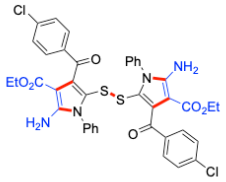
Table 3. Synthesis of pyrrole disulfides **3** catalyzed by lipase.



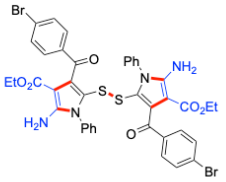
1 **2** **3**



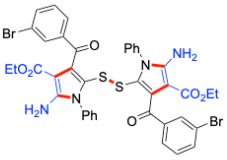
3a: 88%



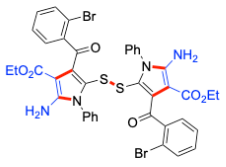
3b: 79%



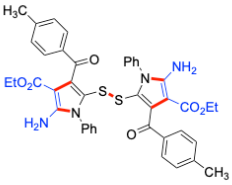
3c: 90%



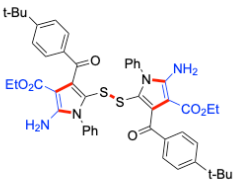
3d: 75%



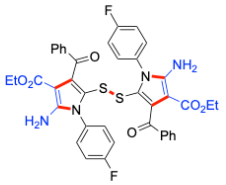
3e: 70%



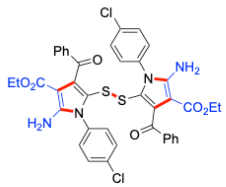
3f: 82%



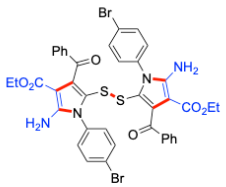
3g: 71%



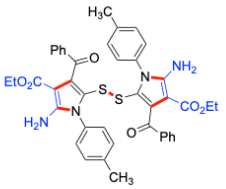
3h: 85%



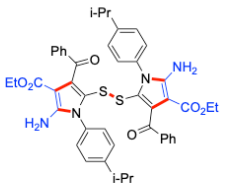
3i: 87%



3j: 84%

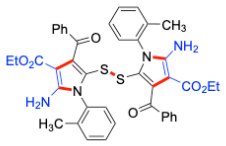


3k: 80%

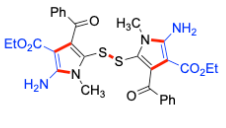


3l: 76%

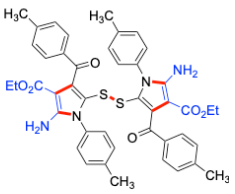
3m: 72%



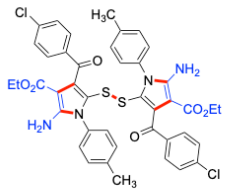
3n: 77%



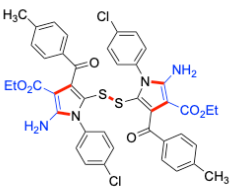
3o: 75%



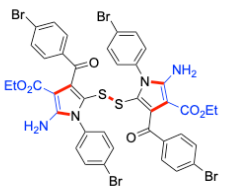
3p: 73%



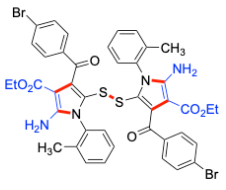
3q: 77%



3r: 80%



3s: 81%

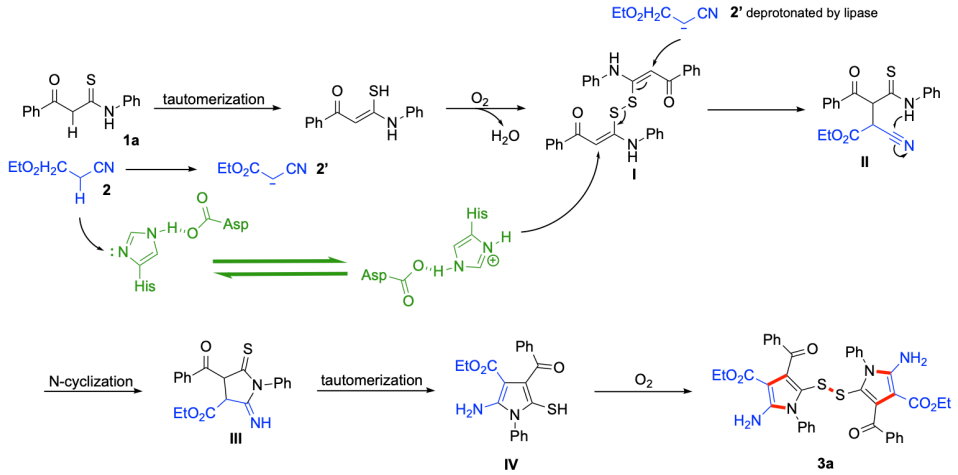


3t: 79%

Reaction conditions: **1** (1 mmol), **2** (1 mmol), EtOH (5 mL), PPL (200 U), 40 °C, 24 h. In compound 3, the black and blue colors represent the source of the atoms (black from 1, blue from 2), while the red color indicates newly formed chemical bonds.

2.6. Mechanistic Speculation

Based on our initial findings and previous literature, we proposed a possible mechanism for this enzymatic reaction (Scheme 2) [21,41,42]. First, a thioenol is formed from KTA **1a** via tautomerization and then oxidized by the dissolved molecular oxygen to produce a dimeric intermediate **I**. Similarly, substrate **2** is deprotonated by lipase, generating an anion. This anion rapidly reacts with intermediate **I**, undergoing C-C bond modification to form intermediate **II**. The secondary amine in intermediate **II** undergoes N-cyclization with the cyano carbon, producing intermediate **III**. Intermediate **III** then undergoes isomerization, resulting in a fully substituted pyrrole **IV**. Finally, product **3a** is formed after oxidation and dimerization.



Scheme 2. Plausible mechanism for the enzymatic synthesis of pyrrole disulfides.

3. Materials and Methods

3.1. Materials

Novozym 435 (a commercial immobilized *C. antarctica* lipase B, 15,000 U/g, U: one unit of the enzyme activity was defined as the amount of enzyme required to hydrolyze 1 μmol of ethyl cyanoacetate per minute at 30 °C) was purchased from Sigma-Aldrich China Co. (Beijing, China). PPL (porcine pancreas lipase, 5600 U/g), Cal-B (*C. antarctica* lipase B, 10,000 U/mL), and CSL (*Candida* sp. lipase, 6400 U/g) were purchased from Shanghai Yuan Ye Biological Technology Company (Shanghai, China), and MML (Mucor miehei lipase, 7300 U/g) was purchased from Sigma-Aldrich China Co. (Beijing, China). All the other chemical reagents were purchased from commercial suppliers (Bide Pharmatech, Aladdin, Energy Chemical, Beijing, China). HRMS were obtained on an Ultima Global spectrometer with an ESI source. NMR spectra were recorded on Bruker 400 MHz spectrometers (see Figure S1–S20 in the Supplementary Materials). Chemical shifts are in ppm with $CDCl_3$ as the internal standard. NMR data are presented as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), and coupling constant in Hertz (Hz), integration. The experiments were performed triplicate, and all data were obtained based on the average values.

*3.2. General Procedure for Synthesis of **1***

Compounds **1** were synthesized according to the procedure reported in the literature [43]. A mixture of NaH (10 mmol), acetophenone (10 mmol) and 1,4-dioxane (8 mL) was stirred at room temperature. Isothiocyanatobenzene (10 mmol) was added dropwise, and stirring was continued at room temperature for 2 h. The solids were collected using filtration and washed with 1,4-dioxane (10 mL). The solids were dissolved with water and then slowly neutralized via stirring with HCl. After filtration, the filter cake was dried. The obtained substance does not require further purification and can be directly utilized in the subsequent steps.

*3.3. General Procedure for Lipase-Catalyzed Synthesis of **3***

PPL (200 U) was added to a stirred solution of thioamides **1** (1 mmol) and ethyl cyanoacetate **2** (1 mmol) in ethanol (5 mL); the reaction mixture was stirred at 40 °C for 24 h. After completion of the reaction as monitored with TLC, the solvent was concentrated under vacuum and the residue was purified using flash column chromatography on silica gel with petroleum ether/ethyl acetate (3:1, *v*/*v*) as the eluent to yield the desired product **3**.

3.4. Data of Products

1a 3-oxo-N,3-diphenylpropanethioamide

Isolated yield: 50%, yellow solid, mp 99–100 °C (lit. (25) 102–103 °C).

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{16}H_{15}NNaOS$ 292.0772, found 292.0768

^{1}H NMR (400 MHz, CDCl3, δ, ppm) 10.93 (s, 1H), 7.08 (d, J = 7.6 Hz, 2H), 7.55–7.51 (m, 1H), 7.47–7.40 (m, 4H), 7.22–7.20 (m, 2H), 4.65 (s, 2H), 2.37 (s, 3H);

^{13}C NMR (100 MHz, CDCl3, δ, ppm) 197.1, 191.0, 137.0, 134.5, 129.5, 129.0, 128.8, 128.6, 123.6, 54.0, 21.2;

3a diethyl 5,5′-disulfanediylbis (2-amino-4-benzoyl-1-phenyl-1H-pyrrole-3-carboxylate)

Isolated yield: 88% (321 mg), yellow solid, mp: 198–200 °C.

HRMS (ESI–TOF, $[M + H]^+$): calcd for $C_{40}H_{35}N_4O_6S_2$, 731.1993; found, 731.1992.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 7.6 Hz, 2H), 7.49 (t, J = 7.6 Hz, 1H), 7.42 (s, 3H), 7.33 (s, 1H), 7.25–7.16 (m, 2H), 5.19 (s, 2H), 3.97 (d, J = 7.2 Hz, 2H), 0.78 (t, J = 7.2 Hz, 3H).

3b diethyl 5,5′-disulfanediylbis (2-amino-4-(4-chlorobenzoyl)-1-phenyl-1H-pyrrole-3-carboxylate)

Isolated yield: 79% (316 mg), yellow solid, mp: 154–156 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{40}H_{32}N_4O_6NaS_2Cl_2$, 821.1033; found, 821.1038.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 8.0 Hz, 2H), 7.44 (tt, J = 6.8, 3.2 Hz, 4H), 7.37–7.29 (m, 2H), 7.20 (s, 1H), 5.22 (s, 2H), 4.01 (q, J = 7.2 Hz, 2H), 0.85 (t, J = 7.2 Hz, 3H).

3c diethyl 5,5′-disulfanediylbis (2-amino-4-(4-bromobenzoyl)-1-phenyl-1H-pyrrole-3-carboxylate)

Isolated yield: 90% (399 mg), yellow solid, mp: 154–156 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{40}H_{32}N_4O_6NaS_2Br_2$, 909.0028; found, 909.0037.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.2 Hz, 5H), 7.20 (s, 1H), 5.22 (s, 2H), 4.02 (q, J = 7.2 Hz, 2H), 0.86 (t, J = 7.2 Hz, 3H).

3d diethyl 5,5′-disulfanediylbis(2-amino-4-(3-bromobenzoyl)-1-phenyl-1H-pyrrole-3-carboxylate)

Isolated yield: 75% (333 mg), yellow solid, mp: 180–182 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{40}H_{32}N_4O_6NaS_2Br_2$, 909.0028; found, 909.0037.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.71–7.50 (m, 1H), 7.45 (s, 3H), 7.26–7.13 (m, 2H), 5.25 (s, 2H), 4.00 (q, J = 7.2 Hz, 2H), 0.84 (t, J = 7.2 Hz, 3H).

3e diethyl 5,5′-disulfanediylbis (2-amino-4-(2-bromobenzoyl)-1-phenyl-1H-pyrrole-3-carboxylate)

Isolated yield: 70% (311 mg), yellow solid, mp: 180–182 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{40}H_{32}N_4O_6NaS_2Br_2$, 909.0028; found, 909.0037.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.45 (s, 3H), 7.18 (d, J = 8.4 Hz, 2H), 5.25 (s, 2H), 4.00 (q, J = 7.2 Hz, 2H), 0.84 (t, J = 7.2 Hz, 3H).

3f diethyl 5,5′-disulfanediylbis (2-amino-4-(4-methylbenzoyl)-1-phenyl-1H-pyrrole-3-carboxylate)

Isolated yield: 82% (311 mg), yellow solid, mp: 172–174 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{42}H_{38}N_4O_6NaS_2$, 781.2130; found, 781.2135.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 7.6 Hz, 2H), 7.41 (s, 3H), 7.16 (d, J = 23.8 Hz, 4H), 5.18 (s, 2H), 4.00 (q, J = 7.2 Hz, 2H), 2.40 (s, 3H), 0.83 (t, J = 7.2 Hz, 3H).

3g diethyl 5,5′-disulfanediylbis (2-amino-4-(4-(tert-butyl)benzoyl)-1-phenyl-1H-pyrrole-3-carboxylate)

Isolated yield: 71% (299 mg), yellow solid, mp: 188–190 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{48}H_{50}N_4O_6NaS_2$, 865.309; found, 865.314.

^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 8.0 Hz, 2H), 7.48–7.32 (m, 5H), 7.18 (s, 1H), 5.21 (s, 2H), 3.97 (d, J = 7.6 Hz, 2H), 1.37–1.23 (m, 9H), 0.72 (s, 3H).

3h diethyl 5,5′-disulfanediylbis (2-amino-4-benzoyl-1-(4-fluorophenyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 85% (326 mg), yellow solid, mp: 179–181 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{40}H_{32}N_4O_6F_2NaS_2$, 789.1629; found, 789.1637.

^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 7.6 Hz, 2H), 7.51 (t, J = 7.6 Hz, 1H), 7.38–7.28 (m, 1H), 7.25–7.07 (m, 4H), 5.16 (s, 2H), 3.95 (q, J = 7.2 Hz, 2H), 0.76 (t, J = 7.2 Hz, 3H).

3i diethyl 5,5′-disulfanediylbis (2-amino-4-benzoyl-1-(4-chlorophenyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 87% (348 mg), yellow solid, mp: 182–184 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{40}H_{32}N_4O_6NaS_2Cl_2$, 821.1033; found, 821.1038.

^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 7.6 Hz, 2H), 7.51 (t, J = 7.2 Hz, 1H), 7.43–7.39 (m, 2H), 7.35 (d, J = 7.2 Hz, 2H), 7.26–7.09 (m, 2H), 5.19 (s, 2H), 3.95 (d, J = 7.2 Hz, 2H), 0.76 (d, J = 7.2 Hz, 3H).

3j diethyl 5,5′-disulfanediylbis (2-amino-4-benzoyl-1-(4-bromophenyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 84% (373 mg), yellow solid, mp: 238–240 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{40}H_{32}N_4O_6NaS_2Br_2$, 909.0028; found, 909.0037.

^1H NMR (400 MHz, $CDCl_3$) δ 7.99–7.91 (m, 2H), 7.61–7.46 (m, 3H), 7.38–7.31 (m, 2H), 7.12 (s, 2H), 5.19 (s, 2H), 3.95 (q, J = 7.2 Hz, 2H), 0.85–0.72 (m, 3H).

3k diethyl 5,5′-disulfanediylbis (2-amino-4-benzoyl-1-(p-tolyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 80% (303 mg), yellow solid, mp: 210–212 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{42}H_{38}N_4O_6NaS_2$, 781.2130; found, 781.2135.

^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 7.6 Hz, 2H), 7.55–7.42 (m, 1H), 7.34 (dd, J = 10.8, 3.6 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.07 (s, 2H), 5.17 (s, 2H), 3.96 (q, J = 7.2 Hz, 2H), 2.39 (s, 3H), 0.77 (t, J = 7.2 Hz, 2H).

3l diethyl 5,5′-disulfanediylbis (2-amino-4-benzoyl-1-(4-isopropylphenyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 76% (310 mg), yellow solid, mp: 220–222 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{46}H_{46}N_4O_6NaS_2$, 837.277; found, 837.282.

^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 7.6 Hz, 2H), 7.45 (t, J = 7.2 Hz, 1H), 7.29 (d, J = 12.4 Hz, 2H), 5.37 (d, J = 22.8 Hz,2H), 3.87 (d, J = 7.6 Hz, 2H), 1.45 (d, J = 6.8 Hz, 6H), 0.66 (t, J = 7.2 Hz, 3H).

3m diethyl 5,5′-disulfanediylbis (2-amino-4-benzoyl-1-(m-tolyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 72% (273 mg), yellow solid, mp: 212–214 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{42}H_{38}N_4O_6NaS_2$, 781.2130; found, 781.2135.

^1H NMR (400 MHz, $CDCl_3$) δ 8.02–7.95 (m, 2H), 7.52–7.30 (m, 4H), 7.22 (d, J = 8.8 Hz, 1H), 6.99 (s, 2H), 5.20 (s, 2H), 3.97 (q, J = 7.2 Hz, 2H), 2.33 (s, 3H), 0.78 (t, J = 7.2 Hz, 3H).

3n diethyl 5,5′-disulfanediylbis (2-amino-4-benzoyl-1-(o-tolyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 77% (292 mg), yellow solid, mp: 210–212 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{42}H_{38}N_4O_6NaS_2$, 781.2130; found, 781.2135.

^1H NMR (400 MHz, $CDCl_3$) δ 7.79–7.72 (m, 2H), 7.57 (tt, J = 15.2, 7.2 Hz, 3H), 7.43–7.33 (m, 4H), 5.58 (s, 2H), 4.34 (q, J = 7.2 Hz, 2H), 2.21 (s, 3H), 0.85 (t, J = 7.2 Hz, 3H).

3o diethyl 5,5′-disulfanediylbis (2-amino-4-benzoyl-1-methyl-1H-pyrrole-3-carboxylate)

Isolated yield: 75% (227 mg), yellow solid, mp: 190–192 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{30}H_{30}N_4O_6NaS_2$, 629.1970; found, 629.2135.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 7.2 Hz, 3H), 7.35–7.29 (m, 3H), 5.10 (d, J = 13.6 Hz, 2H), 3.89 (dq, J = 9.2, 7.2 Hz, 2H), 3.33 (s, 3H), 0.71 (q, J = 7.6 Hz, 3H).

3p diethyl 5,5′-disulfanediylbis (2-amino-4-(4-methylbenzoyl)-1-(p-tolyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 73% (287 mg), yellow solid, mp: 160–162 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{44}H_{42}N_4O_6NaS_2$, 809.2450; found, 809.2455.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 7.6 Hz, 2H), 7.19 (s, 6H), 5.15 (s, 2H), 3.98 (q, J = 7.2 Hz, 2H), 2.39 (d, J = 3.6 Hz, 6H), 0.82 (d, J = 7.2 Hz, 3H).

3q diethyl 5,5′-disulfanediylbis (2-amino-4-(4-chlorobenzoyl)-1-(p-tolyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 77% (319 mg), yellow solid, mp: 134–136 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{42}H_{36}N_4O_6Cl_2NaS_2$, 849.1351; found, 849.1345.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.58 (s, 1H), 7.46 (t, J = 10.4 Hz, 2H), 7.36 (t, J = 7.2 Hz, 2H), 6.90 (s, 2H), 5.03 (s, 2H), 3.95 (q, J = 7.2 Hz, 2H), 2.28 (s, 3H), 0.80 (t, J = 7.2 Hz, 3H).

3r diethyl 5,5′-disulfanediylbis (2-amino-1-(4-chlorophenyl)-4-(4-methylbenzoyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 80% (331 mg), yellow solid, mp: 134–136 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{42}H_{36}N_4O_6Cl_2NaS_2$, 849.1351; found, 849.1345.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 7.6 Hz, 2H), 7.26–7.05 (m, 6H), 5.16 (s, 2H), 3.98 (q, J = 7.2 Hz, 2H), 2.41 (s, 3H), 0.81 (d, J = 8.4 Hz, 3H).

(3s) diethyl 5,5′-disulfanediylbis (2-amino-4-(4-bromobenzoyl)-1-(4-bromophenyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 81% (423 mg), yellow solid, mp: 188–190 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{40}H_{30}N_4O_6NaS_2Br_4$, 1068.8299; found, 1068.8304.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.0 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H), 7.10 (s, 2H), 5.23 (s, 2H), 3.98 (dd, J = 17.2, 8.0 Hz, 2H), 0.84 (t, J = 7.2 Hz, 3H).

(3t) diethyl 5,5′-disulfanediylbis (2-amino-4-(4-bromobenzoyl)-1-(o-tolyl)-1H-pyrrole-3-carboxylate)

Isolated yield: 79% (362 mg), yellow solid, mp: 181–183 °C.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{42}H_{36}N_4O_6NaS_2Br_2$, 937.0044; found, 937.0049.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 8.0 Hz, 2H), 7.47 (d, J = 8.0 Hz, 2H), 7.31–7.20 (m, 2H), 6.98 (s, 2H), 5.23 (s, 2H), 4.01 (q, J = 7.2 Hz, 2H), 2.33 (s, 3H), 0.86 (t, J = 7.2 Hz, 3H).

4. Conclusions

We successfully developed a lipase-catalyzed method for the synthesis of pyrrole disulfides using KTAs and ethyl cyanoacetate as substrates. As the biocatalyst, PPL offers several advantages such as being environmentally friendly and achieving high yields. This enzymatic method utilized various substrates, and the transformation was completed in ethanol at 40 °C, with yields ranging from 70% to 90%. Moreover, it achieved a promising yield (70%) in a scale-up experiment. Simultaneously, based on control experiments, we identified the crucial role of the active center of lipase in catalyzing this reaction and speculated the reaction mechanism for the synthesis of pyrrole disulfides.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal13121493/s1, Figure S1: ^{1}H NMR of **3a**. Figure S2. ^{1}H NMR of **3b**. Figure S3. ^{1}H NMR of **3c**. Figure S4. ^{1}H NMR of **3d**. Figure S5. ^{1}H NMR of **3e**. Figure S6. ^{1}H NMR of **3f**. Figure S7. ^{1}H NMR of **3g**. Figure S8. ^{1}H NMR of **3h**. Figure S9. ^{1}H NMR of **3i**. Figure S10. ^{1}H NMR of **3j**. Figure S11. ^{1}H NMR of **3k**. Figure S12. ^{1}H NMR of **3l**. Figure S13. ^{1}H NMR of **3m**. Figure S14. ^{1}H NMR of **3n**. Figure S15. ^{1}H NMR of **3o**. Figure S16. ^{1}H NMR of **3p**. Figure S17. ^{1}H NMR of **3q**. Figure S18. ^{1}H NMR of **3r**. Figure S19. ^{1}H NMR of **3s**. Figure S20. ^{1}H NMR of **3t**.

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