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Lipase catalyzed 1,2-addition of thiols to imines under mild conditions

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This is the first report of enzymes used as a biocatalyst for a 1,2-sulfur addition. In this study, we describe the N,S-acetals synthesis using an environmentally friendly process with low catalyst loading and in short reaction times using lipase pancreas porcine, chymosin, and bovine serium albumin (BSA). The hydrogen bond between the enzyme and the N-Boc imine is a key factor in this reaction.

Introduction

Biocatalysis research has exponentially grown over the last past decades owing to discoveries related to the synthesis of many compounds, mainly with biological properties, which are beneficial for the society.[1,2] In addition, environmental concerns have driven many research groups around the world, and even industries, to describe new synthetic protocols that are not harmful for the environment and to consider the subproducts formed by side reactions. This results in the use of catalysts that allow faster reactions using low cost reactants and avoid the wastage of solvents, which have a great impact in the environmental factor (E factor).[3] Thus, many protocols that use enzymes (e.g., lipases) have been reported. Enzymes possess great catalytic characteristics such as high efficiency, biodegradation (unlike heavy metals); in some cases, the chemical transformations occur in aqueous solutions, avoiding the use of polluting solvents[4]. Moreover, such enzymes have great specificity and can be used both in free form or immobilized; they are usually cheap and stable catalysts and can be inserted as heterogeneous catalysts in organic reaction media[4]. Many examples of enzymatic catalyzed reactions are described in the literature such as Mannich[5], aldolic[6], decarboxylative aldol and Knoevenagel[7], Morita-Baylis–Hillman[8], and sulfa-Michael[4b] reaction.

The *N,S*-acetals, which are commonly found in a great range of biological products with medical and biochemical applications, have attracted great attention.[9] An excellent example is penicillin, which belongs to the β-lactamics antibiotic family characterized by the therapeutic treatment. Penicillin prevents several infectious processes and its complications with high efficacy and low cost.[9] Another known antibiotic is Fuzaperazine A, which is naturally produced and broadly used against viruses, bacteria, and carcinogenic cells.[9] Owing to their important applications, over the years, a great number of synthetic strategies aimed to produce new *N,S*-acetals molecules have been developed.[9] Thiols were added to imines using thiourea quaternary ammonium salts as catalysts to obtain *N,S*-acetals derivatives.[9a] *N,S*-acetals were synthesized using isatin and thiols, as well as isoindolinones and thiol derivates with BINOL-phosphoric acid derivative as catalyst[9b,9c]. The synthesis of *N,S*-acetals in high yields and excellent enantiomeric excess using chiral phosphoric acids were also reported[9d]. We published a synthetic protocol to obtain some *N,S*-acetals using $Zn(Pro)_2$ as a heterogeneous catalyst[10]. However, to the best of our knowledge, no procedures are described for the synthesis of *N,S*-acetals using a biological catalyst such as enzymes. Since we have developed synthetic methods that use enzymes as catalyst, for example in the sulfa-Michael reaction[4b], we propose employing enzymes to synthesize some *N,S*-acetals under mild and green reaction conditions herein (Figure 1).

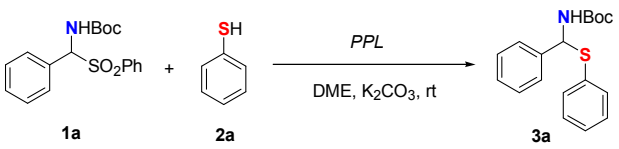

Figure 1. Reaction scheme for the *N,S*-acetal synthesis biocatalyzed by Porcine pancreas lipase (PPL).

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† Footnotes relating to the title and/or authors should appear here.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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Experimental

General methods:

All the chemicals, reagents, and solvents were used as received unless otherwise stated. The respective reactions were monitored via thin layer chromatography (TLC) using MACHEREY-NAGEL silica plates (SIL G/UV254). The compounds were purified via column chromatography on silica gel. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker spectrometer (300 and 75 MHz, respectively). The infrared spectra were recorded on a FT/IR 4100 type A Jasco spectrometer.

General procedure for the *N*-Boc imines synthesis:

The mixture of aldehyde (5.0 mmol), sodium benzenesulfinate (5.0 mmol), *tert*-butyl carbamate (5.0 mmol), and HCO_2H (217 µL) was placed in a 50-mL round-bottom flasks. CH_3OH (5 mL) and water (10 mL) were added, and the mixture was magnetically stirred at room temperature for 24 h. When the reaction was complete, the solid obtained was filtered, washed with ethyl ether, and dried overnight.

General procedure for *N,S*-acetal synthesis:

The mixture of thiol (0.2 mmol), amidosulfones (0.1 mmol), K_2CO_3 (0.5 mmol), and enzyme (0.01 g) in DME (4 mL) was placed in a tube at room temperature. The reaction was stirred using a magnetic bar and monitored using TLC (hexane/EtOAc, 90:10). After the reaction, the catalyst was separated via filtration, and the mixture was extracted with DCM (3 × 10 mL). The organic phases were combined and dried over anhydrous Na_2SO_4; the salt was filtered before concentrating the organic phase under reduced pressure. The product was purified via column chromatography on silica gel (hexane/EtOAc, 90:10).

Results and discussion

We initially evaluated the effectiveness of the biocatalyst in different organic solvents such as ethyl acetate, THF, DCM, DME, and DMSO (Figure 2).

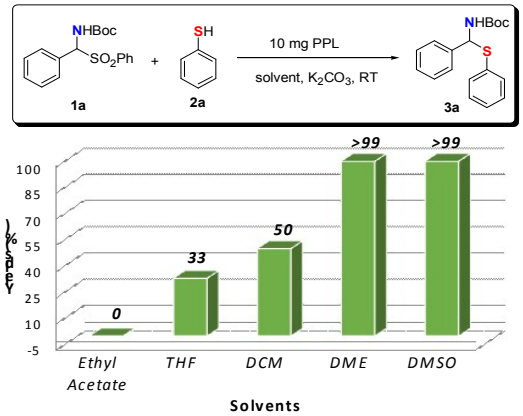
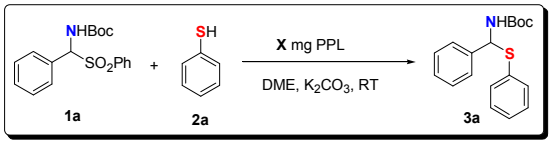


Figure 2. Yields for the *tert*-butyl (phenyl (phenylthio) methyl) carbamate (**3a**) catalyzed by PPL [reaction conditions: **1a** (0.1 mmol), **2a** (0.2 mmol), K_2CO_3 (0.5 mmol), solvent (4 mL), 2 h].

The reaction in ethyl acetate did not afford the desired compound. We concluded that this is likely because of the insolubility of compound 1a in that solvent. Therefore, we also attempted to perform the *N,S*-acetal synthesis in DCM; however, the yield obtained was moderate (50%). To our surprise, when the solvents were DME and DMSO, we achieved a 100% yield. To further evaluate the biocatalyst effectiveness, a blank reaction (without biocatalyst) was performed in those two solvents. The reaction in DMSO occurred without PPL, affording 50% of the desired compound after 2 h. However, reaction in DME did not afford *N,S*-acetal even after 24 h. Therefore, DME was chosen as the solvent for the biocatalyzed reactions. The catalyst loading effect on the yield obtained was studied later (Table 1).

Table 1. PPL catalyst loading and yields for *N,S*-acetal synthesis*.



Entry	Catalyst loading (mg)	Yield (%)
1	-	-
2	2.5	23
3	5.0	52
4	7.5	73
5	***10.0***	***>99***

* Reaction conditions: 1a (0.1 mmol), 2a (0.2 mmol), K_2CO_3 (0.5 mmol), solvent (4 mL), 2 h.

The yield increased with the catalyst loading. As seen in Table 1, increasing the catalyst proportion from 0.0 to 10.0 mg resulted in a yield raise from 0.0 to >99%. These results indicate that a high yield can be obtained even at low catalyst loading (10.0 mg), which clearly demonstrates the effectiveness of the catalyst. Based on these deductions, the following standard protocol was established: N-Boc sulfonylamine (0.1 mmol), thiol derivatives (0.2 mmol), K_2CO_3 (0.5 mmol), and DME (4 mL) for 2 h. This procedure was used for several other reactions involving different N-Boc sulfonylamines and thiols (Table 2).

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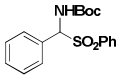
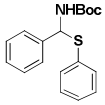
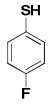
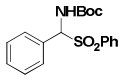
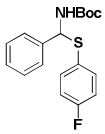
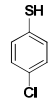
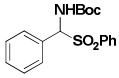
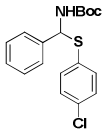
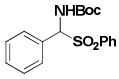
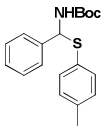
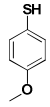
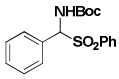
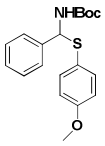
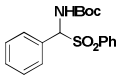
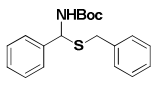
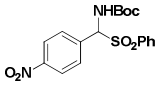
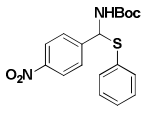
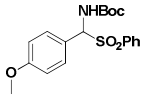
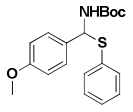
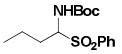
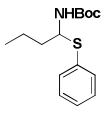
Table 2. Yields for the *N,S*-acetal synthesis biocatalyzed using PPL

Entry	Thiol	Amidosulfone	Product	Yield[a] (%)
1				100
2				100
3				100
4				80
5				57
6				55
7				74
8				43
9				15

[a] Yield calculated for purified compound after column chromatography.

The reactions involving thiols containing either H or electron withdrawing groups at the *para*-position (entries 1–3) afforded the highest yields compared to the ones with electron donor groups (entries 4–5). This fact was not expected; however, this is believed to be caused by the hydrogen bond between the nucleophiles and the acid hydrogen in the enzymes. We concluded that more basic sulfur species resulted in stronger hydrogen bond; thus, the addition of benzenethiol (entry 6), p-OCH_3 (entry 5) or p-CH_3 (entry 4) enables these nucleophiles to create strong hydrogen bond with enzyme's hydrogen acids. Owing to this bond, the nucleophilicity of sulfur species decreased, and the yields for these reactants were low.

For the PPL, the mechanism shown in Figure 3, which is to the one described by Rizzo,[11] is proposed. Therein, the serine portion of the enzyme (hydrogen bond plus imine), which is obtained after the elimination of the sulfinic group in basic media, can activate the interaction for the 1,2-sulfur addition. As seen for the N-Boc sulphonamides derivatives, the hydrogen bond between the imine nitrogen and the enzymatic hydrogen (intermediate #3) made this intermediate more reactive toward the 1,2-sulfur attack. Hence, the derived conclusion is consistent with the data presented in entries 7–9 (Table 2). Moreover, when the imine had an electron withdrawing group, such as nitro, it enabled the attack of the sulfur species easier because of the mesomeric effect. This effect increased the electrophilicity of the imine carbon. Contrastingly, an electron donor group such as OCH_3 bonded at the phenyl group (entry 8, Table 2) or a simple alkyl group such as butyl (entry 9, Table 2) decreased the electrophilicity and resulted in a less effective hydrogen bond in the intermediate #3. It is important to note that certain reports in literature agree with this conclusion[5,7].

The feasibility to reuse the PPL in the *N,S*-acetal synthesis was also studied herein. However, as informed by Rizzo[11], we could not reuse the enzyme because of the presence of sulfinic base was inside the crude reaction. In addition, water could not be used to remove only the sulfinic base because the enzyme was also soluble in water.

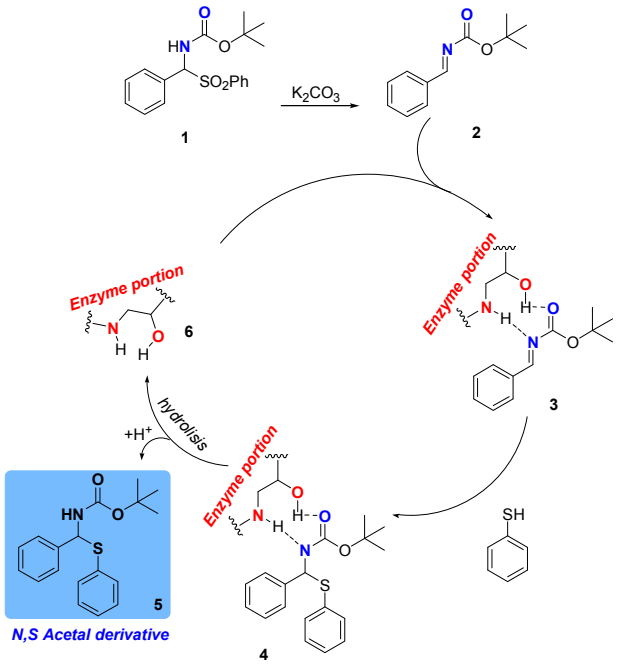


Figure 3. Proposed mechanims for the *N,S*-acetal synthesis biocatalyzed by PPL

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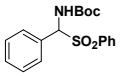
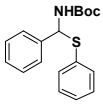
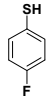
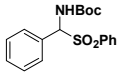
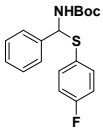
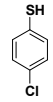
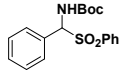
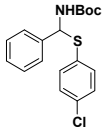
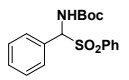
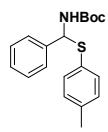
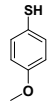
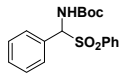
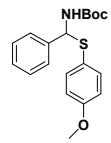
To obtain a more comprehensive analysis of the *N,S*-acetal biosynthesis, we extended the study using two more enzymes such as chymosin and bovine serium albumin (BSA) (Table 3).

Table 3. Yields for the *N,S*-acetal synthesis biocatalyzed by chymosin (CHY) and albumin (ALB).

Entry	Thiol	Amidosulfone	Product	Yield[a] (%)
1				53 (*CHY*) 76 (*ALB*)
2				93 (*CHY*) 100 (*ALB*)
3				56 (*CHY*) 60 (*ALB*)
4				86 (*CHY*) 96 (*ALB*)
5				90 (*CHY*) 84 (*ALB*)

[a] Yield calculated for purified compound after column chromatography.

For these two enzymes, patterns related to the effect of the groups in the thiophenol moiety were not observed. However, we observed that both chymosin and BSA afforded high yields for the OCH_3 and CH_3 groups, contrary to the moderate yields observed in cases wherein PPL biocatalysis was performed. This observation corroborates with the conclusion for PPL regarding to the basicity as for the sulfur species and enzymes. To the best of our knowledge, the albumin structure presents a basic nature in its hydrophobic active site[12]. Therefore, as observed in Table 3, basic sulfur species presented less interaction with enzyme and due to higher nucleophilicity of the sulfur species - as OCH_3 (entry 5) and CH_3 (entry 4) - the yields of these reactions were higher in comparison to the acidic ones, as hydrogen (entry 1). Furthermore, as chymosin presented almost the same results obtained for BSA, we extended the conclusion also for chymosin.

Conclusions

A new, simple, efficient, low cost, and green catalytic enzymatic protocol for the 1,2-sulfur addition to synthesize *N,S*-acetals involving PPL, chymosin, and BSA is described herein. To the best of our knowledge, the use of enzymes has not been reported for this important reaction. Certain important aspects make this protocol a useful alternative to the existing methods for the synthesis of *N,S*-acetals: the reaction was achieved at short reaction times and room temperature, and high yields were obtained.

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PPL, Chymosin and BSA as efficient biocatalysts (low catalyst loading, high yields) in an eco-friendly synthesis of *N,S*-acetal.

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Graphical Abstract