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Bovine serum albumin-catalyzed one-pot synthesis of 2-aminothiophenes via Gewald reaction

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ABSTRACT

A novel bovine serum albumin (BSA)-catalyzed Gewald reaction in one-pot was developed in this work. The influence of reaction conditions including solvent, temperature and catalyst loading was investigated, and 12 multi-substituted 2-aminothiophene derivatives were prepared with moderate to excellent yields. Recycle experiments were designed to demonstrate the reusability of BSA. This novel activity of BSA to catalyze Gewald reaction is of practical significance in expanding the application of biocatalysts.

1. Introduction

Substituted 2-aminothiophenes are a kind of important structures which usually show diverse pharmacological profiles with antimicrobial, anticonvulsant and anti-inflammatory activities [1], and thus are continually used as a scaffold to construct a series of natural products, dyes and agrochemicals [2]. They can also serve as potential JNK2 and JNK3 kinase inhibitor and adenosine agonists [3]. Many other commercial medicines and drug candidates have been increasingly evolved from such scaffold as well [4]. The preparation of 2-aminothiophene derivatives is always achieved by a Gewald reaction (Scheme 1), which is a multicomponent condensation of a ketone with an activated α-hydrogen, a cyanomethylene containing an electron-withdrawing group, e.g., cyanoacetate, and elemental sulfur in the presence of an organic base [5] such as morpholine [6], diethylamine [7], etc. Alternative catalysts like L-proline [8] have also been developed for Gewald reaction. Yet, existing chemical procedures are always associated with all kinds of weaknesses, like high catalyst loading [9], complicated procedures [10], and hazardous solvents [11], which hinder the further development of the Gewald reaction. It is of special significance to investigate new catalysts in terms of environmental sustainability, operational simplicity, and broad substrate scope.

Since the past decade, dynamic efforts to exploit the biocatalysts, like protein (enzyme) [12], DNA [13], whole cell [14], etc. for catalyzing organic reactions have been on the raise due to their simple processing requirements and high selectivity. However, for the Gewald reaction, to the best of our knowledge, no biocatalytic procedures have been reported. This current situation and other relevant reports encourage us to explore the biocatalyzed Gewald reaction. Among all the catalysts tested, bovine serum albumin (BSA) is a powerful one which has been reported with many kinds of catalytic activity. In 2011, Gotor and co-workers reported that BSA could efficiently promote the nitroaldol addition between aromatic aldehydes and 1-nitroal-kanes in aqueous medium [15]; stereoselective thio-Michael addition to chalcones in water catalyzed by BSA was reported by Gaggero in 2011 [16]; BSA could also serve as the catalyst for the one-pot synthesis of benzimidazoles and aldehydes [17]. First bovine serum albumin-promoted synthesis of enones, cinnamic acids and coumarins in ionic liquid and bovine serum albumin-triggered waste-free synthesis of 3,4-dihydropyrimidin-2-(1H)-ones were also developed [18]. In this work, the Gewald reaction could be catalyzed by BSA with satisfying yields.

2. Materials and methods

2.1. Materials

D-Aminoacylase from *Escherichia coli* (10,000 U/mg, 1 U is defined as enzyme quantity which produces 1 μmol of D-amino acid per 30 min) and Acylase "Amano" (AA) from *Aspergillus oryzae*

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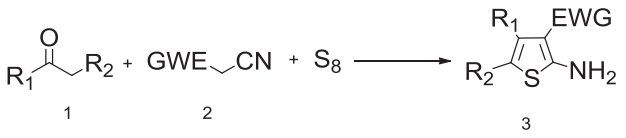

Scheme 1. Gewald reaction.

(≥30,000 U/g, 1 U is defined as enzyme quantity which produces 1 μmol of L-amino acid per 30 min) were purchased from Amano Enzyme Inc. (Japan). Lipase from *Hog pancreas* (2.4 U/mg, 1 U is the amount of immobilized enzyme which forms 1% octyl laurate from 0.5 mmol lauric acid and 1.0 mmol 1-octanol in 10 mL water-saturated isooctane in 1 h at 20 °C), Amano Lipase M from *Mucor javanicus* (≥10,000 U/g enzyme activity, pH 7.0, 40 °C), Lipase immobilized on acrylic resin from *Candida antarctica* (≥10,000 U/g, recombinant, expressed in *A. oryzae*) and lipase from *Porcine pancreas* (30–90 U/mg protein, 1 U will hydrolyze 1.0 mequiv. of triacetin in 1 h at pH 7.7 at 37 °C) were purchased from Sigma (Steinheim, Germany). Lipase AY 30 (700–1500 U/mg solid, 1 U will hydrolyze 1.0 mequiv. of olive oil from a triglyceride in 1 h at pH 7.7 at 37 °C) was purchased from Acros (New Jersey, USA). Lipozyme immobilized from *Mucor miehei* (MML) was purchased from Fluka. Bovine serum albumin (BSA) was obtained from Wuxi Enzyme Co. Ltd., Wuxi, PR China. L-Glutamic acid, L-threonine, L-serine, L-cysteine and L-lysine were obtained from Sinopharm Chemical Reagent Co., Ltd. All other chemicals were of the highest purity commercially available.

2.2. General procedure for the synthesis of 2-aminothiophenes

A mixture of **1** (1 mmol), **2** (1 mmol), elemental sulfur (1 mmol) and BSA (20 mg) was added to 1 mL of DMF. The reaction was incubated at 50 °C and 200 rpm. After the required time, the BSA was filtered off to terminate the reaction. For the products with high yields, the solid crude products precipitated in water, and then followed by filtration and drying. For the products with low yields, the crude residues were purified by flash column chromatography on silica gel using petroleum/ethyl acetate.

The structures of the products were confirmed by IR, ^{1}H NMR and ^{13}C NMR. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$ using TMS (tetramethylsilane) as internal reference. IR spectra were obtained with a Nicolet Nexus 470 FT-IR spectrophotometer. HPLC was carried out using an Agilent 1100 series with an Agilent TC-C18 column (**3a**, **3c**, **3e**, **3g**, **3i**, **3k**: methanol/water ratio = 60/40, 1.0 mL/min and 220 nm; **3b**, **3d**, **3f**, **3h**, **3g**, **3l**: methanol/water ratio = 60/40, 1.0 mL/min and 229 nm).

For all reactions, solvents for column chromatography were distilled before use.

2.2.1. 2-Amino-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carbonitrile (**3a**)

Yellow solid, mp 140–142 °C (lit. [19] mp 144–146 °C). IR (KBr): ν. 3447, 3329, 2198 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.62 (bs, 2H), 2.53–2.46 (m, 4H), 1.85–1.73 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 159.9, 132.4, 120.7, 115.5, 88.8, 24.5, 24.1, 23.4, 22.1.

2.2.2. Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate (**3b**)

Yellow solid, mp 114–115 °C (lit. [19] mp 117–118 °C). IR (KBr): ν. 3403, 3299, 1647 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.93 (bs, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 2.72–2.68 (m, 2H), 2.52–2.46 (m, 2H), 1.82–1.70 (m, 4H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 166.1, 161.2, 132.5, 118.0, 106.1, 59.4, 26.9, 24.6, 23.3, 22.8, 14.5.

2.2.3. 2-Amino-5,6-dihydro-4H-cyclopenta[b] thiophene-3-carbonitrile (**3c**)

Yellow solid. mp 152–153 °C (lit. [20] mp 151 °C). IR (KBr): ν. 3438, 3336, 2194 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.64 (bs, 2H), 2.78–2.66 (m, 4H), 2.41–2.31 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 166.2, 142.9, 125.3, 115.5, 85.6, 29.3, 28.5, 27.4.

2.2.4. Ethyl 2-amino-5,6-dihydro-4H-cyclopenta[b] thiophene-3-carboxylate (**3d**)

Yellow solid. mp 181–182 °C (lit. [21] mp 182–183 °C). IR (KBr): ν. 3413, 3297, 1653 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.85 (bs, 2H), 4.24 (q, *J* = 6.8 Hz, 2H), 2.85–2.78 (m, 2H), 2.75–2.67 (m, 2H), 2.36–2.25 (m, 2H), 1.32 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 166.2, 165.7, 143.1, 122.1, 102.7, 59.5, 30.8, 28.9, 27.2, 14.4.

2.2.5. 2-Amino-5,6,7,8-tetrahydro-4H-cyclohepta[b] thiophene-3-carbonitrile (**3e**)

Yellow solid. mp 125–126 °C (lit. [20] mp 126 °C). IR (KBr): ν. 3443, 3310, 2203 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.50 (bs, 2H), 2.64–2.54 (m, 4H), 1.85–1.77 (m, 2H), 1.68–1.60 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 158.1, 136.9, 123.7, 115.9, 91.7, 31.9, 29.9, 29.4, 28.1, 27.2.

2.2.6. Ethyl 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b] thiophene-3-carboxylate (**3f**)

Yellow solid. mp 87–88 °C (lit. [20] mp 89 °C). IR (KBr): ν. 3397, 3301, 1652 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.76 (bs, 2H), 4.28 (q, *J* = 7.2 Hz, 2H), 2.97 (t, *J* = 5.6 Hz, 2H), 2.57 (t, *J* = 5.6 Hz, 2H), 1.85–1.76 (m, 2H), 1.68–1.56 (m, 4H), 1.34 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 165.9, 159.8, 138.0, 121.3, 107.6, 59.6, 32.1, 29.0, 28.7, 27.8, 26.9, 14.4.

2.2.7. 2-Amino-4-methylthiophene-3-carbonitrile (**3g**)

Yellow solid. mp 118 °C (lit. [22] mp 118–119 °C). IR (KBr): ν. 3419, 3325, 2204 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.96 (s, 1H), 4.75 (bs, 2H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 162.3, 135.8, 115.4, 105.2, 90.8, 15.3.

2.2.8. Ethyl 2-amino-4-methylthiophene-3-carboxylate (**3h**)

Yellow solid. mp 78 °C (lit. [23] mp 76–78 °C). IR (KBr): ν. 3411, 3301, 1651 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 6.03 (bs, 2H), 5.83 (s, 1H), 4.28 (q, *J* = 7.2 Hz, 2H), 2.28 (s, 3H), 1.35 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 172.7, 160.9, 127.3, 114.7, 104.0, 60.6, 20.1, 13.1.

2.2.9. 2-Amino-4,5-dimethylthiophene-3-carbonitrile (**3i**)

Yellow solid. mp 142 °C (lit. [24] mp 141–142 °C). IR (KBr): ν. 3432, 3335, 2200 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.58 (bs, 2H), 2.15 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 158.9, 129.7, 117.3, 115.3, 90.9, 22.71, 13.1.

2.2.10. Ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (**3j**)

Yellow solid. mp 94 °C (lit. [24] mp 91–92 °C). IR (KBr): ν. 3403, 3303, 1648 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.89 (bs, 2H), 4.27 (q, *J* = 7.2 Hz, 2H), 2.16 (s, 3H), 2.15 (s, 3H), 1.35 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 166.1, 161.0, 130.4, 113.9, 107.7, 59.5, 14.8, 14.4, 12.3.

2.2.11. 2-Amino-4-ethyl-5-methylthiophene-3-carbonitrile (**3k**)

Yellow solid. mp 154 °C (lit. [25] mp 155–159 °C). IR (KBr): ν. 3430, 3321, 2199 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.60 (bs, 2H), 2.47 (q, *J* = 7.6 Hz, 2H), 2.16 (s, 3H), 1.14 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 159.6, 136.4, 118.4, 115.7, 89.1, 21.0, 14.3, 12.2.