



## Synthesis, characterization, and antifungal evaluation of novel 1,2,3-triazolium-functionalized starch derivative

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### ABSTRACT

1,2,3-Triazolium-functionalized starch derivative was obtained by straightforward quaternization of the synthesized starch derivative bearing 1,2,3-triazole with benzyl bromide by combining the robust attributes of cuprous-catalyzed azide-alkyne cycloaddition. These novel starch derivatives were characterized by FTIR, UV-vis, <sup>1</sup>H NMR, <sup>13</sup>C NMR, and elemental analysis. Their antifungal activities against *Colletotrichum lagenarium*, *Watermelon fusarium*, and *Phomopsis asparagi* were investigated by hypha measurement *in vitro*. The fungicidal assessment revealed that compared with starch and starch derivative bearing 1,2,3-triazole with inhibitory indices of below 15% at 1.0 mg/mL, 1,2,3-triazolium-functionalized starch derivative had superior antifungal activity with inhibitory rates of over 60%. Especially, the best inhibitory index of 1,2,3-triazolium-functionalized starch derivative against *Colletotrichum lagenarium* attained 90% above at 1.0 mg/mL. The results obviously showed that quaternization of 1,2,3-triazole with benzyl bromide could effectively enhance antifungal activity of the synthesized starch derivatives. The synthetic strategy described here could be utilized for the development of starch as novel antifungal biomaterial.

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## 1. Introduction

As the main carbohydrate storage reserve for plants, starch is composed of anhydroglucose units (AGU) linked together by  $\alpha$ -glucosidic bonds [1–3]. A number of interesting properties such as source universality, renewable, non-toxicity, biodegradable, and biocompatible facilitate a certain degree of applications of starch in biomedicine, biomaterials, and textile areas [4–7]. Rich in free available hydroxyl groups of anhydroglucose units, starch can be functionalized by chemical modification *via* introduction of the individual functional moieties to further improve the bioactivities and broaden application scopes of new valuable biomaterials based on starch [1,5,8–10].

1,2,3-Triazole moiety formed by cuprous-catalyzed azide-alkyne cycloaddition (CuAAC) has emerged as attractive heterocyclic compounds in biochemistry and pharmaceutical research

due to their wide range of biological properties, such as antimicrobial, anticancer, and antimalarial [11–14]. These interesting biological properties can facilitate the chemical modification of starch with 1,2,3-triazoles [15,16]. Zhang et al. prepared a novel oral protein drug delivery made of starch nanoparticles as backbone and poly(L-glutamic acid) as graft chains by click reaction and these copolymer nanoparticles could protect the insulin against harmful gastric environment as well as controlling the drug release [17]. Tan et al. synthesized four different starch-linked-1,2,3-triazole derivatives bearing electron-withdrawing groups *via* 1,3-dipolar cycloaddition and further evaluated for *in vitro* antibacterial potential [18]. Besides, 1,2,3-triazolium cations have recently been developed by quaternization of 1,2,3-triazoles with halogenide [19,20]. However, there are very few reports on synthesis of starch derivatives bearing 1,2,3-triazolium cations, and the effect of quaternization of 1,2,3-triazole moieties with benzyl bromide on the bioactivity of starch derivative was still unknown.

In this paper, 1,2,3-triazolium-functionalized starch was prepared by quaternization of starch derivative bearing 1,2,3-triazole with benzyl bromide. The chemical structures of the derivatives were characterized by FTIR, UV-vis, <sup>1</sup>H NMR, <sup>13</sup>C NMR, and elemental analysis. Three plant-threatening fungi, *Colletotrichum*

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*lagenarium* (*C. lagenarium*), *Watermelon fusarium* (*W. fusarium*), and *Phomopsis asparagi* (*P. asparagi*) were selected to evaluate the antifungal property by hypha measurement *in vitro*.

## 2. Experimental

### 2.1. Material

Soluble starch from potato (granules) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *N*-Bromosuccinimide (NBS), triphenylphosphine (TPP), 3-butyn-1-ol, and benzyl bromide were purchased from the Sigma-Aldrich Chemical Corp (Shanghai, China). The other reagents were all analytical grade and used as received.

### 2.2. Analytical methods

#### 2.2.1. Fourier transform infrared (FTIR) spectroscopy

All spectra were recorded on a Jasco-4100 Fourier Transform Infrared Spectrometer (JASCO Co., Ltd., Japan) at 25 °C in the transmittance mode. About 1 mg of sample with 100 mg of KBr was fully grinded and mixed. The mixed samples were pressed into pills with a compressor and prepared pellets were used for studies. All spectra were scanned against a blank KBr pellet background in the range of 4000–400 cm<sup>-1</sup> with resolution of 4.0 cm<sup>-1</sup>.

#### 2.2.2. Ultraviolet–visible (UV–vis) spectroscopy

The UV–vis spectra were carried out at 200–400 nm using a 3–5 mm quartz cuvette using a TU-1810 UV spectrometer (General Instrument Co., Ltd., China). For this analysis, 5 mL of 50 µg/mL aqueous solution of starch and starch derivatives were put in a cuvette for measurement. All the measurements were carried out at 25 °C.

#### 2.2.3. Nuclear magnetic resonance (NMR) spectroscopy

<sup>1</sup>H Nuclear magnetic resonance (<sup>1</sup>H NMR) and <sup>13</sup>C Nuclear magnetic resonance (<sup>13</sup>C NMR) spectra were all recorded on a Bruker AVIII-500 Spectrometer (Bruker Tech. and Serv. Co., Ltd., Switzerland) at 25 °C using DMSO-*d*<sub>6</sub> or D<sub>2</sub>O as solvent. Chemical shifts (δ ppm) were referenced to tetramethylsilane (TMS).

#### 2.2.4. Elemental analysis

The elemental analyses by combustion were used to quantitatively assess the extent of functionalization (degree of substitution) in starch derivatives. The analyses of elemental carbon, hydrogen, and nitrogen in starch derivatives were performed on a Vario EL III (Elementar, Germany). The degrees of substitution (DS) of starch derivatives were calculated on the basis of the percentages of carbon and nitrogen according to the following equations [21]:

$$DS_1 = \frac{M_C \times n_1}{M_N \times n_2 \times W_{C/N}} \quad (1)$$

$$DS_2 = \frac{M_N \times n_2 \times DS_1 \times W_{C/N} - M_C \times n_1}{n_3 \times M_C} \quad (2)$$

$$DS_3 = \frac{M_N \times n_2 \times DS_1 \times W_{C/N} - M_C \times n_1 - n_3 \times M_C \times DS_2}{n_4 \times M_C} \quad (3)$$

where  $DS_1$ ,  $DS_2$ , and  $DS_3$  represent the degrees of substitution of azido in 6-azido-6-deoxy starch, 1,2,3-triazole in starch derivative bearing 1,2,3-triazole, and 1,2,3-triazolium in starch derivative bearing 1,2,3-triazolium;  $M_C$  and  $M_N$  are the molar mass of carbon and nitrogen,  $M_C = 12$ ,  $M_N = 14$ ;  $n_1$  and  $n_2$  are the number of carbon and nitrogen of 6-azido-6-deoxy starch,  $n_1 = 6$ ,  $n_2 = 3$ ;  $n_3$  is the

number of carbon of 3-butyn-1-ol,  $n_3 = 4$ ;  $n_4$  is the number of carbon of benzyl group,  $n_4 = 7$ ;  $W_{C/N}$  represents the mass ratio between carbon and nitrogen.

### 2.2.5. Thermogravimetric analysis (TGA) and differential thermogravimetry (DTG)

The thermal stability of samples was evaluated using a thermal analyzer (Mettler 5MP/PF7548, Mettler-Toledo, Switzerland) at a heating rate of 10 °C/min with temperature range from 50 °C to 800 °C and nitrogen was used as the purge gas.

### 2.3. Synthesis of starch derivatives

#### 2.3.1. Synthesis of 6-bromo-6-deoxy starch (A) and 6-azido-6-deoxy starch (B)

6-Bromo-6-deoxy starch (A) and 6-azido-6-deoxy starch (B) were prepared according to the previously reported procedure [22]. Briefly, soluble starch (3.24 g, 20 mmol AGU) was suspended in 80 mL of anhydrous DMF and stirred at 120 °C for 1 h. After the slurry was allowed to cool to 90 °C, anhydrous LiBr (3.47 g, 40 mmol) was added. After starch was dissolved completely and cooled to room temperature, *N*-bromosuccinimide (NBS, 14.24 g, 80 mmol) and triphenylphosphine (TPP, 20.99 g, 80 mmol) were added to this solution. The reaction mixture was heated to 80 °C for 3 h under an argon atmosphere. The product was isolated by adding the reaction mixture slowly to 400 mL of absolute ethanol, followed by filtration. Then the precipitate was extracted in a Soxhlet apparatus with ethanol and acetone for 48 h, respectively. The 6-bromo-6-deoxy starch was obtained by freeze-drying overnight in vacuum. For the preparation of 6-azido-6-deoxy starch, 6-bromo-6-deoxy starch (2.25 g, 10 mmol) was dissolved in 40 mL of DMSO, and NaN<sub>3</sub> (1.3 g, 20 mmol) was added to the solution. The solution was heated to 80 °C and stirred for 24 h under an argon atmosphere. The product was isolated by pouring the reaction solution into 200 mL of absolute ethanol. The precipitate was collected by filtration, and washed with acetone. After being dialyzed against deionized water for 2 days to remove the probable remained sodium azide, the 6-azido-6-deoxy starch was obtained by freeze-drying. Yield: 76.52%. Elemental analysis: C(36.87%), N(18.34%), H(5.22%), C/N: 2.01. DS<sub>azido</sub>: 0.85. FTIR: ν 3405, 2923, 2105, 1041 cm<sup>-1</sup>. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>): δ 5.72–3.30 (pyranose rings), 3.77 (CH<sub>2</sub>N<sub>3</sub>) ppm. <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>): δ 100.22–70.26 (pyranose rings), 51.69 (CH<sub>2</sub>N<sub>3</sub>) ppm.

#### 2.3.2. Synthesis of starch derivative bearing 1,2,3-triazole (C)

6-Azido-6-deoxy starch (187 mg, 1 mmol) was dissolved in 20 mL of DMSO, cuprous iodide (19 mg, 0.1 mmol), triethylamine (0.14 mL, 1 mmol), and 3-butyn-1-ol (0.23 mL, 3 mmol) were added, and the solution was stirred at 75 °C for 24 h under an argon atmosphere. The mixture was poured into 100 mL of acetone to precipitate the product. The precipitate was filtered and washed carefully with acetone for three times. The probable remained reagents were dialyzed against deionized water for 2 days, the starch derivative bearing 1,2,3-triazole was obtained by freeze-drying. Yield: 92.08%. Elemental analysis: C(42.20%), N(13.03%), H(6.21%), C/N: 3.12. DS<sub>triazole</sub>: 0.83. FTIR: ν 3401, 2923, 1554, 1045, 794 cm<sup>-1</sup>. The UV λ<sub>max</sub> was 220 nm. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>): δ 7.62 (triazole-5-H), 5.68–3.10 (pyranose rings), 4.69 (CH<sub>2</sub>OH), 3.87 (NCH<sub>2</sub>CH), 2.48 (CH<sub>2</sub>CH<sub>2</sub>OH) ppm. <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>): δ 144.58 (triazole-4-C), 123.99 (triazole-5-C), 99.98–69.76 (pyranose rings), 60.74 (CH<sub>2</sub>OH), 50.44 (NCH<sub>2</sub>CH), 29.21 (CH<sub>2</sub>CH<sub>2</sub>OH) ppm.

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