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Synthesis and Plant-induced Resistance of D-glucosamine Derivatives

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ABSTRACT A series of new D-glucosamine derivatives was designed and synthesized involving five steps procedure. The title D-glucosamine derivatives exhibited moderate fungicidal activity in greenhouse. It was indicated that these D-glucosamine derivatives can cause the plant-induced resistance.

KEYWORDS Synthesis, D-Glucosamine derivatives, Plant-induced resistance, Fungicidal activity.

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INTRODUCTION

Plant can produce resistance when using external elicitors such as physical, chemical, or biological factors.[1,2] More than 100 chemicals have been used as activators, such as plant extracts, proteins, lipid compounds, carbohydrates, amino acids, and their derivatives.[3]

D-Glucosamine is a significant component of various carbohydrates, which has important biochemical effects. It has been reported that D-glucosamine can stimulate the expression of specific genes in plants, which can induce the activation of plant defense responses, inhibiting the formation of disease. Takezawa[4] reported that chitooligosaccharides could enable plants to initiate their own defense system to avoid from fungal infection. In fact, chitooligosaccharides had an effect on Ca^{2+} transmission and induce resistance to fungal pathogens. It had been found that rice which inoculated with chitooligosaccharides showed better resistance against *Pyricularia oryzae*.[5,6] In 2003, Guo *et al*.[7] revealed that bioactivities of phenylalanine ammonia-lyase and superoxide dismutase in cotton cells increased by treating with chitooligosaccharides. The calcium messenger system is involved during the inducement of chitooligosaccharides. Chris[8] treated carrots with chitooligosaccharides and found that chitooligosaccharides could induce carrots to produce antibodies against *Sclerotinia sclerotiorum*. Dai[9] synthesized acetylglucoside and acetolactose esters, and the antiviral (tobacco mosaic virus [TMV]) activity of the target compounds was determined by half leaf spot method in 2009. Their antiviral (TMV) activity was better than 2,4-dioxohexahydro-1,3,5-triazine. Therefore, glucose and lactose derivatives could improve antiviral activity.

Chitinase is well known for its function as pathogenesis-related proteins. The chitin of the pathogen is first hydrolyzed by chitinase into chitooligosaccharides and then further hydrolyzed to N-acetylamino glucose in plant. Although chitin is absent in plants, chitinase is widely found in the vegetative organs, flowers, seeds, and embryos of higher plants. Chitinase was proved to function mainly in the hydrolysis of chitin 1,4-glucosidic bond and obtain the corresponding N-acetylglucosamine derivative. Therefore, the activation of chitinase in plant can destroy bacterial cell

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walls, thus improve plant disease resistance. It is meaningful in plant disease prevention and control.

In view of these facts mentioned above, and also as a part of our work on the synthesis of bioactive lead compounds for drug discovery,[10-35] a series of D-glucosamine analogues was designed and synthesized. The plant immune activating activity was tested using *in vivo* fungicidal activity.

RESULTS AND DISCUSSION

Synthesis

The synthetic route of title D-glucosamine derivatives is outlined in **Scheme 1**. For the synthesis of intermediate **2**, the reaction temperature must be in ice water condition. After the reaction was completed, the mixture was filtered and washed by cold ether. As following step, the acetic anhydride was added dropwise slowly, the higher temperature will oxidize the glucosamine. Then, the mixture was poured into the ice water to give white solid **3**. In the synthesis of intermediate **4**, the sodium acetate needs to grind. If not, the bulk sodium acetate will be entrapped by the rapidly occurring solid product resulting to low yield. It is noted that the synthesis of the target products **5a-5t** was easily carried out after the drying of intermediate **4**.

The structures of target compounds **5a-5t** were confirmed by spectral and elemental analytical data.

Induced resistance test results of 5a-5m

The induced resistance test of compounds **5a-5m** had been tested according to our previous work and the results are listed in **Table 1**. Thiazole zinc, chlorothalonil, and dimethomorph were used as positive control.

Compounds **5i** and **5l** exhibited induced resistance against *P. syringae pv. lachrymans* at concentration of 50 mg/L. Especially, compound **5i** was better than that of control 20% thiazole zinc suspension. Compound **5g** possessed moderate effect against *C. cassiicola* and *P. infestans* at 50 mg/L, which is a little weaker than that of control 75% chlorothalonil WP and 50% dimethomorph WP. Furthermore, compound **5h** possessed good induced resistance against *P. infestans*. From the preliminary data, most of these compounds proved to be efficient in induced resistance against *P. syringae pv. lachrymans*, *C. cassiicola*, and *P. infestans*. It was pointed out that compound in which substituent was on the ortho position displayed better bioactivities against *P. syringae pv. lachrymans*, such as compounds **5g**, **5l**, and **5m**. As for *C. cassiicola* and *P. infestans*, the influence of the position of substituents and the types of the substituents were not obvious. Finally, the induced resistance of compound **5a~5m** against *P. infestans* was better than the other two types of diseases.

EXPERIMENTAL SECTION

Materials

Melting points were determined by an X-4 apparatus (Taike, Beijing, China) and are uncorrected. Proton nuclear magnetic resonance (^{1}HNMR) spectra were measured on a Bruker AV-500 instrument using tetramethylsilane as an internal standard and $CDCl_3$ as the solvent. Fourier transform infrared (FTIR) was obtained on a Nicolet 670 FTIR instrument. All the reagents were of analytical grade or freshly prepared before use. The course of the reactions was monitored by thin-layer chromatography (TLC); analytical TLC was performed on silica gel GF_{254}.

Synthesis of D-glucosamine derivatives

(2R,3R,4S,5S,6R)-3-((E)-benzylideneamino)-6-(hydroxymethyl)tetrahydro-2H-pyran-2,4,5-triol (1)

The benzaldehyde (5.4 mL, 53 mmol) was dropwise added to the mixture of glucosamine hydrochloride (10 g, 46 mmol) and NaOH solution (1.2 mol/L) in ice bath, then stirred for 30 min to give white solid. The crude product was filtered and washed by water, ether and dried. The product **1** was isolated as white solid, yield 90%, m.p. 128–129°C (Ref. Li and Liu[36] M.p. 168–170°C).

(2S,3R,4S,5S,6R)-6-(acetoxymethyl)-3-((E)-benzylideneamino) tetrahydro-2H-pyran-2,4,5-triyl triacetate (2)

To a solution of intermediate **1** (12.06 g, 45 mmol) in pyridine (60 mL), acetic anhydride **4** (40 mL) was added slowly in ice bath. Then, the mixture was stirred for 30 min. The mixture was poured into ice water to obtain white solid **2** after stirring for 6 h, and washed by water and ether in sequence, yield 95%, m.p. 157–159°C. (Ref. Akagi *et al*.[37] M.p. 147–148°C).

(2S,3R,4S,5S,6R)-6-(acetoxymethyl)-3-aminotetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride (3)

To a solution of intermediate **2** (4.4 g, 10 mmol) in anhydrous acetone (100 mL), a mixture of concentrated HCl (1 mL) and methanol (4 mL) was added dropwise at room temperature. Solid appeared immediately and the mixture was stirred vigorously for 1 h, followed by the addition of ether (30 mL). After that, the reaction mixture was stirred for another 1 h in ice bath. The precipitated solid was filtered off, then washed with water, ether and dried to give **3**, yield 87%, m.p. >300°C. (Ref. Lloyd and Stacey[38] M.p. 230°C, decomposition).

(2S,3R,4S,5S,6R)-6-(acetoxymethyl)-3-aminotetrahydro-2H-pyran-2,4,5-triyl triacetate (4)

To a solution of intermediate **3** (3.84 g, 10 mmol) in H_2O (50 mL) was added sodium acetate (1.64 g, 20 mmol) at room temperature. White solid was separated out immediately and then mixture was stirred for 1 h. The reaction mixture was extracted with chloroform (20 mL×3). The combined extract was distilled on a rotary evaporator to give crude white solid. The crude solid was washed and dried to give the intermediate **4**, yield 86%, m.p 127–129°C. (Ref. Inoue *et al*.[39] M.p.132°C).

General procedure for the preparation of glucosamine derivatives (5a-5t)

To a solution of intermediate **4** (2.11 g, 5.5 mmol) and DCC (1.14 g, 5.5 mmol) in dimethylformamide (DMF)

(20 mL), substituted benzoic acid (5 mmol) was added slowly. The mixture was stirred at room temperature for 24 h, then precipitate was removed by filtration. To the filtrate was added acetic acid (1 mL), and mixture was stirred at room temperature until no more precipitate was formed. Subsequently, the precipitate was filtered off at low temperature and washed using dichloromethane (3 mL). The crude product was recrystallized with anhydrous ethanol to afford the corresponding glucosamine derivatives **5a-5t**.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(4-(tert-butyl) benzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5a)

White solid, yield 47.5%, m.p. 171–172°C; IR (KBr) υ (cm^{-1}): 3401, 739 (NH). ^{1}HNMR (500 MHz, CDCl$_3$) δ: 1.32 (s, 9H, -CH$_3$), 2.00 (s, 3H, -CH$_3$), 2.02 (s, 3H, -CH$_3$), 2.05 (s, 3H, -CH$_3$), 2.08 (s, 3H, -CH$_3$), 3.81–3.87 (m, 1H, -CH), 4.14–4.18 (m, 1H, -CH$_2$-), 4.28–4.31 (m, 1H, -CH$_2$-), 4.49–4.54 (m, 1H, -CH), 5.20–5.25 (m, 2H, -CH), 5.79 (d, *J* = 8.0 Hz, 1H, -CH), 6.22 (d, *J* = 4.5 Hz, 1H, -NH), 7.41 (d, *J* = 8.4 Hz, 2H, Ph-H), 7.47 (d, *J* = 8.4 Hz, 2H, Ph-H). ESI-MS: 508.2 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-benzamidotetrahydro-2H-pyran-2,4,5-triyl triacetate (5b)

White solid, yield 48.0%, m.p. 233–234°C; IR (KBr) υ (cm^{-1}): 3350, 725 (NH). ^{1}HNMR (500 MHz, CDCl$_3$) δ: 2.02 (s, 3H, -CH$_3$), 2.09 (s, 3H, -CH$_3$), 2.10 (s, 3H, -CH$_3$), 2.14 (s, 3H, -CH$_3$), 3.86–3.90 (m, 1H, -CH), 4.17–4.20 (m, 1H, -CH$_2$-), 4.31–4.35 (m, 1H, -CH$_2$-), 4.51–4.66 (m, 1H, -CH), 5.23–5.30 (m, 2H, -CH),5.82 (d, *J* = 8.0 Hz, 1H, -CH), 6.24 (d, *J* =2.6 Hz, 1H, -NH), 7.43 (t, *J* = 7.2 Hz, 2H, Ph-H), 7.53–7.56 (m, 1H, Ph-H), 7.69 (d, *J* = 7.1 Hz, 2H, Ph-H). ESI-MS: 452.1 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(3-nitrobenzamido) tetrahydro-2H-pyran-2,4,5-triyl triacetate (5c)

White solid, yield 53.1%, m.p. 196–197°C; IR (KBr) υ (cm^{-1}): 3350, 722 (NH). ^{1}HNMR (500 MHz, CDCl$_3$) δ: 2.07 (s, 3H, -CH$_3$), 2.10 (s, 3H, -CH$_3$), 2.12 (s, 3H, -CH$_3$), 2.13 (s, 3H, -CH$_3$), 3.96–4.00 (m, 1H, -CH), 4.19–4.23 (m, 1H, -CH$_2$-), 4.32–4.36 (m, 1H, -CH$_2$-), 4.62–4.70 (m, 1H, -CH), 5.25–5.30 (m, 1H, -CH),5.43–5.49 (m, 1H, -CH), 5.85 (d, *J* = 8.8 Hz, 1H, -CH), 6.97–7.03 (m, 1H, -NH), 7.62 (t, *J* = 8.2 Hz, 1H, Ph-H), 8.17 (d, *J* = 7.7 Hz, 1H, -CH), 8.31 (d, *J* = 8.2 Hz, 1H, Ph-H), 8.52 (s, 1H, Ph-H). ESI-MS: 497.1 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(3-chlorobenzamido) tetrahydro-2H-pyran-2,4,5-triyl triacetate (5d)

White solid, yield 49.3%, m.p. 194–195°C;IR (KBr) υ (cm^{-1}): 3346, 759 (NH), 698 (C-Cl). ^{1}HNMR (500 MHz, CDCl$_3$) δ: 2.04 (s, 3H, -CH$_3$), 2.10 (s, 3H, -CH$_3$), 2.11 (s, 3H, -CH$_3$), 2.13 (s, 3H, -CH$_3$), 3.88–3.92 (m, 1H, -CH), 4.17–4.21 (m, 1H, -CH$_2$-), 4.30–4.35 (m, 1H, -CH$_2$-), 4.57–4.60 (m, 1H, -CH), 5.22–5.36 (m, 2H, -CH), 5.80 (d, *J* = 8.6 Hz, 1H, -CH), 6.44 (d, *J* = 9.0 Hz, 1H, -NH), 7.35 (t, *J* = 7.8 Hz, 1H, Ph-H), 7.48 (d, *J* = 7.1 Hz, 1H, Ph-H), 7.56 (d, *J* = 7.8 Hz, 1H, Ph-H), 7.72 (s, 1H, Ph-H). ESI-MS: 487.1 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(3-fluorobenzamido) tetrahydro-2H-pyran-2,4,5-triyl triacetate (5e)

White solid, yield 63.2%, m.p. 222–223°C; IR (KBr) υ (cm^{-1}): 3342, 755 (NH), 1115 (C-F). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 2.04 (s, 3H, -CH$_3$), 2.10 (s, 3H, -CH$_3$), 2.11 (s, 3H, -CH$_3$), 2.13 (s, 3H, -CH$_3$), 3.88–3.92 (m, 1H, -CH), 4.17–4.21 (m, 1H, -CH), 4.30–4.35 (m, 1H, -CH), 4.57–4.60 (m, 1H, -CH), 5.22–5.36 (m, 2H, -CH$_2$-), 5.80 (d, *J* =8.6 Hz, 1H, -CH), 6.44 (d, *J* = 9.0 Hz, 1H, -NH), 7.35 (t, *J* = 7.8 Hz, 1H, Ph-H), 7.48 (d, *J* =7.1 Hz, 1H, Ph-H), 7.56 (d, *J* = 7.8 Hz, 1H, Ph-H), 7.72 (s, 1H, Ph-H). ESI-MS: 470.1 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(3-butylbenzamido) tetrahydro-2H-pyran-2,4,5-triyl triacetate (5f)

White solid, yield 56.3%, m.p. 203–204°C; IR (KBr) υ (cm^{-1}): 3338, 759 (NH), 1437(-CH$_2$-). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 0.89 (t, *J*=6.7 Hz, 3H, -CH$_3$), 1.32–1.35 (m, 4H, -CH$_2$-),1.61–1.65 (m, 2H, -CH$_2$-), 2.02 (s, 3H, CH$_3$), 2.09 (s, 3H, -CH$_3$), 2.10 (s, 3H, -CH$_3$), 2.14 (s, 3H, -CH$_3$), 2.63 (t, *J*=7.8 Hz, 2H, -CH$_2$-), 3.86–3.89 (m, 1H, -CH), 4.16-4.21 (m, 1H, -CH$_2$-), 4.30-4.35 (m, 1H, -CH$_2$-), 4.55–4.62 (m, 1H, -CH), 5.22-5.31 (m, 2H, -CH), 5.81 (d, *J*=8.8 Hz, 1H, -CH), 6.16–6.19 (m, 1H, -NH), 7.24 (d, *J*=8.0 Hz, 2H, Ph-H), 7.61 (d, *J*=8.2 Hz, 2H, Ph-H). ESI-MS: 522.2 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(2-methoxybenzamido) tetrahydro-2H-pyran-2,4,5-triyl triacetate (5g)

White solid, yield 62.1%, m.p. 184–185°C; IR (KBr) υ (cm^{-1}): 3354, 637 (NH). ^{1}HNMR (500 MHz, CDCl$_3$) δ: 1.98 (s, 3H, -CH$_3$), 2.05 (s, 3H, -CH$_3$), 2.08 (s, 3H, -CH$_3$), 2.12 (s, 3H, -CH$_3$), 3.95 (s, 3H, -CH$_3$), 3.89 (s, 2H, -CH$_2$-), 4.15–4.18 (m, 1H, -CH), 4.29–4.32 (m, 1H, -CH), 4.56-4.62 (m, 1H, -CH), 5.18 (t, *J* = 9.7 Hz, 1H, -CH), 5.32 (d, *J* = 9.4 Hz, 1H, -CH), 5.81 (d, *J* = 8.8 Hz, 1H, -NH), 6.96 (t, *J* = 18.1 Hz, 1H, Ph-H), 7.45–7.48 (m, 1H, Ph-H), 7.77 (d, *J* = 9.2 Hz, 1H, Ph-H). ESI-MS: 482.2 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(3-nitrobenzamido) tetrahydro-2H-pyran-2,4,5-triyl triacetate (5h)

White solid, yield 61.5%, m.p. 214–215°C; IR (KBr) υ (cm^{-1}): 3305, 694 (NH), 1490, 1303(-NO$_2$). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 2.04 (s, 3H, -CH$_3$), 2.09 (s, 3H, -CH$_3$), 2.10 (s, 3H, -CH$_3$), 2.11 (s, 3H, -CH$_3$), 2.14 (s, 3H, -CH$_3$),3.87–3.90 (m, 1H, -CH), 4.17-4.21 (m, 1H, -CH$_2$-), 4.30–4.35 (m, 1H, -CH$_2$-), 4.55–4.58 (m, 1H, -CH), 5.26-5.28 (m, 2H, -CH), 5.83 (d, *J*= 8.7 Hz, 1H, -CH), 6.47 (d, *J* = 9.2 Hz, 1H, -NH), 7.87 (d, *J* = 8.8 Hz, 2H, Ph-H), 8.29 (d, *J* = 8.7 Hz, 2H, Ph-H). ESI-MS: 497.1 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(3-methylbenzamido) tetrahydro-2H-pyran-2,4,5-triyl triacetate (5i)

White solid, yield 58.4%, m.p. 161–162°C; IR (KBr) υ (cm^{-1}): 3326, 751 (NH). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 2.01 (s, 3H, -CH$_3$), 2.05 (s, 3H, -CH$_3$), 2.09 (s, 3H, -CH$_3$),2.13 (s, 3H, -CH$_3$), 2.40 (s, 3H, -CH$_3$), 3.87-3.90 (m, 1H, -CH), 4.16–4.20 (m, 1H, -CH$_2$-), 4.30–4.34 (m, 1H, -CH$_2$-), 4.56–4.63 (m, 1H, -CH), 5.25–5.31 (m, 2H, -CH), 5.80 (d, *J* = 8.8 Hz, 1H, -CH), 6.27 (d, *J* = 9.5 Hz,1H, -NH), 7.22 (d, *J* = 8.0 Hz, 2H, Ph-H),7.60 (d, *J* = 8.2 Hz, 2H, Ph-H). ESI-MS: 466.2 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(3-aminobenzamido) tetrahydro-2H-pyran-2,4,5-triyl triacetate (5j)

White solid, yield 65.3%, m.p. 151–152°C; IR (KBr) υ (cm^{-1}): 3460, 3239 (-NH$_2$), 3350, 620 (NH). ^{1}HNMR (500

MHz, CDCl$_3$) δ: 2.01 (s, 3H, -CH$_3$), 2.03 (s, 6H, -CH$_3$), 2.05 (s, 3H, -CH$_3$), 2.06 (s, 3H, -CH$_3$), 3.89–3.94 (m, 1H, -CH), 4.13–4.19 (m, 2H, -CH$_2$-), 4.32–4.35 (m, 1H, -CH), 4.57–4.65 (m, 1H, -CH), 5.27–5.33 (m, 1H, -CH), 5.82 (d, *J*= 10.5 Hz, 1H, -CH), 6.29 (d, *J*=9.8 Hz, 1H, -NH), 7.21 (d, *J*=8.0 Hz, 2H, Ph-H), 7.59 (d, *J*=8.2 Hz, 2H, Ph-H). ESI-MS: 467.2 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(2-methylbenzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5k)

White solid, yield 66.1%, m.p. 122–123^{o}C;IR (KBr) υ (cm^{-1}): 3346, 714 (NH). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 2.00 (s, 3H, -CH$_3$), 2.07 (s, 3H, -CH$_3$), 2.09 (s, 3H, -CH$_3$), 2.13 (s, 3H, -CH$_3$), 2.11 (s, 3H, -CH$_3$), 2.14 (s, 3H, -CH$_3$), 3.89 (s, 1H, -CH), 3.96 (s, 3H, -CH$_3$), 4.13–4.22 (m, 1H, -CH$_2$-), 4.31–4.35 (m, 1H, -CH$_2$-), 4.58–4.65 (m, 1H, -CH), 5.19–5.24 (m, 1H, -CH), 5.30–5.35 (m, 1H, -CH), 5.82 (d, *J*=8.8 Hz, 1H, -CH), 6.95–7.02 (m, 1H, -NH), 7.07–7.10 (m, 1H, Ph-H), 7.46–7.50 (m, 1H, Ph-H), 7.77 (d, *J*=9.2 Hz, 1H, Ph-H), 8.12 (d, *J*=6.0 Hz, 1H, Ph-H). ESI-MS: 466.2 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(2-hydroxybenzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5l)

White solid, yield 54.7%, m.p. 201–202^{o}C; IR (KBr) υ (cm^{-1}): 3440, 1037(-OH), 3333, 743 (NH). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 2.06 (s, 3H, -CH$_3$), 2.08 (s, 3H, -CH$_3$), 2.12 (s, 3H, -CH$_3$), 2.16 (s, 3H, -CH$_3$), 3.47–3.50 (m, 1H, -OH), 3.70–3.75 (m, 1H, -CH),4.15–4.18 (m, 1H, -CH$_2$-), 4.29–4.32 (m, 1H, -CH$_2$-), 4.48–4.54 (m, 2H, -CH), 4.78 (d, *J* = 12.1 Hz,1H, -CH), 5.19–5.29 (m, 2H, -CH), 5.80 (d, *J*= 8.8 Hz, 1H, -CH), 5.94 (d, *J* = 9.6 Hz, 1H, -NH), 7.16–7.22 (m, 3H, Ph-H), 7.31-7.34 (m, 1H, Ph-H). ESI-MS: 468.1 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(2,4-dichlorobenzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5m)

White solid, yield 58.9%, m.p. 206–207^{o}C; IR (KBr) υ (cm^{-1}): 3293, 674 (NH), 788 (C-Cl). ^{1}HNMR (500 MHz, CDCl$_3$) δ: 2.05 (s, 3H, -CH$_3$), 2.06 (s, 3H, -CH$_3$), 2.11 (s, 3H, -CH$_3$), 2.14 (s, 3H, -CH$_3$), 3.87–3.90 (m, 1H, -CH), 4.14–4.17 (m, 1H, -CH$_2$-), 4.29–4.32 (m, 1H, -CH$_2$-), 4.45–4.51 (m, 1H, -CH), 5.15 (t, *J* = 9.8 Hz, 1H, -CH), 5.30–5.34 (m, 1H, -CH), 5.84 (d, *J*= 8.8 Hz, 1H, -CH), 6.35 (d, *J* = 9.4 Hz, 1H, -NH), 7.27–7.30 (m, 1H, Ph-H), 7.37–7.40 (m, 2H, Ph-H). ESI-MS: 521.3 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(4-fluorobenzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5n)

White solid, yield 67.0%, m.p. 177–178^{o}C; IR (KBr) υ (cm^{-1}): 3309, 686 (NH), 1151 (C-F). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 1.84 (s, 3H, -CH$_3$), 1.89 (s, 3H, -CH$_3$), 1.95 (s, 3H, -CH$_3$), 2.01 (s, 3H, -CH$_3$), 3.12–3.18 (m, 1H, -CH$_2$-), 3.41–3.45 (m, 1H, -CH$_2$-), 3.96–4.20 (m, 1H, -CH), 4.11–4.17 (m, 1H, -CH), 4.20–4.24 (m, 1H, -CH), 4.96–5.00 (m, 1H, -CH), 5.31–5.35 (m, 1H, -CH), 5.87–5.89 (m, 1H, -NH), 7.18–7.22 (m, 2H, Ph-H), 7.48–7.51 (m, 2H, Ph-H). ESI-MS: 470.4 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(2,3-dichlorobenzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5o)

White solid, yield 58.2%, m.p. 175–176^{o}C; IR (KBr) υ (cm^{-1}): 3280, 702 (NH), 616 (C-Cl). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 2.06 (s, 3H, -CH$_3$), 2.08 (s, 3H, -CH$_3$), 2.12 (s, 3H, -CH$_3$), 2.17 (s, 3H, -CH$_3$),3.85–3.88 (m, 1H, -CH), 4.15 (dd, *J* = 2 Hz, 2 Hz, H, -CH$_2$-), 4.29 (dd, *J* = 4.5 Hz, 4.6 Hz, 1H, -CH), 4.46 (q, *J* = 10.4 Hz, 1H, -CH), 5.17 (t, *J* = 9.8 Hz, 1H, -CH), 5.27 (t, *J* = 9.5 Hz, 1H, -CH-), 5.83 (d, *J*= 8.8 Hz, 1H, -CH-), 6.13 (d, *J* = 9.5 Hz, 1H, -NH), 7.23–7.25 (m, 2H, Ph-H), 7.50–7.55 (m, 1H, Ph-H). ESI-MS: 521.3 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(4-methoxybenzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5p)

White solid, yield 65.9%, m.p. 146–147^{o}C; IR (KBr) υ (cm^{-1}): 3334, 759 (NH), 1254, 1172, 1078, 1025(-C-O-C-). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 2.02 (s, 3H, -CH$_3$), 2.04 (s, 3H, -CH$_3$), 2.06 (s, 3H, -CH$_3$), 2.09 (s, 3H, -CH$_3$),3.49–3.52 (m, 1H, -CH), 3.85 (s, 3H, -OCH$_3$), 4.11–4.16 (m, 2H, -CH$_2$-), 4.15–4.29 (m, 1H, -CH), 4.56–4.60 (m, 1H, -CH), 5.23–5.29 (m, 1H, -CH), 5.80 (q, *J* = 9.5 Hz, 1H, -CH), 6.02 (d, *J* = 7.3 Hz, 1H, -NH), 6.89–6.91 (m, 2H, Ph-H), 7.54–7.56 (m, 2H, Ph-H). ESI-MS: 482.4 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(3-methylbenzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5q)

White solid, yield 66.3%, m.p. 188–189^{o}C; IR (KBr) υ (cm^{-1}): 3338, 690 (NH). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 2.00 (s, 3H, -CH$_3$), 2.08 (s, 3H, -CH$_3$), 2.09 (s, 3H, -CH$_3$), 2.12 (s, 3H, -CH$_3$), 2.38 (s, 3H, -CH$_3$), 3.85–3.89 (m, 1H, -CH), 4.16 (dd, *J* = 4.7 Hz, 2.1, 1H, -CH$_2$-), 4.29 (dd, *J* = 9.3 Hz, 7.8, 1H, -CH$_2$-), 4.56 (q, *J* = 9.4 Hz, 1H, -CH), 5.21 (t, *J* = 9.6 Hz, 1H, -CH), 5.28 (t, *J* = 10.5 Hz, 1H, -CH), 5.80 (d, *J* = 8.8 Hz, 1H, -CH), 6.31 (d, *J* = 9.5 Hz, 1H, -NH), 7.30–7.33 (m, 2H, Ph-H), 7.44 (d, *J* = 7.3 Hz, 1H, Ph-H), 7.54 (s, 1H, Ph-H). ESI-MS: 466.4 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(4-hydroxybenzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5r)

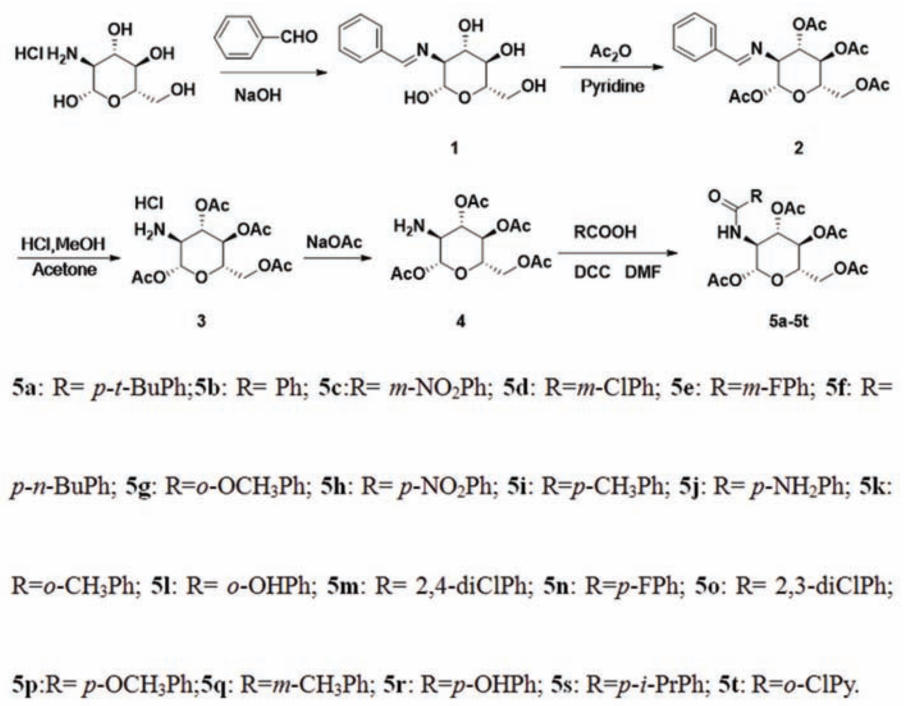
White solid, yield 65.3%, m.p. 179–180^{o}C; IR (KBr) υ (cm^{-1}): 3440, 1172(-OH), 3321, 649 (NH). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 2.00 (s, 3H, -CH$_3$), 2.02 (s, 3H, -CH$_3$), 2.04 (s, 3H, -CH$_3$), 2.05 (s, 3H, -CH$_3$), 2.14 (s, 3H, -CH$_3$), 3.81–3.87 (m, 1H, -CH$_2$-), 4.15–4.19 (m, 1H, -CH$_2$-), 4.31–4.34 (m, 1H, -CH), 4.50–4.53 (m, 1H, -CH), 5.25–5.27 (m, 2H, -CH), 5.33 (s, 1H, -OH), 5.80 (d, *J*=8.8 Hz, 1H, -CH), 6.45 (d, *J*=9.5 Hz, 1H, -NH), 7.24–7.27 (m, 2H, Ph-H), 7.46–7.51 (m, 2H, Ph-H). ESI-MS: 468.4 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(4-isopropylbenzamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5s)

White solid, yield 58.9%, m.p. 144–145^{o}C; IR (KBr) υ (cm^{-1}): 3301, 718 (NH). ^{1}HNMR (500 MHz, CDCl$_3$)δ: 0.93 (s, 6H, -CH$_3$), 1.22–1.28 (m, 1H, -CH), 1.96 (s, 3H, -CH$_3$), 1.99 (s, 3H, -CH$_3$), 2.01 (s, 3H, -CH$_3$), 2.04 (s, 3H, -CH$_3$), 3.81–3.87 (m, 1H, -CH), 4.11–4.13 (m, 1H, -CH$_2$-), 4.30–4.33 (m, 1H, -CH$_2$-), 4.51–4.54 (m, 1H, -CH), 5.26–5.28 (m, 2H, -CH), 5.82 (d, *J*=8.7 Hz, 1H, -CH), 6.47 (d, *J*=9.0 Hz, 1H, -NH), 7.21–7.23 (m, 2H, Ph-H), 7.43–7.48 (m, 2H, Ph-H). ESI-MS: 494.5 [M+H]$^+$.

(2S,3R,5S,6R)-6-(acetoxymethyl)-3-(2-chloronicotinamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (5t)

White solid, yield 54.3%, m.p. 221–222°C; IR (KBr) υ (cm^{-1}): 3297, 649 (NH), 747 (C-Cl). ^{1}HNMR (500 MHz, $CDCl_3$)δ: 2.06 (s, 3H, $-CH_3$), 2.07 (s, 3H, $-CH_3$), 2.11 (s, 3H, $-CH_3$), 2.15 (s, 3H, $-CH_3$), 3.88–3.91 (m, 1H, -CH), 4.15–4.18 (m, 1H, $-CH_2-$), 4.30–4.33 (m, 1H, $-CH_2-$), 4.44–4.49 (m, 1H, -CH), 5.17 (t, *J* = 9.7 Hz, 1H, -CH), 5.33 (q, *J* = 8.1 Hz, 1H, -CH), 5.88 (d, *J*=8.8 Hz, 1H, -CH), 6.48 (d, *J*=9.3 Hz, 1H, -NH), 7.33 (q, *J* = 4.7 Hz, 1H, Py-H), 7.84 (q, *J* =



5a: R= *p*-*t*-BuPh;**5b**: R= Ph; **5c**:R= *m*-NO_2Ph; **5d**: R=*m*-ClPh; **5e**: R=*m*-FPh; **5f**: R= *p*-*n*-BuPh; **5g**: R=*o*-OCH_3Ph; **5h**: R= *p*-NO_2Ph; **5i**: R=*p*-CH_3Ph; **5j**: R= *p*-NH_2Ph; **5k**: R=*o*-CH_3Ph; **5l**: R= *o*-OHPh; **5m**: R= 2,4-diClPh; **5n**: R=*p*-FPh; **5o**: R= 2,3-diClPh; **5p**:R= *p*-OCH_3Ph;**5q**: R=*m*-CH_3Ph; **5r**: R=*p*-OHPh; **5s**: R=*p*-*i*-PrPh; **5t**: R=*o*-ClPy.

Scheme 1: The synthetic route of D-glucosamine Derivatives 5a-5t

Table 1: The screening results of compound 5a-5m in induced resistance

Compound	*Pseudomonas syringae pv. lachrymans*		*Corynespora cassiicola*		*Phytophthora infestans*	
	Average infection index	Efficacy	Average infection index	Efficacy	Average infection index	Efficacy
5a	39.02	24.79	62.78	19.73	60.57	36.86
5b	48.14	7.21	55.48	29.06	59.38	38.11
5c	43.18	16.77	71.06	9.15	64.27	33.01
5d	57.32	−10.49	65.96	15.67	63.69	33.61
5e	49.83	3.94	83.95	−7.34	77.02	19.72
5f	42.82	17.46	62.45	20.15	77.85	18.85
5g	36.11	30.39	36.64	53.15	38.04	60.35
5h	38.52	25.75	58.06	25.77	50.43	47.43
5i	13.70	73.60	44.63	42.94	64.18	33.10
5j	40.60	21.74	70.71	9.59	65.26	31.98
5k	46.04	11.25	68.06	12.98	94.70	1.29
5l	31.09	40.08	78.06	0.20	85.45	10.93
5m	35.89	30.82	72.31	7.54	82.85	13.64
Solvent	50.07	3.49	72.41	7.42	89.00	7.23
Thiazole zinc	24.39	52.99	—	—	—	—
Chlorothalonil	—	—	21.21	72.88	—	—
Dimethomorph	—	—	—	—	27.84	70.98
Clean water	51.88	—	78.21	—	95.94	—
Empty	0.00	—	0.00	—	0.00	—

2 Hz, 1H, Py-H), 8.47 (q, J = 2 Hz, 1H, Py-H). ESI-MS: 487.1 $[M+H]^+$.

Induced resistance of glucosamine derivatives 5a-5m

The induced resistance of new compounds was tested using *in vivo* fungicidal activity. The original compounds were dissolved in acetone and DMF into 2.5% liquor. The 2.5% liquor was suitably diluted with sterilized distilled water. After that, the test concentration of **5a-5m** compounds was evenly sprayed to both sides of leaves. The plants were induced every 7 days carried out for 3 times. Inoculation was conducted 24 h after the third inducement. The test targets in this study, including *Pseudomonas syringae pv. lachrymans*, *Corynespora cassiicola*, and *Phytophthora infestans*, were inoculated by spraying.

CONCLUSIONS

Various substituted benzoic acids were introduced into D-glucosamine to afford D-glucosamine derivatives. D-glucosamine derivatives displayed good plant-induced resistance against *P. syringae pv. lachrymans*, *C. cassiicola*, and *P. infestans*, especially compounds **5g**, **5h**, **5i,** and **5l**. This study provides a meaningful and promising method for environmentally friendly pesticides.

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