An efficient synthesis of *N,N*-bis[2-(2-nitrophenylamino)ethyl]glycine glycosyl esters and antiviral activity

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N,N-bis[2-(2-nitrophenylamino)ethyl]glycine glycosyl esters were synthesised in good yields at room temperature using DMAP/Et_3N. All of the new synthesised compounds were of β-configuration. The compounds show good antiviral activity against tobacco mosaic virus(TMV).

Keywords: *N,N*-bis[2-(2-nitrophenylamino)ethyl]glycine, glycosyl esters, DMAP/Et_3N

Saccharide ester compounds possess broad biological activities.[1] A number of authors have reported the bioactivity of saccharide esters, and of the aryloxy carboxylic acids. They are applied widely in agricultural and medicinal chemistry, as well as plant virus inhibitors.

DMAP (4-dimethylaminopyridine) has been applied widely in organic synthesis.[2,3] DMAP can efficiently catalyse esterification reactions, but it has been rarely reported as a catalyst for the synthesis of *N,N*-bis[2-(2-nitrophenylamino)ethyl] glycine glycosyl esters by using *α*-*O*-acetylglycosyl bromide as the active group and *N,N*-bis [2-(2-nitrophenylamino)ethyl]glycine as lead compounds.

In order to find new plant virucides, we designed and synthesised a series of *O*-acetylglycosyl saccharide esters,[4,5] some of which displayed improved anti-TMV activity. Tobacco mosaic virus (TMV) is a phytovirus, that can harm plants. Considering the wide application of saccharide ester compounds and their potential to serve as antiviral agents, and in conjunction with our recent work on the search for new phytoantiviral agents,[4,5,6] we decided to develop a simpler method for the synthesis substituted saccharide ester compounds and designed novel *N,N*-bis[2-(2-nitrophenylamino)ethyl] glycine glycosyl esters. We selected DMAP as catalyst, Et_3N (triethylamine) was used as deacidification reagent, four new saccharide esters were synthesised in the system of DMAP/Et_3N. Experimental results show that DMAP is an efficient catalyst with yields of over 60%. The biological test results indicate that the antiviral activities of the synthesised compounds against tobacco mosaic virus (TMV) are up to 65.3%. The synthetic route is as follows (Scheme 1).

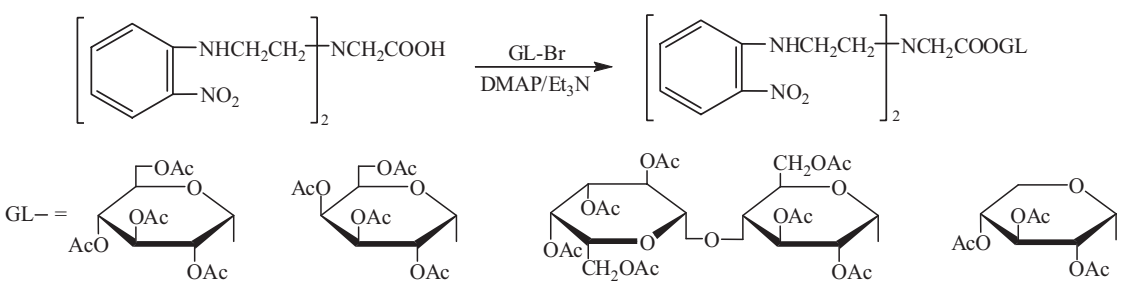
We found that the reaction with DMAP/Et_3N proceeded quickly and smoothly at room temperature under mild conditions and with a substantially increased yield and decrease in the reaction time compared with the phase transfer catalytic method (Table 2).

The preliminary biological tests showed that antiviral activities of the products are moderate, the antiviral activities of the synthesised compounds are up to 65.3%. The anti-TMV data given in Table 2 indicate that *O*-acetylglycosyls affect anti-TMV activity of the compounds, the inhibition rate of lactopyranosyl derivatives is higher than glucopyranosyl and galactopyranosyl analogues. The inhibiting percentage against TMV was expressed by calculating the withered spot number in the tobacco leaf, with a dosage of 0.001% and NS-83[7] (its inhibiting percentage reaches 80%) as control.

The products were confirmed by IR, ^1H NMR, MS and elemental analysis. The compounds **1–4** are of *β*-configuration in the system of DMAP/Et_3N. Their structure is same as compared with a phase transfer catalytic method. In the IR spectra of compounds **1–4**, two strong and wide bi-shoulder peaks of pyranose ring, appear at 1000–1100 cm^{-1} and 1200–1300 cm^{-1}, and the absorbance at 1720 cm^{-1} or so is attributed to the carbonyl group. In the ^1H NMR spectra, the assignments of the hydrogen atoms in peaks are very obvious, the coupling constant J_{1-2} between C_1–H_1 and C_2–H_2 is 7.20–7.92 Hz. Generally, the structures of saccharide esters can be determined according to chemical shift of C_1–H_1 NMR of pyranose and coupling constant of H_1–H_2. Because the coupling constant between C_1–H_1 and C_2–H_2 of *β*-configuration of saccharide ester is 7–10 Hz,[8] therefore, the synthesised compounds **1–4** have *β*-configuration (Table 1).

As far as we know, this was the first example of *N,N*-bis[2-(2-nitrophenylamino)ethyl] glycine glycosyl esters using DMAP/Et_3N method. The mechanism of the reaction is shown in Scheme 2.

The molecular ions $(M + H)^+$ peak were obtained by FAB (fast atom bombardment) of the saccharide ester, whereas by the EI (electron impact) method, no molecular ion peaks were obtained except for a 331 (*m/z*) fragment peak, which corresponding to a *O*-tetra-acetylglucopyranosyl fragment, or *O*-tetra-acetylgalactopyranosyl fragment, and a 259 (*m/z*) fragment peak, which corresponding to a *O*-acetylxylopyranosyl fragment and a 619 (*m/z*) fragment peak, which corresponding to the *O*-octa-acetyllactosyl fragment and some fragment peaks (242, 271, 229, 169, 109



Scheme 1

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Table 1 Structure of the compounds **1–4**

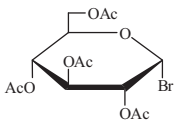
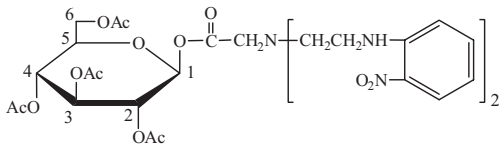
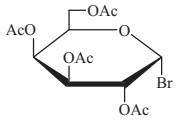
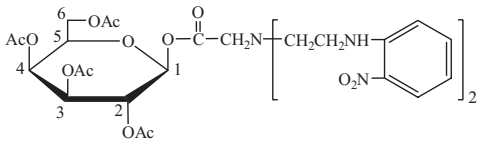
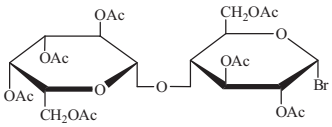
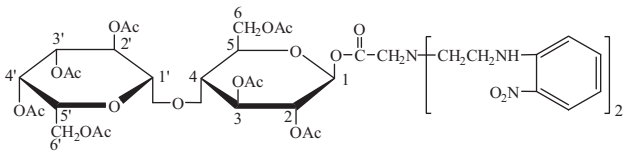
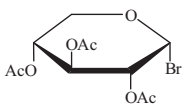
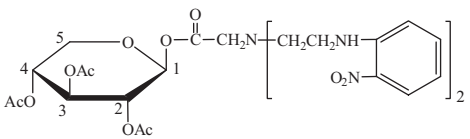
Compound	GL–Br	Product
1		
2		
3		
4		

Table 2 Reaction time, yield and antiviral activity of the compounds **1–4**

Compound	Reaction time/h		Yield[a]/%	Yield[b]/%	Activity/%
	Method a	Method b			
1	2.0	5.5	86	82	57.8
2	2.0	5.5	84	80	58.2
3	1.5	4.5	73	54	65.3
4	1.5	4.5	61	43	53.5

Method a: DMAP/Et_3N, reaction temperature 20–25 °C. Method b: the phase transfer catalyst (Bu_4NBr), reaction temperature 50–60 °C. Yield[a] obtained using Method a. Yield[b] obtained using Method b. Activity(%) is antiviral activity against tobacco mosaic virus.

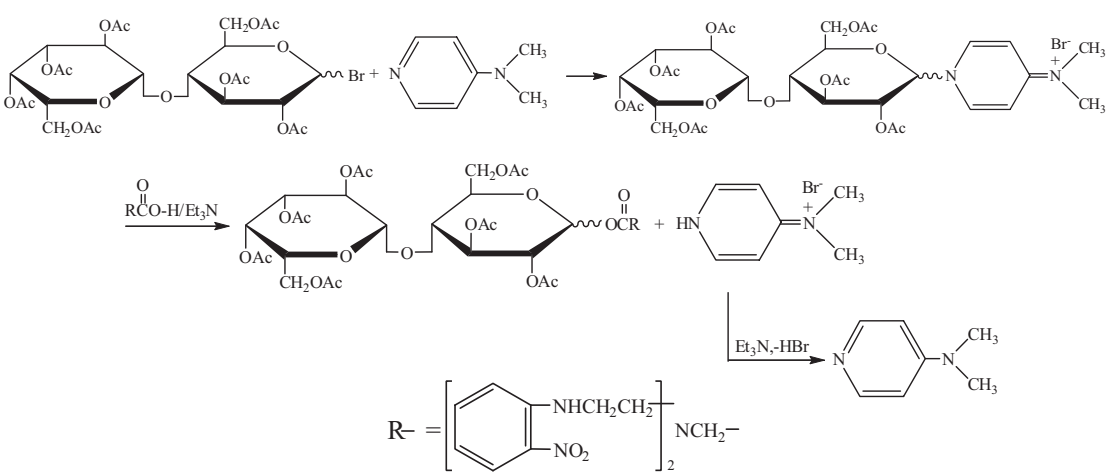
m/z) of pyranose ring were obtained. Moreover, some fragment peak (184, 142, 77 *m/z*) of *N,N*-bis[2-(2-nitrophenylamino) ethyl]glycine was obtained by both FAB and EI methods.

Experimental

IR spectra were obtained using a Shimadzu IR-435 IR spectrometer. The elemental analyses were recorded on an Immunomedia MT-3 elemental analyser. ^1H NMR spectra were recorded in acetone-d_6 on a Varian Mercury-VX300 (300 MHz) NMR instrument, using TMS, as internal standard. FAB mass spectra of the complexes were recorded using a JEOL SX-120 instrument.

General procedure for the preparation of N,N-bis[2-(2-nitrophenylamino)ethyl]glycine glycosyl esters (compounds **1–4**)

1.25 mmol *N,N*-bis[2-(2-nitrophenylamino)ethyl]glycine, 1.25 mmol *α-O*-acetylglycosyl bromide, 0.10 mmol DMAP, 40 ml DMF were added into a four-necked, round-bottomed flask. The temperature was



Scheme 2

kept constant at 0 °C, 0.25 ml triethylamine was slowly dropped into reaction system under stirring, and the reaction kept at 20–25 °C for 1.5–2.0 h. As soon as the reaction finished, the solution was filtrated. After separation, organic phase was washed to neutrality with water and dried with anhydrous magnesium sulfate. The dense liquid was obtained by the vapourisation of solvent under condition of vacuum distillation. After this the semifinished product was dissolved in ethyl acetate and separated through silica gel chromatography using ethyl acetate as eluant, *N,N*-bis[2-(2-nitrophenylamino)ethyl]glycine glycosyl esters were obtained by the evaporation of the solvent.

1: Light yellow dense liquid, ^{1}H NMR data, δ_H 7.98 (2 × t, 2H, ArH), 7.44 (m, 2H, ArH), 6.95 (d, 2H, ArH), 6.60 (m, 2H, ArH), 5.92–5.98 (d, J = 7.92 Hz 1H) (C_1-H_1 of pyranose ring), 4.75–5.40 (m, 3H, $C_{2\text{-}4}$–$H_{2\text{-}4}$ of pyranose ring), 3.45 (m, 1H, C_5–H_5 of pyranose ring), 4.18 (m, 2H, C_6–H_6 of pyranose ring), 3.55 (s, 2H, –NCH_2C=O), 3.15 (m, 2H, N–H); 2.10 (m, 4H, N–CH_2–C) 2.05 (m, 12H, CH_3C=O) 1.05 (t, 4H, –CH_2N); ν/cm^{-1}: 3350 (N–H), 2925 (CH_2), 1740, 1700(C=O), 1610, 1510 (C_6H_6), 1570 (–NO_2), 1020–1090, 1210–1290 (bi-shoulder peaks of pyranose ring); Found: C, 52.19; H, 5.09; N, 9.73; Required for $C_{32}H_{39}N_5O_{15}$: C, 52.39; H, 5.32; N, 9.54%; FAB-MS (*m/z,*%),734 ([M + H]$^+$, 5.6).

2: Light yellow dense liquid, ^{1}H NMR data, δ_H 7.98 (2 × t, 2H, ArH), 7.44 (m, 2H, ArH), 6.95 (d, 2H, ArH), 6.60 (m, 2H, ArH), 5.89–5.93 (d, J = 7.87 Hz 1H) (C_1–H_1 of pyranose ring), 5.20–5.48 (m, 3H, $C_{2\text{-}4}$–$H_{2\text{-}4}$ of pyranose ring), 3.95 (m, 1H, C_5–H_5 of pyranose ring), 4.10 (m, 2H, C_6–H_6 of pyranose ring), 3.42 (s, 2H, –N–CH_2C=O), 3.72 (m, 2H, N–H); 2.10 (m, 4H, N–CH_2–C) 1.95 (m, 12H, CH_3C=O) 1.15 (t, 4H, –CH_2N); ν/cm^{-1}: 3350 (N–H), 2950 (CH_2), 1740, 1710 (C=O), 1615, 1510 (C_6H_6), 1570 (–NO_2), 1020–1080, 1210–1280 (bi-shoulder peaks of pyranose ring); Found: C, 52.50; H, 5.06; N, 9.08; Required for $C_{32}H_{39}N_5O_{15}$: C, 52.39; H, 5.32; N, 9.54%; FAB-MS (*m/z,*%),734 ([M + H]$^+$, 6.2).

3: Light yellow dense liquid; ^{1}H NMR data, δ_H 7.95 (2 × t, 2H, ArH), 7.44 (m, 2H, ArH), 6.95 (d, 2H, ArH), 6.60 (m, 2H, ArH), 5.90–5.92 (d, J = 7.20 Hz 1H) (C_1–H_1 of pyranose ring), 5.33–4.75 (m, 6H, $C_{2',3',4'}$–$H_{2',3',4'}$, $C_{2,3,4}$–$H_{2,3,4}$ of pyranose ring), 3.72 (m, 1H, C_5–H_5 of pyranose ring), 4.35 (m, 1H, $C_{5'}$–$H_{5'}$ of pyranose ring), 4.15 (m, 4H, $C_{6'}$–$H_{6'}$, C_6–$H_6$of pyranose ring), 3.40 (s, 2H, –N–CH_2C=O), 3.15 (m, 2H, N–H); 2.10 (m, 4H, N–CH_2–C) 2.05 (m, 21H, CH_3C=O) 0.98 (t, 4H, –CH_2N); ν/cm^{-1}: 3400 (N–H), 2950 (CH_2), 1745, 1670 (C=O), 1610, 1510 (C_6H_6), 1570 (–NO_2), 1030–1080, 1210–1250 (bi-shoulder peaks of pyranose ring); Found: C, 52.03; H, 5.60; N, 6.59; Required for $C_{44}H_{55}N_5O_{23}$: C, 51.71; H, 5.39; N, 6.86%; FAB-MS (*m/z,*%), 1022 ([M + H]$^+$, 3.5).

4: Yellow dense liquid, ^{1}H NMR data, 7.98 (2 × t, 2H, ArH), 7.44 (m, 2H, ArH), 6.95 (d, 2H, ArH), 6.60 (m, 2H, ArH), 5.90–5.92 (d, J = 7.20 Hz 1H) (C_1–H_1 of pyranose ring), 3.51–3.21 (m, 5H, $C_{2\text{-}5}$–$H_{2\text{-}5}$ of pyranose ring), 3.64 (s, 2H, –N–CH_2C=O), 1.40 (m, 4H, N–CH_2–C), 3.12 (m, 2H, –NH–), 2.05 (m, 9H, CH_3C=O) 0.95 (t, 4H, C–CH_2N–); ν/cm^{-1}: 3350 (N–H), 2900 (CH_2), 1760, 1720 (C=O), 1620, 1510 (C_6H_6), 1570 (–NO_2), 1010–1070, 1200–1300 (bi-shoulder peaks of pyranose ring); Found: C, 52.23; H, 5.12; N, 10.21; Required for $C_{29}H_{35}N_5O_{13}$: C, 52.65; H, 5.30; N, 10.59%; FAB-MS (*m/z,*%), 662 ([M + H]$^+$, 4.3).

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