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Short communication

Synthesis of berbamine acetyl glycosides and evaluation of antitumor activity

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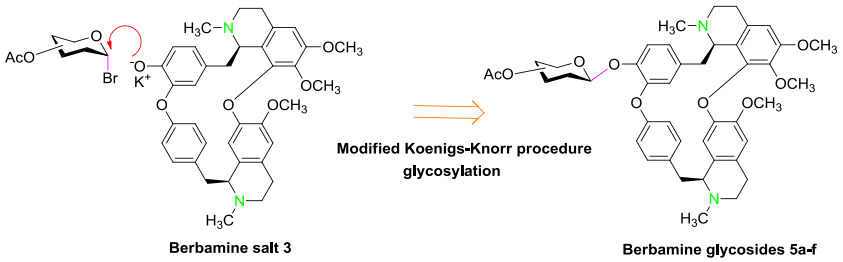
HIGHLIGHTS

- A series of berbamine (natural alkaloid) glycosides was synthesized.
- The new glycosides were evaluated for their antitumor activity in vitro.
- Most of the berbamine glycosides manifested potent cytotoxic activity.
- The most potent compound showed an IC_{50} of 0.30 μM against L1210 leukemia cell line.

GRAPHICAL ABSTRACT



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ABSTRACT

A series of berbamine glycosides was designed, synthesized and evaluated as a new class of antitumor agents. An efficient glycosylation route was developed for berbamide derivatives. The newly synthesized glycosides were evaluated for their cytotoxic activity in vitro against a human leukemia cell line K562, a human lung adenocarcinoma cell line A549 and mouse lymphocytic leukemia cells L1210. In contrast to berbamine most of its glycosides manifested potent cytotoxic activities. The acetyl glycosyl berbamine **5a**, **5d** caused distinct improvement against K562, A549 and L1210. It is suggested that the acetyl D-glucose residue has affinity to these cancer cells.

1. Introduction

From ancient to modern times medicine has been closely linked to the use of traditional medicines and natural products (NPs). Currently roughly half (49%) of the New Chemical Entities (NCEs) introduced are natural products, semi-synthetic natural product analogs or synthetic compounds based on natural products. Utilizing scaffolds of natural products combined with synthetic modifications is clearly an advantageous strategy in drug design [1]. Berbamine is a natural product derived from the plant Berberis amurensis (xiaoboan), which has been used extensively in Asia and Europe for the treatment of various ailments. Due to its bis-benzylisoquinoline alkaloid structure, berbamine has been demonstrated to possess a number of interesting and potent biological activities [2–6], such as an agent against human breast cancer cells, inducing apoptosis in human myeloma cells and suppression of human lung cancer cell growth. It was found by our group that berbamine can selectively induce cell death of both Gleevec sensitive and resistant-Phþ chronic myeloid leukemia (CML) cells [7] and selectively induce caspase-3-dependent apoptosis of leukemia

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NB4 cells via the survivin-mediated pathway [8]. In order to explore the effects and mechanism of berbamine on antitumor activity, we endeavored to make synthetic analogs of berbamine. A handful of the synthetic berbamine derivatives have been reported [9–12]. However, the glycosylation derivatives of berbamine for antitumor activity to the best of our knowledge have not been reported.

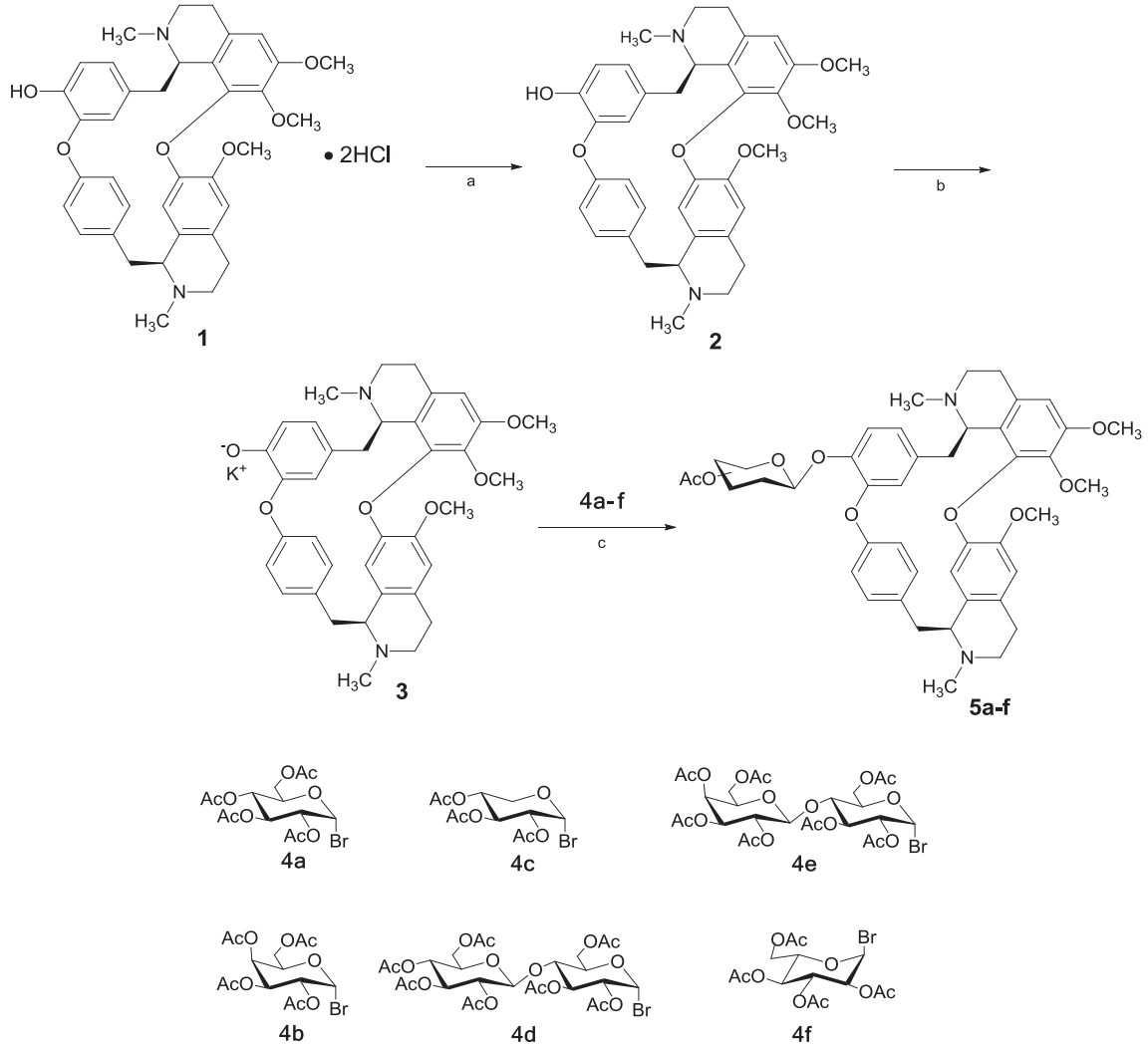
With their high density of defined spatial orientations and their relative rigidity, carbohydrates provide excellent platforms upon which to explore unique features for the drug-discovery process [13]. Substituted carbohydrate derivatives are generally quite stable and usually display reasonable stability to gastric acids and liver metabolism, which is in contrast to unsubstituted saccharides that usually undergo rapid metabolism in a biological environment [14]. There is a number of acetyl glycosides lead compounds that demonstrate more active than their unsubstituted carbohydrate analogs [15,16]. We aimed to synthesize the acetyl glycosides of berbamine for potential therapeutic treatment.

2. Results and discussions

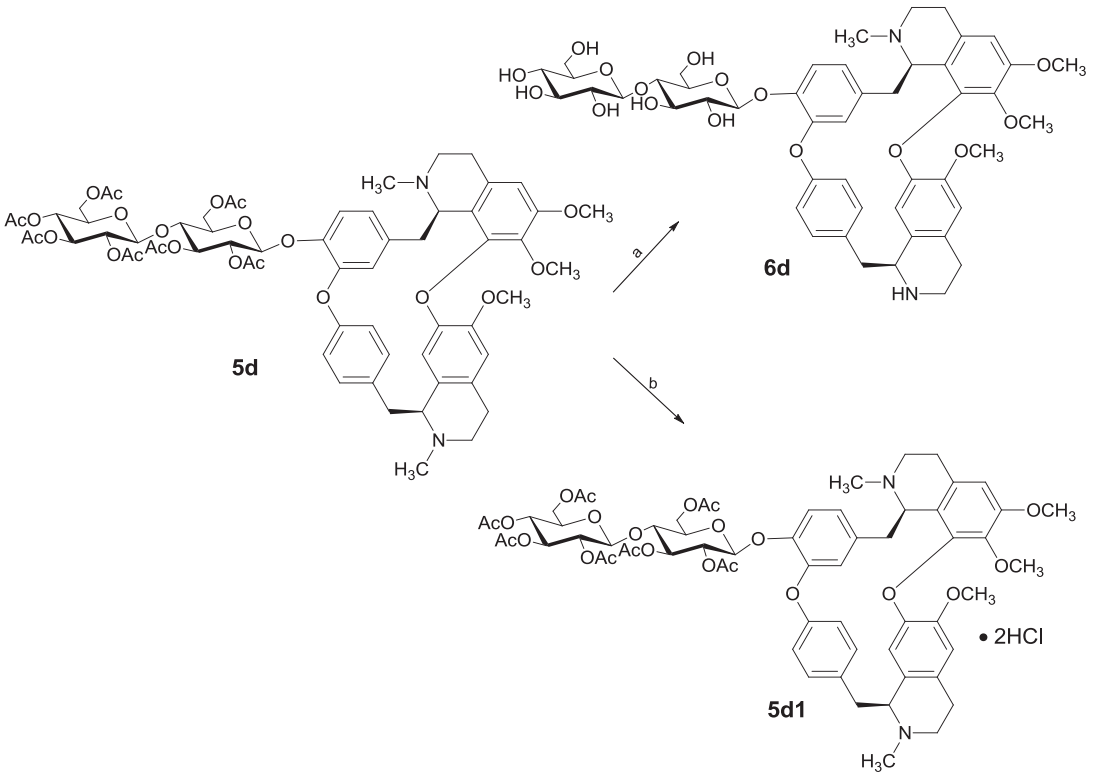
2.1. Chemistry

The synthesis of the berbamine glycosides is illustrated and outlined in Schemes 1 and 2. Pharmaceutical grade berbamine dihydrochloride **1** was neutralized and alkalified to afford the solid berbamine salt **3** using sodium bicarbonate and solid KOH.

The critical step in our analog production was optimizing the formation of the berbamine phenolate. Carbohydrates carrying an aromatic aglycon are important natural products and thus key synthetic targets. The glycosylation of the phenols in an alkaloid is a challenge. First the phenolichydroxyl phenols are ambident nucleophiles in the glycosylation. A second problem is steric hindrance from substituents on the alkaloid compound (Fig. 1) [17,18]. Compared to acetate and trichloroacetimidate glycosyl donors, a glycosyl halide is better in an alkaline acceptor system. Initially we used the Michael procedure [19] which is predominantly used for the glycosylation of the phenols (i.e., the use of a glycosyl halide in combination with a phenolate dissolved in aprotic solvents under phase-transfer catalyst conditions). After many trials it was proved that the procedure was not suitable for berbamine. For our glycosylation route a modified Koenigs–Knorr procedure was finally chosen. The glycopyranosyl bromides **4a**–**f** were prepared according to the methods described elsewhere [20] and then the abovementioned glycosyl bromide reacted with berbamide salt **3** via the modified Koenigs–Knorr procedure to afford the corresponding berbamide glycosides **5a**–**f**. After the screening of catalysts and solvents, silver oxide and acetonitrile were chosen and proved to be effective for the berbamine glycosylation.



Scheme 1. Synthesis of berbamine glycosides **5a**–**f**. Reagents and conditions: (a) sat $NaHCO_3$ aq, CH_2Cl_2, neutralization. (b) KOH, water, pH = 13, 5 h, 40 °C. (c) **4a**–**f**, Ag_2O, dry CH_3CN, 4 Å ms, 40 °C, overnight.



Scheme 2. Reaction of berbamine glycoside **5d**. Reagents and conditions: (a) 0.05 M NaOMe (CH_2Cl_2:MeOH = 1:1 volume), rt, 24 h, 90%. (b) **5d**: HCl (3%, water) = 1:2 (mol ratio), quantitatively.

A class of berbamine glycosides **5a**–**f** containing D-glucose, D-galactose, D-xylose, D-cellobiose, D-lactose and L-glucose residues respectively was made. Glycosyl bromides are usually isolated as the thermodynamically favored α-anomer and due to participating groups (e.g., esters) at sugar C2 glycosylation generally results in β-glycosides. The yields of **5a**–**f** glycosides were 62%, 50%, 50%, 65%, 37% and 21% respectively under our conditions. The preliminary experiments show that acetyl D-cellobiose derivative **5d** improved their cytotoxicity against K562, L1210 and A549 distinctly. To compare the different **5d** derivatives' influence to biological activity compound **6d** (deacetylation of **5d**) and compound **5d1** (chloride salt form of **5d**) were produced. The procedures were shown in Sections 4.1.3, 4.1.4 and Scheme 2.

The chemical structures of the compounds were confirmed by ^{1}H NMR, ^{13}C NMR spectroscopy and HRMS. The results are presented in Section 4.3. The ^{1}H NMR chemical shifts of the berbamine glycosides **5a**–**f** showed the formation of the β-glycosides.

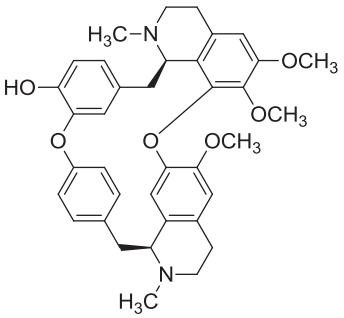


Fig. 1. The structure of berbamine.

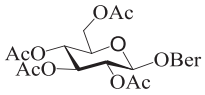
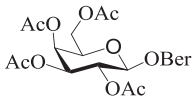
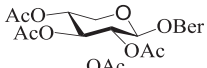
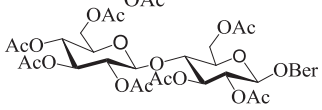
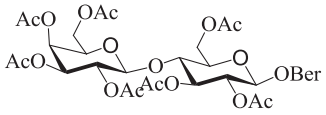
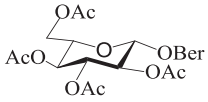
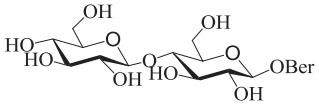
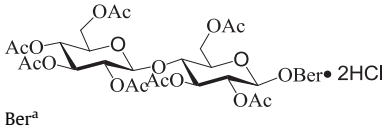
2.2. Biological activities

Three kinds of cancer cells were evaluated, namely, a human leukemia cell line K562, a human lung adenocarcinoma cell line A549 and mouse lymphocytic leukemia cells L1210. The results are summarized in Table 1.

As shown in Table 1, most compounds exhibited antitumor activities. From the IC_{50} data of these compounds, we notice that acetyl D-glucose derivative **5a** and acetyl D-cellobiose derivative **5d** improved their cytotoxicity against K562, L1210 and A549 distinctly. Interestingly, the cellobiose derivative **5d** showed an IC_{50} of 0.30 μM against L1210 leukemia cell-line compared with native berbamine IC_{50} of 2.73 μM. Considering the acetyl D-glucose derivative's similar increase in activity suggests that the acetyl D-glucose residue has affinity to these cancer cells. The acetyl D-galactose and acetyl D-lactose derivatives' cytotoxicity against A549 and L1210 corresponding cancer cells was similar to the native or decreased probably due to the acetyl D-galactose residues. The addition of acetyl glycosyl groups affected the biology of berbamine analogs greatly. The degree of influence achieved from smallest to largest was A549, K562 and L1210 cells respectively. The best activity against K562, A549 and L1210 respectively belonged to acetyl D-xylose derivative **5c**, acetyl D-cellobiose derivative **5d**, and acetyl D-cellobiose derivative **5d**.

In contrast with acetyl D-glucose derivative **5a** and acetyl L-acetyl glucose derivative **5f**, D-glucose derivative showed more potent cytotoxicity than L-glucose residue. It was reported that a good number of active natural products owned unsubstituted carbohydrate moiety, then the deacetylation structure **6d** demonstrated less active than its acetyl carbohydrate analog **5d**. Additionally the hydrochloride salt **5d1** of D-cellobiose derivative **5d** and berbamine hydrochloride salt **1** have similar solubility, but protonation decreased the cytotoxicity. Work to uncover the molecular mechanism of berbamine's cytotoxicity is ongoing.

Table 1
Berbamine glycosides and their IC_{50} values for antitumor.

Compounds **5**	Compounds **5** structures	Compounds **5** sugar residues	A549 IC_{50} (μM)	L1210 IC_{50} (μM)	K562 IC_{50} (μM)
5a		Acetyl-D-Glucose	29.51	0.57	1.96
5b		Acetyl-D-Galactose	42.99	2.17	2.03
5c		Acetyl-D-Xylose	NA	1.27	1.55
5d		Acetyl-D-cellobiose	27.75	0.30	2.23
5e		Acetyl-D-Lactose	NA	2.03	3.56
5f		Acetyl-L-glucose	NA	2.74	NA
6d		D-cellobiose	NA	10.62	NA
5d1		Acetyl-D-cellobiose	NA	1.85	NA
1	Ber[a]	/	39.72	2.73	4.00

Ber = berbamine; Ber[a] = berbamine dihydrochloride; NA: IC50 ≥ 50 (μM).

3. Conclusions

An efficient glycosylation route of berbamine (a natural alkaloid) was found and a series of berbamine glycosides was achieved. The novel glycosyl derivatives of berbamine were evaluated for their cytotoxic activity in vitro against three cancer cell lines (K562, A549 and L1210). Most of the glycosyl derivatives of berbamine exhibited potent cytotoxicities, several showed more significant cytotoxicity than native berbamine. The acetyl glycosides of berbamine **5a**, **5d** caused distinct improvement against K562, A549 and L1210. It is suggested that the acetyl D-glucose residue has affinity to these cancer cells.

4. Experimental protocols

Electrospray ionization (ESI) mass spectra were recorded on an Esquire 3000 plus mass spectrometer. NMR spectra (^1H, ^{13}C) were recorded on a Bruker DRX-400 instrument (Karlsruhe, Germany) at 400 MHz for ^1H NMR and at 100 MHz for ^{13}C NMR and tetramethylsilane was used as an internal standard; coupling constants are represented in hertz. Reactions were monitored by thin-layer chromatography (TLC) on an aluminum sheet precoated with Silica gel 60 (Merck, Germany) and detected by charring with 10% sulfuric acid in ethanol solution. Column chromatography was performed on silica gel (300–400 mesh; Qingdao Marine Chemical Ltd., Qingdao, PRC). CH_3CN and CH_2Cl_2 were distilled from P_2O_5. The other commercial reagents were used directly without further purification.

4.1. General procedure for berbamine derivatives

4.1.1. Procedure for berbamine salt *3*

Pharmaceutical grade berbamine dihydrochloride (**1**, 1.4944 g, 2.19 mmol) was washed with saturated sodium bicarbonate solution and then washed with CH_2Cl_2. The organic phase was concentrated in vacuum to afford neutral berbamine (**2**, 1.2842 g, 2.11 mmol). Neutral berbamine dissolved in 21.1 mL water was adjusted to pH = 13 with solid KOH. After 5 h stirring at 40 °C, the solution was evaporated in vacuum with ethanol to afford the solid berbamine salt **3**. It could be directly used for the next reaction step.

4.1.2. Procedure for 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl berbamine *5a*

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4a**, 100 mg, 0.24 mmol), the berbamine salt (**3**, 52.4 mg, 0.08 mmol) and the activated 4 Å molecular sieve (50 mg) were put into 4 mL anhydrous acetonitrile. The solution was stirred at room temperature for 15 min in inert atmosphere, then silver oxide (56.4 mg, 0.24 mmol) was slowly added into the system with the reaction temperature to 40 °C overnight (avoiding light). TLC (R_f = 0.6, CH_2Cl_2:MeOH = 10:1, containing 0.01% Et_3N) was used to detect the reaction completion. The solution was filtered with kieselguhr, concentrated in vacuum,

the residue was purified by silica gel chromatography (CH_2Cl_2:MeOH = 20:1, containing 0.01% Et_3N) to afford the desired product **5a** (yield 62%).

4.1.3. Procedure for β-D-cellobiopyranosyl berbamine **6d**

Compound **5d** (243.5 mg, 0.198 mmol) was added into 10 mL of 0.05 M NaOMe (MeOH:CH_2Cl_2 = 1:1 volume) solution. The mixture was stirred at room temperature under argon for 24 h. The solution was neutralized by 732 cation exchange resins, then was filtered and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2:MeOH = 1:1, containing 0.01% Et_3N) to afford **6d** (yield 90%).

4.1.4. Procedure for 2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-D-cellobiopyranosyl berbamine dihydrochloride **5d1**

Compound **5d** (60 mg, 0.049 mmol) was mixed with 3 mL water. The 3% HCl (0.098 mmol HCl) was added dropwise to the mixture. The resulting mixture was stirred for 30 min and filted. The filtrate was concentrated. The resulting yellow solid was washed by 3 mL CH_2Cl_2 and dried under reduced pressure to afford **3d1** (yield 60%).

4.2. Biology

To evaluate the cytotoxicity of compounds used in this experiment, a MTT assay was performed. Cells were seeded into 96-well micro-culture plates at a concentration of 5×10^3 cells per well, and then cells were exposed to compounds at concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 μM, while positive and blank controls were set. After incubation (37 °C, 5% CO_2) for 2 days, 20 μL of MTT solution (5 mg/mL) was transferred to each well to yield a final assay volume of 220 μL/well. Plates were incubated for 4 h at 37 °C and 5% CO_2. After incubation, supernatants were removed, and 150 μL of dimethyl sulfoxide (DMSO) was added. The solution was at room temperature for 20 min. The optical density (OD) was then recorded at 570 nm using a spectrophotometer. The mean OD 570 of the control cells exposed to test compound-free culture medium was set to represent 100% of viability and the results were expressed as a percentage of these controls. The average 50% inhibitory concentration (IC_{50}) was evaluated by MTT tetrazolium dye assay. Each experiment was performed three times.

4.3. Analytical data for compounds **5a**, **5b**, **5c**, **5d**, **5e**, **5f**, **6d** and **5d1**

4.3.1. 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl berbamine (**5a**)

Light yellow powder. HRMS (TOF-EI) Calcd for $C_{51}H_{58}N_2O_{15}$ $[M]^+$: 938.3837, Found: 938.3828. ^1H NMR (400 MHz, $CDCl_3$): δ 7.31–7.25 (m, 1H), 7.09 (d, J = 8.0 Hz, 1H), 7.07–6.99 (m, 1H), 6.79–6.70 (m, 1H), 6.61–6.52 (m, 1H), 6.55 (s, 1H), 6.45–6.36 (m, 2H), 6.28 (s, 1H), 5.96 (s, br, 1H), 5.34–5.26 (m, 1H), 5.30 (d, J = 8.0 Hz, 1H, anomeric proton), 5.17 (t, J = 7.6 Hz, 1H), 4.98 (d, J = 5.6 Hz, 1H), 4.30 (dd, J_1 = 4.0 Hz, J_2 = 9.6 Hz, 1H), 4.19 (dd, J_1 = 1.6 Hz, J_2 = 9.6 Hz, 1H), 3.94–3.81 (m, 2H), 3.79 (s, 3H), 3.61 (s, 3H), 3.53–3.24 (m, 4H), 3.14 (s, 3H), 3.05–3.77 (m, 7H), 2.60 (s, 4H), 2.38–2.23 (m, 4H), 2.09 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.65, 170.25, 169.59, 169.40, 154.10, 151.89, 151.17, 150.02, 149.83, 149.66, 144.26, 143.58, 137.07, 135.35, 135.21, 132.36, 130.15, 128.77, 127.50, 123.96, 123.02, 122.22, 121.19, 120.61, 119.72, 116.50, 111.27, 105.61, 101.92 (anomeric carbon), 74.58, 72.63, 71.93, 71.20, 68.73, 63.57, 61.96, 60.44, 55.73, 55.52, 45.97, 45.93, 42.67, 42.48, 38.81, 37.48, 29.64, 25.53, 20.69, 20.65, 20.59, 20.56.

4.3.2. 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl berbamine (**5b**)

Light yellow powder. HRMS (TOF-EI) Calcd for $C_{51}H_{58}N_2O_{15}$ $[M]^+$: 938.3837, Found: 938.3827. ^1H NMR (400 MHz, $CDCl_3$): δ 7.42 (dd, J = 8.0 Hz, 1H), 7.02 (d, J = 8.0 Hz, 1H), 6.97 (dd, J_1 = 1.6 Hz, J_2 = 8.0 Hz, 1H), 6.90 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 6.74 (dd, J = 8.0 Hz, 1H), 6.64–6.60 (m, 1H), 6.59 (s, 1H), 6.40–6.35 (m, 1H), 6.30 (dd, J = 8.4 Hz, 1H), 6.28 (s, 1H), 5.58–5.41 (m, 2H), 5.09 (dd, J_1 = 3.2 Hz, J_2 = 10.4 Hz, 1H), 4.88 (d, J = 8.0 Hz, 1H, anomeric proton), 4.24 (dd, J_1 = 6.8 Hz, J_2 = 11.2 Hz, 1H), 4.20–4.14 (m, 2H), 3.97–3.92 (m, 1H), 3.78 (s, 3H), 3.63 (s, 3H), 3.40–3.29 (m, 2H), 3.17 (s, 3H), 3.10–2.61 (m, 10H), 2.56 (s, 3H), 2.53 (br, 1H), 2.40–2.25 (m, 3H), 2.18 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.21, 170.15, 170.00, 169.57, 153.91, 151.74, 151.02, 149.63, 148.16, 144.20, 143.45, 139.31, 136.96, 135.35, 132.25, 130.06, 128.80, 127.56, 125.15, 122.85, 121.84, 121.07, 120.43, 120.22, 119.58, 116.40, 111.16, 105.49, 102.34 (anomeric carbon), 70.76, 70.64, 68.57, 66.88, 63.62, 61.72, 61.21, 60.30, 55.60, 55.39, 46.00, 43.95, 42.68, 42.40, 38.72, 37.57, 29.54, 25.60, 20.64–20.44.

4.3.3. 2,3,4-Tri-O-acetyl-β-D-xylopyranosyl berbamine (**5c**)

Light yellow powder. HRMS (TOF-EI) Calcd for $C_{48}H_{54}N_2O_{13}$ $[M]^+$: 866.3625, Found: 866.3632. ^1H NMR (400 MHz, $CDCl_3$): δ 7.29 (dd, J_1 = 1.6 Hz, J_2 = 8.0 Hz, 1H), 7.09–7.03 (m, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.75 (dd, J = 8.0 Hz, 1H), 6.58 (dd, J = 6.8 Hz, 1H), 6.54 (s, 1H), 6.40 (s, br, 2H), 6.28 (s, 1H), 5.95 (s, br, 1H), 5.26–5.20 (m, 2H), 5.15–5.10 (m, 1H), 5.01 (d, J = 7.6 Hz, 1H, anomeric proton), 4.26 (dd, J_1 = 4.8 Hz, J_2 = 12.0 Hz, 1H), 3.91–3.80 (m, 3H), 3.75 (s, 3H), 3.60 (s, 3H), 3.53–3.40 (m, 2H), 3.32–3.22 (m, 2H), 3.13 (s, 3H), 3.07–2.75 (m, 6H), 2.67–2.58 (m, 1H), 2.56 (s, 3H), 2.47–2.31 (m, 1H), 2.26 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.90, 169.72, 169.43, 154.08, 151.75, 150.96, 149.74, 148.02, 143.73, 143.47, 138.34, 136.97, 134.36, 132.18, 130.06, 128.51, 127.11, 124.94, 122.97, 121.59, 121.12, 120.96, 120.26, 119.62, 116.52, 111.13, 105.48, 100.73 (anomeric carbon), 71.47, 70.04, 69.43, 62.32, 61.83, 61.71, 60.29, 55.60, 55.39, 45.59, 45.35, 42.49, 42.43, 38.51, 37.69, 25.17, 25.12, 20.60, 20.57, 20.54.

4.3.4. 2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-D-cellobiopyranosyl berbamine (**5d**)

Light yellow powder. HRMS (ESI) Calcd for $C_{63}H_{74}N_2O_{23}$ $[M + H]^+$: 1227.4760, Found: 1227.4718. ^1H NMR (400 MHz, $CDCl_3$): δ 7.28 (dd, J = 8.0 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 7.04–6.98 (m, 1H), 6.73 (dd, J = 8.0 Hz, 1H), 6.58 (dd, J = 7.2 Hz, 1H), 6.54 (s, 1H), 6.43 (s, br, 2H), 6.27 (s, 1H), 5.95 (s, br, 1H), 5.29 (dd, J_1 = 4.4 Hz, J_2 = 9.2 Hz, 1H), 5.21 (d, J = 8.0 Hz, 1H, anomeric proton), 5.16 (t, J = 8.8 Hz, 1H), 5.07 (t, J = 9.6 Hz, 1H), 4.98–4.92 (m, 2H), 4.56 (d, J = 9.6 Hz, 1H, anomeric hydrogen) 4.57–4.53 (m, 1H), 4.38 (dd, J_1 = 4.4 Hz, J_2 = 12.4 Hz, 1H), 4.16 (dd, J_1 = 5.2 Hz, J_2 = 12.0 Hz, 1H), 4.05 (d, J = 10.8 Hz, 1H), 3.92–3.78 (m, 3H), 3.75 (s, 3H), 3.72–3.65 (m, 2H), 3.61 (s, 3H), 3.45–3.33 (m, 1H), 3.34–3.19 (m, 2H), 3.13 (s, 3H), 3.05–2.75 (m, 7H), 2.61 (s, 1H), 2.57 (s, 3H), 2.41–2.30 (m, 1H), 2.27 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.12, 170.09, 170.05, 169.98, 169.89, 169.72, 169.42, 154.13, 151.78, 150.97, 149.78, 148.02, 143.77, 143.51, 138.82, 136.93, 134.92, 132.19, 130.07, 129.08, 128.45, 127.13, 123.02, 121.60, 121.12, 120.22, 120.08, 119.63, 116.66, 111.16, 105.51, 100.73 (anomeric carbon), 95.59 (anomeric carbon), 71.47, 70.53, 70.06, 69.44, 69.06, 68.62, 67.33, 66.11, 65.85, 63.53, 62.33, 61.66, 60.29, 55.62, 55.40, 45.59, 44.33, 42.49, 42.38, 38.50, 37.62, 29.49, 25.12, 20.87, 20.59–20.48.

4.3.5. 2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-D-lactopyranosyl berbamine (**5e**)

Light yellow powder. HRMS (ESI) Calcd for $C_{63}H_{74}N_2O_{23}$ $[M + H]^+$: 1227.4760, Found: 1227.4745. ^1H NMR (400 MHz, $CDCl_3$): δ 7.28 (dd, J = 8.0 Hz, 1H), 7.08–6.98 (m, 2H), 6.73 (dd, J = 7.6 Hz, 1H), 6.58 (dd, J = 6.8 Hz, 1H), 6.54 (s, 1H), 6.43 (s, br, 2H), 6.27 (s, 1H), 5.95 (s, br, 1H), 5.35 (d, J = 3.6 Hz, 1H), 5.30 (t, J = 9.2 Hz, 1H),

5.20 (dd, J_1 = 8.0 Hz, J_2 = 9.6 Hz, 1H), 5.13 (dd, J_1 = 7.6 Hz, J_2 = 10.0 Hz, 1H), 4.97 (d, J = 7.2 Hz, 1H, anomeric proton), 4.97 (dd, J_1 = 3.6 Hz, J_2 = 10.8 Hz, 1H), 4.52 (d, J = 8.0 Hz, 1H, anomeric proton), 4.55–4.50 (m, 1H), 4.19–4.06 (m, 3H), 3.93–3.86 (m, 2H), 3.86–3.78 (m, 2H), 3.75 (s, 3H), 3.72–3.64 (m, 1H), 3.61 (s, 3H), 3.44–3.33 (m, 1H), 3.33–3.18 (m, 2H), 3.13 (s, 3H), 3.06–2.73 (m, 7H), 2.61 (br, 1H), 2.57 (s, 3H), 2.37–2.19 (m, 4H), 2.16 (s, 3H), 2.10 (s, 3H), 2.06 (s, 9H), 1.97 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.32, 170.26, 170.08, 169.97, 169.79, 169.68, 169.00, 153.99, 151.83, 151.08, 149.74, 148.26, 144.15, 143.53, 139.40, 137.03, 135.54, 132.28, 130.15, 129.38, 128.89, 127.76, 122.95, 121.88, 121.17, 120.37, 120.23, 119.68, 116.51, 111.24, 105.56, 101.56 (anomeric carbon), 100.99 (anomeric carbon), 76.23, 72.71, 72.57, 71.56, 70.95, 70.67, 69.08, 68.65, 66.61, 63.73, 61.95, 60.80, 60.40, 55.69, 55.49, 46.13, 46.00, 42.77, 42.51, 38.75, 37.70, 28.47, 25.69, 20.74–20.42.

4.3.6. 2,3,4,6-Tetra-O-acetyl-β-L-glucopyranosyl berbamine (**5f**)

Light yellow powder. MS (ESI) Calcd for $C_{51}H_{58}N_2O_{15}$ $[M + H]^+$: 939.3915, Found: 939.3876. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31–7.25 (m, 1H), 7.08 (dd, J = 8.1 Hz, 1H), 6.85 (d, J = 7.9 Hz, 1H), 6.77 (d, J = 7.8 Hz, 1H), 6.63 (d, J = 7.8 Hz, 1H), 6.53 (s, 1H), 6.52 (d, J = 4.1 Hz, 1H), 6.45–6.37 (m, 1H), 6.29 (s, 1H), 5.99 (s, br, 1H), 5.31–5.26 (m, 2H), 5.17 (t, J = 9.3 Hz, 1H), 4.98 (d, J = 7.2 Hz, 1H, anomeric proton), 4.29 (dd, J_1 = 5.0 Hz, J_2 = 12.2 Hz, 1H), 4.15 (d, J = 11.3 Hz, 1H), 3.92–3.78 (m, 3H), 3.76 (s, 3H), 3.60 (s, 3H), 3.49–3.19 (m, 4H), 3.12 (s, 3H), 3.08–3.72 (m, 7H), 2.58 (s, 3H), 2.45–2.33 (m, 1H), 2.33 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.57, 170.21, 169.46, 169.30, 153.87, 151.80, 151.06, 149.74, 148.20, 143.91, 143.27, 137.79, 136.95, 135.40, 132.10, 130.25, 128.72, 128.06, 127.53, 123.21, 121.87, 121.23, 120.57, 119.75, 116.87, 116.44, 111.06, 105.45, 101.70 (anomeric carbon), 72.56, 71.83, 71.18, 68.41, 63.56, 62.03, 61.87, 60.40, 55.66, 55.38, 46.00, 44.50, 42.79, 42.66, 38.85, 37.57, 30.22, 25.62, 20.63–20.49.

4.3.7. β-D-Cellobiopyranosyl berbamine (**6d**)

Light yellow powder. HRMS (ESI) Calcd for $C_{49}H_{60}N_2O_{16}$ $[M + H]^+$: 933.4016, Found: 933.4022. ^{1}H NMR (400 MHz, pyridine-*d*5): δ 7.48 (d, J = 8.0 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 6.99 (d, J = 4.0 Hz, 1H), 6.81–6.67 (m, 1H), 6.77 (s, 1H), 6.45 (s, 1H), 6.41–6.24 (s, br, 3H), 5.29 (dd, J_1 = 4.4 Hz, J_2 = 9.2 Hz, 1H), 5.21 (d, J = 8.0 Hz, 1H, anomeric proton), 5.16 (t, J = 8.8 Hz, 1H), 5.07 (t, J = 9.6 Hz, 1H), 4.98–4.92 (m, 2H), 4.56 (d, J = 9.6 Hz, 1H, anomeric proton) 4.57–4.53 (m, 1H), 4.38 (dd, J_1 = 4.4 Hz, J_2 = 12.4 Hz, 1H), 4.16 (dd, J_1 = 5.2 Hz, J_2 = 12.0 Hz, 1H), 4.05 (d, J = 10.8 Hz, 1H), 3.92–3.78 (m, 3H), 3.75 (s, 3H), 3.72–3.65 (m, 2H), 3.61 (s, 3H), 3.45–3.33 (m, 1H), 3.34–3.19 (m, 2H), 3.13 (s, 3H), 3.05–2.75 (m, 7H), 2.61 (s, 1H), 2.57 (s, 3H), 2.41–2.30 (m, 1H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, pyridine-*d*5): δ 153.46, 151.78, 149.77, 149.03, 148.43, 144.50, 143.43, 137.23, 136.14, 135.98, 132.25, 131.19, 129.51, 128.73, 128.16, 123.45, 123.25, 121.76, 120.87, 120.43, 119.48, 115.58, 111.85, 106.71, 103.52 (anomeric carbon), 100.57 (anomeric carbon), 80.33, 77.16, 76.77, 75.53, 75.29, 73.63, 73.27, 70.36, 63.02, 62.04, 61.35, 60.45, 60.06, 55.94, 55.62, 46.53, 45.85, 42.92, 42.46, 38.66, 36.21, 26.02, 21.86.

4.3.8. 2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-D-cellobiopyranosyl berbamine dihydrochloride (**5d1**)

Light yellow powder. HRMS (ESI) Calcd for $C_{63}H_{74}N_2O_{23}$ $[M + H]^+$: 1227.4760, Found: 1227.4715. ^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.55–7.39 (m, 1H), 7.27–7.07 (m, 3H), 6.82 (dd, J = 11.0 Hz, 1H), 6.64 (d, J = 12.0 Hz, 1H), 6.59–6.49 (m, 1H), 6.43 (s, 1H), 6.40–6.29 (m, 1H), 6.12 (dd, J = 12.0 Hz, 1H), 5.34 (d, J = 8.0 Hz, 1H), 5.28 (d, J = 8.0 Hz, 1H, anomeric proton), 5.28–5.22 (m, 1H), 5.99 (t, J = 8.2 Hz, 1H), 4.92–4.81 (m, 2H), 4.65 (t, J = 8.2 Hz, 1H), 4.38 (d, J = 8.0 Hz, 1H, anomeric hydrogen), 4.28–4.20 (m, 1H), 4.15–3.99 (m, 3H), 3.94 (d, J = 12.0 Hz, 1H), 3.86 (dd, J_1 = 8.0 Hz, J_2 = 12.0 Hz, 1H), 3.72 (s, 3H), 3.65–3.36 (m, 6H), 3.32–3.08 (m, 6H), 3.04 (s, 3H), 2.96 (s, 3H), 2.75 (s, 1H), 2.50 (s, 6H), 2.36 (s, 1H), 2.41–2.30 (m, 1H), 2.05 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.96 (s, 6H), 1.91 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6): δ 170.54, 170.38, 169.96, 169.77, 169.57, 169.40, 169.31, 154.13, 151.78, 150.97, 149.78, 148.02, 143.77, 143.51, 138.82, 136.93, 134.92, 132.19, 130.07, 129.08, 128.45, 127.13, 123.02, 121.60, 121.12, 120.22, 120.08, 119.63, 116.45, 111.95, 106.66, 99.88 (anomeric carbon), 99.41 (anomeric carbon), 76.79, 72.56, 72.33, 72.16, 71.54, 71.26, 70.81, 68.04, 65.10, 61.83, 61.37, 60.59, 60.30, 56.90, 56.01, 45.02, 44.29, 43.04, 42.20, 38.07, 37.59, 24.61, 23.95, 21.42, 20.97, 20.29, 20.77, 20.69, 20.60, 20.56.

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