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Total synthesis of the proposed structures of gladiosides I and II

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ABSTRACT

Burkholderia gladioli is a Gram-negative bacterium that biosynthesizes a cocktail of potent antimicrobial compounds, including the antifungal phenolic glycoside sinapigladioside. Herein, we report the total synthesis of the proposed structures of gladiosides I and II, two structurally related phenolic glycosides previously isolated from *B. gladioli* OR1 cultures. Importantly, the physical and analytical data of the synthetic compounds were in significant discrepancies with the natural products suggesting a misassignment of the originally proposed structures. Furthermore, we have uncovered an acid-catalyzed fragmentation mechanism converting the α,β-unsaturated methyl carbamate-containing gladioside II into the aldehyde-containing gladioside I. Our results lay the foundation for the expeditious synthesis of derivatives of these *Burkholderia*-derived phenolic glycosides, which would enable to decipher their biological roles and potential pharmacological properties.

1. Introduction

Bacteria represent an unparalleled source of antimicrobial compounds for drug discovery [1–3]. As such, a large proportion of clinically-relevant antibiotics are derived from bacteria. Because of optimization from natural evolution, bacteria-derived compounds are especially highly active and specific towards their cellular targets [3]. *Burkholderia* species, a vast group of Gram-negative bacteria (GNB) found in diverse ecological niches, offer a tantalizing potential in drug discovery owing to their capacity of biosynthesizing highly potent, abundant, and extremely diverse in structure primary [4] and secondary [5] metabolites.

Burkholderia gladioli, formerly known as *Pseudomonas marginata*, is an aerobic GNB that causes diseases in both plants [6] and animals [7] but can also live in symbiosis with them [8]. *B. gladioli* biosynthesizes structurally diverse and potent antimicrobial compounds including gladiolin [9], toxoflavin [10], bongkrekic acid [11], caryonencin [12], lagriamide [13], and lagriene [14]. By studying the symbiotic interactions between *Lagria villosa* beetles and *B. gladioli*, Florez and co-workers recently identified sinapigladioside (**3**, Fig. 1A) as a novel antifungal secondary metabolite biosynthesized by *B. gladioli* HKI0739 (syn. *B. gladioli* Lv-StA) [14,15]. The cocktail of antimicrobial compounds produced by *B. gladioli* helps *L. villosa* female beetles to protect their eggs against fungal pathogens. Gladioside I (**1**) and gladioside II (**2**) (Fig. 1A), sharing structural similarities with sinapigladioside (**3**), were previously identified by Khan et al. [16] from an antimicrobial methanolic extract of *B. gladioli* OR1,[17] however their biological activity remains to be explored.

From a structural point of view, gladioside I (**1**), gladioside II (**2**), and sinapigladioside (**3**) are low molecular weight phenolic glycosides. The glycone moiety is reported to be an unprecedented 3-*O*-methyl-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranoside. The aglycone residue is a *para*-substituted phenol bearing either an aldehyde functionality for gladioside I (**1**) [16], an (*E*)-α,β-unsaturated methyl carbamate for gladioside II (**2**) [16], or an (*E*)-α,β-unsaturated isothiocyanate for sinapigladioside (**3**) [14,15]. The potential antimicrobial activity of these phenolic glycosides combined with our long-standing research interest in the synthesis of *Burkholderia*-derived metabolites [18–24] prompted us to devise a synthetic route towards them. As a first step towards this aim, we present herein the total synthesis of the proposed structures of gladioside I (**1**) and gladioside II (**2**) and show that these natural products may have been originally misassigned. We uncover an acid-catalyzed fragmentation mechanism allowing the one-pot conversion of vinyl carbamate-containing gladioside II (**2**) into

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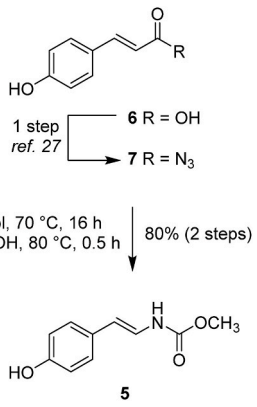
aldehyde-containing gladioside I (**1**). We also present the preliminary antimicrobial activity evaluation of these synthetic compounds.

2. Results and discussion

Gladiosides I (**1**) and II (**2**) can be retrosynthetically disconnected into a protected and activated (1 → 4)-linked disaccharide and 4-hydroxybenzaldehyde (**4**) or methyl (*E*)-(4-hydroxystyryl)carbamate (**5**) as the phenolic aglycones, respectively (Fig. 1B). The protected disaccharide would come from the glycosylation between an activated 3-*O*-methylxylopyranoside donor and a rhamnose acceptor bearing a free hydroxyl group at the C4 position. The presence of anchimeric ester groups at C2 would favor the formation of the 1,2-*trans*-glycosidic bonds in the target natural compounds while taking advantage of robust thioglycoside [25] and trichloroacetimidate (TCA) [26] activation chemistries. The glycosyl donor and acceptor would be obtained from commercially available D-xylose and L-rhamnose monosaccharides, respectively, according to regioselective protecting group schemes.

We first focused our attention on the preparation of phenolic α,β-unsaturated carbamate **5**. Therefore, *p*-coumaric acid **6** was readily transformed into the corresponding acyl azide derivative **7** following a procedure reported by Manzo and co-workers (Scheme 1) [27]. The subsequent Curtius rearrangement was performed by heating acyl azide **7** in hot (70 °C) toluene providing an isocyanate intermediate [28], which was *in situ* converted into methyl carbamate **5** by adding excess MeOH. This synthetic pathway allowed us to reach target carbamate **5** in 80% for two steps from acyl azide **7**.

We next sought to accomplish the synthesis of gladioside I (**1**). As depicted in Scheme 2, tolyl 2,4-di-*O*-acetyl-3-*O*-methyl-1-thio-β-D-xylopyranoside (**8**) was synthesized in six steps from D-xylose inspired from previous studies. Briefly, D-xylose was regioselectively transformed into α-xylofuranoside 1,2-*O*-acetonide [29] and the 4-OH position was protected with a trityl group [30]. Then, the free C3 position was methylated, both trityl and acetonide groups were cleaved under acidic conditions, the resulting 3-*O*-methylxylose was peracetylated, and an STol activating group was inserted at the anomeric position [31]. Resulting thioglycoside **8** [31] was reacted with rhamnosyl acceptor **9** [32] under the promotion of NIS/AgOTf [33] in DCM at −15 °C furnishing fully protected disaccharide **10** in 84% yield in the exclusive β-configuration owing to neighbouring group participation of an ester group at C2. The allyl group of disaccharide **10** was isomerized using an iridium-based catalyst [34,35] and hemiacetal **11** was formed following treatment of the resulting 1-propenyl with iodine in aqueous THF [36]. Hemiacetal **11** was then activated into TCA derivative **12** in 78% yield. Phenol O-glycosylation of TCA **12** with 4-hydroxybenzaldehyde (**4**) in the presence of catalytic amounts of $BF_3{\cdot}OEt_2$ in anhydrous DCM, as optimized by Yu et al. [37], provided protected phenolic glycoside **13** in a good 71% yield. Acidic cleavage of the isopropylidene group followed by Zemplén deacetylation were uneventful providing synthetic



Scheme 1. Three-step synthesis of carbamate **5** from acyl azide **6** through Curtius rearrangement.

gladioside I (**1**) in 91% yield over two steps. A 2D NMR undecoupled HSQC experiment confirmed the formation of the α-L-rhamnopyranosidic linkage ($^1J_{C,H}$ = 169 Hz). The physical and analytical data of synthetic gladioside I (**1**), *i.e.*, $[\alpha]_D$ and ^1H and ^{13}C NMR data, were not in good agreement with the reported values for the natural compound [16] (see Table S2 for NMR data comparison and discussion later in the text for details).

Our next task was to pursue the synthesis of gladioside II (**2**) using a similar strategy than for gladioside I (**1**) (Scheme 3). Phenolic glycosylation between TCA derivative **12** and carbamate **5** under the catalysis of $BF_3{\cdot}OEt_2$ in anhydrous DCM only allowed us to detect traces of phenolic glycoside **14**. Other solvents (THF, CH_3CN, and toluene) were screened for improving the reaction yield (see Table S1 for details). We found that conducting the glycosylation in a mixture of DCM/THF 4:1 using $BF_3{\cdot}OEt_2$ as the catalyst gave the best results (71%). Performing the reaction at higher (0 °C) or lower (−78 °C) temperatures or using TMSOTf as the promoter did not provide any yield improvement. Protected phenolic glycoside **14** was then deacetylated and the isopropylidene group removed by treatment with aqueous acetic acid. However, to our utmost surprise, gladioside I (**1**) rather than gladioside II (**2**) was isolated from the reaction mixture (72% yield over two steps).

To shed light on this intriguing transformation, non-glycosylated phenolic carbamate **5** was subjected to both deprotection conditions (Scheme 4A). While basic Zemplén deacetylation did not affect carbamate **5**, treatment with aqueous acetic acid at high temperatures (50 or 80 °C) or with *p*-toluenesulfonic acid in aqueous DCM led to the quantitative formation of 4-hydroxybenzaldehyde (**4**). We suggest that under aqueous acidic conditions, the hydrolysis of methyl carbamate **2** or **5** generates an instable carbamate, which decarboxylates to form an enamine (Scheme 4B). This enamine would be attacked by a water molecule at the electron-deficient benzylic position [38] yielding a

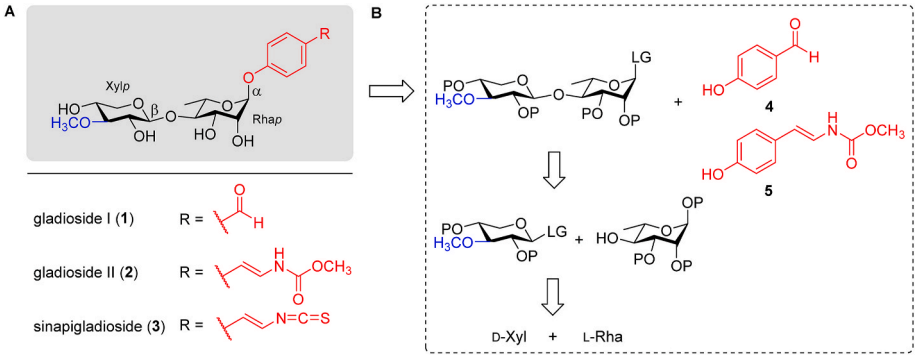
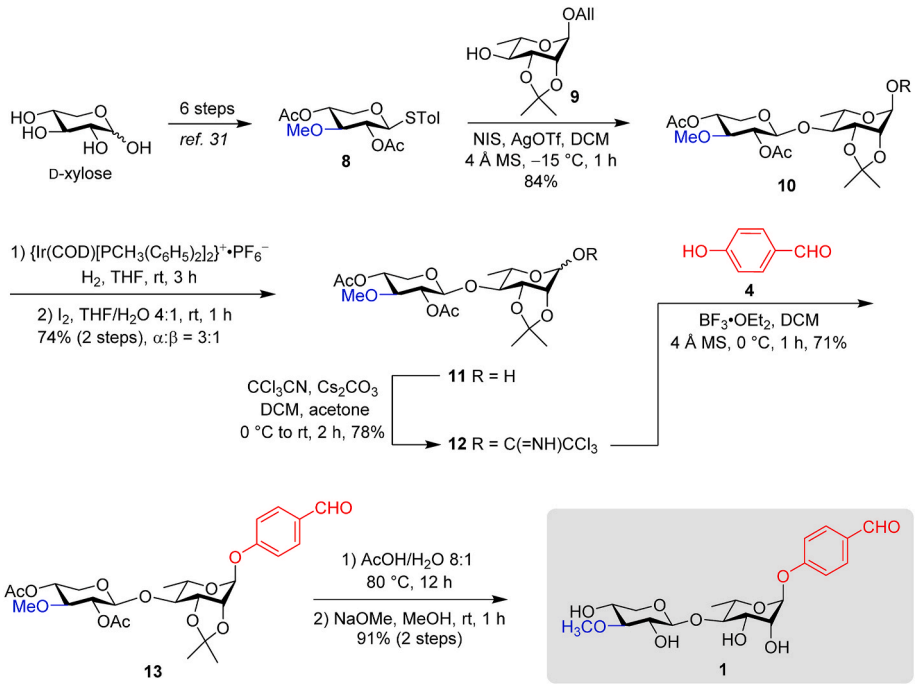
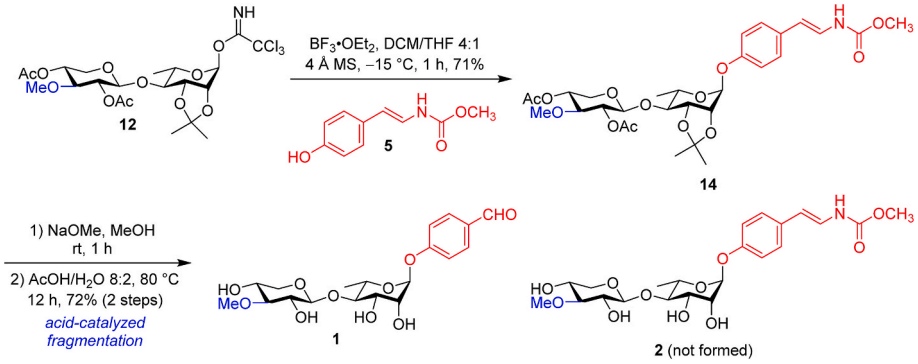


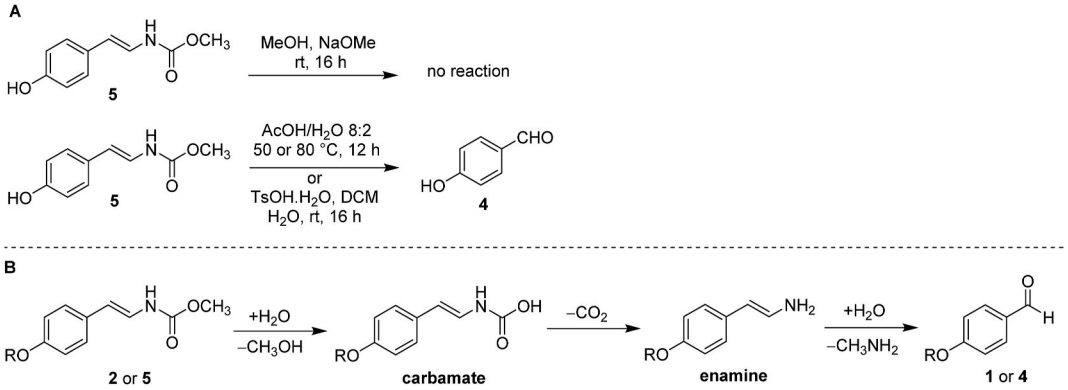
Fig. 1. Proposed structures of the phenolic glycosides gladioside I (**1**), gladioside II (**2**), and sinapigladioside (**3**) isolated from *Burkholderia gladioli* organic extracts (**A**); Retrosynthetic analysis of gladioside I (**1**) and gladioside II (**2**) (**B**). LG = leaving group; P = protecting group.



Scheme 2. Total synthesis of gladioside I (**1**).



Scheme 3. Attempt to synthesize gladioside II (**2**).



Scheme 4. Control experiments for the conversion of carbamate **5** into aldehyde **4** (**A**); Plausible reaction sequence for the acid-catalyzed fragmentation of carbamate **2** or **5** into aldehyde **1** or **4** (**B**).

phenylethanolamine, which undergoes acidic cleavage resulting in the formation of benzaldehyde **1** or **4** along with methylamine [39]. Further experimental details are however needed to fully decipher this acid-catalyzed fragmentation mechanism.

Having understood that the removal of the isopropylidene group was problematic due to the instability of the phenolic α,β-unsaturated methyl carbamate functionality towards acidic conditions, we then sought to implement an alternative protecting groups strategy in order to reach

synthetic gladioside II (**2**). Therefore, as shown in Scheme 5, the isopropylidene group of disaccharide **10** was removed and replaced with benzoyl esters. Resulting disaccharide **15** was transformed into corresponding hemiacetal **16** in 88% yield over two steps. Following its conversion into a TCA derivative, glycosylation of disaccharide **17** with carbamate **5** led to the formation of phenolic glycoside **18** in 60% yield using our previously optimized conditions. Zemplén deacetylation of phenolic glycoside **18** finally provided synthetic gladioside II (**2**) uneventfully. Formation of the α-L-rhamnopyranosidic linkage was confirmed at this stage with the help of a 2D NMR undecoupled HSQC experiment ($^1J_{C,H}$ = 174 Hz). As for gladioside I (**1**), the physical and analytical data of synthetic gladioside II (**2**), *i.e.*, $[\alpha]_D$ and ^{1}H and ^{13}C NMR data, were not in good agreement with the reported values for the natural compound [16] (see Table S3 for NMR data comparison).

As mentioned above, significant discrepancies have been found between the physical and analytical data of the synthetic and natural gladiosides I and II ($[\alpha]_D$ values and NMR data) pointing towards a structural misassignment of these natural products [40]. Comparison of the ^{1}H and ^{13}C NMR data reveals important chemical shift differences for the sugar residues of the molecules (see Tables S2 and S3). As 2D NMR spectra (COSY, HSQC, and HMBC) show very similar correlation patterns between the natural and synthetic gladiosides, we hypothesize our synthetic compounds (**1** and **2**) correspond to diastereoisomeric congeners of the originally described natural products. Evidence in support of this hypothesis comes from the fact that, as mentioned by Khan et al. [16], the L- and D-configuration of rhamnose and xylose residues, respectively, were not experimentally determined due to the paucity of the isolated natural products. Similarly, the chiral analysis of the sugar residues was not reported for the structural determination of sinapigladioside (**3**) [14,15] as well as for structurally related phenolic glycosides such as byelyankacin [41], a melanogenesis inhibitor isolated from *Enterobacter* sp. B20. We believe it is especially important to reinvestigate the exact configuration of the sugar residues in these naturally occurring phenolic glycosides as different strains of *B. gladioli* (ICMP11096 and NCPPB 1891) [42–44] were shown to produce polysaccharides incorporating rhamnose residues in both L- and D-configurations and in either α- or β-glycosidic linkages. That being said, the anomeric linkage of the xylose residue is confirmed to be 1,2-*trans* with $^3J_{H1,H2}$ values of 7.8 and 7.6 Hz for natural gladiosides I and II, respectively. As for the rhamnose residue, a 0.1 ppm difference for the anomeric proton between the synthetic and natural compounds in ^{1}H NMR is unlikely indicative of two different anomers. However, as the $^1J_{C1,H1}$ values were not originally reported, it is difficult to discriminate beyond any doubt between both α- and β-anomers without having access to the natural compounds.

As a preliminary biological evaluation, synthetic gladiosides I (**1**) and II (**2**) were evaluated for their antifungal potential against *Candida albicans* and *Candida auris* as well as for their antibacterial activity against a range of bacteria. Unfortunately, the synthetic compounds were not active against these microorganisms at the maximum tested concentrations.

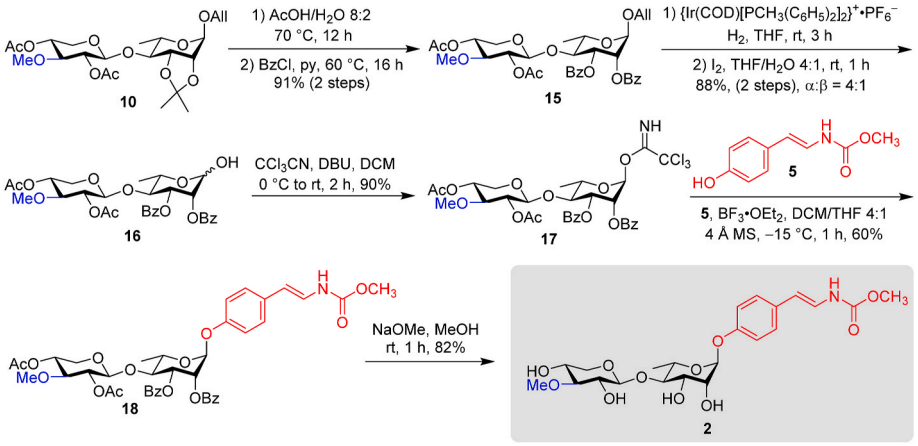
3. Conclusion

In summary, we have accomplished the total synthesis of the proposed structures of gladiosides I (**1**) and II (**2**), two phenolic glycosides isolated from *B. gladioli* methanolic extracts. The two 1,2-*trans*-glycosidic bonds were achieved using neighbouring group participating of a C2 ester group or *via* the steric constraint imposed by the presence of a 2,3-*O*-isopropylidene substituent. We have shown that the structures of these natural products may have been originally misassigned, suggesting the presence of diastereoisomeric congeners. We have uncovered an unprecedented acid-catalyzed fragmentation mechanism converting the α,β-unsaturated methyl carbamate-containing gladioside II (**2**) into the aldehyde-containing gladioside I (**1**). Synthetic gladiosides (**1** and **2**) were not able to inhibit the growth of the tested bacteria and fungi. Our results lay the foundation for the expeditious synthesis of derivatives of these bacterial phenolic glycosides to decipher their biological roles and pharmacological properties. Work is currently in progress in our laboratory to reinvestigate the structures of gladioside I (**1**), gladioside II (**2**), and sinapigladioside (**3**) from *B. gladioli* extracts and to achieve their total synthesis.

4. Experimental

4.1. General methods

All starting materials and reagents were purchased from commercial sources and used as received without further purification. Unless otherwise stated, all reactions were performed under an Ar atmosphere using anhydrous solvents that were either prepared from commercial solvents and dried over heat-gun activated 4 Å molecular sieves or supplied over molecular sieves and used as received. Reagents were introduced via an anhydrous syringe. When required, reaction mixtures were heated using an oil bath. Thin-layer chromatography (TLC) were performed with silica gel (60 F_{254} 0.25 mm) pre-coated aluminum foil plates. Compounds were visualized by using UV_{254} lamp and/or by staining with an orcinol (1.0 mg•mL^{-1} in 10% aq. H_2SO_4) or a ninhydrin (15 mg•mL^{-1} in 3% acetic acid/isopropanol) solution with heating. Normal phase column chromatography was performed on silica gel 60 Å (15–40 μm). NMR spectra were recorded at 297 K in the indicated solvent ($CDCl_3$ and CD_3OD) with 400 or 600 MHz instruments, employing standard software's given by the manufacturer. ^{1}H and ^{13}C NMR spectra were referenced to tetramethylsilane (TMS, $\delta_H = \delta_C = 0.00$ ppm) as



Scheme 5. Successful synthesis of gladioside II (**2**).

internal reference for spectra in MeOD and $CDCl_3$. Assignments were based on ^1H, ^{13}C, COSY, HSQC, HMBC, and undecoupled HSQC experiments. High resolution mass spectra (HRMS) were recorded on an ESI-Q-TOF mass spectrometer. Optical rotation $[\alpha]^{20}{}_D$ data was measured using an Anton Paar polarimeter.

4.2. Allyl 2,4-di-O-acetyl-3-O-methyl-β-D-xylopyranosyl-(1 → 4)-2,3-O-isopropylidene-α-L-rhamnopyranoside (10)

Compound **8** [31] (1.0 g, 2.82 mmol, 1.0 equiv), acceptor **9** [32] (620 mg, 2.53 mmol, 0.9 equiv), and NIS (762 mg, 3.38 mmol, 1.2 equiv) were dried together under high vacuum for 1 h. Activated powdered molecular sieves (4 Å, 4.00 g) and anhydrous DCM (33 mL) were successively added and the mixture was stirred under Ar for 1 h. The reaction flask was cooled to 0 °C and protected from light using aluminum foil. AgOTf (145 mg, 0.564 mmol, 0.2 equiv) was added and the mixture was stirred under Ar for 2 h at 0 °C. Et_3N (1.0 equiv) was added, the yellow suspension was filtered over Celite, and the solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (100% toluene to 90:10 toluene/EtOAc) to give disaccharide **10** (1.00 g, 84%) as a white amorphous solid: R_f 0.5 (toluene/EtOAc 7:3); $[\alpha]^{20}{}_D = -53$ (*c* 0.7, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 5.90 (ddd, $^3J_{CH\text{-}OCH2a} = 5.4$ Hz, $^3J_{CH\text{-}OCH2b} = 6.2$ Hz, $^3J_{CH = CH2c} = 10.4$ Hz, $^3J_{CH = CH2d} = 15.8$ Hz, 1H, C*H*$_{All}$), 5.29 (dd, $^3J_{CH2d = CH} = 15.7$ Hz, $^2J_{CH2d\text{-}CH2c} = 1.5$ Hz, 1H, C=C*H*$_{2All}$), 5.23 (dd, $^3J_{CH2c = CH} = 10.3$ Hz, $^2J_{CH2c\text{-}CH2d} = 1.1$ Hz, 1H, C=C*H*$_{2All}$), 5.00 (br s, 1H, H-1$_{Rha}$), 5.00 (d, $^3J_{H1\text{-}H2} = 6.0$ Hz, 1H, H-1$_{Xyl}$), 4.91–4.87 (m, 2H, H-2$_{Xyl}$, H-4$_{Xyl}$), 4.16 (ddt, $^3J_{CH2a\text{-}CH} = 5.3$ Hz, $^2J_{CH2a\text{-}CH2b} = 1.3$ Hz, 1H, OC*H*$_{2All}$), 4.13–4.09 (m, 3H, H-5a$_{Xyl}$, H-2$_{Rha}$, H-3$_{Rha}$), 3.99 (ddt, $^3J_{CH2b\text{-}CH} = 6.3$ Hz, $^2J_{CH2b\text{-}CH2a} = 1.2$ Hz, 1H, OC*H*$_{2All}$), 3.67 (dq, $^3J_{H5\text{-}H4} = 9.9$ Hz, $^3J_{H5\text{-}CH3} = 6.2$ Hz,1H, H-5$_{Rha}$), 3.54 (dd, $^3J_{H4\text{-}H5} = 9.9$ Hz, $^3J_{H4\text{-}H3} = 6.9$ Hz, 1H, H-4$_{Rha}$), 3.49 (t, $^3J_{H3\text{-}H4} = {}^3J_{H3\text{-}H2} = 7.4$ Hz, 1H, H-3$_{Xyl}$), 3.46 (s, 3H, C*H*$_3$O$_{Xyl}$), 3.34 (dd, $^2J_{H5a\text{-}H5b} = 12.0$ Hz, $^3J_{H5a\text{-}H4} = 7.2$ Hz, 1H, H-5b$_{Xyl}$), 2.13 (s, 3H, C*H*$_{3Ac}$), 2.11 (s, 3H, C*H*$_{3Ac}$), 1.53 (s, 3H, C(C*H*$_3$)$_2$), 1.35 (s, 3H, C(C*H*$_3$)$_2$), 1.27 (d, $^3J_{CH3\text{-}H5} = 6.2$ Hz, 3H, C*H*$_{3Rha}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.0 (CO$_{Ac}$), 169.6 (CO$_{Ac}$), 133.6 (C-2$_{All}$), 117.9 (C-3$_{Allyl}$), 109.3 (*C*(CH$_3$)$_2$), 98.8 (C-1$_{Xyl}$), 96.0 (C-1$_{Rha}$), 78.7 (C-3$_{Xyl}$), 78.4, (C-3$_{Rha}$), 78.1 (C-4$_{Rha}$), 76.1 (C-2$_{Rha}$), 70.8 (C-2$_{Xyl}$), 69.9 (C-4$_{Xyl}$), 68.0 (C-1$_{All}$), 64.1 (C-5$_{Rha}$), 61.2 (C-5$_{Xyl}$), 58.9 (*C*H$_3$O$_{Xyl}$), 27.9 (C(*C*H$_3$)$_2$), 26.4 (C(*C*H$_3$)$_2$), 21.0 (2 × CH$_{3Ac}$), 17.5 (CH$_{3Rha}$). HRMS calcd for $C_{22}H_{38}NO_{11}$ $[M + NH_4]^+$ 492.2445; found 492.2428.

4.3. 2,4-Di-O-acetyl-3-O-methyl-β-D-xylopyranosyl-(1 → 4)-2,3-O-isopropylidene-L-rhamnopyranose (11)

H_2 was bubbled for 10 min in a suspension of $\{Ir(COD)[PCH_3(C_6H_5)_2]_2\}^+ \bullet PF_6^-$ complex (53 mg, 0.063 mmol, 0.1 equiv) in anhydrous THF (6.3 mL). The red suspension became a pale-yellow solution indicating the activation of iridium complex. H_2 was then degassed and replaced by Ar. A solution of compound **10** (300 mg, 0.63 mmol, 1.0 equiv) in anhydrous THF (3.1 mL) was added to the above Ir complex solution under Ar. After stirring at rt for 3.5 h, TLC analysis (toluene/EtOAc 8:2) showed the conversion of the starting material. To the former mixture, a solution of I_2 (321 mg, 1.26 mmol, 2.0 equiv) in THF/H_2O 4:1 (1.9 mL) was added. After stirring at rt for 1 h, TLC analysis (hexanes/EtOAc 1:1) showed that the starting material was totally consumed. Then, the reaction was quenched with a freshly prepared 10% aq. $Na_2S_2O_3$ solution (50 mL). The aqueous layer was extracted with EtOAc (3 × 60 mL). The organic layers were combined, washed with brine, and dried over $MgSO_4$. The solvents were evaporated under reduced pressure. The residue was purified by silica gel column chromatography (100% hexanes to hexanes/EtOAc 75:25) to give hemiacetal **11** (202 mg, 74%, α/β ratio = 3:1) as a pale-yellow oil. R_f 0.3 (hexanes/EtOAc 1:1); $[\alpha]^{20}{}_D = -67$ (*c* 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, α-anomer) δ 5.37 (d, $^3J_{H1\text{-}H2} = 3.2$ Hz, 1H, H-1$_{Rha}$), 5.00 (d, $^3J_{H1\text{-}H2} = 5.7$ Hz, 1H, H-1$_{Xyl}$), 4.91–4.85 (m, 2H, H-2$_{Xyl}$, H-4$_{Xyl}$), 4.18 (dd, $^3J_{H3\text{-}H4} = 7.3$ Hz, $^3J_{H3\text{-}H2} = 5.9$ Hz, 1H, H-3$_{Rha}$), 4.13 (m, 2H, H-5a$_{Xyl}$, H-2$_{Rha}$), 3.89 (dq, $^3J_{H5\text{-}H4} = 9.5$ Hz, $^3J_{H5\text{-}CH3} = 6.2$ Hz, 1H, H-5$_{Rha}$), 3.54 (dd, $^3J_{H4\text{-}H5} = 9.5$ Hz, $^3J_{H4\text{-}H3} = 7.3$ Hz, 1H, H-4$_{Rha}$), 3.48 (t, $^3J_{H3\text{-}H4} = {}^3J_{H3\text{-}H2} = 7.4$ Hz, 1H, H-3$_{Xyl}$), 3.45 (s, 3H, OC*H*$_{3Xyl}$), 3.33 (dd, $^2J_{H5b\text{-}H5a} = 12.0$ Hz, $^3J_{H5b\text{-}H4} = 7.2$ Hz, 1H, H-5b$_{Xyl}$), 3.07 (d, $^3J_{OH\text{-}H1} = 3.6$ Hz, 1H, O*H*$_{Rha}$), 2.13 (s, 3H, C*H*$_{3Ac}$), 2.11 (s, 3H, C*H*$_{3Ac}$), 1.53 (s, 3H, C(C*H*$_3$)$_2$), 1.36 (s, 3H, C(C*H*$_3$)$_2$), 1.27 (d, $^3J_{CH3\text{-}H5} = 6.2$ Hz, 3H, C*H*$_{3Rha}$). ^{13}C NMR (151 MHz, $CDCl_3$, α-anomer) δ 170.0 (CO$_{Ac}$), 169.6 (CO$_{Ac}$), 109.3 (*C*(CH$_3$)$_2$), 98.8 (C-1$_{Xyl}$), 91.9 (C-1$_{Rha}$), 78.6 (C-3$_{Xyl}$), 77.8 (C-4$_{Rha}$, C-3$_{Rha}$), 76.1 (C-2$_{Rha}$), 70.8 (C-2$_{Xyl}$), 69.8 (C-4$_{Xyl}$), 64.5 (C-5$_{Rha}$), 61.2 (C-5$_{Xyl}$), 58.8 (CH$_3$O$_{Xyl}$), 27.8 (C(*C*H$_3$)$_2$), 26.4 (C(*C*H$_3$)$_2$), 21.0 (2 × CH$_{3Ac}$), 17.7 (CH$_{3Rha}$). HRMS calcd for $C_{19}H_{34}NO_{11}$ $[M + NH_4]^+$ 452.2132; found 452.2129.

4.4. 2,4-Di-O-acetyl-3-O-methyl-β-D-xylopyranosyl-(1 → 4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl 2,2,2-trichloroacetimidate (12)

To a cooled solution of hemiacetal **11** (190 mg, 0.437 mmol, 1.0 equiv) in acetone (2.3 mL) and DCM (4.5 mL) were added Cs_2CO_3 (285 mg, 0.87 mmol, 2 equiv) and CCl_3CN (0.43 mL, 4.3 mmol, 10 equiv). The mixture was stirred for 1 h at rt, then the suspension was filtered over Celite and rinsed with DCM. The solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (hexanes/EtOAc + 1% Et_3N 99:1 to 92:8) to give trichloroacetimidate **12** (197 mg, 78%) as a white foam: R_f 0.6 (hexanes/EtOAc 7:3 + 1% Et_3N); $[\alpha]^{20}{}_D = -74$ (*c* 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.68 (s, 1H, NH), 6.45 (s, 1H, H-1$_{Rha}$), 5.03 (d, $^3J_{H1\text{-}H2} = 5.5$ Hz, 1H, H-1$_{Xyl}$), 4.89 (dd, $^3J_{H2\text{-}H3} = 7.3$ Hz, $^3J_{H2\text{-}H1} = 5.6$ Hz, 1H, H-2$_{Xyl}$), 4.87 (dd, $^3J_{H4\text{-}H5b} = {}^3J_{H4\text{-}H3} = 7.0$ Hz, $^3J_{H4\text{-}H5a} = 4.4$ Hz, 1H, H-4$_{Xyl}$), 4.24 (m, 2H, H-3$_{Rha}$, H-2$_{Rha}$), 4.12 (dd, $^2J_{H5a\text{-}H5b} = 12.0$ Hz, $^3J_{H5a\text{-}H4} = 4.3$ Hz, 1H, H-5a$_{Xyl}$), 3.84 (dq, $^3J_{H5\text{-}H4} = 10.0$ Hz, $^3J_{H5\text{-}CH3} = 6.2$ Hz, 1H, H-5$_{Rha}$), 3.60 (dd, $^3J_{H4\text{-}H5} = 10.0$ Hz, $^3J_{H4\text{-}H3} = 6.8$ Hz, 1H, H-4$_{Rha}$), 3.50 (t, $^3J_{H3\text{-}H4} = {}^3J_{H3\text{-}H2} = 7.1$ Hz, 1H, H-3$_{Xyl}$), 3.47 (s, 3H, OC*H*$_{3Xyl}$), 3.37 (dd, $^2J_{H5b\text{-}H5a} = 12.0$ Hz, $^3J_{H5b\text{-}H4} = 6.9$ Hz, 1H, H-5b$_{Xyl}$), 2.14 (s, 3H, C*H*$_{3Ac}$), 2.11 (s, 3H, C*H*$_{3Ac}$), 1.56 (s, 3H, C(C*H*$_3$)$_2$), 1.38 (s, 3H, C(C*H*$_3$)$_2$), 1.30 (d, $^3J_{CH3\text{-}H5} = 6.2$ Hz, 3H, C*H*$_{3Rha}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.0 (CO$_{Ac}$), 169.6 (CO$_{Ac}$), 160.3 (C=NH), 109.9 (*C*(CH$_3$)$_2$), 99.0 (C-1$_{Xyl}$), 95.1 (C-1$_{Rha}$), 78.5 (C-3$_{Xyl}$), 78.0 (C-3$_{Rha}$), 77.6 (C-4$_{Rha}$), 74.9 (C-2$_{Rha}$), 70.7 (C-2$_{Xyl}$), 69.7 (C-4$_{Xyl}$), 67.4 (C-5$_{Rha}$), 61.1 (C-5$_{Xyl}$), 58.9 (OCH$_{3Xyl}$), 27.9 (C(*C*H$_3$)$_2$), 26.5 (C(*C*H$_3$)$_2$), 21.0 (2 × CH$_{3Ac}$), 17.4 (CH$_{3Rha}$).

4.5. 4′-Formylphenyl 2,4-di-O-acetyl-3-O-methyl-β-D-xylopyranosyl-(1 → 4)-2,3-O-isopropylidene-α-L-rhamnopyranoside (13)

To a solution of trichloroacetimidate **12** (230 mg, 0.398 mmol, 1.0 equiv) and 4-hydroxybenzaldehyde (**4**, 49 mg, 0.398 mmol, 1.0 equiv) in anhydrous DCM (4 mL) under Ar was added activated molecular sieves (4 Å, 920 mg). The mixture was stirred for 1 h at rt, then the suspension was cooled at −15 °C and $BF_3 \cdot OEt_2$ (10 μL, 0.080 mmol, 0.2 equiv) was added and the reaction mixture was stirred at the same temperature for 1 h. After 1 h, the reaction mixture was filtered over Celite and rinsed with DCM. The solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (hexanes/EtOAc 99:1 to 76:24) to give compound **13** (152 mg, 71%) as a white amorphous powder: R_f 0.4 (hexanes/EtOAc 6:4); $[\alpha]^{20}{}_D = -100$ (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 9.91 (s, 1H, C*H*O), 7.86–7.84 (m, 2H, C*H*$_{Ar}$), 7.18–7.17 (m, 2H, C*H*$_{Ar}$), 5.80 (s, 1H, H-1$_{Rha}$), 5.02 (d, $^3J_{H1\text{-}H2} = 5.6$ Hz, 1H, H-1$_{Xyl}$), 4.89 (dd, $^3J_{H2\text{-}H3} = 7.3$ Hz, $^3J_{H2\text{-}H1} = 5.7$ Hz, 1H, H-2$_{Xyl}$), 4.86 (dd, $^3J_{H4\text{-}H5b} = {}^3J_{H4\text{-}H3} = 7.0$ Hz, $^3J_{H4\text{-}H5a} = 4.5$ Hz, 1H, H-4$_{Xyl}$), 4.33 (d, $^3J_{H2\text{-}H3} = 5.7$ Hz, 1H, H-2$_{Rha}$), 4.29 (dd, $^3J_{H3\text{-}H4} = 7.1$ Hz, $^3J_{H3\text{-}H2} = 5.8$ Hz, 1H, H-3$_{Rha}$), 4.11 (dd, $^2J_{H5a\text{-}H5b} = 12.0$ Hz, $^3J_{H5a\text{-}H4} = 4.3$ Hz, 1H, H-5a$_{Xyl}$), 3.71 (dq, $^3J_{H5\text{-}H4} = 10.0$ Hz, $^3J_{H5\text{-}CH3} = 6.2$ Hz, 1H, H-5$_{Rha}$), 3.60 (dd, $^3J_{H4\text{-}H5} = 10.0$ Hz, $^3J_{H4\text{-}H3} = 7.2$ Hz, 1H, H-4$_{Rha}$), 3.49 (t, $^3J_{H3\text{-}H4} = {}^3J_{H3\text{-}H2} = 7.2$ Hz, 1H, H-3$_{Xyl}$), 3.45 (s, 3H, OC*H*$_{3Xyl}$), 3.36 (dd, $^2J_{H5b\text{-}H5a} = 12.0$ Hz, $^3J_{H5b\text{-}H4} = 6.9$ Hz, 1H, H-

5b$_{Xyl}$), 2.15 (s, 3H, CH_{3Ac}), 2.10 (s, 3H, CH_{3Ac}), 1.57 (s, 3H, C(CH_3)$_2$), 1.39 (s, 3H, C(CH_3)$_2$), 1.20 (d, $^3J_{CH3\text{-}H5}$ = 6.2 Hz, 3H, CH_{3Rha}). ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.8 (CHO), 170.0 (CO_{Ac}), 169.6 (CO_{Ac}), 160.9 (CH_{Ar}), 131.9 (2 × CH_{Ar}), 131.1 (CH_{Ar}), 116.4 (2 × CH_{Ar}), 109.8 (*C*(CH$_3$)$_2$), 98.8 (C-1$_{Xyl}$), 95.1 (C-1$_{Rha}$), 78.5 (C-3$_{Xyl}$), 78.2 (C-3$_{Rha}$), 77.8 (C-4$_{Rha}$), 75.7 (C-2$_{Rha}$), 70.7 (C-2$_{Xyl}$), 69.7 (C-4$_{Xyl}$), 65.6 (C-5$_{Rha}$), 61.1 (C-5$_{Xyl}$), 58.9 (CH_3O_{Xyl}), 27.9 (C(*C*H$_3$)$_2$), 26.5 (C(*C*H$_3$)$_2$), 21.0 (CH_{3Ac}), 21.0 (CH_{3Ac}), 17.5 (CH_{3Rha}). HRMS calcd for $C_{26}H_{34}NaO_{12}$ $[M + Na]^+$ 561.1948; found 561.1915.

4.6. Synthetic gladioside I (1)

Phenolic disaccharide **13** (50 mg, 0.093 mmol, 1.0 equiv) was dissolved in a mixture of AcOH:H_2O (8:2 *v/v*, 1 mL) and the reaction was heated at 80 °C for 12 h. Then, the reaction mixture was co-evaporated with toluene, dried over high vacuum, and used for the next step without purification. The resulting diol was dissolved in anhydrous MeOH (0.8 mL), a 25% NaOMe solution in MeOH (0.040 mmol, 8 μL, 0.5 equiv) was added, and the reaction mixture was stirred at rt for 1 h. Then, the solvents were evaporated and the residue was purified by silica gel flash chromatography (100% DCM to DCM:MeOH 95:5) to give synthetic gladioside I (**1**, 30 mg, 91%) as a white amorphous powder: R_f 0.3 (DCM/MeOH 9:1); $[\alpha]^{20}{}_D = -168$ (*c* 0.8, MeOH); ^{1}H NMR (600 MHz, MeOD) δ 9.88 (s, 1H, C*H*O), 7.88–7.87 (m, 2H, CH_{Ar}), 7.23–7.21 (m, 2H, CH_{Ar}), 5.59 (d, $^3J_{H1\text{-}H2}$ = 1.6 Hz, 1H, H-1$_{Rha}$), 4.61 (d, $^3J_{H1\text{-}H2}$ = 7.8 Hz, 1H, H-1$_{Xyl}$), 4.09 (dd, $^3J_{H3\text{-}H4}$ = 9.0 Hz, $^3J_{H3\text{-}H2}$ = 3.4 Hz, H-3$_{Rha}$), 4.07 (dd, $^3J_{H2\text{-}H3}$ = 3.4 Hz, $^3J_{H2\text{-}H1}$ = 1.8 Hz, H-2$_{Rha}$), 3.84 (dd, $^2J_{H5a\text{-}H5b}$ = 11.4 Hz, $^3J_{H5a\text{-}H4}$ = 5.5 Hz, 1H, H-5a$_{Xyl}$), 3.69 (t, $^3J_{H4\text{-}H5}$ = $^3J_{H4\text{-}H3}$ = 9.3 Hz, 1H, H-4$_{Rha}$), 3.65 (s, 3H, OC*H*$_{3Xyl}$), 3.63 (dd, $^3J_{H5\text{-}H4}$ = 9.5 Hz, $^3J_{H5\text{-}CH3}$ = 6.1 Hz, 1H, H-5$_{Rha}$), 3.55 (dd, $^3J_{H4\text{-}H5b}$ = 10.3 Hz, $^3J_{H4\text{-}H3}$ = 8.9 Hz, $^3J_{H4\text{-}H5a}$ = 5.5 Hz, 1H, H-4$_{Xyl}$), 3.27 (dd, $^3J_{H2\text{-}H3}$ = 9.0 Hz, $^3J_{H2\text{-}H1}$ = 7.9 Hz, 1H, H-2$_{Xyl}$), 3.20 (dd, $^2J_{H5b\text{-}H5a}$ = 11.3 Hz, $^3J_{H5b\text{-}H4}$ = 10.6 Hz, 1H, H-5b$_{xyl}$), 3.08 (t, $^3J_{H3\text{-}H4}$ = $^3J_{H3\text{-}H2}$ = 9.0 Hz, 1H, H-3$_{Xyl}$), 1.25 (d, $^3J_{CH3\text{-}H5}$ = 6.1 Hz, 3H, C*H*$_{3rha}$). ^{13}C NMR (151 MHz, MeOD) δ 191.5 (CHO), 161.3 (CH_{Ar}), 131.6 (2 × CH_{Ar}), 131.0 (CH_{Ar}), 116.3 (2 × CH_{Ar}), 104.9 (C-1$_{Xyl}$), 98.0 (C-1$_{Rha}$, $^1J_{C1\text{-}H1}$ = 169 Hz), 86.3 (C-3$_{Xyl}$), 81.3 (C-4$_{Rha}$), 74.1 (C-2$_{Xyl}$), 70.9 (C-3$_{Rha}$), 70.3 (C-2$_{Rha}$), 69.4 (C-4$_{Xyl}$), 68.2 (C-5$_{Rha}$), 65.7 (C-5$_{Xyl}$), 59.6 (CH_3O_{Xyl}), 16.7 (CH_{3Rha}). HRMS calcd for $C_{19}H_{26}NaO_{10}$ $[M + Na]^+$ 437.1424; found 437.1409.

4.7. Methyl (E)-styryl carbamate 2,4-di-O-acetyl-3-O-methyl-β-D-xylopyranosyl-(1 → 4)-2,3-O-isopropylidene-5-methyl-α-L-rhamnopyranoside (14)

To a solution of trichloroacetimidate **12** (20 mg, 0.034 mmol, 1.0 equiv) and carbamate **5** (8 mg, 0.041 mmol, 1.2 equiv) in anhydrous DCM:THF (4:1, 0.8 mL) under Ar was added activated molecular sieves (4 Å, 80 mg). The mixture was stirred for 1 h at rt, then the suspension was cooled at −15 °C and $BF_3{\cdot}OEt_2$ (0.9 μL, 0.006 mmol, 0.2 equiv) was added and the reaction mixture was stirred at the same temperature for 1 h. After 1 h, reaction mixture was filtered over Celite and rinsed with DCM. The solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (hexanes/EtOAc 99:1 to 70:30) to give compound **14** (15 mg, 71%) as a white gummy solid: R_f 0.5 (hexanes/EtOAc 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.24–7.22 (m, 2H, 2 × CH_{Ar}), 7.13 (dd, $^3J_{H1b\text{-}H1a}$ = 14.6 Hz, $^3J_{H1b\text{-}NH}$ = 10.9 Hz, 1H, H-1b), 6.99–6.98 (m, 2H, 2 × CH_{Ar}), 6.57 (d, $^3J_{NH\text{-}H1b}$ = 10.7 Hz, 1H, NH), 5.95 (d, $^3J_{H1a\text{-}H1b}$ = 14.5 Hz, 1H, H-1a), 5.68 (s, 1H, H-1$_{Rha}$), 5.05 (d, $^3J_{H1\text{-}H2}$ = 5.6 Hz, 1H, H-1$_{Xyl}$), 4.91 (dd, $^3J_{H2\text{-}H3}$ = 7.3 Hz, $^3J_{H2\text{-}H1}$ = 5.6 Hz, 1H, H-2$_{Xyl}$), 4.88 (td, $^3J_{H4\text{-}H5b}$ = $^3J_{H4\text{-}H3}$ = 6.9 Hz, $^3J_{H4\text{-}H5a}$ = 4.4 Hz, 1H, H-4$_{Xyl}$), 4.31 (d, $^3J_{H2\text{-}H3}$ = 5.6 Hz, 1H, H-2$_{Rha}$), 4.29 (dd, $^3J_{H3\text{-}H4}$ = 7.0 Hz, $^3J_{H3\text{-}H2}$ = 5.7 Hz, 1H, H-3$_{Rha}$), 4.13 (dd, $^2J_{H5a\text{-}H5b}$ = 12.1 Hz, $^3J_{H5a\text{-}H4}$ = 4.4 Hz, 1H, H-5a$_{Xyl}$), 3.82–3.75 (m, 4H, H-5$_{rha}$, OC*H*$_{3carbamate}$), 3.60 (dd, $^3J_{H4\text{-}H5}$ = 10.0 Hz, $^3J_{H4\text{-}H3}$ = 7.0 Hz, 1H, H-4$_{Rha}$), 3.51 (t, $^3J_{H3\text{-}H4}$ = $^3J_{H3\text{-}H2}$ = 7.2 Hz, 1H, H-3$_{Xyl}$), 3.47 (s, 3H, OC*H*$_{3Xyl}$), 3.37 (dd, $^2J_{H5b\text{-}H5a}$ = 12.1 Hz, $^3J_{H5b\text{-}H4}$ = 6.9 Hz, 1H, H-5b$_{Xyl}$), 2.16 (s, 3H, CH_{3Ac}), 2.12 (s, 3H, CH_{3Ac}), 1.57 (s, 3H, C(CH_3)$_2$), 1.40 (s, 3H, C(CH_3)$_2$), 1.22 (d, $^3J_{CH3\text{-}H5}$ = 6.2 Hz, 3H, CH_{3Rha}). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.0 (CO_{Ac}), 169.6 (CO_{Ac}), 154.7 (CH_{Ar}), 154.1($CO_{carbamate}$), 130.5 (CH_{Ar}), 126.4 (2 × CH_{Ar}), 123.0 (C-1b), 116.7 (2 × CH_{Ar}), 110.2 (C-1a), 109.6 (*C*(CH$_3$)$_2$, 98.7 (C-1$_{Xyl}$), 95.4 (C-1$_{Rha}$), 78.4 (C-3$_{Xyl}$), 78.3 (C-4$_{Rha}$), 77.9 (C-2$_{Rha}$), 75.9 (C-3$_{Rha}$), 70.6 (C-2$_{Xyl}$), 69.8 (C-4$_{Xyl}$), 65.1 (C-5$_{Rha}$), 61.1 (C-5$_{Xyl}$), 58.8, (CH_3O_{Xyl}), 52.7 ($CH_3O_{carbamate}$), 27.9 (C(*C*H$_3$)$_2$), 26.5 (C(*C*H$_3$)$_2$), 21.0 (CH_{3Ac}), 21.0 (CH_{3Ac}), 17.6 (CH_{3Rha}).

4.8. Allyl 2,4-di-O-acetyl-3-O-methyl-β-D-xylopyranosyl-(1 → 4)-2,3-di-O-benzoyl-α-L-rhamnopyranoside (15)

Disaccharide **10** (255 mg, 0.537 mmol, 1.0 equiv) was dissolved in a mixture of AcOH:H_2O (8:2 *v/v*, 6 mL) and was heated at 70 °C for 12 h. Then, the reaction mixture was co-evaporated with toluene, dried over high vacuum, and the residue was purified by silica gel flash chromatography. The resulting diol (100 mg, 0.230 mmol, 1.0 equiv) was dissolved in anhydrous pyridine (2.3 mL) and cooled at 0 °C under an Ar atmosphere. DMAP (0.230 mmol, 28 mg, 1.0 equiv) and BzCl (160 L, 1.38 mmol, 6.0 equiv) were added sequentially and the reaction mixture was heated at 60 °C for 22 h. Then, the reaction mixture was cooled to rt, diluted with DCM (30 mL), and washed with 1 N HCl (20 mL). The organic layer was collected, dried over $MgSO_4$, and the residue was purified by silica gel flash chromatography (100% hexanes to hexanes: EtOAc 68:32) to give compound **15** (134 mg, 91%, 2 steps) as a white gummy solid: R_f 0.4 (hexanes/EtOAc 1:1); $[\alpha]^{20}{}_D = +8$ (*c* 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02–8.01 (m, 2H, 2 × CH_{Bz}), 7.93–7.91 (m, 2H, 2 × CH_{Bz}), 7.62–7.59 (m, 1H, CH_{Bz}), 7.56–7.54 (m, 1H, CH_{Bz}), 7.48–7.46 (m, 2H, 2 × CH_{Bz}), 7.41–7.38 (m, 2H, 2 × CH_{Bz}), 5.95 (dd, $^3J_{CH\text{-}CH2d}$ = 17.2 Hz, $^3J_{CH\text{-}CH2c}$ = 10.8 Hz, $^3J_{CH\text{-}CH2a}$ = $^3J_{CH\text{-}CH2b}$ = 5.7 Hz, 1H, CH_{All}), 5.67 (dd, $^3J_{H3\text{-}H4}$ = 9.1 Hz, $^3J_{H3\text{-}H2}$ = 3.5 Hz, 1H, H-3$_{Rha}$), 5.59 (dd, $^3J_{H2\text{-}H3}$ = 3.4 Hz, $^3J_{H2\text{-}H1}$ = 1.7 Hz, 1H, H-2$_{Rha}$), 5.36 (dd, $^3J_{CH2d\text{-}CH}$ = 17.2 Hz, $^2J_{CH2d\text{-}CH2c}$ = 1.4 Hz, 1H, C═CH_{2All}), 5.24 (dd, $^3J_{CH2c\text{-}CH}$ = 10.5 Hz, $^2J_{CH2c\text{-}CH2d}$ = 0.8 Hz, 1H, C═CH_{2All}), 4.95 (d, $^3J_{H1\text{-}H2}$ = 1.4 Hz, 1H, H-1$_{Rha}$), 4.85 (td, $^3J_{H4\text{-}H5b}$ = $^3J_{H4\text{-}H3}$ = 7.8 Hz, $^3J_{H4\text{-}H5a}$ = 4.8 Hz, 1H, H-4$_{Xyl}$), 4.81 (dd, $^3J_{H2\text{-}H3}$ = 8.5 Hz, $^3J_{H2\text{-}H1}$ = 6.6 Hz, 1H, H-2$_{Xyl}$), 4.70 (d, $^3J_{H1\text{-}H2}$ = 6.6 Hz, 1H, H-1$_{Xyl}$), 4.26 (ddt, $^2J_{CH2a\text{-}CH2b}$ = 12.9 Hz, $^3J_{CH2a\text{-}CH}$ = 5.2 Hz, $^4J_{CH2a\text{-}CH2c}$ = 1.5 Hz, 1H, OCH_{2All}), 4.14 (dd, $^2J_{H5a\text{-}H5b}$ = 12.0 Hz, $^3J_{H5a\text{-}H4}$ = 4.8 Hz, 1H, H-5a$_{Xyl}$), 4.08 (ddt, $^2J_{CH2b\text{-}CH2a}$ = 12.9 Hz, $^3J_{CH2b\text{-}CH}$ = 6.3 Hz, $^4J_{CH2b\text{-}CH2c}$ = 1.5 Hz, 1H, OCH_{2All}), 3.98–3.92 (m, 2H, H-4$_{Rha}$, H-5$_{Rha}$), 3.29 (dd, $^2J_{H5b\text{-}H5a}$ = 11.9 Hz, $^3J_{H5b\text{-}H4}$ = 7.8 Hz, 1H, H-5b$_{Xyl}$), 3.28 (s, 3H, OC*H*$_{3Xyl}$), 3.19 (t, $^3J_{H3\text{-}H4}$ = $^3J_{H3\text{-}H2}$ = 8.2 Hz, 1H, H-3$_{Xyl}$), 2.07 (s, 3H, CH_{3Ac}), 1.80 (s, 3H, CH_{3Ac}), 1.43 (d, $^3J_{CH3\text{-}H5}$ = 5.8 Hz, 3H, CH_{3rha}). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.9, 169.5, 165.4, 165.1, 133.4, 133.4, 133.2, 129.8, 129.7, 129.6, 129.6, 128.5, 128.4, 118.1, 101.2, 96.5, 79.5, 76.8, 72.7, 70.9, 70.8, 70.3, 68.4, 67.0, 62.1, 58.7, 20.9, 20.4, 17.9. HRMS calcd for $C_{33}H_{42}NO_{13}$ $[M + NH_4]^+$ 660.2656; found 660.2648.

4.9. 2,4-Di-O-acetyl-3-O-methyl-β-D-xylopyranosyl-(1 → 4)-2,3-di-O-benzoyl-α-L-rhamnopyranose (16)

H_2 was bubbled for 10 min in a suspension of {Ir(COD)[$PCH_3(C_6H_5)_2$]$_2^+$•PF_6^- complex (8 mg, 0.009 mmol, 0.1 equiv) in anhydrous THF (1 mL). The red suspension became a pale-yellow solution indicating the activation of iridium complex. H_2 was then degassed and replaced by Ar. A solution of compound **15** (60 mg, 0.093 mmol, 1.0 equiv) in anhydrous THF (0.5 mL) was added to the above Ir complex solution under Ar. After stirring at rt for 3.5 h, a solution of I_2 (47 mg, 0.87 mmol, 2.0 equiv) in THF/H_2O 4:1 (0.3 mL) was added. After stirring at rt for 1 h, TLC analysis (hexanes/EtOAc 1:1) showed that the starting material was totally consumed. Then, the reaction was quenched with a freshly prepared 10% aq. $Na_2S_2O_3$ solution (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). The organic layers were combined, washed with brine, and dried over $MgSO_4$. The solvents were evaporated under reduced pressure. The residue was

purified by silica gel column chromatography (100% hexanes to hexanes/EtOAc 70:30) to give hemiacetal **16** (44.8 mg, 87%, α/β ratio = 4:1) as a pale-yellow oil. R_f 0.4 (hexanes/EtOAc 1:1); $[\alpha]^{20}{}_D = -29$ (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, α-anomer) δ 8.03–8.01 (m, 2H, 2 × C*H*$_{Bz}$), 7.93–7.92 (m, 2H, 2 × C*H*$_{Bz}$), 7.63–7.60 (m, 1H, C*H*$_{Bz}$), 7.57–7.54 (m, 1H, C*H*$_{Bz}$), 7.49–7.46 (m, 2H, 2 × C*H*$_{Bz}$), 7.41–7.39 (m, 2H, 2 × C*H*$_{Bz}$), 5.73 (dd, $^3J_{H3\text{-}H4} = 9.6$ Hz, $^3J_{H3\text{-}H2} = 3.4$ Hz, 1H, H-3$_{Rha}$), 5.61 (dd, $^3J_{H2\text{-}H3} = 3.4$ Hz, $^3J_{H2\text{-}H1} = 1.9$ Hz, 1H, H-2$_{Rha}$), 5.34 (dd, $^3J_{H1\text{-}OH} = 3.4$ Hz, $^3J_{H1\text{-}H2} = 1.9$ Hz, 1H, H-1$_{Rha}$), 4.85 (td, $^3J_{H4\text{-}H5b} = {}^3J_{H4\text{-}H3} = 7.8$ Hz, $^3J_{H4\text{-}H5a} = 4.9$ Hz, 1H, H-4$_{Xyl}$), 4.82 (dd, $^3J_{H2\text{-}H3} = 8.6$ Hz, $^3J_{H2\text{-}H1} = 6.7$ Hz, 1H, H-2$_{Xyl}$), 4.71 (d, $^3J_{H1\text{-}H2} = 6.6$ Hz, 1H, H-1$_{Xyl}$), 4.20 (dq, $^3J_{H5\text{-}H4} = 9.5$ Hz, $^3J_{H5\text{-}CH3} = 6.2$ Hz, 1H, H-5$_{Rha}$), 4.14 (dd, $^2J_{H5a\text{-}H5b} = 11.9$ Hz, $^3J_{H5a\text{-}H4} = 4.7$ Hz, 1H, H-5a$_{Xyl}$), 3.96 (t, $^3J_{H4\text{-}H5} = {}^3J_{H4\text{-}H3} = 9.5$ Hz, 1H, H-4$_{Rha}$), 3.29 (dd, $^2J_{H5b\text{-}H5a} = 11.8$ Hz, $^3J_{H5b\text{-}H4} = 7.9$ Hz, 1H, H-5b$_{Xyl}$), 3.28 (s, 3H, OC*H*$_{3Xyl}$), 3.19 (t, $^3J_{H3\text{-}H4} = {}^3J_{H3\text{-}H2} = 8.2$ Hz, 1H, H-3$_{Xyl}$), 3.08 (d, $^3J_{OH\text{-}H1} = 3.7$ Hz, 1H, O*H*), 2.08 (s, 3H, C*H*$_{3Ac}$), 1.81 (s, 3H, C*H*$_{3Ac}$), 1.42 (d, $^3J_{CH3\text{-}H5} = 6.2$ Hz, 3H, C*H*$_{3Rha}$). ^{13}C NMR (151 MHz, $CDCl_3$, α-anomer) δ 169.9, 169.5, 165.4, 165.1, 133.4, 133.2, 129.8, 129.6, 128.5, 128.4, 101.1, 92.3, 79.4, 76.7, 72.2, 71.1, 70.7, 70.2, 67.1, 62.1, 58.6, 20.9, 20.4, 18.0. HRMS calcd for $C_{30}H_{38}NO_{13}$ $[M + NH_4]^+$ 620.2343; found 620.2325.

4.10. 2,4-Di-O-acetyl-3-O-methyl-β-D-xylopyranosyl-(1 → 4)-2,3-di-O-benzoyl-α-L-rhamnopyranosyl 2,2,2- trichloroacetimidate (17)

To a cooled solution of hemiacetal **16** (45 mg, 0.074 mmol, 1.0 equiv) in DCM (1 mL) were added DBU (2.2 μL, 0.015 mmol, 0.2 equiv) and CCl_3CN (74 μL, 0.74 mmol, 10 equiv). The mixture was stirred for 1 h at rt, then the suspension was filtered over Celite and rinsed with DCM. The solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (hexanes/EtOAc + 1% Et_3N 99:1 to 82:18) to give trichloroacetimidate **17** (49.9 mg, 90%) as a white foam: R_f 0.6 (hexanes/EtOAc 1:1 + 1% Et_3N); $[\alpha]^{20}{}_D = +3.6$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.77 (s, 1H, N*H*), 8.03–8.02 (m, 2H, 2 × C*H*$_{Bz}$), 7.93–7.92 (m, 2H, 2 × C*H*$_{Bz}$), 7.64–7.61 (m, 1H, C*H*$_{Bz}$), 7.58–7.55 (m, 1H, C*H*$_{Bz}$), 7.50–7.47 (m, 2H, 2 × C*H*$_{Bz}$), 7.42–7.39 (m, 2H, 2 × C*H*$_{Bz}$), 6.39 (d, $^3J_{H1\text{-}H2} = 2.0$ Hz, 1H, H-1$_{Rha}$), 5.80 (dd, $^3J_{H2\text{-}H3} = 3.4$ Hz, $^3J_{H2\text{-}H1} = 2.1$ Hz, 1H, H-2$_{Rha}$), 5.70 (dd, $^3J_{H3\text{-}H4} = 9.5$ Hz, $^3J_{H3\text{-}H2} = 3.5$ Hz, 1H, H-3$_{Rha}$), 4.85 (td, $= {}^3J_{H4\text{-}H5b} = {}^3J_{H4\text{-}H3} = 7.6$ Hz, $^3J_{H4\text{-}H5a} = 4.7$ Hz, 1H, H-4$_{Xyl}$), 4.82 (dd, $^3J_{H2\text{-}H3} = 8.5$ Hz, $^3J_{H2\text{-}H1} = 6.5$ Hz, 1H, H-2$_{Xyl}$), 4.75 (d, $^3J_{H1\text{-}H2} = 6.5$ Hz, 1H, H-1$_{Xyl}$), 4.15 (dq, $^3J_{H5\text{-}H4} = 9.5$ Hz, $^3J_{H5\text{-}CH3} = 6.2$ Hz, 1H, H-5$_{Rha}$), 4.12 (dd, $^2J_{H5a\text{-}H5b} = 11.9$ Hz, $^3J_{H5a\text{-}H4} = 4.7$ Hz, 1H, H-5a$_{Xyl}$), 4.05 (t, $^3J_{H4\text{-}H5} = {}^3J_{H4\text{-}H3} = 9.5$ Hz, 1H, H-4$_{Rha}$), 3.31 (dd, $^2J_{H5b\text{-}H5a} = 12.0$ Hz, $^3J_{H5b\text{-}H4} = 7.6$ Hz, 1H, H-5b$_{Xyl}$), 3.29 (s, 3H, OC*H*$_{3Xyl}$), 3.23 (t, $^3J_{H3\text{-}H4} = {}^3J_{H3\text{-}H2} = 8.1$ Hz, 1H, H-3$_{Xyl}$), 2.08 (s, 3H, C*H*$_{3Ac}$), 1.78 (s, 3H, C*H*$_{3Ac}$), 1.48 (d, $^3J_{CH3\text{-}H5} = 6.2$ Hz, 3H, C*H*$_{3Rha}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.9, 169.4, 165.1, 165.1, 160.3, 133.6, 133.4, 129.9, 129.6, 129.4, 129.2, 129.0, 128.6, 128.5, 128.2, 125.3, 101.1, 94.9, 79.4, 76.0, 72.1, 70.7, 70.2, 70.0, 69.2, 62.1, 58.7, 20.9, 20.3, 18.0. HRMS calcd for $C_{32}H_{34}Cl_3NNaO_{13}$ $[M + Na]^+$ 768.0993; found 768.0887.

4.11. Methyl (E)-(4-hydroxystyryl) carbamate (5)

p-Coumaric acid **6** (100 mg, 0.60 mmol, 1 equiv), triphenylphosphine (399 mg, 1.52 mmol, 2.5 equiv), and sodium azide (59 mg, 0.914 mmol, 1.5 equiv) were dissolved in anhydrous acetonitrile (2 mL) under argon. Trichloroacetonitrile (152 μL, 1.52 mmol, 2.5 equiv) was added dropwise at room temperature. The reaction mixture was stirred for 1.5 h and evaporated under reduced pressure. The residue was purified by silica gel chromatography (100% hexanes to hexanes:EtOAc 95:5) to give compound **7** (102 mg, 87%) as a pale yellow solid. Acyl azide **7** (100 mg, 0.529 mmol) was dissolved in anhydrous toluene (2 mL) and added dropwise to hot anhydrous toluene (2 mL) and heated overnight at 68 °C. Then, the reaction mixture was cooled at rt for 5 min, MeOH (1 mL) was added dropwise, and the reaction was heated at 80 °C for 30 min. After complete consumption of the starting material, the reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel flash chromatography (100% hexanes to hexanes: EtOAc 97:6) to give carbamate **5** (82 mg, 80%) as a white amorphous powder. ^{1}H NMR (600 MHz, MeOD) δ 7.12 (d, $J = 8.5$ Hz, 2H, H-2$_{Ar}$, H-6$_{Ar}$), 7.01 (d, $J = 14.6$ Hz, 1H, H-1b), 6.71 (d, $J = 8.5$ Hz, 2H, H-3$_{Ar}$, H-5$_{Ar}$), 6.00 (d, $J = 14.6$ Hz, 1H, H-1a), 3.73 (s, 3H, CH_3O). ^{13}C NMR (151 MHz, MeOD) δ 155.5, 130.1, 128.3, 127.6, 126.0, 121.9, 115.1, 114.7, 114.5, 110.8, 48.5. HRMS calcd for $C_{10}H_{12}NO_3$ $[M + H]^+$ 194.0817; found 194.0810.

4.12. Methyl (E)-(4-O-styryl) carbamate 2,4-di-O-acetyl-3-O-methyl-β-D-xylopyranosyl-(1 → 4)-2,3-O-benzoyl-α-L-rhamnopyranoside (18)

To a solution of trichloroacetimidate **17** (40 mg, 0.054 mmol, 1.0 equiv) and carbamate **5** (12 mg, 0.064 mmol, 1.2 equiv) in anhydrous DCM:THF (4:1, 0.8 mL) under Ar was added activated molecular sieves (4 Å, 120 mg). The mixture was stirred for 1 h at rt, then the suspension was cooled at −15 °C and $BF_3{\cdot}OEt_2$ (1.3 μL, 0.011 mmol, 0.2 equiv) was added. The reaction mixture was stirred at the same temperature for 1 h. Then, the reaction mixture was filtered over Celite and rinsed with DCM. The solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (hexanes/EtOAc 99:1 to 75:25) to give compound **18** (25 mg, 60%) as a white gummy solid: R_f 0.5 (hexanes/EtOAc 6:4); $[\alpha]^{20}{}_D = -164$ (*c* 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05–8.03 (m, 2H, C*H*$_{Bz}$), 7.96–7.94 (m, 2H, C*H*$_{Bz}$), 7.64–7.61 (m, 1H, C*H*$_{Bz}$), 7.58–7.56 (m, 1H, C*H*$_{Bz}$), 7.50–7.48 (m, 2H, C*H*$_{Bz}$), 7.43–7.40 (m, 2H, C*H*$_{Bz}$), 7.23–7.22 (m, 2H, C*H*$_{Ar}$, C*H*$_{Ar}$), 7.15 (m, 1H, H-1b), 7.06–7.04 (m, 2H, 2 × C*H*$_{Ar}$), 6.69 (d, $^3J_{NH\text{-}H1b} = 10.7$ Hz, 1H, NH), 5.96 (d, $^3J_{H1a\text{-}H1b} = 14.5$ Hz, 1H, H-1a), 5.84 (dd, $^3J_{H3\text{-}H4} = 9.4$ Hz, $^3J_{H3\text{-}H2} = 3.5$ Hz, 1H, H-3$_{Rha}$), 5.76 (dd, $^3J_{H2\text{-}H3} = 3.5$ Hz, $^3J_{H2\text{-}H1} = 1.9$ Hz, 1H, H-2$_{Rha}$), 5.59 (d, $^3J_{H1\text{-}H2} = 1.7$ Hz, 1H, H-1$_{Rha}$), 4.85 (td, $^3J_{H4\text{-}H5b} = {}^3J_{H4\text{-}H3} = 7.6$ Hz, $^3J_{H4\text{-}H5a} = 4.7$ Hz, 1H, H-4$_{Xyl}$), 4.82 (dd, $^3J_{H2\text{-}H3} = 8.5$ Hz, $^3J_{H2\text{-}H1} = 6.5$ Hz, 1H, H-2$_{Xyl}$), 4.75 (d, $^3J_{H1\text{-}H2} = 6.4$ Hz, 1H, H-1$_{Xyl}$), 4.12 (dd, $^2J_{H5a\text{-}H5b} = 12.0$ Hz, $^3J_{H5a\text{-}H4} = 4.7$ Hz, 1H, H-5a$_{Xyl}$), 4.08 (dq, $^3J_{H5\text{-}H4} = 9.4$ Hz, $^3J_{H5\text{-}CH3} = 6.0$ Hz, 1H, H-5$_{Rha}$), 4.03 (t, $^3J_{H4\text{-}H5} = {}^3J_{H4\text{-}H3} = 9.4$ Hz, 1H, H-4$_{Rha}$), 3.77 (s, 3H, C*H*$_3$O$_{carbamate}$), 3.31 (dd, $^2J_{H5b\text{-}H5a} = 12.0$ Hz, $^3J_{H5b\text{-}H4} = 7.6$ Hz, 1H, H-5b$_{Xyl}$), 3.30 (s, 3H, C*H*$_3$O$_{Xyl}$), 3.22 (t, $^3J_{H3\text{-}H4} = {}^3J_{H3\text{-}H2} = 8.0$ Hz, 1H, H-3$_{Xyl}$), 2.08 (s, 3H, C*H*$_{3Ac}$), 1.81 (s, 3H, C*H*$_{3Ac}$), 1.40 (d, $^3J_{CH3\text{-}H5} = 6.0$ Hz, 3H, C*H*$_{3Rha}$). ^{13}C NMR (151 MHz, CDCl3) δ 169.9 (CO_{Ac}), 169.4 (CO_{Ac}), 165.4 (CO_{Bz}), 165.2 (CO_{Bz}), 154.6 (CH_{Ar}), 154.2 ($CO_{carbamate}$), 133.5 (CH_{Bz}), 133.3 (CH_{Bz}), 130.9 (CH_{Ar}), 129.9, 129.6, 129.6, 129.4, 128.6, 128.5, 126.4 (2 × CH_{Ar}), 123.2 (C-1b), 116.9 (2 × CH_{Ar}), 110.1 (C-1a), 101.1 (C-1$_{Xyl}$), 95.9 (C-1$_{Rha}$), 79.2 (C-3$_{Xyl}$), 76.6 (C-4$_{Rha}$), 72.4 (C-3$_{Rha}$), 70.7 (C-2$_{Rha,}$ C-2$_{Xyl}$), 70.2 (C-4$_{Xyl}$), 67.8 (C-5$_{Rha}$), 62.0 (C-5$_{Xyl}$), 58.6 (CH_3O_{Xyl}), 52.7 ($CH_3O_{carbamate}$), 20.9 (CH_{3Ac}), 20.4 (CH_{3Ac}), 17.9 (CH_{3Rha}). HRMS calcd for $C_{40}H_{47}N_2O_{15}$ $[M + NH_4]^+$ 795.2976; found 795.2961.

4.13. Synthetic gladioside II (2)

Phenolic disaccharide **18** (19 mg, 0.024 mmol, 1.0 equiv) was dissolved in anhydrous methanol (400 μL) and NaOMe 25% in MeOH (0.024 mmol, 4 μL, 1 equiv) was added and the reaction mixture was stirred at rt for 1 h. After 1 h, the reaction mixture was evaporated and the residue was purified by silica gel flash chromatography (100% DCM to DCM:MeOH 94:6) to give synthetic gladioside II (**2**, 9.6 mg, 82%) as a white amorphous powder: R_f 0.3 (DCM/MeOH 9:1); $[\alpha]^{20}{}_D = -102$ (*c* 0.6, MeOH); ^{1}H NMR (600 MHz, MeOD) δ 7.21–7.20 (m, 2H, 2 × C*H*$_{Ar}$), 7.10 (d, $^3J_{H1b\text{-}H1a} = 14.6$ Hz, 1H, H-1b), 6.97–6.95 (m, 2H, 2 × C*H*$_{Ar}$), 6.03 (d, $^3J_{H1a\text{-}H1b} = 14.6$ Hz, 1H, H-1a), 5.39 (d, $^3J_{H1\text{-}H2} = 1.7$ Hz, 1H, H-1$_{Rha}$), 4.60 (d, $^3J_{H1\text{-}H2} = 7.7$ Hz, 1H, H-1$_{Xyl}$), 4.07 (dd, $^3J_{H3\text{-}H4} = 9.0$ Hz, $^3J_{H3\text{-}H2} = 3.4$ Hz, 1H, H-3$_{Rha}$), 4.02 (dd, $^3J_{H2\text{-}H3} = 3.4$ Hz, $^3J_{H2\text{-}H1} = 1.8$ Hz, 1H, H-2$_{Rha}$), 3.84 (dd, $^2J_{H5a\text{-}H5b} = 11.4$ Hz, $^3J_{H5a\text{-}H4} = 5.5$ Hz, 1H, H-5a$_{Xyl}$), 3.74 (s, 3H, CO_2CH_3), 3.68 (dq, $^3J_{H5\text{-}H4} = 9.5$ Hz, $^3J_{H5\text{-}CH3} = 6.0$ Hz, 1H, H-5$_{Rha}$), 3.67–3.65 (m, 1H, H-4$_{Rha}$), 3.65 (s, 3H, OC*H*$_{3Xyl}$), 3.54

(qd, $^{3}J_{H4\text{-}H5b} = 10.4$ Hz, $^{3}J_{H4\text{-}H3} = 8.9$ Hz, $^{3}J_{H4\text{-}H5a} = 5.5$ Hz, 1H, H-4_{Xyl}), 3.26 (dd, $^{3}J_{H2\text{-}H3} = 9.1$ Hz, $^{3}J_{H2\text{-}H1} = 7.8$ Hz, 1H, H-2_{Xyl}), 3.21 (dd, $^{2}J_{H5b\text{-}H5a} = 11.3$ Hz, $^{3}J_{H5b\text{-}H4} = 10.6$ Hz, 1H, H-$5b_{Xyl}$), 3.07 (t, $^{3}J_{H3\text{-}H4} = {}^{3}J_{H3\text{-}H2} = 9.0$ Hz, 1H, H-3_{Xyl}), 1.26 (d, $^{3}J_{CH3\text{-}H5} = 6.0$ Hz, 3H, CH_{3Rha}). ^{13}C NMR (151 MHz, MeOD) δ 155.5 ($CO_{carbamate}$), 154.8 (CH_{Ar}), 131.1 (CH_{Ar}), 125.8 (2 × CH_{Ar}), 123.2 (C-1b), 116.4 (2 × CH_{Ar}), 110.0 (C-1a), 105.0 (C-1_{Xyl}), 98.4 (C-1_{Rha}, $^{1}J_{C1\text{-}H1} = 174$ Hz), 86.4 (C-3_{Xyl}), 81.6 (C-4_{Rha}), 74.2 (C-2_{Xyl}), 71.0 (C-3_{Rha}), 70.6 (C-2_{Rha}), 69.5 (C-4_{Xyl}), 67.7 (C-5_{Rha}), 65.7 (C-5_{Xyl}), 59.5 (CH_3O_{Xyl}), 51.5 ($CH_3O_{carbamate}$), 16.6 (CH_{3Rha}). HRMS calcd for $C_{22}H_{31}NNaO_{11}$ $[M + Na]^+$ 508.1795; found 508.1787.

4.14. HPLC analysis of synthetic gladiosides I and II

HPLC analyses were performed on a Thermo Fisher Scientific Ultimate 3000 HPLC-UV-CAD system equipped with a Dionex LPG-3400SD pump, a WPS-3000SL autosampler, a TCC-3000SD column oven, a Dionex Ultimate 3000 DAD detector and a charged aerosol detector (CAD) Corona Veo. The power function value was set at 1.0, the filter at 1 s, the data collection rate at 10 Hz, and the evaporator temperature at 35 °C. Nitrogen (57.2 psi) was used for nebulization. All data were analyzed using the Thermo Fischer Chromeleon 7.2.9 software. A reverse phase column Hypersil Gold (250 × 4.6 mm) was used with a mobile phase consisting of a CH_3CN/H_2O gradient. Prior to the injection, the column was equilibrated for 10 min with 5% CH_3CN. The injection volume was 50 μL. The elution gradient started from 5 to 50% CH_3CN for 20 min, then to 100% CH_3CN within the next 2 min and held for 3 min. HPLC flow rate was set to 1.0 mL•min^{-1} and the oven temperature was set at 28 °C. A flow splitter was used after the column to deliver 20% of the mixture to the CAD detector and the remaining to the DAD detector. Solutions of synthetic gladiosides I (**1**) and II (**2**) were prepared in MeOH with a concentration of 1 mg•mL^{-1}.

4.15. Antifungal inhibition assay

Strains used in this study were *Candida albicans* SC5314 [45] and *Candida auris* B11220 (BioSample ID SAMN05379608). For general propagation and maintenance, the strains were cultured at 30 °C in yeast extract-peptone-dextrose (YPD) medium supplemented with uridine (2% Bacto™ peptone, 1% yeast extract, 2% dextrose, and 50 mg•mL^{-1} uridine). Working stock solutions of gladiosides I and II (50 mg•mL^{-1}) were prepared in DMSO. For growth assays in liquid YPD, SC complete (0.67% yeast nitrogen base with ammonium sulfate, 2.0% glucose, and 0.079% complete supplement mixture) and YP-Glycerol (2% Bacto™ peptone, 1% yeast extract, 2% glycerol, and 50 mg•mL^{-1} uridine) media were used. Overnight cultures of *C. albicans* and *C. auris* were resuspended in fresh appropriate medium at an OD_{600} of 0.05 and added to a flat-bottom of a 96-well plate in a total volume of 100 μL per well in addition of the tested compounds at different concentrations (4–128 μg μL^{-1}). For each experiment, a compound-free growth control and a cell-free negative control were included. Growth assay curves were performed in triplicate in 96-well plates using a Sunrise plate-reader (Tecan) at 30 °C under constant agitation with OD_{600} readings taken every 10 min for 48 h.

4.16. Antibacterial inhibition assay

Strains used in this study were *Pseudomonas aeruginosa* PA14, *Staphylococcus aureus* strains Newman and ED711 (MRSA), *Enterococcus faecalis* ED4019, *Escherichia coli* CFT073, *Bacillus subtilis* PY79, *Acinetobacter baumannii* IRSST B95, *Enterobacter cloacae* IAF25, and *Enterobacter aerogenes* ATCC 13048. Stocks of compounds were prepared in HPLC-grade MeOH at a concentration of 4 mM. Screening for antibacterial activity was performed using a Kirby-Bauer disk diffusion assay [23]. Briefly, bacteria were grown overnight at 37 °C with shaking in tryptic soy broth. Then, cells were spread onto Mueller-Hinton agar (MHA) plates using a sterile cotton swab. Blank antimicrobial susceptibility disks (Oxoid) were soaked with fixed volumes of each compound, reaching a maximum of 1.5 mg for gladioside I and 0.9 mg for gladioside II. MeOH was used as control. Once dried out, the disks were placed onto the surface of an agar plate. Plates were then incubated at 37 °C for 24 h.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2021.108373.

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