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Identification of Neuroprotective Spoxazomicin and Oxachelin Glycosides via Chemoenzymatic Glycosyl-Scanning

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Abstract

The assessment of glycosyl-scanning to expand the molecular and functional diversity of metabolites from the underground coal mine fire-associated *Streptomyces* sp. RM-14-6 is reported. Using the engineered glycosyltransferase OleD Loki and a 2-chloro-4-nitrophenylglycoside-based screen, six metabolites were identified as substrates of OleD Loki, from which 12 corresponding metabolite glycosides were produced and characterized. This study highlights the first application of the 2-chloro-4-nitrophenylglycoside-based screen toward an unbiased set of unique microbial natural products and the first reported application of the 2-chloro-4-nitrophenylglycoside-based transglycosylation reaction for the corresponding preparative synthesis of target glycosides. Bioactivity analysis (including antibacterial, antifungal, anticancer, and EtOH damage neuroprotection assays) revealed glycosylation to attenuate the neuroprotective potency of **4**, while glycosylation of the structurally related inactive spoxazomicin C (**3**) remarkably invoked neuroprotective activity.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00949. Chemical structures of compounds **10–20**; HRMS/NMR spectra of compounds **3a–c**, **4a**, **5a,b**, **6a,b**, **7a,b**, and **8a,b** (PDF)

Natural products (NPs) discovery efforts continue to expose pharmacophores of notable scientific, clinical, and commercial value^{1–5} that often inspire synthetic organic chemists^{6–12} and reveal unique metabolic pathways that offer new opportunities in mechanistic enzymology, synthetic biology, and biocatalysis.^{13–22} For the latter, permissive catalysts involved in late-stage NP tailoring modifications (acylation, alkylation, glycosylation, oxidation) have been particularly enabling in NP core scaffold diversification.^{5,15,23–30} The demonstrated impact of glycosylation within this context is diverse and includes influencing NP or small-molecule drug potency, mechanism, pharmacodynamics, pharmacokinetics, and ADME properties^{29–39} where the advent of permissive chemoenzymatic strategies complement and/or circumvent key limitations of conventional chemical glycosylation as a medicinal chemistry tool.^{29,40–51} Such chemoenzymatic strategies have benefited from the development of donor/acceptor permissive glycosyltransferases via directed evolution and a corresponding screening platform enabled by the use of simple activated glycoside donors that drive the equilibrium of glycosyltransferase-catalyzed reactions toward product formation and provide real-time indicators of sugar-nucleotide utilization in parallel (Figure 1).^{52–60} In the current study, we assess the potential of this permissive chemoenzymatic platform for glycosylation of oxazole carboxamide and oxachelin analogues isolated from the Ruth Mullins underground coal mine isolate *Streptomyces* sp. RM-14-6.⁶¹ This analysis revealed the permissive engineered OleD Loki to catalyze the glycosylation of spoxazomicin C [an antitrypanosomal NP first reported from *Streptosporangium oxazolinicum* K07-0460(T)]⁶² and oxachelin (a metal chelator first reported from *Streptomyces* sp. GW9/1258),⁶³ providing the first reported corresponding glycosides of naturally occurring phenol/oxazolines. Subsequent evaluation of the glycosides produced revealed 2-*O*- β -D-glucosylspoxazomicin C (**3a**) as neuroprotective to EtOH-induced damage in rat hippocampal-derived primary cell cultures, while the parent NP (**3**) and glycosides 2-*O*-(2'-amino-2'-deoxy- β -D-glucosyl)spoxazomicin C (**3c**) and 12-*O*- β -D-glucosylspoxazomicin C (**3b**) lacked activity. In addition, glycosylation of spoxazomicin D led to marked decrease in potency over the parent NP comparator in the same assay, while oxachelin (**5**) glycosylation had no apparent effect on bioactivity. Cumulatively, this study further extends the demonstrated substrate scope of OleD Loki and, given the impact of glucose conjugation on improving blood–brain barrier transport,^{64–68} may also offer a convenient strategy to modulate the properties of novel NPs to treat neurological deficits resulting from alcohol abuse.^{61,69–71}

RESULTS AND DISCUSSION

Using a 2-chloro-4-nitrophenylglycoside-driven colorimetric screen for the identification of putative OleD Loki substrates (Figure 1),^{56,57,60} preliminary assays were accomplished with 2 mM (2-chloro-4-nitrophenyl)- β -D-glucoside (**1**) or (2-chloro-4-nitrophenyl)-2-amino-2-deoxy- β -D-glucoside (**2**) as donor, 1 mM acceptor, 0.1 mM UDP, and enzyme (0.26 μ M Loki for donor **1**; 2.6 μ M Loki for donor **2**) in a 384-well plate format. Putative substrates evaluated included 17 recently characterized metabolites from *Streptomyces* sp. RM-14-6 (**3–7** and **9**; **10–20**; Figures 2 and S1),⁶¹ five of which [spoxazomicin C (**3**), spoxazomicin D (**4**), *N*-salicyloyl-2-aminopropane-1,3-diol (**6**) (2*R*)-*N*-salicyloyl-2-aminopropan-1-ol (**7**), and *o*-hydroxybenzamide (**9**); Figure 2] were identified as putative substrates (Figure 3A and B). Subsequent hit validation confirmed the results from the colorimetric screen with turnovers ranging from 6% to 68% based on HPLC integration, where, in some cases (e.g., **3**), the multiple products observed were attributed to putative variant glycoside regioisomers as previously reported for OleD-catalyzed reactions.^{72,73} Interestingly, this secondary analysis also revealed oxachelin (**5**) as a false negative with 45% turnover indicated by HPLC peak integration, the product of which was also confirmed by HRMS. Importantly, this analysis highlights the utility of the 2-chloro-4-nitrophenylglycoside-based screen as a rapid first-pass assay to identify putative substrates for engineered OleDs and highlights the first application of donor **2** (2-chloro-4-nitrophenyl-GlcNH₂) within this context.

For full product characterization and biological evaluation, chemoenzymatic scale-up reactions were conducted with spoxazomicin C (**3**), oxachelin (**5**), *N*-salicyloyl-2-aminopropane-1,3-diol (**6**), (2*R*)-*N*-salicyloyl-2-aminopropan-1-ol (**7**), and (2*S*)-*N*-salicyloyl-2-aminopropan-1-ol (**8**) to give 2-*O*- β -D-glucosylspoxazomicin C (**3a**, 1.2 mg), 12-*O*- β -D-glucosylspoxazomicin C (**3b**, 0.5 mg), 2-*O*-(2'-amino-2'-deoxy- β -D-glucosyl)spoxazomicin C (**3c**, 3.9 mg), 23-*O*- β -D-glucosyloxachelin (**5a**, 1.5 mg), 23-*O*-(2'-amino-2'-deoxy- β -D-glucosyl)-oxachelin (**5b**, 2.0 mg), *N*-(6-*O*- β -D-glucosyl)salicyloyl-2-aminopropane-1,3-diol (**6a**, 2.0 mg), *N*-[6-*O*-(2'-amino-2'-deoxy- β -D-glucosyl)]salicyloyl-2-aminopropane-1,3-diol (**6b**, 0.8 mg), (2*R*)-*N*-(6-*O*- β -D-glucosyl)salicyloyl-2-aminopropan-1-ol (**7a**, 1.6 mg), (2*R*)-*N*-[6-*O*-(2'-amino-2'-deoxy- β -D-glucosyl)]salicyloyl-2-aminopropan-1-ol (**7b**, 4.6 mg), (2*S*)-*N*-(6-*O*- β -D-glucosyl)salicyloyl-2-aminopropan-1-ol (**8a**, 1.8 mg), and (2*S*)-*N*-[6-*O*-(2'-amino-2'-deoxy- β -D-glucosyl)]salicyloyl-2-aminopropan-1-ol (**8b**; 4.8 mg), respectively. This study is among the first to highlight the utility of the 2-chloro-4-nitrophenyl-glycoside-based platform as a means for preparative synthesis of novel glycosides.

Structure Elucidation

The physicochemical properties of the new glycosylated compounds **3a–c**, **4a**, **5a/b**, **6a/b**, **7a/b**, and **8a/b** are summarized in the Experimental Section. Compounds **3a–c** were obtained as white solids. HRMS of **3a–c** revealed a common molecular formula for **3a** and **3b** (C₁₆H₂₁NO₈; consistent with **1** as donor) and a distinct molecular formula for **3c** (C₁₆H₂₂N₂O₇; consistent with **2** as donor). Comparison of ¹H and ¹³C NMR spectral features of **3a–c** to those of the parent aglycone [spoxazomicin C (**3**)] and, in particular, observed ³JHMBC sugar H-1' to aglycon C-2/C-12 correlations (for **3a** and **3c**) and large

sugar anomeric proton coupling constants (δ_{H} 5.02–5.30 ppm, d; J = 7.8–9.0 Hz) confirmed the corresponding **3a** C2-*O*- β -D-glucosyl (β -D-Glc; Figure 2, GI), **3b** C12-*O*- β -D-glucosyl, and **3c** C2-*O*-2'-amino-2'-deoxy- β -D-glucosyl (β -D-GlcNH₂; Figure 2, GII) moieties. Similarly, compounds **5a/b** were obtained as white solids with molecular formulas consistent with monoglycosides (C₃₃H₄₇N₇O₁₆, C₃₃H₄₈N₈O₁₅, respectively), where NMR confirmed the C23 phenolic oxygen of **5** as the point of attachment for β -D-Glc (Figure 2; GI, **5a**) and β -D-GlcNH₂ (Figure 2; GII, **5b**). Likewise, compounds **6a/b**, **7a/b**, and **8a/b** were confirmed by HRMS and 1D and 2D NMR to be β -D-Glc- and β -D-GlcNH₂-derived C6-*O*-monoglycosides of aglycones **6**, **7**, and **8**, respectively (Figure 2). Given the lack of observed OleD Loki turnover with spoxazomicin D (**4**), the corresponding C2-*O*- β -D-Glc comparator for biological evaluation (Figure 2; **4a**) was generated via Koenigs–Knorr glycosylation following conventional protocols.^{74,75}

Biological Activity

All glycosides were evaluated in standard antibacterial, antifungal, and anticancer activity assays, and, based on the reported EtOH damage neuroprotective activity of spoxazomicin D (**4**),⁶¹ a select set of glycosides (**3a–c** and **4a**, Figure 2) were also evaluated in an EtOH damage neuroprotection assay.^{71,76,77} For the latter, compounds (0.01–1.0 μ M) were assessed for their ability to protect against EtOH cytotoxicity of rat hippocampal-derived primary cell cultures exposed to 100 mM EtOH as determined by propidium iodide uptake, a highly polar nucleic acid intercalating agent that labels compromised cells (Figure 4A–D). Mean increases of approximately 70% of ethanol-naïve control levels were observed with each replication. Compound **3a** was found to reduce EtOH-induced increases in propidium iodide uptake. Post hoc analyses revealed that this reversal of propidium iodide uptake by compound **3a** was observed at 0.01 and 0.10 μ M (Figure 4E). In EtOH-naïve medium, coexposure to compound **3a** was also found to induce significant propidium iodide uptake at 1 μ M. Notably, coexposure of compounds **3b** and **3c** did not significantly attenuate EtOH-induced cytotoxicity. Compound **4a** was also found to decrease EtOH-induced increases in propidium iodide uptake. Post hoc analyses demonstrated this reduction in propidium iodide by **4a** was observed at 0.10 and 1.0 μ M (Figure 4F; $\sim 10^3$ less potent than the parent **4**⁶¹). In EtOH-naïve medium, coexposure to compound **4a** did reveal significant increases in propidium iodide uptake at 0.01 and 0.10 μ M. In contrast, glycosylation had no apparent effect on antibacterial, antifungal, or anticancer activity, as both the parental NPs and all corresponding glycosides were inactive at the concentrations tested (60–120 μ M antibacterial/fungal; 20 μ M anticancer). This study is the first to demonstrate glycosylation as a means to modulate the neuroprotective properties of small molecules in the context of EtOH-induced cytotoxicity.

Discussion

Permissive engineered OleDs in conjunction with UDP-Glc as the donor have been successfully used in the preparative synthesis of select pharmacophore glucosides where product distribution highlighted the catalyst's ability to afford distinct glycosidic regioisomers and, in some cases, disaccharide-substituted analogues via iterative glycosylation.^{72,78,79} The advent of a 2-chloro-4-nitrophenylglycoside-based platform

within this context extended the utility of engineered OleDs for sugar nucleotide synthesis and rapid identification of new substrates (referred to as “glycosyl-scanning”).^{55–57,59,60} The current study notably builds on the prior precedent in two primary ways. First, this study is the first application of the 2-chloro-4-nitrophenylglycoside-based screen toward an unbiased set of unique microbial natural products as well as the first application of 2-chloro-4-nitrophenyl-GlcNH₂ as a donor in “glycosyl-scanning”. The corresponding 35% hit rate further highlights the unique permissivity of the OleD Loki GT and its potential in natural product diversification. Second, this study is the first application of a 2-chloro-4-nitrophenylglycoside-based transglycosylation reaction for the preparative synthesis of target glycosides where the recycling of the nucleotide diphosphate (UDP) byproduct (a known glycosyltransferase inhibitor) to drive reaction equilibrium and the use of a simple inexpensive synthetic donor (i.e., **2**) are advantageous. Consistent with prior studies of engineered OleDs,^{53,54,72,73,78–80} analysis of the glycosides formed in this study reveals a propensity toward phenolic glycosylation, where the observed aliphatic β -D-*O*-Glc **3b** stands as an exception. Lack of OleD turnover with **4** (and the corresponding poor reactivity of the **4** C2-OH based on synthetic yields in chemical glycosylation) may implicate prohibitive acceptor intramolecular interactions not present in extended structures such as **5**. Finally, this study also further highlights the potential of glycosylation to modulate the bioactivity of a given pharmacophore, where glycosylation invokes (in the case of **3**) or slightly reduces (in the case of **4**) the desired neuroprotective effects of the parent natural products.^{29–39} While the fundamental mechanism(s) of neuroprotection remain to be determined, this study presents important structure–activity relationships that may support future target identification (e.g., via incorporation of sugars bearing reactive handles for affinity pull-down studies) and/or, given the impact of glucose conjugation on improving blood–brain barrier activity transport,^{64–68} may also enhance biodistribution in the context of advancing leads to treat neurological deficits resulting from alcohol abuse.^{61,69–71}

EXPERIMENTAL SECTION

General Experimental Procedures

All reagents were reagent grade and purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without purification unless otherwise noted. All solvents used were of ACS grade and purchased from Pharmco-AAPER (Brookfield, CT, USA). Donors (2-chloro-4-nitrophenyl)- β -D-glucoside (**1**) and (2-chloro-4-nitrophenyl)-2-amino-2-deoxy- β -D-glucoside (**2**) were synthesized as previously described.^{55,57} All studies used pure OleD Loki (95%; an engineered P67T/I112P/T113M/S132F/A242I OleD variant), the overproduction and affinity purification for which have been previously described.⁵⁶ The isolation, purification, and characterization of *Streptomyces* sp. RM-14-6 metabolites (**3–7** and **9–20**; Figures 2 and S1) used for this study are reported elsewhere.⁶¹ Analytical TLC was performed using Sorbent Technologies silica gel glass TLC plates (EMD Chemical Inc., PA, USA). Visualization was accomplished with UV light (254 nm) followed by staining with a diluted sulfuric acid solution (5% in EtOH) and heating. NMR spectra were measured using Varian (Palo Alto, CA, USA) Vnmr 500 (¹H, 500 MHz; ¹³C, 125.7 MHz) and Vnmr 400 (¹H, 399.8 MHz; ¹³C, 100.5 MHz) spectrometers using 99.8% D₂O with 0.05% v/v tetramethylsilane (TMS) or 99.8% CD₃OD from Cambridge Isotopes (Cambridge Isotope

Laboratories, MA, USA), where δ -values were referenced to TMS. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), and m (multiplet). Chemical shifts are reported in parts per million (ppm), and coupling constants J are given in Hz. High-resolution electrospray ionization (HRESI) mass spectra were obtained on a Waters (Milford, MA, USA) LCT time-of-flight (TOF) spectrometer or an AB SCIEX Triple TOF 5600 system (AB Sciex, Framingham, MA, USA). *Staphylococcus aureus*, *Salmonella enterica*, and *Saccharomyces cerevisiae* strains and A549 cells were obtained from ATCC (Manassas, VA, USA); *Micrococcus luteus* and *Escherichia coli* were obtained from NRRL (Peoria, IL, USA).

Chromatography Methods

Analytical reversed-phase HPLC was accomplished using an Agilent 1260 system (Santa Clara, CA, USA) equipped with a photodiode diode array detector [method A: Phenomenex Luna C₁₈ (Torrance, CA, USA), 4.6 × 250 mm, 5 μ m; solvent A: H₂O/0.1% TFA, solvent B: CH₃CN; flow rate: 1 mL min⁻¹; 0–20 min, 1–75% B; 20–23 min, 75–100% B; 23–31 min, 100% B; 31–32 min, 100–5% B; 32–34 min, 5% B; A_{215/254}]. Semipreparative reversed-phase HPLC was accomplished using a Varian ProStar model 210 equipped with a photodiode diode array detector [method B: Phenomenex Gemini C₁₈, 10 × 250 mm, 5 μ m; solvent A: H₂O/0.1% TFA, solvent B: CH₃CN; flow rate: 4.5 mL min⁻¹; 0–25 min, 5–55% B; 25–27 min, 55–100% B; 27–33 min, 100% B; 33–34 min, 100–5% B; 34–38 min, 5% B; A₂₅₄].

Colorimetric Glycosyl-Scanning Assays

Reactions were conducted in triplicate in a 384-well plate format containing 2 mM donor [(2-chloro-4-nitrophenyl)- β -D-glucoside (**1**) or (2-chloro-4-nitrophenyl)-2-amino-2-deoxy- β -D-glucoside (**2**)], 1 mM acceptor, 0.1 mM UDP, and enzyme (0.26 μ M Loki for donor **1**; 2.6 μ M Loki for donor **2**) in a final total volume of 20 μ L of Tris-HCl buffer (25 mM, 5 mM MgCl₂, pH 8.0). Reactions were initiated with the addition of enzyme and incubated at 30 °C for 8 h with continuous monitoring (A_{410}) using a Fluostar Omega microplate reader (BMG LABTECH GmbH, Ortenberg, Germany). Reactions that displayed a A_{410} of three standard deviations above that of controls lacking aglycone (DMSO vehicle) were identified as preliminary substrates and subsequently quenched via the addition of 100 μ L of 50% MeOH and filtered (MultiScreen filter plate; Millipore, Billerica, MA, USA) according to manufacturer's instructions. Then the filtrate was analyzed by HPLC (method A) and LC-MS.

General Procedure for Preparative Chemoenzymatic Glycoside Syntheses

Reactions were conducted in 2 dram vials (total volume 4 mL) and contained acceptor (3 mM), donor (5 mM UDP-glucose or 3 mM 2-chloro-4-nitrophenyl-GlcNH₂ with 0.1 mM UDP) in 50 mM Tris HCl, and 5 mM MgCl₂, pH 8.0. Reactions were initiated via the addition of catalyst (OleD Loki, 2.6 μ M) and incubated at 25 °C for up to 12 h with stirring and periodically monitored via A_{410} , TLC, and/or analytical HPLC (method A). Upon completion, reactions were quenched by the addition of 2 mL of MeOH and filtered using Amicon Ultra-15 centrifugal filter units (3 kDa cutoff; Millipore), and the filtrate was

subsequently frozen and lyophilized. The corresponding concentrated crude reaction debris was resuspended in 1 mL of DMSO and centrifuged (18000g, 20 min, 4 °C), and the resulting crude supernatant was resolved by preparative HPLC (method B). Product-containing fractions were subsequently collected and lyophilized, and the corresponding products advanced for spectroscopic characterization.

Antibacterial, Antifungal, and Cancer Cell Line Viability Assays

Antibacterial (*Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* NRRL B-287, *Escherichia coli* NRRL B-3708, *Salmonella enterica* ATCC 10708), antifungal (*Saccharomyces cerevisiae* ATCC 204508), and cell line cytotoxicity (non-small-cell lung A549) assays were accomplished in triplicate following our previously reported protocols.^{61,81}

EtOH Damage Neuroprotection Assay

Assays to assess modulation of EtOH damage were accomplished following previously reported protocols.^{71,76,77}

2-O- β -D-Glucosylspoxazomicin C (3a)

Using the general preparative scale methodology, spoxazomicin C (**3**; 3 mg, 0.015 mmol) and UDP-Glc (6 mg, 0.018 mmol) yielded **3a** (1.2 mg, 3.3 μ mol, 20% yield) and **3b** (0.5 mg, 1.4 μ mol, 10% yield) as white solids: ¹H NMR (CD₃OD, 500 MHz) δ 7.90 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.49 (m, 1H), 7.36 (d, *J* = 8.3 Hz, 1H), 7.15 (t, *J* = 7.5 Hz, 1H), 5.02 (d, *J* = 7.8 Hz, 1H), 4.16 (t, *J* = 5.4 Hz, 1H), 3.90 (dd, *J* = 12.2, 2.0 Hz, 1H), 3.67–3.81 (m, 5H), 3.53–3.59 (m, 1H), 3.38–3.51 (m, 3H); (+)-HRESIMS *m/z* 356.1340 [M + H]⁺ (calcd for C₁₆H₂₂NO₈, 356.1340).

12-O- β -D-Glucosylspoxazomicin C (3b)

¹H NMR (D₂O, 500 MHz) δ 7.73 (d, *J* = 7.3 Hz, 1H), 7.50 (t, *J* = 8.8 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 7.08 (t, *J* = 7.6 Hz, 1H), 5.07 (dd, *J* = 4.6, 2.7 Hz, 1H), 4.44–4.49 (m, 1H), 4.37 (m, 1H), 3.69–3.83 (m, 4H), 3.59–3.67 (m, 2H), 3.48 (m, 2H), 3.38 (d, *J* = 8.8 Hz, 1H); (+)-HRESIMS *m/z* 356.1334 [M + H]⁺ (calcd for C₁₆H₂₂NO₈, 356.1340).

2-O-(2'-Amino-2'-deoxy- β -D-glucosyl)spoxazomicin C (3c)

Using the general preparative scale methodology, spoxazomicin C (**3**; 3 mg, 0.015 mmol) and (2-chloro-4-nitrophenyl)-2-amino-2-deoxy- β -D-glucoside (**2**; 5 mg, 0.015 mmol) yielded **3c** (3.9 mg, 11 μ mol, 71% yield) as a white solid: ¹H NMR (CD₃OD, 400 MHz) δ 7.77 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.70–7.74 (m, 1H), 7.44 (t, *J* = 15.3 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 5.30 (d, *J* = 9.0 Hz, 1H), 4.07–4.16 (m, 1H), 3.82–3.90 (m, 4H), 3.70–3.81 (m, 2H), 3.66 (dd, *J* = 12.1, 5.9 Hz, 1H), 3.42–3.49 (m, 1H), 3.12–3.23 (m, 2H); (+)-HRESIMS *m/z* 355.1498 [M + H]⁺ (calcd for C₁₆H₂₃N₂O₇, 355.1500).

23-O- β -D-Glucosyloxachelin (5a)

Using the general preparative scale methodology, oxachelin (**5**; 5 mg, 0.007 mmol) and UDP-Glc (4 mg, 0.012 mmol) yielded **5a** (1.5 mg, 1.9 μ mol, 24% yield) as a white solid: ¹H

NMR (400 MHz, CD₃OD) δ 8.22 (s, 1H), 7.70 (dd, J = 7.8, 1.6 Hz, 1H), 7.43 (t, J = 7.8 Hz, 1H), 6.85–7.02 (m, 2H), 5.10 (d, J = 7.8 Hz, 1H), 4.58–4.73 (m, 4H), 4.28–4.49 (m, 3H), 3.82–3.95 (m, 4H), 3.64–3.68 (m, 2H), 3.28–3.46 (m, 9H), 2.33–2.36 (m, 3H), 1.83–1.60 (m, 8H); (+)-HRESIMS m/z 798.3162 [M + H]⁺ (calcd for C₃₃H₄₈N₇O₁₆, 798.3152).

23-O-(2'-Amino-2'-deoxy- β -D-glucosyl)oxachelin (5b)

Using the general preparative scale methodology, oxachelin (**5**; 5 mg, 0.007 mmol) and (2-chloro-4-nitrophenyl)-2-amino-2-deoxy- β -D-glucoside (**2**; 3 mg, 0.009 mmol) yielded **5b** (2.0 mg, 2.5 μ mol, 32% yield) as a white solid: ¹H NMR (400 MHz, CD₃OD) δ 8.36 (d, J = 2.7 Hz, 1H), 8.21 (dd, J = 9.0, 2.7 Hz, 1H), 7.49 (d, J = 9.4 Hz, 1H), 6.96 (d, J = 8.6 Hz, 1H), 6.86–6.93 (m, 1H), 5.46 (d, J = 8.6 Hz, 1H), 5.06 (dd, J = 10.6, 7.8 Hz, 1H), 5.00 (m, 1H), 4.64–4.71 (m, 1H), 4.61 (dd, J = 8.0, 3.3 Hz, 1H), 4.36–4.44 (m, 4H), 3.82–3.92 (m, 3H), 3.39–3.77 (m, 12H), 3.10–3.13 (m, 1H), 2.34–2.37 (m, 2H), 1.96–2.04 (m, 3H), 1.81–1.83 (m, 2H), 1.60–1.66 (m, 3H); (+)-HRESIMS m/z 797.3308 [M + H]⁺ (calcd for C₃₃H₄₉N₈O₁₅, 797.3312).

N-(6-O- β -D-Glucosyl)salicyloyl-2-aminopropane-1,3-diol (6a)

Using the general preparative scale methodology, *N*-salicyloyl-2-aminopropane-1,3-diol (**6**; 6 mg, 0.028 mmol) and UDP-glucose (15 mg, 0.028 mmol) yielded **6a** (2.0 mg, 5.4 μ mol, 19% yield) as a white solid: ¹H NMR (400 MHz, CD₃OD) δ 7.88 (dd, J = 7.0, 1.6 Hz, 1H), 7.43–7.51 (m, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.14 (t, J = 7.6 Hz, 1H), 5.01 (d, J = 7.4 Hz, 1H), 4.15 (q, J = 5.3 Hz, 1H), 3.88 (dd, J = 11.9, 1.8 Hz, 1H), 3.61–3.79 (m, 5H), 3.52–3.58 (m, 1H), 3.33–3.51 (m, 3H); HRESIMS m/z 374.1446 [M + H]⁺ (calcd for C₁₆H₂₄NO₉, 374.1446).

N-[6-O-(2'-Amino-2'-deoxy- β -D-glucosyl)]salicyloyl-2-aminopropane-1,3-diol (6b)

Using the general preparative scale methodology, *N*-salicyloyl-2-aminopropane-1,3-diol (**6**; 6 mg, 0.028 mmol) and (2-chloro-4-nitrophenyl)-2-amino-2-deoxy- β -D-glucoside (**2**; 5 mg, 0.015 mmol) yielded **6b** (0.8 mg, 2.2 μ mol, 15% yield) as a white solid: ¹H NMR (400 MHz, CD₃OD) δ 7.65 (dd, J = 7.4, 1.6 Hz, 1H), 7.50 (td, J = 7.4, 1.6 Hz, 1H), 7.38 (d, J = 8.2 Hz, 1H), 7.20 (t, J = 7.2 Hz, 1H), 5.20 (d, J = 8.2 Hz, 1H), 4.16 (q, J = 5.7 Hz, 1H), 3.87–3.93 (m, 1H), 3.67–3.78 (m, 3H), 3.64–3.66 (m, 1H), 3.54–3.63 (m, 2H), 3.44–3.52 (m, 2H), 3.24 (dd, J = 10.6, 8.6 Hz, 1H); HRESIMS m/z 373.1607 [M + H]⁺ (calcd for C₁₆H₂₅N₂O₈, 373.1605).

(2*R*)-N-(6-O- β -D-Glucosyl)salicyloyl-2-aminopropan-1-ol (7a)

Using the general preparative scale methodology, (2*R*)-*N*-salicyloyl-2-aminopropan-1-ol (**7**; 6 mg, 0.031 mmol) and UDP-glucose (17 mg, 0.031 mmol) yielded **7a** (1.6 mg, 4.5 μ mol, 15% yield) as a white solid: ¹H NMR (400 MHz, CD₃OD) δ 7.81 (dd, J = 7.6, 1.4 Hz, 1H), 7.42–7.52 (m, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.13 (t, J = 7.4 Hz, 1H), 5.00 (d, J = 7.4 Hz, 1H), 4.17 (dq, J = 12.1, 6.7 Hz, 1H), 3.79–3.96 (m, 1H), 3.70 (dd, J = 11.9, 5.3 Hz, 1H), 3.58–3.64 (m, 2H), 3.33–3.57 (m, 4H), 1.23 (d, J = 6.7 Hz, 3H); HRESIMS m/z 358.1497 [M + H]⁺ (calcd for C₁₆H₂₄NO₈, 358.1496).

(2*R*)-*N*-[6-*O*-(2'-Amino-2'-deoxy- β -D-glucosyl)]salicyloyl-2-aminopropan-1-ol (7b)

Using the general preparative scale methodology, (2*R*)-*N*-salicyloyl-2-aminopropan-1-ol (**7**; 6 mg, 0.031 mmol) and (2-chloro-4-nitrophenyl)-2-amino-2-deoxy- β -D-glucoside (**2**; 10 mg, 0.031 mmol) yielded **7b** (4.6 mg, 12.9 μ mol, 42% yield) as a white solid: ^1H NMR (400 MHz, CD_3OD) δ 7.56 (dd, J = 7.8, 1.2 Hz, 1H), 7.49 (t, J = 8.2 Hz, 1H), 7.39 (d, J = 8.2 Hz, 1H), 7.19 (t, J = 7.4 Hz, 1H), 5.17 (d, J = 8.6 Hz, 1H), 4.16 (dq, J = 12.3, 6.5 Hz, 1H), 3.90 (dd, J = 11.9, 1.4 Hz, 1H), 3.72–3.78 (m, 1H), 3.54–3.65 (m, 3H), 3.42–3.51 (m, 2H), 3.18 (dd, J = 10.2, 9.0 Hz, 1H), 1.20 (d, J = 6.7 Hz, 3H); HRESIMS m/z 357.1661 [$\text{M} + \text{H}$] $^+$ (calcd for $\text{C}_{16}\text{H}_{25}\text{N}_2\text{O}_7$, 357.1656).

(2*S*)-*N*-(6-*O*- β -D-Glucosyl)salicyloyl-2-aminopropan-1-ol (8a)

Using the general preparative scale methodology, (2*S*)-*N*-salicyloyl-2-aminopropan-1-ol (**8**; 6 mg, 0.031 mmol) and UDP-glucose (17 mg, 0.031 mmol) yielded **8a** (1.8 mg, 5.1 μ mol, 16% yield) as a white solid: ^1H NMR (400 MHz, CD_3OD) δ 7.86 (dd, J = 7.8, 1.6 Hz, 1H), 7.46 (td, J = 8.2, 2.0 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.09–7.18 (m, 1H), 4.98 (d, J = 7.8 Hz, 1H), 4.18 (dq, J = 12.1, 6.7 Hz, 1H), 3.90 (dd, J = 11.9, 2.2 Hz, 1H), 3.70 (dd, J = 11.9, 5.7 Hz, 1H), 3.60 (d, J = 5.1 Hz, 2H), 3.43–3.56 (m, 3H), 3.39 (t, J = 9.0 Hz, 1H), 1.25 (d, J = 6.7 Hz, 3H); HRESIMS m/z 358.1500 [$\text{M} + \text{H}$] $^+$ (calcd for $\text{C}_{16}\text{H}_{24}\text{NO}_8$, 358.1496).

(2*S*)-*N*-[6-*O*-(2'-Amino-2'-deoxy- β -D-glucosyl)]salicyloyl-2-aminopropan-1-ol (8b)

Using the general preparative scale methodology, (2*S*)-*N*-salicyloyl-2-aminopropan-1-ol (**8**; 6 mg, 0.031 mmol) and (2-chloro-4-nitrophenyl)-2-amino-2-deoxy- β -D-glucoside (**2**; 10 mg, 0.031 mmol) yielded **8b** (4.8 mg, 13.5 μ mol, 44% yield) as a white solid: ^1H NMR (400 MHz, CD_3OD) δ 7.54 (dd, J = 7.6, 1.4 Hz, 1H), 7.45–7.52 (m, 1H), 7.38–7.42 (m, 1H), 7.19 (t, J = 7.4 Hz, 1H), 5.15 (d, J = 8.6 Hz, 1H), 4.17 (sxt, J = 6.3 Hz, 1H), 3.92 (dd, J = 11.9, 1.8 Hz, 1H), 3.75 (dd, J = 11.9, 5.3 Hz, 1H), 3.53–3.65 (m, 3H), 3.50 (d, J = 5.9 Hz, 2H), 3.21 (dd, J = 10.4, 8.8 Hz, 1H), 1.22 (d, J = 7.0 Hz, 3H); HRESIMS m/z 357.1667 [$\text{M} + \text{H}$] $^+$ (calcd for $\text{C}_{16}\text{H}_{25}\text{N}_2\text{O}_7$, 357.1656).

2-*O*- β -D-Glucosylspoxazomicin D (4a)

To the solution of 1-bromo- α -D-glucose tetraacetate (60 mg, 0.15 mmol) in anhydrous CH_3CN (5 mL) was added **4** (15 mg, 0.07 mmol) and 4 Å molecular sieves. After stirring the mixture for 30 min at room temperature, Ag_2O (35 mg, 0.15 mmol) was added and the reaction allowed to continue for 24 h. The reaction was filtered through Celite and concentrated under vacuum, and the recovered crude material resuspended in 1 mL of MeOH, centrifuged, and resolved by preparative HPLC (method B). The product-containing fractions were subsequently collected and lyophilized to provide the desired product as an anomeric mixture (2:1 β : α), which was purified by HPLC (method A) to give the desired β -anomer (5 mg, 0.01 mmol). The β -anomer was dissolved in MeOH (1 mL), and global deprotection was accomplished via the addition of K_2CO_3 (5 mg, 0.04 mmol) followed by stirring at room temperature while monitoring the reaction via TLC and HPLC (method A). Upon completion (~6 h), the reaction was quenched by adding Amberlite 120 (H^+) (4 equiv), stirred for 30 min, and filtered through Celite, and the crude reaction was dried under vacuum. The recovered crude material was subsequently dissolved in 1 mL of MeOH and

resolved via preparative HPLC (method B). Product-containing fractions were subsequently collected and lyophilized to provide the desired **4a** (white solid, 2 mg, 0.005 mmol, 8% yield in two steps): ¹H NMR (CD₃OD, 400 MHz) δ 7.94 (d, J = 7.4 Hz, 1H), 7.46–7.56 (m, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.14 (t, J = 9.8 Hz, 1H), 5.06 (d, J = 8.2 Hz, 1H), 4.63 (t, J = 4.3 Hz, 1H), 3.97 (dd, J = 4.3, 11.0 Hz, 1H), 3.80–3.93 (m, 2H), 3.71 (dd, J = 5.3, 11.9 Hz, 1H), 3.65 (t, J = 8.6 Hz, 1H), 3.45–3.51 (m, 2H), 3.41 (t, J = 8.6 Hz, 1H); ¹³C NMR (CD₃OD, 100 MHz) δ 173.7 (Cq-12), 165.9 (Cq-7), 155.9 (Cq-2), 133.0 (CH-4), 130.6 (CH-6), 122.4 (CH), 122.1 (CH), 116.0 (Cq-1), 102.2 (CH-1'), 77.0 (CH), 76.2 (CH), 73.2 (CH), 69.6 (CH), 61.9, 60.9, 55.5 ppm; HRESIMS m/z 387.1404 [M + H₃O]⁺ (calcd for C₁₆H₂₃N₂O₉, 387.1398).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

General glycosyl-scanning scheme. In this strategy, activated 2-chloro-4-nitrophenylglycoside donors help drive the OleD Loki-catalyzed transglycosylation reaction toward desired product formation where the resulting production of colorimetric 2-chloro-4-nitrophenol affords a real-time indicator of sugar-nucleotide production and utilization as an indirect measure of target glycoside production.

Figure 2.
 Structures of validated OleD Loki substrates identified and corresponding glycosides generated via preparative OleD Loki-catalyzed chemoenzymatic synthesis.

Figure 3.

OleD Loki-catalyzed colorimetric glycosyl-scanning results using 2-chloro-4-nitrophenyl-Glc (**1**) (A) or 2-chloro-4-nitrophenyl-GlcNH₂ (**2**) (B) as donor for all *Streptomyces* sp. RM-14-6 metabolites confirmed to turnover based on LC-MS [positive control, 4-methylumbelliferone (4-MeU); negative control, no acceptor (DMSO)]. No turnover was observed with compounds **10–20** (Supporting Information, Figure S1). The turnover of compound **8** (the synthetic enantiomer of metabolite **7**) was identical to **7**.

Figure 4.

EtOH damage neuroprotection assay (propidium iodide uptake in rat-derived organotypic hippocampal slice primary cell cultures). (A) DMSO control; (B) 48 h exposure to 100 mM EtOH; (C) 48 h exposure to 100 mM EtOH and 10 nM **3a**; (D) 48 h exposure to 100 mM EtOH and 1 μ M **4a**; (E) dose-response with 48 h exposure to EtOH (100 mM) in the absence or presence of **3a** on propidium iodide uptake; (F) Dose-response with 48 h exposure to EtOH (100 mM) in the absence or presence of **4a** on propidium iodide uptake.

* $p < 0.001$ vs control; ** $p < 0.001$ vs EtOH.