Extended scaffold glucuronides:

*en route* to universal synthesis of O-aryl glucuronide prodrugs

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1 General methods and remarks

All chemicals were purchased from commercial vendors (Sigma-Aldrich, Acros, TCI). SN-38 was purchased from ApiChem. Deuterated solvents were supplied from Euriso-Top. All moisture and air sensitive reactions were performed in flame-dried glassware under positive pressure of argon or N₂. Dichloromethane (CH₂Cl₂), acetonitrile (MeCN), tetrahydrofuran (THF) and toluene (PhMe) were acquired through a MBraun SPS-800 solvent purification system. Methanol (MeOH) were purchased in an anhydrous state from Sigma-Aldrich. Thin layer chromatography (TLC) analysis was carried out on silica coated aluminum foil plates (Merck Kieselgel 60 F254). The TLC plates were visualized by UV irradiation (254 nm) and/or by staining with KMnO₄. Flash column chromatography was carried out using silica gel (230-400 mesh particle size, 60 Å pore size) as the stationary phase. Mass spectra (High Resolution Mass Spectrometry - HRMS) was recorded on a Bruker Daltonics LC-TOF spectrometer with positive electrospray ionization, or negative ionization when stated. Analytical HPLC was performed on a Shimadzu LC-2010A HT equipped with a Ascentis® Express Peptide ES-C18 column with 2.7 μm particles, a length of 150 mm and an internal diameter of 3.0 mm from Supelco Analytical. Mobile phase A comprised of ultrapure H₂O supplemented with 0.1% TFA (v/v) and mobile phase B acetonitrile (MeCN) supplemented with 0.1% TFA (v/v); flow rate was 0.4 mL min⁻¹ at a temperature of 40°C. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 400 MHz spectrometer, running at 400 and 101 MHz for ¹H and ¹³C, respectively, or on a Bruker Avance III spectrometer, running at 500 and 126 MHz for ¹H and ¹³C, respectively. Chemical shifts (δ) are reported in ppm relative to the residual solvent. When Schlenk-technique was used, glass ware was heated with a heat gun (under vacuum) and subsequently let cool down to room temperature while purging with argon (3 cycles), liquids were added through septa with a syringe, and solid chemicals were added through a counterflow of argon or nitrogen. Fluorescence measurements were performed on an EnSpire 2300 Multilabel Reader (PerkinElmer) in black 96-well plates (732-2700, Nunc.). Ultrapure water was dispensed from MilliQ Direct 8 (Millipore) [18.2 MΩ • cm].
2 HPLC enzymatic prodrug hydrolysis

Hydrolysis of the prodrugs 9 and 10 was carried out by incubating the prodrugs at a final concentration of (20 µg mL⁻¹) with *Escherichia coli* (*E. coli*) β-glucuronidase (Sigma, G 7646) (1.0 µg mL⁻¹) in 10 mM PBS buffer solution pH = 7.4. Aliquots were taken out at given time points (30 min, 120 min, 1 d), enzyme was precipitated in cold MeOH (2.0 mL), and the samples were centrifuged (1400 rcf, 4°C, 5 min). The supernatant was transferred, the solvent was removed *in vacuo*, and the sample dissolved in 50 µL H₂O/MeCN (95/05 v/v %), and analyzed via HPLC. Elution was performed starting with solvent B 5% to B 100% over 15 min at T = 40°C. Detection was performed by UV detector (254 nm and 280 nm). Stability tests were carried out according to the hydrolysis protocol, without the addition of enzyme.

Hydrolysis of prodrugs 11 and 12 was carried out at a final prodrug concentration of 100 µM, and in presence or absence of β-glu (Sigma-Aldrich, G7646)* (1 µg mL⁻¹) and aliquots were taken at the indicated time points. Enzyme was removed via spinfilters (MWCO = 2k) and the supernatant was analyzed for prodrug 11 with Method A and 12 with Method B. Release of drug was compared to authentic SN-38 reference.

**Method A:** Elution was performed starting with B 5% to B 60% over 15 min, then B 60% to B 100% in 2 min, hold 3 min. Detection at 254 and 280 nm. Retention time SN-38 \( t_r = 11.84 \) min; 11 \( t_r = 13.14 \) min.

**Method B:** Elution was performed starting with B 5% to B 40% over 11 min, hold 3 min, then B 40% to B 100% in 4 min, hold 2 min. Detection at 254 and 280 nm. Retention time SN-38 \( t_r = 12.72 \) min; 12 \( t_r = 13.21 \) min.

**Determination of enzyme kinetic parameters:** Prodrugs were incubated at various different concentrations and fluorescence (for 9 and 10 \( \lambda_{ex} = 570 \text{ nm}/\lambda_{em} = 585 \text{ nm} \), and for 11 and 12 was \( \lambda_{ex} = 400 \text{ nm}/\lambda_{em} = 503 \text{ nm} \) measured at 2 min intervals. The mean ± SD of triplicates was plotted against time (N=3). The linear part of the fluorescence-time curve determined the rate. The data was plotted and analyzed in GraphPad Prism 8. Fluorescence was converted into concentration based on a standard curve.

**General remark:** The kinetics of resorufin or SN-38 prodrugs were recorded side-by-side, which allows for direct comparison of the kinetic parameters. For general comparison, the determined values should be used with caution due to an estimation of the enzyme concentration based on the recommendations of Sigma-Aldrich (~25% based on biuret) – independently determined based on UV (absorption at 280 nm; ~22 ± 4%).

**The given enzyme concentration corresponds to the bulk material provided from Sigma-Aldrich and contains sucrose as stabilizer.**
3 **In vitro characterization of prodrugs**

2000 HeLa human cervical cancer cells (passage 14-17) were seeded in 100 µL complete growth medium* in 96 well plates (353872, Corning) and allowed to attach for 4 h at 37 °C in humidified air containing 5% CO₂. Next, serial dilutions of SN-38 prodrugs 11, 12, SN-38-glu (S589980, Toronto Research Chemicals) and pristine SN-38 (H0165, Sigma-Aldrich) were prepared, to reach final concentrations ranging from 10 µM to 5.1 pM in fresh growth medium (± 15 µg/mL β-Glu (G7646, Sigma-Aldrich)) containing >0.5% DMSO. Seeding-medium was aspirated and the (pro)drug solutions added in a final volume of 100 µL per well. After (pro)drug administration, the cells were left to incubate for 72 h at 37 °C in humidified air containing 5% CO₂. Finally, the relative viabilities were quantified using PrestoBlue® Cell Viability Reagent (A13262, Invitrogen), as described by the manufacturer. In short, the reagent was initially diluted 1:10 via direct addition into the culture medium of the wells. After 30 min incubation at 37 °C in humidified air containing 5% CO₂, 100 µL of each sample was transferred to black opaque 96 well plates (732-2700, Nunc.) in which the resorufin signals were quantified at λ<sub>ex</sub>/λ<sub>em</sub> 560/590 nm using an EnSpire 2300 Multilabel Reader (PerkinElmer). The experiment was reproduced at least 3 times with at least 3 replicates per time.

*Complete growth medium: Minimum Essential Medium Eagle (MEME, M2279, Sigma-Aldrich) supplemented with 10% FBS (7524, Sigma-Aldrich), penicillin (100 units ml<sup>-1</sup>) and streptomycin (100 µg ml<sup>-1</sup>) (P4333-100ML, Sigma-Aldrich), 2 mM L-glutamine (G7513, Sigma-Aldrich), and 1% Non-essential amino acids (NEAA; M7145, Sigma-Aldrich). The HeLa cells were kindly provided by Senior Research Fellow Dr. Thomas Breitenbach (Department of Chemistry, Aarhus University).
4 Supporting Figures and Tables

Supporting Table S1. Substrate scope of Ag$_2$O promoted glycosylation of phenols. Reagents and conditions: 1 (1.0 equiv), phenol (2.0 equiv.), Ag$_2$O (2.0 equiv.), 3 Å mol sieves, MeCN, r.t., dark.

<table>
<thead>
<tr>
<th>entry</th>
<th>$pK_a$[¹]</th>
<th>Yield (%)[²]</th>
<th>$\alpha:\beta$ ratio[c]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>10.28</td>
<td>n.r.[³]</td>
<td>-</td>
</tr>
<tr>
<td>4b</td>
<td>9.38</td>
<td>n.r.[³]</td>
<td>-</td>
</tr>
<tr>
<td>4c</td>
<td>7.95</td>
<td>26</td>
<td>02:98</td>
</tr>
<tr>
<td>4d</td>
<td>7.61</td>
<td>45</td>
<td>00:100</td>
</tr>
<tr>
<td>4e</td>
<td>7.15</td>
<td>52</td>
<td>00:100</td>
</tr>
<tr>
<td>4f</td>
<td>~5.00</td>
<td>98</td>
<td>00:100</td>
</tr>
<tr>
<td>4g</td>
<td>4.07</td>
<td>80</td>
<td>00:100</td>
</tr>
<tr>
<td>SN-38[⁴]</td>
<td>9.68</td>
<td>&lt;5</td>
<td>complex mixture</td>
</tr>
</tbody>
</table>

[¹] no reaction observed; [²] isolated; [³] determined by ¹H-NMR; [⁴] previously reported protocol used 1 in presence of K$_2$CO$_3$ in acetone with a reported yield of 3.4%.[²]
### Supporting Table S2: Various glycosylation conditions with glucuronyl bromide 1.\(^\text{[a]}\)

<table>
<thead>
<tr>
<th>entry</th>
<th>Promoter (2.0 equiv.)</th>
<th>additive</th>
<th>solvent</th>
<th>comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Ag₂O</td>
<td>-</td>
<td>MeCN</td>
<td>n.r.</td>
</tr>
<tr>
<td>b</td>
<td>Ag₂O</td>
<td>K₂CO₃</td>
<td>acetone</td>
<td>29% glycal</td>
</tr>
<tr>
<td>c</td>
<td>Ag₂O</td>
<td>K₂CO₃</td>
<td>MeCN</td>
<td>35% glycal</td>
</tr>
<tr>
<td>d</td>
<td>Ag₂CO₃</td>
<td>-</td>
<td>MeCN</td>
<td>6% glycal</td>
</tr>
<tr>
<td>e</td>
<td>Ag₂O</td>
<td>-</td>
<td>quinoline</td>
<td>8% product (α:β 00:100); 25% glycal</td>
</tr>
<tr>
<td>f</td>
<td>AgOTf</td>
<td>2,6-lutidine</td>
<td>CH₂Cl₂</td>
<td>35% orthoester</td>
</tr>
</tbody>
</table>

\(^{[a]}\) All reactions were conducted on 0.25 mmol scale, reaction time 24h; yields are reported after F.C; n.r. = no reaction observed.
Supporting Figure S1. Synthesis overview to the different benzyl alcohol glucuronide precursors. Reagents and conditions: i) NaBH₄, silica, CHCl₃/i-PrOH, quant.; ii) TBS-Cl, imidazole, DMAP, DMF, 79%; iii) HCOOH.NH₃, Pd/C, EtOH, r.t., 88-95%, iv) Ac₂O, TEA, CH₂Cl₂, 0°C – r.t. quant.; v) TEA.3HF, THF, 86 %.

Removal of TBS-protecting group:

We note that deprotection with commonly used TBAF in THF (1 M) resulted in a complex mixture of partly deacetylated sugar and the Δ₄,5 dehydro analogues. Presumably, this originates from the hygroscopic nature of TBAF solutions in THF or other solvents which contain normally copious amounts of H₂O and are not easily dried. In presence of H₂O, the fluoride anion can react with H₂O to generate OH⁻ which might explain the observed outcome of the reaction. [3]

Supporting Figure S2: Alternative synthetic routes for the formation of alkyl-aryl ethers. Conditions and reagents: i) MsCl, TEA, CH₂Cl₂, 0°C, 81%; ii) CCl₃CN, CH₂Cl₂, Cs₂CO₃, r.t., 54%; iii) S₄, resorufin, Cs₂CO₃, DMF, r.t., 22%; S₅, BF₃·OEt₂, CH₂Cl₂, 0°C – r.t. no formation of 8k. Ms = methanesulfonyl.
Supporting Table S3. Screen of reaction conditions for the formation of precursor resorufin turn-on probe 8k and discussion about side product formation.

Common side product formation under basic conditions generating Δ4,5-dehydro glucuronide II. 1H-NMR spectrum indicating characteristic downfield shift of the Me-and Acetyl-ester signals upon E1cb as well as loss of 1 acetyl-signal; green trace Δ4,5-dehydro glucuronide 8k-II, orange trace product 8k of generic structure III.

<table>
<thead>
<tr>
<th>entry</th>
<th>Conditions (equiv.)</th>
<th>solvent</th>
<th>T (°C)</th>
<th>I (%)[a]</th>
<th>II (%)[a]</th>
<th>III (8k) (%)[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>ADDP (6.0), TPB (6.0), resorufin (1.2)</td>
<td>THF</td>
<td>r.t.</td>
<td>n.d.</td>
<td>30[c]</td>
<td>27[c]</td>
</tr>
<tr>
<td>b</td>
<td>ADDP (6.0), TPB (6.0), resorufin (1.2)[b]</td>
<td>THF</td>
<td>r.t.</td>
<td>n.d.</td>
<td>12[c]</td>
<td>28[c]</td>
</tr>
<tr>
<td>c</td>
<td>ADDP (2.0), TPB (2.0), resorufin (1.2)</td>
<td>THF</td>
<td>r.t.</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>d</td>
<td>ADDP (2.0), TPB (2.0), resorufin (1.2)</td>
<td>toluene</td>
<td>r.t.</td>
<td>60.9</td>
<td>4.3</td>
<td>34.8</td>
</tr>
<tr>
<td>e</td>
<td>ADDP (2.0), TPB (2.0), resorufin (1.2)</td>
<td>CH₂Cl₂</td>
<td>r.t.</td>
<td>72.9</td>
<td>0.6</td>
<td>26.4</td>
</tr>
<tr>
<td>f</td>
<td>ADDP (2.0), TPB (2.0), resorufin (1.2)</td>
<td>toluene</td>
<td>60 °C</td>
<td>66.1</td>
<td>4.3</td>
<td>29.6</td>
</tr>
<tr>
<td>g</td>
<td>DIAD (2.0), TPP (2.0), resorufin (1.2)</td>
<td>toluene</td>
<td>60 °C</td>
<td>76.8</td>
<td>0.9</td>
<td>22.3</td>
</tr>
<tr>
<td>h</td>
<td>ADDP (2.0), TPB (2.0), resorufin (5.0)</td>
<td>toluene</td>
<td>60 °C</td>
<td>50.2</td>
<td>2.5</td>
<td>47.3</td>
</tr>
<tr>
<td>i</td>
<td>DIAD (2.0), TPP (2.0), resorufin (5.0)</td>
<td>toluene</td>
<td>60 °C</td>
<td>75.3</td>
<td>1.2</td>
<td>23.5</td>
</tr>
</tbody>
</table>

[a] based on 1H-NMR analysis of the worked up reaction crude after 15 h; [b] inverse addition: Addition of benzyl alcohol into a suspension of reactants; [c] isolated yield; ADDP = 1,1’-(Azodicarbonyl)dipiperidine; TPB = tributylphospine; DIAD = diisopropyl azodicarboxylate; TPP = triphenylphospine; n.d. – not determined, r.t. = room temperature.
Supporting Figure S3. Deprotection of the prodrug precursors. Reagents and conditions: i) 8k,l, NaOMe (0.1 equiv.), MeOH, r.t., 2-5 h; ii) 2 M NaOH, H₂O, 0°C, 15 min; iii) Ba(OH)₂·8H₂O, MeOH, r.t, 86 and 48 % for 11 and 12 respectively.
Supporting Figure S4. Normalized HPLC traces for the hydrolysis of 12 (100 µM) in PBS in presence (1 µg mL⁻¹) or absence of β-glucuronidase at 37°C.
Supporting Figure S5. Monitoring of fluorescence ($\lambda_{ex} = 400$ nm; $\lambda_{em} = 503$ nm) of prodrugs 11 (♦) and 12 (●) at a final concentration of 100 µM in PBS at a final β-glu concentration of 1 µg mL$^{-1}$ at 37°C.
Supporting Figure S6. Dose-response curves of SN-38 and its native and extended scaffold glucuronides against human cervical cancer HeLa cells in the presence and absence of β-Glu over 72 h exposure. Presented data are shown as mean ± SD based on three independent experiments.
5 Detailed procedures for syntheses and characterization

General procedure for Ag₂O catalyzed glycosylation (A)
Glycosyl bromide 1 was azeotroped with toluene and dried under high vacuum prior to use. In a flame dried Schlenk flask under an atmosphere of argon 1 (0.2 mmol, 1.0 equiv.), phenol (0.4 mmol, 2.0 equiv.), and powdered 3Å mol sieves (0.2 g) were suspended in dry MeCN (5.0 mL) and left to stir under an atmosphere of argon at r.t. for 30 min. Then Ag₂O was added and the suspension was left to stir at r.t. in the dark for 12 h. The reaction crude was filtered over Celite®, washed with CH₂Cl₂, and the filtrate was evaporated under reduced pressure. The crude product was purified by column chromatography over silica gel.

For the Koenigs Knorr glycosylations from Figure 1 and Supporting Table 2. Procedure was as above with 1 (0.25 mmol, 1.0 equiv.) instead of (0.2 mmol), 4-chlorophenol (2.0 equiv.), and silver salt (1.5 equiv.). Additives were added (1.1 equiv.) and solvent was 5 mL in presence of 3Å mol (1/1 w/w % compared to glycosyl donor).

General procedure for BF₃·OEt₂ catalyzed glycosylation of anomeric acetate 2 (B)
In a flame dried Schlenk flask under an atmosphere of argon 2 (0.27 mmol, 1.0 equiv.), phenol (0.29 mmol, 1.1 equiv.), and powdered 3Å mol sieves (0.2 g) were suspended in dry CH₂Cl₂ (2.0 mL) and left to stir under an atmosphere of argon at r.t. for 30 min. Then BF₃·OEt₂ (0.29 mmol, 1.1 equiv.) was added and the suspension was left to stir at r.t. for 12 h. The reaction crude was diluted with CH₂Cl₂/MeOH, washed with sat. NaHCO₃ (3 x 10 mL), the organic layers were pooled, dried over MgSO₄, and filtered. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography over silica gel.

General procedure for BF₃·OEt₂ catalyzed glycosylation of Schmidt donor 3 (C)
In a flame dried Schlenk flask was added imidate 3 (70 mg, 0.12 mmol, 1.25 equiv.) and 4-chlorophenol (13 mg, 0.10 mmol, 1.0 equiv.) in CH₂Cl₂ (2.5 mL). The solution was cooled to -40°C and a solution of BF₃·OEt₂ (0.02 mmol, 0.25 equiv.) in CH₂Cl₂ (0.5 mL) was added dropwise. The reaction was left for 2 h at -40°C and then let warm up to r.t. TLC generally shows no difference after 2.5 h and 24 h. The reaction was worked up as above.

General procedure for reduction of aldehyde derivatives (D)
In a flame dried flask aldehyde, derivative (0.23 mmol, 1.0 equiv.) was dissolved in CHCl₃/i-PrOH (2.0 mL/0.5 mL) and cooled to 0°C under an atmosphere of N₂. Then silica gel (100 mg) was added and left to stir for 10 min. NaBH₄ (0.46 mmol, 2.0 equiv.) is added in one scoop. The reaction was monitored by TLC. The reaction was completed usually within 30-45 min, in case it was not, additional NaBH₄ (0.23 mmol, 1.0 equiv.) was added. Upon completion, the reaction was diluted with CH₂Cl₂ and filtered over a pluck of Celite®, and washed with CH₂Cl₂. The filtrate was washed with brine (3 x 20 mL), dried over MgSO₄, filtered and the solvent removed under reduced pressure. The product was isolated as off white solid. Generally, no further purification was required.
General procedure for Mitsunobu reaction screen
Compound 5 (5 mg, 0.01 mmol, 1.0 equiv.), resorufin, and ADDP or DIAD (0.02 mmol, 2.0 equiv.) were dissolved in solvent (0.9 mL). Then TBP or TPP (0.02 mmol, 2.0 equiv.) in solvent (0.3 mL) was added and the mixture was stirred at temperature under an atmosphere of N₂ for 12 h. The reaction crude was diluted with ethyl acetate, washed with sat. NaHCO₃ (2 x 10 mL), brine (1 x 10 mL), dried over Na₂SO₄, and filtered. The solvent was removed under reduced pressure and the crude was analyzed via ¹H-NMR.

General procedure for modified Mitsunobu (E)
Benzyl alcohol (0.10 mmol, 1.0 equiv.) was dissolved in dry toluene (0.5 mL) and added to a solution of phenol (0.52 mmol, 5.0 equiv.), ADDP (52 mg, 0.21 mmol, 2.0 equiv.) in dry toluene (1.5 mL). A solution of TBP (42 mg, 0.05 mL, 0.21 mmol, 2.0 equiv.) in dry toluene (1.0 mL) was added slowly. The suspension was heated to 60 °C under an atmosphere of N₂ and left overnight. The solvent was evaporated, and the crude product was dissolved in ethyl acetate and washed with sat. NaHCO₃ (3 x 20 mL), brine (2 x 20 mL), dried over Na₂SO₄, filtered, and the solvent was evaporated under reduced pressure. The crude product was subjected to column chromatography over silica gel.

General protocol for deprotection (F)
The glucuronide prodrugs (0.05 mmol) were suspended in dry MeOH (2.0 mL) and a solution of NaOMe (0.1 equiv. in case of prodrugs containing no carboxylic acids, 1.1 equiv. in case prodrugs containing free carboxylic acid) was added dropwise and the suspension was left to stir under an atmosphere of N₂ at r.t. The reaction was monitored via TLC (CH₂Cl₂:MeOH 90:10) and the deacetylation was finished within 30-60 min, and in case it was not additional NaOMe (0.1 equiv.) was added. Upon completion, the solution was cooled to 0 °C, H₂O (2.0 mL) and 2 M NaOH (50 ul) were added subsequently. The methyl ester hydrolysis was finished within 10 min and the reaction solution was acidified with amberlite 120H⁺ ion exchange resin to a pH ~4-5. The mixture was filtered and washed with H₂O, and the filtrate was lyophilized, yielding the desired deprotected glucuronide prodrugs. Preparative HPLC or further column chromatography was necessary for several products and is stated in the experimental section for the specific prodrugs.
The product has been synthesized through a modified protocol reported in literature.\(^{[4]}\) (1.0 g, 2.66 mmol) of \(2\) was dissolved in \(\text{CH}_2\text{Cl}_2\) (5 mL) and \(\text{HBr}/\text{AcOH}\) (10 mL) was added dropwise to the solution. The brown solution was left to stir at r.t. under an atmosphere of \(\text{N}_2\). TLC (EtOAc:Pen 1:1) shows the consumption of the starting material and appearance of a faster moving spot after 3-4 h. The crude reaction solution is poured on ice and the aqueous phase is extracted with \(\text{CH}_2\text{Cl}_2\) (3 x 30 mL). The combined organic layers were washed with ice water (3 x 30 mL), sat. \(\text{NaHCO}_3\) (3 x 30 mL) and the organic layer is dried over \(\text{MgSO}_4\). The organic layer is filtered and the solvent is removed under reduced pressure to yield the desired product as a brown syrup (995 mg, 2.66 mmol, 94%). The crude product was used without further purification.

\[\text{C}_{13}\text{H}_{17}\text{BrO}_9\]

1

The product has been obtained through a modified protocol reported in literature.\(^{[4]}\) 2.0 g (11.4 mmol, 1.0 equiv.) D-glucurono-6,3-lactone \(1\) was suspended in dry methanol (20mL) and 2 mL of a \(\text{NaOMe}\) solution (12 mmol, 1.1 equiv.) was added to the suspension. The suspension was left to stir overnight at r.t. under an atmosphere of \(\text{N}_2\). TLC (EtOAc:Pen 1:1) shows consumption of starting material. The solvent was removed under reduced pressure and the crude syrup was dissolved in dry pyridine (6mL) and the solution is cooled to 0°C. Then acetic anhydride (8mL) was added to the solution and left to stir overnight under an atmosphere of \(\text{N}_2\). TLC indicates consumption of the methyl ester intermediate and the dark brown reaction solution was poured onto ice. The aqueous layer was extracted with EtOAc (3 x 50 mL). Then the combined organic layers were washed with \(\text{H}_2\text{O}\) (3 x 30 mL), 1 M \(\text{HCl}\) (3 x 30 mL), \(\text{H}_2\text{O}\) (1 x 30 mL), and sat. \(\text{NaHCO}_3\) (3 x 30 mL), and dried over \(\text{MgSO}_4\). The solvent was removed under reduced pressure and the resulting crude product was recrystallized from abs. \(\text{EtOH}\) to give the desired product as colorless needles (1.36 g, 5.68 mmol, 64 %). \[^{1}\text{H-NMR}\] (400 MHz, CDCl\(_3\)) \(\delta\) (ppm): 5.70 (d, \(J = 7.8\) Hz, 1H, \(H-1\)), 5.21 (dt, \(J = 27.6, 9.3\) Hz, 2H), 5.08 (dd, \(J = 8.8, 7.9\) Hz, 1H), 4.09 (d, \(J = 11.9\) Hz, 1H, \(H-5\)), 3.68 (s, 3H, COOC\(_2\)H\(_3\)), 1.98 (s, 6H, 2 x OAc), 1.97 (s, 3H, OAc). \[^{13}\text{C NMR}\] (101 MHz, CDCl\(_3\)) \(\delta\) (ppm): 170.05, 169.56, 169.32, 168.98, 166.93, 91.48, 73.13, 71.95, 70.26, 69.04, 53.17, 20.92, 20.71, 20.69, 20.62. \[^{1}\text{HR-MS}\] (ESI): \([\text{C}_{15}\text{H}_{20}\text{O}_{11}+\text{NH}_4]^+\) calcd. 394.1344; found 394.1349.\(^{[4]}\)
was synthesized as previously reported. In brief, 2 (1.0 g, 2.66 mmol, 1.0 equiv.) was dissolved in DMF. Hydrazine acetate (318 mg, 3.46 mmol, 1.3 equiv.) was added in one scoop and the solution was left to stir under N₂ for 5-6 h. TLC showed consumption of starting material and appearance of a slower moving spot (EtOAc: pentane 50:50). The solution was diluted with water, and the aqueous phase was extracted with EtOAc. The combined organic phase was washed with 1 M HCl (3 x 30 mL), water (1 x 30 mL), brine (2 x 30 mL), dried over MgSO₄, filtered and the solvent removed in vacuo. The crude material was azeotroped with toluene and the hemi-acetal was obtained as slightly off white foam as a mixture of anomers (α:β 76:24) (571 mg, 1.71 mmol, 64%). The crude product was used without further purification. ^1H-NMR (400 MHz, CDCl₃) for the α-anomer δ (ppm): 5.56 – 5.43 (m, 2H, H-1, H-3), 5.13 (dd, J = 10.1, 9.3 Hz, 1H, H-4), 4.85 (dd, J = 10.1, 3.6 Hz, 1H, H-2), 4.52 (d, J = 10.1 Hz, 1H, H-5), 3.69 (s, 3H, COOC₂H₅), 2.02 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.97 (s, 3H, OAc). ^13C NMR (101 MHz, CDCl₃) δ (ppm): 170.18, 170.10, 169.77, 168.37, 90.46, 70.78, 69.58, 69.10, 68.28, 53.08, 20.84, 20.76, 20.70. HR-MS (ESI): [C₁₃H₁₈O₁₀⁺NH₄]⁺ calcd. 352.1238; found 352.1240.*contains residual toluene.

The hemiacetal (200 mg, 0.6 mmol, 1.0 equiv.) was dissolved in anhydrous CH₂Cl₂ (5.0 mL) and NaH (16 mg, 0.66 mmol, 1.1 equiv.) was added, followed by a dropwise addition of trichloroacetonitrile (0.3 mL, 3.0 mmol, 5.0 equiv.). The reaction was left to stir at r.t. for 3 h upon which TLC analysis shows conversion. The crude reaction mixture was filtered over Celite, and the pad rinsed thoroughly with CH₂Cl₂. The crude product was purified via column chromatography over silica gel (EtOAc: pentane 33:66) and isolated as crude oil (225 mg, 0.5 mmol, 79%). ^1H NMR (400 MHz, CDCl₃): δ 8.73 (s, 1H), 6.64 (d, J = 3.6 Hz, 1H), 5.63 (t, J = 2.5 Hz, 1H), 5.28 (t, J = 9.9 Hz, 1H), 5.15 (dd, J = 10.2, 3.6 Hz, 1H), 4.50 (d, J = 10.3 Hz, 1H), 3.75 (s, 3H), 2.06 – 2.05 (m, 6H), 2.02 (s, 3H).

Characterized glycal in the glycosylation reactions. ^1H NMR (400 MHz, Chloroform-d) δ 6.84 (s, 1H), 5.48 (t, J = 2.4 Hz, 1H), 5.40 (dd, J = 2.5, 1.3 Hz, 1H), 4.84 (dd, J = 2.3, 1.3 Hz, 1H), 3.81 (s, 3H), 2.16 (s, 3H), 2.12 (s, 3H), 2.02 (s, 3H). HR-MS (ESI): [C₁₃H₁₆O₉+H]⁺ calcd. 317.0867, found 317.0868. Spectroscopic data matches reported.
Characterized orthoester in the glycosylation reactions. Reported are the signals of the major isomer: 1H NMR (400 MHz, Chloroform-d) δ 7.26 (d, J = 8.8 Hz, 2H), 7.07 (d, J = 8.9 Hz, 2H), 5.83 (d, J = 4.8 Hz, 1H), 5.81 (d, J = 5.3 Hz, 1H), 5.25 (t, J = 2.6 Hz, 1H), 5.16 (ddd, J = 7.9, 2.2, 1.1 Hz, 1H), 4.30 (d, J = 7.8 Hz, 1H), 4.22 (ddd, J = 4.8, 2.8, 1.1 Hz, 1H), 3.78 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 1.81 (s, 3H). Similar reported spectra for the 4-bromophenol analogue.[8]

According to B or C, the phenol-glycoside was obtained after column chromatography over silica gel with ethyl acetate : pentane (25:75 to 33:66) as a mixture of anomers (α:β 05:95) (67 mg, 0.16 mmol, 60%). 1H-NMR (400 MHz, Acetone-d6) δ (ppm) β-anomer: 7.12 (d, J = 7.8 Hz, 2H, Ar-H), 6.95 (d, J = 8.6 Hz, 2H, Ar-H), 5.48 (d, J = 7.8 Hz, 1H, H-1), 5.43 (t, J = 9.6 Hz, 1H, H-3), 5.24 – 5.17 (m, 2H, H-2, H-4), 4.56 (d, J = 9.9 Hz, 1H, H-5), 3.69 (s, 3H, COOC2H5), 2.26 (s, 3H, CH3), 2.02 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.99 (s, 3H, OAc). 13C-NMR (101 MHz, Acetone) δ (ppm): 170.21, 169.91, 169.65, 167.89, 155.84, 133.34, 130.81, 117.64, 99.59, 72.80, 72.52, 71.81, 70.28, 52.93, 20.54, 20.52, 20.44. HR-MS (ESI): [C20H24O10+Na]+ calcd. 447.1262, found 447.1262.[9]
Acetone) $\delta$ (ppm): 170.20, 169.92, 169.66, 167.79, 156.56, 130.35, 128.53, 119.33, 99.24, 72.82, 72.39, 71.68, 70.15, 52.97, 20.51, 20.44. **HR-MS** (ESI): $[C_{19}H_{21}ClO_{10}+NH_4]^+$ calcd. 462.1162, found 462.1169.$^{[4,5]}$

![C$_{20}$H$_{21}$NO$_{10}$](image)

4c

According to A, the phenol-glycoside was obtained after column chromatography over silica gel with ethyl acetate: pentane (33:66) as a mixture of anomers (\(\alpha: \beta 02:98\)) (26 mg, 0.06 mmol, 26%). **$^1$H-NMR** (400 MHz, Acetone-$d_6$) $\delta$ (ppm) $\beta$-anomer: 7.76 (d, $J = 8.9$ Hz, 2H, $Ar-H$), 7.26 (d, $J = 8.8$ Hz, 2H, $Ar-H$), 5.74 (d, $J = 7.7$ Hz, 1H, $H-1$), 5.47 (t, $J = 9.6$ Hz, 1H, $H-3$), 5.30 – 5.18 (m, 2H, $H-2$, $H-4$), 4.67 (d, $J = 9.8$ Hz, 1H, $H-5$), 3.68 (s, 3H, COOCH$_3$), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc). **$^{13}$C-NMR** (101 MHz, Acetone) $\delta$ (ppm): 170.19, 169.94, 169.66, 167.69, 160.71, 135.04, 119.10, 118.16, 107.32, 98.23, 72.87, 72.24, 71.52, 70.02, 53.00, 20.51, 20.49, 20.43. **HR-MS** (ESI): $[C_{20}H_{21}NO_{10}+Na]^+$ calcd. 458.1058, found 458.1059.

![C$_{20}$H$_{22}$O$_{11}$](image)

4d

According to A, the phenol-glycoside was obtained after column chromatography over silica gel with ethyl acetate: pentane (25:75 to 33:66) (1.13 g, 2.58 mmol, 51%). **$^1$H-NMR** (400 MHz, Acetone-$d_6$) $\delta$ (ppm): 9.95 (s, 1H, CHO), 7.92 (d, $J = 8.8$ Hz, 2H, $Ar-H$), 7.26 (d, $J = 8.7$ Hz, 2H, $Ar-H$), 5.76 (d, $J = 7.8$ Hz, 1H, $H-1$), 5.49 (t, $J = 9.6$ Hz, 1H, $H-3$), 5.34 – 5.18 (m, 2H, $H-2$, $H-4$), 4.68 (d, $J = 9.9$ Hz, 1H, $H-5$), 3.68 (s, 3H, COOCH$_3$), 2.02, 2.01, 1.98 (s, 9H, 3 x OAc). **$^{13}$C-NMR** (101 MHz, Acetone) $\delta$ (ppm): 206.14, 191.35, 170.20, 169.93, 169.66, 167.72, 162.08, 133.04, 132.42, 117.57, 98.26, 72.89, 72.31, 71.60, 70.08, 53.00, 20.51, 20.50, 20.44. **HR-MS** (ESI): $[C_{20}H_{22}O_{11}+H]^+$ calcd. 439.1235, found 439.1237.$^{[10]}$
According to A, the phenol-glycoside was obtained after column chromatography over silica gel with ethyl acetate : pentane (25:75 to 50:50) (52 mg, 0.11 mmol, 52%).\(^1\)\text{H-NMR} (400 MHz, Acetone-\textit{d}6) \(\delta\) (ppm): 8.25 (d, \(J = 9.3\) Hz, 2H, \(\text{Ar-H}\)), 7.31 (d, \(J = 9.3\) Hz, 2H, \(\text{Ar-H}\)), 5.80 (d, \(J = 7.7\) Hz, 1H, \(\text{H-J}\)), 5.49 (t, \(J = 9.5\) Hz, 1H, \(\text{H-3}\)), 5.32 – 5.21 (m, 2H, \(\text{H-2, H-4}\)), 4.68 (d, \(J = 9.8\) Hz, 1H, \(\text{H-5}\)), 3.69 (s, 3H, \(\text{COOC}_3\text{H}_3\)), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc).\(^2\)\text{C-NMR} (101 MHz, Acetone) \(\delta\) (ppm): 170.20, 169.95, 169.67, 167.67, 162.25, 144.10, 140.37, 126.57, 117.64, 98.29, 73.11, 72.91, 72.19, 71.51, 69.98, 53.02, 20.51, 20.49, 20.44. \text{HR-MS} (ESI): [\text{C}_{19}\text{H}_{21}\text{NO}_{12}+\text{Na}]^+ calcd. 478.0956, found 478.0959.\(^{[11]}\)

According to A, the phenol-glycoside was obtained after column chromatography over silica gel with ethyl acetate : pentane (50:50) (117 mg, 0.24 mmol, 96%).\(^1\)\text{H-NMR} (400 MHz, Acetone-\textit{d}6) \(\delta\) (ppm): 10.05 (s, 1H, \(\text{COH}\)), 8.40 (d, \(J = 2.0\) Hz, 1H, \(\text{Ar-H}\)), 8.21 (dd, \(J = 8.7, 2.0\) Hz, 1H, \(\text{Ar-H}\)), 7.78 (d, \(J = 8.7\) Hz, 1H, \(\text{Ar-H}\)), 5.87 (d, \(J = 7.4\) Hz, 1H, \(\text{H-J}\)), 5.46 (t, \(J = 9.2\) Hz, 1H, \(\text{H-3}\)), 5.36 – 5.23 (m, 2H, \(\text{H-2, H-4}\)), 4.73 (d, \(J = 9.4\) Hz, 1H, \(\text{H-5}\)), 3.68 (s, 3H, \(\text{COOC}_3\text{H}_3\)), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc).\(^2\)\text{C-NMR} (101 MHz, Acetone) \(\delta\) (ppm): 190.26, 170.15, 169.93, 169.42, 167.60, 153.67, 141.95, 135.13, 132.57, 126.81, 119.00, 99.19, 72.97, 71.67, 70.86, 69.60, 53.05, 20.50, 20.47, 20.45. \text{HR-MS} (ESI): [\text{C}_{20}\text{H}_{21}\text{NO}_{13}+\text{Na}]^+ calcd. 506.0905, found 506.0920.\(^{[10]}\)

According to A, the phenol-glycoside was obtained after column chromatography over silica gel with ethyl acetate : pentane (50:50) (87 mg, 0.17 mmol, 80%).\(^1\)\text{H-NMR} (400 MHz, Acetone-\textit{d}6) \(\delta\) (ppm):
According to C, 4d (100 mg, 0.23 mmol, 1.0 equiv.) were dissolved in CHCl₃/i-PrOH (2.0 mL/0.5 mL), 100 mg silica gel was added, and the suspension was left to stir at 0 °C under an atmosphere of N₂. NaBH₄ was added in one scoop and the reaction was monitored by TLC (ethyl acetate: pentane 50:50). Upon completion the reaction was worked up accordingly. The product was isolated as colorless solid (91 mg, 0.21 mmol, 91%). ¹H-NMR (400 MHz, Acetone-de₆) δ (ppm): δ 7.31 (d, J = 8.6 Hz, 2H, Ar-H), 7.02 (d, J = 8.6 Hz, 2H, Ar-H), 5.53 (d, J = 7.9 Hz, 1H, H-1), 5.45 (t, J = 9.6 Hz, 1H, H-3), 5.29 – 5.13 (m, 2H, H-2, H-4), 4.64 – 4.50 (m, 3H, H-5, CH₂), 3.69 (s, 3H, COOCH₃), 2.02 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.99 (s, 3H, OAc). ¹³C-NMR (101 MHz, Acetone) δ (ppm): 170.21, 169.91, 169.67, 167.88, 156.82, 156.82, 138.36, 128.81, 117.44, 99.43, 72.81, 72.51, 72.51, 71.80, 70.27, 64.13, 52.94, 20.53, 20.52, 20.44. HR-MS (ESI): [C₂₀H₂₄O₁₁+Na]⁺ calcd. 463.1211, found 463.1214.[10]

According to C, 4f (100 mg, 0.23 mmol, 1.0 equiv.) were dissolved in CHCl₃/i-PrOH (2.0 mL/0.5 mL), 100 mg silica gel was added, and the suspension was left to stir at 0 °C under an atmosphere of N₂. NaBH₄ was added in one scoop and the reaction was monitored by TLC (ethyl acetate: pentane 50:50). Upon completion the reaction was worked up accordingly. The product was isolated as colorless solid (91 mg, 0.21 mmol, 91%). ¹H-NMR (400 MHz, CDCl₃) δ (ppm): 7.80 (d, J = 2.1 Hz, 1H, Ar-H), 7.53 (dd, J = 8.6, 2.2 Hz, 1H, Ar-H), 7.35 (d, J = 8.5 Hz, 1H, Ar-H), 5.41 – 5.23 (m, 3H, H-2, H-3, H-4), 5.18 (d, J = 6.8 Hz, 1H, H-1), 4.71 (d, J = 5.5 Hz, 2H, CH₂), 4.20 (d, J = 9.2 Hz, 1H, H-3), 3.74 (s, 3H, COOCH₃), 2.12 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.05 (s, 3H, OAc). ¹³C-NMR (101 MHz, CDCl₃) δ (ppm): 170.20, 169.51, 166.49, 166.87, 148.30, 141.39, 137.49,
Imidazole (168 mg, 2.47 mmol, 6.0 equiv.), and DMAP (13 mg, 0.10 mmol, 0.25 equiv.) was added over a solution of 6 (200 mg, 0.41 mmol, 1.0 equiv.) in DMF (2.0 mL) under an atmosphere of N₂. The solution was stirred for 5 min, then a solution of TBSCl (373 mg, 2.47 mmol, 6.0 equiv.) in DMF (1.4 mL) was added and the reaction solution was stirred at r.t. under an atmosphere of N₂ overnight. Upon completion, the reaction mixture was diluted with CH₂Cl₂, and the organic phase was washed with NH₄Cl (3 x 20 mL), brine (2 x 20 mL) and the combined organic layers were dried over Na₂SO₄, filtered and the solvent removed in vacuo. The crude product was purified via column chromatography over silica gel to yield the product as colorless solid (187 mg, 0.31 mmol, 76%).

**1H-NMR** (400 MHz, Chloroform-d) δ (ppm): 7.75 (d, J = 2.1 Hz, 1H, Ar-H), 7.48 (dd, J = 8.6, 2.1 Hz, 1H, Ar-H), 5.41 – 5.27 (m, 3H, H-2, H-3, H-4), 5.17 (d, J = 6.9 Hz, 1H, H-1), 4.19 (d, J = 8.8 Hz, 1H, H-5), 3.75 (s, 3H, COOC₃H₇), 2.06 (s, 3H, OAc), 2.05 (s, 3H, OAc), 0.94 (s, 9H, TBS), 0.11 (s, 6H, TBS).

**13C-NMR** (101 MHz, CDCl₃) δ (ppm): 170.19, 169.49, 169.46, 166.87, 147.92, 141.37, 138.22, 131.21, 122.56, 120.30, 100.19, 72.70, 71.31, 70.33, 68.94, 63.50, 53.23, 26.01, 20.77, 20.73, 20.68, 18.49, -5.18.

**HR-MS** (ESI): [C₂₇H₃₇NO₁₃Si+Na]⁺ calcd. 622.1926, found 622.1940.

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**S1**

S1 (388 mg, 0.65 mmol, 1.0 equiv.) was suspended in abs. EtOH (65 mL) and the suspension was purged with argon for 10 min. Then Pd/C (52 mg) and ammonium formate (163 mg, 2.59 mmol, 4.0 equiv.) was added in one scoop and the suspension was stirred under an atmosphere of argon at r.t. overnight. TLC (EtOAc:Pen 60:40) shows consumption of starting material. The suspension was filtered over a pluck of Celite and the filtrate was reduced in vacuo. The crude syrup is taken up by ethylacetate and washed with brine (3 x 20mL) and dried over Na₂SO₄, filtered and the solvent is removed under reduced pressure to yield the pure product as a colorless solid (350 mg, 0.62 mmol, 95%). **1H-NMR** (400 MHz, Chloroform-d) δ (ppm): 6.87 (d, J = 8.2 Hz, 1H, Ar-H), 6.68 (d, J = 1.9 Hz, 1H, Ar-H), 6.61 (dd, J = 8.2, 1.9 Hz, 1H, Ar-H), 5.38 – 5.23 (m, 3H, H-2, H-3, H-4), 5.00 (d, J = 7.4 Hz, 1H, H-1), 4.59 (s, 2H, CH₂), 4.14 (d, J = 9.3 Hz, 1H, H-5), 3.75 (s, 3H, COOCH₃), 2.08 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.04 (s, 3H, OAc), 0.92 (s, 9H, TBS), 0.08 (s, 6H, TBS). **13C-NMR**
(101 MHz, CDCl3) δ (ppm): 170.18, 169.79, 169.55, 166.95, 137.93, 137.63, 116.55, 116.00, 113.83, 100.91, 72.66, 71.77, 71.08, 69.39, 64.71, 53.16, 26.11, 20.93, 20.78, 20.66, 18.58, -5.10. **HR-MS** (ESI): [C27H39NO11Si+Na]+ calcd. 592.2185, found 592.2189.

S2 (200 mg, 0.35 mmol, 1.0 equiv) was dissolved in CH2Cl2 (5 mL), then DIEA (0.3 mL, 1.8 mmol, 5.0 equiv) was added dropwise, followed by acetic anhydride (0.16 mL, 1.8 mmol, 5.0 equiv). The reaction was left to stir for 2 h when TLC showed consumption of the starting material. The organic phase was washed with sat. NaHCO3 (3 x 10mL) and dried over Na2SO4. The crude syrup was azeotroped with toluene (3 x 30mL) and dried under high vacuum to yield a colorless solid (210 mg, 0.34 mmol, 98%). **1H NMR** (400 MHz, Chloroform-d) δ (ppm): 8.30 (d, J = 2.0 Hz, 1H), 7.92 (s, 1H), 7.06 – 7.01 (m, 1H), 6.90 (d, J = 8.3 Hz, 1H), 5.49 – 5.20 (m, 3H), 5.02 (d, J = 7.8 Hz, 1H), 4.68 (s, 2H), 4.17 (d, J = 9.7 Hz, 1H), 3.75 (d, J = 0.7 Hz, 3H), 2.21 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 0.93 (s, 9H), 0.09 (s, 6H). **13C NMR** (101 MHz, CDCl3) δ (ppm): 170.24, 169.97, 169.56, 168.88, 166.68, 144.13, 137.87, 129.25, 121.28, 118.34, 114.95, 100.68, 72.60, 71.20, 71.17, 69.32, 64.70, 53.27, 26.09, 24.72, 20.96, 20.74, 20.63, 18.54, -5.12. **HR-MS** (ESI): [for C28H41NO12Si +Na]+ calcd. 634.2290, found 634.2311.

S3 (200 mg, 0.33 mmol, 1.0 equiv) was dissolved in THF (5 mL) and cooled to 0°C. Then TEA.3HF (0.07 mL, 0.43 mmol, 1.3 equiv) was added dropwise and the reaction was left to stir overnight. The crude reaction was diluted with CH2Cl2, washed with sat. NH4Cl solution (3 x 20 mL), brine (1 x 20mL) and the organic phase was dried over MgSO4. The suspension was filtered and the organic phase was evaporated in vacuo and the crude product was purified via column chromatography over silica gel to give the product as colorless solid (140 mg, 0.28 mmol, 86%). **1H NMR** (400 MHz, Chloroform-d) δ (ppm): 8.32 (d, J = 2.1 Hz, 1H), 7.95 (s, 1H), 7.05 (dd, J = 8.3, 2.1 Hz, 1H), 6.92 (d, J = 8.3 Hz, 1H), 5.40 (t, J = 9.5 Hz, 1H), 5.36 – 5.23 (m, 2H), 5.04 (d, J = 7.6 Hz, 1H), 4.61 (s, 2H), 4.19 (d, J = 9.7 Hz, 1H), 3.75 (s, 3H), 2.21 (s, 3H), 2.08 (s, 3H), 2.06 (s, 4H), 2.05 (s, 3H). **13C NMR** (101 MHz, CDCl3) δ (ppm): 170.29, 169.98, 169.58, 169.13, 166.69, 144.64, 137.36, 129.33, 122.50, 119.57, 115.17, 100.48, 72.54, 71.18, 71.16, 69.27, 64.70, 53.27, 24.64, 20.95, 20.73, 20.62. **HR-MS** (ESI): [C22H27NO12 +Na]+ calcd. 520.1445, found 520.1442.
According to E, the product was obtained as off yellow solid (17 mg, 0.03 mmol, 68%). $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ (ppm): 8.23 (d, $J = 9.2$ Hz, 2H, Ar-H), 7.90 (d, $J = 2.1$ Hz, 1H, Ar-H), 7.61 (dd, $J = 8.6$, 2.2 Hz, 1H, Ar-H), 7.43 (d, $J = 8.6$ Hz, 1H, Ar-H), 7.03 (d, $J = 9.3$ Hz, 2H, Ar-H), 5.43 – 5.26 (m, 3H, H-2, H-3, H-4), 5.24 (d, $J = 7.3$ Hz, 1H, H-1'), 5.15 (s, 2H, CH$_2$), 4.23 (d, $J = 8.8$ Hz, 1H, H-5), 3.74 (s, 3H, COOC$_3$H$_7$), 2.13 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc). $^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ (ppm): 186.32, 170.03, 169.34, 169.27, 166.71, 161.71, 149.66, 149.03, 146.15, 145.55, 141.31, 134.74, 134.45, 132.69, 131.81, 131.70, 128.75, 124.20, 120.30, 113.90, 106.92, 101.10, 99.61, 72.58, 70.89, 70.10, 68.95, 68.60, 53.14, 20.63, 20.60, 20.55. HR-MS (ESI): [C$_{26}$H$_{26}$N$_2$O$_{15}$+Na]$^+$ calcd. 629.1225, found 629.1238.

According to E, the product was obtained as colorless solid (17 mg, 0.03 mmol, 67%), ethyl acetate minor impurity. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ (ppm): 9.86 (s, 1H, CHO), 7.91 (d, $J = 2.1$ Hz, 1H, Ar-H), 7.63 (dd, $J = 8.7$, 2.2 Hz, 1H, Ar-H), 7.48 – 7.32 (m, 3H, Ar-H, Ar-H), 6.97 (d, $J = 8.1$ Hz, 1H, Ar-H), 5.40 – 5.25 (m, 6H, ), 5.21 (d, $J = 7.2$ Hz, 3H, H-1', CH$_2$), 4.21 (d, $J = 8.8$ Hz, 1H, H-5), 3.95 (s, 3H, vanillin-OCH$_3$), 3.73 (s, 3H, COOCH$_3$), 2.12 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.05 (s, 3H, OAc). $^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ (ppm): 190.87, 170.03, 169.34, 169.27, 166.69, 152.78, 150.11, 148.87, 141.31, 132.62, 132.39, 130.87, 126.40, 124.06, 120.34, 112.49, 109.54, 99.74, 72.57, 71.00, 70.12, 69.17, 68.67, 56.06, 53.46, 53.12, 20.63, 20.59, 20.55. HR-MS (ESI): [C$_{30}$H$_{29}$NO$_{13}$+Na]$^+$ calcd 642.1429, found 642.1432.
According to E, the product was obtained as colorless solid (15 mg, 0.03 mmol, 60%). $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ (ppm): 7.95 (d, $J = 2.1$ Hz, 1H, Ar-H), 7.83 – 7.70 (m, 3H, Nap-H), 7.66 (dd, $J = 8.6$, 2.2 Hz, 1H, Ar-H), 7.48 - 7.43 (m, 1H, Nap-H), 7.41 (d, $J = 8.6$ Hz, 1H, Ar-H), 7.38 – 7.34 (m, 1H, Nap-H), 7.25 – 7.12 (m, 2H, Nap-H), 5.44 – 5.25 (m, 3H, H-2, H-3, H-4), 5.22 (d, $J = 6.7$ Hz, 1H, H-I), 5.18 (s, 2H, CH$_2$), 4.21 (d, $J = 8.8$ Hz, 1H, H-5), 3.74 (s, 3H, COOC$_3$H$_3$), 2.13 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc).

13C-NMR (101 MHz, CDCl$_3$) $\delta$ (ppm): 170.18, 169.48, 169.43, 166.83, 156.13, 148.76, 141.42, 134.44, 133.50, 132.77, 129.90, 129.37, 127.84, 126.93, 126.73, 124.19, 124.17, 120.41, 118.84, 107.29, 99.94, 72.69, 71.17, 70.26, 68.82, 68.26, 53.23, 20.76, 20.72, 20.68. HR-MS (ESI): [C$_{30}$H$_{29}$NO$_{13}$+Na]$^+$ calcd. 634.1531, found 634.1537.

According to E, the product was obtained as colorless solid (14 mg, 0.02 mmol, 56%), ethyl acetate minor impurity. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ (ppm): 8.80 (dd, $J = 4.4$, 1.6 Hz, 1H, quinoline-H), 8.11 – 7.99 (m, 2H, quinoline-H), 7.95 (d, $J = 2.1$ Hz, 1H, Ar-H), 7.67 (dd, $J = 8.6$, 2.2 Hz, 1H, Ar-H), 7.48 – 7.34 (m, 3H, Ar-H, quinoline-H), 7.13 (d, $J = 2.8$ Hz, 1H, quinoline-H), 5.46 – 5.28 (m, 3H, H-2, H-3, H-4), 5.23 (d, $J = 6.8$ Hz, 1H, H-I), 5.19 (s, 2H, CH$_2$), 4.22 (d, $J = 8.8$ Hz, 1H, H-5), 3.74 (s, 3H, COOC$_3$H$_3$), 2.13 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc).

13C-NMR (101 MHz, CDCl$_3$) $\delta$ (ppm): 170.17, 169.47, 169.42, 166.83, 156.29, 148.90, 148.51, 144.68, 141.43, 135.12, 132.99, 132.79, 131.37, 129.27, 124.22, 122.42, 121.73, 120.43, 106.71, 99.88, 72.71, 71.13, 70.26, 68.80, 68.55, 53.25, 20.76, 20.73, 20.68. HR-MS (ESI): [C$_{29}$H$_{28}$N$_2$O$_{13}$+H]$^+$ calcd. 613.1664, found 613.1668.
Protocol A:

5 (50 mg, 0.11 mmol, 1.0 equiv.) was dissolved in dry THF (0.5 mL) and added to a suspension of resorufin (29 mg, 0.14 mmol, 1.2 equiv.), ADDP (173 mg, 0.68 mmol, 6.0 equiv.), TBP (138 mg, 0.17 mL, 0.68 mmol, 6.0 equiv.) in dry THF (2.0 mL). The suspension was left to stir under N₂ for 90 min. The solvent was evaporated, and the crude product was dissolved in ethyl acetate and washed with sat. NaHCO₃ (3 x 20 mL), brine (2 x 20 mL), dried over Na₂SO₄, filtered and the solvent was evaporated under reduced pressure. The crude product was subjected to column chromatography over silica gel with ethyl acetate:pentane (50:50 to 66:33), then CH₂Cl₂ : MeOH (100:00 to 100:05). The product was isolated as orange solid (21 mg, 0.03 mmol, 28%). Note: There was the dehydro product isolated as orange solid (20 mg, 0.04 mmol, 31%).

Protocol B:

5 (50 mg, 0.11 mmol, 1.0 equiv.) was dissolved in CH₂Cl₂ (3.0 mL) and cooled to 0°C under an atmosphere of N₂. Then methanesulfonyl chloride (16 mg, 10.6 ul, 0.14 mmol, 1.2 equiv.), and TEA (14 mg, 19 ul, 0.14 mmol, 1.2 equiv.) were added dropwise. The reaction was monitored via TLC (ethyl acetate:pentane 2:1), upon completion the reaction was diluted with CH₂Cl₂ and washed with sat. NaHCO₃ (3 x 20 mL), dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude syrup was dissolved in dry DMF (0.6 mL) and added to a solution of resorufin (26.6 mg, 0.13 mmol, 1.1 equiv.) and Cs₂CO₃ (40.7 mg, 0.13 mmol, 1.1 equiv.) in dry DMF (1.4 mL). The reaction was left overnight under an atmosphere of N₂ at r.t. The reaction was diluted with ethyl acetate and the organic phase was washed with sat. NaHCO₃ (2 x 20 mL), brine (1 x 20 mL), and dried over Na₂SO₄. The mixture was filtered, the solvent was removed under reduced pressure and the crude product was purified via column chromatography over silica gel with ethyl acetate:pentane (50:50 to 66:33). The product was isolated as orange solid (12 mg, 0.02 mmol, 17%).

Protocol C:

5 (50 mg, 0.11 mmol, 1.0 equiv.) was dissolved in dry THF (3.0 mL) and resorufin (29 mg, 0.14 mmol, 1.2 equiv.), ADDP (173 mg, 0.68 mmol, 6.0 equiv.), TBP (138 mg, 0.17 mL, 0.68 mmol, 6.0 equiv.) were added subsequently. The reaction suspension was stirred at r.t. under an atmosphere of N₂ for 90 min. The crude reaction mixture was worked up according to Protocol A. The crude product was purified by column chromatography over silica gel with ethyl acetate:pentane (50:50 to 66:33). The product was isolated as orange-red solid (20 mg, 0.03 mmol, 28 %). Again, the dehydro product was isolated as major side product (8 mg, 0.01 mmol, 12%).

1H-NMR (400 MHz, CDCl₃) δ (ppm): 7.70 (d, J = 8.9 Hz, 1H, reso-H), 7.41 (d, J = 9.8 Hz, 1H, reso-H), 7.38 (d, J = 8.6 Hz, 2H, Ar-H), 7.04 (d, J = 8.6 Hz, 2H, Ar-H), 6.98 (dd, J = 8.9, 2.6 Hz, 1H, reso-H), 6.86 (d, J = 2.6 Hz, 1H, reso-H), 6.83 (dd, J = 9.8, 2.0 Hz, 1H, reso-H), 6.31 (d, J = 2.1 Hz, 1H, reso-H), 5.39 – 5.31 (m, 2H, H-3, H-4), 5.31 – 5.24 (m, 1H, H-2), 5.17 (d, J = 7.2 Hz, 1H, H-1), 5.11 (s, 2H, CH₂), 4.19 (d, J = 9.5 Hz, 1H, H-1), 3.67 (s, 3H, CH₃), 3.07 – 2.92 (m, 2H, CH₂), 2.95 – 2.75 (m, 2H, CH₂), 2.73 – 2.55 (m, 2H, CH₂), 2.52 – 2.30 (m, 2H, CH₂), 2.02 – 1.75 (m, 2H, CH₂), 1.72 – 1.45 (m, 2H, CH₂), 1.41 – 1.23 (m, 2H, CH₂), 1.22 (s, 3H, CH₃), 0.91 (t, J = 7.2 Hz, 3H, CH₃).
Hz, 1H, H-5), 3.72 (s, 3H, COOHCH$_3$), 2.05 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.04 (s, 3H, OAc).
$^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ (ppm): 186.42, 171.28, 170.22, 169.46, 169.32, 166.93, 162.63, 156.91, 149.89, 145.84, 145.70, 134.82, 134.39, 131.74, 130.57, 129.38, 128.62, 117.45, 114.34, 106.89, 101.15, 99.09, 77.48, 77.36, 77.16, 76.84, 72.82, 71.90, 71.15, 70.48, 69.17, 53.16, 21.19, 20.76, 20.65. HR-MS (ESI): [C$_{32}$H$_{29}$NO$_{13}$+H]$^+$ calcd. 636.1712, found 636.1715.

![C$_{30}$H$_{28}$NO$_{11}$](image)

8k - II

Isolated as major side product from previous reaction. $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ (ppm): 7.71 (d, $J$ = 8.9 Hz, 1H), 7.42 (d, $J$ = 9.8 Hz, 1H), 7.39 (d, $J$ = 8.7 Hz, 2H), 7.15 (d, $J$ = 8.6 Hz, 2H), 6.99 (dd, $J$ = 8.9, 2.6 Hz, 1H), 6.86 (d, $J$ = 2.6 Hz, 1H), 6.84 (dd, $J$ = 9.8, 2.1 Hz, 1H), 6.35 – 6.26 (m, 2H), 5.85 (dd, $J$ = 2.5, 1.3 Hz, 1H), 5.41 – 5.22 (m, 3H), 5.12 (s, 2H), 3.81 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H). HR-MS (ESI): [C$_{30}$H$_{25}$NO$_{11}$+H]$^+$ calcd. 576.1500, found 576.1503.

![C$_{32}$H$_{28}$N$_2$O$_{15}$](image)

8l

According to E, 7 (50 mg, 0.10 mmol, 1.0 equiv.) was dissolved in dry toluene (0.5 mL) and added to a suspension of resorufin (110 mg, 0.52 mmol, 5.0 equiv.), ADDP (52 mg, 0.21 mmol, 2.0 equiv.) in dry toluene (1.5 mL). A solution of TBP (42 mg, 0.05 mL, 0.21 mmol, 2.0 equiv.) in dry toluene (1.0 mL) was added slowly. The suspension was heated to 60 $^\circ$C under an atmosphere of N$_2$ and left overnight. The solvent was evaporated, and the crude product was dissolved in ethyl acetate and washed with sat. NaHCO$_3$ (3 x 20 mL), brine (2 x 20 mL), dried over Na$_2$SO$_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was subjected to column chromatography over silica gel with ethyl acetate : pentane (33:66 to 66:33). The product was isolated as orange solid (20 mg, 0.03 mmol, 35%). $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ (ppm): 7.91 (d, $J$ = 2.2 Hz, 1H, Ar-H), 7.74 (d, $J$ = 8.9 Hz, 1H, reso-H), 7.62 (dd, $J$ = 8.7, 2.2 Hz, 1H, Ar-H), 7.49 – 7.42 (m, 2H, Ar-H, reso-H), 7.00 (dd, $J$ = 8.9, 2.7 Hz, 1H, reso-H), 6.86 (t, $J$ = 2.5 Hz, 1H, reso-H), 6.83 (d, $J$ = 2.1 Hz, 1H,
reso-H), 6.33 (d, J = 2.1 Hz, 1H, reso-H), 5.43 – 5.22 (m, 4H, H-1, H-2, H-3, H-4), 5.16 (s, 2H, CH2), 4.23 (d, J = 8.9 Hz, 1H, H-5), 3.74 (s, 3H, COOCH3), 2.13 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc). 13C-NMR (101 MHz, CDCl3) δ (ppm): 186.32, 170.03, 169.34, 169.27, 166.71, 161.71, 149.66, 149.03, 146.15, 145.55, 141.31, 134.45, 132.79, 131.81, 131.70, 128.75, 124.20, 120.30, 113.90, 106.92, 101.10, 99.61, 72.58, 70.89, 70.10, 68.95, 68.60, 53.14, 20.63, 20.60, 20.55. HR-MS (ESI): [C32H28N2O15+H]+ calcd. 681.1562, found 681.1563.

According to E, instead of 5.0 equiv. phenol, 1.1 equiv. were used. SN-38 was dissolved in minimal amounts of DMF and azeotroped with tolene (3 x 10 mL) prior to addition to reaction mixture. The product was obtained after trituration with ice cold EtOAc to remove excess phosphine oxide as pale yellow solid (46 mg, 0.08 mmol, 65%). 1H-NMR (400 MHz, CDCl3) δ (ppm): δ 8.17 (d, J = 9.2 Hz, 1H, SN-38), 7.97 (d, J = 2.1 Hz, 1H, Ar-H), 7.69 (dd, J = 8.7, 2.2 Hz, 1H, Ar-H), 7.62 (s, 1H, SN-38), 7.49 (dd, J = 9.3, 2.7 Hz, 1H, SN-38), 7.44 (d, J = 8.6 Hz, 1H, Ar-H), 7.34 (d, J = 2.7 Hz, 1H, SN-38), 5.72 (d, J = 16.3 Hz, 1H, CH2a), 5.44 – 5.14 (m, 9H, H-1, H-2, H-3, H-4, 2 x CH2, CH3b), 4.24 (d, J = 8.7 Hz, 1H, H-5), 4.00 (s, 1H), 3.74 (s, 3H, COOCH3), 3.12 (q, J = 7.6 Hz, 2H, CH2), 2.13 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.88 (ddt, J = 16.5, 14.2, 7.1 Hz, 2H, CH2), 1.36 (t, J = 7.6 Hz, 3H, CH3), 1.01 (t, J = 7.4 Hz, 3H, CH3). 13C-NMR (101 MHz, CDCl3) δ (ppm): 174.07, 170.15, 169.48, 169.41, 166.85, 157.76, 157.33, 150.31, 149.01, 147.18, 145.63, 144.01, 141.37, 132.88, 132.57, 128.12, 127.55, 124.33, 122.43, 120.44, 118.17, 103.41, 99.80, 97.75, 72.94, 72.66, 71.06, 70.23, 68.76, 68.72, 66.44, 53.25, 49.55, 31.68, 23.30, 20.75, 20.71, 20.67, 13.76, 7.98. HR-MS (ESI): [C42H41N3O17+Na]+ calcd. 882.2332, found 882.2329.
According to E, instead of 5.0 equiv. phenol, 1.1 equiv. were used. SN-38 was dissolved in minimal amounts of DMF and azeotroped with tolene (3 x 10 mL) prior to addition to reaction mixture. The product was obtained after trituration with ice cold EtOAc to remove excess phosphine oxide as pale yellow solid (contains residuals phosphine oxide) (40 mg, 0.05 mmol, 46%).

\[ ^1H \text{NMR} \ (400 \text{ MHz, Chloroform-}d) \ \delta \ 8.57 \ (d, J = 2.1 \text{ Hz, } 1H, Ar-H), 8.09 \ (d, J = 9.2 \text{ Hz, } 1H, SN-38), 8.01 \ (s, 1H, N\text{H}), 7.61 \ (s, 1H), 7.44 \ (d, J = 9.2, 2.6 \text{ Hz, } 1H, SN-38), 7.30 \ (d, J = 2.7 \text{ Hz, } 1H, SN-38), 7.16 \ (dd, J = 8.3, 2.1 \text{ Hz, } 1H, Ar-H), 6.98 \ (d, J = 8.4 \text{ Hz, } 1H, Ar-H), 5.70 \ (d, J = 16.2 \text{ Hz, } 1H, CH_2a), 5.47 - 5.36 \ (m, 1H, H-3), 5.36 - 5.21 \ (m, 3H, CH_2b, H-2, H-4), 5.18 \ (s, 2H, CH_2), 5.14 \ (s, 2H, CH_2), 5.07 \ (d, J = 7.6 \text{ Hz, } 1H, H-1), 4.22 \ (d, J = 9.7 \text{ Hz, } 1H, H-5), 3.74 \ (s, 3H, COOC_2H_5), 3.08 \ (q, J = 7.6 \text{ Hz, } 2H, CH_2), 2.24 \ (s, 3H, NHAc), 2.08 \ (s, 3H, OAc), 2.07 \ (s, 3H, OAc), 2.05 \ (s, 3H, OAc), 1.96 - 1.78 \ (m, 2H, CH_2), 1.31 \ (t, J = 7.6 \text{ Hz, } 3H, CH_3), 0.99 \ (t, J = 7.3 \text{ Hz, } 3H, CH_3). \ ^13C \text{NMR} \ (101 \text{ MHz, CDCl}_3) \ \delta \ 173.96, 170.30, 169.89, 169.49, 169.13, 166.64, 158.33, 157.88, 157.73, 149.47, 147.05, 145.08, 144.93, 144.27, 132.27, 131.87, 129.67, 128.20, 127.40, 120.02, 118.13, 115.10, 103.37, 100.27, 97.83, 72.93, 72.63, 71.27, 71.11, 70.23, 69.30, 66.39, 53.25, 45.06, 31.71, 25.61, 23.30, 20.92, 20.68, 20.57, 13.62, 7.94. \text{HR-MS (ESI): } [C_{44}H_{45}N_3O_{16}+Na]^+ \text{ calcd. } 894.2692, \text{ found } 894.2669.\]

Fmoc-Tyr(OH)-OAll was synthesized according to literature procedure. According to E, instead of 5.0 equiv. phenol, 1.1 equiv. were used. Fmoc-Tyr(OH)-OAll was dissolved in minimal amounts
of DMF and azeotroped with tolene (3 x 10 mL) prior to addition to reaction mixture. The product was obtained as colorless solid (22 mg, 0.02 mmol, 59%). 

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ (ppm): 7.86 (d, $J = 2.1$ Hz, 1H, Ar-H), 7.77 (d, $J = 7.6$ Hz, 2H, Fmoc-H), 7.61 – 7.50 (m, 3H, Fmoc-H, Ar-H), 7.44 – 7.35 (m, 2H, Fmoc-H, Ar-H), 7.35 – 7.27 (m, 2H, Fmoc-H), 7.03 (d, $J = 8.5$ Hz, 2H, Tyr-Ar-H), 6.85 (d, $J = 8.6$ Hz, 2H, Tyr-Ar-H), 6.01 – 5.78 (m, 1H, -C=), 5.41 – 5.23 (m, 6H, H-2, H-3, H-4, CH$_2$-allyl, =C=H$_2$), 4.74 – 4.62 (m, 2H, =C=H$_2$), 4.45 (dd, $J = 10.6$, 7.0 Hz, 1H, Fmoc-C$\equiv$H), 4.33 (dd, $J = 10.6$, 7.0 Hz, 1H, Fmoc-C$\equiv$H), 4.24 – 4.17 (m, 2H, H-5, Fmoc-H), 3.74 (s, 3H, COOC$_3$H$_3$), 3.12 (dd, $J = 14.0$, 5.7 Hz, 1H, Tyr-C$\equiv$H), 3.05 (dd, $J = 14.0$, 6.0 Hz, 1H, Tyr-C$\equiv$H), 2.13 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc).

$^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ (ppm): 171.32, 170.17, 169.47, 169.42, 166.82, 157.41, 155.64, 148.73, 143.91, 143.86, 141.42, 141.38, 133.53, 132.72, 131.49, 130.73, 128.72, 127.87, 127.18, 125.25, 125.17, 124.11, 120.36, 120.15, 120.12, 119.36, 114.96, 99.92, 72.67, 71.17, 70.25, 68.82, 68.29, 67.06, 66.28, 54.97, 53.23, 47.28, 37.54, 20.76, 20.72, 20.67. HR-MS (ESI): [C$_{47}$H$_{46}$N$_2$O$_{17}$+NH$_4$]$^+$ calcd. 928.3135, found 928.3143.

The compound was synthesized from 8k, according to general protocol F. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm): 7.77 (d, $J = 8.9$ Hz, 1H, reso-H), 7.53 (d, $J = 9.8$ Hz, 1H, reso-H), 7.41 (d, $J = 8.7$ Hz, 2H, Ar-H), 7.19 (d, $J = 2.7$ Hz, 1H, reso-H), 7.12 (dd, $J = 8.9$, 2.6 Hz, 1H, reso-H), 7.05 (d, $J = 8.6$ Hz, 2H, Ar-H), 6.78 (dd, $J = 9.8$, 2.1 Hz, 1H, reso-H), 6.27 (d, $J = 2.0$ Hz, 1H, reso-H), 5.21 (s, 2H, CH$_2$), 4.85 (d, $J = 7.3$ Hz, 1H, H-1), 3.52 – 3.02 (m, 5H, H-2, H-3, H-4, H-5). $^{13}$C-NMR (101 MHz, DMSO) $\delta$ (ppm): 185.36, 162.41, 157.54, 149.80, 145.26, 145.18, 134.95, 133.71, 131.34, 129.60, 128.96, 127.98, 116.32, 114.43, 105.64, 101.24, 100.13, 79.22, 76.76, 73.51, 73.13, 72.13, 70.13, 67.05. HR-MS (ESI): [C$_{25}$H$_{21}$NO$_{10}$+H]$^+$ calcd. 928.3135, found 928.3143.

The compound was synthesized from 8l, according to general protocol F. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm): 8.02 (d, $J = 2.2$ Hz, 1H), 7.79 (d, $J = 8.9$ Hz, 1H), 7.76 (dd, $J = 8.8$, 2.2 Hz, 1H), 7.53 (d, $J = 9.7$ Hz, 1H), 7.37 (d, $J = 8.8$ Hz, 1H), 7.23 (d, $J = 2.7$ Hz, 1H), 7.15 (dd, $J = 8.9$, 2.7 Hz,
1H), 6.78 (dd, J = 9.8, 2.1 Hz, 1H), 6.28 (d, J = 2.1 Hz, 1H), 5.30 (s, 2H), 5.03 (d, J = 7.2 Hz), 3.52 – 3.09 (m, 5H). 

$^{13}$C-NMR (126 MHz, DMSO) $\delta$ (ppm): 185.36, 171.41, 166.01, 161.98, 149.75, 149.60, 148.55, 145.41, 139.69, 136.21, 134.94, 133.77, 129.46, 128.12, 124.27, 122.16, 117.47, 117.27, 116.87, 105.67, 101.34, 100.22, 76.86, 73.50, 73.01, 71.90, 68.75, 61.41. 

HR-MS (ESI): [C$_{25}$H$_{20}$N$_{2}$O$_{10}$-H]$^{-}$ calcd. 593.0943, found 593.0944.

Glucuronide prodrug 8m (0.06 mmol, 50 mg) was suspended in dry MeOH (2.0 mL) and a solution of NaOMe (0.1 equiv.) was added dropwise and the suspension was left to stir under an atmosphere of N$_2$ at r.t. The reaction was monitored via TLC (CH$_2$Cl$_2$:MeOH 90:10) and the deacetylation was finished within 30-60 min, in case it was not additional NaOMe (0.1 equiv.) was added. The reaction did not show full conversion, but was nevertheless diluted with CH$_2$Cl$_2$ and the crude reaction mixture was adsorbed onto Celite and purified by flash column chromatography over silica gel with CH$_2$Cl$_2$:MeOH from 100:00 to 90:10, then EtOAc:MeOH:MeCN:H$_2$O 70:10:10:10. Starting material was recovered as well as the desired product S6 isolated (28 mg, 0.04 mmol, 71%). The completely deprotected glucuronide prodrug was also isolated (0.3 mg, 0.00, 0.8%). 

$^{1}$H-NMR (400 MHz, DMSO-$_d$$_6$) $\delta$ 8.17 – 8.00 (m, 2H), 7.84 (dd, J = 8.8, 2.2 Hz, 1H), 7.63 – 7.54 (m, 2H), 7.50 (d, J = 8.9 Hz, 1H), 7.26 (s, 1H), 6.52 (s, 1H), 5.57 (d, J = 4.6 Hz, 1H), 5.52 (d, J = 5.6 Hz, 1H), 5.42 (s, 2H), 5.38 (s, 2H), 5.34 (t, J = 6.4 Hz, 2H), 5.29 (s, 2H), 4.14 (d, J = 9.6 Hz, 1H), 3.65 (s, 3H), 3.44 - 3.36 (m 1H), 3.33 – 3.25 (m, 2H), 3.22 - 3.11 (m, 2H), 1.86 (hept, J = 7.1 Hz, 2H), 1.25 (t, J = 7.6 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H). 

HR-MS (ESI): [C$_{36}$H$_{43}$_N$_{3}$O$_{14}$+H]$^{+}$ calcd. 734.2191, found 734.2373.

Glucuronide prodrug methyl ester S6 (0.007 mmol, 5 mg) was suspended in dry MeOH (1.0 mL) and Bariumhydroxide octahydrate (1.9 mg, 0.01 mmol, 1.6 equiv.) was added in one scoop and the suspension was left to stir under an atmosphere of N$_2$ at r.t for 2 days. The crude reaction mixture was adsorbed onto Celite and purified by flash column chromatography over silica gel with CH$_2$Cl$_2$:MeOH from 100:00 to 90:10, then EtOAc:MeOH:MeCN:H$_2$O 70:10:10:10. The product was isolated as pale yellow solid (4.2 mg, 0.006 mmol, 86%). Later isolation and purification was performed by preparative HPLC as well. 

$^{1}$H-NMR (400 MHz, DMSO-$_d$$_6$) $\delta$ 8.15 - 8.08 (m, 2H), 7.81 (dd, J = 8.9, 2.2 Hz, 1H), 7.67 – 7.56 (m, 2H), 7.47 (d, J = 8.8 Hz, 1H), 7.27 (s, 1H), 5.43 (s, 2H), 5.42 (d, J = 4.6 Hz, 1H), 5.38 (s, 2H), 5.34 (t, J = 6.4 Hz, 2H), 5.29 (s, 2H), 4.14 (d, J = 9.6 Hz, 1H), 3.65 (s, 3H), 3.44 - 3.36 (m 1H), 3.33 – 3.25 (m, 2H), 3.22 - 3.11 (m, 2H), 1.86 (hept, J = 7.1 Hz, 2H), 1.25 (t, J = 7.6 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H). 

HR-MS (ESI): [C$_{36}$H$_{43}$_N$_{3}$O$_{14}$+H]$^{+}$ calcd. 734.2191, found 734.2373.
5.38 (s, 2H), 5.31 (s, 2H), 5.08 (d, \( J = 7.1 \) Hz, 1H), 3.48 (d, \( J = 9.9 \) Hz, 1H), 3.31 – 3.05 (m, 4H), 1.95 – 1.80 (m, 2H), 1.35 – 1.13 (m, 3H), 0.87 (t, \( J = 7.4 \) Hz, 3H). \( \text{HR-MS (ESI): [C}_{35}\text{H}_{33}\text{N}_{3}\text{O}_{14}\text{-H]} \) calcd. 718.1890, found 718.1933.

\[
\text{HOOC} \quad \text{AcNH} \quad \text{HOHO} \quad \text{O} \quad \text{O} \\
\text{N} \quad \text{N} \quad \text{O} \\
\text{HO} \quad \text{O} \quad \text{O} \\
\text{C} 37 \\
\text{H} 37 \\
\text{N} 3 \\
\text{O} 13
\]

Glucuronide prodrug 8n (0.05 mmol, 40 mg) was suspended in dry MeOH (2.0 mL) and a solution of NaOMe (0.2 equiv.) was added dropwise and the suspension was left to stir under an atmosphere of \( \text{N}_2 \) at r.t. The reaction was monitored via TLC (\( \text{CH}_2\text{Cl}_2: \text{MeOH} 90:10 \)) and the deacetylation was finished within 30-60 min, in case it was not additional NaOMe (0.2 equiv.) was added. The crude reaction mixture was adsorbed onto Celite and purified by flash column chromatography over silica gel with \( \text{CH}_2\text{Cl}_2: \text{MeOH} \) from 100:00 to 90:10. Starting material was recovered as well as the desired product \( \text{S7} \) isolated (22 mg, 0.03 mmol, 63%). The elimination product (\( \Delta_{4,5}\text{-S7} \)) was also isolated (5 mg, 0.007, 15%). \( \text{^1H NMR (400 MHz, Methanol-}d_4) \) \( \delta \) 8.24 (d, \( J = 2.0 \) Hz, 1H), 7.80 (d, \( J = 9.3 \) Hz, 1H), 7.40 (s, 1H), 7.27 (dd, \( J = 9.2 , 2.6 \) Hz, 1H), 7.12 (d, \( J = 8.33 \) Hz, 1H), 7.09 – 7.03 (m, 2H), 5.48 (d, \( J = 16.03 \) Hz, 2H), 5.26 (d, \( J = 16.1 \) Hz, 1H), 4.94 (d, \( J = 7.90 \) Hz, 2H), 4.86 (d, \( J = 6.17 \) Hz, 2H), 4.76 (d, \( J = 7.7 \) Hz, 1H), 3.97 (d, \( J = 9.7 \) Hz, 1H), 3.77 (s, 3H), 3.66 – 3.43 (m, 3H), 3.35 (s, 1H), 2.97 (q, \( J = 7.6 \) Hz, 2H), 2.19 (s, 3H), 1.90 (q, \( J = 7.4 \) Hz, 2H), 1.23 (t, \( J = 7.6 \) Hz, 3H), 0.99 (t, \( J = 7.3 \) Hz, 3H). \( \text{^13C NMR (101 MHz, cd}_3\text{od)} \) \( \delta \) 174.81, 171.67, 170.86, 158.99, 158.83, 152.52, 150.31, 147.77, 147.68, 145.75, 133.91, 131.97, 131.16, 129.23, 128.86, 124.29, 124.15, 121.55, 119.98, 119.63, 104.85, 104.45, 98.88, 77.05, 76.83, 74.53, 74.16, 72.96, 70.77, 66.76, 54.79, 52.96, 50.60, 32.26, 24.22, 23.86, 13.79, 8.20. \( \text{HR-MS (ESI): [C}_{38}\text{H}_{39}\text{N}_{3}\text{O}_{13}+\text{Na}^+] \) calcd. 768.2375, found 768.2385.

\( \Delta_{4,5}\text{-S7} \) \( \text{^1H NMR (400 MHz, Methanol-}d_4) \) \( \delta \) 8.32 (d, \( J = 2.1 \) Hz, 1H), 7.96 (d, \( J = 9.3 \) Hz, 1H), 7.53 (s, 1H), 7.45 (dd, \( J = 9.3 , 2.6 \) Hz, 1H), 7.34 (d, \( J = 2.6 \) Hz), 7.31 (d, \( J = 8.4 \) Hz, 1H), 7.22 (dd, \( J = 8.3 , 2.0 \) Hz, 1H), 6.21 (d, \( J = 4.3 \) Hz, 1H), 5.75 (d, \( J = 4.2 \) Hz, 1H), 5.55 (d, \( J = 16.2 \) Hz, 1H), 5.33 (d, \( J = 16.2 \) Hz, 1H), 5.14 (s, 2H), 5.13 (s, 3H), 4.20 (t, \( J = 4.0 \) Hz, 1H), 4.06 (t, \( J = 4.1 \) Hz, 1H), 3.69 (s, 3H), 3.11 (q, \( J = 7.4 \) Hz, 2H), 2.14 (s, 3H), 1.94 (t, \( J = 7.8 \) Hz, 2H), 1.28 (t, \( J = 7.6 \) Hz, 3H), 1.00 (t, \( J = 7.3 \) Hz, 3H). \( \text{HR-MS (ESI): [C}_{38}\text{H}_{39}\text{N}_{3}\text{O}_{13}+\text{Na}^+] \) calcd. 750.2269, found 750.2273.

Glucuronide prodrug methyl ester \( \text{S7} \) (0.017 mmol, 13 mg) was suspended in dry MeOH (1.0 mL) and Bariumhydroxide octahydrate (4-8 mg, 0.028 mmol, 1.6 equiv.) was added in one scoop and the suspension was left to stir under an atmosphere of \( \text{N}_2 \) at r.t 4. TLC showed consumption of starting
material. The solution was acidified with amberlite 120H+ upon which the solution turned cloudy. The solvent was removed and the crude product was purified via prep. HPLC. Interestingly, HPLC indicated not complete consumption of starting material which contradicts TLC analysis. The product was isolated as pale yellow solid (2.1 mg, 0.003 mmol, 28%; 48% based on recovered starting material). ¹H NMR (400 MHz, DMSO-d₆) δ 9.15 (s, 1H), 8.36 (d, J = 2.1 Hz, 1H), 8.08 (d, J = 9.2 Hz, 1H), 7.60 (d, J = 2.7 Hz, 1H), 7.55 (dd, J = 9.2, 2.7 Hz, 1H), 7.27 (s, 1H), 7.23 (dd, J = 8.3, 2.2 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.54 (d, J = 17.4 Hz, 4H), 5.94 (s, 1H), 5.43 (s, 2H), 5.30 (s, 3H), 5.29 (s, 2H), 4.85 (d, J = 7.6 Hz, 1H), 3.89 (d, J = 9.5 Hz, 1H), 3.18 (d, J = 8.0 Hz, 1H), 2.11 (s, 2H), 1.87 (dq, J = 15.0, 7.0 Hz, 2H), 1.25 (t, J = 7.6 Hz, 4H), 0.87 (t, J = 7.3 Hz, 3H). HR-MS (ESI): [C₃₇H₃₇N₃O₁₃-H] calecd. 732.2399, found 732.2404.
6 NMR Spectra

![NMR Spectra](image-url)

**MeOOC**

**AcOAcO**

**OAc**

**OAc**

2
*contains residual EtOAc.
*contains residual EtOAc.
*contains residual EtOAc
Walther et al. – Supporting Information

MeOOC

AcO

AcO

OAc

O

CHO

4d
$4f$

Chemical structure of compound $4f$ with labeled groups:

- MeOOC
- AcOAcO
- OAc
- CHO
- NO$_2$

NMR spectra and chemical shifts for $4f$.
COSY

HSQC
*contains residual EtOAc and pentane
*contains 8% elimination product.
*contains 13% elimination product.
COSY
HPLC traces of synthesized prodrugs 11 and 12 indicating high purity and absence of residual SN-38.
8 REFERENCES


