Supporting Information

Bypassing stereoselectivity in the early steps of indole alkaloid biosynthesis

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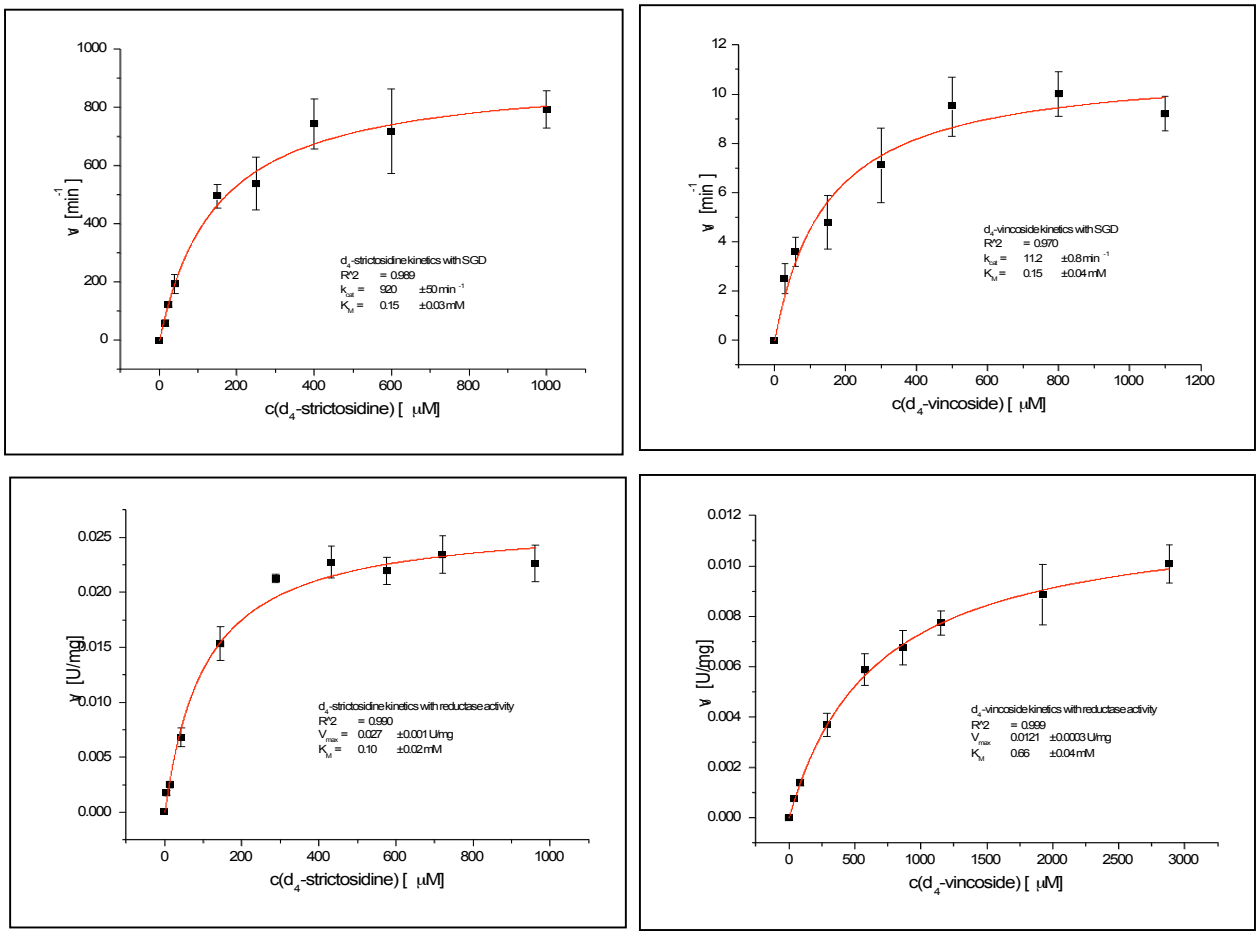
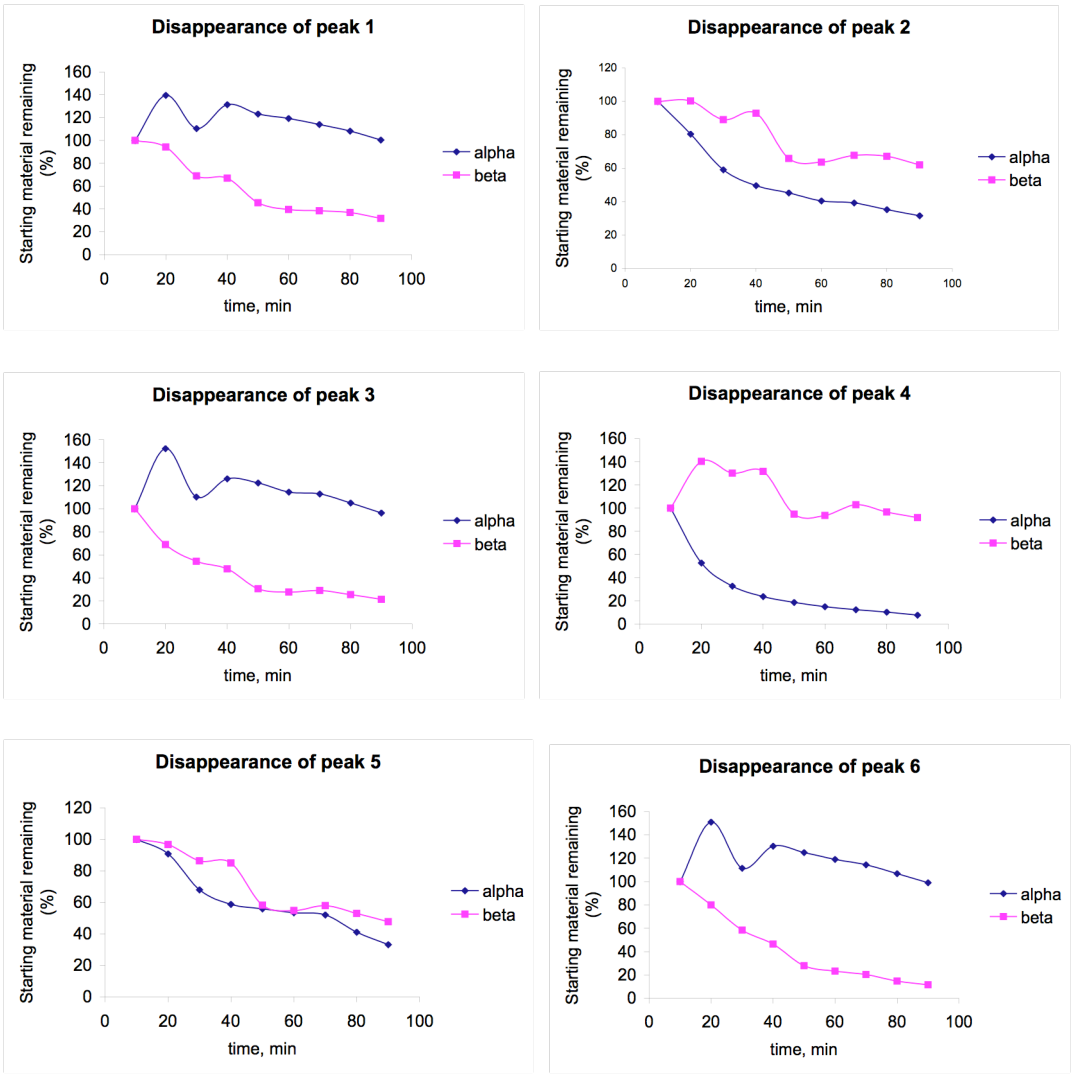
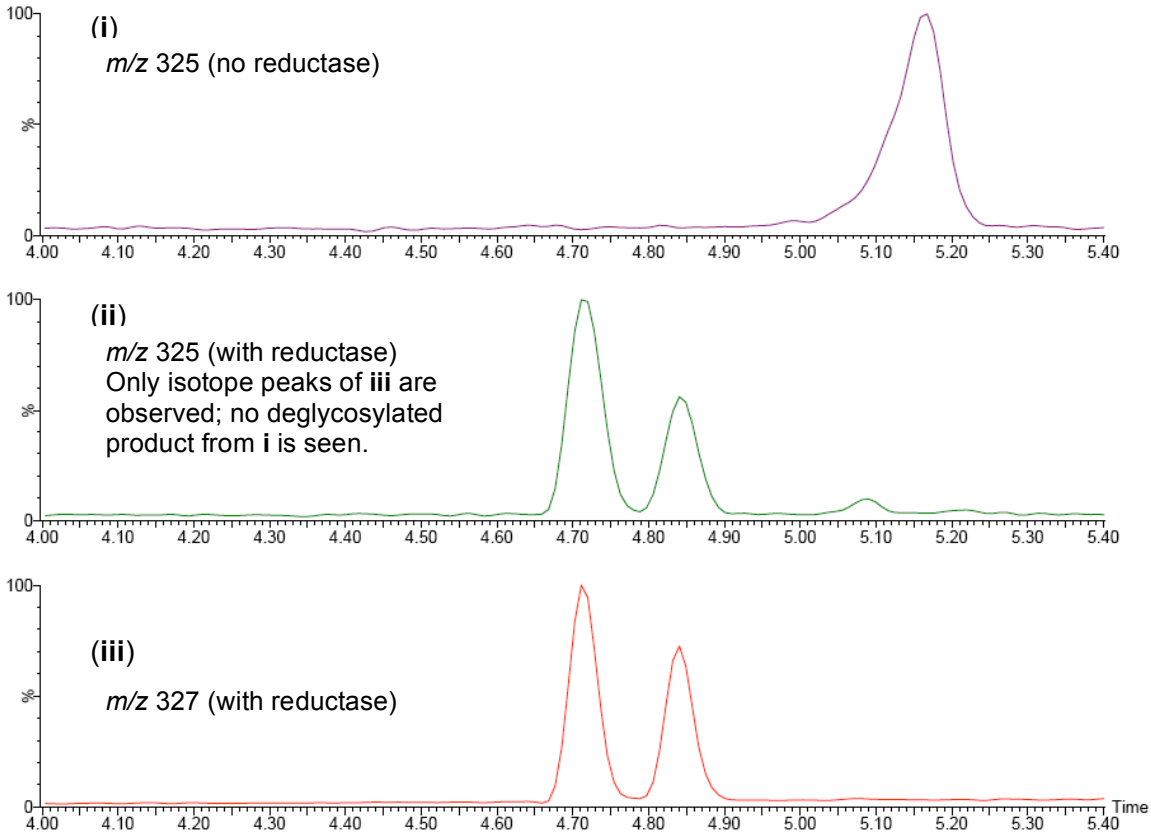
1. Figure S1. Data fit for kinetics of strictosidine glucosidase (SGD) and reductase activity using d_4-strictosidine (d_4-**3a**) or d_4-vincoside (d_4-**3b**) as substrates.



2. Figure S2. Deglucosylation of 18,19-nor-strictosidine isomers **12**. Graphs showing the relative rate of disappearance of UPLC peaks 1-6 using *B. stearothermophilus* α-glucosidase (alpha, blue points) or almond β-glucosidase (beta, pink points). Based on these data, pk 1, pk 3, and pk 6 contain mainly β-anomers, while pk 2 and pk 4 contain mainly α-anomers. Pk 5 appears to contain both α- and β-anomers. Each data point is the average value of three experiments. The error of each point is <20%.



3. Figure S3. Deglucosylated 18,19-nor-strictosidine **12** (**i**, *m/z* = 325) is completely converted by the reductase activity to form **13** (**iii**, *m/z* = 327), as evidenced by the absence of *m/z* 325 in the reductase-supplemented reaction (**ii**). (The two peaks with *m/z* 325 observed in trace (**ii**) correspond to the isotope peaks of *m/z* 327.) Trace (**i**) shows the product of incubation of **12** with *B. stearothermophilus* α-glucosidase, almond β-glucosidase, and *C. roseus* strictosidine-β-D-glucosidase, without reductase. Traces (**ii**) and (**iii**) are extracted masses from the same reaction, where reductase-has been added.



4. Experimental methods

General methods

All chemicals were used without further purification as received from the supplier. For chemical synthesis, all glassware was oven dried, evacuated, and filled with argon. Strictosidine **3a** and strictosidine analogs were prepared enzymatically using strictosidine synthase (STS) and purified by preparative HPLC. Alternatively, strictosidine analogs were prepared chemically by reacting tryptamine **1** and secologanin **2**, or secologanin analogs, in aqueous maleic acid buffer (0.1 M, pH 2.0). STS and SGD were expressed in *E. coli* BL21(DE3) and purified by affinity chromatography using a hexa-His tag as previously described (*13, 23*). Secologanin was purified from *Lonicera tatarica* as previously described (*15*). α-glucosidase from *B. stearothermophilus* (104 U mg^{-1}, where 1 U = 1 μmol D-glucose from *p*-nitrophenyl α-D-glucoside per minute at pH 6.8 at 37 °C) and β-glucosidase from almonds (26 U mg^{-1}, where 1 U = 1 μmol of salicin per minute at pH 5.0 at 37 °C) were purchased from Sigma-Aldrich (St. Louis, MO).

UPLC analysis was performed using an Acquity Ultra Performance BEH C18 column with a 1.7 μm particle size, 2.1 x 100 mm dimension, and a flow rate of 0.6 mL min^{-1}. The column elution was coupled to MS analysis carried out using a Micromass LCT Premier TOF Mass Spectrometer with an ESI source. Both modules are from Waters Corporation (Milford, MA). The capillary and sample cone voltages were 3000 V and 30 V, respectively. The desolvation and source temperatures were 300 and 100 °C, respectively. The cone and desolvation gas flow rates were 60 and 800 L h^{-1}. Analysis was performed with MassLynx 4.1 and integrations were carried out using the QuantLynx tool. Proton nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on Varian 300 MHz, Varian 500 MHz, or Brucker 400 MHz

spectrometers. Chemical shifts (δ) were referenced to residual protium in the NMR solvent.

Kinetics of SGD

Steady-state kinetic constants for strictosidine **3a** (15-1000 μM) or vincoside **3b** (30 to 1100 μM) were determined in citrate-phosphate buffer (0.15 M, pH 6.0) at 30 °C (0.200 mL reaction volume) with SGD concentrations appropriate for measuring the initial rate of the reaction (0.005-0.2 μM). Aliquots (10 μL) were quenched in 1 mL methanol, containing yohimbine (0.5 μM) as an internal standard, at appropriate time points. The samples were centrifuged (13,000*g*, 1 min) to remove particulates and then analyzed by LC-MS. The starting material and products were separated using a gradient of 10-40% acetonitrile in water (containing 0.1% formic acid) over a period of 5 minutes. The disappearance of strictosidine **3a** or vincoside **3b** was monitored by peak integration and normalized to the internal standard. Seven substrate concentrations were tested for each substrate, and the amount of **3a** or **3b** remaining was correlated to peak area by standard curves. Each concentration was assayed at least three times and the averaged value is reported with standard deviations. The data were fitted using non-linear regression to the Michaelis-Menten or Northrop equations using OriginPro 7 (OriginLab, Northhampton, MA).

*Deglucosylation of **12***

Deglucosylation of 18,19-nor-strictosidine isomers **12** (0.24 mM final concentration) was carried out in citrate-phosphate buffer (0.15 M, pH 6.0) at 30 °C in the presence of either SGD (20 μM), almond β-glucosidase (5 U), or *B. stearothermophilus* α-glucosidase (15 U) in a 0.145 mL reaction volume. Aliquots (5 μL) were quenched at appropriate time

points and treated as described above. Peaks corresponding to starting material were integrated and normalized to the yohimbine internal standard.

Isolation of reductase activity from cell suspension culture

Cells were recovered from two-week-old *C. roseus* PC510 cell suspension cultures (DSMZ, Germany) by filtration through cheesecloth. The cells were suspended in borate buffer (0.1 M, pH 7.0) containing polyvinylpyrrolidone (PVP, 0.3% w/v) and β-mercaptoethanol (20 mM) and ground in a blender (4 °C, 10 min). The cell extract was centrifuged (6,000*g*, 10 min) and subjected to ammonium sulfate precipitation near 0 °C. The 40-70% fraction was recovered and desalted by passing the solution through a Sephadex G25 column and eluting with sodium phosphate buffer (30 mM, pH 7.0), supplemented with sodium chloride (50 mM) and glycerol (10% v/v). The desalted eluent was concentrated using a centrifugal filter device (AmiconUltra 10-kDa cut-off, Millipore Corp., Billerica, MA), aliquoted for single-use fractions, and stored at -20 °C.

Kinetic analysis of the reductase activity

Steady-state kinetic constants were obtained by performing a coupled assay with isotopically labeled strictosidine d_4-**3a** or vincoside d_4-**3b** in the presence of SGD. The isotope-labeled versions of the substrates were used to ensure that no trace amounts of alkaloid present in the cell free extract would interfere with kinetic analyses. Briefly, d_4-**3a** or d_4-**3b** (5 to 3000 μM) were diluted in citrate-phosphate buffer (0.15 M, pH 6.0, 0.05 mL final volume) and SGD (7.3 μM) was added. Under these conditions, the reactions with the highest concentration of d_4-**3a** and d_4-**3b** are completely deglucosylated after 30 min at 30 °C. After this time, deglucosylated d_4-**3a** or d_4-**3b** (50 μL volume) was added to a solution containing NADPH (2.4 mM final concentration) and cell free extract (prepared as described above, 3.3 mg total protein mL^{-1}) to a total volume of 200 μL. The reaction

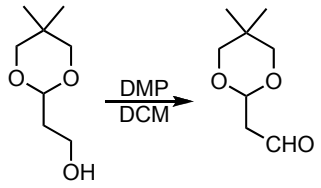
mixture was incubated at 30 °C and aliquots (15 µL) were quenched at appropriate time points and treated as described for the SGD kinetic analysis. The increase in the area under the product peak was used to obtain the initial rates of the reduction reaction. Each concentration was assayed at least three times and the averaged value is reported with standard deviations. The data were fitted by the same method as described for SGD kinetics.

Cell suspension culture feeding studies

d_4-**3a**, d_4-**3b**, **12a**, and **12**, and the deglycosylated versions of these substrates, (0.15 mM) were each added to the media (75 mL) of separate flasks containing cells from the PC510 cell suspension culture line. The cell cultures were incubated (125 rpm) at 25 °C for two weeks on a 17/7 h light-dark cycle. The cells were then recovered by filtration using cheesecloth. The plant tissue pulp was suspended in methanol and sonicated to permeabilize the cells. After the debris settled, the methanol extract was filtered through a 0.45-µm filter, concentrated *in vacuo* and passed through a C18 cartridge (Honeywell Burdick&Jackson, Morristown, NJ). The samples were then subjected to LC-MS analysis.

5. Procedures for chemical synthesis

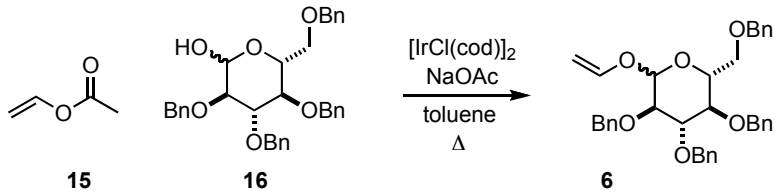
2-(5,5-dimethyl-1,3-dioxan-2-yl)ethanal **7**



14 **7**

To a solution of 2-(5,5-dimethyl-1,3-dioxan-2-yl)ethanol **14** (1.6 g, 10 mmol, 1.0 eq) in DCM (100 mL) at 0 °C was added Dess-Martin periodinane (DMP, 5.1 g, 12 mmol, 1.2 eq). The reaction was stirred at 0 °C for 5 min, warmed to room temperature and then stirred for an additional 45 min. The reaction was diluted with DCM (100 mL) and quenched by the successive addition of a saturated aqueous solution of sodium bicarbonate (50 mL) and a concentrated aqueous solution of sodium thiosulfate (50 mL). The layers were separated and the aqueous layer was extracted with DCM (3 x 20 mL). The combined organic layers were washed with a (1:1) mixture of sodium bicarbonate/sodium thiosulfate and dried over anhydrous sodium sulfate. After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromtography (SiO_2, hex/ether 3:1) to afford 2-(5,5-dimethyl-1,3-dioxan-2-yl)ethanal **7** (1.5 g, 9.7 mmol, 97%) as a clear oil. The analytical data matched those reported in literature.[1] r_f = 0.69 (hex/ether 1:3); ^{1}H NMR ($CDCl_3$): δ 9.81 (t, 1H, *J* = 2.4), 4.86 (t, 1H, *J* = 4.5), 3.61 (d, 2H, *J* = 10.0), 3.45 (d, 2H, *J* = 10.5), 2.68 (dd, 1H, *J* = 2.4, 4.6), 1.17 (s, 3H), 0.72 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 199.57, 97.81, 77.17, 48.48, 29.99, 22.84, 21.75; ESI-MS($C_8H_{15}O_3^+$): *m/z* calculated: 159.1016, found: 159.1014.

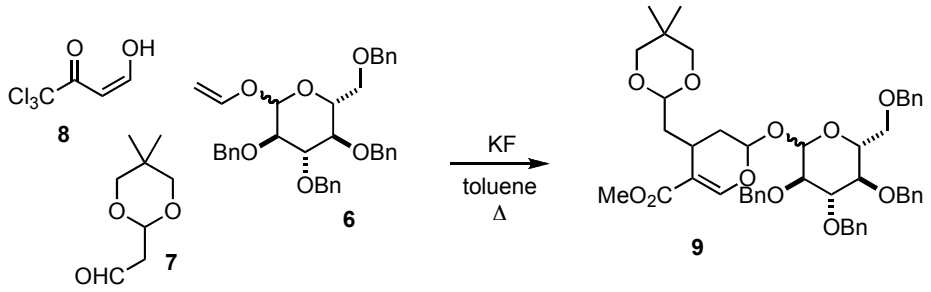
α/β-vinyl-2,3,4,6-tetra-O-benzyl-glucopyranose **6**



15 **16** **6**

In a Schlenk flask under a stream of argon, $[IrCl(cod)]_2$ (0.050 g, 0.074 mmol, 0.02 eq) was added to a toluene solution (7.5 mL) of vinyl acetate **15** (0.64 g, 7.4 mmol, 2.0 eq), 2,3,4,6-tetra-*O*-benzylglucopyranose **16** (2.0 g, 3.7 mmol, 1.0 eq), and sodium acetate (0.36 g, 4.4 mmol, 1.2 eq). The mixture was heated to 95 °C and stirred at this temperature for 90 min. A second portion of vinyl acetate **15** was then added (0.32 g, 3.7 mmol, 1.0 eq) and the reaction was allowed to continue for a total of 2 h. The reaction was diluted with wet ether (40 mL) and filtered through SiO_2 eluting with ether. After evaporation of the solvent, the residue was purified by flash column chromatography (SiO_2, hex/EtOAc, 4:1) to afford vinyl-2,3,4,6-tetra-O-benzyl-glucopyranose **6** (1.7 g, 3.0 mmol, 80%) as an oil (0.62:0.38 mixture of α- and β-anomers), which solidified upon standing. ^1H NMR ($CDCl_3$): δ 7.44-7.05 (m, 20H), 6.48 (dd, 0.62H, *J* = 6.4, 13.9), 6.36 (dd, 0.38H, *J* = 6.5, 14.1), 5.10-4.39 (m, 10H), 4.24 (ddd, 1H, *J* = 1.7, 6.5, 16.4), 3.87-3.41 (m, 6H); ESI-MS($C_{36}H_{39}O_6^+$): *m/z* calculated: 567.2741, observed: 567.2752.

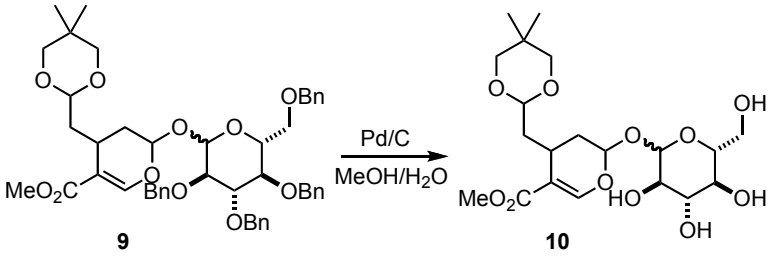
Methyl 4-((5,5-dimethyl-1,3-dioxan-2-yl)methyl)-2-(a/b-glucopyranose)-3,4-dihydro-2H-pyran-5-carboxylate **9**



Substrate **8** was synthesized as described in: [P. Shi-Qi, E. Winterfeldt, Synthesis of Malonaldehyde Monoacetals, *Liebigs Ann. Chem.* **1989**, 1045-1047.] 2-(5,5-dimethyl-1,3-dioxan-2-yl)ethanal **7** (1.3 g, 8.2 mmol, 2.2 eq) and 1-vinyl-2,3,4,6-tetra-*O*-benzyl-glucopyranose **6** (2.1 g, 3.7 mmol, 1.0 eq) were dissolved in toluene (7.5 mL) at 0 °C. Potassium fluoride was added (0.19 g) and the resulting mixture quickly changed color to orange. 1,1,1-trichloro-4-hydroxybut-3-en-2-one **8** (1.4 g, 7.4 mmol, 2.0 eq) was added drop-wise and the resulting reaction was heated up to 100 ºC over a period of 1 h. After a total reaction time of 8 h, the reaction was diluted with ether and filtered through water-deactivated aluminum oxide (pH 9.5-10.5). Evaporation of the solvent and purification of the residue by flash column chromatography (SiO_2, hex/MTBE, 8:1 to 5:1) afforded partially purified cycloadduct (six stereoisomers by NMR, four major stereoisomers). The cycloadducts were dissolved in anhydrous methanol (10 mL) and DBU was added (0.1 mL). After 20 min stirring at room temperature, the solvent was evaporated and the residue was purified by flash column chromatography (SiO_2, hex/MTBE, 5:1 to 3:1) to afford analytically pure methyl 4-((5,5-dimethyl-1,3-dioxan-2-yl)methyl)-2-(α/β-glucopyranose)-3,4-dihydro-2H-pyran-5-carboxylate **9** (1.2 g, 1.4 mmol, 38%) as a mixture of diastereomers. ^{1}H NMR ($CDCl_3$): δ 7.48-7.06 (m, 1H), 7.37-7.06 (m, 20H), 5.58 (t, 0.20H, *J* = 2.9), 5.51-5.45 (m, 0.31H), 5.40 (t, 0.17H, *J* = 2.9), 5.34-5.27 (m, 0.42H), 5.02-4.64 (m, 4H), 4.64-4.39 (m, 5H), 3.80-3.22 (m, 8H), 2.93-2.75 (m,

1H), 2.37-1.75 (m, 4H), 1.66-1.50 (m, 1H), 1.25-1.03 (m, 3H), 0.74-0.52 (m, 3H); ESI-MS($C_{48}H_{57}O_{11}^+$): *m/z* calculated: 809.3895 $[M+H]^+$, found: 809.3880 $[M+H]^+$.

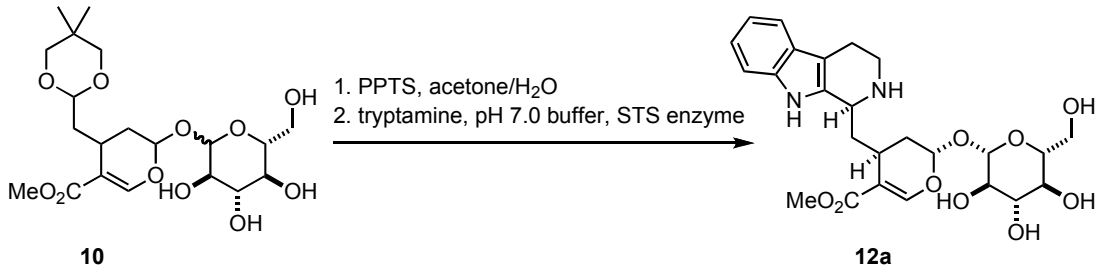
Methyl 4-((5,5-dimethyl-1,3-dioxan-2-yl)methyl)-2-(a/b-glucopyranose)-3,4-dihydro-2H-pyran-5-carboxylate **10**



To a solution of the tetrabenzylated starting material **9** (1.1 g, 1.4 mmol) in MeOH (50 mL) was added water (1.5 mL) and then Pd/C (cat.). The flask was evacuated and filled with an H_2-atmosphere. The resulting heterogenous mixture was stirred for 2 h at room temperature or until TLC (DCM/MeOH, 9:1) indicated complete conversion. Filtration through celite and evaporation of the solvents afforded a yellow oil that was subjected to flash column chromatography (SiO_2, DCM/MeOH, 9:1) to afford the debenzylated product **10** as a white foam (0.45 g, 1.0 mmol, 74%); ^{1}H NMR (MeOD): δ 7.51-7.45 (m, 1H), 5.67 (t, 0.26H, *J* = 2.5), 5.59-5.54 (m, 0.38H), 5.50 (t, 0.22H, *J* = 2.5), 5.47 (dd, 0.11H, *J* = 2.3, 9.2), 5.26 (d, 0.12H, *J* = 3.7), 5.16 (d, 0.21H, *J* = 3.7), 4.72 (d, 0.17H, *J* = 7.9), 4.67-4.60 (m, 1H), 4.57 (t, 0.38H, *J* = 4.3), 4.52 (d, 0.24H, *J* = 7.8), 3.92-3.77 (m, 1H), 3.72-3.68 (m, 3H), 3.69-3.40 (m, 6H), 3.26-3.09 (m, 1H), 2.94-2.73 (m, 1H), 2.37-1.79 (m, 4H), 1.62 (ddd, 0.3H, *J* = 4.8, 9.7, 14.2), 1.17 (s, 3H), 0.73 (s, 3H); ESI-MS($C_{20}H_{33}O_{11}^+$): *m/z* calculated: 449.2019 $[M+H]^+$, found: 449.2025 $[M+H]^+$.

Synthesis of 18,19-nor-strictosidine **12a** and **12**

*Enzymatic formation of **12a**:*



To a solution of methyl 4-((5,5-dimethyl-1,3-dioxan-2-yl)methyl)-2-(glucopyranose)-3,4-dihydro-2H-pyran-5-carboxylate **10** (0.35 g, 0.78 mmol) was added PPTS (1.56 mmol, 2 eq) to make a 0.2 M solution in acetone/water (2:1). The mixture was heated to 65 °C for 35 h, after which it was quenched by addition of solid sodium carbonate (0.5 g). After filtration, the solvents were evaporated in the presence of toluene. Purification by normal phase flash column chromatography (SiO_2, DCM/MeOH, 85:15) followed by reverse-phase flash column chromatography ($C_{18}SiO_2$, packed in MeOH, equilibrated to H_2O, run with H_2O/MeOH, 99:1 to 8:2) afforded partially purified aldehyde **11** (68 mg, est. 20% yield). The aldehyde product could not be purified to analytical purity. The NMR signals at δ 9.80-9.60 (m) are indicative of the aldehyde functional group. The signals associated with the dioxane protective group are absent in this NMR spectrum. The aldehyde is expected to exist partially in its hydrated form, due to the C18 column chromatography system. This compound was used directly in the next reaction with tryptamine **1**.

To a solution of the partially purified 9,10-nor-secologanin **11** (68 mg, 0.17 mmol, 8.5 mM, 1.0 eq)) in sodium phosphate buffer (20 mL, 50 mM, pH 7.0) was added tryptamine hydrochloride **1** (44 mg, 0.22 mmol, 11 mM, 1.3 eq) and *Catharanthus roseus* strictosidine synthase (crSTS, 0.25 mL, 1 mol-%). The reaction was followed by HPLC until no further product was formed. MeOH was added (200 mL) and the resulting

precipitate was removed by filtration. After evaporation of most of the solvent the enzymatic product was purified by preparative HPLC (detection at 238 nm; solvent system: 25-50% MeCN in water-0.1% TFA over 20 min; rt(SM) = 4.5 min, rt(product) = 8.5 min). The mobile phase was evaporated *in vacuo* and dried to yield an amorphous solid (2 mg, 0.004 mmol), which consisted of a single diastereomer. ^{1}H NMR (MeOD): δ 7.76 (s, 1H, H-17), 7.45 (d, 1H, *J* = 7.8, H-12), 7.30 (d, 1H, *J* = 8.1, H-9), 7.13 (dt, 1H, *J* = 1.0, 7.1, H-10), 7.02 (dt, 1H, *J* = 0.9, 8.0, H-11), 5.75 (dd, 1H, *J* = 2.9, 9.2, H-2), 4.77 (d, 1H, *J* = 7.8, H-1'), 4.65 (d, 1H, *J* = 8.1, H-3), 3.93 (dd, 1H, *J* = 1.9, 11.7), 3.75 (s, 3H, OMe), 3.59 (dd, 1H, *J* = 7.0 11.8), 3.52-2.95 (m, 9H), 2.48 (ddd, 1H, *J* = 3.7, 10.0, 13.9), 2.06-1.92 (m, 3H, H-14); ESI-MS ($C_{25}H_{33}N_2O_9^+$), calc. *m/z* 505.2181 $[M+H]^+$, obsd. *m/z* 505.2180 $[M+H]^+$.

*Chemical formation of **12**:*

9,10-nor-secologanin **11** was reacted with tryptamine hydrochloride **1** in maleic acid buffer (10 mM, pH 2.0) for 48 h at 37 °C. The reaction products were collectively purified by prep-HPLC as described for the enzymatic product to afford an amorphous solid. Since the reaction products were complex mixtures of multiple diastereomers, complete structural characterization could not be performed. However, several representative peaks in each ^{1}H NMR spectrum could be used to assign certain structural elements.

Peak 1: Two stereoisomers with 2,4-*cis* configuration (s_{app} or t at δ ~5 ppm) and are β-anomers (d, *J* = 8.0 Hz). This compound is turned over by SGD and almond β-glucosidase (Figure S2).

Peak 2: One stereoisomer with 2,4-*cis* configuration (s_{app} or t at δ ~ 5 ppm) and α-anomeric configuration (d, *J* = 3.8 Hz). This compound is turned over by α-glucosidase (Figure S2).

Peaks 3-4: Several stereoisomers of both 2,4-*cis* (s_{app} or t at δ ~5 ppm) and 2,4-*trans* configuration (dd at δ ~5 ppm). The anomeric region is crowded, but both alpha and beta anomers are present. See Figure S2, where peak 4 is exclusively converted by α-glucosidase, and where peak three is converted converted by SGD and almond β-glucosidase.

Peaks 5-6: Several stereoisomers, of which there are both 2,4-*trans* and 2,4-*cis* configurations, as evidenced by the br-s and dd in the 5-6 ppm region. 2,4-*cis* appears to be dominating in these two peaks. The anomeric region is also crowded here, but both apha and beta anomers are present. See Figure S2, where peak 5 is converted by both β- and α-glucosidases, and where peak 6 is converted by almond β-glucosidase.

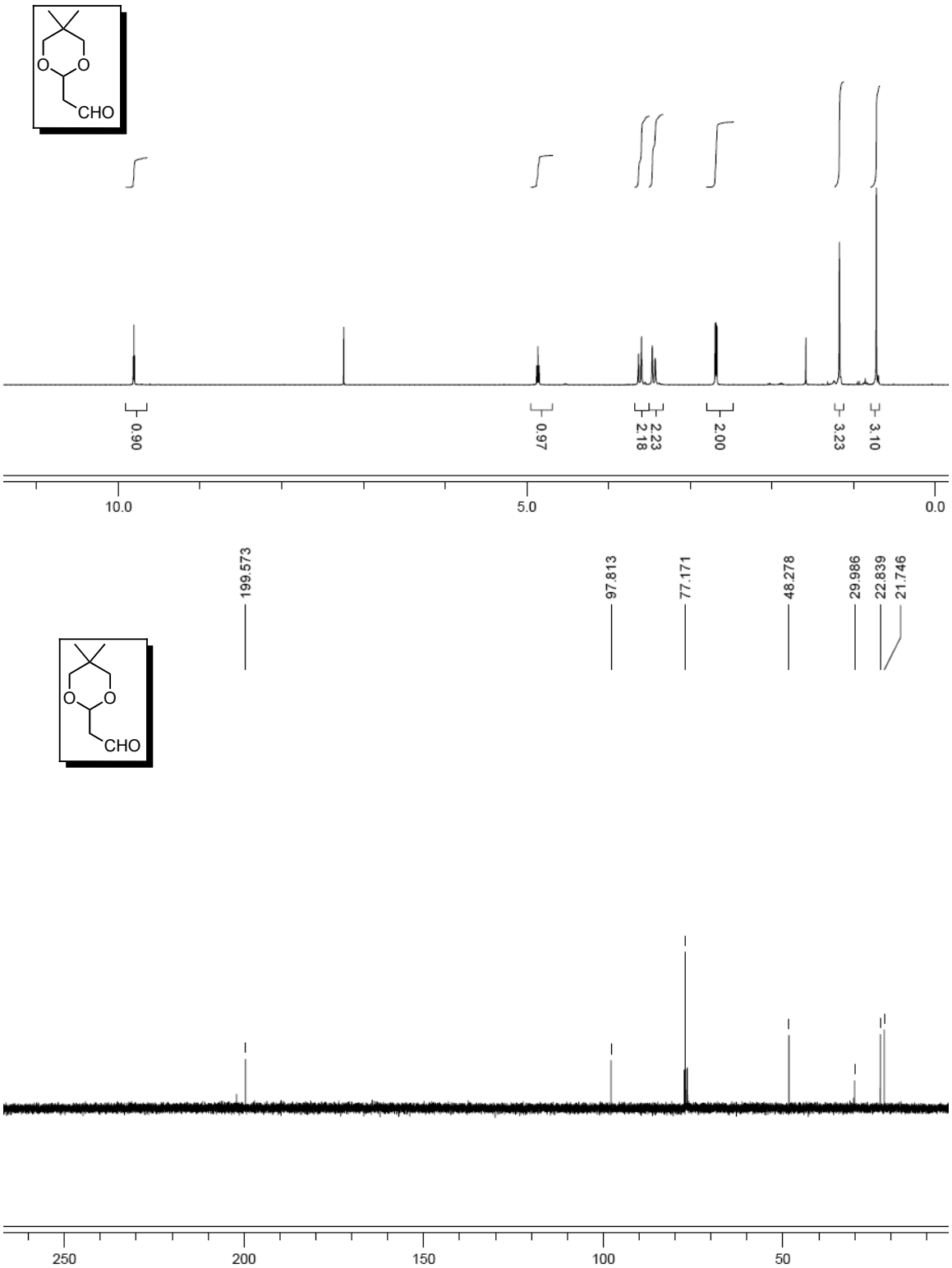
Methyl 3-hydroxy-2-((2*R*,12b*S*)-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizin-2-yl)acrylate **13a**

Description:

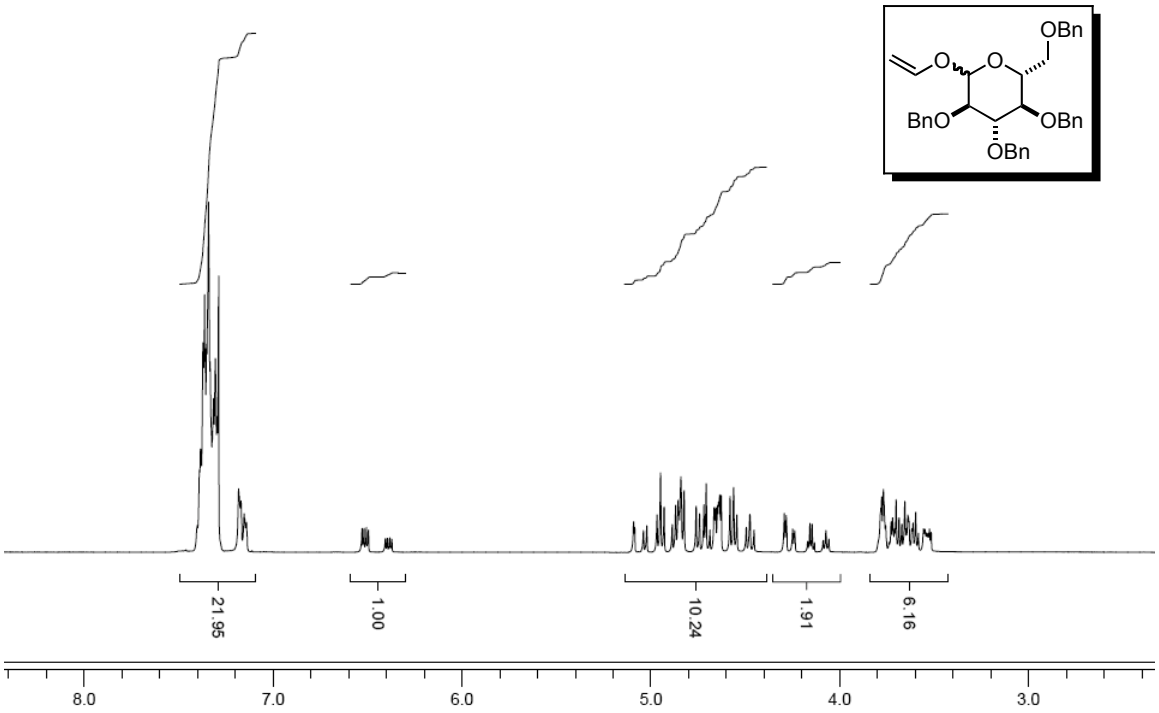
^{1}H NMR (MeOD): δ 7.59 (s, 1H), 7.45 (d, *J* = 7.9, 1H), 7.34 (td, *J* = 0.8, 8.1, 1H), 7.11 (ddd, *J* = 1.2, 7.1, 8.2, 1H), 7.04 (ddd, *J* = 1.0, 7.1, 8.0, 1H), 4.62-4.55 (m, 1H), 3.77-3.72 (m, 1H), 3.62 (s, 3H), 3.61-3.53 (m, 3H), 2.89-2.73 (m, 3H), 2.39 (ddd, *J* = 1.9, 3.7, 13.3, 1H), 1.69-1.64 (m, 2H), 1.57 (ddd, *J* = 5.0, 12.1, 13.2, 1H)

6. NMR spectra

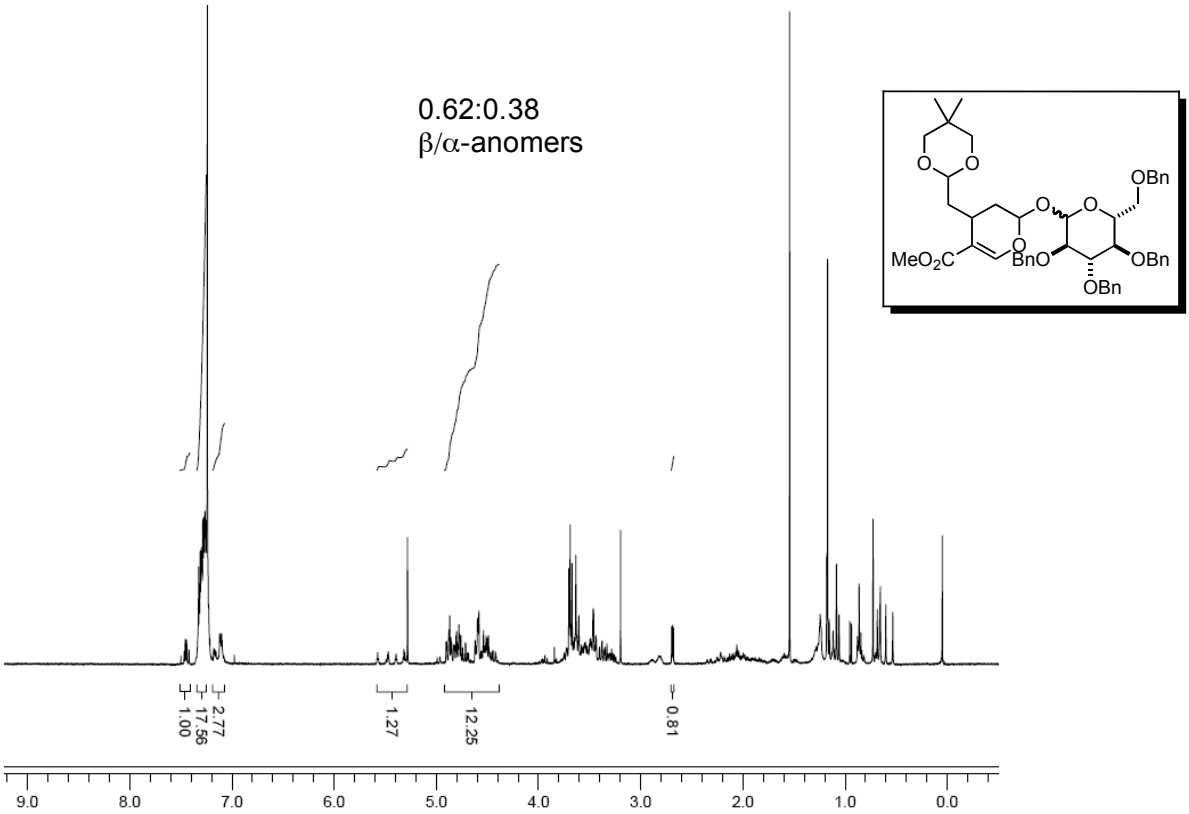
Compound 7



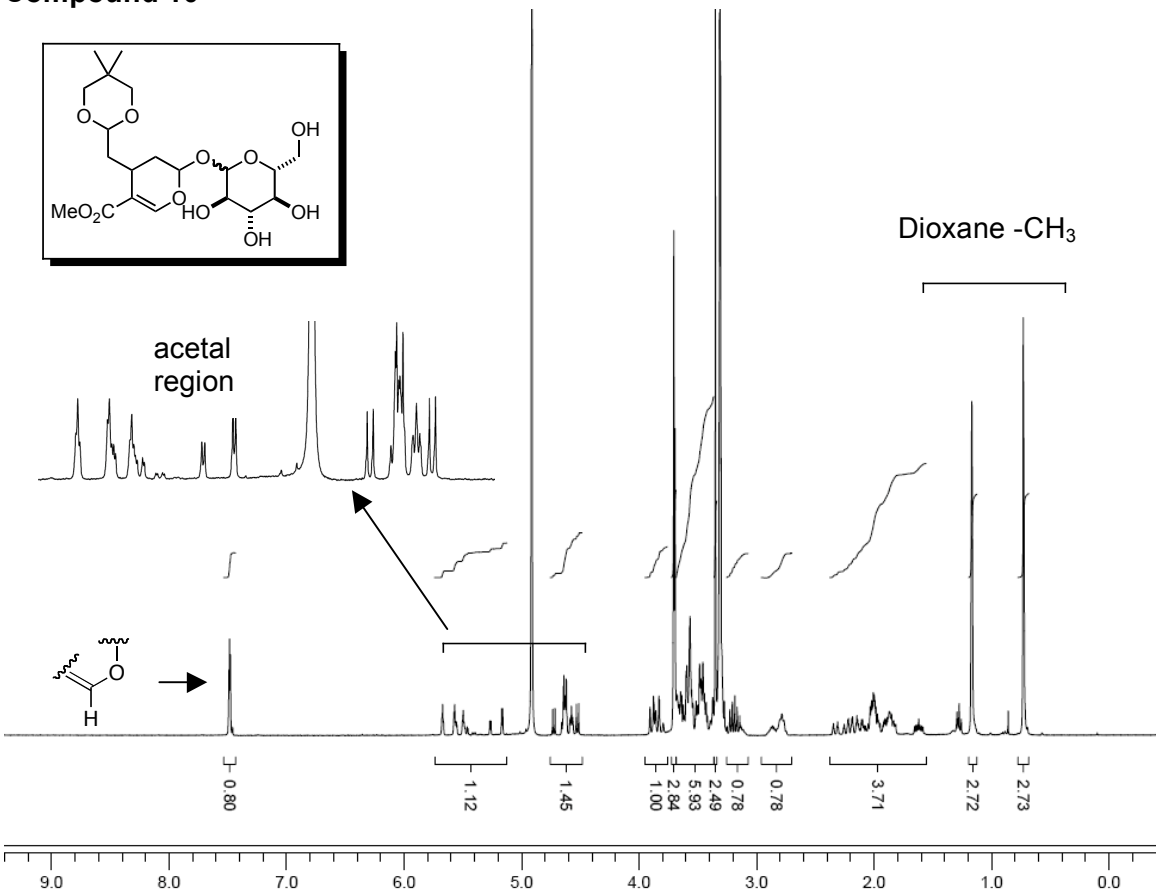
Compound 6



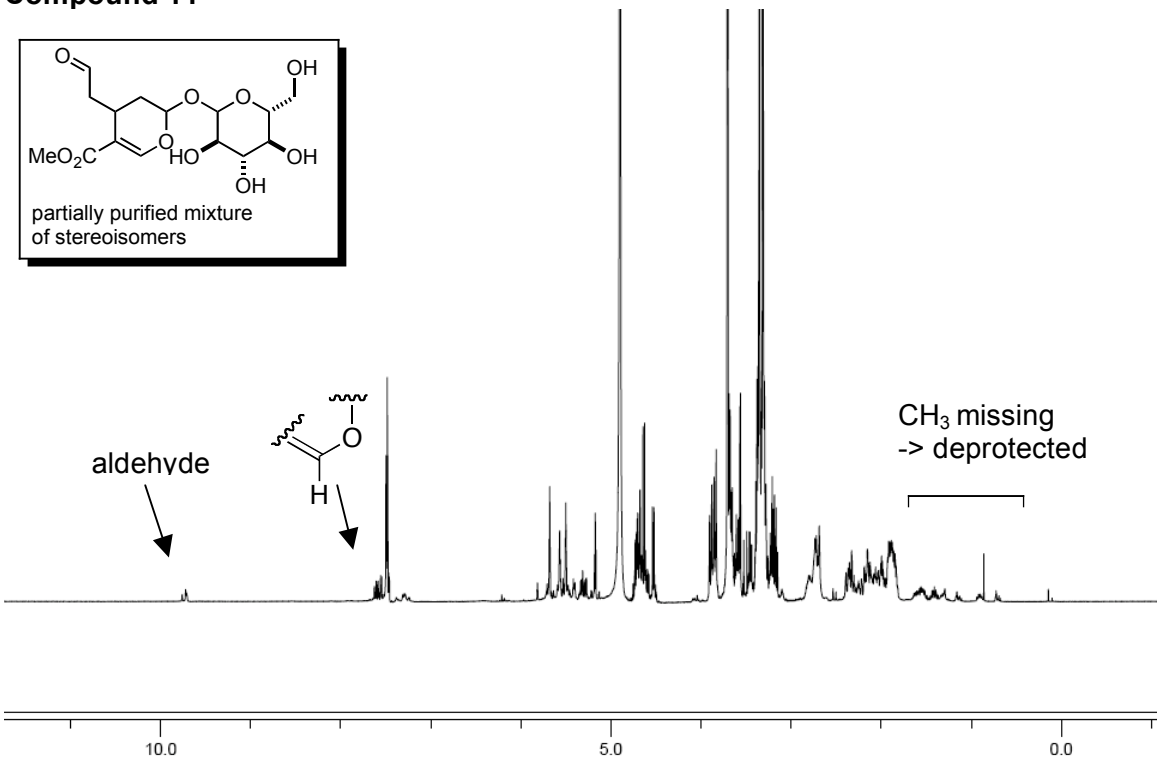
Compound 9



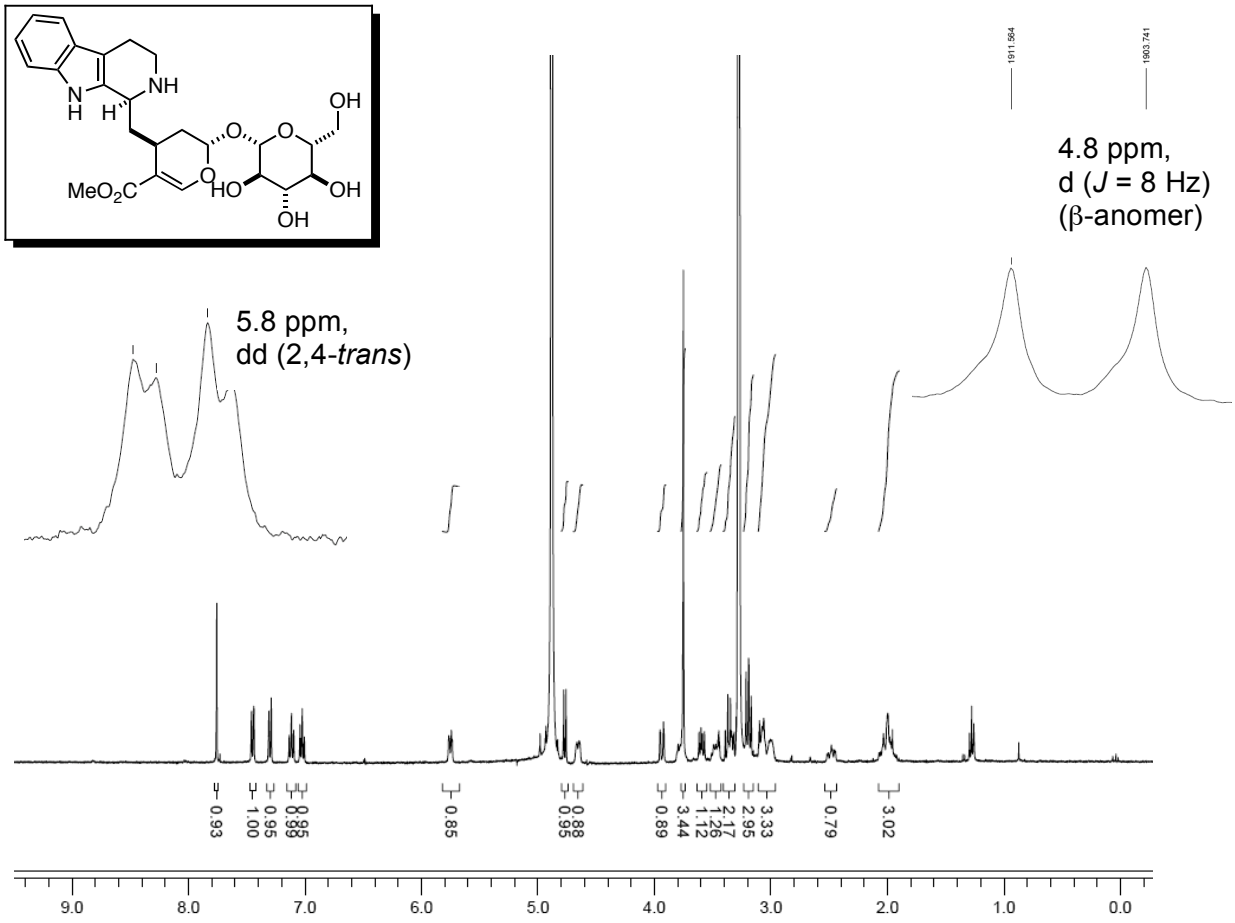
Compound 10



Compound 11

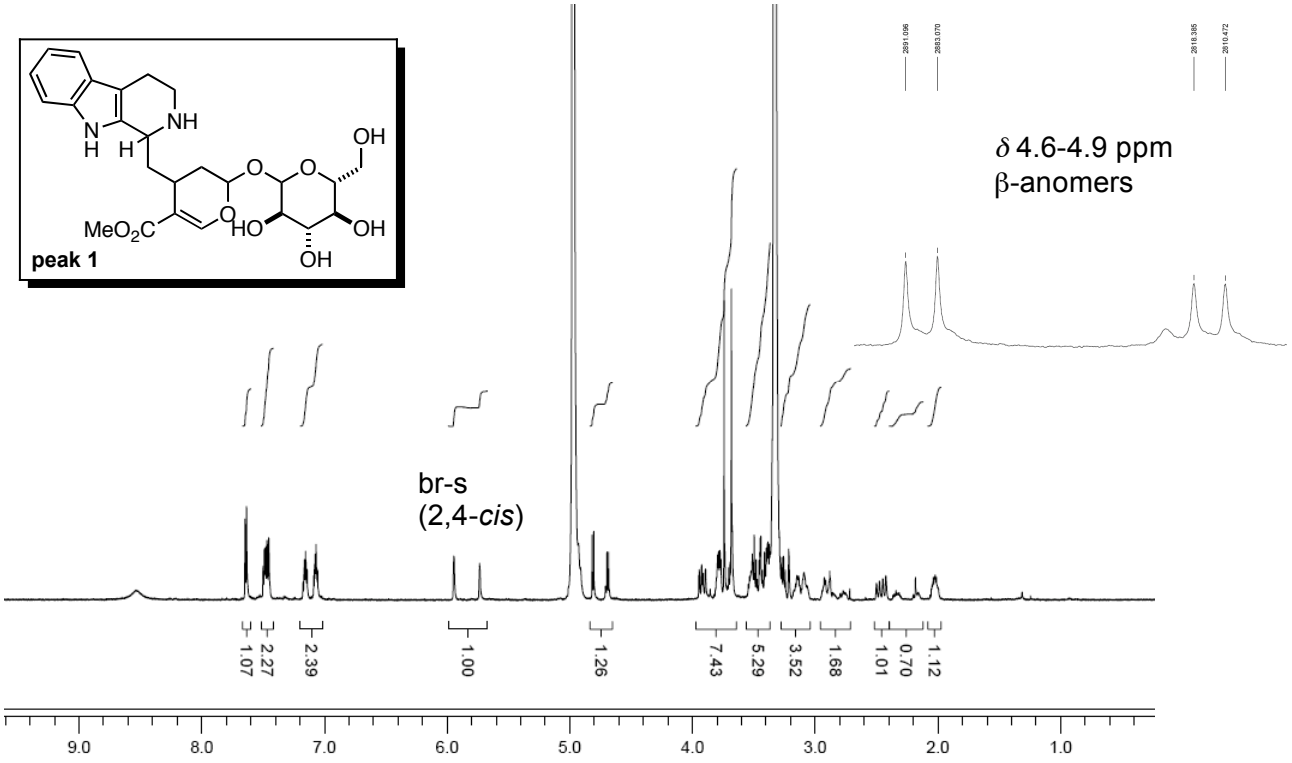


Compound 12a (generated enzymatically from STS, **1** and **11**)

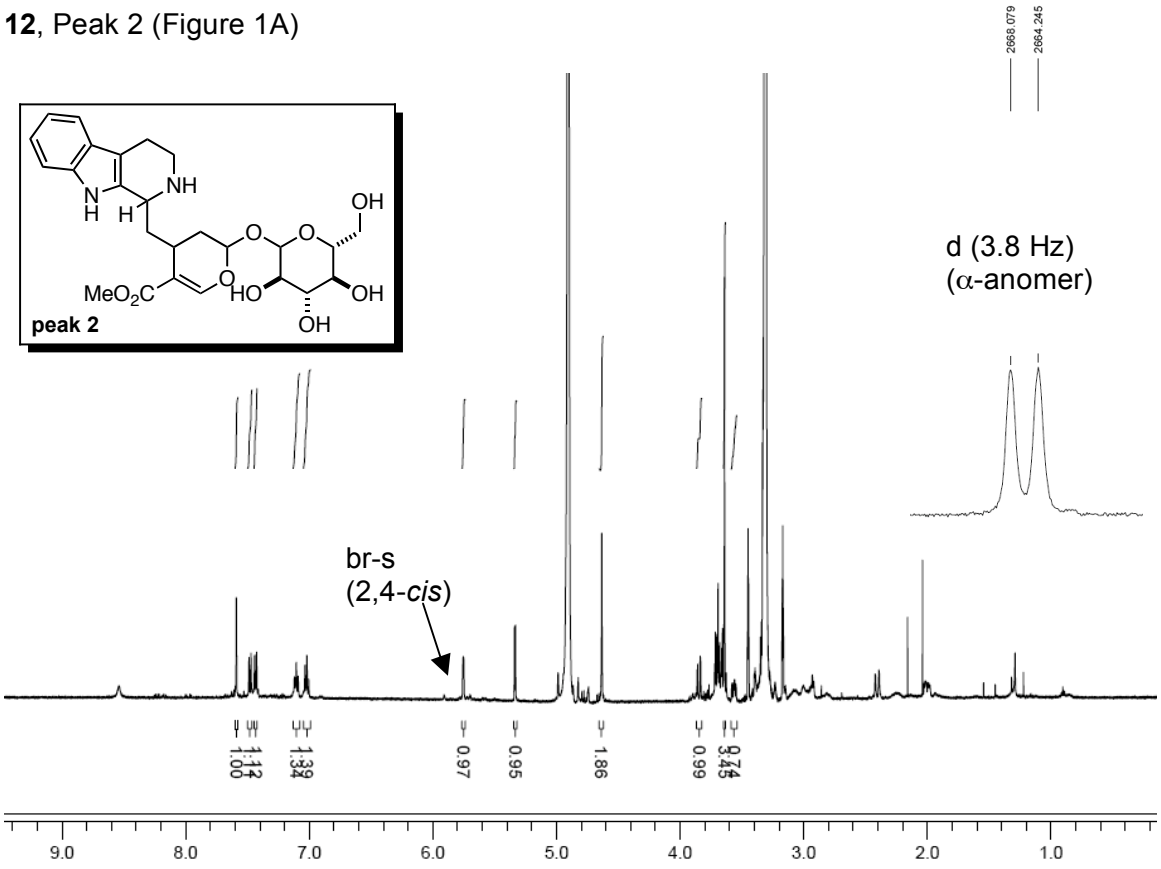


Compound 12 (generated from chemical reaction of **1** and **11**)

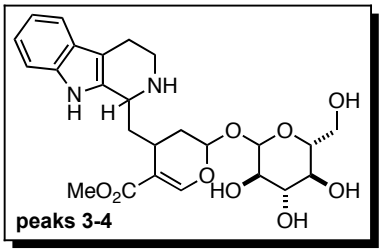
12, Peak 1 (Figure 1A)



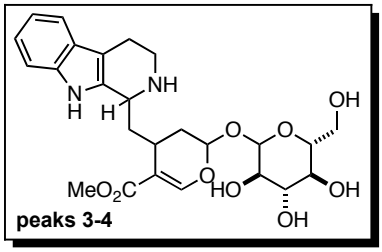
12, Peak 2 (Figure 1A)

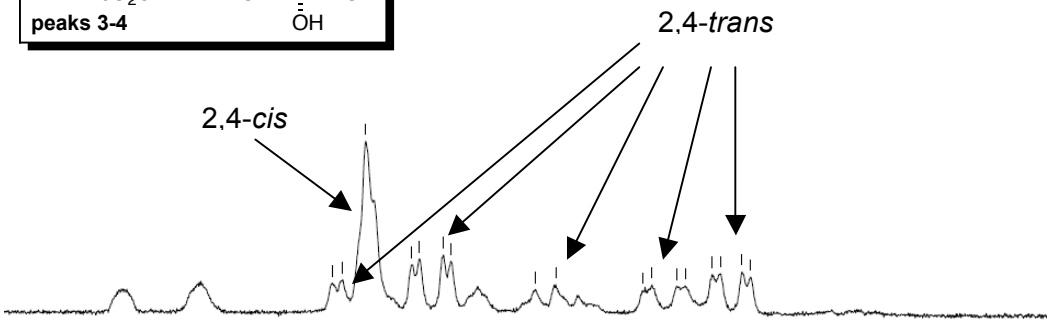


12, Peak 3 and 4 (Figure 1A)

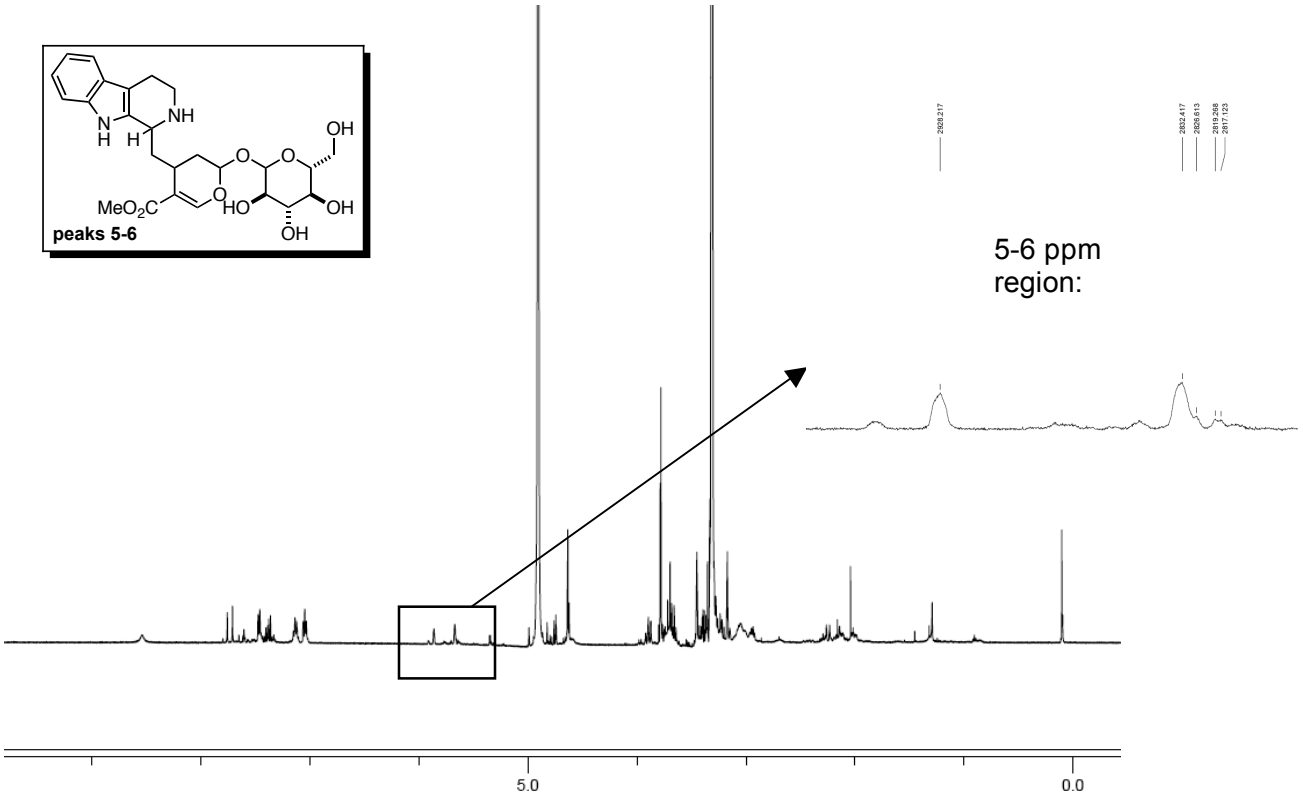


5-6 ppm region:

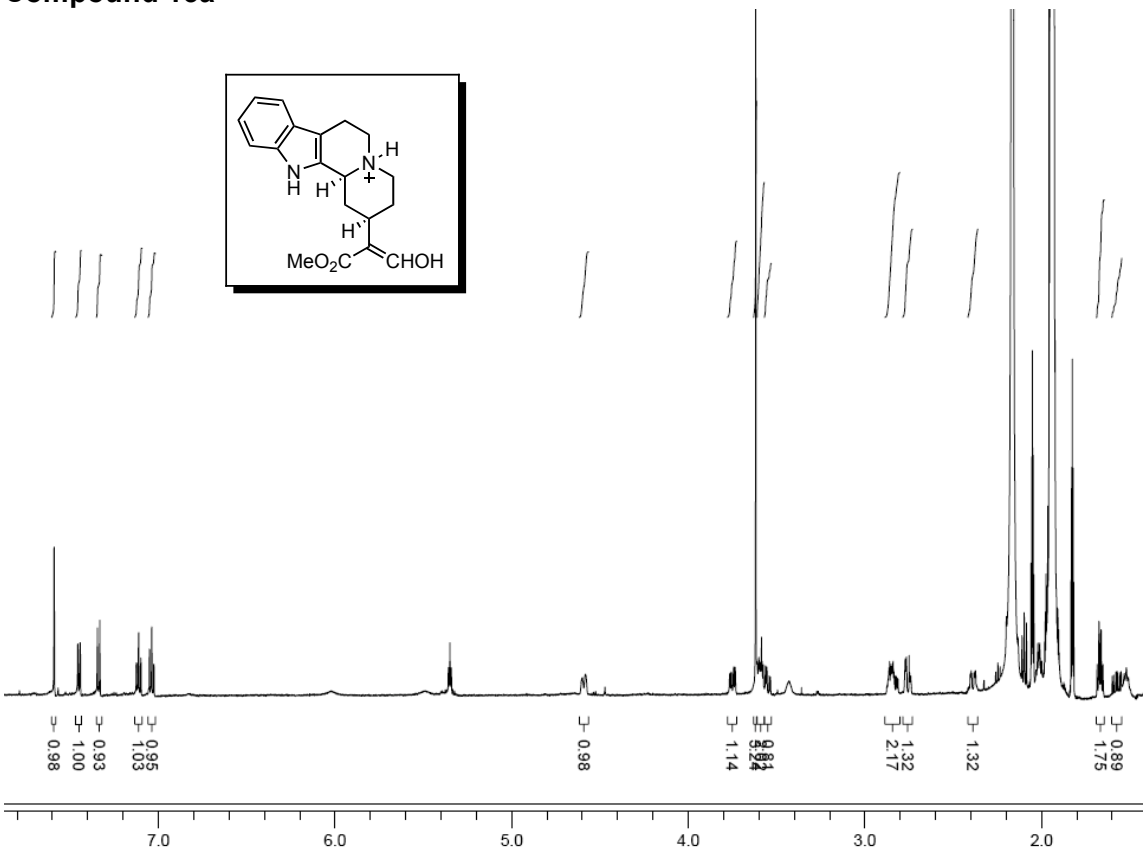




12, Peaks 5 and 6 (Figure 1A)



Compound 13a



2-D ^{1}H-^{1}H COSY of **13a:**