Supporting Information

Combretastatin A4-β-Galactosyl Conjugates for an Ovarian Cancer Prodrug Monotherapy

Tomohiro Doura[*‡], Kazuaki Takahashi[‡], Yasumitsi Ogra, Noriyuki Suzuki[*]

Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan.
E-mail: n-suzuki@chiba-u.jp (N.S.), doura_t@yamaguchi-u.ac.jp (T.D.)

[‡]T.D. and K.T. contributed equally.

Supplemental Figures

Supplemental Figures

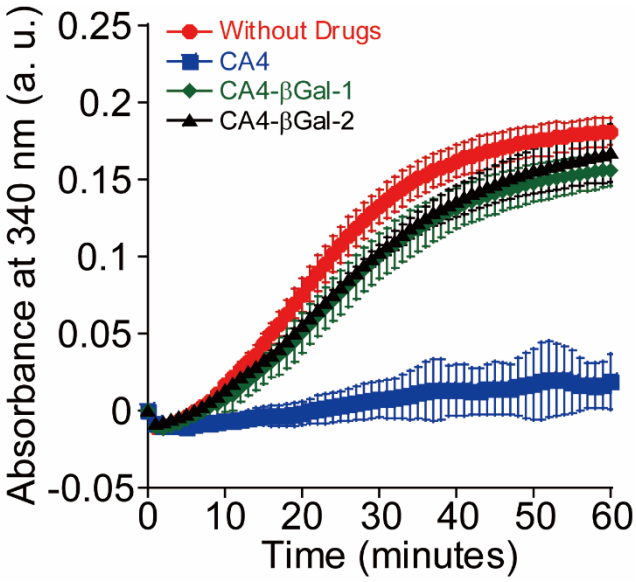


Figure S1. Tubulin polymerization inhibitory activities of CA4-βGals. As a control compound, CA4, a potent tubulin polymerization inhibitor, was used. The absorbance at 340 nm was monitored at 37 °C every 1 minute for 1 hour. Concentrations of test compounds were 5 μM.

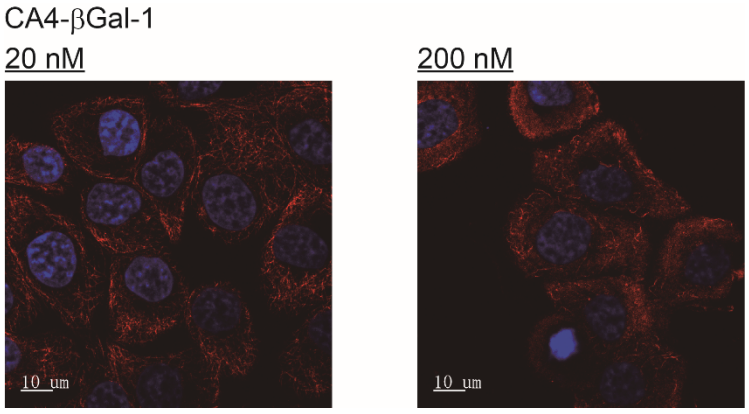
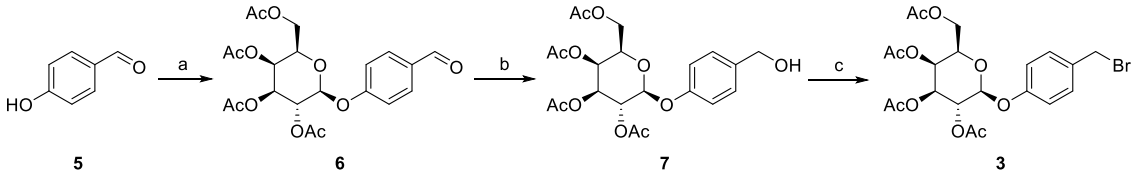
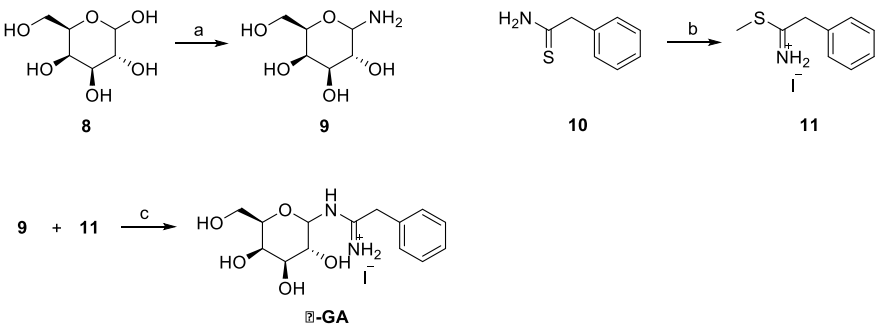


Figure S2. Fluorescence images of an ovarian cancer cell line, OVCAR3 treated with CA4-βGal-1 (left: 20 nM and right: 200 nM). Nucleus was directly stained with DAPI (blue) and α-tubulin was detected with Alexa Fluor 647-(red) conjugated with the secondary antibody. Scale bars, 10 μm.



Scheme S1. Synthetic route to **3**. Reagents and conditions: (a) **1**, Cs_2CO_3, THF/DMF (9:1), RT, 22.5 hours, yield 43%; (b) $LiAlH(OtBu)_3$, THF, 0 °C, 1 hour, yield 96%; (c) PBr_3, Et_2O, 0 °C, 2 hours, yield 91%.



Scheme S2. Synthetic route to β-GA[S1]. Reagents and conditions: (a) NH_3, AcOH, AcOH/NH_3, MeOH/H_2O, RT, 27.5 hours, yield 45%; (b) MeI, acetone, RT, overnight, yield 97%; (c) pyridine, RT, overnight, yield 82%.

Procedures.

CA4, CA4-βGal-1 and CA4-βGal-2 were dissolved in DMSO to obtain 27, 17 and 6.8 mM stock solutions.

In vitro tubulin polymerization assay (Figure S1). Inhibitory activities of CA4, CA4-βGal-1 and CA4-βGal-2 against tubulin polymerization were evaluated using HTS-tubulin polymerization assay biochemistry kit (Cytoskeleton Inc., Denver, CO, USA) according to the instructions. Tubulin solution (4.0 mg/ml) dissolved in G-PEM buffer (80 mM PIPES pH 6.9, 1.0 mM GTP, 2 mM $MgCl_2$, and 0.5 mM EGTA) was used as a working solution. Concentrations of test compounds were 5 μM. Tubulin polymerization dynamics was monitored by measuring the change in absorbance at 340 nm every 1 minute for 60 minutes at 37 °C using an E max Precision Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

HPLC analyses of *in vitro* enzymatic reaction (Figure 2). Stock solutions of CA4, CA4-βGal-1 and CA4-βGal-2 were added to 0.2 M PBS(-) to obtain 500 μL of 0.2 mM solutions. For enzyme reaction, β-galactosidase (0.1 U) was added and the solution was incubated for 1 hour at 37 °C. HPLC analyses of CA4, CA4-βGal-1 and CA4-βGal-2 were performed under an isocratic condition (A: H_2O; B: CH_3CN; A/B = 65/35 for 30 minutes; flow speed: 1 mL per minute). Absorbance at 300 nm was monitored. Identifications of CA4-derived peaks and yields of CA4 were determined based on the peak of CA4 as an authentic sample. All analytical reversed-phase HPLC using a COSMOSIL 4.6 mm x 250 mm column, $5C_{18}$-P-MS Waters (Nacalai) was run on an intelligent HPLC pump system (a degasser DG-1580-53, a HPLC pump PU1580, a column oven CO-1565, and a multi wavelength detector MD-1510) (JASCO).

Cell lines and culture. OVCAR3 and OVK18 were obtained from RIKEN BRC. OVCAR3 was cultured in RPMI 1640 containing 10 % fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. OVK18 was cultured in MEM containing 10 % fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. Reagents were purchased from Wako, Sigma-Aldrich Japan and Biosera. All cell lines were maintained at 37 °C in 5% CO_2.

Cytotoxicity assay (Table 1, Figure 3a). Cells were seeded on 96-well microplates at 6500 cells per well. After 24 hours, cells were treated with the test compounds. In these experiments, <0.5% DMSO was present as cosolvent. After 72 hours of treatment, the solution of water-soluble tetrazolium salt WST-8 (Cell Counting Kit-8, Dojindo) was added to each well, and cells were cultured for 2 hours. The absorbance of WST-8 formazan was determined with at 450 nm with a

precision microplate reader (Molecular Devices). Results are given from 6 experiments performed in triplicates. Graphical representations and statistical analysis of the data were performed using KaleidaGraph (Hulinks). The EC_{50} values were calculated according to the Hill equation:

$$Y = A + (B - A)/(1 + (X/EC_{50})^{\wedge}C)$$

Where Y = observed absorbance, A = minimum absorbance, B = maximum absorbance, C = Hill coefficient, X = concentration of a test compound.

Cytotoxicity assay with β-GA (Figure 3b). Cells were seeded on 96-well microplates at 6000 cells per well. After 24 hours, cells were treated with 200 nM CA4-βGal-1 or 10 nM CA4-βGal-2 and/or 1 mM β-GA as a β-galactosidase inhibitor. In these experiments, <0.005% DMSO was present as cosolvent. After 48 hours of treatment, the solution of water-soluble tetrazolium salt WST-8 was added to each well, and cells were cultured for 2 hours. The absorbance of WST-8 formazan was determined with at 450 nm with a precision microplate reader (Molecular Devices). Results are given from 6 experiments performed in triplicates.

Confocal imaging of fixed cells (Figure 4 and S2). Cells were seeded on an 8-chamber plates (Ibidi, μ-slide) at 6500 cells per plate and cultured for 24 hours. CA4-βGal-1 (20 nM or 200 nM) or CA4-βGal-2 (1 nM or 10 nM) or CA4 (10 nM) was added and cells were cultured for 24 hours. The medium containing a test compound was removed, and ice-cold MeOH was added and cells were incubated for 10 minutes. Following the remove of MeOH, cells were washed with PBS(-) and incubated with PBS(-) containing 0.01% (w/v) NaN_3 and 1% (w/v) BSA at 4 °C for 1 hour. Following the remove of blocking solution, immunostaining was performed using 1 μg/mL mouse monoclonal antibody to α-tubulin (ab80779, Abcam) at 4 °C overnight. Following the remove of solution, cells were washed with PBS(-) and incubated with 2 μg/mL goat anti-mouse polyclonal antibody conjugated with Alexa Fluor 647 (ab150115, Abcam) and 2 μM DAPI at room temperature for 30 minutes. Following 1 wash with PBS(-), cells were covered with 200 μL of PBS(-). Fluorescence images were acquired with a confocal fluorescence microscope system (LSM 780, Zeiss) equipped with an objective lens (Plan-Apochromat 40x/1.3 Oil DIC M27, Zeiss) and a ZEN software (Zeiss). Excitation and emission wavelengths were 633 nm/638-747 nm for Alexa Fluor 647 and 405 nm/410-585 nm for DAPI.

Synthesis and characterization

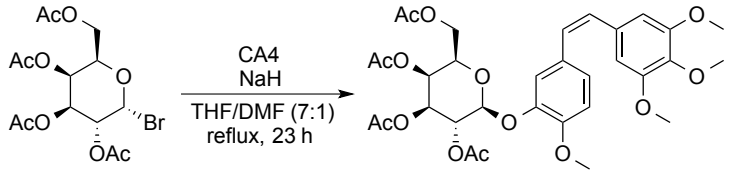
Materials and general information

All chemical reagents and dry solvents for synthesis were purchased from commercial suppliers (Wako Pure Chemical, Tokyo Chemical Industries, Sigma-Aldrich Japan, Nacalai Tesque, Kanto Chemical) and were used without further purification. β-Galactosidase was purchased from Sigma-Aldrich Japan. The composition of mixed solvents is given as volume ratio (v/v). The reaction progress was monitored on a TLC Silica gel 60 F254 (Merck). Preparative thin-layer chromatograpy was performed with a 1 mm PLC Silica gel 60 F254 (Merck). ^{1}H and proton-decoupled ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a JNM-ECZ400 (JEOL, 400 MHz for ^{1}H NMR) or a JNM-ECZ600 (JEOL, 600 MHz for ^{1}H NMR, 151 MHz for ^{13}C NMR). Chemical shifts of ^{1}H NMR spectra were reported in parts per million (ppm) relative to tetramethylsilane (0 ppm) in $CDCl_3$ or the residual protio solvent in dimethylsulfoxide-d_6 (DMSO-d_6, 2.50 ppm). Splitting patterns were designated as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m), broad singlet (brs), broad doublet (brd), broad triplet (brt), and broad multiplet (brm). Coupling constants were reported in hertz (Hz). Chemical shifts of ^{13}C NMR spectra were reported in ppm using the solvent peak as an internal standard ($CDCl_3$, 77.16 ppm and DMSO-d_6, 39.52 ppm). Mass spectra (MS, ESI-TOF) were measured with a MicrOTOF (Bruker) or a JMS-T100LP (JEOL). High-resolution MS (HRMS) was measured using sodium formate as an external standard.

Abbreviations

TLC, thin-layer chromatography; ESI, electrospray ionization; TOF, time of flight; $TMSCH_2N_2$, trimethylsilyldiazomethane; THF, tetrahydrofuran; RT, room temperature; DIPEA, *N*,*N*-diisopropylethylamine; DME, 1,2-dimethoxyethane; TMSBr, bromotrimethylsilane; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide.

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(2-methoxy-5-((*Z*)-3,4,5-trimethoxystyryl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**2**)

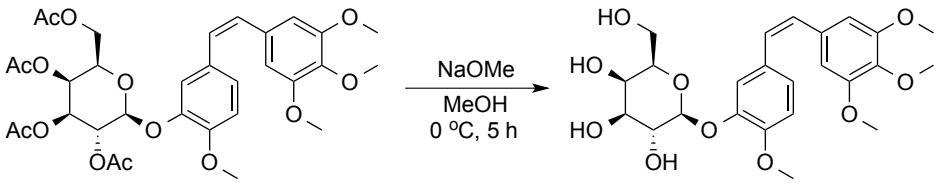


1 **2**

CA4 and compound **1** were synthesized according to reported schemes, respectively[S2,S3]. Sodium hydride (11 mg, 0.458 mmol) was added to THF (0.7 mL) at 0 °C. To the mixture, CA4 (75 mg, 0.238 mmol) dissolved in THF/DMF (7/3, 1 mL) was dropped for 10 minutes. After warming to room temperature, THF (0.7 mL) containing compound **1** (119 mg, 0.290 mmol) was dropped to the mixture for 10 minutes. After stirring at reflux temperature for 23 hours, the mixture was washed with saturated NH_4Cl aqueous solution (3 x) and brine (1 x), and the organic phase was dried with sodium sulfate and evaporated. The residue was purified by flash chromatography (*n*-hexane/EtOAc = 1/1) to obtain compound **2** as a yellow oil (45 mg, yield 40%).

^{1}H NMR (600 MHz, $CDCl_3$): *δ*. 7.09 (d, 1H, *J* = 1.8 Hz), 7.00 (dd, 1H, *J* = 1.8, 8.3 Hz), 6.78 (d, 1H, *J* = 8.3 Hz), 6.50 (s, 2H), 6.47 (d, 1H, *J* = 12.4 Hz), 6.44 (d, 1H, *J* = 12.4 Hz), 5.48 (dd, 2H, *J* = 8.2, 11.0 Hz), 5.41 (d, 1H, *J* = 3.1 Hz), 5.06 (dd, 1H, *J* = 3.1, 11.0 Hz), 4.76 (d, 1H, *J* = 8.2 Hz), 4.08-4.07 (m, 2H), 3.85 (s, 3H), 3.84 (t, 1H, *J* = 6.9 Hz), 3.80 (s, 3H), 3.69 (s, 6H), 2.16 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): *δ*. 170.47, 170.41, 170.25, 169.58, 153.08 (2 x), 149.68, 146.31, 137.36, 132.70, 130.20, 129.50, 129.18, 125.15, 120.04, 112.41, 106.23 (2 x), 101.56, 70.95, 70.86, 68.79, 66.96, 61.27, 61.03, 56.27, 56.09 (2 x), 20.85, 20.81, 20.78, 20.77. HRMS-ESI (*m*/*z*): calculated for $C_{32}H_{38}NaO_{14}$ ($[M + Na]^+$) 669.2154, found 669.2167.

(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(hydroxymethyl)-6-(2-methoxy-5-((*Z*)-3,4,5-trimethoxystyryl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triol (**CA4-βGal-1**)

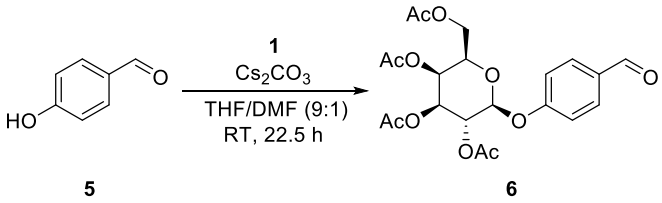


2 **CA4-βGal-1**

Compound **2** (45 mg, 0.0940 mmol) and sodium methoxide (5.0 mg, 0.0926 mmol) were dissolved in MeOH (2 mL) and the solution was stirred at 0 °C for 5 hours. After evaporation of MeOH, the residue was purified by preparative thin-layer chromatography (EtOAc/MeOH = 9/1) to obtain **CA4-βGal-1** as a yellow crystal (18 mg, yield 41%).

^{1}H NMR (600 MHz, DMSO-d_6): δ. 7.01 (brs, 1H), 6.91 (d, 1H, J = 8.2 Hz), 6.88 (d, 1H, J = 8.2 Hz), 6.55 (s, 2H), 6.48 (d, 1H, J = 12.4 Hz), 6.44 (d, 1H, J = 12.4 Hz), 5.17 (brs, 1H, OH), 5.05 (brs, 1H, OH), 4.67 (brs, 2H, OH), 4.57 (brd, 1H, J = 7.6 Hz), 3.74 (s, 3H), 3.66 (brs, 1H), 3.65 (s, 3H), 3.61 (s, 6H), 3.53 (brt, 1H, J = 8.6 Hz), 3.48-3.46 (brm, 1H), 3.29-3.25 (brm, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ. 152.63 (2 x), 148.31, 146.36, 136.69, 132.41, 129.34, 129.28, 128.66, 122.39, 115.79, 112.34, 105.96 (2 x), 100.98, 75.08, 73.56, 70.11, 67.69, 60.02, 59.85, 55.71, 55.65 (2 x). HRMS-ESI (m/z): calculated for $C_{24}H_{30}NaO_{10}$ ([M + Na]$^+$) 501.1731, found 501.1754.

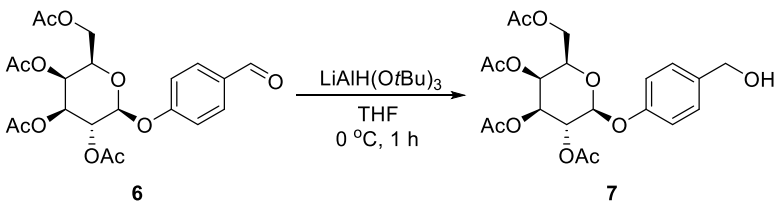
(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(4-formylphenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**6**)



5 **6**

Cesium carbonate (2.70 g, 8.29 mmol) was added to DMF/THF (1/9, 10 mL). To the mixture, DMF/THF (1/9, 7 mL) containing compound **5** (250 mg, 2.04 mmol) was dropped for 10 minutes. In addition, compound **1** (999 mg, 2.44 mmol) dissolved in DMF/THF (1/9, 7 mL) was dropped to the mixture for 10 minutes. After stirring at room temperature for 22.5 hours, the mixture was washed with saturated $NaHCO_3$ aqueous solution (3 x) and brine (1 x), and the organic phase was dried with sodium sulfate and evaporated. The residue was purified by flash chromatography (*n*-hexane/EtOAc = 4/3) to obtain compound **6** as a yellow solid (283 mg, yield 43%).
^{1}H NMR (400 MHz, $CDCl_3$): δ. 9.93 (s, 1H), 7.86 (d, 2H, J = 8.7 Hz), 7.12 (d, 2H, J = 8.7 Hz), 5.52 (dd, 1H, J = 7.8, 10.5 Hz), 5.48 (d, 1H, J = 3.2 Hz), 5.16 (d, 1H, J = 7.8 Hz), 5.14 (dd, 1H, J = 3.2, 10.5 Hz), 4.11-4.26 (m, 3H), 2.20 (s, 3H), 2.08 (s, 6H), 2.03 (s, 3H).

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(4-(hydroxymethyl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**7**)



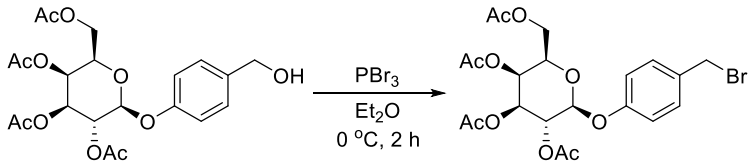
6 **7**

Compound **6** (229 mg, 0.506 mmol) was dissolved in dry THF (5 mL), and 1.0 M $LiAlH(OtBu)_3$ in THF (1.0 mL, 1.0 mmol) was added to the solution at 0 °C. After stirring at 0 °C for 1 hour, saturated NH_4Cl aqueous solution (5 mL) and EtOAc (10 mL) were added to the mixture,

and the mixture was stirred at room temperature for 1 hour. To the mixture, saturated Rochelle salt aqueous solution was added, and crude products were extracted with $CHCl_3$ (3 x). The organic phase was dried with sodium sulfate and evaporated. The residue was purified by flash chromatography (*n*-hexane/EtOAc = 3/4) to obtain compound **7** as a white solid (220 mg, yield 96%).

^{1}H NMR (400 MHz, $CDCl_3$): *δ*. 7.31 (d, 2H, *J* = 8.7 Hz), 7.00 (d, 2H, *J* = 8.7 Hz), 5.50 (dd, 1H, *J* = 7.8, 10.5 Hz), 5.46 (d, 1H, *J* = 3.0 Hz), 5.11 (dd, 1H, *J* = 3.0, 10.5 Hz), 5.04 (d, 1H, *J* = 7.8 Hz), 4.66 (d, 2H, *J* = 5.5 Hz), 4.26-4.14 (m, 2H), 4.06 (t, 1H, *J* = 6.4 Hz), 2.19 (s, 3H), 2.07 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H).

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(4-(bromomethyl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**3**)

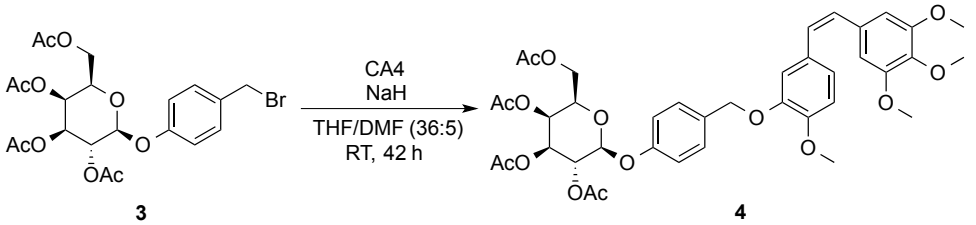


7 **3**

Compound **7** (99 mg, 0.219 mmol) was dissolved in dry Et_2O (2 mL), and phosphorus tribromide (10 μL, 0.105 mmol) was added to the solution at 0 °C. After stirring at 0 °C for 2 hours, the solution was washed with saturated $NaHCO_3$ aqueous solution (3 x) and brine (1 x). The organic phase was dried with sodium sulfate and evaporated to obtain compound **3** as a white oil (102 mg, yield 91%).

^{1}H NMR (400 MHz, $CDCl_3$): *δ*. 7.34 (d, 2H, *J* = 8.7 Hz), 6.97 (d, 2H, *J* = 8.7 Hz), 5.49 (dd, 1H, *J* = 7.7, 10.5 Hz), 5.46 (d, 1H, *J* = 3.5 Hz), 5.11 (dd, 1H, *J* = 3.5, 10.5 Hz), 5.05 (d, 1H, *J* = 7.7 Hz), 4.49 (s, 2H), 4.25-4.14 (m, 2H), 4.07 (t, 1H, *J* = 6.7 Hz), 2.19 (s, 3H), 2.07 (s, 6H), 2.02 (s, 3H).

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(4-((2-methoxy-5-((*Z*)-3,4,5-trimethoxystyryl)phenoxy)methyl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**4**)

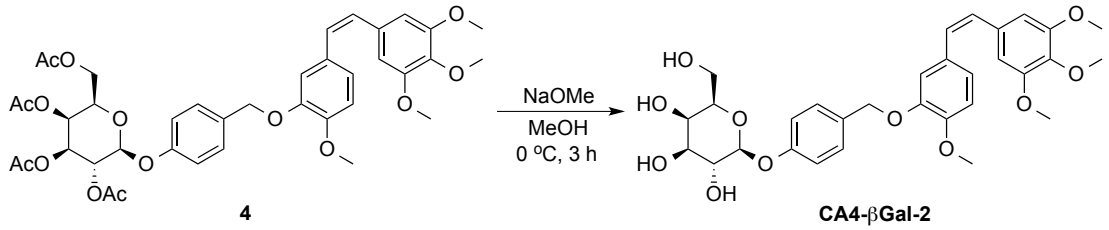


3 **4**

Sodium hydride (5.9 mg, 0.245 mmol) was added to dry THF (1.2 mL), and CA4 (61 mg, 0.192 mmol) in dry THF (1.2 mL) and dry DMF (0.5 mL) was dropped to the mixture at 0 °C for 10 minutes. The mixture was heated to room temperature, and compound **3** (96 mg, 0.185 mmol) in dry THF (1.2 mL) was dropped to the mixture for 10 minutes. After stirring at room temperature for 42

hours, the solution was washed with saturated NH_4Cl aqueous solution (3 x) and brine (1 x). The organic phase was dried with sodium sulfate and evaporated. The residue was purified by flash chromatography (*n*-hexane/EtOAc = 1/1) to obtain compound **4** as a white solid (98 mg, yield 71%). ^{1}H NMR (600 MHz, $CDCl_3$): *δ*. 7.25 (d, 2H, *J* = 8.9 Hz), 6.96 (d, 2H, *J* = 8.9 Hz), 6.88-6.86 (m, 2H), 6.79 (d, 1H, *J* = 9.0 Hz), 6.51 (s, 2H), 6.46 (d, 1H, *J* = 12.1 Hz), 6.43 (d, 1H, *J* = 12.1 Hz), 5.48 (dd, 1H, *J* = 8.2, 10.3 Hz), 5.46 (d, 1H, *J* = 3.4 Hz), 5.13 (dd, 1H, *J* = 3.4, 10.3 Hz), 5.06 (d, 1H, *J* = 8.2 Hz), 4.86 (s, 2H), 4.21-4.18 (m, 2H), 4.09 (t, 1H, *J* = 6.2 Hz), 3.85 (s, 3H), 3.83 (s, 3H), 3.69 (s, 6H), 2.18 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): *δ*. 170.32, 170.24, 170.08, 169.38, 156.59, 152.95 (2 x), 148.87, 147.54, 137.02, 132.96, 131.81, 129.73, 129.60, 128.81, 128.81 (2 x), 122.55, 116.91 (2 x), 114.35, 111.36, 105.90 (2 x), 99.58, 70.97, 70.81, 70.32, 68.64, 66.90, 61.33, 60.85, 55.92, 55.90 (2 x), 20.63, 20.63, 20.57. HRMS-ESI (*m*/*z*): calculated for $C_{39}H_{44}NaO_{15}$ ($[M + Na]^+$) 775.2572, found 775.2598.

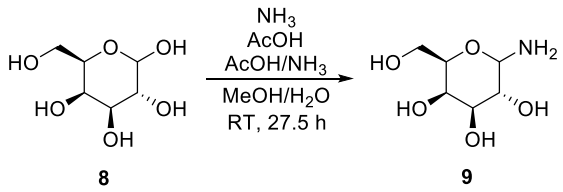
(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(hydroxymethyl)-6-(4-((2-methoxy-5-((*Z*)-3,4,5-trimethoxystyryl)phenoxy)methyl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triol (**CA4-βGal-2**)



4 **CA4-βGal-2**

Compound **4** (20 mg, 0.0309 mmol) was dissolved in MeOH (4 mL), and sodium methoxide (5.0 mg, 0.0926 mmol) was added to the solution at 0 °C. After stirring at 0 °C for 3 hours, the solution was evaporated. The residue was purified by preparative thin-layer chromatography (EtOAc/MeOH = 8/1) to obtain **CA4-βGal-2** as a white crystal (16 mg, yield 89%). ^{1}H NMR (600 MHz, DMSO-d_6): *δ*. 7.22 (d, 2H, *J* = 9.0 Hz), 7.00 (d, 2H, *J* = 9.0 Hz), 6.93 (brs, 1H), 6.90 (d, 1H, *J* = 8.3 Hz), 6.85 (d, 1H, *J* = 8.3 Hz), 6.57 (s, 2H), 6.49 (d, 1H, *J* = 12.4 Hz), 6.46 (d, 1H, *J* = 12.4 Hz), 4.82 (d, 1H, *J* = 5.2 Hz), 4.76 (s, 2H), 3.73 (s, 3H), 3.71 (brs, 1H), 3.63 (s, 6H), 3.62 (s, 3H), 3.57-3.53 (brm, 3H), 3.48-3.46 (brm, 1H), 3.40 (dd, 1H, *J* = 2.8, 9.7 Hz). ^{13}C NMR (151 MHz, DMSO-d_6): *δ*. 162.30, 157.23, 152.68 (2 x), 148.51, 147.32, 136.70, 132.59, 129.93, 129.44, 129.14 (2 x), 128.54, 121.94, 116.10 (2 x), 113.86, 111.85, 105.96 (2 x), 100.90, 75.47, 73.34, 70.29, 69.55, 68.00, 60.25, 60.04, 55.69 (2 x), 55.54. HRMS-ESI (*m*/*z*): calculated for $C_{31}H_{36}NaO_{11}$ ($[M + Na]^+$) 607.2150, found 607.2150.

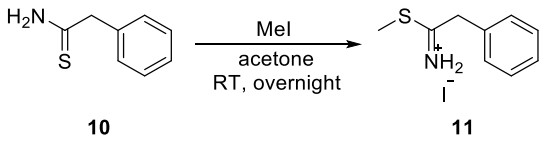
(3*R*,4*S*,5*R*,6*R*)-2-amino-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (galactosylamine, **9**)



Compound **8** (1.01 g, 5.59 mmol), AcOH (63 μL), AcOH/NH_3 (5.0 mg, 0.0649 mmol), H_2O (0.22 mL) were dissolved in MeOH (4.5 mL), and the solution was bubbled with injection of NH_3 gas at room temperature for 24 hours. MeOH (2 mL) was added to the solution, and the solution was bubble with injection of NH_3 gas for 3.5 hours. EtOH (40 mL) was added to the solution, and the solution was incubated at 4 °C overnight. The precipitate was filtered and dried to obtain compound **9** as a white solid (446 mg, yield 45%).

MS-ESI (*m*/*z*): $[M + H]^+$ calculated for 180, found 180.

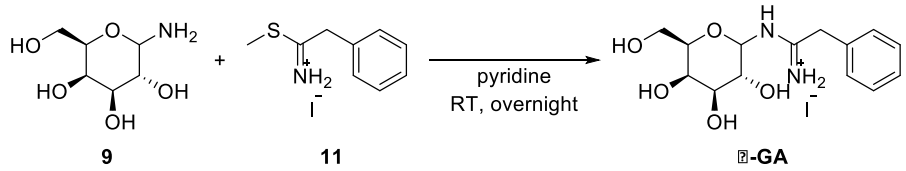
Methyl 2-phenylethanimidothioate iodate (**11**)



Compound **10** (88 mg, 0.580 mmol) was dissolved in acetone (8 mL), and methyl iodide (0.16 mL, 2.57 mmol) was added to the solution at room temperature. After stirring at room temperature for 15 hours, the solution was evaporated and dried to obtain compound **11** as a white solid (165 mg, yield 97%).

^{1}H NMR (400 MHz, $CDCl_3$): *δ*. 7.46-7.37 (m, 5H), 4.41 (s, 2H), 2.88 (s, 3H, S-CH_3).

2-Phenyl-*N*-((3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)acetimidamide iodate (**β-GA**)



Compound **9** (78 mg, 0.433 mmol) and compound **11** (165 mg, 0.564 mmol) were dissolved in dry pyridine (2.5 mL) at room temperature. After stirring at room temperature for 15 hours, diethyl ether (10 mL) was added to the solution and the product was extracted with H_2O. The aqueous solution was lyophilized to obtain **β-GA** as a yellow crystal (151 mg, yield 82%).

MS-ESI (*m*/*z*): $[M + H]^+$ calculated for 297, found 297.

Supporting References:

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