Supporting Information

Deciphering the origins of molecular toxicity for combretastatin A4 and its glycoconjugates: Interactions with major drug transporters and their safety profiles *in vitro* and *in vivo*

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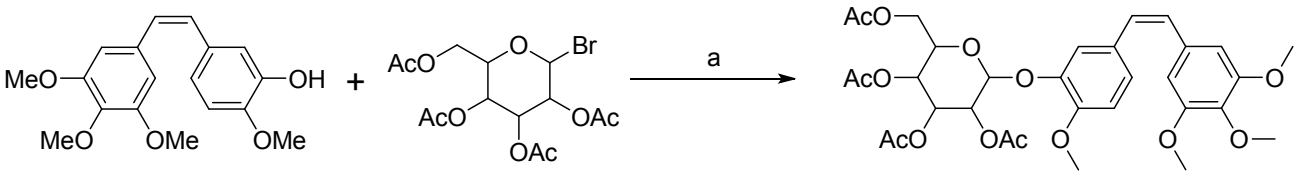
Table of Contents:

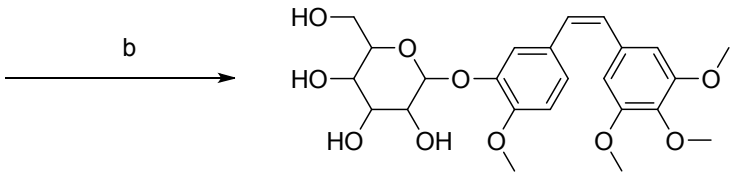
General Procedures

All reagents were purchased from commercial companies and directly used unless stated otherwise. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer at the School of Pharmaceutical Science and Technology of Tianjin University, all chemical shift values are reported as δ ppm. The IR spectra were measured on a Bruker Tensor 27 FT-IR spectrometer. High resolution mass spectra were recorded on a Bruker MicroTOF spectrometer using positive (ESI+) or negative electrospray ionization (ESI-).

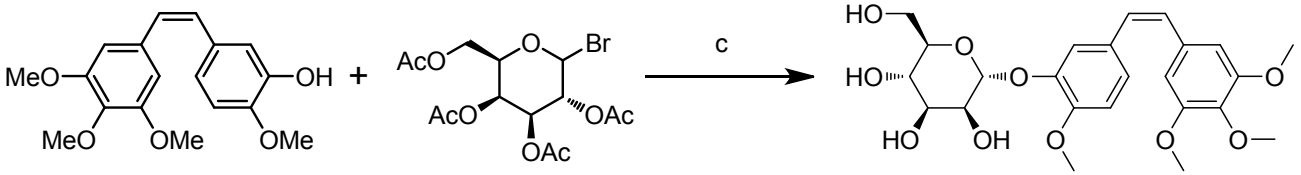


1: 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl bromide
2: 2,3,4,6-tetra-O-acetyl-D-galactopyranosyl bromide

1a
2a



Glu-CA4 **Gala-CA4**



Man-CA4

Reagents and conditions: (a) TBAB, NaOH, $CHCl_3$, H_2O,12 h; b) NaOH, MeOH, 1 h; (c) LiOH, MeOH, 2 h.

Preparation of Glu-CA4: A solution of 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl bromide (37.0 mg, 0.09 mmol) and tetrabutylammonium bromide (10.0 mg, 0.03 mmol) in $CHCl_3$ (5.5 mL) was added dropwise to a solution of CA4 (25.0 mg, 0.08 mmol) and NaOH (4.5 mg, 0.11 mmol) in $CHCl_3$ and H_2O. The two-phase reaction mixture was vigorously stirred at rt for 12h. EtOAc was added, and the

resulting organic phase was successively washed with H_2O and dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (n-hexane/EtOAc = 3:1) to afford **1a** (24.0 mg, 47%). Compound **1a** (26.0 mg, 0.04 mmol) was dissolved in MeOH and H_2O (v/v : 3:1), NaOH (4.8mg, 0.12mmol) was added at rt for 2 h, the solvent was removed in vacuo and the residue was dissolved in DCM (100 mL) and saturated NH_4Cl (aq.) (100 mL). The organic layers were separated and the aqueous layer was extracted with DCM (2 × 70 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash chromatography on silica gel (DCM : MeOH = 10 : 1) afforded **Glu-CA4** (15.2 mg, 79%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 7.04 (s, 1H), 6.90 (s, 2H), 6.57 (s, 2H), 6.49 (d, J = 12.2 Hz, 1 H), 6.44 (d, J = 12.2 Hz, 1 H), 5.23 (d, J = 4.9 Hz, 1H, exchanges with D_2O, OH), 5.07 (d, J = 4.4 Hz, 1H, exchanges with D_2O, OH), 4.96 (d, J = 4.4 Hz, 1H, exchanges with D_2O, OH), 4.57 (d, J = 7.5 Hz, 1H), 4.43 (t, J = 5.7 Hz, 1H, exchanges with D_2O, OH), 3.74 (s, 3H), 3.66 (s, 3H), 3.62 (s, 6H), 3.52 – 3.38 (m, 2H), 3.24 – 3.10 (m, 3H), 2.96 – 2.90 (m, 1H); ^{13}C NMR (100 MHz, DMSO) δ 153.13, 148.73, 146.65, 137.13, 132.98, 129.75, 129.60, 128.95, 123.23, 116.09, 112.67, 106.37, 100.94, 77.25, 77.17, 73.49, 69.55, 60.62, 60.60, 56.17, 56.13. IR (KBr): 3438, 3002, 2938, 2836, 1578, 1514, 1463, 1420, 1324, 1272, 1237, 1123, 1070, 1009, 846, 834 cm^{-1}; HRMS (ESI+): calculated for $C_{24}H_{30}O_{10}$ [M+H$^+$]: 479.1912; found: 479.1918.

Preparation of Man-CA4: CA4 (20.0 mg, 0.06 mmol) and 2,3,4,6-tetra-O-acetyl--D-mannopyranosyl bromide (52.0 mg, 0.12 mmol) were dissolved in MeOH, LiOH (6 mg, 0.25 mmol) was added at rt for 2 h, the solvent was removed in vacuo and the residue was dissolved in DCM (100 mL) and saturated NH_4Cl (aq.) (100 mL). The organic layers were separated and the aqueous layer was extracted with DCM (2 × 70 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash chromatography on silica gel (DCM : MeOH = 10 : 1) afforded **Man-CA4** (6.7 mg, 23.2%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO) δ 7.08 (s, 1H), 6.93 (s, 2H), 6.54 (s, 2H), 6.50 (d, J = 12.2 Hz, 1H), 6.44 (d, J = 12.2 Hz, 1H), 5.15 (d, J = 1.5 Hz, 1H), 4.93 (d, J = 4.4 Hz, 1H, exchanges with D_2O, OH), 4.79 (d, J = 5.2 Hz, 1H, exchanges with D2O, OH), 4.72 (d, J = 5.7 Hz, 1H, exchanges with D_2O, OH), 4.34 (t, J = 5.6 Hz, 1H, exchanges with D_2O, OH), 3.83 – 3.78 (m, 1H), 3.75 (s, 3H), 3.68 – 3.65 (m, 1H), 3.64 (s, 3H), 3.60 (s, 6H), 3.54 – 3.41 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ 153.01, 149.90, 145.64, 137.22, 132.66, 130.01, 129.65, 129.36, 123.69, 119.43, 112.89, 106.48, 100.56, 75.37, 71.10, 70.59, 66.90, 61.21, 60.55, 56.27, 56.06. IR (KBr): 3551, 3476, 3414, 3003, 2931, 2831, 1617, 1583, 1514, 1462, 1403, 1323, 1265, 1232, 1126, 1072, 1011, 870 cm^{-1}; HRMS (ESI+): calculated for $C_{24}H_{30}O_{10}$ [M+H$^+$]: 479.1912; found: 479.1916.

Preparation of Gala-CA4: The synthetic route of **Gala-CA4** is similar to **Glu-CA4** from CA4. The crude intermediate was purified by flash column chromatography (n-hexane/EtOAc = 3:1) to give **2a** (43%). Deprotection of **2a** by NaOH gave **Gala-CA4** (76%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 7.01 (s, 1H), 6.94 – 6.84 (m, 2H), 6.56 (s, 2H), 6.49 (d, J = 12.2 Hz, 1H), 6.43 (d, J = 12.2 Hz, 1H), 4.57 (d, J = 7.7 Hz, 1H), 3.74 (s, 3H), 3.67 – 3.65 (m, 1H), 3.64 (s, 3H), 3.61 (s, 6H), 3.57 – 3.45 (m, 2H), 3.31 – 3.23 (m, 3H); ^{13}C NMR (100 MHz, DMSO) δ 153.08, 148.73, 146.77, 137.08, 132.89, 129.80, 129.71, 129.11, 122.86, 116.18, 112.71, 106.36, 101.41, 75.54, 74.01, 70.54, 68.18, 60.49, 60.33, 56.13, 56.09; IR (KBr): 3460, 3001, 2935, 2838, 2074, 1656, 1581, 1513, 1460, 1407, 1327, 1259, 1231, 1128, 1077, 1013, 868, 802 cm^{-1}; HRMS (ESI+): calculated for $C_{24}H_{30}O_{10}$ [M+H^{+}]: 479.1912; found: 479.1915.

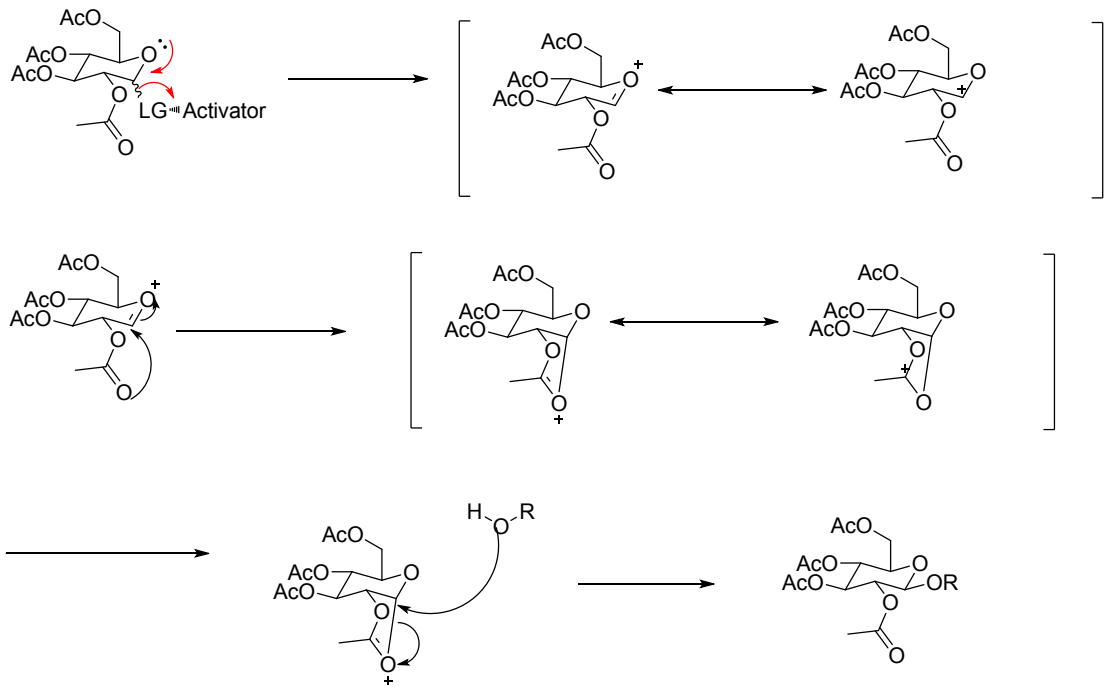


Fig. S1. Neighbouring group participation effect of acetobromo-D-glucose as glycosyl donor to form β-glycosides of CA4 (for Glu-CA4 and Gal-CA4). The similar effect will lead to the formation of α-glycosides of CA4 for acetobromo-D-mannose.

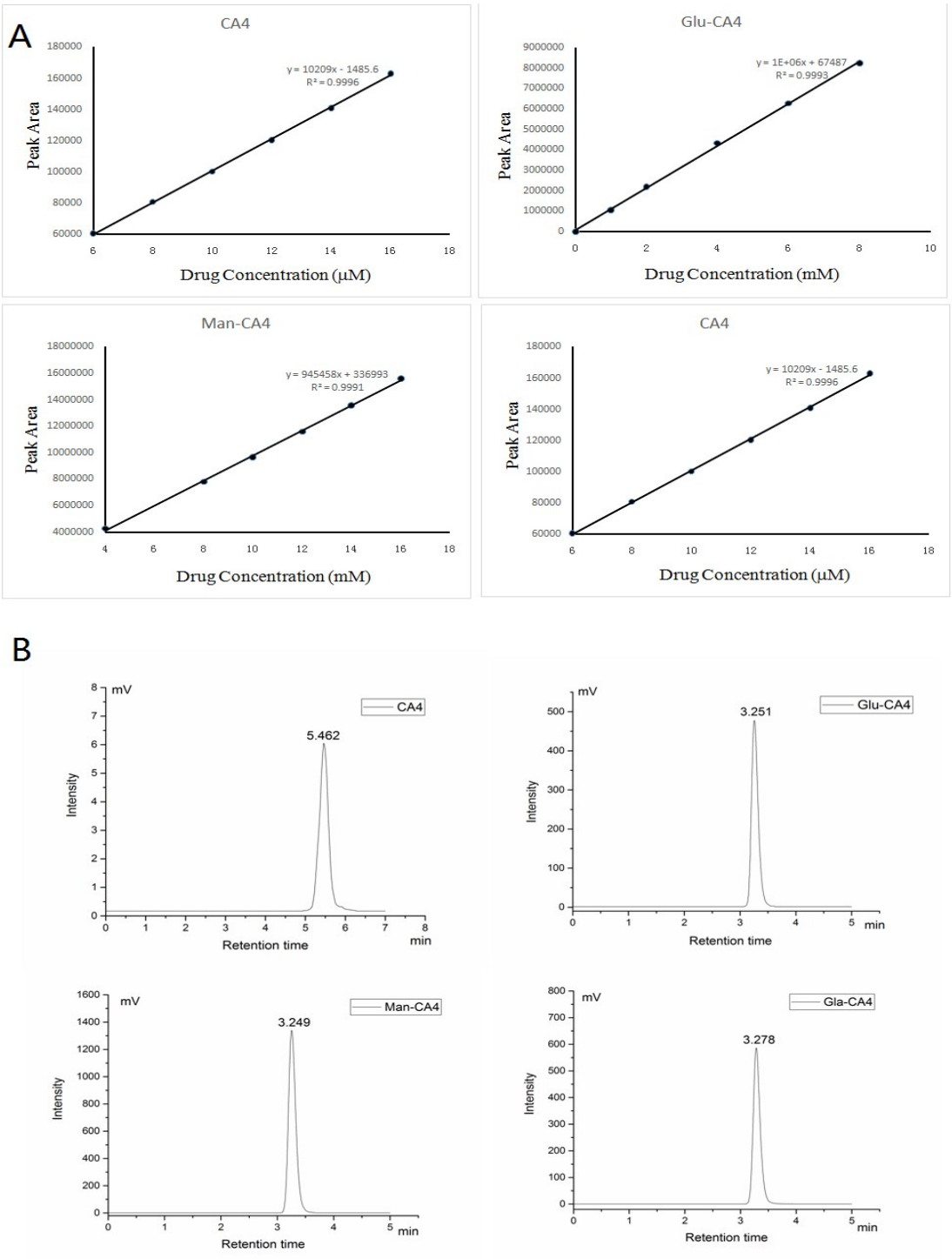


Fig. S2. A) Calibration curve of the analytes. MeOH was used as solvent; B) HPLC charts from the solubility test of the samples in deionized water. Waters HPLC system with UV detector at 294 nm. A reversed phase column (Zorbax SB-C18, 5 µm, 4.6 x 150 mm, Agilent) was used at room temperature for all analyses. The mobile phase consisted of methanol and water (70:30, v/v), and the flow rate was 1.0 mL/min. The injection volume was 20 µL for CA4 and 2 µL for the conjugates. No internal standard was used but all analysis were performed in three replicates. All experiments were performed in dark.

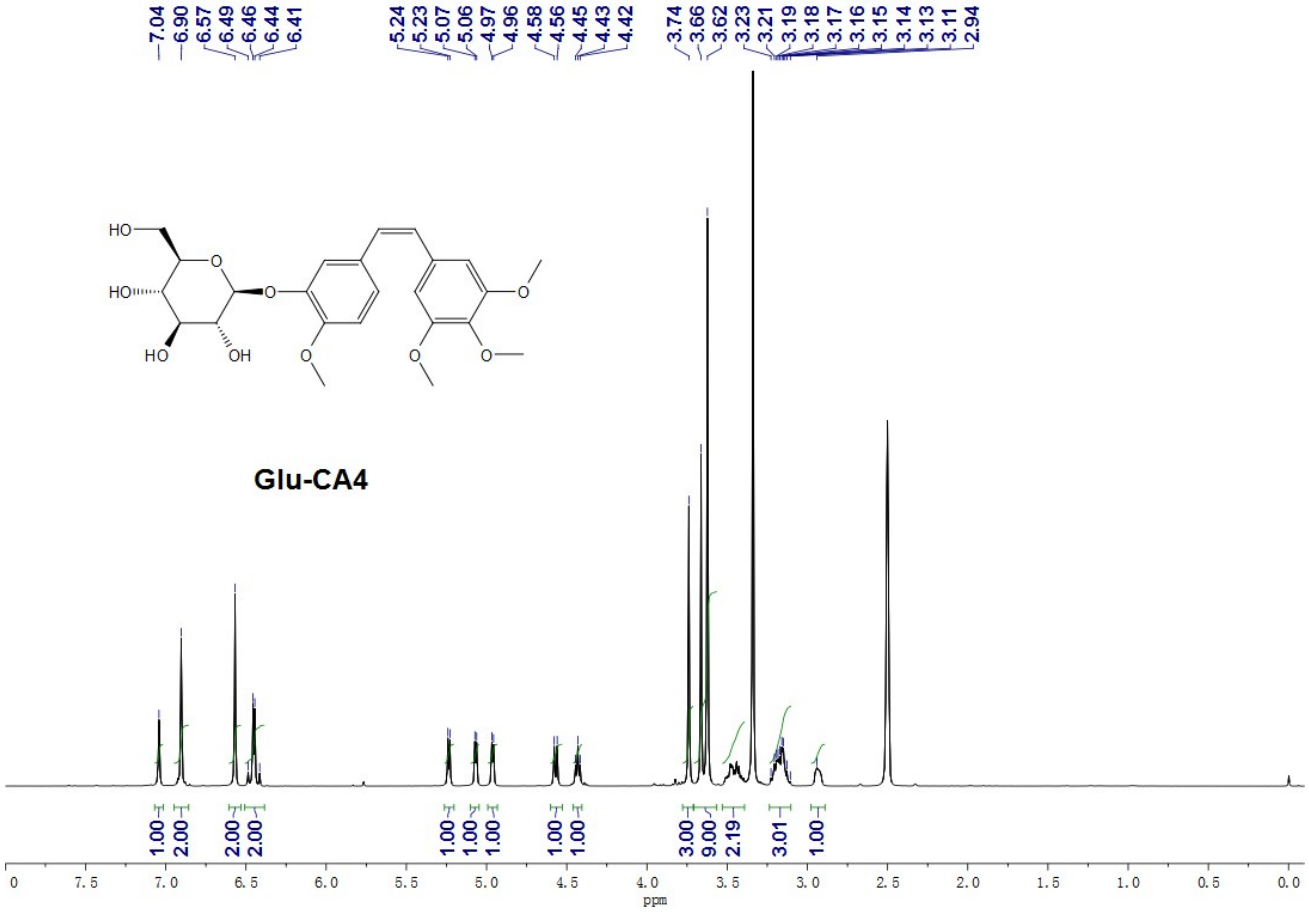


Fig. S3. ^{1}H-NMR of compound **Glu-CA4**.

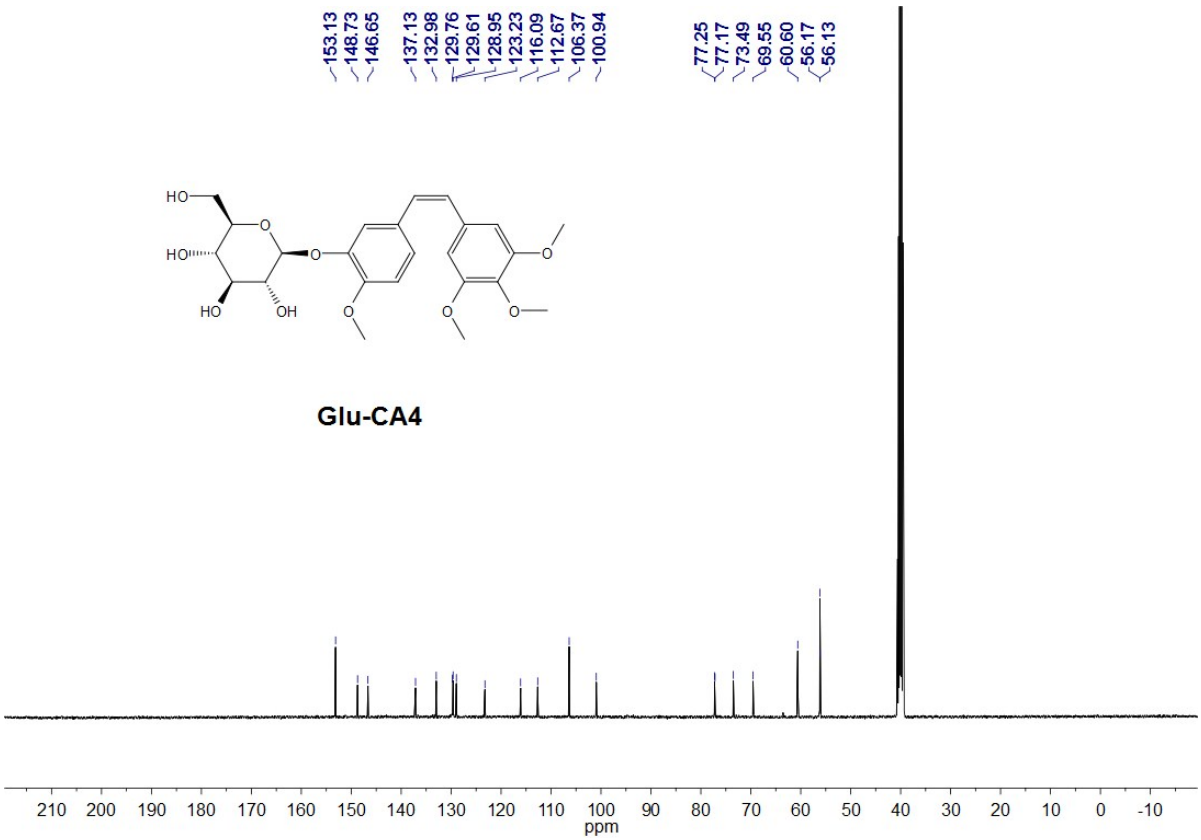


Fig. S4. ^{13}C-NMR of compound **Glu-CA4**.

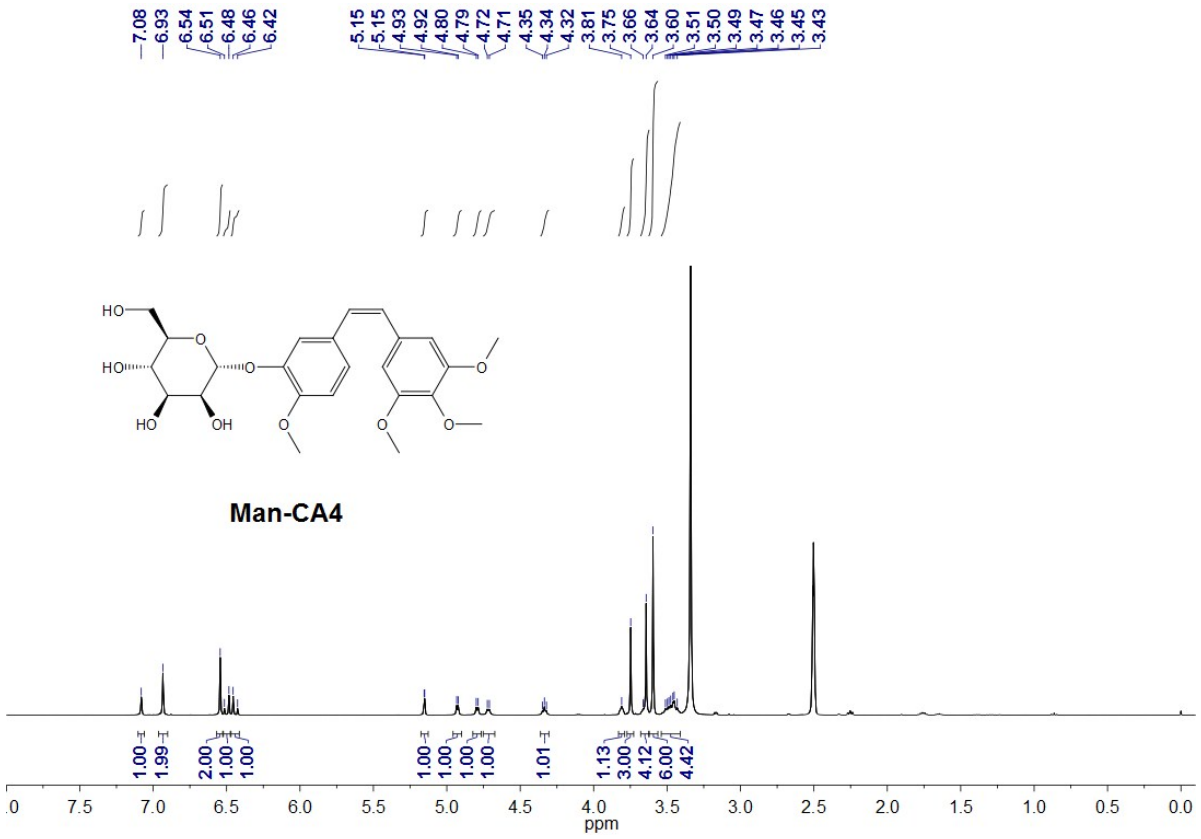


Fig. S5. ^{1}H-NMR of **Man-CA4**.

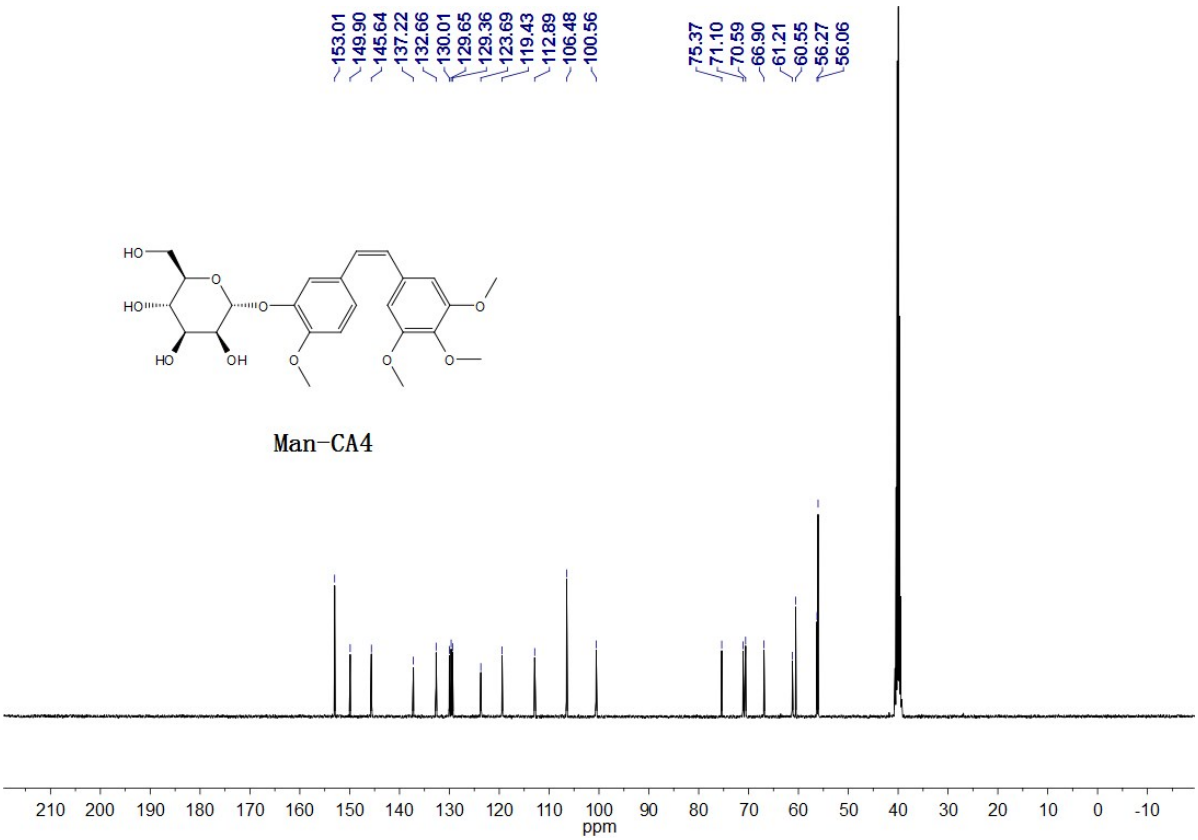


Fig. S6. ^{13}C-NMR of **Man-CA4**.

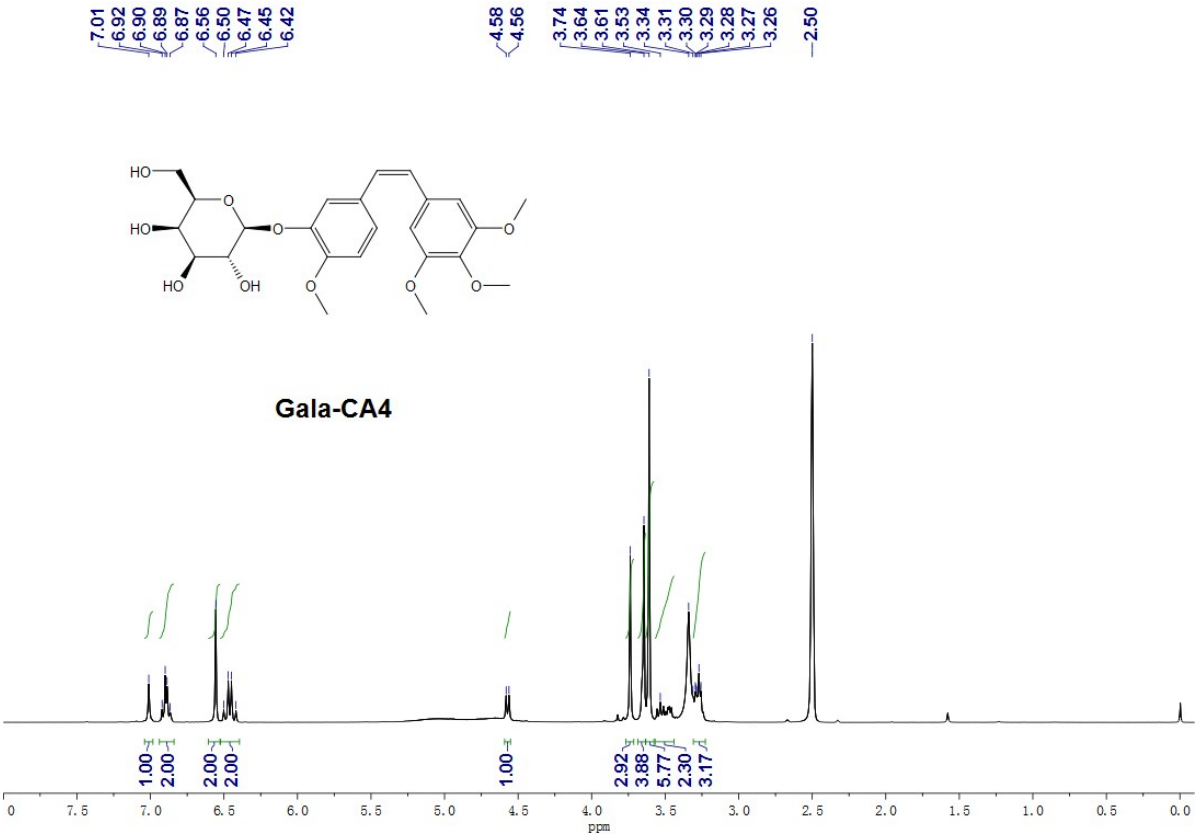


Fig. S7. ^{1}H-NMR of compound **Gala-CA4**.

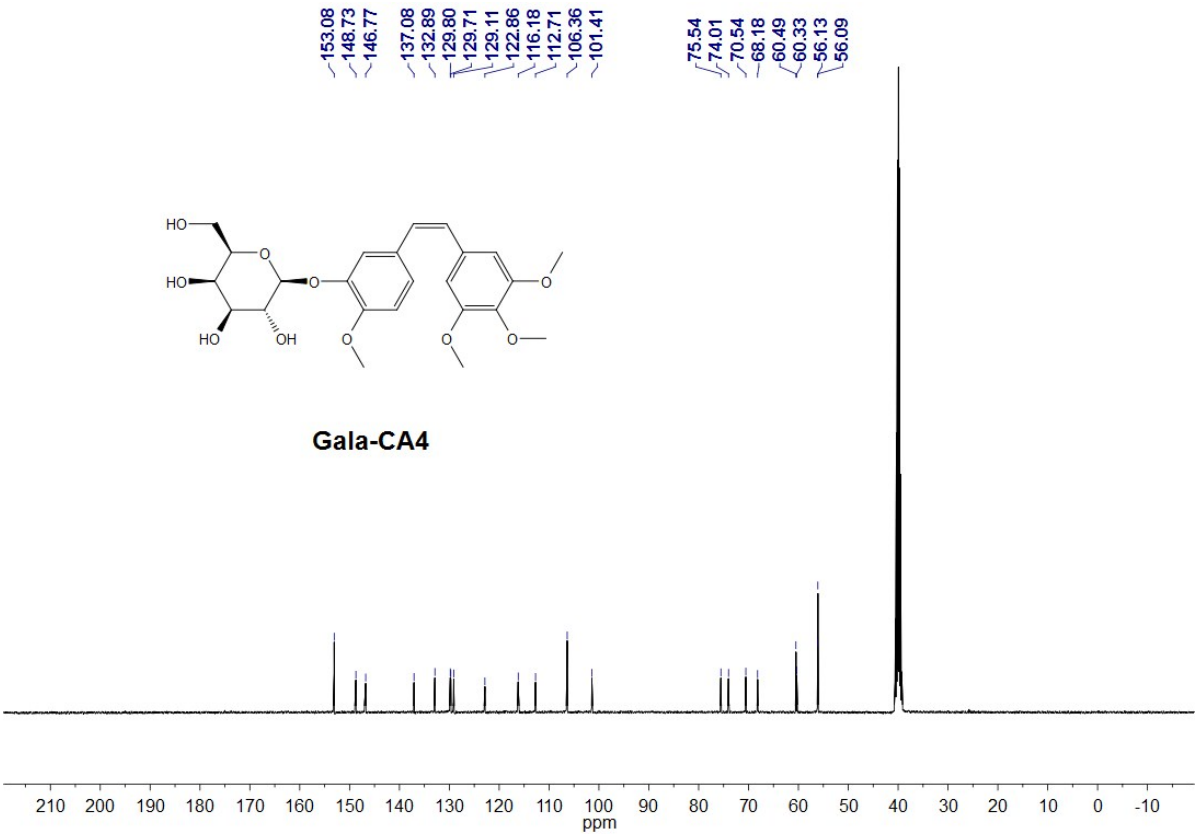


Fig. S8. ^{13}C-NMR of compound **Gala-CA4**