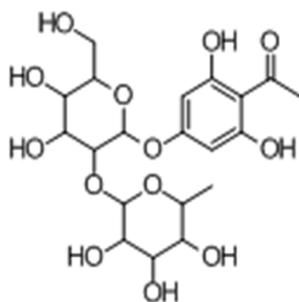


Supplementary Information

NMR Experiments [^1H , ^{13}C , distortionless enhancement by polarization transfer (DEPT), correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC)] were performed using a Bruker AVANCE 300 spectrometer equipped with a 5 mm QNP probe. Chemical shifts are reported in ppm with the solvent resonance as the internal standard (CD_3OD : 3.31 ppm (^1H), 49.00 ppm (^{13}C)). Data are reported as follows for ^1H spectra: chemical shift, integration, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet) and coupling constant (Hz).

Reagents were purchased from Sigma Aldrich and Fisher Scientific. Chromatography was performed on Purasil silica gel (60 Å, 40-63 μm) or Supelco Diaion HP-20 resin (260 Å, 250-850 μm). Thin-layer chromatography (TLC) was performed on precoated 0.25 mm silica gel 60 F₂₅₄ TLC plates.

Experimental Procedures



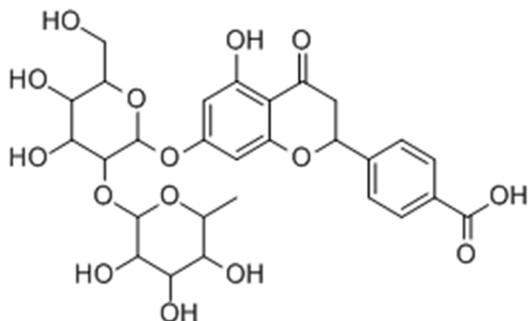
Phloracetophenone 4'-neohesperidoside, **A**¹

To a three necked flask containing a magnetic stirbar and fitted with a condenser was added naringin (50 g, 0.0861 mol) and 400 mL 20% KOH in distilled H_2O . The mixture was allowed to stir at 25 °C for 90 min. The temperature was raised to reflux and stirred for an additional 90 min. The reaction was allowed to cool to room temperature then 200 g of crushed ice was added. The pH was adjusted to 6 with 12 N HCl and the resulting precipitate was collected by vacuum filtration. The crude product was recrystallized from water to yield a yellow powder (22.7 g, 55% yield).

^1H NMR (300 MHz, CD_3OD): δ 6.05 (2H, s), 5.26-5.25 (1H, d, $J = 2.0$ Hz), 5.04-5.01 (1H, d, $J = 7.5$ Hz), 3.94-3.87 (3H, m), 3.73-3.55 (4H, m), 3.47-3.35 (3H, m), 2.62 (3H, s), 1.32-1.30 (3H, d, $J = 6.0$ Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 205.25,

¹ Krbechek et al. (1968) *J. Agr. Food Chem.*, 16(1):108-112

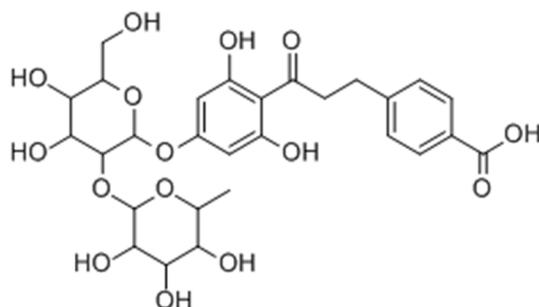
165.50, 164.83, 107.14, 102.47, 99.38, 96.14, 79.07, 78.89, 78.15, 73.97, 72.22, 72.16, 71.26, 69.94, 62.32, 32.98, 18.20.



4-((7-neohesperidoside)-3-oxy-5-hydroxy-4-oxochroman-2-yl)benzoic acid, **B**

To a three necked flask containing a magnetic stirbar and fitted with a condenser, phloracetophenone 4'-neohesperidoside (10.0 g, 0.021 mol) and 4-carboxybenzaldehyde (3.15 g, 0.021 mol) was added to 100 mL of absolute EtOH. The mixture was stirred to homogeneity then pyrrolidine (5.26 mL, 0.063 mol) and acetic acid (0.88 mL) were added. The mixture was stirred at reflux until the reaction was complete by TLC analysis (CHCl₃/MeOH, 70/30 (v/v), 2 - 4 h). Silica gel (5 g) was added to the warm solution and mixture was concentrated by rotary evaporation for purification by flash chromatography (0-50% MeOH in CH₂Cl₂). Fractions were identified by TLC and dried via rotary evaporation to afford orange crystals (7.93 g, 71% yield).

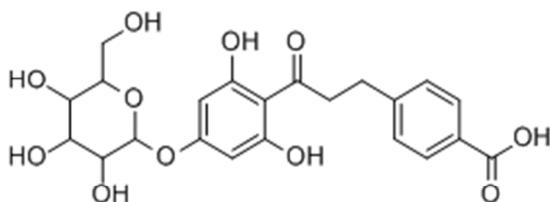
¹H NMR (300 MHz, CD₃OD): δ 7.99-7.97 (2H, d, *J* = 8.0 Hz), 7.51-7.48 (2H, d, *J* = 8.0 Hz), 6.23-6.22 (1H, d, *J* = 2.0 Hz), 6.17-6.16 (1H, d, *J* = 2.0 Hz), 5.54-5.53 (1H, d, *J* = 2.5 Hz), 5.26-5.25 (1H, dd, *J* = 5.0 Hz, *J* = 1.6 Hz), 5.13-5.09 (1H, t, *J* = 12.5 Hz), 3.94-3.85 (4H, m), 3.64-3.57 (4H, m), 3.49-3.38 (3H, m), 3.15-3.10 (2H, m), 1.93-1.88 (3H, m); ¹³C NMR (75 MHz, CD₃OD): δ 197.99, 174.86, 166.63, 166.56, 165.00, 164.38, 142.15, 142.08, 139.32, 130.59, 129.65, 126.81, 126.78, 104.96, 102.55, 102.47, 99.40, 99.34, 98.01, 96.82, 80.34, 80.27, 79.16, 78.94, 78.12, 73.90, 72.18, 72.15, 71.22, 69.99, 62.23, 25.12, 18.22.



4-(3-oxo-3-(2,6-dihydroxy-4-neohesperidoside phenyl)propyl)benzoic acid, **C**

To a round bottom flask containing a stirbar, 4-((7-neohesperidoside)-3-oxy-5-hydroxy-4-oxochroman-2-yl)benzoic acid (7.0 g, 0.013 mol) and 1 N NaOH (250 mL) was added and mixed to homogeneity. 10% Pd/C (2.0 g, 30 mol%) was added to the reaction. The air was immediately removed *in vacuo* and the vacuum released with H₂. The reaction was stirred at room temperature under H₂ for 48 h or until complete by TLC analysis (BuOH/PrOH/H₂O, 10/5/4 (v/v)), replacing the Pd/C every 24 h. The palladium catalyst was removed from the reaction mixture via gravity filtration and the reaction mixture was acidified to pH = 5.5. The solvent level was reduced by half via rotary evaporation. The remaining solution was left to crystallize at 4 °C overnight. The crude product was recrystallized from water to provide light brown crystals (4.40 g, 55.4% yield).

¹H NMR (300 MHz, CD₃OD): δ 7.95-7.92 (2H, d, *J* = 8.0 Hz), 7.36-7.33 (2H, d, *J* = 8.0 Hz), 6.06 (2H, s), 5.27-5.26 (1H, d, *J* = 1.5 Hz), 5.05-5.02 (1H, d, *J* = 7.0 Hz), 3.98-3.88 (3H, m), 3.74-3.57 (4H, m), 3.48-3.38 (4H, m), 3.32-3.30 (2H, q, *J* = 1.5 Hz), 3.05-3.00 (2H, t, *J* = 7.5 Hz), 1.33-1.30 (3H, d, *J* = 6.0 Hz); ¹³C NMR (75 MHz, CD₃OD): δ 206.09, 169.97, 165.31, 164.65, 148.88, 130.88, 129.56, 129.51, 106.80, 102.41, 99.34, 96.29, 79.01, 78.84, 78.09, 73.97, 72.18, 72.14, 71.25, 69.90, 62.32, 46.40, 31.73, 18.20.



Hapten - 4-(3-oxo-3-(2,6-dihydroxy-4-glucoside phenyl) propyl)benzoic acid

A jacketed flask equipped with a magnetic stirbar was equilibrated to 65 °C using a temperature controlled water circulator. To the flask was added a buffer solution (50 mL, 0.1 M KH_2PO_4 , 0.2 M Na_2HPO_4 , pH = 6.6) and naringinase (1.5 g). The reaction was stirred for 2 h at 65 °C. 4-(3-oxo-3-(2,6-dihydroxy-4-neohesperidoside phenyl)propyl)benzoic acid (1.2 g, 0.002 mol) was dissolved in 10 mL of distilled H_2O and added to the stirred reaction. The reaction was cooled to 45 °C with stirring for 24 h or until complete by TLC analysis (BuOH/PrOH/ H_2O , 10/5/4 (v/v)). The reaction was heated to 90 °C for 15 min to deactivate the enzyme. The enzyme was removed from the reaction solution by vacuum filtration. Purification was performed with HP-20 resin (0-60% MeOH in H_2O) followed by HPLC (5-50% MeOH with 0.01% Formic Acid in H_2O). Fractions were identified by NMR analysis and lyophilized to provide a light yellow powder (0.092 g, 9.9% yield). NMR analysis provided an estimated 90% pure product which was deemed acceptable for use in this assay.

^1H NMR (300 MHz, CD_3OD): δ 7.86-7.84 (2H, d, J = 8.0 Hz), 7.26-7.23 (2H, d, J = 8.0 Hz), 5.96 (2H, s), 4.94-4.91 (1H, d, J = 7.0 Hz), 3.91 (1H, d, J = 11.0 Hz), 3.76-3.73 (1H, m), 3.44-3.40 (4H, m), 3.32-3.30 (2H, q, J = 7.0 Hz, J = 1.5 Hz), 3.00-2.96 (2H, t, J = 15.0 Hz, J = 7.5 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 206.22, 165.71, 164.98, 148.39, 148.23, 130.81, 129.42, 106.82, 105.24, 101.12, 96.44, 78.23, 77.86, 74.60, 71.14, 64.36, 62.34, 46.49, 31.76.

Figure S1. ^1H NMR spectrum of phloracetophenone 4'-neohesperidoside, **A**

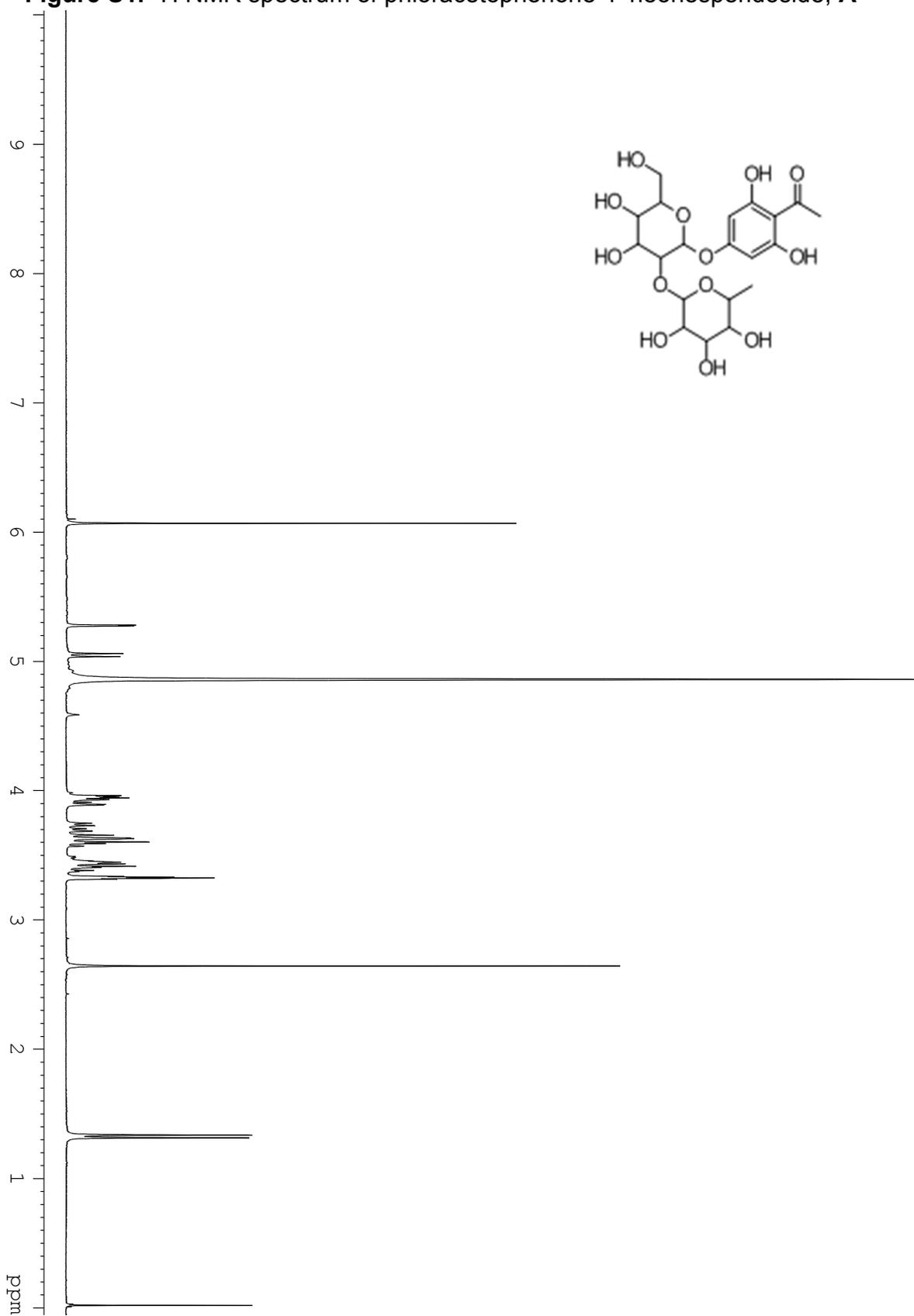


Figure S2a. ^1H NMR spectrum of 4-((7-neohesperidoside)-3-oxy-5-hydroxy-4-oxochroman-2-yl)benzoic acid, **B**

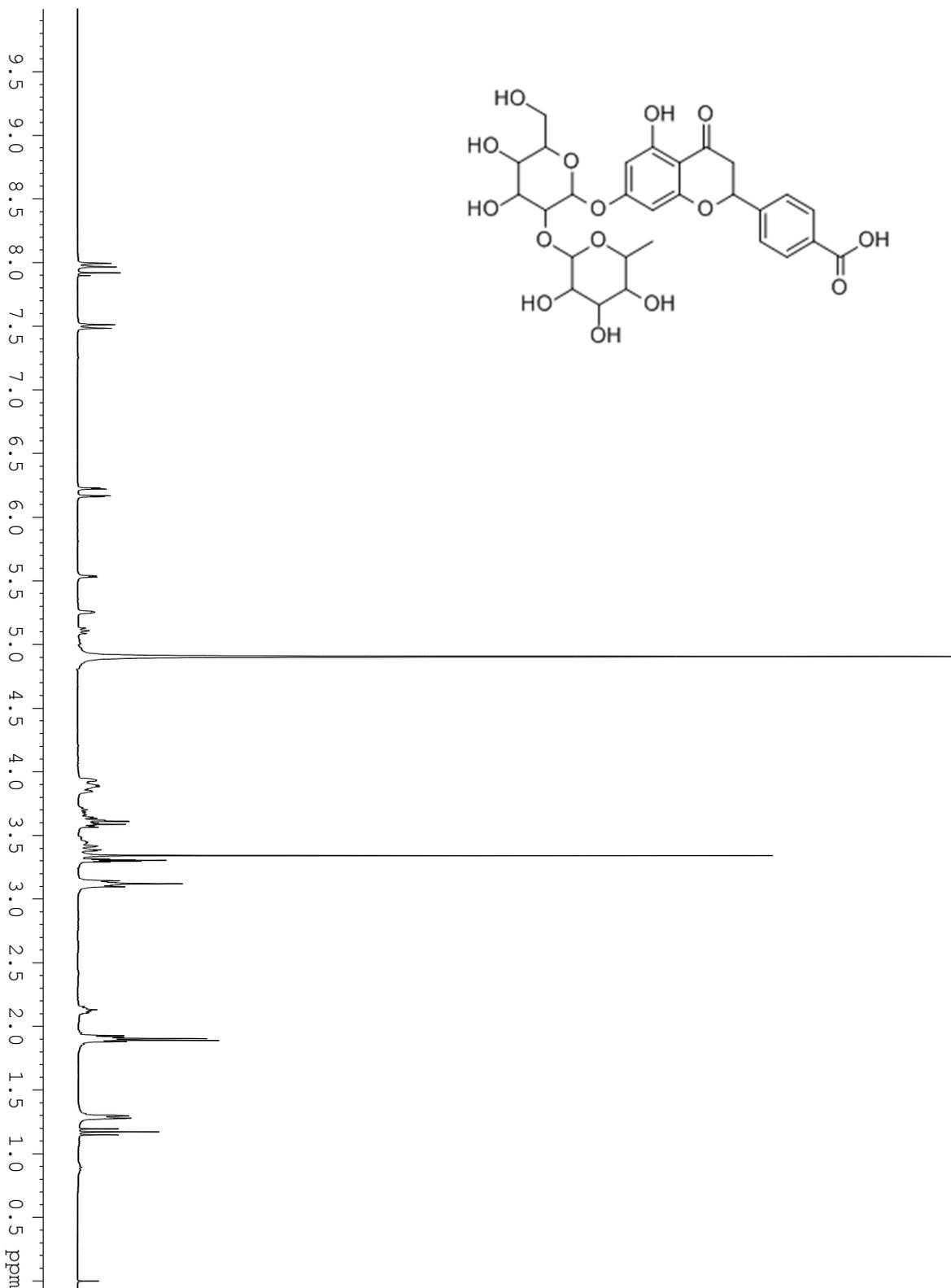


Figure S2b. ^{13}C NMR spectrum of 4-((7-neohesperidoside)-3-oxy-5-hydroxy-4-oxochroman-2-yl)benzoic acid, **B**

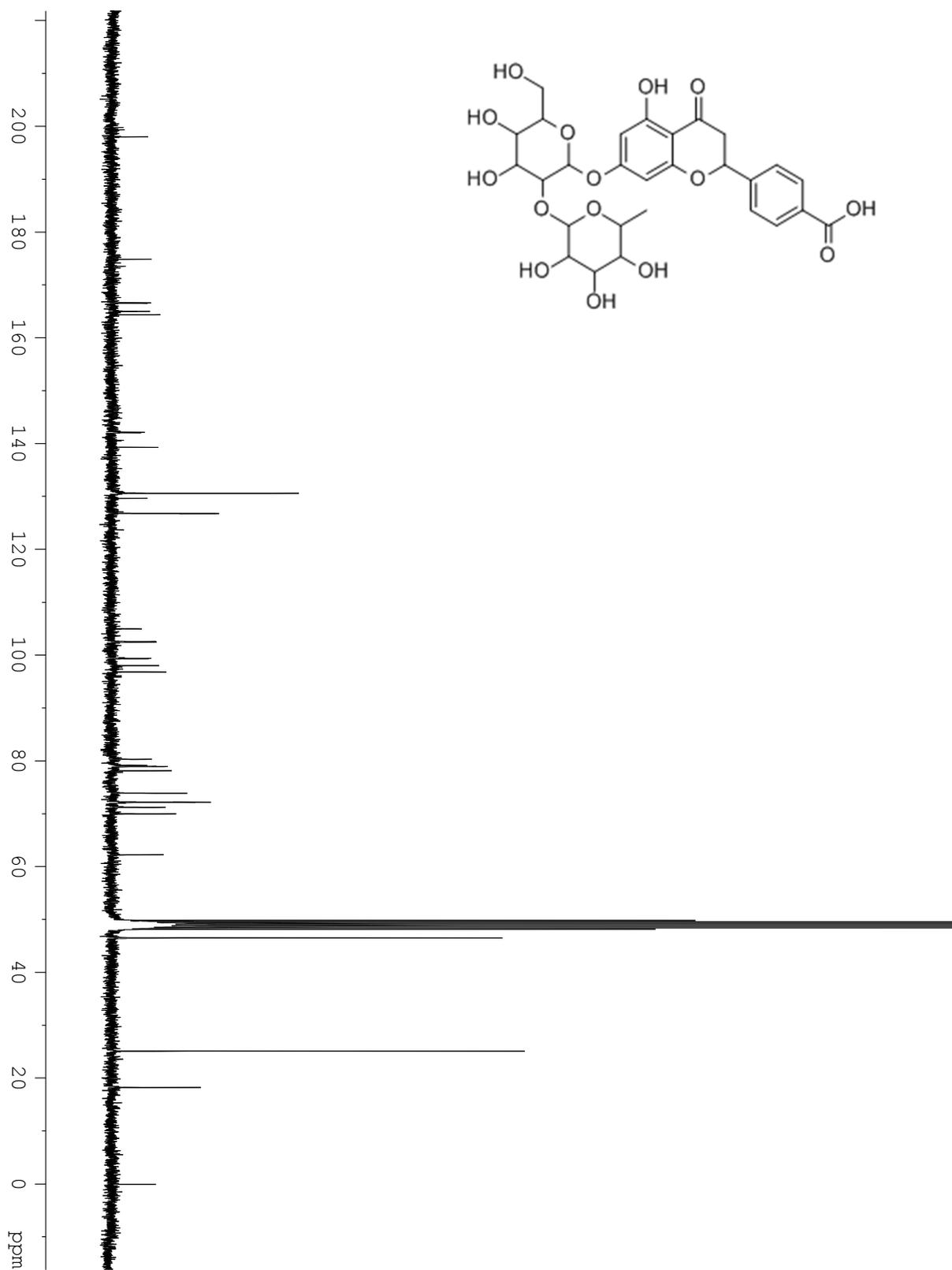


Figure S3a. ^1H NMR spectrum of 4-(3-oxo-3-(2,6-dihydroxy-4-neohesperidoside phenyl)propyl)benzoic acid, **C**

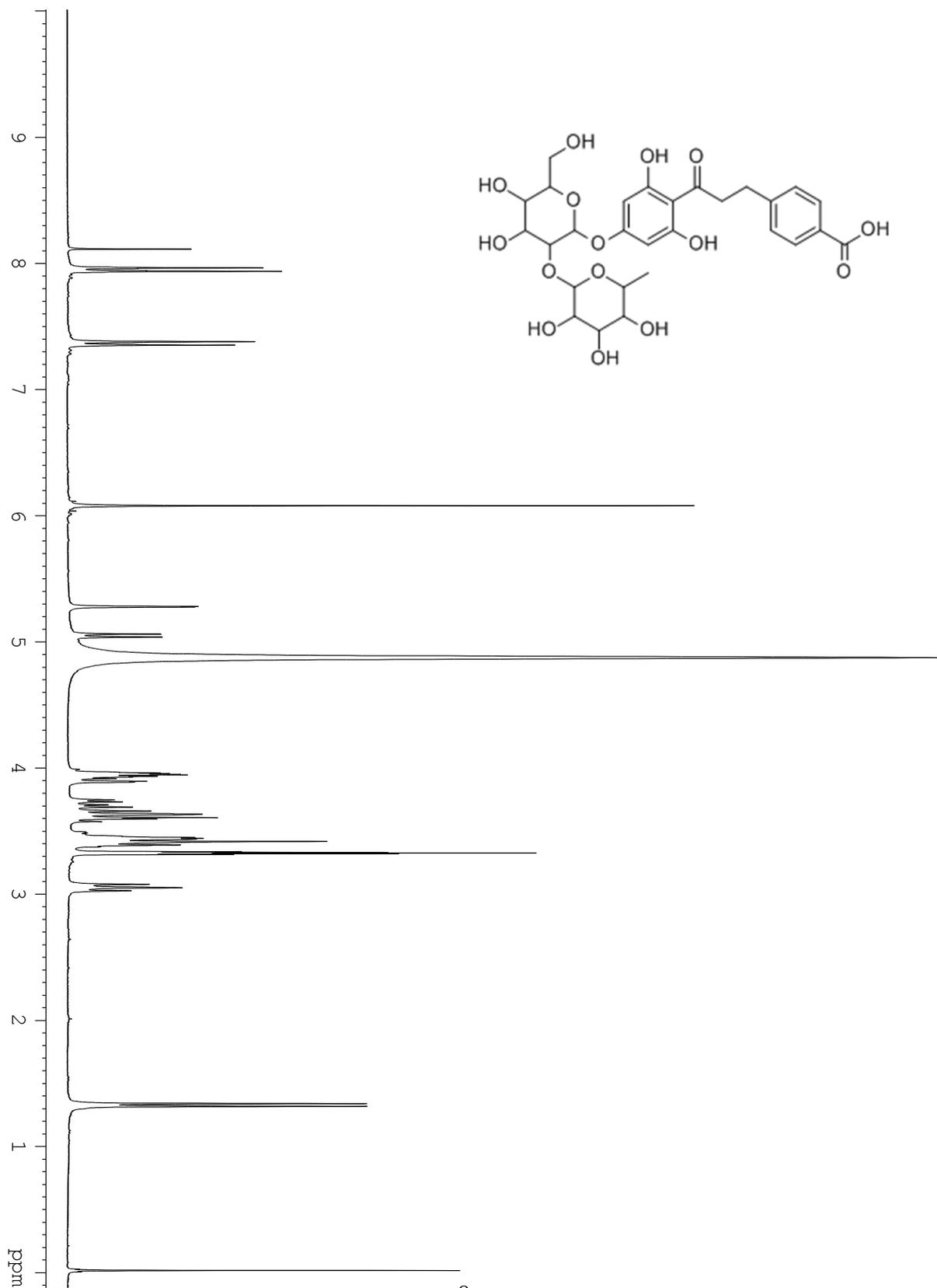


Figure S3b. ^{13}C NMR spectrum of 4-(3-oxo-3-(2,6-dihydroxy-4-neohesperidoside phenyl)propyl)benzoic acid, **C**

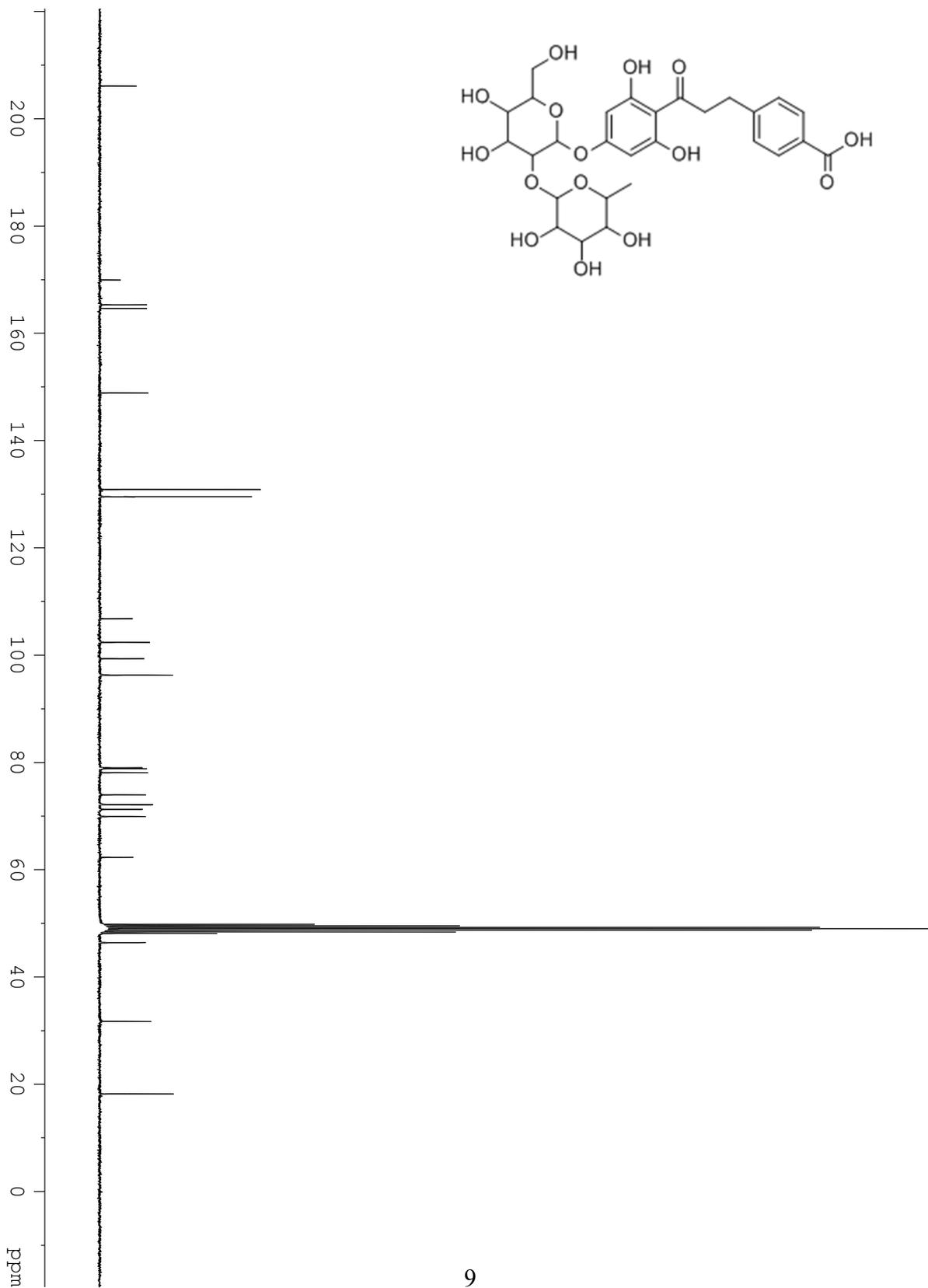


Figure S4a. ^1H NMR spectrum of hapten - 4-(3-oxo-3-(2,6-dihydroxy-4-glucoside phenyl) propyl)benzoic acid.

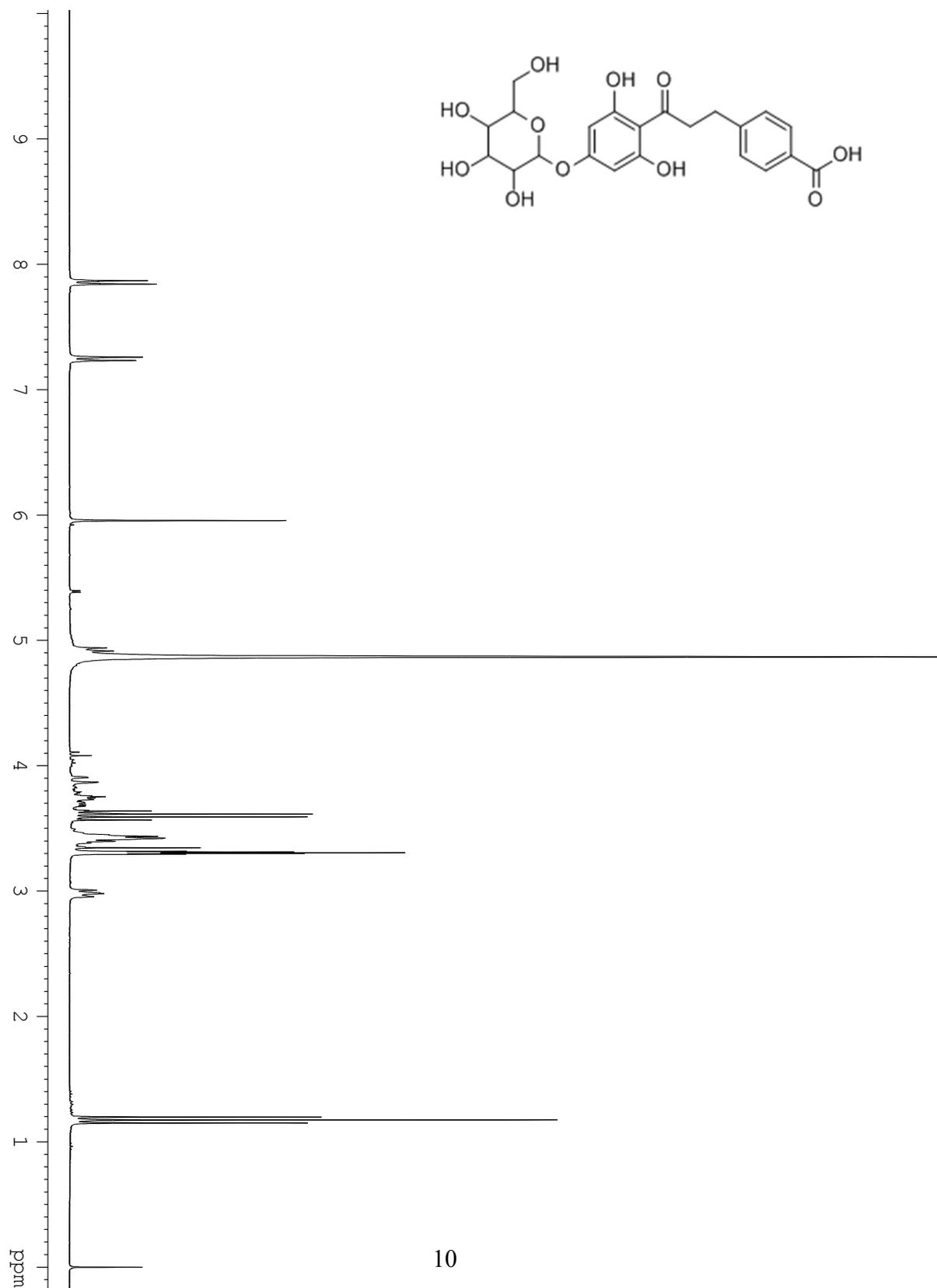


Figure S4b. ^{13}C NMR spectrum of hapten - 4-(3-oxo-3-(2,6-dihydroxy-4-glucoside phenyl) propyl)benzoic acid.

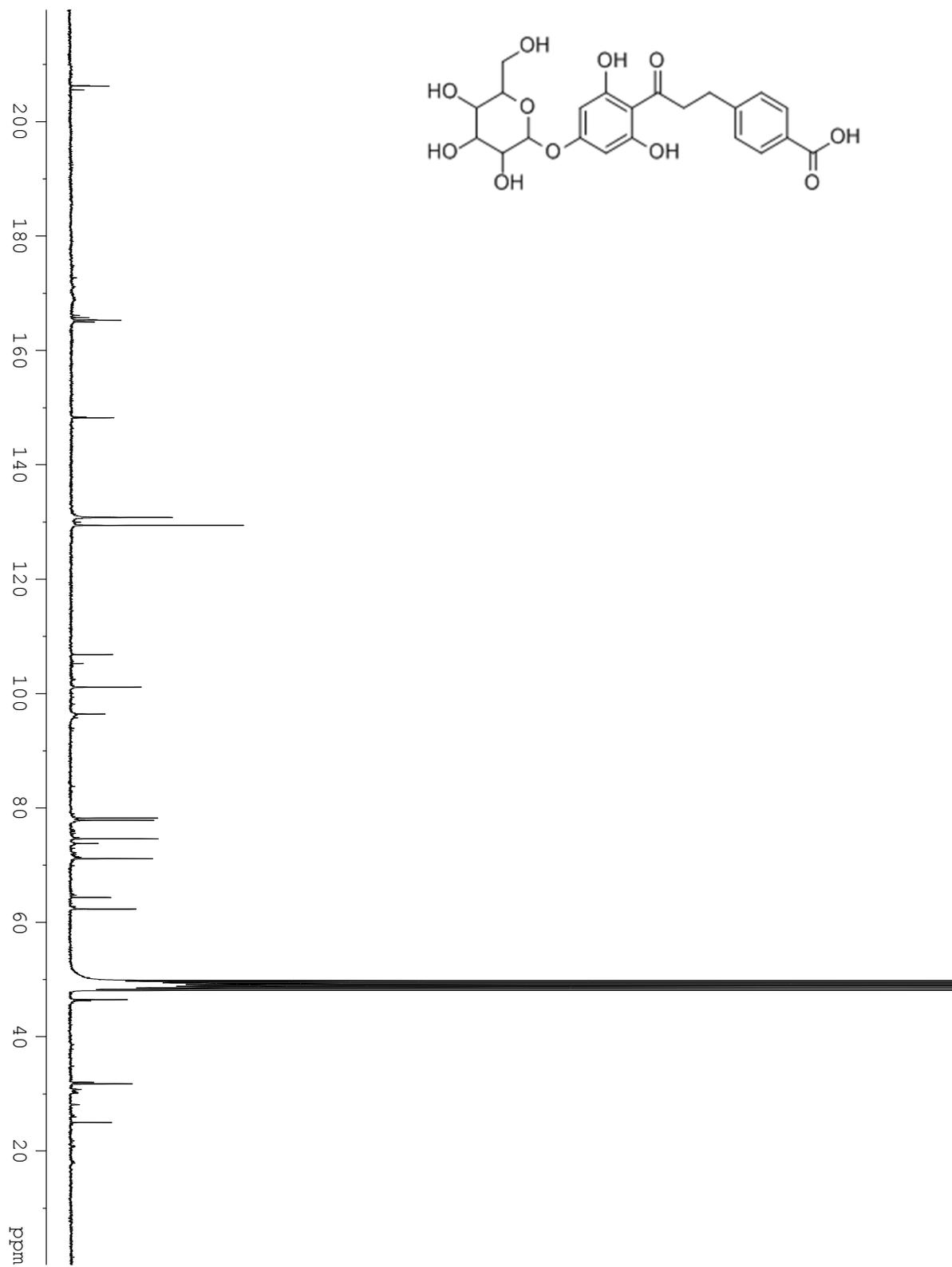


Table S1. Competitive indirect ELISA screening data.^a

Target	Ab	A _{max}	slope	IC ₅₀	A _{min}	A _{max} /A _{min}
1	<i>M6122</i>	0.92 ±0.01	0.57 ±0.04	27.8	0.06 ±0.02	16.1
2	<i>M6122</i>	0.77 ±0.02	0.54 ±0.07	21.8	0.06 ±0.03	13.8

^aStandard solutions were prepared in 10% DMSO in PBS. n = 3 with 4-well replicates. Hapten used for immunogen and coating antigen was -(3-oxo-3-(2,6-dihydroxy-4-glucoside phenyl) propyl)benzoic acid.

Figure S5. Testing cross-reactivity (CR) of Abs *M6123* and *M6124* to hesperetin dihydrochalcone-2'-glucoside.

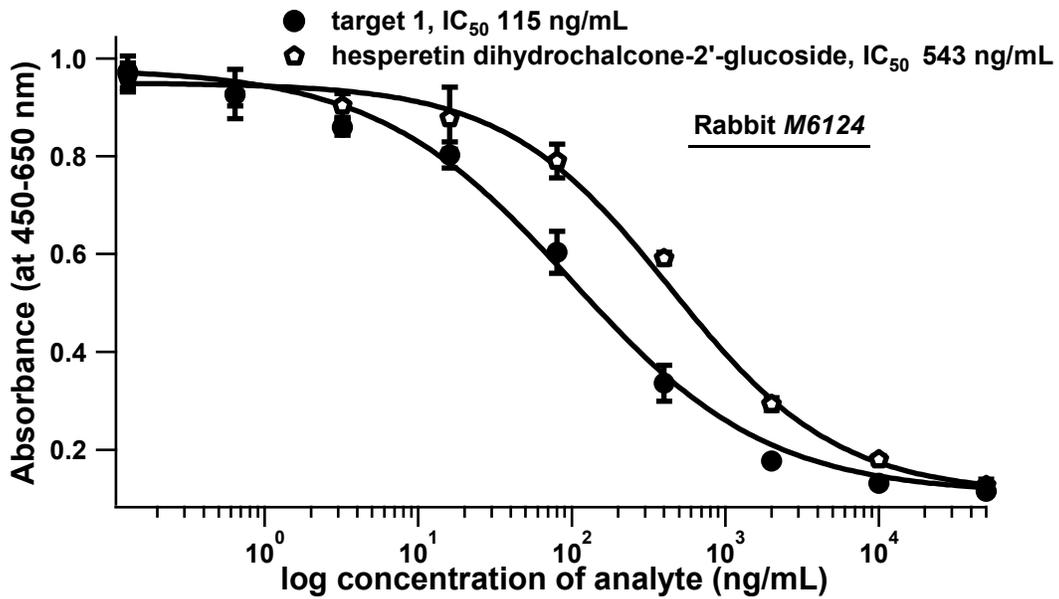
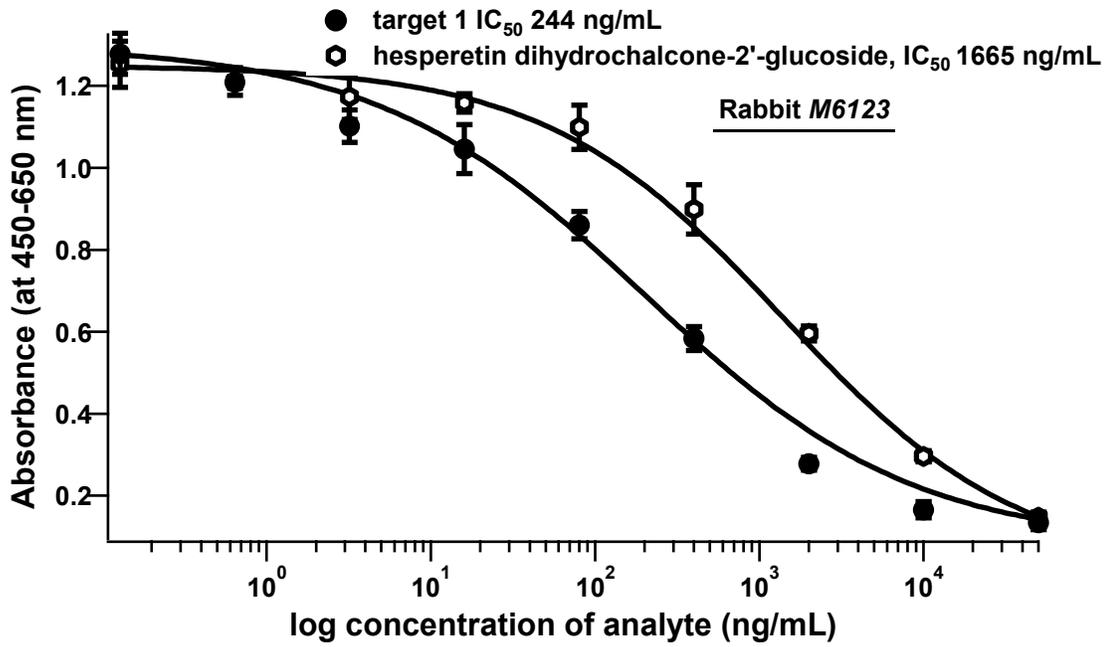


Figure S6. Standard curves obtained for target **1** in PBS buffer containing different concentrations of DMSO. Reagent concentration: antiserum *M6122*, 1/10,000 (final dilution in wells); coating antigen - hapten-BSA, 0.1 $\mu\text{g}/\text{mL}$ (100 $\mu\text{L}/\text{well}$), secondary Ab – goat anti-rabbit HRP, 1/25,000 dilution. Standards prepared in 20%, 40%, 60% and 80% DMSO-PBS (final concentration in wells depicted in figure legend). Each data point represents the mean of quadruplicates.

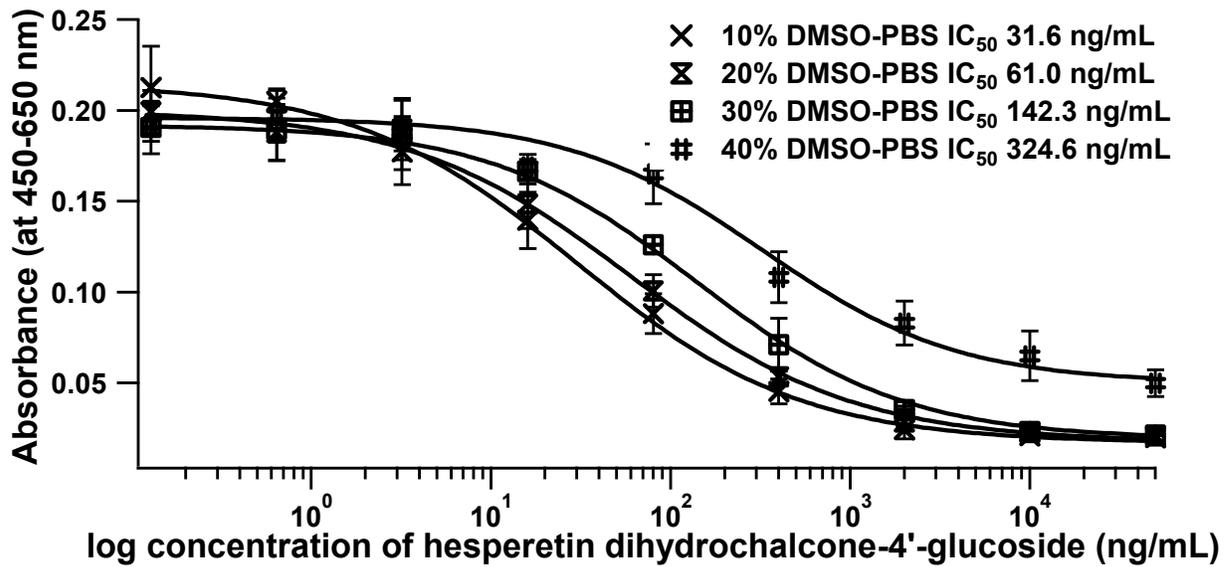


Figure S7. Percent coefficient of variation (% CV) against analyte concentration (ng/mL) for (a) intra- and (b) inter-plate variations for target 1 (hesperetin dihydrochalcone-4'-glucoside).

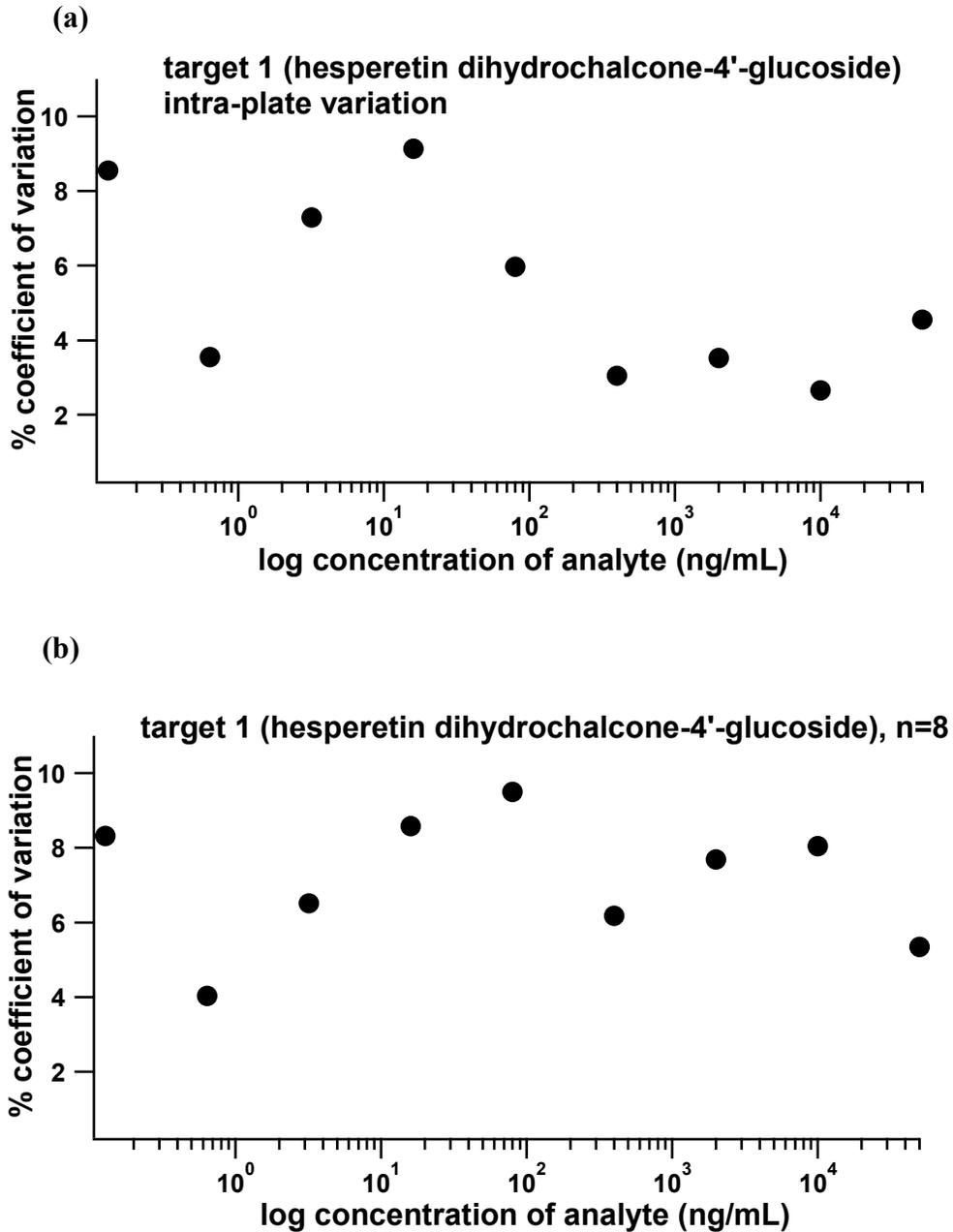


Figure S8. Percent coefficient of variation (% CV) against analyte concentration (ng/mL) for (a) intra- and (b) inter-plate variations for target 2 (trilobatin). “n” designates number of ELISA plates.

