

## Supporting Information

For

### **Synthesis of Montbretin A Analogues Yields Potent Competitive Inhibitors of Human Pancreatic $\alpha$ -Amylase.**

Christina R. Tysoe<sup>[a]</sup>, Sami Caner<sup>[b]</sup>, Matthew B. Calvert<sup>[a]</sup>, Anna Win-Mason<sup>[a]</sup>, Gary D. Brayer<sup>[b]</sup> and Stephen G. Withers<sup>[a]</sup>

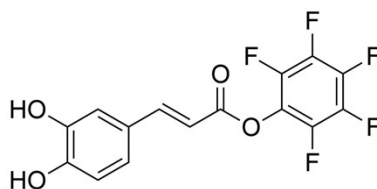
---

[a] C. R. Tysoe, M. B. Calvert A. Win-Mason, S. G. Withers  
Department of Chemistry  
University of British Columbia  
2036 Main Mall, Vancouver, B.C., Canada V6T 1Z1  
E-mail: [withers@chem.ubc.ca](mailto:withers@chem.ubc.ca)

[b] Dr. S. Caner, Prof. Dr. G. D. Brayer  
Department of Biochemistry and Molecular Biology  
University of British Columbia  
2350 Health Sciences Mall, Vancouver, B.C., Canada V6T 1Z3

## Synthesis of Analogues M01 to M11

**General Materials.** All reagents and solvents were purchased from commercial suppliers (Sigma-Aldrich® or Thermo Fisher Scientific®) unless otherwise specified. Dichloromethane was dried by distillation with calcium hydride. Deionized water was prepared using a Millipore-Direct QTM 5 Ultrapure Water System. Analytical thin layer chromatography (TLC) was performed on Merck pre-coated 0.2 mm aluminum-backed sheets of Silica gel 60F<sub>254</sub>. TLC plates were visualized with UV light (254 nm) and stained with 1% ferric chloride in 50% water 50% methanol, or with 10% ammonium molybdate in 2 M H<sub>2</sub>SO<sub>4</sub>, followed by heating. Flash chromatography was performed with silica gel (pore size 60 Å, 220-440 mesh particle size) from Sigma-Aldrich. High performance liquid chromatography (HPLC) was performed on an Agilent 1260 Infinity Bio-inert Quaternary LC using an Agilent Eclipse XDB-C18 column (9.4 x 250 mm, 5 μm) at room temperature using acetonitrile and water with a flow rate of 4 mL/min. Elution of material was monitored by UV/Vis at 210, 280, and 350 nm. <sup>1</sup>H NMR, <sup>13</sup>C NMR, HMBC, and HSQC spectra were acquired on a Bruker 300 MHz, 400 MHz, or 600 MHz spectrometer. Low resolution mass spectra were acquired on a Waters ZQ Mass Detector equipped with an ESCI ion source and Waters 2695 HPLC. High resolution mass spectra were acquired on a Waters/Micromass LCT ESI-TOF.



*(E)*-Perfluorophenyl 3-(3,4-dihydroxyphenyl)acrylate (**1**). Caffeic acid (0.798 g, 4.38 mmol) was dissolved in 10 mL of DMF. Pyridine (0.57 mL, 7.05 mmol) was added, followed by 1.21 mL (7.05 mmol) of pentafluorophenyl trifluoroacetate<sup>1</sup>. The reaction mixture was stirred at room temperature for 2 hours then diluted with DCM and washed 4x with 1 M HCl. The material was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The crude material was purified by silica gel chromatography using an eluent system of pet. ether/EtOAc (6:4). Pentafluorocaffeic ester **1** was isolated as a pale yellow powder upon evaporation of collected fractions (1.41 g, 4.08 mmol, 93% yield); <sup>1</sup>H NMR (300 MHz, acetone-d<sub>6</sub>) δ 8.59 (s, 1H), 8.41 (s, 1H), 7.88 (d, J = 15.9 Hz, 1H), 7.30 (d, J = 1.9 Hz, 1H), 7.21 (dd, J = 8.1 Hz, J = 2.0 Hz, 1H), 6.93 (d, J = 8.2 Hz, 1H), 6.60 (d, J = 15.9 Hz, 1H); <sup>19</sup>F NMR (300 MHz, acetone-d<sub>6</sub>) δ -155.56 (d, J = 18.4 Hz, 2F), -161.33 (t, 1F), -165.51 (m, 2F); <sup>13</sup>C NMR (150 MHz, acetone-d<sub>6</sub>) δ 163.7, 151.0, 150.1, 146.4, 143.1, 141.5, 141.0, 139.7, 139.4, 138.0, 126.9, 123.9, 116.5, 115.8, 111.0; ESI-MS: m/z: 345 [M-H]<sup>-</sup>

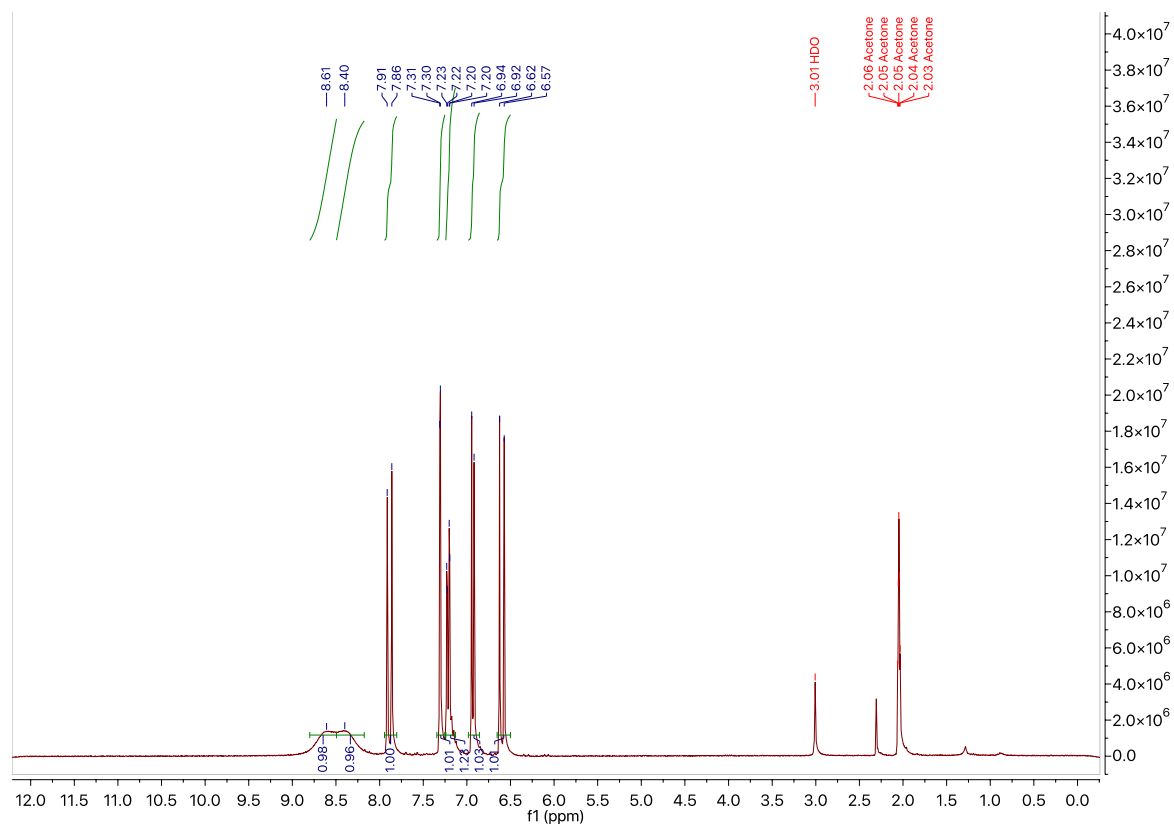


Figure S1.  $^1\text{H}$  NMR of intermediate (1)

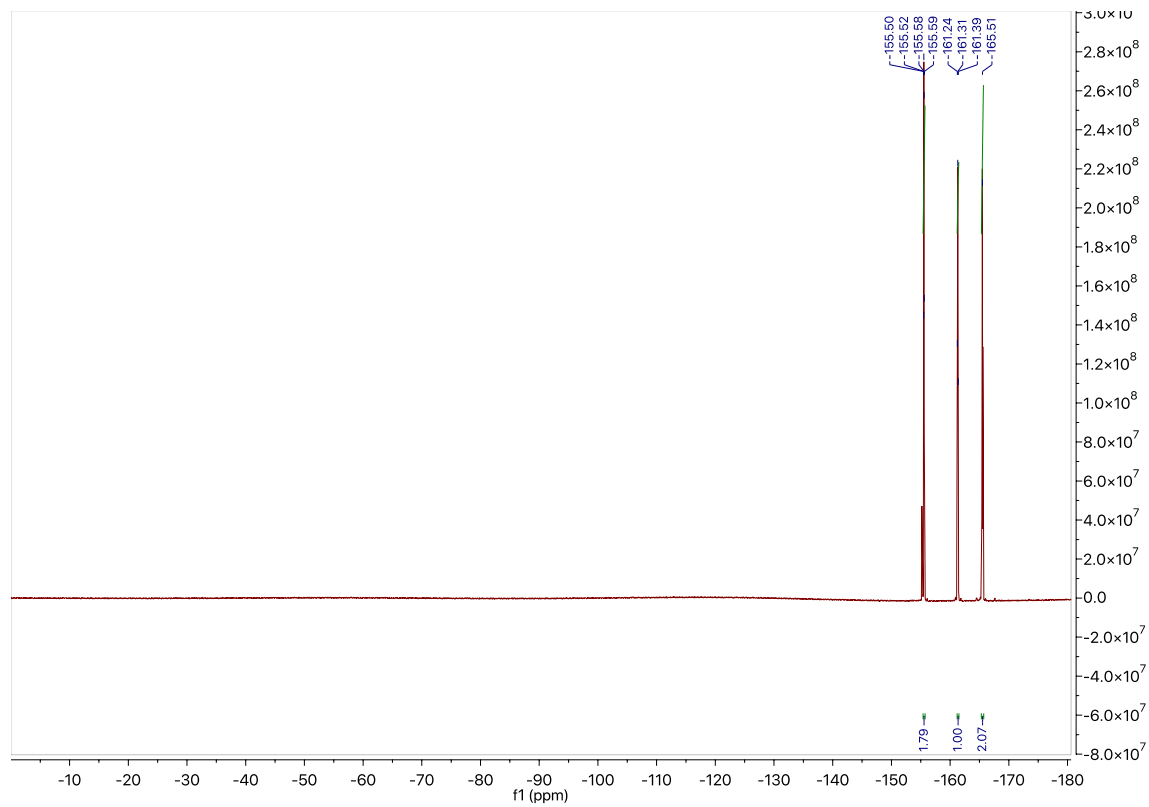
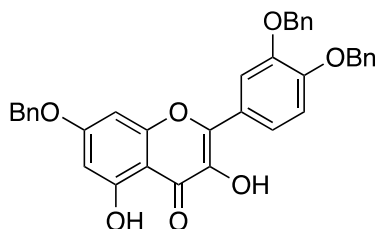


Figure S2.  $^{19}\text{F}$  NMR of intermediate (1)



**7-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-3,5-dihydroxy-4H-chromen-4-one (2).**

This protocol was based on work described by Huang *et al.*<sup>2</sup>. Rutin hydrate (20 g, 32.7 mmol) was added to a 500 mL RB flask, dissolved in 150 mL of DMF and 31 g (224 mmol) of K<sub>2</sub>CO<sub>3</sub> added. The mixture was stirred and heated to 70°C, 27 mL (224 mmol) of BnBr was added and the reaction was stirred vigorously at 70°C for 18 hours. The reaction mixture was then cooled to room temperature and 10% AcOH was added until pH 5 was achieved, causing the benzylated intermediate to precipitate out of solution as a thick brown oil. The thick orangey brown oil was collected and redissolved in 200 mL EtOH. HCl (30 mL) was added and the mixture was stirred at 70°C for two hours when a bright yellow precipitate had formed. The reaction mixture was cooled to room temperature and the yellow precipitate was collected and washed with cold EtOH. The product could be purified by recrystallization with EtOH yielding **2** as a yellow powder (13 g, 22.9 mmol, 70% yield). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 7.85 (m, 2H), 7.43 (m, 15H), 7.27 (d, J = 9.0 Hz, 1H), 6.57 (m, 2H), 5.24 (s, 4H), 5.21 (s, 2H); ESI-MS: m/z: 573 [M+H]<sup>+</sup>, 595 [M+Na]<sup>+</sup>, 571 [M-H]<sup>-</sup>

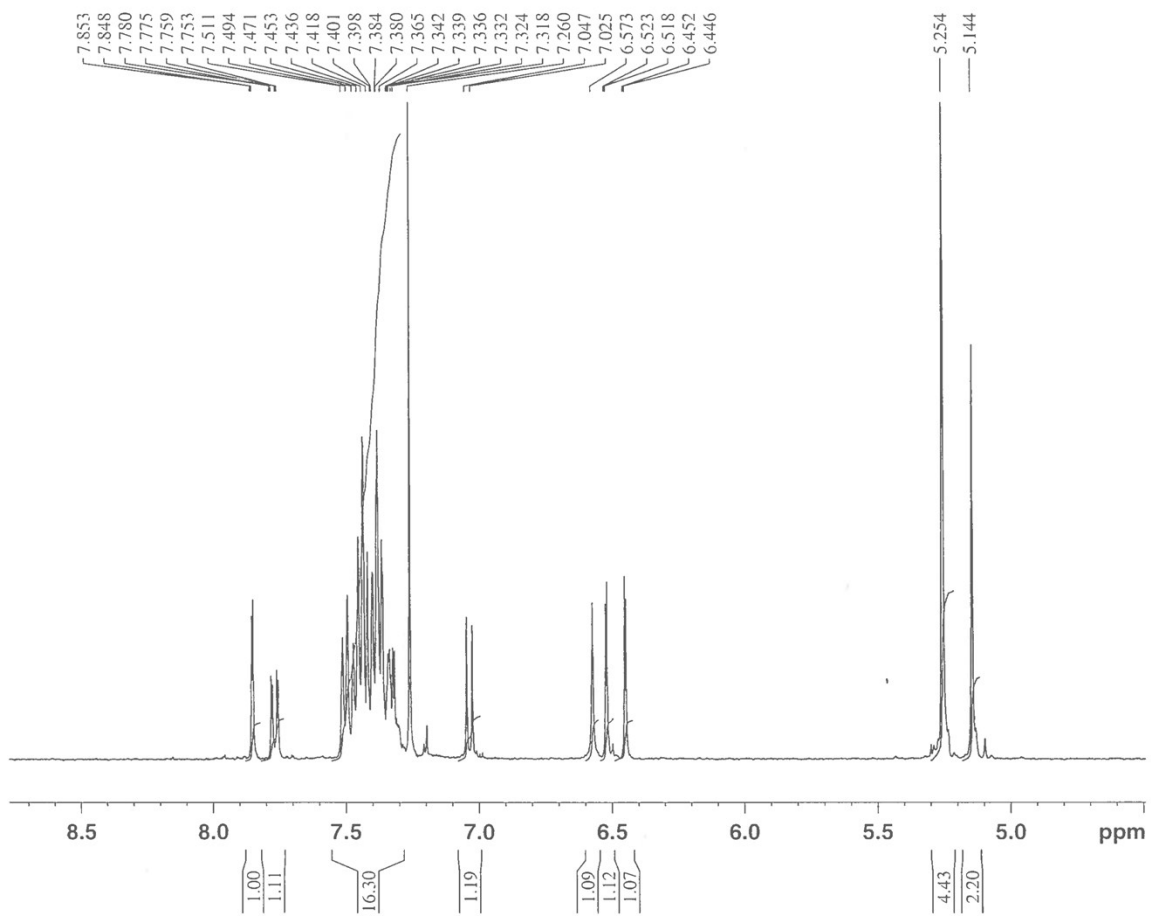
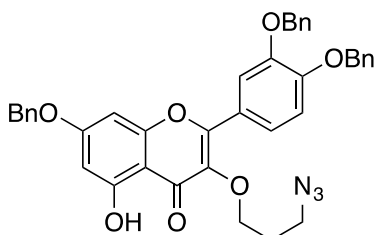


Figure S3. <sup>1</sup>H NMR of intermediate (2)



3-(3-Azidopropoxy)-7-(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-4H-chromen-4-one (**3 Propyl**). Alcohol **2** (10 g, 17.5 mmol) was added to a 250 mL RB flask then dissolved in 50 mL of DMF and 25 mL of THF.  $K_2CO_3$  (3.6 g, 26.25 mmol) was added and the mixture was stirred while 2.6 mL (26.25 mmol) of 1-bromo-3-chloropropane was added, then stirred at rt overnight. The mixture was then diluted with DCM and washed 4x with 1 M HCl, dried over  $MgSO_4$ , and concentrated *in vacuo*. A solution of  $NaN_3$  (5.2 g, 79.8 mmol) in water (10 mL) was added to tetrabutylammonium hydroxide (40% aqueous solution, 10.4 g, 39.9 mmol). After one minute, dichloromethane (50 mL) was added, and the organic layer was separated. The aqueous layer was extracted three times with dichloromethane. The organic layers were dried over  $MgSO_4$ , and concentrated *in vacuo*<sup>3</sup>. The resulting colourless oil was then immediately added to a 250 mL RB flask containing the chloride intermediate dissolved in DMF (70 mL). The reaction mixture was stirred vigorously at 50°C overnight. The mixture was then cooled to rt, diluted with DCM and washed 4x with 1 M HCl, dried over  $MgSO_4$ , and concentrated *in vacuo*. The material was purified by silica gel chromatography with 5% EtOAc/toluene yielding azide (**3 Propyl**) as a yellow crystalline solid (7.9 g, 12.0 mmol, 68% yield over two steps).  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  7.64 (m, 2H), 7.39 (m, 15H), 7.03 (d, J = 8.2 Hz, 1H), 6.44 (m, 2H), 5.26 (s, 2H), 5.23 (s, 2H), 5.12 (s, 2H), 4.07 (t, J = 6.0 Hz, 2H), 3.61 (t, J = 6.0 Hz, 2H), 2.08 (p, J = 6.0 Hz, 2H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$  178.5, 164.6, 162.2, 156.8, 156.2, 151.5, 148.6, 138.0, 137.1, 136.7, 135.9, 128.9, 128.8, 128.7, 128.5, 128.2 (x2), 128.1, 127.6, 127.5, 127.4, 127.3, 123.4 (x2), 123.0 (x2), 115.3, 114.5, 113.9, 113.7, 106.3, 98.7, 93.2 (x2), 71.6, 71.0, 70.6, 69.6, 66.2, 41.7; ESI-MS: m/z: 656  $[M+H]^+$ , 678  $[M+Na]^+$



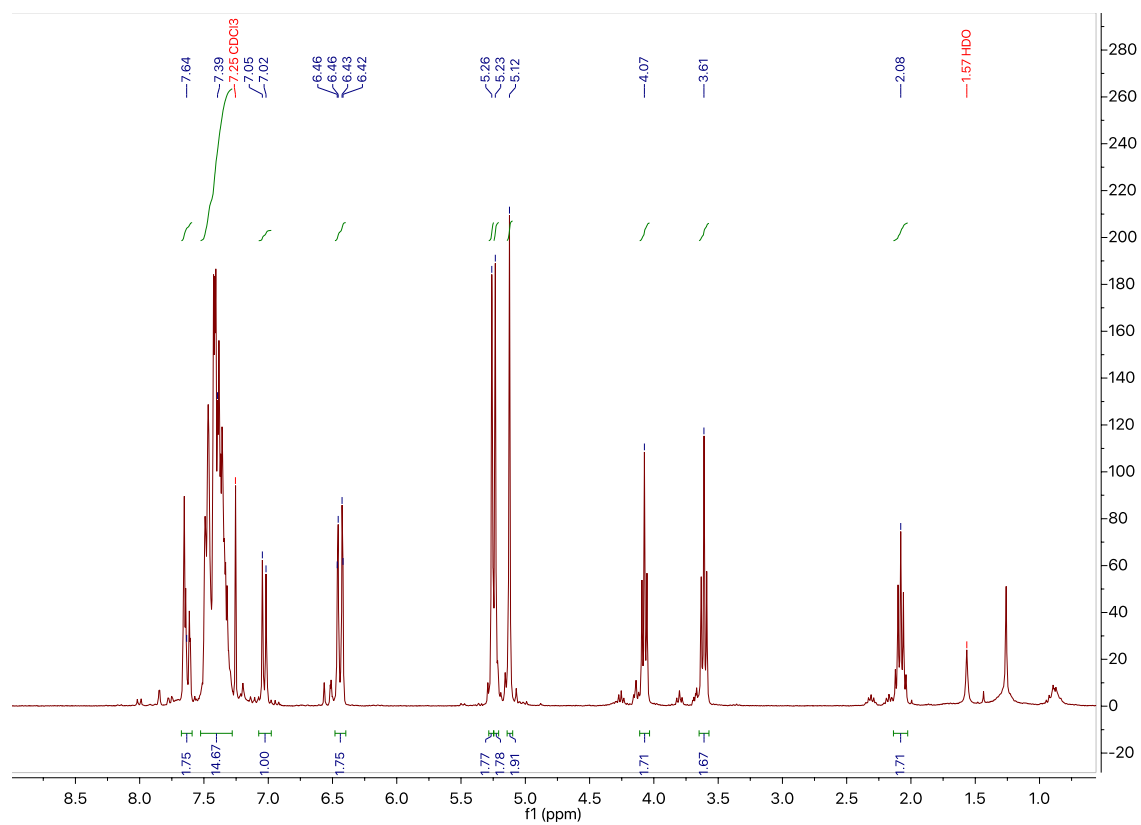


Figure S4. <sup>1</sup>H NMR of intermediate (3 Propyl)

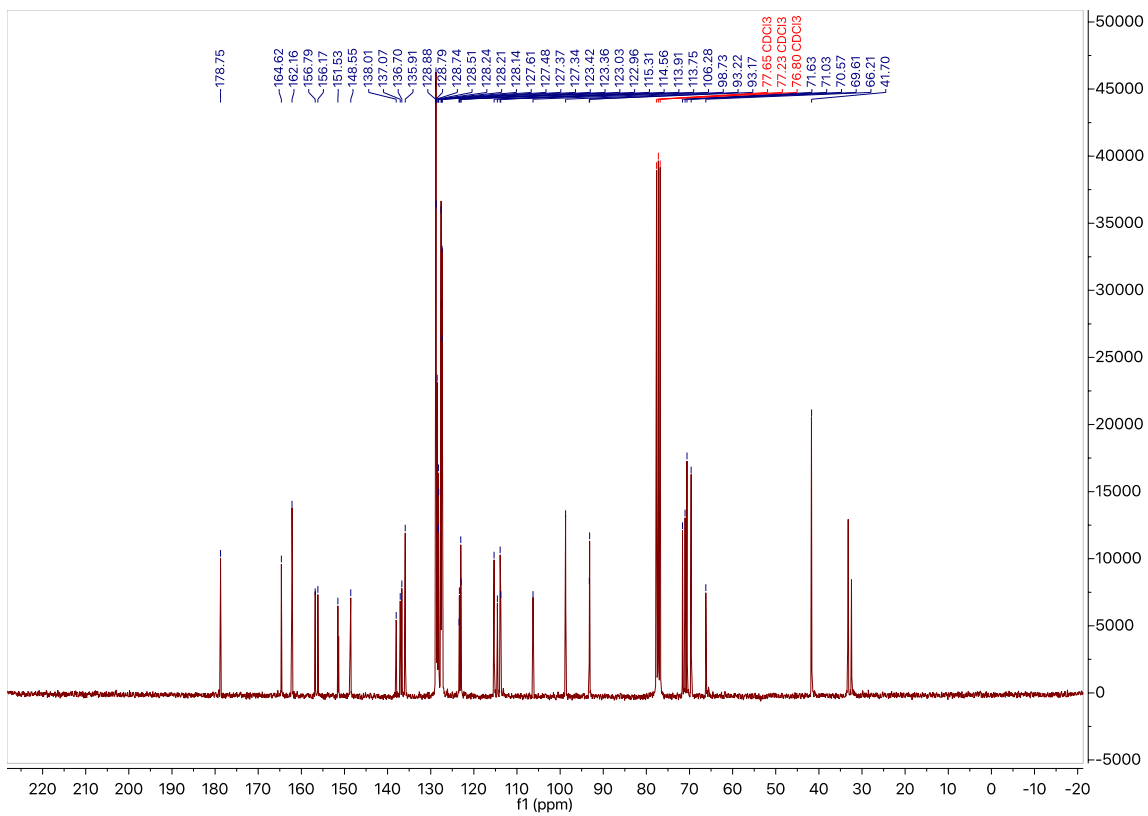
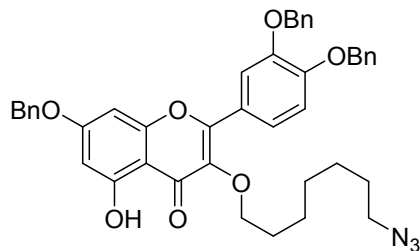


Figure S5.  $^{13}\text{C}$  NMR of intermediate (3 Propyl)



3-((7-azidoheptyl)oxy)-7-(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-4H-chromen-4-one (**3 Heptyl**). Alcohol **2** (193 mg, 0.34 mmol) was dissolved in DMF (3.6 mL) and anhydrous THF (1.8 mL).  $K_2CO_3$  (70 mg, 0.51 mmol) and MsO-Heptyl- $N_3$  (100  $\mu$ L, 0.44 mmol)<sup>4</sup> were added to give a dark yellow mixture and the reaction was stirred at 40°C for 20 h. The reaction mixture was diluted with DCM (10 mL), washed with water and  $NaHCO_3$ , dried with  $MgSO_4$ , filtered and concentrated *in vacuo*. The material was purified by recrystallisation (MeOH/DCM), to give azide (**3 Heptyl**) as a yellow solid (148 mg, 0.21 mmol, 62% yield).  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  12.69 (s, 1H), 7.78 (d,  $J$  = 2.0 Hz, 1H), 7.68 (dd,  $J$  = 8.7, 2.0, 1H), 7.51-7.32 (m, 15H), 7.04 (d,  $J$  = 8.7 Hz, 1H), 6.47 (d,  $J$  = 2.2 Hz, 1H), 6.43 (d,  $J$  = 2.2 Hz, 1H), 5.26 (s, 2H), 5.23 (s, 2H), 5.12 (s, 2H), 3.96 (t,  $J$  = 6.8 Hz, 2H), 3.22 (t,  $J$  = 7.0 Hz, 2H), 1.68 (p,  $J$  = 7.0 Hz, 2H), 1.56 (p,  $J$  = 7.0 Hz, 2H), 1.41-1.28 (m, 6H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  178.9, 164.5, 162.1, 156.7, 155.9, 151.4, 148.4, 138.3, 137.0, 136.7, 135.9, 128.8 (x2), 128.71 (x2), 128.67 (x2), 128.4, 128.13, 128.08, 127.5 (x2), 127.4 (x2), 127.3 (x2), 123.6, 122.9, 115.4, 113.7, 106.3, 98.6, 93.1, 72.9, 71.6, 71.0, 70.5, 51.5, 30.1, 29.0, 28.8, 26.7, 25.8. HRMS (ESI-TOF):  $m/z$  calc'd for  $C_{43}H_{41}N_3O_7Na$ : 734.2842  $[M+Na]^+$ ; found: 734.2835.

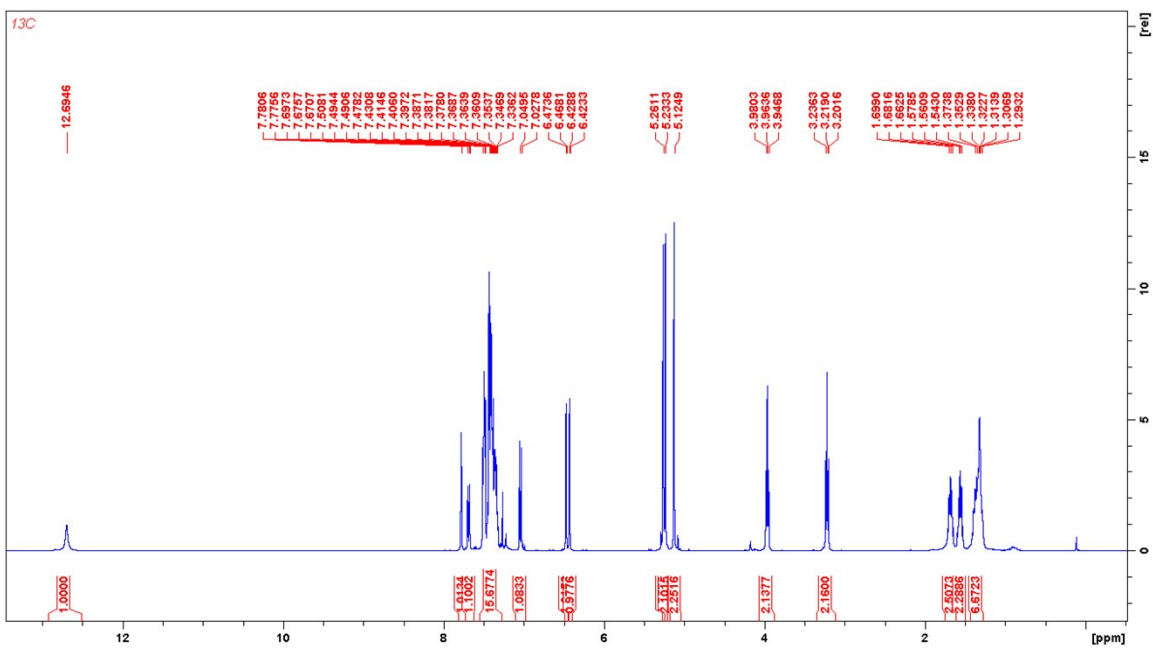


Figure S6. <sup>1</sup>H NMR of intermediate (3 Heptyl)

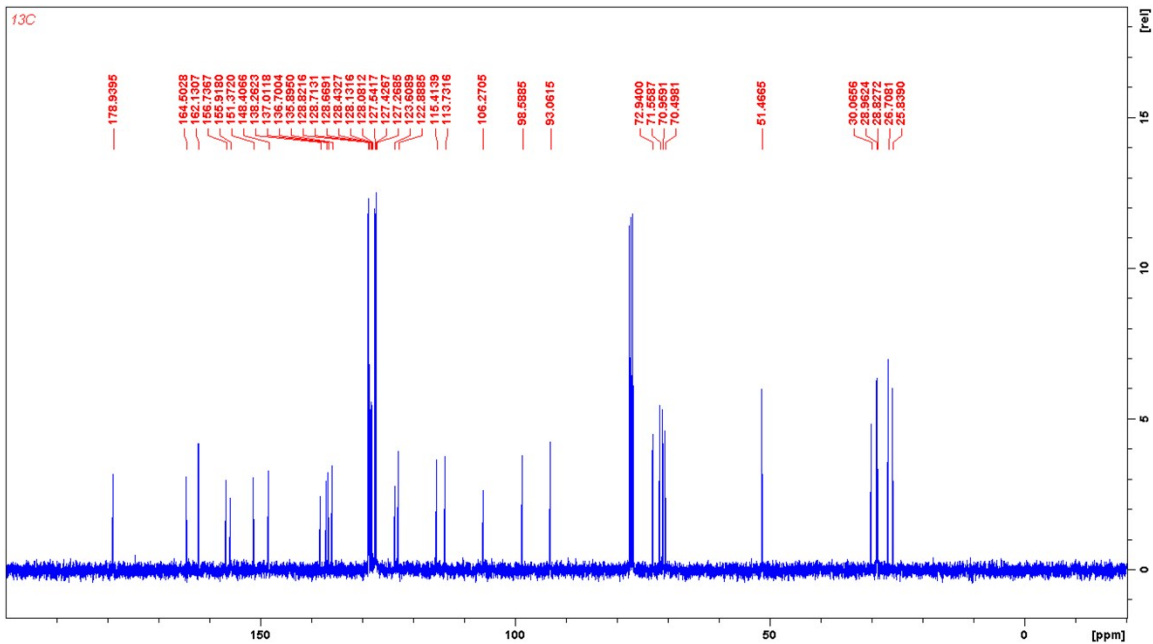
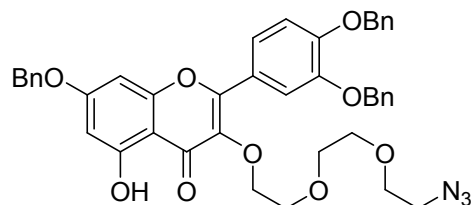


Figure S7. <sup>13</sup>C NMR of intermediate (3 Heptyl)



3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-7-(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-4H-chromen-4-one (**3 TEG**). Alcohol **2** (100 mg, 0.17 mmol) was dissolved in DMF (2 mL) and anhydrous THF (1 mL).  $K_2CO_3$  (35 mg, 0.25 mmol) and Br-TEG- $N_3$  (55  $\mu$ L, 50 mg, 0.21 mmol)<sup>4</sup> were added to give a dark yellow mixture and the reaction was stirred at room temperature for 40 h. The reaction mixture was diluted with DCM (10 mL) and washed with water (10 mL). The aqueous layer was back-extracted with DCM (3 x 10 mL) and the combined organics were pooled, dried with  $MgSO_4$ , filtered and concentrated *in vacuo*. The material was purified by silica gel chromatography with a gradient of 20:1 to 1:1 petroleum ether/ethyl acetate to give azide (**3 TEG**) as a yellow solid (92 mg, 0.13 mmol, 72% yield).  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  12.65 (s, 1H), 7.84 (d,  $J$  = 1.8 Hz, 1H), 7.75 (dd,  $J$  = 8.6, 1.9, 1H), 7.52-7.31 (m, 15H), 7.03 (d,  $J$  = 8.7 Hz, 1H), 6.47 (d,  $J$  = 2.0 Hz, 1H), 6.42 (d,  $J$  = 2.0 Hz, 1H), 5.26 (s, 4H), 5.13 (s, 2H), 4.20 (t,  $J$  = 4.5 Hz, 2H), 3.69 (t,  $J$  = 4.5 Hz, 2H), 3.54 (t,  $J$  = 5.1 Hz, 2H), 3.51 (s, 4H), 3.28 (t,  $J$  = 5.1, 2H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  178.7, 164.5, 162.1, 156.7, 155.8, 151.3, 148.3, 138.0, 137.1, 136.7, 135.9, 128.8 (x2), 128.72 (x2), 128.66 (x2), 128.4, 128.1, 128.0, 127.6 (x2), 127.5 (x2), 127.3 (x2), 123.5, 123.0, 115.6, 113.7, 106.2, 98.6, 93.1, 71.6, 71.5, 70.9, 70.6, 70.54, 70.52, 70.49, 70.0, 50.7. HRMS (ESI-TOF):  $m/z$  calc'd for  $C_{42}H_{39}N_3O_9Na$ : 752.2584  $[M+Na]^+$ ; found: 752.2574.

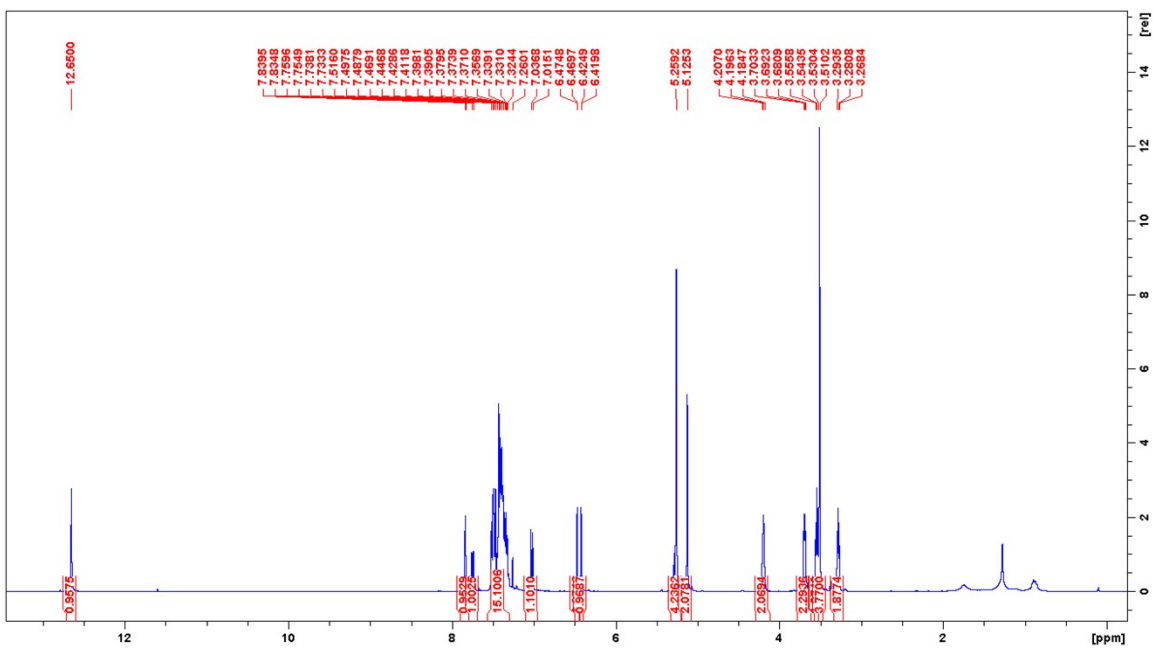


Figure S8. <sup>1</sup>H NMR of intermediate (3 TEG)

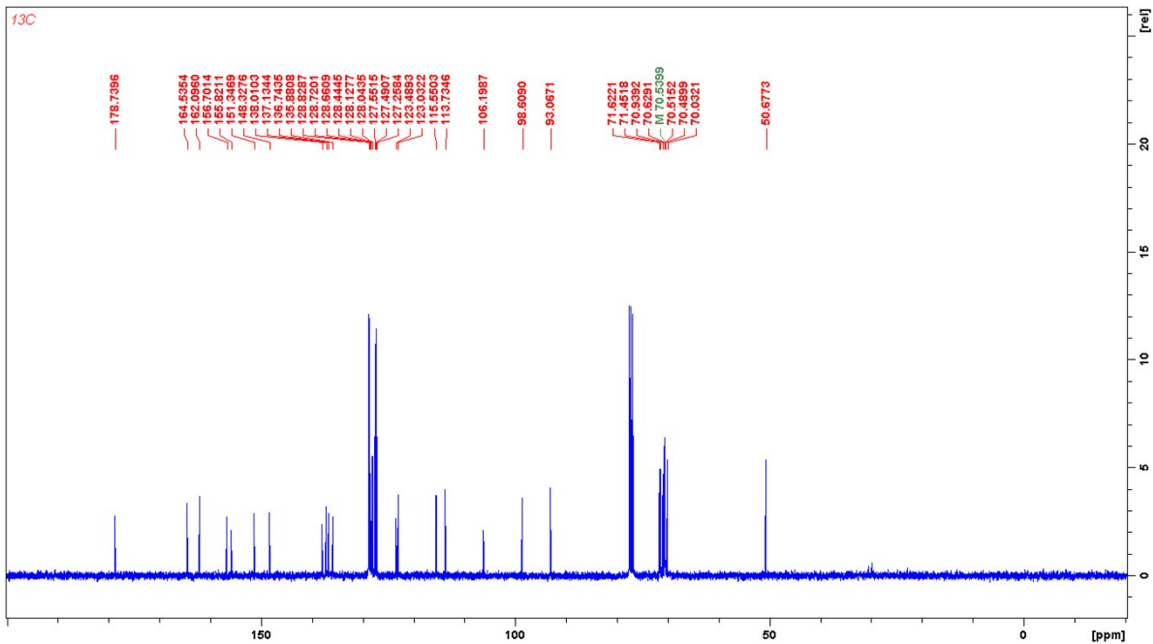
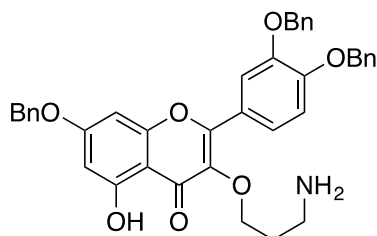


Figure S9. <sup>13</sup>C NMR of intermediate (3 TEG)



3-(3-Aminopropoxy)-7-(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-4H-chromen-4-one (**4**). Azide (**3 Propyl**) (7.9 g, 12.0 mmol) was added to a 250 mL RB flask then dissolved in THF (60 mL). 0.05 M Aqueous NaOH (12 mL) was added. 1 M PMe<sub>3</sub> in THF (36 mL, 36.0 mmol) was added, then stirred at rt overnight. The reaction mixture was then concentrated *in vacuo*. The material was purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/H<sub>2</sub>O yielding amine (**4 Propyl**) as a yellow solid (4.5 g, 7.2 mmol, 60% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.62 (m, 2H), 7.35 (m, 15H), 6.98 (d, J = 9.0 Hz, 1H), 6.32 (m, 2H), 5.19 (s, 2H), 5.18 (s, 2H), 4.97 (s, 2H), 3.85 (t, J = 6.0 Hz, 2H), 3.28 (t, J = 6.0 Hz, 2H), 2.05 (p, J = 6.0 Hz, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 178.3, 164.7, 161.4, 156.5, 156.1, 151.9, 148.5, 137.4, 137.1, 136.6, 135.7, 128.9, 128.7 (x3), 128.4, 128.1, 128.0, 127.7, 127.6, 127.4 (x2), 123.2, 123.1, 122.4, 115.9, 114.5, 113.8, 113.0, 105.5, 98.8, 93.1 (x2), 71.5, 70.8, 70.4, 69.9, 53.7, 38.5; ESI-MS: m/z: 630 [M+H]<sup>+</sup>

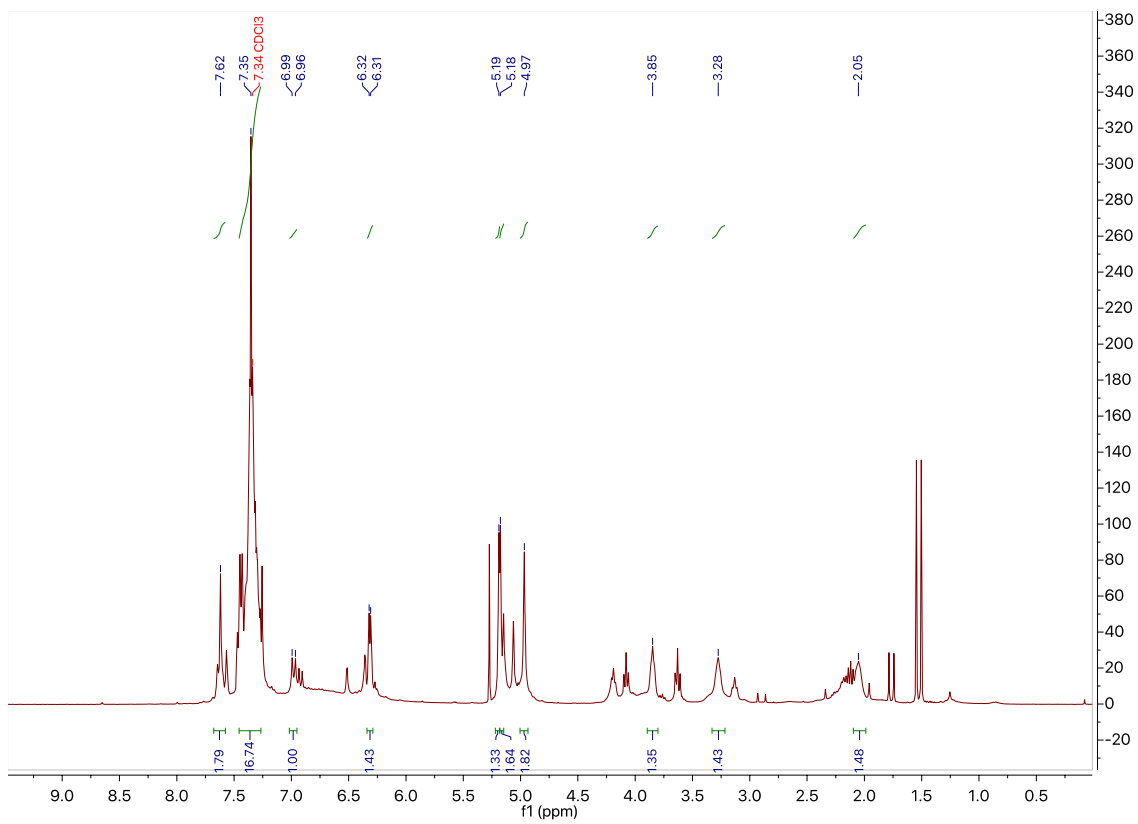


Figure S10. <sup>1</sup>H NMR of intermediate (4 Propyl)



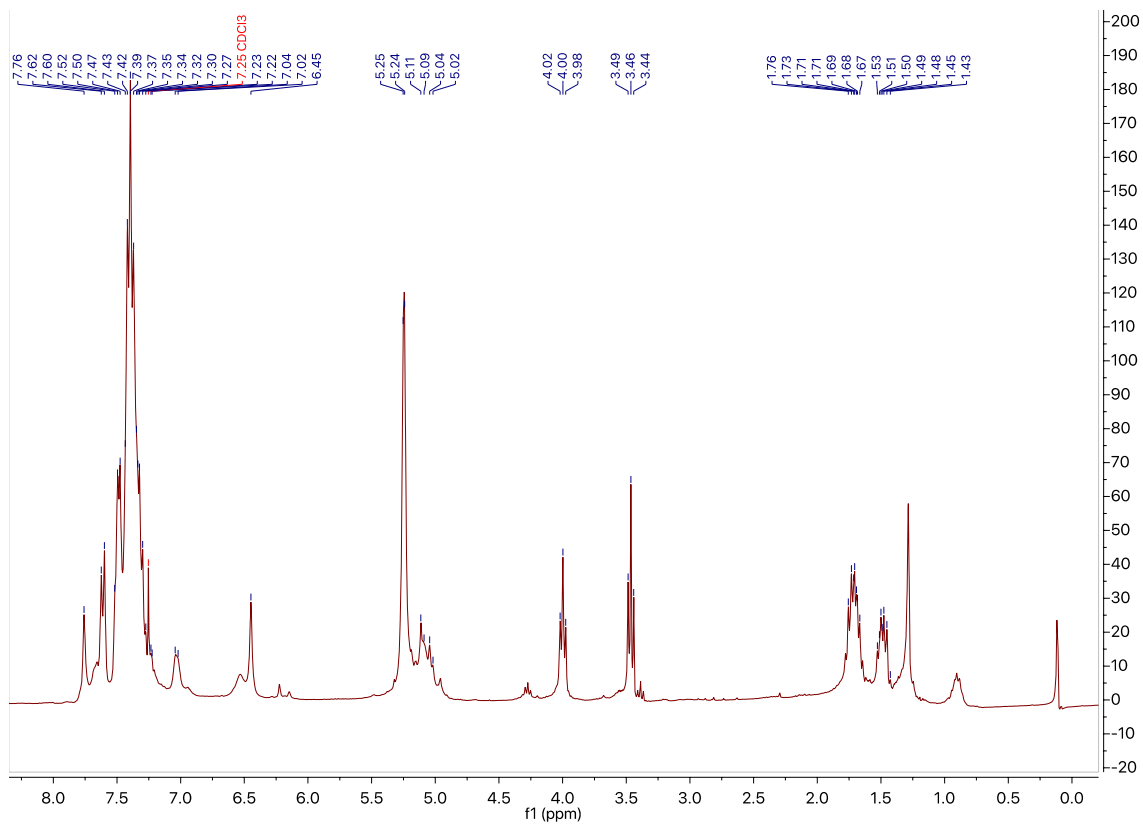


Figure S11. <sup>1</sup>H NMR of intermediate (4 Pentyl)

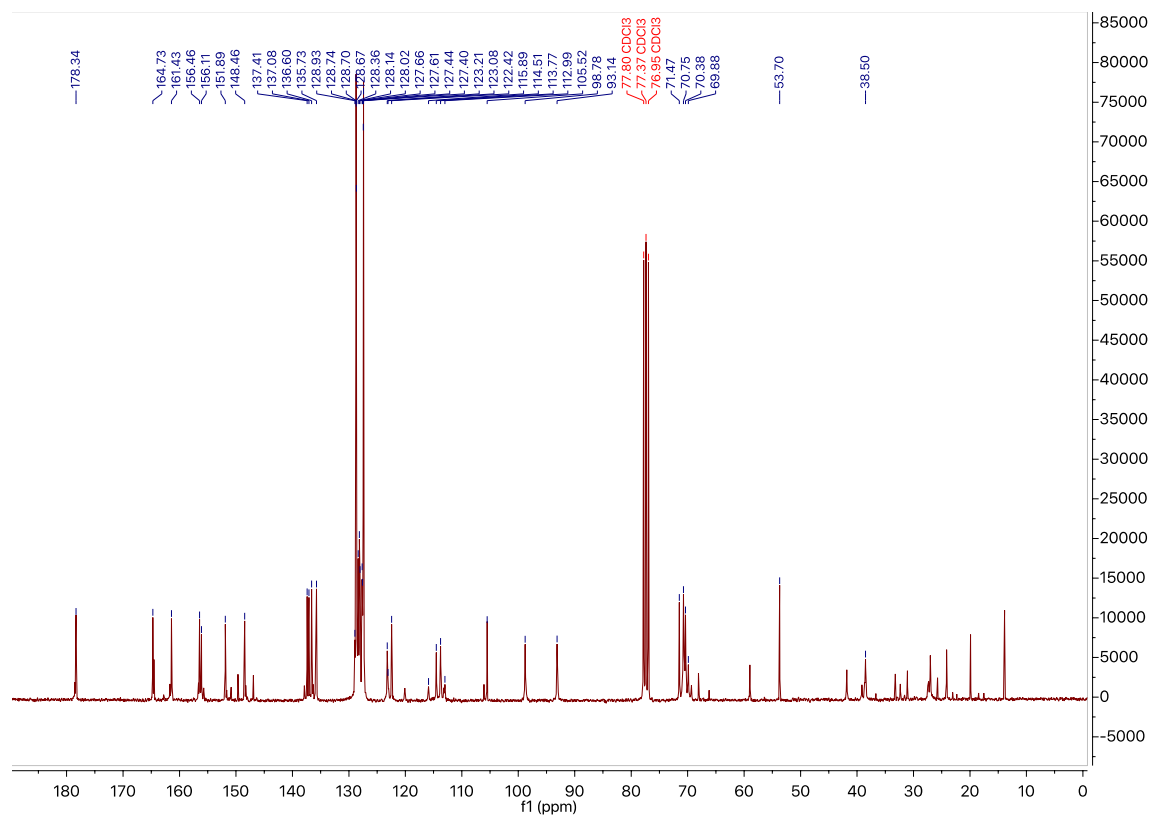
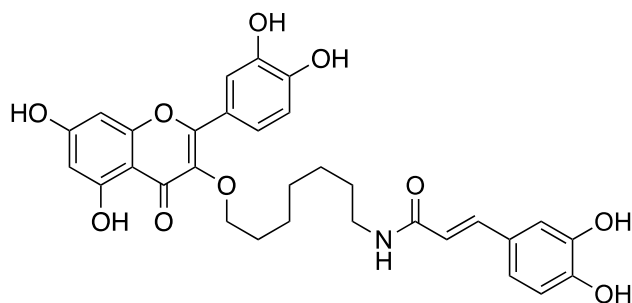


Figure S12. <sup>13</sup>C NMR of intermediate (4 Propyl)



(*E*)-3-(3,4-dihydroxyphenyl)-*N*-(5-((2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)oxy)heptyl)acrylamide (**M01**). Azide **3 Heptyl** (103 mg, 0.14 mmol) was dissolved in THF (1.2 mL), then NaOH aq (0.05 M, 240  $\mu$ L) was added to give a yellow clear solution. A solution of PMe<sub>3</sub> in THF (1 M, 720  $\mu$ L, 0.72 mmol) was added and the reaction sealed and stirred vigorously for 23 hrs. The reaction mixture was diluted with DCM (5 mL) and washed with water (5 mL). The aqueous layer was back-extracted with DCM (3 x 5 mL), and the combined organics were dried over MgSO<sub>4</sub>, filtered and concentrated to yield crude amine (**4 Heptyl**). The crude amine was treated with anhydrous DMF (2 mL) and dry pyridine (100  $\mu$ L), followed by pentafluorophenyl caffeic ester **1** (1.2 eq, 56 mg, 0.16 mmol). The reaction mixture was stirred vigorously at 40 °C for 48 hrs. The reaction was diluted with ethyl acetate (10 mL) and washed with 0.5 M HCl (10 mL), and brine (2 x 10 mL). The organic layer was dried over MgSO<sub>4</sub>, filtered and concentrated. The material was purified by silica gel chromatography, using a gradient of DCM/[DCM/MeOH/EtOH/NH<sub>3</sub> aq 30%], 200/1 – 10/1 to give amide (**4.1 Heptyl**) as a yellow solid (80 mg, 0.094 mmol, 66% yield over 2 steps). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  12.62 (s, 1H), 8.26 (s, 1 H), 7.72 (d, *J* = 1.9, 1H), 7.65 (dd, *J* = 8.5, 1.7, 1H), 7.47 - 7.24 (m, 16H), 7.08 (s, 1H), 7.00 (d, *J* = 8.8 Hz, 1H), 6.82-6.78 (m, 2H), 6.44 (d, *J* = 2.1 Hz, 1H) 6.38 (d, *J* = 2.1 Hz, 1H), 6.29 (s, 1H), 6.16 (d, *J* = 15.5 Hz, 1H), 5.17 (s, 2H), 5.16 (2, 2H), 5.06 (s, 2H), 3.86 (t, *J* = 6.4, 2H), 3.23-3.17 (m, 2H), 1.58 (p, *J* = 6.7, 2H), 1.42-1.33 (m, 2H), 1.28-1.12 (m, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  179.0, 167.6, 164.6, 161.9, 156.8, 156.3, 151.4, 148.3, 147.0, 144.8, 141.8, 138.2, 136.8, 136.4, 135.8, 128.8 (x2), 128.71 (x2), 128.67 (x2), 128.4, 128.21, 128.18, 127.6 (x2), 127.5

(x2), 127.4 (x2), 127.2, 123.4, 123.1, 121.6, 117.5, 115.5, 115.3, 114.5, 113.6, 106.2, 98.7, 93.2, 73.2, 71.6, 71.0, 70.5, 40.1, 30.0, 29.3, 29.0, 26.9, 25.8.  
HRMS (ESI-TOF): m/z calc'd for C<sub>52</sub>H<sub>49</sub>NO<sub>10</sub>Na: 870.3254 [M+Na]<sup>+</sup>; found: 870.33239.

Under argon, amide **4.1 Heptyl** (80 mg, 0.094 mmol) was dissolved in dry DCM (1.5 mL), cooled to -78 °C for 5- 10 mins and a solution of BBr<sub>3</sub> in DCM (1 M, 290 uL, 0.29 mmol) was added. After vigorous stirring for an hour a second portion of BBr<sub>3</sub> (250 uL, 0.25 mmol) was added to make a total of 7 equivalents BBr<sub>3</sub>. The reaction mixture was kept at -78 °C for another 5.5 hrs with vigorous stirring. The reaction was quenched with sat. NaHCO<sub>3</sub>. The mixture was extracted 3 times with EtOAc, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/H<sub>2</sub>O. This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/H<sub>2</sub>O to 95% ACN/ H<sub>2</sub>O over 50 minutes to yield **M01** as yellow powder. <sup>1</sup>H NMR (400 MHz, Acetone-d<sub>6</sub>) δ 7.63 (d, J = 2.0 Hz, 1H), 7.52 (dd, J = 2.4 Hz, J = 8.0 Hz, 1H), 7.43 (d, J = 15.6 Hz, 1H), 7.13 (d, J = 2.0 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.91 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.51 (d, J = 15.6 Hz, 1H), 6.51 (d, J = 2.0 Hz, 1H), 6.45 (d, J = 2.0 Hz, 1H), 4.01 (t, J = 6.0 Hz, 2H), 3.30 (m, 4H), 1.66 (m, 2H), 1.49 (m, 4H), 1.39 (m, 2H). HRMS (ESI-TOF): m/z calc'd for C<sub>31</sub>H<sub>31</sub>NO<sub>10</sub>Na: 600.1846 [M+Na]<sup>+</sup>; found: 600.1866.

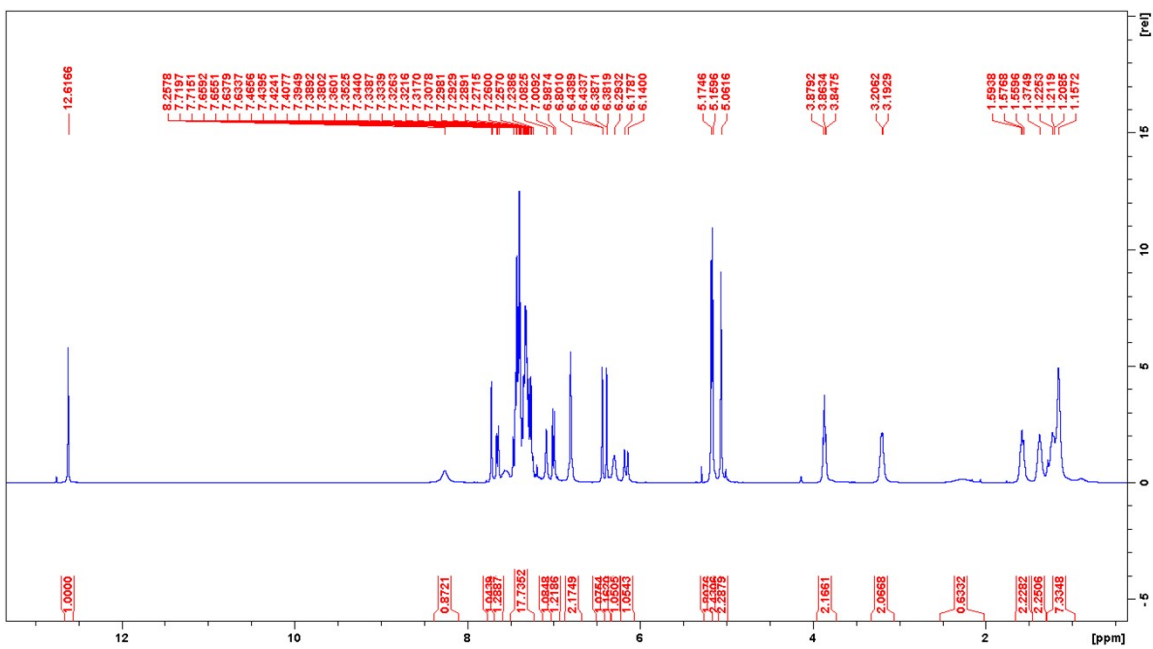


Figure S13. <sup>1</sup>H NMR of intermediate (4.1 Heptyl)

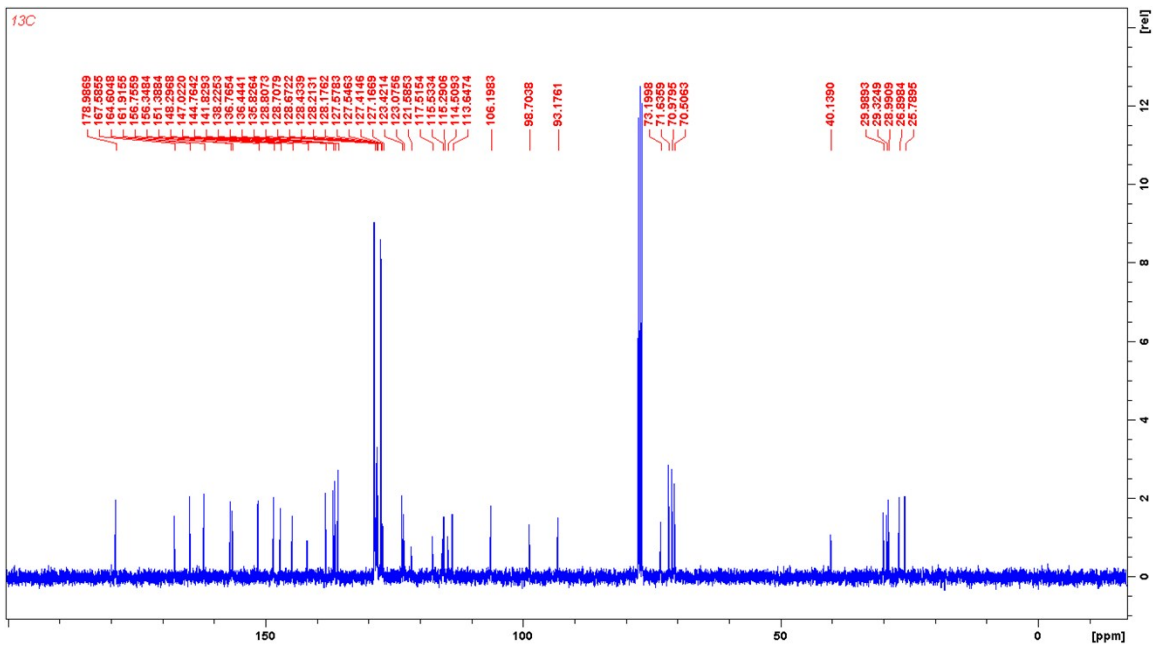


Figure S14. <sup>13</sup>C NMR of intermediate (4.1 Heptyl)

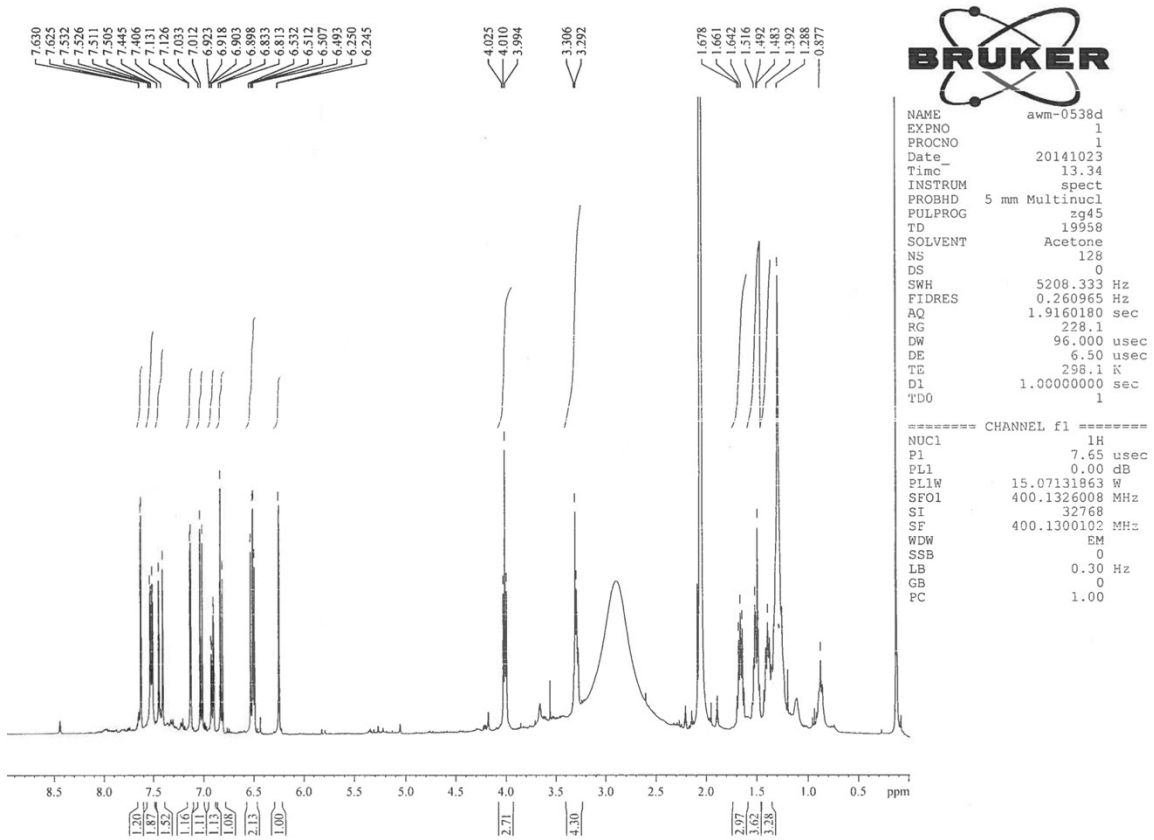
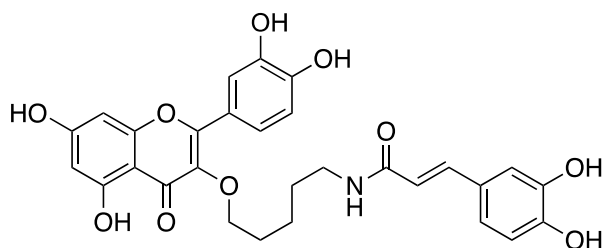


Figure S15. <sup>1</sup>H NMR of M01



(*E*)-3-(3,4-Dihydroxyphenyl)-*N*-(5-((2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)oxy)pentyl)acrylamide (**M02**). Pentyl amine (**4 Pentyl**) (108 mg, 0.165 mmol) was added to a 5 mL RB flask. DCM (2 mL) and pyridine (20  $\mu$ L, 0.247 mmol) were added followed by pentafluorophenyl caffeic ester **1** (63 mg, 0.181 mmol) in THF (1 mL). The reaction mixture was stirred at rt for 23 hours then diluted with DCM and the reaction washed with 3 x 1 M HCl, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was purified by silica gel chromatography with 78:10:10:2 DCM/Acetone/MeOH/H<sub>2</sub>O to yield the intermediate (**4.1 Pentyl**) as a yellow solid (100 mg, 0.122 mmol, 74% yield). This material was then dissolved in anhydrous DCM and added to a 5 mL RB flask kept under nitrogen. The mixture was cooled to -78°C in an acetone dry ice bath. 488  $\mu$ L (0.488 mmol) of 1 M BBr<sub>3</sub> in DCM was added. After 2.5 hours another 488  $\mu$ L aliquot of 1 M BBr<sub>3</sub> was added for a total of 0.976 mmol. The reaction was stirred for 2.5 hours before being warmed to rt. The reaction was quenched with sat. NaHCO<sub>3</sub>, extracted 3 times with EtOAc, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/H<sub>2</sub>O. This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/H<sub>2</sub>O to 95% ACN/ H<sub>2</sub>O over 50 minutes to yield **M02** as yellow powder (11 mg, 0.02 mmol, 16% yield). <sup>1</sup>H NMR (600 MHz, MeOH-d<sub>4</sub>)  $\delta$  7.59 (d, *J* = 2.2 Hz, 1H), 7.50 (dd, *J* = 8.4 Hz, *J* = 2.2 Hz, 1H), 7.38 (d, *J* = 15.7 Hz, 1H), 7.00 (d, *J* = 2.2 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.89 (dd, *J* = 8.3 Hz, *J* = 2.1 Hz, 1H), 6.75 (d, *J* = 8.2, 1H), 6.37 (d, *J* = 2.0, 1H), 6.36 (d, *J* = 15.7 Hz, 1H), 6.18 (d, *J* = 2.0 Hz, 1H), 3.93 (t, *J* = 6.2 Hz, 2H), 3.46 (m, 2H), 1.76 (p, *J* = 7.2 Hz, 2H), 1.56 (p, *J* = 7.4 Hz, 2H), 1.48 (p, *J* = 7.2 Hz, 2H); <sup>13</sup>C NMR (HSQC, 600 MHz, MeOH-d<sub>4</sub>)  $\delta$  142.2 122.4, 122.0, 118.2,

116.7, 116.3, 116.2, 114.8, 99.8, 94.7, 73.7, 40.4, 30.7, 30.0, 24.5; ESI-MS: m/z: 572 [M+Na]<sup>+</sup>, 548 [M-H]<sup>-</sup>; HRMS (ESI-TOF): m/z calc'd for C<sub>29</sub>H<sub>27</sub>NO<sub>10</sub>Na: 572.1527 [M+Na]<sup>+</sup>; found: 572.1518

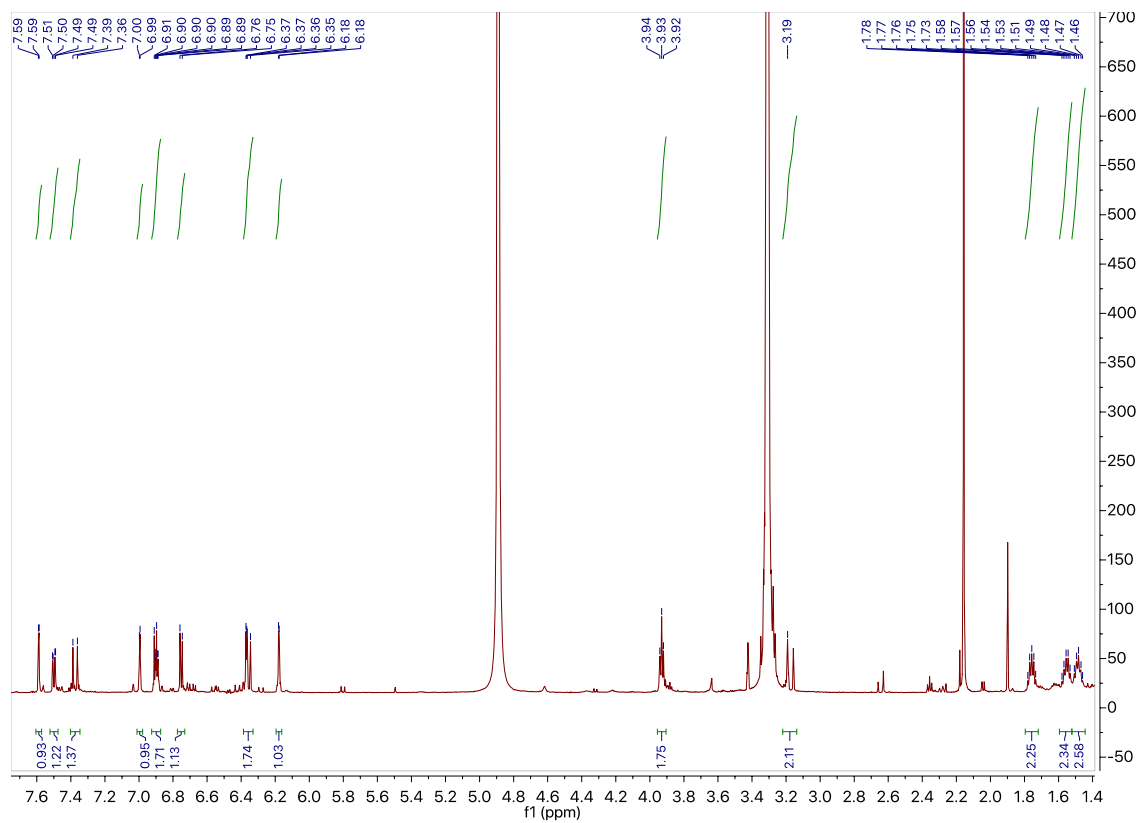


Figure S16. <sup>1</sup>H NMR of M02



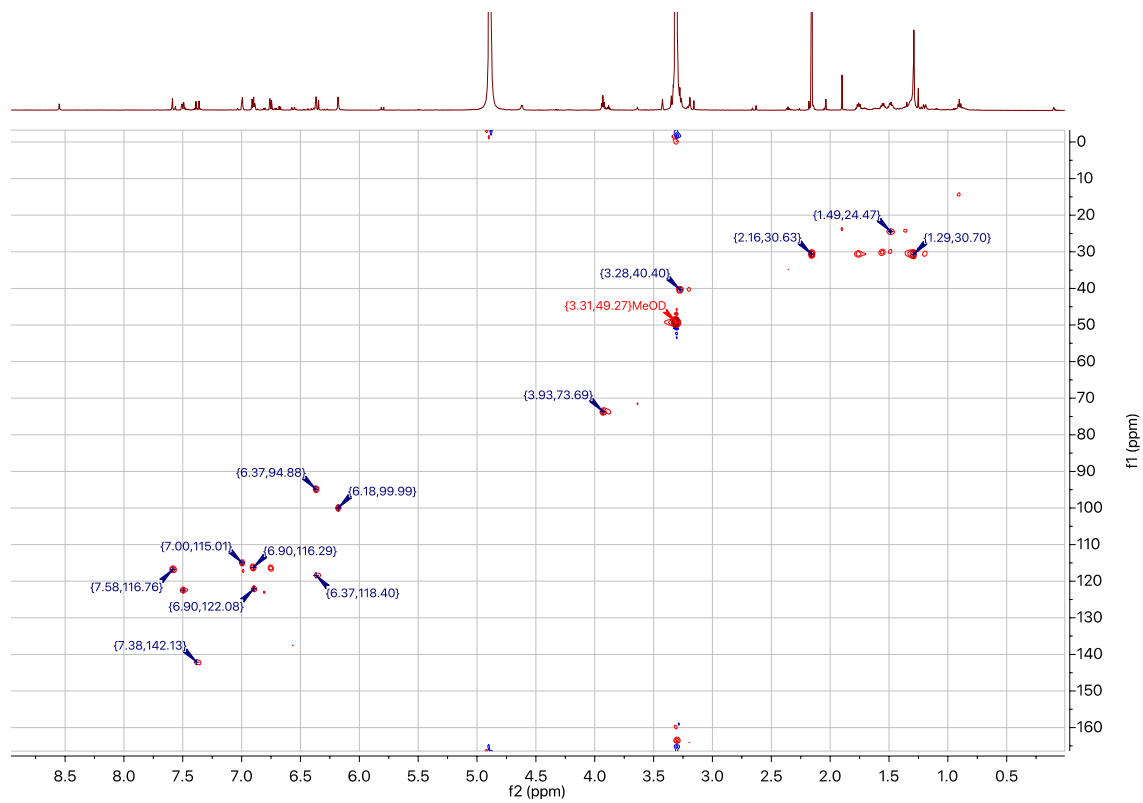
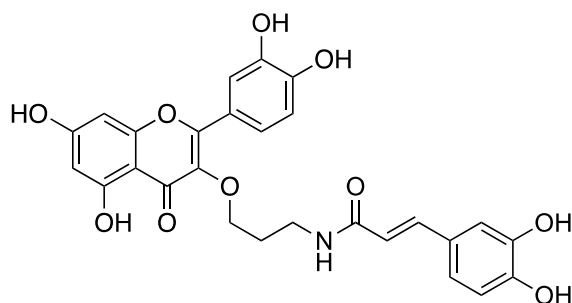
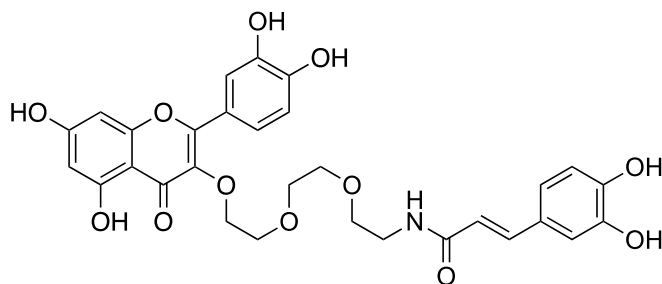


Figure S17. HSQC NMR of M02



(*E*)-3-(3,4-Dihydroxyphenyl)-*N*-(3-((2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)oxy)propyl)acrylamide (**M03**). (150 mg, 0.238 mmol) of Propyl amine (**4 Propyl**) was added to a 10 mL RB flask. DCM (2 mL) was added. Pyridine (29  $\mu$ L, 0.358 mmol) was added. Pentafluorocaffeic ester **1** (91 mg, 0.262 mmol) in THF (1 mL) was added to this solution, then stirred at rt for 23 hours. The reaction was diluted with DCM and the reaction was washed with 3 x 1 M HCl, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was purified by silica gel chromatography with 78:10:10:2 DCM/Acetone/MeOH/H<sub>2</sub>O to give intermediate (**4.1 Propyl**) as a yellow solid (122 mg, 0.154 mmol, 65% yield) . This material was then dissolved in anhydrous DCM and added to a 5 mL RB flask kept under nitrogen. The mixture was cooled to – 78°C in an acetone dry ice bath. 616  $\mu$ L (0.616 mmol) of BBr<sub>3</sub> in 1 M DCM was added. After 2.5 hours another 616  $\mu$ L aliquot of 1 M BBr<sub>3</sub> was added for a total of 1.232 mmol. The reaction was stirred for 2.5 hours before being warmed to rt. The reaction was quenched with sat. NaHCO<sub>3</sub>. The mixture was extracted 3 times with EtOAc, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/H<sub>2</sub>O. This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/H<sub>2</sub>O to 95% ACN/ H<sub>2</sub>O over 50 minutes to give of **M03** as yellow powder (10 mg, 0.019 mmol, 12% yield). <sup>1</sup>H NMR (600 MHz, MeOH-d<sub>4</sub>)  $\delta$  7.58 (d, *J* = 2.0 Hz, 1H), 7.47 (dd, *J* = 8.4 Hz, *J* = 2.2 Hz, 1H), 7.38 (d, *J* = 15.2 Hz, 1H), 7.03 (d, *J* = 2.2 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.87 (dd, *J* = 8.4 Hz, *J* = 2.1 Hz, 1H), 6.75 (d, *J* = 8.2, 1H), 6.37 (d, *J* = 2.0, 1H), 6.34 (d, *J* = 15.7 Hz, 1H), 6.17 (d, *J* = 2.0 Hz, 1H), 3.93 (m, 2H), 3.19 (m, 2H), 1.79 (p, *J* = 7.2 Hz, 2H); <sup>13</sup>C NMR (HSQC, 600 MHz, MeOH-d<sub>4</sub>)  $\delta$

142.5, 122.2, 122.0, 118.2, 116.7, 116.3 (x2), 114.8, 99.8, 94.7, 73.7, 40.9, 36.7;  
ESI-MS: m/z: 544 [M+Na]<sup>+</sup>, 520 [M-H]<sup>-</sup>; HRMS (ESI-TOF): m/z calc'd for  
C<sub>27</sub>H<sub>23</sub>NO<sub>10</sub>Na: 544.1214 [M+Na]<sup>+</sup>; found: 544.1236



**M04.** Azide **3 TEG** (70 mg, 0.095 mmol) was dissolved in THF (750  $\mu$ L), then NaOH aq (0.05 M, 150  $\mu$ L) was added to give a yellow clear solution. A solution of  $\text{PMe}_3$  in THF (1 M, 480  $\mu$ L, 0.48 mmol) was added and the reaction sealed and stirred vigorously for 23 hrs. The reaction mixture was diluted with DCM (5 mL) and washed with water (5 mL). The aqueous layer was back-extracted with DCM (3 x 5 mL), and the combined organics were dried over  $\text{MgSO}_4$ , filtered and concentrated to yield crude amine (**4 TEG**). The crude amine was dissolved in anhydrous DMF (1 mL) and dry pyridine (100  $\mu$ L), then pentafluorophenyl caffeate **1** (1.2 eq, 45 mg, 0.13 mmol) added and the reaction mixture stirred vigorously at 40  $^\circ\text{C}$  for 5 days. The reaction mixture was stirred vigorously at 40  $^\circ\text{C}$  for 5 days. The reaction was diluted with ethyl acetate (10 mL) and washed with 0.5 M HCl (10 mL), and brine (2 x 10 mL). The organic layer was dried over  $\text{MgSO}_4$ , filtered and concentrated. The material was purified by silica gel chromatography, using a gradient of DCM/[DCM/MeOH/EtOH/ $\text{NH}_3$  aq 30%], 200/1 – 10/1 to give amide (**4.1 TEG**), in 65% yield over 2 steps from azide (**3 TEG**).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  12.61 (s, 1H), 7.97 (s, 2H), 7.46 - 7.28 (m, 21H), 7.07 (d,  $J = 2.0$  Hz, 1H), 7.00 (d,  $J = 8.4$  Hz), 6.77 (m, 4H), 6.44 (d,  $J = 8.0$  Hz, 1H), 6.40 (d,  $J = 2.0$  Hz, 1H), 6.14 (d,  $J = 16.0$  Hz, 1H), 5.20 (s, 5H), 5.09 (s, 3H), 4.14 (m, 2H), 3.68 (m, 2H), 3.48 (m, 4H), 3.44 (m, 2H), 2.17 (m, 2H).  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  178.8, 167.2, 164.2, 161.7, 156.8, 156.6, 151.7, 148.4, 146.9, 144.4, 141.4, 137.8, 137.0, 136.6, 135.8, 128.9, 128.8, 128.7, 128.5, 128.2 (x2), 127.6 (x2), 127.4 (x2), 123.4, 123.1, 122.3, 117.8, 115.5, 115.4, 113.8, 106.1, 99.0, 93.3, 71.7 (x2), 70.9, 70.6, 70.5, 70.3, 70.0 (x2), 39.5. HRMS (ESI-TOF):  $m/z$  calc'd for  $\text{C}_{51}\text{H}_{47}\text{NO}_{12}\text{Na}$ : 888.2996  $[\text{M}+\text{Na}]^+$ ; found: 888.3014

Under argon, amide (**4.1 TEG**) (53 mg, 0.061 mmol) was dissolved in dry DCM

(1.2 mL), cooled to -78 °C for 5- 10 mins and a solution of BBr<sub>3</sub> in DCM (1 M, 230 uL, 0.23 mmol) was added. After vigorous stirring for an hour a second portion of BBr<sub>3</sub> (200 uL, 0.20 mmol) was added to make a total of 7 equivalents BBr<sub>3</sub>. The reaction mixture was kept at -78 °C for another 4.5 hrs with vigorous stirring. The reaction was quenched with sat. NaHCO<sub>3</sub>. The mixture was extracted 3 times with EtOAc, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/H<sub>2</sub>O. This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/H<sub>2</sub>O to 95% ACN/ H<sub>2</sub>O over 50 minutes to yield **M04** as yellow powder. HRMS (ESI-TOF): m/z calc'd for C<sub>30</sub>H<sub>29</sub>NO<sub>12</sub>Na: 618.1587 [M+Na]<sup>+</sup>; found: 618.1578.

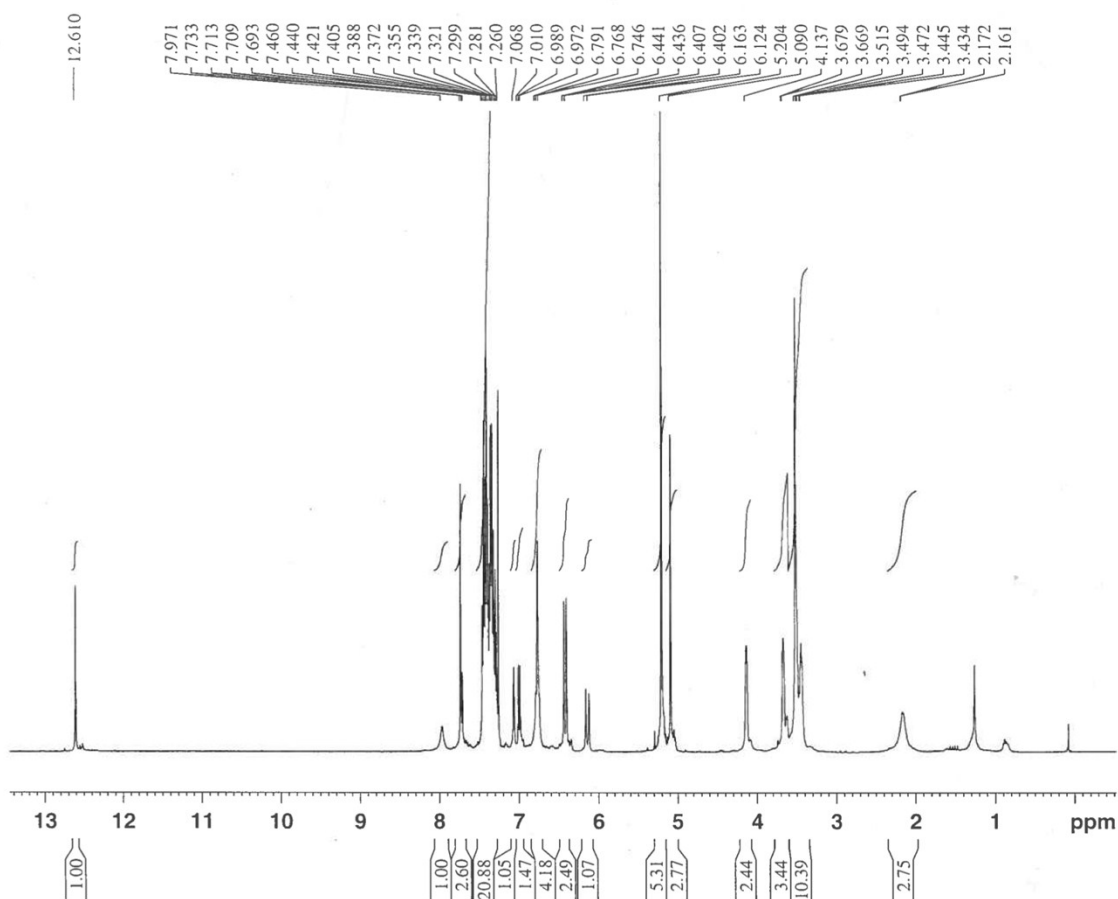


Figure S18. <sup>1</sup>H NMR of intermediate (4.1 TEG)

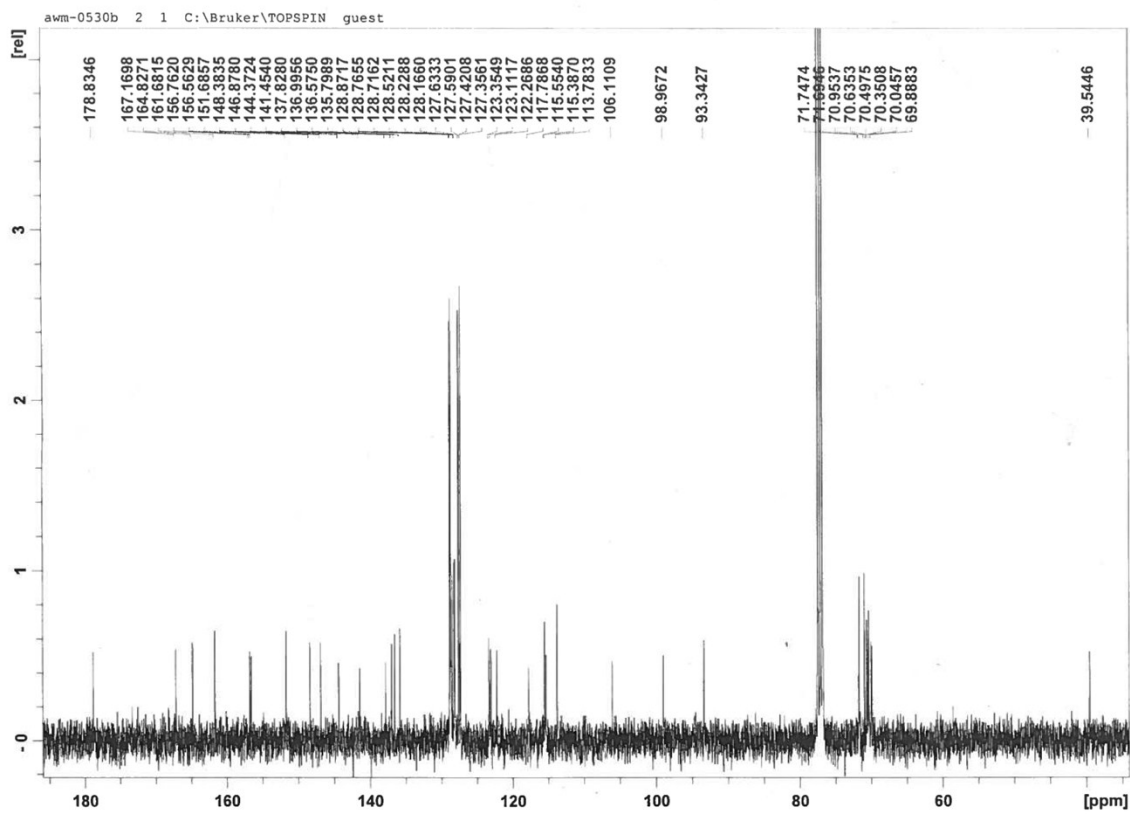
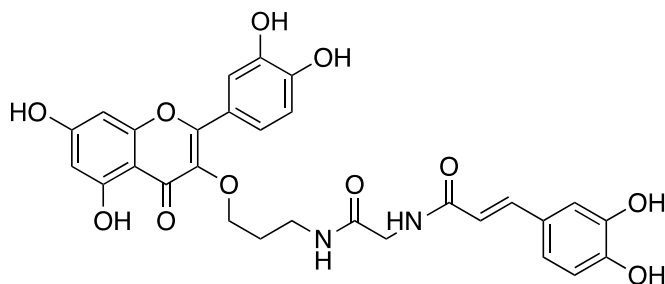


Figure S19. <sup>13</sup>C NMR of intermediate (4.1 TEG)

**General procedure for synthesis of 4 via use of PFP activated amino acids.**

Propyl amine (**4 Propyl**) (0.5 g, 0.793 mmol) was added to a 10 mL RB flask and dissolved in DCM (5 mL). Pyridine (128  $\mu$ L, 1.586 mmol) was added. Fmoc-L-Xaa-PFP with appropriate side chain protecting groups (0.872 mmol) was dissolved in DCM (1 mL) and this solution was added to the reaction. The mixture was stirred at room temperature overnight. The reaction was then washed with 3 x 1 M HCl, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was purified by silica gel column chromatography using an eluent system of 78:2:10:10 DCM/H<sub>2</sub>O/Acetone/MeOH. An average of 0.674 mmol (85% yield) of **5** was isolated as a yellow solid.

**General Procedure for synthesis of (4.1).** Fmoc deprotection of **5**: 0.5 mmol of **5** was dissolved in DCM (2 mL). Piperidine (0.5 mL) was added. The mixture was stirred for 1.5 hr at room temperature. The reaction was quenched with 1 M HCl and concentrated *in vacuo*. This material was then dissolved in DCM (4 mL), added to a 10 mL RB flask and pyridine (60  $\mu$ L, 0.75 mmol) added. Pentafluorocaffeic ester **1** (190 mg, 0.55 mmol) dissolved in THF (1 mL) was added to the reaction. The reaction was stirred overnight at room temperature. The reaction was then washed with 3 x 1 M HCl, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was purified by silica gel column chromatography using an eluent system of 78:2:10:10 DCM/H<sub>2</sub>O/Acetone/MeOH. An average of 0.325 mmol (65% yield) of **5.1** was isolated as a yellow solid.



(*E*)-3-(3,4-Dihydroxyphenyl)-*N*-(2-((3-((2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)oxy)propyl)amino)-2-oxoethyl)acrylamide (**M05**). **5.1 Gly** (152 mg, 0.179 mmol) was dissolved in anhydrous DCM and added to a 5 mL RB flask kept under nitrogen. The mixture was cooled to  $-78^{\circ}\text{C}$  in an acetone dry ice bath then 717  $\mu\text{L}$  (0.717 mmol) of 1 M  $\text{BBr}_3$  in DCM was added. After 2.5 hours another 717  $\mu\text{L}$  aliquot of  $\text{BBr}_3$  was added for a total of 1.433 mmol. The reaction was stirred for 2.5 hours before being warmed to rt then quenched with sat.  $\text{NaHCO}_3$ . The mixture was extracted 3 times with EtOAc, dried over  $\text{MgSO}_4$ , and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/ $\text{H}_2\text{O}$ . This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/ $\text{H}_2\text{O}$  to 95% ACN/ $\text{H}_2\text{O}$  over 50 minutes to give **M05** as yellow powder (5.2 mg, 0.0089 mmol, 5% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{MeOH-d}_4$ )  $\delta$  7.75 (d,  $J = 8.7$  Hz, 1H), 7.64 (d,  $J = 8.6$  Hz, 1H), 7.33 (m, 4H), 7.15 (d,  $J = 15.2$  Hz, 1H), 7.03 (d,  $J = 8.7$  Hz, 1H), 6.89 (d,  $J = 8.6$  Hz, 1H), 6.38 (d,  $J = 16.0$  Hz, 1H), 4.20 (t, 1H) 3.16 (d,  $J = 2.6$  Hz, 1H), 2.77 (m, 2H), 1.76 (m, 2H), 1.50 (m, 2H);  $^{13}\text{C}$  NMR (HSQC, 600 MHz,  $\text{MeOH-d}_4$ )  $\delta$  146.7, 122.9, 118.3, 117.9, 116.4, 115.6, 115.2, 115.0, 88.1, 61.4, 49.2, 28.1 26.9; ESI-MS:  $m/z$ : 601  $[\text{M}+\text{Na}]^+$ , 577  $[\text{M}-\text{H}]^-$ ; HRMS (ESI-TOF):  $m/z$  calc'd for  $\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_{11}\text{Na}$ : 601.1429  $[\text{M}+\text{Na}]^+$ ; found: 601.1447



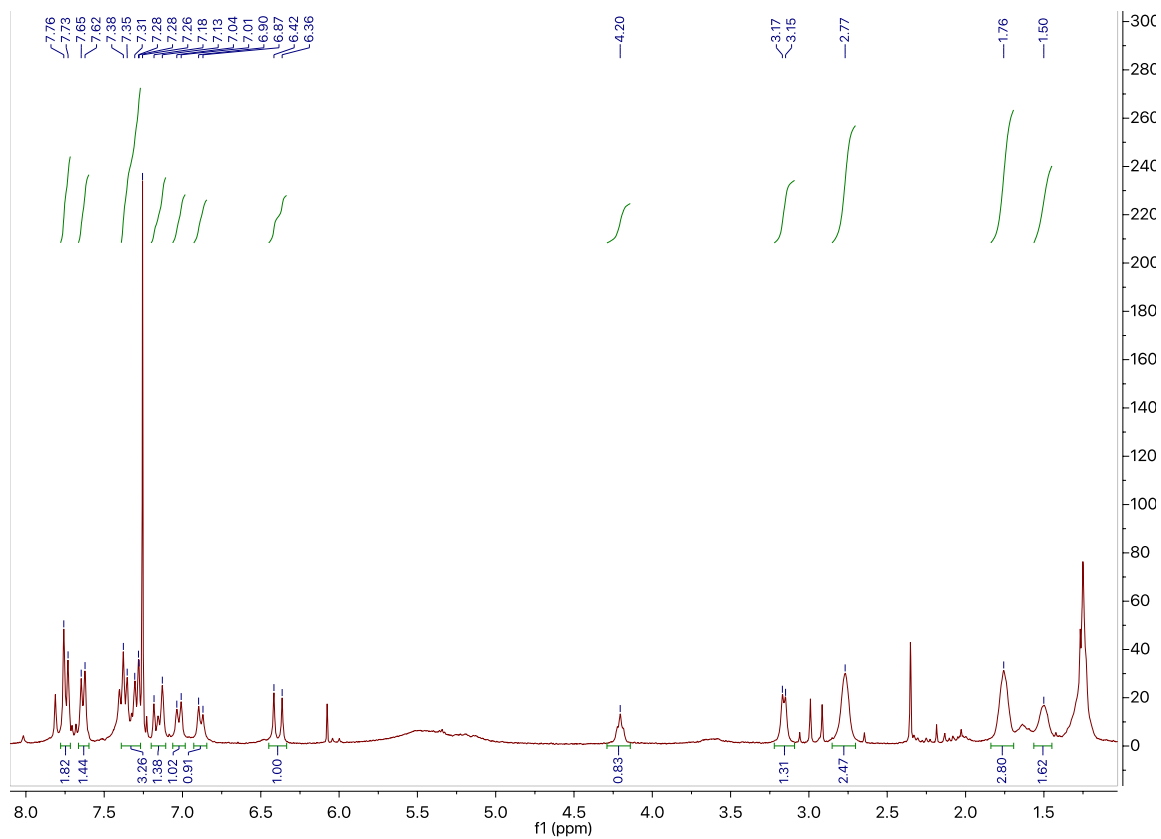


Figure S20. <sup>1</sup>H NMR of M05

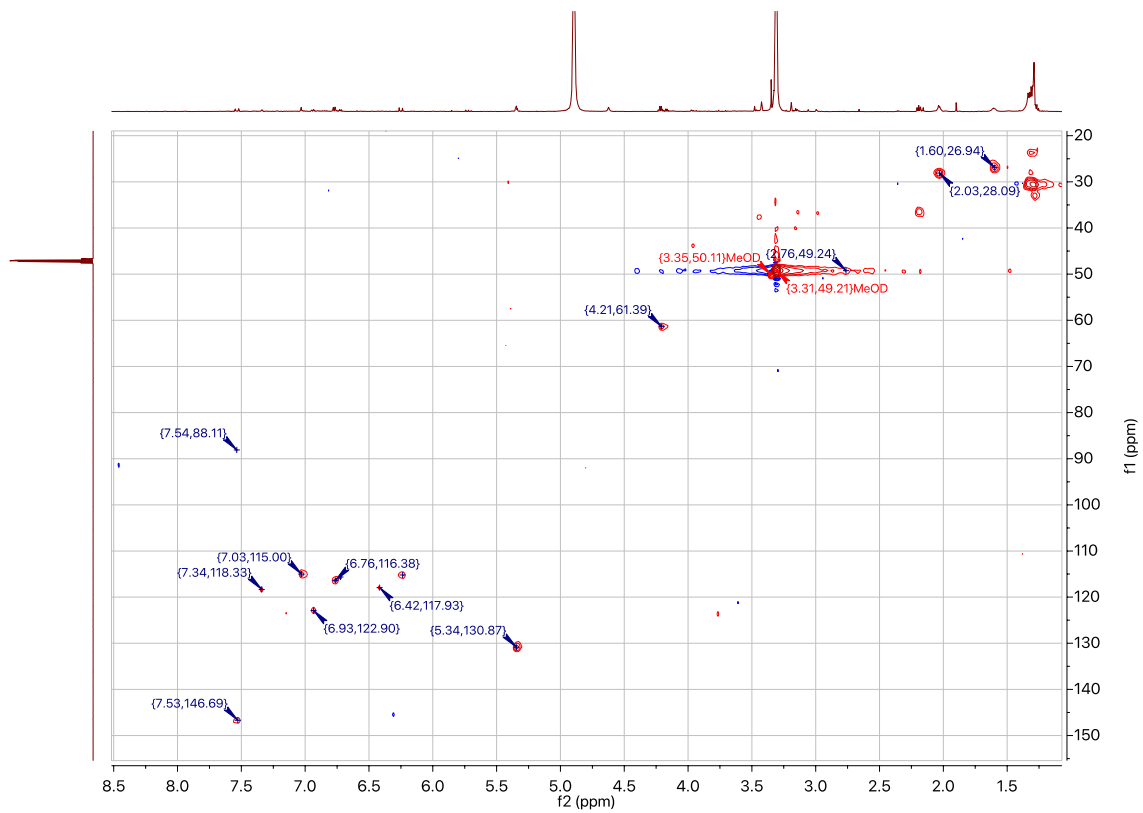
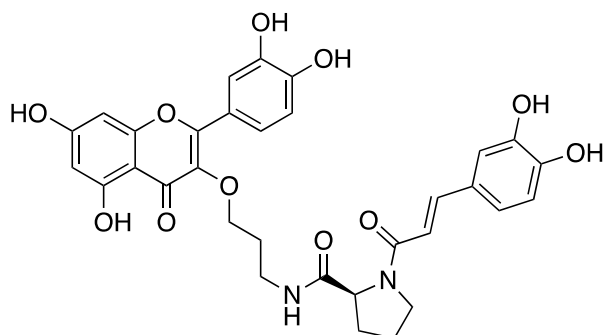


Figure S21. HSQC of M05



(*S,E*)-*N*-(3-((2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)oxy)propyl)-1-(3-(3,4-dihydroxyphenyl)acryloyl)pyrrolidine-2-carboxamide (**M06**). **5.1 Pro** (67 mg, 0.075 mmol) was dissolved in anhydrous DCM and added to a 5 mL RB flask kept under nitrogen. The mixture was cooled to  $-78^{\circ}\text{C}$  in an acetone dry ice bath. 302  $\mu\text{L}$  (0.302 mmol) of 1 M  $\text{BBr}_3$  in DCM was added. After 2.5 hours another 302  $\mu\text{L}$  aliquot of 1 M  $\text{BBr}_3$  was added for a total of 0.604 mmol. The reaction was stirred for 2.5 hours before being warmed to rt. Then quenched with sat.  $\text{NaHCO}_3$ . The mixture was extracted 3 times with EtOAc, dried over  $\text{MgSO}_4$ , and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/ $\text{H}_2\text{O}$ . This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/ $\text{H}_2\text{O}$  to 95% ACN/  $\text{H}_2\text{O}$  over 50 minutes to give **M06** as yellow powder (9.3 mg, 0.015 mmol, 20% yield).  $^1\text{H}$  NMR (600 MHz, MeOH- $d_4$ )  $\delta$  7.62 (d,  $J$  = 1.8 Hz, 2H), 7.46 (dd,  $J$  = 8.3 Hz,  $J$  = 2.0 Hz, 1H), 7.24 (d,  $J$  = 15.4 Hz, 1H), 6.89 (1H), 6.73 (1H), 6.60 (2H), 6.49 (d,  $J$  = 15.6 Hz, 1H), 6.18 (d,  $J$  = 2.0 Hz, 1H), 6.13 (d,  $J$  = 2.1 Hz), 4.52 (dd,  $J$  = 8.7 Hz,  $J$  = 3.2 Hz, 1H), 3.87 (m, 4H), 3.72 (m, 2H), 2.21 (m, 2H), 2.07 (m, 2H), 1.87 (m, 2H);  $^{13}\text{C}$  NMR (150 MHz, MeOH- $d_4$ )  $\delta$  180.2, 174.4, 168.1, 166.0, 162.7, 158.2, 150.0, 149.1, 146.5, 146.4, 144.7, 138.7, 127.8, 123.0, 122.3, 122.2, 116.5, 116.5, 116.3, 115.3, 115.0, 105.8, 99.9, 94.9, 94.8, 72.3, 62.5, 49.9, 38.4, 30.8, 30.4, 25.6; ESI-MS:  $m/z$ : 641  $[\text{M}+\text{Na}]^+$ , 617  $[\text{M}-\text{H}]^-$ ; HRMS (ESI-TOF):  $m/z$  calc'd for  $\text{C}_{32}\text{H}_{30}\text{N}_2\text{O}_{11}\text{Na}$ : 641.1742  $[\text{M}+\text{Na}]^+$ ; found: 641.1747

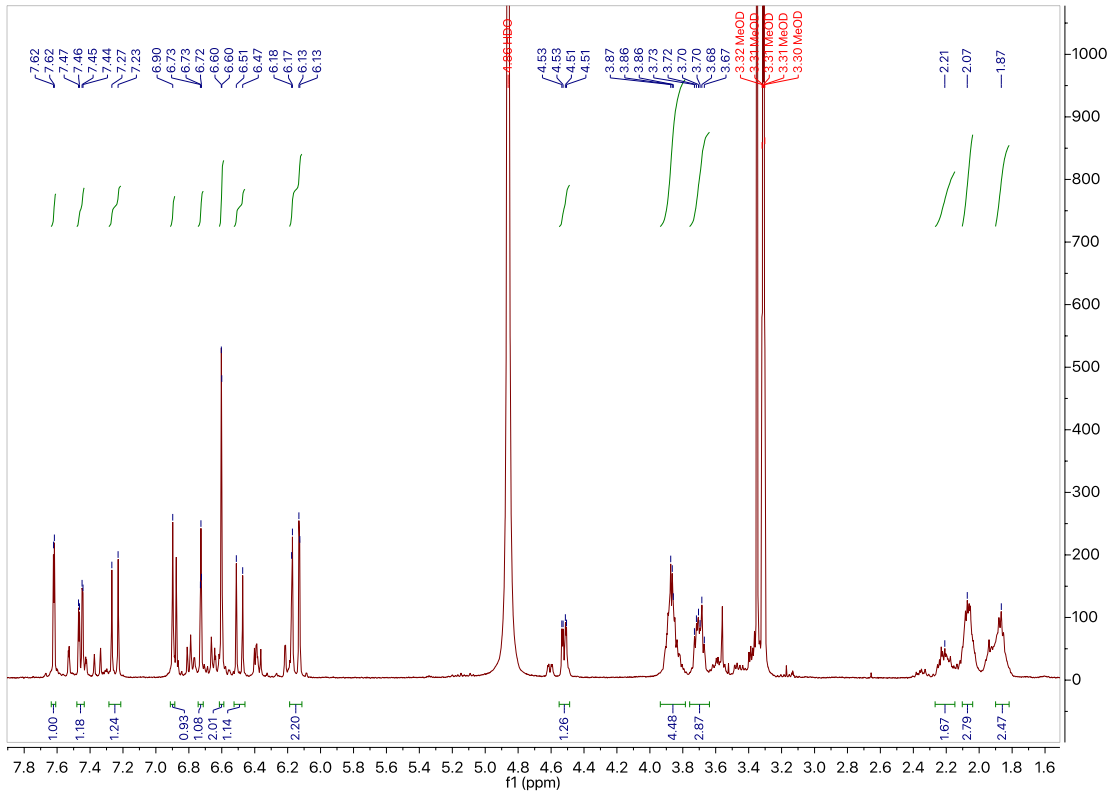


Figure S22.  $^1\text{H}$  NMR of M06

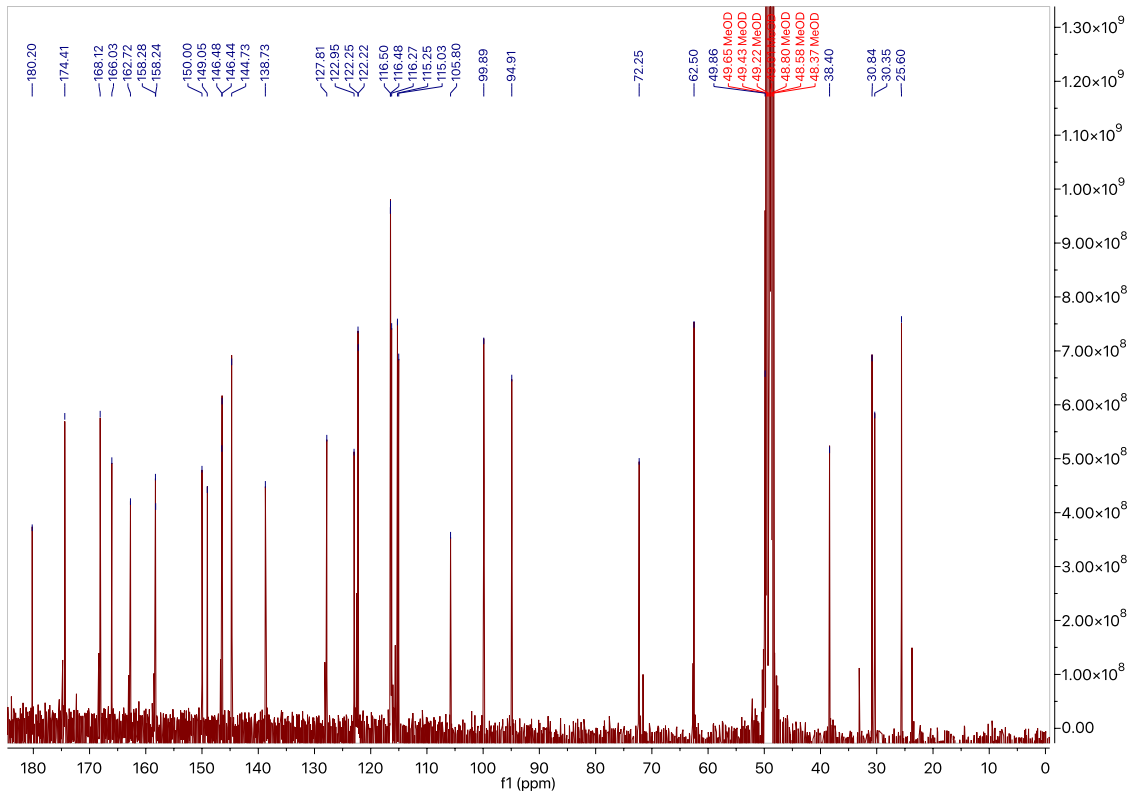


Figure S23. <sup>13</sup>C NMR of M06

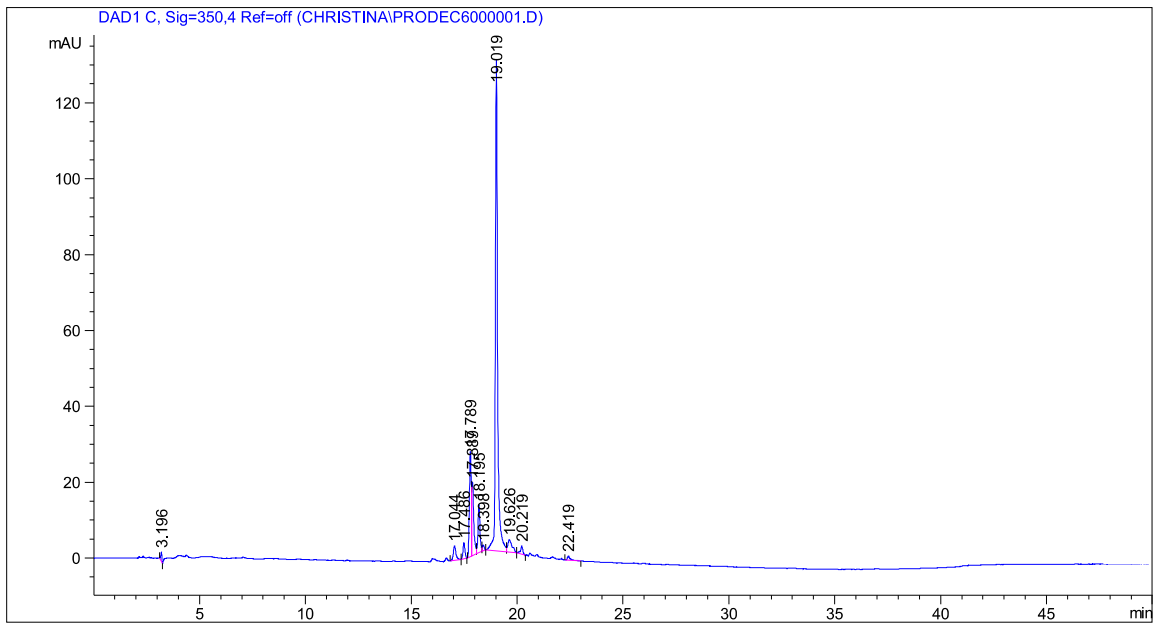
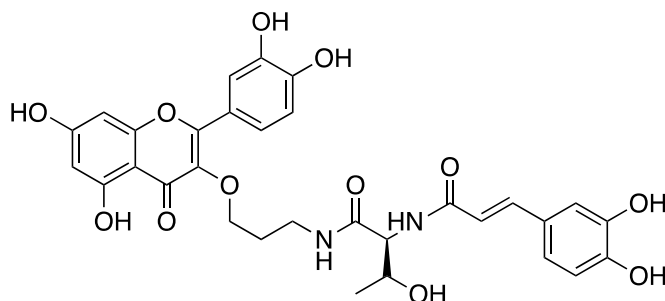


Figure S24. HPLC of M06 used for X-ray crystallography



(2S,3R)-N-(3-((2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl)oxy)propyl)-2-((E)-3-(3,4-dihydroxyphenyl)acrylamido)-3-hydroxybutanamide (**M07**). **5.1 Thr** (36 mg, 0.038 mmol) was dissolved in anhydrous DCM and added to a 5 mL RB flask kept under nitrogen. The mixture was cooled to -78°C in an acetone dry ice bath. 152  $\mu$ L (0.152 mmol) of 1 M BBr<sub>3</sub> in DCM was added. After 2.5 hours another 152  $\mu$ L aliquot of 1 M BBr<sub>3</sub> was added for a total of 0.304 mmol. The reaction was stirred for 2.5 hours before being warmed to rt. The reaction was quenched with sat. NaHCO<sub>3</sub>. The mixture was extracted 3 times with EtOAc, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 7:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/H<sub>2</sub>O. This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/H<sub>2</sub>O to 95% ACN/ H<sub>2</sub>O over 50 minutes to give **M07** as yellow powder (3.5 mg, 0.0057 mmol, 15% yield). <sup>1</sup>H NMR (600 MHz, MeOH-d<sub>4</sub>)  $\delta$  7.62 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H), 7.52 (dd, J = 8.5 Hz, J = 2.1 Hz, 1H), 7.37 (d, J = 1.8 Hz, 1H), 7.23 (d, J = 16.0 Hz, 1H), 6.90 (d, J = 8.6 Hz, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.40 (d, J = 15.2 Hz, 1H), 6.38 (d, J = 2.0 Hz, 1H), 6.20 (d, J = 2.1 Hz, 1H), 6.19 (d, J = 1.9 Hz, 1H), 4.27 (d, J = 3.6 Hz, 1H), 3.94 (m, 4H), 3.46 (m, 2H), 1.91 (m, 2H), 1.34 (3H); <sup>13</sup>C NMR (protected material, 150 MHz, MeOH-d<sub>4</sub>)  $\delta$  178.86, 173.69, 164.70, 162.12, 156.81, 156.21, 151.61, 148.57, 143.47, 140.26, 138.14, 138.06, 137.04, 136.68, 135.89, 129.18, 128.87, 128.78, 128.73, 128.50, 128.36, 128.21, 128.14, 127.59, 127.42, 127.34, 127.31, 127.18, 123.35, 122.96, 121.13, 119.87, 115.22, 113.89, 107.91, 106.26, 98.74, 93.20, 77.65, 77.23, 76.81, 74.00, 71.65, 71.00, 70.57, 68.18, 60.51, 55.05, 44.62, 35.98, 29.88, 28.70, 28.42, 22.74, 20.35; ESI-

MS: m/z: 645 [M+Na]<sup>+</sup>, 621 [M-H]<sup>-</sup>; HRMS (ESI-TOF): m/z calc'd for C<sub>31</sub>H<sub>30</sub>N<sub>2</sub>O<sub>12</sub>Na: 645.1691 [M+Na]<sup>+</sup>; found: 645.1680

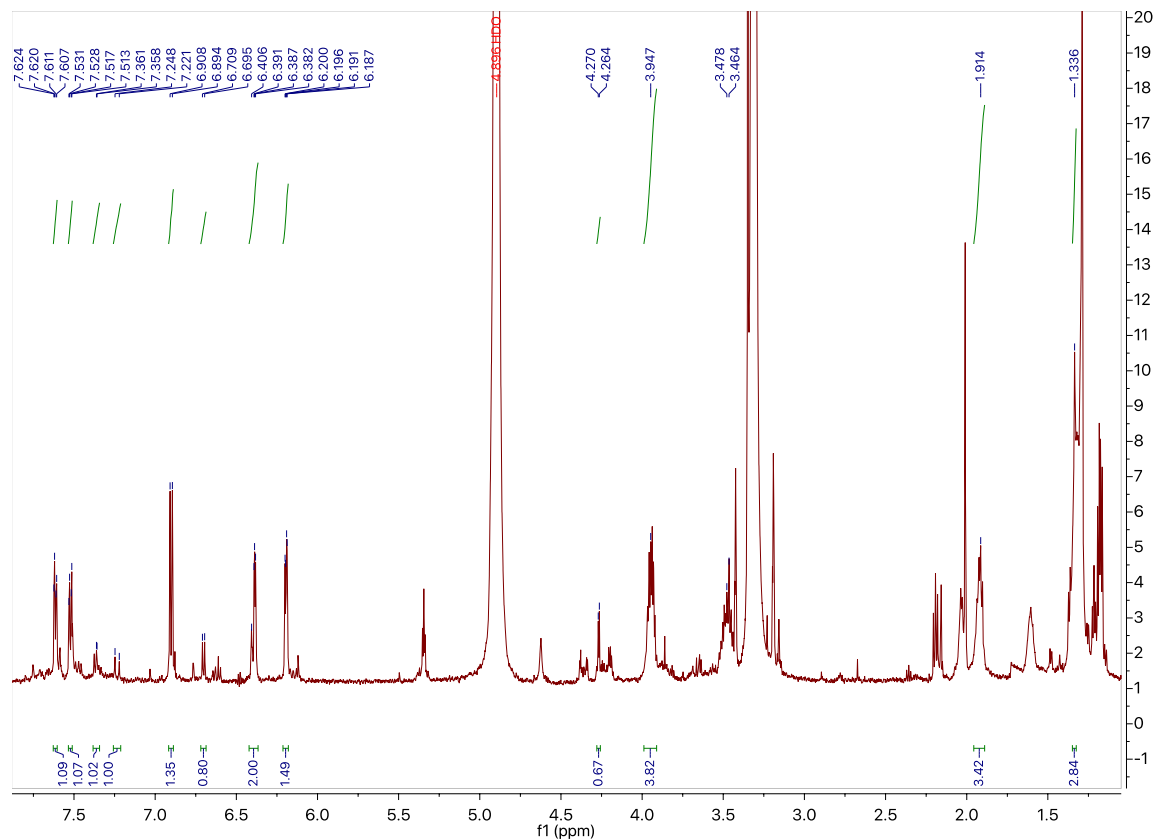


Figure S25. <sup>1</sup>H NMR of M07

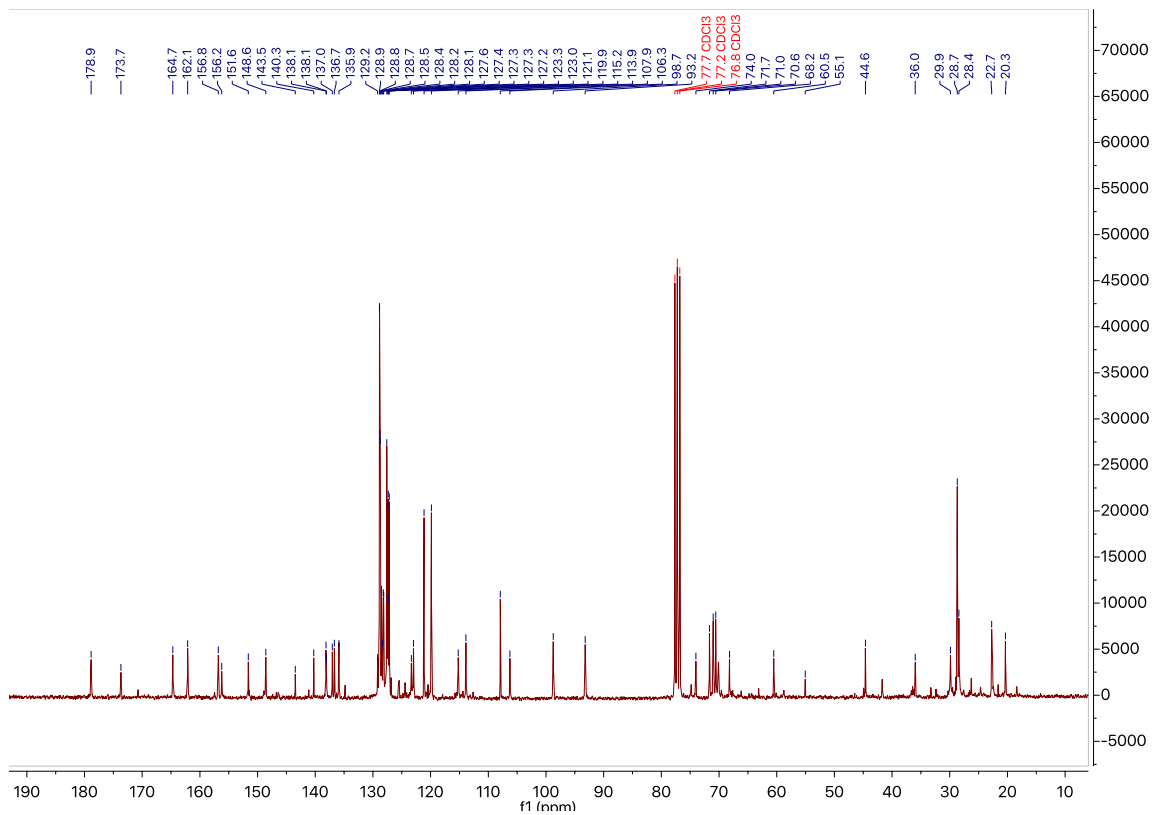
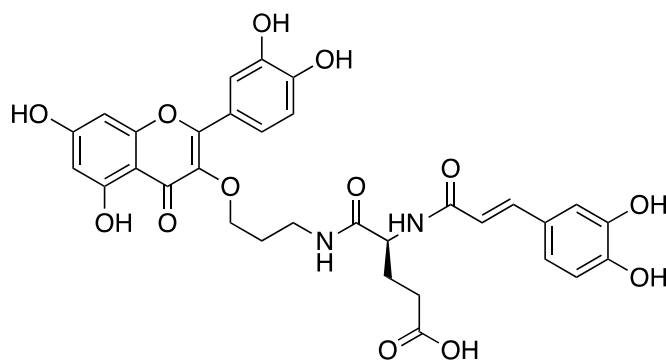


Figure S26.  $^{13}\text{C}$  NMR of intermediate (5.1 Thr)





(*S,E*)-5-((3-((2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)oxy)propyl)amino)-4-(3-(3,4-dihydroxyphenyl)acrylamido)-5-oxopentanoic acid (**M08**). **5.1 Glu** (50 mg, 0.051 mmol) was dissolved in anhydrous DCM and added to a 5 mL RB flask kept under nitrogen. The mixture was cooled to  $-78^{\circ}\text{C}$  in an acetone dry ice bath. 204  $\mu\text{L}$  (0.204 mmol) of 1 M  $\text{BBr}_3$  in DCM was added. After 2.5 hours another 204  $\mu\text{L}$  aliquot of 1 M  $\text{BBr}_3$  was added for a total of 0.408 mmol. The reaction was stirred for 2.5 hours before being warmed to rt. The reaction was quenched with sat.  $\text{NaHCO}_3$ . The mixture was extracted 3 times with EtOAc, dried over  $\text{MgSO}_4$ , and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/ $\text{H}_2\text{O}$ . This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/ $\text{H}_2\text{O}$  to 95% ACN/  $\text{H}_2\text{O}$  over 50 minutes to give of **M08** was obtained as yellow powder (5.2 mg, 0.008 mmol, 16% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{MeOH-d}_4$ )  $\delta$  7.62 (d,  $J = 2.2$  Hz, 1H), 7.45 (dd,  $J = 8.6$  Hz,  $J = 2.1$  Hz, 1H), 7.21 (d,  $J = 15.7$  Hz, 1H), 6.89 (d,  $J = 8.2$  Hz, 1H), 6.78 (d,  $J = 1.8$  Hz, 1H), 6.63 (d,  $J = 8.4$  Hz, 1H), 6.62 (d,  $J = 8.4$  Hz, 1H), 6.31 (d,  $J = 15.6$  Hz, 1H), 6.21 (d,  $J = 2.0$  Hz, 1H), 6.12 (d,  $J = 2.1$  Hz, 1H), 4.35 (dd,  $J = 9.4$  Hz,  $J = 4.5$  Hz, 1H), 3.87 (m, 2H), 3.62 (m, 2H), 2.37 (m, 2H), 2.03 (m, 2H), 1.90 (m, 2H);  $^{13}\text{C}$  NMR (HSQC and HMBC, 600 MHz,  $\text{MeOH-d}_4$ )  $\delta$  179.6, 169.3, 165.3 158.4, 157.7, 149.9, 148.7, 146.3, 142.7, 127.7, 122.9, 122.2, 122.1, 117.6, 116.5, 116.2, 114.9, 105.9, 99.8, 94.7, 72.3, 55.7, 38.2, 34.0, 30.3, 28.9; ESI-MS:  $m/z$ : 673  $[\text{M}+\text{Na}]^+$ , 649  $[\text{M}-\text{H}]^-$ ; HRMS (ESI-TOF):  $m/z$  calc'd for  $\text{C}_{32}\text{H}_{30}\text{N}_2\text{O}_{13}\text{Na}$ : 673.1640  $[\text{M}+\text{Na}]^+$ ; found: 673.1621

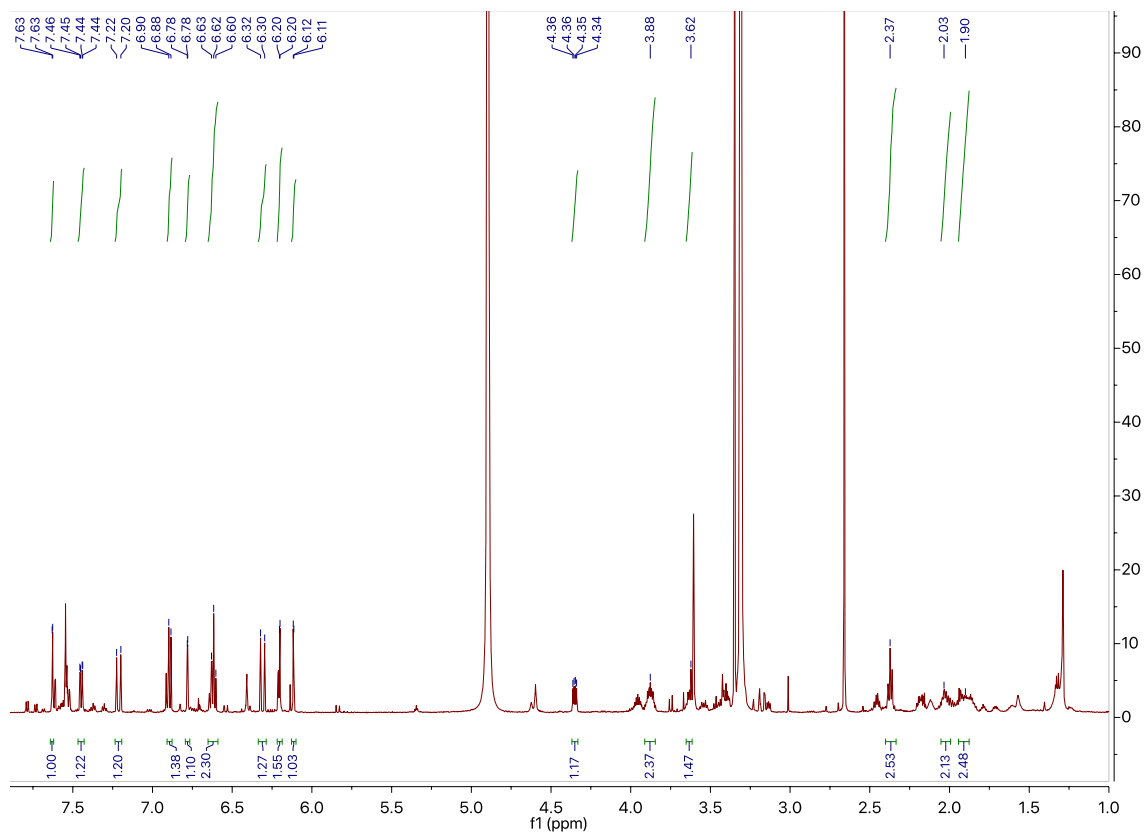


Figure S27. <sup>1</sup>H NMR of M08

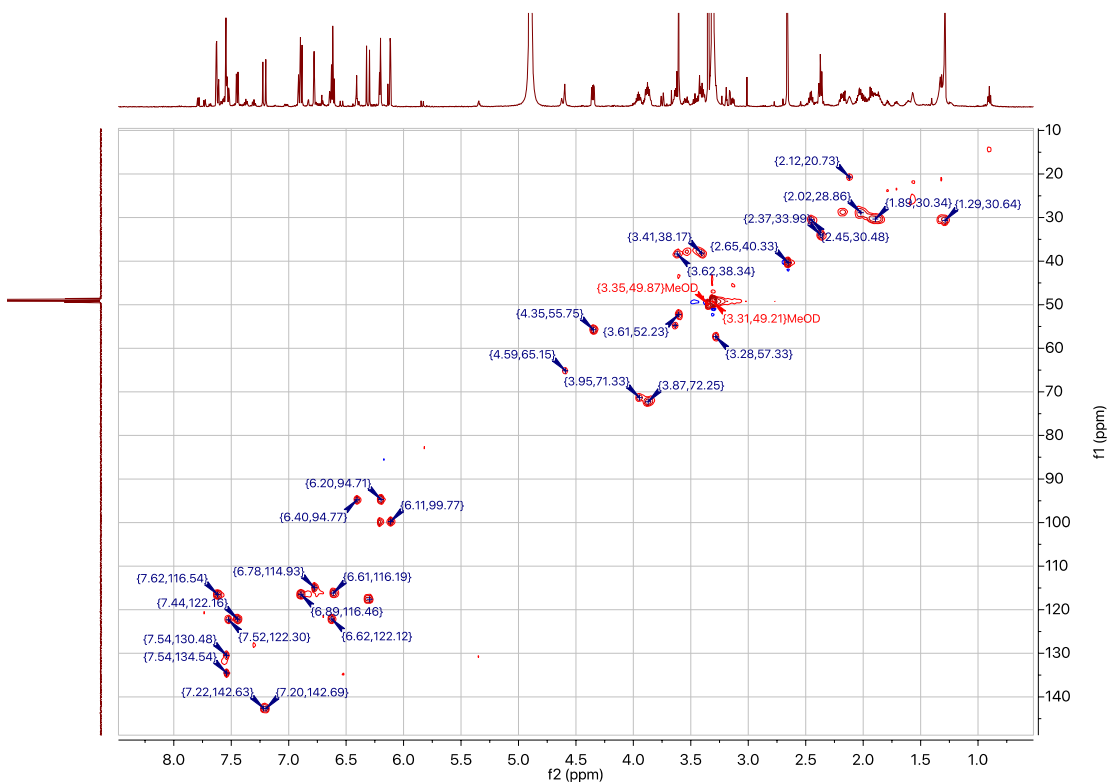


Figure S28. HSQC NMR of M08

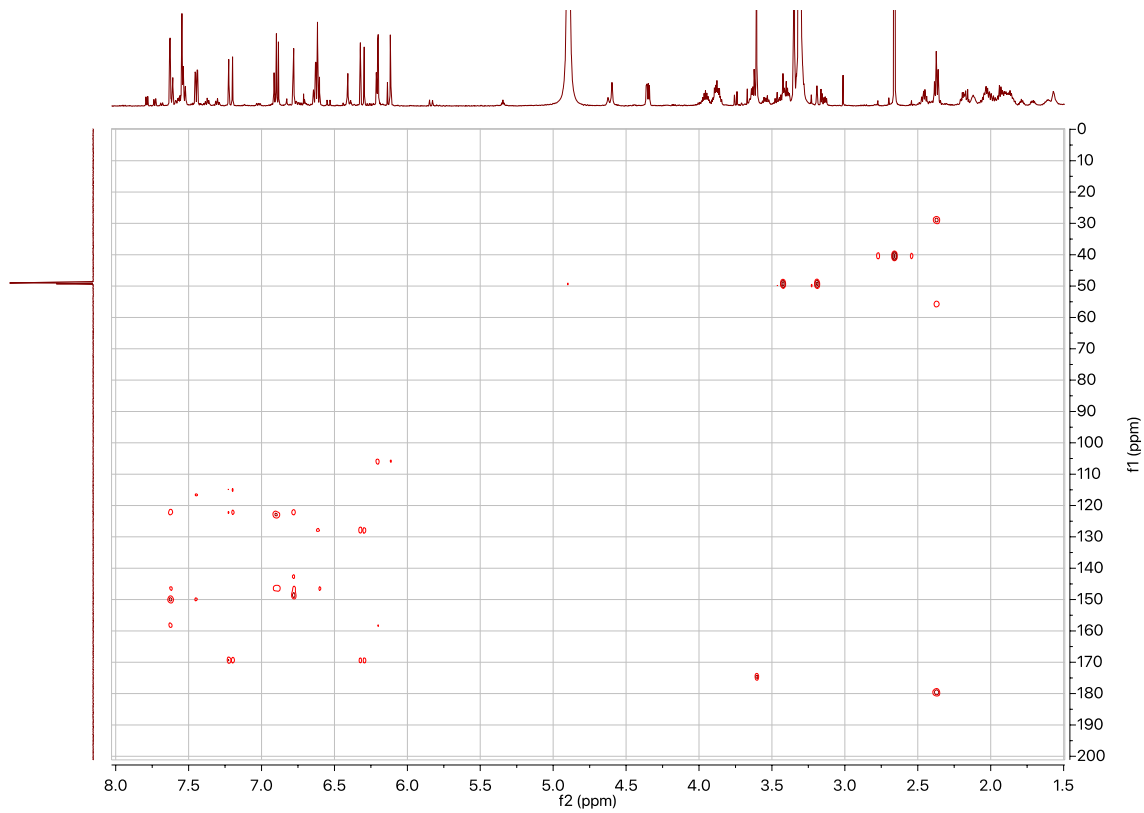
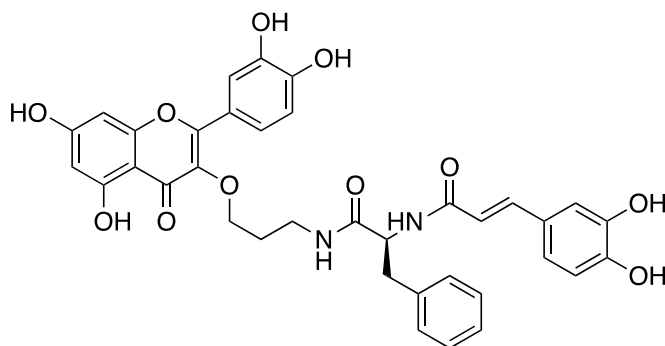


Figure S29. HMBC NMR of M08



(*S,E*)-3-(3,4-Dihydroxyphenyl)-*N*-(1-((3-((2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)oxy)propyl)amino)-1-oxo-3-phenylpropan-2-yl)acrylamide (**M09**). **5.1 Phe** (44 mg, 0.047 mmol) was dissolved in anhydrous DCM and added to a 5 mL RB flask kept under nitrogen. The mixture was cooled to -78°C in an acetone dry ice bath and 187  $\mu$ L (0.187 mmol) of 1 M BBr<sub>3</sub> in DCM was added. After 2.5 hours another 187  $\mu$ L aliquot of 1 M BBr<sub>3</sub> was added for a total of 0.375 mmol. The reaction was stirred for 2.5 hours before being warmed to rt. then quenched with sat. NaHCO<sub>3</sub>. The mixture was extracted 3 times with EtOAc, dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/H<sub>2</sub>O. This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/H<sub>2</sub>O to 95% ACN/ H<sub>2</sub>O over 50 minutes to give **M09** as a yellow powder (7.5 mg, 0.0113 mmol, 24% yield). <sup>1</sup>H NMR (600 MHz, MeOH-d<sub>4</sub>)  $\delta$  7.58 (d, *J* = 2.2 Hz, 1H), 7.46 (dd, *J* = 8.4 Hz, *J* = 2.2 Hz, 1H), 7.25 (m, 2H), 7.21 (m, 3H), 7.13 (m, 1H), 6.88 (d, *J* = 8.5, 1H), 6.80 (d, *J* = 2.1 Hz, 1H), 6.68 (dd, *J* = 8.3 Hz, *J* = 2.1 Hz, 1H), 6.64 (d, *J* = 8.2 Hz, 1H) 6.29 (d, *J* = 15.7 Hz, 1H), 6.21 (d, *J* = 2.0 Hz, 1H), 6.13 (dd, *J* = 2.0 Hz, *J* = 8.2 Hz, 1H), 4.69 (dd, *J* = 8.6 Hz, *J* = 6.4 Hz, 1H), 3.73 (m, 2H), 3.60 (m, 2H), 3.19 (dd, *J* = 13.0 Hz, *J* = 6.6 Hz, 1H), 2.97 (dd, *J* = 12.8 Hz, *J* = 8.6 Hz, 1H), 1.80 (m, 2H); <sup>13</sup>C NMR (HSQC, 600 MHz, MeOH-d<sub>4</sub>)  $\delta$  142.9, 130.1 (x3), 129.9 (x2), 127.8, 122.1, 117.5, 116.5, 116.4, 115.2, 109.3, 100.2, 95.3, 70.6, 58.2, 56.9, 38.8, 30.0; ESI-MS: *m/z*: 691 [M+Na]<sup>+</sup>, 667 [M-H]<sup>-</sup>; HRMS (ESI-TOF): *m/z* calc'd for C<sub>36</sub>H<sub>32</sub>N<sub>2</sub>O<sub>11</sub>Na: 691.1898 [M+Na]<sup>+</sup>; found: 691.1898

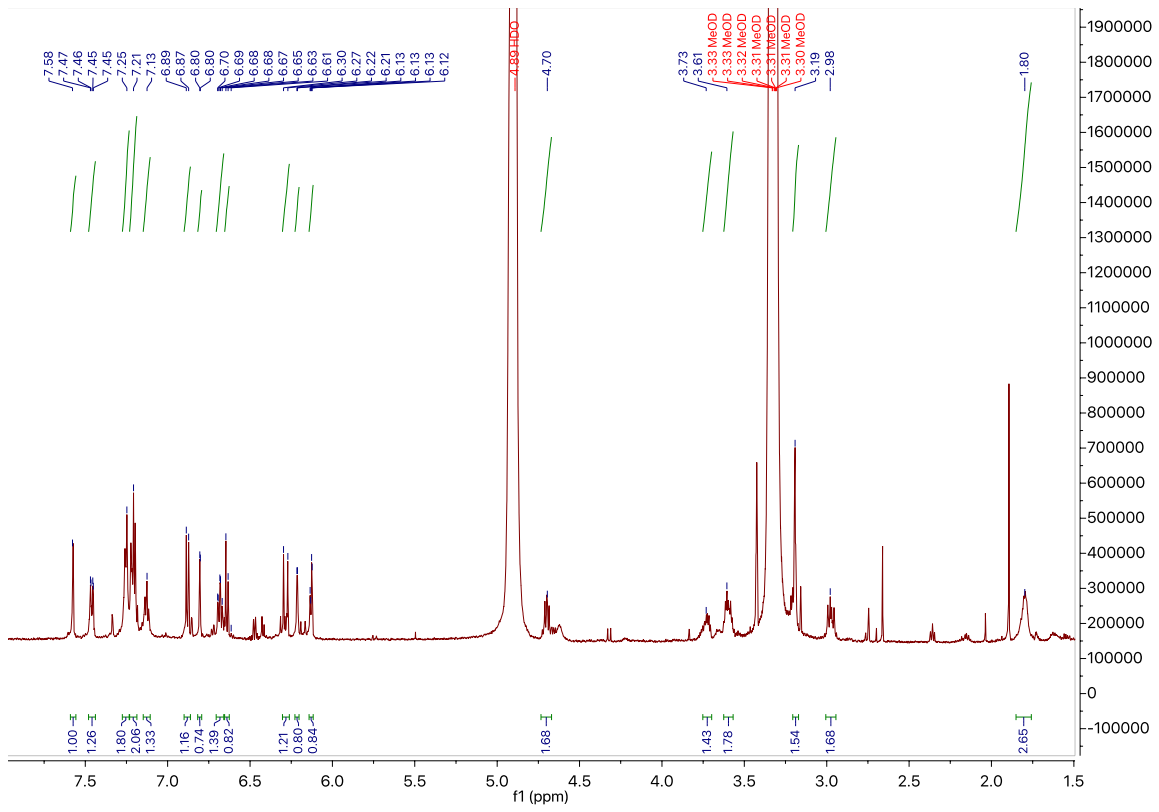


Figure S30. <sup>1</sup>H NMR of M09

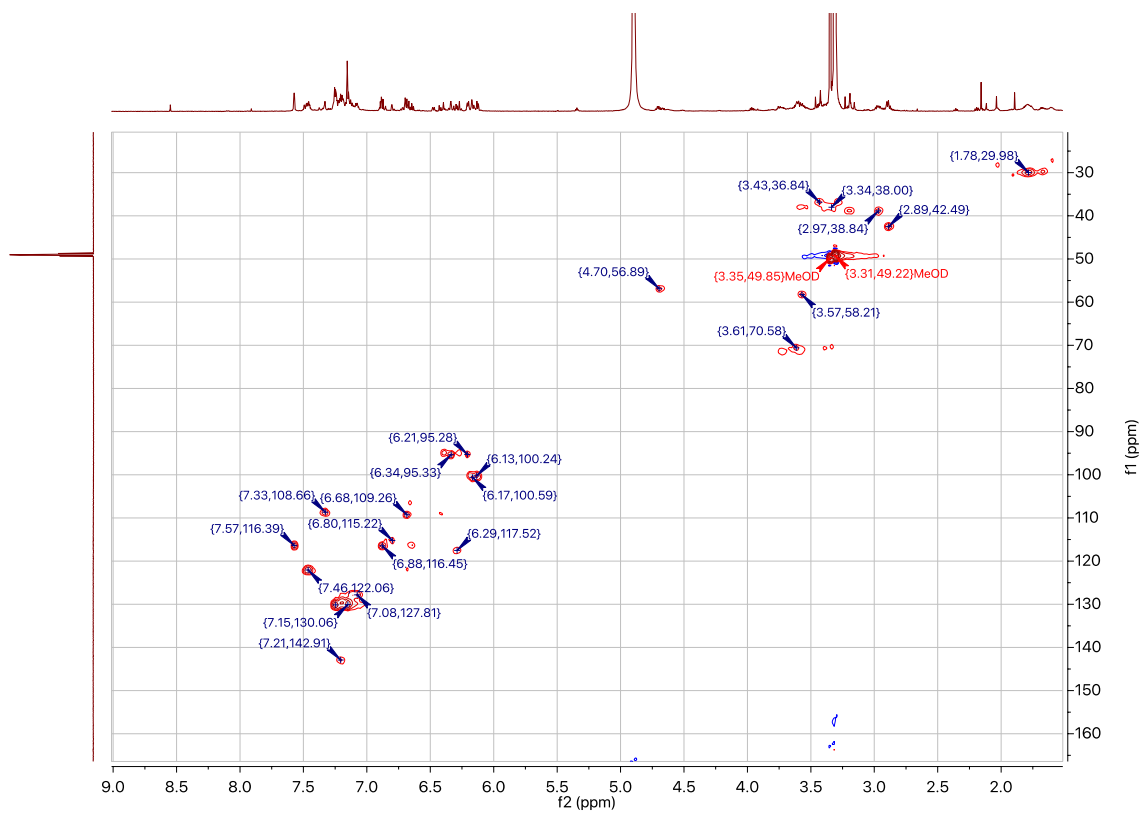
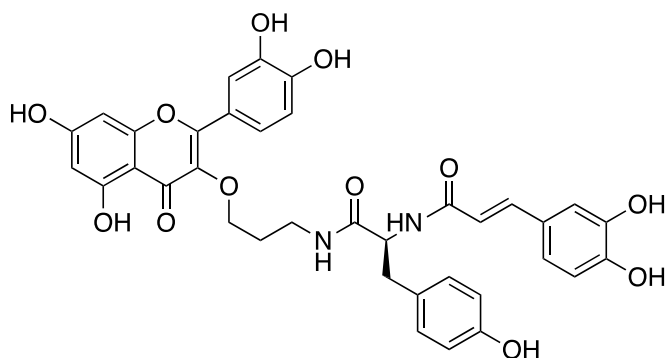


Figure S31. HSQC NMR of M09



(*S,E*)-3-(3,4-Dihydroxyphenyl)-*N*-(1-((3-((2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)oxy)propyl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)acrylamide (**M10**). **5.1 Tyr** (56 mg, 0.055 mmol) was dissolved in anhydrous DCM and added to a 5 mL RB flask kept under nitrogen. The mixture was cooled to  $-78^{\circ}\text{C}$  in an acetone dry ice bath and 220  $\mu\text{L}$  (0.22 mmol) of 1 M  $\text{BBr}_3$  in DCM was added. After 2.5 hours another 220  $\mu\text{L}$  aliquot of 1 M  $\text{BBr}_3$  was added for a total of 0.44 mmol. The reaction was stirred for 2.5 hours before being warmed to rt. then quenched with sat.  $\text{NaHCO}_3$ . The mixture was extracted 3 times with EtOAc, dried over  $\text{MgSO}_4$ , and concentrated *in vacuo*. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/ $\text{H}_2\text{O}$ . This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/ $\text{H}_2\text{O}$  to 95% ACN/ $\text{H}_2\text{O}$  over 50 minutes to give **M10** as yellow powder (11 mg, 0.016 mmol, 29% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{MeOH-d}_4$ )  $\delta$  7.59 (d,  $J = 2.2$  Hz, 1H), 7.47 (dd,  $J = 8.1$  Hz,  $J = 1.8$  Hz, 1H), 7.22 (d,  $J = 15.6$  Hz, 1H), 7.06 (d,  $J = 8.4$  Hz, 2H), 6.89 (d,  $J = 8.4$  Hz, 1H), 6.81 (d,  $J = 2.1$  Hz, 1H), 6.69 (dd,  $J = 7.8$  Hz,  $J = 2.4$  Hz, 1H), 6.64 (d,  $J = 8.4$  Hz, 1H), 6.63 (d,  $J = 8.4$  Hz, 2H), 6.30 (d,  $J = 15.6$  Hz, 1H), 6.26 (d,  $J = 2.2$  Hz, 1H) 6.16 (d,  $J = 2.1$  Hz, 1H), 4.62 (dd,  $J = 8.4$ ,  $J = 6.6$  Hz, 1H), 3.73 (p,  $J = 5.2$  Hz, 2H), 3.60 (m, 2H), 3.09 (dd,  $J = 13.6$  Hz,  $J = 6.6$  Hz, 1H), 2.89 (dd,  $J = 13.6$  Hz,  $J = 8.4$  Hz, 1H), 1.79 (m, 2H);  $^{13}\text{C}$  NMR (150 MHz,  $\text{MeOH-d}_4$ )  $\delta$  180.0, 173.6, 169.0, 166.1, 162.9, 158.4, 158.2, 157.3, 150.0, 148.8, 146.5, 146.4, 142.9, 138.6, 131.2, 129.3, 128.0, 123.0, 122.3, 122.0, 117.6, 116.50, 116.46, 116.3, 116.2, 115.2, 105.9, 99.9, 94.9, 71.6, 57.2, 38.2, 37.9, 30.1; ESI-MS:  $m/z$ :

707 [M+Na]<sup>+</sup>, 683 [M-H]<sup>-</sup>; HRMS (ESI-TOF): m/z calc'd for C<sub>36</sub>H<sub>32</sub>N<sub>2</sub>O<sub>12</sub>Na:  
707.1847 [M+Na]<sup>+</sup>; found: 707.1841

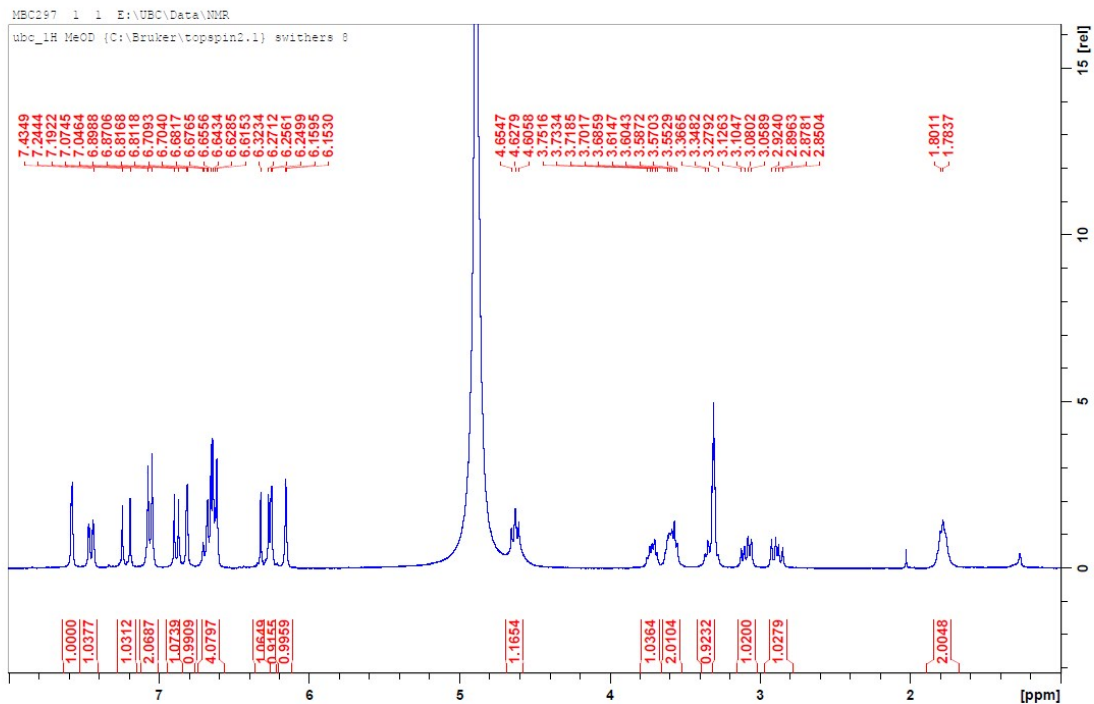


Figure S32. <sup>1</sup>H NMR of M10



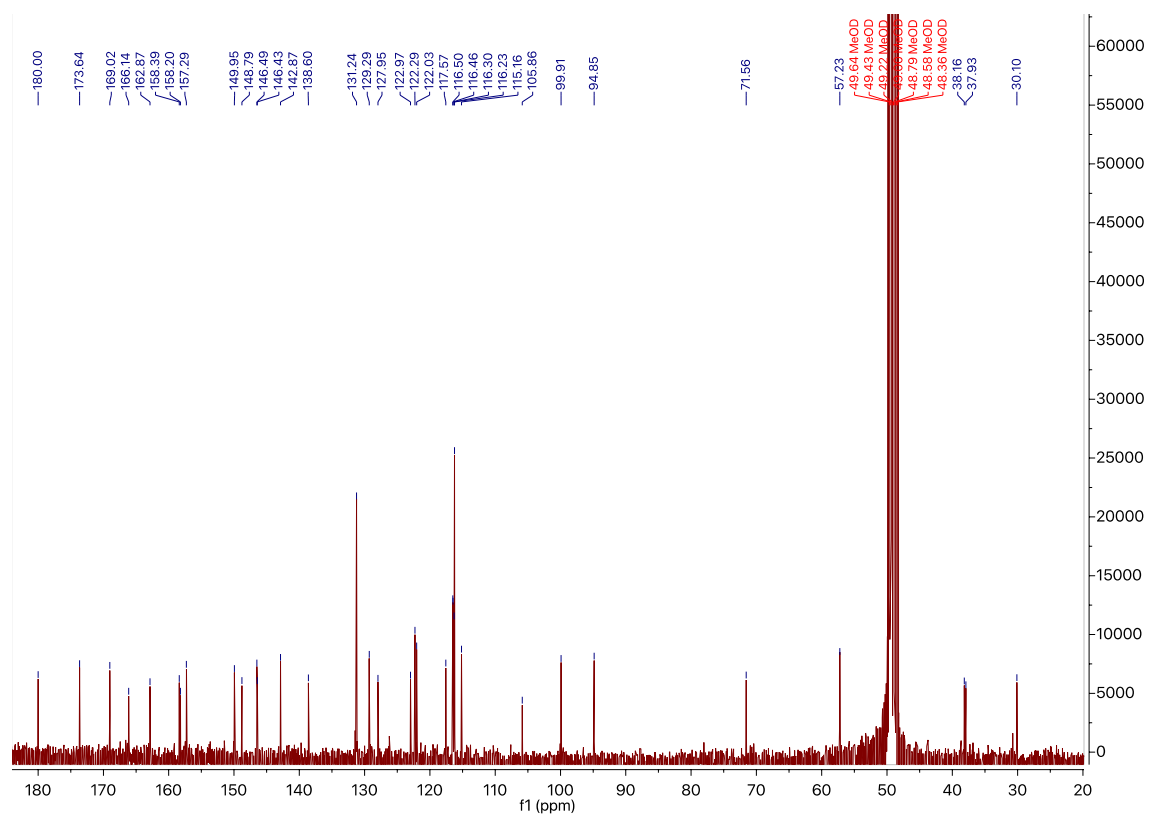


Figure S33.  $^{13}\text{C}$  NMR of M10

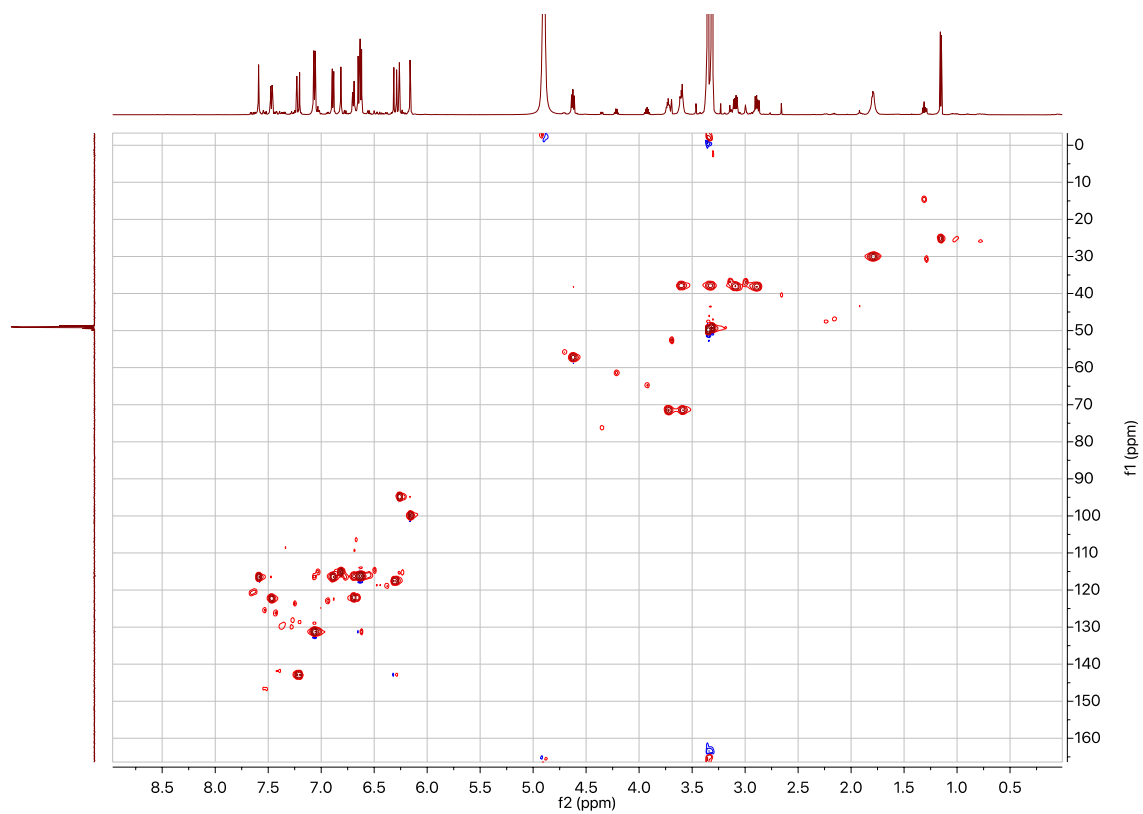


Figure S34. HSQC NMR of M10

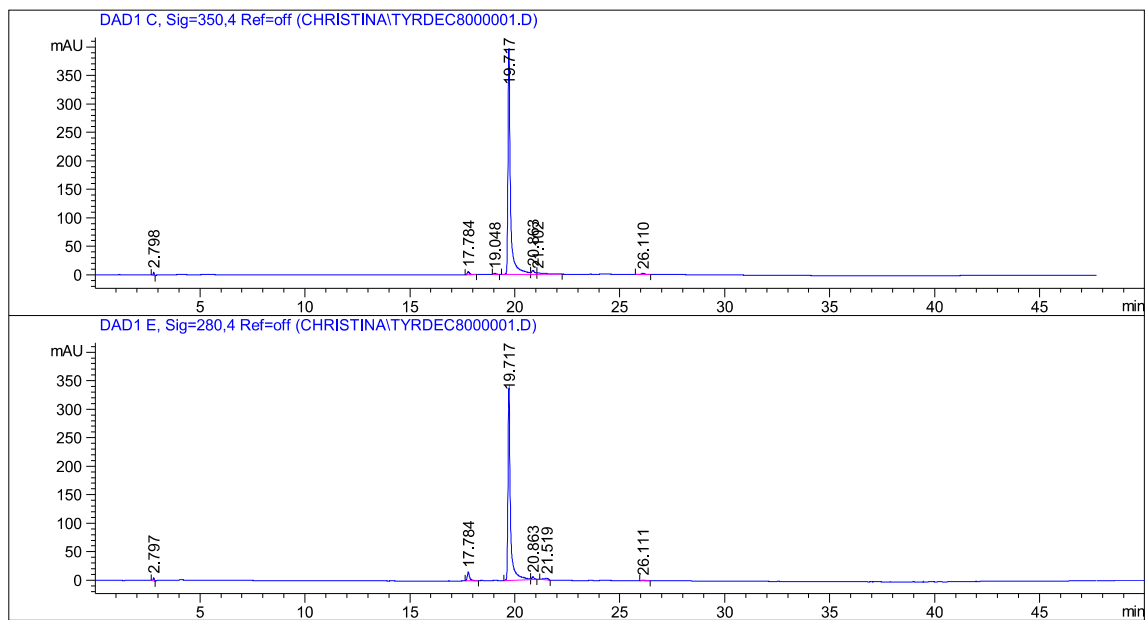
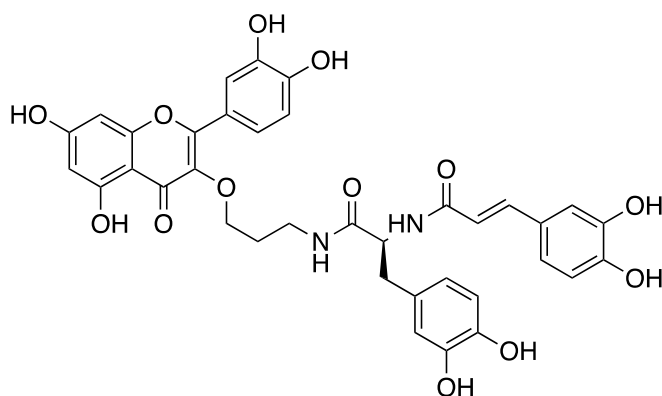


Figure S35. HPLC trace of M10



(*S,E*)-3-(3,4-Dihydroxyphenyl)-*N*-(3-(3,4-dihydroxyphenyl)-1-((3-((2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)oxy)propyl)amino)-1-oxopropan-2-yl)acrylamide (**M11**). **5.1 Dopa** (34 mg, 0.034 mmol) was dissolved in anhydrous DCM and added to a 5 mL RB flask kept under nitrogen. The mixture was cooled to  $-78^{\circ}\text{C}$  in an acetone dry ice bath and 134  $\mu\text{L}$  (0.134 mmol) of 1 M  $\text{BBr}_3$  in DCM was added. After 2.5 hours another 134  $\mu\text{L}$  aliquot of 1 M  $\text{BBr}_3$  was added for a total of 0.268 mmol. The reaction was stirred for 2.5 hours before being warmed to rt. then quenched with sat.  $\text{NaHCO}_3$ . The mixture was extracted 3 times with EtOAc, dried over  $\text{MgSO}_4$ , and concentrated *in vacuo*. The acetonide protecting group of the L-DOPA was removed by 1 hr treatment in 10% TFA/DCM. The material was first purified by silica gel chromatography with a gradient of 78:10:10:2 to 30:30:30:10 DCM/Acetone/MeOH/ $\text{H}_2\text{O}$ . This was followed by purification by HPLC with a reversed phase C-18 column and gradient of 5% ACN/ $\text{H}_2\text{O}$  to 95% ACN/ $\text{H}_2\text{O}$  over 50 minutes to give **M11** as yellow powder (1.8 mg, 0.007 mmol, 7.5% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{MeOH-d}_4$ )  $\delta$  7.59 (d,  $J = 2.1$  Hz, 1H), 7.46 (dd,  $J = 8.5$  Hz,  $J = 2.1$  Hz, 1H), 7.22 (d,  $J = 15.6$  Hz, 1H), 6.89 (d,  $J = 8.5$  Hz, 1H), 6.82 (d,  $J = 1.8$  Hz, 1H), 6.71-6.56 (m, 5H), 6.30 (d,  $J = 15.6$  Hz, 1H), 6.25 (d,  $J = 2.0$  Hz, 1H) 6.15 (d,  $J = 2.0$  Hz, 1H), 4.61 (dd,  $J = 8.0$ ,  $J = 6.7$  Hz, 1H), 3.77-3.72 (m, 1H), 3.66-3.57 (m, 2H), 3.36-3.32 (m, 1H), 3.04 (dd,  $J = 13.8$  Hz,  $J = 6.5$  Hz, 1H), 2.89 (dd,  $J = 13.6$  Hz,  $J = 7.9$  Hz, 1H), 1.83-1.76 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{MeOH-d}_4$ )  $\delta$  180.0, 173.6, 169.0, 165.9, 162.9, 158.3, 158.2, 149.9, 148.8, 146.5, 146.4, 146.2, 145.2, 142.9,

138.6, 130.0, 127.9, 123.0, 122.3, 122.0, 121.6, 117.6, 117.3, 116.50, 116.45, 116.32, 116.29, 115.2, 105.9, 99.9, 94.8, 71.5, 57.2, 38.4, 37.9, 30.2. ESI-MS: m/z: 723 [M+Na]<sup>+</sup>, 699 [M-H]<sup>-</sup>; HRMS (ESI-TOF): m/z calc'd for C<sub>36</sub>H<sub>32</sub>N<sub>2</sub>O<sub>13</sub>Na: 723.1797 [M+Na]<sup>+</sup>; found: 723.1818.

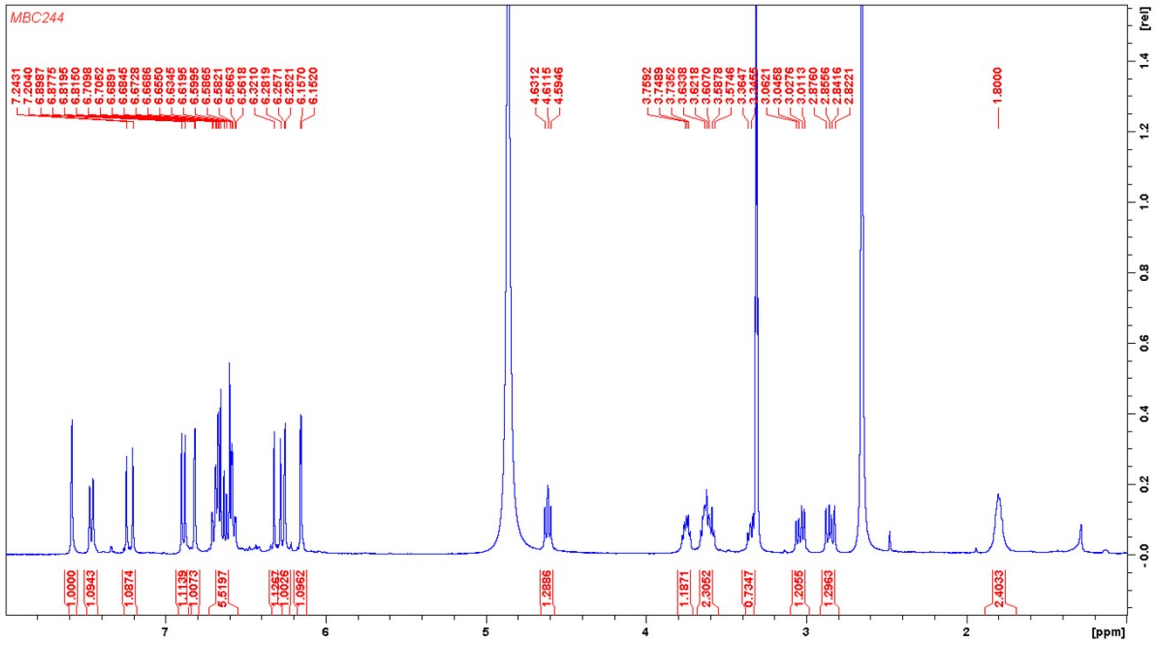


Figure S36. <sup>1</sup>H NMR of M11 (racemate)

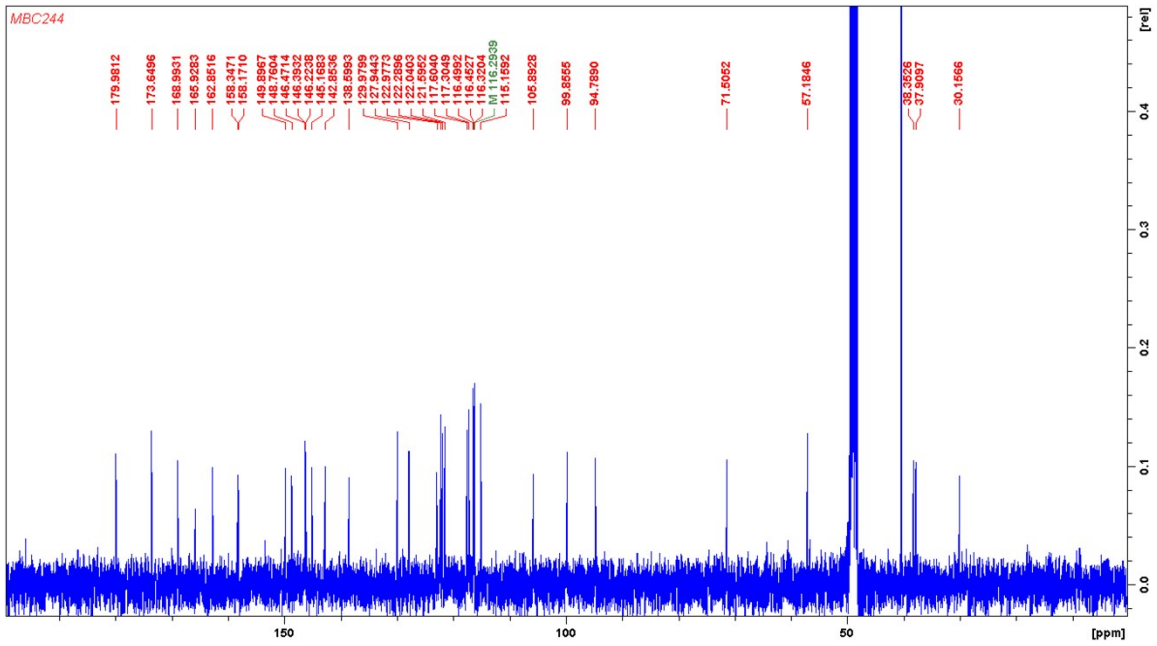


Figure S37. <sup>13</sup>C NMR of M11 (racemate)

**Improved synthesis of M10.** A solution of azide (**3 Propyl**) (100 mg, 153  $\mu\text{mol}$ ) and tyrosine derivative **6** (87 mg, 153  $\mu\text{mol}$ ) in DMF (3 mL) was treated with Lindlar catalyst (60 mg), placed under hydrogen pressure (1 atm) and stirred for 22 hours at room temperature. The reaction mixture was filtered through celite, and the celite washed with DMF (1 mL) to give a solution of amide **7**. This solution was treated with 5% palladium on alumina (30 mg) and pentafluorophenol (112 mg, 0.61 mmol), placed under hydrogen pressure (1 atm) and stirred for 20 hours at room temperature. The reaction mixture was filtered through celite, and the celite washed with DMF (1 mL) to give a solution of amine **8**. This solution was evacuated-backfilled with argon, cooled to 0 °C and treated with **1** (108 mg, 0.3 mmol) and triethylamine (42  $\mu\text{L}$ , 0.3 mmol). The reaction mixture was stirred at room temperature for 22 hours, whereupon further triethylamine (10.5  $\mu\text{L}$ , 75  $\mu\text{mol}$ ) was added at 0 °C. The reaction mixture was stirred for a further 6 hours at room temperature, cooled to 0 °C and diluted with ethyl acetate (30 mL) and 0.1 M HCl (30 mL). The layers were separated and the organic layer further washed with 0.1 M HCl (2 x 30 mL) and brine (20 mL). The pooled aqueous washes were back-extracted with ethyl acetate (10 mL) and the combined organics were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (79:10:10:1 DCM:Acetone:MeOH:H<sub>2</sub>O + 0.1% HCOOH to 73:12.5:12.5:2 DCM:Acetone:MeOH:H<sub>2</sub>O + 0.1% HCOOH) gave **M10** (51 mg, 74  $\mu\text{mol}$ , 49%) as a yellow powder. Analytical data as reported above.

## Kinetic Analysis of Inhibitors

2-Chloro-4-nitrophenyl  $\alpha$ -maltotrioside (CNP3) was purchased from Sekisui Enzymes. All other chromogenic substrates were purchased from Sigma-Aldrich. *R. inulinivorans*  $\alpha$ -amylase A, *B. fibrisolvens*  $\alpha$ -amylase B and *Agrobacterium*  $\beta$ -glucosidase were expressed in *E. coli*<sup>5,6</sup>. N-terminal maltase-glucoamylase, C-terminal maltase-glucoamylase and C-terminal sucrose-isomaltase were generous gifts from Prof. David Rose's lab at the University of Waterloo. Porcine pancreatic  $\alpha$ -amylase, Green coffee bean  $\alpha$ -galactosidase, Bovine liver  $\beta$ -galactosidase, Jack bean  $\alpha$ -mannosidase, *S. cerevisiae*  $\alpha$ -glucosidase, Almond  $\beta$ -glucosidase, and *E. coli*  $\beta$ -galactosidase were purchased from Sigma-Aldrich.

The release of 2-chloro-nitrophenol ( $\epsilon = 17,200 \text{ M}^{-1}\text{cm}^{-1}$ ) or 4-nitrophenol ( $\epsilon = 18\,400 \text{ M}^{-1}\text{cm}^{-1}$  for phenolate;  $\epsilon = 200 \text{ M}^{-1}\text{cm}^{-1}$  for phenol<sup>7</sup>) resulting from glycosidase-catalyzed hydrolysis of chromogenic substrates was monitored at 400 nm. All assays were performed on a Varian Cary 300 UV/Vis spectrophotometer. 200  $\mu\text{L}$  reactions were monitored in blackout quartz cuvettes. Reactions were monitored over 5 minutes to measure the initial reaction rate. Reactions were run at 30°C in 50 mM sodium phosphate, 100 mM sodium chloride (pH 7.0). Reactions were run using two to five different [CNP3] (2 mM and 4 mM; 2 mM, 4 mM, and 6 mM; 2 mM, 3 mM, 4 mM, 6 mM and 8 mM) for each range of inhibitor concentrations. Inhibitor concentrations generally ranging from 1/7 to 7x  $K_i$  were used. For each reaction, HPA was incubated with varying [I] at 30°C for 1 minute. The reaction was then initiated by addition of CNP3. These data were fit to a competitive inhibition model using nonlinear regression analysis, as performed by the GraFit 7.0 program to provide a value for  $K_i$ . Dixon and Lineweaver-Burke plots of each data set validated the use of a competitive inhibition model.  $\Delta G_{\text{binding}}$  was calculated using the equation  $\Delta G_{\text{binding}} = RT \ln(K_i)$ , where  $RT = 0.592 \text{ kcal mol}^{-1}$ .

For specificity analysis of M10, kinetic studies were performed at 37°C in an appropriate buffer (specific conditions listed below). The enzyme was

incubated with different concentrations of inhibitor for two minutes before initiating the reaction by the addition of substrate. Initial reaction rates were measured by monitoring  $\Delta A_{400\text{nm}}$  over the course of five minutes. If inhibition was observed at the highest  $[I]$  tested an  $IC_{50}$  analysis was carried out using  $[S] \sim K_M$ .

**R. inulinivorans  $\alpha$ -amylase A:** 50 mM sodium phosphate, 100 mM sodium chloride (pH 7). CNPG3,

**B. fibrisolvens  $\alpha$ -amylase B:** 50 mM sodium phosphate, 100 mM sodium chloride (pH 7). CNPG3,

**N-terminal maltase-glucoamylase:** 50 mM sodium phosphate buffer (pH 7). PNP  $\alpha$ -Glc,  $K_M = 12$  mM.

**C-terminal maltase-glucoamylase:** 50 mM sodium phosphate buffer (pH 7). PNP  $\alpha$ -Glc, 2 mM.

**C-terminal sucrose-isomaltase:** 50 mM sodium phosphate buffer (pH 7). PNP  $\alpha$ -Glc, 4 mM.

**Agrobacterium  $\beta$ -glucosidase (Abg):** 50 mM sodium phosphate buffer (pH 7). PNP  $\beta$ -Gal,  $K_M = 4.1$  mM.

**E.coli  $\beta$ -galactosidase:** 50 mM sodium phosphate, 1.0 mM  $MgCl_2$  (pH 7). PNP  $\beta$ -Gal,  $K_M = 60$   $\mu$ M.

**Green coffee bean  $\alpha$ -galactosidase:** 50 mM sodium phosphate buffer (pH 7). PNP  $\alpha$ -Gal,  $K_M \sim 0.5$  mM.

**Jack bean  $\alpha$ -mannosidase:** 50 mM sodium phosphate buffer (pH 7). PNP  $\alpha$ -mann,  $K_M = 2.5$  mM.

### **Expression of HPA in *Pichia pastoris*.**

A detailed protocol for the expression and purification of HPA can be found in the work done by Rydberg *et al.*<sup>8</sup>. Expression was carried out in *P. pastoris*. Colonies were grown in 60 mL BMGY media at 30°C 200 RPM. 20 mL of the overnight culture was added to 600 mL BMGY. After 16 hours the cells were transferred to 300 mL BMMY. After 24 hrs 2 mL of 50% methanol was added. The culture was left overnight. The culture supernatant was collected and purified via Phenyl Sepharose and Hitrap™ Q columns.



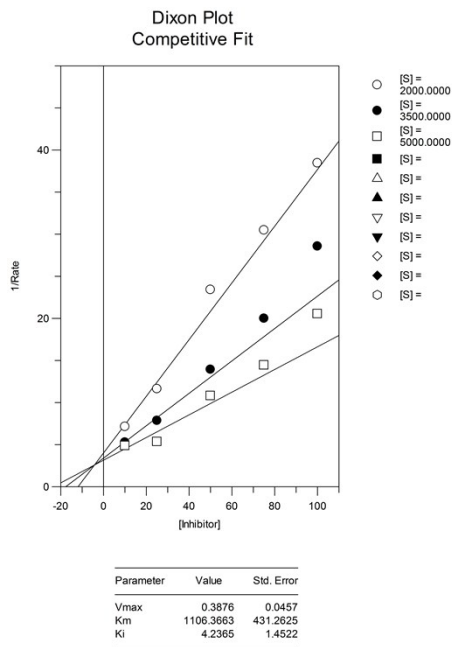


Figure S38. Dixon plot of M01 vs HPA

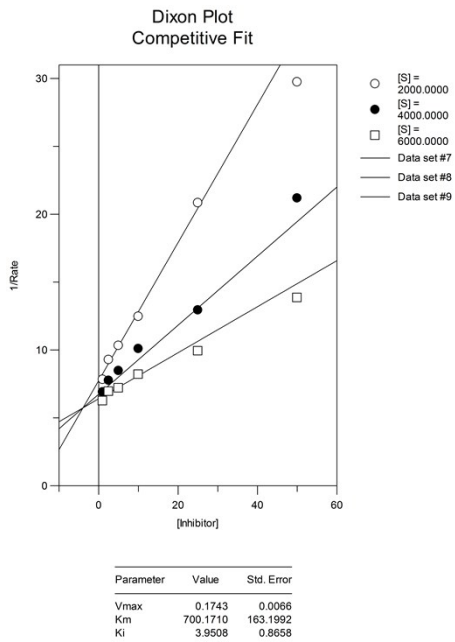


Figure S39. Dixon plot of M02 vs HPA

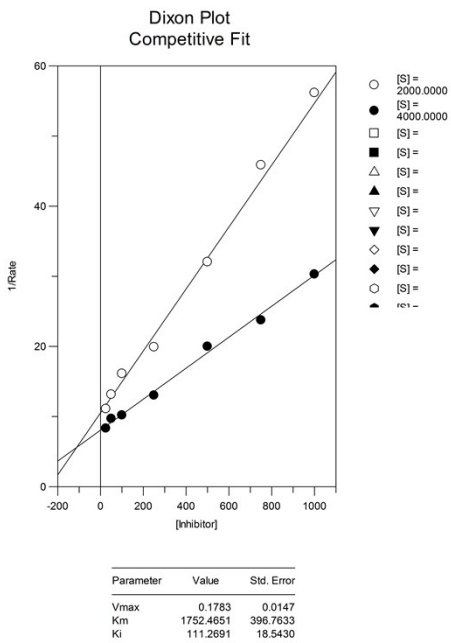


Figure S40. Dixon plot of M03 vs HPA

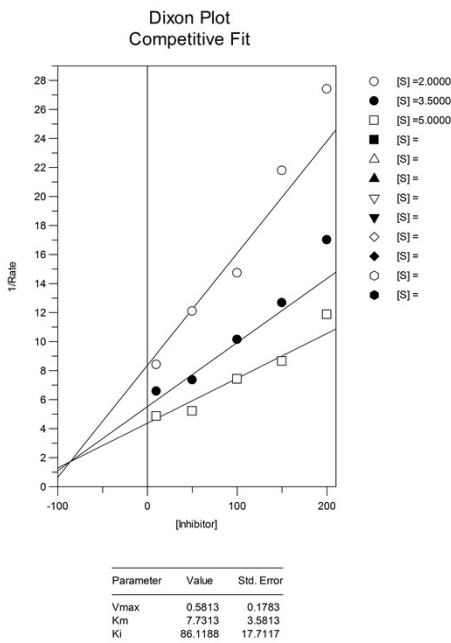


Figure S41. Dixon plot of M04 vs HPA

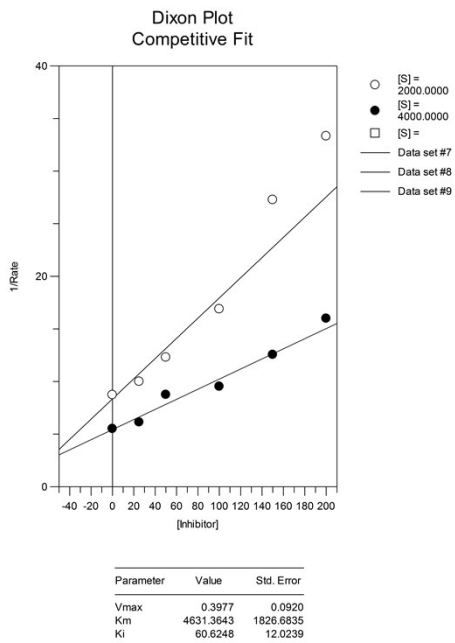


Figure S42. Dixon plot of M05 vs HPA

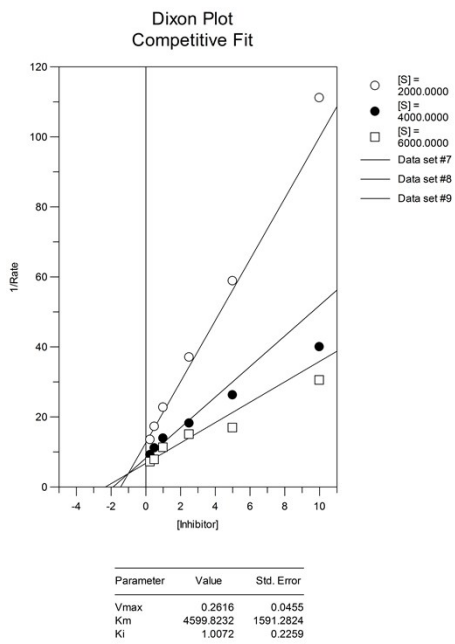


Figure S43. Dixon plot of M06 vs HPA

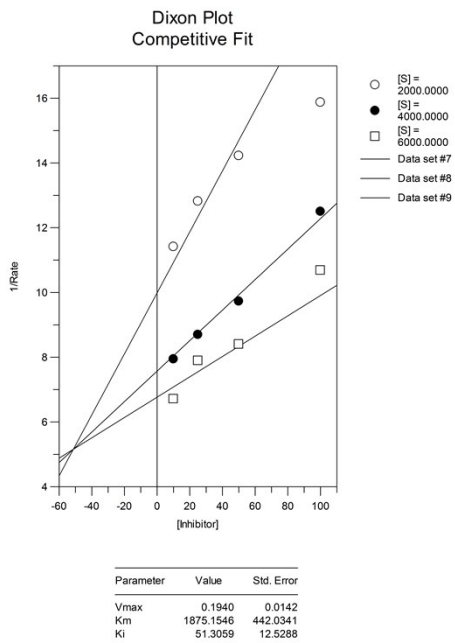


Figure S44. Dixon plot of M07 vs HPA

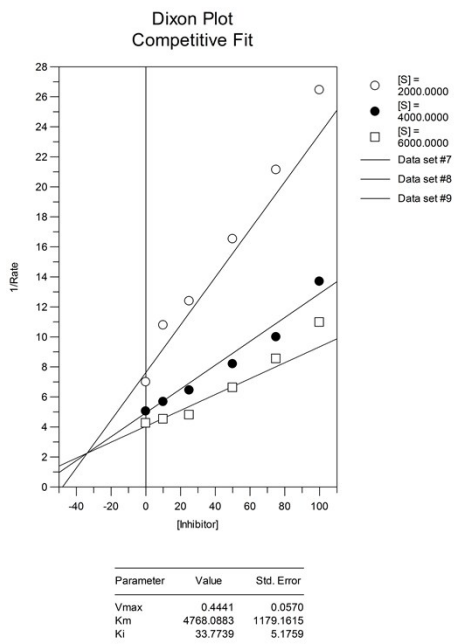


Figure S45. Dixon plot of M08 vs HPA

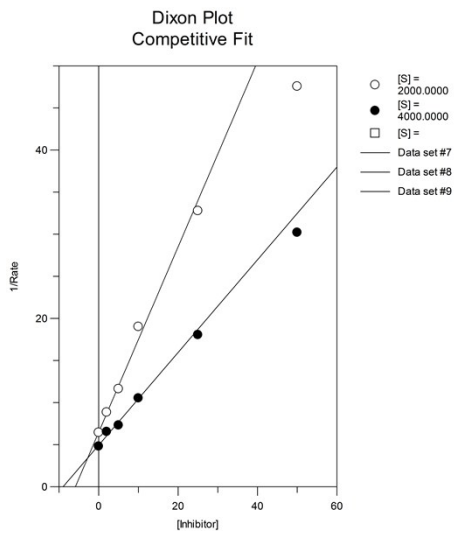


Figure S46. Dixon plot of M09 vs HPA

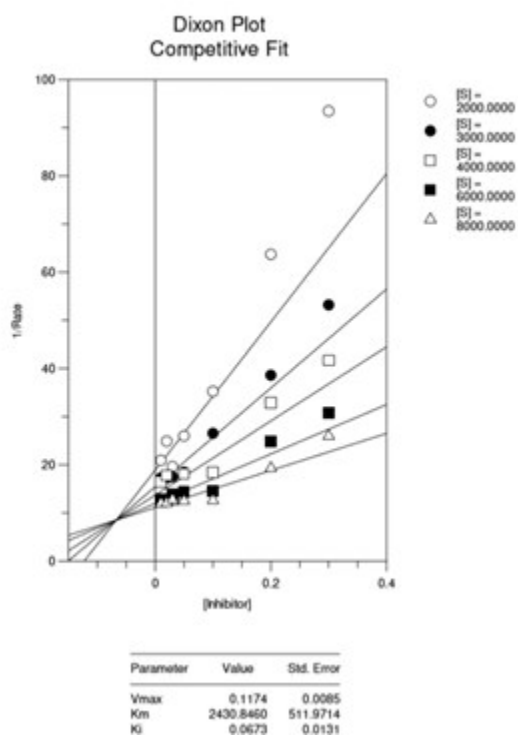


Figure S47. Dixon plot of M10 vs HPA

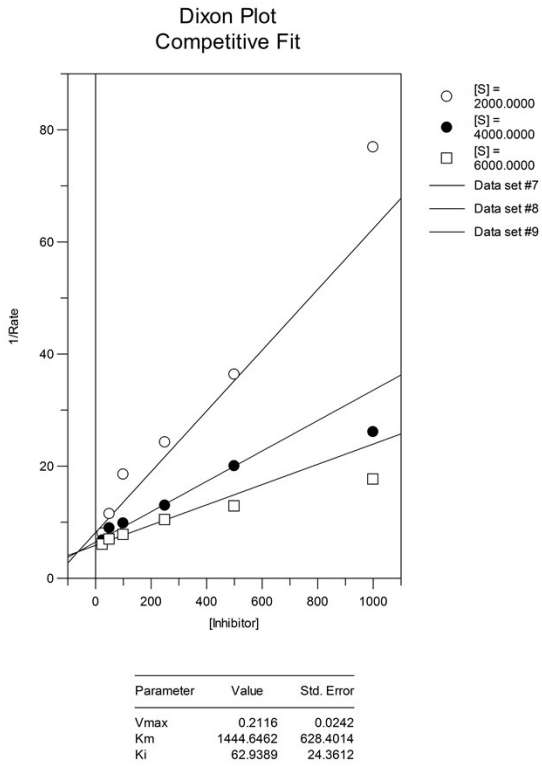


Figure S48. Dixon plot of M11 vs HPA

20190306-MiniMbA-Ki.GFF [Dixon plot, competitive]

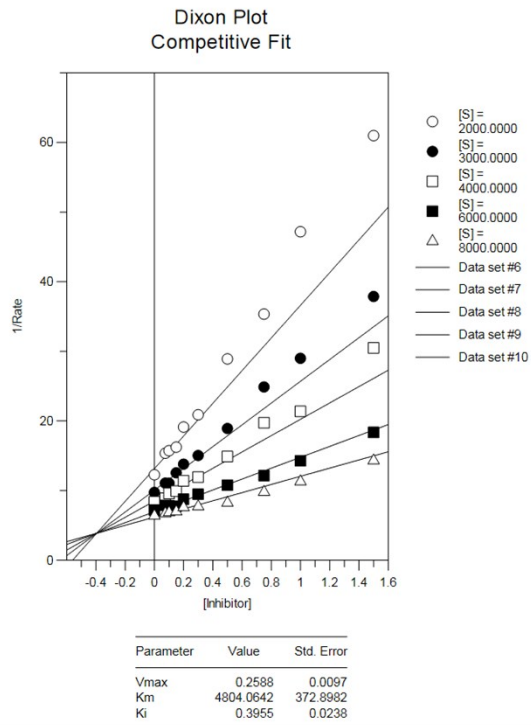


Figure S49. Dixon plot of Mini-MbA vs HPA

**Table S1:** Ligand efficiencies of Montbretin A and analogues

<b>Compound</b>	<b>Ligand efficiency (kcal/mol per non- hydrogen atom)</b>
<b>Montbretin A</b>	0.130
<b>Mini MbA</b>	0.158
<b>myricetin</b>	0.241
<b>Ethyl caffeate</b>	0.266
<b>M01</b>	0.177
<b>M02</b>	0.187
<b>M03</b>	0.144
<b>M04</b>	0.130
<b>M05</b>	0.139
<b>M06</b>	0.185
<b>M07</b>	0.132
<b>M08</b>	0.132
<b>M09</b>	0.157
<b>M10</b>	0.198
<b>M11</b>	0.195

## **X-ray Crystallographic Analysis of M06/HPA and M10/HPA Complexes**

### **Crystallization and Soaking Procedures**

Crystallization of HPA was performed using the sitting drop vapor diffusion method. Sitting drops were composed in a 1:1 ratio of 3-5  $\mu\text{L}$  of a solution of 20 mg/mL protein containing 0.1 M sodium phosphate buffer at pH 7.5 and 3-5  $\mu\text{L}$  of reservoir solution (52%-58% MPD, 0.1 M Na Cacodylate, pH 7.0). Crystals appeared after 3 days and grew up to 3 months at room temperature to reach full size.

Complexes of mini-MbA and M06 were obtained by soaking HPA crystals with 2  $\mu\text{L}$  of the compound solution at saturating concentrations. Crystals were incubated at room temperature for up to two weeks to allow complex formation. In the case of M10, co-crystallization was performed by mixing HPA and M10 in a 1:10 molar ratio, respectively. Prior to data collection, crystals were mounted into nylon cryo-loops (Hampton Research) and flash frozen in liquid nitrogen.

### **Data Collection and Structure Determination**

Crystallographic data were collected at cryogenic temperature (100 K) using a PILATUS 6M detector (Dectris) on beamline BL-12 at the Stanford Synchrotron Radiation Lightsource, Stanford, USA. The diffraction data obtained were indexed and integrated using the program XDS<sup>9</sup>. Data truncation was performed according to a split half correlation CC(1/2) criterion of  $\sim 75\%$  and a sigma  $I/\sigma$  cut-off criterion of  $\sim 2$ <sup>10</sup>.

To obtain optimal structural models of the studied HPA ligand complexes, an improved structure of wild type HPA was determined at 0.95 Å resolution (PDB 5U3A). This wild-type protein template served as the search model in the molecular replacement process for M06 and M10 complexed structures. The Mini-MbA-HPA complex was solved at an earlier time point using the highest resolution wild type structure of HPA (1.07 Å; PDB 4X9Y) available at the time. For all HPA ligand complexes, the molecular replacement procedure employed



the program PHASER<sup>11</sup>. Subsequent refinement of the HPA-inhibitor complex structures was accomplished by using the program PHENIX<sup>12</sup>. The refinement parameters included geometry and individual isotropic (mini-MbA) or anisotropic (M10 and M06) atomic displacement parameter restraints.

Additionally, all mini-MbA inhibitor analogs were defined as a constrained group in the occupancy refinement. In the case of M06 two nearby and hydrogen bonded water molecules (water 690 and water 691) were included in the constrained group of the inhibitor. These water molecules are only present in the active site of HPA upon binding this inhibitor. For the alternative, unoccupied form of M06, water molecule 693 (occupancy 26%) is present in the space between water 690 and 691. As the distances between these alternating water molecule conformations is below 2 Å, a remark in the PDB has been added to address this rare case of alternative water conformation that depends on ligand occupancy.

The validity of water molecules in all complexes was monitored on the basis of hydrogen bonding potential to protein and inhibitor atoms. Hydrogen atoms were added to all the structures, although their coordinates were not refined individually but as 'riding' in the PHENIX refinement protocol. Where necessary, obtained models were optimized by iterative model building using the program COOT<sup>13</sup>. Initially, ligand structure coordinates were created with the PRODRG server<sup>14,15</sup>. Prior to modeling the ligand into the active site of HPA, chemical restraints for each ligand were generated using the program eLBOW or Jligand. To confirm the presence of ligand binding, simulated annealing omit maps of the putative ligand position in the HPA active site were calculated. The resulting electron density maps were evaluated at the 3  $\sigma$  level for appropriate continuous ligand fit. Details of the refinement statistics are summarized in Table S1.

**Table S2:** Diffraction Data Collection and Structural Refinement Statistics\*

	HPA/M06-MbA	HPA/M10-MbA
Wavelength (Å)	0.97946	0.97946
Resolution range (Å)	34.0 - 1.15	36.6 - 1.30
Highest shell	(1.188 - 1.15)	(1.35 - 1.30)
Space group	P 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	P 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit cell a,b,c	52.3 68.0 130.5	52.4 68.2 130.3
$\alpha, \beta, \gamma$	90° 90° 90°	90° 90° 90°
Total reflections	1366530 (92149)	971638 (97410)
Unique reflections	162652 (13252)	113320 (11124)
Multiplicity	8.4 (7.0)	8.6 (8.8)
Completeness (%)	97.5 (80.2)	98.09 (97.24)
Mean I/sigma(I)	17.54 (3.72)	19.73 (6.73)
Wilson B-factor	10.74	11.78
R-merge	0.06 (0.40)	0.06 (0.27)
R-meas	0.0655	0.06766
CC1/2	0.999 (0.933)	0.998 (0.976)
CC*	1 (0.983)	1 (0.994)
R-work	0.0988 (0.1238)	0.0987 (0.0860)
R-free	0.1166 (0.1502)	0.1227 (0.1281)
No. of non-hydrogen atoms	4667	4633
macromolecules	4073	4042
ligands	61	74
water	533	517
Protein residues	496	496
RMS(bonds)	0.009	0.009
RMS(angles)	1.46	1.46
Ramachandran favored (%)	98	98
Ramachandran allowed (%)	2	2
Ramachandran outliers (%)	0	0
Average B-factor	14.4	15.8
macromolecules	12.8	14.2
ligands	20.9	18.9
water	26.6	28.5

\*Values in parenthesis refer to the highest resolution shell.

## References

1. M. Green, J. Berman, *Tetrahedron Letters* **1990**, 31, 2.
2. H. Huang et al., *Eur J Med Chem* **2009**, 44, 1982-1988.
3. R. W. Bates, R. N. Khanizeman, H. Hirao, Y. S. Tay, P. Sae-Lao, *Org Biomol Chem* **2014**, 12, 4879-4884.
4. Q. H. Sodji, V. Patil, J. R. Kornacki, M. Mrksich, A. K. Oyelere, *J Med Chem* **2013**, 56, 9969-9981.
5. J. Shim and S. G. Withers, *Food Sci and Biotech* **2013**, 22, 269-273
6. E. Rumbak et al., *J Bacteriol* **1991**, 173, 4203-4211
7. G. N. Bowers et al., *Clinical Chemistry* **1980**, 26, 724-729
8. E. H. Rydberg et al., *Protein Sci* **1999**, 8, 635-643.
9. W. Kabsch, *Acta crystallographica. Section D, Biological crystallography* **2010**, 66, 125-132.
10. P. A. Karplus, K. Diederichs, *Science* **2012**, 336, 1030-1033.
11. A. J. McCoy, *Acta crystallographica. Section D, Biological crystallography* **2007**, 63, 32-41.
12. P. D. Adams et al., *Acta crystallographica. Section D, Biological crystallography* **2010**, 66, 213-221.
13. P. Emsley, K. Cowtan, *Acta crystallographica. Section D, Biological crystallography* **2004**, 60, 2126-2132.
14. D. M. van Aalten et al. *Journal of computer-aided molecular design* **1996**, 10, 255-262.
15. A. W. Schuttelkopf, D. M. van Aalten, *Acta crystallographica. Section D, Biological crystallography* **2004**, 60, 1355-1363