

Supplemental Material

General Methods

Unless otherwise indicated, all anhydrous solvents were commercially obtained and stored under nitrogen. Reactions were performed under an atmosphere of dry nitrogen in oven dried glassware and were monitored for completeness by thin layer chromatography (TLC) using silica gel 60 F-254 (0.25 mm) plates with detection under UV light. ¹H-NMR spectra were recorded on dilute solutions in CDCl₃, CD₃OD or DMSO-d₈ at 300 MHz. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS). Coupling constants (*J*) are reported in Hz. Electrospray ionization mass spectra were acquired on a Bruker Esquire LC00066. Flash chromatography was carried out with silica gel (40-63 micron). Preparative reverse phase HPLC was performed on an automated Varian Prep star system using a gradient of 20% MeOH to 100% MeOH (with 0.1% trifluoroacetic acid) at 12 mL/min over 30 min using a YMC S5 ODS column (20x100 mm, Waters Inc.).

Synthesis of Reagents for MPS-IVA assay

(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl triacetate (9)

To a stirred ice-cooled solution of 33% hydrobromic acid (50 mL) in acetic acid under nitrogen was added β -D-galactose pentaacetate (4.68 g, 12 mmol) and stirring continued for 15 minutes. Then the reaction mixture was brought to room temperature and stirred for 2 hrs. The mixture was diluted with toluene and rotary evaporated to a residue which was then diluted with 250 mL of ethyl acetate followed by successive washing with 250 mL of cold saturated NaHCO₃ and 150 mL of cold brine solutions. The organic layer was dried over MgSO₄, filtered and concentrated under vacuum to yield the crude bromide derivative which was used as such for the next step.

Tert-butyl 5-(2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetamido) pentylcarbamate (6b)

To a stirred ice-cooled mixture of 7-hydroxycoumarin-4-acetic acid (2.20 g, 10 mmol) and N-Boc-1,5-diaminopentane (2.22 g, 11 mmol) in DMF (2 mL) and anhydrous dichloromethane (20 mL) was added 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (3.43 g, 18 mmol) and the stirring was continued overnight at room temperature. TLC in CHCl₃ : MeOH : AcOH : H₂O (96 : 12 : 4 : 1) and detection with UV light showed the complete consumption of the acid.

The reaction mixture was diluted with 10 mL CH₂Cl₂ and 20 mL acetone and washed successively with water (20 mL, pH 8), 1 M HCl : H₂O (10 mL : 20 mL, pH 1), 5% NaHCO₃ : water (10 mL : 10 mL, pH 9) and finally with water (20 mL, pH 7). The aqueous layer was back extracted with DCM and dried over MgSO₄, filtered and rotary evaporated to a solid residue which was successively washed with acetone to yield the desired product (2.0 g, 4.95 mmol) in 50 % yield. ¹H NMR (300 MHz, DMSO-d₈) δ 8.15 (brs, 1H), 7.59 (d, *J* = 11.9 Hz, 1H), 6.80-6.73 (m, 2H), 6.11 (s, 1H), 3.62 (s, 2H), 3.04 (m, 2H), 2.86 (m, 2H), 1.37 (s, 13H), 1.21 (m, 2H).

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(2-(5-(tert-butoxy carbonyl amino) pentyl amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yloxy)tetrahydro-2H-pyran-3,4,5-triyl tri acetate

7-Hydroxycoumarin-4-acetamide **6b** (1.70 g, 4.20 mmol), acetobromogalactose **9** (0.844 g, 2.10 mmol) and tetrabutyl ammonium hydrogen sulfate (0.709 g, 2.10 mmol) were stirred in equimolar ratio of CH₂Cl₂ (4.5 mL) and 1 M NaOH (4.5 mL) at room temperature for 50 minutes. The reaction mixture was diluted with ethyl acetate (30 mL) and successively washed the organic phase with 30 mL of 1 M NaOH (10 mL × 3), 60 mL of water (30 mL × 2) and finally with 15 mL of saturated brine solution. The organic phase was dried over MgSO₄, filtered and rotary evaporated to a yellow semisolid. Column chromatography over silica gel in CHCl₃ : EtOAc : (CH₃)₂CHOH (91 : 6 : 3) afforded the desired product (1.60 g, 2.18 mmol) in 52 % yield. ¹H NMR (300 MHz, DMSO-d₈) δ 8.17 (brs, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.06 (d, *J* = 2.2 Hz, 1H), 6.98 (d, *J* = 9.0 Hz, 1H), 6.74 (brs, 1H), 6.31 (s, 1H), 5.65 (d, *J* = 6.7 Hz, 1H), 5.37 (s, 1H), 5.25 (d, *J* = 5.7 Hz, 2H), 4.50 (t, *J* = 6.4 Hz, 1H), 4.11 (d, *J* = 6.2 Hz, 2H), 3.68 (s, 2H), 3.03 (t, *J* = 5.8 Hz, 2H), 2.87 (t, *J* = 6.3 Hz, 2H), 2.15 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.95 (s, 3H), 1.36 (s, 13H), 1.23-1.20 (m, 2H).

Tert-butyl 5-(2-(2-oxo-7-((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yloxy)-2H-chromen-4-yl)acetamido)pentylcarbamate (10)

To an ice-cooled stirred solution of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(2-(5-(tert-butoxy carbonyl amino) pentyl amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yloxy)tetrahydro-2H-pyran-3,4,5-triyl tri acetate (1.46 g, 2.0 mmol) in anhydrous methanol (10 mL) was added a solution of 0.5 M sodium methoxide in methanol (66.7 μL, 1.0 mmol) dropwise and then stirring continued at 0 °C for 1.5 hrs. The reaction mixture was neutralized with amberlite IR-120 (H⁺), filtered and concentrated to a solid residue. Column chromatography of the latter over silica gel in CH₂Cl₂ : MeOH (9 : 1) afforded the desired compound (0.60 g, 1.06 mmol) in 53 % yield. ¹H NMR (300 MHz, DMSO-d₈) δ 8.17 (brs, 1H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.05-7.01 (m, 2H), 6.74 (brs, 1H), 6.26 (s, 1H), 5.23 (d, *J* = 5.1 Hz, 1H), 4.98 (d, *J* = 7.5 Hz, 1H), 4.89 (d, *J* = 5.5 Hz, 1H), 4.68 (s, 1H), 4.54 (d, *J* = 4.3 Hz, 1H), 3.67 (s, 2H), 3.51 (s, 2H), 3.04 (t, *J* = 6.3 Hz, 2H), 2.88 (t, *J* = 6.6 Hz, 2H), 1.36 (s, 13H), 1.23 (brs, 2H).

Sodium ((2R,3R,4S,5R,6S)-6-(4-(2-(5-(tert-butoxycarbonylamino)pentylamino)-2-oxoethyl)-2-oxo-2H-chromen-7-yloxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)methyl sulfate (2)

A mixture of compound **10** (0.096 g, 0.17 mmol) and SO₃ – pyridine complex (0.056 g, 0.35 mmol) in dry pyridine (5 mL) was stirred at 40 °C for 5 hrs. The reaction mixture was quenched with MeOH (8 mL) and solution passed through a column of dowex 50WX8 (Na⁺ form) and eluted with methanol. The eluent was concentrated under vacuum to an oil which was chromatographed over silica gel in CHCl₃ : MeOH : H₂O (8 : 5 : 1) to yield the target compound **2** (0.051 g, 0.076 mmol) in 45% yield. ¹H NMR (300 MHz, DMSO-d₈) δ 7.55 (d, *J* = 8.4 Hz, 1H), 7.01-6.91 (m, 2H), 6.13 (s, 1H), 4.52 (d, *J* = 5.1 Hz, 1H), 4.06 (d, *J* = 7.5 Hz, 1H), 3.93 (d, *J* = 5.1 Hz, 1H), 3.83 (d, *J* = 3.6 Hz, 1H),

3.68 (d, $J = 8.4$ Hz, 1H), 3.60 (d, $J = 6.8$ Hz, 2H), 3.51 (s, 2H), 3.06 (t, $J = 6.4$ Hz, 2H), 2.86 (t, $J = 6.3$ Hz, 2H), 1.29-1.27 (m, 13H), 1.18 (m, 2H). ESI-MS⁺ m/z 645.4 (M + H)⁺.

Internal Standard Synthesis

Tert-butyl 6-(2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetamido)hexylcarbamate (6a)

The compound was prepared by the analogous procedure described for the synthesis of compound **6b**. 7-hydroxycoumarin-4-acetamide (2.20 g, 10 mmol) and N-Boc-1, 6-diaminohexane (2.38 g, 11 mmol) were treated with 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (3.43 g, 18 mmol) to afford the product (1.67 g, 3.99 mmol) in 40% yield. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.15 (t, $J = 10.7$ Hz, 1H), 7.59 (d, $J = 8.7$ Hz, 1H), 6.76 (dd, $J = 7.9, 2.2$ Hz, 1H), 6.71 (d, $J = 2.2$ Hz, 1H), 6.14 (s, 1H), 3.61 (s, 2H), 3.04 (t, $J = 6.0$ Hz, 2H), 2.87 (t, $J = 6.3$ Hz, 2H), 1.36 (s, 13H), 1.21 (m, 4H).

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(2-(6-(tert-butoxy carbonylamino) hexyl amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yloxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate

The compound was prepared by the analogous procedure described for the synthesis of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(2-(5-(tert-butoxy carbonyl amino) pentyl amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yloxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate. 7-hydroxycoumarin-4-acetamide **6a** (1.50 g, 3.59 mmol) and acetobromogalactose (0.72 g, 1.80 mmol) were reacted with tetrabutyl ammonium hydrogen sulfate (1.22 g, 3.60 mmol) to yield the product (1.40 g, 1.87 mmol) in 52% yield. ¹H NMR (300 MHz, CDCl₃) δ 7.97 (s, 1H), 7.66 (d, $J = 9.5$ Hz, 1H), 6.94 (s, 1H), 6.30 (s, 1H), 6.10 (brs, 1H), 5.49 (d, $J = 7.7$ Hz, 1H), 5.16-5.12 (m, 2H), 4.59 (brs, 1H), 4.18-4.12 (m, 3H), 3.66 (s, 2H), 3.22 (dd, $J = 6.5, 5.7$ Hz, 2H), 3.08 (q, $J = 6.4$ Hz, 2H), 2.18 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 1.55 (s, 6H), 1.43 (s, 9H), 1.27 (brs, 2H).

Tert-butyl 6-(2-(2-oxo-7-((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yloxy)-2H-chromen-4-yl)acetamido)hexylcarbamate (4)

The compound was prepared by the analogous procedure described for the synthesis of compound **10**. To an ice-cooled stirred solution of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(2-(6-(tert-butoxycarbonylamino)hexylamino)-2-oxoethyl)-2-oxo-2H-chromen-7-yloxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (20 mg, 0.027 mmol) in anhydrous methanol (3 mL) was added a solution of 0.5 M sodium methoxide in methanol (1.66 μ L, 0.025 mmol) dropwise to yield the product (10 mg, 0.017 mmol) in 65% yield. ¹H NMR (300 MHz, CD₃OD) δ 7.72 (d, $J = 9.3$ Hz, 1H), 7.12-7.11 (m, 2H), 6.52 (brs, 1H), 6.29 (s, 1H), 5.01 (d, $J = 7.7$ Hz, 1H), 4.57 (s, 1H), 3.94-3.82 (m, 2H), 3.78-3.75 (m, 3H), 3.63 (s, 2H), 3.21 (t, $J = 6.8$ Hz, 2H), 3.02 (t, $J = 6.0$ Hz, 2H), 1.52 (m, 2H), 1.44 (s, 11H), 1.32 (m, 4H). ESI-MS⁺ m/z 581.3 (M + H)⁺.

Synthesis of reagents for MPV-VI assay

Synthesis of *tert*-butyl 6-(7-hydroxy-coumarin-4-acetamido)hexylcarbamate (**6a**)

Coumarin **5** (3.86 g, 17.5 mmol) and *N*-Boc-1,6-hexanediamine (4.64 g, 21.6 mmol, 1.2 equiv) were dissolved in DCM (35 mL) and DMF (3.5 mL) and cooled in an ice bath. *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (6.00 g, 31.3 mmol, 1.8 equiv) were added and the solution was allowed to warm to rt. After 16 h, the solution was diluted with DCM (10 mL) and acetone (20 mL) and washed with H₂O (20 mL), aq. HCl (0.33 M, 30 mL), aq. NaHCO₃ (2.5%, 20 mL), and H₂O (20 mL). Each of the aqueous layers were extracted a second time with a mixture of DCM (40 mL) and acetone (10 mL). The combined organic layers were evaporated followed by evaporation with acetone to afford a yellow solid. This material was recrystallized in from acetone to yield coumarin **6a** as a white crystalline solid (2.79 g, 38%). Recrystallization of the mother liquor afforded additional product as a white crystalline solid (1.00 g, 14%, 52% total) *R*_f 0.33 (5% MeOH in CH₂Cl₂); ¹H NMR (500 MHz, DMSO): δ 10.58 (1H, br s), 8.19 (1H, t, *J*= 5.4 Hz), 7.60 (1H, d, *J*= 8.7 Hz), 6.78 (1H, dd, *J*= 8.7, 2.5 Hz), 6.74 (1H, br s), 6.72 (1H, d, *J*= 2.5 Hz), 6.15 (1H, s), 3.62 (2H, s), 3.04 (2H, td, *J*= 6.4, 6.0 Hz), 2.87 (2H, ddd, *J*= 6.8, 6.4, 6.2 Hz), 1.36 (9H, s), 1.42-1.28 (4H, m), 1.25-1.18 (4H, m); ESI-MS: *m/z* 419.3 (M + H)⁺

Synthesis of *tert*-butyl 5-(7-hydroxy-coumarin-4-acetamido)pentylcarbamate (**6b**)

Coumarin **5** (1.10 g, 5.0 mmol) and *N*-Boc-1,5-pentanediamine (1.12 g, 5.5 mmol, 1.1 equiv) were dissolved in DCM (10 mL) and DMF (1 mL) and cooled in an ice bath. *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1.73 g, 9.0 mmol, 1.8 equiv) were added and the solution was allowed to warm to rt. After 16 h, the solution was diluted with DCM (5 mL) and acetone (10 mL) and washed with H₂O (5 mL), aq. HCl (0.33 M, 5 mL), aq. NaHCO₃ (2.5%, 5 mL), and H₂O (5 mL). Each of the aqueous layers were extracted a second time with a mixture of DCM (20 mL) and acetone (5 mL). The combined organic layers were evaporated followed by evaporation with acetone to afford a yellow solid. This material was recrystallized from acetone to yield coumarin **6b** as a white crystalline solid (1.25g, 62%). *R*_f 0.33 (5% MeOH in CH₂Cl₂); ¹H NMR (300 MHz, DMSO): δ 10.59 (1H, br s), 8.18 (1H, t, *J*= 5.4 Hz), 7.59 (1H, d, *J*= 8.8 Hz), 6.78 (1H, d, *J*= 9.8 Hz), 6.75 (1H, dd, *J*= 5.7, 5.4 Hz), 6.72 (1H, br s), 6.15 (1H, s), 3.62 (2H, s), 3.03 (1H, app q, *J*= 6.3 Hz), 2.87 (1H, app q, *J*= 6.3 Hz), 1.36 (9H, s), 1.42-1.30 (4H, m), 1.24-1.17 (2H, m); ESI-MS: *m/z* 405.2 (M + H)⁺

Synthesis of 7-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl) *tert*-butyl 6-(7-hydroxy-coumarin-4-acetamido)hexylcarbamate (**A**)

A solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glycopyranosyl chloride (**7a**, 1.10 g, 3.0 mmol), coumarin **6a** (2.51 g, 6.0 mmol, 2 equiv) and tetrabutyl ammonium hydrogen sulfate (1.02 g, 3.0 mmol, 1 equiv) in DCM (10 mL) was vigorously stirred and aqueous NaOH was added (1 M, 10 mL). The solution turned bright yellow and solid

began precipitating. After 45 min, no glycosyl chloride remained by TLC (H₂SO₄ stain). The precipitate was filtered and washed 3X with DCM (20 mL) and H₂O (20 mL). The precipitate was recrystallized from 1-butanol to afford glycoside **A** as an off-white powder (1.058 g, 47%). The layers of the filtrate from the reaction were then saturated with citric acid and extracted with DCM (20 mL, 3X). The layers were separated, and the organic layers were evaporated to afford recovered coumarin **6a** which was recrystallized from acetone (1.26 g, 50% of starting coumarin recovered). *R_f* 0.19 (5% MeOH in DCM); ¹H NMR (500 MHz, DMSO-d₆): δ 8.16 (1H, t, *J*= 6.1 Hz), 8.11 (1H, d, *J*= 9.2 Hz) 7.70 (1H, d, *J*= 8.8 Hz), 7.12 (1H, d, *J*= 2.6 Hz), 6.98 (1H, dd, *J*= 8.8, 2.6 Hz), 6.75 (1H, t, *J*= 5.3 Hz), 6.30 (1H, s), 5.50 (1H, d, *J*= 8.4 Hz), 5.22 (1H, dd, *J*= 10.3, 9.5 Hz), 4.93 (1H, dd, *J*= 9.9, 9.5 Hz), 4.25 (1H, ddd, *J*= 5.3, 2.4 Hz), 4.19 (1H, dd, *J*= 11.9, 5.3 Hz), 4.10-4.01 (2H, m), 3.68 (2H, s), 3.04 (1H, ddd, *J*= 7.0, 6.8, 6.1 Hz), 2.88 (1H, dd, *J*= 6.8, 6.6, 6.2 Hz), 2.10 (3H, s), 2.10 (3H, s), 1.95 (3H, s), 1.77 (3H, s), 1.36 (9H, s), 1.43-1.29 (4H, m), 1.25-1.18 (4H, m); ESI-MS: *m/z* 770.2 (M + Na)⁺

Synthesis of 7-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl) tert-butyl 6-(7-hydroxy-coumarin-4-acetamido)hexylcarbamate (8**)**

Sodium methoxide in methanol (0.5 M, 0.23 mL) was added to a solution of triacetyl glycoside **A** (871 mg, 1.16 mmol) in methanol (24 mL) and chloroform (12 mL). After 4 h, no starting material remained by TLC, and DOWEX 50WX8 (H⁺ form) was added until solution was neutral by litmus paper. The solution was filtered, rinsing with methanol and the combined organic layers were evaporated to afford the trialcohol **8** as a white solid (687 mg, 95%). *R_f* 0.32 (20% MeOH in DCM); ¹H NMR (500 MHz, DMSO-d₆): δ 8.16 (1H, dd, *J*= 5.8, 5.4 Hz), 7.81 (1H, d, *J*= 8.8 Hz), 7.68 (1H, d, *J*= 8.1 Hz), 7.00 (1H, d, *J*= 2.5 Hz), 6.93 (1H, dd, *J*= 8.8, 2.5 Hz), 6.73 (1H, t, *J*= 5.2 Hz), 6.27 (1H, s), 5.13 (2H, dd, *J*= 6.9, 1.3 Hz), 5.10 (1H, d, *J*= 5.4 Hz), 4.62 (1H, t, *J*= 5.7 Hz), 3.76-3.68 (2H, m), 3.67 (2H, s) 3.52-3.38 (3H, m), 3.19 (1H, ddd, *J*= 9.1, 5.7, 3.5 Hz), 3.04 (1H, ddd, *J*= 6.9, 6.6, 6.0 Hz), 2.88 (1H, td, *J*= 6.6, 6.3 Hz), 1.81 (3H, s), 1.36 (9H, s), 1.42-1.31 (4H, m), 1.27-1.17 (4H, m); ESI-MS: *m/z* 622.3 (M + H)⁺

Synthesis of 7-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl) tert-butyl 5-(7-hydroxy-coumarin-4-acetamido)pentylcarbamate (B**)**

A solution of 2-acetamido-3,4,5-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl chloride (**7b**, 460 mg, 1.26 mmol), coumarin **6b** (1.02 g, 2.5 mmol, 2 equiv) and tetrabutyl ammonium hydrogen sulfate (427 g, 1.26 mmol, 1 equiv) in DCM (4.2 mL) was vigorously stirred and aqueous NaOH was added (1 M, 4.2 mL). The solution turned bright yellow and solid began precipitating. After 1 h 15 min, no glycosyl chloride remained by TLC (H₂SO₄ stain). The precipitate was filtered and washed 3X with DCM (20 mL) and H₂O (20 mL) to afford glycoside **B** as an off-white powder (513 mg, 56%). The layers of the filtrate from the reaction were then saturated with citric acid and extracted with DCM (20 mL, 3X), a filtrate formed during the extraction and was isolated to afford recovered coumarin **6b** (286 mg, 28% of starting coumarin recovered). The layers were separated, and the organic layers were evaporated to afford recovered coumarin # and product. The residue was taken up in MeOH and filtered. The solid was glycoside # (180 mg, 20%: 76% total). *R_f* 0.19 (5% MeOH in DCM); ¹H NMR (500 MHz, DMSO-d₆): δ 8.18 (1H, dd, *J*= 5.5, 5.1 Hz), 8.00 (1H, d, *J*= 9.2 Hz) 7.71 (1H, d, *J*= 9.0 Hz), 7.10 (1H, d, *J*= 2.2

Hz), 6.96 (1H, dd, $J= 9.0, 2.2$ Hz), 6.76 (1H, dd, $J= 5.5, 4.5$ Hz), 6.29 (1H, s), 5.39 (1H, d, $J= 8.4$ Hz), 5.32 (1H, d, $J= 3.3$ Hz), 5.10 (1H, dd, $J= 11.2, 3.3$ Hz), 4.43 (1H, dd, $J= 6.6, 5.9$ Hz), 4.22 (1H, ddd, $J= 10.8, 9.2, 9.0$ Hz), 4.15-4.04 (2H, m), 3.68 (2H, s), 3.03 (1H, ddd, $J= 6.8, 6.6, 6.1$ Hz), 2.87 (1H, dd, $J= 6.6, 6.4, 6.2$ Hz), 2.14 (3H, s), 2.02 (3H, s), 1.93 (3H, s), 1.79 (3H, s), 1.36 (9H, s), 1.42-1.31 (4H, m), 1.31-1.15 (4H, m); ESI-MS: m/z 734.3 (M + H)⁺

Synthesis of 7-*O*-(2-acetamido-2-deoxy- β -D-galactopyranosyl) *tert*-butyl 5-(7-hydroxy-coumarin-4-acetamido)pentylcarbamate (3)

Sodium methoxide in methanol (0.5 M, 0.14 mL) was added to a solution of triacetyl glycoside **B** (508 mg, 0.69 mmol) in methanol (14 mL) and chloroform (7 mL). After 4 h, no starting material remained by TLC, and DOWEX 50WX8 (H⁺ form) was added until solution was neutral by litmus paper. The solution was filtered, rinsing with methanol (~400 mL) and the combined organic layers were evaporated to afford the trialcohol **3** as a white solid (351 mg, 83%). R_f 0.32 (20% MeOH in DCM); ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.18 (1H, t, $J= 5.7$ Hz), 7.76 (1H, d, $J= 9.2$ Hz), 7.68 (1H, d, $J= 9.0$ Hz), 7.01 (1H, d, $J= 2.6$ Hz), 6.94 (1H, dd, $J= 9.0, 2.6$ Hz), 6.77 (1H, t, $J= 5.7$ Hz), 6.27 (1H, s), 5.10 (1H, d, $J= 8.3$ Hz), 4.79 (1H, d, $J= 6.4$ Hz), 4.72-4.68 (2H, m) 4.01 (1H, ddd, $J= 10.1, 9.2, 8.6$ Hz), 3.73 (1H, t, $J= 3.9$ Hz) 3.66 (2H, s), 3.66-3.62 (1H, m), 3.61-3.49 (3H, m), 3.03 (2H, ddd, $J= 6.8, 6.1, 5.7$ Hz), 2.87 (2H, ddd, $J= 6.8, 6.6, 5.7$ Hz) 1.80 (3H, s), 1.36 (9H, s), 1.42-1.31 (4H, m), 1.26-1.18 (2H, m); ESI-MS: m/z 630.2 (M + Na)⁺

Synthesis of 7-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy- β -D-glucopyranosyl) *tert*-butyl 6-(7-hydroxy-coumarin-4-acetamido)hexylcarbamate (9)

Triol **8** (425 mg, 0.68 mmol) was taken up in pyridine (6.8 mL) and cooled in an ice bath. Added benzoyl chloride (79 μ L, 0.68 mmol) and solution was stirred for 1 h until most of the solid had dissolved. More benzoyl chloride (95 μ L, 0.82 mmol) was added and the solution was stirred for an additional 2 h. Methanol (10 mL) was then added and the solvent was evaporated. The residue was purified by column chromatography (2.5% MeOH in DCM – 5% MeOH in DCM) to afford dibenzoate **9** as a white solid (334 mg, 59%). R_f 0.22 (5% MeOH in DCM); ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.20-8.15 (2H, m), 8.03 (2H, d, $J= 6.9$ Hz) 7.99 (2H, d, $J= 7.3$ Hz), 7.70-7.64 (3H, m), 7.60-7.53 (4H, m), 7.22 (1H, d, $J= 2.2$ Hz), 7.00 (1H, dd, $J= 8.8, 2.5$ Hz), 6.75 (1H, dd, $J= 5.7, 5.4$ Hz), 6.33 (1H, s), 5.89 (1H, d, $J= 6.0$ Hz), 5.55 (1H, d, $J= 8.5$ Hz), 5.31 (1H, dd, $J= 10.1, 9.5$ Hz), 4.69 (1H, d, $J= 10.4$ Hz), 4.38 (1H, dd, $J= 12.0, 6.9$ Hz), 4.27-4.19 (2H, m), 3.78 (1H, ddd, $J= 9.5, 6.0, 5.7$ Hz), 3.68 (1H, d, $J= 16.7$ Hz), 3.67 (1H, d, $J= 16.7$ Hz), 3.05 (1H, td, $J= 6.6, 6.3$ Hz), 2.89 (1H, td, $J= 6.6, 6.3$ Hz), 1.68 (3H, s), 1.37 (9H, s), 1.42-1.30 (4H, m), 1.26-1.19 (4H, m); ESI-MS: m/z 852.3 (M + Na)⁺

Synthesis of 7-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy- β -D-galactopyranosyl) *tert*-butyl 6-(7-hydroxy-coumarin-4-acetamido)hexylcarbamate (10)

Alcohol **9** (334 mg, 0.40 mmol) was taken up in pyridine (2.6 mL) and DCM (8 mL) and cooled in an ice bath. Added triflic anhydride (85 μ L, 0.5 mmol) and the solid dissolved and the solution turned orange. After 1 h additional triflic anhydride was added (85 μ L, 0.5 mmol). After an additional 2 h, the reaction poured over aq. HCl (0.1 M) and diluted with CHCl₃. The layers were separated and the organic layer was washed with water, aq.

NaHCO₃ (sat.), and water. The solvent was then evaporated to afford an orange oil that was used in the next reaction without further purification. The triflate was taken up in DMF (0.4 mL) and sodium nitrite (222 mg, 3.2 mmol) was added. The solution was stirred overnight, then poured over water and diluted with chloroform. The layers were separated and the aqueous layer was extracted with chloroform. The combined organic layers were washed with aq. HCl (0.1 M) and brine, dried (MgSO₄) and evaporated. The residue was recrystallized from MeOH to afford galactopyranoside **10** as a white crystalline solid (167 mg, 50%). *R_f* 0.22 (5% MeOH in DCM); ¹H NMR (500 MHz, DMSO-d₆): δ 8.15 (1H, dd, *J* = 5.7, 5.4 Hz), 8.03-7.97 (5H, m), 7.70-7.63 (3H, m), 7.59-7.53 (4H, m), 7.20 (1H, d, *J* = 2.5 Hz), 6.99 (1H, dd, *J* = 8.8, 2.5 Hz), 6.73 (1H, t, *J* = 5.0 Hz), 6.31 (1H, s), 5.59 (1H, d, *J* = 6.0 Hz), 5.45 (1H, d, *J* = 8.5 Hz), 5.07 (1H, dd, *J* = 11.0, 3.2 Hz), 4.60 (1H, dd, *J* = 19.9, 9.0 Hz), 4.48 (1H, d, *J* = 7.3 Hz), 4.44 (1H, d, *J* = 8.2 Hz), 4.42 (1H, d, *J* = 8.2 Hz), 4.22 (1H, dd, *J* = 5.7, 3.2 Hz), 3.68 (1H, d, *J* = 15.3 Hz), 3.65 (1H, d, *J* = 15.3 Hz), 3.04 (1H, ddd, *J* = 6.9, 6.6, 6.0 Hz), 2.88 (1H, td, *J* = 6.6, 6.3 Hz), 1.74 (3H, s), 1.36 (9H, s), 1.41-1.30 (4H, m), 1.25-1.18 (4H, m); ESI-MS: *m/z* 852.3 (M + Na)⁺

Synthesis of 7-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-sulfonato-β-D-galactopyranosyl) *tert*-butyl 6-(7-hydroxy-coumarin-4-acetamido)hexylcarbamate sodium salt (C)

Alcohol **10** (87 mg, 0.11 mmol) was taken up in pyridine (2.6 mL) and SO₃·pyridine (33 mg, 0.21 mmol) was added. After 3 h, MeOH (1 mL) was added and the solution was applied to a plug of DOWEX 50WX8 (Na⁺ form) and rinsed with MeOH (20 mL). The eluent was collected and evaporated. The residue was purified by column chromatography (10% MeOH in DCM – 20% MeOH in DCM) to afford sulfate **C** as a white powder (55 mg, 56%). *R_f* 0.71 (20% MeOH in DCM); ¹H NMR (500 MHz, DMSO-d₆): δ 8.17 (1H, t, *J* = 5.5 Hz), 8.04-7.94 (5H, m), 7.65-7.59 (3H, m), 7.56 (2H, dd, *J* = 7.9, 7.5 Hz), 7.49 (2H, dd, *J* = 7.7, 7.5 Hz), 7.16 (1H, d, *J* = 2.4 Hz), 6.97 (1H, dd, *J* = 8.6, 2.4 Hz), 6.76 (1H, t, *J* = 6.0 Hz), 6.30 (1H, s), 5.45 (1H, d, *J* = 8.4 Hz), 5.13 (1H, dd, *J* = 11.2, 3.3 Hz), 4.79 (1H, d, *J* = 3.2 Hz), 4.59 (1H, dd, *J* = 11.7, 3.1 Hz), 4.52-4.37 (3H, m), 3.67 (1H, d, *J* = 15.2 Hz), 3.63 (1H, d, *J* = 15.2 Hz), 3.04 (1H, ddd, *J* = 6.8, 6.6, 6.0 Hz), 2.87 (1H, dt, *J* = 7.0, 6.2 Hz), 1.74 (3H, s), 1.36 (9H, s), 1.40-1.31 (4H, m), 1.28-1.18 (4H, m); ESI-MS: *m/z* 908.9 (M-H)⁻

Synthesis of 7-*O*-(2-acetamido-2-deoxy-4-*O*-sulfonato-β-D-galactopyranosyl) *tert*-butyl 6-(7-hydroxy-coumarin-4-acetamido)hexylcarbamate sodium salt (1)

Compound **C** (105 mg, 0.11 mmol) was taken up in MeOH (4 mL) and sodium methoxide (0.22 mL, 0.5 M, 0.11 mmol) was added. The reaction was stirred and monitored by MS (ESI-). After 5 h, all dibenzoate (908 *m/z*) and monobenzoate (804 *m/z*) were converted to diol (700 *m/z*). The reaction was quenched by adding Amberlite IRC-50 (H⁺ form) until the solution was neutral by litmus paper. The solution was filtered and solvent evaporated to afford a yellow solid. Purification by column chromatography (silica gel, CHCl₃:MeOH with 5% H₂O, 0% to 30%) afforded the deprotected galactopyranoside **1** as a white solid (62 mg, 77%). *R_f* 0.10 (CHCl₃:MeOH with 5% H₂O, 3:1); ¹H NMR (500 MHz, DMSO-d₆): δ 8.16 (1H, dd, *J* = 5.8, 5.5 Hz), 7.81 (1H, d, *J* = 9.2 Hz), 7.68 (1H, d, *J* = 8.9 Hz), 7.01 (1H, d, *J* = 2.4 Hz), 6.93 (1H, dd, *J* = 9.2, 2.4 Hz), 6.76 (1H, t, *J* = 5.8 Hz), 6.27 (1H, s), 5.10 (1H, d, *J* = 8.5 Hz), 4.82 (1H, d, *J* =

6.7 Hz), 4.53 (1H, dd, $J = 7.6, 5.2$ Hz), 4.51 (1H, d, $J = 3.1$ Hz), 3.93 (1H, ddd, $J = 10.7, 8.9, 8.5$ Hz), 3.86 (1H, dd, $J = 7.0, 5.8$ Hz), 3.74 (1H, ddd, $J = 10.7, 6.7, 3.1$ Hz), 3.66 (2H, br s), 3.56 (1H, ddd, $J = 11.3, 5.8, 5.2$ Hz) 3.49 (1H, ddd, $J = 11.3, 7.3, 7.0$ Hz) 3.04 (1H, app dt, $J = 7.0, 6.7$ Hz), 2.88 (1H, app td, $J = 7.0, 6.1$ Hz), 1.79 (3H, s), 1.36 (9H, s), 1.41-1.30 (4H, m), 1.25-1.18 (4H, m); ESI-MS: m/z 700.8 (M-H)⁻