Supporting information

Discovery of novel FabF ligands inspired by platensimycin by integrating structure-based design with diversity-oriented synthetic accessibility

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1. De novo design methods

The design procedure using the SPROUT de novo design program has been reported extensively elsewhere\textsuperscript{1-4}. In order to integrate the synthetic strategy with the design procedure some alternations were made; these are reported here.

1.1 Overview of the design process

Using the CANGAROO module the binding site was determined as any amino acid residue within 10 angstroms of platensimycin in the 2GFX crystal structure. Next, after examination of the binding site in the HIPPO module, Arg 206, Thr270 and Ala 205 were identified as potential residues to form H-bonding interactions close to the position of the cage region. These procedures complete, fragments could then be docked to target sites in the ELEFANT module and linked together in the SPIDER module. The ALIGATOR module was used to remove compounds that could not be made via our chosen approach. The top scoring skeletons were then selected and rescored in the HIPPO module then energy minimised in Maestro and rescored in HIPPO. Potential ligand designs which maintained a score close to that achieved by platensimycin after this process were selected for further investigation in Glide.

1.2 Fragment preparation

1.2.1 Preparation of the headgroup fragments

The structure of the ligand fragment including the headgroup was drawn in Maestro. It was ensured the headgroup portion of the fragment had the same co-ordinates as the platensimycin headgroup. This allowed the fragment to be placed within the platensimycin binding site of FabF and use Maestro to generate a series of different rotomers which could all potentially exist within the protein structure. Various rotomers generated (Figure S1.1) were then imported into the SPROUT library module and treated as described in section 1.2.3. For rotamer generation an MM2 force field was used with water as a solvent at pH 8 ± 0.5, headgroup atoms were fixed in position using the “fix atom” tool. All water molecules external to the platensimycin binding site were removed and the protein residues fixed in position using the “fix atom” tool.
1.2.2 Preparation of DOS fragments

The simpler fragments, described in Figure 3 (main paper) were derived from molecules made previously within the group (Figure S1.2).

Figure S1.1: Rotomer generation in Maestro. Panel A) fragment structure. Panel B) Different rotational structures generated, the headgroup portion of the molecule was fixed.

Figure S1.2. Origin of connection rules and DOS fragments Panel A) known compounds synthesised using our synthetic strategy. Panel B) Example fragments generated from these molecules.

For fragments which contained chiral centres all enantiomers and diastereomers were generated and incorporated into the library. Again rotomers were generated using the same...
procedure, however, unlike the headgroup fragments the protein structure was not used to guide acceptable fragments. Once the rotomers were generated they were imported into the SPROUT library module.

1.2.3 Treatment of fragments in the SPROUT library module

All the fragments used in the design process were converted for use in SPROUT using the library design module in SPROUT. This module was used to define some atoms on the fragment as “joinable” or “non-joinable”. Joinable atoms are able to make covalent interactions with other atoms on different fragments during the design process. Similarly, all carbon atoms were defined as hydrophobic and heteroatoms were defined as H-bond donors or acceptors as appropriate. The weighting of how hydrophobic an atom or the predicted strength for particular functional groups to make hydrogen bond was determined through algorithms within the program.

1.3 Minimisation of platensimycin analogues

All fragments designed within the SPROUT de novo design program were energy minimised in Glide in the context of the FabF active site. The position of the amino acids was fixed artificially to the positions from the 2GFX crystal structure. For minimisation an MM2 force field was used with water as a solvent at pH 8 ± 0.5. All water molecules external to the platensimycin binding site were removed and the protein residues fixed in position using the “fix atom” tool. Once minimisation was complete the ligand was imported back into SPROUT and scored in the HIPPO module.

1.4 Design process in Glide

Glide was selected for the addition of substitution as it was best able to reproduce the platensimycin binding pose (Figure S1.3). In order to reproduce the correct structure the interaction between the acid molecule and the histidine residues of the FabF were fixed. Attempts to reproduce the platensimycin binding pose in Glide without this resulted in poor overlap with the known binding pose. Similarly, attempts to reproduce the binding pose in Autodock and eHiTS failed. During the Glide design process the pH was set to 8, the program
was allowed to generate tautomers and up to 4 low energy ring conformations. The runs were allowed to generate 10 poses per molecule per run within a range of 0.5 kcalmol$^{-1}$.

**Figure S1.3**: reproduction of the platensimycin binding pose in Glide.

### 1.5 Predicted interactions of designed analogues.

The predicted binding poses with hydrogen bonding interactions highlighted of the substituted designs from Glide, which were subsequently synthesised, are shown in Figure 6 (main paper) and Figure S1.4
Figure S1.4: Hydrogen bonding interactions of ligands with the C163Q mutant of FabF. Panel A: Known interactions of platensimycin 6; its interaction with Ala309 has been omitted for clarity. Panel B: Predicted interactions of the sulfurea 9d. Panel C: Predicted interactions of the urea 9g.
A series of polycyclic designs with substitution were also designed but which were unable to be synthesised. The predicted binding affinities are shown in Table S1.1 and a predicted binding pose in Figure S1.5.

**Table S1.1**: Predicted affinities of compounds containing the polycyclic molecular scaffold.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Glide score</th>
<th>SPROUT score</th>
</tr>
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<td>-9.03</td>
</tr>
<tr>
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<td><img src="image5" alt="Structure 5" /></td>
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<td>-8.68</td>
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</tbody>
</table>
Figure S1.5 Top scoring substituted ligand from molecular scaffold. The predicted binding pose (top), and predicted hydrogen bonding interactions (bottom) are shown.
2. Synthetic chemistry

2.1 General Experimental

All non-aqueous reactions were carried out under an atmosphere of nitrogen. Water-sensitive reactions were performed in oven or flame-dried glassware cooled under nitrogen before use. An amorphous solid refers to a compound that was isolated as a non-crystalline solid or one which could not be re-crystallised in order to obtain an accurate melting point. Fluorous tagged compounds were typically isolated as foams and were not crystalline; because, of this melting points were not obtained.

Solvents were removed under reduced pressure using either a Büchi rotary evaporator and a Vacuubrand PC2001 Vario diaphragm pump, or a Genevac HT-4 evaporation system. Magnesium turnings were dried before use by heating with a heat gun under a flow of nitrogen. Solvents were distilled before use when necessary and possible according to scale. Tetrahydrofuran was freshly distilled from sodium, using benzophenone as a self-indicator. Dichloromethane (DCM), toluene and propan-2-ol were freshly distilled from calcium hydride. Anhydrous N,N-dimethylformamide (DMF) was obtained from Sigma-Aldrich. MTBE refers to methyl tertiarybutyl ether, ether refers to diethyl ether and petrol refers to petroleum spirit (b.p. 40-60 °C) unless otherwise stated.

Flash column chromatography was carried out using silica (35-70 μm particles) according to the method of Still, Kahn and Mitra. Thin layer chromatography was carried out on commercially available pre-coated glass or aluminium plates (Merck silica 2 8 8 0 Kieselgel 60F254).

Fluorous–Solid Phase Extraction (F-SPE) was carried out using pre-packed FluoroFlash® cartridges purchased from Fluorous Technologies Inc. Cartridges were washed extensively with DMF and MeOH and were pre-conditioned with 60:40 MeOH–H2O. Crude reaction mixtures were loaded onto cartridges using DMF and eluted using first 60:40 MeOH–H2O, then 80:20 MeOH–H2O (if required) as the fluorophobic eluent then methanol as the fluorophilic solvent. Methanol–NEt3 (200:1) was used an eluent in the presence of amines.

Strong-cation exchange (SCX) was carried out with Discovery DSC-SCX cartridges purchased from Sigma-Aldrich. Before use cartridges were activated by repeated washing with distilled water, crude mixtures were loaded onto cartridges using water or methanol eluting with water then
methanol to remove neutral or negatively charges ions then saturated ammonia methanol to remove the deprotonated amine. After repeated use cartridges were cleaned by eluting with 0.5 M trifluoromethanesulfonic acid followed by water.

Proton and carbon NMR spectra were recorded on a Bruker Advance DPX 300, Advance 500 or DRX500 spectrophotometer using an internal deuterium lock. Carbon NMR spectra were recorded with composite pulse decoupling using the waltz 16 pulse sequence. DEPT, COSY, HMQC and HMBC pulse sequences were routinely used to aid the assignment of spectra and example spectra. Chemical shifts are quoted in parts per million downfield of tetramethylsilane, and coupling constants \( (J) \) are given in Hz. NMR spectra were recorded at 300 K unless otherwise stated. Melting points were determined on a Reichert hot stage microscope and are uncorrected. Signals for fluorinated carbons in \( ^{13}\text{C} \) NMR spectra were weak and so were routinely not observed or reported. Quaternary carbons were also often weak and not observed; where this is the case it has been noted in the spectra assignment.

Infrared spectra were recorded on a Perkin Elmer spectrum One FT-IR spectrophotometer. Nominal mass spectrometry was routinely performed on a Waters-Micromass ZMD spectrometer using electrospray (+) ionization. Nominal and accurate mass spectrometry using electrospray ionisation was carried out by staff in the School of Chemistry at the University of Leeds, using either a Micromass LCT-KA111 or Bruker MicroTOF mass spectrometer. Field Desorption Ionisation mass spectra were acquired on a Waters-Micromass GCT premier spectrometer equipped with a Linden LIFDI probe. Melting points were determined on a Reichert hot stage microscope and are uncorrected.

Compounds which had been synthesized previously are referenced in the title, in all cases key spectral information matched that reported previously.

### 2.2 Compound numbering key

Figure S2.1 shows the compound numbering scheme used for all platensimycin analogues and all the intermediates on route to these compounds. With the exception of the fluorous ester protecting groups are not numbered and labelled by their abbreviated name *e.g.* Ns, MOM or TBDPS.
2.3 General Methods

General method 1: Deprotection of headgroup

LiOH (10 equiv) was added to a solution of the fluorous-tagged compound in 4:1 THF—H₂O and stirred at 45 °C. Once complete the reaction was diluted with 1M HCl and extracted with ether, the solvent was removed under vacuum. The residue was then dissolved in 4:1 THF—1M HCl and stirred at 45 °C. Once complete the reaction was diluted with 1M HCl and extracted with ether, the solvent was removed under reduced pressure and the crude product purified by column chromatography.

General method 2: Removal of the o-nitro phenylsulfonyl group

Thiophenol (3 equiv) was added to a stirred solution of the Ns-protected amine (1 equiv) and potassium carbonate (6 equiv) in anhydrous DMF (0.1 M). The resulting yellow solution was stirred until complete conversion of the starting material was observed by TLC. The solution was then quenched with HCl, diluted with water (0.2 ml) and loaded directly onto a cat-ion exchange column washing with water, then MeOH, then saturated ammonia MeOH to elute the deprotonated amine, the solvent was then removed under vacuum.
**General Method 3: Mitsunobu reaction of fluorous-tagged amines**

Diisopropyl azodicarboxylate (3-4 equiv) was added to a solution of the sulfonamide 23 (1 equiv), PPh₃ (3-4 equiv) and the alcohol (3-4 equiv) in anhydrous THF (0.1 M) and allowed to stir at room temperature until complete by TLC. The THF was then removed under vacuum to give a bright yellow oil which was loaded directly onto an F-SPE column of appropriate size with DMF.

**General method 4: Metathesis in methyl tertiarybutyl ether (MTBE)**

Hoveyda—Grubbs II catalyst (5 mol%) was added to a solution of the diene and 1,4 benzoquinone (10 mol%) in MTBE (0.01 M) and heated to 50 °C until no starting material could be seen by TLC. Once complete trishydroxymethyl phosphine (50 equiv) and NEt₃ (50 equiv) were added and stirred for 30 mins then silica 5.0 g was added and stirred again for a further 30 mins after which the mixture is filtered over celite and washed with 98:2 EtOAc—NEt₃ the solvent was then removed under vacuum, and the crude product purified by column chromatography.

### 2.4 Experimental

**2, 4-Dihydroxy-3-propionamidobenzoic acid**

![Chemical structure](image)

By general method 1 LiOH (147 mg, 3.6 mmol) was added to a solution of the ester 34 (120 mg, 0.36 mmol) in 4:1 THF—H₂O (5 mL) and heated to 45 °C overnight. Then 2M HCl (750 μL) was added and stirred at 45 °C for 4 hrs. Once complete the solvent was removed under reduced pressure and the resulting crude product was purified by column chromatography eluting with 40:59:1 Petrol—EtOAc—Acetic acid. The acid 7 was isolated as a brown amorphous solid (40 mg, 50%); νₘₘₙ/cm⁻¹ 3500-2941 (broad), 1643, 1579; δₜ (300 MHz; MeOD) 8.05-8.23 (2H, bs, Phenol), 7.55 (1H, d, J 7.5, H-6), 6.31 (1H, d, J 7.5, H-5), 2.42 (2H, q, J 7.4, H-2'), 1.15 (3H, t, J 7.4, H-3'); δ_c (75 MHz; MeOD) 177.3, 159.3, 158.3, 130.7, 114.2, 109.2, 30.4, 10.7; m/z (ES⁺) 226.1 ([M + H]⁺, 100%); HRMS found [MH]⁺ 226.0701, C₁₀H₁₁NO₅ requires MH 226.0704.

2 quaternary carbons were not observed.
2,4-Dihydroxy-3-(3′-(1″,2″,5″,6″-tetrahydropyridin-3″-yl)propanamido)benzoic acid

By general method 2 thiophenol (18 µL, 0.16 mmol) was added to a suspension of potassium carbonate (45 mg, 0.33 mmol) and the sulfonamide 9h (20 mg, 0.032 mmol) in DMF (1 mL) and stirred for 1 hour. Once complete by TLC the reaction was quenched with 2M aqueous HCl (200 µL) and purified by solid cation-exchange chromatography. The amine 8 was isolated as a colourless amorphous solid (10 mg, 80%); ν\text{max}/cm⁻¹ 3500-2800 (broad), 1638 and 1571; δ\text{H} (300 MHz; d₆DMSO) 7.38 (1H, d, J 8.5, H-6), 6.05 (1H, d, J 8.5, H-5), 5.66 (1H, s, H-4″), 3.57 (2H, s, H-2″), 3.10 (2H, t, J 6.1, H-6″), 2.59 (2H, t, J 7.1, H-2″), 2.31 (2H, t, J 7.1, H-3″), 2.18-2.23 (2H, bs, H-5″); δ\text{C} (75 MHz; d₆DMSO) 172.2, 171.5, 131.4, 128.1, 120.0, 111.1, 104.9, 43.6, 33.7, 30.6, 21.9; m/z (ES⁺) 307.1 ([M + H]⁺, 100%); HRMS found [MH]⁺ 307.1288, C₁₅H₁₈N₂O₅ requires MH 307.1292.

2 quaternary carbons were not observed

2,4-Dihydroxy-3-(3′-(phenylsulfonyl)-1″,2″,5″,6″-tetrahydropyridin-3″-yl)propanamido)benzoic acid

By general method 1 LiOH (22 mg, 0.56 mmol) was added to a solution of the fluorous ester S40a (55 mg, 0.056 mmol) in 4:1 THF—H₂O (5 mL) and heated to 45 °C overnight. Then 2M HCl (1 mL) was added and stirred at 45 °C for 4 hrs. Once complete by TLC the solvent was removed under reduced pressure and the resulting crude product was purified by column chromatography eluting with 20:79:1 Petrol—EtOAc—Acetic acid. The acid 9c was isolated as a brown amorphous solid, (9 mg, 36%), ν\text{max}/cm⁻¹ (film) 3100-2600 (broad), 1652, 1533; δ\text{H} (300 MHz; CDCl₃) 7.96 (1H, s, amide), 7.84 (2H, ad, J 7.7, H-6 and Ph), 7.53-7.71 (4H, m, Ph), 6.59 (1H, d, J 8.9, H-5), 5.60-5.66 (1H, bs, H-4″), 3.56 (2H, s, H-2″), 3.18 (2H, t, J 5.8, H-6″), 2.68 (2H, t, J 7.4, H-2″), 2.39-2.48 (2H, m, H-3″), 2.21-2.30 (2H, bs, H-5″); δ\text{C} (75 MHz; CDCl₃)
173.6, 172.8, 155.7, 154.3, 136.1, 132.9, 131.8, 129.1, 128.6, 127.6, 120.8, 114.2, 114.2, 111.7, 47.0, 42.5, 34.9, 30.0, 25.1; m/z (ES\(^+\)) 447.1 ([M + H]\(^+\), 100%); HRMS found [MH]\(^+\) 447.1220, C\(_{21}\)H\(_{22}\)N\(_2\)O\(_7\)S requires MH 447.1213.

2 quaternary carbons were not observed

3-(3′-(N-(tert-butoxycarbonyl)sulfamoyl)-1″,2″,5″,6″-tetrahydropyridin-3″-yl)propanamido)-2,4-dihydroxybenzoic acid

By general method 1 LiOH (10 mg, 0.25 mmol) was added to a stirred solution of the ester S40b (50 mg, 0.49 mmol) in 4:1 THF—H\(_2\)O (1 mL) and heated to 45 °C for 5 hrs. Then 2M HCl (250 µL) was added and stirred at 45 °C for 3 hrs. Once complete by TLC the solvent was removed under reduced pressure and the resulting crude product was purified column chromatography eluting with 40:59:1 Petrol—EtOAc—Acetic acid. The acid 9d was isolated as a yellow foam, 13 mg, 54%. \(\nu_{\text{max}}/\text{cm}^{-1}\) 3400-2800 (broad), 1651 and 1544; \(\delta_{\text{H}}\) (500 MHz; MeOD) 7.67 (1H, d, \(J = 8.9\), H-6), 6.42 (1H, d, \(J = 8.9\), H-5), 5.70-5.74 (1H, bs, H-4″), 3.87-3.90 (2H, bs, H-2″), 3.44 (2H, t, \(J = 5.8\), H-6″), 2.67 (2H, t, \(J = 7.2\), H-2′), 2.44 (2H, t, \(J = 7.2\), H-3′), 2.21-2.27 (2H, bs, H-5″), 1.49 (9H, s, tBu); \(\delta_{\text{C}}\) (125 MHz; MeOD) 175.2, 165.5, 159.1, 158.4, 152.6, 134.5, 134.3, 130.5, 121.4, 121.3, 113.9, 109.0, 83.4, 44.4, 35.2, 31.5, 35.1, 30.8, 28.3, 26.0 m/z (ES\(^+\)) 508.1 ([M + H]\(^+\), 100%); HRMS found [MNa]\(^+\) 508.1360, C\(_{20}\)H\(_{27}\)N\(_3\)O\(_9\)S requires MNa 508.1346.

2,4-Dihydroxy-3-(3′-(sulfamoyl-1″,2″,5″,6″-tetrahydropyridin-3″-yl)propanamido)benzoic acid

Sodium iodide (16 mg, 0.11 mmol) was added to a stirred solution of the crude acid 9d (15 mg, 0.026 mmol) in 10:1 acetone—water (295 µL) the suspension was then refluxed at 80 °C for 30 mins. The solvent was removed under reduced pressure, the crude product loaded onto silica with methanol and purified by column chromatography eluting with 40:59:1 petrol—EtOAc—acetic acid. The sulfonyl urea 9e was isolated as a yellow amorphous solid.
(4.6 mg, 46%). $\nu_{max}$/cm$^{-1}$ 3500-2800 (broad), 1653 and 1540; $\delta_\text{n}$ (500 MHz; MeOD) 7.68 (1H, d, $J$ 8.9, H-6), 6.45 (1H, d, $J$ 8.9, H-5), 5.68-5.74 (1H, bs, H-4$''$), 3.61-3.66 (2H, bs, H-2$''$), 3.20 (2H, t, $J$ 6.1, H-6$'$), 2.66 (2H, t, $J$ 8.1, H-2$'$), 2.44 (2H, t, $J$ 8.1, H-3$'$), 2.23-2.30 (2H, bs, H-5$'$); $\delta_\text{C}$ (75 MHz; MeOD) 179.5, 177.8, 136.9, 123.6, 111.8, 80.2, 51.2, 46.6, 37.8, 34.2; $m/z$ (ES$^+$) 408.1 ([M + H]$^+$, 100%); HRMS Found: 408.0836, C$_{15}$H$_{19}$N$_3$O$_7$S requires MNa 408.0831.

4 quaternary carbons were not observed

2,4-Dihydroxy-3-(3$'$-{(isopropylcarbamoyl)-1$'$,2$'$,5$'$,6$''$-tetrahydropyridin-3$''$-yl)propanamido}benzoic acid

![Structural formula of the compound](image)

**By general method 1** LiOH (11 mg 0.028 mmol) was added to a solution of the ester $S_{40c}$ (48 mg, 0.052 mmol) in 4:1 THF—H$_2$O (600 µL) and heated to 45 °C for 5 hrs. Then 2M HCl (100 µL) was added and stirred at 45 °C for 4 hrs. Once complete by TLC the solvent was removed under reduced pressure and the resulting crude product was purified by column chromatography eluting with 30:69:1 Petrol—EtOAc—Acetic acid. The acid 9f was isolated as a red foam, (15.1 mg, 74%). $\nu_{max}$/cm$^{-1}$ 3500-2800 (broad), 1621, 1538; $\delta_\text{H}$ (500 MHz; MeOD) 8.04 (1H, s, amide), 7.55 (1H, d, $J$ 8.8, H-6), 6.31 (1H, d, $J$ 8.8, H-5), 5.60-5.64 (1H, bs, H-4$''$), 3.81 (1H, aq, $J$ 6.5, CH(Me)$_2$), 3.73 (2H, s, H-2$''$), 3.34 (2H, t, $J$ 5.7, H-6$''$), 2.25 (2H, t, $J$ 7.5, H-2$'$), 2.32 (2H, t, $J$ 7.5, H-3$'$), 2.00-2.04 (2H, bs, H-5$''$), 1.04 (6H, d, $J$ 6.5, $^1$Pr); $\delta_\text{C}$ (125 MHz; MeOD) 177.9, 176.7, 162.1, 161.9, 161.3, 137.8, 133.1, 124.4, 116.3, 111.6, 110.2, 80.0, 49.5, 46.3, 38.0, 34.1, 33.3, 28.5, 25.9; $m/z$ (ES$^+$) 447.1 ([M + H]$^+$, 100%); HRMS found [MH]$^+$ 392.1821, C$_{19}$H$_{25}$N$_3$O$_6$ requires MH 392.1816.

2,4-Dihydroxy-3-(3$'$-{(pyridin-3$'''$-ylcarbamoyl)-1$'$,2$'$,5$'$,6$''$-tetrahydropyridin-3$''$-yl)propanamido}benzoic acid

![Structural formula of the compound](image)

**By general method 1** LiOH (10 mg, 0.26 mmol) was added to a stirred solution of the ester $S_{40d}$ (49 mg, 0.051 mmol) in 4:1 THF—H$_2$O (600 µL) heated to 45 °C for 5 hrs. Then 2M HCl
(150 µL) was added and stirred at 45 °C for 3 hrs. Once complete by TLC the solvent was removed under reduced pressure and the resulting crude product was purified by column chromatography eluting with 99:1 EtOAc—Acetic acid the acid 9g was isolated as a yellow foam, (10.6 mg, 49%), \( \nu_{\text{max}} / \text{cm}^{-1} \): 3500-2800 (broad), 1647 and 1540; \( \delta_{\text{H}} \) (500 MHz; \( d_6 \)DMSO) 9.27 (1H, bs, Urea or amide), 8.91 (1H, d, \( J = 4.5 \), Urea or amide), 8.72-8.75 (1H, bs, H-2'''), 8.14 (1H, d, \( J = 4.5 \), H-6'''), 7.95 (1H, d, \( J = 8.6 \), H-4''), 7.42 (1H, d, \( J = 8.6 \), H-6), 7.25 (1H, dd, \( J = 8.1, 4.5 \), H-5'''), 6.11 (1H, d, \( J = 8.6 \), H-5), 5.63-5.67 (1H, bs, H-4''), 3.98 (2H, s, H-2''), 3.54 (2H, t, \( J = 5.7 \), H-6''), 2.63 (2H, t, \( J = 7.6 \), H-2'), 2.32 (2H, t, \( J = 7.6 \), H-3'), 2.09-2.14 (2H, bs, H-5'); \( \delta_{\text{C}} \) (125 MHz; \( d_6 \)DMSO) 172.1, 171.9, 163.2, 154.8, 154.7, 142.5, 142.5, 137.4, 134.4, 127.7, 126.6, 127.7, 123.1, 119.5, 113.2, 110.7, 47.5, 40.1, 33.7, 30.2, 24.7; \( m/z \) (ES+): 427.2 ([M + H]+, 100%); HRMS found [MH]+ 427.1593, \( C_{24}H_{21}N_4O_6 \) requires MH 427.1612

4 quaternary carbons were not observed

2,4-Dihydroxy-3-(3′-{1″-2″-nitrophenylsulfonyl}-1″,2″,5″,6″-tetrahydropyridin-3″-yl)propanamido)benzoic acid

By general method 1 LiOH (50 mg, 1.26 mmol) was added to a solution of the cyclic 29 (130 mg, 0.126 mmol) in 4:1 THF—H₂O (2 mL) and heated to 45 °C overnight after which 2 M HCl (500 µL) was then added and stirred at 45 °C for 4 hrs. Once complete by TLC the solvent was removed under reduced pressure and the resulting crude product was purified column chromatography eluting with 40:59:1 Petrol—EtOAc—Acetic acid. The acid 9h was isolated as a colourless gum (30 mg, 50%). \( \nu_{\text{max}} / \text{cm}^{-1} \) (film) 3400-2851 (broad), 1649, 1545; \( \delta_{\text{H}} \) (300 MHz; MeOD) 7.93 (1H, ad, \( J = 7.8 \), Ns), 7.60-7.71 (3H, m, Ns), 7.57 (1H, d, \( J = 8.8 \), H-6), 6.35 (1H, d, \( J = 8.8 \), H-5), 5.55-5.61 (1H, bs, alkene), 3.71-3.76 (2H, bs, H-2''), 3.33 (2H, t, \( J = 5.7 \), H-6''), 2.53 (2H, t, \( J = 7.2 \), H-2''), 2.33 (2H, t, \( J = 7.2 \), H-3'), 2.06-2.14 (2H, bs, H-5); \( \delta_{\text{C}} \) (75 MHz; MeOD) 175.5, 159.8, 150.2, 135.6, 134.5, 133.2, 132.7, 132.0, 130.9, 125.6, 121.8, 114.0, 109.7, 105.0, 35.5, 48.4, 44.0, 31.7, 26.4; \( m/z \) (ES+): 492.1 ([M + H]+, 100%) HRMS found [MH]+ 492.1593, \( C_{24}H_{21}N_3O_9S \) requires MH 492.1612
2,4-Dihydroxy-3-(3′-(2″,5″,6″,7″-tetrahydro-1H-azepin-3″-yl)propanamido)benzoic acid

By general method 2 thiophenol (5 µL, 0.047 mmol) was added to a suspension of potassium carbonate (7.6 mg, 0.063 mmol) and the sulfonamide 10b (8 mg, 0.016 mmol) in DMF (500 µL) and stirred for 1 hour. Once complete by TLC the reaction was quenched with 2M aqueous HCl (200 µL) and purified by solid cation exchange chromatography. The amine 10a was isolated as a colourless amorphous solid (4 mg, 79%); ν\textsubscript{max}/cm\textsuperscript{-1} 3400-2500 (broad) and 1646; δ\textsubscript{H} (300 MHz; d\textsubscript{6}DMSO) 7.35 (1H, d, J 8.6, H-6), 6.00 (1H, d, J 8.6, H-5), 5.82 (1H, t, J 5.7, H-4″), 3.70 (2H, s, H-2″), 3.23 (2H, t, J 5.2, H-7″), 2.58 (2H, t, J 6.9, H-2′), 2.35 (2H, t, J 6.9, H-3′), 2.15-2.25 (2H, bs, H-5″), 1.66-1.78 (2H, bs, H-6″); δ\textsubscript{C} (75 MHz; d\textsubscript{6}DMSO) 173.0, 171.6, 135.3, 132.2, 128.2, 114.1, 104.5, 49.5, 46.9, 43.6, 34.3, 26.1, 23.9; m/z (ES\textsuperscript{+}) 321.1 ([M + H]\textsuperscript{+}, 100%); HRMS Found [MH]\textsuperscript{+} 321.1445, C\textsubscript{16}H\textsubscript{20}N\textsubscript{2}O\textsubscript{5} requires MH 321.1459.

3 quaternary carbons were not observed

2,4-Dihydroxy-3-(3′-(1″-(2″-nitrophenylsulfonyl)-2″,5″,6″,7″-tetrahydro-1H-azepin-3″-yl)propanamido)benzoic acid

By general method 1 LiOH (35 mg, 0.87 mmol) was added to a solution of the cyclic 30 (90 mg, 0.087 mmol) in 4:1 THF—H\textsubscript{2}O (1.8 mL) and heated to 45 °C overnight. 2 M HCl (500 µL) was then added and stirred at 45 °C for 4 hrs. Once complete by TLC the solvent was removed under reduced pressure and the resulting crude product was purified column chromatography eluting with 40:59:1 Petrol—EtOAc—Acetic acid. The acid 10b was isolated as a colourless gum (17 mg, 68%). ν\textsubscript{max}/cm\textsuperscript{-1} (film) 3400-2800, 1648 and 1543; δ\textsubscript{H} (300 MHz; CDCl\textsubscript{3}) 8.01-8.06 (1H, m, Ns), 7.64–7.75 (4H, m, H-6 and Ns), 6.59 (1H, d, J 8.9, H-5), 5.76 (1H,
t, J 5.6, H-4'), 3.95 (2H, t, J 5.9, H-7''), 2.78 (2H, t, J 7, H-2'), 2.53 (2H, t, J 7, H-3'), 2.28-2.36 (2H, m, H-5''), 1.88 (2H, aq, J 5.9, H-6''); δ_C (75 MHz; CDCl_3) 174.21, 156.5, 155.2, 148.4, 137.2, 133.9, 133.4, 132.1, 131.1, 130.2, 129.0, 124.5, 114.8, 112.0, 103.6, 50.7, 49.2, 35.6, 33.5, 30.1, 27.4, 26.7, 14.6; m/z (ES+ 100%)

2,4-Dihydroxy-3-(3′-((R*)-5′-(((S*)-1′′′,2′′′,5′′′,6′′′-tetrahydropyridin-2′′-yl)methyl)-1″,2″,5″,6″-tetrahydropyridin-3″-yl)propanamido)benzoic acid

By general method 2 thiophenol (14 µL, 0.12 mmol) was added to a suspension of potassium carbonate (14 mg, 0.12 mmol) and the sulfonamide 13d (17 mg, 0.022) in DMF (500 µL) and stirred for 6 hrs. Once complete by TLC the reaction was quenched with 2M aqueous HCl (200 µL) and purified using solid cation exchange chromatography. The diamine 12 was isolated as a yellow amorphous solid (8.2 mg, 91%). v_max/cm⁻¹ (film) 3300-2800 (broad), 2298, 2007, 1789, 1693, and 1606; δ_H (500 MHz; MeOD) 7.66 (1H, d, J 8.7, H-6), 6.38 (1H, d, J 8.7, H-5), 5.94-6.01 (1H, m, H-4''), 5.83-5.87 (1H, m, H-4''), 5.64 (1H, add, J 10.5, 2.0, H-3''), 3.98-4.04 (1H, bs, H-2''), 3.81 (1H, d, J 16.8, H-2''), 3.68 (1H, d, J 16.8, H-2''), 3.47-3.57 (1H, m, H-6''_a), 3.40-2.45 (1H, m, H-6''_b), 3.13-3.22 (1H, m, H-6''_b), 2.97 (1H, dd, J 12.3, 9.1, H-6''_b), 2.81-2.88 (1H, bs, H-5''), 2.68-2.74 (2H, m, H-2''), 2.41-2.60 (3H, m, H-3' and H-5''), 2.28-2.37 (2H, m, H-5''_b), 1.75-1.88 (2H, adt, J 8.6, 7.4, methylene); δ_C (125 MHz; MeOD) 174.4, 173.8, 157.7, 155.7, 131.4, 129.2, 126.0, 124.2, 123.5, 112.0, 110.5, 106.6, 50.2, 44.5, 43.8, 40.0, 36.0, 33.2, 29.9, 28.4, 21.2; m/z (ES+) 402.2 ([M + H]^+, 60%) HRMS found 402.2023, C_{22}H_{23}N_O_S requires MH 402.060.
2,4-Dihydroxy-3′-(3′′-((R*)-1′′-(2-nitrophenylsulfonyl)-5′′-((S*)-1′′-(2-nitrophenylsulfonyl)-1′′,2′′,5′′,6′′-tetrahydropyridin-2′′-yl)methyl)-1′′,2′′,5′′,6′′-tetrahydropyridin-3′′-yl)propanamido)benzoic acid

By general method 1 LiOH (19 mg, 0.46 mmol) was added to a solution of the cyclic 31 (65 mg, 0.091 mmol) in 4:1 THF—H₂O (800 µL) and heated to 45 °C then after 3 hrs 2 M HCl (200 µL) was added and stirred for 4 hrs. Once complete by TLC the solvent was removed under reduced pressure and the resulting crude product was purified column chromatography eluting with 40:59:1 Petrol—EtOAc—Acetic acid. The acid 13d was isolated as a colourless foam, (17 mg, 49%). v_m/ ν/cm⁻¹ (film) 3600-2800 (broad), 1652, 1537; δ_H (500 MHz; CDCl₃) 7.93-8.15 (3H, m, Ns), 7.59-7.75 (6H, m, Ns, NH and H-6), 7.55 (1H, dd, J 7.6, 1.4, Ns), 6.53 (1H, dd, J 9.1, H-5), 5.82-5.86 (1H, bs, H-4''), 5.57-5.65 (2H, m, H-3'' and H-4''), 4.43-4.50 (1H, bs, 2'''), 3.96 (1H, dd, J 14.9, 5.5, H-6'''), 3.88 (1H, d, J 16.5, H-2'''), 3.71 (1H, d, J 16.5, H-2''b), 3.41 (1H, dd, J 12.6, 4.5, H-6'''), 3.26-3.36 (2H, m, H-6''s and H-6''b), 2.76 (2H, asx, J 7.7, H-2'), 2.44-2.57 (3H, m, H-3' and H-5'''), 1.80-1.99 (2H, m, H-5'''s and H-5'''b), 1.68-1.76 (1H, m, CH₃ methylene ), 1.56-1.65 (1H, m, CH₆ methylene); δ_C (75 MHz; CDCl₃) 173.4, 173.1, 155.6, 154.5, 148.3, 148.1, 133.8, 133.6, 132.1, 131.8, 131.6, 131.3, 130.9, 130.2, 128.6, 127.7, 124.9, 124.8, 124.1, 123.9, 114.3, 111.4, 52.4, 50.8, 48.0, 47.1, 38.3, 38.0, 34.7, 31.7, 29.7, 23.0; m/z (ES⁺) 772.2 ([M+H]^+ 100%); HRMS found [MH]^+ 772.1590, C₃₃H₃₅N₅O₁₃S₂ requires MNα 772.1589.

Methyl 2,4-bis(methoxymethoxy)-3-nitrobenzoate⁶

N,N-Diisopropylethylamine (3.3 mL, 19.2 mmol) was added to a stirred solution of chloromethoxymethylether (0.9 mL, 11.0 mmol) and the ester S36 (0.5 g, 2.4 mmol) in anhydrous
DCM at −15 °C. The solution was allowed to warm to room temperature and then heated at reflux for 45 mins. The organic layer was washed with saturated NaHCO₃ (2 × 20 mL) water (20 mL), 1M HCl (2 × 20 mL) then brine (20 mL) dried (MgSO₄) and the solvent removed under reduced pressure. The crude product was purified by column chromatography eluting with 75:25 petrol—EtOAc. The protected catechol 14 was isolated as pale yellow needles (606 mg, 84%); m.p. 65-67 °C (toluene) [lit. 66-68 °C]; νₑ₅ₑₒₓ/cm⁻¹ (film) 3100, 3002, 2833, 1727, 1714, and 1672 δₜ (300 MHz; CDCl₃) 7.97 (1H, d, J 9.0, H-6), 7.07 (1H, d, J 9.0, H-5), 5.28 (2H, s, MOM), 5.15 (2H, s, MOM), 3.89 (3H, s, COOMe), 3.49 (6H, m, MOM); δₑ (75 MHz; CDCl₃) 164.7, 154.9, 154.4, 152.7, 151.2, 138.9, 111.1, 102.6, 95.3, 58.3, 57.3, 52.9; m/z (ES⁺) 324.1 ([M + Na]⁺, 100%).

2-Hydroxy-4-(methoxymethoxy)-3-nitrobenzoic acid

LiOH (403 mg, 9.6 mmol) was added to a stirred solution of the ether 14 (500 mg, 1.6 mmol) in THF—Water (4:1) and stirred for 24 hrs at room temperature. Once complete by TLC the reaction mixture was diluted with EtOAc (15 mL) then quenched with 0.1 M HCl to pH 3. The aqueous layer was then extracted with EtOAc (2 × 20 mL) and the combined organic layers washed with 0.1 M aqueous HCl, dried (MgSO₄) and the solvent removed under reduced pressure. The crude product was loaded onto silica and purified by column chromatography eluting with 90:10 petrol—EtOAc the acid 15 was isolated as colourless plates (350 mg, 87%); m.p. 162-164 °C (toluene); νₑ₅ₑₒₓ/cm⁻¹ 3200-2557, 1667 and 1614; δₜ (300 MHz; MeOD) 7.84 (1H, d, J 8.98, H-6), 6.70 (1H, d, J 8.98, H-1), 5.21 (2H, s, MOM), 3.21 (3H, s, MOM); δₑ (75 MHz; CDCl₃) 173.1, 156.2, 154.8, 134.1, 110.8, 106.6, 96.4, 57.3; m/z (ES⁺) 242.0 ([M + H]⁺, 100%).

3',3',4',4',5',5',6',6',7',7',8',8',9',9',10',10'−Heptadecafluorodecyl 2-hydroxy-4-(methoxymethoxy)-3-nitrobenzoate
1H, 1H, 2H, 2H Perfluro decanol (13.9 g, 30.0 mmol) was added to a stirred solution of the acid 15 (5.2 g, 21.4 mmol), DCC (6.17 g, 30.0 mmol) and DMAP (261 mg, 10 mol%) in 50:50 THF—MeCN (60 mL) at −10 °C, the bright yellow suspension was then stirred for 72 hrs. The reaction mixture was diluted with EtOAc (100 mL) filtered and loaded onto silica, then purified by column chromatography eluting with 80:20 DCM—Petrol. The fluorous ester 16 was isolated as a colourless plates, (7.2 g, 63%); m.p. 124–126 °C, νmax/cm−1 (film) 3110, 2915, 2254, 1692, 1627 and 1586; δH (300 MHz; CDCl3) 11.13 (1H, s, OH), 7.86 (1H, d, J 9.1, H-6), 6.80 (1H, d, J 9.1, H-5), 5.28 (2H, s, MOM), 4.68 (2H, t, J 6.4, H-1'), 3.49 (3H, s, OMe), 2.62 (2H, m, H-2'); δC (75 MHz; CDCl3) 168.8, 155.1, 154.8, 132.7, 107.5, 106.5, 95.3, 58.0, 57.3, 30.93 (t, J 21.1); m/z (ES+) 712.0 ([M + Na]+ 100%).

13C NMR signals from the fluorous tag and 1 quaternary carbon were not observed

N,N-Diisopropylethylamine (2.5 mL, 16.5 mmol) then chloro-methoxymethyl ether (1.5 mL, 18.7 mmol) were added to a stirred solution of the fluorous ester 16 (5.2 g, 7.5 mmol) in anhydrous DCM (250 mL) at −15 °C then allowed to warm to room temperature and stirred for 4 hrs. The organic layer was washed with water (250 mL), saturated NaHCO3 (200 mL), 0.1M HCl (100 mL) and brine (50 mL) then dried (MgSO4) and the solvent removed under reduced pressure. The MOM protected 17 was isolated without further purification as a pale yellow oil (5.2 g, 93%); νmax/cm−1 (film) 3155, 2967, 2254, 1794 and 1727; δH (500 MHz; CDCl3) 7.96 (1H, d, J 9.0, H-6), 7.10 (1H, d, J 9.0, H-5), 5.29 (2H, s, MOM), 5.15 (2H, s, MOM), 4.60 (2H, t, J 6.4, H-1'), 3.40 (6H, m, MOM), 2.60 (2H, m, H-2'); δC (125MHz; CDCl3) 163.1, 152.7, 151.1, 134.0, 117.2, 110.7, 102.3, 94.9, 57.9, 57.2, 56.9, 30.5 (t, J 22.1 Hz); m/z (ES+) 751.1 [M + NH4]+, 100%; HRMS found 751.0942 [MNH4]+ C21H16F17NO8 requires MNH4 751.0943.

13C NMR signals from the fluorous tag and 1 quaternary carbon were not observed
3',3',4',4',5',5',6',6',7',7',8',8',9',9',10',10'-Heptadecafluorodecyl 3-amino-2,4-
bis(methoxymethoxy)benzoate

Pd/C (55 mg) was added to a stirred solution of the fluorous ester 17 (400 mg, 0.54 mmol) in EtOAc (5 mL) under a hydrogen atmosphere for 24 hrs. The reaction mixture was then diluted with EtOAc (5 mL) and filtered through celite. The solvent was removed under reduced pressure and the crude mixture purified by column chromatography eluting with 75:25 Petrol—EtOAc. The analine 18 was isolated as a clear colourless oil (350 mg, 91%); ν\textsubscript{max}/cm\textsuperscript{-1} (film) 3155, 2253, 1793, 1718; δ\textsubscript{H} (300 MHz; CDCl\textsubscript{3}) 7.24 (1H, d, J 8.4, H-6), 6.86 (1H, d, J 8.4, H-5), 5.25 (2H, s, MOM), 5.10 (2H, s, MOM), 4.56 (2H, t, J 6.4, H-1'), 4.23 (2H, bs, NH\textsubscript{2}), 3.61 (3H, s, MOM), 3.49 (3H, s, MOM), 2.48-2.67 (2H, bm, H-2'); δ\textsubscript{C} (75 MHz; CDCl\textsubscript{3}) 165.3, 149.4, 146.1, 132.2, 120.7, 117.6, 109.9, 101.6, 95.2, 58.0, 56.7, 41.4, 31.1 (t, 22.1 Hz); m/z (ES\textsuperscript{+}) 704.1 [M + H]\textsuperscript{+}, 40%; HRMS found 704.0939 [MH]\textsuperscript{+} C\textsubscript{21}H\textsubscript{18}F\textsubscript{17}NO\textsubscript{6} requires MH \textsuperscript{704.0935}.

4-((tert-Butyldiphenylsilyloxy)methyl)pent-4-enoic acid

LiOH (1.7 g, 40.5 mmol) was added to a stirred solution of the ester S38 (5.3 g, 13 mmol) in THF—H\textsubscript{2}O (4:1, 20 mL). The reaction mixture was then diluted with water (50 ml), extracted with ethyl acetate (3 × 70 mL) washed with brine (50 mL) and the solvent removed under reduced pressure. The crude product was purified by column chromatography eluting with 90:10 Petrol—EtOAc. The acid 19 was isolated as colourless plates (3.6 g, 78%). ν\textsubscript{max}/cm\textsuperscript{-1} (film) 3100-2800 (broad), 2159, 1975 and 1710; δ\textsubscript{H} (300 MHz; CDCl\textsubscript{3}) 7.70-7.77 (4H, m, TBDPS), 7.40-7.53 (6H, m, TBDPS), 5.22-5.25 (1H, m, H\textsubscript{a} alkene), 4.93-4.95 (1H, m, H\textsubscript{b} alkene), 4.15-4.17 (2H, bs, H-5), 2.52-2.58 (2H, m, H-2), 2.35-2.42 (2H, m, H-3), 1.11 (9H, s, TBDPS); δ\textsubscript{C}
HATU (7.5 g, 17.4 mmol) was added to a stirred solution of the acid 19 (7.5 g, 0.29 mmol), N,N-Diisopropylethylamine (1.5 mL, 24.9 mmol) and aniline 18 (3.9 g, 11.6 mmol) in anhydrous DMF (80 mL). The reaction mixture was allowed to stir for 72 hrs at room temperature. Once complete by TLC the reaction mixture was diluted with water (500 mL), extracted with EtOAc (3 × 150 mL) and dried (MgSO₄). The solvent was removed under reduced pressure and the crude material purified by column chromatography eluting with 75:25 Petrol—EtOA. The amide 20 was isolated a clear colourless gel which solidified to a colourless amorphous solid (8.1 g, 91%); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2932, 2858, 2253, 1722 and 1600; \( \delta_{\text{H}} \) (300 MHz; CDCl₃) 7.78 (1H, d, \( J = 8.5 \), H-6), 7.67 (4H, d, \( J = 6.1 \), TBDPS), 7.32 - 7.46 (7H, m, Ph and NH), 7.03 (1H, d, \( J = 8.5 \), H-5), 5.21 (1H, bs, alkene), 5.19 (2H, s, MOM), 5.05 (2H, s, MOM), 4.95 (1H, bs, alkene), 4.57 (2H, t, \( J = 6.1 \), H-1'), 4.14 (2H, bs, H-2", H-3" or H-5"), 3.52 (3H, s, MOM), 3.45 (3H, s, MOM), 2.37-2.67 (6H, bm, H-2' and H-2", H-3" or H-5"), 1.06 (9H, s, tBu); \( \delta_{\text{C}} \) (75 MHz; CDCl₃) 164.1, 157.1, 146.8, 135.5, 133.5, 129.7, 127.7, 117.1, 110.9, 109.3, 101.6, 94.7, 66.4, 57.3, 56.6, 56.5, 30.5 , (t, \( J = 22.3 \), 26.8 , 19.3; \( m/z \) (ES+) 1054.2 ([M + H]^+), 100%) HRMS found MH^+ 1054.2457, \( C_{44}H_{44}F_{17}NO_{10}Si \) requires MH 1054.2472.

\(^{13}\text{C} \) NMR signals from the fluorous tag and 6 quaternary carbons were not observed

\[ 3\',3\',4\',4\',5\',5\',6\',6\',7\',7\',8\',8\',9\',9\',10\',10\',10\'-\text{Heptadecafluorodecyl} \ 3''-(5'')-(\text{hydroxymethyl})-2''-\text{oxohex-5''-enyl}-2,4-bis(\text{methoxymethoxy})\text{benzoate} \]

Tetrabutylammoniumfluoride, 1M in THF, (7.5 mL, 7.5 mmol) was added to a stirred solution of silyl ether 20 (5 g, 4.7 mmol) in anhydrous THF (50 mL) at 0 °C, the mixture was then...
allowed to warm to room temperature and stirred for a further 20 mins. The reaction mixture was quenched with acetic acid (450 µL) diluted with water (100 mL), extracted with EtOAc (3 × 100 mL), washed with saturated NaHCO₃ (100 mL), brine (50 mL) and dried (MgSO₄). The solvent was removed under reduced pressure and purified by column chromatography eluting with 20:80 Petrol—EtOAc. The alcohol 21 was isolated as colourless plates (3.14 g, 82%); m.p. 76-78 °C (EtOAc); ν max/cm⁻¹ (solid) 3000-3300 (broad), 2511, 2029, 1718, and 1650; δₜ (300 MHz; CDCl₃) 7.81 (1H, d, J 9, H-6), 7.51 (0.8H, bs, NH), 7.05 (1H, d, J 9, H-5), 5.24 (2H, s, MOM), 5.06 (3H, bs, MOM and alkene), 4.96 (1H, bs, alkene), 4.60 (2H, t, J 6.4, H-1'), 4.11 (2H, bs, H-2'', H-3'' or H-5''), 3.59 (3H, s, MOM), 3.47 (3H, s, MOM), 2.46-2.66 (7H, m, H-2', H-2'', H-3'' or H-5'', OH); δₜ (75 MHz; CDCl₃) 164.1, 157.2, 147.9, 111.9, 111.4, 101.9, 94.8, 66.4, 57.8, 57.1, 57.0, 30.9 (t, J 21 Hz); m/z (ES⁺) 816.1 ([M + H]⁺, 100%); HRMS found MH⁺ 816.1460, C₂₇H₂₆F₁₇NO₈ requires MH⁺ 816.1446

¹³C NMR signals from the flouorous tag and 8 quaternary carbons were not observed

3',3',4',4',5',5',6',6',7',7',8',8',9',9',10',10'-Heptadecafluorodecyl 3-(5'"-((N-(tert-butoxycarbonyl)-2-nitrophenylsulfonamido)methyl)-2"'-oxohex-5'"-enyl)-2,4-bis(methoxymethoxy)benzoate.

Diisopropyl azodicarboxylate (470 µL, 2.3 mmol) was added to a solution of the alcohol 21 (500 mg, 0.6 mmol), tert-butyl 2-nitrophenylsufonlycarbamate (772 mg 2.5 mmol) and PPh₃ (629 mg, 2.4 mmol) in THF (25 mL) and stirred at room temperature for 3 hrs. The solvent was removed under reduced pressure and the crude product purified by F-SPE followed by a silica plug eluting with 20:80 Petrol—EtOAc. The carbamate 22 was isolated as a colourless amorphous solid (626 mg, 95%); ν max/cm⁻¹ (film) 3234, 2981, 2484, 1722 and 1680; δₜ (300 MHz; CDCl₃) 8.37-8.42 (1H, m, Ns), 7.76-7.86 (4H, m, Ns and H-6), 7.40-7.52 (1H, bs, NH), 7.75 (1H, d, J 9, H-5), 5.27 (2H, s, MOM), 5.15-5.22 (1H, bs, alkene), 5.08-5.14 (3H, s, MOM), 4.65 (2H, t, J 6.6, H-1'), 4.37-4.46 (2H, bs, H-5''), 3.62 (3H, s, MOM), 3.53 (3H, s, MOM), 2.52-2.71 (6H, m, H-2', H-2'', H-3''), 1.38 (9H, s, boc); δₜ (75 MHz; CDCl₃) 164.3, 157.2,
Imidazole (18 mg, 0.27 mmol) was added to a suspension of the carbamate 22 (200 mg 0.18 mmol) and Cs$_2$CO$_3$ (88 mg, 0.27 mmol) in anhydrous MeCN (2 ml) the slurry was then heated at 70 °C for 5 hrs. The reaction mixture was then diluted with EtOAc and filtered over celite then loaded onto silica and purified by column chromatography eluting with 50:50 Petrol—EtOAc then 20:80 Petrol—EtOAc. The sulfonamide 23 was isolated as a colourless foam (129 mg, 72%); $\nu_{\text{max}}$/cm$^{-1}$ (film) 3246, 2482, 1716 and 1620; $\delta_H$(300 MHz; CDCl$_3$) 8.135 (1H, dd, $J$ 3.4, 5.76, Ns), 7.73-7.79 (4H, m, Ns), 7.80-7.89 (3H, m, Ns and C-6), 7.48-7.46 (1H, bs, amide), 7.05 (1H, dd, $J$ 8.4, H-5), 5.82 (1H, t, $J$ 6.2, sulfonamide ), 5.28 (2H, s, MOM), 5.11 (2H, s, MOM), 5.05-5.08 (1H, bs, alkene), 4.95-5.01 (1H, bs, alkene), 4.61 (2H, t, $J$ 6.4, H-1"), 3.74-3.83 (2H, bs, H-5"), 3.60 (3H, s, MOM), 3.53 (3H, s, MOM), 2.53-2.72 (4H, m, H-2", H-2'" or H-3"), 2.41-2.52 (2H, bs, H-2" or H-3"), $\delta_C$ (75 MHz; CDCl$_3$) 164.1, 157.2,148.0, 143.1, 133.9, 133.5, 132.7, 131.1, 125.3, 113.8, 110.9, 101.7, 94.8, 77.3, 57.4, 56.7, 56.6, 48.5, 30.4 (t, $J$ 21); $m/z$ (ES$^+$) 1000.1 ([M + H]$^+$, 100%); HRMS found [MH]$^+$ 1000.1408, C$_{33}$H$_{30}$F$_{17}$N$_3$O$_{11}$S requires MH$^+$ 1000.1402.

$^{13}$C NMR signals from the fluorous tag and 6 quaternary carbons were not observed.

Dibromoethane (20 µL) was added to a stirred suspension of magnesium turnings (500 mg) and chloromethyl(isopropoxy)dimethylsilane (300 µL, 1.8 mmol) in dry THF (5 mL). The
suspension was then heated with a heat gun until vigorous bubbling occurred, the exothermic reaction was sustained by dropwise addition of chloromethyl(isopropoxy)dimethylsilane (1.5 mL, 9.0 mmol) in dry THF (16 mL). Once addition was complete the suspension was refluxed for 30 mins then cooled to 0 °C in a salt/ice bath and added to a suspension of Cul (142 mg, 0.75 mmol) in THF (5 mL) and stirred for 20 mins. To this solution was added 4-hydroxycyclopent-2-enyl acetate (700 mg, 5.0 mmol) in THF (3 mL) which was stirred for a further 3 hrs at 0 °C. Once complete by TLC the reaction was quenched with ammonium chloride (10 mL) and aqueous ammonia (10 mL) then extracted with EtOAc (3 × 100 mL) and dried (MgSO₄). The solvent was removed under reduced pressure and the crude product was purified by column chromatography eluting with 60:40 Petrol—DCM, the silane 24 was isolated as a colourless oil (170 mg 16 %); νₓmax/cm⁻¹ (film) 3550-3100 and 2960, 1712; δₓ (300 MHz; CDCl₃) 5.82 (1H, dd, J 5.5, 2.1, H-3), 5.71 (1H, dt, J 5.5, 2.1, H-2), 4.70-4.74 (1H, m, H-1), 3.82-3.89 (1H, m, iPr), 2.85-2.92 (1H, m, H-4), 1.88 (1H, ddd, J 13.8, 7.1, 2.4, H-5a), 1.62 (1H, ddd, J 13.8, 7.1, 5.5, H-5b), 1.28-1.41 (1H, bs, OH); 0.66 (1H, dd, J 14.8, 8.5, CH₃Si(Me)₂OiPr), 0.49 (1H, dd, J 14.8, 6.6, CH₃Si(Me)₂OiPr), 0.00 (3H, s, Me), -0.01 (3H, s, Me); δₓ (75 MHz; CDCl₃) 143.7, 132.0, 80.5, 77.9, 65.6, 44.5, 39.8, 26.6, 25.0, 0.2, 0.0.

\[
\text{N-(But-3'-enyl)-N-((15\,S,\,4\,R\,\ast)}-4-((\text{isopropoxydimethylsilyl)methyl)cyclopent-2-ethyl)-2-nitrobenzenesulfonamide}
\]

Diisopropyl azodicarboxylate (368 µL, 1.8 mmol) was added to a stirred solution of triphenylphosphine (477 mg, 1.8 mmol), N-(but-3'-enyl)-2-nitrobenzenesulfonamide 25 (258 mg, 1.8 mmol) and the alcohol 24 (130 mg, 0.6 mmol) in THF (6 mL) and left overnight. Once complete by TLC, the reaction mixture was loaded onto silica and purified by column chromatography eluting with 90:10 Petrol—EtOAc. The diene 26 was isolated as a colourless oil (200 mg, 73%). νₓmax/cm⁻¹ (film) 3392, 3055, 2981 and 1707; δₓ (300 MHZ; CDCl₃) 7.94-8.00 (1H, m, Ns), 7.50-7.62 (3H, m, Ns), 5.79 (1H, dt, J 5.6, 2.2, H-2), 5.63 (1H, ddt, J 17.2, 10.6, 6.8, H-4'), 5.42 (1H, dt, J 5.6, 2.2, H-3), 4.83-5.01 (3H, m, H-5' and H-1), 3.83-3.95 (1H, m,
CH(Pr), 3.19-3.29 (1H, m, H-5a), 3.00-3.11 (1H, m, H-5b), 2.56-2.63 (1H, m, H-4), 2.20-2.42 (2H, m, H-2'), 1.13-1.33 (2H, m, H-3'), 1.03 (6H, d, J 6.0, 'Pr), 0.73-0.86 (1H, m, CHaSi(Me2)OiPr), 0.55 (1H, dd, J 14.7, 8.5, CHbSi(Me2)O'iPr), 0.00 (6H, s, Me); δC (75 MHz; CDCl3) 142.4, 135.3, 134.0, 132.1, 131.6, 132.1, 129.1, 124.7, 117.7, 80.6, 65.5, 44.3, 39.9, 38.7, 36.4, 26.5, 25.3, 22.7, 0.00, -0.2; m/z (ES+), 475.2 ([M + Na]+, 100%); HRMS found [MNa]+ 475.1709, C21H32N2O5SSi requires MNa 475.1693.

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\text{3',3',4',4',5',5',6',6',7',7',8',8',9',9',10',10'-Heptadecafluorodecyl 3-(4''-((N-(But-3''-enyl)-2-nitrophenylsulfonamido)methyl)pent-4''-enamido)-2,4-bis(methoxymethoxy)benzoate}
\]

By general method 3 diisopropyl azodicarboxylate (120 µL, 0.6 mmol) was added to a solution of 3-buten-1-ol (50 µL, 0.6 mmol), triphenylphosphate (157 mg, 0.6 mmol), and the sulfonamide 23 (150 mg, 0.15 mmol) in THF (2 mL) and stirred for 4 hrs. Once complete by TLC the solvent was removed under reduced pressure and purified by F-SPE, followed by a silica plug eluting with 50:50 Petrol—EtOAc. The diene 27 was isolated as a colourless foam (130 mg, 82%); νmax/cm⁻¹ (film) 3234, 2930, 1715 and 1658; δH (300 MHz; CDCl3) 8.11-8.44 (1H, m, Ns), 7.83 (1H, d, J 9.0, H-6), 7.65-7.75 (3H, m, Ns), 7.41-7.50 (1H, bs, NH), 7.07 (1H, d, J 9.0, H-5), 5.52-5.68 (1H, ddt, J 17.1, 10.2, 6.8, H-3''), 5.27 (2H, s, MOM), 5.08-5.14 (3H, m, MOM, 4'' alkene or H-4''), 5.01-5.03 (1H, m, 4'' alkene or H-4'''), 4.95-4.98 (1H, m, 4'' alkene or H-4'''), 4.93-4.95 (1H, m, 4'' alkene or H-4''), 4.61 (2H, t, J 6.0, H-1'), 4.00-4.09 (2H, bs, H-5''), 3.60 (3H, s, MOM), 3.52 (3H, s, MOM), 3.36 (2H, t, J 7.6, H-1''), 2.46-2.71 (6H, m, H-2',H-2', H-3''), 2.17-2.28 (2H, m, H-2''); δC (75 MHz; CDCl3) 164.6, 157.6, 148.3, 142.9, 134.5, 134.2, 133.9, 132.1, 131.4, 124.7, 117.7, 117.5, 115.6, 111.2, 101.9, 95.1, 57.8, 57.0, 56.9, 52.8, 46.6, 32.3, 30.9 (t, J 21); m/z (ES') 1054.2 ([M + H]⁺, 100%); HRMS found [MH⁺] 1054.1872, C38H38F17N3O11S requires MH 1054.1851.

\[^{13}C\text{ NMR signals from the fluorous tag and 6 quaternary carbons were not observed}\]
By general method 3 diisopropyl azodicarboxylate (363 µL, 1.8 mmol) was added to a solution of 4-penten-1-ol (154 µL, 1.8 mmol), triphenylphosphine (472 mg, 1.8 mmol), and the sulfonamide 23 (600 mg, 0.6 mmol) in THF (6 mL) then stirred for 4 hrs. Once complete by TLC the solvent was removed under reduced pressure and the crude oil purified by F-SPE, followed by a silica plug eluting with 50:50 Petrol—EtOAc. The diene 28 was isolated as a colourless foam,(595 mg, 93%); ν<sub>max</sub>/cm<sup>-1</sup> (film) 3243, 2159 and 1717; δ<sup>1</sup>H (300 MHz; CDCl<sub>3</sub>) 8.04-8.10 (1H, m, Ns), 7.82 (1H, d, J 9.1, H-6), 7.64-7.75 (3H, m, Ns), 7.41-7.50 (1H, bs, NH), 7.07 (1H, d, J 9.1, H-5), 5.60-5.75 (1H, ddt, J 16.3, 9.7, 6.6, H-4‴), 5.27 (2H, s, MOM), 5.06-5.14 (4H, m, MOM, 4‴<sub>a</sub> alkene and H-5‴<sub>a</sub>), 4.96-5.01 (1H, m, H-5‴<sub>b</sub>), 4.92-4.96 (1H, m, 4‴<sub>b</sub> alkene), 4.60 (2H, t, J 6.6, H-1″), 3.99-4.05 (2H, bs, H-5‴<sub>b</sub>), 3.60 (3H, s, MOM), 3.52 (3H, s, MOM), 3.28 (2H, t, J 7.6, H-1‴), 2.39-2.72 (6H, m, H-2″, H-2‴ and H-3″), 1.96 (2H, aq, J 14.2, 7.0, H-2‴), 1.50-1.63 (2H, m, H-3‴); δ<sup>13</sup>C (75 MHz; CDCl<sub>3</sub>) 164.6, 157.6, 148.3, 142.9, 134.5, 134.2, 133.9, 132.1, 131.4, 124.7, 117.7, 117.5, 115.6, 111.2, 101.9, 95.1, 57.8, 57.0, 52.8, 46.6, 32.3, 30.9 (t, J 21), 30.6; m/z (ES<sup>+</sup>) 1068.2 ([M + H]<sup>+</sup>, 100%); HRMS found [MH]<sup>+</sup> 1068.2028, C<sub>37</sub>H<sub>36</sub>F<sub>17</sub>N<sub>3</sub>O<sub>11</sub>S requires MH 1068.2039.

<sup>13</sup>C NMR signals from the fluorous tag and 7 quaternary carbons were not observed
**By general method 4** Hoveyda—Grubbs II catalyst (17.5 mg, 5 mol%), was added to a stirred solution of diene 27 (590 mL, 0.5 mmol) and 1,4-benzoquinone (6 mg, 10 mol%) in MTBE (300 mL) and heated to 50 °C. Once complete by TLC, addition of trishydroxymethyl phosphine (178 mg) and triethylamine (179 µL) gave the crude product which was purified by column chromatography eluting with 50:50 Petrol—EtOAc. The **cyclic sulfonamide 29** was isolated as a colourless foam (570 mg, 99%). \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 3155, 2904, 1793, 1722 and 1599. \( \delta_{\text{H}} \) (300 MHz; CDCl\(_3\)) 8.00–8.07 (1H, m, Ns), 7.83 (1H, d, J 9.2, H-6), 7.62-7.75 (3H, m, Ns), 7.47-7.55 (1H, bs, NH), 7.08 (1H, d, J 9.2, H-5), 5.62-5.71 (1H, bs, alkene), 5.27 (2H, s, MOM), 5.10 (2H, s, MOM), 5.52 (3H, s, MOM), 3.42 (2H, t, J 6.4, H-1'), 2.15-2.25 (4H, m, H-2' and H-2'' or H-3''), 2.38-2.50 (2H, bs, H-2'' or H-3''), 2.20-2.28 (2H, bs, H-5'''), \( \delta_{\text{C}} \) (75 MHz; CDCl\(_3\)) 164.5, 157.8, 148.7, 134.0, 132.4, 132.0, 131.3, 124.5, 117.4, 111.4, 102.1, 95.2, 57.8, 57.1, 56.9, 47.2, 42.9, 30.9 (t, J 21.1), 25.5; m/z (ES\(^+\)) 1048.2 ([M + Na]\(^+\), 100%); HRMS found [MNa]\(^+\) 1048.1378, \( \text{C}_{35}\text{H}_{32}\text{F}_{17}\text{N}_{3}\text{O}_{11}\text{S} \) requires MNa 1048.1413.

\(^{13}\text{C} \) NMR signals from the fluorous tag and 7 quaternary carbons were not observed.

3',3',4',4',5',5',6',6',7',7',8',8',9',9',10',10'-Heptadecafluorodecyl 2,4-bis(methoxymethoxy)-3-(3''-(1''''-(2-nitrophenylsulfonyl)-2'''',5'''',6'''',7''''-tetrahydro-1H-azepin-3''''-yl)propanamido)benzoate

**By general method 4** Hoveyda—Grubbs II catalyst (1 mg, 5 mol%) was added to a stirred solution of diene 28 (84 mg, 0.08 mmol), in MTBE (40 mL) and heated to 50 °C for 48 hrs. Once complete by TLC, addition of trishydroxymethyl phosphine (14 mg) and triethylamine (50 µL) to give the crude product which was purified by column chromatography eluting with 50:50 petrol—EtOAc. The **cyclic sulfonamide 30** was isolated as a colourless gum (82 mg, 97%); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 3244, 2922, 1715, 1657, 1599 and 1539; \( \delta_{\text{H}} \) (300 MHz; CDCl\(_3\)) 7.99-8.06 (1H, m, Ns), 7.83 (1H, d, J 8.9, H-6), 7.62-7.76 (3H, m, Ns), 7.47-7.54 (1H, bs, NH), 7.08 (1H, d, J 8.9, H-5), 5.66-5.78 (1H, bs, alkene), 5.28 (2H, s, MOM), 5.11 (2H, s, MOM), 4.60 (2H, t, J 6.1, H-1'), 3.90-3.98 (2H, bs, H-2'''), 3.62 (3H, s, MOM), 3.49-3.57 (5H, m, MOM and H-7'''), 2.43-2.73 (4H, m, H-2' and H-2'' or H-3''), 2.26-2.36 (2H, m, H-2'', H-3''), 1.84-1.96 (2H, bs, H-...
5''' or H-6'''); δC (75 MHz; CDCl₃) 174.4, 163.9, 157.0, 147.8, 137.6, 133.2, 132.9, 131.4, 130.3, 123.8, 116.9, 110.6, 101.4, 94.5, 57.2, 56.3, 49.8, 49.0, 30.3 (t, J 22), 29.5, 26.9, 25.9; m/z (ES⁺) 1040.2 ([M + H]⁺, 100%), HRMS found [MH⁺] 1040.1715, C₃₆H₃₄F₁₇N₃O₁₁S requires MH 1040.1707.

¹³C NMR signals from the fluorous tag and 5 quaternary carbons were not observed

3',3',4',4',5',5',6',6',7',7',8',8',9',9',10',10'-Heptadecafluorodecyl 3-(4''-((1''R*,4''S*)-4''-((N-(but-3''-enyl)-2''-nitrophensulfonamidocyclopent-2''-eny) methy)-2-nitrophensulfonamido) methyl)pent-4''-enamido)-2,4-bis(methoxymethoxy)benzoate

Hydrogen peroxide 30% (1 mL) was added dropwise to a stirred suspension of potassium fluoride (60 mg, 0.66 mmol), potassium hydrogen carbonate (88 mg, 0.88 mmol) and the diene 26 (200 mg, 0.44 mmol) in 50:50 THF—MeOH (3 mL) at 0 °C. Once addition was complete the mixture was allowed to warm to room temperature and stirred overnight. The reaction was then quenched with saturated sodium thiosulfate (10 mL) and extracted with EtOAc (3 × 50 mL), washed with brine (20 mL) and dried (MgSO₄). The solvent was removed under reduced pressure and the crude product was subjected to column chromatography eluting with 50:50 Petrol—EtOAc. The crude alcohol was isolated as a pale yellow oil (115 mg) and proved difficult to purify further and so was used crude in the next step. v_max/cm⁻¹ (film) 3500-3100, 3054, 1710 and 1546; δH (500 MHz; CDCl₃) 7.96-8.01 (1H, m, Ns), 7.52-7.65 (3H, m, Ns), 5.82 (1H, dt, J 5.6, 2.2, H-2), 5.55-5.70 (2H, m, H-3 amd H-4'), 4.98-5.08 (3H, m, H-5' and H-1), 3.45-3.65 (2H, m, RCH₂OH), 3.07-3.34 (2H, m, H-5a and H-5b), 2.69-2.79 (1H, m, H-4), 2.21-2.34 (2H, m, H-2'), 1.40 (2H, qn, J 7.1, H-3'), 1.34-1.42 (1H, bs, OH); δC (75 MHz; CDCl₃) 135.8, 134.6, 133.5, 132.2, 131.6, 130.9, 124.1, 117.0, 80.0, 65.5, 64.5, 46.8, 43.6, 35.6, 30.7, 22.0; m/z (ES⁺) 375.1 ([M + Na]⁺, 100%); HRMS found [MNa⁺] 375.0993, C₁₆H₂₆N₂O₅S requires MNa 375.0983.
Diisopropyl azodicarboxylate (185 µL, 0.9 mmol) was added to a solution of the sulfonamide 23 (494 mg, 0.49 mmol), the crude alcohol (115 mg c.a. 0.32 mmol) and triphenylphosphine (251 mg, 0.9 mmol) in THF (3.5 mL) then stirred overnight. Once complete by TLC the solvent removed under reduced pressure and the crude oil was loaded onto a F-SPE column followed by a silica plug eluting with 90:10 Petrol—EtOAc. The triene 31 was isolated as a colourless oil (155 mg, 28% yield over 2 steps). $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 3267, 3094, 2938, 1724, 1598; $\delta_{\text{H}}$ (500 MHz; CDCl$_3$) 7.90-7.98 (2H, m, Ns), 7.68-7.75 (1H, m, H-6), 7.52-7.65 (6H, m, Ns), 7.28-7.38 (1H, bs, NH), 6.96 (1H, d, $J$ 9.1, H-5), 5.52-5.67 (3H, H-2‴′, H-3‴′ and H-4‴′), 5.15 (2H, s, MOM), 4.86-5.02 (6H, m, MOM and H-5‴′, 4‴ alkene), 4.50 (2H, t, $J$ 6.8, H-1″), 3.87-3.95 (2H, bs, H-5″), 3.47 (3H, s, MOM), 3.40 (3H, s, MOM), 3.29 (1H, dd, $J$ 13.9, 7.5, CH$_a$ methylene), 3.08-3.22 (2H, m, H-2‴″$_a$ and CH$_b$ methylene), 2.88-2.95 (1H, m, H-2‴″$_b$), 2.75-2.83 (1H, bs, H-4‴′), 2.41-2.58 (4H, m, H-2′, and H-2″), 2.27-2.37 (2H, bs, H-3″), 2.15-2.26 (3H, m, H-3‴′ and H-5‴″$_b$), 1.65-1.70 (1H, bs, H-5‴″$_a$), 1.05-1.22 (1H, m, H-1‴″); $\delta_{\text{C}}$ (75 MHz; CDCl$_3$) 170.2, 157.2, 148.0, 147.8, 135.3, 134.5, 134.0, 133.7, 133.2, 132.4, 132.0, 131.9, 131.5, 130.9, 124.9, 124.1, 117.3, 117.1, 115.2, 115.0, 110.8, 101.5, 101.4, 94.8, 72.5, 64.3, 57.4, 56.6, 55.5, 53.0, 51.7, 43.9, 42.6, 35.5, 32.7, 30.5, 28.5; m/z (ES$^+$) 1351.3 (M + NH$_4^+$, 100%); HRMS Found [MNH$_4^+$]: 1351.2646, C$_{49}$H$_{52}$F$_{17}$N$_6$O$_{15}$S$_2$, requires MNH$_4$ 1351.2655.

$^{13}$C NMR signals from the fluorous tag and 4 quaternary carbons were not observed

3′,3′,4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,9′,9′,10′,10′-Heptadecafluorodecyl 2,4-bis(methoxymethoxy)-3-(3′′-((R*)-1′′′-[(2-nitrophenylsulfonyl)-5′′′-(((S*)-1′′′-2-nitrophenylsulfonyl)-1′′′-2″′′,5″′′,6″′′-tetrahydropyridin-2″′′-yl)methyl]-1′′′,2″′′,5″′′,6″′′-tetrahydropyridin-3″′-yl)propanamido)benzoate

By general method 4 Hoveyda—Grubbs II catalyst (3.5 mg, 5 mol%), was added to a solution of triene 31 (140 mg, 0.1 mmol) and 1,4 benzoquinone (1.5 mg) in MTBE (120 mL) and heated to 50 °C for 18 hrs. Once complete by TLC, addition of trishydroxymethyl phosphate (31 mg) and triethylamine (50 µL) gave the crude product which was purified by column
chromatography eluting with 30:70 Petrol—EtOAc and dried by azeotroping with methanol. The cyclic 32 was isolated as a colourless foam, (120 mg, 87%). ν$_{\text{max}}$/cm$^{-1}$ (film) 3260, 3097, 2912, 1725, 1680; δ$_{\text{H}}$ (500 MHz; CDCl$_3$) 7.88-7.94 (2H, m, Ns), 7.69-7.74 (1H, m, H-6), 7.51-7.65 (5H, m, Ns and NH), 7.48 (1H, dd, J 7.4, 1.7, Ns), 6.97 (1H, d, J 9.1, H-5), 5.68-5.76 (1H, bs, H-4''), 5.54-5.63 (2H, m, H-3''' and H-4'''), 5.16 (2H, s, MOM), 4.99 (2H, s, MOM), 4.49 (2H, t, J 6.5, H-1'), 4.36-4.43 (1H, bs, H-2'''), 3.90 (1H, dd, J 14.9, 5.6, H-6''', a), 3.71-3.79 (1H, m, H-2''''), 3.57-3.66 (1H, m, H-2'''), 3.49 (3H, s, MOM), 3.41 (3H, ad, J 5.6, MOM ), 3.20-3.34 (3H, m, H-6''', a, H-6''', b and H-6''', br'), 2.42-2.57 (4H, m, H-2' and H-2'''), 2.30-2.40 (3H, bs, H-3', H-6'''', br'), 1.75 - 1.92 (2H, m, H-5''''', a, H-5''''', b), 1.50-1.70 (3H, m, Ha methylene, Hb methylene and H-5''); δ$_{\text{C}}$ (75 MHz; CDCl$_3$) 157.2, 148.4, 148.1, 133.9, 133.7, 133.6, 133.0, 131.7, 131.2, 130.9, 130.3, 127.9, 124.0, 123.9, 123.7, 117.0, 110.9, 101.7, 94.8, 80.3, 57.4, 56.6, 52.4, 48.0, 38.4, 38.1, 31.7, 30.5, 30.2, 23.0; m/z (ES') 1323.2 (M+NH$_4^+$, 100%); HRMS Found: [MNH$_4^+$]$^+$ 1323.2342, C$_{47}$H$_{44}$F$_{17}$N$_5$O$_{15}$S$_2$ requires MNH$_4$1323.238;

$^{13}$C NMR signals from the fluorous tag and 9 quaternary carbons were not observed

Methyl 3-amino-2,4-bis(methoxymethoxy)benzoate$^6$

Pd/C (50 mg) was added to a stirred solution of the ester 14 (600 mg, 2 mmol) in EtOAc (20 mL) under a hydrogen atmosphere for 24 hrs. The reaction mixture was then diluted with EtOAc (5 mL) and filtered through celite. The solvent was removed under reduced pressure and the crude mixture purified by column chromatography eluting with 75:25 Petrol—EtOAc. The analine 33 was isolated as a clear colourless oil (460 mg, 85%); ν$_{\text{max}}$/cm$^{-1}$ (film) 3475, 3373, 2951, 2828, 1718 and 1596; δ$_{\text{H}}$ (500 MHz; CDCl$_3$) 7.26 (1H, d, J 8.9, H-6), 6.86 (1H, d, J 8.9, H-5), 5.25 (2H, s, MOM), 5.11 (2H, s, MOM), 4.15-4.22 (2H, bs, NH$_2$), 3.86 (3H, s, OMe), 3.61 (3H, s, MOM), 3.49 (3H, s, MOM); δ$_{\text{C}}$ (75 MHz; CDCl$_3$) 166.2, 148.6, 145.4, 131.7, 120.1, 117.9, 109.5, 101.2, 94.8, 57.7, 56.3, 51.9; m/z (ES') 294.1 ([M+Na]$^+$, 90%) HRMS found [MNa]$^+$ 294.0944, C$_{13}$H$_{17}$NO$_6$ requires MNa 294.0948.

S33
Methyl 2,4-bis(methoxymethoxy)-3-propionamidobenzoate

\[
\text{MeOOC} \quad \text{OMOM} \quad \text{OMOM} \quad \text{N-MeOOC} \quad \text{OMOM} \quad \text{OMOM} \quad \text{OMOM}
\]

\(N,N\)-Diisopropylethylamine (0.6 mL, 3.2 mmol) was added to a stirred solution of the aniline 33 (220 mg, 0.81 mmol), HATU (760 mg, 2.0 mmol) and propanoic acid (0.1 mL, 1.6 mmol) in DMF (8 mL) and stirred for 4 days at room temperature. Once complete by TLC the reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3 × 100 mL), washed with 2M HCl (100 mL), saturated NaHCO\(_3\) (100 mL), brine (50 mL) and dried (MgSO\(_4\)). The solvent was removed under reduced pressure and the crude product purified by column chromatography eluting with 50:50 Petrol—EtOAc. The ester 34 was isolated as a colourless foam (130 mg, 59%); \(\nu_{\text{max}}/\text{cm}^{-1}\) (film) 3424, 2954, 1718 and 1600; \(\delta_{\text{H}}\) (500 MHz; CDCl\(_3\)) 7.83 (1H, d, \(J=8.7\), H-6), 7.35-7.42 (1H, bs, NH), 7.07 (1H, d, \(J=8.7\), H-5), 5.27 (2H, s, MOM), 5.12 (2H, s, MOM), 3.91 (3H, s, OMe), 3.61 (3H, s, MOM), 3.54 (3H, s, MOM), 2.36-2.56 (2H, bs, 2'), 1.20-1.37 (3H, bs, 3'); \(\delta_{C}\) (75 MHz; CDCl\(_3\)) 165.8, 157.3, 131.0, 122.3, 118.3, 111.3, 102.1, 95.2, 57.8, 56.9, 52.5, 30.3, 10.1; \(m/z\) (ES\(^{+}\)) 328.1 ([M + H]\(^{+}\), 100%); HRMS found [MH\(^{+}\)] 328.1391, \(C_{15}H_{21}NO_7\) requires MH 328.1402.

Methyl 2,4-dihydroxy-3-nitrobenzoate\(^6\)

\[
\text{MeOOC} \quad \text{OH} \quad \text{H} \quad \text{H} \quad \text{MeOOC} \quad \text{OH} \quad \text{NO}_2
\]

A solution of nitric acid (550 μL, 12.9 mmol) in acetic acid (6.8 mL) was added dropwise over one minute to a stirred solution of 2,4, dihydroxybenzoate (2.0 g, 11.8 mmol) in acetic acid (14.4 mL) and acetic anhydride (7.41 mL) at 0 °C. The reaction was allowed to warm to room temperature, stirred for 20 mins, then water (30 mL) was added and the solution was left to stand for 30 mins. The resulting slurry was then filtered and the filtrate extracted with ether (3 × 30 mL) dried (MgSO\(_4\)) and the solvent removed under reduced pressure. The crude product was then loaded onto silica and purified by column chromatography eluting with 60:40 petrol—EtOAc. The ester S36 was isolated as bright yellow needles (590 mg 25%); m.p. 124-126 °C (toluene) [lit. 125-126 °C]; \(\nu_{\text{max}}/\text{cm}^{-1}\) 3100-2800 (broad), 1679, 1624 and 1588; \(\delta_{\text{H}}\) (300 MHz; CDCl\(_3\)) 12.87 (1H, s, OH), 11.17 (1H, s, OH), 7.99 (1H, d, \(J=8.9\), 6-H), 6.63 (1H, d, J
8.9, 5-H), 3.98 (1H, s, OMe); δ C (75 MHz; CDCl₃) 170.3, 160.3, 161.2, 137.2, 109.6, 106.16, 53.3; m/z (ES⁺) 212.0 ([M+H]⁺ 20%).

2-((Tert-butyldiphenylsilyloxy)methyl)prop-2-en-1-ol

NaH 60% in mineral oil (2.25 g, 56 mmol) was added to a solution of 2-Methylene-1,3-propanediol (5.0 g, 56 mmol) in anhydrous THF (140 mL) at 0 °C and stirred for 1 hour whereupon TBDPS chloride (14.5 ml, 56 mmol) was added and the mixture stirred overnight at room temperature. The reaction was then diluted with water (200 mL), extracted with ethyl acetate (3 × 100 mL) and washed with saturated NaHCO₃ (100 mL) then dried (MgSO₄). The solvent was removed under reduced pressure, then purified by column chromatography eluting with 90:10 petrol—EtOAc. The alcohol **S37** was isolated as a colourless oil (13.5 g, 73%). νmax/cm⁻¹ (film) 3500-3150 (broad), 3071, 2930, 2857, 1960, 1891 and 1824; δH (300 MHz; CDCl₃) 7.72 (4H, dd, J 2.0, 7.9, TBDPS), 7.40-7.48 (6H, m, TBDPS), 5.15-5.20 (2H, m, alkene), 4.26-4.31 (2H, bs, H-3), 4.22 (2H, d, J 6.2, H-1), 1.83 (1H, t, J 6.2, OH), 1.11 (9H, s, Boc); δ C (75 MHz; CDCl₃) 147.1, 135.5, 133.2, 129.8, 127.7, 111.2, 65.6, 64.6, 26.8, 19.2; m/z (ES⁺) 327.2 ([M + H]⁺ 100%).

Ethyl 4-((tert-butyldiphenylsilyloxy)methyl)pent-4-enoate

Propanoic acid (88 µL, 6 mol%) was added to a stirred solution of the alcohol **S37** (6.8 g, 20 mmol) in triethyl orthoacetate (35 mL). The reaction mixture was then heated at 150 °C, in such a way as to allow ethanol to be removed by distillation, for 90 mins or until complete by TLC. The solvent was removed under reduced pressure and the crude mixture purified by column chromatography eluting with 90:10 Petrol—EtOAc. The ester **S38** was isolated as a clear colourless oil (5.3 g, 66%); νmax/cm⁻¹ (film) 3050, 3072, 2932, 2857 and 1736; δH (300 MHz; CDCl₃) 7.68 (4H, d, J 6.8, TBDPS), 7.36-7.44 (6H, m, TBDPS), 5.18 (1H, bs, alkene), 4.87.
(1H, d, J 1.7, alkene), 4.11 (4H, m, H-5, OEt), 2.44 (2H, dd, J 6.0, 8.5, H-2 or H-3), 2.32 (2H, t, J 8.5, H-2 or H-3), 1.21 (3H, t, J 6.8, OEt), 1.02 (9H, s, TBDPS); δ_C (75 MHz; CDCl_3) 178.0, 146.2, 135.5, 133.4, 129.7, 127.7, 109.6, 77.2, 66.4, 32.2, 27.4, 26.8, 14.6; m/z (ES^+) 387.2 ([M + H]^+ 100%).

3′,3′,4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,9′,9′,10′,10′-Heptadecafluorodecyl 2,4-bis(methoxymethoxy)-3-(3′′-1′′′,2′′′,5′′′,6′′′-tetrahydropyridin-3′′-yl)propanamido)benzoate

Thiophenol (190 µL, 1.7 mmol), was added to a stirred suspension of potassium carbonate (261 mg, 2.2 mmol) and the sulfonamide 29 (570 mg, 0.55 mmol) in DMF (5 mL) for 1 hour. Once complete by TLC water (500 µL) was added and the resulting suspension loaded directly onto an F-SPE column. The amine S39 was isolated as a yellow foam (380 mg, 82%, purity 99% by ^1^HNMR). ν_max/cm\(^-1\) (film) 2700-3300 (broad), 1714, 1654, 1599 and 1534; δ_H (500 MHz; CDCl_3) 7.82 (1H, d, J 8.8, H-6), 7.70-7.76 (0.5H, m, amide), 7.07 (1H, d, J 8.8, H-5), 5.63-5.76 (1H, bs, alkene), 5.27 (2H, s, MOM), 5.10 (2H, s, MOM), 4.65 (2H, t, J 6.5, H-1′), 3.75-4.43 (2H, bs, NH), 3.60 (3H, s, MOM), 3.53 (3H, s, MOM), 3.06-3.15 (2H, bs, H-5′′ or H-4′′), 2.50-2.60 (4H, m, H-2′ and H-2′′ or H-3′′), 2.37-2.47 (2H, bs, H-2′′ or H-3′′), 2.15-2.34 (4H, m, H-2′′ and H-5′′ or H-4′′); δ_C (75 MHz; CDCl_3) 163.1, 156.1, 120.6, 116.1, 110.0, 100.4, 93.7, 56.5, 55.8, 55.5, 44.5, 40.8, 29.2 (t, J 22), 22.4; m/z (ES^+) 842.2 ([M + H]^+, 100%) HRMS found [MH]^+ 841.1776, C_{29}H_{32}F_{17}N_2O_7 requires MH 841.1764.

^13^C NMR signals from the fluorous tag and 7 quaternary carbon were not observed
Benzene sulfonyl chloride (32 µL, 0.24 mmol) was added to a solution of the amine S39 (50 mg, 0.06 mmol) and DMAP (36 mg, 0.29 mmol) in DCM (1 mL) and stirred overnight at room temperature. Once complete by TLC the solvent was removed under reduced pressure and the crude product purified by F-SPE, followed by a silica plug eluting with 40:60 Petrol—EtOAc. The sulfonamide S40a was isolated as a colourless foam (55 mg 93 %). \(\nu_{\text{max}}/\text{cm}^{-1}\) (film) 3243, 2915, 1720, 1651; \(\delta_H\) (300 MHz; CDCl\(_3\)) 7.30 (3H, ad, \(J_7.4\), H-6 and Ph), 7.53-7.67 (3H, m, Ph), 7.40-7.50 (1H, bs, NH), 7.08 (1H, d, \(J_9\), H-5), 5.53-5.57 (1H, bs, Alkene), 5.27 (2H, s, MOM), 5.10 (2H, s, MOM), 4.61 (2H, t, \(J_6.4\), H-1'), 3.58 (3H, s, MOM), 3.48-3.56 (5H, bs, MOM, H-2''), 3.17 (2H, t, \(J_5.8\), H-6''), 2.50-2.72 (4H, m, H-2' and H-2'' or H-3''), 2.30-2.47 (2H, bs, H-2'' or H-3''), 2.18-2.31 (2H, bs, H-5''); \(\delta_C\) (75 MHz; CDCl\(_3\)) 157.5, 136.6, 133.2, 133.1, 129.5, 128.0, 122.2, 120.1, 111.4, 102.1, 95.2, 80.0, 57.8, 57.0, 47.5, 42.9, 30.7 (t, \(J_21\) Hz), 30.1, 25.5; \(m/z\) (ES\(^+\)) 981.2 ([M + H]\(^+\), 100%); HRMS found [MH]\(^+\) 981.1708, \(C_{35}H_{33}F_{17}N_{2}O_{9}\) requires \(MH\) 981.1734.

\(^{13}\)C NMR signals from the fluorous tag and 8 quaternary carbons were not observed

Inner salt S41 (27 mg, 0.085 mmol) was added to a stirred solution of the amine S39 (60 mg 0.071 mmol) in DCM and stirred overnight at room temperature. Once complete by TLC the
crude mixture was loaded onto silica and purified by column chromatography eluting with 40:60 petrol—EtOAc. The carbamate S40b was isolated as a clear colourless gum (50 mg, 70\%). $\nu_{\text{max}}$/cm$^{-1}$ (film) 3251, 2923, 1715, 1658; $\delta_H$/<br>$\left(300 \text{ MHz;} \text{CDCl}_3\right)$ 7.83 (1H, d, $J$ 8.8, H-6), 7.45-7.54 (1H, bs, amide), 7.27 (1H, s, carbamate), 7.08 (1H, d, $J$ 8.8, H-5), 5.61-5.73 (1H, bs, alkene), 5.28 (2H, s, MOM), 5.11 (2H, s, MOM), 4.61 (2H, t, $J$ 6.4, H-1'), 3.84-3.94 (2H, bs, H-2''), 3.60 (3H, s, MOM), 3.54 (3H, s, MOM), 3.48 (2H, t, $J$ 5.2, H-6''), 2.52-2.72 (4H, m, H-2' and H-2'' or H-3''), 2.40-2.51 (2H, bs, H-2'' or H-3''), 2.21-2.32 (2H, bs, H-5''); $\delta_C$/<br>$\left(75 \text{ MHz;} \text{CDCl}_3\right)$ 170.7, 164.1, 157.2, 151.4, 149.9, 133.0, 119.9, 110.9, 94.8, 83.4, 56.6, 47.4, 43.3, 30.5 (J 21), 29.7, 28.0, 25.0, 0.0; m/z (ES$^+$) 1042.2 ([M + Na]$^+$, 100%); HRMS found [MNa]$^+$ 1042.1848, C$_{35}$H$_{38}$F$_{17}$N$_3$O$_{11}$S requires MNa 1042.1863.

$^{13}$C NMR signals from the fluorous tag and 8 quaternary carbon were not observed

3',3',4',4',5',5',6',6',7',7',8',8',9',9',10',10'-Heptadecafluorodecyl 3-(3''-(1'''-isopropylcarbamoyl)-1''''-2'''',5''''-tetrahydropyridin-3''''-yl)propanamido)-2,4-bis(methoxymethoxy)benzoate

Isopropyl isocyanate (9 µL, 0.09 mmol) was added to a stirred solution of the amine S39 (50 mg, 0.06 mmol) in DCM (600 µL) for 4 hrs. Once complete by TLC the solvent was removed under reduced pressure, dissolved in DMF and purified by F-SPE, followed by a silica plug eluting with 99:1 EtOAc—acetic acid to give the urea S40c as a colourless foam (50 mg, 90%)

$\delta_H$/<br>$\left(500 \text{ MHz;} \text{CDCl}_3\right)$ 7.79-7.86 (1H, m, H-6), 7.42-7.54 (1H, bs, amide), 7.10 (1H, d, $J$ 9, H-5), 5.65-5.75 (1H, bs, alkene), 5.26 (2H, s, MOM), 5.10 (2H, s, MOM), 4.60 (2H, t, $J$ 6.1, H-1'), 4.26 (1H, d, $J$ 6.9, Urea), 4.02 (1H, dq, $J$ 6.9, 6.6, OCH(Me)$_2$), 3.73-3.82 (2H, bs, H-2'''), 3.59 (3H, s, MOM), 3.52 (3H, s, MOM), 3.44 (2H, t, $J$ 5.5, H-6''), 2.54-2.67 (4H, m, H-2' and H-2'' or H-3''), 2.40-2.49 (2H, bs, H-2'' or H-3''), 2.12-2.22 (2H, bs, H-5'''), 1.18 (6H, d, $J$ 6.6, iPr); $\delta_C$/<br>$\left(75 \text{ MHz;} \text{CDCl}_3\right)$ 170.7, 164.1, 157.1, 151.4, 149.9, 133.0, 119.9, 110.7, 101.7, 94.8, 83.4, 56.6, 47.4, 43.3, 30.5 (J 21), 29.7, 28.0, 25.0, 0.0; m/z (ES$^+$) 981.2 ([M + Na]$^+$, 100%); HRMS found [MNa]$^+$ 1042.1848, C$_{35}$H$_{38}$F$_{17}$N$_3$O$_{11}$S requires MNa 1042.1863.

$^{13}$C NMR signals from the fluorous tag and 7 quaternary carbons were not observed
3',3',4',4',5',5',6',6',7',7',8',8',9',9',10',10'-Heptadecafluorodecyl 2,4-bis(methoxymethoxy)-3'(3''-1''-(pyridin-3''''-ylcarbamoyl)-1''',2''',5''',6'''-tetrahydropyridin-3''''-yl)propanamido)benzoate

Pyridine isocyanate (11 mg, 0.09 mmol) was added to a stirred solution of the amine S39 (50 mg, 0.06 mmol) in DCM (600 µL) for 4 hrs. Once complete by TLC the solvent removed under reduced pressure, dissolved in DMF and purified by F-SPE, followed by a silica plug eluting with 99:1 EtOAc—acetic acid. The urea S40d was isolated as a colourless foam (48 mg, 85%).

δH (500 MHz; CDCl3) 8.47-8.52 (1H, bs, H-2'''), 8.25 (1H, d, J 4.4, H-6''''), 7.93-8.00 (1H, bs, H-5'''''), 7.82 (1H, d, J 8.7, H-6), 7.60-7.70 (1H, bs, amide), 7.20 (1H, dd, J 7.8, 4.4, H-3''''), 7.08-7.12 (1H, bs, urea), 7.05 (1H, d, J 8.7, H-5), 5.70-5.79 (1H, bs, alkene), 5.23 (2H, s, MOM), 5.10 (2H, s, MOM), 4.58 (2H, t, J 6.4, H-1'), 3.95-4.02 (2H, bs, H-2'''), 3.59 (2H, t, J 5.5, H-6'''''), 3.57 (3H, s, MOM), 3.49 (3H, s, MOM), 2.54-2.68 (4H, m, H-2' and H-2'' or H-3''), 2.42-2.52 (2H, bs, H-2'' or H-3'''), 2.17-2.27 (2H, bs, H-5'''''); δC (75 MHz; CDCl3) 164.0, 157.1, 154.8, 143.8, 141.3, 133.9, 127.2, 121.2, 111.0, 101.7, 94.9, 57.4, 56.6, 46.5, 40.4, 37.1, 29.7(J 21), 24.9; m/z (ES') 961.2 ([M + H]+, 100%) HRMS found [MH]+ 961.2100, C35H33F17N4O8 requires MH 961.2102.

13C NMR signals from the fluorous tag and 8 quaternary carbon were not observed

N-([tert-Butoxycarbonyl]-N-[4-(dimethylazaniumylidene)-1,4-dihydropyridin-1-ylsulfonyl] Azanide8

Chlorosulfonyl isocyanate (1.2 mL, 13.6 mmol) was added dropwise to a solution of tBuOH (1.3 ml, 13.6 mmol) in DCM (10 mL) at 0 °C. Once addition was complete DMAP (3.45 g, 27.2 mmol) was added in 1 portion and stirred for one hour at room temperature. The reaction mixture was then quenched with water (20 ml) washed again with water (2 x 20 mL), brine (10 mL) and dried (MgSO4). The solvent was removed under reduced pressure and the crude
product was then recrystallised from MeCN. The inner salt S41 was isolated as a colourless plates (2.1 g, 50%). m.p. 179-183 °C MeCN [lit. 178-180]; νmax/cm⁻¹ (film) 3097, 2971, 1787, 1653; δH (500 MHz; d6DMSO) 8.47 (2H, d, J 8, DMAP), 6.99 (2H, d, J 8, DMAP), 3.24 (6H, s, DMAP), 1.27 (9H, s, ′Bu); δC (75 MHz; d6DMSO) 28.7, 78.6, 107.0, 107.7, 139.2, 157.3, 157.5; m/z (ES⁺) 302.1 ([M + H]+, 100%); HRMS found [MH]+ 302.1169, C12H19N3O4S requires MH 302.1181.
3. WaterLOGSY assay

3.1 Materials and methods

3.1.1 Instrumentation

Bacterial cultures were grown using a labnet 311DS shaker incubator or in a KuhnerSHAKERX shaker incubator. Agar plates were grown in a binder incubator. Media and glassware were sterilised using a Prestige Medical bench top autoclave or LTR Touchclave-R autoclave. Centrifugation was done using a Heraeus pico17 centrifuge, a Heraeus Superdex 200 26/60 multifuge 3 s-r, or a Beckman Coulter Avanti J-301 Centrifuge using Beckman JA-25.50 and Beckman JLA-9.1000 rotors. Electroporation was carried using a Harvad Apparatus BTX ECM 630 Electrocell manipulator.

3.1.2 Materials

Analytical grade reagents were supplied by Sigma–Aldrich, Fisher Scientific, Melford, Alfa Aesar, and VWR international. All solutions were prepared to 1 L with 18 MΩ water purified using an Elga purelab system unless otherwise stated.

Buffers used in His-tag purification: Lysis buffer (100 ml); NaH$_2$PO$_4$.2H$_2$O (700 mg), NaCl (1.75 g), imidazole (70 mg) adjusted to pH 8 with 2M NaOH. Wash buffer (100 ml); NaH$_2$PO$_4$.2H$_2$O (700 mg), NaCl (1.75 g), imidazole (136 mg) adjusted to pH 8 with 2M NaOH. Elution buffer (100 ml); NaH$_2$PO$_4$.2H$_2$O (700 mg), NaCl (1.75 g), imidazole (1.7 mg) adjusted to pH 8 with 2M NaOH.

Buffers used for protein analysis and storage: Tris Storage Buffer; 25 mM Tris (3 g), 230 mM NaCl (13.4 g), 2.5 mM EDTA (730 mg), 2 mM DTT (310 mg) 9% (v/v) glycerol adjusted to pH 8. Phosphate storage buffer; 25 mM Sodium phosphate (210 mg NaH$_2$PO$_4$, 4.15 g Na$_2$HPO$_4$), 450 mM (26.5 g), 2 mM DTT (310 mg), 1% (v/v) glycerol adjusted to pH 8. Phosphate NMR buffer; 25 mM Sodium phosphate (210 mg NaH$_2$PO$_4$, 4.15 g Na$_2$HPO$_4$), 450 mM (26.5 g), 1 mM DTT (155 mg), adjusted to pH 8

Bacteria were always grown in Lysogeny Broth Media (LB); 1% (w/v) Tryptone (10 g), 1% (w/v) NaCl (10 g), 0.5% (w/v). Other reagents were made up to 1.5 mL as 1000x stock solutions and stored at
−20 °C. IPTG; 1 M Isopropyl β-D-1-thiogalactopyranoside, Ampicillin; 100 mg/mL (w/v) Ampicillin, Kanamycin; 50 mg/mL (w/v) Kanamycin, Glycerol stocks; were prepared from 0.5 mL of overnight culture of e.coli to 0.5 mL of sterile glycerol and storing at −80 °C.

3.2 Protein Methods

3.2.1 Sequence of C163Q FabF

MRGSHHHHHHSACVSRRVVTGLGMLSPVGTVESTWKALLAGQSGISLIDHFDTSAYATKFAGLVDKFNCEDIIISRKEQRMDAFQYGYIVAVGQAMQDSGLETENATRIGAAIAGSGIGGLGIEENHTSLMNGPPKISPFVPS
TIVNMVAGHLTIMYLGPSISATAQTSGVHNGHAARIIAYGDADVMMVAGGAEKASTPLGVGGFAARALSTR
NDNPGQASRPWDKEDGFVLDGAGMLVEEEYEHAKRGRGAKIYAEVGFMSSDAYHMSTPPENGAGAALAMANALRDAEGIEASQIGVNAHGTSTPAGDKAEAQVTIFGEAASRVLSSTKSMTGHLLGAAGAVESIYSILAR
DQAVPPTINLDPDEGCDDLFPVHEARQVSGMEYTLCSFGGGTGNSLIFKIKI

The proteins identity was confirmed by mass spectrometry, predicted mass 44611 Da, observed mass 44623 Da. Further analysis by mass spectrometry from a tryptic digest of the protein identified diagnostic peptides (predicted using expasy “peptide mass program”) including a peptide fragment which contained the C163Q mutation. The mutant was developed from the wild type protein native to E. coli. as described by Wang et. al.

3.2.2 Transformation by electroporation

An aliquot (1 µL) of pQE-30 plasmids (obtained from Dr Stephen M. Soisson Merck research Laboratories) were mixed with competent 100 µL M15 [pREP4] cells (obtained from Qiagen) suspended in n/100 glycerol and incubated for 10 minutes on ice in pre-chilled cuvettes. The cells were electroporated at 25 µF 1.5-1.8 KV then immediately diluted with 900 µL pre-warmed LB media, transferred to a sterile epindorf and incubated for 45 minutes at 37 °C. Finally the mixture was plated out to restrictive media (kanamycin and ampicillin) and grown overnight at 37 °C.

3.2.3 Over-expression and purification of C163Q FabF

E. coli cells expressing C163Q FabF were picked from a single colony from a agar plate supplemented with kanamycin and ampicillin or from a glycerol stock and grown overnight in
restrictive LB media (20 mL) at 37 °C. The overnight culture was then added to 1 L restrictive LB media and grown at 37 °C until an OD$_{600}$ of 1.0 is reached, whereupon IPTG (1 mL) was added and the culture incubated for a further hour. Cells were centrifuged at 5000 rpm for 45 minutes and the supernatant discarded, then re-suspended and transferred to a 50 mL falcon tube centrifuged at 8000 rpm for 20 minutes and stored at −20 °C for later purification.

### 3.2.4 Protein purification of His tagged C163Q FabF

Bacterial pellets were re-suspended by incubation on ice with occasional mixing in lysis buffer for 20 minutes. Cells were then lysed at 0 °C by sonication for 15 minutes (40% power, 40% cycle) the lysate was cleaned by centrifugation at 8000 rpm for 20 minutes and the supernatant was decanted in preparation for nickel affinity chromatography.

Protein samples were loaded onto a gravity flow column of Ni-NTA agrose. The column was first washed with methanol (10 mL), then lysis buffer (20 mL). The protein solution was then added and passed through the column 3 times. The column was then washed with lysis buffer (15 mL), wash buffer (10 mL) and elution buffer (2 × 15 mL). The protein was then dialysed overnight into Tris storage buffer (Section 3.13) at 4 °C and stored for up to 1 week at 4 °C. Fractions were analysed by SDS page (Figure S3.1).

![Figure S3.1: Typical SDS page gel of fractions from His tag purification. L1-3 = loading fractions, W1 = wash with lysis buffer W2 = Wash with wash buffer E1-2 wash with elution buffer.](image-url)
Protein concentration was determined by UV absorption irradiating with 280 nM UV light. Theoretical extinction coefficients were obtained using the Expasy program. The extinction coefficient of C163Q FabF is 25900 M$^{-1}$ cm$^{-1}$. Proteins were separated by SDS-polyacrylamide gel electrophoresis using 15 % resolving gel with a 5 % of the following composition.

3.3 WaterLOGSY experiment

3.3.1 Preparation of standard solutions

The protein was dialysed from the tris storage buffer into the phosphate storage buffer overnight at 4 °C. The protein was then dialysed again into the phosphate NMR buffer at 4 °C. Once in the phosphate NMR buffer the protein was rapidly precipitated if stored in plastic falcon tubes, as a result it was stored in glass sample tubes at 0 °C or transferred directly into an NMR tube were it would typically remain soluble for 8-10 days.

All ligands were stored in stock solutions of DMSO. The reference ligand 7 was stored at a concentration of 20 mM, the ligands 8, 10a, 12, and 9c-g were all dissolved in d$_6$DMSO to a concentration 10 mM. DSS was dissolved in water to a concentration of 10 mM.

3.3.2 NMR experimental parameters

All experiments were carried out with a 500 MHz Varian Inova spectrometer at 25 °C. WaterLOGSY experiments employed a 15 ms 5% truncated Gaussian inversion pulse at the water frequency and a NOE mixing time of 1.5 seconds. The number of scans per experiment varied depending on the method used (Sections 3.3.3 and 3.3.4) with a relaxation delay of 2.5 seconds for a total acquisition time per experiment of 23 min. Solvent suppression was achieved using excitation sculpting.$^{9}$ WaterLOGSY intensities were taken as the difference between the WaterLOGSY signals recorded with and without protein in the sample. Protein background signals were suppressed with a 15 ms spinlock added to the sequence.
Signal intensity was measured using MestReNova version 6.0.4. Spectra were first aligned using DSS signal normalised to 0.00 ppm. Spectra were then baseline corrected using the Whittickar polynomial and stacked into a single spectrum. The signal intensity of the methyl peak on the reference ligand was then measured for at all concentrations simultaneously using the data analysis function.

### 3.3.3 Titration experiment

The reference ligand 7 was analysed by the titration waterLOGSY experiment. The ligand was prepared by adding 9 µL, 13.6 µL, 17.4 µL, 40.4 µL, 67.4 µL of the stock solution to separate vials and removing the DMSO on a GT-4 series 2 Genevac then re-dissolved in 10 µL of d6DMSO.

Samples were then prepared to a volume of 610 µL; containing 520 µL of protein solution in phosphate buffer, 60 µL of D2O, 20 µL of DSS solution and 10 µL of DMSO used in a solution containing the reference ligand 7; the final protein concentration was 33.2 µM. An 1H NMR spectra (nt = 32) and waterLOGSY spectra (nt = 401) were obtained for each sample at ligand concentrations of 300 µM, 450 µM, 600 µM, 1500 µM, 2500 µM. Analysis to obtain a dissociation constant was done by non-linear curve fitting in Origin (Figure S3.2)

Selectivity for the platensimycin binding site was confirmed by addition of platensimycin as a solution in DMSO to the NMR tube to a final concentration of 33 µM i.e. identical to protein concentration. Complete suppression of the signal for the reference ligand 7 was observed (Figure S3.3)
Figure S3.2: Determination of the dissociation constant of the reference ligand 7. Panel A) Structure of the reference ligand 7. Panel B) An expansion of the WaterLOGSY spectra of the reference ligand 7. Positive signals from the CH$_2$ and CH$_3$ groups are highlighted. Panel C) The intensity of the waterLOGSY signal of the CH$_3$ group as a function of ligand concentration. The green line is a line of best fit for the ligand in the absence of protein. The red line is the uncorrected nonlinear curve fit. The black line is a non-linear curve fit the data corrected for the negative signal observed in the absence of protein.
Panel A

Panel B

**Figure S3.3**: NMR spectra taken in the presence and absence of platensimycin. Spectra were taken at 500 MHz at 300 K, protein concentration was 30 μM, the ligand 7 concentration was 1.5 mM. Panel A) The structure of the reference ligand 7. Panel B) Spectrum 3 (blue) $^1$H NMR of the mixture. The signals corresponding to the reference ligand 7 are highlighted. Other signals are from components of the buffer; DSS, DTT and DMSO. Spectrum 2 (green) Signals corresponding to the reference ligand 7 are positive, signals from the buffer are negative. Spectrum 1 (red) the waterLOGSY spectrum in the presence of platensimycin (30 μM), the positive signals corresponding to the reference ligand 7 have been almost completely eliminated.

3.3.4 Competition experiment method 1

The competitor ligands 9d and 9f were analysed by the competition waterLOGSY experiment. The ligand was prepared by adding 7.6 μL, 18 μL, 27.2 μL, 34.8 μL of the 10 mM stock solution into separate vials and removing the DMSO on a GT-4 series 2 Genevac then redissolved in 10 μL of d$_6$DMSO.

Samples were then prepared to a volume of 627 μL; containing 520 μL of protein solution in
phosphate buffer, 60 µL of D₂O, 20 µL of DSS solution and 10 µL of DMSO used to containing the competitor ligand 9d or 9f and 17.4 µL of the reference ligand 7 in a DMSO stock solution; the final protein concentration was 22.6 µM. An 1H NMR spectra (nt = 32) and waterLOGSY spectra (nt = 289) were obtained for each sample at ligand concentrations of 145 µM, 295 µM, 438 µM, 583 µM. Analysis to obtain a dissociation constant was done by non-linear curve fitting in Origin.

For competitor ligand 7 (Figure S3.4) the reduction of the signal for the reference ligand 7 and the increase in the signal of the competitor ligand was easily observed due to the presence of the highly protic Pr group.
Panel A

Panel B

**Figure S3.4:** Determination of the dissociation constant of the ligand 9f. Experiments were carried out at 300 K on a 500 MHz spectrometer. The protein concentration was 22.6 µM, and the ligand concentration is labelled on the spectra. Panel A) Structure of the reference ligand 7 and the ligand 9f. Panel B) Part of the waterLOGSY spectrum in which key signals corresponding to both ligands are highlighted.

### 3.3.5 Competition experiment method 2

The competitor ligands 8, 10a, 9c, 9e, 9g and 12 were also analysed by the competition waterLOGSY experiment. 7.6 µL of the appropriate ligand in 10 mM stock solution was added directly into the NMR tube containing 520 µL of protein solution in phosphate buffer, 60 µL of D2O, 20 µL of DSS solution and 17.4 µL of the reference ligand 7 in a DMSO stock solution. Concentration of the ligand was increased by adding a further 7.6 µL of the ligand stock solution directly into the NMR tube. An 1H NMR spectra (nt = 32) and waterLOGSY spectra (nt = 289) were obtained for each sample at ligand concentrations between 122 µM and 569 µM.
4.0 Scanned Spectra of Novel Compounds

![Graph showing the scanned spectra of a novel compound.](image-url)
7, 75MHz, $^{13}$C NMR, MeOD
$^1$HNMR, 300MHz, d$_6$-DMSO
$^{13}$CNMR, 75MHz, DMSO
9c, 300 MHz, $^{13}$CNMR, CDCl$_3$
$9c$, 300 MHz, $^{13}$CNMR, CDCl$_3$
9d, 500 MHz, $^1$HNMR, MeOD
9d, 125 MHz, $^{13}$C{NMR}, MeOD
**9e, 125 MHz, $^{13}$CNMR, MeOD**

![Chemical Structure](image)

**S59**
9f, 500 MHz, $^1$H NMR, MeOD
9f, 125 MHz, $^{13}$CNMR, MeOD
9g, 500 MHz, $^1$HNMR, $d_6$-DMSO
9g, 125 MHz, $^{13}$CNMR, $d_6$-DMSO
9h, 300 MHz, $^1$H NMR, MeOD
9h, 75 MHz, $^{13}$C NMR, MeOD
10a, 300 MHz, $^1$HNMR, d$_6$-DMSO
10a, 300 MHz, $^{13}$CNMR, d$_6$.
DMSO
12, 500 MHz, $^1$HNMR, MeOD
$13d$, 500 MHz, $^1$HNMR, CDCl$_3$
$^{13}$C NMR, CDCl$_3$
$16$, 300 MHz, $^1$HNMR, CDCl$_3$, 

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$^{13}$CNMR, 75 MHz, CDCl$_3$, C$_8$F$_{17}$OMOM
17, 500 MHz, $^1$HNMR, CDCl$_3$
$\text{C}_8\text{F}_{17}$

17, 125 MHz, $^{13}$C NMR, CDCl$_3$
18, $^1$HNMR, 300MHz, CDCl$_3$,
$^{13}$C NMR, CDCl$_3$, 75 MHz, 18,
$^{19,13}$CNMR, 75 MHz, CDCl$_3$
$^{1}$H NMR, 300MHz, CDCl$_3$, 

![NMR spectrum](image-url)
20. $^{13}$CNMR, 75 MHz, CDCl$_3$
21, $^1$HNMR, 300 MHz, CDCl$_3$, 

![Chemical Structure Image]
22, $^1$HNMR, 300 MHz, CDCl$_3$, 

![Chemical structure diagram]
22, $^{13}$CNMR, 75 MHz, CDCl$_3$
$^{1}$HNMR, 75 MHz, CDCl$_3$
$^{13}$CNMR, 75 MHz, CDCl$_3$. 

![NMR Spectrum of Compound 23]
24, 300 MHz, $^1$HNMR, CDCl$_3$
24, 75 MHz, $^{13}$CNMR, CDCl$_3$
26, 300 MHz, $^1$HNMR, CDCl$_3$
26, 75 MHz, $^{13}$CNMR, CDCl$_3$
27, 300 MHz, $^1$HNMR, CDCl$_3$
27, 300 MHz, $^1$HNMR, CDCl$_3$
28, $^1$HNMR, 300 MHz, CDCl$_3$
$^{13}$CNMR, 75 MHz, CDCl$_3$, 

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29, $^1$HNMR, 300 MHz, CDCl$_3$, 

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$^{29,^{13}}$CNMR, 75 MHz, CDCl$_3$. 

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$^1$HNMR, 75 MHz, CDCl$_3$, 30

S98
$^{13}$CNMR, 75 MHz, CDCl$_3$
$31$, 500 MHz, $^1$HNMR, CDCl$_3$
31, 125 MHz, $^{13}$CNMR, CDCl$_3$
32, 500 MHz, $^1$HNMR, CDCl$_3$
32, 125 MHz, $^{13}$C NMR, CDCl$_3$
34, 500 MHz, $^1$HNMR, CDCl$_3$
34, 125 MHz, $^{13}$CNMR, CDCl$_3$
40a, 300 MHz, $^1$H NMR, CDCl$_3$
$^{13}$CNMR, CDCl$_3$
**40b, 300MHz, $^1$HNMR, CDCl$_3$**

![NMR Spectrum](image_url)

*S108*
40c, 300 MHz, $^1$H NMR, CDCl$_3$
$^{13}$CNMR, CDCl$_3$, 75 MHz, 40c
40d, 500 MHz, $^1$HNMR, CDCl$_3$
References


