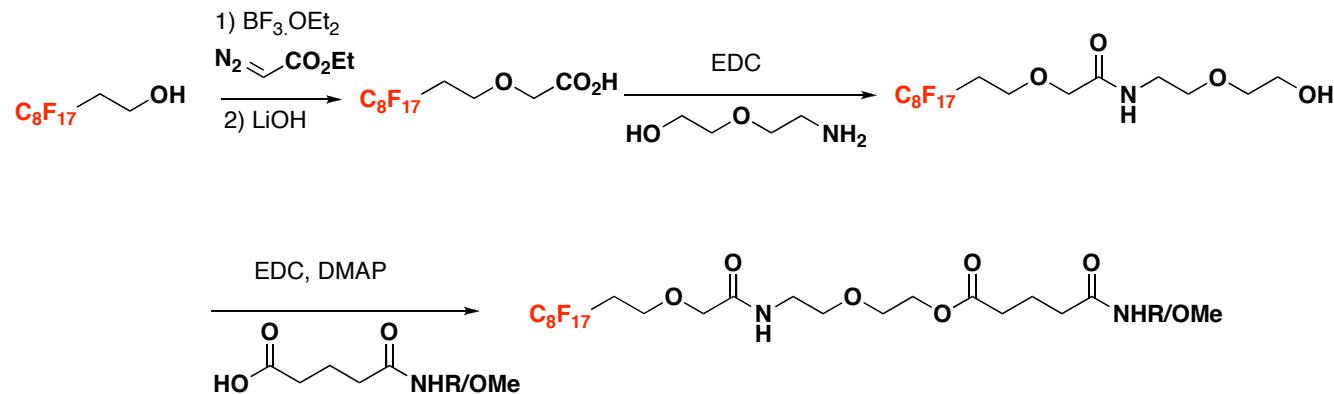


Electronic Supplementary Information

Fluorous Tagged Small Molecule Microarrays Rebecca L. Nicholson, Mark L. Ladlow and David R. Spring

Preparation of fluorous tagged small molecules shown in Fig. 2

The compounds were prepared as outlined in the following scheme.



Procedures for the preparation of fluorous tagged library

Boron trifluoride (catalytic, 0.05 ml) was added to a stirred solution of *1H,1H,2H,2H*-perfluorodecanol (1 equiv.) and ethyl diazoacetate (1 equiv.) in anhydrous dichloromethane at 0°C. After stirring for 18 hours, the reaction mixture was quenched with saturated sodium carbonate, extracted with dichloromethane, dried (MgSO_4) and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica (eluent: 5:1 30–40 °C petroleum ether: diethyl ether). The resultant ester (1 equiv.) was dissolved in 66% aqueous methanol and treated with LiOH (2 equiv.). After 18 hours, the solvents were evaporated and the residue re-dissolved in dichloromethane, washed with 1N hydrochloric acid, dried and concentrated *in vacuo* to afford 2-(*1H,1H,2H,2H*-perfluorodecyloxy)ethanoic acid as a white solid that was used without further purification.

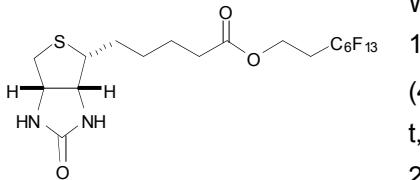
EDC (1.1 equiv.) was added to a stirred solution of 2-(*1H,1H,2H,2H*-perfluorodecyloxy)ethanoic acid (1 equiv.) and 2-(2-aminoethoxy)ethanol (1 equiv.) in anhydrous dichloromethane at room temperature. After stirring for 18 hours, the reaction mixture was diluted with dichloromethane, washed with 1N hydrochloric acid, dried and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica (eluent: 9:1 $\text{CH}_2\text{Cl}_2 : \text{MeOH}$) to afford 2-(*1H,1H,2H,2H*-perfluorodecyloxy)-N-(2-(2-hydroxyethoxy)ethyl)ethanamide as a pale yellow oil.

EDC (1.1 equiv.) was added to a stirred solution of the requisite acid (1 equiv.), 2-(*1H,1H,2H,2H*-perfluorodecyloxy)-N-(2-(2-hydroxyethoxy)ethyl)ethanamide (1 equiv.) and DMAP (0.6 equiv.) in anhydrous dichloromethane at room temperature. After stirring for 18 hours, the reaction mixture was diluted with dichloromethane, washed with 1N hydrochloric acid, dried and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica to afford the fluorous tagged small molecules.

Instruments

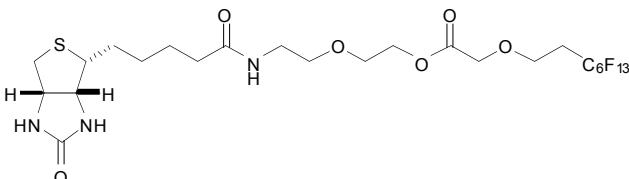
The ^1H and ^{13}C spectra shown were recorded on a Bruker DPX-400 spectrometer. Chemical shifts (δ_{H} and δ_{C}) are reported in parts per million (ppm) and are referenced to the residual solvent peak. Coupling constants (J) are measured in hertz. Infrared spectra were recorded on a Perkin-Elmer Spectrum One spectrometer with internal referencing as neat films. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}) and the following abbreviations are used: w, weak; m, medium; s, strong; br, broad. Low resolution mass spectra (m/z) were recorded on a Waters ZQ (ESCI) instrument. Major peaks are listed with intensities quoted as percentages of the base peak. Melting points were recorded on a Gallenkamp Hot Stage apparatus and are uncorrected.

1H,1H,2H,2H-Perfluoroctyl 2-((3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)ethanoate (1)



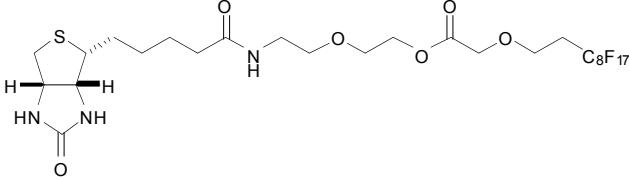
White solid; m.p. 94°C ($\text{CH}_2\text{Cl}_2/\text{MeOH}$); ν_{max} (neat/ cm^{-1}) 1753w, 1694m, 1678m, 1649m, 1549w, 1478w, 1465w, 1326w, 1198s, 1144s, 1117s; δ_{H} (400MHz, d_6 -DMSO) 1.26-1.63 [6H, m, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 2.29 [2H, t, J 7.3, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 2.55 [1H, app. d, J 12.5, $\text{CH}_A\text{H}_B\text{S}$], 2.57-2.69 [2H, m, $\text{OCH}_2\text{CH}_2\text{C}_6\text{F}_{13}$], 2.79 [1H, dd, J 5.2, 12.5, $\text{CH}_A\text{H}_B\text{S}$], 3.04-3.09 [1H, m, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 4.09-4.11 [1H, m, C(O)NHCH_A], 4.26-4.31 [3H, m, C(O)NHCH_B & $\text{OCH}_2\text{CH}_2\text{C}_6\text{F}_{13}$], 6.32 [1H, s, NH], 6.39 [1H, s, NH]; δ_{C} (100MHz, CD_3SOCD_3) 24.8, 28.4, 29.9, 33.6, 40.1, 56.2 [CH₂], 55.7, 59.5, 61.4 [CH], 163.1, 173.0 [C]; m/z (ES+) 591 [100%, MH^+].

2-(2-((3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)ethanamido)ethoxyethyl 2-(1H,1H,2H,2H-perfluoroctyl oxy)ethanoate (2)



White solid; m.p. 107°C (EtOAc); ν_{max} (neat/ cm^{-1}) 1669m, 1656m, 1649m, 1505w, 1438w, 1406w, 1387m, 1256w, 1093m; δ_{H} (400MHz, CDCl_3) 1.40-1.48 [2H, m, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 1.61-1.78 [4H, m, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 2.22 [2H, t, J 7.3, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 2.41-2.54 [2H, m, $\text{OCH}_2\text{CH}_2\text{C}_8\text{F}_{17}$], 2.72 [1H, app. d, J 12.9, $\text{CH}_A\text{H}_B\text{S}$], 2.91 [1H, dd, J 4.9, 12.9, $\text{CH}_A\text{H}_B\text{S}$], 3.11-3.16 [1H, m, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 3.40-3.43 [2H, m, $\text{NHCH}_2\text{CH}_2\text{O}$], 3.54-3.56 [2H, m, $\text{NHCH}_2\text{CH}_2\text{O}$], 3.66-3.69 [2H, m, $\text{OCH}_2\text{CH}_2\text{O}$], 3.85 [2H, t, J 6.6, $\text{OCH}_2\text{CH}_2\text{C}_6\text{F}_{13}$], 4.14 [2H, s, $\text{C(O)CH}_2\text{OCH}_2\text{CH}_2\text{C}_6\text{F}_{13}$], 4.29-4.32 [3H, m, C(O)NHCH_A & $\text{OCH}_2\text{CH}_2\text{O}$], 4.49-4.51 [1H, m, C(O)NHCH_B], 5.59 [1H, s, NH], 6.49 [1H, s, NH]; δ_{C} (100MHz, CDCl_3) 22.4, 25.5, 28.1, 35.9, 39.0, 40.5, 60.1, 68.3, 68.6, 69.9 [CH₂], 55.6, 61.8, 63.6 [CH], 170.0, 177.3, 197.3 [C]; m/z (ES+) 736 [100%, MH^+].

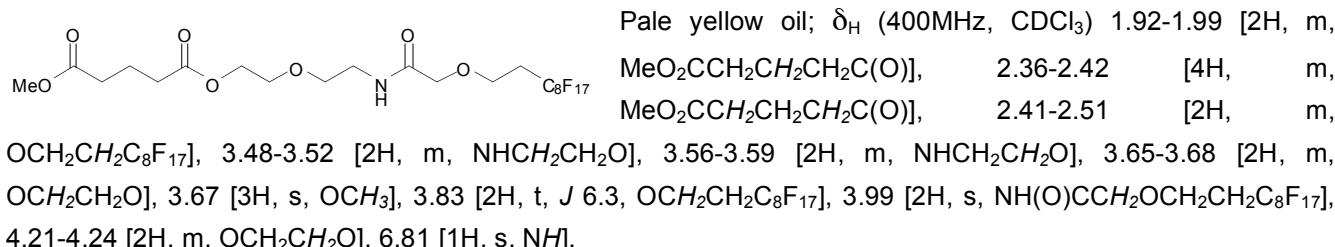
2-(2-((3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)ethanamido)ethoxyethyl 2-(1H,1H,2H,2H-perfluorodecyloxy)ethanoate (3)



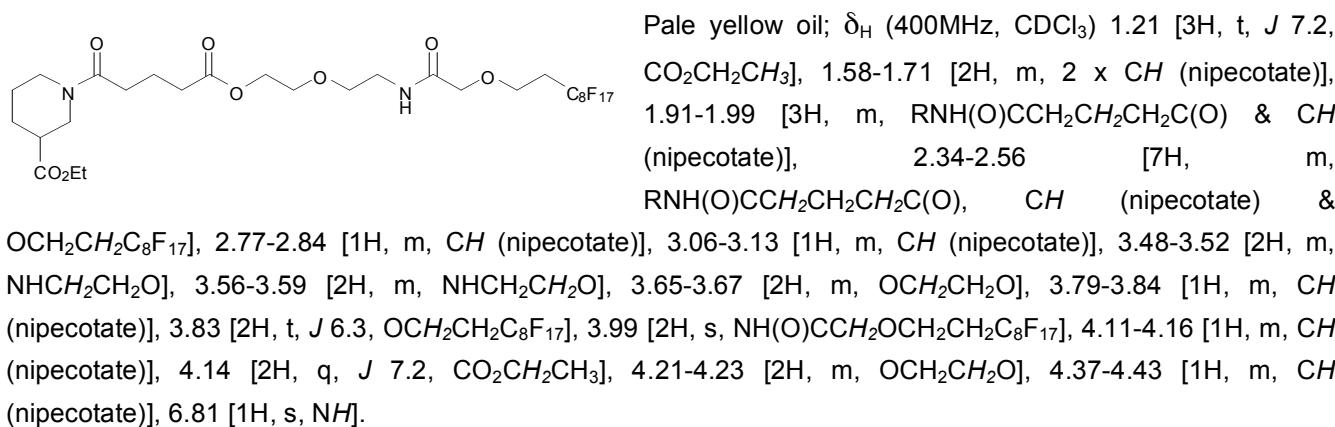
White solid; m.p. 114°C (EtOAc); ν_{max} (neat/ cm^{-1}) 1754w, 1697m, 1678m, 1654m, 1547w, 1478w, 1464w, 1417w, 1372w, 1328w, 1197s, 1144s, 1116m; δ_{H} (400MHz, CDCl_3) 1.40-1.48 [2H, m, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 1.63-1.75 [4H, m, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 2.22 [2H, t, J 7.3, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 2.43-2.52 [2H, m, $\text{OCH}_2\text{CH}_2\text{C}_8\text{F}_{17}$], 2.72 [1H, app. d, J 12.9, $\text{CH}_A\text{H}_B\text{S}$], 2.90 [1H, dd, J 4.9, 12.9, $\text{CH}_A\text{H}_B\text{S}$], 3.11-3.16 [1H, m, $\text{SCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)}$], 3.40-3.44 [2H, m, $\text{NHCH}_2\text{CH}_2\text{O}$], 3.56 [2H, m, $\text{NHCH}_2\text{CH}_2\text{O}$], 3.68 [2H, m,

OCH₂CH₂O], 3.86 [2H, t, J 7.0, OCH₂CH₂C₈F₁₇], 4.15 [2H, s, C(O)CH₂OCH₂CH₂C₈F₁₇], 4.29-4.33 [3H, m, C(O)NHCH_A & OCH₂CH₂O], 4.48-4.51 [1H, m, C(O)NHCH_B]; δ_C (100MHz, CDCl₃) 22.4, 25.5, 28.1, 35.9, 39.0, 60.1, 68.7, 69.9 [CH₂], 55.2, 61.8, 63.7 [CH], 163.8, 170.0, 173.3 [C]; *m/z* (ES+) 836 [100%, MH⁺].

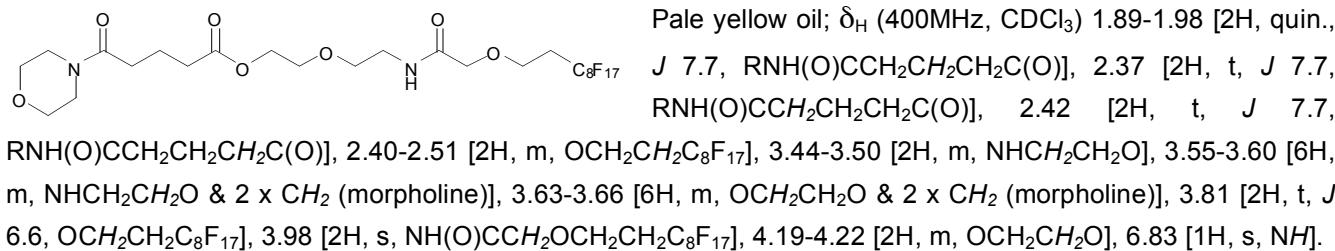
2-(1*H*,1*H*,2*H*,2*H*-perfluorodecyloxy)-*N*-(2-(2-methoxyethoxy)ethyl)ethanamide (B)



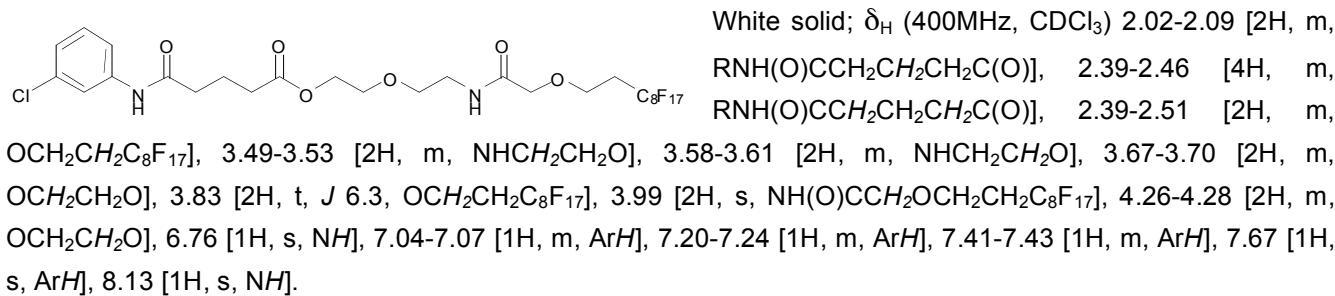
2-(2-(1*H*,1*H*,2*H*,2*H*-perfluorodecyloxy)ethanamido)ethoxyethyl 3-oxo-3-(ethylcarboxyl)piperidin-1-ylpropanoate (C)



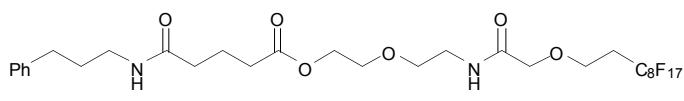
2-(2-(1*H*,1*H*,2*H*,2*H*-perfluorodecyloxy)ethanamido)ethoxyethyl 3-morpholino-3-oxopropanoate (E)



2-(2-(1*H*,1*H*,2*H*,2*H*-perfluorodecyloxy)ethanamido)ethoxyethyl 3-oxopropanoate (F)

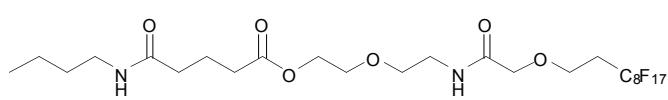


2-(2-(2-(1H,1H,2H,2H-perfluorodecyloxy)ethanamido)ethoxyethyl 3-(benzylamino)-3-oxopropanoate (G)



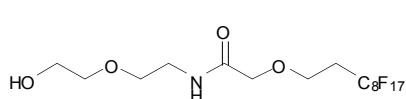
Pale yellow oil; δ_H (400MHz, CDCl₃) 1.79-1.87 [2H, m, PhCH₂CH₂NHC(O)], 1.93-1.98 [2H, m, RNH(O)CCH₂CH₂CH₂C(O)], 2.18 [2H, t, J 7.0, PhCH₂CH₂NHC(O)], 2.38 [2H, t, J 7.3, RNH(O)CCH₂CH₂CH₂C(O)], 2.38-2.50 [2H, m, OCH₂CH₂C₈F₁₇], 2.65 [2H, t, J 7.3, RNH(O)CCH₂CH₂CH₂C(O)], 3.28 [2H, q, J 7.0, PhCH₂CH₂NHC(O)], 3.47-3.50 [2H, m, NHCH₂CH₂O], 3.55-3.59 [2H, m, NHCH₂CH₂O], 3.64-3.66 [2H, m, OCH₂CH₂O], 3.81 [2H, t, J 6.3, OCH₂CH₂C₈F₁₇], 3.97 [2H, s, NH(O)CCH₂OCH₂CH₂C₈F₁₇], 4.21-4.23 [2H, m, OCH₂CH₂O], 5.70 [1H, s, NH], 6.78 [1H, s, NH], 7.16-7.20 [3H, m, PhH], 7.26-7.29 [2H, m, PhH].

2-(2-(2-(1H,1H,2H,2H-perfluorodecyloxy)ethanamido)ethoxyethyl 3-(ethylamino)-3-oxopropanoate (H)



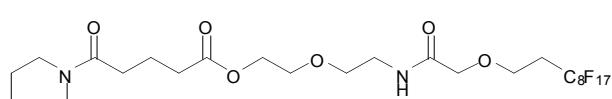
Pale yellow oil; δ_H (400MHz, CDCl₃) 0.91 [3H, t, J 7.3, CH₃CH₂CH₂NHC(O)], 1.29-1.38 [2H, m, CH₃CH₂CH₂NHC(O)], 1.43-1.50 [2H, m, CH₃CH₂CH₂CH₂NHC(O)], 1.96 [2H, quin., J 7.3, RNH(O)CCH₂CH₂CH₂C(O)], 2.21 [2H, t, J 7.3, RNH(O)CCH₂CH₂CH₂C(O)], 2.39 [2H, t, J 7.3, RNH(O)CCH₂CH₂CH₂C(O)], 2.39-2.50 [2H, m, OCH₂CH₂C₈F₁₇], 3.33 [2H, q, J 7.3, CH₃CH₂CH₂CH₂NHC(O)], 3.47-3.52 [2H, m, NHCH₂CH₂O], 3.55-3.59 [2H, m, NHCH₂CH₂O], 3.65-3.67 [2H, m, OCH₂CH₂O], 3.83 [2H, t, J 6.6, OCH₂CH₂C₈F₁₇], 3.98 [2H, s, NH(O)CCH₂OCH₂CH₂C₈F₁₇], 4.21-4.23 [2H, m, OCH₂CH₂O], 5.66 [1H, s, NH], 6.80 [1H, s, NH].

2-(1H,1H,2H,2H-perfluorodecyloxy)-N-(2-(2-hydroxyethoxy)ethyl)ethanamide (I)



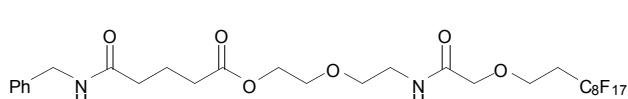
Pale yellow oil; δ_H (400MHz, CDCl₃) 1.97 [1H, t, J 5.9, OH], 2.46 [2H, tt, J 7.0, 18.5, OCH₂CH₂C₈F₁₇], 3.50-3.54 [2H, m, NHCH₂CH₂O], 3.58-3.61 [4H, m, NHCH₂CH₂O & OCH₂CH₂OH], 3.72-3.75 [2H, m, OCH₂CH₂OH], 3.83 [2H, t, J 6.3, OCH₂CH₂C₈F₁₇], 3.99 [2H, s, NH(O)CCH₂OCH₂CH₂C₈F₁₇], 6.81 [1H, s, NH].

2-(2-(2-(1H,1H,2H,2H-perfluorodecyloxy)ethanamido)ethoxyethyl 3-oxo-3-(piperidin-1-yl)propanoate (J)



Pale yellow oil; δ_H (400MHz, CDCl₃) 1.54-1.57 [4H, m, 2 x CH₂ (piperidine)], 1.61-1.66 [2H, m, CH₂ (piperidine)], 1.91-1.99 [2H, m, RNH(O)CCH₂CH₂CH₂C(O)], 2.37 [2H, t, J 7.3, RNH(O)CCH₂CH₂CH₂C(O)], 2.42 [2H, t, J 7.3, RNH(O)CCH₂CH₂CH₂C(O)], 2.39-2.51 [2H, m, OCH₂CH₂C₈F₁₇], 3.37-3.40 [2H, m, NHCH₂CH₂O], 3.47-3.61 [6H, m, NHCH₂CH₂O & 2 x CH₂ (piperidine)], 3.64-3.67 [2H, m, OCH₂CH₂O], 3.83 [2H, t, J 6.3, OCH₂CH₂C₈F₁₇], 3.99 [2H, s, NH(O)CCH₂OCH₂CH₂C₈F₁₇], 4.21-4.23 [2H, m, OCH₂CH₂O], 6.82 [1H, s, NH].

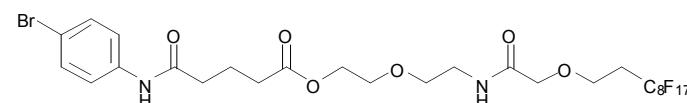
2-(2-(2-(1H,1H,2H,2H-perfluorodecyloxy)ethanamido)ethoxyethyl 3-(benzylamino)-3-oxopropanoate (K)



Pale yellow oil; δ_H (400MHz, CDCl₃) 1.93-2.03 [2H, m, RNH(O)CCH₂CH₂CH₂C(O)], 2.25-2.30 [2H, m, RNH(O)CCH₂CH₂CH₂C(O)], 2.37-2.43 [2H, m, RNH(O)CCH₂CH₂CH₂C(O)], 2.39-2.51 [2H, m, OCH₂CH₂C₈F₁₇], 3.43-3.47 [2H, m, NHCH₂CH₂O], 3.52-3.54 [2H, m, NHCH₂CH₂O], 3.62-3.64 [2H, m, OCH₂CH₂O], 3.80 [2H, t, J 6.3, OCH₂CH₂C₈F₁₇], 3.93 [2H, s, NH].

$\text{NH}(\text{O})\text{CCH}_2\text{OCH}_2\text{CH}_2\text{C}_8\text{F}_{17}$], 4.20-4.22 [2H, m, $\text{OCH}_2\text{CH}_2\text{O}$], 4.42 [2H, d, J 5.6, CH_2Ph], 6.10 [1H, s, NH], 6.76 [1H, s, NH], 7.26-7.34 [5H, m, PhH].

2-(2-(2-(1*H*,1*H*,2*H*,2*H*-perfluorodecyloxy)ethanamido)ethoxyethyl oxopropanoate (L)



Cream solid; δ_{H} (400MHz, CDCl_3) 2.02-2.09 [2H, m, RNH(O) $\text{CCH}_2\text{CH}_2\text{CH}_2\text{C}(\text{O})$], 2.39-2.46 [4H, m, RNH(O) $\text{CCH}_2\text{CH}_2\text{CH}_2\text{C}(\text{O})$], 2.39-2.51 [2H, m, $\text{OCH}_2\text{CH}_2\text{C}_8\text{F}_{17}$], 3.48-3.52 [2H, m, $\text{NHCH}_2\text{CH}_2\text{O}$], 3.57-3.60 [2H, m, $\text{NHCH}_2\text{CH}_2\text{O}$], 3.67-3.69 [2H, m, $\text{OCH}_2\text{CH}_2\text{O}$], 3.83 [2H, t, J 6.3, $\text{OCH}_2\text{CH}_2\text{C}_8\text{F}_{17}$], 3.98 [2H, s, NH(O) $\text{CCH}_2\text{OCH}_2\text{CH}_2\text{C}_8\text{F}_{17}$], 4.25-4.27 [2H, m, $\text{OCH}_2\text{CH}_2\text{O}$], 6.76 [1H, s, NH], 7.39-7.41 [2H, m, ArH], 7.45-7.47 [2H, m, ArH], 8.11 [1H, s, NH].

Data used to generate values shown in Figure 1.

Cpd 1	Cy5 Mean	Cy5 Bg Mean	Cpd 2	Cy5 Mean	Cy5 Bg Mean	Cpd 3	Cy5 Mean	Cy5 Bg Mean
440	45			1039	0		2068	27
466	30			739	1		1922	24
598	40			798	8		1793	43
893	33			712	2		2997	60
430	41			900	10		1745	18
537	1			641	3		1750	16
476	392			1003	6		1844	7
720	19			905	11		1728	14
156	19			1540	51		1670	12
173	15			684	28		1878	9
311	27			844	9		1785	26
2	0			575	3		1535	17
117	22			821	16		1237	2
267	6			692	16		1393	3
164	24			902	2		1784	6
156	9			929	2		1388	4
Sum	5906	723	Sum	13724	168	Sum	28517	288
Average	369	45	Average	858	11	Average	1782	18
SD	244	94	SD	224	13	SD	389	16
SNR (1)	3		SNR (1)	65		SNR (1)	113	
SNR (2)	67		SNR (2)	6673		SNR (2)	9804	

$$\text{SNR (1)} = (\text{Cy5 Mean} - \text{Cy5 Bg Mean}) / \text{Bg SD}$$

$$\text{SNR (2)} = (\text{Cy5 Mean} / \text{Cy5 Bg Mean})^2$$

