Supporting Information

Solid phase synthesis of functionalised SAM-forming alkanethiol-oligoethyleneglycols

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1. Synthesis and characterisation of compounds

1.1 General Remarks

All chemicals, including N-Fmoc amino acids (with Boc, ^{t}Bu or Trt side chain protection), and coupling reagents, were purchased from major chemical suppliers. 2-Chlorotrityl chloride resin (1.2 mmol/g) was purchased from VWR International. All non-aqueous solution phase reactions were carried out under a N_2 atmosphere, using solvents from a solvent purification system (Innovative Technology Inc. PureSolv®). DMF used was of analytical grade and purchased from Sigma-Aldrich and used as provided. Solid phase reactions were performed in fritted solid phase extraction tubes (Grace and Co.) and agitated on a Stuart rotator. Lyophilisation of compounds was performed using a Virtis Benchtop K freeze dryer.

Proton and carbon NMR spectra were recorded on a Bruker Advance 500 or DRX500. Chemical shifts are quoted in parts per million downfield of tetramethylsilane and referenced to residual solvent peaks, and coupling constants (*J*) are given in Hz. Assignment of spectra was aided by the use of COSY, DEPT, TOCSY, HSQC and HMBC experiments where appropriate. ^{1}HNMR and ^{13}CNMR spectra have been provided for all novel compounds and for compounds for which no NMR has previously been published; for all other compounds a reference has been provided. Low resolution electrospray ionisation (ESI+) mass spectra were obtained on a Bruker HCT Ultra mass spectrometer; high resolution electrospray (ESI+) were performed on a Bruker Daltonics MicroTOF mass spectrometer. Infrared spectra were recorded on a Perkin Elmer Fourier-Transfer spectrometer.

1.2 Reagent cocktails for solid phase chemistry

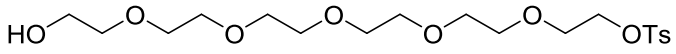
Capping cocktail A for inactivation of 2-chlorotrityl chloride residues: MeOH (10% v/v) and DIPEA (1% v/v) in DCM.

Cleavage cocktail B for cleavage of LCAT-OEG azide **13**, amine **15** and methylene blue derivative **18**: TFA (5% v/v) and TES (2% v/v) in DCM.

Cleavage cocktail C for cleavage of LCAT-OEG-OH **11a** and **11b**: TFA (65% v/v) and TES (2% v/v) in DCM.

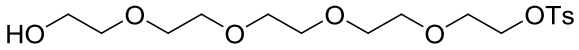
Cleavage cocktail D for cleavage of LCAT-OEG-peptides **17a** and **17b**: H_2O (2.5% v/v) and TES (2.5% v/v) in TFA.

17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (4a)[1]



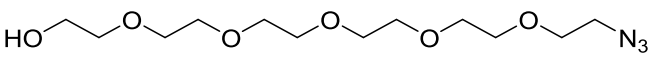
Silver (I) oxide (3.7 g, 15.9 mmol), and potassium iodide (352 mg, 2.12 mmol) were added to a stirred solution of hexaethylene glycol (3.0 g, 10.6 mmol) in DCM (80 mL) at 0 °C. A solution of 4-toluenesulfonyl chloride (2.2 g, 11.7 mmol) in DCM (20 mL) was then added drop-wise and the reaction mixture stirred at 0 °C until all starting material had been consumed (LC-MS). The reaction was quenched by filtering through a pad of silica, with a mobile phase of EtOAc (250 mL) and the solvents evaporated. The crude tosylate was subjected to flash chromatography (SiO_2; EtOAc–MeOH 95:5) to afford the title compound as a colourless oil (3.4g, 78%). $\mathbf{R_f}$ **=** 0.16 (EtOAc–MeOH, 95:5); **^{1}H NMR:** (500 MHz, $CDCl_3$, 298K) δ 7.79 (d, ^3J = 8.3 Hz, 2H, ArH), 7.33 (d, ^3J = 8.1 Hz, 2H, ArH), 3.78–3.53 (m, 22H. C$\underline{H_2}$-O-), 4.15 (s, 2H, C$\underline{H_2}$-OH), 3.47 (s, 2H, C$\underline{H_2}$-OTs), 2.44 (s, 3H, tosyl-C$\underline{H_3}$); **ESI-LC-MS:** Calcd. for $C_{19}H_{33}O_9S$: *m/z* 437.1 $[M+H]^+$; found 437.1; R_t = 1.66 min.

15-hydroxy-3,6,9,12-pentaoxaheptadecyl 4-methylbenzenesulfonate (4b)[1]



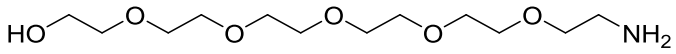
Prepared analogously to **4a**; starting from pentaethylene glycol (3.0 g, 12.6 mmols) gave **4b** (3.65g, 74%) as a colourless oil; $\mathbf{R_f}$ = 0.28 DCM–MeOH (96:4); **^{1}H NMR:** (500 MHz, $CDCl_3$ 298K) δ 7.80 (d, *J* = 8.1 Hz, 2H, Ar), 7.34 (d, *J* = 8.1 Hz, 2H, Ar), 4.26–4.03 (m, 2H, C$\underline{H_2}$-OH), 3.75–3.51 (m, 18H, OEG), 2.45 (s, 3H, tosyl-C$\underline{H_3}$); **ESI-LC-MS:** Calcd. for $C_{17}H_{29}O_8S$: *m/z* 393.1 $[M+H]^+$; found 393.1; R_t = 1.64 min.

17-azido-3,6,9,12,15-pentaoxaheptadecan-1-ol (5a)[2]



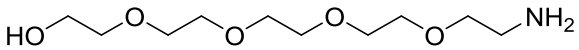
A solution of **4a** (3.18 g, 7.28 mmol) in anhydrous DMF was added to a flask containing sodium azide. The mixture was stirred and heated to 50 °C for 16 hours, then left to cool to room temperature. The DMF was removed by coevaporation with toluene at 50 °C, the residue re-suspended in EtOAc and filtered over a pad of SiO_2. The EtOAc was removed under reduced pressure to afford the product as a colourless oil (2.2g, 98%). $\mathbf{R_f}$ = 0.24 (EtOAc–MeOH 10:1); **^{1}H NMR:** (500 MHz, $CDCl_3$) δ 3.72 (t, *J* = 4.3 Hz, 2H, -C$\underline{H_2}$-OH), 3.69–3.63 (m, 18H, OEG), 3.60 (t, *J* = 4.3 Hz, 2H, -C$\underline{H_2}$-CH_2OH), 3.39 (t, *J* = 5.3 Hz, 2H, C$\underline{H_2}$-N_3). **ESI-LC-MS:** Calcd. for $C_{12}H_{25}N_3NaO_6$: *m/z* = 330.1 $[M+Na]^+$; found 330.1; R_t = 1.39 min.

17-amino-3,6,9,12,15-pentaoxaheptadecan-1-ol (6a)[2]



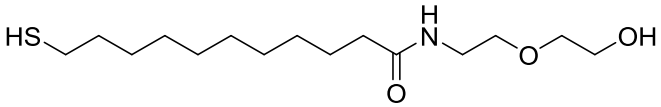
Triphenylphosphine (2.36 g, 9.02 mmol) was added to a stirred solution of **3** (2.51 g, 8.2 mmol) in THF (30 mL) at 0 °C. The reaction mixture was and stirred at 0 °C for 5 hours, diluted into water (30 mL) and washed with toluene (2 × 20 mL); the aqueous layer was concentrated in vacuo (rotary evaporator) to yield a colourless oil (2.18g, 95%); $\mathbf{R_f}$ = 0.7 (DCM–MeOH–NH_3 (aq) 3:3:1); **^{1}H NMR:** (500 MHz, D_2O, 298K) δ 3.76–3.68 (m, 22H, C$\underline{H_2}$), 3.65 (t, ^3J = 5.9, Hz, 2H, C$\underline{H_2}$OH), 3.60 (t, ^3J = 5.3 Hz, 2H, C$\underline{H_2}$$NH_2$); **ESI-LC-MS:** Calcd. for $C_{12}H_{28}NO_6$, *m/z* 282.1 $[M+H]^+$: found 282.1; R_t = 0.25 min.

15-amino-3,6,9,12,-pentaoxaheptadecan-1-ol (6b)[2]



Prepared analogously to **5a** and **6**a; starting from **4b** (1.21 g, 3.09 mmols) gave **6b** (0.69 g, 94% over two steps) as a colourless oil. **^{1}H NMR:** (500 MHz, $CDCl_3$) δ 3.72 (t, *J* = 4.8 Hz, 2H, -C$\underline{H_2}$-CH_2OH), 3.69-3.62 (m, 12H, OEG), 3.60 (t, *J* = 4.9 Hz, 2H, -C$\underline{H_2}$OH), 3.56 (t, *J* = 5.0 Hz, 2H, OC$\underline{H_2}$-CH_2NH_2), 2.88 (t, *J* = 5.0 Hz, 2H, OCH_2-C$\underline{H_2}$-NH_2); **ESI-LC-MS:** Calcd. for $C_{10}H_4NO_5$: *m/z* 238.2 [M+H]; found 238.2; R_t = 0.20min.

N-5-hydroxy-3-oxapentyl-11-mercaptounadecamide (11a)



2-Chlorotrityl chloride resin (100 mg, 0.12 mmol) was swelled in DCM for 15 min then the SPE-tube was drained. A solution of 11-mercaptounadecanoic acid (56 mg, 0.24) in DCM (1.5 mL) was added

to the resin and allowed to stir for 16 hours. The resin was washed with DCM (3 × 1.5 mL), after which the remaining, unreacted, 2-chlorotrityl chlorides groups were inactivated using capping cocktail A (1 mL, 2 × 2 min). The resin was washed with DCM (3 x 1.5 mL) and DMF (3 x 1.5 mL). A solution of 2-(2'-aminoethoxy)-ethanol (34mg, 0.36 mmol), HOBt (49 mg, 0.36 mmol), DIC (56 µL, 0.36 mmol) and DIPEA (70 µL, 0.36 mmol) was *preincubated* for five minutes in DMF (1.5 mL*)*, then added to the resin and the reaction mixture stirred for 16 hours. The resin was washed with DMF (3 × 1.5 mL), then DCM (3 × 1.5 mL). The resin-bound alcohol was washed with diethyl ether (1 × 1.5 mL), after which the resin was dried under high vacuum for 2 hours and then treated with washes of cleavage cocktail C (1.5 mL, 3 × 2 min). The filtrates were combined and concentrated *in vacuo*. The residue was heated at reflux in water for two hours to hydrolyse trifluoroacetate esters of **11a**). The water was removed (rotary evaporator) and the residue purified by column chromatography (SiO_2; DCM-MeOH, 94:6) to yield a colourless solid (28 mg, 62%). $\mathbf{R_f}$ = 0.2 (DCM – MeOH, 94:6); **^{1}H NMR** (500 MHz, $CDCl_3$, 298K) δ 5.96 (br s, 1H, O-H) 3.76 (t, 2H, ^3J = 4.6 Hz, -CH_2-OH), 3.65–3.53 (m, 4H, -CH_2-O-CH_2-), 3.47 (q (ap), ^3J = 5.3 Hz, 2H, CONH-CH2-), 2.52 (q (ap), ^3J = 7.4 Hz, 2H, -CH_2-CONH), 2.18 (t, 2H, ^3J = 7.4 Hz, HS-CH_2), 1.68–1.56 (m, 4H, HSCH_2-CH_2- and -CH_2-CH_2CONH), 1.42–1.24 (m, 13H, alkyl CH_2 and S-H); **^{13}C NMR** (125 MHz, $CDCl_3$, 298K) δ 173.39(-CONH-), 72.20 (O-CH_2-CH_2OH), 70.03(CONHCH$_2$-CH_2-O), 61.78(-CH_2-OH), 39.18 (CONH-CH_2-), 36.78 (HS-CH_2), 34.01(HSCH$_2$-CH_2-), 29.43(alkyl), 29.38(alkyl), 29.31(alkyl), 29.28(alkyl), 29.02(alkyl), 28.34 (alkyl), 25.71 (-CH_2-CH_2CONH), 24.64 (-CH_2-CONH); **ESI-MS:** Calcd. for $C_{15}H_{32}NNaO_3S$: *m/z* 328.1922 $[M+Na]^+$; found 328.1929.

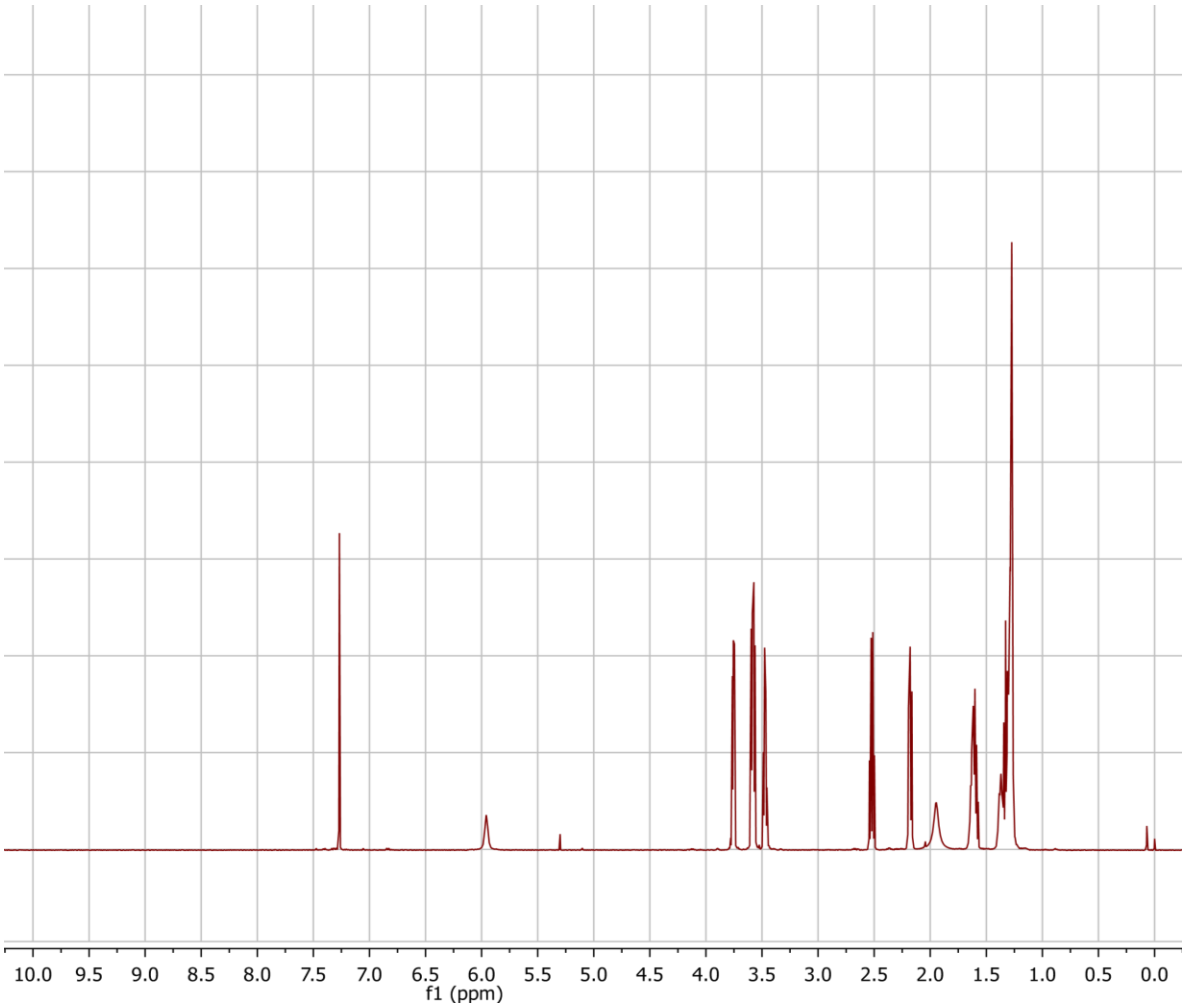


Figure S1.1: ^{1}HNMR spectrum (500 MHz, $CDCl_3$) of **11a**.

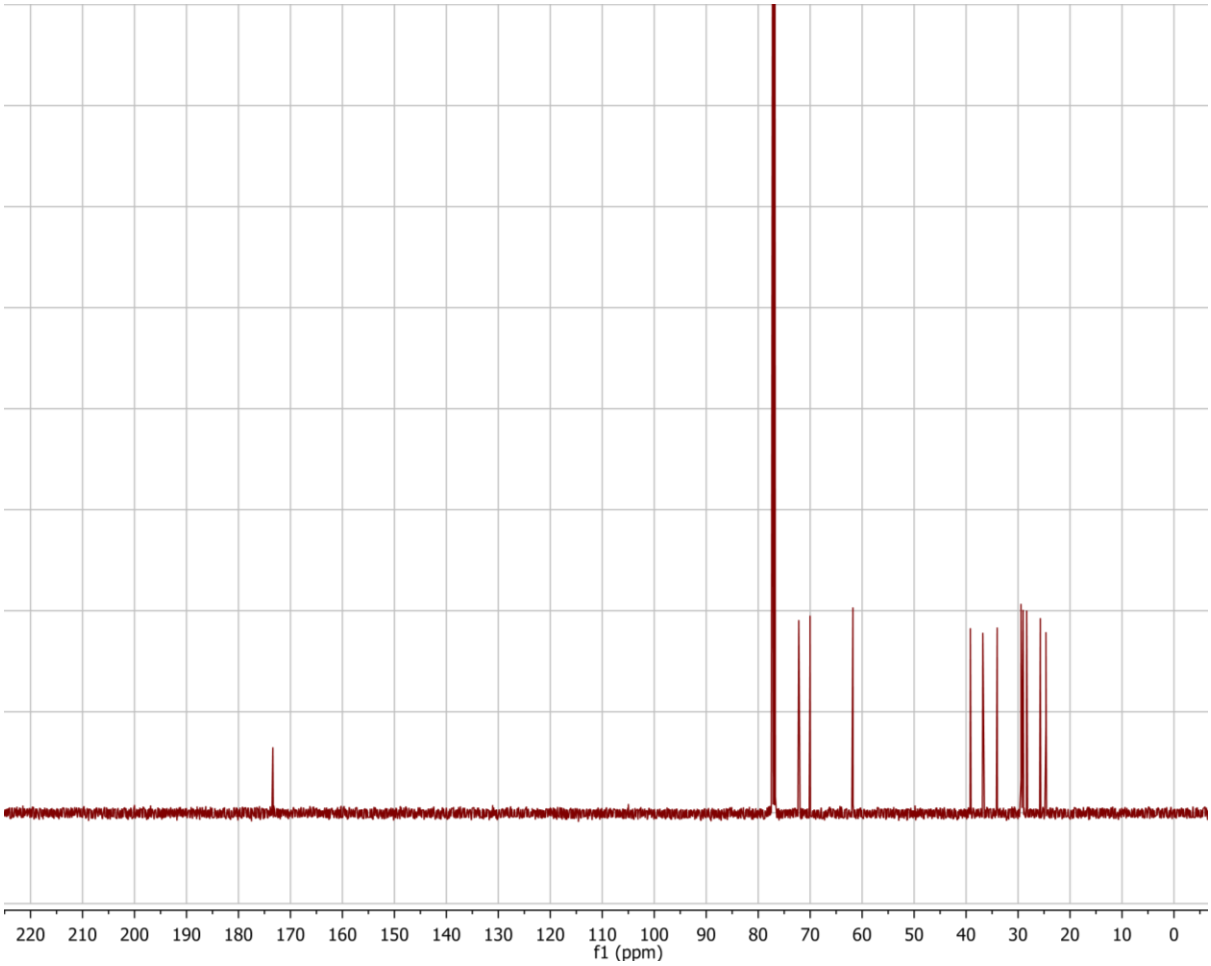
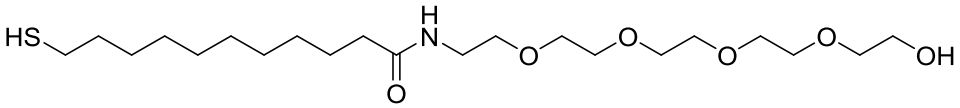


Figure S1.2: ^{13}CNMR spectrum (125 MHz, $CDCl_3$) of **11a**.

N-15-hydroxy-3,6,9,11-tetraoxapentadecyl-11-mercaptounadecamide (11b)



2-Chlorotrityl chloride resin (100 mg, 0.12 mmol) was swelled in DCM for 15 min and the SPE-tube drained. A solution of 11-mercaptounadecanoic acid (56 mg, 0.24 mmol) in DCM (1.5 mL) was added to the resin and allowed to stir for 16 hours. The resin was washed with DCM (3 × 1.5 mL) after which the remaining, unreacted, 2-chlorotrityl chlorides groups were inactivated using capping cocktail A (1 mL, 2 × 2 min). The resin was washed with DCM (3 × 1.5 mL) and DMF (3 × 1.5 mL). A solution of amine **6a**, (85 mg, 0.36 mmol), HOBt (49 mg, 0.36 mmol), DIC (56 µL, 0.36 mmol) and DIPEA (70 µL, 0.36 mmol) was *preincubated* for five minutes in DMF (1.5 mL), then added to the resin and the reaction mixture stirred for 16 hours. The resin was washed with DMF (3 × 1.5 mL), then DCM (3 × 1.5 mL). The resin-bound alcohol was washed diethyl ether (1 × 1.5 mL) the resin was then dried under high vacuum for 2 hours and then treated with washes of cleavage cocktail C 1.5 mL, 3 × 2 min). The filtrates were combined and concentrated *in vacuo*. The residue was heated at reflux in water for two hours to hydrolyse trifluoroacetate esters of **11b**. The water was removed (rotary evaporator) and the residue purified by column chromatography ($CHCl_3$–MeOH–AcOH, 96:4:0.1) and purified product dissolved in water and lyophilised to yield an off-white semi-solid (32 mg, 62%). $\mathbf{R}_f$ = 0.2 ($CHCl_3$–MeOH–AcOH, 96:4:0.1); **^{1}H NMR** (500 MHz, $CDCl_3$, 298K) δ 3.71 (br s), 2H, C$\underline{H_2}$OH) 3.69–3.58 (m, 14H, OEG), 3.54 (d, *J* = 4.8 Hz, 2H), 3.43 (q (ap), *J* =4.9 Hz, 2H, -CONHC$\underline{H_2}$-), 2.50 (q (ap), *J* = 7.4 Hz, 2H, -C$\underline{H_2}$-CONH), 2.17 (t, *J* = 7.6 Hz, 2H, -C$\underline{H_2}$-SH), 1.65–

1.54 (m, 4H, $HSCH_2$-$C\underline{H_2}$- and -$C\underline{H_2}$-CH_2CONH), 1.29 (m, 13H, alkyl + S-$\underline{H}$); **^{13}C NMR:** (125 MHz, $CDCl_3$, 298K) δ 173.56 (CONH), 72.59 (OEG), 70.50 (OEG), 70.47 (2C, OEG), 70.21 (OEG), 70.13 (2C, OEG), 65.84 (OEG), 61.62 (-$\underline{C}H_2OH$), 39.17 (-$CONH\underline{C}H_2$), 36.61 (-$\underline{C}H_2SH$), 34.03 ($HSCH_2$-$\underline{C}H_2$), 29.45 (alkyl), 29.41 (alkyl), 29.35 (alkyl), 29.34 (alkyl), 29.04 (alkyl), 28.35(alkyl), 25.76 (-$\underline{C}H_2$-CH_2CONH), 24.64(-$\underline{C}H_2$-CONH); **ESI-MS:** Calcd. for $C_{21}H_{43}NNaO_6S$: *m/z* 460.2709 $[M+Na]^+$; found 460.2728; **IR:** ν_{max} (neat) 3608-3100 (O-H), 3082, 2926, 2855, 2556 (S-H weak), 1650 (HNC=O).

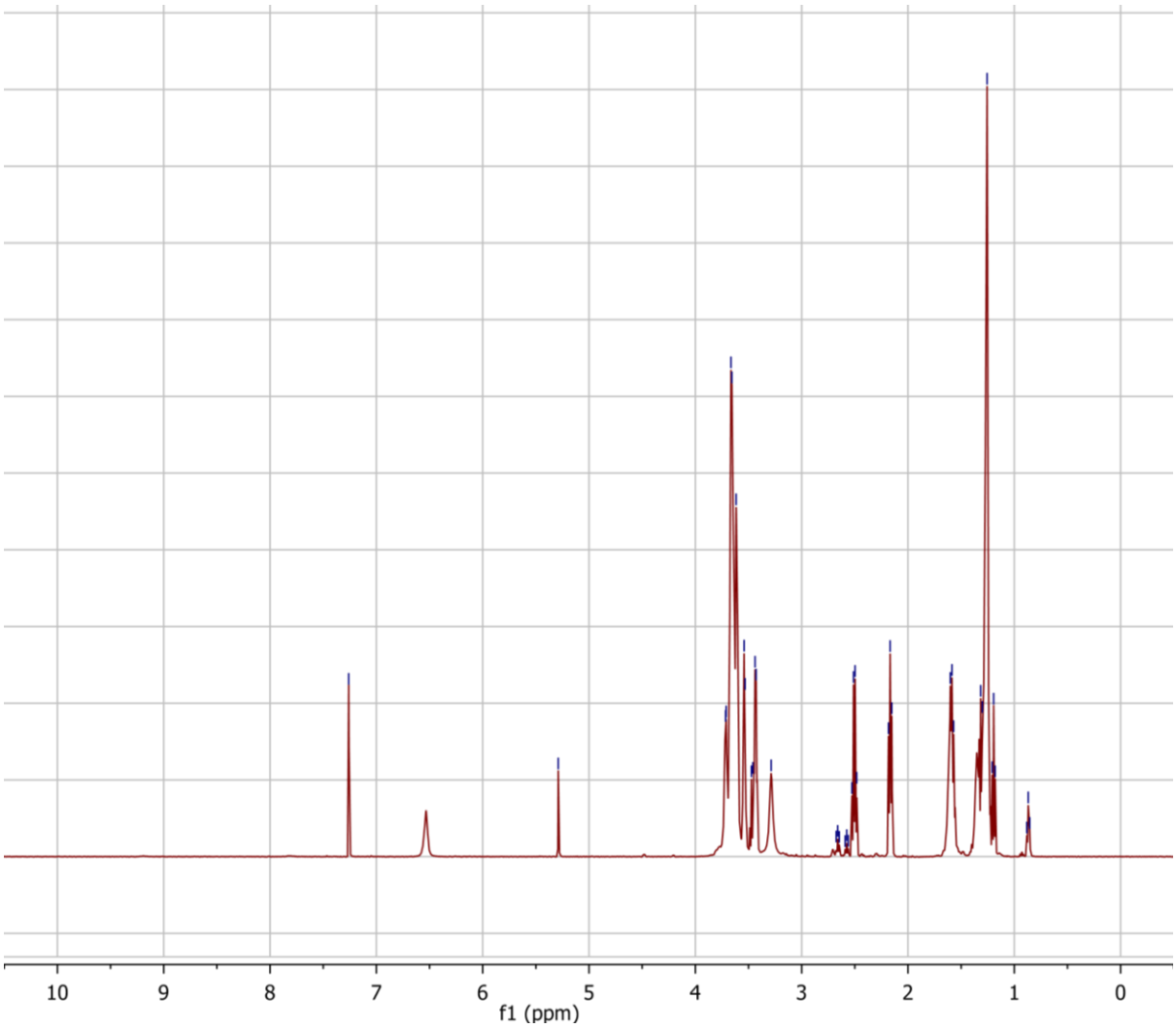


Figure S1.3: ^{1}HNMR spectrum (500 MHz, $CDCl_3$) of **11b**.

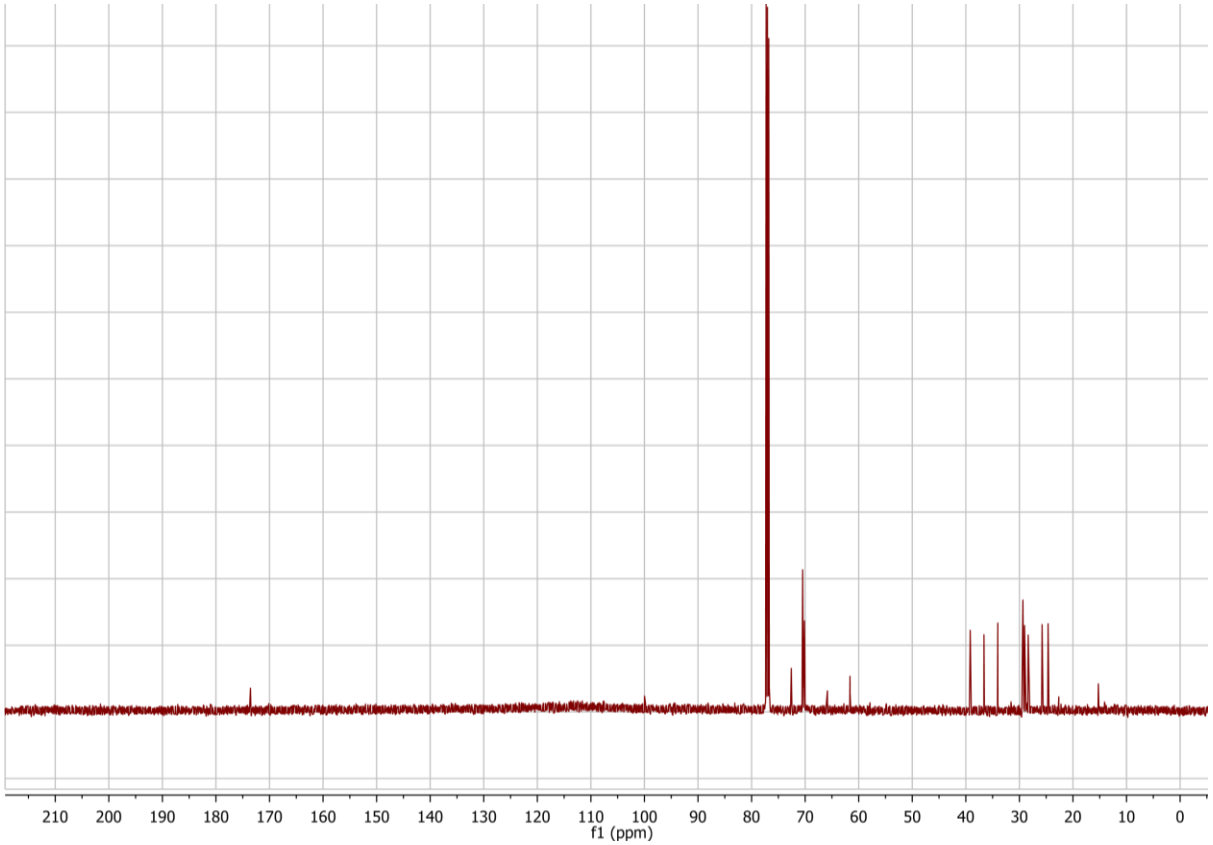
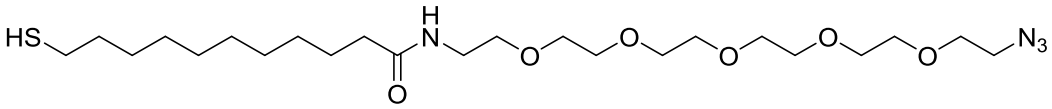


Figure S1.4: ^{13}CNMR spectrum (125 MHz, $CDCl_3$) of **11b**.

N-18-azido-3,6,9,11,15-pentaoxaheptadecyl-11-mercaptounadecamide (13)



2-Chlorotrityl chloride resin (100 mg, 0.12 mmol) was swelled in DCM for 15 min and the SPE-tube drained. A solution of 11-mercaptounadecanoic acid (56 mg, 0.24) in DCM (1.5 mL) was added to the resin and allowed to stir for 16 hours. The resin was washed with DCM (3 × 1.5 mL) after which the remaining, unreacted, 2-chlorotrityl chlorides groups were inactivated using capping cocktail A (1 mL, 2 × 2 min). The resin was washed with DCM (3 × 1.5 mL) and DMF (3 × 1.5 mL). A solution of amine **6**, (101 mg, 0.36 mmol), HOBt (49 mg, 0.36 mmol), DIC (56 µL, 0.36 mmol) and DIPEA (70 µL, 0.36 mmol) was *preincubated* in for five minutes in DMF (1.5 mL), then added to the resin and the reaction mixture stirred for 16 hours. The resin was washed with DMF (3 × 1.5 mL) and then DCM (3 × 1.5 mL). A solution of 4-toluenesulphonyl chloride (228 mg, 1.2 mmol), DMAP (29 mg, 0.24 mmol) and triethylamine (167 µL, 1.2 mmol) in DCM (1.5 mL) was added to the resin, and the reaction mixture stirred overnight. During this time, the liquid phase went from pale yellow to orange-red. The mixture was stirred overnight, then drained, and the resin washed with DCM (3 × 1.5 mL); the resin was treated with a freshly prepared tosylation solution and allowed to react overnight again. The resin was washed with DCM (3 × 1.5 mL) and then *thoroughly* with DMF (5 × 1.5 mL) to remove all traces of DCM. A suspension of sodium azide (78 mg, 1.2 mmol) in DMF (1.5 mL) was then added to the resin and the reaction mixture stirred for 72 hours. The excess sodium azide was removed by washing with DMF-H_2O (5 × 1.5 mL) and then the remaining water washed out with DMF (5 × 1.5 mL). The resin was then washed with DCM (3 × 1.5 mL). The resin-bound azide **12**

was washed with diethyl ether (1 × 1.5 mL), after which the resin was dried under high vacuum for 2 hours and then treated with washes of cleavage cocktail B (1.5 mL, 3 × 2 min) and the combined washes were concentrated *in vacuo* and subjected to reverse phase chromatography, starting at 100% H_2O (+0.1% TFA) and moving to 100% MeOH (+0.1% TFA) in 5% increments, collecting each solvent mixture as a separate 10 mL fraction. Each fraction was analysed by TLC (potassium permanganate stain) and the purity of the positive fractions was assessed by LC-MS. All pure fractions containing the title compound were combined, the MeOH removed *in vacuo* and the water removed by lyophilisation to yield the azide as a colourless semi-solid (21mg, 35%). **^{1}H NMR:** (500 MHz, $CDCl_3$, 298K) δ 3.71–3.62 (m, 18H, OEG CH_2) 3.58 (t, ^3J = 4.9 Hz, 2H, N_3CH2-C$\underline{H_2}$-O), 3.48 (q (ap), ^3J = 4.9 Hz, 2H, CONH-C$\underline{H_2}$-), 3.39 (t, ^3J = 5.0 Hz, 2H, CONH-C$\underline{H_2}$-), 2.52 (q (ap), ^3J = 7.4 Hz, 2H, -C$\underline{H_2}$-CONH), 2.27 (t, ^3J = 7.5 Hz, 2H, HS-C$\underline{H_2}$), 1.66–1.56 (m, 4H, $HSCH_2$-C$\underline{H_2}$- and -C$\underline{H_2}$-CH_2CONH), 1.41–1.24 (m, 13H, alkyl CH_2 and -S$\underline{H}$); **^{13}C NMR:** (125 MHz, $CDCl_3$, 298K) δ 175.58 (-$\underline{C}$ONH-), 70.61(OEG), 70.58 (OEG), 70.52 (OEG), 70.44 (2C, OEG), 70.41 (OEG), 70.40 (OEG), 70.12 (OEG), 70.00 (OEG), 69.47 (N_3CH_2-$\underline{C}H_2$-O), 50.66 ($CONHCH_2$-$\underline{C}H_2$-O), 39.70 ($CONH\underline{C}H_2$), 36.25(HS-$\underline{C}H_2$), 34.01 ($HSCH_2$-$\underline{C}H_2$-), 29.41 (alkyl), 29.34 (alkyl), 29.20 (alkyl), 29.16(alkyl), 29.01 (alkyl), 28.33 (alkyl), 25.82 (-$\underline{C}H_2$-CH_2CONH), 24.62(-$\underline{C}H_2$-CONH); **ESI-MS:** Calcd. for $C_{23}H_{46}N_4O_6S$: *m/z* 529.3036 $[M+Na]^+$; found 529.3048; **FT-IR**: ν_{max} (neat) 3447 (CON-H), 3011, 2928, 2401 (-S-H, weak), 2107 (N_3), 1659 (HNC=O).

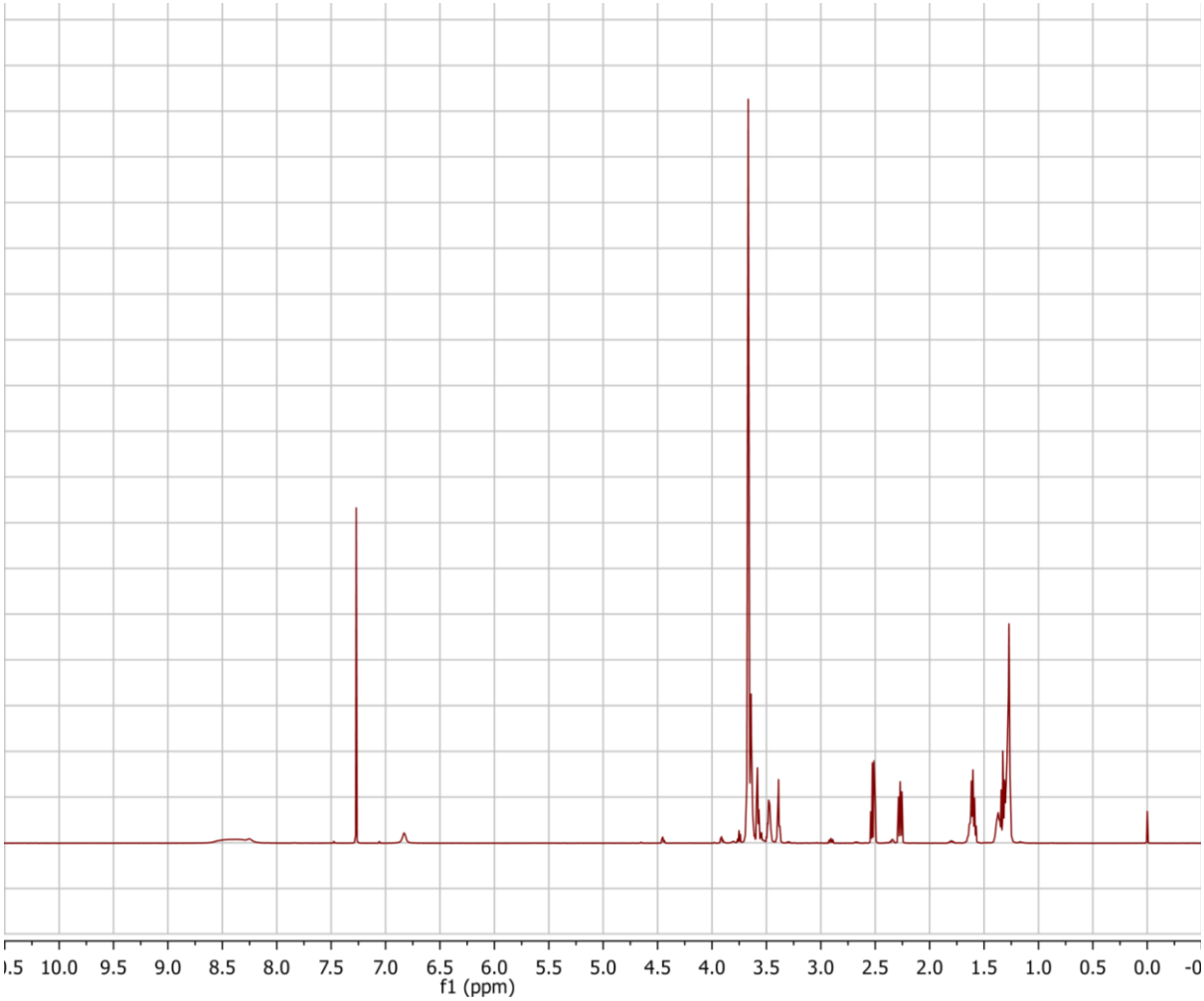


Figure S1.5: ^{1}HNMR spectrum (500 MHz, $CDCl_3$) of **13**.

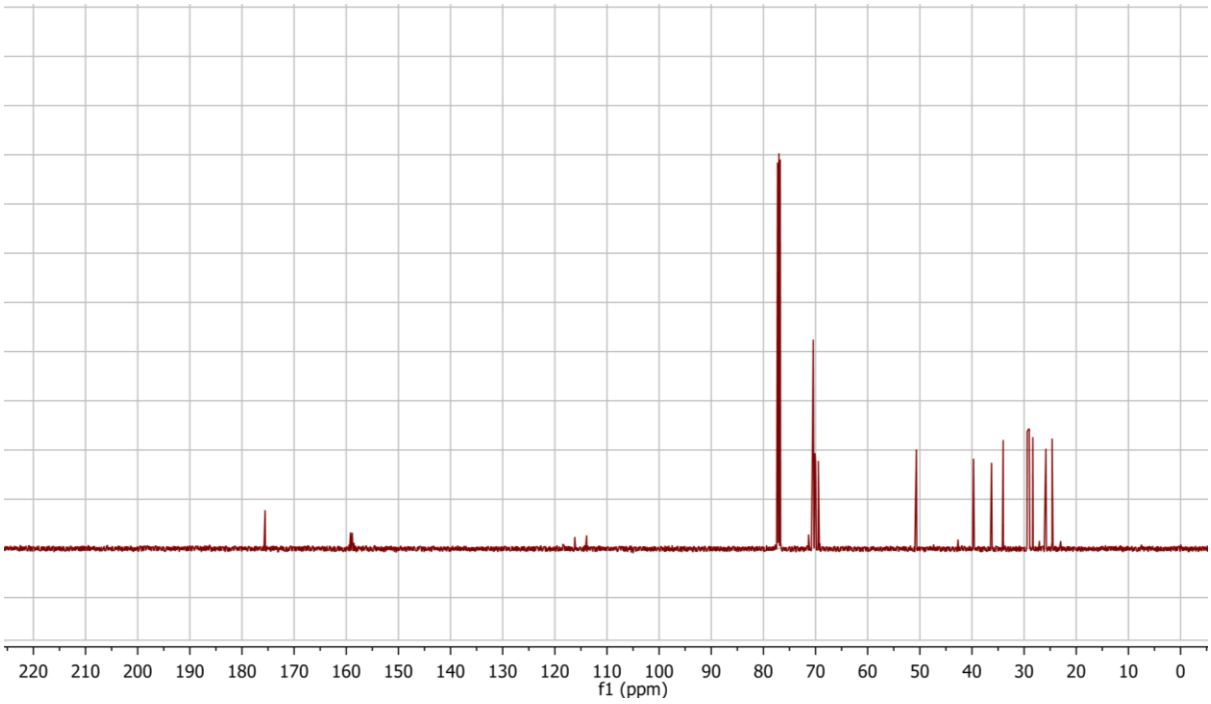
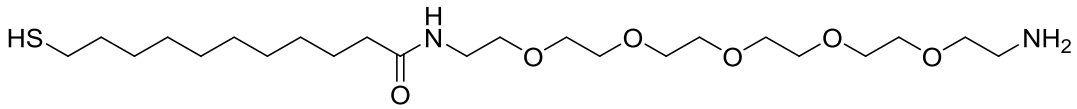


Figure S1.6: ^{13}CNMR spectrum (125 MHz, $CDCl_3$) of **13**.

N-18-amino-3,6,9,11,15-pentaoxaheptadecyl-11-mercaptounadecamide (15)



Resin-bound azide **12** (0.12 mmol) was washed with THF (3 × 1.5 mL), dried under suction and then transferred to a Schlenk vessel. A solution of anhydrous $SnCl_2$ (157 mg, 0.83 mmol) in THF (4 mL) was added to the vessel. The vessel was fitted with a charcoal scrubber, then thiophenol (246 µL, 3.32 mmol) was added followed by DIPEA (786 µL, 4.15 mmol). The solution was stirred gently with a stirrer bar for 1 hour, during which the bubbles could be seen, indicating the release of nitrogen gas. The reaction was quenched by filtering the resin, and the filtrate was collected in a bleach bath (10% in H_20). The resin was washed with THF-H_2O (2:1, 3 × 10 mL), then THF (3 × 10 mL), and finally DCM (3 × 10 mL). The resin-bound amine **14** was transferred back to an SPE tube, washed with diethyl ether (1 × 1.5 mL), dried under high vacuum for 2 hours and then treated with washes of cleavage cocktail B (1.5 mL, 3 × 2 min). The combined washes were concentrated *in vacuo* and subjected to reverse phase chromatography, starting at 100% H_2O (+0.1% TFA) and moving to 100% MeOH (+ 0.1% TFA) in 5% increments, collecting each solvent mixture as a separate 10 mL fraction. Each fraction was analysed by TLC (ninhydrin stain) and the purity of the positive fractions was assessed by LC-MS. All pure fractions containing the title compound were combined, the MeOH removed *in vacuo* and the remaining water removed by lyophilisation to yield the amine as a colourless oil (21 mg, 37 %). **^{1}H NMR:** (500 MHz, $CDCl_3$, 298K) δ 7.89 (br s, 2H, -N$\underline{H}_2$), 7.59 (br s, 1H, -CON$\underline{H}$-), 3.83 (br s, 2H, H_2N-C$\underline{H}_2$-), 3.74 (br s, 2H, OEG), 3.70 – 3.56 (br s, 16H, OEG), 3.41 (br s, 2H, CONH-C$\underline{H}_2$-), 3.12 (br s, 2H, H_2NCH_2-C$\underline{H}_2$-O), 2.51 (q (ap), ^3J = 7.3 Hz, 2H, -C$\underline{H}_2$-CONH), 2.21 (t, ^3J = 7.5 Hz, 2H, HS-C$\underline{H}_2$), 1.61 (t, ^3J = 6.9 Hz, 2H, -C$\underline{H}_2$-CH_2CONH), 1.57 (t, ^3J = 7.5 Hz, 2H, $HSCH_2$-C$\underline{H}_2$-) 1.40–1.25 (m, 13H, CH_2 alkyl and -S$\underline{H}$); **^{13}C NMR:** (125 MHz, $CDCl_3$, 298K) δ 174.90 (C$\underline{O}$NH), 70.21 (OEG), 70.15 (OEG), 70.10 (OEG), 69.97 (OEG), 69.90 (OEG), 69.85 (OEG), 69.83 (OEG), 69.57 (OEG), 69.17 (OEG), 67.05 (H_2N-C$\underline{H}_2$-), 40.18 (H_2NCH_2-C$\underline{H}_2$-O), 38.88 (CONH-C$\underline{H}_2$), 36.19(alkyl), 34.05 ($HSCH_2$-C$\underline{H}_2$-), 29.43(alkyl), 29.40 (alkyl), 29.32(alkyl),

29.27(alkyl), 29.03(alkyl), 28.36 (alkyl), 25.84(-$\underline{C}H_2$-CH_2CONH), 24.64(-C$\underline{H_2}$-CONH); **ESI-MS:** Calcd. for $C_{23}H_{49}N_2O_6S$: *m/z* 481.3311 $[M+H]^+$; found 481.3312 **FT-IR**: ν_{max} (neat) 3321 (CON-H), 3287 (NH_2) 3104 (NH_2), 2927, 2857 (CH), 1646 (HNC=O).

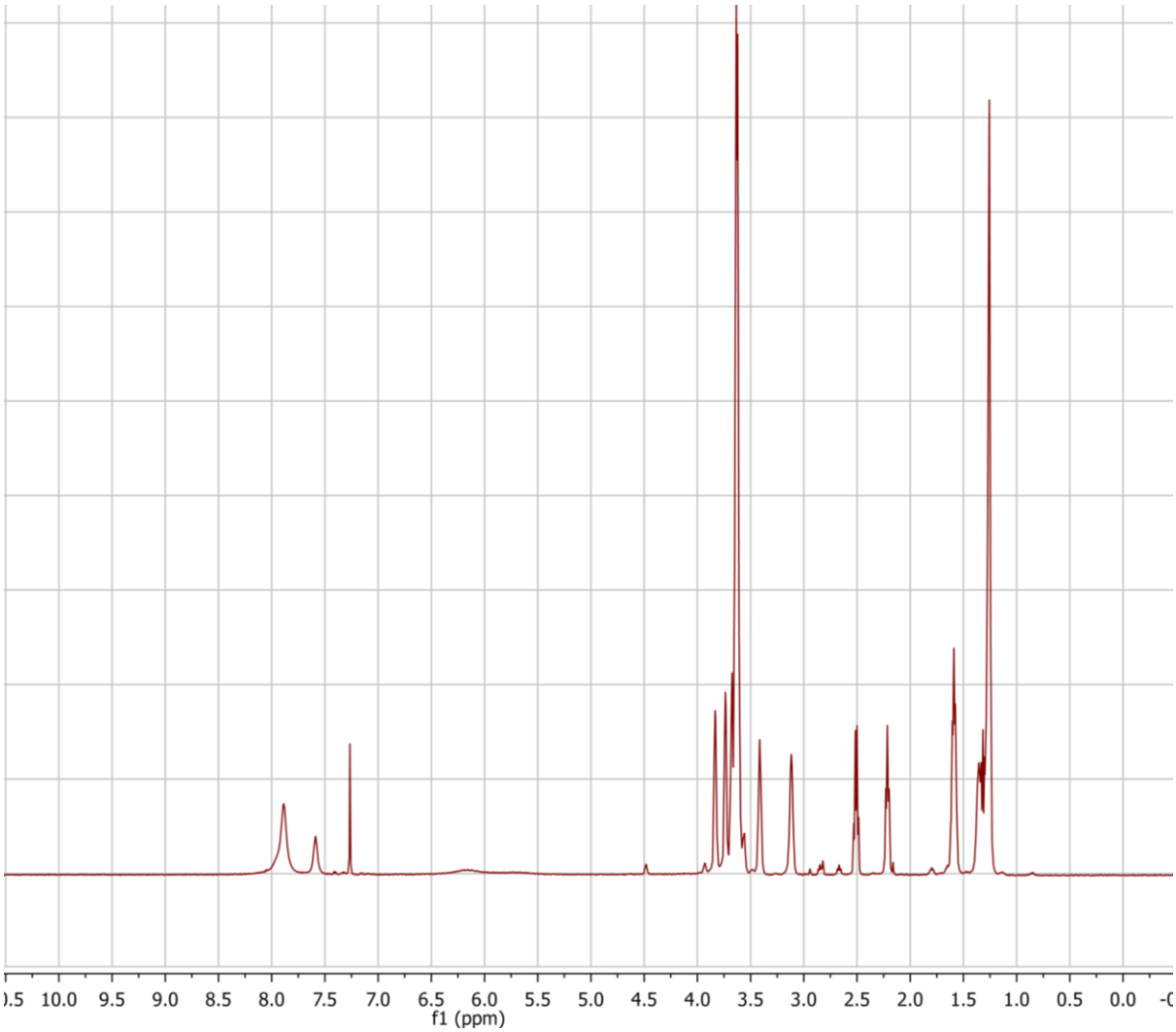


Figure S1.7: ^{1}HNMR spectrum (500 MHz, $CDCl_3$) of **15**.

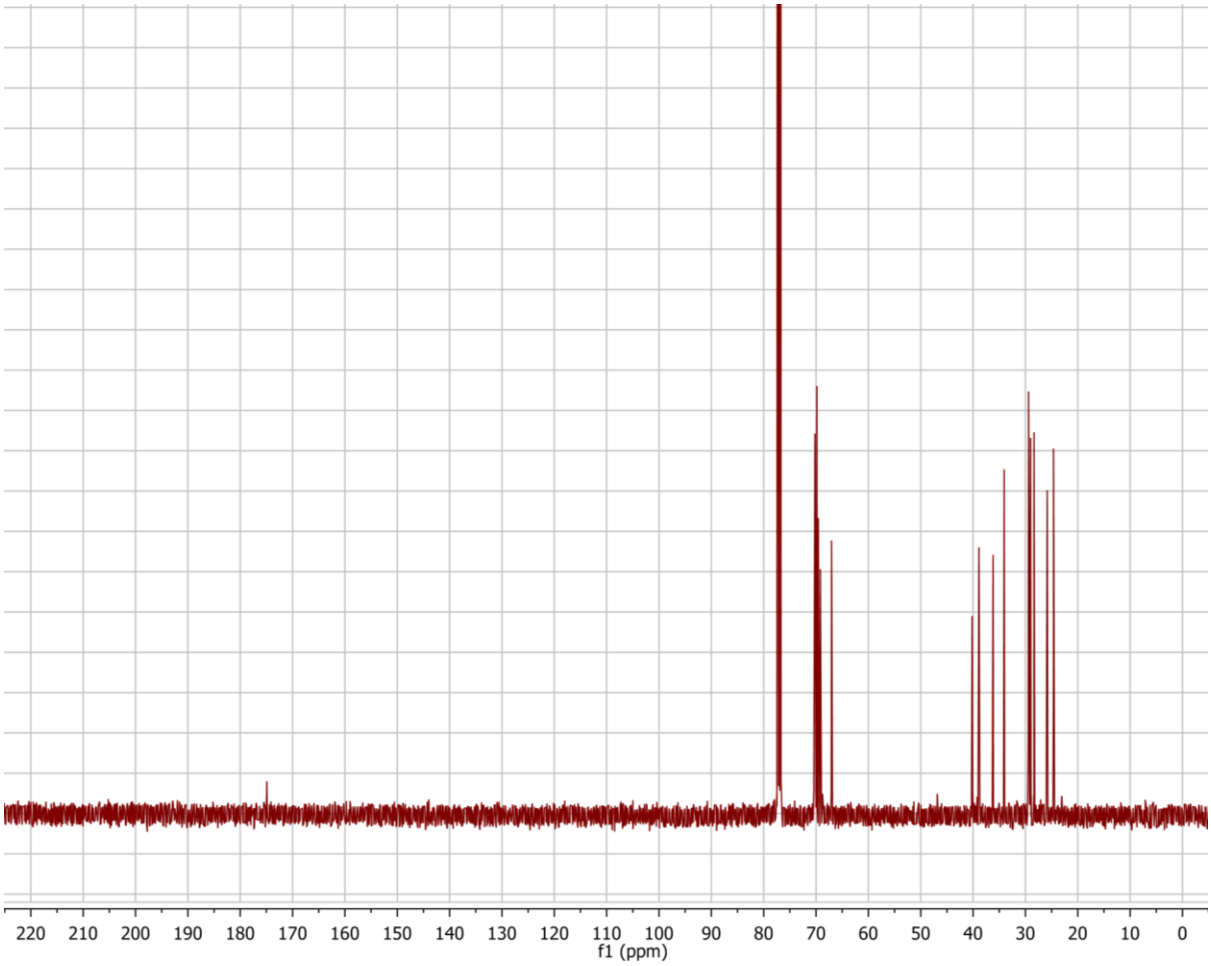
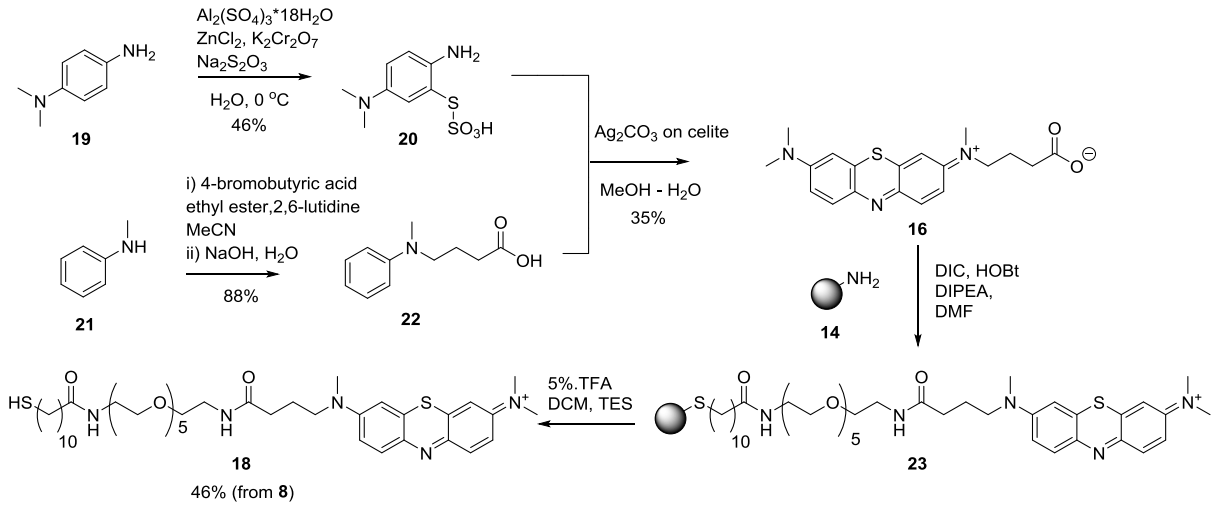
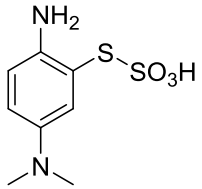


Figure S1.8: ^{13}CNMR spectrum (125 MHz, $CDCl_3$) of **15**.



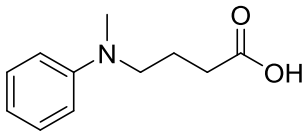
Scheme S1.1: Synthesis of MB-functionalised LCAT-OEG **18**.

2-Amino-5-(dimethylamino)phenylthiosulfonic acid[3] **(20)**



Aluminium sulfate octahydrate (45.0 g, 65 mmol), sodium thiosulfate (22.0 g 140 mmol), and zinc(II) chloride (8.8 g, 63 mmol) were dissolved separately into 100 mL, 80 mL and 12 mL of water respectively. These solutions were then added to a flask charged with N,N-dimethylphenylenediamine **19** (10.0 g, 73 mmol) and the mixture cooled to 0 °C under continuous stirring. A solution of potassium dichromate (5.0 g, 17 mmol) in water (30 mL) was added dropwise over 30 min. The reaction mixture was stirred at 0 °C for a further 2 hours, then allowed to warm to room temperature. The precipitate was isolated by filtration; the solid was washed with water, acetone, then ether to afford the title compound as a lilac solid (8.1 g, 46%). **^{1}H NMR:** (500 MHz, DMSO-d6) δ 8.59 (br s, 1H, -SO_3-H,), 7.31–6.75 (m, 3H, Ar), 2.98 (s, 6H, -N-$(Me)_2$); **ESI-MS:** Calcd. for $C_8H_{13}N_2O_3S_2$: *m/z* 249.0368 $[M+H]^+$; found 249.0362.

N-Methyl-N-(carboxypropyl)aniline[4] **(22)**



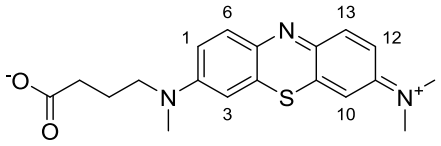
N-Methylaniline **21** (10.1 mL, 93 mmol), 2,6-lutidine (11.3 mL, 100 mmol) and 4-bromobutyric acid ethyl ester (15.0 mL, 100 mmol) were refluxed in MeCN for 16 hours. The MeCN was removed *in vacuo* to leave an indigo residue, which was dissolved in EtOAc (50 mL) and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4) and concentrated to yield an indigo oil. Starting materials and reagents were removed from the residue by vacuum distillation (1 mm/Hg, 60 °C). The residue from the distillation was hydrolysed with NaOH (2.5 M, 30 mL) at reflux for two hours. The mixture was allowed to cool, then acidifed to pH 5 (conc. HCl), then extracted with EtOAc (3 × 30 mL), and dried ($NaSO_4$). Concentraqtion *in vacuo* gave the title compound as a colourless oil (15.1g, 88%). **^{1}H NMR:** (500 MHz, $CDCl_3$, 298K) δ 8.70-7.78 (br s), COOH), 7.31–7.22 (m, 2H, Ar), 6.80 – 6.72 (m, 3H, Ar), 3.40 (t, 2H, ^3J = 7.3 Hz, N-CH_2-), 2.96 (s, 3H, N-CH_3) 2.44 (t, ^3J = 7.2 Hz, 2H, -CH_2-CO_2H), 1.96 (p (ap), ^3J = 7.3 Hz, 2H, CH_2-CH_2-CH_2); **^{13}C NMR:** (125 MHz, $CDCl_3$, 298K) δ 178.79 (CO_2H), 149.23(Ar ispo, NMe-), 129.23 (2C, Ar meta), 116.71 (Ar para), 112.63 (2C, Ar ortho), 52.10(ArN(CH_3)-CH_2-), 38.53(N-CH_3), 31.49(-CH_2-CO_2H), 21.96 (CH_2-CH_2-CH_2); **ESI-MS:** Calcd. for $C_{11}H_{16}NO_2$: *m/z* 194.1181 $[M+H]^+$; found 194.1178.

N-(carboxypropyl)methylene blue[3] **(16)**

Compounds **20 (**2.4 g, 9.7 mmol) and **22** (1.9 g, 9.7 mmol) were dissolved in a mixture of MeOH–H_2O (200:80 mL). The mixture was heated to ~50 °C and Fétizon's reagent* (Ag_2CO_3 on celite; 11.4g, 19 mmol) was added portion wise over 15 min. The mixture was then heated at reflux for 2 hours. The solid-supported reagent was removed by filtration; the filtrate was concentrated *in vacuo* to yield a dark blue residue, which was purified on a short SiO_2 column. The impurities were eluted with EtOAc, after which the target compound was eluted with DCM-MeOH (9:1) to afford the target

* Fétizon's reagent was prepared as described by Fétizon *et al.*[12]

compound as a indigo-violet glassy solid (1.2 g, 35%). LC-MS was consistent with data reported by Pheeney and co-workers. Compound **16** was characterised further as follows:



R_f = 0.15 (DCM – MeOH, 9:1); **^{1}H NMR:** (500 MHz, CD_3OD, 298K) δ 7.97 (d, ^3J = 9.6 Hz, 2H, C6-H and C13-H), 7.57–7.53 (m, 1H, C12-H), 7.50 (d, ^3J = 9.0 Hz, 1H, C1-H), 7.45–7.40 (m, 1H, C10-H), 7.37 (d, ^4J = 2.1 Hz, 1H, C3-H), 3.79 (t, ^3J = 7.4 Hz, 2H, N(Me)-C$\underline{H_2}$-), 3.72 (s, 6H, Ar(CH_3)$_2$), 3.43 (s, 3H, N-C$\underline{H_3}$), 2.49 (t, ^3J = 6.1 Hz, 2H, -C$\underline{H_2}$-CO_2), 2.04 (p, ^3J = 6.8 Hz, 2H, CH_2-C$\underline{H_2}$-CH_2); **ESI-MS:** Calcd. for $C_{19}H_{22}N_3O_2S$, *m/z* 356.1433 $[M+H]^+$; found *m/z* 356.1446; **ESI-LC-MS:** Calcd. for $C_{19}H_{22}N_3O_2S$: *m/z* 356.1 $[M+H]^+$; found 356.1; R_t = 1.47 min.

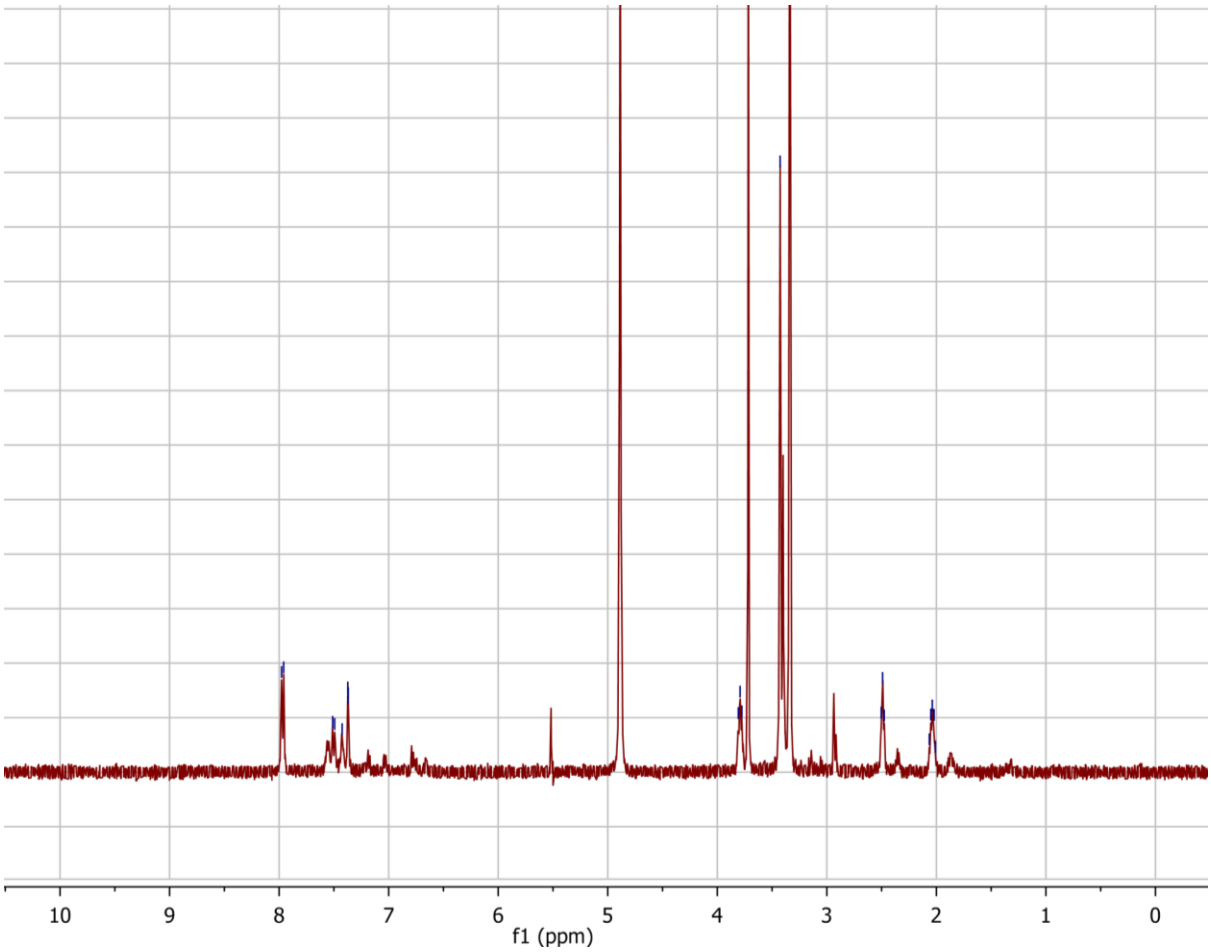
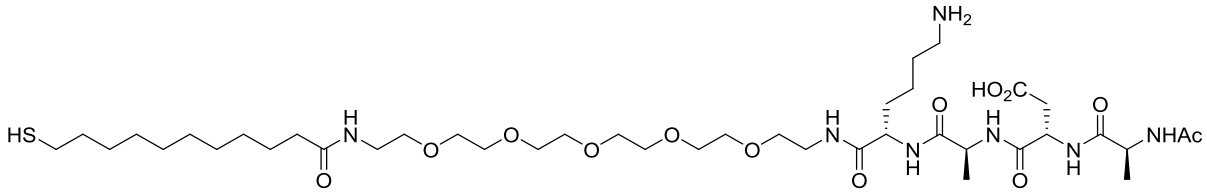


Figure S1.9: ^{1}HNMR spectrum (500 MHz, CD_3OD) of **16**.

N-18-(Lys-Ala-Asp-Ala-NAc)-3,6,9,11,15-pentaoxaheptadecyl-11-mercaptounadecamide (17a)



Resin-bound amine **14** (0.12 mmol) was swelled in DMF. A solution of N-Fmoc-Lys-N'-Boc-OH (168 mg, 0.36 mmol), DIC (55 µL, 0.36 mmol), HOBt (49 mg, 0.36 mmol) and collidine (48 µL, 0.36 mmol) was dissolved in DMF (1 mL) and then added to the resin and the reaction mixture stirred overnight. N-terminal Fmoc deprotection was completed by washes with 20% piperidine in DMF (1.5 mL; 3 × 2 min), after which the resin was waqshed with DMF (1.5 mL; 3 x 2 min). The peptide coupling step was achieved by completely dissolving the requisite protected amino acid (0.6 mmol) and HCTU (248 mg, 0.6 mmol) in DMF (~1.0 mL), then adding DIPEA (222 µL,1.2 mmol) and pre-incubating for five minutes before adding this mixture to the resin. The coupling reaction was allowed to proceed for 1 hour, then ceased by draining and washing with DMF (3 × 1.5 mL, 2 min). The deprotection and coupling steps were repeated until the desired peptide with an unprotected N-terminus was obtained, which was then capped (as the NH-Ac) by incubation with acetic anhydride (60 µL, 0.6 mmol) and DIPEA (222 µL, 1.2 mmol) in DMF (1.0 mL) for 90 min. The solution was drained, then washed with DMF (3 × 1.5 mL, 2 min), DCM (3 × 1.5 mL, 2 min).

The resin-bound peptide was washed with ether (1 × 1.5 mL) and the resin dried under high vacuum for two hours. Then, cleavage cocktail D (1.5 mL) was added, the reaction mixture allowed to stir for 90 min, and the wash collected. Two further washes (1.5 mL, 5 min) were performed to ensure complete cleavage from the resin (indicated by the beads remaining yellow when treated with cleavage cocktail D). The combined washes were concentrated *in vacuo* and subjected to reverse phase chromatography, starting at 100% H_2O (+0.1% TFA) and moving to 100% MeOH (+0.1% TFA) in 5% increments, collecting each solvent mixture as a separate 10 mL fraction. Each fraction was analysed by TLC (ninhydrin stain) and the purity of the positive fractions was assessed by LC-MS. All pure fractions containing the title compound were combined, the MeOH removed *in vacuo* and the remaining water removed by lyophilisation to yield peptide **17a** as a colourless oil (28 mg, 26%). **^{1}H NMR:** (500 MHz, CD_3OD, 298K) δ 4.66 (t (ap), ^3J = 6.6 Hz, 1H, Asp αCH), 4.33 (dd, ^3J = 9.5, 4.8 Hz, 1H, Lys αCH), 4.26 (q, ^3J = 7.0 Hz, 1H, Ala α), 4.20 (q, ^3J = 7.0 Hz, 1H, Ala α), 3.68–3.60 (m, 18H, OEG), 3.58–3.51 (m, 4H, OEG), 3.40–3.29 (2H, m, OEG), 2.98–2.88 (m, 3H, Lys εCH_2 + Asp βCH'), 2.84–2.77 (m, 1H, Asp βCH'), 2.49 (t, ^3J = 7.1 Hz, 2H, AT-C$\underline{H_2}$CONH), 2.20 (t, ^3J = 7.5 Hz, 2H, HS-C$\underline{H_2}$-), 2.01 (s, 3H, NCOC$\underline{H_3}$), 1.94–1.85 (m, 1H, Lys βCH), 1.85–1.80 (m, 1H,), 1.80–1.72 (m, 1H, Lys βCH'), 1.71–1.64 (m, 2H, Lys- δ CH_2), 1.65–1.54 (m, 4H, HSCH_2-C$\underline{H_2}$- and -C$\underline{H_2}$-CH_2CONH), 1.51–1.23 (m, 20H {includes 1.41 (d, ^3J = 7.0 Hz, 3H), 1.36 (d, ^3J = 7.0 Hz, 3H)}, Lys γCH_2, and alkane thiol); **^{13}C NMR:** (126 MHz, MeOD) δ 176.26 (CO_2H), 175.50 (C=O), 174.69 (C=O), 174.12 (C=O), 173.83 (C=O), 173.79 (C=O), 173.06 (C=O), 71.42 (OEG), 71.38 (OEG), 71.37 (2C, OEG), 71.35 (OEG), 71.32 (OEG), 71.10 (OEG), 71.00 (OEG), 70.49 (OEG), 70.15 (OEG), 51.58 (Lys αC), 51.49 (Ala αC), 51.33 (Ala αC), 40.47 (AT-CONH-C$\underline{H_2}$-OEG), 40.21(Lys εC), 40.16 (Asp αC), 36.92 (HS-C$\underline{H_2}$-), 35.94 (Asp βC) , 35.03 (HSCH_2-C$\underline{H_2}$), 31.93 (alkyl), 30.42 (alkyl), 30.38 (alkyl), 30.24 (alkyl), 30.10 (alkyl), 30.01 (alkyl), 29.22 (Lys γC), 27.65 (Lys- δC), 26.86 (-C$\underline{H_2}$-CH_2CONH), 24.79 (C$\underline{H_2}$CONH), 23.41 (Lys βC), 22.30 (NCOC$\underline{H_3}$), 17.14 (2C Ala βC). One oligoethyleneglycol carbon not observed; **ESI-MS:** Calcd. for $C_{41}H_{78}N_7O_{13}S$: *m/z* 908.5372 $[M+H]^+$; found 908.5380 $[M+H]^+$; Calcd. for $C_{41}H_{79}N_7O_{13}S$: *m/z* 454.7723 $[M+2H]^{2+}$; found 454.7731.

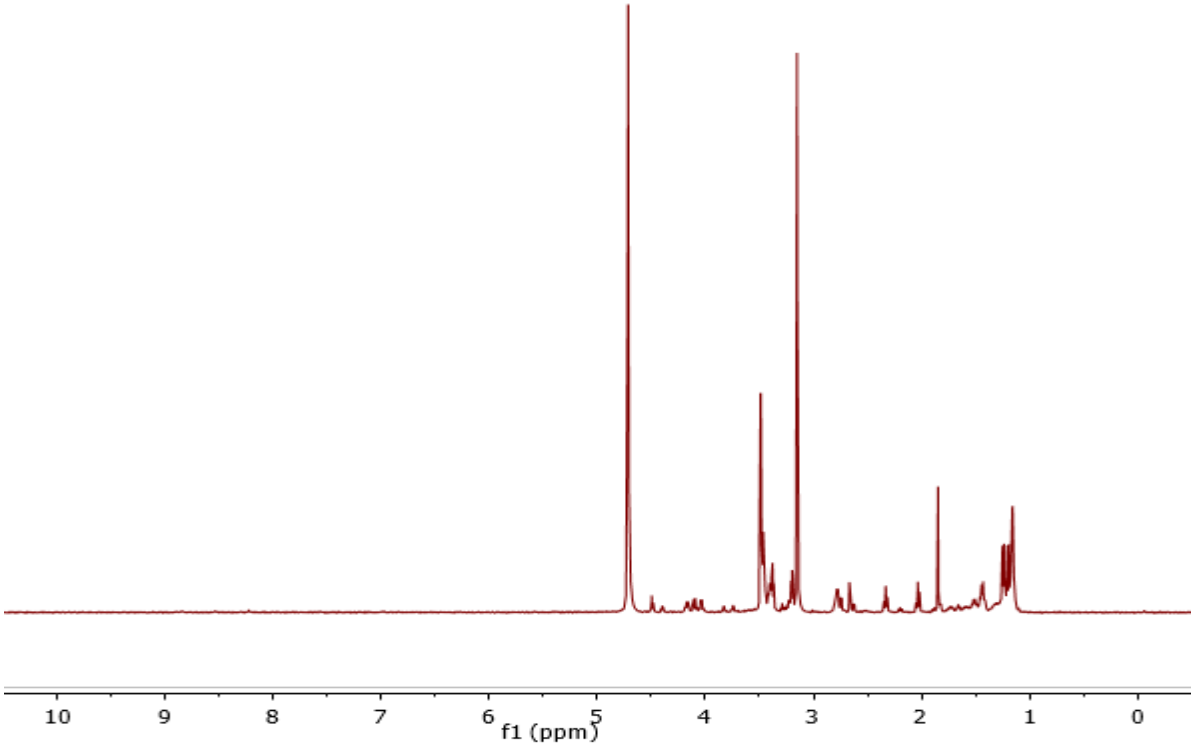


Figure S1.10: ^{1}HNMR spectrum (500 MHz, CD_3OD) of **17a**.

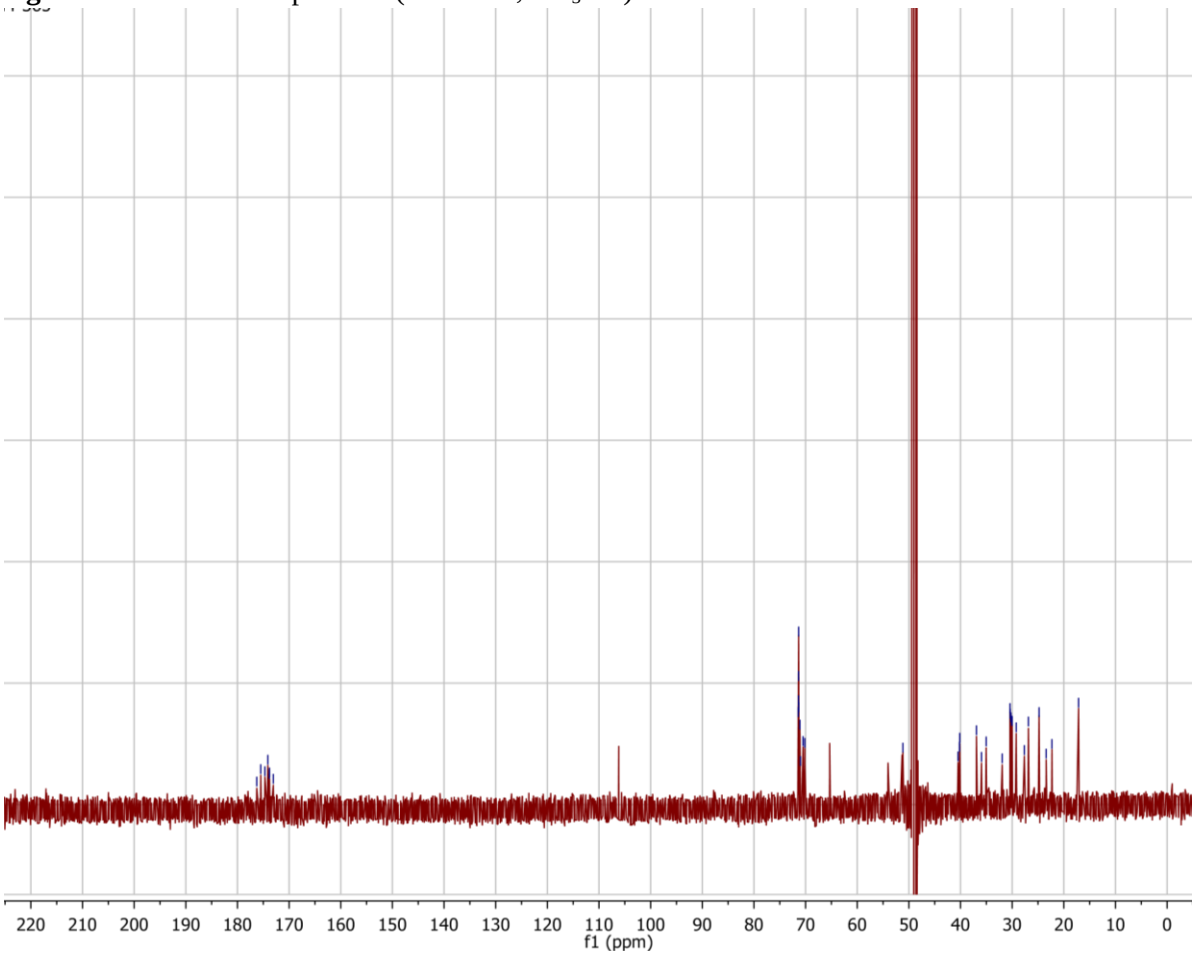
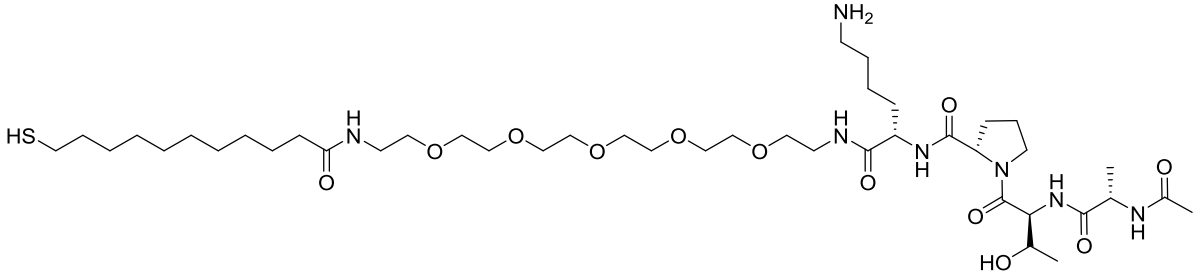


Figure S1.11: ^{13}CNMR spectrum (125 MHz, $CDCl_3$) of **17a**.

N-18-(Lys-Pro-Thr-Ala-NAc)-3,6,9,11,15-pentaoxaheptadecyl-11-mercaptounadecamide (17b)



Prepared analogously to **17a**[†]; isolated as a colourless glassy solid (37 mg, 34%). **^{1}H NMR:** (500 MHz, CD_3OD, 298K) δ 4.62 (d, ^{3}J = 4.5 Hz, 1H, Thr αCH), 4.47–4.40 (m, 1H, Pro αCH), 4.40–4.32 (m, 2H, Ala α, and Lys αCH), 4.23–4.09 (m, 1H,Thr βCH), 3.93–3.87 (t, 2H, ^{3}J = 4.5 Hz, OEG), 3.85 (dq (ap), ^{2}J = 10.3, ^{3}J = 6.3 Hz, 1H, Pro δCH'), 3.73 (dt (ap), ^{3}J = 10.0, ^{2}J = 6.8 Hz, 1H, Pro δCH), 3.68–3.60 (m, 18H, OEG), 3.58–3.52 (m, 2H, -C$\underline{H_2}$-NHCO-Lys, and Pro γCH'), 3.46–3.39 (m, 1H, Pro γCH), 3.35 (t, ^{3}J = 5.6 Hz, 2H, AT-CONH-C$\underline{H_2}$-OEG), 2.95 (t, ^{3}J = 6.7 Hz, 2H, Lys εCH_2), 2.49 (t, ^{3}J = 7.2 Hz, 2H, -C$\underline{H_2}$CONH), 2.26 (ddd, ^{2}J = 12.1, ^{3}J = 8.4, 6.1 Hz, 1H, Pro -βCH'), 2.19 (t, ^{3}J = 7.2 Hz, 2H, HS-C$\underline{H_2}$-), 2.08 (ddd ^{2}J = 12.1, ^{3}J = 6.4, 5.7 Hz, 1H, Pro -βCH), 1.99 (s, 3H, NAc), 1.93–1.77 (m, 2H, Pro, γCH and γCH'), 1.76–1.61 (m, 2H, Lys- δ CH_2), 1.63–1.54 (m, 4H, HSCH_2-C$\underline{H_2}$- and -C$\underline{H_2}$-CH_2CONH), 1.54–1.45 (m, 2H, Lys βCH and βCH'), 1.44–1.36 (m, 2H, Lys γCH_2), 1.36–1.27 (m, 16H, alkane thiol, Ala βCH_3), 1.24 (d, ^{3}J = 6.4 Hz, 3H, Thr-CH_3); **^{13}C NMR:** (125 MHz, CD_3OD, 298K) δ 176.44 (C=O), 175.14 (C=O), 174.32 (C=O), 173.91 (C=O), 173.38 (C=O), 171.42 (C=O), 71.58 (OEG), 71.54 (OEG), 71.53 (OEG), 71.51 (2C, OEG), 71.48 (OEG), 71.27 (OEG), 71.13 (OEG), 70.66 (OEG), 70.37 (-$\underline{C}H_2$-NHCO-Pep), 68.12 (Thr βC), 61.93 (Pro αC), 57.75 (Thr -αC), 54.02 (Lys -αC), 50.39 (Ala -αC), 49.00 (Pro δC, under solvent peak), 40.69 (AT-CONH-$\underline{C}H_2$-OEG), 40.32(Lys εC), 37.09 (HS-C$\underline{H_2}$-), 35.19 (HSCH_2-$\underline{C}H_2$), 32.51 (alkyl), 30.59 (2C, alkyl), 30.54 (Pro βC), 30.41(alkyl), 30.27 (alkyl), 30.18 (alkyl), 29.38 (Lys γC), 27.69 (Lys- δC), 27.03 (-C$\underline{H_2}$-CH_2CONH), 26.15 (Pro γC), 24.96 (C$\underline{H_2}$CONH), 23.49 (Lys βC), 22.39 (NCO$\underline{C}H_3$), 20.01(Thr γC), 17.46 (Ala βC). One oligoethyleneglycol carbon not observed; **ESI-MS:** Calcd. for $C_{43}H_{82}N_7O_{12}S$: *m/z* 920.5742 $[M+H]^+$; found 920.5751; **ESI-LC-MS:** Calcd. for $C_{43}H_{81}N_7NaO_{12}S$: *m/z* 920.6 $[M+H]^+$; found 920.5, R_t = 1.55 min.

[†] In our hands N-Fmoc-Thr-O-Trt-OH was much easier to deprotect than N-Fmoc-Thr-O-^{t}Bu-OH.

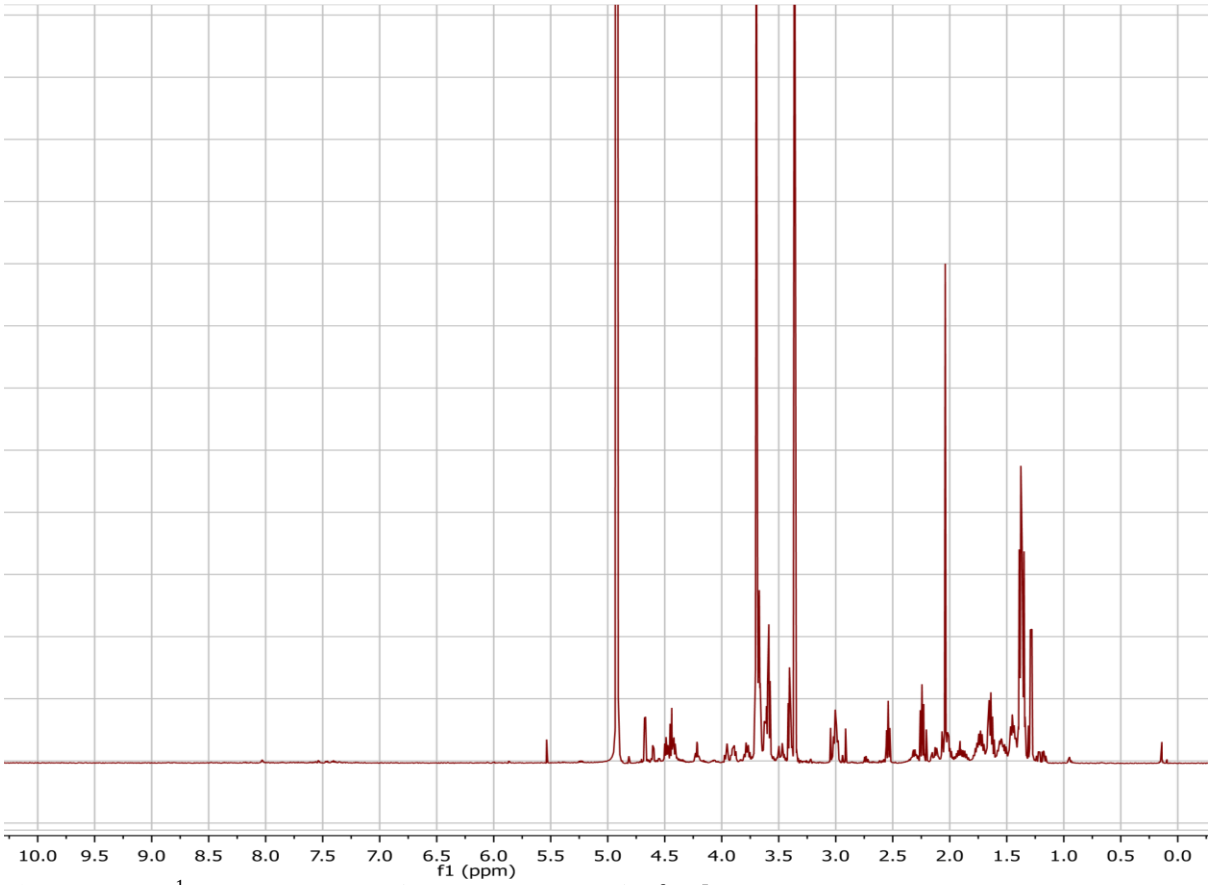


Figure S1.12: ^{1}HNMR spectrum (500 MHz, CD_3OD) of **17b**.

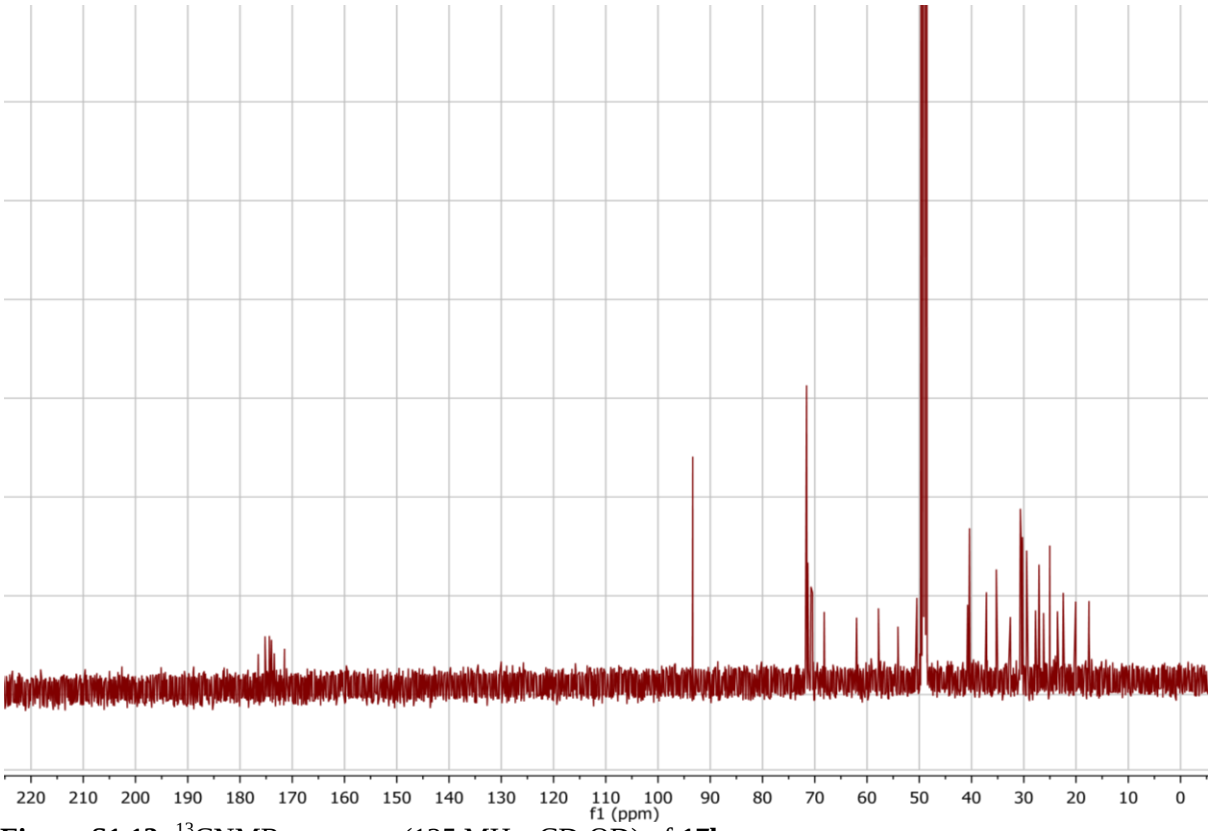
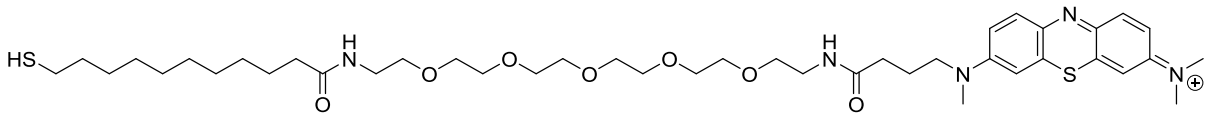


Figure S1.13: ^{13}CNMR spectrum (125 MHz, CD_3OD) of **17b**.

N-18-(N'-(carboxypropyl) methylene blue)-3,6,9,11,15-pentaoxaheptadecyl-11-mercaptounadecamide (18)



Resin-bound amine **14** (0.18 mmol) was swelled in DMF. A solution of **16** (128 mg, 0.36 mmol), DIC (56 µL, 0.36 mmol), HOBt (49 mg, 0.36 mmol) and collidine (54µL, 0.36 mmol) was dissolved in DMF and then added to the resin. The reaction mixture was protected from light (by wrapping the SPE tube inaluminium foil) and stirred overnight. The solution was drained, then washed with DMF (3 × 1.5 mL), DCM (3 × 1.5 mL).

Resin-bound methylene blue derivative **18** was washed diethyl ether (1 × 1.5 mL), dried under high vacuum for 2 hours and then treated with washes of cleavage cocktail B (1.5 mL, 3 × 2 min). The combined washes were concentrated to approx. 0.5 mL and precipitated into cold diethyl ether (25 mL; −20 °C) and left in a freezer overnight. The precipitate was collected by centrifugation (2 min, 40,000 rpm) and subjected to reverse phase chromatography, starting at 100% H_2O (+0.1% TFA) and moving to 100% MeOH (+0.1% TFA) in 5% increments, collecting each solvent mixture as a separate 10 mL fraction. Each fraction was analysed by TLC and the purity of the most intensely blue spots were assessed by LC-MS. All pure fractions containing the target compound were combined and the solvent removed *in vacuo* to yield methylene blue derivative **18** as a dark blue solid (75 mg, 45 %). **^{1}H NMR:** (500 MHz, CD_3OD, 298K) δ 8.01 (dd, ^3J = 9.7 Hz, ^4J = 1.0 Hz, 2H, Ar), 7.49–7.43 (m, 2H, Ar), 7.47–7.41 (m, 2H, Ar), 3.80 (t, ^3J = 7.6 Hz, 2H, ArNMe-C$\underline{H_2}$-), 3.70–3.62 (m, 18H, OEG), 3.58 (m, 4H, OEG), 3.45 (s, 6H, ArN-$\underline{Me_2}$), 3.44–3.43 (m, 2H, OEG), 3.42 (s, 3H, ArN-$\underline{Me}$), 2.70 (t, ^3J = 7.3 Hz, 2H, HNCO-C$\underline{H_2}$-C_2H_6-MB), 2.41 (t, ^3J = 6.9 Hz, 2H, C$\underline{H_2}$CONH), 2.22 (t, ^3J = 7.5 Hz, 2H, HS-C$\underline{H_2}$), 2.09 (p (ap), ^3J = 7.4 Hz, 2H, MB-alkyl), 1.77–1.57 (m, 4H, $HSCH_2$-C$\underline{H_2}$- and -C$\underline{H_2}$-CH_2CONH,), 1.51–1.24 (m, 13H, alkyl and SH); **ESI-LC-MS:** Calcd. for $C_{42}H_{68}N_5O_7S_2$; *m/z* 818.4 $[M]^+$; found 818.0; R_t = 1.47 min.

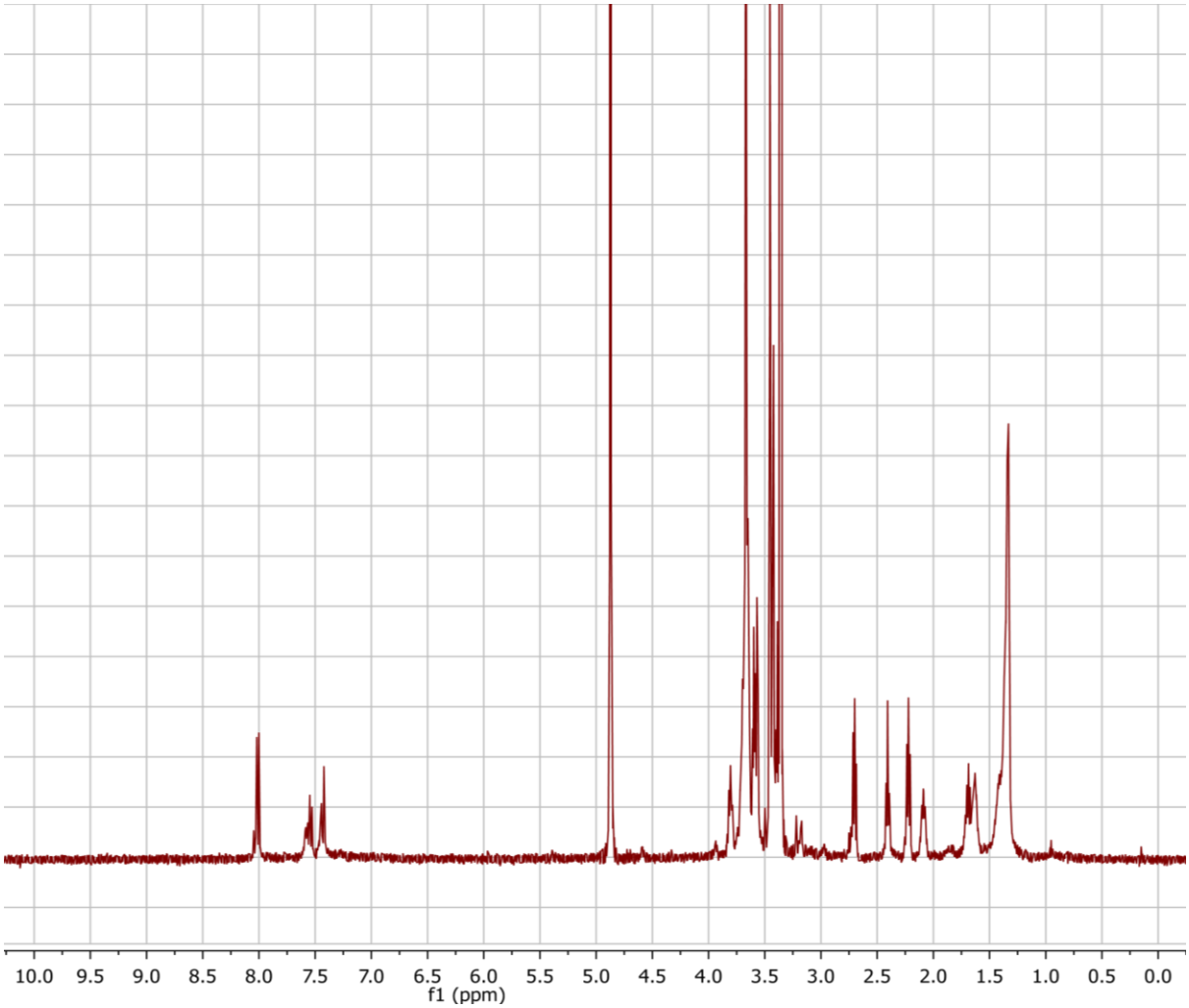
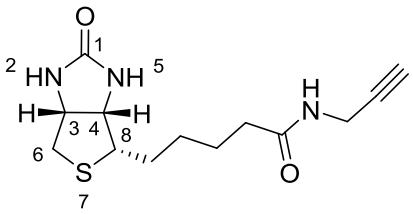


Figure S1.10: ^{1}HNMR spectrum (500 MHz, CD_3OD) of **18**

Propargyl Biotin[5]

Biotin (200 mg, 0.8 mmol), N-hydroxysuccinimde (102 mg, 0.88 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (184 mg, 0.88 mmol) were dissolved in DMF (10 mL) and the reaction mixture stirred for 18 hours at room temperature. The DMF was removed by co-evaporation with toluene and the resultant white solid washed with cold MeOH to afford the succinimide ester (161 mg, 60%), which was used without further purification. Biotin succinimide (150 mg, 0.45 mmol) and propargylamine hydrochloride (62 mg, 0.68 mmol) were dissolved in DMF (5 mL) at 0 °C. Triethylamine (194 µL, 1.35 mmol) was added and mixture allowed to warm to room temperature overnight. The DMF was removed by co-evaporation with toluene to afford the crude material as an off-white solid, which was then purified by column chromatography (SiO_2; $CHCl_3$–MeOH, 6:1) to afford propargyl biotin (86 mg, 68%) as an off-white solid.



R_f = 0.2 ($CHCl_3$ – MeOH, 6:1); **^{1}H NMR:** (500 MHz, CD_3OD, 298K) δ 4.49 (dd, ^3J = 7.9, 4.8 Hz, 1H, C^3-H), 4.31 (dd, ^3J = 7.9, 4.4 Hz, 1H, C^4-H), 3.94 (d, ^3J = 2.6 Hz, 2H, CONH-CH_2CCH), 3.28–3.31 (m, 1H, C^8H), 2.93 (dd, ^2J = 12.7, ^3J = 5.0 Hz, 1H, C^6-H_{eq}), 2.70 (d, ^2J = 12.8 Hz, 1H, C^6-H_{ax}), 2.57 (t, ^3J = 2.5 Hz, 1H, CH_2CC-H), 2.21 (t, ^3J = 7.4 Hz, 2H, CH_2-CONH), 1.80–1.51 (m, 4H, alkyl), 1.44 (p (ap), ^3J = 7.7, 2H, alkyl); **^{13}C NMR:** (125 MHz, CD_3OD, 298K) δ 175.70 (C=O), 175.35 (C=O), 80.82 (-CH2-CCH), 72.16 (-CCH), 63.43(C^4), 61.71 (C^3), 57.03 (C^8), 41.15 (C^6), 36.58 (-CH_2-CONH), 29.78 (alkyl), 29.46 (alkyl), 26.78 (alkyl), 26.39 (alkyl); **ESI-MS:** Calcd. for $C_{13}H_{19}N_3NaO_2S$: *m/z* 304.1096 $[M+Na]^+$; found 304.1091.

2. X-Ray Photoelectron Spectroscopy

Piranha-cleaned glass slides were thermally coated with 5 nm chromium adhesion layer and 150 nm of gold (at 0.1 nm s^{-1} rate and base pressure of $\sim 1 \times 10^{-6}$ mbar) using Edwards Auto 306 thermal evaporator. SAMs were formed by immersing gold substrates in a pure or mixed 0.2–1 mM ethanolic solution of **11a**, **11b** and **13** for 18–20 hours at room temperature. The samples were removed from solution, rinsed with copious amounts of ethanol, dried with a nitrogen stream, rinsed with Milli-Q water, and dried again. XPS high-resolution spectra obtained on Thermo Electron Corporation ESCA Lab 250 at 20 eV pass energy with 0.2 eV resolution were processed with CasaXPS software. For quantitative analysis, peaks were normalised to alkyl carbon, which binding energy was referenced to 284.9 eV. Three to five areas were analysed on at least two samples of each SAMs type. For all SAMs, we found S $2p_{3/2}$ and S $2p_{1/2}$ respectively to have peak area ratio 2:1 and binding energies of ~161.8 eV and ~163.0 eV, which are consistent with thiolates on gold (Figure S2.1A).[6] Three peaks present in C 1s region are assigned to alkyl chain (284.9 eV), the OEG chain (286.9 eV), and carbonyl (288.3 eV) (Figure 2A in the main text). Peaks in the O 1s region are attributed to carbonyl (531.8eV) and OEG chain (533.4 eV) (Figure S2.1C). The nitrogen 1s peak at 400.1 eV present in all SAMs corresponds to the amide group, while an additional two peaks in SAMs of **13** are consistent with that expected for the azide moiety (Figure S2.1B). The peak at 401.7 eV is consistent with the expected binding energy of the lateral nitrogen atoms of the azide group. The peak at 405.2 eV is at the expected position for the central electron-deficient nitrogen and is 2.1 ± 0.3 times smaller in area than the peak at 401.7 eV. These are consistent with previous XPS reports on azide groups[7,8]. Rapid degradation of the azide group (up to 60% degradation under standard XPS conditions) was reduced to just 20% by optimising acquisition parameters i.e. minimising scan time.

Ratios of chemical elements in reference SAMs of **11a**, **11b** and **13** are consistent with molecular structures and were used to determine actual proportions of the compounds in mixed SAMs. Data analysis of SAMs, formed in 1:1 and 1:4 mixtures of **13:11a** respectively, show a 7–18% deviation from solution concentration, while mixed SAMs with **11b** instead of **11a** give up to 10% deviation. These values are within the experimental error. However, the N 1s region was used to determine mixing of **11b** and **13** due to very similar molecular structures, while calculations of **11a** and **13** mixing also included carbon and oxygen atoms in OEG chains.

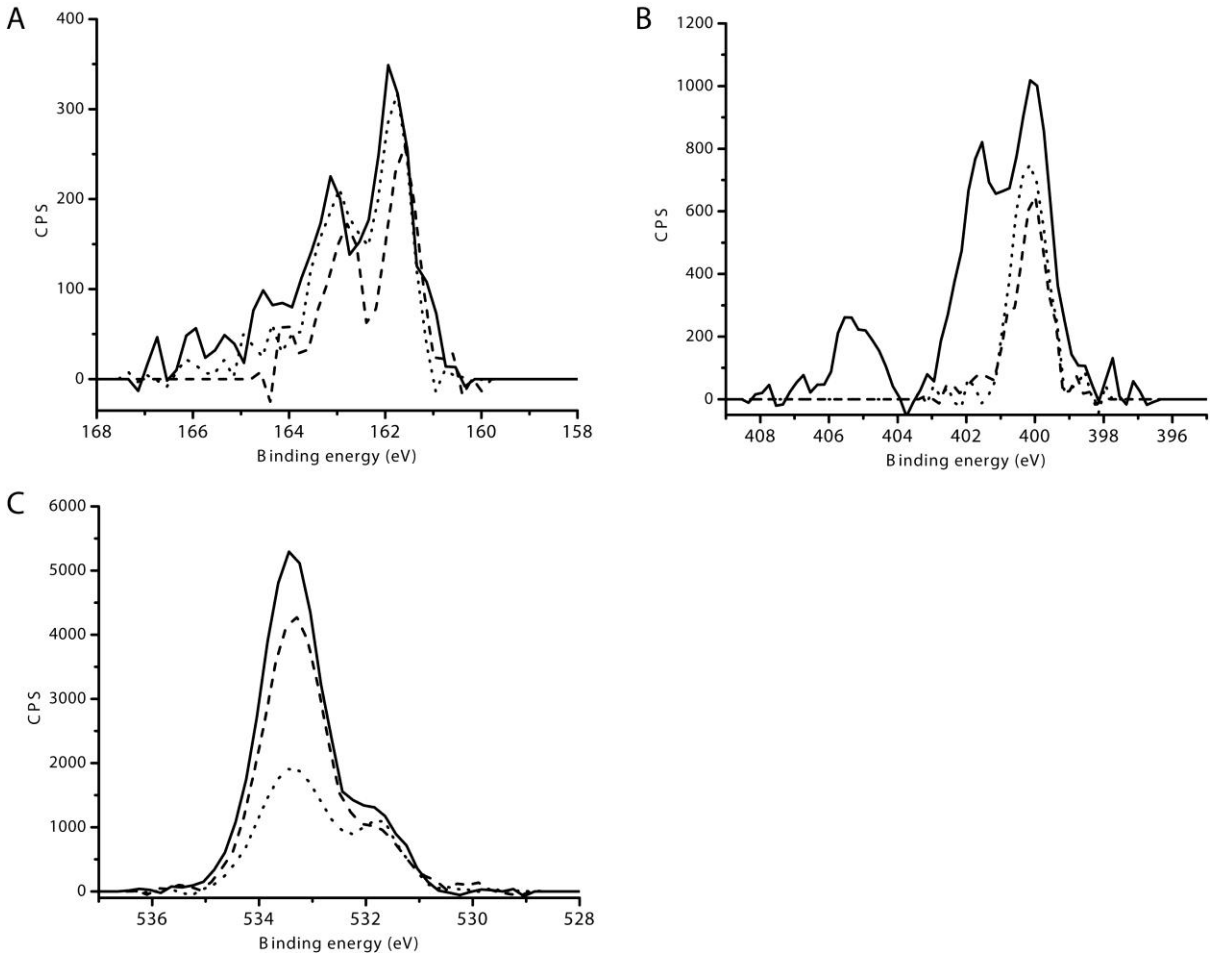


Figure S2.1 XPS spectra of S 2p (A), N 1s (B) and O 1s (C) of reference SAMs **11a** (dotted), **11b** (dashed) and **13** (solid) are shown above. All spectra were referenced to alkyl carbon at 284.9 eV and baseline corrected. Graphs were not normalised to account for different beam spot size.

3. Electrochemical Impedance Spectroscopy

EIS measurements were performed using the BioLogic potentiostat with EC-lab software. A three electrode electrochemical cell was employed with a SAM modified electrode as a working electrode, a platinum counter electrode and a Ag/AgCl reference electrode.

The insulating properties of mixed SAMs (diluent/amine and diluent/peptide terminated) in different ratios 1:1, 1:9 and 9:1 immobilised on a clean gold substrates were characterised by EIS-Bode plot (phase angle vs. frequency). The measurements were carried out over a frequency range of 0.01 Hz to 100 kHz at applied DC potential of 0 V and a modulation voltage of 10 mV. Phase angle values were analysed at 0.1 Hz. All measurements were done in 100 mM phosphate buffer (PB) at pH 7.1.

The minimum phase angles of –88˚ to –83˚ for amine-containing mixed SAMs (**11a**:**15**, Figure S3.1) and of –88˚ to –87˚ for peptide-containing mixed SAMs (**11a**:**17**, Figure S3.2), measured at 0.1 Hz, correspond to the formation of well-packed and insulating monolayers, which are almost devoid of pinholes and collapsed sites effects. The SAMs show very high stability. The three readings of the phase angle for each ratio were taken within 1 hour with 20 min intervals. The phase angle shifts were less than 0.5 degrees.

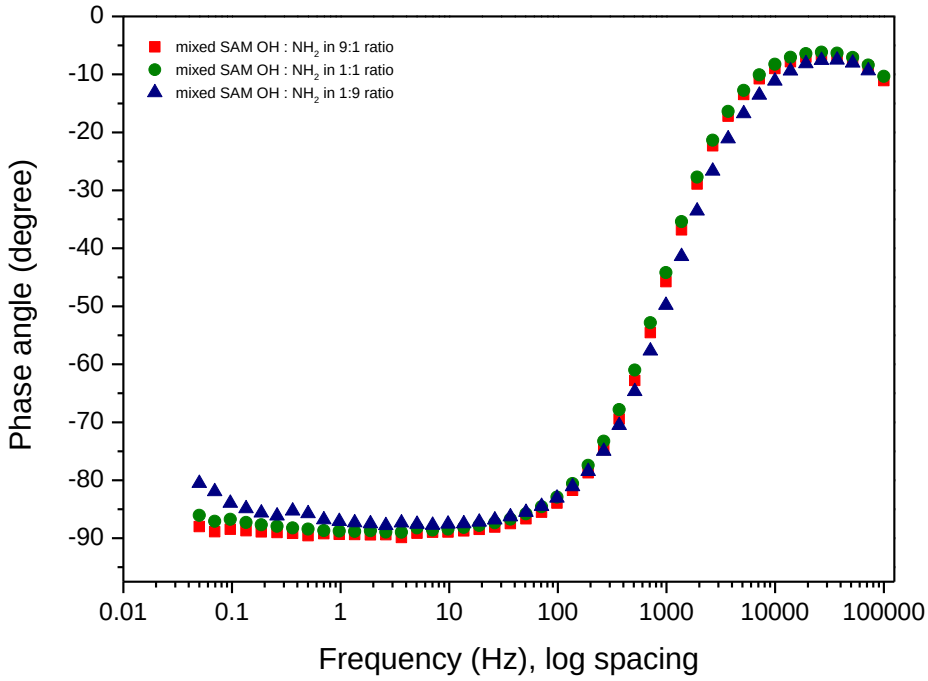


Figure S3.1: Electrochemical impedance spectra of gold electrodes modified with 0.5 mM mixed SAM (OH/NH_2-terminated, **11a:15**) in different ratios. Solid squares – 9: 1 ratio, solid circles – 1: 1 ratio and solid triangles – 1: 9 ratio. EIS performed in 100 mM PB at pH 7.1.

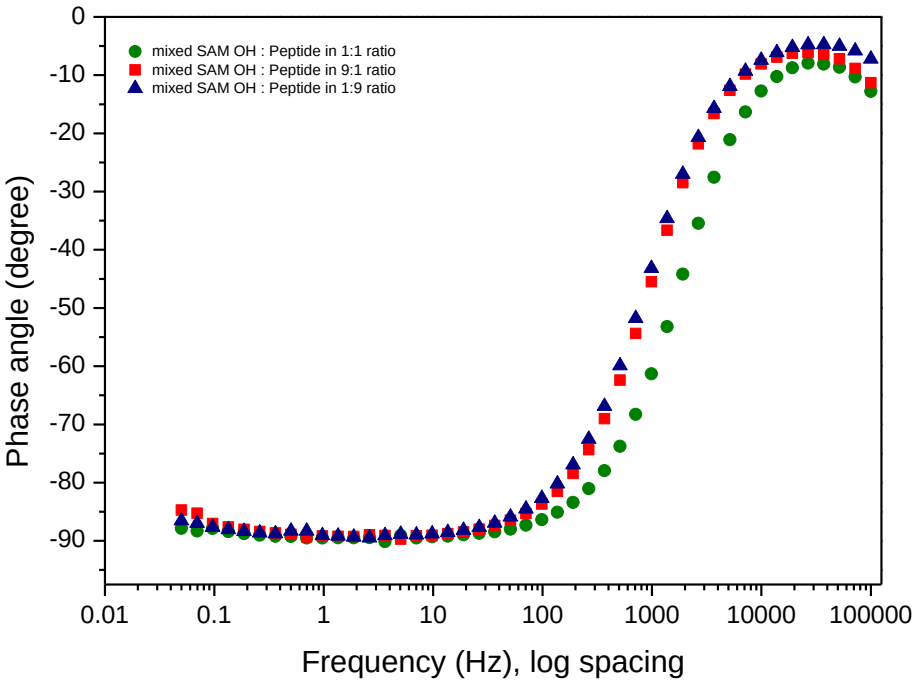


Figure S3.2 Electrochemical impedance spectra of gold electrodes modified with 0.5 mM mixed SAM (OH/peptide-terminated, **11a**:**17a**) in different ratios. Solid squares – 9: 1 ratio, solid circles – 1: 1 ratio and solid triangles – 1: 9 ratio. EIS performed in 100 mM PB at pH 7.1

4. Contact Angle Measurements

Advancing and receding Milli-Q water droplet contact angles were measured using a First-Ten-Ångstrom 2000 goniometer. Pure SAMs of **11a** and **11b** diluents were formed in 1mM, while SAMs of **13** were formed in 0.2mM ethanolic solutions. Such solutions were mixed to give 1:1 and 1:4 molar ratios of **13** and diluents respectively. More detailed sample preparation described in the XPS section. Four to five measurements on two samples of each type were analysed using the FTA32 video software. The water contact angles for pure and mixed SAMs of **11a, 11b** and **13** formed on gold are summarised in Table S4.1. All SAMs formed in this study produced hydrophilic surfaces consistent with the presence of the OEG moieties. SAMs of **13** produced the highest contact angles (both advancing and receding) and this most likely reflects a reduced ability of the water molecules to hydrogen bond with azides. Azide-terminated alkanethiol SAMs reportedly have a sessile drop contact angle of 77°.[7] In this study, we observed both advancing and receding contact angles to be much lower, but this is most likely due the effect of having an OEG chain below the azide tail group as opposed to an alkane chain. In the case of mixed SAMs of **11a** (or **11b**) and **13**, a linear change in the cosine of the contact angle with the molar ratio of **11a**:**13 (**and **11b**:**13)** was measured. This linear behaviour indicates similar molar ratio of **13** and diluents on surface as in solution.

Molar fraction (%) of 13	Diluent 11a		Diluent 11b	
	Advancing angle, θa (˚)	Receding angle, θr (˚)	Advancing angle, θa (˚)	Receding angle, θr (˚)
0	38.5 ± 0.9	28.0 ± 0.5	37.6 ± 0.3	29.8 ± 0.9
	36.6 ± 1.1	22.1 ± 1.7	40.6 ± 1.0	31.0 ± 0.8
19	41.7 ± 1.1	21.2 ± 2.2	44.3 ± 0.5	30.5 ± 1.1
	44.9 ± 1.2	21.2 ± 0.9	46.8 ± 0.9	30.7 ± 1.0
50	50.2 ± 1.0	39.8 ± 2.3	50.2 ± 1.3	38.5 ± 0.3
	49.6 ± 2.2	39.6 ± 1.0	50.0 ± 1.5	39.0 ± 0.5
100	59.9 ± 0.7	47.4 ± 0.7	59.9 ± 0.7	47.4 ± 0.7
	55.8 ± 2.2	44.5 ± 1.7	55.8 ± 2.2	44.5 ± 1.7

Table S4.1. Advancing and receding angles of pure and mixed SAMs of **11a**, **11b** and **13**. Errors refer to standard deviation of 4–5 measurements per sample.

5. Atomic Force Microscopy

AFM measurements were carried out on self-prepared template stripped gold (TSG). Such surfaces are known to be smooth and atomically flat. TSG were prepared by deposition of 150 nm of gold on a polished silicon wafer using an E-Beam evaporator. The clean microscope glass slides were cut into (1 cm^2) pieces and glued to the gold surface with epoxy glue. They were cured for 120 min at 120 °C. After cooling, the slides were mechanically separated from the silicon wafer to expose the TSG surface. AFM measurements were performed using an Veeco Nanoscope IV instrument in tapping mode. The cantilever was made of silicon nitride. The AFM images revealed that the surface of the TSG layer had a mean roughness of 0.2 nm over areas as large as several square micrometers (Figure S5.1A). These fresh and smooth TSG were then immediately immersed in 0.5 mM ethanolic solutions of mixed SAMs (**11a**:**17**) – at ratios of 1:9, 1:1 and 9:1 for 24 h to reduce contamination of the exposed surface. After immobilisation the surfaces were rinsed with ethanol and dried with nitrogen. The mixed SAMs formed a very densely packed, flat layer covering the gold surface (Figure S5.1B, C, and D). Macroscopic island formation by different LCAT-OEGs in SAMs was not observed. The 1:1 mixed SAM shows the area of 3 μm^2 with an average roughness of 0.3 nm (Figure S5.1B).

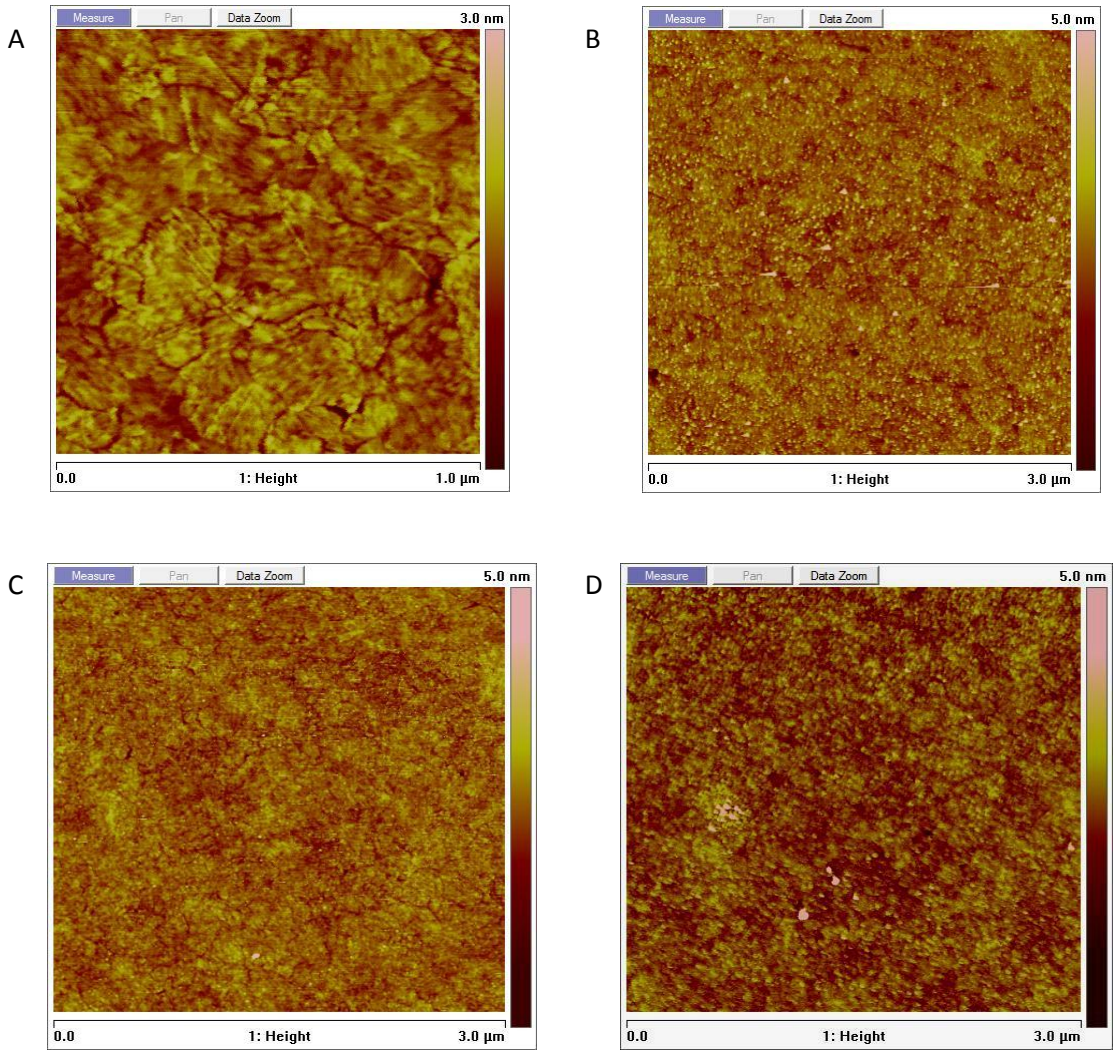


Figure S5.1: The AFM images show the TSG surface modified with mixed peptide/OH terminated SAMs in A) Bare TSG B) 1:1 ratio, C) 1:9 ratio, D) 9:1 ratio.

6. Cyclic Voltammetry

6.1 Cyclic voltammetry of mixed SAMs

To show that our LCAT-OEGs form well-ordered SAMs of high capacitance, we performed CV measurements on SAMs made of compounds **11b** and **15** (Figures S6.1, S6.2 and S6.3). CVs were performed with 2 mM redox probe $[Fe(CN)6]^{3-/4}$ in 100 mM phosphate buffer (pH 7.1) using a conventional three-electrode electrochemical cell. The voltammograms were recorded at potentials in the range –0.2 V to +0.5 V vs Ag/ AgCl reference electrode. The virtually flat CV plots for SAMs formed from three different ratios of **11b** and **15** (black) indicate insulation of the electrodes by the SAMs. Using the electrode surface area of 0.08 cm^2 and the scan rate of 62 mV s^{-1}, we were able to calculate capacitances of $4.4 \cdot 10^{-6}$ F cm^{-1}. This compare well with the capacitance of simple alkanethiol monolayers,[9] suggesting our SAMs pack well, likely driven by intermolecular interactions between the alkane components of LCAT-OEGs. Furthermore, the lack of distinct reduction of oxidation peaks (black CV plots) is consistent with the formation of a well-packed and pin-hole free insulating molecular monolayer. Redox peaks were recovered following desorption of the SAMs from the gold electrode (red CV plots) by electrochemical reduction of the gold-thiol bond (performed by changing the potential range from -1.5 V to –0.5 V).

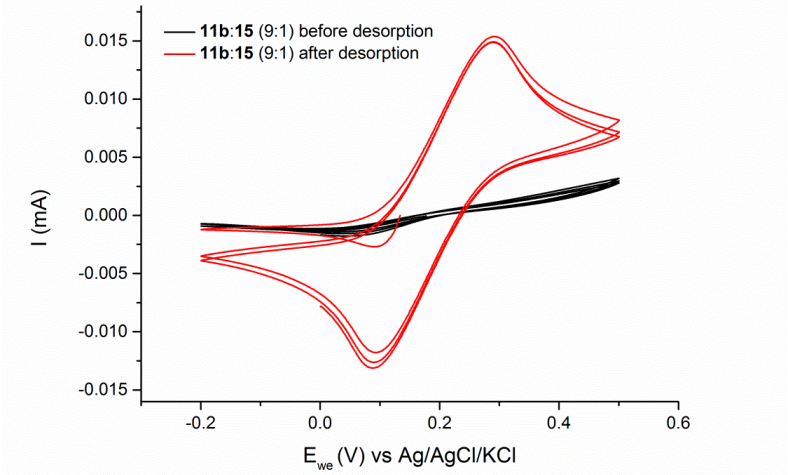


Figure S6.1: CV voltammograms (3 cycles each) of SAMs formed from **11b** and **15** (9:1) (black) and of the electrode after SAM desorption (red).

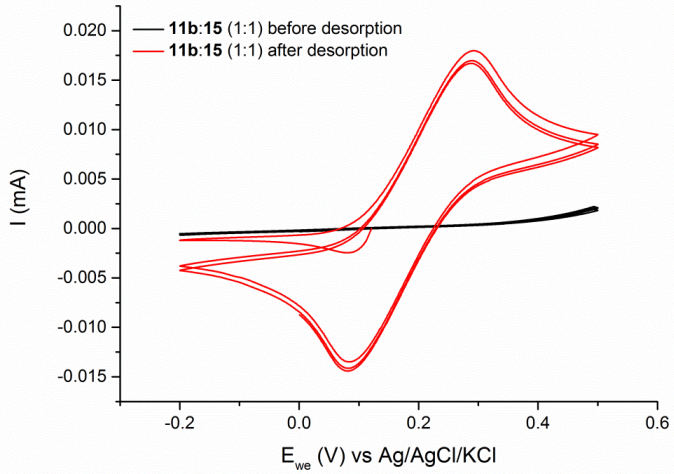


Figure S6.2: CV voltammograms (3 cycles each) of SAMs formed from **11b** and **15** (1:1) (black) and of the electrode after SAM desorption (red).

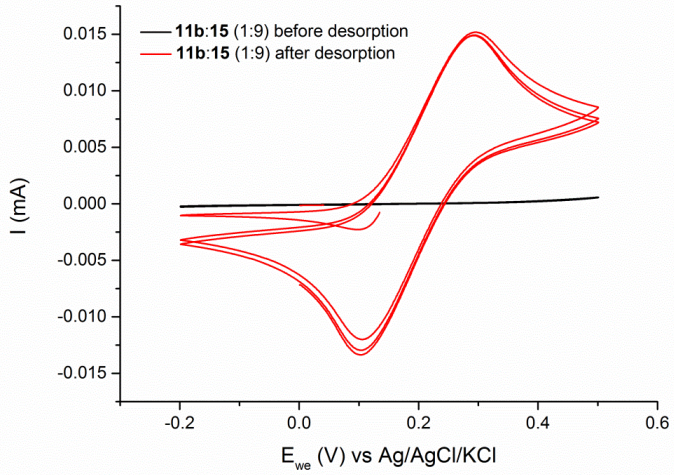


Figure S6.3: CV voltammograms (3 cycles each) of SAMs formed from **11b** and **15** (1:9) (black) and of the electrode after SAM desorption (red).

6.2 Cyclic voltammetry of redox-active methylene blue-containing SAMs

CV measurements were also used to characterise SAMs containing the redox probe methylene blue (MB). Figure 2C of the main paper shows a typical cyclic voltammogram (CV) for a monolayer assembled from MB-functionalised alkanethiol-OEG **18**. CVs were performed in 100 mM phosphate buffer (pH 7) using a standard three-electrode electrochemical cell. Clear oxidation and reduction peaks associated with the methylene blue moiety are observed around -130 mV vs Ag/AgCl.

The FWHM of the oxidation and reduction peaks was found to be 37 mV (at 200 mV/s). While this is lower than the theoretical ideal (45.3 mV for a 2 electron process) we note that deviations in the FWHM are not uncommon in densely packed redox active monolayers due to electrostatic interactions between adjacent charged species.

Using the Laviron method[10] we extracted rates of electron transfer from the linear region of a trumpet plot (Figure S6.4). which gives a $\mathbf{k_{et} = 8\ s^{-1}}$; this value is similar to the k_{et} of a C16 redox-active monolayer, with ferrocene as the redox-active group.[11] However, it should be noted that this method does not work well for densely packed redox active monolayers, and that our monolayers incorporate a flexible OEG spacer.

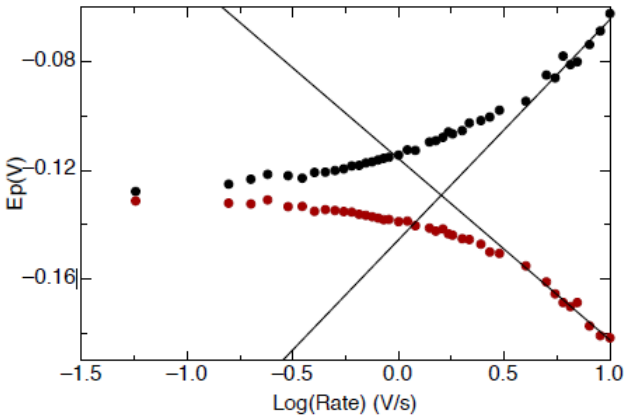


Figure S6.4: Trumpet plot analysis; the electron transfer rate was extracted from the linear region using Laviron's method.

7. Colorimetry

1 cm^2 gold coated square silicon wafers (80 nm gold on 12 nm Ti) were sonicated in acetone for 15 min, then washed with EtOH (3×) and dried under a stream of N_2. The gold substrates were then incubated in $H_2O_2 – H_2SO_4$ (30:70) for 15 min; then washed with dd H_2O (3×) and EtOH (3×). Stock solutions in ethanol of **11a** and **13** (2 mM) were prepared and mixed to give a 1:1 solution of the SAM solution (1 mM, 1 mL). The gold squares were incubated in the SAM solution for 24 hours, in a petri dish back-filled with N_2, sealed with parafilm.. After incubation the gold surface was rinsed with EtOH (3×) then dried under a stream of N_2.

Stock solutions of copper(I) iodide (1.5 mM), triethylamine, and propargyl biotin (20 mM) were prepared in DMSO–H_2O (3:1). Copper(I) iodide required several min sonication for complete dissolution. Two solutions were prepared: 1. Containing triethylamine (150 µM), copper(I) iodide (150 µM), and propargyl biotin (2 mM); and 2. containing triethylamine (150 µM), and propargyl biotin (2 mM). Solutions 1. and 2. were spotted (3 µL) on separate areas of the gold and left for 24 hours in a sealed petri dish, in the dark. The spots were adsorbed carefully onto filter paper, and the residue on the surface washed away with ddH_2O. The surface was blocked with Tween-20 by incubating for one hour in Tris-buffered saline (TBS) (25 mM Tris.HCl, 150 mM NaCl, pH 7.5, with Tween 20 0.5% v/v). The gold was rinsed with TBS (6 × 1 mL); then the gold surface was covered with streptavidin-alkaline phosphatase solution (6 µL in 15 mL of TBS) for 15 min. The gold surface was washed with TBS (6 × 1mL). The entire surface was then incubated with western blue, in the dark. Staining occurred over spot 1. after 30 min. The gold was rinsed with water (3 × 1mL) and dried under N_2. The edge of spot 1 was imaged on optical microscope (Figure S7.1); background staining was also imaged (Figure S7.2).

Colorimetry (optical microscope, Carl Zeiss Axio Scope A1. Objective 10 X, in a clean room)

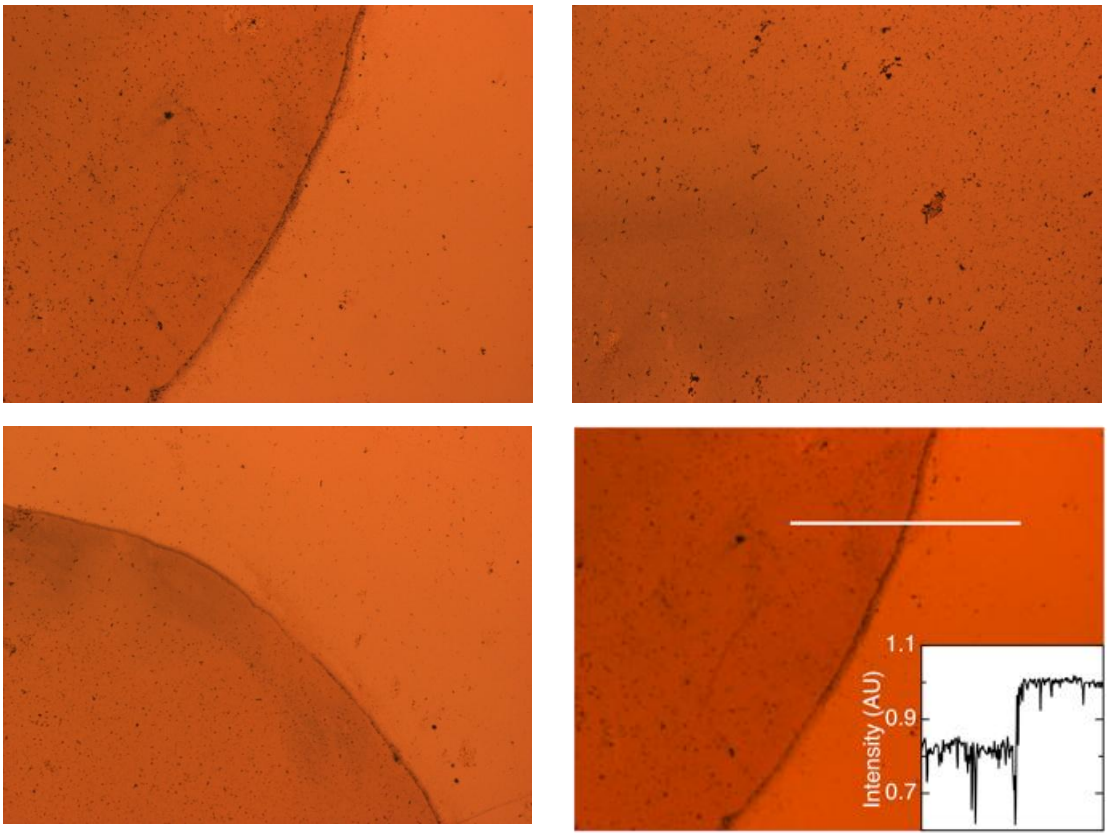


Figure S7.1: Images of colorimetric detection of immobilised biotin taken at three different areas of spot treated with solution 1, and quantification relative to background staining (bottom right).

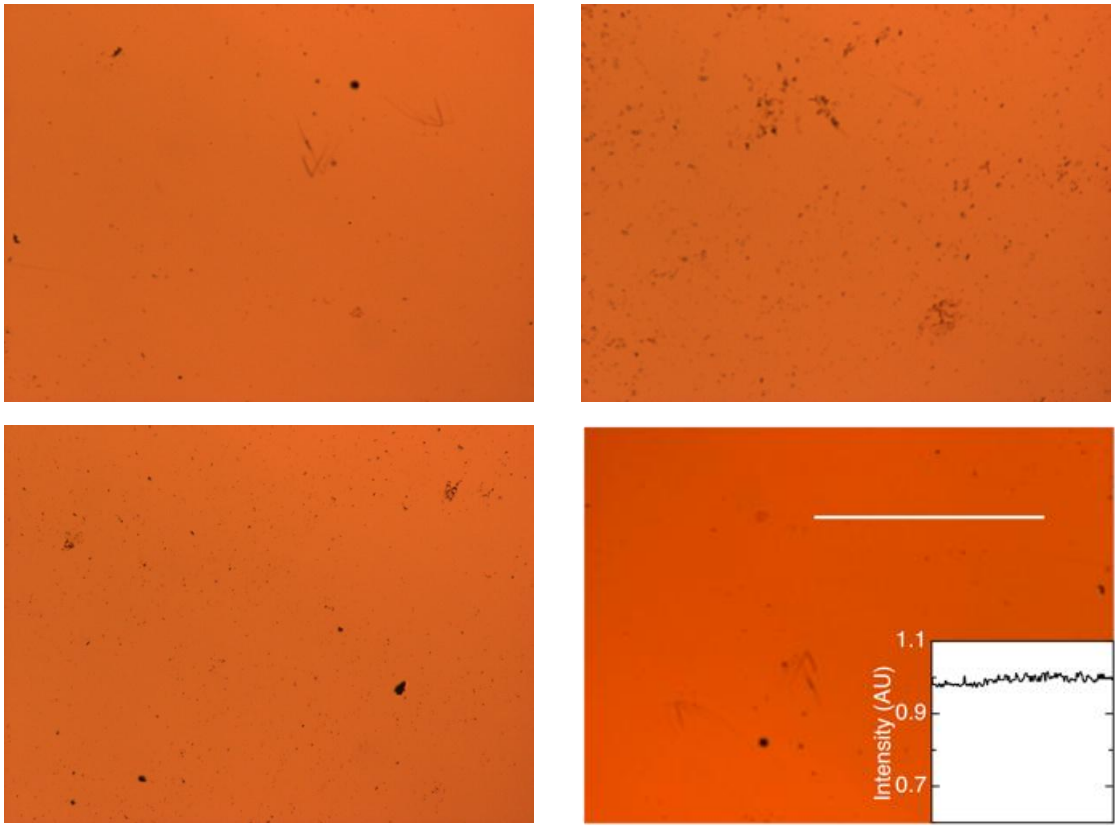
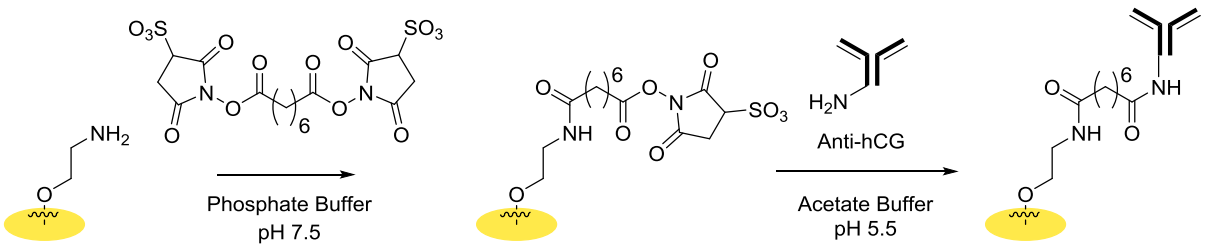


Figure S7.2: Images taken at four different spots treated with solution 2 (the negative control).

8. Surface Plasmon Resonance

SPR experiments were performed on a Biacore 3000 system (GE Healthcare, Sweden) using a bare gold sensor chip (SIA Kit Au, GE Healthcare, Sweden).

First, the gold chip was cleaned using ethanol (absolute), followed by incubation in Piranha solution (sulfuric acid and hydrogen peroxide (70:30)) for 30 min. The gold chip was then washed with copious amount of water and dried under a stream of nitrogen. The gold chip was then immersed into a 1 mM solution of amine **15** and dilutent **11a** (1:1), in ethanol and incubated for 24 hours. The chip was washed with ethanol and dried under a stream of nitrogen. The chip was mounted into the holder as per the manufacturer's instructions. After docking, the surface was equilibrated with 100 mM phosphate buffer at pH 7.5 (PB, filtered and degassed) at a flow rate of 10 µL/min. The surface was activated by treatment with bis-sulfosuccinimidyl suberate (BS3) (10 mM, 100 µL) in PB. The buffer was exchanged for 10 mM acetate buffer pH 5.5 and the system re-primed. Anti-hCG (0.01 µg/mL, 300 µL) was injected in acetate buffer to immobilise the antibody (Scheme S8.1). Any remaining activated sites were blocked with 1 M ethanolamine hydrochloride at pH 8.5, to complete preparation of the sensor surface. As a control a channel was prepared analogously, but was not treated with BS3 to show the difference between covalently and non-covalently attached antibody (Figure S8.1).



Scheme S8.1: Preparation of the sensor surface.

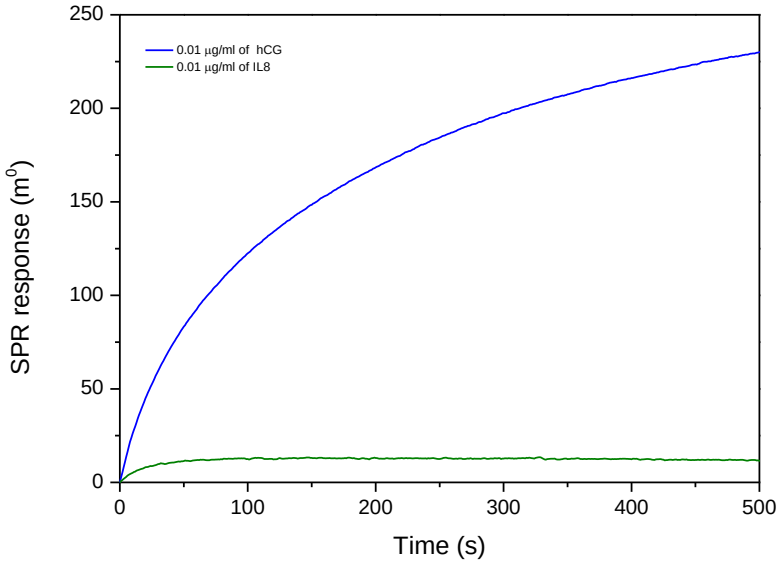


Figure S8.1: Binding studies of hCG (0.01 µg/ mL; blue line) and IL8 (0.01 µg/ mL; green line).

The binding studies were performed in PB (25 mM, 150 mM NaCl, pH 7.5) as a flow rate of 10 µL/min. hCG and IL8 (both 100 µL @ 0.01µg/mL) were injected to observe the binding (Figure 8.1). The sensor surface could be regenerated by pulsed 20 µL injections of NaOH (10 mM), data not shown.

9. References

1. A. Bouzide and G. Sauve, *Org. Lett.*, 2002, **4**, 8781–8783.

2. S. Svedhem, C. a Hollander, J. Shi, P. Konradsson, B. Liedberg, and S. C. Svensson, *J. Org. Chem.*, 2001, **66**, 4494–4503.

3. C. G. Pheeney and J. K. Barton, *Langmuir*, 2012, **28**, 7063–7070.

4. G. W. Dombrowski, J. P. Dinnocenzo, P. A. Zielinski, S. Farid, Z. M. Wosinska, and I. R. Gould, *J. Org. Chem.*, 2005, **70**, 3791–3800.

5. P.-C. Lin, S.-H. Ueng, S.-C. Yu, M.-D. Jan, A. K. Adak, C.-C. Yu, and C.-C. Lin, *Org. Lett.*, 2007, **9**, 2131–2134.

6. P. E. Laibinis, G. M. Whitesides, D. L. Allara, Y. T. Tao, A. N. Parikh, and R. G. Nuzzo, *J. Am. Chem. Soc.*, 1991, **113**, 7152–7167.

7. J. P. Collman, N. K. Devaraj, T. P. a Eberspacher, and C. E. D. Chidsey, *Langmuir*, 2006, **22**, 2457–2464.

8. A. C. Gouget-Laemmel, J. Yang, M. A. Lodhi, A. Siriwardena, D. Aureau, R. Boukherroub, J.-N. Chazalviel, F. Ozanam, and S. Szunerits, *J. Phys. Chem. C*, 2012, **117**, 368–375.

9. M. S. Góes, H. Rahman, J. Ryall, J. J. Davis, and P. R. Bueno, *Langmuir*, 2012, **28**, 9689–9699.

10. E. Laviron, *J. Electroanal. Chem. Interfacial Electrochem.*, 1979, **101**, 19–28.

11. J. F. Smalley, H. O. Finklea, C. E. D. Chidsey, M. R. Linford, S. E. Creager, J. P. Ferraris, K. Chalfant, T. Zawodzinsk, S. W. Feldberg, and M. D. Newton, *J. Am. Chem. Soc.*, 2003, **125**, 2004–2013.

12. M. Fetizon, V. Balogh, and M. Golfier, *J. Org. Chem.*, 1971, **36**, 1339–1341.