A new *Pseudomonas* Quinolone Signal (PQS) binding partner: MexG.

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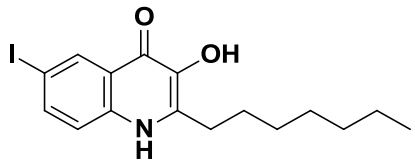
Chemical and analytical methods

^{1}H NMR spectra were recorded on Bruker DPX 400 or 500 spectrometers in deuterated chloroform or deuterated dimethyl sulfoxide or deuterated methanol operating at 400 and 500 MHz respectively. ^{13}C NMR spectra were recorded on Bruker 400 or 500 spectrometers operating at 100 and 125 MHz respectively. Chemical shifts are quoted relative to residual solvent (7.26 ppm for deuterated chloroform and 77.0 ppm for ^{13}C of deuterated chloroform, 2.54 ppm for deuterated dimethyl sulfoxide and 40.45 ppm for ^{13}C of deuterated dimethyl sulfoxide, 3.31 for deuterated methanol and 49.01 for ^{13}C deuterated methanol) and coupling constants (*J*) are given in Hz. The following abbreviations are used singularly or in combination to indicate the multiplicity of signals: s singlet, d doublet, t triplet, q quartet, m multiplet and b broad. NMR spectra were acquired at 300 K unless otherwise indicated. All coupling constants are reported to the nearest 0.5 Hz. High resolution mass spectroscopic (HRMS) analyses were measured on a Micromass Q-TOF or a Micromass LCT Premier spectrometer. Infra-red spectra were recorded on a Perkin-Elmer 1600 FT IR spectrometer. The sample was prepared neat or as a solution in the solvent indicated. Selected absorption maxima (νmax) are reported in wavenumbers (cm^{-1}).Melting points were determined on a Buchi B-545 melting point apparatus and are uncorrected. Reactions were carried out in oven-dried glassware under an atmosphere of nitrogen with dry, freshly distilled solvents. Freeze / thaw degassed solvent was frozen with liquid nitrogen and allowed to partially thaw under vacuum. This was repeated 3 times. Tetrahydrofuran was distilled from $LiAlH_4$ with triphenylmethane as indicator. Rt (rt) refers to ambient temperature. Temperatures of 0°C were maintained using an ice-water bath and temperatures below 0°C were maintained using an acetone-card ice bath. Reactions involving microwave irradiation were performed in 10

cm^3 or 35 cm^3 microwave tubes with clip lids using CEM Discover® microwave apparatus. Flash column chromatography was carried out using Merck 9385 Keiselgel 60 SiO_2 (230-400 mesh) unless otherwise stated.

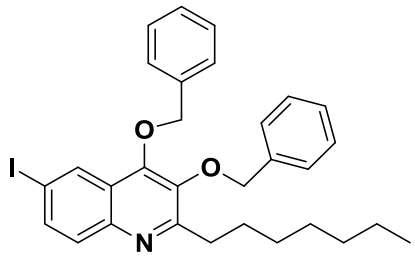
6-Iodo-2-heptyl-3-hydroxyquinolin-4-(1H)-one (**3**)



Di*iso*propylethylamine (0.73 mL, 5.41 mM) and 1-chlorononan-2-one (0.739 g, 4.18 mM) prepared as previously described[1] were added in sequence to a solution of commercially available 2-amino-5-iodobenzoic acid (1.0 g, 3.8 mM) in anhydrous NMP (10 mL) contained in a 35 mL microwave vial. The solution was then heated under microwave irradiation to 200 ^oC for 30-60 min. The reaction mixture was then allowed to cool to rt and added to an ice/H_2O mix and left to settle for 20 min. The precipitate thus formed was then isolated by filtration, dried under high vacuum overnight and the crude product was purified by trituration in hot acetonitrile to yield a dark purple powder 0.88 g (60%).

δ_H (400 MHz, d_6-DMSO) 11.52 (1H br s, NH), 8.35 (1H, d, *J* = 2.0 Hz ,C<u>H</u>CCO), 8.31 (1H, br s, OH), 7.75 (1H, dd, *J* = 9.0, 2.0 Hz, aryl CIC<u>H</u>CH), 7.33 (2H, d, *J*= 9.0 Hz, CICHC<u>H</u>), 2.70 (2H, t, *J* = 7.5 Hz NHCC<u>H</u>$_2$), 1.61-1.64 (2H, m, NHCCH$_2$C<u>H</u>$_2$), 1.40-1.20 (8H, m, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$CH$_3$), 0.81 (3H, t, *J* = 7.0 Hz CH_3); **δ_C** (125 MHz; d_6-DMSO) 167.5 (C=O), 138.4 (COH), 137.89 (CH), 136.4 (CNH), 136.3 (CNH), 133.0 (CH), 124.3 (CH<u>C</u>CO), 120.4 (<u>C</u>HCNH), 85.72 (<u>C</u>I), 31.2 (CH_2), 28.9 (CH_2), 28.5 (CH_2), 28.2 (CH_2), 27.8 (CH_2), 22.1 (CH_2), 14.0 (CH_3); **ν_{max}** (neat)/cm^{-1} 3390 br (OH), 2925, 2854 (CH), 1649 (C=O), **HRMS** (ESI^+) *m/z* found $[M+H]^+$ 386.0617, $[C_{16}H_{21}NO_2I]^+$ required 386.0632 Spectroscopic data consistent with previous literature[2]

6-Iodo-2-heptyl-3,4-bis(benzyloxy) quinoline (**4**)

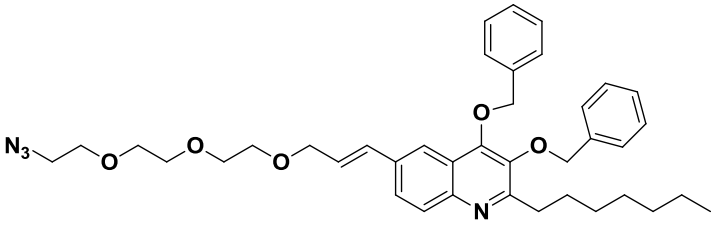


NaH (60% in mineral oil 0.60 g, 15.16 mM) was added to 6-Iodo-1-(*H*)-2-heptyl-3-hydroxy-quinolone **3** (2.78 g, 7.22 mM) in DMF (55 mL) at 0 ^{o}C. The reaction was left to stir for 15 min, benzyl bromide (1.7 mL, 14.44 mM) was then added. The reaction was allowed to warm to rt and stirred for a further 2 hr. Water (100 mL) was added followed by Et_2O (100 mL) and the aqueous phase was extracted a further two times with Et_2O (2 x 150 mL), in some cases filtration was required to aid extraction. The organic phases were combined, dried ($MgSO_4$), filtered and the solvent was removed by spin evaporation under vacuum. The product was purified by column chromatography,[Et_2O: 30-40 Pet ether], gradient (8:92). 6-Iodo-2-heptyl-3,4-bis(benzyloxy) quinoline **4** was isolated as a yellow solid 2.68 g (64 %).

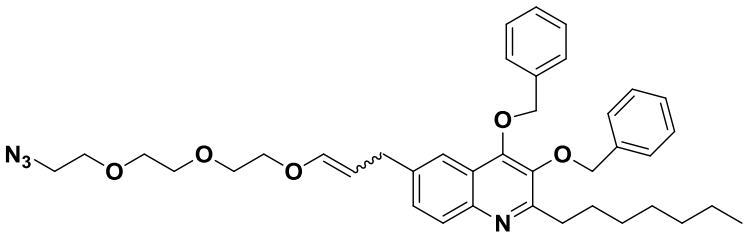
δ_H (400 MHz, d_6-DMSO) 8.27 (1H, d, *J* = 2.0 Hz ,CC<u>H</u>CI), 7.85 (1H, dd, *J* = 9.0, 2.0 Hz CIC<u>H</u>CH), 7.67 (1H, d, *J* = 9.0 Hz, CICHC<u>H</u>) 7.47-7.34 (10H, m, C<u>H</u>CN, benzyl aromatic CH), 5.35 (2H, s, OC<u>H</u>$_2$) 5.14 (2H, s, OC<u>H</u>$_2$), 2.82 (2H, t, *J* = 8.0 Hz NCC<u>H</u>$_2$), 1.64 (2H, app quintet, *J* = 7.5 Hz NCCH$_2$C<u>H</u>$_2$) 1.28-1.17 (8H, m, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$CH$_3$), 0.84 (3H, t, *J* = 7.0 Hz C<u>H</u>$_3$), **δ_C** (125 MHz; d_6-DMSO) 161.3 (NC<u>C</u>O), 150.0 (NCC<u>C</u>O), 141.8 (CH<u>C</u>N) 141.8 (N<u>C</u>CH$_2$) 136.8 (<u>C</u>HCHCN) 136.7 (CH$_2$<u>C</u> benzyl), 136.4 (CH$_2$<u>C</u> benzyl), 130.3 (<u>C</u>HC), 130.2 (<u>C</u>HC), 128.7 (CH benzyl), 128.6 (CH benzyl), 128.6 (CH benzyl), 128.6 (CH benzyl), 128.5 (CH benzyl), 128.5 (CH benzyl), 125.8 (CH<u>C</u>CO), 75.0 (<u>C</u>H$_2$O), 75.0 (<u>C</u>H$_2$O) 33.1 (CH$_2$), 31.3 (CH$_2$), 29.1 (CH$_2$), 28.6 (CH$_2$), 27.9 (CH$_2$), 22.2 (CH$_2$), 14.0 (CH$_3$); **ν_{max}** (neat)/cm^{-1},

2916, 2872, 2835 (CH), **HRMS (**ESI^+**)** *m/z* found $[M+H]^+$ 566.1542, $[C_{30}H_{33}NO_2I]^+$ required 566.1556, **mp** 60-63 ^oC (Et_2O)

(E)-6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)prop-1-enyl)-3,4-bis(benzyloxy)-2-heptylquinoline (**6**)



(Z)-6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)allyl)-3,4-bis(benzyloxy)-2-heptylquinoline and(E)-6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)allyl)-3,4-bis(benzyloxy)-2-heptylquinoline were isolated as a mixture (**7**)



3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)prop-1-ene **5** (0.56 g, 2.6 mM), $NaHCO_3$ (0.29 g, 3.44 mM) and 6-Iodo-2-heptyl-3,4-bis(benzyloxy) quinoline **4** (0.97 g, 1.7 mM) were suspended in DMF (30 mL) and the solution was degassed by bubbling through with nitrogen for 20 min. $PdCl_2(PPh_3)_2$ (120 mg, 0.172 mM) was added and the reaction was heated to 110 ^oC and left to stir at this temperature under a nitrogen atmosphere for 13 hr. The reaction mixture was allowed to cool to rt and the majority of DMF was removed by spin evaporation under vacuum. The remaining residue was partitioned between Et_2O (60 mL) and water (20 mL) and stirred for 15 min. The organic phase was collected and the aqueous phase was extracted again with Et_2O (2 x 50 mL). The organic phases were combined, dried (Na_2SO_4), filtered and the solvent was removed by spin evaporation under vacuum to give 1.4 g of the crude

product as a orange/brown oil. TLC analysis indicated three products which were purified by column chromatography, EtOAc : Pet ether 30-40, (1 : 3)

(E)-6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)prop-1-enyl)-3,4-bis(benzyloxy)-2-heptylquinoline (**6**) was isolated as a pale yellow amorphous solid 150 mg (15%)

δ_H (400 MHz, d_6-DMSO) 7.90 (1H, d, *J* = 1.0 Hz ,C<u>H</u>CCO), 7.84 (1H, dd, *J* = 9.0, 2.0 Hz C<u>H</u>CHCN), 7.81 (1H, d, *J* = 9.0 Hz, CHC<u>H</u>CN), 7.51 (2H, dd, *J* = 8.0, 1.5 Hz, benzyl aromatic CH) 7.47 (2H, dd, *J* = 7.5. 1.5 Hz benzyl aromatic CH) 7.44-7.33 (6H, m, C<u>H</u>CN, benzyl aromatic CH), 6.79 (1H, *J* = 16.0 Hz, C<u>H</u>CHCH$_2$OCH$_2$) 6.47 (1H, dt, *J* = 16.0, 5.5 Hz, CHC<u>H</u>CH$_2$OCH$_2$) 5.37 (2H, s, OC<u>H$_2$</u>) 5.14 (2H, s, OC<u>H$_2$</u>), 4.18 (2H, app dd, *J* = 5.5, 1.0 Hz, CHCHC<u>H$_2$</u>OCH$_2$) 3.63-3.57 (10H, m, CH$_2$O x 5), 3.39 (2H, t, *J* = 5.0 Hz, CH$_2$N$_3$) 2.85 (2H, t, *J* = 8.0 Hz NHCC<u>H$_2$</u>), 1.67 (2H, app quintet, *J* = 7.5 Hz NHCCH$_2$C<u>H$_2$</u>) 1.30-1.21 (8H, m, C<u>H$_2$</u>C<u>H$_2$</u>C<u>H$_2$</u>C<u>H$_2$</u>CH$_3$), 0.85 (3H, t, *J* = 7.0 Hz, C<u>H$_3$</u>), **δ_C** (125 MHz; d_6-DMSO) 162.1 (NC<u>C</u>O), 151.3 (NCC<u>C</u>O), 144.9 (CH<u>C</u>N) 141.9 (N<u>C</u>CH$_2$), 136.9 (CH$_2$<u>C</u> benzyl), 136.6 (CH$_2$<u>C</u> benzyl), 133.8 (<u>C</u>CHCHCH$_2$O) 131.0 (CC<u>H</u>CHCH$_2$O), 128.7 (CH benzyl or <u>C</u>HCN), 128.7 (CH benzyl or <u>C</u>HCN), 128.6 (CH benzyl or <u>C</u>HCN), 128.5 (CH benzyl or C<u>H</u>CN), 128.4 (CH benzyl or <u>C</u>HCN), 128.0 (CCH<u>C</u>HCH$_2$O), 126.0 (<u>C</u>HCHCN), 124.0 (CCH<u>C</u>CO), 119.8 (C<u>C</u>HCCO), 74.9 (<u>C</u>H$_2$O benzyl), 74.7 (<u>C</u>H$_2$O benzyl), 70.8 (CH$_2$O), 70.0 (CH$_2$O), 70.0 (CH$_2$O), 69.9 (CH$_2$O), 69.4 (CH$_2$O), 69.2 (CH$_2$O), 50.1 (CH$_2$N$_3$), 33.1 (CH$_2$), 31.3 (CH$_2$), 29.1 (CH$_2$), 28.6 (CH$_2$), 28.1 (CH$_2$), 22.2 (CH$_2$), 14.1 (CH$_3$); **ν_{max}** (neat)/cm^{-1}, 2924, 2854, (CH), 2098 (N$_3$) **HRMS (**ESI$^+$**)** *m/z* found $[M+H]^+$ 653.3720, $[C_{39}H_{49}N_4O_5]^+$ required 653.3703

(Z)-6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)allyl)-3,4-bis(benzyloxy)-2-heptylquinoline and(E)-6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)allyl)-3,4-bis(benzyloxy)-2-heptylquinoline were isolated as a mixture (**7**) 370 mg (33%)

(Z)-6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)allyl)-3,4-bis(benzyloxy)-2-heptylquinoline

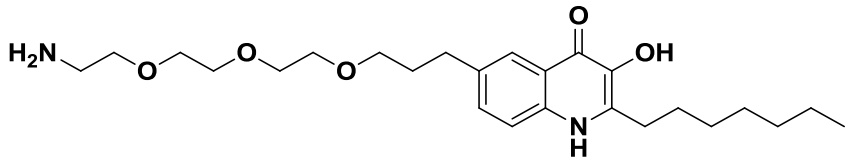
δ_H (500 MHz, d_6-DMSO) 7.68-7.73 (3H, m, quinoline CH), 7.48-7.31 (10H, m, benzyl CH), 6.25 (1H, d, *J* = 6.0 Hz, OC<u>H</u>CHCH$_2$), 5.29 (2H, s, OCH$_2$ benzyl), 5.11 (2H, s, OCH$_2$ benzyl), 4.52 (1H, app q, *J* = 6.0 Hz, OCHC<u>H</u>CH$_2$), 3.90 (2H, t, *J* = 5.0 Hz, C<u>H$_2$</u>OCHCHCH$_2$), 3.62-3.50 (8H, m, CH2O x 4), 3.45 (2H,d, *J* = 7.5 Hz, OCHCHC<u>H$_2$</u>), 3.32 (2H, app t, *J* = 5.0 Hz, CH$_2$N$_3$), 2.80 (2H, t, *J* = 7.5 Hz, NCC<u>H$_2$</u>CH$_2$), 1.63 (2H, app quintet, *J* = 7.5 Hz, NCCH$_2$C<u>H$_2$</u>), 1.28-1.17 (8H, m, C<u>H$_2$</u>C<u>H$_2$</u>C<u>H$_2$</u>C<u>H$_2$</u>CH$_3$), 0.82 (3H, t, *J* = 6.5 Hz, CH$_3$) **δ_C** (125 MHz; d_6-DMSO) 159.3 (NC<u>C</u>O), 151.0 (CHC<u>C</u>O), 144.1 (O<u>C</u>HCHCH$_2$), 141.6 (CH<u>C</u>N), 138.9 (N<u>C</u>CH$_2$), 138.9 (<u>C</u>CH$_2$CHCHOCH$_2$), 136.7 (<u>C</u>CH$_2$O benzyl), 136.7 (<u>C</u>CH$_2$O benzyl), 129.7 (C<u>H</u>CHCN), 128.7 (CH benzyl), 128.6 (CH benzyl), 128.6 (CH benzyl), 128.6 (CH benzyl), 128.4 (CH benzyl), 128.4 (CH benzyl), 128.3 (<u>C</u>HCN), 123.7 (CH<u>C</u>CO), 119.5 (C<u>C</u>HCCO), 104.0 (OCH<u>C</u>HCH$_2$), 74.9 (OCH$_2$ benzyl), 74.7 (OCH$_2$ benzyl), 71.1 (<u>C</u>H$_2$OCHCHCH$_2$), 70.1 (OCH$_2$), 69.8 (OCH$_2$), 69.8 (OCH$_2$), 69.4 (OCH$_2$), 50.1 (CH$_2$N$_3$), 33.01 (CH$_2$), 31.3 (CH$_2$), 29.8 (OCHCHC<u>H$_2$</u>), 29.1 (CH$_2$), 28.6 (CH$_2$), 28.1 (CH$_2$), 221.2 (CH$_2$) 14.1 (CH$_3$)

(E)-6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)allyl)-3,4-bis(benzyloxy)-2-heptylquinoline

δ_H (500 MHz, d_6-DMSO) 7.68-7.73 (3H, m, quinoline CH), 7.48-7.31 (10H, m, benzyl CH), 6.49 (1H, d, *J* = 12.5 Hz, OC<u>H</u>CHCH$_2$), 5.29 (2H, s, OCH$_2$ benzyl), 5.11 (2H, s, OCH$_2$ benzyl), 4.90 (1H, dt, *J* = 12.5, 7.5 Hz, OCHC<u>H</u>CH$_2$), 3.76 (2H, t, *J* = 5.0 Hz, C<u>H$_2$</u>OCHCHCH$_2$), 3.62-3.50 (8H, m, CH$_2$O x 4), 3.32 (2H, app t, *J* = 5.0 Hz, CH$_2$N$_3$), 2.80 (2H, t, *J* = 7.5 Hz, NCC<u>H$_2$</u>CH$_2$), 1.63 (2H, app quintet, *J* = 7.5 Hz, NCCH$_2$C<u>H$_2$</u>), 1.28-1.17 (8H, m, C<u>H$_2$</u>C<u>H$_2$</u>C<u>H$_2$</u>C<u>H$_2$</u>CH$_3$), 0.82 (3H, t, *J* = 6.5 Hz, CH$_3$) **δ_C** (125 MHz; d_6-DMSO) 159.3 (NC<u>C</u>O), 151.0 (CHC<u>C</u>O), 146.5 (O<u>C</u>HCHCH$_2$), 141.6 (CH<u>C</u>N), 138.9 (N<u>C</u>CH$_2$), 139.5 (<u>C</u>CH$_2$CHCHOCH$_2$), 136.7 (<u>C</u>CH$_2$O benzyl), 136.7 (<u>C</u>CH$_2$O benzyl), 129.7

(CHCHCN), 128.7 (CH benzyl), 128.6 (CH benzyl), 128.6 (CH benzyl), 128.6 (CH benzyl), 128.4 (CH benzyl), 128.4 (CH benzyl), 128.3 (CHCN), 123.7 (CHCCO), 119.5 (CCHCCO), 102.2 (OCHCHCH$_2$), 74.9 (OCH$_2$ benzyl), 74.7 (OCH$_2$ benzyl), 68.3 (CH$_2$OCHCHCH$_2$), 70.1 (OCH$_2$), 69.8 (OCH$_2$), 69.8 (OCH$_2$), 69.4 (OCH$_2$), 50.1 (CH$_2$N$_3$), 33.7 (OCHCHCH$_2$), 33.01 (CH$_2$), 31.3 (CH$_2$), 29.1 (CH$_2$), 28.6 (CH$_2$), 28.1 (CH$_2$), 221.2 (CH$_2$) 14.1 (CH$_3$)

6-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propyl)-2-heptyl-3-hydroxyquinolone (**8**)

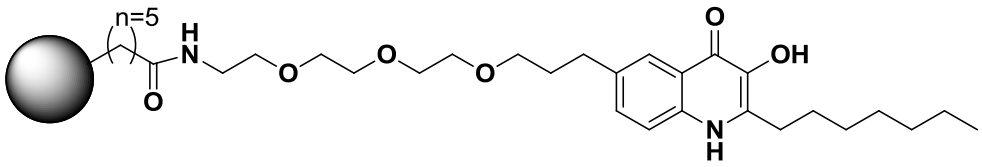


Intermediates **6** and **7** were combined (0.37 g, 0.57 mM) and dissolved in methanol (25 mL) in a round bottom flask (100 mL). 10% Palladium on activated carbon (50 mg) was added and the air from the round bottom flask was evacuated under vacuum and replaced with hydrogen gas from a balloon, this process was repeated 3 times. The reaction mixture was then vigorously stirred for 7 hrs. The reaction mixture was filtered through celite and the organic phase was spin evaporated under vacuum. ^{1}H NMR analysis indicated a very minor amount of what seemed to be benzyl protected intermediate. The oil was dissolved in methanol and the above reaction process was repeated for a further 18 hr. The reaction mixture was filtered through celite, the solvent was removed by spin evaporation under vacuum to yield an amorphous solid 1H NMR analysis indicated no benzyl protected intermediate present. The product was then purified by trituration in Et$_2$O (4 x 1-2 mL washes) with decanting to remove the Et$_2$O containing impurities. The desired product **8** was isolated as a beige amorphous solid 222 mg (87 %).

δH (400 MHz, d$_6$-DMSO) 7.88 (1H, d *J* = 1.5 Hz, CHCCO), 7.47 (1H, d *J* = 8.5 Hz, CHCHCN), 7.40 (1H, dd, *J* = 8.5, 2.0 Hz, CHCHCN), 3.55-3.35 (12H, m, CH$_2$O x 6 + wa-

ter), 2.71 (4H, t, *J* = 7.5 Hz, NCC$\underline{H}_2$, OCH$_2$CH$_2$CH$_2$C), 2.66 (2H, t *J* = 5.5 Hz, NH$_2$C$\underline{H}_2$), 1.83 (2H, app quintet, *J* = 7.0 Hz, OCH$_2$C$\underline{H}_2$CH$_2$C), 1.66 (2H, quintet *J* = 7.0 Hz, NCCH$_2$C$\underline{H}_2$CH$_2$), 1.35-1.20 (8H, m, C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$CH$_3$), 0.85 (3H, t, *J* = 7.0 Hz, C$\underline{H}_3$) **δC** (125 MHz; d$_6$-DMSO) 168.7 (CO), 137.9 (COH), 135.9 (C), 135.3 (C), 135.0 (C), 131.0 (C$\underline{H}$CHCN), 123.0 (C$\underline{H}$CCO), 122.2 (CH$\underline{C}$CO), 117.9 (C$\underline{H}$CN), 72.5 (CH$_2$O), 69.9 (CH$_2$O), 69.9 (CH$_2$O), 69.7 (CH$_2$O), 69.6 (CH$_2$O), 69.5 (CH$_2$O), 41.2 (CH$_2$NH$_2$), 31.4 (CH$_2$), 31.3 (CH$_2$), 31.0 (CH$_2$), 28.9 (CH$_2$), 28.6 (CH$_2$), 28.2 (CH$_2$), 27.9 (CH$_2$), 22.1 (CH$_2$), 14.0 (CH$_3$) **νmax** (neat)/cm^{-1} 3249 br (OH, NH$_2$), 2926, 2857 (CH), 1642 (CO) **HRMS** (ESI$^+$) m/z found [M+H]$^+$ 449.3036, $C_{25}H_{41}N_2O_5^+$ required 449.3015

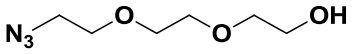
PQS affinity probe



N-Hydroxysuccinimidyl-Sepharose beads (4% beaded agarose) containing a 6 atom linker between the beads and the *N*-Hydroxysuccinimidyl group were purchased from Sigma Aldrich; the beads were in an isopropanol suspension with a binding capacity between (16-23 µM)/mL. Intermediate **8** (3 mg, 6.7 x 10-6 M) was dissolved in anhydrous DMF (1 mL). The sepharose beads (1 mL) in an isopropanol suspension were filtered and washed with DMF (3 x 2 mL). The beads were then added to the DMF solution of **8** followed by triethylamine (10 µL). The reaction mixture was left to stir at rt for 2 hr, and the phases were allowed to settle, LCMS analysis of the solution indicated that **8** was no longer present in solution. Ethanolamine (15 µL) was added to block the remaining NHS sites on the beads and the reaction mixture was left to stir for a further 2 hr. Water (3 mL) was added to the reaction mixture and the beads were filtered and washed with water (3 x 3 mL) to remove residual DMF. The beads

were then collected and suspended in isopropanol (1 mL) and stored at 4^{o}C until use for biological assays.

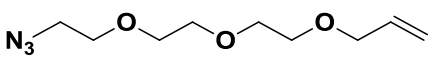
2-(2-(2-azidoethoxy)ethoxy)ethanol



Sodium azide (2.0 g, 31.7 mM) was added to commercially available 2-2-(Chloroethoxy)ethoxy)ethanol (4 g, 23.8 mM) dissolved in anhydrous DMF (20 mL) at rt. The reaction mixture was heated to 100 ^{o}C for 5 hr with stirring under nitrogen. The reaction mixture was allowed to cool to rt and the DMF was removed by spin evaporation under vacuum. The remaining residue was portioned between water (20 mL) and CH_2Cl_2 (20 mL) and left to stir for 10 min. The aqueous phase was extracted again with CH_2Cl_2 (2 x 50 mL). The organic phases were combined dried ($MgSO_4$), filtered and the solvent was removed by spin evaporation under vacuum to give the desired product as a pale yellow oil 4.1 g (100 %).

δ_H (500 MHz, $CDCl_3$) 3.73 (2H, t, *J* = 4.5 Hz CH_2O), 3.66-3.69 (6H, m, CH_2O x 3), 3.61 (2H, t, *J* = 5.0 Hz, CH_2O), 3.40 (2H, t, *J* = 5.0 Hz, CH_2N_3), **δ_C** (125 MHz; $CDCl_3$) 72.5 (CH_2O), 70.7 (CH_2O), 70.4 (CH_2O), 70.65 (CH_2O), 61.8 (CH_2OH), 50.7 (CH_2N_3) **ν_{max}** (neat)/cm^{-1} 3414.3 (OH), 2869 (CH), 2096 (N_3) Spectroscopic data consistent with previous literature[3]

3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)prop-1-ene (**5**)

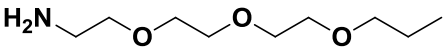


NaH (60 % in mineral oil 0.178 g, 4.3 mM) was added to 2-2-(azidomethoxy)ethoxy)ethanol (0.5 g, 2.9 mM), prepared by the protocol described below, in DMF (20 mL) at 0 ^{o}C under nitrogen. The reaction mixture was left to stir for 20 min at 0 ^{o}C and allyl bromide was added (0.37 mL, 4.3 mM). The reaction mixture was warmed to rt and left to stir for 16 hr. The majority of the DMF was removed by spin evaporation under vacuum and the remaining

residue was partitioned between water (20 mL) and Et_2O (50 mL) and stirred for 10 min. The organic phase was collected and the aqueous phase was extracted a further two times with Et_2O (2 x 75 mL). The organic phases were combined dried (Na_2SO_4), filtered and the solvent was removed by spin evaporation under vacuum. The crude product was purified by column chromatography, Et_2O : pet ether 30-40, (3 : 7) and the desired product **5** was isolated as a colourless oil 0.47 g (77 %).

$\boldsymbol{\delta_H}$ (500 MHz, $CDCl_3$) 5.90 (1H, ddt, *J* = 17.0, 10.5, 5.5 Hz, $CH_2C\underline{H}CH_2O$), 5.26 (1H, app dq, *J* = 17.0, 1.5 Hz, $C\underline{H}_2CHCH_2O$), 5.17 (1H, app dq, *J* = 10.5, 1.5 Hz, $C\underline{H}_2CHCH_2O$) 4.01 (2H, app dt, *J* = 5.5, 1.5 Hz, $CH_2CHC\underline{H}_2O$) 3.68-3.65 (8H, m, CH_2O x 4), 3.61- 3.58 (2H, m, CH_2O) 3.38 (2H, t, *J* = 5.0 Hz, CH_2N_3), $\boldsymbol{\delta_C}$ (125 MHz; $CDCl_3$) 134.7 (CH), 117.1 ($\underline{C}H_2CH$) 72.2 (CH_2O), 70.7 (CH_2O x 2), 70.7 (CH_2O), 70.0 (CH_2O), 69.4 (CH_2O) 50.7 (CH_2N_3) $\boldsymbol{\nu_{max}}$ (neat)/cm^{-1} 2865 (CH), 2097 (N_3) **HRMS (**ESI^+**)** *m/z* found $[M+H]^+$ 216.1358 $[C_9H_{18}N_3O_3]^+$, required 216.1348

<u>2-(2-(2-propoxyethoxy)ethoxy)ethanamine</u>

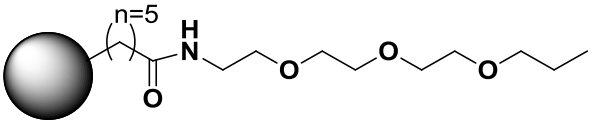


The same procedure for 6-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propyl)-2-heptyl-3-hydroxyquinolone (**8**) was followed except the reaction was left to stir overnight and the product was purified by column chromatography on alumina, (MeOH : CH_2Cl_2) increasing methanol gradient (3:197), (3:97) and (1:9). The desired product was isolated as colourless oil 0.165 g, (62 %).

$\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$) 3.62-3.55 (6H, m, CH_2O x 3), 3.45-3.51 (2H, m CH_2O), 3.44 (2H, t *J* = 5.5 Hz CH_2O), 3.36 (2H, t, *J* = 7.0 Hz CH_2O), 2.80 (2H, t, *J* = 5.0 Hz, $C\underline{H}_2NH_2$), 1.55 (2H, app sextet, *J* = 7.0 Hz, $OCH_2C\underline{H}_2CH_3$), 0.84 (3H, t, *J* = 7.5 Hz, $OCH_2CH_2C\underline{H}_3$) $\boldsymbol{\delta_C}$ (125 MHz;

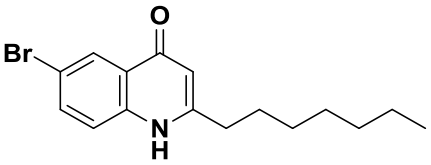
$CDCl_3$) 73.5 (CH_2O), 73.1 (CH_2O), 70.6 (CH_2O), 70.6 (CH_2O), 70.3 (CH_2O), 70.0 (CH_2O), 41.8 (CH_2NH_2), 22.8 (CH_2CH_3), 10.5 (CH_3) ν_{max} (neat)/cm^{-1} 2959, 2862 (CH) **HRMS (**ESI^+**)** *m/z* found $[M+H]^+$ 192.1598, $C_9H_{22}N_2O_3^+$ required 192.1600

Linker only control



Attachment to the sepharose beads was carried out as described for the PQS affinity probe.

6-Bromo-2-heptylquinolin-4(1H)-one (**9**)

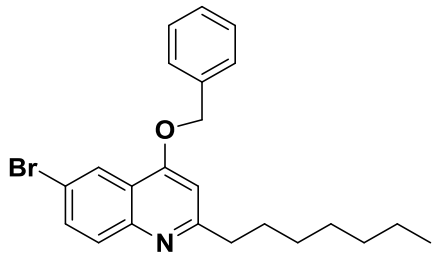


6-Bromo-2-heptylquinolin-4(1H)-one **9** was prepared by a Conrad-Limpach cyclisation similar to the procedure described for HHQ by Woschek et. al.[3] A solution of methyl 3-oxodecanoate (2.00 g, 10mmol, 1eq.), 4-bromoaniline (1.72 g, 10 mmol, 1 eq.) and PTSA (40 mg, 0.23 mmol, 0.023 eq.) in hexane (11.8 mL) was heated at reflux with stirring using a dean and stark water separator for 5 hr. The solvents were removed under reduced pressure and the residue was added dropwise to refluxing diphenyl ether (2.4 mL). After 30 min the MeOH was removed under reduced pressure and diethyl ether (10 mL) was added. The precipitate was filtered, washed with diethyl ether (2x 20 mL) and dried in vacuum to give **9** (1.27 g, 3.95 mmol, 39%) as a pink powder which was used without further purification.

δ_H(400 MHz, MeOD): 8.31 (1H, d, *J* = 2.5 Hz, CBrC<u>H</u>CCO), 7.77 (1H, dd, *J* = 9.0, 2.5 Hz, CHC<u>H</u>CBr), 7.50 (1H, d, *J* = 9.0 Hz, C<u>H</u>CHCBr), 6.22 (1H, s, CH2CC<u>H</u>CO), 4.59 (1H, brs, N<u>H</u>), 2.69 (2H, t, *J* = 8.0 Hz, NHCC<u>H</u>$_2$), 1.77-1.71 (2H, m, NHCCH$_2$C<u>H</u>$_2$), 1.43-1.27 (8H, m, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$CH$_3$), 0.88 (3H, t, *J* = 7.0 Hz, C<u>H</u>$_3$) δ_C (125 MHz, MeOD): 179.2 (C=O),

157.6 (NH<u>C</u>CH_2), 140.4 (CH<u>C</u>NH), 136.3 (CBr<u>C</u>HCH), 128.5 (CBr<u>C</u>HCCO), 126.9 (CBrCH<u>C</u>CO), 121.3 (CBrCH<u>C</u>H or CO<u>C</u>HC), 118.3 (BrC), 109.3 (CBr<u>C</u>HCH or CO<u>C</u>HC), 35.0 (NC<u>C</u>H_2), 32.9 (CH_2), 30.1 (CH_2), 30.1 (CH_2), 30.1 (CH_2), 23.7 (CH_2), 14.4 (CH_3); $\boldsymbol{\nu_{max}}$ (neat)/cm^{-1} : 2953, 2924, 2853 (s, C-H), 1634 (s, C=O), 1588 (s, C=C aromatic) **HRMS** (ESI^+) *m/z*: found $[M+H]^+$ 322.0821 $[C_{16}H_{21}NOBr]^+$ calculated: 322.07283;**mp**: 221-224^oC (Et_2O).

<u>(Benzyloxy)-6-bromo-2-heptylquinoline (**10**)</u>

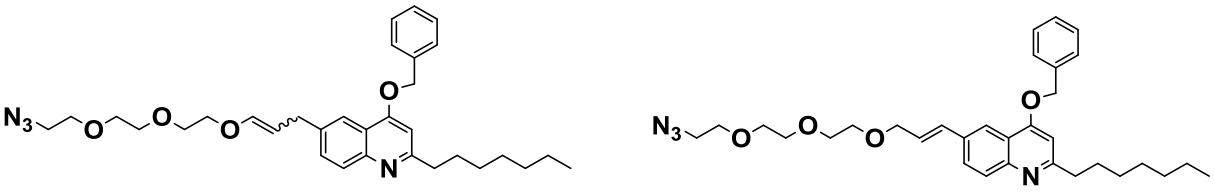


A solution of **9** (500 mg, 1.56 mmol, 1 eq.) in DMF (10 mL) was cooled to 0^oC and NaH (60% dispersion in mineral oil, 93 mg, 2.33 mmol, 1.5 eq.) was added. After 15 min benzyl bromide (0.18 mL, 1.56 mmol, 1 eq.) was added and the reaction was allowed to warm to room temperature and stirred for a further 2 hr. The solvent was removed under reduced pressure and the residue partitioned between Et_2O (20 mL) and H_2O (20 mL). The aqueous phase was extracted twice more with ether (2 x 20 mL), dried over Mg_2SO_4 and the solvent removed under reduced pressure. The resulting pale yellow residue was purified by column chromatography (Et_2O/40-60 PE 1:9) to yield **10** (339 mg, 0.82 mmol, 53%) as a clear sticky solid.

δH (500 MHz, $CDCl_3$): 8.33 (1H, d, *J* = 2.5 Hz, CBrC<u>H</u>CCO), 7.83 (1H, d, *J* = 9.0 Hz, C<u>H</u>CHCBr), 7.72 (1H, dd, *J* = 9.0, 3.0 Hz, CHC<u>H</u>CBr), 7.50 (2H, app d, *J* = 7.2 Hz, 2 x CC<u>H</u>CH benzyl), 7.47-7.43 (2H, app m, 2 x CCHC<u>H</u>CH benzyl), 7.42-7.38 (1H, app m, CCHCHC<u>H</u> benzyl), 6.72 (1H, s, COC<u>H</u>C), 5.27 (2H, s, C<u>H</u>$_2$ 74 benzyl), 2.89 (2H, t, *J* = 8.0 Hz, NHCC<u>H</u>$_2$), 1.77 (2H, app q, *J* = 6.0 Hz, NHCCH$_2$C<u>H</u>$_2$), 1.39-1.23 (8H, m,

C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$CH$_3$), 0.88 (3H, t, *J* = 7.0 Hz, CH$_3$); **δ**C (125 MHz, $CDCl_3$): 164.9 (N$\underline{C}$CH$_2$), 160.5 (C$\underline{C}$OCH), 147.7 ($\underline{C}$NCCH$_2$), 135.5 (CH$_2\underline{C}$ benzyl), 133.2 (CBr$\underline{C}$HCH, CH$\underline{C}$HCN or $\underline{C}$H benzyl), 130.2 (CBr$\underline{C}$HCH, CH$\underline{C}$HCN or $\underline{C}$H benzyl), 129.0 (2 x CH benzyl), 128.7 (CBr$\underline{C}$HCH, CH$\underline{C}$HCN or CH benzyl), 127.8 (2 x CH benzyl), 124.4 CBr$\underline{C}$HCCO), 121.5 (CBr), 118.8 (CH$\underline{C}$CO), 101.8 (CO$\underline{C}$HCN), 70.5 (OCH$_2$ benzyl), 40.1 (NC$\underline{H}_2$), 31.9 (NCCH$_2\underline{C}$H$_2$), 30.2 (CH$_2$), 29.7 (CH$_2$), 29.4 (CH$_2$), 22.8 (CH$_2$), 14.3 (CH$_3$); **νmax** (neat)/cm^{-1}: 2955, 2917, 2848 (s, C-H), 1611 (s, C=O), 1590 (s, C=C aromatic) 1559 (s, C=C aromatic); **HRMS** (ESI+) *m/z*: found $[M+H]^+$ 412.1281 $[C_{23}H_{27}NOBr]^+$ calculated: 412.1276.

(E/Z)-6-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)allyl)-4-(benzyloxy)-2-heptylquinoline (**12**) and (E)-6-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)prop-1-en-1-yl)-4-(benzyloxy)-2-heptylquinoline (**11**)



A solution of compound **10** (Benzyloxy)-6-bromo-2-heptylquinoline (268 mg, 0.65 mmol, 1 eq.), compound **5** (216 mg, 1 mmol, 1.5 eq.) and sodium bicarbonate (83 mg, 98 mmol, 1.5 eq.) in DMF (8 mL) was degassed with N_2 for 15 min. $PdCl_2(PPh_3)_2$ (58 mg, 0.065 mmol, 0.1 eq.) was added and the reaction mixture heated to 100^{o}C for 12 hr. The solvent was then removed under reduced pressure and the residue was purified by column chromatography (stepwise gradient of EtOAc in 40-60 PE) to yield (E)-6-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)prop-1-en-1-yl)-4-(benzyloxy)-2-heptylquinoline **11** (94.7 mg, 0.17 mmol, 27%) as a colourless residue and a mixture of (E/Z)-6-(3-(2-(2-(2-

Azidoethoxy)ethoxy)ethoxy)allyl)-4-(benzyloxy)-2-heptylquinoline **12** (80 mg, 0.15 mmol, 23%) as a pale yellow residue.

(E)-6-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)prop-1-en-1-yl)-4-(benzyloxy)-2-heptylquinoline (**11**)

δH (500 MHz, $CDCl_3$): 8.09 (1H, d, *J* = 2.0 Hz, CHCCO), 7.91 (1H, d, *J* = 9.0 Hz, CCHC<u>H</u>CN), 7.78 (1H, dd, *J* = 9.0, 2.0 Hz, C<u>H</u>CHCN), 7.51 (2H, app d, *J* = 6.8 Hz, 2 x CH benzyl), 7.45 (2H, app t, *J* = 7.4 Hz, 2 x CH benzyl), 7.41-7.37 (1H, m, CH benzyl), 6.77 (1H, d, *J* = 16.0 Hz, $CH_2OCH_2CHC\underline{H}$), 6.69 (1H, s, NCC<u>H</u>CO), 6.40 (1H, dt, *J* = 16.0, 6.0 Hz, $CH_2OCH_2C\underline{H}CH$), 5.28 (2H, s, OCH_2 benzyl), 4.23 (2H, app dd, *J* = 6.0, 1.5 Hz, $CH_2OC\underline{H}_2CHCH$), 3.72-3.59 (10H, m, $C\underline{H}_2OC\underline{H}_2C\underline{H}_2OC\underline{H}_2C\underline{H}_2O$), 3.37 (2H, t, *J* = 5.0 Hz, N_3CH_2), 2.88 (2H, t, *J* = 8.0 Hz, $NCCH_2$), 1.80-1.73 (2H, m, $NCCH_2C\underline{H}_2$), 1.40-1.12 (8H, m, $C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_3$), 0.89-0.86 (3H, m, $CH_2C\underline{H}_3$); **δ**C (125MHz, $CDCl_3$): 164.3 (CCO or $CN\underline{C}CH_2$), 161.5 (CCO or $CN\underline{C}CH_2$), 148.7 (CHCH<u>C</u>N), 136.0 (CH_2C benzyl), 133.4 ($CH_2CHCH\underline{C}$), 132.4 ($OCH_2CH\underline{C}HC$), 128.9 (2 x CH benzyl), 128.7 (<u>C</u>HCHCN, <u>C</u>HCN or CH benzyl), 128.6 (<u>C</u>HCHCN, <u>C</u>HCN or <u>C</u>H benzyl), 127.7 (2 x CH benzyl), 127.6 (CHCHCN, CHCN or CH benzyl), 126.7 ($CH_2\underline{C}HCHC$), 120.3 (C<u>C</u>HCCO), 120.2 (CCHCCO), 101.5 (C<u>C</u>HCCH), 72.1 ($O\underline{C}H_2C$ benzyl), 70.9 (CH_2O), 70.9 (CH_2O), 70.3(CH2O), 70.2 (CH_2O), 69.7 (CH_2O), 50.8 (N_3CH_2), 40.1 ($\underline{C}H_2CHCHC$), 31.9 ($NC\underline{C}H_2$), 30.3 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 22.8 (CH_2), 14.3 (CH_3); **νmax** (neat)/cm^{-1}: 2925, 2857 (s, C-H), 2103 (s, N_3), 1663 (s, C=C), 1595 (s, C=C aromatic), 1566 (s, C=C aromatic), 1500 (s, C=C aromatic); **HRMS** (ESI+) m/z: found [M+H]+: 547.3284 $[C_{32}H_{43}N_4O_4]^+$ calculated 547.3284.

(E)-6-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)allyl)-4-(benzyloxy)-2-heptylquinoline and (Z)-6-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)allyl)-4-(benzyloxy)-2-heptylquinoline were isolated as a 1:3 mixture (ratio determined by NMR) (**12**):

(Z)-6-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)allyl)-4-(benzyloxy)-2-heptylquinoline:

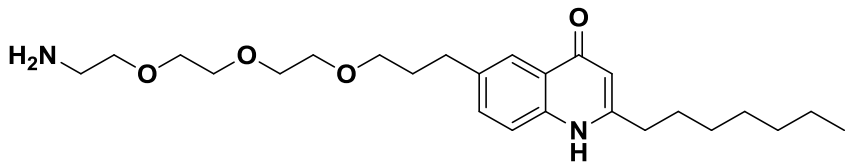
δH (500 MHz, $CDCl_3$): 7.99 (1H, d, *J* = 1.5 Hz, CHCCO), 7.89 (1H, d, *J* = 8.5 Hz, CHC<u>H</u>CN), 7.56 (1H, dd, *J* = 8.5, 2.0 Hz, C<u>H</u>CHCN), 7.51 (2H, app d, *J* = 7.5 Hz, 2 x CH benzyl), 7.44 (2H, app t, *J* = 7.5 Hz, 2 x CH benzyl), 7.41-7.36 (1H, m, CH benzyl), 6.67 (1H, s, NCCHCO), 6.13 (1H, dt, *J* = 6.0, 1.5 Hz, $CH_2OC\underline{H}CHCH_2$), 5.28 (2H, s, OCH_2 benzyl), 4.63 (1H, dt, *J* = 6.0, 7.5 Hz, $CH_2OCHC\underline{H}CH_2$), 3.73-3.62 (10H, m, $C\underline{H}_2OC\underline{H}_2C\underline{H}_2OC\underline{H}_2C\underline{H}_2O$), 3.57 (2H, d, *J* = 7.5 Hz, $CH_2OCHCHC\underline{H}_2$), 3.34 (2H, t, *J* = 5.0 Hz, N_3CH_2), 2.87 (2H, t, *J* = 6.5 Hz, $NCCH_2$), 1.82-1.71 (2H, m, $NCCH_2C\underline{H}_2$), 1.46-1.07 (8H, m, $C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_3$), 0.87 (3H, t, *J* = 7.0 Hz, $CH_2C\underline{H}_3$); **δ**C (125MHz, $CDCl_3$): 163.6 (CHCCO), 161.2 ($CN\underline{C}CH_2$), 147.9 ($\underline{C}NCCH_2$), 147.4 ($O\underline{C}HCH$), 138.3 ($CH_2\underline{C}$ benzyl), 136.2 ($CH_2\underline{C}CHC$), 131.0 ($C\underline{C}HCHCN$), 128.9 (2 x benzyl CH), 128.4 ($CH\underline{C}HCN$ or CH benzyl), 128.3 ($\underline{C}HCN$ or CH benzyl), 127.6 (2 x CH benzyl), 120.2 ($CCHCH\underline{C}CO$), 120.0 ($C\underline{C}HCCO$), 103.3 ($OCH\underline{C}HCH_2$), 101.2 ($OC\underline{C}HCCH_2$), 70.9 (CH_2O or $O\underline{C}H_2C$ benzyl), 70.8 (CH_2O or $O\underline{C}H_2C$ benzyl), 70.2 (CH_2O or $O\underline{C}H_2C$ benzyl), 70.2 (CH_2O or $O\underline{C}H_2C$ benzyl), 70.1 (CH_2O or $O\underline{C}H_2C$ benzyl), 66.0 ($\underline{C}H_2OCHCH$), 50.8 (N_3CH_2), 34.4 ($CHCH\underline{C}H_2C$), 31.9 (CH_2), 30.5 (CH_2), 30.3 (CH_2), 29.7 (CH_2), 29.4 (CH_2),22.8 (CH_2), 14.3 (CH_3).

(E)-6-(3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)allyl)-4-(benzyloxy)-2-heptylquinoline:

δH (500 MHz, $CDCl_3$): 7.97 (1H, d, *J* = 1.5 Hz, CHCCO), 7.89 (1H, d, *J* = 8.5 Hz, CHC<u>H</u>CN), 7.53-7.52 (1H, m, C<u>H</u>CHCN) 7.54-7.37 (5H, m, 5 x CH benzyl), 6.68 (1H, s, NCC<u>H</u>CO), 6.42 (1H, d, *J*=12.5 Hz, $CH_2OC\underline{H}CHCH_2$), 5.28 (2H, s, OCH_2 benzyl), 4.98 (1H,

dt, *J*=12.5, 7.5 Hz, $CH_2OCHC\underline{H}CH_2$), 3.73-3.62 (10H, m, $C\underline{H}_2OC\underline{H}_2C\underline{H}_2OC\underline{H}_2C\underline{H}_2O$), 3.41 (2H, d, *J* = 7.5 Hz, $CH_2OCHCHC\underline{H}_2$), 3.37 (2H, t, *J* = 5.0 Hz, N_3CH_2), 2.87 (2H, t, *J* = 6.5 Hz, $NCCH_2$), 1.82-1.71 (2H, m, $NCCH_2C\underline{H}_2$), 1.46-1.07 (8H, m, $C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_3$), 0.87 (3H, t, *J* = 7.0 Hz, CH_2CH_3); **δC** (126MHz, $CDCl_3$): 163.4 ($CHC\underline{C}O$), 161.2 ($CN\underline{C}CH2$), 147.8 ($\underline{C}NCCH2$), 145.9 ($O\underline{C}HCHCH_2$), 138.7 ($CH_2\underline{C}$ benzyl), 136.2 ($CH_2\underline{C}CHC$), 131.2 ($C\underline{C}HCHCN$), 128.7 (2 x CH benzyl), 128.4 ($CH\underline{C}HCN$ or CH benzyl), 128.3 ($\underline{C}HCN$ or CH benzyl), 127.6 (2 x CH benzyl), 120.2 ($CCHCH\underline{C}CO$), 120.0 ($C\underline{C}HCCO$), 105.8 ($OCH\underline{C}HCH_2$), 101.1 ($OC\underline{C}HCCH_2$), 71.7 (CH_2O or OCH_2C benzyl), 71.0 (CH_2O or OCH_2C benzyl), 70.9 (CH_2O or OCH_2C benzyl), 70.6 (CH_2O or OCH_2C benzyl), 70.2 (CH_2O or OCH_2C benzyl), 68.5 ($\underline{C}H_2OCHCH$), 50.8 (N_3CH_2), 40.0 ($CHCH\underline{C}H_2C$), 31.9 (CH_2), 30.5 (CH_2), 30.4 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 22.8 (CH_2), 14.3 (CH_3).

6-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propyl)-2-heptylquinolin-4(1H)-one (**13**)

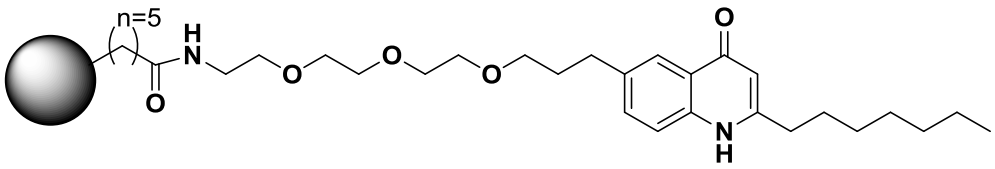


To **11** and **12** (140 mg, 0.26 mmol) in MeOH (12.5 mL) was added 10% palladium on activated carbon (52.0 mg). The flask was evacuated under vacuum and refilled with H_2 gas three times to ensure a hydrogen atmosphere. The reaction mixture was then vigorously stirred overnight. The mixture was filtered through a pad of celite and the solvent removed under reduced pressure to yield **13** (78 mg, 0.18 mmol, 70%) as a pale pink waxy solid which was used without further purification.

δH (500 MHz, CD_3OD): 8.02 (1H, d, *J* = 2.0 Hz, $C\underline{H}CCO$), 7.57 (1H, dd, *J* = 8.5, 2.0 Hz, $CC\underline{H}CHCN$), 7.51 (1H, d, *J* = 8.5 Hz, $CHC\underline{H}CN$), 6.20 (1H, s, $NCC\underline{H}CO$), 4.64 (2H, bs, $N\underline{H}_2CH_2$), 3.67-3.56 (8H, m, $OC\underline{H}_2C\underline{H}_2OC\underline{H}_2C\underline{H}_2O$), 3.55 (2H, t, *J* = 5.5 Hz,

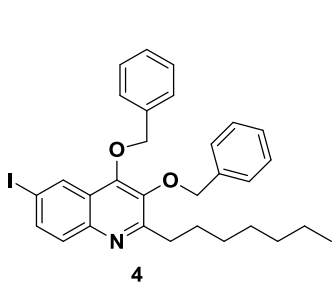
$H_2NCH_2C\underline{H_2}O$), 3.49 (2H, t, *J* = 6.5 Hz, $OC\underline{H_2}CH_2C\underline{H_2}$), 2.86-2.79 (4H, m, $H_2NC\underline{H_2}CH_2O$ and $NCC\underline{H_2}$), 2.70 (2H, t, *J* = 7.7 Hz, $OCH_2CH_2CH_2$), 1.94 (2H, app dq, *J* = 8.5, 6.5 Hz, $OCH_2C\underline{H_2}CH_2$), 1.75 (2H, app p, *J* = 8.5, 6.5 Hz, $NCCH_2C\underline{H_2}$), 1.45-1.24 (8H, m, $C\underline{H_2}C\underline{H_2}C\underline{H_2}C\underline{H_2}CH_3$), 0.88 (3H, t, *J* = 7.0 Hz, $CH_2C\underline{H_3}$); **δC** (125 MHz, CD_3OD): 180.4 ($\underline{C}O$), 156.8 ($NH\underline{C}CH_2$), 140.0 ($CH\underline{C}NH$), 139.5 ($CH_2\underline{C}CHCCO$), 134.4 ($C\underline{C}HCHCNH$), 125.4 ($CH\underline{C}CO$), 124.7 ($\underline{C}HCCO$), 119.2 ($CH\underline{C}HCNH$), 108.7 ($CO\underline{C}HCNH$), 72.0 ($O\underline{C}H_2$), 71.6 ($O\underline{C}H_2$), 71.6 ($O\underline{C}H_2$), 71.6 ($O\underline{C}H_2$), 71.2 ($O\underline{C}H_2$), 71.1 ($O\underline{C}H_2$), 41.7 ($NH_2\underline{C}H_2$), 35.0 ($NHC\underline{C}H_2$), 32.9 ($OCH_2\underline{C}H_2CH_2$), 32.9 ($OCH_2CH_2\underline{C}H_2$), 32.5 ($\underline{C}H_2$), 30.9 ($\underline{C}H_2$), 30.2 ($\underline{C}H_2$), 30.2 ($\underline{C}H_2$), 30.1 ($\underline{C}H_2$), 23.7 ($\underline{C}H_2$), 14.4 ($\underline{C}H_3$); **νmax** (neat)/$cm^{-1}$: 3250 (br m, N-H), 2953, 2921, 2857 (s, C-H) 1637 (s, C=O), 1595 (s, C=C aromatic), 1551 (s, C=C aromatic); **HRMS** (ESI^+) m/z: found [M+H]: 433.3064 $[C_{25}H_{41}N_2O_4]^+$ required: 433.3069.

<u>HHQ affinity probe</u>



N-Hyroxysuccinimidyl-sepharose bead suspension in isopropanol (1 mL, 4% beaded sepharose, 16-23 μM/mL) was filtered, washed with dry DMF (3 x 1 mL) and added to a solution of the free amine **13** (6 μM) in anhydrous DMF (1 mL). Triethylamine (10 μL) was added and the reaction mixture was left to stir at room temperature. After 3 hrs LCMS analysis of the eluent showed that there was no amine present in the reaction, ethanolamine (15 μL) was added and the slurry was left to stir for a further 2 hr. Water (3 mL) was added and the beads were collected by filtration, washed with water (3 x 2 mL), isopropanol (2 x 2 mL), collected by filtration, suspended in isopropanol (1 mL) and stored at 4 ^oC until use.

NMR spectra



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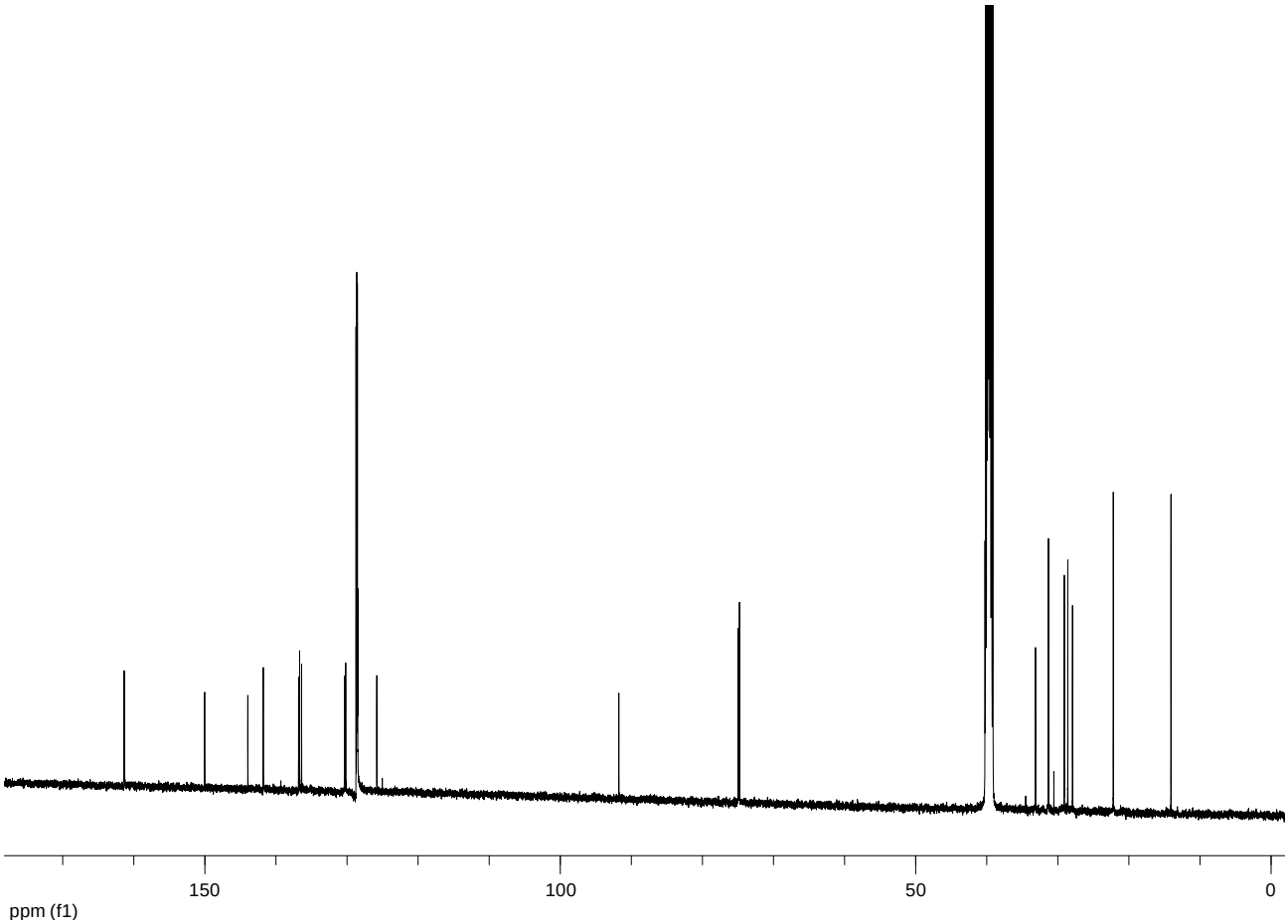

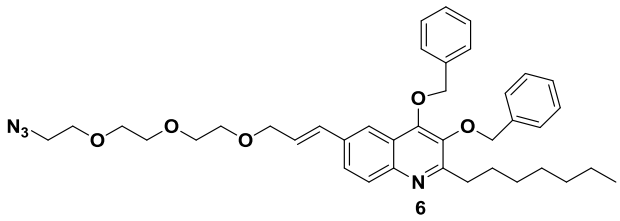

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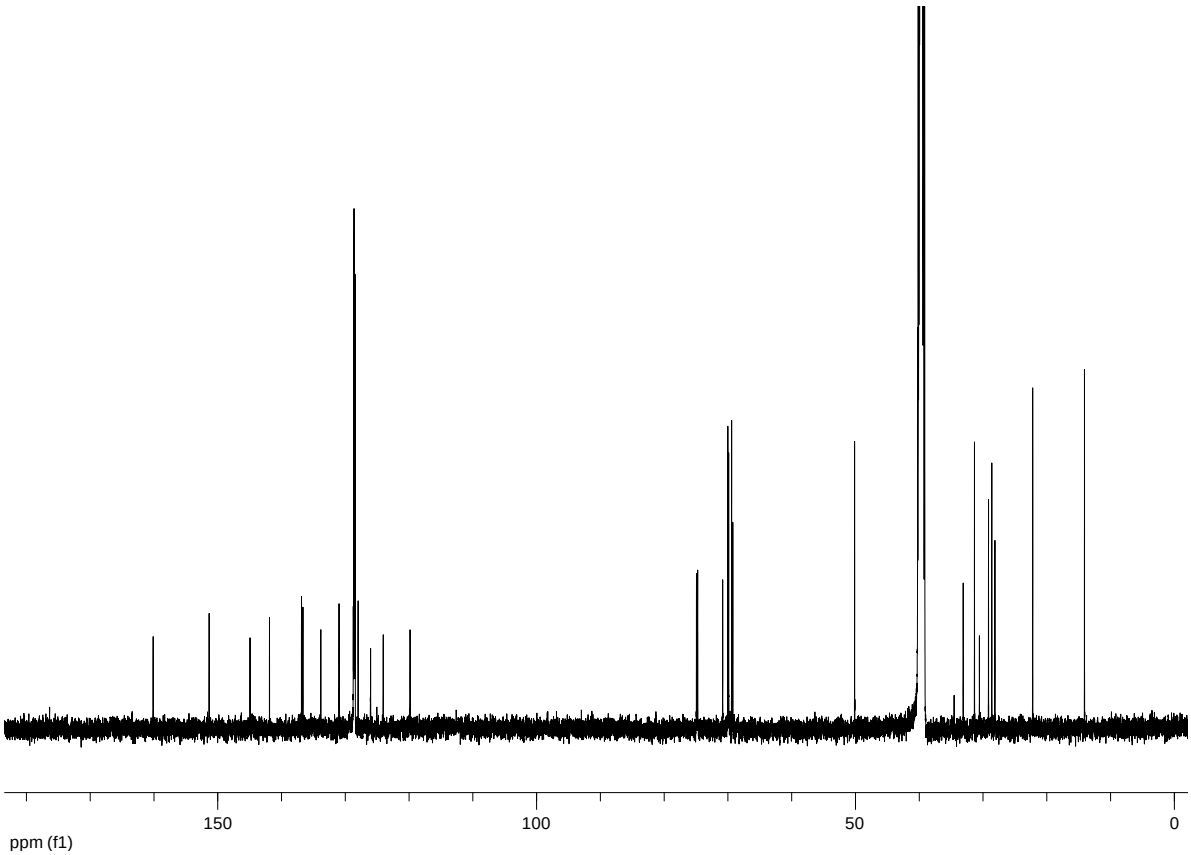

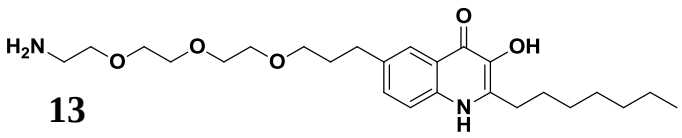

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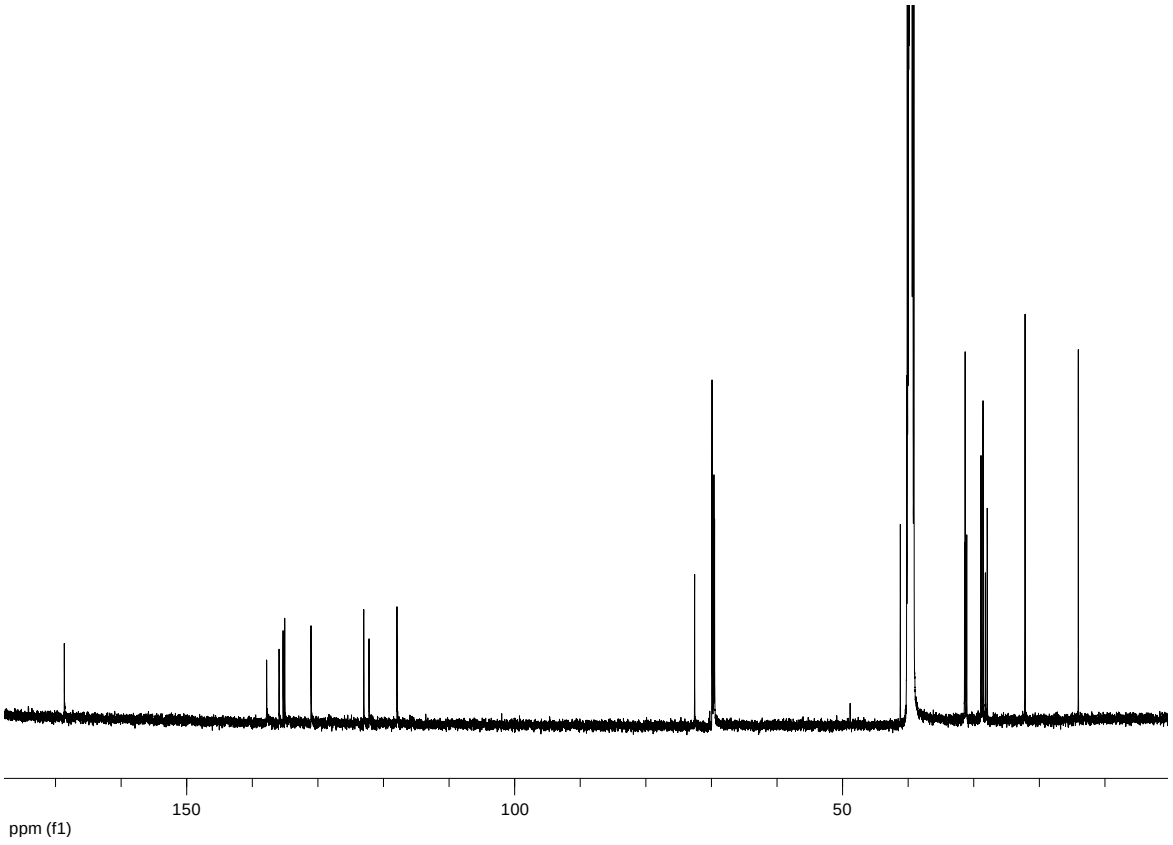

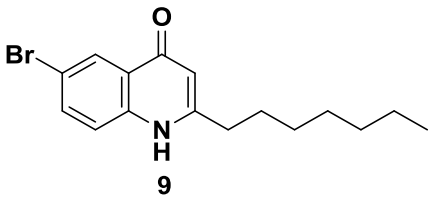
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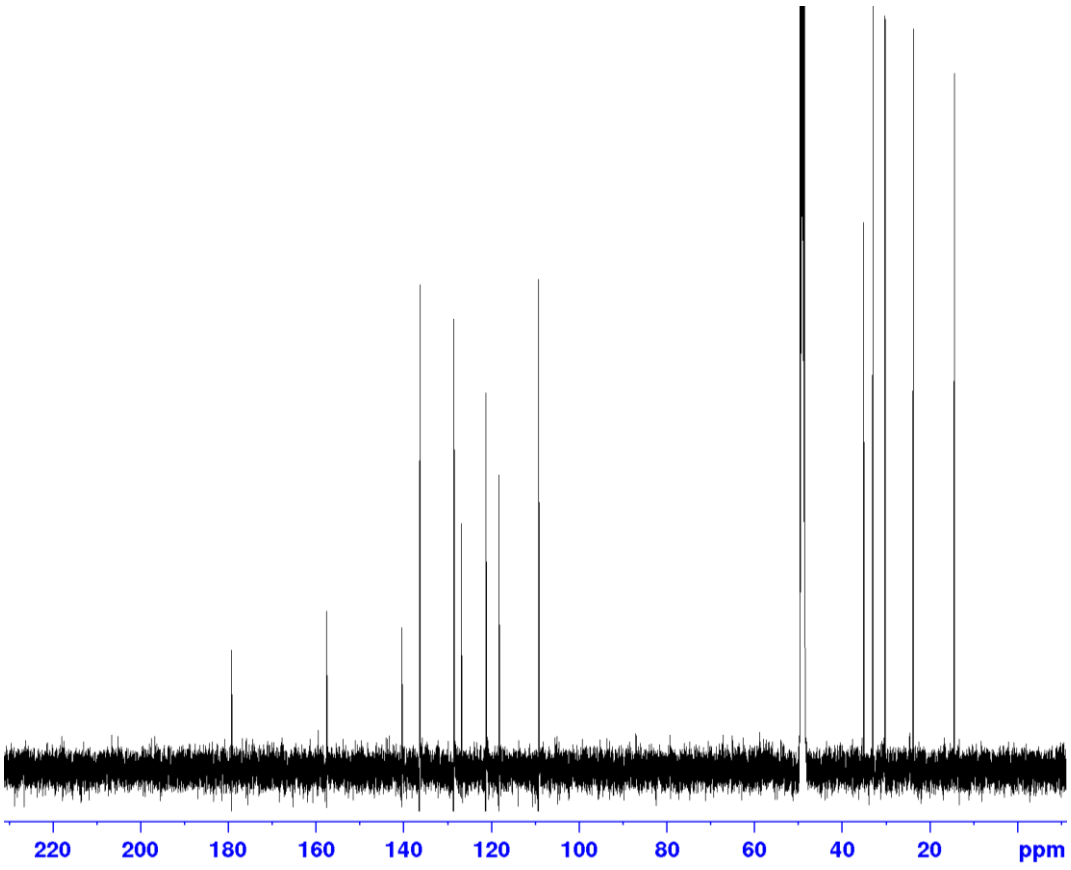
Br
N
H

9

12
11
10
9
8
7
6
5
4
3
2
1
ppm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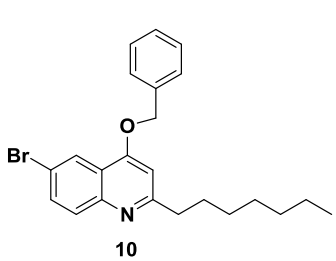
20
ppm


Br
O
N

10

12
11
10
9
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7
6
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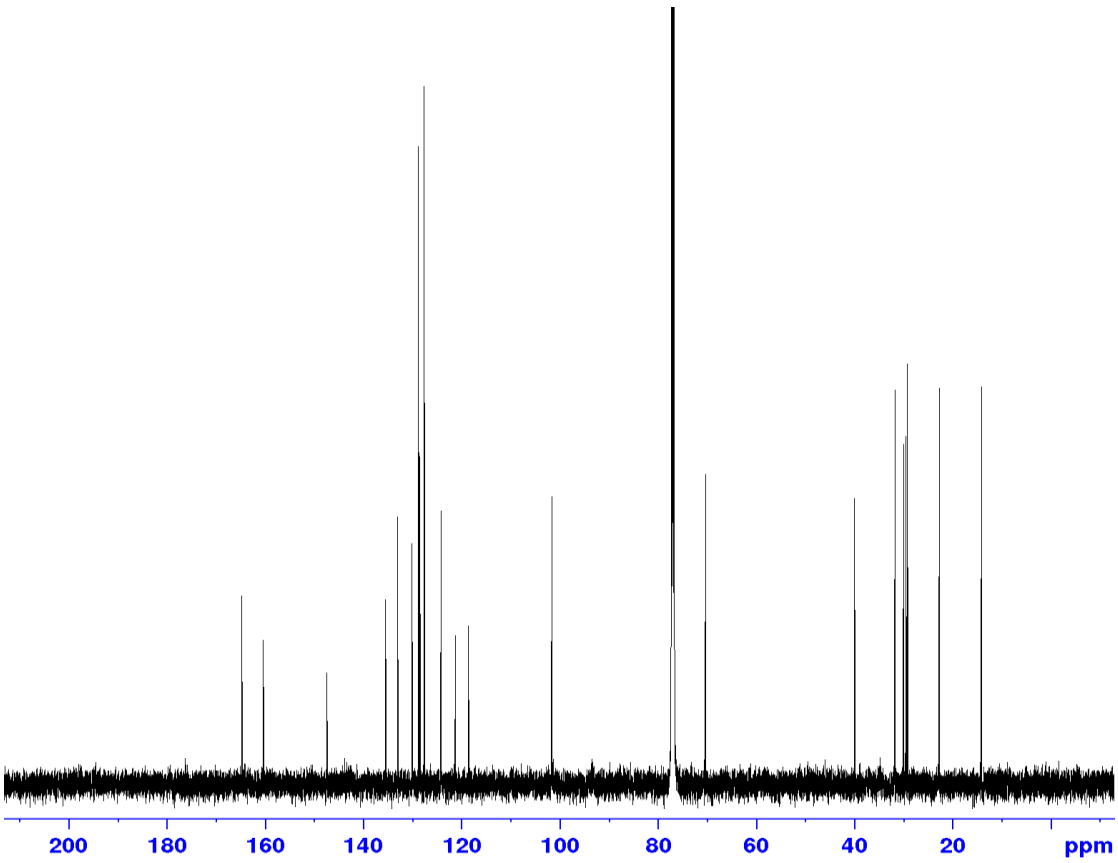
4
3
2
1
ppm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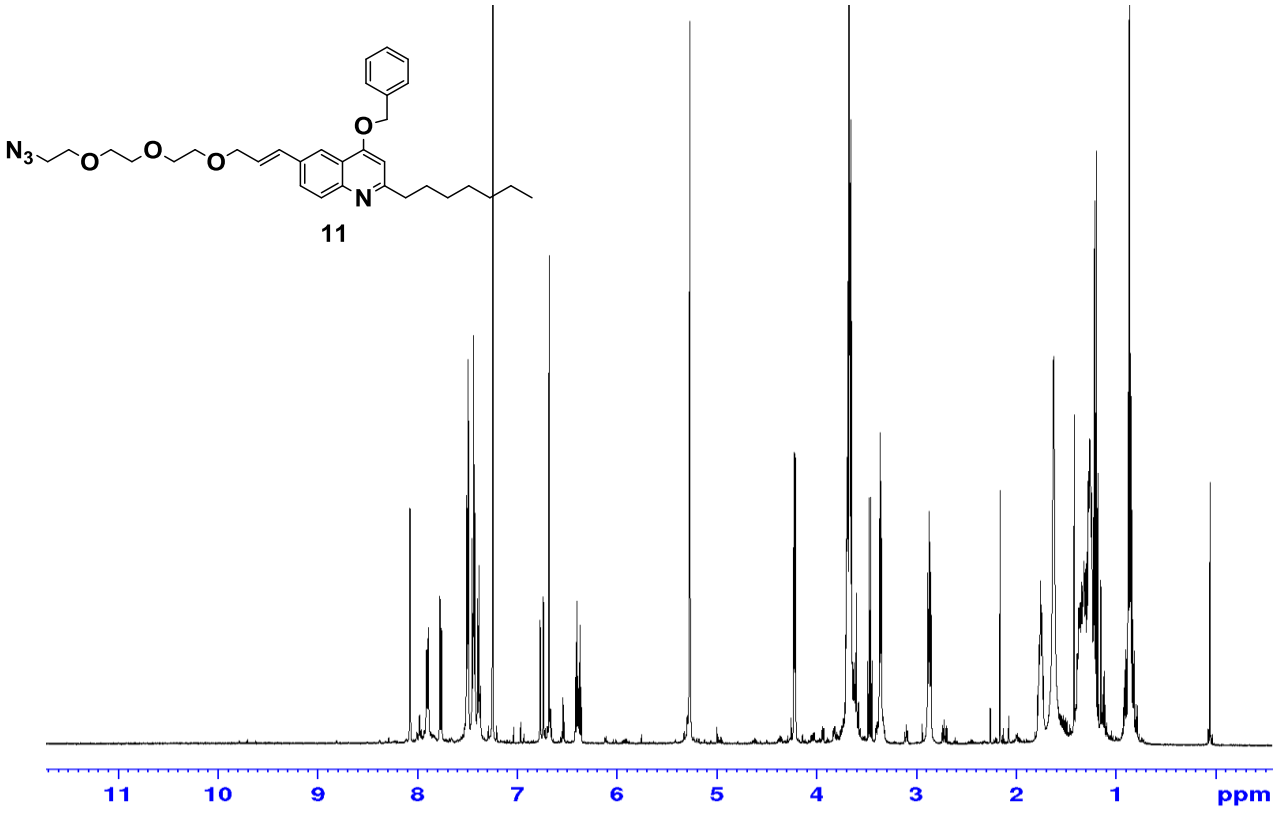
N3
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O
O
O
N
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10
9
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4
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2
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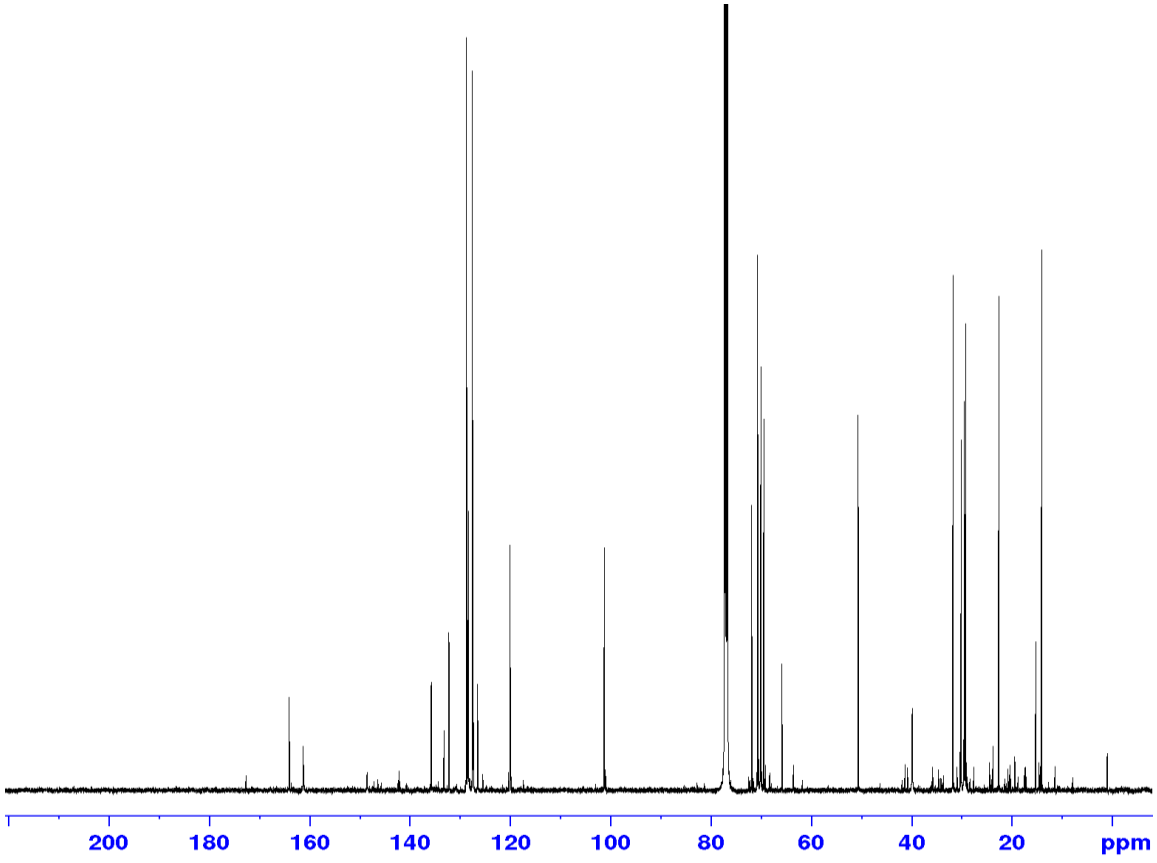
ppm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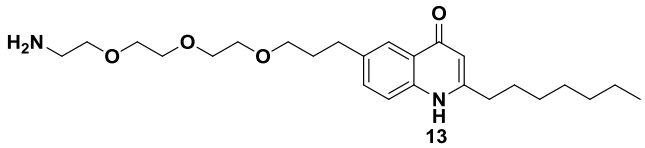
ppm


H2N
O
O
O
O
N
H

13

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11
10
9
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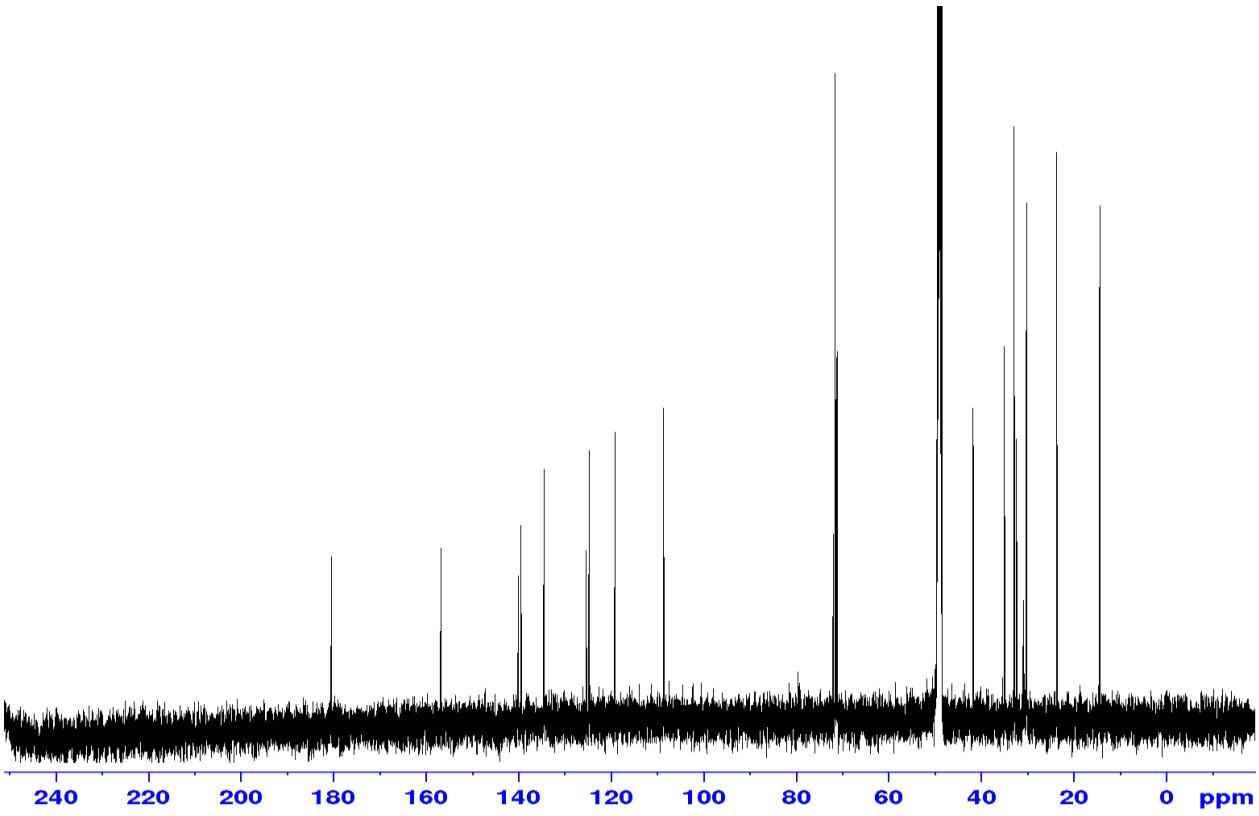
4
3
2
1
ppm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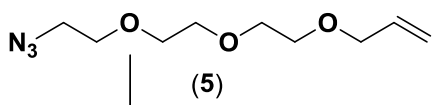
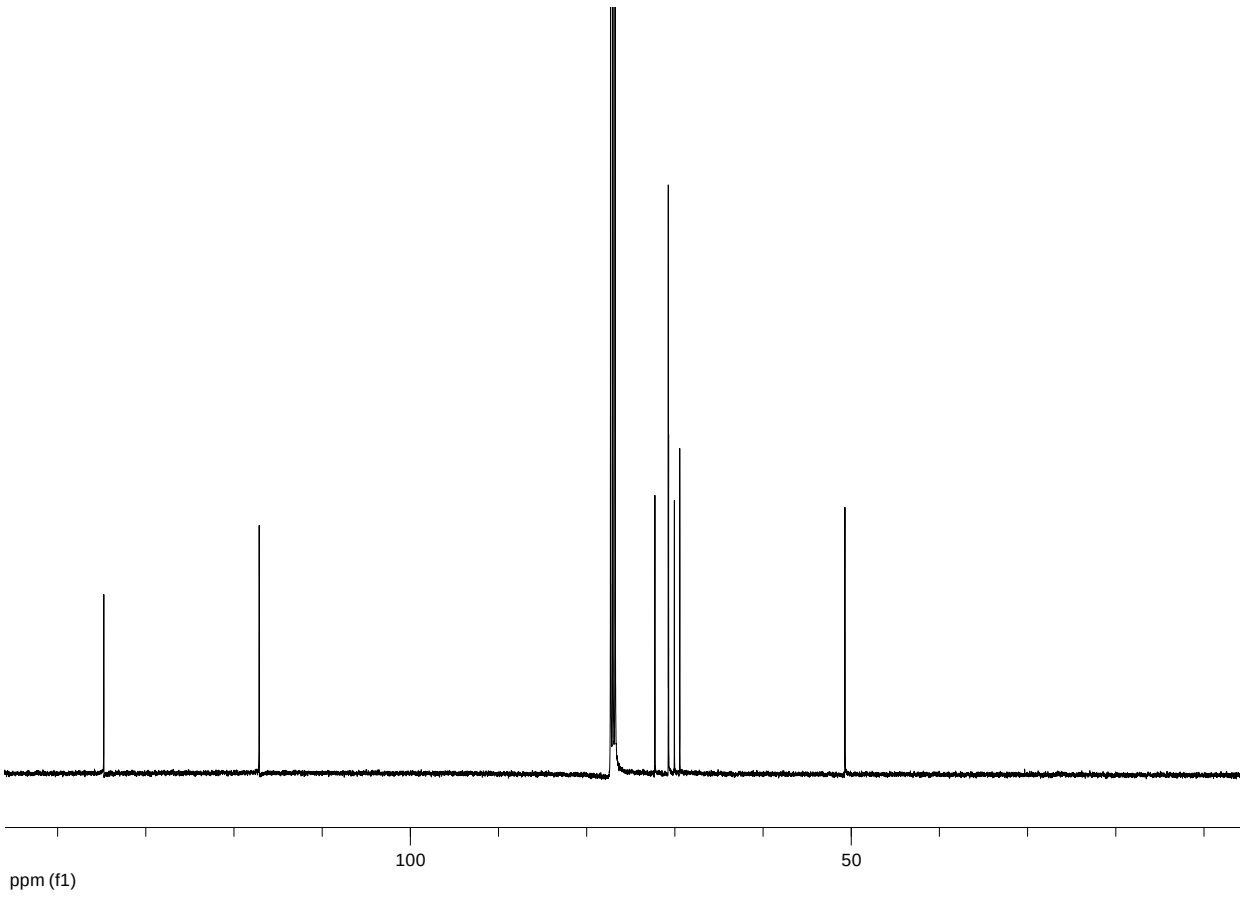
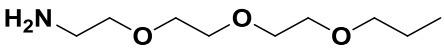


(**5**)





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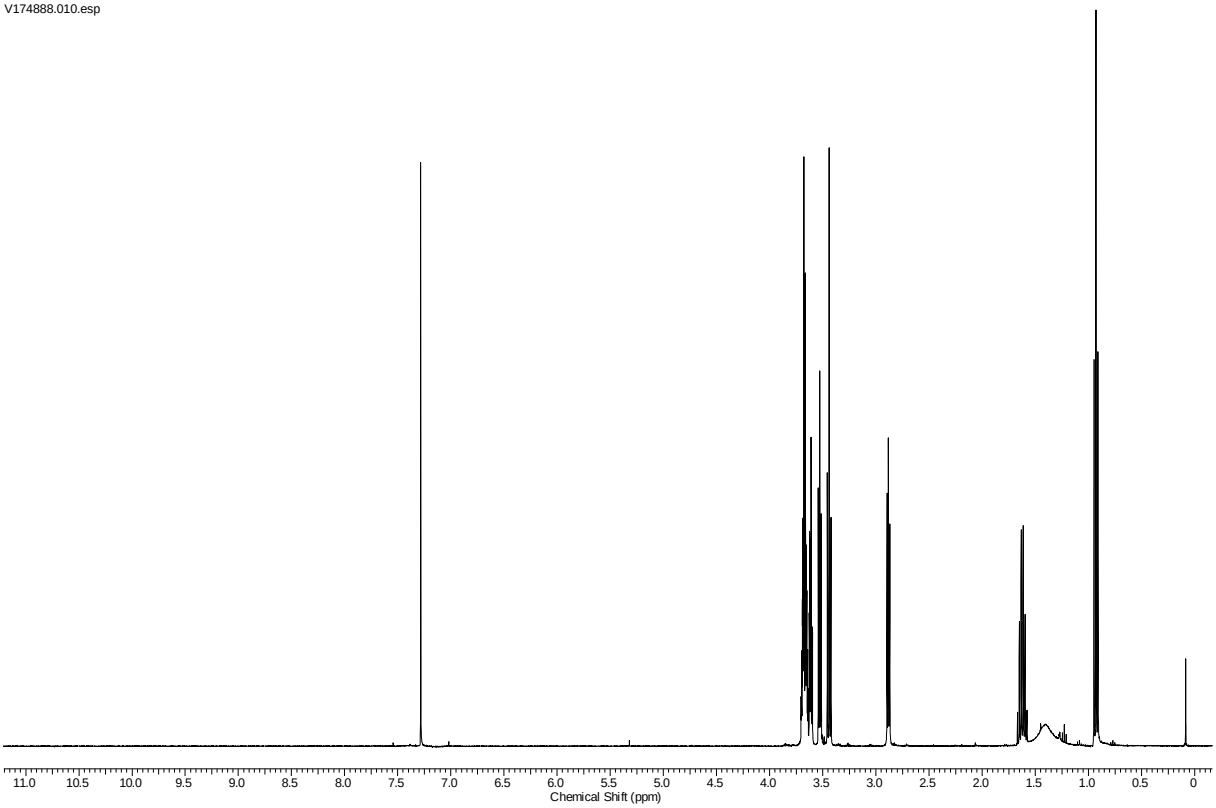

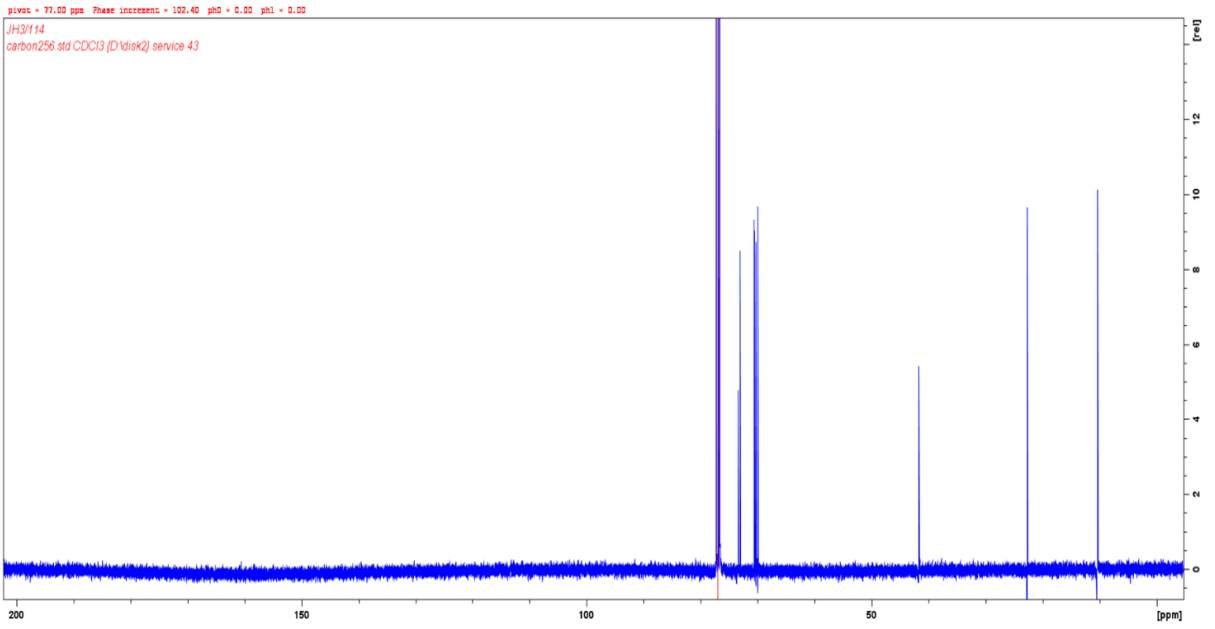

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1. Hodgkinson J.T., Galloway W.R.D.J, Saraf S., Baxendale I.R., Ley S.V., Ladlow M., Welch M., and Spring D.R. (2011) Microwave and flow syntheses of *Pseudomonas* quinolone signal (PQS) and analogues. Org. Biomol. Chem. 9, 57-61.

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