Biologically Targeted Probes for Zn\(^{2+}\): A Diversity Oriented Modular “Click-S\(_{\text{NAr}}\)-Click” Approach

J. Pancholi,\(^a\) D. J. Hodson,\(^b\) K. Jobe,\(^a\) G. A. Rutter,\(^b\) S. M. Goldup,\(^a\) and M. Watkinson\(^a\)

\(^a\)School of Biological and Chemical Science, Queen Mary University of London, Mile End Road, London, E1 4NS, U.K.
\(^b\)Section of Cell Biology, Department of Medicine, Imperial College London, Exhibition Road, London SW7 2AZ, U.K.
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1) General Experimental Information

All commercially available reagents, unless otherwise stated, and solvents for column chromatography were used as supplied without further purification. EDTA refers to ethylenediaminetetraacetic acid. EtOH was stored over 4 Å-molecular sieves. Et₃N was stored over KOH under a N₂ atmosphere. Reaction solvents were supplied dry from an MBRAUN MB SPS-800 solvent purification system. Petrol refers to the fraction of petroleum ether boiling in the range 40-60 °C. All glassware and needles were oven dried and cooled under an inert atmosphere prior to experimental use.

Infrared spectra were recorded in the range 4000-600 cm⁻¹, obtained directly from the compound as a solid or neat liquid on a Bruker Tensor 37 FTIR spectrometer. ¹H and ¹³C NMR spectra were recorded on a Bruker AV400 or AVIII 400 NMR spectrometer. Chemical shifts were reported in δ (ppm) and referenced to residual solvent. Multiplicities of signals are reported using standard abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad, and coupling constants measured in Hertz (Hz) and reported to 1 d.p.. Electrospray ionisation mass spectrometry was carried out by the EPSRC National Mass Spectrometry Service, University of Wales, Swansea on a Thermofisher LTQ Orbitrap XL. Melting points were measured on a Stuart SMP3 melting point apparatus and are uncorrected.

Flash column chromatography, unless stated otherwise was performed using VWR silica gel 60 (220-240 mesh), and TLC was carried out using pre-coated aluminium backed plates with Merck Kieselgel 60 F254. The plates were visualised under a UV lamp (254 nm), or by staining with basified aqueous KMnO₄ solution followed by gentle heating.

Fluorescence spectroscopic/spectrophotometric studies for characterisation were performed using a Jobin Yvon Horiba Fluorormax*-3 machine, in a 1 cm path length cell without an incident ray filter. Solutions of deprotected sensor were prepared from a stock solution in DMSO such that each 3 mL testing sample contained 1% DMSO. All aqueous testing was carried out using HEPES buffered water (0.1 mM, pH 7.4) at ambient temperature. Testing in MeCN was carried out using HPLC grade MeCN (VWR) which was used without any further purification.

Dynamic light scattering (DLS) measurements to determine average aggregate size of solutions of sensors 8 and 9b were obtained on a Malvern Zetasizer Nano ZS instrument equipped with a 633 nm laser. The measurement angle was 173 degrees. Samples were prepared as for the fluorescence measurements.
Tri-Boc protected cyclam (S1), tri-Boc protected cyclam acetylene (S2), propargyl-dipicolylamine (S3), diethyl iminodiacetate (S4), (4-azidobutyl)triphenylphosphonium bromide (S5), 3-azido-1-aminopropane (S6), 2-chloro-N-(2-diethylamino-ethyl)acetamide hydrochloride (S7), and 1-propargyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (S8), were all prepared following previously reported literature procedures. All spectroscopic data obtained matched those previously reported.
2) Experimental Procedures

6-Bromo-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (2):

Following a modified literature procedure, b 4-bromo-1,8-naphthalic anhydride (1.0 g, 4.1 mmol) was dissolved in 1,4-dioxane (30 mL). One portion of propargyl amine (0.26 mL, 4.1 mmol) was added to the solution. This was stirred at r.t. for 1 h, and then heated at 70 °C for 2 h. After cooling the reaction mixture to r.t., a further portion of propargyl amine (0.05 mL, 0.8 mmol) was added, and the reaction left to stir at 70 °C overnight. After cooling to r.t, the reaction mixture was slowly poured into iced water (120 mL). The resulting precipitate was collected by suction filtration and dried \textit{in vacuo} to give bromide 2 as a brown solid (1.1 g, 96%). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.70 (d, \(J = 7.3\), 1H, \(H_c\)), 8.60 (d, \(J = 8.5\), 1H, \(H_a\)), 8.46 (d, \(J = 7.9\), 1H, \(H_g\)), 8.06 (d, \(J = 7.9\), 1H, \(H_f\)), 7.87 (dd, \(J = 8.4, 7.4\), 1H, \(H_d\)), 4.95 (d, \(J = 2.4\), 2H, \(H_b\)), 2.20 (t, \(J = 2.4\), 1H, \(H_a\)); \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 162.9, 162.9, 133.8, 132.5, 131.7, 131.2, 130.9, 130.8, 129.1, 128.2, 122.8, 121.9, 78.3, 70.7, 29.6; IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)) 3391, 3257, 2923, 2106, 1700, 1659, 778; M.p. (°C) 177-179; HRMS (EI) calcd for C\textsubscript{15}H\textsubscript{8}BrNO\textsubscript{2} [M + H]\textsuperscript{+} 313.9811, found: 313.9809. UV: \(\lambda_{\text{max}}(\text{CH}_2\text{Cl}_2)/\text{nm} (\varepsilon / \text{mol}^{-1}\text{cm}^{-1}\text{dm}^{3})\) 357 (8379), 343 (10173), 328 (6896).
2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-6-bromo-1H-benzo[de]isoquinoline-1,3(2H)-dione (S9):

Acetylene 3 (1.0 g, 3.2 mmol), benzyl azide (0.43 g, 3.2 mmol), and Cul (60 mg, 10 mol%) were combined in a sealed CEM microwave vial which was purged with N₂. Anhydrous NMP (16 mL) was added, and the reaction stirred at r.t. for 1 h. The reaction mixture was diluted with a saturated solution of EDTA in 17.5% aqueous NH₃ (30 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (4:1 to 1:1 CH₂Cl₂/EtOAc) to yield triazole S9 as a pale yellow powder (1.35 g, 95%). ¹H NMR (400 MHz, CDCl₃) δ: 8.64 (d, J = 7.3, 1H, H₂), 8.55 (d, J = 8.5, 1H, H₁), 8.40 (d, J = 7.8, 1H, H₃), 8.02 (d, J = 7.8, 1H, H₄), 7.82 (dd, J = 8.5, 7.3, 1H, H₅), 7.56 (s, 1H, H₆), 7.37-7.29 (m, 4H, H₇ and H₈), 7.26-7.22 (m, 1H, H₉), 5.46 (s, 4H, H₁₀ and H₁₁); ¹³C NMR (101 MHz, CDCl₃) δ: 207.1, 163.5, 163.4, 134.7, 133.6, 132.5, 131.6, 131.2, 130.8, 130.8, 129.2, 128.8, 128.3, 128.2, 123.3, 123.1, 122.2, 54.3, 35.5, 31.1; IR: (νmax/cm⁻¹) 3133, 1700, 1653, 1586, 1337, 1049, 959, 779. M.p. (°C) 128-131. HRMS (El) calcd for C₂₂H₁₆⁷⁹BrN₄O₂ [M + H]⁺ 447.0451, found: 447.0450. UV: λmax(CH₂Cl₂)/nm (ε / mol⁻¹cm⁻¹dm³) 358 (3459), 343 (4066), 328 (2753).
2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-6-azido-1H-benzo[de]isoquinoline-1,3(2H)-dione (S10):

Bromo-triazole S9 (1.4 g, 3.2 mmol), and NaN₃ (0.25 g, 3.80 mmol) were combined in a sealed CEM microwave vial which was purged with N₂ and wrapped in foil. Anhydrous NMP (16 mL) was added, and the reaction stirred at r.t. for 24 h. The reaction mixture was diluted with water (30 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (1:1 Petrol/EtOAc) to yield azide S10 as a yellow powder (0.72 g, 55%).

**¹H NMR** (400 MHz, CDCl₃) δ, 8.64 (d, J = 7.2, 1H, H₈), 8.59 (d, J = 8.1, 1H, H₉), 8.44 (d, J = 8.4, 1H, H₇), 7.73 (dd, J = 7.4, 8.4, 1H, H₅), 7.55 (s, 1H, H₄), 7.46 (d, J = 8.0, 1H, H₆), 7.35-7.31 (m, 3H, H₃ and H₆), 7.26-7.22 (m, 2H, H₄ and H₅).

**¹³C NMR** (101 MHz, CDCl₃) δ, 174.6, 163.8, 163.3, 143.8, 134.7, 132.6, 132.2, 129.3, 129.1, 128.8, 128.3, 126.9, 124.5, 123.3, 122.5, 118.8, 114.8, 54.2, 35.4.

**IR:** (v max/cm⁻¹) 3142, 2923, 2120, 1655, 1579, 1325, 1233, 1046, 780. M.p. (°C) 160-162. HRMS (El) calcd for C₂₂H₁₆N₇O₂ [M + H]^⁺ 410.1360, found: 410.1358. UV: λ max(CH₂Cl₂)/nm (ε / mol⁻¹cm⁻¹dm³) 384 (9103), 367 (11295), 344 (7235).
2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-6-(4-phenyl-1H-1,2,3-triazol-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4):

![Chemical Structure](image)

Triazole-azide **S10** (0.050 g, 0.12 mmol), phenylacetylene (0.014 mL, 0.12 mmol), Cul (2.3 mg, 10 mol%) and NaOAc (1.1 mg, 10 mol%) were combined in a sealed CEM microwave vial which was purged with N₂. Anhydrous NMP (0.61 mL) was added, and the reaction stirred at r.t. for 16 h. The reaction mixture was diluted with a saturated solution of EDTA in 17.5% aqueous NH₃ (5 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (100% CH₂Cl₂ to 1:1 CH₂Cl₂/EtOAc) to yield bis-triazole 4 as a pale yellow powder (0.051 g, 81%).

**¹H NMR** (400 MHz, CDCl₃) δ, 8.75-8.68 (m, 2H, H₉ and H₈), 8.33 (d, J = 8.5, 1H, H₆), 8.25 (s, 1H, H₅), 7.96 (d, J = 7.4, 2H, H₄), 7.88 (d, J = 7.8, 1H, H₃), 7.86-7.82 (m, 1H, H₆), 7.39 (s, 1H, H₄), 7.52-7.48 (m, 2H, H₃), 7.43-7.40 (m, 1H, H₂), 7.36-7.31 (m, 3H, H₃ and H₂), 7.27-7.23 (m, 2H, H₁), 5.51 (s, 2H, H₂), 5.49 (s, 2H, H₁).

**¹³C NMR** (101 MHz, CDCl₃) δ, 163.5, 162.9, 148.7, 143.7, 138.5, 134.7, 132.7, 131.1, 129.9, 129.7, 129.4, 129.3, 129.2, 129.0, 128.8, 128.7, 128.3, 126.7, 126.1, 123.9, 123.6, 123.3, 123.0, 121.9, 54.3, 35.6. IR: (ν_max/cm⁻¹) 3128, 3066, 1705, 1658, 1584, 1401, 1121, 1023, 953, 850, 781, 761, 718. M.p. (°C) >230. HRMS (EI) calcd for C₃₀H₂₂N₇O₂ [M + H]^+ 512.1829, found: 512.1829. UV: λ_max(CH₂Cl₂)/nm (ε/mol⁻¹cm⁻¹dm³) 344 (9099).
2-(3-Azidopropyl)-6-bromo-1H-benzo[de]isoquinoline-1,3(2H)-dione (3):

4-Bromo-1,8-naphthalic anhydride (0.25 g, 0.91 mmol) was dissolved in EtOH (30 mL). 3-azido-1-aminopropane S6 (0.46 g, 4.6 mmol) was added to the solution and the reaction left to stir at reflux overnight. After slow cooling to r.t, the resulting precipitate was collected by suction filtration and purified by flash column chromatography (4:1 CH₂Cl₂/EtOAc) to give bromide 3 as a brown solid (0.26 g, 79%). ¹H NMR (400 MHz, CDCl₃) δ: 8.67 (d, J = 7.2, 1H, Hₐ), 8.59 (d, J = 8.5, 1H, Hₖ), 8.42 (d, J = 7.8, 1H, H₈), 8.05 (d, J = 7.8, 1H, H₉), 7.86 (m, 1H, H₇), 4.28 (t, J = 7.0, 2H, H₄), 3.44 (t, J = 6.8, 2H, H₃), 2.04 (app. quint, J = 6.8, 2H, H₂). ¹³C NMR (101 MHz, CDCl₃) δ: 163.8, 163.8, 133.6, 132.4, 132.4, 131.5, 131.3, 131.3, 130.8, 130.6, 129.2, 128.3, 123.1, 122.2, 49.6, 38.2, 27.7. M.p. (°C) 92-93. HRMS (EI) calcd for C₁₅H₉₁Br₇N₇O₂ [M + H]^⁺ 359.0138, found 359.0141. UV: λ_max(CH₂Cl₂)/nm (ε / mol⁻¹cm⁻¹dm³) 357 (11646), 343 (13675), 327 (9214).
6-Bromo-2-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (S11):

Azide 3 (0.20 g, 0.56 mmol), phenylacetylene (0.062 mL, 0.56 mmol), and Cul (10 mg, 10 mol%) were combined in a sealed CEM microwave vial which was purged with N₂. Anhydrous NMP (2.8 mL) was added, and the reaction stirred at r.t. for 1 h. The reaction mixture was diluted with a saturated solution of EDTA in 17.5% aqueous NH₃ and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (4:1 to 1:1 CH₂Cl₂/EtOAc) to yield triazole S11 as a pale yellow powder (0.17 g, 67%). ¹H NMR (400 MHz, CDCl₃) δ H 8.65 (d, J = 7.3, 1H, Hₐ), 8.56 (d, J = 8.5, 1H, Hₖ), 8.40 (d, J = 7.9, 1H, Hₙ), 8.02 (d, J = 7.9, 1H, Hₙ), 7.94 (s, 1H, Hₙ), 7.83 (dd, J = 8.3, 7.4, 1H, Hₖ), 7.75 (m, 2H, Hₙ), 7.42-7.36 (m, 2H, Hₙ), 7.34-7.28 (m, 1H, Hₙ), 4.54 (t, J = 6.9, 2H, Hₙ), 4.33 (t, J = 6.6, 2H, Hₙ), 2.48 (app. quint, J = 6.8, 2H, Hₙ). ¹³C NMR (101 MHz, CDCl₃) δ C 163.9, 163.9, 147.8, 133.7, 132.4, 131.6, 131.3, 130.8, 130.7, 129.1, 128.9, 128.3, 128.2, 125.7, 122.9, 122.0, 120.0, 48.6, 37.9, 28.8; IR: (v max/cm⁻¹) 3142, 3061, 1698, 1659, 1586, 1568, 1460, 1345, 1225, 1055, 970, 918, 810, 767, 696. M.p. (°C) 200-202. HRMS (EI) calcd for C₂₃H₁₇⁷⁹BrN₄O₂ [M + H]⁺ 461.0608, found 461.0604. UV: λ max(CH₂Cl₂)/nm (ε / mol⁻¹cm⁻³dm³) 358 (20486), 343 (23859), 327 (15782).
6-Azido-2-{3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione (S12):

Bromo-triazole S11 (50 mg, 0.11 mmol), and NaN₃ (9.0 mg, 1.2 equiv., 0.13 mmol) were combined in a sealed CEM microwave vial which was purged with N₂ and wrapped in foil. Anhydrous NMP (0.54 mL) was added, and the reaction stirred at r.t. for 24 h. The reaction mixture was diluted with H₂O (5 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (1:1 Petrol/EtOAc) to yield azide S12 as a yellow powder (35 mg, 76%). ¹H NMR (400 MHz, CDCl₃) δ, 8.63 (d, J = 7.3, 1H, Hₙ), 8.56 (d, J = 8.0, 1H, Hₜ), 8.44 (d, J = 8.4, 1H, Hₖ), 7.96 (s, 1H, H₂), 7.77-7.71 (m, 2H, H₉ and H₈), 7.45-7.35 (m, 4H, H₇ and H₆), 7.34-7.28 (m, 1H, H₅), 4.54 (t, J = 7.0, 2H, Hₚ), 4.33 (t, J = 6.6, 2H, Hₚ), 2.48 (app. quint, J = 6.8, 2H, Hₕ). Due to the instability of S12 we were unable to obtain further characterisation data and it as used in subsequent reactions immediately.
6-(4-Phenyl-1H-1,2,3-triazol-1-yl)-2-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5):

Triazole-azide S12 (64 mg, 0.15 mmol), phenylacetylene (0.02 mL, 0.15 mmol), CuI (2.8 mg, 10 mol%) and NaOAc (1.2 mg, 10 mol%) were combined in a sealed CEM microwave vial which was purged with N₂. Anhydrous NMP (0.76 mL) was added, and the reaction stirred at r.t. for 16 h. The reaction mixture was diluted with a saturated solution of EDTA in 17.5% aqueous NH₃ (10 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (100% CH₂Cl₂ to 1:1 CH₂Cl₂/EtOAc) to yield bis-triazole 5 as a pale yellow powder (64 mg, 81%). ¹H NMR (400 MHz, CDCl₃) δ 8.72 (d, J = 7.8, 1H), 8.71 (d, J = 7.2, 1H), 8.32 (d, J = 8.5, 1H), 8.21 (s, 1H), 7.97 (d, J = 7.3, 2H), 7.94 (s, 1H), 7.89-7.81 (m, 2H), 7.73 (d, J = 7.3, 2H), 7.55-7.49 (m, 2H), 7.47-7.35 (m, 3H), 7.34-7.28 (m, 1H), 4.57 (t, J = 6.8, 2H), 4.38 (t, J = 6.6, 2H), 2.53 (app. quint, J = 6.6, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 158.9, 158.9, 132.7, 131.1, 130.0, 129.7, 129.3, 129.1, 128.9, 128.8, 128.2, 126.2, 125.7, 123.6, 121.9, 120.0, 48.6, 38.2, 28.7. IR: (ν max/cm⁻¹) 3126, 3064, 2923, 2112, 1698, 1654, 1583, 1517, 1465, 1432, 1400, 1348, 1232, 1188, 1152, 1057, 976, 898, 783. M.p. (°C) 185-188. HRMS (EI) calcd for C₃₁H₂₃N₇O₂ [M + H]⁺ 526.1986, found 526.1979. UV: λ max(CH₂Cl₂)/nm (ε / mol⁻¹cm⁻³dm³) 345 (30104).
2-Chloro-N,N-didodecylacetamide (S13):

A solution of didodecylamine (2.00 g, 5.65 mmol) and NEt₃ (1.97 mL, 14.1 mmol) in dry CH₂Cl₂ (100 mL) was added in a slow dropwise manner to a solution of chloroacetyl chloride (0.54 mL, 6.79 mmol) in anhydrous CH₂Cl₂ (50 mL) at 0 °C under N₂. When the addition was complete, the red solution was warmed to r.t. and stirred for 24 h. The solution was filtered and the solid washed with chloroform (30 mL). The filtrate was further diluted with chloroform and washed with H₂O (2 x 200 mL), Na₂CO₃ solution (1 M, 2 x 200 mL), HCl solution (1 M, 2 x 200 mL) and brine (2 x 200 mL). The organic phase was dried over MgSO₄ and the solvent was removed in vacuo. The crude oil was purified by flash column chromatography (4:1 CH₂Cl₂/EtOAc) to yield chloride S13 as a clear oil (1.86 g, 76%). ¹H NMR (400 MHz, CDCl₃) δ: 4.05 (s, 2H, Hₐ), 3.36-3.22 (m, 4H, H₉), 1.65-1.47 (m, 4H, H₉), 1.36-1.17 (m, 36H, alkyl-H), 0.92-0.84 (m, 6H, H₉). ¹³C NMR (101 MHz, CDCl₃) δ: 166.2, 48.4, 46.3, 41.5, 32.0, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 27.5, 27.1, 27.0, 22.8, 14.3. IR: (νₑₛₑₑ/cm⁻¹) 2921, 2852, 1653, 1458, 1376, 1302, 1122, 925, 790. HRMS (EI) calcd for C₂₆H₅₂ClNO [M + H]⁺ 430.3810, found 430.3810.
2-Azido-N,N-didodecylacetamide (S14):

Chloride S13 (1.86 g, 4.31 mmol) and NaN₃ (1.68 g, 25 mmol) were combined in a flask purged with N₂, dissolved in dry DMF (60 mL) and stirred for 24 h at 65 °C. After cooling to r.t, the reaction mixture was slowly poured into icy H₂O (200 mL). The resulting precipitate was extracted with EtOAc (3 x 100 mL), the organic extracts combined and washed with saturated NaHCO₃ solution (2 x 200 mL), brine (2 x 200 mL) and H₂O (2 x 100 mL). The organic phase was dried over MgSO₄ and the solvent was removed in vacuo to give azide S14 as a viscous yellow oil (1.90 g, 98%). ¹H NMR (400 MHz, CDCl₃) δ, 3.87 (s, 2H, Hₐ), 3.31 (t, J = 7.6, 2H, Hₖ), 3.11 (t, J = 7.6, 2H, Hₖ), 1.58-1.48 (m, 4H, Hₙ), 1.35-1.20 (m, 36H, alkyl-H), 0.91-0.84 (m, 6H, Hₐ). ¹³C NMR (101 MHz, CDCl₃) δ, 166.9, 50.4, 47.6, 46.6, 32.0, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.2, 27.8, 27.1, 26.9, 22.8, 14.2. IR: (υ max/cm⁻¹) 2921, 2852, 2103, 1654, 1458, 1425, 1376, 1273, 720. HRMS (EI) calcd for C₂₆H₆₅N₄O [M + H]⁺ 437.4214, found 437.4215.
(4-Azidobutyl)triphenylphosphonium tetrafluoroborate (S15):

(4-Azidobutyl)triphenylphosphonium bromide S5 (2.27 g, 5.15 mmol) was dissolved in CH₂Cl₂ (40 mL). This solution was thoroughly washed with a saturated aqueous solution of ammonium tetrafluoroborate (50 mL). The organic phase was dried over MgSO₄ and the solvent was removed in vacuo to yield tetrafluoroborate S15 as a white solid (2.18 g, 95%). ¹H NMR (400 MHz, CDCl₃) δ 7.86-7.65 (m, 15H, Hₐ), 3.46-3.28 (m, 4H, Hₐ and Hₜ), 1.96-1.85 (m, 2H, Hₗ), 1.78-1.63 (m, 2H, Hₗ). ¹³C NMR (101 MHz, CDCl₃) δ 135.0 (J₉₋₃ = 2.9), 133.2 (J₉₋₃ = 9.9), 130.4 (J₉₋₃ = 12.7), 117.9 (J₉₋₃ = 86.4), 50.1, 28.8 (J₉₋₃ = 16.9), 21.2 (J₉₋₃ = 52.0), 19.5 (J₉₋₃ = 3.9). IR: (ν max/cm⁻¹) 2943, 2923, 2877, 2098, 1588, 1486, 1438, 1342, 1276, 1239, 1025, 749, 722, 689. M.p. (°C) 115-118. HRMS (EI) calcd for C₂₂H₂₃N₃P₁ [M − BF₄]⁺ 360.1624, found 360.1627.
2-Azido-N-(2-diethylamino-ethyl)acetamide (S16):

![Chemical Structure]

2-Chloro-N-(2-diethylamino-ethyl)acetamide hydrochloride S7 (0.83 g, 2.50 mmol) was dissolved in H₂O (12.5 mL), NaN₃ (1.61 g, 15.0 mmol) was added to the flask, and the reaction mixture stirred for 48 h at 65 °C. After cooling, the solution was adjusted to pH 10 using aqueous NaHCO₃ (1.0 M), and extracted with EtOAc (3 x 20 mL). The remaining aqueous layer was adjusted to pH 14 with aqueous NaOH (1.0 M), and extracted with EtOAc (3 x 20 mL). All organic extracts were combined, dried over MgSO₄ and the solvent was removed in vacuo (400 mbar – volatile product) to give azide S16 as a pale yellow oil (0.31 g, 72%).

\[
\begin{align*}
\text{H NMR (400 MHz, CDCl₃) } & \delta_{H} 6.82 \text{ (br s, 1H, H₆)}, 3.96 \text{ (s, 2H, Hₑ)}, 3.34 \text{ (q, J = 5.9, 2H, H₄)}, 2.42 \text{ (t, J = 6.0, 2H, H₂)}, 2.23 \text{ (s, 6H, Hₐ)}. \\
\text{¹³C NMR (101 MHz, CDCl₃) } & \delta_{C} 166.8, 57.8, 52.8, 45.3, 36.9. \\
\text{IR: } (\nu \text{ max/cm}^{-¹}) & 3292 (b), 3079, 2978, 2948, 2872, 2783, 2101, 1658, 1541, 1461, 1252, 1189, 1166, 1040, 906, 849, 776, 645. \\
\text{HRMS (EI) calcd for C₉H₁₃N₃O [M + H]} & \text{ found 172.1191.}
\end{align*}
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Diethyl 2,2’-(prop-2-yn-1-ylazanediyl)diacetate (S17):

Diethyl iminodiacetate S4 (1.00 g, 5.28 mmol) was dissolved in MeCN (30 mL). K₂CO₃ (1.46 g, 10.5 mmol) was added, and propargyl bromide (0.94 mL, 6.34 mmol) was added slowly to the stirring reaction mixture and refluxed for 16 h. After cooling, the suspension was filtered, and the solvent removed in vacuo. The crude oil was purified by flash column chromatography (2:8 EtOAc/Petrol) to yield alkyne S17 as a colourless oil (0.71 g, 58%). ¹H NMR (400 MHz, CDCl₃) δ 4.17 (q, J = 7.1, 4H, H₄), 3.66 (d, J = 2.3, 2H, H₂), 3.54 (s, 4H, H₃), 2.25 (t, J = 2.4, 1H, H₁), 1.27 (t, J = 7.1, 6H, H₆). ¹³C NMR (101 MHz, CDCl₃) δ 170.5, 78.4, 73.8, 60.8, 54.4, 43.4. IR: (ν max/cm⁻¹) 3278, 2978, 2870, 2105, 1733, 1444, 1372, 1190, 1027, 990. HRMS (EI) calcd for C₁₁H₁₈N₂O₄ [M + H]⁺ 228.1230, found 228.1224.
General procedure A: a one-pot synthesis of a sensor from building block 2.

Acetylene 2 (314 mg, 1.0 mmol), an azide (1.0 mmol), CuI (19.0 mg, 10 mol%) and NaOAc (8.2 mg, 10 mol%) were combined in a sealed CEM microwave vial which was wrapped in foil and purged with N₂. Anhydrous NMP was added, and the reaction was stirred at r.t. for 1 h. Upon complete consumption of 2 (determined by ¹H NMR spectroscopy), NaN₃ (78 mg, 1.2 mmol) was added in a single portion to the reaction vial, which was resealed and the reaction stirred for a further 24 h. Upon completion of azide formation (determined by ¹H NMR spectroscopy), an acetylene (1 mmol) and EtOH (1:1 w/ NMP) were added to the reaction vial, and the final mixture was stirred for a further 16 h, at which point the reaction was complete (determined by ¹H NMR spectroscopy).
General procedure B: a one-pot synthesis of a sensor from building block 3.

Azide 3 (359 mg, 1.0 mmol), an acetylene (1.0 mmol), CuI (19.0 mg, 10 mol%) and NaOAc (8.2 mg, 10 mol%) were combined in a sealed CEM microwave vial which was wrapped in foil and purged with N₂. Anhydrous NMP was added, and the reaction was stirred at r.t. for 1 h. Upon complete consumption of 3 (determined by ¹H NMR spectroscopy), NaN₃ (78 mg, 1.2 mmol) was added in a single portion to the reaction vial, which was resealed and the reaction stirred for a further 24 h. Upon completion of azide formation (determined by ¹H NMR spectroscopy), an acetylene (1 mmol) and EtOH (1:1 w/ NMP) were added to the reaction vial, and the final mixture was stirred for a further 16 h, at which point the reaction was complete (determined by ¹H NMR spectroscopy).
2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-6-(4-phenyl-1H-1,2,3-triazol-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4):

**General procedure A** was employed with 2 (0.31 g, 1.0 mmol), benzyl azide (0.13 g, 1.0 mmol) in NMP (3.33 mL), followed by phenylacetylene (0.11 mL, 1.0 mmol) and EtOH (3.33 mL). The crude product was obtained via precipitation with a saturated solution of EDTA in 17.5% aqueous NH$_3$ (20 mL) and purified by flash column chromatography (100% CH$_2$Cl$_2$ to CH$_2$Cl$_2$/EtOAc 1:1) to yield bis-triazole 4 as a pale yellow powder (0.43 g, 85%). All spectroscopic data in accordance with double triazole 4 **vide supra**.
General procedure B was employed with 3 (0.36 g, 1.0 mmol), phenylacetylene (0.11 mL, 1.0 mmol) in NMP (3.33 mL), followed by a second portion of phenylacetylene (0.11 mL, 1.0 mmol) and EtOH (3.33 mL). The crude product was obtained via precipitation with a saturated solution of EDTA in 17.5% aqueous NH₃ (20 mL) and purified by flash column chromatography (100% CH₂Cl₂ to CH₂Cl₂/EtOAc 1:1) to yield bis-triazole 5 a pale yellow powder (0.37 g, 72%). All spectroscopic data in accordance with double triazole 5 *vide supra*. 
2-(4-{6-[(bis(Pyridin-2-ylmethyl)amino)methyl]-1H-1,2,3-triazol-1-yl}-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)methyl]-1H-1,2,3-triazol-1-yl)-N,N-didodecylacetamide (6):

**General procedure A** was employed with 2 (314 mg, 1.00 mmol), azide S14 (0.48 g, 1.0 mmol) in NMP (3.33 mL), followed by propargyl dipicolylamine S3 (0.29 g, 1.1 mmol) and EtOH (3.33 mL).

Crude product obtained after dilution of the reaction mixture with CH$_2$Cl$_2$ (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH$_3$ (3 x 15 mL). The organic phase was dried over MgSO$_4$, concentrated *in vacuo* and purified by flash column chromatography on alumina (99:1 CH$_2$Cl$_2$/MeOH) to yield sensor 6 as a brown oil (0.78 g, 82%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.68 (d, J = 7.8, 1H, H$_h$), 8.67 (d, J = 7.1, 1H, H$_g$), 8.55-8.51 (m, 2H, H$_r$), 8.25 (d, J = 8.5, 1H, H$_j$), 8.15 (s, 1H, H$_l$), 7.91 (s, 1H, H$_e$), 7.82-7.75 (m, 2H, H$_i$ and H$_k$), 7.66 (app. td, J = 7.7, 1.6, 2H, H$_p$), 7.62-7.57 (m, 2H, H$_q$), 7.18-7.12 (m, 2H, H$_o$), 5.52 (s, 2H, H$_f$), 5.14 (s, 2H, H$_d$), 4.06 (s, 2H, H$_m$), 3.94 (s, 4H, H$_n$), 3.31-3.23 (m, 4H, H$_c$), 1.64-1.43 (m, 4H, H$_b$), 1.34-1.12 (m, 36H, alkyl-H), 0.85 (app. t, 6H, H$_a$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 164.3, 163.4, 162.9, 159.1, 149.3, 145.2, 143.4, 138.5, 136.6, 132.5, 131.1, 129.9, 129.3, 128.5, 126.5, 125.7, 125.3, 123.7, 123.5, 122.9, 122.3, 59.9, 50.8, 48.6, 47.9, 46.8, 35.5, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 27.6, 27.1, 26.9, 22.8, 14.2. IR: (v$_{max}$/cm$^{-1}$) 2922, 2852, 1703, 1661, 1587, 1467, 1430, 1369, 1233, 1040, 995, 783. HRMS (EI) calcld for C$_{56}$H$_{76}$N$_{11}$O$_{3}$ [M + H]$^+$ 950.6127, found 950.6117. UV: $\lambda_{max}$(CH$_2$Cl$_2$)/nm ($\epsilon$/ mol$^{-1}$cm$^{-1}$dm$^{3}$) 344 (20365)
Diethyl 2,2'-([(1-(2-didodecylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl]-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-1H-1,2,3-triazol-4-yl)methyl]azanediyl]diacetate (7):

**General procedure A** was employed with 2 (314 mg, 1.00 mmol), azide S14 (0.48 g, 1.0 mmol) in NMP (3.33 mL), followed by acetylene S17 (0.59 g, 1.1 mmol) and EtOH (3.33 mL). Crude product obtained after dilution of the reaction mixture with CH2Cl2 (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH3 (3 x 15 mL). The organic phase was dried over MgSO4, concentrated *in vacuo* and purified by flash column chromatography (4:1 CH2Cl2/EtOAc to EtOAc to 4:1 EtOAc/MeOH) to yield 7 as an orange foam (0.72 g, 76%). 1H NMR (400 MHz, CDCl3) δ 8.70 (d, J = 7.8, 1H, Hj), 8.69 (d, J = 7.2, 1H, Hg), 8.28 (d, J = 8.6, 1H, Hi), 8.13 (s, 1H, Hl), 7.91 (s, 1H, Hm), 7.92-7.78 (m, 2H, Hn and Hk), 5.54 (s, 2H, Hd), 5.15 (s, 2H, Hn), 4.23 (s, 2H, Hm), 4.18 (q, J = 7.1, Hn), 3.69 (s, 4H, Hp), 3.33-3.22 (m, 4H, Hc), 1.65-1.41 (m, 4H, Hb), 1.35-1.14 (m, 42H, Hp (6H) and alkyl-H (36H)), 0.90-0.80 (m, 6H, Ha). 13C NMR (101 MHz, CDCl3) δc 171.2, 164.3, 163.4, 162.9, 146.4, 143.5, 138.5, 132.6, 131.1, 129.9, 129.3, 128.6, 126.5, 125.7, 125.4, 123.9, 123.6, 122.9, 65.9, 60.8, 55.1, 50.9, 49.3, 48.0, 46.9, 35.6, 32.0, 29.8, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 27.6, 27.1, 26.9, 22.8, 15.4, 14.4, 14.2. IR: (ν max/cm⁻¹) 3145, 2921, 2852, 1739, 1704, 1659, 1585, 1465, 1369, 1232, 1184, 1026, 949, 783. M.p. (°C) 48-51. HRMS (EI) calcd for C52H78N9O7 [M + H]+ 940.6019, found 940.6010. UV: λ max(CH2Cl2)/nm (ε / mol⁻¹cm⁻³dm³) 344 (18804) .
Tri-tert-butyl 11-((1-(2-(1-(2-(didodecylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-1H-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (8a):

**General procedure A** was employed with 2 (314 mg, 1.00 mmol), azide S14 in NMP (3.33 mL), followed by cyclam acetylene S2 (0.59 g, 1.1 mmol) and EtOH (3.33 mL). Crude product was obtained by dilution of the reaction mixture with CH$_2$Cl$_2$ (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH$_3$ (3 x 15 mL). The organic phase was dried over MgSO$_4$, concentrated in vacuo and purified by flash column chromatography (4:1 CH$_2$Cl$_2$/EtOAC to EtOAc to 4:1 EtOAc/MeOH) to yield 8a as a brown oil (0.93 g, 75%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 8.67-8.59 (m, 2H, H$_g$ and H$_j$), 8.20 (app. br s, 1H, H$_i$), 7.98-7.72 (m, 4H, H$_e$ and H$_h$, H$_l$ and H$_k$), 5.47 (s, 2H, H$_f$), 5.09 (s, 2H, H$_d$), 3.89 (br s, 2H, H$_m$), 3.50-3.08 (m, 16H, H$_c$ and cyclam-H (12H)), 2.70-2.62 (m, 2H, H$_o$), 2.54-2.48 (m, 2H, H$_p$), 1.92-1.78 (m, 2H, H$_q$), 1.76-1.66 (m, 2H, H$_n$), 1.60-0.93 (m, 67H, H$_b$ (4H), $^3$Bu (27H) and alkyl-H (36H)), 0.86-0.72 (m, 6H, H$_a$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$: 164.2, 163.4, 162.8, 155.9, 155.6, 143.4, 138.4, 132.5, 131.1, 129.8, 129.9, 129.2, 128.7, 126.5, 125.3, 123.7, 123.5, 122.9, 79.7, 50.8, 47.9, 46.8, 35.5, 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.6, 28.5, 27.6, 27.0, 26.9, 22.7, 14.2. IR: (v$_{max}$/cm$^{-1}$) 2927, 2855, 1691, 1588, 1466, 1414, 1365, 1236, 1166, 782. HRMS (EI) calcd for C$_{29}$H$_{48}$N$_3$O$_2$ [M + H]$^+$ 1251.8149, found 1251.8577. UV: $\lambda_{max}$(CH$_2$Cl$_2$)/nm ($\varepsilon$ / mol$^{-1}$cm$^{-1}$dm$^3$) 344 (25178).
(4-{4-{1,3-Dioxo-6-{4-({4,8,11-tris(tert-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecan-1-yl}methyl)-1H-1,2,3-triazol-1-yl}-1H-benzo[d]isoquinolin-2(3H)-ylmethyl}-1H-1,2,3-triazol-1-yl}butyl)triphenylphosphonium (9a):

General procedure A was employed with 2 (314 mg, 1.00 mmol), azide S15 (0.45 g, 1.0 mmol) in NMP (5 mL), followed by cyclam acetylene S2 (0.59 g, 1.1 mmol) and EtOH (5 mL). Crude product obtained by dilution of the reaction mixture with CH₂Cl₂ (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH₃ (3 x 15 mL). The organic phase was dried over MgSO₄, concentrated in vacuo and triturated with EtOAc (3 x 30 mL). The resulting suspension was filtered, the filtrate concentrated in vacuo and purified by flash column chromatography (CH₂Cl₂/MeOH 99:1 to 9:1) to yield 9a as a yellow foam (0.84 g, 66%). ¹H NMR (400 MHz, CDCl₃) δ H, 8.65-8.61 (m, 2H, H₇ and Hᵢ), 8.26-8.21 (m, 1H, Hᵢ), 8.02 (br s, 1H, Hᵢ), 7.88-7.83 (m, 1H, Hᵦ), 7.80 (dd, J = 7.4, 8.4, 1H, Hᵦ), 7.75 (s, 1H, Hᵦ), 7.75-7.59 (m, 15H, 3 x Ph), 5.41 (s, 2H, Hᵦ), 4.38 (t, J = 6.5, 2H, Hᵦ), 3.93 (br s, 2H, Hᵦ), 3.45-3.25 (m, 14H, cyclam-H (12H) and Hᵦ), 2.74-2.68 (m, 2H, Hᵦ), 2.55 (t, J = 5.5, 2H, Hᵦ), 2.16 (quint, J = 6.7, 2H, Hᵦ), 1.93-1.86 (m, 2H, Hᵦ), 1.80-1.72 (m, 2H, Hᵦ), 1.63-1.51 (m, 2H, Hᵦ), 1.48-1.30 (m, 27H, 3 x Boc). ¹³C NMR (101 MHz, CDCl₃) δ C, 163.5, 163.0, 155.7, 143.6, 138.6, 135.3, 135.3, 133.6, 133.6, 132.6, 131.2, 130.7, 130.6, 129.3, 128.7, 128.7, 123.7, 123.7, 122.9, 118.4, 117.5, 79.8, 48.8, 35.7, 30.0, 29.8, 28.7, 28.6, 21.5, 21.0, 19.4, 19.3 (overlapping signals). IR: (ν max/cm⁻¹) 2981, 2930, 1684, 1664, 1588, 1478, 1441, 1411, 1364, 1237, 1157, 1111, 1050, 1049, 882. HRMS (EI) calcd for C₉₅H₉₈N₅O₂ [M – BF₄]⁺ 1174.6002, found 1174.5983. UV: λ max(CH₂Cl₂)/nm (ε / mol⁻¹ cm⁻¹ dm³) 344 (3139)
tri-tert-Butyl 11-((1-(2-((1-(2-((2-(dimethylamino)ethyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-1H-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (10a):

General procedure A was employed with 2 (314 mg, 1.0 mmol), azide S16 (0.17 g, 1.0 mmol) in NMP (5 mL), followed by cyclam acetylene S2 (0.59 g, 1.1 mmol) and EtOH (5 mL). The crude product was obtained by dilution of the reaction mixture with CH₂Cl₂ (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH₃ (3 x 15 mL). The organic phase was dried over MgSO₄, concentrated in vacuo and purified by flash column chromatography on alumina (EtOAc to 9:1 EtOAc/MeOH) to yield 10a as a brown oil (0.67 g, 68%). ¹H NMR (400 MHz, CDCl₃) 8.75-8.69 (m, 2H, Hh and Hk), 8.34-8.26 (m, 1H, Hj), 7.98-7.82 (m, 4H, Hi, Hl, Hm and Hn), 6.49 (br s, 1H, Hd), 5.56 (s, 2H, Hg), 4.99 (s, 2H, He), 3.96 (br s, 2H, Hn), 3.49-3.25 (m, 12H, cyclam-H), 3.29 (q, J = 5.8, 2H, Ha), 3.20 (t, J = 5.2, 2H, Ha), 2.58 (t, J = 5.8, 2H, Ha), 2.35 (t, J = 6.0, 2H, Ha), 2.15 (s, 6H, Hs (2 x CH₃), 1.95-1.70 (m, 4H, Hb + Hq), 1.49-1.35 (m, 27H, 3 x tBu). δ₁³C NMR (101 MHz, CDCl₃) 164.9, 163.5, 163.0, 143.9, 132.7, 131.2, 130.2, 130.1, 130.0, 129.4, 128.8, 126.6, 124.9, 123.7, 122.9, 79.8, 57.4, 53.2, 49.6, 45.1, 37.2, 35.5, 30.8, 28.7, 28.6, 17.8 (overlapping signals). IR: (ν max/cm⁻¹) 3409, 2966, 2919, 2850, 1691, 1688, 1593, 1476, 1420, 1369, 1260, 1165, 1096, 1017, 864, 801. HRMS (EI) calcd for C₄₉H₇₁N₁₃O₉ [M + H]+ 986.5570, found 986.5562. UV: λ max(CH₂Cl₂)/nm (ε / mol⁻¹ cm⁻¹ dm³) 344 (43812).
(2R,3R,4S,5R,6R)-2-(Acetoxy)methyl]-6-[[1-(3-6-(4-(bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl]-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl]-1H-1,2,3-triazol-4-yl)methoxy]tetrahydro-2H-pyran-3,4,5-triyl triacetate (11a):

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\begin{align*}
&\text{General procedure B was employed with 3 (359 mg, 1.00 mmol), propargyl-glucose S8 (0.36 g, 1.0 mmol) in NMP (3.33 mL), followed by propargyl dipicolylamine S3 (0.26 g, 1.1 mmol) and EtOH (3.33 mL). Crude product obtained by dilution of the reaction mixture with CH}_2\text{Cl}_2 (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH}_3 (3 x 15 mL). The organic phase was dried over MgSO}_4, concentrated in vacuo and purified by flash column chromatography on alumina (gradient from 99:1 to 90:10 CH}_2\text{Cl}_2/MeOH) to yield 11a as a yellow foam (0.58 g, 61%). 1H NMR (400 MHz, CDCl}_3): \delta 8.75-8.70 (m, 2H, H}_l+H}_o), 8.58-8.54 (m, 2H, H}_w), 8.32 (dd, J = 8.6, 1.0, 1H, H}_n), 8.19 (s, 1H, H}_q), 7.88-7.82 (m, 2H, H}_m+H}_p), 7.76 (s, 1H, H}_h), 7.69 (td, J = 7.7, 1.8, 2H, H}_u), 7.63-7.59 (m, 2H, H}_t), 7.30-7.16 (m, 2H, H}_v), 5.22 (t, J = 9.5, 1H, H}_d), 5.10 (t, J = 9.5, 1H, H}_c), 5.03 (dd, J = 9.5, 8.0, 1H, H}_e), 4.94 (d, J = 12.7, 1H, one of H}_g), 4.84 (d, J = 12.7, 1H, one of H}_h), (4.69 (d, J = 7.9, 1H, H)_i), 4.56-4.48 (m, 2H, H}_s), 4.33-4.24 (m, 3H, H and one of H}_o), 4.17 (dd, J = 12.2, 2.2, 1H, one of H}_j), 4.08 (s, 2H, H}_h), 3.96 (s, 4H, H}_o), 3.80-3.74 (m, 1H, H}_b), 2.43 (app. quint, J = 6.5, 2H, H}_j), 2.09 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.99 (s, 3H, Ac). 13C NMR (101 MHz, CDCl}_3): \delta 170.8, 170.3, 169.5, 163.9, 163.4, 159.1, 149.3, 145.1, 144.1, 138.8, 136.7, 132.6, 131.2, 130.3, 129.3, 128.7, 126.6, 125.8, 123.6, 123.6, 123.3, 122.7, 122.4, 99.6, 72.9, 72.0, 71.4, 68.6, 62.9, 62.0, 59.9, 53.5, 48.6, 48.4, 37.9, 29.0, 20.9, 20.8, 20.7 (overlapping signals). IR: (\nu_max/cm^-1) 3454 (weak), 3138, 3066, 2958, 1753, 1704, 1660, 1588, 1435, 1361, 1218, 1038, 995, 905, 785, 755. HRMS (EI) calcd for C_{47}H_{48}N_{10}O_{12}[M + H]^+ 945.3526, found 945.3517. UV: \lambda_{max} (CH}_2\text{Cl}_2)/nm (\epsilon/ mol^-1 cm^-1 dm^3) 344 (16280)
2,2’-(((1-(2-(1-(2-(Didodecylamino)-2-oxoethyl)-1H,1,2,3-triazol-4-yl)methyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-1H,1,2,3-triazol-4-yl)methyl)azanediyl)diacetic acid (7b):

To a stirred solution of 7a (28 mg, 0.030 mmol) in THF (0.15 mL) was added an aqueous solution of LiOH (2 equiv., 0.15 mL). The reaction mixture was stirred for 18 h at r.t., and the solvent removed in vacuo. The residue was re-suspended in CHCl₃ (5 mL), washed with aqueous citric acid solution (5% w/v, 5 mL), the organic phase dried over MgSO₄ and the solvent removed in vacuo to yield deprotected sensor 7b as an orange residue (14.0 mg, 53%). ¹H NMR (400 MHz, MeOD) δ H 8.72-8.66 (m, 2H, H₉ and H₇), 8.24 (d, J = 5.6, 1H, H₈), 7.98 (s, 1H, H₂), 7.96 (d, J = 5.2, 1H, H₄), 7.91-7.86 (m, 1H, H₆), 5.49 (s, 2H, H₅), 5.34 (s, 2H, H₃), 4.35 (s, 2H, H₉), 3.73 (s, 4H, H₄), 3.39-3.11 (m, 2H, H – obscured by solvent peak), 1.68-1.46 (m, 4H, H₉), 1.37-1.13 (m, 36H, alkyl-H), 0.89-0.84 (m, 6H, H₈).

¹³C NMR (101 MHz, MeOD) δ C 166.5, 164.5, 164.0, 156.7, 144.3, 140.3, 133.1, 132.2, 131.8, 131.4, 131.0, 130.6, 130.1, 129.6, 128.4, 128.3, 127.6, 127.1, 125.0, 124.9, 123.8, 121.2, 51.8, 49.6, 47.7, 35.9, 32.8, 30.5, 30.4, 30.4, 30.2, 30.1, 29.6, 28.3, 27.7, 27.6, 23.5, 14.4. IR: (ν max/cm⁻¹) 3357 (broad), 2923, 2854, 1705, 1588, 1465, 1423, 1377, 1235, 1120, 1052, 952, 785. HRMS (EI) calcd for C₄₈H₇₀N₁₀O₇ [M + H]⁺ 884.5393, found 884.5391. UV: λ max(CH₂Cl₂)/nm (ε / mol⁻¹cm⁻¹dm³) 344 (9173)
2-(4-((6-(1,4,8,11-Tetraazacyclotetradecan-1-yl)-1H-1,2,3-triazol-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)methyl)-1H-1,2,3-triazol-1-yl)-N,N-didodecylacetamide (8b):

Compound 8a (50 mg, 0.040 mmol) was dissolved in a solution of TFA (20%) in CH$_2$Cl$_2$ (1 mL), and for 6 h at r.t. The reaction mixture was concentrated in vacuo, re-suspended in CHCl$_3$ (5 mL) and washed with aqueous NaOH (1 M, 5 mL). The organic phase was dried over MgSO$_4$ and the solvent was removed in vacuo to yield deprotected sensor 8b as a yellow residue (34.0 mg, 89%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.72-8.64 (m, 2H, H$_g$ and H$_i$), 8.30 (d, $J$ = 8.5, 1H, H$_j$), 8.15 (s, 1H, H$_l$), 7.90 (s, 1H, H$_e$), 7.85 (d, $J$ = 7.8, 1H, H$_d$), 7.83-7.78 (m, 1H, H$_o$), 5.52 (s, 2H, H$_f$), 5.14 (s, 2H, H$_d$), 3.98 (s, 2H, H$_m$), 3.33-3.20 (m, 4H, H$_c$), 2.84-2.62 (m, 12H, cyclam-H), 2.00-1.87 (m, 12H, H$_n$, H$_q$, H$_p$), 1.70-1.42 (m, 6H, H$_o$, H$_r$, H$_u$), 1.36-1.12 (m, 40H, H$_b$ (4H), and alkyl-H (36H)), 0.90-0.82 (m, 6H, H$_a$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 164.3, 163.4, 162.9, 145.8, 143.5, 138.6, 132.5, 131.1, 129.9, 129.3, 128.6, 126.5, 125.3, 125.2, 123.8, 123.6, 123.0, 55.2, 53.8, 51.0, 50.9, 50.0, 49.4, 48.8, 48.2, 48.0, 47.8, 47.4, 46.9, 35.6, 32.0, 29.7, 29.7, 29.7, 29.6, 29.4, 29.3, 29.2, 28.8, 27.6, 27.1, 26.9, 26.3, 22.8, 14.2. IR: ($\tilde{v}$ max/cm$^{-1}$) 2927, 2850, 1695, 1578, 1460, 1403, 1368, 1244, 1156, 807, 782. HRMS (El) calcd for C$_{54}$H$_{88}$N$_{12}$O$_3$ [M + H]$^+$ 951.7019, found 951.7022. UV: $\lambda_{\text{max}}$(CH$_2$Cl$_2$)/nm ($c$ / mol$^{-1}$ cm$^{-1}$ dm$^3$) 345 (8389)
(4-{4-{6-{4-{1,4,8,11-Tetraazacyclotetradecan-1-yl}methyl}-1H-1,2,3-triazol-1-yl}-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl}methyl)-1H-1,2,3-triazol-1-yl}butyl)triphenylphosphonium trifluoroacetate (9b):

Compound 9a (20 mg, 0.016 mmol) was dissolved in a solution of TFA (20%) in CH$_2$Cl$_2$ (1 mL), and for 6 h at r.t. The reaction mixture was concentrated in vacuo, re-suspended in CHCl$_3$ (5 mL) and washed with aqueous NaOH (1 M, 5 mL). The organic phase was dried over MgSO$_4$ and the solvent was removed in vacuo to yield deprotected sensor 9b as a yellow residue (12.0 mg, 74%) $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 8.71-8.64 (m, 2H, H$_g$ and H$_j$), 8.30 (dd, $J = 8.6$, 0.9, 1H, H$_i$), 8.16 (s, 1H, H$_l$), 7.87 (d, $J = 7.8$, 1H, H$_k$), 7.83-7.58 (m, 16H, H$_n$ and 3 x Ph), 7.80 (s, 1H, H$_e$), 5.46 (s, 2H, H$_f$), 4.47 (t, $J = 6.3$, 2H, H$_d$), 3.98 (s, 2H, H$_m$), 3.64-3.55 (m, 2H, H$_c$), 2.87-2.64 (m, 16H, cyclam-H (12H) and H$_p$ + H$_o$), 2.22 (app. quint, $J = 6.6$, 2H, H$_b$), 1.99-1.90 (m, 2H, H$_q$), 1.74-1.65 (m, 2H, H$_a$), 1.63-1.52 (m, 2H, H$_a$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$: 163.5, 163.1, 145.6, 143.6, 138.7, 138.2, 135.2, 135.2, 133.7, 133.7, 132.6, 131.2, 130.7, 130.6, 129.4, 128.7, 127.2, 126.7, 125.4, 123.8, 123.7, 123.0, 118.5, 117.9, 53.9, 50.9, 49.4, 48.8, 48.7, 47.7, 44.0, 35.8, 29.8, 29.8, 21.2, 19.4. $^{19}$F NMR (376MHz, CDCl$_3$) $\delta$: -75.0. $^{31}$P NMR (162 MHz, CDCl$_3$) $\delta$: 24.3. IR: ($\nu_{max}$/cm$^{-1}$) 3509 (b), 2966, 2943, 1663, 1585, 1482, 1443, 1419, 1363, 1325, 1232, 1167, 1108, 1030, 863, 802. HRMS (EI) submitted calcd for [M–anion]$^+$ 874.4429, found 874.4425. UV: $\lambda_{max}$(CH$_2$Cl$_2$)/nm ($\epsilon$ / mol$^{-1}$cm$^{-1}$dm$^3$) 345 (10549)

S30
2-(4-{[6-{[1,4,8,11-Tetraazacyclotetradecan-1-yl]methyl}-1H-1,2,3-triazol-1-yl]-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl]methyl}-1H-1,2,3-triazol-1-yl}-N-[2-(dimethylamino)ethyl]acetamide (10b):

Compound 10a (20.0 mg, 0.016 mmol) was dissolved in a solution of TFA (20%) in CH₂Cl₂ (1 mL), and for 6 h at r.t.. The reaction mixture was concentrated in vacuo, re-suspended in CHCl₃ (5 mL) and washed with aqueous NaOH (1 M, 5 mL). The organic phase was dried over MgSO₄ and the solvent was removed in vacuo to yield deprotected sensor 10b as a yellow residue (8.9 mg, 63%) ¹H NMR (400 MHz, CDCl₃) δ, 8.72-8.66 (m, 2H, Hₗ + Hₘ), 8.31 (d, J = 6.5, 1H, Hₖ), 8.15 (s, 1H, Hₜ), 7.87 (d, J = 7.8, 1H, Hₚ), 7.84 (s, 1H, Hₚ), 7.81 (dd, J = 7.4, 8.5, 1H, H, and Hₘ), 6.57 (br s, 1H, Hₙ), 5.53 (s, 2H, Hₚ), 4.99 (s, 2H, Hₘ), 3.98 (s, 2H, Hₙ), 3.28 (q, J = 5.7, 2H, Hₕ), 2.85-2.60 (m, 16H, Hₗ and Hₚ and cyclam-H), 2.33 (t, J = 6.0, 2H, Hₔ), 2.13 (s, 6H, Hₕ (2 x CH₃)), 1.96-1.89 (m, 2H, Hₕ), 1.70-1.62 (m, 2H, Hₕ). ¹³C NMR (101 MHz, CD₃CN) δ, 168.4, 164.4, 163.8, 160.9, 160.7, 160.5, 144.1, 138.8, 132.9, 131.6, 130.4, 129.6, 129.6, 127.1, 127.0, 126.6, 124.9, 118.3, 116.0, 58.5, 55.5, 52.9, 50.7, 49.0, 47.9, 47.7, 45.9, 45.5, 44.1, 36.1, 35.7, 31.0, 24.7, 23.4, 18.2. IR: (ν max/cm⁻¹). UV: λ max(CH₂Cl₂)/nm (ε / mol⁻¹ cm⁻¹ dm³) 345 (11800)
6-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-2-(3-(4-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (11b):

Following a modified literature procedure, sensor 11a (25 mg, 0.026 mmol) was dissolved in MeOH (0.5 mL) and NaOMe (0.3 eq) was added to the solution. The reaction mixture was stirred for 4 h, and evaporated to dryness in vacuo to yield deprotected sensor 11b as a yellow residue (16.0 mg, 79%). $^1$H NMR (400 MHz, MeOD) $\delta$: 8.84-8.77 (m, 2H, H$_l$ + H$_o$), 8.60 (s, 1H, H$_q$), 8.58-8.55 (m, 2H, H$_w$), 8.33 (d, J = 8.6, 1H, H$_i$), 8.17 (s, 1H, H$_n$), 8.07 (d, J = 7.8, 1H, H$_q$), 8.04-7.98 (m, 1H, H$_m$), 7.90 (td, J = 7.7, 1.6, 2H, H$_i$), 7.86-7.81 (m, 2H, H$_j$), 7.41-7.35 (m, 2H, H$_m$), 5.01 (d, J = 12.5, 1H, one of H$_g$), 4.86-4.82 (m, 1H, one of H$_g$ – obscured by solvent peak, identified by cross peak on COSY), 4.66 (t, J = 6.8, 2H, H$_i$), 4.48 (d, J = 7.7, 1H, H$_i$), 4.39 (t, J = 7.0, 2H, H$_i$), 4.16 (s, 2H, H$_i$), 4.06 (s, 4H, H$_i$), 4.00 (dd, J = 12.1, 1.6, 1H, one of H$_g$), 3.80 (dd, J = 11.8, 5.3, 1H, one of H$_g$), 3.50-3.40 (m, 3H, H$_c$ and H$_d$ and H$_e$ – obscured by solvent peak, identified by cross peak on COSY), 3.34 (dd, J = 9.0, 7.8, 1H, H$_b$), 2.54 (quint, J = 7.0, 2H, H$_j$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$: 159.8, 149.3, 145.3, 139.4, 138.5, 133.2, 131.8, 130.1, 127.5, 125.2, 124.8, 123.7, 120.7, 103.1, 95.6, 77.7, 77.6, 74.7, 71.4, 62.7, 62.6, 60.5, 49.8, 49.6, 38.7. IR: (v$_{max}$/cm$^{-1}$) 3378 (broad), 2929, 2831, 1699, 1659, 1623, 1592, 1476, 1434, 1394, 1352, 1268, 1233, 1103, 1082, 1046, 996, 842. HRMS (EI) calcd for C$_{36}$H$_{40}$N$_{10}$O$_8$ [M + H]$^+$ 777.3103, found 777.3104. UV: $\lambda_{max}$(CH$_2$Cl$_2$)/nm ($\varepsilon$/mol$^{-1}$cm$^{-1}$dm$^3$) 345 (13126).
3) UV and Fluorescence Spectroscopic Characterisation

a) Sensor 6 - for full aggregation phenomena studies see Section 4.

![UV-vis absorption spectrum of sensor 6 (MeCN)](image1)

*Fig. S1* UV-vis absorption spectrum of sensor 6 (MeCN)

![Fluorescence response of sensor 6 (50 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).](image2)

*Fig. S2* Fluorescence response of sensor 6 (50 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).
**Fig. S3** Fluorescence response of sensor 6 (100 μM) with increasing amounts of Zn\(^{2+}\) in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, \(\lambda_{ex} = 347\) nm).

**Fig. S4** Fluorescence response of sensor 6 (100 μM) with increasing amounts of Zn\(^{2+}\) in MeCN (ambient temperature, \(\lambda_{ex} = 347\) nm).
b) Sensor 7b

Fig. S5  UV-vis absorption spectrum of sensor 7b (MeCN)

Fig. S6 Fluorescence response of sensor 7b (100 μM) with increasing amounts of Zn$^{2+}$ in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, $\lambda_{ex} = 347$ nm).
Fig. S7 Fluorescence response of sensor 7b (100 μM) with increasing amounts of Zn²⁺ in MeCN (ambient temperature, $\lambda_{ex} = 347$ nm).

c) Sensor 8b

Fig. S8 UV-vis absorption spectrum of sensor 8b (MeCN)
Fig. S9 Fluorescence response of sensor 8b (50 μM) with increasing amounts of Zn$^{2+}$ in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, $\lambda_{ex} = 347$ nm).

Fig. S10 Fluorescence response of sensor 8b (100 μM) with increasing amounts of Zn$^{2+}$ in MeCN (ambient temperature, $\lambda_{ex} = 347$ nm).

d) Sensor 9b
Fig. S11 UV-vis absorption spectrum of sensor 9b (MeCN)

Fig. S12 Fluorescence response of sensor 9b (50 μM) with increasing amounts of Zn$^{2+}$ in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, $\lambda_{ex} = 347$ nm}
Fig. S13 Fluorescence response of sensor 9b (100 μM) with increasing amounts of Zn$^{2+}$ in MeCN (ambient temperature, $\lambda_{ex} = 347$ nm).

d) Sensor 10b

Fig. S14 UV-vis absorption spectrum of sensor 10b (MeCN)
**Fig. S15** Fluorescence response of sensor 10b (100 μM) with increasing amounts of Zn$^{2+}$ in aqueous buffer (10 mM HEPES, pH 7.4, ambient temperature, $\lambda_{ex} = 347$ nm).

**Fig. S16** Fluorescence response of sensor 10b (100 μM) with increasing amounts of Zn$^{2+}$ in MeCN (ambient temperature, $\lambda_{ex} = 347$ nm).
f) Sensor 11b

*Fig. S17* UV-vis absorption spectrum of sensor 11b (MeCN)

*Fig. S18* Fluorescence response of sensor 11b (50 μM) with increasing amounts of Zn$^{2+}$ in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, $\lambda_{ex} = 347$ nm).
**Fig. S19** Fluorescence response of sensor 11b (100 μM) with increasing amounts of Zn$^{2+}$ in MeCN (ambient temperature, λ$_{ex}$ = 347 nm).

**g) Determination of Kd values for sensors 8b, 9b, 10b, and 11b**

Fluorescence titration was used to determine the dissociation constant (K$_d$) and F(max) of probes 8b, 9b, 10b, and 11b in HEPES buffer (1% DMSO). Each sensor was titrated at a constant sensor concentration of 50 μM with increasing equivalents of Zn$^{2+}$ and the fluorescence output (F) measured. The relative increase in fluorescence (F/F(0)) was plotted against the ratio of probe to Zn$^{2+}$ (CM/CL) and fit to the 1:1 binding isotherm (equation shown in Fig. S20) using the non-linear regression function of Graphpad Prism 5. The values obtained are tabulated in Table S1. In the case of probes 9b and 10b that target the lysosome and mitochondria respectively titrations were also performed at the relevant pH of the organelle. The poor fitting of sensors 9b and 11b is currently under investigation.

$$\frac{F}{F(0)} = 1 + \left(\frac{F(\text{max})-F(0)}{2F(0)} \right) \times \left(1 + \frac{CM}{CL} + \frac{K_d}{CL} - \left[\left(1 + \frac{CM}{CL} + \frac{K_d}{CL}\right)^2 - 4\frac{CM}{CL}\right]^{0.5}\right)$$

**Fig. S20** Equation used to determine K$_d$ values for sensors 8b, 9b, 10b and 11b where CM and CL concentrations of Zn$^{2+}$ and probe respectively, F is the observed fluorescence, F(0) is the observed fluorescence of the probe alone (ie CM = 0). Non-linear regression analysis was used to determine F(max)/F(0) and K$_d$. 
**Fig. S21** Example titration plot and non-linear curve fitting of Sensor 8b.

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<th>Sensor</th>
<th>$K_d$ (calculated)</th>
<th>$F_{\text{max}}/F(0)$</th>
<th>$R^2$</th>
<th>$K_d$ Lit. value$^{2,10}$</th>
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<td>8b</td>
<td>$2.96 \times 10^{-6}$ (pH 7.4)</td>
<td>4.3</td>
<td>0.9926</td>
<td>$4.3 \times 10^{-8}$ M$^{-1}$</td>
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<td>9b</td>
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<td>0.7438</td>
<td>$4.3 \times 10^{-8}$ M$^{-1}$</td>
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<td></td>
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<td>0.9223</td>
<td>$4.3 \times 10^{-8}$ M$^{-1}$</td>
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<td></td>
<td>$1.38 \times 10^{-7}$ (pH 5.8)</td>
<td>10.0</td>
<td>0.9019</td>
<td></td>
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<tr>
<td>11b</td>
<td>$2.12 \times 10^{-7}$ (pH 7.4)</td>
<td>2.6</td>
<td>0.6482</td>
<td>$1.2 \times 10^{-8}$ M$^{-1}$</td>
</tr>
</tbody>
</table>

**Table S1** Values for $K_d$ and $F_{\text{max}}/F(0)$ obtained by non-linear regression analysis.
4) Aggregation Studies of Sensor 6

The aggregation of probe 6 was studied by investigating the effect of concentration on its response to Zn\(^{2+}\), the effect of solvent composition and dynamic light scattering.

a) Effect of Probe Concentration of the Fluorescence Response of Probe 6

![Graph](image)

**Fig. S22** Fluorescence response of the titration of sensor 6 (100 \(\mu\)M) with increasing amounts of Zn\(^{2+}\) in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, \(\lambda_{ex} = 347\) nm).

![Graph](image)

**Fig. S23** Fluorescence response of the titration of sensor 6 (50 \(\mu\)M) with increasing amounts of Zn\(^{2+}\) in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, \(\lambda_{ex} = 347\) nm).
Fig. S24 Fluorescence response of the titration of sensor 6 (10 μM) with increasing amounts of Zn²⁺ in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ<sub>ex</sub> = 347 nm).

Fig. S25 Graphical representation of the red-shift in λ<sub>em</sub> upon binding of 1 equiv. Zn²⁺ with sensor 6 (100 μM) in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ<sub>ex</sub> = 347 nm).
**Fig. S26** HEPES/MeCN titration of sensor 6 (100 μM) (0.1 mM HEPES, pH 7.4, ambient temperature, $\lambda_{ex} = 347$ nm).

**Fig. S27** HEPES/MeCN titration of sensor 6 (100 μM) with 5 equiv. of Zn$^{2+}$ (0.1 mM HEPES, pH 7.4, ambient temperature, $\lambda_{ex} = 347$ nm).

**b) Dynamic Light Scattering Study**

Dynamic light scattering (DLS) experiments were performed at a sensor concentration of 30 μM in HEPES buffer (1% DMSO). Sensor 6 demonstrates aggregation phenomena in aqueous solution, as demonstrated by the large particle size observed in these samples. The aggregates are at their largest with no Zn$^{2+}$ present; in the presence of Zn$^{2+}$ the particle size decreases greatly, coinciding
with an increase in fluorescence emission upon addition of Zn$^{2+}$ to the sensor. Samples containing 11b on the other hand, structurally similar to 6 but with a different biological targeting unit, show no significant aggregation as demonstrated by the low degree of scattering and poor signal quality, demonstrating the difference small structural changes can have on the behaviour of these probes.

- Sensor 6

Size Distribution Report by Intensity
v2.2

Sample Details
Sample Name: JP2107 Water 30uM 0 1
SOP Name: mansettings.nano
General Notes: Average result created from record number(s): 1 2 3

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<th>Water</th>
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<td>Dispersant RI:</td>
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<td>Material RI:</td>
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<td>Material Absorption:</td>
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<td>Measurement Date and Time:</td>
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Measurement Position (mm): 1.25
Cell Description: Disposable sizing cuvette
Attenuator: 7

Results

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<td>Intercept:</td>
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<td>Peak 2:</td>
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Result quality: Refer to quality report

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Record 16: JP2107 Water 30uM 0 1
Malvern

Sample Details
Sample Name: JP2-107 30uM 1 zinc 1
SOP Name: mansettings.nano

File Name: ZET0002894.dts
Record Number: 2
Material ID: 1.59
Material Absorption: 0.010

Dispersant Name: Water
Dispensant RI: 1.330
Viscosity (cP): 0.9781
Measurement Date and Time: 17 October 2013 14:38:36

System
Temperature (°C): 21.0
Duration Used (s): 70
Count Rate (kcps): 191.6
Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette
Attenuator: 7

Results

Size (d.nm): | % Intensity: | St Dev (d.nm):
---|---|---
Z-Average (d.nm): 260.6 | Peak 1: 290.0 | 113.5
Pdb: 0.165 | Peak 2: 0.000 | 0.000
Intercept: 0.968 | Peak 3: 0.000 | 0.000

Result quality: Good

Size Distribution by Intensity

Intensity (Record): 16
Size (d.nm): 0.1 1 10 100 1000 10000

Record 2: JP2-107 30uM 1 zinc 1
Sensor 6 + 5 equiv. Zn$^{2+}$:

Size Distribution Report by Intensity
v2.2

Sample Details
Sample Name: JP2-107 30uM 5 zinc 1
SOP Name: mansettings.nano

General Notes:

File Name: ZET0002894.dls
Record Number: 3
Material Ref: 1.59
Material Absorption: 0.010

Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.9781
Measurement Date and Time: 17 October 2013 14:45:15

System
Temperature (°C): 21.0
Duration Used (s): 80
Count Rate (kcps): 150.6
Measurement Position (mm): 4.65
Cell Description: Disposable cuvette
Attenuator: 7

Results

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Result quality: Good

Size Distribution by Intensity

Record 3: JP2-107 30uM 5 zinc 1
Sensor 6 + 15 equiv. Zn$^{2+}$:

Size Distribution Report by Intensity
v2.2

Sample Details
- Sample Name: JP2-107 30uM 15 zinc 1
- SOP Name: mansettings.nano
- General Notes:

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System
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- Duration Used (s): 70
- Count Rate (kcps): 185.7
- Measurement Position (mm): 4.65
- Cell Description: Disposable cuvette
- Attenuator: 7

Results
- Z-Average (d.nm): 247.2
- Pdl: 0.187
- Intercep: 0.972
- Peak 1: 279.7 100.0 116.4
- Peak 2: 0.000 0.0 0.000
- Peak 3: 0.000 0.0 0.000

Result quality: Good

Size Distribution by Intensity

Record 4: JP2-107 30uM 15 zinc 1
Sensor 11b blank:

Size Distribution Report by Intensity
v2.2

Sample Details
Sample Name: JP2-156D 30µM 0 zinc 1
SOP Name: marnsetings.nano
General Notes:

File Name: ZET0002894.dts
Dispansant Name: Water
Record Number: 5
Dispansant RI: 1.330
Material RI: 1.59
Viscosity (cP): 0.9781
Material Absorption: 0.010
Measurement Date and Time: 17 October 2013 14:56:37

System
Temperature (C): 21.0
Count Rate (kcps): 116.7
Count Rate Used (kcps): 70
Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette
Attenuator: 10

Results

<table>
<thead>
<tr>
<th>Size (d.nm)</th>
<th>% Intensity</th>
<th>St Dev (d.nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-Average (d.nm): 552.7</td>
<td>Peak 1: 144.9</td>
<td>15.18</td>
</tr>
<tr>
<td>PDI: 0.638</td>
<td>Peak 2: 16.53</td>
<td>8.5</td>
</tr>
<tr>
<td>Intercept: 1.03</td>
<td>Peak 3: 0.000</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Result quality: Refer to quality report

Size Distribution by Intensity

Record 5: JP2-156D 30µM 0 zinc 1
• Sensor 11b + 1 equiv. Zn$^{2+}$:

Size Distribution Report by Intensity

Sample Details

- Sample Name: JP2-156D 30uM 1 zinc 1
- SOP Name: mansettings.nano
- General Notes:

- File Name: ZET0002894.dts
- Dispersant Name: Water
- Record Number: 6
- Material R#: 1.59
- Material Absorption: 0.010
- Measurement Date and Time: 17 October 2013 15:02:53

System

- Temperature (°C): 21.0
- Duration Used (s): 60
- Count Rate (kcps): 337.7
- Measurement Position (mm): 4.65
- Cell Description: Disposable sizing cuvette
- Attenuator: 10

Results

- Z-Average (d.nm): 452.2
- Peak 1: 226.4 100.0 27.00
- PdI: 0.468
- Peak 2: 0.000 0.0 0.000
- Intercept: 1.01
- Peak 3: 0.000 0.0 0.000

Result quality: Refer to quality report

Size Distribution by Intensity

Record 6: JP2-156D 30uM 1 zinc 1
Sensor **11b + 5 equiv. Zn**²⁺:

Size Distribution Report by Intensity

v2.2

Sample Details

- **Sample Name:** JP2-1560 30uM 5 zinc 1
- **SOP Name:** mansettings.nano
- **General Notes:**

<table>
<thead>
<tr>
<th>File Name</th>
<th>Dispersant Name</th>
<th>Dispersant RI</th>
<th>Viscosity (cP)</th>
<th>Measurement Date and Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZET0002894.dts</td>
<td>Water</td>
<td>1.330</td>
<td>0.9781</td>
<td>17 October 2013 15:08:06</td>
</tr>
</tbody>
</table>

**Record Number:** 7
**Material RI:** 1.59
**Material Absorbance:** 0.010

System

- **Temperature (°C):** 21.0
- **Count Rate (kcps):** 302.3
- **Measurement Position (mm):** 4.65
- **Cell Description:** Disposable sizing cuvette
- **Attenuator:** 10

Results

<table>
<thead>
<tr>
<th>Size (d.nm)</th>
<th>% Intensity</th>
<th>St Dev (d.nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-Average</td>
<td>1.791</td>
<td>10.0</td>
</tr>
<tr>
<td>PdI</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.25</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Peak 1:** 214.1
**Peak 2:** 0.000
**Peak 3:** 0.000

Result quality: Refer to quality report

Size Distribution by Intensity
Sensor 11b + 15 equiv. Zn$^{2+}$:

Size Distribution Report by Intensity

Sample Details:
- Sample Name: JP2-1560 30uM 15 zinc 1
- SOP Name: msettings.nano
- General Notes:

- File Name: ZET0002894.dts
- Dispersant Name: Water
- Record Number: 8
- Dispersant RI: 1.330
- Material RI: 1.59
- Viscosity (cP): 0.9781
- Material Absorption: 0.010
- Measurement Date and Time: 17 October 2013 15:14:23

System:
- Temperature (°C): 21.0
- Duration Used (s): 60
- Count Rate (kcps): 279.3
- Measurement Position (mm): 4.65
- Cell Description: Disposable size cuvette
- Attenuator: 10

Results:
- Z-Average (d.nm): 1679
  - Peak 1: 216.2 100.0 10.22
  - Peak 2: 1.000 0.000 0.0 0.000
  - Peak 3: 1.29 0.000 0.0 0.000
- Result quality: Refer to quality report

Result quality: Refer to quality report

Size Distribution by Intensity

Record ID: JP2-1560 30uM 15 zinc 1
5) Response of Probe 6 to Other Metal Ions

![Graph showing fluorescence response of sensor 6 (100 μM) in HEPES buffer (1% DMSO) to 5 equiv of metal ions (cations were used as the perchlorate salt).](image)

**Fig. S29** Fluorescence response of sensor 6 (100 μM) in HEPES buffer (1% DMSO) to 5 equiv of metal ions (cations were used as the perchlorate salt).

6) Experimental Procedures for the Evaluation of Sensors in Pancreatic Islets

Female CD1 mice (8-12 weeks of age) were housed under specific pathogen free conditions with *ad libitum* access to food and water. Animals were euthanized by cervical dislocation before isolation of islets by collagenase digestion, as previously described (PMID: 20204627). Animal procedures were approved by the Home Office according to the Animals (Scientific Procedures) Act 1986 of the United Kingdom (PPL 70/7349). Following 24-48 h culture, islets were incubated for 60 min. with the sensor under study before imaging using a Zeiss Axiovert 200 inverted widefield stereomicroscope. Illumination was delivered through a 20× 0.4NA objective (LD Plan Neofluar) using a halogen light source and a DAPI filter set (λ<sub>ex</sub> = 365/12, λ<sub>em</sub> = 447/60). Emitted signals were detected using a highly-sensitive 1344 x 1024 CCD camera (Hamamatsu ORCA-ER). Throughout, islets were incubated at 36 °C and irrigated with HEPES-bicarbonate buffer (120 mM NaCl, 4.8 mM KCl, 24 mM NaHCO<sub>3</sub>, 0.5 mM Na<sub>2</sub>HPO<sub>4</sub>, 5 mM HEPES, 2.5 mM CaCl<sub>2</sub> and 1.2 mM MgCl<sub>2</sub>) saturated with 95% O<sub>2</sub>/5% CO<sub>2</sub> and adjusted to pH 7.4. Offline signal analysis was performed using Volocity (Perkin Elmer) and Igor Pro (WaveMetrics) softwares. For two-photon imaging of probe distribution, islets were incubated with sensor as above before imaging using a Leica SP5 multiphoton microscope equipped with a 25× 0.95NA water-dipping objective adjusted for infrared wavelengths (HCX IRAPO). Two-photon excitation was achieved using a Spectraphysics Mai Tai femtosecond-pulsed laser (λ<sub>ex</sub> = 850 nm) and emitted signals were collected using a PMT (λ<sub>em</sub> = 400-550 nm). Image analysis was performed using Image J (NIH).

a) Cytotoxicity Assay

Islets were incubated with 3 μM calcein-AM (Life Technologies) and 2.5 μM propidium iodide (PI; Sigma-Aldrich) before detection of absorbance/emission at 491/525nm and 561/620nm, respectively. Calcein AM is a vital stain and requires cleavage by intracellular esterases for
fluorescence. Conversely, PI is a necrosis stain which only enters the nucleus in membrane-compromised cells. The islet area occupied by dead cells was expressed as a unitary ratio versus that occupied by live cells. Non-multifactorial pairwise comparisons were performed using Student’s t-test. Interactions between multiple treatments were assessed using Kruskal-Wallis test followed by pairwise comparisons using Dunn’s post-hoc test. In all cases, analysis was performed using Graphpad Prism (Graphpad Software) and results considered significant at P<0.05.

**Fig. S3**: Cytotoxicity assays for probes 6, 9b and 10b. A. Incubation of mouse islets with probes 6, 9b and 10b does not significantly induce cell death (necrosis) versus DMSO-alone (1:333) (NS, non-significant; P<0.54). B. Representative images showing calcein and PI staining in islets treated with DMSO and probes 6, 9b and 10b. Below is a positive control (Triton X-100; to permeabilise the membrane).

**a) Live imaging of dye co-localisation**

MIN6 beta cells were incubated for 1-2 h with each Zn⁡²⁺-binding probe (30 μM for probes 6 and 9b; 300 μM for probe 10b) before 30 min incubation with either 200 nM Mitotracker Red FM or 200 nM Lysotracker DND-99 (both Life Technologies). Cells were washed three times with PBS before live-imaging using a confocal microscope equipped with an acousto optical beam splitter (AOBS) and spectral detectors (Leica TCS SP5). The Zn²⁺-binding probes were excited using a 405 nm diode laser and emissions collected at 460/70 nm (63 x oil-immersion objective; NA 1.4). The organelle-specific dyes were excited using a 543 HeNe laser and emitted light captured using PMTs centred on 650/50 nm and 595/50 nm for Mitotracker and Lysotracker, respectively. Analysis of co-localisation was performed after background fluorescence correction using the Pearson’s correlation coefficient embedded within the Intensity Correlation Analysis (ICA) plugin for ImageJ (NIH). Uniform linear adjustments were applied to contrast/brightness to improve image quality for analysis/presentation purposes.
**Fig. S31:** Cellular distribution of probes 6, 9b and 10b. A. Probe 9b, expected to sequester in mitochondria, is co-localised with Mitotracker. B. Probe 6, expected to aggregate at the plasma membrane, does not co-localise with Mitotracker. C. Probe 10b, expected to aggregate in lysosomes, does not co-localise with Mitotracker. D. Probe 10b co-localises with Lysotracker, a marker of acidic organelles. E. Probe 9b does not co-localise with Lysotracker, further confirming its mitochondrial specificity. Scale bar represents 10 µm.
Image analysis demonstrates that the signals from probe 9b and Mitotracker are strongly correlated. By contrast, fluorescence from both probe 10b and probe 6 poorly correlates with that of the organelle-specific dye (*P<0.05 and **P<0.01 versus probe 9b) (Kruskal-Wallis test). Note that some co-localisation is still apparent due to the inability to fully resolve sub-cellular structures close to the diffraction limit. We also observe a strong correlation between probe 10b and Lysotracker DND-99, but not probe 9b and the organelle-specific dye (**P<0.001 probe 11b versus probe 10b) (Student’s t-test).

**Fig. S32** Statistical analysis of probe localisation with a) mito- and b) lyso-tracker.
7) NMR Spectroscopic Characterisation
a) Compounds reported in the manuscript

$^1$H NMR of 2

$^{13}$C NMR of 2
$^{1}H$ NMR of 3

$^{13}C$ NMR of 3
$^1$H NMR of 4

$^{13}$C NMR of 4
$^1$H NMR of 6

$^{13}$C NMR of 6
$^1$H NMR of 8a

$\text{C NMR of 8a}$
$^1$H NMR of 9a

$^{13}$C NMR of 9a
$^{31}\text{P}$ of 9a:

$^{19}\text{F}$ of 9a:
$^1$H NMR of 10a

$^{13}$C NMR of 10a
1H NMR of 11a

13C NMR of 11a
$^1$H NMR of 7b

$^{13}$C NMR of 7b
$^1$H NMR of 8b:

$^{13}$C NMR of 8b:
$^{31}$P of 9b:

$^{19}$F of 9b:
$^1$H NMR of 10b:

$^{13}$C NMR of 10b:
1H NMR of 11b:

13C NMR of 11b:
b) Starting materials, intermediates and other compounds

$^1$H NMR of S9

$^{13}$C NMR of S9
$^1$H NMR of S10

$^{13}$C NMR of S10
$^1$H NMR of S11

$^{13}$C NMR of S11
$^1$H NMR of S12
$^{1}H$ NMR of S13

$^{13}C$ NMR of S13
$^1$H NMR of \textbf{S14}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{s14_hnmr}
\caption{$^1$H NMR spectrum of S14.}
\end{figure}

$^{13}$C NMR of \textbf{S14}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{s14_cnmr}
\caption{$^{13}$C NMR spectrum of S14.}
\end{figure}
$^{31}$P of S15:

$^{19}$F of S15:
1H NMR of S16

13C NMR of S16
References: