Electronic Supplementary Information (ESI)

Biocompatible Well-Defined Chromophore-Polymer Conjugates for Photodynamic Therapy and Two-Photon Imaging

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1. Materials.

All chemicals were purchased from Sigma Aldrich, ACROS or Alfa-Aesar at the highest purity available and used without further purification. All reactions were routinely performed under argon. THF was dried and degassed on a solvent station by passage through an activated alumina column followed by argon flush. Other solvents were used as received from the supplier without further purification.

2. Analytical techniques.

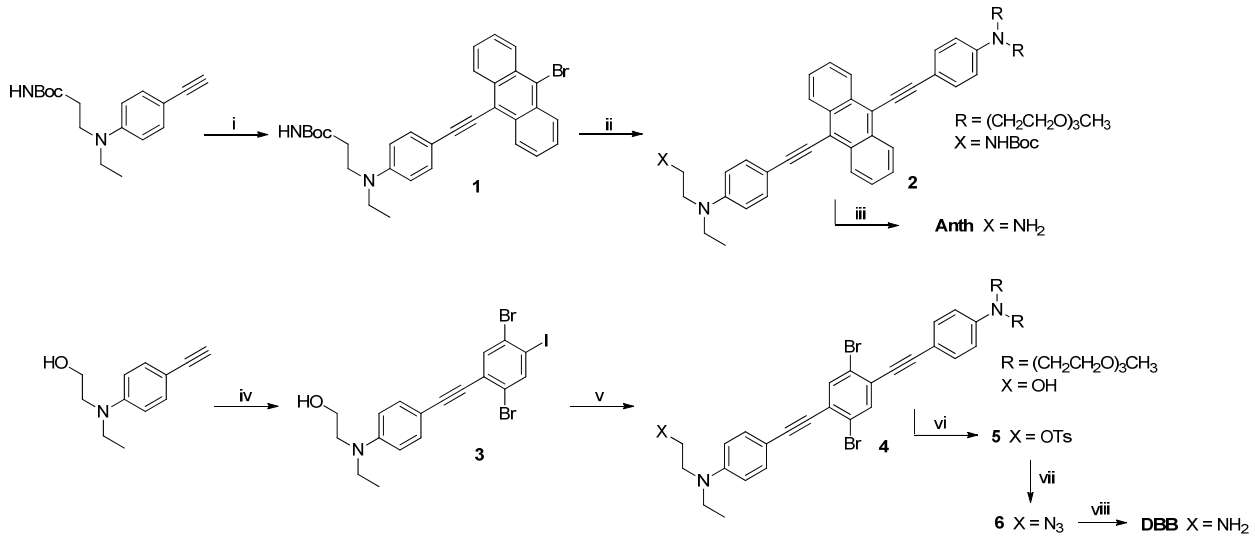
2.1. NMR. Spectra (^{1}H, ^{13}C) were recorded at 298K on a BRUKER® AC 200 operating at 200.13 and 50.32 MHz for ^{1}H and ^{13}C respectively and on a BRUKER® 500 Ultra Shield operating at 500.1 and 126.3 MHz for ^{1}H and ^{13}C respectively. Data are listed in parts per million (ppm) and are reported relative to tetramethylsilane (^{1}H, ^{13}C), residual solvent peaks being used as internal standard ($CHCl_3$ ^{1}H: 7.26 ppm, ^{13}C: 77.36 ppm).

2.2. Mass Spectrometry. HRMS measurements were performed by ESI-TOF and low resolution MS by MALDI TOF. In the case of ESI-TOF a Bruker Daltonics® Micro TOF-Q II was used with a resolution of 8000, in positive mode with a capillary tension of 4500V, a source temperature of 180°C, and a cone tension of 60V. The internal reference used for calibration was sodium formate.

2.3. Elemental Analyses. Elemental analyses were performed on a Flash EA1112 CHNS/O Thermo Electron micro-analyzer.

3. Two-Photon Chromophores synthesis.

Scheme S1 Synthesis of Anth and DBB compound

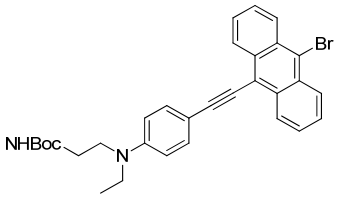


i) 9,10-dibromobenzene (1.1 eq), $PdCl_2(PPh_3)_2$, CuI, Et_3N/THF, 55°C, 1 night, 35%; **ii)** Alkyne (1.8 eq), $PdCl_2(PPh_3)_2$, CuI, Et_3N/THF, 55°C, 1 night, 47%; **iii)** HCl, MeOH, rt, 44 h, quant; **iv)** 1,4-diiodo-2,5-dibromobenzene (3.0 eq.), $PdCl_2(PPh_3)_2$, CuI, Et_3N/THF, RT, 12 h, 64%; **v)** Alkyne (1.0 eq), $PdCl_2(PPh_3)_2$, CuI, Et_3N/THF, RT, 12 h, 80%; **vi)** Ts-Cl (1.2 eq.), NaOH, , quant.; **vii)** NaN_3 (1.2 eq.), DMF, 60°C, 3 h, 89%; **viii)** PPh_3 (1.2 eq.), toluene, reflux, 7 h, H_2O/THF, RT, 1 night 90%.

Starting alkynes were synthesized using published procedures.[1,2]

3.1. Anth.

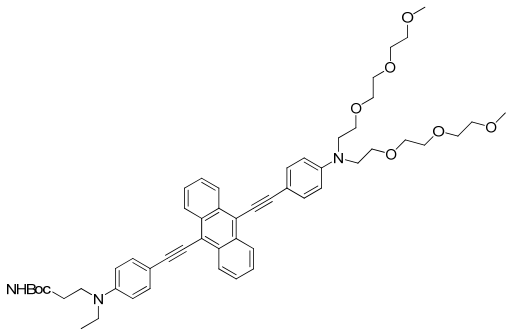
1



9,10-Dibromoanthracene (710 mg, 2.1 mmol) was dissolved in a triethylamine/THF mixture (v/v = 15/50 mL), the product was solubilized by heating at 50°C. A solution of NH-Boc protected *N*-ethyl,*N*-aminoethylethynylaniline (540 mg, 1.87 mmol) in THF (10 mL) was added. The solution was degassed by bubbling argon for 20 min, and $Pd(PPh_3)_2Cl_2$ (20 mg, 15 % mol) and CuI (6 mg, 15% mol) were

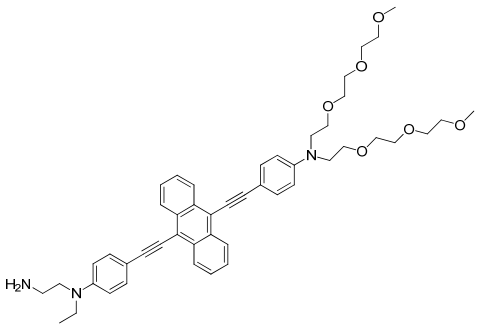
added, and the reaction was stirred overnight at 55°C. After cooling down to RT, the solvent was evaporated *in vacuo*. The residue was dissolved in dichloromethane and washed with a saturated aqueous solution of NH_4Cl, and brine. After drying on Na_2SO_4 the solvent was evaporated under reduced pressure. The crude material was purified by flash chromatography on silica gel (CH_2Cl_2) to provide a red solid (350 mg, 35 %). ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 8.70 (m, 2H), 8.55 (m, 2H), 7.66 (m, 6H), 6.75 (d, *J* = 8.8 Hz, 2H), 4.71 (br, 1H), 3.40 (m, 6H), 1.43 (s, 9H), 1.20 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 156.3, 148.2,133.3, 132.9, 130.5, 128.3, 127.8, 127.6, 126.6, 123.0, 119.7, 111.9, 110.0, 103.7, 84.8, 79.7, 49.9, 45.4, 38.5, 28.6, 12.3. HRMS (ESI) calculated for [$C_{31}H_{32}BrN_2O_2$ + H^+] 553.1622, found 543.1642.

2



1 (300 mg, 0.55 mmol) and N,N-$(PEG)_2$-ethynylaniline (415 mg, 1.0 mmol) were dissolved in a triethylamine/THF mixture (v/v = 15/50 mL). The solution was degassed by bubbling argon for 20 min, and $Pd(PPh_3)_2Cl_2$ (81mg) and CuI (45mg) were added, the reaction was stirred overnight at 65°C. After cooling down to RT, the solvent were evaporated *in vacuo*, the residue was dissolved in CH_2Cl_2 and washed with a saturated solution of NH_4Cl, and brine. After drying on Na_2SO_4 the solvent was evaporated under reduced pressure. The crude material was purified by flash chromatography on silica gel (CH_2Cl_2) to provide a red solid. (225 mg, 47%). ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 8.67 (m, 4H), 7.60 (m, 6H), 7.46 (m, 2H), 6.74 (d, *J* = 9.0 Hz, 4H), 4.73 (br, 1H), 3.63-3.40 (m, 30H), 3.37 (s, 6H), 1.45 (s, 9H), 1.19 (t, *J* = 8.8 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 156.2, 148.0, 148.0, 133.1, 133.0, 127.4, 126.4, 118.5, 111.7, 111.6, 110.1, 110.1, 103.8, 103.8, 85.0, 79.5, 71.9, 70.8, 70.7, 70.6, 68.5, 59.1, 51.0, 49.7, 45.3, 38.4, 28.5, 12.2. HRMS (ESI) calculated for [$C_{53}H_{65}N_3O_8$ + H^+] 872.4844, found 872.4810.

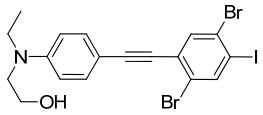
Anth



2 (20.5 mg, 2.7×10^{-5} mol) was dissolved in MeOH-d4 (5 mL) 10 drops of DCl 37% were added and the mixture was stirred in the dark, the reaction was followed 44 h by NMR. After neutralization by addition of KOH 1M solution, the solvent was evaporated *in vacuo*. The product was dissolved in CH_2Cl_2 and washed with brine. After drying on Na_2SO_4 the solvent was evaporated under reduced pressure to provide a red solid (20 mg, 100%). ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 8.67 (m, 2H), 7.67 (m, 8H), 6.74 (d, J = 9.0 Hz, 2H), 3.63-3.40 (m, 30H), 3.37 (s, 6H), 1.19 (t, J = 8.8 Hz, 3H). HRMS (ESI) calculated for $[C_{48}H_{58}N_3O_6 + H^+]$ 772.4320, found 772.4329.

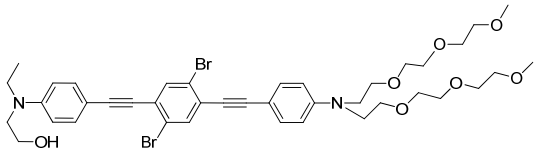
3.2. DBB.

3



N-ethyl,N-hydroxyethylethynylaniline (511 mg, 2.7 mmol) was dissolved in a mixture of THF/Et_3N (1/1, 20 mL), 3,6-dibromo-1,4-diiodobenzene (4.0 g, 8.1 mmol) was added and the solution was degassed by bubbling argon for 20 min. $Pd(PPh_3)_2Cl_2$ (98 mg, 0.14 mmol) and CuI (27 mg, 0.14 mmol) were then added to the stirred solution at RT. The reaction was monitored by TLC (SiO_2; Et_2O/pentane, 9/1) and after full conversion (*ca* 12 h). Diethylether was added (15 mL). The solution was filtered on celite and solvents were removed under vacuum. After a chromatography column on silica gel (Et_2O/pentane, from 0/1 to 9/1), the desired compound was obtained as a yellowish solid (956 mg, 64 %). ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 7.98 (1H, s), 7.65 (1H, s), 7.33 (2H, d, *J* = 8.8 Hz), 6.62 (2H, d, *J* = 8.8 Hz), 3.74 (2H, d, *J* = 5.3 Hz), 3.41 (4H, m), 1.14 (3H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm 148.7, 142.5, 135.2, 133.3, 128.2, 123.9, 111.9, 99.7, 98.1, 60.3, 52.3, 45.6, 11.9. HRMS (ESI) calculated for $[M+Na]^+$ 569.8536, found 569.8536. Elemental analysis calculated for $C_{18}H_{16}Br_2INO$, H_2O: C, 38.13; H, 3.20; N, 2.47. Found: C, 38.57; H, 2.82; N, 2.51.

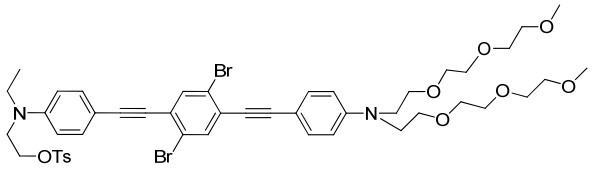
4



N,N-$(PEG)_2$-ethynylaniline (201 mg, 0.49 mmol) was dissolved in a mixture of THF/Et_3N (1/1, 20 mL), **3** (270 mg, 0.49 mmol) was added and the solution was degassed by bubbling argon during 20 min. $Pd(PPh_3)_2Cl_2$ (18 mg, 0.025 mmol) and CuI (5 mg, 0.025 mmol) were then added. The reaction was stirred at RT and monitored by TLC (SiO_2 Et_2O/THF, 7/3). After full conversion (*ca* 12 h) diethylether was added (15 mL). The solution was filtered on celite and solvents were removed under vacuum. After a chromatography column on silica gel (Et_2O, then Et_2O/THF: 7/3), the desired compound was obtained as a yellowish oil (324 mg, 80 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.73 (2H, s), 7.44 (2H, d, *J* = 9.2 Hz), 7.42 (2H, d, *J* = 9.2 Hz), 6.72 (2H, d, *J* = 9.2 Hz), 6.70 (2H, d, *J* = 9.2 Hz), 3.84 (2H, t, *J* = 5.5 Hz), 3.65 (20H, m), 3.56 (4H, m), 3.54 (2H, t, *J* = 6.4 Hz), 3.50 (2H, m), 3.41 (6H, s) 1.23 (3H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 148.5, 148.3, 135.3, 133.2, 133.1, 132.1, 132.0, 131.9, 128.5, 128.4,

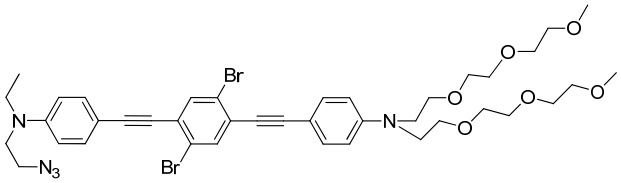
126.3, 126.2, 123.1, 111.8, 111.4, 109.1, 108.8, 98.1, 97.9, 85.9, 85.6, 71.9, 70.7, 70.6, 70.6, 68.4, 65.8, 60.18, 59.0, 52.2, 50.8, 45.5, 15.3, 11.9. HRMS (ESI) calculated for $[M+Na]^+$ 851.1877, found 851.1873.

5



A solution of TsCl (115 mg, 0.6mmol) in THF (5 mL) was added dropwise to a solution of **4** (419 mg, 0.50 mmol) and sodium hydroxide (100 mg, 2.50 mmol) in a mixture of THF/H_2O (5 mL + 2 mL) at 40°C. After addition, the mixture was stirred at 40°C overnight. The mixture was cooled to RT and brine was added. The aqueous layer was extracted with diethyl ether and the organic layer was dried with sodium sulfate. After removing the solvent under vacuum, the product was obtained as a yellowish oil (497 mg, 100%).%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 7.63 (4H, m), 7.25 (m, 6H), 6.60 (2 H, d, *J* = 3.5 Hz), 6.40 (2 H, d, *J* = 2.9 Hz), 4.07 (2 H, t, *J* = 6.2 Hz), 3.47 (26 H, m), 3.31 (6 H, s), 3.30 (2H, t, *J* = 6.2 Hz), 2.34 (3H, s), 1.13 (3H, t, *J* = 6.8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm 148.3, 147.6, 135.4, 135.4, 133.4, 133.2, 132.1, 132.1, 128.5, 126.4, 126.2, 123.4, 123.00, 111.6, 111.4, 109.5, 108.8, 98.2, 97.8, 85.7, 85.6, 71.9, 70.8, 70.7, 70.6, 68.4, 59.1, 50.9, 49.4, 48.9, 48.6, 45.5, 45.3, 29.7, 29.4, 12.2. HRMS (ESI) calculated for $[M+Na]^+$ 1005.1966, found 1005.1963. Elemental analysis calculated for $C_{47}H_{56}Br_2N_2O_9S$, H_2O: C, 56.29; H, 5.83; Br, 15.94; N, 2.79; S, 3.20. Found: C, 56.25; H, 5.73; N, 2.72; S, 2.81.

6

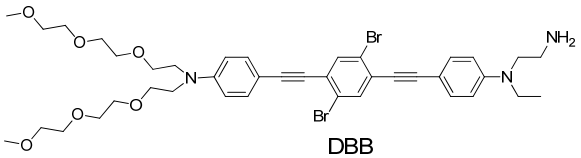


Sodium azide (NaN_3, 28 mg, 0.42 mmol) was added to a solution of **5** (348 mg, 0.35 mmol) in DMF (10 mL) at 60°C and the solution was stirred for 3 h. Brine was then added and the aqueous layer was extracted with ethyl acetate. The organic layer was dried with sodium sulfate and solvents were removed under vacuum. A chromatography column on silica gel (Et_2O, then Et_2O/CH_3CN: 7/3) gave the desired compound as a pale yellowish oil (266 mg, 89 %). ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 7.63 (2

H, s), 7.36 (2 H, d, *J* = 3.5 Hz), 7.30 (2 H, d, *J* = 2.9 Hz), 6.62 (2 H, d, *J* = 3.5 Hz), 6.57 (2 H, d, *J* = 2.9 Hz), 3.57 (2 H, m), 3.47 (20 H, m), 3.40 (8 H, m), 3.31 (6 H, s), 1.13 (3 H, t, *J* = 6.8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm 148.4, 135.4, 133.4, 133.2, 123.3, 123.2, 111.7, 111.5, 108.9, 98.2, 97.9, 85.7, 85.6, 72.9, 70.8, 70.7, 68.4, 59.1, 50.9, 49.5, 48.9, 45.5, 11.9.

HRMS (ESI) calculated for $[M+Na]^+$ 876.1942, found 876.1942. Elemental analysis calculated for $C_{40}H_{49}Br_2N_5O_6$, H_2O: C, 54.99; H, 5.88; N, 8.02; S, 0.00. Found: C, 55.14; H, 5.64; N, 7.74; S, 0.00.

DBB



DBB

To a solution of **6** (113 mg, 0.13 mmol) in toluene (5 mL) was added a solution of PPh_3 (39 mg, 0.15 mmol) in toluene (5 mL). The mixture was then stirred for 7 h under reflux. Water (1 mL) and THF (15 mL) were added, and the mixture was stirred at RT overnight. Brine was then added and the aqueous layer was extracted with ethyl acetate, the organic layer was dried with sodium sulfate and solvents were removed under vacuum. A chromatography column on a short pad of silica gel (Ethylacetate/MeOH/NH_4OH) from 9/1/0 to 8.5/1/0.5) gives the desired compound as pale yellowish oil (97 mg, 90 %).

Note : A short pad of silica gel was preferred to a large column, since the product tends to interact with the silica stationary phase in a difficultly reversible way. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.67 (2 H, s), 7.37 (2 H, d, *J* = 4.7 Hz), 7.33 (2 H, d, *J* = 4.7 Hz), 6.64 (4 H, m), 3.60 (20 H, m), 3.52 (4 H, m), 3.42 (4 H, m), 3.36 (6 H, s), 2.93 (2 H, br.), 1.15 (2 H, d, *J* = 7.1 Hz) ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 148.4, 147.6, 135.4, 133.4, 133.2, 123.2, 111.6, 111.5, 109.5, 108.9, 98.2, 97.9, 85.7, 85.6, 72,0, 70.8, 70.7, 68.4, 59.1, 50.9, 49.5, 48.9, 45.5, 12.2. HRMS (ESI) calculated for $[M+Na]^+$ 850.2037, found 850.2039. Elemental analysis calculated for $C_{40}H_{51}Br_2N_3O_6H_2O$: C, 56.68; H, 6.30; N, 4.96; S, 0.00. Found: C, 56.84; H, 6.10; N, 4.68; S, 0.00.

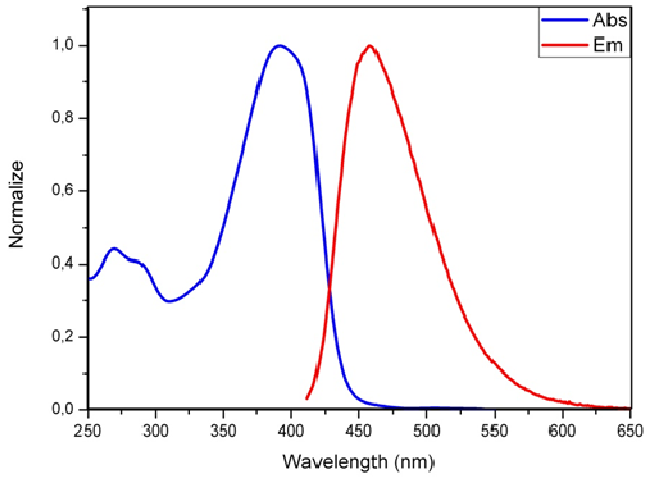


Fig.S1 Normalized absorption (blue) and emission (red) of **DBB** in CH_2Cl_2 (note: there is no shift in $CHCl_3$) at 298 K. λ_{exc} = 396 nm.

Table S1 Spectroscopic data in CH_2Cl_2 (same in $CHCl_3$).

λ_{max}/nm (ε/L.cm^{-1}.mol^{-1})	λ_{em}/nm λ_{exc} = 396 nm (ϕ)[a]
396 (58 000)	458 (0.23)

[a] Calculated taking coumarine 153 in MeOH as reference

4. Additional conjugate characterization

4.1. Spectroscopic parameters for 6Anth-H conjugate in various solvents

Table S2 **Photophysical parameters for 6Anth-H in various solvents**

Solvent	Dielectric constant	λmax (abs ; em)	Stokes shifts (cm^{-1})	ϕ
Dioxane	2.25	476 ; 554	2958	0.07
EtOH	24.5	475 ; 574	3630	0.07
MeOH	32.7	474 ; 580	3856	0.05
DMF	36.7	483 ; 615	4444	0.13
Water	80.1	476 ; 609	4588	0.01

$\lambda_{max\ (abs\ ;\ em)}$: maximal absorption and emission wavelengths (nm); ϕ: fluorescence quantum yield.

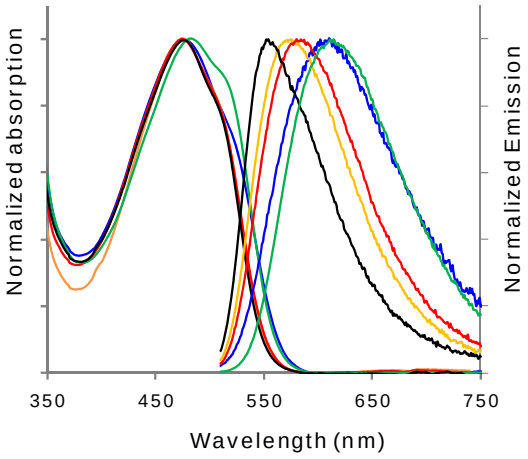


Fig. S2 Absorption and emission spectra of **6Anth-H** conjugate in water (blue), methanol (red), ethanol (orange), dimethylformamide (green), dioxane (black).

4.2. NMR

Although the conjugates were highly soluble in D_2O, only peaks corresponding to the hydrophilic polymer backbone were observed on the ^1H NMR spectra (even by increasing the number of scans). No peak corresponding to the hydrophobic chromophores were observed. Conjugates were then mainly characterized using UV and fluorescence spectroscopy.

4. Biological evaluation.

4.1. Flow cytometry analyses.

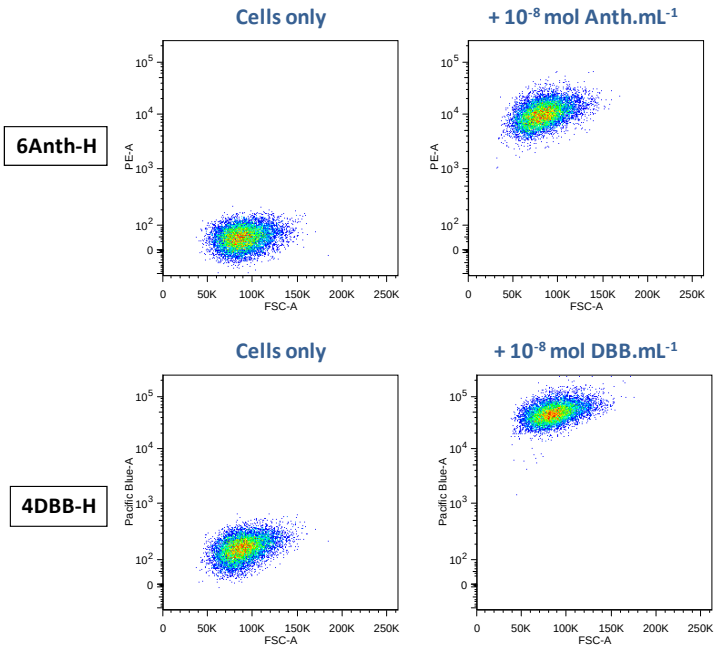


Fig. S3 Detection of **6Anth-H** and **4DBB-H** cell uptake by flow cytometry. Baf3 cells were incubated with **6Anth-H** or **4DBB-H** (10^{-5} mol.L^{-1} chromophore) for 24 h. Cells were analyzed by flow cytometry. Density dot plot displays two parameters: fluorescence (Y-axis) and cell size (X-axis). The colors range from blue (low cell density) to red (high cell density).

4.2. 6Anth-H-associated cytotoxicity.

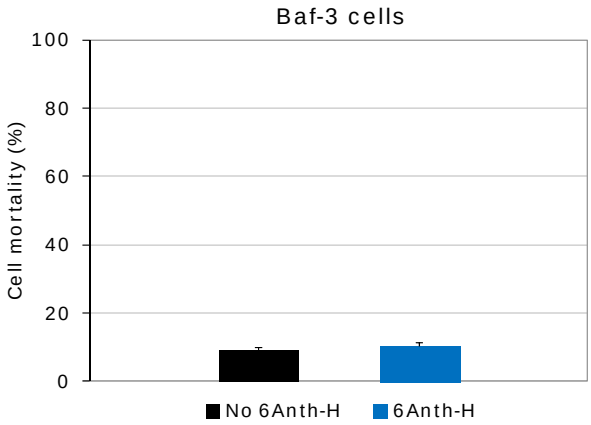


Fig. S4 Baf3 cells were incubated for 24 h without 6Anth-H (Black) or with **6Anth-H** (10^{-5} mol.L^{-1} **Anth**) (Blue). The percentage of mortality was assessed by flow cytometry analysis using a Forward Scatter (FSC)/Side Scatter (SSC) gate to discriminate between live and dead cells. Results show the mean mortality ± SD of 2 independent experiments.

4.3. Kinetics of 6Anth-H cell uptake.

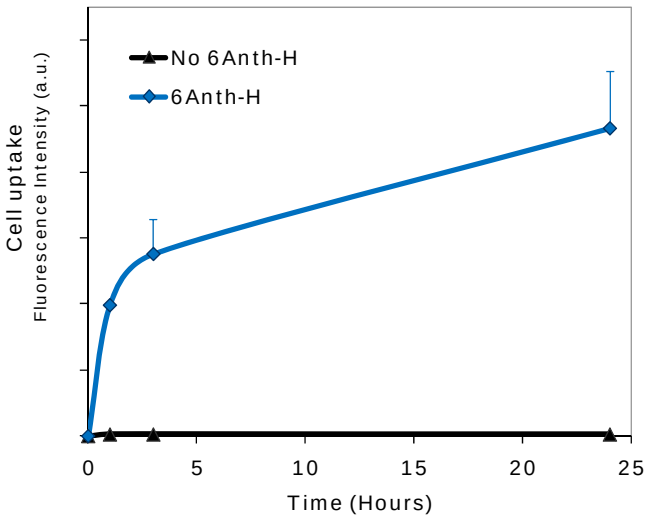


Fig. S5 Kinetics of **6Anth-H** uptake. Baf3 cells were incubated without (black line) or with **6Anth-H** (blue line, 10^{-5} mol.L^{-1} **Anth**) for the indicated period of time. Uptake was measured by flow cytometry. Results are expressed as the average of the mean fluorescence intensity ± SD of 2 independent experiments.

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