Aquatic host-guest complex between a supramolecular G-quadruplex and the anticancer drug doxorubicin

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A. General Experimental Procedures

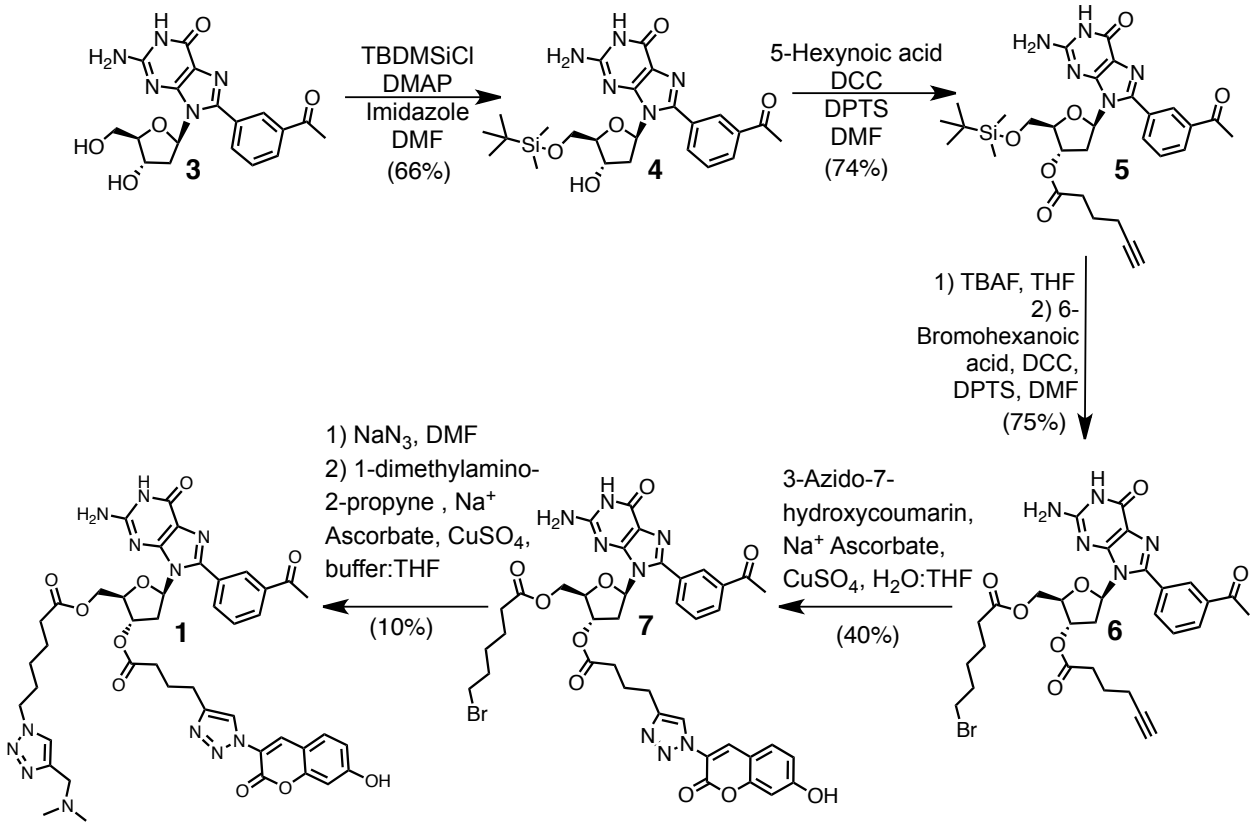
DMF was purchased anhydrous from Aldrich and used without further treatment. MeOH (1 L) was distilled from Magnesium (5 g) and iodine (0.5 g). All other reagents were from commercial sources and used without further purification. Unless otherwise noted, all compounds were purified by column chromatography on silica gel 60, 0.04-0.063 mm, and TLC and PTLC (from Sorbent Technologies) were performed using EMD silica gel 60 F_{254} glass backed plates from Sorbent Technologies. Visualization of spots was effected with UV light and iodine. Reactions requiring anhydrous conditions were carried out using flamed-dried glassware under Argon.

B. Characterization of the target compounds

^{1}H and ^{13}C NMR spectra were recorded on Bruker AV-500 spectrometer, with nominal frequencies of 500.13 MHz for proton or 125.77 MHz for carbon respectively. ^{1}H NMR and ^{13}C NMR chemical shifts are reported in parts per million relative to the undeuterated solvent as an internal reference. The following abbreviations are used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiple; b, broad. FT-IR analyses were performed on a Bruker Tensor 27 Infrared Spectrometer equipped with a Helios Attenuated Total Reflectance (ATR) accessory with a diamond crystal. Melting temperatures were determined using Fisher brand electro-thermal digital melting point apparatus from Fisher Scientific. High-resolution electrospray ionization mass spectrometry (ESI MS) was recorded on a Q-Tof Ultima Global mass spectrometer (Micromass) equipped with a Z-spray source. Electrospray ionization was achieved in the positive mode by 3 kV on the needle. Compound **2** was synthesized as previously reported in our group.[1]

[1] García-Arriaga, M.; Hobley, G.; Rivera, J. M., "Isostructural Self-assembly of 2'-deoxyguanosine Derivatives in Aqueous and Organic Media." *J. Am. Chem. Soc.* **2008,** 130, (32), 10492.

C. Synthetic scheme and procedures for the preparation of 1



Scheme S1. *Synthetic strategy for the preparation of the fluorescent derivative* ***1****.*

- ***Synthesis of 8-(3-acetylphenyl)-2-amino-9-((2R,4S,5R)-5-((tert-butyldimethylsilyloxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-1H-purin-6(9H)-one (4)***

3 (500 mg, 1.30 mmol), imidazole (266 mg, 3.90 mmol) and 4-dimethylaminopyridine (16 mg, 0.130 mmol) were pre-dried under vacuum for 1h in a flame-dried round bottom flask. Anhydrous DMF was then added to the solids under Ar atmosphere. This was followed by the addition of t-butyldimethylsilylchloride (254 mg, 1.69 mmol) to the suspension. The reaction was stirred at rt under Ar atmosphere for 5 h. The solvent from the reaction mixture was evaporated, followed by the addition of water, sonication and filtration. The resulting solid was dry loaded with silica for purification by flash column chromatography (EtOAc-MeOH, 93:7) affording the target compound as a white solid with a yield of 66% (429 mg).

White solid, mp 167 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.75 (s, 1H), 8.23 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.92 (d, *J* = 7.6 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 6.47 (s, 2H), 6.06 (t, *J* = 6.8 Hz, 1H), 5.14 (d, 1H), 4.35 (s, 1H), 3.76 (m, 3H), 3.22 (m, 1H), 2.64 (s, 3H), 2.05 (m, 1H), 0.82 (s, 9H), 0.02 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.29, 156.53, 152.99, 152.02, 146.22, 137.20, 133.59, 130.72, 128.82, 117.42, 87.40, 84.17, 70.92, 63.49, 36.19, 26.81, 25.78, 17.99, -5.28, -5.34. IR (ν_{max}): 3327, 3149, 2932, 2860, 1685, 1680, 1629, 1595, 1358, 1256, 1076, 837, 781 cm^{-1}. HR-MS $[M+H]^+$ 500.6334 m/z.

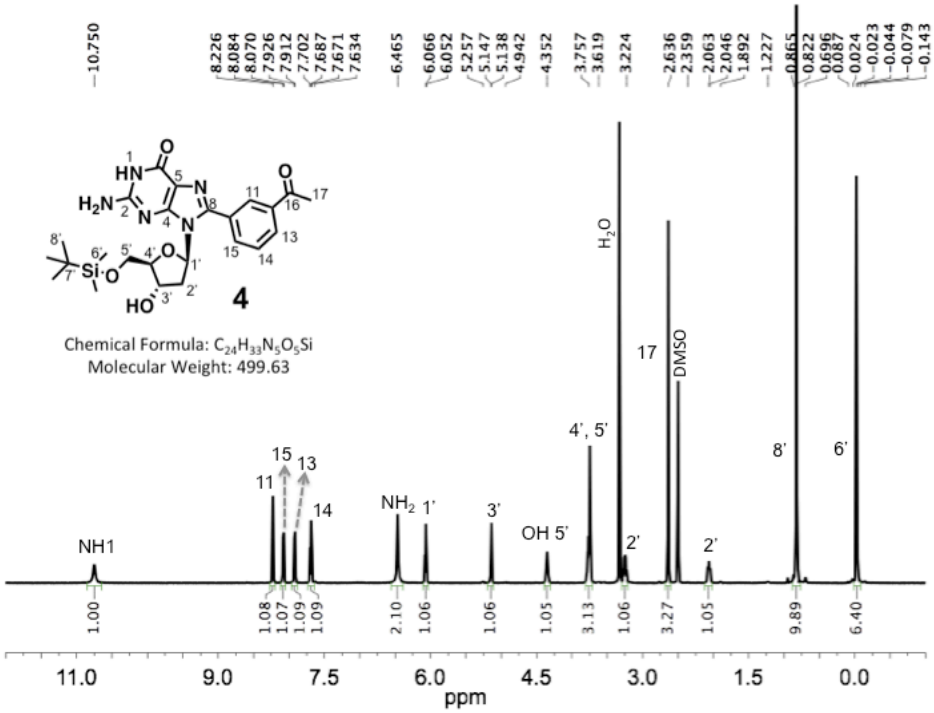


Figure S1. ^{1}H NMR (DMSO–d_6, 500 MHz, 298 K) of **4**.

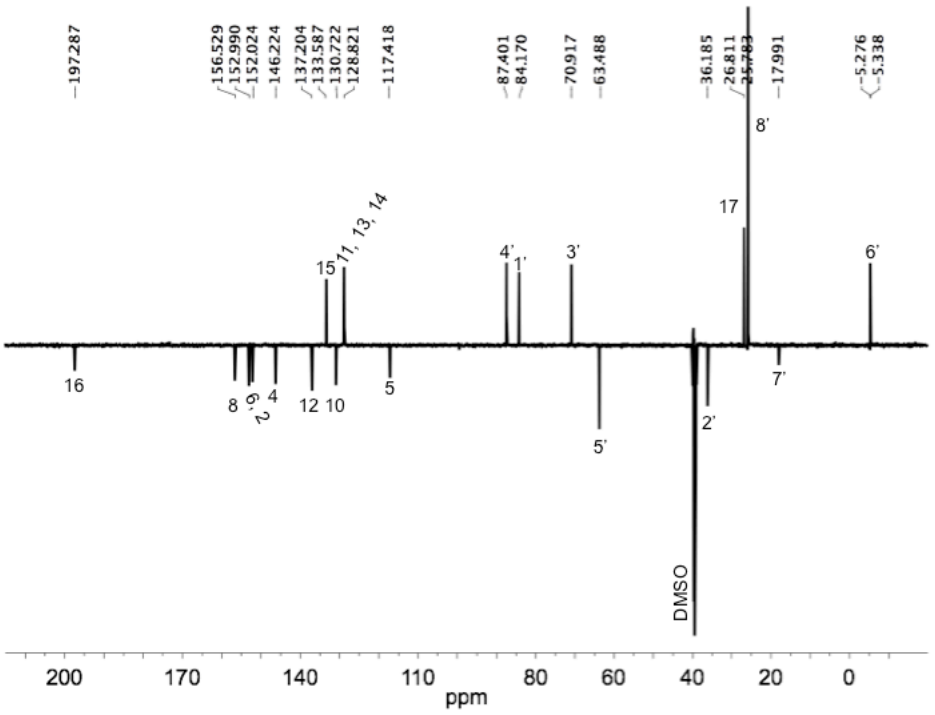


Figure S2. ^{13}C DEPTQ-135 NMR (DMSO–d_6, 125 MHz, 298 K) of **4**.

- ***Synthesis of (2R,3S,5R)-5-(8-(3-acetylphenyl)-2-amino-6-oxo-1H-purin-9(6H)-yl)-2-((tert-butyldimethylsilyloxy)methyl)tetrahydrofuran-3-yl hex-5-ynoate (5)***

4 (400 mg, 0.801 mmol), a 1:1 complex of 4-dimethylaminopridine: 4-toluenesulphonic acid (DPTS) (240 mg, 0.801 mmol) and 5-hexynoic acid (179 mg, 1.60 mmol) were pre-dried under vacuum for 1 h in a flame-dried round bottom flask. Under an Ar atmosphere anhydrous DMF was added to the solids followed by the addition of Dicyclohexylcarbodiimide (DCC) (330 mg, 1.60 mmol). The reaction was stirred at rt until TLC analysis (EtOAc-MeOH, 9:1) showed complete conversion of starting material to product. The reaction mixture was quenched by the addition of an excess of MeOH and this was followed by evaporation of the solvent. Water was poured to the solid, sonicated and filtered. The resulting solid material was dry-loaded into silica for purification by flash column chromatography (EtOAc-MeOH, 96:4) affording the target compound **5** as a white solid with a yield of 74% (350 mg).

White solid, mp 103-105 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.93 (s, 1H), 8.24 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 7.7 Hz, 1H), 7.70 (t, *J* = 7.8 Hz, 1H), 6.55 (s, 2H), 6.09 (t, *J* = 6.9 Hz, 1H), 5.37 (m, 1H), 4.01 (d, *J* = 7.2 Hz, 1H), 3.84 (m, 3H), 3.59 (dt, *J* = 7.1 Hz, 14.3, 1H), 2.79 (d, *J* = 2.6 Hz, 1H), 2.64 (s, 3H), 2.39 (t, *J* = 7.2 Hz, 2H), 2.33 (m, 1H), 2.18 (m, 2H), 1.65 (m, 2H) 0.80 (s, 9H), -0.08 (d, *J* = 19.3 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.4, 172.0, 156.7, 153.1, 152.0, 146.2, 137.0, 133.3, 130.6, 129.1, 128.8, 128.8, 117.2, 85.1, 84.6, 74.9, 71.8, 63.5, 33.8, 32.3, 26.8, 25.8, 23.3, 17.9, 17.0, -5.4. IR (ν_{max}): 3300, 3139, 2935, 2858, 2360, 1734, 1686, 1628, 1595, 1360, 1252, 1082, 837, 781, 692, 592 cm^{-1}. HR-MS $[M+Na]^+$ 617.7342 m/z.

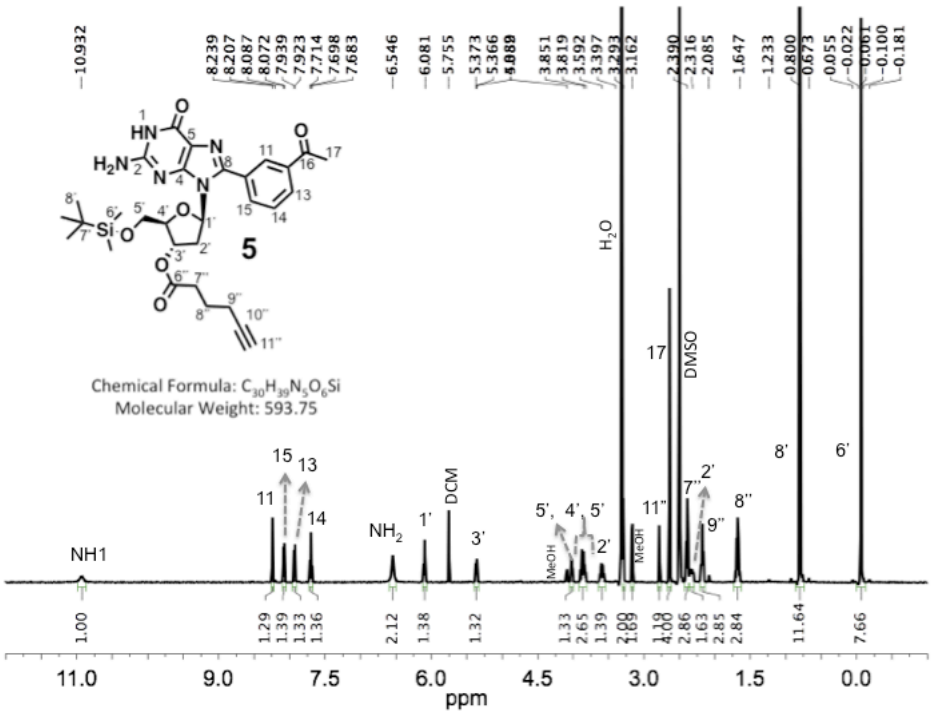


Figure S3. ^{1}H NMR (DMSO–d_6, 500 MHz, 298 K) of **5**.

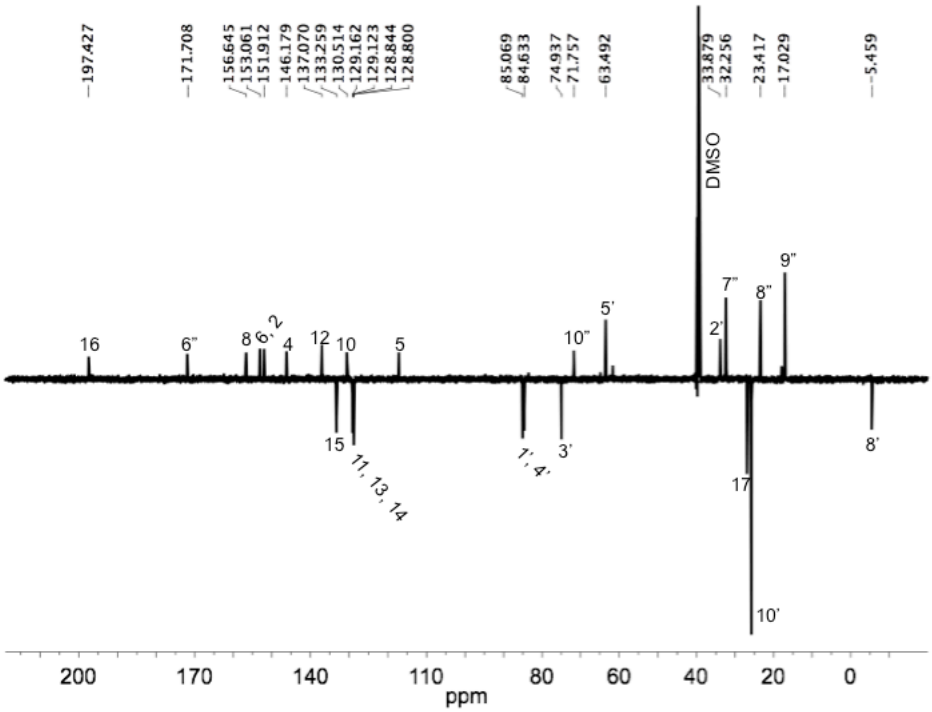


Figure S4. ^{13}C DEPTQ-135 NMR (DMSO–d_6, 125 MHz, 298 K) of **5**.

- ***Synthesis of (2R,3S,5R)-5-(8-(3-acetylphenyl)-2-amino-6-oxo-1H-purin-9(6H)-yl)-2-((6-bromohexanoyloxy)methyl)tetrahydrofuran-3-yl hex-5-ynoate (6)***

The following reactions were performed under Argon atmosphere. Tetra-n-butylammoniumfloride (1 M solution in THF, 0.766 mmol) was added to a solution of **5** (350 mg, 0.589 mmol) in THF. The reaction was stirred at rt until TLC (EtOAc-MeOH, 9:1) showed complete conversion of starting material to products. The reaction mixture was quenched by the addition of an excess of MeOH and this was followed by solvent evaporation. The solid crude was poured into water, sonicated and filtered. The resulting white solid (used without further purification) (200 mg, 0.417 mmol), DPTS (125 mg, 0.417 mmol) and 6-bromohexanoic acid (163 mg, 0.834 mmol) were pre-dried under vacuum for 1h in a flamed round bottom flask. Anhydrous DMF was added to the solids. Dicyclohexylcarbodiimide (DCC) (172 mg, 0.834 mmol) was added to this suspension. The reaction was stirred at rt under Ar atmosphere until TLC (EtOAc-MeOH, 9:1) showed complete conversion of starting material to product. The reaction mixture was quenched by the addition of an excess of MeOH and this was followed by solvent evaporation. Water was poured into the solid crude, sonicated and filtered. The resulting solid material was dry-loaded with silica for purification by flash column chromatography (EtOAc-MeOH, 96:4) affording the target compound **6**, yielding 177 mg (75%) of a white solid.

White solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.83 (s, 1H), 8.21 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.90 (d, *J* = 7.6 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 6.51 (s, 2H), 6.11 (t, *J* = 6.9 Hz, 1H), 5.46 (m, 1H), 4.44 (m, 1H), 4.28 (dd, *J* = 7.4 Hz, 11.5, 1H), 4.18 (m, 1H), 4.05 (m, 1H), 3.52 (m, 1H), 3.48 (t, *J* = 7.1 Hz, 1H), 2.79 (s, 1H) 2.64 (s, 3H), 2.40 (m, 3H), 2.30 (t, *J* =7.5 Hz, 2H), 2.18 (m, 2H), 1.76 (m, 2H), 1.68 (m, 2H), 1.51 (m, 2H), 1.35 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.5, 172.6, 172.0, 156.6, 153.1, 152.0, 146.0, 137.1, 133.3, 130.4, 129.2, 128.9, 128.8, 117.1, 84.7, 81.7, 74.8, 71.8, 63.6, 35.0, 33.9, 33.5, 32.3, 31.9, 31.8, 26.9, 26.8, 23.6, 23.4, 16.9 IR (ν_{max}): 3292, 3117, 2961, 2932, 2359, 1732, 1682, 1645, 1595, 1356, 1248, 1153, 1080, 802, 689, 635 cm^{-1}. HR-MS $[M+K]^+$ 695.6216 m/z.

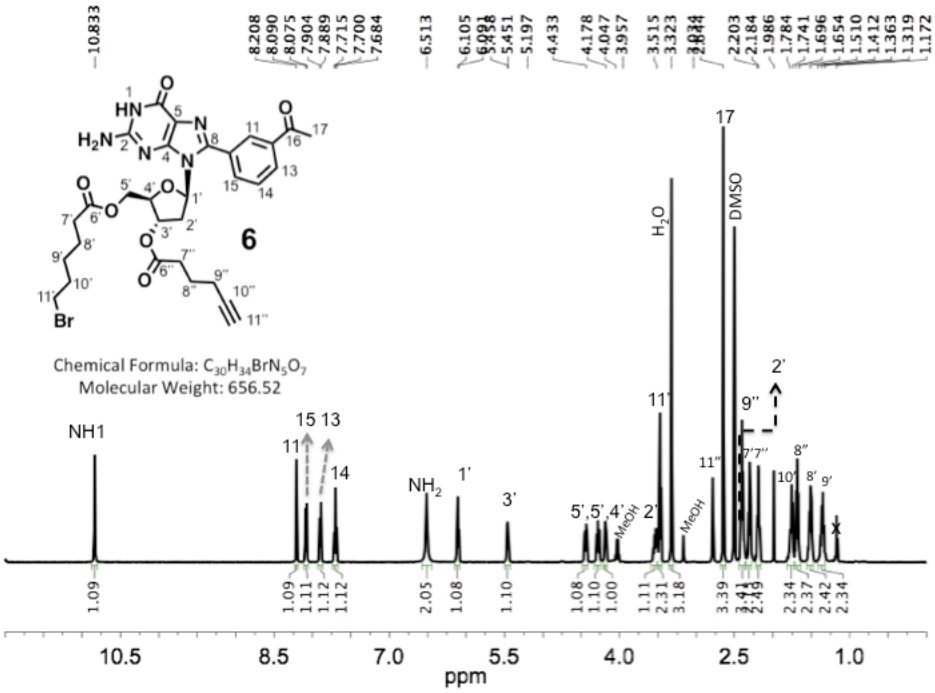


Figure S5. ^{1}H NMR (DMSO–d_6, 500 MHz, 298 K) of **6**.

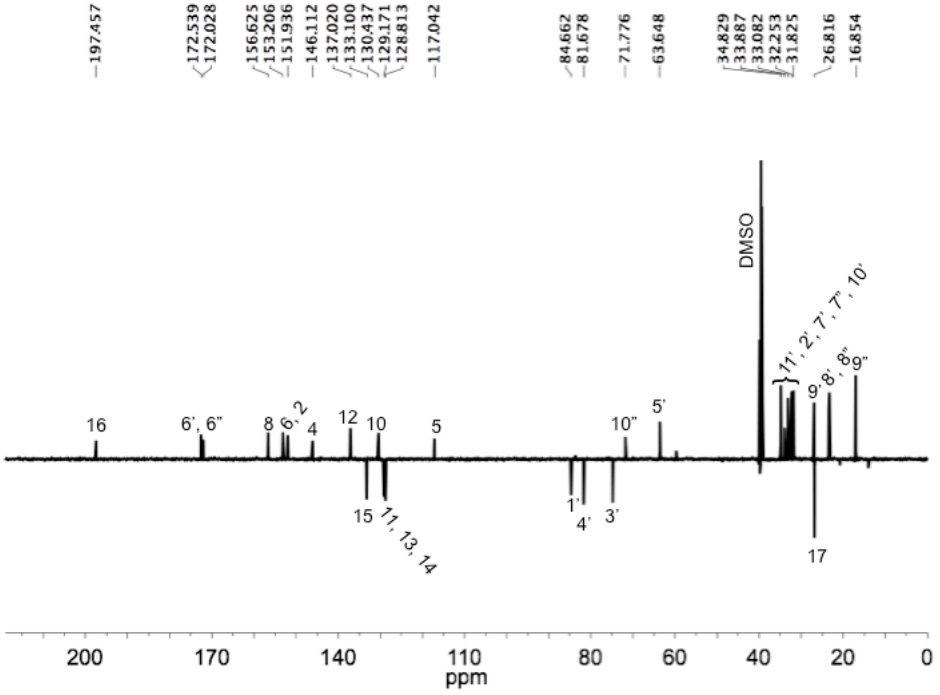


Figure S6. ^{13}C DEPTQ-135 NMR (DMSO–d_6, 125 MHz, 298 K) of **6**.

- ***Synthesis of ((2R,3S,5R)-5-(8-(3-acetylphenyl)-2-amino-6-oxo-1H-purin-9(6H)-yl)-3-(4-(1-(7-hydroxy-2-oxo-2H-chromen-3-yl)-1H-1,2,3-triazol-4-yl)butanoyloxy)tetrahydrofuran-2-yl)methyl 6-bromohexanoate (7)***

The starting material **6**, (400 mg, 0.609 mmol) and 3-azido-7-hydroxycoumarin[2] (186 mg, 0.914 mmol) were dispersed in THF: H_2O (3:1, 20 mL). To this solution sodium ascorbate (36 mg, 0.183 mmol) and $CuSO_4 \cdot H_2O$ (0.1 M solution, 0.0609 mmol) were added. The reaction was followed by TLC (9:1, EtOAc:MeOH) until the starting material disappeared. This was followed by solvent evaporation. Water was added and the product was sonicated and filtered. The resulting solid was dry-loaded with silica for purification by flash column chromatography (9:1, EtOAc:MeOH), affording the target compound **5** as a yellow solid, yielding 210 mg (40%).

Yellow Solid, mp 167.6 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.10 (s, 1H), 8.39 (s, 1H), 8.26 (s, 1H), 8.21 (s, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 7.4 Hz, 1H), 7.68 (t, *J* = 7.7 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 6.65 (s, 2H), (m, 3H), 6.58 (s, 1H), 6.12 (t, *J* = 6.9 Hz, 1H), 5.46 (m, 1H), 4.44 (m, 1H), 4.28 (dd, *J* = 7.4 Hz, 11.5 Hz, 1H), 4.19 (m, 1H), 3.54 (m, 1H), 3.46 (t, *J* = 6.6 Hz, 1H), 2.72 (t, *J* = 7.2 Hz, 1H), 2.63 (s, 3H), 2.40 (m, 3H), 2.29 (t, *J* = 7.1 Hz, 2H), 1.89 (m, 2H), 1.74 (m, 2H), 1.49 (m, 2H), 1.34 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): 197.5, 172.6, 172.2, 156.8, 153.3, 152.0, 146.0, 137.1, 136.5, 133.2, 130.5, 129.1, 128.8, 123.0, 117.2, 102.4, 84.7, 81.7, 74.7, 63.6, 34.8, 33.9, 33.0, 32.7, 31.8, 26.9, 26.8, 24.1, 23.4. IR (ν_{max}): 3306, 3099, 2961, 2930, 2345, 2122, 1726, 1682, 1607, 1578, 1357, 1256, 1231, 1097, 1040, 800, 694, 588, 525, 463 cm^{-1}. HR-MS $[M+Na]^+$ 882.6645 m/z.

[2] Prepared as previously reported by Sivakumar K.; Xie, F.; Cash, B.M.; Long S.; Barnhill, H.; Wang, Q., "A Fluorogenic 1,3-Dipolar Cycloaddition Reaction of 3-Azidocoumarins and Acetylenes." *Org. Lett.* **2004**, 6, 4603- 4606.

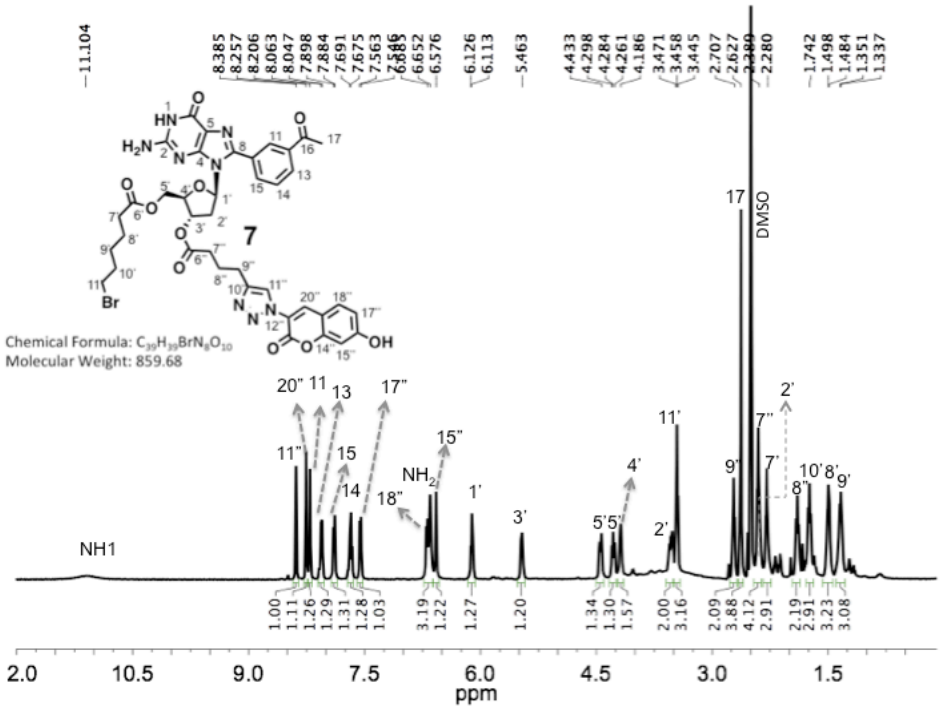


Figure S7. ^{1}H NMR (DMSO–d_6, 500 MHz, 298 K) of **7.**

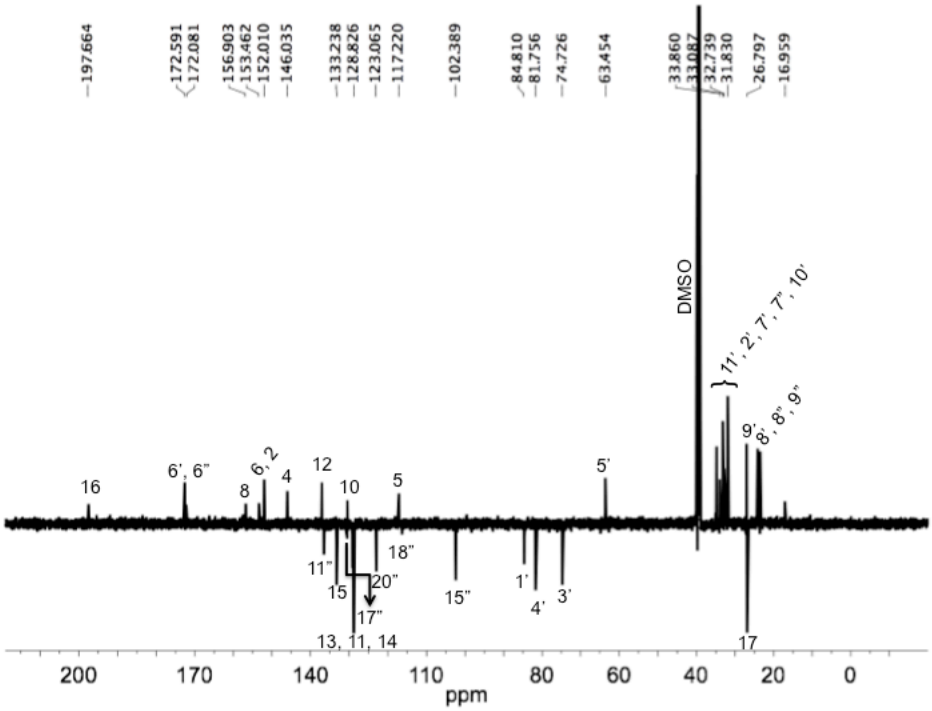


Figure S8. ^{13}C DEPTQ-135 NMR (DMSO–d_6, 125 MHz, 298 K) of **7.**

- ***Synthesis of ((2R,3S,5R)-5-(8-(3-acetylphenyl)-2-amino-6-oxo-1H-purin-9(6H)-yl)-3-(4-(1-(7-hydroxy-2-oxo-2H-chromen-3-yl)-1H-1,2,3-triazol-4-yl)butanoyloxy)tetrahydrofuran-2-yl)methyl 6-(4-((dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)hexanoate (1)***

The following reaction was performed under Ar atmosphere. NaN_3 (47 mg, 0.722 mmol) was added to a solution of **7** (200 mg, 0.232 mmol) in DMF. The reaction was stirred and refluxed at 80 °C overnight. This was followed by solvent evaporation. Water was poured into the solid crude, which was sonicated and filtered. The resulting yellow solid (used without further purification) (198 mg, 0.241 mmol) and 1-dimethylamino-2-propyne (40 mg, 0.482 mmol) were dispersed in THF: PBS (7.4 pH) (3:1, 20 mL). To this solution sodium ascorbate (14 mg, 0.0723 mmol) and $CuSO_4 \bullet H_2O$ (0.1 M solution, 0.0241 mmol) were added. The reaction was monitored by TLC (80:20:1, DCM: MeOH: Et_3N) until the starting material was consumed. The solvent was removed under reduced pressure and water was added to the product, followed by sonication and filtration. The resulting solid was dry-loaded into silica and purified by flash column chromatography (EtOAc:MeOH, 90:10, followed by 89:10:1 EtOAc:MeOH:Et_3N), then a preparative TLC (80:20:1, DCM: MeOH: Et_3N) was done to afford the target compound **1**, to yield 22 mg (10 %) of a yellow solid.

Yellow solid, mp 153.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.94 (s, 1H), 8.55 (s, 1H), 8.31 (s, 1H), 8.20 (s, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 7.93 (s, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.35 (d, J = 9.0 Hz, 2H), 7.67 (t, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 9.5 Hz, 1H), 6.85 (s, 1H), 6.54 (b, 2H), 6.11 (t, J = 7.0 Hz, 1H), 5.47 (m, 1H) 4.45 (m, 1H), 4.27 (t, *J* = 7.5 Hz, 3 H), 3.51 (m, 1H), 3.47 (s, 2H), 3.17 (s, 6H), 2.74 (t, *J* = 7.5, 2H), 2.50 (s, 3H), 2.39 (m, 3H), 2.27 (t, *J* = 6 Hz, 2H), 1.91 (m, 2H), 1.77(m, 2H), 1.49 (m, 2H) 1.23 (b, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.81, 172.55, 172.21, 162.49, 156.34, 156.30, 154.54, 153.36, 152.76, 152.07, 146.26, 145.94, 137.18, 136.90, 135.55, 132.97, 131.01, 130.46, 129.19, 128.90, 128.44, 122.63, 119.99, 117.12, 114.48, 110.25, 101.87, 84.35, 82.40, 81.56, 74.91, 63.61, 48.66, 45.40, 33.76, 33.09, 30.15, 29.99, 27.15, 25.15, 23.70. IR (ν_{max}): 3292, 3117, 2961, 2932, 2359, 1732, 1682, 1645, 1595, 1356, 1248, 1153, 1080, 802, 689, 635 cm^{-1}. HR-MS $[M+H]^+$ 906.9263 m/z

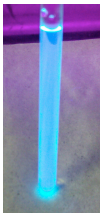


Figure S9. A solution of **1** in DMSO-d_6 exposed to UV irradiation showing its blue luminescence.

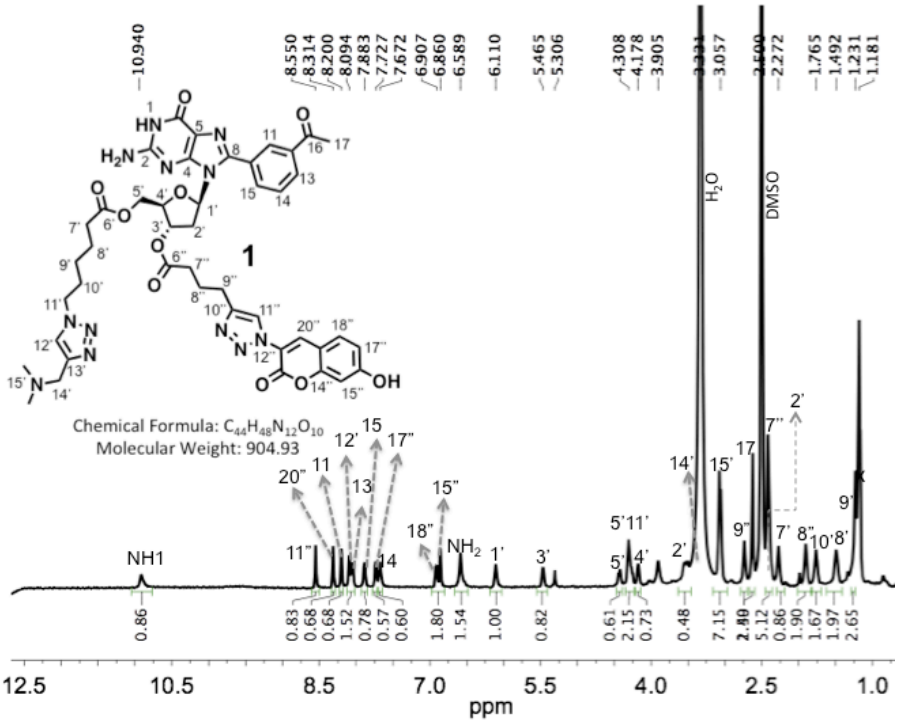


Figure S10. ^{1}H NMR (DMSO–d_6, 500 MHz, 298 K) of **1**.

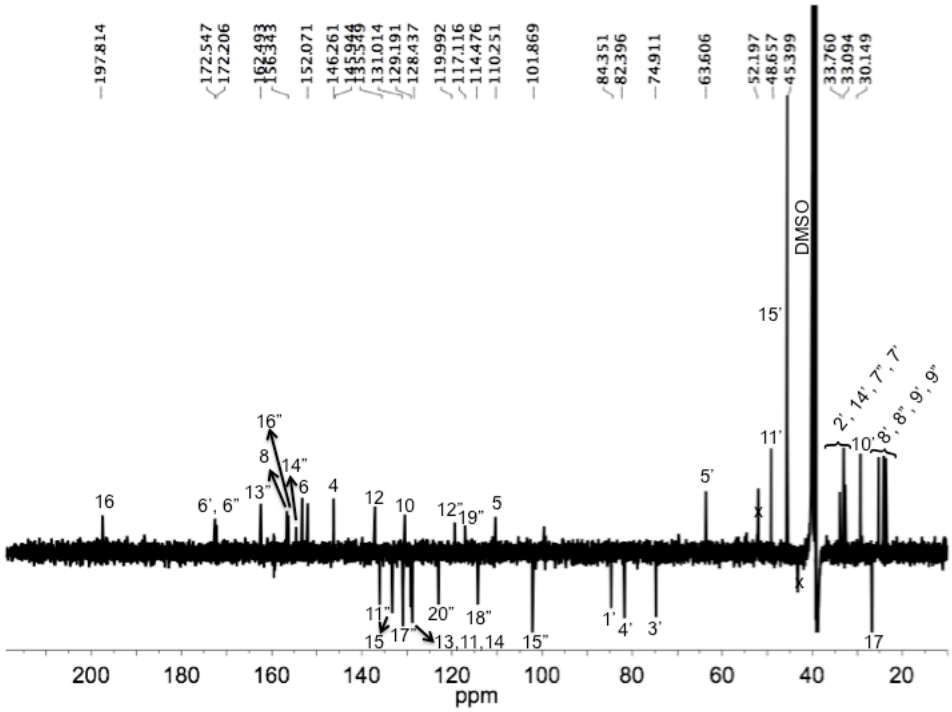
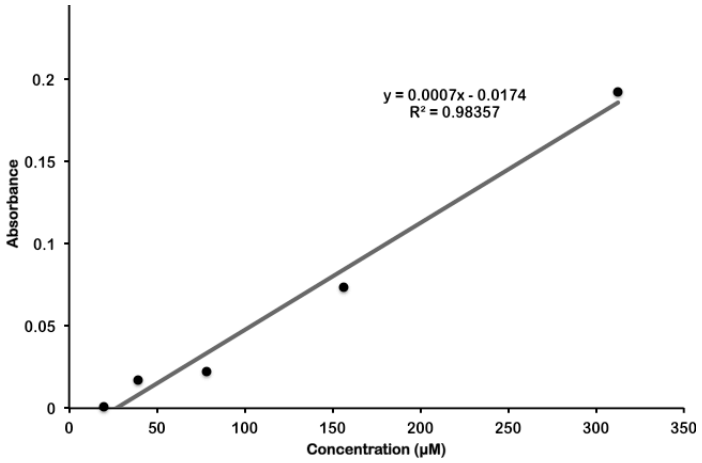


Figure S11. ^{13}C DEPTQ-135 NMR (DMSO–d_6, 125 MHz, 298 K) of **1.**

F. Photophysical properties

- ***UV-visible Characterization***

UV/Vis absorption spectra of **1** at the specific conditions (390 nm) were recorded with a Varian Cary 1E UV-visible spectrophotometer. The solutions were contained in quartz cells (0.1 cm optical path) closed with a plastic cap. The molar absorption coefficient (ε_{max}) was calculated using the Beer-Lambert law (Graph S1) and calculated to be ε_{max} = 7,000 $M^{-1}cm^{-1}$. Different concentrations of a mixture of 0.0625 equiv of **1** to 0.9375 equiv of **2** were prepared in a lithium phosphate buffer (LiPB, pH 7.4). For each run the corresponding concentration of **2** (LiPB, pH 7.4) was measured as a baseline and subtracted to the mixture sample to obtain the absorbance corresponding to **1**. The absorption band of guanine moiety in **1** lies mostly below 300 nm with no detectable absorption at the absorption/excitation and emission wavelengths of the coumarin moiety. Therefore, the former is unlikely to interfere with the latter.



Graph S1. Variable concentration of **1** as a function of its absorbance (390 nm).

- ***Fluorescence Characterization***

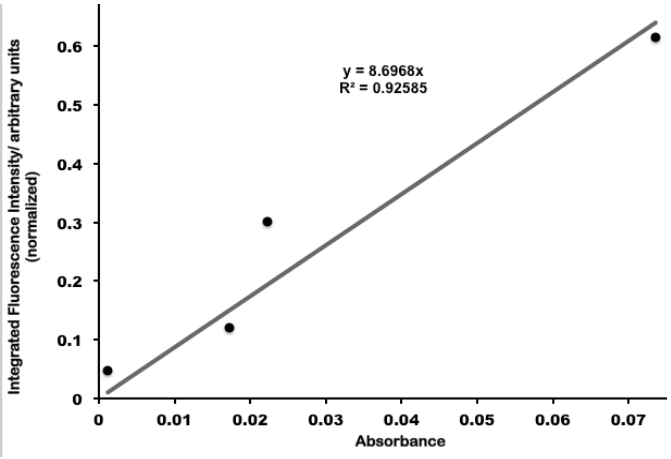
Fluorescent experiments were made in Varian Cary Eclipse fluorescence spectrophotometer. The samples were excited at 390 nm with an excitation slit, 5 nm/emission; slit, 10 nm, emission monitored at 477 nm, at 500 V. Data was normalized, and curve fitted using Microsoft® Excel® for Mac 2011, version 14.1.0 and KaleidaGraph, version 3.6.2.

The quantum yield was calculated as describe by Arce et al.[3] Different concentrations of stoichiometric mixtures of **1** (0.0625 equiv) and **2** (0.9375 equiv) were prepared in LiPB (pH 7.4, η_{ST} = 1.3285). Emission spectra of the solutions showing absorbance with less than 0.1 at the excitation wavelength (390 nm) were recorded, to avoid non-linear effects. Coumarin 120[4], Φ= 0.51 in methanol (η_{ST} = 1.328) was used as the standard. Plots of the integrated/normalized fluorescence intensity as a function of the absorbance were done, the straight-line intercept was set to 0 and the slope (graphs S2 and S3) was used to determine the relative fluorescence quantum yield using the following equation.

$\Phi_1 = \Phi_{ST}\,(slope_6/\ slope_{ST})(\eta^2_x/\eta^2_{ST}) = 0.32$

Brightness[5] = $\Phi_1 \bullet \varepsilon_{max}$ = 2240

Stoke shift = 87 nm

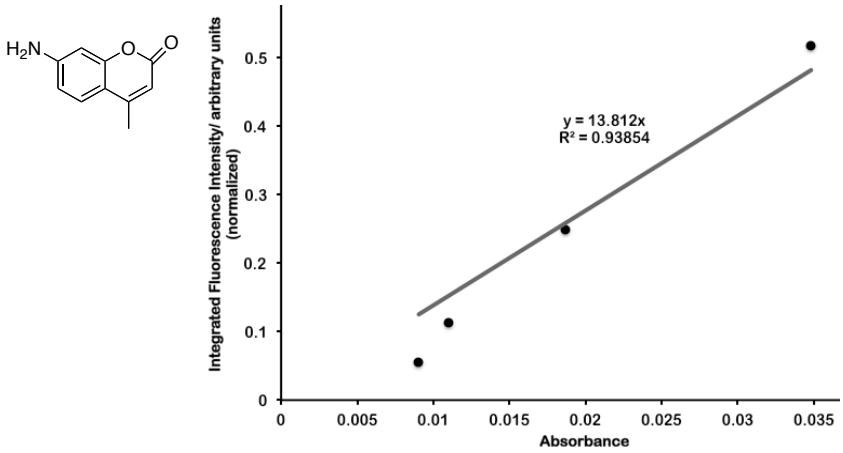


Graph S2. Integrated fluorescence intensity (477 nm) as a function of the absorbance (390 nm) of samples at different concentrations of **1** LiPB**.**

[3] Arce, R.; Pino, E. F.; Valle, C.; Ágreda, J., "Photophysics and Photochemistry of 1-Nitropyrene." *J. Phys. Chem. A* **2008,** 112, 10294-10304.

[4] Pal, H.; Nad, S.; Kumbhakar, M., "Photophysical properties of coumarin-120: Unusual behavior in nonpolar solvents" *J. Chem. Phys.* **2003,** 119, 443-452.

[5] Cuppoletti, A.; Cho, Y.; Park, J.-S.; Strässler, C.; Kool, E. T., "Oligomeric Fluorescent Labels for DNA." *Bioconjugate Chem.* **2005,** 16, 528-534.



Graph S3. Integrated fluorescence intensity (477 nm) as a function of the absorbance (390 nm) of samples at different concentrations of coumarin 120 in methanol.

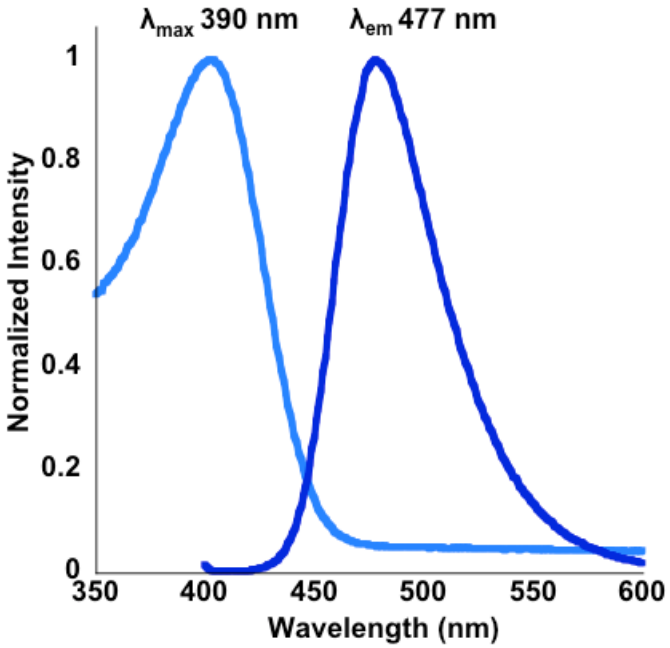


Figure S12. Overlay spectra of **1** showing the absorbance with a maximum at 390 nm (baby blue) and the emission at 477 nm (navy blue).

- ***Doxorubicin (DOX): guest acceptor molecule***

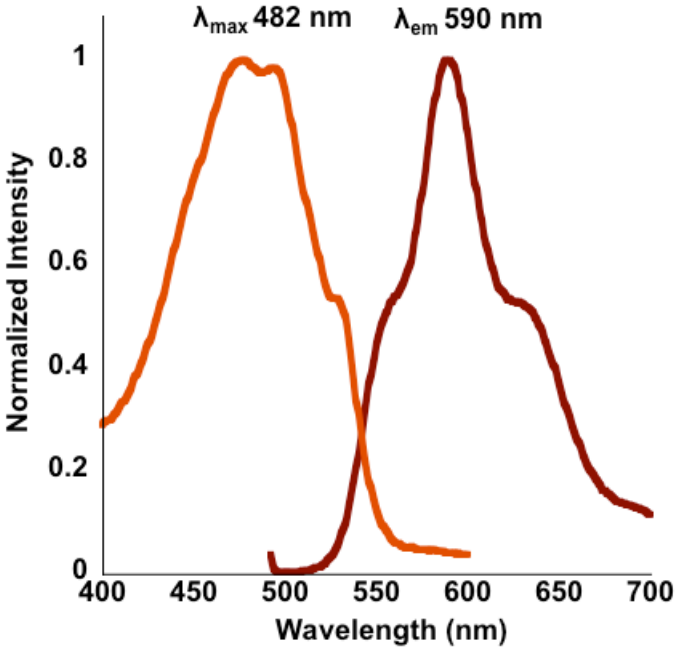


Figure S13. Overlay spectra of **DOX** showing the absorbance with a maximum at 482 nm (orange) and the emission at 590 nm (burgundy).

G. ^{1}H-NMR experiments: self-assembly

The studies were carried out using a Bruker AV-500 NMR spectrometer, equipped with a 5 mm TABBO probe. In water, a conventional 1D pre-saturation pulse sequence with the excitation pulse set over the water peak at 4.8 ppm was used. Sodium 3-(trimethylsilyl)propionate-2,2,3,3-*d*4 (Aldrich) was used as the internal standard for the NMR experiments performed in H_2O:D_2O (9:1) and used to calibrate all spectra to 0 ppm. Self-assembly studies were performed, using a 5 mM total solution concentration of derivatives (0.0625 equiv of **1** and 0.9375 equiv of **2**) in 650 µL of LiPB (pH 7.4)-D2O (9:1). All the spectra were processed using MestReNova, version: 5.2.1-3586, applying a signal suppression at 4.82 ppm, followed by a Whitaker Smoother baseline correction and smoothing along the F1 (Figures S14 and S15).

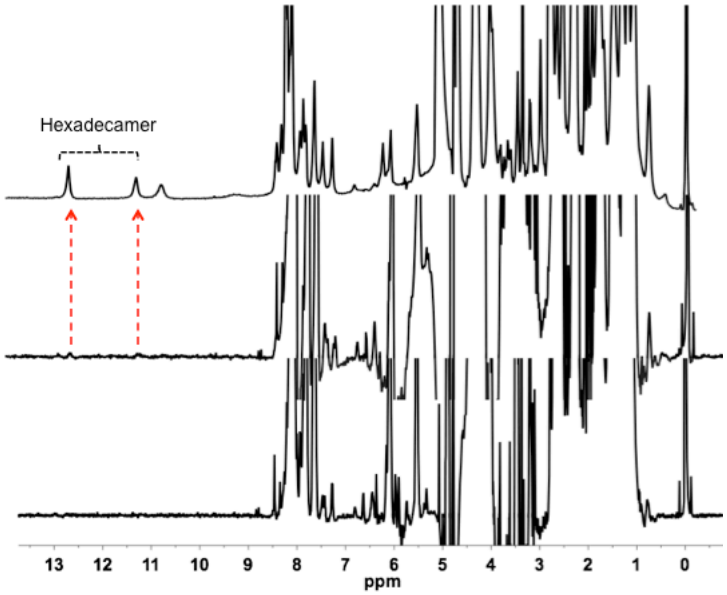


Figure S14. ^{1}H NMR spectra for: (bottom) for a mixture of **1** and **2** (0.0625 equiv of **1** and 0.9375 equiv of **2**; 5 mM total concentration) in LiPB with 10% D_2O; (middle) same as previous with 0.0625 equiv of **DOX** without KCl; and (top) with 1 M KCl.

H. Energy transfer studies: DOX titration experiments

Fluorescent experiments were made in Varian Cary Eclipse fluorescence spectrophotometer. The emission was measured exciting the samples at 390 nm with an excitation slit of 5 nm and emission slit of 10 nm, with the detector set to 500V (400V for control experiments). The excitation was measured at 590 nm, under the same conditions. Data was processed, and curve fitted using Microsoft® Excel® for Mac 2011, version 14.1.0 and KaleidaGraph, version 3.6.2.

- ***Emission spectra sample with KCl***

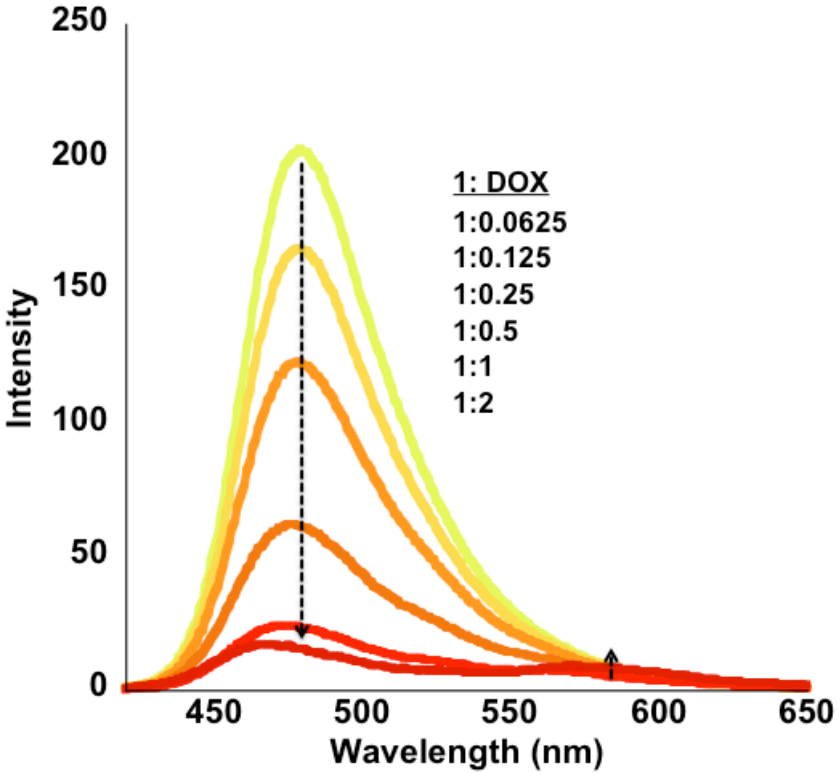


Figure S15. Overlaid fluorescence emission spectra (excited at 390 nm) of a mixture of **1** and **2** (0.0625 equiv of **1** and 0.9375 equiv of **2**; 5 mM total concentration of derivatives) in LiPB with 1 M KCl with increasing amount of **DOX**, showing the donor quenching as the amount of acceptor is increased.

- ***Emission spectra with no potassium***

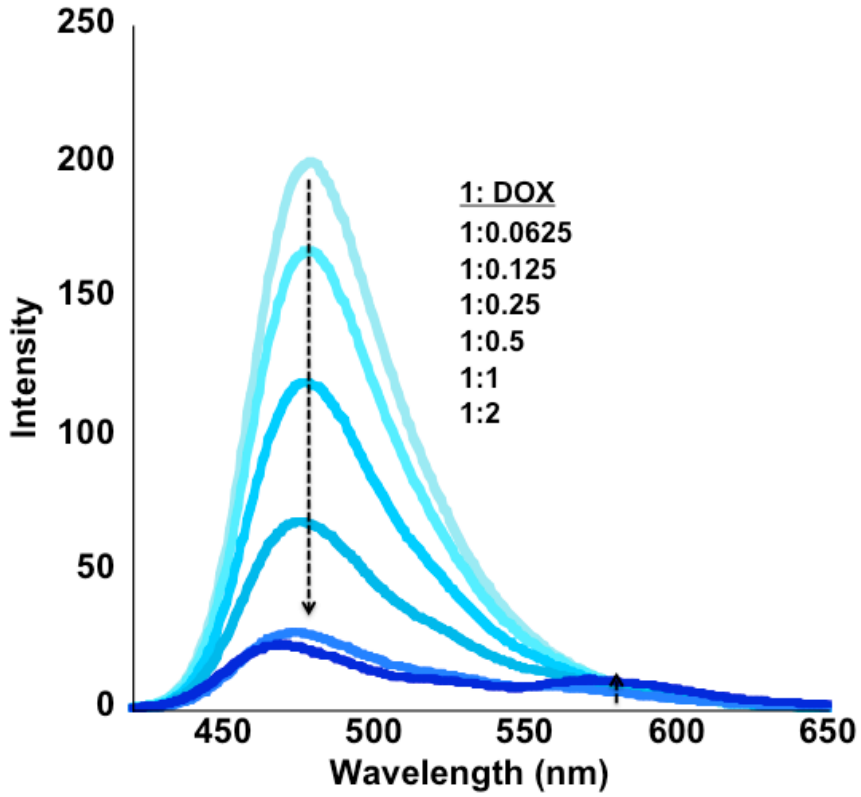
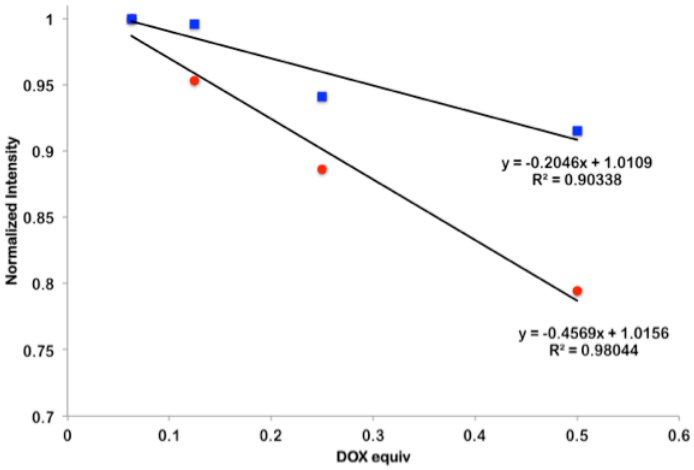


Figure S16. Overlaid fluorescence emission spectra (excited at 390 nm) of a mixture of **1** and **2** (0.0625 equiv of **1** and 0.9375 equiv of **2**; 5 mM total concentration of derivatives) in LiPB with no potassium and with increasing amount of **DOX**, showing the donor quenching as the amount of acceptor is increased.



Graph S4. Correlation for the first four points of the titration with **DOX**, corresponding to the excitation intensity of the sample with 1 M KCl (red circle) and the sample with no KCl (blue square).

I. Variable temperature experiments measured by fluorescence spectroscopy

Variable temperature fluorescent experiments were made in a Varian Cary Eclipse fluorescence spectrophotometer equipped with a Harrick series 989 temperature controller. The donor emission was recorded every 5 degrees, starting from 25 °C up to 80 °C. The samples were measured exciting at 390 nm with an excitation slit of 5 nm and emission slit of 10 nm, with the detector set to 500 V. Data was processed, and curve fitted using Microsoft® Excel® for Mac 2011, version 14.1.0 and KaleidaGraph, version 3.6.2. First derivative calculation was done in Prism 4 for Macintosh version 4.0a after data smoothing in KaleidaGraph.

- ***Sample with KCl***

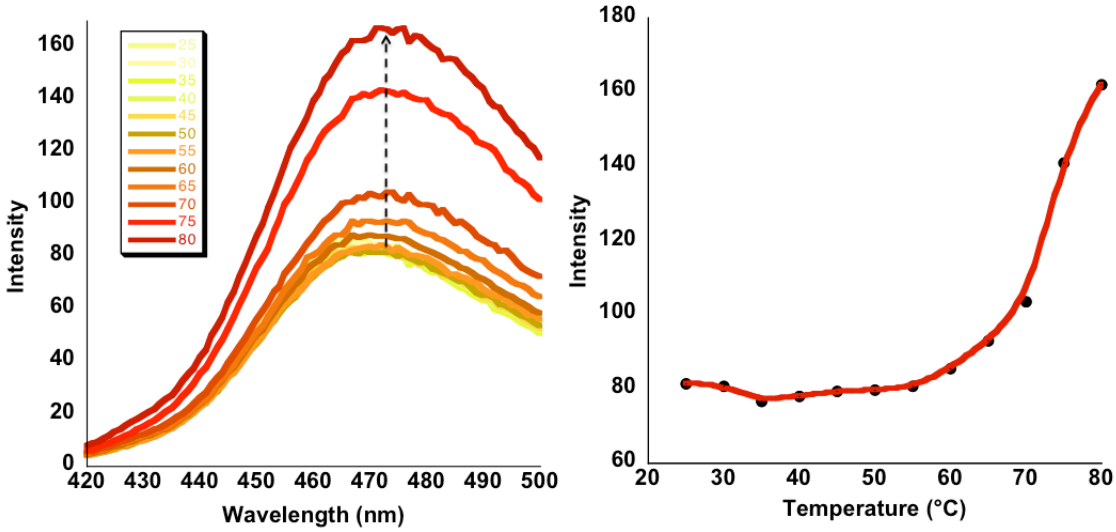


Figure S17. Overlaid fluorescence emission spectra of a mixture of **1** and **2** (0.0625 equiv of **1** and 0.9375 equiv of **2**; 5 mM total concentration of derivatives) in LiPB with 0.125 equiv of **DOX** and 1 M of KCl at different temperatures. To the right a plot showing the emission intensity sigmoidal tendency of **1** as a function of temperature.

- ***Sample with no potassium***

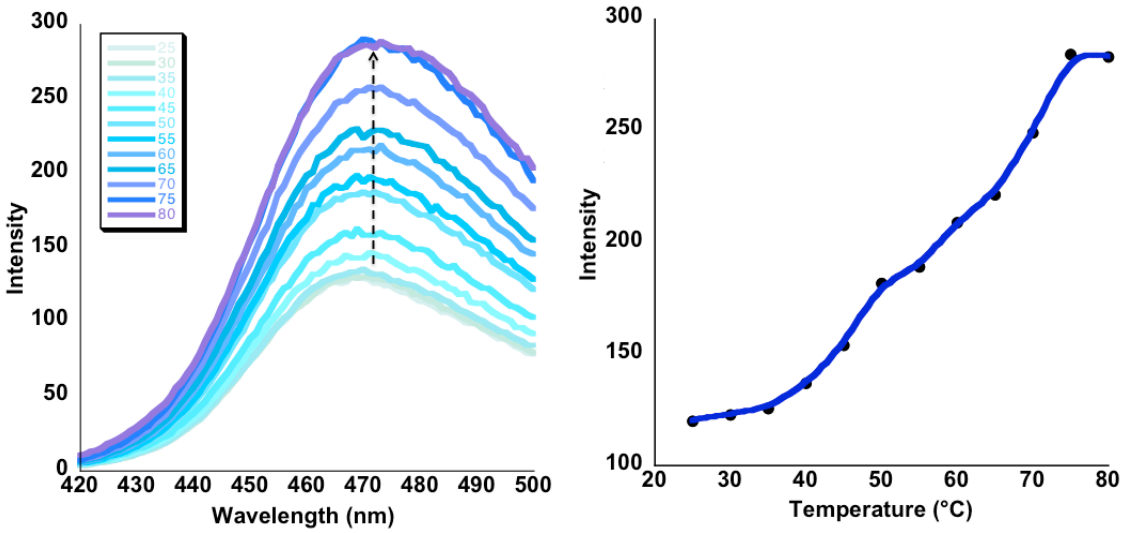


Figure S18. Overlaid fluorescence emission spectra of a mixture of **1** and **2** (0.0625 equiv of **1** and 0.9375 equiv of **2**; 5 mM total concentration of derivatives) in LiPB with 0.125 equiv of **DOX** at different temperatures. To the right a plot showing the gradual trend in emission intensity of **1** as a function of temperature.

J. Control Experiments using 8 and DOX

- **Titration of 8 with DOX**

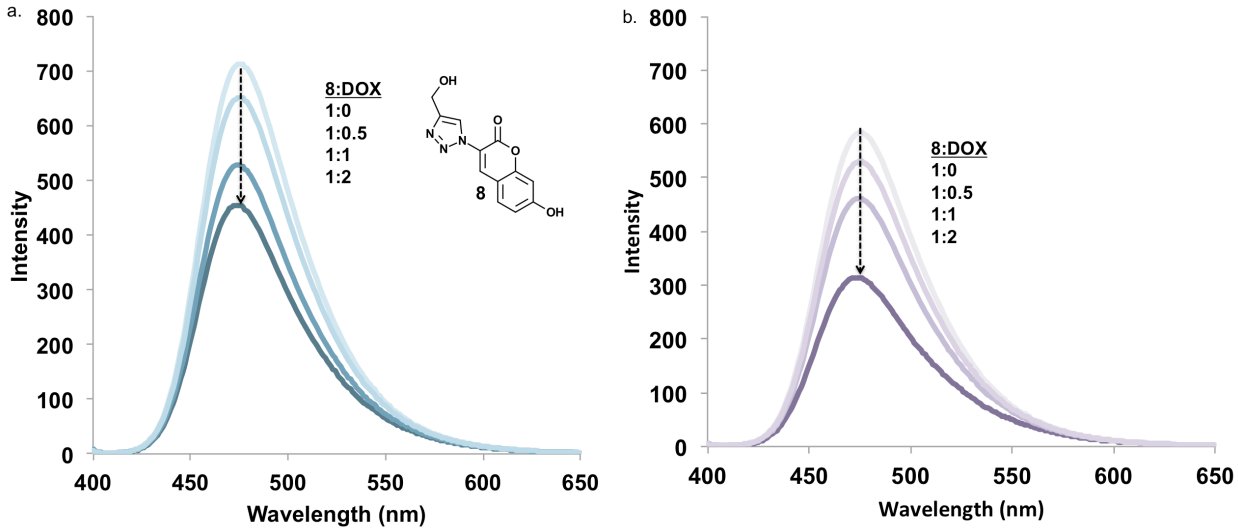


Figure S19. *Titration of **8** with **DOX** in the presence of a) LiPB and b) LiPB with 1 M KCl. **8** is quenched by about 20% in 1 M KCl but the efficiency of the quenching (slope in the curve in the plot shown in Fig. S20) remains essentially the same.*

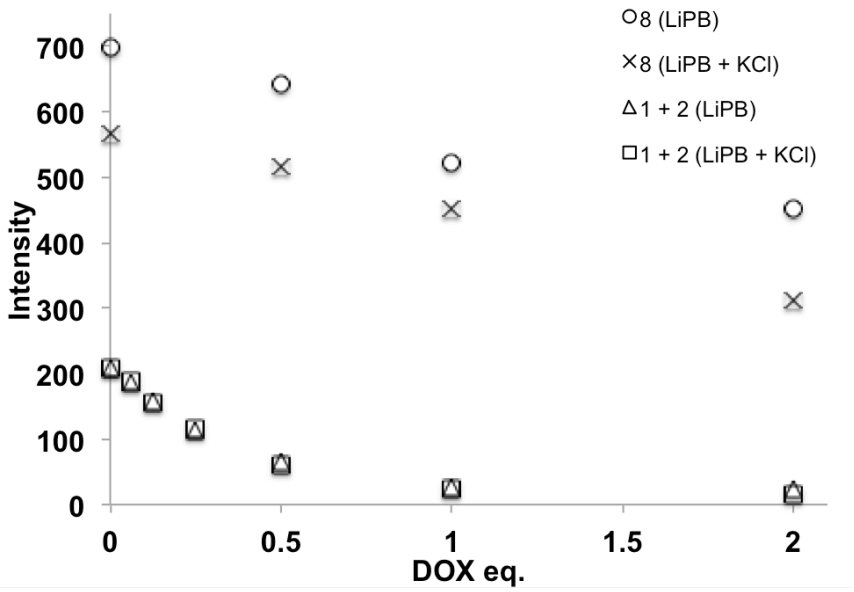


Figure S20. *Non-normalized version of Figure 5*

- ***Variable temperature Control experiment***

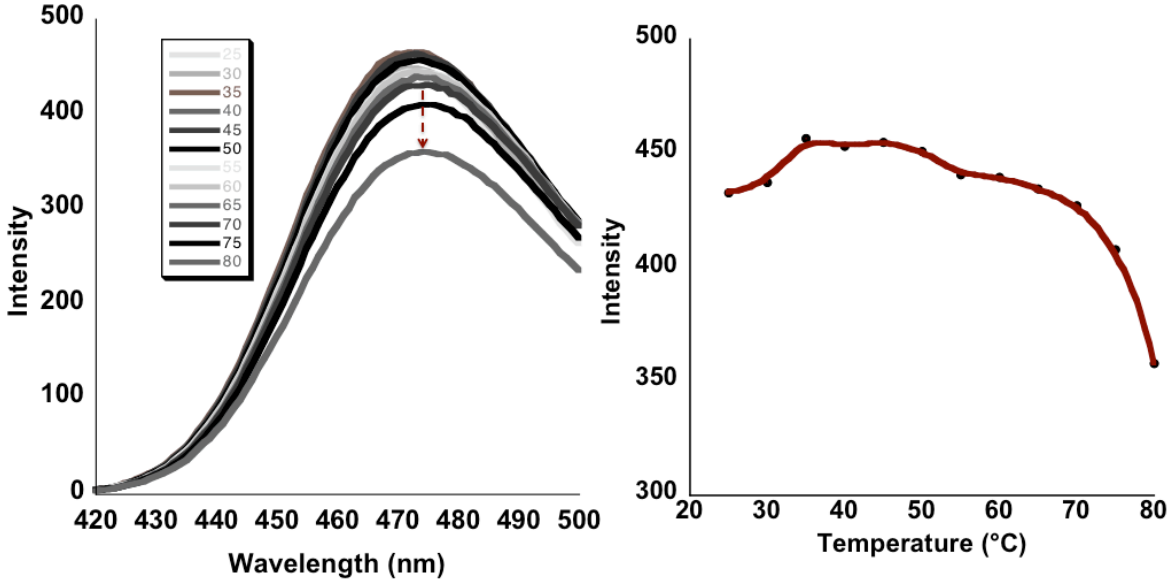


Figure S21. (Left) Overlaid fluorescence emission spectra of **8** (0.0625 equiv) with **DOX** (2 equiv) in LiPB at different temperatures. (Right) Plot showing the relatively constant emission intensity of **8** (up to ca. 70 °C) as a function of temperature.

K. Differential Scanning Colorimetry (DSC) Data

The total heat required for the dissociation of the hexadecamer and the complex was measured in LiPB (pH 7.4, 1 M KCl), in a temperature range of 20–100 °C, using a heating rate of 1 °C min^{-1}. The T_m value is taken as the maxima in the DSC curve. The curves were generated using Origin v7.

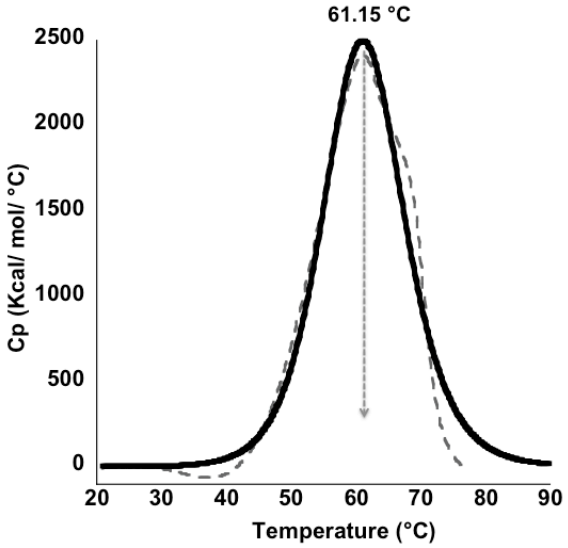


Figure S22. DSC melting curve of a mixture of **1** and **2** (0.0625 equiv of **1** and 0.9375 equiv of **2**; 5 mM total concentration of derivatives) in LiPB with 1M KCl.

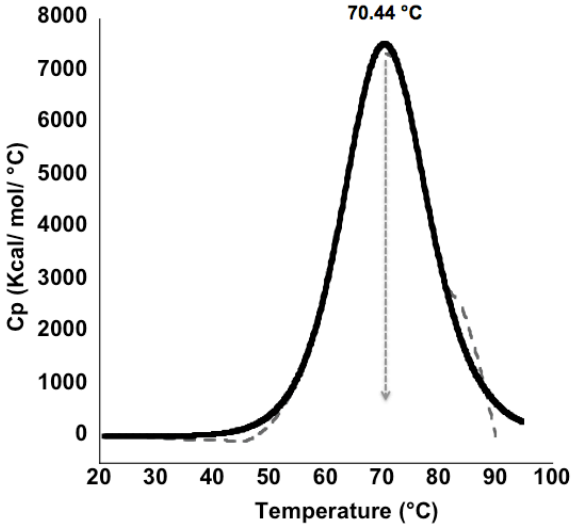


Figure S23. DSC melting curve of a mixture of **1** and **2** (0.0625 equiv of **1** and 0.9375 equiv of **2**; 5 mM total concentration of derivatives) with 0.125 equiv of **DOX** in LiPB with 1 M KCl.

L. Molecular Modeling Studies

The molecular model shown below was minimized using: *AMBER* (MacroModel)*, Version 9.5, Maestro 9.1.207; Schrödinger, LLC: New York, 2007, representing water as a continuum solvent. This model was built upon a previously published model of $\mathbf{2}_{16}$•$3K^+$.[6] Compound **1** replaced a molecule from one of the outer tetrads of the hexadecamer of **2**. The resulting structure **hSGQ** ($\mathbf{1}_1\mathbf{2}_{15}$•$3K^+$) was minimized constraining the K^+ ions and the guanine core up to the 1' carbon of the sugar, allowing free movement of the side chains. Finally, a previously constructed and minimized molecule of **DOX** was placed on the outer tetrad opposite to the tetrad containing **1**. The model of the complex **hSGQ•DOX** ([$\mathbf{1}_1\mathbf{2}_{15}$•$3K^+$]•**DOX**) in Figure 2 was constructed and minimized using the same protocol just described.

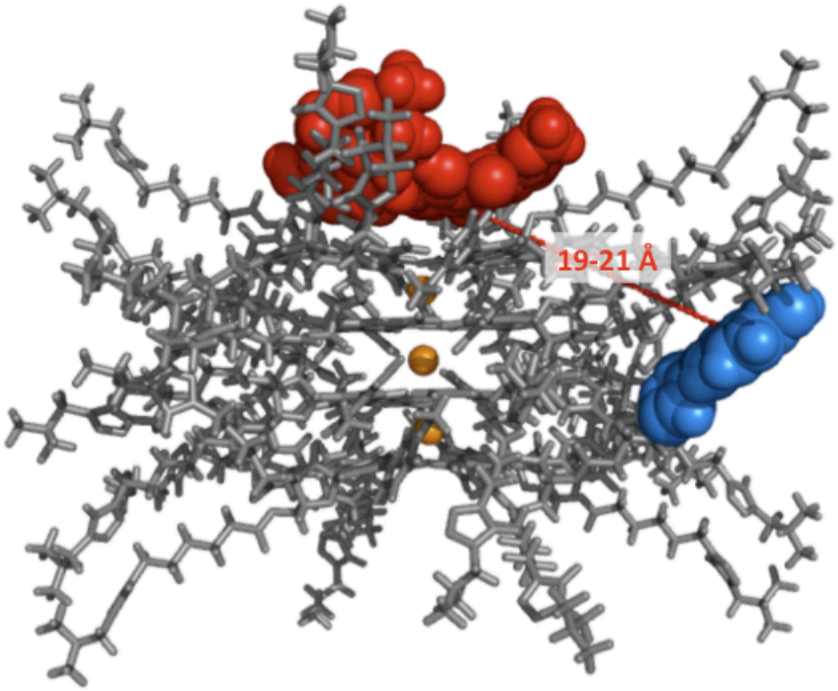


Figure S24. Molecular model of a **hSGQ•DOX** complex showing one of the many possible configurations where the coumarin moiety and the **DOX** are at the opposite ends of the **hSGQ**. Other isomers of this **hSGQ** could place the coumarin group and DOX at closer average distances.

[6] García-Arriaga, M.; Hobley, G.; Rivera, J. M., "Isostructural Self-assembly of 2'-deoxyguanosine Derivatives in Aqueous and Organic Media." *J. Am. Chem. Soc.* **2008,** 130, (32), 10492.

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