Drug Encapsulation within Self-assembled Microglobules Formed by Thermoresponsive Supramolecules

José E. Betancourt,[a] Chandramouleeswaran Subramani,[b] José L. Serrano-Velez,[c] Eduardo Rosa-Molinar,[c] Vincent M. Rotello[b] and José M. Rivera[a]*

[a] Department of Chemistry, University of Puerto Rico Río Piedras Campus, San Juan, PR. 00931, USA.
[b] Department of Chemistry, University of Massachusetts Amherst, 710 North Pleasant Street, Amherst, MA 01003, USA.
[c] Department of Biology, University of Puerto Rico Río Piedras Campus, San Juan, PR. 00931, USA.

Supporting Information

Disclaimer: The project described in this manuscript was supported by Award Number SC1GM093994 from the National Institute Of General Medical Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of General Medical Sciences or the National Institutes of Health.

A. General Experimental Procedures

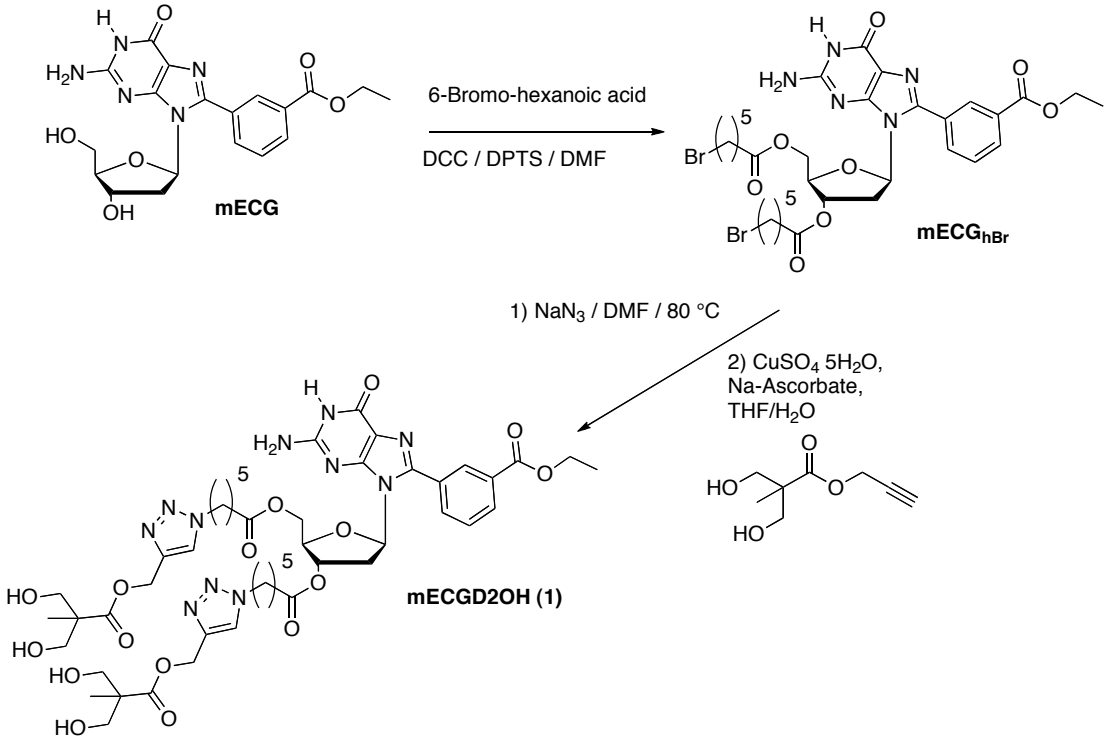
Dimethylformamide (DMF) was purchased anhydrous from Aldrich and used without further treatment. All other reagents were from commercial sources and used without further purification. Unless otherwise noted, all compounds were purified by column chromatography on silica gel 60, 0.04-0.063 mm, and TLC 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, iodine, 3,5-dinitrophenylhydrazine, and phosphomolybdic acid in ethanol stains. Reactions requiring anhydrous conditions were carried out using flame-dried glassware under Argon. 8-(3-ethoxycarbonylphenyl)-2'-deoxyguanosine (**mECG**) was synthesized according to the procedures outlined elsewhere.[1]

B. Characterization of the target compounds and Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-500 or AV-500 (TopSpin v 2.0) 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. Sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (Aldrich) was used as the internal standard for the NMR experiments performed in D_2O or H_2O:D_2O (9:1). All NMR experiments were performed at 298.2 K unless otherwise stated. The following abbreviations are used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiple; br, broad. 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. 10 mM solutions of monomer in acetonitrile at room temperature, were directly and continuously infused at a flow rate of 5 μL/min with a syringe pump. The source block temperature was maintained at 60 °C and the desolvation gas was heated to 80 °C. Argon was used as the collision gas and the cone voltage was set to 35 V. The mass spectrometer was operated in the mass range 0-4000 amu. 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. The transmittance experiments were measured at 500 nm or 800 nm using a Varian UV-visible spectrometer, Model Cary Bio-100. The heating rate was adjusted at 2.0 °C/min using Cary temperature controller apparatus from Varian. Dynamic light scattering (DLS) was used to measure the hydrodynamic size of the assemblies as a function of temperature in a 25–75 °C range. Dynamic Light Scattering (DLS) experiments were performed in a DynaPro Titan (Wyatt Technology Corporation) with temperature control and a microsampler. Samples were dispersed in a buffer solution at pH 7.4 and filtered with a 0.45 μm Nylon filter using a Fisherbrand 10 μm o.d. glass tubes prior to the experiment.

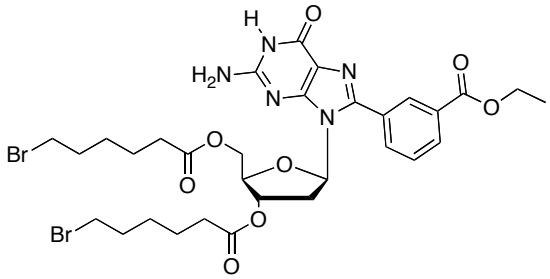
C. Synthesis.



Scheme S1 Synthesis of **1**.

Representative procedures for the synthesis of 1.

*General method for the esterification of **mECG**.* The starting material, powder **mECG** (500 mg, 500 µmol), pre-dried by suspension in acetonitrile and solvent evaporation (3x), was suspended in anhydrous DMF. To this suspension, DPTS (0.5 equivalents), 6-Bromo hexanoic acid (2.5 equivalents) and DCC (2.5 equivalents) were added. The reaction mixture was stirred until TLC (DCM/MeOH, 80:20) showed complete conversion of starting material. The reaction mixture was quenched by adding excess of MeOH followed by solvent evaporation. The resulting solid material was dissolved in EtOAc and washed with 10% $NaHCO_3$ (2 x 25 mL) and brine (1 x 25 mL). The organic phase was separated, dried over $MgSO_4$ and evaporated into silica gel. Dry loading of the silica column followed by flash chromatography (DCM/MeOH, 95:5) afforded the target compound **mECGhBr** as solids.



Chemical Formula: $C_{31}H_{39}Br_2N_5O_8$
Molecular Weight: 769.4781

8-(3-ethoxycarbonylphenyl)-(3',5'-bis-O-(6-bromohexanoyl))-2'-deoxyguanosine (**mECGhBr**) Light yellow powder, mp (decomposition) 165.2-167.5 °C. TLC (DCM:MeOH, 8:2): R_F = 0.3; 90 % yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.86 (s, 1H), 8.26 (s, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 7.7 Hz, 1H), 7.69 (t, *J* = 7.8 Hz, 1H), 6.54 (br-s, 2H), 6.11 (t, *J* = 7.0 Hz, 1H), 5.44 (d, *J* = 6.9, Hz, 1H), 4.45 (dd, *J* = 11.6, 4.8 Hz, 1H), 4.32 (dd, *J* = 12.8, 7.1 Hz, 3H), 4.22 - 4.13 (m, 1H), 3.54 (m, 1H), 3.46 (dd, *J* = 11.9, 6.4 Hz, 4H), 2.39 - 2.23 (m, 5H), 1.82 - 1.67 (m, 4H), 1.51 (q, *J* = 7.3 Hz, 4H), 1.42 - 1.27 (m, 7H). ^{13}C NMR (125.8 MHz, DMSO-d_6): δ 172.6, 172.4, 165.2, 156.7, 153.2, 145.9, 133.3, 130.5, 130.4, 129.9, 129.7, 117.2, 84.7, 81.8, 74.7, 63.6, 61.0, 34.8, 34.7, 33.2, 31.8, 26.9, 26.9, 23.5, 23.4, 14.1. IR (ν_{max}): 3347, 2931, 1684, 1635, 1678, 1593, 1573, 1257, 1173, 1123, 1048 cm^{-1}. ESI-MS (*m*/*z*): $[M + 1]^+$ calcd for $C_{31}H_{39}Br_2N_5O_8$, 770.4781; found, 770.4542 $[M + 1]^+$, 790.1338 $[M + Na]^+$.

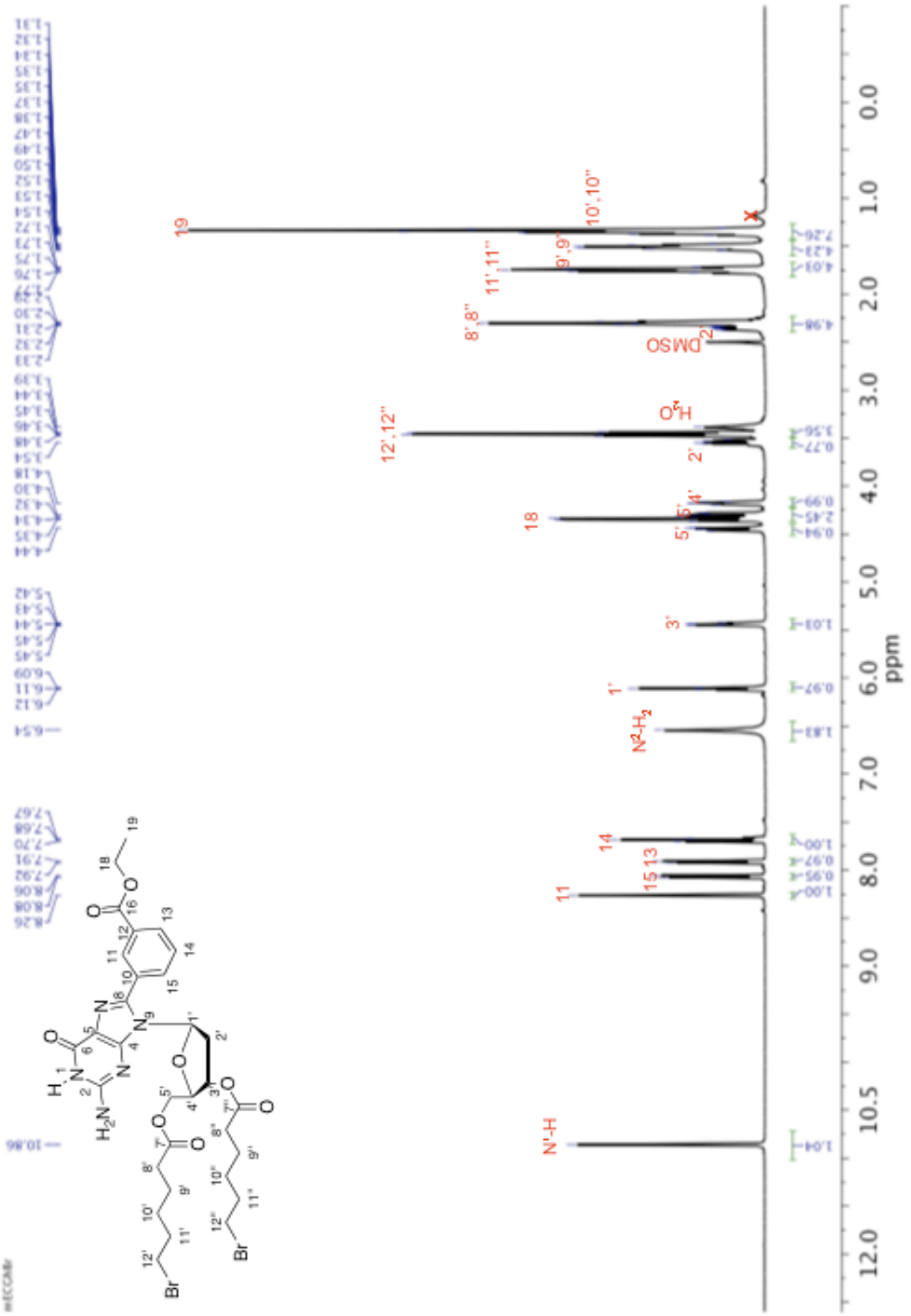


Figure S1 .^{1}H NMR (DMSO – d_6, 500 MHz) of **mECGhBr**.

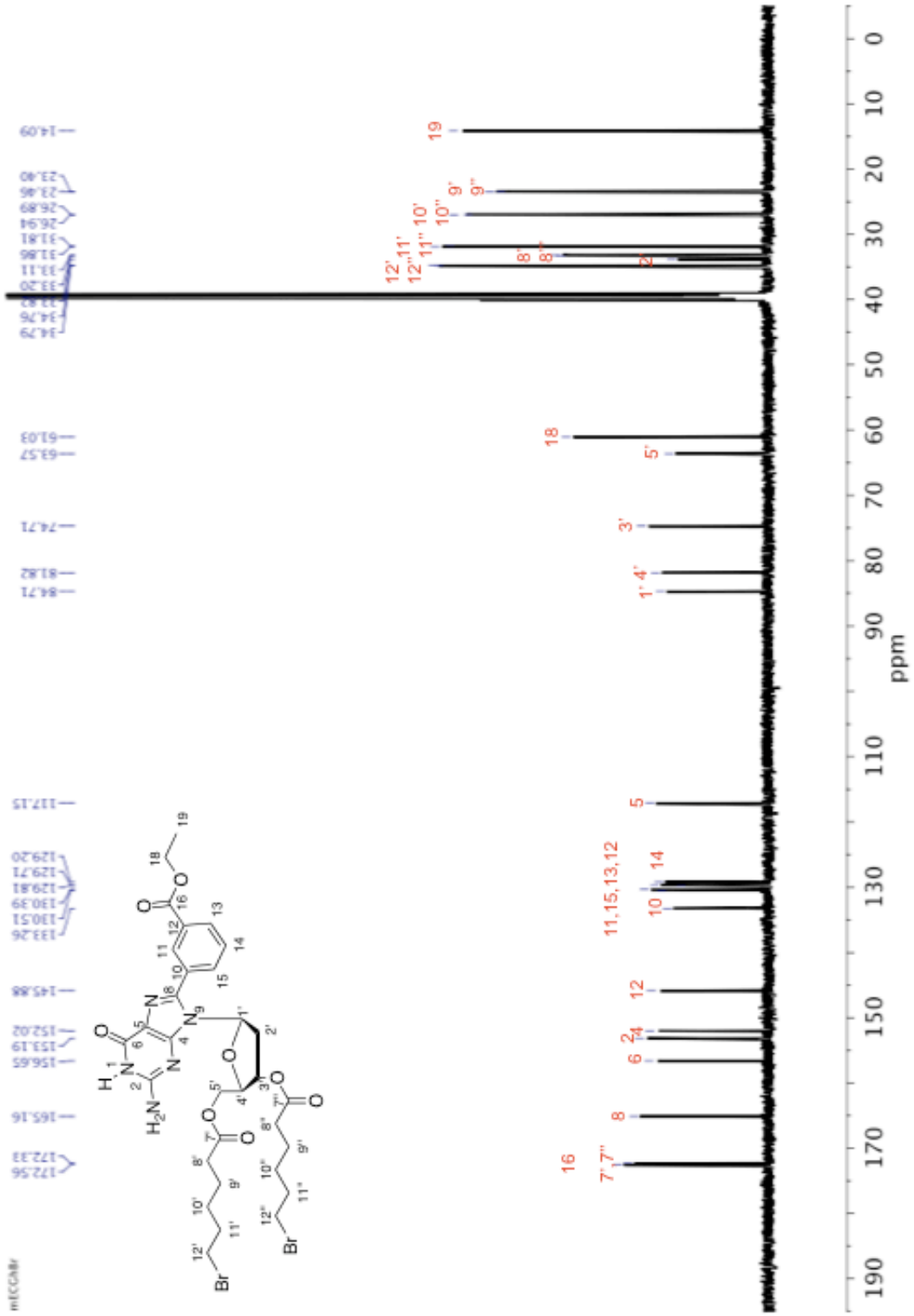
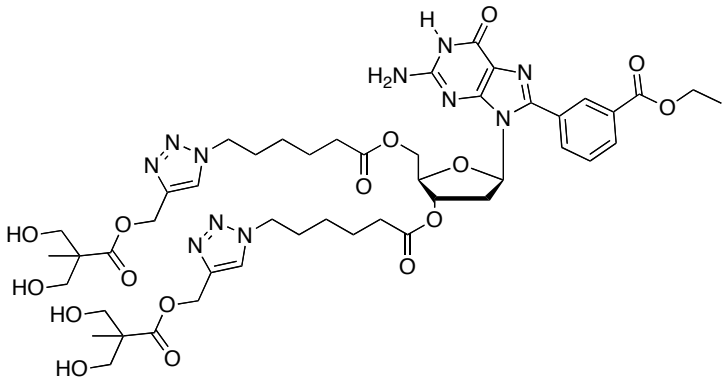


Figure S2 . ^{13}C NMR (DMSO – d_6, 125 MHz) of **mECGhBr**.

General method for the copper-catalyzed azide/alkyne cycloaddition

To a solution of alkyne-**Dg$_n$** and **mECGhaz** in THF:phosphate buffer (pH 7.4; 3:1, 10 mL) were added sodium ascorbate (15% mol) and $CuSO_4 \cdot 5H_2O$ (5% mol). The reaction mixture was then allowed to stir at room temperature until completion as determined by TLC (DCM:MeOH, 90:10). The solvents were evaporated and the crude product was purified by dry-loading of the silica column followed by flash chromatography, eluting with DCM and gradually increasing the polarity with methanol (DCM:MeOH; 70:30), to give pure **mECGD2OH** (**1**) as a solid. Yield >75 %



Chemical Formula: $C_{47}H_{63}N_{11}O_{16}$
Molecular Weight: 1038.0672

Light yellow powder, mp (decomposition) 249.5–251.2 °C. TLC (DCM:MeOH, 8:2) R_F = 0.2; 75 % yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.09 (s, 1H), 8.26 (s, 1H), 8.06 (m, 3H), 7.91 (d, *J* = 7.2 Hz, 1H), 7.69 (t, *J* = 7.2 Hz, 1H), 6.66 (s, 2H), 6.09 (t, *J* = 5.8 Hz, 1H), 5.43 (s, 1H), 5.10 (s, 4H), 4.71 (m, 4H), 4.43 (d, *J* = 8.2 Hz, 1H), 4.32 (dd, *J* = 20.1, 5.5 Hz, 6H), 4.16 (m, 1H), 4.02 (dd, *J* = 13.9, 6.9 Hz, 1H), 3.45 (m, 5H), 2.29 (m, 5H), 1.77 (d, *J* = 4.6 Hz, 4H), 1.51 (d, *J* = 6.9 Hz, 6H), 1.33 (t, *J* = 6.4 Hz, 3H), 1.27 - 1.10 (m, 5H), 1.03 (s, 6H); ^{13}C NMR (125.8 MHz, DMSO-d_6): δ 174.5, 172.6, 172.4, 165.2, 156.8, 153.4, 152.1, 145.9, 142.3, 142.2, 133.3, 130.6, 129.8, 129.7, 129.3, 124.1, 117.2, 84.7, 81.8, 74.7, 63.8, 63.6, 61.1, 59.8, 57.3, 50.3, 49.2, 33.9, 33.2, 33.1, 29.4, 29.3, 25.3, 25.2, 23.7, 23.6, 20.8, 16.8, 14.1, 14.1. IR (ν_{max}): 3347, 2926, 1732, 1678, 1635, 1593, 1573, 1257, 1173, 1123, 1048 cm^{-1}. ESI-MS (*m*/*z*): $[M + 1]^+$ calcd for $C_{47}H_{63}N_{11}O_{16}$, 1039.0672; found, 1039.0772 $[M + 1]^+$, 1061.0584 $[M + Na]^+$

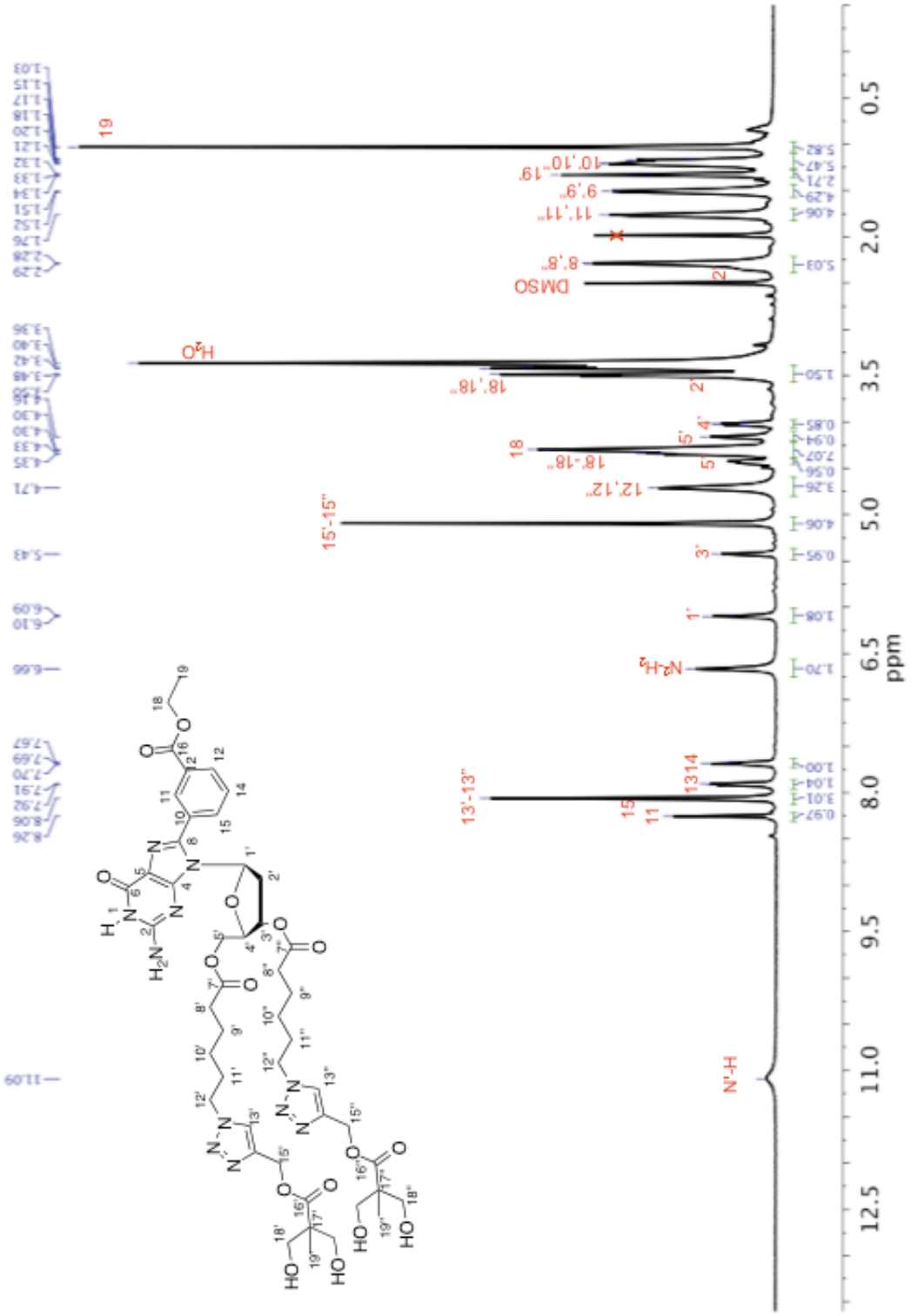


Figure S3. ^{1}H NMR (DMSO – d_6, 500 MHz) of **mECGD2OH** (**1**).

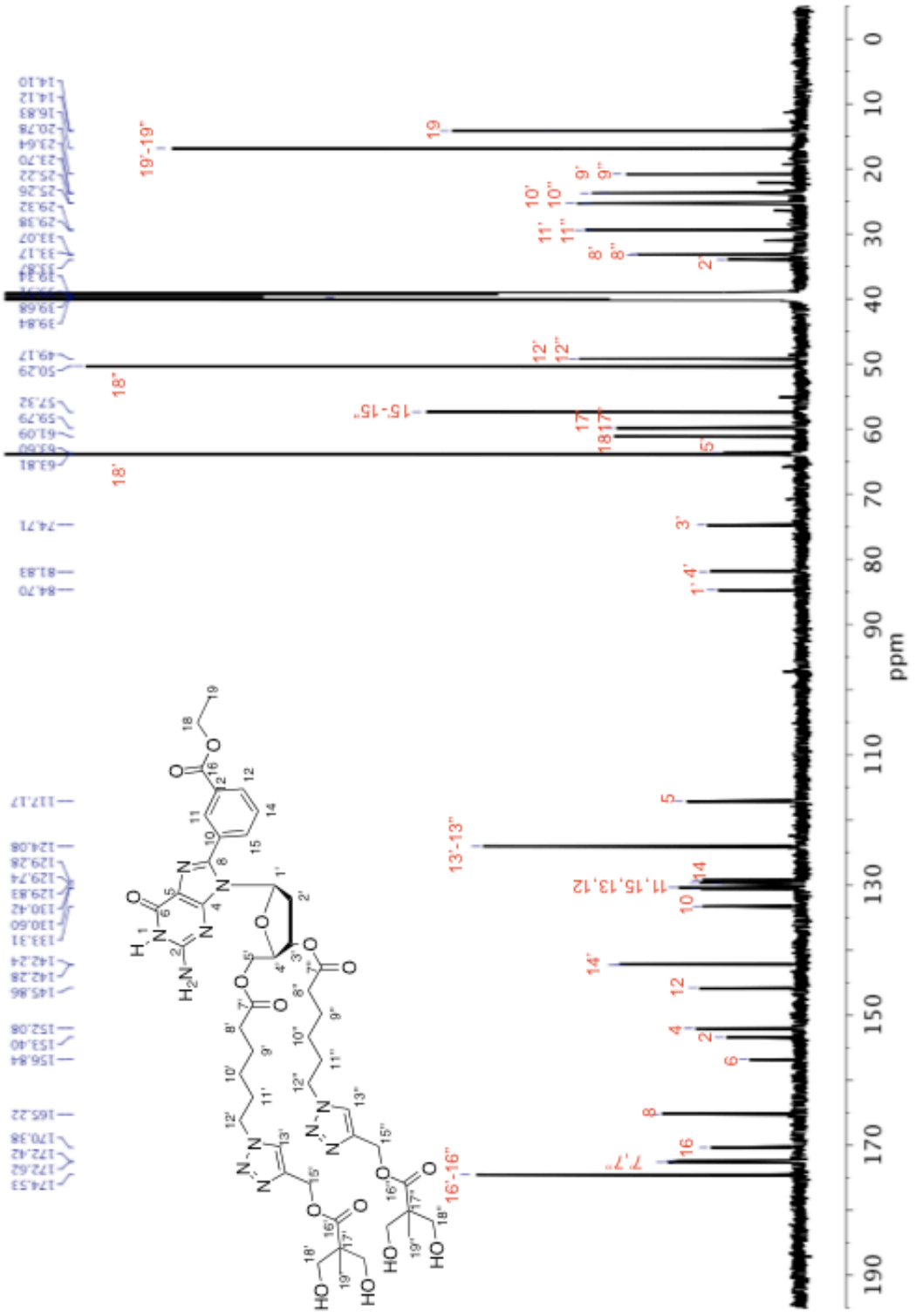


Figure S4 . ^{13}C NMR (DMSO – d_6, 125 MHz) of **mECGD2OH** (**1**).

D. Self-assembly NMR studies

Self-assembly studies were carried out using a Bruker DRX-500 NMR spectrometer, equipped with a 5 mm BBO probe. In water, a conventional 1D presaturation pulse sequence with the excitation pulse set over the water peak at 4.7 ppm was used. A standard proton sequence was used for experiments in D_2O. Self-assembly studies were performed, for example, using a 10 mM solution of **1** in 650 µL of H_2O-D_2O (9:1, potassium buffer, 2 M KI). For the NOESY experiment a phase-sensitive 2D NOESY pulse sequence with presaturation (noesyphpr) from Bruker was used.

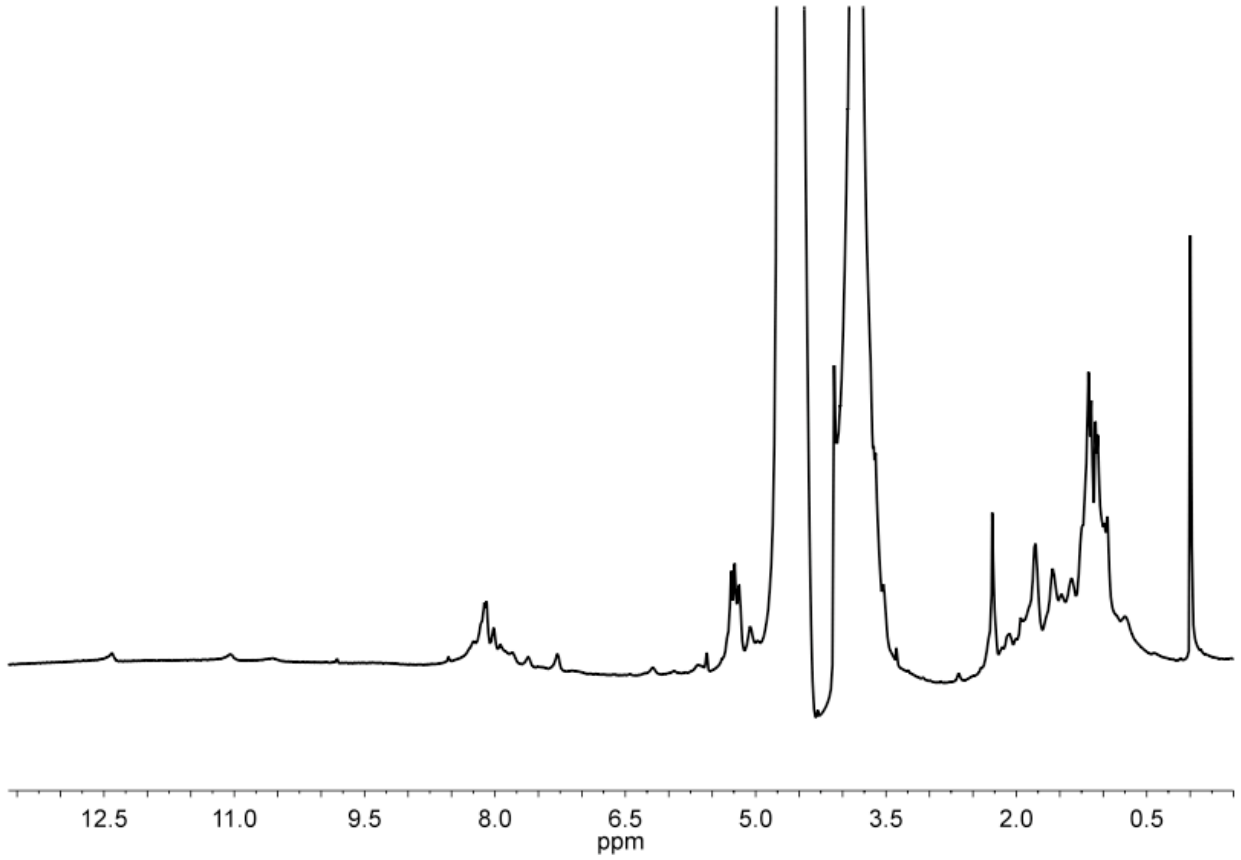


Figure S5 ^{1}H-NMR for $\mathbf{1}_{16}$ (10 mM) in H_2O-D_2O (9:1) with 2 M KI, shows peaks that are characteristic of the hexadecamer in aqueous media.[2]

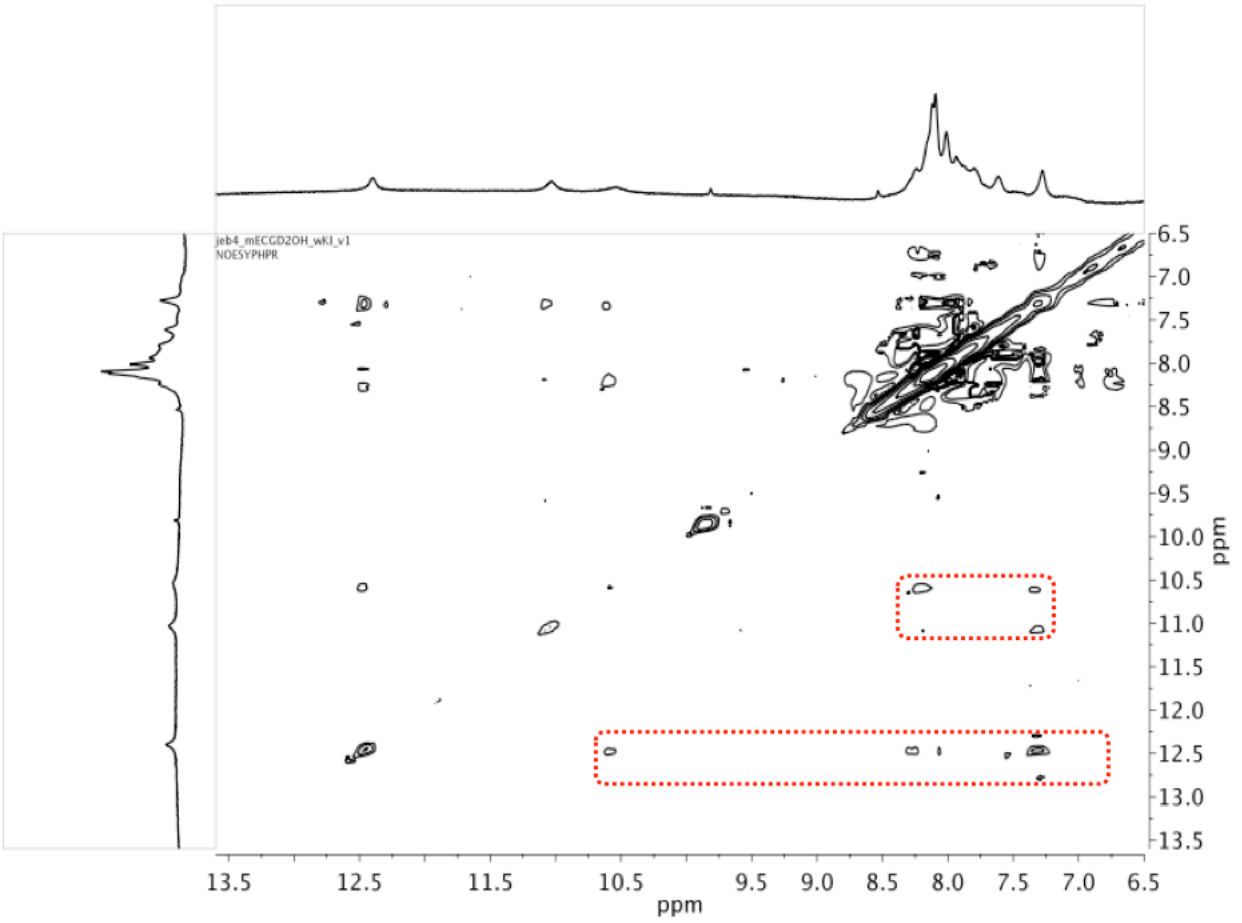


Figure S6 Partial NOESY spectra (500 MHz, τ_m = 500 msec, H_2O-D_2O (9:1), potassium buffer, pH 7.4, 2 M KI) for $\mathbf{1}_{16}$. The red squares point to the signature cross peaks that are characteristic of the hexadecamer formed in aqueous media.[2]

E. Turbidity Measurements

Turbidity measurements were performed at 500 nm using a Varian UV-visible spectrometer, Model Cary Bio-100. The heating rate was adjusted at 2.0 °C/min using Cary temperature controller apparatus from Varian. The **1** (10 mM, 2 M KI) derivatives were dissolve in a potassium buffer (pH 7.4) and filtered with a 0.45 μm Nylon filter using a Fisherbrand 10 mm o.d. glass tubes prior to the experiment. All the transmittance measurements have an error of ± < 1 nm based on their standard deviation.

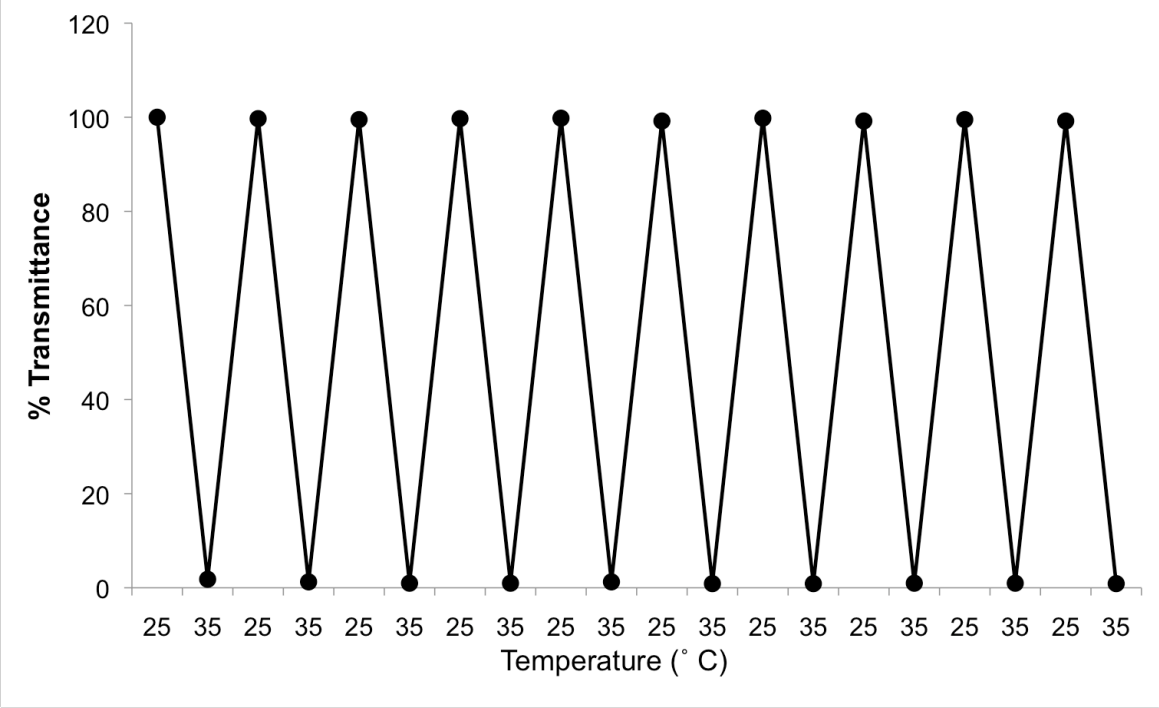


Figure S7 Changes in optical transmittance as a function of temperature for an aqueous solution of $\mathbf{1}_{16}$ (10 mM, 2 M KI, pH 7.4, potassium buffer). The solution was equilibrated for 20 min at the setting temperature before each measurement.

G. Dynamic Light-Scattering (DLS) Experiments

Dynamic light scattering (DLS) was used to measure the hydrodynamic size of the particles as a function of temperature from 25–75 °C. The measurements were performed with samples (7–10 mM) dissolved in phosphate-buffered solution at pH 7.4.

For these propose we used a DynaPro Titan (Wyatt Technology Corporation) with temperature controlled and microsampler with a diode laser at 90° of scattering angle, a wavelength at 657 nm, and a power of 15 mW. Samples were dispersed in a buffer solution at pH 7.4 and filtered with a 0.45 μm Nylon filter using a Fisherbrand 10 mm o.d. glass tubes prior to the experiments.

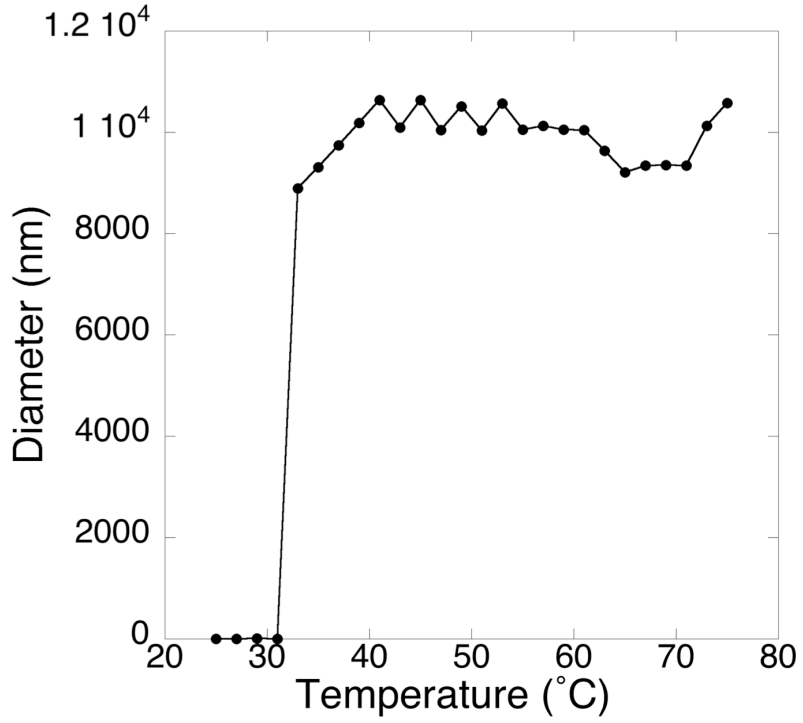


Figure S8 Average hydrodynamic diameters (D_H) of $\mathbf{1}_{16}$ as a function of temperature measured by DLS.

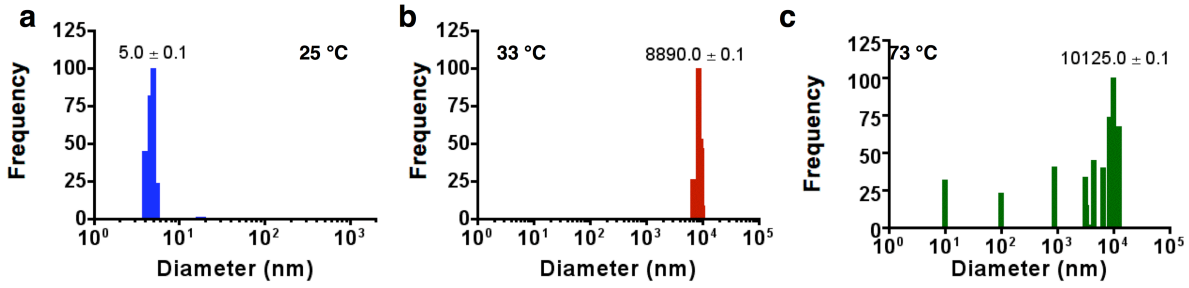


Figure S9 Size distributions for selected temperatures from the DLS curve shown on Figure S8: a) 25 °C, b) 33 °C, c) 73 °C.

H. Optical and Fluorescence Microscopy Images.

Nikon Eclipse E800 wide-field fluorescence microscope with differential interference contrast and motorized stage (x and y), fine-coarse control (z), CFI 60 Plan Apochromat objectives (2X[0.10 N.A.; 8.50 W.D.]; 10X[0.45 N.A.; 4.00 W.D.]; 60X oil [1.40 N.A.; 0.21 W.D.]; 100X oil [1.40 N.A.; 0.17 W.D.], and CFI 60 Plan Fluor objectives (ELWD 40XC [0.60 N.A.; 3.7-2.7 W.D.] The Nikon E800 is coupled to a Retiga EXi digital monochromatic camera with Red/Green/Blue liquid crystal filters. The Nikon E800 is controlled by MetaMorph (Molecular Devices Corporation, Downingtown, PA) complete with image analysis capabilities and control and acquisition drivers for the digital camera, Z-axis motorized focus drivers for coarse and fine focus, XY-axis motorized drivers for the mechanical stage, stage micrometer, and 10 position filter wheel. The entire Nikon E800 microscope sits on an air table in a custom designed temperature/humidity-controlled enclosure.[3]

The measurements were performed with $\mathbf{1}_{16}$ (10 mM) dissolved in phosphate-buffered solution at pH 7.4 wit 2 M KI. We use 100 µL of this solution over a Fisherbrand Hanging Drop Slides (0.5 mm deep). For the **DOX** experiment we used 100 µL (0.625 mM) of **DOX** mixed with $\mathbf{1}_{16}$ (10 mM) incubated at 40 °C for 45 min before visualization with the microscope at 37 °C.

References.

1. Betancourt, J.E., Martín-Hidalgo,M., Gubala, V. and Rivera, J.M., *J. Am. Chem. Soc.*, **2009**, *131*, 3186–3188.
2. García-Arriaga, M., Hobley, G., Rivera, J.M., *J. Am. Chem. Soc.* **2008**, 130, 10492–10493.
3. http://pisces.cnnet.clu.edu/erm-lab