

Non-polymeric Thermosensitive Supramolecules

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Supporting Information

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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 F₂₅₄ 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-Acetylphenyl)-(3',5'-bis-O-(6-azidohexanoyloxy))-2'-deoxyguanosine (**mAGhaz**) was synthesized according to the procedures outlined elsewhere.¹

B. Characterization of the target compounds and Instrumentation

¹H and ¹³C NMR spectra were recorded on Bruker DRX-500 (TopSpin v 2.0) with nominal frequencies of 500.13 MHz for proton or 125.77 MHz for carbon respectively. ¹H NMR and ¹³C 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-d₄ (Aldrich) was used as the internal standard for the NMR experiments performed in D₂O or H₂O:D₂O (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; b, broad. High-resolution mass spectral data were obtained from Emory University Mass Spectrometry Center on a Micromass VG AutoSpec magnetic sector mass spectrometer (70 eV). 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 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 particles as a function of temperature in a 24–74 °C range. For this purpose we used a Brookhaven Instrument BI-90 Plus Particle Size 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 7.1 and filtered with a 0.45 μm nylon filter using a Fisherbrand 10 mm o.d. glass tubes prior to the experiment. Differential Scanning Calorimetry (DSC) analyses were performed on a VP-DSC Micro-Calorimeter from MicroCal and Origin (v. 7) was used for data processing. Operating temperature range 20–100 °C. Transmission electron microscopy (TEM) measurements were performed using a JEOL-100S TEM at 100 KV.

C. Synthesis.

General method for the azide/alkyne cycloaddition catalyzed by CuSO₄ and sodium ascorbate.

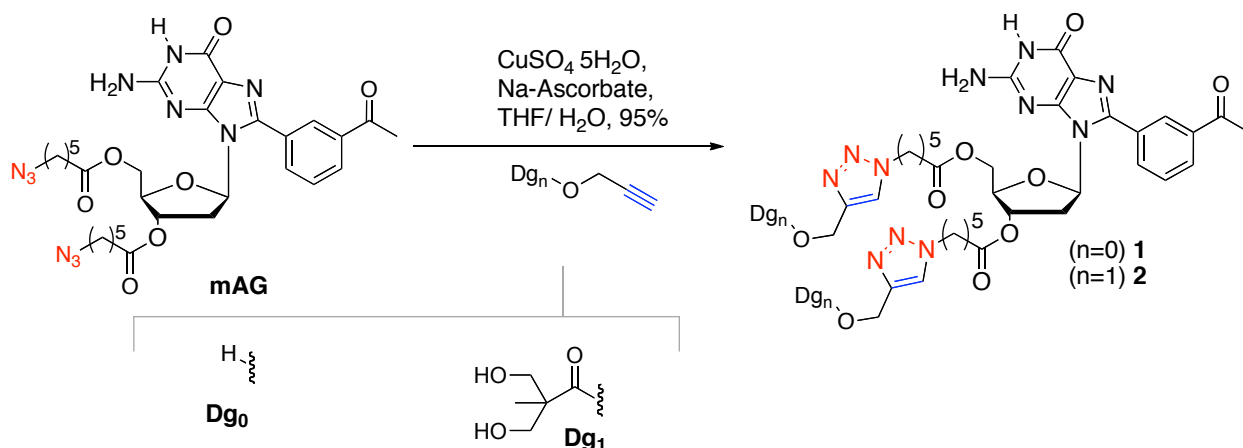
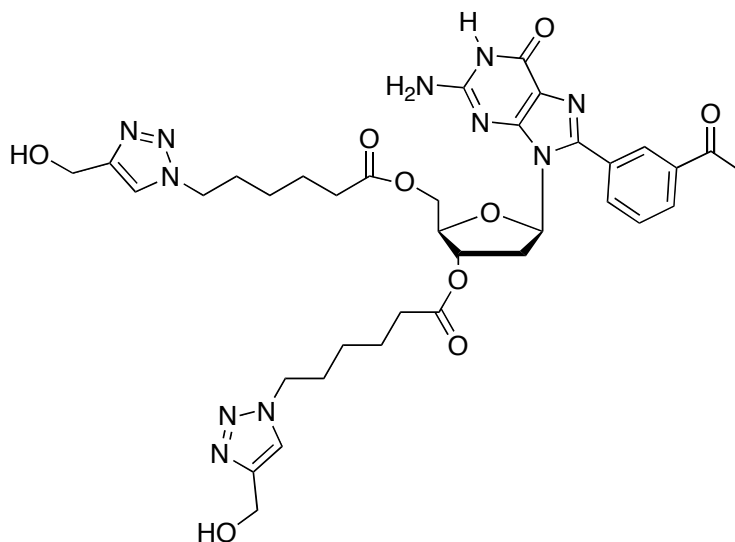


Figure S1 Synthesis of **1** and **2**.

Representative procedure for the synthesis of 1. The starting material, **mAGhaz** (500 mg, 753 μmol) and propargyl alcohol (126 mg, 134 μL, 2.26 μmol) were dispersed in THF: 66 mM sodium phosphate buffer (pH 7.1) (3:1, 40.0 mL). To this was added sodium ascorbate (44.7 mg, 22.6 μmol) and then CuSO₄ (775 μL of a 100 mM solution, 77.5 μmol). When the starting material was consumed as determined by TLC (2:8, MeOH-CHCl₃) the solvent was removed under reduced pressure then dispersed in chloroform and loaded onto a chromatography column. The product was eluted using a

gradient of MeOH-CHCl₃ (0:10) to (3:7). The oil thus obtained was dispersed dichloromethane (DCM), precipitated onto the walls of a round-bottomed flask by adding Et₂O, then Et₂O-DCM. The solvent was decanted off and the residue dried under reduced pressure. This process was repeated until a pale yellow foam was obtained.



Chemical Formula: C₃₆H₄₅N₁₁O₉
 Exact Mass: 775.3402
 Molecular Weight: 775.8108

1. Light yellow powder, mp (decomposition) 145.2-147.5 °C. TLC (CHCl₃:MeOH, 8:2): *R_F* = 0.43; 90 % yield. ¹H NMR (500 MHz, DMSO-*d*₆): δ 10.89 (s, 1H), 8.20 (s, 1H), 8.07 (d, *J* = 7.5 Hz, 1H), 7.93 (s, 1H), 7.92 (s, 1H) 7.89 (d, *J* = 7.5, 1H) 7.69 (t, *J* = 7.6, 1H), 6.55 (bs, 2H), 6.10 (t, *J* = 6.6, 1H), 5.44 (s, 1H), 5.13 (s, 2H), 4.49 (s, 4H) 4.42 – 4.37 (m, 1H), 4.33 – 4.21 (m, 5H), 4.15 (bs, 1H), 3.54 (m, 1H), 2.63 (s, 3H), 2.35 (m, 1H), 2.29 (q, *J* = 6.5 Hz, 4H), 1.77 (q, *J* = 6.5 Hz, 4H), 1.52 (q, *J* = 6.5 Hz, 4H), 1.21 (q, *J* = 6.5 Hz, 4H); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 197.48, 172.58, 172.36, 156.71, 152.00, 147.89, 147.86, 146.07, 137.10, 133.27, 129.18, 128.89, 128.80, 122.49, 117.16, 84.69, 81.73, 79.22, 79.16, 78.96, 78.69, 74.63, 63.57, 55.04, 48.95, 48.94, 33.89, 33.15, 33.06, 29.41, 29.36, 26.81, 25.25, 25.21, 23.68, 23.64. IR (*v*_{max}): 3304, 2926, 1732, 1678, 1593, 1573, 1257, 1173, 1123, 1048 cm⁻¹. HRMS (*m/z*): [M + 1]⁺ calcd for C₃₆H₄₅N₁₁O₉, 776.3402; found, 776.3491

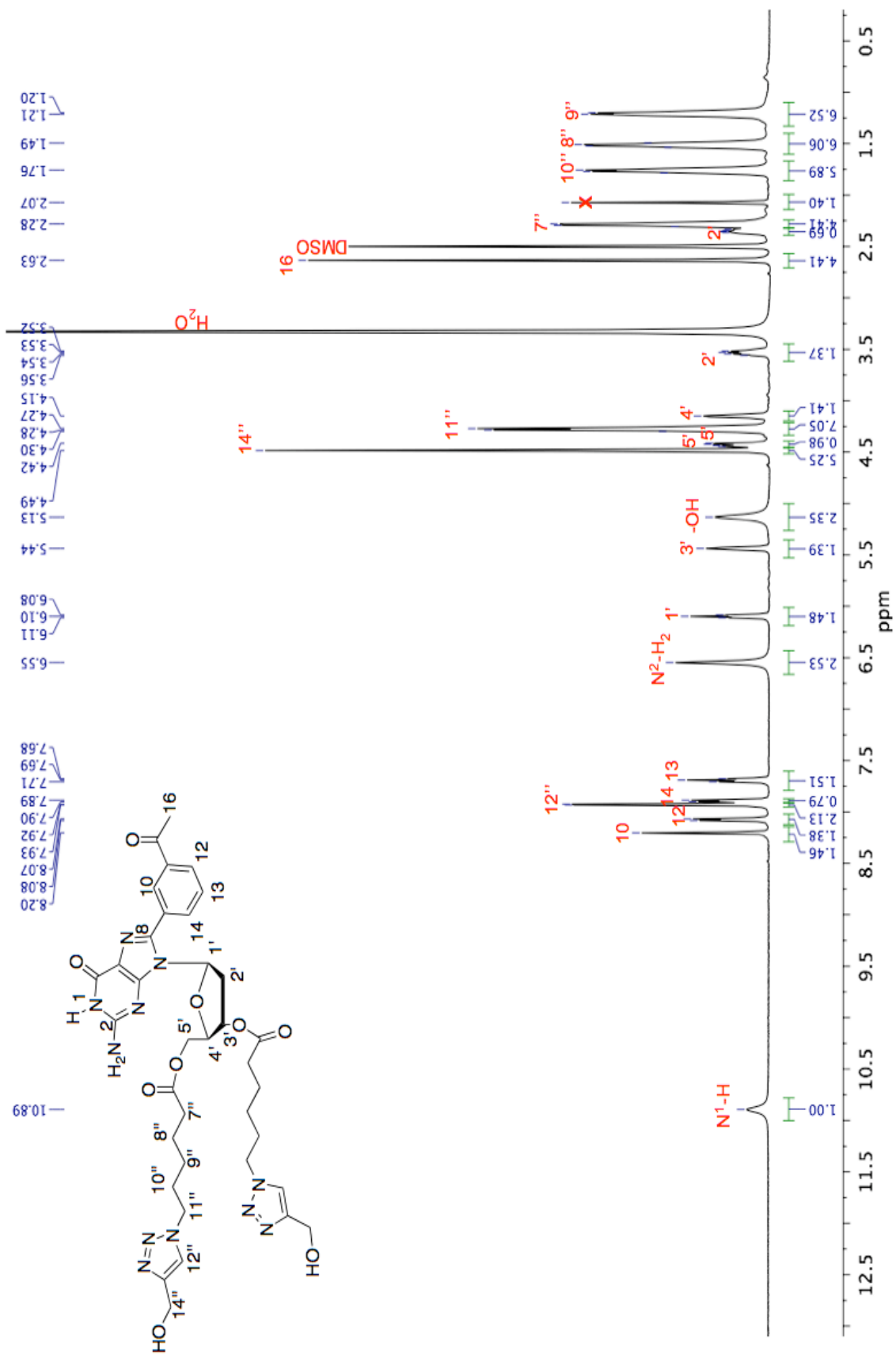


Figure S2 ¹H-NMR of 1 in DMSO-d₆.

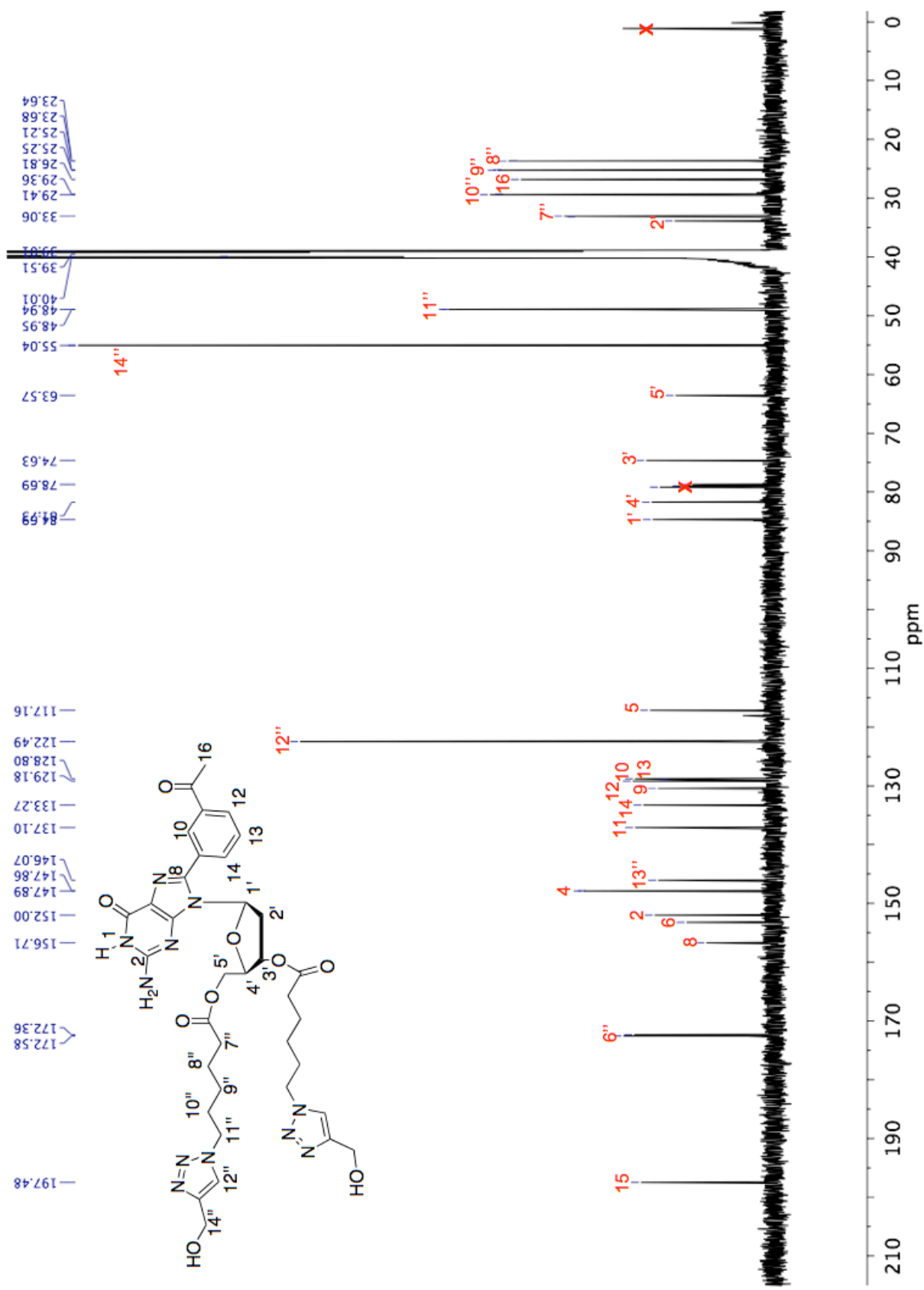
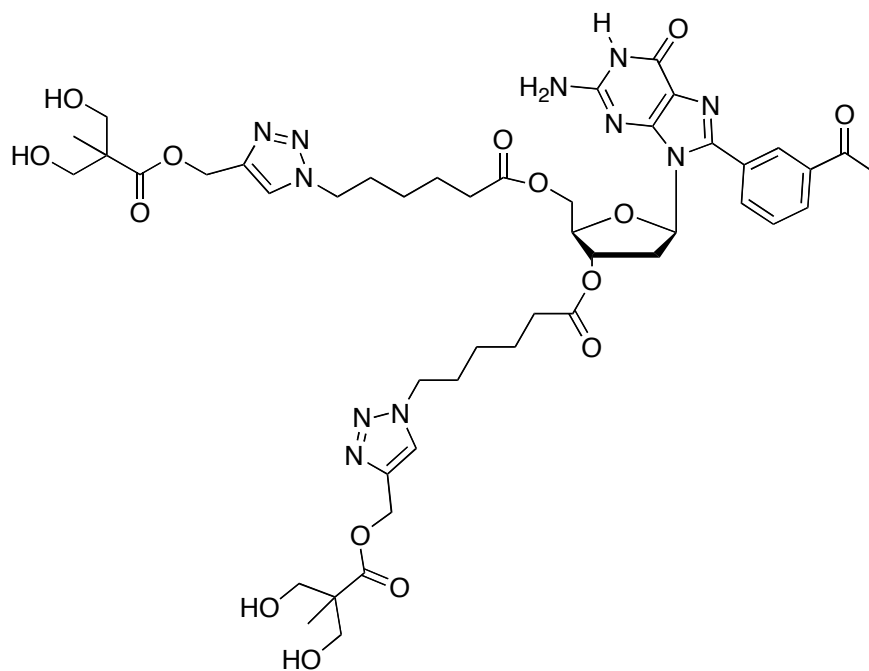


Figure S3 ^{13}C -NMR of 1 in $\text{DMSO-}d_6$.



Chemical Formula: $C_{46}H_{61}N_{11}O_{15}$
 Exact Mass: 1007.4349
 Molecular Weight: 1008.0412

2. Light yellow powder, mp (decomposition) 239.5–241.2 °C. TLC ($CHCl_3$:MeOH, 8:2) R_F = 0.53; 85 % yield. 1H NMR (500 MHz, $DMSO-d_6$): δ 10.85 (s, 1H), 8.20 (s, 1H), 8.07 (m, 3H), 7.89 (d, J = 7.5 Hz, 1H), 7.69 (t, J = 7.7 Hz, 1H), 6.52 (s, 2H), 6.10 (s, 1H), 5.44 (s, 1H), 5.09 (s, 4H), 4.67 (t, J = 5.2 Hz, 4H), 4.43 (d, J = 7.2 Hz, 1H), 4.3 (m, 5H), 4.15 (s, 1H), 3.45 (m, 9H), 2.63 (s, 3H), 2.31 (m, 5H), 1.77 (dd, J = 6.4, 12.8 Hz, 4H), 1.51 (dd, J = 7.4, 14.7 Hz, 4H), 1.21 (m, 4H), 1.03 (s, 6H); ^{13}C NMR (125 MHz, $DMSO-d_6$): δ 197.50, 174.50, 172.61, 172.39, 156.68, 153.17, 152.01, 146.13, 142.27, 142.23, 137.11, 133.29, 130.46, 129.20, 128.93, 128.80, 124.05, 117.17, 84.71, 81.77, 74.65, 63.84, 63.61, 57.31, 54.91, 50.28, 49.15, 33.92, 33.15, 33.06, 29.35, 29.30, 26.82, 25.24, 25.20, 23.68, 23.63, 16.81. IR (ν_{max}): 3304, 2926, 1732, 1678, 1593, 1573, 1257, 1173, 1123, 1048 cm^{-1} . HRMS (m/z): $[M + 1]^+$ calcd for $C_{46}H_{61}N_{11}O_{15}$, 1008.4349; found, 1008.4443

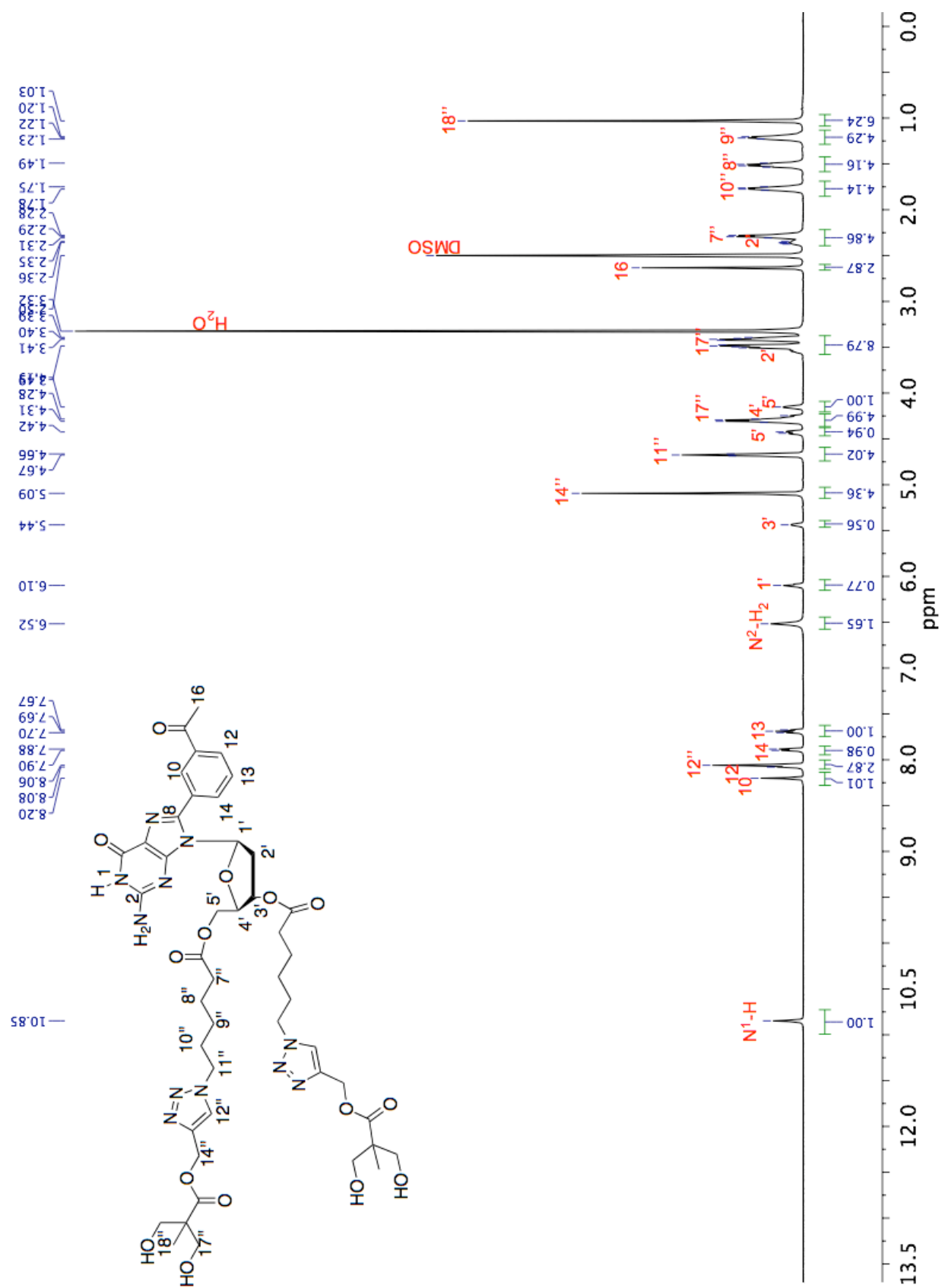
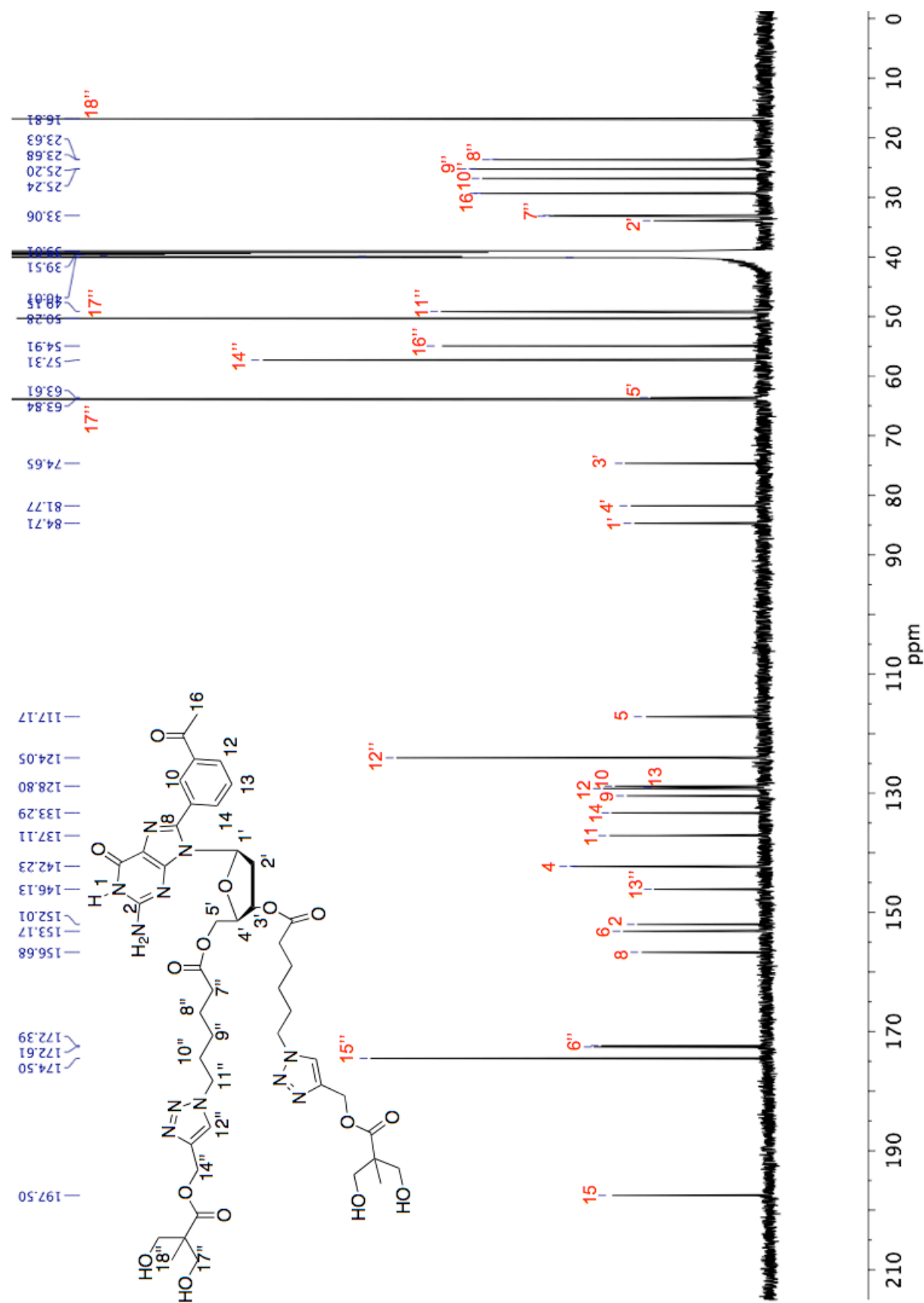


Figure S4 $^1\text{H-NMR}$ of **2** in $\text{DMSO-}d_6$.



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 D₂O. Self-assembly studies were performed, for example, using a 20 mM solution of **1** or **2** in 650 μ L of H₂O-D₂O (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.

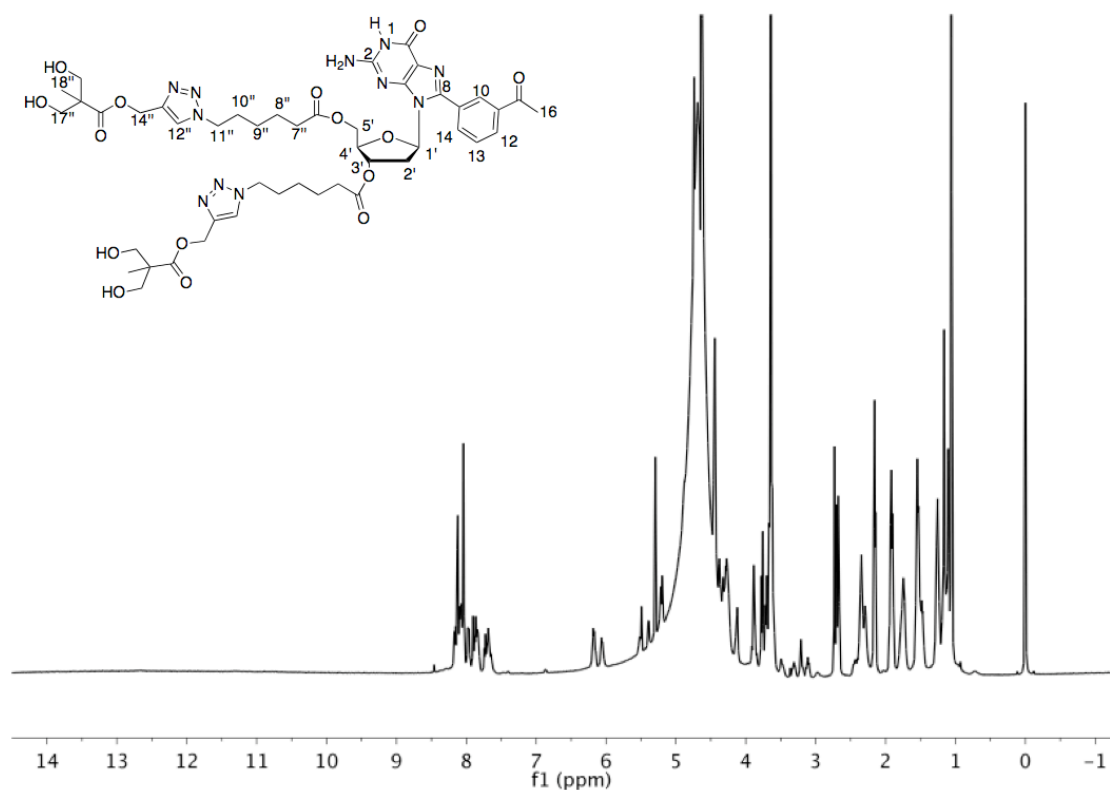


Figure S6 ¹H-NMR of **2** (20 mM) in H₂O-D₂O (9:1) with 2 M LiI, shows no detectable presence of a hexadecamer.

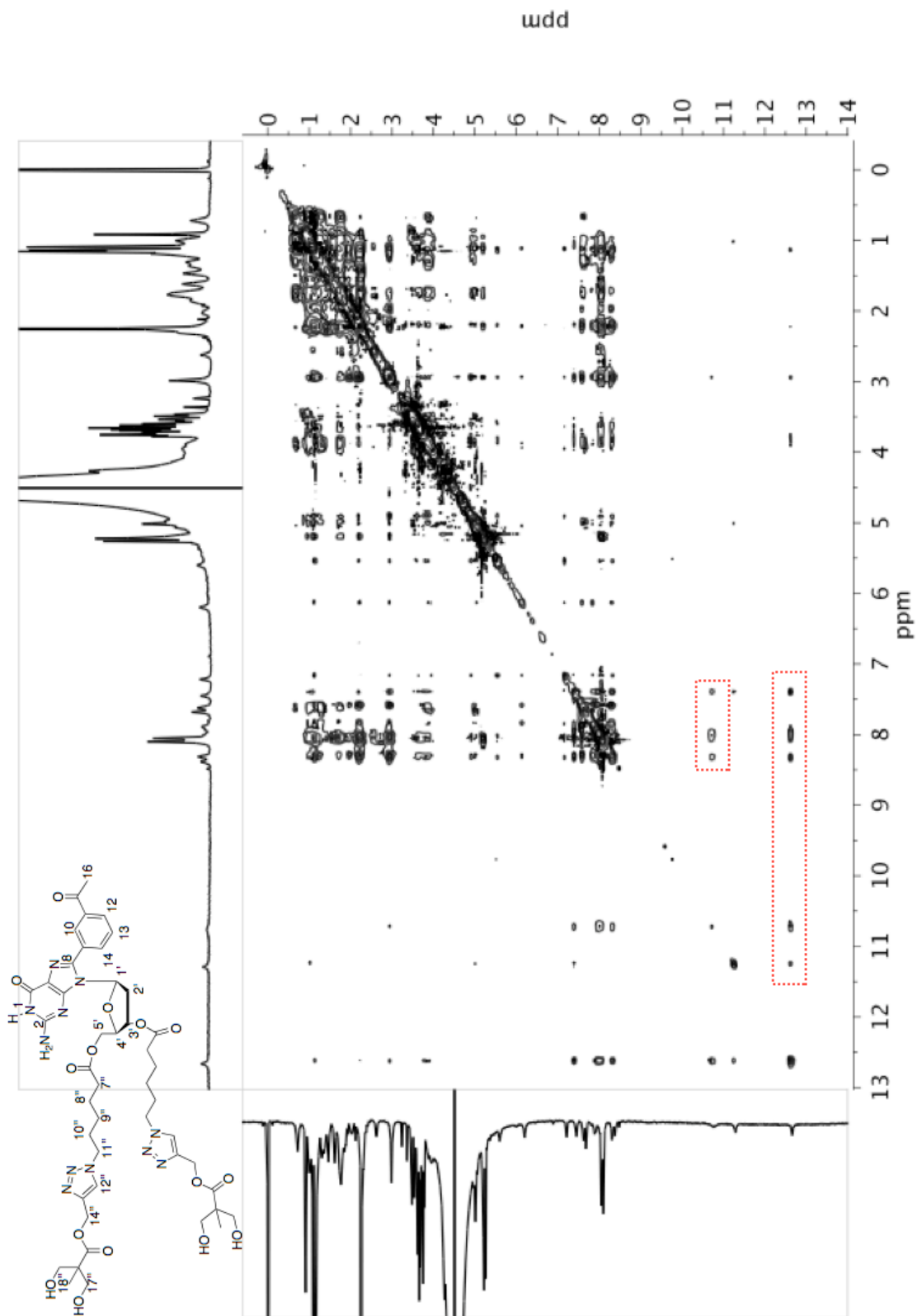


Figure S7 NOESY spectra (500 MHz, $\tau_m = 500$ msec, H₂O-D₂O (9:1), potassium buffer, pH 7.1) for $2_{16} \cdot 3KI$. The red squares point to the signature cross peaks that are characteristic of the hexadecamer formed by the **mAG** scaffold in aqueous media.³

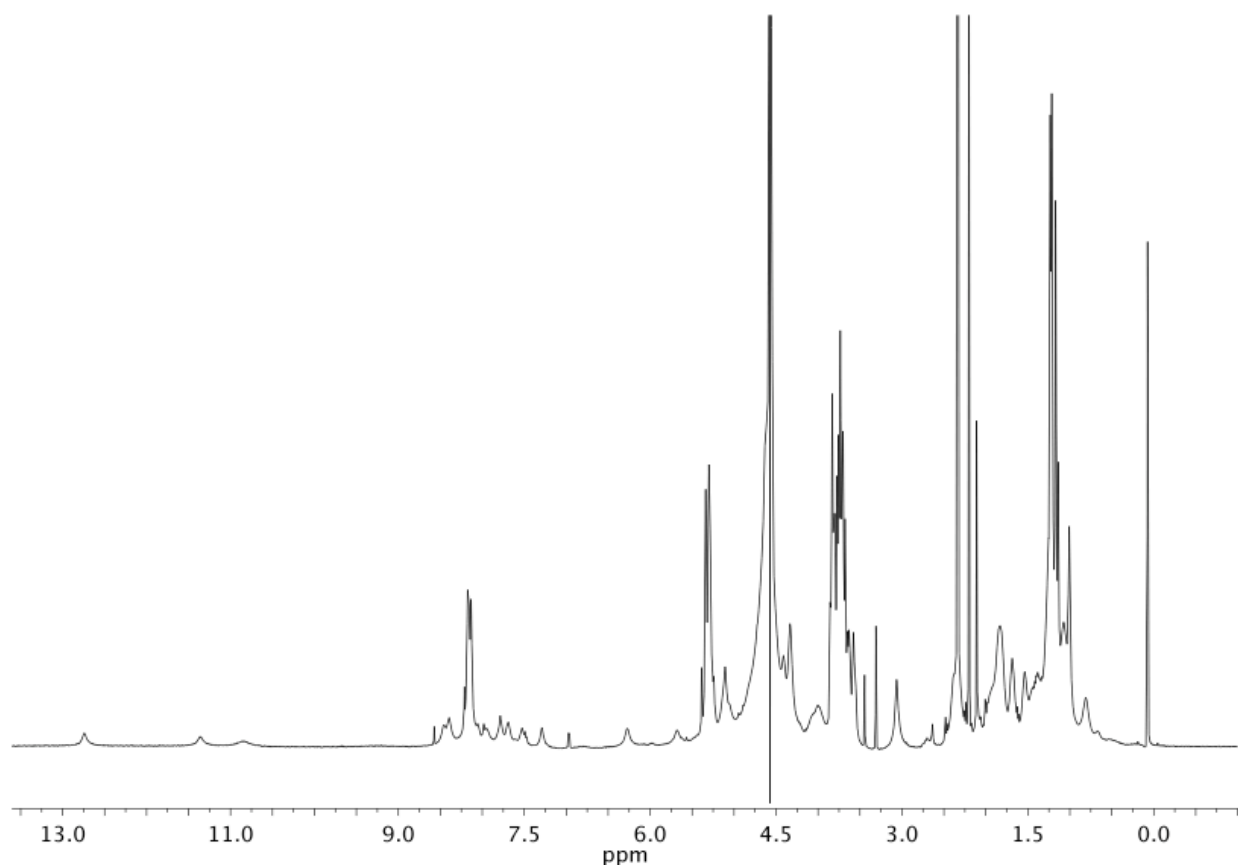


Figure S8 $^1\text{H-NMR}$ for $\mathbf{1}\cdot\mathbf{2}_{15}$ (20 mM) in $\text{H}_2\text{O-D}_2\text{O}$ (9:1) with 2 M KI, shows peaks that are characteristic of the hexadecamer formed by the **mAG** scaffold in aqueous media.³

E. Turbidity Measurements

The 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** and **2** (20 mM, 2 M KI) derivatives were dissolve in a potassium buffer (pH 7.1) 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 $\pm < 1$ nm based on their standard deviation.

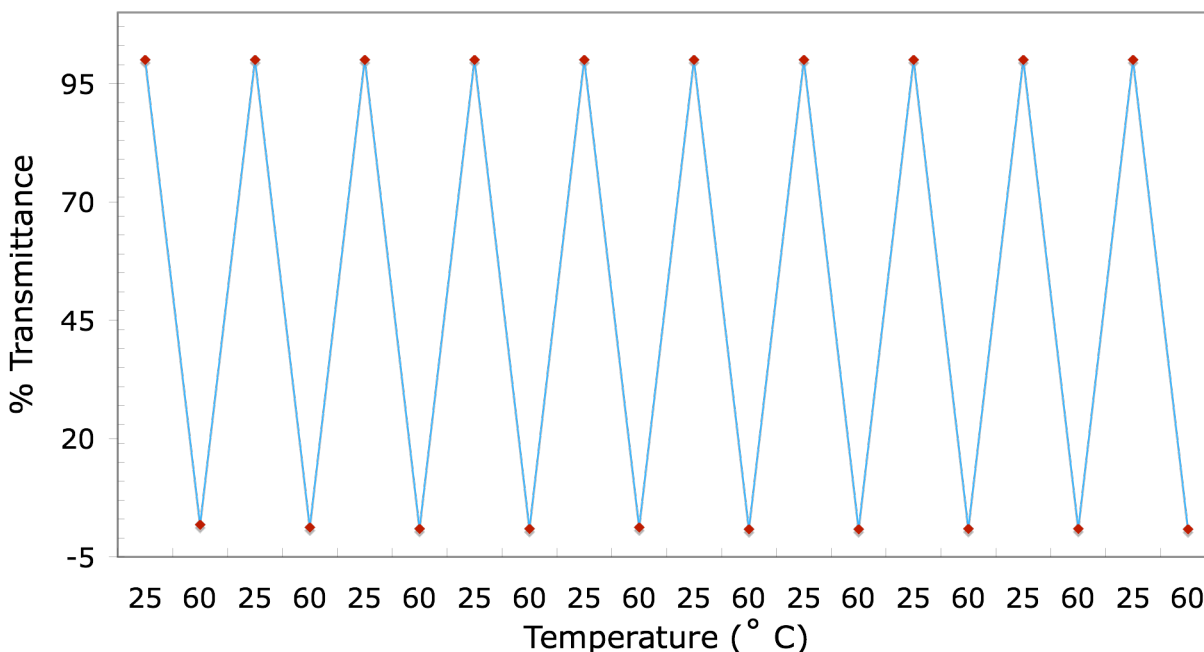


Figure S9 Reversible changes of optical transmittance against temperature fluctuation for **2₁₆** (20 mM, 2 M KI, pH 7.1) in aqueous solution. The solution was equilibrated for 20 min at the setting temperature before each measurement.

F. Diffusion NMR studies

Pulsed field gradient diffusion experiments were carried out with a Bruker DRX-500, equipped with a 5 mm BBO probe, spectrometer using the Stimulated Echo Pulse Gradient sequence (stebpgp1s) in FT mode. To improve homogeneity a “13 interval pulse sequence” was used with two pairs of bipolar gradients. The shape of the gradient used was sine and the temperature was actively controlled at 25.0 ± 0.5 °C. Diffusion coefficients were derived using integration of the desired peaks to a single exponential decay, using the Bruker software package T1/T2 Relaxation (TopSpin v 2.0). Calculation of the hydrodynamic radius in D₂O used the viscosity value ($\eta = 1.103 \times 10^{-3}$ Kg m⁻¹ s⁻¹, 298.15 K) reported in the literature.² The hydrodynamic radii of the species in the NMR tube were calculated according to the spherical approximation using the Einstein-Stokes equation:

$$D = \frac{k_B T}{3\pi\eta D_H}$$

where T denotes the temperature, η is the viscosity of the solvent at the given temperature, k_B is the Boltzmann-Constant, D is measured diffusion constant and D_H is the hydrodynamic diameter. The data were further processed by the Bruker software package.

Table S1 Diffusion coefficients (D_s) and hydrodynamic diameter (D_H) of the assemblies formed by **1** and **2** determined by PFG-NMR in D_2O and KI (2 M) at 298 K.

<i>Supramolecule</i>	$D \times 10^{11} \text{ (m}^2 \text{ s}^{-1}\text{)}$	$D_H \text{ (nm)}$
1 ₁₆ •3KI	9.33 ± 0.31	4.26 ± 0.14
2 ₁₆ •3KI	9.11 ± 0.23	4.40 ± 0.12

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 24–74 °C. The measurements were performed with samples (7–10 mM) dissolved in phosphate-buffered solution at pH 7.1. For this propose we used a Brookhaven Instrument BI-90 Plus Particle Size 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 7.1 and filtered with a 0.45 µm nylon filter using a Fisherbrand 10 mm o.d. glass tubes prior to the experiments.

Table S2 Dynamic light scattering (DLS) to measure the hydrodynamic diameter (D_H) of the assemblies formed by **1** and **2** in solution at 25 °C.

<i>Supramolecule</i>	D_H (nm)
1 ₁₆ •3KI	4.2 ± 0.4
2 ₁₆ •3KI	4.6 ± 0.2

H. Transmission Electron Microscopy (TEM) Measurements.

TEM measurements were performed using a JEOL-100S TEM at 100KV. Samples were prepared by dipping copper EM grids (pre-coated with holey carbon from TED PELLA, INC.) in aqueous solutions of the **2** (20 mM, potassium buffer, 2 M KI, 60 °C) and dried at room temperature and visualized under TEM.

I. Variable temperature ¹H NMR

A solution of **1** and **2** (20 mM) containing an excess of KI (2 M) was placed in a threaded cap sealed NMR tube. The ¹H-NMR was recorded in H₂O-D₂O (9:1, potassium buffer, 2 M KI) at 25 °C and increased up to 80 °C. The fraction of ordered species was determined by the integration of the area under selected peaks (H1' in the monomer and hexadecamer) and reported as the ratio between the monomer and the assembly.

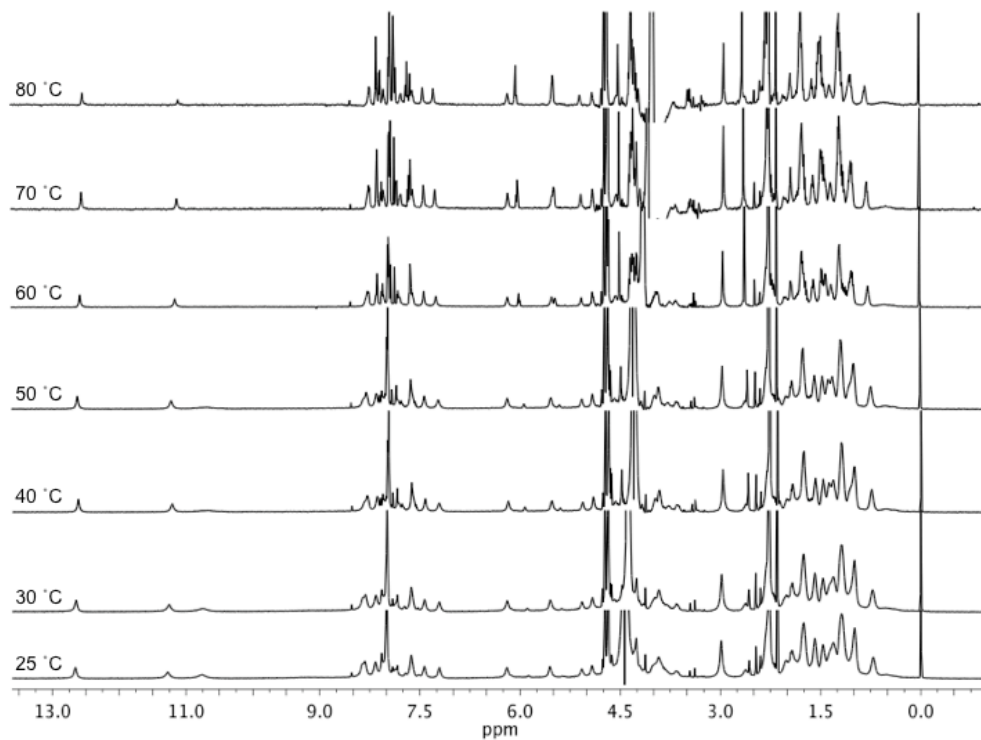


Figure S10 Variable temperature $^1\text{H-NMR}$ for **1**₁₆ (20 mM) in $\text{H}_2\text{O-D}_2\text{O}$ (9:1) with 2 M KI.

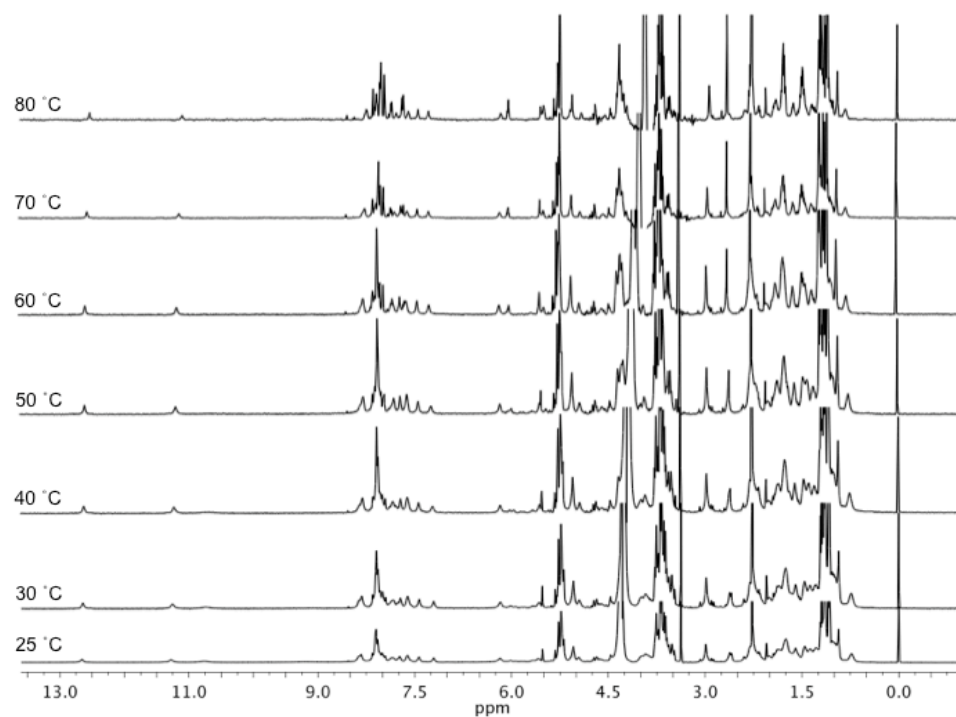


Figure S11 Variable temperature $^1\text{H-NMR}$ for **2**₁₆ (20 mM) in $\text{H}_2\text{O-D}_2\text{O}$ (9:1) with 2 M KI.

Table S3 Thermal stability of the **1** and **2** in H₂O-D₂O (9:1, potassium buffer, 2 M KI) as determined by ¹H NMR (500 MHz).

Supramolecule	Molecularity	T _m * [°C]
1 ₁₆ •3KI	Hexadecamer	79.7
2 ₁₆ •3KI	Hexadecamer	76.7

* The T_m is the inflexion point in the melting profiles as determined by the minimum in the respective first derivative plots.

Table S4 Relative abundances of the hexadecamers and the monomers for both **1** and **2** as a function of temperature and their respective normalized intensities (I_N).

T (°C)	1			2		
	% H	% M	I _N	% H	% M	I _N
25	96.0	4.0	1.0	97.3	2.7	1.0
30	95.9	4.1	1.0	95.2	4.8	0.9
35	--	--	--	95.1	4.9	0.9
40	90.6	9.4	0.9	91.7	8.3	0.9
50	82.8	17.2	1.0	90.7	9.3	1.0
55	--	--	--	85.1	14.9	1.2
60	77.2	22.8	1.1	79.6	20.4	1.5
70	67.7	32.3	1.5	61.8	38.2	1.4
80	49.6	50.4	1.3	46.8	53.2	1.5

% H, % M = Percentage of hexadecamer and monomer, respectively; I_N = Normalized Intensity

The normalized intensity (I_N) was calculated as follows:

$$I_N = [I_{t(T)} \div I_{STD(T)}] / I_{t(25^\circ\text{C})}$$

Where STD refers to sodium 3-(trimethylsilyl)propionate-2,2,3,3-d₄ and the total intensity at a given temperature (T) is given by:

$$I_{t(T)} = I_{H(T)} + I_{M(T)}$$

The sum of the intensity of the H1' integrals (∫) for the hexadecamers at a given temperature (T):

$$I_{H(T)} = \int \text{H1}'_{\text{(subunits in the outer tetrads)}} + \int \text{H1}'_{\text{(subunits in the inner tetrads)}}$$

The intensity of the H1' integral (∫) for the monomers at a given temperature (T):

$$I_{M(T)} = \int \text{H1}'$$

The intensity of the CH₃ integrals (∫) for the standard at a given temperature (T):

$$I_{STD(T)} = \int \text{STD}$$

The normalized intensity (I_N) provides a measure of how much compound remains visible by NMR. In principle, if a substance remains in solution at all temperatures (e.g. **1**) this value should remain constant at around 1. A large decrease in this number would be indicative of the molecules reduced tumbling rate, which is commonly seen in the formation of precipitates, gels, etc. Curiously, as shown in Table S4, for both **1** and **2** the value remains close or above to 1 (We hypothesize that the apparent increase is related to the interference of some peaks that overlap with the H1' signals used to calculate this number). The expectation for the latter was a large decrease for this number above 60 °C. We hypothesize that this indicates a high level of hydration in the globules where the tumbling rates of the hexadecamers inside is very similar to those in solution.

J. Differential Scanning Calorimetry (DSC)

The total heat required for the dissociation of the hexadecamer was measured in PBS (pH 7.1, 2 M KI), 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 deconvolution curves were generated using Origin v7. Standard thermodynamic parameters were obtained from the DSC experiment using the relationships $\Delta H_{cal} = \int \Delta C_P(T) dT$, $\Delta S_{cal} = \int \Delta C_P(T)/T dT$ and the Gibbs equation, $\Delta G_{cal} = \Delta H_{cal} - T\Delta S_{cal}$, where C_P is the heat capacity of the solution during the disassembly process.

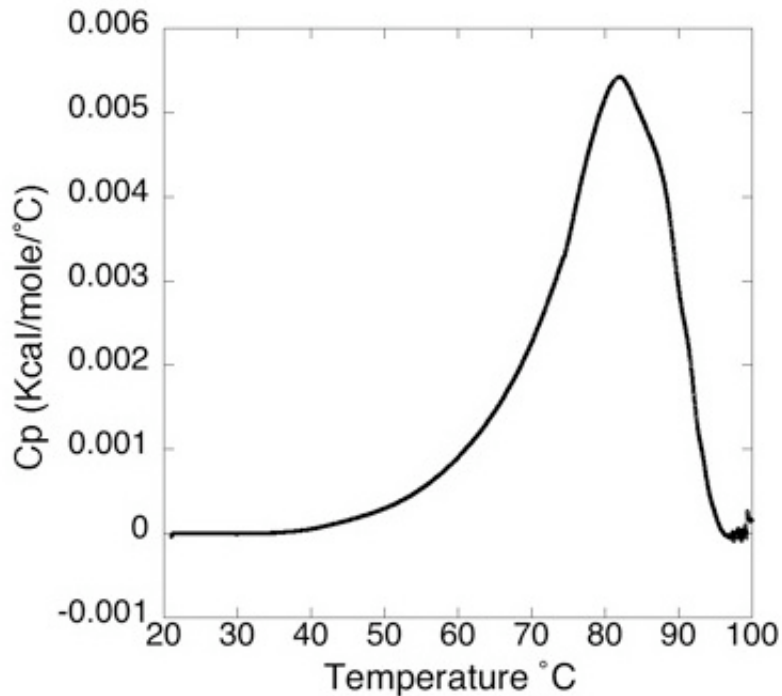


Figure S12 Microcalorimetric endotherms for $1_{16}\cdot 3\text{KI}$ dissolved in phosphate-buffered solution (pH 7.1, 2 M KI).

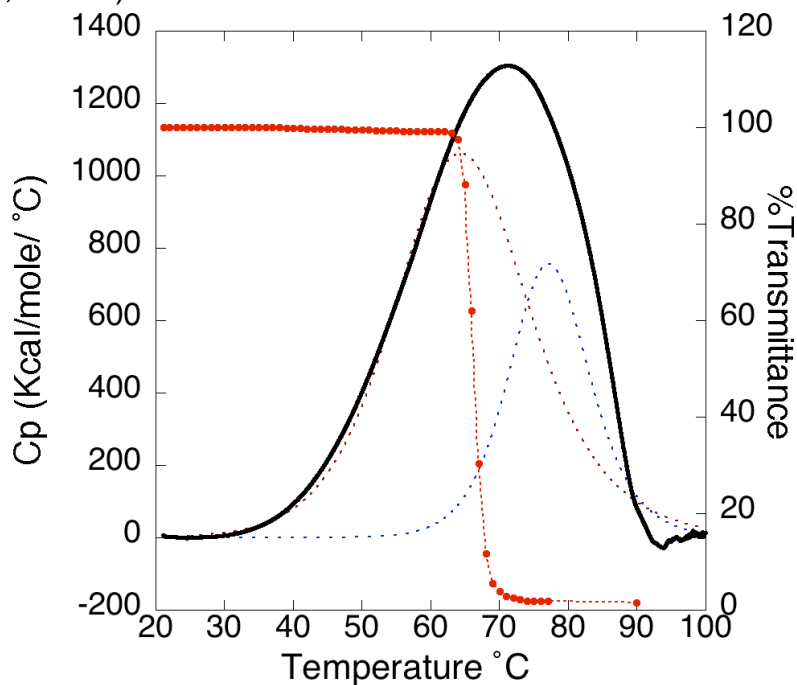


Figure S13 DSC endotherm for $1\cdot 2_{15}$ with the two resulting deconvolution peaks (dotted lines) representing the T_t , left, and the T_m , right. The overlaid turbidity curve (●) shows excellent correlation with the DSC peak that corresponds to the T_t . Microcalorimetric endotherms dissolved in phosphate-buffered solution (pH 7.1, 2 M KI).

Table S5 Thermodynamic parameters for the LCST phenomenon and for the formation of **1**₁₆ and **1**•**2**₁₅ in aqueous solution as determined by DSC. The ΔG_{20} , ΔH_{cal} , $T\Delta S_{\text{cal}}$ values reported in this table correspond to those calculated for the assembly of the hexadecamers at 20 °C.

Sample	T_t (°C)	T_m (°C)	ΔG_{20} (kcal/mol)	ΔH_{cal} (kcal/mol)	$T\Delta S_{\text{cal}}$ (kcal/mol)
1	---	77.19	- 0.63	- 114.70	- 114.06
2	58.54*	72.74	- 15.42	- 49.87	- 34.45
mixture	64.88**	77.35	- 14.19	- 121.7	- 107.51

* $\Delta H_{\text{cal}} = 45.85$ kcal/mol, ** $\Delta H_{\text{cal}} = 28.56$ kcal/mol

References.

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2. Harris, K. R., Woolf L. A., *J. Chem. Eng. Data* **2004**, 49, 1064–1069.
3. García-Arriaga, M., Hogley, G., Rivera, J.M., *J. Am. Chem. Soc.* **2008**, 130, 10492–10493.