Aquatic Self-Assembly of Sixteen Subunits into a 39-kDa Dendrimer

*Luis R. Rivera, José E. Betancourt and José M. Rivera**

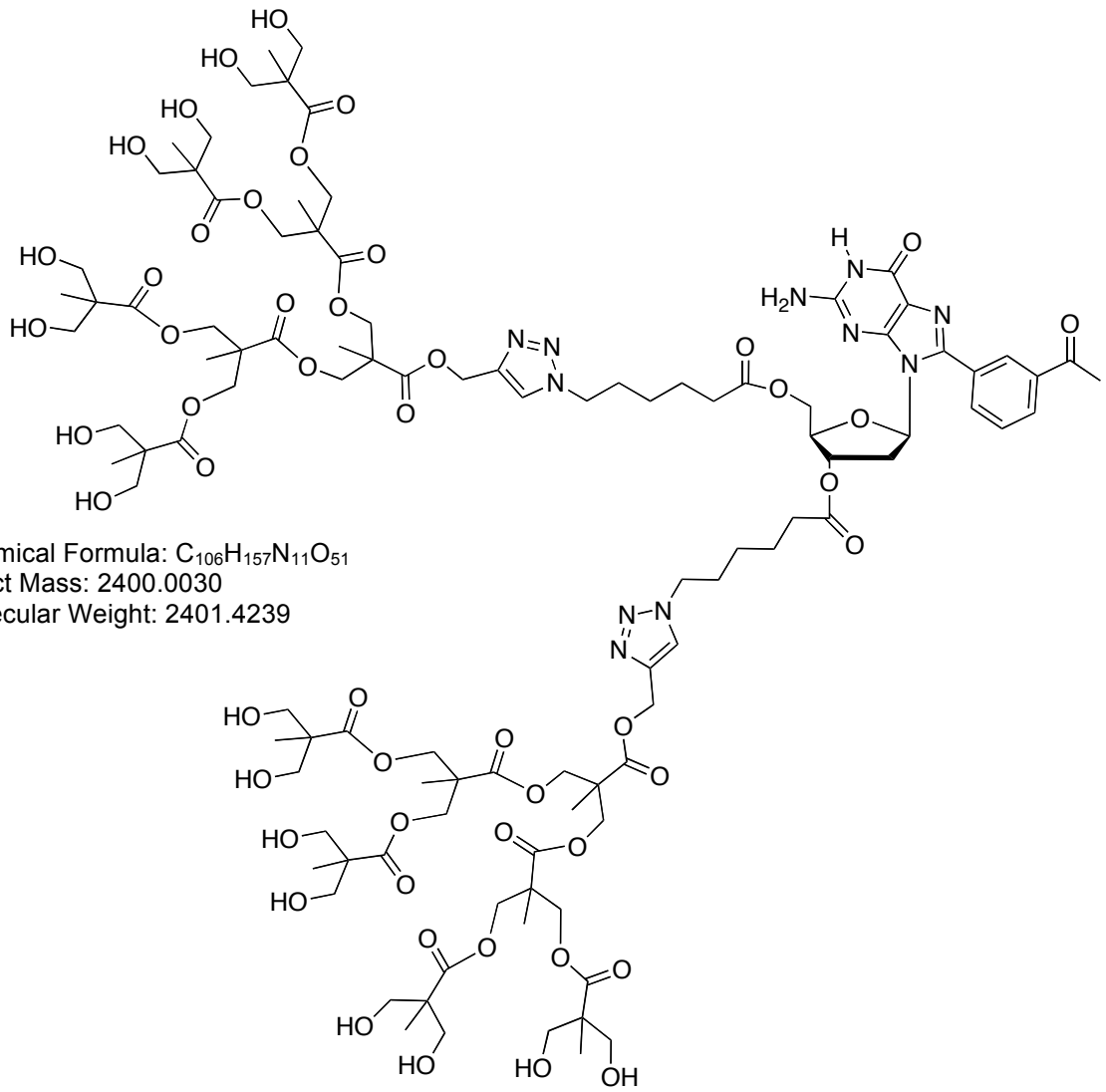
Department of Chemistry,

University of Puerto Rico at Río Piedras, San Juan, Puerto Rico, 00931

*To whom correspondence should be addressed. Telephone: 1-787-764-0000 ext. 2906 Fax: 787-756-8242. E-mail: jmrivortz@mac.com.

TABLE OF CONTENTS

A. High Resolution Mass Spectrometry



Chemical Formula: $C_{106}H_{157}N_{11}O_{51}$
Exact Mass: 2400.0030
Molecular Weight: 2401.4239

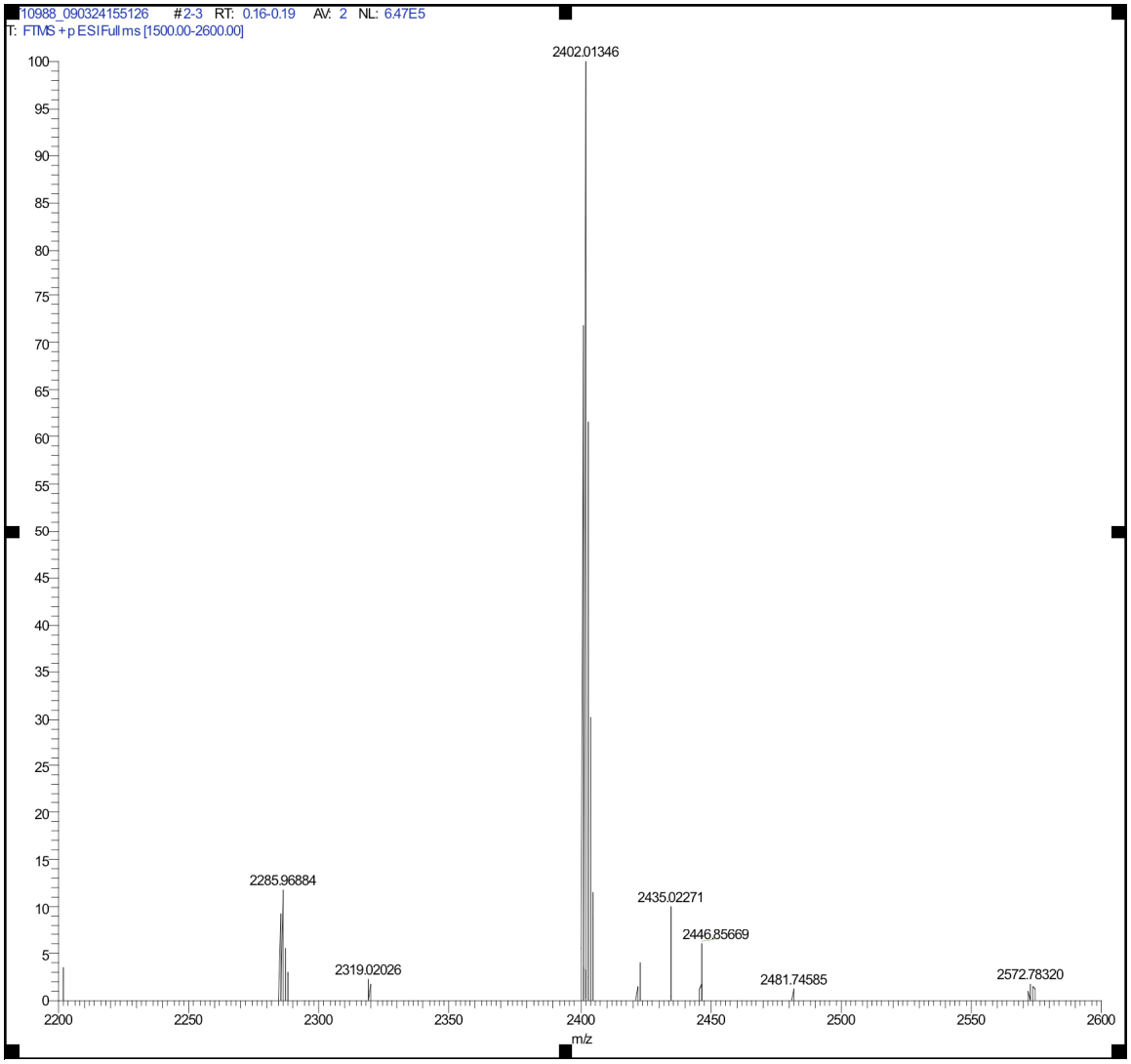


Figure S1. FT-MS pESI full MS [1.5-2.6 kDa] of **3**.

B. NMR Spectra for monomer 3

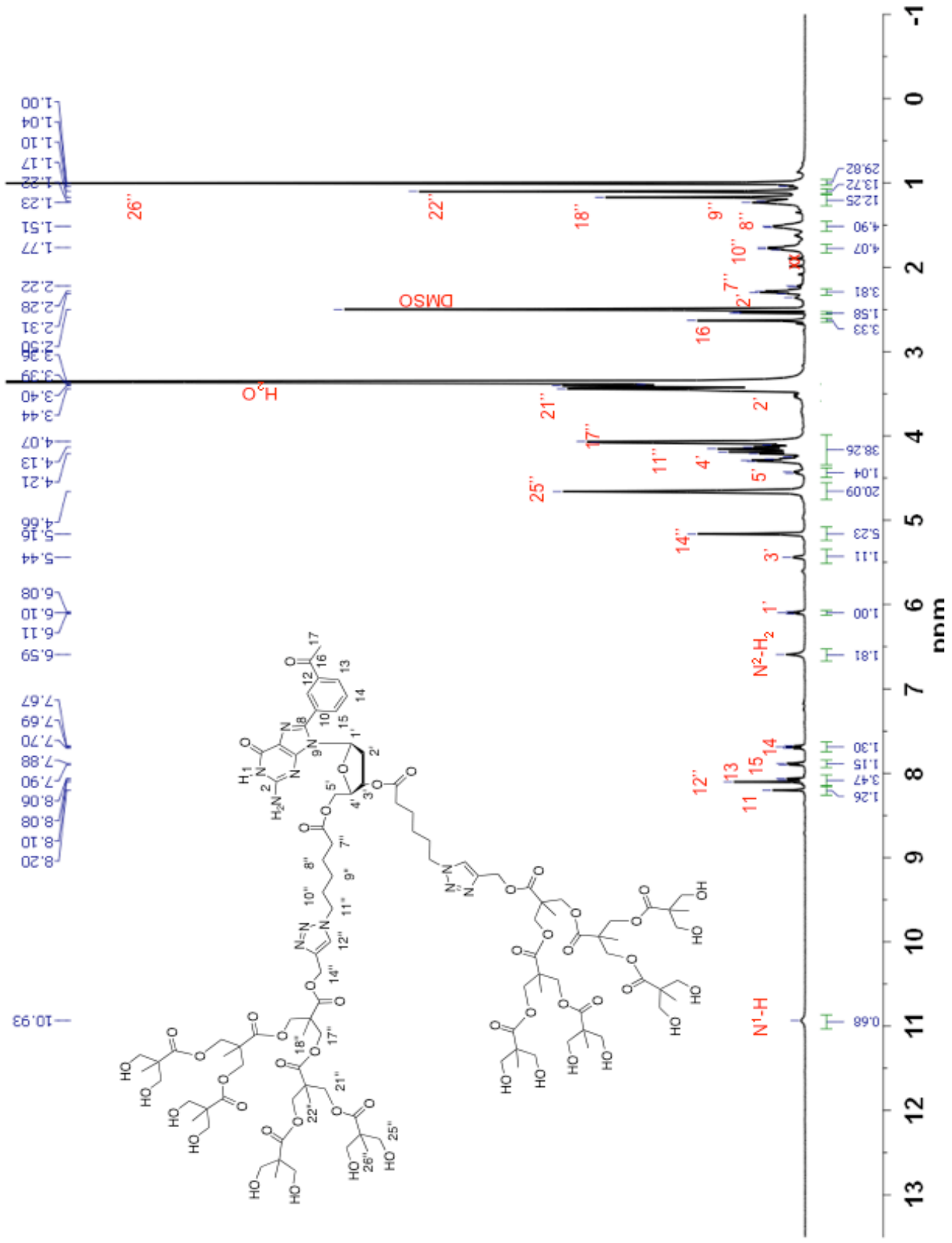


Figure S2. ^{1}H NMR spectrum (500 MHz) of **3** in DMSO-d_6.

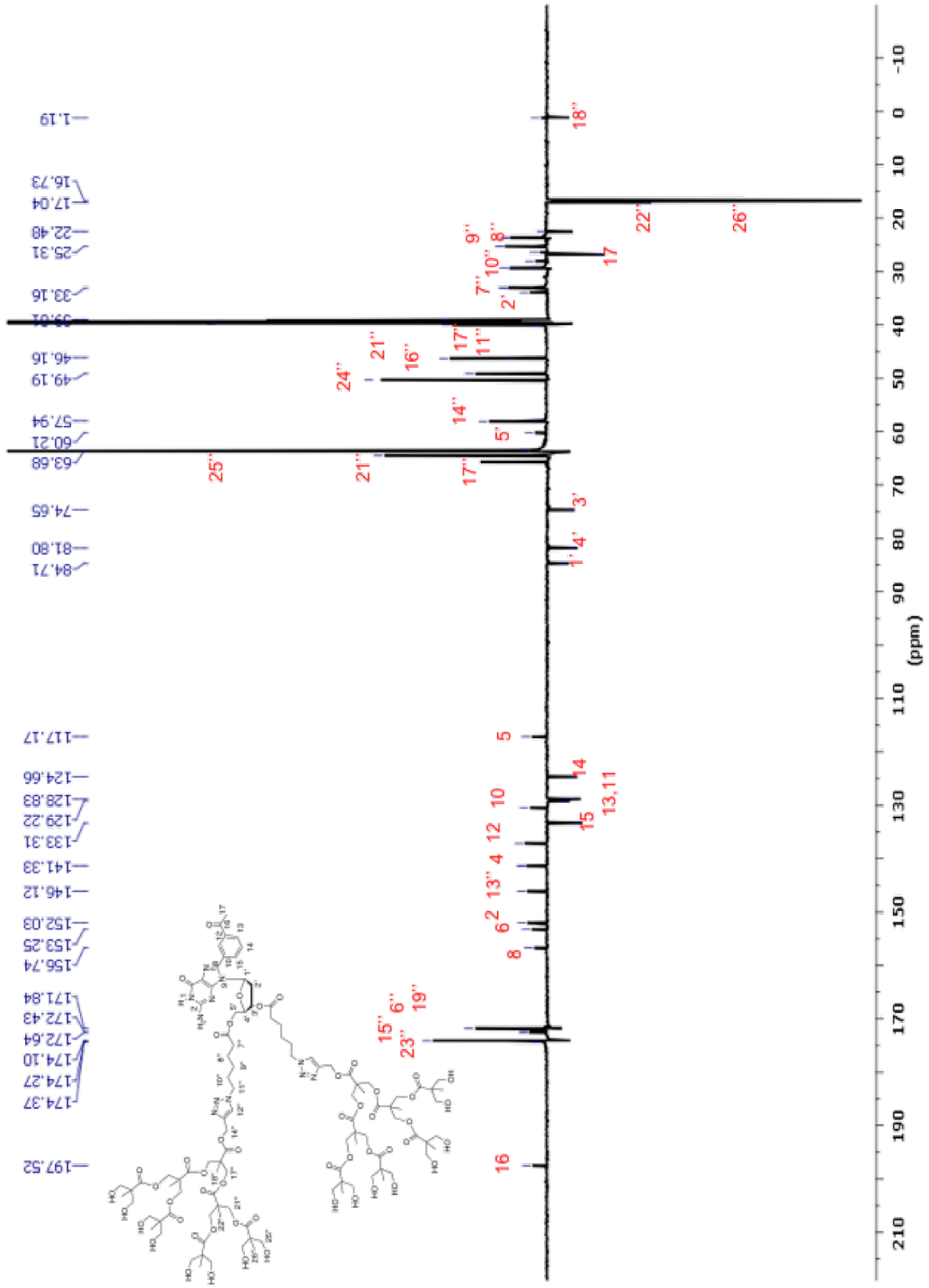


Figure S3. ^{13}C NMR spectrum (125 MHz) of **3** in DMSO-d_6.

C. NMR spectra for 3_{16}

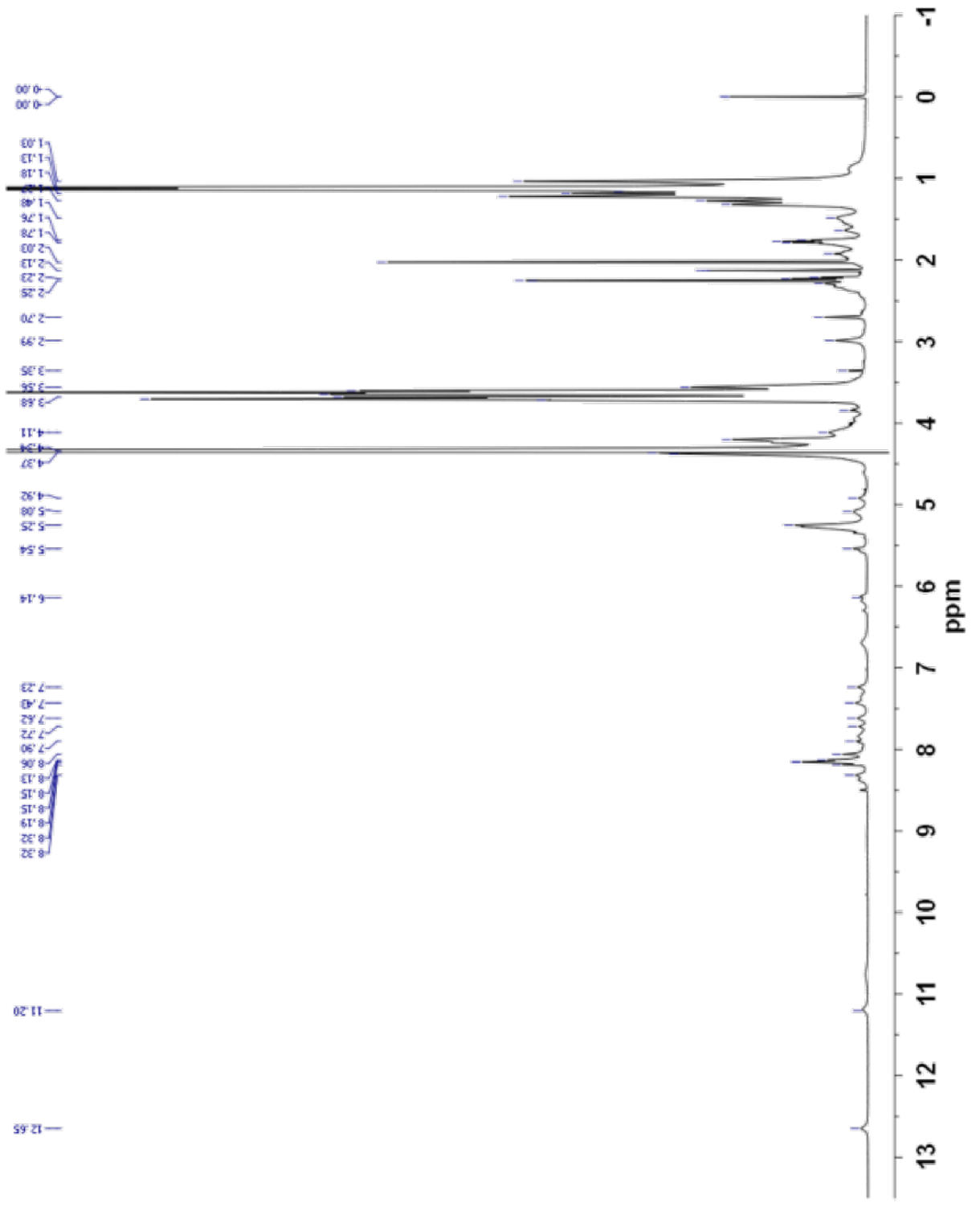


Figure S4. ^{1}H-NMR of $\mathbf{3}_{16}$ (500 MHz; H_2O-D_2O (9:1); 0.1 M PBS, pH 7.1; 3 M KI; 10 mM in **3**).

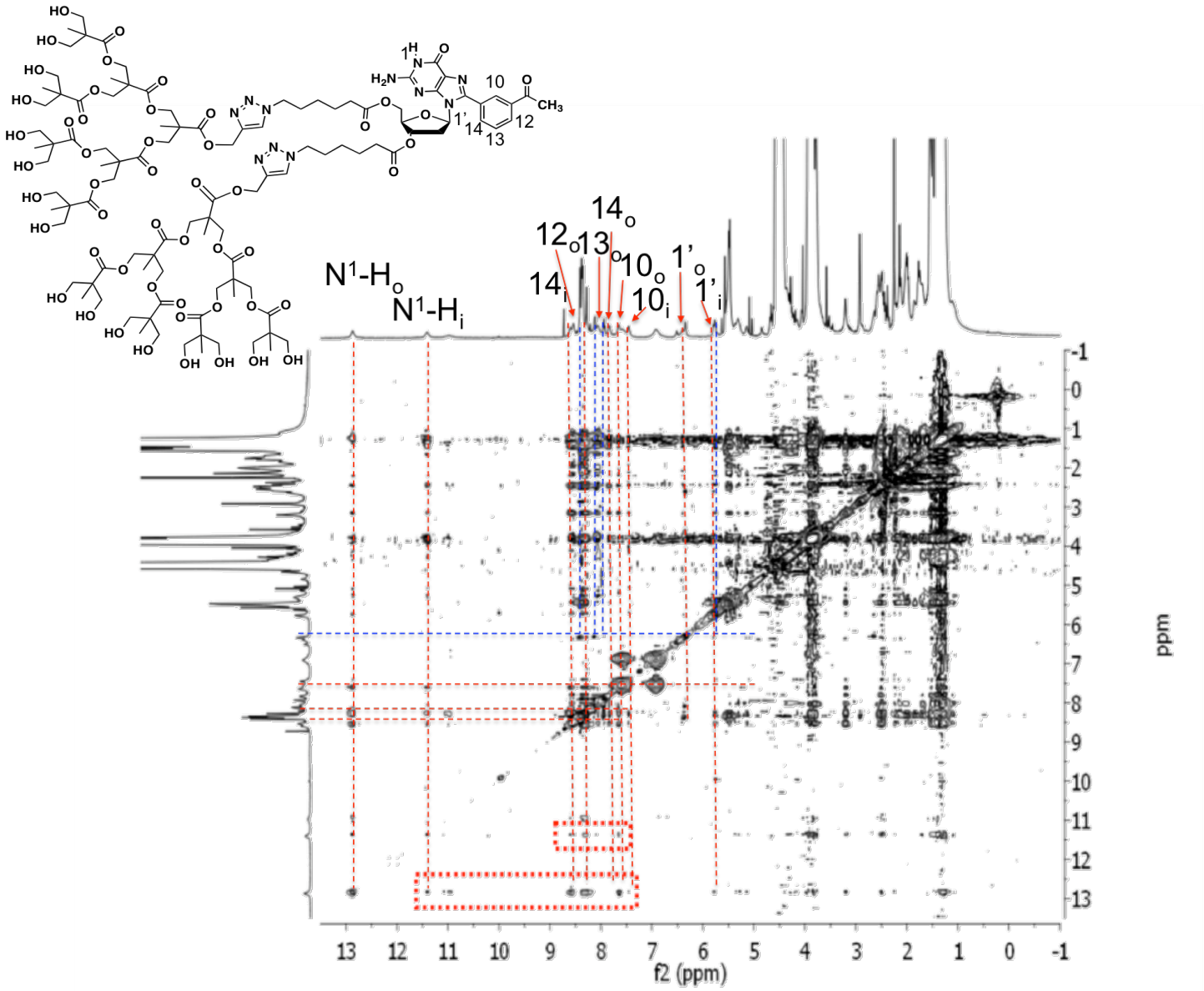


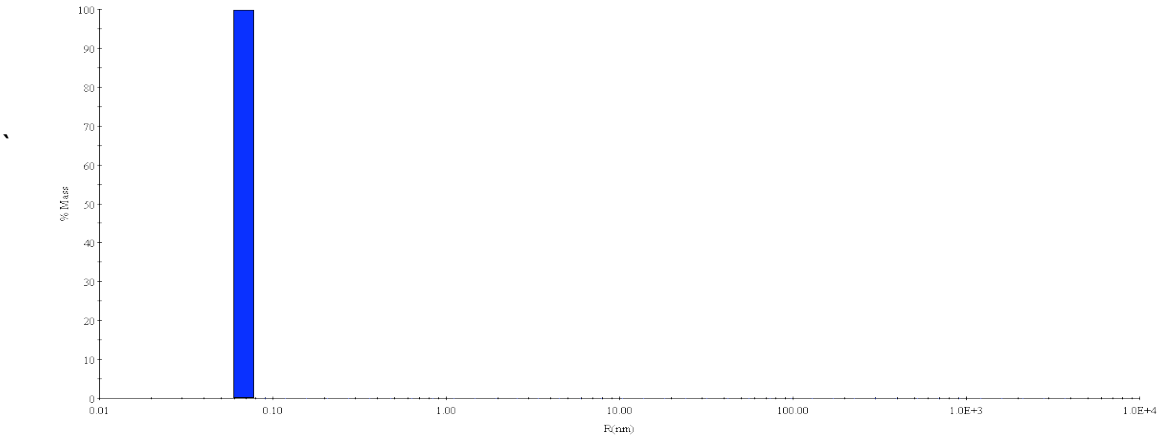
Figure S5. NOESY spectrum of $\mathbf{3}_{16}$ (500 MHz; τ_m = 500 msec; H_2O-D_2O (9:1); 0.1 M PBS, pH 7.1; 3 M KI; 10 mM in **3**). The red lines show the correlations between the protons of the assembly; the blue lines show correlation between monomeric species; and red squares point to the signature cross peaks characteristic of hexadecameric assemblies formed by the **mAG** scaffold in aqueous media.[1,2]

D. Dynamic Light Scattering (DLS) Data

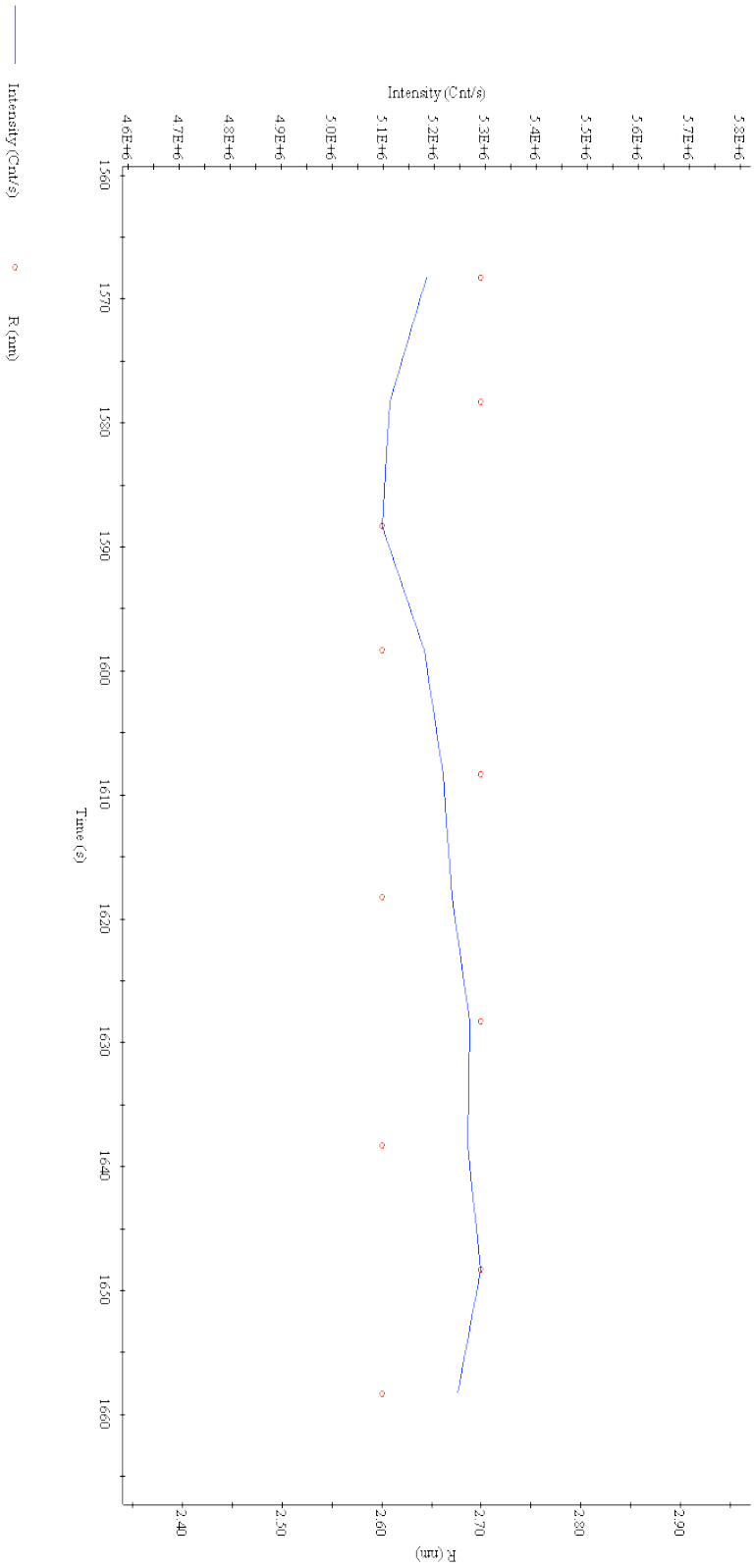
Table S1. DLS raw data for $\mathbf{3}_{16}$ with 99% LASER Power, 10 scans of 10 s each.

Acq #	Time (s)	Temp (C)	Intensity (Cnt/s)	R (nm)	% PD	MW-R (kDa)	Amp	Baseline	SOS
1	1568.3	25	5185610	2.7	23.8	18	0.146	1	0.509
2	1578.3	25	5114500	2.7	36.5	18	0.150	1	0.498
3	1588.3	25	5099330	2.6	20.7	17	0.150	1	0.425
4	1598.3	25	5181560	2.6	22.5	17	0.147	1	0.570
5	1608.3	25	5218710	2.7	36.7	18	0.143	1	0.541
6	1618.3	25	5234680	2.6	33.2	17	0.142	1	0.305
7	1628.3	25	5269420	2.7	15.2	18	0.140	1	0.318
8	1638.3	25	5266750	2.6	22.9	17	0.141	1	0.253
9	1648.3	25	5291180	2.7	30.2	18	0.149	1	0.786
10	1658.3	25	5245270	2.6	36.2	17	0.149	1	0.459
Average				**2.65**	**27.79**				
StdDev				**0.053**	**7.72**				

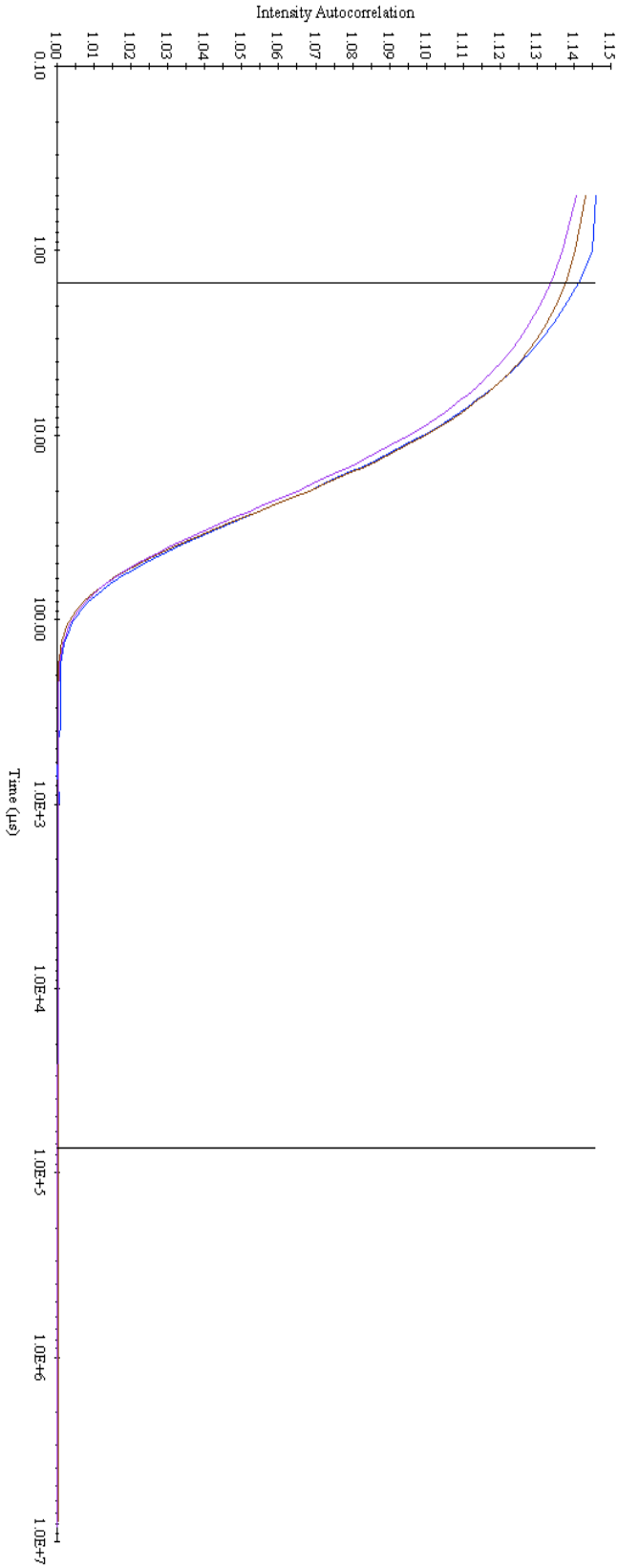
Graph S1. Histogram of the size distributions for $\mathbf{3}_{16}$.



Graph S2. Size and signal intensity changes as function of the detection time for $\mathbf{3}_{16}$.



Graph S3. Correlation values for the data shown in Graphs S1-2.



E. Computational Analysis

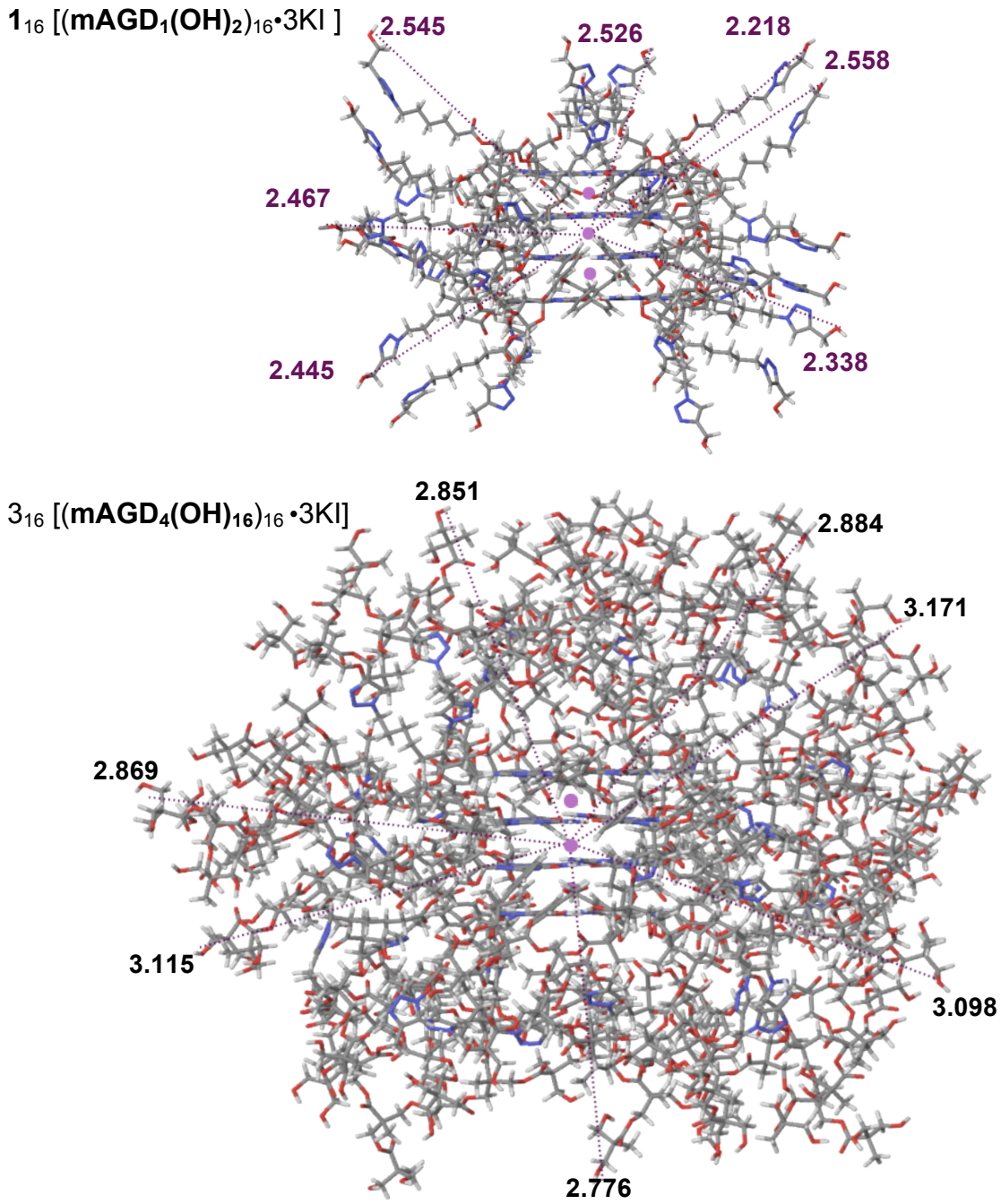


Figure S6. Hydrodynamic radii were determined using a molecular model of $\mathbf{1}_{16}$ and $\mathbf{3}_{16}$ as constructed using *OPLS2005* (MacroModel, Version 9.5, Maestro 8.0.315, Schrödinger, LLC, New York, NY, 2007) representing water as a continuum solvent.* The purple spheres represent potassium cations.[1]

F. T_m calculation from VT-NMR data

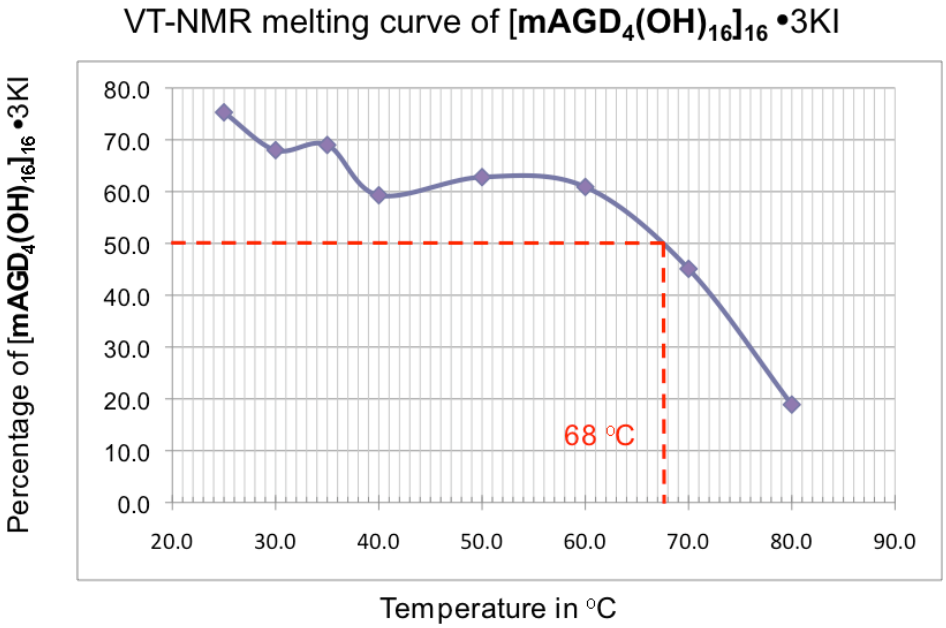


Figure S7. Melting curve of $\mathbf{3}_{16}$ (10 mM in **3**) in D_2O-H_2O (9:1), 0.1 M PBS, pH 7.1 and 3M KI constructed with VT-NMR. The average of the integrations of four peaks (corresponding to protons at: 8.0 ppm, 7.8 ppm, 7.6 ppm, and 6.5 ppm for the assembled specie and 8.2 ppm, 8.1 ppm, and 6.4 ppm for the monomer) were used to calculate the ration of $\mathbf{3}_{16}$:**3** with the T_m defined here as the temperature at which this ratio is 1:1. NOTE: $[mAGD_X(OH)_{2x}]_{16}$•3KI refers to $\mathbf{3}_{16}$ as stated in the main text (Ref. 23).

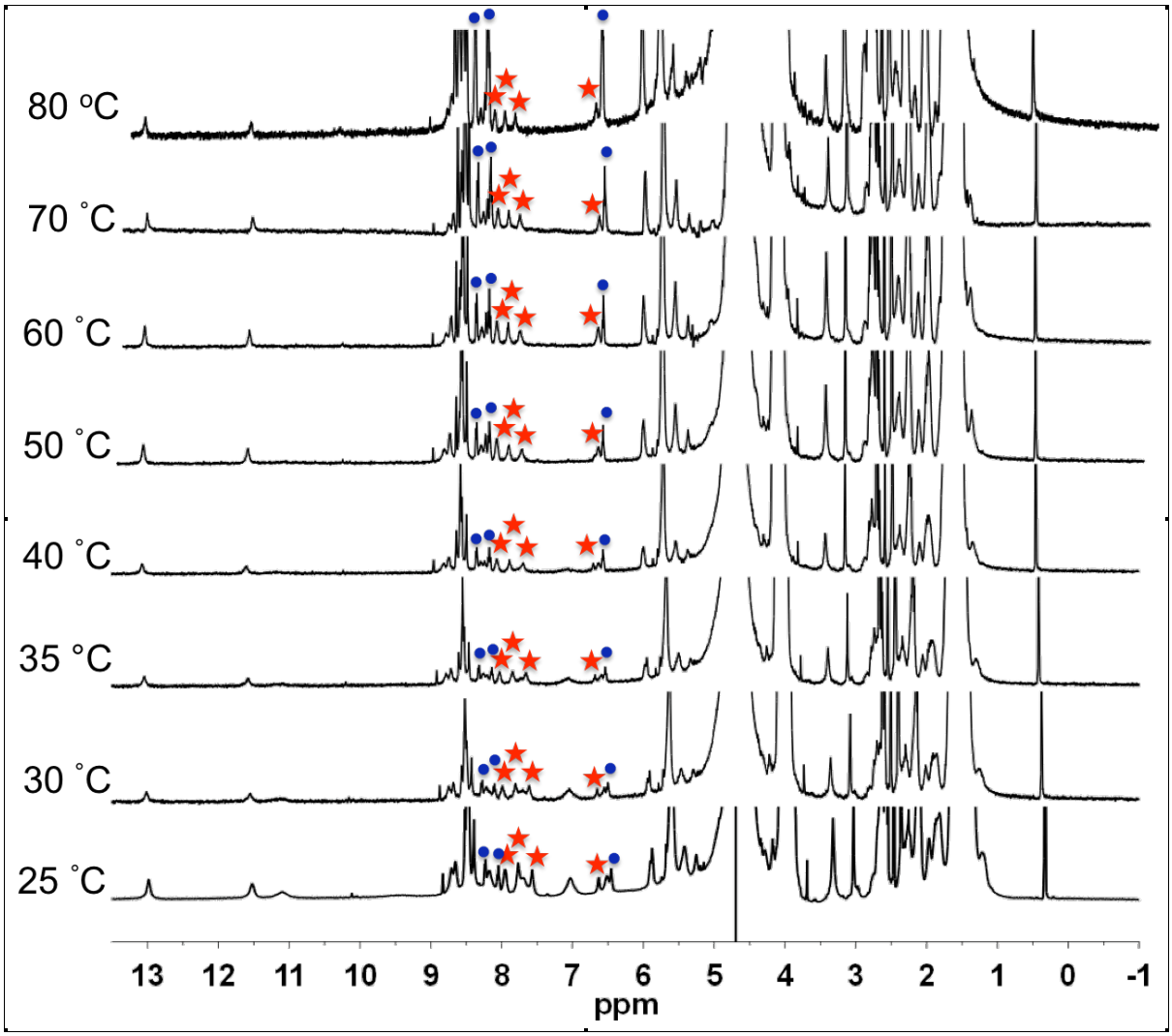


Figure S8. Variable temperature (VT) ^{1}H-NMR (500 MHz) spectra for $\mathbf{3}_{16}$ in H_2O-D_2O (9:1; 10 mM in **3**; 3 M KI; 0.1 M PBS; pH 7.1). The amounts of $\mathbf{3}_{16}$ as a function of temperature are (from bottom to top): 75%, 68%, 69%, 59%, 63%, 61%, 45%, and 19%. The percentage of assembly ($\mathbf{3}_{16}$) were calculated from the average values obtained by integrating the marked peaks corresponding to $\mathbf{3}_{16}$ (red stars, ★) and **3** (blue circles, •). This is a version of Figure 3 (from the main text) showing a wider spectral window (13.5 to -1 ppm).

Table S2. Integration values for assembled specie obtained from the peak integration of the VT-NMR experiments.

GQ Tetrad Proton	**8.0 ppm** 14 outer	**7.8 ppm** 11 outer	**7.6 ppm** 11 inner	**6.5 ppm** 1' outer	**Average of Hexadecamer**
Temperature (°C)	Integration	Integration	Integration	Integration	
25.0	1.79	1.30	1.30	0.65	2.52
30.0	1.18	0.91	0.58	0.47	1.57
35.0	1.32	1.25	0.80	0.52	1.95
40.0	1.08	0.97	0.53	0.28	1.43
50.0	1.22	1.11	0.50	0.43	1.63
60.0	1.10	1.02	0.53	0.78	1.72
70.0	0.64	0.46	0.37	0.29	0.88
80.0	0.18	0.12	0.11	0.10	0.26

Table S3. Integration values for monomeric species obtained from the peak integration of the VT-NMR experiments and the fraction of hexadecamer in solution.

Monomer Proton	**8.2 ppm** 13	**8.1 ppm** 14	**6.4 ppm** 1'	**Average of Monomer**	**Fraction of hexadecamer**
Temperature (°C)	Integration	Integration	Integration		
25.0	0.85	1.00	0.63	0.827	0.753
30.0	0.65	1.00	0.57	0.740	0.680
35.0	0.83	1.00	0.79	0.873	0.690
40.0	0.96	1.00	0.98	0.980	0.593
50.0	0.95	1.00	0.95	0.967	0.628
60.0	1.20	1.00	1.11	1.103	0.609
70.0	1.09	1.00	1.12	1.070	0.451
80.0	1.15	1.00	1.13	1.093	0.189

G. References

1. García-Arriaga, M., Hobley, G., Rivera, J.M., *J. Am. Chem. Soc.* **2008**, *130*, 10492–10493.

2. Betancourt, J.E., Rivera, J.M., *J. Am. Chem Soc.* **2009**, *131*, 16666–16668.