Electronic Supplementary Material (ESI) for Chemical Communications

Metallo-responsive switching between hexadecameric and octameric supramolecular G-quadruplexes

Mariana Martín-Hidalgo & José M. Rivera

Department of Chemistry, University of Puerto Rico at Río Piedras, San Juan, PR 00931
jmrivortz@mac.com

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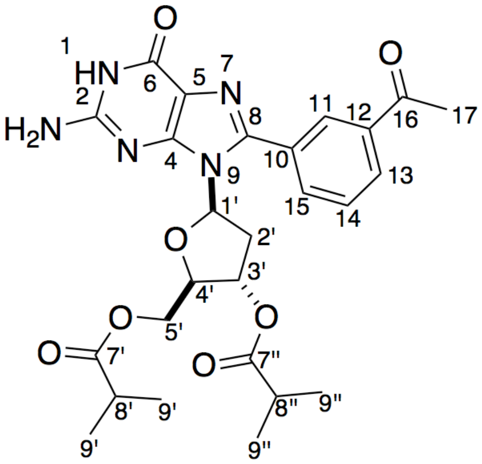
A. Characterization of the target compound

NMR spectra were recorded on Bruker DRX-500 or AV-500 spectrometer, equipped with either a 5 mm BBO or a TXI probe and with nominal frequencies of 500.13 MHz for proton and 125.77 MHz for carbon respectively. All NMR experiments were performed at 298.15 K unless otherwise stated. All the assemblies were characterized with ^{1}H NMR and 2D-NMR techniques such as COSY and NOESY.

The synthesis and characterization for **1** was reported in Gubala, V.; Betancourt, J. E.; Rivera, J. M. *Org. Lett*. **2004**, *6*, 4735-4738.



B. ^{1}H NMR studies of 1 using potassium and strontium salts

A monovalent and a divalent cation were used to promote the cation-templated self-assembly of **1**. The addition of 0.5 equiv of KI and 0.125 equiv of SrI_2,[1] into 30 mM solutions of the **1** in CD_3CN yield the formation of different GQs, different molecularities with high fidelity were observed (Table S1).[2]

Table S1 *Type (molecularity) and relative amounts (fidelity) of quadruplexes formed by **1** (30 mM) determined by ^{1}H NMR (0.5 equiv of KI, and 0.125 equiv of SrI_2 at 298.2 K) in CD_3CN.*

Cation	Assembly (%)		
	O_{D4}	H	UA
K^+	-	90	10
Sr^{2+}	100	-	-

Note: O_{D4} = Octamer (D4 symmetry); H = Hexadecamer; UA = Unidentified Assembly
All measurements have an estimated 5% error

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[1] The equivalents of salt reported are calculated realtive to the monomeric subunits. For example, 0.5 equiv of KI relative to **1** represents 8 equiv (or an excess) of K^+ relative to $\mathbf{1}_{16}$.

[2] Fidelity refers to the percentage of the desired supramolecule when there are two or more potential outcomes. For more information see: Todd, E. M.; Quinn, J. R.; Park, T.; Zimmerman, S. C., Fidelity in the supramolecular assembly of triply and quadruply hydrogen-bonded complexes. *I. J. Chem.* **2005,** *45*, 381-389.

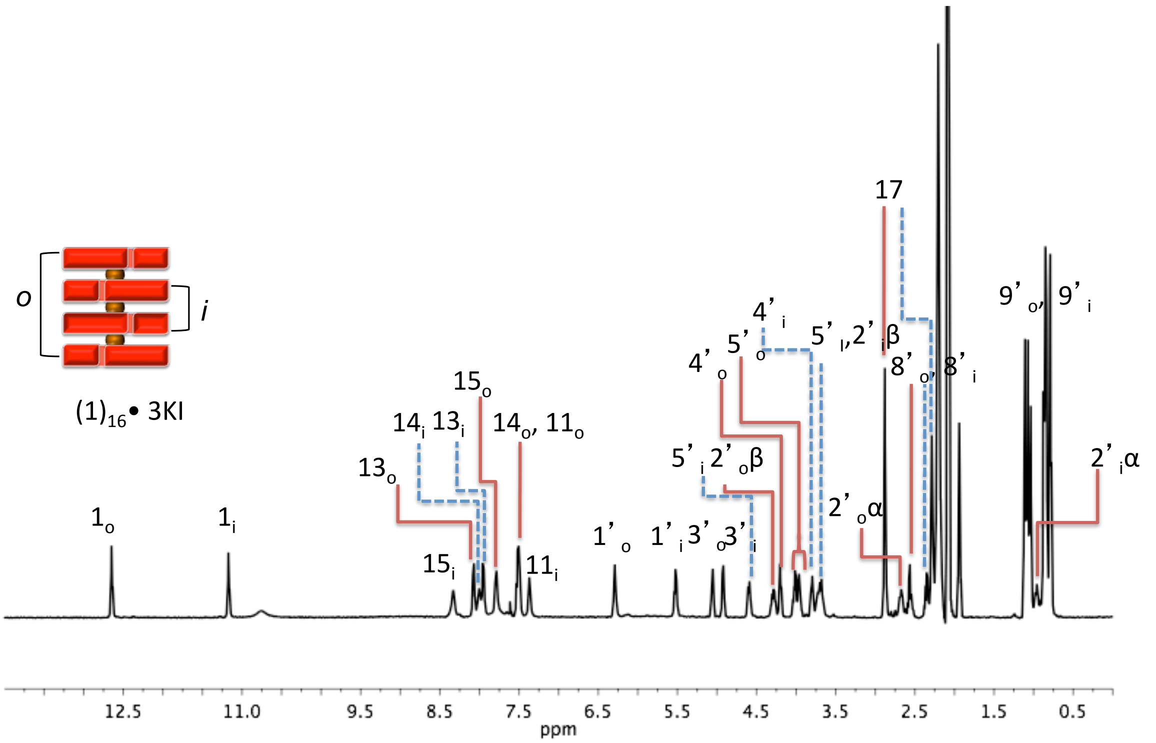


Fig. S1 *^{1}H NMR (500 MHz, CD$_3$CN, 298.2 K) of **1** (30 mM) with 0.5 equiv of KI. The signals corresponding to the hexadecamer's inner (i) and outer (o) tetrads are highlighted.*

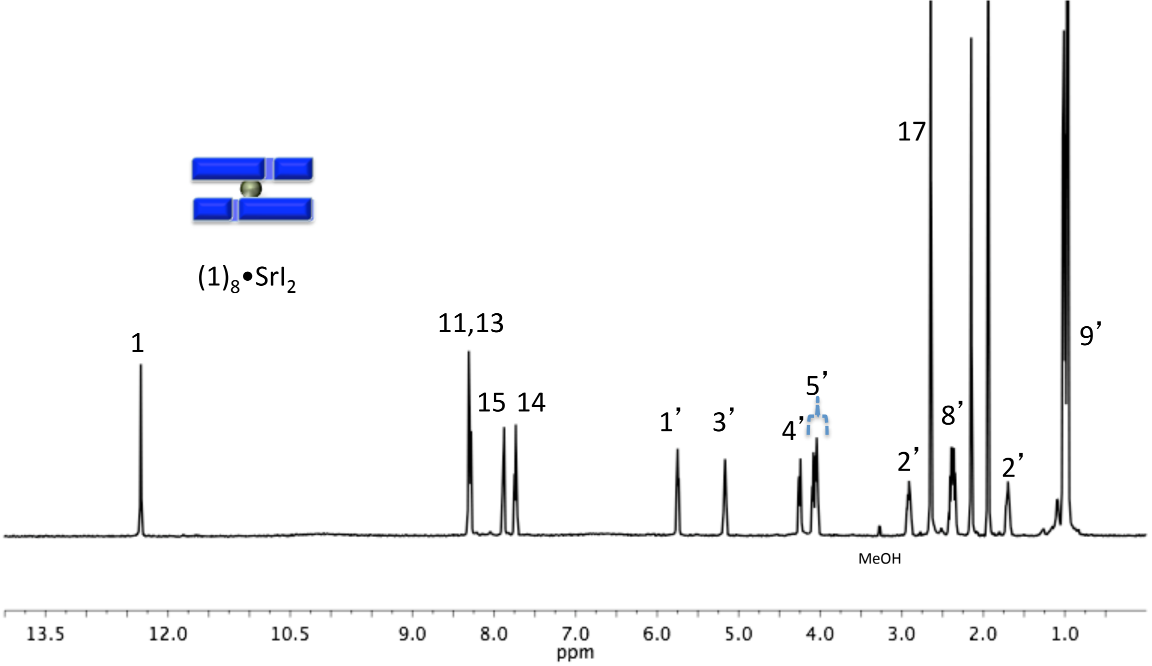


Fig. S2 *^{1}H NMR (500 MHz, CD$_3$CN, 298.2 K) of **1** (30 mM) with 0.125 equiv of SrI$_2$.*

C. Vapor Pressure Osmometry Studies

Vapor Pressure Osmometer (VPO) model Knauer K-7000 with the corresponding software EuroOsmo® version 1.3 was used. The calibration of the instrument was performed, using a standard of formula $C_{54}H_{57}N_9O_{12}$ and a molecular weight of 1024.08 g/mol. Solutions with concentrations ranging from (0.02 - 0.05) molal were prepared, after the calibration procedure, a calibration curve and calibration constant (K_{calib}) value were obtained. For each solution at different concentrations, 3-5 measured values were obtained with good to excellent standard deviation. Validation of the calibration method showed a molecular weight for the standard of 1010 g/mol, which compared to the theoretical value of 1024.08 g/mol, represents a discrepancy of 1.4%. Solutions of **1** (45 - 25 mM) in dry acetonitrile containing 0.5 equiv of the potassium and 0.125 equiv of the strontium salt. Again for each of these solutions 3-5 measurements were obtained with good to excellent standard deviation. The start gain was set to 128, the chamber temperature to 40 °C and the head temperature to 45 °C. To obtain a stable baseline, drops of solvent were added to the thermistors and the system was auto-zeroed several times together with a stabilization time of about an hour after obtaining the best possible baseline. Input of the concentrations in g/kg prior sample injection lead to computer calculated average molecular weight for a given sample. VPO experiments were performed for assemblies that show 90% of fidelity or higher.

Table S2 *Molecular weight (MW) as determined by VPO in CH_3CN.*

Sample	Solution composition	MW (Da)		
		Calculated*	Experimental	Discrepancy
1	$\mathbf{1}_{16}$•3(KI)	8907	8661	246
	$\mathbf{1}_8$•SrI_2	4546	4566	20

* Calculated assuming 100% fidelity in CH_3CN.

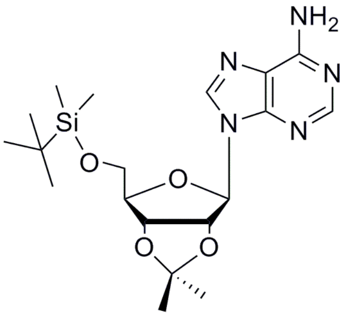
For VPO standard synthesis and preparation see: Rivera-Sánchez, M. d. C.; Andújar-de-Sanctis, I.; García-Arriaga, M.;Gubala, V.; Hobley, G.; Rivera, J. M. *J. Am. Chem. Soc.* **2009**,*131*,10403–10405

D. Diffusion NMR studies

Diffusion NMR experiments were carried out in a Bruker DRX-500 spectrometer equipped with a 5 mm BBO probe, 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, all samples were prepared in Shigemi tubes (Shigemi, Inc., Allison Park, PA) and the temperature was actively controlled at (25.0 ± 0.5) °C. Diffusion coefficients (***D***) 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). The supramolecules were assumed to be spherical[3] and their hydrodynamic radii were calculated with the Stokes-Einstein equation:

$$D = \frac{k_B T}{6 \pi \eta r};$$

where *T* denotes the temperature, *η* is the viscosity of the solvent at the given temperature, k_B is the Boltzmann-Constant, ***D*** is the measured diffusion constant and ***r*** is the hydrodynamic radii. For the calculations of the hydrodynamic radii in CD_3CN the value ($\eta = 0.341 \times 10^{-3}$ $Kg \cdot m^{-1} \cdot s^{-1}$, 298.15 K) reported elsewhere was used. All the measurements were performed in triplicate and the uncertainty is given as the standard deviation. An internal adenosine standard was used to validate the experiments, $D = (12.7 \pm 1) \times 10^{-10}$ $m^2 s^{-1}$ and $r = (5.0 \pm 0.5)$ Å at 298.15 K.



Adenosine Standard

Table S3 *Diffusion coefficients (D_s) and hydrodynamic radii (R_H) of the assemblies formed by* ***1*** *, determined by PFG–NMR in CD_3CN.*

Assembly	D_s (10^{-10} m^2/s) ± SD	(R_H) (Å)
$\mathbf{1}_{16} \cdot 3(KI)$	6.4 ± 0.1	10.0 ± 0.2
$\mathbf{1}_8 \cdot SrI_2$	7.0 ± 0.2	9.2 ± 0.3

E. Mass Spectrometry

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. 10mM solutions of monomer **1** in acetonitrile with 0.5 equiv of KI and 0.125 equiv of SrI_2 were prepared. At room temperature these samples were directly and continuously infused at a flow rate of 5 µL/min with a syringe pump. The source block temperature was maintained at 60º C and the desolvation gas was heated to 80º 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.

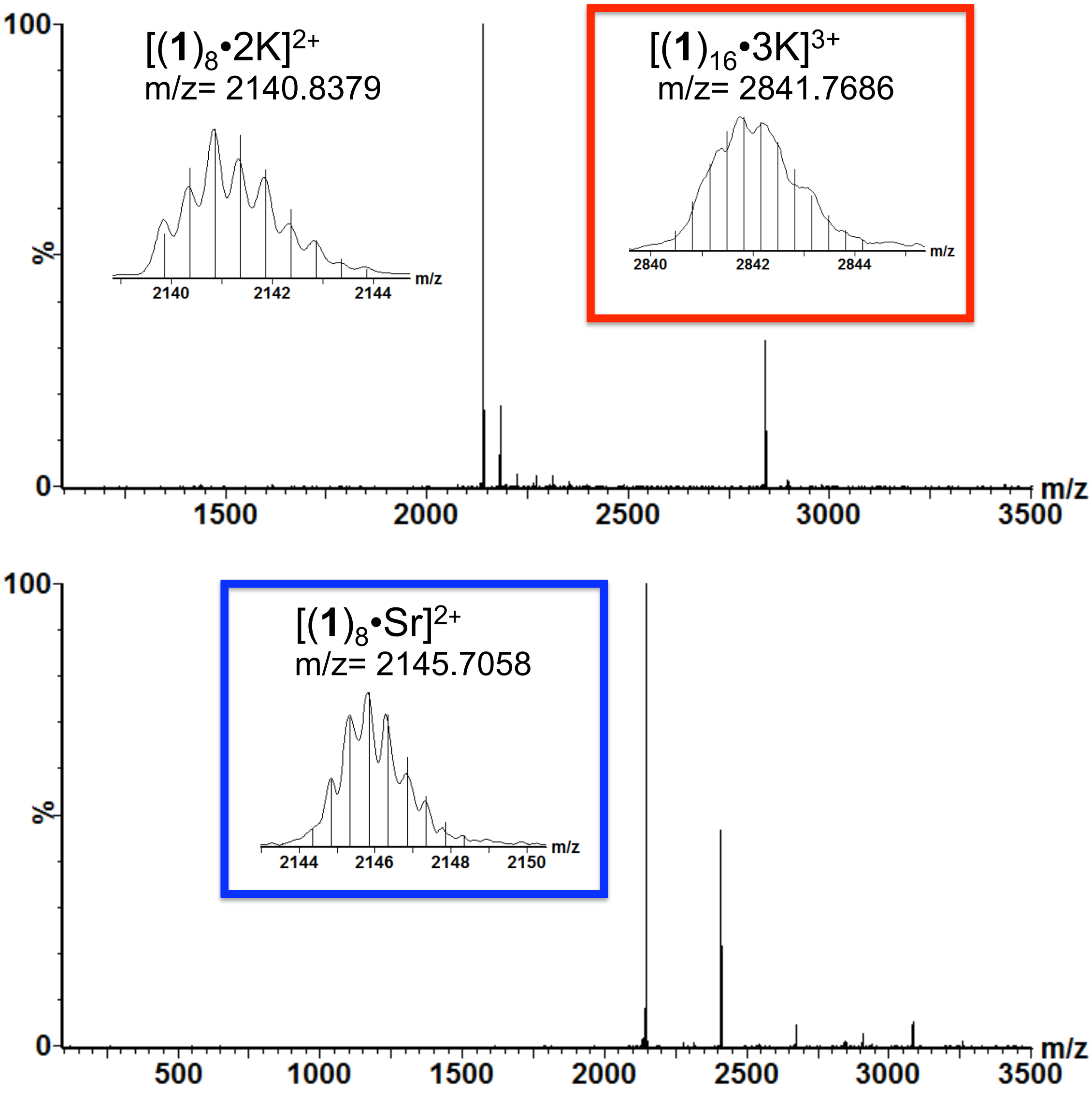


Fig. S3 *MS spectra of* ***1*** *(10 mM) solutions with 0.5 equiv of KI (top) and 0.125 equiv of* SrI_2 *(bottom) in* CH_3CN*.*

F. Thermal stability studies

Variable temperature experiments were performed at 5 mM of **1**. At this particular concentration there is enough monomer and self-assembled species to enable the construction of a melting profile. Integration of the area under the H1' peaks and the first derivative calculations allows a good approximation of the melting temperature for the assemblies. The fraction (f) of assembly (fidelity) $f = X_{SA}/X_T$, is reported as the ratio between the total self-assembled species (X_{SA}) and the total concentration of **1** (X_T), see Fig.S4 for more information. Value for the thermal stability of the hexadecamer form by **1** in KI was taken from Betancourt, J. E.; Martín-Hidalgo, M.; Gubala, V.; Rivera, J. M., *J. Am. Chem. Soc.* **2009**, *131*, 3186-3188.

Table S4 *Melting temperatures for the assemblies of* ***1*** *(5mM) as a function of different metal salts.*

Metal salt	T_m (K)
KI	329.2
SrI_2	>333.2

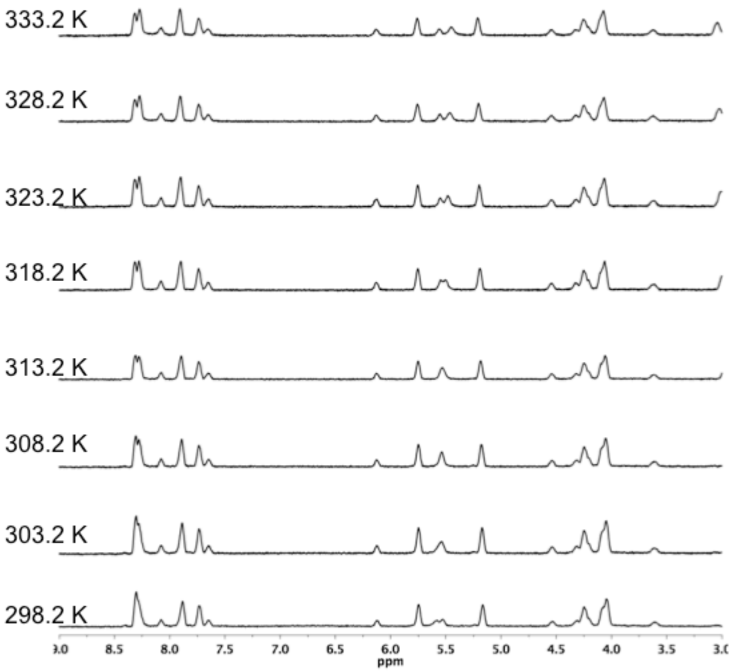


Fig. S4 *Variable temperature ^{1}H NMR (500 MHz, CD$_3$CN) spectra* $\mathbf{1}_8$•*Sr^{2+}* (5 mM in **1**, *with 0.125 equiv of SrI$_2$).*

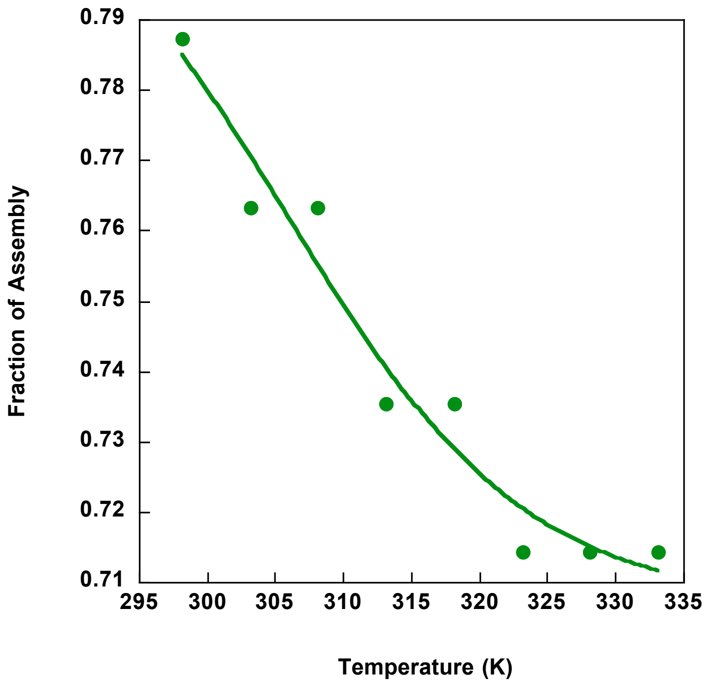


Fig. S5 Melting profile as determined by VT-NMR measurements for $\mathbf{1}_8$•Sr^{2+} (5 mM in **1**, 0.125 equiv. SrI_2).

G. ^{1}H NMR titration experiments of 1 with KI and SrI_2

1 (30 mM) was assembled into the hexadecamer $\mathbf{1}_{16}$•3K$^+$ by adding 0.5 equiv of KI in CD_3CN. Upon incremental addition of SrI_2 the signals corresponding to $\mathbf{1}_{16}$•3K$^+$ decreased with the concomitant increase of those corresponding to $\mathbf{1}_8$•Sr^{2+} reaching ~100% of fidelity at 0.125 equiv of SrI_2 (Fig. S6). Similarly, **1** was assembled with 0.125 equiv of SrI_2 and upon incremental addition of KI the supramolecule remains as an octamer (Fig. S7). Only small peaks corresponding to a putative hexadecamer are detected (ca. 5 %).

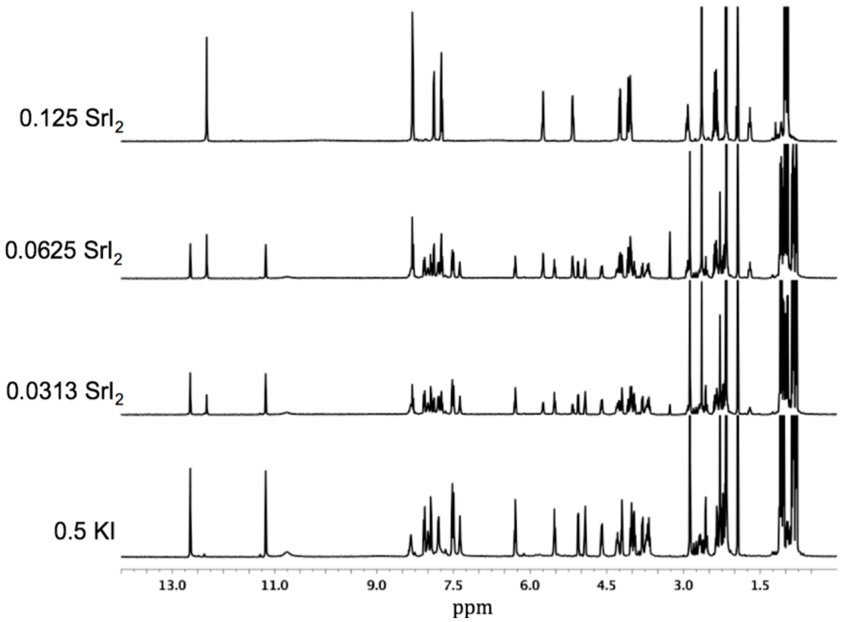


Fig. S6 *^{1}H NMR (500 MHz, CD_3CN, 298.2 K) spectra of **1** (30 mM, with 0.5 equiv of KI) followed by those with increasing amounts of SrI_2 (until reaching 0.125 equiv).*

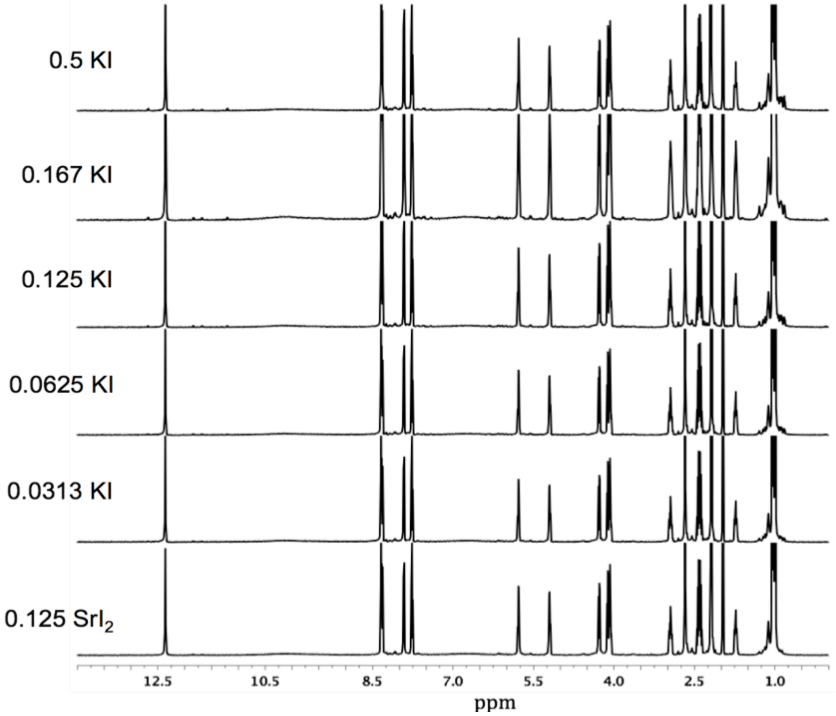


Fig. S7 *^{1}H NMR (500 MHz, CD_3CN, 298.2 K) spectra of **1** (30 mM) with SrI_2 (0.125 equiv) followed by those with increasing amounts of KI (until reaching 0.5 equiv).*

H. Switching experiments

The switching studies were performed using a solution of the hexadecamer of **1** (30 mM, 0.5 equiv KI) in deuterated acetonitrile**,** after adding 0.0625 equiv of SrI_2 an interconvertion from $\mathbf{1}_{16}$•$3K^+$ to $\mathbf{1}_8$•Sr^{2+} was observed (Fig. S8). The controlled addition of K_2SO_4 caused the precipitation of $SrSO_4$ with the simultaneous switching of $\mathbf{1}_8$•Sr^{2+} back to $\mathbf{1}_{16}$•$3K^+$ (Fig. S9). The subsequent filtration of the precipitate and further addition of SrI_2 regenerated the octamer $\mathbf{1}_8$•Sr^{2+}. The process was repeated three times with no significant loss in fidelity. The cycle can be started from the octamer of SrI_2 followed by the addition of 0.5 equiv of K_2SO_4. On the other hand **1** in K_2SO_4 only promotes the formation of an octamer ($\mathbf{1}_8$•K^+) with a moderate fidelity of 66% (Fig. S10).

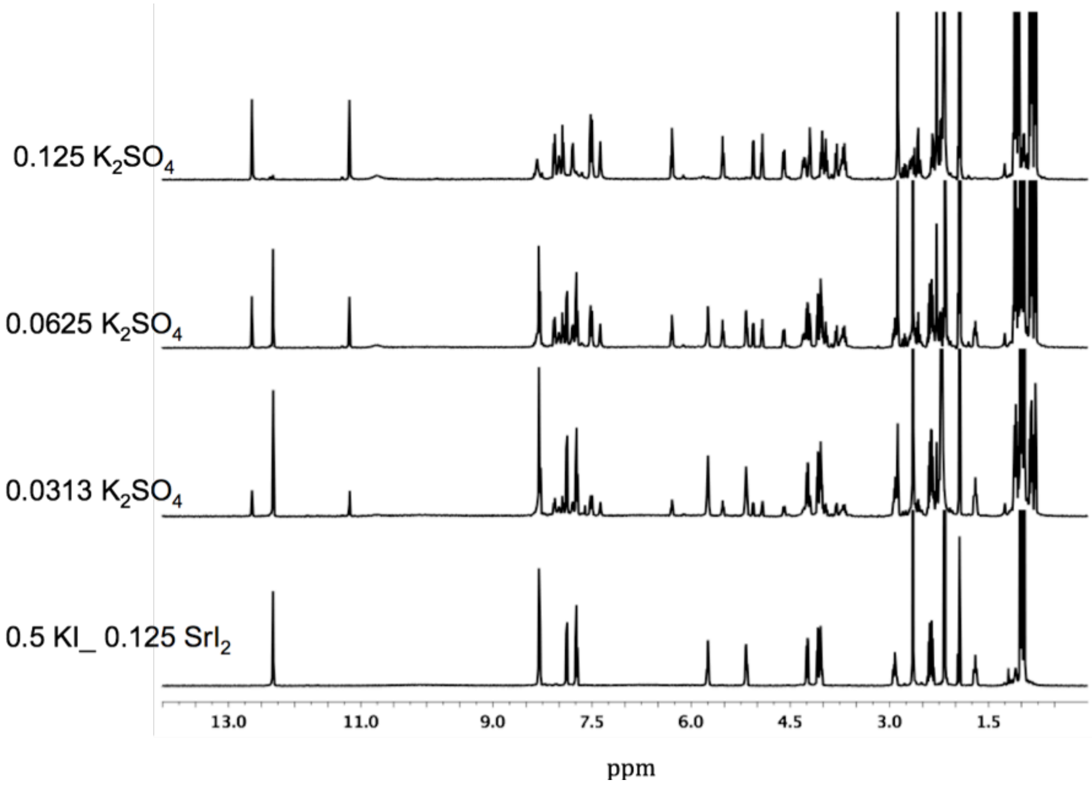


Fig. S8 *^1H NMR (500 MHz, CD_3CN, 298.2 K) spectra of **1** (30 mM) with 0.5 equiv of KI and 0.125 equiv of SrI_2, followed by the addition of increasing amounts of K_2SO_4 until reaching 0.125 equiv of K_2SO_4.*

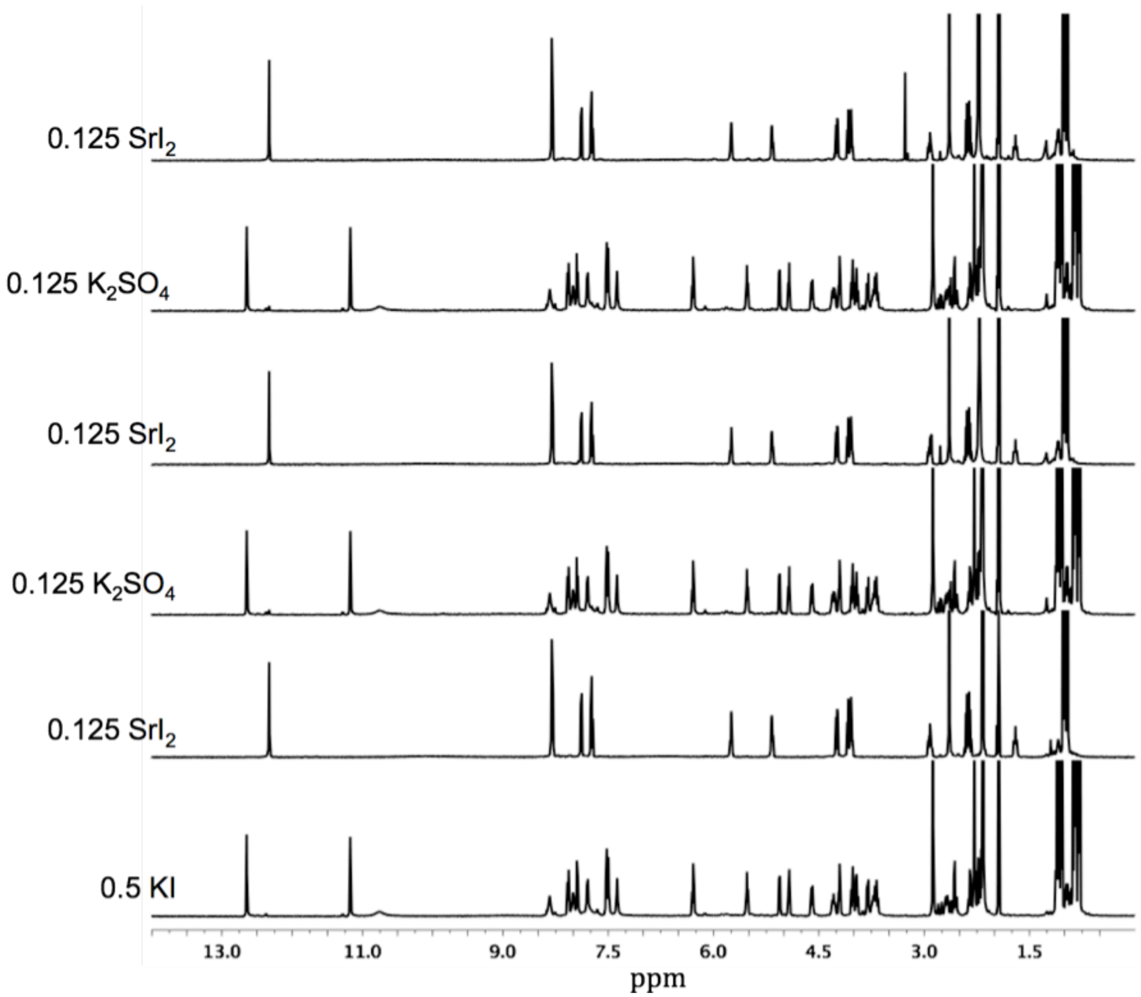


Fig. S9 *^{1}H NMR (500 MHz, CD_3CN, 298.2 K) spectra of* ***1*** *(30 mM) with 0.5 equiv of KI followed by the addition of 0.125 equiv of SrI_2. The eventual addition of 0.125 equiv of K_2SO_4 followed by the further addition of 0.125 equiv of SrI_2 induces the switching between the octameric and hexadecameric states.*

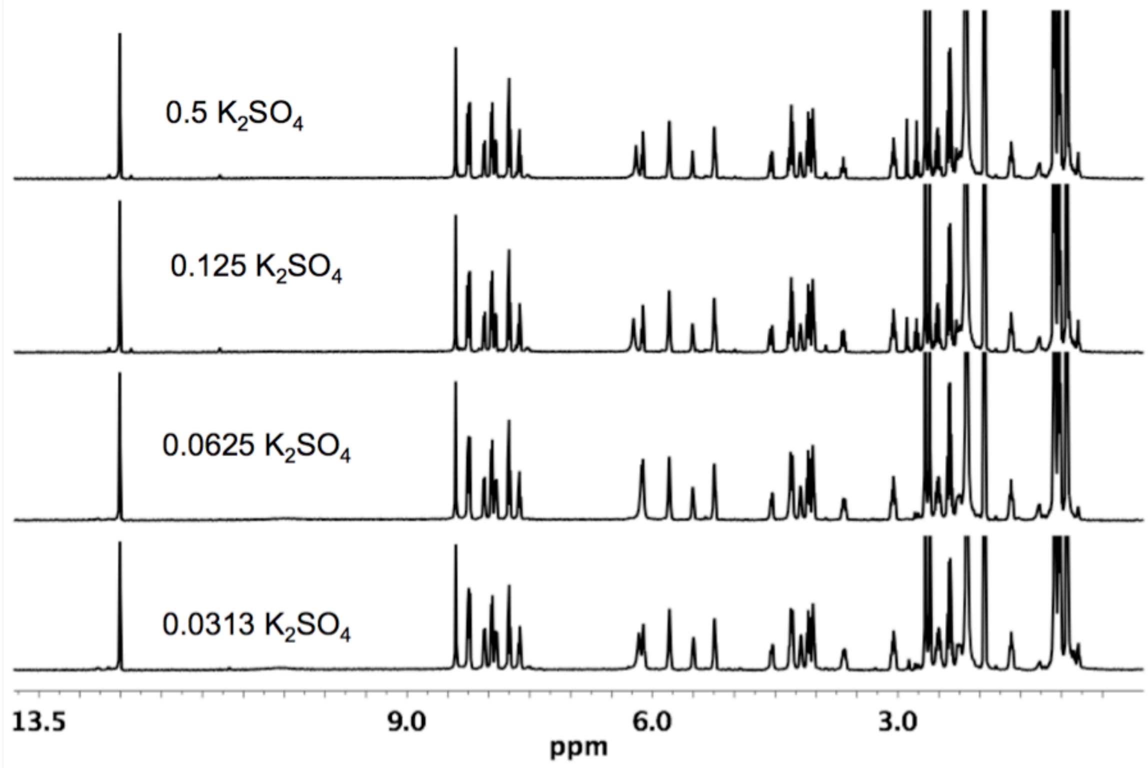


Fig. S10. *Titration of **1** (30 mM) solution with K_2SO_4 as measured by ^{1}H NMR (500 MHz, CD_3CN, 298.2 K).*

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