Supporting Information for:

G_4-Quartet Hydrogels from 5'-Hydrazino-Guanosine for Non-Covalent and Covalent Remediation of Contaminants from Water

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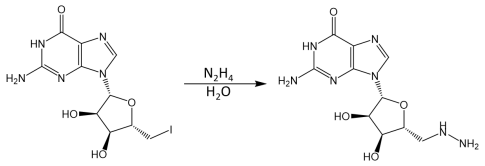
General Experimental: NMR spectra were recorded on Bruker DRX-400, Bruker DRX-500, or Bruker AVIII-600 spectrometers. Chemical shifts are reported in ppm relative to the residual solvent peak. Deuterated solvents were purchased from Cambridge Isotope Labs. CD spectroscopy was performed on a Jasco J-810 spectropolarimeter. ESI-MS experiments were done with a JEOL AccuTOF spectrometer. UV-visible spectroscopy measurements were made on a Varian Cary 100 spectrometer. Rheological data was collected with an AR2000 stress-controlled rheometer from TA Instruments. IR spectra were collected on a Thermo Nicolet Nexus 670 FT-IR with ATR module at room temperature. PXRD experiments were performed with a Bruker D8 Advance Bragg-Brentano Diffractometer. SEM images were obtained on a Hitachi SU-70 High Resolution Analytical SEM.

Materials. Reagents and solvents were purchased from Acros, Sigma-Aldrich, Alfa Aesar, Fisher, Carbosynth or Santa Cruz Biotechnology and used without further purification. D_2O was purchased from Cambridge Isotope Laboratories. Guanosine (G **1**) and guanosine 5′-monophosphate (GMP **6**) were purchased from Sigma-Aldrich. The following compounds were synthesized according to literature procedures and their ^1H and ^{13}C NMR matched published values: 5′-deoxy-5′-hydrazinoguanosine (HG **2**),[1] 5′-hydrazide (LG **3**),[2] 5′-deoxy-5′-aminoguanosine (NH_2G **4**),[3] and 5′-deoxy-5′-hydroxylaminoguanosine (NHOHG **5**). The last step in the synthesis of HG **2** is also described below.

Preparation of 5′-deoxy-5′-hydrazinoguanosine (HG 2).[1]



HG **2**

5′-deoxy-5′-hydrazinoguanosine (HG **2**). Hydrazine hydrate (30 mL of a 50 % NH_2NH_2 solution in water) was added to a 100 mL round-bottom flask containing 5′-deoxy-5′-iodoguanosine[3] (3.0 g, 7.5 mmol). The reaction mixture was stirred at 40 ^oC for 18 h. Methanol (75 mL) was added to the reaction mixture and the mixture was cooled on ice, causing precipitation of a white solid. The solid was collected by filtration, and washed thoroughly with methanol (50 mL). After methanol was removed by evaporation, the solid was heated in deionized water (25 mL) until a clear solution resulted and hot filtration was performed to remove any insoluble material. Then, methanol (50 mL) was added to the filtrate and cooled on ice for 30 min and the precipitate was collected by vacuum filtration and dried on a lyophilizer to afford HG **2** as an off-white solid (1.03 g, 45.4 %). ^1H NMR (DMSO-d_6) δ: 2.80-2.89 (2 H, m, 5′-CH_2), 3.94 (1 H, dd, 4′-CH), 4.05 (1 H, dd, 3′-CH), 4.45 (1 H, dd, 2′-CH), 5.64 (1 H, d, J=6.1 Hz 1′-CH), 6.46 (2 H, s, NH_2), 7.89 (1 H, s, 8-CH), 10.21 (1 H, s br, N1H). ^{13}C NMR (DMSO-d_6) δ: 57.00 (C5′), 71.62 (C3′), 72.87 (C2′), 82.62 (C4′), 86.75 (C1′), 116.90 (C5), 136.00 (C8), 151.19 (C4), 153.54 (C2), 156.72 (C6).

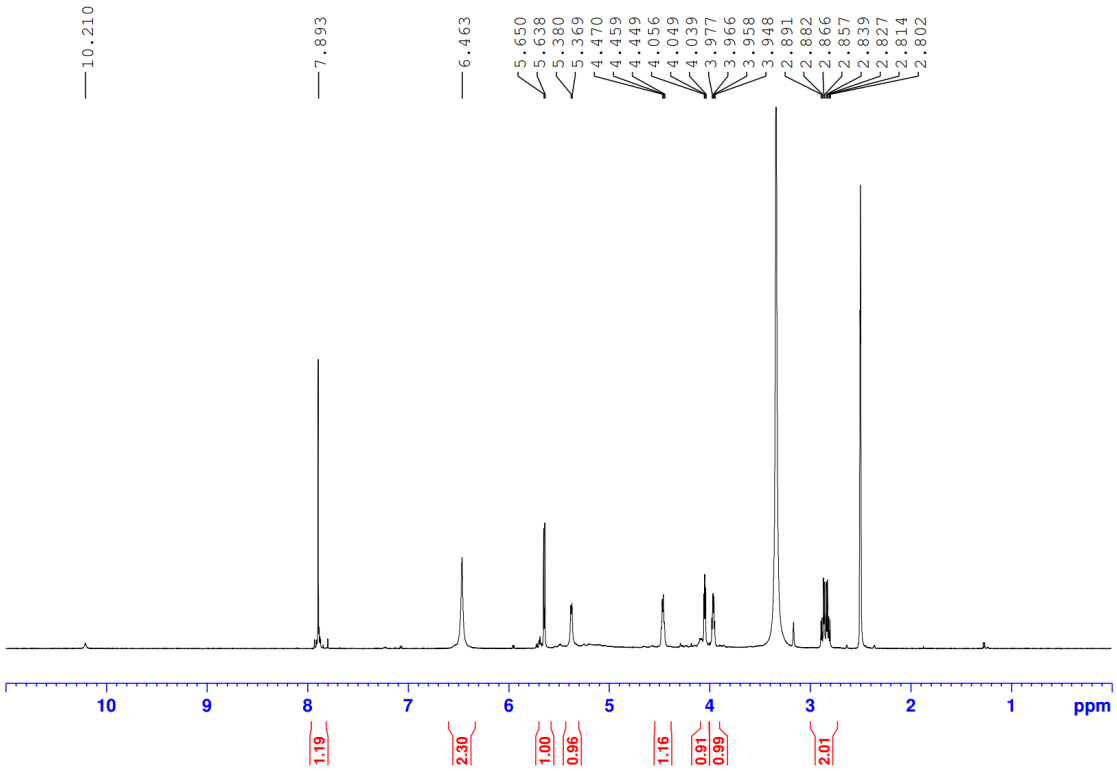


Figure S1: ^{1}H NMR spectrum of HG **3** in DMSO-d_6

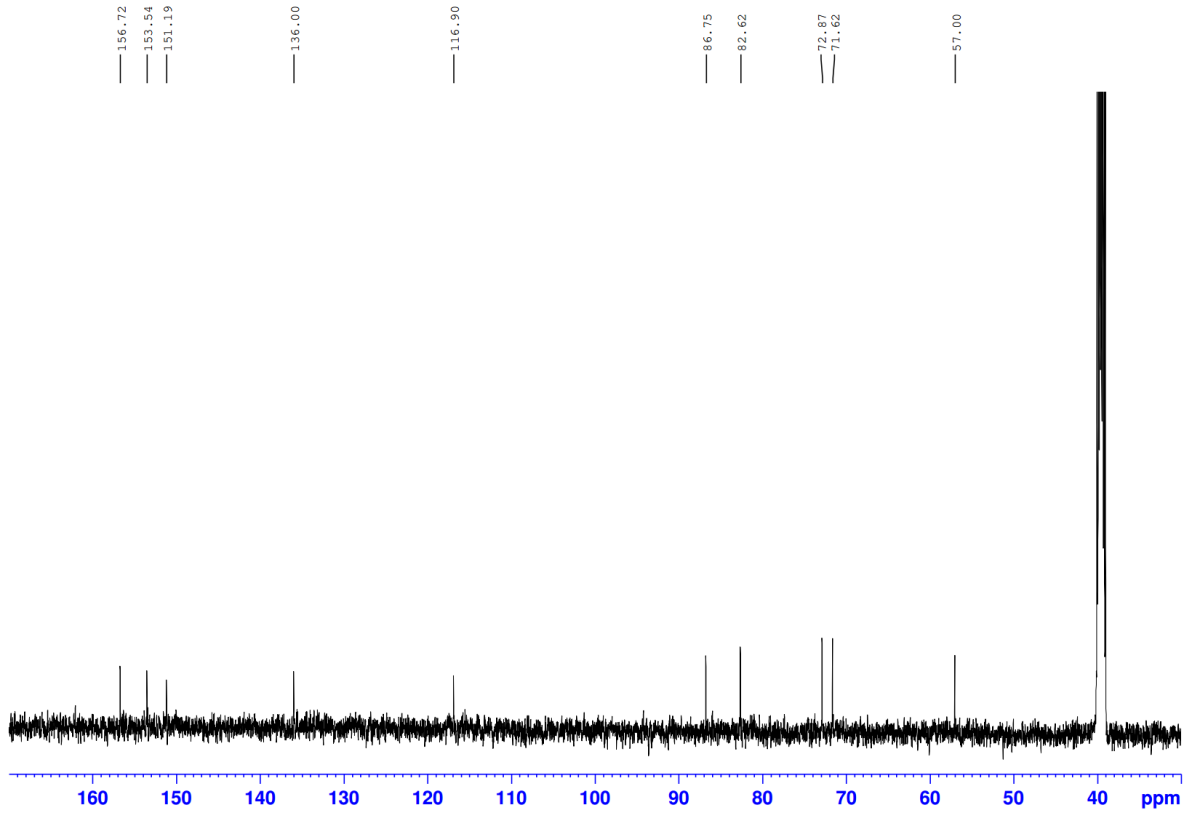
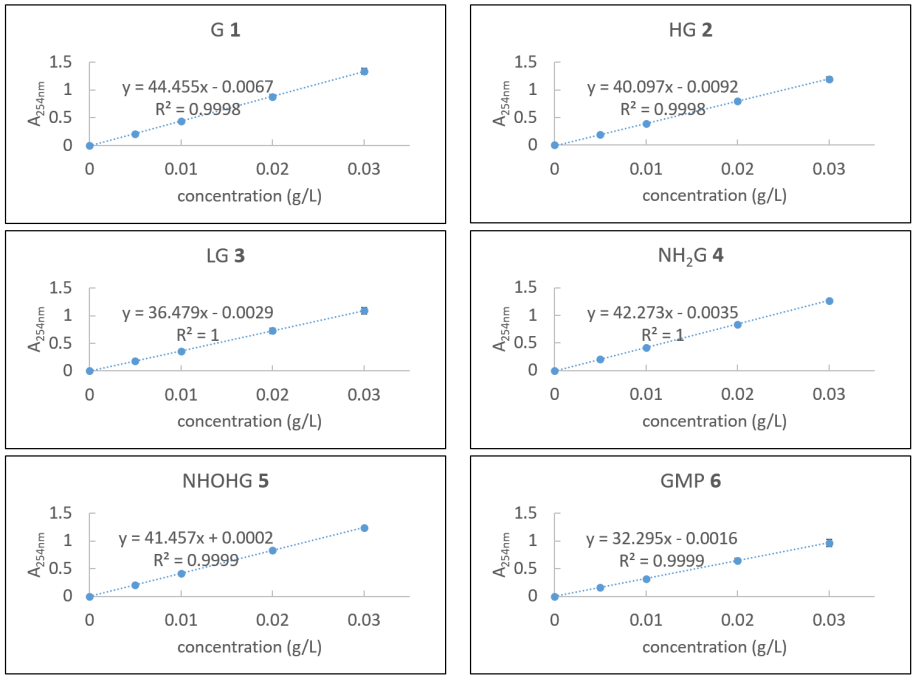


Figure S2: ^{13}C NMR spectrum of HG **2** in DMSO-d_6.

Determination of water solubility of guanosine derivatives 1-6.

Working curve and determination of molar extinction coefficients: For each guanosine analog, dilute aqueous solutions at different concentrations were prepared (0, 0.005, 0.01, 0.02, 0.03 g/L). The absorbance of each sample at λ= 254 nm was measured by UV-Vis spectroscopy at 22 ^{o}C. The experiments were done in triplicate and the absorbance at λ= 254 nm at 22 ^{o}C was plotted against the concentration of G analog (g/L) to give working curves and for determining the water solubility of G analogs **1-6** (see Figure S3 for molar extinction coefficients).

Solubility determination: To separate vials 10 mg of each G analog **1-5** was added to 1 mL of deionized water (for highly soluble GMP **6**, 90 mg was added to 1 mL of water). The mixtures were sonicated and stirred at 22 ^{o}C for 1 h before ultracentrifugation was done to remove insoluble particles. Then, 10 µL of the supernatant was diluted with 3 mL of DI water (for GMP **6**, 10 µL of supernatant was diluted with 1 mL of water, and 10 µL of that new stock solution was diluted with 3 more mL of water). The absorbance at λ= 254 nm at 22 ^{o}C was then measured by UV spectroscopy for all final diluted solutions of **1-6**. All experiments were done in triplicate and the absorbance at λ= 254 nm was used to calculate the concentration of each saturated solution of analogs **1-6**.



	ε (L·mol^{-1}·cm^{-1})
NHOHG **5**	12364.96
NH_2 G **4**	11931.98
G **1**	12591.43
LG **3**	11354.45
HG **2**	11920.04
GMP **6**	11730.19

Figure S3: (left) Plots of A_{254} values vs. concentration for determining water solubility of each G derivative **1-6**. (right) The molar extinction coefficient for G analogs **1-6**.

Procedures for Preparation and Characterization of the HG 2•KCl Hydrogel

General Gel Preparation Procedure: A weighed amount of guanosine derivatives G **1**-**6** was added to deionized water. The vial was sonicated until any large insoluble particles were broken up. The resulting suspension was then heated with a heat-gun until a clear solution was obtained. The vial was removed from the heat and the appropriate amount of KCl or other salt solution was immediately added. The resulting mixture was then vigorously shaken and heated to a boil again. The mixture was then allowed to cool at room temperature. Experiments, measurements observations on the resulting hydrogels were typically done 1 day after preparation, unless otherwise noted.

While preparing the hydrogels, we found that GMP **6** was easily dissolved, even at rt, whereas extended heating at high temperatures (~ 100 ^{o}C) was required to dissolve G **1**, NH_2G **4** and NHOHG **5**. For HG **2** and LG **3**, milder heating (60-80 ^{o}C) was needed to obtain a clear solution. As shown below in Fig. S4, the critical gelation concentration for the HG **2**•KCl gel with 0.5 eq KCl is between 0.8 wt % 27 mM) and 0.9 wt % (30 mM).

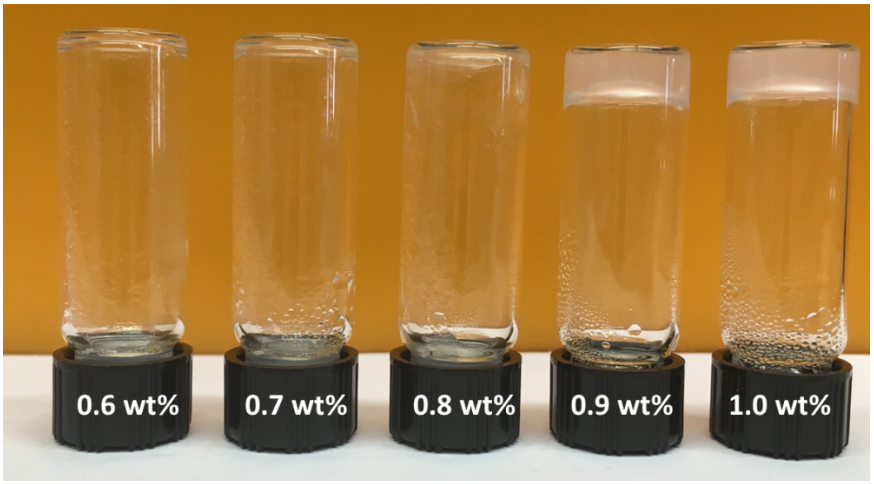


Figure S4: The critical gelation concentration for the gel with 0.5 eq KCl is between 0.8 wt % and 0.9 wt %. Picture was taken 1 day after preparation of the hydrogels.

Powder X-ray Diffraction Procedure: A 1 wt % HG **2**•KCl hydrogel (34 mM HG **2**, 17 mM KCl) was prepared using the general gel preparation procedure. After sitting for 1 day at rt the hydrogel sample was lyophilized to give a white powder. X-ray powder diffraction measurements were performed with a Cu radiation source at 20 °C using a Bruker D8 Advance Bragg-Brentano diffractometer equipped with a LynxEye detector. Data are shown in Figure 4A of the paper.

Infrared Spectroscopy Procedure: A 1 wt % HG **2**•KCl hydrogel (34 mM HG **2**, 17 mM KCl) was prepared using the general gel preparation procedure. After sitting for 1 day at rt the hydrogel sample was lyophilized to give a white powder. Infrared spectroscopy was done using a Thermo Nicolet Nexus 670 FT-IR with ATR module at room temperature. Spectra were recorded for both the lyophilized HG **2**•KCl hydrogel and for "monomeric" HG **2** in the absence of any added KCl. Spectra for the 2 samples are shown in Figure 4B of the paper.

Circular Dichroism Procedure: A 2 wt % HG **2**•KCl hydrogel (68 mM HG **2**, 1 equiv KCl) was prepared using the general gel preparation procedure. The CD spectra were recorded at 25 °C with a Hellma 106-QS quartz cell with an optical path length of 0.01mm (scanning speed 200 nm/min; response time 2 seconds). The experiment was repeated at least 3 times, and the curves were smoothed and averaged. The spectra are shown in Figure 4C of the paper.

SEM Procedure: A 2 wt % HG **2**•KCl hydrogel (68 mM, 0.5 eq KCl) was prepared using the general gel procedure. A small amount of the hydrogel was loaded onto an AL holder after which 0.3 µL of 3% ionic liquid (HILEM 1000 for Hitachi EM) was pipetted on top of the gel sample. The sample was allowed to equilibrate overnight at room temperature before SEM images were taken. A representative SEM images is shown in Figure 4D of the paper.

Rheology Procedure: Gels were made following the general procedure. Experiments were performed on an AR2000 stress-controlled rheometer from TA Instruments at 20 °C with a 20 mm diameter parallel plate geometry. Strain sweeps of 2 wt % HG **2**•KCl hydrogel (68 mM) made with different amounts of KCl were taken at a constant angular frequency of 10 rad/s 1 day after gel preparation. Rheology data is shown in Figure 5 of the paper.

The *in situ* formation of self-assembled colloidal suspensions by HG 2•KCl and use for separation of anionic dye Naphthol Blue Black.

Procedure for *in situ* generation of self-assembled colloidal suspensions by HG 2•KCl*:* G **1** or HG **2** was added to deionized water to give a suspension containing the nucleoside at a concentration of 10 mg/mL. The mixture was sonicated, stirred for 1 h at rt and ultracentrifuged to remove insoluble particles. The clear supernatant contained a saturated solution of either G **1** (2.17 mM) or HG **2** (14.53 mM). Then, varying amounts of a KCl stock solution was added to the saturated solution of G **1** or HG **2** and the solution was well mixed. Unless otherwise noted, all observations or experiments were performed 1 hour after KCl addition. As shown below in Fig. S5 a milky white precipitate formed when 100 mM KCl was added to HG **2**.

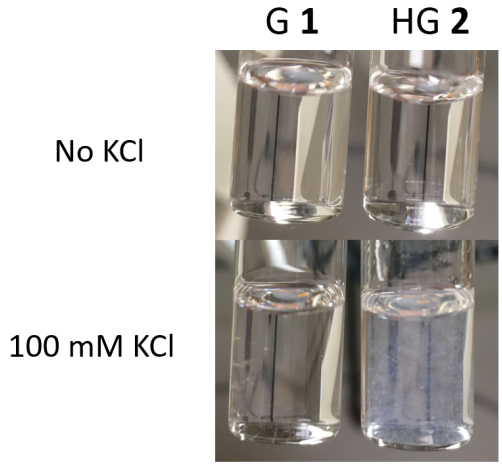


Figure S5: Addition of KCl (to give a final KCl concentration of 100 mM) into a saturated solution of HG **2** (14.53 mM) generated milky white aggregates (lower right photo), whereas a saturated solution of G **1** remained clear in the presence of the same concentration of KCl.

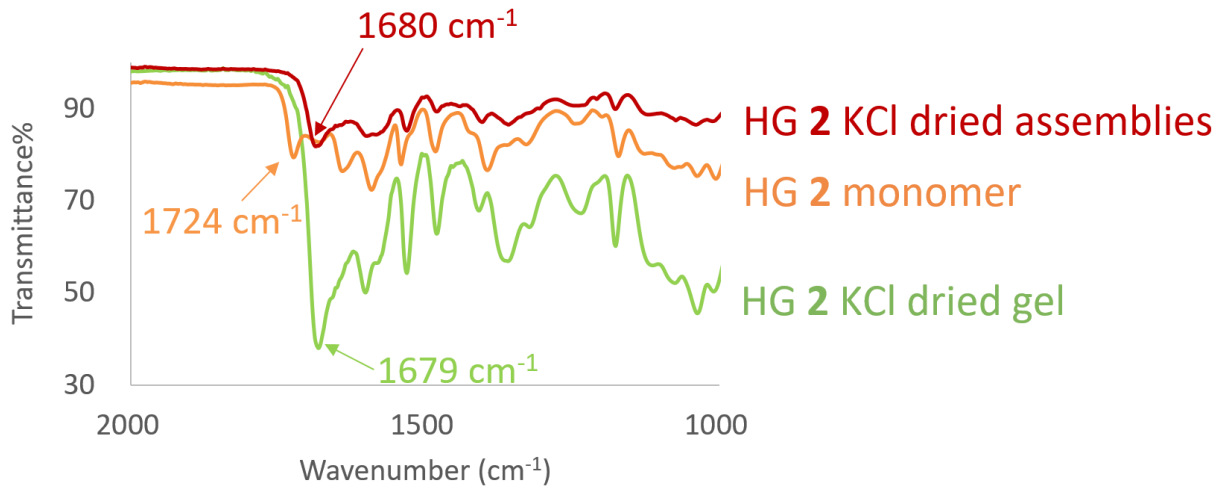


Figure S6: IR spectra show that the carbonyl wavenumber (red spectrum, 1680 cm^{-1}) of the freeze-dried assemblies colloidal assemblies made from addition of KCl to a saturated solitiuon of HG **2** is similar to that for a dried powder from a 1 wt % HG **2**•KCl hydrogel (34 mM HG **2**, 17 mM KCl) (green spectrum, 1679 cm^{-1}).

Procedure for UV Quantification of Precipitation of Nucleoside by KCl: Each vial depicted in Figure S7 was charged with 1 mL of a saturated solution of G **1** (2.17 mM) or HG **2** (14.53 mM), and a specified amount of a 4 M KCl stock solution was added to each vial. The resulting solutions were mixed well and allowed to sit at rt for 1 h. Then, the vials were ultracentrifuged to remove any insoluble particles and 10 µL of supernatant was pipetted into a vial containing 3 mL of deionized water. The sample was well-mixed and the amount of G species in solution was quantified using UV spectroscopy by measuring the guanine absorbance at λ= 254 nm . These A_{254} measurements are plotted below in Figure S7B.

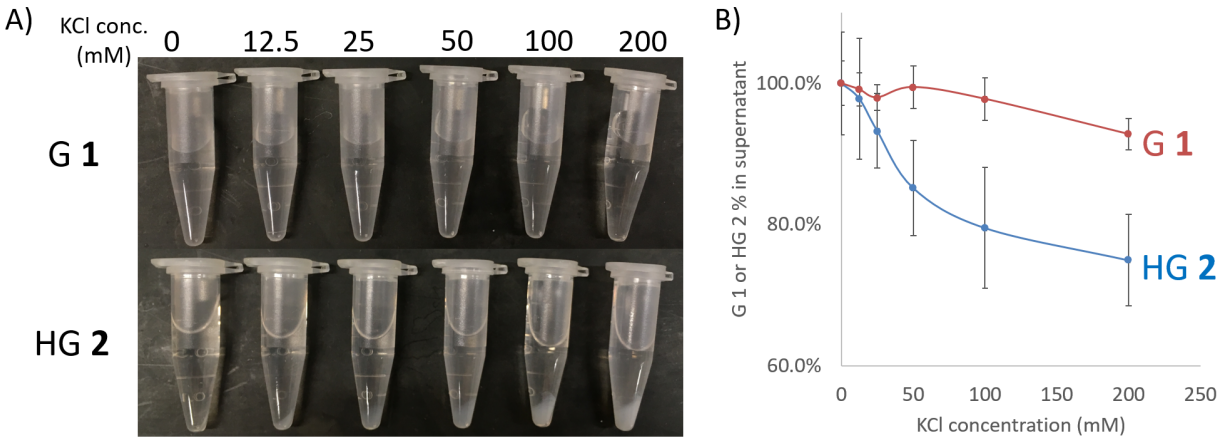


Figure S7: A) Photos showing that addition of higher concentrations of KCl generates more solid phase on the bottom of the vials for HG **2**, whereas G **1** shows no obvious precipitation. B) UV-Vis quantification of G concentration in the supernatant decreases more for HG **2** with increasing addition of KCl, indicating that HG **2** is more effective generation of assemblies than G **1** (at 200 mM KCl: 93% G **1** vs 75% HG **2** in supernatant).

Qualitative Dye Binding Procedure: Two different HG **2**•KCl hydrogel (2 wt %, 68 mM, 2 eq KCl) were prepared, one containing the cationic dye CV and one containing the anionic dye NBB. While the solution of HG **2**•KCl was still hot, 200 μM final concentration of CV or 200 μM of NBB dye solution was added to each solution and the vials were shaken. Then the solutions were immediately transferred to a mold made from parafilm and gels were allowed to form. After cooling for 1 hour at rt, the gel cubes were placed into a vial containing 5 mL of 155 mM KCl solution. The vials were gently shaken after 1 hour and the pictures shown in Figure S8 were taken.

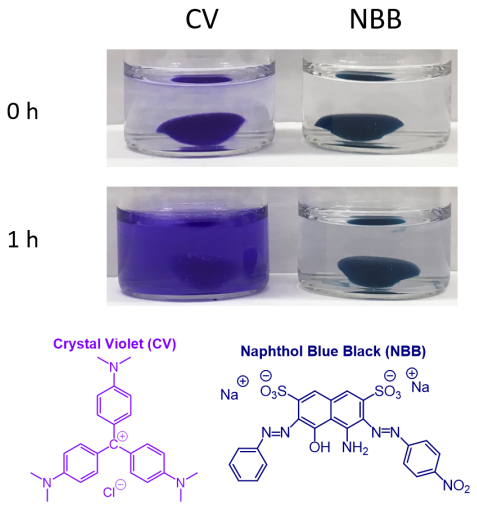


Figure S8: The HG **2**•KCl hydrogel (2 wt %, 68 mM, 2 eq KCl) binds anionic NBB while releasing cationic CV into 155 mM KCl. The selective binding indicates that the cationic G_4•K^+ hydrogel is selective for electrostatic binding of anions vs. cationic dyes.

Set up and Characterization of propionaldehyde uptake experiments

Procedure for gas-phase uptake of propionaldehyde: The small vial (no cap) containing 0.5 mL of a hydrogel made from HG **2**•KCl (2 wt %, 68 mM, 0.5 eq KCl) was placed in the large vial, and a 1.0 equiv of neat propionaldehyde was pipetted into the outside vial. The large vial was immediately capped and sealed with parafilm. The small vial was taken out 1 day later and lyophilized to give a white powder, which was dissolved in 0.6 mL of DMSO-d_6 and the reaction products were analyzed by ^{1}H and ^{13}C NMR.

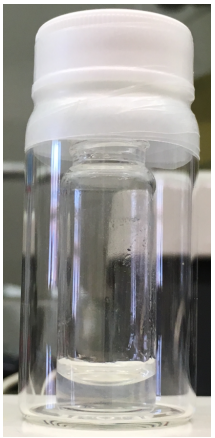


Figure S9: Experimental setup for gas phase uptake of propionaldehyde by HG **2**•KCl hydrogel.

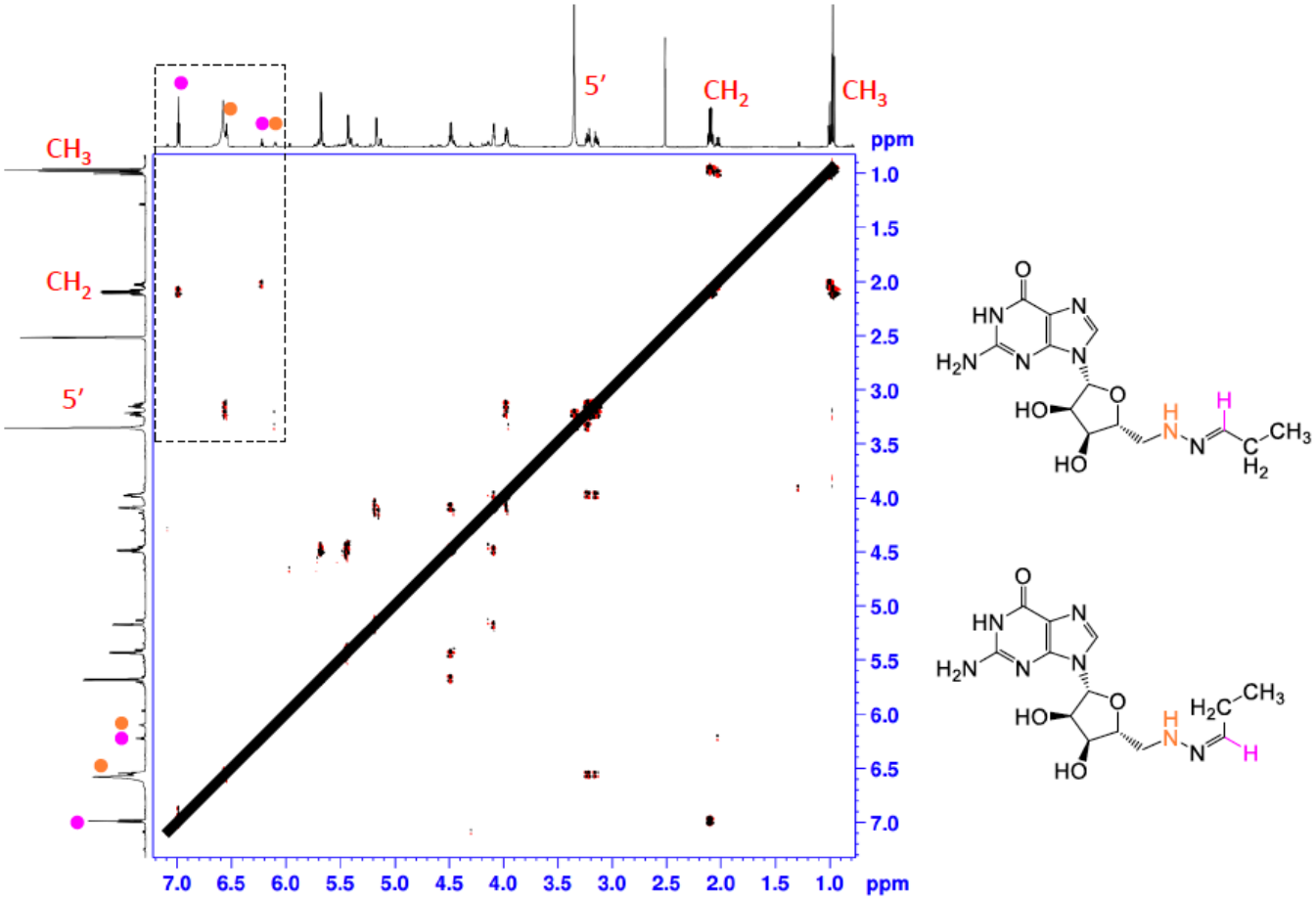


Figure S10. ^{1}H-^{1}H COSY 2D NMR showing assignments for hydrazone products trans-PG **7** (major) and cis-PG **7** (minor) from the gas phase reaction of HG **2**•KCl hydrogel with propionaldehyde. The box highlights ^{3}J coupling correlations: NH (orange) and 5′-CH_2 correlation; imino protons (pink) and CH_2 (originally from propionaldehyde) correlation.

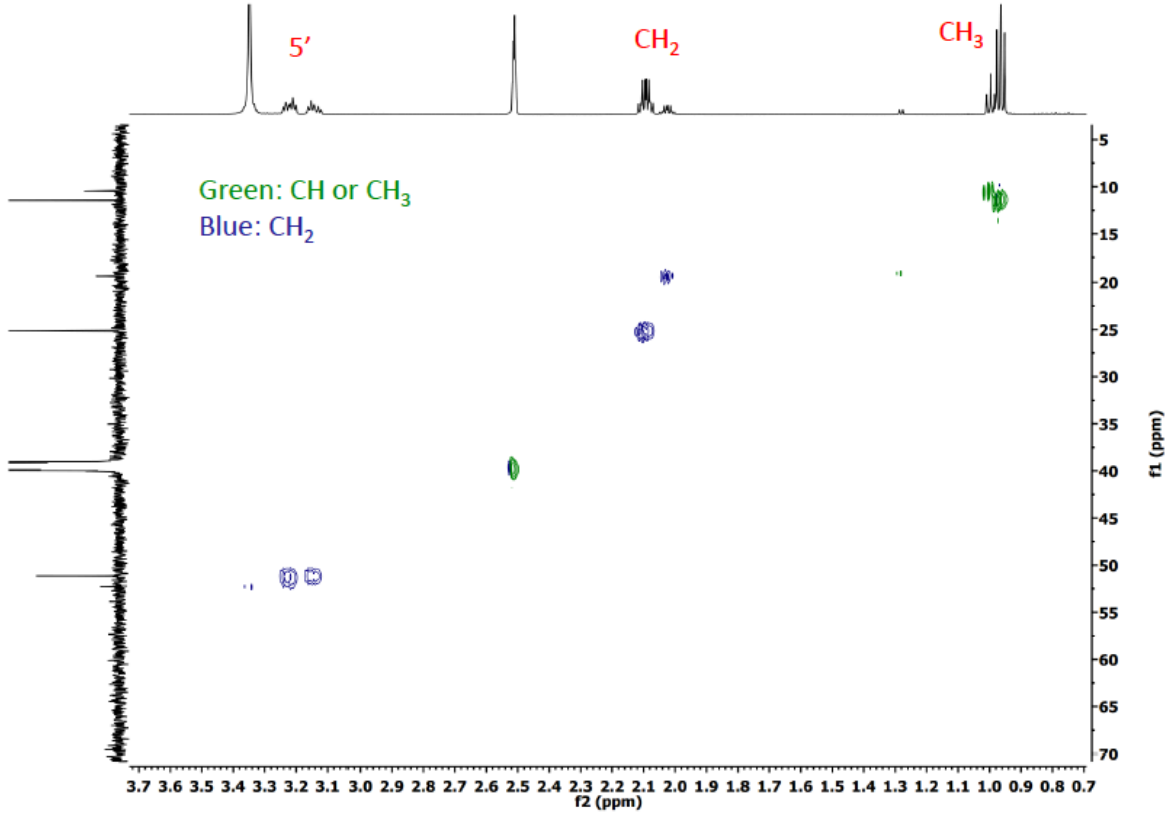


Figure S11. ^{1}H-^{13}C HSQC 2D NMR showing 2 sets of correlations for the hydrazone products trans-PG **7** (major) and cis-PG **7** (minor) from the gas phase reaction of HG **2**•KCl hydrogel with propionaldehyde.

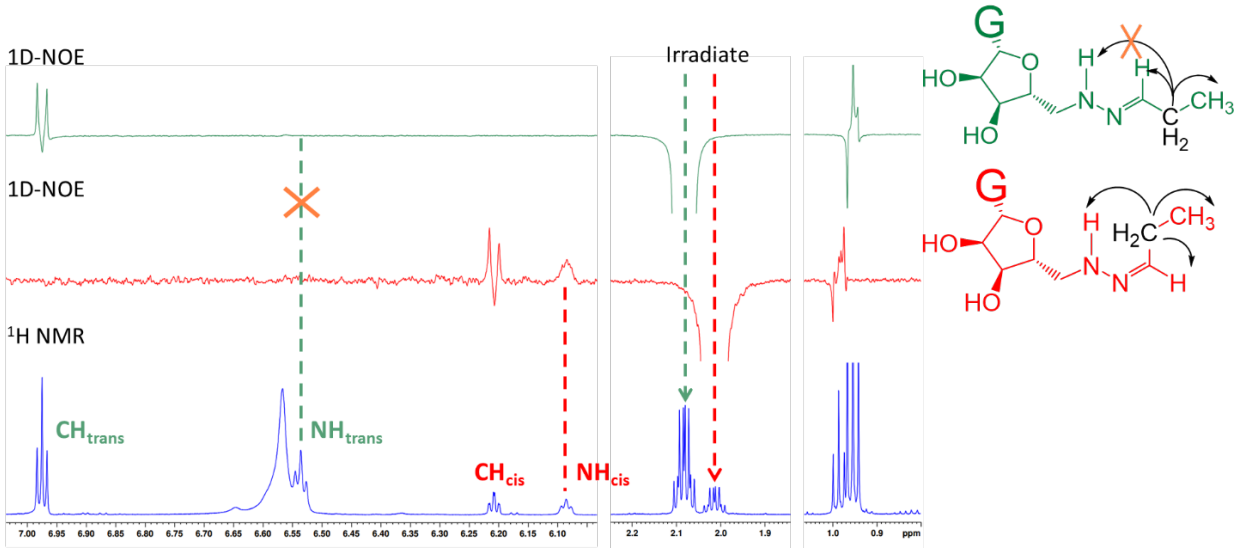


Figure S12. 1D NOE experiments that confirm assignment of hydrazone products trans-PG **7** (green structure) as the major product and cis-PG **7** (red structure) as the minor product from the gas phase reaction of HG **2**•KCl hydrogel with propionaldehyde.

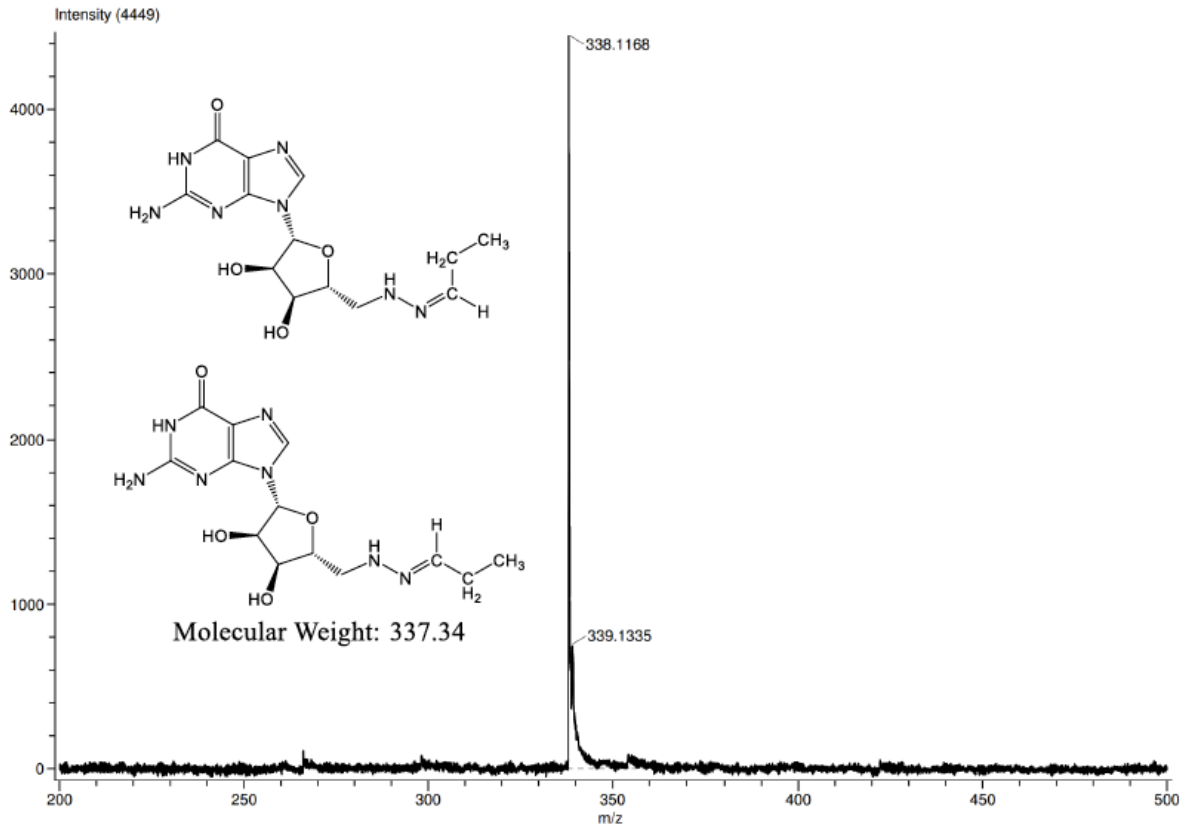


Figure S13. ESI-MS analysis of products from the gas phase reaction of HG **2**•KCl hydrogel with propionaldehyde.

References

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3 D. K. Dean, *Synth. Commun.*, 2002, **32**, 1517–1521.