SUPPORTING INFORMATION

A G₄•K⁺ hydrogel made from 5ʹ-hydrazinoguanosine for remediation of α,β-unsaturated carbonyls

Songjun Xiao and Jeffery T. Davis*

Department of Chemistry and Biochemistry
University of Maryland
College Park, MD 20742 USA
Email: jdavis@umd.edu

Table of Contents

General Experimental 3
General Procedure for Preparation of G₄•K⁺ Hydrogels 3
Procedure for Gas Phase Uptake of α,β-unsaturated Carbonyls by G₄•K⁺ Hydrogels 4
Figure S1 The Experimental Setup for Gas Phase Uptake 4
Section on Acrolein (AC) Remediation pages 5-15
Preparation and NMR data of 5ʹ-deoxy-5ʹ-pyrazolinoguanosine 4
Figure S2 ¹H NMR Spectrum of 4 5
Figure S3 ¹³C NMR Spectrum of 4 6
Figure S4 ¹H-¹³C HSQC Spectrum of 4 7
Figure S5 ¹H-¹H COSY Spectrum of 4 8
Figure S6 ESI-MS of 4 9
Figure S7 ¹H NMR Spectrum of Adducts between 2 and AC 10
Figure S8 ¹H-¹³C HSQC Spectrum of Adducts between 2 and AC 11
Figure S9 ¹H-¹H COSY Spectrum of Adducts between 2 and AC 12
Figure S10 ESI-MS of Adducts between 2 and AC
Procedure for Aqueous Phase Uptake of AC by G₄•K⁺ Hydrogels
Procedure for AC Binding with 3•KCl Hydrogel in Aqueous Phase
Figure S11 AC Binding with 3•KCl Hydrogel

Section on Methyl Vinyl Ketone (MVK) Uptake

Figure S12. A) Pyrazoline 5 from Michael addition/cyclization of MVK with hydrazine 3 G₄•K⁺ hydrogel. B) % MVK (3.37 mM) in 5 mL solution of 155 mM KCl in D₂O, pD 6.3 after addition of hydrogels (0.5 mL, 68 mM) made from 1-3.
NMR Data of 5ʹ-deoxy-5ʹ-methylpyrazolinoguanosine 5

Figure S13 ¹H NMR Spectrum of 5
Figure S14 ¹³C NMR Spectrum of 5
Figure S15 ¹H-¹³C HSQC Spectrum of 5
Figure S16 ¹H-¹H COSY Spectrum of 5
Figure S17 ESI-MS of 5

Figure S18 ESI-MS of Adducts between 2 and MVK
Procedure for Aqueous Phase Uptake of MVK by G₄•K⁺ Hydrogels
Procedure for MVK Binding with 3•KCl Hydrogel in Aqueous Phase
Figure S19 MVK Binding with 3•KCl Hydrogel

Section on Methyl Acrylate (MA) Uptake

Figure S20 ¹H-¹³C HSQC Spectrum of Adducts between 3 and MA
Figure S21 Proposed Product Distribution of 1:1 Adducts between 3 and MA
Figure S22 ESI-MS of Adduct between 3 and MA
Figure S23 ¹H NMR Spectrum of 2•KCl Hydrogel after MA Uptake
Figure S24 ESI-MS of 2•KCl Hydrogel after MA Uptake

Procedure for Aqueous Phase Uptake of MA by G₄•K⁺ Hydrogels

References
**General Experimental:** Guanosine 1 was purchased from Sigma-Aldrich. The 5′-hydrazido guanosine 2, and the 5′-hydrazinoguanosine 3, were synthesized according to literature procedures and their 1H and 13C NMR spectra matched published results. 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. ESI-MS experiments were done with a JEOL AccuTOF spectrometer. Chemicals and solvents were purchased from Acros, Aldrich, Alfa Aesar, Fisher, Carbosynth, or Santa Cruz Biotechnology.

**General Procedure for Preparation of G₄•K⁺ hydrogels:** The desired amount of guanosine derivatives 2 or 3 was weighed into a vial and the appropriate amount of deionized water was added (e.g. 2 wt% hydrazine 3 (68 mM), 0.5 eq KCl (34 mM)). The resulting suspension was sonicated until large aggregates were broken up to give a fine suspension. The suspension was then heated to boil with a heat gun until a clear solution resulted. The vial was removed from the heat and the appropriate amount of KCl was immediately added to the hot solution. The mixture was then vigorously shaken and heated to boil again. The mixture was then allowed to cool at room temperature.
Procedure for gas phase uptake of $\alpha,\beta$-unsaturated carbonyls by G$_4$•K$^+$ hydrogels: A small vial, without a cap, containing 0.5 mL of a G$_4$•K$^+$ hydrogel made from either 5’-hydrazidoguanosine 2 or 5’-hydrazinoguanosine 3 (2 wt%, 0.5 eq KCl) was placed inside the larger vial, and 1.0 equiv of neat $\alpha,\beta$-UC (either AC, MVK or MA) was added to the outside, larger vial (see Fig. S1). The large vial was immediately capped and sealed with parafilm and the reaction mixture was allowed to stand at RT. The small vial was removed after 2 days, the hydrogel was lyophilized to give a white powder, which was dissolved in 0.6 mL of DMSO-d$_6$ and analyzed by $^1$H and $^{13}$C NMR and by ESI-MS.

Figure S1: The experimental setup for gas phase uptake of $\alpha,\beta$-UC by G$_4$•K$^+$ hydrogels.
Section on Acrolein (AC) Uptake

Preparation of 5'-deoxy-5'-pyrazolinoguanosine (4).

5'-deoxy-5'-pyrazolinoguanosine 4. Using the experimental protocol for gas phase uptake described above, pyrazoline 4 was prepared from AC and 5'-deoxy-5'-hydrazinoguanosine 3. The lyophilized hydrogel, a white powder containing 4, was analyzed by $^1$H and $^{13}$C NMR and by ESI-MS. $^1$H NMR (DMSO-d$_6$) $\delta$: 2.51-2.56 (2 H, m, CH$_2$), 2.84-2.98 (2 H, m, CH$_2$), 3.02-3.08 (1 H, dd, 5'-CH$_2$, J=6.5 Hz, J=6.7 Hz), 3.21-3.26 (1 H, dd, 5''-CH$_2$, J=8.3 Hz, J=5.0 Hz), 4.07 (1 H, m, 4'-CH), 4.12 (1 H, m, 3'-CH), 4.50 (1 H, m, 2'-CH), 5.22 (1 H, d, J=5.0 Hz, 3'-OH), 5.45 (1 H, d, J=6.0 Hz, 2'-OH), 5.68 (1 H, d, J=6.0 Hz, 1'-CH), 6.61 (2 H, s, NH$_2$), 6.81 (1 H, t, J=1.6 Hz, CH), 7.91 (1 H, s, 8-CH), 10.77 (1 H, s, br, N1H); $^{13}$C NMR (DMSO-d$_6$) $\delta$: 33.90 (CH$_2$), 55.23 (CH$_2$), 58.36 (C5'), 71.55 (C4'), 72.73 (C2'), 82.51 (C3'), 86.35 (C1'), 116.69 (C5), 135.60 (C8), 142.81 (C3), 151.39 (C4), 153.75 (C2), 156.66 (C6); ESI-MS m/z=335.98 (MW of 4: 335.32)

Figure S2: $^1$H NMR spectrum of pyrazoline 4
Figure S3: The $^{13}$C NMR spectrum for pyrazoline 4 shows 13 peaks, consistent with the formation of an adduct between 5'-hydrazinoguanosine 3 and acrolein.
Figure S4: $^1$H-$^{13}$C HSQC spectrum of 4. NMR signals for the CH$_2$ and CH groups in the 5'-pyrazoline ring are indicated by colored dots (pink, orange and red).
Figure S5: $^1$H-$^1$H COSY spectrum of 4. The signal for the CH$_2$ group at δ 2.51 ppm (orange dot) has correlation peaks with both the triplet at δ 6.81 ppm (blue dot) and the multiplet CH$_2$ at δ 2.90 ppm (pink dot). Combining this data with the HSQC spectrum in Figure S4, a CH$_2$-CH$_2$-CH linkage can be identified in pyrazoline 4.
**Mass Spectrometry Procedure:** 0.1 mL DMSO-$d_6$ solution of the freeze-dried hydrazine 2 or hydrazide 3 hydrogel (2 wt%, 0.5 eq KCl) after uptake of electrophile was diluted with 0.5 mL H$_2$O and then injected into the JEOL AccuTOF-CS Spectrometer.

**Figure S6:** ESI-MS of pyrazoline 4. No peak corresponding to the precursor hydrazine 3 (mw =297.28) was detected.
Figure S7: Top: A region of the $^1$H NMR spectrum of reaction products formed by gas phase uptake of 1.0 eq of AC by the G₄•K⁺ hydrogel made from 5′-hydrazidoguanosine 2 (2 wt%, 65 mM, 0.5 eq KCl) shows formation of a cis/trans mixture of acylhydrazones 8 and some cyclic adduct 9 (see also Figure S8). The region of the spectrum shown contains H₈ (pink) and the acylhydrazone CH (orange) in the acylhydrazones 8. Bottom: The spectrum of a sample obtained by performing a gas phase uptake of 1.0 eq of AC with a 2 wt% solution of 2 in DMSO-d₆ for 2 days at RT. This sample gives a cleaner spectrum that shows formation of only cis/trans hydrazones 8. All NMR signals were unambiguously assigned using 2D $^1$H, $^1$H-COSY and $^1$H, $^{13}$C-HSQC spectra (not shown).
Figure S8: $^1$H-$^{13}$C HSQC spectrum of reaction products formed by gas phase uptake of 1.0 eq of AC by the G$_4$•K$^+$ hydrogel made from 5'-hydrazidoguanosine 2 (2 wt%, 65 mM, 0.5 eq KCl) shows formation of a cis/trans mixture of acylhydrazones 8 and some cyclic adduct 9 (see also Figure S8). Blue crosspeaks represent CH$_2$ groups while green crosspeaks represent CH or CH$_3$ groups. Three CH$_2$ signals are identified: X and Y result from a CH$_2$-CH$_2$ linkage in the cyclic adduct 9 (a crosspeak is observed between X and Y in the $^1$H, $^1$H-COSY spectrum in Figure S9), while the crosspeak Z represents 2 different terminal alkenes, namely the acylhydrazones cis-8 and trans-8, which show 2 separate $^{13}$C signals peaks (f1) at $\delta \sim$126 ppm.
Figure S9: $^1$H-$^1$H COSY spectrum of reaction products formed by gas phase uptake of 1.0 eq of AC by the G$_4$•K$^+$ hydrogel made from 5’-hydrazidoguanosine 2 (2 wt%, 65 mM, 0.5 eq KCl) shows a CH$_2$-CH$_2$-CH linkage (yellow CH$_2$ has crosspeaks with both pink CH$_2$ and blue CH), indicating formation of cyclic acylhydrazone 9.
Figure S10: ESI-MS shows dehydration products formed between 1.0 eq of AC and the G₄·K⁺ hydrogel made from 5ʹ-hydrazidoguanosine 2 (2 wt%, 65 mM, 0.5 eq KCl). The major signal at m/z=350.07 is consistent with the structures of the 2 acyclic acylhydrazones (cis/trans-8) and the cyclic acylhydrazones 9. A small amount of unreacted hydrazide 3 is also found at m/z=312.13.
This following experiment shows that AC in the solution is taken up from the solution:

**Procedure for aqueous phase uptake of AC by G₄•K⁺ hydrogels:** Hydrogels made from either 5′-hydrazidoguanosine 2 or 5′-hydrazinoguanosine 3 (2 wt%, 0.5 eq KCl) in D₂O were prepared according to the general preparation method. The G₄•K⁺ hydrogel made from guanosine 1 (2 wt%, 72 mM) and KB(OH)₄ (0.5 equiv) was prepared according to literature procedure.³ After heating the mixtures to boiling we then added 0.5 mL of the hot solutions into a parafilm-lined mold and allowed the solution to cool at RT for 1 h. The resulting hydrogels were then placed into a vial containing 5 mL of 155 mM KCl in D₂O that contained AC (3.37 mM, overall 0.5 eq of AC relative to the amount of nucleoside 1-3 in the hydrogel). Aliquots of the solution (0.6 mL) were removed periodically into a small vial and 10 µL of a 200 mM internal standard (3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid sodium salt in D₂O) was added to each vial. These solutions were well mixed and transferred to NMR tubes after which ¹H NMR spectra were acquired (32 scans). The integration of the internal standard was set to 1.0 and the integration of the three olefin peaks in the AC (~ 6.5 ppm) was used for quantification. Experiments were done in triplicate. The amount of AC in solution at t hour was determined according to the following equation:

\[
\frac{\text{AC integration}(t)}{\text{AC integration}(0\ h)} \times 100\%
\]

This following experiment shows that AC in the solution is taken up into the 3•KCl hydrogel:
Procedure for AC Binding with 3•KCl Hydrogel in Aqueous Phase: 2 wt% hydrazine 3 (68 mM) hydrogels with 2.0 eq KCl were prepared according to the general gel preparation method in H₂O. 0.5 mL of the hot gels were then pipetted into a parafilm mold and allowed to cool at room temperature for 1 h. The hydrogels were then placed into a vial containing 5 mL of freshly prepared H₂O solution of AC (3.37 mM, overall 0.5 eq relative to the amount of hydrazine 3 in the gel) in 155 mM KCl. After 4 hours, the vial was gently shaken and the outside solution was removed. Then another 5 mL of 155 mM KCl solution was added to the vial in order to rinse the hydrogel. The solution was completely removed and the remaining hydrogel was lyophilized, dissolved in DMSO-d₆ and studied by ¹H NMR.

Figure S11: Acrolein reacts with the G₄•K⁺ hydrogel containing hydrazine 3 (2 wt%, 68 mM, 2 eq KCl) in aqueous solution by forming the cyclic pyrazoline 4. ¹H NMR of the lyophilized gel (middle) shows peaks for both pyrazoline 4 (top) and unreacted hydrazine 3 (bottom). This experiment shows that AC is taken up from the solution into the hydrogels.
Section on Methyl Vinyl Ketone (MVK) Uptake

Fig. S12. A) Pyrazoline 5 from Michael addition/cyclization of MVK with hydrazine 3 G4•K+ hydrogel. B) % MVK (3.37 mM) in 5 mL solution of 155 mM KCl in D2O, pD 6.3 after addition of hydrogels (0.5 mL, 68 mM) made from 1-3.
5'-deoxy-5'-methylpyrazolinoguanosine 5. $^1$H NMR (DMSO-d$_6$): $\delta$ 1.86 (3 H, s, CH$_3$), 2.45-2.50 (2 H, m, CH$_2$), 2.84-2.98 (2 H, m, CH$_2$), 2.91-2.98 (1 H, dd, 5'-CH$_2$), 3.10-3.16 (1 H, dd, 5''-CH$_2$, J=7.7 Hz, J=5.3 Hz), 4.05 (1 H, m, 4'-CH), 4.11 (1 H, m, 3'-CH), 4.50 (1 H, m, 2'-CH), 5.20 (1 H, d, J=5.0 Hz, 3'-OH), 5.43 (1 H, d, J=6.0 Hz, 2'-OH), 5.67 (1 H, d, J=6.0 Hz, 1'-CH), 6.60 (2 H, s, NH$_2$), 7.91 (1 H, s, 8-CH), 10.76 (1 H, s, br, N1H), $^{13}$C NMR (DMSO-d$_6$) $\delta$: 15.70, 36.61 (CH$_2$), 55.00 (CH$_2$), 58.84 (C5'), 71.61 (C4'), 72.74 (C2'), 82.59 (C3'), 86.23 (C1'), 116.68 (C5), 135.63 (C8), 151.44 (C4), 151.45 (C4), 153.75 (C2), 156.69 (C6); ESI-MS m/z=350.13 (MW of 5: 349.35)

Figure S13: $^1$H NMR spectrum of methylpyrazoline 5.
Figure S14: The $^{13}$C NMR spectrum for methylpyrazoline 5 shows 14 peaks, consistent with the formation of an adduct between 5'-hydrazinoguanosine 3 and MVK. Inset shows two overlapping peaks at $\delta$ 151.45.
Figure S15: $^1$H-$^{13}$C HSQC spectrum of methylpyrazoline 5. The blue crosspeaks represent CH$_2$ groups while green crosspeaks represent CH or CH$_3$ groups. NMR signals for the CH$_2$ groups in the 5'-pyrazoline ring are indicated by colored dots (pink and orange).
Figure S16: $^1$H-$^1$H COSY spectrum of methylpyrazoline 5. Combining this data with the HSQC spectrum in Figure S14, a CH$_2$-CH$_2$ linkage (pink and yellow) for the pyrazoline ring can be identified.
Figure S17: ESI-MS spectrum of methylpyrazoline 5. No peak corresponding to hydrazine 2 (MW=297.28) was detected.
Figure S18: ESI-MS of gas-phase uptake reaction between 1.0 eq of MVK and the G₄⁺K⁺ hydrogel containing hydrazide 2 (2 wt%, 65 mM, 0.5 eq KCl). Only peaks for unreacted 2 (m/z=312.13) and 1:2 Michael adduct (m/z=452.15) are observed. Signals for the 1:1 Michael adduct and 1:1 acylhydrazone adduct are not observed. A putative structure is shown for the 1:2 adduct. Addition of the first MVK molecule would likely increase the nucleophilicity of the terminal nitrogen, leading to a second Michael addition.
This following experiment shows that MVK in the solution is taken up from the solution:

**Procedure for aqueous phase uptake of MVK by G₄•K⁺ hydrogels:** Hydrogels made from either 5'-hydrazidoguanosine 2 or 5'-hydrazinoguanosine 3 (2 wt%, 0.5 eq KCl) in D₂O were prepared according to the general preparation method. The G₄•K⁺ hydrogel made from guanosine 1 (2 wt%, 72 mM) and KB(OH)₄ (0.5 equiv) was prepared according to literature procedure.³ After heating the mixtures to boiling we then added 0.5 mL of the hot solutions into a parafilm-lined mold and allowed the solution to cool at RT for 1 h. The resulting hydrogels were then placed into a vial containing 5 mL of 155 mM KCl in D₂O that contained MVK (3.37 mM, overall 0.5 eq of MVK relative to the amount of nucleoside 1-3 in the hydrogel). Aliquots of the solution (0.6 mL) were removed periodically into a small vial and 10 μL of a 200 mM internal standard (3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid sodium salt in D₂O) was added to each vial. These solutions were well mixed and transferred to NMR tubes after which ¹H NMR spectra were acquired (32 scans). The integration of the internal standard was set to 1.0 and the integration of the three olefin peaks in the MVK (~ 6.5 ppm) was used for quantification. Experiments were done in triplicate. The amount of MVK in solution at t hour was determined according to the following equation:

\[
\frac{\text{MVK integration}(t)}{\text{MVK integration}(0 \text{ h})} \times 100\%
\]
This following experiment shows that MVK in the solution is taken up into 3•KCl hydrogel:

**Procedure for MVK Binding with 3•KCl Hydrogel in Aqueous Phase:** 2 wt% hydrazine 3 (68 mM) hydrogels with 2.0 eq KCl were prepared according to the general gel preparation method in H2O. 0.5 mL of the hot gels were then pipetted into a parafilm mold and allowed to cool at room temperature for 1 h. The hydrogels were then placed into a vial containing 5 mL of freshly prepared H2O solution of MVK (3.37 mM, overall 0.5 eq relative to the amount of hydrazine 3 in the gel) in 155 mM KCl. After 4 hours, the vial was gently shaken and the outside solution was removed. Then another 5 mL of 155 mM KCl solution was added to the vial in order to rinse the hydrogel. The solution was completely removed and the remaining hydrogel was lyophilized, dissolved in DMSO-d6 and studied by 1H NMR.

![Figure S19: Methyl vinyl ketone (MVK) reacts with the G4•K+ hydrogel containing hydrazine 3 (2 wt%, 68 mM, 2 eq KCl) in aqueous solution by forming the cyclic pyrazoline 4. 1H NMR of the lyophilized gel (middle) shows peaks for both methylpyrazoline 5 (top) and unreacted hydrazine 3 (bottom).]
Figure S20: $^1$H-$^{13}$C HSQC spectrum of reaction products formed by gas phase uptake of 1.0 eq of MA by the G$_4$•K$^+$ hydrogel made from 5’-hydrazinoguanosine 3 (2 wt%, 68 mM, 0.5 eq KCl) shows that the Michael acceptor end of MA has all reacted to form 7. Blue crosspeaks represent CH$_2$ groups while green crosspeaks represent CH or CH$_3$ groups. In the pink region (from δ 3.7-7.5 ppm) where alkene protons are expected, no CH$_2$ crosspeaks for unreacted terminal alkene (-CH=CH$_2$) are found. Meanwhile, multiple CH$_2$ crosspeaks are identified in the blue region (from δ 2.0-3.7 ppm), indicating different sp$^3$ CH$_2$ groups in the resulting adducts. Also, the -OMe peaks around δ 3.6 ppm shows that cyclization must be a slower step than Michael addition. This result indicates that the Michael acceptor of MA is more electrophilic than the ester end (inset) toward hydrazine 3.
Figure S21: Proposed product distribution of 1:1 adduct between hydrazine 3 and MA. Due to the higher nucleophilicity of the alkylated nitrogen of hydrazine, the 1,1-dialkyl hydrazine product (in the box) is favored during absorption, which influences the regioselectivity of linear and cyclic products.
Figure S22: (left) ESI-MS spectra of hydrazine 3 hydrogel (2 wt%, 0.5 eq KCl) after 2 and 4 days of MA uptake (1.0 eq) from the gas phase at room temperature. (right) Structures of adducts formed in this reaction. MS shows that 1:1 acyclic adduct 7 (m/z=384.01) is slowly converted into cyclic adducts 6 (m/z=352.00), by losing a molecule of methanol (mw=32). Small amounts of 1:2 adducts, labeled A and B, were also observed (A: m/z=438.02; B: m/z=470.06).
Figure S23: $^1$H NMR shows no reaction of MA with hydrazide 2 gel (2 wt%, 0.5 eq KCl) after 2 days at RT. The gel was lyophilized and dissolved in DMSO-d$_6$. This result shows that hydrazide 2 hydrogel is far less reactive toward MA than it is toward AC or MVK.
**Figure S24:** ESI-MS shows no covalent adduct formation between hydrazide 2 hydrogel (2 wt%, 0.5 eq KCl) and MA in gas phase uptake experiments after 2 days at RT.
Procedure for aqueous phase uptake of MA by $G_3$•$K^+$ hydrogels: 2 wt% $G_3$ (68 mM) and $G_2$ (65 mM) hydrogels with 2.0 eq KCl were prepared according to the general gel preparation method in D$_2$O. 0.5 mL of the hot gels were then pipetted into a parafilm lined mold and allowed to cool at room temperature for 1 h. A MA solution in 155 mM D$_2$O solution without any hydrogels served as a blank control. The hydrogels were then placed into a vial containing 5.5 mL of freshly prepared D$_2$O solution of MA (3.37 mM, overall 0.5 eq relative to the amount of $G_3$ in the gel) in 155 mM KCl. Before the addition of hydrogels ($t=0$ h), 0.5 mL of MA solution was pipetted into a vial which was used for NMR experiments at $t=0$ h. After 24 h of incubation at room temperature, the vials were gently shaken before 0.5 mL of the outside solution (0.5 mL) were removed into a small vial and 10 µL of a 200 mM internal standard (3-(trimethylsilyl)propionic-2,2,3,3-d$_4$ acid sodium salt in D$_2$O) was added to each vial. The solutions were well mixed and transferred to NMR tubes after which $^1$H NMR spectra were acquired (16 scans). The integration of internal standard was set to 1.0 and the integration of three olefin peaks (~ 6.5 ppm) was used for quantification. Experiments were done in triplicates. The amount of MA in solution at 24 h was determined according to the following equation:

\[
\frac{\text{MA integration}(24 \text{ h})}{\text{MA integration}(0 \text{ h})} \times 100\%
\]

The % MA that disappeared in the blank is subtracted from samples containing $G_3$ and $G_2$ hydrogels.
References


