Electronic Supporting Information

Toward Potential New Supramolecular Tissue Engineering Scaffolds Based on Guanosine Derivatives

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Experimental

Materials. All synthetic materials were purchased from Fisher and Sigma-Aldrich and used without further purification except where noted. DMEM was purchased from Fisher (Hyclone SH30243.02) and to it was added 10% FBS (Hyclone SH3008803) and 1% Penicillin-Streptomycin (MP Biomedicals 1670249). The C166 and C166 GFP+ cells (mouse endothelial progenitor) were purchased from ATCC.org. Camptothecin was purchased from Sigma-Aldrich. For the MTT assay, the Promega CellTiter96 Non-Radioactive Cell Proliferation Assay (G4000) was utilized. For the apoptosis assay, the BD Pharmingen PE Annexin V Apoptosis Detection Kit I (559763) was utilized.

Synthesis of 8-Bromoguanosine (3, 8BrG).[1] In one flask, 3 mL of bromine was added to 300 mL of distilled water and stirred for approximately 15 minutes to dissolve. In another flask, 10 g of guanosine was suspended in 60 mL of distilled water and to it was added the bromine solution in 10-20 mL aliquots at a rate where the reaction was allowed to return to a colorless state between additions. Once the reaction remained a yellow color, the resulting solid was filtered and washed with 60 mL cold water and 30 mL cold acetone. The product was recrystallized from distilled water in 20 mL vials, where approximately ¼ of the vial was filled with product and the rest with water. Quantitative yield. ^{1}H NMR (DMSO-D_6): δ 10.78 (s, 1H, NH), 6.47 (s, 2H, NH_2), 5.66 (d, 1H, CH), 5.42 (d, 1H, OH), 5.06 (d, 1H, OH), 4.98 (t, 1H, OH), 4.89 (m, 1H, CH), 4.11 (m, 1H, CH), 3.83 (m, 1H, CH), 3.62 (m, 1H, CH_2), 3.50 (m, 1H, CH_2). ^{13}C NMR (DMSO-D_6): δ 156.1, 154.2, 152.7, 121.8, 118.2, 90.3, 86.5, 71.2, 71, 62.7.

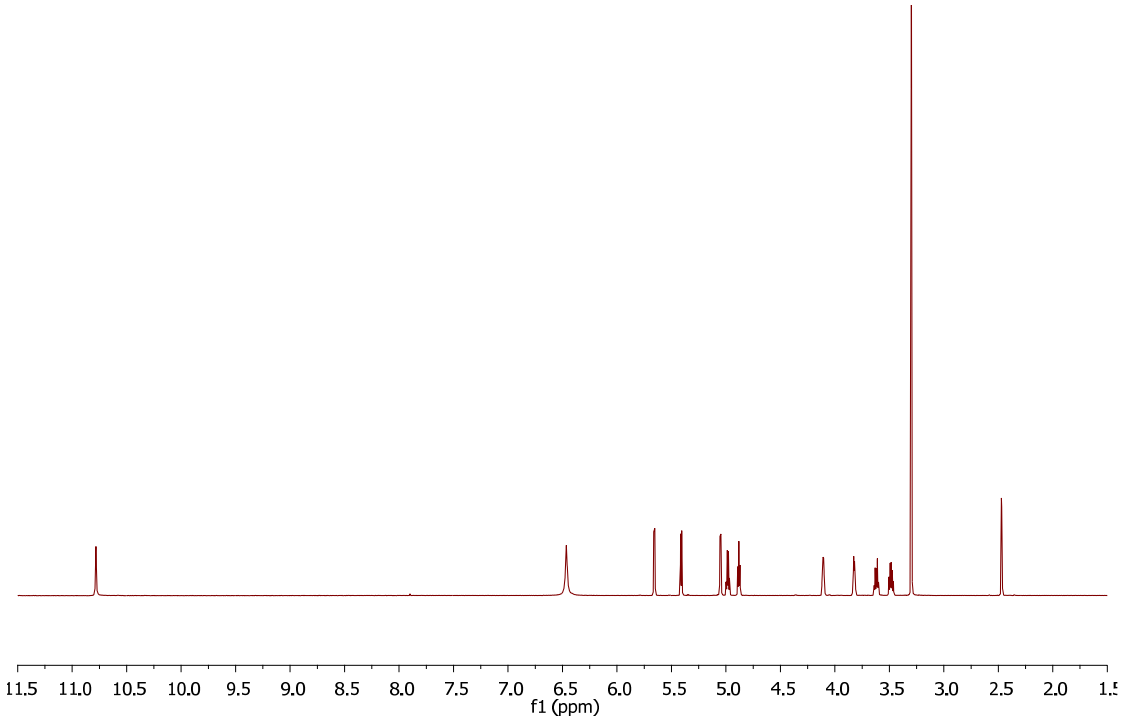

Figure S1. ^{1}H NMR of 8-bromoguanosine (**3**) in DMSO.

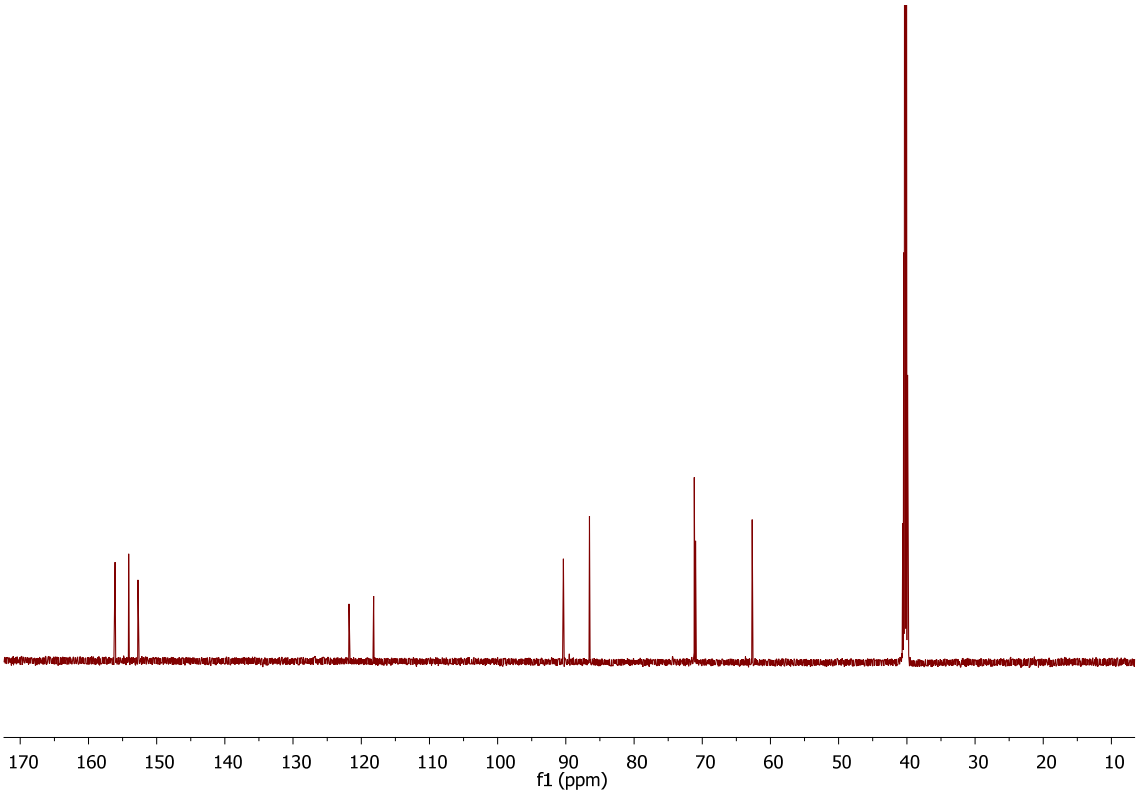

Figure S2. ^{13}C NMR of 8-bromoguanosine (**3**) in DMSO.

Synthesis of 8-Methoxyguanosine (4, 8OMeG).[1] 730 mg 8-bromoguanosine was placed in a flask and purged with argon. 15 mL DMSO was added, and the mixture was stirred until the 8-bromoguanosine dissolved. A suspension of 900 mg sodium methoxide in 8 mL of methanol was added to the reaction, and the reaction was allowed to proceed for 18 hours at 65 °C under argon. The reaction was then cooled to room temperature and neutralized with approximately 1.5 mL of acetic acid. The neutralized solution was added dropwise to 400 mL of diethyl ether, and the resulting precipitate was collected by filtration and washed with cold acetone. After drying, the product was recrystallized from distilled water in 20 mL vials, where approximately ¼ of the vial was filled with product and the rest with water. 67% yield. ^{1}H NMR (DMSO-D_6): δ 10.53 (s, 1H, NH), 6.27 (s, 2H, NH_2), 5.57 (d, 1H, CH), 5.28 (d, 1H, OH), 4.98 (d, 1H, OH), 4.82 (t, 1H, OH), 4.69 (m, 1H, CH), 4.02 (m, 1H, CH), 3.93 (s, 3H, OCH_3), 3.75 (m, 1H, CH), 3.53 (m, 1H, CH_2), 3.42 (m, 1H, CH_2). ^{13}C NMR (DMSO-D_6): δ 156.3, 153.7, 152.3, 151, 111.6, 86.7, 85.8, 71.2, 62.7, 57.1.

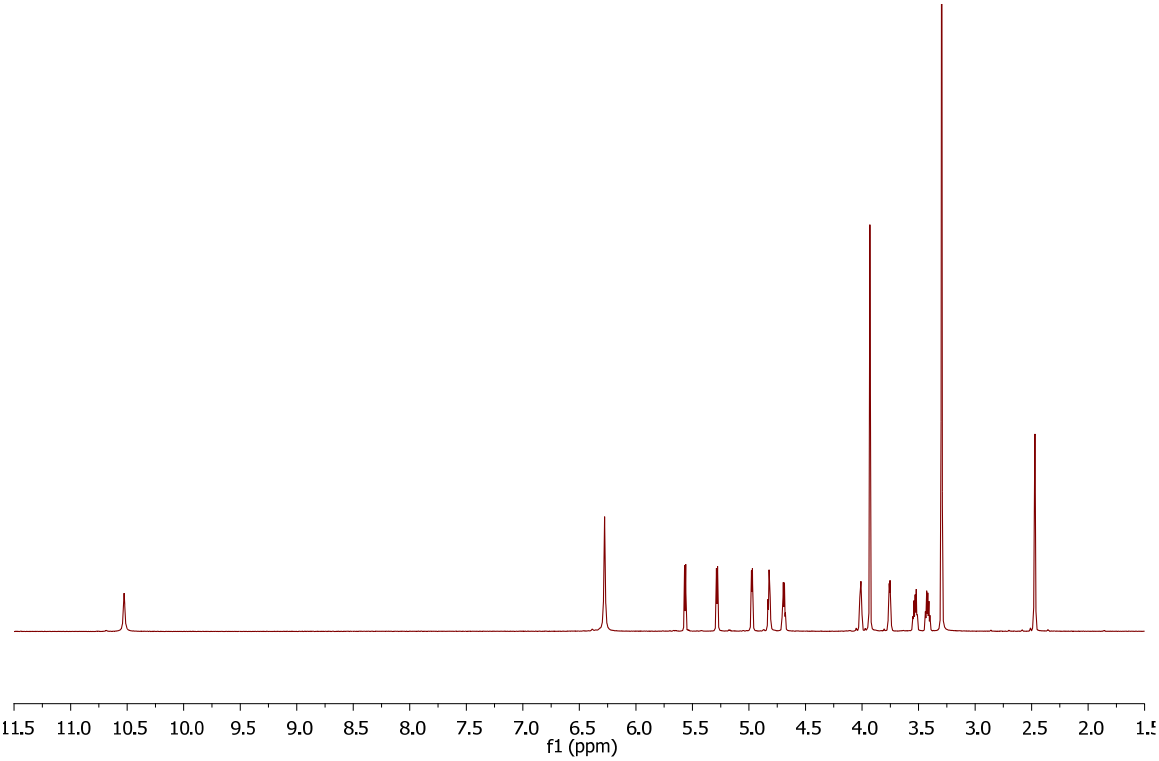

Figure S3. ^{1}H NMR of 8-methoxyguanosine (**4**) in DMSO.

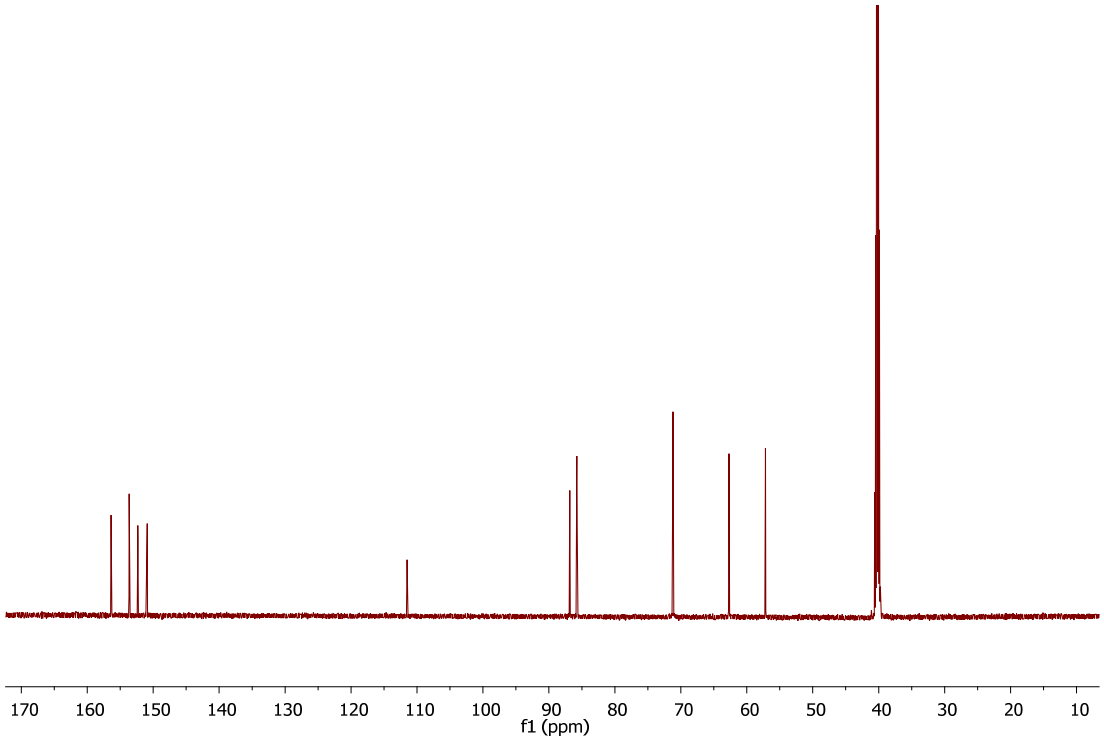

Figure S4. ^{13}C NMR of 8-methoxyguanosine (**4**) in DMSO.

Synthesis of 8-Methoxy-2',3',5'-Tri-O-Acetylguanosine (6, 8OMeTAcG).[2] 250 mg 8-methoxyguanosine and 10 mg 4-dimethylaminopyridine were placed in a flask and purged with argon. 10 mL of acetonitrile was added, followed by 668 µL of triethylamine. The mixture was cooled to 0 °C, and 236 µL of acetic anhydride was added. The reaction was allowed to proceed for 1 hour at 0 °C and 3 hours at room temperature, followed by quenching with 600 µL of methanol, and evaporation of the solvent. The residue was dried and recrystallized from approximately 10 mL of 2-propanol. 83% yield. ^{1}H NMR (DMSO-D_6): δ 10.6 (s, 1H, NH), 6.4 (s, 2H, NH_2), 5.86 (t, 1H, CH), 5.78 (d, 1H, CH), 5.46 (t, 1H, CH), 4.32 (m, 1H, CH), 4.23 (m, 1H, CH_2), 4.13 (m, 1H, CH_2), 3.97 (s, 3H, OCH_3), 2.07 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$).^{13}C NMR (DMSO-D_6): δ 170.7, 170, 156.3, 154, 151.5, 150.6, 112, 84.2, 79.3, 71.5, 70.6, 63.3, 57.6, 21.1, 21, 20.9. MALDI-MS (α-cyano/NaCl): 461.5 [M+Na].

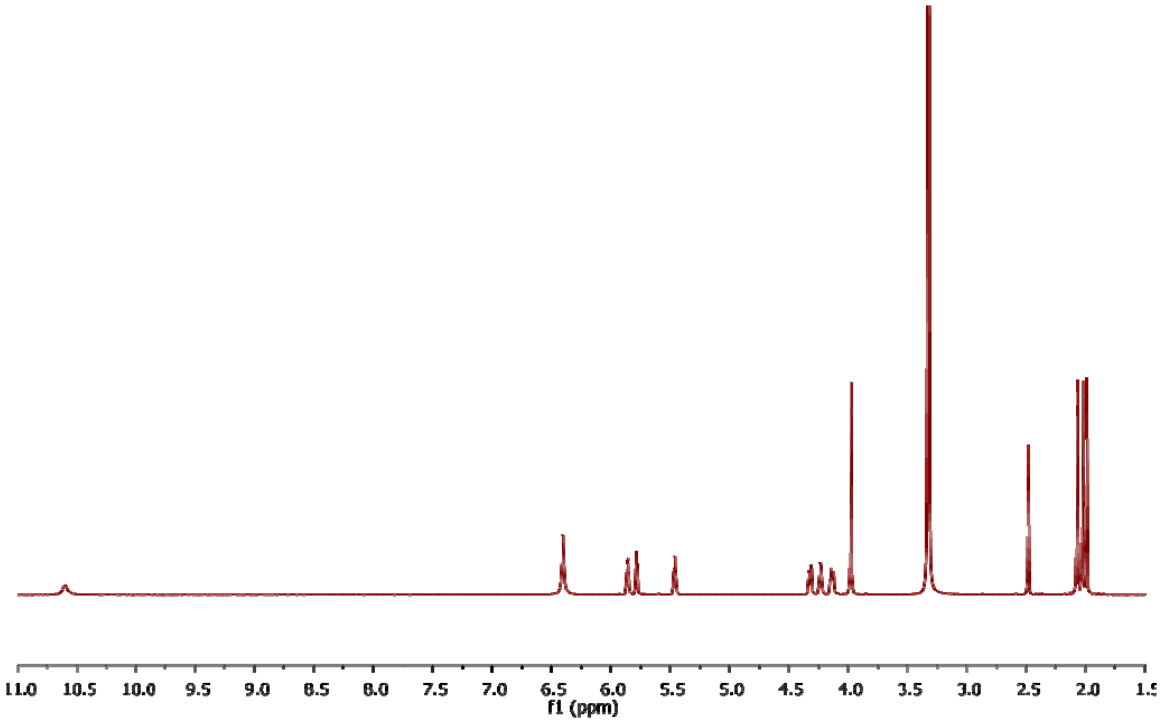

Figure S5. ^{1}H NMR spectrum of 8-Methoxy-2',3',5'-Tri-O-Acetylguanosine (**6**) in DMSO.

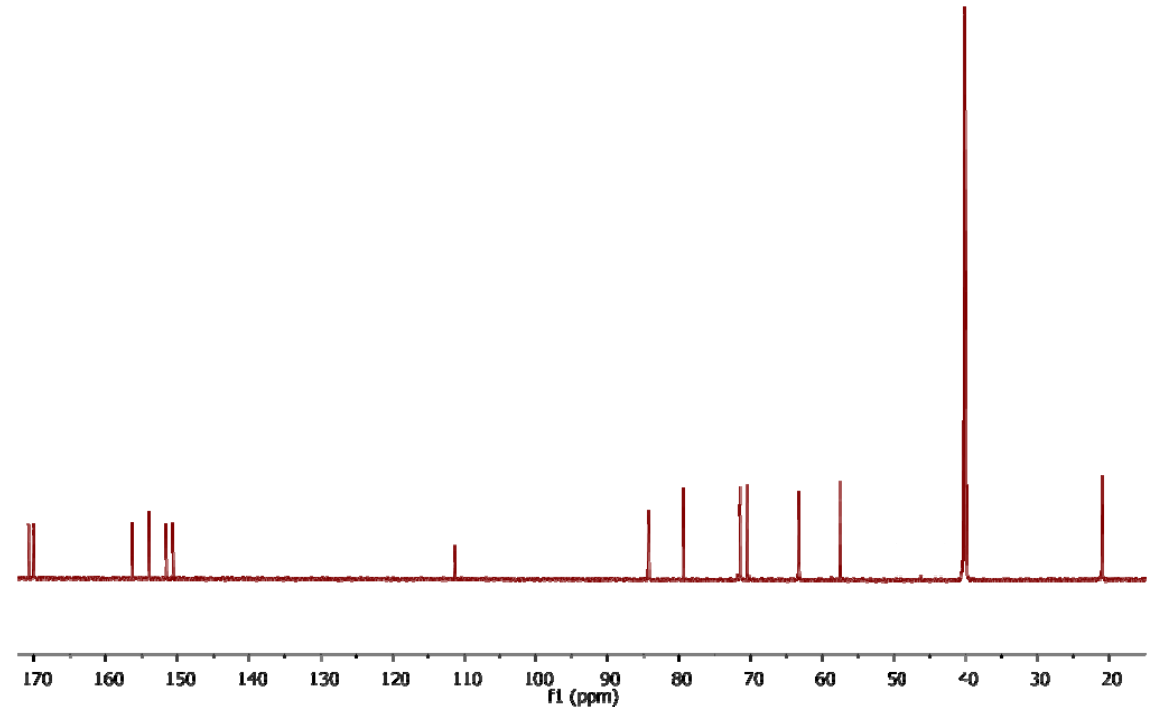

Figure S6. ^{13}C NMR spectrum of 8-Methoxy-2',3',5'-Tri-O-Acetylguanosine (**6**) in DMSO.

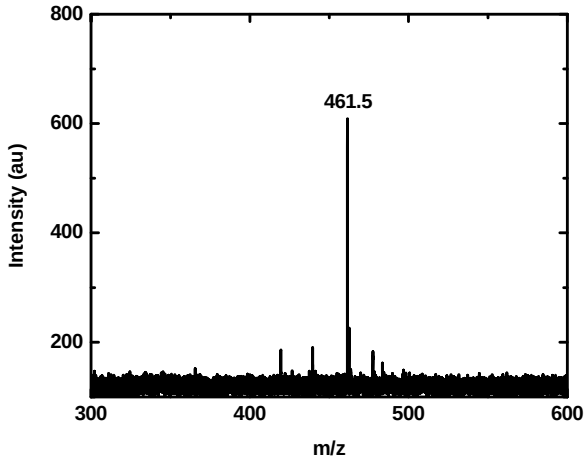

Figure S7. MALDI-MS (α-cyano/NaCl) of **8OMeTAcG** + 1Na+.

General procedure for testing of gelation ability. 20 mg of a molecule or molecules of interest was/were suspended in 1 mL of distilled water (or aqueous solution) in a 1 Dram vial. The suspension was heated to approximately 100 °C until dissolution was achieved (about one minute maximum). The sample was then allowed to cool to room temperature upon which gelation, and/or crystallization was observed. To make the gels with 1% gelatin for cell culture, a heated solution of 4 wt % 8OMeTAcG in DMEM was mixed with an equal volume of a heated 2 wt % solution of gelatin. The resulting solution was mixed via shaking over heat and allowed to cool.

Calculation of *syn*:*anti* ratio of 8OMeG. It has been shown literature that *syn*:*anti* conformer populations can be determined by the chemical shift of the ribose H-2' protons.[3] Under rapid exchange between these two conformations on the NMR timescale the observed chemical shift is the weighted mean of the chemical shift of the fixed *syn* (δ_{syn}) and fixed *anti* (δ_{anti})

Thus $\delta_{obs} = P_{syn}\delta_{syn} + P_{anti}\delta_{anti}$

where P_{syn} and P_{anti} are the equilibrium populations of the two conformations.

The chemical shift of the H-2' protons in the fixed *syn* and *anti* conformations were determined form model compounds[3] and from that data it was calculated that **8BrG**, with a chemical shift of the H2' = 4.89 ppm in d^6-DMSO, is ca. 90% syn conformation while for guanosine (**G**) the chemical shift of H2' = 4.36 ppm corresponds to ca. 40 % syn conformation.[4] Using these values and assuming a linear correlation it can be estimated that the syn:anti ratio for 8MeOG to be ca. 70 syn based on the chemical shift of the H2' = 4.69 ppm. It is important to note that the estimated error in the syn anti ratio from the original papers is ca. 10%.

Rheology. Rheological measurements were taken on a TA AR2000ex rheometer with a Peltier temperature control system and a 20 mm cone (1° angle) with a solvent trap. For the oscillatory

stress sweep studies, the samples were pre-sheared at 500% strain and an angular frequency of 30 rad/s and then were allowed to recover for 60 minutes under 0.2% strain at an angular frequency of 6.28 rad/s. Following recovery, the samples were subjected to a continuous shear ramp from 0.1 to 1000 Pa at a frequency of 6.28 rad/s. A pre-shear, as opposed to a thermal conditioning, was utilized to ensure that the concentration of each sample did not change as a result of evaporation after being loaded into the rheometer. In the case of the shear recovery experiment, the gels were melted followed by monitoring of their recovery at a stress of 0.1 Pa for 30 minutes. The gels were then subjected to a stress of 250 Pa, which resulted in yielding, and their subsequent recovery was again monitored at 0.1 Pa for 30 minutes. All portions of the experiment were done with $\omega = 6.28$ rad/s and T = 25 °C. For the temperature sweep study, a 20 mm parallel plate with a solvent trap was utilized with the Peltier temperature control system. The samples were pre-sheared at 500% strain and an angular frequency of 30 rad/s and then were allowed to recover for 60 minutes under 0.2% strain at an angular frequency of 6.28 rad/s. Following recovery, the samples were heated from 20 °C to 80 °C at a rate of 2 °C/minute under 0.2% strain and an angular frequency of 6.28 rad/s.

TEM. Transmission Electron Microscope measurements were carried out on a Zeiss Libra 200EF microscope utilizing carbon-coated 200 square mesh copper grids. To prepare the samples for analysis, a piece of gel was placed on the grid and allowed to remain for five minutes. The piece was then gently removed with filter paper and the grid allowed to air dry overnight. The following day, the samples were sputter-coated under vacuum with a palladium layer approx. 1 nm in thickness for visualization purposes by utilizing a sputter setpoint of 75%, a rotation setpoint of 25%, a sputter time of 5 s, and a pressure of 22 mtorr.

Circular Dichroism. 0.3 or 1 wt % solutions or gels were placed into a 0.1 mm quartz cuvette and analyzed on a Jasco J-810 CD spectrometer from 325-200 nm with 5 scans per sample.

MTT Cell Proliferation Assay. 50 μL of appropriately concentrated 8OMeTAcG/DMEM/(Gelatin) gel (or straight DMEM in the case of the negative controls) was plated into a 96-well plate so that each sample was done in triplicate. The wells on the periphery of all four sides of the plate were not used and instead filled with DMEM or PBS so as to prevent evaporation over the course of the experiment due to the small well size. The plate was allowed to equilibrate at 37 °C for 30 minutes, after which C166 cells were injected at a density of 5,000 cells/well (in a minimal amount (10 μL) of DMEM) into the gel or DMEM, covered with an additional 100 μL of DMEM, and incubated for 48 hours at 37 °C. Next, 15 μL of the dye solution was added to each well (mixing the contents of each well with the pipette during addition), and the plate was incubated for an additional 4 hours at 37 °C. 100 μL of the solubilization/stop mix was then added to each well, and the plate was wrapped in foil and allowed to incubate in the sterile hood for 1 hour. The contents of each well were then mixed and their absorbance read with a Tecan Safire plate reader (XFLUOR4 Software, COS96ft Plate Definition File, 4 reads/well, 10 flashes, 100 ms between move and read) at a wavelength of 570 nm with a reference wavelength of 650 nm. To convert absorbance values into the normalized

absorbance (and hence % viability), the values from each set of 3 wells were averaged and divided by the averaged absorbance value of the negative control.

FACS Apoptosis Assay. 1 mL of appropriately concentrated 8OMeTAcG/DMEM/(Gelatin) gel (or straight DMEM in the case of the negative and positive controls) was plated into a 6-well plate allowed to equilibrate at 37 °C for 30 minutes, after which C166 cells were injected at a density of 500,000 cells/well (in a minimal amount (100 µL) of DMEM) into the gel or DMEM, covered with an additional 2 mL of DMEM, and incubated for 24 hours at 37 °C. 15 µL of a 1mM stock solution of Camptothecin (5 µM final concentration) was added to the positive control well(s), and the plate was incubated for an additional 24 hours at 37 °C. The cells were then trypsinized (.25% Trypsin) and placed into individual 15 mL centrifuge tubes, washed twice with PBS via centrifugation, and resuspended in 1X Binding Buffer at a concentration of 10^6 cells/mL. 100 µL of the resulting suspensions were transferred to each of two microcentrifuge tubes, where 5 µL of PE Annexin V was added to one, and 5 µL of 7-AAD was added to the other to yield a total of 8 samples. Each tube was gently vortexed, wrapped in foil, and left in the sterile hood for 15 minutes after which an additional 400 µL of 1X Binding Buffer was added to each. The resulting suspensions were transferred into 5 mL culture tubes via pipetting through their cell-strainer caps and analyzed immediately on a BD FACSCalibur flow cytometer. Data was collected using 10,000 counts after gating was optimized for each sample via observation of the FSC-SSC plots. PE Annexin V was read on the FL2 channel with a voltage for 350, and 7-AAD was read on the FL3 channel also with a voltage of 350.

Optical Microscopy. 100 µL of appropriately concentrated 8OMeTAcG/DMEM/(Gelatin) gel (or straight DMEM in the case of the negative control) was plated into a 4-well Nunc Lab-Tek Permanox chamber slide and allowed to equilibrate at 37 °C for 30 minutes, after which GFP+ C166 cells were injected at a density of 10,000 cells/well (in a minimal amount (10 µL) of DMEM) into the gel or DMEM, covered with an additional 200 µL of DMEM, and incubated for 24 hours at 37 °C. The cells were then imaged on a Nikon Eclipse TE300 microscope.

Confocal Microscopy. GFP+ C166 cells were plated in the same manner as what was done for optical microscopy analysis. Confocal microscopy images were taken a Leica TCS SP2 AOBS filter-free UV/spectral confocal laser scanner on an inverted DM IRE2 microscope using wavelengths optimized for GFP.

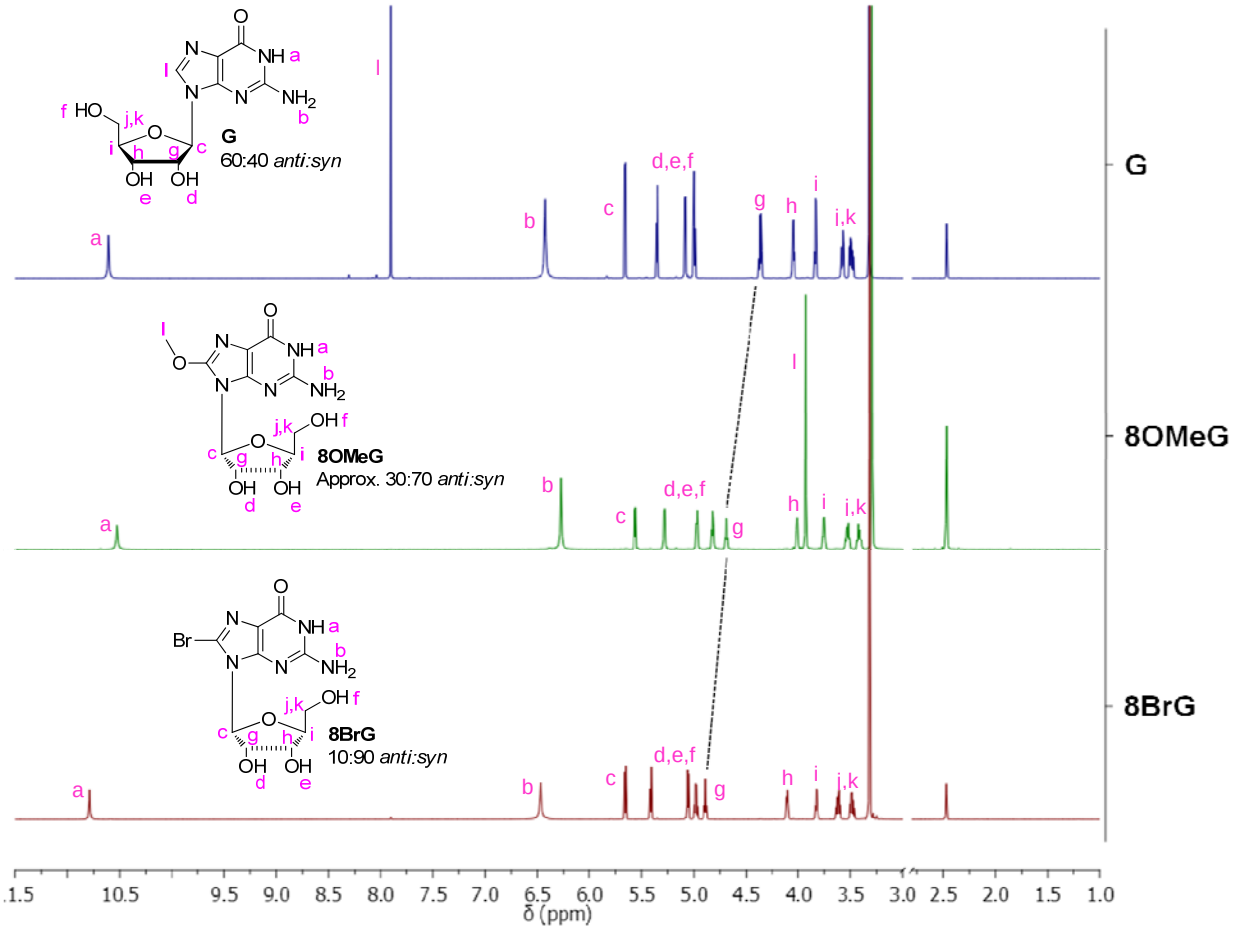

Figure S8. ^{1}H NMR of **G**, **8OMeG**, and **8BrG** in DMSO. The peaks from the protons at the 2'-position are highlighted by the dotted lines showing the steady conversion of the anti conformation to the syn conformation.

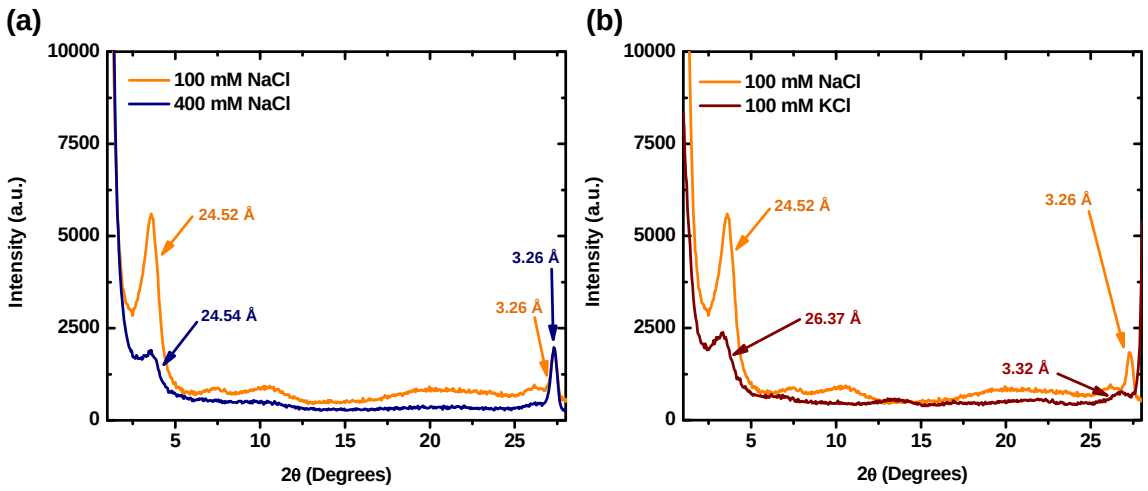

Figure S9. (a) WAXD of **8OMeTAcG** in 100 and 400 mM NaCl. The peaks ca. 24 Å correspond to the width of a single **8OMeTAcG** stack, while the peaks ca. 3 Å correspond to the vertical distance between quartets. The decrease in intensity of the peak ca. 24 Å with increasing salt concentration suggests the increased presence of aggregates too large to be seen in WAXD. (b) WAXD of **8OMeTAcG** in 100 mM NaCl and 100 mM KCl. The width of the stacks decreases from 26.37 to 24.53 Å when switching from potassium to sodium to allow gelation by the smaller sodium ion.

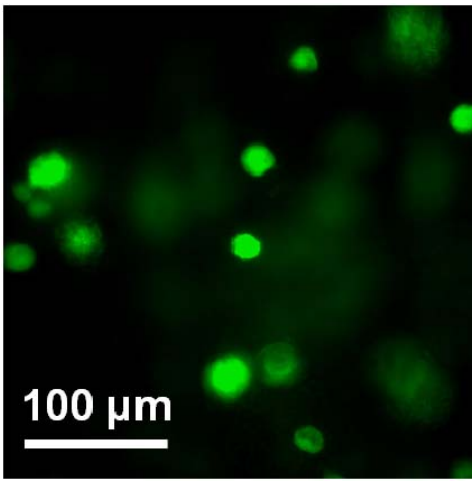

Figure S10. Fluorescence optical microscopy image of GFP+ C166 cells in a 2 wt % **8OMeTAcG**/DMEM gel without gelatin.

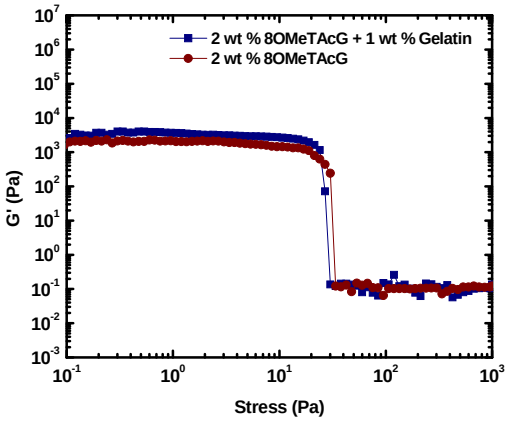

Figure S11. Stress sweep of a 2 wt % **8OMeTAcG** gel compared with a 2 wt % **8OMeTAcG**/1 wt % gelatin gel in DMEM showing the addition of gelatin does not affect the mechanical properties of the gel, where ω = 6.28 rad/s, T = 25 °C, and γ = 0.2%.

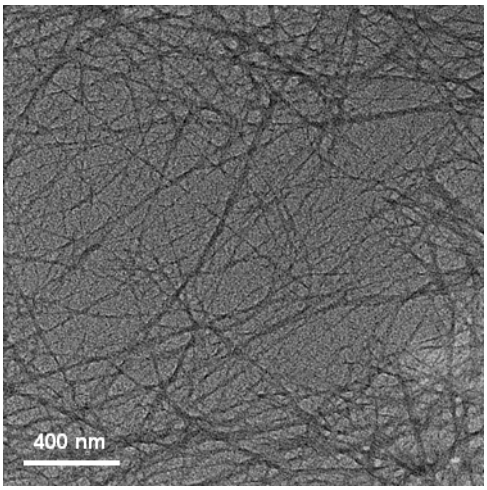

Figure S12. TEM image of a **8OMeTAcG**:**TAcG** gel in 100 mM NaCl with 1 wt % **8OMeTAcG** and 0.2wt% **TAcG**. There is no evident morphological difference between this sample and those of 8OMeTAcG alone.

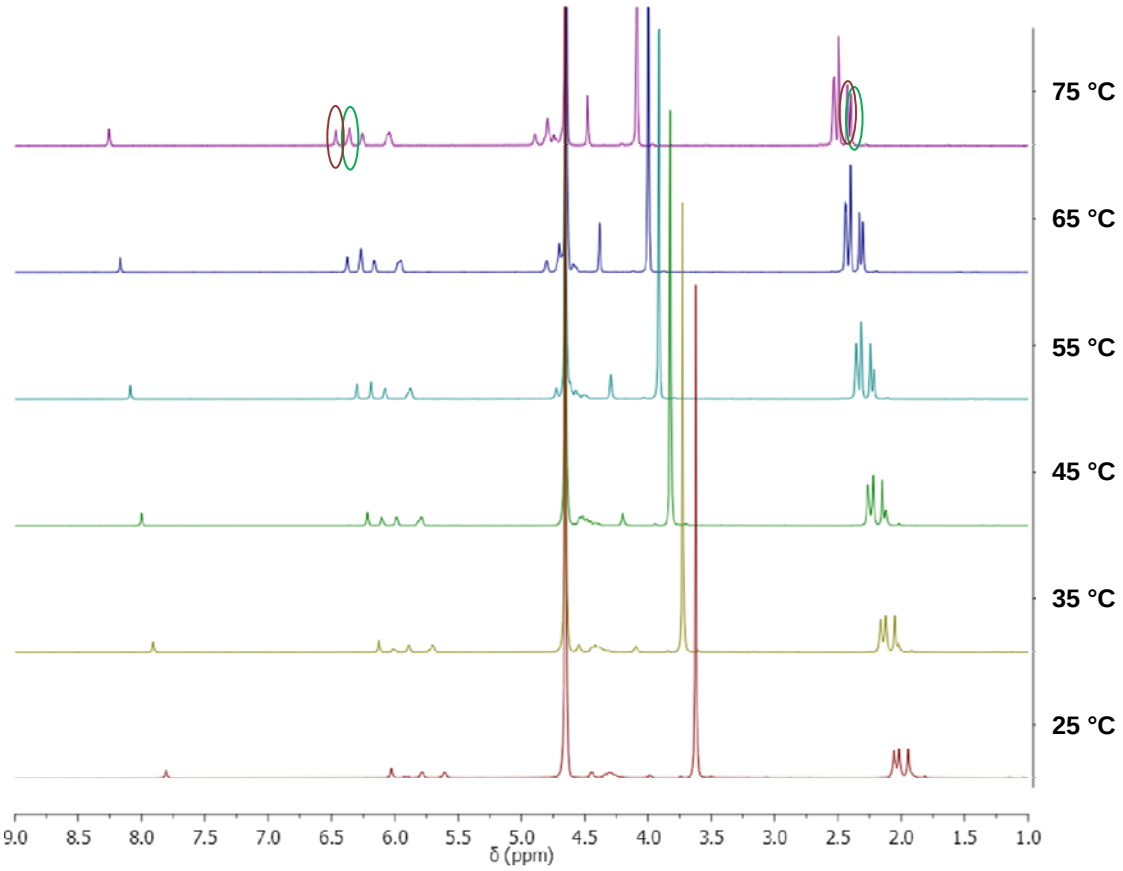

Figure S13. VT-NMR data of a 2 wt % 50/50 **8OMeTAcG**/**TAcG** gel in D_2O. 20 µL of dioxane was added as an internal standard, and thus, by comparing the integrations of the **TAcG** peaks circled in red and the **8OMeTAcG** peaks circled in green with the dioxane, it was established that the **TAcG** is over 50% incorporated into the gel state at 25 °C, while the **8OMeTAcG** is approximately 95% incorporated at 25 °C.

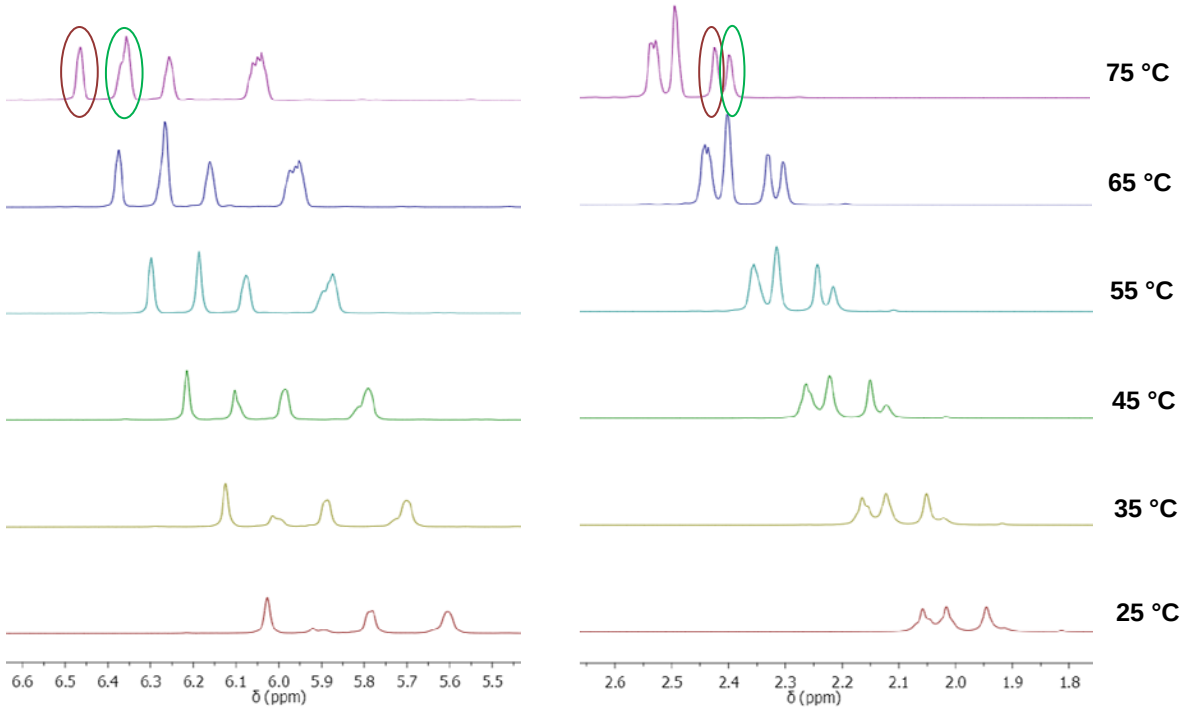

Figure S14. Zoomed VT-NMR data from Figure S14 of a 2 wt % 50/50 **8OMeTAcG/TAcG** gel in 100mM NaCl D_2O solution. 20 µL of dioxane was added as an internal standard, and thus, by comparing the integrations of the **TAcG** peaks circled in red and the **8OMeTAcG** peaks circled in green with the dioxane, it was established that the **TAcG** is over 50% incorporated into the gel state at 25 °C, while the **8OMeTAcG** is approximately 95%

Table S1. Peak integration values for the acetyl peaks (approx. 2 ppm) in the VT-NMR experiment shown in **Figures S14 and S15**.

Temperature	Percent Gelation According to **8OMeTAcG**	Percent Gelation According to **TAcG**
75 °C	0% (assumed)	0% (assumed)
65 °C	2.5%	3.5%
55 °C	42.6%	16.9%
45 °C	65.8%	31.4%
35 °C	83.1%	42.7%
25 °C	93.1%	51.4%

Table S2. Peak integration values for the CH peaks (approx. 6 ppm) in the VT-NMR experiment shown in **Figure S14 and S15**.

Temperature	Percent Gelation According to **8OMeTAcG**	Percent Gelation According to **TAcG**
75 °C	0% (assumed)	0% (assumed)
65 °C	1.8%	3.9%
55 °C	47.7%	14.0%
45 °C	79.5%	39.5%
35 °C	96.5%	54.1%
25 °C	Negligible Integration	59.6%

References

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(2) (a) W. Adam and A. Treiber, *J. Am. Chem. Soc.* 1995, **117**, 2686-2693. (b) W. Luo, J.G. Muller and C.J. Burrows *Org. Lett.* 2001, **3**, 2801-2804.

(3) R. Stolarski, L. Dudycz and D. Shugar, *Eur. J. Biochem*. 1980, **108**, 111-121.

(4) P. Lassota, R. Stolarski and D. Shugar, *Z. Naturforsch.* 1984, **39c**, 55-63.